SUPPLEMENTAL MATERIALS

Neoantigen screening identifies broad TP53 mutant immunogenicity in patients with epithelial cancers.

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MATERIALS AND METHODS

Cell culture

One or more metastatic lesions were resected for TIL growth as previously described (1-3). Briefly, tumor segments were plated in 24 fragments each containing 6000 IU/mL IL-2 in complete media (RPMI-1640 with L-glutamine, penicillin/streptomycin (Lonza, Basel Switzerland) and 10% human AB serum). Cultures were fed every 2-3 days with complete media and IL-2 until growth was observed in multiple wells of 24 well plate. In some cases, T cells were expanded by rapid expansion protocol (REP) using irradiated PBL feeders, 30 ng/mL OKT3 antibody and 3000 IU/mL IL-2 in 50/50 media (complete media mixed 1:1 with AIM-V media (Gibco)). Fragment cultures were sent to the research lab as a fraction of the original culture and were expanded for ~1 week before testing. In some cases, the TIL were cryopreserved, and in these instances the TIL cultures were thawed and rested with IL-2 at least one day prior to co-culture. All tumor cell lines were purchased from American Type Culture Collection (ATCC; Manassas, VA) and were grown in RPMI-1640, L-glutamine, 10% fetal bovine serum, penicillin/streptomycin and amphotericin B.

Generation of universal *TP53* "hotspot" mutation screening reagents

Each *TP53* "hotspot" mutation (R175H, Y220C, G245S, G245D, R248L, R248Q, R248W, R249S, R273C, R273H, R273L and R282W) was constructed into TMG containing genes with mutated

codon in the middle with 12 normal codons upstream and downstream (**Supplemental Figure 1**). A similar sequence was derived in the same antigen order but with the wild type sequences. Plasmids encoding the TMGs were synthesized as DNA and cloned in frame to LAMP signal sequence and DC-LAMP localization sequence and *in vitro* transcribed to mRNA using mMESSAGE mMACHINE T7 Ultra Kit according to manufacturer's instructions (Thermo-Fisher; Waltham, MA). In addition, 25 amino acid peptides containing the mutant amino acid in the middle flanked by 12 wild type amino acids for each *TP53* "hotspot" mutation along with the wild type counterpart were synthesized and purified to >95% by high-performance liquid chromatography (Genscript; Piscataway, NJ). Minimal epitope peptide and 15 amino acid peptides were crude preparations during screening and were synthesized at >95% purity for subsequent experiments. All peptides were reconstituted in DMSO.

TP53 **"hotspot" mutation screening method**

Whole-exome sequencing and RNA-seq analysis were performed on metastatic epithelial tumors in line with the clinical protocols. Following identification of one of the twelve *TP53* mutations of interest, autologous dendritic cells were produced by adherence methods using GM-CSF and IL-4 as previously described (4-6). After 4-6 days of *in vitro* culture, immature DCs were harvested in PBS, 5 mM EDTA and electroporated in OptiMEM (Gibco) with TMG mRNA (80 µg/mL) using ECM 830 square electroporator (BTX; Holliston, MA) at 150V, 10 ms and 1 pulse. Electroporated APCs were rested in DC media (RPMI-1640, L-glutamine and 5% human AB serum) containing IL-4 and GM-CSF for 18-24 hours at 37ºC. In parallel, individual p53 peptides were pulsed on autologous APCs at 10 µg/mL and incubated at 37ºC for 2-4 hours. TIL ($2x10^4$) were then co-cultured in 50/50 media in a 96 well IFNγ ELISPOT plate with autologous APCs

(8×10^4) for 18-24 hours at 37℃. Following incubation, cells were transferred to new 96 well plate for staining and flow cytometry and the ELISPOT plate was processed according to manufacturer's instructions (Mabtech; Cincinnati, OH). Immunospot (Cellular Technology Ltd.; Shaker Heights, OH) was used to quantify spots on the plates. Cells were acquired on BD FACS Canto II and FlowJo (v10) was used to analyze flow cytometry data. A list of antibodies used, antigen specificity, clone name, catalog number and vendors can be found in **Supplemental Table 8**.

Minimal peptide assays

To discern the minimal epitopes for HLA Class-I alleles, netMHC peptide binding affinity algorithm (v3.4) was used (7). Candidate 8-11 amino acid peptides were pulsed on autologous APCs for 1-2 hours prior to co-culture with T cells. To characterize the minimal determinants of CD4$^+$ T cell reactivities, 15 amino acid peptides overlapping 14 amino acids were synthesized, pulsed for 2-4 hours on autologous APCs and co-cultured with T cells. Readouts of responses were 41BB expression and IFNγ secretion by ELISPOT or ELISA.

HLA restriction mapping

COS7 tumor cell line was plated at 2.5×10^4 cells/well in RPMI-1640, L-glutamine and 10% fetal bovine serum in flat-bottom 96 well plates and incubated overnight at 37℃. Patient specific individual HLA Class-I alleles (300 ng/well) or both HLA Class-II α and β chains (150 ng/well each) plasmids were transfected with Lipofectamine 2000 according to manufacturer's instructions (Thermo Fisher) and incubated at 37℃ overnight. The following day, transfection media was removed, peptides or DMSO were pulsed for 2-4 hours, wells were washed 3 times with 50/50 media and T cells were added in 50/50 media then co-cultured overnight at 37℃. In some

instances, TMGs were co-transfected with HLA and were not pulsed with peptides. The TMGs used for these experiments were irrelevant, mutated *TP53* TMG or the mutated *TP53* TMG reverted to wild type only at the position of interest, *e.g.,* R175H changed from histidine to arginine, while the remaining *TP53* mutations were intact. Secretion of IFNγ and expression of 41BB were used to assay reactivities.

TCR identification and expression

Identification of p53 neoantigen-specific TCRs was achieved by co-culturing TIL with p53 neoepitopes, sorting 41BB expressing T cells into a single wells per cell in 96 well plates using a BD FACS Aria II, amplification of TCRs with TCRα and TCRβ gene-specific primers and reverse transcriptase PCR and Sanger sequencing, as was performed in other studies (2, 8, 9). *De novo* gene synthesis was performed to generate DNA constructs with TCRα-linker-TCRβ conformation where the linker was composed of RAKR furin cleavage site, SGSG flexible linker and P2A ribosomal slip sequence. Each human variable region was fused to the corresponding murine constant chain such that introduced TCRs could be detected with mTCRβ-specific antibody. Full-length murinized TCRs were cloned into a *Sleeping Beauty* transposon for expression in human peripheral blood T cells according to previously established protocols (10). One difference to the previous *Sleeping Beauty* TCR protocol is that mTCR$^+$ T cells were sorted with the SH800 cell sorter (Sony Biotechnology) and stimulated by REP as described above.

Generation of tumor cell lines and co-culture with T cells

Tumor fragments were taken directly after resection and were implanted into immunocompromised NSG mice (NOD.Cg-Prkdc^{scid}Il2r^{gtm1Wjl}/SzJ; The Jackson Laboratory; Bar

Harbor, ME) to grow autologous xenograft tumor cell lines. Following serial passage of the established xenograft, tumors were resected from the mice and grown *in vitro* as tissue culture. Established tumor cell lines were purchased from American Type Culture Collection (Saos2: HTB-85; SKMEL5: HTB-70; HCC2935: CRL-2869) or Deutsche Sammlung von Mikroorganismen und Zellkulturen (U698M: ACC 4). The identity of the human tumor cells in the xenografted tissue culture line was verified by morphology, human CD47 and HLA expression, and DNA sequence verification of human *TP53* mutation autologous to the patient (**Supplemental Figure 16**). For HLA blocking experiments the antibody clones W6/32 (Class-I) and IVA12 (Class-II) were incubated with tumor cells at 10 µg/mL for one hour at 37°C then added to an equal volume of T cells. In order to overexpress *TP53* variants in Saos2 cells, full-length *TP53* genes with R175H or Y220C codon changes were fused to a RAKR-SGSG-P2A linker and truncated CD19 then synthesized *de novo* and cloned into the *Sleeping Beauty* transposon for non-viral stable transgene expression. Saos2 cells were co-transfected with *TP53* transposon and SB11 transposase DNA plasmids with Lipofectamine 2000 and were selected the following day with CD19 microbeads (Miltenyi Biotec, Germany). A second CD19 microbead enrichment was performed 14 days later to create the cell lines for T cell co-culture. Tumor cell lines were harvested and co-cultured at 1:1 ratio with T cells ($2x10^5$ total cells) overnight in round-bottom 96 well plates. Co-culture supernatants were harvested to analyze IFNγ secretion and cells were stained to assess 41BB expression. Intracellular cytokine staining experiments were performed similarly, except that $2x10^4$ T cells and 10^5 tumor cells were co-cultured, GolgiSTOP and GolgiPLUG (BD Biosciences) were added to block exocytosis of cytokines, CD107a antibody was pre-incubated for 30 minutes with T cells and was maintained during the co-culture and cells were fixed and permeabilized with BD Cytofix/CytoPerm kit then stained for IL-2, IFNγ and TNFα.

Expression percentile and copy number analyses

To generate expression percentiles, FPKMs were 1st determined for all genes using Cufflinks (11) under default settings. FPKM values were normalized using python sklearn module robust scaler. For patients with multiple samples a median of normalized expression was used for each gene. All genes with the minimal normalized value were removed as these genes are unexpressed. The remaining expressed genes were used to determine percentiles and genes were assigned to the appropriate percentile. All unexpressed genes were assigned to percentile 1. WES data was used in the copy number analysis. The data analysis was performed in the R statistical environment, version 3.4.0. The segmented copy number, cellularity and ploidy were determined using Sequenza v2.1.2 with normal sample as references and hg19 coordinates.

Data Availability

Sequencing data will be made available at bioproject Accession of PRJNA342632.

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FIGURES

YKQSQHMTEVVR**H**CPHHERCSDSDGSGNLLGRNSFEV**H**VCACPGRDRRTEYMCNSSCMGGMN**L**RPILTIITLEDS

R175H   R273H   R248L

FEVRVCACPGRD**W**RTEEEENLRKKGESGNLLGRNSFEV**C**VCACPGRDRRTEHYNYMCNSSCMG**S**MNRRPILTIITL

R282W   R273C   G245S

YMCNSSCMGGMN**Q**RPILTIITLEDSHYNYMCNSSCMG**D**MNRRPILTIITLSGNLLGRNSFEV**L**VCACPGRDRRTE

R248Q   G245D   R273L

YMCNSSCMGGMN**W**RPILTIITLEDSDRNTFRHSVVVP**C**EPPEVGSDCTTIMCNSSCMGGMNR**S**PILTIITLEDSS

R248W   Y220C   R249S

Supplemental Figure 1. *TP53* **"hotspot" mutation TMG.** Amino acid sequence for mutated

TP53 tandem minigene (TMG) with mutation in bolded underline.
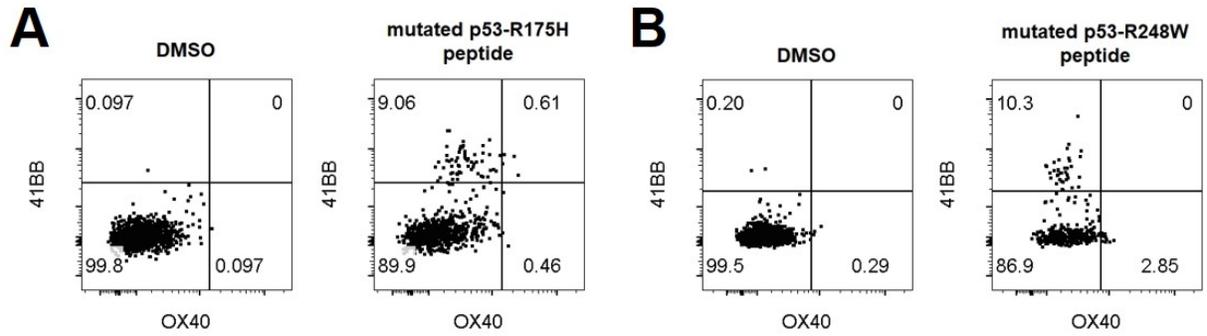
Supplemental Figure 2. Examples of 41BB and OX40 expression on CD4⁺ T cells reactive to mutated p53 neoepitopes. (**A**) Fragment culture 4285-F9 from Patient 4 versus autologous APCs pulsed with DMSO (peptide vehicle) or mutated p53^{R175H} peptide (25 amino acids). (**B**) Fragment culture 4273-F15 from Patient 10 versus autologous APCs pulsed with DMSO (peptide vehicle) or mutated p53^{R248W} peptide (25 amino acids). The gating strategy was lymphocytes → live (PI negative) → CD3+ (T cells) → CD8-CD4+.
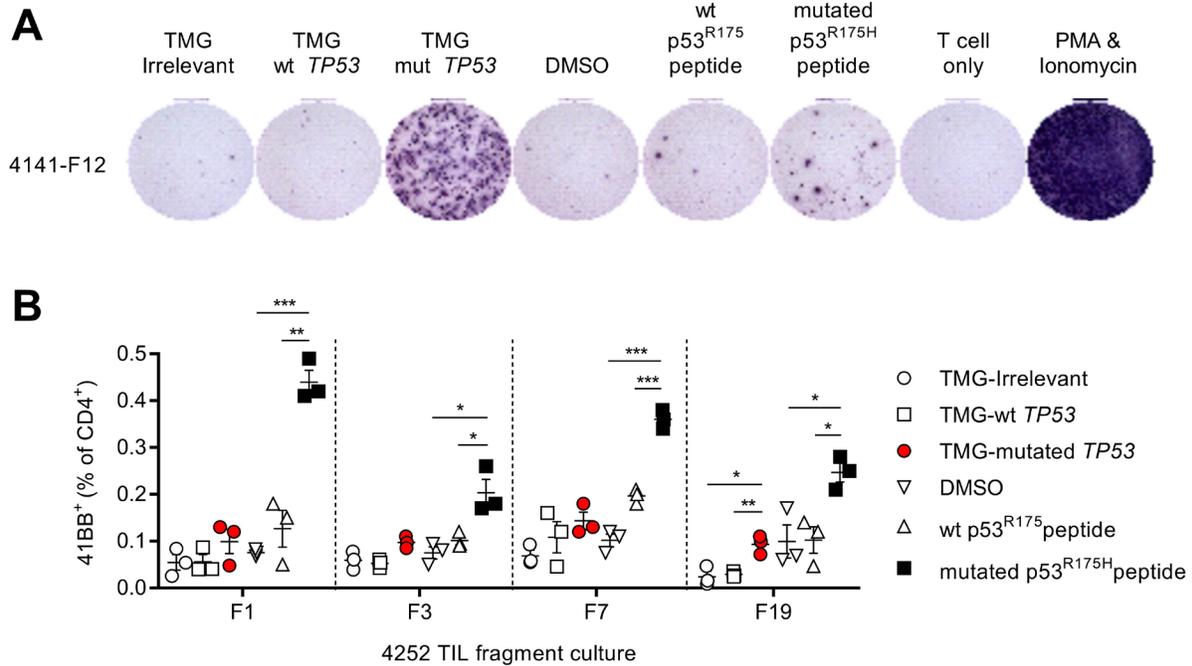
Supplemental Figure 3. T-cell responses to p53^R175H by TIL from Patients 1 and 3. (**A**) Interferon-γ secretion as measured by ELISPOT assay from co-cultures of TIL fragment culture 4141-F1 from Patient 1 with autologous antigen presenting cells either (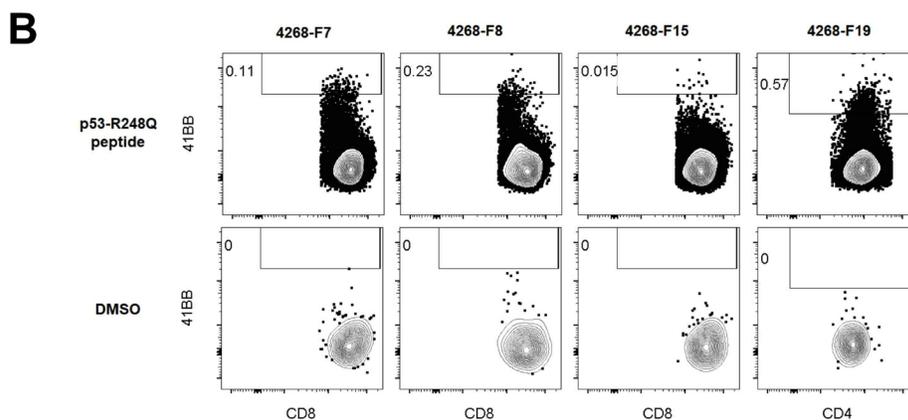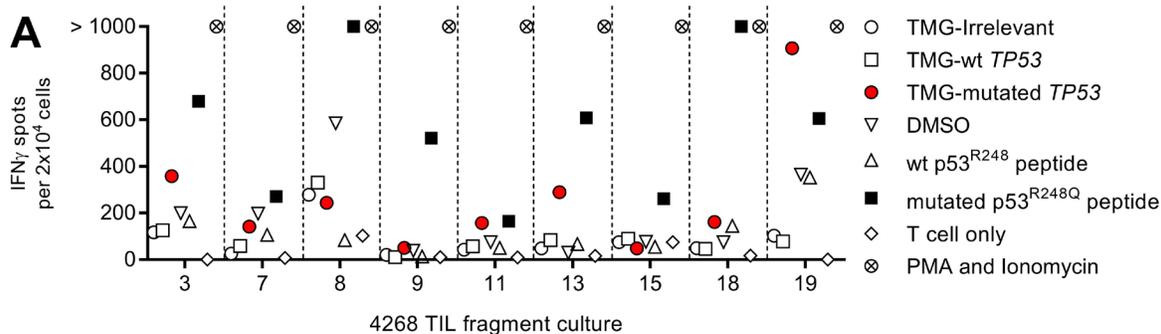1) electroporated with tandem minigenes (TMG) encoding irrelevant mutations or *TP53* wild type (wt) or mutated (mut) sequences or (2) pulsed with DMSO or 25 amino acid peptides with wild type p53^R175 mutated p53^R175H sequences at 10 μg/mL concentration. Negative and positive controls were T cells only (media) and PMA and Ionomycin, respectively. Results are representative of 2 independent experiments. (**B**) The same screening co-culture strategy as in (A) but showing expression of 41BB on CD4$^+$ T cells within selected TIL fragments from Patient 3. Data are mean ± SEM (n=3) and are representative of 2 independent experiments. Data shown are mean ± SEM (n=3). Student's t-tests were performed between indicated groups by line which were twice the value of the comparison group. *p<0.05, **p<0.01, ***p<0.001
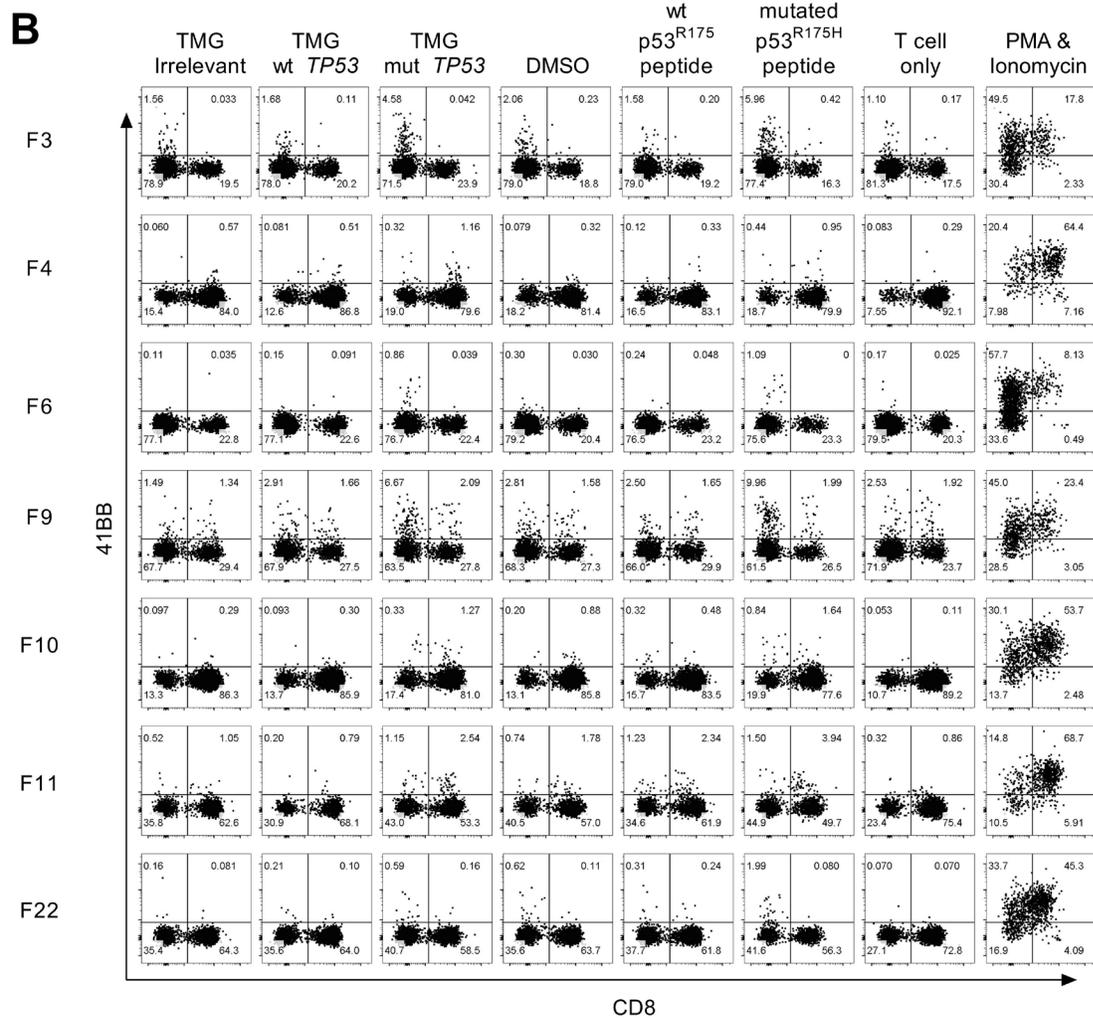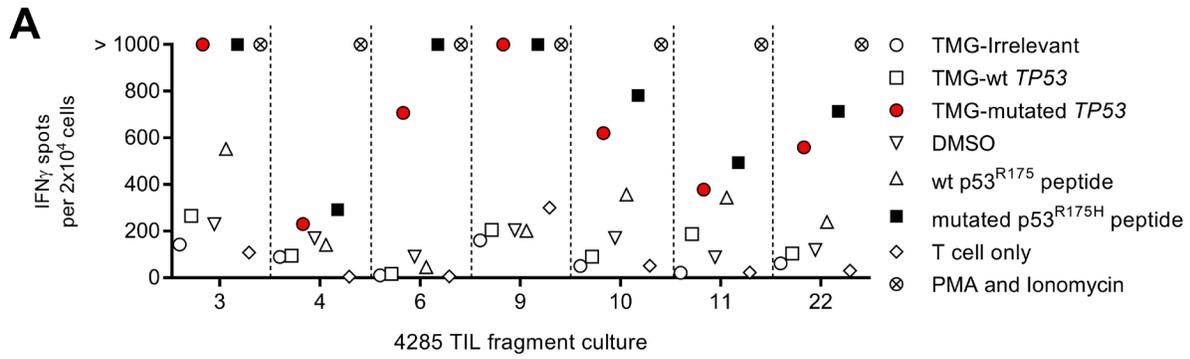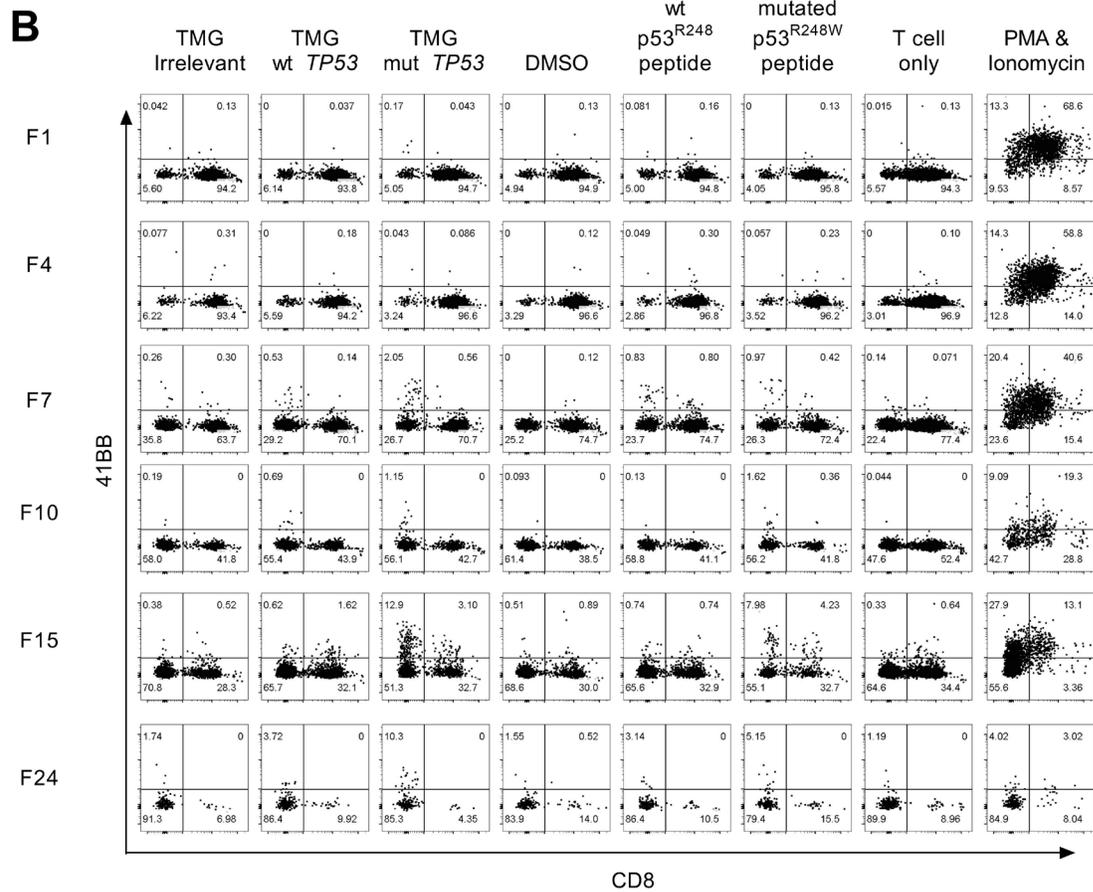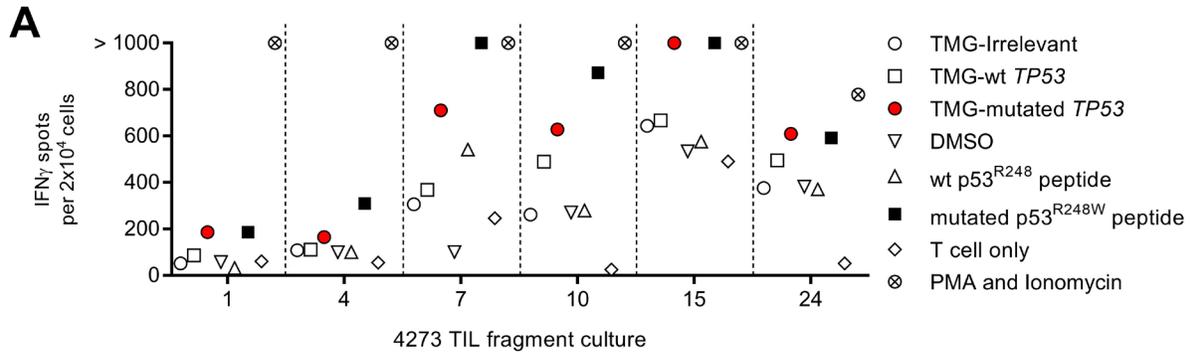
Supplemental Figure 4. T-cell responses to p53^{R175H} by TIL from Patient 4. (A) Interferon-γ secretion as measured by ELISPOT assay from co-cultures of selected TIL fragment cultures from Patient 4 with autologous antigen presenting cells either (1) electroporated with tandem minigenes

(TMG) encoding irrelevant mutations or *TP53* wild type (wt) or mutated (mut) sequences or (2) pulsed with DMSO or 25 amino acid peptides with wild type p53^{R175} mutated p53^{R175H} sequences at 10 µg/mL concentration. Negative and positive controls were T cells only (media) and PMA and Ionomycin, respectively. The assay was limited to counting less than 1000 spots per co-culture. (B) Upregulation of 41BB on selected co-cultures described in (A). Data are representative of at least 2 independent experiments.
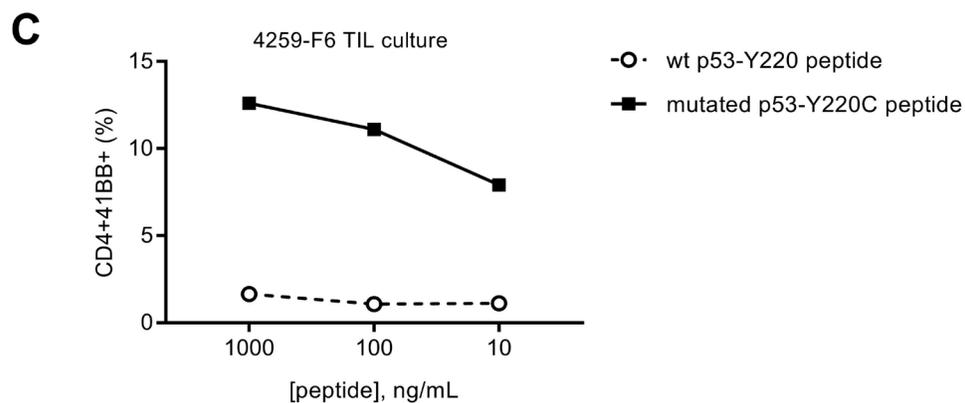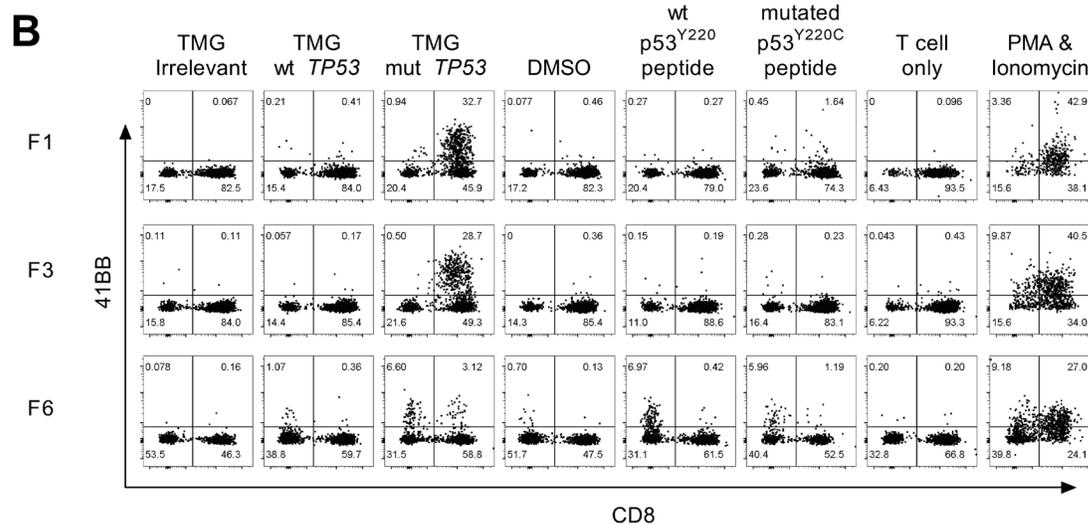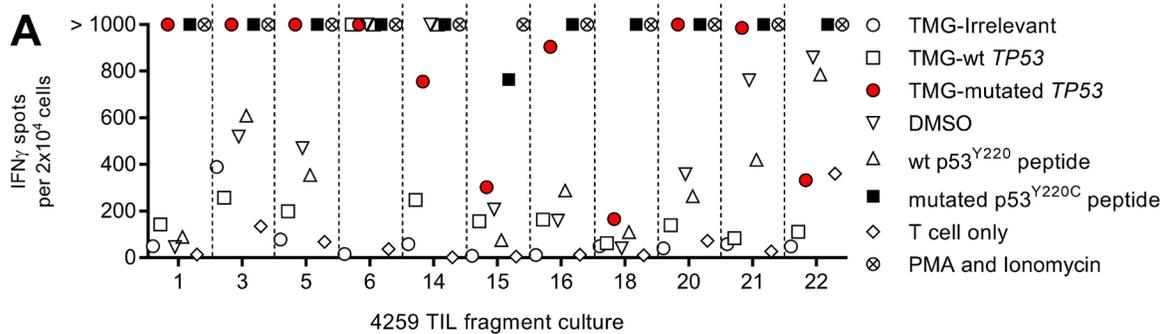
Supplemental Figure 5. T-cell responses to p53^{Y220C} by TIL from Patient 5. (A) Interferon-γ

secretion as measured by ELISPOT assay from co-cultures of selected TIL fragment cultures from

Patient 5 with autologous antigen presenting cells either (1) electroporated with tandem minigenes

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(TMG) encoding irrelevant mutations or *TP53* wild type (wt) or mutated (mut) sequences or (2) pulsed with DMSO or 25 amino acid peptides with wild type p53^{Y220} mutated p53^{Y220C} sequences at 10 µg/mL concentration. Negative and positive controls were T cells only (media) and PMA and Ionomycin, respectively. The assay was limited to counting less than 1000 spots per co-culture. (B) Upregulation of 41BB on selected co-cultures described in (A) above. (C) Serial dilutions of p53^{Y220} (wild type) or p53^{Y220C} (mutated) peptides pulsed on autologous antigen presenting cells and co-cultured with TIL fragment culture 4259-F6 from Patient 5. Data presented in (A-C) are representative of at least 2 independent experiments.
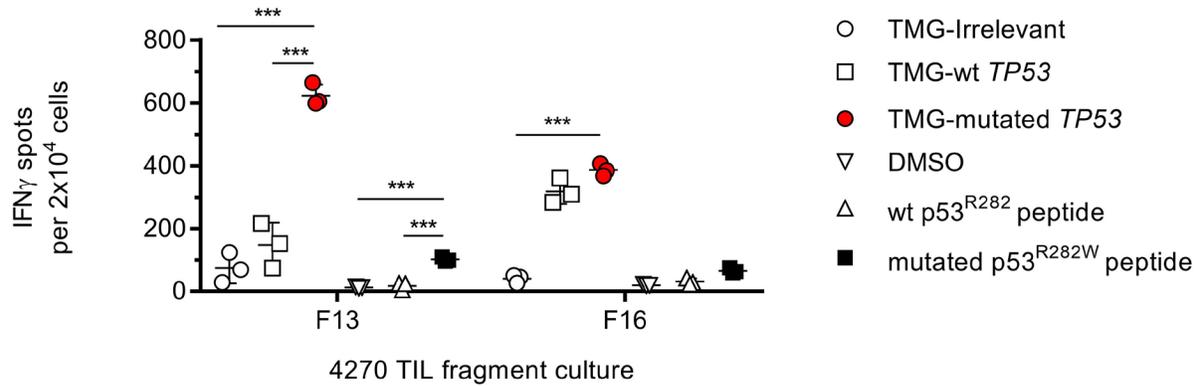
Supplemental Figure 6. T-cell responses to p53^{R248Q} by TIL from Patient 8. (A) Interferon-γ

secretion as measured by ELISPOT assay from co-cultures of selected TIL fragment cultures from

Patient 8 with autologous antigen presenting cells either (1) electroporated with tandem minigenes

(TMG) encoding irrelevant mutations or *TP53* wild type (wt) or mutated (mut) sequences or (2)

pulsed with DMSO or 25 amino acid peptides with wild type p53^{R248} mutated p53^{R248Q} sequences

at 10 μg/mL concentration. Negative and positive controls were T cells only (media) and PMA

and Ionomycin, respectively. The assay was limited to counting less than 1000 spots per co-culture.

(B) Upregulation of 41BB on gated CD8 (4268-F7, 4268-F8 and 4268-F15) or CD4 (4268-F19) T

cells following co-culture with autologous APCs pulsed with DMSO (bottom) or p53^{R248Q} peptide

(top). Data from (A) and (B) were acquired from independent experiments.

Supplemental Figure 7. T-cell responses to p53^{R248W} by TIL from Patient 10. (A) Interferon-γ secretion as measured by ELISPOT assay from co-cultures of selected TIL fragment cultures from Patient 10 with autologous antigen presenting cells either (1) electroporated with tandem minigenes (TMG) encoding irrelevant mutations or *TP53* wild type (wt) or mutated (mut) sequences or (2) pulsed with DMSO or 25 amino acid peptides with wild type p53^{R248} mutated

p53^{R248W} sequences at 10 µg/mL concentration. Negative and positive controls were T cells only (media) and PMA and Ionomycin, respectively. The assay was limited to counting less than 1000 spots per co-culture. (B) Upregulation of 41BB on selected co-cultures described in (A) above. Data presented are representative of at least 2 independent experiments.

Supplemental Figure 8. T-cell responses to p53^{R282W} by TIL from Patient 11. Interferon-γ secretion as measured by ELISPOT assay from co-cultures of TIL fragment cultures 4270-F13 and 4270-F16 from Patient 11 with autologous antigen presenting cells either (1) electroporated with tandem minigenes (TMG) encoding irrelevant mutations or *TP53* wild type (wt) or mutated (mut) sequences or (2) pulsed with DMSO or 25 amino acid peptides with wild type p53^{R282} mutated p53^{R282W} sequences at 10 µg/mL concentration. Negative and positive controls were T cells only (media) and PMA and Ionomycin, respectively. Results are representative of 2 independent experiments. Data shown are mean ± SEM (n=3). Student's t-tests were performed between indicated groups by line which were twice the value of the comparison group. ***p<0.001
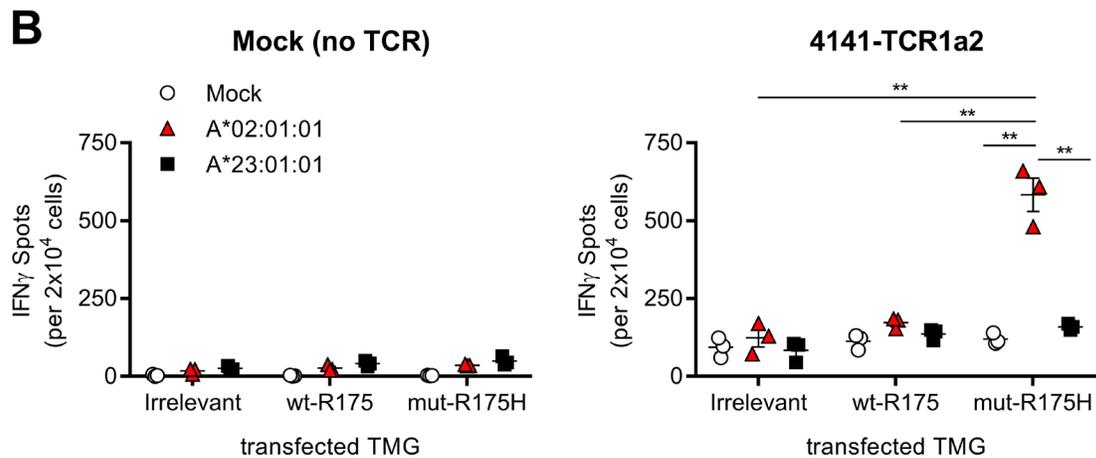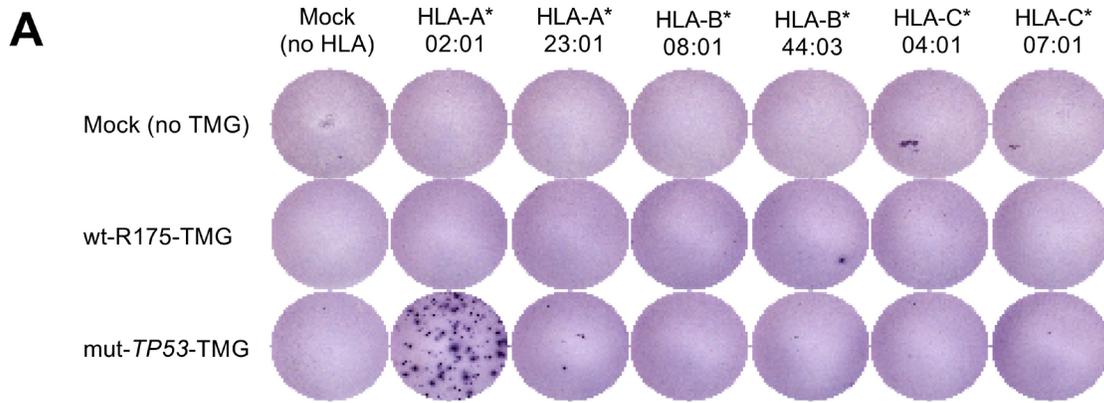
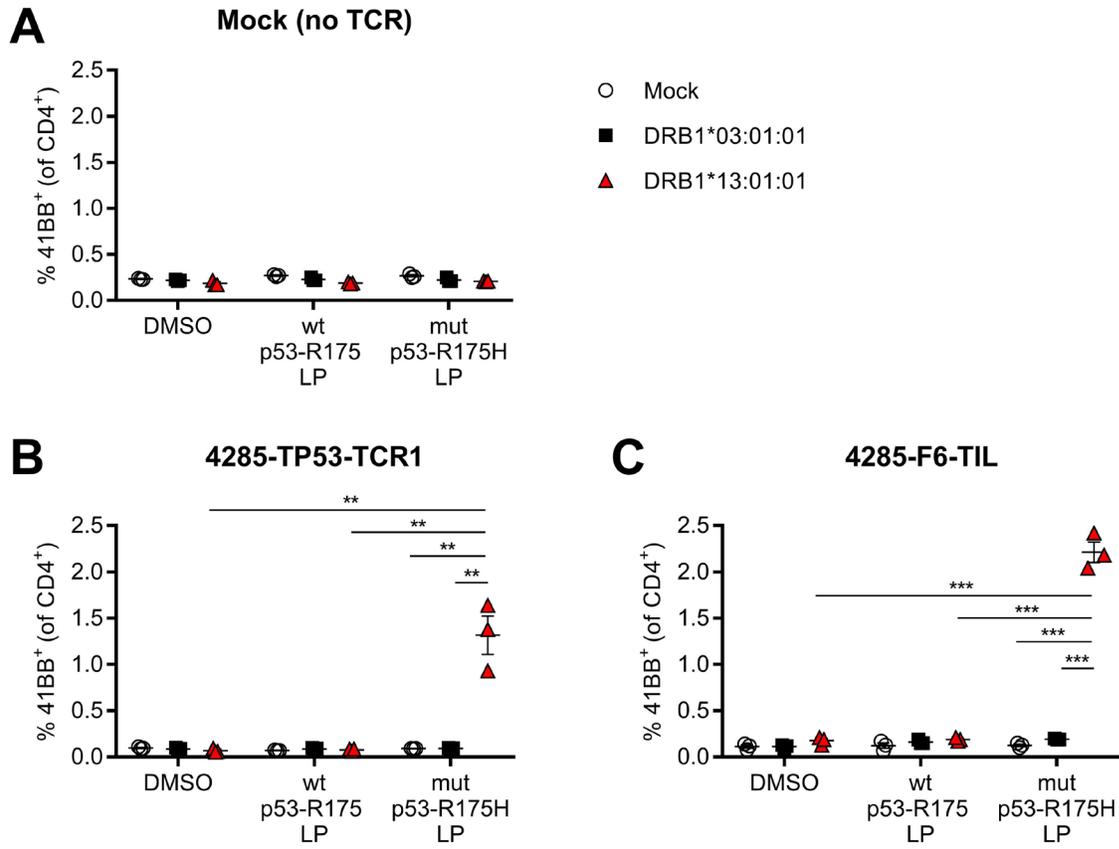**Supplemental Figure 9. Identification of HLA restriction element for p53^{R175H} neoantigen response by TIL from Patient 1.** COS7 cells transfected with DNA plasmids encoding HLA Class-I alleles from Patient 1 and either (i) nothing else (mock), (ii) mutated *TP53* TMG plasmid DNA reverted to wild type at R175 or (iii) mutated *TP53* TMG plasmid DNA. The following day, T cells were added and co-cultured overnight with transfected COS7 cells. Interferon-γ secretion was measured by ELISPOT assay. (**A**) TIL fragment culture 4141-F12 co-cultured with all HLA Class-I alleles from Patient 1. (**B**) Peripheral blood T cells transposed with nothing (mock; no TCR; left graph) or 4141-TCR1a2 (right graph) with HLA-A alleles. Data shown are mean ± SEM (n=3). Student's unpaired, 2-tailed t-tests performed for statistical analysis between HLA and TMG groups. **p<0.01
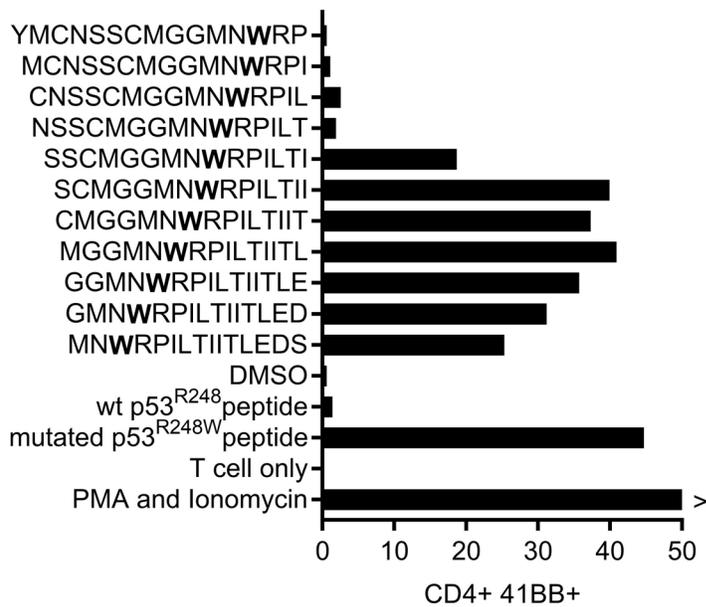
**Supplemental Figure 10. Minimal epitope determination for p53^{R248W} neoantigen in Patient 10.** Autologous antigen presenting cells were pulsed with 15 amino acid peptides overlapping 14 amino acids from the 25 amino acid p53^{R248W} neoantigen (amino acid substitution in bold). DMSO (peptide vehicle), wild type p53^{R248} peptide (25 amino acids) and T cell only (media) were negative controls. PMA and Ionomycin and mutated p53^{R248W} peptide (25 amino acids) were positive controls. Upregulation of 41BB expression by flow cytometry is shown following gating for lymphocytes → live cells (propidium iodide negative) → CD3+ (T cells).

Supplemental Figure 11. Identification of HLA Class-II restriction element for p53^{R175H} neoantigen recognized by TIL from Patient 4. COS7 cells were transfected with DNA plasmids encoding HLA-DRB1 alleles from Patient 4 and pulsed with DMSO, wild type p53^{R175} long peptide (YKQSQHMTEVVR**R**CPHHERCSDSDG) or p53^{R175H} long peptide (YKQSQHMTEVVR**H**CPHHERCSDSDG). The following day, T cell co-cultures were initiated with (**A**) mock transposed (no TCR) peripheral blood T cells, (**B**) peripheral blood T cells transposed with 4285-TP53-TCR1 or (**C**) a selected TIL fragment culture (4285-F6-TIL) from Patient 4. After overnight co-culture, 41BB upregulation was evaluated on CD4$^+$ T cells (gate: lymphocytes\live\CD3$^+$\CD4$^+$CD8$^-$). Data shown are mean ± SEM (n=3). Student's unpaired, 2-tailed t-tests performed for statistical analysis between peptide and HLA groups. **p<0.01 and ***p<0.001
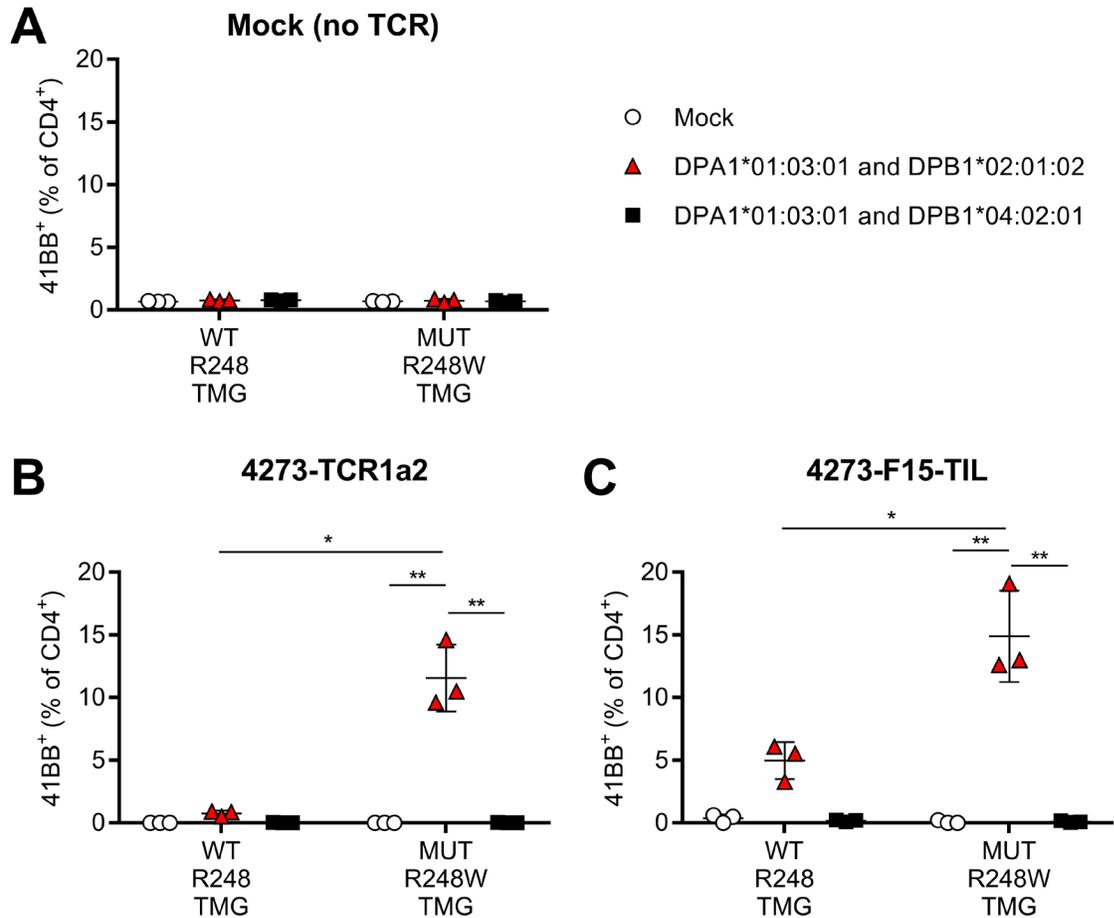
Supplemental Figure 12. Identification of HLA Class-II restriction element for p53^{R248W} neoantigen recognized by TIL from Patient 10. COS7 cells were transfected with DNA plasmids encoding HLA-DP alleles from Patient 10 and a TMG encoding wild type p53^{R248} or mutated p53^{R248W} neoepitopes. The following day, T cell co-cultures were initiated with (**A**) mock transposed (no TCR) peripheral blood T cells, (**B**) peripheral blood T cells transposed with 4273-TCR1a2 or (**C**) a selected TIL fragment culture (4273-F15-TIL) from Patient 10. After overnight co-culture, 41BB upregulation was evaluated on CD4$^+$ T cells (gate: lymphocytes\live\CD3$^+$\CD4$^+$CD8$^-$). Data shown are mean ± SEM (n=3). Student's unpaired, 2-tailed t-tests performed for statistical analysis between peptide and HLA groups. *p<0.05 and **p<0.01
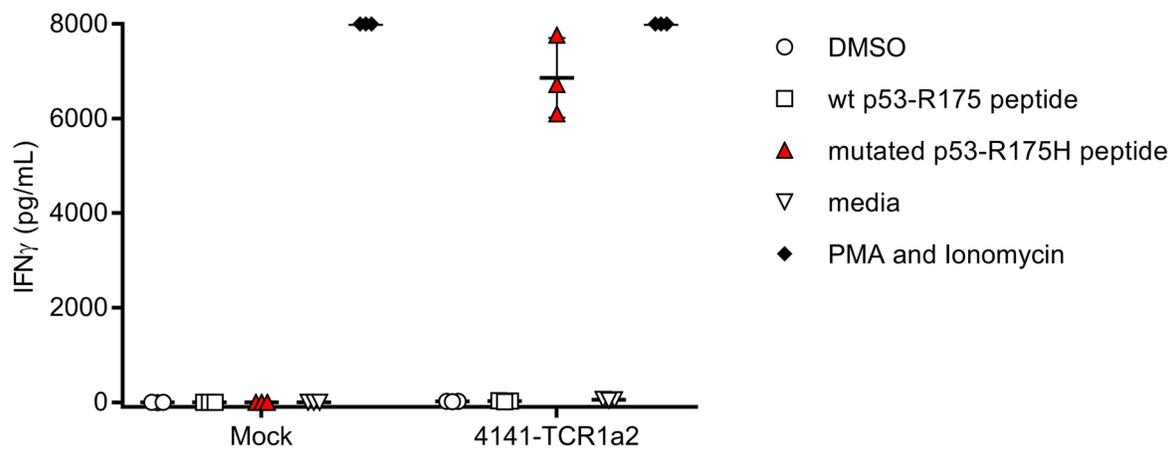
Supplemental Figure 13. The specificity of TIL towards HLA-A*02:01/p53^{R175H} from Patient 1 was mediated by 4141-TCR1a2. T2 cells, which express endogenous HLA-A*02:01, were pulsed with DMSO, wild type p53^{R175} peptide (wt; HMTEVVR**R**C) or mutated p53^{R175H} peptide (HMTEVVR**H**C). Peripheral blood T cells expressing mock (no TCR) or 4141-TCR1a2 were co-cultured with pulsed T2 cells. Negative and positive controls were T cells only (media) and PMA and Ionomycin, respectively. Secretion of interferon-γ (IFNγ) into co-culture supernatants was measured by ELISA. Data shown are mean ± SEM (n=3).
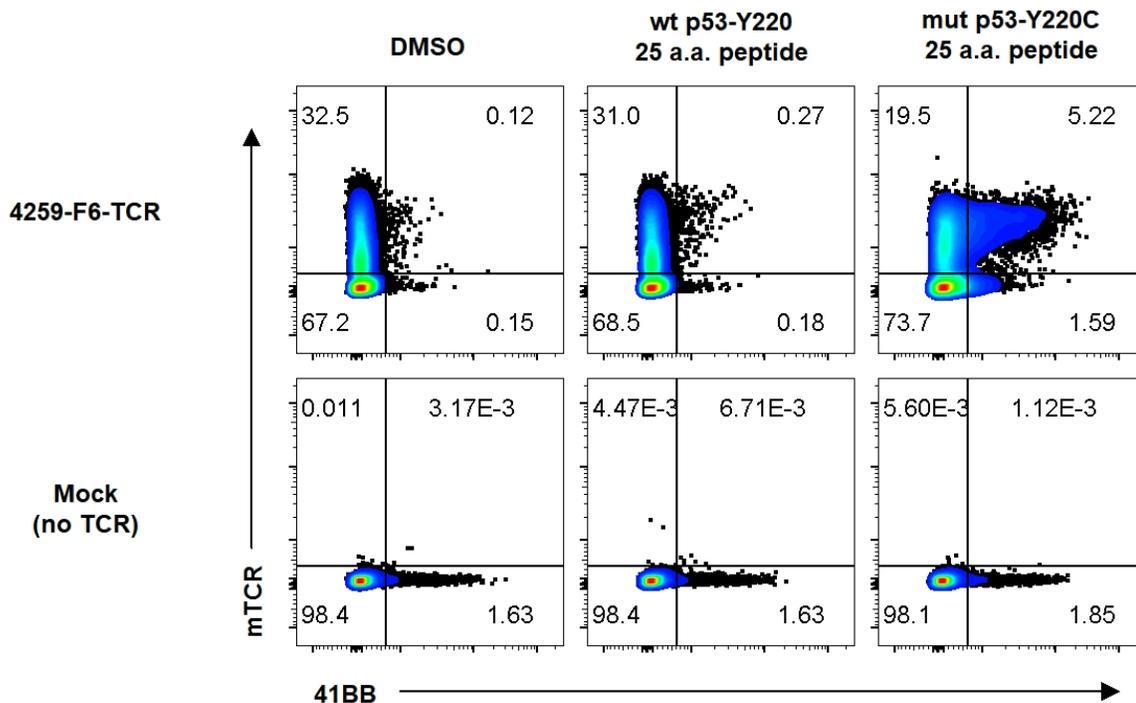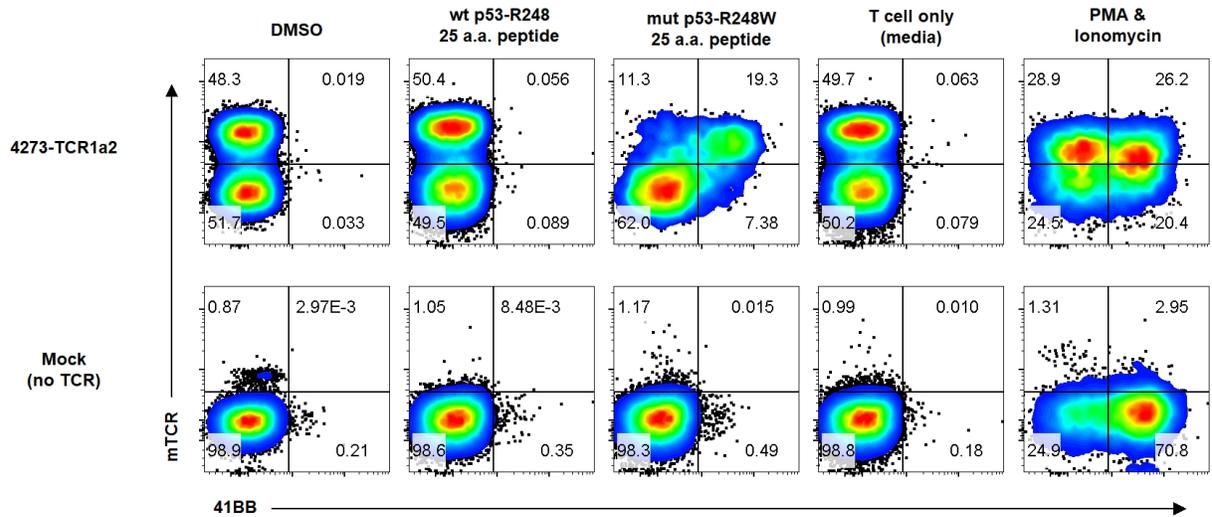
Supplemental Figure 14. The specificity of TIL towards p53^{Y220C} from Patient 5 was mediated by 4259-F6-TCR. Autologous antigen presenting cells from Patient 5 were pulsed with DMSO, wild type p53^{Y220} or mutated p53^{Y220C} peptides then co-cultured overnight at 37°C with peripheral blood T cells genetically modified with nothing (mock; no introduced TCR; bottom plots) or 4259-F6-TCR (top plots). Upregulation of 41BB expression on T cells expressing introduced TCRs (as measured by mTCR expression) by flow cytometry is shown following gating for lymphocytes → live cells (propidium iodide negative) → CD3+ (T cells).
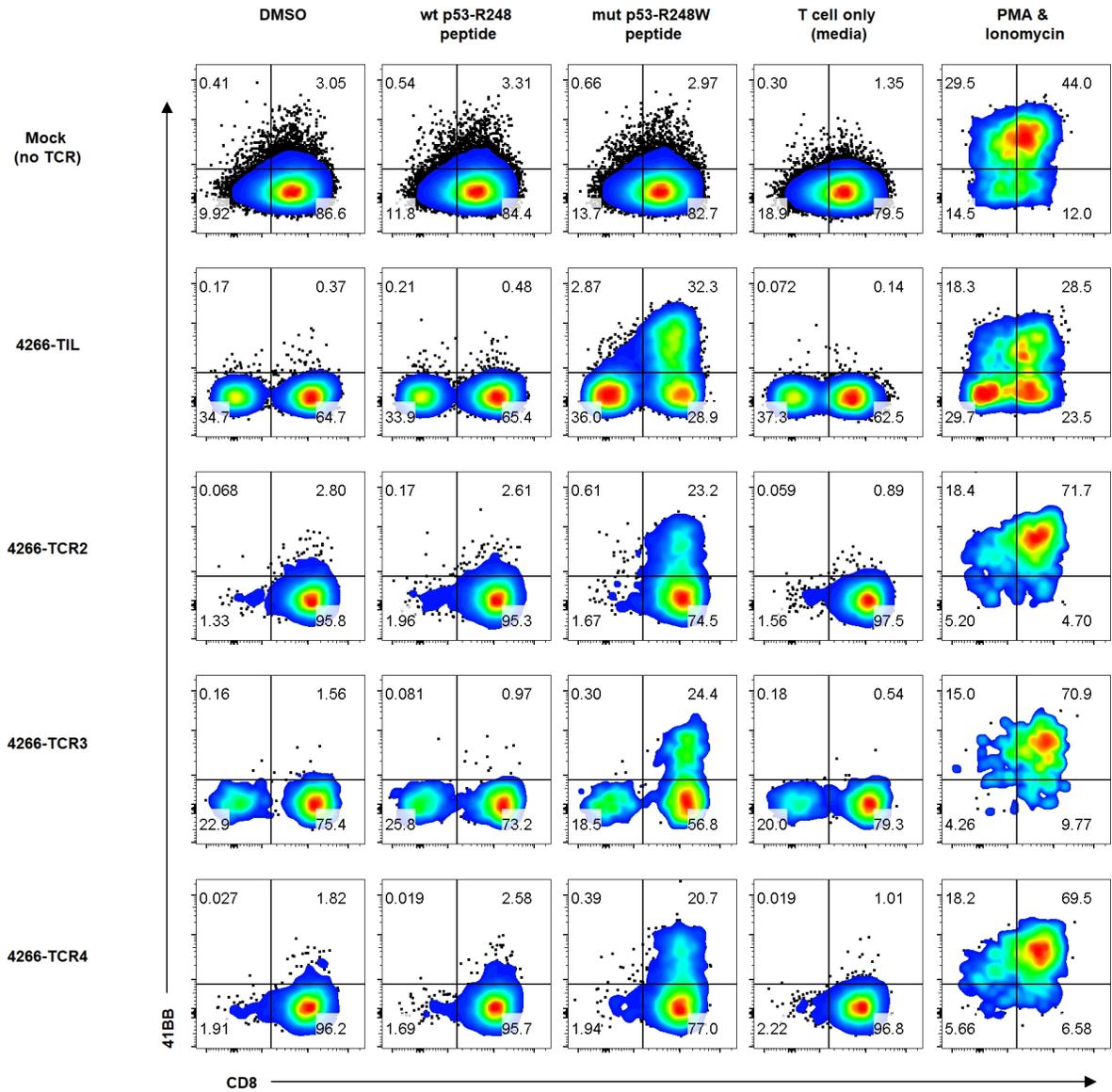
Supplemental Figure 15. The specificity of TIL towards p53^{R248W} from Patient 9 was mediated by three TCRs. Autologous antigen presenting cells from Patient 9 were pulsed with DMSO, wild type p53^{R248} or mutated p53^{R248W} peptides. Peripheral blood T cells genetically modified with nothing (mock; no introduced TCR), 4266-TCR2, 4266-TCR3 or 4266-TCR4 and p53^{R248W} neoantigen-reactive TIL (4266-TIL) were co-cultured with peptide-pulsed APCs. Negative and positive controls were T cells only (media) and PMA and Ionomycin, respectively.

Upregulation of 41BB expression on T cells by flow cytometry is shown following gating for lymphocytes → live cells (propidium iodide negative) → CD3+ (T cells).
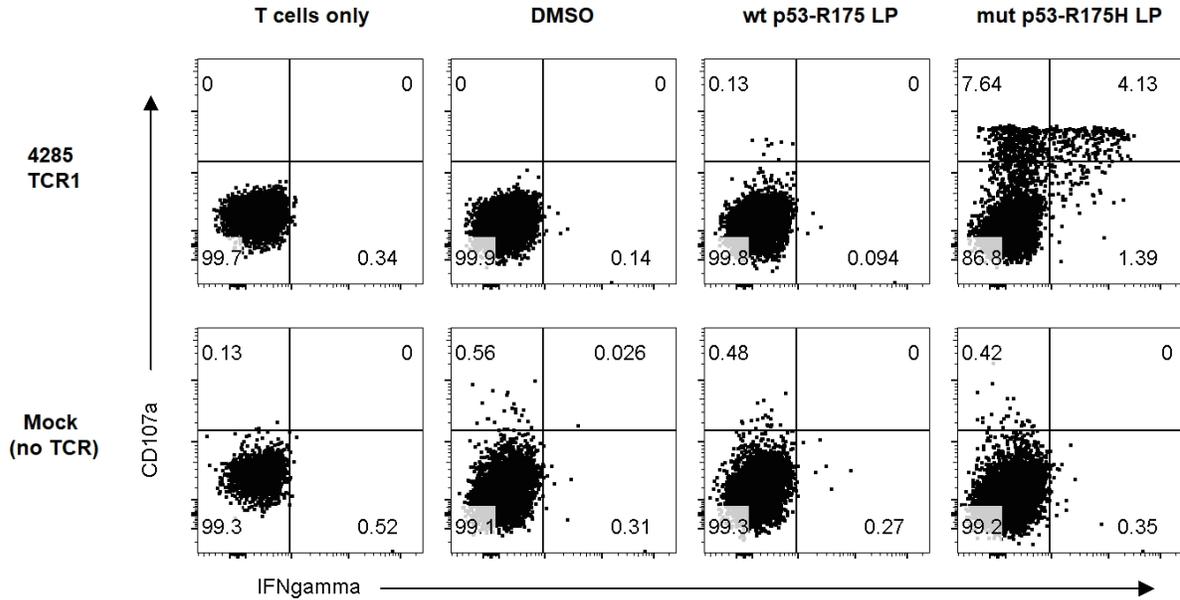
Supplemental Figure 16. The specificity of TIL towards p53^{R248W} from Patient 10 was mediated by 4273-TCR1a2. Autologous antigen presenting cells from Patient 10 were pulsed with DMSO, wild type p53^{R248} or mutated p53^{R248W} peptides then co-cultured overnight at 37°C with peripheral blood T cells genetically modified with nothing (mock; no introduced TCR; bottom plots) or 4273-TCR1a2 (top plots). Negative and positive controls were T cells only (media) and PMA and Ionomycin, respectively. Upregulation of 41BB expression on T cells expressing introduced TCRs (as measured by mTCR expression) by flow cytometry is shown following gating for lymphocytes → live cells (propidium iodide negative) → CD3+ (T cells).
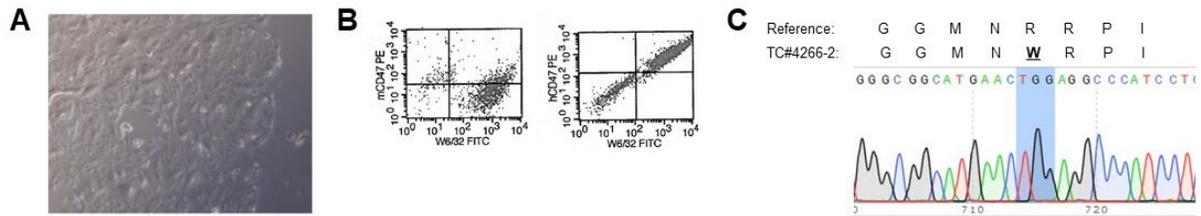
Supplemental Figure 17. Tumor cell line TC#4266-2 morphology, phenotype and genotype.
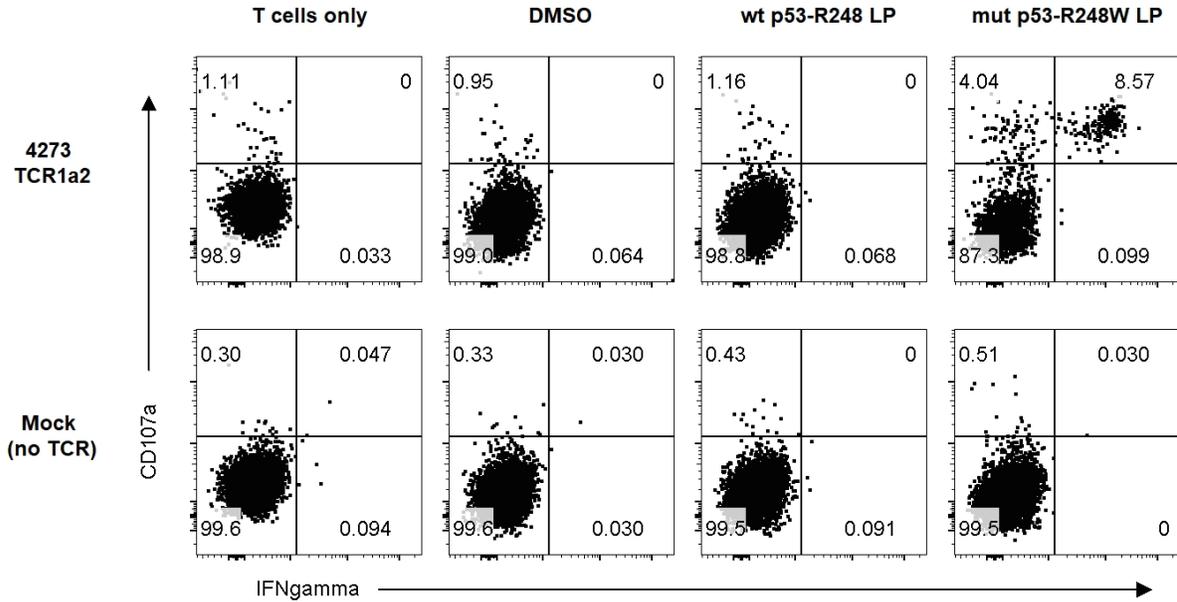(A) Morphology of TC#4266-2 under light microscopy. (B) Flow cytometry analyzing expression of murine CD47 (mCD47), human CD47 (hCD47) and HLA (W6/32). (C) RNA was isolated from TC#4266-2, reverse transcribed to mRNA, *TP53* was amplified by PCR and Sanger sequencing is displayed. The translation of the sequence is displayed above the chromatograms for TC#4266-2 and the wild type reference translation.

	T cells only	DMSO	wt p53-R175 LP	mut p53-R175H LP

4285 TCR1 (top plots); **Mock (no TCR)** (bottom plots). Y-axis: CD107a; X-axis: IFNgamma.

Top row (4285 TCR1):
- T cells only: 0 / 0 / 99.7 / 0.34
- DMSO: 0 / 0 / 99.9 / 0.14
- wt p53-R175 LP: 0.13 / 0 / 99.8 / 0.094
- mut p53-R175H LP: 7.64 / 4.13 / 86.8 / 1.39

Bottom row (Mock no TCR):
- T cells only: 0.13 / 0 / 99.3 / 0.52
- DMSO: 0.56 / 0.026 / 99.1 / 0.31
- wt p53-R175 LP: 0.48 / 0 / 99.3 / 0.27
- mut p53-R175H LP: 0.42 / 0 / 99.2 / 0.35

Supplemental Figure 18. Degranulation of CD4$^+$ T cells expressing 4285-TCR1 in response to p53^{R175H} neoantigen. Autologous antigen presenting cells from Patient 4 were pulsed with DMSO, wild type p53^{R175} or mutated p53^{R175H} peptides then co-cultured for 6 hours at 37°C with peripheral blood T cells genetically modified with nothing (mock; no introduced TCR; bottom plots) or 4285-TCR1 (top plots). Brefeldin A (GolgiPlug) and monensin (GolgiStop) were added to co-cultures to inhibit secretion of cytokines. Upregulation of interferon-gamma (x-axes) or CD107a (y-axes; marker of degranulation) on T cells by flow cytometry is shown following gating for lymphocytes → CD3+ (T cells). T cell populations used for co-culture were >98% CD3$^+$CD4$^+$.
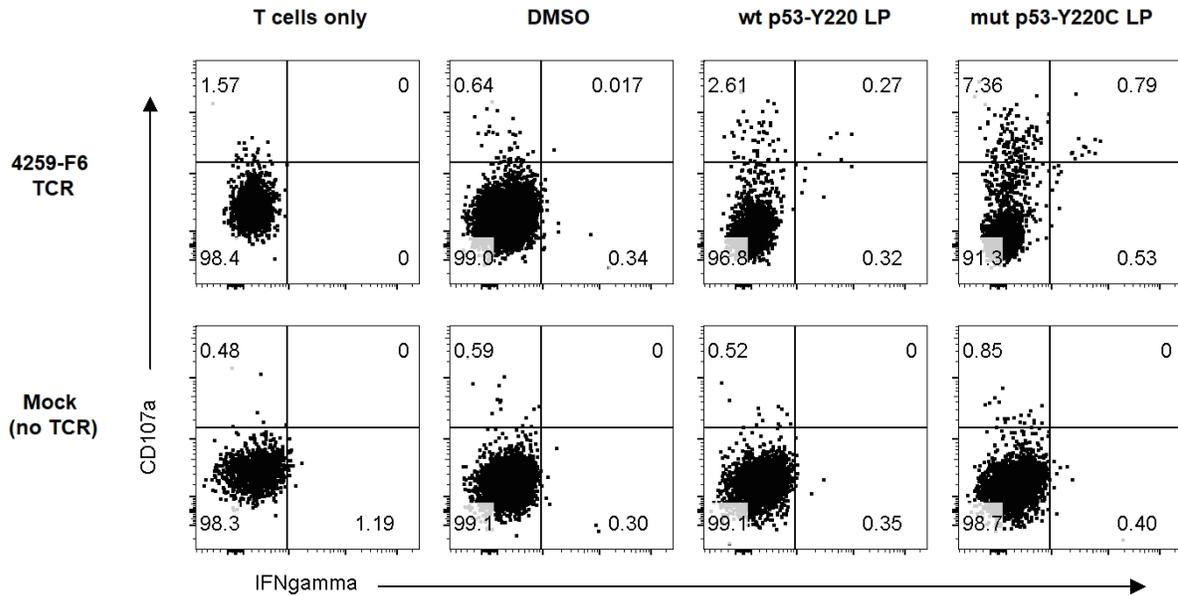
Supplemental Figure 19. Degranulation of CD4$^+$ T cells expressing 4259-F6-TCR in response to p53^{Y220C} neoantigen. Autologous antigen presenting cells from Patient 5 were pulsed with DMSO, wild type p53^{Y220} or mutated p53^{Y220C} peptides then co-cultured for 6 hours at 37ºC with peripheral blood T cells genetically modified with nothing (mock; no introduced TCR; bottom plots) or 4259-F6-TCR (top plots). Brefeldin A (GolgiPlug) and monensin (GolgiStop) were added to co-cultures to inhibit secretion of cytokines. Upregulation of interferon-gamma (x-axes) or CD107a (y-axes; marker of degranulation) on T cells by flow cytometry is shown following gating for lymphocytes → CD3+ (T cells). T cell populations used for co-culture were >98% CD3$^+$CD4$^+$.

Supplemental Figure 20. Degranulation of CD4⁺ T cells expressing 4273-TCR1a2 in response to p53^{R248W} neoantigen. Autologous antigen presenting cells from Patient 10 were pulsed with DMSO, wild type p53^{R248} or mutated p53^{R248W} peptides then co-cultured for 6 hours at 37°C with peripheral blood T cells genetically modified with nothing (mock; no introduced TCR; bottom plots) or 4259-F6-TCR (top plots). Brefeldin A (GolgiPlug) and monensin (GolgiStop) were added to co-cultures to inhibit secretion of cytokines. Upregulation of interferon-gamma (x-axes) or CD107a (y-axes; marker of degranulation) on T cells by flow cytometry is shown following gating for lymphocytes → CD3+ (T cells). T cell populations used for co-culture were >98% CD3⁺CD4⁺.