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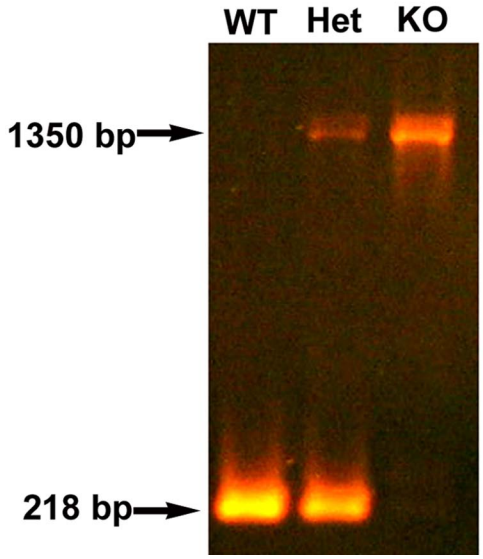
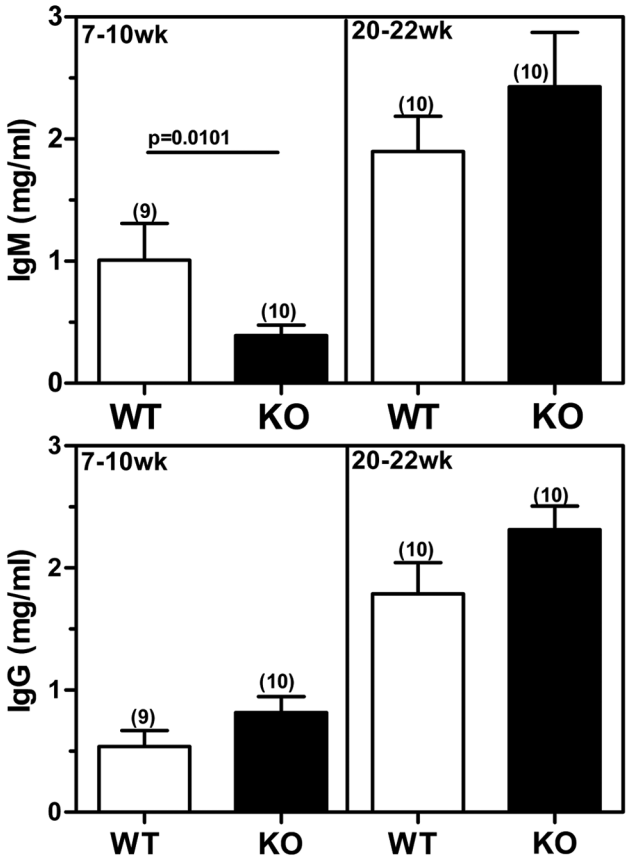
Type I Interferon Receptor Deficiency Prevents Murine Sjögren's Syndrome

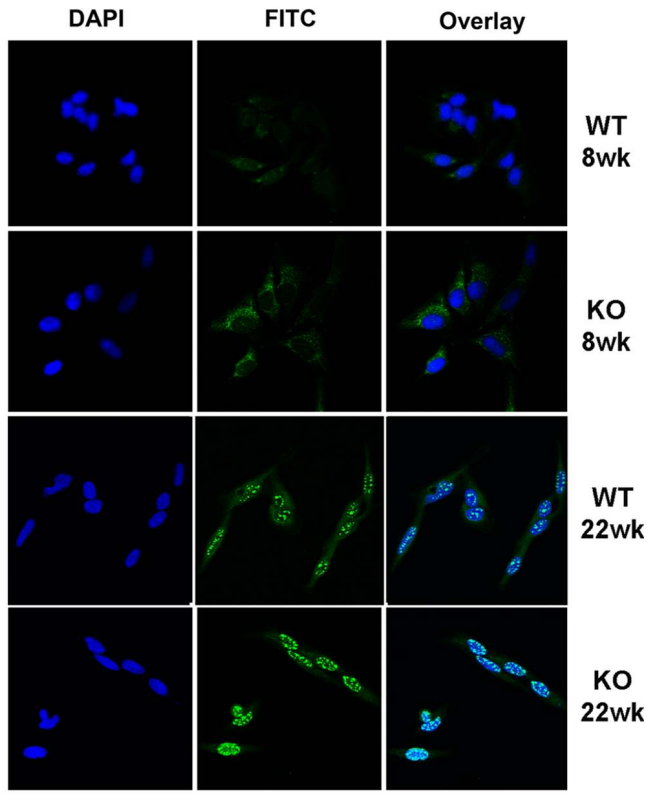
APPENDIX



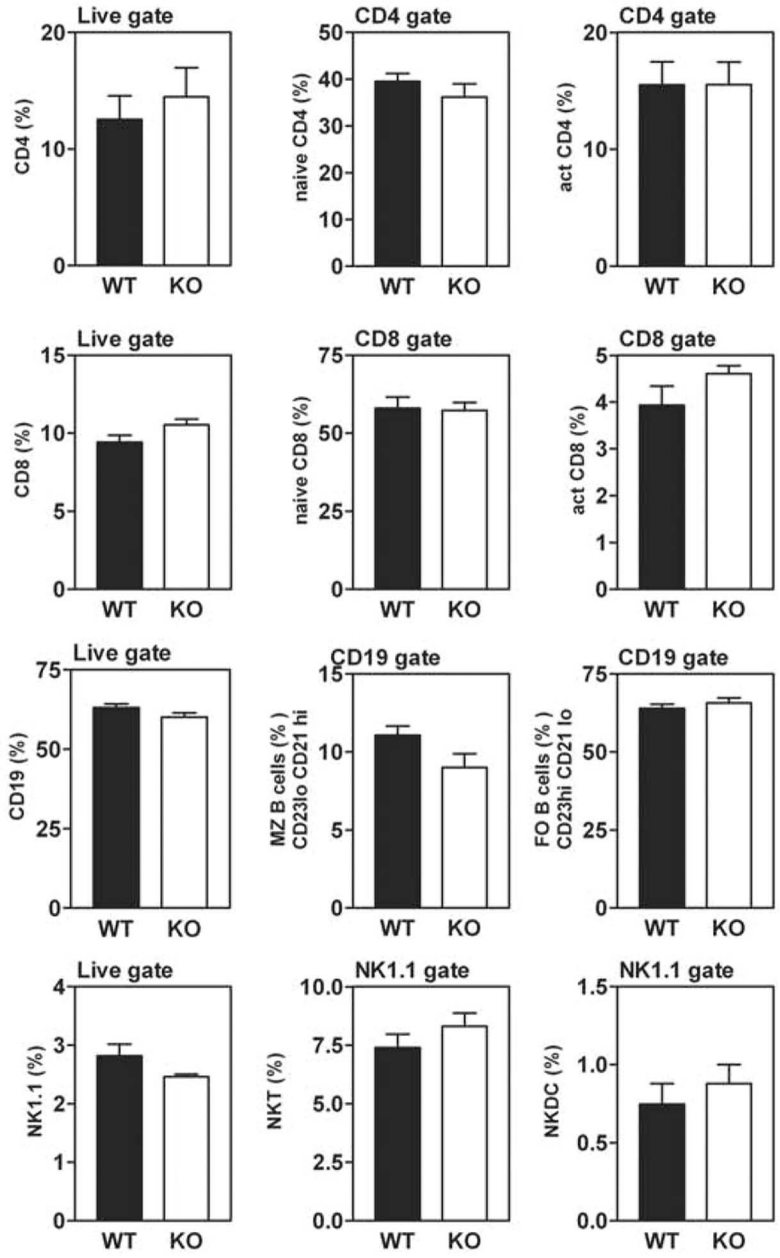
Appendix Figure 1. PCR to detect mutant *Ifnar1*. The original *Ifnar1*$^{-/-}$ mouse was generated by the insertion of the neomycin gene resistance cassette in Exon 3 of *Ifnar1* gene (Müller *et al.,* 1994). To follow the mutant *Ifnar1* gene, we developed a PCR so that progeny could be screened with purified genomic tail DNA. The following primers were obtained from Integrated DNA Technologies (Coralville, IA, USA): (forward primer) 5′-AAAGACGAGGCGAAGTGGTT-3′ (primes in Exon 3); and (reverse primer) 5′-GGAAGAGAGGAACAATATAGCCAGCC-3′ (primes in introns 3-4). The conditions for PCR were: 94°C, 3 min, followed by 30 cycles of 92°C for 22 sec, 59°C for 30 sec, and 72°C for 30 sec, followed by an elongation step at 72°C for 6 min. The PCR products were resolved on a 1% agarose gel and stained with ethidium bromide. The following product sizes could distinguish among the WT, Het, and KO mice: WT, 218-bp product only; KO, 1350-bp product only; and Het, 218- and 1350-bp products.



Appendix Figure 2. Serum IgM and IgG levels between WT and KO mice are similar. Mice were bled at different time-points, and sera were used to determine total IgM and IgG levels by sandwich ELISA. The capture and detection antibodies and immunoglobulin standards were obtained from SouthernBiotech (Birmingham, AL, USA): goat anti-mouse IgM (μ chain-specific), AP-conjugated goat anti-mouse IgM (μ chain-specific), goat anti-mouse IgG (γ chain-specific), and AP-conjugated goat anti-mouse IgG (γ chain-specific). IgM and IgG concentrations were determined from standard curves generated with Four Parameter Logistic Fit, available at http://www.myassays.com. Statistical significance was calculated by unpaired Student's *t* test. At 7 to 10 wks of age, the mean IgM level in WT mice was higher than that in the KO mice. However, at 20 to 22 wks of age, mean IgM levels were comparable between the WT and KO mice. The difference in mean total IgG levels between the KO and WT mice at both time-points was not statistically significant.



Appendix Figure 3. Both WT and KO mice show the presence of ANA in serum at older ages. Representative images of ANA staining are shown in sera obtained at early (8 wks) and late time-points (22 wks). All sera were used at a 1:50 dilution, and bound antibodies were detected with FITC-conjugated goat anti-mouse IgG (green). Nuclei (blue) were stained with DAPI, and the overlay shows nuclear *vs.* cytoplasmic staining.



Appendix Figure 4. Comparison of immune cell populations between WT and KO mice. To determine whether IFNAR deficiency alters immune cell populations, we removed spleens from young 8- to 10-week-old WT and KO females and prepared single-cell suspensions. Cells were incubated with anti-CD16/32 followed by staining with fluorochrome-conjugated antibodies to identify T-cell [CD4 (RM4-5), CD8 (53-6.7), CD62L (Mel-14), and CD44 (IM7)], B-cell [CD19 (eBio1D3), CD21 (eBio8D9), and CD23 (B3B4)], and NK cell [NK1.1 (PK136), CD3 (145-2C11), and CD11c (N418)] subsets. Incubation with biotin-conjugated anti-mouse IFNAR-1 antibody (BioLegend, San Diego, CA, USA) followed by streptavidin-Alexa647 (Life Technologies, Grand Island, NY, USA) was used to detect IFNAR. Incubation with biotin-conjugated mouse IgG and streptavidin-Alexa647 was used as isotype control. Stained cells were acquired on a BD FACSCalibur with Cytek dxP8 software (Cytek Technologies, Freemont, CA, USA). Data were analyzed with FlowJo Software 9.1 (Tree Star Inc., Ashland, OR, USA). No significant difference in frequencies of immune cell populations was seen between WT and KO mice. Further, the absolute cell numbers for total splenocytes and for each subset were also comparable between the 2 groups (data not shown).

APPENDIX REFERENCE

Müller U, Steinhoff U, Reis LFL, Hemmi S, Pavlovic J, Zinkernagel RM, *et al*. (1994). Functional role of Type I and Type II interferons in antiviral defense. *Science* 264:1918-1921.