Cell Reports Medicine, Volume *5*

Supplemental information

Assessing personalized responses to anti-PD-1 treatment using patient-derived lung tumor-on-chip

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Supplemental Information

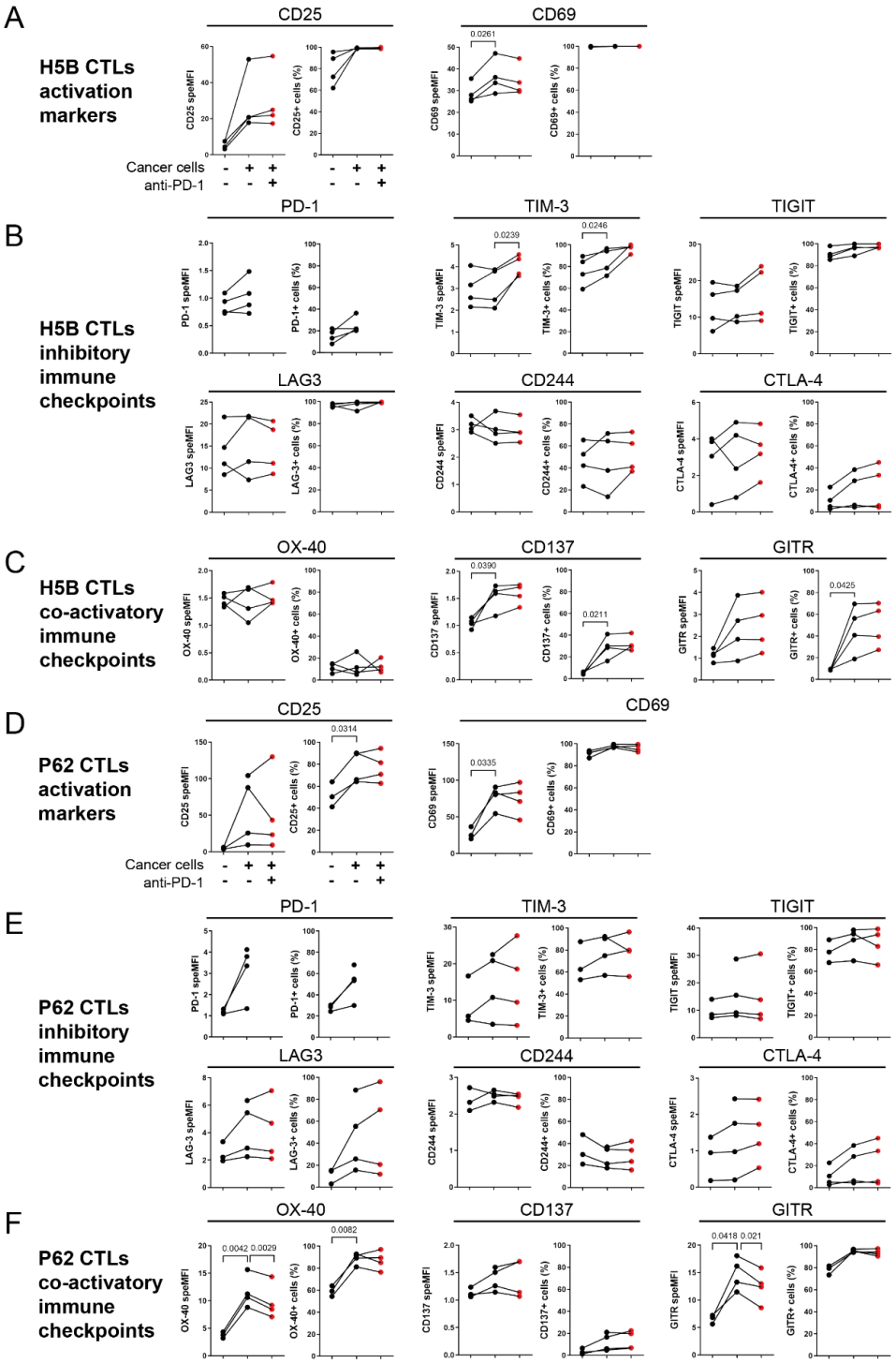

Figure S1. Analysis of T-cell plasticity in ToC co-cultures. Related to Figure 3.
Three conditions were assessed: CTLs only, with cancer cells without anti-PD-1, with cancer cells with anti-PD-1. **A-C**. H5B T cells. Specific MFI and percentage of positive cells for activation markers (A) immune checkpoints (B), and co-stimulatory receptors (C). **D-E.** P62 T cells. Specific MFI and percentage of positive cells for activation markers (D) immune checkpoints (E), and co-stimulatory receptors (F). Wilcoxon test was used to determine statistical significance, from 2 to 4 independent experiments. Red points represent the condition treated with anti-PD-1. It was not possible to measure PD-1 marker in presence of anti-PD-1 treatment because of antibody competition.

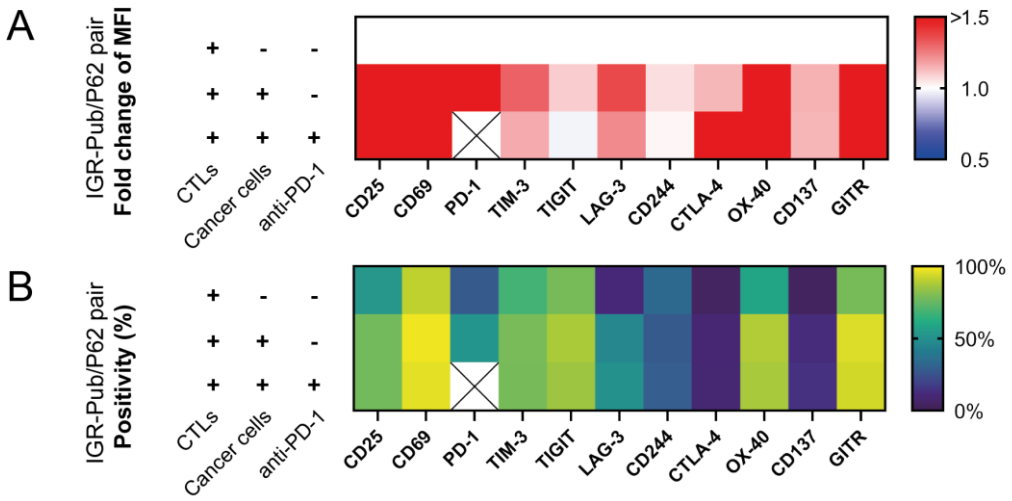

Figure S2. Analysis of T-cell plasticity in ToC co-cultures (P62 clone). Related to Figure 3. **A.** Fold change of specific MFI for CTL markers of P62 cells. The specific MFI for the condition CTLs only is set as 1. **B.** Percentage of positive P62 cells for CTL markers.

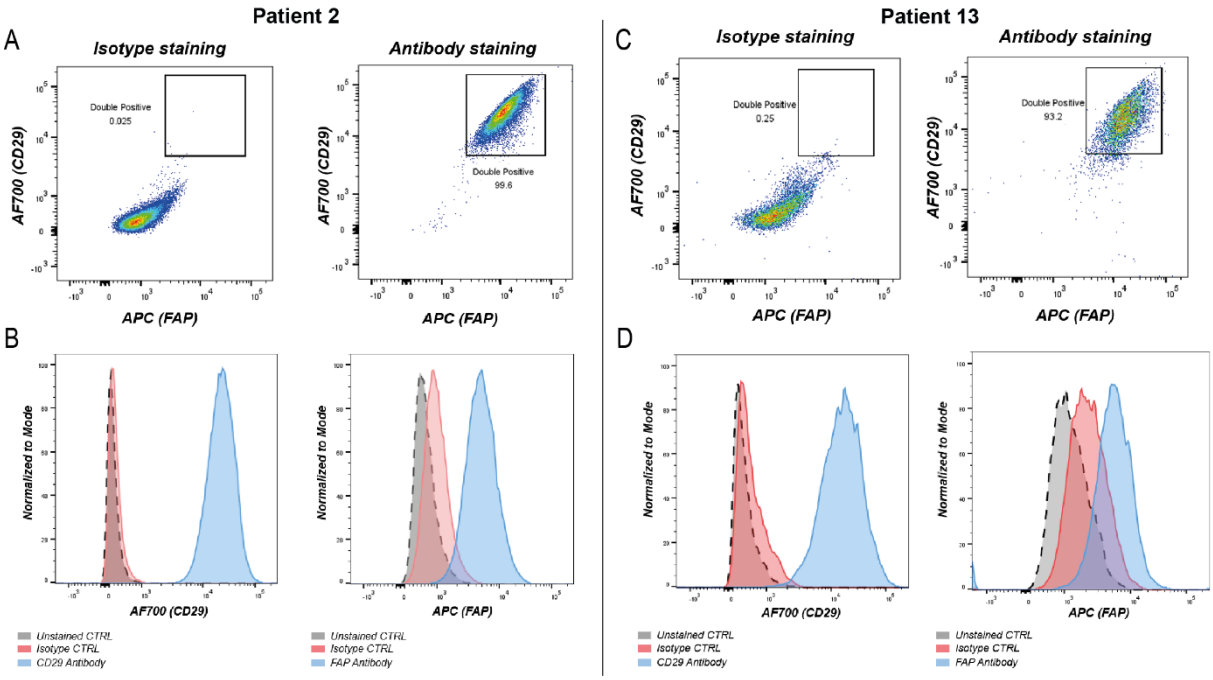

Figure S3. Flow cytometry analysis of patient-derived CAFs. Related to Figures 4 and 5. **A-B.** Analysis of FAP and CD29 markers for CAFs isolated form patient #2. **C-D.** Analysis of FAP and CD29 markers for CAFs isolated form patient #13.

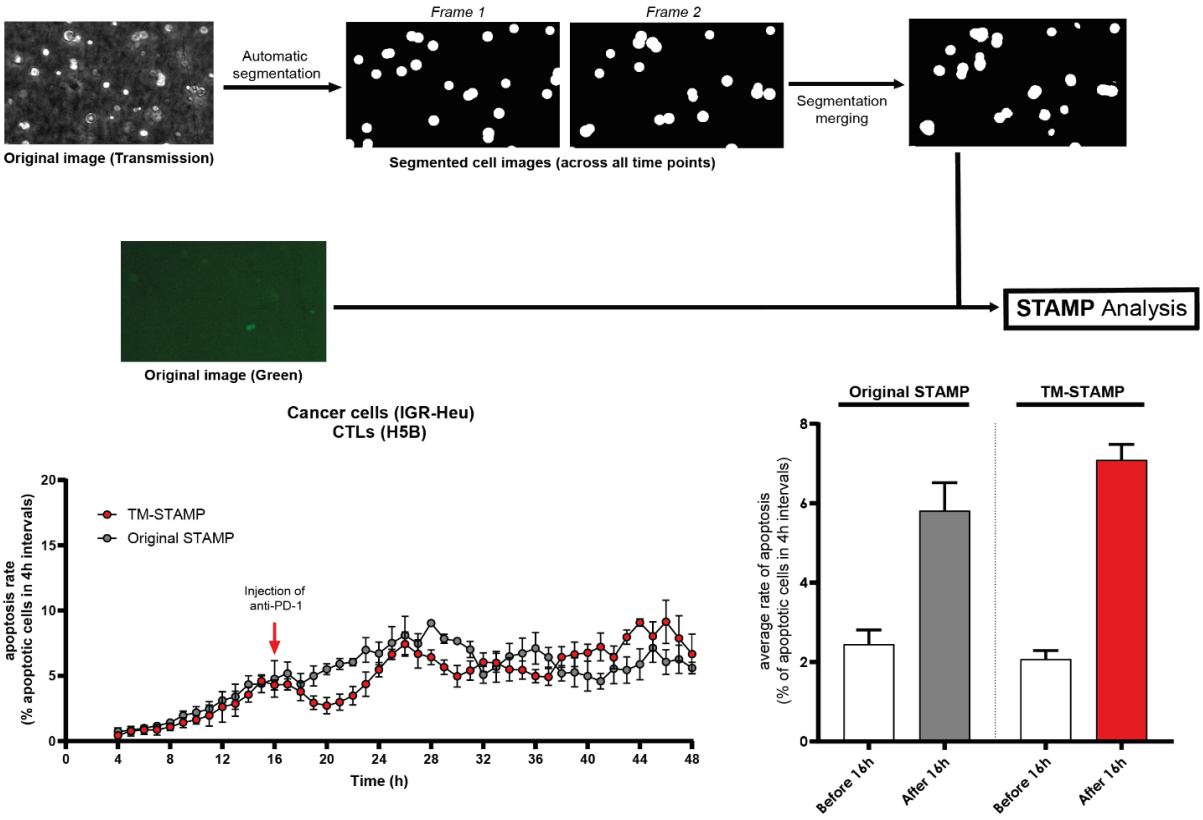

Figure S4. Validation of TM-STAMP method. Related to Figures 7.

In TM-STAMP areas occupied by tumor cell are automatically identified and segmented based on contrast differences in the transmission channel, instead of red channel used for original STAMP. The segmented cell images are then merged to form the total areas of cellular activity. This unique segmentation mask was applied along the entire video analysis by the STAMP algorithm (see Materials and Methods).

The original STAMP and TM-STAMP methods were compared using videos from one ToC experiment generated using the IGR-Heu/H5B cancer- immune cell pair. The apoptosis rates of cancer cells were computed over 28 h (left graph) and averaged (right graph) over the 16 h before drug injection and over the 16 h after drug injection. The graphs report means +/- SEM from n=4 view fields.

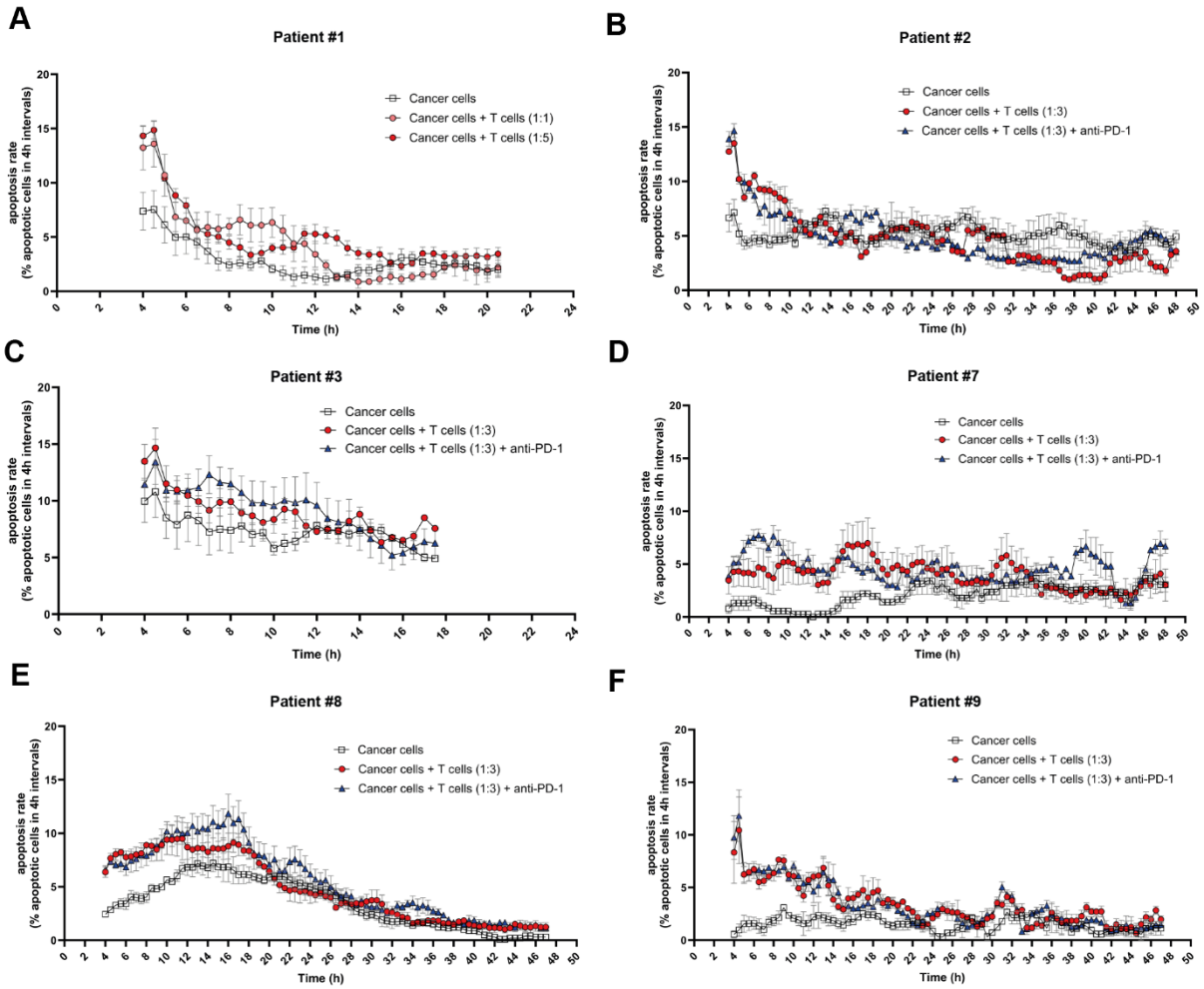

Figure S5. ToC analysis data for all patients. Related to Figures 7.

A. Apoptosis rates for Patient 1. **B.** Apoptosis rates for Patient 2. **C.** Apoptosis rates for Patient 3. **D.** Apoptosis rates for Patient 7. **E.** Apoptosis rates for Patient 8. **F.** Apoptosis rates for Patient 9.

The percentage of cancer cells dying in 4 h-time-intervals were computed using the TM-STAMP method. The averages were computed every 1h using a 4 h-sliding-window. The observation times varied from 24 h to 48 h, depending on the patient. The graphs report means +/- SEM from 4 view fields.