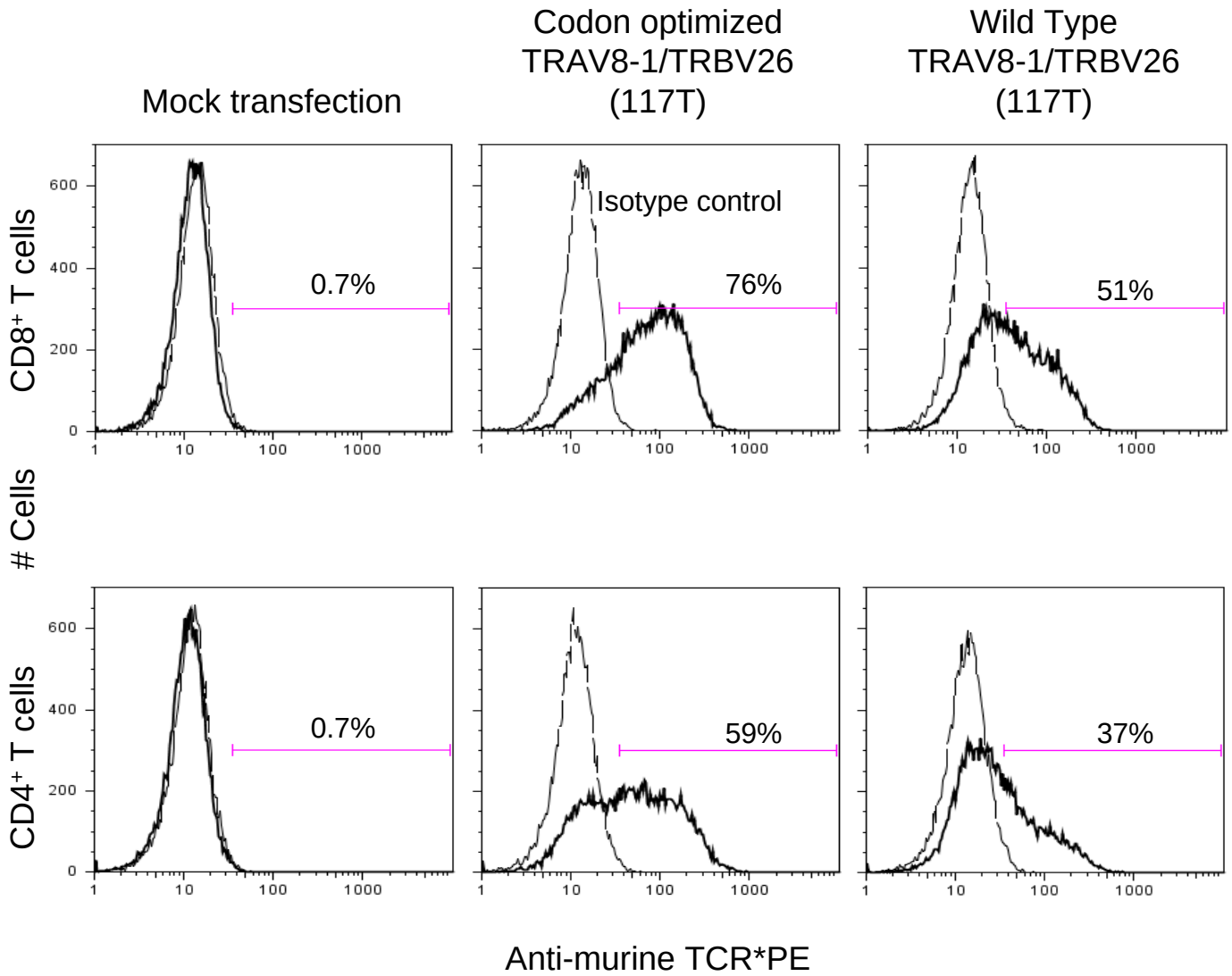
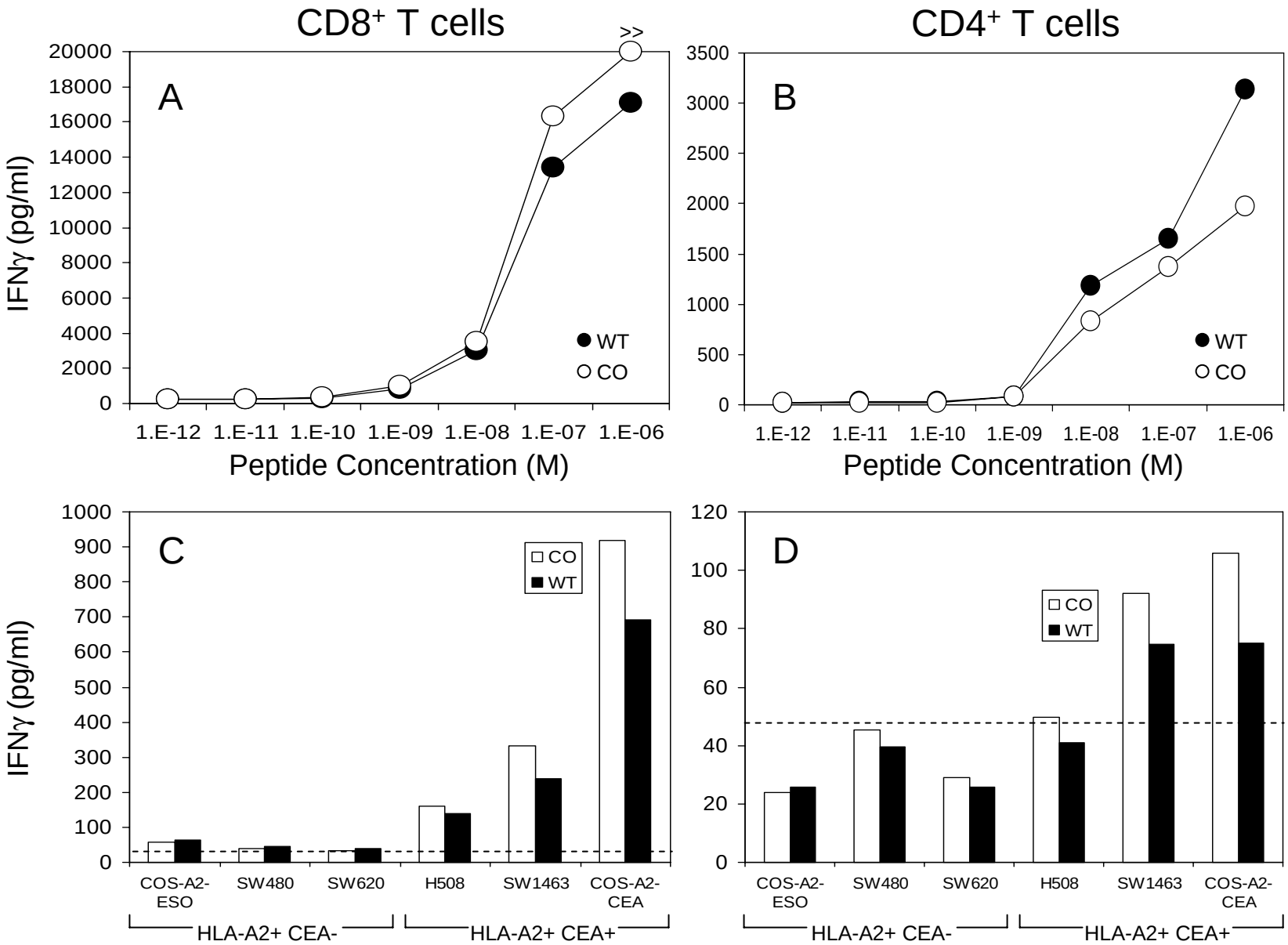
CDR3 region variations of TRAV8 α and TRBV26 β chains:

TRBV26*01 CDR3 (Clones 1,2,4; 117T):	C	A	S	S	L	G	**T**	G	D	Y	E	Q
	TGT	GCC	AGC	AGT	**CTC**	**GGG**	**ACA**	GGG	GAC	TAT	GAA	CAG
TRBV26*01 CDR3 (Clone 3; 117L):	C	A	S	S	L	G	**L**	G	D	Y	E	Q
	TGT	GCC	AGC	AGT	**CTG**	**GGA**	**CTG**	GGG	GAC	TAT	GAA	CAG
TRAV8-1*01 CDR3 (Clone 1; GAT):	C	A	T	D	L	T	S	G	G	N	Y	K
	TGT	GCT	ACT	**GAT**	**CTA**	ACC	TCA	GGA	GGA	AAC	TAC	AAA
TRAV8-1*01 CDR3 (Clones 2,3,4; GAC):	C	A	T	D	L	T	S	G	G	N	Y	K
	TGT	GCT	ACT	**GAC**	**CTC**	ACC	TCA	GGA	GGA	AAC	TAC	AAA

Supplemental Figure 1: Genetic variation in TCR α and β chain CDR3 regions from CEA:691-699 reactive murine T cell populations. HLA-A2.1 transgenic mice were immunized with CEA:691-699. Splenocytes were subsequently stimulated with peptide in vitro, and reactive cultures were restimulated with peptide under limiting dilution conditions. From four tumor reactive murine T cell populations that were identified, TCR a and b chains were identified using a 5' RACE technique followed by DNA sequencing. Two α chains (TRAV7-D-3*01 TRAJ15*01 and TRAV8-1*01 TRAJ6*01) and two β chains (TRBV26*01 TRBD1*01 TRBJ2-7*01 TRBC2 and TRBV3*01 TRBD1*01 TRBJ1-5*01 TRBC1) were identified. Additional sequencing revealed the presence of 3 α chains and 3 β chains since two genetic variations were identified within the CDR3 regions of both the TRBV26 β chain and the TRAV8-1 α chain as shown.



Supplemental Figure 2: Expression of murine TCR by T cells electroporated with CEA:691-699 reactive murine TCR α and β chain RNAs. RNAs encoding wild type and codon optimized TCR α and β chains were used to electroporate human CD8+ and CD4+ T cells previously stimulated to proliferate with anti-CD3 and IL-2. TCR expression was evaluated approximately 24 hours later by FACS using a monoclonal antibody against the constant region of the murine β chain.

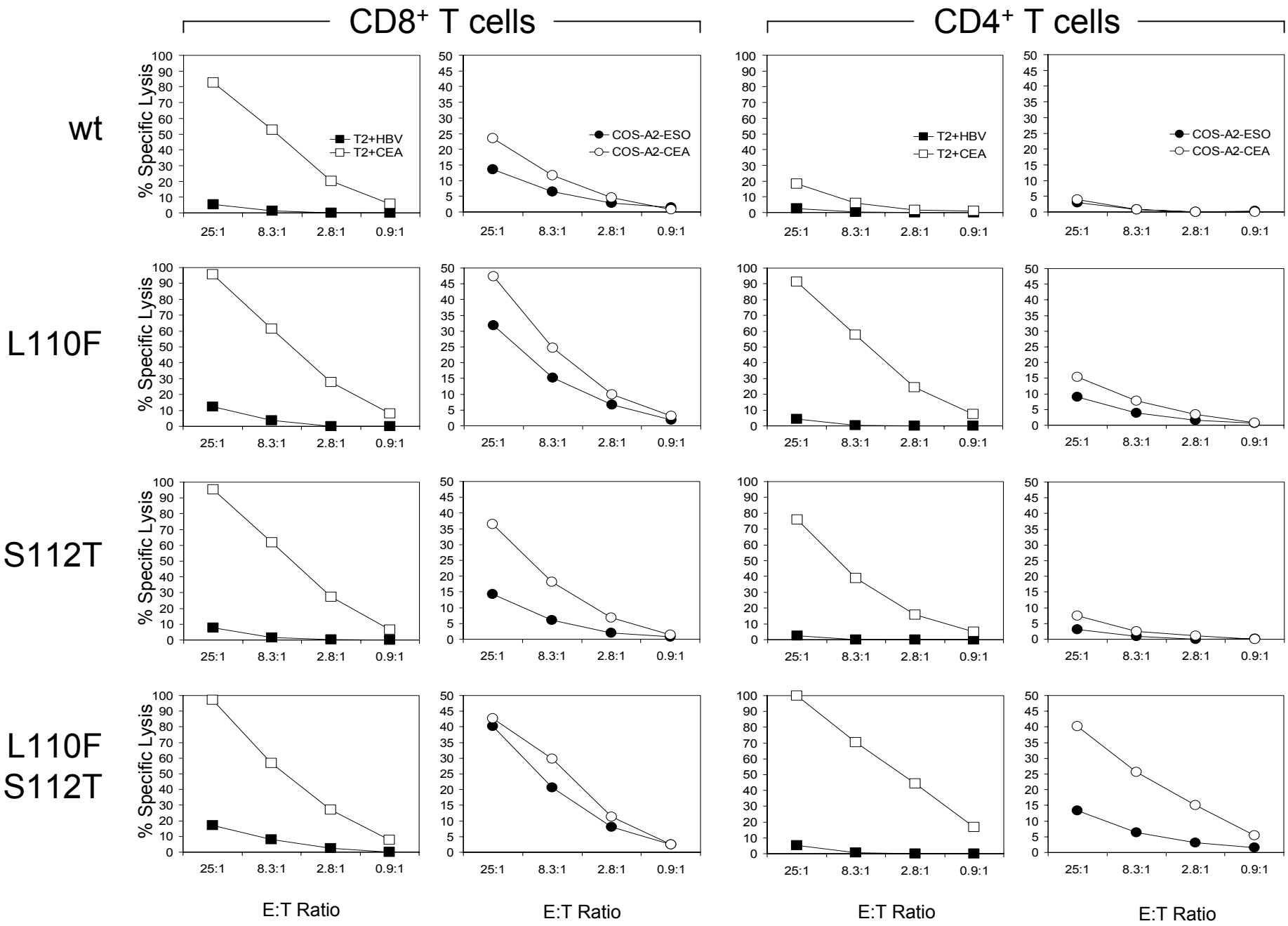


Supplemental Figure 3: Recognition of peptide and target cells expressing HLA-A2.1 and CEA by CD8+ and CD4+T cells transfected with RNAs encoding CEA:691-699 reactive TCR α chains and β chains. RNAs encoding wild type (WT) and codon optimized (CO) TCR α and β chains were used to electroporate human CD8+ (A and C) and CD4+ (B and D) T cells previously stimulated to proliferate with anti-CD3 and IL-2. Two to four hours later, T cells were cocultured with various target cells. Approximately 20 hours later, IFNγ secretion was measured in response to CEA:691-699 peptide-pulsed T2 cells (A and B) and HLA-A2.1+ CEA+ tumor cells or genetically engineered COS cells (C and D).

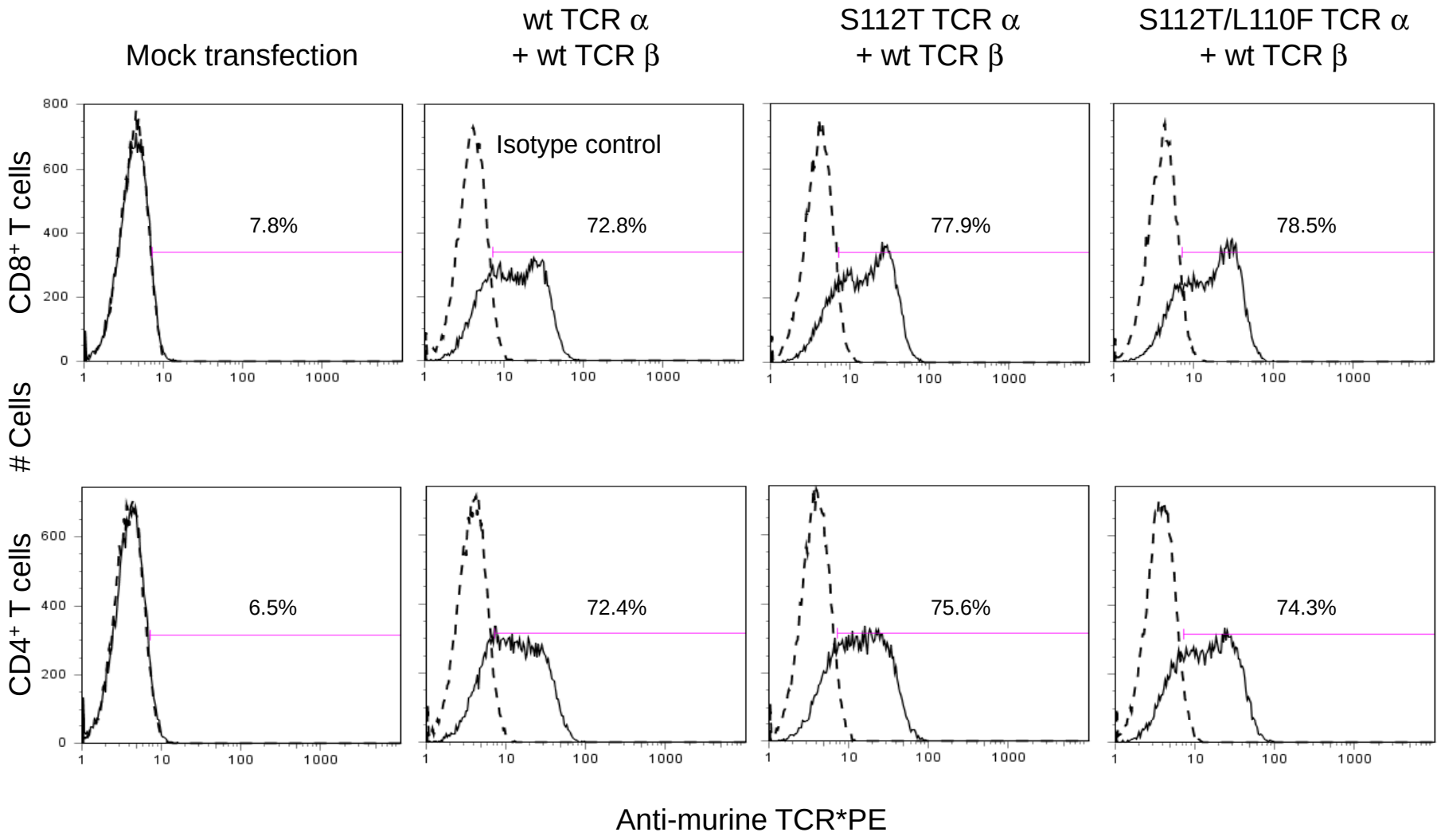
CDR3 mutations in TRAV8-1 α chain:

Wt	C	A	T	D	L	T	S	G	G	N	Y	K
L110A					**A**	T	S	G	G			
T111A					L	**A**	S	G	G			
S112A					L	T	**A**	G	G			
G113A					L	T	S	**A**	G			
G114A					L	T	S	G	**A**			
L110C					**C**	T	S	G	G			
L110G					**G**	T	S	G	G			
L110M					**M**	T	S	G	G			
L110I					**I**	T	S	G	G			
L110V					**V**	T	S	G	G			
L110F					**F**	T	S	G	G			
T111C					L	**C**	S	G	G			
T111S					L	**S**	S	G	G			
T111P					L	**P**	S	G	G			
T111D					L	**D**	S	G	G			
T111H					L	**H**	S	G	G			
T111K					L	**K**	S	G	G			
S112T					L	T	**T**	G	G			
S112N					L	T	**N**	G	G			
S112K					L	T	**K**	G	G			

Supplemental Figure 4: List of amino acid substitutions evaluated in the CDR3 region of the CEA:691-699 reactive murine TCR α chain (TRAV8-1*01 TRAJ6*01).


CD8+ T cells
CD4+ T cells
wt
L110F
S112T
L110F
S112T
% Specific Lysis
T2+HBV
T2+CEA
COS-A2-ESO
COS-A2-CEA
25:1
8.3:1
2.8:1
0.9:1
E:T Ratio

Supplemental Figure 5: Recognition of peptide and target cells expressing HLA-A2.1 and CEA by CD8+ and CD4+T cells transfected with RNAs encoding CEA:691-699 reactive TCR modified α chains and wild type (wt) β chains. RNAs encoding TCR amino-acid substituted α and wild type β chains were used to electroporate human CD8+ and CD4+ T cells previously stimulated to proliferate with anti-CD3 and IL-2. Approximately 24 hours later, T cell function was evaluated in a 4 hour ^{51}Cr release lysis assay in response to CEA:691-699 peptide-pulsed T2 cells and COS-7 cells genetically engineered to express HLA-A2.1 and full-length CEA.



Supplemental Figure 6: Expression of murine TCR by T cells electroporated with CEA:691-699 reactive murine TCR α and β chain RNAs. RNAs encoding wild type TCR α and β chains and amino acid substituted TCR α chains with wild type β chains were used to electroporate human CD8+ and CD4+ T cells previously stimulated to proliferate with anti-CD3 and IL-2. TCR expression was evaluated approximately 24 hours later by FACS using a monoclonal antibody against the constant region of the murine β chain.

Supplemental Table 1: Cross-recognition of homologous peptides from other CEA protein family members by CD8+ T cells transfected with RNAs encoding modified TCR α chains and wt β chains derived from CEA:691-699 reactive murine T cell clones[*]

	CEACAM5 691-699 (IMIGVLVGV)	CEACAM3/21: 240-248 (ILIGVLVGS)	CEACAM4:36/3: 156-164 (IVTGVLVGV)	CEACAM8: 338-347 (IMIGVLARV)	CEACAM7/2a: 254-262 (IMIGVLAGM)	HBVc: 18-27(23Y) (FLPSDYFPSV)
wt						
T2+10^{-12} M	96	54	59	47	52	-
T2+10^{-11} M	80	46	62	60	49	-
T2+10^{-10} M	842[†]	44	32	48	46	-
T2+10^{-9} M	>1000	40	48	53	58	-
T2+10^{-8} M	>1000	60	56	48	53	-
T2+10^{-7} M	>1000	322	87	45	77	-
T2+10^{-6} M	>1000	895	160	60	57	44
S112T						
T2+10^{-12} M	128	66	52	44	62	-
T2+10^{-11} M	505	45	44	40	78	-
T2+10^{-10} M	>1000	77	76	70	71	-
T2+10^{-9} M	>1000	353	>1000	55	67	-
T2+10^{-8} M	>1000	>1000	>1000	312	75	-
T2+10^{-7} M	>1000	>1000	>1000	>1000	67	-
T2+10^{-6} M	>1000	>1000	>1000	>1000	54	81
L110F S112T						
T2+10^{-12} M	283	170	128	286	186	-
T2+10^{-11} M	>1000	317	146	218	213	-
T2+10^{-10} M	>1000	>1000	188	172	252	-
T2+10^{-9} M	>1000	>1000	351	167	303	-
T2+10^{-8} M	>1000	>1000	>1000	393	720	-
T2+10^{-7} M	>1000	>1000	>1000	>1000	>1000	-
T2+10^{-6} M	>1000	>1000	>1000	>1000	>1000	199

[*] IFNγ secretion (pg/ml) in 20 hr coculture supernatants of target cells with electroporated human CD8+ T cells

[†] Underlined values for peptide-pulsed targets indicate that IFNγ release in response to T2 cells pulsed with the indicated concentration of CEA:691-699 was $\geq$50 pg/ml and at least twice background with media and T2 cells preloaded with 1 μg/ml of the negative control peptide HBVc:18-27(23Y).