

Supplementary Methods and Materials

The construction of minigene and transfection of tumor cell lines

Two 51 aa long minigenes which encompassing the mutant peptides TP53-R267P, NFE2L2-D13N, PCLO-E4090Q or the corresponding wild type sequence, flank on each side by 8 amino acids of the WT sequence, were cloned using EcoRI and BamHI restriction sites in the lentiviral vector pLVX-IRES-ZsGreen1(Ampicillin) upstream of an IRES sequence preceding a GFP tag. Sequence of each minigene contain a 5'Kozak (GCCACC) sequence, a 5' START codon (ATG) and a 3' STOP codon (TGA):

Minigene of MUT peptides TP53-R267P, NFE2L2-D13N, PCLO-E4090Q

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gac tcc agt ggt aat cta ctg gga cgg aac agc ttt gag gtg cgt gtt tgt gac ata ctt tgg agg caa gat ata
D S S G N L L G R N S F E V R V C D I L W R Q D I
gat ctt gga gta agt cga gaa gta ttt acc act gag aca cgc cgg tct caa gaa gtg aca gat ttc cta gca cct tta
D L G V S R E V F T T E T R R S Q E V T D F L A P L

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Minigene of WT peptides TP53-R267P, NFE2L2-D13N, PCLO-E4090Q

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gac tcc agt ggt aat cta ctg gga cg aac agc ttt gag gtg cgt gtt tgt gac ata ctt tgg agg caa gat ata
D S S G N L L G R N S F E V R V C D I L W R Q D I
aat ctt gga gta agt cga gaa gta ttt acc act gag aca cgc egg tct caa caa gtg aca gat ttc cta gca cct tta
D L G V S R E V F T T E T R R S Q E V T D F L A P L

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Each lentivirus vector was produced upon HEK293-T packaging cells and then were respectively transfected into KYSE140 (HLA-A2 $^+$) and KYSE150 (HLA-A2 $^-$) tumor cell lines to get KYSE140-MUT (HLA-A2 $^+$, MUT peptide $^+$), KYSE140-WT (HLA-A2 $^+$, MUT peptide $^-$), KYSE150-WT (HLA-A2 $^-$, MUT peptide $^-$) and KYSE150-MUT (HLA-A2 $^-$, MUT peptide $^+$) cell lines.

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1 **Supplementary Tables**

2 **Table S1** Data of ESI-MS and the HLA-A*02 binding affinity and stability of other
3 mutant peptides

Gene	Position	Peptide	ESI-MS[M+H] ⁺		FI ^a	DC ₅₀ ^b
			Calculated	Observed		
ABCA13	D1303H	NLHSINDFL	1072.19	1072.85	0.24	Nd ^c
DNAH5	S3587Y	GLPNDDL YI	1019.12	1020.1	0.68	Nd ^c
	D4110N	FM N ELMDII	1025.38	1126.54	0.38	Nd ^c
	L4406H	RMQRVLSHV	1125.36	1126.30	0.30	Nd ^c
	M4495T	FLTATRQEI	1078.23	1079.22	0.21	Nd ^c
KMT2D	F4722L	ILGEEAPRL	997.16	998.31	0.29	Nd ^c
LRP1B	C2479Y	Y LLTPNGRV	1032.21	1033.31	0.39	Nd ^c
	R3362L	GLFQCGTGL	895.05	895.83	0.09	Nd ^c
	P3707L	A LDMCVKFL	1039.33	1040.11	0.37	Nd ^c
LRP2	D1744Y	CLRD Y QPFL	1154.35	1154.92	0.20	Nd ^c
MUC16	Q5024H	LMSRIP HDV	1067.28	1068.41	0.14	Nd ^c
MUC17	T3809M	T MSERSTLL	1037.22	1037.81	0.12	Nd ^c
NEB	D3282V	VISDYKYKV	1114.31	1115.01	0.09	Nd ^c
NFE2L2	I28T	ILWRQDTDL	1159.31	1159.98	0.79	Nd ^c
NOTCH1	G1995V	RMHD V TTP	1069.25	1070.42	0.42	Nd ^c
	S2202F	GMLSPVDFL	978.18	979.24	0.13	Nd ^c
PCDH15	S628L	T L TATVNIV	931.1	931.83	0.43	Nd ^c
SYNE1	A65S	KLL S LLEV	1027.31	1028.46	0.08	Nd ^c
TP53	C135F	ALNKMFFQL	1111.37	1112.57	0.12	Nd ^c
	G244V	YMCNSSCM V	1037.27	1038.17	0.49	Nd ^c
	G266A	LL ARNSFEV	1048.21	1049.41	0.16	Nd ^c
	V272L	LLGRNSFEL	1048.21	1049.44	0.53	Nd ^c

4 ^aFI= (MFI of the given peptide- MFI of the PBS control group without peptides)/ MFI of the PBS
5 control group without peptides.

6 ^bDC₅₀ was calculated as follow: [MFI of 0 h-MFI of (2, 4 or 6 h)]/MFI of 0 h × 100%

7 ^c Not determined.

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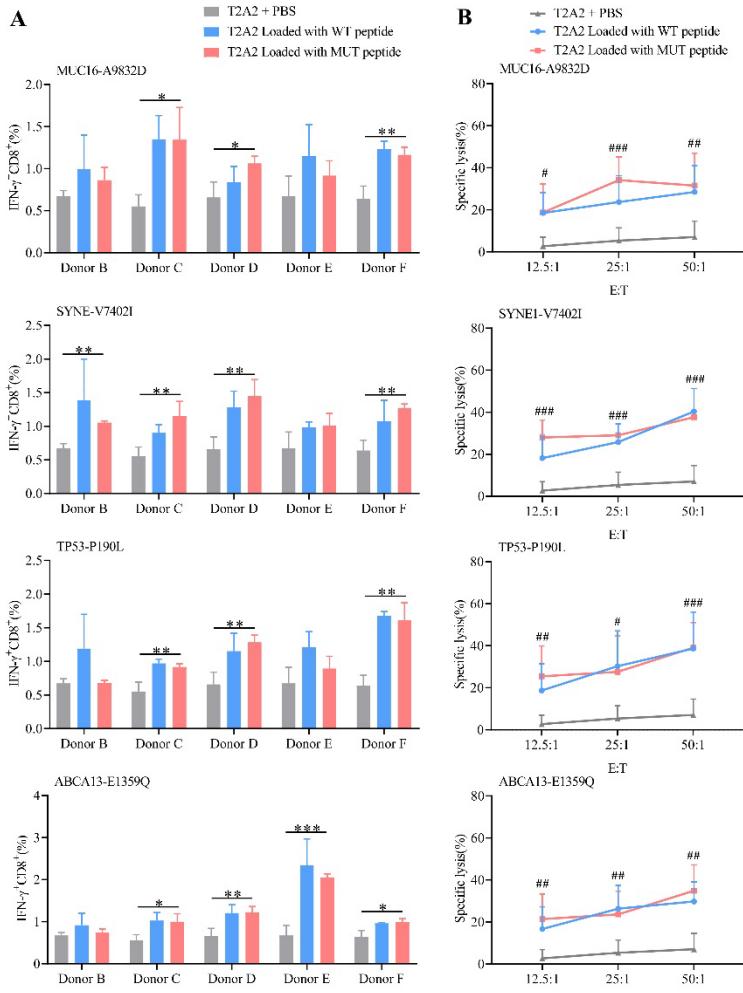
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1 **Supplementary Figure**

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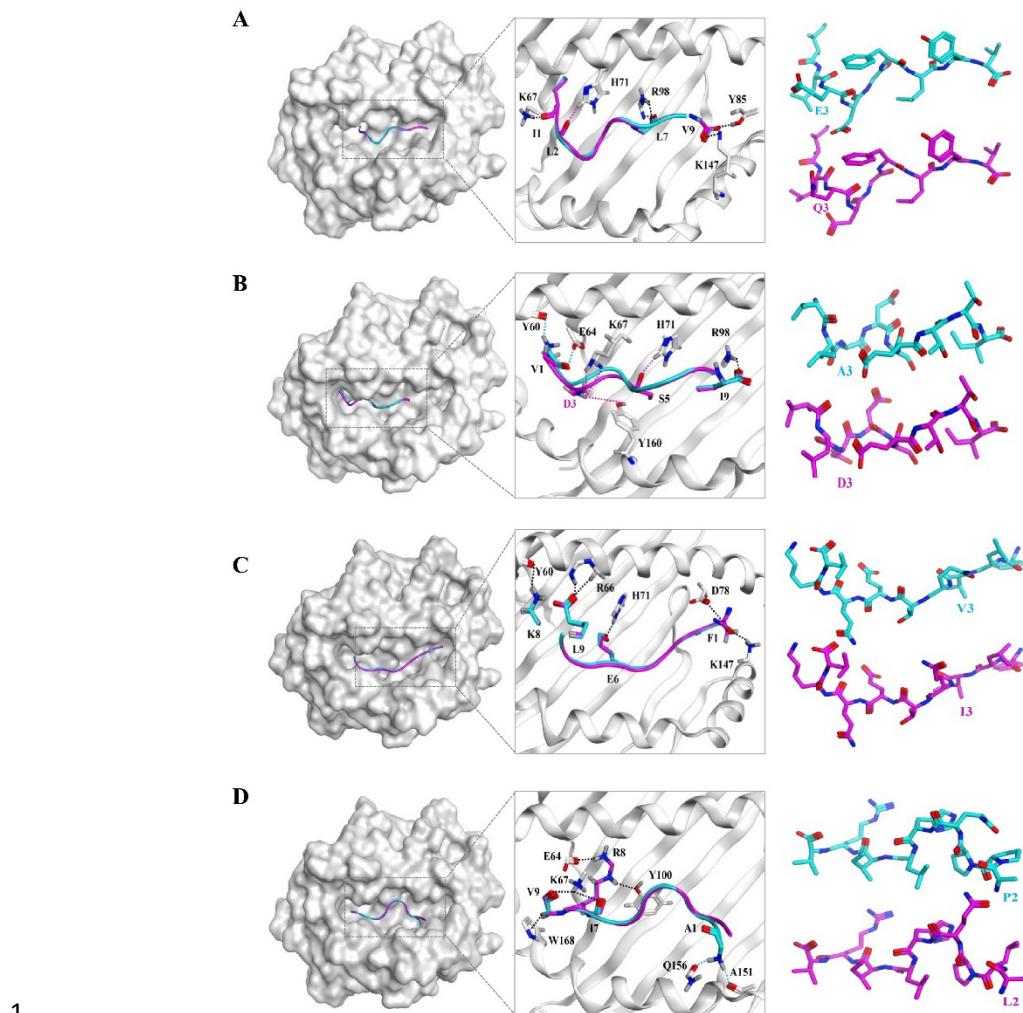
Fig S1



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4 **Figure S1. The immunogenicity of the rest mutant peptides induced T cells to**
5 **peptide-pulsing T2A2 cells *in vitro*.** PBMCs isolated from five healthy HLA-A2⁺
6 donors (donor B-F) were induced by mature DCs pulsed by MUT peptide
7 MUC16-A9832D, SYNE1-V7402I, TP53-P190L or ABCA13-E1359Q (10 µg/mL)
8 once a week. After three rounds stimulated by MUT peptides, CTLs were collected
9 and co-cultured with T2A2 cells loaded with MUT or WT peptides and then were
10 detected for IFN- γ release (A, n = 3) and lysis cytotoxicity (B, n = 5). T2A2 + PBS
11 cells group served as negative control. Statistical significance was determined by
12 Student's t-test. *p < 0.05, **p < 0.01, ***p < 0.001 represented the significances of
13 T2A2 cells loaded with MUT peptide group versus T2A2 cells loaded with WT
14 peptide group, #p < 0.05, ##p < 0.01, ###p < 0.001 represented the significances of
15 T2A2 cells loaded with MUT peptide group versus T2A2 + PBS cells group.

Fig S2



2 **Figure S2. The possible structural models of the other MUT peptide and**
3 **HLA-A*0201 molecule.** The structures of the WT peptides and MUT peptides was
4 predicted by PEP-Fold. WT Peptide (blue, A: ABCA13-WT; B: MUC16-WT; C:
5 SYNE1-WT; D: TP53-WT) or MUT peptide (magenta, A: ABCA13-E1359Q; B:
6 MUC16-A9832D; C: SYNE1-V7402I; D: TP53-P190L) was docked with
7 HLA-A*0201 molecule (gray) (PDB ID: 5YXN) by MOE (Molecular Operating
8 Environment software). The binding sites of the peptides to HLA-A*0201 molecules
9 were labeled.