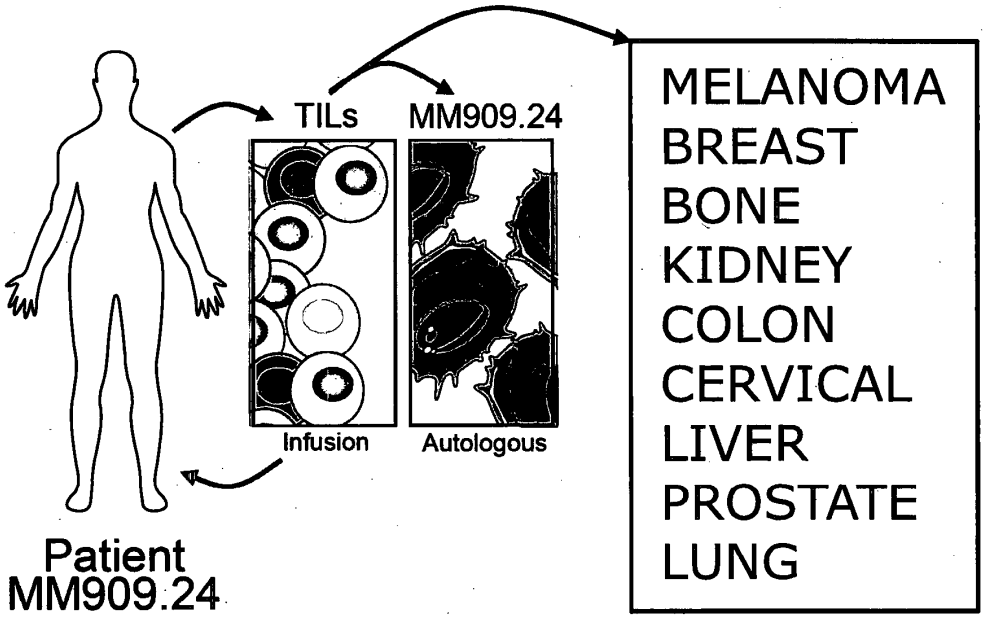


Figure 1A

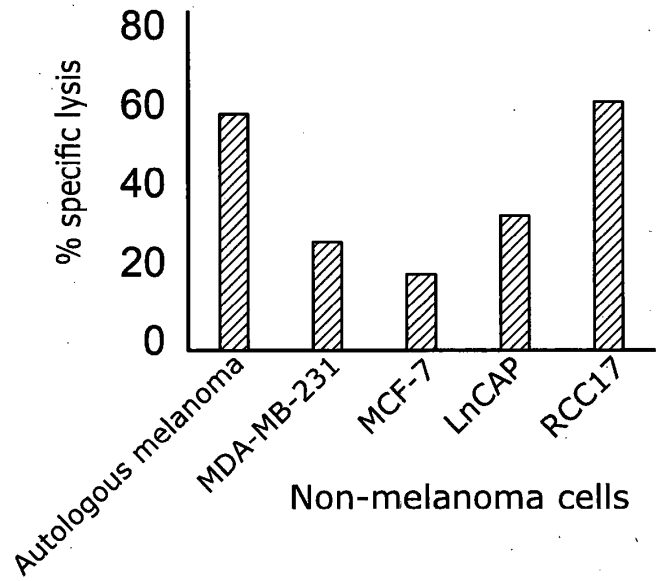


Figure 1B

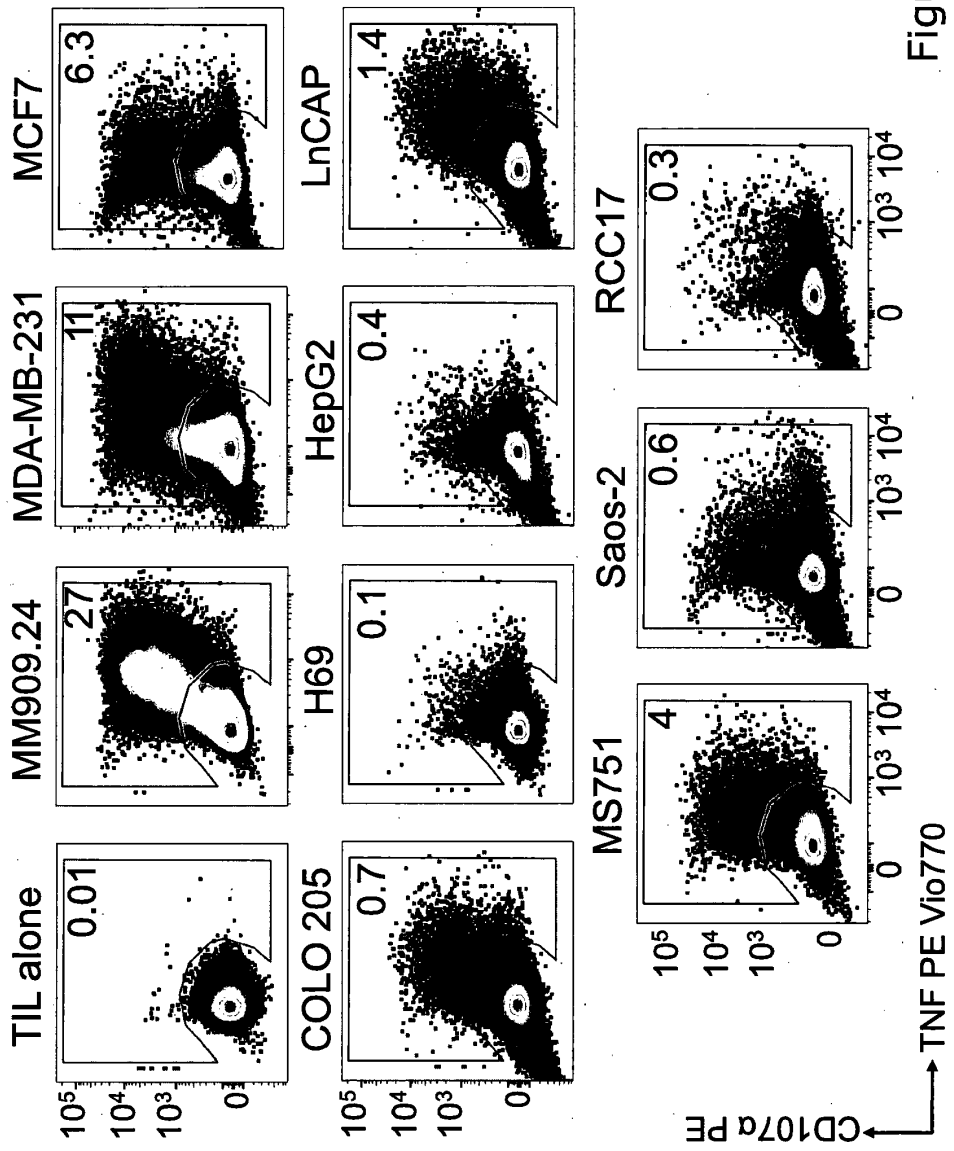

TIL alone
0.01
MM909.24
27
MDA-MB-231
11
MCF7
6.3
COLO 205
0.7
H69
0.1
HepG2
0.4
LnCAP
1.4
MS751
4
Saos-2
0.6
RCC17
0.3
CD107a PE
TNF PE Vio770

Figure 1C

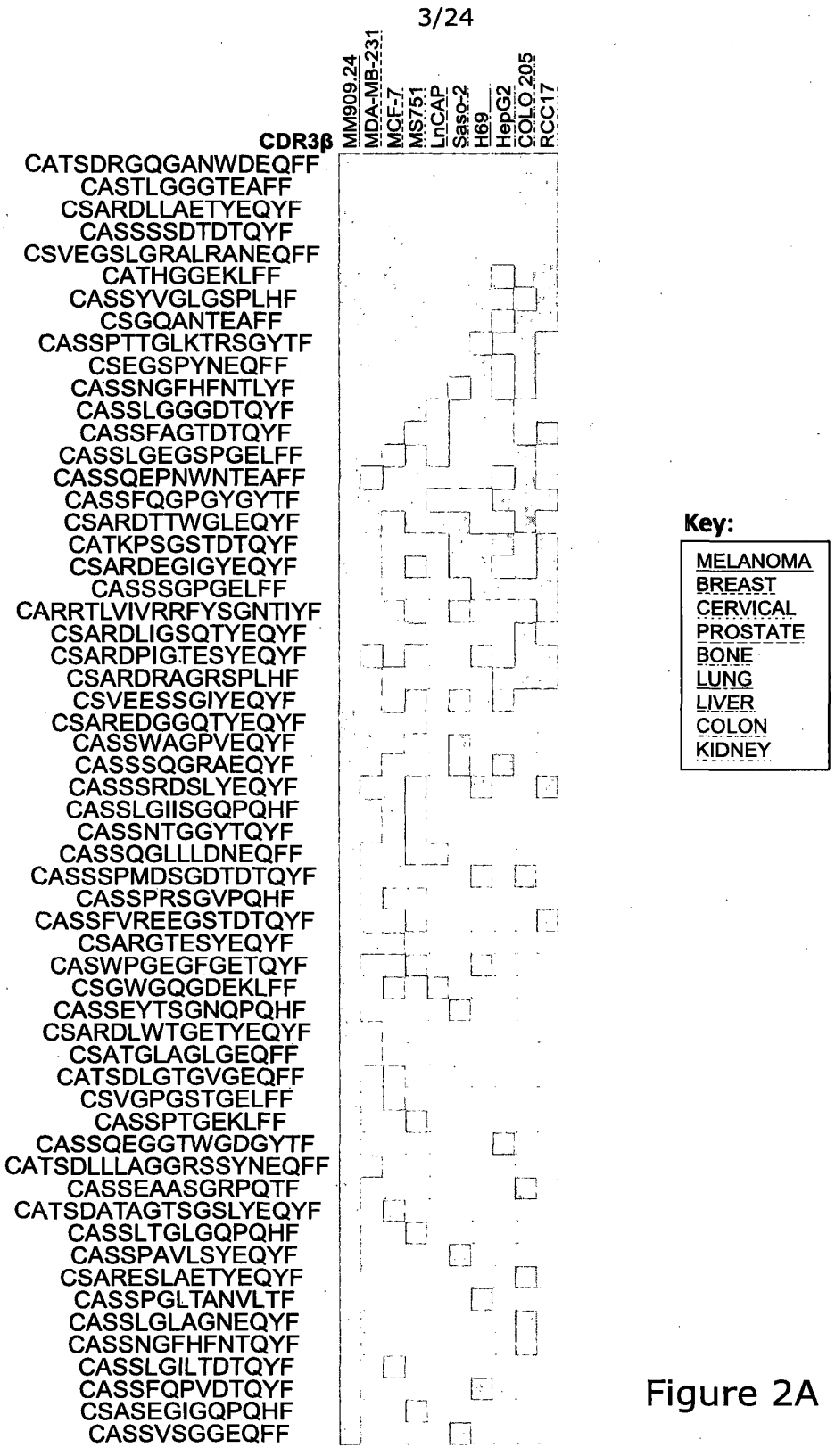
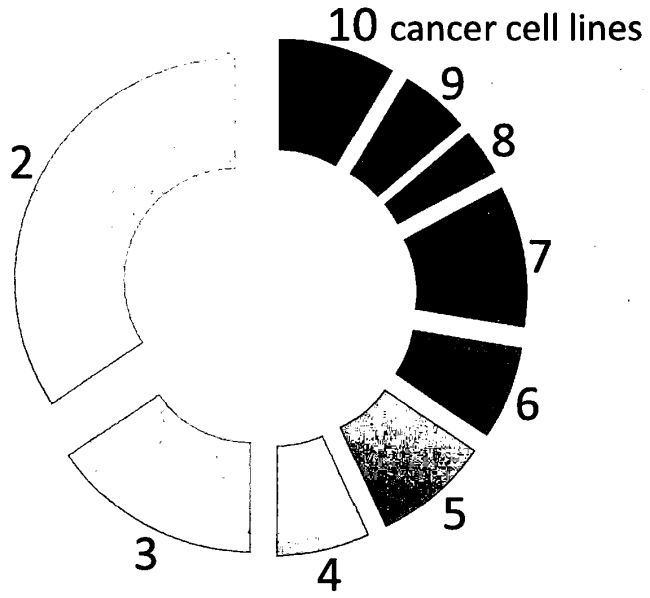

CDR3β
MM909.24
MDA-MB-231
MCF-7
MS751
LnCAP
Saso-2
H69
HepG2
COLO 205
RCC17
CATSDRGQGANWDEQFF
CASTLGGGTEAFF
CSARDLLAETYEQYF
CASSSSDTDTQYF
CSVEGSLGRALRANEQFF
CATHGGEKLFF
CASSYVGLGSPLHF
CSGQANTEAFF
CASSPTTGLKTRSGYTF
CSEGSPYNEQFF
CASSNGFHFNTLYF
CASSLGGGDTQYF
CASSFAGTDTQYF
CASSLGEGSPGELFF
CASSQEPNWNTEAFF
CASSFQGPGYGYTF
CSARDTTWGLEQYF
CATKPSGSTDTQYF
CSARDEGIGYEQYF
CASSSGPGELFF
CARRTLVIVRRFYSGNTIYF
CSARDLIGSQTYEQYF
CSARDPIGTESYEQYF
CSARDRAGRSPLHF
CSVEESSGIYEQYF
CSAREDGGQTYEQYF
CASSWAGPVEQYF
CASSSQGRAEQYF
CASSSRDSLYEQYF
CASSLGIISGQPQHF
CASSNTGGYTQYF
CASSQGLLLDNEQFF
CASSSPMDSGDTDTQYF
CASSPRSGVPQHF
CASSFVREEGSTDTQYF
CSARGTESYEQYF
CASWPGEGFGETQYF
CSGWGQGDEKLFF
CASSEYTSGNQPQHF
CSARDLWTGETYEQYF
CSATGLAGLGEQFF
CATSDLGTGVGEQFF
CSVGPGSTGELFF
CASSPTGEKLFF
CASSQEGGTWGDGYTF
CATSDLLLAGGRSSYNEQFF
CASSEAASGRPQTF
CATSDATAGTSGSLYEQYF
CASSLTGLGQPQHF
CASSPAVLSYEQYF
CSARESLAETYEQYF
CASSPGLTANVLTF
CASSLGLAGNEQYF
CASSNGFHFNTQYF
CASSLGILTDTQYF
CASSFQPVDTQYF
CSASEGIGQPQHF
CASSVSGGEQFF
Key:
MELANOMA
BREAST
CERVICAL
PROSTATE
BONE
LUNG
LIVER
COLON
KIDNEY

Figure 2A



The pie chart shows the proportion of TCRs (segments) able to recognise multiple cancer cell lines (numbers).

Figure 2B

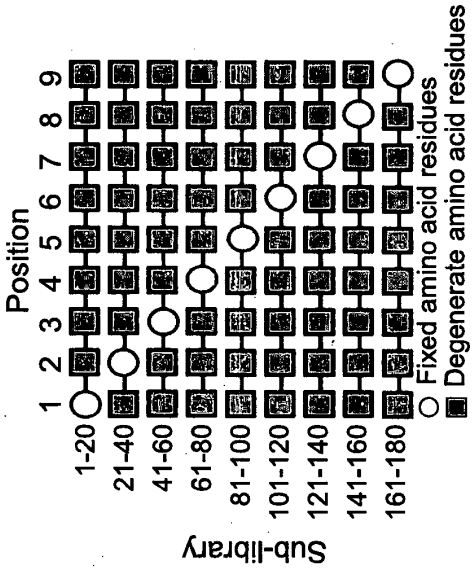


Figure 3B CPL screening

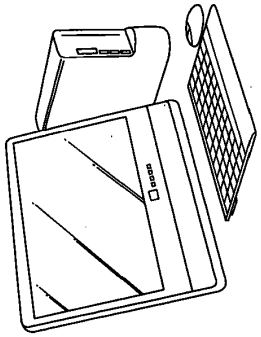


Figure 3C Cancer antigen database

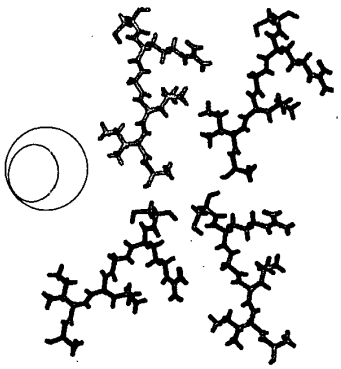


Figure 3D Testing candidate peptides

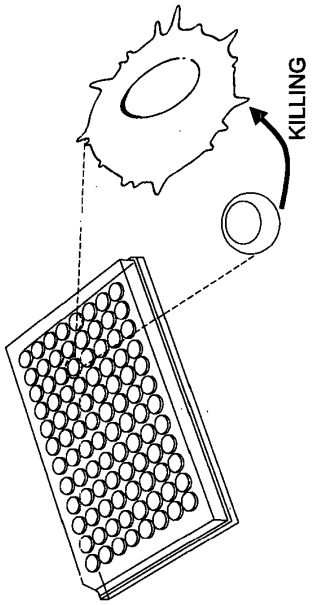


Figure 3A Cloning and cytotoxicity screening

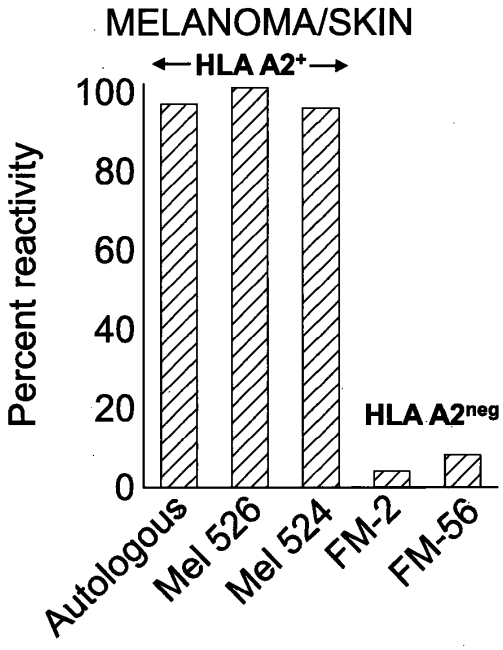


Figure 4A

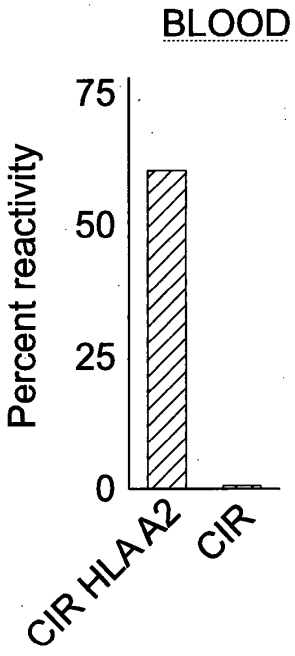


Figure 4B

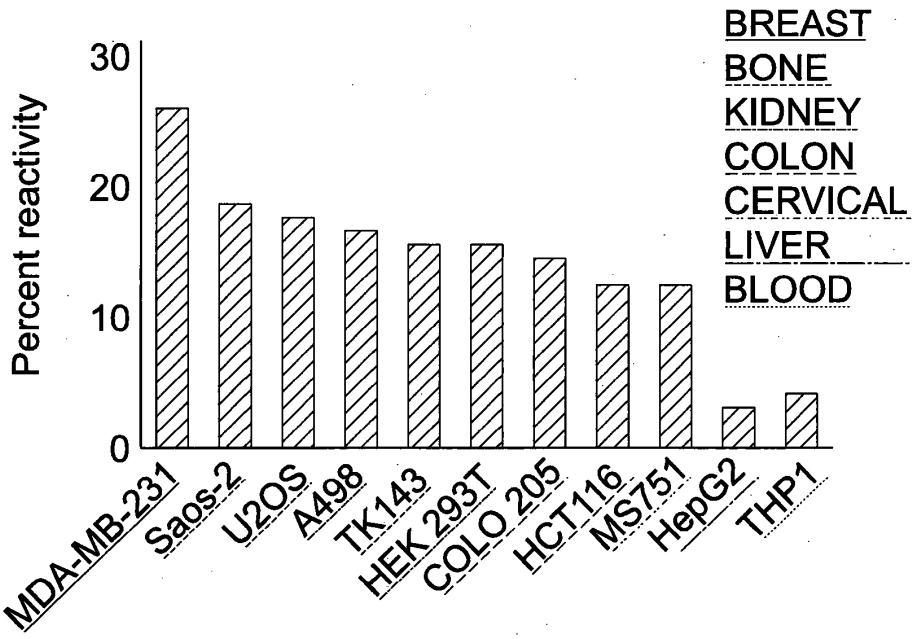


Figure 4C

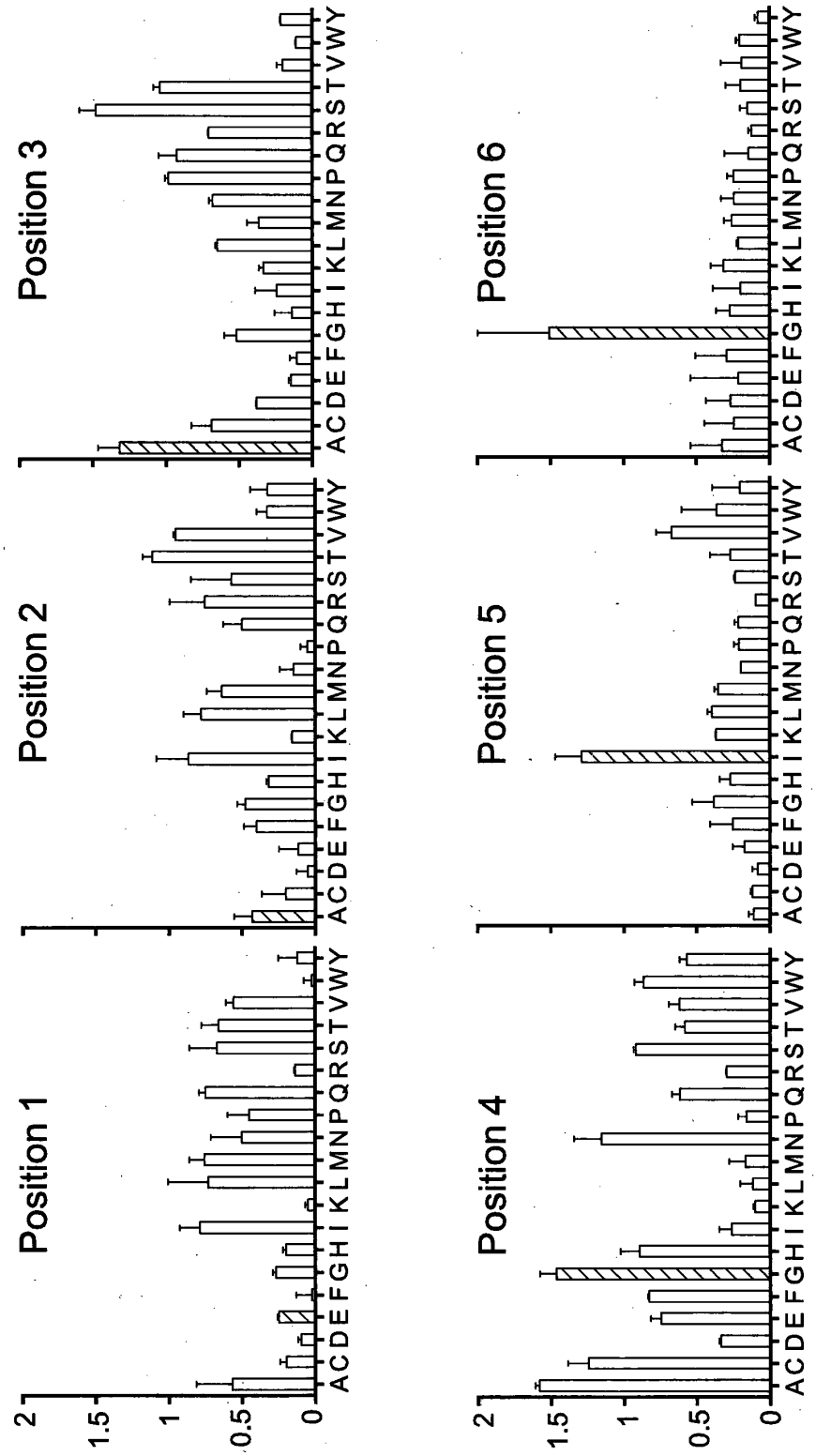

Position 1
Position 2
Position 3
Position 4
Position 5
Position 6
2
1.5
1
0.5
0
A C D E F G H I K L M N P Q R S T V W Y

Figure 5

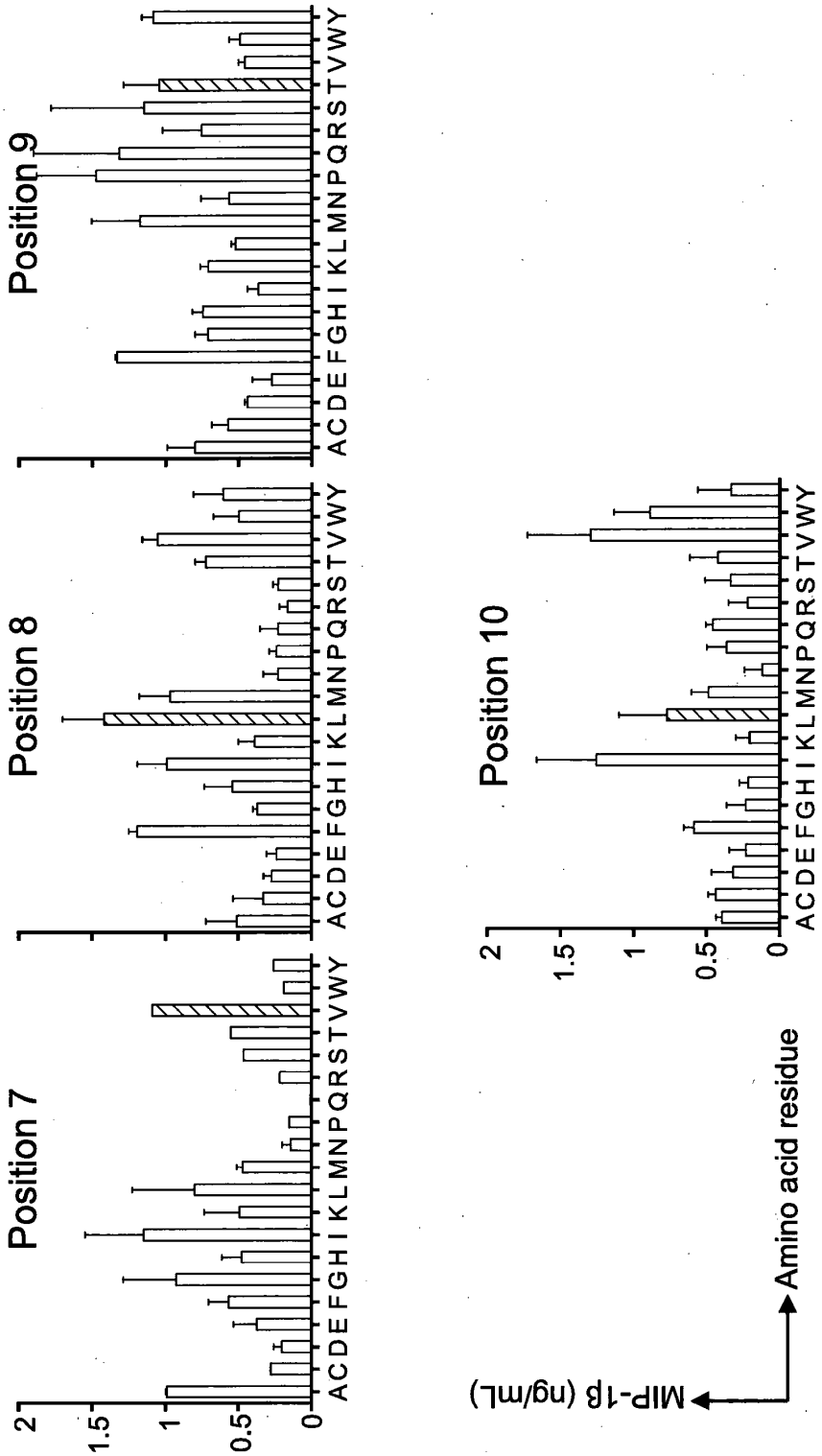


Figure 5 (continued)

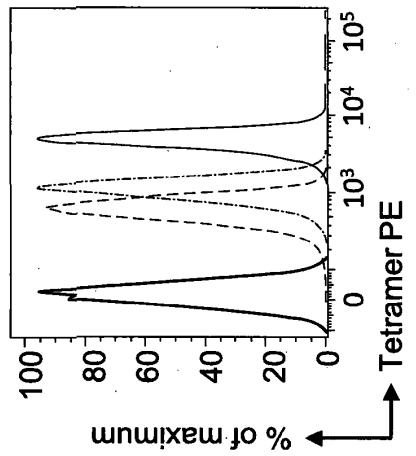


Figure 6B

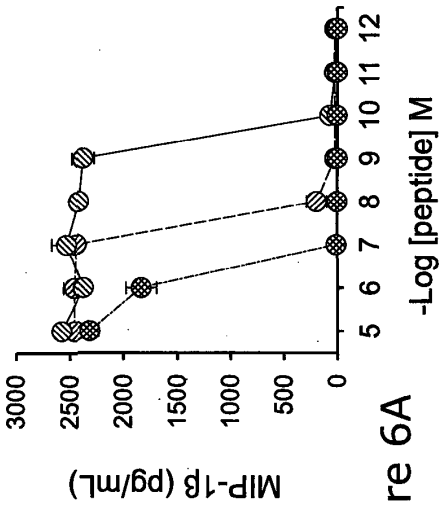


Figure 6A

Key:

Melan A (residues 26-35)
EAAGIGILTV

BST2 (residues 22-31)
LLLGIGILVL

IMP2 (residues 367-376)
NLSALGIFST

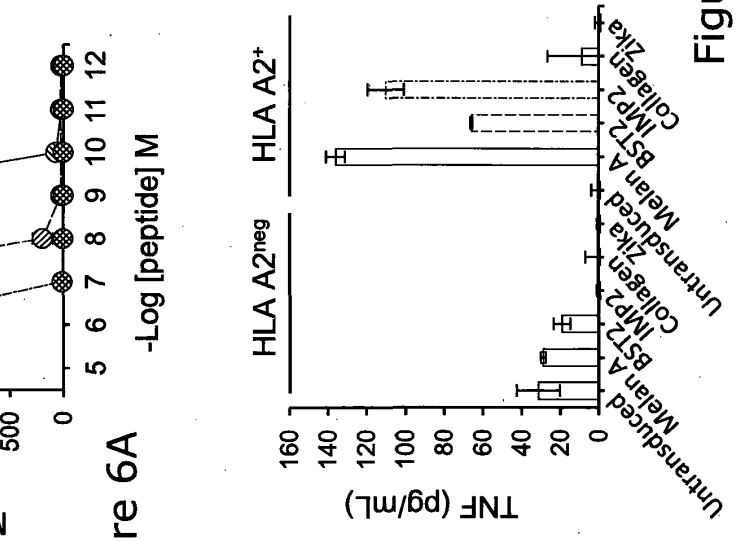


Figure 6C

Wild-type MPREDAHFIYGYPKKGHGHSYTTAEEAAGIGILTVILGVLLLIGCWYCRRRNGYRALMD
KSLHVGTQCALTRRCPQEGFDHRDSKVSLQEKNCEPVVPNAPPAYEKLSAEQSPPPYSP

Melan A KO MPREDAHFIYGYPKKGHGHSYNHG**Stop**

Figure 7A

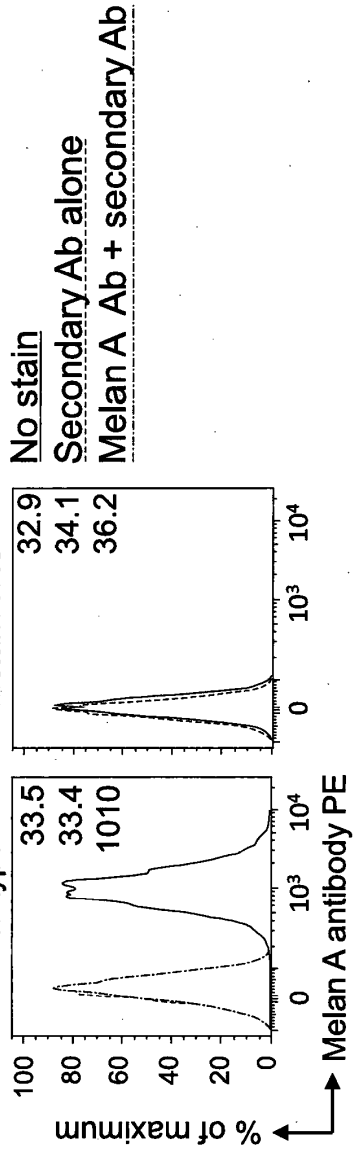
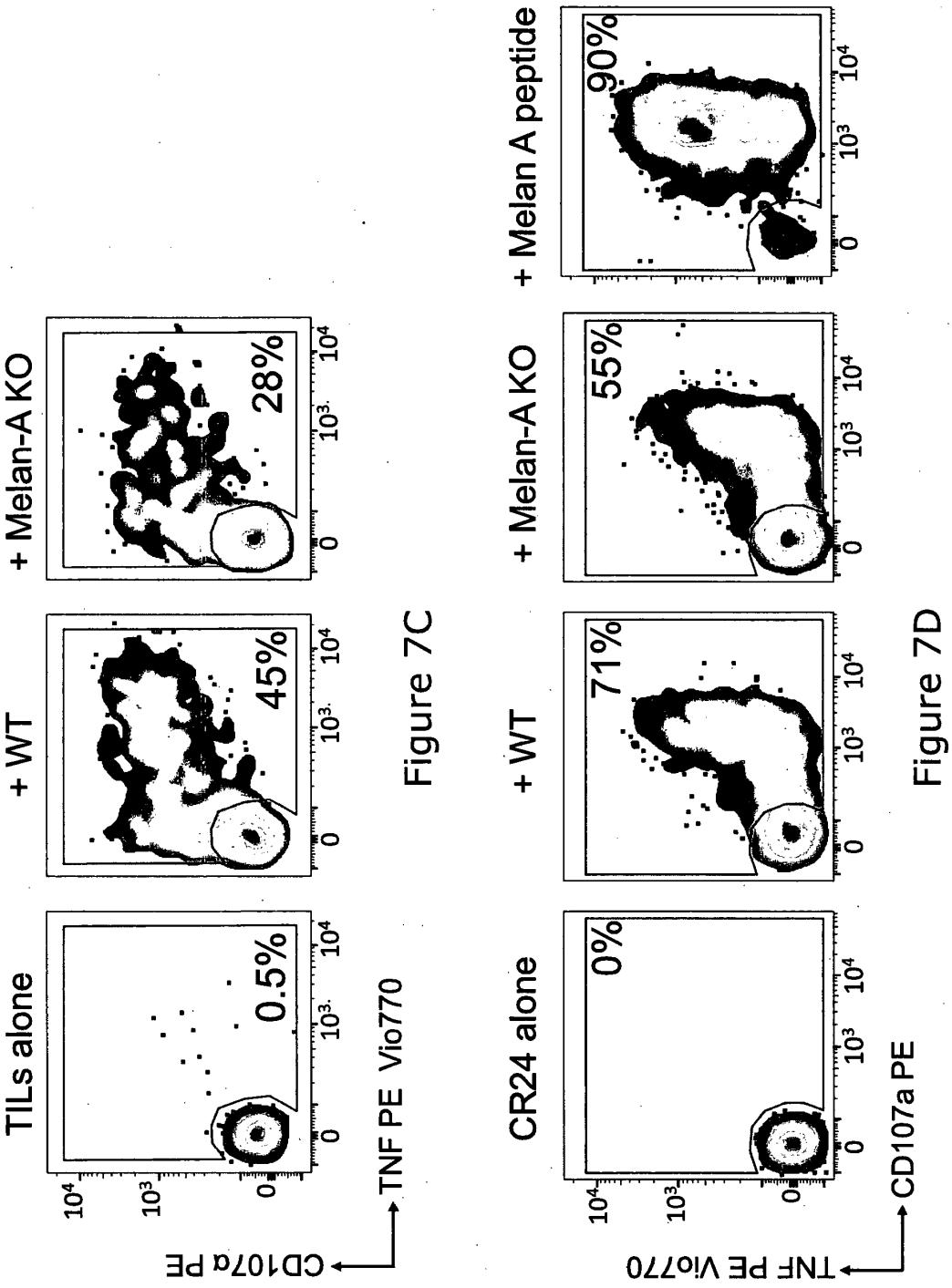


Figure 7B


TILs alone
+ WT
+ Melan-A KO
0.5%
45%
28%
CD107a PE
TNF PE Vio770
Figure 7C
CR24 alone
+ WT
+ Melan-A KO
+ Melan A peptide
0%
71%
55%
90%
TNF PE Vio770
CD107a PE
Figure 7D

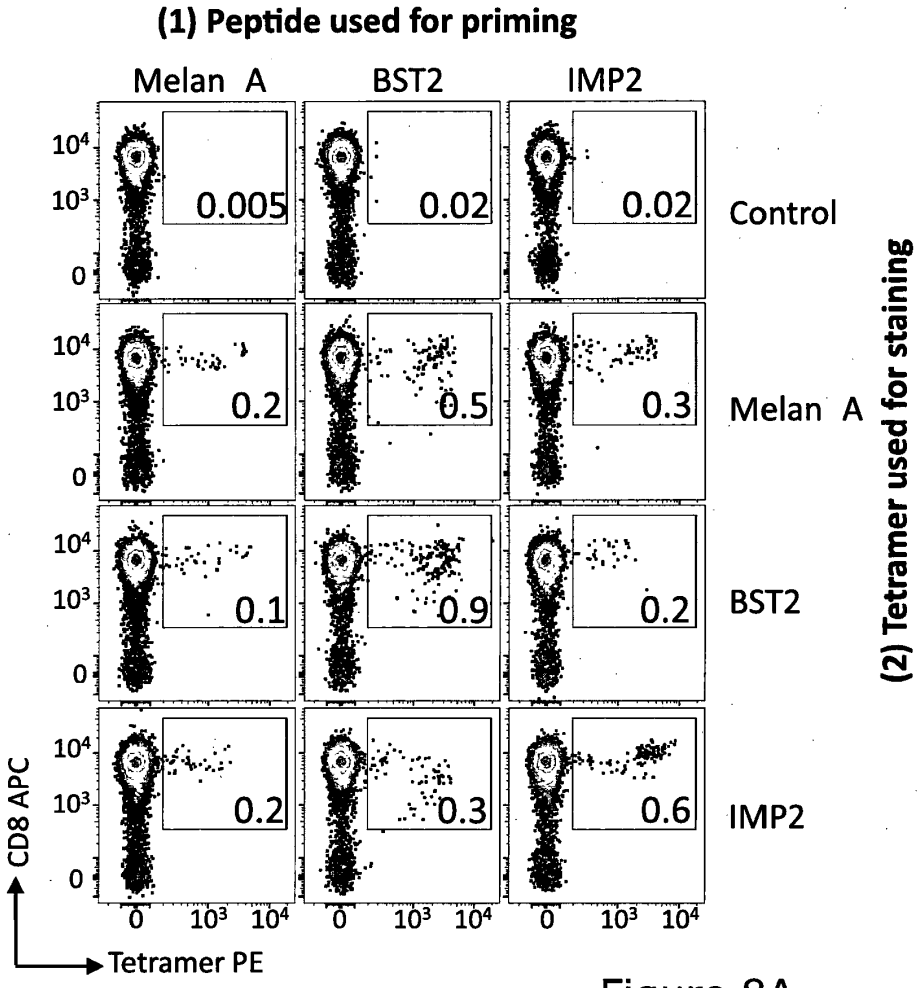


Figure 8A

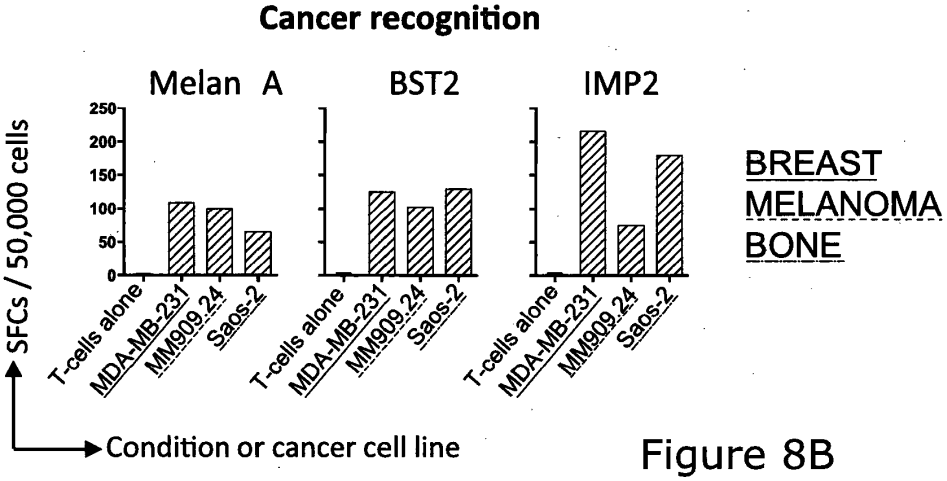


Figure 8B

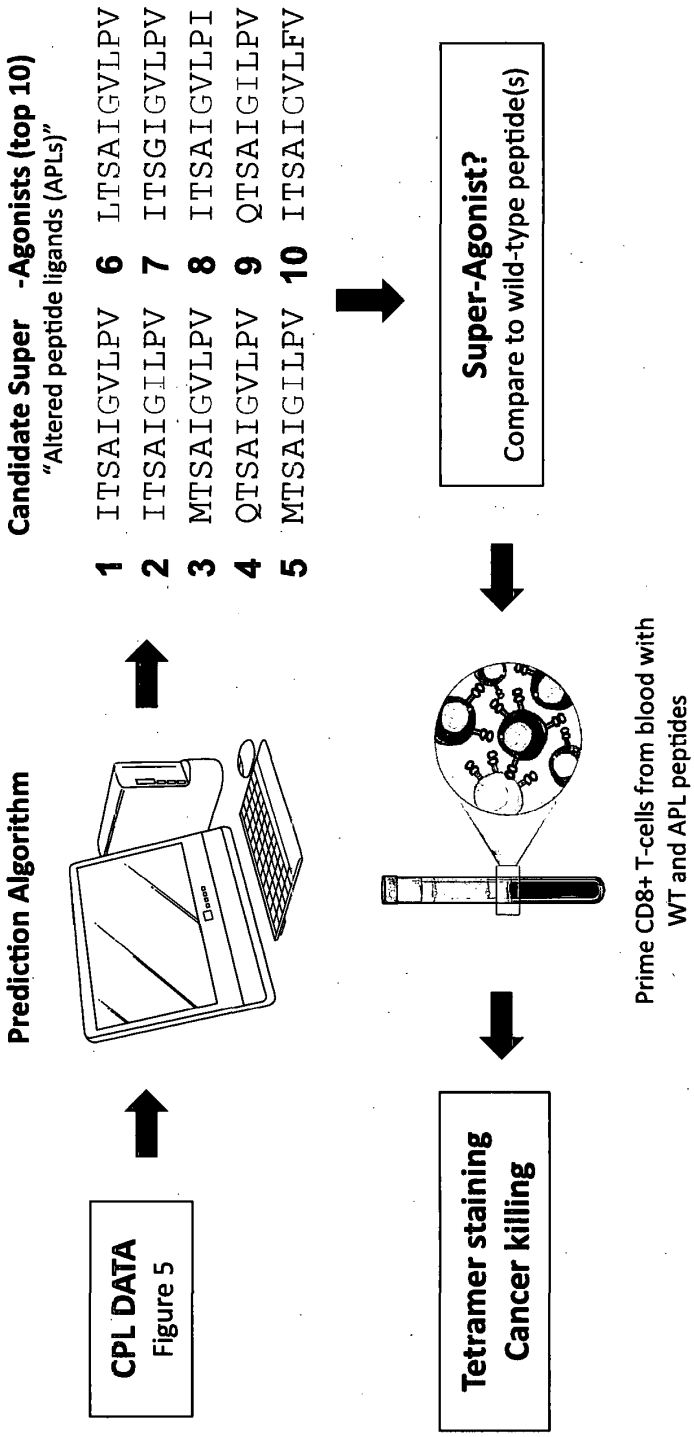

CPL DATA
Figure 5
Prediction Algorithm
Candidate Super -Agonists (top 10)
"Altered peptide ligands (APLs)"
1 ITSAIGVLPV
2 ITSAIGILPV
3 MTSAIGVLPV
4 QTSAIGVLPV
5 MTSAIGILPV
6 LTSAIGVLPV
7 ITSGIGVLPV
8 ITSAIGVLPI
9 QTSAIGILPV
10 ITSAICVLFV
Super-Agonist?
Compare to wild-type peptide(s)
Prime CD8+ T-cells from blood with WT and APL peptides
Tetramer staining
Cancer killing

Figure 9A

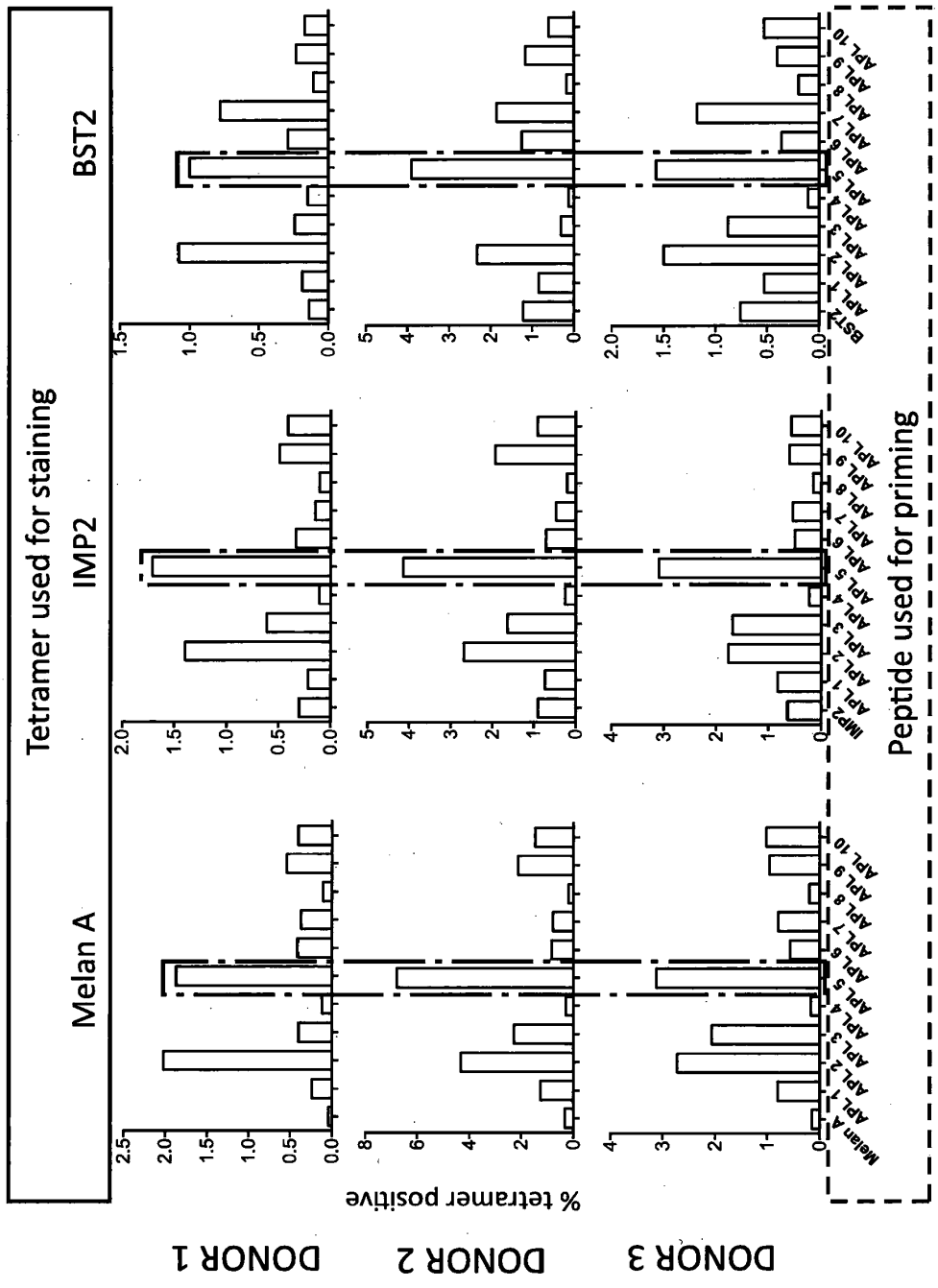

Tetramer used for staining
Melan A
IMP2
BST2
Peptide used for priming
% tetramer positive
DONOR 1
DONOR 2
DONOR 3

Figure 9B

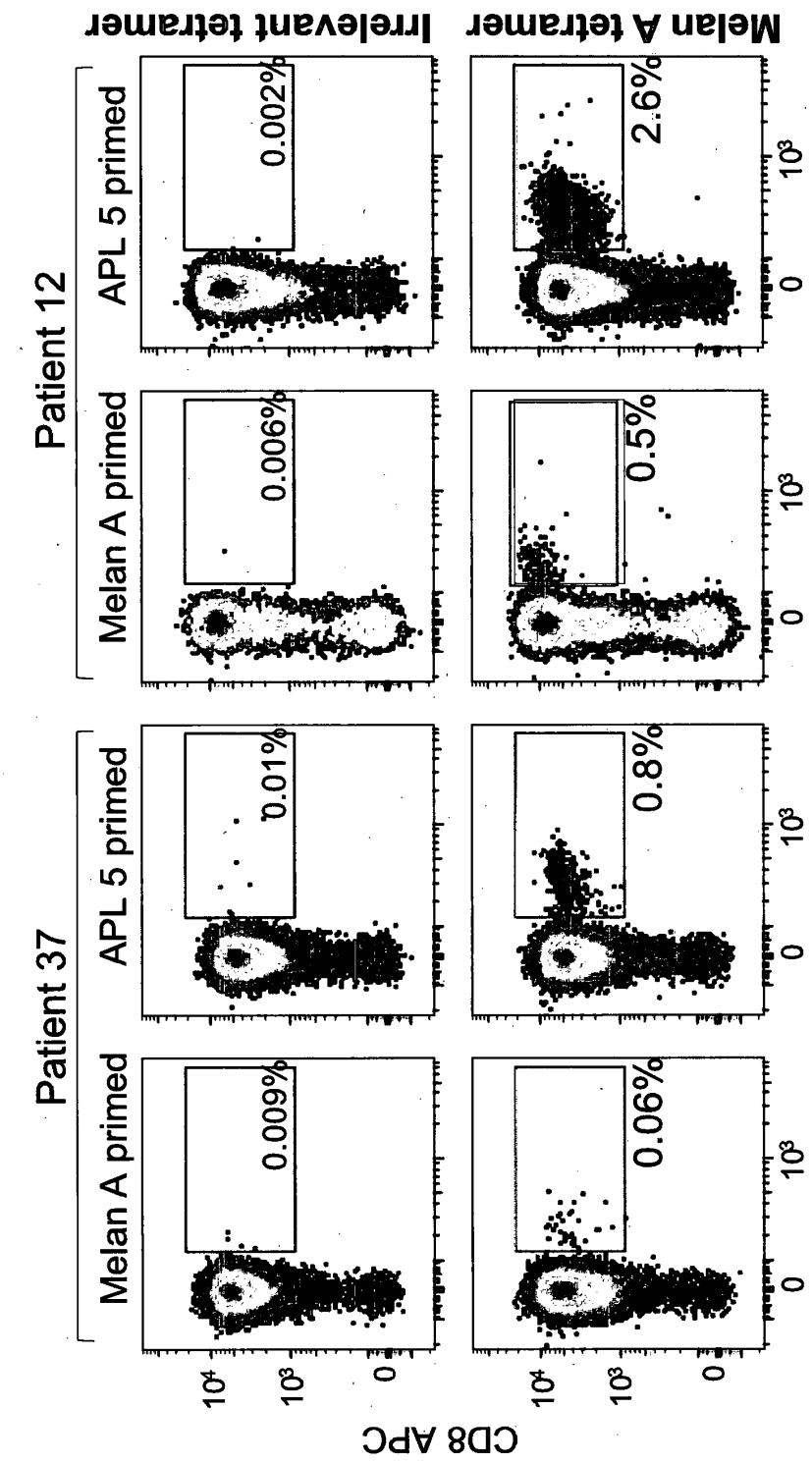

Irrelevant tetramer
Melan A tetramer
Patient 12
Patient 37
APL 5 primed
Melan A primed
0.002%
2.6%
0.006%
0.5%
0.01%
0.8%
0.009%
0.06%
CD8 APC
Tetramer PE

Figure 10A

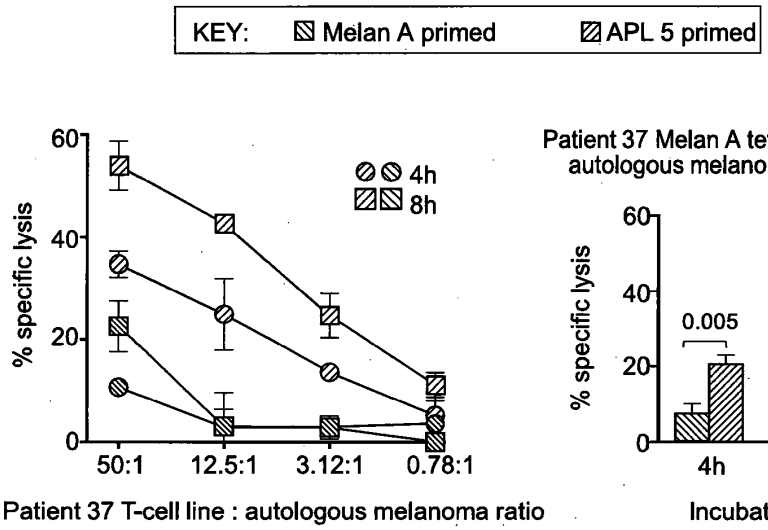


Figure 10B

Figure 10C

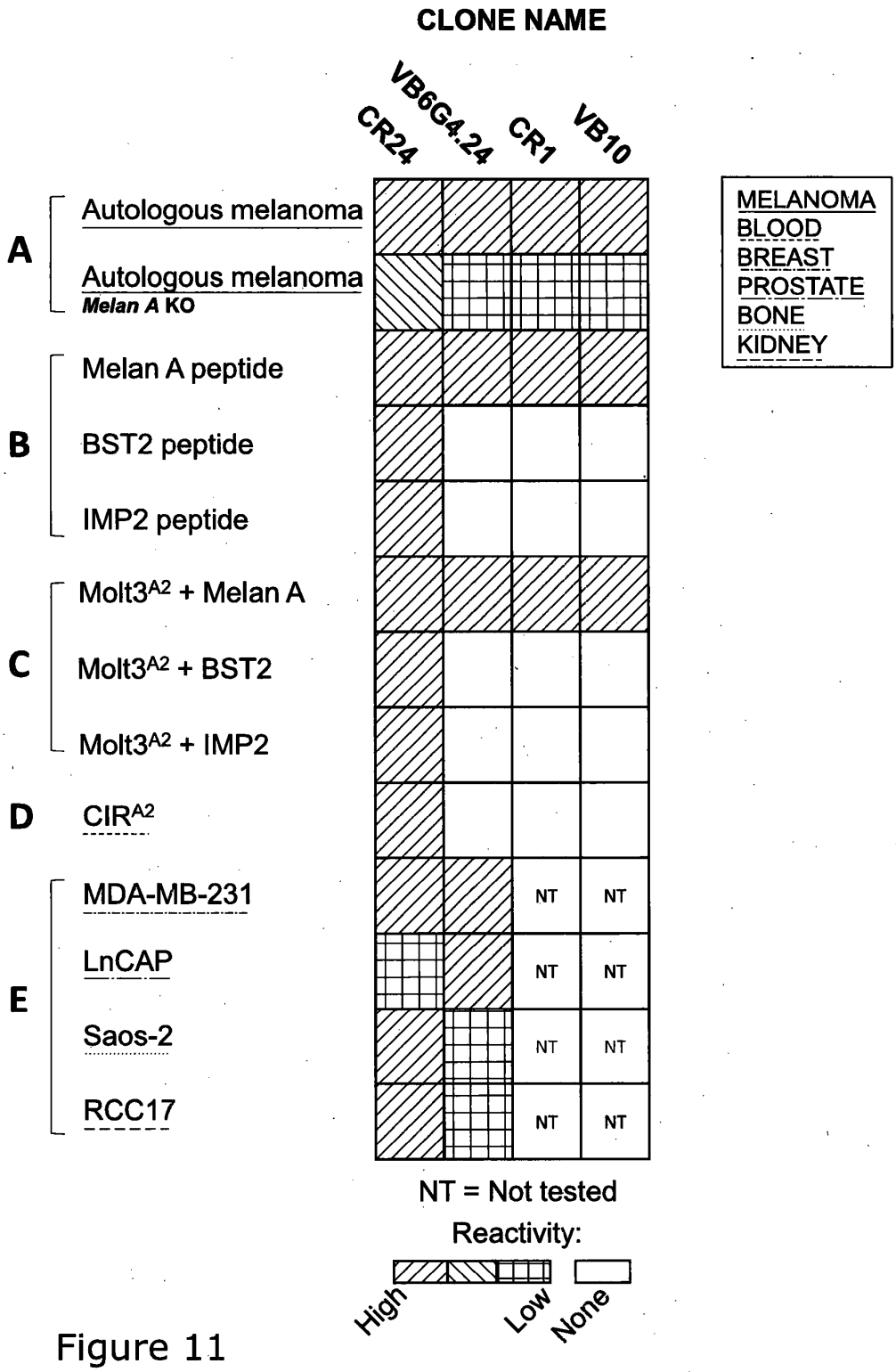

CLONE NAME
CR24
VB6G4.24
CR1
VB10
A
Autologous melanoma
Autologous melanoma
Melan A KO
B
Melan A peptide
BST2 peptide
IMP2 peptide
C
Molt3A2 + Melan A
Molt3A2 + BST2
Molt3A2 + IMP2
D
CIRA2
E
MDA-MB-231
LnCAP
Saos-2
RCC17
NT
NT = Not tested
Reactivity:
High
Low
None
MELANOMA
BLOOD
BREAST
PROSTATE
BONE
KIDNEY

Figure 11

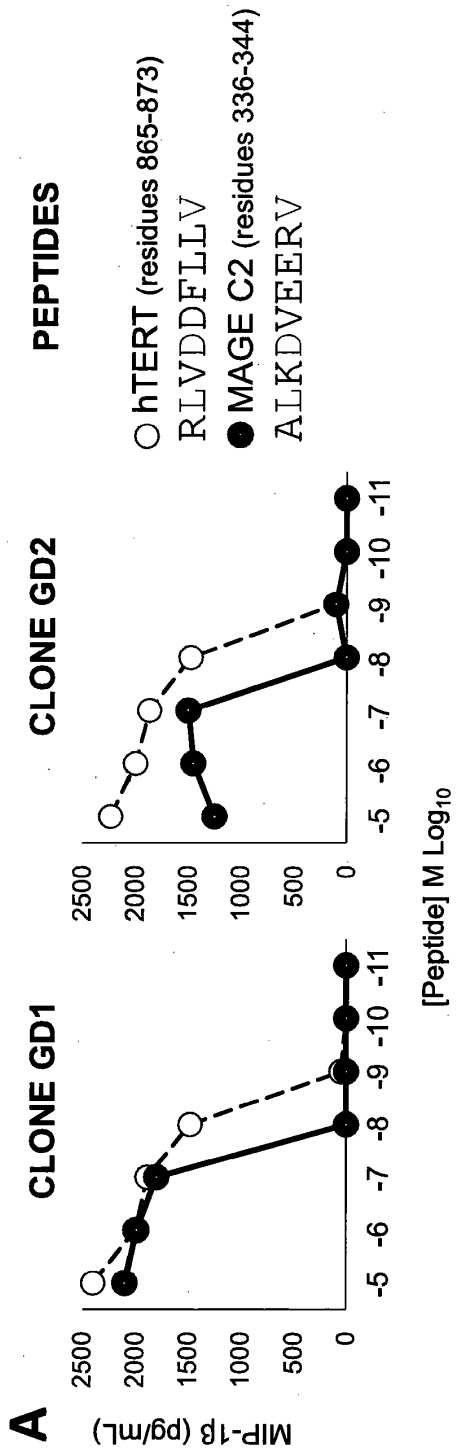

A
MIP-1β (pg/mL)
CLONE GD1
CLONE GD2
PEPTIDES
○ hTERT (residues 865-873)
RLVDDFLLV
● MAGE C2 (residues 336-344)
ALKDVEERV
[Peptide] M Log10
2500
2000
1500
1000
500
0
-5 -6 -7 -8 -9 -10 -11

Figure 12A

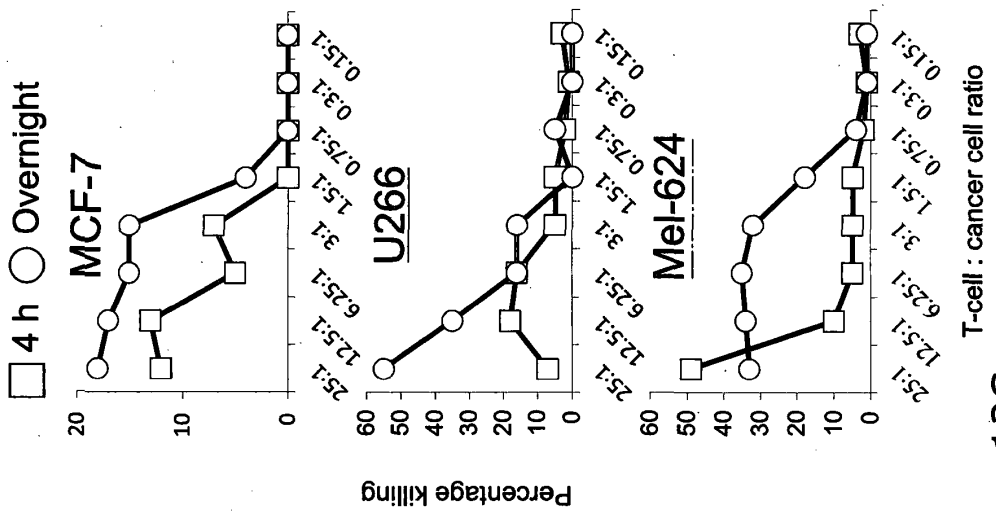


Figure 12C

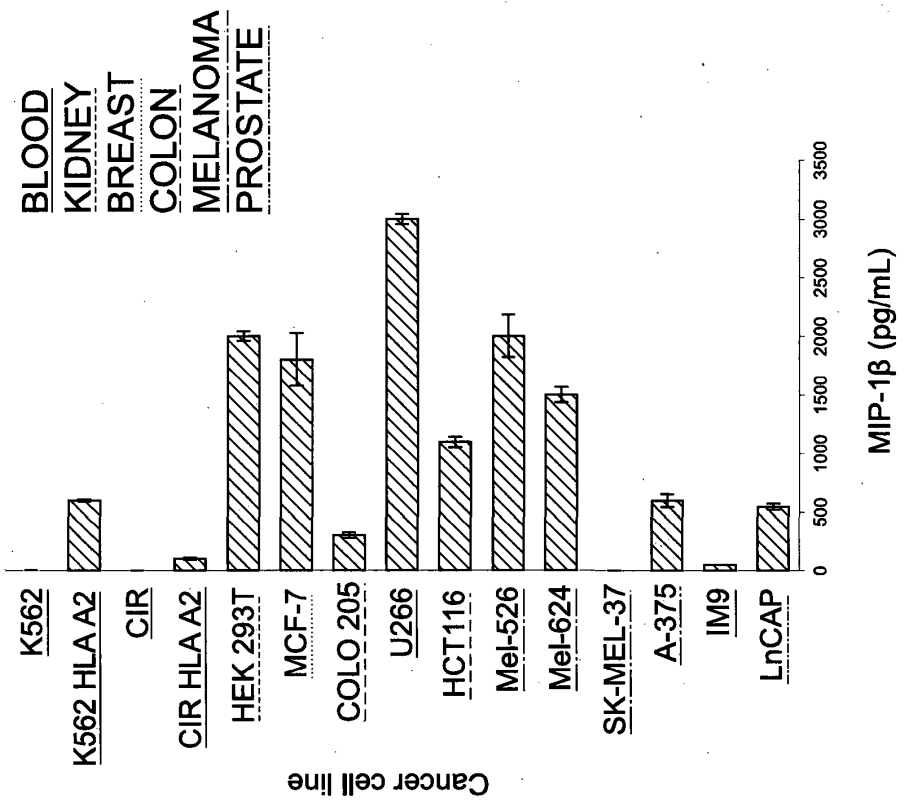


Figure 12B

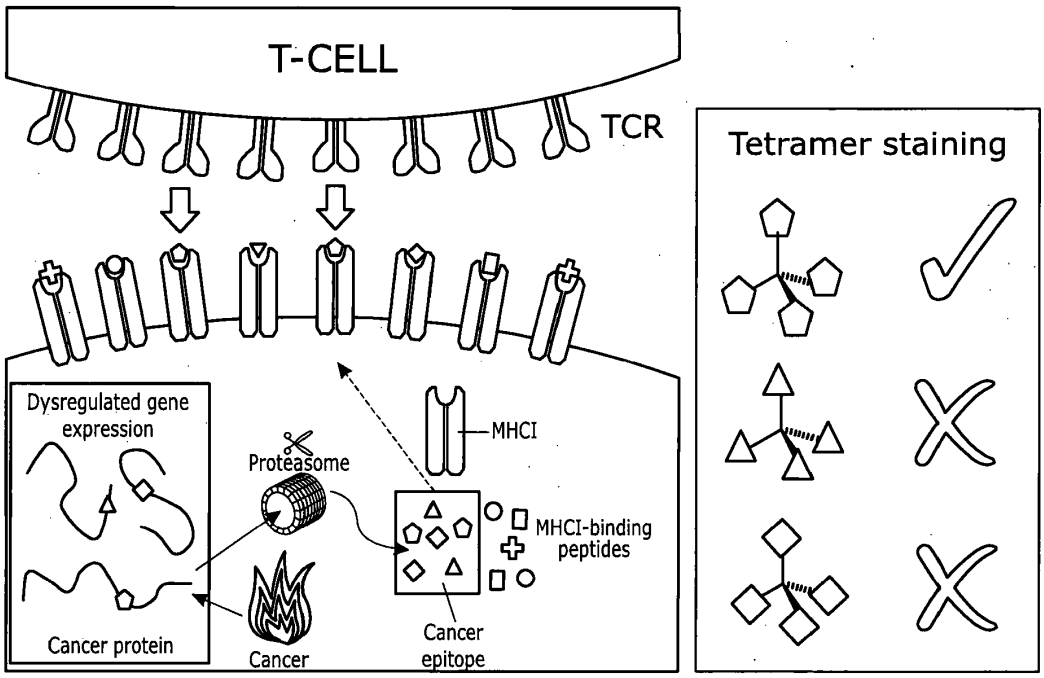


Figure 13A Normal Cancer-specific T-cell (recognizes one cancer peptide)

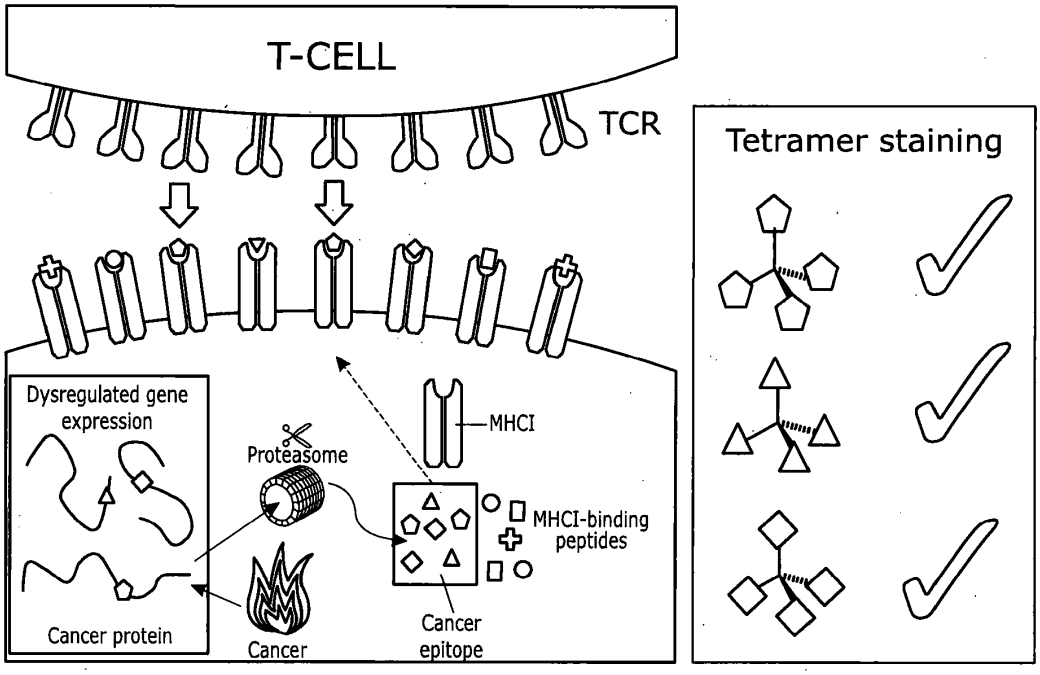


Figure 13B Multipronged Cancer-specific T-cell (recognizes more than one cancer peptide)

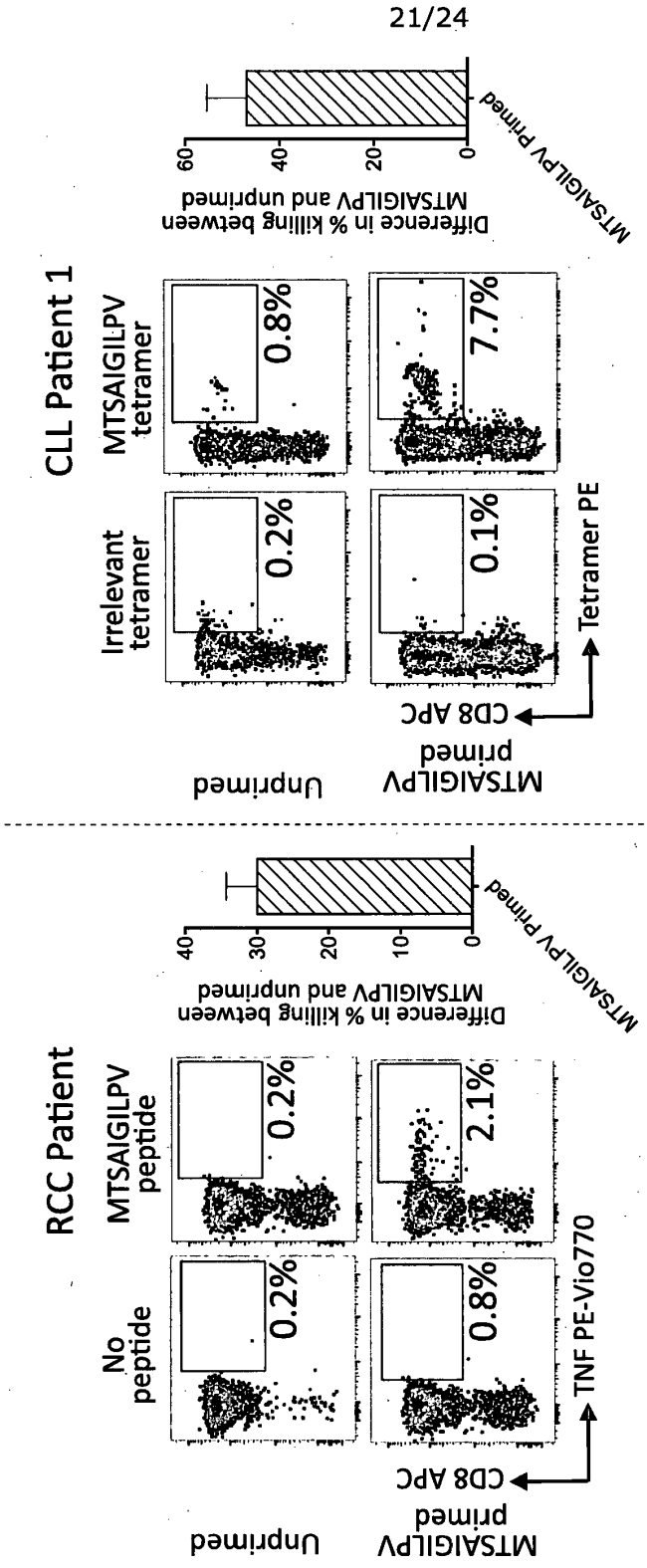

CLL Patient 1
MTSAIGILPV tetramer
Irrelevant tetramer
0.8%
7.7%
0.2%
0.1%
Tetramer PE
CD8 APC
MTSAIGILPV primed
Unprimed
Difference in % killing between MTSAIGILPV and unprimed
0
20
40
60
MTSAIGILPV Primed
RCC Patient
MTSAIGILPV peptide
No peptide
0.2%
2.1%
0.2%
0.8%
TNF PE-Vio770
CD8 APC
MTSAIGILPV primed
Unprimed
Difference in % killing between MTSAIGILPV and unprimed
0
10
20
30
40
MTSAIGILPV Primed

Figure 14A

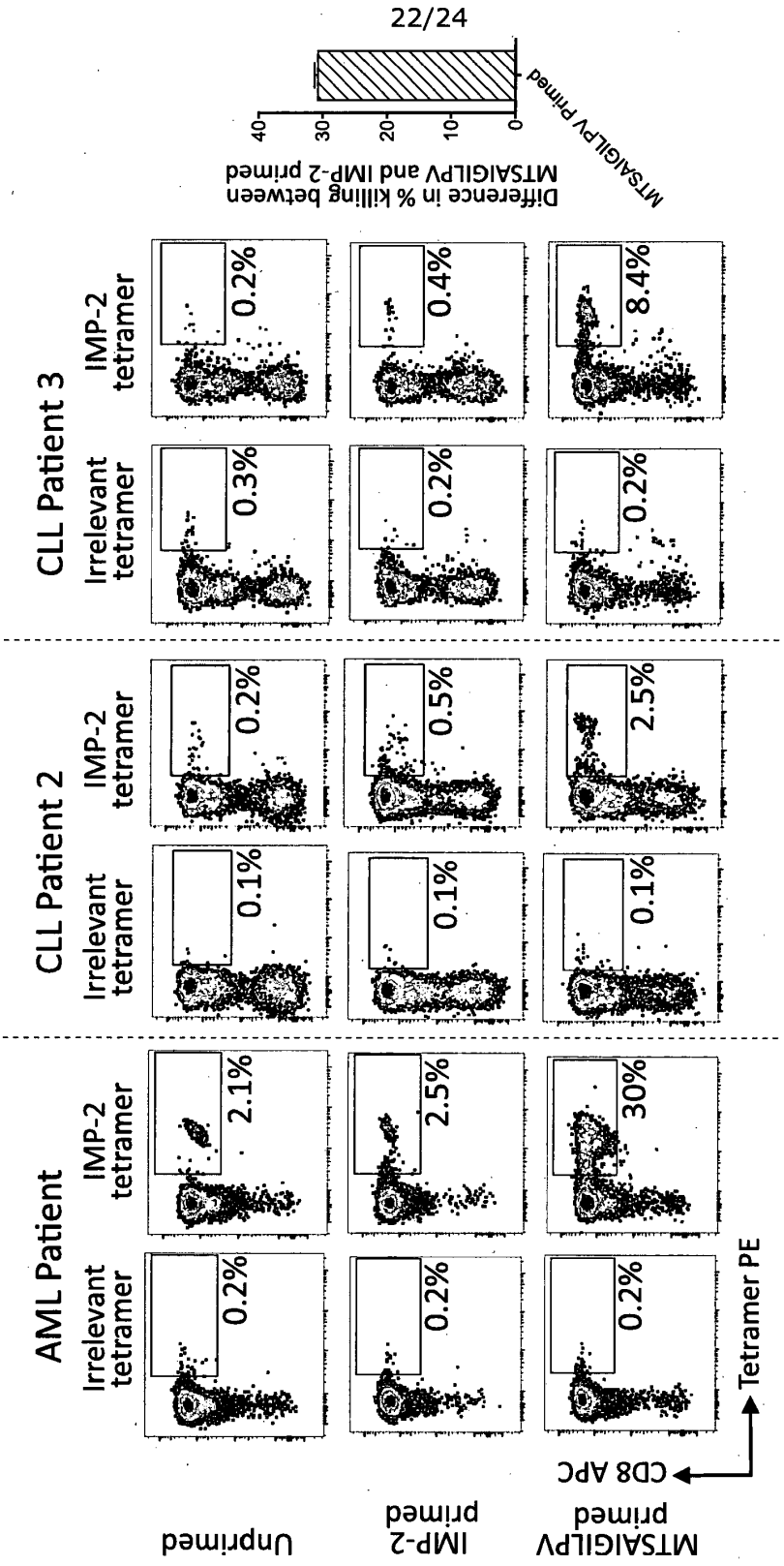

MTSAIGILPV primed
IMP-2 primed
Unprimed
CD8 APC
Tetramer PE
AML Patient
Irrelevant tetramer
IMP-2 tetramer
0.2%
2.1%
0.2%
2.5%
0.2%
30%
CLL Patient 2
Irrelevant tetramer
IMP-2 tetramer
0.1%
0.2%
0.1%
0.5%
0.1%
2.5%
CLL Patient 3
Irrelevant tetramer
IMP-2 tetramer
0.3%
0.2%
0.2%
0.4%
0.2%
8.4%
Difference in % killing between MTSAIGILPV and IMP-2 primed
0
10
20
30
40
MTSAIGILPV Primed

Figure 14 B

Multipronged T-cells recognise a *plurality* of different peptides derived from *different* cancer-specific antigens at the surface of the same cancer cell

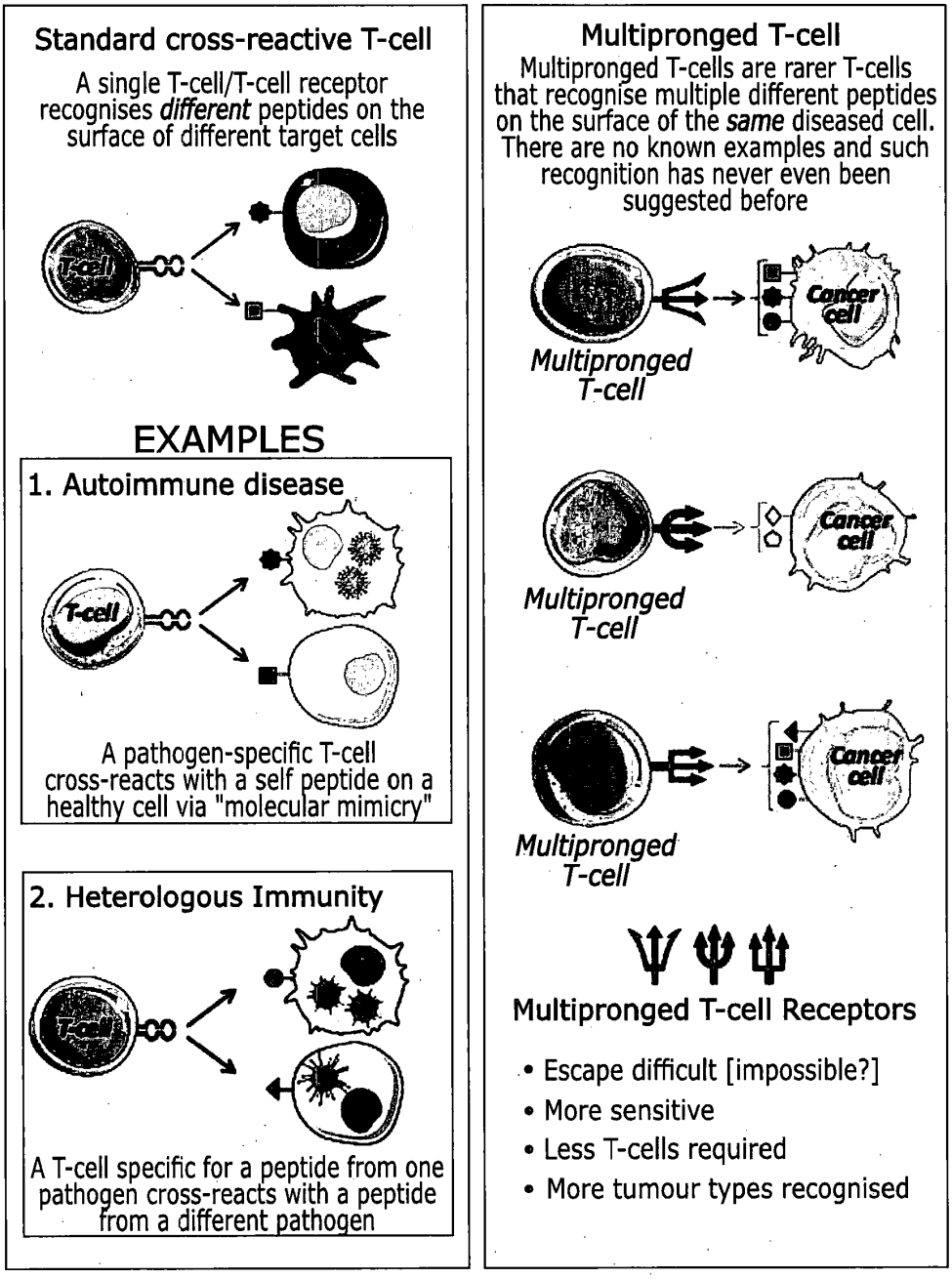


Figure 15

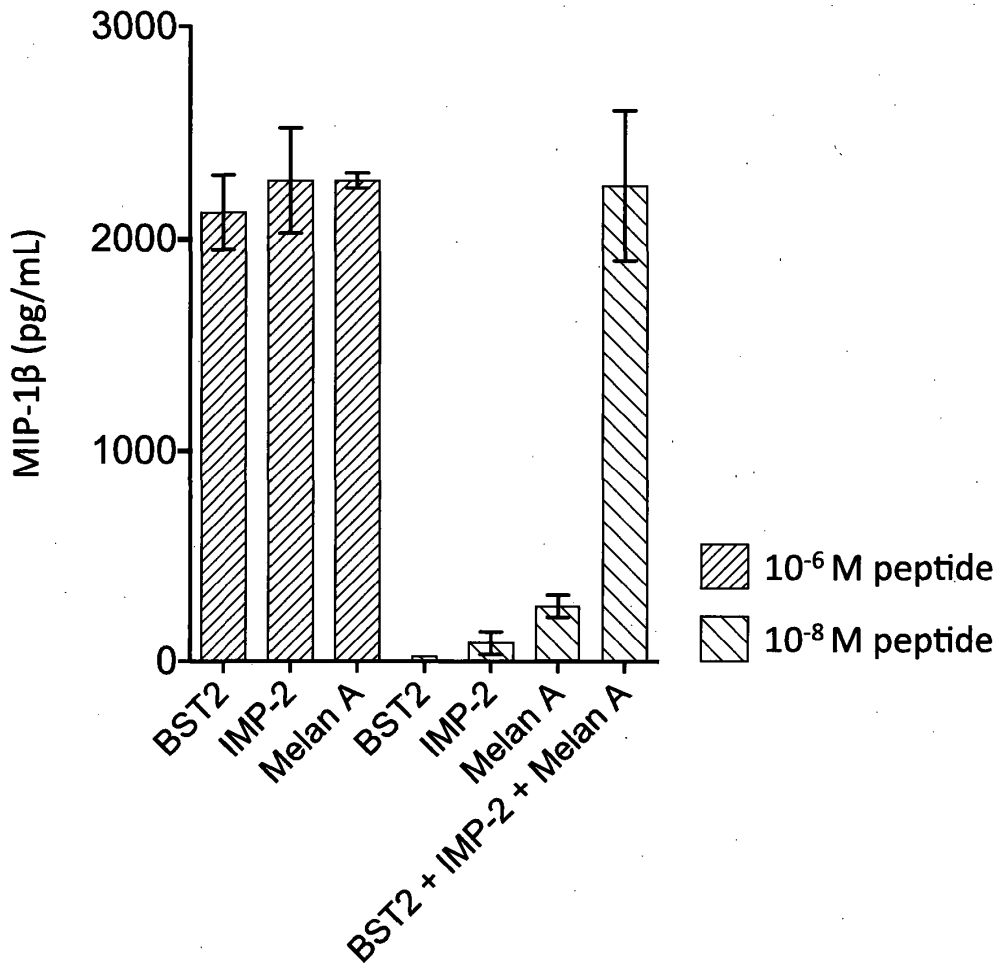

3000
2000
1000
0
MIP-1β (pg/mL)
BST2
IMP-2
Melan A
BST2
IMP-2
Melan A
BST2 + IMP-2 + Melan A
10-6 M peptide
10-8 M peptide

Figure 16