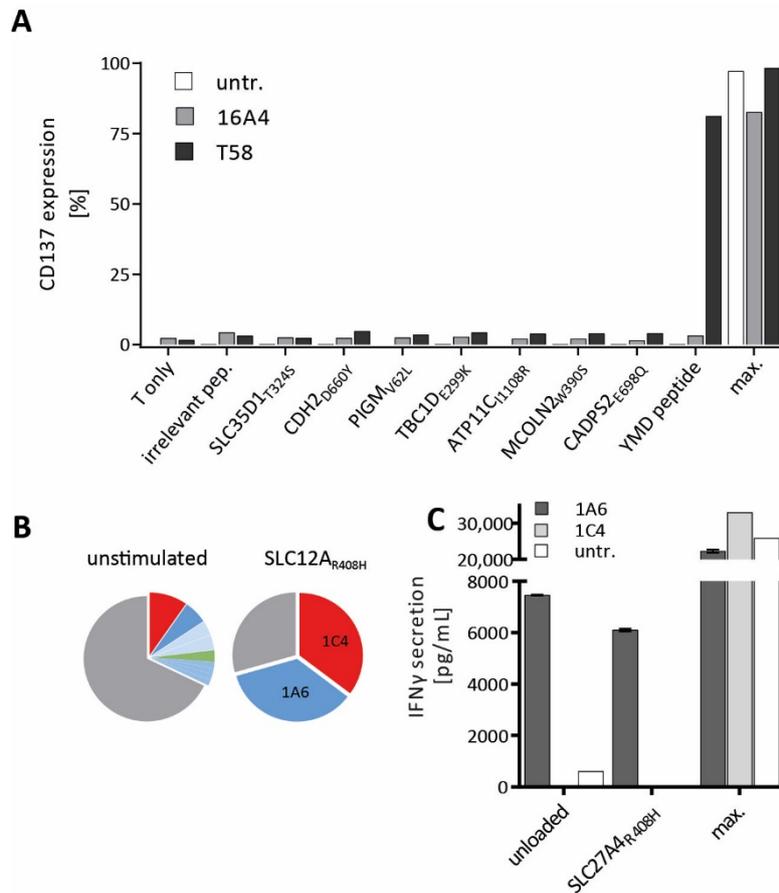
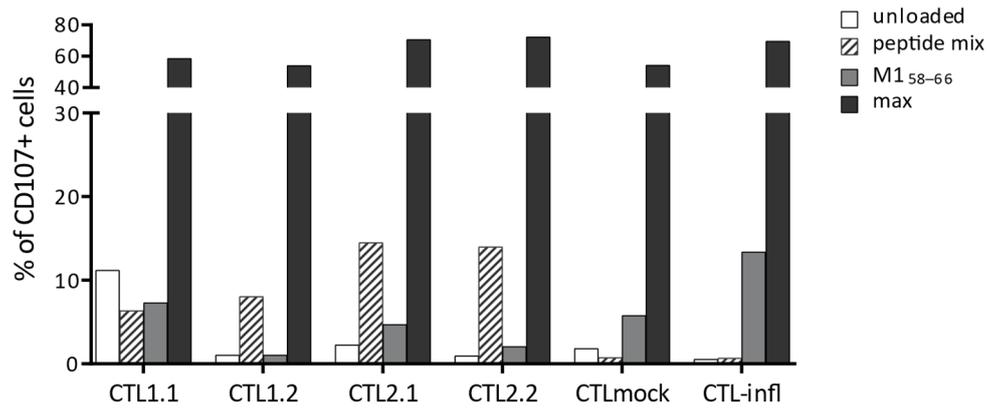


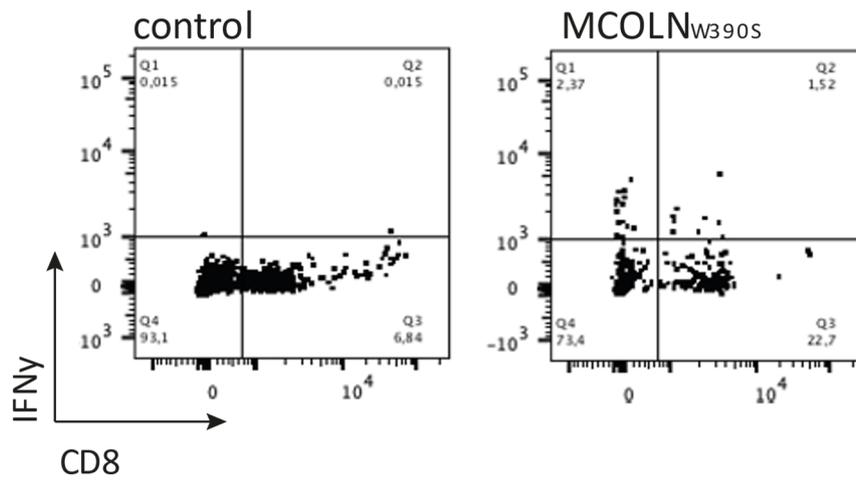
## Supplementary Figures



**Figure S1.** Test of patient derived TCRs from single-cell sorting from patients BIH146 and BIH56. **(A)** Peptide recognition assay of peptide-loaded target cells by TCR 16A4-transduced (16A4-td) PBLs identified for patient BIH146. Peptide reactivity was measured by surface expression of activation marker CD137 on 16A4-td PBLs after a 20h co-culture with peptide-loaded target cells. Activation marker expression from one donor is shown. Alive, single cells were gated on CD8<sup>+</sup>, mTCR $\beta$ <sup>+</sup>. **(B)** Single-cell sequencing of peptide-stimulated PBMCs of patient BIH56. Pie charts show frequencies of detected TCR clones of unstimulated and neoepitope candidate SLC12A<sub>R408H</sub> stimulated T cells. TCRs of 1C4 and 1A6 were identified, grey segments represent all TCR $\alpha/\beta$  rearrangements which were detected only once per sample. **(C)** TCR 1C4 and 1A6 were expressed on PBLs and tested for reactivity in a co-culture with peptide-loaded target cells. IFN $\gamma$ -release was measured after an overnight co-culture in duplicates, error bars show standard deviation. Untr.: untransduced; T58: control TCR specific for Tyrosinase peptide (YMD: YMDGTMSQV); max.: PMA/Ionomycin for maximal; unspecific activation.

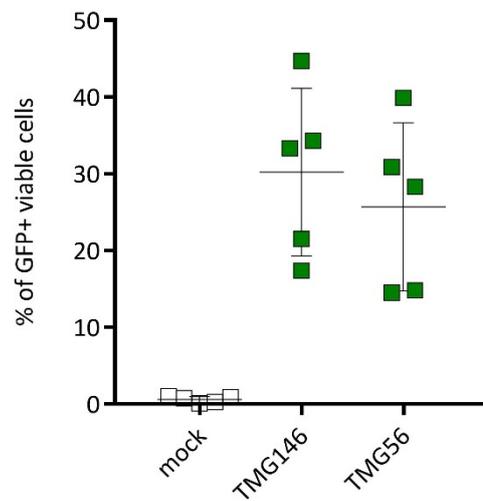


**Figure S2.** Degranulation assay of cytotoxic T cell lines (CTLs). CTLs were generated by stimulation of bulk CD8<sup>+</sup> T cells from healthy donor A with a peptide pool of three candidate neoantigen peptides (CDH2<sub>D660Y</sub>, SLC35D1<sub>T324S</sub> and PIGM<sub>V62L</sub>). Peptide reactivity was assessed by measuring expression of degranulation marker CD107 after incubation with peptide-loaded target cells by flow cytometry. The surface expression of CD107 on CTLs is shown for 4 generated CTL lines from donor A. CTLs generated against influenza peptide M1<sub>58-66</sub> were used as an assay control. CTL were gated on single, viable, CD8<sup>+</sup> cells.



0.0	0.0	2.4	1.5
93.1	6.8	73.4	22.7

**Figure S3.** Generation of neopeptide candidate specific TCRs in ABabDII mice for patient BIH146. Splenocytes of immunized ABabDII mice were cultured to expand peptide reactive CD8<sup>+</sup> cells. Expanded cells were sorted after an IFN $\gamma$  capture assay and IFN $\gamma$ <sup>+</sup> cells were sorted. Cells were gated on CD3<sup>+</sup>CD8<sup>+</sup>IFN $\gamma$ <sup>+</sup> cells. Cells cultured without peptide served as a negative control. Plots are shown for peptide SLC12A<sub>R408H</sub> stimulated splenocytes, from which TCR m875 was identified.



**Figure S4:** Nucleofection of multiple myeloma cell line U266 with TMG-constructs. U266 cells were nucleofected with neopeptide-encoding Tandem Minigenes (TMG). Nucleofection efficiency was determined 16-20 h post nucleofection by detection of eGFP expression via flow-cytometry of viable cells (n=5). Mock cells were nucleofected with PBS. Cells were immediately used as target cells in co-culture experiments. TMG146 – TMG encoding for predicted neopeptide candidates for patient BIH146; TMG56 - TMG encoding for predicted neopeptide candidates for patient BIH56.