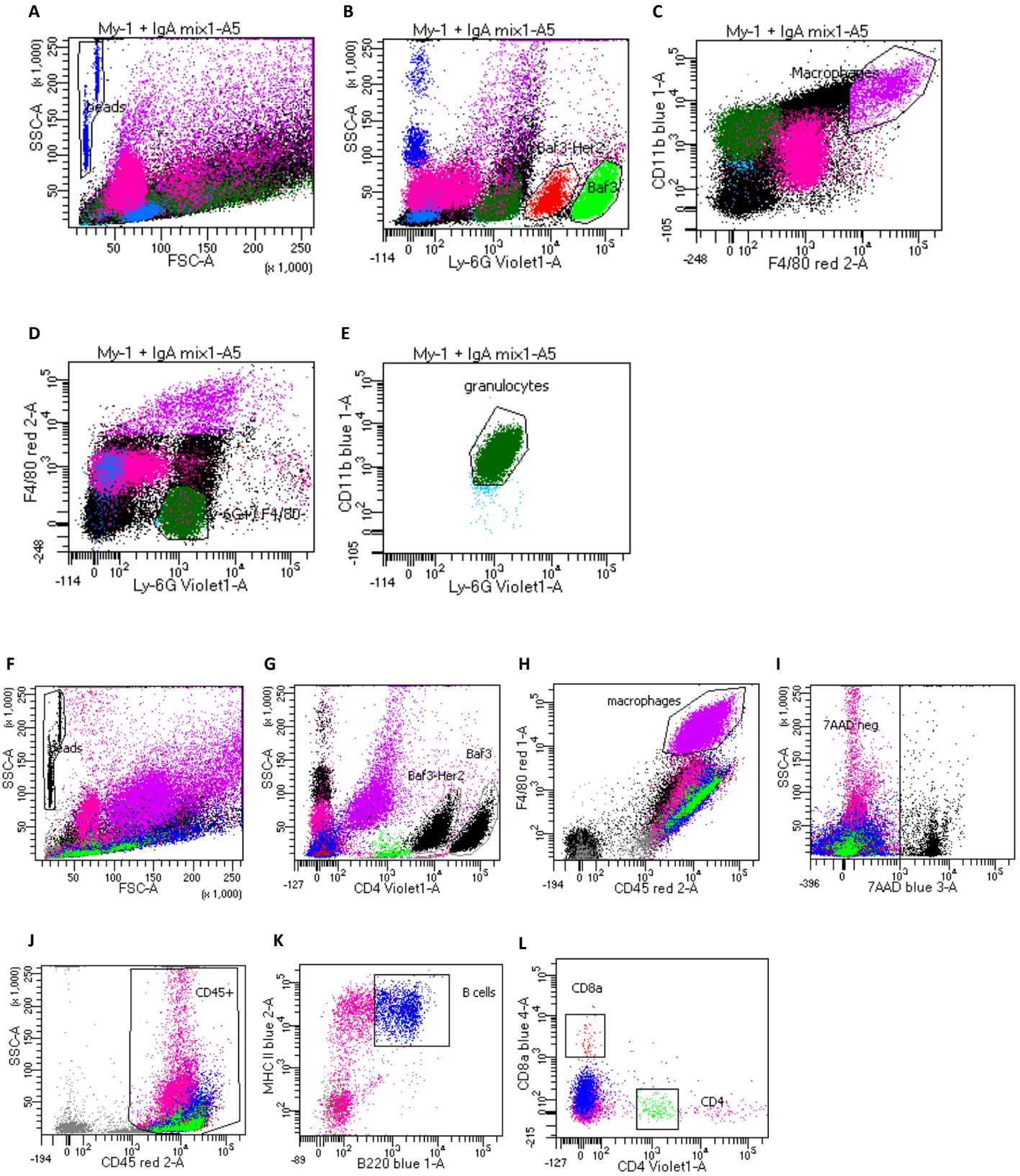
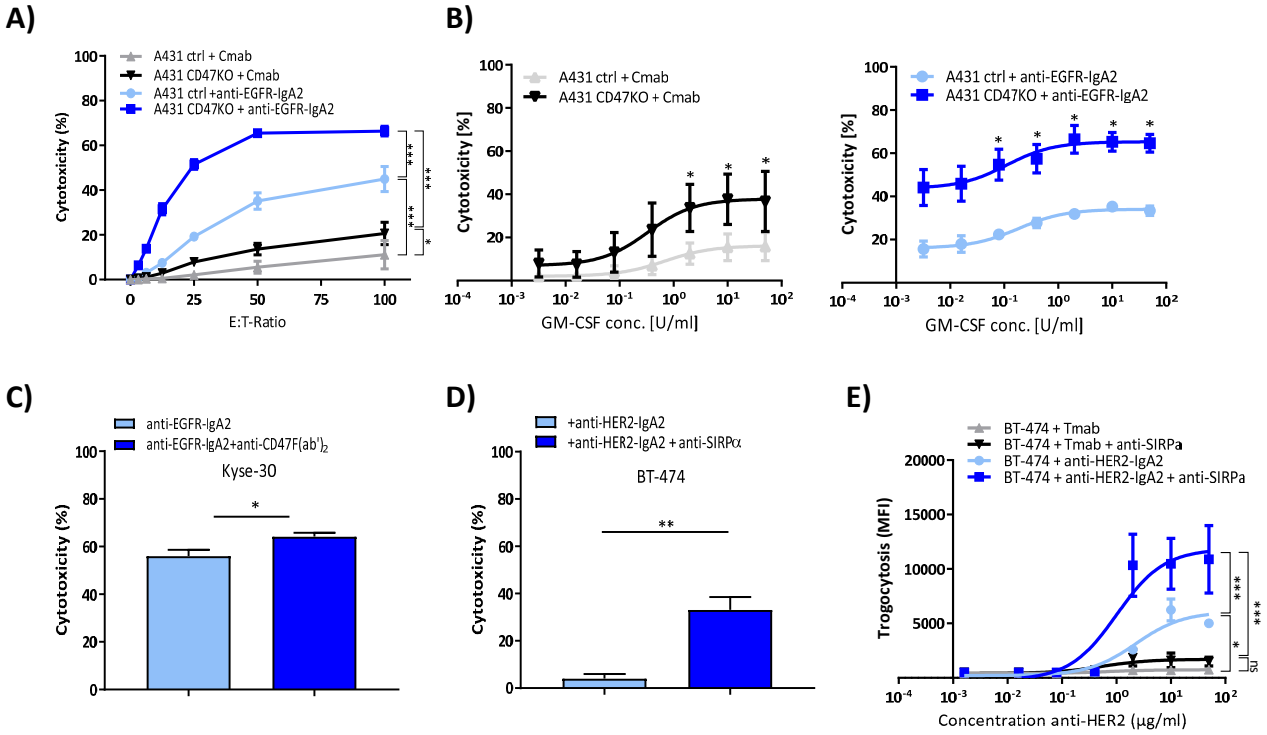
Supplemental figure 1



Supplemental figure 1

Gating strategy for defining Ba/F3, and immune cells subsets. (A) Beads are gated based on FCS/SSC in the upper left corner (dark blue events). (B) Gating on the separate populations of Ba/F3 Her2 positive and negative cells was possible by differential CellTrace violet labeling. The violet1 high population are the Ba/F3 cells (light green events) and the Ba/F3 Her2 are the violet1 low cells (red events). (C) Macrophages are gated based on the markers F4/80 and CD11b. Granulocytes were defined as being (D) Ly-6G^{+} and F4/80^{-} followed by (E) gating on CD11b^{+}. (F) Beads are gated based on FCS/SSC in the upper left corner (black events). (G) The separately CellTrace Violet labeled Ba/F3 Her2 and Ba/F3 populations can be gated as violet1 low and violet high populations alike Fig. S1A (H) Macrophages are gated based on the markers F4/80 and CD45 being positive for both. (I) After omitting beads, Ba/F3 cells and macrophages we additionally excluded dead cells and (J) continued with the CD45^{+} events. (K) B cells were selected based on the B220 and MHC II markers followed by (L) CD4 and CD8a selection.

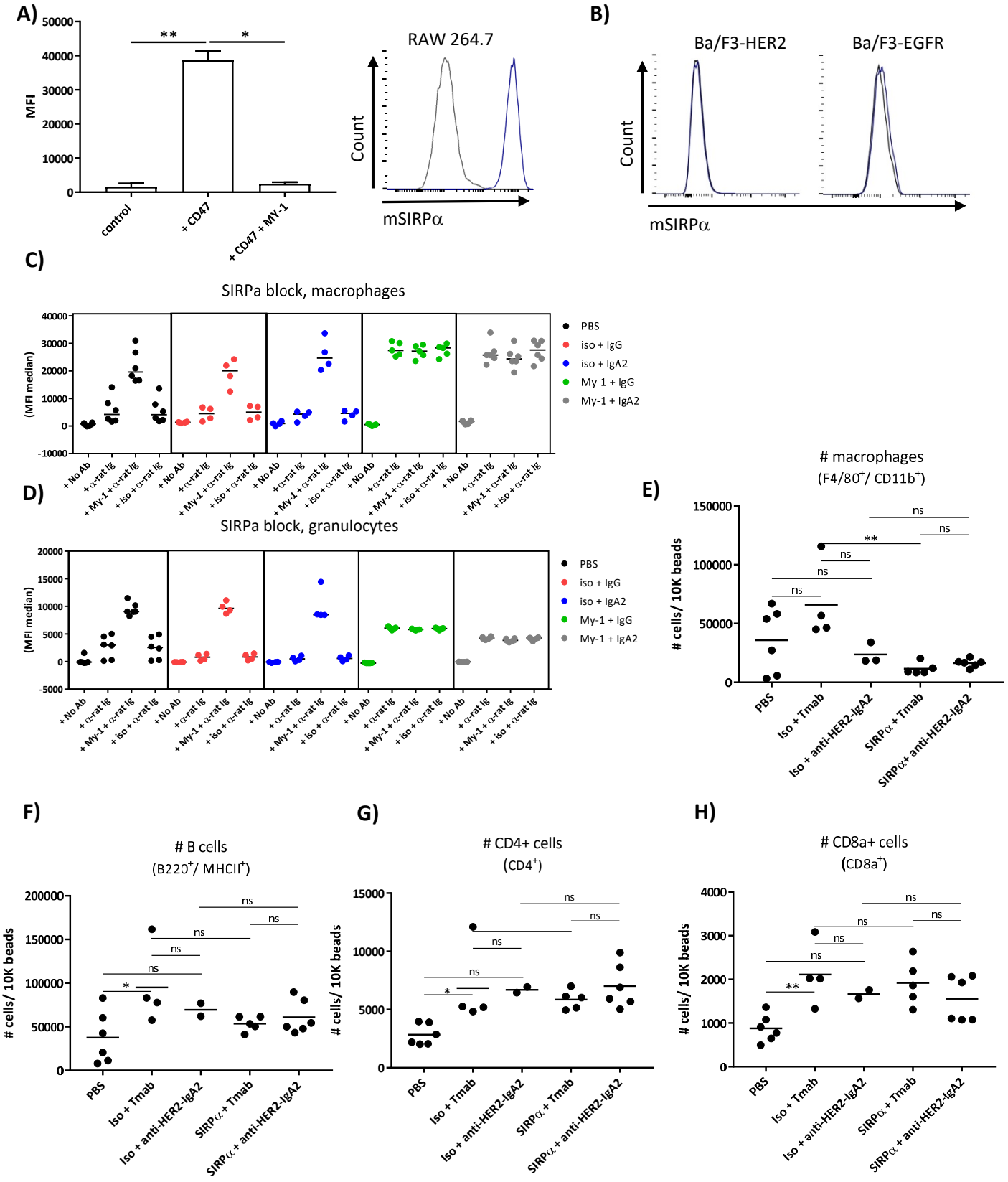
Supplemental figure 2



Supplemental figure 2

Improvement of neutrophil-mediated ADCC by disruption of CD47-SIRPα interaction is not target cell dependent. (A) Neutrophil mediated ADCC of A431 and A431-CD47KO cancer cells using increasing effector-to-target ratios. Cetuximab is depicted by black lines, while anti-EGFR-IgA2 is indicated by blue lines (both 1 µg/mL). (B) Effect of increasing GM-CSF concentrations (0-50 U/mL) on neutrophil-mediated ADCC by EGFR-directed IgG1 or IgA2 antibodies (left and right panels, respectively; antibody concentration 1 µg/mL; E:T ratio 25:1) without or with CD47-SIRPα axis disruption using A431 or A431-CD47KO cancer cells as targets. ADCC (C-D) and trogocytosis (E) of additional tumor cell lines by freshly isolated neutrophils using anti-EGFR-IgA2 (C), or anti-HER2-IgA2 (D-E) with and without interference of CD47-SIRPα by anti-CD47 $F(ab')_2$ (Kyse-30) or by anti-SIRPα (BT-474). Data shown are means ± SEM pooled from 2-3 experiments with 3-4 individual donor samples per experiment with n=3 (A), n=3 (B), n=3 (C), n=3 (D), n=4 (E). Statistics shown in (A) are for the highest antibody concentrations or highest E:T ratio using two-way ANOVA with Tukey's correction for multiple tests. Statistics shown in (B) were done by two-way ANOVA with Bonferroni´s post test correction. Statistics in (C) – (E) were done by paired t-test. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

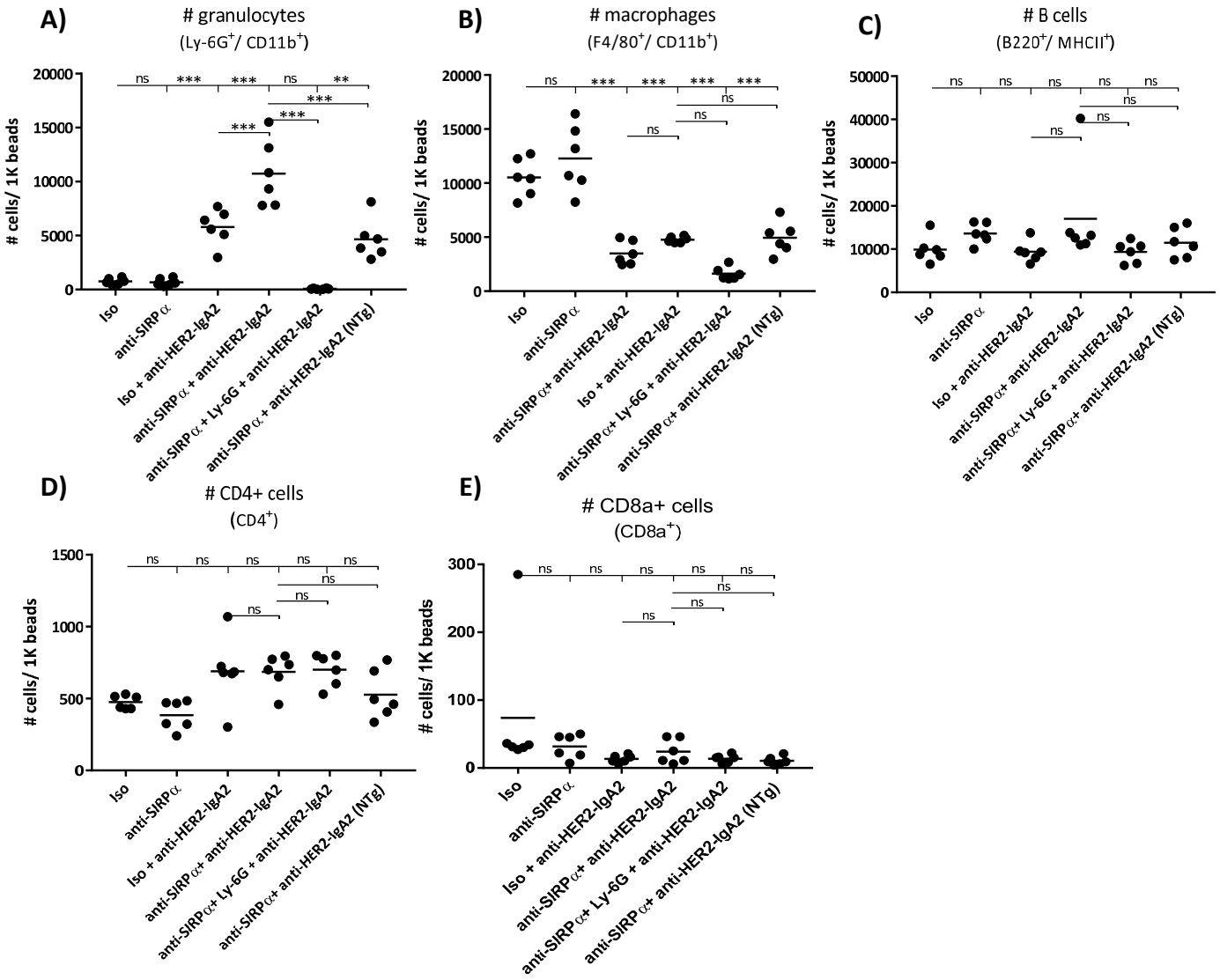
Supplemental figure 3



Supplemental figure 3

No influx of other effector cells than PMN after SIRPα inhibition *in vivo*. (A) MY-1 inhibits mouse SIRPα binding to CD47, using a beads-binding assay and RAW 264.7 cells expressing mouse SIRPα. MFI = mean fluorescent intensity of mouse CD47-Fc- goat anti-human Alexa 647 complex as measured by flow cytometry. (B) Expression of mouse SIRPα on Ba/F3-EGFR cells and Ba/F3-HER2. (C-D) The saturation levels of MY-1 during the *in vivo* experiment was determined on (C) macrophages and (D) neutrophils isolated from the peritoneal cavity. (E-H) Cells present after 16h in the peritoneal cavity with (E) macrophages ($F4/80^+/CD11b^+$), (F) B cells ($B220^+/MHCII^+$), (G) $CD4^+$ T cells ($CD4^+$), (H) $CD8a^+$ T cells ($CD8a^+$). Data shown are means from 1 experiments with 6 mice per group, with (C) N=4-6, (D) N=4-6. (E) N=3-6, (F) N=2-6, (G) N=2-6, (H) N=2-6, Statistics were performed by one way ANOVA with Sidak's post-test for (A)-(H). ns= non-significant, $*p < 0.05$, and $**p < 0.01$.

Supplemental figure 4



Supplemental figure 4

Granulocytes were successfully depleted during in vivo experiments. Cells present after 16h in the peritoneal cavity with (A) granulocytes (Ly-6G$^+$/CD11b$^+$), (B) macrophages (F4/80$^+$/CD11b$^+$), (C) B cells (B220$^+$/MHCII$^+$), (D) CD4$^+$ T cells (CD4$^+$), (E) CD8a$^+$ T cells (CD8a$^+$). Data shown are means from 1 experiments with 6-8 mice per group, with (A) N=6, (B) N=6, (C) N=6, (D) N=6, (E) N=6. Statistics were performed by one way ANOVA with Sidak's post-test for (A)-(E), ns= non-significant, *$p < 0.05$, **$p < 0.01$.