

**Dominant neoantigen verification in hepatocellular carcinoma by a
single-plasmid system coexpressing patient HLA and antigen**

AUTHORS

Pu Chen^{1†}, Dongbo Chen^{1†*}, Dechao Bu², Jie Gao³, Wanying Qin⁴, Kangjian Deng⁴,
Liyang Ren¹, Shaoping She¹, Wentao Xu⁴, Yao Yang¹, Xingwang Xie^{1,5*}, Weijia
Liao^{4*} and Hongsong Chen^{1*}

¹Peking University People's Hospital, Peking University Hepatology Institute, Beijing
Key Laboratory of Hepatitis C and Immunotherapy for Liver Disease, Beijing
International Cooperation Base for Science and Technology on NAFLD Diagnosis,
Beijing 100044, China;

²Research Center for Ubiquitous Computing Systems, Institute of Computing
Technology, Chinese Academy of Sciences, Beijing 100190, China;

³Department of Hepatobiliary Surgery, Peking University People's Hospital, Beijing
100044, China;

⁴Laboratory of Hepatobiliary and Pancreatic Surgery, Affiliated Hospital of Guilin
Medical University, Guilin 541001, Guangxi, China;

⁵Corregene Biotechnology Co., Ltd, Beijing 102206, China.

[†]These authors contributed equally to this work.

^{*}These authors contributed equally to this work and shared senior authorship.

***CORRESPONDENCE**

chenhongsong@bjmu.edu.cn (H.S. Chen), chendongbo338@bjmu.edu.cn (D.B.
Chen), liaoweijia288@163.com (W.J. Liao), xiexingwang@corregene.com (X.W.
Xie).

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SUPPLEMENTAL MATERIALS AND METHODS

1. Patients and sample collection

We enrolled 14 patients with hepatocellular carcinoma (HCC) who underwent curative resection, including 6 patients from the Peking University People's Hospital and 8 patients from the Affiliated Hospital of Guilin Medical University. All cases were confirmed by pathology reports. The study was approved by the Research Ethics Committees of the Peking University People's Hospital and the Affiliated Hospital of Guilin Medical University. All patients provided written informed consent. Tumor tissues, nontumor liver tissues (nearby tissues > 2 cm away from tumor tissue margin) and portal vein tumor thrombi (PVTs) were collected and frozen in liquid nitrogen for further processing. Peripheral blood mononuclear cells (PBMCs) were isolated from fresh peripheral blood of patients or healthy donors by Ficoll density gradient centrifugation. They were immediately frozen in -80°C and transferred to liquid nitrogen the next day. COS-7 cells, HEK293T cells, PLC/PRF/5 cells, Hep3B cells and HepG2 cells were purchased from American Type Culture Collection (ATCC). Huh-7 cells were purchased from Cell Resource Center, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences.

2. Extraction and next-generation sequencing (NGS) of DNA and RNA

2.1 Extraction and quality control of DNA and RNA

DNA and RNA were extracted from fresh-frozen samples using AllPrep DNA/RNA Mini Kit (QIAGEN) following the protocol of the manufacturer. The quantity and quality of DNA and RNA were determined by Qubit Fluorometer (Invitrogen) and agarose gel electrophoresis generally. Fragment Analyzer 5400 (Agilent) was used for

accurate verification. At least 1.5 µg high quality genomic DNA and 1.0 µg high quality RNA were prepared for subsequent experiments.

2.2 Whole exome sequencing (WES)

WES was performed using SureSelect Human All Exon V6 (Agilent) according to the manufacturer's instructions. We first used the Covaris to randomly fragment DNA sample to an average of 180 - 280 bp. Fragments were end-repaired, and an extra A base was added to the 3' end. Illumina adaptors were ligated to the fragments. Subsequently, we used SPRI beads to purify and acquire the library. After the solution hybridization of the library and biotin-labeled probes, we used beads to capture exome and then performed PCR amplification. The quality was determined by Qubit Fluorometer (Invitrogen) and Fragment Analyzer 5400 (Agilent). Furthermore, the effective concentration of the library was accurately quantified by quantitative real time PCR (qPCR). The library was sequenced by Illumina NovaSeq 6000 platform. Finally, raw data was filtered to obtain high-quality clean reads for further analysis.

2.3 RNA-sequencing (RNA-seq)

The library was acquired by NEBNext Ultra™ RNA Library Prep Kit for Illumina. First, we used beads to capture mRNA from total RNA and fragment it. Then, we used M-MuLV Reverse Transcriptase and random hexamer primer to synthesize first strand cDNA and used RNase H and DNA Polymerase I to perform second strand cDNA synthesis. Remaining overhangs were converted into blunt ends via exonuclease/polymerase activities. After adenylation of 3' ends of DNA fragments, NEBNext Adaptor with hairpin loop structure were ligated to prepare for hybridization. The library fragments were purified with AMPure XP system

(Beckman Coulter). Subsequently, 3 μ l USER Enzyme (NEB) was used with size-selected, adaptor-ligated cDNA at 37°C for 15 min followed by incubation at 95°C for 5 min. Furthermore, we performed PCR to acquire products and used AMPure XP system to purify them. Fragment Analyzer 5400 (Agilent) were used to quantitate the products. The clustering of the index-coded samples was performed on a cBot Cluster Generation System using TruSeq PE Cluster Kit v3-cBot-HS (Illumina). After cluster generation, the library was sequenced on Illumina NovaSeq 6000. Finally, raw data was filtered to obtain high-quality clean reads for subsequent analysis.

3. Variation calling

3.1 Base substitution, mutational signatures, mutated genes and evolutionary tree of the samples

For single nucleotide variants (SNVs), we analyzed the number of 6 different types of base substitutions including A>C, A>G, A>T, C>A, C>G and C>T, and calculated their proportions. Further, each of the substitutions was examined by incorporating information on the bases immediately 5' and 3' to each mutated base generating 96 possible triplet nucleotides (6 types of substitution \times 4 types of 5' base \times 4 types of 3' base). We calculated the proportion of each triplet nucleotide and identified the 30 mutational signatures from COSMIC database (<https://cancer.sanger.ac.uk/cosmic>) by the R package “deconstructSigs”. Subsequently, we analyzed the abundance of each mutational signatures.

The gene that had at least one mutation was defined as mutated gene. And we focused our analysis on the high-frequency mutated genes, pan-cancer driver mutated genes^[1], HCC driver mutated genes (according to previous reports and NCG7.0 database,

<http://ncg.kcl.ac.uk/index.php>)^[2-6], immune-related mutated genes (according to GSEA, <https://www.gsea-msigdb.org/gsea/index.jsp>) and cytotoxic T lymphocyte (CTL)-evasion mutated genes^[7]. The TMB for each sample was calculated as the number of mutations per 36 megabase (Mb) of gene, and the mutated frequency for each gene was the proportion of patients who had mutated genes in all patients.

We constructed an $M \times N$ matrix based on the occurrence of mutations in different samples. M was the number of mutations and N was the number of samples. The status of the mutation was 0 or 1, where 0 was unmutated and 1 was mutated. In addition, a negative control sample was added as negative control (NC), and the mutation frequency of all sites was defined as 0. Then phylograms were inferred using the R package “phangorn”. The parsimony ratchet method was used to search for the best tree and acctran function was used to get edge length according to the ACCTRAN criterion^[8].

3.2 Base substitution and mutated genes of HCC in TCGA database and reference mutational signatures in COSMIC database

Effective mutation analysis is the basis of neoantigen prediction. We used other database to verify the reliability of mutations from our samples. The data for analysis of base substitution and mutated genes of HCC were downloaded from the GDC data portal of TCGA database (<https://www.cancer.gov/>) and the used R package was “maftools”. Reference mutational signatures were downloaded from COSMIC database.

4. Neoantigen prediction

4.1 Affinity prediction between neoantigens and human leukocyte antigens (HLAs)

We used OptiType^[9], a new algorithm based on integer linear programming, to

analyze the HLA types of patients from WES data of nontumor liver tissues or PBMCs and used LOHHLA^[10] to predict potential loss of heterozygosity (LOH) of HLAs from WES data of tumor tissues. At the same time, we used Variant Effect Predictor (VEP)^[11] to predict mutated proteins from somatic mutations and disassembled the mutated proteins to potential mutated peptides. Then, we used Genome Analysis Toolkit (GATK) ASEReadCounter^[12] to detect mutation count (MutCount) of potential mutated peptide from RNA sequencing (RNA-seq) data. Subsequently, the NetMHCpan-4.1 algorithm^[13] was used to determine the affinity of the mutated peptides to the patient's HLAs. Finally, we filtered the data based on mutated peptide affinity (MutAff) ≤ 200 nM or referenced peptide affinity (RefAFF) / MutAff ≥ 10 in the case of excluding duplication and LOH of HLAs and predicted potential neoantigens.

4.2 Selection of indexes and filter values

Prediction with computer algorithms aimed at assessing affinity between neoantigens and HLAs is currently the main strategy for clinical neoantigen screening^[14]. The common indexes for filtering neoantigens are MutAff, RefAFF / MutAff and the rank of mutated peptide affinity (Mutrank).

The common filter value of MutAff is 500 nM or lower, the RefAFF / MutAff is 1 or higher and the Mutrank is 2 or lower^[15-16]. Because neoantigens have high heterogeneity in different tumors and population, the selection of indexes and filter values should be personalized^[17].

Single nucleotide polymorphisms (SNPs) are one of the most common types of genetic variation among people. The mutated peptides from SNPs could not induce a strong immune response. We used VEP to predict mutated proteins from SNPs in

nontumor liver tissues and disassembled the mutated proteins to potential mutated peptides as negative dataset^[18]. The frequency of the SNPs is 10% or more and the MutCount of potential mutated peptide is 5 or more. Positive dataset consisted of the neoantigens with high confidence collected by Tan et al^[19], and the neoantigens without the paired wild types were eliminated. Then we used the negative dataset and the positive dataset to determine appropriate indexes and filter values. We found the increasing filter values of MutAff and Mutrank or the declining filter value of RefAFF / MutAff could induce more candidate neoantigens and the real neoantigens but less proportion of real neoantigens in candidate neoantigens (supplemental figure 6A-C). When the same amount of neoantigens were screened by different indexes, neoantigen proportions screened by MutAff and RefAFF / MutAff were higher than those screened by Mutrank (supplemental figure 7A-C). In our study, we excluded inferior Mutrank and selected $\text{MutAff} \leq 200$ nM and $\text{RefAFF} / \text{MutAff} \geq 10$ to balance the number and the proportion of the screened neoantigen for these HCC samples.

5. Establishment, validation and application of the Co-HA system

5.1 Construction of the *KRAS* G12D TCR-T cells

The starting vector was lenti-BSD-T2A-EGFP. This vector was constructed by CorreGene. The vector map is shown in supplemental figure 1A (supplemental plasmid sequence NO. 1). The gene segment of *EGFP* in vector were released from the vector by cleavage with restriction enzymes BfuAI (NEB) and EcoRI (NEB), and the vector without *EGFP* was recovered from the gel. Meanwhile, we entrusted Synbio Technologies to synthesize the gene segment of the *KRAS* G12D TCR, including TK412 V β , Cys mTRBC1, P2A, TK412 V α and LVL_Cys mTRAC, as

described by Wang et al^[20]. Gibson Assembly was then performed to assemble the TCR gene segment into the viral vector without *EGFP* to construct the TCR plasmid lenti-BSD-T2A-TK412 (supplemental figure 1A, supplemental plasmid sequence NO. 2). The 3589 bp gene segment was successfully excised from the TCR plasmid by restriction enzymes NotI (NEB) and BamHI (NEB) for verification (supplemental figure 1B). PBMCs from healthy donors were cultured in X-VIVO 15 medium (Lonza) supplemented with 5% Human AB serum (GemCell), 100 U/ml penicillin (Gibco), 100 µg/ml streptomycin (Gibco), 100 IU/ml IL-2 (R&D) and 10 µL T Cell TransAct EA (Miltenyi). According to the methods of production and infection of lentivirus in the previous research of our group^[21], the TCR plasmid and assistant plasmid pVSVg (AddGene 8454) and psPAX2 (AddGene 12260) were cotransfected into HEK293T cells. After 48 hours, the supernatant of the virus was collected and centrifuged for 10 min with 2,000 g. The debris was removed by 0.45 µm filter (Millipore). The lentivirus was concentrated with Macrosep Advance Centrifugal Device (100K, PALL) and then the titer was determined by p24 enzyme-linked immunosorbent assay (ELISA). PBMCs were infected by the lentivirus with 20 multiplicity of infection (MOI). After 14 days of cultivation, TCR-T cells recognizing the *KRAS* G12D neoantigen were acquired. DNA was extracted from the TCR-T cells by TIANamp Genomic DNA Kit (TIANGEN) following the protocol of the manufacturer. Sanger sequencing was used to determine the key sequence of the TCR. The sequence of primer was shown as SEQ ID NO. 1 in supplemental table 5. In supplemental figure 1C, the crimson part of the picture represented the matching sequence, and the arrow represented the sequencing direction. In addition, the expression of the TCR was detected by flow cytometry (FCM). The antibodies were PE anti-mouse TCR β chain Antibody (BioLegend), FITC Mouse Anti-Human CD8 (BD) and Cell Viability

Solution (BD).

It is challenging to identify the TCRs of T cells for newfound neoantigens. In general, we produced the specific tetramers to identify the neoantigen-specific T cells. In order to get the sequences of the TCRV α and the TCRV β of the neoantigen-specific T cells, we need to sort the neoantigen-specific T cells for Sanger sequencing. Besides the TCR-T cells, antigen-specific T cells in peripheral blood and tumor infiltrating lymphocytes (TILs) of patients could be as effector cells in the Co-HA system.

5.2 Construction of the HLA vectors

The HLA vector was the main component of the Co-HA system that contained the HLA subtype linker region, the antigen linker region and the tag sequence. At first, the HLA-A*11:01 vector containing HLA-A*11:01 gene was constructed with the lenti-PURO-T2A-EGFP vector (supplemental figure 3A, supplemental plasmid sequence NO. 3). The steps of constructing the HLA-A*11:01 vector were described in detail as follows. (1) To prevent disturbing subsequent restriction enzyme digestion, the recognition site of restriction enzyme BsmBI on the lenti-PURO-T2A-EGFP was synonymously mutated. (2) We simultaneously entrusted Synbio Technologies to synthesize the gene segment including HLA-A*11:01, E2A, Tag-A*11:01, P2A and *EGFP* we designed. The sequence of HLA-A*11:01 was acquired from the IPD-IMGT/HLA database (<https://www.ebi.ac.uk/ipd/imgt/hla/>), and the recognition site of restriction enzyme BsmBI in HLA-A*11:01 was synonymously mutated similarly. (3) The gene segment of *EGFP* was excised by restriction enzymes BamHI (NEB) and EcoRI (NEB), and the main part of the vector was recovered from the gel. Then, the synthesized gene segment was assembled into the main part of the vector by Gibson Assembly, and the HLA-A*11:01 vector was constructed and named

lenti-PURO-T2A-(HLA-A*11:01)-E2A-(Tag-A*11:01)-P2A-EGFP (supplemental figure 3A, supplemental plasmid sequence NO. 4). (4) The 2611 bp gene segment was excised from the HLA-A*11:01 vector by restriction enzymes BamHI (NEB) and XhoI (NEB) for verification (supplemental figure 3B). (5) Subsequently, the HLA-A*11:01 vector, and assistant plasmid pVSVg (AddGene 8454) and psPAX2 (AddGene 12260) were cotransfected into HEK293T cells for producing lentivirus. The *EGFP* in vector was used to as reporter gene. Then, COS-7 cells were infected with the lentivirus and cultured with 4 μ g/ml puromycin (Sigma) for one week to establish stable cell lines. DNA was extracted from the COS-7 cells by TIANamp Genomic DNA Kit (TIANGEN) following the protocol of the manufacturer. The key sequence of the HLA-A*11:01 vector was analyzed with Sanger sequencing. The sequences of primers were shown as SEQ ID NO. 2-3 in supplemental table 5. In supplemental figure 3C, the crimson parts of the picture represented the matching sequences sequenced by Sanger sequencing, and the arrows represented the sequencing directions. In addition, the expression of HLA-A*11:01 was detected by FCM. The antibodies were FITC Mouse Anti-Human HLA-A (BD) and Cell Viability Solution (BD).

We designed the recognition sites of restriction enzymes BamHI (NEB) and AfeI (NEB) in the HLA-A*11:01 vector to replace the gene segment of the HLA with its tag to construct various HLA vectors. Then, we provided an innovative scheme to design the tag sequences of various HLA vectors. The length of the tag sequences was 15 bp. In order to ensure each tag unique, tag sequence was derived from the corresponding part of the various HLA. In addition, the design of the tag sequences for HLA-A, HLA-B and HLA-C respectively were different and described below. The tag of HLA-A* α : β designed was derived from the first sequence at positions ($\alpha \times 3 +$

1) to $(\alpha \times 3 + 3)$ and the second sequence at positions $(\beta \times 3 + 1)$ to $(\beta \times 3 + 12)$ of HLA-A* α : β sequence; The tag of HLA-B* α : β designed was derived from the first sequence at positions $(\alpha \times 3 + 1)$ to $(\alpha \times 3 + 6)$ and the second sequence at positions $(\beta \times 3 + 1)$ to $(\beta \times 3 + 9)$ of HLA-B* α : β sequence; The tag of HLA-C* α : β designed was derived from the first sequence at positions $(\alpha \times 3 + 1)$ to $(\alpha \times 3 + 9)$ and the second sequence at positions $(\beta \times 3 + 1)$ to $(\beta \times 3 + 6)$ of HLA-C* α : β sequence. For instance, the tag of HLA-A*11:01 was derived from the first sequence at positions 34 $(11 \times 3 + 1)$ to 36 $(11 \times 3 + 3)$ and the second sequence at positions 4 $(01 \times 3 + 1)$ to 15 $(01 \times 3 + 12)$ of the HLA-A*11:01 sequence. Thirty-nine HLA vectors (supplemental table 1), including HLA-A*24:02 and HLA-A*02:01, were constructed for future use.

5.3 Construction of the target cells

We designed overlapping ends on both sides of the antigen linker region (supplemental table 2 SEQ ID NO. 3-4), and different gene segments of antigens can be seamlessly assembled to the HLA vectors at the same time with only one cutting and joining procedure. Specifically, we designed 2 recognition sites of restriction enzyme BsmBI on the HLA vectors for replacing the gene segment of *EGFP* with the gene segment of antigen, which can be used to construct the plasmid with coexpressing patient HLA and antigen.

We first entrusted Synbio Technologies to synthesize the gene segments of the *KRAS* G12D neoantigen (supplemental table 2 SEQ ID NO. 1) and its paired wild antigen (supplemental table 2 SEQ ID NO. 2). Then, we excised the gene segment of *EGFP* by restriction enzyme BsmBI (NEB) and recovered the HLA-A*11:01 vector without *EGFP* from the gel (supplemental figure 4B). Gibson Assembly was performed to

assemble the gene segments of the *KRAS* G12D neoantigen and its paired wild antigen to the HLA-A*11:01 vector without *EGFP* respectively. The target plasmids lenti-(HLA-A*11:01)-*KRAS*-M and lenti-(HLA-A*11:01)-*KRAS*-W were constructed (supplemental figure 4A, supplemental plasmid sequence NO. 5-6). The 3290 bp gene segments were excised from the target plasmids respectively by restriction enzymes EcoRI (NEB) and NotI (NEB) for verification (supplemental figure 4C). Subsequently, the target plasmids and assistant plasmid pVSVg (AddGene 8454) and psPAX2 (AddGene 12260) were cotransfected into HEK293T cells for producing lentivirus. Then, COS-7 cells were infected with the lentivirus and cultured with 4 µg/ml puromycin (Sigma) for one week to establish stable target cells. DNA was extracted from the target cells by TIANamp Genomic DNA Kit (TIANGEN) following the protocol of the manufacturer. Sanger sequencing was further performed to analyze the key sequence of the target plasmids. The sequence of primer was shown as SEQ ID NO. 3 in supplemental table 5. In supplemental figure 4D, the crimson parts of the picture represented the matching sequences sequenced by Sanger sequencing, and the arrows represented the sequencing directions.

5.4 Validation and application of the Co-HA system

First, the expression of HLA-A*11:01, HLA-A*24:02, HLA-A*02:01 molecules in the Co-HA system was validated by FCM. The antibodies used to detect the expression of HLA-A*11:01 were FITC Mouse Anti-Human HLA-A (BD) and Cell Viability Solution (BD). The antibodies used to detect the expression of HLA-A*24:02 were Anti-HLA-A24 (Human) mAb-FITC (MBL) and Cell Viability Solution (BD). The antibodies used to detect the expression of HLA-A*02:01 were Mouse Anti-Human HLA-A2 (BD) and Cell Viability Solution (BD). Subsequently, we cocultured 1×10^5 T cells (including 1×10^3 CD8⁺ TCR-T cells) and 1×10^4 COS-7

cells for 3 days. The COS-7 cells had 3 types: WT COS-7 cells, COS-7 cells expressing the *KRAS* G12D neoantigen and COS-7 cells expressing its paired WT antigen. Then, the specific proliferation of CD8⁺ TCR-T cells was detected by FCM. The antibodies were PE anti-mouse TCR β chain Antibody (BioLegend), FITC Mouse Anti-Human CD8 (BD) and Cell Viability Solution (BD). In addition, we performed a series of cytotoxicity tests to detect the immunogenicity of the *KRAS* G12D neoantigen in the Co-HA system. The effector cells (1×10^5 TCR-T cells) and the target cells (1×10^4 COS-7 cells) were cocultured for 12 hours. Then, the specific secretion of IFN- γ by the TCR-T cells against the *KRAS* G12D neoantigen was detected by enzyme-linked immunospot assay (ELISPOT) and ELASA. The effector cells (1×10^5 TCR-T cells) and the target cells (1×10^4 COS-7 cells) were cocultured for 3 days. Then, the specific lysis of the COS-7 cells generated by the *KRAS* G12D neoantigen in the Co-HA system was detected by Sanger sequencing. The effector cells (1×10^6 TCR-T cells) and the target cells (1×10^5 COS-7 cells) were cocultured following the protocol of the CytoTox 96® Non-Radioactive Cytotoxicity Assay (Promega). Then, the specific cytotoxicity against COS-7 cells generated by the *KRAS* G12D neoantigen in the Co-HA system was detected by ELISA for LDH levels.

For verification of new HCC-dominant neoantigens by the Co-HA system, the effector cells were T cells amplified by potential neoantigens with PBMCs from peptides. Other steps were same as above.

6. Amplification of neoantigen-specific T cells in peripheral blood

We produced the potential neoantigen peptides in GenScript and coincubated them with 2×10^6 PBMCs/well to promote specific T-cell proliferation. They were cultured in X-VIVO 15 medium (Lonza) supplemented with 5% Human AB serum (GemCell),

100 U/ml penicillin (GIBCO), 100 µg/ml streptomycin (Gibco), and 10 IU/ml IL-2 (R&D) for 3 weeks. Medium was replenished twice a week and 4 µM/ml peptides were replenished weekly.

7. Tetramer staining

7.1 Generation of new specific tetramer using peptide exchange

New specific tetramers were generated by QuickSwitch Quant Tetramer Kit (MBL) following the protocol of peptide exchange experiments provided by the manufacturer. The predicted potential neoantigen peptides were loaded onto the tetramer at 2 mM according to standard procedure. The reference peptide was loaded onto the same tetramer at 1 mM.

7.2 Quantification of peptide exchange using flow cytometric sandwich immunoassay

All control samples were acquired following the protocol of the manufacturer. Then, the following procedure to quantify the peptide exchange efficiency was performed by flow cytometry (FCM): (1) 5 µl of Magnetic Capture Beads were pipetted to a flow cytometer tube containing 200 µl 1× Assay Buffer and run as a “beads” control; (2) Control #1 was bead-captured QuickSwitch Tetramer; (3) Control #2 was beads that have not captured any tetramer and stained with exiting peptide antibody, and control #3 (negative control) was beads that have captured the QuickSwitch Tetramer and stained with exiting peptide antibody. (4) Peptide exchange samples were run, the MFI_{FITC} of each was noted. Peptide-exchanged tetramers displayed various Exiting Peptide amounts, which were inversely proportional to the newly loaded peptide on the HLA molecules. Control #4 was the reference peptide; (5) The percentages of peptide exchange of each peptide exchange samples were calculated. Tetramers bound to T-cell receptors via three HLA/peptide monomers^[22-23]. Therefore, the minimal

recommended peptide exchange percentage should be 75%.

7.3 Detection of the neoantigen-specific T cells in peripheral blood by Tetramer staining

The cells tested were re-suspended at a concentration of 2×10^7 cells/ml. 50 μ l of samples was prepared for tetramer staining. 10 μ l Clear Back (MBL), a human FcR blocking reagent for reducing nonspecific staining, was added to each tube. They were incubated for 5 min at room temperature. 10 μ l of tetramer was added and vortexed gently. They were protected from light and incubated for 30 min at room temperature. FITC Mouse Anti-Human CD8 (BD) was added and vortexed gently. They were protected from light and incubated for 20 min at 4 °C. PBS was used to rinse for 3 times. The cells were resuspended in 500 μ l of PBS with Cell Viability Solution (BD) and tested by FCM immediately.

8. Antitumor efficacy of *ENTPD6* neoantigen-specific T cells in vivo

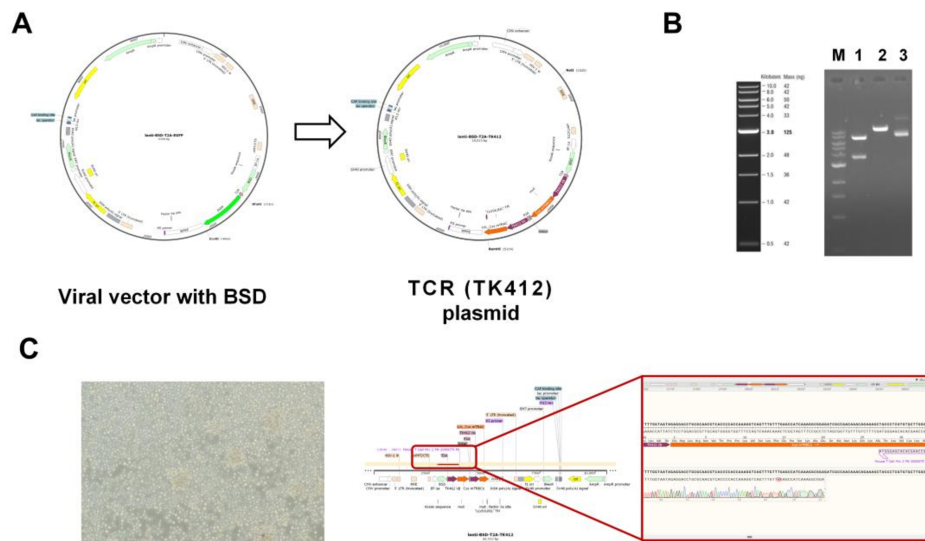
The *ENTPD6* neoantigen-specific T cells from patient 04 were sorted by FCM after tetramer staining (supplemental figure 10A). The survival rate of the neoantigen-specific T cells sorted by FCM and cultivated in vitro (supplemental figure 10B). The Huh-7 cells coexpressing HLA-A*24:02 and *ENTPD6* neoantigen were selected as the target cells (supplemental figure 9A), and then implanted into the B-NDG-*B2m^{tm1}Fcrn^{tm1(mB2m)}*/Bcgen mouse model. When tumor size was approximately 150 ± 50 mm³, they were treated with the neoantigen-specific T cells (0.5×10^6) through intratumor injection every 2 days. In addition, the key sequences of the *ENTPD6* in tumors were detected by Sanger sequencing.

9. Quantification and statistical analysis

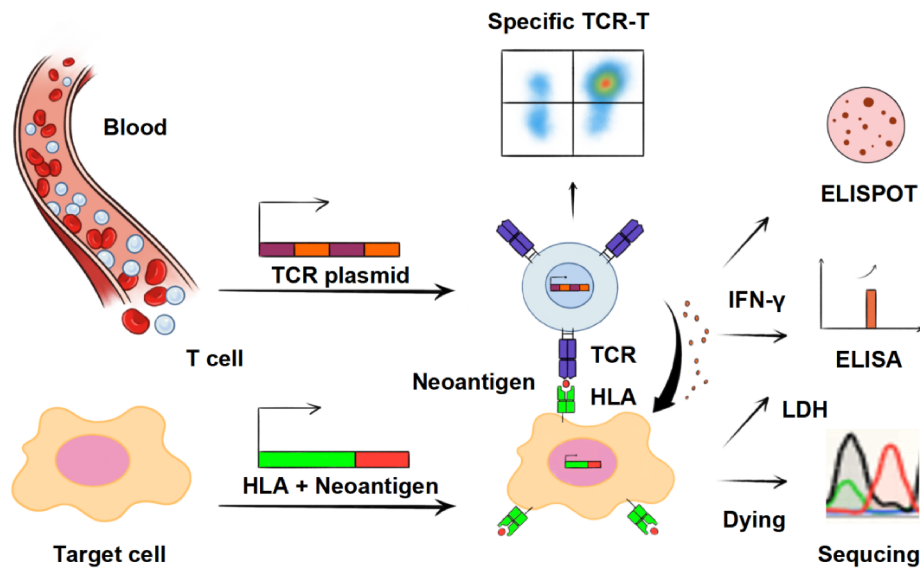
Statistical analyzes were conducted using GraphPad Prism 8.0.2 software. A 2-tailed *P*

value < 0.05 was considered significant. The method for two group comparison is 2-tailed unpaired t test.

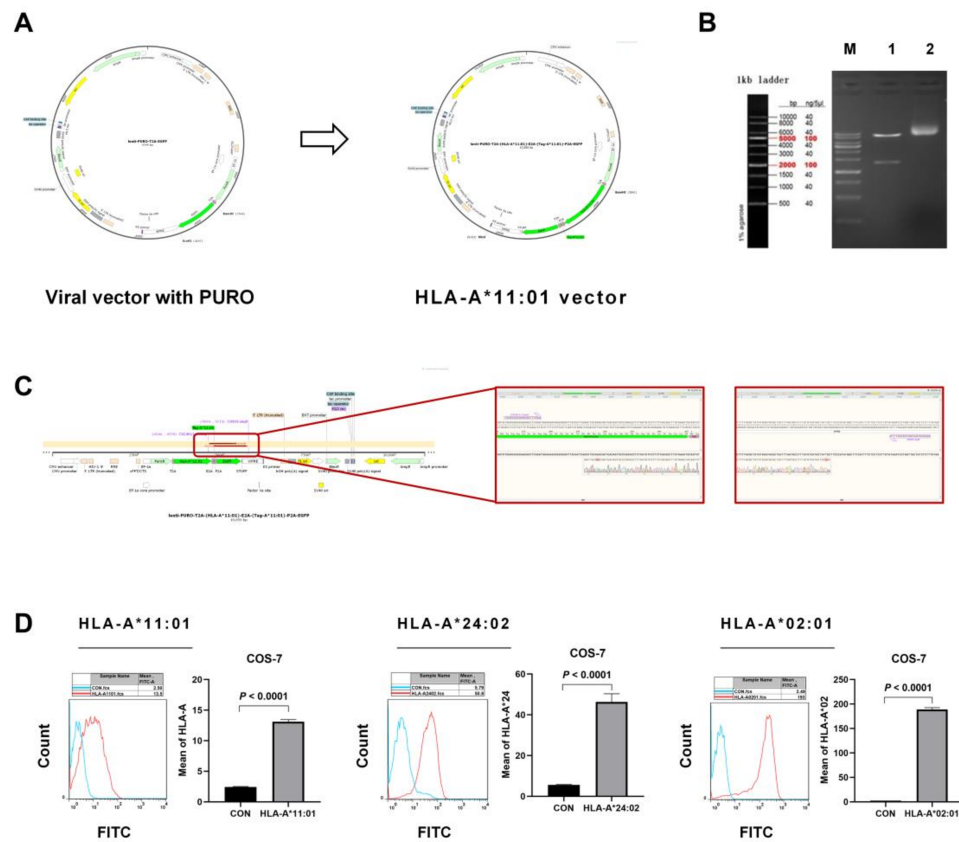
SUPPLEMENTAL FIGURES



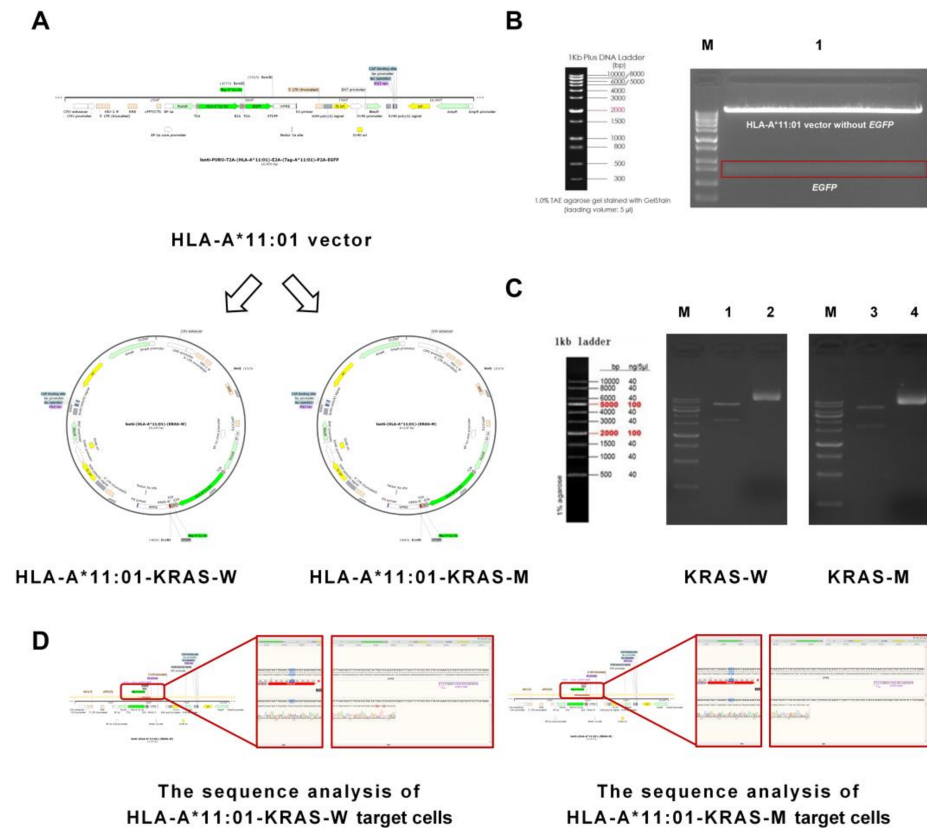
Supplemental figure 1 The construction of the *KRAS* G12D TCR plasmid. (A) The map of the *KRAS* G12D TCR plasmid. (B) Validation of the *KRAS* G12D TCR plasmid by gel electrophoresis. Lane M shows the DNA marker, lane 1 shows the TCR plasmid digested by BamHI and NotI, lane 2 shows the TCR plasmid digested by BamHI, and lane 3 shows the TCR plasmid without digestion. (C) The established *KRAS* G12D TCR-T cells and the key sequence of the TCR-T cells analyzed with Sanger sequencing.



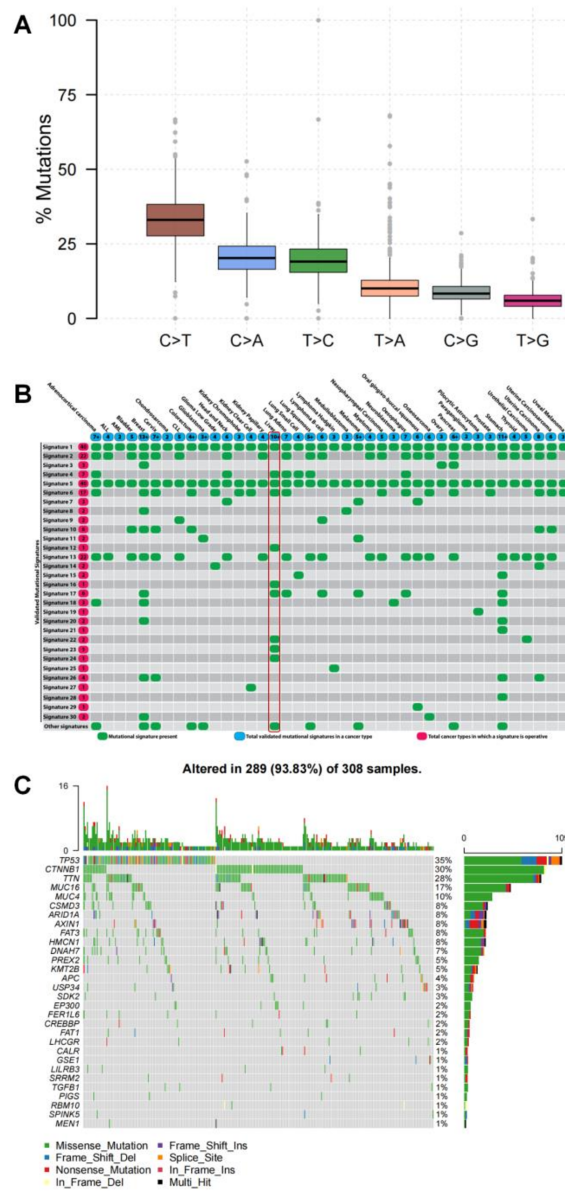
Supplemental figure 2 Schematic for the establishment and validation of the Co-HA system by the *KRAS* G12D TCR-T cells. HLA, human leukocyte antigen; IFN- γ , interferon- γ ; LDH, lactate dehydrogenase; ELISPOT, enzyme-linked immunospot assay; ELISA, enzyme-linked immunosorbent assay.



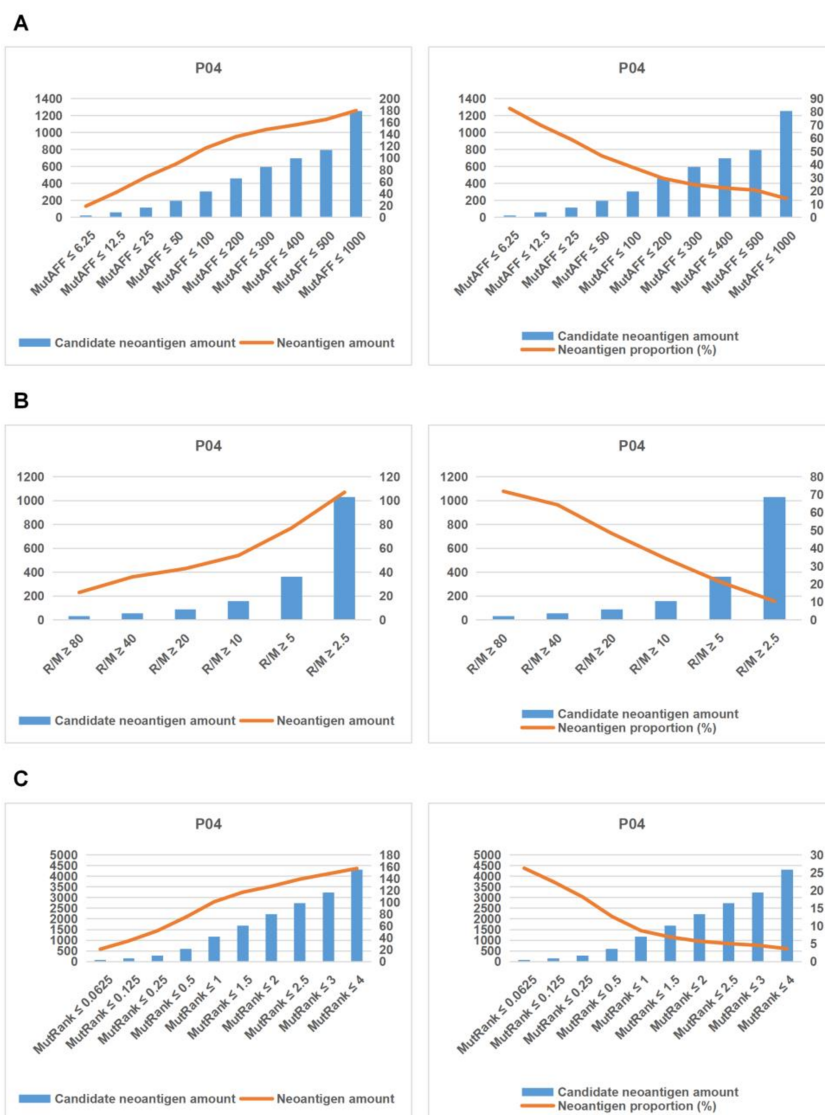
Supplemental figure 3 The construction of the HLA vectors. (A) The map of the HLA-A*11:01 vector. (B) Validation of the HLA-A*11:01 vector by gel electrophoresis. Lane M shows the DNA Marker, lane 1 shows the HLA-A*11:01 vector digested by BamHI and XhoI, and lane 2 shows the HLA-A*11:01 vector without digestion. (C) The key sequence of the HLA-A*11:01 vector analyzed with Sanger sequencing. (D) The expression of prevalent HLA molecules in the COS-7 cells constructed by the Co-HA system. HLA, human leukocyte antigen.



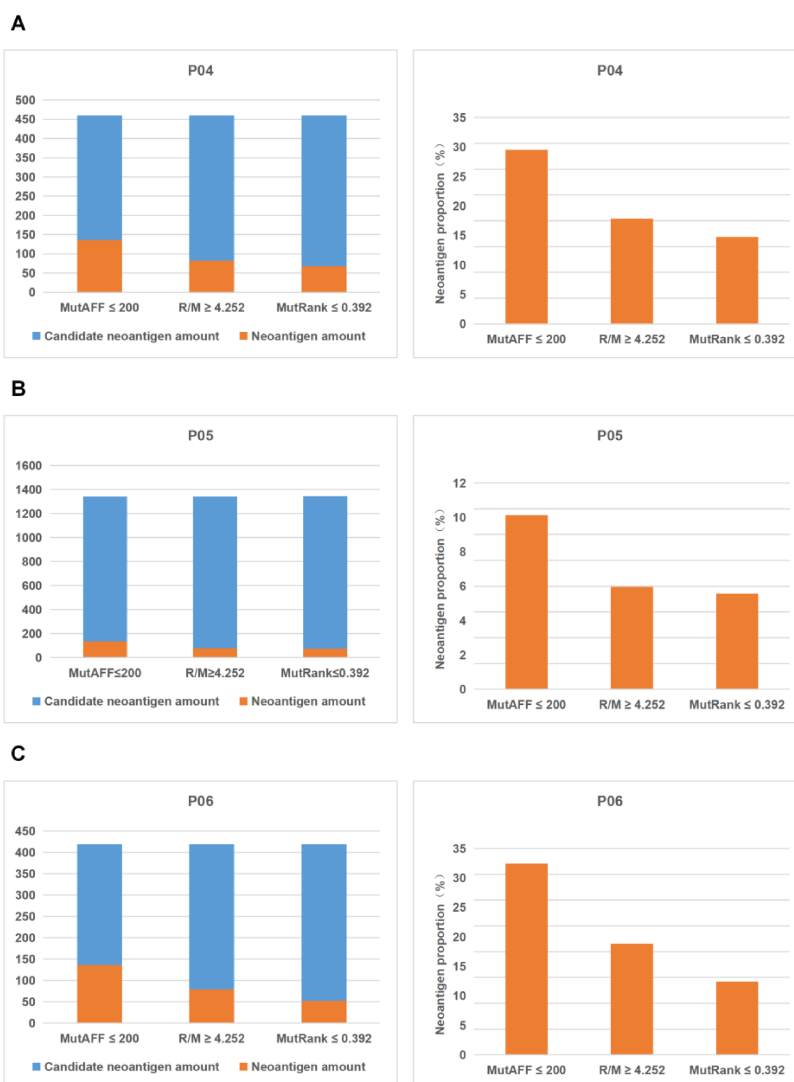
Supplemental figure 4 The construction of target cells coexpressing HLA-A*11:01 and antigens. (A) The maps of the target cell plasmids. (B) The construction of HLA*11:01 vector without *EGFP*. To insert the gene segment of the antigen, the gene segment of *EGFP* in the vector was released from the vector by cleavage with the restriction enzyme BsmBI. Lane M shows the DNA Marker and lane 1 shows the HLA-A*11:01 vector digested by BsmBI. (C) Validation of the target cell plasmids by gel electrophoresis. Lane M shows the DNA marker, lanes 1 and 3 show the target cell plasmids digested by EcoRI and NotI, and lanes 2 and 4 shows the target cell plasmids without digestion. (D) The key sequences of HLA-A*11:01-KRAS-W or HLA-A*11:01-KRAS-M in the COS-7 cells analyzed with Sanger sequencing. HLA, human leukocyte antigen.



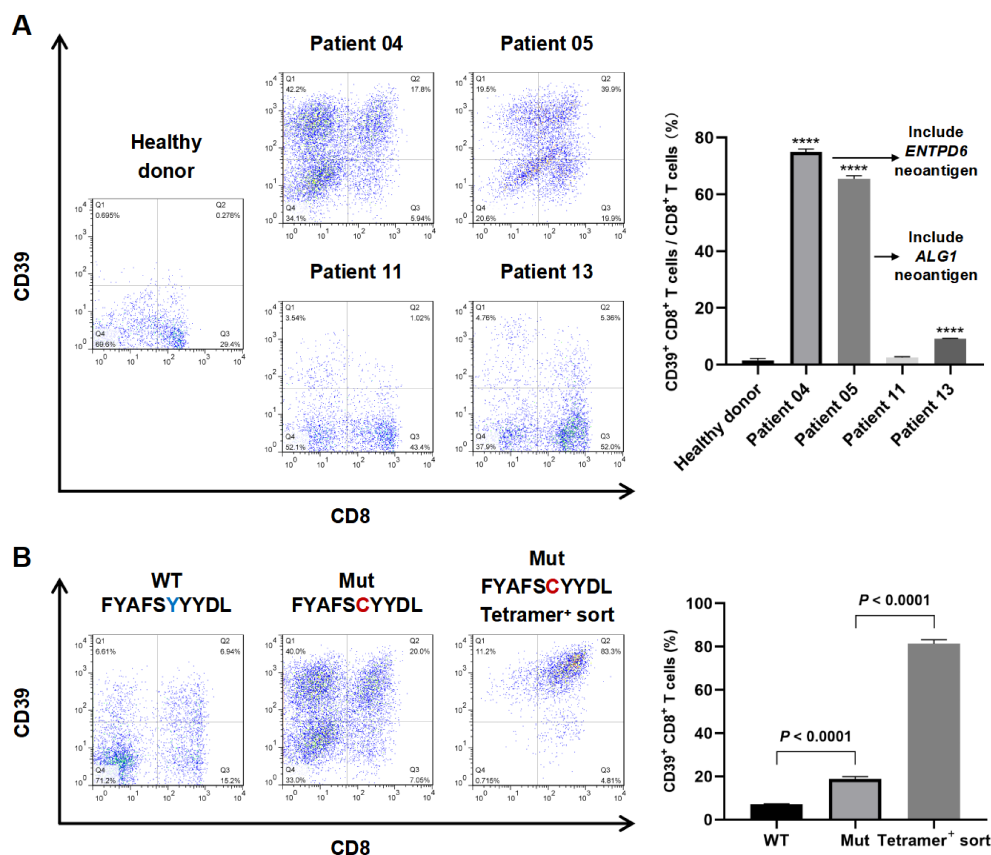
Supplemental figure 5 The mutation spectrum of tumor tissues of HCC patients in databases. (A) The proportions of base substitution subtypes of the samples in TCGA-LIHC dataset. (B) Reference of mutational signatures of tumors in COSMIC database. (C) The key mutated genes and the TMB of the samples in TCGA-LIHC dataset. TCGA-LIHC, The Cancer Genome Atlas Liver Hepatocellular Carcinoma; COSMIC, Catalogue Of Somatic Mutations In Cancer.



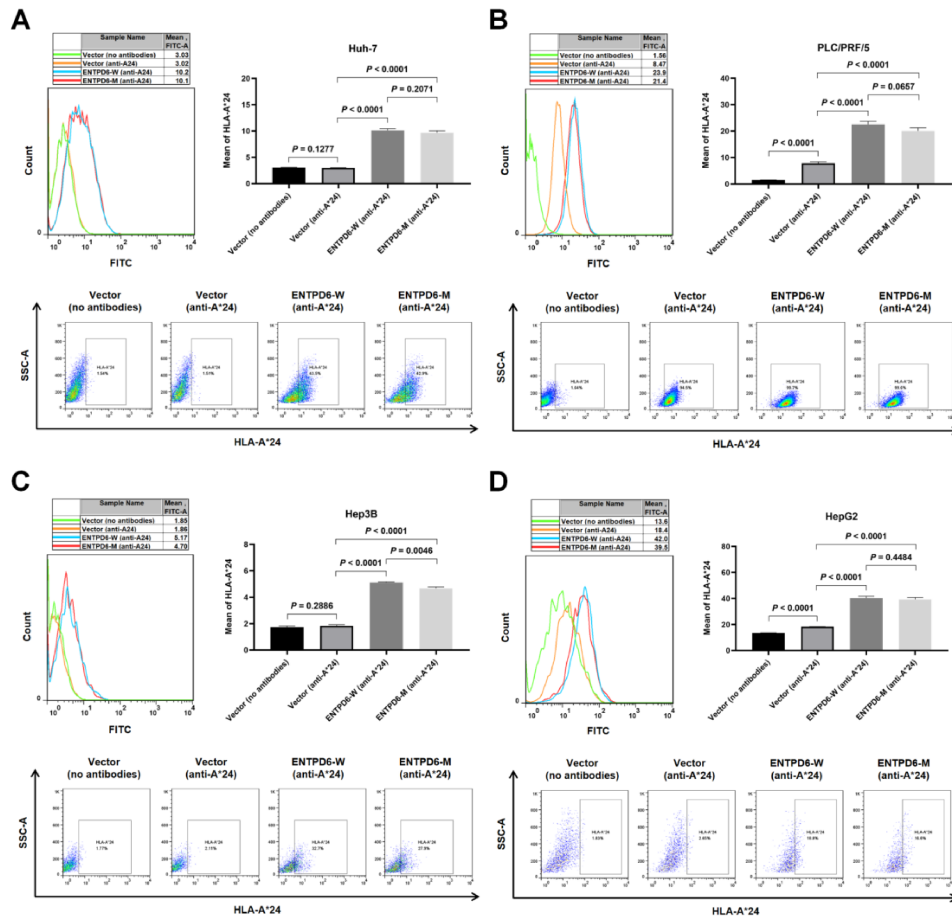
Supplemental figure 6 The screening efficiency of neoantigens screened with different filter values of MutAFF, R/M and MutRank. (A) The candidate neoantigen amount, neoantigen amount and neoantigen proportion screened with different filter values of MutAFF (nM). (B) The candidate neoantigen amount, neoantigen amount and neoantigen proportion screened with different filter values of R/M. (C) The candidate neoantigen amount, neoantigen amount and neoantigen proportion screened with different filter values of MutRank (%). The candidate neoantigen amount is the amount of screened neoantigens. Neoantigen amount is the amount of screened real neoantigens. Neoantigen proportion is the proportion of real neoantigens in candidate neoantigens. MutAFF, mutated peptide affinity; R/M, referenced peptide affinity / mutated peptide affinity. MutRank, the rank of mutated peptide affinity.



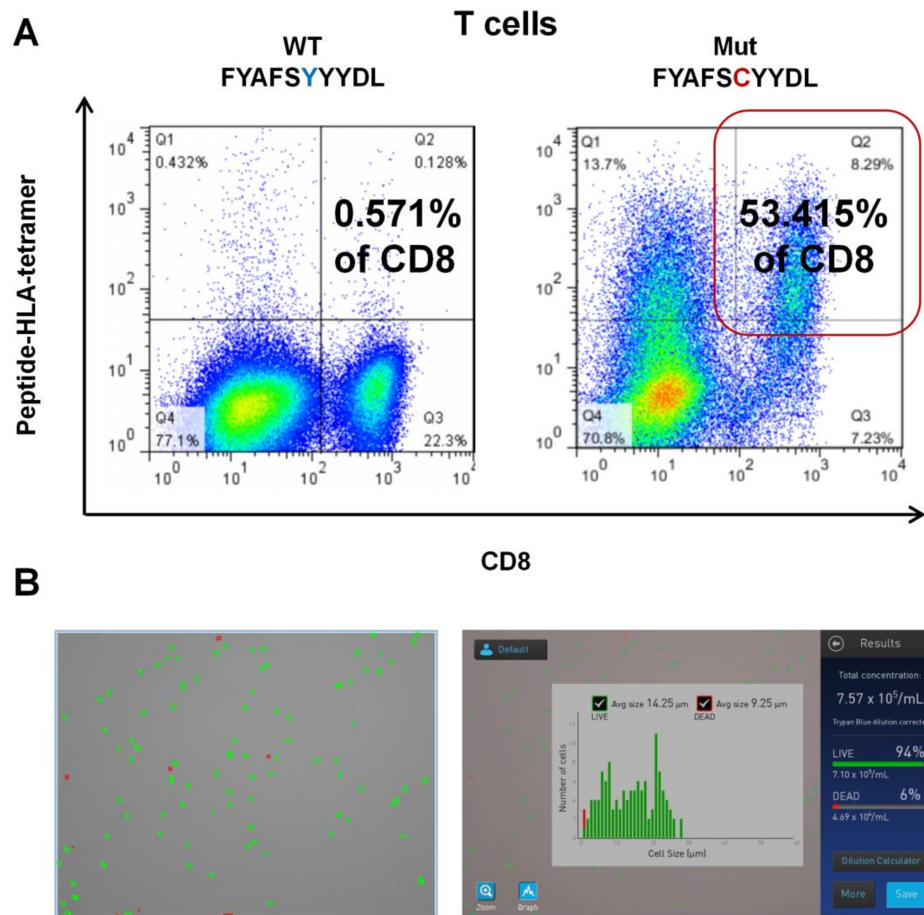
Supplemental figure 7 The comparison of screening efficiency of neoantigens by MutAFF, R/M and MutRank. (A) The comparison of screening efficiency of neoantigens in Patient 04 by MutAFF, R/M and MutRank. (B) The comparison of screening efficiency of neoantigens in Patient 05 by MutAFF, R/M and MutRank. (C) The comparison of screening efficiency of neoantigens in Patient 06 by MutAFF, R/M and MutRank. The candidate neoantigen amount is the amount of screened neoantigens. Neoantigen amount is the amount of screened real neoantigens. Neoantigen proportion is the proportion of real neoantigens in candidate neoantigens. MutAFF, mutated peptide affinity; R/M, referenced peptide affinity / mutated peptide affinity. MutRank, the rank of mutated peptide affinity.



Supplemental figure 8 The high frequency of CD39⁺ CD8⁺ T cells in HCC patients with dominant neoantigens. (A) The frequency of CD39⁺ CD8⁺ T cells after stimulation with predicted neoantigen peptides in HCC patients. (B) The frequency of CD39⁺ CD8⁺ T cells in patient 04 induced by *ENTPD6* neoantigen peptides or its paired WT peptide. The high frequency of CD39⁺ CD8⁺ T cells in the tetramer⁺ *ENTPD6* neoantigen-specific T cells sorted by FCM. FCM, flow cytometry.



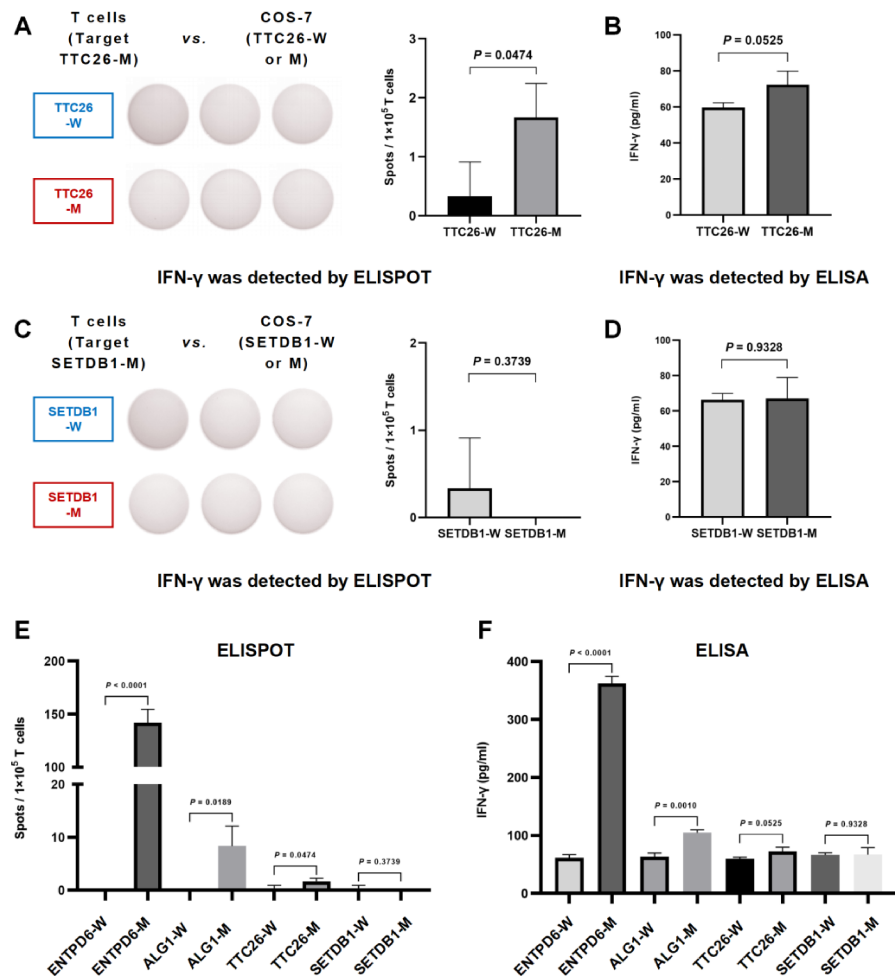
Supplemental figure 9 The construction of target cells in HCC cell lines coexpressing HLA-A*24:02 and *ENTPD6* neoantigen. (A) The expression of HLA-A*24:02 on Huh-7 cells. (B) The expression of HLA-A*24:02 on PLC/PRF/5 cells. (C) The expression of HLA-A*24:02 on Hep3B cells. (D) The expression of HLA-A*24:02 on HepG2 cells. HLA, human leukocyte antigen.



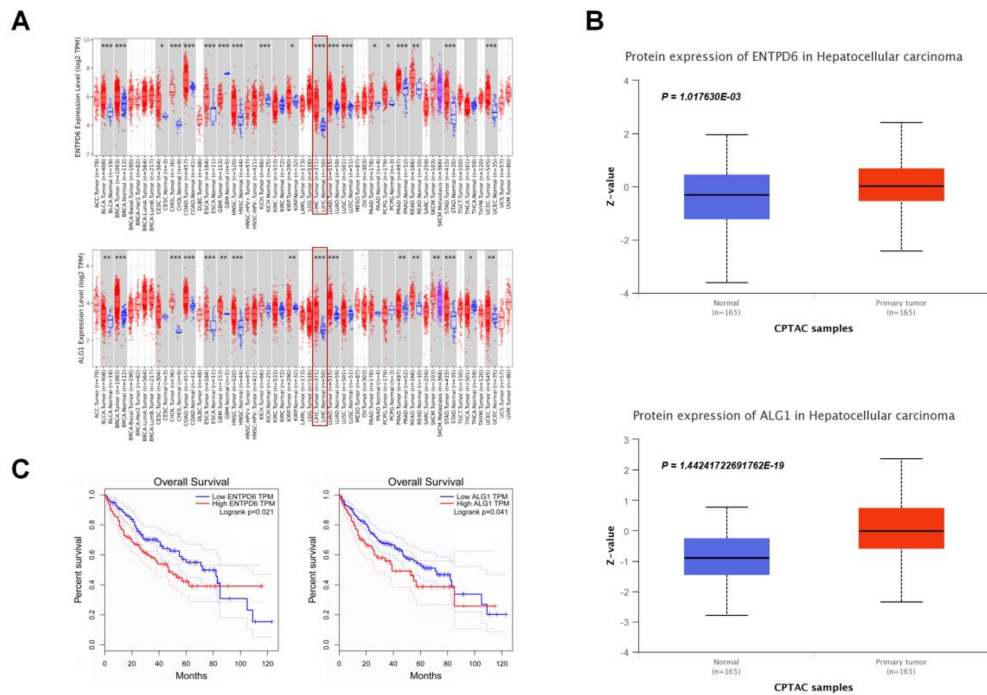
Supplemental figure 10 The *ENTPD6* neoantigen-specific T cells from patient 04. (A) Tetramer staining of PBMCs from patient 04 after stimulation with the *ENTPD6* neoantigens and paired WT peptides. (B) The survival rate of the *ENTPD6* neoantigen-specific T cells acquired by FCM and cultivated in vitro. PBMCs, peripheral blood mononuclear cells; FCM, flow cytometry.



Supplemental figure 11 The frequency of the new HCC-dominant neoantigens in our HCC cohort. (A) The frequency of the neoantigen 5'-FYAFSCYYDL-3' in our HCC cohort determined by Sanger sequencing. (B) The frequency of the neoantigen 5'-WVWCMSPTI-3' in our HCC cohort determined by Sanger sequencing. HLA, human leukocyte antigen.



Supplemental figure 12 Two predicted potential neoantigens are nondominant neoantigens verified in the Co-HA system. (A) The secretion of IFN- γ by T cells from PBMCs of patient 05 against the *TTC26* mutated antigen and the paired WT antigen in the Co-HA system detected by ELISPOT. (B) The secretion of IFN- γ by T cells from PBMCs of patient 05 against the *TTC26* mutated antigen and the paired WT antigen in the Co-HA system detected by ELISA. (C) The secretion of IFN- γ by T cells from PBMCs of patient 05 against the *SETDB1* mutated antigen and the paired WT antigen in the Co-HA system detected by ELISPOT. (D) The secretion of IFN- γ by T cells from PBMCs of patient 05 against the *SETDB1* mutated antigen and the paired WT antigen in the Co-HA system detected by ELISA. (E) The comparison between the dominant neoantigens and nondominant neoantigens detected by ELISPOT in the Co-HA system. (F) The comparison between the dominant neoantigens and nondominant neoantigens detected by ELISA in the Co-HA system. PBMCs, peripheral blood mononuclear cells; ELISPOT, enzyme-linked immunospot assay; ELISA, enzyme-linked immunosorbent assay; IFN- γ , Interferon gamma.



Supplemental figure 13 The expression level and the clinical correlation analysis of *ENTPD6* and *ALG1*. (A) The differential expression between tumor and adjacent normal tissues for *ENTPD6* and *ALG1* in various cancers of TCGA database. Distributions of gene expression levels are displayed using box plots. The statistical significance computed by the Wilcoxon test is annotated by the number of stars ($P < 0.05$; $**P < 0.01$; $***P < 0.001$). (B) The expression of *ENTPD6* and *ALG1* in the CPTAC-LIHC dataset. (C) Overall survival analysis of HCC patients with high and low expression levels of *ENTPD6* or *ALG1* in TCGA-LIHC dataset. TCGA, The Cancer Genome Atlas; LIHC, Liver Hepatocellular Carcinoma; CPTAC, Clinical Proteomic Tumor Analysis Consortium.

SUPPLEMENTAL TABLES

Supplemental table 1 Summary of constructed HLA vectors

HLA-A		HLA-B		HLA-C	
A*11:01	A*03:01	B*40:01	B*27:01	C*01:02	C*15:02
A*24:02	A*32:01	B*15:01	B*55:01	C*07:02	C*03:03
A*02:01	A*02:06	B*46:01	B*39:01	C*03:04	C*07:04
A*33:01		B*13:01	B*38:01	C*08:01	C*08:01
A*30:01		B*51:01	B*52:01	C*04:01	
A*31:01		B*58:01	B*44:02	C*14:02	
A*26:01		B*35:01	B*15:18	C*06:02	
A*01:01		B*54:01	B*67:01	C*12:02	

Supplemental table 2 Sequence list of antigens and overlaps

SEQ ID	Name	Sequence
NO. 1	KRAS-M	GTAGTTGGAGCTGATGGCGTAGGCAAG
NO. 2	KRAS-W	GTAGTTGGAGCTGGTGGCGTAGGCAAG
NO. 3	Overlap-1	GATGTCGAAGAGAATCCTGGACCG
NO. 4	Overlap-2	TAGCCTAAGAATTCGATATCAAGC

Supplemental table 3 The characteristics of 37 paired mutated and WT peptides restricted by HLA-A*24:02, HLA-A*02:01 or HLA-A*11:01

Patient	Gene	Mut	HLA	MutPeptide	MutAFF (nM)	MutRank	RefPeptide	RefAFF (nM)	RefRank
04	<i>ENTPD6</i>	Y/C	HLA-A24:02	FYAFSCYYDL	82.16	1.957	FYAFSYYDL	51.58	1.101
04	<i>VPS13D</i>	Y/F	HLA-A24:02	RFIDTCMVIF	52.99	0.33	RYIDTCMVIF	9.54	0.048
04	<i>VPS13D</i>	Y/F	HLA-A24:02	RFIDTCMVI	124.72	0.752	RYIDTCMVI	13.08	0.144
05	<i>CTNNB1</i>	D/H	HLA-A02:01	YLHSGIHSGA	93.29	0.397	YLDSGIHSGA	55.69	0.215
05	<i>SEPT1</i>	N/S	HLA-A02:01	FLSLRRMLV	63.97	1.192	FLNLRRLV	69.82	1.119
05	<i>HEATR6</i>	I/M	HLA-A02:01	MLMSLEDKSL	56.44	1.958	ILMSLEDKSL	124.57	1.457
05	<i>ALG1</i>	G/V	HLA-A02:01	YSIMGLVHV	102.37	1.064	YSIMGLVHG	17767.11	36.417
05	<i>ALG1</i>	A/S	HLA-A02:01	WVWCMSPTI	41.2	3.375	WVWCMAPTI	72.26	4.695
05	<i>TTC26</i>	G/A	HLA-A02:01	KMAQFYISA	11.98	0.602	KMGQFYISA	35.01	1.321
05	<i>SETDB1</i>	L/I	HLA-A02:01	KQIAELETWV	113.57	1.531	KQLAELETWV	30.33	0.661
11	<i>TMEM176B</i>	R/T	HLA-A11:01	CTAYMQMLR	41.27	0.948	CRAYMQMLR	11832.89	12.191
11	<i>TMEM176B</i>	R/T	HLA-A11:01	CTAYMQMLRK	22.17	1.604	CRAYMQMLRK	277.17	3.489
11	<i>HNRNPA2B1</i>	D/G	HLA-A11:01	GPVDKIVLQK	172.23	0.31	DPVDKIVLQK	3224.26	2.437
11	<i>PPFIBP2</i>	I/K	HLA-A11:01	KLGFSHFGNK	83.75	1.52	KLGFSHFGNI	19571.99	33
11	<i>TMEM176B</i>	R/T	HLA-A11:01	TAYMQMLRK	16.58	0.123	RAYMQMLRK	16.33	0.084
11	<i>HNRNPA2B1</i>	D/G	HLA-A11:01	VTFDDHGPVVK	104.23	0.17	VTFDDHDPVVK	270.42	0.499
11	<i>TUBG1</i>	Q/R	HLA-A11:01	ASIRVALSR	40.43	0.13	ASIQVALSR	73.17	0.112
11	<i>TUBG1</i>	Q/R	HLA-A11:01	ASIRVALSRK	17.27	0.239	ASIQVALSRK	20.52	0.104
11	<i>TUBG1</i>	Q/R	HLA-A11:01	SIRVALSRK	189.1	0.884	SIQVALSRK	25.38	0.151
11	<i>OSER1</i>	S/C	HLA-A11:01	ASSSSQLK	24.82	0.153	ASSSSQLK	18.86	0.038
11	<i>OSER1</i>	S/C	HLA-A11:01	TIASSSSQLK	108.54	0.447	TIASSSSQLK	112.17	0.403
11	<i>OSER1</i>	S/C	HLA-A11:01	SSSSQLKHK	33.3	0.457	SSSSQLKHK	45.62	0.149
11	<i>OSER1</i>	S/C	HLA-A11:01	SSSSQLKHK	143.13	0.714	SSSSQLKHK	89.38	0.141
11	<i>OSER1</i>	S/C	HLA-A11:01	IASSSSQLK	110.36	1.358	IASSSSQLK	104.67	0.826
13	<i>FNDC3A</i>	A/S	HLA-A11:01	LSYSIDFGDK	66.69	1.466	LAYSIDFGDK	189.17	2.481
13	<i>CKAP4</i>	R/S	HLA-A11:01	ASCSRSLGR	186.71	0.734	ASCRRLGR	569.46	1.475
13	<i>SLC25A15</i>	L/V	HLA-A11:01	VSTVWSVIK	83.89	0.777	LSTVWSVIK	123.35	1.287
13	<i>SLC25A15</i>	L/V	HLA-A11:01	KVSTVWSVIK	25.33	0.281	KLSTVWSVIK	71.25	1.013
13	<i>GART</i>	V/F	HLA-A11:01	VTEAVFAGIAK	188.37	1.08	VTEAVVAGIAK	317.35	1.118
13	<i>SDS</i>	H/Y	HLA-A11:01	MSGEPLYVK	43.76	0.162	MSGEPLHVK	67.56	0.143
13	<i>SDS</i>	H/Y	HLA-A11:01	MMSGEPLYVK	29	0.807	MMSGEPLHVK	76.73	0.694
13	<i>SDS</i>	H/Y	HLA-A11:01	GMSGEPLYVK	73.27	0.823	GMSGEPLHVK	106.22	0.572
13	<i>EP400</i>	P/R	HLA-A11:01	KTDPSAAGRK	172.96	0.704	KTDPSAAGRK	84.51	0.154
13	<i>AK3</i>	G/V	HLA-A11:01	ETFSVTETNK	198.61	0.543	ETFSGTETNK	226.36	0.383
13	<i>TMEM219</i>	R/H	HLA-A11:01	TAMCFPHR	183.81	2.211	TAMCFPRR	223.43	2.507
13	<i>TMEM219</i>	R/H	HLA-A11:01	VTAMCFPHR	132.99	2.72	VTAMCFPRR	161.63	3.042
13	<i>DAB2IP</i>	I/V	HLA-A11:01	IVNSYCVFPR	97.83	3.095	IINSYCVFPR	199.66	4.497

Mut, mutation; MutAFF, mutated peptide affinity; MutRank, the rank of mutated peptide affinity. RefAFF, referenced (WT) peptide affinity; RefRank, the rank of referenced (WT) peptide affinity.

Supplemental table 4 The sequence of potential *ENTPD6* specific TCRs

	TCR Vα	TCR Vβ
S5-1-BA1	ATGACACGAGTTAGCTTGTCAAGGTGGTCCACAGCTTCACTGTGG CTAGGACCTGGCAATTCGCCAGAAGTAACCTAAACCCAAACAGGA ATGTTCTGTGCAGAAAAGGAGGTGTGACTCTGGACTGCACATAT GACCACAGTATCCAAGTTATGGTCTATCTGTGACAAAGCAGCCC AGCAGTGGGAAATGATTTTCTTATTTATCAGGGGTCTTATGACC AGCAAATGCAACAGAAAGTCTGCTACTCATTTGAATTCACAGAAGG CAAGAAATCGCCAACTTGTCAITCTCCCTTCACAACTGGGGG ACTCACCAATCTACTTCTCTCAATGGAGAGATGGAACTGGTA ACCAGTCTATTTGGGACAGGGCAAGTTTGGCGGTCAATCCAA AT ATGGAGACCCTCTTGGGGCTGCTATCCTTTGGCTGCAGCTCAAT GGGTGAGCAGAAACAGGAGGTGACACAGATTCTGGAGCTCTGA GTGTCGCCAGAAGGAGAAATCTGGTTCCTAACTGCAGTTTCACTG ATAGCGCTATTACAACTCCAGTGGTTAGCGAGGACCCCTGGGA AGCGTCTGACACTCTGCTTCTATTCCTCAAGTCACTCCAGAGC AAACAAGTGGAAAGCTTAAAGCTCGCTGGATAAATCATCAGGAC GTAGTACTTTATACATTCAGCTCTCAGCCTGGTGACTACGCCAC CTACCTCTGTGCTGTGAACAGGCAAGTCTGTCTGATCTTTGGG AAGGGAACCCTTATCAGTGAAGTCCAAAT ATGGTCTGAAATCTCCGTGTCCATTCTTTGGATTGAGTTGGCAT GGGTGGAGCCAGCTGCTGGAGCAGGACCCCTAGTTTCTAAGCA TCCAAAGGAGGAGAAATCTCACTGTGCTACTGCAACTCTCAAGT TTTITTCCAGCTTACAAGTGTACAGACAGGAGCCCTGGGGAAGTTC CTGTCTCTGTGGACAGTGTACGGGTGGAGAAAGTGAAGAAGC TGAAGAGACTAACCTTTCAGTTTGGTGTACAGAAAGGACAGT CTCTCCACATCACTCGGGCCAGCCTGGTATACAGGCCCTCTACCT CTGTCCAGCGTGTACTGTGGTGTACTAGCTATGGAAAGCT GACATTITGGCAAGGAGCAATCTTACTGTTCCTCAAT GGTGGAGCCAGCTGCTGGAGCAGGAGCCCTAGTTTCTAAGCA TCCAAAGGAGGAGAAATCTCACTGTGACTGCAACTCTCAAGT TTTITTCCAGCTTACAAGTGTACAGACAGGAGCCCTGGGGAAGTTC CTGTCTCTGTGGACAGTGTACGGGTGGAGAAAGTGAAGAAGC TGAAGAGACTAACCTTTCAGTTTGGTGTACAGAAAGGACAGT CTCTCCACATCACTCGGGCCAGCCTGGTATACAGGCCCTCTACCT GACTTITGGCAAGGAGCAATCTTACTGTTCCTCAAT ATGAAGACATTTGTGGATTTCTGTCTGTTTITGGGTGTGAGC TGGACTGTATGAGTAGAGGAGGAGGTGTGAGGACGAGTCTTTCC TGAGTGTCCGAGAGGAGACAGCTCCGTTATAAAGTCACTTACA CAGACAGCTCTCCACCTACTTACTGGTATAAGCAAGAACTGT GAGCAGGTCTCCAGTGTGAGCTATATTTTCAAATATGGACAT GAAACAAGACAAAGACTACTGTCTATTGAATAAAAGGATAA ACATCTCTCTCCGATTTGACAGCAGCAGACTGGGACTCAGC TATCTACTTCTGTCCAGAGAGAGGAGACTGGAGGCTTCAAACAT CTTTGGAGCAGGAACAAGACTATTGTTAAAGCAAAAT ATGGAGAAAATGTTGGAGTGTGCATTCATAGTCTTGGCTCAGC TTGGCTGGTGTGAGTGGAGAAGACCAGTGTACGAGAGTCCCGAGG CCCTGAGACTCCAGGAGGAGAGAGTACAGTCTCAACTGCAGT ACACAGTACGCGTTTAAAGAGGCTGTTCTGTGATAGGCAAGATC CTGGAAAGGCTGTGATTTCTCTCAGCTGTATCACTGGTGGGA AGAAAAGGAGAAAGAAAGGCTAAAGCCACATTAAACAAGAAGG AAAGCTTCTGCACATCACAGCCCTAAACCTGAAGACTCAGCCA CTTATCTGTGCTGTGCAAGGCTCAGCGGAGGAGGAAACAAC TCACTTTGGGACAGGCACTCAGCTAAAGTGGAACTCAAT ATGAGGCTGGTGGCAAGAGTAAGTGTGTTCTGACCTTTGGAACT ATAATTGATGCTAAGCAACCAGCCACTCCATGATTTGGCTT GAAGGAAGAGCTGTGATTTCTCTCAGCTGTATCACTGGATCAGT GGAATGAGTATGTTATGGTATCAGACAGATTCACCTCCAGGGG CCACAGTATATCATTTGTTTAAAACAAGTAAAACAATGAA ATGGCTCTCTGATCATCAGAAAGACAGAAAGTCCAGCACTTG ATCCTGCCCCAGCTACGCTGAGAGACTGTCTGTACTATTGCA TCGACCTGGATGCCAGACTATGTTGGAGATGGAACCTAGCTGG TGGTGAAGCCCAAT	ATGGCCCCGGGCTCTTGCTGTGTGGTCTTGGCTTTAGGAG CAGGCCCTGGAAAGCCAAAGTGAACCCAGAACCAGGATACCTCA TCACAGTGAAGGAAAGTAAAGTAACTGACTGTCTTCTCAGAATAT GAACATGAGTATATGCTTGGTATCGACAAGCCAGGGCTGGG CTTAAAGCAGATCTACTATTCATGAAATGTGGAGTGAAGTAAAG GGAGATGTCTTGAAGGGTACAAGTCTCTCGAAAGAGAGAAGAG AATTTCCCTGTGCTGGAGTGGCCAGGCCAAACAGACTCTC TGTCTCTGTGGCAGCAGTGAAGTAAAGGAGTCCAGATACAGATACGGA GTATTTTGGCCCAGGCCCGGTGACAGTGTCT
SP10-2-BA6	ATGGATATCTGGCTCTCTGCTGTGGCCCTTTGTCTCTGGGAGC AGGCCAGTGGATTCTGGAGTCAACAACCCCAAAAGCAGCTGAT CACAGCACTGGACAGCGGATGACGCTAGATGCTCCCTAGTCT TGGAGACTCTGTGTACTGTTACCAACAGAGACTTGGACAGCGGC CTGAGTATGACAATAAGAAAGTCTTGAATGAGGAGCAACATAT GGGACAGAGGCTATGTATGTTACAAGCAGAAAGCTTAAGAAGCC ACCGAGCTCATGTITGTCTACAGCTATGAGAACTCTATAAAT GAAAGTGTGCAAGTGGTCTTCACTGAATGCCCCAACAGCTCTC TCTTAAACCTTCACTACAGCCCTGCAGCAGAGAACTCAGCCCT GTATCTCTGGCCAGTGGAAACCGGGGAGCTTTTTGGAGAAGCC CTTAGGCTGACCTGACTG ATGTCACAGTGTGCTGCTGGTGTCTGTCTCTGGGAG CAGTCCCATAGACACTGAAGTTACCCAGACACAAACACCTGTG CATGGAAATGACAAAAGAAAGTCTTGAATGTAAGAACTAT TGGGCACAAGAGTGTGCTGTGACCAAGGCTCTGGTCAAGG GCCCCAGTATTTCTCAGTATTATGAGAAAGAGAGAGAAAG AGGAAACTTCCGTGATCGATTCTCAGCTGCCAGTTCCTACTACT AGCTCTGAGTGAATGTGAACCGCTTTGTGCTGGGGACTCGGCC TGTATTTGTGTCAGCAGTGTGGAGTGTGATGAACTGAAAGC TTCTTTTGGCAAGGAGCACTTGAAGTGTGTA ATGGGCACAGCTCTCTGCTGGGCTGTCTGTCTCTGGGAG CAGTCCCATAGACACTGAAGTTACCCAGACACAAACACCTGTG CATGGAAATGACAAAAGAAAGTCTTGAATGTAAGAACTAT TGGGCACAAGGCTATGATTTGTACAAGCAGAAAGCTAAGAAGCC ACCGAGCTCATGTITGTCTACAGCTATGAGAACTCTATAAAT GAAAGTGTGCAAGTGGTCTTCACTGAATGCCCCAACAGCTCTC TCTTAAACCTTCACTACAGCCCTGCAGCAGAGAACTCAGCCCT GTATCTCTGGCCAGTGGAAACCGGGGAGCTTTTTGGAGAAGCC TCTAGGCTGACCTGACTG	ATGGATATCTGGCTCTCTGCTGTGGCCCTTTGTCTCTGGGAGC AGGCCAGTGGATTCTGGAGTCAACAACCCCAAAAGCAGCTGAT CACAGCACTGGACAGCGGATGACGCTAGATGCTCCCTAGTCT TGGAGACTCTGTGTACTGTTACCAACAGAGACTTGGACAGCGGC CTGAGTATGACAATAAGAAAGTCTTGAATGAGGAGCAACATAT GGGACAGAGGCTATGTATGTTACAAGCAGAAAGCTTAAGAAGCC ACCGAGCTCATGTITGTCTACAGCTATGAGAACTCTATAAAT GAAAGTGTGCAAGTGGTCTTCACTGAATGCCCCAACAGCTCTC TCTTAAACCTTCACTACAGCCCTGCAGCAGAGAACTCAGCCCT GTATCTCTGGCCAGTGGAAACCGGGGAGCTTTTTGGAGAAGCC TCTAGGCTGACCTGACTG
S20-1-BA1	ATGGAGAAAATGTTGGAGTGTGCATTCATAGTCTTGGCTCAGC TTGGCTGGTGTGAGTGGAGAAGACCAGTGTACGAGAGTCCCGAGG CCCTGAGACTCCAGGAGGAGAGAGTACAGTCTCAACTGCAGT ACACAGTACGCGTTTAAAGAGGCTGTTCTGTGATAGGCAAGATC CTGGAAAGGCTGTGATTTCTCTCAGCTGTATCACTGGTGGGA AGAAAAGGAGAAAGAAAGGCTAAAGCCACATTAAACAAGAAGG AAAGCTTCTGCACATCACAGCCCTAAACCTGAAGACTCAGCCA CTTATCTGTGCTGTGCAAGGCTCAGCGGAGGAGGAAACAAC TCACTTTGGGACAGGCACTCAGCTAAAGTGGAACTCAAT ATGAGGCTGGTGGCAAGAGTAAGTGTGTTCTGACCTTTGGAACT ATAATTGATGCTAAGCAACCAGCCACTCCATGATTTGGCTT GAAGGAAGAGCTGTGATTTCTCTCAGCTGTATCACTGGATCAGT GGAATGAGTATGTTATGGTATCAGACAGATTCACCTCCAGGGG CCACAGTATATCATTTGTTTAAAACAAGTAAAACAATGAA ATGGCTCTCTGATCATCAGAAAGACAGAAAGTCCAGCACTTG ATCCTGCCCCAGCTACGCTGAGAGACTGTCTGTACTATTGCA TCGACCTGGATGCCAGACTATGTTGGAGATGGAACCTAGCTGG TGGTGAAGCCCAAT	ATGGATATCTGGCTCTCTGCTGTGGGTTGCTGTTTGTCTCTGGGAGC AGGCCAGTGGACTGGAGTCAACAACCTCAACACACACTGAT CAAAAAGAGAGGAGAGCAAGTGAAGTCTGTAGATGCTCTCTACTCT GGGCACAAGAGTGTCTCTGTTACCAAGAGTCTGGGCTGGGCTCAGGGG CTTATGAGAAAAGGAGTATGTAAGAAGAAAGTCTACTATCT GGCAACTTCCCTGATCGATTCTCAGCTGCCAGTTCCTCACTATA GCTCTGAGCTGATGTGAACCGCTTTGTGCTGGGGACTCGGCC GTATCTCTGTGCCAGCAGCTTGGAGTGGATATGAACACTGAAGCT TTCTTTGGACAAGGCCACAGACTCAGACTGTTGA ATGAGCCTGGGCTCTCTGCTGGGCGGCCCTCTGTCTCTGGGAG CAGACTCACAGAAAGTGGAGTTGCCAGTCTCCAGATATAAGA TTATGAGAAAAGGAGTGTCTTGTGAGAAAGAAAGTCTACTATCT TGGCAGTCTACCCTTACTGTTACCAGACAGACTTGGACAGGGC CAAAGCTTCTGATTCAGTGTAGAATACGGGTGTAGTGGATGATT CACAGTGGCTAAGGATCGATTTCTGAGAGAGGCTCAAAGGAT AGACTCCACTCAAGTCCAACCTGCAAGCTGAGGACTCGGCC GTATCTCTGTGCCAGCAGCTTGAAGGACAGGGGAAAGTACGAG CAGTACTCTGGCCGGGACCAAGCTCACGTTGCA ATGGGCACAGCTCTCTGCTGTGGGCTTCTGTCTCTGGGAG CAGTCCCATAGACACTGAAGTTACCCAGACACAAACACCTGTG
S20-1-BA2	ATGGAGAAAATGTTGGAGTGTGCATTCATAGTCTTGGCTCAGC TTGGCTGGTGTGAGTGGAGAAGACCAGTGTACGAGAGTCCCGAGG CCCTGAGACTCCAGGAGGAGAGAGTACAGTCTCAACTGCAGT ACACAGTACGCGTTTAAAGAGGCTGTTCTGTGATAGGCAAGATC CTGGAAAGGCTGTGATTTCTCTCAGCTGTATCACTGGTGGGA AGAAAAGGAGAAAGAAAGGCTAAAGCCACATTAAACAAGAAGG AAAGCTTCTGCACATCACAGCCCTAAACCTGAAGACTCAGCCA CTTATCTGTGCTGTGCAAGGCTCAGCGGAGGAGGAAACAAC TCACTTTGGGACAGGCACTCAGCTAAAGTGGAACTCAAT ATGAGGCTGGTGGCAAGAGTAAGTGTGTTCTGACCTTTGGAACT ATAATTGATGCTAAGCAACCAGCCACTCCATGATTTGGCTT GAAGGAAGAGCTGTGATTTCTCTCAGCTGTATCACTGGATCAGT GGAATGAGTATGTTATGGTATCAGACAGATTCACCTCCAGGGG CCACAGTATATCATTTGTTTAAAACAAGTAAAACAATGAA ATGGCTCTCTGATCATCAGAAAGACAGAAAGTCCAGCACTTG ATCCTGCCCCAGCTACGCTGAGAGACTGTCTGTACTATTGCA TCGACCTGGATGCCAGACTATGTTGGAGATGGAACCTAGCTGG TGGTGAAGCCCAAT	ATGGATATCTGGCTCTCTGCTGTGGGTTGCTGTTTGTCTCTGGGAGC AGGCCAGTGGACTGGAGTCAACAACCTCAACACACACTGAT CAAAAAGAGAGGAGAGCAAGTGAAGTCTGTAGATGCTCTCTACTCT GGGCACAAGAGTGTCTCTGTTACCAAGAGTCTGGTCAAGG GCCCCAGTATTTCTCAGTATTATGAGAAAGAGAGAGAAAG AGGAAACTTCCGTGATCGATTCTCAGCTGCCAGTTCCTACTACT AGCTCTGAGTGAATGTGAACCGCTTTGTGCTGGGGACTCGGCC TGTATTTGTGTCAGCAGTGTGGAGTGTGATGAACTGAAAGC TTCTTTTGGCAAGGAGCACTTGAAGTGTGTA ATGGGCACAGCTCTCTGCTGGGCTGTCTGTCTCTGGGAG CAGTCCCATAGACACTGAAGTTACCCAGACACAAACACCTGTG CATGGAAATGACAAAAGAAAGTCTTGAATGTAAGAACTAT TGGGCACAAGGCTATGATTTGTACAAGCAGAAAGCTAAGAAGCC ACCGAGCTCATGTITGTCTACAGCTATGAGAACTCTATAAAT GAAAGTGTGCAAGTGGTCTTCACTGAATGCCCCAACAGCTCTC TCTTAAACCTTCACTACAGCCCTGCAGCAGAGAACTCAGCCCT GTATCTCTGGCCAGTGGAAACCGGGGAGCTTTTTGGAGAAGCC TCTAGGCTGACCTGACTG
S20-1-BA4	ATGGAGAAAATGTTGGAGTGTGCATTCATAGTCTTGGCTCAGC TTGGCTGGTGTGAGTGGAGAAGACCAGTGTACGAGAGTCCCGAGG CCCTGAGACTCCAGGAGGAGAGAGTACAGTCTCAACTGCAGT ACACAGTACGCGTTTAAAGAGGCTGTTCTGTGATAGGCAAGATC CTGGAAAGGCTGTGATTTCTCTCAGCTGTATCACTGGTGGGA AGAAAAGGAGAAAGAAAGGCTAAAGCCACATTAAACAAGAAGG AAAGCTTCTGCACATCACAGCCCTAAACCTGAAGACTCAGCCA CTTATCTGTGCTGTGCAAGGCTCAGCGGAGGAGGAAACAAC TCACTTTGGGACAGGCACTCAGCTAAAGTGGAACTCAAT ATGAGGCTGGTGGCAAGAGTAAGTGTGTTCTGACCTTTGGAACT ATAATTGATGCTAAGCAACCAGCCACTCCATGATTTGGCTT GAAGGAAGAGCTGTGATTTCTCTCAGCTGTATCACTGGATCAGT GGAATGAGTATGTTATGGTATCAGACAGATTCACCTCCAGGGG CCACAGTATATCATTTGTTTAAAACAAGTAAAACAATGAA ATGGCTCTCTGATCATCAGAAAGACAGAAAGTCCAGCACTTG ATCCTGCCCCAGCTACGCTGAGAGACTGTCTGTACTATTGCA TCGACCTGGATGCCAGACTATGTTGGAGATGGAACCTAGCTGG TGGTGAAGCCCAAT	ATGGATATCTGGCTCTCTGCTGTGGGTTGCTGTTTGTCTCTGGGAGC AGGCCAGTGGACTGGAGTCAACAACCTCAACACACACTGAT CAAAAAGAGAGGAGAGCAAGTGAAGTCTGTAGATGCTCTCTACTCT GGGCACAAGAGTGTCTCTGTTACCAAGAGTCTGGTCAAGG GCCCCAGTATTTCTCAGTATTATGAGAAAGAGAGAGAAAG AGGAAACTTCCCTGATCGATTCTCAGCTGCCAGTTCCTCACTATA GCTCTGAGCTGATGTGAACCGCTTTGTGCTGGGGACTCGGCC GTATCTCTGTGCCAGCAGCTTGGAGTGGATATGAACACTGAAGCT TTCTTTGGACAAGGCCACAGACTCAGACTGTTGA ATGAGCCTGGGCTCTCTGCTGGGCGGCCCTCTGTCTCTGGGAG CAGACTCACAGAAAGTGGAGTTGCCAGTCTCCAGATATAAGA TTATGAGAAAAGGAGTGTCTTGTGAGAAAGAAAGTCTACTATCT TGGCAGTCTACCCTTACTGTTACCAGACAGACTTGGACAGGGC CAAAGCTTCTGATTCAGTGTAGAATACGGGTGTAGTGGATGATT CACAGTGGCTAAGGATCGATTTCTGAGAGAGGCTCAAAGGAT AGACTCCACTCAAGTCCAACCTGCAAGCTGAGGACTCGGCC GTATCTCTGTGCCAGCAGCTTGAAGGACAGGGGAAAGTACGAG CAGTACTCTGGCCGGGACCAAGCTCACGTTGCA ATGGGCACAGCTCTCTGCTGTGGGCTTCTGTCTCTGGGAG CAGTCCCATAGACACTGAAGTTACCCAGACACAAACACCTGTG
S20-1-BA5	ATGGAGAAAATGTTGGAGTGTGCATTCATAGTCTTGGCTCAGC TTGGCTGGTGTGAGTGGAGAAGACCAGTGTACGAGAGTCCCGAGG CCCTGAGACTCCAGGAGGAGAGAGTACAGTCTCAACTGCAGT ACACAGTACGCGTTTAAAGAGGCTGTTCTGTGATAGGCAAGATC CTGGAAAGGCTGTGATTTCTCTCAGCTGTATCACTGGTGGGA AGAAAAGGAGAAAGAAAGGCTAAAGCCACATTAAACAAGAAGG AAAGCTTCTGCACATCACAGCCCTAAACCTGAAGACTCAGCCA CTTATCTGTGCTGTGCAAGGCTCAGCGGAGGAGGAAACAAC TCACTTTGGGACAGGCACTCAGCTAAAGTGGAACTCAAT ATGAGGCTGGTGGCAAGAGTAAGTGTGTTCTGACCTTTGGAACT ATAATTGATGCTAAGCAACCAGCCACTCCATGATTTGGCTT GAAGGAAGAGCTGTGATTTCTCTCAGCTGTATCACTGGATCAGT GGAATGAGTATGTTATGGTATCAGACAGATTCACCTCCAGGGG CCACAGTATATCATTTGTTTAAAACAAGTAAAACAATGAA ATGGCTCTCTGATCATCAGAAAGACAGAAAGTCCAGCACTTG ATCCTGCCCCAGCTACGCTGAGAGACTGTCTGTACTATTGCA TCGACCTGGATGCCAGACTATGTTGGAGATGGAACCTAGCTGG TGGTGAAGCCCAAT	ATGGATATCTGGCTCTCTGCTGTGGGTTGCTGTTTGTCTCTGGGAGC AGGCCAGTGGACTGGAGTCAACAACCTCAACACACACTGAT CAAAAAGAGAGGAGAGCAAGTGAAGTCTGTAGATGCTCTCTACTCT GGGCACAAGAGTGTCTCTGTTACCAAGAGTCTGGTCAAGG GCCCCAGTATTTCTCAGTATTATGAGAAAGAGAGAGAAAG AGGAAACTTCCCTGATCGATTCTCAGCTGCCAGTTCCTCACTATA GCTCTGAGCTGATGTGAACCGCTTTGTGCTGGGGACTCGGCC GTATCTCTGTGCCAGCAGCTTGGAGTGGATATGAACACTGAAGCT TTCTTTGGACAAGGCCACAGACTCAGACTGTTGA ATGAGCCTGGGCTCTCTGCTGGGCGGCCCTCTGTCTCTGGGAG CAGACTCACAGAAAGTGGAGTTGCCAGTCTCCAGATATAAGA TTATGAGAAAAGGAGTGTCTTGTGAGAAAGAAAGTCTACTATCT TGGCAGTCTACCCTTACTGTTACCAGACAGACTTGGACAGGGC CAAAGCTTCTGATTCAGTGTAGAATACGGGTGTAGTGGATGATT CACAGTGGCTAAGGATCGATTTCTGAGAGAGGCTCAAAGGAT AGACTCCACTCAAGTCCAACCTGCAAGCTGAGGACTCGGCC GTATCTCTGTGCCAGCAGCTTGAAGGACAGGGGAAAGTACGAG CAGTACTCTGGCCGGGACCAAGCTCACGTTGCA ATGGGCACAGCTCTCTGCTGTGGGCTTCTGTCTCTGGGAG CAGTCCCATAGACACTGAAGTTACCCAGACACAAACACCTGTG
SP30-2-BA4	ATGGAGAAAATGTTGGAGTGTGCATTCATAGTCTTGGCTCAGC TTGGCTGGTGTGAGTGGAGAAGACCAGTGTACGAGAGTCCCGAGG CCCTGAGACTCCAGGAGGAGAGAGTACAGTCTCAACTGCAGT ACACAGTACGCGTTTAAAGAGGCTGTTCTGTGATAGGCAAGATC CTGGAAAGGCTGTGATTTCTCTCAGCTGTATCACTGGTGGGA AGAAAAGGAGAAAGAAAGGCTAAAGCCACATTAAACAAGAAGG AAAGCTTCTGCACATCACAGCCCTAAACCTGAAGACTCAGCCA CTTATCTGTGCTGTGCAAGGCTCAGCGGAGGAGGAAACAAC TCACTTTGGGACAGGCACTCAGCTAAAGTGGAACTCAAT ATGAGGCTGGTGGCAAGAGTAAGTGTGTTCTGACCTTTGGAACT ATAATTGATGCTAAGCAACCAGCCACTCCATGATTTGGCTT GAAGGAAGAGCTGTGATTTCTCTCAGCTGTATCACTGGATCAGT GGAATGAGTATGTTATGGTATCAGACAGATTCACCTCCAGGGG CCACAGTATATCATTTGTTTAAAACAAGTAAAACAATGAA ATGGCTCTCTGATCATCAGAAAGACAGAAAGTCCAGCACTTG ATCCTGCCCCAGCTACGCTGAGAGACTGTCTGTACTATTGCA TCGACCTGGATGCCAGACTATGTTGGAGATGGAACCTAGCTGG TGGTGAAGCCCAAT	ATGGATATCTGGCTCTCTGCTGTGGGTTGCTGTTTGTCTCTGGGAGC AGGCCAGTGGACTGGAGTCAACAACCTCAACACACACTGAT CAAAAAGAGAGGAGAGCAAGTGAAGTCTGTAGATGCTCTCTACTCT GGGCACAAGAGTGTCTCTGTTACCAAGAGTCTGGTCAAGG GCCCCAGTATTTCTCAGTATTATGAGAAAGAGAGAGAAAG AGGAAACTTCCCTGATCGATTCTCAGCTGCCAGTTCCTCACTATA GCTCTGAGCTGATGTGAACCGCTTTGTGCTGGGGACTCGGCC GTATCTCTGTGCCAGCAGCTTGGAGTGGATATGAACACTGAAGCT TTCTTTGGACAAGGCCACAGACTCAGACTGTTGA ATGAGCCTGGGCTCTCTGCTGGGCGGCCCTCTGTCTCTGGGAG CAGACTCACAGAAAGTGGAGTTGCCAGTCTCCAGATATAAGA TTATGAGAAAAGGAGTGTCTTGTGAGAAAGAAAGTCTACTATCT TGGCAGTCTACCCTTACTGTTACCAGACAGACTTGGACAGGGC CAAAGCTTCTGATTCAGTGTAGAATACGGGTGTAGTGGATGATT CACAGTGGCTAAGGATCGATTTCTGAGAGAGGCTCAAAGGAT AGACTCCACTCAAGTCCAACCTGCAAGCTGAGGACTCGGCC GTATCTCTGTGCCAGCAGCTTGAAGGACAGGGGAAAGTACGAG CAGTACTCTGGCCGGGACCAAGCTCACGTTGCA ATGGGCACAGCTCTCTGCTGTGGGCTTCTGTCTCTGGGAG CAGTCCCATAGACACTGAAGTTACCCAGACACAAACACCTGTG
SP30-2-BA6	ATGGAGAAAATGTTGGAGTGTGCATTCATAGTCTTGGCTCAGC TTGGCTGGTGTGAGTGGAGAAGACCAGTGTACGAGAGTCCCGAGG CCCTGAGACTCCAGGAGGAGAGAGTACAGTCTCAACTGCAGT ACACAGTACGCGTTTAAAGAGGCTGTTCTGTGATAGGCAAGATC CTGGAAAGGCTGTGATTTCTCTCAGCTGTATCACTGGTGGGA AGAAAAGGAGAAAGAAAGGCTAAAGCCACATTAAACAAGAAGG AAAGCTTCTGCACATCACAGCCCTAAACCTGAAGACTCAGCCA CTTATCTGTGCTGTGCAAGGCTCAGCGGAGGAGGAAACAAC TCACTTTGGGACAGGCACTCAGCTAAAGTGGAACTCAAT ATGAGGCTGGTGGCAAGAGTAAGTGTGTTCTGACCTTTGGAACT ATAATTGATGCTAAGCAACCAGCCACTCCATGATTTGGCTT GAAGGAAGAGCTGTGATTTCTCTCAGCTGTATCACTGGATCAGT GGAATGAGTATGTTATGGTATCAGACAGATTCACCTCCAGGGG CCACAGTATATCATTTGTTTAAAACAAGTAAAACAATGAA ATGGCTCTCTGATCATCAGAAAGACAGAAAGTCCAGCACTTG ATCCTGCCCCAGCTACGCTGAGAGACTGTCTGTACTATTGCA TCGACCTGGATGCCAGACTATGTTGGAGATGGAACCTAGCTGG TGGTGAAGCCCAAT	ATGGATATCTGGCTCTCTGCTGTGGGTTGCTGTTTGTCTCTGGGAGC AGGCCAGTGGACTGGAGTCAACAACCTCAACACACACTGAT CAAAAAGAGAGGAGAGCAAGTGAAGTCTGTAGATGCTCTCTACTCT GGGCACAAGAGTGTCTCTGTTACCAAGAGTCTGGTCAAGG GCCCCAGTATTTCTCAGTATTATGAGAAAGAGAGAGAAAG AGGAAACTTCCCTGATCGATTCTCAGCTGCCAGTTCCTCACTATA GCTCTGAGCTGATGTGAACCGCTTTGTGCTGGGGACTCGGCC GTATCTCTGTGCCAGCAGCTTGGAGTGGATATGAACACTGAAGCT TTCTTTGGACAAGGCCACAGACTCAGACTGTTGA ATGAGCCTGGGCTCTCTGCTGGGCGGCCCTCTGTCTCTGGGAG CAGACTCACAGAAAGTGGAGTTGCCAGTCTCCAGATATAAGA TTATGAGAAAAGGAGTGTCTTGTGAGAAAGAAAGTCTACTATCT TGGCAGTCTACCCTTACTGTTACCAGACAGACTTGGACAGGGC CAAAGCTTCTGATTCAGTGTAGAATACGGGTGTAGTGGATGATT CACAGTGGCTAAGGATCGATTTCTGAGAGAGGCTCAAAGGAT AGACTCCACTCAAGTCCAACCTGCAAGCTGAGGACTCGGCC GTATCTCTGTGCCAGCAGCTTGAAGGACAGGGGAAAGTACGAG CAGTACTCTGGCCGGGACCAAGCTCACGTTGCA ATGGGCACAGCTCTCTGCTGTGGGCTTCTGTCTCTGGGAG CAGTCCCATAGACACTGAAGTTACCCAGACACAAACACCTGTG

Supplemental table 5 Sequence list of primers

SEQ ID	Name	Sequence
NO. 1	Mouse T Cell Mix 2 PN-2000079 Pb	GGCCAAGCACACGAGGGTA
NO. 2	CN24613-1VseqF	TGGAGGAGGAAGAGCTCAGA
NO. 3	CN803-seqR	AAGAGACAGCAACCAGGATTTA

SUPPLEMENTAL PLASMID SEQUENCE

Supplemental plasmid sequence No. 1

lenti-BSD-T2A-EGFP:

GTCGACGGATCGGGAGATCTCCCGATCCCTATGGTGCAGTCTCAGTACAATCTGCTCTGATGCCGCATAGTTAAGCCAGTAT
 CTGCTCCCTGCTTGTGTGTGGAGGTGCTGAGTAGTGCGCGAGCAAAATTAAGCTACAACAAGGCAAGGCTTGACCCGAC
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TTGCCGATTTCGGCTATTGGTTAAAAAATGAGCTGATTAAACAAAATTTAACGCGAATTAATTCTGTGGAATGTGTGTCAG
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Supplemental plasmid sequence No. 2

lenti-BSD-T2A-TK412:

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Supplemental plasmid sequence No. 4

lenti-PURO-T2A-(HLA-A*11:01)-E2A-(Tag-A*11:01)-P2A-EGFP:

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Supplemental plasmid sequence No. 5

lenti-(HLA-A*11:01)-KRAS-M:

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Supplemental plasmid sequence No. 6

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