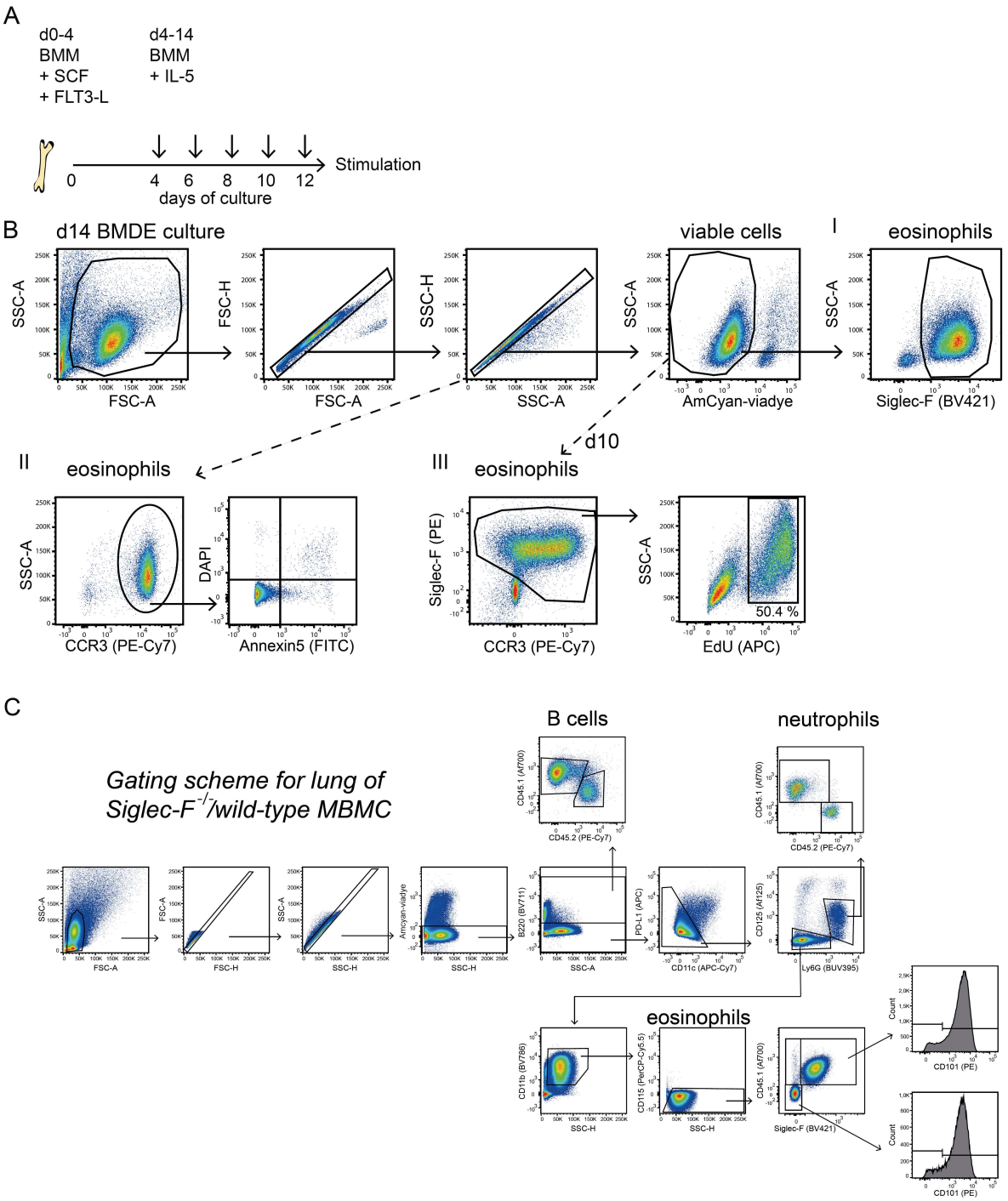
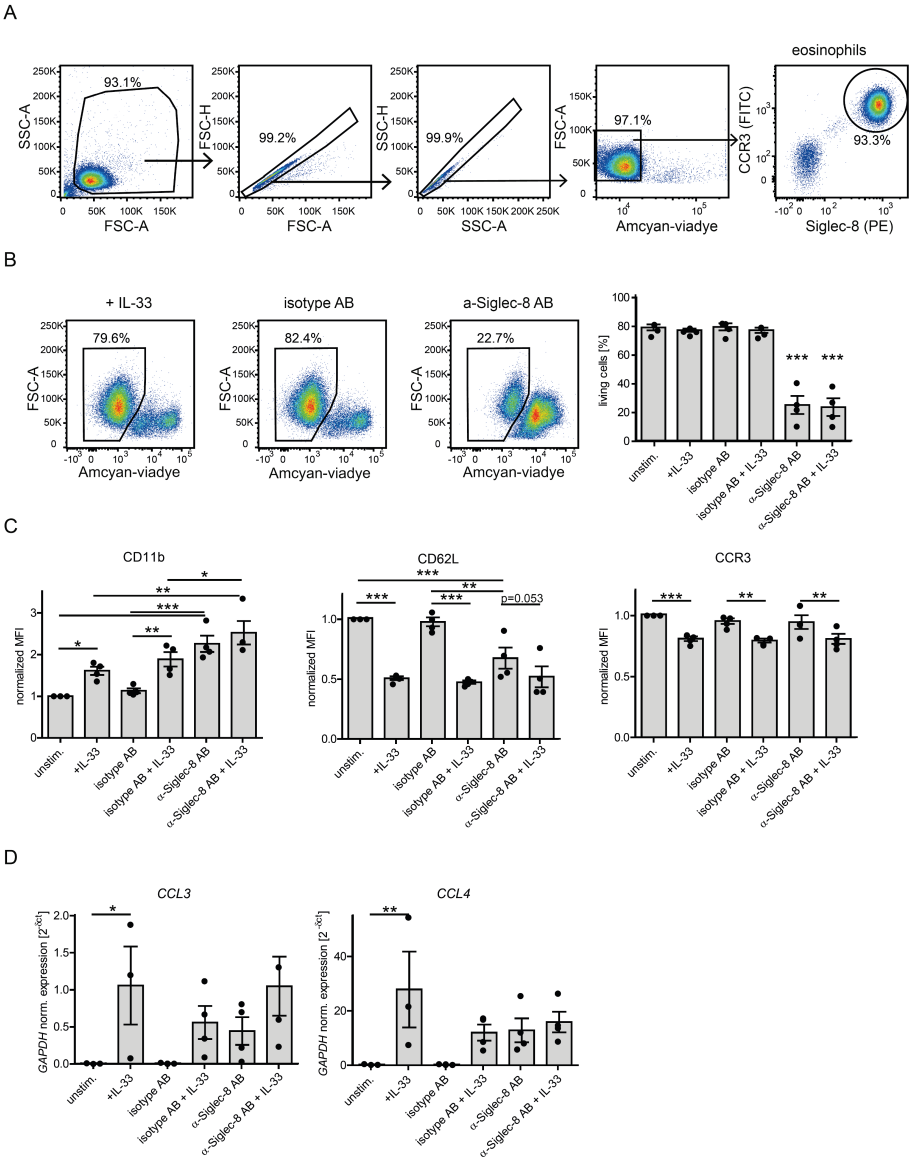
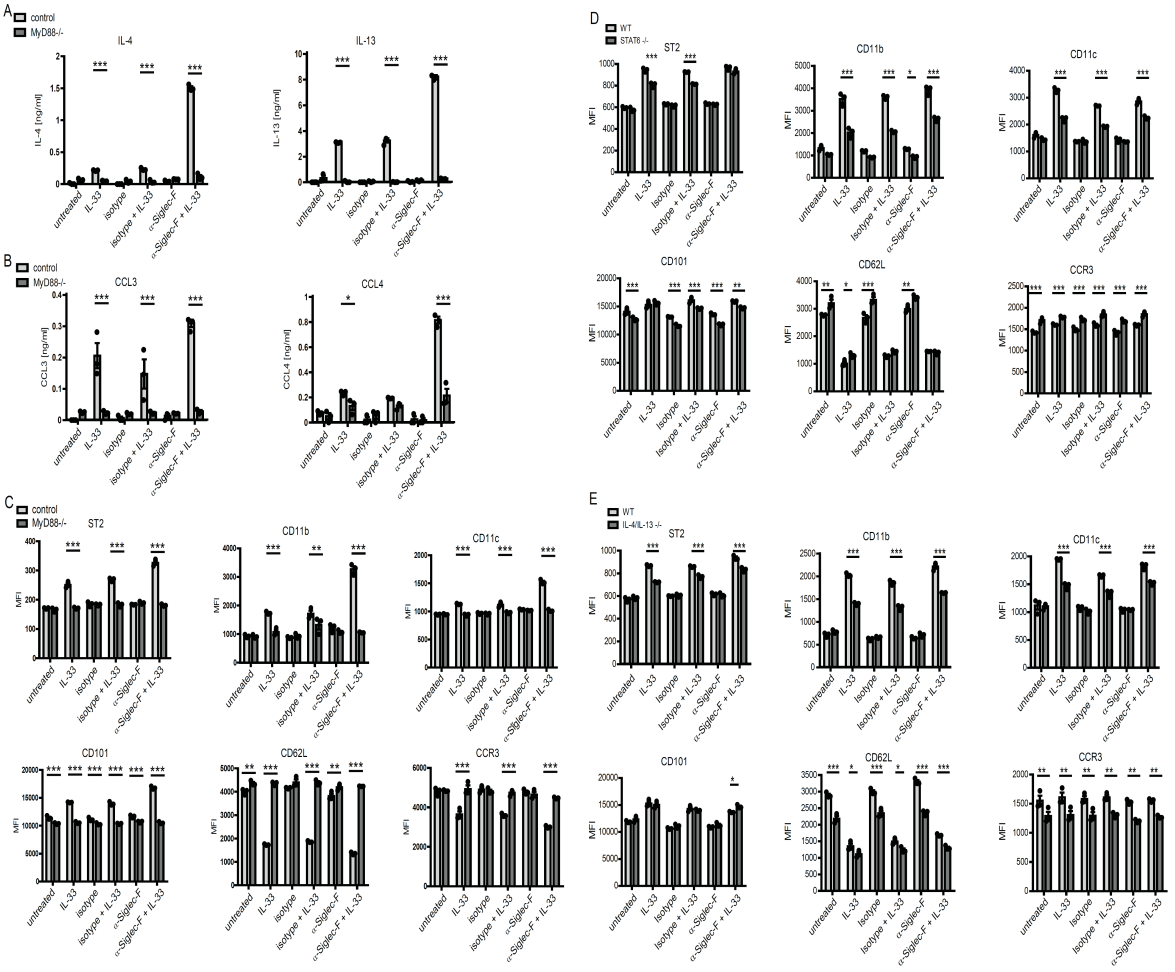


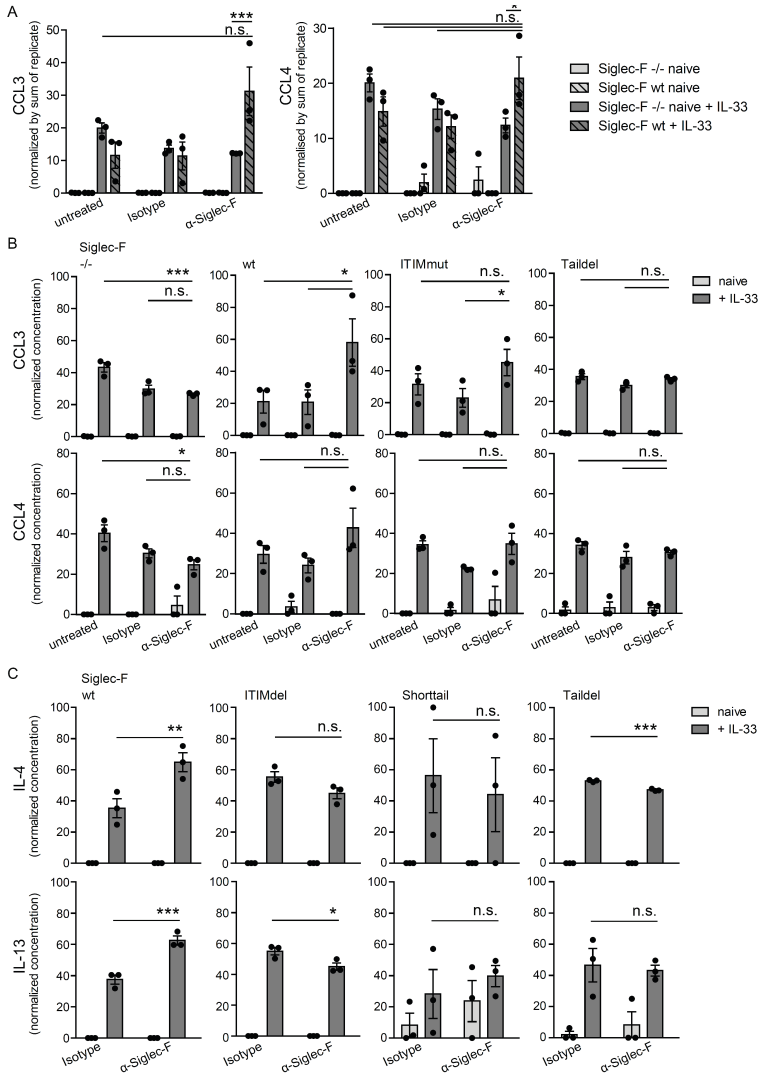
Suppl. Figure 1. Gating schemes for flow cytometry. A) Schematic procedure of BMDE culture. B) Gating strategies for live eosinophils (I), detection of apoptotic/dead eosinophils (II), determination of eosinophil proliferation (EdU+ cells) (III). Examples represent d14 BMDEs except for sub-panel III in which d10 BMDEs are shown. Dashed arrows point from exemplary d14 pre-gating for one experiment to plots that show data from other experiments using the same pre-gating strategy. C) Gating strategy for lung cells from MBMC generated with 50% CD45.2_Siglec-F$^{-/-}$ and 50% CD45.1_wild-type donor bone marrow.



Suppl. Figure 2. Anti-Siglec-8 induces eosinophil apoptosis but also activation marker regulation while additional IL-33 did not synergistically activate beyond single treatments. Human eosinophil were purified from peripheral blood and stimulated *ex vivo*. (A) Gating strategy of human eosinophils ($CCR3^{+}Siglec\text{-}8^{+}$) after purification with duplet and dead cell exclusion. (B) Representative viability gates for human eosinophils stimulated with IL-33, isotype control antibody or anti-Siglec-8 antibody pre-gated on $CCR3^{+}$ cells. Bar graph diagram summarizes mean ± SEM percentage of living cells. Significance symbols indicate a significant difference of the two conditions using anti-Siglec-8 antibody against all other conditions without anti-Siglec-8 antibody. (C) Flow cytometric activation marker analysis (CD11b, CD62L and CCR3) of purified, stimulated human eosinophils further gated as $SSC\text{-}A^{high}$ living singlets for MFI determination. Mean fluorescence intensity (MFI) of stimulation conditions was normalized to the MFI of the unstimulated control of the respective donor and bars show normalized MFI ± SEM. (D) Quantitative real-time PCR for *CCL3* and *CCL4* gene expression of 4 hrs stimulated human eosinophils. Bars show mean ± SEM of *Gapdh* normalized expression. (B-D) Bars show pooled data for four biologically distinct human donors from two experiments and statistical differences were determined by two-way ANOVA with Holm-Sidak posttest *p<0.05, **p<0.01, ***p<0.001.



Suppl. Figure 3. Signaling through the ST2-MyD88-axis is required for synergistic combined IL-33 and anti-Siglec-F effects while STAT6 and IL-4/IL-13 are not essential
(A-C) BMDE were generated from MyD88$^{-/-}$ (MyD88$^{-/-}$) and MyD88$^{+/-}$ (WT) mice and stimulated on d14 with 5 µg/mL isotype control or anti-Siglec-F antibody and optionally 10 ng/mL IL-33 for 24 hrs. A) Cytokine and B) chemokine level in the supernatant measured by ELISA. C) Flow cytometric activation marker analysis and mean fluorescence quantification of ST2, CD11b, CD11c, CCR3, CD62L and CD101 for cells of the living gate. (D-E) BMDE were generated from D) 4get_STAT6$^{-/-}$ and 4get_BALB/c (WT) mice or E) IL-4/IL-13$^{-/-}$ and CD45.1_BALB/c (WT) mice. Stimulation and activation marker analysis as above. (A-E) Bars show the mean ± SEM of triplicates out of one culture representative for two biologically distinct cultures per genotype. Two-way ANOVA with Bonferroni posttests *p<0.05, **p<0.01, ***p<0.001.



Suppl. Figure 4. The cytoplasmic tail of Siglec-F promotes enhanced IL-33 and Siglec-F mediated synergistic cytokine and chemokine production for which Siglec-F ITIM motif phosphorylation is dispensable. Relates to main figure 8. Siglec-F$^{-/-}$ bone marrow cells were transduced with Siglec-F variant-coding MSCV-IRES-Thy1.1 retroviral vectors at the beginning of bone marrow derived eosinophil cultures. ELISA of culture supernatants from sorted Thy1.1^{+} BMDE after 24 hrs of stimulation were performed. Stimulation in ECM-medium with 10 ng/mL IL-5 plus additions as indicated in the following concentrations: Siglec-F 5 µg/mL, Isotype-control 5 µg/mL, 10 ng/mL IL-33. A) CCL3 and CCL4 ELISA for cultures that lack Siglec-F and cultures transduced with wt Siglec-F. B) As A but with additional variant in which tyrosins of the ITIM like and ITIM motif of Siglec-F were mutated (ITIMmut) and a variant in which the cytoplasmic tail was completely deleted (Stop codon behind K463; Taildel). C) IL-4 and IL-13 ELISA of supernatants from cultures transduced with wt or sequential Siglec-F tail truncation variants (Stop codon behind D528: ITIMdel; Stop codon behind S493: Shorttail and Taildel variant). In B) and C) culture conditions are compared individually for each Siglec-F variant. Bars show the mean ± SEM from n=3 independent cultures. One-way ANOVA with Bonferroni posttests was performed to determine significance. Only selected significances that highlight IL-33 and Siglec-F mediated effector release are indicated; n.s. = non significant, *p < 0.05; **p < 0.01; ***p < 0.001.