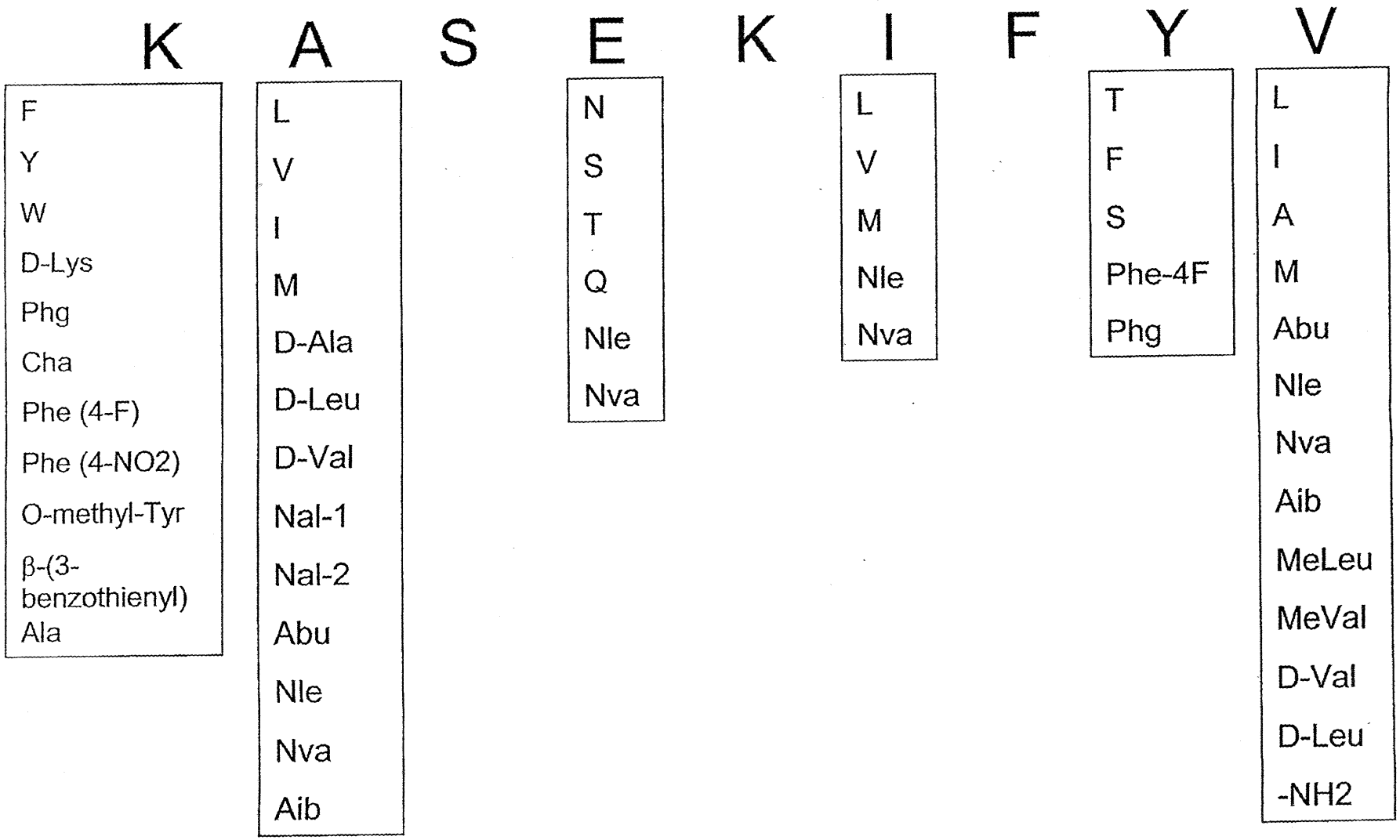


Figure 1A: Overview of the designed nonamer analogs

Figure 1B: Overview of the designed decamer analogs

Figure 2: Methodology

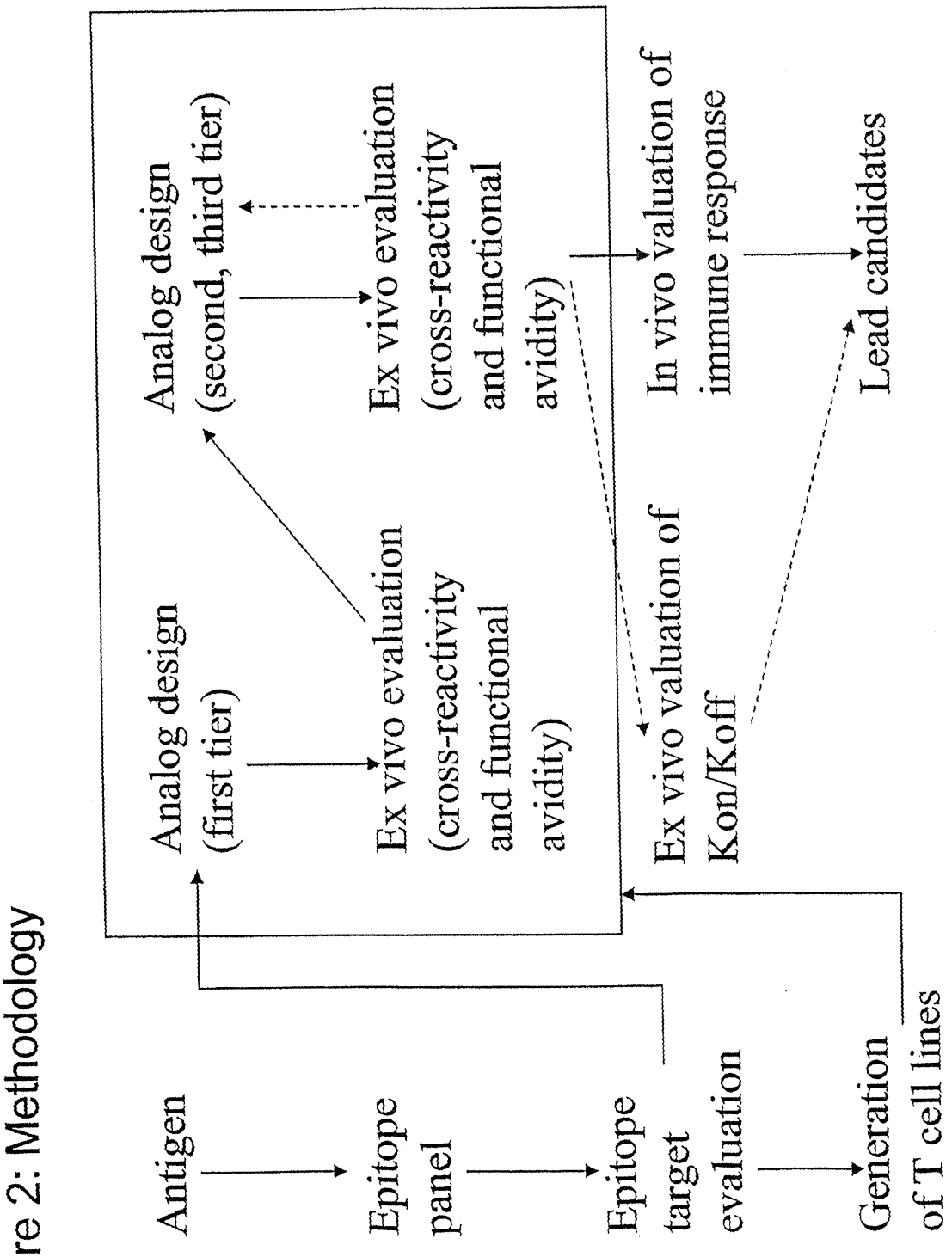


Figure 3: Cross-reactivity and functional avidity of analogs substituted at single position

Category	Number	Peptide name	Sequence	NOTE	Cross-reactivity and fct avidity (native to analogues) *	**Cross-reactivity and fct avidity (Nat to analogues, peak IFNgamma)
Native	1	SSX2 41-49	KASEKIFYV	Native peptide	1	46
N-terminal Primary Anchor	2	SSX2 41-49 (A42L)	KLSEKIFYV		0.03	89
	3	SSX2 41-49 (A42V)	KVSEKIFYV		0.03	65
	4	SSX2 41-49 (A42I)	KISEKIFYV		3	55
	5	SSX2 41-49 (A42M)	KMSEKIFYV		0.1	65
	6	SSX2 41-49 (A42a)	KaSEKIFYV	Small letter stands for the corresponding d-Amino acid	10	26
	7	SSX2 41-49 (A42v)	KvSEKIFYV		3	43
	8	SSX2 41-49 (A42Nal-1)	KNal-1SEKIFYV	Nal-1 is 1-naphtyl L-Ala	10	12
	9	SSX2 41-49 (A42Nal-2)	KNal-2SEKIFYV	Nal-1 is 2-naphtyl L-Ala	3	48
	10	SSX2 41-49 (A42Abu)	KAbuSEKIFYV	Abu is L-α-aminobutyric acid	0.3	60
	11	SSX2 41-49 (A42Nle)	KNleSEKIFYV	Nle is Norleucine	0.1	69
	12	SSX2 41-49 (A42Nva)	KNvaSEKIFYV	Nva is Norvaline	0.1	72
	13	SSX2 41-49 (A42Aib)	KAibSEKIFYV	Aib is α-aminoisobutyric acid	3	70
N-terminal Secondary Anchor	14	SSX2 41-49 (K41F)	FASEKIFYV		0.3	59
	15	SSX2 41-49 (K41W)	WASEKIFYV		10	16
	16	SSX2 41-49 (K41Y)	YASEKIFYV		3	53
	17	SSX2 41-49(K41k)	kASEKIFYV	k is d-Lys	10	10
	18	SSX2 41-49 (K41Phg)	PhgASEKIFYV	Phg is Phenylglycine	0.1	40
	19	SSX2 41-49 (K41Cha)	ChaASEKIFYV	Cha is β-cyclohexylalanine	10	160
	20	SSX2 41-49 (K41Phe-4F)	Phe(4-F)ASEKIFYV	Phe(4-F) is 4-Fluorophenylalanie	3	49
	21	SSX2 41-49 (K41Phe-4NO2)	Phe(4-NO2)ASEKIFYV	Phe(4-NO2) is 4-Nitrophenylalanie	3	46
	22	SSX2 41-49 (K41O-methyl Tyr)	O-methyl-TyrASEKIFYV	O-methyl-Tyr is O-methyl-L-tyrosine	3	41
	23	SSX2 41-49 (K41β-(3-benzothienyl)Ala)	β-(3-benzothienyl)AlaASEKIFYV	β-(3-benzothienyl)Ala:	10	23
C-terminal Primary Anchor	24	SSX2 41-49 (V49I)	KASEKIFYI	Based on 1st round of cross-reactivity data	10	24
C-terminal amide	25	SSX2 41-49-NH2	KASEKIFYV-NH2	Exploring the C-terminus anchor residue	10	12
	26	SSX2 41-49-NH2 (A42L)	KLSEKIFYV-NH2	Exploring the C-terminus anchor residue	3	65
	27	SSX2 41-49-NH2 (A42V)	KVSEKIFYV-NH2	Exploring the C-terminus anchor residue	10	49

* μM; **ng/ml

Figure 4: Cross-reactivity and functional avidity of analogs substituted at two positions

Category	Number	Peptide name	Sequence	NOTE	Cross-reactivity and fct avidity (native to analogues) *	**Cross-reactivity and fct avidity (Nat to analogues, peak IFNgamma)	**Cross-reactivity and fct avidity (analogue conc at peak IFNgamma)
Native	1	SSX2 41-49	KASEKIFYV	Native peptide	1	46	10
N-terminal Primary/Secondary Anchor	2	SSX2 41-49 (K41Y, A42V)	YVSEKIFYV		0.1	69	10
	3	SSX2 41-49 (K41Y, A42M)	YMSEKIFYV		3	44	10
	4	SSX2 41-49 (K41Y, A42I)	YISEKIFYV		1	52	3
	5	SSX2 41-49 (K41F, A42L)	FLSEKIFYV		0.3	50	10
	6	SSX2 41-49 (K41F, A42V)	FVSEKIFYV		0.1	61	10
	7	SSX2 41-49 (K41F, A42M)	FMSEKIFYV		3	36	10
	8	SSX2 41-49 (K41F, A42I)	FISEKIFYV		0.3	51	10
	9	SSX2 41-49 (K41W, A42L)	WLSEKIFYV		0.3	43	10
	10	SSX2 41-49 (K41W, A42V)	WVSEKIFYV		1	54	10
	11	SSX2 41-49 (K41W, A42M)	WMSEKIFYV		1	49	10
	12	SSX2 41-49 (K41W, A42I)	WISEKIFYV		0.1	53	10
	13	SSX2 41-49 (K41k, A42L)	kLSEKIFYV		10	48	10
	14	SSX2 41-49 (K41k, A42V)	kVSEKIFYV		10	52	10
N-terminal Primary Anchor, C-terminal Primary Anchor	16	SSX2 41-49 (A42V, V49I)	KVSEKIFYI		0.3	68	10
	17	SSX2 41-49 (A42L, V49I)	KLSEKIFYI		0.03	68	10
	18	SSX2 41-49 (A42a, V49v)	KaSEKIFYv		[illegible]	[illegible]	[illegible]

* μM; **ng/ml

Figure 5: Cross-reactivity and functional avidity of analogs substituted at more than two positions

Catergory	Number	Peptide name	Sequence	NOTE	Cross-reactivity and fct avidity (native to analogues) *	**Cross-reactivity and fct avidity (Nat to analogues, peak IFNgamma)	**Cross-reactivity and fct avidity (analogue conc at peak IFNgamma)
Native	1	SSX2 41-49	KASEKIFYV	Native peptide	1	46	10
N-terminal Primary/Secondary Anchor, C-terminal Primary Anchor	2	SSX2 41-49 (K41F, A42V, V49L)	FVSEKIFYL	Exploring the C-terminus anchor residue	1>10	20	10
	3	SSX2 41-49 (K41F, A42V, V49I)	FVSEKIFYI	Exploring the C-terminus anchor residue	0.3	49	10
	4	SSX2 41-49 (K41F, A42V, V49A)	FVSEKIFYA	Exploring the C-terminus anchor residue	1	45	10
	5	SSX2 41-49 (K41F, A42V, V49M)	FVSEKIFYM	Exploring the C-terminus anchor residue	10	85	10
	6	SSX2 41-49 (K41F, A42V, V49Nle)	FVSEKIFY(Nle)	Exploring the C-terminus anchor residue	10	0	N/A
	7	SSX2 41-49 (K41F, A42V, V49Nva)	FVSEKIFY(Nva)	Exploring the C-terminus anchor residue	0.1	63	10
	8	SSX2 41-49 (K41F, A42V, V49MeVal)	FVSEKIFY(MeVal)	Exploring the C-terminus anchor residue	10	30	10
	9	SSX2 41-49 (K41F, A42V, V49Aib)	FVSEKIFY(Aib)	Exploring the C-terminus anchor residue	10	0	N/A
	10	SSX2 41-49 (K41F, A42V, V49Abu)	FVSEKIFY(Abu)	Exploring the C-terminus anchor residue	1	48	10
N-terminal Primary/Secondary Anchor, TCR sites	11	SSX2 41-49 (K41F, A42V, E44D)	FVSDKIFYV	Exploring the TCR site	10	0	10
	12	SSX2 41-49 (K41F, A42V, E44N)	FVSNKIFYV	Exploring the TCR site	10	45	10
	13	SSX2 41-49 (K41F, A42V, E44S)	FVSSKIFYV	Exploring the TCR site	10	43	10
	14	SSX2 41-49 (K41F, A42V, E44T)	FVSTKIFYV	Exploring the TCR site	10	19	10
	15	SSX2 41-49 (K41F, A42V, E44Q)	FVSQKIFYV	Exploring the TCR site	10	30	10
	16	SSX2 41-49 (K41F, A42V, E44Nle)	FVS(Nle)KIFYV	Exploring the TCR site	10	42	10
	17	SSX2 41-49 (K41F, A42V, E44Nva)	FVS(Nva)KIFYV	Exploring the TCR site	3	37	10
	18	SSX2 41-49 (K41F, A42V, I46L)	FVSEKLFYV	Exploring the TCR site	3	43	10
	19	SSX2 41-49 (K41F, A42V, I46V)	FVSEKVFYV	Exploring the TCR site	1	53	3
	20	SSX2 41-49 (K41F, A42V, I46M)	FVSEKMFYV	Exploring the TCR site	10	0	N/A
	21	SSX2 41-49 (K41F, A42V, I46Nle)	FVSEK(Nle)FYV	Exploring the TCR site	10	35	10
	22	SSX2 41-49 (K41F, A42V, I46Nva)	FVSEK(Nva)FYV	Exploring the TCR site	1	33	10
	23	SSX2 41-49 (K41F, A42V, Y48T)	FVSEKIFTV	Exploring the TCR site	10	0	N/A
	24	SSX2 41-49 (K41F, A42V, Y48F)	FVSEKIFFV	Exploring the TCR site	10	14	10
	25	SSX2 41-49 (K41F, A42V, Y48S)	FVSEKIFSV	Exploring the TCR site	10	0	N/A
	26	SSX2 41-49 (K41F, A42V, Y48(Phe-4F))	FVSEKIF(Phe-4F)V	Exploring the TCR site	10	25	10
	27	SSX2 41-49 (K41F, A42V, Y48Phg)	FVSEKIF(Phg)V	Exploring the TCR site	10	0	N/A
	28	SSX2 41-49 (K41F, A42V, I46L, Y48T)	FVSEKLFTV	SAR based exploration	10	0	N/A
N-terminal Primary/Secondary Anchor, C-terminal Primary Anchor, TCR sites	30	SSX2 41-49 (K41F, A42V, I46L, Y48T, V49A)	FVSEKLFTA	SAR based exploration	10	0	N/A
	31	SSX2 41-49 (K41F, A42V, I46L, Y48S, V49A)	FVSEKLFSA	SAR based exploration	10	0	N/A

* μM; **ng/ml

Figure 6: Cross-reactivity and functional avidity of decamer analogs encompassing the nominal 41-49 peptide

Catergory	Number	Peptide name	Sequence	NOTE	Cross-reactivity and fct avidity (native to analogues) *	**Cross-reactivity and fct avidity (Nat to analogues, peak IFNgamma)	**Cross-reactivity and fct avidity (analogue conc at peak IFNgamma)
Native	1	SSX2 41-49	KASEKIFYV	Native peptide	1	46	10
Decamer	2	SSX2 41-50	KASEKIFYVY	Native peptide	>10	>20	>10
	3	SSX2 41-50 (Y50I)	KASEKIFYVI		10	25	10
	4	SSX2 41-50 (Y50L)	KASEKIFYVL		10	31	10
	5	SSX2 41-50 (Y50V)	KASEKIFYVV		>10	>20	>10
	6	SSX2 41-50 (Y50 (Nle))	KASEKIFYV(Nle)		>10	>20	>10
	7	SSX2 41-50 (Y50 (Nva))	KASEKIFYV(Nva)		>10	0	NA
	8	SSX2 41-50 (A42V,Y50I)	KVSEKIFYVI		10	44	10
	9	SSX2 41-50 (A42V,Y50L)	KVSEKIFYVL		10	41	10
	10	SSX2 41-50 (A42L,Y50L)	KLSEKIFYVL		3	58	10
	11	SSX2 41-50 (A42V,Y50V)	KVSEKIFYVV		10	25	10
	12	SSX2 41-50 (A42L,Y50V)	KLSEKIFYVV		10	44	10
	13	SSX2 41-50 (A42V,Y50(Nle))	KVSEKIFYV(Nle)		10	55	10
	14	SSX2 41-50 (A42L,Y50(Nle))	KLSEKIFYV(Nle)		10	39	10
	15	SSX2 41-50 (A42V,Y50(Nva))	KVSEKIFYV(Nva)		>10	0	NA
	16	SSX2 41-50 (A42L,Y50(Nva))	KLSEKIFYV(Nva)		10	40	10

* μM; **ng/ml

Figure 7

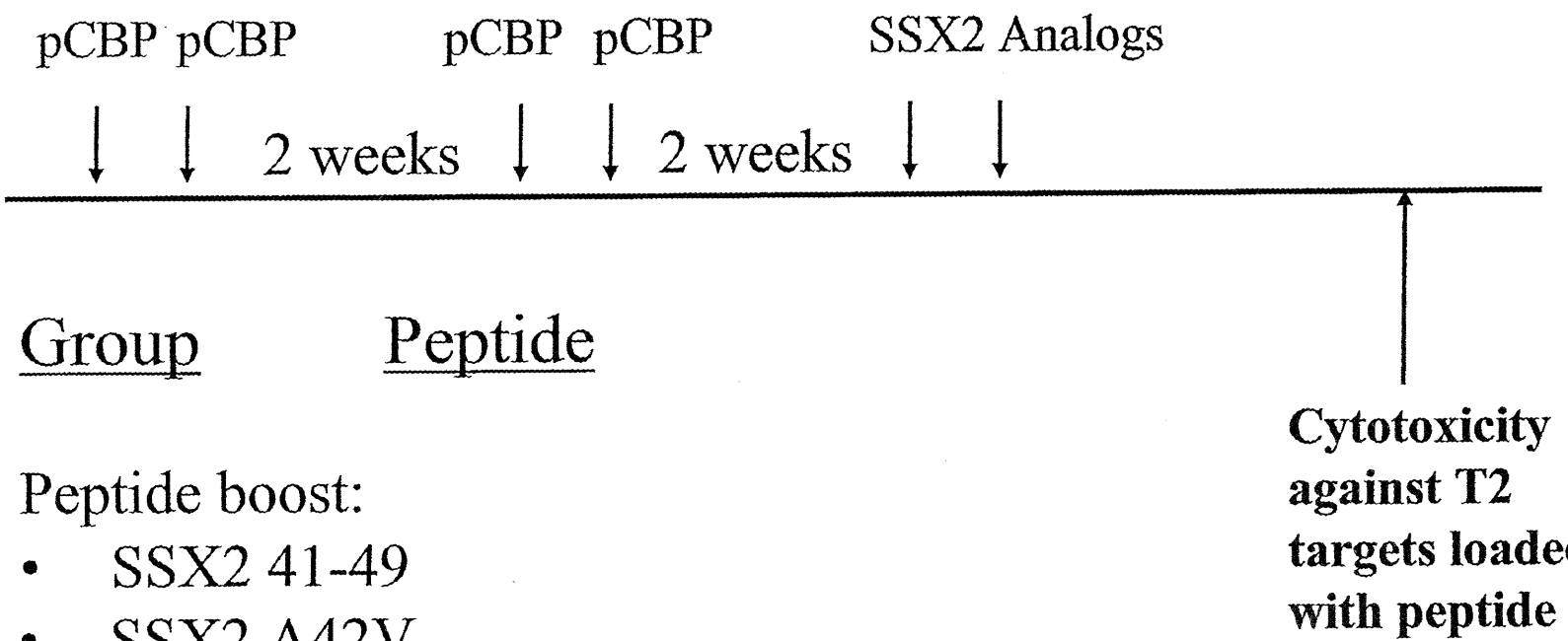

pCBP pCBP
pCBP pCBP
SSX2 Analogs
2 weeks
2 weeks
Group
Peptide
Peptide boost:
• SSX2 41-49
• SSX2 A42V
• SSX2 A42L
Cytotoxicity against T2 targets loaded with peptide

Figure 8:

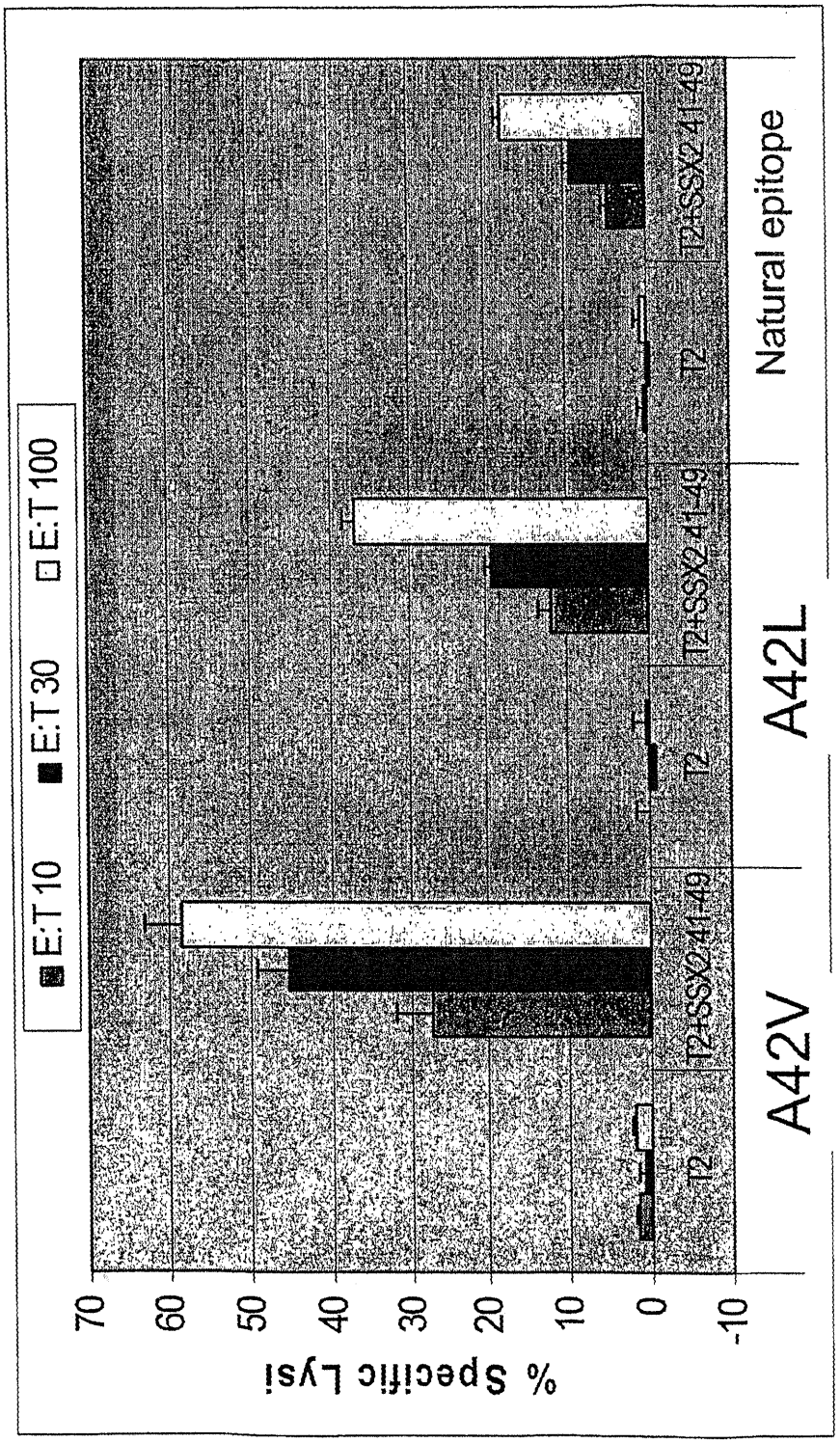
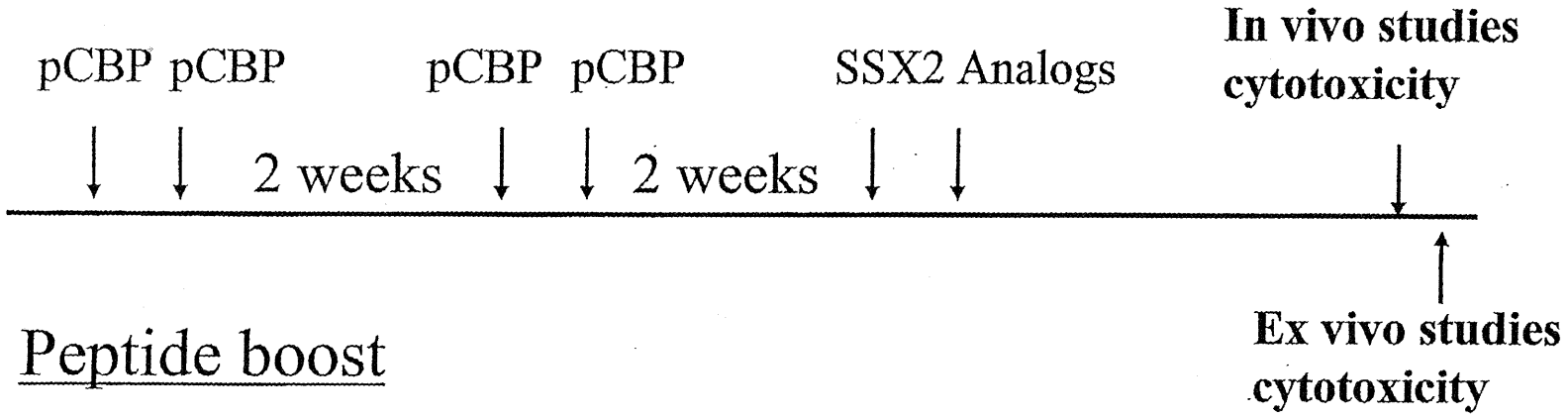

E:T 10
E:T 30
E:T 100
% Specific Lysi
70
60
50
40
30
20
10
0
-10
T2
T2+SSX2 41-49
T2
T2+SSX2 41-49
T2
T2+SSX2 41-49
A42V
A42L
Natural epitope
Binding (1/RA) 2.8 1.6 1.0
Stability (hours) 20 19 11

Figure 9



Peptide boost

- EAA Control
- SSX2 41-49
- SSX2 A42V
- SSX2 K41Y A42V
- SSX2 K41F
- SSX2 K41Y
- SSX2 A42V V49I
- SSX2 K41F A42V E44Nva

Figure 10

In Vivo Specific Lysis

Spleen	SSX2 1ug/ml Mean	SEM	SSX2 20ug/ml Mean	SEM
Control	-0.2	2.8	-0.2	2.8
SSX 41-49 natural	50.8	13.9	71.9	17.1
SSX2 A42V	72.4	4.42	94.7	3.22
SSX2 K41F	68.2	9.5	91.3	5.4
SSX2 K41Y	69.2	14.45	90.7	8.45
SSX2 A42V V49I	56.3	28.29	74.4	27.34
SSX2 K41Y A42V	46.8	23.5	67.2	29.1
SSX2 K41F A42V E44Na	53.0	7.6	70.1	14.7
EAA	0.6	2.85	1.7	1.8

Figure 11

In Vivo Specific Lysis

Blood	SSX2 1ug/ml Mean	SEM	SSX2 20ug/ml Mean	SEM	MHC Binding (1/RA)	MHC Stability (hrs)
Control	-0.01	0.88	0.3	1.61	-	-
SSX 41-49 natural	59.64	15.33	73.33	15.53	1.0	11
SSX2 A42V	79.31	3.91	90.84	0.72	2.8	20
SSX2 K41F	74.28	11.46	89.07	3.20	1.5	>24
SSX2 K41Y	72.49	11.69	88.21	4.02	1.7	>24
SSX2 A42V V49I	61.27	27.62	68.84	27.86	1.1	
SSX2 K41Y A42V	52.60	25.65	66.21	28.18		>24
SSX2 K41F A42V E44Nva	55.38	-	65.98	-	3.9	>24
EAA	3.06	1.26	2.61	3.96		

Figure 12:

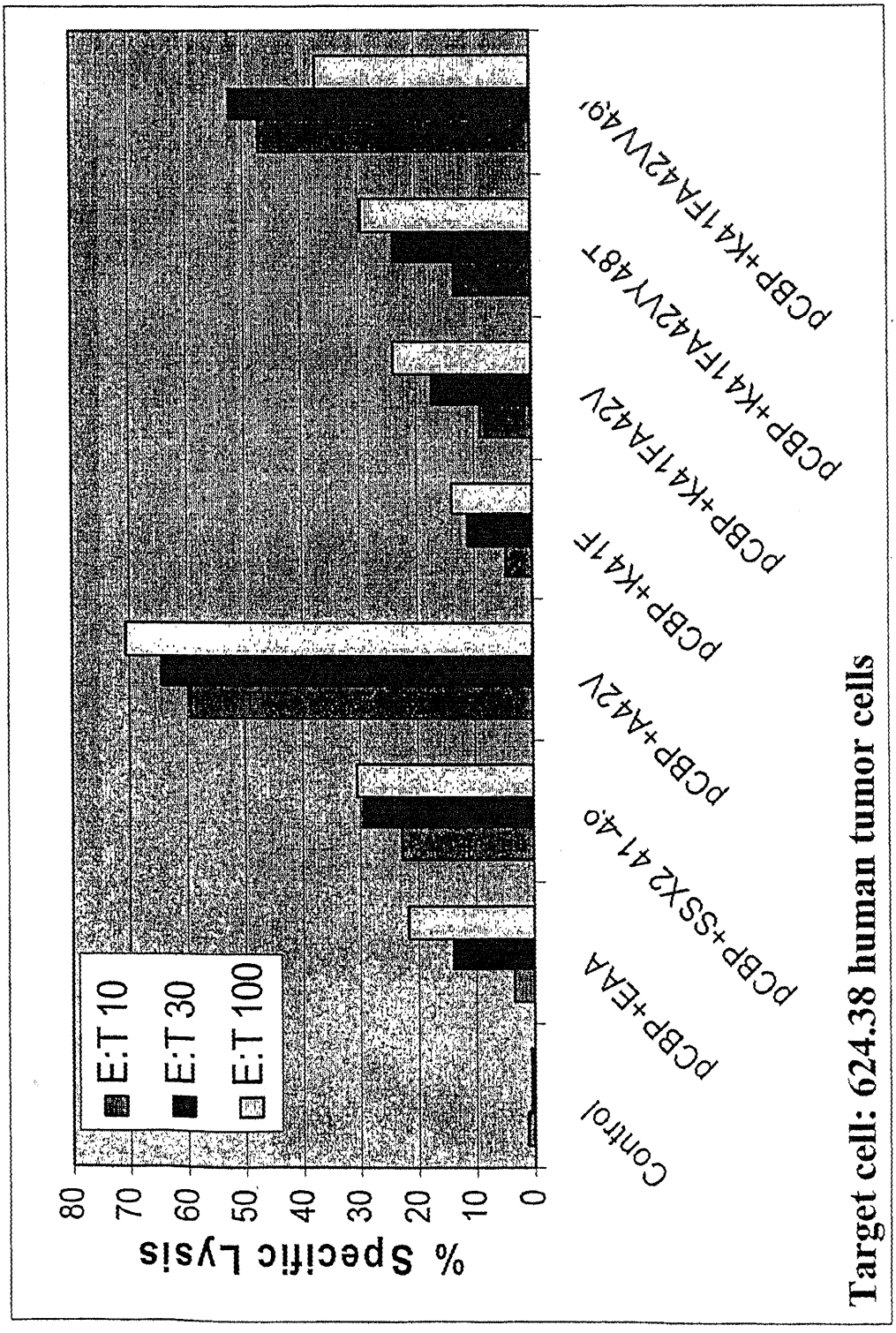

% Specific Lysis
0
10
20
30
40
50
60
70
80
E:T 10
E:T 30
E:T 100
Control
pCBP+EAA
pCBP+SSX2 41 40
pCBP+A42V
pCBP+K41F
pCBP+K41FA42V
pCBP+K41FA42VV48T
pCBP+K41FA42VV49I
Target cell: 624.38 human tumor cells

Figure 13A

* Min conc (uM) analogue for value > 10xbck
** Maximal conc of cytokine (ng/ml); bkg = 0.046+0.011 ng/ml
Comparable with or more potent than L's C165V
More potent than natural, less potent than L's
No significant activity

Catergory	Peptide name	Sequence	NOTE	Half-maximal Binding (μM)	Relative affinity (1/RA)	*Cross-reactivity and fct avidity (Nat to analogues, min conc)	**Cross-reactivity and fct avidity (Nat to analogues, peak IFNgamma)
Native	NY-ESO-1 157-165	SLLMWITQC	Native Peptide	27.89	1.0	0.3	1.83
N-terminal Primary Anchor	NY-ESO-1 157-165 (S157Y)	YLLMWITQC	Anchors explore	26.32	1.1	>10	0.43
	NY-ESO-1 157-165 (S157F)	FLLMWITQC	Anchors explore			10	1.9
	NY-ESO-1 157-165(S157K)	KLLMWITQC	Anchors explore (improve the solubility)			10	1.61
N-terminal Primary/Secondary Anchor	NY-ESO-1 157-165 (S157F, L158V)	FVLMWITQC	Anchors explore				
	NY-ESO-1 157-165 (S157F, L158I)	FILMWITQC	Anchors explore	12.19	2.3	>10	0.08
	NY-ESO-1 157-165 (S157Y, L158V)	YVLMWITQC	Anchors explore	27.07	1.0	>10	0.17
	NY-ESO-1 157-165 (S157Y, L158I)	YILMWITQC	Anchors explore	24.9	1.1	>10	0.28
N-terminal secondary and C-terminal primary anchor	NY-ESO-1 157-165 (S157Y, C165V)	YLLMWITQV	Anchors explore	1.92	14.5	0.3	0.78
	NY-ESO-1 157-165 (S157Y, C165L)	YLLMWITQL	Anchors explore	6.05	4.6	>10	0.45
	NY-ESO-1 157-165 (S157Y, C165A)	YLLMWITQA	Anchors explore	2.26	12.3	>10	0.28
	NY-ESO-1 157-165 (S157Y, C165I)	YLLMWITQI	Anchors explore	2.33	12.0	>10	0.35
	NY-ESO-1 157-165 (S157F, C165V)	FLLMWITQV	Anchors explore	2.95	9.5	0.001	1.67
	NY-ESO-1 157-165 (S157F, C165L)	FLLMWITQL	Anchors explore	1.96	14.2	0.03	0.74
	NY-ESO-1 157-165 (S157F, C165A)	FLLMWITQA	Anchors explore	1.84	15.2	1	1
	NY-ESO-1 157-165 (S157F, C165I)	FLLMWITQI	Anchors explore	2.09	13.3	0.03	0.91
	NY-ESO-1 157-165 (S157K, C165V)	KLLMWITQV	Anchors explore (improve the solubility)			0.001	1.66
	NY-ESO-1 157-165 (S157K, C165L)	KLLMWITQL	Anchors explore (improve the solubility)			0.03	2.77
	NY-ESO-1 157-165 (S157K, C165A)	KLLMWITQA	Anchors explore (improve the solubility)			1	0.68
	NY-ESO-1 157-165 (S157K, C165I)	KLLMWITQI	Anchors explore (improve the solubility)			0.01	1.65
	NY-ESO-1 157-165 (S157Y, C165(Abu))	YLLMWITQ(Abu)	Anchors explore			0.001	1.9
	NY-ESO-1 157-165 (S157Y, C165(Nva))	YLLMWITQ(Nva)	Anchors explore			0.001	1.5
	NY-ESO-1 157-165 (S157Y, C165(Nle))	YLLMWITQ(Nle)	Anchors explore				

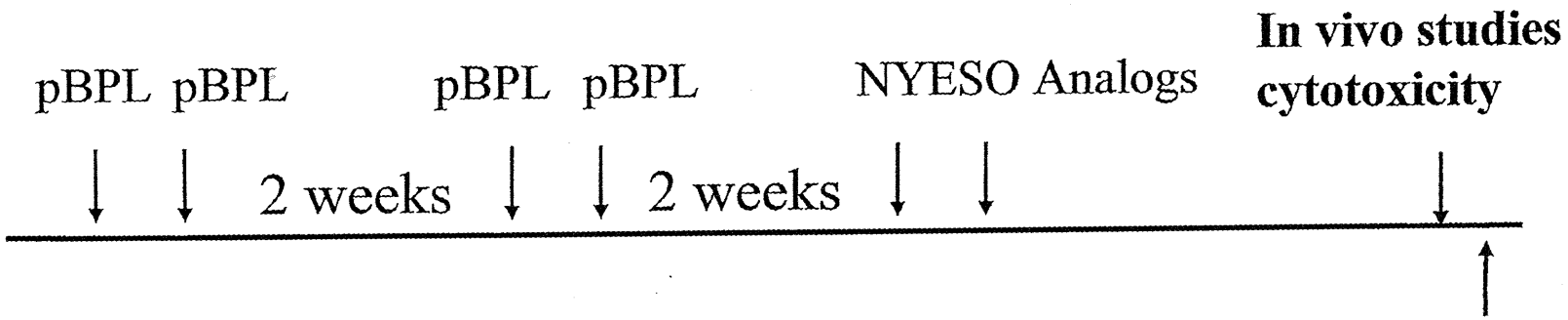
Figure 13B

Catergory	Peptide name	Sequence	NOTE	Half-maximal Binding (μM)	Relative affinity (1/RA)	*Cross-reactivity and fct avidity (Nat to analogues, min conc)	**Cross-reactivity and fct avidity (Nat to analogues, peak IFNgamma)
N-terminal primary/secondary and C-terminal primary anchor	NY-ESO-1 157-165 (S157Y, L158V, C165V)	YVLMMTQV	Anchors explore	2.33	12.0	1	0.72
	NY-ESO-1 157-165 (S157Y, L158V, C165L)	YVLMMTQL	Anchors explore	3.12	8.9	>10	0.08
	NY-ESO-1 157-165 (S157Y, L158V C165A)	YVLMMTQA	Anchors explore	5.44	5.1	>10	0.07
	NY-ESO-1 157-165 (S157Y, L158V C165I)	YVLMMTQI	Anchors explore	5.02	5.6	3	0.59
	NY-ESO-1 157-165 (S157Y, L158I, C165V)	YILMMTQV	Anchors explore	1.13	24.7	0.1	1.51
	NY-ESO-1 157-165 (S157Y, L158I, C165L)	YILMMTQL	Anchors explore	4.25	6.6	3	1.62
	NY-ESO-1 157-165 (S157Y, L158I, C165A)	YILMMTQA	Anchors explore	1.11	25.1	>10	0.12
	NY-ESO-1 157-165 (S157Y, L158I, C165I)	YILMMTQI	Anchors explore	2.31	12.1	3	0.81
	NY-ESO-1 157-165 (S157F, L158V, C165V)	FVLMMTQV	Anchors explore	1.63	17.1	0.3	1.21
	NY-ESO-1 157-165 (S157F, L158V, C165L)	FVLMMTQL	Anchors explore	1.15	24.3	0.3	0.6
	NY-ESO-1 157-165 (S157F, L158V, C165A)	FVLMMTQA	Anchors explore	4.26	6.5	>10	0.13
	NY-ESO-1 157-165 (S157F, L158V, C165I)	FVLMMTQI	Anchors explore	4.21	6.6	3	0.55
	NY-ESO-1 157-165 (S157F, L158I, C165V)	FILMMTQV	Anchors explore	12.28	2.3	0.3	0.81
	NY-ESO-1 157-165 (S157F, L158I, C165L)	FILMMTQL	Anchors explore	6.17	4.5	>10	0.37
	NY-ESO-1 157-165 (S157F, L158I, C165A)	FILMMTQA	Anchors explore	2.98	9.4	1	0.6
	NY-ESO-1 157-165 (S157F, L158I, C165I)	FILMMTQI	Anchors explore	3.55	7.9	>10	0.38
N-terminal secondary and TCR site	NY-ESO-1 157-165 (S157Y, M160A, C165V)	YLLAMTQV	TCR explore			>10	0.14
	NY-ESO-1 157-165 (S157Y, M160V, C165V)	YLLVMTQV	TCR explore	5.62	5.0	>10	0.32
	NY-ESO-1 157-165 (S157Y, M160I, C165V)	YLLIMTQV	TCR explore	6.9	4.0	>10	0.13
	NY-ESO-1 157-165 (S157Y, M160L, C165V)	YLLLMTQV	TCR explore	3.83	7.3	0.1	0.69
	NY-ESO-1 157-165 (S157Y, M160N, C165V)	YLLNMTQV	TCR explore	0.83	33.6	10	0.54
	NY-ESO-1 157-165 (S157Y, I162A, C165V)	YLLMMATQV	TCR explore	6.07	4.6	10	1.1
	NY-ESO-1 157-165 (S157Y, I162L, C165V)	YLLMMLTQV	TCR explore			3	0.58
	NY-ESO-1 157-165 (S157Y, I162T, C165V)	YLLMMTTQV	TCR explore	0.81	34.4	>10	0.08
	NY-ESO-1 157-165 (S157Y, I162V, C165V)	YLLMMVTQV	TCR explore	6.12	4.6	3	0.81
	NY-ESO-1 157-165 (S157Y, I162N, C165V)	YLLMMNTQV	TCR explore	4.53	6.2	10	0.54
	NY-ESO-1 157-165 (S157Y, Q164A, C165V)	YLLMMTAV	TCR explore	5.45	5.1	>10	0.31
	NY-ESO-1 157-165 (S157Y, Q164E, C165V)	YLLMMTEV	TCR explore			10	1.57
	NY-ESO-1 157-165 (S157Y, Q164D, C165V)	YLLMMTDV	TCR explore			10	0.49
	NY-ESO-1 157-165 (S157Y, Q164N, C165V)	YLLMMTNV	TCR explore			>10	0.36
	NY-ESO-1 157-165 (S157Y, Q164T, C165V)	YLLMMTTV	TCR explore			3	0.49
	NY-ESO-1 157-165 (S157Y, Q164S, C165V)	YLLMMTSV	TCR explore			>10	0.37

Figure 13C

Catergory	Peptide name	Sequence	NOTE	Half-maximal Binding (μM)	Relative affinity (1/RA)	*Cross-reactivity and fct avidity (Nat to analogues, min conc)	**Cross-reactivity and fct avidity (Nat to analogues, peak IFNgamma)
C-terminal amide	NY-ESO-1 157-165-NH2 (S157Y, C165V)	YLLMWTQV-NH2	Anchors explore			1	0.6
	NY-ESO-1 157-165-NH2 (S157Y, C165L)	YLLMWTQL-NH2	Anchors explore			3	0.68
	NY-ESO-1 157-165-NH2 (S157Y, C165A)	YLLMWTQA-NH2	Anchors explore			>10	0.04
C-terminal anchor (Ludwig patent)	NY-ESO-1 157-165 (C165V)	SLLMWTQV	Anchors explore	6.13	4.5	0.001	2.46
	NY-ESO-1 157-165 (C165L)	SLLMWTQL	Anchors explore			0.001	2.44
	NY-ESO-1 157-165 (C165A)	SLLMWTQA	Anchors explore	4.29	6.5	0.01	2.2
	NY-ESO-1 157-165 (C165I)	SLLMWTQI	Anchors explore	9.99	2.8	0.001	3.44
Decamer	NY-ESO-1 157-166	SLLMWTQCF	Based on the proposal by Ddiamond			>10	0.19
	NY-ESO-1 157-166 (F166L)	SLLMWTQCL	Based on the proposal by Ddiamond			10	0.78
	NY-ESO-1 157-166 (S157Y, C165V, F166L)	YLLMWTQVL	Based on the proposal by Ddiamond			1	0.95
	NY-ESO-1 157-166 (S157Y, C165V, F166I)	YLLMWTQVI	Based on the proposal by Ddiamond			1	0.65
	NY-ESO-1 157-166 (S157Y, C165V, F166V)	YLLMWTQVV	Based on the proposal by Ddiamond			>10	0.22
	NY-ESO-1 157-166 (S157Y, C165V, F166(Nle))	YLLMWTQV(Nle)	Based on the proposal by Ddiamond			10	1.68
	NY-ESO-1 157-166 (S157Y, C165V, F166 (Nva)	YLLMWTQV(Nva)	Based on the proposal by Ddiamond			>10	0.16
		SVLMWTQL	Additional peptides			0.03	2.93
		SVLMWTQI	Additional peptides			0.03	2.68
		SVLMWTQV	Additional peptides			0.001	3.84
		SILMWTQL	Additional peptides			0.001	3.25
		YNleLMWTQV	Additional peptides			0.003	2.61
		WVLMWTQV	Additional peptides			1	2.35
		SVLMWTQA	Additional peptides			0.3	1.87
		SVLMWTQC	Additional peptides			1	1.47
		TVLMWTQV	Additional peptides			0.01	3.64
		YNvaLMWTQV	Additional peptides			0.001	2.91
		SILMWTQC	Additional peptides			0.1	2.59
		SILMWTQA	Additional peptides			0.03	2.91
		SILMWTQV	Additional peptides			0.001	3.57

Figure 14



A: HBVc (neg ctrl)
B: natural peptide
C: C165V
D: L158V C165V
E: S157K L158V C165V
F: S157F L158V C165V
G: L158V C165Nva
H: L158Nva C165V

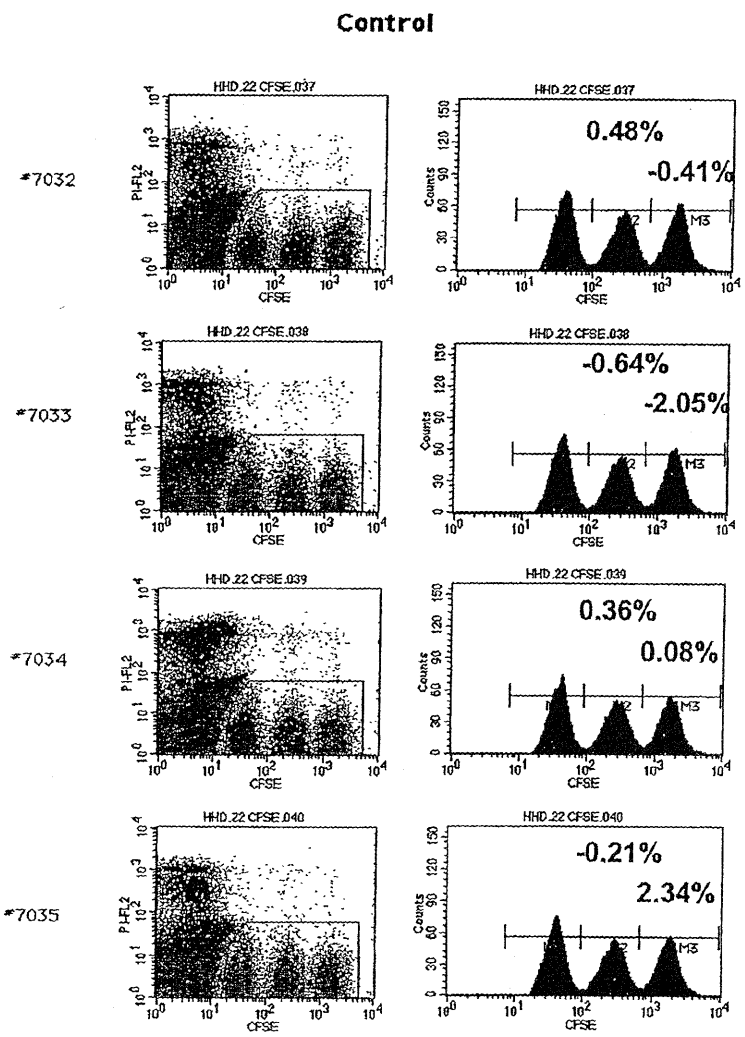
Selected peptides based on binding, stability, avidity, and cross reactivity data

N=4 HHD mice per group

Figure 15A

Immunization protocol for HHD Experiment 22:

2 clusters (4 total injections) of pBPL plasmid followed by 1 cluster of NY-ESO analogues



Targets:

M1 (CFSE Lo)= control (0uM)

M2 (CFSE med)= 20uM natural NY-ESO-1 peptide

M3 (CFSE Hi)= 1uM natural NY-ESO-1 peptide

Flow cytometry results include PI (vertical) and CFSE (horizontal)

Figure 15B

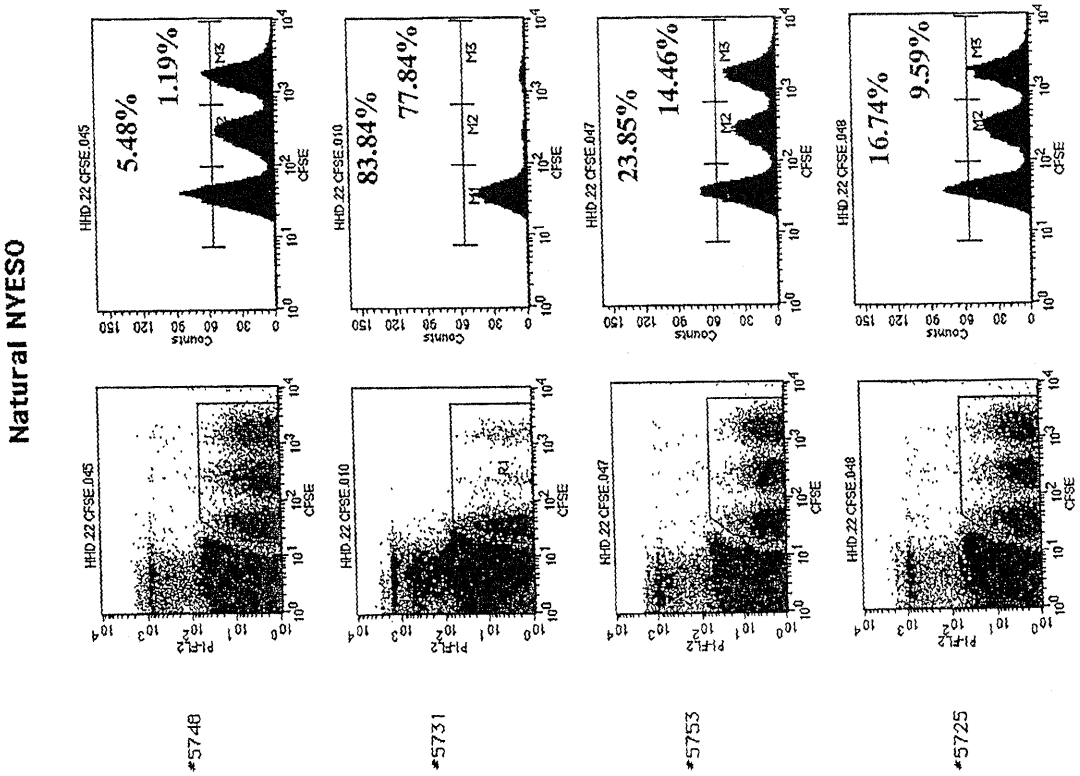

Natural NYESO
HHD.22 CFSE.045
5.48%
1.19%
HHD.22 CFSE.010
83.84%
77.84%
HHD.22 CFSE.047
23.85%
14.46%
HHD.22 CFSE.048
16.74%
9.59%
Counts
CFSE
PI+FL2
#5748
#5731
#5753
#5725

Figure 15C: (L158Nva, C165V)

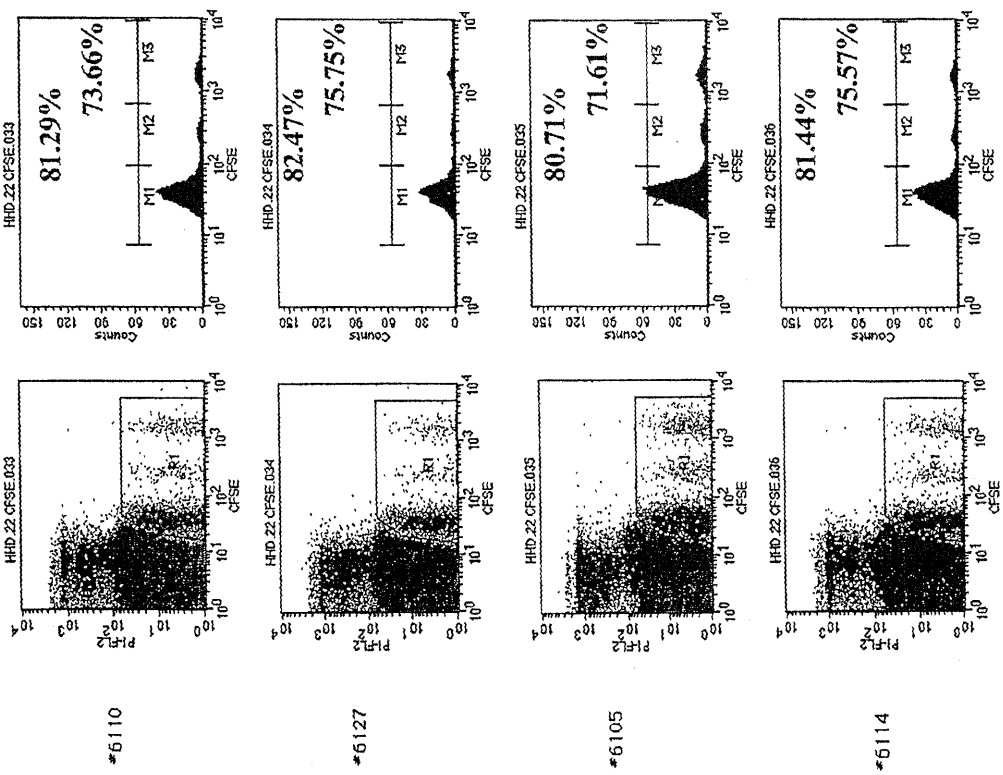


Figure 16A: CFSE *in vivo* cytoxicity assay of NY-ESO-1 prime-boost candidates

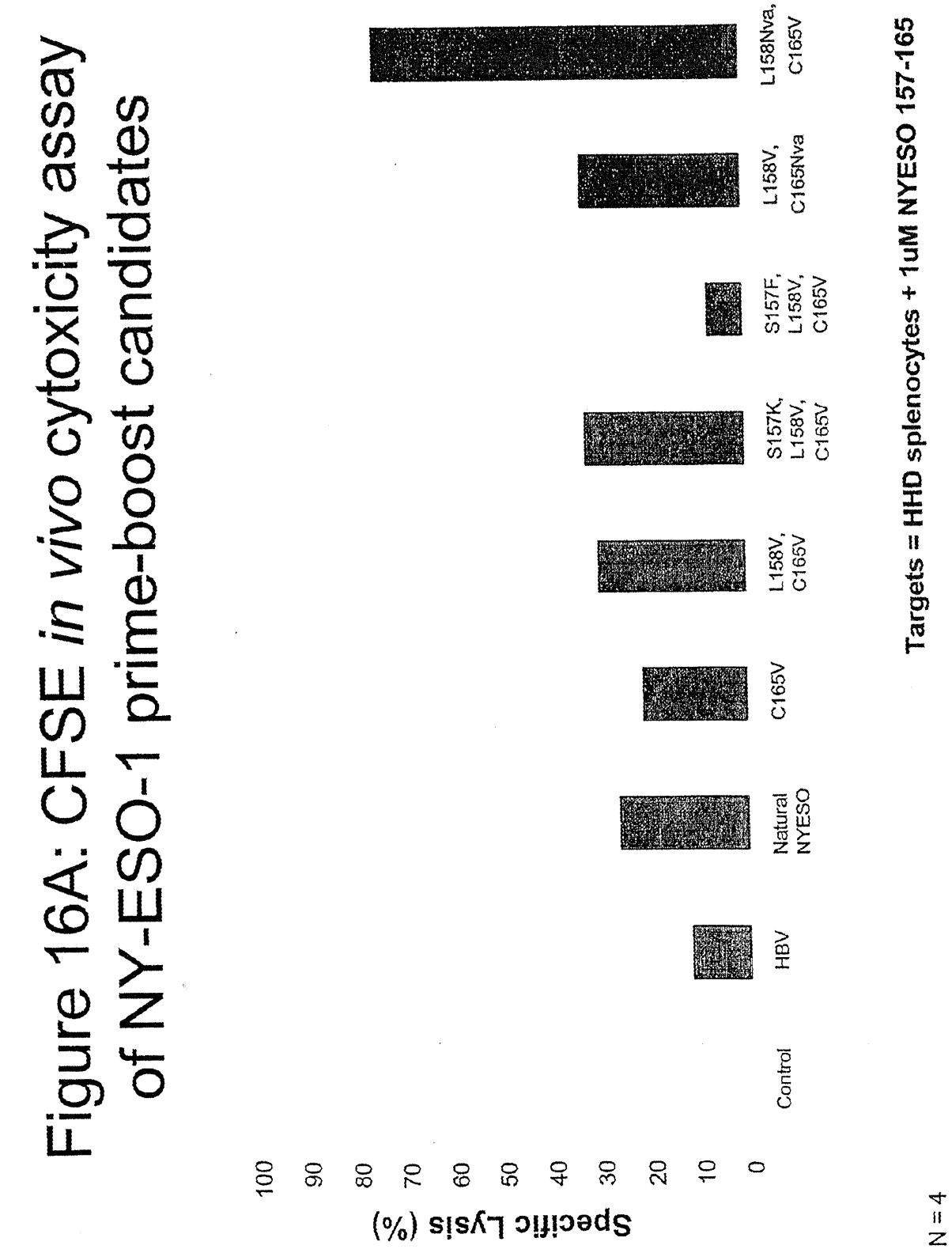


Figure 16B: CFSE *in vivo* cytoxicity assay of NY-ESO-1 prime-boost candidates

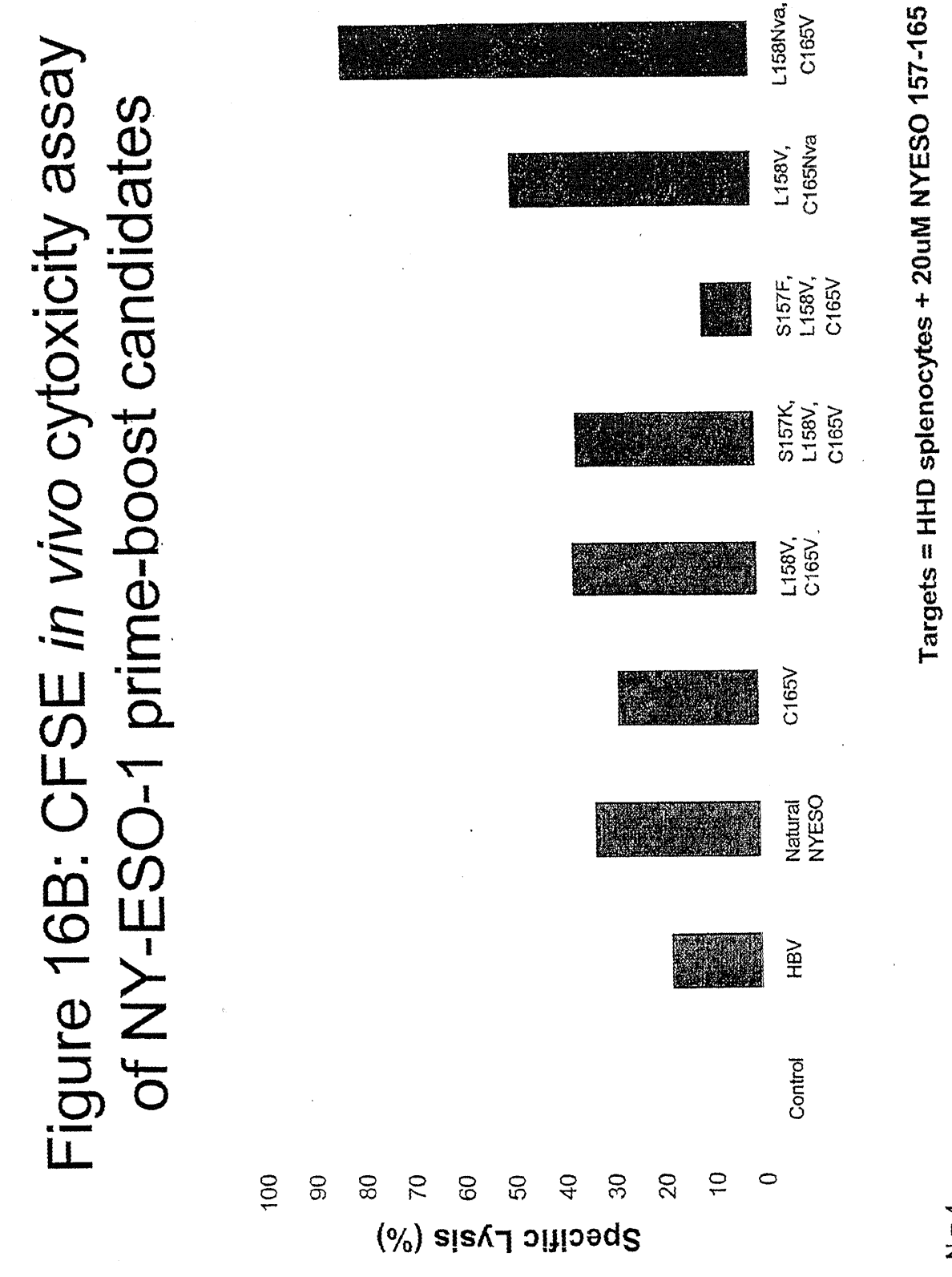


Figure 17A

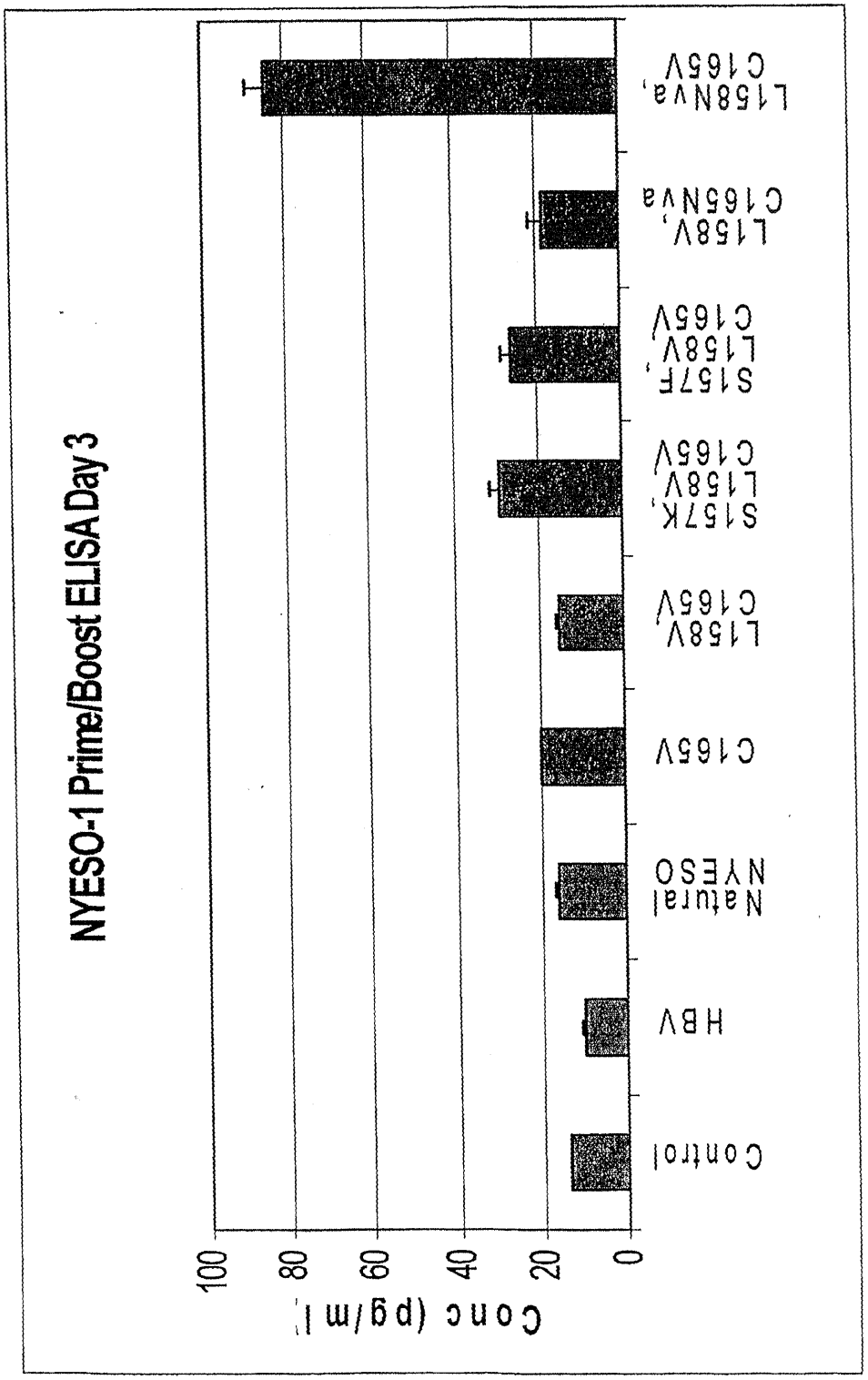

NYESO-1 Prime/Boost ELISA Day 3
Conc (pg/ml)
0
20
40
60
80
100
Control
HBV
Natural NYESO
C165V
L158V C165V
S157K, L158V C165V
S157F, L158V C165V
L158V, C165NVa
L158NVa, C165V

Figure 17B

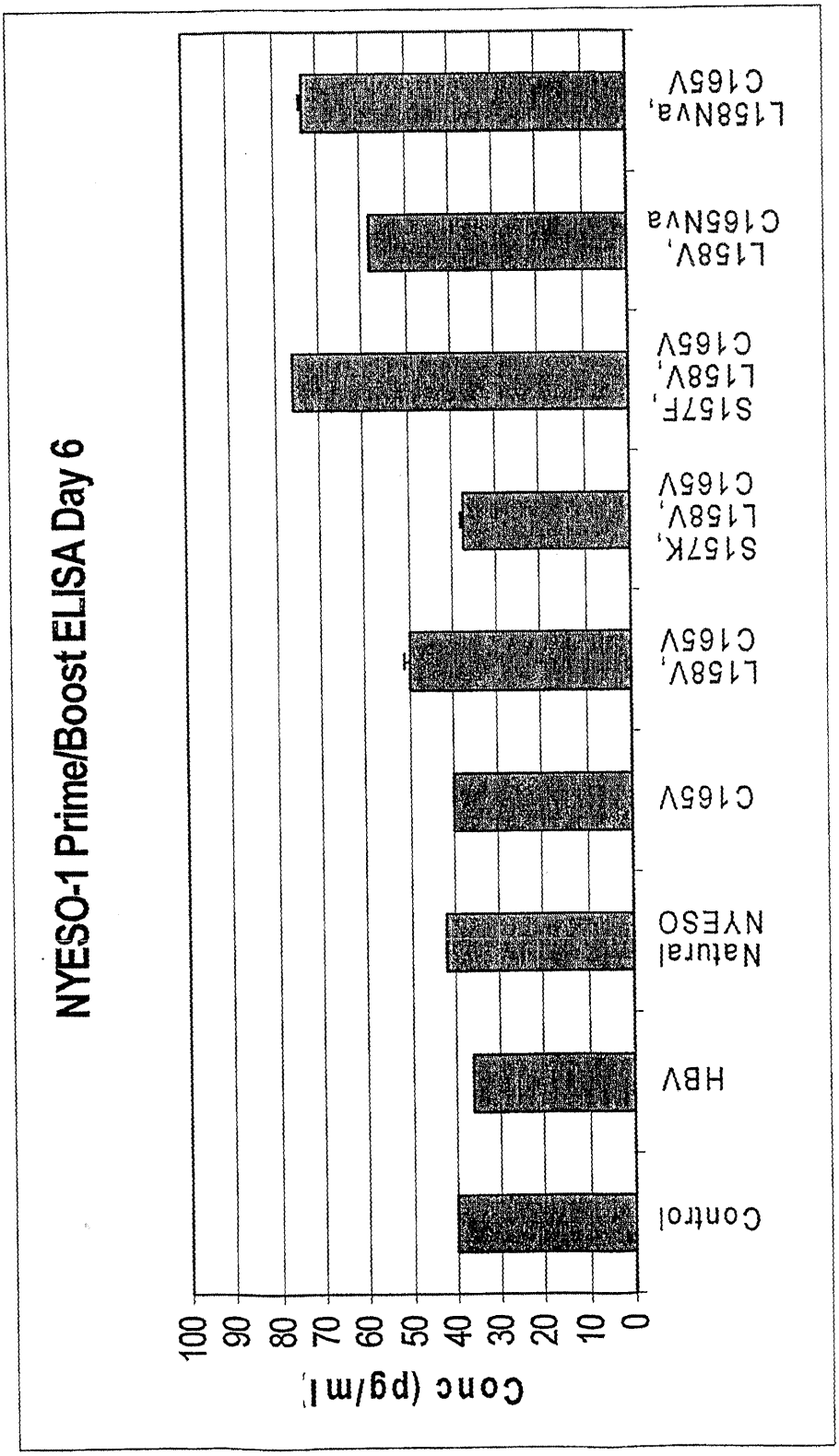
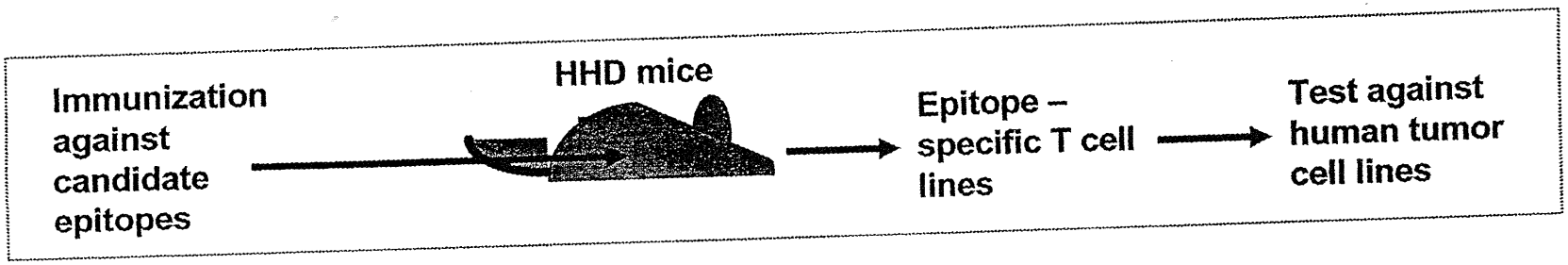

NYESO-1 Prime/Boost ELISA Day 6
Conc (pg/ml)
0
10
20
30
40
50
60
70
80
90
100
Control
HBV
Natural NYESO
C165V
L158V, C165V
S157K, L158V, C165V
S157F, L158V, C165V
L158V, C165NVa
L158NVa, C165V

Figure 18: $PSMA_{288-297}$ Epitope Validation –
Lysis of Human Tumor Cells by Specific T Cell Lines



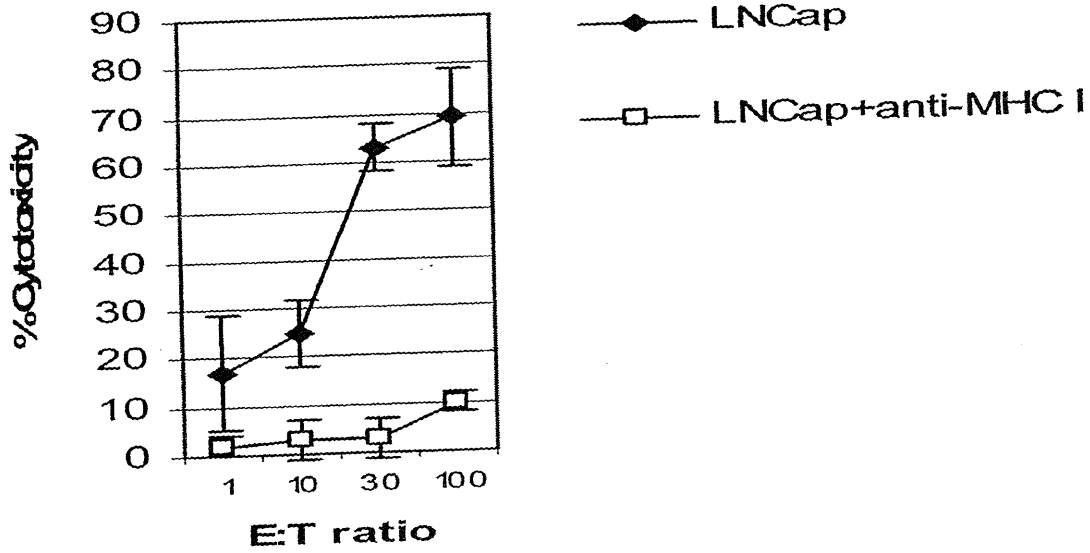


Figure 19: Single substitution analogs of $PSMA_{288-297}$

Category	SEQ ID No.	Peptide name	Sequence	% Binding	ED50 (M)	Stability (t1/2) (hr)	IScore	reactivity (IFN-γ)
Native	1	PSMA 288-297	GLPSIPVHPI	86.3	1.330E-06	5.341	0.693	27.29
N-terminal primary anchor	2	PSMA 288-297 (L289M)	GMPSIPVHPI	93.2	1.072E-06	3.340	0.605	-4.99
	3	PSMA 288-297 (L289I)	GIPSIPVHPI	75.3	1.314E-06	0.346	0.159	4.39
	4	PSMA 288-297 (L289Q)	GQPSIPVHPI	84.9	6.687E-07	1.445	0.402	-1.65
	5	PSMA 288-297 (L289V)	GVPSIPVHPI	69.9	2.414E-06	0.212	0.105	18.86
	6	PSMA 288-297 (L289(Nva))	G(Nva)PSIPVHPI					15.26
	7	PSMA 288-297 (L289(Nle))	G(Nle)PSIPVHPI	100.0	5.946E-07	4.342	0.81	5.36
	8	PSMA 288-297 (L289(Abu))	G(Abu)PSIPVHPI	65.9	1.234E-06	0.594	.189	13.85
N-terminal secondary anchor	9	PSMA 288-297 (G288A)	ALPSIPVHPI	74.4	9.806E-07	9.331	0.858	8.60
	10	PSMA 288-297 (G288S)	SLPSIPVHPI	87.7		4.337	0	12.55
	11	PSMA 288-297 (G288(Sar))	(Sar)GLPSIPVHPI	90.4	6.851E-07	8.786	1.037	6.41
	12	PSMA 288-297 (G288(Abu))	(Abu)LPSIPVHPI	101.4	4.083E-07	7.815	1.173	15.26
C-terminal primary anchor	13	PSMA 288-297 (I297V)	GLPSIPVHPV	98.6	8.344E-07	13.123	1.312	59.90
	14	PSMA 288-297 (I297L)	GLPSIPVHPL	83.6	1.166E-06	3.258	0.54	11.67
	15	PSMA 288-297 (I297(Nva))	GLPSIPVHP(Nva)	92.4	1.221E-06	10.062	1.02	40.19
	16	PSMA 288-297 (I297(Nle))	GLPSIPVHP(Nle)	97.3	8.525E-07	4.860	0.787	11.32
C-terminal secondary anchor/TCR exploration	17	PSMA 288-297 (P296A)	GLPSIPVHAI	90.4	8.160E-07	8.506	0.989	6.41
	18	PSMA 288-297 (P296L)	GLPSIPVHLI	86.3	5.859E-07	1.894	0.477	11.58
	19	PSMA 288-297 (P296S)	GLPSIPVHSI	83.6	4.468E-07	4.053	0.712	1.12
	20	PSMA 288-297 (P296T)	GLPSIPVHTI	82.2	4.629E-07	2.361	0.532	3.19
TCR expolration/ secondary anchor	21	PSMA 288-297 (P290W)	GLWSIPVHPI	102.7	7.770E-07	10.768	1.243	15.19

Figure 20: Double substitution analogs of $PSMA_{288-297}$

Category	SEQ ID No.	Peptide name	Sequence	% Binding	ED50 (M)	Stability (t1/2) (hr)	iScore	reactivity (IFN-γ)
Native	1	PSMA 288-297	GLPSIPVHPI	88.3	1.330E-06	5.341	0.693	27.29
N-terminal primary anchor and C-terminal primary	22	PSMA 288-297 (L289(Nva), I297(Nle))	G(Nva)PSIPVHP(Nle)	82.2	3.438E-07	1.044	0.374	6.37
	23	PSMA 288-297 (L289(Nle), I297(Nva))	G(Nle)PSIPVHP(Nva)	86.3	5.443E-07	4.308	0.728	13.85
	24	PSMA 288-297 (L289(Nva), I297(Nva))	G(Nva)PSIPVHP(Nva)	97.3	1.187E-06	6.356	0.849	10.81
	25	PSMA 288-297 (L289(Nle), I297(Nle))	G(Nle)PSIPVHP(Nle)	100.0	6.243E-07	4.548	0.647	6.52
	26	PSMA 288-297 (L289(Nva), I297V)	G(Nva)PSIPVHPV	94.6	2.195E-06	13.685	1.094	68.32
	27	PSMA 288-297 (L289(Nle), I297V)	G(Nle)PSIPVHPV	92.4	2.271E-06	13.332	1.053	57.46
	28	PSMA 288-297 (L289V, I297V)	GVPSIPVHPV	78.3	3.323E-06	1.624	0.301	8.43
	29	PSMA 288-297 (L289I, I297V)	GIPSIPVHPV	94.3	1.146E-06	2.478	0.634	0.00
	30	PSMA 288-297 (L289V, I297(Nva))	GVPSIPVHP(Nva)	96.6	8.236E-07	14.942	1.736	
	31	PSMA 288-297 (L289I, I297(Nva))	GIPSIPVHP(Nva)	81.6	2.692E-07	2.685	0.502	0.00
N-terminal secondary anchor and C-terminal primary anchor	32	PSMA 288-297 (G288A, I297V)	ALPSIPVHPV	88.0	5.967E-06	14.157	0.882	72.33
	33	PSMA 288-297 (G288A, I297(Nva))	ALPSIPVHP(Nva)	81.6	1.006E-06	10.329	0.924	48.11
	34	PSMA 288-297 (G288(Abu), I297V)	(Abu)LPSIPVHPV	85.1	7.888E-07	13.618	1.185	66.58
	35	PSMA 288-297 (G288(Abu), I297(Nva))	(Abu)LPSIPVHP(Nva)	92.0	9.469E-07	10.894	1.141	56.01
C-terminal primary anchor and TCR exploration	35	PSMA 288-297 (P290W, I297V)	GLWSIPVHPV	89	1.244E-06	12.082	1.082	10.83
	37	PSMA 288-297 (P290W, I297(Nva))	GLWSIPVHP(Nva)	102.2	8.329E-07	13.693	1.379	28.59
N-terminal primary anchor and TCR exploration	38	PSMA 288-297 (L289(Nva), P290W)	G(Nva)WSIPVHPI	101.1	1.411E-06	13.688	1.247	41.79
	39	PSMA 288-297 (L289V, P290W)	GVWSIPVHPI	83.7	2.184E-06	2.316	0.408	8.45
	40	PSMA 288-297 (L289(Nle), P290W)	G(Nle)WSIPVHPI	105.4	1.242E-06	10.527	1.156	21.2
	41	PSMA 288-297 (L289I, P290W)	GIWSIPVHPI	86.7	1.639E-07	9.667	1.202	0.00

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Figure 21: Triple substitution analogs of $PSMA_{288-297}$

Category	SEQ ID No.	Peptide name	Sequence	% Binding	ED50 (M)	Stability (t1/2) (hr)	iScore	reactivity (IFN-γ)
Native	1	PSMA 288-297	GLPSIPVHPI	86.3	1.330E-06	5.341	0.693	27.29
N-terminal primary/secondary anchor and TCR exploration	42	PSMA 288-297 (G288A, L289(Nva), P290W)	A(Nva)WSIPVHPI	96.6	1.307E-06	13.559	1.582	26.59
	43	PSMA 288-297 (G288A, L289V, P290W)	AVWSIPVHPI	85.1	8.419E-07	4.626	0.662	6.26
	44	PSMA 288-297 (G288A, L289(Nle), P290W)	A(Nle)WSIPVHPI	108.7	2.484E-06	13.775	1.2	71.97
	45	PSMA 288-297 (G288A, L289I, P290W)	AIWSIPVHPI	90.8	1.340E-07	5.124	0.841	0.00
N-terminal primary/secondary anchor and C-terminal primary anchor	46	PSMA 288-297 (G288A, L289(Nva), I297V)	A(Nva)PSIPVHPV	85.9	1.264E-06	13.517	1.108	119.1
	47	PSMA 288-297 (G288A, L289V, I297V)	AVPSIPVHPV	78.3	1.082E-06	2.765	0.477	16.06
	48	PSMA 288-297 (G288A, L289V, I297(Nva))	AVPSIPVHP(Nva)	98.9	2.854E-07	16.057	1.68	
N- and C-terminal primary anchor and TCR	49	PSMA 288-297 (L289(Nva), P290W, I297V)	G(Nva)WSIPVHPV	102.2	1.372E-06	17.756	1.439	60.91
	50	PSMA 288-297 (L289V, P290W, I297V)	GVWSIPVHPV	78	1.917E-06	9.562	0.803	0.00
	51	PSMA 288-297 (L289(Nva), P290W, I297(Nva))	G(Nva)WSIPVHP(Nva)	78.2	3.655E-07	0.707	0.239	0.00
	52	PSMA 288-297 (L289V, P290W, I297(Nva))	GVWSIPVHP(Nva)	92.4	2.747E-06	3.706	0.537	8.1
N-terminal secondary, C-terminal primary anchor and TCR	53	PSMA 288-297 (G288A, P290W, I297V)	ALWSIPVHPV	102.3	4.937E-07	13.530	1.672	6.19
	54	PSMA 288-297 (, G288A, P290W, I297(Nva))	ALWSIPVHP(Nva)	106.5	4.332E-06	13.715	1.069	6.6

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Figure 22: Immunogenicity of various $PSMA_{288-297}$ Analogs Measured by Elispot

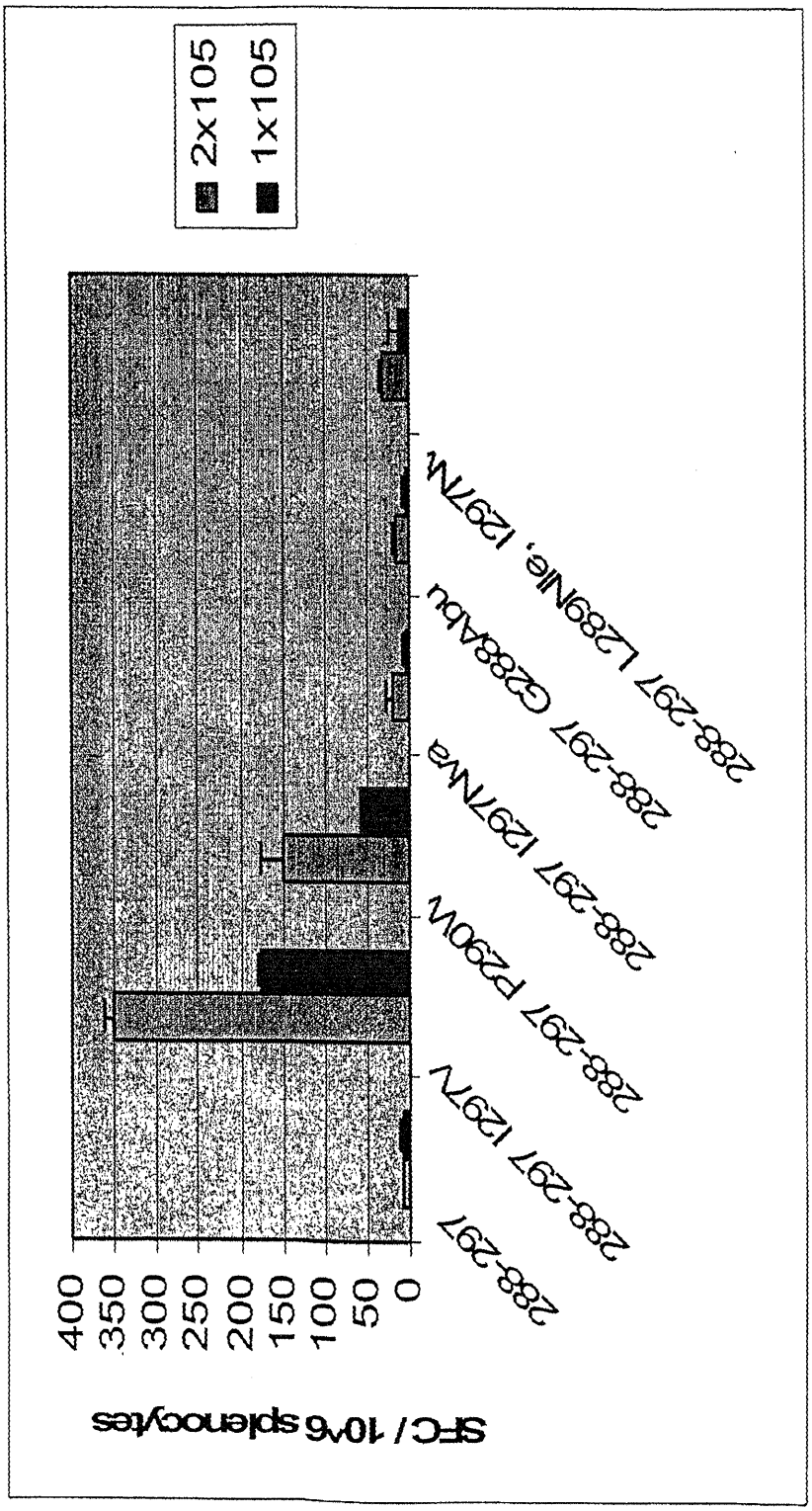


Figure 23: Amplification of anti-PSMA$_{288-297}$ response by the I297V Analog, Measured by Elispot.

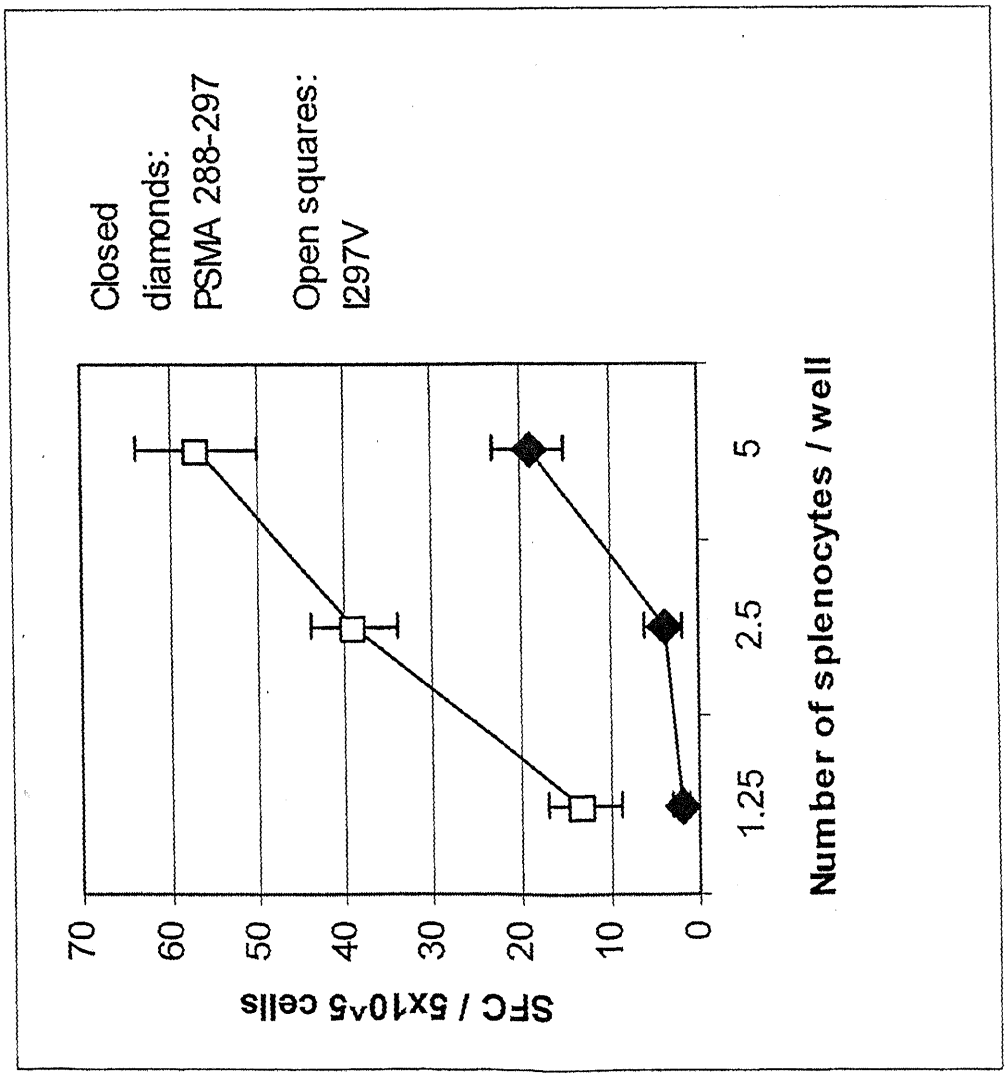


Figure 24: Boosting with I297V Epitope Analog Results in Cytotoxic Immunity against a PSMA+ Human Tumor Cell Line.

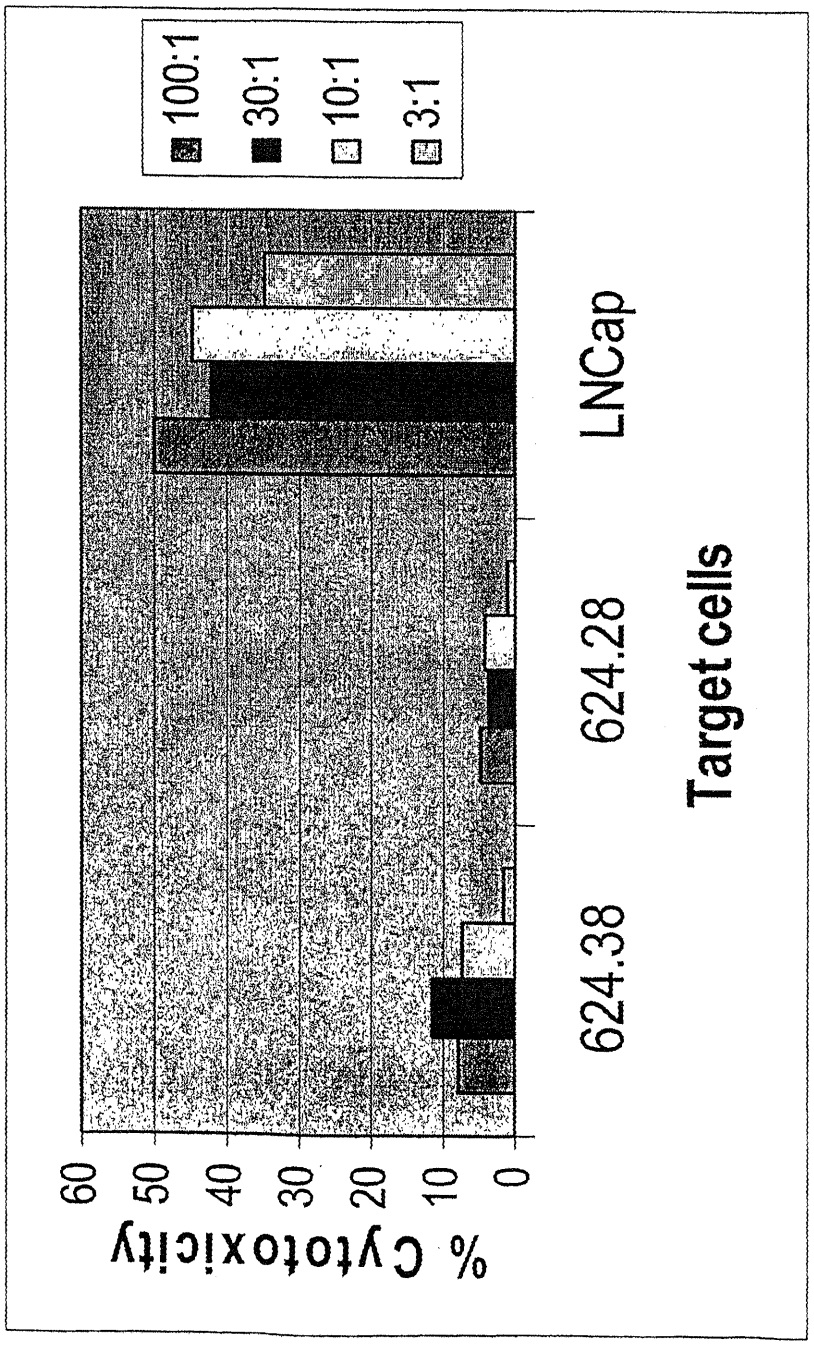
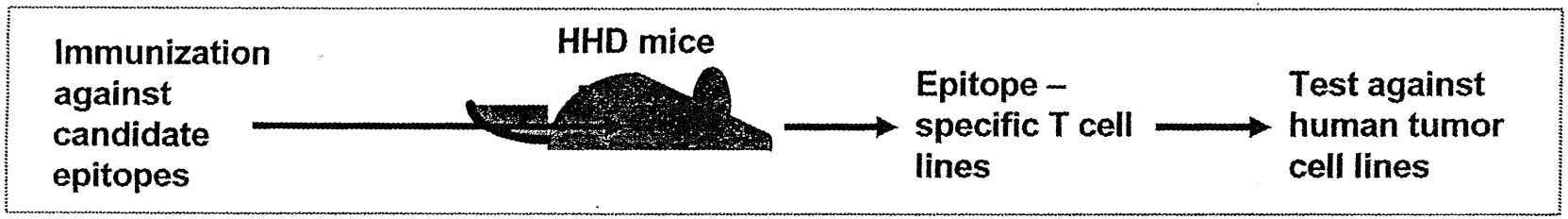


Figure 25: $PRAME_{425-433}$ Epitope Validation – Lysis of Human Tumor Cells by Specific T Cell Lines



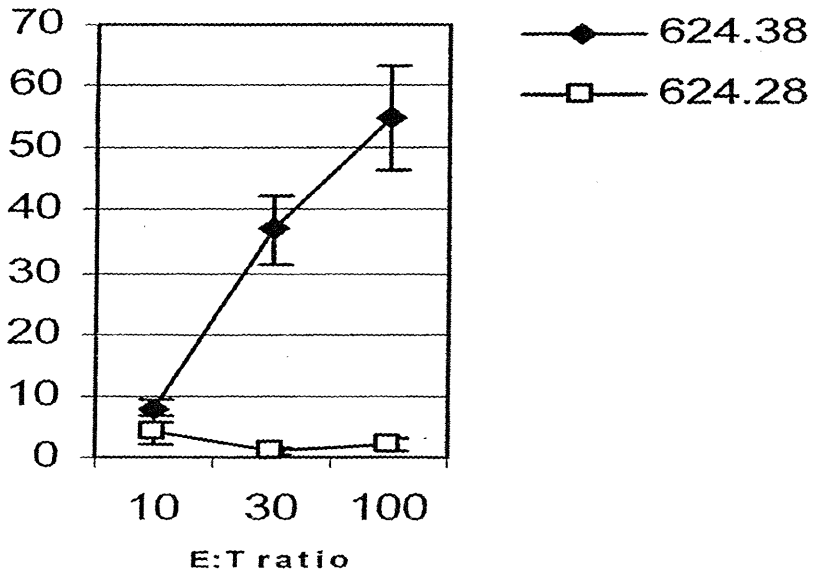


Figure 26: Single substitution analogs of PRAME 425-433

Category	SEQ ID No.	Peptide name	Sequence	% Binding (A0201)	Stability (t1/2) (hr) (A0201)	ED50 (mM) (A0201)	iScore (A0201)	Cross-reactivity as normalized IFN-gamma production
Native	1	Prame 425-433	SLLQHLIGL	81	12.2	7.158E-07	1.17	1.00
N-terminal primary anchor	2	Prame 425-433 (L426V)	SVLQHLIGL	66	7.1	6.178E-06	0.49	0.4
	3	Prame 425-433 (L426M)	SMLQHLIGL	100	10.3	9.884E-07	1.14	0.6
	4	Prame 425-433 (L426I)	SILQHLIGL	76	9.4	1.628E-06	0.80	*
	5	Prame 425-433 (L426(Nle))	S(Nle)LQHLIGL	93	12.6	2.423E-06	1.01	0.55
	6	Prame 425-433 (L426(Nva))	S(Nva)LQHLIGL	92	11.4	1.911E-06	1.00	0.8
	7	Prame 425-433 (L426(Abu))	S(Abu)LQHLIGL	73	8.1	4.849E-06	0.56	*
N-terminal secondary anchor	8	Prame 425-433 (S425K)	KLLQHLIGL	82	10.8	4.412E-06	0.77	0.4
	9	Prame 425-433 (S425F)	FLLQHLIGL	110	17.7	4.335E-07	1.86	0.52
	10	Prame 425-433 (S425Y)	YLLQHLIGL	99	11.8	5.496E-07	1.34	0.8
	11	Prame 425-433 (S425T)	TLLQHLIGL	88	9.3	1.452E-06	0.92	0.81
	12	Prame 425-433 (S425(Orn) (Orn is L-ornithine)	(Orn)LLQHLIGL	89	10.0	2.252E-06	0.89	0.55
	13	Prame 425-433 (S425(Hse) (Hse is L-Homoserine)	(Hse)LLQHLIGL	90	10.6	1.360E-06	1.01	0.85
C-terminal primary anchor	14	Prame 425-433 (L433V)	SLLQHLIGV	80	12.6	7.310E-06	0.74	*
	15	Prame 425-433 (L433I)	SLLQHLIGI	75	11.9	3.349E-06	0.79	*
	16	Prame 425-433 (L433A)	SLLQHLIGA	80	13.6	8.430E-06	0.85	*
	17	Prame 425-433 (L433(Nle))	SLLQHLIG(Nle)	98	14.8	7.768E-07	1.40	0.72
	18	Prame 425-433 (L433(Nva))	SLLQHLIG(Nva)	92	17.2	1.663E-06	1.26	0.52
	19	Prame 425-433 (L433(Abu))	SLLQHLIG(Abu)	81	12.3	3.411E-06	0.85	*
C-terminal amide	20	Prame 425-433-NH2	SLLQHLIGL-NH_2	89	3.0	1.384E-06	0.53	0.6
TCR exploration	21	Prame 425-433 (G432A)	SLLQHLIAL	88	12.4	1.728E-06	1.02	*
	22	Prame 425-433 (G432S)	SLLQHLISL	71	10.7	4.357E-06	0.69	*
	23	Prame 425-433 (G432(Sar)) Sar is sarcosine	SLLQHLI(Sar)L	74	9.2	2.368E-06	0.73	*
	24	Prame 425-433 (L427(Nle))	SL(Nle)QHLIGL	81	12.3	2.493E-06	0.90	*
	25	Prame 425-433 (L427(Nva))	SL(Nva)QHLIGL	78	11.7	3.153E-06	0.81	0.24
	26	Prame 425-433 (L427(Abu))	SL(Abu)QHLIGL	74	10.1	9.258E-06	0.60	*
	27	Prame 425-433 (L430(Nle))	SLLQH(Nle)IGL	81	11.2	4.740E-06	0.77	*
	28	Prame 425-433 (L430(Nva))	SLLQH(Nva)IGL	74	11.4	7.265E-06	0.66	0.61
	29	Prame 425-433 (L430(Abu))	SLLQH(Abu)IGL	76	10.6	5.140E-06	0.70	0.44

Figure 27A: Double substitution analogs of PRAME 425-433

Category	SEQ ID No.	Peptide name	Sequence	% Binding (A0201)	Stability (t1/2) (hr) (A0201)	ED50 (mM) (A0201)	iScore (A0201)	Cross-reactivity as normalized IFN-gamma production
Native	1	Prame 425-433	SLLQHLIGL	81	12.2	7.158E-07	1.17	1.00
N-terminal primary/secondary anchor	30	Prame 425-433 (S425F, L426(Nva))	F(Nva)LQHLIGL	106	11.7	4.518E-07	1.46	*
	31	Prame 425-433 (S425Y, L426(Nva))	Y(Nva)LQHLIGL	96	9.4	1.660E-06	0.97	*
	32	Prame 425-433 (S425T, L426(Nva))	T(Nva)LQHLIGL	87	10.7	2.386E-06	0.92	*
	33	Prame 425-433 (S425(Hse), L426(Nva))	(Hse)(Nva)LQHLIGL	90	13.6	1.449E-06	1.12	*
	34	Prame 425-433 (S425(Orn), L426(Nva))	(Orn)(Nva)LQHLIGL	88	12.3	5.019E-06	0.85	*
	35	Prame 425-433 (S425F, L426(Nle))	F(Nle)LQHLIGL	118	11.4	5.661E-07	1.52	*
	36	Prame 425-433 (S425Y, L426(Nle))	Y(Nle)LQHLIGL	110	11.5	5.572E-07	1.44	*
	37	Prame 425-433 (S425T, L426(Nle))	T(Nle)LQHLIGL	100	12.6	2.554E-06	1.07	*
	38	Prame 425-433 (S425(Hse), L426(Nle))	(Hse)(Nle)LQHLIGL	81	10.9	2.546E-06	0.84	*
	39	Prame 425-433 (S425(Orn), L426(Nle))	(Orn)(Nle)LQHLIGL	80	9.9	4.704E-06	0.83	*
	40	Prame 425-433 (S425F, L426M)	FMLQHLIGL	100	12.8	1.160E-06	1.24	*
	41	Prame 425-433 (S425Y, L426M)	YMLQHLIGL	109	11.7	2.861E-07	1.62	*
	42	Prame 425-433 (S425T, L426M)	TMLQHLIGL	83	9.2	2.011E-06	0.89	*
	43	Prame 425-433 (S425(Hse), L426M)	(Hse)MLQHLIGL	93	11.7	4.674E-06	0.88	*
	44	Prame 425-433 (S425(Orn), L426M)	(Orn)MLQHLIGL	86	10.5	2.951E-06	0.90	*
	45	Prame 425-433 (S425F, L426I)	FILQHLIGL	98	10.3	1.152E-07	1.63	*
	46	Prame 425-433 (S425Y, L426I)	YILQHLIGL	87	10.1	7.155E-07	1.07	*
	47	Prame 425-433 (S425T, L426I)	TILQHLIGL	75	8.1	3.321E-06	0.65	*
	48	Prame 425-433 (S425(Hse), L426I)	(Hse)ILQHLIGL	75	9.9	2.774E-06	0.72	*
	49	Prame 425-433 (S425(Orn), L426I)	(Orn)ILQHLIGL	84	8.8	1.952E-06	0.81	*

Figure 27B: Double substitution analogs of PRAME 425-433

Category	SEQ ID No.	Peptide name	Sequence	% Binding (A0201)	Stability (t1/2) (hr) (A0201)	ED50 (mM) (A0201)	iScore (A0201)	Cross-reactivity as normalized IFN-gamma production
Native	1	Prame 425-433	SLLQHLIGL	81	12.2	7.158E-07	1.17	1.00
N-terminal primary anchor and C-terminal primary anchor	50	Prame 425-433 (L426(Nva),L433(Nva))	S(Nva)LQHLIG(Nva)	91	13.2	2.936E-06	0.99	*
	51	Prame 425-433 (L426(Nva),L433(Nle))	S(Nva)LQHLIG(Nle)	93	13.7	8.475E-07	1.27	0.69
	52	Prame 425-433 (L426(Nva),L433V)	S(Nva)LQHLIGV	88	16.0	2.980E-06	1.06	*
	53	Prame 425-433 (L426(Nle),L433(Nva))	S(Nle)LQHLIG(Nva)	90	12.5	1.125E-06	1.13	*
	54	Prame 425-433 (L426(Nle),L433(Nle))	S(Nle)LQHLIG(Nle)	100	12.4	9.950E-07	1.25	*
	55	Prame 425-433 (L426(Nle),L433V)	S(Nle)LQHLIGV	93	13.9	2.481E-06	1.07	*
	56	Prame 425-433 (L426M,L433(Nva))	SMLQHLIG(Nva)	99	13.4	6.878E-07	1.37	*
	57	Prame 425-433 (L426M,L433(Nle))	SMLQHLIG(Nle)	104	11.2	6.148E-07	1.34	*
	58	Prame 425-433 (L426M,L433V)	SMLQHLIGV	97	15.5	9.401E-07	1.38	*
N-terminal secondary anchor and C-terminal primary anchor	59	Prame 425-433 (S425K, L433V)	KLLQHLIGV	77	14.2	9.038E-06	0.74	*
	60	Prame 425-433 (S425F, L433V)	FLLQHLIGV	110	16.3	7.180E-07	1.64	*
	61	Prame 425-433 (S425Y, L433V)	YLLQHLIGV	91	14.3	2.816E-06	1.19	*
	62	Prame 425-433 (S425T, L433V)	TLLQHLIGV	85	17.2	3.722E-06	1.02	*
	63	Prame 425-433 (S425(Orn), L433V)	(Orn)LLQHLIGV	79	12.9	2.740E-06	0.89	*
	64	Prame 425-433 (S425(Hse), L433V)	(Hse)LLQHLIGV	77	10.2	5.849E-06	0.67	*
	65	Prame 425-433 (S425F, L433(Nle))	FLLQHLIGNle	88	23.3	1.538E-07	2.14	1.16
	66	Prame 425-433 (S425T, L433(Nle))	TLLQHLIGNle	88	13.9	1.251E-06	1.15	0.75

Figure 28: Triple substitution analogs of PRAME$_{425-433}$

Category	SEQ ID No.	Peptide name	Sequence	% Binding	ED50 (M)	Stability (t1/2) (hr)	iScore	Cross-reactivity as normalized IFN-γ production
Native	1	Prame 425-433	SLLQHLIGL	81	7.158E-07	12.2	1.17	1.00
N-terminal primary/secondary anchor and C-terminal primary anchor	67	Prame 425-433 (S425F, L426(Nva), L433(Nle))	FNvaLQHLIGNle	108	1.158E-07	25.3	2.78	0.82
	68	Prame 425-433 (S425T, L426(Nva), L433(Nle))	TNvaLLQHLIGNle	118	2.844E-07	19.6	2.24	0.91
	69	Prame 425-433 (S425F, L426M, L433(Nle))	FMLQHLIGNle	124	1.803E-07	13.1	2.05	ND
	70	Prame 425-433 (S425T, L426M, L433(Nle))	TMLQHLIGNle	97	6.802E-07	13.5	1.36	ND

Figure 29: Immunogenicity of a **PRAME**$_{425-433}$ Analog Measured by Elispot

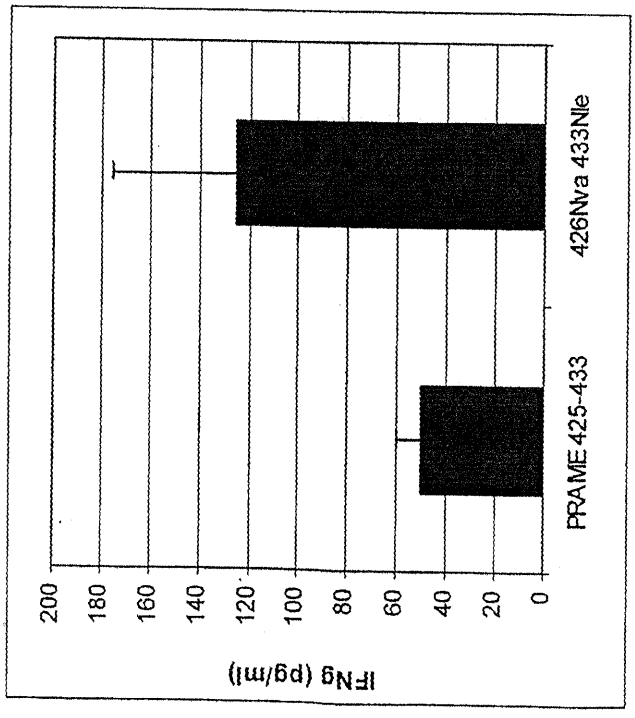


Figure 30: Boosting with the L426Nva L433Nle Epitope Analog Results in *in vivo* Cytotoxicity Against Native Epitope Coated Cells.

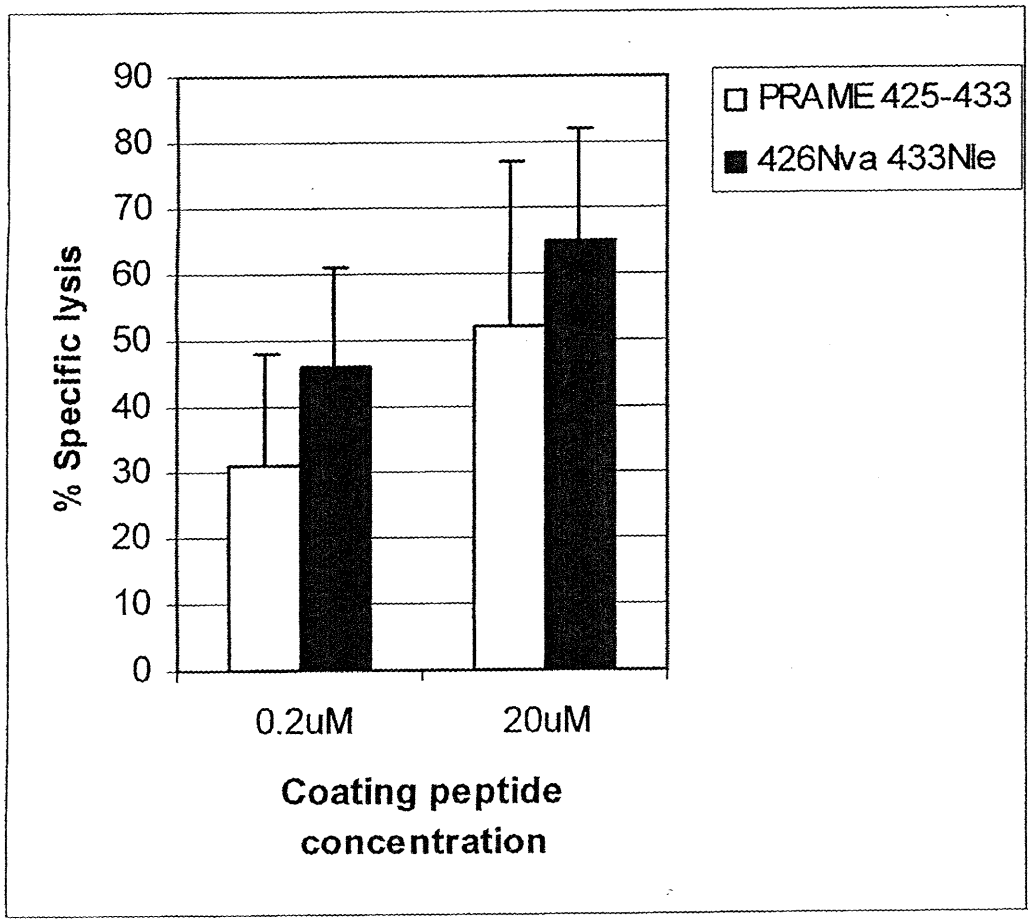


Figure 31: *In Vivo* Evaluation of PRAME Analogs

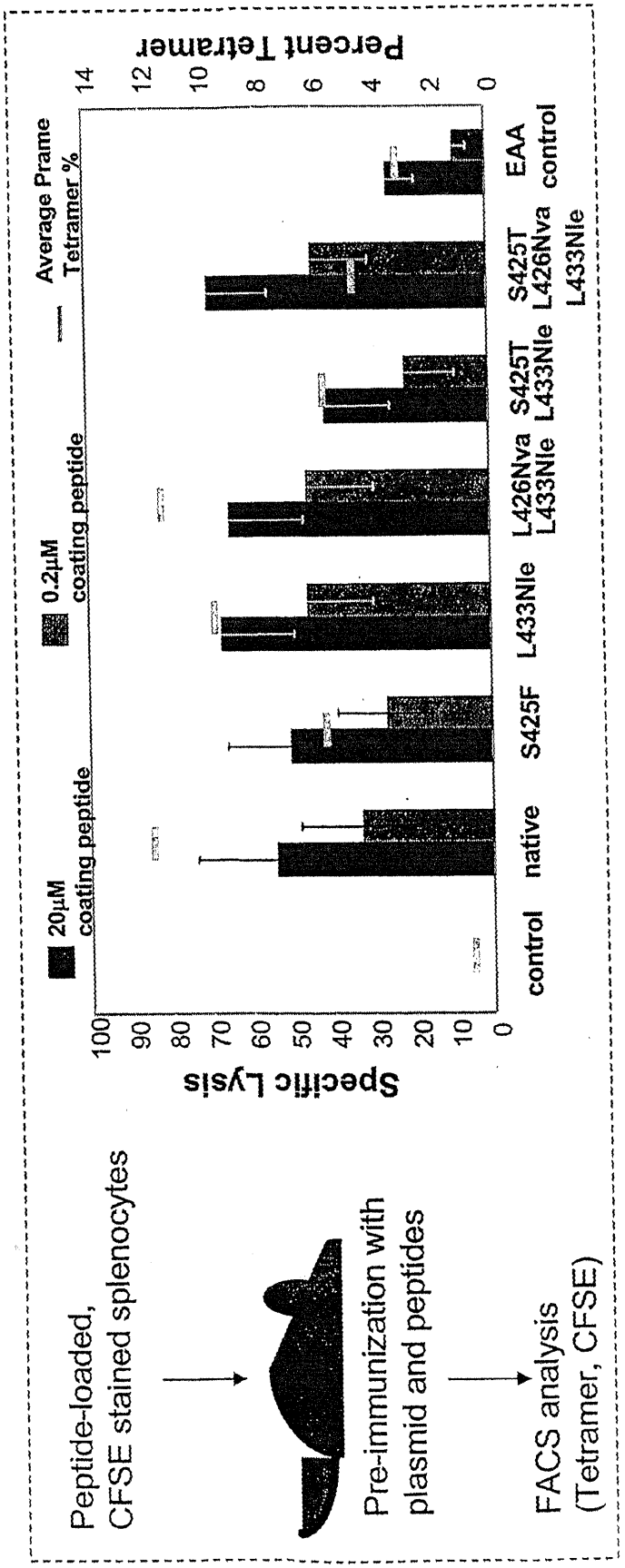


Figure 32: *Ex vivo* cytokine production of analog-induced, native epitope re-stimulated T cells.



Immunization with plasmid and boost with peptides

↓

Ex vivo stimulation (native peptide, dose-effect) and ELISA

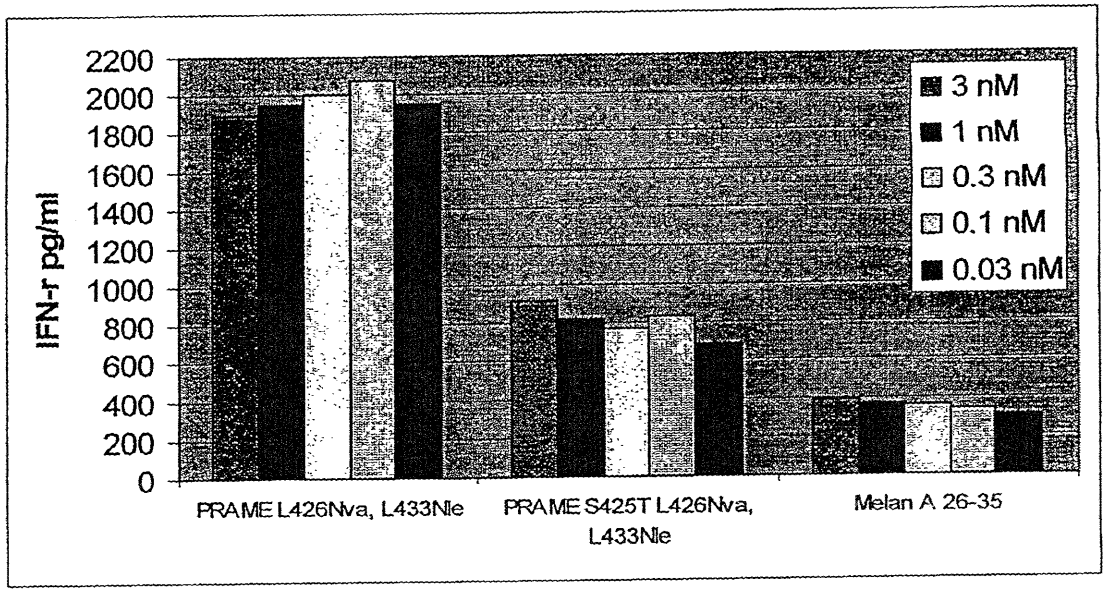
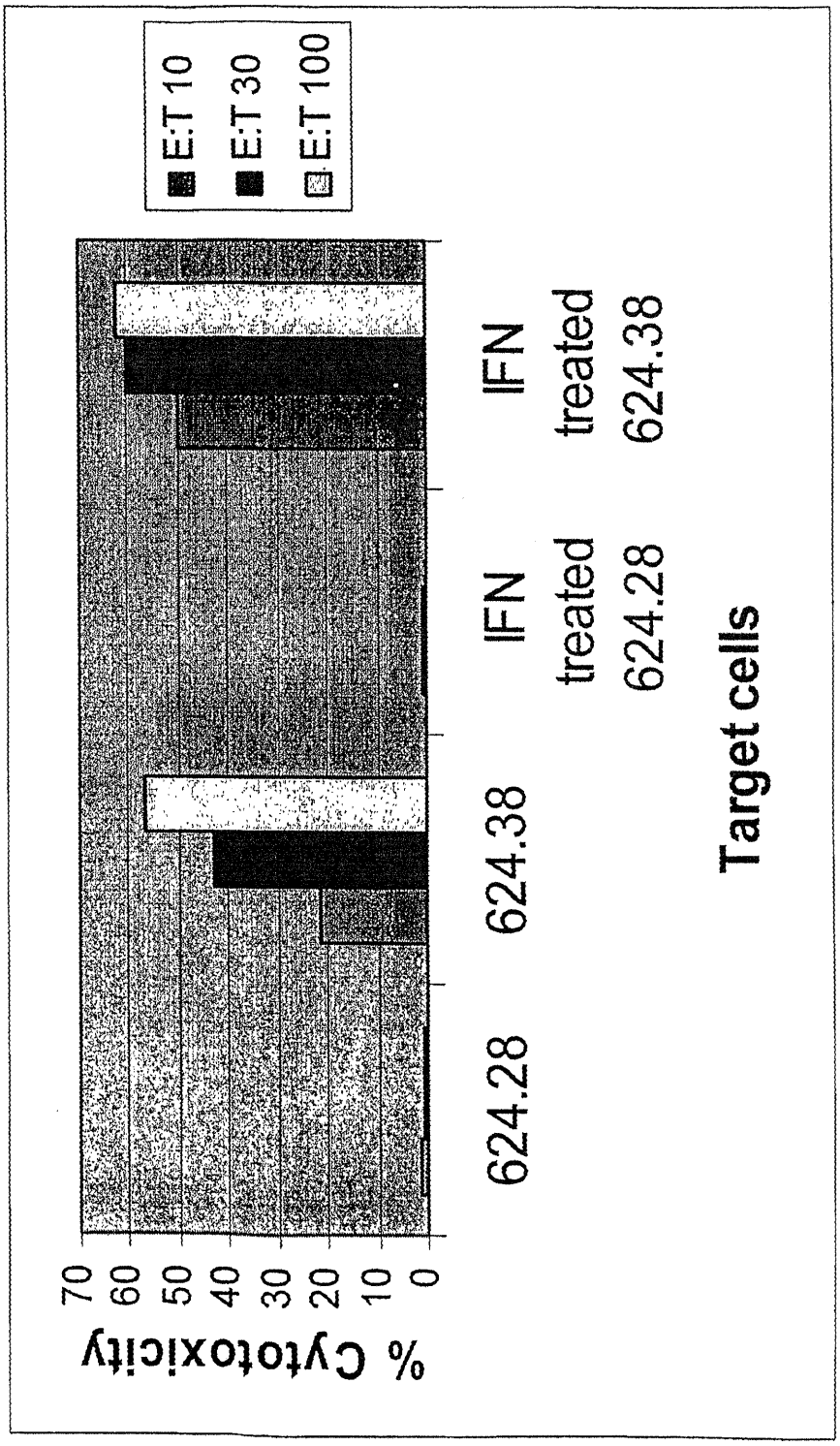


Figure 33: Boosting with the **L426Nva L433Nle** Analogue Results in Cytotoxic Immunity against a Human Tumor Cell Line



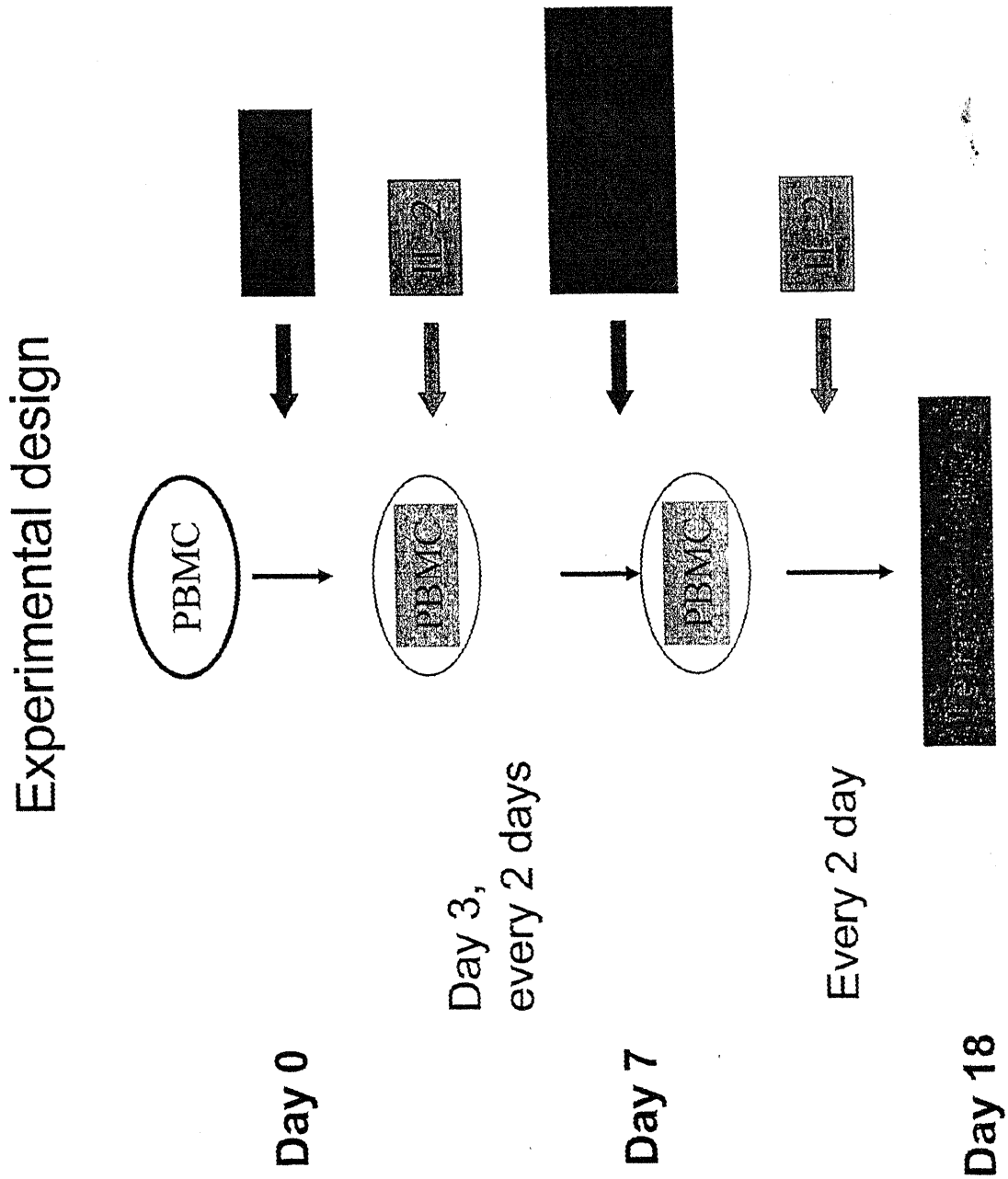


Figure 34: In vitro immunization

Figure 35: *In vitro* Immunization with a PRAME Analog Generates Tetramer$^+$ Cells

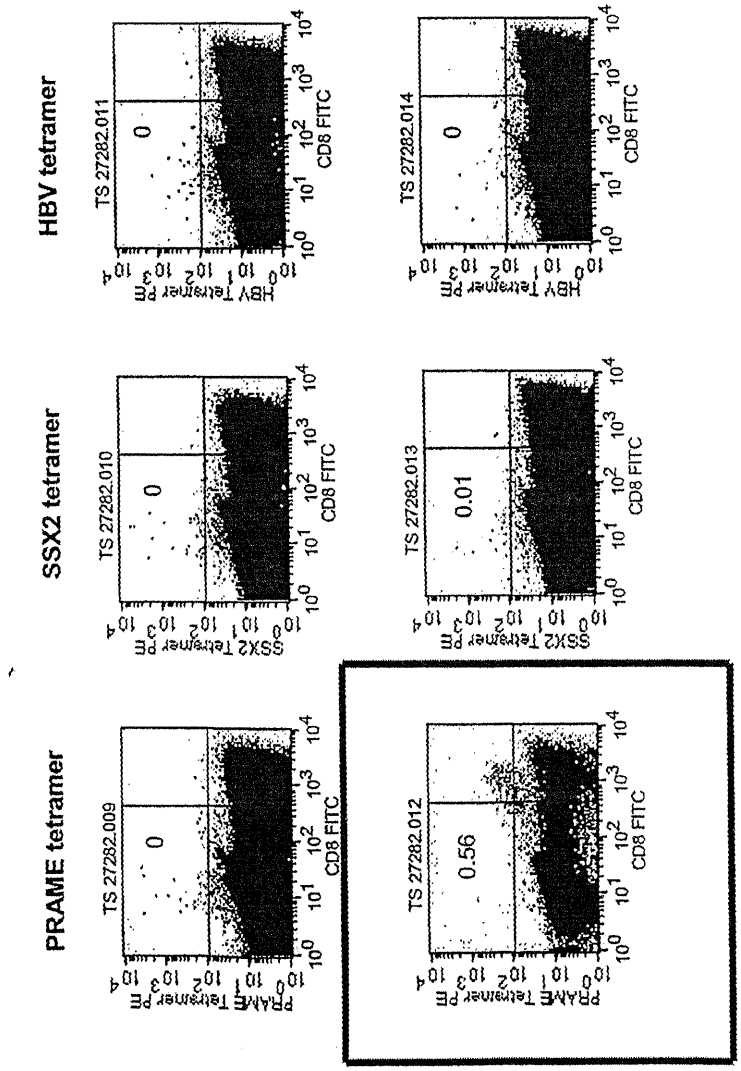


Figure 36: pCTLR2, A Plasmid expressing the **PRAME**$_{425-433}$ Epitope

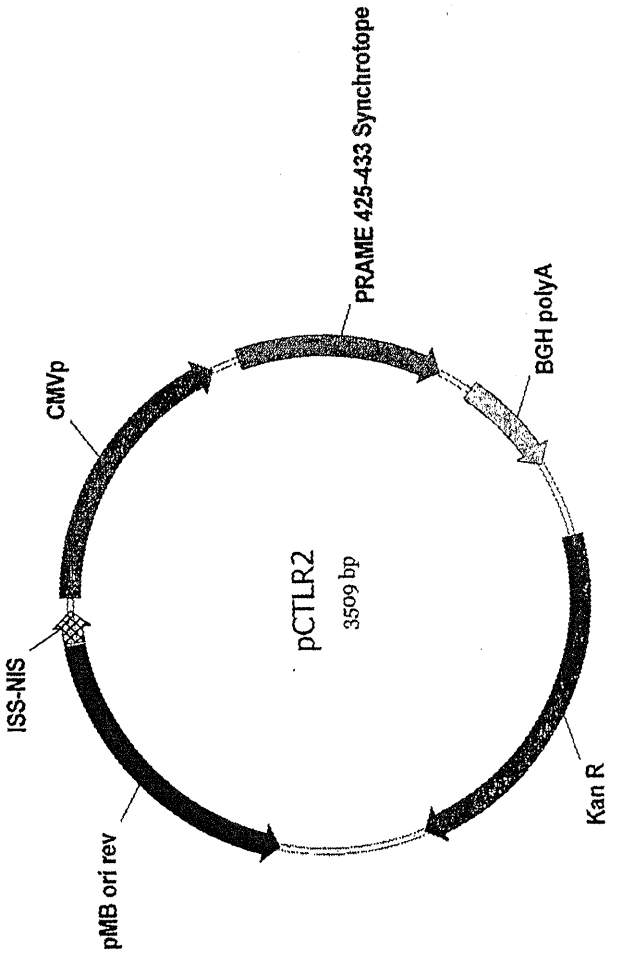


Figure 37: **Lysis of 624.38 cells by T cells primed with plasmid DNA and boosted with peptide**

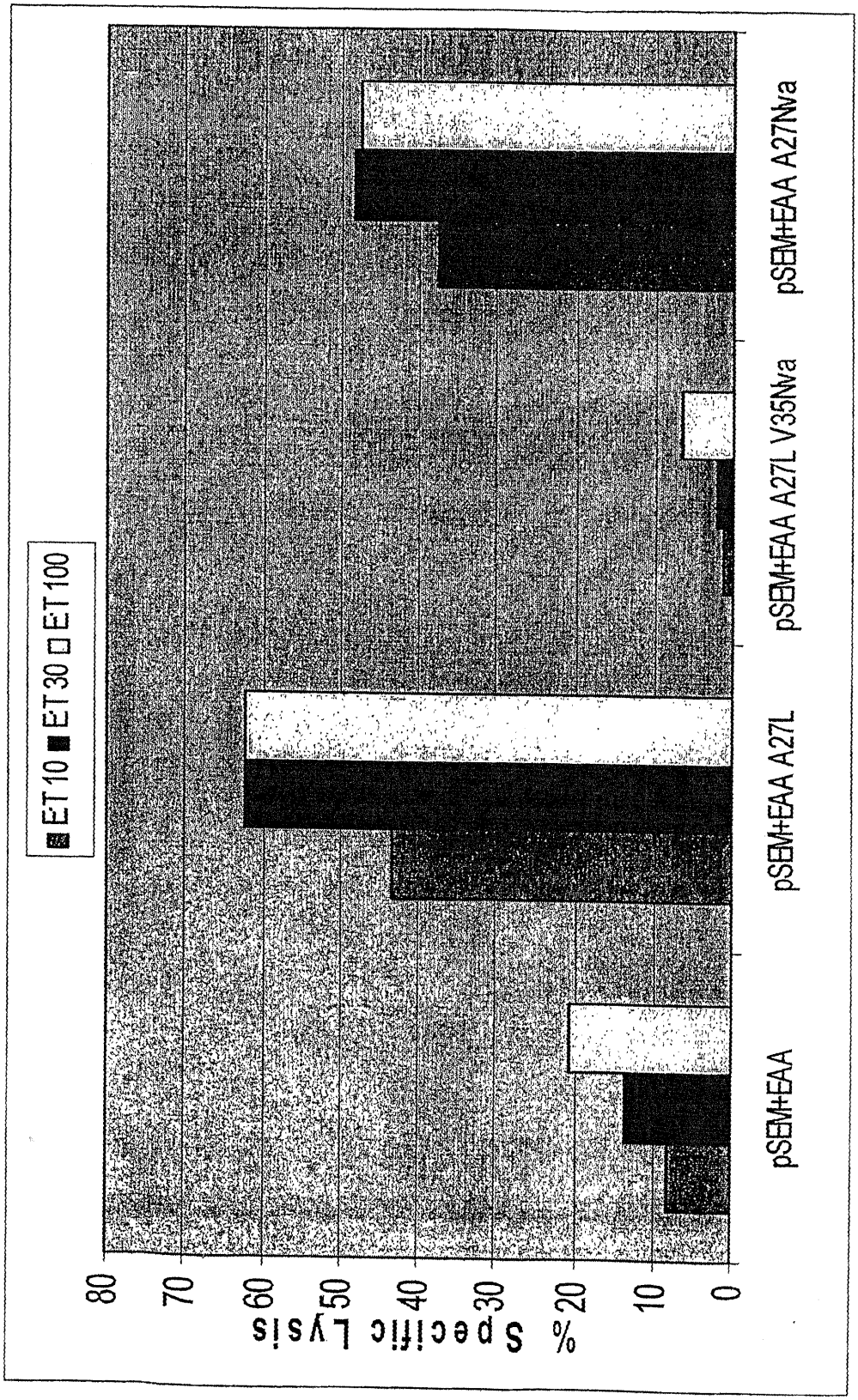


Figure 38: $Tyr_{369-377}$ Tetramer Staining (plasmid prime, peptide boost)

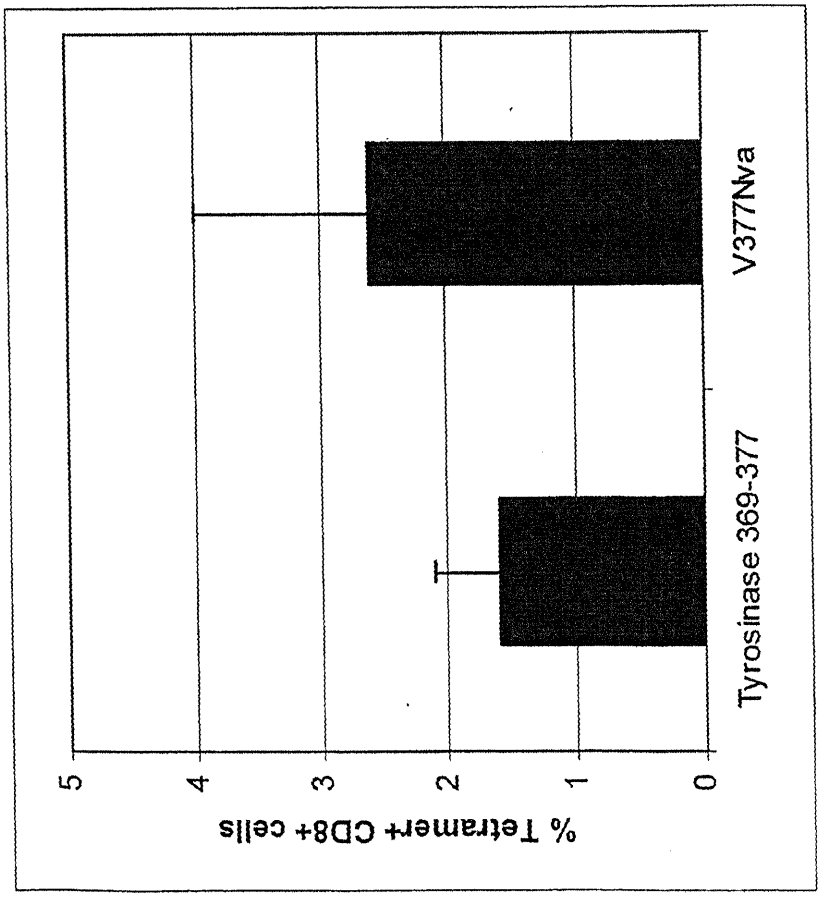


Figure 39: CFSE *in vivo* challenge with multivalent Melan A A27Nva/Tyrosinase analog immunized animals

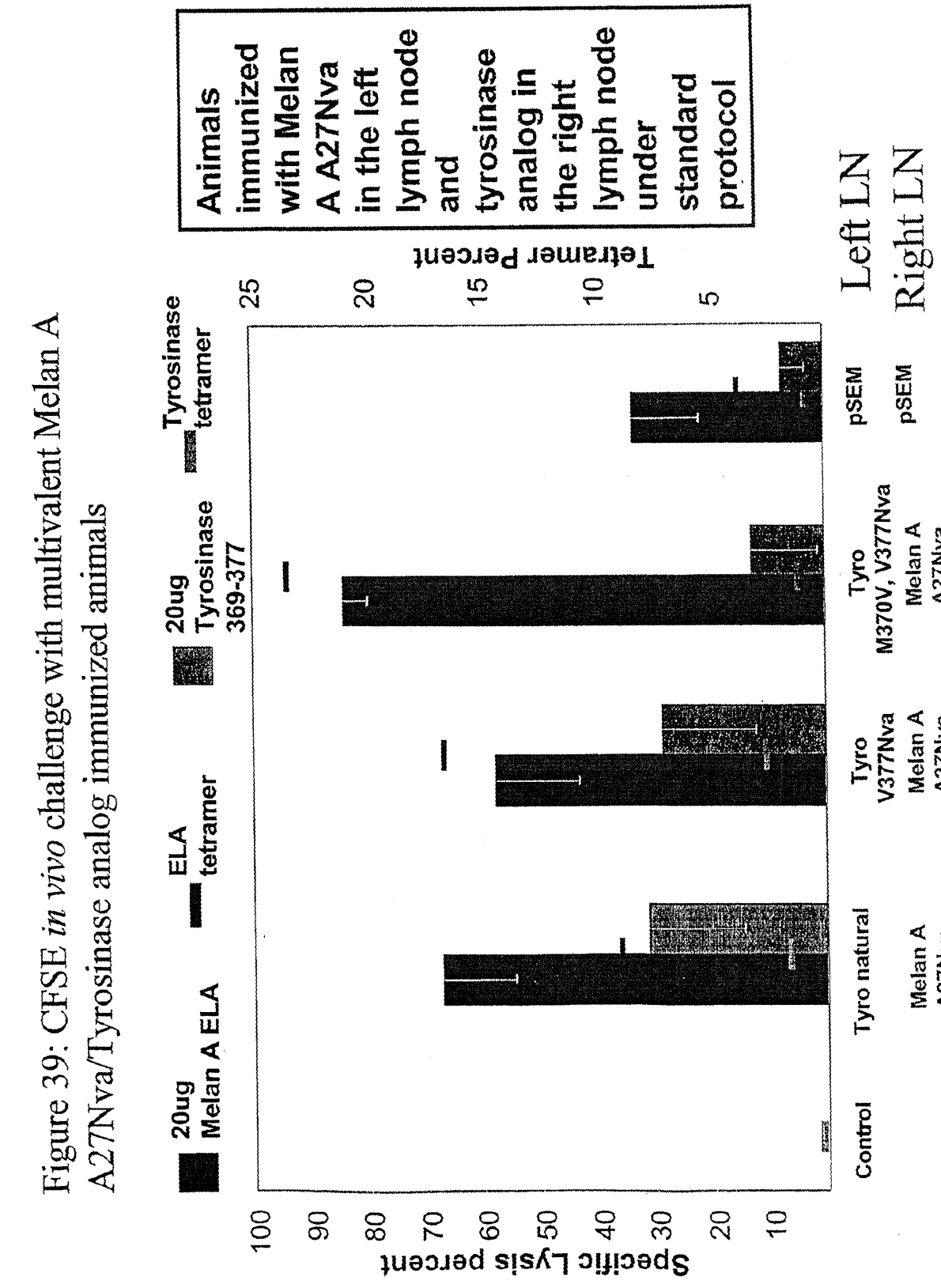


Figure 40: Schematic of Analog Immunogenicity Evaluation Protocol

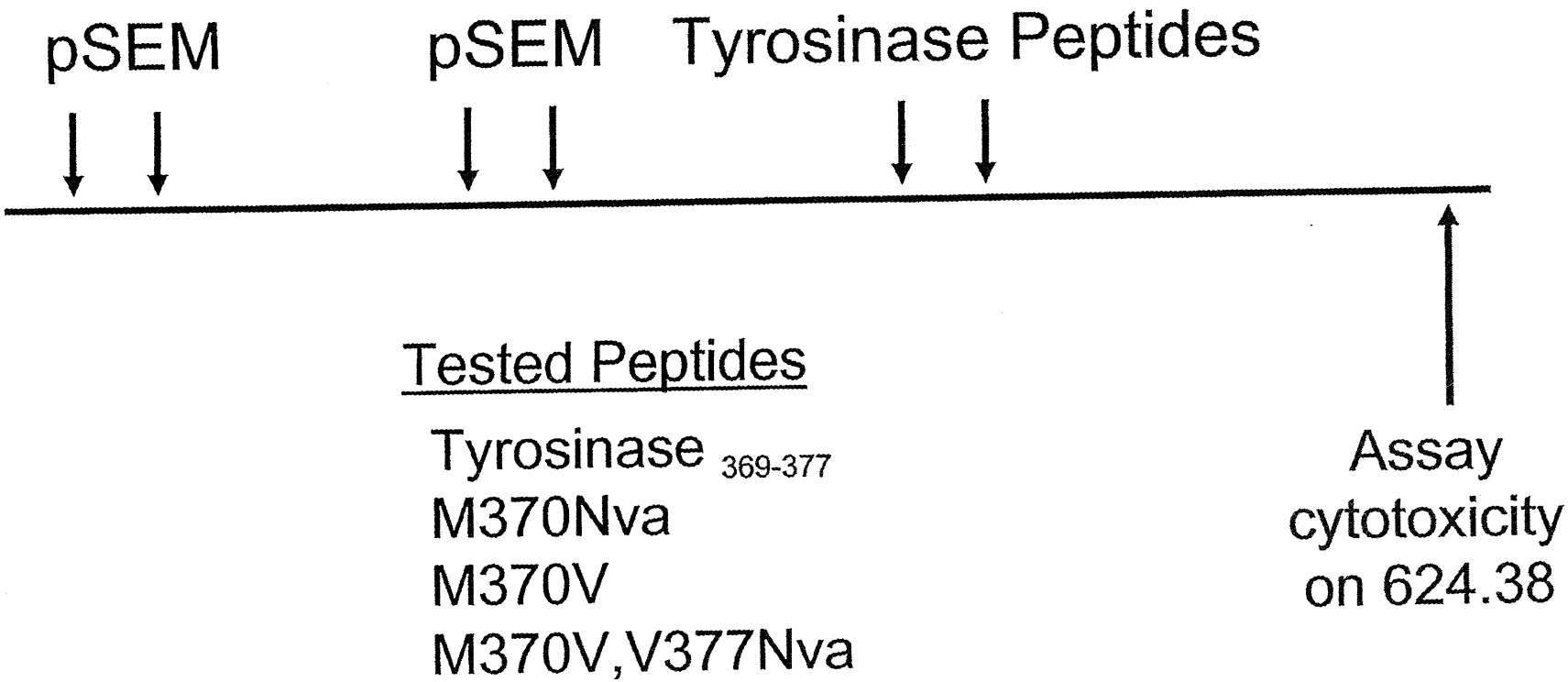


Figure 41: **Immune Response against 624.38 Cells Effector Cells from HHD Primed with pSEM and Boosted with $Tyr_{369-377}$ Analogs**

