SUPPLEMENTAL INFORMATION

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SUPPLEMENTARY METHODS

Flow Cytometry and Antibodies

FACS Ariar (BD Bioscience) and Accuri™ C6 (BD Bioscience) were used to perform fluorescent expression analysis, the FlowJo software (Treestar, USA) was used for data interpretation. PBMCs, DCs or T Cells were harvested and stained with mouse anti-human antibody labeled by fluorescence for 30 min 4°C in darks as follows: CD3-PerCP-CY5.5(OKT-3,eBioscience) or CD3-FITC(HIT3a, BD Bioscience); CD4-APC(RPA-T4, BD Bioscience) or CD4-PerCP-CY5.5(RPA-T4, BD Bioscience); CD8-PE (HIT8a, BD Bioscience) or CD8-APC (RPA-T8, BD Bioscience) or CD8-PE-CY7(RPA-T8, eBioscience); CD137-PE(4B4-1, BD Bioscience) or CD137-FITC(4B4-1, eBioscience); CD54-PE (HA58, BD Bioscience); CD86-APC(FUN-1, BD Bioscience); HLA-DR-PerCP-CY5.5 (G46-6, BD Bioscience); CD11c-FITC(B-ly6, BD Bioscience); CD45RO-APC(UCHL1, BD Bioscience); CD62L-PE(DREG-56, BD Bioscience) or CD62L-FITC(DREG-56, BD Bioscience); HLA-A2(BB7.2, Medical & Biological Laboratories); CD19-PE(HIB19, BD Bioscience); CD16-PE(3G8, BD Bioscience); CD56-APC(B159, BD Bioscience); CD279-PE(MIH4, BD Bioscience); CD223-PE(T47-530, BD Bioscience); CD366-PE(7D3, BD Bioscience); CD27-PE(M-T271, BD Bioscience); CD28-APC(CD28.2, BD Bioscience); IFN-γ-PE(B27, eBioscience); TNF-α-PE(Mab11, eBioscience).

Intracellular Cytokine Staining

In brief, the enriched neoantigen specific T cells (1×10^5) were cocultured with corresponding mutant peptide-pulsed irradiated K562-A11 cells (2.5×10^4) for 18 hours in 96-well plates. And then, 1 ul/ml GolgiPlug (BD Bioscience) was added to the culture. After 5 hours, cells were stained for CD137 and costained for CD8. Cells were fixed, permeabilized, and stained with antibodies against IFN-γ and TNF-α, respectively, using Cytofix/Cytoperm^{TM} and Perm/Wash^{TM} Buffer (BD Bioscience), and subsequently the stained cells were subjected to flow cytometry assay.

SUPPLEMENTARY TABLES

Supplemental Table 1. Overview of identified neoantigens by targeted sequencing guided de novo synthesis model.

Patient ID	Somatic mutations[a]	Selected mutations by first filtering[b]	Predicted neoantigens[c]	Selected neoantigens for in vitro identification by second filtering[d]	Immunogenic neoantigen validated in vitro	Tumor regression[e]
A004	n=51, tumor; n=82, cfDNA;	n=16	n=25	n=8	1/8	—
A008	n=31, tumor;	n=21	n=20 (HLA class I); n=36 (HLA class II)	n=7 (HLA class I) n=2 (HLA class II)	2/9	—

A015	n=42, tumor 1;
	n=49, tumor 2;
	n=52, cfDNA;
		n=22	n=59	n=12	0/12	—

A017	n=91, tumor;	n=42	n=44	n=3	1/3	CR

[a]As determinded by targeted sequencing panel of 416 cancer-related genes. Somatic mutations listed including non-synonymous single nucleotide variants (SNV), and insertions/deletions (indels).

[b]Tumor VAF>2% were selected for patient A008 and A017. For patient A004 and A015, mutations shared in tumor and cfDNA samples were selected, and then VAF > 2% was filtered.

[c]HLA-binding affinities of mutant peptides were predicted by NetMHC 4.0/NetMHCpan 3.0 for HLA class I alleles, and NetMHCII 2.2 for HLA class II alleles. Peptides with an IC50 <500nM or %Rank < 2.0 were predicted to be MHC binders.

[d]The predicted neo-epitopes were ranked, and prioritized the peptides according to the following criteria: i) Strong binders with IC50<50nM or %Rank < 0.5; ii) Mutations with higher tumor VAF; iii) A peptide was predicted to bind two or more HLA molecules; iv) MHC class I binding peptides can also be predicted by NetMHC3.4, and MHC class II binding peptides can also be predicted by IEDB software.

[e]Tumor regression assessment after immunotherapy.

Supplemental Table 2. HLA-I and HLA-II alleles in 17 patients with refractory solid tumors

Patient ID	*Age*	*Sex*	*Primary tumor type*	*HLA class I alleles*			*HLA class II alleles*	
A001	34	M	Embryonal	A*02:07, A*11:01	B*46:01, B*54:01	C*01:02, C*01:02	DRB1* 07:01, DRB1* 08:03	DQB1*06:01, DQB1*03:03
A002	65	M	Hepatocellular	A*01:01, A*24:02	B*35:01, B*57:01	C*03:03, C*06:02	DRB1* 04:04, DRB1* 07:01	DQB1*03:02, DQB1*03:03
A003	61	M	Colon	A*02:01, A*11:01	B*35:01, B*40:06	C*03:03, C*08:01	DRB1* 08:02, DRB1* 12:01	DQB1*03:01, DQB1*04:02
A004	65	M	Gastric	A*33:01, A*33:03	B*14:02, B*15:18	C*07:04, C*08:02	DRB1* 01:02, DRB1* 09:01	DQB1*05:01, DQB1*03:03
A005	50	F	Endometrium	A*11:01, A*24:02	B*40:01, B*40:02	C*03:03, C*07:02	DRB1* 04:05, DRB1* 09:01	DQB1*03:03, DQB1*04:01
A006	40	M	Gastric	A*02:01, A*30:01	B*13:02, B*40:06	C*06:02, C*08:01	DRB1* 07:01, DRB1* 09:01	DQB1*02:02, DQB1*03:03
A007	63	M	Pancreatic	A*02:07, A*32:01	B*46:01, B*52:01	C*01:02, C*12:02	DRB1* 09:01, DRB1* 15:02	DQB1*06:01, DQB1*03:03
A008	51	F	Pancreatic	A*30:01, A*30:01	B*13:02, B*13:02	C*06:02, C*06:02	DRB1* 07:01, DRB1* 07:01	DQB1*02:02, DQB1*02:02
A009	50	F	Ovarian	A*02:06, A*11:01	B*15:02, B*58:01	C*03:02, C*08:01	DRB1* 03:01,1 DRB1* 5:01	DQB1*06:01, DQB1*02:01
A010	24	M	Glioma	A*02:01, A*29:01	B*13:02, B*48:01	C*06:02, C*08:03	DRB1* 07:01, DRB1* 09:01	DQB1*02:02, DQB1*03:03
A011	66	M	Pancreatic	A*01:01, A*02:01	B*40:01, B*57:01	C*06:02, C*07:02	DRB1* 08:02, DRB1* 12:02	DQB1*03:01, DQB1*04:02
A012	43	F	Hepatocellular	A*24:02, A*24:02	B*40:01, B*48:01	C*07:02, C*08:22	DRB1* 11:01, DRB1* 15:01	DQB1*11:01, DQB1*15:01
A013	56	F	Ovarian	A*24:02, A*31:01	B*40:06, B*46:01	C*01:02, C*08:01	DRB1* 09:01, DRB1* 15:01	DQB1*06:02, DQB1*03:03
A014	37	F	Gastric	A*02:01, A*11:01	B*40:01, B*40:01	C*07:02, C*14:02	DRB1* 08:03, DRB1* 09:01	DQB1*06:01, DQB1*03:03
A015	62	M	Gastric	A*03:01, A*11:01	B*44:02, B*55:02	C*01:02, C*05:01	DRB1* 04:04, DRB1* 13:01	DQB1*06:03, DQB1*03:02
A016	62	F	Pancreatic	A*11:01, A*24:02	B*15:07, B*40:01	C*03:03, C*03:04	DRB1* 04:06, DRB1* 15:01	DQB1*06:02, DQB1*03:02
A017	53	M	Thymoma	A*02:01 A*24:02	B*35:01 B*40:01	C*03:03 C*03:04	DRB1*09:01, DRB1*15:01	DQB1*03:03, DQB1*06:02

Supplemental Table 3. Candidate HLA-I and HLA-II alleles binding peptides for patient A008

Peptide ID	*Mutant peptide*[a]	*Protein*	*Amino acid change*	*Allele frequency*	*HLA allele*	*IC50 (nM)*[b]
TP53-V25G-1	R**G**RAMAIYK	TP53	V25G	5.34%	A*3001	2.2
MTAP-V56I	KIKNVDC**I**L	MTAP	V56I	26.67%	A*3001	63.6
TP53-V25G-2	GTR**G**RAMAI	TP53	V25G	5.34%	A*3001	15.7
BMPR1A-K257N	KWRGE**N**VAV	BMPR1A	K257N	2.72%	A*3001	47.2
SUFU-G11R	GAP**R**PTAPPA	SUFU-	G11R	7.33%	A*3001	134.1
BMPR1A-A357T	KP**T**IAHRDLK	BMPR1A	A357T	6.92%	A*3001	101.9
MEN1-A68P	LTFQPSP**P**P	MEN1	A68P	5.74%	A*3001	354.5
DIS3L2-I777V (15-mers)	MVMG**V**LKQAF DVLVL	DIS3L2	I777V	12.09%	DRB1*0701	18.3
KRAS-G12D (15-mers)	MTEYKLVVVG A**D**GVG	KRAS	G12D	2.44%	DRB1*0701	121.5

[a] Mutant residues are underlined and in bold.

[b] HLA-binding affinities of peptides, as predicted by NetMHC4.0 and NetMHCII 2.2. Peptides with an IC50 < 500nM are predicted to be MHC binders. Peptides with IC50 <50nM are considered as strong binders.

Supplemental Table 4. Candidate HLA-A*0201-binding epitopes for patient A017

Peptide ID	*Mutant peptide*[a]	*Protein*	*Amino acid change*	*Allele frequency*	*HLA* **allele**	*IC50 (nM)*[b]	*Rank%*[c]
CDC73-Q254E-1	NIFAIL**E**SV	CDC73	Q254E	12.96%	A*0201	48.1	0.2
CDC73-Q254E-2	KNIFAIL**E**SV	CDC73	Q254E	12.96%	A*0201	70.2	0.6
FH-S24L	A**L**APGLGGAAV	FH	S24L	14.43%	A*0201	253.7	1

[a] Mutated residues are underlined and in bold. HLA-binding affinities of peptides, as predicted by NetMHC4.0 [b] and NetMHCpan 3.0 [c]. Peptides with an IC50 < 500nM or Rank% < 2 are predicted to be MHC binders. Peptides with IC50 <50nM or Rank% < 0.5 are considered as strong binders.

Supplemental Table 5. HLA-A*02-restricted irrelevant mutant peptides for patient A017

Peptide ID	*Mutant peptide*[a]	*Protein*	*Amino acid change*	*IC50 (nM)*[b]
KRAS(A02)-G12D	KLVVVGA***D***GV	KRAS	G12D	498
KRAS(A02)-G13D-1	VVVGAG***D***V	KRAS	G13D	495
KRAS(A02)-G13D-2	KLVVVGAG***D***V	KRAS	G13D	506.9
KRAS(A02)-G12V	KLVVVGA***V***GV	KRAS	G12V	300.2
KRAS(A02)-G12C	KLVVVGA***C***GV	KRAS	G12C	373.6
KRAS(A02)-G12R	KLVVVGA***R***GV	KRAS	G12R	506.9
CTNNB1(A02)-T41A	GIHSGAT***A***TA	CTNNB1	T41A	83
TP53(A02)-R249S	GMNR***S***PILTI	TP53	R249S	349
GNAS(A02)-R201H	LLRC***H***VLTS	GNAS	R201H	249

[a] Mutated residues are underlined and in bold. HLA-binding affinities of peptides, as predicted by NetMHC4.0[b]

Supplemental Table 6.

Predicted HLA-A*0201-binding affinity.

Peptide ID	***Sequence***	***IC50(nM)*** [a]
EBV-LMP2a-356	FLYALALLL	8.25
KRAS-A11-G12C	VVGACGVGK	29376.91
CDC73-MT	NIFAILESV	48
CDC73-WT	NIFAILQSV	36.86

[a]HLA-binding affinities of peptides, as predicted by NetMHC4.0. Peptides with an IC50 < 500nM are predicted to be MHC binders. Peptides with IC50 <50nM are considered as strong binders.

Supplemental Table 7. Candidate HLA-binding peptides for patient A004

Peptide ID	*Mutant peptide*[a]	*Protein*	*AA change*	*Allele frequency*[b]	*HLA binding affinity (Rank%)*[c]
PMS2-L236F-1	FGQKQ**F**QSL	PMS2	L236F	11.06%	B*1402(0.4); C*0704(0.8); C*0802(2)
CDA-A88T	RAIAI**T**SDM	CDA	A88T	20.31%	B*1518(0.7); C*0802(0.7)
SUFU-G11R	ELRPSGAP**R**	SUFU	G11R	16.32%	A*3301(0.18); A*3303(0.15)
PMS2-L236F-2	KQ**F**QSLIPF	PMS2	L236F	11.06%	B*1402(1.5); B*1518(0.06)
CYP2A6-V80M-1	HLGPRRVV**M**	CYP2A6	V80M	2.17%	B*1402(0.4); C*0704(0.7)
CYP2A6-V80M-2	**M**LCGHDAVR	CYP2A6	V80M	2.17%	A*3301(0.8); A*3303(0.6)
CYP2A6-N438Y	KR**Y**CFGEGL	CYP2A6	N438Y	13.33%	B*1402(1.1); C*0704(0.7)
TP53-S96fs	**SRKPTRAATV**	TP53	S96fs (c.285_297delTTC TGTCCCTTCC)	32.93%	C*0704(0.8)

[a]Mutated residues are underlined and in bold. [b] Listed are variant allele frequency (AF) in tumor sample.

[c] HLA-binding affinities of peptides are predicted by NetMHCpan 3.0. Peptides with Rank% < 2 are predicted to be MHC binders. Peptides with Rank% < 0.5 are considered as strong binders.

Supplemental Table 8. Candidate HLA-binding peptides for patient A015

Peptide ID	*Mutant peptide*[a]	*Protein*	*AA change*	*Allele frequency*[b]	*HLA binding affinity (Rank%)*[c]
PDE11A-921insS-1	SS***S***SPASVM	PDE11A	921insS (c.2761 _2763insTCC)	82.35%	C*0102(0.4); C*0501(0.3)
TUBB2A-A185T	VVEPYN***T***TL	TUBB2A	A185T	27.14%	C*0102(0.04); C*0501(0.25)
CYP2D6-G322S	FGDIVPL***S***V	CYP2D6	G322S	7.30%	C*0102(1); C*0501(0.08)
SDHA-I579V	T***V***YGAEARK	SDHA	I579V	1.51%	A*0301(0.5); A*1101(0.4)
TUBB2A-A185T	EPYN***T***TLSV	TUBB2A	A185T	27.14%	B*5502(0.25)
ATM-E2351A	AETCL***A***NPA	ATM	E2351A	5.53%	B*4402(0.4)
CYP2D6-G322S-2	IVPL***S***VTHM	CYP2D6	G322S	7.30%	C*0102(0.4)
CYP2D6-G322S-3	VPL***S***VTHMT	CYP2D6	G322S	7.30%	B*5502(0.3)
CYP2D6-V319I	FGDI***I***PLGV	CYP2D6	V319I	6.46%	C*0501(0.125)
SDHA-Y606H	RIDEYD***H***SK	SDHA	Y606H	4.09%	A*1101(0.9)
PDE11A-921insS-2	***S***SPASVMVAK	PDE11A	921insS (c.2761_2763insTCC)	82.35%	A*0301(0.5); A*1101(0.03)
ATM-NPAVIM2353delinsK	AETCLE***K***QTY	ATM	NPAVIM2353delinsK (c.7056_7070delTCCT GCGGTCATCAT)	5.14%	B*4402(0.05)

[a] Mutated residues are underlined and in bold. [b] Listed are variant allele frequency (AF) in tumor sample.

[c] HLA-binding affinities of peptides are predicted by NetMHCpan 3.0. Peptides with Rank% < 2 are predicted to be MHC binders. Peptides with Rank% < 0.5 are considered as strong binders.

Supplemental Table 9. High-frequency mutant genes in common solid tumors (COSMIC)

Tumor types	Alteration frequency of mutated gene				
Stomach Adenocarcinoma	TP53 (26 %)	ARID1A (11 %)	FAT4 (16 %)	KMT2C (10 %)	LRP1B (16 %)
	PIK3CA (9 %)	PREX2 (10 %)			
Pancreas carcinoma	KRAS (56 %)	CDKN2A (11 %)	SMAD4 (12 %)	TP53 (33 %)	GNAS (15%)
Oesophagus Squamous cell	TP53 (57 %)	CDKN2A (13 %)	FAT1 (15 %)	KMT2D (19 %)	LRP1B (12 %)
carcinoma	NFE2L2 (11 %)	NOTCH1 (18 %)	PIK3CA (9 %)		
Ovary Carcinoma	TP53 (47 %)	PIK3CA (11 %)	KRAS (9%)		
Liver Hepatocellular carcinoma	TP53 (27 %)	TERT (25 %)	CTNNB1 (19 %)		
Lung Adenocarcinoma	EGFR (36 %)	TP53 (30 %)	KRAS (17 %)		
Lung Squamous cell carcinoma	TP53 (44 %)	LRP1B (16 %)			
Large intestine adenocarcinoma	APC (46 %)	BRAF (11 %)	FAT4 (15 %)	KMT2C (11 %)	KRAS (34 %)
	LRP1B (13 %)	PIK3CA (13 %)	SMAD4 (10 %)	TP53 (44 %)	
Cervix Squamous cell carcinoma	PIK3CA (18 %)				

Supplemental Table 10. Alteration frequency of 21 mutant genes in nine common solid tumors (TCGA)

GENE	*Cervical (TCGA)*	*Colorectal (TCGA)*	*ESCC (UCLA 2014)*	*Liver (TCGA)*	*Lung adeno (TCGA)*	*Lung squ (TCGA)*	*Ovarian (TCGA)*	*Pancreas (QCMG 2016)*	*Stomach (TCGA)*
TP53	4.60%	53.80%	60.60%	30.80%	46.10%	72.30%	87.00%	66.10%	48.10%
KRAS	5.70%	43.00%	0.00%	1.60%	32.60%	1.10%	0.60%	89.80%	9.40%
LRP1B	6.70%	17.90%	10.90%	8.80%	29.60%	39.00%	4.10%	5.70%	26.30%
KMT2D	11.30%	5.80%	18.20%	5.60%	8.30%	18.60%	0.30%	5%	17.50%
APC	3.60%	71.70%	2.20%	3.20%	3.90%	4.50%	2.20%	1.30%	12.40%
KMT2C	14.90%	5.40%	8%	5.40%	18.30%	16.40%	2.20%	5.50%	13.90%
PIK3CA	27.30%	14.80%	8%	3.50%	6.50%	15.30%	0.60%	1.60%	16.50%
FAT4	5.20%	17.50%	0%	5.10%	14.80%	14.70%	0.90%	2.90%	21.50%
ARID1A	7.70%	9.40%	1.50%	8.60%	7%	5.10%	0.90%	7.60%	25.80%
FAT1	5.20%	5.80%	11.70%	2.40%	10.90%	11.90%	3.50%	1.80%	8.60%
CDKN2A	1.50%	0%	2.90%	2.70%	3.90%	11.90%	0%	18.50%	4.30%
SMAD4	3.60%	11.70%	0%	1.10%	3.50%	2.80%	0%	22.50%	7.10%
PREX2	1.50%	4.90%	0%	5.10%	3.90%	12.40%	0.60%	1.80%	12.70%
CTNNB1	2.10%	4.90%	0%	26%	3.90%	2.30%	0.60%	0.80%	6.60%
NOTCH1	5.70%	0%	8%	1.90%	4.30%	7.30%	1.30%	0.50%	7.10%
NFE2L2	6.20%	0.90%	5.80%	3.50%	1.70%	14.70%	0%	0%	0.50%
EGFR	2.10%	4.50%	0.70%	1.60%	14.30%	3.40%	2.20%	0%	4.80%
BRAF	1%	9.90%	1.50%	0%	9.60%	4.50%	0.60%	0.50%	4.60%
GNAS	1.50%	2.70%	0.00%	1.60%	3.00%	1.70%	0.90%	2.60%	5.30%
TERT	0.50%	0.40%	0%	0.50%	0.40%	1.70%	0.30%	0.50%	2.80%
MDM2	0.50%	0.90%	0%	0.30%	0.90%	1.10%	0%	0.30%	1.80%

Supplemental Table 11. Alteration frequency of hotspot mutations in common solid tumors (COSMIC)

Tumor types	*Alteration frequency of mutated genes and hotspot mutations*					
Stomach Adenocarcinoma	***TP53 (26%)***		***PIK3CA (9%)***			
	AA change	***MF***	***AA change***	***MF***		
	R175H	1.72%	E542K	1.22%		
	R248Q	1.12%	E545K	1.27%		
	R248W	0.80%	H1047R	2.54%		
	R249S	0.16%				
	R273C	0.08%				
	R273H	0.59%				
	R282W	0.80%				
	Y163C	0.16%				
	Y220C	0.16%				
	G245S	1.13%				
Pancreas Tumor	***KRAS (56%)***		***TP53 (33%)***		***GNAS (15%)***	
	AA change	***MF***	***AA change***	***MF***	***AA change***	***MF***
	G12A	0.77%	R249S	0.11%	R201H	4.62%
	G12C	1.49%	R175H	0.98%	R201C	5.91%
	G12D	26.55%	R248Q	0.95%		
	G12R	6.97%	R248W	0.80%		
	G12S	0.90%	R273C	0.91%		
	G12V	16.97%	R273H	1.64%		
	G13D	0.79%	R282W	1.35%		
	Q61H	0.79%	V157F	0.22%		
			Y163C	0.29%		
			Y220C	0.51%		
			G245S	0.54%		
Oesophagus Squamous cell carcinoma	***TP53 (57 %)***		***PIK3CA (9%)***			
	AA change	***MF***	***AA change***	***MF***		
	R175H	0.00%	E542K	1.15%		
	R248Q	0.00%	E545K	2.17%		
	R248W	0.97%	H1047R	1.02%		
	R249S	0.25%				
	R273C	0.64%				
	R273H	0.97%				
	R282W	1.82%				
	Y163C	0.32%				
	Y220C	1.25%				
	G245S	0.52%				
Ovary Carcinoma	***TP53 (47 %)***		***PIK3CA (11 %)***		***KRAS (9%)***	
	AA change	***MF***	***AA change***	***MF***	***AA change***	***MF***
	R175H	0.00%	E542K	1.22%	G12A	0.61%

	R248Q	0.00%	E545K	1.48%	G12C	0.28%
	R248W	1.05%	H1047R	4.01%	G12D	3.34%
	R249S	0.20%			G12R	0.45%
	R273C	1.22%			G12S	0.14%
	R273H	1.90%			G12V	3.05%
	R282W	0.56%			G13D	0.56%
	Y163C	0.49%			Q61H	0.16%
	Y220C	1.61%				
	G245S	0.86%				
Liver Hepatocellular carcinoma	***TP53 (27%)***		***CTNNB1 (19%)***			
	AA change	***MF***	***AA change***	***MF***		
	R248W	0.10%	S45P	1.77%		
	R249S	7.65%	T41A	2.26%		
	R273C	0.42%				
	R273H	0.12%				
	R282W	0.15%				
	Y163C	0.07%				
	Y220C	0.25%				
	G245S	0.07%				
Lung Adenocarcinoma	***EGFR (36 %)***		***TP53 (30%)***		***KRAS (17 %)***	
	AA change	***MF***	***AA change***	***MF***	***AA change***	***MF***
	T790M	2.55%	Y163C	0.27%	G12A	1.47%
	L858R	9.65%	Y220C	0.31%	G12C	5.93%
			R248W	0.36%	G12D	3.30%
			R249S	0.44%	G12R	0.24%
			R273C	0.09%	G12S	0.50%
			R273H	0.85%	G12V	4.06%
			R282W	0.31%	G13D	0.49%
			G245S	0.18%	Q61H	0.22%
Lung Squamous cell carcinoma	***TP53 (44 %)***					
	AA change	***MF***				
	R248W	0.77%				
	R249S	0.70%				
	R273C	0.35%				
	R273H	0.98%				
	R282W	0.49%				
	Y163C	0.63%				
	Y220C	0.56%				
	G245S	0.07%				
Large intestine adenocarcinoma	***TP53 (44 %)***		***KRAS (34%)***		***PIK3CA (13%)***	
	AA change	***MF***	***AA change***	***MF***	***AA change***	***MF***
	R248W	2.27%	G12A	2.03%	E542K	2.27%

	R249S	0.04%	G12C	2.66%	E545K	3.50%
	R273C	2.00%	G12D	11.75%	H1047R	2.53%
	R273H	2.34%	G12R	0.41%		
	R282W	2.31%	G12S	1.93%		
	Y163C	0.16%	G12V	7.40%		
	Y220C	0.33%	G13D	6.42%		
	G245S	1.68%	Q61H	0.22%		
	BRAF (11%)					
	AA change	***MF***				
	V600E	10.59%				
Cervix Squamous cell carcinoma	***PIK3CA (18%)***					
	AA change	***MF***				
	E542K	4.24%				
	E545K	8.92%				
	H1047R	0.45%				

MF: mutational frequency; AA change: amino acid changes.

Supplemental Table 12. Peptides eliciting IFN-γ release from PBMC measured by ELISPOT and CBA using shared neoantigen peptide library

Patient ID	Tumor type	Missense mutation	HLA alleles	Epitope peptides[a]	ELISPOT[b] peptide/control (fold change)	CBA[b] peptide/control (fold change)	Results[c]
A020	Pancreatic	KRAS G12D	A*1101	VVGA**D**GVGK	178/65 (2.74)	NA	+
A005	Endometrial	KRAS G12C	A*1101	VVGA**C**GVGK	172/42 (4.1)	NA	+
A011	Pancreatic	KRAS G12V	A*0201	KLVVVGA**V**GV	NA	129.82/39.09 (3.32)	+
A007	Pancreatic	KRAS G12V	A*0201	KLVVVGA**V**GV	NA	152.62/186.79 (0.84)	-
A018	Lung	KRAS G12A	A*1101	VVGA**A**GVGK	NA	57.3/8.16 (7.02)	+
A019	Lung	TP53 G245S	A*1101	SSCMG**S**MNR	NA	623.31/601.75 (1.04)	-
A021	Gastric	TP53 R248W	A*0201	GMN**Q**RPILTI	0/0	NA	-
B001	Pancreatic	KRAS G12V	A*1101	VVGA**V**GVGK	39/36 (1.1)	NA	-
B002	Pancreatic	KRAS G12V	A*0201	KLVVVGA**V**GV	19/21 (0.9)	5.81/10.63 (0.55)	-
B003	Pancreatic	KRAS G12D	A*0201	KLVVVGA**D**GV	0/0	6.27/8.80 (0.71)	-
C001	Gastric	BRAF V600E[d]	DRB1*1101	EDLTVKIGDFGLAT**E**K SRWSGSHQFEQLS	NA	264.31/245.55 (1.08)	-
C002	Pancreatic	KRAS G12V	A*1101	VVGA**V**GVGK	NA	90.23/20.68 (4.36)	+
C003	Pancreatic	KRAS G12D	A*0201	KLVVVGA**D**GV	NA	1272.56/390.35 (3.26)	+

[a]Mutated residues are underlined and in bold. [b] The secretion of IFN-γ was measured by ELISPOT（spots/10^5 cells） and Cytometric Bead Array (CBA, pg/ml) to identify immunogenic neoantigen. Values indicate the number of spots per 10^5PBMCs reactive with the corresponding peptides (left value) and negative control (right value) in IFN-γ ELISPOT. [c] "+" represent IFN-γ levels greater than twice the negative control, which was considered positive T cell reactivity. In contrast, " - " represent a negative T cell reactivity. NA, not assessed.
[d]The HLA-DRB1*1101 restricted long peptide of mutant BRAF-V600E, which had been identified as immunogenic peptide, was also stored in our inventory peptide library.

Supplemental Table 13. Phenotypic characteristics of the adoptive transfer cells (bulk T cells and DCs)

			Patient ID					
	Phenotype	**Markers of monitoring**	**A005**	**A011**	**A017**	**A020**	**C002**	**C003**
Composition of bulk T cells	CD8+T Cells	CD3+CD8+	60.3%	43.4%	67.2%	36.7%	30.1%	25.0%
	CD4+T Cells	CD3+CD4+	36.3%	42.6%	25.2%	60.6%	47.6%	53.8%
	B Cells	CD3-CD19+	0.5%	0.7%	0.3%	0.2%	2.5%	7.80%
	NK Cells	CD56+CD16+	3.1%	13.8%	10.6%	1.5%	6.8%	10.5%
	NKT Cells	CD3+CD56+CD16+	0.3%	3.8%	1.1%	0.0%	0.4%	1.2%
Costimulatory molecules on T cells[a]	PD-1 (CD279)	CD8+CD279+	23.8%	4.2%	2.1%	1.4%	1.6%	2.3%
		CD4+CD279+	15.4%	1.1%	0.6%	0.6%	4.5%	3.9%
	LAG-3 (CD223)	CD8+CD223+	67.3%	49.0%	75.5%	58.9%	72.6%	54.8%
		CD4+CD223+	43.3%	11.8%	44.7%	13.2%	29.6%	28.2%
	TIM-3 (CD366)	CD8+CD366+	58.9%	78.5%	64.6%	89.2%	78.9%	34.9%
		CD4+CD366+	28.8%	14.5%	19.7%	49.5%	48.5%	10.7%
	CD27	CD8+CD27+	59.4%	20.4%	58.0%	95.6%	49.0%	57.4%
		CD4+CD27+	38.6%	51.2%	49.2%	80.7%	31.1%	35.9%
	CD28	CD8+CD28+	49.0%	22.5%	72.3%	81.2%	45.6%	73.8%
		CD4+CD28+	75.7%	66.6%	79.2%	98.2%	82.0%	75.7%
DC maturity	CD11C+	CD11C+	81.1%	81.7%	93.7%	97.8%	90.8%	83.7%
	HLA-DR+	CD11C+HLA-DR+	99.5%	98.2%	99.7%	61.1%	99.7%	93.9%
	CD86+	CD11C+CD86+	97.7%	78.4%	98.3%	12.8%	80.9%	88.6%
	CD54+	CD11C+CD54+	97.5%	99.7%	99.5%	99.6%	99.9%	99.3%

[a]gated on CD3. The represent data are depicted.

Supplemental Table 14. Clinical characteristics of 6 patients receiving personalized immunotherapy

Patient ID	Age	sex	Primary tumor	Pathology	Grade	TNM (Stage)	Metastatic Sites	Prior Therapy	PS[a]
A020	51	F	Pancrease	Adenocarcinoma	G2	T2N0M1 (IV)	Liver, Para-aortic lymph nodes	Surgery, S-1, Gemcitabine+S-1	1
C002	69	M	Pancrease	Adenocarcinoma	G3	T4N0 M0 (III, R2)	Local advanced	Surgery	1
C003	35	F	Pancrease	Adenocarcinoma	G3	T1N2 M1 (IV)	Lung, retroperitoneal and mediastinal metastatic lymph nodes	NO	2
A005	50	F	Endometrium	Clear cell carcinoma	G3	T4 N1 M1 (IV)	Left clavicle lymph nodes, Lung, Liver	Surgery, Albumin paclitaxel + paraplatin, Albumin paclitaxel	1
A017	52	M	Thymus	Adenocarcinoma	G2	T4 N0 M1 (IV)	Lung	Surgery, 3-dimensional conformal radiotherapy, Docetaxel +cisplatin, S-1	0
A011	66	M	Pancrease	Adenocarcinoma	G2-3	T2 N1 M0 (IIb, R1)	Resection margin positive	Surgery	0

[a] PS: Performance status: ECOG, Eastern Cooperative Oncology Group.

Supplemental Table 15. Treatment scheme and clinical responses after personalized immunotherapy

Patient ID	Preconditioning Regimens		Treatment Cycles	Total No. of Infusion Cells		PFS (days)	iRR	PS[c] after 2 cycles of treatment
	Chemotherapy	Radiotherapy[b]		DCs（$\times 10^7$）	CTLs（$\times 10^{10}$）			
A020	Gem+Abraxane+CTX	0.5Gy bid*2d	10	21.378	11.968	289	SD	0
C002	Gem+CTX	60Gy/15f	5	14.36	5.372	146	SD	0
C003	Gem+CTX	0.5Gy bid*2d	4	7.88	2.356	159	PR	0
A005	Gem+CTX	40Gy/20f	3	8.75	2.124	229	SD	0
A017	Gem+CTX	—	5	14.86	3.612	895[a]	CR	0
A011	Gem+CTX	55Gy/25f	2	4.64	1.924	454	ND	0

[a] (The last follow up: Oct. 15, 2018).

[b] The patients with locally advanced unresectable solid tumor received stereotactic body radiotherapy (SBRT) with a total dose of 40–60 Gy during the first immunotherapy cycle. For patients with metastases, partial lesions received a low-dose radiation (0.5 Gy bid for 2 days) before the infusion of NRTs in each immunotherapy cycle.

[c] PS: Performance status: ECOG, Eastern Cooperative Oncology Group.

Gem, Gemcitabine; CTX, Cyclophosphamid; iRR, immune-related response, according to irRECIST1.1.

Supplemental Table 16. Information of TAA-derived peptides used in the present study.

Peptide name	Original protein	Position of peptide	Amino acid sequence	HLA type	References
CEA571	CEA	571-579	YLSGANLNL	HLA-A2	[1]
CEA-691-H5	CEA	691-699	IMIGHLVGV	HLA-A2	[2]
VEGFR2-773	VEGFR2	773-781	VIAMFFWL	HLA-A2	[3]
Sur1M2-96	Survivin	96-104	LMLGEFLKL	HLA-A2	[4]
HER2-369 v2v9	Her2/neu	369-377	KVFGSLAFV	HLA-A2	[5, 6]
HER2-444	Her2/neu	444-453	TLQGLGISWL	HLA-A2	[7]
HER2-689	Her2/neu	689-697	RLLQETELV	HLA-A2	[8]
HER2-776	Her2/neu	776-790	GVGSPYVSRLLGICL	HLA-A2	[9, 10]
WT p53M2-149	p53	149-157	SMPPPGTRV	HLA-A2	[11]
WT p53-264	p53	264-272	LLGRNSFEV	HLA-A2	[12]
MUC1-12	MUC1	12-20	LLLLTVLTV	HLA-A2	[13]
CA125-13272	MUC16, CA125	13272-13281	YTLDRDSLYV	HLA-A2	[14]
AGR2-11	AGR2	11--19	LLVALSYTL	HLA-A2	[15]
AGR2-127	AGR2	127-135	RIMFVDPSL	HLA-A2	[15]
EGFR-479	EGFR	479-488	KLFGTSGQKT	HLA-A2	[16]
hTERT-540	TERT	540–548	ILAKFLHWL	HLA-A2	[9, 17]
SART3-302	SART3	302-310	LLQAEAPRL	HLA-A2	[18]
SART3-309	SART3	309-317	RLAEYQAYI	HLA-A2	[19]
WT1-126	WT1	126-134	RMFPNAPYL	HLA-A2	[20]
NY-ESO1-161	NY-ESO-1	161-180	WITQCFLPVFLAQPPSGQRR	HLA-A2	[21]
NY-ESO1-157	NY-ESO-1	157–165	SLLMWITQV	HLA-A2	[9]

TAA: tumor associated antigens.

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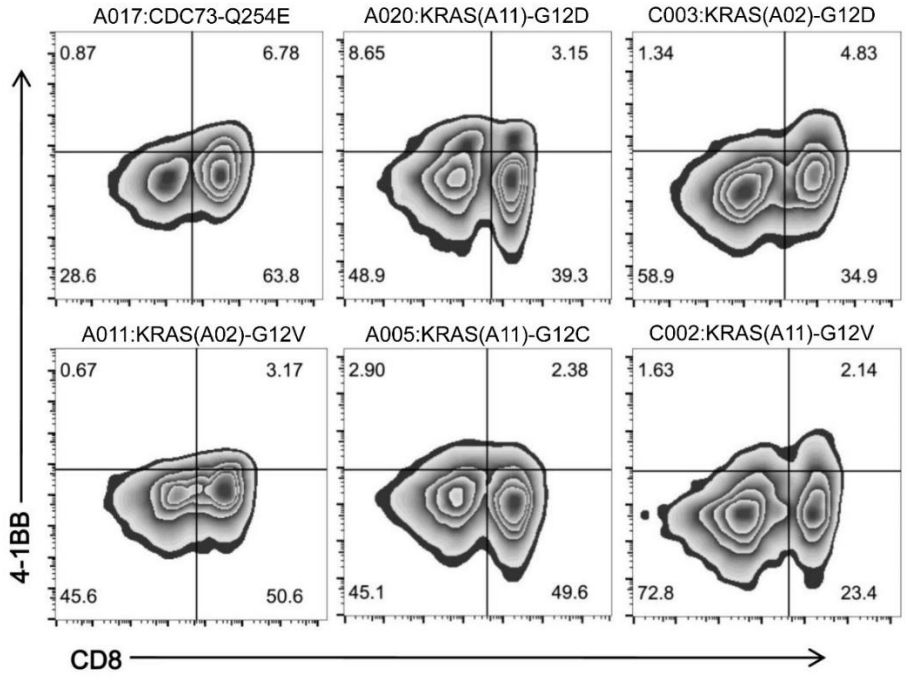
Supplemental Table 17. Side effects of neoantigen based personalized immunotherapy.

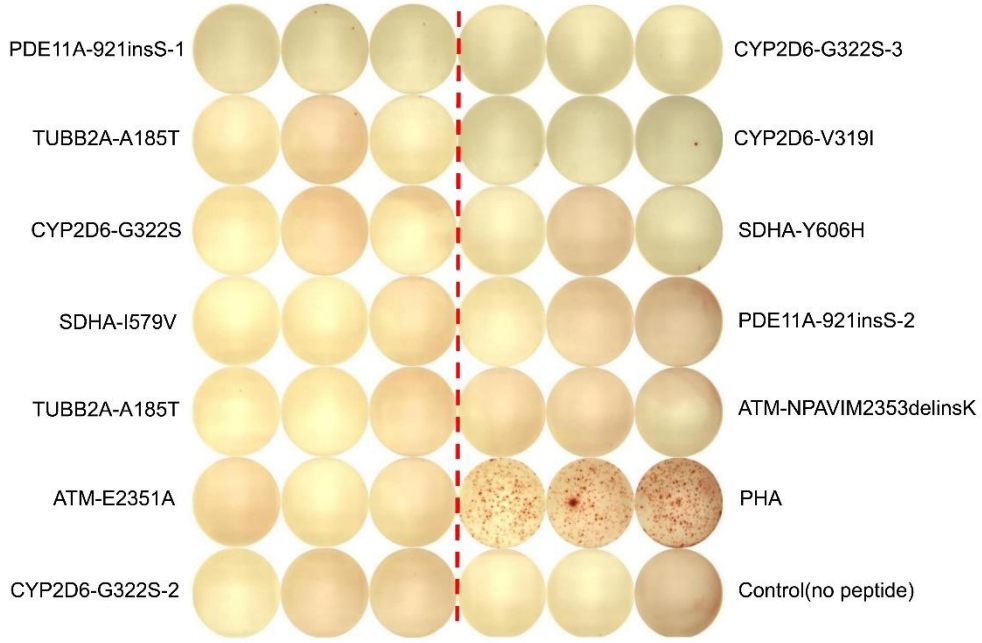
	Grade 1	Grade 2	Grade 3	Grade 4
Constitutional symptom				
Fever and chills	1	1	0	0
Tumor pain	0	0	0	0
Rash	1	0	0	0
Diarrhea	0	0	0	0
Nausea and vomiting	1	0	0	0
Respiratory				
Dyspnea	0	0	0	0
Hypoxia	0	0	0	0
Neurological				
CNS cerebrovascular ischemia	0	0	0	0
Blood/bone marrow				
Anemia	3	0	0	0
Neutropenia	2	0	0	0
Lymphocytopenia	0	0	0	0
Thrombocytopenia	3	0	0	0
Metabolic and laboratory				
AST elevation	1	0	0	0
ALT elevation	1	0	0	0
Scr elevation	0	0	0	0
BUN elevation	0	0	0	0

SUPPLEMENTARY FIGURES

Supplemental Figure 1. Proportion of neoantigen-reactive CD8+CD137+T cells in the infusion T-cells. Flow cytometric analysis of 4-1BB expression on the clinical grade infusion T-cells after overnight co-culture with DCs pulsed with mutant peptides (gated on CD3). We depicted a representative data out of three experiments yielding similar results at the first treatment cycle.

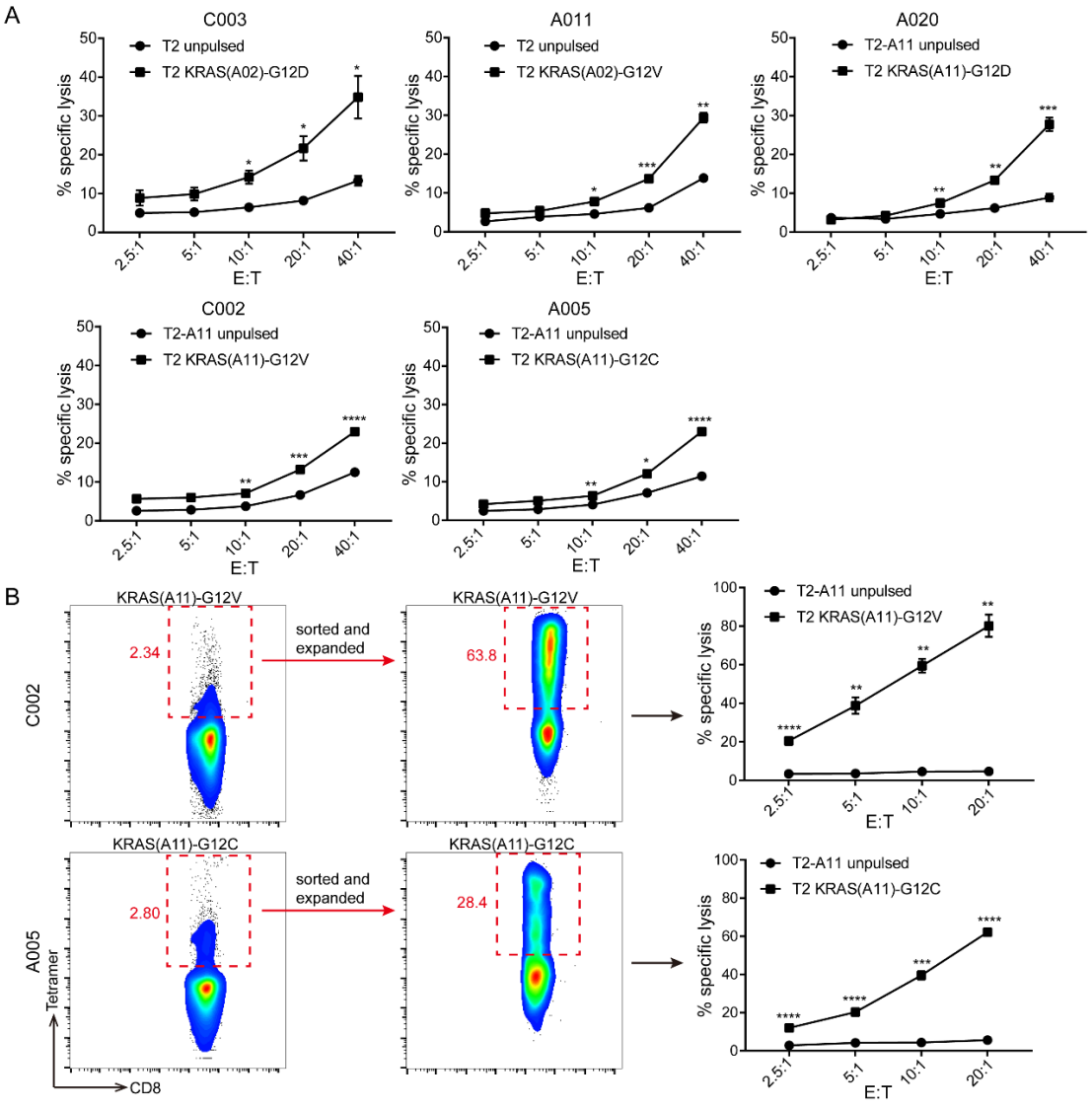


Supplemental Figure 2. Identification of personalized neoantigens for patient A015 with advanced gastric cancer. Autologous PBMCs were stimulated with twelve candidate mutant peptides for 10 days, after which IFN-γ ELISPOT assays were performed to assess the T-cell specific antigen response. PHA was used as positive control, and no-peptide stimulation was tested as negative control.



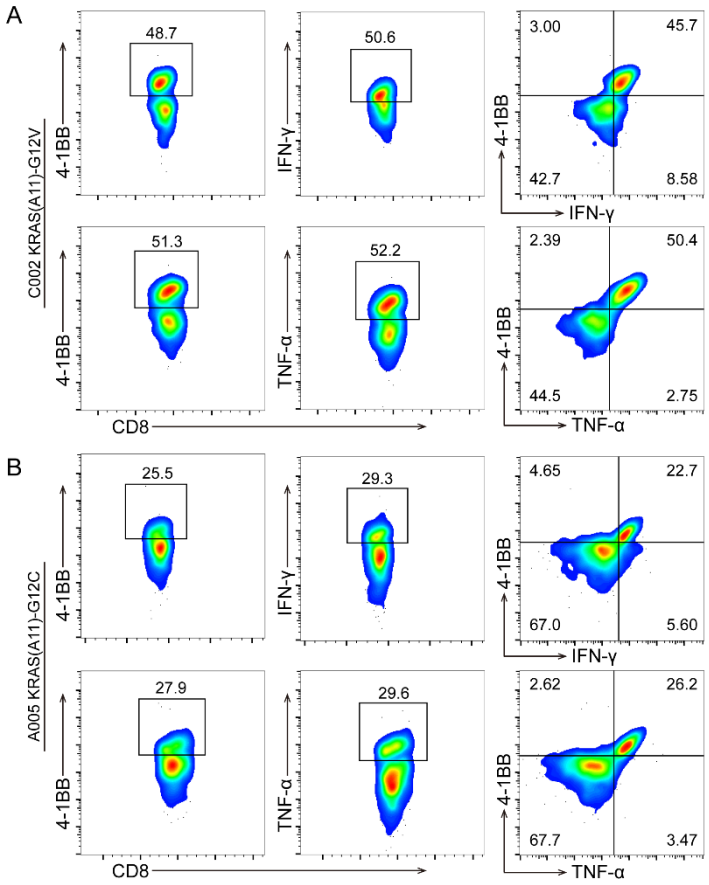
Supplemental Figure 3. Antigen specific cytotoxicity of clinical grade neoantigen reactive T cells.

(A) Clinical grade NRTs (bulk T cells) from five patients were each cocultured with CFSE labeled T2/T2-A11 cells that pulsed with corresponding mutant peptides, at ratios (E:T) of 2.5:1, 5:1, 10:1, 20:1, 40:1, respectively. After 6 h, the PI+ CFSE+ T cells were analyzed by FACS. No peptide-pulsed T2/T2-A11 cells were used as a negative control. (B) Tetramer+CD8+T of bulk T cells from patient C002 and A005 were isolated using FACSAria sorter, and the purified T cells were expanded to large numbers with IL-2, anti-CD3 Ab, and irradiated feeder cells(K562-A11). After sorting and expansion, antigen specific cytotoxicity of enriched T cells were performed at ratios (E:T) of 2.5:1, 5:1, 10:1, 20:1, respectively. All Cytotoxicity data are presented as mean ± S.E.M., n=3, *P < 0.05, **P < 0.01, ***P < 0.001, **** P < 0.0001, by 2-tailed Student t-test.



Supplemental Figure 4. Correlation between CD137 and intracellular cytokine expression.

The enriched KRAS(A11)-G12V specific T cells (63.8% of tetramer+CD8+T cells, see Supplemental Figure 3) from patient C002 (A) and KRAS(A11)-G12C specific T cells (28.4% of tetramer+CD8+T cells, see Supplemental Figure 3) from patient A005 (B) were cocultured with K562-A11 cells pulsed with the corresponding neoantigen peptides at an E:T ratio of 5:1 for 24h, respectively. CD137 and intracellular IFN-γ or TNF-α staining were performed in parallel (gated on CD8). Experiments were performed in triplicates, and the representative data was shown.



Supplemental Figure 5. The memory and activation phenotype analysis of infusion T-cells.

Before T-cell infusion, FACS was performed to characterize the memory and activation phenotype. CD45RO+CD62L+, CD45RO+CD62L- and CD45RO-CD62L+ cells were analyzed by gating on CD3+ cells. The representative data are depicted. Data are representative experiments depicted (n=3).