Variable region sequence of murine antibody ABTIM3

Heavy chain (118 aa; SEQ ID NO: 1)

QVQLQQPGAE LVKPGASVKM SCKASGYTFT SYNMHWIKQT PGQGLEWIGD IYPGNGDTSY
NQKFKGKATL TADKSSSTVY MQLSSLTSED SAVYYCARVG GAFPMDTWGQ GTSVTVSS

Light chain (111 aa; SEQ ID NO: 2)

DIVLTQSPAS LAVSLGQRAT ISCRASESVE YYGTSLMQWY QQKPGQPPKL LIYAASNVES
GVPARFSGSG SGTDFSLNIH PVEEDDIAIY FSQQSRKDPS TFGGGTKLEI K

Fig. 1A

Sequence alignment of murine antibody ABTIM3 versus murine germline antibody sequences

Heavy chain (118 aa)

V-gene: 94.1% identity (271/288 nt) with IGHV1-12*01F
J-gene: 90.57% identical (48/53) with IGHJ4*01F
Total of 10 amino acid differences

```
ABTIM3 QVQLQQPGAE LVKPGASVKM SCKASGYTFT SYNMHWIKQT PGQGLEWIGD IYPGNGDTSY
G1     -AY---S--- --R------- ---------- -----V--- -R-------A ----------

ABTIM3 NQKFKGKATL TADKSSSTVY MQLSSLTSED SAVYYCARVG GAFPMDYWGQ GTSVTVSS
G1     ---------- -V------A- ---------- ----F---
```

Light chain (111 aa)

V-gene: 99.66% identical (290/291 nt) with IGKV3-1*01F
J-gene: 97.06% identity with IGKJ1*01F
Total of 2 amino acid differences

```
ABTIM3 DIVLTQSPAS LAVSLGQRAT ISCRASESVE YYGTSLMQWY QQKPGQPPKL LIYAASNVES
g1     ---------- ---------- ---------- --------   ---------- ---------

ABTIM3 GVPARFSGSG SGTDFSLNIH PVEEDDIAIY FCQQSRKDPS TFGGGTKLEI K
G1     ---------- ---------- --------M- ---------- ---------- -
```

Fig.1B

	BIAcore K_D (nM)	TIM-3-300.19 K_D (nM)	Cyno cell K_D (nM)
anti-TIM-3#2	0.459	1.57	7.4
ABTIM3	0.042	0.16	0.68

Fig. 2A

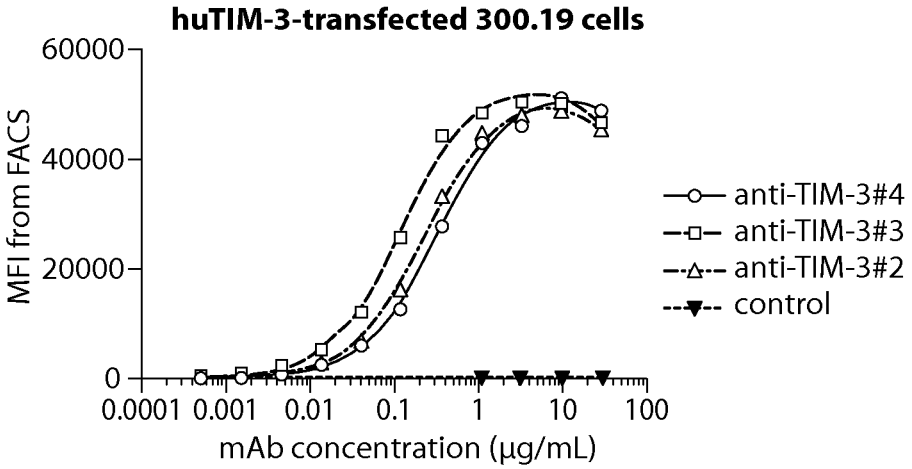


Fig. 2B

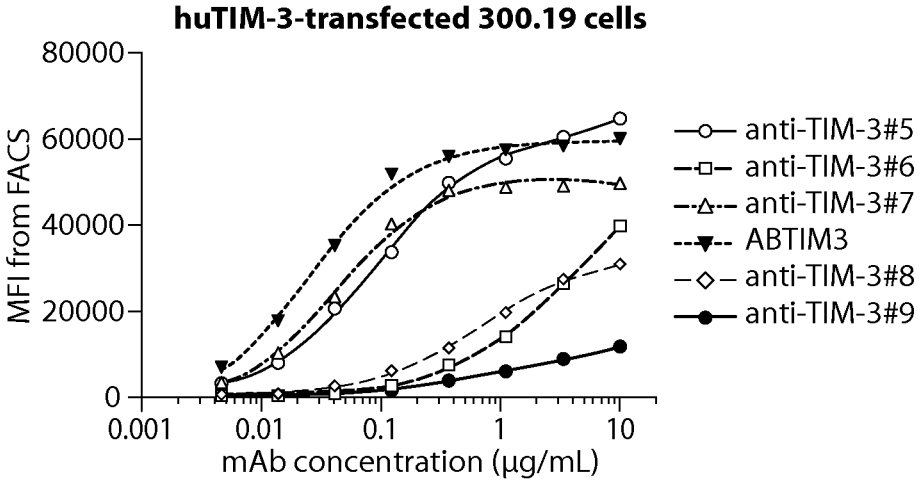


Fig. 2C

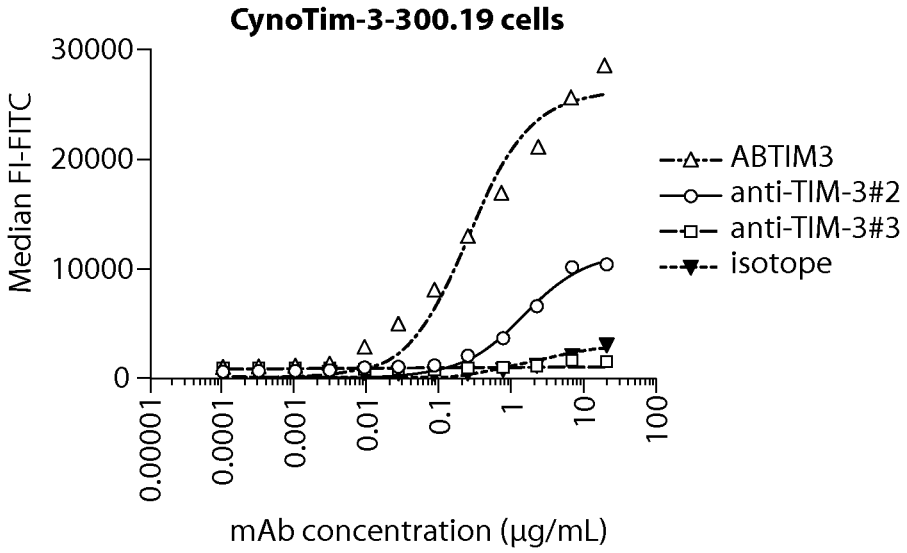

CynoTim-3-300.19 cells
Median FI-FITC
30000
20000
10000
0
0.00001
0.0001
0.001
0.01
0.1
1
10
100
mAb concentration (µg/mL)
ABTIM3
anti-TIM-3#2
anti-TIM-3#3
isotope

Fig. 2D

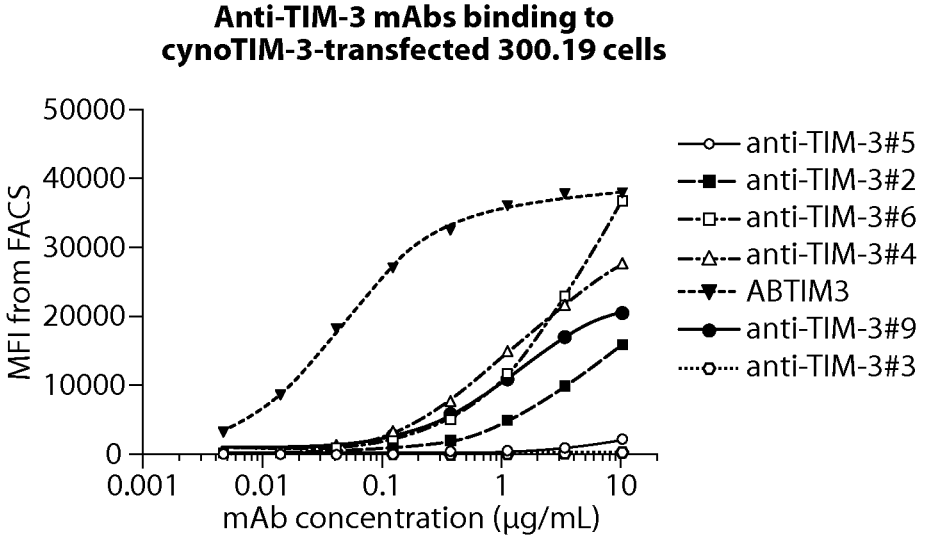

Anti-TIM-3 mAbs binding to
cynoTIM-3-transfected 300.19 cells
MFI from FACS
50000
40000
30000
20000
10000
0
0.001
0.01
0.1
1
10
mAb concentration (µg/mL)
anti-TIM-3#5
anti-TIM-3#2
anti-TIM-3#6
anti-TIM-3#4
ABTIM3
anti-TIM-3#9
anti-TIM-3#3

Fig. 2E

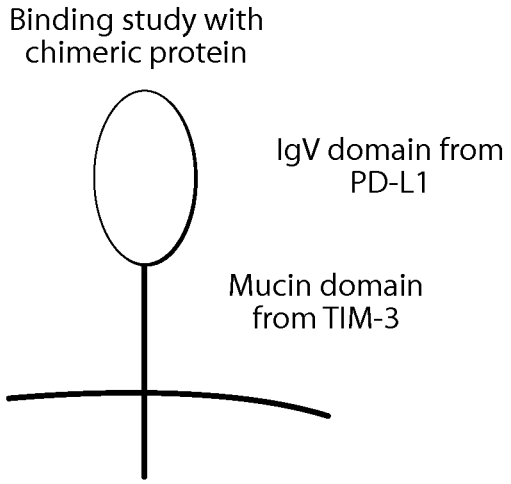

Binding study with chimeric protein
IgV domain from PD-L1
Mucin domain from TIM-3

Fig. 3A

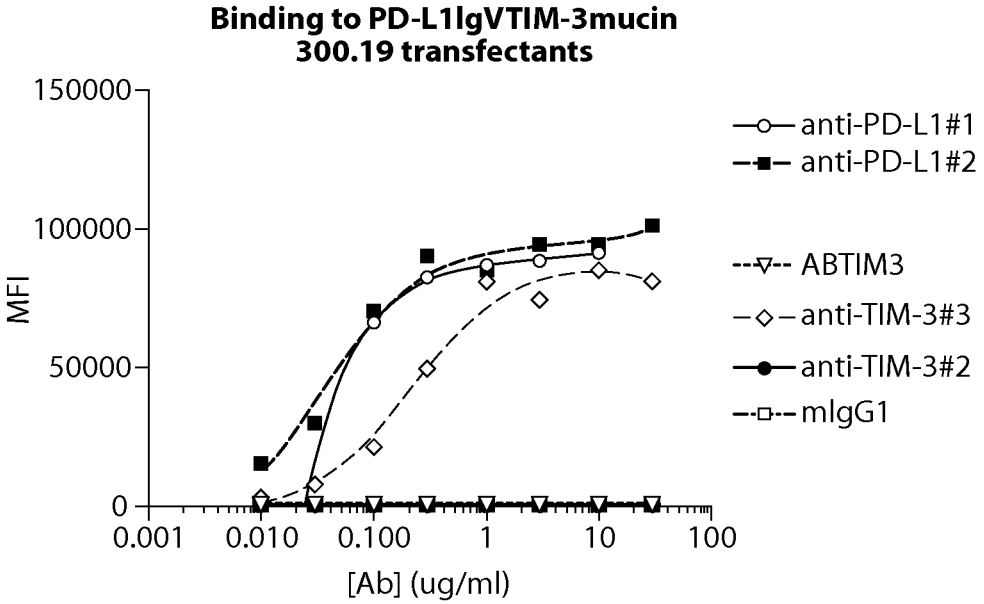

Binding to PD-L1IgVTIM-3mucin 300.19 transfectants
150000
100000
50000
0
MFI
0.001
0.010
0.100
1
10
100
[Ab] (ug/ml)
anti-PD-L1#1
anti-PD-L1#2
ABTIM3
anti-TIM-3#3
anti-TIM-3#2
mIgG1

Fig. 3B

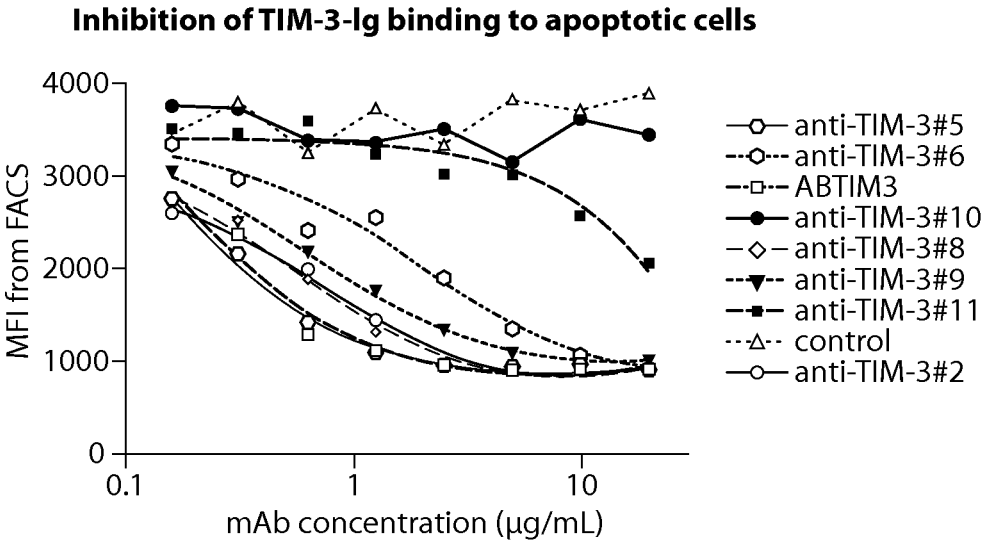

Inhibition of TIM-3-Ig binding to apoptotic cells
MFI from FACS
4000
3000
2000
1000
0
0.1
1
10
mAb concentration (µg/mL)
anti-TIM-3#5
anti-TIM-3#6
ABTIM3
anti-TIM-3#10
anti-TIM-3#8
anti-TIM-3#9
anti-TIM-3#11
control
anti-TIM-3#2

Fig. 4

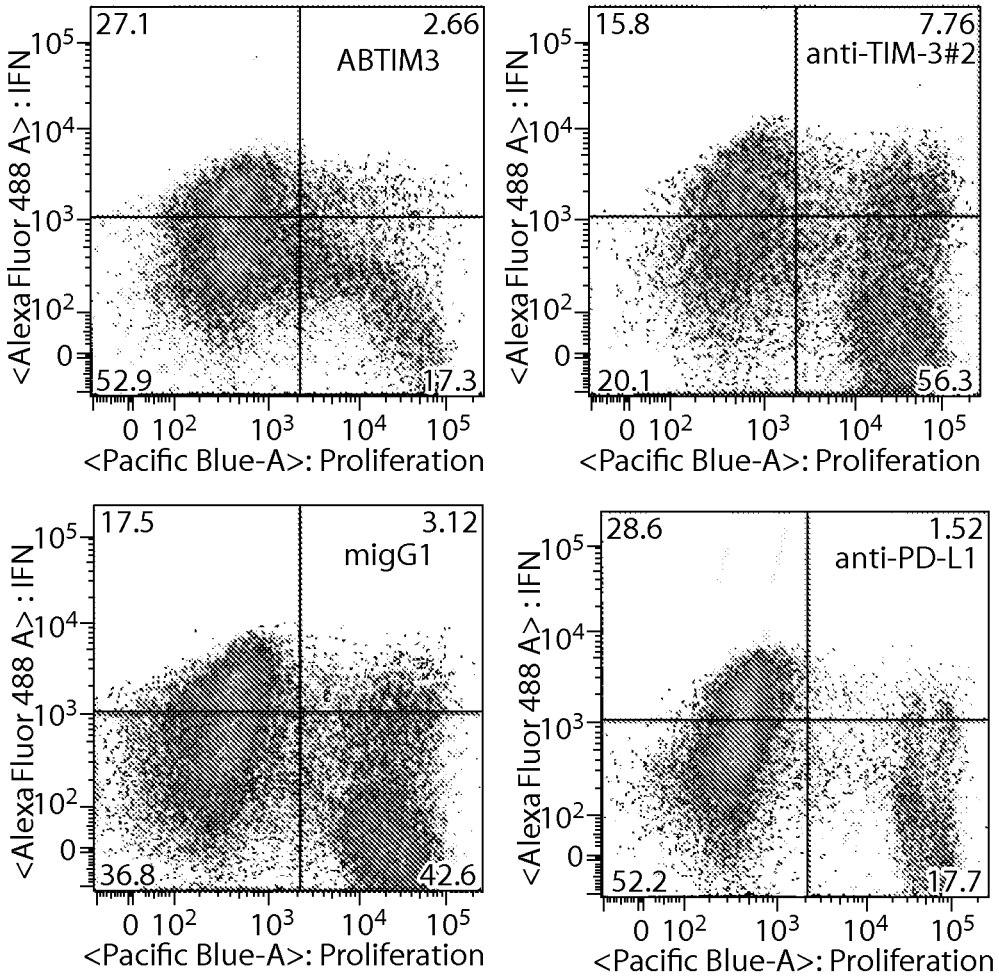

27.1
2.66
ABTIM3
52.9
17.3
15.8
7.76
anti-TIM-3#2
20.1
56.3
17.5
3.12
migG1
36.8
42.6
28.6
1.52
anti-PD-L1
52.2
17.7
<Alexa Fluor 488 A>: IFN
<Pacific Blue-A>: Proliferation

Fig. 5A

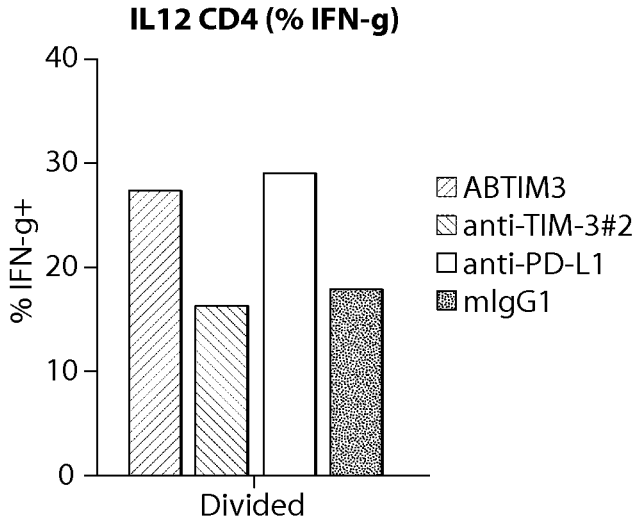

IL12 CD4 (% IFN-g)
% IFN-g+
Divided
ABTIM3
anti-TIM-3#2
anti-PD-L1
mIgG1

Fig. 5B

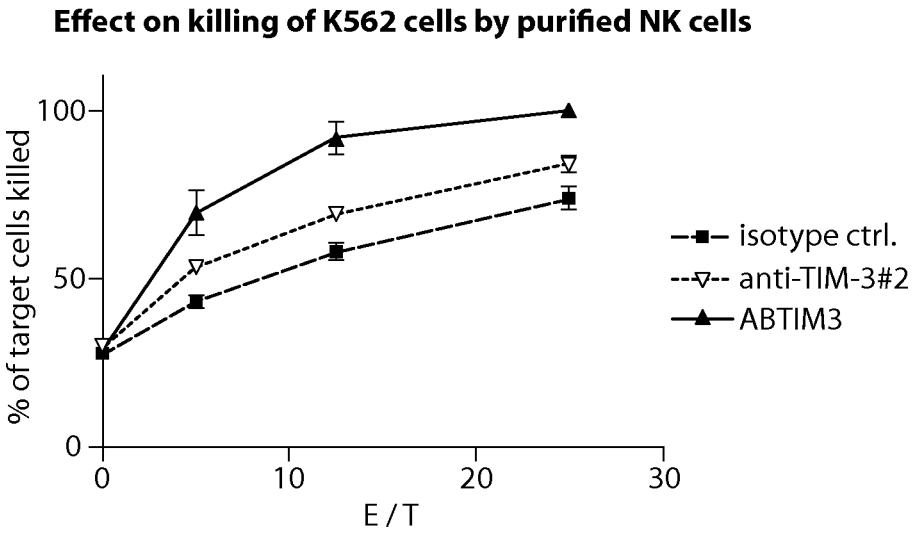

Effect on killing of K562 cells by purified NK cells
% of target cells killed
100
50
0
0
10
20
30
E / T
isotype ctrl.
anti-TIM-3#2
ABTIM3

Fig. 6

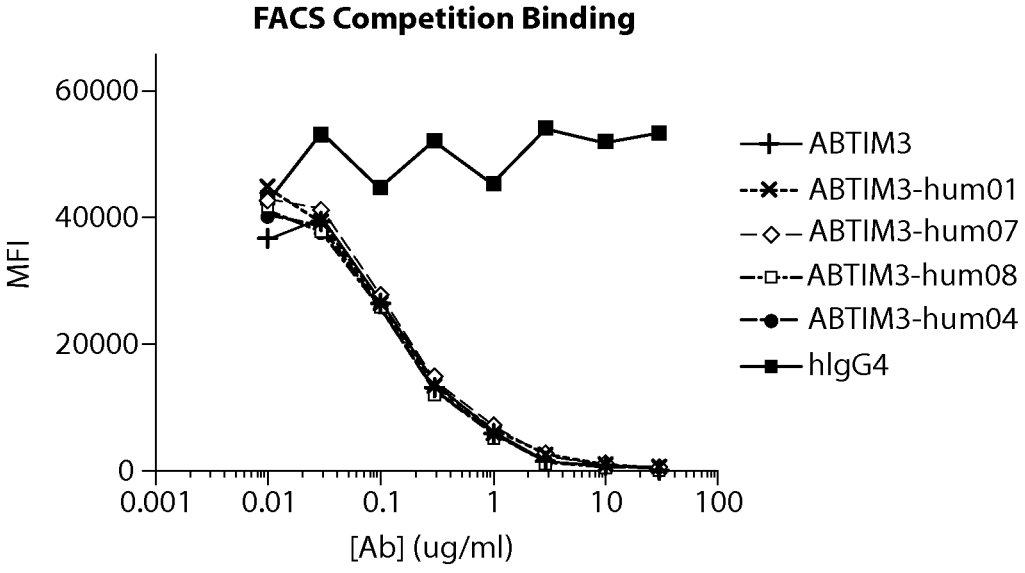

FACS Competition Binding
MFI
60000
40000
20000
0
0.001
0.01
0.1
1
10
100
[Ab] (ug/ml)
ABTIM3
ABTIM3-hum01
ABTIM3-hum07
ABTIM3-hum08
ABTIM3-hum04
hIgG4

Fig. 7

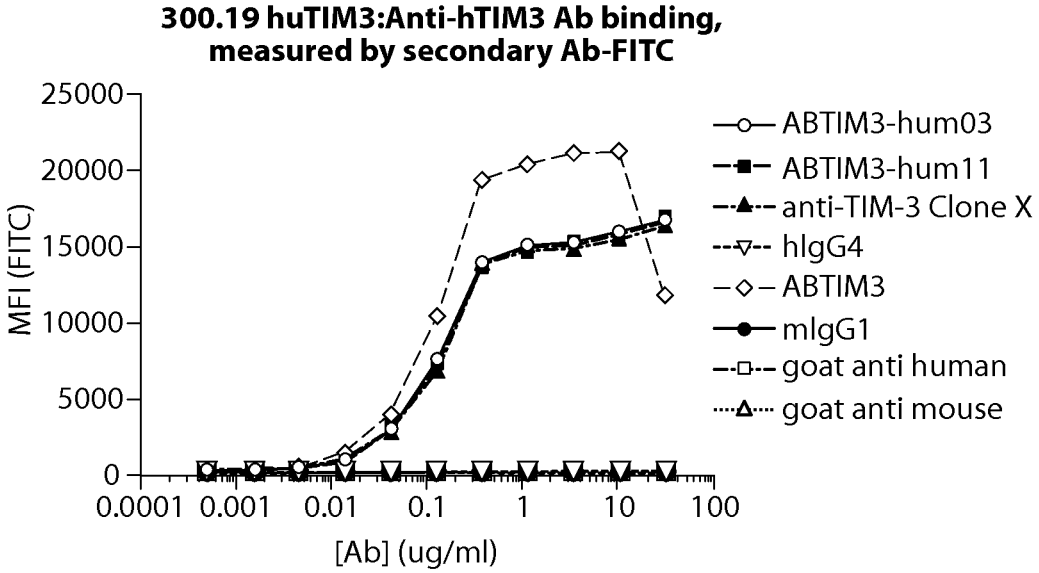

300.19 huTIM3:Anti-hTIM3 Ab binding, measured by secondary Ab-FITC
MFI (FITC)
25000
20000
15000
10000
5000
0
0.0001
0.001
0.01
0.1
1
10
100
[Ab] (ug/ml)
ABTIM3-hum03
ABTIM3-hum11
anti-TIM-3 Clone X
hIgG4
ABTIM3
mIgG1
goat anti human
goat anti mouse

Fig. 8A

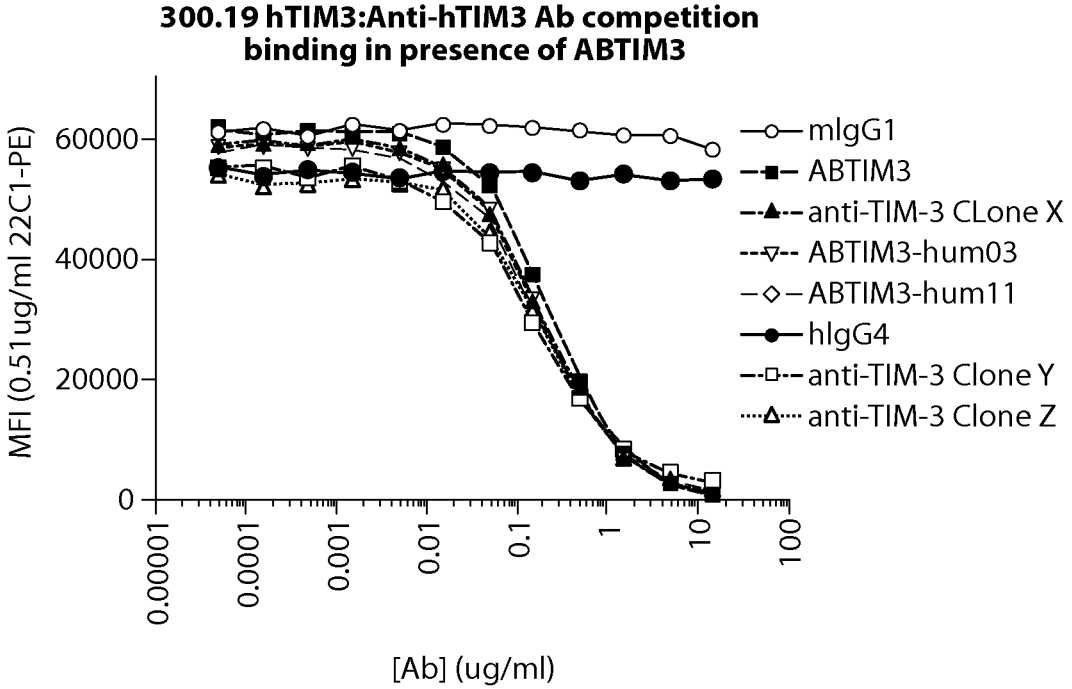

300.19 hTIM3:Anti-hTIM3 Ab competition binding in presence of ABTIM3
MFI (0.51ug/ml 22C1-PE)
60000
40000
20000
0
0.00001
0.0001
0.001
0.01
0.1
1
10
100
[Ab] (ug/ml)
mIgG1
ABTIM3
anti-TIM-3 CLone X
ABTIM3-hum03
ABTIM3-hum11
hIgG4
anti-TIM-3 Clone Y
anti-TIM-3 Clone Z

Fig. 8B

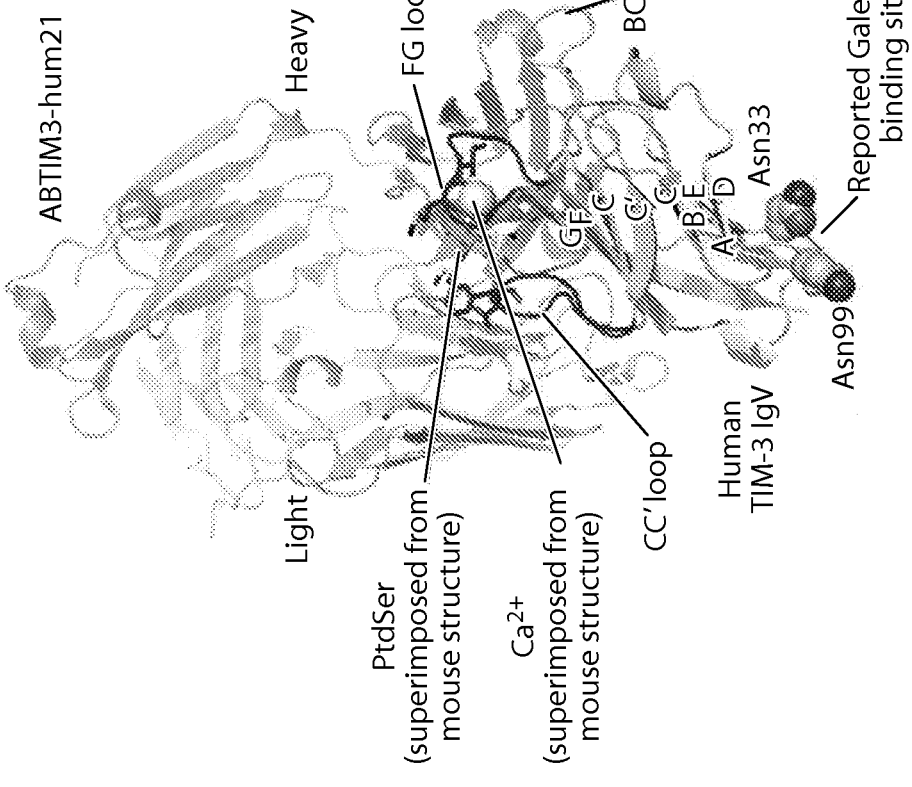

ABTIM3-hum21
Heavy
Light
FG loop
BC loop
PtdSer
(superimposed from
mouse structure)
Ca2+
(superimposed from
mouse structure)
CC' loop
Human
TIM-3 IgV
Asn33
Asn99
Reported Galectin-9
binding sites

Fig. 9A

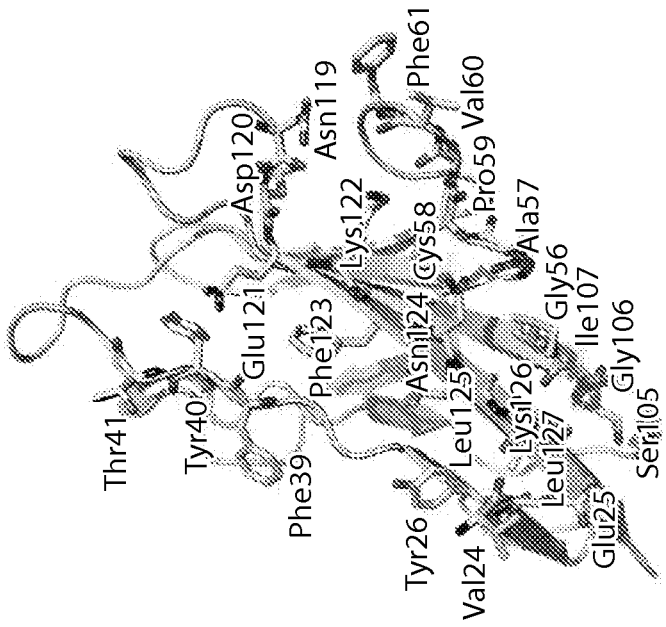
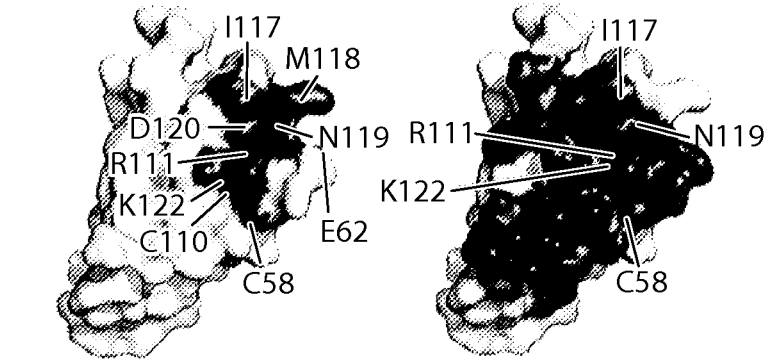

Thr41
Tyr40
Glu121
Asp120
Asn119
Phe39
Phe123
Lys122
Phe61
Val60
Pro59
Cys58
Ala57
Gly56
Ile107
Gly106
Ser105
Asn124
Leu125
Lys126
Leu127
Glu25
Tyr26
Val24

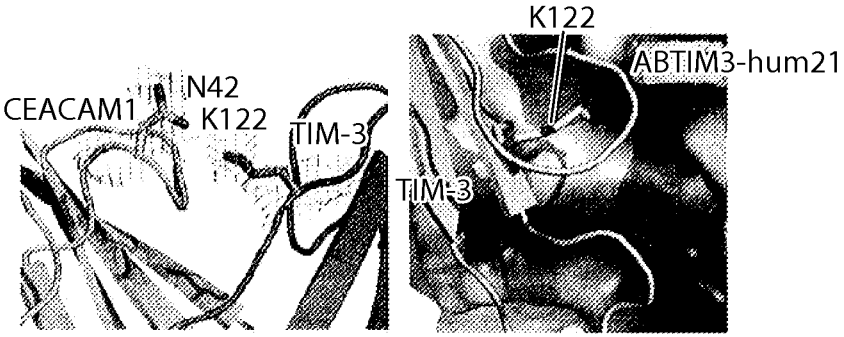
Fig. 9B



CEACAM1-binding residues on human TIM-3

ABTIM3-hum21 epitope on human TIM-3

Fig. 10A



K122 of human TIM-3 forms hydrogen bond with CEACAM1 (Huang *et al.*, (2014) Nature)

K122 of human TIM-3 is completely blocked by ABTIM3-hum21

Fig. 10B

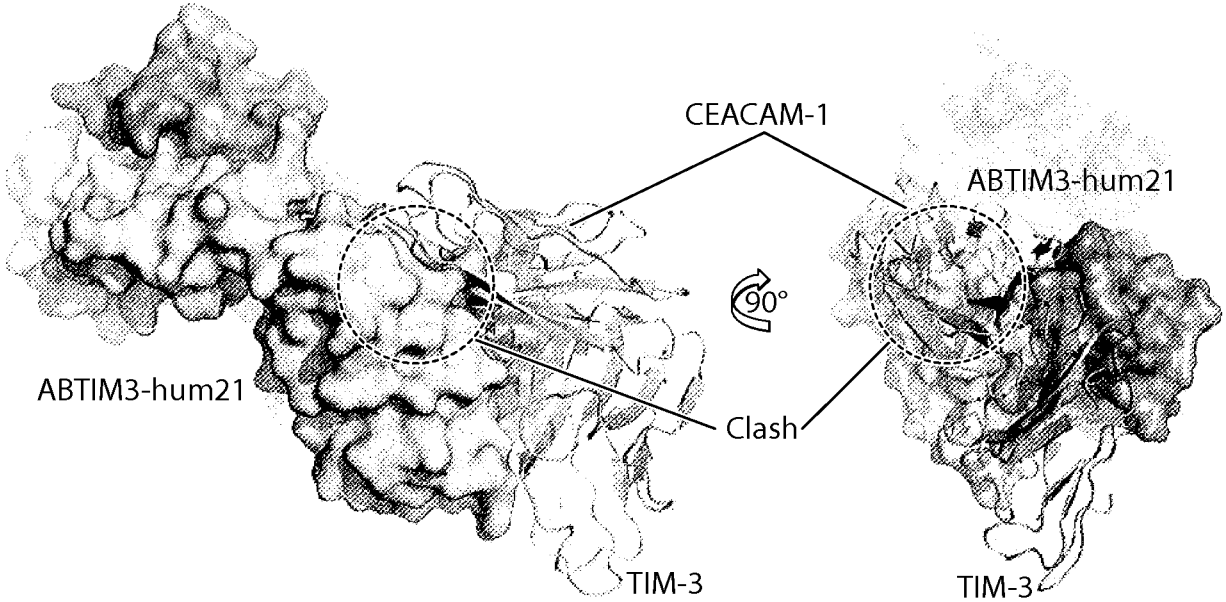
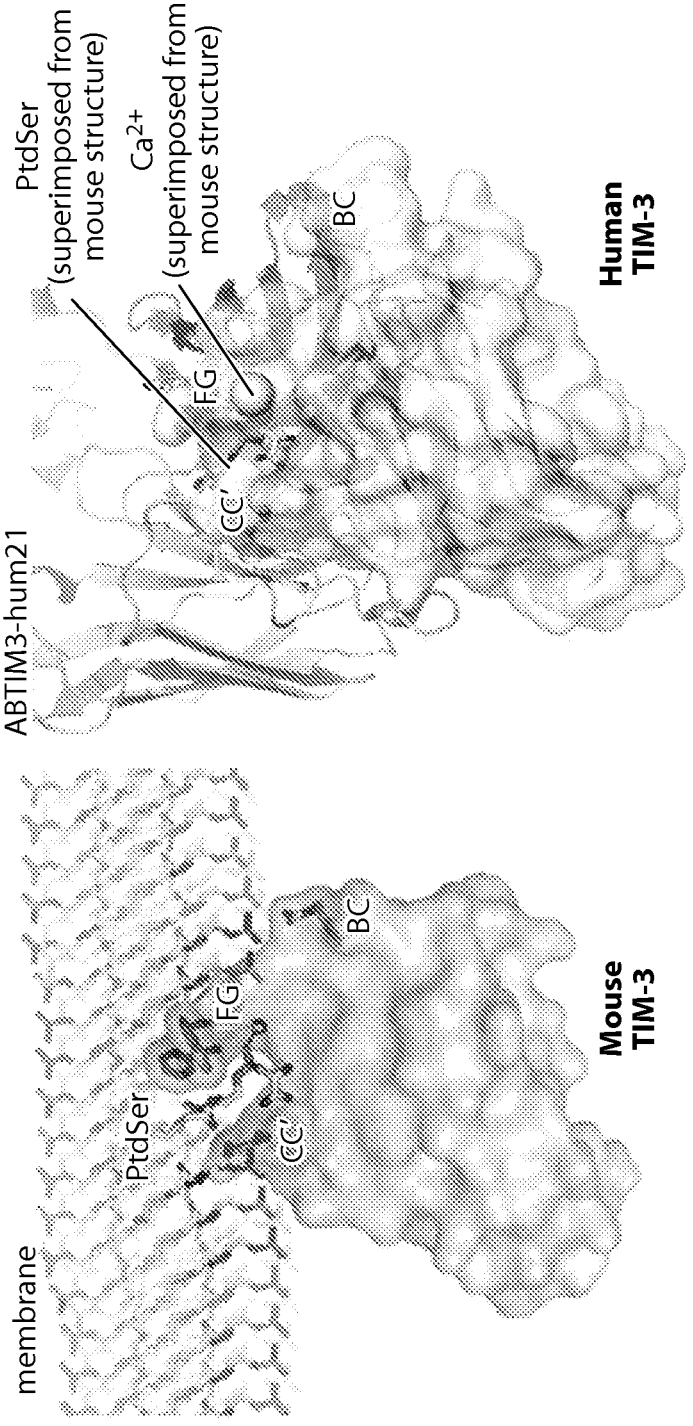


Fig. 10C



DeKruyff, *et al.*, (2010) J Immunol. 184(4):1918-1930

Fig. 11

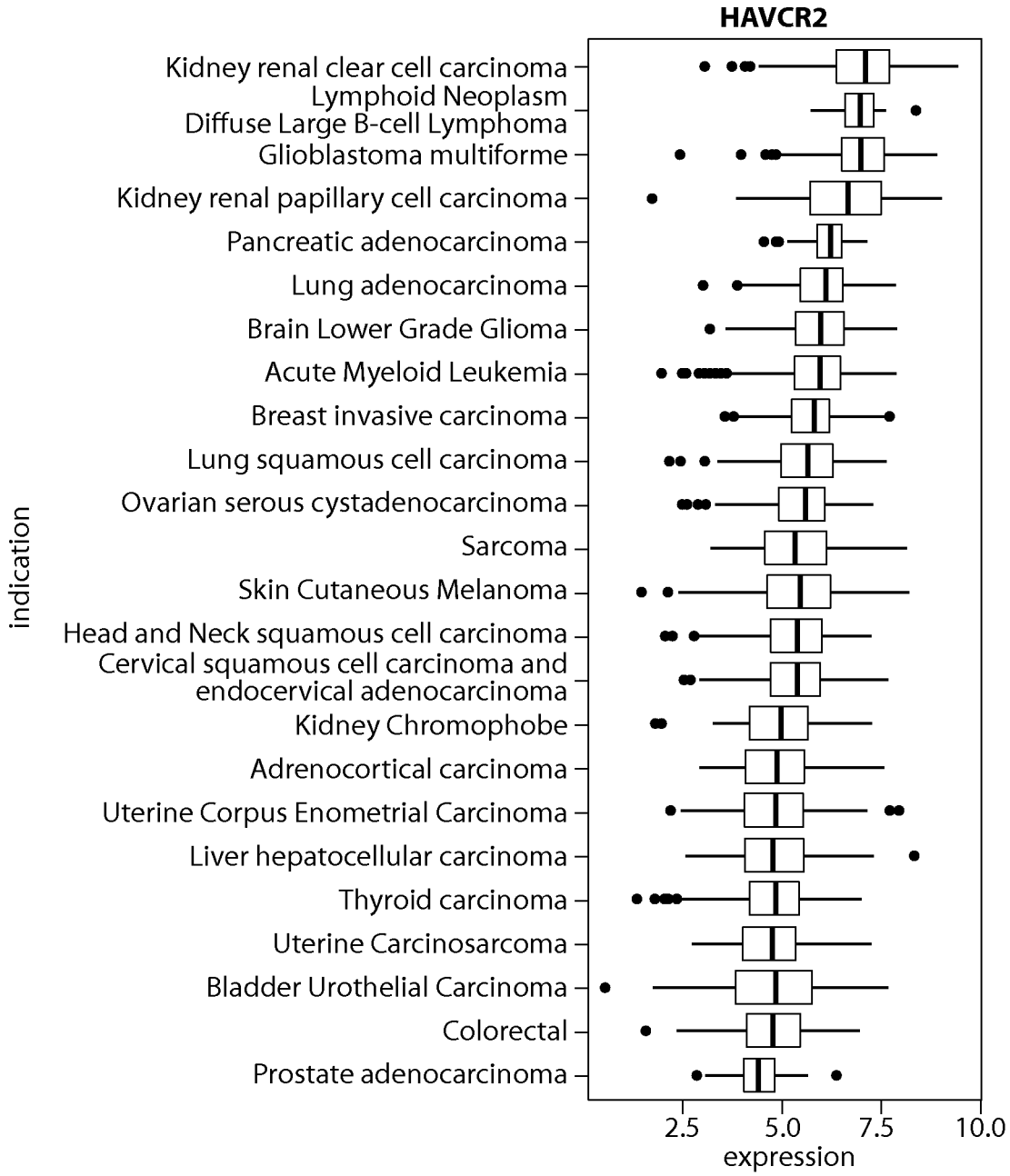

HAVCR2
indication
Kidney renal clear cell carcinoma
Lymphoid Neoplasm Diffuse Large B-cell Lymphoma
Glioblastoma multiforme
Kidney renal papillary cell carcinoma
Pancreatic adenocarcinoma
Lung adenocarcinoma
Brain Lower Grade Glioma
Acute Myeloid Leukemia
Breast invasive carcinoma
Lung squamous cell carcinoma
Ovarian serous cystadenocarcinoma
Sarcoma
Skin Cutaneous Melanoma
Head and Neck squamous cell carcinoma
Cervical squamous cell carcinoma and endocervical adenocarcinoma
Kidney Chromophobe
Adrenocortical carcinoma
Uterine Corpus Enometrial Carcinoma
Liver hepatocellular carcinoma
Thyroid carcinoma
Uterine Carcinosarcoma
Bladder Urothelial Carcinoma
Colorectal
Prostate adenocarcinoma
2.5
5.0
7.5
10.0
expression

Fig. 12

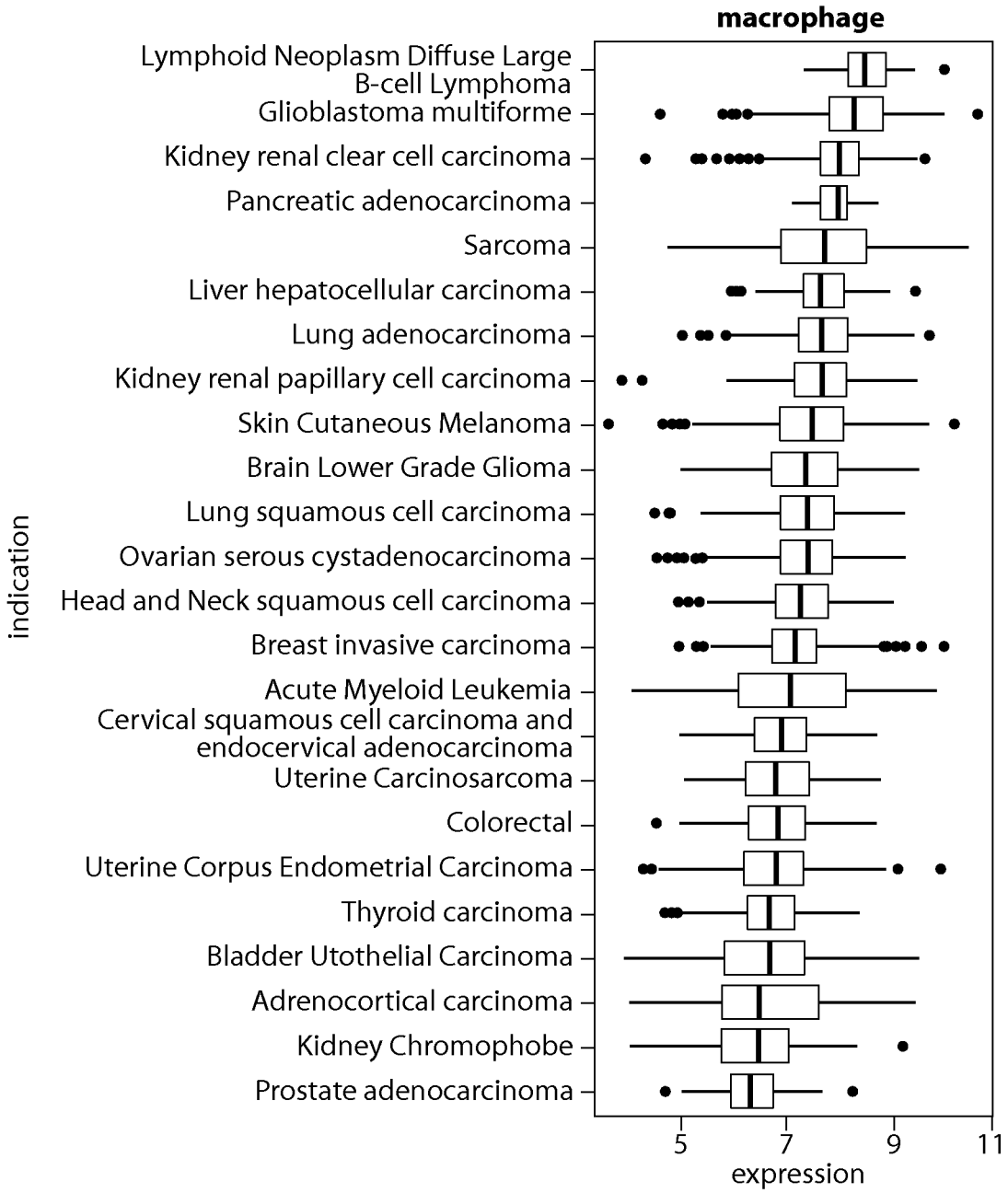

macrophage
Lymphoid Neoplasm Diffuse Large B-cell Lymphoma
Glioblastoma multiforme
Kidney renal clear cell carcinoma
Pancreatic adenocarcinoma
Sarcoma
Liver hepatocellular carcinoma
Lung adenocarcinoma
Kidney renal papillary cell carcinoma
Skin Cutaneous Melanoma
Brain Lower Grade Glioma
Lung squamous cell carcinoma
Ovarian serous cystadenocarcinoma
Head and Neck squamous cell carcinoma
Breast invasive carcinoma
Acute Myeloid Leukemia
Cervical squamous cell carcinoma and endocervical adenocarcinoma
Uterine Carcinosarcoma
Colorectal
Uterine Corpus Endometrial Carcinoma
Thyroid carcinoma
Bladder Utothelial Carcinoma
Adrenocortical carcinoma
Kidney Chromophobe
Prostate adenocarcinoma
indication
5
7
9
11
expression

Fig. 13

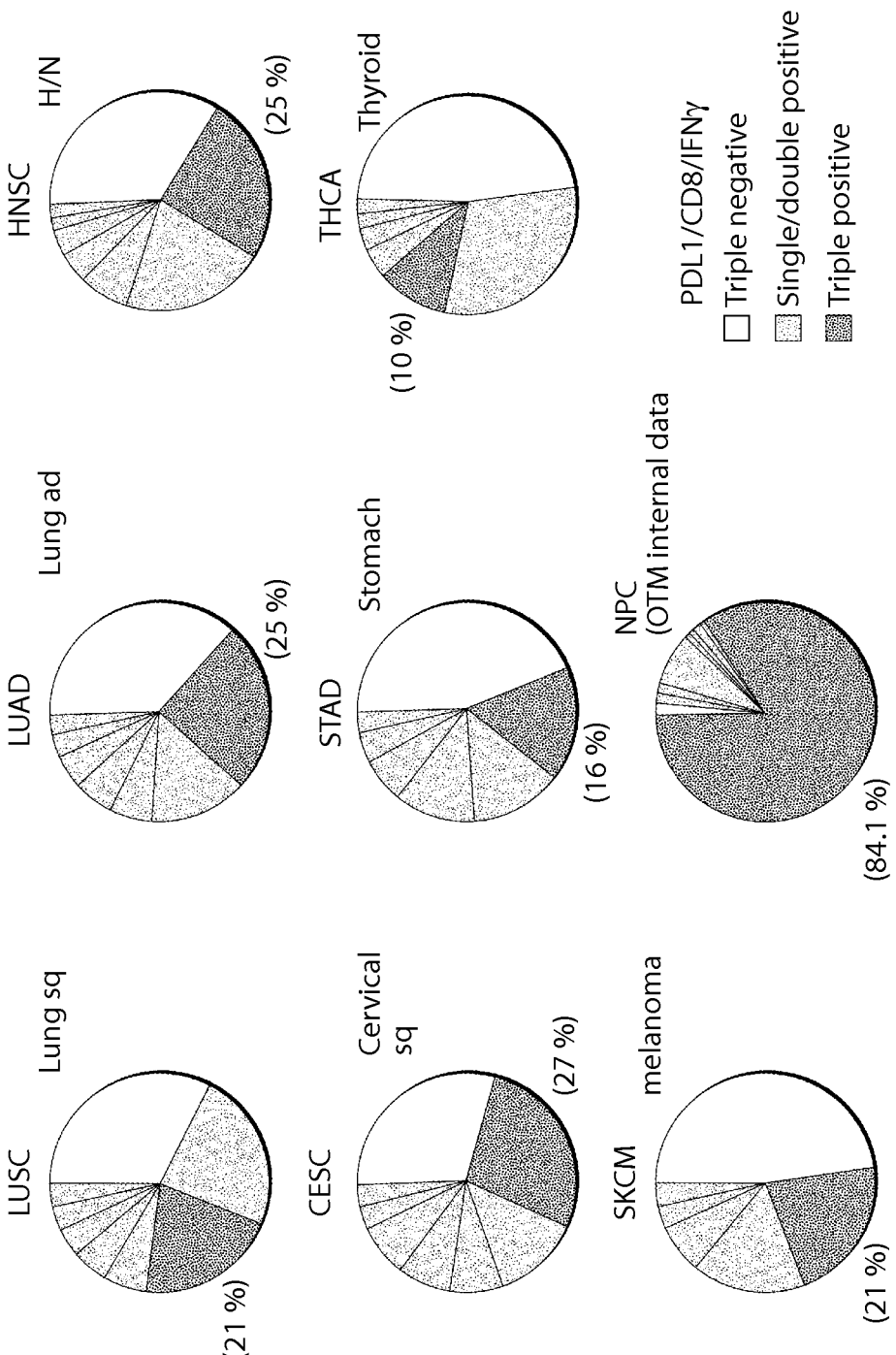

HNSC
H/N
(25 %)
THCA
Thyroid
(10 %)
LUAD
Lung ad
(25 %)
STAD
Stomach
(16 %)
NPC
(OTM internal data
(84.1 %)
LUSC
Lung sq
(21 %)
CESC
Cervical sq
(27 %)
SKCM
melanoma
(21 %)
PDL1/CD8/IFNγ
Triple negative
Single/double positive
Triple positive

Fig. 14

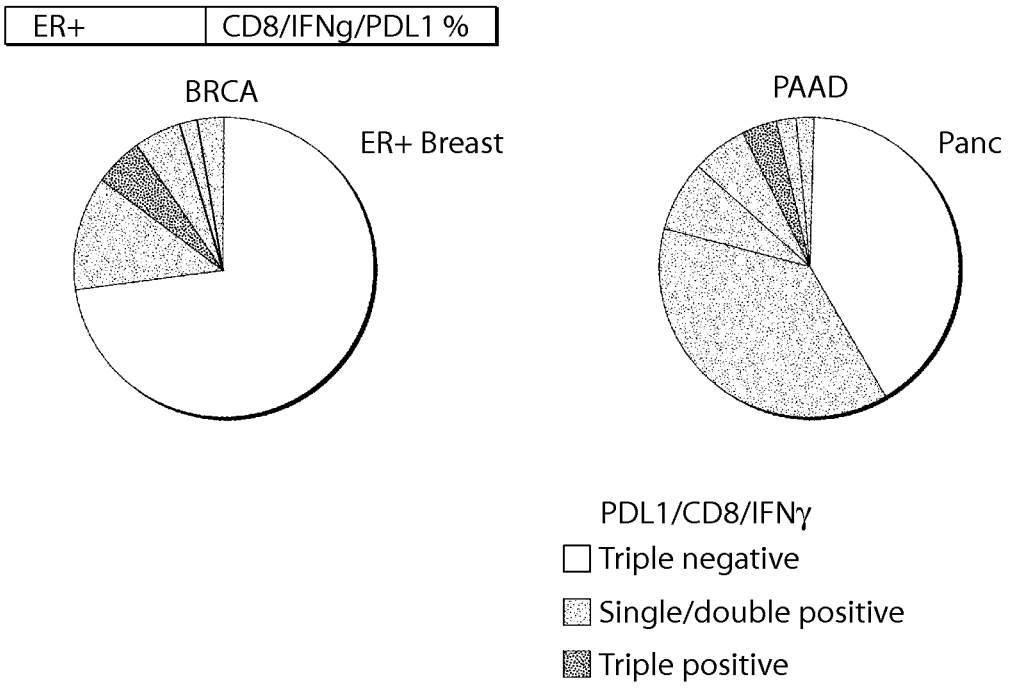

ER+
CD8/IFNg/PDL1 %
BRCA
ER+ Breast
PAAD
Panc
PDL1/CD8/IFNγ
Triple negative
Single/double positive
Triple positive

Fig. 15

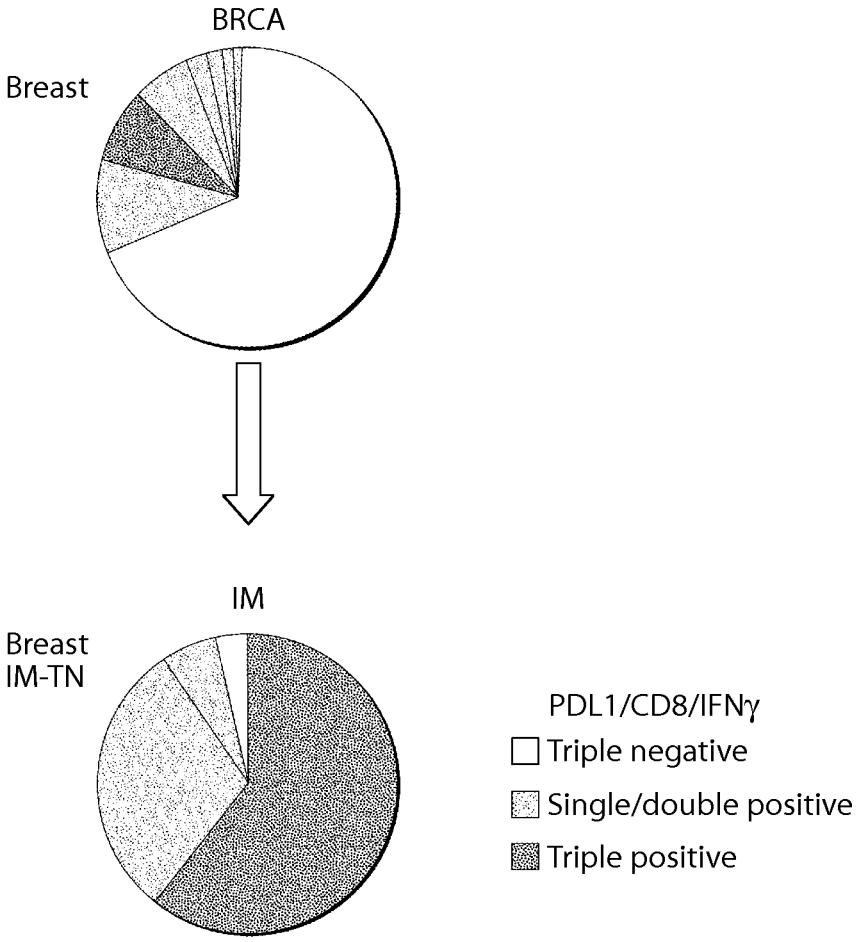

BRCA
Breast
IM
Breast
IM-TN
PDL1/CD8/IFNγ
Triple negative
Single/double positive
Triple positive

Fig. 16

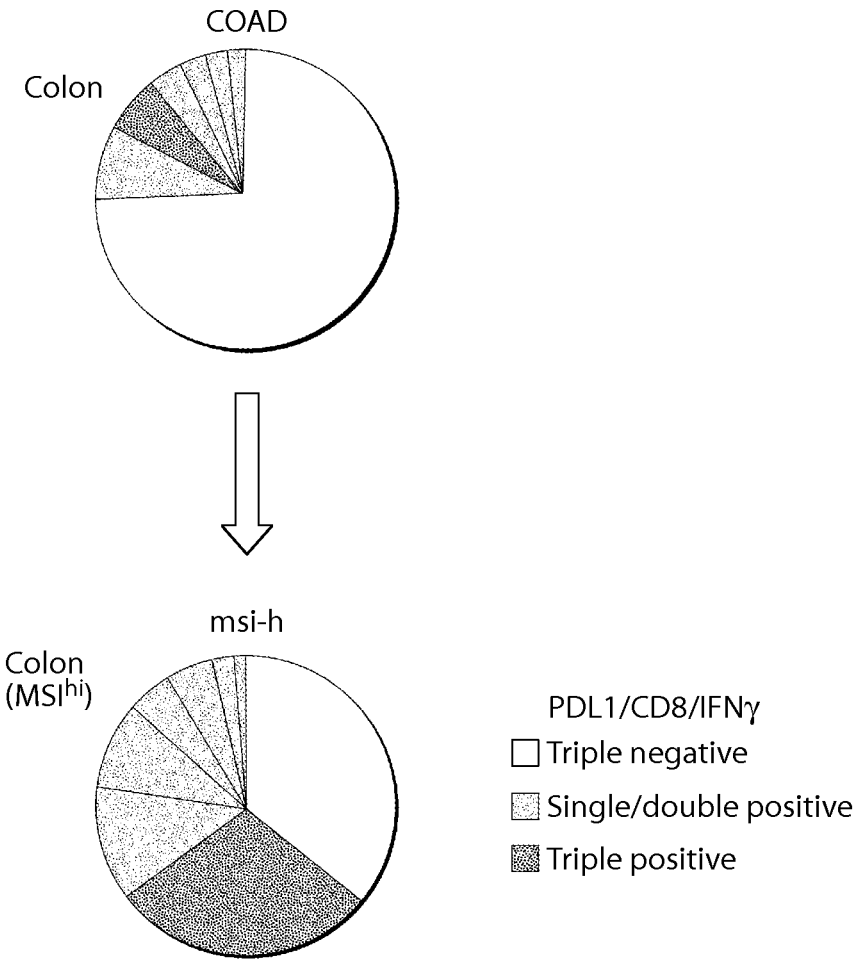

COAD
Colon
msi-h
Colon
(MSI^hi)
PDL1/CD8/IFNγ
Triple negative
Single/double positive
Triple positive

Fig. 17

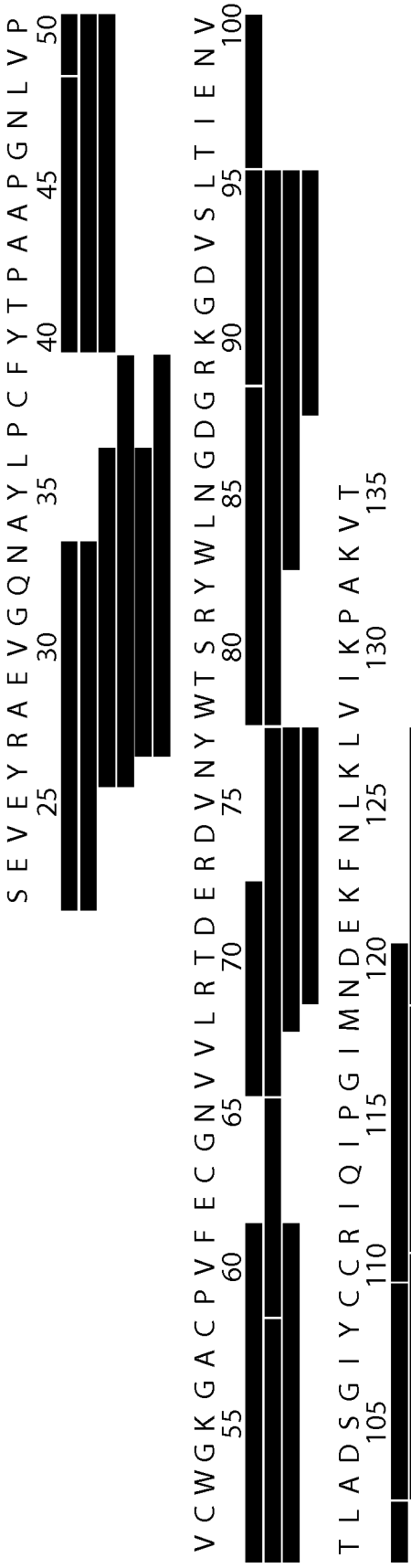

S E V E Y R A E V G Q N A Y L P C F Y T P A A P G N L V P
25 30 35 40 45 50
V C W G K G A C P V F E C G N V V L R T D E R D V N Y W T S R Y W L N G D G R K G D V S L T I E N V
55 60 65 70 75 80 85 90 95 100
T L A D S G I Y C C R I Q I P G I M N D E K F N L K L V I K P A K V T
105 110 115 120 125 130 135

Fig. 18

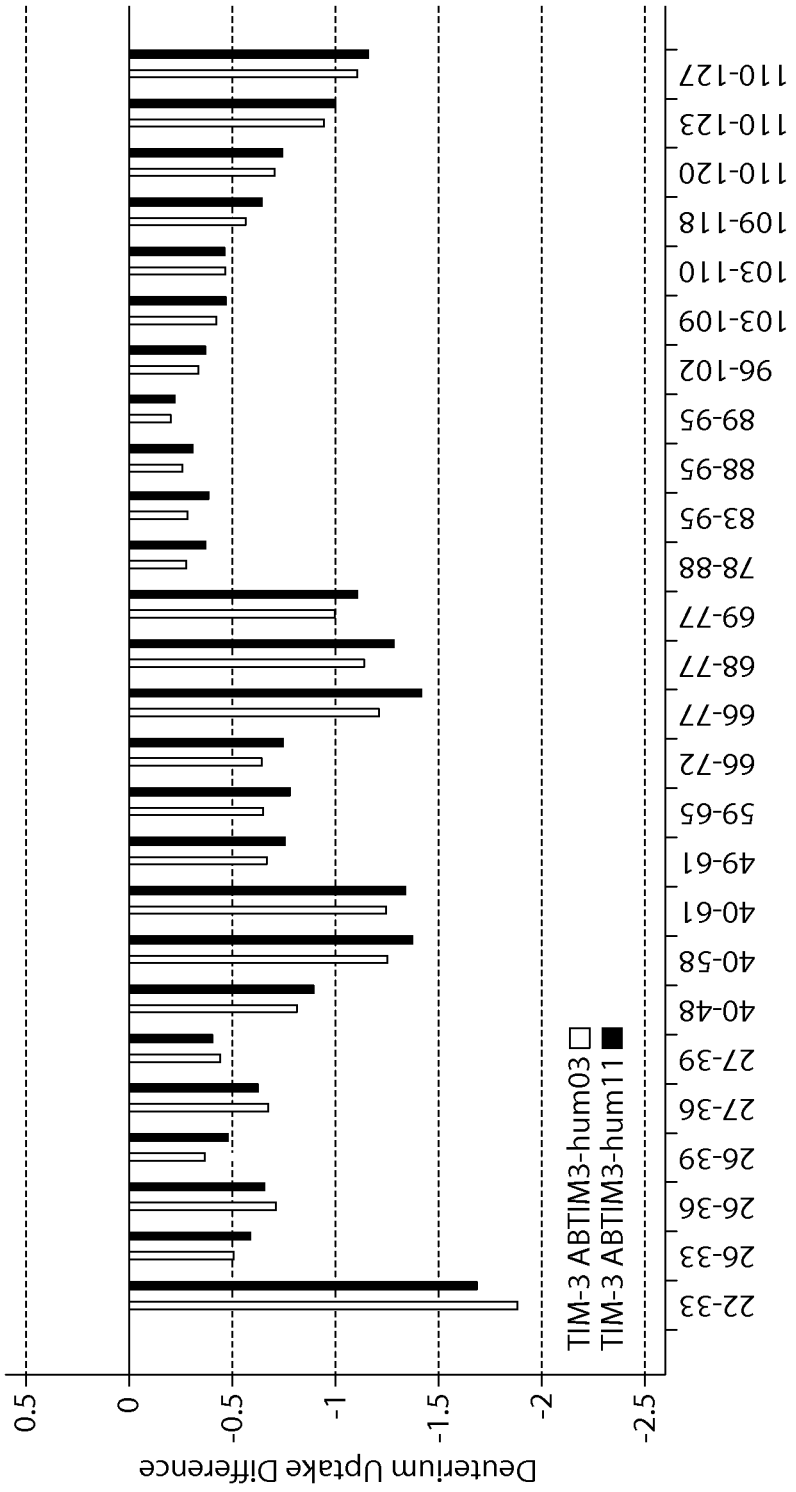

Deuterium Uptake Difference
0.5
0
-0.5
-1
-1.5
-2
-2.5
TIM-3 ABTIM3-hum03 □
TIM-3 ABTIM3-hum11 ■
Peptide ID (Uniprot Numbering)
22-33
26-33
26-36
26-39
27-36
27-39
40-48
40-58
40-61
49-61
59-65
66-72
66-77
68-77
69-77
78-88
83-95
88-95
89-95
96-102
103-109
103-110
109-118
110-120
110-123
110-127

Fig. 19

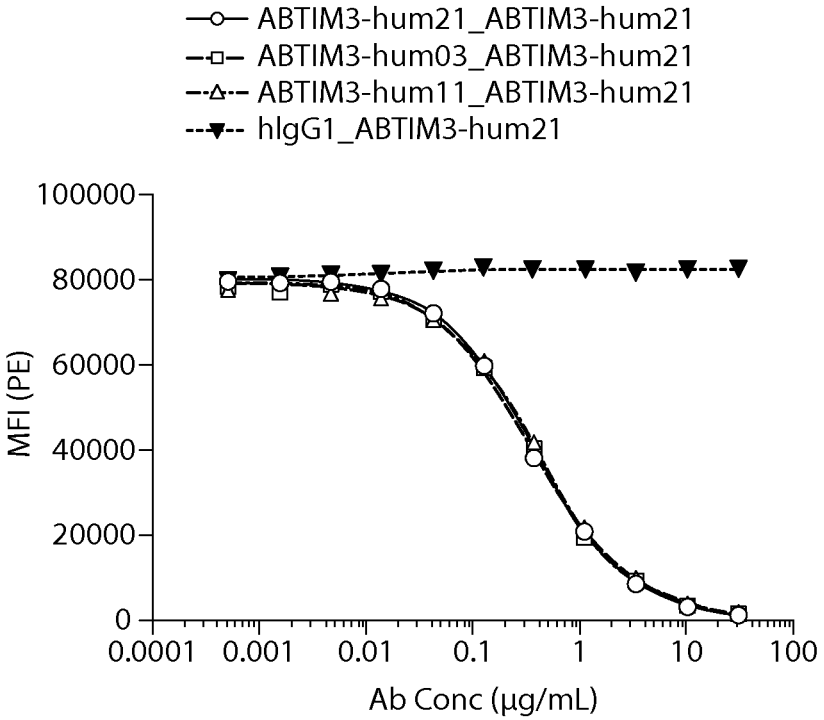

ABTIM3-hum21_ABTIM3-hum21
ABTIM3-hum03_ABTIM3-hum21
ABTIM3-hum11_ABTIM3-hum21
hIgG1_ABTIM3-hum21
100000
80000
60000
40000
20000
0
MFI (PE)
0.0001
0.001
0.01
0.1
1
10
100
Ab Conc (µg/mL)

Fig. 20

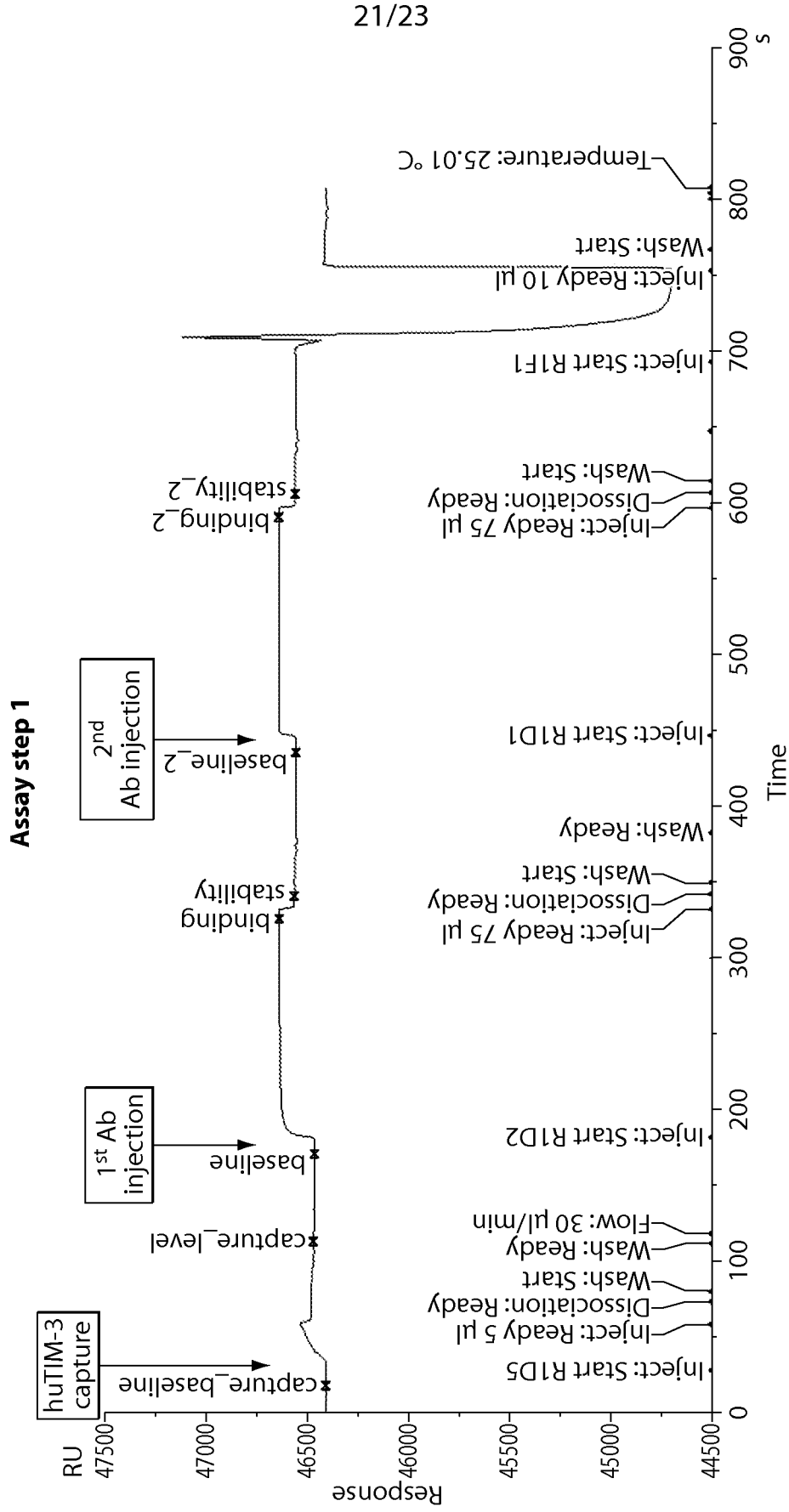

Assay step 1
huTIM-3 capture
capture_baseline
capture_level
1st Ab injection
baseline
binding
stability
2nd Ab injection
baseline_2
binding_2
stability_2
Response
RU
47500
47000
46500
46000
45500
45000
44500
0
100
200
300
400
500
600
700
800
900
s
Time
Inject: Start R1D5
Inject: Ready 5 µl
Dissociation: Ready
Wash: Start
Wash: Ready
Flow: 30 µl/min
Inject: Start R1D2
Inject: Ready 75 µl
Dissociation: Ready
Wash: Start
Wash: Ready
Inject: Start R1D1
Inject: Ready 75 µl
Dissociation: Ready
Wash: Start
Inject: Start R1F1
Inject: Ready 10 µl
Wash: Start
Temperature: 25.01 °C

Fig. 21

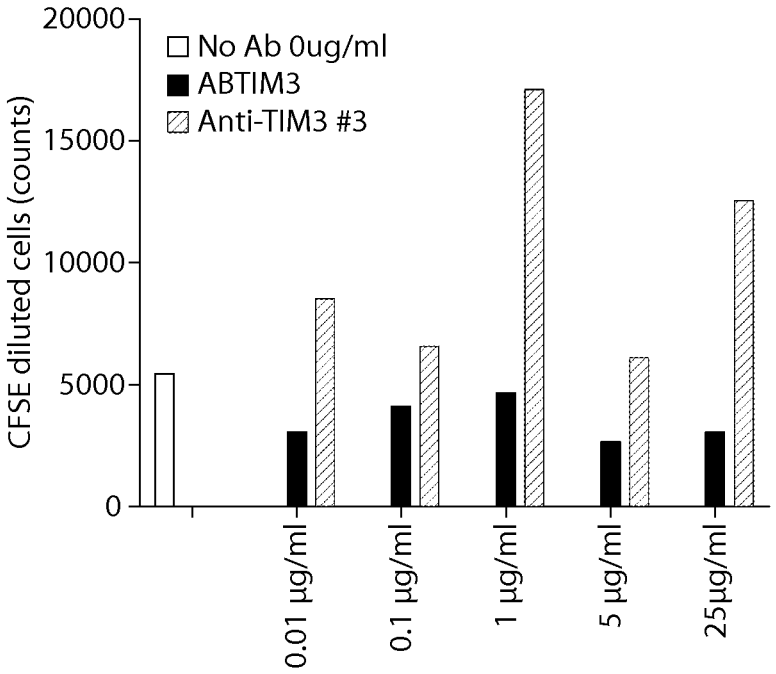

20000
15000
10000
5000
0
CFSE diluted cells (counts)
No Ab 0ug/ml
ABTIM3
Anti-TIM3 #3
0.01 µg/ml
0.1 µg/ml
1 µg/ml
5 µg/ml
25µg/ml

Fig. 22

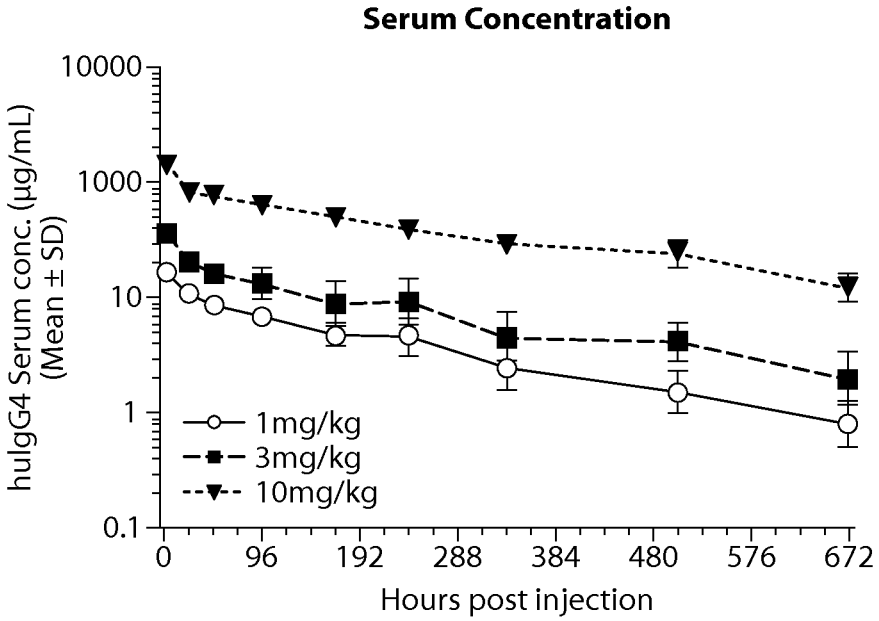


Fig. 23A

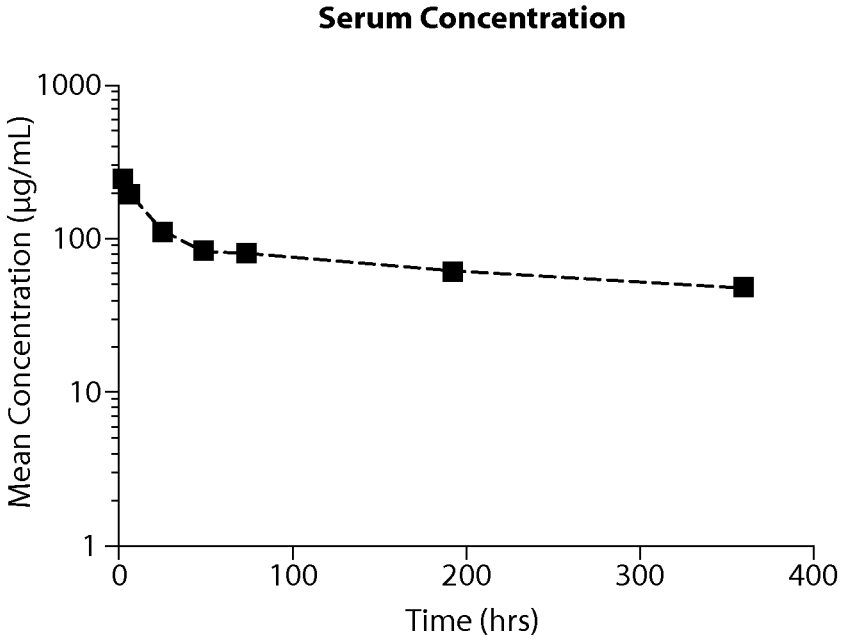


Fig. 23B