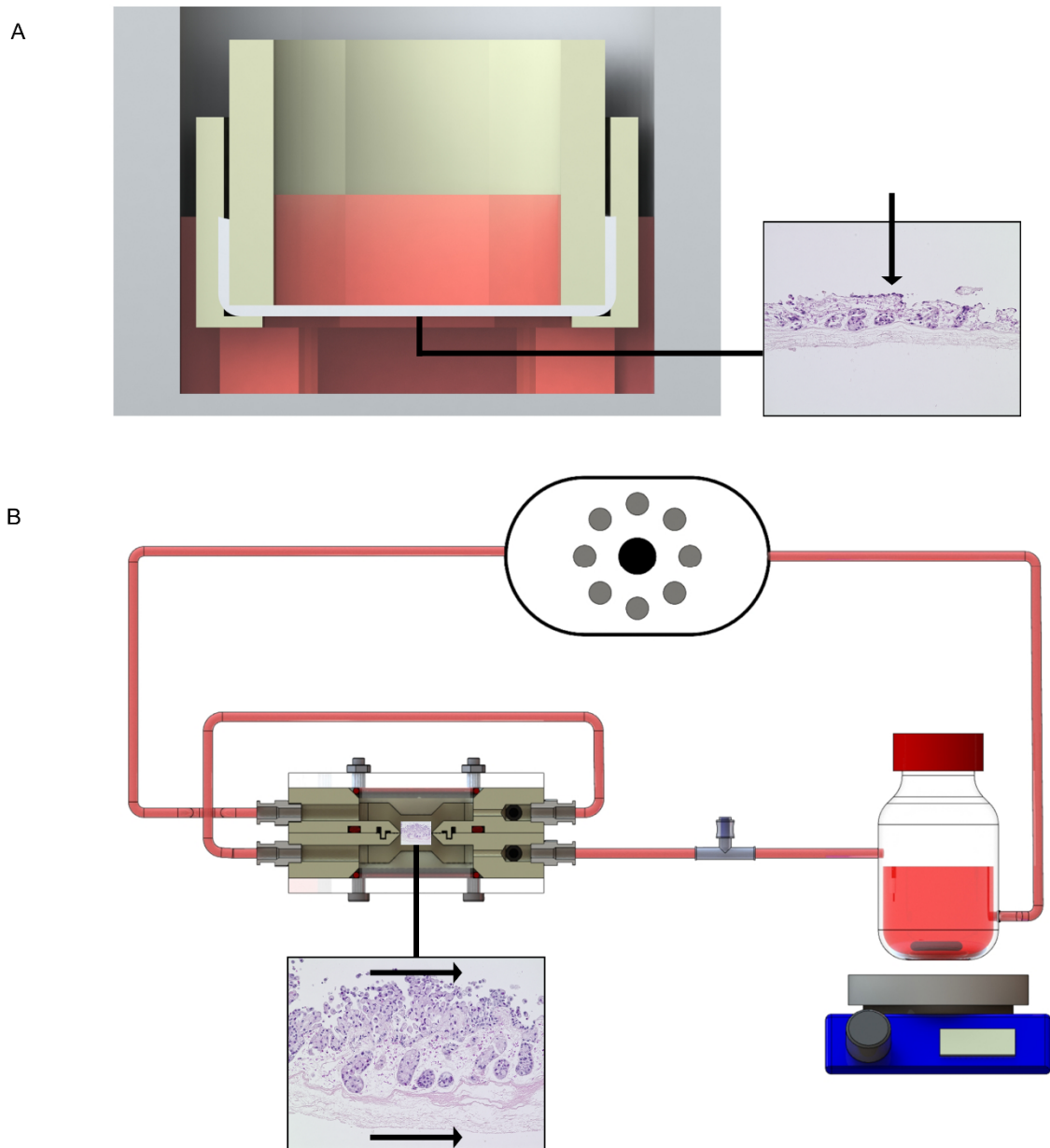
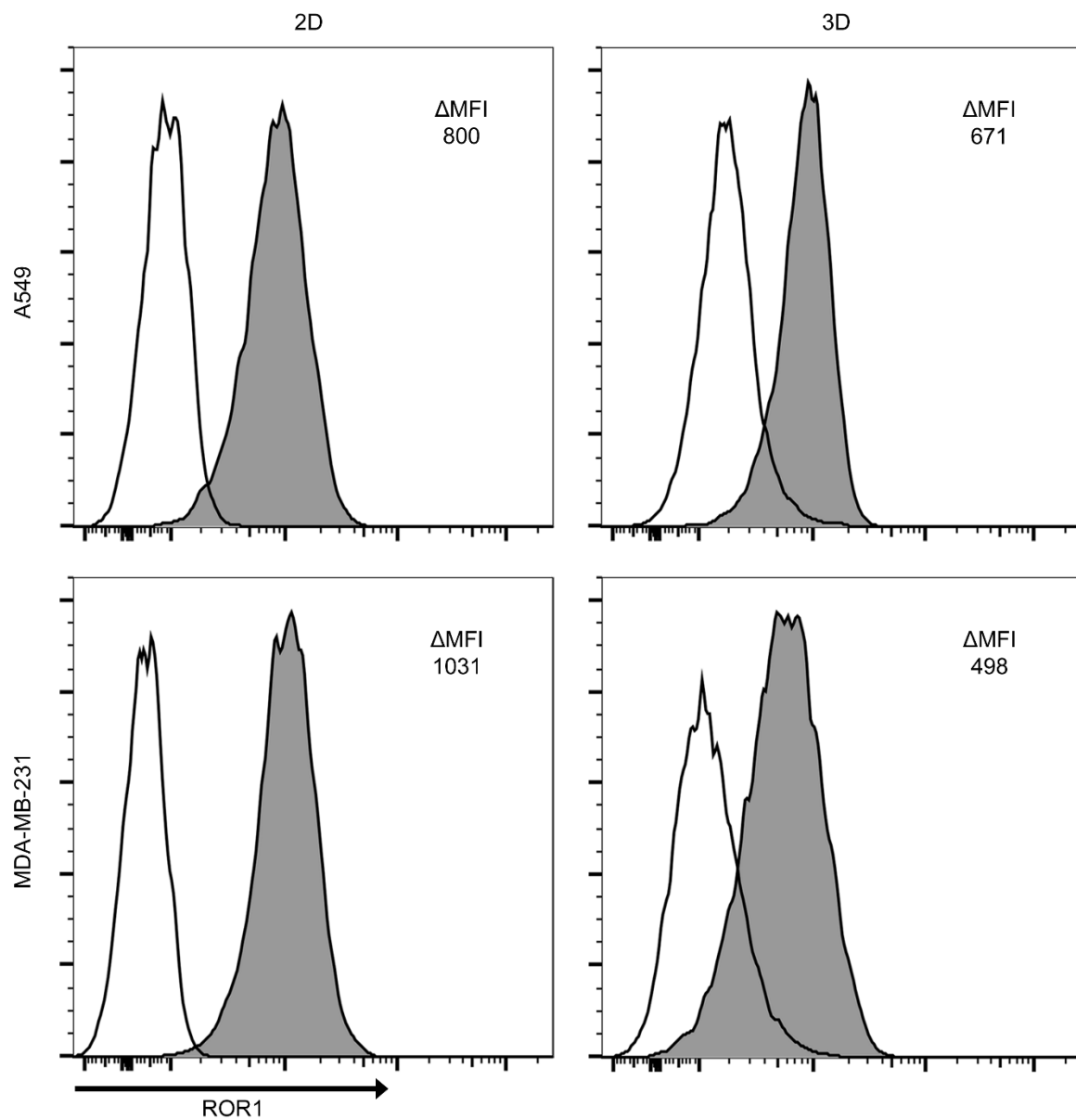


Supplement

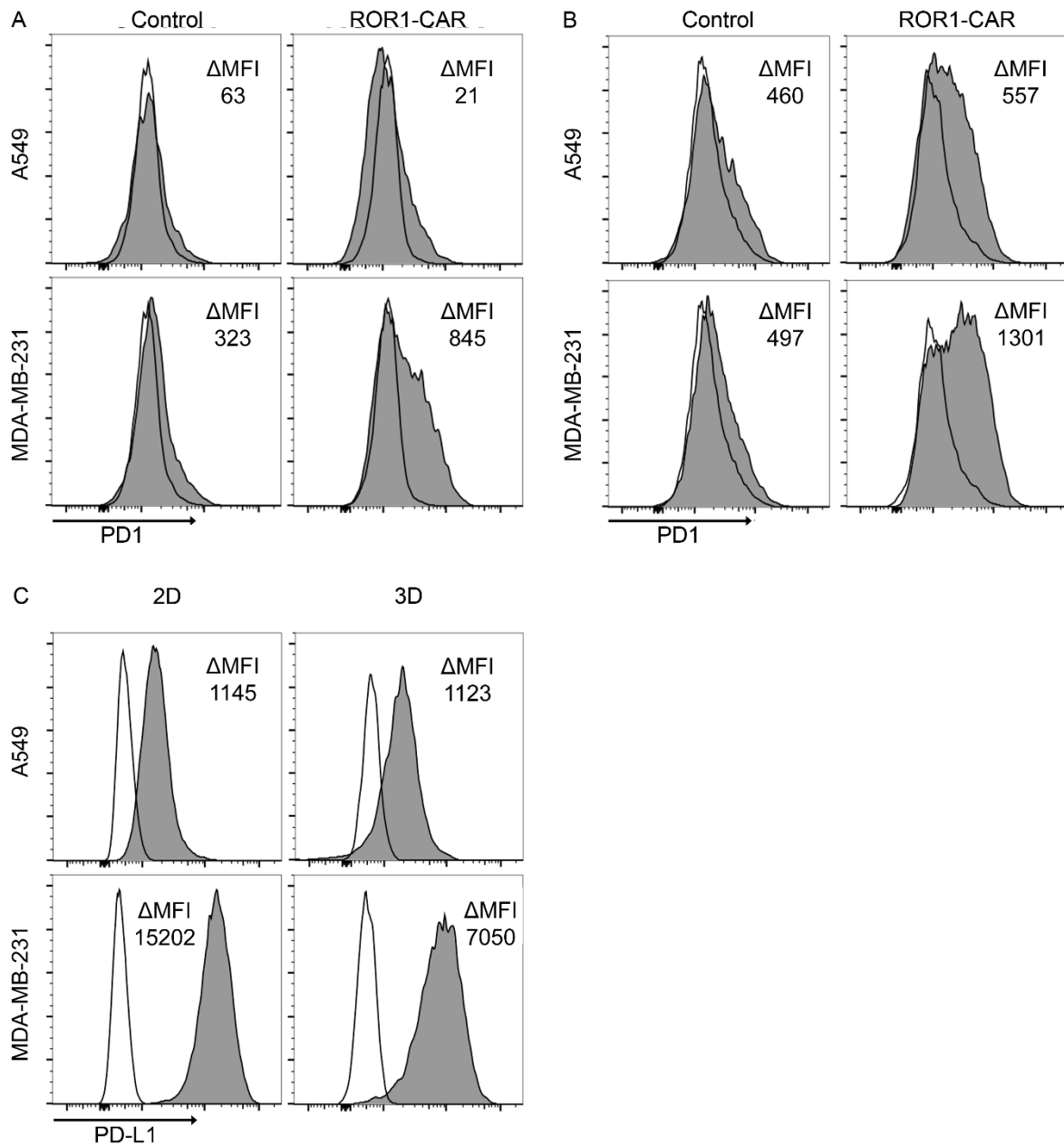
Supplemental Figures and Supplemental Figure legends



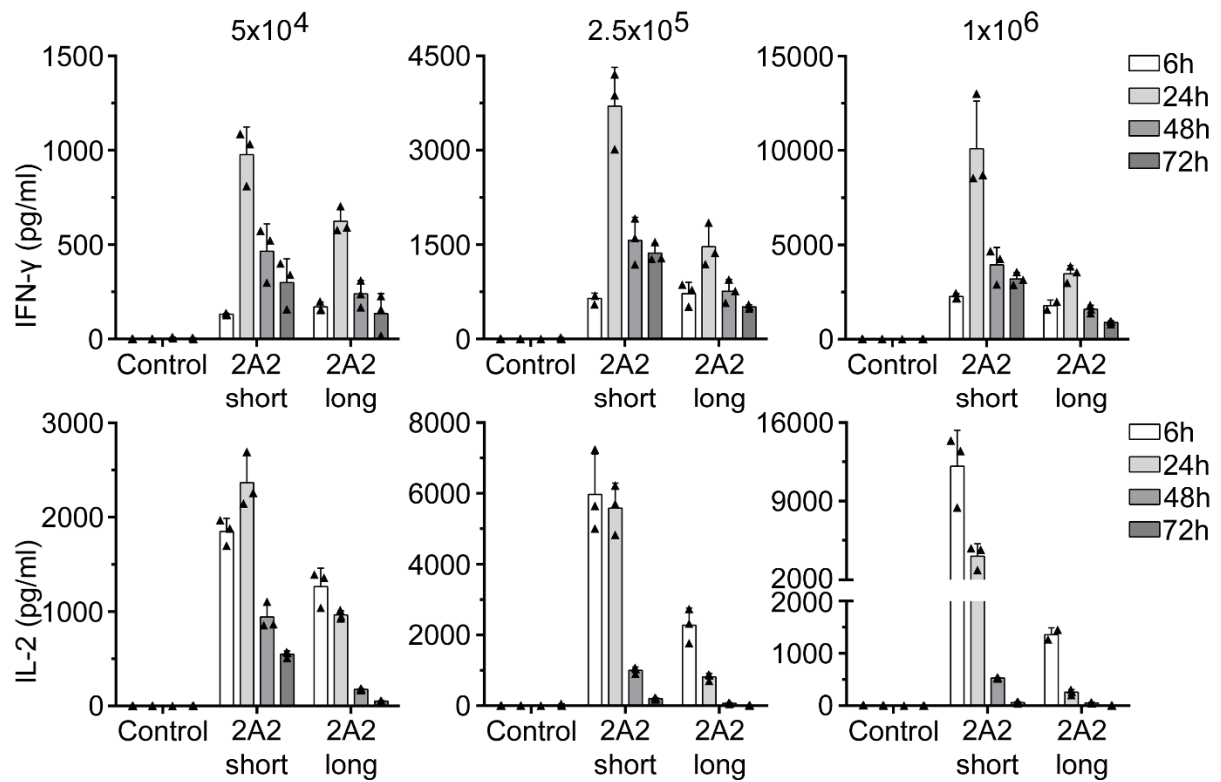
Supplemental Figure 1. Experimental set-up of static and dynamic 3D lung cancer and breast cancer models. A, In static culture, the SISmuc scaffold is fixed between two tailored metal rings (cell crown). T-cells are applied to the inner compartment of the cell crown and thus reach the 3D tumor cells from the top (arrow). **B,** In dynamic culture, medium is continuously pumped through the tubing set by a peristaltic pump. T-cells are applied to the medium reservoir, and can assess the tumor tissue through the tubing system.



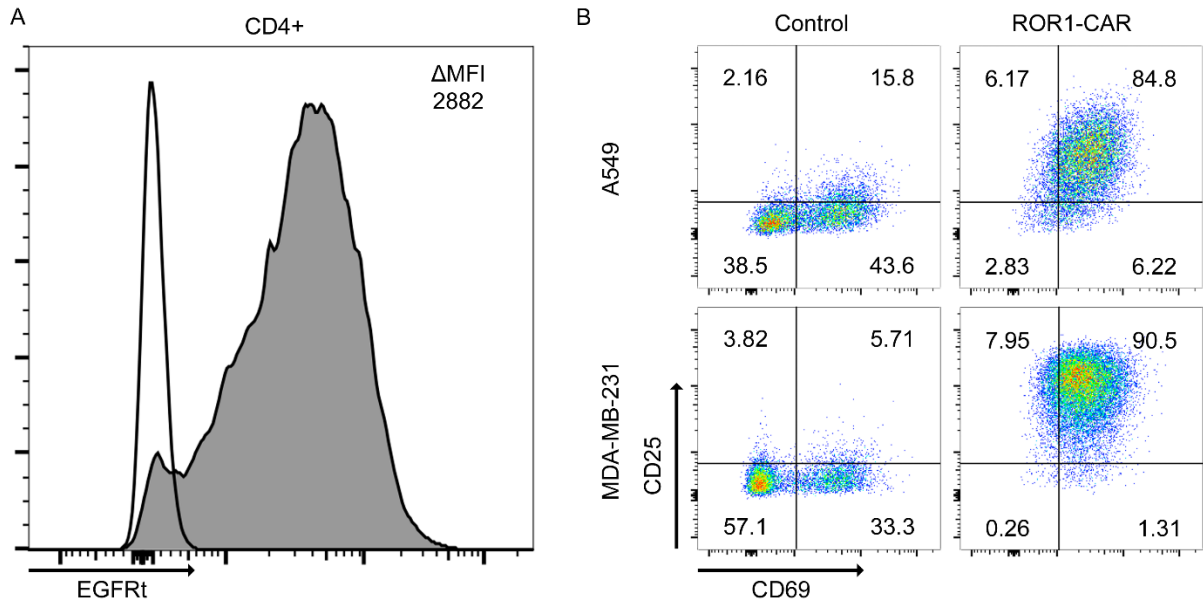
Supplemental Figure 2. ROR1 expression on tumor cell lines in 2D and 3D conditions. ROR1 expression on A549 and MDA-MB-231 tumor cells under conventional 2D culture conditions (2D) or static 3D culture (3D) was assessed by flow cytometric analysis. Δ MFI depicts the difference between fluorochrome conjugated anti-ROR1 antibody and isotype control. One representative of two experiments is shown.



Supplemental Figure 3. PD-1 expression on T-cells and PD-L1 expression on tumor cells. A, Expression of PD-1 on CD8⁺ T-cells and **B,** on CD4⁺ T-cells after 72 h treatment in static 3D culture. n=1, Δ MFI depicts the difference in geometric mean fluorescence intensity between T-cells from 3D culture and T-cells in medium. **C,** Expression of PD-L1 on tumor cells obtained from conventional 2D culture (left histograms) and tumor cells that were re-isolated from static 3D tumor models (right histograms) was analyzed by flow cytometry. n=1, Δ MFI depicts the difference between fluorochrome-conjugated anti-PD-L1 antibody and isotype control.



Supplemental Figure 4. Variations in ROR1-CAR spacer design affect anti-tumor function in 3D lung cancer models. ELISA-based quantification of IFN- γ and IL-2 from supernatants collected at the indicated time point from static tumor models treated for 72 h with increasing numbers of CD8⁺ unmodified control T-cells or ROR1-CAR T-cells with 2A2 targeting domain and either a short (IgG4-Fc hinge) or long (IgG4-Fc hinge-C_{H2}-C_{H3}) spacer domain. Data are presented as arithmetic mean of three cell crowns from n=1 experiment + SD.



Supplemental Figure 5. Phenotypic analysis of CD4⁺ T-cells. **A**, Expression of EGFRt transduction marker on CD4⁺ T-cells prior to functional testing. Δ MFI depicts the difference in geometric mean fluorescence intensity between unmodified control T-cells and ROR1-CAR T-cells. **B**, After five days of treatment, CD4⁺ T-cells from supernatants were analyzed for expression of activation markers (CD25 and CD69) by flow cytometry. One representative plot of n=4 experiments is shown.