

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

```

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

```

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

```

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
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

```

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.

17

18

19

20

21

22

25

27

—

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.

8

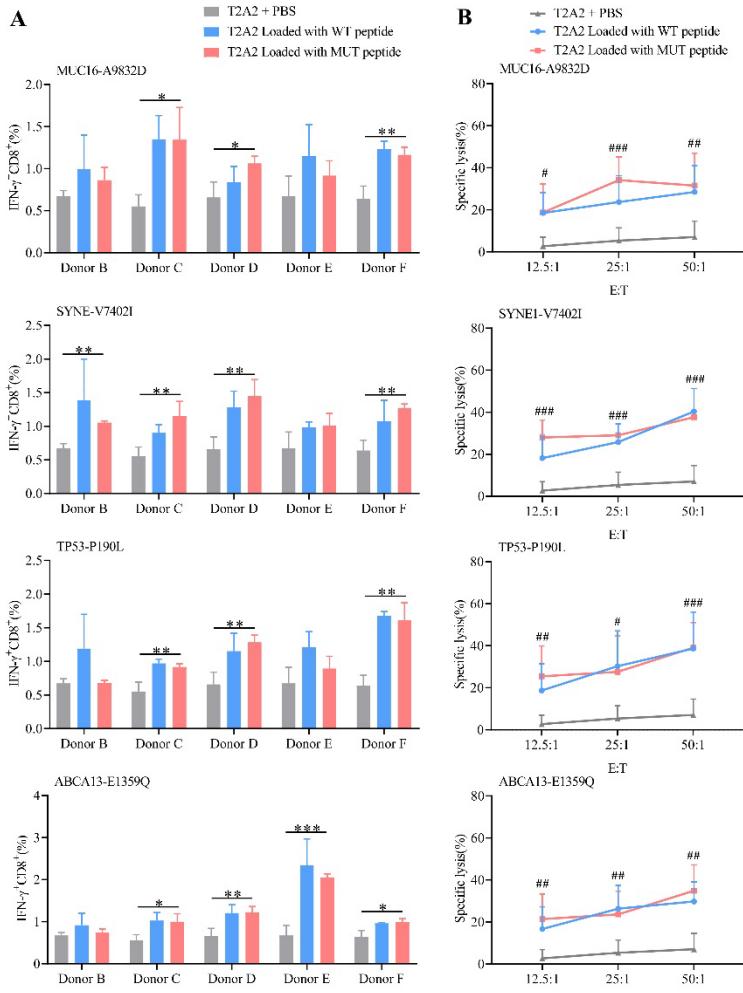
9

10

1 **Supplementary Figure**

2

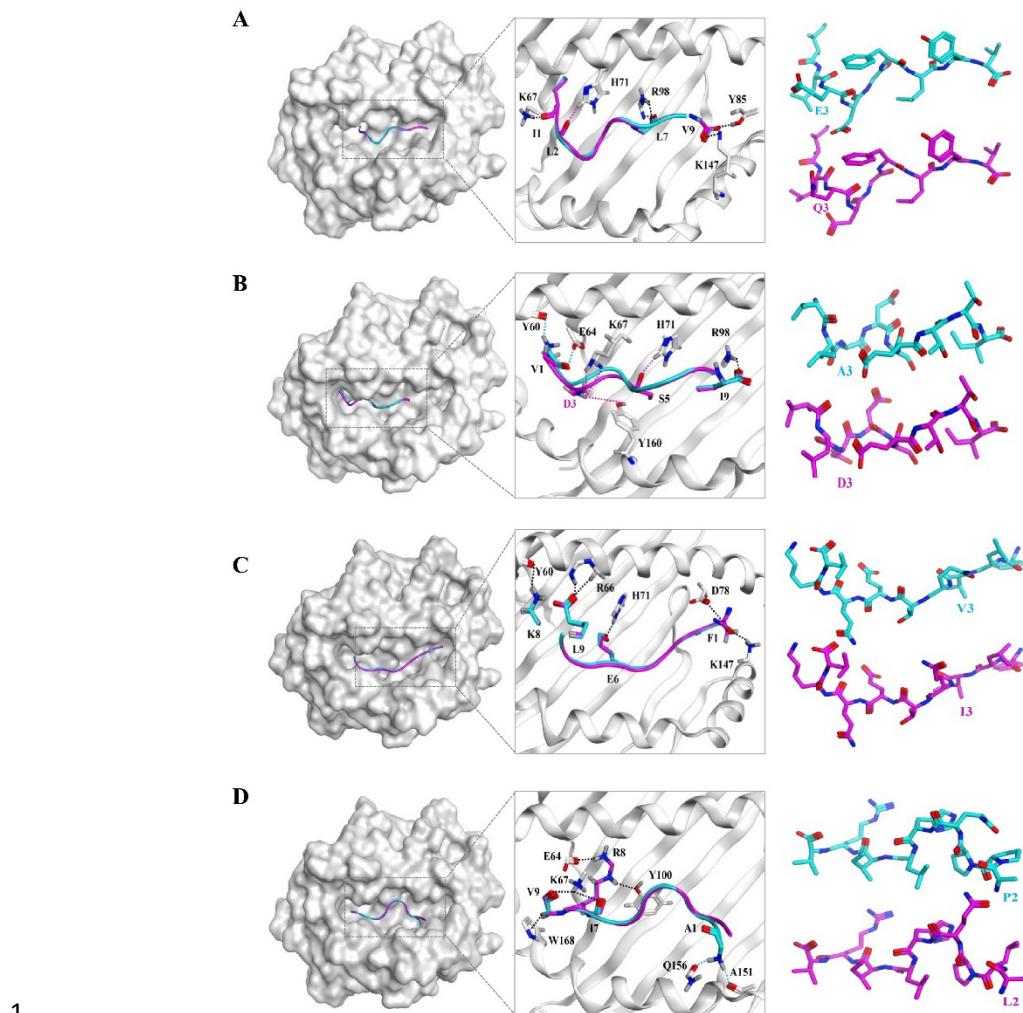
Fig S1



3

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.