Supplementary methods

Western blot

$5x10^6$-$1x10^7$ cells were used for protein extraction with cell lysis buffer (Mammalian Cell Lysis Kit, Sigma). Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed with 50 ug protein per sample, which was heated before at 70°C for 10 min in lithium dodecyl sulfate (LDS) sample buffer and sample reducing agent. Samples were run in a 10% polyacrylamide gel (bis-tris) in 2-morpholinoethanesulfonic acid (MES) SDS running buffer. Proteins were blotted onto a methanol-activated polyvinylidene difluoride (PVDF) membrane in transfer buffer (all buffer and components from Novex, Life Technologies). After blocking of the membrane, it was incubated with anti-NY-ESO-1 antibody 1:750 (E978, Sigma) at 4°C overnight. Then the membrane was incubated with goat anti-mouse IgG conjugated to horse radish peroxidase (HRP; Santa Cruz Biotechnology) 1:5000 and chemiluminescence was detected by Lumi-Imager F1 (Roche) after application of Western blotting Luminol Reagent (Santa Cruz). For loading control, actin was analyzed after incubation of the membrane with anti-β-actin-HPR (AC-15, Sigma) 1:30000.

Figure S1

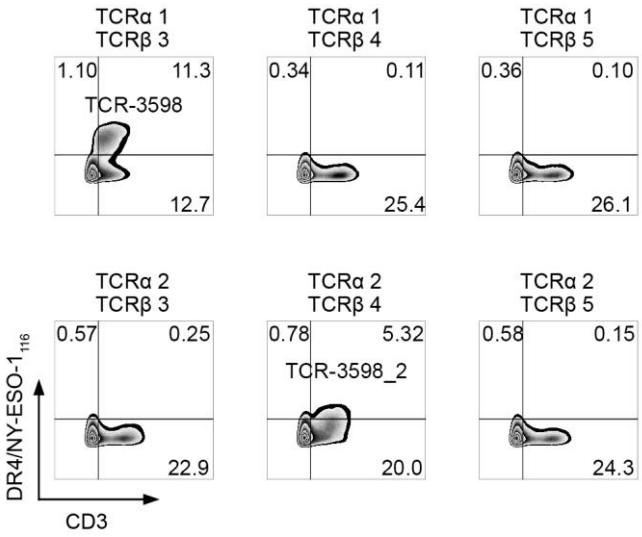

Figure S1 Combinatorial expression of TCRα and β chains isolated from NY-ESO-1-reactive CD4 T cells from ABabDR4 mouse 3598 revealed two DR4/NY-ESO-1_{116} tetramer binding TCRs. Two TCRα and three TCRβ chains isolated from NY-ESO-1-reactive CD4 T cells (Figure 1B) were expressed in different combinations in TCR-deficient Jurkat76/CD4 cells and stained for CD3 and with DR4/NY-ESO-1_{116} tetramer.

Figure S2

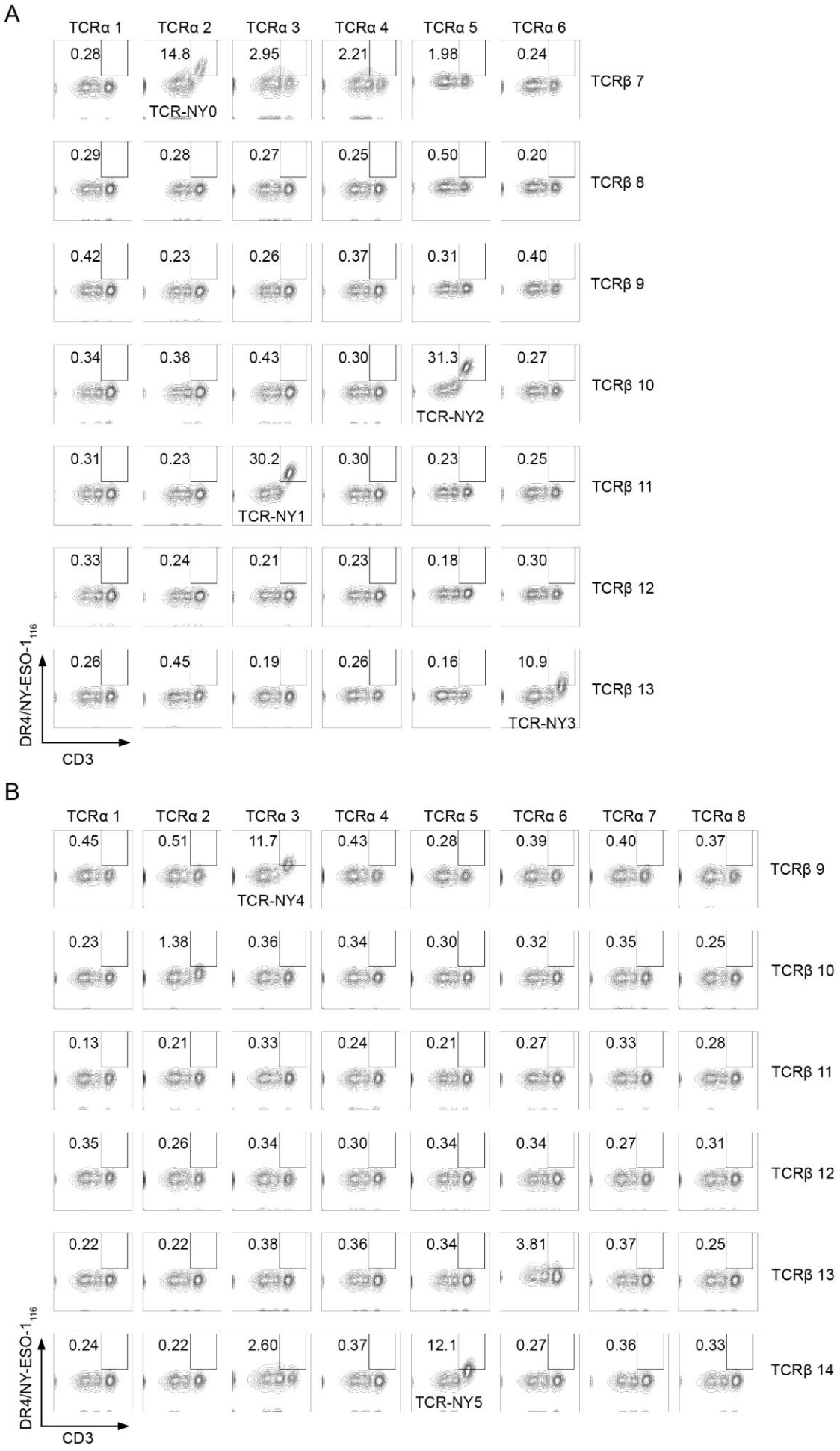
A
TCRα 1
TCRα 2
TCRα 3
TCRα 4
TCRα 5
TCRα 6
0.28
14.8
TCR-NY0
2.95
2.21
1.98
0.24
TCRβ 7
0.29
0.28
0.27
0.25
0.50
0.20
TCRβ 8
0.42
0.23
0.26
0.37
0.31
0.40
TCRβ 9
0.34
0.38
0.43
0.30
31.3
TCR-NY2
0.27
TCRβ 10
0.31
0.23
30.2
TCR-NY1
0.30
0.23
0.25
TCRβ 11
0.33
0.24
0.21
0.23
0.18
0.30
TCRβ 12
0.26
0.45
0.19
0.26
0.16
10.9
TCR-NY3
TCRβ 13
DR4/NY-ESO-1116
CD3
B
TCRα 1
TCRα 2
TCRα 3
TCRα 4
TCRα 5
TCRα 6
TCRα 7
TCRα 8
0.45
0.51
11.7
TCR-NY4
0.43
0.28
0.39
0.40
0.37
TCRβ 9
0.23
1.38
0.36
0.34
0.30
0.32
0.35
0.25
TCRβ 10
0.13
0.21
0.33
0.24
0.21
0.27
0.33
0.28
TCRβ 11
0.35
0.26
0.34
0.30
0.34
0.34
0.27
0.31
TCRβ 12
0.22
0.22
0.38
0.36
0.34
3.81
0.37
0.25
TCRβ 13
0.24
0.22
2.60
0.37
12.1
TCR-NY5
0.27
0.36
0.33
TCRβ 14
DR4/NY-ESO-1116
CD3

Figure S2 Combinatorial expression of TCRα and β chains isolated from human PBL cultures revealed DR4/NY-ESO-1_{116} tetramer binding TCRs. TCRα and β chains isolated from two PBL in vitro cultures (A and B) were expressed in different combinations in TCR-deficient 58/CD4 cells and stained for CD3 and with DR4/NY-ESO-1_{116} tetramer. TCR chains were considered if they occurred at least twice in 31 or 32 clones sequenced. TCR NY0 was not considered in further experiments, because initial tetramer staining technically failed.

Figure S3

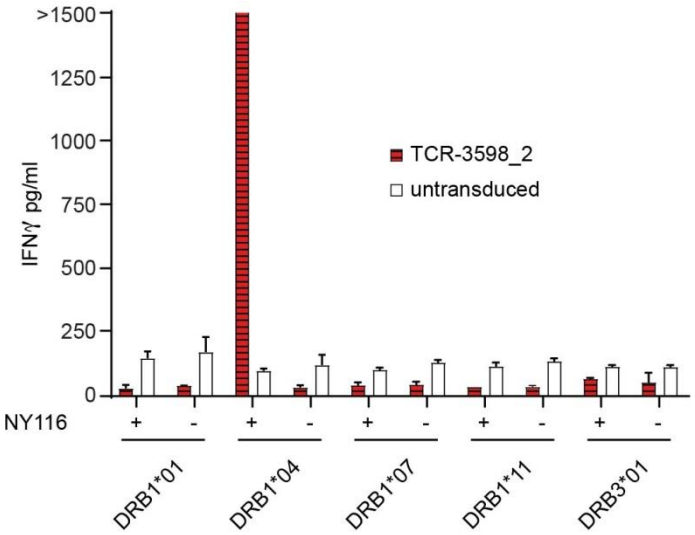

Figure S3 TCR-3598_2 is exclusively restricted to HLA-DR4. TCR-transduced CD4 T cells were co-cultured with K562 cells expressing different HLA-DR molecules. NY-ESO-1_{116} peptide (NY116) was added where indicated. After overnight incubation IFNγ was measured in the supernatant. Mean values of intra-assay duplicates with standard deviation are shown. The results are representative of two independent experiments.

Figure S4

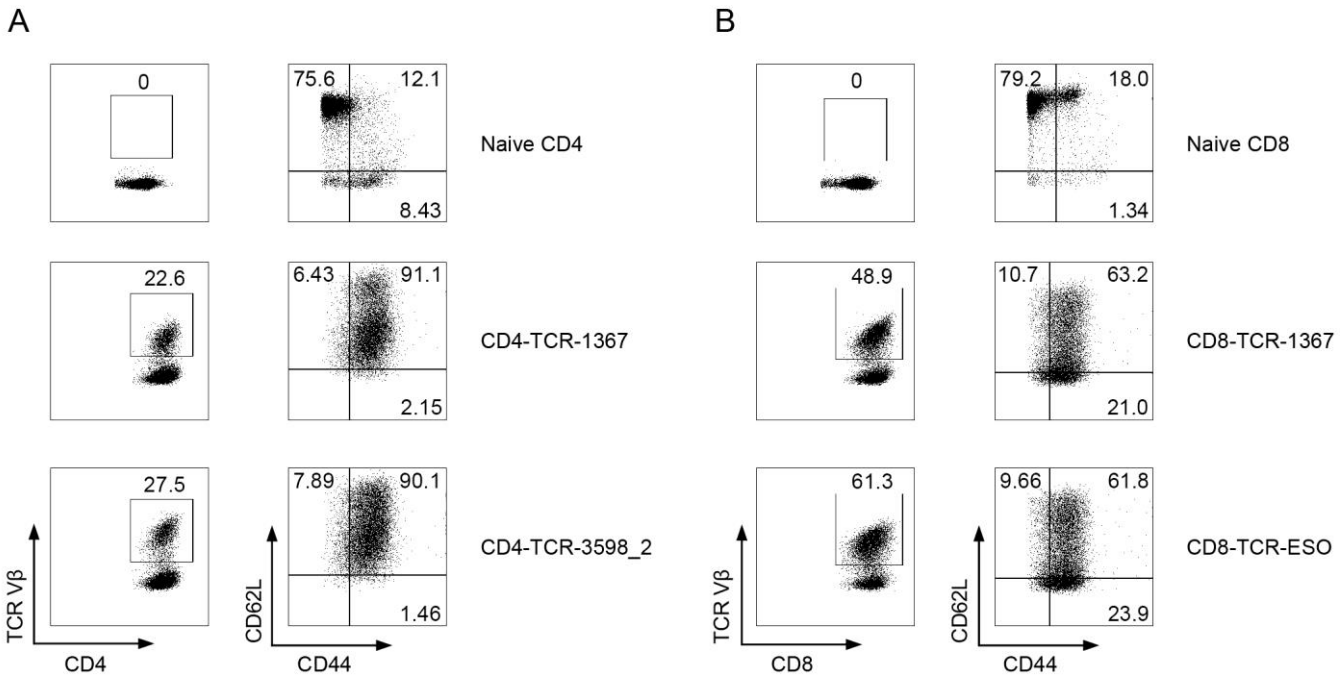

Figure S4 Phenotype of T cells used for adoptive transfer. (A, B) CD4 (A) and CD8 T cells (B) used for adoptive transfer were stained for CD4 or CD8, respectively, as well as for Vβ of the transduced TCR (TRBV2 for TCR-3598_2, TRBV12 for TCR-ESO, TRBV28 for TCR-1367), and CD62L and CD44. Splenocytes from a naive B6 mouse were taken as control. Displayed cells in the second columns were gated on the stained Vβ or total CD4 or CD8 T cells for naïve T cells. The results are representative of two experiments.

Figure S5

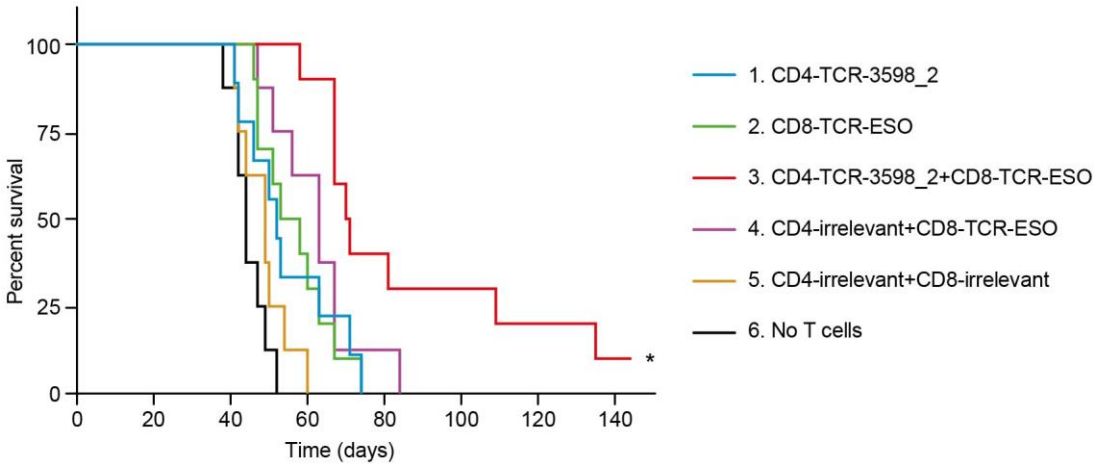

Figure S5 Combined treatment with CD4 and CD8 T cells achieved longest survival. Tumor-bearing mice were treated with TCR-3598_2-transduced CD4 T cells and/or TCR-ESO-transduced CD8 T cells at day 30, when the tumors were palpable. TCR-1367-transduced CD4 and/or CD8 T cells were injected as controls (CD4-/CD8-irrelevant) where indicated. Displayed is percent survival of the indicated treatment groups with an endpoint of 500 mm^3 tumor volume. Survival was compared by Log-rank (Mantel-Cox) test. *P<0.05.

Figure S6

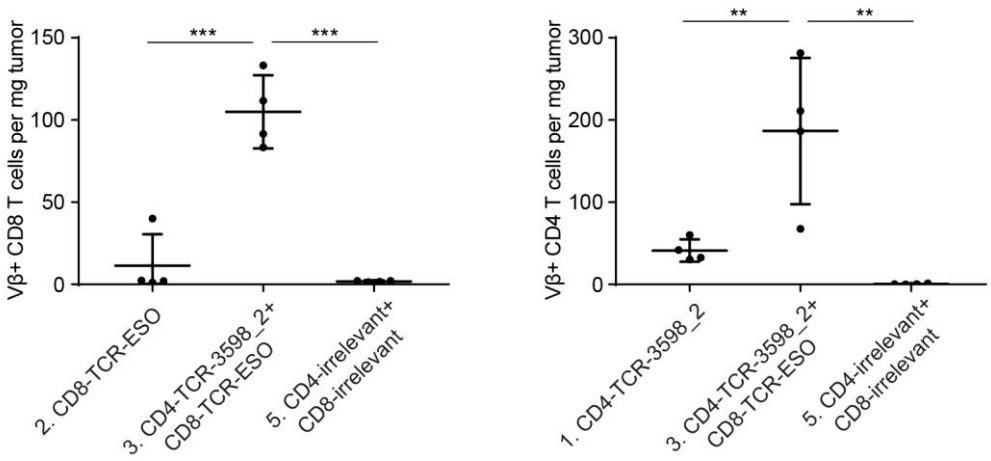

Figure S6 More T cells were found in tumors of mice that were treated with CD4 and CD8 T cells in combination. Tumor infiltrating lymphocytes were analyzed in tumor lysates prepared on day 5 following adoptive T cell therapy from indicated treatment groups. Group numbers refer to Figure 7. Cells were stained for CD3, CD4, CD8 and Vβ of the transduced TCRs. Each dot represents data derived from one individual mouse. One-way ANOVA followed by Bonferroni post-hoc test was performed for statistical analysis. ***P*<0.01, ****P*<0.005.

Figure S7

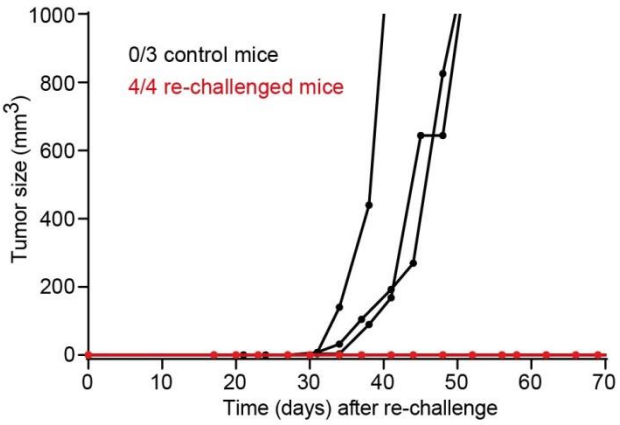

Figure S7 Mice previously treated with CD4 and CD8 T cells in combination rejected tumor cells upon re-challenge. Mice that were treated with TCR-3598_2-transduced CD4 T cells and TCR-ESO-transduced CD8 T cells (group 3 in Figure 7) were injected with tumor cells in the opposite flank on day 37 (3 mice) or day 190 (1 mouse) after adoptive T cell therapy. Control mice were not treated previously.

Figure S8

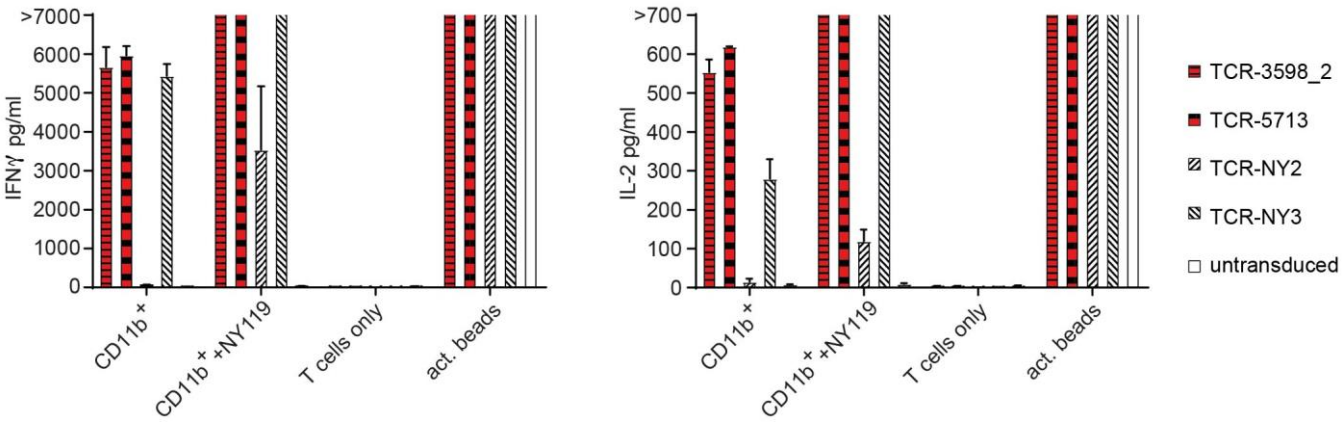

Figure S8 Tumoral macrophages were recognized by TCR-transduced CD4 T cells. $CD11b^+$ cells were isolated from tumor material and co-cultured with TCR-transduced CD4 T cells. As positive controls, $CD11b^+$ cells were loaded with NY-ESO-1_{119} (NY119) or anti-CD3/CD28 activator beads were added to the T cells. Mean values of intra-assay duplicates with standard deviation are shown. The results are representative of three independent experiments.

Table S1

HLA allotypes of the LCL panel and the blood donation used for TCR isolation.

B-LCL	A*		B*		C*		DRB1*		DRB3*	DRB4*	DRB5*	DQA1*		DQB1*		DPA1*		DPB1*	
AMAI	6802		5301		0401		1503				0101	010201		0602		0301		0402	
AMALA	021701		15010101		0303		1402		0101			0501		0301		010301		0402	9401
BSM	020101		15010101		030401		040101			01030101		03		030201		01		020102	
DJS	0201	0301	3501	3702	0401	0602	0101	1601				0101	0102	0501	0502			0401	0402
DUCAF	3002		1801		0501		0301		0202			050101		0201		0103		0202	
HOR	330301		440301		1403		130201		030101			0102		0604				0401	
KAS011	010101		3701		0602		160101				0202	010202	010201	050201		020101	010301	1401	040101
KAS116	24020101		5101		1203		0101					010101		050101		0201		1301	
KE	0201	2902	4403	4405	0202	1601													
MT14B	3101		4001		0304		0404			0101		03		0302				0402	
RML	0204		510101		1502		160201				0202	0501		0301		0103		0402	
SA	2E+07		070201		0702		0101					0101		050101				0402	
SPO	0201		4402		0501		1101		0202			010202		0502		01		020102	
TISI	24020101		3508		0401		1103		0202			0505		0301		0103		0402	
WIN	0101		570101		0602		0701			0101		0201		0202	030302	0103	020102	0401	1301
Blood donor	020101	310101	440201		050101		040101			0103		0303		030101		0103		040101	

Table S2

Peptide sequences containing the TCR-3598_2 recognition motif X-X-X-X-L-K-E-F-X-X-X-X-X-X-X.

Protein	Peptide[A]	IC50 (nM)
Neuroserpin	EFSFLKEFSNMVTAK	11,40
Folliculin-interacting protein 2	CQRFLKEFTLLIEQI	20,00
Cytosol aminopeptidase	AAAFLKEFVTHPKWA	20,50
Gamma-parvin	FFLHLKEFYLTPNSP	33,50
DNA mismatch repair protein Msh3	IIKYLKEFNLEKMLS	39,50
Protein Lines homolog	RPLVLKEFDTAFSFD	68,90
Ankyrin and armadillo repeat-containing protein	NPAFLKEFQMQQTLV	73,70
Piwi-like protein 3	RHHTLKEFINTLQDN	93,90
Protein LAP2	QLSGLKEFWMDANRL	95,80
Apolipoprotein L1	RNWFLKEFPRLKSEL	101,10
Formin-like protein 2	HNTLLKEFILNNEGK	105,40
Apolipoprotein L2	RQWFLKEFPRLKREL	107,60
Formin-like protein 1	DCMVLKEFLRANSPT	109,40
Transcriptional-regulating factor 1	CSICLKEFKNLPALN	120,20
Separin	LLPALKEFLSNPPAG	134,80
Netrin-G2	SAKGLKEFFTLTDLR	139,90
Discoidin domain-containing receptor 2	EPDDLKEFLQIDLHT	149,80
Apolipoprotein L4	REWFLKEFPQIRWKI	151,30
Protein Jumonji	LYLSLKEFKNSQKRQ	153,50
Apolipoprotein L3	REWFLKEFPQVKRKI	154,10
Tripartite motif-containing protein 59	IFYLLKEFVWKIVSH	161,00
Cell cycle checkpoint control protein RAD9A	ITFCLKEFRGLLSFA	165,10
Putative E3 ubiquitin-protein ligase UNKL	HYRYLKEFRTEQCPL	180,20
NACHT, LRR and PYD domains-containing protein 1	KKEELKEFQLLLANK	181,80
Alpha-hemoglobin-stabilizing protein	ISAGLKEFSVLLNQQ	194,30
Peroxisome biogenesis factor 1	TKDGLKEFSLSIVHS	202,00
Probable small intestine urate exporter	WNETLKEFKAMSGIL	202,60
X-ray radiation resistance-associated protein 1	AKRLLKEFQARYRQL	205,90
Protein-arginine deiminase type-4	QLFKLKEFSKAEAFF	210,40
Vacuolar protein sorting-associated protein 13A	ANAFLKEFCLKCPEY	221,40
Coiled-coil domain-containing protein 127	ARLLLKEFEAVLTER	222,00
Probable small intestine urate exporter	WNETLKEFKAMAPAY	224,90
Breast cancer type 1 susceptibility protein	NTSELKEFVNPSLPR	231,80
Fibrous sheath-interacting protein 2	INSLLKEFSDAQIKV	238,40
Dystonin	FYSKLKEFSILLQKA	241,10
Ropporin-1B	LPKMLKEFAKAAIRA	261,50
Nucleotide exchange factor SIL1	SHQNLKEFALTNPEK	286,90
Sodium channel modifier 1	RQMALKEFSSVYSEE	298,70
Poly(A) RNA polymerase, mitochondrial	LNTLLKEFQLTEENT	318,90
Phosducin-like protein	GKMTLKEFAIMNEDQ	330,50
Ropporin-1A	LPKMLKEFAKAAIRV	387,30
Collagen alpha-3(VI) chain	GFPLLKEFVQRVVES	390,30
Gem-associated protein 4	PDEVLKEFVLPFLRL	392,00
Olfactory receptor 10X1	NQTILKEFILVGFSV	455,30
Spectrin alpha chain, erythrocytic 1	SEETLKEFSTIYKHF	459,00
Protein NYNRIN	FKRALKEFIFLHGKK	459,70
UPF0565 protein C2orf69	YPEVLKEFAQTGIIV	463,60
Leucine-rich repeats and immunoglobulin-like domains protein 3	LPEHLKEFQSLETLD	487,70
Epididymal-specific lipocalin-10	SFQSLKEFMDACDIL	492,40
Required for meiotic nuclear division protein 1 homolog	MLKPLKEFENTTCST	497,00

[A] Peptides were included if they had a predicted affinity to HLA-DR4 of below 500 nM and are present in the human but not the mouse proteome.