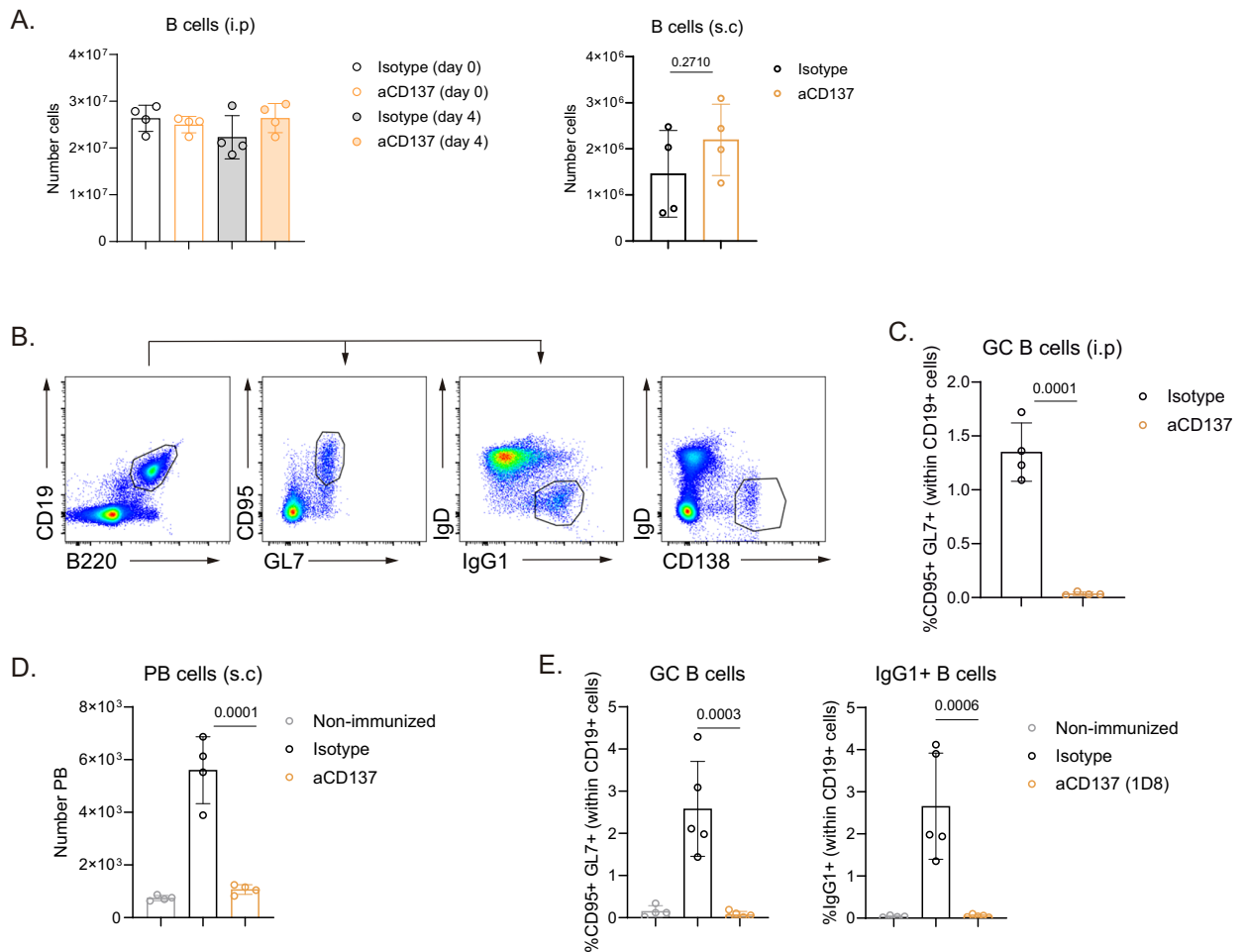


Expanded View Figures

**Figure EV1. Methodology and analysis of B-cell responses upon anti-CD137 treatment.**

(A) Number of B cells in the spleen (left) or dLNs (right) of mice immunized with ovalbumin mixed with Alhydrogel intraperitoneally (100 µg) or subcutaneously (10 µg/site). Mice immunized intraperitoneally were treated at day 0 or 4 with anti-CD137 or its isotype control (100 µg i.p.). Mice immunized subcutaneously were treated at day 0 with anti-CD137 or its isotype control (100 µg i.p.) ($n = 4$ mice; representative of two independent experiments). (B) Flow cytometry gating strategy for B cells, GC B cells, IgG1 CS B cells and PB. (C) Percentage of GC B cells in the spleen of mice immunized i.p. with ovalbumin mixed with Alhydrogel and treated at day 4 with anti-CD137 or its isotype control (100 µg i.p.) ($n = 4$ mice; representative of two independent experiments). (D) Number of PB in the dLNs of non-immunized or immunized mice treated at day 0 with anti-CD137 or its isotype control (100 µg i.p.) ($n = 4$ mice). (E) Percentage of GC B cells (left graph) and IgG1+ B cells (right graph) in non-immunized or immunized mice treated with isotype or anti-CD137 1D8 mAb ($n = 5$ mice; 1 independent experiment). All quantitative data were shown as means $\pm$ SD and analyzed using one-way ANOVA with multiple comparisons or t -tests.

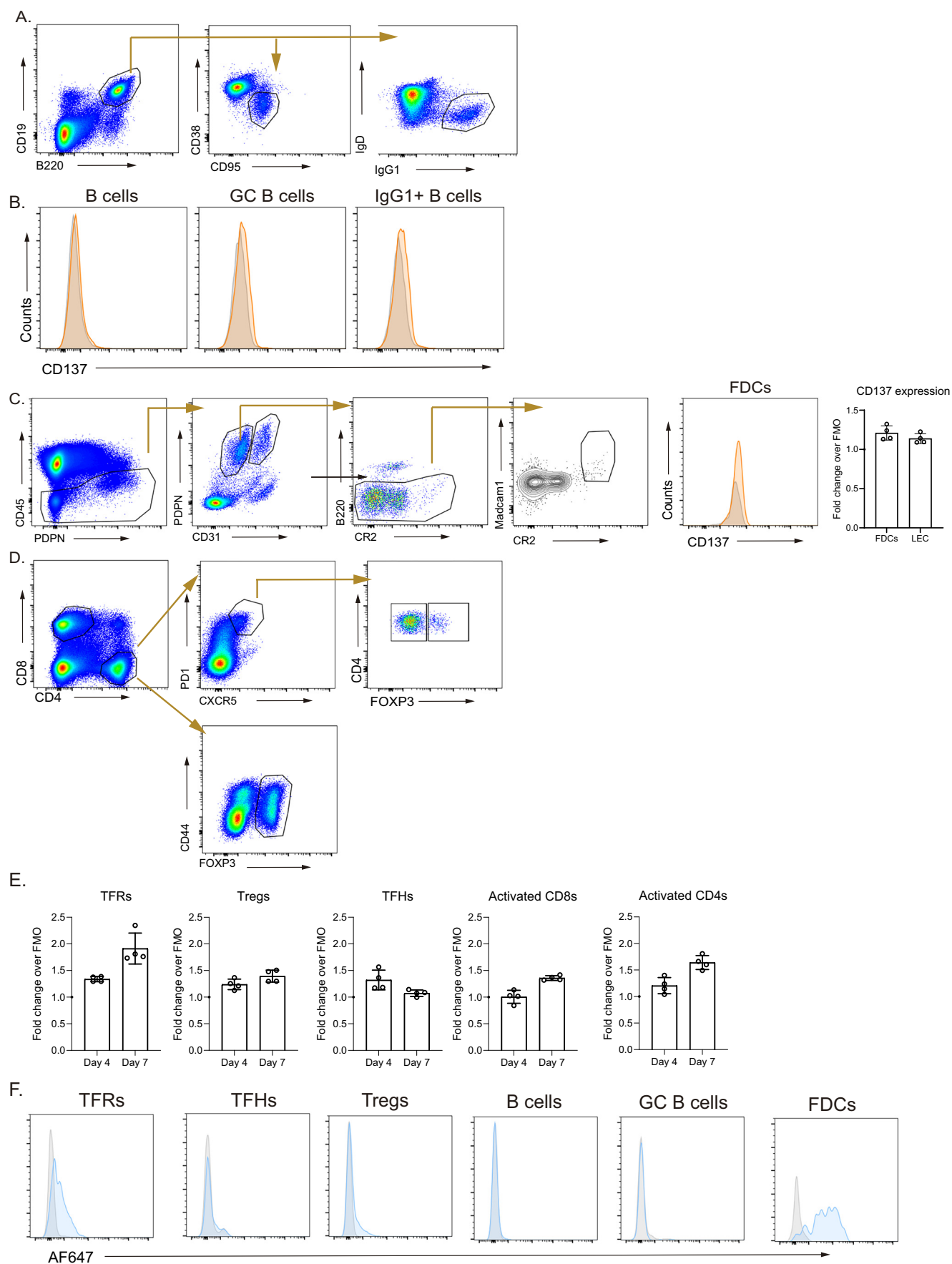
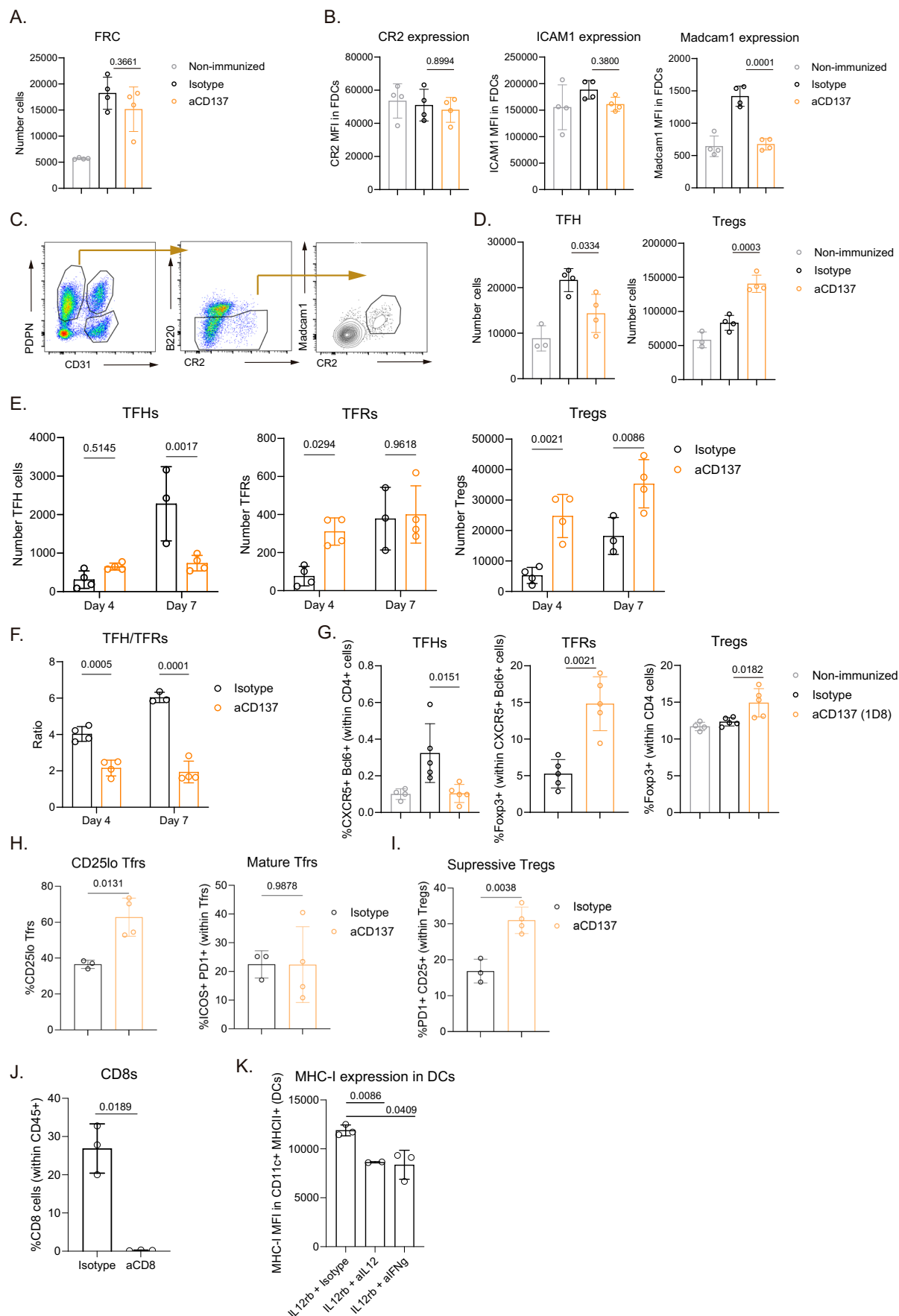


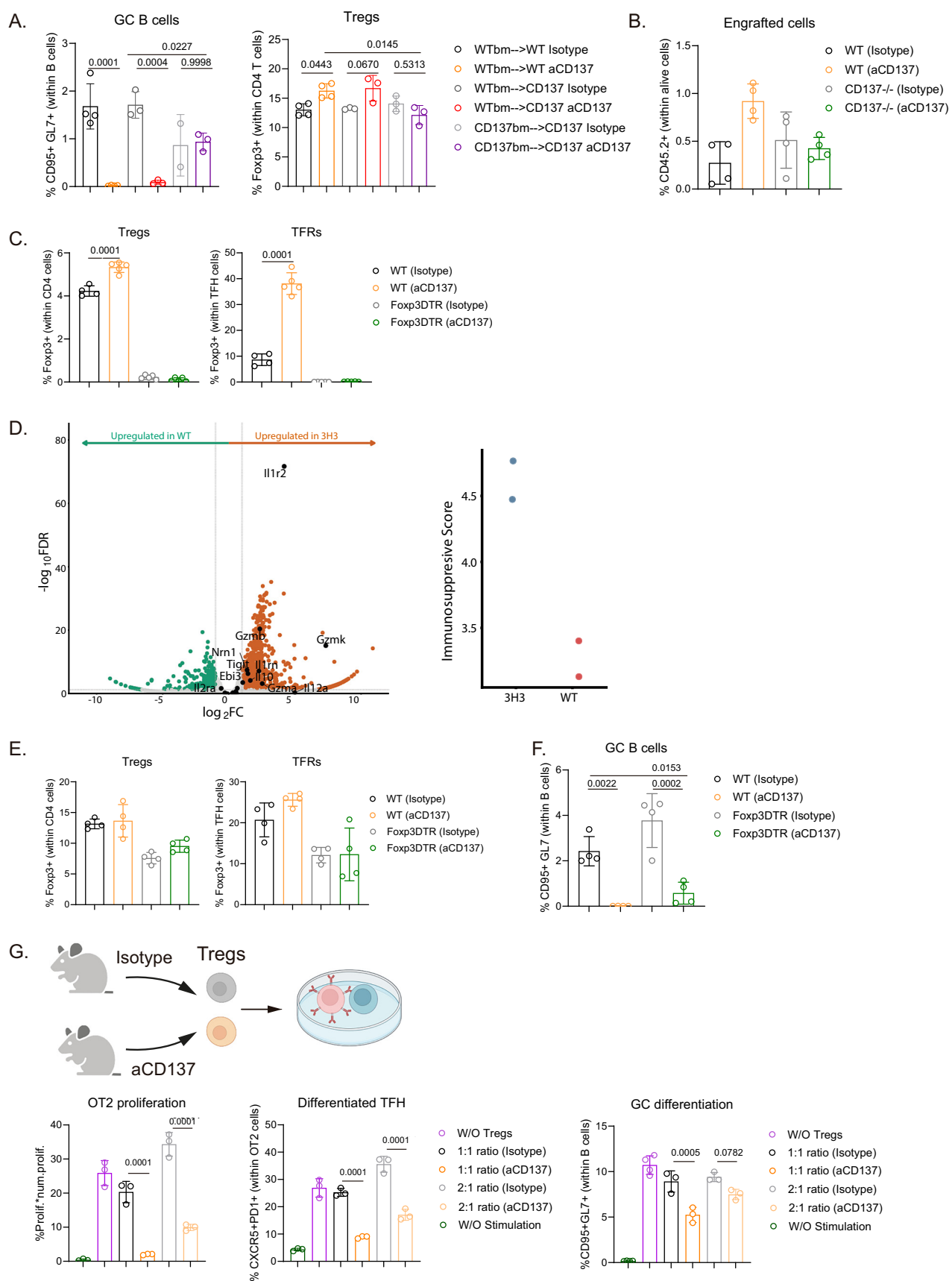
Figure EV2. Analysis of CD137 expression and in vivo anti-CD137 targeting to lymphocyte populations and FDCs.

(A) Flow cytometry strategy to analyze B cells, GC B cells and IgG1 CS B cells. (B) Histograms of CD137 expression (orange) and FMO (gray) on total B cells, GC and IgG1⁺ B cells. (C) Gating strategy to analyze FDCs. CD137 expression (orange) and FMO (gray) on FDCs. Quantification of CD137 expression (MFI) in mice immunized with ovalbumin in Alhydrogel. Graph shows the fold change in MFI relative to normalized FMO controls ($n = 4$ mice; 1 independent experiment). (D) Gating strategy to select Tfh (CXCR5 + PD-1⁺), Tfrs (CXCR5 + PD-1⁺ Foxp3⁺) and Tregs (CD4⁺ Foxp3⁺). (E) Quantification of CD137 expression (MFI) in mice immunized with ovalbumin in Alhydrogel for 4 and 7 days. Graph shows the fold change in MFI relative to normalized FMO controls (dotted line = 1) ($n = 4$ mice; 1 independent experiment). (F) Binding of AF647-labeled anti-CD137 (blue) and AF647-labeled IgG isotype (gray) to different dLNs immune populations. All quantitative data are shown as means $\pm$ SD.



◀ **Figure EV3. CD137-agonism in vivo modifies Treg and Tfh numbers in a manner independent of CD8-T cells, IFN γ and IL12.**

(A) Number of FRCs on the dLNs of non-immunized and immunized mice treated with anti-CD137 or its isotype-matched control. (B) Expression (MFI) of CR2, ICAM1, and MAdCAM1 on FDCs ($n = 4$ mice/group). (C) Gating strategy used to analyze FDCs on Fig. 3. (D) Number of Tfh and Treg cells on the dLNs of non-immunized and immunized mice treated with anti-CD137 or its isotype control ($n = 4$ mice/group). (E) Number of Tfh, Tfrs and Tregs in immunized mice for 4 and 7 days treated with anti-CD137 or its isotype-matched control ($n = 3-4$ mice; 1 independent experiment). (F) Ratio Tfh/Tfrs at day 4 and 7 post-immunization ($n = 4$ mice). (G) Quantification of the percentage of Tfh, Tfrs, and Tregs in non-immunized or immunized mice treated with anti-CD137 1D8 mAb or its isotype-matched control ($n = 5$ mice; 1 independent experiment). (H) Quantification of the percentage of Tfrs expressing low CD25 on the membrane (left graph) and ICOS + PD-1+ double-positive cells in mice immunized and treated with anti-CD137 or its isotype-matched control ($n = 3-4$ mice/group; 1 independent experiment). (I) Quantification of the percentage of PD-1 + CD25+ double-positive Tregs in mice treated as in (H). (J) Quantification of the percentage of CD8-T cells in the LNs of mice treated with the anti-CD8 β depleting antibody or its isotype control ($n = 3$ mice/group; 1 independent experiment). (K) Quantification of the MFI of MHC-I expressed by DCs in mice injected with recombinant IL-12 (10 μ g) and treated with neutralizing anti-IL12, anti-IFN γ or isotype control at days -1 and 1 ($n = 2-3$ mice/group; 1 independent experiment). All quantitative data are shown as mean $\pm$ SD and analyzed using one-way ANOVA with multiple comparisons or Welch's *t*-test.



◀ **Figure EV4. CD137-agonism acting in bone marrow-derived cells increases Treg/Tfr immunosuppressive activity.**

(A) Percentage of GC B cells and Tregs in the indicated bone marrow reconstituted mice ($n = 2-4$ mice/group; 1 independent experiment). (B) Percentage of engrafted CD45.2+ cells on CD45.1+ host mice 14 days after immunization ($n = 4$ mice/group; 1 independent experiment). (C) Percentage of Tregs and Tfrs on the LNs of WT and Foxp3-DTR mice injected with anti-CD137 or its isotype and 660 ng of DTx ($n = 4$ mice/group; representative of two independent experiments). (D) Volcano plot showing the magnitude of the transcriptional changes ($\log_2$ fold change) together with the statistical significance ($-\log_{10}$ FDR) of the genes expressed by Tregs from isotype- or anti-CD137-treated mice. In black, highlight those genes that participate in the immunosuppressive gene score. The right graph shows the immunosuppressive score of the sequenced Foxp3+ cells. (E) Percentage of Tregs and Tfrs on the LNs of WT and Foxp3-DTR mice injected with anti-CD137 or its isotype and 66 ng of DTx ($n = 4$ mice/group; one independent experiment). (F) WT and Foxp3-DTR mice treated as in (C) but with 10 $\times$ lower DTx dose (66 ng/dose) ($n = 4$ mice/group). (G) Co-culture assay workflow. Foxp3-GFP mice were injected with anti-CD137 or isotype control; 4 days later, GFP+ cells were sorted and co-cultured for 3 days with purified B cells, CTV-labeled OT-II CD4 T cells, and 1 μ m beads coated with anti-IgM F(ab')₂ and ovalbumin. Graphs show OT-II proliferation, as well as Tfh (CXCR5 + PD-1 +) and GC B cells (CD95 + GL7 +) percentages. Tregs were added at CD4 T:Treg ratios of 1:1 or 2:1; cultures without Tregs (magenta) and without bead stimulation (green) served as controls (representative of two independent experiments). All quantitative data were shown as means $\pm$ SD and analyzed using one-way ANOVA with multiple comparisons.

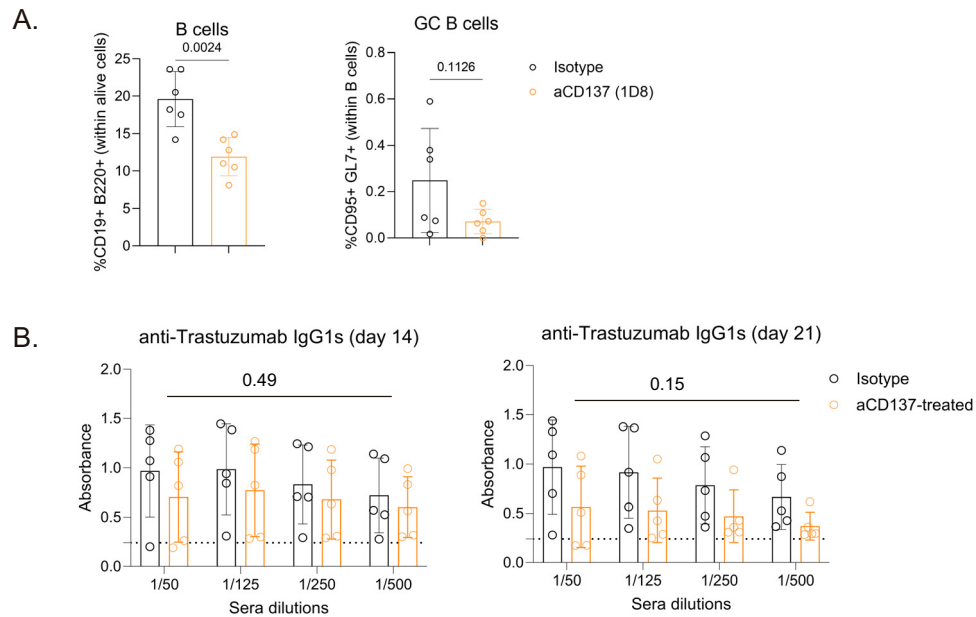


Figure EV5. Anti-CD137 disrupts tumor-associated TLS and ADA-associated humoral responses.

(A) Quantification of lung B cells and GC B cells in mice bearing KPAR tumors treated with isotype or anti-CD137 1D8 mAb ($n = 5-6$ mice/group; 1 independent experiment). (B) Absorbance levels of IgG1 anti-Trastuzumab antibodies in the sera of mice immunized with Trastuzumab and treated with isotype or anti-CD137 antibody after 14 days or 21 days post-immunization. The dotted line shows the background level of antibodies from non-immunized mice ($n = 5$ mice/group; 1 independent experiment). Quantitative data were shown as means $\pm$ SD and analyzed using two-way ANOVA or t -tests.