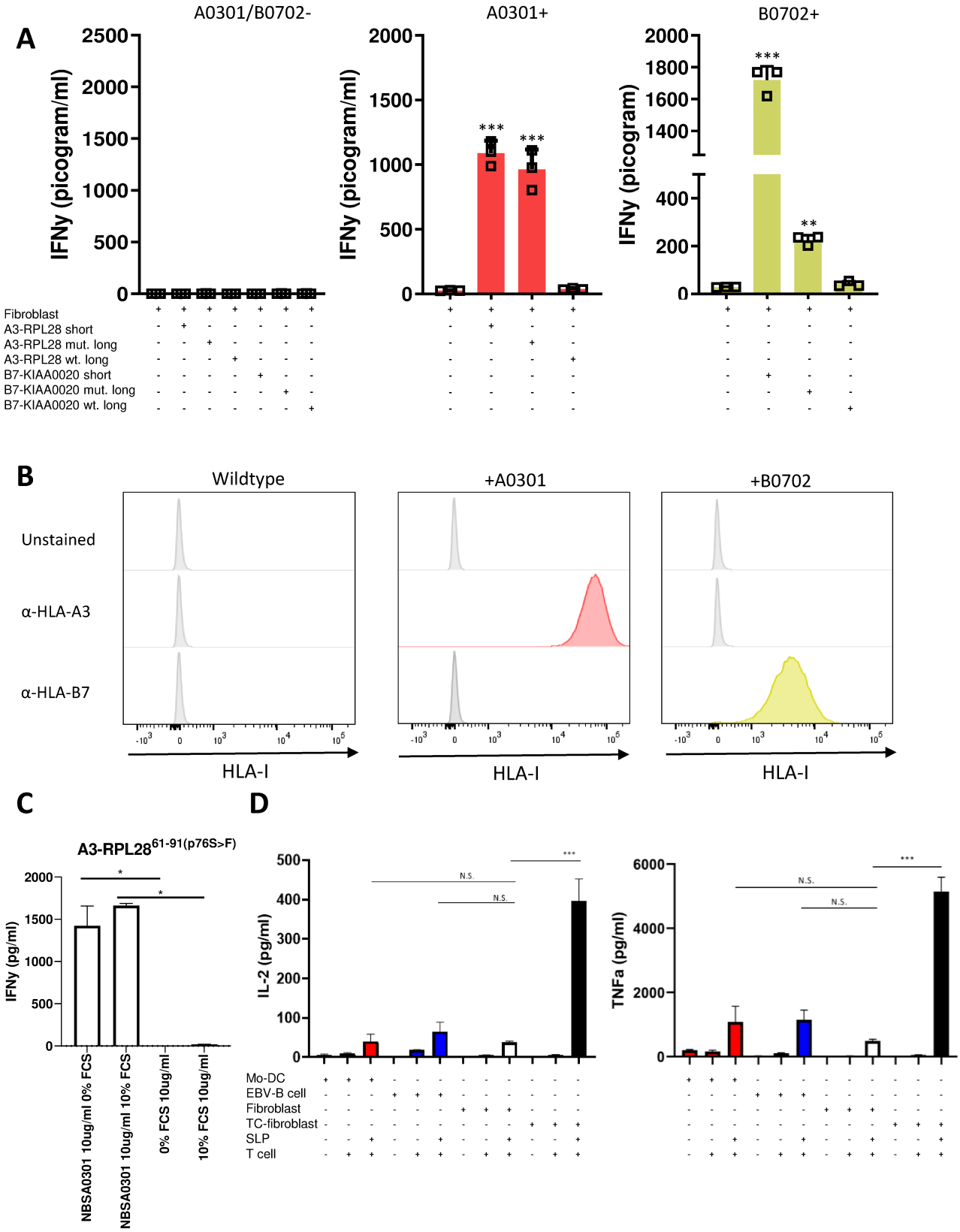
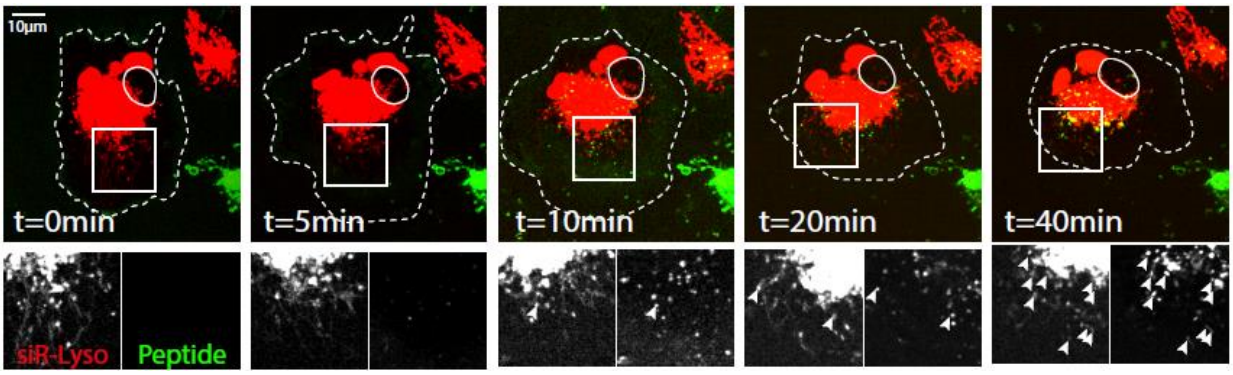
Supplementary Figure 1



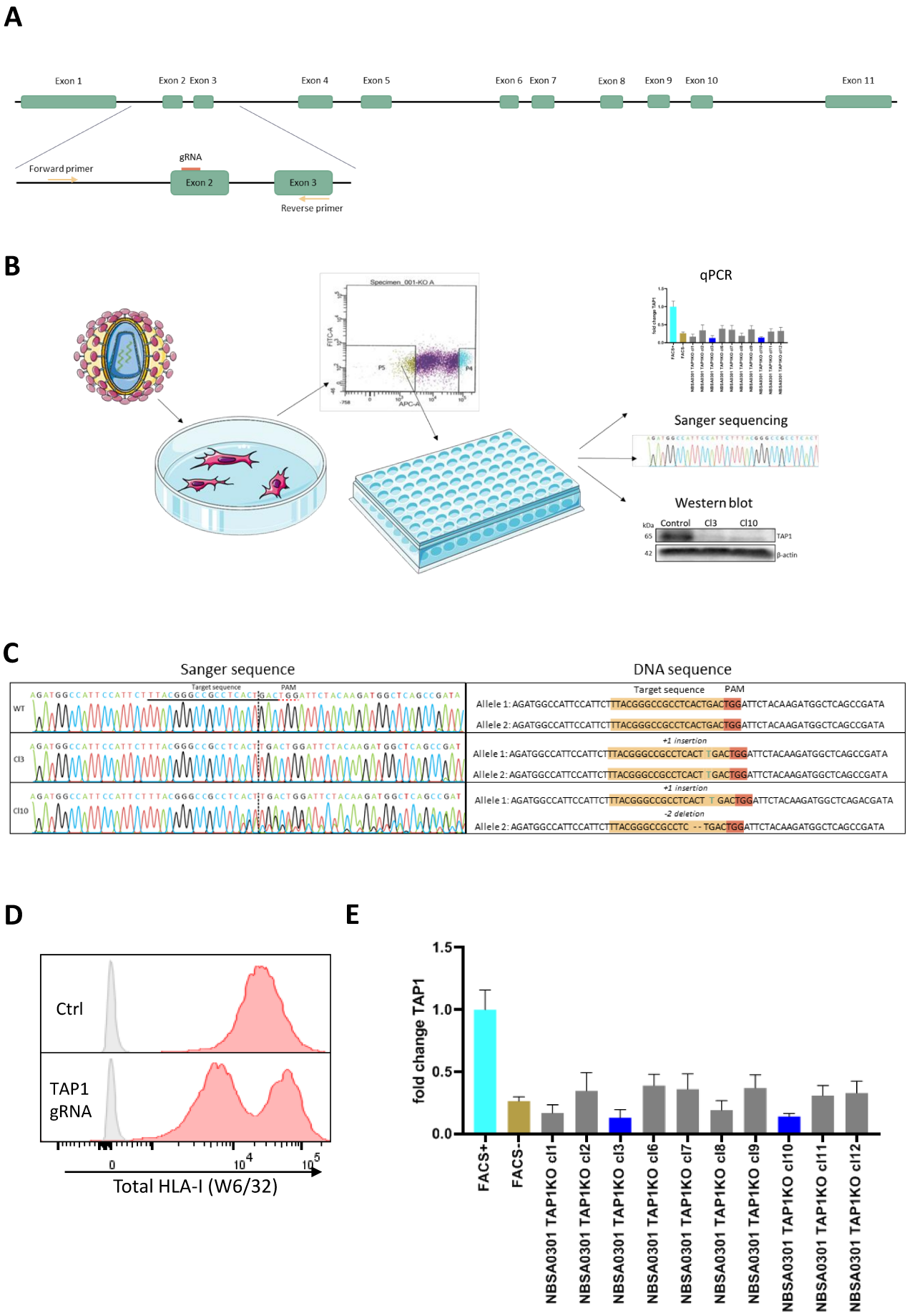
Supplementary figure 1. A IFNγ production by neoantigen-specific T cells (T-cell bulk reactive to both the A3 and B7-epitopes) after 24h co-incubation with antigen presenting human normal skin-derived fibroblasts (20 µg/ml SLP, 2 µg/ml SSP). The different panels show different normal skin-derived CAFs with differential HLA-haplotypes. Experimental conditions are compared to the T cell – fibroblast only condition with 1-way ANOVA ***=p≤0.0001, **=p≤0.001 **B** HLA-A3-/B7- NBS fibroblasts were retrovirally transduced with HLA-A3 or HLA-B7 and expression was confirmed by FACS. **C** IFNγ production by neoantigen-specific T cells after 24h co-incubation with either A3-RPL28$^{61-91(p76S>F)}$ loaded-fibroblasts or A3-RPL28$^{61-91(p76S>F)}$ only, under serum-containing and serum-free conditions. Means with SD are plotted from representative experiments (n=2). Student's t test *=p≤0.05 **D** IL-2 and TNFα expression by T cells after A3-RPL28$^{61-91(p76S>F)}$ presentation by either professional APCs (Mo-DC & EBV-B cell) and (tumor-conditioned) NBS fibroblasts. Mo-DC=Monocyte-derived Dendritic Cell, EBV-B cell=Epstein-Barr Virus immortalized B cell, TC-fibroblast=tumor-conditioned fibroblast N.S= not significant, ***p≤0.0001

Supplementary Figure 2



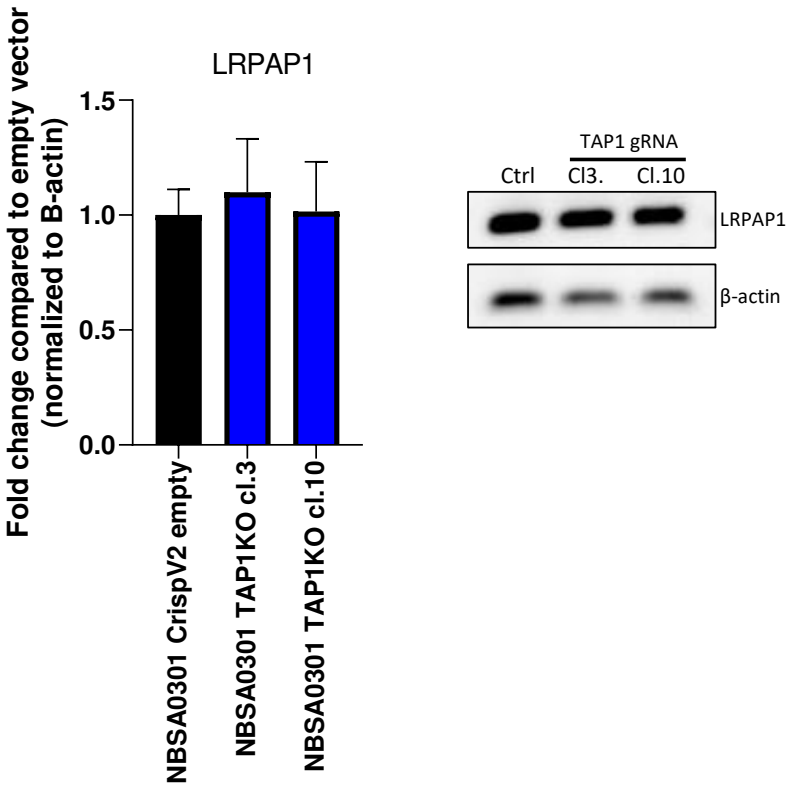
Supplementary figure 2. Spinning confocal microscopy shows co-localization of the internally quenched A3-RPL28$^{61\text{-}91(p76S>F)}$ SLP (green) and the lysosome compartment (red) in professional APCs (Mo-DCs). Dashed line indicates the outlines of the fibroblast. Closed line indicates the location of the nucleus.

Supplementary Figure 3



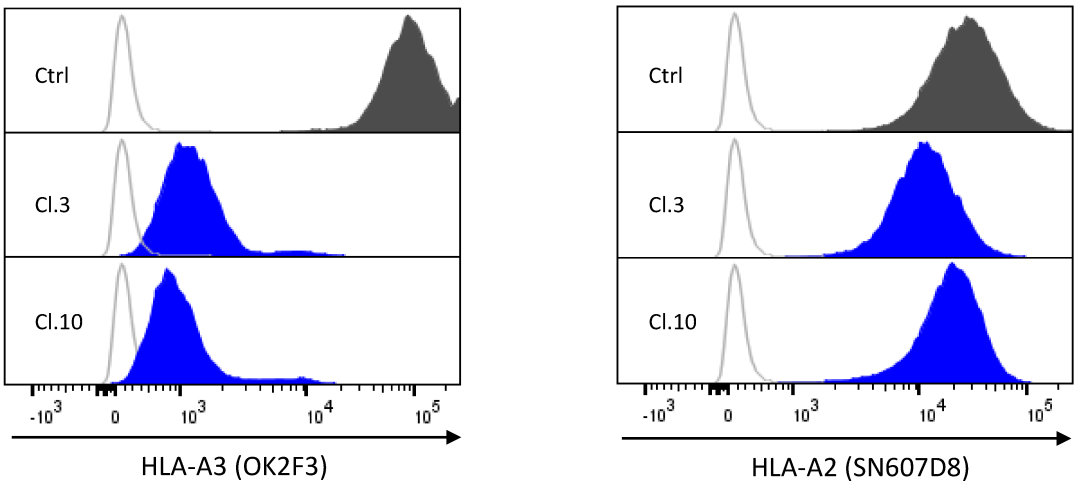
Supplementary figure 3. Strategy and verification of TAP1KO in NBS fibroblasts. **A** gRNA design (exon 2) and PCR strategy to detect TAP1KO. **B** FACS sort strategy and TAP1KO verification. **C** Sanger sequence chromatograms of TAP1KO fibroblast clones and vector control. **D** HLA-I expression in NBS fibroblast bulk after transduction with either TAP1-targeting gRNA or an empty vector control. **E** Relative expression of TAP1 mRNA in HLA-low sorted fibroblast clones compared to the HLA-high fibroblast-sorted population. Clones in blue were used for TAP1KO validation and subsequent experiments.

Supplementary Figure 4



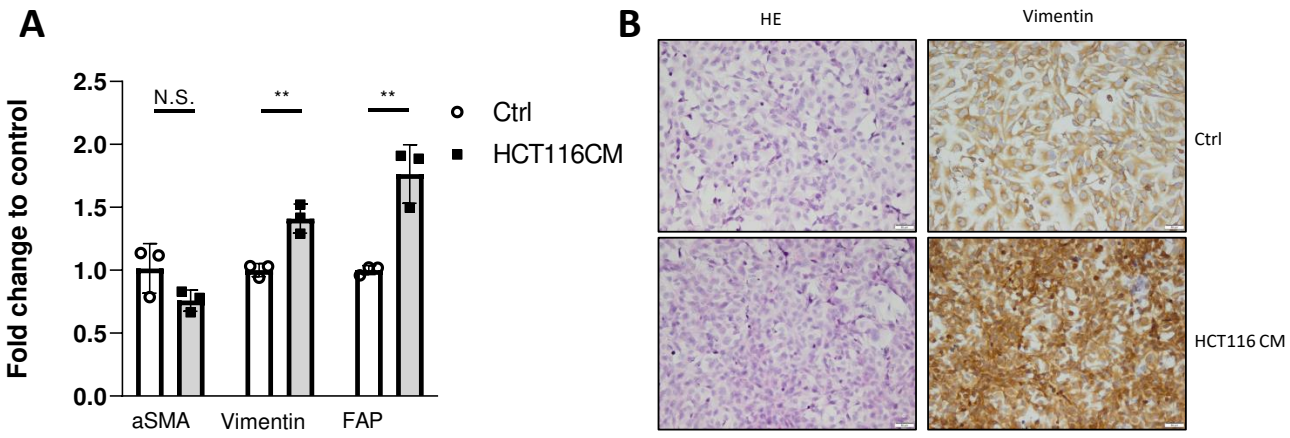
Supplementary figure 4. LRPAP1 expression in TAP1KO fibroblasts. Expression of LRPAP1 in TAP1KO (blue bars) and vector control (black bar) conditions (left panel). After RT-qPCR, amplicons were subjected to gel electrophoresis and imaged (right panel).

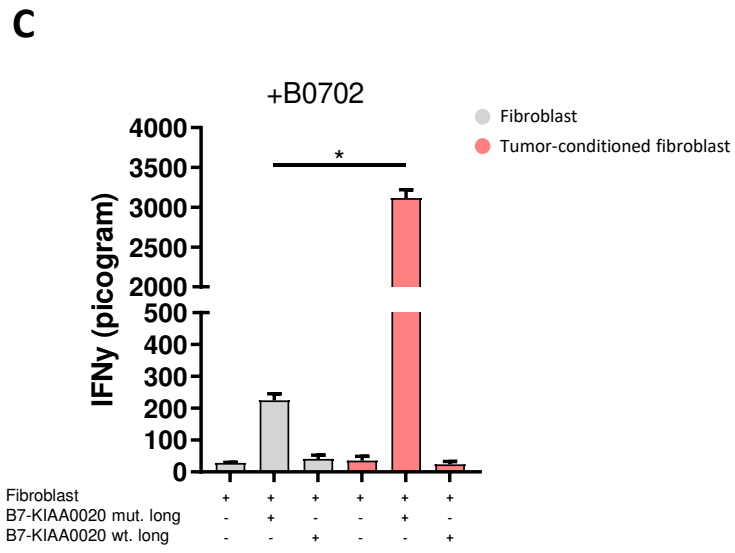
Supplementary Figure 5



Supplementary figure 5. Expression of HLA-A3 and HLA-A2 on TAP1KO fibroblasts. Expression of surface HLA-A3 and HLA-A2 in TAP1KO and vector control conditions determined by FACS.

Supplementary Figure 6



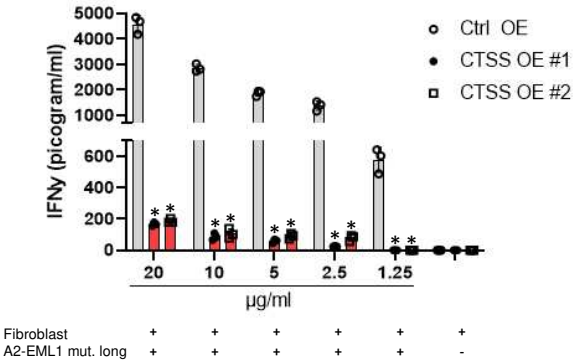


D

	IFNg	GM-CSF	IL-1 beta	Il-2	IL-4	IL-5	IL-6	IL-8	IL-10	TNF alpha
BLMwt	0,0	500,7	9,2	10,7	27,6	10,3	25310,2	16241,2	0,0	28,8
FM3	0,0	0,0	0,0	0,0	0,0	0,0	0,0	284,5	0,0	0,0
Mel0401	0,0	0,0	0,0	0,0	0,0	0,0	0,0	272,7	8,5	0,0
Mel1207	0,0	0,0	0,0	0,0	0,0	0,0	0,0	1638,4	0,0	0,0
518A2	0,0	22,8	2,0	5,7	3,0	0,0	4702,3	1813,6	0,0	13,3
HT29	0,0	0,0	0,0	0,0	0,0	0,0	0,0	198,7	0,0	0,0
SW480	0,0	22,8	2,0	5,7	13,8	10,3	147,3	7274,5	0,0	4,6
LS180	0,0	12,2	2,0	0,0	13,8	10,3	119,9	7395,5	0,0	0,0
HCT116	0,0	114,3	0,0	11,4	20,1	0,0	154,5	7128,7	0,0	0,0

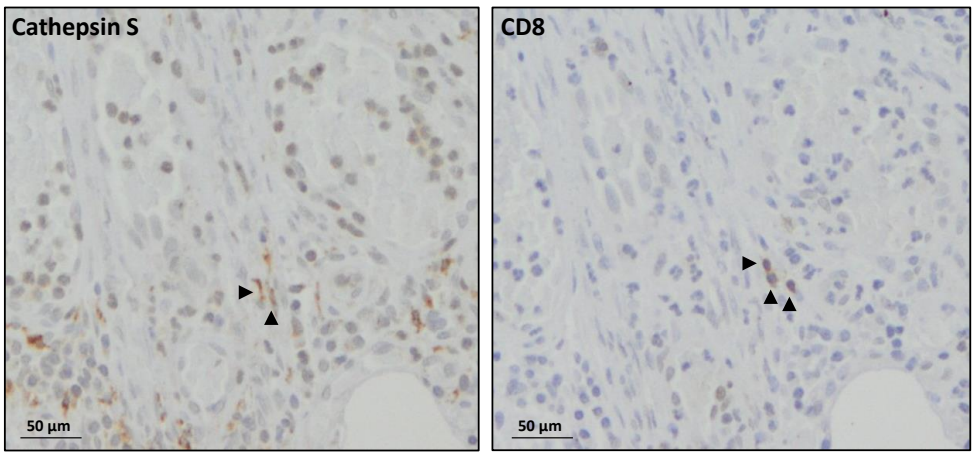
Supplementary figure 6. Expression of CAF-markers after CRC-conditioning of fibroblasts. **A** NBS fibroblasts were exposed to CRC-derived (HCT116) conditioned medium and the expression of αSMA, vimentin and FAP was determined. **B** NBS fibroblasts were seeded on cell-culture slips and were exposed to CRC-conditioned medium or control medium. After 48 hours, cells were stained for vimentin expression. **C** IFNγ production by neoantigen-specific T cells after 24h co-incubation with NBS fibroblast (grey) or CRC-conditioned NBS fibroblasts (red) that are loaded with mutated B7-KIAA0020$^{433\text{-}471(p451P>L)}$ or B7-KIAA0020$^{433\text{-}471(wildtype)}$ at 20 µg/ml. Student's t test *=p≤0.05 **D** LUMINEX data showing expression of inflammatory cytokines in CRC and melanoma-derived conditioned media.

Supplementary Figure 7



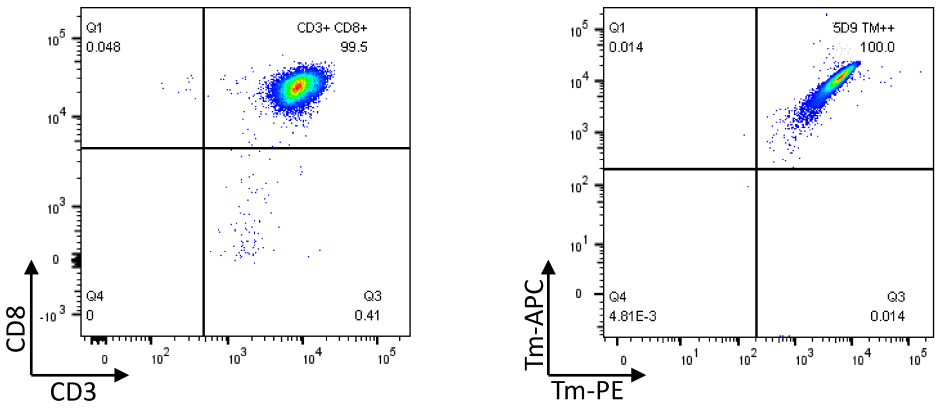
Supplementary figure 7. IFNγ production by neoantigen-specific T cells after 24h co-incubation with vector control (grey bars) and CTSS overexpressing NBS fibroblasts (red bars) that have been loaded with A2-EML1$^{50\text{-}80(p64R>W)}$ at indicated concentrations. Means with SD are plotted from representative experiments (n=2). Two-tailed ANOVA with correction for multiple testing *=P≤0.01

Supplementary Figure 8



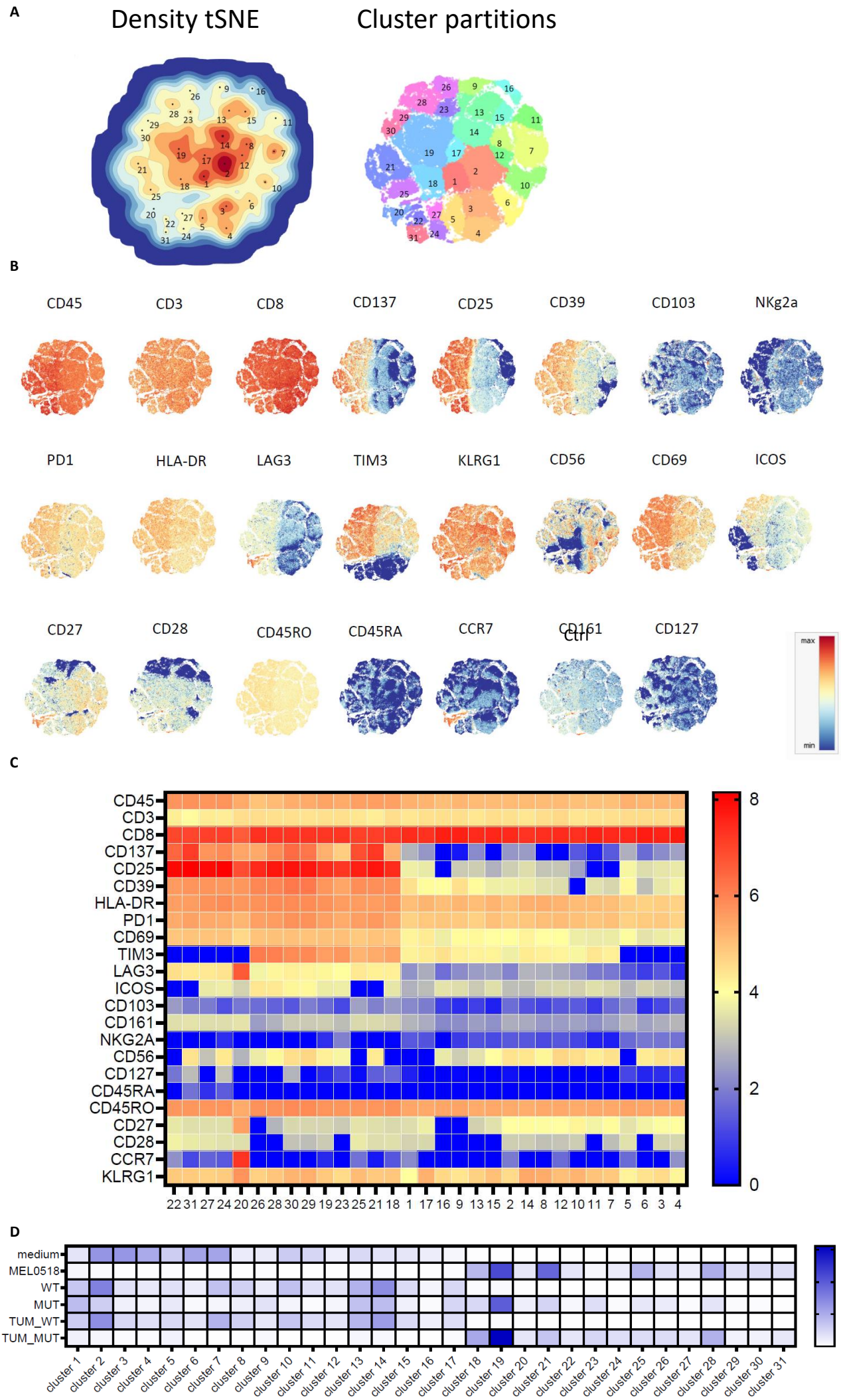
Supplementary figure 8. Co-localization of Cathepsin S-expressing fibroblasts and CD8+ T cells on sequential CRC slides. Arrowheads point to Cathepsin S+-fibroblasts (left panel) or CD8+ T cells (right panel).

Supplementary Figure 9



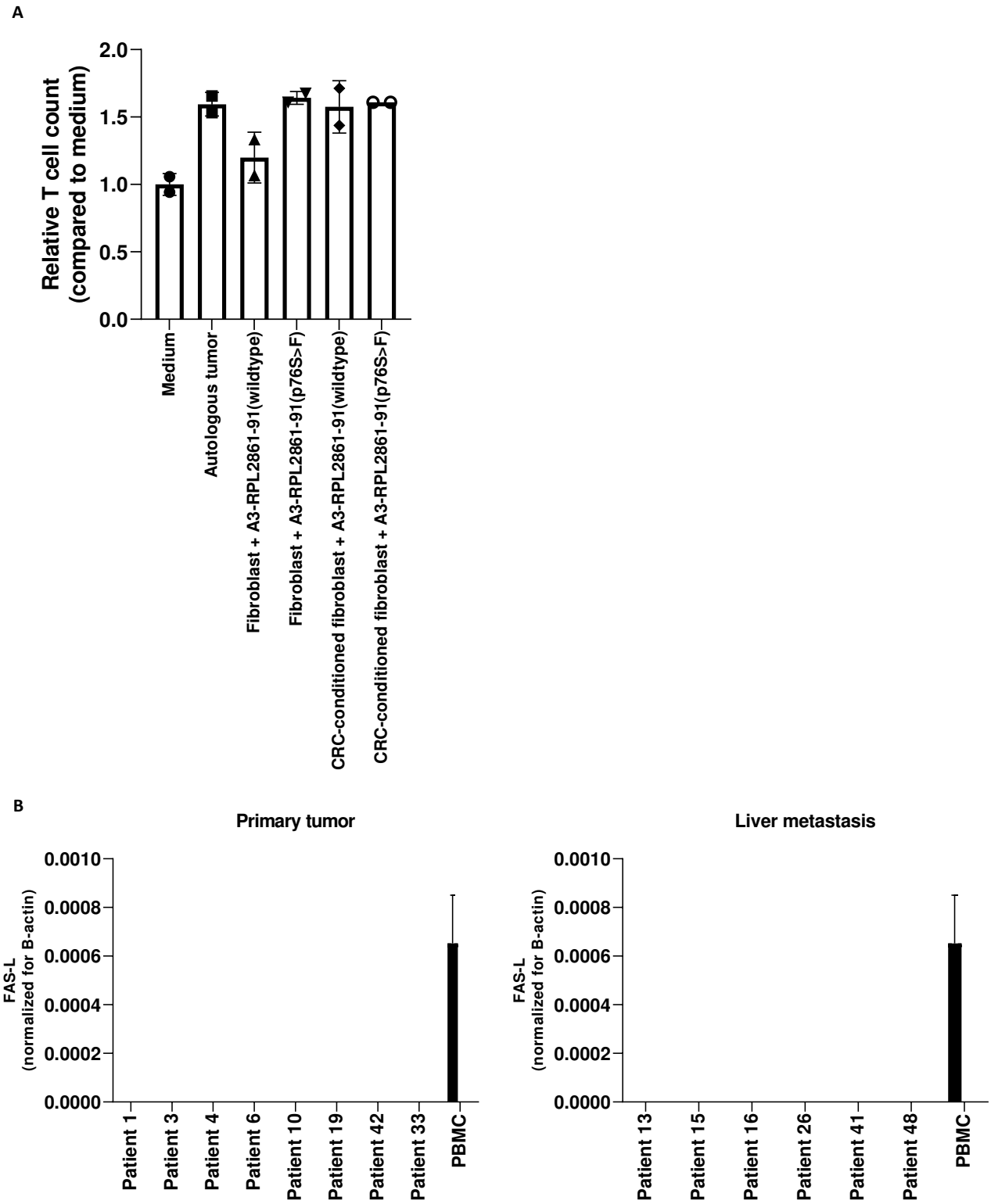
Supplementary figure 9. A3-RPL28$^{74\text{-}84\ (p76S>F)}$-sorting of T cells and marker expression. Sorting strategy for A3-RPL28$^{74\text{-}84\ (p76S>F)}$-specific T cells using both PE and APC-labeled tetramers.

Supplementary Figure 10



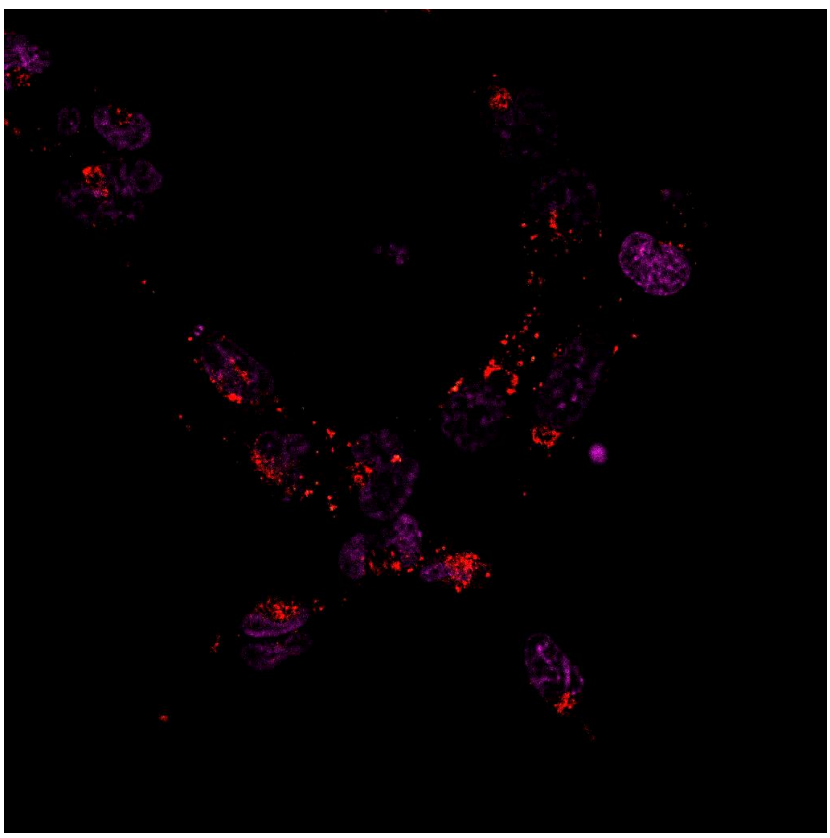
Supplementary figure 10. Effect of fibroblast-mediated antigen presentation on T cell marker expression. **A** Density tSNE and cluster partitioning of A3-RPL28$^{74\text{-}84\ (p76S>F)}$-specific T cells for all conditions combined. **B** tSNE plots of Individual marker expression for the entire panel of activating and inhibitory receptors. **C** Relative abundance of markers present in the cluster partitioning. **D** Percentage of T cells in the separate clusters for individual conditions.

Supplementary Figure 11



Supplementary figure 11. No evidence for FAS-L mediated killing of neoantigen-specific T cells. **A** Relative T cell count of A3-RPL28$^{74\text{-}84\ (p76S>F)}$-specific T cells after pre-incubation with different target conditions for 24 hours. **B** FAS-L ligand expression of primary CRC and CRC-liver metastasis derived CAFs and matched normal fibroblasts. No expression was detected in any of the fibroblasts, peripheral blood mononuclear cells (PBMC) served as a positive control for efficacy of FAS-L specific primers.

Supplementary Video 1



Supplementary video 1. Imaging of A3-RPL28$^{61\text{-}91(p76S>F)}$ processing in fibroblasts. 1-hour clip of A3-RPL28$^{61\text{-}91(p76S>F)}$ (green) processing in NBS fibroblasts (purple nuclei) with labeling of the lysosomal compartment (red).