Supplemental Figure 1

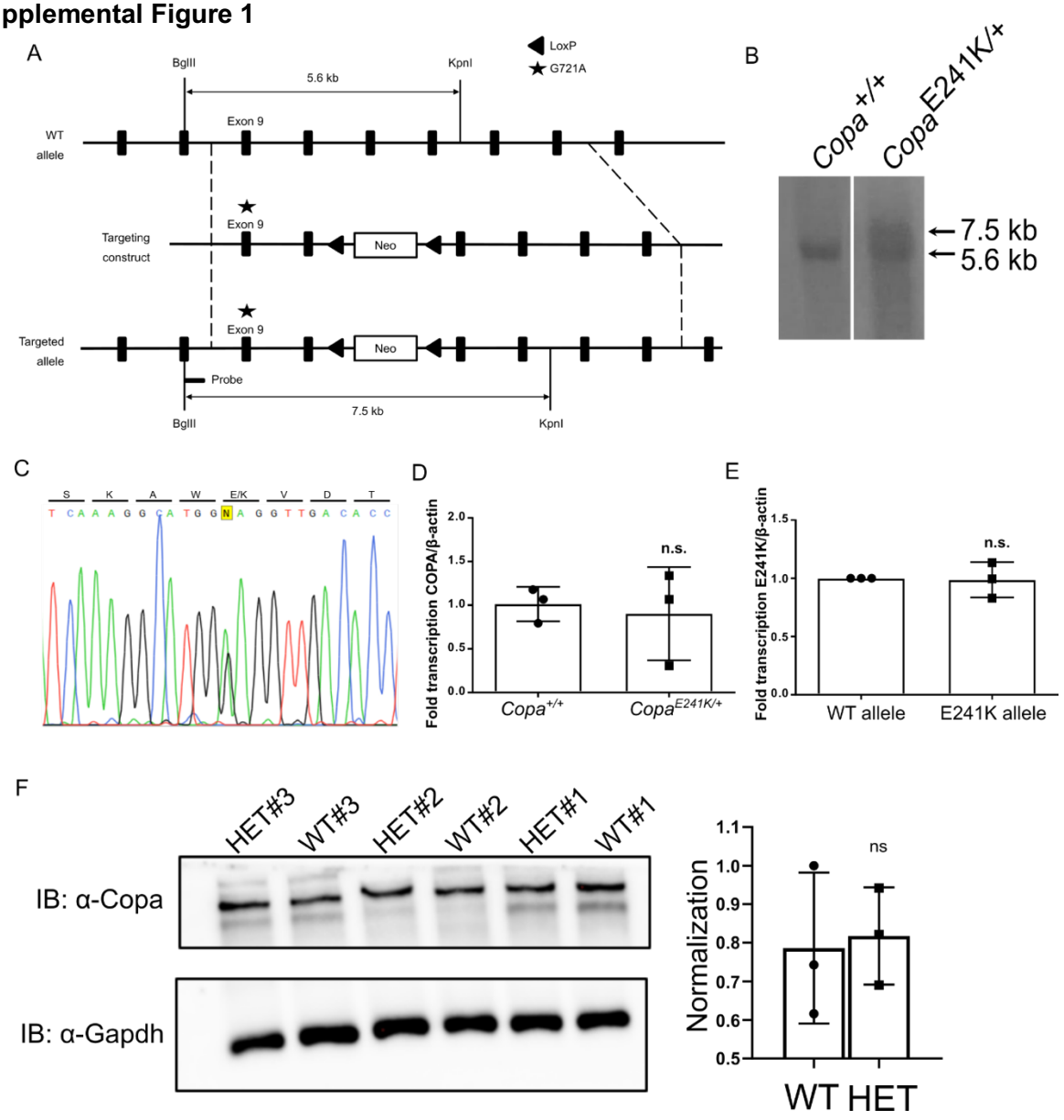

Fig. S1. Schematic for generation of *Copa*$^{E241K/+}$ mice and validation of gene targeting. A) Schematic of the targeting vector used to knock-in the E241K mutation into exon 9 of the *Copa* gene. The Neomycin cassette is flanked by LoxP sites. The dashed lines indicate the ends of the targeting construct's homology. **B)** Southern blot of genomic DNA from ES cells targeted with the E241K/G721A knock-in construct and digested with BglII and KpnI. **C)** The presence of the mutation was confirmed by Sanger sequencing in ES cells that were positive by Southern blot. **D)** Real time PCR measurement of total *Copa* mRNA level in WT and *Copa*$^{E421K/+}$ mice (WT, $n = 3$; HET, $n = 3$). **E)** mRNA level of wild type *Copa* and *Copa*^{E241K} alleles in heterozygous *Copa*$^{E421K/+}$ mice ($n = 3$). **F)** left: Western blot showing the Copa protein level in the thymocytes from indicated mice (WT, $n = 3$; HET, $n = 3$). right: Quantification of the western blot. Data are mean ± *SD*. Unpaired, parametric, two-tailed Student's *t*-test was used for statistical analysis. $p < 0.05$ is considered statistically significant. ns: not significant.

Supplemental Figure 2

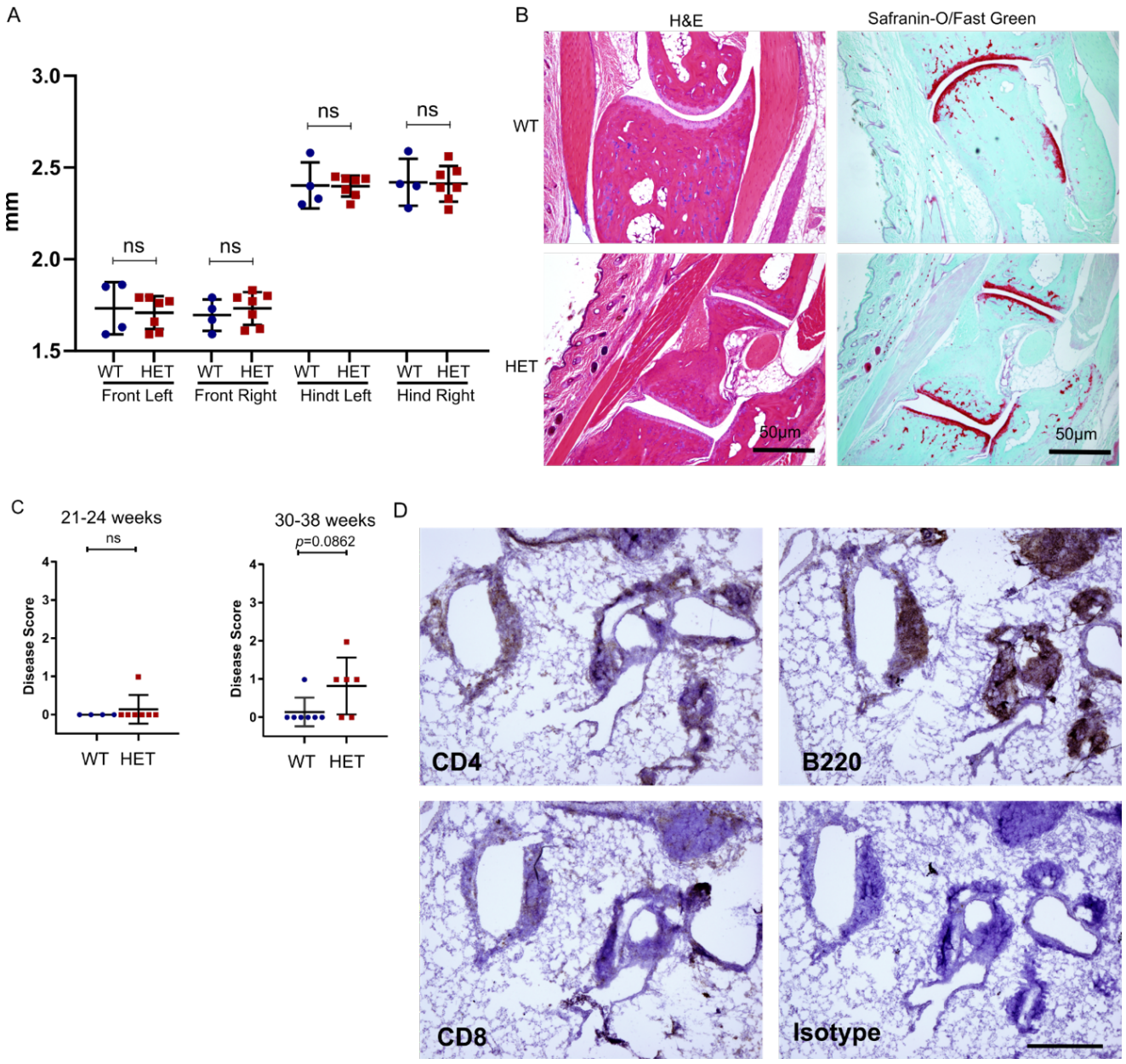

Fig. S2: *Copa*$^{E241K/+}$ mice develop lymphocytic infiltration of lung but no joint disease. **A)** Ankle thickness of the wild type and *Copa*$^{E241K/+}$ mice (6-month-old litter mates: WT, n = 4; HET, n = 7). **B)** left: H&E staining of the ankle sections of the hind legs. right: Safranin-O/Fast green staining of the ankle sections of the hind legs. **C)** Disease scores of lung sections from the 5-6-month-old (Littermates: WT, n = 4; HET, n = 7) and 7-9-month-old (Littermates: WT, n = 7; HET, n = 6) mice. **D)** Representative image of the IHC staining of the lung section from *Copa*$^{E241K/+}$ mice. Data are mean ± *SD*. Unpaired, parametric, two-tailed Student's *t*-test was used for statistical analysis in A. $p < 0.05$ is considered statistically significant. ns: not significant. B and D taken at 4x magnification, scale bar = 50µm.

Supplemental Figure 3

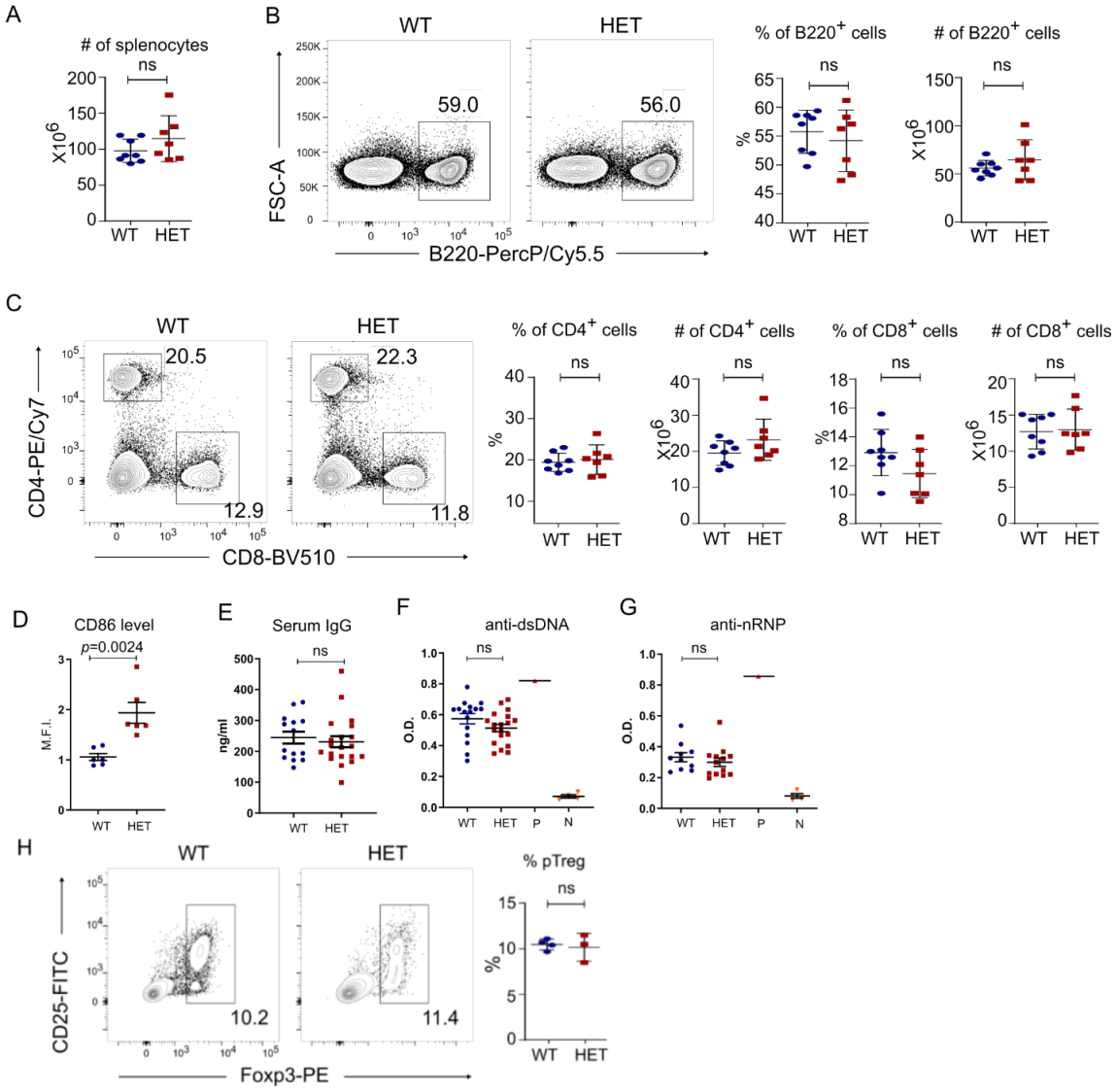

Fig. S3: *Copa*$^{E241K/+}$ mice have normal percentages of splenic B and T cells and no autoantibodies. **A)** Cell counts of splenocytes (3-month-old littermates: WT, $n = 8$; HET, $n = 7$). **B)** left: Representative flow plots showing percentage of B cells based on B220 expression. right: Percentage and cell counts of B220$^+$ cells among splenocytes (3-month-old littermates: WT, $n = 8$; HET, $n = 7$). **C)** left: Representative flow plots showing the percentages of CD4$^+$ and CD8$^+$ T cells. right: Percentages and cell counts of CD4$^+$ and CD8$^+$ T cells among splenocytes (3-month-old littermates: WT, $n = 8$; HET, $n = 7$). **D)** Quantification of the M.F.I. of CD86 on B cells. (3-month-old littermates: WT, $n = 6$; HET, $n = 6$) **E)** Measurement of serum IgG level by Elisa (9-10-month-old littermates: WT, $n = 14$; HET, $n = 20$) **F)** Serum anti-dsDNA IgG level by Elisa (9-10-month-old littermates: WT, $n = 16$; HET, $n = 19$). P: serum from a Lyn knock out mouse. N: PBS. **G)** Serum anti-nRNP IgG level by Elisa (9-10-month-old littermates: WT, $n = 10$; HET, $n = 13$). P: serum from a Lyn knock out mouse. N: PBS. **H)** left: Intracellular Foxp3 in total splenic CD4+ T cells (gated on CD4+TCRβ+). right: Percentages of Foxp3+ CD4+ T cells among total splenic CD4+ T cells. Data are mean ± *SD*. Unpaired, parametric, two-tailed Student's *t*-test was used for statistical analysis. $p < 0.05$ is considered statistically significant. ns: not significant.

Supplemental Figure 4

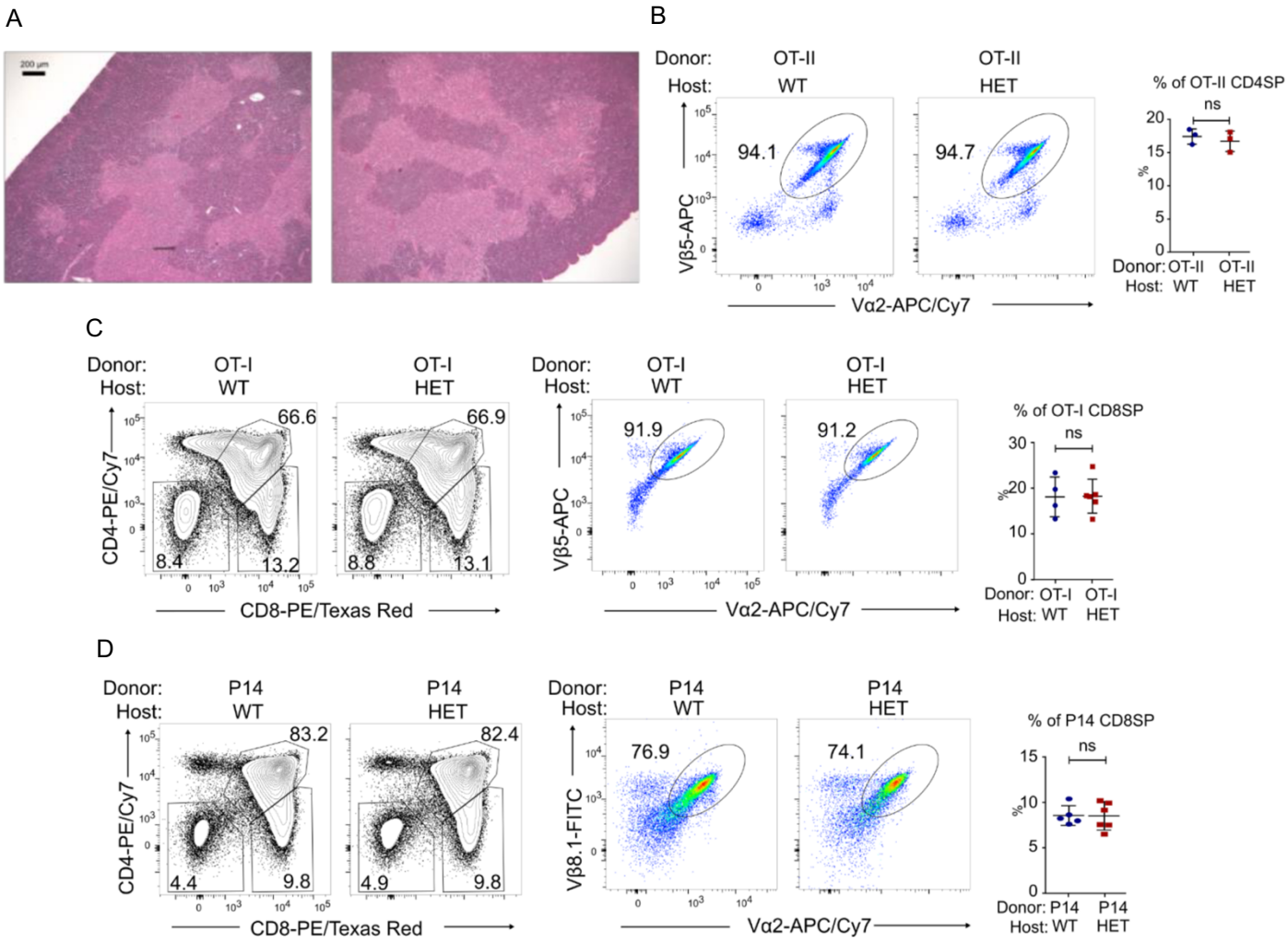

Fig. S4. Mutant Copa in thymic stroma causes an increase in SP thymocytes despite apparent normal positive selection in *Copa*$^{E241K/+}$ mice. A) Representative H&E staining of thymic architecture in WT and CopaE241/+ littermates (n = 3 per genotype). **B)** left: Representative flow plots of Vα2 and Vβ5 on OT-II CD4SP thymocytes in WT and HET hosts. right: Percentages of OT-II CD4SP in the indicated hosts (WT, $n = 3$; HET, $n = 3$). **C)** left: Representative CD4 versus CD8 flow plots of OT-I thymocytes in WT and HET hosts. middle: Flow cytometric measurement of Vα2 and Vβ5 on CD8SP thymocytes. right: Percentage of CD8SP in the indicated hosts (WT, $n = 4$; HET, $n = 5$). **D)** left: Representative CD4 versus CD8 flow plots of P14 thymocytes in WT and HET hosts. middle: Flow cytometric measurement of Vα2 and Vβ8.1 on CD8SP thymocytes. right: Percentage of CD8SP in the indicated hosts (WT, $n = 5$; HET, $n = 5$). Data are mean ± *SD*. Unpaired, parametric, two-tailed Student's *t*-test was used for statistical analysis. $p < 0.05$ is considered statistically significant. ns: not significant.