Regnase-1 degradation is crucial for interleukin-33- and interleukin-25-mediated group-2 innate lymphoid cell activation

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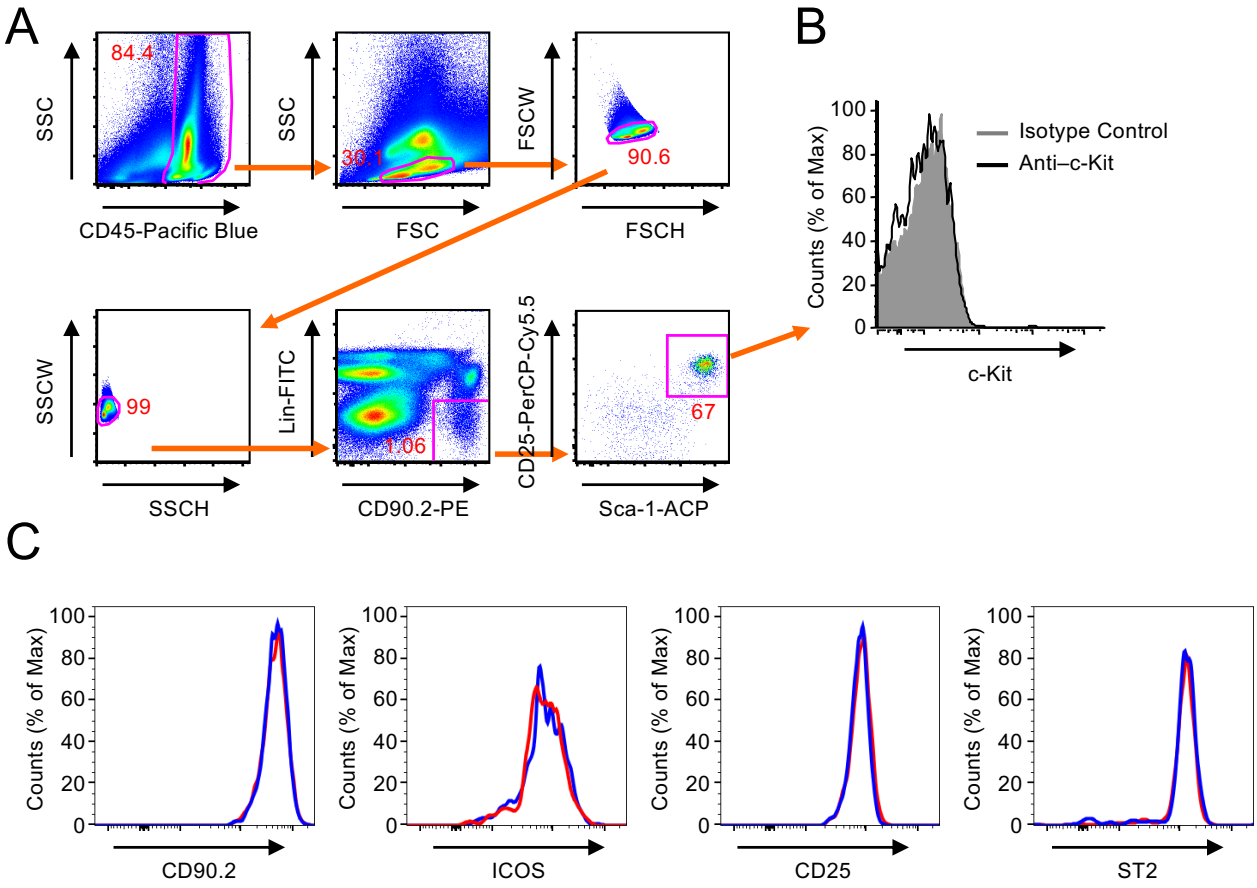
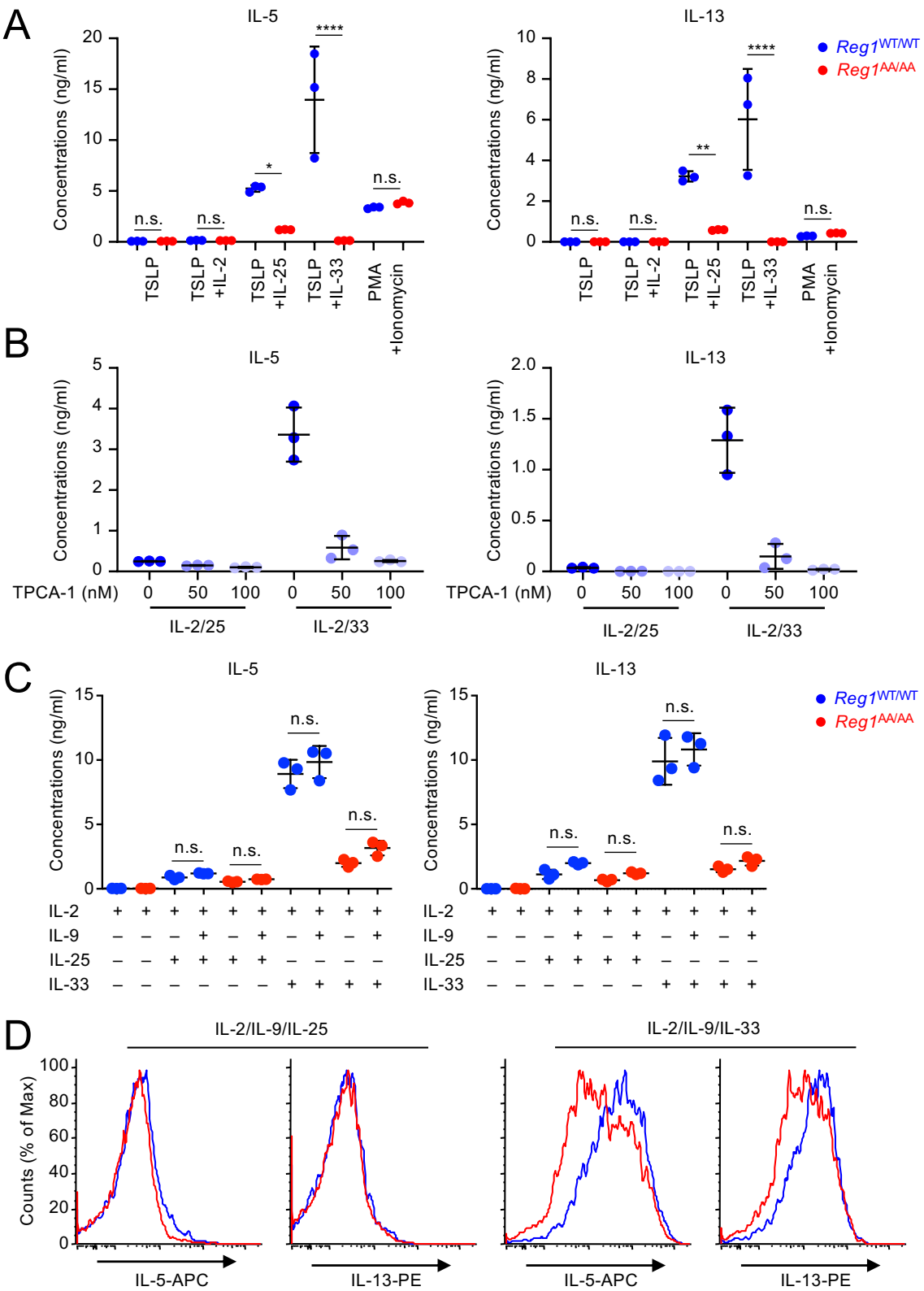
Supplemental Data

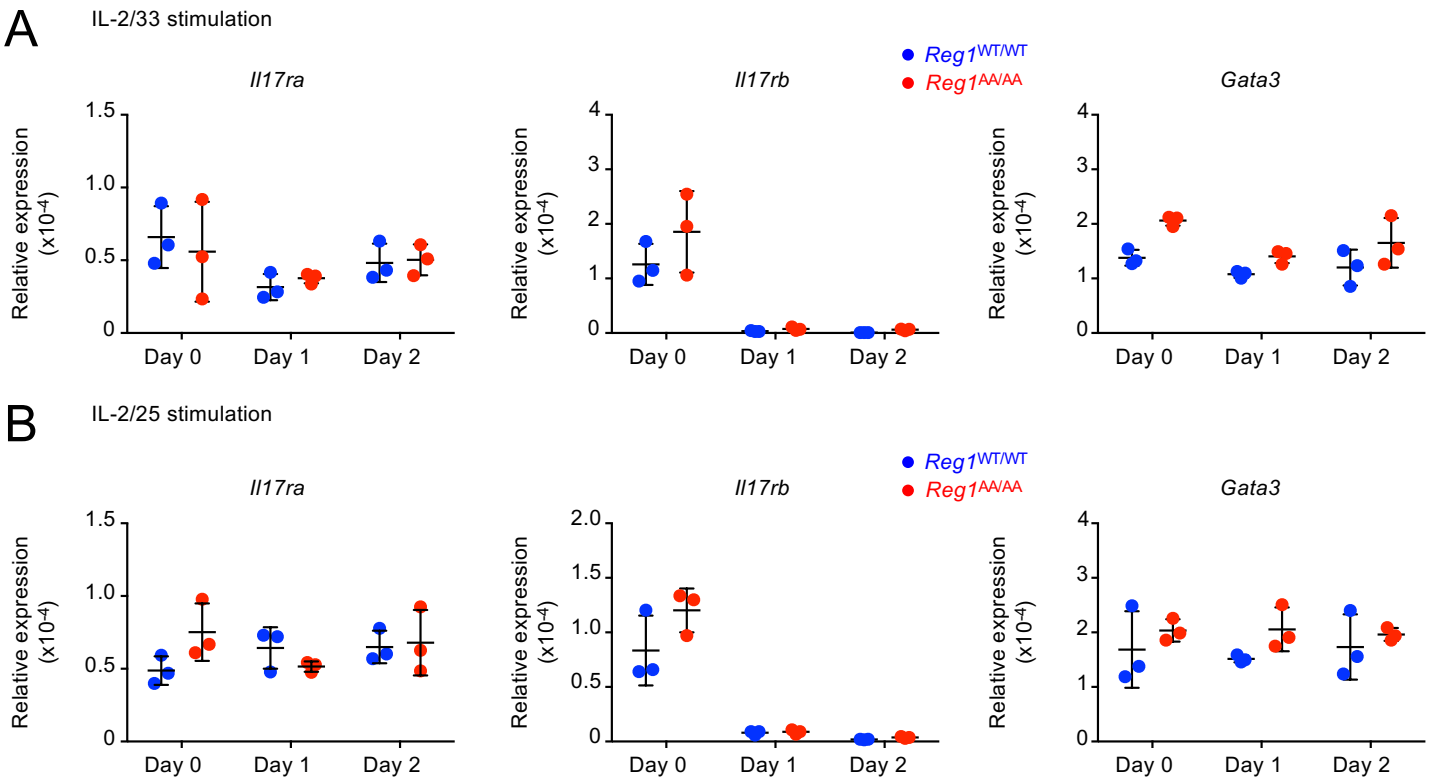


Supplementary Figure 1. The gating strategy for sorting ILC2s from bone-marrow cells.
(**A**) Lineage-negative cell enriched bone marrow (BM) cells were stained with lineage markers (B220, CD3, CD4, CD8, CD11b, CD11c, CD49b, and FcεRI) as well as CD90.2, CD25, and Sca-1. $Lin^{-}CD90.2^{+}CD25^{+}Sca\text{-}1^{+}$ cells were sorted as BM ILC2s. (**B**) The expression of c-Kit on sorted BM ILC2s was examined. (**C**) The expression of CD90.2, ST2, CD25, and ICOS on BM ILC2s from *Regnase-1*$^{WT/WT}$ (blue) and *Regnase-1*$^{AA/AA}$ (red) mice were examined.

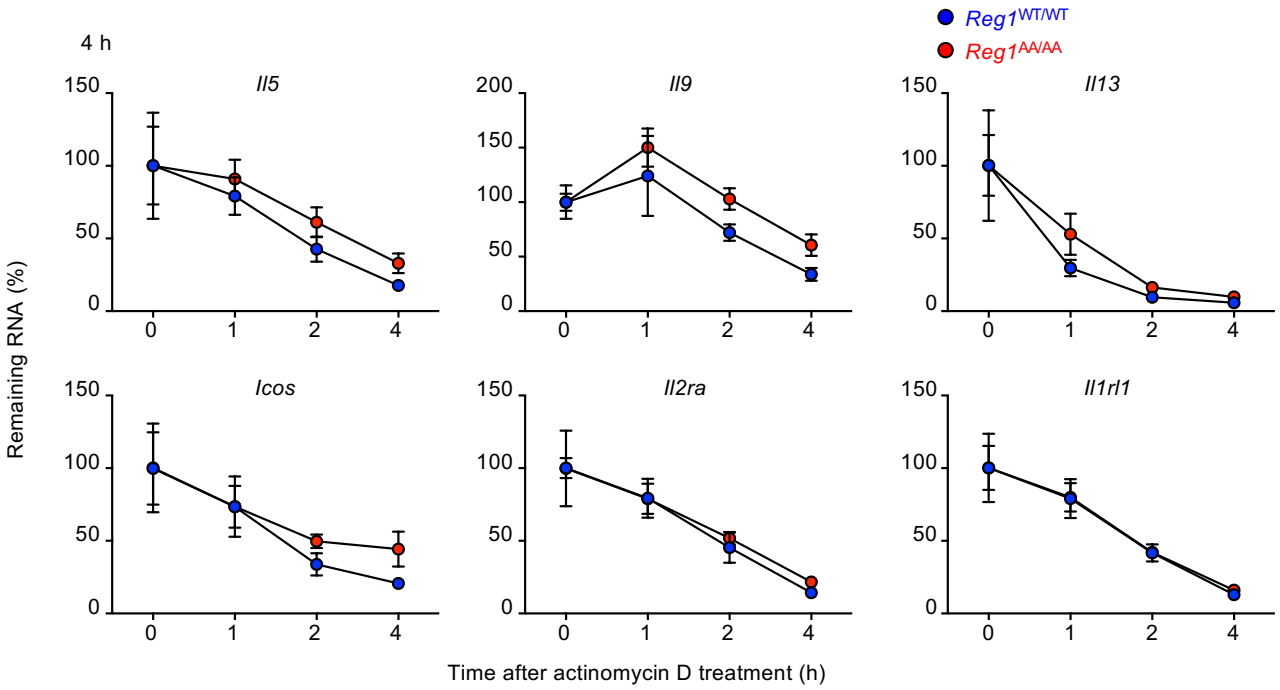


Supplementary Figure 2. Cytokine production from bone marrow ILC2s.

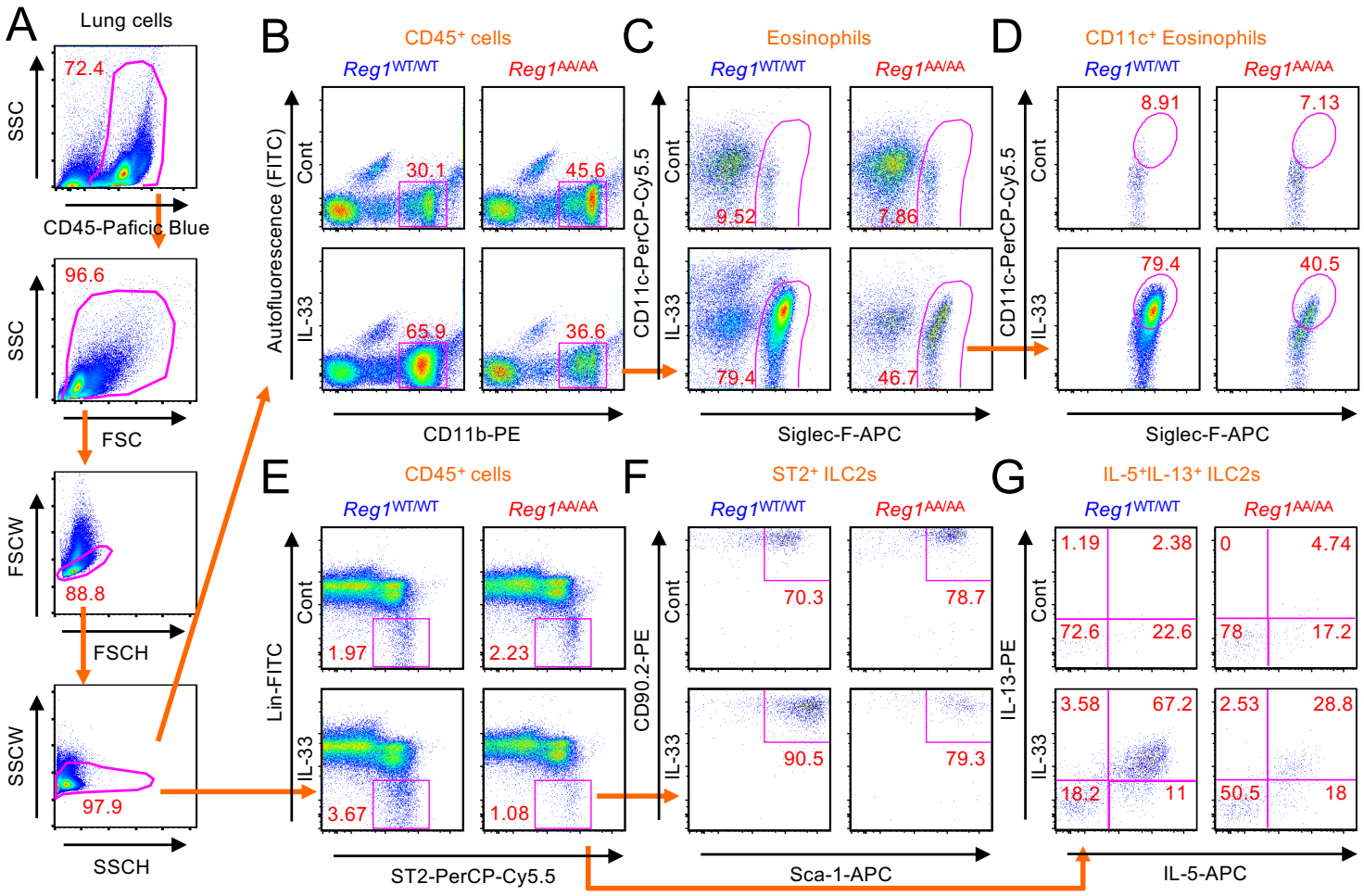
(**A**) Bone marrow (BM) ILC2s from *Regnase-1*$^{WT/WT}$ (blue) and *Regnase-1*$^{AA/AA}$ (red) mice were stimulated with combinations of TSLP, IL-2, IL-25, and IL-33, or PMA plus Ionomycin for 3 days. The concentrations of IL-5 and IL-13 in culture supernatants were determined by ELISA. (**B**) BM ILC2s from WT mice were stimulated with IL-2 plus IL-25 or IL-2 plus IL-33 in the presence or absence of TPCA-1 for 3 days. The concentrations of IL-5 and IL-13 in culture supernatants were determined by ELISA. (**C** and **D**) BM ILC2s from *Regnase-1*$^{WT/WT}$ (blue) and *Regnase-1*$^{AA/AA}$ (red) mice were stimulated with combinations of IL-2, IL-9, IL-25, and IL-33 for 3 days. (**C**) The concentrations of IL-5 and IL-13 in culture supernatants were determined by ELISA. (**D**) Golgi-Stop was added for the final 4 h of a 3-day culture, and intracellular cytokine levels were determined by FACS. Data are representative of two independent experiments. Mean (n=3) ± SD (**A**–**C**) and representative flow cytometry plots (**D**) are shown. Significance was determined by one-way ANOVA followed by Tukey's test; *P<0.05, **P<0.01, ****P<0.0001. n.s. not significant.



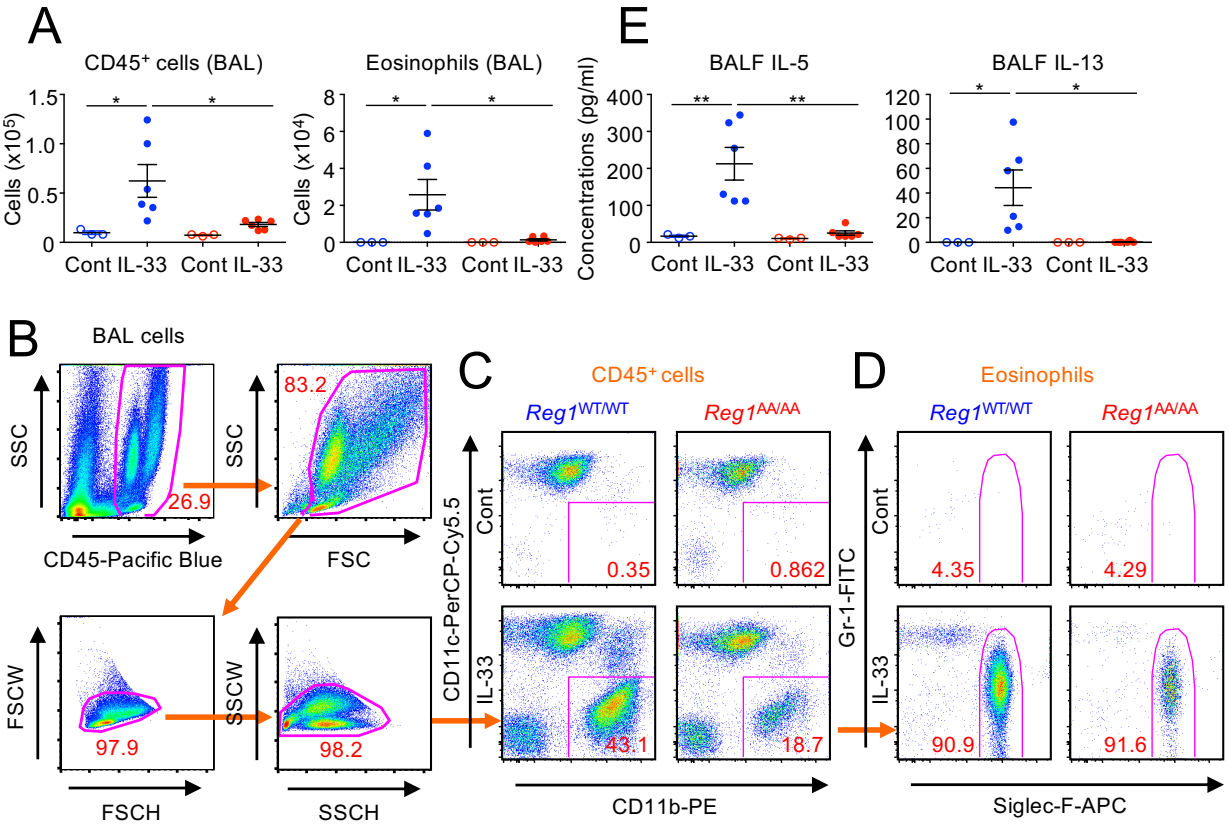
Supplementary Figure 3. *Il17ra*, *Il17rb*, and *Gata3* mRNA levels are not reduced in *Regnase-1*$^{AA/AA}$ ILC2s.
Bone marrow ILC2s from *Regnase-1*$^{WT/WT}$ (blue) and *Regnase-1*$^{AA/AA}$ (red) mice were stimulated with IL-2 plus IL-33 (IL-2/33; **A**) or IL-2 plus IL-25 (IL-2/25; **B**) for 1 or 2 days. mRNA levels for *Il17ra*, *Il17rb*, and *Gata3* were determined by quantitative PCR. Data are representative of two independent experiments. Mean (n=3) ± SD are shown.



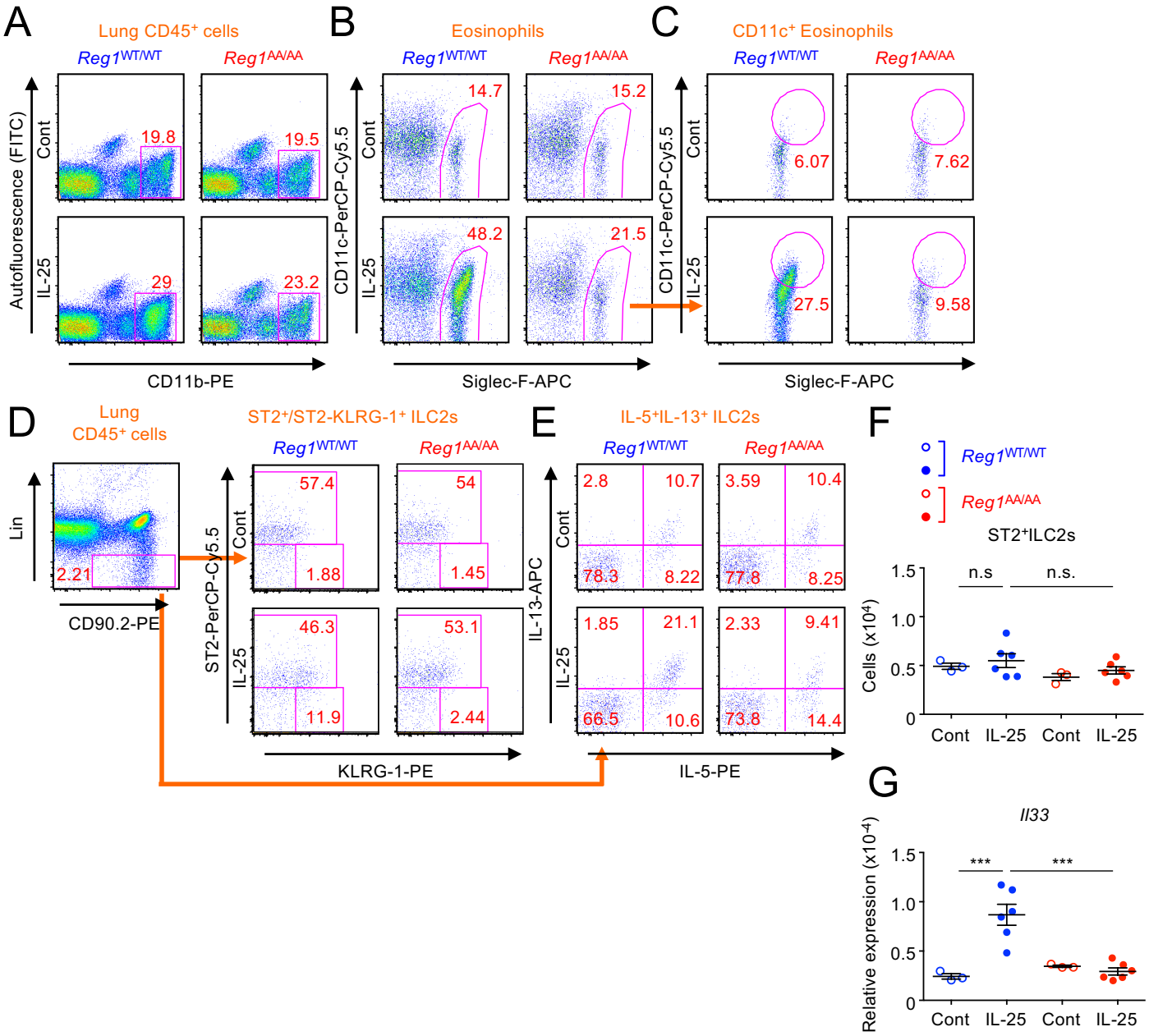
Supplementary Figure 4. Normal mRNA half-lives in 4 h IL-33–stimulated *Regnase-1*$^{AA/AA}$ ILC2s.
Bone marrow ILC2s from *Regnase-1*$^{WT/WT}$ (blue) and *Regnase-1*$^{AA/AA}$ (red) mice were stimulated with IL-2 and IL-33 for 4 h and then treated with actinomycin D for the indicated periods. mRNA levels for *Il5*, *Il9*, *Il13*, *Icos*, *Il2ra*, and *Il1rl1* were determined by quantitative PCR and remaining mRNAs after actinomycin D treatment (relative to 0 h) were calculated. Data are representative of two independent experiments. Mean (n=3) ± SD are shown.



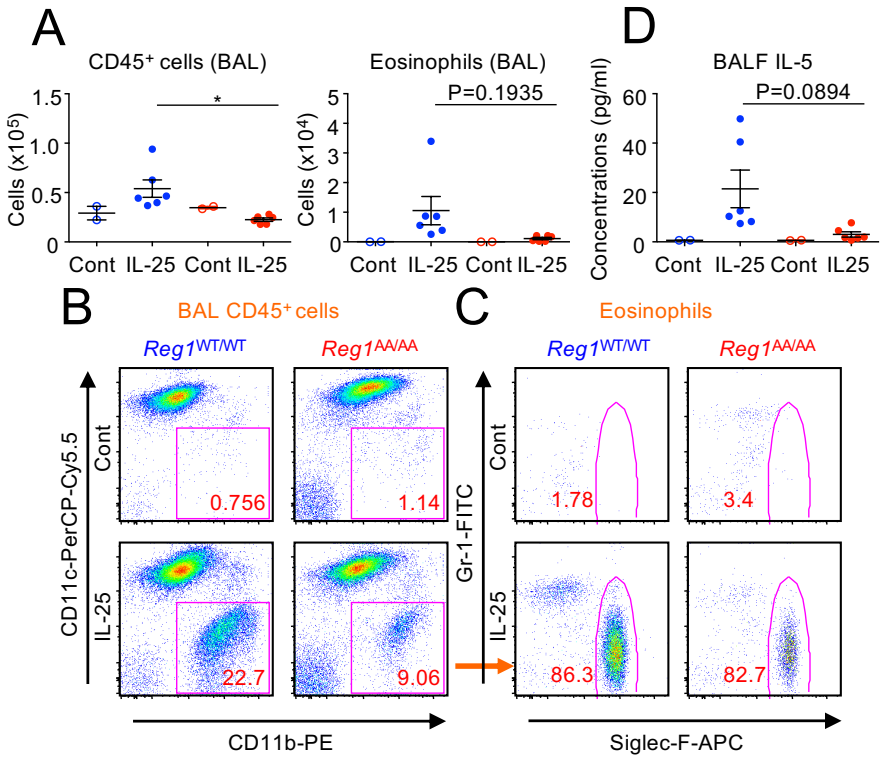
Supplementary Figure 5. Attenuated IL-33–induced pulmonary inflammation in *Regnase-1*$^{AA/AA}$ mice (Lungs). *Regnase-1*$^{WT/WT}$ (blue) and *Regnase-1*$^{AA/AA}$ (red) mice were intranasally administered with IL-33 (100 ng/dose) or PBS (control; Cont) for 4 consecutive days. The gating strategy to define lung CD45$^+$ singlet cells (**A**), and representative flow cytometry plots of lung CD45$^+$ cells (**B**), eosinophils (CD45$^+$Autofluorescence$^-$CD11b$^+$Siglec-F$^+$) (**C**), CD11c expression on eosinophils (**D**), ST2$^+$ILC2s (CD45$^+$Lin$^-$ST2$^+$CD90.2$^+$Sca-1$^+$) (**E** and **F**), and IL-5/IL-13 producing ILC2s (CD45$^+$Lin$^-$ST2$^+$IL-5$^+$IL13$^+$) (**G**) are shown. Data are representative of three independent experiments.



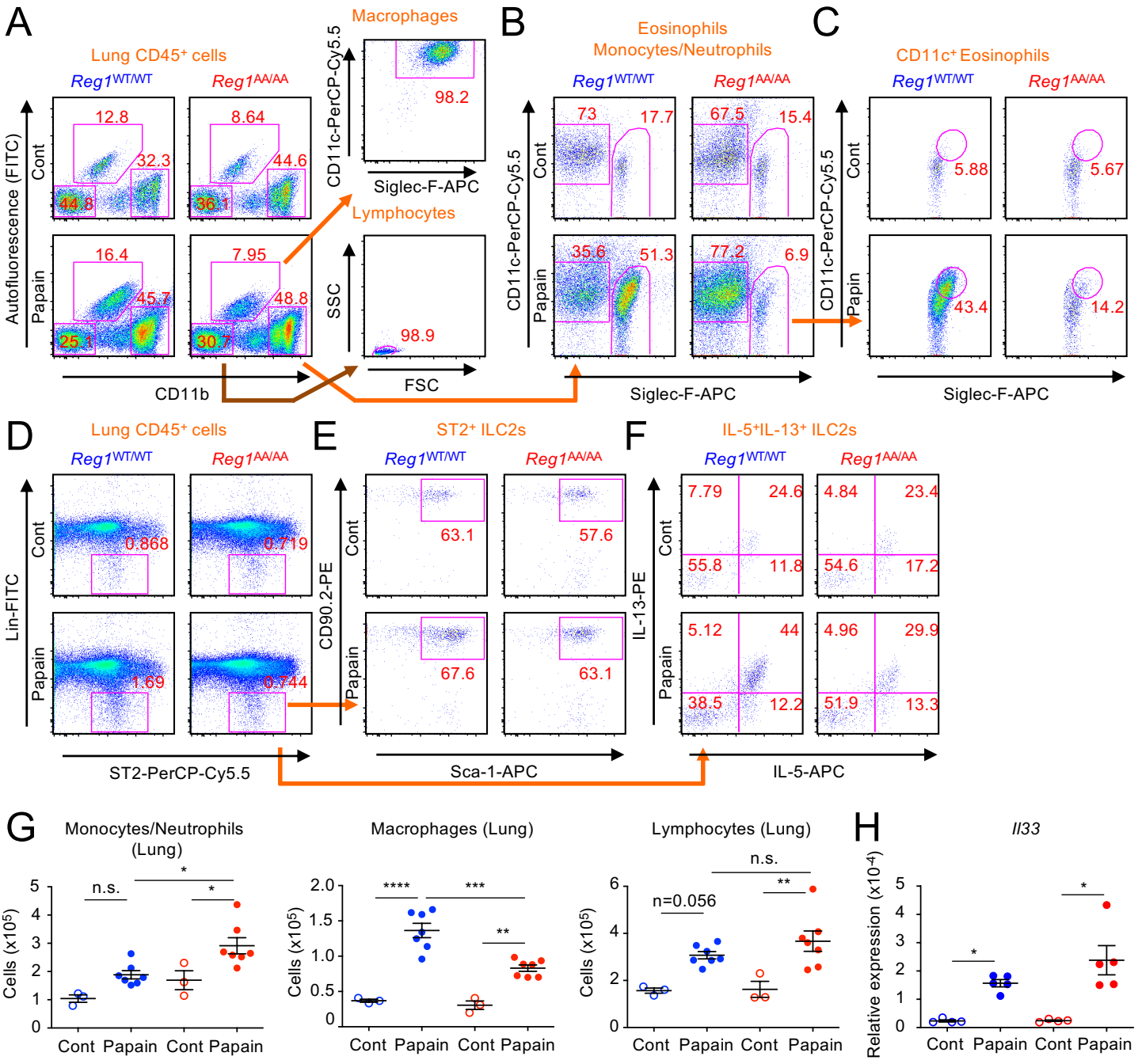
Supplementary Figure 6. Attenuated IL-33–induced pulmonary inflammation in *Regnase-1*$^{AA/AA}$ mice (BALs). *Regnase-1*$^{WT/WT}$ (blue) and *Regnase-1*$^{AA/AA}$ (red) mice were intranasally administered with IL-33 (100 ng/dose) or PBS (control; Cont) for 4 consecutive days. (**A**) Total numbers of CD45$^+$ cells (CD45$^+$ singlet cells) and eosinophils (CD45$^+$CD11b$^+$ CD11c$^-$ Siglec-F$^+$) in bronchoalveolar lavages (BALs) were quantified by FACS. (**B**–**D**) The gating strategy to define BAL CD45$^+$ singlet cells (**B**) and representative flow cytometry plots of BAL CD45$^+$ singlet cells (**C**) and eosinophils (CD45$^+$CD11b$^+$CD11c$^-$Siglec-F$^+$) (**D**) are shown. (**E**) The concentrations of IL-5 and IL-13 in the BAL fluids (BALFs) were determined by ELISA. Data are representative of two independent experiments. Representative flow cytometry plots (**B**–**D**) and mean (n=3 for control and n=6 for IL-33) ± SEM (**A** and **E**) are shown. Significance was determined by one-way ANOVA followed by Tukey's test; *P<0.05, **P<0.01.



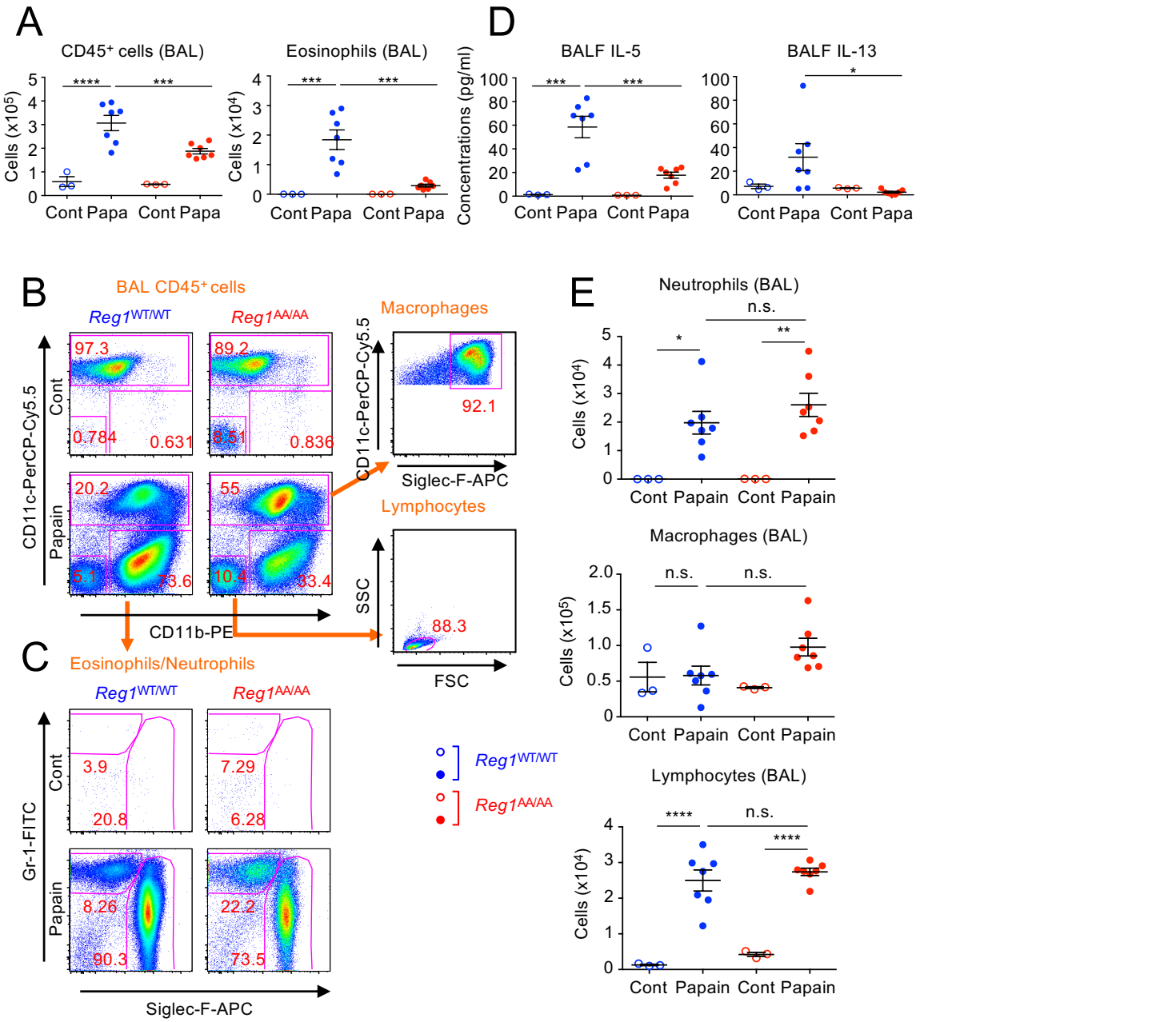
Supplementary Figure 7. Attenuated IL-25–induced pulmonary inflammation in *Regnase-1*$^{AA/AA}$ mice (Lungs). *Regnase-1*$^{WT/WT}$ (blue) and *Regnase-1*$^{AA/AA}$ (red) mice were intranasally administered with IL-25 (100 ng/dose) or PBS (control; Cont) for 4 consecutive days. (**A**–**E**) Representative flow cytometry plots of lung CD45$^+$ cells (**A**), eosinophils (CD45$^+$Autofluorescence$^-$CD11b$^+$Siglec-F$^+$) (**B**), CD11c expression on eosinophils (**C**), ST2$^+$ILC2s (CD45$^+$Lin$^-$CD90.2$^+$ST2$^+$) and ST2$^-$KLRG-1$^+$ILC2s (CD45$^+$Lin$^-$CD90.2$^+$ST2$^-$KLRG-1$^+$) (**D**), and IL-5/IL-13 producing ILC2s (CD45$^+$Lin$^-$CD90.2$^+$IL-5$^+$IL13$^+$) (**E**) are shown. (**F**) Total numbers of lung ST2$^+$ILC2s (CD45$^+$Lin$^-$CD90.2$^+$ST2$^+$) were quantified from (**D**). (**G**) mRNA levels for *Il33* in the lungs were determined by quantitative PCR. Data are representative of two independent experiments. Representative flow cytometry plots (**A**–**E**) and mean (n=3 for control and n=6 for IL-25) ± SEM (**F** and **G**) are shown. Significance was determined by one-way ANOVA followed by Tukey's test; ***P<0.001, n.s. not significant.



Supplementary Figure 8. Attenuated IL-25–induced pulmonary inflammation in *Regnase-1*$^{AA/AA}$ mice (BALs). *Regnase-1*$^{WT/WT}$ (blue) and *Regnase-1*$^{AA/AA}$ (red) mice were intranasally administered with IL-25 (100 ng/dose) or PBS (control; Cont) for 4 consecutive days. (**A**) Total numbers of CD45$^+$ cells (CD45$^+$ singlet cells) and eosinophils (CD45$^+$CD11b$^+$ CD11c$^-$ Siglec-F$^+$) in the bronchoalveolar lavages (BALs) were quantified by FACS. (**B** and **C**) The representative flow cytometry plots of BAL CD45$^+$ cells (**B**) and eosinophils (CD45$^+$CD11b$^+$CD11c$^-$Siglec-F$^+$) (**C**) are shown. (**D**) The concentrations of IL-5 in the BAL fluids (BALFs) were determined by ELISA. Data are representative of two independent experiments. Representative flow cytometry plots (**B** and **C**) and mean (n=2 for control and n=6 for IL-25) ± SEM (**A** and **D**) are shown. Significance was determined by one-way ANOVA followed by Tukey's test; *P<0.05, n.s. not significant.



Supplementary Figure 9. Attenuated Papain-induced pulmonary inflammation in *Regnase-1*$^{AA/AA}$ mice (Lungs). *Regnase-1*$^{WT/WT}$ (blue) and *Regnase-1*$^{AA/AA}$ (red) mice were intranasally administered with papain (Papa; 10 μg/dose) or PBS (control; Cont) for 4 consecutive days. (**A**–**F**) Representative flow cytometry plots of lung CD45$^+$ cells, macrophages (CD45$^+$Autofluorescence$^+$CD11c^{high}Siglec-F^{high}), and lymphocytes (CD45$^+$Autofluorescence$^-$CD11b$^-$FSC^{low}SSC^{low}) (**A**), eosinophils (CD45$^+$Autofluorescence$^-$CD11b$^+$Siglec-F$^+$) and monocytes/neutrophils (CD45$^+$Autofluorescence$^-$CD11b$^+$CD11c^{int}Siglec-F$^-$) (**B**), CD11c expression on eosinophils (**C**), ST2$^+$ILC2s (CD45$^+$Lin$^-$ST2$^+$CD90.2$^+$Sca-1$^+$) (**D** and **E**), and IL-5/IL-13 producing ILC2s (CD45$^+$Lin$^-$ST2$^+$IL-5$^+$IL13$^+$) (**F**) are shown. (**G**) The total numbers of lung neutrophils, macrophages, and lymphocytes quantified from (**A**) and (**B**). (**H**) mRNA levels for *Il33* in the lungs 6 hours after a single papain administration. Data are representative of two independent experiments. Representative flow cytometry plots (**A**–**F**) and mean (n=3 for control and n=7 for papain (**G**) and n=4 for control and n=5 for papain (**H**)) ± SEM (**G** and **H**) are shown. Significance was determined by one-way ANOVA followed by Tukey's test; *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001. n.s. not significant.



Supplementary Figure 10. Attenuated Papain-induced pulmonary inflammation in *Regnase-1*$^{AA/AA}$ mice (BALs). *Regnase-1*$^{WT/WT}$ (blue) and *Regnase-1*$^{AA/AA}$ (red) mice were intranasally administered with papain (Papa; 10 μg/dose) or PBS (control; Cont) for 4 consecutive days. (**A**) Total numbers of CD45$^+$ cells (CD45$^+$ singlet cells) and eosinophils (CD45$^+$CD11b$^+$CD11c$^-$Siglec-F$^+$) in the bronchoalveolar lavages (BALs) were quantified by FACS. (**B** and **C**) Representative flow cytometry plots of bronchoalveolar lavage (BAL) CD45$^+$ cells, macrophages (CD45$^+$CD11c^{high}Siglec-F^{high}), and lymphocytes (CD45$^+$CD11b$^-$CD11c$^-$FSC^{low}SSC^{low}) (**B**), and eosinophils (CD45$^+$CD11b$^+$CD11c$^-$Siglec-F$^+$) and neutrophils (CD45$^+$CD11b$^+$CD11c$^-$Gr-1^{high}Siglec-F$^-$) (**C**) are shown. (**D**) The concentrations of IL-5 and IL-13 in the BAL fluids (BALFs) were determined by ELISA. (**E**) The total numbers of BAL neutrophils, macrophages, and lymphocytes quantified from (**B**) and (**C**). Data are representative of two independent experiments. Representative flow cytometry plots (**B** and **C**) and mean (n=3 for control and n=7 for papain) ± SEM (**A**, **D** and **E**) are shown. Significance was determined by one-way ANOVA followed by Tukey's test; *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001. n.s. not significant.