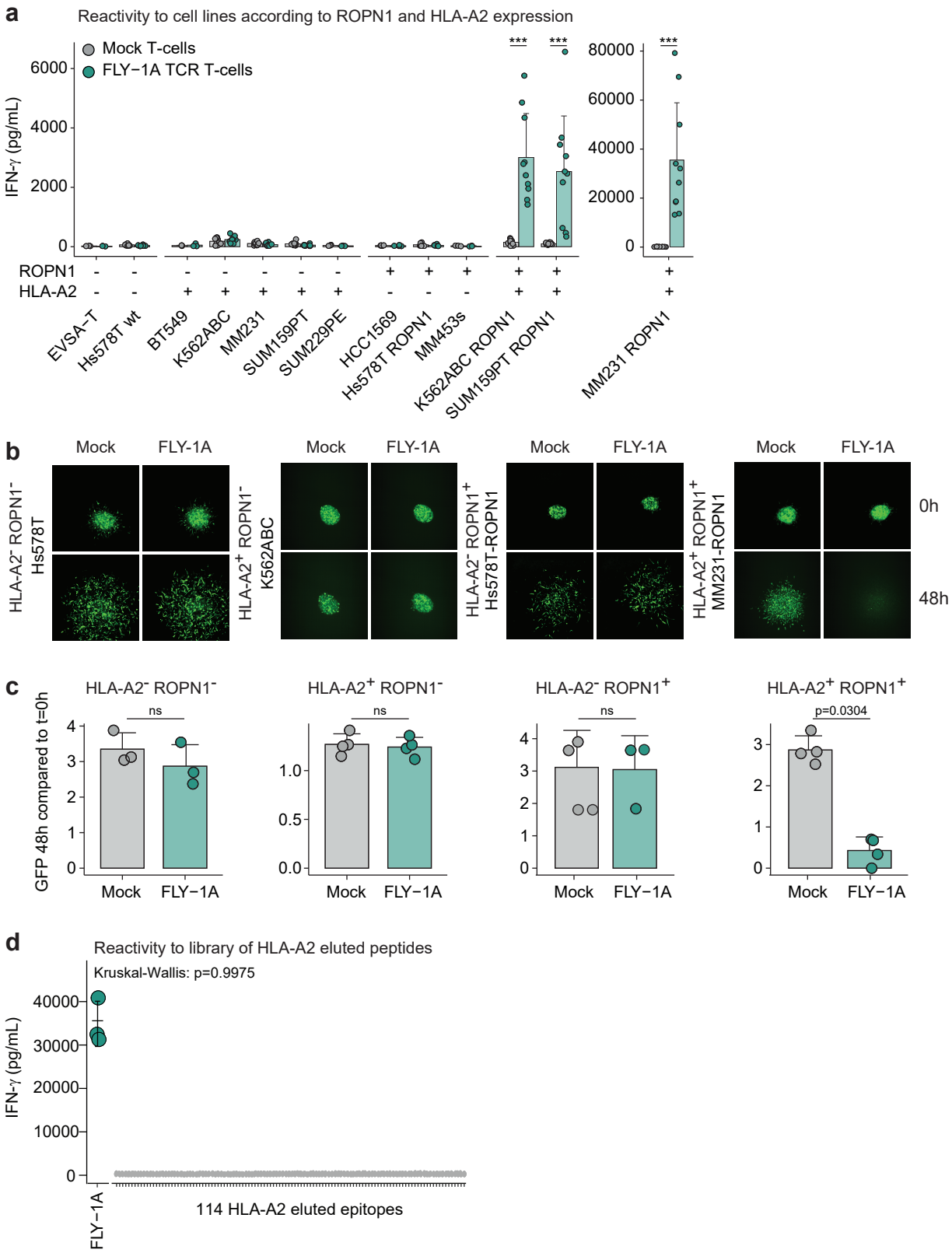
Supplementary Figure 4.



Supplementary figure 4. FLY-1A TCR T-cells do not recognize ROPN1-negative and/or HLA-A2-negative tumor cells. a) Bar graphs display no enhanced IFN-γ production by FLY-1A TCR T-cells upon co-culture with tumor cell lines that lack ROPN1 and/or HLA-A2 (n=6-10 replicates, n=5 donors). Supernatants were collected O/N and used to measure IFN-γ levels as described in legend to **Figure 2h**. Mock T-cells were used as a negative control (grey). Cell lines expressing ROPN1 and HLA-A2 were used as positive controls. The Wilcoxon signed-rank test was performed to test significance between FLY-1A TCR versus Mock T-cells and only significant differences are shown. **b)** Representative confocal fluorescence microscopy images demonstrate no killing by FLY-1A TCR T-cells upon co-culture with organoids derived from tumor cell lines that lack ROPN1 and/or HLA-A2 (n=3-4 per cell line). Green colors represent GFP-expressing organoids following imaging at 0 and 48h after co-culture with T-cells. Mock T-cells were included as a negative control. Organoids derived from the MM231 cell line expressing ROPN1 and HLA-A2 were used as positive controls. **c)** Bar graphs display GFP signals from organoids derived from cell lines from c (n=3-4 per cell line). Organoids were monitored at 48h after addition of Mock or FLY-1A TCR T-cells and signals were expressed relative to 0h as described in legend to Figure 4b. The Wilcoxon signed-rank test was performed to test significance between FLY-1A TCR versus Mock T-cells. **d)** IFN-γ production by FLY-1A TCR T-cells upon co-culture with BSM cells that were loaded with a library of 114 HLA-A2-eluted peptides. IFN-γ production was depicted as FC compared to the cognate epitope (n=3). Kruskal-Wallis: p=0.9975. Individual points, mean and SD are shown.