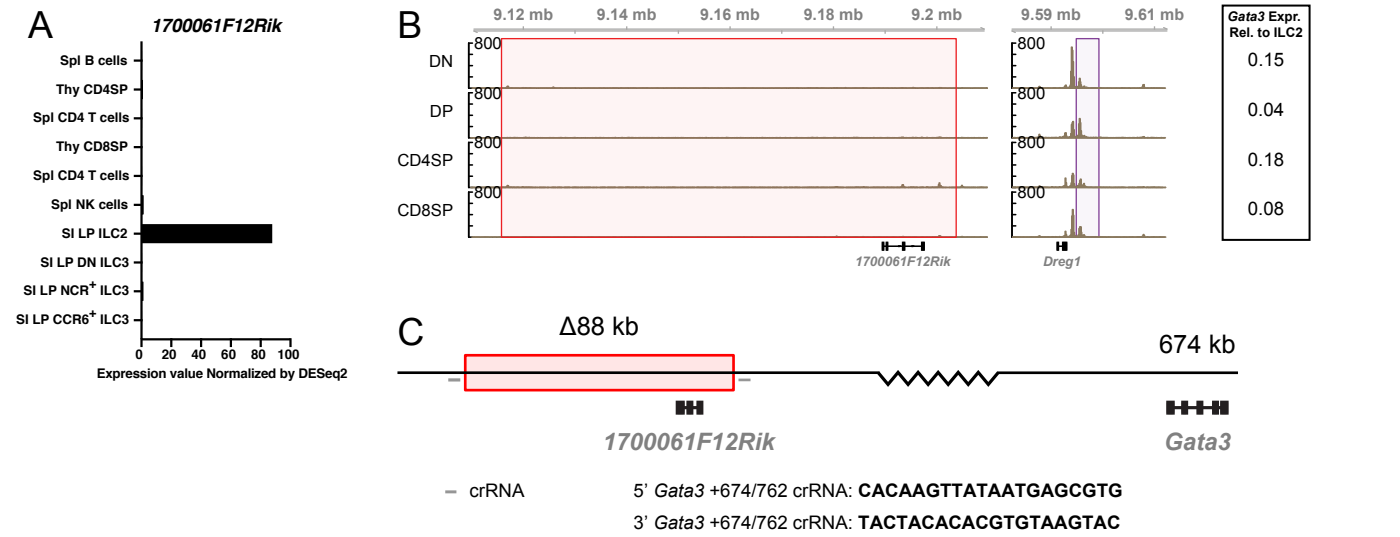
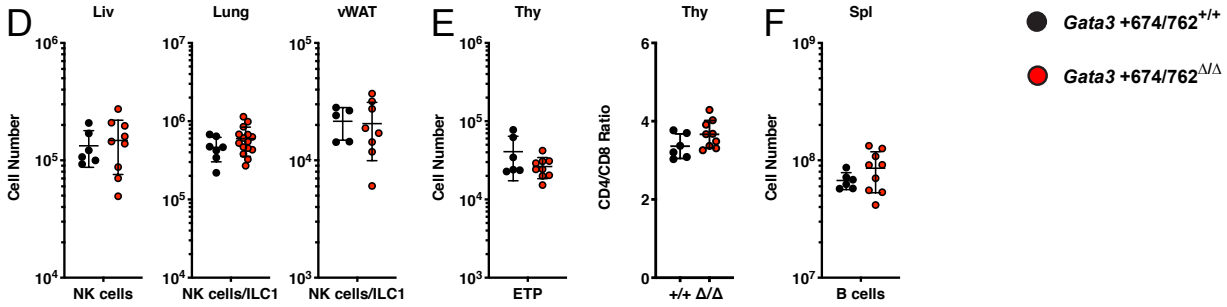
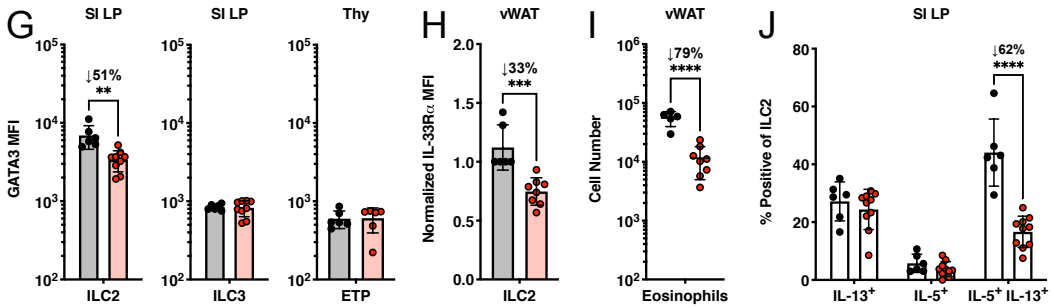
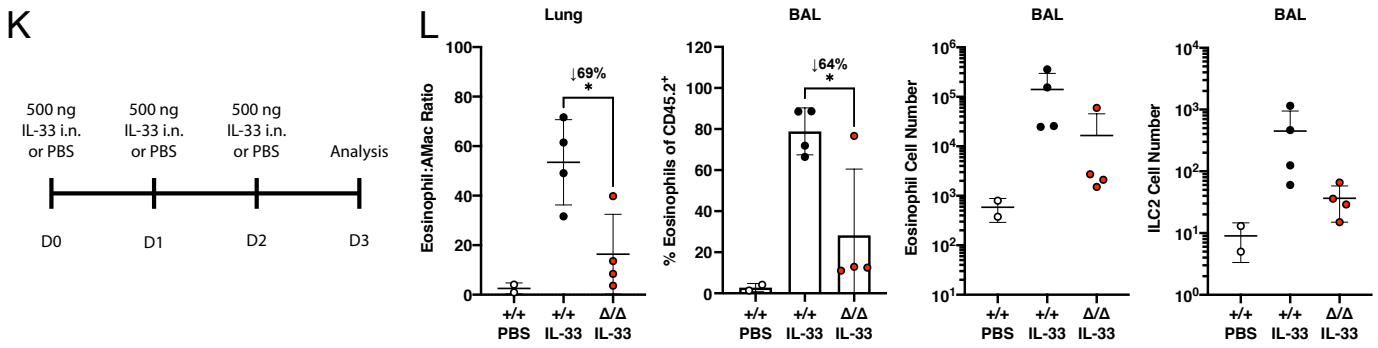
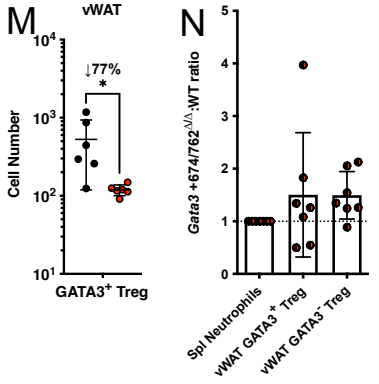
Supplemental Figure 1











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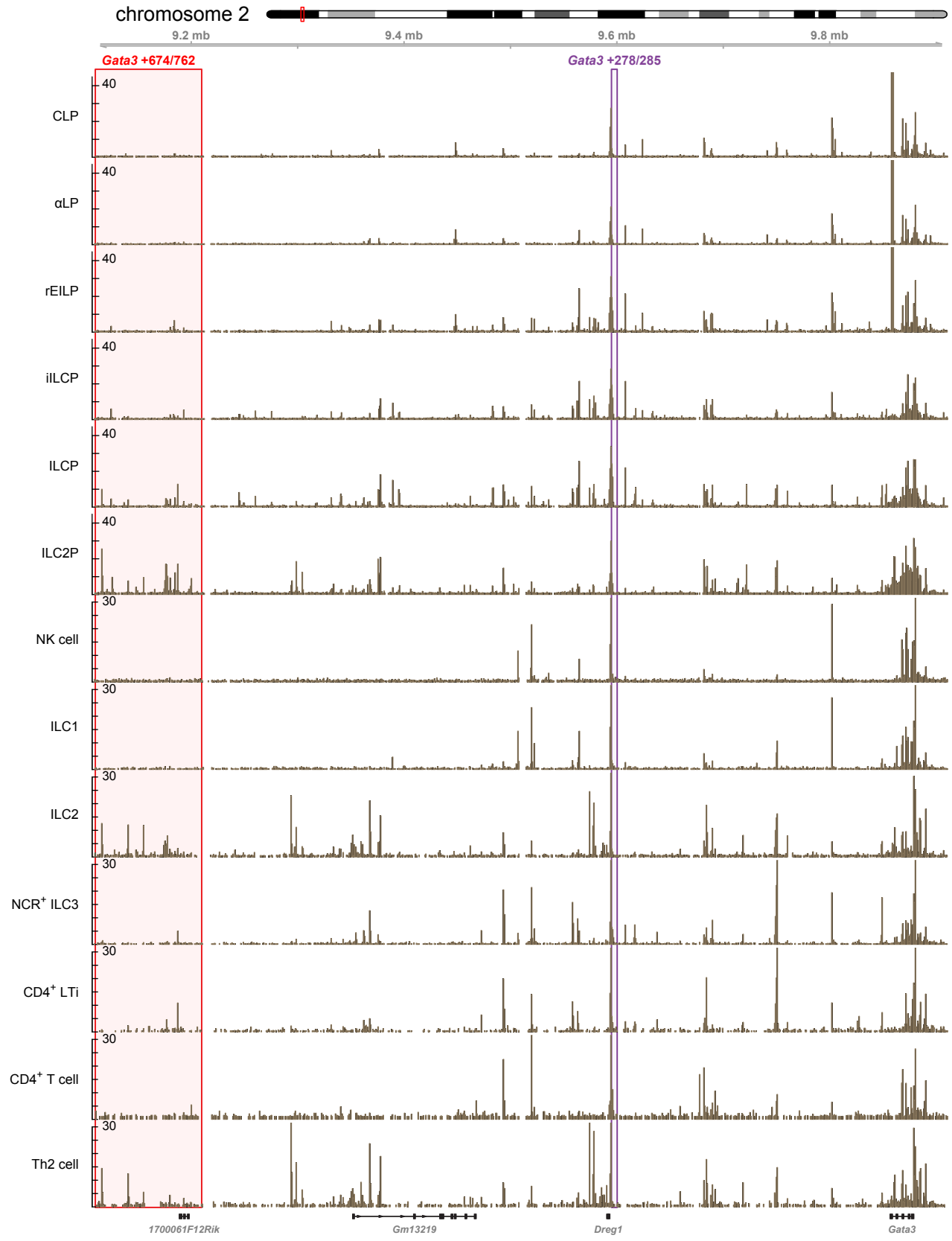
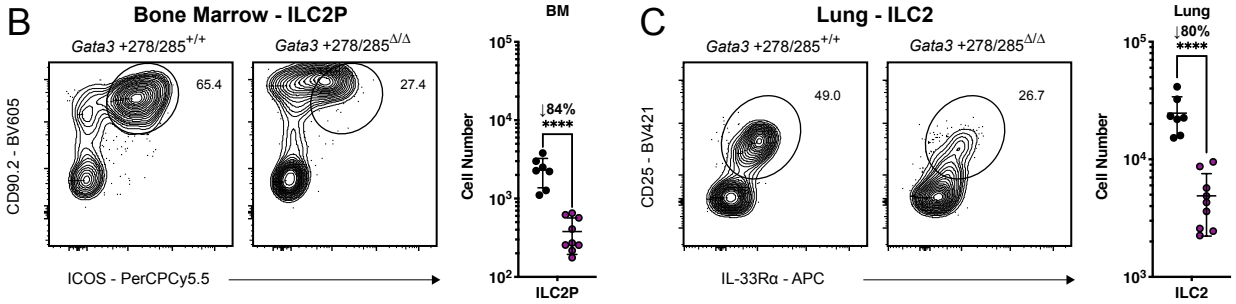
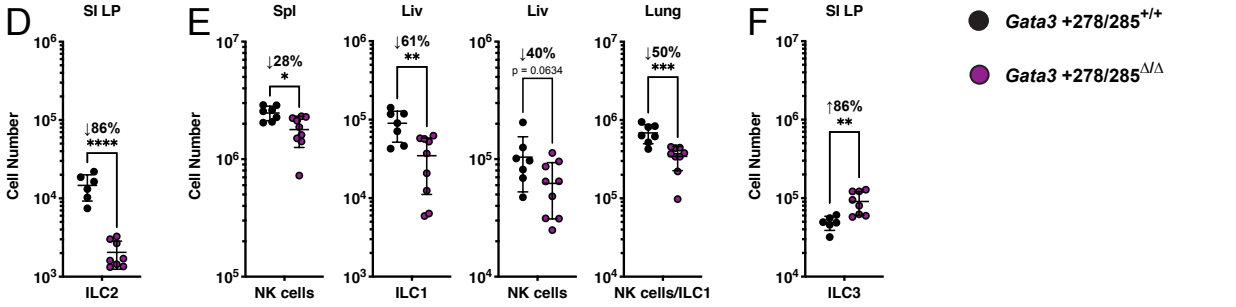
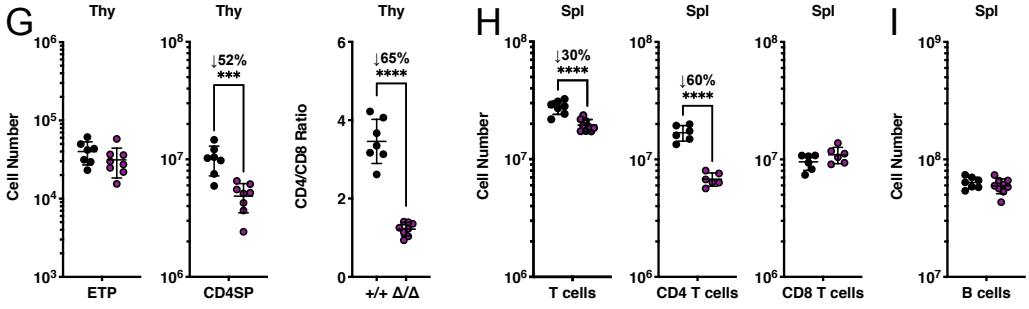
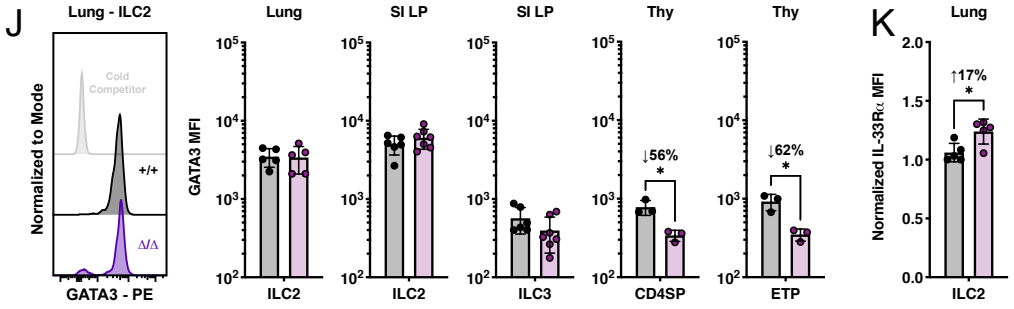


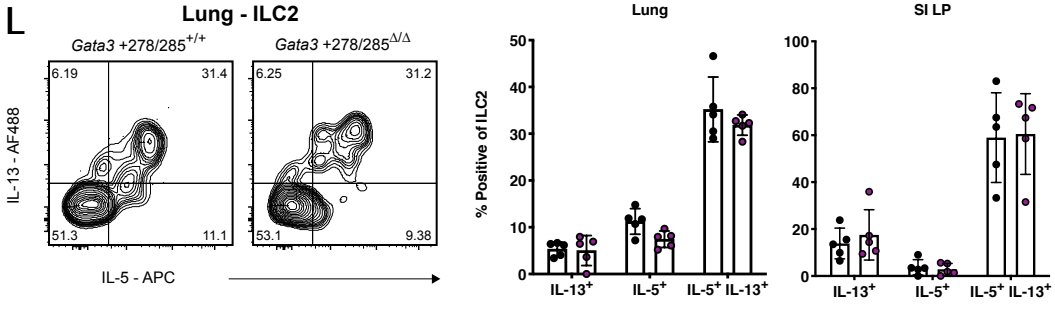
Supplemental Figure 3



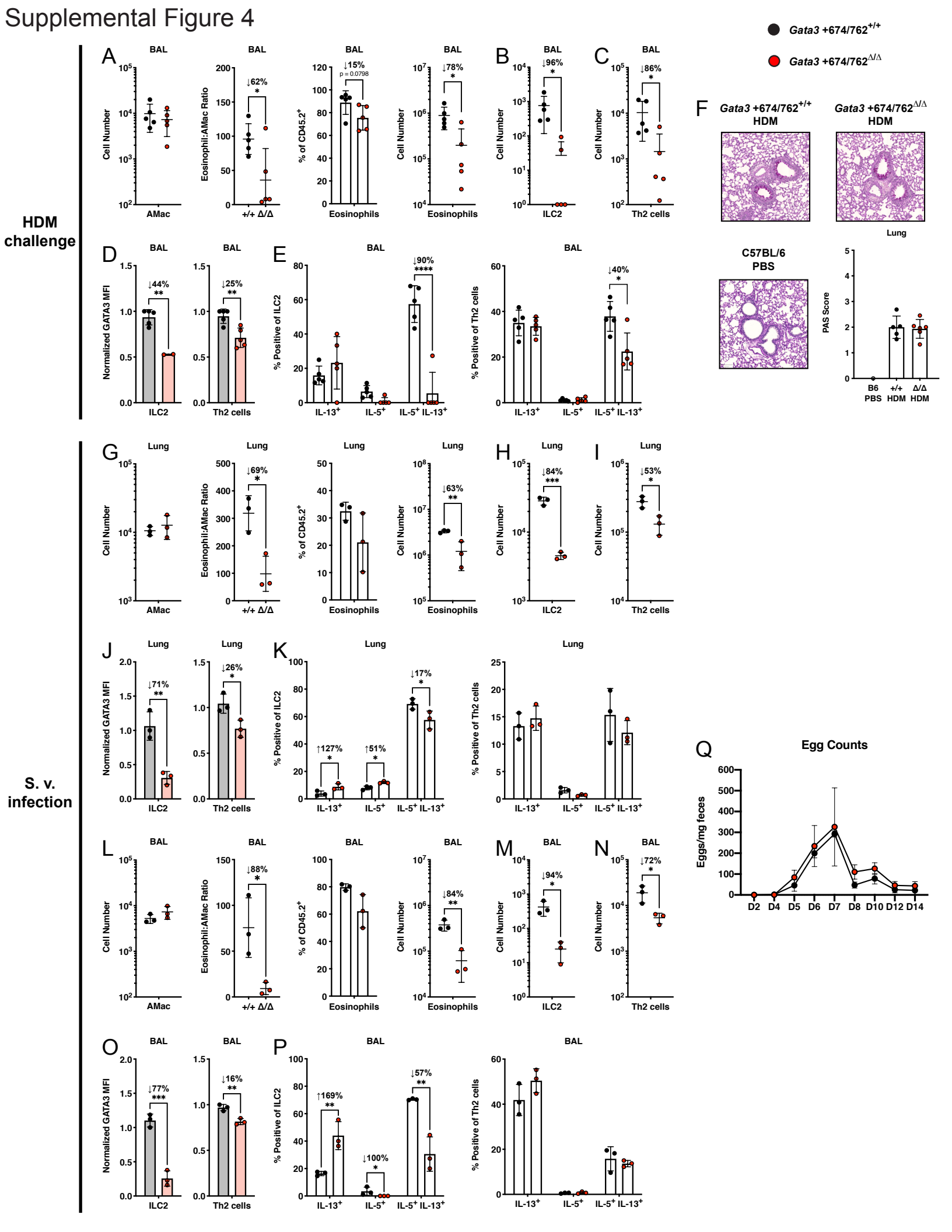




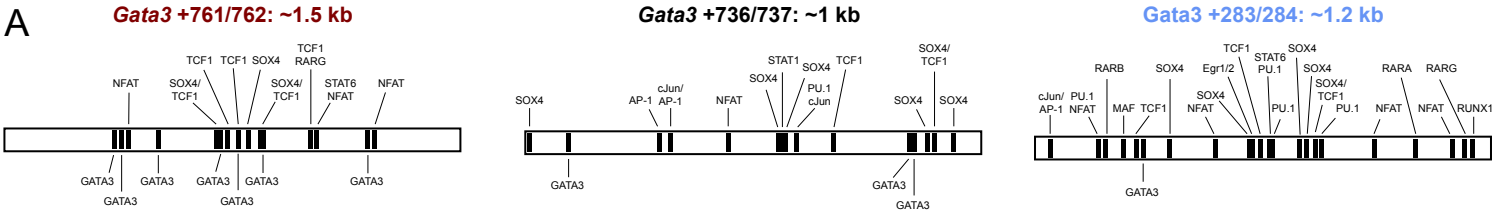


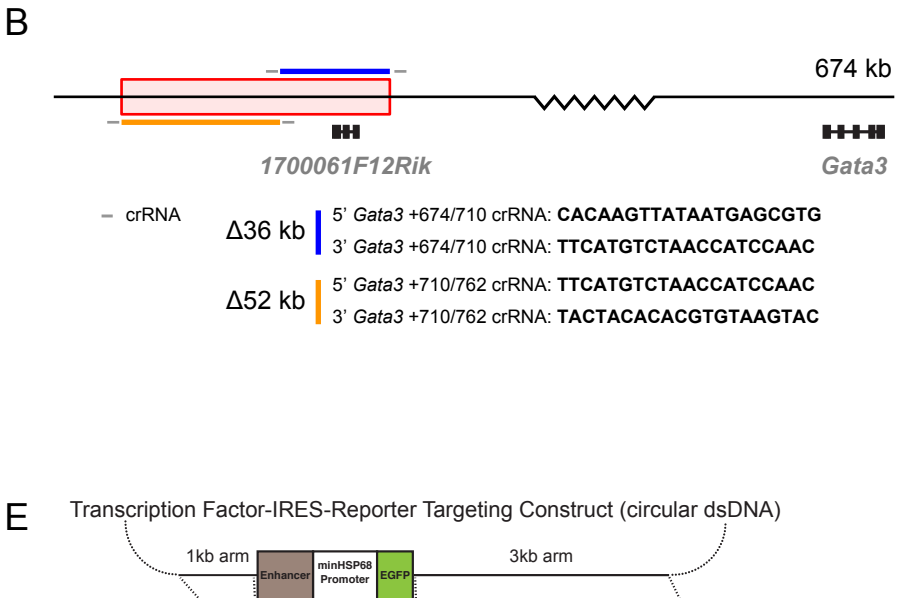


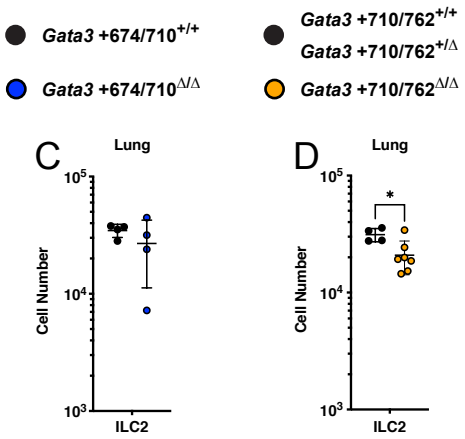
Supplemental Figure 4

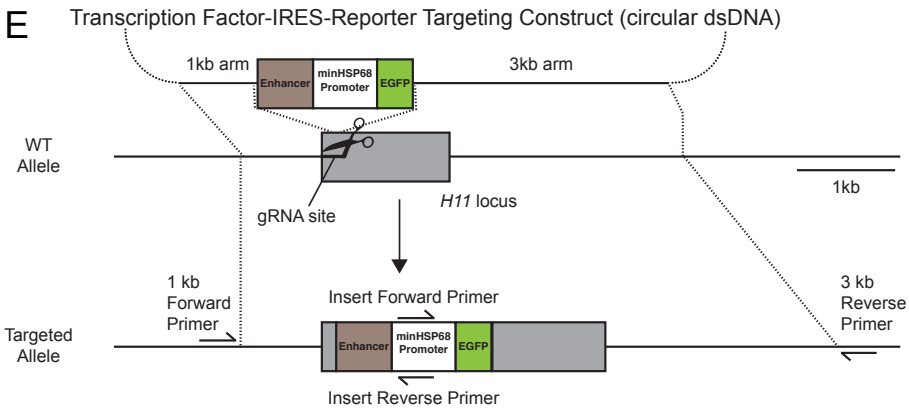


Supplemental Figure 5









H11 locus crRNA: **CCTTACCTTACTACCACTGT**

Supplemental Figure 1. Deletion of *Gata3* +674/762 and profiling of immune cells, related to Figure 1 and 2. (A) *1700061F12Rik* expression data in the indicated populations from Immgen (32). **(B)** Zoomed in ATAC-seq accessibility coverage tracks for the *Gata3* +674/762 and *Gata3* +278/285 regions in double negative (DN), double positive (DP), CD4SP, and CD8 single positive (CD8SP) thymocytes. Expression levels of *Gata3*-Citrine relative to lung ILC2 are shown to the right of **(B)** (18). **(C)** CRISPR/Cas9 deletion strategy for *Gata3* +674/762 with crRNA sequences. Summary data of cell numbers for **(D)** liver NK cells, lung NK/ILC1, and vWAT NK/ILC1; **(E)** ETPs and CD4/CD8 thymocyte ratio; and **(F)** splenic B cells. **(G)** Summary bar graphs of GATA3 MFI in SI LP ILC2, SI LP ILC3, and ETPs. **(H)** Summary bar graph of normalized IL-33Rα MFI on vWAT ILC2. **(I)** Summary data of vWAT eosinophil cell numbers. **(J)** Summary data for frequency of IL-5 and IL-13 cytokine production from SI LP ILC2s stimulated *in vitro* with PMA and ionomycin for 4 hours. **(K)** Schematic for i.n. challenge with IL-33. **(L)** Summary data for eosinophils in the lung and BAL and ILC2 numbers in the BAL from IL-33 challenged mice. (**M**) Summary data of GATA3$^+$ Treg cell numbers in the vWAT. (**N**) *Gata3* +674/762$^{\Delta/\Delta}$:WT reconstitution ratio for the indicated populations in mixed bone marrow chimeric mice. Dots represent individual mice; n ranging from 4-15 in different groups pooled from multiple independent experiments; data are presented as mean ± SEM. Statistical comparison was performed via unpaired t-test or multiple unpaired t-test. *, P < 0.05; **, P < 0.01; ***, P < 0.001; ****, P < 0.0001.

Supplemental Figure 2. Chromatin accessibility at the *Gata3* locus. ATAC-seq accessibility coverage tracks in BM CLP, αLP, rEILP, iILCP, ILCP, ILC2P, NK cell, ILC1, ILC2, NCR$^+$ ILC3, CD4$^+$ LTi, CD4$^+$ T cell, and Th2 cell (2). Red and purple windows represent *Gata3* +674/762 and *Gata3* +278/285 regions respectively.

Supplemental Figure 3. Deletion of *Gata3* +278/285 and profiling of lymphocyte subsets and homeostatic ILC2 function. (A) CRISPR/Cas9 deletion strategy for *Gata3* +278/285 with crRNA sequences. Representative flow cytometry plots and summary data of cell numbers in WT vs. *Gata3* +278/285$^{\Delta/\Delta}$ mice for **(B)** BM ILC2P (pre-gated on Lin$^-$α4β7$^+$IL-7Rα$^+$) and **(C)** lung ILC2 (pre-gated on CD45.2$^+$CD19$^-$CD11c$^-$CD3ε$^-$TCRβ$^-$IL-7Rα$^+$CD90.2$^+$). Summary data of cell numbers for **(D)** SI LP ILC2; **(E)** spleen NK cells, liver ILC1, liver NK cells, and lung NK/ILC1; **(F)** SI LP ILC3; **(G)** ETPs, CD4SP thymocytes, and CD4/CD8 thymocyte ratio; **(H)** splenic T cells and split CD4$^+$ and CD8$^+$ T cells, and **(I)** splenic B cells in WT vs. *Gata3* +278/285$^{\Delta/\Delta}$ mice. **(J)** Representative histogram and summary bar graph of GATA3 MFI in lung ILC2 and summary bar graph for SI LP ILC2, SI LP ILC3, CD4SP thymocytes, and ETPs. White histogram denotes lung ILC2 GATA3 stain blocked with unlabeled antibody (cold competitor). **(K)** Summary bar graph of normalized IL-33Rα MFI on lung ILC2. **(L)** Representative flow cytometry plots of lung ILC2s (pre-gated on CD45.2$^+$CD19$^-$CD11c$^-$CD3ε$^-$TCRβ$^-$IL-7Rα$^+$CD90.2$^+$CD25$^+$IL-33Rα$^+$) and summary data for frequency of IL-5 and IL-13 cytokine production from lung ILC2s and SI LP ILC2s stimulated *in vitro* with PMA and ionomycin for 4 hours. Dots represent individual mice; n ranging from 3-9 in different groups pooled from multiple independent experiments; data are presented as mean ± SEM. Statistical comparison was performed via unpaired t-test or multiple unpaired t-test. *, P < 0.05; **, P < 0.01; ***, P < 0.001; ****, P < 0.0001.

Supplemental Figure 4. Response of *Gata3* +674/762$^{\Delta/\Delta}$ mice to type 2 inflammatory challenge, related to Figure 3. (A) Summary data for AMac and eosinophils in the BAL of HDM challenged mice. Summary data of **(B)** ILC2 and **(C)** Th2 cell numbers in the BAL from HDM challenged mice. **(D)** Summary plots of GATA3 MFI in BAL ILC2s and Th2 cells from HDM challenged mice. **(E)** Summary data for frequency of IL-5 and IL-13 cytokine production from BAL ILC2s and Th2 cells from HDM challenged mice stimulated *in vitro* with PMA and ionomycin for 4 hours. **(F)** PAS staining of lung sections at 40X magnification with summary bar graph of PAS scores. **(G, L)** Summary data for AMac and eosinophils in the lung or BAL from *S. venezuelensis* challenged mice. Summary data of **(H, M)** ILC2 and **(I, N)** Th2 cell numbers in the lung or BAL from *S. venezuelensis* challenged mice. **(J, O)** Summary bar graphs of GATA3 MFI in lung and BAL ILC2s and Th2 cells from *S. venezuelensis* challenged mice. **(K, P)** Summary data for frequency of IL-5 and IL-13 cytokine production from lung or BAL ILC2s and Th2 cells from *S. venezuelensis* challenged mice stimulated *in vitro* with PMA and ionomycin for 4 hours. **(Q)** Time course of *S. venezuelensis* egg counts in the feces of infected mice. Dots represent individual mice; data are pooled from multiple independent experiments (HDM; n = 5) or one experiment (*S. venezuelensis*; n = 3); data are presented as mean ± SEM. Values of 0 were converted to a value of 1 on a log-scale. Statistical comparison was performed via unpaired t-test or multiple unpaired t-test. *, P < 0.05 ; **, P < 0.01; ***, P < 0.001.

Supplemental Figure 5. CRISPR/Cas9-mediated dissection of *Gata3* +674/762 sub-domains. (A) TRANSFAC predicted immune specific transcription factor binding sites for *Gata3* +761/762, *Gata3* +736/737, and *Gata3* +283/284 regions (59). **(B)** CRISPR/Cas9 deletion strategy for *Gata3* +674/710 and *Gata3* +710/762 with crRNA sequences. Summary data of lung ILC2 numbers in WT vs. **(C)** *Gata3* +674/710$^{\Delta/\Delta}$ and **(D)** *Gata3* +710/762$^{\Delta/\Delta}$ mice. **(E)** Design of CRISPR/Cas9 knock-in strategy for enhancer reporter insertion at the *H11* locus. Dots represent individual mice; n ranging from 4 to 8 in multiple independent experiments; data are presented as mean ± SEM. *Gata3* +710/762$^{\Delta/\Delta}$ and respective control mice were pups from the cross breeding of F_0 mice.