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Immune recognition of somatic mutations leading to complete durable regression in metastatic breast cancer

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Supplementary Table 1: List of the 71 mutated peptides (62 mutations) screened for recognition by the 4136 TIL.

| gene name | Mutation type | cDNA change | AA change | Mutated peptide sequence | Tumor variant freq. (RNA) | Tumor variant freq. (exome) | variant key | exon | TMG/pp ¹ |
|-----------|---------------|-------------|-----------|-----------------------------|---------------------------|-----------------------------|----------------------------|--------|---------------------|
| LMNA | NS SNV | c.T1550G | p.V517G | ASSVTVTRSYRSGGGSGGGSGFDNL | 20 | 7 | 1:156108466-156108466 T>G | exon11 | 1 |
| TMEI53 | NS SNV | c.C100T | p.R34W | NSPSPGGKEAETWQPVVILLGWGGC | 67 | 46 | 1:45125929-45125929 G>A | exon2 | 1 |
| ZNF239 | NS SNV | c.C1077A | p.S359R | YKCGECGKGFQSRNSLHHCRIHTG | 18 | 10 | 10:44052451-44052451 G>T | exon1 | 1 |
| ZNF239 | NS SNV | c.T1071A | p.S357R | RPYKCGECGKGFQSRNSLHHCRIHTG | 25 | 21 | 10:44052457-44052457 A>T | exon1 | 1 |
| FAM21C | NS SNV | c.A1562C | p.Y521S | NKARAEEKVTLSSSKNLKPSSETKT | 100 | 86 | 10:46254776-46254776 A>C | exon17 | 1 |
| SLC3A2 | NS SNV | c.A281C | p.K94T | LLASSDPALASTNAEVTGTMSQDT | 50 | 38 | 11:62639124-62639124 A>C | exon3 | 1 |
| SLC3A2 | NS SNV | c.A188C | p.K63T | LLASSDPALASTNAEVTGTGFHH | 50 | 38 | 11:62639124-62639124 A>C | exon2 | 1 |
| SLC3A2 | NS SNV | c.A281C | p.K94T | LLASSDPALASTNAEVTGTGFHHV | 50 | 38 | 11:62639124-62639124 A>C | exon3 | 1 |
| C11orf24 | NS SNV | c.G427T | p.A143S | SAPPTAASSTTVSSIAPTTAASSMT | 15 | 20 | 11:68030036-68030036 C>A | exon4 | 1 |
| ATP2A2 | NS SNV | c.A428G | p.Y143C | VHGGSWIRGAIYCFKIAVALAVAAI | 33 | 44 | 12:110765611-110765611 A>G | exon4 | 1 |
| KMT2D | NS SNV | c.T2509C | p.S837P | SPQPPEESHLSPPQPEECLSPRPPEES | 20 | 17 | 12:49444957-49444957 A>G | exon10 | 1 |
| PABPC3 | NS SNV | c.G1271A | p.R424Q | NHAAAYPPSQIAQLRPSRWTAQGA | 100 | 11 | 13:25671607-25671607 G>A | exon1 | 1 |
| FLJ00385 | NS SNV | c.A134T | p.Y45F | VHNAKTKPREEQFNSTRVVSVLTV | 52 | 40 | 14:106236128-106236128 T>A | exon5 | 2 |
| LTKE | NS SNV | c.G1367A | p.R456H | VRCVGLSLRATPHLILMELMSGGDM | 100 | 70 | 15:41797669-41797669 C>T | exon12 | 2 |
| BC108660 | NS SNV | c.C9G | p.S3R | MARPSGSSEATGKPR | 50 | 64 | 16:52899896-52899896 C>G | exon1 | 2 |
| BC108660 | NS SNV | c.C29T | p.A10V | MASPSGSSEVTGKPRGRDGRPR | 50 | 58 | 16:52899916-52899916 C>T | exon1 | 2 |
| TUBB3 | NS SNV | c.G10A | p.V4I | MDSIRSGAFGHLFRPD | 38 | 69 | 16:89999935-89999935 G>A | exon3 | 2 |
| TUBB3 | NS SNV | c.G226A | p.V76I | ILVDLEPGTMDSIRSGAFGHLFRPD | 38 | 69 | 16:89999935-89999935 G>A | exon3 | 2 |
| LRR37A2 | NS SNV | c.A1286C | p.H429P | GTISENTNHNHPPEDASGATFNLG | 33 | 12 | 17:44626773-44626773 A>C | exon3 | 2 |
| PCTP | NS SNV | c.T7G | p.S3A | MDADYRKQWDQYVKE | 35 | 36 | 17:53844777-53844777 T>G | exon2 | 2 |
| PCTP | NS SNV | c.T223G | p.S75A | CSPTLLADIWDADYRKQWDQYVKE | 35 | 36 | 17:53844777-53844777 T>G | exon2 | 2 |
| ZNF506 | NS SNV | c.G998T | p.R333I | AFNRSSNLTKHKIIHTGDVPHYKDE | 17 | 17 | 19:19905698-19905698 C>A | exon4 | 2 |
| ZNF93 | NS SNV | c.G1139C | p.W380S | HYKCEECGKAFISSVLTNRHKRVHT | 25 | 7 | 19:20044903-20044903 G>C | exon4 | 2 |
| ZNF486 | NS SNV | c.T1100A | p.M367K | CGKAFTRSSLTKHKIIHTGEPYKTK | 20 | 7 | 19:20308619-20308619 T>A | exon4 | 2 |
| ZNF708 | NS SNV | c.A678C | p.K226N | KPYKCEECGKAFNQSSNLNTHKKIHI | 20 | 13 | 19:21476898-21476898 T>G | exon4 | 3 |
| ZNF43 | NS SNV | c.T2011A | p.C671S | HKKIHTGEQPKYSEECGKAFNYSSH | 10 | 6 | 19:21990633-21990633 A>T | exon3 | 3 |
| ZNF43 | NS SNV | c.T1814A | p.I605K | AFKWSSTLTKHKKIHTGEPYKCEE | 11 | 8 | 19:21990830-21990830 A>T | exon3 | 3 |
| ZNF43 | NS SNV | c.T1582C | p.F528L | LTTHKKIHTGELKLYKCEECGKAFQT | 20 | 13 | 19:21991062-21991062 A>G | exon3 | 3 |
| ZNF479 | NS SNV | c.A1043G | p.N348S | KPYKCEECGKAFVSSVTLQHKRIHI | 33 | 19 | 19:23406004-23406004 T>C | exon4 | 3 |
| ZNF681 | NS SNV | c.G1512T | p.Q504H | PYQCCEECGKAFNHSSHLTRHKRIHT | 67 | 12 | 19:23926633-23926633 C>A | exon3 | 3 |
| ZNF180 | NS SNV | c.G1033T | p.A345S | CGKSFVSSSHLVSHQRTHTGEKPYE | 11 | 6 | 19:44981590-44981590 C>A | exon4 | 3 |
| ZNF880 | NS SNV | c.C1216A | p.Q406K | CLTNHHRMHTGEPYKCNCEGKFR | 17 | 11 | 19:52888049-52888049 C>A | exon4 | 3 |
| ZNF880 | NS SNV | c.C1216A | p.Q406K | CLTNHHRMHTGEPYKCNCEGKAFR | 17 | 11 | 19:52888049-52888049 C>A | exon4 | 3 |
| ZNF880 | NS SNV | c.A1217G | p.Q406R | CLTNHHRMHTGERPYKCNCEGKAFR | 17 | 11 | 19:52888050-52888050 A>G | exon4 | 3 |
| ZNF880 | NS SNV | c.A1217G | p.Q406R | CLTNHHRMHTGERPYKCNCEGKAFR | 17 | 11 | 19:52888050-52888050 A>G | exon4 | 3 |
| ZNF83 | NS SNV | c.C475A | p.H159N | KPYKCNCEGKVFNNMSHLAQHRIHI | 11 | 8 | 19:53117343-53117343 G>T | exon4 | 3 |
| ZNF600 | NS SNV | c.T1615A | p.S539T | KCNCEGKSFQRTYVHCHRRRLHSGE | 20 | 11 | 19:53269394-53269394 A>T | exon1 | 4 |
| ZNF761 | NS SNV | c.T697A | p.C233S | CGKTFQSSTLTSRRLHTGEPYK | 17 | 7 | 19:53958620-53958620 T>A | exon5 | 4 |
| ZNF761 | NS SNV | c.A1361C | p.Y454S | CNCEGKTFSRKSSLTCHHRLHTGKE | 15 | 8 | 19:53959284-53959284 A>C | exon5 | 4 |
| ZNF813 | NS SNV | c.G217A | p.V73I | RPLVRRHPLHAIIDFILERNLSSVM | 33 | 9 | 19:53994523-53994523 G>A | exon1 | 4 |
| ZNF813 | NS SNV | c.G1037A | p.R346H | KTFQSSTLTSRRLHTGEPYKPKFN | 33 | 9 | 19:53994523-53994523 G>A | exon4 | 4 |
| ZNF551 | NS SNV | c.T1629A | p.S543R | YECSECGKSFQSRASLIHQHRVHTG | 25 | 9 | 19:58199356-58199356 T>A | exon3 | 4 |
| ZNF814 | NS SNV | c.T1094A | p.V365D | ECGCEGKGSFKYDYSFNHQRVHTGK | 17 | 11 | 19:58385664-58385664 A>G | exon3 | 4 |
| ZNF132 | NS SNV | c.A1384T | p.S462C | QKVTHTGERPFECCEGGRDVSQSSHL | 50 | 8 | 19:58945427-58945427 T>A | exon3 | 4 |
| WALSHP2 | NS SNV | c.A544G | p.M182V | QAGGIGAKLRVSKKELKKKQKE | 65 | 43 | 2:114355167-114355167 A>G | exon4 | 4 |
| ALMS1 | NS SNV | c.T3653C | p.L1218S | ELPHFTEAGLKSIRVPPADQKGTGI | 20 | 8 | 2:73679146-73679146 T>C | exon1 | 4 |
| ANKRD36C | NS SNV | c.C2836G | p.H946D | KDSVLNIARGKDKGKTRRVSSHKQ | 25 | 33 | 2:96557434-96557434 G>C | exon46 | 4 |
| ANKRD36C | NS SNV | c.G2828A | p.G943E | SDEKDSVNLNIAREKKHGEKTRRVSS | 14 | 31 | 2:96557442-96557442 C>T | exon46 | 4 |
| RRBP1 | NS SNV | c.T1415G | p.V472G | KKAEGAQNQGKKGGAQNQGKKAEG | 13 | 57 | 20:17639738-17639738 A>C | exon1 | 5 |
| OPA1 | NS SNV | c.C1063G | p.L355V | PETISLVNKGPGQVRMLVLDLPGVI | 67 | 36 | 3:193361192-193361192 C>G | exon11 | 5 |
| FYCO1 | NS SNV | c.A4483C | p.S1495R | HLTVDRPVIYDGRDFL | 100 | 38 | 3:45963271-45963271 T>G | exon18 | 5 |
| MST1 | NS SNV | c.G323A | p.R108H | PWVTQHSPTRLRHSGRCDLFLQKDY | 33 | 12 | 3:49725021-49725021 C>T | exon3 | 5 |
| MST1 | NS SNV | c.G55A | p.V19I | HGLNTRPTRGCGILGAVTSSRRKPK | 33 | 12 | 3:49725021-49725021 C>T | exon3 | 5 |
| MST1 | NS SNV | c.G281A | p.R94H | PWVTQHSPTRLRHSGRCDLFLQKGT | 33 | 12 | 3:49725021-49725021 C>T | exon3 | 5 |
| MST1 | NS SNV | c.A310T | p.T104S | QQLLPWTQHSPTSLRHSGRCDLFLQ | 33 | 16 | 3:49725034-49725034 T>A | exon3 | 5 |
| MST1 | NS SNV | c.C38G | p.P13R | MVANCCGHLNTRRTRGCVLGAVTS | 25 | 15 | 3:49725038-49725038 G>C | exon3 | 5 |
| NIPBL | NS SNV | c.T1794A | p.D598E | GESRPETPKQKSEGHPEPKQKGDG | 13 | 6 | 5:36985439-36985439 T>A | exon6 | 5 |
| GPBP1 | NS SNV | c.A353G | p.H118R | SSIHFAGKSGQGLRENNIPDNETGRK | 41 | 36 | 5:56527069-56527069 A>G | exon4 | 5 |
| HLA-DRB1 | NS SNV | c.A320T | p.Y107F | DILEQARAAVDTFCRHNHYGVVSEFT | 17 | 13 | 6:32551936-32551936 T>A | exon2 | 5 |
| HLA-DRB1 | NS SNV | c.T319G | p.Y107D | DILEQARAAVDTFCRHNHYGVVSEFT | 17 | 14 | 6:32551937-32551937 A>C | exon2 | 5 |
| HLA-DQA2 | NS SNV | c.A143T | p.Y48F | VNFYQSHGSPGQTFHEHDGFEFV | 57 | 19 | 6:32712996-32712996 A>T | exon2 | 6 |
| HLA-DQA2 | NS SNV | c.C208A | p.Q70K | FYVDLETETVWKLPMFSKFISFDP | 33 | 11 | 6:32713061-32713061 C>A | exon2 | 6 |
| CADPS2 | NS SNV | c.G3797A | p.R1266H | TLNSKTYDTVHRHLTVEEATSVSE | 45 | 42 | 7:121960313-121960313 C>T | exon30 | 6 |
| STK17A | NS SNV | c.G298C | p.D100H | AAKFMRKRRKGQHCRIEMIEIAVL | 17 | 38 | 7:43635591-43635591 G>C | exon2 | 6 |
| STK17A | NS SNV | c.G387C | p.E129D | DNPWWINLHEVYDTASEMILVLEYA | 50 | 36 | 7:43635680-43635680 G>C | exon2 | 6 |
| ZNF12 | NS SNV | c.C1185A | p.F395L | GEKPYECYICGLFSQMSYLTIHHR | 13 | 5 | 7:6730902-6730902 G>T | exon5 | 6 |
| CTS8 | NS SNV | c.G475C | p.D159H | GYNSYSVSNSEKIHMAEIKNGPVE | 49 | 37 | 8:11704642-11704642 C>G | exon6 | 6 |
| PHF20L1 | NS SNV | c.A260T | p.E87V | ERKWFVPSKKEVTSTCIATPDVEK | 14 | 11 | 8:133816206-133816206 A>T | exon4 | 6 |
| ZC3H3 | NS SNV | c.G2563A | p.A855T | TPSSAALTAATAVAPPCHPGGASAP | 60 | 52 | 8:14452463-14452463 C>T | exon11 | 6 |
| KIAA0368 | NS SNV | c.C565T | p.R189C | MPYGVVNLNSQSCQNSSAQAQSSSN | 47 | 20 | 9:114199363-114199363 G>A | exon7 | 6 |
| KIAA0368 | NS SNV | c.C557T | p.S186F | VLLMPYGVVNLNSQSCQNSSAQAQSS | 39 | 20 | 9:114199371-114199371 G>A | exon7 | 6 |

NS SNV: non-synonymous single nucleotide variant. ¹Tandem minigene (TMG) and peptide pool (PP) designation for each mutated peptide

Supplementary Table 2: Phenotypic characterization of the 24 TIL fragments.

| TIL fragment ¹ | F1 | F2 | F3 | F4 | F5 | F6 | F7 | F8 | F9 | F10 | F11 | F12 |
|---------------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| CD3+CD4+ (%) | 11 | 21 | 10 | 20 | 21 | 14 | 21 | 22 | 22 | 18 | 15 | 39 |
| CD3+CD8+ (%) | 11 | 19 | 42 | 5 | 22 | 13 | 10 | 13 | 13 | 11 | 13 | 19 |
| CD56+CD3- (%) | 73 | 56 | 41 | 71 | 53 | 70 | 64 | 57 | 62 | 67 | 69 | 38 |
| | | | | | | | | | | | | |
| TIL fragment | F13 | F14 | F15 | F16 | F17 | F18 | F19 | F20 | F21 | F22 | F23 | F24 |
| CD3+CD4+ (%) | 9 | 23 | 16 | 13 | 19 | 19 | 15 | 10 | 31 | 33 | 30 | 40 |
| CD3+CD8+ (%) | 8 | 7 | 13 | 7 | 10 | 9 | 17 | 14 | 10 | 17 | 11 | 10 |
| CD56+CD3- (%) | 79 | 68 | 69 | 75 | 67 | 70 | 64 | 75 | 57 | 47 | 56 | 47 |

¹ All cells were gated on lymphocytes/single/live/

Supplementary Table 3: Eight TCR clonotypes recognize neoantigens SLC3A2 and KIAA0368 in patient 4136.

| Label (TCR-) ^a | CDR3 Sequence | TRBV | CDR3 Sequence | TRAV | Neoantigen recognized |
|---------------------------|-----------------|-----------|-------------------|-----------|-----------------------|
| A | CASSASTGRNQPHF | TRBV4-1 | CVVSAAQAGTALIF | TRAV10 | SLC3A2 |
| B | CASSLGADNEQFF | TRBV7-6 | CAVRSTGTASKLTF | TRAV20 | |
| C | CASSLARRQYGYTF | TRBV7-9 | CAMSAGANTGNQFYF | TRAV12-3 | SLC3A2 |
| D | CASSSQGFYNIQYF | TRBV7-9 | CILRAPSGNTPLVF | TRAV26-2 | SLC3A2 |
| E | CASSPGSPSSYEQYF | TRBV18 | CAVTASGGSYIPTF | TRAV8-1 | |
| F | | | CVVSPSGGSYIPTF | TRAV10 | SLC3A2 |
| G | CASSIRNYSNQPHF | TRBV19 | CVPGGGGYQKVTF | TRAV2 | |
| H | | | CALSDPQIKAAGNKLTF | TRAV9-2 | SLC3A2 |
| I | CASSEPGWETQYF | TRBV25-1 | CVVNIGSDMRF | TRAV12-1 | |
| K^b | CASSLRTGQNTEAFF | TRBV19 | CAVAPSQAGTALIF | TRAV2 | SLC3A2 |
| K1^b | | | CAVIRLGAAGNKLTF | TRAV8-6 | |
| K2^c | | | CAFMKRTNRDDKIIF | TRAV38-1 | |
| L^b | CASSGGTPYNSPLHF | TRBV5-1 | CAFMKRTNRDDKIIF | TRAV38-1 | SLC3A2 |
| L1^c | | | CAVIRLGAAGNKLTF | TRAV8-6 | |
| L2^c | | | CAVAPSQAGTALIF | TRAV2 | |
| R^b | CASRPWTGANEKLF | TRBV06-05 | CAVGTSYDKVIF | TRAV21-01 | KIAA0368 |

^a A total of 16 TCR pairs were synthesized, expressed and tested for recognition of the neoantigens SLC3A2 and KIAA0368. These TCR pairs were identified by high-frequency-TRBV-based sort and 4-1BB+ enrichment (see Supplementary Fig. 1) and/or single cell sequencing of reactive (4-1BB^{hi}) T cells.

^b TCR K, K1, L and R were suggested by both high-frequency-TRBV-based sort/4-1BB+ enrichment (see Supplementary Fig. 1) and single cell sequencing approaches

^c TCR K2, L1 and L2 were suggested only by the high-frequency-TRBV-based sort/4-1BB+ enrichment approach.

Supplementary Table 4: Three TCR clonotypes recognize neoantigens CADPS2 and CTSB in patient 4136.

| Label (TCR-) ^a | CDR3 Sequence | TRBV | CDR3 Sequence | TRAV | Neoantigen recognized |
|---------------------------|-----------------|--------|-----------------|----------|-----------------------|
| J | CASSLDREDEQYF | TRBV28 | ILRDVGNYQLI | TRAV26-2 | CADPS2 |
| M | CASTPQVNYGYTF | TRBV28 | CVVFTGGGNKLTf | TRAV12-1 | CTSB |
| N | CASRGSQGENYGYTF | TRBV28 | CAVSEKGGSEKLVF | TRAV8-6 | |
| O | CASTLQANYGYTF | TRBV28 | CVVFGGNNARLMF | TRAV12-1 | CTSB |
| P | CASRPPLDNYGYTF | TRBV28 | CAMRERGTGNQFYF | TRAV14 | |
| Q | | | CAVSGEETSGSRLTF | TRAV41 | |

^a A total of 6 TCR pairs were synthesized, expressed and tested for recognition of the neoantigens CADPS2 and CTSB. These TCR pairs were suggested by single cell PCR (TCR J) or single cell sequencing of the reactive (4-1BB+) T cells

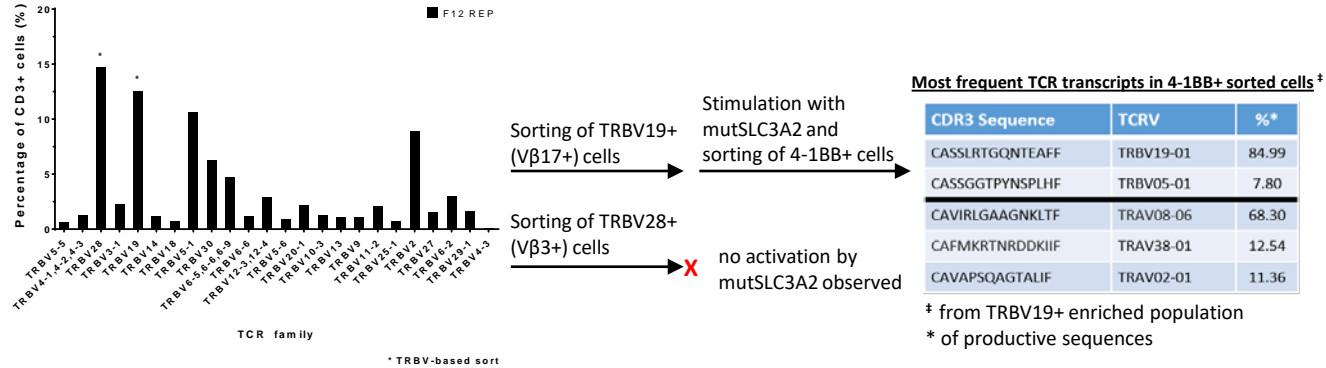
Supplementary Table 5: Shared HLA alleles between 4136 and other patients.

| # | Patient ID ^a | HLA-class I | | | HLA-class II | | |
|----|-------------------------|--------------------------|--------------------------|--------------------------|--------------------------------|--------------------------------|---------------|
| | | A*24:02:01 A*29:02:01 | B*35:01:01 B*57:01:01 | C*04:01:01 C*06:02:01 | DRB1*04:01:01 DRB1*07:01:01 | DQB1*03:02:01 DQB1*03:03:02 | DPB1*04:01:01 |
| 1 | 4136 | A*24:02:01 A*29:02:01 | B*35:01:01 B*57:01:01 | C*04:01:01 C*06:02:01 | DRB1*04:01:01 DRB1*07:01:01 | DQB1*03:02:01 DQB1*03:03:02 | DPB1*04:01:01 |
| 2 | 4035 | | | | DRB1*07:01:01 | | DPB1*04:01:01 |
| 3 | 3926 | | | C*06:02:01 | DRB1*07:01:01 | | |
| 4 | 3784 | | | | DRB1*07:01:01 | | DPB1*04:02:01 |
| 5 | 1612 | | | | DRB1*04:01 | DQB1*03:01 | not known |
| 6 | 1088 | | | | DRB1*04:01 | DQB1*03:01 | DPB1*04:01 |
| 7 | 3737 | | | | DRB1*04:05 | DQB1*03:01 | DPB1*04:01 |
| 8 | 4180 | | B*35:01 | C*04:01 | DRB1*04:01:01 | DQB1*03:01:01 | DPB1*04:02:01 |
| 9 | 4131 | A*24:02:01 | | | | | |
| 10 | 4051 | A*24 | | | | | DPB1*04:01:01 |
| 11 | 4125 | A*24 | B*57 | C*06 | | DQB1*03 | DPB1*04 |

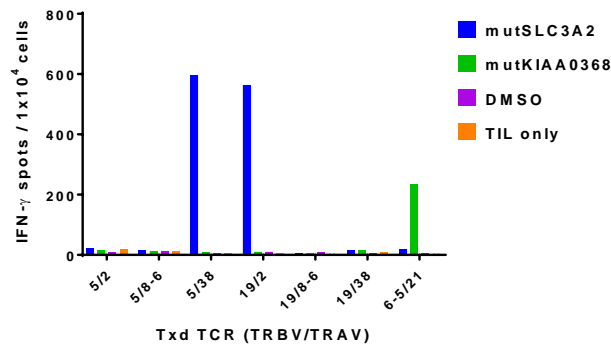
^a Antigen presenting cells (B cells) from ten allogeneic donors were used in the HLA-mismatched experiments for determination of the SLC3A2 and CTSB HLA-restriction.

Supplementary Fig. 1

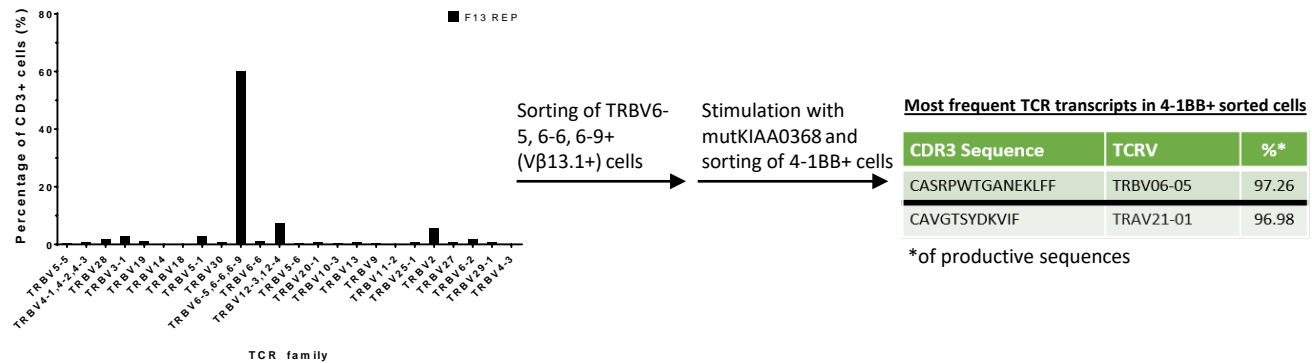
a.



b.



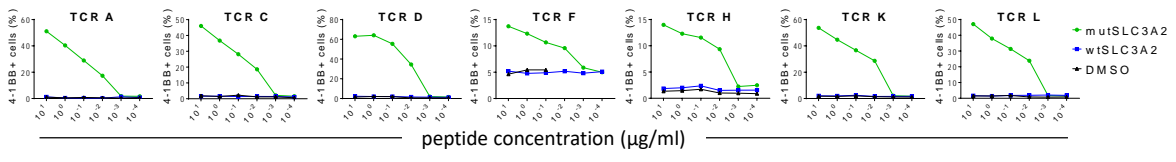
c.



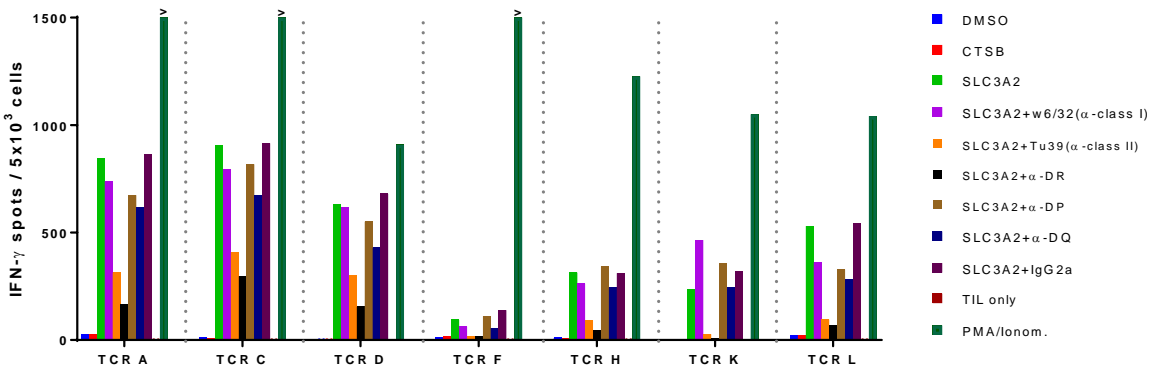
Supplementary Fig. 1: Identification of neoantigen-reactive TCR clonotypes based on FACS sort of higher frequency TRBV families and 4-1BB+ enrichment. **a.** The two highest frequency families, TRBV19 and TRBV28, were sorted by FACS from the fragment F12, following evaluation of the fragment's TCR repertoire by flow cytometry. After screening to determine which population recognizes the mutSLC3A2, TRBV19 enriched T cells were stimulated with mutSLC3A2 and 4-1BB+ cells were subjected to TCR targeted high throughput sequencing, revealing an oligoclonal population of reactive cells. **b.** A predominant TRBV6-5, -6, -9+ T cell population was sorted from the fragment F13, following evaluation of the T cell repertoire of the fragment. Following recognition of the mutKIAA0368 by the sorted cells, 4-1BB+ T cells were sorted and high throughput sequencing was performed, revealing a nearly monoclonal population of reactive cells. **c.** Synthesis, expression and screening of the candidate TCR pairs demonstrated the recognition of the mutSLC3A2 neoantigen by two TCR pairs and the KIAA0368 neoantigen by one TCR pair.

Supplementary Fig. 2

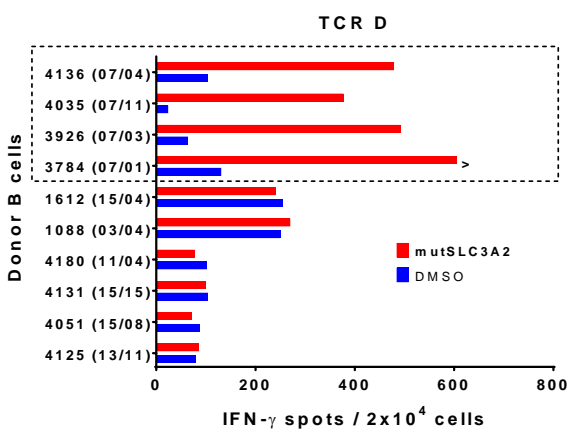
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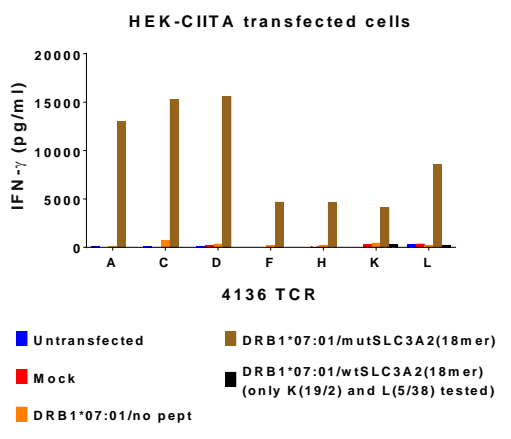
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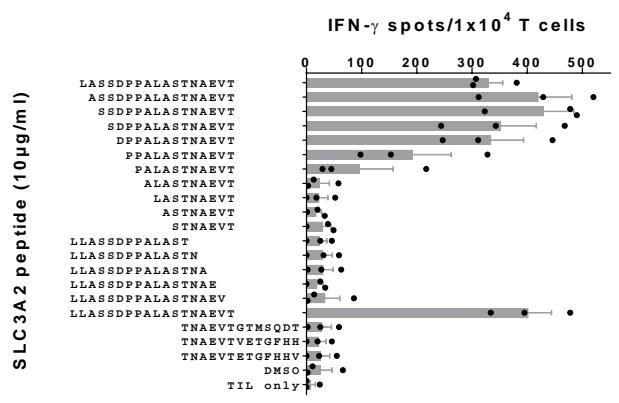
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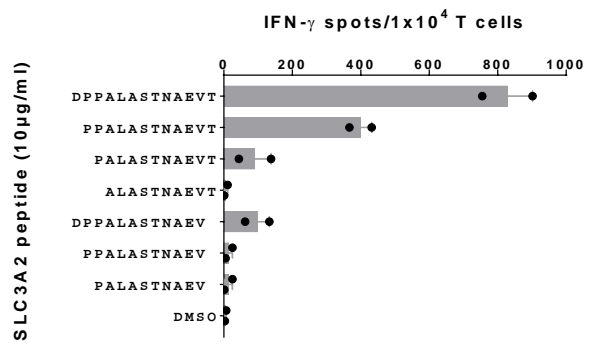
d.



e.



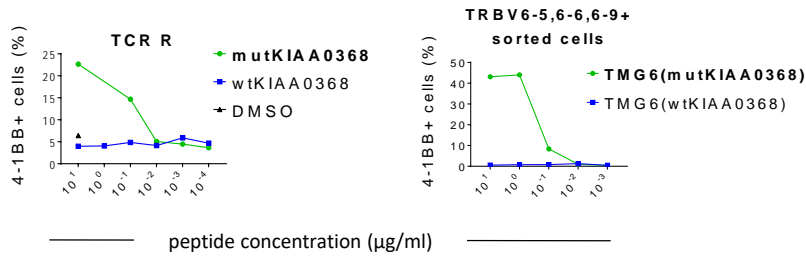
f.



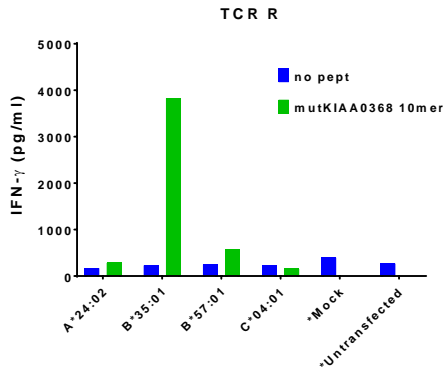
Supplementary Fig. 2: SLC3A2-reactive TCRs confer mutation-specific recognition. **a.** Titration of the mutated(mut) and wild-type(wt) SLC3A2 peptide demonstrated specific recognition of the mutSLC3A2 by the seven identified SLC3A2 reactive TCRs, with no recognition of the wtSLC3A2. All cells were gated on lymphocytes/single/live/CD3+ cells. **b.** Recognition of mutSLC3A2 by mutation-specific TCR pairs was blocked by anti-HLA-class II and anti-HLA-DR blocking antibodies. TCR transduced T cells were co-cultured overnight with APCs pulsed with mutSLC3A2 or irrelevant peptide (CTSB) in the presence or absence of the indicated HLA blocking antibodies. Positive controls for the function of each antibody were performed (data not shown). **c.** SLC3A2 reactive TCR recognizes the mutated epitope (DPPALASTNAEVT) only when presented by APCs (B cells) expressing the HLA-DRB1*07:01:01 molecule. In parentheses, the DRB1 alleles of each donor are shown (See Supplementary Table 5 for a complete list of shared HLA molecules). Data representative for one of the seven SLC3A2 reactive TCR, is shown. All seven TCRs demonstrated similar results. **d.** Transfection of HEK-CIITA cells with the HLA-DRB1*07:01:01 encoding plasmid confers presentation and recognition of the mutated SLC3A2 by the seven SLC3A2 reactive TCRs. **e.** A series of truncated peptides, derived from the 25AA-long mutated SLC3A2 peptide were screened for recognition by SLC3A2-reactive TCRs. The epitope core sequence was determined and **f.** additional screens with the candidate minimal epitopes were performed. The minimal epitope is likely contained within the core sequence DPPALASTNAEVT among all seven SLC3A2 reactive TCRs. Data shown in **e** are from three independent experiments and those in **f** are from two independent experiments. Values are means and error bars represent \pm s.e.m.. >: Too numerous to count; mutSLC3A2: LLASSDPPALASTNAEVT, wtSLC3A2: LLASSDPPALASKNAEVT.

Supplementary Fig. 3

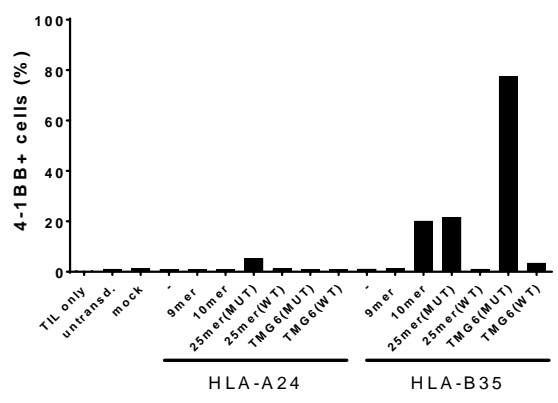
a.



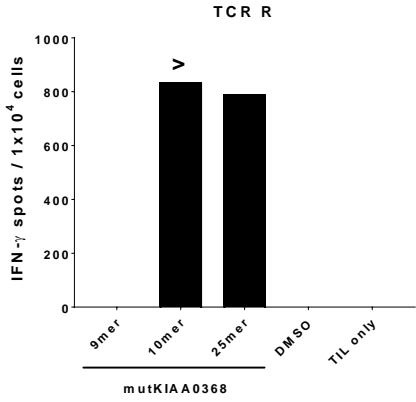
b.



c.



d.

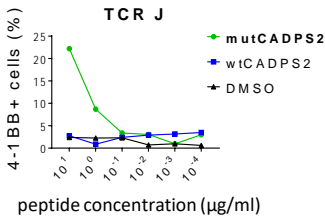


Supplementary Fig. 3: A KIAA0368-reactive TCR confers mutation-specific recognition.

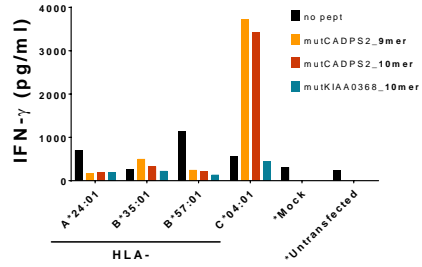
a. mutKIAA0368 peptide is specifically recognized by the KIAA0368-reactive TCR. TRBV6-5,-6,-9 sorted TIL cells demonstrate specific recognition against the TMG6 which encodes the mutated KIAA0368, but not the wild type peptide. All cells were gated on lymphocytes/single/live/CD3+ cells. **b.** Transfection of COS-7 cells with the autologous HLA-A*24:02 and HLA-B*35:01 molecules, demonstrated that the recognition of the minimal epitope, 25mer peptide or TMG6 by TRBV6-5, 6-6, 6-9+ sorted TIL is restricted by the HLA class I molecule HLA-B*35:01, as shown by upregulation of 4-1BB. **c.** High levels of IFN- γ secretion by TCR R transduced T cells was observed only when the mutated KIAA0368 epitope was presented by HLA-B*35:01 transfected COS-7 cells and not other putative autologous HLA-class I molecules. **d.** APCs pulsed with the putative HLA-B*35 epitopes PYGYVLNEF (9mer) and MPYGYVLNEF (10mer), as predicted by the epitope prediction algorithm netMHCpan 3.0, were screened. The 10mer was found to be the minimal epitope.

Supplementary Fig. 4

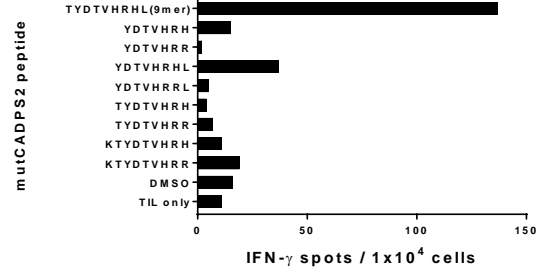
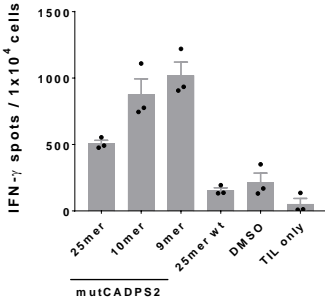
a.



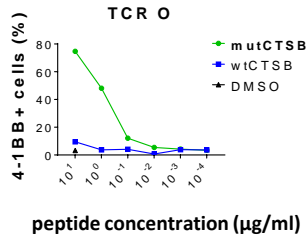
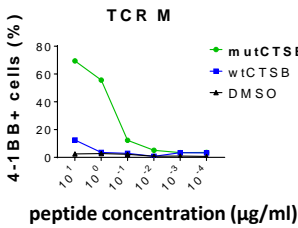
b.



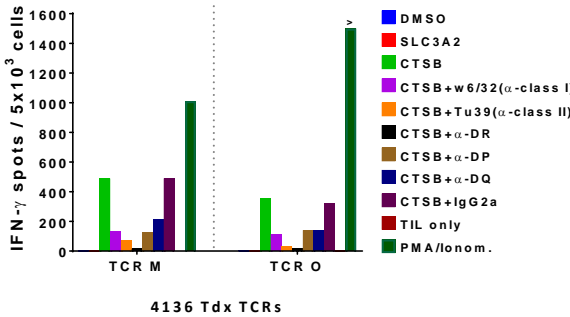
c.



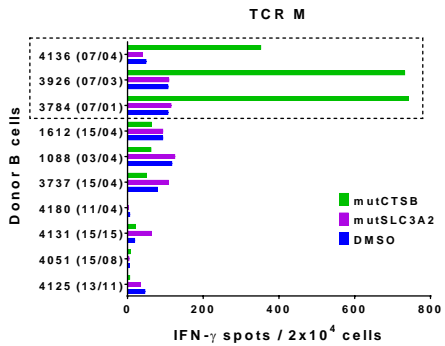
d.



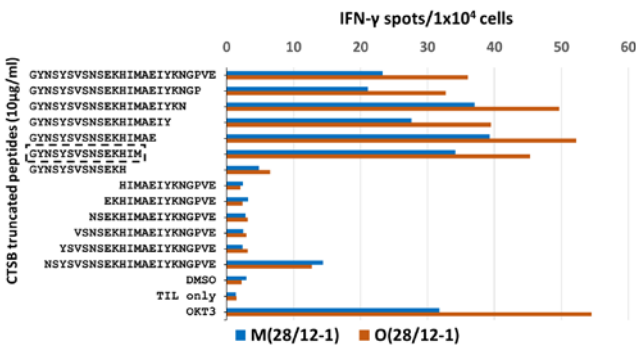
e.



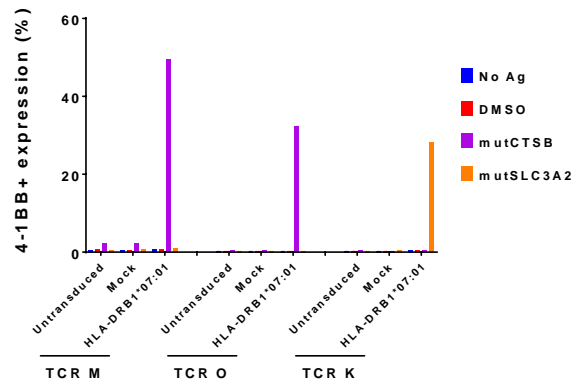
f.



g.



h.



Supplementary Fig. 4: Mutation-reactive TCRs isolated from post-treatment peripheral blood confer specific reactivity to mutated CADPS2 and mutated CTSB. **a.** Titration of the 25mer mutated CADPS2 peptide demonstrated a specific recognition of the mutated and not the wild-type peptide by the TCR J mutCADPS2-reactive TCR. **b.** Transfection of COS-7 cells with autologous HLA-class I molecules demonstrated that mutCADPS2 is recognized in a HLA-C*04:01-restricted manner. **c.** mutCADPS2-reactive T cells showed recognition of both the predicted HLA-C minimal epitopes KTYDTVHRHL(10mer) and TYDTVHRHL(9mer) (prediction algorithm netMHCpan 3.0), after peptide pulsing onto autologous APCs. Data shown are from 3 independent experiments. Values are means and error bars represent \pm s.e.m.. Further testing with the indicated shorter minimal epitopes for recognition by the CADPS2 specific TCR recapitulates the 9mer epitope TYDTVHRHL as the minimal epitope of the neoantigen CADPS2. **d.** Titration of the 25mer mutated CTSB peptide demonstrated a specific recognition of the mutCTSB by the TCR M and O. **e.** Recognition of mutCTSB by the mutation-specific TCR M and O was blocked by anti-HLA-class II and anti-HLA-DR blocking antibodies. TCR transduced T cells were co-cultured overnight with APCs pulsed with mutCTSB or irrelevant peptide (SLC3A2) in the presence or not of the indicated HLA blocking antibodies **f.** CTSB reactive TCR recognizes the mutated epitope only when presented by APCs (B cells) expressing the HLA-DRB1*07:01:01 molecule. In parentheses, the DRB1 alleles of each donor are shown. (See Supplementary Table 5 for a complete list of shared HLA molecules). **g.** Truncated peptides derived from the 25mer mutCTSB were used to determine the minimal epitope core sequence, which is contained within the 15AA long peptide GYNSYSVSNSEKHIM. **h.** Transfection of HEK-CIITA cells with the HLA-DRB1*07:01:01 encoding plasmid confers presentation and recognition of the mutated CTSB peptide by the mutCTSB-specific TCR M and O. >: Too numerous to count; mutCADPS2: TLNSKTYDTVHRHLTVEEATASVSE, wtCADPS2: TLNSKTYDTVHRRLTVEEATASVSE, mutCTSB: GYNSYSVSNSEKHIMAEIYKNGPVE, wtCTSB: GYNSYSVSNSEKDIMAEIYKNGPVE. All cells in the FACS data are gated on lymphocytes/single/live/CD3+ cells.