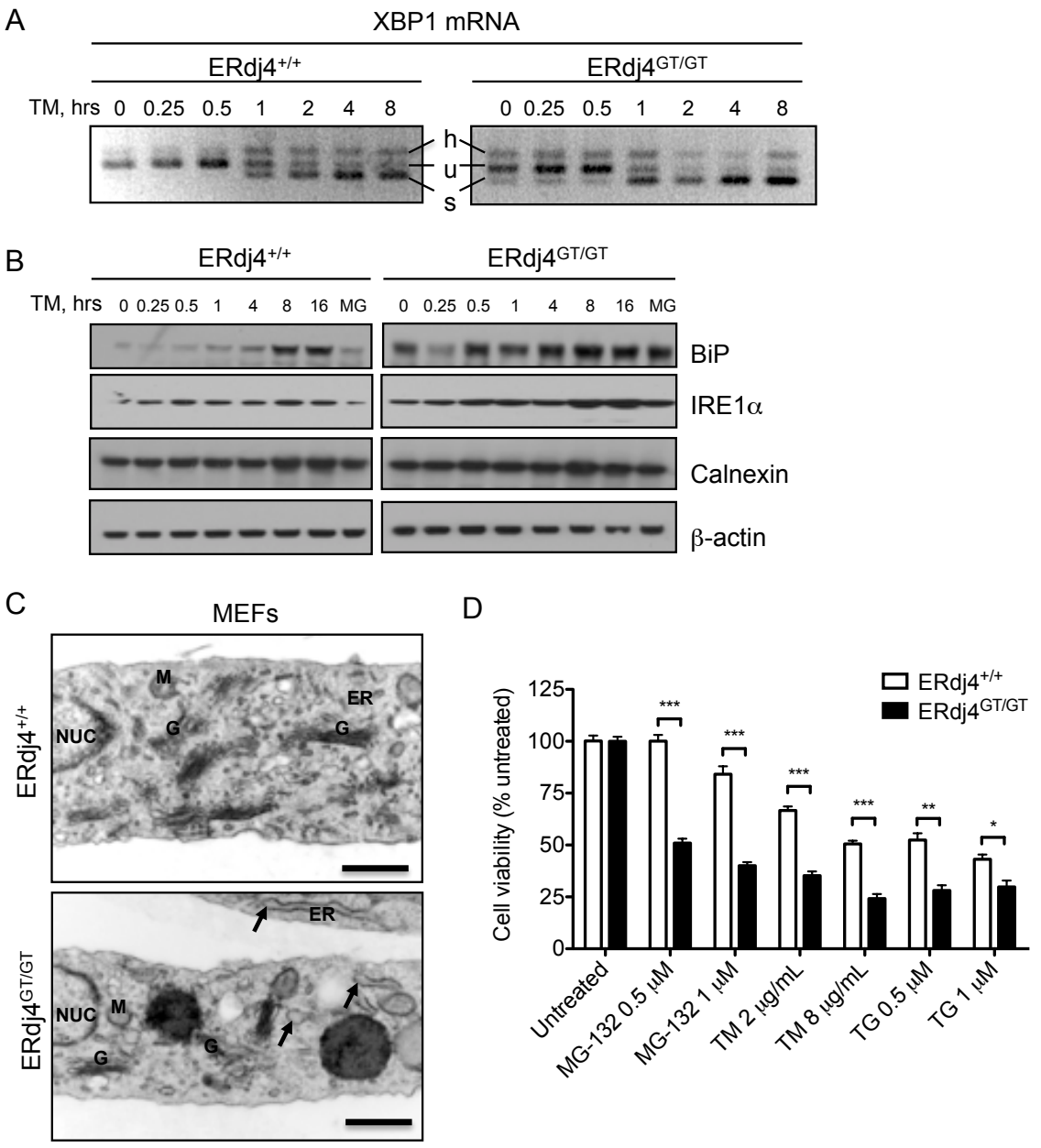
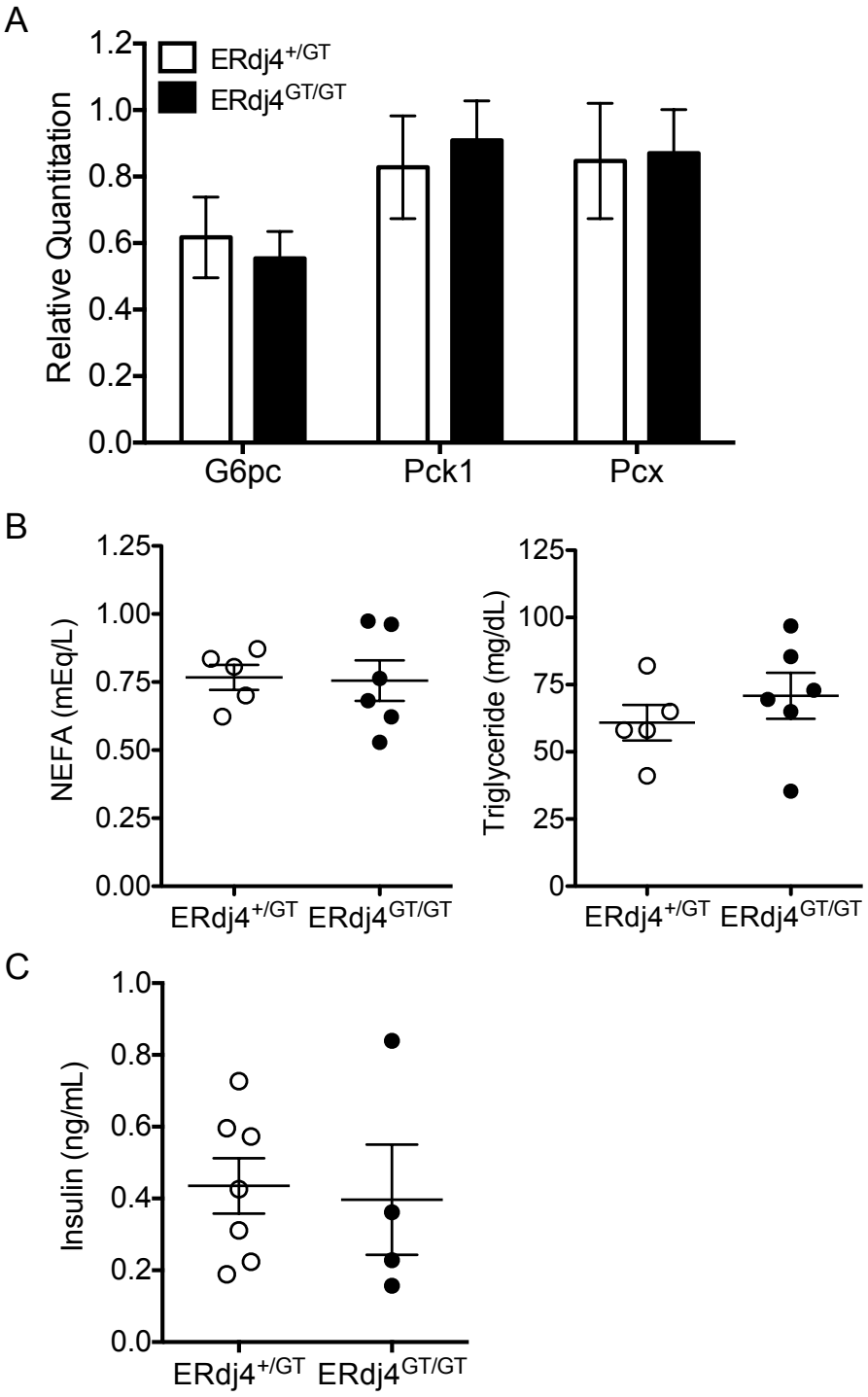
Supplemental Materials

Molecular Biology of the Cell

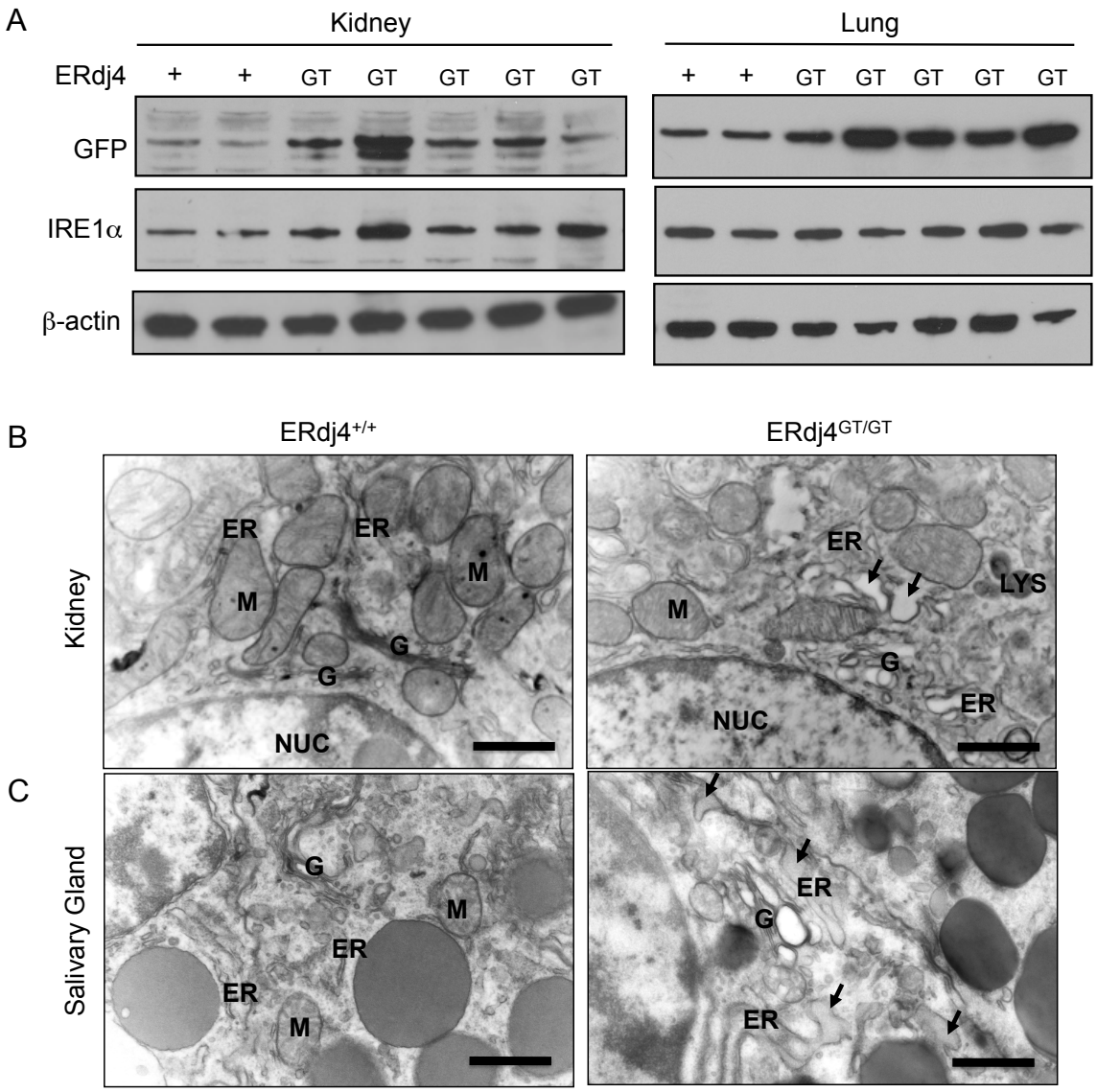
Fritz et al.



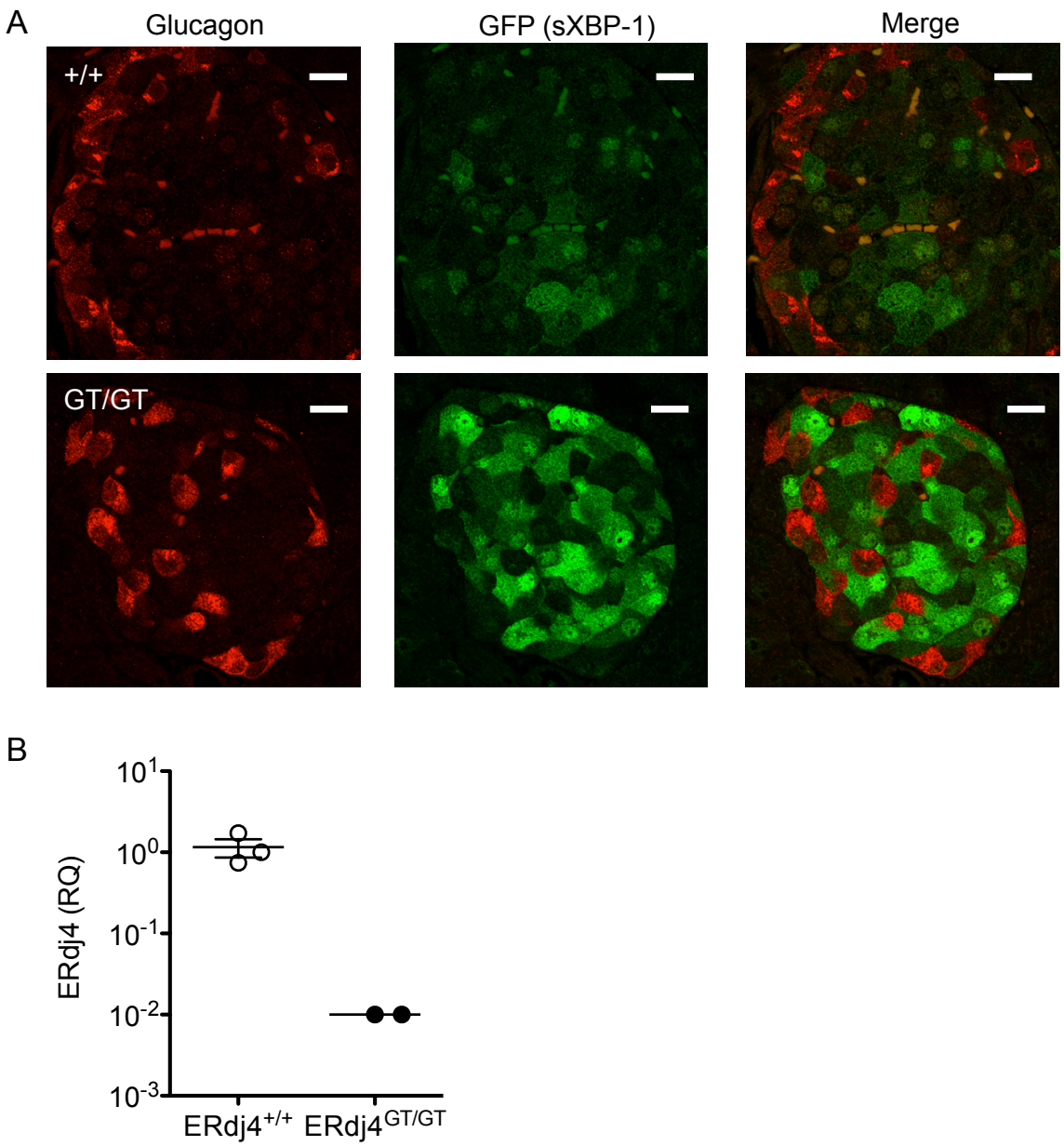
SUPPLEMENTAL FIGURE 1. Constitutive ER stress and decreased cell viability in ERdj4$^{GT/GT}$ MEFs. (A) RT-PCR of XBP1 mRNA in MEFs treated with tunicamycin (10 μg/ml) for the indicated periods of time. The PCR products represent hybrid (h), unspliced (u) and spliced (s) XBP1 species. (B) Western blot analyses of BiP, IRE1α, calnexin and β-actin (loading control) in MEFs treated with tunicamycin (TM, 2 μg/mL) for the indicated periods of time or MG-132 (MG, 5 nM, 4 hours). (C) Electron micrographs of MEFs. Note the dilated ER (arrows) in ERdj4$^{GT/GT}$ MEFs. ER: endoplasmic reticulum; G: Golgi complex; M: mitochondria; NUC: nucleus. (D) Cell viability of MEFs treated with indicated doses of MG-132, tunicamycin (TM) or thapsigargin (TG) for 48 hours. Relative values were determined by setting the absorbance of untreated ERdj4$^{+/+}$ MEFs to 100%. n = 3 samples/group.



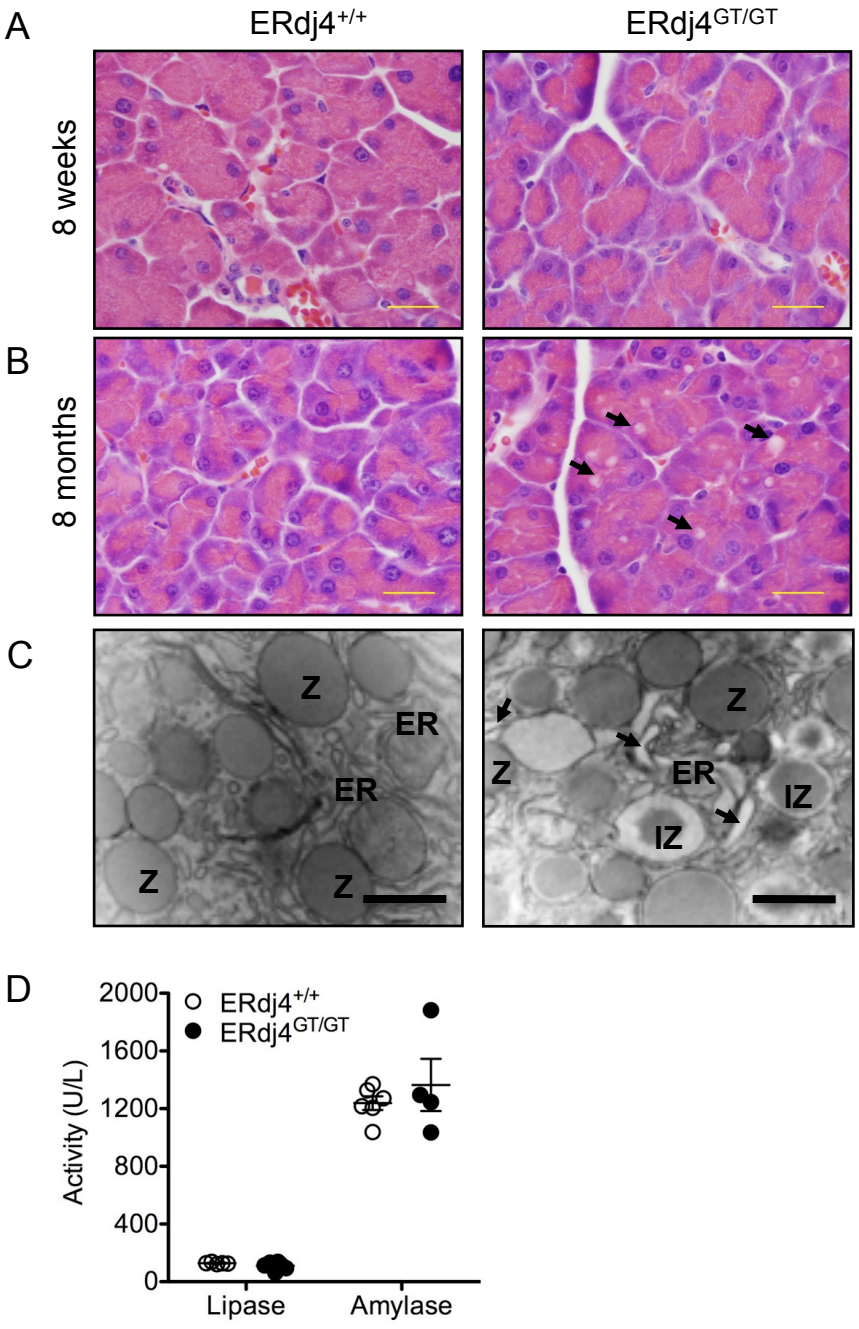
SUPPLEMENTAL FIGURE 2. Normal levels of gluconeogenic enzymes and lipids in neonatal ERdj4$^{GT/GT}$ mice. (A) qRT-PCR of glucose-6-phosphatase (G6pc), phosphoenolpyruvate carboxykinase (Pck1) and pyruvate carboxylase (Pcx) in livers of neonatal mice. Samples were normalized to eukaryotic 18S rRNA. *n* = 5-6 mice/genotype. (B) Non-esterified fatty acids (NEFAs) and triglycerides in plasma of neonatal mice. *n* = 5-6 mice/genotype. (C) Plasma insulin in neonatal mice. *n* = 4-7 mice/genotype.



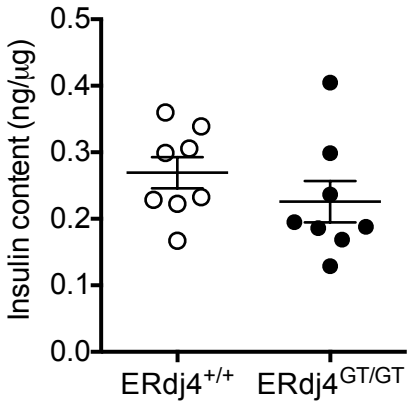
SUPPLEMENTAL FIGURE 3. Elevated ER stress in tissues of adult ERdj4$^{GT/GT}$ mice. (A) Western blot analyses of GFP (reporter for XBP1 splicing), IRE1α and β-actin (loading control) proteins in kidney and lung homogenates from 8-week-old ERdj4$^{+/+}$/ERAI (left panel, *n* = 2) and ERdj4$^{GT/GT}$/ERAI (right panel, *n* = 5) mice. (B) Electron micrographs of renal tubular epithelial cells in the kidneys of mice. Note the dilated ER (arrows) in ERdj4$^{GT/GT}$ cells. Scale bars are 1 μm. *n* = 2 mice/genotype. (C) Electron micrographs of serous cells in the salivary glands of mice. Note the dilated ER (arrows) in ERdj4$^{GT/GT}$ cells. Scale bars are 1 μm. *n* = 2 mice/genotype. ER: endoplasmic reticulum; G: Golgi complex; LYS: lysosome; M: mitochondria; NUC: nucleus.



SUPPLEMENTAL FIGURE 4. GFP (sXBP1) and ERdj4 expression in pancreatic islets. (A) Immunofluorescence of glucagon (red) and GFP (green) proteins in pancreatic islets of 16-20-week-old ERdj4$^{+/+}$/ERAI (top panel) and ERdj4$^{GT/GT}$/ERAI (bottom panel) mice. Autofluorescence (yellow in merged panel) was detected in red blood cells. Scale bars are 10 μm. *n* = 5 mice/genotype. (B) qRT-PCR of ERdj4 mRNA in islets isolated from 6-week-old, C57BL/6 littermates; samples were normalized to β-actin. *n* = 2-3 samples/genotype.



SUPPLEMENTAL FIGURE 5. ER stress in pancreatic acinar cells of ERdj4$^{GT/GT}$ mice does not impair function. (A-B) H&E staining of pancreatic tissue sections from 8-week-old (A) or 8-month-old (B) mice. Note the cytoplasmic vacuolation (arrows) in acinar cells of ERdj4$^{GT/GT}$ mice. Scale bars are 20 µm. *n* = 3-5 mice/genotype. (C) Electron microscopy of pancreatic acinar cells from 8-week-old mice. Note ER dilation (arrows) and immature zymogen granules (IZ) in ERdj4$^{GT/GT}$ mice. Scale bars are 1 µm. *n* = 2 mice/genotype. ER: endoplasmic reticulum; Z: zymogen granules; IZ: immature zymogen granules. (D) Amylase and lipase enzyme activity in the serum of 8-week-old mice. *n* = 4-6 mice/genotype.



SUPPLEMENTAL FIGURE 6. Total insulin protein in pancreatic extracts of 12-week-old mice. $n = 8$ mice/genotype.