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Supplemental Information

Interleukin-27 Is Essential for Type 1 Diabetes Development and Sjögren Syndrome-like Inflammation

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A

Partial sequence of the 4th coding exon of *Il27*

WT TGCTTCCTCGCTACCACACTTCGGCCCTTCCCTGCCATGCTGGGAGGGCTGGGGACCCA

Il27-/- TGCTTCCTCGCTACCACACTTCGGCCCT~~-~~CCCTGCCATGCTGGGAGGGCTGGGGACCCA

Partial sequence of the 2nd coding exon of *Il27ra*

WT CGTCGGTCCCCTGGGAATCCTGAACTGCTCCTGGGAACCTTTGGGCGACCTGGAGA

Il27ra-/- CGTCGGTCC~~CCTGGGAATCCTGAACTGCTCCTGGGAACCTTTG~~GGCGACCTGGAGA

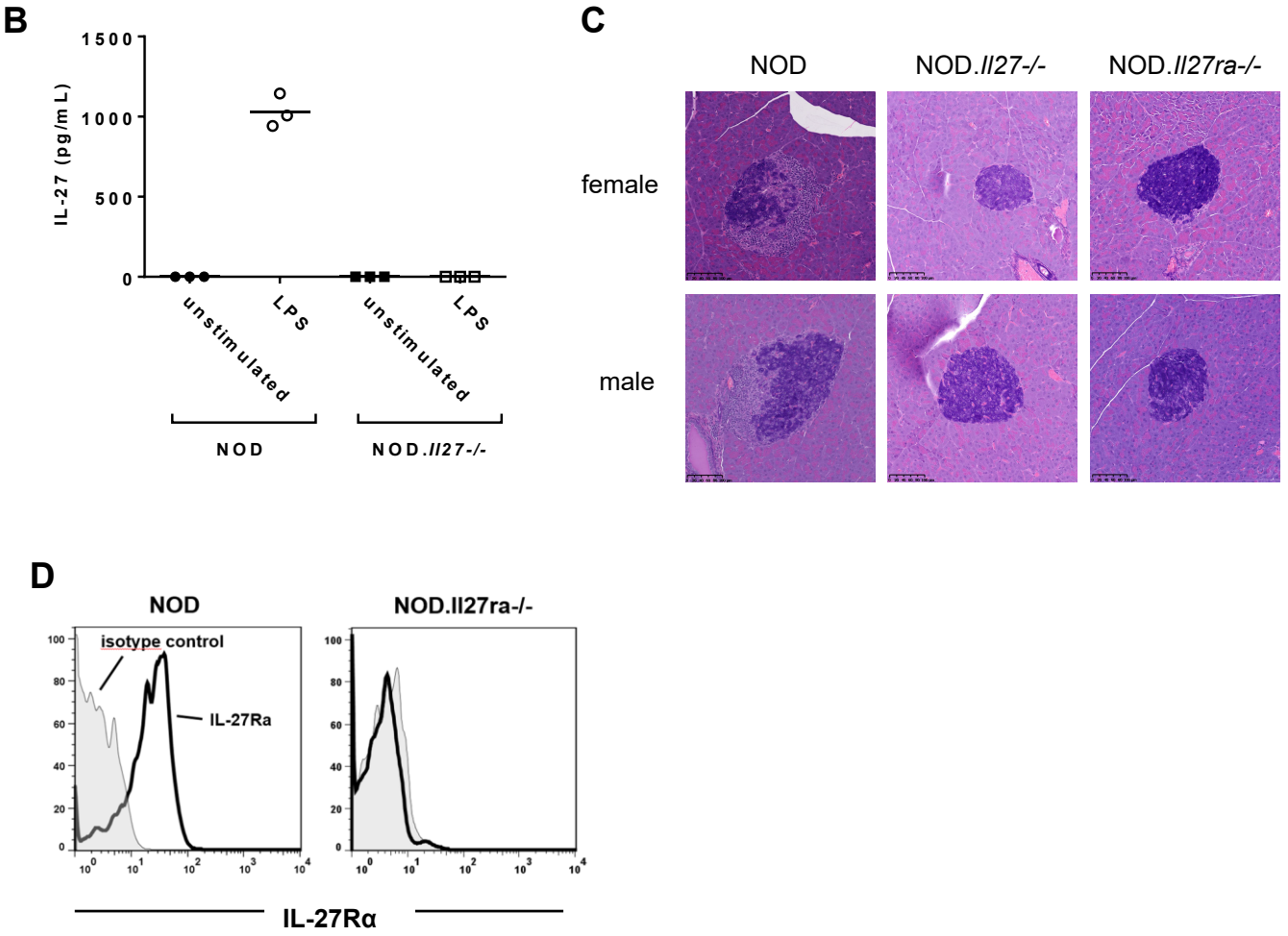

Figure S1. Related to Figures 1 and 4. Generation and characterization of NOD.*Il27*$^{-/-}$ and NOD.*Il27ra*$^{-/-}$ mice. (A) Partial sequence of the 4th coding exon of *Il27* depicting single base pair deletion (double strikethrough) created by zinc finger nucleases (Top). Partial sequence of the 2nd coding exon of *Il27ra* depicting deletion of 34 base pairs (double strikethrough) created by the CRISPR/Cas9 technology. (B) Bone marrow derived macrophages from NOD or NOD.*Il27*$^{-/-}$ mice were stimulated for 24 hours with 100ng/mL LPS. IL-27 protein was measured in the supernatant by ELISA. (C) Pancreatic sections from 10-week-old non-diabetic mice were stained with aldehyde fuchsin followed by H&E. Representative images depict islet infiltration in mice of the indicated sex and strain. Scale bar is 100 μm. (D) Representative flow cytometry histograms depicting IL-27Rα expression in splenic CD8 T cells of NOD or NOD.*Il27ra*$^{-/-}$ mice. Shaded area indicates isotype control.

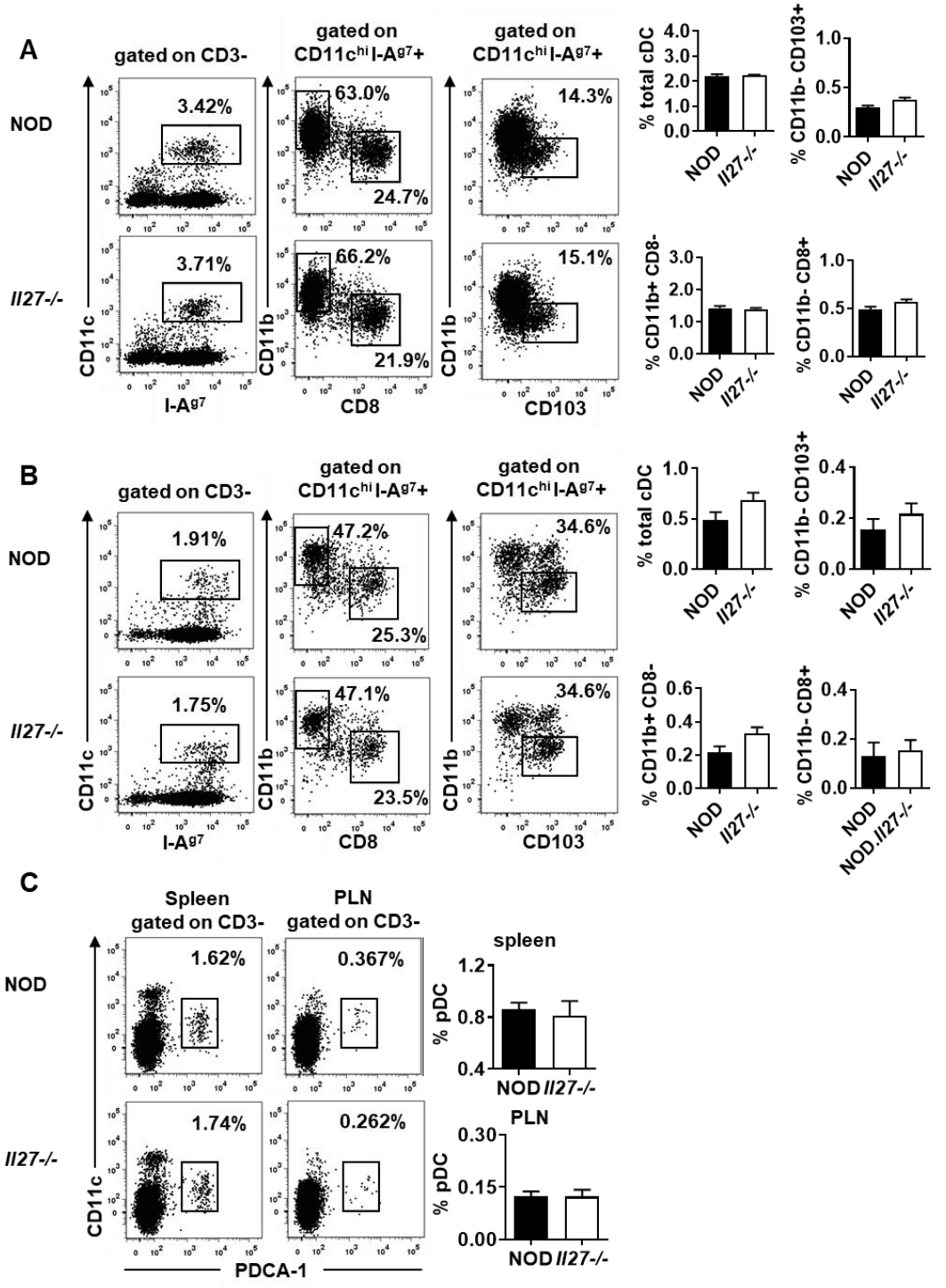

Figure S2. Related to Figure 2. Splenic and PLN DC subsets in NOD and NOD.*Il27*$^{-/-}$ mice. (A) Analyses of splenic DC subsets. Representative flow cytometry plots depicting splenic DC subsets in 10-week-old female NOD and NOD.*Il27*$^{-/-}$ mice (Left). Summarized data from at least two independent experiments (Right). The frequencies in the summarized results depict the percentages of total DCs or their subsets among total splenocytes. (B) Analyses of PLN DC subsets. Representative flow cytometry plots depicting PLN DC subsets in 10-week-old NOD or NOD.*Il27*$^{-/-}$ females (Left). Summarized data from at least two independent experiments (Right). The frequencies in the summarized results depict the percentages of total DCs or their subsets among total PLN cells. (C) Representative flow cytometry plots (Left) and the summarized results (Right) depicting the frequencies of splenic and PLN plasmacytoid DCs in 10-week-old NOD females. The frequencies in the summarized results depict the percentages of pDCs among total splenocytes or PLN cells. All error bars are SEM.

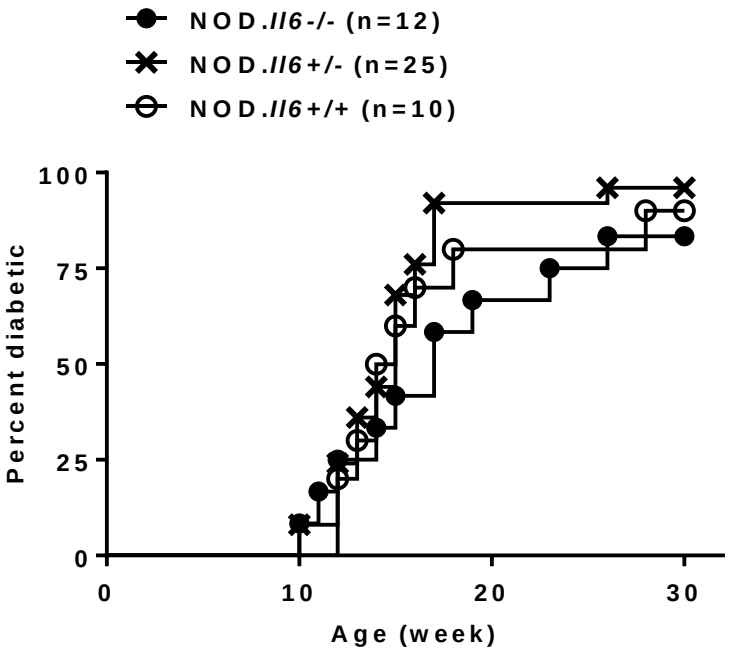

Figure S3. Related to Figure 4. IL-6 does not play an essential role in T1D development. Female littermates of the indicated genotypes were monitored for diabetes development for 30 weeks. Diabetes onset was determined by two consecutive positive readings of glycosuria on a urine test strip (> 250 mg/dl). No difference in diabetes development was found among genotypes.

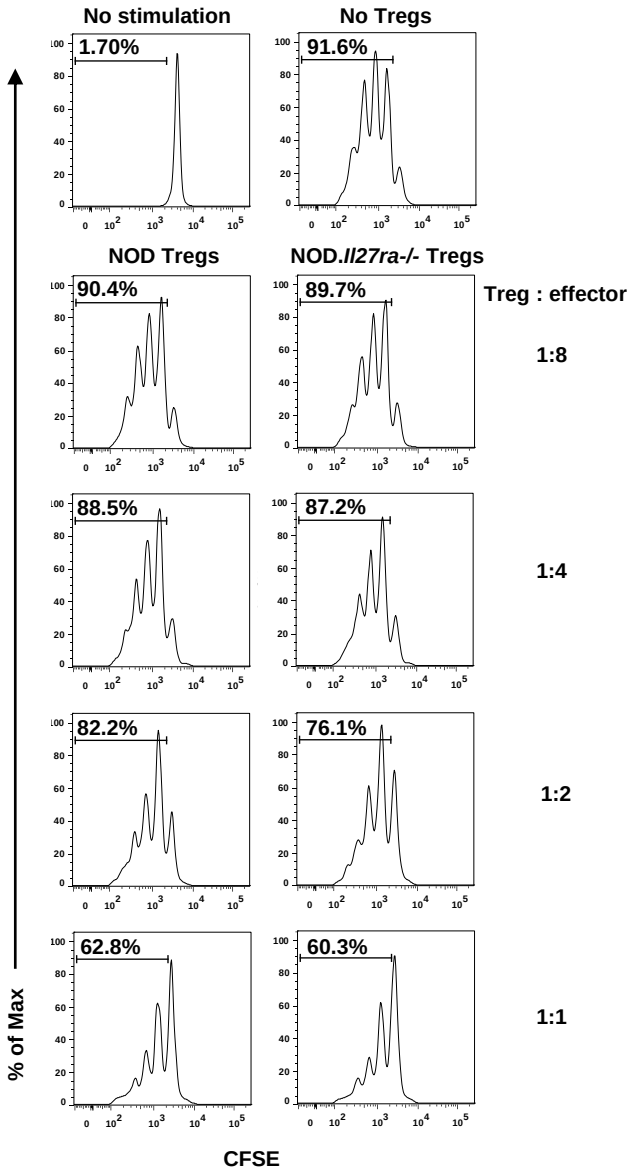

Figure S4. Related to Figure 4. *In vitro* suppression function of IL-27 receptor deficient Tregs. Splenic $CD4^+CD25^-$ T cells were isolated from NOD mice, labeled with CFSE, and cultured either alone or in the presence of unlabeled $CD4^+CD25^+$ NOD or NOD.*Il27ra*$^{-/-}$ Tregs at the indicated ratios. Cells were activated with soluble anti-CD3 in the presence of NOD.*Rag1*$^{-/-}$ splenocytes for three days and proliferation was analyzed by flow cytometry. Representative histograms depicting proliferation of single, viable, $CD4^+$ $CFSE^+$ responder cells are shown. Data are representative of 3 independent experiments.

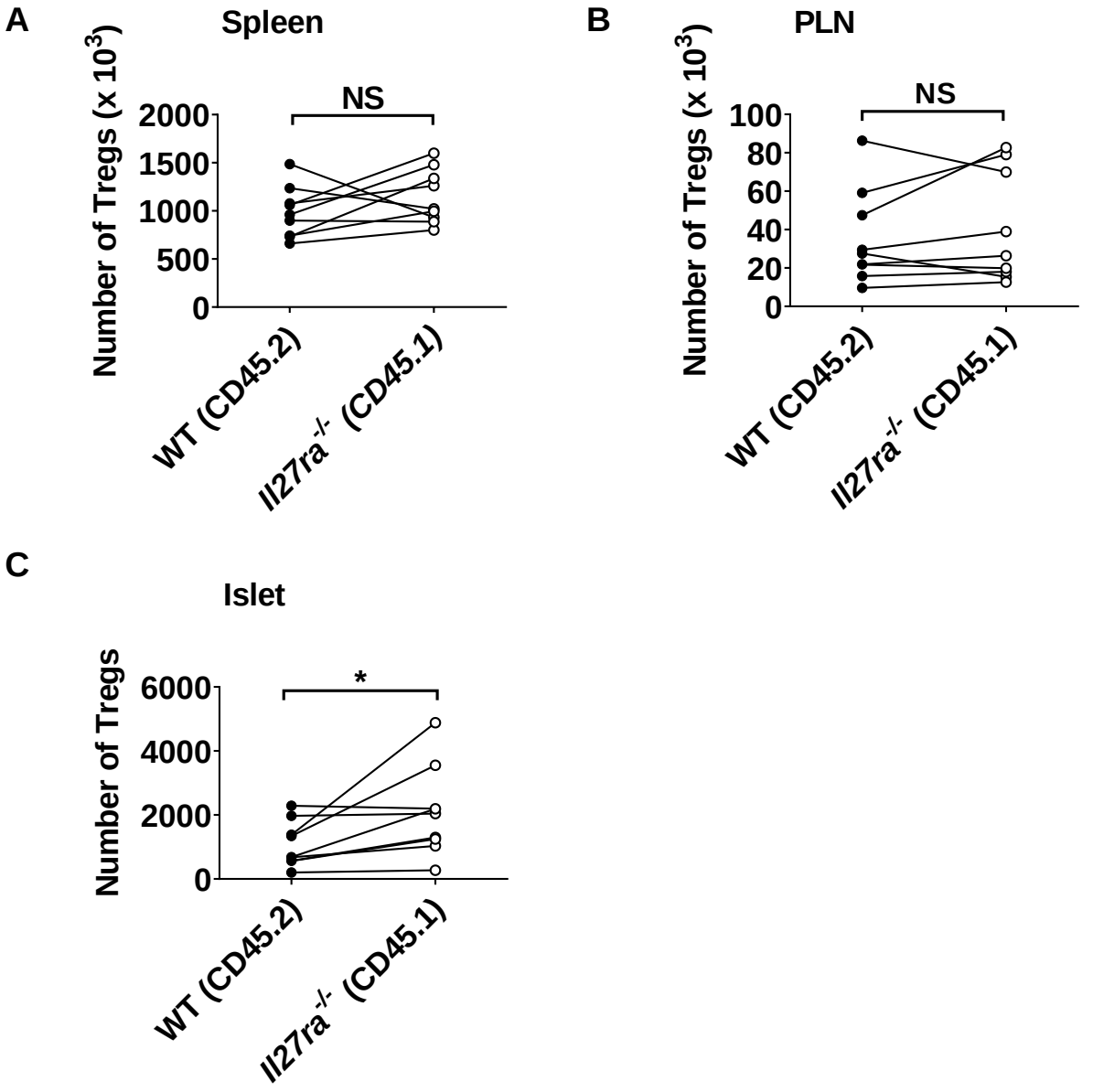

Figure S5. Related to Figure 5. Differential accumulation of wildtype and IL-27Rα-deficient Tregs in mixed BM chimeras. Lethally irradiated (NOD x NOD.*Cd45.2*)F1 mice were infused with equal numbers of T cell-depleted BM cells from NOD.*Cd45.2* and NOD.*Il27ra*$^{-/-}$ donors. Pre-diabetic recipients were analyzed for wildtype (CD45.2^{+}) and IL-27Rα-deficient (CD45.1^{+}) T cell subsets 10-12 weeks after BM reconstitution. (A-C) Summarized results pooled from 2 independent experiments depict absolute number of CD3^{+}CD4^{+}Foxp3^{+} Tregs in the spleens (A), PLNs (B), and islets (C) of the mixed BM chimeras. Statistical significance was determined with Wilcoxon matched-pairs signed rank test. *$p < 0.05$. NS: not significant.