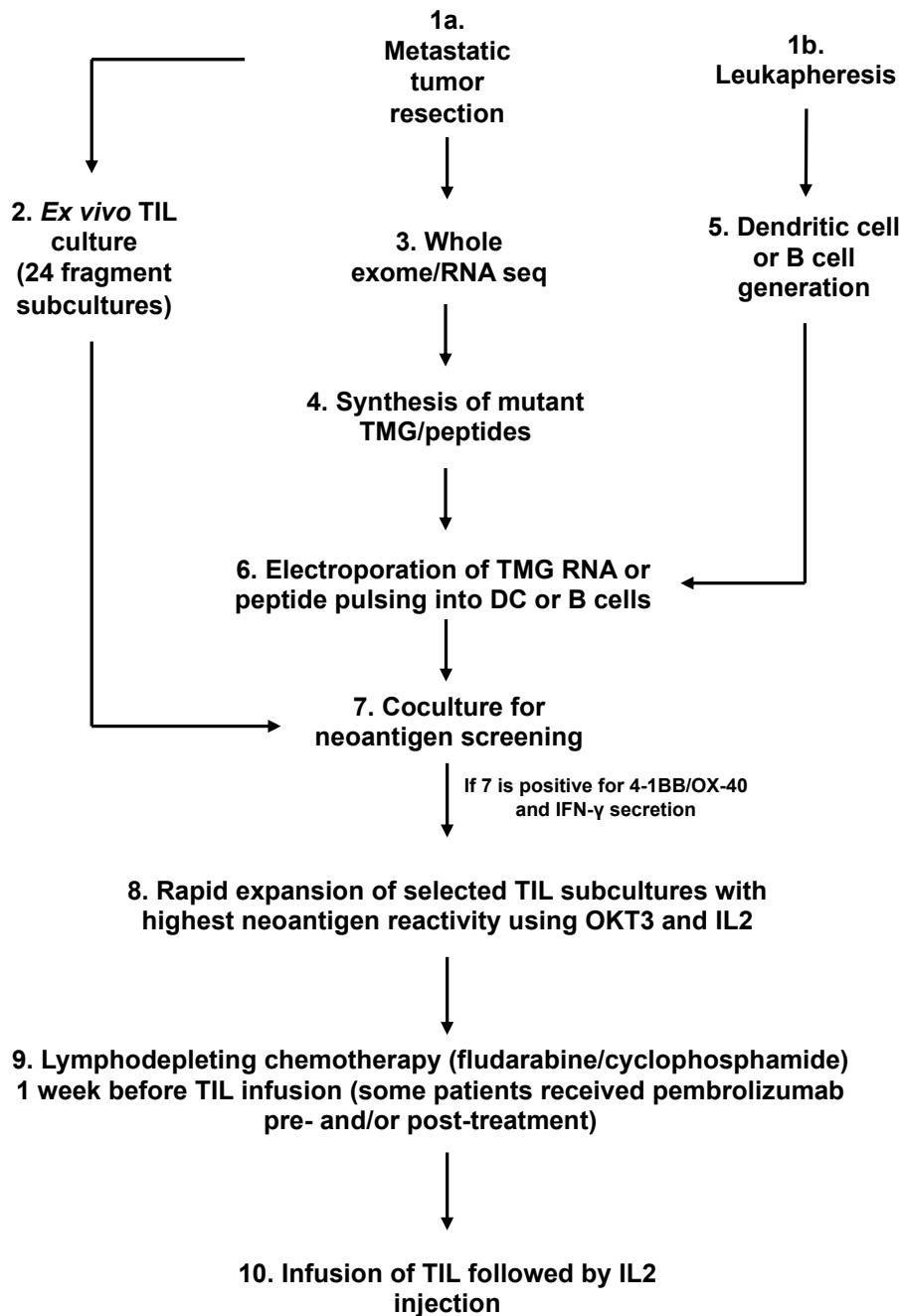
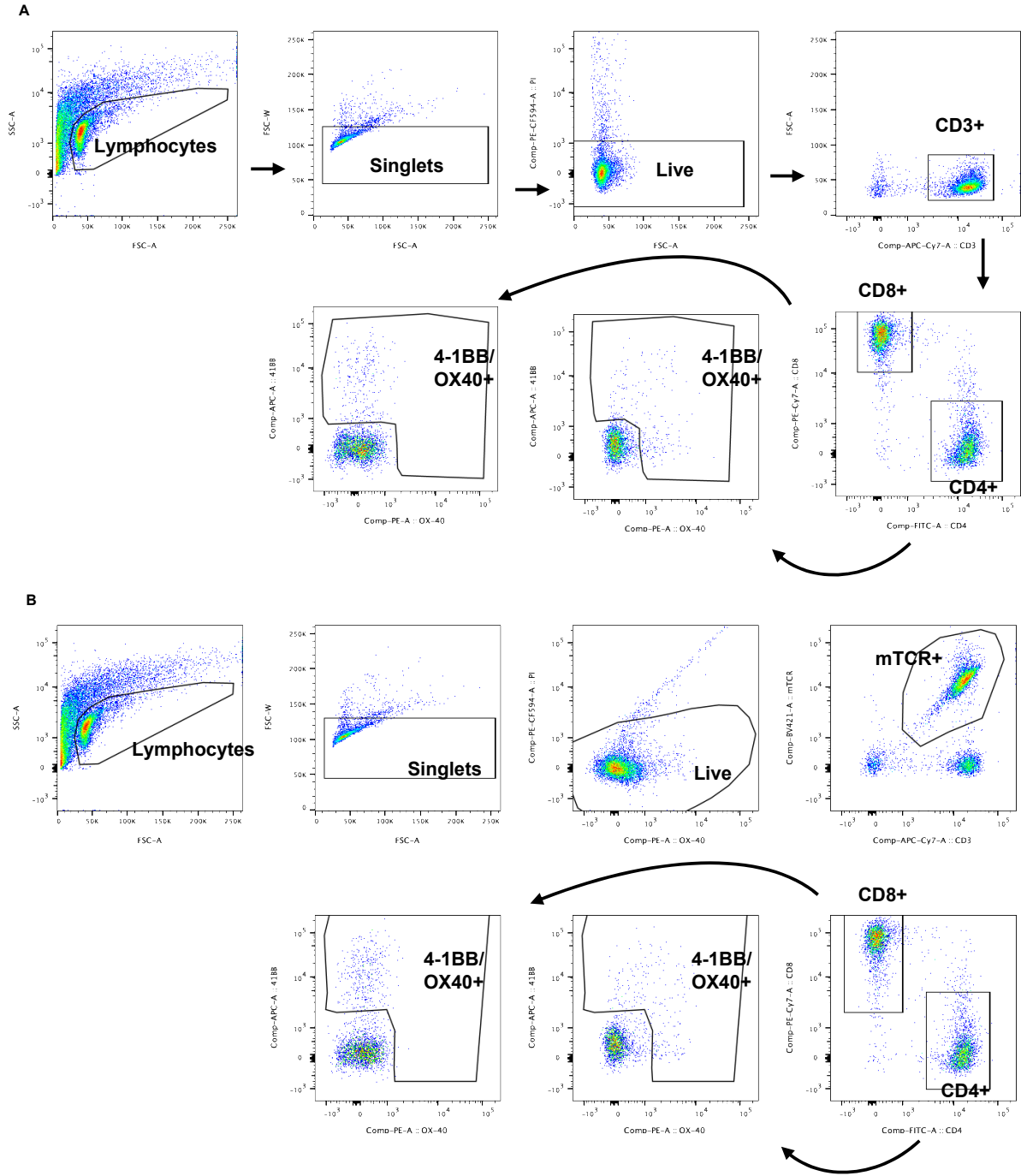


## Supplementary Figures for Kim et al.

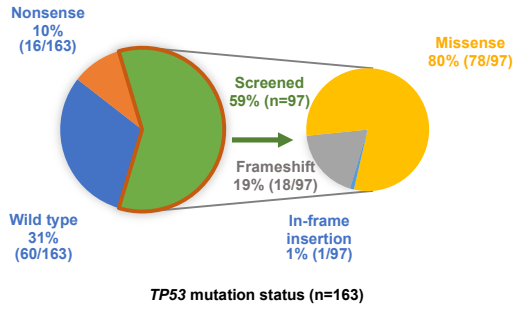


**Supplementary Figure S1. Processes for development and treatment with autologous TIL infusion products targeting p53 neoantigens.** Diagram showing the process for TIL infusion product development and patient treatment. See also Fig. 1A.

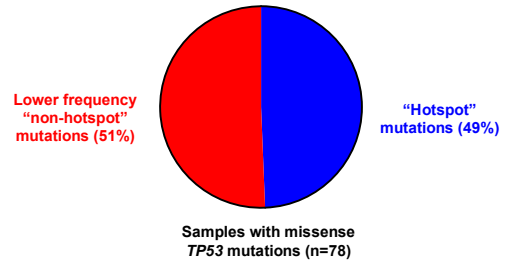


**Supplementary Figure S2. Gating strategy for flow cytometric analysis of TIL (A) and PBLs engineered with a TCR (B).**

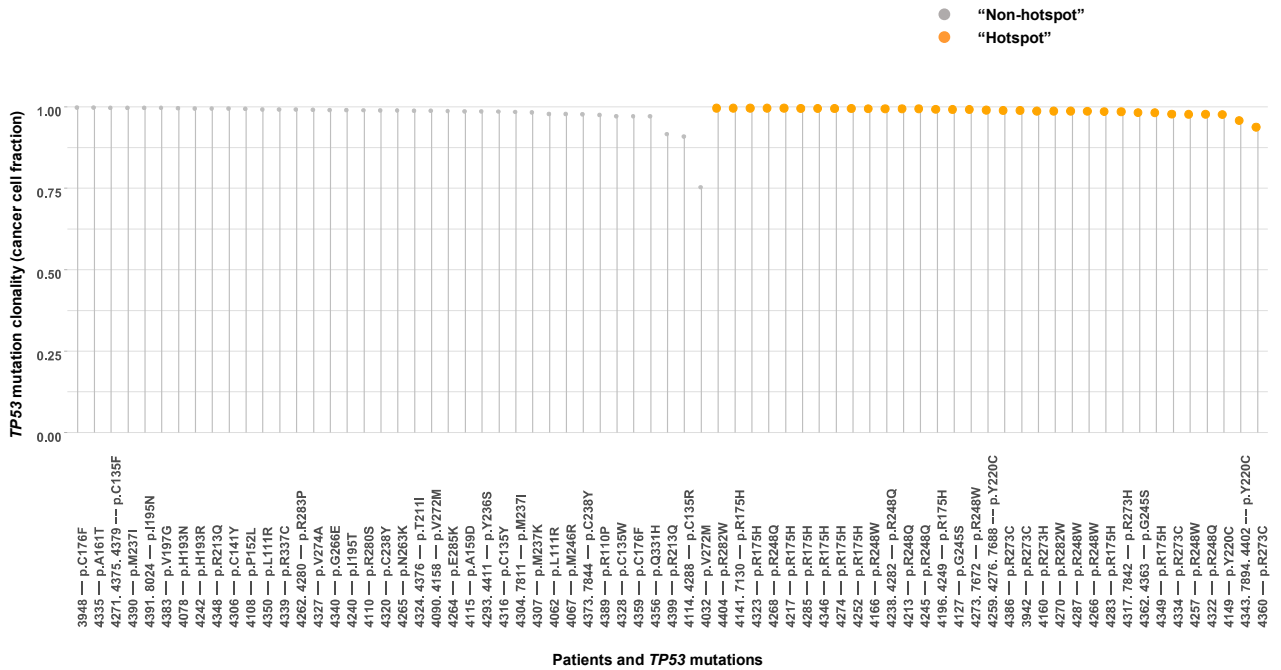
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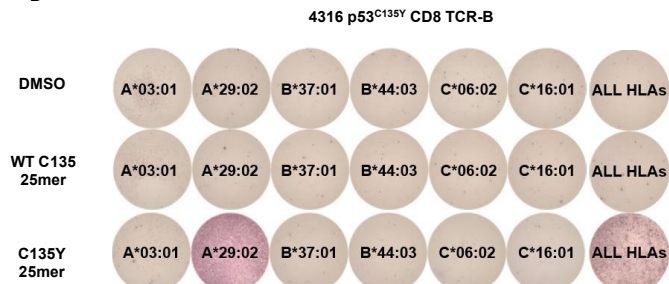
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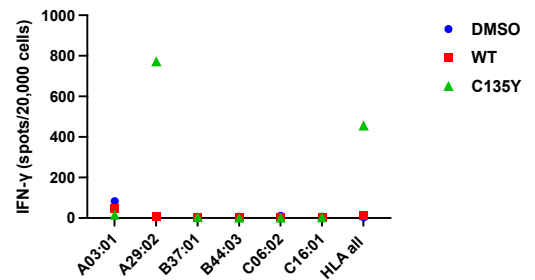
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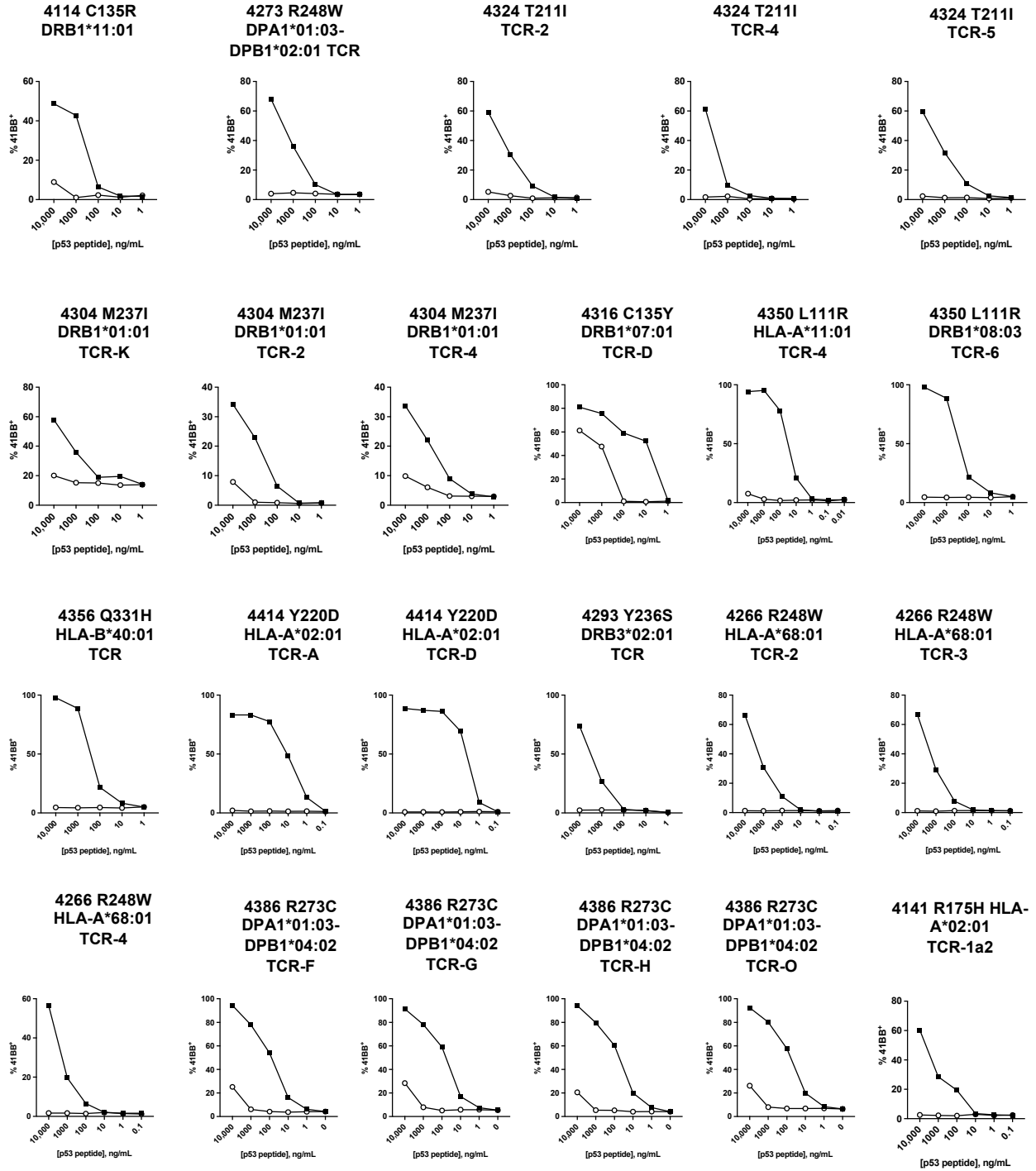
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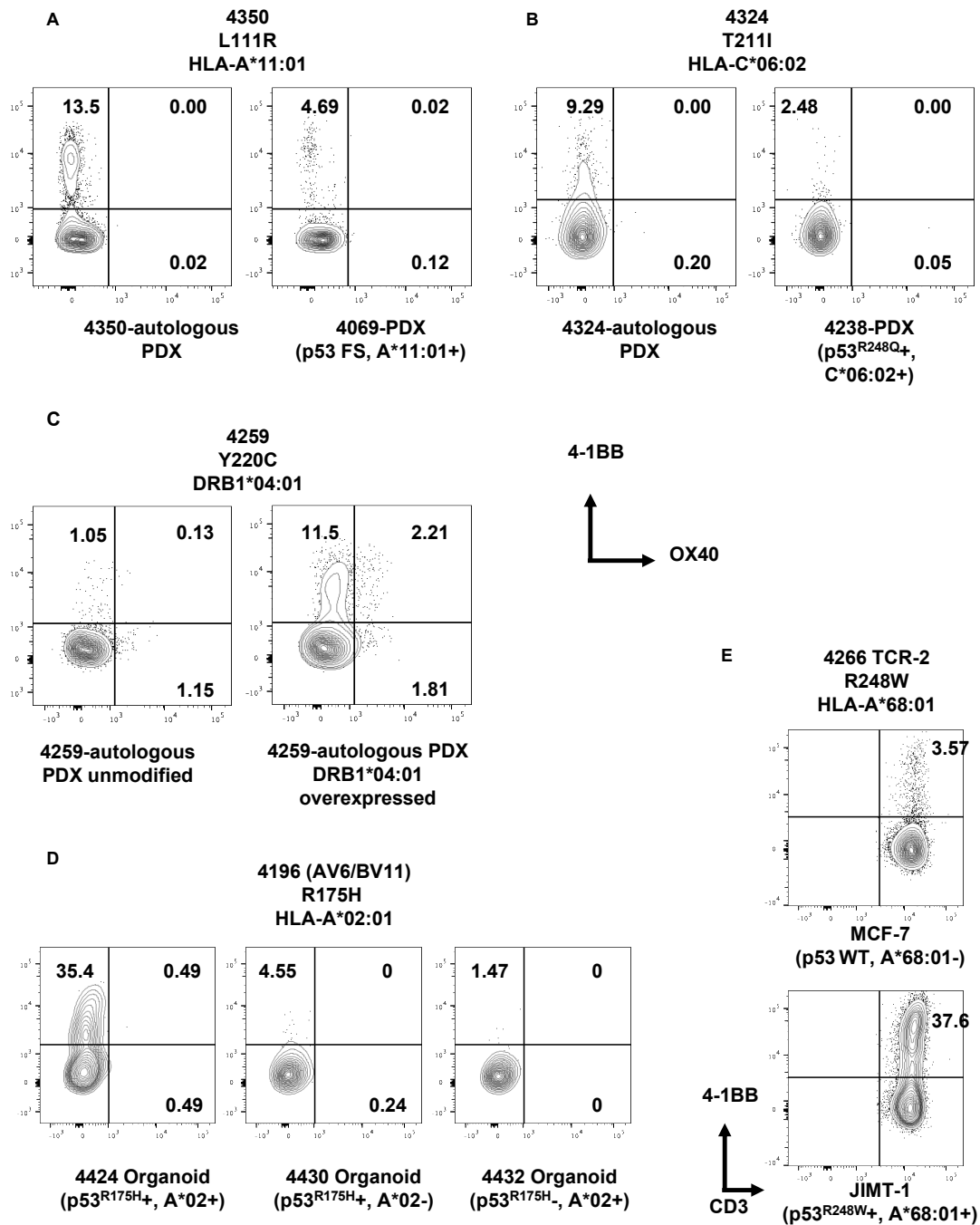
**Supplementary Figure S3. Characteristics of *TP53* mutations in the Surgery Branch cohort and HLA restriction of CD8 TCR-B isolated from patient 4316.**

**A**, Pie chart showing the frequencies of somatic *TP53* mutations within the Surgery Branch patient cohort (n=163). Patients with missense, frameshift and in-frame insertion mutations in *TP53* that consisted 59% of the patients were subjected to the immunogenicity screening for *TP53* mutations. **B**, Pie chart showing the frequencies of “hotspot” and “non-hotspot” *TP53* missense mutations within the Surgery Branch cohort (n=78). **C**, Clonality of “hotspot” and “non-hotspot” *TP53* mutations. The cancer cell fraction values for *TP53* mutations generated from the whole exome sequencing results are plotted. Each dot represents the average clonality for three or more tumor fragments from a single patient. **D**, COS7 cells individually transfected with patient 4316’s own HLA class I were pulsed with DMSO, wild-type or p53C135Y 25mer peptides and co-cultured with CD8 TCR-B-expressing healthy donor PBLs. ELISpot measurement of IFN- $\gamma$  secretion is shown (N=1). **E**, IFN- $\gamma$  spots in **D** are quantified.

○ Wild-type  
 ■ Mutant p53



**Supplementary Figure S4. Avidity of the library of the anti-mutant p53 TCRs targeting p53 neoantigens.** *TCRA/B* sequences were reconstructed into an MSGV1 retroviral backbone and retrovirus was generated for the individual TCRs from Table 1. Healthy donor PBLs were transduced to express the TCRs. Exogenous TCR expression was validated by positive staining of an H57-597 antibody against the mouse *TCRB* constant region (mTCR). Finally, TCR-engineered PBLs were titrated against imDC or B cells pulsed with a serially diluted wild-type or mutant peptide (either 25mer or minimal epitopes of 9 to 12mer). The frequencies of 4-1BB<sup>+</sup> cells of mTCR<sup>+</sup>/CD4<sup>+</sup> or mTCR<sup>+</sup>/CD8<sup>+</sup> cells are shown. Data for the TCRs that were previously reported are not shown. N=1, the experiments in A-E were independently repeated at least once.

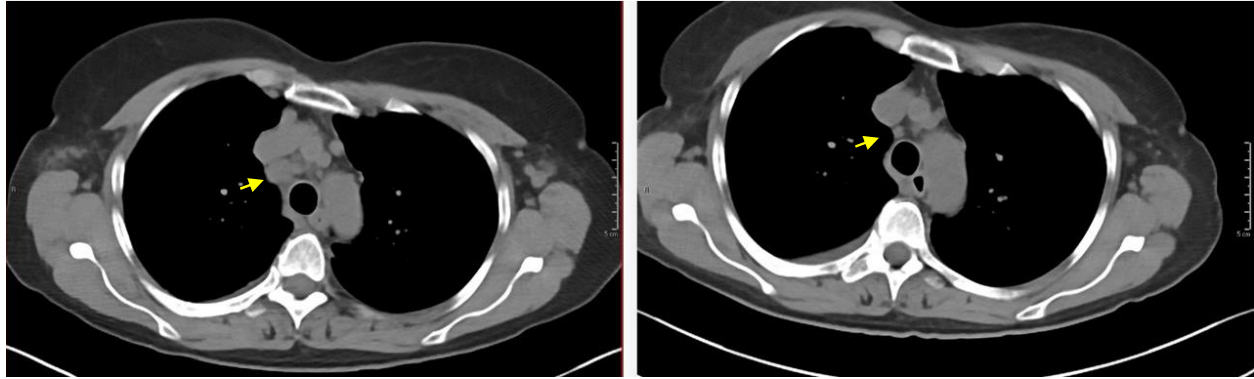


**Supplementary Figure S5. Recognition of tumor cells by neoantigen-reactive T cells.**

**A-E**, Autologous or healthy donor T cells expressing above listed TCRs were cocultured with autologous PDX cells or allogeneic cells with matching *TP53* mutations and HLA for 18 to 24 hours. Irrelevant PDX cells or commercially available cell lines without corresponding *TP53*

mutations or HLA were included as negative controls. The flow cytometry data are pre-gated for CD8+ (**A**, **B**, **D** and **E**) or CD4 (**C**), and 4-1BB and OX40 expression is shown (**E** is plotted for 4-1BB and CD3). **C**, Unmodified 4259 autologous PDX cells or 4259 PDX cells transduced with DRA1/DRB1\*04:01 were used. FS, frame-shift mutations. N=1, the experiments in A-E were independently repeated at least once.



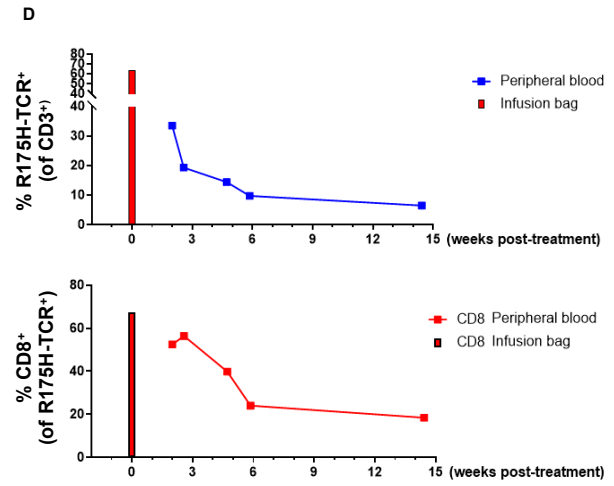
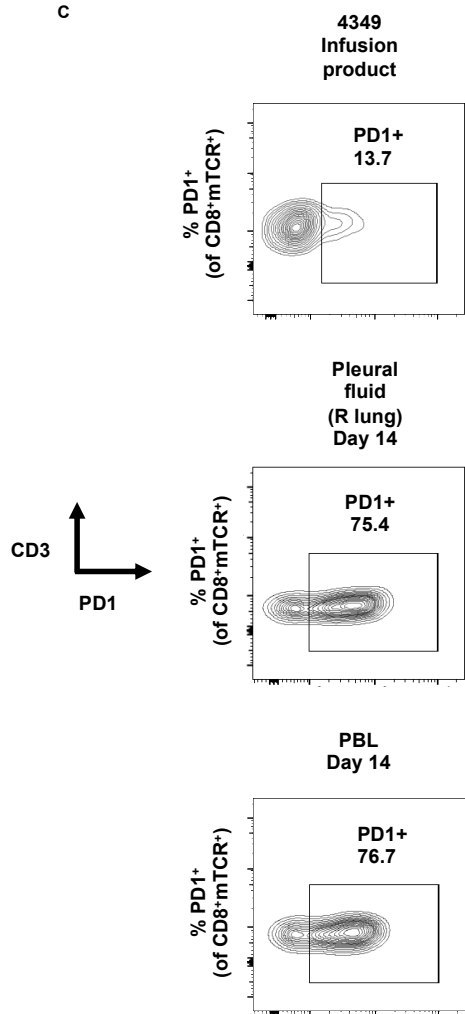
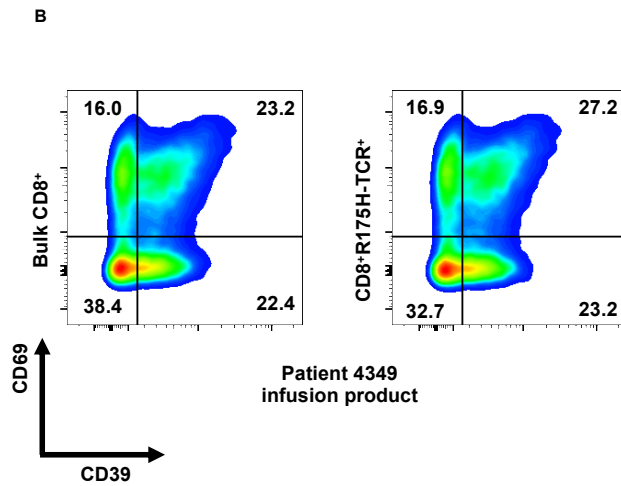
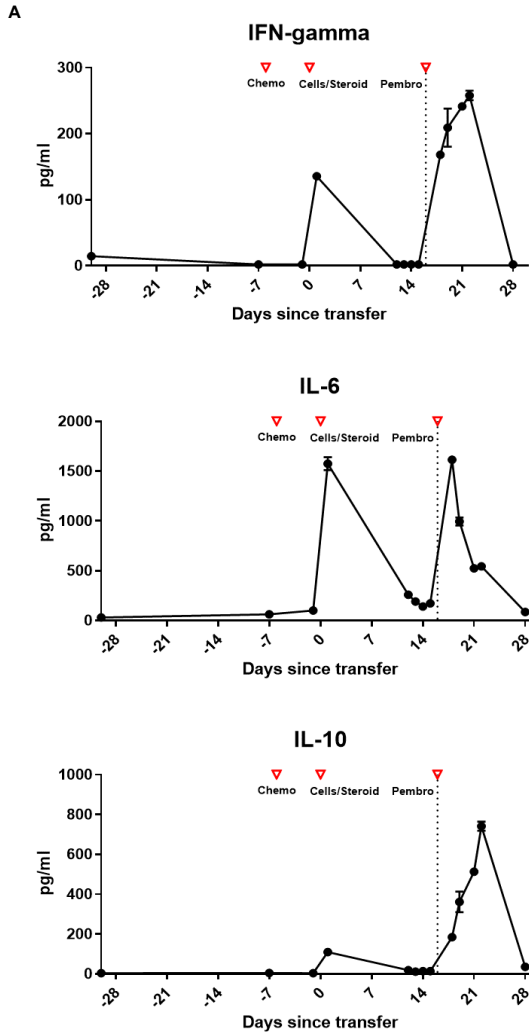


**Pre-treatment**

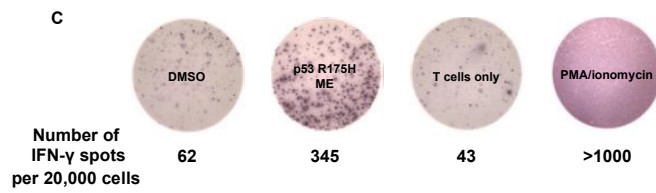
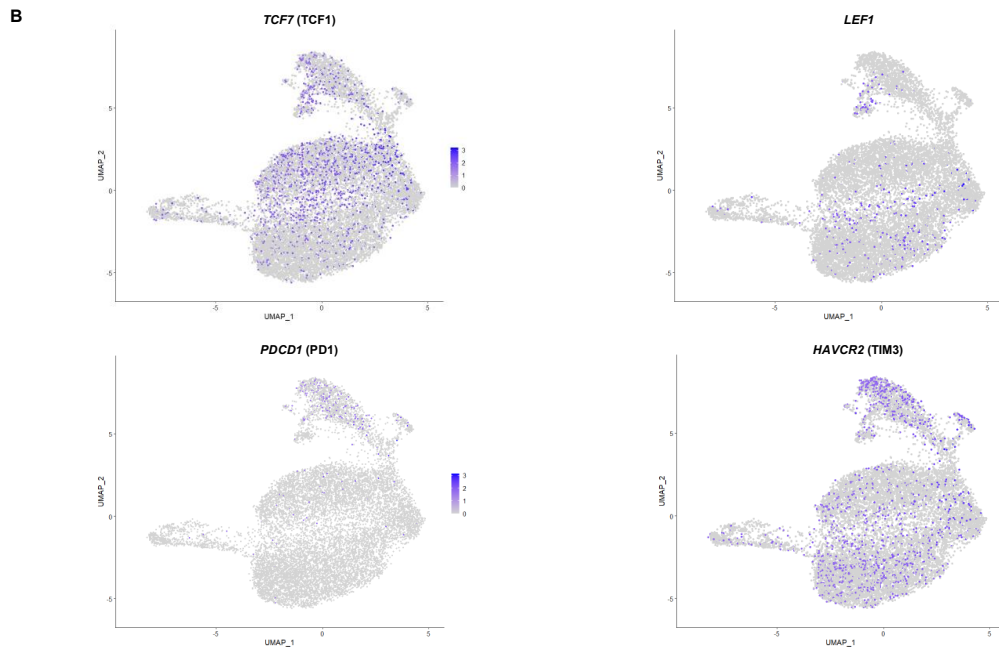
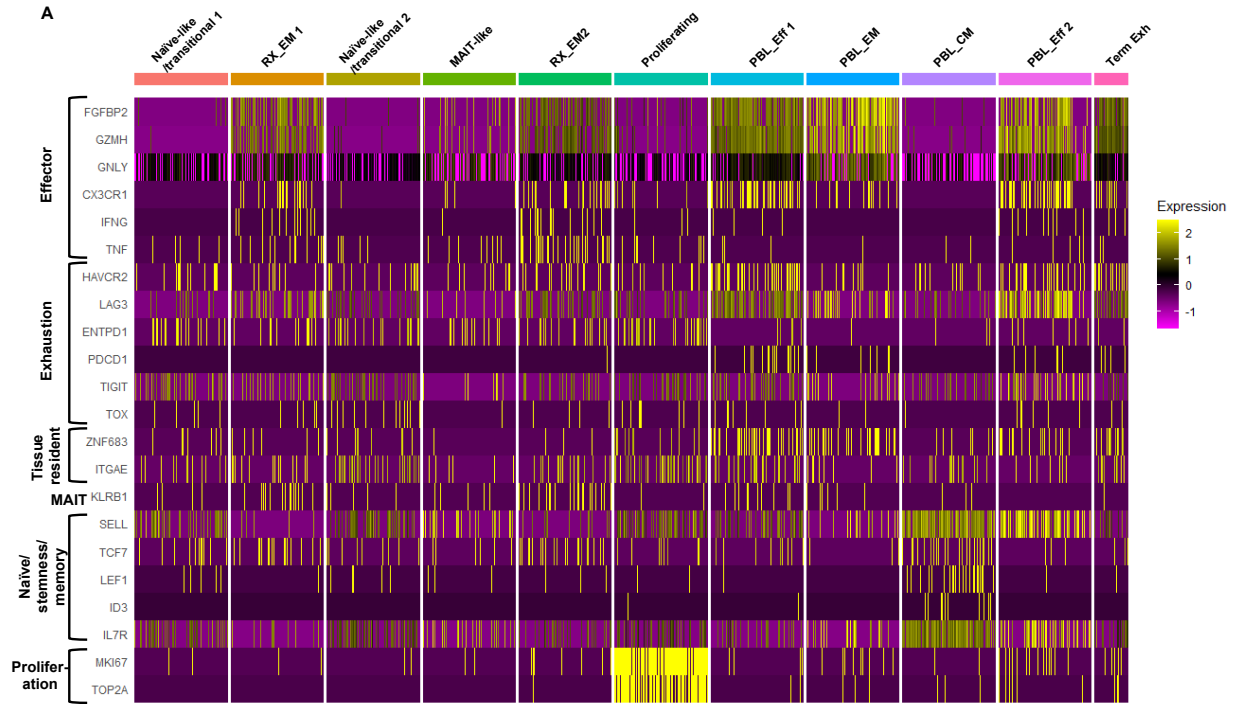
**3 months post-treatment**

**Supplementary Figure S6. Computed tomography of chest of patients 4127.**

Contrast-enhanced computed tomographic scans of the chest of patient 4127 before (left) and approximately 3 months after the infusion of the autologous TIL (right). Arrows highlight the right paratracheal lymph node before and after therapy.

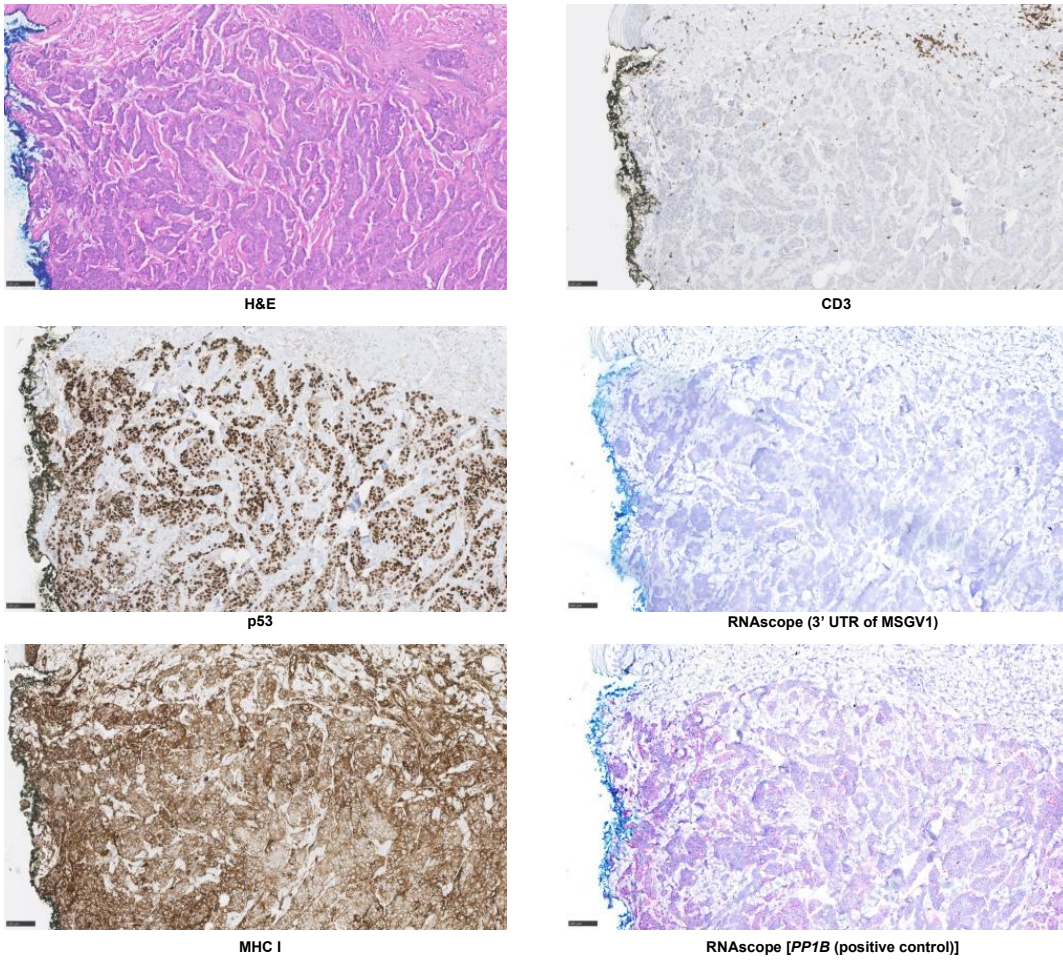


**Supplementary Fig. S7. Analysis of T cells and cytokines for patient 4349.** **A**, Phenotypic analysis of antigen-specific (R175H-TCR<sup>+</sup>) or bulk CD8<sup>+</sup> T cells from the infusion products for patient 4349 by flow cytometry. Bulk CD8<sup>+</sup> T cells (left) or CD8<sup>+</sup>R175H-TCR<sup>+</sup> cells (right) were stained for CD39 and CD69. **B**, Serum cytokine levels of IFN- $\gamma$  (top), IL-6 (middle), and IL-10 (bottom) were measured by a flow cytometry-based LEGENDplex assay. **C**, Flow cytometric analysis of patient 4349's infusion product (top), pleural fluid at day 14 (middle), and PBL at day 14 (bottom) for PD1 expression. **D**, Persistence of R175H-TCR-expressing T cells by flow cytometric detection of R175H-TCR<sup>+</sup> T cells (upper panel) and CD8<sup>+</sup> T cells of R175H-TCR<sup>+</sup> T cells (bottom panel).

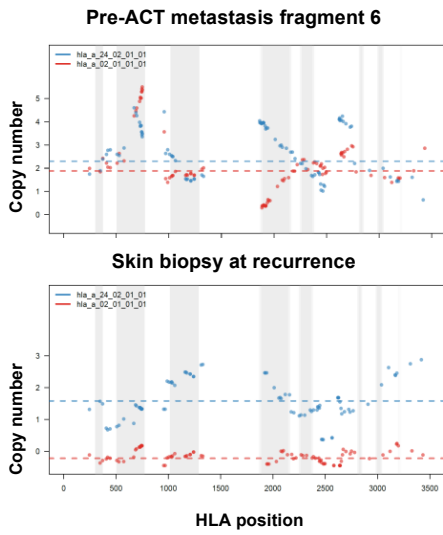


**Supplementary Fig. S8. Analysis of patient 4349's infusion product (RX) and PBL samples post-ACT.** **A**, Heatmap of selected genes across the clusters in Fig. 4F. Expression from each single cell was down sampled to 100 cells per cluster for visibility. **B**, Expression of indicated genes overlaid on the UMAP projection of RX cells and PBL\_6w cells. **C**, Functional analysis of PBL sample at 4 months post-ACT. Peripheral blood mononuclear cells were isolated by Ficoll and were subject to co-culture with autologous imDC pulsed with the p53<sup>R175H</sup> ME or DMSO. T cells only and PMA/ionomycin conditions were included as negative and positive controls, respectively.

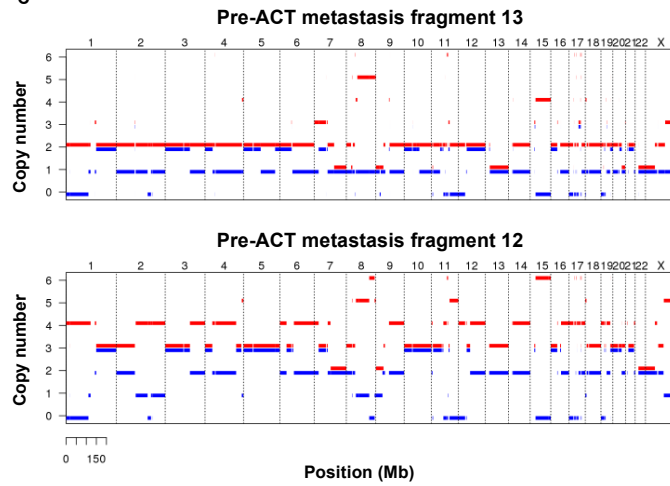
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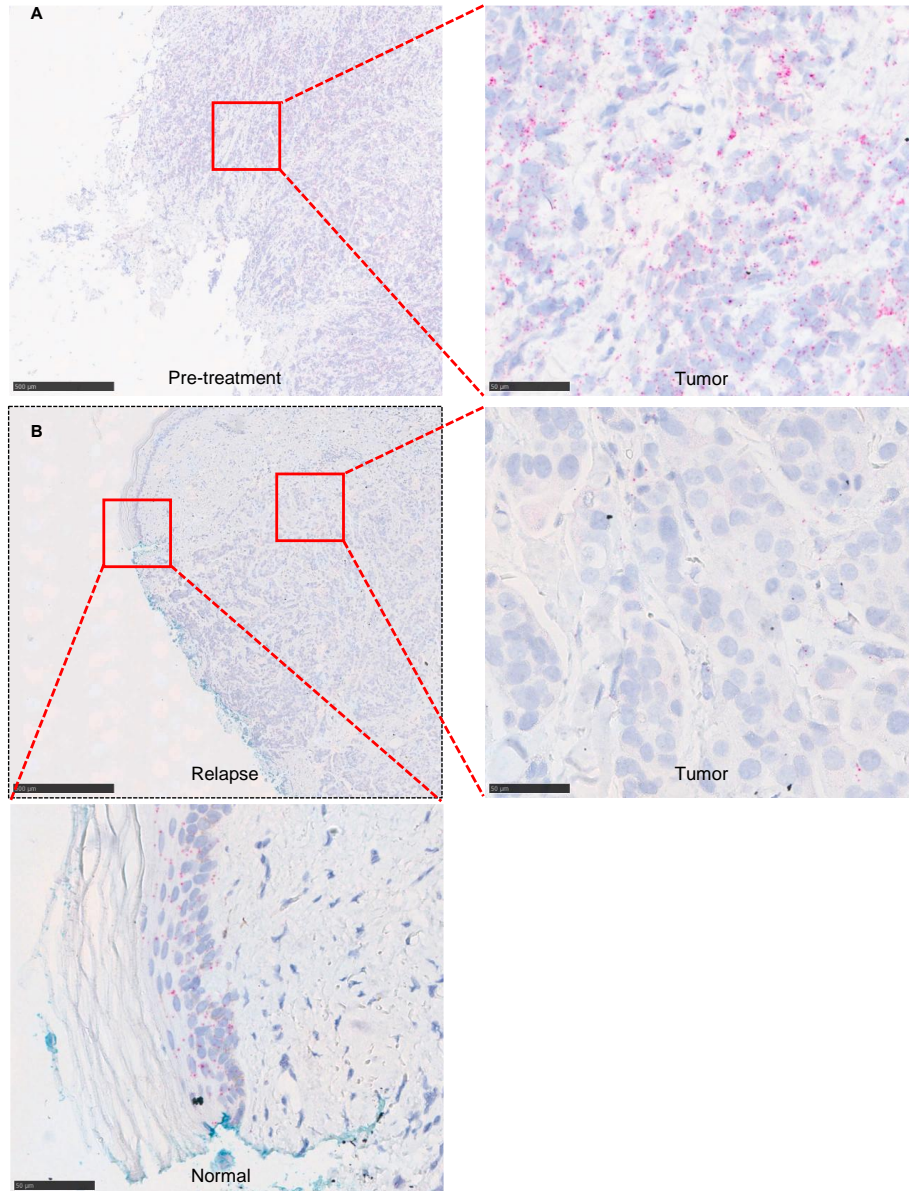
B



C



**Supplementary Fig. S9. Immunohistochemical and genomic analysis of the skin biopsies of patient 4349's progressing lesion and the pre-ACT metastases.** **A**, Skin biopsies from patient 4349 at day 209 post-ACT were analyzed by an immunohistochemical and RNAscope analysis. RNAscope against *PPIB* was included as a positive control to ensure the quality of RNA of the specimen. Scale bar, 100  $\mu$ m. **B**, Copy number analysis at the chromosome level. **C**, Haplotype specific copy number analysis for the HLA-A locus. Exons are shaded in gray and each dot represents a copy number for a single nucleotide polymorphism corrected for Log R ratio, B allele frequency, tumor purity and tumor ploidy. Dotted lines represent the median allele copy number.



**Supplementary Fig. S10. Detection of HLA-A\*02:01 by RNAscope in the pre-ACT and progressing lesion biopsies from patient 4349.** Skin biopsies from patient 4349 before the ACT (A) and at recurrence at day 209 post-ACT (B) were analyzed by an RNAscope analysis using a probe against HLA-A\*02:01. B, From the relapse biopsy, the normal epidermis with intact HLA-A\*02:01 expression (bottom panel) and the tumor cells with a lack of HLA-A\*02:01 expression (right panel) are shown. Scale bar, left panel in A and top left panel in B: 500  $\mu\text{m}$ ; right panel in A and top right and bottom panel in B: 50  $\mu\text{m}$ .