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Asimakopoulos et al.

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(54) **VERSIKINE FOR INDUCING AND POTENTIATING AN IMMUNE RESPONSE**

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(73) Assignee: **WISCONSIN ALUMNI RESEARCH FOUNDATION**, Madison, WI (US)

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(21) Appl. No.: **15/454,496**

(22) Filed: **Mar. 9, 2017**

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Related U.S. Application Data

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(51) **Int. Cl.**

A61K 39/39	(2006.01)
C07K 14/47	(2006.01)
A61K 39/00	(2006.01)

(52) **U.S. Cl.**

CPC ***A61K 39/39*** (2013.01); ***C07K 14/4725*** (2013.01); *A61K 2039/55516* (2013.01); *A61K 2039/572* (2013.01); *C07K 2319/00* (2013.01)

(58) **Field of Classification Search**

None

See application file for complete search history.

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(74) *Attorney, Agent, or Firm* — Quarles & Brady LLP

(57) **ABSTRACT**

Disclosed are methods, kits, polypeptides, and pharmaceutical compositions for inducing an immune response in a subject, which may include a T-cell mediated immune response. The methods comprise administering to the subject, or to explanted cells of the subject, a pharmaceutical composition comprising an effective amount of versikine or a variant of versikine that induces the T-cell mediated immune response. The methods, kits, polypeptides, and pharmaceutical compositions may be used, in particular, to treat a subject having a cell proliferative disease or disorder.

6 Claims, 24 Drawing Sheets

Specification includes a Sequence Listing.

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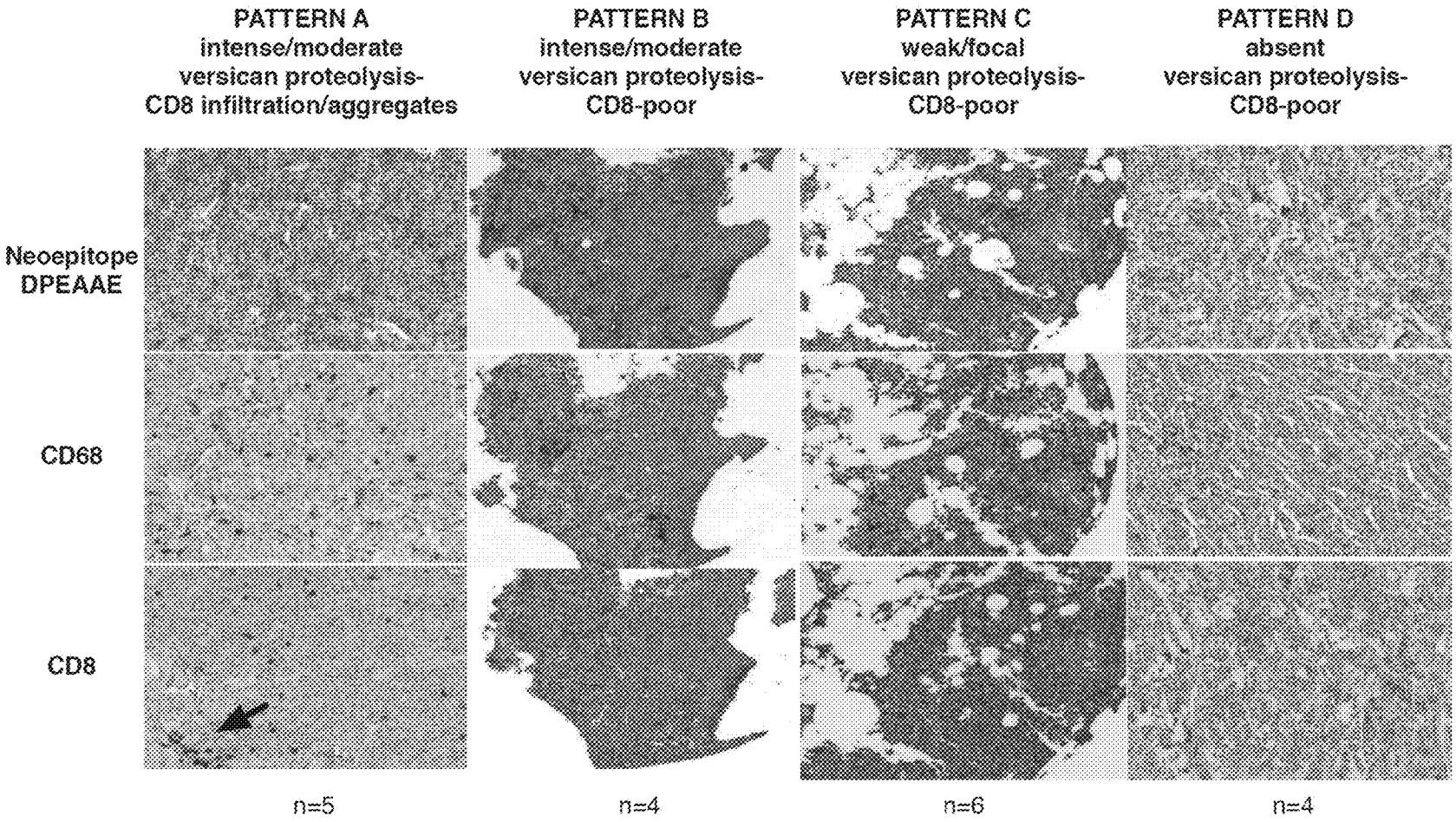
Figure 1


PATTERN A
intense/moderate
versican proteolysis-
CD8 infiltration/aggregates
PATTERN B
intense/moderate
versican proteolysis-
CD8-poor
PATTERN C
weak/focal
versican proteolysis-
CD8-poor
PATTERN D
absent
versican proteolysis-
CD8-poor
Neoepitope
DPEAAE
CD68
CD8
n=5
n=4
n=6
n=4

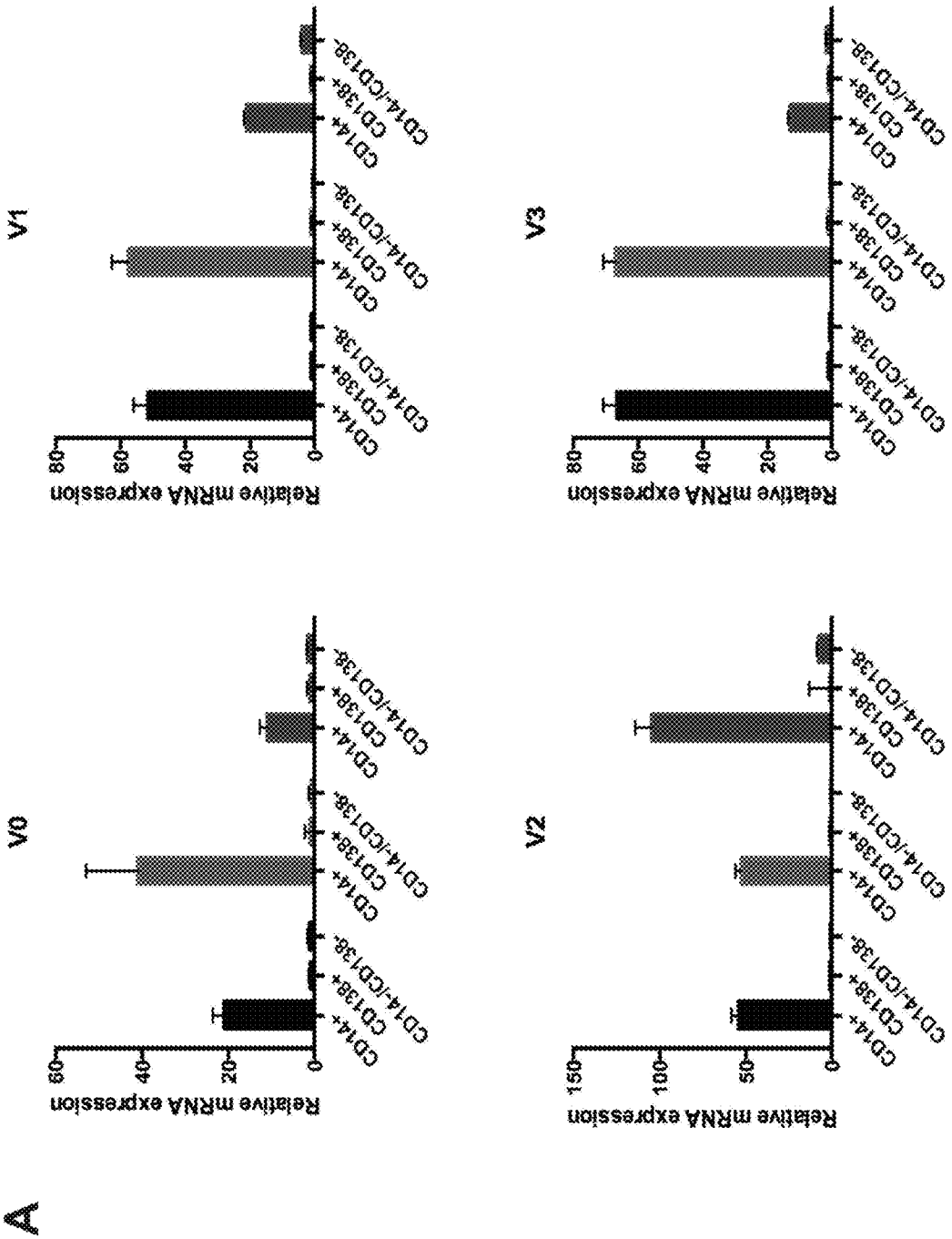

A
V0
V1
V2
V3
Relative mRNA expression
CD14+
CD14+/CD138+
CD14-/CD138+
CD14-/CD138-

Figure 2

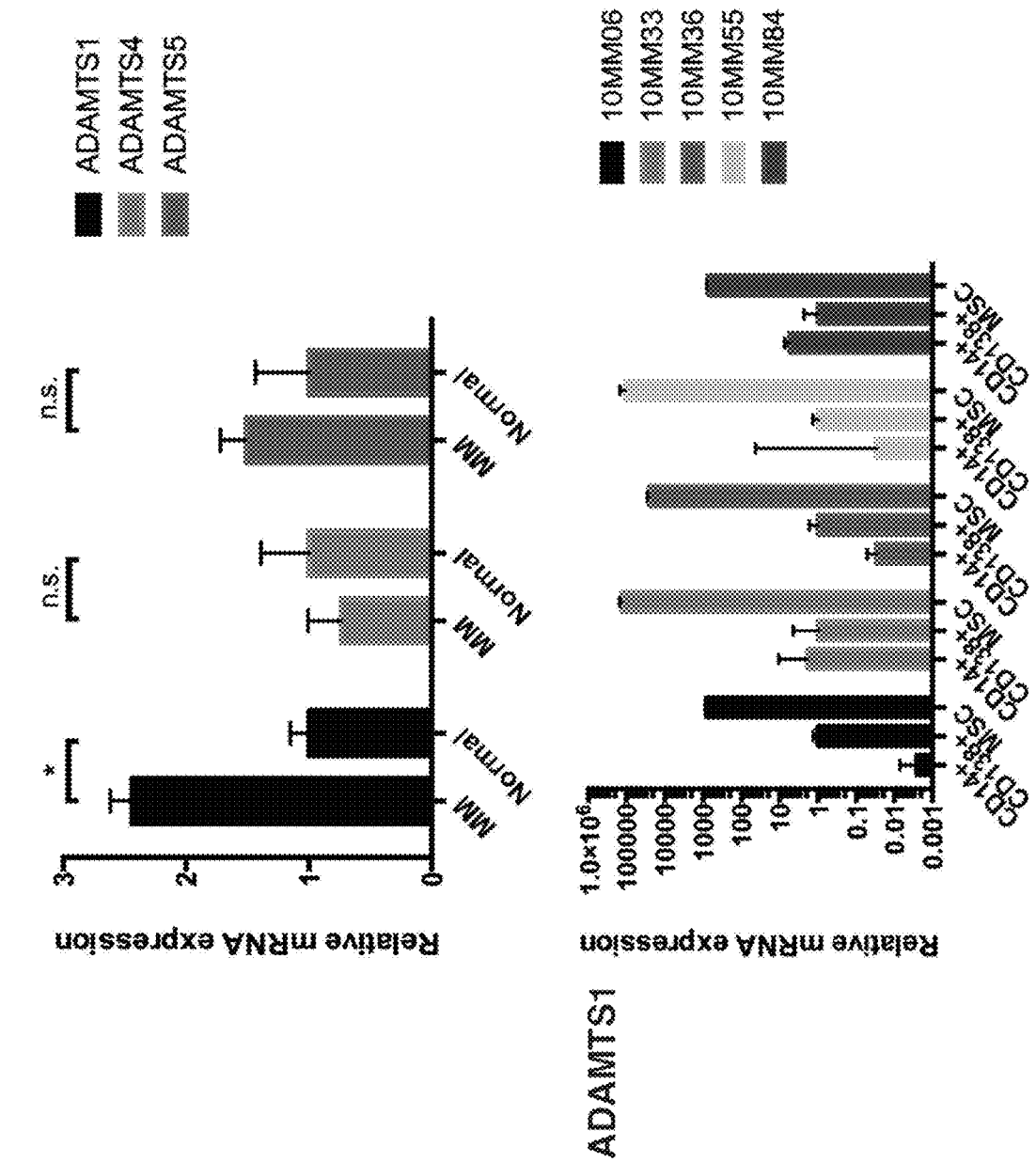

B
ADAMTS1
ADAMTS4
ADAMTS5
Relative mRNA expression
3
2
1
0
*
n.s.
n.s.
MM
Normal
MM
Normal
MM
Normal
C
ADAMTS1
10MM06
10MM33
10MM36
10MM55
10MM84
Relative mRNA expression
1.0×10^6
100000
10000
1000
100
10
1
0.1
0.01
0.001
CD14+CD138+ MSC
CD14+CD138+ MSC
CD14+CD138+ MSC
CD14+CD138+ MSC
CD14+CD138+ MSC

Figure 2

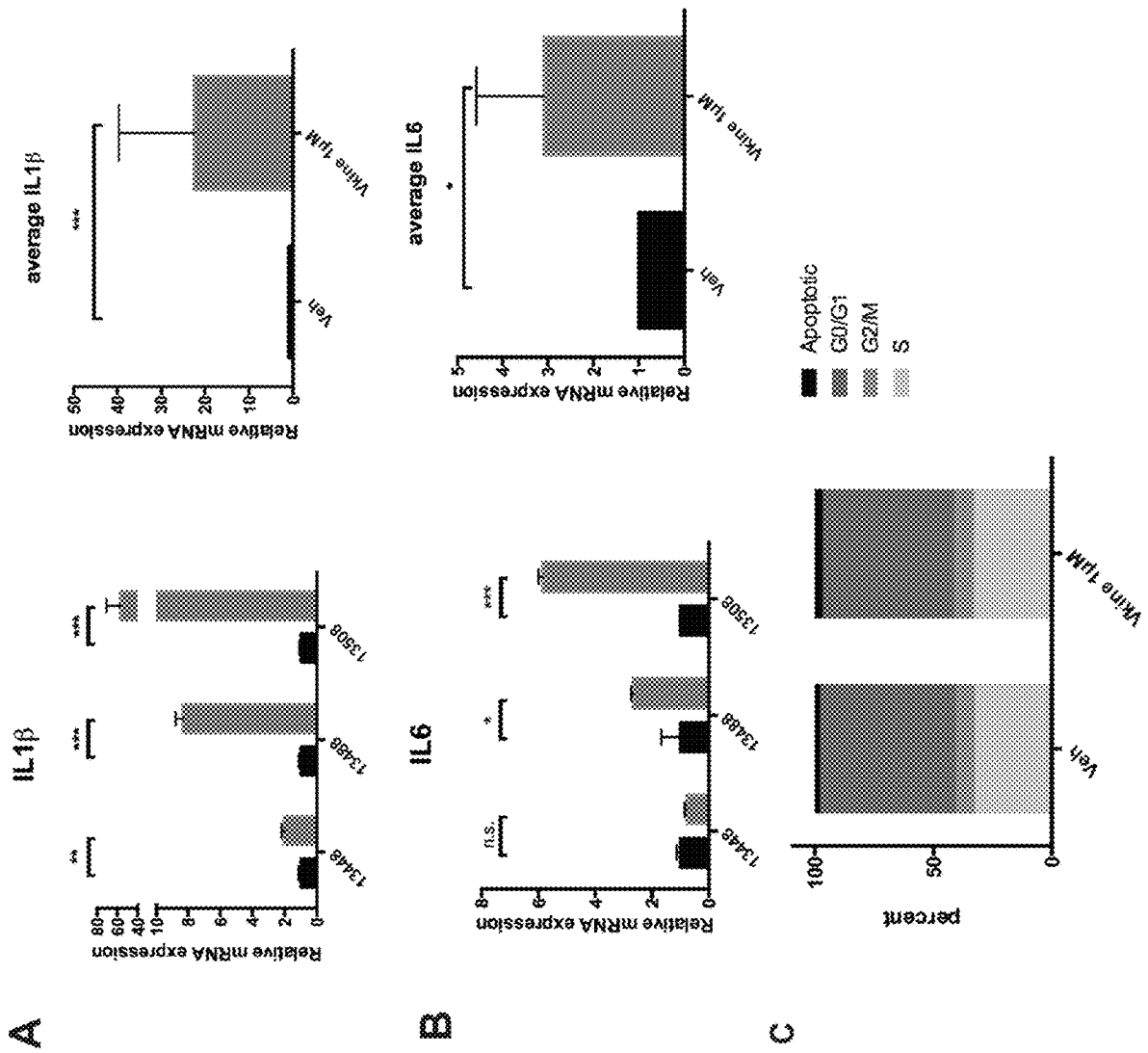

A
IL1β
Relative mRNA expression
1348
13488
13508
B
IL6
Relative mRNA expression
n.s.
1348
13488
13508
C
percent
Veh
Vkine 1μM
Apoptotic
G0/G1
G2/M
S
average IL1β
Relative mRNA expression
Veh
Vkine 1μM
average IL6
Relative mRNA expression
Veh
Vkine 1μM

Figure 3

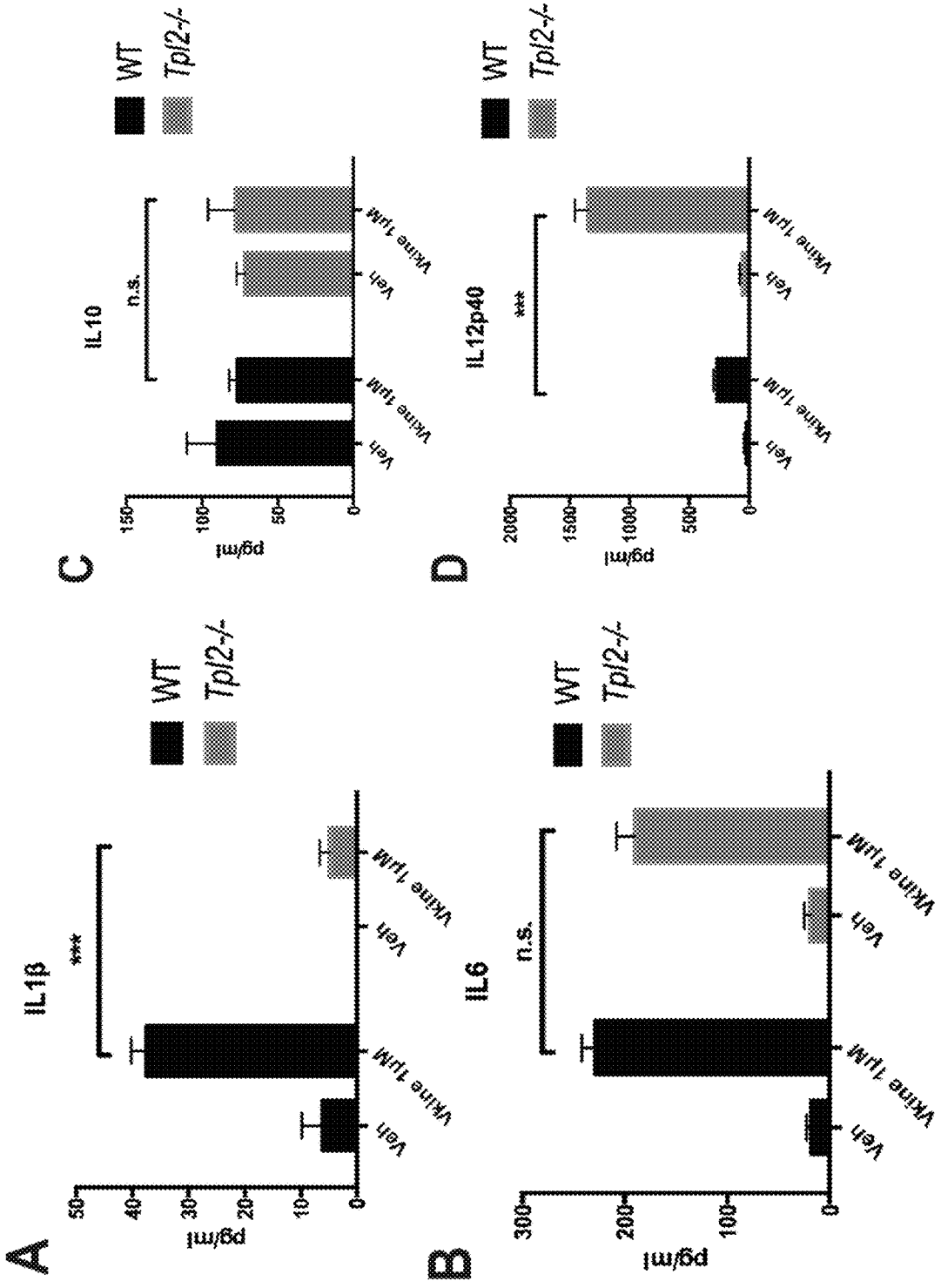

A
IL1β
***
pg/ml
50
40
30
20
10
0
Veh
Vkine 1μM
B
IL6
n.s.
pg/ml
300
200
100
0
Veh
Vkine 1μM
C
IL10
n.s.
pg/ml
150
100
50
0
Veh
Vkine 1μM
D
IL12p40
***
pg/ml
2000
1500
1000
500
0
Veh
Vkine 1μM
WT
Tpl2-/-

Figure 4

Figure 4

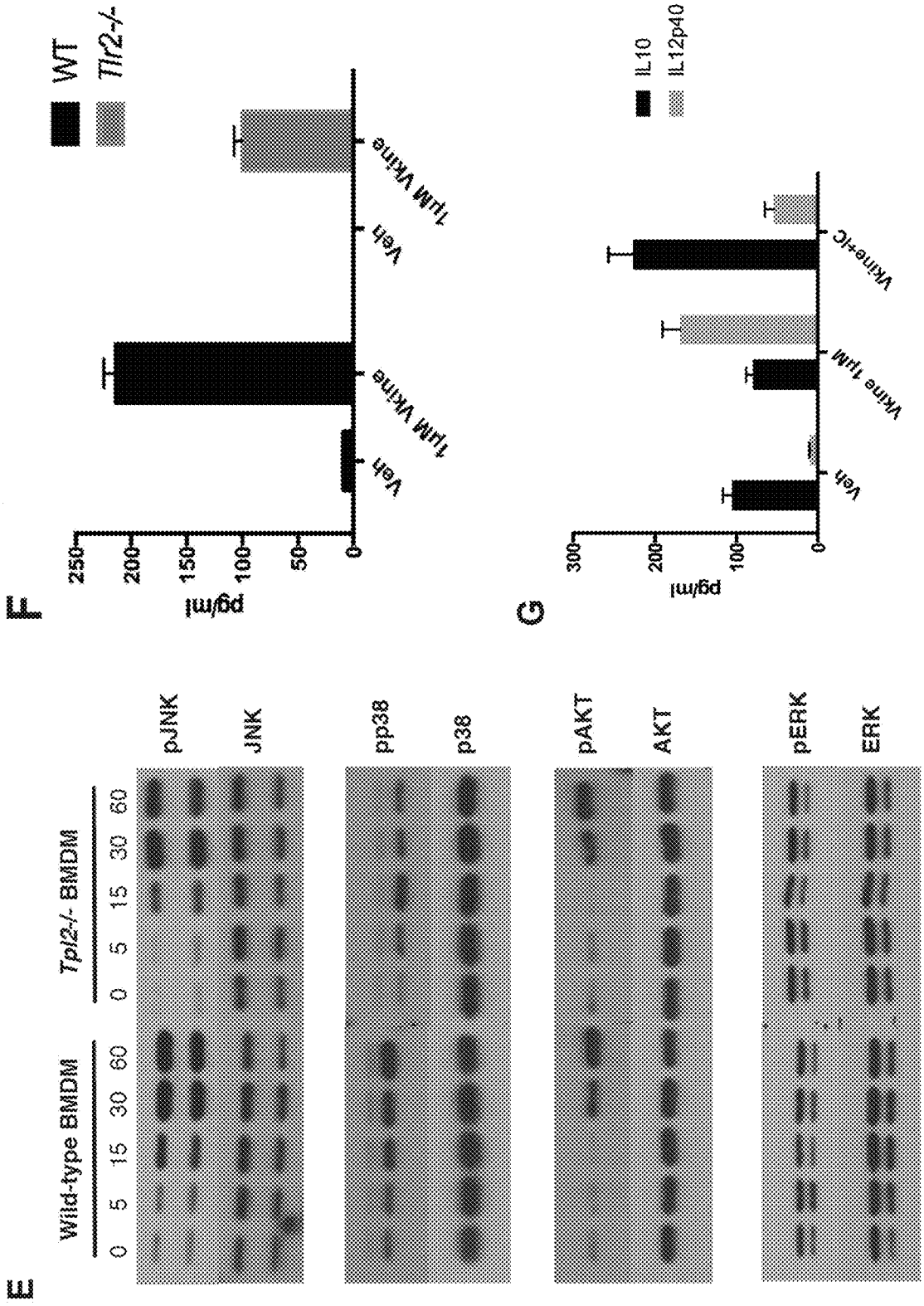

E
Wild-type BMDM
Tpl2-/- BMDM
0 5 15 30 60 0 5 15 30 60
pJNK
JNK
pp38
p38
pAKT
AKT
pERK
ERK
F
WT
Tlr2-/-
pg/ml
250
200
150
100
50
0
Veh
1μM Vkitne
Veh
1μM Vkitne
G
IL10
IL12p40
pg/ml
300
200
100
0
Veh
Vkitne 1μM
Vkitne+IC

Figure 5

A

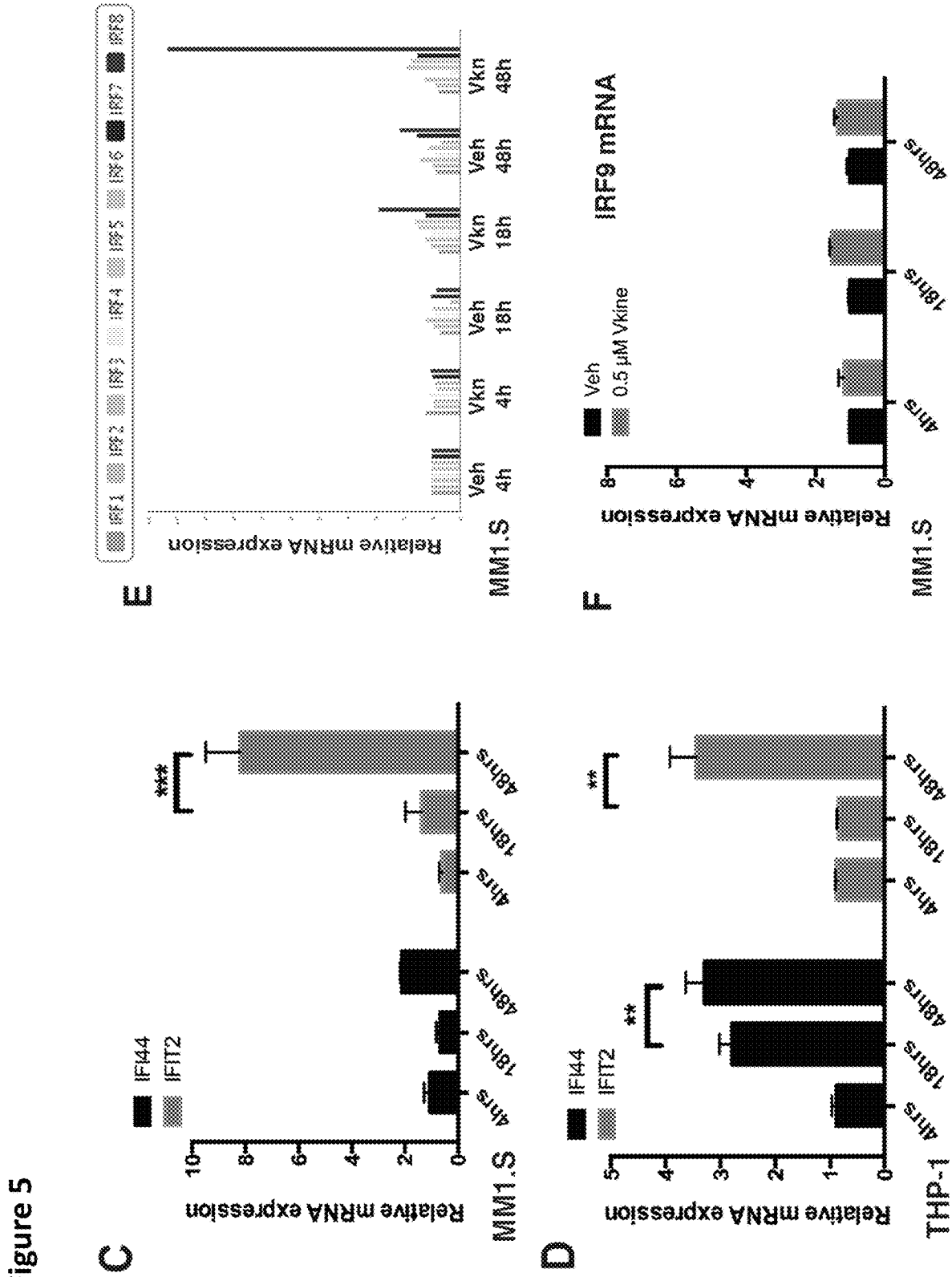
Genes	Log^2 fold
TNFAIP8L2-SCNM1	2.771
VCAN	1.629
MX1	1.560
IFI44L	1.481
IFI44	1.447
THBD	1.393
IFITM1	1.392
TRIM22	1.342
CCL2	1.295
LOC154092	1.293
IL8	1.281
MMP9	1.271
XAF1	1.243
IFIT1	1.214
ZNF618	1.198
GLIS3	1.140
MX2	1.102
IFI6	1.092
PLAU	1.010
OAS3	0.887
OAS2	0.534
PARP14	0.485
STAT1	0.436

B

Genes (1-20)	Log^2 fold
VCAN	3.074
MTHFS	2.723
SIGLEC1	1.719
IFI44L	1.637
PLVAP	1.624
ISG15	1.587
ICAM5	1.583
RSAD2	1.541
IFI44	1.460
OASL	1.454
TRIM22	1.432
ABHD8	1.367
CD14	1.351
MAP1S	1.333
IFIT1	1.323
PHLDA2	1.312
MMRN2	1.295
CCL2	1.277
MMP9	1.249
MX1	1.205
ABCA1	1.174
IFITM1	1.171

Genes (20-40)	Log^2 fold
PCDHGA9	1.150
CCL7	1.140
COL6A2	1.113
LIF	1.080
SIGLEC11	1.077
CMPK2	1.076
ANGPTL6	1.069
ASRGL1	1.038
FCGBP	1.020
LRRC18	1.011
COLEC12	1.011
C9	1.008
GAA	1.002
EBI3	1.151
NBPF11	-8.617
ARL2-SNX15	-4.327
AGPHD1	-1.194
MPDZ	-1.180

Figure 5


C
IFI44
IFIT2
Relative mRNA expression
MM1.S
4hrs
18hrs
48hrs
***
D
IFI44
IFIT2
Relative mRNA expression
THP-1
**
**
E
IRF 1
IRF 2
IRF 3
IRF 4
IRF 5
IRF 6
IRF 7
IRF 8
Relative mRNA expression
MM1.S
Veh
Vkn
4h
18h
48h
F
IRF9 mRNA
Veh
0.5 μM Vkine
Relative mRNA expression
MM1.S
4hrs
18hrs
48hrs

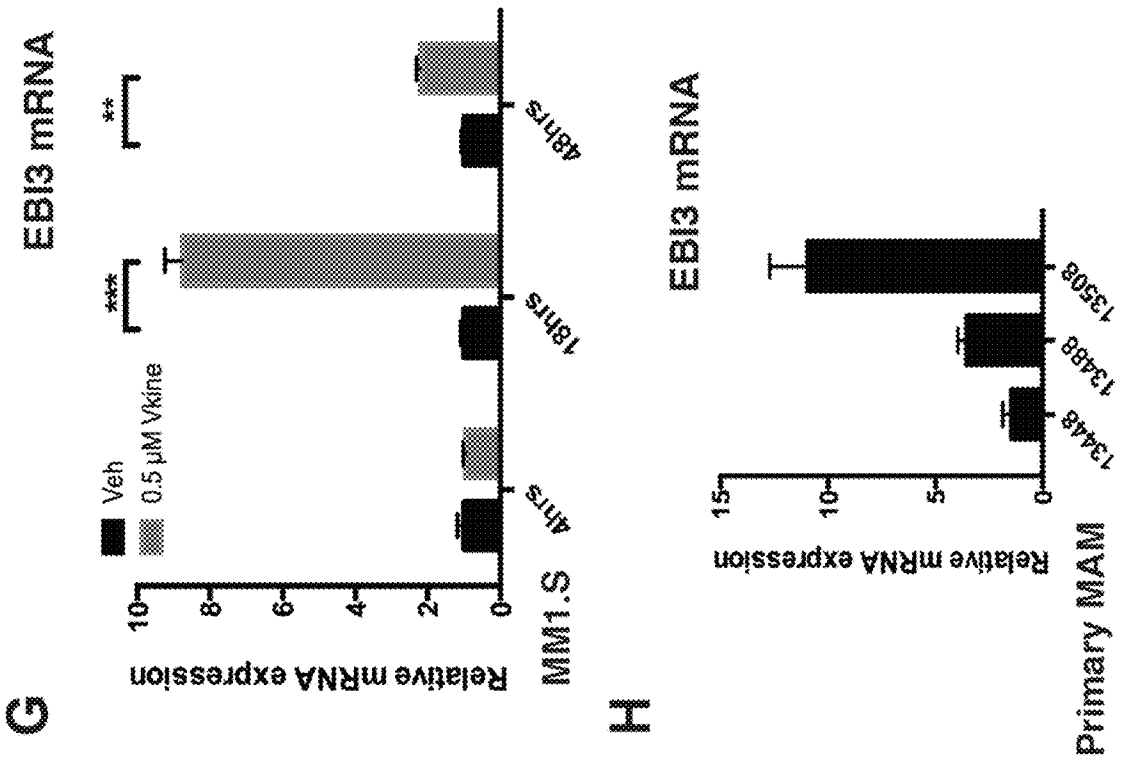

G
EBI3 mRNA
Veh
0.5 μM Vkine
***
**
Relative mRNA expression
10
8
6
4
2
0
4hrs
18hrs
48hrs
MM1.S
H
EBI3 mRNA
Relative mRNA expression
15
10
5
0
13448
13488
13508
Primary MAM

Figure 5

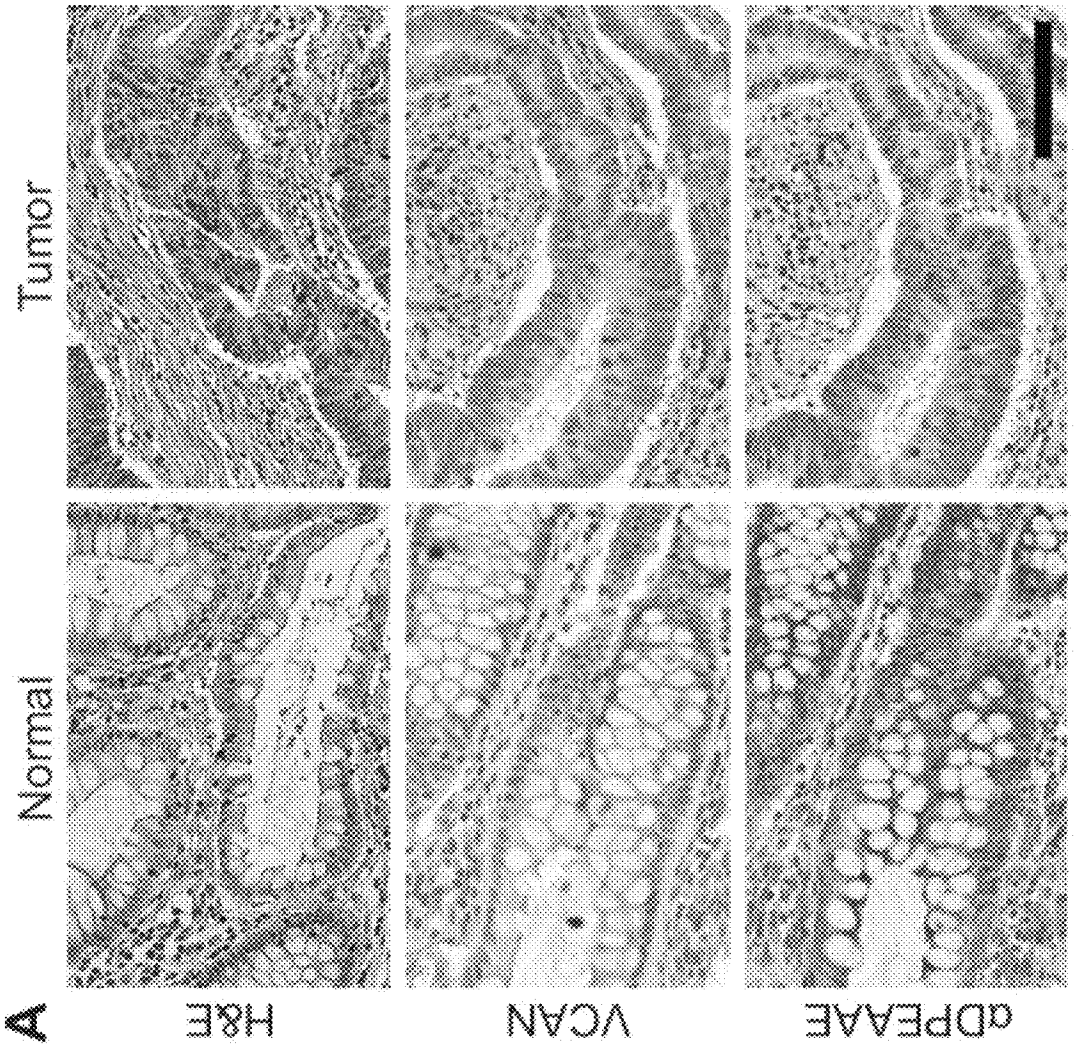


Figure 6

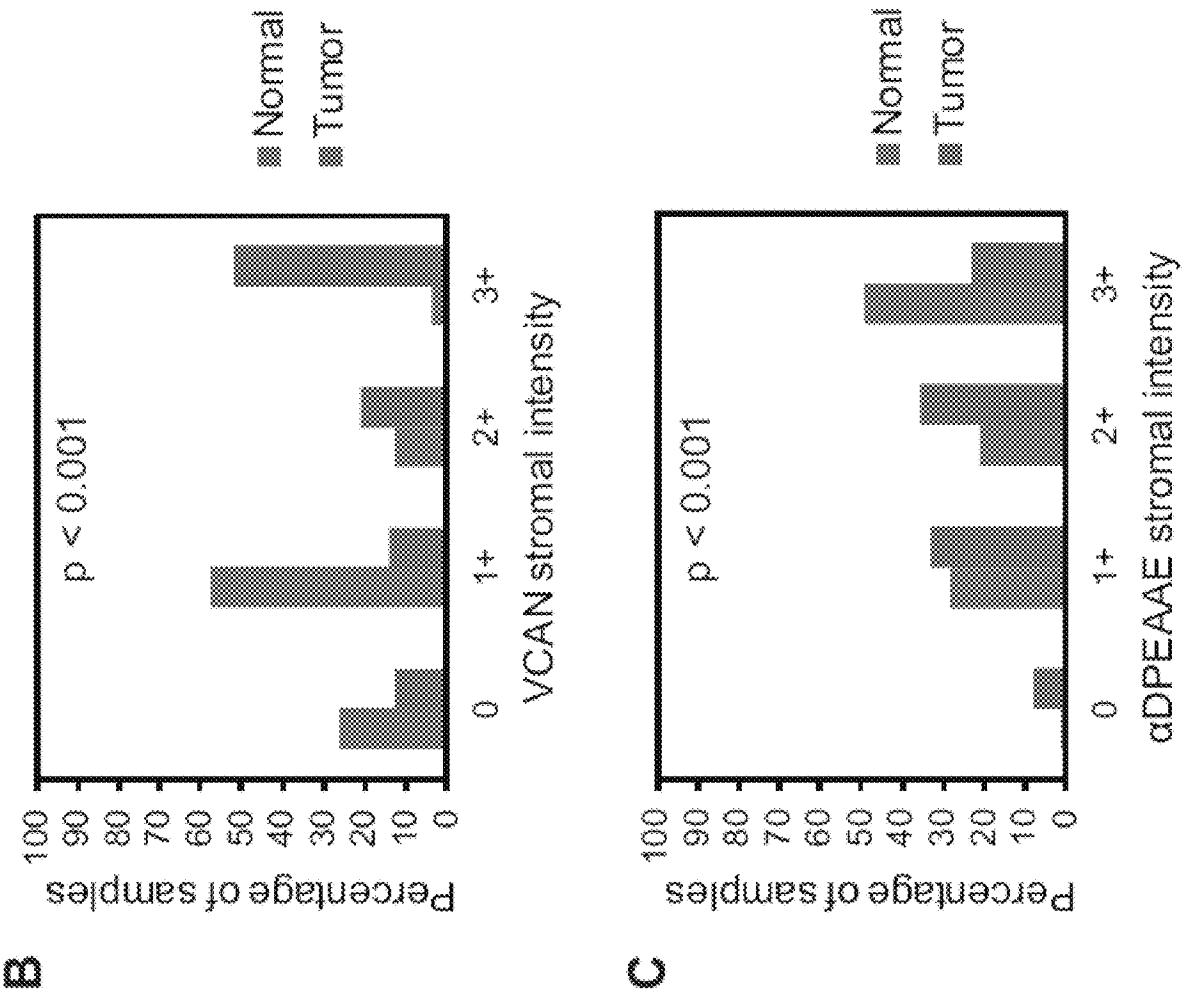

B
Normal
Tumor
p < 0.001
Percentage of samples
100
90
80
70
60
50
40
30
20
10
0
0
1+
2+
3+
VCAN stromal intensity
C
Normal
Tumor
p < 0.001
Percentage of samples
100
90
80
70
60
50
40
30
20
10
0
0
1+
2+
3+
αDPEAAE stromal intensity

Figure 6

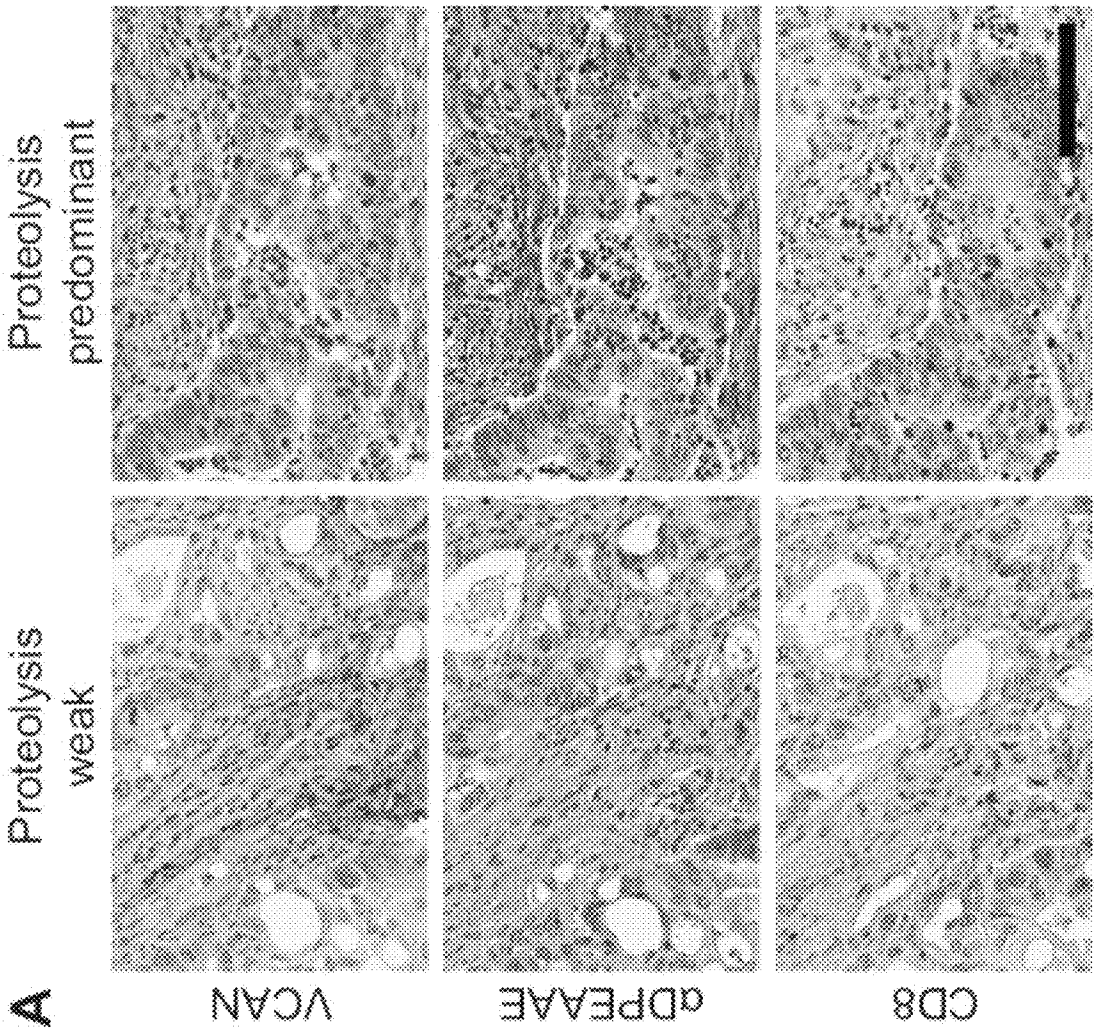

A
VCAN
αDPEAAE
CD8
Proteolysis weak
Proteolysis predominant

Figure 7

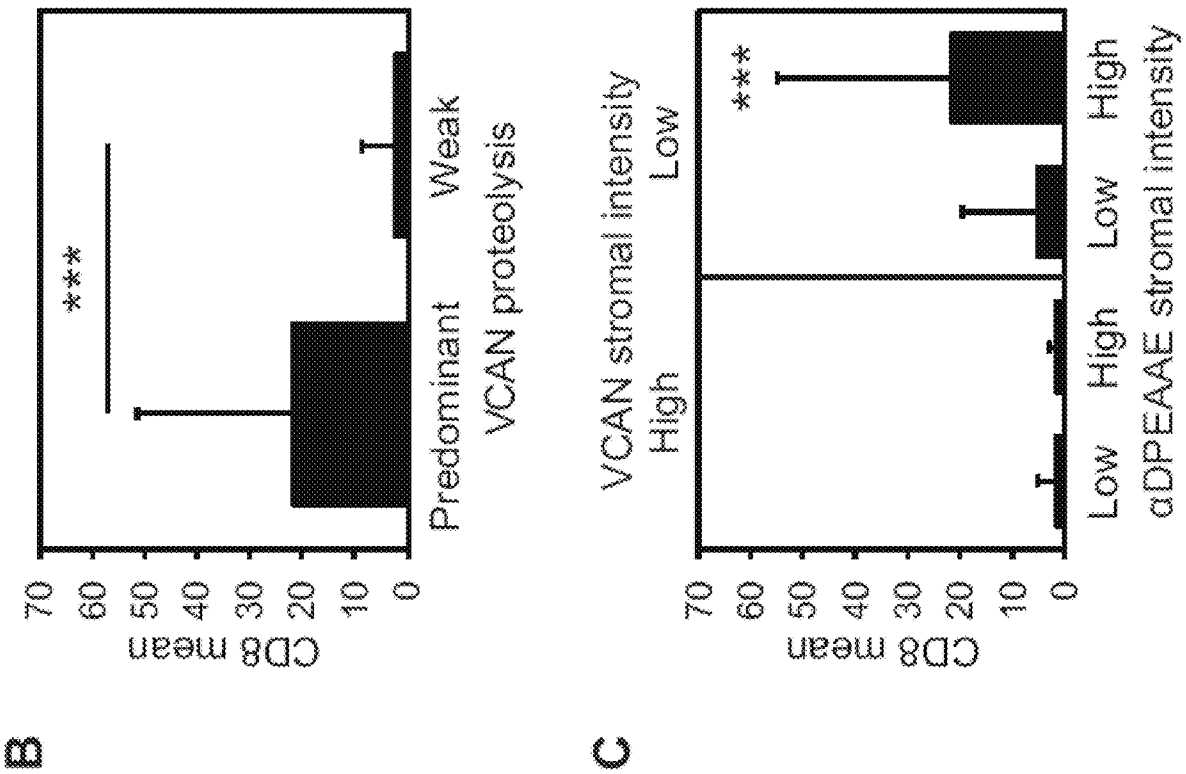
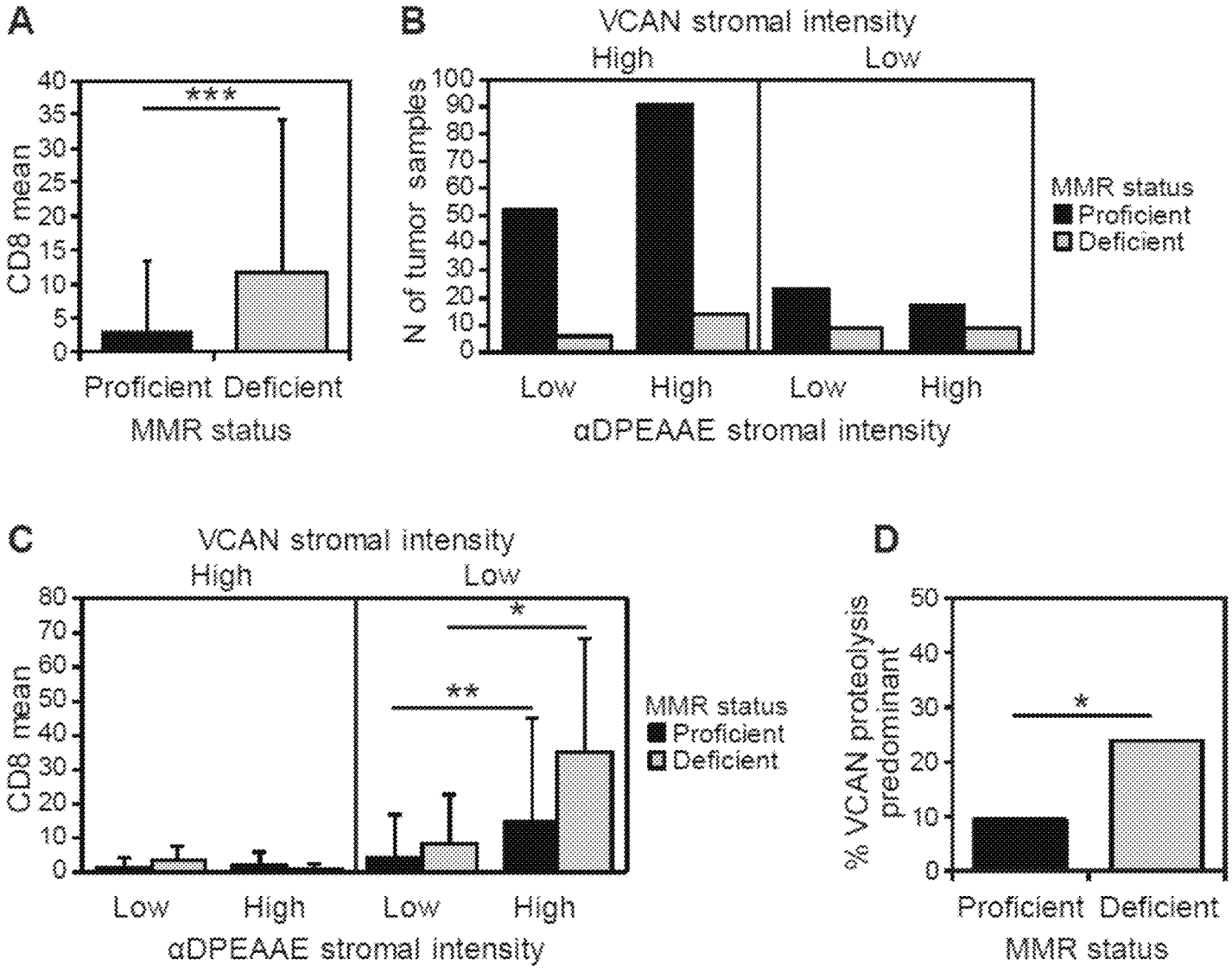

B
CD8 mean
70
60
50
40
30
20
10
0
***
Predominant
Weak
VCAN proteolysis
C
CD8 mean
70
60
50
40
30
20
10
0
***
VCAN stromal intensity
High
Low
Low
High
Low
High
αDPEAAE stromal intensity

Figure 7

Figure 8


A
CD8 mean
***
Proficient Deficient
MMR status
B
VCAN stromal intensity
High
Low
N of tumor samples
MMR status
Proficient
Deficient
Low
High
Low
High
αDPEAAE stromal intensity
C
VCAN stromal intensity
High
Low
CD8 mean
*
**
MMR status
Proficient
Deficient
Low
High
Low
High
αDPEAAE stromal intensity
D
% VCAN proteolysis predominant
*
Proficient Deficient
MMR status

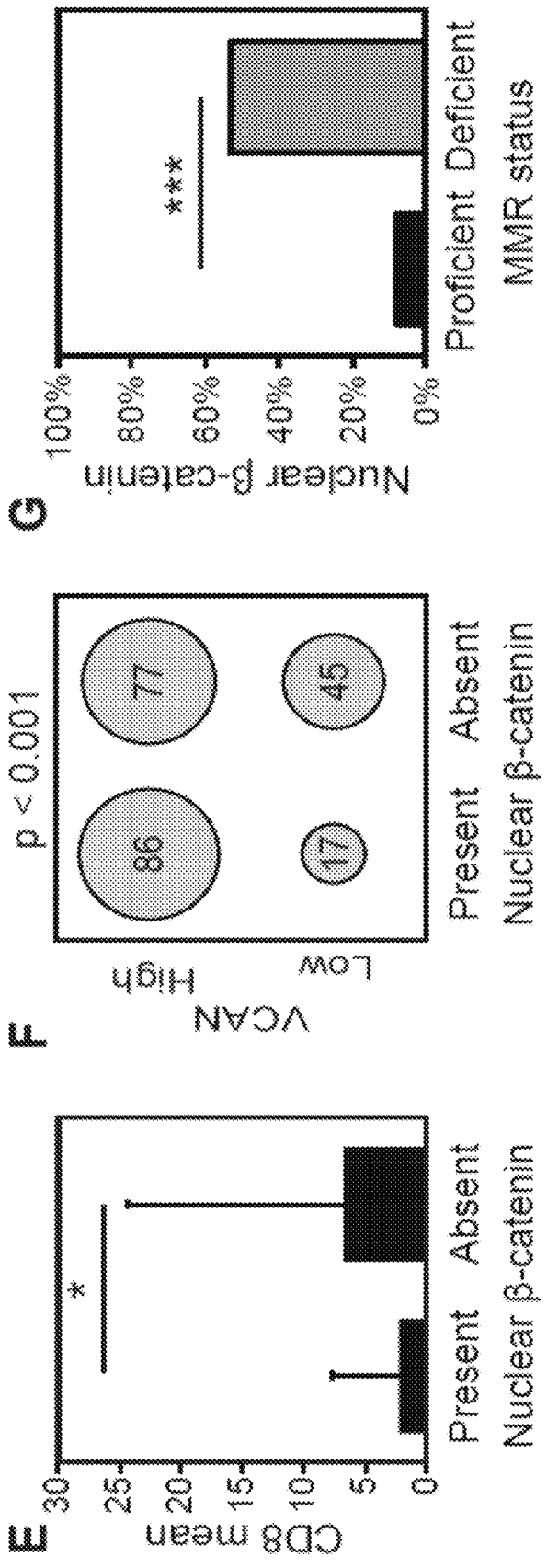
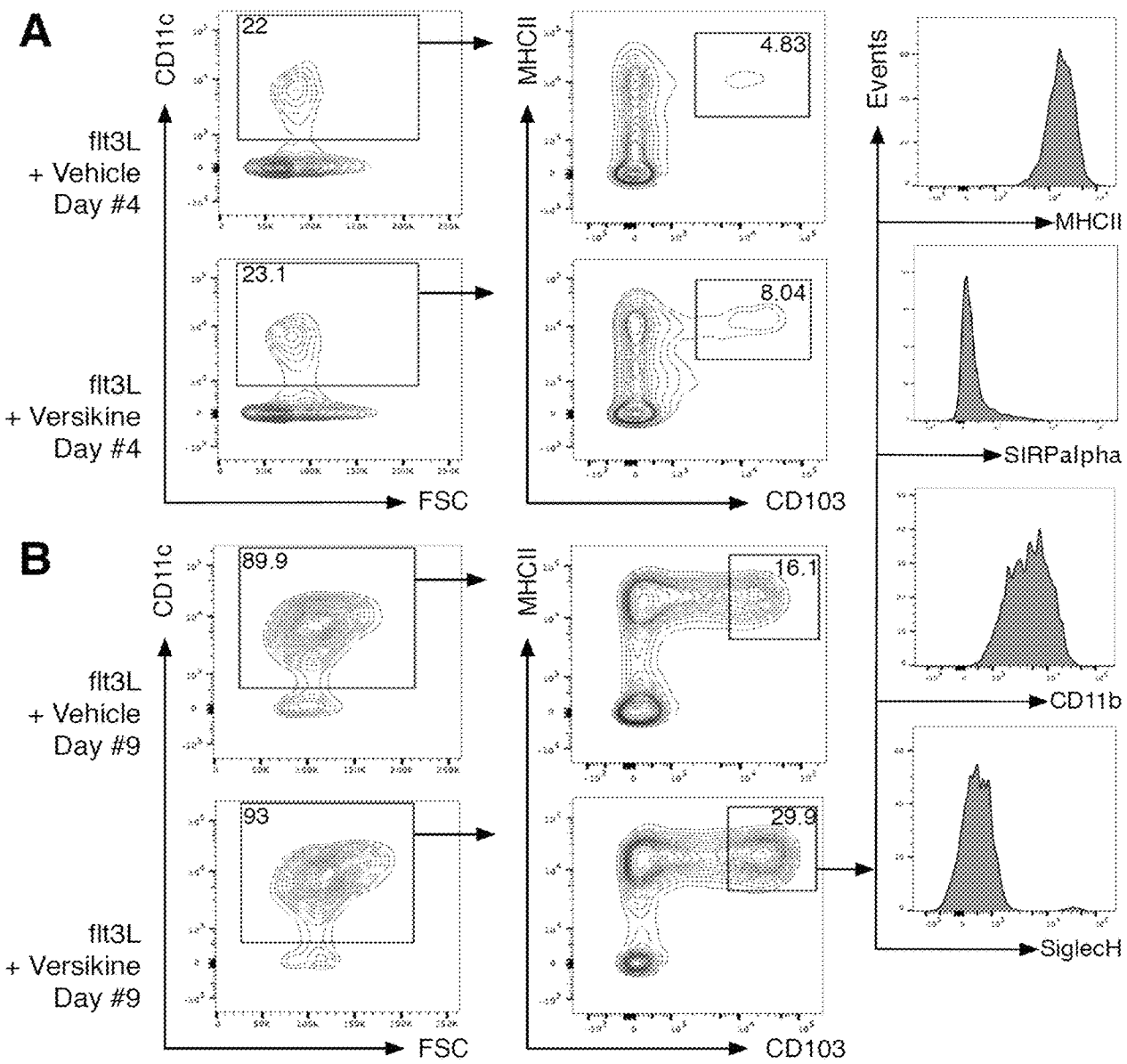

E
CD8 mean
30
25
20
15
10
5
0
*
Present Absent
Nuclear β-catenin
F
VCAN
High
Low
p < 0.001
86
17
77
45
Present Absent
Nuclear β-catenin
G
Nuclear β-catenin
100%
80%
60%
40%
20%
0%
***
Proficient Deficient
MMR status

Figure 8

Figure 9


A
CD11c
22
MHCII
4.83
Events
flt3L
+ Vehicle
Day #4
MHCII
23.1
8.04
flt3L
+ Versikine
Day #4
SIRPalpha
FSC
CD103
B
CD11c
89.9
MHCII
16.1
flt3L
+ Vehicle
Day #9
CD11b
93
29.9
flt3L
+ Versikine
Day #9
SiglecH
FSC
CD103

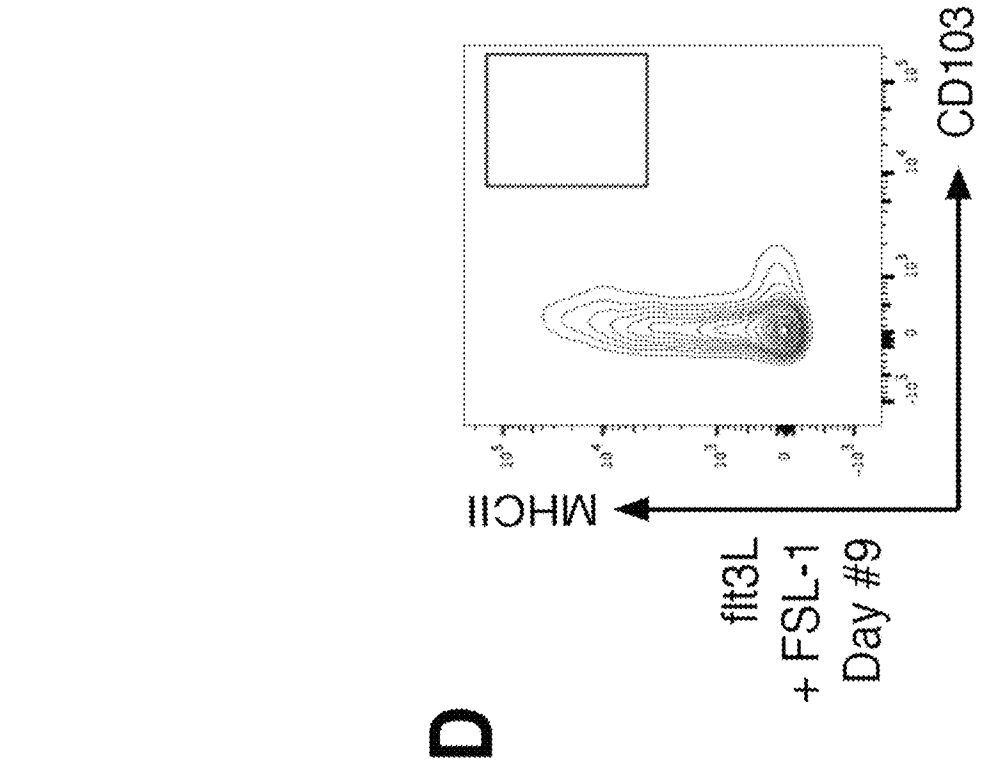


FIGURE 4

Figure 9

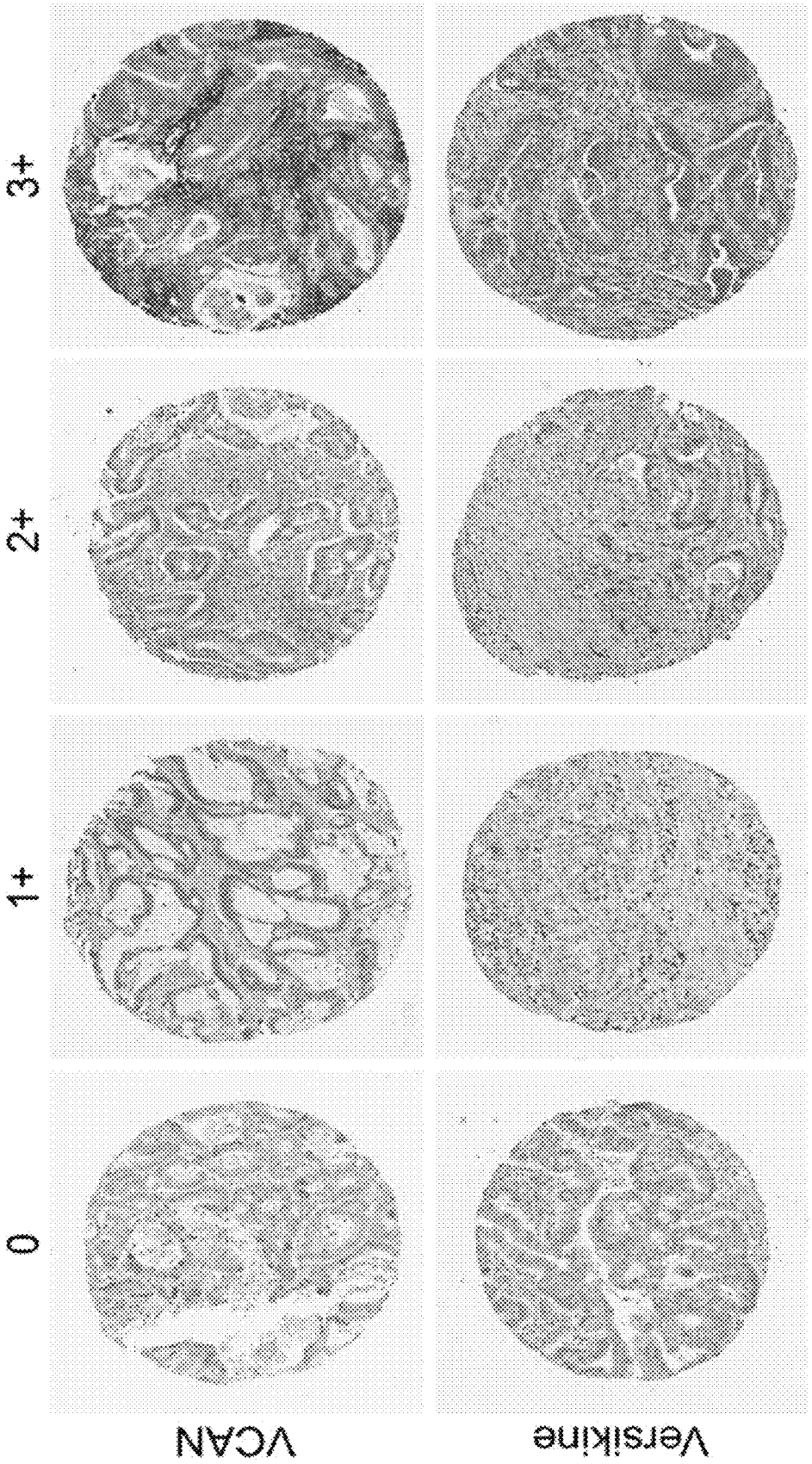
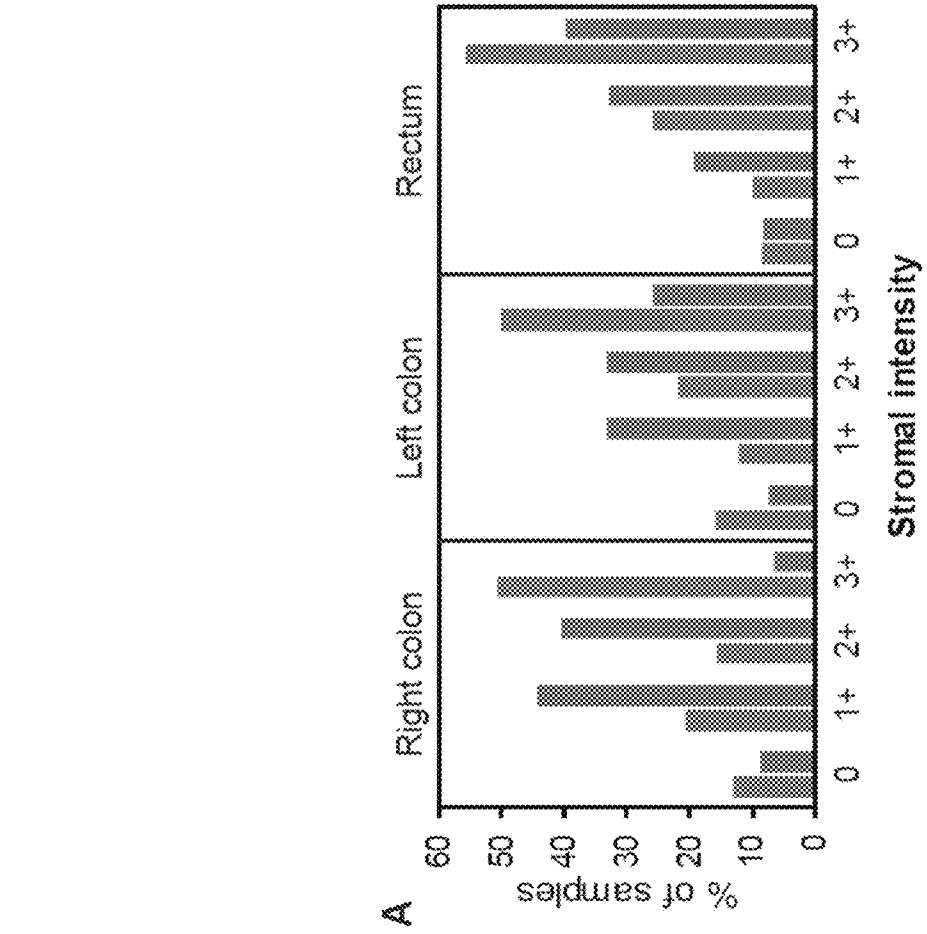


Figure 10

Figure 11



Chi-square test
Table VCAN_location BINNED

Location	Low	High	Total
LeftColon	23	59	82
Rectum	13	57	70
RightColon	26	51	77
Total	62	167	229

X2 = 4.349, P = 0.1137 (2 df)

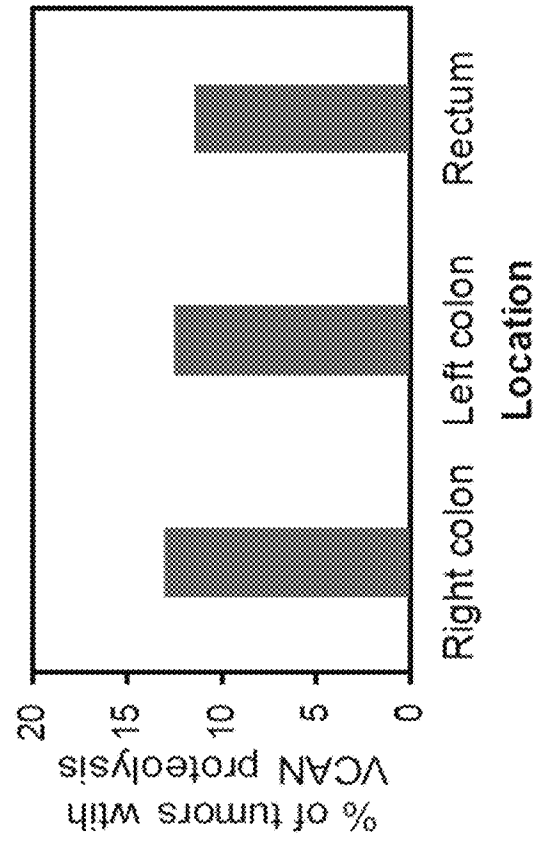
Chi-square test
Table Vkine_location BINNED

Location	Low	High	Total
RightColon	42	37	79
LeftColon	33	48	81
Rectum	21	52	73
Total	96	37	232

X2 = 9.333, P = 0.009405 (2 df)

Figure 11

B



Chi-square test

Table VCANprot_v_location

Location	No	Yes	Total
LeftColon	70	10	80
Rectum	62	8	70
RightColon	67	10	77
Total	199	28	227

X2 = 0.08547, P = 0.9582 (2 df)

Chi-square test

Table VCANFrot_v_stage

SimpleStage	No	Yes	Total
I	48	11	59
II	47	5	52
III	54	8	62
IV	50	4	54
Total	199	28	227

X2 = 3.759, P = 0.2887 (3 df)

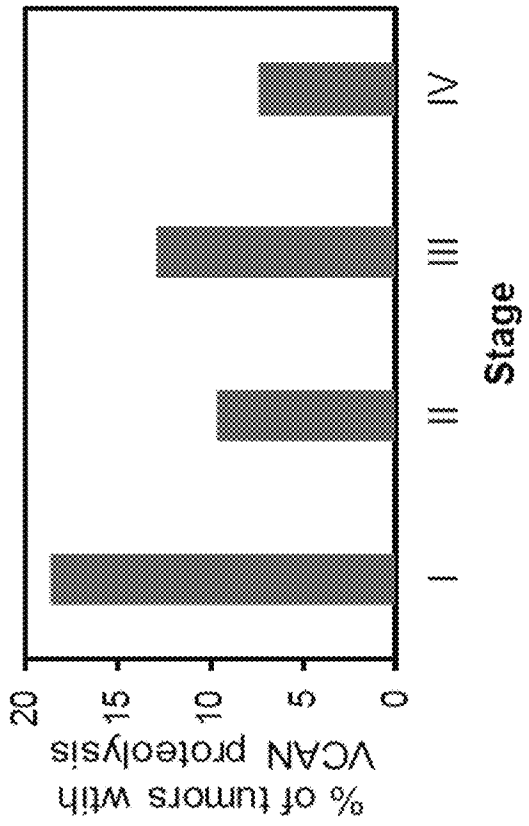
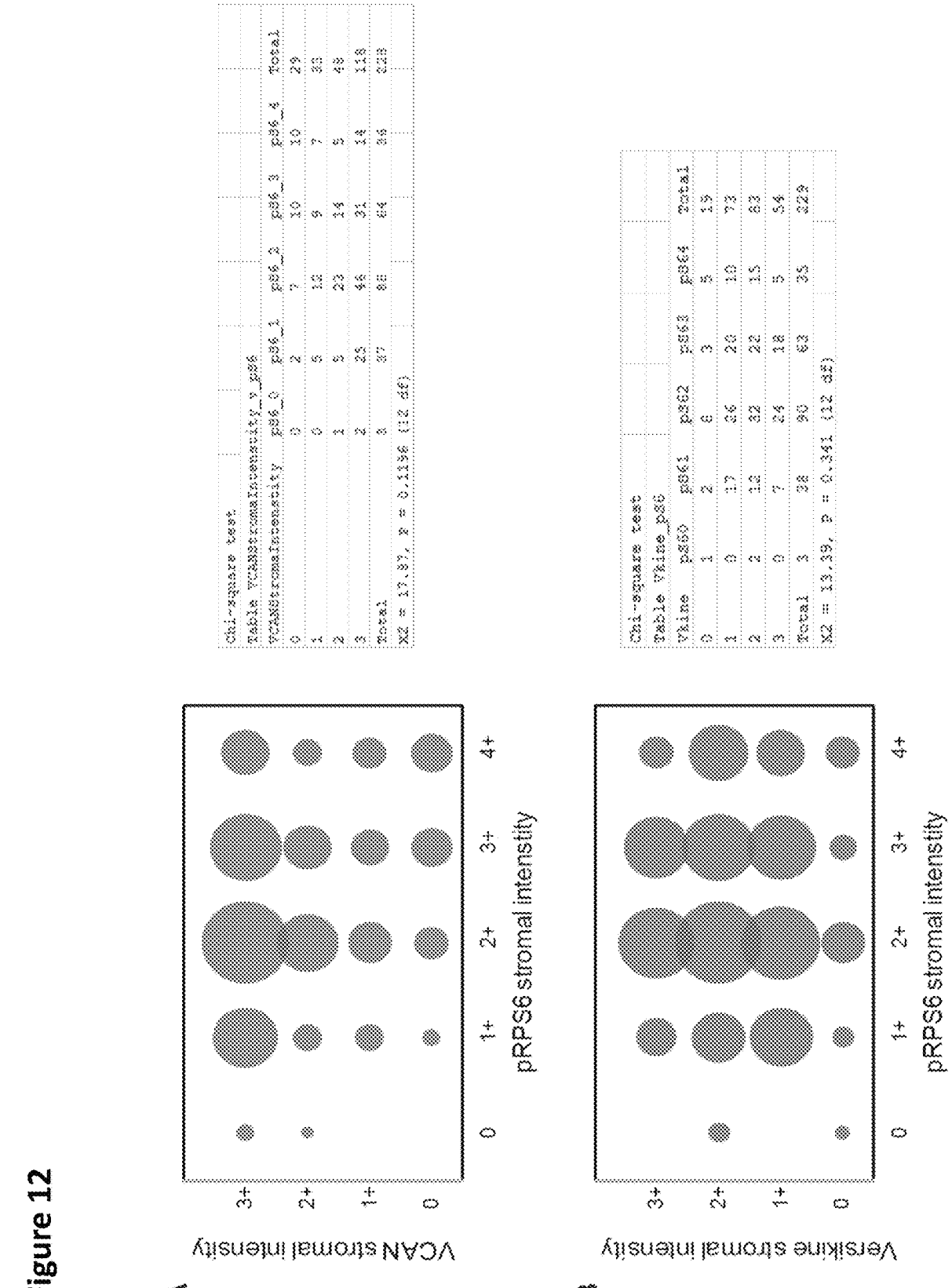


Figure 11

Figure 12



Chi-square test

Table VCANStromalIntensity_v_p86

VCANStromalIntensity	p86_0	p86_1	p86_2	p86_3	p86_4	Total
0	0	2	7	10	10	29
1	0	6	12	9	7	34
2	1	5	23	14	5	48
3	2	25	46	31	14	118
Total	3	37	88	64	36	228

X2 = 17.87, p = 0.1196 (12 df)

Chi-square test

Table VkLine_p86

VkLine	p860	p861	p862	p863	p864	Total
0	1	2	8	3	5	19
1	0	17	26	20	10	73
2	2	12	32	22	15	83
3	0	7	24	18	5	54
Total	3	38	90	63	35	229

X2 = 13.39, p = 0.341 (12 df)

Figure 12

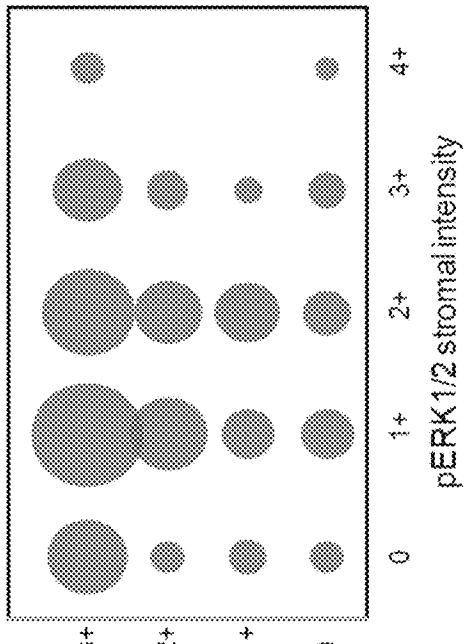
C

Chi-square test

Table Vcan_ERK

VCAN	ERK_0	ERK_1	ERK_2	ERK_3	ERK_4	Total
0	4	10	8	5	4	25
1	5	10	15	3	0	33
2	4	24	16	6	0	46
3	22	45	30	17	4	118
Total	35	87	69	31	6	228

X2 = 12.71, P = 0.3901 (12 df)



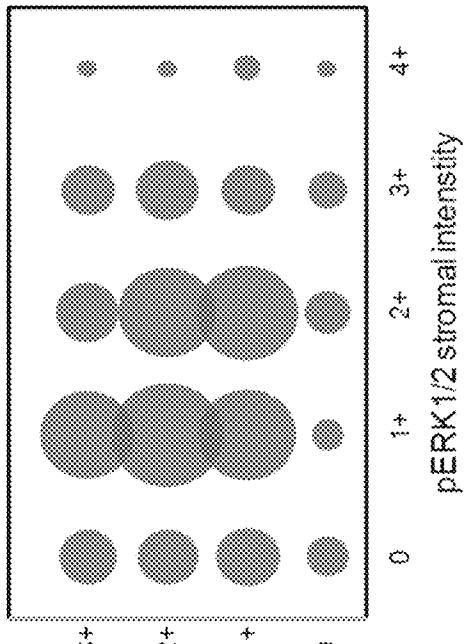
D

Chi-square test

Table Vkine_pERK

VKinestromalIntensity	pERK0	pERK1	pERK2	pERK3	pERK4	Total
0	3	3	6	4	1	19
1	11	27	28	8	2	76
2	10	35	24	11	1	83
3	9	24	11	8	1	53
Total	35	89	71	31	5	231

X2 = 11.23, P = 0.5095 (12 df)



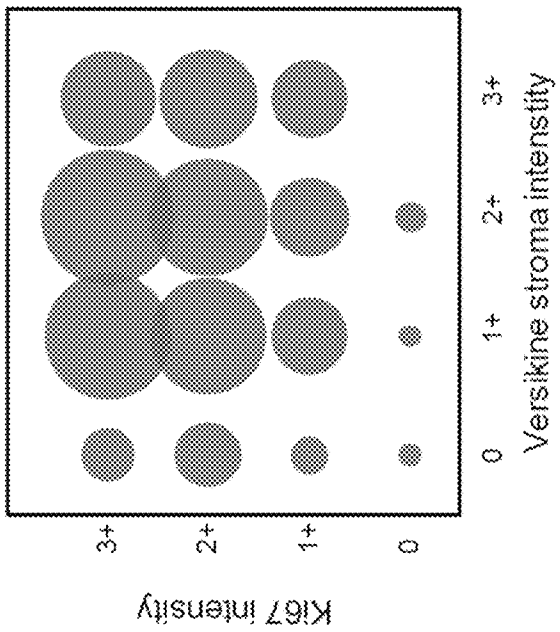


Figure 13

Chi-square test

Table Vkine_Ki67

Vkine	Ki67_0	Ki67_1	Ki67_2	Ki67_3	Total
0	1	3	9	6	19
1	1	12	28	33	74
2	2	13	29	37	81
3	0	12	20	19	51
Total	4	40	86	95	225

X2 = 5.419, P = 0.7964 (9 df)

VERSIKINE FOR INDUCING AND POTENTIATING AN IMMUNE RESPONSE

CROSS-REFERENCE TO RELATED PATENT APPLICATIONS

The present application claims the benefit of priority under 35 U.S.C. § 119(e) to U.S. Provisional Application No. 62/437,418, filed on Dec. 21, 2016, and to U.S. Provisional Application No. 62/343,414, filed on May 31, 2016, and to U.S. Provisional Application No. 62/305,761, filed on Mar. 9, 2016, the contents of which are incorporated herein by reference in their entireties.

BACKGROUND

The field of the invention relates to methods and compositions for inducing and/or potentiating an immune response. In particular, the field of the invention relates to methods and compositions that utilize and/or include versikine for inducing and/or potentiating a T-cell mediated immune response.

Versican, also known by the synonyms PG-M and CSPG2, was identified first in the culture of labeled fibroblasts. (See Coster et al., (1979)). Versican is a chondroitin sulfate (CS) proteoglycan that belongs to a family of hyaluronan (HA) binding proteins. The human versican gene is located on chromosome 5q and contains 15 exons. The versican glycoprotein comprises three major functional domains including: an N-terminal globular domain that mediates HA binding via two linking sub-domains, one or two alternatively spliced glycosaminoglycan (GAG) attachment domains referred to as GAGα and GAGβ, and a C-terminal G3 domain. (See Zimmermann et al., (1989)). Five different splice variants result in five different versican isoforms referred to as V0, V1, V2, V3, and V4. (See Dours-Zimmermann, et al., (1994)). Versican V0 contains both GAGα and GAGβ attachment exons and is the largest isoform, containing up to 23 CS chains; versican V1 contains only exon 8 and has up to 15 CS chains; versican V2 contains only exon 7 and has up to 8 GAG attachment sites; versican V3 does not contain either large exon and thus lacks CS chains; versican V4 has a truncated GAGβ domain from utilization of a cryptic splice site in exon 8 and 5 predicted CS attachment sites. (See id.; see also, Kischel et al., 2010).

Versican has been shown to bind to Toll-like receptor-2 (TLR2) receptor complexes on tumor-infiltrating myeloid cells and regulate inflammatory cytokine production (Kim et al., 2009), promote tolerogenic polarization of antigen-presenting cells (Tang et al., 2015), and promote the mesenchymal-epithelial transition in the carcinoma metastatic niche (Gao et al., 2012). Versican is proteolytically cleaved by ADAMTS-type proteases in a highly-regulated manner that involves CS chains. A cleavage product generated by disruption of a Glu-Ala bond at position 441 of versican's V1 isoform, has been previously termed versikine (Nandadasa et al., 2014). Versikine has been shown to be bioactive in development (McCulloch et al., 2009). However, the roles of versican proteolysis and/or versikine in immunomodulation remain unknown.

SUMMARY

Disclosed are methods and compositions for inducing and/or potentiating an immune response. The present inventors have determined that versikine can be administered in order to induce and/or potentiate, in particular, a T-cell mediated immune response, which may be characterized by a T-cell inflamed phenotype. As such, the inventors have determined that versikine can be administered to potentiate T-cell activating immunotherapies, including chimeric antigen receptor (CAR) T-cell therapies, tumor infiltrating lymphocyte (TIL) therapies, and other cellular therapies utilized for treating cell proliferative diseases or disorders. The inventors also have determined that versikine can be administered to potentiate other therapies utilized for treating cell proliferative diseases or disorders whose efficacy is linked to a T-cell inflamed phenotype, including, but not limited to, conventional chemotherapies, targeted therapies, oncolytic viral therapies, and radiotherapy.

The disclosed methods include methods for inducing an immune response in a subject in need thereof. The immune response induced and/or potentiated by the disclosed methods may include a T-cell mediated immune response, optionally characterized by a type 1 interferon signature (i.e., a type 1 interferon expression profile), expression of chemokines that attract T-cells (e.g., CCL2), expression of T-cell specific transcripts, and expression of macrophage-activation markers. The disclosed methods may include administering to the subject in need thereof a pharmaceutical composition comprising an effective amount of versikine or a variant thereof that induces the T-cell mediated immune response. The pharmaceutical composition may be administered by any suitable route including, for example, systemically or by injecting the pharmaceutical composition directly into tissue (e.g., tumor tissue).

The disclosed methods also may include administering the pharmaceutical composition comprising an effective amount of versikine or a variant thereof that induces and/or potentiates a T-cell mediated immune response to explanted cells from a subject, for example, in a method in which the explanted cells are treated with the pharmaceutical composition ex vivo. The explanted cells thus treated may then be administered back to the subject, for example, by re-infusion. The explanted cells may include immune cells (e.g., T-cells or dendritic cells), which optionally are treated, contacted, or primed with an antigen (e.g., a tumor antigen), either before, concurrently with, or after treatment with the pharmaceutical composition comprising an effective amount of versikine or a variant thereof. The explanted cells may include tumor cells.

The disclosed methods include methods for treating cell proliferative diseases and disorders such as cancers in a subject by administering to the subject a pharmaceutical composition comprising an effective amount of versikine or a variant thereof that induces and/or potentiates a T-cell mediated immune response. As such, cancers treated by the disclosed methods may include cancers that are characterized by an impaired T-cell mediated immune response, and in particular, an impaired T-cell inflamed phenotype. As an example, the disclosed methods may include methods of administering versikine or a variant thereof to a subject having a non-T-cell inflamed tumor. In the disclosed methods for treating cancer in a subject, the methods further may include administering to the subject cancer therapy before, concurrently with, or after administering the pharmaceutical composition comprising versikine or the variant thereof. Suitable cancer therapies may include, but are not limited to, administering chemotherapeutic agents.

Also disclosed are methods for determining whether a subject will benefit from a method that includes administering to the subject a pharmaceutical composition comprising an effective amount of versikine or a variant thereof that induces and/or potentiates a T-cell mediated immune response. The methods may include determining the con-

centration of versikine in a biological sample from the subject (e.g., a blood product), and if the determined level is determined to be below a selected baseline, then administering the pharmaceutical composition comprising versikine or the variant thereof that induces and/or potentiates a T-cell mediated immune response.

Also disclosed are kits comprising components that optionally may be utilized to perform the methods disclosed herein. The kits may include one or more of (a) versikine or a variant thereof, where the versikine or the variant thereof optionally is provided as a pharmaceutical composition; and (b) a reagent for detecting the concentration of versikine in a biological sample (e.g., an anti-versikine antibody which optionally is labelled with a detectable label).

Also disclosed are isolated polypeptides which may include non-naturally occurring isolated polypeptides. The isolated polypeptides typically exhibit one or more biological activities associated with versikine, which include, but are not limited to, inducing and/or potentiating a T-cell mediated immune response. The isolated polypeptides may be formulated as a pharmaceutical composition, which preferably comprises an effective amount of the polypeptides for inducing and/or potentiating a T-cell mediated immune response in a subject in need thereof. The isolated polypeptides may be fused and/or conjugated to other therapeutic polypeptides, including but not limited to, therapeutic antibodies, therapeutic bi-specific antibodies, and/or therapeutic ligands.

Also disclosed are isolated polynucleotides encoded any of the isolated polypeptides disclosed herein. The isolated polynucleotide may be present in vectors for replication of the polynucleotides or for expression of the encoded polypeptides, for example, where the polynucleotides are operably linked to a promoter. Also disclosed are isolated cells that comprise the isolated polynucleotides, particular the isolated polynucleotides as present in the disclosed vectors. Also disclosed are methods for expressing the encoded polypeptides, which methods include culturing the isolated cells as disclosed herein.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1**. CD8+ aggregate infiltration in myeloma bone marrows with active versican proteolysis. Staining of bone marrow biopsies with antibodies against neoepitope DPEAAE generated by V1-versican cleavage at Glu^{441}-Ala^{442}, macrophage marker CD68 and T-cell marker CD8. Four patterns of staining were observed in 19 informative punches, as shown. Arrow points at a CD8+ lymphocytic aggregate (>5 CD8+ cells in cluster). CD8−"poor" pattern refers to single CD8+ cells (occasionally doublets) sparsely distributed within tumor.

FIG. **2**. Cellular origin of versican and versican-degrading proteases in the myeloma microenvironment. A. Isoform-specific primers detected expression of versican isoforms by MAM (CD14+) but not myeloma tumor cells (CD138+) or myeloma BM-MSC isolated from the cases indicated. B. Relative expression of ADAMTS1, ADAMTS4, and ADAMTS5 mRNA in myeloma bone marrow stromal cells (BM-MSC) compared to normal BM-MSC. C. ADAMTS1 mRNA was robustly expressed in BM-MSC (note logarithmic scale). * $p<0.05$.

FIG. **3**. Versikine stimulates inflammatory cytokine production by primary MAM. A. and B. Freshly explanted MAM were exposed to 1 μM versikine for 12 hours. Relative expression of IL6 and IL1β transcripts is shown. C. MM1.S myeloma cells were exposed to 1 μM versikine overnight and labeled with BrdU for 30 minutes prior to analysis. Anti-BrdU/PI FACS analysis was employed to determine the relative proportion of cells in each phase of the cell cycle. * $p<0.05$; ** $p<0.01$; *** $p<0.001$.

FIG. **4**. Tpl2 and Tlr2 are implicated in versikine signaling. Tpl2−/− bone-marrow-derived macrophages (BMDM) were treated with 1 μM versikine and cytokine concentrations were measured in the culture supernatant at 12 hours post-exposure. A., IL1β; B., IL6; C., IL10; D, IL12p40. E. Signaling mediators induced by versikine stimulation of BMDM were evaluated. BMDM were collected following stimulation with versikine at designed timepoints (each number reflects minutes) and subjected to immunoblot analysis with the antibodies shown. F. Tlr2−/− BMDM were stimulated by versikine for 12 hours and IL6 protein was measured in the supernatant. G. Versikine modulates macrophage polarization. BMDM were exposed to versikine alone (A) or versikine+OVA/anti-OVA immune complexes (IC), as previously described (Edwards et al., 2006). Versikine exposure resulted in M1-like phenotype ($IL12^{hi}$, $IL10^{lo}$) in the absence of concurrent Fcγ ligation. Versikine+IC promoted macrophage polarization towards an M2b-like, immunoregulatory phenotype ($IL12^{lo}$, $IL10^{hi}$). ***$p<0.001$.

FIG. **5**. Versikine induces upregulation of IRF8 and interferon-stimulated-genes (ISG). A. RNA-seq analysis of MM1.S myeloma cells exposed to versikine-producing macrophages for 48 h. Only 23 genes were differentially expressed and all were upregulated. 13 of 23 upregulated genes were interferon-stimulated-genes (ISG, highlighted yellow). VCAN gene transcription changes are highlighted in blue. B. RNA-seq analysis of THP-1 cells expressing versikine following co-culture with MM1.S cells for 48 hours. Genes shown were differentially expressed at least 2-fold (log^2 fold change > or equal to 1 for overexpressed, < or equal to 1 for underexpressed) with a threshold false discovery rate (FDR) of 0.05. ISGs are highlighted in yellow; VCAN gene transcription changes are highlighted in blue; EBI3 transcription changes are highlighted in grey (FDR for EBI3 was 0.053). C and D. MM1.S and THP-1-derived macrophage co-cultures were exposed to 0.5 μM of purified versikine for 4, 18 or 48 hours. RNA was collected from each cell type and analyzed by RT-PCR using an interferon signaling RT-PCR array (see Materials and Methods). Representative ISG transcription is shown for MM1.S (FIG. **5**C) and THP-1 cells (FIG. **5**D). E. Interferon regulatory factor (IRF) transcription in MM1.S cells following treatment with versikine (Vkn) versus vehicle (Veh). Expression is normalized to Veh-only levels at 4 h. F. IRF9 mRNA levels in MM1.S cells co-cultured with macrophages and treated with versikine and compared to vehicle-only control at each timepoint. G. EBI3 transcription in MM1.S cells co-cultured with macrophages and treated with versikine (grey bars) or vehicle (black bars) for designated time-lengths. H. RT-PCR analysis for EBI3 transcripts in patient-derived, freshly-explanted MAM treated with 0.5 μM versikine for 12 hours. Relative expression is normalized to vehicle-only control (=1). * $p<0.05$; ** $p<0.01$; *** $p<0.001$.

FIG. **6**. VCAN accumulation and processing in colorectal cancer. A tissue microarray containing matched cores from colorectal cancers and the tumor-associated normal colon was stained for total VCAN and αDPEAAE, a neoepitope generated from VCAN cleavage at Glu^{441}-Ala^{442} (V1-enumeration) (A). VCAN staining was observed variably within the stroma of CRCs, however overall an increase in the intensity of VCAN staining was observed in the tumor tissues compared to the normal colon (Chi-square test,

p<0.001, A and B). VCAN proteolysis, as determined by αDPEAAE staining, was extensive in the stroma of normal tissue and markedly reduced in numerous CRCs (Chi-square test, p<0.001, A and C). Scale bar in A=100 μm.

FIG. **7**. Robust CD8+ T-cell infiltration in "VCAN proteolysis-predominant" tumors. Colorectal cancers were classified as "VCAN proteolysis-predominant" if their staining for total VCAN was weak (≤1+) and staining for versican proteolysis was strong (αDPEAAE intensity ≥2+). Tumors that did not meet those criteria were classified as "proteolysis-weak" (A). Given the immunoregulatory properties of VCAN and the immunostimulatory properties of its proteolytic product, versikine, CD8+ T-cell infiltration was assessed comparing VCAN proteolysis-predominant cancers versus proteolysis-weak cancers. Proteolysis-predominant tumors display 10-fold higher CD8 scores on average than proteolysis-weak tumors (Wilcoxon rank sum test, p<0.001; B). CD8+ T-cell infiltration is greatest in cancers with intensive VCAN proteolysis and low total VCAN (Wilcoxon rank sum test, p<0.001, C). Scale bar in A=100 μm.

FIG. **8**. Impact of VCAN proteolysis on CD8+ T-cell infiltration in MMR proficient and deficient cancers. Identification of cases within the TMA with MMR deficiency was performed by IHC analysis for MLH1, MSH2, PMS2 and MSH6. Loss of staining for any of these proteins confirmed MMR deficiency. Non-tumor cells were utilized as an internal control. Increased CD8+ T-cell infiltration in dMMR cancers was confirmed in the TMA CRC cores with a mean of 11.7 CD8+ T-cells per HPF in dMMR tumors compared to 3.1 per HPF in pMMR (Wilcoxon rank sum test, p<0.001; A). The intensity of staining for both VCAN and αDPEAAE varied across both dMMR and pMMR cancers with a trend toward more intense VCAN stromal staining in pMMR cancers (B). In both pMMR and dMMR cancers, the VCAN proteolysis predominant cancers had the greatest infiltration of CD8+ T-cells (Wilcoxon rank sum test, dMMR p=0.031, pMMR p=0.006; C). Comparing the VCAN proteolysis-predominant tumors, the dMMR cancers had increased CD8+ T-cell infiltration compared to the pMMR cancers (Wilcoxon rank sum test, p=0.04; C). The proportion of VCAN proteolysis predominant tumors varies depending on the MMR status with this being more common in dMMR tumors (Wilcoxon rank sum test, p=0.01; D). Truncating mutations in APC are commonly encountered in CRC and activation of WNT signaling has demonstrated immunoregulatory properties (20). To examine the impact of activation of WNT signaling, IHC staining for β-catenin was performed and the presence of nuclear localization of β-catenin was assessed. Those tumors with nuclear β-catenin had a significant reduction in CD8+ T-cell infiltration (Wilcoxon rank sum test, p=0.01; E). In addition, those tumors with nuclear localization of β-catenin had a higher rate of intense staining for VCAN (Chi-square test, p<0.001; F). Nuclear β-catenin was more common in the pMMR cancers (8 vs. 53%, Chi-square test, p<0.001, G).

FIG. **9**. Versikine, a product of VCAN proteolysis, promotes CD103+CD11c^{hi}MHCII^{hi} DC generation from flt3L-mobilized bone marrow progenitors. A. Bone marrow (BM) from C57BL/6J animals was isolated and cultured in the presence of 200 ng/mL flt3L for 9 days, as previously described (19). At conclusion of culture, a mixture of DC precursors and mature DC is obtained in this well-characterized system. Addition of versikine (1 mM) at D#0, alongside flt3L, resulted in reproducible expansion of CD103+CD11c^{hi}MHCII^{hi} DC (at least 5 independent experiments). Although the total number of CD11c+ cells was similar between vehicle- and versikine-supplemented cultures, there was a consistent skewing towards CD103+ differentiation, measurable at both earlier culture timepoints (4 days, A) and later culture timepoints (9 days, B). CD103+ MHCII^{hi} cells were SIRPa^{lo}, CD11b$^{lo\text{-}int}$ and SiglecH^{lo} confirming their identity as CD103+ conventional DC (cDC) (B). Versikine-supplemented flt3L-mobilized BM cultures demonstrate increased expression of the CD103+DC terminal selector, Irf8, as well as transcription factor Batf3 (C). Intact VCAN acts through TLR2/6 heterodimers. Addition of the TLR2/6 ligand, FSL-1, to flt3L-supplemented cultures results in a disadvantage to CD103+MHCII^{hi} expansion, suggesting that versikine acts through mechanisms distinct from intact VCAN (D).

FIG. **10**. VCAN and αDPEAAE staining intensity scoring. The normal colon tissue and CRCs on the TMA were stained for VCAN and αDPEAAE. The staining intensity of each core was categorized as 0 for no staining, 1 for low/weak staining, 2 for moderate staining and 3 for strong/intense staining.

FIG. **11**. VCAN and αDPEAAE staining across tumor locations and stages. There was no correlation between total VCAN staining and location of primary tumor (A). Increased αDPEAAE staining was observed in the rectum compared to the left or right colon (Chi-square test, p=0.009; A). Despite a greater staining for αDPEAAE being identified within the rectum, there was no significant correlation between the VCAN proteolysis-predominant classification and tumor location (Chi-square test, p=0.96; B). A trend toward an increased prevalence staining for the VCAN proteolysis-predominant classification was seen in colon cancers of earlier stage, albeit not statistically significant (Chi-square test, p=0.28; C).

FIG. **12**. Association of VCAN and αDPEAAE staining with phosphorylation of RPS6 and ERK1/2. There was not a significant correlation between stromal intensity of VCAN or αDPEAAE staining and phosphorylation of RPS6 (A and B) or phosphorylation of ERK1/2 (C and D).

FIG. **13**. αDPEAAE stromal intensity and cellular proliferation. Ki67 was staining was categorized by the percent of cells with nuclear staining for Ki67. No correlation was identified between αDPEAAE staining and the percent of Ki67 positive nuclei (Chi-square test, p=0.9).

DETAILED DESCRIPTION

The present invention is described herein using several definitions, as set forth below and throughout the application.

Definitions

Unless otherwise specified or indicated by context, the terms "a", "an", and "the" mean "one or more." For example, "a polypeptide" should be interpreted to mean "one or more polypeptides."

As used herein, "about," "approximately," "substantially," and "significantly" will be understood by persons of ordinary skill in the art and will vary to some extent on the context in which they are used. If there are uses of these terms which are not clear to persons of ordinary skill in the art given the context in which they are used, "about" and "approximately" will mean plus or minus ≤10% of the particular term and "substantially" and "significantly" will mean plus or minus ≥10% of the particular term.

As used herein, the terms "include" and "including" have the same meaning as the terms "comprise" and "compris-

ing." The terms "comprise" and "comprising" should be interpreted as being "open" transitional terms that permit the inclusion of additional components further to those components recited in the claims. The terms "consist" and "consisting of" should be interpreted as being "closed" transitional terms that do not permit the inclusion of additional components other than the components recited in the claims. The term "consisting essentially of" should be interpreted to be partially closed and allowing the inclusion only of additional components that do not fundamentally alter the nature of the claimed subject matter.

As used herein, a "subject" may be interchangeable with "patient" or "individual" and means an animal, which may be a human or non-human animal, in need of treatment. Non-human animals may include dogs, cats, horses, cows, pigs, sheep, and the like.

A "subject in need thereof" may include a patient having a disease, disorder, or condition that is characterized by the lack of, or by a deficient or impaired T-cell mediated immune response, which may include, but is not limited to a T-cell response characterized as a T-cell inflamed phenotype. A T-cell inflamed phenotype may include, but is not limited to a type 1 interferon signature (i.e., a type 1 interferon expression profile), expression of chemokines that attract T-cells such as T_{regs} (i.e., FoxP3$^+$ cells) or CD8$^+$ T-cells into tumor sites (e.g., CCL2, CCL3, CCL, 4, CCL, 5, CCL22, CXCL9, ad CXCL10), expression of T-cell specific transcripts, and/or expression of macrophage-activation markers. Diseases characterized by the lack of, or by a deficient or impaired T-cell mediated immune response, may include but are not limited to cell proliferative diseases and disorders (e.g., cancer).

A "subject in need thereof" may include a subject having a cell proliferative disease or disorder such as cancer. Cancer types may include, but are not limited to adenocarcinoma, leukemia, lymphoma, melanoma, myeloma, sarcoma, and teratocarcinoma. Cancer types may include, but are not limited to cancers of the adrenal gland, bladder, blood, bone, bone marrow, brain, breast, cervix, gall bladder, ganglia, gastrointestinal tract, heart, kidney, liver, lung, muscle, ovary, pancreas, parathyroid, prostate, skin, testis, thymus, and uterus. A "subject in need thereof" may include a subject having a cancer that is characterized by a non-T-cell inflamed tumor microenvironment. (See Gajewski, "The Next Hurdle in Cancer Immunotherapy: Overcoming the Non-T-Cell-Inflamed Microenvironment," Seminars in Oncology, Vol. 42, No. 4, August 2015, pp 663-671, the content of which is incorporated herein by reference in its entirety).

Reference is made herein to polypeptides and pharmaceutical compositions comprising polypeptides such as versikine and variants of versikine. An exemplary polypeptide may comprise the amino acid sequence of any of SEQ ID NOs:1-27, or may comprises an amino acid sequence having at least about 80%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to any of SEQ ID NOs:1-27. Variant polypeptides may include polypeptides having one or more amino acid substitutions, deletions, additions and/or amino acid insertions relative to a reference polypeptide. Also disclosed are nucleic acid molecules that encode the disclosed polypeptide (e.g., polynucleotides that encode the polypeptide of any of SEQ ID NOs:1-27 or variants thereof).

SEQ ID NOs:1-27 provide amino acid sequences as follows: SEQ ID NO:1—full length versican V1 including signal peptide sequence (i.e., aa 1-2339); SEQ ID NO:2—full length versican V1 minus signal peptide sequence (i.e., aa 21-2339); SEQ ID NO:3—full length versican V1 minus signal peptide sequence, plus N-terminal methionine; SEQ ID NO:4—non-versikine sequence of versican V1 (i.e., aa 442-2339); SEQ ID NO:5—full length versican including signal peptide sequence (i.e., aa 1-441); SEQ ID NO:6—full length versikine minus signal peptide sequence (i.e., aa 21-441); SEQ ID NO:7—full length versikine minus signal peptide sequence, plus N-terminal methionine; SEQ ID NO:8—Ig-like domain of versikine including signal peptide sequence (i.e., aa 1-146); SEQ ID NO:9—Ig-like domain of versikine minus signal peptide sequence (i.e., aa 21-146); SEQ ID NO:10—Ig-like domain of versikine minus signal peptide sequence, plus N-terminal methionine; SEQ ID NO:11—Linker domain 1 of versikine (i.e., aa 150-245); SEQ ID NO:12—Linker domain 1 of versikine plus N-terminal methionine; SEQ ID NO:13—Linder domain 2 of versikine (i.e., aa 251-347); SEQ ID NO:14—Linker domain 2 of versikine plus N-terminal methionine; SEQ ID NO:15—Portion of GAG-β domain in versikine (i.e., aa 349-441); SEQ ID NO:16—N-terminal portion of versikine including signal peptide sequence, Ig-like domain, and Linker domain 1 (i.e., aa 1-245); SEQ ID NO:17—N-terminal portion of versikine including Ig-like domain and Linker domain 1 (i.e., aa 21-245); SEQ ID NO:18—N-terminal portion of versikine including Ig-like domain and Linker domain 1 plus N-terminal methionine; SEQ ID NO:19—N-terminal portion of versikine including signal peptide sequence, Ig-like domain, Linker domain 1, and Linker domain 2 (i.e., aa 1-347); SEQ ID NO:20—N-terminal portion of versikine including Ig-like domain, Linker domain 1, and Linker domain 2 (i.e., aa 21-347); SEQ ID NO:21—N-terminal portion of versikine including Ig-like domain, Linker domain 1, and Linker domain 2, plus N-terminal methionine; SEQ ID NO:22—Internal portion of versikine including Linker domain 1 and Linker domain 2 (i.e., aa 150-347); SEQ ID NO:23—Internal portion of versikine including Linker domain 1 and Linker domain 2, plus N-terminal methionine (i.e., aa 150-347); SEQ ID NO:24—C-terminal portion of versikine including Linker domain 1, Linker domain 2, and portion of Gag-β domain (i.e., aa 150-441); SEQ ID NO:25—C-terminal portion of versikine including Linker domain 1, Linker domain 2, and portion of Gag-β domain, plus N-terminal methionine; SEQ ID NO:26—C-terminal portion of versikine including Linker domain 2 and portion of Gag-β domain (i.e., aa 251-441); SEQ ID NO:27—C-terminal portion of versikine including Linker domain 2 and portion of Gag-β domain, plus N-terminal methionine.

The disclosed versikine polypeptides or variant polypeptide preferably exhibit one or more biological activities that include inducing and/or potentiating a T-cell mediated immune response, and in particular, inducing and/or potentiating a T-cell inflamed phenotype. A T-cell inflamed phenotype may be characterized by a number of criteria, including but not limited to a type 1 interferon signature (i.e., a type 1 interferon expression profile), expression of chemokines that attract T-cells such as Tregs (i.e., FoxP3$^+$ cells) or CD8$^+$ T-cells into tumor sites (e.g., CCL2, CCL3, CCL, 4, CCL, 5, CCL22, CXCL9, ad CXCL10), expression of T-cell specific transcripts, and/or expression of macrophage-activation markers. (See, e.g., Gajewski, "The Next Hurdle in Cancer Immunotherapy: Overcoming the Non-T-Cell-Inflamed Tumor Microenvironment, Seminars in Oncology, Vol. 42, No. 4, August 2015, pp. 663-671; Zitvogel et al., "Type 1 interferons in anticancer immunity," Nature Reviews, Vol. 15, July 2015, pp. 405-414; and Harlin et al., "Chemokine Expression in Melanoma Metastases Associated with CD8+ T-cell Recruitment," Cancer Res. 2009 Apr.

1; 69(7)). A type 1 interferon signature can be used to characterize a number of diseases and disorders, including cell proliferative diseases and disorders as well as other diseases and disorder. (See, e.g., Gajewski, "The Next Hurdle in Cancer Immunotherapy: Overcoming the Non-T-Cell-Inflamed Tumor Microenvironment, Seminars in Oncology, Vol. 42, No. 4, August 2015, pp. 663-671; Zitvogel et al., "Type 1 interferons in anticancer immunity," Nature Reviews, Vol. 15, July 2015, pp. 405-414Häupl et al., "The type 1 interferon signature: facts, fads and fallacies," Ann. Rheum Dis 2011; 70:A24; Ronnblom et al., "The interferon signature in autoimmune diseases," Curr Opin. Rheumatol. 2013 March; 25(2):248-53; Ferreira et al., "A type 1 interferon transcriptional signature precedes autoimmunity in children genetically at risk for type 1 diabetes," Diabetes, 2014 July; 63(7):2538-50; Cornabella et al., "A type 1 interferon signature in monocytes is associated with poor response to interferon-beta in multiple sclerosis," Brain 2009 December; 132(Pt 12):3353-65; the contents of which are incorporated herein by reference in their entireties).

The disclosed polynucleotides encoding the disclosed polypeptides may be present in a replication vector and/or expression vector. Suitable vectors may include bacterial, plant, fungal, insect, or animal host cell replication and/or expression vectors that express the disclosed versikine polypeptides or variants thereof. Vectors may be used to transform appropriate host cells (e.g., *E. coli*). The transformed host cell may be cultivated or fermented such that the polypeptide is expressed constitutively or after adding a reagent that induces expression (e.g., via an inducible promoter). Expression vectors as contemplated herein may include control sequences that modulate expression of the encoded polypeptide. Expression control sequences may include constitutive or inducible promoters (e.g., T3, T7, Lac, trp, or phoA), ribosome binding sites, or transcription terminators.

The vectors disclosed herein may be utilized to transform host cells. Suitable host cells include bacterial, plant, fungal, insect, or animal host cell. Suitable bacteria include, but are not limited to: Gram-negative bacteria such as *Escherichia* species (e.g., *E. coli*), other Gram-negative bacteria, (e.g., *Pseudomonas* sp., such as *Pseudomonas aeruginosa*, or *Caulobacter* sp., such as *Caulobacter crescentus*), or Gram-positive bacteria (e.g., *Bacillus* sp., in particular *Bacillus subtlis*). Suitable fungal cells may include yeast (e.g., *Saccharomyces cerevisiae*).

Also disclosed are methods for expressing, preparing, isolating, separating, or purifying the disclosed versikine polypeptides or variants thereof. In some embodiments, the methods may be utilized to produce the versikine polypeptides as disclosed herein. The steps of the methods may include: (i) cultivating or fermenting a transformed host cell (e.g., a bacterial host cell as contemplated herein) which comprises an expression vector (as contemplated herein) which in turn comprises a nucleic acid molecule encoding the disclosed versikine polypeptides or variants thereof (as contemplated herein), wherein cultivation occurs under conditions which cause expression of the versikine polypeptides; and (ii) isolating, separating, or purifying the versikine polypeptide. The transformed bacteria may be cultivated or fermented using methods known in the art in order to express the versikine polypeptide. An exemplary isolation, separation, or purification method may include one or more of the following steps: a cell disruption step, a clarification step (e.g., via centrifugation or filtration), a chromatographic separation step, a dialysis step, and a precipitation step.

The terms "nucleic acid" and "nucleic acid sequence" refer to a nucleotide, oligonucleotide, polynucleotide (which terms may be used interchangeably), or any fragment thereof. These phrases also refer to DNA or RNA of genomic or synthetic origin (which may be single-stranded or double-stranded and may represent the sense or the antisense strand).

The terms "amino acid" and "amino acid sequence" refer to an oligopeptide, peptide, polypeptide, or protein sequence (which terms may be used interchangeably), or a fragment of any of these, and to naturally occurring or synthetic molecules. Where "amino acid sequence" is recited to refer to a sequence of a naturally occurring protein molecule, "amino acid sequence" and like terms are not meant to limit the amino acid sequence to the complete native amino acid sequence associated with the recited protein molecule.

The amino acid sequences contemplated herein may include conservative amino acid substitutions relative to a reference amino acid sequence. For example, a variant versikine polypeptide may include conservative amino acid substitutions relative to the natural versikine polypeptide. "Conservative amino acid substitutions" are those substitutions that are predicted to interfere least with the properties of the reference polypeptide. In other words, conservative amino acid substitutions substantially conserve the structure and the function of the reference protein. Conservative amino acid substitutions may include:

Original Residue	Conservative Substitutions
Ala	Gly, Ser
Arg	His, Lys
Asn	Asp, Gln, His
Asp	Asn, Glu
Cys	Ala, Ser
Gln	Asn, Glu, His
Glu	Asp, Gln, His
Gly	Ala
His	Asn, Arg, Gln, Glu
Ile	Leu, Val
Leu	Ile, Val
Lys	Arg, Gln, Glu
Met	Leu, Ile
Phe	His, Met, Leu, Trp, Tyr
Ser	Cys, Thr
Thr	Ser, Val
Trp	Phe, Tyr
Tyr	His, Phe, Trp
Val	Ile, Leu, Thr

Conservative amino acid substitutions generally maintain (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a beta sheet or alpha helical conformation, (b) the charge or hydrophobicity of the molecule at the site of the substitution, and/or (c) the bulk of the side chain.

A "deletion" refers to a change in the amino acid or nucleotide sequence that results in the absence of one or more amino acid residues or nucleotides relative to a reference sequence. A deletion removes at least 1, 2, 3, 4, 5, 10, 20, 50, 100, or 200 amino acids residues or nucleotides. A deletion may include an internal deletion or a terminal deletion (e.g., an N-terminal truncation or a C-terminal truncation of a reference polypeptide or a 5'-terminal or 3'-terminal truncation of a reference polynucleotide).

A "fragment" is a portion of an amino acid sequence or a polynucleotide which is identical in sequence to but shorter in length than a reference sequence. A fragment may comprise up to the entire length of the reference sequence, minus

at least one nucleotide/amino acid residue. For example, a fragment may comprise from 5 to 1000 contiguous nucleotides or contiguous amino acid residues of a reference polynucleotide or reference polypeptide, respectively. In some embodiments, a fragment may comprise at least (or no more than) 5, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, 150, 250, or 500 contiguous nucleotides or contiguous amino acid residues of a reference polynucleotide or reference polypeptide, respectively. A fragment may comprise a range of contiguous nucleotides or contiguous amino acid residues of a reference polynucleotide or reference polypeptide, respectively, bounded by endpoints selected from any of 5, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, 150, 250, or 500 contiguous nucleotides or contiguous amino acid residues, respectively (e.g., a peptide fragment having 100-150 contiguous amino acid residues of a reference polypeptide). Fragments may be preferentially selected from certain regions of a molecule. The term "at least a fragment" encompasses the full length polynucleotide or full length polypeptide.

Fusion proteins also are contemplated herein. A "verskine fusion protein" refers to a protein formed by the fusion (e.g., genetic fusion) of at least one molecule of versikine (or a fragment or variant thereof) to at least one molecule of a heterologous protein (or fragment or variant thereof), which may include a therapeutic protein. A versikine fusion protein comprises at least a fragment or variant of the heterologous protein and at least a fragment or variant of versikine, which are associated with one another, preferably by genetic fusion (i.e., the versikine fusion protein is generated by translation of a nucleic acid in which a polynucleotide encoding all or a portion of the heterologous protein is joined in-frame with a polynucleotide encoding all or a portion of versikine or a fragment or variant thereof). The heterologous protein and versikine protein, once part of the versikine fusion protein, may each be referred to herein as a "portion", "region" or "moiety" of the versikine fusion protein (e.g., a "a heterologous protein portion" or a "versikine protein portion").

Conjugate proteins also are contemplated herein. A "versikine conjugate protein" refers to a protein formed by the conjugation (i.e., covalently bonding) of at least one molecule of versikine (or a fragment or variant thereof) to at least one molecule of a heterologous protein (or fragment or variant thereof), which may include a therapeutic protein. A versikine conjugate protein comprises at least a fragment or variant of the heterologous protein and at least a fragment or variant of versikine, which are associated with one another by covalent bonding. The heterologous protein and versikine protein, once part of the versikine conjugate protein, may each be referred to herein as a "portion," "region" or "moiety" of the versikine conjugate protein (e.g., "a heterologous protein portion" or a "versikine protein portion").

Suitable heterologous proteins for the contemplated versikine fusion protein and versikine conjugate proteins may include therapeutic antibodies or antigen-binding fragments thereof. Suitable antibodies may include, but are not limited to, antibodies that bind to the protein CD20 (e.g., rituximab or an antigen-binding fragments thereof that binds the protein CD20), antibodies that bind to the protein CD38 (e.g., daraturumab or an antigen-binding fragment thereof that binds the protein CD38), antibodies that bind to the protein CD30 (e.g., brentuximab or an antigen-binding fragment thereof that binds the protein CD30), antibodies that bind to the protein CD19 (e.g., blinatumomab or an antigen-binding fragment thereof that binds the protein CD19), antibodies that bind to the protein CD40 (e.g. ipilimumab or an antigen-binding fragment thereof that binds CD40), antibodies that bind to the protein PD-1 (e.g., nivolumab or an antigen-binding fragment thereof that binds PD-1). Suitable heterologous proteins may also include ligands for receptor present on T-cells and immunoadhesins (e.g., immunoadhesins that target any of CD20, CD38, CD30, CD19, CD40, and/or PD-1).

A "full length" polynucleotide sequence is one containing at least a translation initiation codon (e.g., methionine) followed by an open reading frame and a translation termination codon. A "full length" polynucleotide sequence encodes a "full length" polypeptide sequence.

"Homology" refers to sequence similarity or, interchangeably, sequence identity, between two or more polynucleotide sequences or two or more polypeptide sequences. Homology, sequence similarity, and percentage sequence identity may be determined using methods in the art and described herein.

The phrases "percent identity" and "% identity," as applied to polypeptide sequences, refer to the percentage of residue matches between at least two polypeptide sequences aligned using a standardized algorithm. Methods of polypeptide sequence alignment are well-known. Some alignment methods take into account conservative amino acid substitutions. Such conservative substitutions, explained in more detail above, generally preserve the charge and hydrophobicity at the site of substitution, thus preserving the structure (and therefore function) of the polypeptide. Percent identity for amino acid sequences may be determined as understood in the art. (See, e.g., U.S. Pat. No. 7,396,664, which is incorporated herein by reference in its entirety). A suite of commonly used and freely available sequence comparison algorithms is provided by the National Center for Biotechnology Information (NCBI) Basic Local Alignment Search Tool (BLAST) (Altschul, S. F. et al. (1990) J. Mol. Biol. 215:403 410), which is available from several sources, including the NCBI, Bethesda, Md., at its website. The BLAST software suite includes various sequence analysis programs including "blastp," that is used to align a known amino acid sequence with other amino acids sequences from a variety of databases.

Percent identity may be measured over the length of an entire defined polypeptide sequence, for example, as defined by a particular SEQ ID number, or may be measured over a shorter length, for example, over the length of a fragment taken from a larger, defined polypeptide sequence, for instance, a fragment of at least 15, at least 20, at least 30, at least 40, at least 50, at least 70 or at least 150 contiguous residues. Such lengths are exemplary only, and it is understood that any fragment length supported by the sequences shown herein, in the tables, figures or Sequence Listing, may be used to describe a length over which percentage identity may be measured.

A "variant" of a particular polypeptide sequence is defined as a polypeptide sequence having at least 50% sequence identity to the particular polypeptide sequence over a certain length of one of the polypeptide sequences using blastp with the "BLAST 2 Sequences" tool available at the National Center for Biotechnology Information's website. (See Tatiana A. Tatusova, Thomas L. Madden (1999), "Blast 2 sequences—a new tool for comparing protein and nucleotide sequences", FEMS Microbiol Lett. 174:247-250). Such a pair of polypeptides may show, for example, at least 60%, at least 70%, at least 80%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% or greater sequence identity over a certain defined length of one of the polypeptides. A "variant" may have

substantially the same functional activity as a reference polypeptide. For example, a variant of versikine may exhibit or more biological activities associated with versikine, including inducing of a type 1 interferon signature.

The disclosed polypeptides may be modified so as to comprise an amino acid sequence or modified amino acids, such that the disclosed polypeptides cannot be said to be naturally occurring. In some embodiments, the disclosed polypeptides are modified and the modification is selected from the group consisting of acylation, acetylation, formylation, lipolylation, myristoylation, palmitoylation, alkylation, isoprenylation, prenylation, and amidation. An amino acid in the disclosed polypeptides may be thusly modified, but in particular, the modifications may be present at the N-terminus and/or C-terminus of the polypeptides (e.g., N-terminal acylation or acetylation, and/or C-terminal amidation). The modifications may enhance the stability of the polypeptides and/or make the polypeptides resistant to proteolysis.

The terms "percent identity" and "% identity," as applied to polynucleotide sequences, refer to the percentage of residue matches between at least two polynucleotide sequences aligned using a standardized algorithm. Such an algorithm may insert, in a standardized and reproducible way, gaps in the sequences being compared in order to optimize alignment between two sequences, and therefore achieve a more meaningful comparison of the two sequences. Percent identity for a nucleic acid sequence may be determined as understood in the art. (See, e.g., U.S. Pat. No. 7,396,664, which is incorporated herein by reference in its entirety). A suite of commonly used and freely available sequence comparison algorithms is provided by the National Center for Biotechnology Information (NCBI) Basic Local Alignment Search Tool (BLAST) (Altschul, S. F. et al. (1990) J. Mol. Biol. 215:403 410), which is available from several sources, including the NCBI, Bethesda, Md., at its website. The BLAST software suite includes various sequence analysis programs including "blastn," that is used to align a known polynucleotide sequence with other polynucleotide sequences from a variety of databases. Also available is a tool called "BLAST 2 Sequences" that is used for direct pairwise comparison of two nucleotide sequences. "BLAST 2 Sequences" can be accessed and used interactively at the NCBI website. The "BLAST 2 Sequences" tool can be used for both blastn and blastp (discussed below).

Percent identity may be measured over the length of an entire defined polynucleotide sequence, for example, as defined by a particular SEQ ID number, or may be measured over a shorter length, for example, over the length of a fragment taken from a larger, defined sequence, for instance, a fragment of at least 20, at least 30, at least 40, at least 50, at least 70, at least 100, or at least 200 contiguous nucleotides. Such lengths are exemplary only, and it is understood that any fragment length supported by the sequences shown herein, in the tables, figures, or Sequence Listing, may be used to describe a length over which percentage identity may be measured.

A "variant," "mutant," or "derivative" of a particular nucleic acid sequence may be defined as a nucleic acid sequence having at least 50% sequence identity to the particular nucleic acid sequence over a certain length of one of the nucleic acid sequences using blastn with the "BLAST 2 Sequences" tool available at the National Center for Biotechnology Information's website. (See Tatiana A. Tatusova, Thomas L. Madden (1999), "Blast 2 sequences—a new tool for comparing protein and nucleotide sequences", FEMS Microbiol Lett. 174:247-250). Such a pair of nucleic acids may show, for example, at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% or greater sequence identity over a certain defined length.

Nucleic acid sequences that do not show a high degree of identity may nevertheless encode similar amino acid sequences due to the degeneracy of the genetic code. It is understood that changes in a nucleic acid sequence can be made using this degeneracy to produce multiple nucleic acid sequences that all encode substantially the same protein.

The words "insertion" and "addition" refer to changes in an amino acid or nucleotide sequence resulting in the addition of one or more amino acid residues or nucleotides, respectively. An insertion or addition may refer to 1, 2, 3, 4, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 150, or 200 amino acid residues or nucleotides.

"Operably linked" refers to the situation in which a first nucleic acid sequence is placed in a functional relationship with a second nucleic acid sequence. For instance, a promoter is operably linked to a coding sequence if the promoter affects the transcription or expression of the coding sequence. Operably linked DNA sequences may be in close proximity or contiguous and, where necessary to join two protein coding regions, in the same reading frame.

A "recombinant nucleic acid" is a sequence that is not naturally occurring or has a sequence that is made by an artificial combination of two or more otherwise separated segments of sequence. This artificial combination is often accomplished by chemical synthesis or, more commonly, by the artificial manipulation of isolated segments of nucleic acids, e.g., by genetic engineering techniques such as those described in Sambrook, J. et al. (1989) Molecular Cloning: A Laboratory Manual, 2^{nd} ed., vol. 1 3, Cold Spring Harbor Press, Plainview N.Y. The term recombinant includes nucleic acids that have been altered solely by addition, substitution, or deletion of a portion of the nucleic acid. Frequently, a recombinant nucleic acid may include a nucleic acid sequence operably linked to a promoter sequence. Such a recombinant nucleic acid may be part of a vector that is used, for example, to transform a cell.

"Substantially isolated or purified" nucleic acid or amino acid sequences are contemplated herein. The term "substantially isolated or purified" refers to nucleic acid or amino acid sequences that are removed from their natural environment, and are at least 60% free, preferably at least 75% free, and more preferably at least 90% free, even more preferably at least 95% free from other components with which they are naturally associated.

"Transformation" describes a process by which exogenous DNA is introduced into a recipient cell. Transformation may occur under natural or artificial conditions according to various methods well known in the art, and may rely on any known method for the insertion of foreign nucleic acid sequences into a prokaryotic or eukaryotic host cell. The method for transformation is selected based on the type of host cell being transformed and may include, but is not limited to, bacteriophage or viral infection, electroporation, heat shock, lipofection, and particle bombardment. The term "transformed cells" includes stably transformed cells in which the inserted DNA is capable of replication either as an autonomously replicating plasmid or as part of the host chromosome, as well as transiently transformed cells which express the inserted DNA or RNA for limited periods of time.

A "composition comprising a given polypeptide" and a "composition comprising a given polynucleotide" refer

broadly to any composition containing the given polynucleotide or amino acid sequence. The composition may comprise a dry formulation or an aqueous solution. The compositions may be stored in any suitable form including, but not limited to, freeze-dried form and may be associated with a stabilizing agent such as a carbohydrate. The compositions may be aqueous solution containing salts (e.g., NaCl), detergents (e.g., sodium dodecyl sulfate; SDS), and other components (e.g., Denhardt's solution, dry milk, salmon sperm DNA, and the like).

As used herein, "potentiating" or "enhancing" an immune response means increasing the magnitude and/or the breadth of the immune response. For example, the number of cells that recognize a particular epitope may be increased ("magnitude") and/or the numbers of epitopes that are recognized may be increased ("breadth"). Preferably, a 5-fold, or more preferably a 10-fold or greater, enhancement in an immune response may be obtained by administering the polypeptides and pharmaceutical compositions disclosed herein. In some embodiments, potentiating or enhancing an immune response means overcoming a non-T-cell-inflamed tumor microenvironment in a subject having cancer (e.g., by increasing the number of T-cells that are infiltrating the tumor, by increasing the number of cells that are exhibiting a type 1 interferon signature, and/or by increasing the number of cells that are expressing macrophage-activation markers).

The disclosed pharmaceutical composition may comprise the disclosed versikine polypeptides and variants at any suitable dose. Suitable doses may include, but are not limited to, about 0.01 μg/dose, about 0.05 μg/dose, about 0.1 μg/dose, about 0.5 μg/dose, about 1 μg/dose, about 2 μg/dose, about 3 μg/dose, about 4 μg/dose, about 5 μg/dose, about 10 μg/dose, about 15 μg/dose, about 20 μg/dose, about 25 μg/dose, about 30 μg/dose, about 35 μg/dose, about 40 μg/dose, about 45 μg/dose, about 50 μg/dose, about 100 μg/dose, about 200 μg/dose, about 500 μg/dose, or about 1000 μg/dose. Suitable doses may be within dose ranges bounded by any of about 0.01 μg/dose, about 0.05 μg/dose, about 0.1 μg/dose, about 0.5 μg/dose, about 1 μg/dose, about 2 μg/dose, about 3 μg/dose, about 4 μg/dose, about 5 μg/dose, about 10 μg/dose, about 15 μg/dose, about 20 μg/dose, about 25 μg/dose, about 30 μg/dose, about 35 μg/dose, about 40 μg/dose, about 45 μg/dose, about 50 μg/dose, about 100 μg/dose, about 200 μg/dose, about 500 μg/dose, or about 1000 μg/dose (e.g., about 50 μg/dose to about 100 μg/dose)

The disclosed versikine polypeptides and variants may be administered at any suitable dose level. In some embodiments, a subject in need thereof is administered a versikine polypeptide or variant thereof at a dose level of from about 1 ng/kg up to about 2000 ng/kg. In some embodiments, the versikine polypeptide or variant thereof is administered to the subject in need thereof at a dose level of at least about 1 ng/kg, 2 ng/kg, 5 ng/kg, 10 ng/kg, 20 ng/kg, 50 ng/kg, 100 ng/kg, 200 ng/kg, 500 ng/kg, 1000 ng/kg or 2000 ng/kg. In other embodiments, the versikine polypeptide or variant thereof is administered to the subject in need thereof at a dose level of less than about 2000 ng/kg, 1000 ng/kg, 500 ng/kg, 200 ng/kg, 100 ng/kg, 50 ng/kg, 20 ng/kg, 10 ng/kg, 5 ng/kg, 2 ng/kg, or 1 ng/kg. In further embodiments, the versikine polypeptide or variant thereof is administered to a subject in need thereof within a dose level range bounded by any 1 ng/kg, 2 ng/kg, 5 ng/kg, 10 ng/kg, 20 ng/kg, 50 ng/kg, 100 ng/kg, 200 ng/kg, 500 ng/kg, 1000 ng/kg or 2000 ng/kg (e.g., a dose level range of 100 ng/kg to 200 ng/kg).

The disclosed versikine polypeptides and variants may be administered under any suitable dosing regimen. Suitable dosing regimens may include, but are not limited to, daily regimens (e.g., 1 dose/day for 1, 2, 3, 4, 5, 6, 7 or more days), twice daily regimens (e.g., 2 doses/day for 1, 2, 3, 4, 5, 6, 7 or more days), and thrice daily regiments (e.g., 3 doses/day for 1, 2, 3, 4, 5, 6, 7 or more days). Suitable regiments also may include dosing every other day, 3 times/week, once a week, for 1, 2, 3, 4, or more weeks.

The disclosed versikine polypeptides and variants (or pharmaceutical compositions comprising the disclosed versikine polypeptides and variant) may be administered to a subject in need thereof by any suitable route. In some embodiments, the disclosed versikine polypeptides and variant are administered to a subject in need thereof via an injectable delivery route selected from the group consisting of intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intratumorally, or epidural routes. In another embodiment, the disclosed versikine polypeptides and variant are administered to a subject near a site of a tumor or cancer. The disclosed versikine polypeptides and variants may be administered to cells or tissue that has been explanted from a subject. For example, the explanted cells or tissue may be contacted or treated with the disclosed versikine polypeptides and variants ex vivo, and after treatment/contact, the explanted cells or tissue may be administered to the patient, for example, but re-infusion and/or transplant.

Use of Versikine and Variants Thereof in Treatment Methods

Disclosed are methods and compositions for inducing and/or potentiating an immune response. The present inventors have determined that versikine can be administered in order to induce and or potentiate a T-cell mediated immune response, which may be characterized as a T-cell inflamed phenotype. As such, the inventors have determined that versikine may be administered to potentiate T-cell activating immunotherapies.

The disclosed methods include methods for inducing and/or potentiating an immune response in a subject in need thereof, including a T-cell mediated immune response. The disclosed methods may include administering to the subject in need thereof a pharmaceutical composition comprising an effective amount of versikine or a variant thereof that induces and/or potentiates the T-cell mediated immune response. The pharmaceutical composition may be administered by any suitable route including, for example, systemically (e.g., intervenously) or by injecting the pharmaceutical composition directly into tissue (e.g., tumor tissue).

In some embodiments, the T-cell mediated immune response induced and/or potentiated in the disclosed methods may be characterized by a type 1 interferon signature (i.e., type 1 interferon expression profile). As such, in the disclosed methods versikine or a variant thereof may be administered in order to induce and/or potentiate expression of one or more genes whose expression is observed to be induced by type 1 interferon. In some embodiments, the enhanced expression is observed relative to a baseline or control of one or more genes encoding any of IF16, MX1, XAF1, IFITM1, OAS3, IFI44L, TRIM22, STAT1, IFI44, CCL2, MX2, IFIT1, OAS2, SIGLEC1, TTSAD2, OASL, SIGLEC11, IFITM1, and ISG15. Expression may be measured and assessed by methods known in the art, including methods for detecting mRNA (e.g., via RT-PCR) and methods for detecting encoded proteins (e.g., via immunoassay).

The disclosed versikine polypeptides and variants may be administered in order to induce production of other cytok-

ines. In some embodiments, the disclosed versikine polypeptides and variants may be administered in order to induce or enhance production of IL1β, IL6, or both (e.g., by macrophages). Induction or enhanced production of cytokines may be measured and assessed by methods known in the art (e.g., via immunoassays or via assays that measure biological activity of the cytokines).

The disclosed versikine polypeptides and variants may be administered in order to induce expression of other proteins. In some embodiments, the disclosed versikine polypeptides and variants may be administered in order to induce expression of one or more of EBI3, IRF8, and IL12p40. Expression may be measured and assessed by methods known in the art, including methods for detecting mRNA (e.g., via RT-PCR) and methods for detecting encoded proteins (e.g., via immunoassay).

The disclosed versikine polypeptides and variants may be administered in order to induce phosphorylation of other proteins. In some embodiments, the disclosed versikine polypeptides and variants may be administered to induce phosphorylation of one or more of JNK, p38-MAPK, and AKT.

The disclosed methods include administering versikine polypeptides to a subject in need thereof and also administering variants of versikine to a subject in need thereof. Typically, the variants exhibit one or more biological activities associated with versikine, such as induction of a T-cell mediated immune response. In some embodiments, the versikine polypeptide or variant thereof comprises, consists essentially of, or consists of the amino acid sequence of any of SEQ ID NOs:1-27 or an amino acid sequence having a least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to any of SEQ ID NOs:1-27.

The disclosed versikine polypeptides and variants thereof optionally comprise an N-terminal methionine which optionally may not be present in naturally occurring versikine. In some embodiments, the disclosed versikine polypeptides and variants thereof may comprise, consist essentially of, or consist of the amino acid sequence of any of SEQ ID NOs:3-27 or an amino acid sequence having a least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence to any of SEQ ID NOs:3-27, wherein the versikine polypeptides or variants thereof comprise a non-naturally occurring N-terminal methionine. Exemplary polypeptides include polypeptides comprising, consisting essentially of, or consisting of the amino acid sequence of any of SEQ ID NOs:3, 7, 10, 12, 14, 18, 21, 23, 25 and 27.

The disclosed versikine polypeptides and variants thereof may comprise, consist essentially of, or consist of a fragment of a reference polypeptide. In some embodiments, the disclosed versikine polypeptides and variants thereof comprise, consist essentially of, or consist of a fragment of any of SEQ ID NOs:1-27. In some embodiments, the disclosed versikine polypeptides and variants thereof do not comprise the amino acid sequence of SEQ ID NOs:4 or 8-27. In embodiments where the versikine polypeptides and variants thereof comprise a fragment of a reference polypeptide that does not include the naturally occurring N-terminal methionine, the versikine polypeptides and variants thereof may be modified to include a non-naturally occurring N-terminal methionine.

The disclosed versikine polypeptides and variants thereof may comprise post-translational modifications or may lack post-translation modifications. In some embodiments, the disclosed versikine polypeptides and variants thereof do not have any chondroitin sulfate side chains. In other embodiments, the disclosed versikine polypeptides and variants thereof include one or more amino acid modifications selected from the group consisting of acylation (e.g., N-terminal acylation), acetylation (e.g., N-terminal acetylation), formylation, lipolylation, myristoylation, palmitoylation, alkylation, isoprenylation, prenylation, pegylation, and amidation (e.g., C-terminal amidation).

The disclosed versikine polypeptides and variants thereof may be modified to replace a natural amino acid residue by an unnatural amino acid. Unnatural amino acids may include, but are not limited to an amino acid having a D-configuration, an N-methyl-α-amino acid, a non-proteogenic constrained amino acid, or a β-amino acid.

The disclosed versikine polypeptides and variants thereof may be modified in order to increase the stability of the versikine polypeptides and variants thereof in plasma. For example, the disclosed peptides may modified in order to make the versikine polypeptides and variants thereof resistant to peptidases. The disclosed versikine polypeptides and variants thereof may be modified to replace an amide bond between two amino acids with a non-amide bond. For example, the carbonyl moiety of the amide bond can be replaced by CH2 (i.e., to provide a reduced amino bond: —CH2-NH—). Other suitable non-amide replacement bonds for the amide bond may include, but are not limited to: an endothiopeptide, —C(S)—NH, a phosphonamide, —P(O)OH—NH—), the NH-amide bond can be exchanged by O (depsipeptide, —CO—O—), S (thioester, —CO—S—) or CH_2 (ketomethylene, —CO—CH_2—). The peptide bond can also be modified as follows: retro-inverso bond (—NH—CO—), methylene-oxy bond (—CH_2—), thiomethylene bond (—CH_2—S—), carbabond (—CH_2—CH_2—), hydroxyethylene bond (—CHOH—CH_2—) and so on, for example, to increase plasma stability of the versikine polypeptides and variants thereof (notably towards endopeptidases).

The disclosed versikine polypeptides and variants thereof may include a non-naturally occurring N-terminal and/or C-terminal modification. For example, the N-terminal of the disclosed versikine polypeptides and variants thereof may be modified to include a N-acylation or a N-pyroglutamate modification (e.g., as a blocking modification). The C-terminal end of the disclosed versikine polypeptides and variants thereof may be modified to include a C-amidation.

The disclosed versikine polypeptides and variants thereof may include an N-terminal esterification (e.g., a phosphoester modification) or a pegylation modification, for example, to enhance plasma stability (e.g. resistance to exopeptidases) and/or to reduce immunogenicity.

The disclosed versikine polypeptides and variants thereof may be fused to additional functional polypeptide domains. In some embodiments, the disclosed versikine polypeptides and variants thereof are fused to an antibody or an antigen-binding domain thereof (e.g., one or more scFv or other antigen-binding domains). Optionally, the antigen-binding domain binds to an epitope of a tumor antigen and the versikine/antigen-binding fusion polypeptide is administered to a subject having a cancer for which the tumor antigen is associated in order to target the versikine/antigen-binding fusion polypeptide to the subject's tumor.

The disclosed versikine polypeptides and variants thereof may be conjugated to a resin or a solid support. For example, the disclosed versikine polypeptides and variants thereof maybe conjugated via there N-terminus and/or C-terminus to a solid support, either directly or via a linking moiety that conjugates the peptides to the resin or the solid support. Solid supports may include microparticles or nanoparticles such as polymeric microparticles or polymeric nanoparticles

comprising a biodegradable polymer (e.g., poly(lactic-co-glycolic acid) (PLGA) polylactic acid, and poly(caprolactone)).

The disclosed versikine polypeptides and variants thereof may be formulated as a pharmaceutical composition for use in the methods disclosed herein. Typically, the pharmaceutical compositions contemplated herein will comprise an effective amount of a versikine polypeptide or variant thereof for inducing a T-cell mediated immune response in a subject after the pharmaceutical composition is administered to the subject or in cells or tissues explanted from the subject after the cells or tissues are contacted with the pharmaceutical composition.

The disclosed methods also may include administering the pharmaceutical composition comprising an effective amount of versikine or a variant thereof that induces a T-cell mediated immune response to explanted cells from a subject, for example, in a method in which the explanted cells are treated with the pharmaceutical composition ex vivo. The explanted cells thus treated may then be administered back to the subject, for example, by re-infusion. The explanted cells may include immune cells (e.g., T-cells or dendritic cells), which optionally are treated, contacted, or primed with an antigen (e.g., a tumor antigen), either before, concurrently with, or after treatment with the pharmaceutical composition comprising an effective amount of versikine or a variant thereof. The explanted cells may include tumor cells.

The disclosed methods include methods for treating cell proliferative diseases and disorders such as cancers in a subject by administering to the subject a pharmaceutical composition comprising an effective amount of versikine or a variant thereof that induces a T-cell mediated immune response. As such, cancers treated by the disclosed methods may include cancers that are characterized the absence of, or by a defective or impaired T-cell mediated response (e.g., cancers that are characterized the absence of, or by a defective T-cell inflamed phenotype). Cancers treated by the presently disclosed methods may include tissue type cancers selected from, but not limited to adenocarcinoma, leukemia, lymphoma, melanoma, myeloma, sarcoma, and teratocarcinoma. Cancers treated by the presently disclosed methods may include organ type cancers selected from, but not limited to the adrenal gland, bladder, blood, bone, bone marrow, brain, breast, cervix, gall bladder, ganglia, gastrointestinal tract, heart, kidney, liver, lung, muscle, ovary, pancreas, parathyroid, prostate, skin, testis, thymus, and uterus. In particular, a subject in need thereof may include a subject having a hematological malignancy, including but not limited to myeloma.

In the disclosed methods for treating cancer in a subject, the methods further may include administering to the subject cancer therapy before, concurrently with, or after administering the pharmaceutical composition comprising versikine or the variant thereof. Suitable cancer therapies may include, but are not limited to, administering chemotherapeutic agents. Suitable chemotherapeutic agents for administration in the disclosed methods may include, but are not limited to Abitrexate, Adcetris, Ambochlorin, Aredia (Pamidronate Disodium), Arranon, Asparaginase Erwinia chrysanthemi, Becenum (Carmustine), Beleodaq, Belinostat, Bendamustine Hydrochloride, Bexxar, BiCNU (Carmustine), Blenoxane, Bleomycin, Blinatumomab, Blincyto, Bortezomib, Brentuximab Vedotin, Carfilzomib, Carmubris (Carmustine), Carmustine, Cerubidine, Chlorambucil, Clafen (Cyclophosphamide), Clofarex, Clolar, Cyclophosphamide, Cytarabine, Cytarabine Liposome, Cytosar-U, Cytoxan (Cyclophosphamide), Daratumumab, Darzalex (Daratumumab), Dasatinib, Daunorubicine Hydrochloride, Denileukin Diftitox, DepoCyt, Dexamethasone, Docetaxel, Doxil (Doxorubicin Hydrochloride Liposome), Doxorubicin Hydrochloride Liposome, Dox-SL (Doxorubicin Hydrochloride Liposome), Elotuzumab, Empliciti (Elotuzumab), Epirubicin, Erwinaze, Estramustine, Etoposide, Evacet (Doxorubicin Hydrochloride Liposome), Farydak (Panobinostat), Folex, Folex PFS, Folotyn, Ibrutumomab Tiuxetan, Ibrutinib, Iclusig, Idarubicin, Idelalisib, Imatinib Mesylate, Imbruvica, Intron A, Irinotecan, Istodax, Ixabepilone, Ixazomib Citrate, Kyprolis (Carfilzomib), Lenalidomide, Leukeran, Linfolizin, LipoDox (Doxorubicin Hydrochloride Liposome), Marqibo, Mechlorethamine Hydrochloride, Mercaptopurine, Methotrexate, Methotrexate LPF, Mexate, Mexate-AQ, Mitoxantrone, Mozobil (Plerixafor), Mustargen, Nelarabine, Neosar (Cyclophosphamide), Ninlaro (Ixazomib Citrate), Oncaspar, Ontak, Paclitaxel, Pamidronate Disodium, Panobinostat, Pegaspargase, Plerixafor, Pomalidomide, Pomalyst (Pomalidomide), Ponatinib Hydrochloride, Pralatrexate, Prednisone, Purinethol, Purixan, Recombinant Interferon Alfa-2b, Revlimid (Lenalidomide), Rituxanab, Romidepsin, Rubidomycin, Sprycel, Synovir (Thalidomide), Tarabiune PFS, Teniposide, Thalidomide, Thalomid (Thalidomide), Topotecan, Tositumomab, Treanda, Velban, Velcade (Bortezomib), Vinblastine Sulfate, Vincasar PGS, Vincristine Sulfate, Vincristine Sulfate Liposome, Vinorelbine, Vorinostat, Zevalin, Zoledronic Acid, Zolinza, Zometa (Zoledronic Acid), and Zydelig.

Other suitable cancer therapies that may be administered before, concurrently with, or after administering the pharmaceutical composition comprising versikine or the variant thereof may include therapies in which oncolytic viruses are administered, therapies in which immunomodulatory drugs are administered, therapies in which anthracyclines are administered, and therapies in which check-point blockers are administered. Other suitable cancer therapies that may be administered before, concurrently with, or after administering the pharmaceutical composition comprising versikine or the variant thereof may include therapies in which chimeric antigen receptor (CAR) T-cells are administered, therapies in which tumor infiltrating lymphocyte (TIL) are administered. Other suitable cancer therapies that may be administered before, concurrently with, or after administering the pharmaceutical composition comprising versikine or the variant thereof may include radiation therapy.

Also disclosed are methods for determining whether a subject will benefit from a method that includes administering to the subject a pharmaceutical composition comprising an effective amount of versikine or a variant thereof that induces a T-cell mediated immune response. The methods may include determining the concentration of versikine in a biological sample from the subject (e.g., a blood product), and if the determined level is determined to be below a selected baseline, then administering the pharmaceutical composition comprising versikine or the variant thereof. Suitable blood products as biological samples may include blood itself, plasma, and serum. Suitable tissue samples as biological samples may include biopsies, for example, from a tumor. Immunoassays as known in the art may be utilized to determine the concentration of versikine in the biological sample.

Also disclosed are kits comprising components that optionally may be utilized to perform the methods disclosed herein. The kits may include one or more of (a) versikine or a variant thereof, where the versikine or the variant thereof

optionally is provided as a pharmaceutical composition; and (b) a reagent for detecting the concentration of versikine in a biological sample (e.g., an anti-versikine antibody which optionally is labelled with a detectable label). The kit optionally may include implements for administering the versikine or variant thereof (e.g., a syringe/needle type implement). The kit optionally may include an immunoassay for detecting versikine in a biological sample, for example, where the reagent for detecting the concentration of versikine in a biological sample is an anti-versikine antibody. The anti-versikine antibody may be labelled, or optionally, the kit may include a labelled secondary antibody that binds to the anti-versikine antibody, which functions as the primary antibody. Suitable labels may include fluorescent labels, chemiluminescent labels, enzyme labels, radio labels, and the like.

Also disclosed are isolated polynucleotides encoded any of the versikine polypeptides and variants disclosed herein. The isolated polynucleotide may be present in vectors for replication of the polynucleotides or for expression of the encoded polypeptides, for example, where the polynucleotides are operably linked to a promoter, which optionally may be an inducible promoter. Also disclosed are isolated cells that comprise the isolated polynucleotides, particular the isolated polynucleotides as present in the disclosed vectors. Isolated cells that comprise vectors for expression of the encoded versikine polypeptides and variants may be cultured in methods in order to produce the encoded versikine polypeptides and variants.

ILLUSTRATIVE EMBODIMENTS

The following embodiments are illustrative and are not intended to limit the scope of the claimed subject matter.

Embodiment 1

A method for inducing and/or potentiating a T-cell mediated immune response in a subject in need thereof, the method comprising administering to the subject or to explanted cells of the subject a pharmaceutical composition comprising an effective amount of versikine or a variant thereof that induces and/or potentiates the T-cell mediated immune response.

Embodiment 2

The method of embodiment 1, wherein the T-cell mediated immune response is characterized by a type 1 interferon signature.

Embodiment 3

The method of embodiment 2, wherein the type 1 interferon signature is characterized by increased expression of one or more of IL1β, IL6, EBI3, IRF8, IL12p40, IF16, MX1, XAF1, IFITM1, OAS3, IFI44L, TRIM22, STAT1, IFI44, CCL2, MX2, IFIT1, OAS2, SIGLEC1, TTSAD2, OASL, SIGLEC11, IFITM1, and ISG15.

Embodiment 4

The method of embodiment 2 or 3, wherein the type 1 interferon signature is characterized by increased phosphorylation of one or more of JNK, p38-MAPK, and AKT.

Embodiment 5

The method of any of the foregoing embodiments, wherein the variant comprises the amino acid sequence of any of SEQ ID NOs:1-27 or an amino acid sequence having a least about 80% sequence identity to any of SEQ ID NOs:1-27.

Embodiment 6

The method of any of the foregoing embodiments, wherein the variant comprises an N-terminal methionine.

Embodiment 7

The method of any of the foregoing embodiments, wherein the variant comprises a fragment of any of SEQ ID NOs:1-27.

Embodiment 8

The method of any of the foregoing embodiments, wherein the variant does not comprise the amino acid sequence of SEQ ID NOs:4 or 8-27.

Embodiment 9

The method of any of the foregoing embodiments, wherein the variant does not have any chondroitin sulfate side chains.

Embodiment 10

The method of any of the foregoing embodiments, wherein the variant has one or more amino acid modifications selected from the group consisting of acylation (e.g., N-terminal acylation), acetylation (e.g., N-terminal acetylation), formylation, lipolylation, myristoylation, palmitoylation, alkylation, isoprenylation, prenylation, and amidation (e.g., C-terminal amidation).

Embodiment 11

The method of any of the foregoing embodiments, wherein the variant comprises a fused antigen-binding domain.

Embodiment 12

The method of embodiment 11, wherein the antigen-binding domain binds to an epitope of a tumor antigen.

Embodiment 13

The method of any of the foregoing embodiments, wherein administering comprising intravenously administering to the subject the pharmaceutical composition comprising an effective amount of versikine or a variant thereof.

Embodiment 14

The method of any of the foregoing embodiments, wherein administering comprising injecting locally into tissue of the subject the pharmaceutical composition comprising an effective amount of versikine or a variant thereof.

Embodiment 15

The method of embodiment 14, wherein the tissue is a tumor.

Embodiment 16

The method of any of the foregoing embodiments, wherein administering comprises treating explanted cells of

the subject with the pharmaceutical composition comprising an effective amount of versikine or a variant thereof, and administering the treated explanted cells to the subject.

Embodiment 17

The method of embodiment 16, wherein the explanted cells comprise T-cells or dendritic cells.

Embodiment 18

The method of embodiments 16 or 17, further comprising contacting the explanted cells with an antigen prior to administering the treated cells to the subject.

Embodiment 19

The method of any of the foregoing embodiments, further comprising administering an antigen to the subject before, concurrently with, or after administering the pharmaceutical composition comprising versikine or the variant thereof to the subject.

Embodiment 20

The method of embodiment 18 or 19, wherein the antigen is a tumor antigen.

Embodiment 21

The method of any of the foregoing embodiments, wherein the subject has a cell proliferative disease or disorder such as cancer.

Embodiment 22

The method of embodiment 21, wherein the cancer is selected from the group consisting of adenocarcinoma, leukemia, lymphoma, melanoma, myeloma, sarcoma, and teratocarcinoma.

Embodiment 23

The method of embodiment 21, wherein the cancer is selected from cancers of the adrenal gland, bladder, blood, bone, bone marrow, brain, breast, cervix, gall bladder, ganglia, gastrointestinal tract, heart, kidney, liver, lung, muscle, ovary, pancreas, parathyroid, prostate, skin, testis, thymus, and uterus.

Embodiment 24

The method of any of the foregoing embodiments, wherein the subject has a hematological malignancy.

Embodiment 25

The method of any of the foregoing embodiments, wherein the subject has myeloma.

Embodiment 26

The method of any of embodiments 21-25, further comprising administering to the subject cancer therapy before, concurrently with, or after administering the pharmaceutical composition comprising versikine or the variant thereof.

Embodiment 27

The method of embodiment 26, wherein the cancer therapy comprises administering a chemotherapeutic agent.

Embodiment 28

The method of embodiment 27, wherein the chemotherapeutic agent is selected from a group consisting of Abitrexate, Adcetris, Ambochlorin, Aredia (Pamidronate Disodium), Arranon, Asparaginase Erwinia chrysanthemi, Becenum (Carmustine), Beleodaq, Belinostat, Bendamustine Hydrochloride, Bexxar, BiCNU (Carmustine), Blenoxane, Bleomycin, Blinatumomab, Blincyto, Bortezomib, Brentuximab Vedotin, Carfilzomib, Carmubris (Carmustine), Carmustine, Cerubidine, Chlorambucil, Clafen (Cyclophosphamide), Clofarex, Clolar, Cyclophosphamide, Cytarabine, Cytarabine Liposome, Cytosar-U, Cytoxan (Cyclophosphamide), Daratumumab, Darzalex (Daratumumab), Dasatinib, Daunorubicine Hydrochloride, Denileukin Diftitox, DepoCyt, Dexamethasone, Docetaxel, Doxil (Doxorubicin Hydrochloride Liposome), Doxorubicin Hydrochloride Liposome, Dox-SL (Doxorubicin Hydrochloride Liposome), Elotuzumab, Empliciti (Elotuzumab), Epirubicin, Erwinaze, Estramustine, Etoposide, Evacet (Doxorubicin Hydrochloride Liposome), Farydak (Panobinostat), Folex, Folex PFS, Folotyn, Ibrutumomab Tiuxetan, Ibrutinib, Iclusig, Idarubicin, Idelalisib, Imatinib Mesylate, Imbruvica, Intron A, Irinotecan, Istodax, Ixabepilone, Ixazomib Citrate, Kyprolis (Carfilzomib), Lenalidomide, Leukeran, Linfolizin, LipoDox (Doxorubicin Hydrochloride Liposome), Marqibo, Mechlorethamine Hydrochloride, Mercaptopurine, Methotrexate, Methotrexate LPF, Mexate, Mexate-AQ, Mitoxantrone, Mozobil (Plerixafor), Mustargen, Nelarabine, Neosar (Cyclophosphamide), Ninlaro (Ixazomib Citrate), Oncaspar, Ontak, Paclitaxel, Pamidronate Disodium, Panobinostat, Pegaspargase, Plerixafor, Pomalidomide, Pomalyst (Pomalidomide), Ponatinib Hydrochloride, Pralatrexate, Prednisone, Purinethol, Purixan, Recombinant Interferon Alfa-2b, Revlimid (Lenalidomide), Rituxanab, Romidepsin, Rubidomycin, Sprycel, Synovir (Thalidomide), Tarabiune PFS, Teniposide, Thalidomide, Thalomid (Thalidomide), Topotecan, Tositumomab, Treanda, Velban, Velcade (Bortezomib), Vinblastine Sulfate, Vincasar PGS, Vincristine Sulfate, Vincristine Sulfate Liposome, Vinorelbine, Vorinostat, Zevalin, Zoledronic Acid, Zolinza, Zometa (Zoledronic Acid), and Zydelig.

Embodiment 29

The method of embodiment 26, wherein the cancer therapy comprises one or more of therapies in which oncolytic viruses are administered, therapies in which immunomodulatory drugs are administered, therapies in which anthracyclines are administered, and therapies in which check-point blockers are administered.

Embodiment 30

The method of embodiment 26, wherein the cancer therapy comprises one or more of therapies in which chimeric antigen receptor (CAR) T-cells are administered, and therapies in which tumor infiltrating lymphocyte (TIL) are administered.

Embodiment 31

The method of embodiment 26, wherein the cancer therapy comprises radiation therapy.

Embodiment 32

The method of any of the foregoing embodiments, further comprising, before administering the pharmaceutical composition comprising versikine or the variant thereof, determining the concentration of versikine in a biological sample from the subject.

Embodiment 33

The method of embodiment 32, wherein the biological sample is blood or a blood product.

Embodiment 34

The method of embodiment 32, wherein the biological sample is tissue.

Embodiment 35

The method of embodiment 34, wherein the tissue is obtained from a tumor.

Embodiment 36

A kit, which optionally may be used to perform any of the foregoing methods, the kit comprising one or more of: (a) versikine or a variant thereof, wherein the versikine or the variant thereof optionally is provided as a pharmaceutical composition; and (b) a reagent for detecting the concentration of versikine in a biological sample (e.g., an anti-versikine antibody which optionally is labelled with a detectable label).

Embodiment 37

An isolated polypeptide comprising, consisting essentially of, or consisting of the amino acid sequence of any of SEQ ID NOs:3-27 or an amino acid sequence having a least about 80% sequence identity to any of SEQ ID NOs:3-27, wherein the polypeptide comprises a non-naturally occurring N-terminal methionine and the polypeptide induces expression of an interferon type 1 signature.

Embodiment 38

The isolated polypeptide of embodiment 37 comprising, consisting essentially of, or consisting of the amino acid sequence of any of SEQ ID NOs:3, 7, 10, 12, 14, 18, 21, 23, 25 and 27.

Embodiment 39

The isolated polypeptide of embodiment 37 or 38 comprising, consisting essentially of, or consisting of a fragment of any of SEQ ID NOs:1-27.

Embodiment 40

The isolated polypeptide of any of embodiments 37-39, wherein the polypeptide does not comprise the amino acid sequence of SEQ ID NOs:4 or 8-27.

Embodiment 41

The isolated polypeptide of any of embodiments 37-40, wherein the polypeptide does not have any chondroitin sulfate side chains.

Embodiment 42

The isolated polypeptide of any of embodiments 37-41, wherein the polypeptide has one or more amino acid modifications selected from the group consisting of acylation (e.g., N-terminal acylation), acetylation (e.g., N-terminal acetylation), formylation, lipolylation, myristoylation, palmitoylation, alkylation, isoprenylation, prenylation, and amidation (e.g., C-terminal amidation).

Embodiment 43

The isolated polypeptide of any of embodiments 37-42, wherein the polypeptide comprises a fused antigen-binding domain.

Embodiment 44

The isolated polypeptide of embodiment 43, wherein the antigen-binding domain binds to an epitope of a tumor antigen.

Embodiment 45

A pharmaceutical composition comprising an effective amount of any of the polypeptides of embodiments 37-44 for inducing expression of an interferon type 1 signature in a subject in need thereof.

Embodiment 46

An isolated polynucleotide encoding the isolated polypeptide of any of embodiments 37-44.

Embodiment 47

An expression vector comprising the isolated polynucleotide of embodiment 46 operably linked to a promoter.

Embodiment 48

An isolated cell comprising the expression vector of embodiment 47.

Embodiment 49

An anti-tumor vaccine comprising versikine, optionally wherein the vaccine stimulates and/or activates dendritic cells.

Embodiment 50

A method for treating a subject having a cell proliferative disease or disorder such as cancer or at risk for developing a cell proliferative disease or disorder such as cancer, the method comprising administered to the subject a pharma-

ceutical composition comprising versikine, optionally, wherein the pharmaceutical composition is an anti-tumor vaccine.

Embodiment 51

A method comprising detecting versican proteolysis in a biological sample comprising cancer cells.

Embodiment 52

The method of embodiment 51, wherein detecting versican proteolysis comprises detecting a fragment of versikine.

EXAMPLES

The following Examples are illustrative and are not intended to limit the scope of the claimed subject matter.

Example 1—Immunoregulatory Roles of Versican Proteolysis in the Myeloma Microenvironment

Reference is made to the manuscript: Hope et al., "Immunoregulatory Roles of Versican Proteolysis in the Myeloma Microenvironment," Blood. 2016 Aug. 4; 128(5):680, which is incorporated herein by reference in its entirety.

Abstract

Myeloma immunosurveillance remains incompletely understood. We have demonstrated proteolytic processing of the matrix proteoglycan, versican, in myeloma tumors. Whereas intact versican exerts tolerogenic activities through Toll-like receptor (TLR)-2 binding, the immunoregulatory consequences of versican proteolysis remain unknown. Here we show that human myeloma tumors displaying CD8+ aggregates underwent versican proteolysis at a site predicted to generate a glucosaminoglycan-bereft N-terminal fragment, versikine. Myeloma-associated macrophages (MAM), but not tumor cells, produced V1-versican, the precursor to versikine, whereas stromal cell-derived ADAMTS1 was the most robustly expressed versican-degrading protease. Purified versikine induced early expression of inflammatory cytokines IL1β and IL6 by freshly-explanted MAM. We show that versikine signals through pathways both dependent and independent of Tp12 kinase, a key regulator of NF□B1-mediated MAPK activation in macrophages. Unlike intact versican, versikine-induced IL6 production was partially independent of Tlr2. Versikine stimulated expression of type I-interferon (IFN)-stimulated genes in a model of macrophage-myeloma cell crosstalk without detectable type-I or -III interferon induction. Our data suggest that versikine, generated by ADAMTS proteolysis, constitutes a novel bioactive damage-associated-molecular-pattern (DAMP) that may promote T-cell-inflammation and modulate the tolerogenic consequences of intact versican accumulation. Therapeutic versikine administration may potentiate anti-cancer T-cell-activating immunotherapies.

Introduction

Myeloma is a tumor of plasma cells which are terminally differentiated B lymphocytes that produce antibody (Palumbo and Anderson, 2011). Myeloma plasma cells typically live within the bone marrow microenvironment ("canonical" myeloma niche). However malignant plasma cells can often thrive in extramedullary sites and soft tissues ("non-canonical" niche).

We have hypothesized that infiltrating myeloid cells may exert crucial trophic and immunoregulatory functions in both "canonical" and "non-canonical" niches, in part through their regulation of extracellular matrix composition and remodeling (Asimakopoulos et al., 2013). We and others have previously demonstrated that versican, a chondroitin-sulfate large matrix proteoglycan, accumulates in myeloma lesions and have hypothesized that versican may contribute to the regulation of their inflammatory milieu (Gupta et al., 2015; Hope et al., 2014). Versican has crucial, non-redundant significance in embryonic development (Nandadasa et al., 2014) and emerging roles in cancer inflammation, immunoregulation and metastasis (Gao et al., 2012; Kim et al., 2009; Ricciardelli et al., 2009; Wight et al., 2014). Versican promotes tolerogenic polarization of antigen-presenting cells through TLR2 (Tang et al., 2015). Versican is proteolytically cleaved by ADAMTS-type proteases in a highly-regulated manner (Nandadasa et al., 2014). A cleavage product generated by proteolysis of the Glu^{441}-Ala^{442} bond within the versican V1 isoform, has been termed versikine (Nandadasa et al., 2014). Versikine has been shown to be bioactive (proapoptotic) during interdigital web regression in the mouse embryo (McCulloch et al., 2009); however, the roles of versican proteolysis and/or versikine in tumor immunomodulation or progression remain unknown.

Results and Discussion

Myeloma Tumors Displaying CD8+ Aggregates Undergo Versican Proteolysis.

Because versican exerts tolerogenic activities in the tumor microenvironment, we hypothesized that versican proteolysis may promote "T-cell inflammation". We stained myeloma bone marrow biopsy specimens with antibodies against a versican neoepitope ($DPEAAE^{441}$) generated by Glu^{441}-Ala^{442} cleavage of V1-versican (corresponding to Glu^{1428} in V0-versican). Consecutive sections were stained for CD68, an immunohistochemical marker for tissue-resident macrophages and CD8, a marker of cytotoxic T cells. We observed four patterns of staining in 19 core biopsies arrayed on a UW myeloma tissue array as well as a commercially-obtained myeloma tissue array (FIG. **1**). Myeloma tumors displaying CD8+ aggregates (n=5 out of 19) (Gerard et al., 2013) demonstrated intense/moderate versican proteolysis, as detected by the anti-DPEAAE antibody.

All Four Versican Isoforms are Expressed by CD14+ Monocytic Cells in the Myeloma Microenvironment.

Versican has been variably reported to be expressed by tumor cells or the microenvironment in carcinomas, however its cellular origin in the myeloma niche is unknown. We carried out RT-PCR using versican isoform-specific primers in paired CD138+ malignant plasma cells, CD14+ monocytic cells and bone-marrow stromal cells (BM-MSC) from 3 patients with newly-diagnosed myeloma. We found that CD14+ monocytic cells were the predominant versican producers. Moreover, all four isoforms were expressed by myeloma CD14+ cells (FIG. **2**A). This was somewhat surprising, given the reported tissue-specific distribution of certain versican isoforms (e.g., V2 in neural tissue). Importantly, V1-versican, the precursor to versikine, was expressed robustly by myeloma-associated CD14+ monocytic cells. We next determined the relative expression of mRNAs for ADAMTS proteases that may degrade versican in the myeloma microenvironment, i.e., ADAMTS-1, -4, -5, -15, -19 and -20 (Nandadasa et al., 2014). We readily detected ADAMTS1, ADAMTS4 and ADAMTS5 mRNA expression in mononuclear cell lysate from myeloma bone marrow whereas ADAMTS15, ADAMTS19 and ADAMTS20 mRNAs were undetectable (data not shown). A previous report showed low expression of ADAMTS proteases in components of the myeloma microenvironment with the exception of bone marrow-derived mesenchymal

stromal cells (BM-MSC) (Bret et al., 2011). Therefore, we compared expression levels for ADAMTS1, ADAMTS4 and ADAMTS5 between myeloma and BM-MSC from normal donors and found that ADAMTS1 was expressed at higher levels by myeloma-derived BM-MSC (FIG. **2**B). Within the myeloma microenvironment, BM-MSC expressed much higher levels of ADAMTS1 message than either tumor cells or macrophages (FIG. **2**C).

Versikine Stimulates Inflammatory Cytokine Production by Primary MAM but does not have a Direct Impact on Tumor Cell Turnover.

To determine whether versican degradation products possessed immunoregulatory activity, we exposed freshly explanted primary myeloma CD14+ cells to recombinant purified human versikine (1 μM) overnight. Addition of versikine to primary CD14+ cells resulted in induction of inflammatory cytokines IL1β and IL6 (FIG. **3**A/B). These data demonstrate that versican chondroitin-sulfate side chains are unnecessary for inflammatory cytokine production, since they are not present on versikine. To determine whether versikine has direct effects on tumor cell turnover in a cell-autonomous fashion, we treated MM1.S human myeloma cells as well as primary bone marrow-derived myeloma cells with recombinant versikine. There was no discernible effect on cell cycle profiles of MM1.S myeloma cells treated with 1 μM versikine (FIG. **3**C). Similarly, primary bone marrow-derived, CD138+ myeloma plasma cells did not show detectable changes in cell cycle progression following versikine treatment (not shown). We conclude that versikine does not directly impact on cell cycle progression of human myeloma cells. Our results do not exclude non-cell-autonomous effects on cell cycle progression of myeloma cells through the actions of versikine-induced growth factors, such as IL6.

Versikine Signals Through Tpl2-Dependent and Independent Pathways and May Dispense of Tlr2 for IL6 Production.

Versican stimulates TLR2 complexes to promote immunomodulatory cytokine production (Kim et al., 2009). Signaling downstream of TLRs engages the MAP3K Tp12 (Cot, MAP3K8), a master regulator of macrophage activation and cytokine production in response to TLR or TNF-like stimuli (Hope et al., 2014; Vougioukalaki et al., 2011). Tpl2 loss in primary bone-marrow-derived macrophages (BMDM) abrogated IL1β production in response to purified versikine (FIG. **4**A). However, Tpl2 deletion in macrophages did not significantly affect versikine-induced IL6 (FIG. **4**B). Interestingly, versikine did not induce IL10 production (FIG. **4**C) and Tpl2 was a negative regulator of IL12p40 production in response to versikine (FIG. **4**D), similar to TLR agonists (Jensen et al., 2015).

Our results indicate that versikine may control cytokine production in both a Tp12-dependent and a Tp12-independent manner. To define the signaling cascades induced by versikine, we exposed BMDM to 1 μM purified versikine and collected cell lysate at sequential timepoints post-exposure. Versikine stimulation of wild-type BMDM rapidly induced JNK, p38-MAPK and AKT phosphorylation (FIG. **4**E). JNK and AKT phosphorylation were independent of Tpl2 status, whereas Tpl2 loss affected p38-MAPK phosphorylation (FIG. **4**E).

Intact versican is thought to signal through TLR2 (Tang et al., 2015). To determine whether versikine-induced IL6 required TLR2, we exposed wild-type and Tlr2−/− BMDM to TLR2 agonist Pam2CSK3 as well as versikine. Whereas Tlr2−/− BMDM showed a complete IL6 production defect in response to Pam2CSK3 (data not shown), they were still able to produce IL6 in response to versikine, albeit at 50% levels compared to WT-BMDM (FIG. **4**F). These data demonstrate that versikine signaling pathways may not overlap entirely with those activated by intact versican.

Versikine Modulates Macrophage Polarization.

Addition of versikine to BMDM induced expression of Th1-type cytokine IL12p40 (FIG. **4**G). Concurrent Fcγ receptor ligation through addition of ovalbumin (OVA)/anti-OVA immune complexes promoted IL10 production and induced macrophage polarization towards an immunoregulatory M2b phenotype ($Th12^{lo}$-$IL10^{hi}$) (Edwards et al., 2006). Therefore, versikine can act as an endogenous damage-associated molecular pattern (DAMP) that may modulate macrophage polarization in response to extracellular cues.

Versikine Induces Type-I-Interferon-Regulated Genes in a Model of Macrophage-Myeloma Cell Crosstalk.

Our data suggested that versican proteolysis results in the generation of versikine, a bioactive fragment that may act as a DAMP in the myeloma microenvironment. We then characterized the effects of versikine expression in a model of myeloma-macrophage crosstalk. Human THP-1 monocytic cells can be induced to generate macrophages that provide a functional platform to study macrophage regulation, including polarization by defined stimuli (Genin et al., 2015). We introduced a versikine-expressing plasmid into THP-1 cells prior to macrophage differentiation. Macrophages transduced with versikine-plasmid or empty-vector control, were co-cultured with human myeloma MM1.S cells for 48 hours. RNA-seq analysis was performed on each cell type after 48 h co-culture. Remarkably, only 23 genes were differentially expressed in MM1.S cells exposed to versikine-secreting macrophages versus control and all 23 genes were overexpressed (FIG. **5**A). Of the 23 genes, 13 genes were interferon-stimulated-genes (ISG), suggesting that versikine exposure induced a type I-interferon signature in myeloma cells. Differentially expressed genes did not include those coding for type I- or III-interferons. Interestingly, VCAN transcription was itself induced, suggesting a positive autoregulatory loop responding to cleaved versican. THP-1 macrophages expressing versikine demonstrated differential regulation of 39 genes (4 downregulated, 35 upregulated) when a cutoff of 2-fold expression change was used with a false discovery rate (FDR) threshold of 0.05. 12 upregulated genes defined a type-I-interferon signature (FIG. **5**B), again without overt transcription changes in type I or III-interferon genes.

We subsequently treated myeloma-macrophage co-cultures with recombinant versikine. RNA was extracted from each cell type at defined timepoints and subjected to analysis using a parallel RT-PCR array platform focusing on interferon signaling (Human Interferons and Receptors array; see Materials and Methods). As shown in FIG. **5**C, 0.5 μM versikine induced upregulation of ISGs in MM1.S myeloma cells as well as THP-1 macrophages (FIG. **5**D). IRF8 expression was upregulated at 18 and 48 hours in MM1.S cells (FIG. **5**E), whereas IRF9 expression remained relatively constant (FIG. **5**F). Interestingly, IRF8 was upregulated in MM1.S cells only in the presence of co-cultured macrophages (data not shown). Irf8 expression in transplanted tumor cells has been shown to be inducible through an 1127-dependent mechanism (Mattei et al., 2012). We observed upregulation of the IL27 subunit, EBI3, in tumor cells and in primary MAM treated with versikine (FIGS. **5**G and **5**H).

Versican has been proposed to promote immunosuppression and tolerance in tumor microenvironments through a

TLR2-dependent mechanism (Tang et al., 2015). We previously reported versican proteolysis in myeloma bone marrow tissue (Hope et al., 2014). We hypothesized that the regulated degradation of versican by ADAMTS-type versicanases may modulate its tolerogenic potential by controlling versican bioavailability, disrupting its extracellular matrix networks and/or by generating novel bioactive fragments. Myeloma marrows with CD8 aggregates showed active versican proteolysis. Interestingly, only 5 out of 19 myeloma marrows showed evidence of CD8 infiltration/aggregates. This low preponderance of CD8-infiltrated myeloma tumors is consistent with the reported failure of checkpoint inhibition as monotherapy in myeloma (Suen et al., 2015). Macrophages, on the other hand, were abundant in all biopsies.

We report that versikine, a product of versican proteolysis, possesses immunoregulatory activities that may promote "T-cell-inflammation" (Gajewski, 2015; Zitvogel et al., 2015). The induction of IRF8 transcription in response to versikine is particularly intriguing and could provide a mechanistic link to ISG upregulation. IRF8 is a transcription factor with central non-redundant roles in dendritic cell development and maturation as well as homeostasis of myeloid-derived suppressor cells (Merad et al., 2013; Waight et al., 2013). IRF8 and IL27 can operate in a regulatory loop (Mattei et al., 2012). IL27 subunit EBI3 was induced by versikine. Ebi3−/− animals are prone to impaired anti-tumor T cell responses and accelerated tumor growth (Liu et al., 2015). Taken together, our results suggest that versikine may antagonize the tolerogenic actions of intact versican and thus, may provide a novel anti-tumor strategy. The findings also suggest that, in addition to small leucine-rich proteoglycans, previously shown to act as DAMPs (Schaefer, 2014), fragments of large aggregating proteoglycans may have the capacity to stimulate innate immunity and provide a bridge to adaptive immunity.

Materials and Methods

Patient Sample Collection and Processing.

Bone marrow aspirates were collected with informed consent under a University of Wisconsin IRB-approved protocol (HO07403). Mononuclear cells were separated using Ficoll-Hypaque 1.073 (GE Healthcare Bio-sciences, Piscataway, N.J., USA) and were immune-magnetically sorted using anti-CD138 or anti-CD14 microbeads (Miltenyi Biotec, Auburn, Calif., USA). Purity was over 90% for both CD14+ and CD138+ fractions. For mesenchymal stromal cells, the CD138−/CD14− double-negative fraction was plated in aMEM supplemented with 10% FBS (Hyclone, Logan, Utah, USA). Attached cells were harvested and passaged using TrypLE (Invitrogen, Carlsbad, Calif., USA) until reaching passage 4.

Versikine Production, Purification and Analysis.

Methods for expression and purification of recombinant versikine from mammalian cells have been previously published (Foulcer et al., 2015; Foulcer et al., 2014, the contents of which are incorporated herein by referenece in their entireties). In brief, a plasmid vector for expression of human versikine (residues 1-441 of the V1 isoform) with a C-terminal myc-His6 tag was transfected into CHO-K1 cells. Serum-free medium was collected from stably transfected cells and combined with Ni-NTA agarose. Bound versikine was sequentially washed with 4 M Guanidine-HCl and 500 mM NaCl and eluted with 250 mM imidazole. Purified versikine was extensively dialysed into phosphate buffered saline. Purified versican was characterized by Coomassie Brilliant Blue staining and LC-MS/MS at the Lerner Research Institute Proteomics Core to identify associated bioactive proteins and by fluorophore-assisted carbohydrate electrophoresis (FACE) for co-purifying hyaluronan (at the Lerner Research Institute Program of Excellences in Glycobiology Glycomics Core). Presence of hyaluronan was ruled out by FACE and by solid phase binding assay and size exclusion chromatography.

Each versikine aliquot used in this study tested negative for endotoxin contamination using ToxinSensor Gel Clot kit (Genscript, sensitivity limit 0.25 endotoxin units (EU)/mL) (roughly equivalent to 25-50 pg/mL). The ultra-sensitive Chromogenic LAL Endotoxin Assay Kit (Genscript) was used to obtain a quantitative determination of the endotoxin concentration in versikine stock: it was determined to be 0.1 EU/mL (roughly equivalent to 10-20 pg endotoxin/mL prior to 1/10 dilution in versikine-treated wells). To definitively exclude the possibility of spurious endotoxin-mediated transcriptional changes, we exposed THP-1 macrophages to graded endotoxin concentrations, 10 ng/mL, 1 ng/mL and each of 500/250/100/75/50/25/10/5/1 pg/mL for 12 hours. RNA was isolated and EBI3 transcripts by RT-PCR were compared to a zero-endotoxin control. EBI3 transcriptional induction was not observed at endotoxin concentrations equal or less than 100 pg/mL.

Mice and Primary Cell Culture.

C57BL6/J, Tpl2−/− (Dumitru et al., 2000), Tlr2−/− (Jax stock #004650) mice were housed, cared for, and used in accordance with the *Guide for Care and Use of Laboratory Animals* (NIH Publication 86-23) under an IACUC-approved protocol (M2395). Bone marrow was extracted from spine and long bones as previously described. BMDM were derived and cultured as previously described (Hope et al., 2014).

Cell and Tissue Culture.

MM1.S cells were generously provided by Dr. Constantine Mitsiades (Dana-Farber Cancer Institute, Boston, Mass.). THP-1 (ATCC® TIB-202™) cells were maintained in tissue culture according to ATCC protocols. Cell culture was carried out in complete RPMI 1640 media supplemented with 10% fetal bovine serum (HyClone), a standard antibiotic/antimycotic solution (Life Technologies, 35050-061), and GlutaMax (Mediatech, 30-004-CI). In co-cultures shown in FIG. **5**, the ratio of THP-1 macrophages: MM1.S cells were 5:1 for RNA-seq experiments and 1:1 for recombinant versikine experiments.

RNA Extraction and Real-Time RT-PCR.

RT-PCR was performed using Applied Biosystems® StepOnePlus™ with accompanying software and Power SYBR® Green (Applied Biosystems® No. 4309155). Primer sequences are listed in Supplementary Methods. Human Interferon and Receptor RT^2-Profiler PCR array was obtained from Qiagen (PAHS-064Z). Relative expression was determined by ΔΔCt calculation. All RT-PCR protocols were performed in accordance with MIQE standards.

Immunohistochemistry.

The University of Wisconsin myeloma tissue microarray (TMA) has been previously reported (Hope et al., 2014). A second myeloma TMA was purchased from US Biomax (catalog no. T291b). Slides were deparaffinized using standard xylene/ethanol methods followed by antigen retrieval in citrate (DPEAAE and CD68 detection) or EDTA buffer (CD8 detection). Primary antibodies are listed in the Supplementary File.

Immunoblot Analysis.

Whole cell lysates were prepared by boiling cells in Laemmli Sample buffer (Bio-Rad) supplemented with 100 mM DTT for 10 minutes at a final concentration of 10^7 cells/ml. Protein was quantified using Bradford assay

reagent (BioRad). 10^5 cells or 20 μg protein was resolved by SDS-PAGE and transferred to Immobilon-P PVDF membrane (Millipore). Primary antibodies are listed in the Supplementary File.

Cell Cycle Analysis.

Cells were harvested after exposure to BrdU for 30 minutes. Cells were washed in PBS, fixed and permeabilized in 70% ethanol and stained with anti-BrdU-FITC and propidium iodide per standard protocols. Flow cytometry was carried out on FACSCalibur analyzer (Becton Dickinson). FlowJo software was used for flow data analysis.

Cytokine Measurement.

Cytokine levels were measured in culture supernatant using the bead-based Bio-Plex system (Bio-Rad).

RNA-Seq and Data Analysis.

Total RNA submitted to the University of Wisconsin-Madison Biotechnology Center was verified for purity and integrity via the NanoDrop2000 Spectrophotometer and Agilent 2100 BioAnalyzer, respectively. Samples that met the Illumina sample input guidelines were prepared according the TruSeq® Stranded Total RNA Sample Preparation Guide (Rev. E) using the Illumina® TruSeq® Stranded Total RNA Sample Preparation kits (Illumina Inc., San Diego, Calif., USA) with minor modifications. For each library preparation, lug of total RNA was ribosomally reduced as directed. Ribosomally depleted. RNA samples were purified by paramagnetic beads (AgencourtRNA Clean XP beads, Beckman Coulter, Indianapolis Ind., USA). Subsequently, each rRNA-depleted sample was fragmented using divalent cations under elevated temperature. The fragmented RNA was synthesized into double-stranded cDNA using SuperScript IIReverse Transcriptase (Invitrogen, Carlsbad, Calif., USA) and random primers for first strand cDNA synthesis followed by second strand synthesis using DNA Polymerase land RNAse H for removal of mRNA. Double-stranded cDNA was purified by paramagnetic beads (Agencourt AMPure XP beads, Beckman Coulter). The cDNA products were incubated with Klenow DNA Polymerase to add an 'A' base (Adenine) to the 3' end of the blunt DNA fragments. DNA fragments were ligated to Illumina adapters, which have a single 'T' base (Thymine) overhang at their 3'end. The adapter-ligated DNA products were purified by paramagnetic beads. Adapter ligated DNA was amplified in a Linker Mediated PCR reaction (LM-PCR) for 10 cycles using Phusion™ DNA Polymerase and Illumina's PE genomic DNA primer set and then purified by paramagnetic beads. Quality and quantity of the finished libraries were assessed using an Agilent DNA1000 chip (Agilent Technologies, Inc., Santa Clara, Calif., USA) and Qubit® dsDNA HS Assay Kit (Invitrogen, Carlsbad, Calif., USA), respectively. Libraries were standardized to 2 μM. Cluster generation was performed using standard Cluster Kits (v3) and the Illumina Cluster Station. Single 100 bp sequencing was performed, using standard SBS chemistry (v3) on an Illumina HiSeq2000 sequencer. Images were analyzed using the standard Illumina Pipeline, version 1.8.2. The RNA-seq reads were trimmed and filtered to remove contaminant and low quality bases prior to the analysis. Filtered reads were aligned to the reference genome using open source software STAR followed by RSEM (Li et al., 2010) for reads assignment and expression estimation. We used EdgeR (Robinson et al., 2010) to compare differential expression (DE) between conditions/treatment. The DE gene list can be obtained by filtering result by FDR value and relative fold changes.

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Example 2—Versican Proteolysis Predicts Robust CD8+ T-Cell Infiltration in Human Mismatch Repair-Proficient and -Deficient Colorectal Cancers: Mechanistic Implications

Abstract

Colorectal cancer (CRC) originates within immunologically complex microenvironments. To date the benefits of immunotherapy have been modest except in neoantigen-laden mismatch repair (MMR)-deficient tumors. Approaches to enhance tumor-infiltrating lymphocytes in the tumor bed may substantially augment clinical immunotherapy responses. We recently reported that proteolysis of the tolerogenic matrix proteoglycan versican (VCAN), in myeloma tumors, generates a bioactive fragment, versikine, with putative immunostimulatory activities. Here we report that VCAN proteolysis strongly correlated with CD8+ T-cell infiltration in CRC. Tumors displaying active VCAN proteolysis and low total VCAN were associated with robust (10-fold) CD8+ T-cell infiltration. The correlation between VCAN proteolysis and CD8+ T-cell infiltration was maintained in MMR-proficient and -deficient CRCs. Tumor-intrinsic WNT pathway activation was associated with CD8+ T-cell exclusion and correlated with VCAN accumulation. VCAN proteolytic fragment, versikine, promoted the generation of CD103+CD11c^{hi}MHCII^{hi} conventional dendritic cells (cDC) from flt3L-mobilized primary bone marrow-derived cultures, suggesting that versican proteolysis in the tumor microenvironment may favor differentiation of tumor-seeding DC precursors towards IRF8-expressing CD103+DC, endowed with enhanced tumor antigen presentation capacity. Our findings indicate that VCAN proteolysis may shape CRC immune contexture and provide a rationale for testing VCAN proteolysis as a predictive and/or prognostic immune biomarker.

Significance

This study identifies VCAN proteolysis as a potential key regulator of CD8+ T-cell infiltration in colorectal cancer. Further studies are warranted to determine the role of VCAN proteolysis as an immune biomarker. In addition, therapeutic

manipulation of the VCAN-versikine axis may augment immunotherapy efficacy against CRC.

Introduction

CRC is the second leading cause of cancer-related mortality in the United States (1). The 5-year survival rate for patients with metastatic disease is unacceptably low (12%), generating an impetus for rapid progress to improve outcomes. Recent advances in cancer immunotherapy have only marginally impacted outcomes in CRC (2, 3). The noteworthy exception includes patients with mismatch repair-deficient (dMMR) tumors where genetic instability generates an expanded neo-antigenic repertoire (4). In dMMR cancers, treatment with the anti-PD1 antibodies pembrolizumab and nivolumab result in deep and prolonged therapeutic responses for a large proportion of patients (2, 4, 5). Unfortunately, not all patients with dMMR CRCs respond to these agents indicating that other regulatory factors play a key role in the response of CRCs to checkpoint blockade. In addition, an effective means to utilize immuno-oncology agents for mismatch repair proficient (pMMR) CRCs, which encompass greater than 95% of all metastatic CRCs, has yet to be identified.

The presence of infiltrating lymphocytes (TILs) is linked to favorable clinical outcomes and increased response rates to immune checkpoint inhibition (5, 6). Thus, TIL infiltration possesses both prognostic and predictive biomarker utility. However, at a mechanistic level, the tumor-cell autonomous and non-autonomous networks controlling immune infiltration into the tumor bed are mostly unknown. Approaches to enhance TIL entry/activation could have a major impact on immunotherapy efficacy.

We recently demonstrated that versican (VCAN), a large matrix proteoglycan with immunoregulatory activity, accumulates in the extracellular matrix of multiple myeloma tumors (7). VCAN contributes to cancerous and non-cancerous inflammation by promoting leukocyte-derived elaboration of inflammatory mediators (8-13) but also immunodeficiency through dendritic cell (DC) dysfunction (14). Interestingly, we also detected in situ VCAN proteolysis in a pattern consistent with the activities of a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS)-type proteases (15). Whereas tumor-associated macrophages produce all known VCAN isoforms, tumor-associated mesenchymal stromal cells secrete ADAMTS proteases that cleave VCAN. We hypothesized that VCAN proteolysis serves to generate bioactive fragments. Indeed, we demonstrated a fragment containing VCAN's N-terminal 441 amino acids, "versikine" (16), elicits a transcriptional program that is predicted to promote immunogenicity, and thus, antagonize the tolerogenic actions of its parent, intact VCAN (15). However, it is unclear whether VCAN-dependent immunoregulatory mechanisms are operative in non-myeloma, or indeed non-hematopoietic, settings. We chose to investigate CRC because both myeloma and CRC are driven by chronic inflammatory networks (17) and because better understanding of CRC immunosurveillance mechanisms will likely result in improved outcomes for large patient populations. Here we demonstrate that VCAN proteolysis correlates with CD8+ T-cell infiltration in CRC, regardless of mismatch-repair status and provide mechanistic implications. These results provide strong rationale for investigation of VCAN processing in immunotherapy prognostication and therapy across several solid and liquid tumor types.

Materials and Methods

Colorectal Cancer (CRC) Tissue Microarray (TMA).

A CRC TMA was created through the University of Wisconsin Carbone Cancer Center Translational Science Biocore Biobank. This TMA contains samples from 122 subjects with colorectal cancer across all stages. For each subject, the TMA contains 2 cores from the primary tumor and 1 core of tumor-associated normal tissue. The tumors utilized in the TMA were selected for their location and stage, such that an equal distribution of right, left and rectal tumors and stage I through IV cancers are present.

Immunohistochemical (IHC) Methods and Antibodies.

Unstained 4-5 μm-thick TMA sections were deparaffinized and rehydrated using standard methods. Antigen retrieval was carried out in EDTA buffer (CD8 detection) or citrate (all others). The slides were treated with chondroitinase ABC prior to staining with the total VCAN antibody (18). Primary antibodies included total VCAN (HPA004726, Sigma, St. Louis, Mo.), αDPEAAE (PA1-1748A, Thermo Fisher, Waltham, Mass.), CD8 (c4-0085-80, Ebioscience, San Diego, Calif., USA), phosphorylated ERK 1/2 (Thr202/Tyr204, 4370, Cell Signaling Technology, Danvers, Mass.), phosphorylated ribosomal protein S6 (RPS6) (Ser235/236, 4858, Cell Signaling Technology), and CTNNB1 (β-catenin, 8480, Cell Signaling Technology). The αDPEAAE neoepitope antibody has been previously validated (18).

Scoring and Analysis of Staining Patterns.

Cytoplasmic and membrane staining of the epithelium and stroma was scored for each core sample by a pathologist (K.A.M.) blinded to clinical parameters. Stained slides were examined using an Olympus BX43 microscope with attached Olympus DP73 digital camera (Olympus Corp, Waltham, Mass.). Epithelium and stroma were evaluated separately for total VCAN and αDPEAAE staining. Immunostaining for VCAN, αDPEAAE, phosphorylated ERK1/2, and phosphorylated RPS6 was assessed by scoring staining intensity (0 for no staining, 1 for low/weak staining, 2 for moderate staining and 3 for strong/intense staining) and the percentage of cells staining positive (0 for no staining, 1 for >0-10%, 2 for 11-50%, 3 for 51-75% and 4 for >75% staining; FIG. **10**). For CD8+ detection, the number of tumor infiltrating lymphocytes (TILs) per high-power field (HPF) within the malignant epithelium was calculated using a single area at 400× magnification (ocular 10× with an objective of 40×). Nuclear localization of β-catenin was recorded as present or absent. Tissue cores that were missing, damaged, contained staining artifacts, or had uncertain histology were excluded from the analysis.

Mismatch Repair (MMR) Analyses.

MMR status was determined by IHC for MLH1, MSH6, MSH2, and PMS2. The following prediluted primary antibodies were utilized: MLH1 ((M1) mouse monoclonal, Ventana Medical Systems, Inc, Tucson, Ariz.), MSH6 ((44) mouse monoclonal, Ventana Medical Systems, Inc), MSH2 ((G219-1129) mouse monoclonal, Ventana Medical Systems, Inc), and PMS2 ((EPR3947) rabbit monoclonal, Ventana Medical Systems, Inc). Staining was performed on a BenchMark ULTRA automated slide staining system and detected using the Opitview DAB IHC detection kit. Absence of staining for these proteins was scored by independent pathology review (K.A.M.). Tumor infiltrating leukocytes were utilized as an internal control.

KI67 Proliferation Index.

Immunofluorescence was performed by placing the TMA slides into a humidity chamber after slides were deparaffinized and rehydrated. Slides were blocked with 5% bovine serum albumin in Tris Buffered Saline (TBS) with 0.05%

Tween 20 for one hour at room temperature. Slides were then washed in TBS. The KI67 primary antibody (#11882 (Alexa Fluor 488 conjugate), Cell Signaling Technology) was diluted in PBS and incubated overnight at 4° C. overnight. After incubation, coverslips were washed in TBS and mounted using Prolong Gold DAPI mounting media (#P36931, Invitrogen, Carlsbad, Calif.) and sealed. TMA cores were classified based on the number of KI67 positive nuclei per core.

Generation of Recombinant Versikine.

Recombinant versikine was purified from mammalian cells and endotoxin-tested as previously described (15).

Bone Marrow Harvesting, flt3L-Mobilized Cultures and Flow Cytometry.

Bone marrow (BM) cells were harvested from C57BL/6J mice under IACUC-approved protocol M005476. Total BM cells were cultured for 9 days in the presence of 200 ng/mL flt3L, as previously described (19) with the addition of 1 mM recombinant versikine or vehicle at the beginning of culture. Harvested cells were resuspended in FACS buffer (PBS pH7.4, 2 mM EDTA, 0.5% BSA). Cell viability was established by Trypan Blue exclusion and 2×10^6 live cells were stained the following antibodies: anti-CD11c (N418-PE-Cy7, Tonbo); anti-CD103 (2E7-PE, Biolegend); anti-MHCII (M5/114.152-AlexaFluor 700, Biolegend), anti-SiglecH (551-PerCP-Cy5.5, Biolegend); anti-CD11b (P84-FITC, Biolegend) for 30 minutes on 4° C. Cells were washed and analyzed on a BD LSR II instrument-viability was assessed by DAPI staining. The instrument was calibrated daily according to manufacturer's protocol using the BD FACSDiva (v.6) Cytometer Setting & Tracking software application. Flow cytometry data was analyzed by FlowJo version 9.7.6 software (Tree Star, Ashland, Oreg.).

Immunoblot Analysis.

Whole cell lysates were prepared by boiling cells in Laemmli Sample buffer (Bio-Rad) supplemented with 100 mM DTT for 10 minutes at a final concentration of 10^7 cells/ml. 10^5 cells or 20 μg protein was resolved by SDS-PAGE and transferred to Immobilon-P PVDF membrane (Millipore). Membranes were blocked in 5% Milk in TBS-T [25 mM Tris-HCl (pH 7.4), 0.13M NaCl, 2.7 mM KCl]. Primary antibodies [anti-IRF8 (Cell Signaling Technologies, D20D8), anti-Batf3 (LSBio, B12B125)] were diluted in 5% Milk-TBST and membranes were incubated overnight at 4° C. Secondary antibody-HRP-conjugates as well as anti-GAPDH-HRP conjugate (Genscript A00192) incubations were carried out for 1 hour at room temperature. Signal detection was achieved using Amersham ECL Plus chemiluminescent solution (GE Healthcare). Blots were developed on Classic Blue Autoradiography Flim BX (MidSci).

Statistical Analyses.

Descriptive statistics were utilized to present the data including mean+standard deviation. Wilcoxon rank sum and chi-square analyses were utilized where noted. A p-value of <0.05 is considered statistically significant.

Results

VCAN Accumulation and Proteolysis in Normal and Malignant Colorectal Tissue.

The University of Wisconsin CRC TMA consists of 122 cases with matched cores from colorectal cancer and tumor-associated normal colon tissues. We stained the TMA with antibodies raised against a neoepitope (αDPEAAE) generated through VCAN cleavage at the Glu^{441}-Ala^{442} bond of the V1-VCAN isoform (16). DPEAAE constitutes the C-terminal end of the bioactive VCAN fragment, versikine. Serial tissue TMA sections were stained with an antibody recognizing the immunoglobulin-like domain at VCAN's N-terminal end. The latter would be expected to recognize all intact VCAN isoforms (total VCAN). Although its immunogen sequences are also included within cleaved VCAN, detection of cleaved/mobilized VCAN appears far less sensitive with the latter antibody. Intense total VCAN staining was observed in tumor stroma (FIGS. **6**A and **6**B). By contrast, highest intensity staining for the αDPEAAE neoepitope (2+, 3+) was detected within normal stroma and only variably within tumor stroma (Chi-square test, $p<0.001$; FIGS. **6**A and **6**C; FIG. **10**).

There was no correlation between total VCAN staining and location of primary tumor (left/right colon, rectum) (FIG. **11**). Increased αDPEAAE staining was observed in the rectum compared to the colon (Chi-square test, $p=0.009$). To determine whether VCAN processing correlated with tumor location, tumors were classified according to the degree of VCAN accumulation and processing in their stroma. Tumors were classified as "VCAN proteolysis-predominant" if their staining for total VCAN staining intensity was ≤1+ and staining for VCAN proteolysis (αDPEAAE antibody) was ≥2. Conversely, tumors were classified as "proteolysis-weak" if intact VCAN staining intensity was >1+ or αDPEAAE intensity was <2+. Despite a greater staining for αDPEAAE neoepitope being identified within the rectum, there was no significant correlation between the VCAN proteolysis-predominant classification and tumor location (Chi-square test, $p=0.96$; FIG. **11**B).

"VCAN Proteolysis-Predominant" Tumors Show Robust CD8+ T-Cell Infiltration.

Given the immunosuppressive properties of VCAN and immunostimulatory properties of its proteolytic product, versikine (15), we hypothesized that VCAN proteolysis-predominant tumors are primed for immune infiltration. To determine whether VCAN processing correlated with CD8+ T-cell infiltration, the TMA was stained for the effector T-cell marker, CD8, and correlated with the VCAN proteolysis classification. We detected a statistically significant correlation between proteolysis-predominant status and CD8+ T-cell infiltration. CD8+ scores in "proteolysis-predominant" tumors were on average 10-fold higher than "proteolysis-weak" tumors (mean of 22 CD8+ T-cells per HPF versus 2, respectively; Wilcoxon rank sum test, $p<0.001$; FIG. **7**A-B).

CD8+ T-cell infiltration was highest in tumors that displayed intense VCAN proteolysis together with low amounts of total VCAN (FIG. **7**C). This finding suggests that low VCAN accumulation may not adequately promote T-cell infiltration unless VCAN is actively processed to generate proteolytic fragments. This observation is consistent with our hypothesis that VCAN proteolysis generates bioactive fragments with novel activities. Conversely, in tumors with high total VCAN, CD8+ T-cell infiltration may be impeded through an unfavorable stoichiometry between intact VCAN and VCAN fragments. In summary, these data suggest that VCAN proteolytic fragments are not mere markers of VCAN turnover but are endowed with important novel immunomodulatory activities. We have previously elucidated the immunoregulatory role of the VCAN fragment, versikine (15).

Since tumors with greater degrees of CD8+ T-cell infiltration are known to result in a better prognosis, the association between VCAN proteolysis and tumor stage was assessed. A trend toward an increased prevalence of staining for the VCAN proteolysis-predominant classification was seen in colon cancers of earlier stage, albeit not statistically significant (Chi-square test, $p=0.28$; FIG. **11**C).

CD8+ T-Cell Infiltration Correlates with VCAN Proteolysis Regardless of MMR Status.

dMMR is observed in 15% of localized CRCs and 3-4% of metastatic cases (2, 4, 5). MLH1 and MSH2 are the most commonly lost MMR proteins. These proteins can be lost secondary to somatic or germline mutations or epigenetic silencing. dMMR status has been associated with an improved prognosis and increased response to immune checkpoint blockade (2, 4, 5). Since dMMR is one of the strongest predictors of CD8+ T-cell infiltration, we next examined the potential for a correlation between VCAN proteolysis and MMR status. IHC staining for the MMR proteins MLH1, MSH2, PMS2 and MSH6 was performed to determine MMR status. Consistent with prior reports, CD8+ T-cell infiltration was increased in dMMR tumors (Wilcoxon rank sum test, $p<0.001$; FIG. **8**A). MMR status was then correlated with VCAN and αDPEAAE staining. We observed all potential staining combinations in both pMMR and dMMR cancers (FIG. **8**B). A trend towards increased intensity of VCAN staining in pMMR cancers was observed. No significant differences were observed in the proportions of tumors staining for VCAN and αDPEAAE across dMMR cancers (FIG. **8**B). The correlation between VCAN proteolysis and CD8+ T-cell infiltration was maintained in both pMMR and dMMR (FIG. **8**C). In both pMMR and dMMR, those tumors staining for the VCAN proteolysis-predominant classification had the greatest degree of CD8+ T-cell infiltration (Wilcoxon rank sum tests: pMMR $p=0.006$; dMMR $p=0.03$). Among the VCAN proteolysis-predominant tumors there was a greater degree of CD8+ T cell infiltration in the dMMR cancers compared to pMMR cancers (35 versus 14.8 TILs per HPF, Wilcoxon rank sum test, $p=0.04$).

The VCAN Proteolysis Predominant Phenotype is More Common in dMMR Cancers.

Since the VCAN proteolysis predominant phenotype predicts CD8+ T-cell infiltration in both dMMR and pMMR cancers the prevalence of this phenotype was examined. Of the dMMR tumor samples, 25% possessed the VCAN proteolysis predominant phenotype, while this was observed in only 10% of pMMR samples (FIG. **3**D, Wilcoxon rank sum test, $p=0.01$). In addition, another 25% of dMMR cancers demonstrated 1+ or less staining for both total VCAN and αDPEAAE, while this was observed in an additional 14% of pMMR cancers.

CD8+ T-Cell Exclusion is Associated with WNT Pathway Activation in Tumor Cells.

In a recent report by the Gajewski group (20), WNT signaling activation in melanoma tumor cells correlated with CD8 T-cell exclusion. Because activation of WNT signaling is a frequent molecular event in CRC secondary to the presence of truncating mutations in APC or activation mutations in CTNNB1 (21), we investigated whether analogous mechanisms operated in CRC. Indeed, we detected a statistically significant negative correlation between nuclear CTNNB1 (β-catenin, a marker of active WNT signaling) and CD8+ T-cell infiltration in CRC (Wilcoxon rank sum test, $p=0.014$; FIG. **8**E). In addition, VCAN accumulation correlated with the presence of nuclear β-catenin (Chi-square test, $p<0.001$, FIG. **8**F) and was more common in the pMMR cancers (8 vs. 53%, respectively, Chi-square test, $p<0.001$, FIG. **8**G).

VCAN Accumulation and/or Proteolysis is not Associated with Tumor-Intrinsic Activation of the MAPK and PI3K Pathways, Nor with KI67 Index in CRC.

We investigated a potential correlation between MAPK pathway activation in tumor cells (detected through ERK1/2 phosphorylation), PI3K pathway activation (detected through RPS6 phosphorylation) or tumor cell proliferation (as measured through KI67 staining). The results are shown in FIGS. **12** and **13**. There was no correlation between activation of these key oncogenic pathways and/or KI67 index with VCAN processing.

Versikine Promotes the Generation of CD103+ cDC from flt3L-Mobilized Primary Bone Marrow Cultures.

Versican proteolysis may impact on tumor immune contexture through regulation of intact versican bioavailability and/or the generation of novel bioactive fragments. We have previously shown that versikine, a fragment generated through versican proteolysis at the Glu^{440}-Ala^{441} bond, activates an IRF8-dependent transcriptional program in cultured myeloid cells (15). IRF8 is a terminal selector for CD8a/CD103+ cDC (22), a DC subset with crucial roles in T-cell-mediated immunosurveillance (20, 23, 24).

Flt3L-mobilized BM cultures have long provided a faithful ex vivo model of DC differentiation (19). Addition of recombinant versikine at the onset of culture (together with flt3L) consistently and reproducibly promoted expansion of the CD103+CD11c+$MHCII^{hi}$ DC subset at both early and late culture timepoints (FIG. **9**A/B). These cells were $SIRP\alpha^{lo}$, $CD11b^{lo\text{-}int}$ and $SiglecH^{lo}$ confirming their identity as CD103+ conventional DC (cDC). There was no difference in the prevalence of $SiglecH^{hi}$ cells at Day 4 (data not shown). Versikine-treated cultures displayed increased expression of Irf8 and Batf3, both essential transcription factors for cDC1 development (FIG. **9**C). By contrast, addition of the TLR2/6 ligand, FSL-1 (Pam2CGDPKHPKSF) conferred a disadvantage to CD103+DC development (FIG. **9**D). Because intact VCAN is thought to act through TLR2/6 heterodimers (13), these results suggest that versikine may signal through pathways other than those triggered by intact VCAN. Taken together, our data suggest that tumor-seeding, bone-marrow-derived DC precursors may preferentially develop into immunogenic CD103+ DC in tumor microenvironments undergoing active VCAN proteolysis.

Discussion

Colorectal cancer remains a challenging problem of public health proportions. Recent advances in immunotherapy of solid tumors previously thought to be non-immunogenic, such as lung cancer, raised hopes that CRC patients might also benefit. However, CRC responses to novel immunotherapy modalities have been modest at best, with the exception of a small number of patients with mismatch repair-deficient CRC. Future challenges include the selection of patients most likely to respond (through the identification and validation of novel predictive biomarkers) as well as the devising and testing of innovative combinatorial immunotherapy regimens that augment efficacy with acceptable toxicity. CD8+ T-cell infiltration has been associated with an improved prognosis and response to immune checkpoint blockade, especially in the setting of dMMR. However, the mechanisms regulating immune cell infiltration are largely yet to be determined.

We report here the strong association between VCAN proteolysis and CD8+ T-cell infiltration. At a mechanistic level, proteolysis of intact VCAN can be postulated to produce three alternative consequences, not mutually exclusive: Firstly, proteolysis may regulate the amount and bioavailability of tolerogenic intact VCAN at the tumor site and the resultant degree of DC dysfunction (14). Secondly, proteolysis may disrupt VCAN's complex interactions with other immunoregulatory matrix components, such as hyaluronan or tenascin C (25). Thirdly, VCAN proteolysis generates fragments with novel activities. We recently

showed that versikine, a bioactive fragment generated through VCAN proteolysis, elicits an IRF8-dependent type-I interferon transcriptional program as well as IL12 but not IL10 production from myeloid cells (15). These actions are predicted to enhance immunogenicity and tumor "sensing" by the immune system. Indeed, in a small myeloma panel, VCAN proteolysis was necessary, albeit not sufficient, for CD8+ T-cell infiltration (15). In this manuscript we demonstrate that versikine promotes generation of CD103+ $CD11c^{hi}MHCII^{hi}$ conventional DC from flt3L-mobilized BM progenitors. The data support a model in which DC precursors seeding tumor sites undergoing active versican proteolysis may preferentially differentiate towards CD103+ DC implicated in T-cell mediated immunosurveillance and response to immunotherapies (20, 23, 24).

We observed intense VCAN proteolysis in normal colonic epithelium. The colon constitutes an immunologically active microenvironment that has evolved to cope with the continuous exposure to exogenous antigens provided by food processing as well as intestinal microbiota. The implications of this regulation are profound and bear significance well beyond the confines of the gastrointestinal tract. Importantly, a correlation between the composition of intestinal flora and degree of response to anti-tumor immunotherapy is established and beginning to be clinically exploited (26-28). The mechanisms accounting for the regulation and "fine-tuning" of immune responses in normal colonic epithelium are poorly understood (29). It is tempting to associate VCAN processing, and the resultant generation of bioactive immunoregulatory fragments, with homeostatic DC maturation in normal colon. Because the effects of intestinal microbiota on anti-tumor immunity are thought to be regulated at the level of DCs, we hypothesize that VCAN proteolysis may collaborate with the microbiome to influence immune priming against distally-located tumors. Alternatively or additionally, versican proteolysis may specifically shape the immunological milieu of the normal epithelium located adjacent to the "expanding rim" of colonic cancers.

Moreover, VCAN accumulation and turnover may impact on the local immunoregulation of several types of solid tumors that arise in normally "sterile" sites. For example, in prostate tissue, immunosuppressive signaling from TGFβ increases expression of VCAN, reduces expression of VCAN-cleaving ADAMTS proteases and enhances expression of ADAMTS metalloproteinase inhibitor, TIMP-3 (30). Interestingly, prostate cancer constitutes another common type of solid tumor that has yet to benefit from the recent advances in immunotherapy (31). It is intriguing to hypothesize that the VCAN-versikine axis may regulate immune infiltration across a wide spectrum of solid tumors.

Our data confirm and extend previous findings regarding the mechanisms regulating T-cell infiltration or exclusion from the tumor site. In particular, we confirm previous observations implicating melanoma-intrinsic WNT signaling in T-cell exclusion and extend these findings to CRC (20). Mechanistic analyses in melanoma suggested that WNT signaling acts through CCL4 to regulate tumor infiltration by Batf3-lineage DC (CD103+DC in peripheral tissues). Our data raise the testable hypothesis that WNT signaling enhances VCAN accumulation in the tumor microenvironment, potentially through the recruitment of immunosuppressive, VCAN-producing, macrophages. VCAN promotes DC dysfunction through Toll-like receptor-2 (TLR2) signaling (14). It is tempting to speculate that tumor-intrinsic WNT signaling radically remodels the myeloid immune contexture of the tumor through inhibition of immunogenic, Batf3-expressing DC together with recruitment of immunosuppressive, VCAN-producing, macrophages. We are currently testing these hypotheses.

The data presented in this manuscript suggest that VCAN processing may influence the balance between tolerogenic and immunogenic inflammation in common solid tumors. Further to our earlier work (15), corroborating evidence has lately come from different angles. A recent paper suggested a link between VCAN turnover and anti-viral T-cell responses in mice (32). We speculate the analogous mechanisms may operate during innate immune sensing of tumors (33). VCAN-producing, immunosuppressive macrophages were shown to expand post-therapy in myeloma and inhibit T-cell proliferation (34). The abundance of VCAN in CRCs is likely regulated both at the transcriptional level through WNT signaling and post-translationally, through ADAMTS proteases encoded by loci that are epigenetically regulated upon CRC progression (35). The data provide a rationale for investigating VCAN proteolysis as a novel immune biomarker in solid tumor settings. Moreover, therapeutic manipulation of the VCAN-versikine axis through targeted proteolysis of VCAN or administration of recombinant proteolytic fragment, versikine, could be clinically tested for synergy with modern immunotherapy modalities against CRC regardless of mismatch repair status.

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In the foregoing description, it will be readily apparent to one skilled in the art that varying substitutions and modifications may be made to the invention disclosed herein without departing from the scope and spirit of the invention. The invention illustratively described herein suitably may be practiced in the absence of any element or elements, limitation or limitations which is not specifically disclosed herein. The terms and expressions which have been employed are used as terms of description and not of limitation, and there is no intention that in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention. Thus, it should be understood that although the present invention has been illustrated by specific embodiments and optional features, modification and/or variation of the concepts herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention.

Citations to a number of references are made herein. All of the cited references are incorporated by reference herein in their entireties. In the event that there is an inconsistency between a definition of a term in the specification as compared to a definition of the term in a cited reference, the term should be interpreted based on the definition in the specification.

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 27

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<211> LENGTH: 2409
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

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<400> SEQUENCE: 1

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Val Thr His Ala Leu His Lys Val Lys Val Gly Lys Ser Pro Pro Val
            20                  25                  30

Arg Gly Ser Leu Ser Gly Lys Val Ser Leu Pro Cys His Phe Ser Thr
        35                  40                  45

Met Pro Thr Leu Pro Pro Ser Tyr Asn Thr Ser Glu Phe Leu Arg Ile
    50                  55                  60

Lys Trp Ser Lys Ile Glu Val Asp Lys Asn Gly Lys Asp Leu Lys Glu
65                  70                  75                  80

Thr Thr Val Leu Val Ala Gln Asn Gly Asn Ile Lys Ile Gly Gln Asp
                85                  90                  95

Tyr Lys Gly Arg Val Ser Val Pro Thr His Pro Glu Ala Val Gly Asp
            100                 105                 110

Ala Ser Leu Thr Val Val Lys Leu Leu Ala Ser Asp Ala Gly Leu Tyr
        115                 120                 125

Arg Cys Asp Val Met Tyr Gly Ile Glu Asp Thr Gln Asp Thr Val Ser
    130                 135                 140

Leu Thr Val Asp Gly Val Val Phe His Tyr Arg Ala Ala Thr Ser Arg
145                 150                 155                 160

Tyr Thr Leu Asn Phe Glu Ala Ala Gln Lys Ala Cys Leu Asp Val Gly
                165                 170                 175

Ala Val Ile Ala Thr Pro Glu Gln Leu Phe Ala Ala Tyr Glu Asp Gly
            180                 185                 190

Phe Glu Gln Cys Asp Ala Gly Trp Leu Ala Asp Gln Thr Val Arg Tyr
        195                 200                 205

Pro Ile Arg Ala Pro Arg Val Gly Cys Tyr Gly Asp Lys Met Gly Lys
    210                 215                 220

Ala Gly Val Arg Thr Tyr Gly Phe Arg Ser Pro Gln Glu Thr Tyr Asp
225                 230                 235                 240

Val Tyr Cys Tyr Val Asp His Leu Asp Gly Asp Val Phe His Leu Thr
                245                 250                 255

Val Pro Ser Lys Phe Thr Phe Glu Glu Ala Ala Lys Glu Cys Glu Asn
            260                 265                 270

Gln Asp Ala Arg Leu Ala Thr Val Gly Glu Leu Gln Ala Ala Trp Arg
        275                 280                 285

Asn Gly Phe Asp Gln Cys Asp Tyr Gly Trp Leu Ser Asp Ala Ser Val
    290                 295                 300

Arg His Pro Val Thr Val Ala Arg Ala Gln Cys Gly Gly Gly Leu Leu
305                 310                 315                 320

Gly Val Arg Thr Leu Tyr Arg Phe Glu Asn Gln Thr Gly Phe Pro Pro
                325                 330                 335

Pro Asp Ser Arg Phe Asp Ala Tyr Cys Phe Lys Arg Arg Met Ser Asp
            340                 345                 350

Leu Ser Val Ile Gly His Pro Ile Asp Ser Glu Ser Lys Glu Asp Glu
        355                 360                 365

Pro Cys Ser Glu Glu Thr Asp Pro Val His Asp Leu Met Ala Glu Ile
    370                 375                 380

Leu Pro Glu Phe Pro Asp Ile Ile Glu Ile Asp Leu Tyr His Ser Glu
385                 390                 395                 400

Glu Asn Glu Glu Glu Glu Glu Glu Cys Ala Asn Ala Thr Asp Val Thr
                405                 410                 415
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Thr Thr Pro Ser Val Gln Tyr Ile Asn Gly Lys His Leu Val Thr Thr
            420                 425                 430

Val Pro Lys Asp Pro Glu Ala Ala Glu Ala Arg Arg Gly Gln Phe Glu
        435                 440                 445

Ser Val Ala Pro Ser Gln Asn Phe Ser Asp Ser Ser Glu Ser Asp Thr
    450                 455                 460

His Pro Phe Val Ile Ala Lys Thr Glu Leu Ser Thr Ala Val Gln Pro
465                 470                 475                 480

Asn Glu Ser Thr Glu Thr Thr Glu Ser Leu Glu Val Thr Trp Lys Pro
                485                 490                 495

Glu Thr Tyr Pro Glu Thr Ser Glu His Phe Ser Gly Gly Glu Pro Asp
            500                 505                 510

Val Phe Pro Thr Val Pro Phe His Glu Glu Phe Glu Ser Gly Thr Ala
        515                 520                 525

Lys Lys Gly Ala Glu Ser Val Thr Glu Arg Asp Thr Glu Val Gly His
    530                 535                 540

Gln Ala His Glu His Thr Glu Pro Val Ser Leu Phe Pro Glu Glu Ser
545                 550                 555                 560

Ser Gly Glu Ile Ala Ile Asp Gln Glu Ser Gln Lys Ile Ala Phe Ala
                565                 570                 575

Arg Ala Thr Glu Val Thr Phe Gly Glu Glu Val Glu Lys Ser Thr Ser
            580                 585                 590

Val Thr Tyr Thr Pro Thr Ile Val Pro Ser Ser Ala Ser Ala Tyr Val
        595                 600                 605

Ser Glu Glu Glu Ala Val Thr Leu Ile Gly Asn Pro Trp Pro Asp Asp
    610                 615                 620

Leu Leu Ser Thr Lys Glu Ser Trp Val Glu Ala Thr Pro Arg Gln Val
625                 630                 635                 640

Val Glu Leu Ser Gly Ser Ser Ser Ile Pro Ile Thr Glu Gly Ser Gly
                645                 650                 655

Glu Ala Glu Glu Asp Glu Asp Thr Met Phe Thr Met Val Thr Asp Leu
            660                 665                 670

Ser Gln Arg Asn Thr Thr Asp Thr Leu Ile Thr Leu Asp Thr Ser Arg
        675                 680                 685

Ile Ile Thr Glu Ser Phe Phe Glu Val Pro Ala Thr Thr Ile Tyr Pro
    690                 695                 700

Val Ser Glu Gln Pro Ser Ala Lys Val Val Pro Thr Lys Phe Val Ser
705                 710                 715                 720

Glu Thr Asp Thr Ser Glu Trp Ile Ser Ser Thr Thr Val Glu Glu Lys
                725                 730                 735

Lys Arg Lys Glu Glu Glu Gly Thr Thr Gly Thr Ala Ser Thr Phe Glu
            740                 745                 750

Val Tyr Ser Ser Thr Gln Arg Ser Asp Gln Leu Ile Leu Pro Phe Glu
        755                 760                 765

Leu Glu Ser Pro Asn Val Ala Thr Ser Ser Asp Ser Gly Thr Arg Lys
    770                 775                 780

Ser Phe Met Ser Leu Thr Thr Pro Thr Gln Ser Glu Arg Glu Met Thr
785                 790                 795                 800

Asp Ser Thr Pro Val Phe Thr Glu Thr Asn Thr Leu Glu Asn Leu Gly
                805                 810                 815

Ala Gln Thr Thr Glu His Ser Ser Ile His Gln Pro Gly Val Gln Glu
            820                 825                 830
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Gly Leu Thr Thr Leu Pro Arg Ser Pro Ala Ser Val Phe Met Glu Gln
        835                 840                 845

Gly Ser Gly Glu Ala Ala Ala Asp Pro Glu Thr Thr Thr Val Ser Ser
    850                 855                 860

Phe Ser Leu Asn Val Glu Tyr Ala Ile Gln Ala Glu Lys Glu Val Ala
865                 870                 875                 880

Gly Thr Leu Ser Pro His Val Glu Thr Thr Phe Ser Thr Glu Pro Thr
                885                 890                 895

Gly Leu Val Leu Ser Thr Val Met Asp Arg Val Val Ala Glu Asn Ile
            900                 905                 910

Thr Gln Thr Ser Arg Glu Ile Val Ile Ser Glu Arg Leu Gly Glu Pro
        915                 920                 925

Asn Tyr Gly Ala Glu Ile Arg Gly Phe Ser Thr Gly Phe Pro Leu Glu
    930                 935                 940

Glu Asp Phe Ser Gly Asp Phe Arg Glu Tyr Ser Thr Val Ser His Pro
945                 950                 955                 960

Ile Ala Lys Glu Glu Thr Val Met Met Glu Gly Ser Gly Asp Ala Ala
                965                 970                 975

Phe Arg Asp Thr Gln Thr Ser Pro Ser Thr Val Pro Thr Ser Val His
            980                 985                 990

Ile Ser His Ile Ser Asp Ser Glu  Gly Pro Ser Ser Thr  Met Val Ser
        995                 1000                1005

Thr Ser  Ala Phe Pro Trp Glu  Glu Phe Thr Ser Ser  Ala Glu Gly
    1010                1015                1020

Ser Gly  Glu Gln Leu Val Thr  Val Ser Ser Ser Val  Val Pro Val
    1025                1030                1035

Leu Pro  Ser Ala Val Gln Lys  Phe Ser Gly Thr Ala  Ser Ser Ile
    1040                1045                1050

Ile Asp  Glu Gly Leu Gly Glu  Val Gly Thr Val Asn  Glu Ile Asp
    1055                1060                1065

Arg Arg  Ser Thr Ile Leu Pro  Thr Ala Glu Val Glu  Gly Thr Lys
    1070                1075                1080

Ala Pro  Val Glu Lys Glu Glu  Val Lys Val Ser Gly  Thr Val Ser
    1085                1090                1095

Thr Asn  Phe Pro Gln Thr Ile  Glu Pro Ala Lys Leu  Trp Ser Arg
    1100                1105                1110

Gln Glu  Val Asn Pro Val Arg  Gln Glu Ile Glu Ser  Glu Thr Thr
    1115                1120                1125

Ser Glu  Glu Gln Ile Gln Glu  Glu Lys Ser Phe Glu  Ser Pro Gln
    1130                1135                1140

Asn Ser  Pro Ala Thr Glu Gln  Thr Ile Phe Asp Ser  Gln Thr Phe
    1145                1150                1155

Thr Glu  Thr Glu Leu Lys Thr  Thr Asp Tyr Ser Val  Leu Thr Thr
    1160                1165                1170

Lys Lys  Thr Tyr Ser Asp Asp  Lys Glu Met Lys Glu  Glu Asp Thr
    1175                1180                1185

Ser Leu  Val Asn Met Ser Thr  Pro Asp Pro Asp Ala  Asn Gly Leu
    1190                1195                1200

Glu Ser  Tyr Thr Thr Leu Pro  Glu Ala Thr Glu Lys  Ser His Phe
    1205                1210                1215

Phe Leu  Ala Thr Ala Leu Val  Thr Glu Ser Ile Pro  Ala Glu His
    1220                1225                1230
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Val Val  Thr Asp Ser Pro Ile  Lys Lys Glu Glu Ser  Thr Lys His
    1235                1240                1245

Phe Pro  Lys Gly Met Arg Pro  Thr Ile Gln Glu Ser  Asp Thr Glu
    1250                1255                1260

Leu Leu  Phe Ser Gly Leu Gly  Ser Gly Glu Glu Val  Leu Pro Thr
    1265                1270                1275

Leu Pro  Thr Glu Ser Val Asn  Phe Thr Glu Val Glu  Gln Ile Asn
    1280                1285                1290

Asn Thr  Leu Tyr Pro His Thr  Ser Gln Val Glu Ser  Thr Ser Ser
    1295                1300                1305

Asp Lys  Ile Glu Asp Phe Asn  Arg Met Glu Asn Val  Ala Lys Glu
    1310                1315                1320

Val Gly  Pro Leu Val Ser Gln  Thr Asp Ile Phe Glu  Gly Ser Gly
    1325                1330                1335

Ser Val  Thr Ser Thr Thr Leu  Ile Glu Ile Leu Ser  Asp Thr Gly
    1340                1345                1350

Ala Glu  Gly Pro Thr Val Ala  Pro Leu Pro Phe Ser  Thr Asp Ile
    1355                1360                1365

Gly His  Pro Gln Asn Gln Thr  Val Arg Trp Ala Glu  Glu Ile Gln
    1370                1375                1380

Thr Ser  Arg Pro Gln Thr Ile  Thr Glu Gln Asp Ser  Asn Lys Asn
    1385                1390                1395

Ser Ser  Thr Ala Glu Ile Asn  Glu Thr Thr Thr Ser  Ser Thr Asp
    1400                1405                1410

Phe Leu  Ala Arg Ala Tyr Gly  Phe Glu Met Ala Lys  Glu Phe Val
    1415                1420                1425

Thr Ser  Ala Pro Lys Pro Ser  Asp Leu Tyr Tyr Glu  Pro Ser Gly
    1430                1435                1440

Glu Gly  Ser Gly Glu Val Asp  Ile Val Asp Ser Phe  His Thr Ser
    1445                1450                1455

Ala Thr  Thr Gln Ala Thr Arg  Gln Glu Ser Ser Thr  Thr Phe Val
    1460                1465                1470

Ser Asp  Gly Ser Leu Glu Lys  His Pro Glu Val Pro  Ser Ala Lys
    1475                1480                1485

Ala Val  Thr Ala Asp Gly Phe  Pro Thr Val Ser Val  Met Leu Pro
    1490                1495                1500

Leu His  Ser Glu Gln Asn Lys  Ser Ser Pro Asp Pro  Thr Ser Thr
    1505                1510                1515

Leu Ser  Asn Thr Val Ser Tyr  Glu Arg Ser Thr Asp  Gly Ser Phe
    1520                1525                1530

Gln Asp  Arg Phe Arg Glu Phe  Glu Asp Ser Thr Leu  Lys Pro Asn
    1535                1540                1545

Arg Lys  Lys Pro Thr Glu Asn  Ile Ile Ile Asp Leu  Asp Lys Glu
    1550                1555                1560

Asp Lys  Asp Leu Ile Leu Thr  Ile Thr Glu Ser Thr  Ile Leu Glu
    1565                1570                1575

Ile Leu  Pro Glu Leu Thr Ser  Asp Lys Asn Thr Ile  Ile Asp Ile
    1580                1585                1590

Asp His  Thr Lys Pro Val Tyr  Glu Asp Ile Leu Gly  Met Gln Thr
    1595                1600                1605

Asp Ile  Asp Thr Glu Val Pro  Ser Glu Pro His Asp  Ser Asn Asp
    1610                1615                1620
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Glu Ser  Asn Asp Asp Ser Thr  Gln Val Gln Glu Ile  Tyr Glu Ala
    1625                 1630                 1635

Ala Val  Asn Leu Ser Leu Thr  Glu Glu Thr Phe Glu  Gly Ser Ala
    1640                 1645                 1650

Asp Val  Leu Ala Ser Tyr Thr  Gln Ala Thr His Asp  Glu Ser Met
    1655                 1660                 1665

Thr Tyr  Glu Asp Arg Ser Gln  Leu Asp His Met Gly  Phe His Phe
    1670                 1675                 1680

Thr Thr  Gly Ile Pro Ala Pro  Ser Thr Glu Thr Glu  Leu Asp Val
    1685                 1690                 1695

Leu Leu  Pro Thr Ala Thr Ser  Leu Pro Ile Pro Arg  Lys Ser Ala
    1700                 1705                 1710

Thr Val  Ile Pro Glu Ile Glu  Gly Ile Lys Ala Glu  Ala Lys Ala
    1715                 1720                 1725

Leu Asp  Asp Met Phe Glu Ser  Ser Thr Leu Ser Asp  Gly Gln Ala
    1730                 1735                 1740

Ile Ala  Asp Gln Ser Glu Ile  Ile Pro Thr Leu Gly  Gln Phe Glu
    1745                 1750                 1755

Arg Thr  Gln Glu Glu Tyr Glu  Asp Lys Lys His Ala  Gly Pro Ser
    1760                 1765                 1770

Phe Gln  Pro Glu Phe Ser Ser  Gly Ala Glu Glu Ala  Leu Val Asp
    1775                 1780                 1785

His Thr  Pro Tyr Leu Ser Ile  Ala Thr Thr His Leu  Met Asp Gln
    1790                 1795                 1800

Ser Val  Thr Glu Val Pro Asp  Val Met Glu Gly Ser  Asn Pro Pro
    1805                 1810                 1815

Tyr Tyr  Thr Asp Thr Thr Leu  Ala Val Ser Thr Phe  Ala Lys Leu
    1820                 1825                 1830

Ser Ser  Gln Thr Pro Ser Ser  Pro Leu Thr Ile Tyr  Ser Gly Ser
    1835                 1840                 1845

Glu Ala  Ser Gly His Thr Glu  Ile Pro Gln Pro Ser  Ala Leu Pro
    1850                 1855                 1860

Gly Ile  Asp Val Gly Ser Ser  Val Met Ser Pro Gln  Asp Ser Phe
    1865                 1870                 1875

Lys Glu  Ile His Val Asn Ile  Glu Ala Thr Phe Lys  Pro Ser Ser
    1880                 1885                 1890

Glu Glu  Tyr Leu His Ile Thr  Glu Pro Pro Ser Leu  Ser Pro Asp
    1895                 1900                 1905

Thr Lys  Leu Glu Pro Ser Glu  Asp Asp Gly Lys Pro  Glu Leu Leu
    1910                 1915                 1920

Glu Glu  Met Glu Ala Ser Pro  Thr Glu Leu Ile Ala  Val Glu Gly
    1925                 1930                 1935

Thr Glu  Ile Leu Gln Asp Phe  Gln Asn Lys Thr Asp  Gly Gln Val
    1940                 1945                 1950

Ser Gly  Glu Ala Ile Lys Met  Phe Pro Thr Ile Lys  Thr Pro Glu
    1955                 1960                 1965

Ala Gly  Thr Val Ile Thr Thr  Ala Asp Glu Ile Glu  Leu Glu Gly
    1970                 1975                 1980

Ala Thr  Gln Trp Pro His Ser  Thr Ser Ala Ser Ala  Thr Tyr Gly
    1985                 1990                 1995

Val Glu  Ala Gly Val Val Pro  Trp Leu Ser Pro Gln  Thr Ser Glu
    2000                 2005                 2010
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Arg Pro  Thr Leu Ser Ser Ser  Pro Glu Ile Asn Pro  Glu Thr Gln
    2015                 2020                 2025

Ala Ala  Leu Ile Arg Gly Gln  Asp Ser Thr Ile Ala  Ala Ser Glu
    2030                 2035                 2040

Gln Gln  Val Ala Ala Arg Ile  Leu Asp Ser Asn Asp  Gln Ala Thr
    2045                 2050                 2055

Val Asn  Pro Val Glu Phe Asn  Thr Glu Val Ala Thr  Pro Pro Phe
    2060                 2065                 2070

Ser Leu  Leu Glu Thr Ser Asn  Glu Thr Asp Phe Leu  Ile Gly Ile
    2075                 2080                 2085

Asn Glu  Glu Ser Val Glu Gly  Thr Ala Ile Tyr Leu  Pro Gly Pro
    2090                 2095                 2100

Asp Arg  Cys Lys Met Asn Pro  Cys Leu Asn Gly Gly  Thr Cys Tyr
    2105                 2110                 2115

Pro Thr  Glu Thr Ser Tyr Val  Cys Thr Cys Val Pro  Gly Tyr Ser
    2120                 2125                 2130

Gly Asp  Gln Cys Glu Leu Asp  Phe Asp Glu Cys His  Ser Asn Pro
    2135                 2140                 2145

Cys Arg  Asn Gly Ala Thr Cys  Val Asp Gly Phe Asn  Thr Phe Arg
    2150                 2155                 2160

Cys Leu  Cys Leu Pro Ser Tyr  Val Gly Ala Leu Cys  Glu Gln Asp
    2165                 2170                 2175

Thr Glu  Thr Cys Asp Tyr Gly  Trp His Lys Phe Gln  Gly Gln Cys
    2180                 2185                 2190

Tyr Lys  Tyr Phe Ala His Arg  Arg Thr Trp Asp Ala  Ala Glu Arg
    2195                 2200                 2205

Glu Cys  Arg Leu Gln Gly Ala  His Leu Thr Ser Ile  Leu Ser His
    2210                 2215                 2220

Glu Glu  Gln Met Phe Val Asn  Arg Val Gly His Asp  Tyr Gln Trp
    2225                 2230                 2235

Ile Gly  Leu Asn Asp Lys Met  Phe Glu His Asp Phe  Arg Trp Thr
    2240                 2245                 2250

Asp Gly  Ser Thr Leu Gln Tyr  Glu Asn Trp Arg Pro  Asn Gln Pro
    2255                 2260                 2265

Asp Ser  Phe Phe Ser Ala Gly  Glu Asp Cys Val Val  Ile Ile Trp
    2270                 2275                 2280

His Glu  Asn Gly Gln Trp Asn  Asp Val Pro Cys Asn  Tyr His Leu
    2285                 2290                 2295

Thr Tyr  Thr Cys Lys Lys Gly  Thr Val Ala Cys Gly  Gln Pro Pro
    2300                 2305                 2310

Val Val  Glu Asn Ala Lys Thr  Phe Gly Lys Met Lys  Pro Arg Tyr
    2315                 2320                 2325

Glu Ile  Asn Ser Leu Ile Arg  Tyr His Cys Lys Asp  Gly Phe Ile
    2330                 2335                 2340

Gln Arg  His Leu Pro Thr Ile  Arg Cys Leu Gly Asn  Gly Arg Trp
    2345                 2350                 2355

Ala Ile  Pro Lys Ile Thr Cys  Met Asn Pro Ser Ala  Tyr Gln Arg
    2360                 2365                 2370

Thr Tyr  Ser Met Lys Tyr Phe  Lys Asn Ser Ser Ser  Ala Lys Asp
    2375                 2380                 2385
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Asn Ser  Ile Asn Thr Ser Lys  His Asp His Arg Trp  Ser Arg Arg
    2390                2395                2400

Trp Gln  Glu Ser Arg Arg
    2405


<210> SEQ ID NO 2
<211> LENGTH: 2389
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 2

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Ser Gly Lys Val Ser Leu Pro Cys His Phe Ser Thr Met Pro Thr Leu
            20                  25                  30

Pro Pro Ser Tyr Asn Thr Ser Glu Phe Leu Arg Ile Lys Trp Ser Lys
        35                  40                  45

Ile Glu Val Asp Lys Asn Gly Lys Asp Leu Lys Glu Thr Thr Val Leu
    50                  55                  60

Val Ala Gln Asn Gly Asn Ile Lys Ile Gly Gln Asp Tyr Lys Gly Arg
65                  70                  75                  80

Val Ser Val Pro Thr His Pro Glu Ala Val Gly Asp Ala Ser Leu Thr
                85                  90                  95

Val Val Lys Leu Leu Ala Ser Asp Ala Gly Leu Tyr Arg Cys Asp Val
            100                 105                 110

Met Tyr Gly Ile Glu Asp Thr Gln Asp Thr Val Ser Leu Thr Val Asp
        115                 120                 125

Gly Val Val Phe His Tyr Arg Ala Ala Thr Ser Arg Tyr Thr Leu Asn
    130                 135                 140

Phe Glu Ala Ala Gln Lys Ala Cys Leu Asp Val Gly Ala Val Ile Ala
145                 150                 155                 160

Thr Pro Glu Gln Leu Phe Ala Ala Tyr Glu Asp Gly Phe Glu Gln Cys
                165                 170                 175

Asp Ala Gly Trp Leu Ala Asp Gln Thr Val Arg Tyr Pro Ile Arg Ala
            180                 185                 190

Pro Arg Val Gly Cys Tyr Gly Asp Lys Met Gly Lys Ala Gly Val Arg
        195                 200                 205

Thr Tyr Gly Phe Arg Ser Pro Gln Glu Thr Tyr Asp Val Tyr Cys Tyr
    210                 215                 220

Val Asp His Leu Asp Gly Asp Val Phe His Leu Thr Val Pro Ser Lys
225                 230                 235                 240

Phe Thr Phe Glu Glu Ala Ala Lys Glu Cys Glu Asn Gln Asp Ala Arg
                245                 250                 255

Leu Ala Thr Val Gly Glu Leu Gln Ala Ala Trp Arg Asn Gly Phe Asp
            260                 265                 270

Gln Cys Asp Tyr Gly Trp Leu Ser Asp Ala Ser Val Arg His Pro Val
        275                 280                 285

Thr Val Ala Arg Ala Gln Cys Gly Gly Gly Leu Leu Gly Val Arg Thr
    290                 295                 300

Leu Tyr Arg Phe Glu Asn Gln Thr Gly Phe Pro Pro Pro Asp Ser Arg
305                 310                 315                 320

Phe Asp Ala Tyr Cys Phe Lys Arg Arg Met Ser Asp Leu Ser Val Ile
                325                 330                 335

Gly His Pro Ile Asp Ser Glu Ser Lys Glu Asp Glu Pro Cys Ser Glu
            340                 345                 350
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Glu Thr Asp Pro Val His Asp Leu Met Ala Glu Ile Leu Pro Glu Phe
        355                 360                 365

Pro Asp Ile Ile Glu Ile Asp Leu Tyr His Ser Glu Glu Asn Glu Glu
    370                 375                 380

Glu Glu Glu Glu Cys Ala Asn Ala Thr Asp Val Thr Thr Thr Pro Ser
385                 390                 395                 400

Val Gln Tyr Ile Asn Gly Lys His Leu Val Thr Thr Val Pro Lys Asp
                405                 410                 415

Pro Glu Ala Ala Glu Ala Arg Arg Gly Gln Phe Glu Ser Val Ala Pro
            420                 425                 430

Ser Gln Asn Phe Ser Asp Ser Ser Glu Ser Asp Thr His Pro Phe Val
        435                 440                 445

Ile Ala Lys Thr Glu Leu Ser Thr Ala Val Gln Pro Asn Glu Ser Thr
    450                 455                 460

Glu Thr Thr Glu Ser Leu Glu Val Thr Trp Lys Pro Glu Thr Tyr Pro
465                 470                 475                 480

Glu Thr Ser Glu His Phe Ser Gly Gly Glu Pro Asp Val Phe Pro Thr
                485                 490                 495

Val Pro Phe His Glu Glu Phe Glu Ser Gly Thr Ala Lys Lys Gly Ala
            500                 505                 510

Glu Ser Val Thr Glu Arg Asp Thr Glu Val Gly His Gln Ala His Glu
        515                 520                 525

His Thr Glu Pro Val Ser Leu Phe Pro Glu Glu Ser Ser Gly Glu Ile
    530                 535                 540

Ala Ile Asp Gln Glu Ser Gln Lys Ile Ala Phe Ala Arg Ala Thr Glu
545                 550                 555                 560

Val Thr Phe Gly Glu Glu Val Glu Lys Ser Thr Ser Val Thr Tyr Thr
                565                 570                 575

Pro Thr Ile Val Pro Ser Ser Ala Ser Ala Tyr Val Ser Glu Glu Glu
            580                 585                 590

Ala Val Thr Leu Ile Gly Asn Pro Trp Pro Asp Asp Leu Leu Ser Thr
        595                 600                 605

Lys Glu Ser Trp Val Glu Ala Thr Pro Arg Gln Val Val Glu Leu Ser
    610                 615                 620

Gly Ser Ser Ser Ile Pro Ile Thr Glu Gly Ser Gly Glu Ala Glu Glu
625                 630                 635                 640

Asp Glu Asp Thr Met Phe Thr Met Val Thr Asp Leu Ser Gln Arg Asn
                645                 650                 655

Thr Thr Asp Thr Leu Ile Thr Leu Asp Thr Ser Arg Ile Ile Thr Glu
            660                 665                 670

Ser Phe Phe Glu Val Pro Ala Thr Thr Ile Tyr Pro Val Ser Glu Gln
        675                 680                 685

Pro Ser Ala Lys Val Val Pro Thr Lys Phe Val Ser Glu Thr Asp Thr
    690                 695                 700

Ser Glu Trp Ile Ser Ser Thr Thr Val Glu Glu Lys Lys Arg Lys Glu
705                 710                 715                 720

Glu Glu Gly Thr Thr Gly Thr Ala Ser Thr Phe Glu Val Tyr Ser Ser
                725                 730                 735

Thr Gln Arg Ser Asp Gln Leu Ile Leu Pro Phe Glu Leu Glu Ser Pro
            740                 745                 750

Asn Val Ala Thr Ser Ser Asp Ser Gly Thr Arg Lys Ser Phe Met Ser
        755                 760                 765
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```
Leu Thr Thr Pro Thr Gln Ser Glu Arg Glu Met Thr Asp Ser Thr Pro
    770                 775                 780

Val Phe Thr Glu Thr Asn Thr Leu Glu Asn Leu Gly Ala Gln Thr Thr
785                 790                 795                 800

Glu His Ser Ser Ile His Gln Pro Gly Val Gln Glu Gly Leu Thr Thr
                805                 810                 815

Leu Pro Arg Ser Pro Ala Ser Val Phe Met Glu Gln Gly Ser Gly Glu
            820                 825                 830

Ala Ala Ala Asp Pro Glu Thr Thr Thr Val Ser Ser Phe Ser Leu Asn
        835                 840                 845

Val Glu Tyr Ala Ile Gln Ala Glu Lys Glu Val Ala Gly Thr Leu Ser
    850                 855                 860

Pro His Val Glu Thr Thr Phe Ser Thr Glu Pro Thr Gly Leu Val Leu
865                 870                 875                 880

Ser Thr Val Met Asp Arg Val Val Ala Glu Asn Ile Thr Gln Thr Ser
                885                 890                 895

Arg Glu Ile Val Ile Ser Glu Arg Leu Gly Glu Pro Asn Tyr Gly Ala
            900                 905                 910

Glu Ile Arg Gly Phe Ser Thr Gly Phe Pro Leu Glu Glu Asp Phe Ser
        915                 920                 925

Gly Asp Phe Arg Glu Tyr Ser Thr Val Ser His Pro Ile Ala Lys Glu
    930                 935                 940

Glu Thr Val Met Met Glu Gly Ser Gly Asp Ala Ala Phe Arg Asp Thr
945                 950                 955                 960

Gln Thr Ser Pro Ser Thr Val Pro Thr Ser Val His Ile Ser His Ile
                965                 970                 975

Ser Asp Ser Glu Gly Pro Ser Ser Thr Met Val Ser Thr Ser Ala Phe
            980                 985                 990

Pro Trp Glu Glu Phe Thr Ser Ser  Ala Glu Gly Ser Gly  Glu Gln Leu
        995                 1000                1005

Val Thr  Val Ser Ser Ser Val  Val Pro Val Leu Pro  Ser Ala Val
    1010                1015                1020

Gln Lys  Phe Ser Gly Thr Ala  Ser Ser Ile Ile Asp  Glu Gly Leu
    1025                1030                1035

Gly Glu  Val Gly Thr Val Asn  Glu Ile Asp Arg Arg  Ser Thr Ile
    1040                1045                1050

Leu Pro  Thr Ala Glu Val Glu  Gly Thr Lys Ala Pro  Val Glu Lys
    1055                1060                1065

Glu Glu  Val Lys Val Ser Gly  Thr Val Ser Thr Asn  Phe Pro Gln
    1070                1075                1080

Thr Ile  Glu Pro Ala Lys Leu  Trp Ser Arg Gln Glu  Val Asn Pro
    1085                1090                1095

Val Arg  Gln Glu Ile Glu Ser  Glu Thr Thr Ser Glu  Glu Gln Ile
    1100                1105                1110

Gln Glu  Glu Lys Ser Phe Glu  Ser Pro Gln Asn Ser  Pro Ala Thr
    1115                1120                1125

Glu Gln  Thr Ile Phe Asp Ser  Gln Thr Phe Thr Glu  Thr Glu Leu
    1130                1135                1140

Lys Thr  Thr Asp Tyr Ser Val  Leu Thr Thr Lys Lys  Thr Tyr Ser
    1145                1150                1155

Asp Asp  Lys Glu Met Lys Glu  Glu Asp Thr Ser Leu  Val Asn Met
    1160                1165                1170
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Ser Thr  Pro Asp Pro Asp Ala  Asn Gly Leu Glu Ser  Tyr Thr Thr
    1175                1180                1185

Leu Pro  Glu Ala Thr Glu Lys  Ser His Phe Phe Leu  Ala Thr Ala
    1190                1195                1200

Leu Val  Thr Glu Ser Ile Pro  Ala Glu His Val Val  Thr Asp Ser
    1205                1210                1215

Pro Ile  Lys Lys Glu Glu Ser  Thr Lys His Phe Pro  Lys Gly Met
    1220                1225                1230

Arg Pro  Thr Ile Gln Glu Ser  Asp Thr Glu Leu Leu  Phe Ser Gly
    1235                1240                1245

Leu Gly  Ser Gly Glu Glu Val  Leu Pro Thr Leu Pro  Thr Glu Ser
    1250                1255                1260

Val Asn  Phe Thr Glu Val Glu  Gln Ile Asn Asn Thr  Leu Tyr Pro
    1265                1270                1275

His Thr  Ser Gln Val Glu Ser  Thr Ser Ser Asp Lys  Ile Glu Asp
    1280                1285                1290

Phe Asn  Arg Met Glu Asn Val  Ala Lys Glu Val Gly  Pro Leu Val
    1295                1300                1305

Ser Gln  Thr Asp Ile Phe Glu  Gly Ser Gly Ser Val  Thr Ser Thr
    1310                1315                1320

Thr Leu  Ile Glu Ile Leu Ser  Asp Thr Gly Ala Glu  Gly Pro Thr
    1325                1330                1335

Val Ala  Pro Leu Pro Phe Ser  Thr Asp Ile Gly His  Pro Gln Asn
    1340                1345                1350

Gln Thr  Val Arg Trp Ala Glu  Glu Ile Gln Thr Ser  Arg Pro Gln
    1355                1360                1365

Thr Ile  Thr Glu Gln Asp Ser  Asn Lys Asn Ser Ser  Thr Ala Glu
    1370                1375                1380

Ile Asn  Glu Thr Thr Thr Ser  Ser Thr Asp Phe Leu  Ala Arg Ala
    1385                1390                1395

Tyr Gly  Phe Glu Met Ala Lys  Glu Phe Val Thr Ser  Ala Pro Lys
    1400                1405                1410

Pro Ser  Asp Leu Tyr Tyr Glu  Pro Ser Gly Glu Gly  Ser Gly Glu
    1415                1420                1425

Val Asp  Ile Val Asp Ser Phe  His Thr Ser Ala Thr  Thr Gln Ala
    1430                1435                1440

Thr Arg  Gln Glu Ser Ser Thr  Thr Phe Val Ser Asp  Gly Ser Leu
    1445                1450                1455

Glu Lys  His Pro Glu Val Pro  Ser Ala Lys Ala Val  Thr Ala Asp
    1460                1465                1470

Gly Phe  Pro Thr Val Ser Val  Met Leu Pro Leu His  Ser Glu Gln
    1475                1480                1485

Asn Lys  Ser Ser Pro Asp Pro  Thr Ser Thr Leu Ser  Asn Thr Val
    1490                1495                1500

Ser Tyr  Glu Arg Ser Thr Asp  Gly Ser Phe Gln Asp  Arg Phe Arg
    1505                1510                1515

Glu Phe  Glu Asp Ser Thr Leu  Lys Pro Asn Arg Lys  Lys Pro Thr
    1520                1525                1530

Glu Asn  Ile Ile Ile Asp Leu  Asp Lys Glu Asp Lys  Asp Leu Ile
    1535                1540                1545

Leu Thr  Ile Thr Glu Ser Thr  Ile Leu Glu Ile Leu  Pro Glu Leu
    1550                1555                1560
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Thr Ser  Asp Lys Asn Thr Ile  Ile Asp Ile Asp His  Thr Lys Pro
    1565                1570                1575

Val Tyr  Glu Asp Ile Leu Gly  Met Gln Thr Asp Ile  Asp Thr Glu
    1580                1585                1590

Val Pro  Ser Glu Pro His Asp  Ser Asn Asp Glu Ser  Asn Asp Asp
    1595                1600                1605

Ser Thr  Gln Val Gln Glu Ile  Tyr Glu Ala Ala Val  Asn Leu Ser
    1610                1615                1620

Leu Thr  Glu Glu Thr Phe Glu  Gly Ser Ala Asp Val  Leu Ala Ser
    1625                1630                1635

Tyr Thr  Gln Ala Thr His Asp  Glu Ser Met Thr Tyr  Glu Asp Arg
    1640                1645                1650

Ser Gln  Leu Asp His Met Gly  Phe His Phe Thr Thr  Gly Ile Pro
    1655                1660                1665

Ala Pro  Ser Thr Glu Thr Glu  Leu Asp Val Leu Leu  Pro Thr Ala
    1670                1675                1680

Thr Ser  Leu Pro Ile Pro Arg  Lys Ser Ala Thr Val  Ile Pro Glu
    1685                1690                1695

Ile Glu  Gly Ile Lys Ala Glu  Ala Lys Ala Leu Asp  Asp Met Phe
    1700                1705                1710

Glu Ser  Ser Thr Leu Ser Asp  Gly Gln Ala Ile Ala  Asp Gln Ser
    1715                1720                1725

Glu Ile  Ile Pro Thr Leu Gly  Gln Phe Glu Arg Thr  Gln Glu Glu
    1730                1735                1740

Tyr Glu  Asp Lys Lys His Ala  Gly Pro Ser Phe Gln  Pro Glu Phe
    1745                1750                1755

Ser Ser  Gly Ala Glu Glu Ala  Leu Val Asp His Thr  Pro Tyr Leu
    1760                1765                1770

Ser Ile  Ala Thr Thr His Leu  Met Asp Gln Ser Val  Thr Glu Val
    1775                1780                1785

Pro Asp  Val Met Glu Gly Ser  Asn Pro Pro Tyr Tyr  Thr Asp Thr
    1790                1795                1800

Thr Leu  Ala Val Ser Thr Phe  Ala Lys Leu Ser Ser  Gln Thr Pro
    1805                1810                1815

Ser Ser  Pro Leu Thr Ile Tyr  Ser Gly Ser Glu Ala  Ser Gly His
    1820                1825                1830

Thr Glu  Ile Pro Gln Pro Ser  Ala Leu Pro Gly Ile  Asp Val Gly
    1835                1840                1845

Ser Ser  Val Met Ser Pro Gln  Asp Ser Phe Lys Glu  Ile His Val
    1850                1855                1860

Asn Ile  Glu Ala Thr Phe Lys  Pro Ser Ser Glu Glu  Tyr Leu His
    1865                1870                1875

Ile Thr  Glu Pro Pro Ser Leu  Ser Pro Asp Thr Lys  Leu Glu Pro
    1880                1885                1890

Ser Glu  Asp Asp Gly Lys Pro  Glu Leu Leu Glu Glu  Met Glu Ala
    1895                1900                1905

Ser Pro  Thr Glu Leu Ile Ala  Val Glu Gly Thr Glu  Ile Leu Gln
    1910                1915                1920

Asp Phe  Gln Asn Lys Thr Asp  Gly Gln Val Ser Gly  Glu Ala Ile
    1925                1930                1935

Lys Met  Phe Pro Thr Ile Lys  Thr Pro Glu Ala Gly  Thr Val Ile
    1940                1945                1950
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Thr Thr  Ala Asp Glu Ile Glu  Leu Glu Gly Ala Thr  Gln Trp Pro
    1955                1960                1965

His Ser  Thr Ser Ala Ser Ala  Thr Tyr Gly Val Glu  Ala Gly Val
    1970                1975                1980

Val Pro  Trp Leu Ser Pro Gln  Thr Ser Glu Arg Pro  Thr Leu Ser
    1985                1990                1995

Ser Ser  Pro Glu Ile Asn Pro  Glu Thr Gln Ala Ala  Leu Ile Arg
    2000                2005                2010

Gly Gln  Asp Ser Thr Ile Ala  Ala Ser Glu Gln Gln  Val Ala Ala
    2015                2020                2025

Arg Ile  Leu Asp Ser Asn Asp  Gln Ala Thr Val Asn  Pro Val Glu
    2030                2035                2040

Phe Asn  Thr Glu Val Ala Thr  Pro Pro Phe Ser Leu  Leu Glu Thr
    2045                2050                2055

Ser Asn  Glu Thr Asp Phe Leu  Ile Gly Ile Asn Glu  Glu Ser Val
    2060                2065                2070

Glu Gly  Thr Ala Ile Tyr Leu  Pro Gly Pro Asp Arg  Cys Lys Met
    2075                2080                2085

Asn Pro  Cys Leu Asn Gly Gly  Thr Cys Tyr Pro Thr  Glu Thr Ser
    2090                2095                2100

Tyr Val  Cys Thr Cys Val Pro  Gly Tyr Ser Gly Asp  Gln Cys Glu
    2105                2110                2115

Leu Asp  Phe Asp Glu Cys His  Ser Asn Pro Cys Arg  Asn Gly Ala
    2120                2125                2130

Thr Cys  Val Asp Gly Phe Asn  Thr Phe Arg Cys Leu  Cys Leu Pro
    2135                2140                2145

Ser Tyr  Val Gly Ala Leu Cys  Glu Gln Asp Thr Glu  Thr Cys Asp
    2150                2155                2160

Tyr Gly  Trp His Lys Phe Gln  Gly Gln Cys Tyr Lys  Tyr Phe Ala
    2165                2170                2175

His Arg  Arg Thr Trp Asp Ala  Ala Glu Arg Glu Cys  Arg Leu Gln
    2180                2185                2190

Gly Ala  His Leu Thr Ser Ile  Leu Ser His Glu Glu  Gln Met Phe
    2195                2200                2205

Val Asn  Arg Val Gly His Asp  Tyr Gln Trp Ile Gly  Leu Asn Asp
    2210                2215                2220

Lys Met  Phe Glu His Asp Phe  Arg Trp Thr Asp Gly  Ser Thr Leu
    2225                2230                2235

Gln Tyr  Glu Asn Trp Arg Pro  Asn Gln Pro Asp Ser  Phe Phe Ser
    2240                2245                2250

Ala Gly  Glu Asp Cys Val Val  Ile Ile Trp His Glu  Asn Gly Gln
    2255                2260                2265

Trp Asn  Asp Val Pro Cys Asn  Tyr His Leu Thr Tyr  Thr Cys Lys
    2270                2275                2280

Lys Gly  Thr Val Ala Cys Gly  Gln Pro Pro Val Val  Glu Asn Ala
    2285                2290                2295

Lys Thr  Phe Gly Lys Met Lys  Pro Arg Tyr Glu Ile  Asn Ser Leu
    2300                2305                2310

Ile Arg  Tyr His Cys Lys Asp  Gly Phe Ile Gln Arg  His Leu Pro
    2315                2320                2325

Thr Ile  Arg Cys Leu Gly Asn  Gly Arg Trp Ala Ile  Pro Lys Ile
    2330                2335                2340
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Thr Cys  Met Asn Pro Ser Ala  Tyr Gln Arg Thr Tyr  Ser Met Lys
    2345                2350                2355

Tyr Phe  Lys Asn Ser Ser Ser  Ala Lys Asp Asn Ser  Ile Asn Thr
    2360                2365                2370

Ser Lys  His Asp His Arg Trp  Ser Arg Arg Trp Gln  Glu Ser Arg
    2375                2380                2385

Arg


<210> SEQ ID NO 3
<211> LENGTH: 2390
<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Full length human versican (V1) (minus signal
      peptide, plus N-terminal methionine)

<400> SEQUENCE: 3

Met Leu His Lys Val Lys Val Gly Lys Ser Pro Pro Val Arg Gly Ser
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Leu Ser Gly Lys Val Ser Leu Pro Cys His Phe Ser Thr Met Pro Thr
            20                  25                  30

Leu Pro Pro Ser Tyr Asn Thr Ser Glu Phe Leu Arg Ile Lys Trp Ser
        35                  40                  45

Lys Ile Glu Val Asp Lys Asn Gly Lys Asp Leu Lys Glu Thr Thr Val
    50                  55                  60

Leu Val Ala Gln Asn Gly Asn Ile Lys Ile Gly Gln Asp Tyr Lys Gly
65                  70                  75                  80

Arg Val Ser Val Pro Thr His Pro Glu Ala Val Gly Asp Ala Ser Leu
                85                  90                  95

Thr Val Val Lys Leu Leu Ala Ser Asp Ala Gly Leu Tyr Arg Cys Asp
            100                 105                 110

Val Met Tyr Gly Ile Glu Asp Thr Gln Asp Thr Val Ser Leu Thr Val
        115                 120                 125

Asp Gly Val Val Phe His Tyr Arg Ala Ala Thr Ser Arg Tyr Thr Leu
    130                 135                 140

Asn Phe Glu Ala Ala Gln Lys Ala Cys Leu Asp Val Gly Ala Val Ile
145                 150                 155                 160

Ala Thr Pro Glu Gln Leu Phe Ala Ala Tyr Glu Asp Gly Phe Glu Gln
                165                 170                 175

Cys Asp Ala Gly Trp Leu Ala Asp Gln Thr Val Arg Tyr Pro Ile Arg
            180                 185                 190

Ala Pro Arg Val Gly Cys Tyr Gly Asp Lys Met Gly Lys Ala Gly Val
        195                 200                 205

Arg Thr Tyr Gly Phe Arg Ser Pro Gln Glu Thr Tyr Asp Val Tyr Cys
    210                 215                 220

Tyr Val Asp His Leu Asp Gly Asp Val Phe His Leu Thr Val Pro Ser
225                 230                 235                 240

Lys Phe Thr Phe Glu Glu Ala Ala Lys Glu Cys Glu Asn Gln Asp Ala
                245                 250                 255

Arg Leu Ala Thr Val Gly Glu Leu Gln Ala Ala Trp Arg Asn Gly Phe
            260                 265                 270

Asp Gln Cys Asp Tyr Gly Trp Leu Ser Asp Ala Ser Val Arg His Pro
        275                 280                 285

Val Thr Val Ala Arg Ala Gln Cys Gly Gly Gly Leu Leu Gly Val Arg
    290                 295                 300
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Thr Leu Tyr Arg Phe Glu Asn Gln Thr Gly Phe Pro Pro Pro Asp Ser
305                 310                 315                 320

Arg Phe Asp Ala Tyr Cys Phe Lys Arg Arg Met Ser Asp Leu Ser Val
                325                 330                 335

Ile Gly His Pro Ile Asp Ser Glu Ser Lys Glu Asp Glu Pro Cys Ser
            340                 345                 350

Glu Glu Thr Asp Pro Val His Asp Leu Met Ala Glu Ile Leu Pro Glu
        355                 360                 365

Phe Pro Asp Ile Ile Glu Ile Asp Leu Tyr His Ser Glu Glu Asn Glu
    370                 375                 380

Glu Glu Glu Glu Glu Cys Ala Asn Ala Thr Asp Val Thr Thr Thr Pro
385                 390                 395                 400

Ser Val Gln Tyr Ile Asn Gly Lys His Leu Val Thr Thr Val Pro Lys
                405                 410                 415

Asp Pro Glu Ala Ala Glu Ala Arg Arg Gly Gln Phe Glu Ser Val Ala
            420                 425                 430

Pro Ser Gln Asn Phe Ser Asp Ser Ser Glu Ser Asp Thr His Pro Phe
        435                 440                 445

Val Ile Ala Lys Thr Glu Leu Ser Thr Ala Val Gln Pro Asn Glu Ser
    450                 455                 460

Thr Glu Thr Thr Glu Ser Leu Glu Val Thr Trp Lys Pro Glu Thr Tyr
465                 470                 475                 480

Pro Glu Thr Ser Glu His Phe Ser Gly Gly Glu Pro Asp Val Phe Pro
                485                 490                 495

Thr Val Pro Phe His Glu Glu Phe Glu Ser Gly Thr Ala Lys Lys Gly
            500                 505                 510

Ala Glu Ser Val Thr Glu Arg Asp Thr Glu Val Gly His Gln Ala His
        515                 520                 525

Glu His Thr Glu Pro Val Ser Leu Phe Pro Glu Glu Ser Ser Gly Glu
    530                 535                 540

Ile Ala Ile Asp Gln Glu Ser Gln Lys Ile Ala Phe Ala Arg Ala Thr
545                 550                 555                 560

Glu Val Thr Phe Gly Glu Glu Val Glu Lys Ser Thr Ser Val Thr Tyr
                565                 570                 575

Thr Pro Thr Ile Val Pro Ser Ser Ala Ser Ala Tyr Val Ser Glu Glu
            580                 585                 590

Glu Ala Val Thr Leu Ile Gly Asn Pro Trp Pro Asp Asp Leu Leu Ser
        595                 600                 605

Thr Lys Glu Ser Trp Val Glu Ala Thr Pro Arg Gln Val Val Glu Leu
    610                 615                 620

Ser Gly Ser Ser Ser Ile Pro Ile Thr Glu Gly Ser Gly Glu Ala Glu
625                 630                 635                 640

Glu Asp Glu Asp Thr Met Phe Thr Met Val Thr Asp Leu Ser Gln Arg
                645                 650                 655

Asn Thr Thr Asp Thr Leu Ile Thr Leu Asp Thr Ser Arg Ile Ile Thr
            660                 665                 670

Glu Ser Phe Phe Glu Val Pro Ala Thr Thr Ile Tyr Pro Val Ser Glu
        675                 680                 685

Gln Pro Ser Ala Lys Val Val Pro Thr Lys Phe Val Ser Glu Thr Asp
    690                 695                 700

Thr Ser Glu Trp Ile Ser Ser Thr Thr Val Glu Glu Lys Lys Arg Lys
705                 710                 715                 720
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Glu Glu Glu Gly Thr Thr Gly Thr Ala Ser Thr Phe Glu Val Tyr Ser
                725                 730                 735

Ser Thr Gln Arg Ser Asp Gln Leu Ile Leu Pro Phe Glu Leu Glu Ser
            740                 745                 750

Pro Asn Val Ala Thr Ser Ser Asp Ser Gly Thr Arg Lys Ser Phe Met
        755                 760                 765

Ser Leu Thr Thr Pro Thr Gln Ser Glu Arg Glu Met Thr Asp Ser Thr
    770                 775                 780

Pro Val Phe Thr Glu Thr Asn Thr Leu Glu Asn Leu Gly Ala Gln Thr
785                 790                 795                 800

Thr Glu His Ser Ser Ile His Gln Pro Gly Val Gln Glu Gly Leu Thr
                805                 810                 815

Thr Leu Pro Arg Ser Pro Ala Ser Val Phe Met Glu Gln Gly Ser Gly
            820                 825                 830

Glu Ala Ala Ala Asp Pro Glu Thr Thr Thr Val Ser Ser Phe Ser Leu
        835                 840                 845

Asn Val Glu Tyr Ala Ile Gln Ala Glu Lys Glu Val Ala Gly Thr Leu
    850                 855                 860

Ser Pro His Val Glu Thr Thr Phe Ser Thr Glu Pro Thr Gly Leu Val
865                 870                 875                 880

Leu Ser Thr Val Met Asp Arg Val Val Ala Glu Asn Ile Thr Gln Thr
                885                 890                 895

Ser Arg Glu Ile Val Ile Ser Glu Arg Leu Gly Glu Pro Asn Tyr Gly
            900                 905                 910

Ala Glu Ile Arg Gly Phe Ser Thr Gly Phe Pro Leu Glu Glu Asp Phe
        915                 920                 925

Ser Gly Asp Phe Arg Glu Tyr Ser Thr Val Ser His Pro Ile Ala Lys
    930                 935                 940

Glu Glu Thr Val Met Met Glu Gly Ser Gly Asp Ala Ala Phe Arg Asp
945                 950                 955                 960

Thr Gln Thr Ser Pro Ser Thr Val Pro Thr Ser Val His Ile Ser His
                965                 970                 975

Ile Ser Asp Ser Glu Gly Pro Ser Ser Thr Met Val Ser Thr Ser Ala
            980                 985                 990

Phe Pro Trp Glu Glu Phe Thr Ser  Ser Ala Glu Gly Ser  Gly Glu Gln
        995                 1000                1005

Leu Val  Thr Val Ser Ser Ser  Val Val Pro Val Leu  Pro Ser Ala
    1010                1015                1020

Val Gln  Lys Phe Ser Gly Thr  Ala Ser Ser Ile Ile  Asp Glu Gly
    1025                1030                1035

Leu Gly  Glu Val Gly Thr Val  Asn Glu Ile Asp Arg  Arg Ser Thr
    1040                1045                1050

Ile Leu  Pro Thr Ala Glu Val  Glu Gly Thr Lys Ala  Pro Val Glu
    1055                1060                1065

Lys Glu  Glu Val Lys Val Ser  Gly Thr Val Ser Thr  Asn Phe Pro
    1070                1075                1080

Gln Thr  Ile Glu Pro Ala Lys  Leu Trp Ser Arg Gln  Glu Val Asn
    1085                1090                1095

Pro Val  Arg Gln Glu Ile Glu  Ser Glu Thr Thr Ser  Glu Glu Gln
    1100                1105                1110

Ile Gln  Glu Glu Lys Ser Phe  Glu Ser Pro Gln Asn  Ser Pro Ala
    1115                1120                1125
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Thr Glu  Gln Thr Ile Phe Asp  Ser Gln Thr Phe Thr  Glu Thr Glu
    1130                1135                1140

Leu Lys  Thr Thr Asp Tyr Ser  Val Leu Thr Thr Lys  Lys Thr Tyr
    1145                1150                1155

Ser Asp  Asp Lys Glu Met Lys  Glu Glu Asp Thr Ser  Leu Val Asn
    1160                1165                1170

Met Ser  Thr Pro Asp Pro Asp  Ala Asn Gly Leu Glu  Ser Tyr Thr
    1175                1180                1185

Thr Leu  Pro Glu Ala Thr Glu  Lys Ser His Phe Phe  Leu Ala Thr
    1190                1195                1200

Ala Leu  Val Thr Glu Ser Ile  Pro Ala Glu His Val  Val Thr Asp
    1205                1210                1215

Ser Pro  Ile Lys Lys Glu Glu  Ser Thr Lys His Phe  Pro Lys Gly
    1220                1225                1230

Met Arg  Pro Thr Ile Gln Glu  Ser Asp Thr Glu Leu  Leu Phe Ser
    1235                1240                1245

Gly Leu  Gly Ser Gly Glu Glu  Val Leu Pro Thr Leu  Pro Thr Glu
    1250                1255                1260

Ser Val  Asn Phe Thr Glu Val  Glu Gln Ile Asn Asn  Thr Leu Tyr
    1265                1270                1275

Pro His  Thr Ser Gln Val Glu  Ser Thr Ser Ser Asp  Lys Ile Glu
    1280                1285                1290

Asp Phe  Asn Arg Met Glu Asn  Val Ala Lys Glu Val  Gly Pro Leu
    1295                1300                1305

Val Ser  Gln Thr Asp Ile Phe  Glu Gly Ser Gly Ser  Val Thr Ser
    1310                1315                1320

Thr Thr  Leu Ile Glu Ile Leu  Ser Asp Thr Gly Ala  Glu Gly Pro
    1325                1330                1335

Thr Val  Ala Pro Leu Pro Phe  Ser Thr Asp Ile Gly  His Pro Gln
    1340                1345                1350

Asn Gln  Thr Val Arg Trp Ala  Glu Glu Ile Gln Thr  Ser Arg Pro
    1355                1360                1365

Gln Thr  Ile Thr Glu Gln Asp  Ser Asn Lys Asn Ser  Ser Thr Ala
    1370                1375                1380

Glu Ile  Asn Glu Thr Thr Thr  Ser Ser Thr Asp Phe  Leu Ala Arg
    1385                1390                1395

Ala Tyr  Gly Phe Glu Met Ala  Lys Glu Phe Val Thr  Ser Ala Pro
    1400                1405                1410

Lys Pro  Ser Asp Leu Tyr Tyr  Glu Pro Ser Gly Glu  Gly Ser Gly
    1415                1420                1425

Glu Val  Asp Ile Val Asp Ser  Phe His Thr Ser Ala  Thr Thr Gln
    1430                1435                1440

Ala Thr  Arg Gln Glu Ser Ser  Thr Thr Phe Val Ser  Asp Gly Ser
    1445                1450                1455

Leu Glu  Lys His Pro Glu Val  Pro Ser Ala Lys Ala  Val Thr Ala
    1460                1465                1470

Asp Gly  Phe Pro Thr Val Ser  Val Met Leu Pro Leu  His Ser Glu
    1475                1480                1485

Gln Asn  Lys Ser Ser Pro Asp  Pro Thr Ser Thr Leu  Ser Asn Thr
    1490                1495                1500

Val Ser  Tyr Glu Arg Ser Thr  Asp Gly Ser Phe Gln  Asp Arg Phe
    1505                1510                1515
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Arg Glu  Phe Glu Asp Ser Thr  Leu Lys Pro Asn Arg  Lys Lys Pro
    1520                1525                1530

Thr Glu  Asn Ile Ile Ile Asp  Leu Asp Lys Glu Asp  Lys Asp Leu
    1535                1540                1545

Ile Leu  Thr Ile Thr Glu Ser  Thr Ile Leu Glu Ile  Leu Pro Glu
    1550                1555                1560

Leu Thr  Ser Asp Lys Asn Thr  Ile Ile Asp Ile Asp  His Thr Lys
    1565                1570                1575

Pro Val  Tyr Glu Asp Ile Leu  Gly Met Gln Thr Asp  Ile Asp Thr
    1580                1585                1590

Glu Val  Pro Ser Glu Pro His  Asp Ser Asn Asp Glu  Ser Asn Asp
    1595                1600                1605

Asp Ser  Thr Gln Val Gln Glu  Ile Tyr Glu Ala Ala  Val Asn Leu
    1610                1615                1620

Ser Leu  Thr Glu Glu Thr Phe  Glu Gly Ser Ala Asp  Val Leu Ala
    1625                1630                1635

Ser Tyr  Thr Gln Ala Thr His  Asp Glu Ser Met Thr  Tyr Glu Asp
    1640                1645                1650

Arg Ser  Gln Leu Asp His Met  Gly Phe His Phe Thr  Thr Gly Ile
    1655                1660                1665

Pro Ala  Pro Ser Thr Glu Thr  Glu Leu Asp Val Leu  Leu Pro Thr
    1670                1675                1680

Ala Thr  Ser Leu Pro Ile Pro  Arg Lys Ser Ala Thr  Val Ile Pro
    1685                1690                1695

Glu Ile  Glu Gly Ile Lys Ala  Glu Ala Lys Ala Leu  Asp Asp Met
    1700                1705                1710

Phe Glu  Ser Ser Thr Leu Ser  Asp Gly Gln Ala Ile  Ala Asp Gln
    1715                1720                1725

Ser Glu  Ile Ile Pro Thr Leu  Gly Gln Phe Glu Arg  Thr Gln Glu
    1730                1735                1740

Glu Tyr  Glu Asp Lys Lys His  Ala Gly Pro Ser Phe  Gln Pro Glu
    1745                1750                1755

Phe Ser  Ser Gly Ala Glu Glu  Ala Leu Val Asp His  Thr Pro Tyr
    1760                1765                1770

Leu Ser  Ile Ala Thr Thr His  Leu Met Asp Gln Ser  Val Thr Glu
    1775                1780                1785

Val Pro  Asp Val Met Glu Gly  Ser Asn Pro Pro Tyr  Tyr Thr Asp
    1790                1795                1800

Thr Thr  Leu Ala Val Ser Thr  Phe Ala Lys Leu Ser  Ser Gln Thr
    1805                1810                1815

Pro Ser  Ser Pro Leu Thr Ile  Tyr Ser Gly Ser Glu  Ala Ser Gly
    1820                1825                1830

His Thr  Glu Ile Pro Gln Pro  Ser Ala Leu Pro Gly  Ile Asp Val
    1835                1840                1845

Gly Ser  Ser Val Met Ser Pro  Gln Asp Ser Phe Lys  Glu Ile His
    1850                1855                1860

Val Asn  Ile Glu Ala Thr Phe  Lys Pro Ser Ser Glu  Glu Tyr Leu
    1865                1870                1875

His Ile  Thr Glu Pro Pro Ser  Leu Ser Pro Asp Thr  Lys Leu Glu
    1880                1885                1890

Pro Ser  Glu Asp Asp Gly Lys  Pro Glu Leu Leu Glu  Glu Met Glu
    1895                1900                1905
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Ala Ser  Pro Thr Glu Leu Ile  Ala Val Glu Gly Thr  Glu Ile Leu
    1910                1915                1920

Gln Asp  Phe Gln Asn Lys Thr  Asp Gly Gln Val Ser  Gly Glu Ala
    1925                1930                1935

Ile Lys  Met Phe Pro Thr Ile  Lys Thr Pro Glu Ala  Gly Thr Val
    1940                1945                1950

Ile Thr  Thr Ala Asp Glu Ile  Glu Leu Glu Gly Ala  Thr Gln Trp
    1955                1960                1965

Pro His  Ser Thr Ser Ala Ser  Ala Thr Tyr Gly Val  Glu Ala Gly
    1970                1975                1980

Val Val  Pro Trp Leu Ser Pro  Gln Thr Ser Glu Arg  Pro Thr Leu
    1985                1990                1995

Ser Ser  Ser Pro Glu Ile Asn  Pro Glu Thr Gln Ala  Ala Leu Ile
    2000                2005                2010

Arg Gly  Gln Asp Ser Thr Ile  Ala Ala Ser Glu Gln  Gln Val Ala
    2015                2020                2025

Ala Arg  Ile Leu Asp Ser Asn  Asp Gln Ala Thr Val  Asn Pro Val
    2030                2035                2040

Glu Phe  Asn Thr Glu Val Ala  Thr Pro Pro Phe Ser  Leu Leu Glu
    2045                2050                2055

Thr Ser  Asn Glu Thr Asp Phe  Leu Ile Gly Ile Asn  Glu Glu Ser
    2060                2065                2070

Val Glu  Gly Thr Ala Ile Tyr  Leu Pro Gly Pro Asp  Arg Cys Lys
    2075                2080                2085

Met Asn  Pro Cys Leu Asn Gly  Gly Thr Cys Tyr Pro  Thr Glu Thr
    2090                2095                2100

Ser Tyr  Val Cys Thr Cys Val  Pro Gly Tyr Ser Gly  Asp Gln Cys
    2105                2110                2115

Glu Leu  Asp Phe Asp Glu Cys  His Ser Asn Pro Cys  Arg Asn Gly
    2120                2125                2130

Ala Thr  Cys Val Asp Gly Phe  Asn Thr Phe Arg Cys  Leu Cys Leu
    2135                2140                2145

Pro Ser  Tyr Val Gly Ala Leu  Cys Glu Gln Asp Thr  Glu Thr Cys
    2150                2155                2160

Asp Tyr  Gly Trp His Lys Phe  Gln Gly Gln Cys Tyr  Lys Tyr Phe
    2165                2170                2175

Ala His  Arg Arg Thr Trp Asp  Ala Ala Glu Arg Glu  Cys Arg Leu
    2180                2185                2190

Gln Gly  Ala His Leu Thr Ser  Ile Leu Ser His Glu  Glu Gln Met
    2195                2200                2205

Phe Val  Asn Arg Val Gly His  Asp Tyr Gln Trp Ile  Gly Leu Asn
    2210                2215                2220

Asp Lys  Met Phe Glu His Asp  Phe Arg Trp Thr Asp  Gly Ser Thr
    2225                2230                2235

Leu Gln  Tyr Glu Asn Trp Arg  Pro Asn Gln Pro Asp  Ser Phe Phe
    2240                2245                2250

Ser Ala  Gly Glu Asp Cys Val  Val Ile Ile Trp His  Glu Asn Gly
    2255                2260                2265

Gln Trp  Asn Asp Val Pro Cys  Asn Tyr His Leu Thr  Tyr Thr Cys
    2270                2275                2280

Lys Lys  Gly Thr Val Ala Cys  Gly Gln Pro Pro Val  Val Glu Asn
    2285                2290                2295
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Ala Lys  Thr Phe Gly Lys Met  Lys Pro Arg Tyr Glu  Ile Asn Ser
    2300                2305                2310

Leu Ile  Arg Tyr His Cys Lys  Asp Gly Phe Ile Gln  Arg His Leu
    2315                2320                2325

Pro Thr  Ile Arg Cys Leu Gly  Asn Gly Arg Trp Ala  Ile Pro Lys
    2330                2335                2340

Ile Thr  Cys Met Asn Pro Ser  Ala Tyr Gln Arg Thr  Tyr Ser Met
    2345                2350                2355

Lys Tyr  Phe Lys Asn Ser Ser  Ser Ala Lys Asp Asn  Ser Ile Asn
    2360                2365                2370

Thr Ser  Lys His Asp His Arg  Trp Ser Arg Arg Trp  Gln Glu Ser
    2375                2380                2385

Arg Arg
    2390


<210> SEQ ID NO 4
<211> LENGTH: 1968
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 4

Ala Arg Arg Gly Gln Phe Glu Ser Val Ala Pro Ser Gln Asn Phe Ser
1               5                  10                  15

Asp Ser Ser Glu Ser Asp Thr His Pro Phe Val Ile Ala Lys Thr Glu
            20                  25                  30

Leu Ser Thr Ala Val Gln Pro Asn Glu Ser Thr Glu Thr Thr Glu Ser
        35                  40                  45

Leu Glu Val Thr Trp Lys Pro Glu Thr Tyr Pro Glu Thr Ser Glu His
    50                  55                  60

Phe Ser Gly Gly Glu Pro Asp Val Phe Pro Thr Val Pro Phe His Glu
65                  70                  75                  80

Glu Phe Glu Ser Gly Thr Ala Lys Lys Gly Ala Glu Ser Val Thr Glu
                85                  90                  95

Arg Asp Thr Glu Val Gly His Gln Ala His Glu His Thr Glu Pro Val
            100                 105                 110

Ser Leu Phe Pro Glu Glu Ser Ser Gly Glu Ile Ala Ile Asp Gln Glu
        115                 120                 125

Ser Gln Lys Ile Ala Phe Ala Arg Ala Thr Glu Val Thr Phe Gly Glu
    130                 135                 140

Glu Val Glu Lys Ser Thr Ser Val Thr Tyr Thr Pro Thr Ile Val Pro
145                 150                 155                 160

Ser Ser Ala Ser Ala Tyr Val Ser Glu Glu Glu Ala Val Thr Leu Ile
                165                 170                 175

Gly Asn Pro Trp Pro Asp Asp Leu Leu Ser Thr Lys Glu Ser Trp Val
            180                 185                 190

Glu Ala Thr Pro Arg Gln Val Val Glu Leu Ser Gly Ser Ser Ser Ile
        195                 200                 205

Pro Ile Thr Glu Gly Ser Gly Glu Ala Glu Glu Asp Glu Asp Thr Met
    210                 215                 220

Phe Thr Met Val Thr Asp Leu Ser Gln Arg Asn Thr Thr Asp Thr Leu
225                 230                 235                 240

Ile Thr Leu Asp Thr Ser Arg Ile Ile Thr Glu Ser Phe Phe Glu Val
                245                 250                 255

Pro Ala Thr Thr Ile Tyr Pro Val Ser Glu Gln Pro Ser Ala Lys Val
            260                 265                 270
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Val Pro Thr Lys Phe Val Ser Glu Thr Asp Thr Ser Glu Trp Ile Ser
        275                 280                 285

Ser Thr Thr Val Glu Glu Lys Lys Arg Lys Glu Glu Glu Gly Thr Thr
    290                 295                 300

Gly Thr Ala Ser Thr Phe Glu Val Tyr Ser Ser Thr Gln Arg Ser Asp
305                 310                 315                 320

Gln Leu Ile Leu Pro Phe Glu Leu Glu Ser Pro Asn Val Ala Thr Ser
                325                 330                 335

Ser Asp Ser Gly Thr Arg Lys Ser Phe Met Ser Leu Thr Thr Pro Thr
            340                 345                 350

Gln Ser Glu Arg Glu Met Thr Asp Ser Thr Pro Val Phe Thr Glu Thr
        355                 360                 365

Asn Thr Leu Glu Asn Leu Gly Ala Gln Thr Thr Glu His Ser Ser Ile
    370                 375                 380

His Gln Pro Gly Val Gln Glu Gly Leu Thr Thr Leu Pro Arg Ser Pro
385                 390                 395                 400

Ala Ser Val Phe Met Glu Gln Gly Ser Gly Glu Ala Ala Ala Asp Pro
                405                 410                 415

Glu Thr Thr Thr Val Ser Ser Phe Ser Leu Asn Val Glu Tyr Ala Ile
            420                 425                 430

Gln Ala Glu Lys Glu Val Ala Gly Thr Leu Ser Pro His Val Glu Thr
        435                 440                 445

Thr Phe Ser Thr Glu Pro Thr Gly Leu Val Leu Ser Thr Val Met Asp
    450                 455                 460

Arg Val Val Ala Glu Asn Ile Thr Gln Thr Ser Arg Glu Ile Val Ile
465                 470                 475                 480

Ser Glu Arg Leu Gly Glu Pro Asn Tyr Gly Ala Glu Ile Arg Gly Phe
                485                 490                 495

Ser Thr Gly Phe Pro Leu Glu Glu Asp Phe Ser Gly Asp Phe Arg Glu
            500                 505                 510

Tyr Ser Thr Val Ser His Pro Ile Ala Lys Glu Glu Thr Val Met Met
        515                 520                 525

Glu Gly Ser Gly Asp Ala Ala Phe Arg Asp Thr Gln Thr Ser Pro Ser
    530                 535                 540

Thr Val Pro Thr Ser Val His Ile Ser His Ile Ser Asp Ser Glu Gly
545                 550                 555                 560

Pro Ser Ser Thr Met Val Ser Thr Ser Ala Phe Pro Trp Glu Glu Phe
                565                 570                 575

Thr Ser Ser Ala Glu Gly Ser Gly Glu Gln Leu Val Thr Val Ser Ser
            580                 585                 590

Ser Val Val Pro Val Leu Pro Ser Ala Val Gln Lys Phe Ser Gly Thr
        595                 600                 605

Ala Ser Ser Ile Ile Asp Glu Gly Leu Gly Glu Val Gly Thr Val Asn
    610                 615                 620

Glu Ile Asp Arg Arg Ser Thr Ile Leu Pro Thr Ala Glu Val Glu Gly
625                 630                 635                 640

Thr Lys Ala Pro Val Glu Lys Glu Glu Val Lys Val Ser Gly Thr Val
                645                 650                 655

Ser Thr Asn Phe Pro Gln Thr Ile Glu Pro Ala Lys Leu Trp Ser Arg
            660                 665                 670

Gln Glu Val Asn Pro Val Arg Gln Glu Ile Glu Ser Glu Thr Thr Ser
        675                 680                 685
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Glu Glu Gln Ile Gln Glu Glu Lys Ser Phe Glu Ser Pro Gln Asn Ser
    690                 695                 700

Pro Ala Thr Glu Gln Thr Ile Phe Asp Ser Gln Thr Phe Thr Glu Thr
705                 710                 715                 720

Glu Leu Lys Thr Thr Asp Tyr Ser Val Leu Thr Thr Lys Lys Thr Tyr
                725                 730                 735

Ser Asp Asp Lys Glu Met Lys Glu Glu Asp Thr Ser Leu Val Asn Met
            740                 745                 750

Ser Thr Pro Asp Pro Asp Ala Asn Gly Leu Glu Ser Tyr Thr Thr Leu
        755                 760                 765

Pro Glu Ala Thr Glu Lys Ser His Phe Phe Leu Ala Thr Ala Leu Val
    770                 775                 780

Thr Glu Ser Ile Pro Ala Glu His Val Val Thr Asp Ser Pro Ile Lys
785                 790                 795                 800

Lys Glu Glu Ser Thr Lys His Phe Pro Lys Gly Met Arg Pro Thr Ile
                805                 810                 815

Gln Glu Ser Asp Thr Glu Leu Leu Phe Ser Gly Leu Gly Ser Gly Glu
            820                 825                 830

Glu Val Leu Pro Thr Leu Pro Thr Glu Ser Val Asn Phe Thr Glu Val
        835                 840                 845

Glu Gln Ile Asn Asn Thr Leu Tyr Pro His Thr Ser Gln Val Glu Ser
    850                 855                 860

Thr Ser Ser Asp Lys Ile Glu Asp Phe Asn Arg Met Glu Asn Val Ala
865                 870                 875                 880

Lys Glu Val Gly Pro Leu Val Ser Gln Thr Asp Ile Phe Glu Gly Ser
                885                 890                 895

Gly Ser Val Thr Ser Thr Thr Leu Ile Glu Ile Leu Ser Asp Thr Gly
            900                 905                 910

Ala Glu Gly Pro Thr Val Ala Pro Leu Pro Phe Ser Thr Asp Ile Gly
        915                 920                 925

His Pro Gln Asn Gln Thr Val Arg Trp Ala Glu Glu Ile Gln Thr Ser
    930                 935                 940

Arg Pro Gln Thr Ile Thr Glu Gln Asp Ser Asn Lys Asn Ser Ser Thr
945                 950                 955                 960

Ala Glu Ile Asn Glu Thr Thr Thr Ser Ser Thr Asp Phe Leu Ala Arg
                965                 970                 975

Ala Tyr Gly Phe Glu Met Ala Lys Glu Phe Val Thr Ser Ala Pro Lys
            980                 985                 990

Pro Ser Asp Leu Tyr Tyr Glu Pro  Ser Gly Glu Gly Ser  Gly Glu Val
        995                 1000                1005

Asp Ile  Val Asp Ser Phe His  Thr Ser Ala Thr Thr  Gln Ala Thr
    1010                1015                1020

Arg Gln  Glu Ser Ser Thr Thr  Phe Val Ser Asp Gly  Ser Leu Glu
    1025                1030                1035

Lys His  Pro Glu Val Pro Ser  Ala Lys Ala Val Thr  Ala Asp Gly
    1040                1045                1050

Phe Pro  Thr Val Ser Val Met  Leu Pro Leu His Ser  Glu Gln Asn
    1055                1060                1065

Lys Ser  Ser Pro Asp Pro Thr  Ser Thr Leu Ser Asn  Thr Val Ser
    1070                1075                1080

Tyr Glu  Arg Ser Thr Asp Gly  Ser Phe Gln Asp Arg  Phe Arg Glu
    1085                1090                1095
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Phe Glu  Asp Ser Thr Leu Lys  Pro Asn Arg Lys Lys  Pro Thr Glu
    1100                1105                1110

Asn Ile  Ile Ile Asp Leu Asp  Lys Glu Asp Lys Asp  Leu Ile Leu
    1115                1120                1125

Thr Ile  Thr Glu Ser Thr Ile  Leu Glu Ile Leu Pro  Glu Leu Thr
    1130                1135                1140

Ser Asp  Lys Asn Thr Ile Ile  Asp Ile Asp His Thr  Lys Pro Val
    1145                1150                1155

Tyr Glu  Asp Ile Leu Gly Met  Gln Thr Asp Ile Asp  Thr Glu Val
    1160                1165                1170

Pro Ser  Glu Pro His Asp Ser  Asn Asp Glu Ser Asn  Asp Asp Ser
    1175                1180                1185

Thr Gln  Val Gln Glu Ile Tyr  Glu Ala Ala Val Asn  Leu Ser Leu
    1190                1195                1200

Thr Glu  Glu Thr Phe Glu Gly  Ser Ala Asp Val Leu  Ala Ser Tyr
    1205                1210                1215

Thr Gln  Ala Thr His Asp Glu  Ser Met Thr Tyr Glu  Asp Arg Ser
    1220                1225                1230

Gln Leu  Asp His Met Gly Phe  His Phe Thr Thr Gly  Ile Pro Ala
    1235                1240                1245

Pro Ser  Thr Glu Thr Glu Leu  Asp Val Leu Leu Pro  Thr Ala Thr
    1250                1255                1260

Ser Leu  Pro Ile Pro Arg Lys  Ser Ala Thr Val Ile  Pro Glu Ile
    1265                1270                1275

Glu Gly  Ile Lys Ala Glu Ala  Lys Ala Leu Asp Asp  Met Phe Glu
    1280                1285                1290

Ser Ser  Thr Leu Ser Asp Gly  Gln Ala Ile Ala Asp  Gln Ser Glu
    1295                1300                1305

Ile Ile  Pro Thr Leu Gly Gln  Phe Glu Arg Thr Gln  Glu Glu Tyr
    1310                1315                1320

Glu Asp  Lys Lys His Ala Gly  Pro Ser Phe Gln Pro  Glu Phe Ser
    1325                1330                1335

Ser Gly  Ala Glu Glu Ala Leu  Val Asp His Thr Pro  Tyr Leu Ser
    1340                1345                1350

Ile Ala  Thr Thr His Leu Met  Asp Gln Ser Val Thr  Glu Val Pro
    1355                1360                1365

Asp Val  Met Glu Gly Ser Asn  Pro Pro Tyr Tyr Thr  Asp Thr Thr
    1370                1375                1380

Leu Ala  Val Ser Thr Phe Ala  Lys Leu Ser Ser Gln  Thr Pro Ser
    1385                1390                1395

Ser Pro  Leu Thr Ile Tyr Ser  Gly Ser Glu Ala Ser  Gly His Thr
    1400                1405                1410

Glu Ile  Pro Gln Pro Ser Ala  Leu Pro Gly Ile Asp  Val Gly Ser
    1415                1420                1425

Ser Val  Met Ser Pro Gln Asp  Ser Phe Lys Glu Ile  His Val Asn
    1430                1435                1440

Ile Glu  Ala Thr Phe Lys Pro  Ser Ser Glu Glu Tyr  Leu His Ile
    1445                1450                1455

Thr Glu  Pro Pro Ser Leu Ser  Pro Asp Thr Lys Leu  Glu Pro Ser
    1460                1465                1470

Glu Asp  Asp Gly Lys Pro Glu  Leu Leu Glu Glu Met  Glu Ala Ser
    1475                1480                1485
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Pro Thr  Glu Leu Ile Ala Val  Glu Gly Thr Glu Ile  Leu Gln Asp
    1490                1495                1500

Phe Gln  Asn Lys Thr Asp Gly  Gln Val Ser Gly Glu  Ala Ile Lys
    1505                1510                1515

Met Phe  Pro Thr Ile Lys Thr  Pro Glu Ala Gly Thr  Val Ile Thr
    1520                1525                1530

Thr Ala  Asp Glu Ile Glu Leu  Glu Gly Ala Thr Gln  Trp Pro His
    1535                1540                1545

Ser Thr  Ser Ala Ser Ala Thr  Tyr Gly Val Glu Ala  Gly Val Val
    1550                1555                1560

Pro Trp  Leu Ser Pro Gln Thr  Ser Glu Arg Pro Thr  Leu Ser Ser
    1565                1570                1575

Ser Pro  Glu Ile Asn Pro Glu  Thr Gln Ala Ala Leu  Ile Arg Gly
    1580                1585                1590

Gln Asp  Ser Thr Ile Ala Ala  Ser Glu Gln Gln Val  Ala Ala Arg
    1595                1600                1605

Ile Leu  Asp Ser Asn Asp Gln  Ala Thr Val Asn Pro  Val Glu Phe
    1610                1615                1620

Asn Thr  Glu Val Ala Thr Pro  Pro Phe Ser Leu Leu  Glu Thr Ser
    1625                1630                1635

Asn Glu  Thr Asp Phe Leu Ile  Gly Ile Asn Glu Glu  Ser Val Glu
    1640                1645                1650

Gly Thr  Ala Ile Tyr Leu Pro  Gly Pro Asp Arg Cys  Lys Met Asn
    1655                1660                1665

Pro Cys  Leu Asn Gly Gly Thr  Cys Tyr Pro Thr Glu  Thr Ser Tyr
    1670                1675                1680

Val Cys  Thr Cys Val Pro Gly  Tyr Ser Gly Asp Gln  Cys Glu Leu
    1685                1690                1695

Asp Phe  Asp Glu Cys His Ser  Asn Pro Cys Arg Asn  Gly Ala Thr
    1700                1705                1710

Cys Val  Asp Gly Phe Asn Thr  Phe Arg Cys Leu Cys  Leu Pro Ser
    1715                1720                1725

Tyr Val  Gly Ala Leu Cys Glu  Gln Asp Thr Glu Thr  Cys Asp Tyr
    1730                1735                1740

Gly Trp  His Lys Phe Gln Gly  Gln Cys Tyr Lys Tyr  Phe Ala His
    1745                1750                1755

Arg Arg  Thr Trp Asp Ala Ala  Glu Arg Glu Cys Arg  Leu Gln Gly
    1760                1765                1770

Ala His  Leu Thr Ser Ile Leu  Ser His Glu Glu Gln  Met Phe Val
    1775                1780                1785

Asn Arg  Val Gly His Asp Tyr  Gln Trp Ile Gly Leu  Asn Asp Lys
    1790                1795                1800

Met Phe  Glu His Asp Phe Arg  Trp Thr Asp Gly Ser  Thr Leu Gln
    1805                1810                1815

Tyr Glu  Asn Trp Arg Pro Asn  Gln Pro Asp Ser Phe  Phe Ser Ala
    1820                1825                1830

Gly Glu  Asp Cys Val Val Ile  Ile Trp His Glu Asn  Gly Gln Trp
    1835                1840                1845

Asn Asp  Val Pro Cys Asn Tyr  His Leu Thr Tyr Thr  Cys Lys Lys
    1850                1855                1860

Gly Thr  Val Ala Cys Gly Gln  Pro Pro Val Val Glu  Asn Ala Lys
    1865                1870                1875
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Thr Phe  Gly Lys Met Lys Pro  Arg Tyr Glu Ile Asn  Ser Leu Ile
    1880                1885                1890

Arg Tyr  His Cys Lys Asp Gly  Phe Ile Gln Arg His  Leu Pro Thr
    1895                1900                1905

Ile Arg  Cys Leu Gly Asn Gly  Arg Trp Ala Ile Pro  Lys Ile Thr
    1910                1915                1920

Cys Met  Asn Pro Ser Ala Tyr  Gln Arg Thr Tyr Ser  Met Lys Tyr
    1925                1930                1935

Phe Lys  Asn Ser Ser Ser Ala  Lys Asp Asn Ser Ile  Asn Thr Ser
    1940                1945                1950

Lys His  Asp His Arg Trp Ser  Arg Arg Trp Gln Glu  Ser Arg Arg
    1955                1960                1965


<210> SEQ ID NO 5
<211> LENGTH: 441
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 5

Met Phe Ile Asn Ile Lys Ser Ile Leu Trp Met Cys Ser Thr Leu Ile
1               5                  10                  15

Val Thr His Ala Leu His Lys Val Lys Val Gly Lys Ser Pro Pro Val
            20                  25                  30

Arg Gly Ser Leu Ser Gly Lys Val Ser Leu Pro Cys His Phe Ser Thr
        35                  40                  45

Met Pro Thr Leu Pro Pro Ser Tyr Asn Thr Ser Glu Phe Leu Arg Ile
    50                  55                  60

Lys Trp Ser Lys Ile Glu Val Asp Lys Asn Gly Lys Asp Leu Lys Glu
65                  70                  75                  80

Thr Thr Val Leu Val Ala Gln Asn Gly Asn Ile Lys Ile Gly Gln Asp
                85                  90                  95

Tyr Lys Gly Arg Val Ser Val Pro Thr His Pro Glu Ala Val Gly Asp
            100                 105                 110

Ala Ser Leu Thr Val Val Lys Leu Leu Ala Ser Asp Ala Gly Leu Tyr
        115                 120                 125

Arg Cys Asp Val Met Tyr Gly Ile Glu Asp Thr Gln Asp Thr Val Ser
    130                 135                 140

Leu Thr Val Asp Gly Val Val Phe His Tyr Arg Ala Ala Thr Ser Arg
145                 150                 155                 160

Tyr Thr Leu Asn Phe Glu Ala Ala Gln Lys Ala Cys Leu Asp Val Gly
                165                 170                 175

Ala Val Ile Ala Thr Pro Glu Gln Leu Phe Ala Ala Tyr Glu Asp Gly
            180                 185                 190

Phe Glu Gln Cys Asp Ala Gly Trp Leu Ala Asp Gln Thr Val Arg Tyr
        195                 200                 205

Pro Ile Arg Ala Pro Arg Val Gly Cys Tyr Gly Asp Lys Met Gly Lys
    210                 215                 220

Ala Gly Val Arg Thr Tyr Gly Phe Arg Ser Pro Gln Glu Thr Tyr Asp
225                 230                 235                 240

Val Tyr Cys Tyr Val Asp His Leu Asp Gly Asp Val Phe His Leu Thr
                245                 250                 255

Val Pro Ser Lys Phe Thr Phe Glu Glu Ala Ala Lys Glu Cys Glu Asn
            260                 265                 270

Gln Asp Ala Arg Leu Ala Thr Val Gly Glu Leu Gln Ala Ala Trp Arg
        275                 280                 285
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Asn Gly Phe Asp Gln Cys Asp Tyr Gly Trp Leu Ser Asp Ala Ser Val
    290                 295                 300

Arg His Pro Val Thr Val Ala Arg Ala Gln Cys Gly Gly Gly Leu Leu
305                 310                 315                 320

Gly Val Arg Thr Leu Tyr Arg Phe Glu Asn Gln Thr Gly Phe Pro Pro
                325                 330                 335

Pro Asp Ser Arg Phe Asp Ala Tyr Cys Phe Lys Arg Arg Met Ser Asp
            340                 345                 350

Leu Ser Val Ile Gly His Pro Ile Asp Ser Glu Ser Lys Glu Asp Glu
        355                 360                 365

Pro Cys Ser Glu Glu Thr Asp Pro Val His Asp Leu Met Ala Glu Ile
    370                 375                 380

Leu Pro Glu Phe Pro Asp Ile Ile Glu Ile Asp Leu Tyr His Ser Glu
385                 390                 395                 400

Glu Asn Glu Glu Glu Glu Glu Glu Cys Ala Asn Ala Thr Asp Val Thr
                405                 410                 415

Thr Thr Pro Ser Val Gln Tyr Ile Asn Gly Lys His Leu Val Thr Thr
            420                 425                 430

Val Pro Lys Asp Pro Glu Ala Ala Glu
        435                 440


<210> SEQ ID NO 6
<211> LENGTH: 421
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 6

Leu His Lys Val Lys Val Gly Lys Ser Pro Pro Val Arg Gly Ser Leu
1               5                   10                  15

Ser Gly Lys Val Ser Leu Pro Cys His Phe Ser Thr Met Pro Thr Leu
            20                  25                  30

Pro Pro Ser Tyr Asn Thr Ser Glu Phe Leu Arg Ile Lys Trp Ser Lys
        35                  40                  45

Ile Glu Val Asp Lys Asn Gly Lys Asp Leu Lys Glu Thr Thr Val Leu
    50                  55                  60

Val Ala Gln Asn Gly Asn Ile Lys Ile Gly Gln Asp Tyr Lys Gly Arg
65                  70                  75                  80

Val Ser Val Pro Thr His Pro Glu Ala Val Gly Asp Ala Ser Leu Thr
                85                  90                  95

Val Val Lys Leu Leu Ala Ser Asp Ala Gly Leu Tyr Arg Cys Asp Val
            100                 105                 110

Met Tyr Gly Ile Glu Asp Thr Gln Asp Thr Val Ser Leu Thr Val Asp
        115                 120                 125

Gly Val Val Phe His Tyr Arg Ala Ala Thr Ser Arg Tyr Thr Leu Asn
    130                 135                 140

Phe Glu Ala Ala Gln Lys Ala Cys Leu Asp Val Gly Ala Val Ile Ala
145                 150                 155                 160

Thr Pro Glu Gln Leu Phe Ala Ala Tyr Glu Asp Gly Phe Glu Gln Cys
                165                 170                 175

Asp Ala Gly Trp Leu Ala Asp Gln Thr Val Arg Tyr Pro Ile Arg Ala
            180                 185                 190

Pro Arg Val Gly Cys Tyr Gly Asp Lys Met Gly Lys Ala Gly Val Arg
        195                 200                 205

Thr Tyr Gly Phe Arg Ser Pro Gln Glu Thr Tyr Asp Val Tyr Cys Tyr
    210                 215                 220
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Val Asp His Leu Asp Gly Asp Val Phe His Leu Thr Val Pro Ser Lys
225                 230                 235                 240

Phe Thr Phe Glu Glu Ala Ala Lys Glu Cys Glu Asn Gln Asp Ala Arg
                245                 250                 255

Leu Ala Thr Val Gly Glu Leu Gln Ala Ala Trp Arg Asn Gly Phe Asp
            260                 265                 270

Gln Cys Asp Tyr Gly Trp Leu Ser Asp Ala Ser Val Arg His Pro Val
        275                 280                 285

Thr Val Ala Arg Ala Gln Cys Gly Gly Gly Leu Leu Gly Val Arg Thr
    290                 295                 300

Leu Tyr Arg Phe Glu Asn Gln Thr Gly Phe Pro Pro Pro Asp Ser Arg
305                 310                 315                 320

Phe Asp Ala Tyr Cys Phe Lys Arg Arg Met Ser Asp Leu Ser Val Ile
                325                 330                 335

Gly His Pro Ile Asp Ser Glu Ser Lys Glu Asp Glu Pro Cys Ser Glu
            340                 345                 350

Glu Thr Asp Pro Val His Asp Leu Met Ala Glu Ile Leu Pro Glu Phe
        355                 360                 365

Pro Asp Ile Ile Glu Ile Asp Leu Tyr His Ser Glu Glu Asn Glu Glu
    370                 375                 380

Glu Glu Glu Glu Cys Ala Asn Ala Thr Asp Val Thr Thr Thr Pro Ser
385                 390                 395                 400

Val Gln Tyr Ile Asn Gly Lys His Leu Val Thr Thr Val Pro Lys Asp
                405                 410                 415

Pro Glu Ala Ala Glu
            420


<210> SEQ ID NO 7
<211> LENGTH: 422
<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Full length human versikine (minus signal
      peptide, plus N-terminal methionine)

<400> SEQUENCE: 7

Met Leu His Lys Val Lys Val Gly Lys Ser Pro Pro Val Arg Gly Ser
1               5                   10                  15

Leu Ser Gly Lys Val Ser Leu Pro Cys His Phe Ser Thr Met Pro Thr
            20                  25                  30

Leu Pro Pro Ser Tyr Asn Thr Ser Glu Phe Leu Arg Ile Lys Trp Ser
        35                  40                  45

Lys Ile Glu Val Asp Lys Asn Gly Lys Asp Leu Lys Glu Thr Thr Val
    50                  55                  60

Leu Val Ala Gln Asn Gly Asn Ile Lys Ile Gly Gln Asp Tyr Lys Gly
65                  70                  75                  80

Arg Val Ser Val Pro Thr His Pro Glu Ala Val Gly Asp Ala Ser Leu
                85                  90                  95

Thr Val Val Lys Leu Leu Ala Ser Asp Ala Gly Leu Tyr Arg Cys Asp
            100                 105                 110

Val Met Tyr Gly Ile Glu Asp Thr Gln Asp Thr Val Ser Leu Thr Val
        115                 120                 125

Asp Gly Val Val Phe His Tyr Arg Ala Ala Thr Ser Arg Tyr Thr Leu
    130                 135                 140

Asn Phe Glu Ala Ala Gln Lys Ala Cys Leu Asp Val Gly Ala Val Ile
145                 150                 155                 160
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Ala Thr Pro Glu Gln Leu Phe Ala Ala Tyr Glu Asp Gly Phe Glu Gln
                165                 170                 175

Cys Asp Ala Gly Trp Leu Ala Asp Gln Thr Val Arg Tyr Pro Ile Arg
            180                 185                 190

Ala Pro Arg Val Gly Cys Tyr Gly Asp Lys Met Gly Lys Ala Gly Val
        195                 200                 205

Arg Thr Tyr Gly Phe Arg Ser Pro Gln Glu Thr Tyr Asp Val Tyr Cys
    210                 215                 220

Tyr Val Asp His Leu Asp Gly Asp Val Phe His Leu Thr Val Pro Ser
225                 230                 235                 240

Lys Phe Thr Phe Glu Glu Ala Ala Lys Glu Cys Glu Asn Gln Asp Ala
                245                 250                 255

Arg Leu Ala Thr Val Gly Glu Leu Gln Ala Ala Trp Arg Asn Gly Phe
            260                 265                 270

Asp Gln Cys Asp Tyr Gly Trp Leu Ser Asp Ala Ser Val Arg His Pro
        275                 280                 285

Val Thr Val Ala Arg Ala Gln Cys Gly Gly Gly Leu Leu Gly Val Arg
    290                 295                 300

Thr Leu Tyr Arg Phe Glu Asn Gln Thr Gly Phe Pro Pro Pro Asp Ser
305                 310                 315                 320

Arg Phe Asp Ala Tyr Cys Phe Lys Arg Arg Met Ser Asp Leu Ser Val
                325                 330                 335

Ile Gly His Pro Ile Asp Ser Glu Ser Lys Glu Asp Glu Pro Cys Ser
            340                 345                 350

Glu Glu Thr Asp Pro Val His Asp Leu Met Ala Glu Ile Leu Pro Glu
        355                 360                 365

Phe Pro Asp Ile Ile Glu Ile Asp Leu Tyr His Ser Glu Glu Asn Glu
    370                 375                 380

Glu Glu Glu Glu Glu Cys Ala Asn Ala Thr Asp Val Thr Thr Thr Pro
385                 390                 395                 400

Ser Val Gln Tyr Ile Asn Gly Lys His Leu Val Thr Thr Val Pro Lys
                405                 410                 415

Asp Pro Glu Ala Ala Glu
            420


<210> SEQ ID NO 8
<211> LENGTH: 146
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 8

Met Phe Ile Asn Ile Lys Ser Ile Leu Trp Met Cys Ser Thr Leu Ile
1               5                   10                  15

Val Thr His Ala Leu His Lys Val Lys Val Gly Lys Ser Pro Pro Val
            20                  25                  30

Arg Gly Ser Leu Ser Gly Lys Val Ser Leu Pro Cys His Phe Ser Thr
        35                  40                  45

Met Pro Thr Leu Pro Pro Ser Tyr Asn Thr Ser Glu Phe Leu Arg Ile
    50                  55                  60

Lys Trp Ser Lys Ile Glu Val Asp Lys Asn Gly Lys Asp Leu Lys Glu
65                  70                  75                  80

Thr Thr Val Leu Val Ala Gln Asn Gly Asn Ile Lys Ile Gly Gln Asp
                85                  90                  95

Tyr Lys Gly Arg Val Ser Val Pro Thr His Pro Glu Ala Val Gly Asp
            100                 105                 110
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Ala Ser Leu Thr Val Val Lys Leu Leu Ala Ser Asp Ala Gly Leu Tyr
        115                 120                 125

Arg Cys Asp Val Met Tyr Gly Ile Glu Asp Thr Gln Asp Thr Val Ser
    130                 135                 140

Leu Thr
145


<210> SEQ ID NO 9
<211> LENGTH: 126
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 9

Leu His Lys Val Lys Val Gly Lys Ser Pro Pro Val Arg Gly Ser Leu
1               5                   10                  15

Ser Gly Lys Val Ser Leu Pro Cys His Phe Ser Thr Met Pro Thr Leu
            20                  25                  30

Pro Pro Ser Tyr Asn Thr Ser Glu Phe Leu Arg Ile Lys Trp Ser Lys
        35                  40                  45

Ile Glu Val Asp Lys Asn Gly Lys Asp Leu Lys Glu Thr Thr Val Leu
    50                  55                  60

Val Ala Gln Asn Gly Asn Ile Lys Ile Gly Gln Asp Tyr Lys Gly Arg
65                  70                  75                  80

Val Ser Val Pro Thr His Pro Glu Ala Val Gly Asp Ala Ser Leu Thr
                85                  90                  95

Val Val Lys Leu Leu Ala Ser Asp Ala Gly Leu Tyr Arg Cys Asp Val
            100                 105                 110

Met Tyr Gly Ile Glu Asp Thr Gln Asp Thr Val Ser Leu Thr
        115                 120                 125


<210> SEQ ID NO 10
<211> LENGTH: 127
<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Ig-like domain of human versikine (minus signal
      peptide, plus N-terminal methionine)

<400> SEQUENCE: 10

Met Leu His Lys Val Lys Val Gly Lys Ser Pro Pro Val Arg Gly Ser
1               5                   10                  15

Leu Ser Gly Lys Val Ser Leu Pro Cys His Phe Ser Thr Met Pro Thr
            20                  25                  30

Leu Pro Pro Ser Tyr Asn Thr Ser Glu Phe Leu Arg Ile Lys Trp Ser
        35                  40                  45

Lys Ile Glu Val Asp Lys Asn Gly Lys Asp Leu Lys Glu Thr Thr Val
    50                  55                  60

Leu Val Ala Gln Asn Gly Asn Ile Lys Ile Gly Gln Asp Tyr Lys Gly
65                  70                  75                  80

Arg Val Ser Val Pro Thr His Pro Glu Ala Val Gly Asp Ala Ser Leu
                85                  90                  95

Thr Val Val Lys Leu Leu Ala Ser Asp Ala Gly Leu Tyr Arg Cys Asp
            100                 105                 110

Val Met Tyr Gly Ile Glu Asp Thr Gln Asp Thr Val Ser Leu Thr
        115                 120                 125
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<210> SEQ ID NO 11
<211> LENGTH: 96
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 11

Val Val Phe His Tyr Arg Ala Ala Thr Ser Arg Tyr Thr Leu Asn Phe
1 5 10 15

Glu Ala Ala Gln Lys Ala Cys Leu Asp Val Gly Ala Val Ile Ala Thr
20 25 30

Pro Glu Gln Leu Phe Ala Ala Tyr Glu Asp Gly Phe Glu Gln Cys Asp
35 40 45

Ala Gly Trp Leu Ala Asp Gln Thr Val Arg Tyr Pro Ile Arg Ala Pro
50 55 60

Arg Val Gly Cys Tyr Gly Asp Lys Met Gly Lys Ala Gly Val Arg Thr
65 70 75 80

Tyr Gly Phe Arg Ser Pro Gln Glu Thr Tyr Asp Val Tyr Cys Tyr Val
85 90 95

<210> SEQ ID NO 12
<211> LENGTH: 97
<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Linker domain 1 of human versikine (plus N-terminal methionine)

<400> SEQUENCE: 12

Met Val Val Phe His Tyr Arg Ala Ala Thr Ser Arg Tyr Thr Leu Asn
1 5 10 15

Phe Glu Ala Ala Gln Lys Ala Cys Leu Asp Val Gly Ala Val Ile Ala
20 25 30

Thr Pro Glu Gln Leu Phe Ala Ala Tyr Glu Asp Gly Phe Glu Gln Cys
35 40 45

Asp Ala Gly Trp Leu Ala Asp Gln Thr Val Arg Tyr Pro Ile Arg Ala
50 55 60

Pro Arg Val Gly Cys Tyr Gly Asp Lys Met Gly Lys Ala Gly Val Arg
65 70 75 80

Thr Tyr Gly Phe Arg Ser Pro Gln Glu Thr Tyr Asp Val Tyr Cys Tyr
85 90 95

Val

<210> SEQ ID NO 13
<211> LENGTH: 97
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 13

Asp Val Phe His Leu Thr Val Pro Ser Lys Phe Thr Phe Glu Glu Ala
1 5 10 15

Ala Lys Glu Cys Glu Asn Gln Asp Ala Arg Leu Ala Thr Val Gly Glu
20 25 30

Leu Gln Ala Ala Trp Arg Asn Gly Phe Asp Gln Cys Asp Tyr Gly Trp
35 40 45

Leu Ser Asp Ala Ser Val Arg His Pro Val Thr Val Ala Arg Ala Gln
50 55 60

Cys Gly Gly Gly Leu Leu Gly Val Arg Thr Leu Tyr Arg Phe Glu Asn
65 70 75 80

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Gln Thr Gly Phe Pro Pro Pro Asp Ser Arg Phe Asp Ala Tyr Cys Phe
                85                  90                  95

Lys


<210> SEQ ID NO 14
<211> LENGTH: 98
<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Linker domain 2 of human versikine (plus N-
      terminal methionine)

<400> SEQUENCE: 14

Met Asp Val Phe His Leu Thr Val Pro Ser Lys Phe Thr Phe Glu Glu
1               5                   10                  15

Ala Ala Lys Glu Cys Glu Asn Gln Asp Ala Arg Leu Ala Thr Val Gly
            20                  25                  30

Glu Leu Gln Ala Ala Trp Arg Asn Gly Phe Asp Gln Cys Asp Tyr Gly
        35                  40                  45

Trp Leu Ser Asp Ala Ser Val Arg His Pro Val Thr Val Ala Arg Ala
    50                  55                  60

Gln Cys Gly Gly Gly Leu Leu Gly Val Arg Thr Leu Tyr Arg Phe Glu
65                  70                  75                  80

Asn Gln Thr Gly Phe Pro Pro Pro Asp Ser Arg Phe Asp Ala Tyr Cys
                85                  90                  95

Phe Lys


<210> SEQ ID NO 15
<211> LENGTH: 93
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 15

Arg Met Ser Asp Leu Ser Val Ile Gly His Pro Ile Asp Ser Glu Ser
1               5                   10                  15

Lys Glu Asp Glu Pro Cys Ser Glu Glu Thr Asp Pro Val His Asp Leu
            20                  25                  30

Met Ala Glu Ile Leu Pro Glu Phe Pro Asp Ile Ile Glu Ile Asp Leu
        35                  40                  45

Tyr His Ser Glu Glu Asn Glu Glu Glu Glu Glu Glu Cys Ala Asn Ala
    50                  55                  60

Thr Asp Val Thr Thr Thr Pro Ser Val Gln Tyr Ile Asn Gly Lys His
65                  70                  75                  80

Leu Val Thr Thr Val Pro Lys Asp Pro Glu Ala Ala Glu
                85                  90


<210> SEQ ID NO 16
<211> LENGTH: 245
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 16

Met Phe Ile Asn Ile Lys Ser Ile Leu Trp Met Cys Ser Thr Leu Ile
1               5                   10                  15

Val Thr His Ala Leu His Lys Val Lys Val Gly Lys Ser Pro Pro Val
            20                  25                  30

Arg Gly Ser Leu Ser Gly Lys Val Ser Leu Pro Cys His Phe Ser Thr
        35                  40                  45
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Met Pro Thr Leu Pro Pro Ser Tyr Asn Thr Ser Glu Phe Leu Arg Ile
    50                  55                  60

Lys Trp Ser Lys Ile Glu Val Asp Lys Asn Gly Lys Asp Leu Lys Glu
65                  70                  75                  80

Thr Thr Val Leu Val Ala Gln Asn Gly Asn Ile Lys Ile Gly Gln Asp
                85                  90                  95

Tyr Lys Gly Arg Val Ser Val Pro Thr His Pro Glu Ala Val Gly Asp
            100                 105                 110

Ala Ser Leu Thr Val Val Lys Leu Leu Ala Ser Asp Ala Gly Leu Tyr
        115                 120                 125

Arg Cys Asp Val Met Tyr Gly Ile Glu Asp Thr Gln Asp Thr Val Ser
    130                 135                 140

Leu Thr Val Asp Gly Val Val Phe His Tyr Arg Ala Ala Thr Ser Arg
145                 150                 155                 160

Tyr Thr Leu Asn Phe Glu Ala Ala Gln Lys Ala Cys Leu Asp Val Gly
                165                 170                 175

Ala Val Ile Ala Thr Pro Glu Gln Leu Phe Ala Ala Tyr Glu Asp Gly
            180                 185                 190

Phe Glu Gln Cys Asp Ala Gly Trp Leu Ala Asp Gln Thr Val Arg Tyr
        195                 200                 205

Pro Ile Arg Ala Pro Arg Val Gly Cys Tyr Gly Asp Lys Met Gly Lys
    210                 215                 220

Ala Gly Val Arg Thr Tyr Gly Phe Arg Ser Pro Gln Glu Thr Tyr Asp
225                 230                 235                 240

Val Tyr Cys Tyr Val
                245


<210> SEQ ID NO 17
<211> LENGTH: 225
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 17

Leu His Lys Val Lys Val Gly Lys Ser Pro Pro Val Arg Gly Ser Leu
1               5                   10                  15

Ser Gly Lys Val Ser Leu Pro Cys His Phe Ser Thr Met Pro Thr Leu
            20                  25                  30

Pro Pro Ser Tyr Asn Thr Ser Glu Phe Leu Arg Ile Lys Trp Ser Lys
        35                  40                  45

Ile Glu Val Asp Lys Asn Gly Lys Asp Leu Lys Glu Thr Thr Val Leu
    50                  55                  60

Val Ala Gln Asn Gly Asn Ile Lys Ile Gly Gln Asp Tyr Lys Gly Arg
65                  70                  75                  80

Val Ser Val Pro Thr His Pro Glu Ala Val Gly Asp Ala Ser Leu Thr
                85                  90                  95

Val Val Lys Leu Leu Ala Ser Asp Ala Gly Leu Tyr Arg Cys Asp Val
            100                 105                 110

Met Tyr Gly Ile Glu Asp Thr Gln Asp Thr Val Ser Leu Thr Val Asp
        115                 120                 125

Gly Val Val Phe His Tyr Arg Ala Ala Thr Ser Arg Tyr Thr Leu Asn
    130                 135                 140

Phe Glu Ala Ala Gln Lys Ala Cys Leu Asp Val Gly Ala Val Ile Ala
145                 150                 155                 160

Thr Pro Glu Gln Leu Phe Ala Ala Tyr Glu Asp Gly Phe Glu Gln Cys
                165                 170                 175
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Asp Ala Gly Trp Leu Ala Asp Gln Thr Val Arg Tyr Pro Ile Arg Ala
            180                 185                 190

Pro Arg Val Gly Cys Tyr Gly Asp Lys Met Gly Lys Ala Gly Val Arg
        195                 200                 205

Thr Tyr Gly Phe Arg Ser Pro Gln Glu Thr Tyr Asp Val Tyr Cys Tyr
    210                 215                 220

Val
225


<210> SEQ ID NO 18
<211> LENGTH: 226
<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: N-terminal portion of human versikine including
      Ig-like domain and Linker domain 1 (minus signal peptide, plus N-
      terminal methionine)

<400> SEQUENCE: 18

Met Leu His Lys Val Lys Val Gly Lys Ser Pro Pro Val Arg Gly Ser
1               5                   10                  15

Leu Ser Gly Lys Val Ser Leu Pro Cys His Phe Ser Thr Met Pro Thr
            20                  25                  30

Leu Pro Pro Ser Tyr Asn Thr Ser Glu Phe Leu Arg Ile Lys Trp Ser
        35                  40                  45

Lys Ile Glu Val Asp Lys Asn Gly Lys Asp Leu Lys Glu Thr Thr Val
    50                  55                  60

Leu Val Ala Gln Asn Gly Asn Ile Lys Ile Gly Gln Asp Tyr Lys Gly
65                  70                  75                  80

Arg Val Ser Val Pro Thr His Pro Glu Ala Val Gly Asp Ala Ser Leu
                85                  90                  95

Thr Val Val Lys Leu Leu Ala Ser Asp Ala Gly Leu Tyr Arg Cys Asp
            100                 105                 110

Val Met Tyr Gly Ile Glu Asp Thr Gln Asp Thr Val Ser Leu Thr Val
        115                 120                 125

Asp Gly Val Val Phe His Tyr Arg Ala Ala Thr Ser Arg Tyr Thr Leu
    130                 135                 140

Asn Phe Glu Ala Ala Gln Lys Ala Cys Leu Asp Val Gly Ala Val Ile
145                 150                 155                 160

Ala Thr Pro Glu Gln Leu Phe Ala Ala Tyr Glu Asp Gly Phe Glu Gln
                165                 170                 175

Cys Asp Ala Gly Trp Leu Ala Asp Gln Thr Val Arg Tyr Pro Ile Arg
            180                 185                 190

Ala Pro Arg Val Gly Cys Tyr Gly Asp Lys Met Gly Lys Ala Gly Val
        195                 200                 205

Arg Thr Tyr Gly Phe Arg Ser Pro Gln Glu Thr Tyr Asp Val Tyr Cys
    210                 215                 220

Tyr Val
225


<210> SEQ ID NO 19
<211> LENGTH: 348
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
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-continued

<400> SEQUENCE: 19

Met Phe Ile Asn Ile Lys Ser Ile Leu Trp Met Cys Ser Thr Leu Ile
1 5 10 15

Val Thr His Ala Leu His Lys Val Lys Val Gly Lys Ser Pro Pro Val
20 25 30

Arg Gly Ser Leu Ser Gly Lys Val Ser Leu Pro Cys His Phe Ser Thr
35 40 45

Met Pro Thr Leu Pro Pro Ser Tyr Asn Thr Ser Glu Phe Leu Arg Ile
50 55 60

Lys Trp Ser Lys Ile Glu Val Asp Lys Asn Gly Lys Asp Leu Lys Glu
65 70 75 80

Thr Thr Val Leu Val Ala Gln Asn Gly Asn Ile Lys Ile Gly Gln Asp
85 90 95

Tyr Lys Gly Arg Val Ser Val Pro Thr His Pro Glu Ala Val Gly Asp
100 105 110

Ala Ser Leu Thr Val Val Lys Leu Leu Ala Ser Asp Ala Gly Leu Tyr
115 120 125

Arg Cys Asp Val Met Tyr Gly Ile Glu Asp Thr Gln Asp Thr Val Ser
130 135 140

Leu Thr Val Asp Gly Val Val Phe His Tyr Arg Ala Ala Thr Ser Arg
145 150 155 160

Tyr Thr Leu Asn Phe Glu Ala Ala Gln Lys Ala Cys Leu Asp Val Gly
165 170 175

Ala Val Ile Ala Thr Pro Glu Gln Leu Phe Ala Ala Tyr Glu Asp Gly
180 185 190

Phe Glu Gln Cys Asp Ala Gly Trp Leu Ala Asp Gln Thr Val Arg Tyr
195 200 205

Pro Ile Arg Ala Pro Arg Val Gly Cys Tyr Gly Asp Lys Met Gly Lys
210 215 220

Ala Gly Val Arg Thr Tyr Gly Phe Arg Ser Pro Gln Glu Thr Tyr Asp
225 230 235 240

Val Tyr Cys Tyr Val Asp His Leu Asp Gly Asp Val Phe His Leu Thr
245 250 255

Val Pro Ser Lys Phe Thr Phe Glu Glu Ala Ala Lys Glu Cys Glu Asn
260 265 270

Gln Asp Ala Arg Leu Ala Thr Val Gly Glu Leu Gln Ala Ala Trp Arg
275 280 285

Asn Gly Phe Asp Gln Cys Asp Tyr Gly Trp Leu Ser Asp Ala Ser Val
290 295 300

Arg His Pro Val Thr Val Ala Arg Ala Gln Cys Gly Gly Gly Leu Leu
305 310 315 320

Gly Val Arg Thr Leu Tyr Arg Phe Glu Asn Gln Thr Gly Phe Pro Pro
325 330 335

Pro Asp Ser Arg Phe Asp Ala Tyr Cys Phe Lys Arg
340 345

<210> SEQ ID NO 20
<211> LENGTH: 328
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

-continued

<400> SEQUENCE: 20

Leu His Lys Val Lys Val Gly Lys Ser Pro Pro Val Arg Gly Ser Leu
1 5 10 15

Ser Gly Lys Val Ser Leu Pro Cys His Phe Ser Thr Met Pro Thr Leu
20 25 30

Pro Pro Ser Tyr Asn Thr Ser Glu Phe Leu Arg Ile Lys Trp Ser Lys
35 40 45

Ile Glu Val Asp Lys Asn Gly Lys Asp Leu Lys Glu Thr Thr Val Leu
50 55 60

Val Ala Gln Asn Gly Asn Ile Lys Ile Gly Gln Asp Tyr Lys Gly Arg
65 70 75 80

Val Ser Val Pro Thr His Pro Glu Ala Val Gly Asp Ala Ser Leu Thr
85 90 95

Val Val Lys Leu Leu Ala Ser Asp Ala Gly Leu Tyr Arg Cys Asp Val
100 105 110

Met Tyr Gly Ile Glu Asp Thr Gln Asp Thr Val Ser Leu Thr Val Asp
115 120 125

Gly Val Val Phe His Tyr Arg Ala Ala Thr Ser Arg Tyr Thr Leu Asn
130 135 140

Phe Glu Ala Ala Gln Lys Ala Cys Leu Asp Val Gly Ala Val Ile Ala
145 150 155 160

Thr Pro Glu Gln Leu Phe Ala Ala Tyr Glu Asp Gly Phe Glu Gln Cys
165 170 175

Asp Ala Gly Trp Leu Ala Asp Gln Thr Val Arg Tyr Pro Ile Arg Ala
180 185 190

Pro Arg Val Gly Cys Tyr Gly Asp Lys Met Gly Lys Ala Gly Val Arg
195 200 205

Thr Tyr Gly Phe Arg Ser Pro Gln Glu Thr Tyr Asp Val Tyr Cys Tyr
210 215 220

Val Asp His Leu Asp Gly Asp Val Phe His Leu Thr Val Pro Ser Lys
225 230 235 240

Phe Thr Phe Glu Glu Ala Ala Lys Glu Cys Glu Asn Gln Asp Ala Arg
245 250 255

Leu Ala Thr Val Gly Glu Leu Gln Ala Ala Trp Arg Asn Gly Phe Asp
260 265 270

Gln Cys Asp Tyr Gly Trp Leu Ser Asp Ala Ser Val Arg His Pro Val
275 280 285

Thr Val Ala Arg Ala Gln Cys Gly Gly Gly Leu Leu Gly Val Arg Thr
290 295 300

Leu Tyr Arg Phe Glu Asn Gln Thr Gly Phe Pro Pro Pro Asp Ser Arg
305 310 315 320

Phe Asp Ala Tyr Cys Phe Lys Arg
325

<210> SEQ ID NO 21
<211> LENGTH: 329
<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: N-terminal portion of human versikine including Ig-like domain, Linker domain 1, and Linker domain 2 (minus signal peptide, plus N-terminal methionine)

-continued

<400> SEQUENCE: 21

Met Leu His Lys Val Lys Val Gly Lys Ser Pro Pro Val Arg Gly Ser
1 5 10 15

Leu Ser Gly Lys Val Ser Leu Pro Cys His Phe Ser Thr Met Pro Thr
20 25 30

Leu Pro Pro Ser Tyr Asn Thr Ser Glu Phe Leu Arg Ile Lys Trp Ser
35 40 45

Lys Ile Glu Val Asp Lys Asn Gly Lys Asp Leu Lys Glu Thr Thr Val
50 55 60

Leu Val Ala Gln Asn Gly Asn Ile Lys Ile Gly Gln Asp Tyr Lys Gly
65 70 75 80

Arg Val Ser Val Pro Thr His Pro Glu Ala Val Gly Asp Ala Ser Leu
85 90 95

Thr Val Val Lys Leu Leu Ala Ser Asp Ala Gly Leu Tyr Arg Cys Asp
100 105 110

Val Met Tyr Gly Ile Glu Asp Thr Gln Asp Thr Val Ser Leu Thr Val
115 120 125

Asp Gly Val Val Phe His Tyr Arg Ala Ala Thr Ser Arg Tyr Thr Leu
130 135 140

Asn Phe Glu Ala Ala Gln Lys Ala Cys Leu Asp Val Gly Ala Val Ile
145 150 155 160

Ala Thr Pro Glu Gln Leu Phe Ala Ala Tyr Glu Asp Gly Phe Glu Gln
165 170 175

Cys Asp Ala Gly Trp Leu Ala Asp Gln Thr Val Arg Tyr Pro Ile Arg
180 185 190

Ala Pro Arg Val Gly Cys Tyr Gly Asp Lys Met Gly Lys Ala Gly Val
195 200 205

Arg Thr Tyr Gly Phe Arg Ser Pro Gln Glu Thr Tyr Asp Val Tyr Cys
210 215 220

Tyr Val Asp His Leu Asp Gly Asp Val Phe His Leu Thr Val Pro Ser
225 230 235 240

Lys Phe Thr Phe Glu Glu Ala Ala Lys Glu Cys Glu Asn Gln Asp Ala
245 250 255

Arg Leu Ala Thr Val Gly Glu Leu Gln Ala Ala Trp Arg Asn Gly Phe
260 265 270

Asp Gln Cys Asp Tyr Gly Trp Leu Ser Asp Ala Ser Val Arg His Pro
275 280 285

Val Thr Val Ala Arg Ala Gln Cys Gly Gly Gly Leu Leu Gly Val Arg
290 295 300

Thr Leu Tyr Arg Phe Glu Asn Gln Thr Gly Phe Pro Pro Pro Asp Ser
305 310 315 320

Arg Phe Asp Ala Tyr Cys Phe Lys Arg
325

<210> SEQ ID NO 22
<211> LENGTH: 198
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 22

Val Val Phe His Tyr Arg Ala Ala Thr Ser Arg Tyr Thr Leu Asn Phe
1 5 10 15

Glu Ala Ala Gln Lys Ala Cys Leu Asp Val Gly Ala Val Ile Ala Thr
20 25 30

-continued

```
Pro Glu Gln Leu Phe Ala Ala Tyr Glu Asp Gly Phe Glu Gln Cys Asp
        35                  40                  45

Ala Gly Trp Leu Ala Asp Gln Thr Val Arg Tyr Pro Ile Arg Ala Pro
    50                  55                  60

Arg Val Gly Cys Tyr Gly Asp Lys Met Gly Lys Ala Gly Val Arg Thr
65                  70                  75                  80

Tyr Gly Phe Arg Ser Pro Gln Glu Thr Tyr Asp Val Tyr Cys Tyr Val
                85                  90                  95

Asp His Leu Asp Gly Asp Val Phe His Leu Thr Val Pro Ser Lys Phe
            100                 105                 110

Thr Phe Glu Glu Ala Ala Lys Glu Cys Glu Asn Gln Asp Ala Arg Leu
        115                 120                 125

Ala Thr Val Gly Glu Leu Gln Ala Ala Trp Arg Asn Gly Phe Asp Gln
    130                 135                 140

Cys Asp Tyr Gly Trp Leu Ser Asp Ala Ser Val Arg His Pro Val Thr
145                 150                 155                 160

Val Ala Arg Ala Gln Cys Gly Gly Gly Leu Leu Gly Val Arg Thr Leu
                165                 170                 175

Tyr Arg Phe Glu Asn Gln Thr Gly Phe Pro Pro Pro Asp Ser Arg Phe
            180                 185                 190

Asp Ala Tyr Cys Phe Lys
        195


<210> SEQ ID NO 23
<211> LENGTH: 199
<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Internal portion of human versikine including
      Linker domain 1 and Linker domain 2 (plus N-terminal methionine)

<400> SEQUENCE: 23

Met Val Val Phe His Tyr Arg Ala Ala Thr Ser Arg Tyr Thr Leu Asn
1               5                   10                  15

Phe Glu Ala Ala Gln Lys Ala Cys Leu Asp Val Gly Ala Val Ile Ala
            20                  25                  30

Thr Pro Glu Gln Leu Phe Ala Ala Tyr Glu Asp Gly Phe Glu Gln Cys
        35                  40                  45

Asp Ala Gly Trp Leu Ala Asp Gln Thr Val Arg Tyr Pro Ile Arg Ala
    50                  55                  60

Pro Arg Val Gly Cys Tyr Gly Asp Lys Met Gly Lys Ala Gly Val Arg
65                  70                  75                  80

Thr Tyr Gly Phe Arg Ser Pro Gln Glu Thr Tyr Asp Val Tyr Cys Tyr
                85                  90                  95

Val Asp His Leu Asp Gly Asp Val Phe His Leu Thr Val Pro Ser Lys
            100                 105                 110

Phe Thr Phe Glu Glu Ala Ala Lys Glu Cys Glu Asn Gln Asp Ala Arg
        115                 120                 125

Leu Ala Thr Val Gly Glu Leu Gln Ala Ala Trp Arg Asn Gly Phe Asp
    130                 135                 140

Gln Cys Asp Tyr Gly Trp Leu Ser Asp Ala Ser Val Arg His Pro Val
145                 150                 155                 160

Thr Val Ala Arg Ala Gln Cys Gly Gly Gly Leu Leu Gly Val Arg Thr
                165                 170                 175
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-continued

```
Leu Tyr Arg Phe Glu Asn Gln Thr Gly Phe Pro Pro Pro Asp Ser Arg
            180                 185                 190

Phe Asp Ala Tyr Cys Phe Lys
        195
```

<210> SEQ ID NO 24
<211> LENGTH: 292
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 24

```
Val Val Phe His Tyr Arg Ala Ala Thr Ser Arg Tyr Thr Leu Asn Phe
1               5                   10                  15

Glu Ala Ala Gln Lys Ala Cys Leu Asp Val Gly Ala Val Ile Ala Thr
            20                  25                  30

Pro Glu Gln Leu Phe Ala Ala Tyr Glu Asp Gly Phe Glu Gln Cys Asp
        35                  40                  45

Ala Gly Trp Leu Ala Asp Gln Thr Val Arg Tyr Pro Ile Arg Ala Pro
    50                  55                  60

Arg Val Gly Cys Tyr Gly Asp Lys Met Gly Lys Ala Gly Val Arg Thr
65                  70                  75                  80

Tyr Gly Phe Arg Ser Pro Gln Glu Thr Tyr Asp Val Tyr Cys Tyr Val
                85                  90                  95

Asp His Leu Asp Gly Asp Val Phe His Leu Thr Val Pro Ser Lys Phe
            100                 105                 110

Thr Phe Glu Glu Ala Ala Lys Glu Cys Glu Asn Gln Asp Ala Arg Leu
        115                 120                 125

Ala Thr Val Gly Glu Leu Gln Ala Ala Trp Arg Asn Gly Phe Asp Gln
    130                 135                 140

Cys Asp Tyr Gly Trp Leu Ser Asp Ala Ser Val Arg His Pro Val Thr
145                 150                 155                 160

Val Ala Arg Ala Gln Cys Gly Gly Gly Leu Leu Gly Val Arg Thr Leu
                165                 170                 175

Tyr Arg Phe Glu Asn Gln Thr Gly Phe Pro Pro Pro Asp Ser Arg Phe
            180                 185                 190

Asp Ala Tyr Cys Phe Lys Arg Arg Met Ser Asp Leu Ser Val Ile Gly
        195                 200                 205

His Pro Ile Asp Ser Glu Ser Lys Glu Asp Glu Pro Cys Ser Glu Glu
    210                 215                 220

Thr Asp Pro Val His Asp Leu Met Ala Glu Ile Leu Pro Glu Phe Pro
225                 230                 235                 240

Asp Ile Ile Glu Ile Asp Leu Tyr His Ser Glu Glu Asn Glu Glu Glu
                245                 250                 255

Glu Glu Glu Cys Ala Asn Ala Thr Asp Val Thr Thr Thr Pro Ser Val
            260                 265                 270

Gln Tyr Ile Asn Gly Lys His Leu Val Thr Thr Val Pro Lys Asp Pro
        275                 280                 285

Glu Ala Ala Glu
    290
```

<210> SEQ ID NO 25
<211> LENGTH: 293
<212> TYPE: PRT
<213> ORGANISM: Artificial

-continued

<220> FEATURE:
<223> OTHER INFORMATION: C-terminal portion of human versikine including
Linker domain 1, Linker domain 2, and portion of Gag-beta domain
(plus N-terminal methionine)

<400> SEQUENCE: 25

Met Val Val Phe His Tyr Arg Ala Ala Thr Ser Arg Tyr Thr Leu Asn
1 5 10 15

Phe Glu Ala Ala Gln Lys Ala Cys Leu Asp Val Gly Ala Val Ile Ala
20 25 30

Thr Pro Glu Gln Leu Phe Ala Ala Tyr Glu Asp Gly Phe Glu Gln Cys
35 40 45

Asp Ala Gly Trp Leu Ala Asp Gln Thr Val Arg Tyr Pro Ile Arg Ala
50 55 60

Pro Arg Val Gly Cys Tyr Gly Asp Lys Met Gly Lys Ala Gly Val Arg
65 70 75 80

Thr Tyr Gly Phe Arg Ser Pro Gln Glu Thr Tyr Asp Val Tyr Cys Tyr
85 90 95

Val Asp His Leu Asp Gly Asp Val Phe His Leu Thr Val Pro Ser Lys
100 105 110

Phe Thr Phe Glu Glu Ala Ala Lys Glu Cys Glu Asn Gln Asp Ala Arg
115 120 125

Leu Ala Thr Val Gly Glu Leu Gln Ala Ala Trp Arg Asn Gly Phe Asp
130 135 140

Gln Cys Asp Tyr Gly Trp Leu Ser Asp Ala Ser Val Arg His Pro Val
145 150 155 160

Thr Val Ala Arg Ala Gln Cys Gly Gly Gly Leu Leu Gly Val Arg Thr
165 170 175

Leu Tyr Arg Phe Glu Asn Gln Thr Gly Phe Pro Pro Pro Asp Ser Arg
180 185 190

Phe Asp Ala Tyr Cys Phe Lys Arg Arg Met Ser Asp Leu Ser Val Ile
195 200 205

Gly His Pro Ile Asp Ser Glu Ser Lys Glu Asp Glu Pro Cys Ser Glu
210 215 220

Glu Thr Asp Pro Val His Asp Leu Met Ala Glu Ile Leu Pro Glu Phe
225 230 235 240

Pro Asp Ile Ile Glu Ile Asp Leu Tyr His Ser Glu Glu Asn Glu Glu
245 250 255

Glu Glu Glu Glu Cys Ala Asn Ala Thr Asp Val Thr Thr Thr Pro Ser
260 265 270

Val Gln Tyr Ile Asn Gly Lys His Leu Val Thr Thr Val Pro Lys Asp
275 280 285

Pro Glu Ala Ala Glu
290

<210> SEQ ID NO 26
<211> LENGTH: 191
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 26

Asp Val Phe His Leu Thr Val Pro Ser Lys Phe Thr Phe Glu Glu Ala
1 5 10 15

Ala Lys Glu Cys Glu Asn Gln Asp Ala Arg Leu Ala Thr Val Gly Glu
20 25 30

-continued

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Leu Gln Ala Ala Trp Arg Asn Gly Phe Asp Gln Cys Asp Tyr Gly Trp
        35                  40                  45

Leu Ser Asp Ala Ser Val Arg His Pro Val Thr Val Ala Arg Ala Gln
    50                  55                  60

Cys Gly Gly Gly Leu Leu Gly Val Arg Thr Leu Tyr Arg Phe Glu Asn
65                  70                  75                  80

Gln Thr Gly Phe Pro Pro Pro Asp Ser Arg Phe Asp Ala Tyr Cys Phe
                85                  90                  95

Lys Arg Arg Met Ser Asp Leu Ser Val Ile Gly His Pro Ile Asp Ser
            100                 105                 110

Glu Ser Lys Glu Asp Glu Pro Cys Ser Glu Glu Thr Asp Pro Val His
        115                 120                 125

Asp Leu Met Ala Glu Ile Leu Pro Glu Phe Pro Asp Ile Ile Glu Ile
    130                 135                 140

Asp Leu Tyr His Ser Glu Glu Asn Glu Glu Glu Glu Glu Glu Cys Ala
145                 150                 155                 160

Asn Ala Thr Asp Val Thr Thr Thr Pro Ser Val Gln Tyr Ile Asn Gly
                165                 170                 175

Lys His Leu Val Thr Thr Val Pro Lys Asp Pro Glu Ala Ala Glu
            180                 185                 190


<210> SEQ ID NO 27
<211> LENGTH: 192
<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: C-terminal portion of human versikine including
      Linker domain 2 and portion of Gag-beta domain (plus N-terminal
      methionine)

<400> SEQUENCE: 27

Met Asp Val Phe His Leu Thr Val Pro Ser Lys Phe Thr Phe Glu Glu
1               5                   10                  15

Ala Ala Lys Glu Cys Glu Asn Gln Asp Ala Arg Leu Ala Thr Val Gly
            20                  25                  30

Glu Leu Gln Ala Ala Trp Arg Asn Gly Phe Asp Gln Cys Asp Tyr Gly
        35                  40                  45

Trp Leu Ser Asp Ala Ser Val Arg His Pro Val Thr Val Ala Arg Ala
    50                  55                  60

Gln Cys Gly Gly Gly Leu Leu Gly Val Arg Thr Leu Tyr Arg Phe Glu
65                  70                  75                  80

Asn Gln Thr Gly Phe Pro Pro Pro Asp Ser Arg Phe Asp Ala Tyr Cys
                85                  90                  95

Phe Lys Arg Arg Met Ser Asp Leu Ser Val Ile Gly His Pro Ile Asp
            100                 105                 110

Ser Glu Ser Lys Glu Asp Glu Pro Cys Ser Glu Glu Thr Asp Pro Val
        115                 120                 125

His Asp Leu Met Ala Glu Ile Leu Pro Glu Phe Pro Asp Ile Ile Glu
    130                 135                 140

Ile Asp Leu Tyr His Ser Glu Glu Asn Glu Glu Glu Glu Glu Glu Cys
145                 150                 155                 160

Ala Asn Ala Thr Asp Val Thr Thr Thr Pro Ser Val Gln Tyr Ile Asn
                165                 170                 175

Gly Lys His Leu Val Thr Thr Val Pro Lys Asp Pro Glu Ala Ala Glu
            180                 185                 190
```

We claim:

1. A method for inducing and/or potentiating a T-cell mediated immune response in a subject in need thereof, the method comprising administering to the subject a pharmaceutical composition comprising an effective amount of a molecule comprising a versican fragment or variant thereof, wherein the versican fragment or variant thereof consists of the amino acid sequence of SEQ ID NO: 5, SEQ ID NO:6, or SEQ ID NO: 7; and wherein the molecule induces and/or potentiates the T-cell mediated immune response.

2. The method of claim 1, wherein the molecule does not have any chondroitin sulfate side chains.

3. The method of claim 1, wherein administering comprises injecting locally into tumor tissue of the subject the pharmaceutical composition comprising an effective amount of the molecule.

4. A method for inducing and/or potentiating a T-cell mediated immune response in a subject in need thereof, wherein the subject has a cell proliferative disease or disorder, the method comprising administering to the subject a pharmaceutical composition comprising an effective amount of a molecule comprising a versican fragment or variant thereof, wherein the versican fragment or variant thereof consists of the amino acid sequence of SEQ ID NO: 5, SEQ ID NO:6, or SEQ ID NO: 7; and wherein the molecule induces and/or potentiates the T-cell mediated immune response.

5. The method of claim 4, wherein the molecule does not have any chondroitin sulfate side chains.

6. The method of claim 4, wherein administering comprises injecting locally into tumor tissue of the subject the pharmaceutical composition comprising an effective amount of the molecule.

* * * * *