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Supplemental Information

T Cell-Activating Mesenchymal Stem Cells as a Biotherapeutic for HCC

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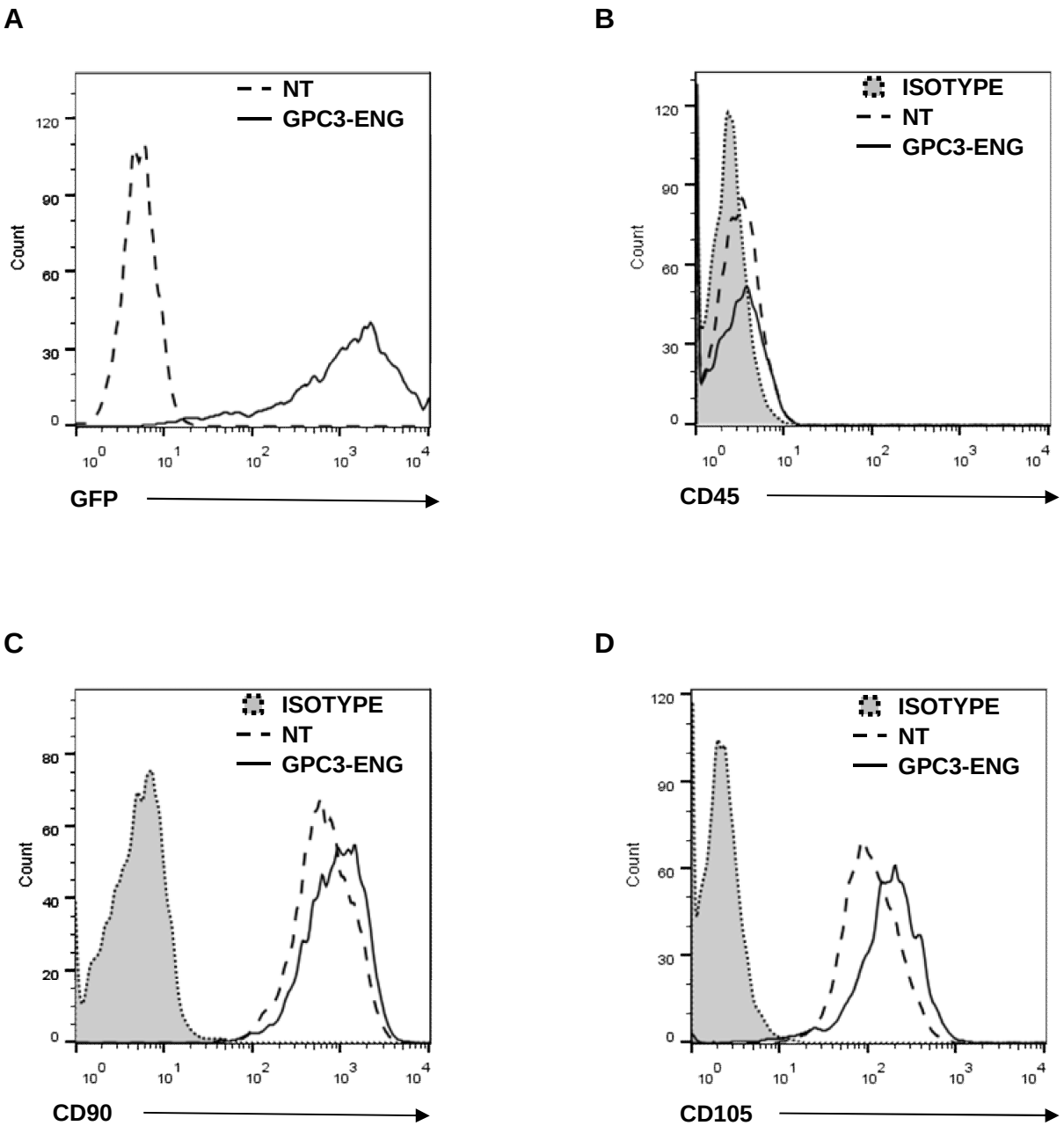
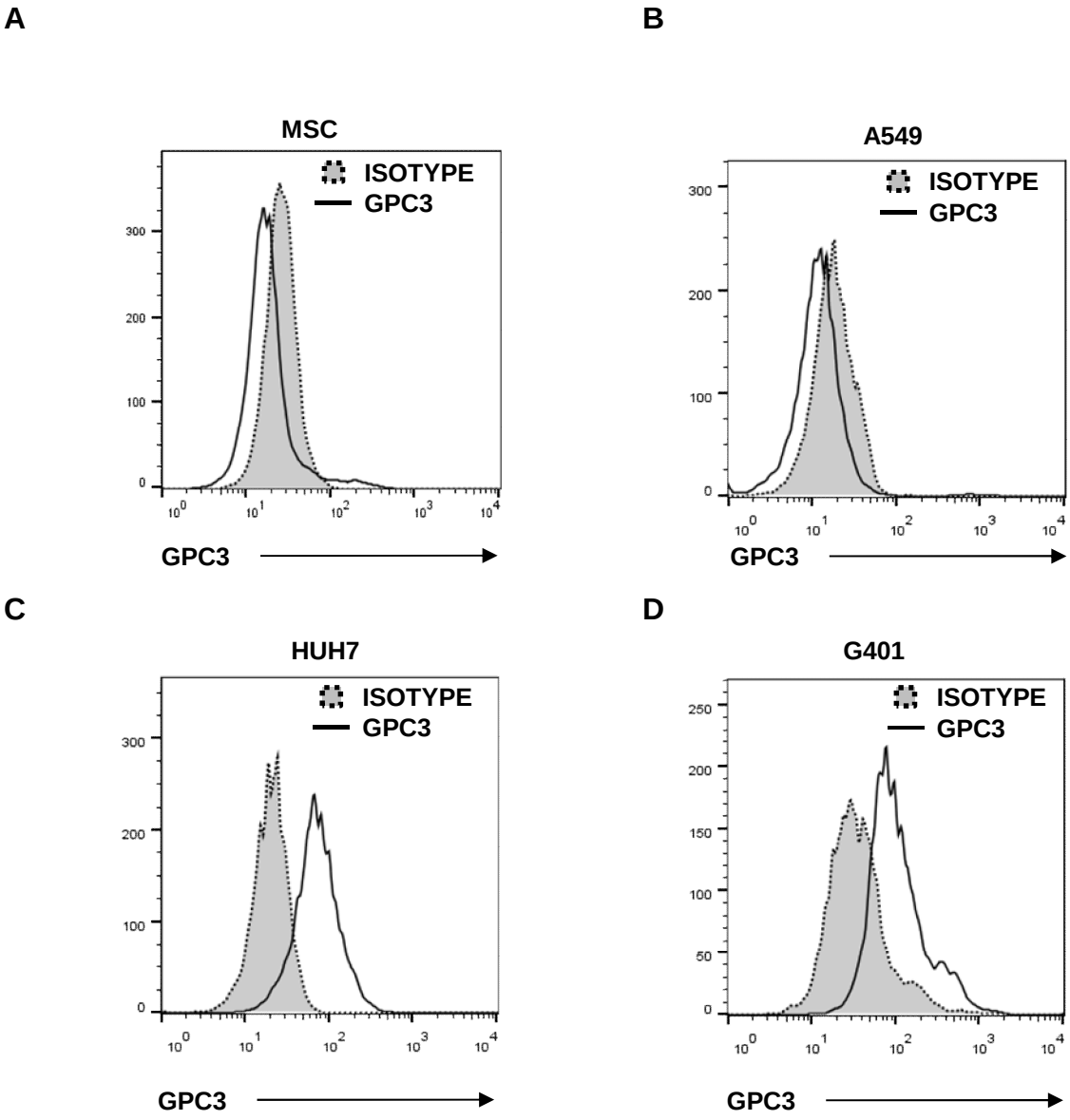


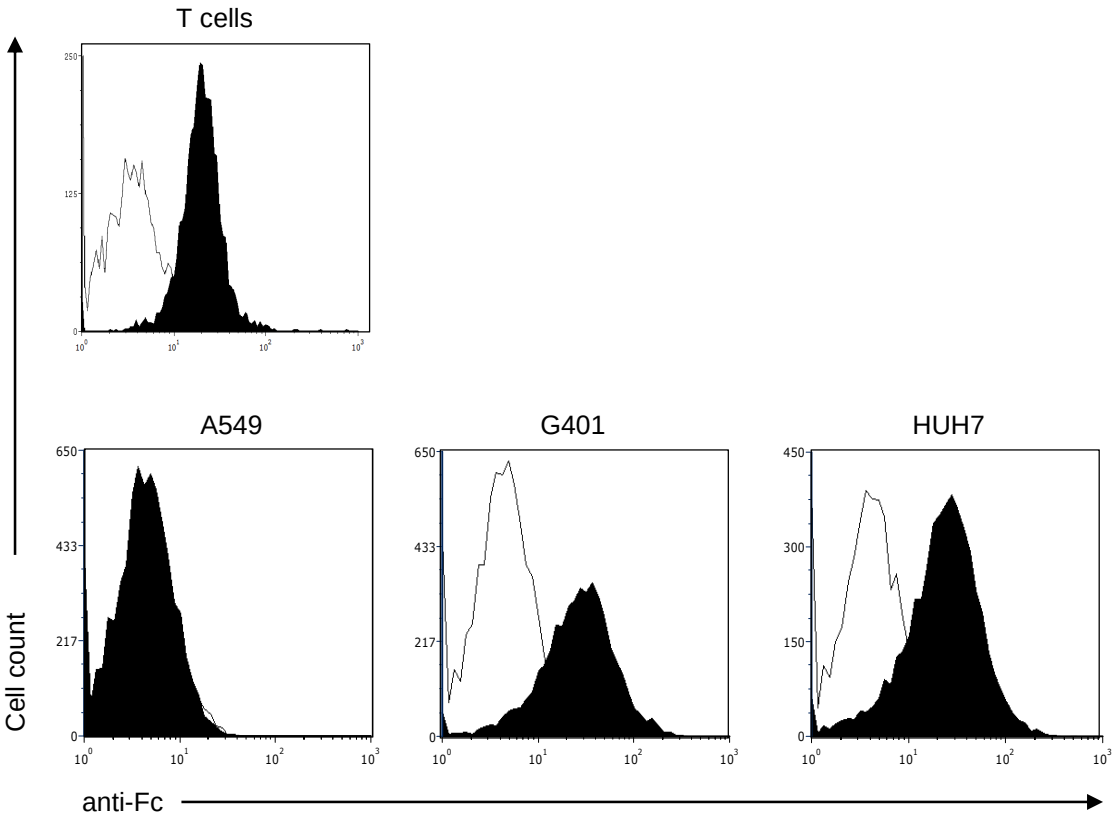
Supplementary Figure 1: Phenotypic analysis of GPC3-ENG MSCs. Representative FACS diagram of (**a**) GFP, (**b**) CD45, (**c**) CD90 and (**d**) CD105 expression of GPC3-ENG and NT MSCs.

Supplementary Figure 1



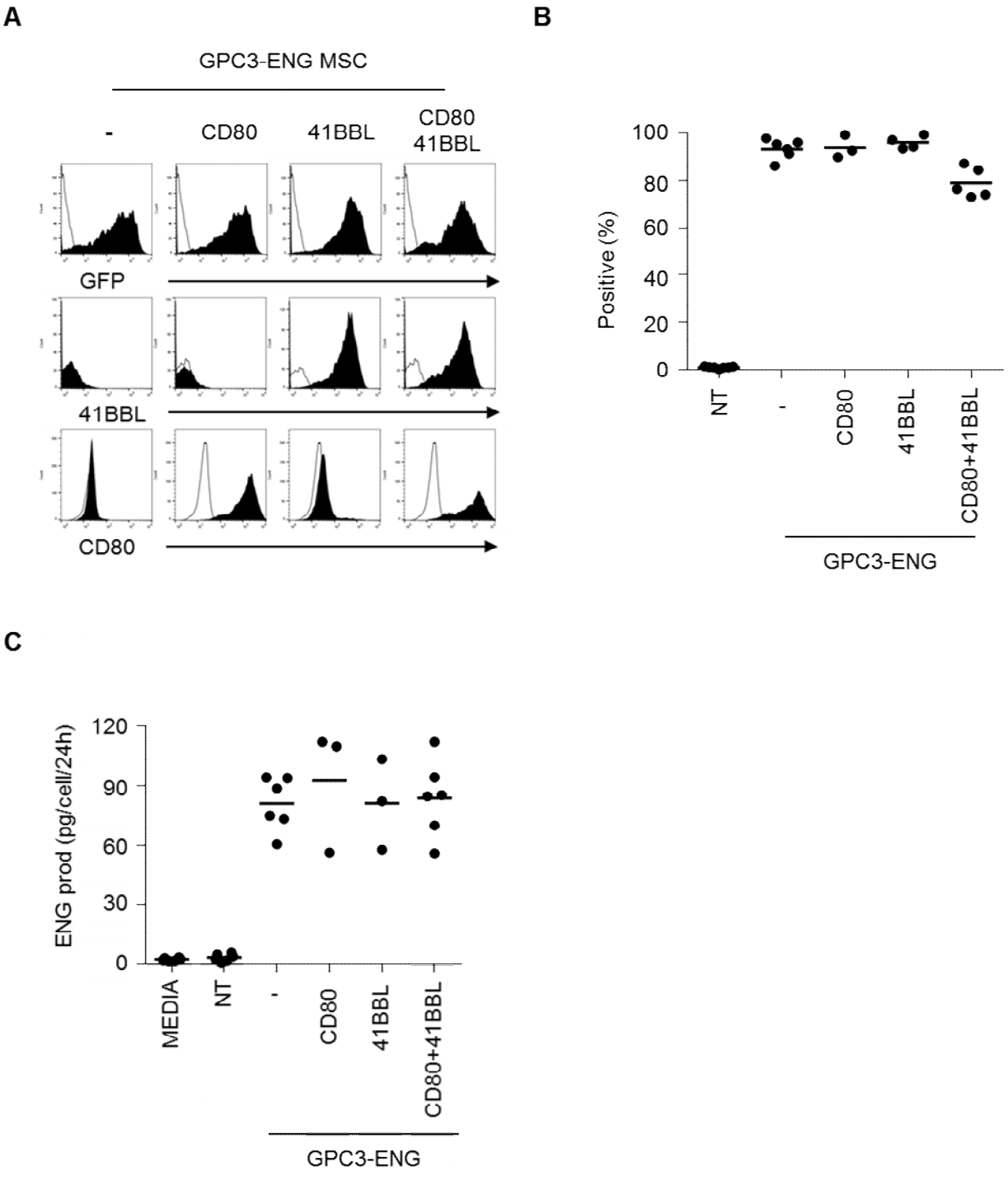
Supplementary Figure 2: GPC3 expression of MSCs and tumor cell lines. Representative FACS diagram of GPC3 expression of (**a**) MSC, (**b**) A549, (**c**) HUH7 and (**d**) G401 cells.

Supplementary Figure 2



Supplementary Figure 3: Target specific binding of GPC3-ENGs. T cells and GPC3-positive (G401; HUH7) or negative (A549) target cells were incubated with supernatant from GPC3-ENG or non-transduced (NT) MSCs. After 24 hours cells were then washed, and incubated with recombinant GPC3-Fc (R&D Systems; for T cells) or CD3e-Fc (Creative BioMart; for tumor cells) protein. Bound recombinant protein was detected with an Fc antibody (GPC3-ENG MSC supernatant: filled curve; NT MSC supernatant: open curve.

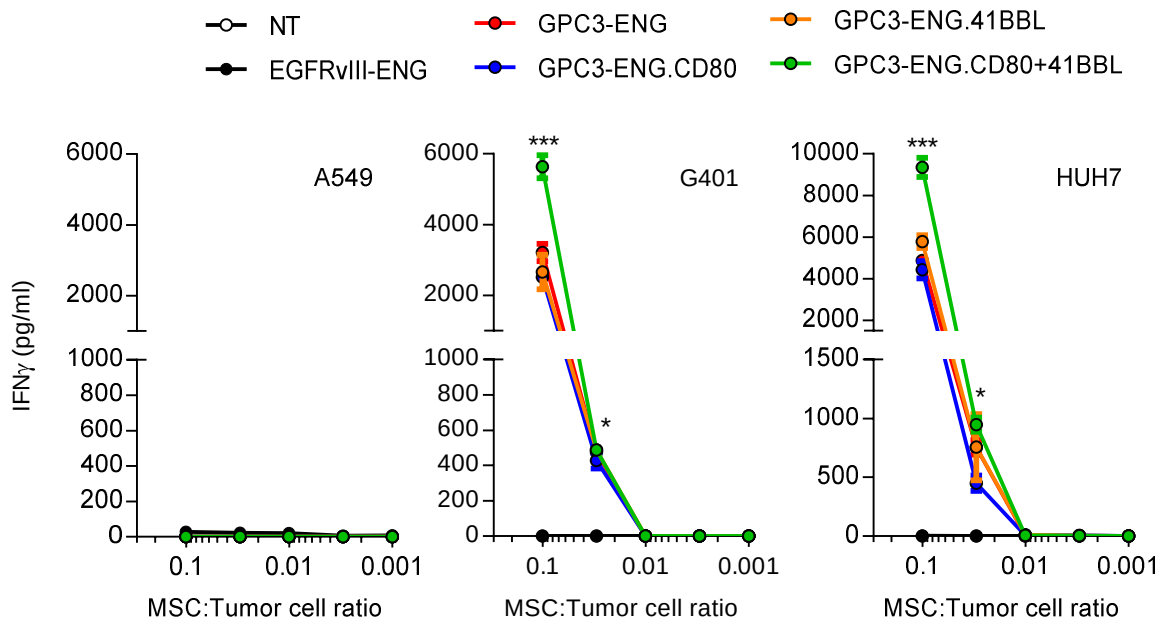
Supplementary Figure 3



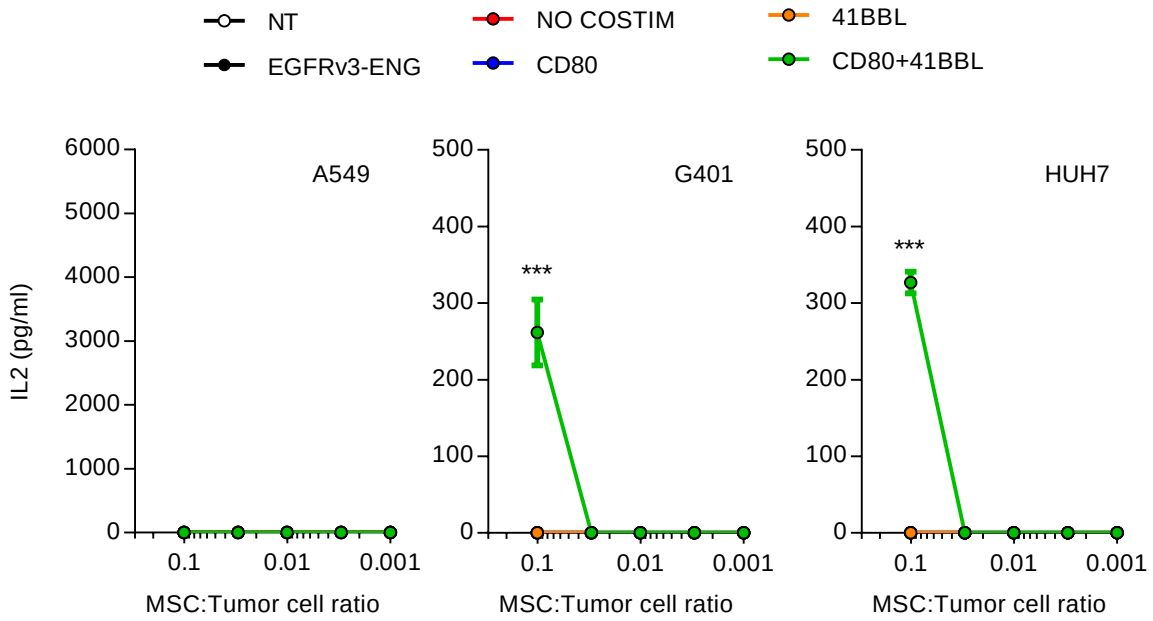
Supplementary Figure 4: Generation of GPC3-ENG MSCs expressing CD80 and 41BBL costimulatory molecules. (**a,b**) Representative FACS diagram and summary data. (**c**) Detection of GPC3-ENG protein in media of GPC3-ENG, GPC3-ENG.CD80, GPC3-ENG.41BBL and GPC3-ENG.CD80+41BBL MSCs after 24 hours of culture (GPC3-ENG.CD80, GPC3-ENG.41BBL MSCs (n=3); NT MSCs (n=4); GPC3-ENG, GPC3-ENG.CD80+41BBL MSCs (n =6; performed in duplicates).

Supplementary Figure 4

A

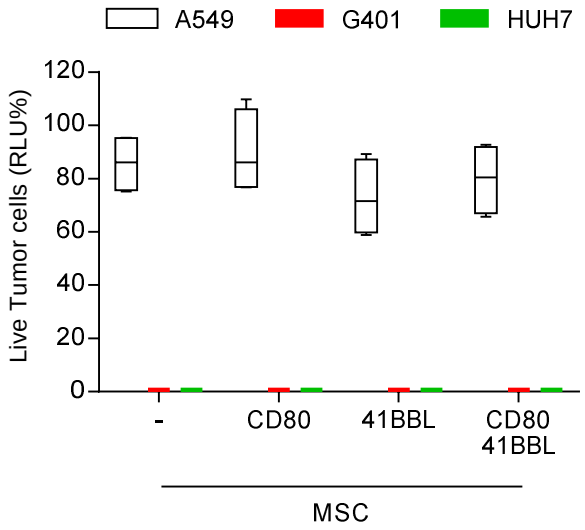


B

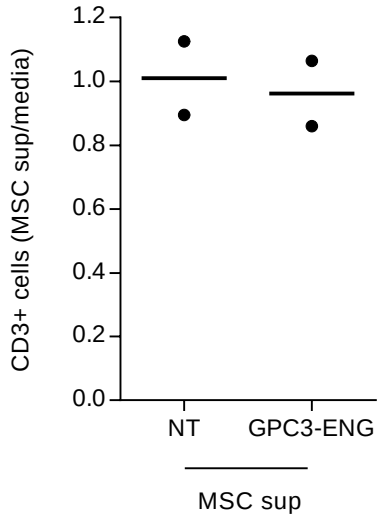


Supplemental Figure 5: GPC3-ENG MSCs at a MSC to tumor cell ratio of 1:100 induce IFNγ production. GPC3-ENG, GPC3-ENG.CD80, GPC3-ENG.41BBL or GPC3-ENG.CD80+41BBL MSCs were cocultured with GPC3-positive (G401, HUH7) or -negative (A549) cell lines at a 1:10, 1:30, 1:100, 1:300, 1:1000 MSC to tumor cell ratio in the presence of human T cells (10:1 T-cell to tumor cell ratio). After 24 hours, IFNγ (**a**) or IL-2 (**b**) was determined by ELISA (n = 2, assay performed in duplicates, p*<0.05, ***p<0.001).

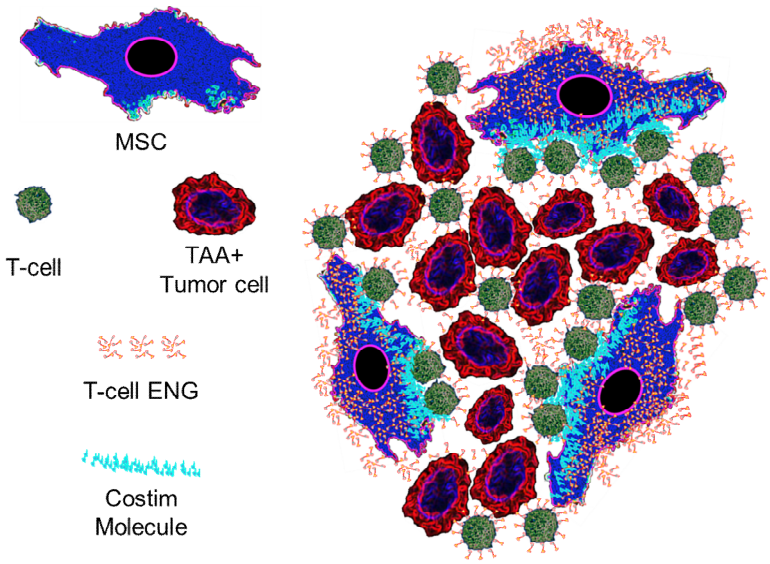
Supplementary Figure 5



Supplementary Figure 6: GPC3-ENG MSCs expressing CD80 and/or 41BBL eliminate GPC3+ target cells after 48 hours in coculture. GPC3-ENG, GPC3-ENG.CD80, GPC3-ENG.41BBL or GPC3-ENG.CD80+41BBL MSCs were cocultured with GPC3-positive (G401.ffluc; HUH7.ffluc) or -negative (A549.ffluc) cell lines in 1:10 MSC to tumor cell ratios in the presence of human T cells (10:1 T-cell to tumor cell ratio). After 48 hours a luciferase-based cytotoxicity assay was performed (n=2; assay performed in duplicate).

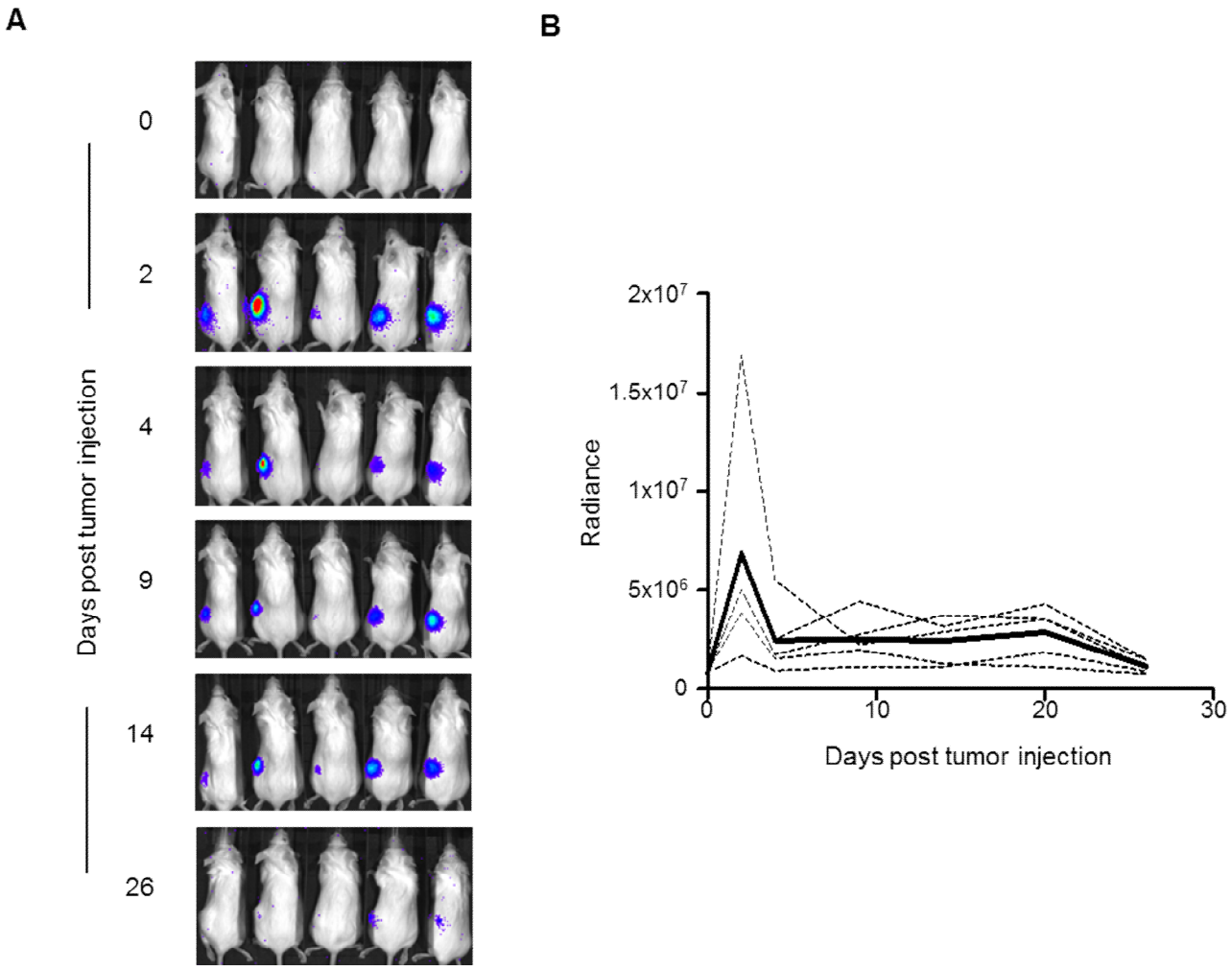


Supplementary Figure 7: GPC3-ENG protein does not induce T cell proliferation in absence of GPC3-positive tumor cells. T cells were incubated with supernatants from GPC3-ENG or NT MSCs, or media. After 72 hours absolute number of CD3-positive cells was determined by FACS analysis. Relative ratio of MSC sup/media is shown (n=2).



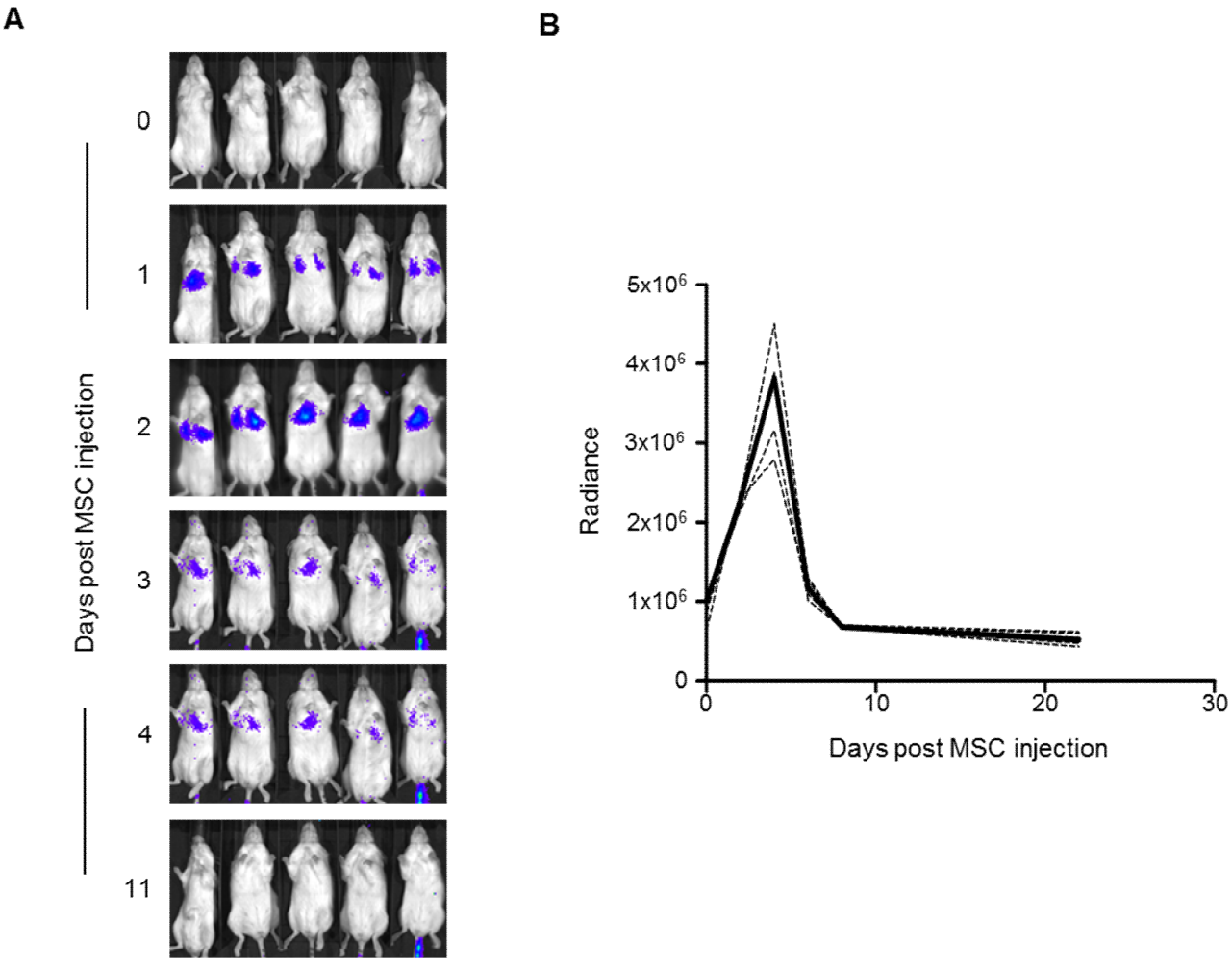
Supplementary Figure 8: Schematic model of T-cell activating MSCs. For details see text.

Supplementary Figure 8



Supplementary Figure 9: Persistence of GPC3-ENG MSCs after subcutaneous injection. Mice were injected s.c. with 5×10^6 HUH7 cells and 5×10^5 GPC3-ENG.eGFP.ffLuc MSCs (n=5) on their left lower flank. MSC persistence was followed by bioluminescence imaging. (**a**) Representative images of animals. (**b**) Quantitative bioluminescence imaging results (solid lines: mean; dotted lines: individual mice; radiance=photons/sec/cm^2/sr).

Supplementary Figure 9



Supplementary Figure 10: Persistence of GPC3-ENG MSCs after intravenous injection. Mice were injected i.v. in their tail vein with 5×10^5 GPC3-ENG.eGFP.ffLuc MSCs (n=5). MSC persistence was followed by bioluminescence imaging. (**a**) Representative images of animals. (**b**) Quantitative bioluminescence imaging results (solid lines: mean; dotted lines: individual mice; radiance=photons/sec/cm^2/sr).

Supplementary Figure 10