SUPPLEMENTARY MATERIAL

T-cell responses to *TP53* "hotspot" mutations and unique neoantigens expressed by human ovarian cancers

Materials and Methods

Growth of TIL from resected metastases

One or more metastatic lesions were resected for TIL growth and sequencing of DNA and RNA. For each patient, 24 fragments from the resected metastasis were plated into one well each of 24 well plates in 2 mL complete media (RPMI-1640 media with L-glutamine (Lonza, Basel Switzerland), 10% human AB serum (NCI Surgery Branch) and penicillin/streptomycin (Lonza)) supplemented with 6000 IU/mL IL-2, consistent with previous studies (1,2). Cultures were fed every 2-3 days with complete media and IL-2 until they reached growth of at least 4 wells of the 24 well plate or 30 days had elapsed since tumor resection. Each TIL fragment culture was frozen until the time of screening. Following a positive screening result, selected TIL fragments were stimulated by REP with irradiated PBL feeder cells, 30 ng/mL OKT3 (agonistic pan-CD3 antibody) and 3000 IU/mL IL-2 in GREX-100 flasks (Wilson Wolf Corp. St. Paul, MN) as was done in other clinical trials at the NCI Surgery Branch (3,4). Leukapheresis was performed to obtain PBL for (i) normal tissue sequencing, (ii) generation of autologous APCs and (iii) rapid expansion protocols to generate TIL therapy.

Whole-exome sequencing (WES) and RNASeq library prep, next-generation sequencing and data analysis.

Genomic DNA and total RNA was purified using the QIAGEN AllPrep DNA/RNA (cat #80204) for fresh tumor and matched normal PBL samples following manufacturer's suggestions. Whole-exome library construction and exon capture of approximately 20,000 coding genes was prepared using Agilent Technologies SureSelectXT Target Enrichment System (cat# 5190-8646) for paired-end libraries coupled with Human All Exon V6 RNA bait (cat# 5190-8863) (Agilent Technologies, Santa Clara, CA, USA). WES libraries were subsequently sequenced on a NextSeq 500/550 desktop sequencer (Illumina, San Diego, CA, USA). The library was prepared using gDNA (3 µg) isolated from the fresh tumor tissue following manufacturer's protocol. Paired-end sequencing was done with an Illumina High-output flow cell kit (300 cycles) (cat# FC-404-2004) using version 2 of reagent/flow cell kit. Furthermore, RNASeq libraries were prepared using 2 µg of total RNA with the Illumina TruSeq RNA Stranded library prep kit following the manufacturer's protocol. RNA-Seq libraries were paired-end sequenced on NextSeq 500/550 desktop sequencer (Illumina, San Diego, CA, USA) again using the same mechanism described above to generate 25+ million paired-end reads.

WES Alignment, processing and variant calling

Alignments were performed using novoalign MPI from novocraft (http://www.novocraft.com/) to human genome build hg19. Duplicates were marked using Picard's (http://broadinstitute.github.io/picard/) MarkDuplicates tool. Indel realignment and base recalibration was carried out according to the GATK best practices workflow

(https://www.broadinstitute.org/gatk/). Post cleanup of data, samtools (http://samtools.sourceforge.net) was used to create pileup files and Varscan2, (http://varscan.sourceforge.net), SomaticSniper (http://gmt.genome.wustl.edu/packages/somatic-sniper/), Strelka (https://sites.google.com/site/strelkasomaticvariantcaller/) and Mutect (https://www.broadinstitute.org/gatk/). Following callers VCF files were merged using GATK CombineVariants tools and annotated using Annovar (http://annovar.openbioinformatics.org). Filtering of called variants was done by requiring tumor and normal coverage of greater than 10, a variant allele frequency of 7% or above, variant read counts of 4 or above, and two of the four callers identifying mutations for snps. For indels the same requirements needed to be met except only detection by a single caller was required.

RNA-seq alignment, processing, variant calling and FPKM

Alignments were performed using the STAR (https://github.com/alexdobin/STAR) two pass method to human genome build hg19. Duplicates were marked and sorted using Picard's MarkDuplicates tool. Reads were then split and trimmed using GATK SplitNTrim tool. After which In/Del realignment and base recalibration were performed using GATK toolbox. A pileup file was created using the final recalibrated bam file and variants were called using Varscan. FPKMs are generated from the bam files after duplicate reads have been marked. Cufflinks was used to generate FPKMs using default parameters (5). GTF used was obtained from illumine igenomes Homo_sapiens_Ensembl_GRCh37.gtf (available at ftp://igenome:G3nom3s4u@ussd-ftp.illumina.com/Homo_sapiens/Ensembl/GRCh37/Homo_sapiens_Ensembl_GRCh37.tar.gz). Cufflinks default parameters were used to generate FPKMs for all transcripts.

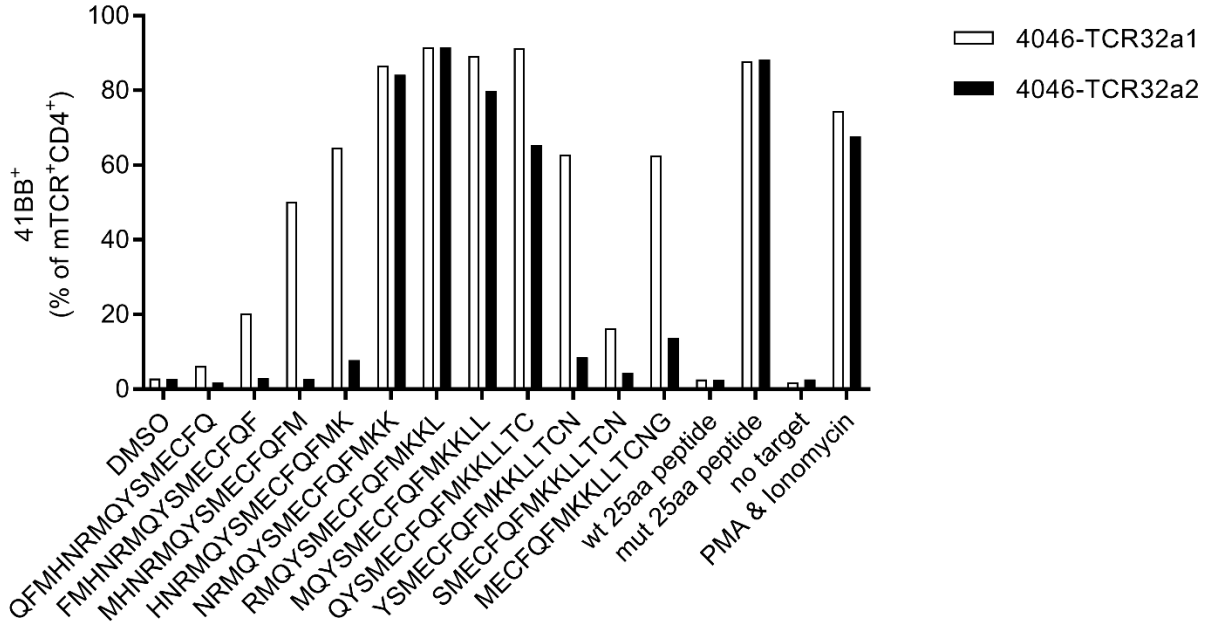

Figure S1. CD4$^+$ T cells responded to USP9X^{Y2009C} minimal epitope. T cells transduced with 4046-TCR32a1 or 4046-TCR32a2 were co-cultured with autologous APCs pulsed with DMSO or USP9X^{Y2009C} wild type (wt) or mutated (mut) LP (25 amino acids) or mutated minimal peptides (15 amino acids overlapping 14 amino acids). Media (no target) and PMA & Ionomycin were negative and positive controls, respectively. Expression of 41BB in gated CD4$^+$mTCR$^+$ T cells is displayed.

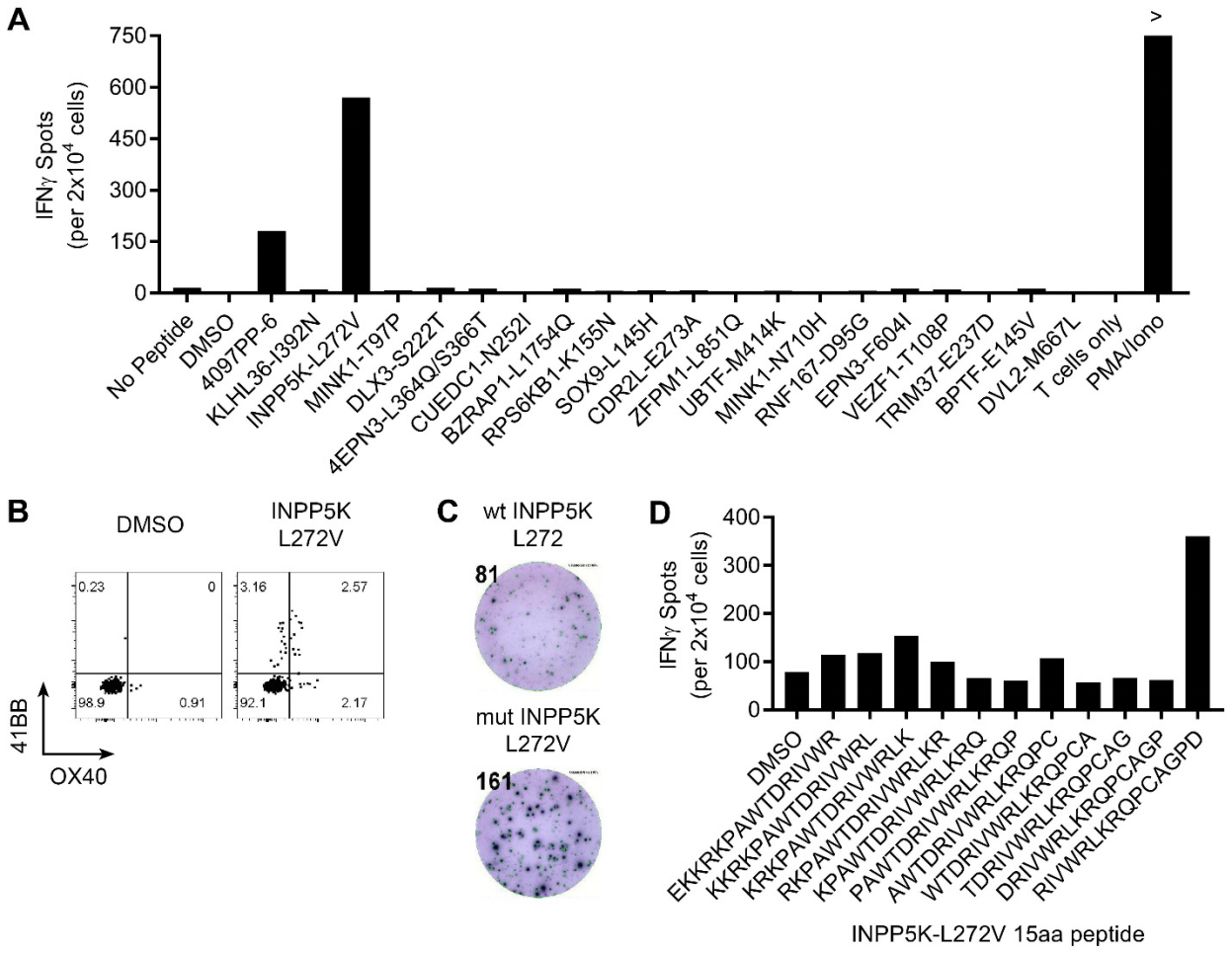

Figure S2. CD4$^+$ T cells recognized INPP5K^{L272V} neoantigen. (**A**) 4097-F23 was co-cultured with autologous APCs pulsed with DMSO, 4097-PP6 or individual LP within 4097-PP6. T cells only (media) and PMA & Ionomycin were negative and positive controls, respectively. Secretion of IFNγ was evaluated by ELISPOT. (**B**) Expression of 41BB and/or OX40 by gated CD4$^+$ T cells from 4097-F23 co-cultured with INPP5K^{L272V} LP. (**C**) Co-culture of 4097-F23 with autologous APCs pulsed with wild type (wt) or mutated (mut) INPP5K^{L272V} LP. Secretion of IFNγ was evaluated by ELISPOT. (**D**) 4097-F23 was co-cultured with autologous APCs pulsed with DMSO or 15 amino acid INPP5K^{L272V} peptides overlapping 14 amino acids. Secretion of IFNγ was evaluated by ELISPOT.

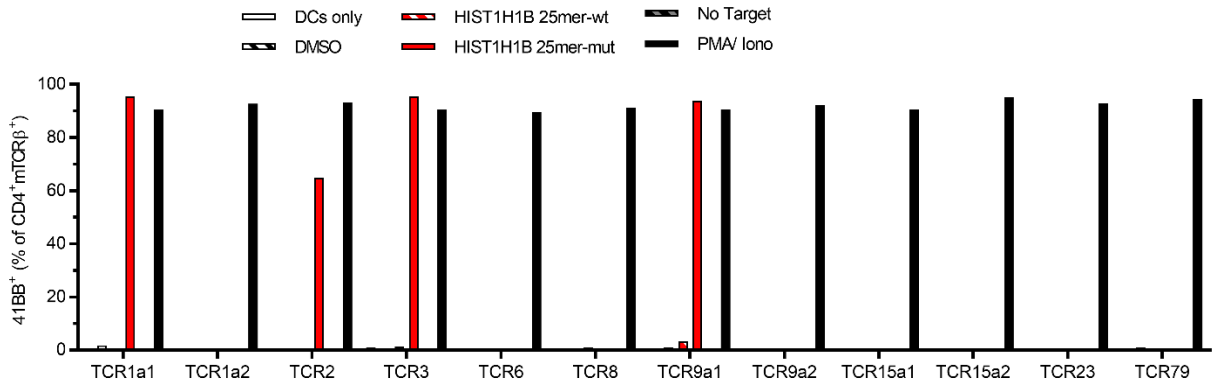

Figure S3. T cells transduced with TCRs recognize Histone H1.5^{A71D} neoantigen. Putative Histone H1.5^{A71D} reactive TCRs were transduced into T cells and co-cultured with autologous APCs pulsed with (i) nothing (DCs only), (ii) DMSO or (iii) wild type (wt) or mutated (mut) Histone H1.5^{A71D} LP. T cells only (media; no target) and PMA & Ionomycin were negative and positive controls, respectively. Expression of 41BB was evaluated in gated CD4^{+}mTCR^{+} T cells.

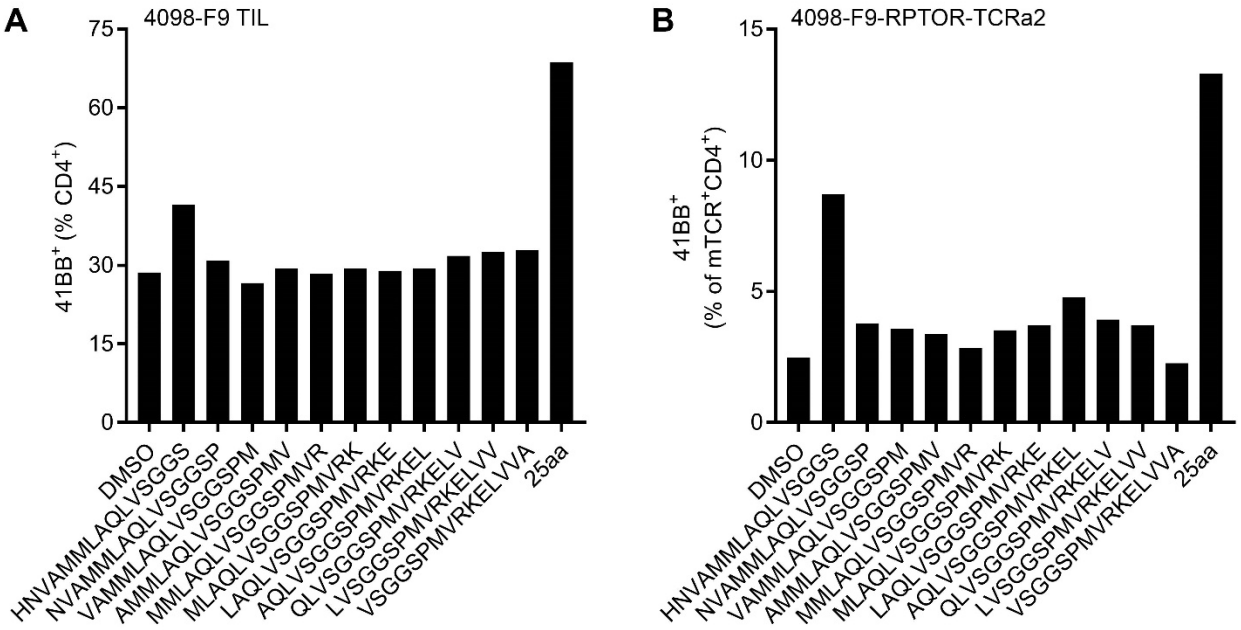

Figure S4. CD4$^+$ T cells responded to RAPTOR^{D654G} minimal neoepitope. (**A**) 4098-F9 TIL or (**B**) T cells transposed with 4046-F9-RAPTOR-TCRa2 were co-cultured with autologous APCs pulsed with DMSO or mutated RAPTOR^{D654G} LP (25 amino acids) or minimal peptides (15 amino acids overlapping 14 amino acids). Expression of 41BB in gated CD4$^+$ (4098-F9 TIL) or CD4$^+$mTCR$^+$ (4098-F9-RAPTOR-TCRa2) T cells is displayed.

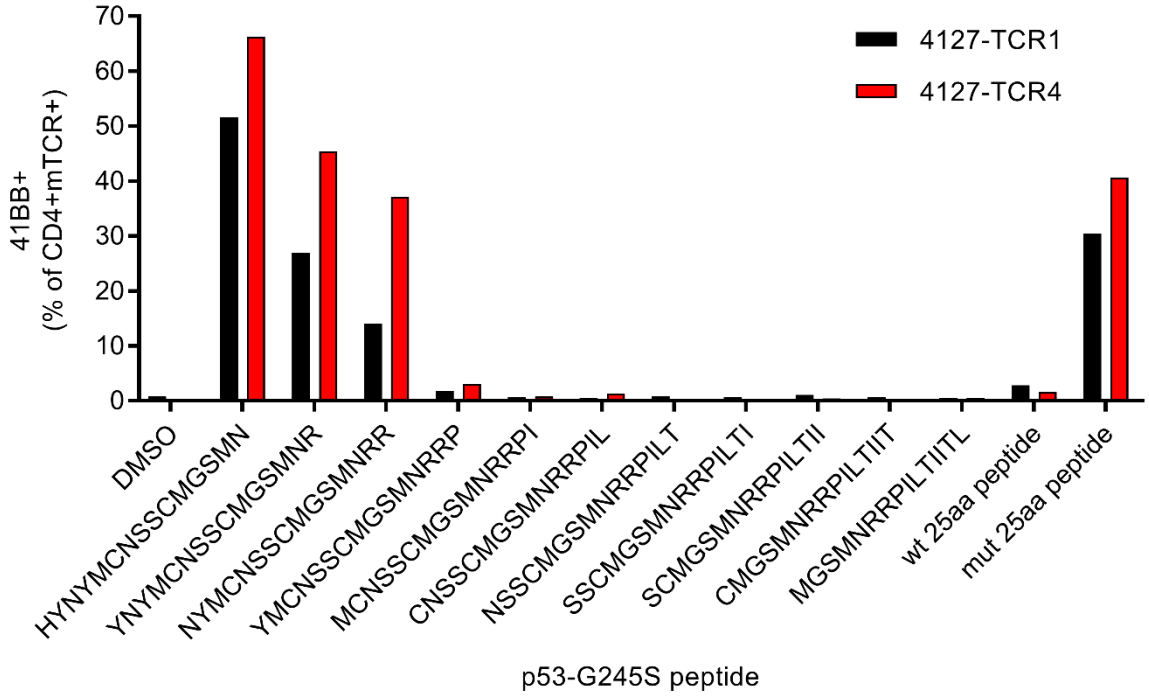

Figure S5. The p53^{G245S} minimal neoepitope was mapped to the N-terminus of the 25 amino acid neoantigen. T cells transduced with 4127-TCR1 or 4127-TCR2 were co-cultured with autologous APCs pulsed with DMSO or p53^{G245S} wild type (wt) or mutated (mut) LP (25 amino acids) or mutated minimal peptides (15 amino acids overlapping 14 amino acids). Expression of 41BB in gated CD4^{+}mTCR^{+} T cells is displayed.

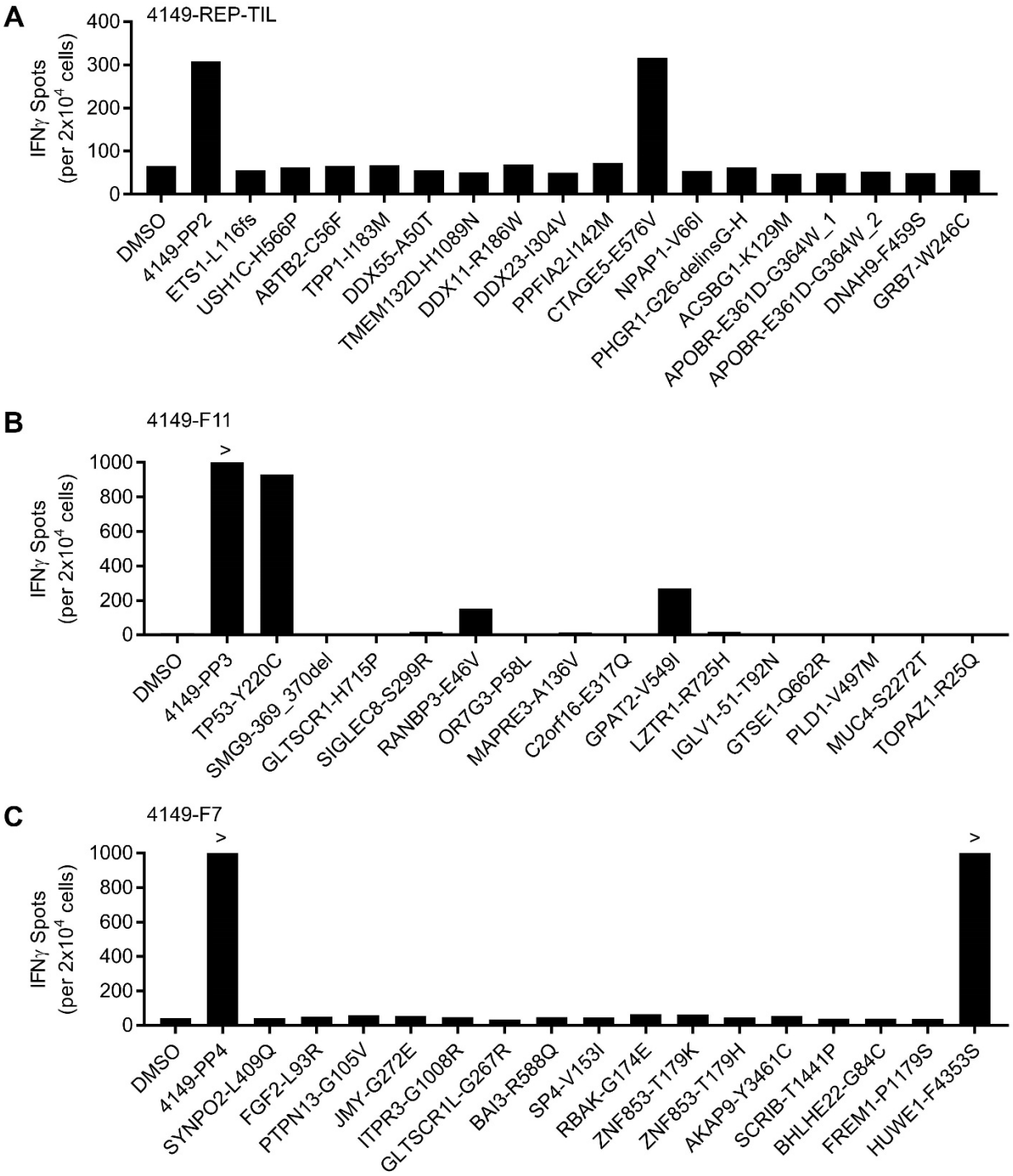

Figure S6. Peptide parsing from Patient 7 demonstrated CTAGE5^{E576V}, p53^{Y220C} and HUWE1^{F4353S} responses. T cells were co-cultured with autologous APCs pulsed with DMSO, a pool of peptides (PP) or each of the individual peptides from the PP. Secretion of IFNγ was

evaluated by ELISPOT. (**A**) 4149-REP-TIL versus 4149-PP2. (**B**) 4149-F11 versus 4149-PP3. (**C**) 4149-F7 TIL versus 4149-PP4.

References

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