SUPPLEMENTARY MATERIAL AND METHODS

Clinical course of the melanoma patient

A 56-year-old patient was diagnosed with malignant melanoma in 2008 and underwent resection of the primary tumor in the same year. In 2013 metastatic disease with pulmonary and intestinal metastases were detected. A biopsy from the lung metastasis (B_{Lung}) was performed for histologic confirmation of metastatic status of malignant melanoma. Subsequently, the patient underwent two cycles of chemotherapy resulting in a mixed response with regression of the intestinal metastasis (M_{Int}) but progression of lung metastasis (M_{Lung}). In November 2013, the patient became eligible for administration of anti-CTLA-4-antibody Ipilimumab for a total of four cycles. In March 2014, restaging revealed response of M_{Lung}, whereas M_{Int} progressed and was therefore surgically removed, together with two non-malignant lymph nodes (M_{Int}-LN1 and -LN2). The residual M_{Lung} previously targeted by biopsy, initially regressed, but later progressed and was therefore surgically removed in January 2016, together with one adjacent non-malignant lymph node (M_{Lung}-LN). Treatment with the PD-1-directed monoclonal antibody Pembrolizumab was subsequently applied for one year and the patient is in complete remission since then (Supplementary Fig. S1).

Cultivation of cell lines and primary human cells

All target cell lines were maintained in RPMI 1640, MEM or DMEM (Invitrogen), according to manufacturer's instructions, supplemented with Penicillin and streptomycin antibiotics (Pen/Strep), L-glutamine, non-essential amino acids (NEAA), sodium pyruvate (NaP) and 10% heat-inactivated fetal calf serum (FCS). T cells were cultured in RPMI supplemented with: L-glutamine, 5% human serum and 5% FCS, Hepes, Pen/Strep, NEAA, NaP and gentamycin.

T-cell antigen-experience recall responses

For stimulation with peptides, PBMCs were cultured as previously described[1 2]. Briefly, frozen PBMCs were thawed and cultured in AIM-V medium supplemented with 100 ng/ml of IL-4 and 100 ng/ml GM-CSF. After 24h, Poly:IC (20 µg/ml), IL-7 (0.5 ng/ml) and peptide pools (1 µM/peptide) were added to the culture. After 20-24h incubation, PBMCs were transferred on a pre-coated IFN-γ ELISpot plate, which was developed after additional 20-24h, prior transfer of PBMCs to culture for expansion of T cells in RPMI medium supplemented with IL-7 (5 ng/ml) and IL-15 (5 ng/ml) for 10-15 days. IFN-γ ELISpot assay was repeated on expanded T cells co-cultured for 72h with T2-A3 cells (E:T = 1:1) pulsed with single peptides from sub-pool A4 shared by Pool 4 and 9.

Arrangement of peptide pools for immunogenicity assessment of predicted ligands

The first 25 predicted binders to each allele were synthetized and used for stimulation assays, excluding previously identified neoantigens (NCAPG2^{P333L}, ranked 24th and SYTL4^{S363F} ranked 6th). In total 48 peptides were assembled in 11 pools. Each pool contained 10 peptides: 5 HLA-A03:01-predicted binders (sub-pool A) and 5 HLA-B27:05-predicted binders (sub-pool B) were arranged as depicted in Supplementary Table S3. Sub-pools A5 and B2 contained 9 peptides, due to the exclusion of known immunogenic peptides[1]. Pool 11 contained peptide SYTL4^{S363F} and served as positive control for peptide pool approach. Following screening of peptide pools, T cells were expanded for two weeks and co-cultured with single-peptide-pulsed target T2 cells to define the peptides eliciting reactivity in the pool.

IC_{50} in vitro measurement of predicted peptide candidates and neoantigens

Binding of selected peptides to HLA was further investigated through a peptide exchange assay. HLA-A03:01, HLA-B27:05, and human β2m (hβ2m) were expressed in E. coli and purified from inclusion bodies as described previously[3]. Purified proteins were stored at −80 °C until use. Folding of heavy chain–hβ2m–peptide complexes was performed by diluting purified denatured proteins to a final concentration of 30 µg/mL of heavy chain and 30 µg/mL hβ2m folding buffer (100 mM Tris·Cl, pH 7.5, 0.4 M arginine, 2 mM EDTA, 0.5 mM oxidized glutathione, 5 mM reduced glutathione, and 0.5 mM PMSF) and stirring at 4 °C (2 days for HLA-A03:01, 14 days for HLA-B27:05) with 10 µM high-affinity peptide (KLIETYFJK for HLA-A03:01, and RRKJRRWHL for HLA-B27:05, where J is the photolabile aa residue)[4]. After folding, samples were ultracentrifuged at 15,000 × g for 20 min and further purified by gel filtration. For peptide exchange reaction, 0.5 µM HLA class I monomers (HLA–hβ2m– photolabile peptide complex) were exposed to UV radiation (345 nm, 1000 W) for 5 min, followed by incubation for 18 h at 4 °C with 10nM fluorophore-labeled peptide (KLIE-FITC-YFSK for HLA-A3:01 and RRKW-FITC-RWHL for HLA-B27:05, FITC λex = 494 nm, λem = 517 nm) and varying concentration of the measured peptide. The binding was measured by fluorescence anisotropy using a Victor 3V reader (Perkin Elmer) in a black 96-well plate (Corning). Data were fitted and IC_{50} values were calculated by using GraphPad Prism 7.

TCR in situ hybridization

RNA in situ hybridization for identification of neoantigen-specific TCR on FFPE tumor slides was performed using BaseScope™ Detection Reagent Kit v2 - RED (ACD). TCR-specific Probes were designed to anneal on the TCR CDR3 sequence. Probes against PPIB (Cyclophilin B) and a bacterial protein (DapB, Bacillus subtillis) served as respective positive and negative controls. For each paraffin tumor block, slides of 4 µm were trimmed

with a microtome. Tissue slides were deparaffinized, pretreated using Protease IV (ACD) and stained with target probe, negative control and positive control probes following the manufacturer's instructions on an automated immunostainer. For localization of T-cell infiltration, Immunohistochemistry (IHC) was performed using anti-CD3-antibody (clone MRQ, Cell Marque Cat# 103R) as previously described[1]. Slides were scanned on a Leica AT2 (Leica, Wetzlar, Germany) system and visualized using Aperio ImageScope software (v12.4.3).

HLA-peptide complex structural modelling and SASA calculation

Each model was solvated in a rectangular box of TIP3P[5] water with a minimum distance of 12 Å between solute and box boundary, neutralized, and energy minimized. All modelling steps were performed using AMBER17[6]. The force field FF14SB was selected[7]. Subsequently, all 6 models were progressively heated up to 300 K over 3 ns gradually releasing initial positional restraints from the solute (Supplementary Table S4). The last heat-up step was performed in constant number, pressure and temperature (NPT) thermodynamic ensemble whereas the other steps were performed in constant number, volume and temperature (NVT). Temperature was controlled using a Langevin thermostat with a collision frequency (gamma_ln) of 4.00 ps^{-1}. Pressure was controlled using a Berendsen barostat[8] with default settings. SHAKE algorithm[9] was applied for all bonds including hydrogen atoms. Particle mesh Ewald (PME) method[10] was used to compute long range electrostatic interactions. A cut-off for non-bonded interactions of 12 Å was applied. After equilibration models were simulated for 200 ns at 300 K using the NPT ensemble with a 1 fs time step. For each model 3 replicas were simulated resulting in a cumulative simulation time of 600 ns per system.

To quantitatively analyse the HLA-p interface, solvent-accessable surface area (SASA) was calculated for each peptide residue and the electrostatic potential of the interaction surface

as previously described[11 12]. Briefly, frames were extracted every 200 ps from the last 180 ns of each replicon and analyzed. To select representative structures for visualization, frames were clustered, and central structure of the highest populated cluster was picked. The clustering was based on non-hydrogen peptide atoms using the hierarchical agglomerative algorithm with an epsilon of 2.0 and default settings. Trajectory processing and clustering was conducted with the CPPTRAJ[13] tool provided in AmberTools17[6]. Molecule images were generated using PyMOL (version 1.7.X, Schrödinger, LCC). Electrostatic potential was computed with PyMOL plugin APBS Tools 2.1[14] using default settings. Hydrogen bonds were identified using the VMD software HBonds plugin (version 1.2)[15].

Molecular cloning and retroviral transduction of TCRs, minigenes and HLA

TCR repertoire PCR analysis followed by Sanger sequencing was performed to determine variable alpha and beta chain usage of neoantigen reactive T-cell clones[16 17]. V-D-J rearrangements of complementarity-determining regions (CDR3) were investigated using IMGT/V-Quest (http://www.imgt.org/IMGT_vquest/vquest) and the complete sequences were in silico reconstructed with Ensembl database. TCR sequences were modified by in silico murinization, insertion of an additional cysteine bridge and codon optimization (BioCat)[18 19]. Optimized TCR constructs were retrovirally transduced in $CD8^+$ healthy donor derived T cells as described before and expanded for 7 to 10 days with IL-7 and IL-15 (5ng/mL; Peprotech) before functional characterization[16 20].

SYTL4 and NCAPG2 mutated and WT minigenes were cloned as previously described[1]. KIF2C minigenes were in silico designed, comprising 100 bp up- and downstream of mutated position, synthetized (Genscript) and cloned into pMP71 backbone. Additionally, tandem constructs were in silico designed, synthetized (Genscript) and cloned into the pMP71 backbone with single or tandem constructs for minigenes containing mutated

sequences of all three neoantigens and WT counterparts separated in two different vectors. All minigene vectors included a reporter gene dsRed ExpressII to allow sorting of transgenic cells. Neoantigen-coding and WT counterpart minigenes were transduced in different cell lines as LCL-1, U-698-M, A2058 and MDST8 as previously described[1] with small changes for adherent cell lines omitting RetroNectin (Clontech). LCL-1 was transduced with single minigenes, while other cell lines with tandem constructs, followed by single clone selection.

TCR beta and alpha chains as well as tandem constructs of minigenes were separated by a self-cleaving P2A element[16]. All vectors were amplified using NEB® 5-alpha Competent E. coli (New England BioLabs) and purified with NucleoBond® Xtra Midi/Maxi (Macherey-Nagel).

T2 were retrovirally transduced with the HLA restriction elements HLA-A03:01 and B27:05 as described before[16].

In-vitro cytotoxicity assessment

For monitoring of T-cell mediated killing in vitro, two different adherent cell lines were chosen according to the naturally expressed HLA allotypes. MDST8 cell line expressing HLA-B27:05 was selected for SYTL4^{S363F}-specific TCRs; A2058 expressing HLA-A03:01 for KIF2C^{P13L} and NCAPG2^{P333L}-specific TCRs. Killing assays were performed with impedance-based xCELLigence assays (ACEA BioSciences)[21]. Target cell culturing media was added to each well of 96 well E-Plates (ACEA Biosciences) for background impedance measurement[22]. Dissociated adherent target cells were seeded on E-Plate at different densities depending on the cell line (A2058 – 50,000/well; MDST8 – 20,000/well). Cell density on plates is measured within the RTCA MP instrument inside a cell culture incubator for 24h. For addition of T cells, data acquisition was paused and effector cells were added. Data recording was initiated immediately at 15-min intervals for the first 8 h

and then at 30-min intervals for remaining 16 h. Target cells only as well as target cells and non-transduced T cells served as controls. For calculation of percentage of cytolysis, the following formula was adopted: $Cytotoxicity\ (\%) = 100 - \left(\frac{CI_{TCR}}{CI_{ntd}}\right) \times 100$.

Activation/dysfunction patterns of stimulated and restimulated enriched TCR-transgenic T cells

A near-infrared fluorescent protein (iRFP) linked with a T2A element was cloned downstream of TCR constructs KIF2-PBC2 and SYTL4-TIL1 to allow for flow-cytometry based enrichment of TCR-transduced T cells. Retroviral transduction was performed as stated above and iRFP-positive T cells were sorted on a FACSAria III Cell Sorter (BD Biosciences). Sorted T cells were coincubated with minigene-transduced U-698-M in an E:T ratio of 1:1. To analyze repeated antigen exposure, transduced and enriched T cells were co-cultured with irradiated U-698-M expressing mutated tandem minigene (E:T = 1:1), fed with IL-7 and IL-15 (5 ng/ml each) every three days and restimulated with respective target cells after 11 days. For flow cytometry assessment, T cells were stained with anti-CD8, TCRmu and Zombie UV Fixable Viability Kit (BioLegend). Expression of activation/dysfunction markers was assessed using anti-PD-1-antibody (BioLegend, Cat# 329930) and, in combination with True-Nuclear Transcription Factor buffer set (BioLegend), anti-T-bet (BioLegend, Cat# 644816). Proliferation after 6 days of target stimulation was assessed with CellTrace Violet Cell Proliferation Kit, (ThermoFisher) according to the manufacturer's recommendations. Flow cytometry data acquisition and analysis was performed as stated in respective section within the main manuscript.

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SUPPLEMENTARY FIGURE LEGENDS

Figure S1.
Clinical course of disease of the melanoma patient. He was diagnosed with malignant melanoma in 2008 and underwent resection of the primary tumor that year. In 2013, pulmonary and intestinal metastases were detected and a lung biopsy (B_{Lung}) was performed for histological analysis. Carboplatin and paclitaxel were administered, resulting in a mixed response of metastatic disease. The patient was subsequently treated with the anti-CTLA-4-antibody Ipilimumab. In 2014, the progressing abdominal lesion (M_{Int}) and two non-malignant lymph nodes (LNs) were surgically removed. In 2016, one primarily responding lung metastasis (M_{Lung}) progressed and was resected with one adjacent non-malignant LN. Treatment with Pembrolizumab was subsequently administered for 18 months. The patient is currently in complete remission without therapy. Analyses performed on tissue samples and time points of blood withdrawals are depicted.

Figure S2.
Detection of immunogenicity of predicted neoantigen KIF2C^{P13L} in autologous PBMCs of the melanoma patient. **A**, PBMCs derived from time point 925 were cultured in presence of IL-4 and GM-CSF following addition of Poly:IC, IL-7 and peptide pools (1 µM/peptide). After 24h, IFN-γ ELISpot assay was performed. **B**, IFN-γ ELISpot assay with T cells expanded after stimulation with Pool 4 and 9 (Supplemental Table 3). Expanded T cells were incubated with T2-A3 cells (E:T = 1:1) pulsed with single peptides of subpool A4 shared by Pool 4 and 9 for 72h. Subpool B4 containing peptides only present in Pool 4 but not Pool 9, served as negative control.

Figure S3.
Expression of transgenic neoantigen-specific TCRs on the surface of human CD8+ T cells assessed by TCRmu staining. Representative flow cytometry plots of single-cell suspension of TCR-transduced-CD8+ T cells stained (7-10 days after transduction) for CD8, TCRmu, and 7-AAD; the dot plots were gated on living cells (7-AAD-) and CD8-TCRmu+ T cells. The numbers in the gate represent the percentage of CD8+-T cells expressing the murinized TCR. Gates were set on the basis of isotype control for TCRmu-Ab, for each single TCR. Transduction efficiency was assessed for all transgenic T cells showing consistent results for the different TCR constructs.

Figure S4.
Expression of transgenic neoantigen-specific TCRs on the surface of $CD8^+$ T cells assessed by multimer staining. Representative flow cytometry plots show stainings with indicated multimers: HLA-B27:05-SYTL4 (**A**), HLA-A03:01-KIF2C (**B**) and HLA-A03:01-NCAPG2 (**C**). TCR-transduced-$CD8^+$ T cells were then stained for CD8 and 7-AAD; the dot plots were gated on living cells (7-AAD^-). The values in the gate represent the percentage of multimer-positive $CD8^+$-T cells. The quadrants were set on the basis of non-transduced T cells and isotype control. One representative out of three experiments with T cells from two different healthy donors is shown.

Figure S5.
Functional avidity of neoantigen-specific TCRs. Representative plots of IFN-γ secretion of TCR-transduced T cells upon stimulation with T2 target cells pulsed with titrated peptide concentrations of SYTL4^{S363F} (**A**), KIF2C^{P13L} (**B**) and NCAPG2^{P333L} (**C**). EC_{50} values of all experiments were calculated by fitting a non-linear curve and results are summarized in Fig. 1D.

Figure S6.
TCR-transduced CD8^{+} T cells mediate specific lysis of target cells expressing defined neoantigens. **A**, **B** and **C**, growth of target cells transduced with MUT/WT minigenes is monitored for ca. 24h (impedance measurement every 15 min) before addition of transgenic T cells to the culture and observation of cytolysis. MDST8MUT/WT cells were seeded at density 20,000/well and 40,000 TCR-T cells/well were added at time 0 (**A**). A2058MUT/WT cells were seeded at density 50,000/well and 100,000 TCR-T cells/well were added (**B** and **C**). Cell index values of target cell lines are depicted on left y axis. Percentage of target cell lysis, calculated on non-transduced T cell, is depicted on right y axis and indicated by the colored line. **D**, Comparison of cytolysis mediated by all TCRs. TCR transduction efficiency is indicated in the figure legend.

Figure S7.
Investigation of peptide-dependent and – independent HLA alloreactivity of neoantigen-specific TCR. Reactivity of all defined TCRs was tested to different LCLs expressing common HLA allotypes, pulsed with 1 µM mutated peptides SYTL4^{S363F}, KIF2C^{P13L} and NCAPG2^{P333L} (pLCL) in comparison to non-pulsed condition (LCL). All experiments were performed three times on two different healthy-donor-derived sets of transduced T cells showing consistent results. For co-cultures, E:T ratio was 1:1 and read-out consisted of IFN-γ secretion, assessed by ELISA on culture supernatants after 20h incubation

Figure S8.
Venn diagrams with distribution of TCR CDR3 sequences. **A**, Overlap of TCR CDR3 clonotypes obtained from TCR-β sequencing on B_{Lung}, M_{Int} and M_{Lung}. **B**, Overlap of TCR CDR3 clonotypes from removed metastases and associated lymph nodes. Arrows indicate shared compartments in which defined TCRs with validated neoantigen-specificity were detectable. The overlap was calculated using the Venn diagram tool from Bioinformatics & Systems Biology available online (http://bioinformatics.psb.ugent.be/webtools/ Venn/).

Figure S9.
RNA in situ hybridization of neoantigen-specific T cells within tumor tissue M_{Lung}. **A**, CDR3-specific probe visualizes T cells expressing TCR KIF2C-PBC2, which show polarization of detected RNA within single cells. **B**, anti-CD3 staining show an inhomogenous T-cell infiltrate. **C**, Negative control probe DapB (Bacillus subtillis). **D**, positive control probe Cyclophilin B.

Figure S10.
Expanded TIL from M_{Lung}^{1} were analyzed for specific responses against mutated peptide ligands. IFN-γ secretion upon stimulation with peptide-pulsed autologous LCL line and analyzed by IFN-γ ELISpot.

Figure S11.
Longitudinal monitoring of activation of TCR-transgenic T cells upon neoantigen encountering. **A-B**, percentage of T-bet positive cells within TCRmu^{+}CD8^{+} fraction of TCR-transduced T cells after 24 and 96 hours of co-culture with U-698-M expressing the mutated (**A**) or wildtype (**B**) tandem minigene. **C-D**, T-bet expression of SYLT4-TIL1-iRFP and KIF2C-PBC2-iRFP transduced T cells as shown in Fig. 6C including analysis after 96 hours after coincubation with mutated (**C**) or wildtype (**D**) transduced U-698-M.

SUPPLEMENTARY TABLES

Rank	Gene	Peptide seq (aa)	Mutation (aa)	Predicted affinity (nM)	Experimental IC_{50} (nM)
HLA-A03:01					
1	*CRMP1*	KIFNFYPRK	L > F	6.8	11.5
2	*FUT9*	KMKNFFFTK	S > F	7.4	135.7
3	*ANO7*	RMLRRRAQK	E > K	8.7	791.3
4	*ADAMDEC1*	TLYSPRGEK	E > K	9.2	416.9
5	*ASAH2*	AMYQRAKLK	S > L	9.5	27.1
6	*SLC5A3*	SLLTPPSTK	P > S	9.6	211.4
7	*CRYBB1*	RLMFFRPIK	S > F	9.8	57.6
8	*ZNF234*	SLYLKIHLK	L > K	11	--
9	*ZNF234*	KIYAAGTFY	H > Y	11.2	975
10	*DNAH5*	YLFFIQGYK	S > F	12.5	932.8
11	*TENM1*	TTYSPIGEK	G > E	14.5	81.51
12	*NCAPG2*	RLYKLILWR	P > L	14.7	707.1
13	*TGM5*	KTYPCKIFY	S > F	16.6	248.1
14	*FHDC1*	SLQPRGSFK	P > S	18.2	36.0
15	*CCDC7*	KVINLSPFK	E > K	18.6	474.3
16	*PCDHGA10*	CLFFGIPWK	S > I	19.2	2391
17	*SGK3*	KQFSAMALK	P > S	21.4	208.2
18	*KIF2C*	RLFLGLAIK	P > L	21.6	277.4
19	*AKAP6*	KLKLPIIMK	M > I	23.3	35.1
20	*FREM1*	LLINRGFSK	D > N	25.2	931.2
21	*UVSSA*	RLKCPFYGK	H > Y	26.1	249
22	*USP37*	KVMTDPSRK	A > V	28.7	16,29
23	*ARHGEF17*	RIAGKALKK	P > L	31.5	669
24	*NCAPG2*	KLILWRGLK	P > L	32.6	263.9
25	*ZFN226*	KLYQCNECK	S > L	32.7	3502

Table S1. Measured binding activity of top 25 neoantigen candidates for HLA-A03:01 predicted by NetMHC4.0.

Rank	Gene	Peptide seq (aa)	Mutation (aa)	Predicted affinity (nM)	Experimental IC_{50} (nM)
HLA-B27:05					
1	*TECTB*	R**R**FSSLYSF	G > R	11.5	48.0
2	*CLDN24*	RRLLILG**R**I	G > R	11.5	311.0
3	*KAT2A*	FRMF**L**TQGF	P > L	15	2.0
4	*TMPRSS11E*	ARWTA**F**FGV	S > F	17.1	525.0
5	*SDK2*	GRWALHSA**F**	S > F	17.5	68.0
6	*SYTL4*	GRIAF**F**LKY	S > F	18.4	224.0
7	*Ac087289.2, MRPL38, Ac087289.3*[a]	KRF**L**HRQPL	P > L	20.2	73.0
8	*B3GAT1*	A**R**FAVNLRL	G > R	20.7	8.0
9	*ITLN1*	WRNS**F**LLRY	S > F	24	29.0
10	*Af241726.2, CYBB*[a]	YR**I**YDIPPK	V > I	24	60.0
11	*KIF2C*	ARLF**L**GLAI	P > L	25.7	--
12	*P2RX1*	YRHLFKVF**R**	G > R	26.6	70.0
13	*SCN2A*	FRFFTR**K**SL	E > K	26.9	4.0
14	*RNF216*	RRHCRSY**N**R	D > N	27.6	55.0
15	*CLDN24*	KRRLLILG**R**	G > R	28.0	111.0
16	*ZFN234*	FRQSLYL**K**I	L > K	29.2	7.0
17	*NEK10*	RRTQRYFM**K**	E > K	29.3	46.0
18	*ERCC5*	FRI**C**PIFVF	R > C	32.3	877.0
19	*DSG1*	**K**RTNVGILK	E > K	33.3	136.0
20	*LIPH*	LRILR**I**KLR	M > I	35.6	109.0
21	*SI*	KR**H**EVPVPL	Y > H	36.8	907.0
22	*MBOAT1*	HRY**F**FFVAM	S > F	37.5	353.0
23	*SCN4A*	FRF**F**ATPAL	S > F	38.3	13.0
24	*INO80C*	LRFS**I**IEEF	T > I	45.9	--
25	*STON2*	SRVILF**S**PL	N > S	46.0	--

a) Overlapping genes

Table S2. Measured binding activity of top 25 neoantigen candidates for HLA-B27:05 predicted by NetMHC4.0.

Subpool A	A03:01 binders	Subpool B	B27:05 binders	Pools
A1	KIFN**F**YPRK	B1	R**R**FSSLYSF	Pool 1
	KMKNFF**F**TK		RRLLILG**R**I	
	RMLRRRAQ**K**		FRMF**L**TQGF	
	TLYSPRGE**K**		ARWTA**F**FGV	
	AMYQRAK**L**K		GRWALHSA**F**	
A2	SLLTPP**S**TK	B2	-	Pool 2
	RLM**F**FRPIK		KRF**L**HRQPL	
	SLYL**K**IHLK		A**R**FAVNLRL	
	KI**Y**AAGTFY		WRNS**F**LLRY	
	YL**F**FIQGYK		YR**I**YDIPPK	
A3	TTYSPIG**E**K	B3	ARLF**L**GLAI	Pool 3
	RLYK**L**ILWR		YRHLFKVF**R**	
	KTYPCKI**F**Y		FRFFTR**K**SL	
	SLQPRGSFK		RRHCRSY**N**R	
	KVINLSPF**K**		KRRLLILG**R**	
A4	CLFFG**I**PWK	B4	FRQSLYL**K**I	Pool 4
	KQF**S**AMALK		RRTQRYFM**K**	
	RLF**L**GLAIK		FRI**C**PIFVF	
	KLKLP**I**IMK		**K**RTNVGILK	
	LLI**N**RGFSK		LRILR**I**KLR	
A5	RLKCPF**Y**GK	B5	KR**H**EVPVPL	Pool 5
	K**V**MTDPSRK		HRY**F**FFVAM	
	RIAGKA**L**KK		FRF**F**ATPAL	
	-		LRFS**I**IEEF	
	K**L**YQCNECK		SRVILF**S**PL	
A1	KIFN**F**YPRK	B2	-	Pool 6
	KMKNFF**F**TK		KRF**L**HRQPL	
	RMLRRRAQ**K**		A**R**FAVNLRL	
	TLYSPRGE**K**		WRNS**F**LLRY	
	AMYQRAK**L**K		YR**I**YDIPPK	
A2	SLLTPP**S**TK	B3	ARLF**L**GLAI	Pool 7
	RLM**F**FRPIK		YRHLFKVF**R**	
	SLYL**K**IHLK		FRFFTR**K**SL	
	KI**Y**AAGTFY		RRHCRSY**N**R	
	YL**F**FIQGYK		KRRLLILG**R**	
A3	TTYSPIG**E**K	B4	FRQSLYL**K**I	Pool 8
	RLYK**L**ILWR		RRTQRYFM**K**	
	KTYPCKI**F**Y		FRI**C**PIFVF	
	SLQPRGSFK		**K**RTNVGILK	
	KVINLSPF**K**		LRILR**I**KLR	
A4	CLFFG**I**PWK	B5	KR**H**EVPVPL	Pool 9
	KQF**S**AMALK		HRY**F**FFVAM	
	RLF**L**GLAIK		FRF**F**ATPAL	
	KLKLP**I**IMK		LRFS**I**IEEF	
	LLI**N**RGFSK		SRVILF**S**PL	
A5	RLKCPF**Y**GK	B1	R**R**FSSLYSF	Pool 10
	K**V**MTDPSRK		RRLLILG**R**I	
	RIAGKA**L**KK		FRMF**L**TQGF	
	-		ARWTA**F**FGV	
	K**L**YQCNECK		GRWALHSA**F**	
A1	SLLTPP**S**TK	B2	GRIAF**F**LKY	Pool 11
	RLM**F**FRPIK		KRF**L**HRQPL	
	SLYL**K**IHLK		A**R**FAVNLRL	
	KI**Y**AAGTFY		WRNS**F**LLRY	
	YL**F**FIQGYK		YR**I**YDIPPK	

Table S3. Predicted peptide pool arrangement for immunogenicity assessment.

Step	Time (ps)	Restraint atoms	Restraint force constant (kcal mol-1 Å-2)	Initial temperature (K)	Target temperature (K)	Thermodynamic ensemble[d]	Barostat
1	10	all[a]	2.39	0	0	NVT	-
2	50	all[a]	2.39	2.5	5	NVT	-
3	50	all[a]	2.39	5	10	NVT	-
4	50	all[a]	2.39	10	20	NVT	-
5	50	bb[b]	2.39	25	50	NVT	-
6	100	bb[b]	2.39	50	100	NVT	-
7	100	bb[b]	2.39	100	200	NVT	-
8	100	bb[b]	0.24	100	200	NVT	-
9	200	mh-bb[c]	0.24	100	200	NVT	-
10	200	mh-bb[c]	0.24	150	300	NVT	-
11	590	-	-	150	300	NVT	-
12	1500	-	-	150	300	NPT	Berendsen

a) All MHC and peptide atoms
b) Heavy (C,CA,O,N) backbone (bb) atoms of MHC and peptide
c) Heavy backbone atoms of MHC (mh-bb)
d) NVT: constant number, volume and temperature; NPT: constant number, pressure and temperature

Table S4. Heat-up parameters used for MD simulations.

Name cell line	Alias	HLA-A	HLA-B	HLA-C
HOM2	LCL 01	03:01	27:05:00	01:02
SWEIG007	LCL 02	29:02:00	40:02:00	02:02
AMALA	LCL 03	02:17	15:01	03:03
OZB	LCL 04	02:09/03:01	35:01/38:01	04:01/12:03
RSH	LCL 05	68:02/30:01	42:01:00	17:01
KLO	LCL 06	02:08/01:01	50:01/08:01	07:01/06:02
LWAGS	LCL 07	33:01:00	14:02	08:02
-	LCL 08	02:01	07:02/15:01	30:4/12:03
BM21	LCL 09	01:01	41:01:00	17:01

Table S5. HLA-allotypes of lymphoblastoid cell lines

Table S6. Characteristics of neoantigen-specific TCRs.

Specificity	Source	TCR	TRAV	TRAJ	CDR3 alpha	TRBV	TRBJ	TRBD	CDR3 beta
SYTL4^{S363F}	TILs	SYTL4-TIL1	38-2/DV8*01	42*01	CAYRSARGSQGNLIF	7-8*01	2-5*01	2*01	CASSLGNYYQETQYF
		SYTL4-TIL2	35*02	52*01	CAAGGTSYGKLTF	27*01	2-3*01	2*01	CASSWGGAVGDTQYF
	PBMCs	SYTL4-PBC1	9-2*02	34*01	CALYTFYNTDKLIF	6-2*01	2-5*01	2*01	CASTIAASGYQETQYF
		SYTL4-PBC2	8-3*01	43*01	CAVGVLRGDMRF	12-3*01	2-3*01	1*01	CASSSRGPTDTQYF
KIF2C^{P13L}		KIF2C-PBC1	14/DV4*02F	31*01F	CAMREQNNNARLMF	7-6*01F	2-1*01F	2*01F	CASSLTRMGDRGEFF
		KIFC-PBC2	12-2*01F	6*01F	CAVKERASGGSYIPTF	10-3*02F	2-3*01F	1*01F	CAISDTSGGLWTDTQYF
NCAPG2^{P333L}		NCAPG2-PBC1	12-2*02	30*01	CPTGGDDKIIF	15*02	1-6*02	1*01	CATSRGDRPLHF

TCR	Recognition motif[a]	Number of antigens[b,c]
SYTL4-TIL1	X-R-I-A-F-F-X-X-X	6
SYTL4-TIL2	X-R-I-A-F-F-X-X-X	6
SYTL4-PBC1	X-R-I-A-F-F-X-X-X	6
SYTL4-PBC2	X-R-I-A-F-F-X-X-X	6
KIF2C-PBC1	X-X-X-L-X-L-A-I-K	60
KIF2C-PBC2	X-X-X-L-X-L-X-I-K	> 400
NCAPG2-PBC1	K-X-X-L-W-R-X-X-K	4

a) Recognition motifs are defined through T cell IFN-γ production in response to alanine/threonine scanned cognate epitopes
b) Number of human proteins containing matching recognition motif according to ScanProSite
c) Results derived from protein sequence database UniprotKB, Swiss-Prot (splice variants included)

Table S7. Number of human proteins potentially identified by neoantigen-specific TCR according to their recognition motif.

TCR	M_{Int} (%)	M_{Int}-LN1 (%)	M_{Int}-LN2 (%)	M_{Lung} (%)	M_{Lung}-LN (%)
SYTL4-TIL1	0.041	0.036	0.012	0.039	0.005
SYTL4-TIL2	0.079	0.022	0.019	0.017	0.026
SYTL4-PBC1	0.152	-	0.019	0.024	0.034
SYTL4-PBC2	0.029	0.014	-	0.020	0.021
KIF2C-PBC1	0.560	0.029	0.037	0.629	0.023
KIF2C-PBC2	0.115	0.007	0.019	0.800	0.070
NCAPG2-PBC1	0.003	0.007	-	-	-

Table S8. Productive frequency (%) of neoantigen-specific clonotypes in tumor metastases and lymph nodes detected by TCR-β sequencing.

TCR	d142 (%)	d546 (%)	d796 (%)	d945 (%)	d1120 (%)	d1519 (%)
SYTL4-TIL1	0.0018	0.0016	0.0022	0.0020	0.0008	0.0010
SYTL4-TIL2	-	-	0.0015	0.0007	0.0008	0.0010
SYTL4-PBC1	0.0053	0.0132	0.0112	0.0099	0.0115	0.0094
SYTL4-PBC2	-	0.0008	0.0015	0.0007	0.0008	-
KIF2C-PBC1	0.1489	0.1672	0.3273	0.5449	0.4715	0.2505
KIF2C-PBC2	0.0132	0.0311	0.0509	0.0365	0.0376	0.0136
NCAPG2-PBC1	0.0247	0.0156	0.0187	0.0205	0.0230	0.0167

Table S9. Productive frequency (%) of neoantigen-specific clonotypes detected by TCR-β sequencing on peripheral blood collected at different time points indicated as days (d) after start of Ipilimumab treatment.