SUPPLEMENTAL MATERIALS

Ishimaru et al., http://www.jem.org/cgi/content/full/jem.20080174/DC1

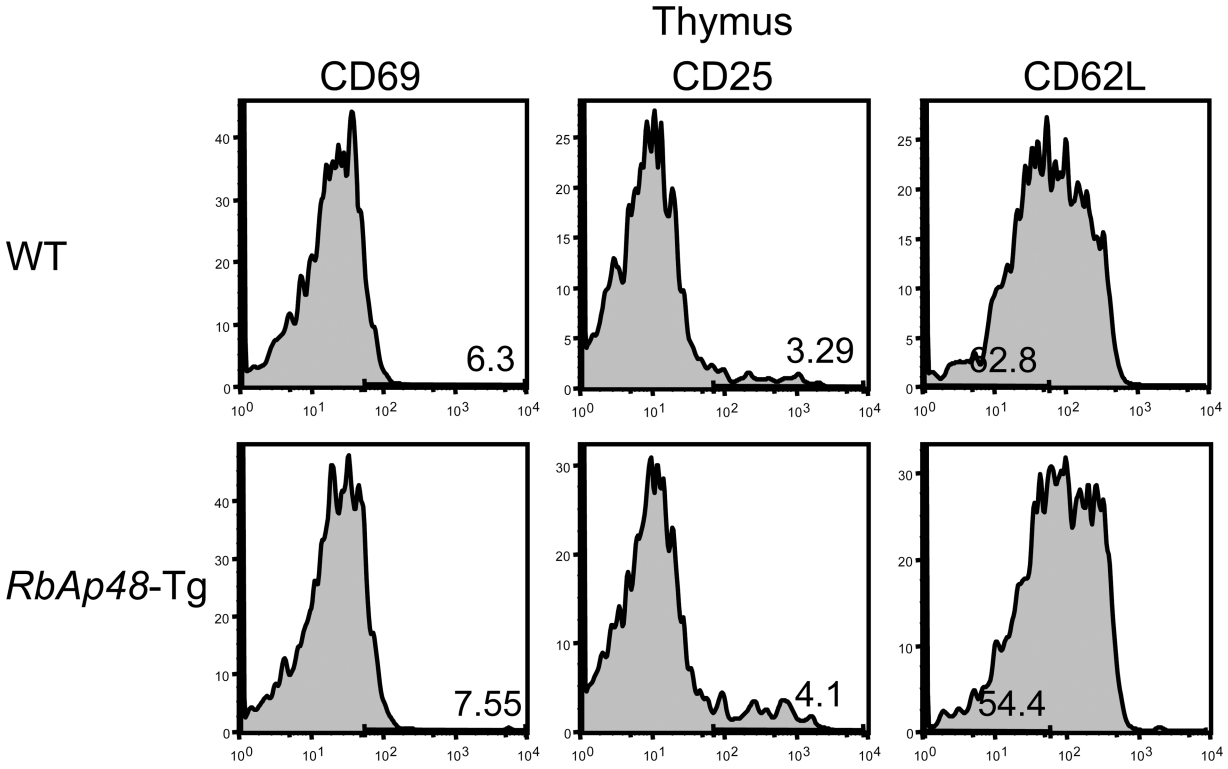

Figure S1. Surface phenotypes of CD4$^+$CD8$^-$ thymocytes from WT and *RbAp48*–Tg mice were analyzed by flow cytometry. Results are representative of 3–5 mice at 28 wk of age in 2 independent experiments.

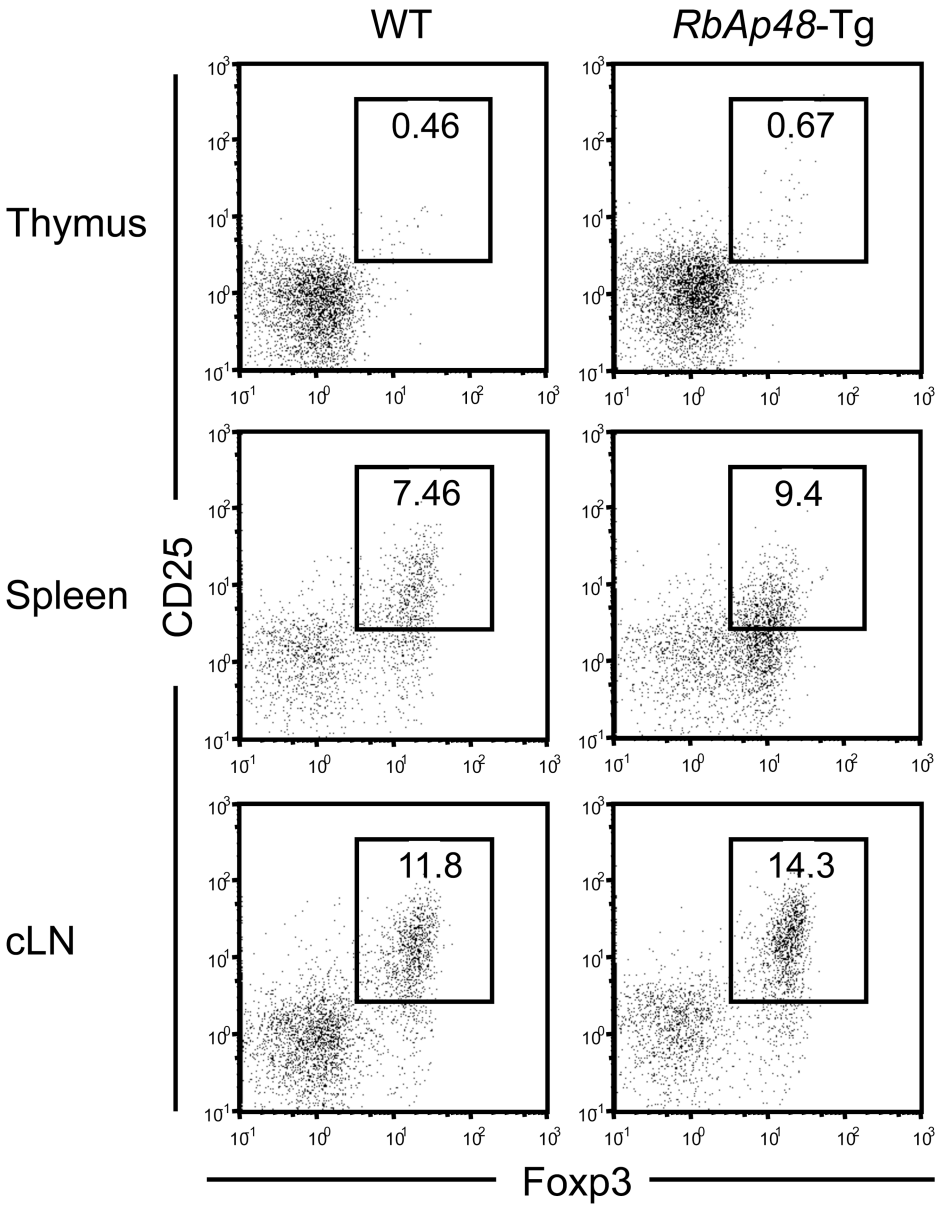

Figure S2. Foxp3 expressions of CD4+CD25+ T cells of thymus, spleen, and cLNs from WT and *RbAp48*-Tg mice at 28 wk of age were detected by intracellular flow cytometric analysis. Results are representative of three mice in two independent experiments.

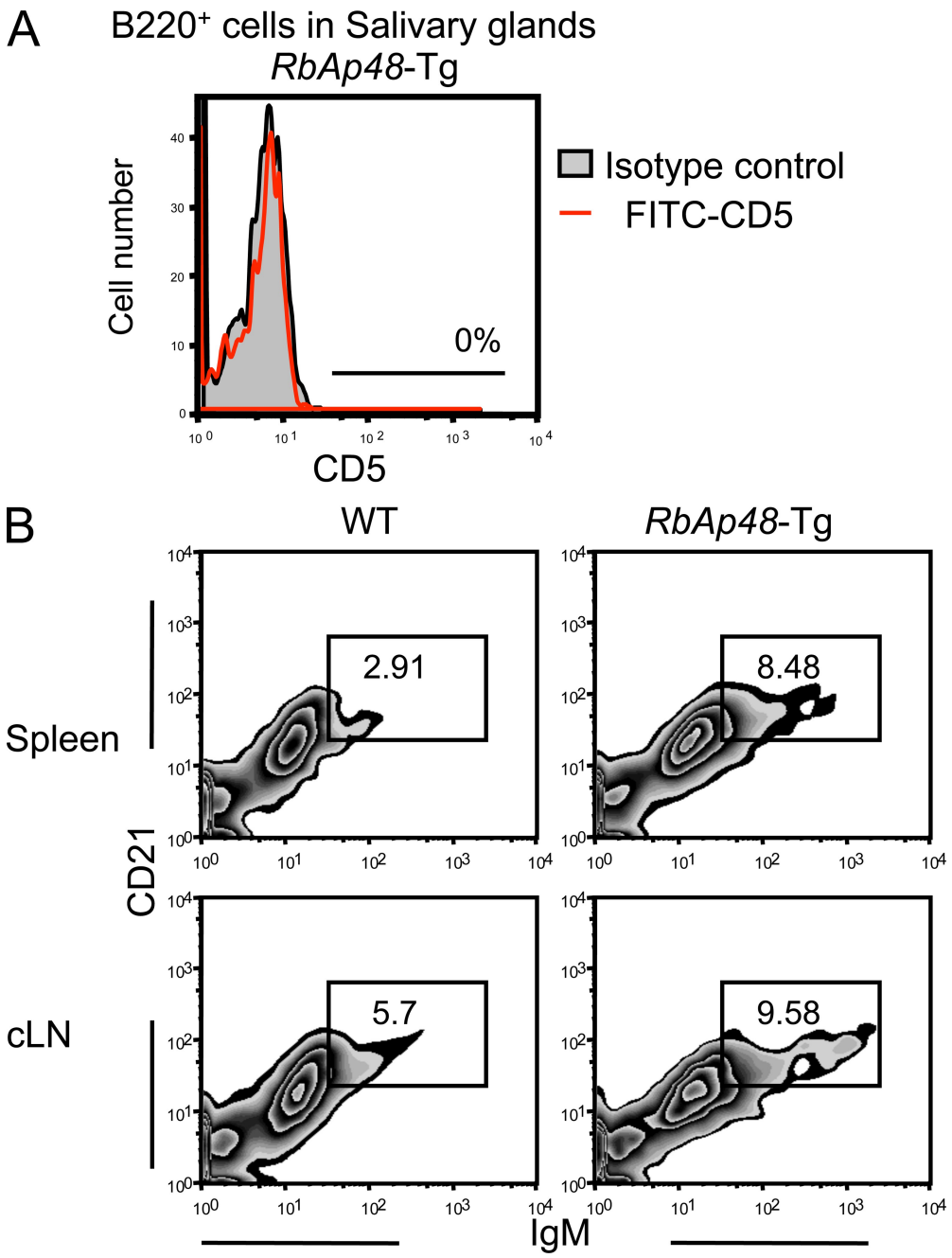

Figure S3. B1 cells in salivary glands and marginal B cells of spleen and cLN from *RbAp48*-Tg and WT mice. (A) B220^{+}CD5^{+} B1 cells in salivary glands from *RbAp48*-Tg mice were analyzed by flow cytometry. Results are representative of three mice in three independent experiments. (B) CD21^{high}IgM^{high}B220^{+} (marginal zone) B cells of spleen and cLNs from WT and *RbAp48*-Tg mice at 30 wk of age were detected by flow cytometry. Results are representative of three mice in two independent experiments.

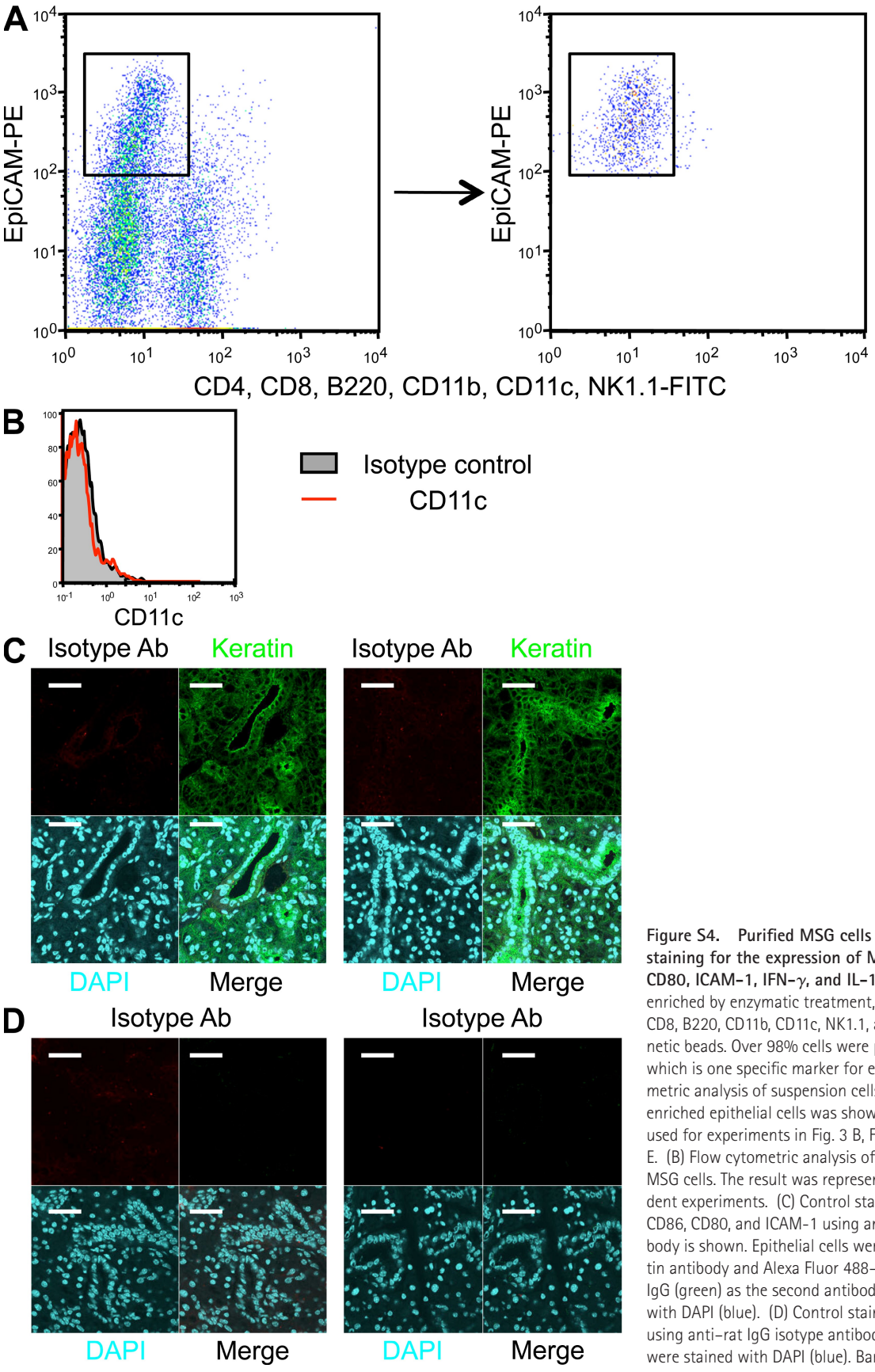

Figure S4. Purified MSG cells and images of control staining for the expression of MHC class II, CD86, CD80, ICAM-1, IFN-γ, and IL-18. (A) MSG cells were enriched by enzymatic treatment, several antibodies (CD4, CD8, B220, CD11b, CD11c, NK1.1, and EpiCAM), and magnetic beads. Over 98% cells were positive for EpiCAM, which is one specific marker for epithelium. The flow cytometric analysis of suspension cells before enrichment and enriched epithelial cells was shown. The MSG cells were used for experiments in Fig. 3 B, Fig. 4 (A and B), and Fig. 5 E. (B) Flow cytometric analysis of CD11c on the purified MSG cells. The result was representative of three independent experiments. (C) Control staining for MHC class II, CD86, CD80, and ICAM-1 using anti-rat IgG isotype antibody is shown. Epithelial cells were stained with anti-keratin antibody and Alexa Fluor 488–conjugated anti-rabbit IgG (green) as the second antibody. The nuclei were stained with DAPI (blue). (D) Control staining for IFN-γ and IL-18 using anti-rat IgG isotype antibody is shown. The nuclei were stained with DAPI (blue). Bars: (C and D) 50 µm.

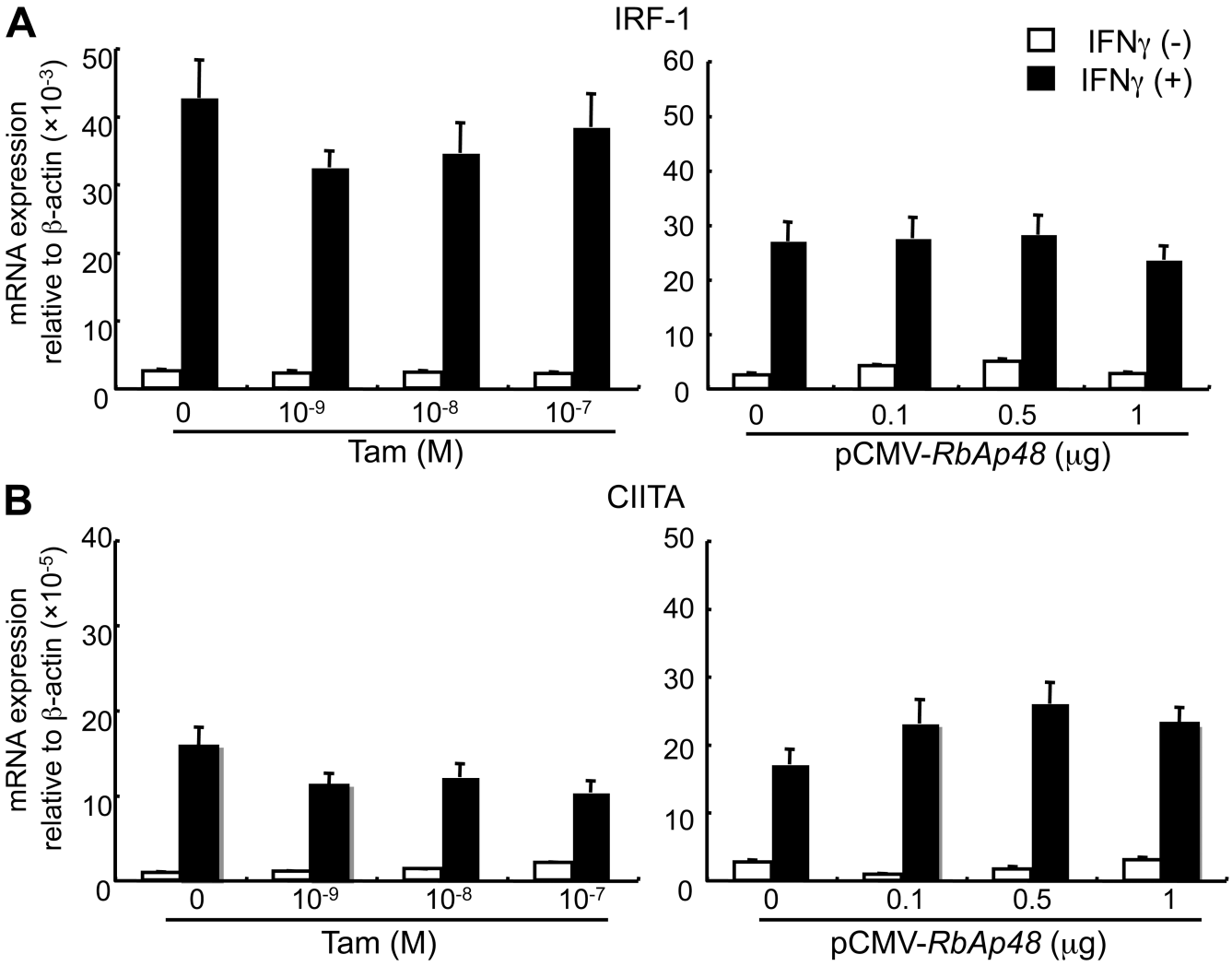

Figure S5. IRF-1 and CIITA mRNA of MCF-7 cells stimulated with Tam or transfected with pCMV-*RbAp48*. (A) IRF-1 and CIITA mRNA expressions of MCF-7 cells stimulated with Tam (10^{-9}∼10^{-7} M) in the presence of IFN-γ (5 ng/ml) were detected by real-time PCR. Data are shown as means ± SE (SE) relative to β-actin mRNA of two independent experiments. (B) IRF-1 and CIITA mRNA expressions of MCF-7 cells stimulated with Tam (10^{-9}–10^{-7} M) or transfected with pCMV-*RbAp48* (0–1 μg) in the presence of IFN-γ (5 ng/ml) for 2 h were detected by real-time PCR. Data are shown as means ± SE relative to β-actin mRNA of two independent experiments.

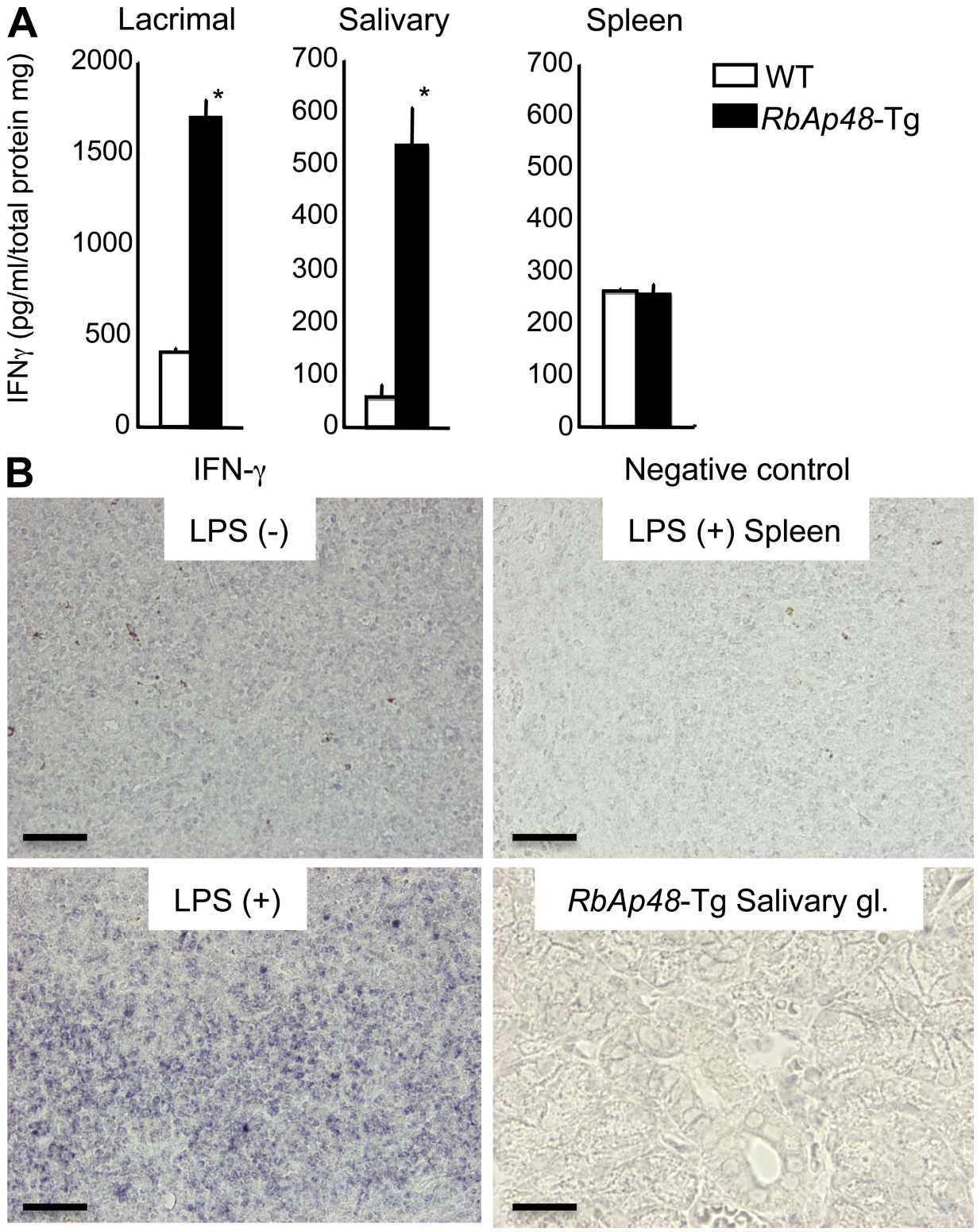

Figure S6. IFN-γ concentration of tissue homogenates of lacrimal glands, salivary glands, and spleen from *RbAp48*-Tg and WT mice, and control sections for in situ hybridization of IFN-γ mRNA. (A) IFN-γ concentrations in the tissue homogenates of lacrimal glands, salivary glands, and spleen from WT and *RbAp48*-Tg mice at 28 wk of age were detected by ELISA. Data are shown as means ± SE of four to five mice in two independent experiments. *,$P < 0.05$, WT versus *RbAp48*-Tg mice. (B) Negative (antisense probe) and positive controls for in situ hybridization of IFN-γ mRNA (sense probe) are shown. LPS or PBS was intraperitoneally injected into C57BL/6 mice, and 3 h later the spleen was removed. The paraffin-embedded sections of spleen of the mice were used for ISH. The intensive signal of IFN-γ RNA was observed in the spleen section from LPS-injected mice, but not from PBS-injected mice. Images are representative of two independent experiments. Bars, 40 µm.

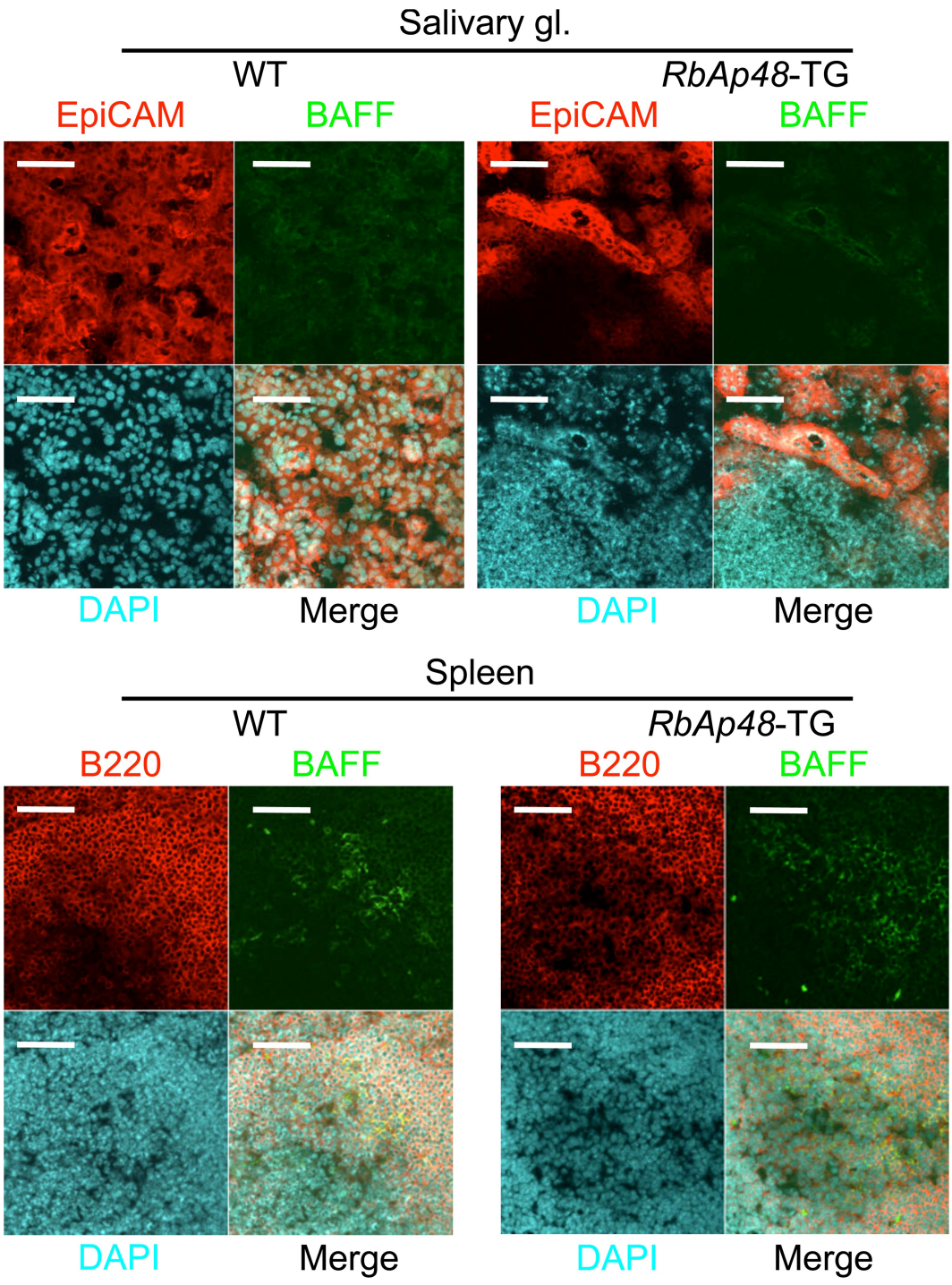

Figure S7. BAFF expressions of salivary glands and spleen from WT and *RbAp48*-Tg mice were detected by confocal microscopic analysis. FITC-conjugated anti-BAFF mAb and PE-conjugated anti-EpiCAM mAb were used for staining. The nuclei were stained with DAPI. The photos are representative of three mice of each group in two independent experiments. Bars: 50 μm.

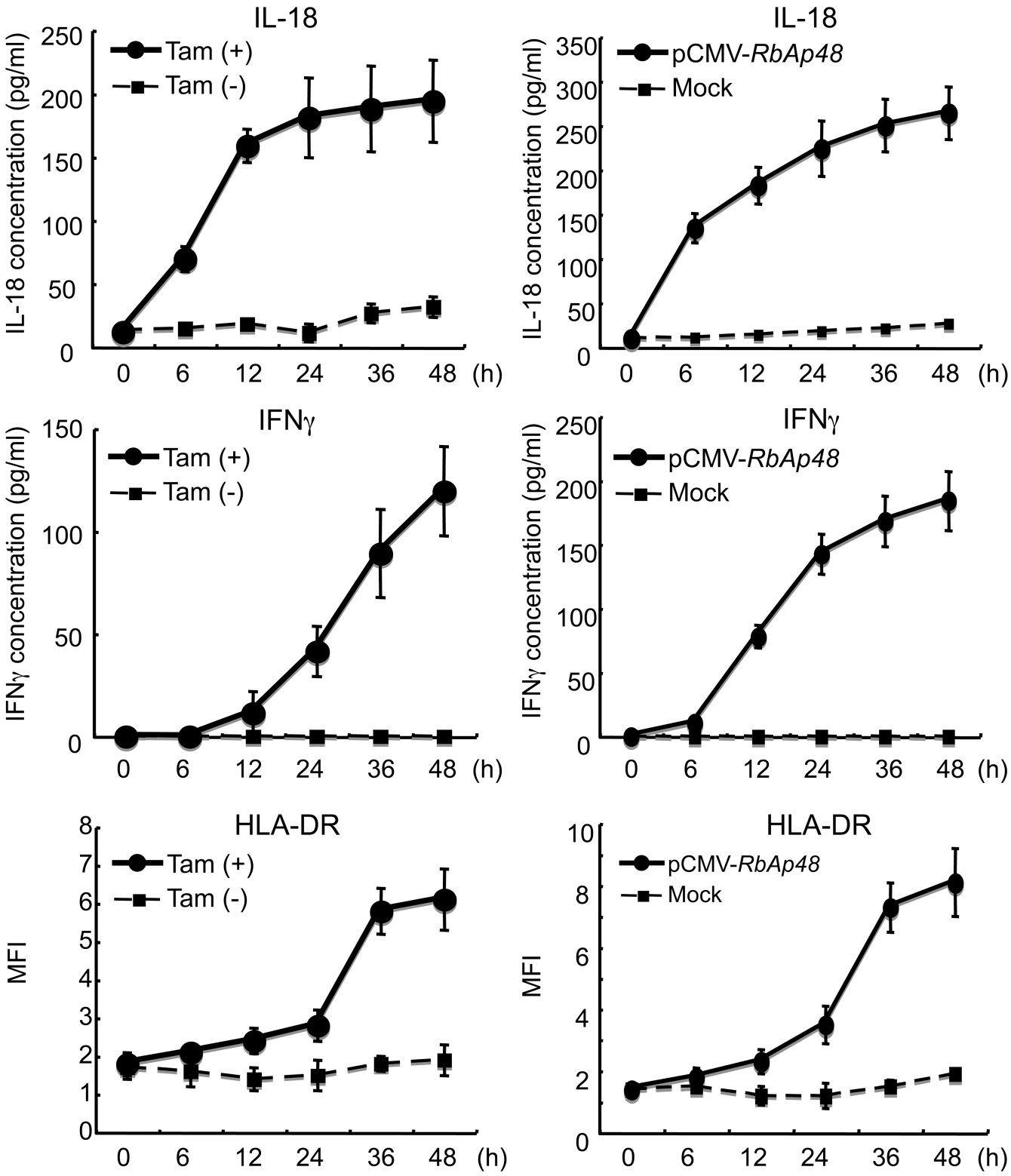

Figure S8. IL-18, IFN-γ, and HLA-DR expressions of Tam-stimulated or pCMV-*RbAp48*-trasfected HSG cells were analyzed 48 h later. IL-18 and IFN-γ productions of the culture supernatants were detected by ELISA. HLA-DR on the cells was detected by flow cytometry, and the results are shown as means ± SE of mean fluorescence intensity in triplicates in two independent experiments.

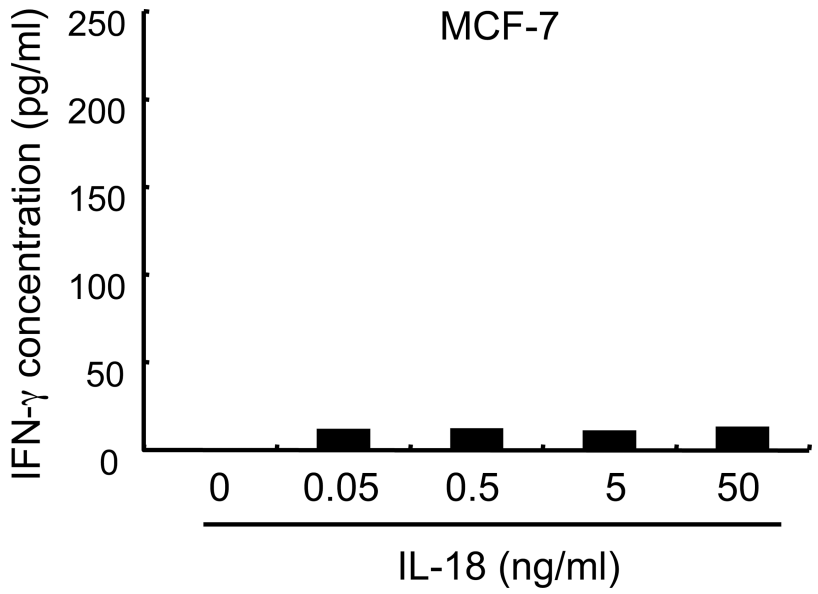

Figure S9. IFN-γ secretion of MCF-7 cells by the addition of recombinant IL-18 was analyzed by ELISA. Data are representative of three independent experiments.

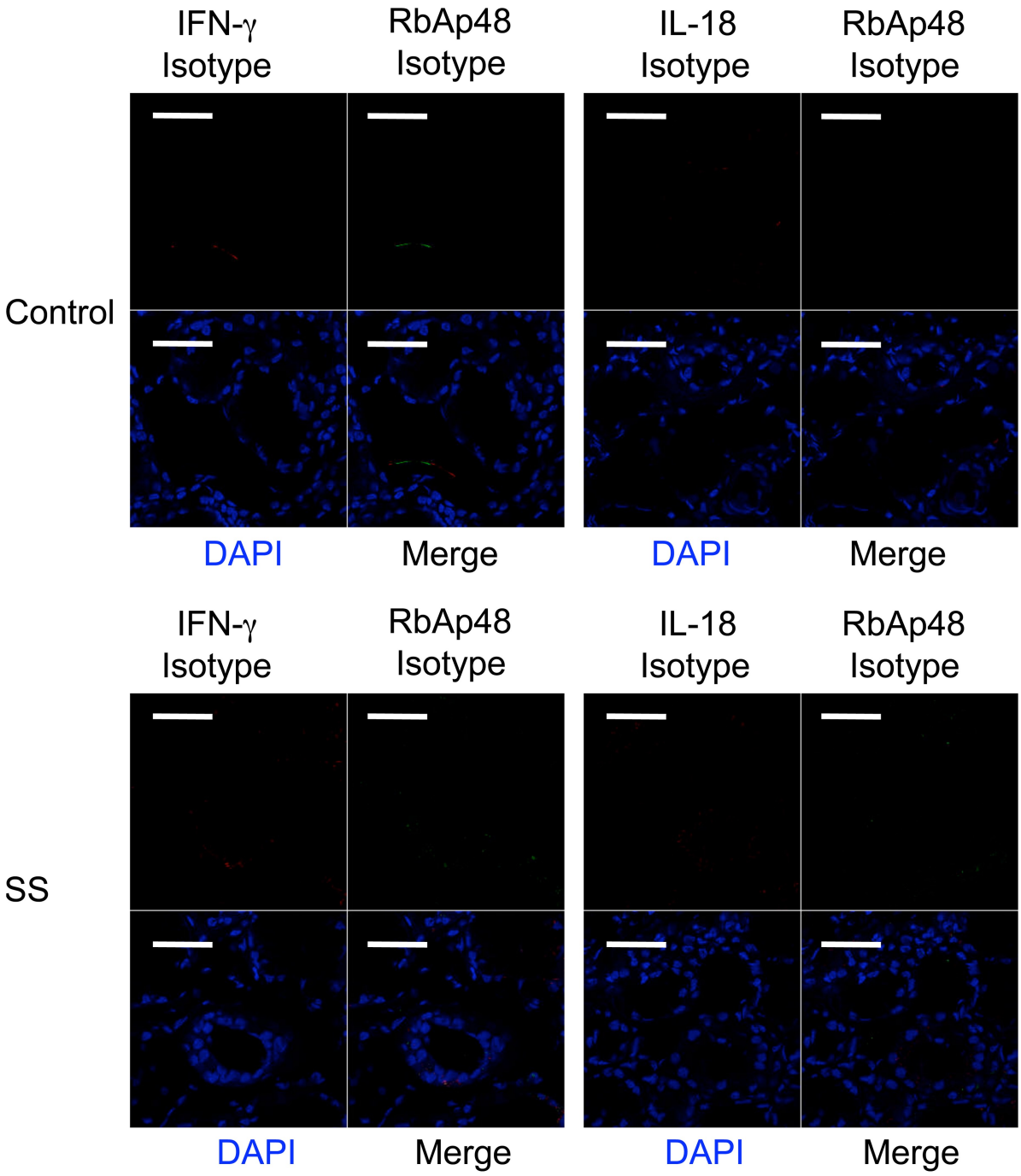

Figure S10. Isotype-matched controls of staining for IFN-γ or IL-18 and RbAp48 were shown in samples from SS patients and controls. The nuclei were stained with DAPI. The control stainings were performed for all experiments. Photos representative of three independent experiments. Bars, 50 µm.