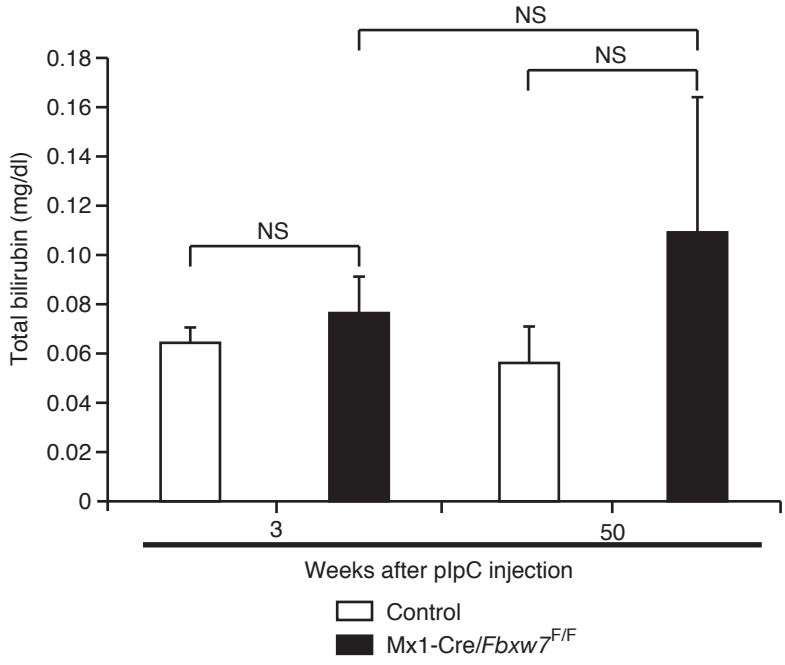
Supplemental Information

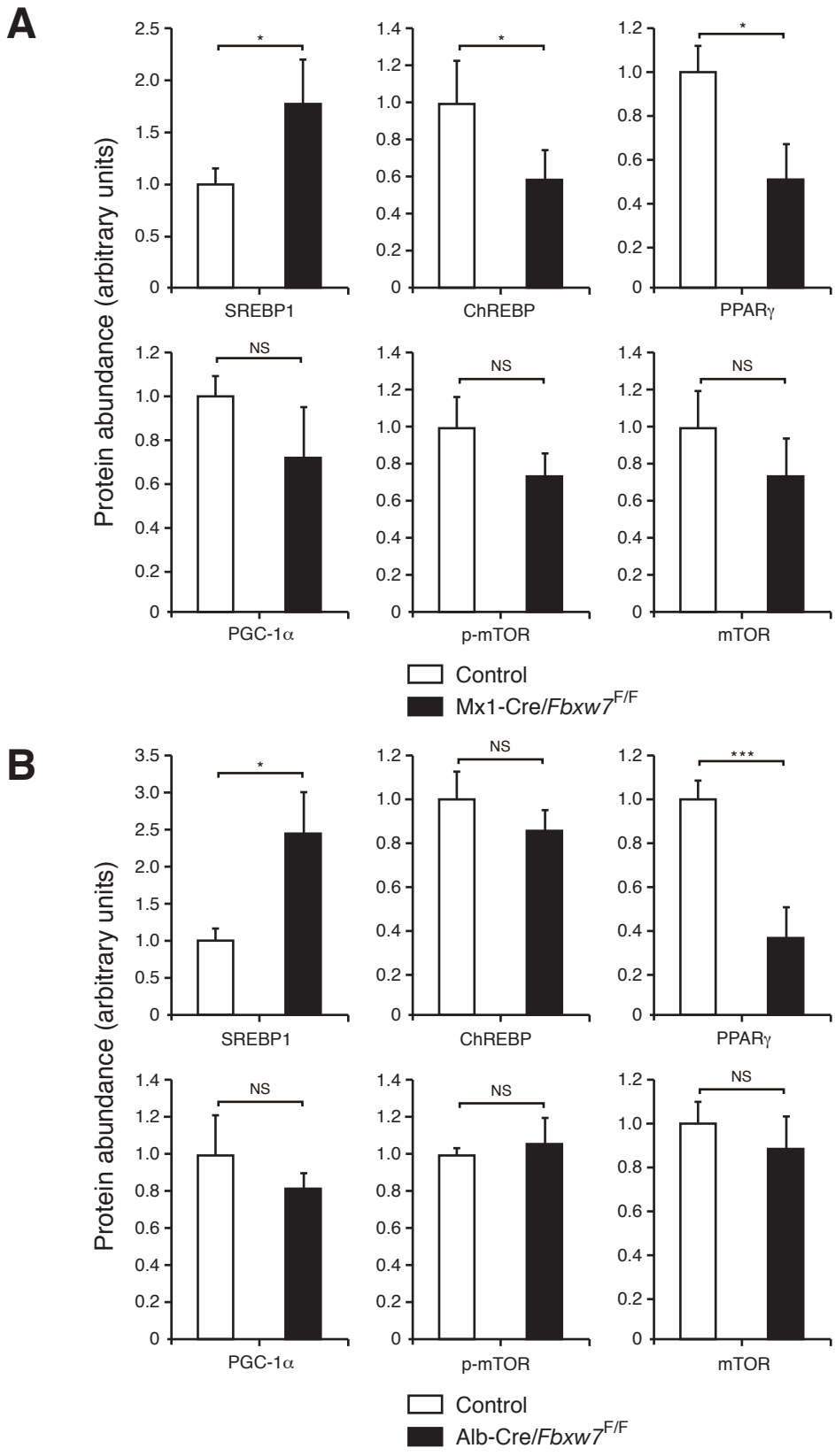
Fbxw7 controls lipid metabolism and cell fate decision in the liver in mice

Ichiro Onoyama, Atsushi Suzuki, Akinobu Matsumoto, Kengo Tomita, Hideki Katagiri, Yuichi Oike, Keiko Nakayama, and Keiichi I. Nakayama

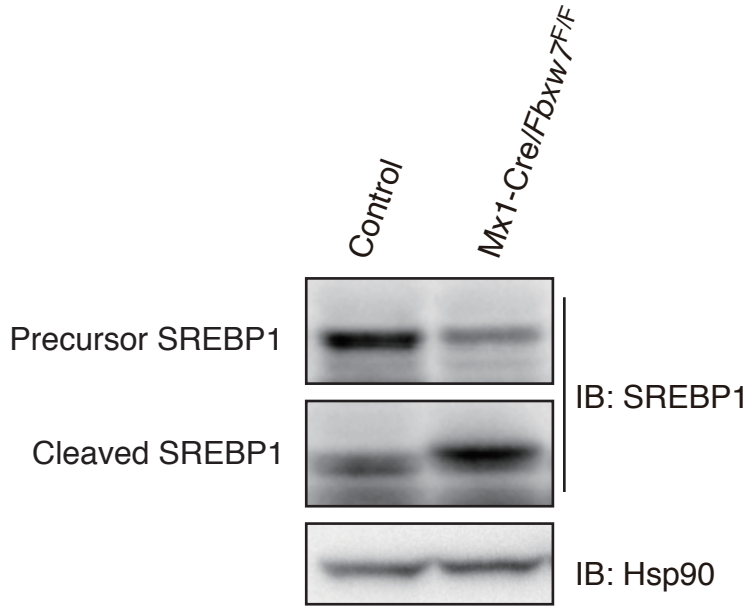
Supplemental Figures 1–7



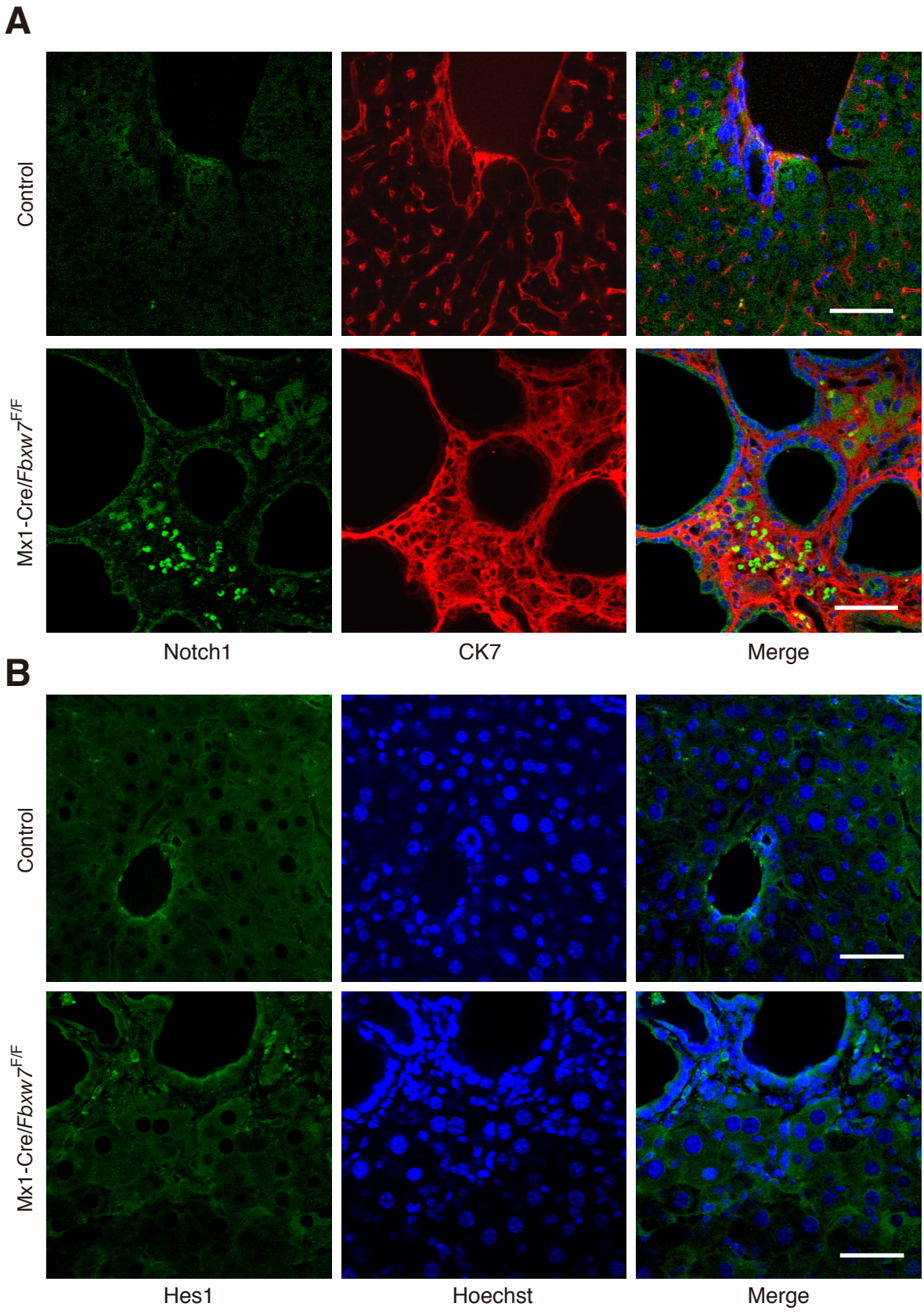
Supplemental Figure 1. Serum concentration of total bilirubin in Mx1-Cre/*Fbxw7*$^{F/F}$ mice. Serum levels of total bilirubin in Mx1-Cre/*Fbxw7*$^{+/F}$ (control) and Mx1-Cre/*Fbxw7*$^{F/F}$ mice were determined at 3 or 50 weeks after the final injection of pIpC beginning at 8 weeks of age. Data are means ± SD from three animals of each genotype.



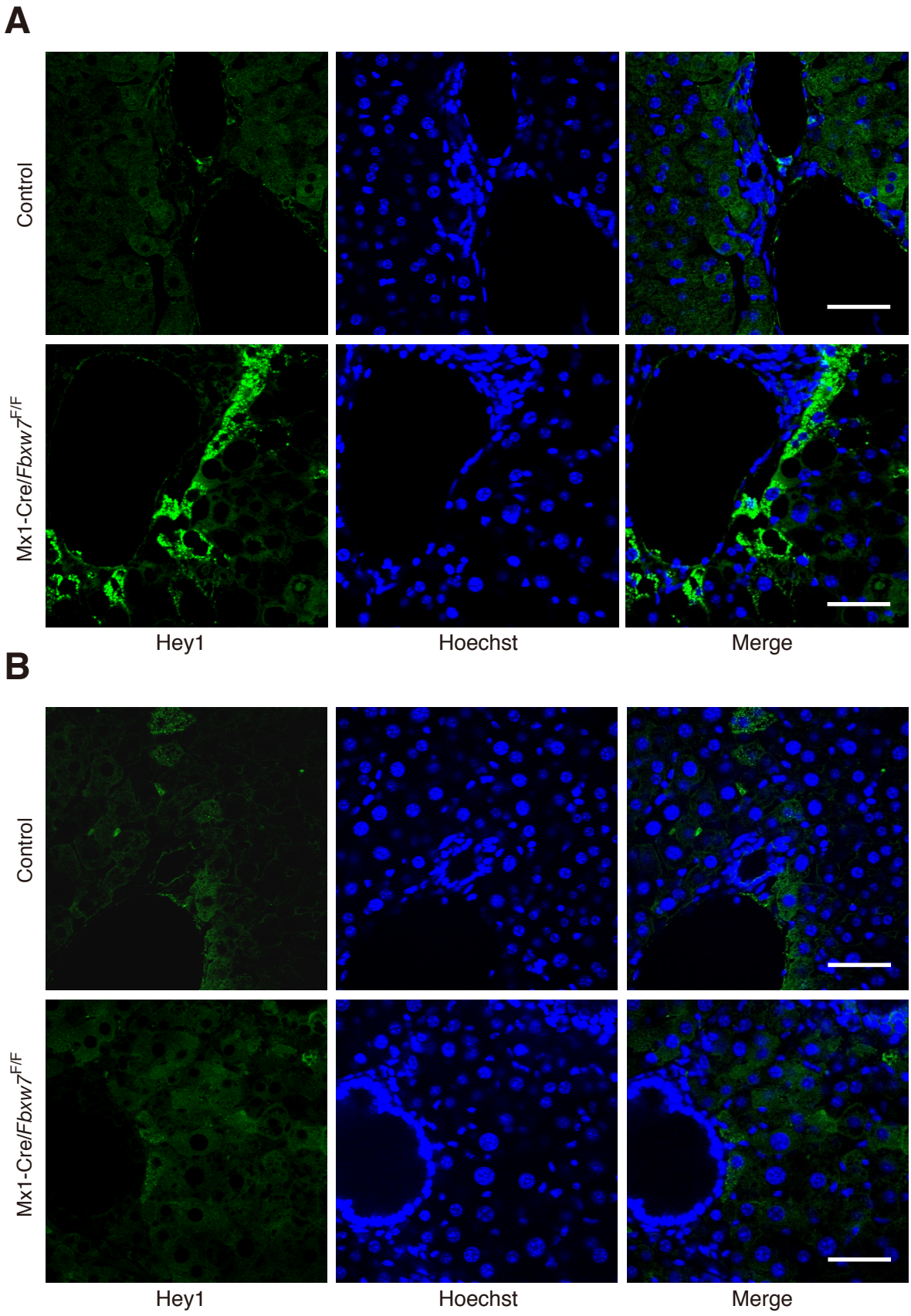
Supplemental Figure 2. Quantitation of protein abundance in the liver for the IB analysis shown in Figure 3B. The intensity of the bands corresponding to the indicated proteins in the liver of Mx1-Cre/*Fbxw7*$^{+/F}$ (control) and Mx1-Cre/*Fbxw7*$^{F/F}$ mice (**A**) or of *Fbxw7*$^{F/F}$ (control) and Alb-Cre/*Fbxw7*$^{F/F}$ mice (**B**) was normalized by that of the band for Hsp90 and then expressed relative to the corresponding value for control mice. Data are means ± SD from three animals of each genotype. *P < 0.05. ***P < 0.005.



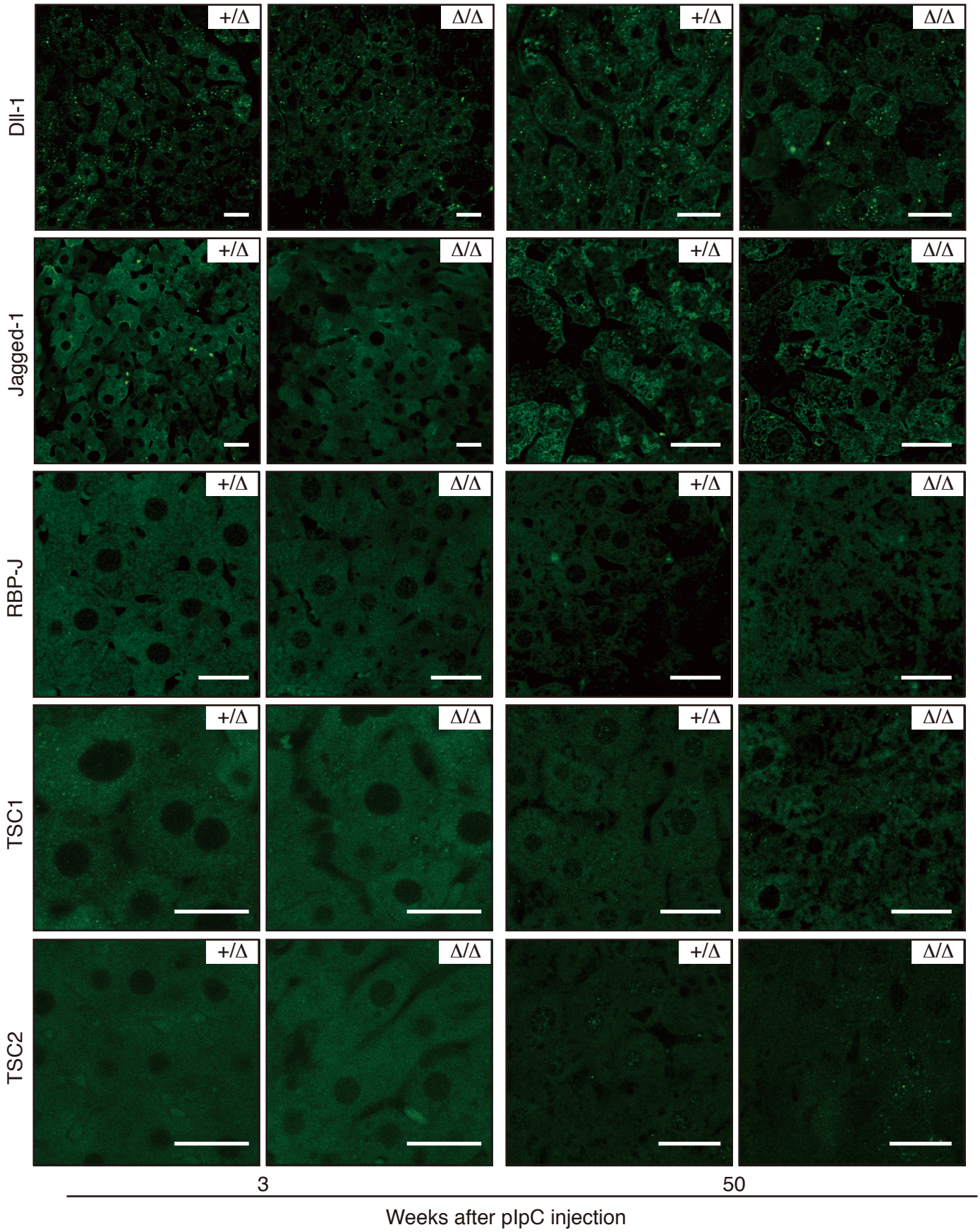
Supplemental Figure 3. Protein extracts of the liver of Mx1-Cre/*Fbxw7*$^{+/F}$ (control) and Mx1-Cre/*Fbxw7*$^{F/F}$ mice at 3 weeks after the final injection of pIpC beginning at 8 weeks of age were subjected to IB analysis with antibodies to SREBP1. Cleaved SREBP1 (nuclear SREBP1) accumulated in Fbxw7-deleted liver, although precursor SREBP1 was decreased.



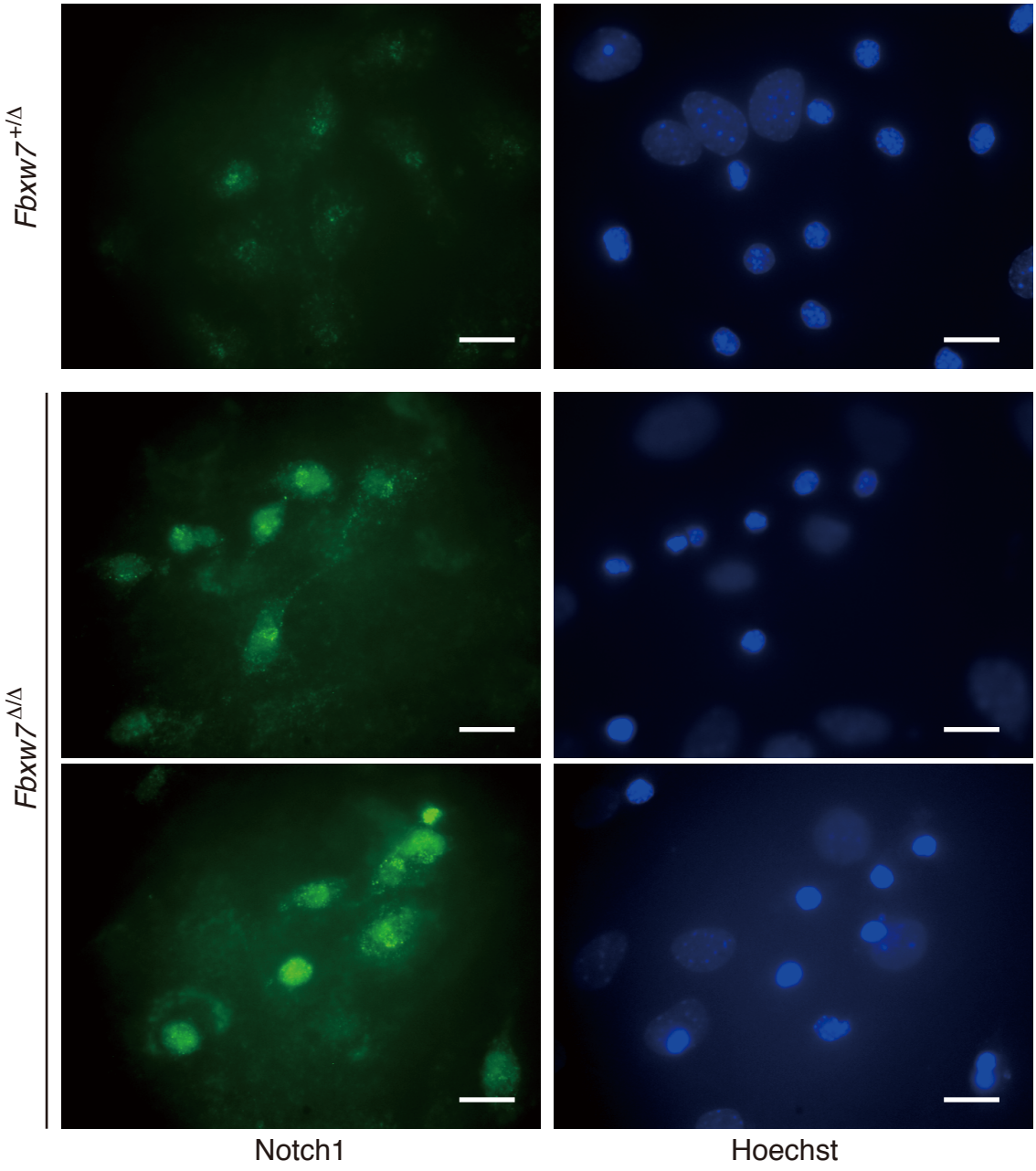
Supplemental Figure 4. (**A**) Immunofluorescence staining for Notch1 and CK7 in the liver of Mx1-Cre/*Fbxw7*$^{+/F}$ (control) and Mx1-Cre/*Fbxw7*$^{F/F}$ mice at 50 weeks after the final injection of pIpC beginning at 8 weeks of age. (**B**) Immunofluorescence staining for Hes1 in the liver of Mx1-Cre/*Fbxw7*$^{+/F}$ (control) and Mx1-Cre/*Fbxw7*$^{F/F}$ mice treated as in (**A**). Nuclei are stained blue. Bar, 50 μm.



Supplemental Figure 5. Immunofluorescence staining for Hey1 (ab22614, abcam) in the liver of Mx1-Cre/*Fbxw7*$^{+/F}$ (control) and Mx1-Cre/*Fbxw7*$^{F/F}$ mice at 3 (**A**) or 50 (**B**) weeks after the final injection of pIpC beginning at 8 weeks of age. Fbxw7-deficient hepatocytes around portal area express Hey1 excessively at 3 weeks after Fbxw7 deletion (**A**). Bar, 50 μm.



Supplemental Figure 6. Immunofluorescence staining for Dll-1 (F-15, Santa Cruz Biotechnology), Jagged-1 (C-20, Santa Cruz Biotechnology), RBP-J (T6709, Institute of Immunology, Tokyo, Japan), TSC1 (Cell Signaling Technology), and TSC2 (Y320, Abcam) in the liver of Mx1-Cre/*Fbxw7*$^{+/F}$ (+/Δ) and Mx1-Cre/*Fbxw7*$^{F/F}$ (Δ/Δ) mice at 3 or 50 weeks after the final injection of pIpC beginning at 8 weeks of age. Ablation of Fbxw7 had no apparent effect on the expression of these proteins in the liver.



Supplemental Figure 7. Notch1 accumulation in Fbxw7-deficient hepatic stem cells. Primary cultured liver cells of the indicated genotypes were subjected to immunofluorescence staining with antibodies to Notch1. Nuclei were also stained with Hoechst 33258. Notch1 accumulation was apparent in *Fbxw7*$^{\Delta/\Delta}$ cells. Bar, 10 μm.