Cell Reports

Supplemental Information

Disruption of O-linked N-Acetylglucosamine Signaling Induces ER Stress and β Cell Failure

Emilyn U. Alejandro, Nadejda Bozadjieva, Doga Kumusoglu, Sarah Abdulhamid, Hannah Levine, Leena Haataja, Suryakiran Vadrevu, Leslie S. Satin, Peter Arvan, and Ernesto Bernal-Mizrachi

Supplemental to Figure 1: Supplemental Experimental Procedure 1, Generation of mice harboring β-cell specific deletion of OGT. (A) Breeding schemes for the generation of β-cell specific βOGT-KO mice. **B)** Immunofluorescence staining of GFP (green) and insulin (blue) in 4-week-old $\beta OGT^{-/y}$ mice, indicating efficiency of the RIPCre enzyme. **(C)** OGT mRNA expression in islets corrected to actin in βOGT-KO mice. **(D-E)** OGT protein levels in hypothalamus (D) and liver (E) lysates from βOGT-KO and control mice. Quantification showing the ratio of OGT and Vinculin. **(F)** IPGTT was performed in 4- week-old $\beta OGT^{-/y}$ and control mice. $*P < 0.05$, $\beta OGT^{-/-}$ vs. control, n=3.

Supplemental to Figure 2: Supplemental to Main Figure 2, Female $\beta OGT^{-/-}$ mice exhibit reduced β-cell mass and calcium signaling. (A) Reduced β-cell mass in 20-week-old $\beta OGT^{-/-}$ mice compared to control. **(B)** Measurement of intracellular Ca^{2+} in islets of $\beta OGT^{-/-}$ mice in various ages as shown.

Supplemental Figure 3. Supplemental to Main Figure 3, Transcription levels in normoglycemic in βOGTKO and GFP expression in inducible $i\beta OGT^{-/y}$ mice. (A,B) Insulin content corrected to DNA in 4-week-old $\beta OGT^{-/y}$ (A) or 6-8 week-old $\beta OGT^{-/-}$ (F) mice and control. **(C-I)** Quantitative PCR was performed for *Ins1, Ins2, Pdx-1,* and *NeuroD1* mRNA expression in islets of 4-5 week-old $\beta OGT^{-/y}$ (C,E,G,I) $\beta OGT^{-/-}$ (D,F,H) mice and control. Transcript levels were normalized to β-actin. **(J)** Immunofluorescence staining of GFP (green), insulin (blue), and glucagon (red) in inducible $i\beta OGT^{-/y}$ (MIP-CreERTM;CAG-ZsGreen;OGT^{f-y}) and control (MIP-CreERTM;CAG-ZsGreen) mice. **(K)** Quantification of GFP and insulin expressing cells in islets of $i\beta OGT^{-/y}$ and control mice. $*P < 0.05$ vs. $i\beta OGT^{-/y}$ or $\beta OGT^{-/-}$ and control, n=3-4.

Supplemental Figure 4. Supplemental to Main Figure 4, Phloridzin, partially ameliorates the development of diabetes in 10-week-old mice lacking OGT. (A)

IPGTT was performed in 10-week-old βOGT$^{-/y}$ mice before Phloridzin (PHZ) treatment. **(B)** Glucose levels during a 12-hour fasting period on 20 of PHZ treatment. **(C)** Fed glucose levels in βOGT$^{-/y}$ and control mice treated with PHZ. **(D)** IPGTT was performed on day 20 of PHZ treatment. **(E)** IPGTT was performed in 12-week-old βOGT$^{-/y}$ mice with or without 20 days of PHZ treatment. **(F)** Quantification of area under curve (AUC) of A. **(G-H)** Fed (G) and fasting (H) insulin levels measured on day 15 and 20 of PHZ treatment respectively. *P < 0.05, βOGT$^{-/y}$ and control, n=5.

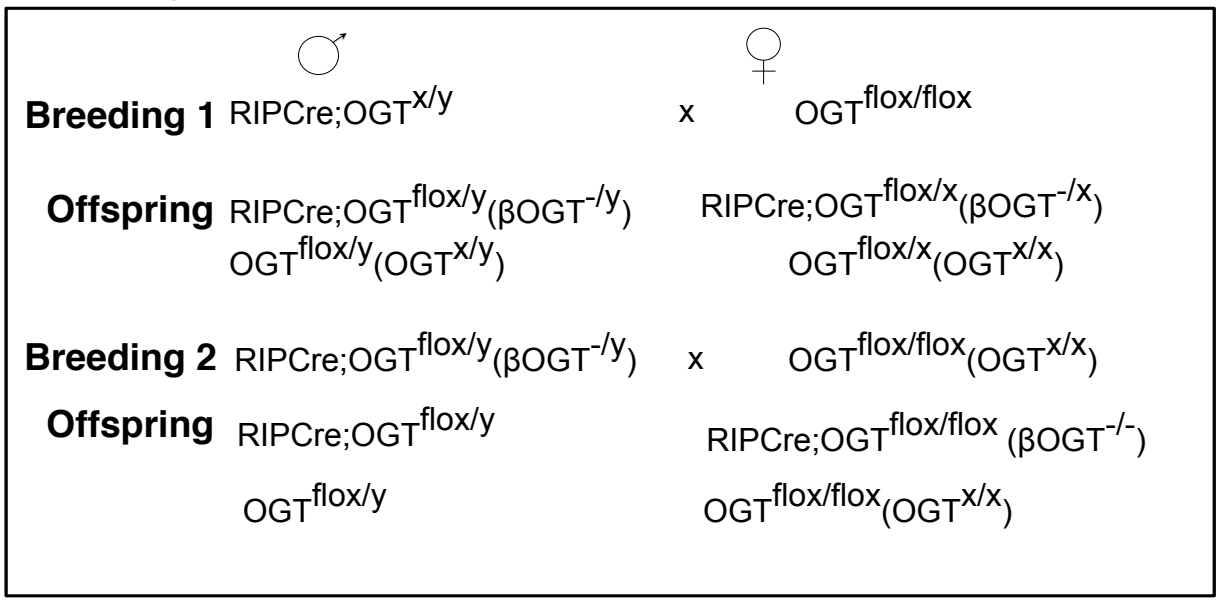
Supplemental Figure 5. Supplemental to Main Figure 5, Increased Proinsulin levels in young and normoglycemic mice lacking OGT. (A) Fed insulin levels in 4-week-old βOGT$^{-/y}$ mice. **(B-C)** Proinsulin level (B) and Proinsulin/insulin (C) ratio in 4-week-old βOGT$^{-/y}$ mice. **(D)** Quantification of proinsulin localizing in the ER (arrowhead with tail) or Golgi (arrowhead). **(E)** Confocal images of islets from 4-week-old male βOGT$^{-/y}$ mice; insulin (blue), proinsulin (red), and calnexin (green). **(F)** ER stress marker BiP protein expression in islets from 8-week-old βOGT$^{-/-}$ and control mice. *P < 0.05 vs. control, n=4-5.

Supplemental Figure 6. Supplemental to Main Figure 6, Phosphorylated GSK3 staining in mice overexpressing Akt and lacking OGT. (A) Immunofluorescence images of islets from βOGT$^{-/-}$ and βOGT$^{-/-}$;Akt(Tg); insulin (green), PhosphoGSK3β(Ser9) (red), and Dapi (blue). Images shown are taken in 40x magnification. **(B)** O-GlcNAc level, a read out of OGT activity, in islets from 8-week-old βOGT$^{-/-}$;Akt(Tg), βOGT$^{-/-}$ and control mice.

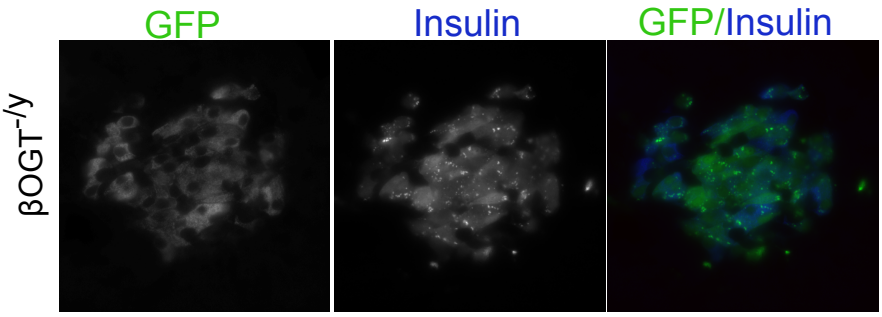
Supplemental Figure 7. Supplemental to Main Figure 6, Proinsulin staining in mice overexpressing Akt and lacking OGT. (A) Confocal images of islets from control, βOGT$^{-/-}$ and βOGT$^{-/-}$;Akt(Tg) mice; insulin (blue), proinsulin (red), and calnexin (green).

Supplemental Figure 1

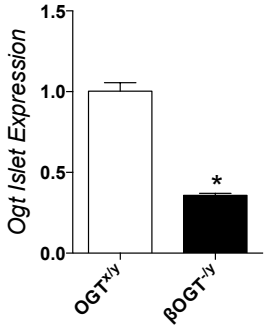
A



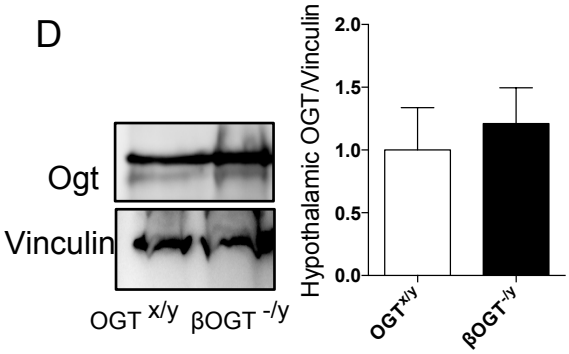
B



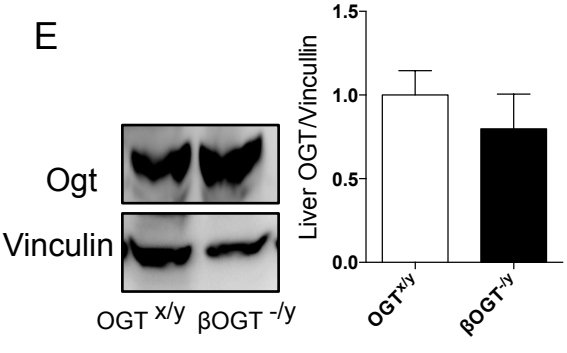
C



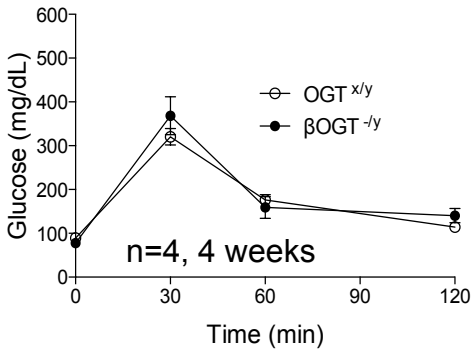
D



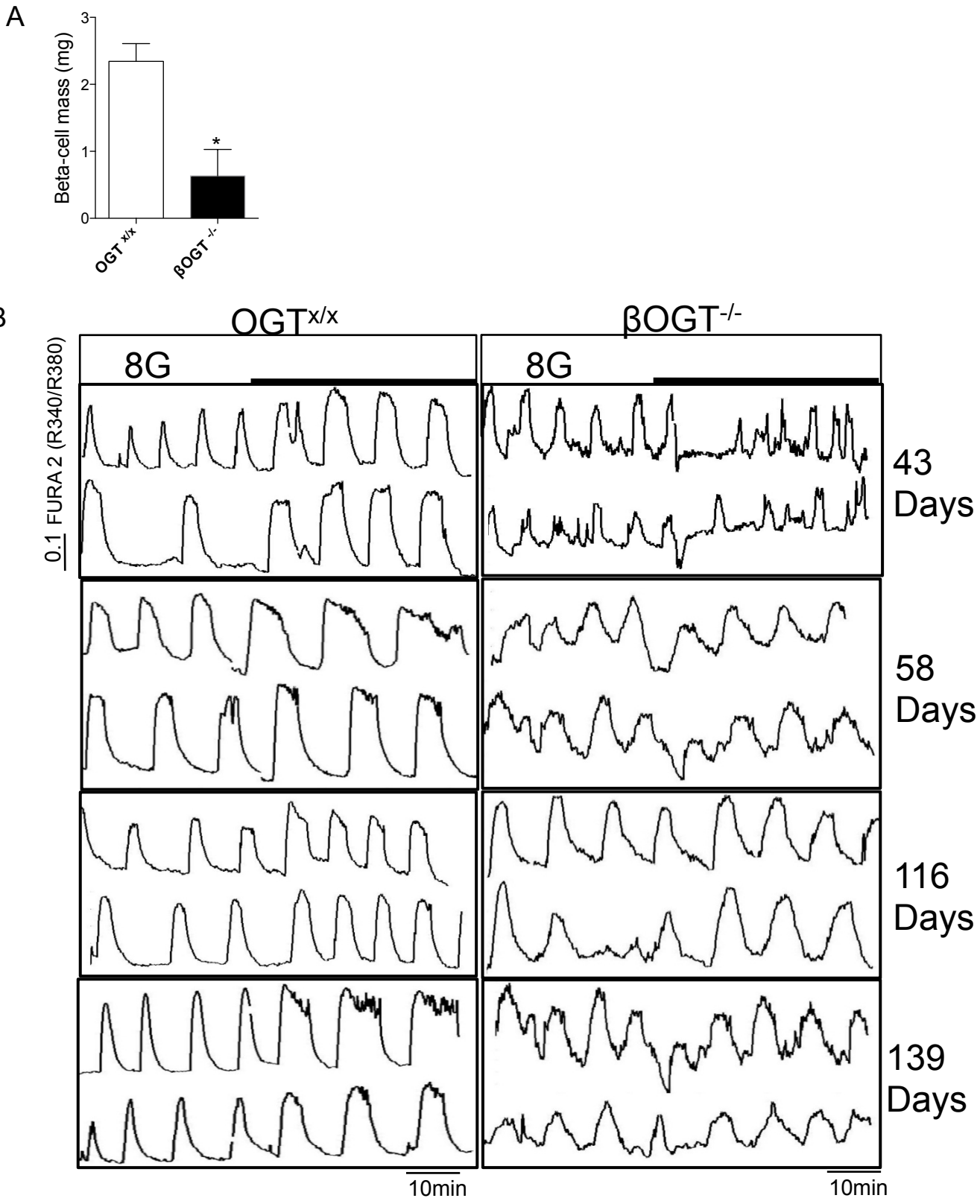
E



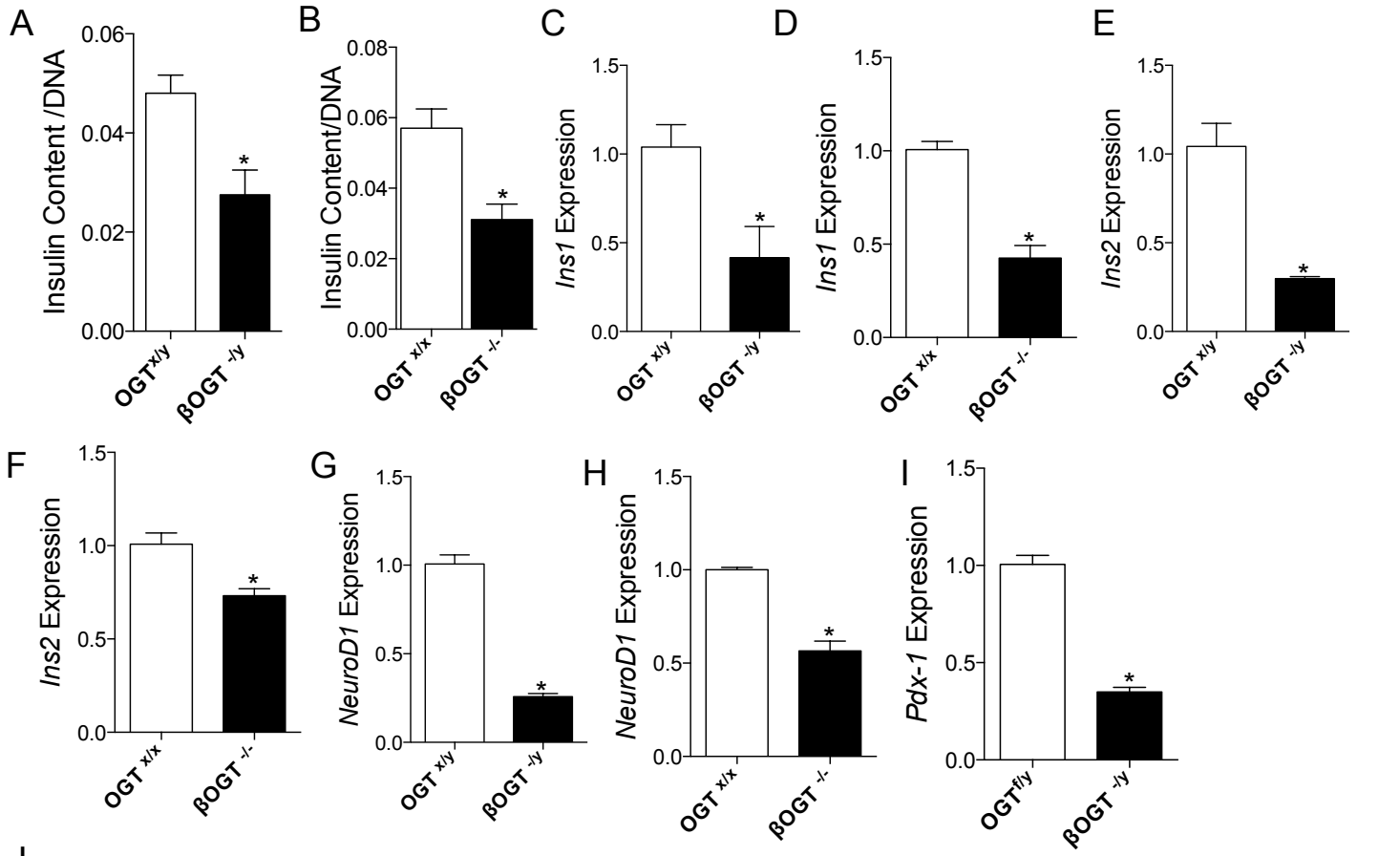
F

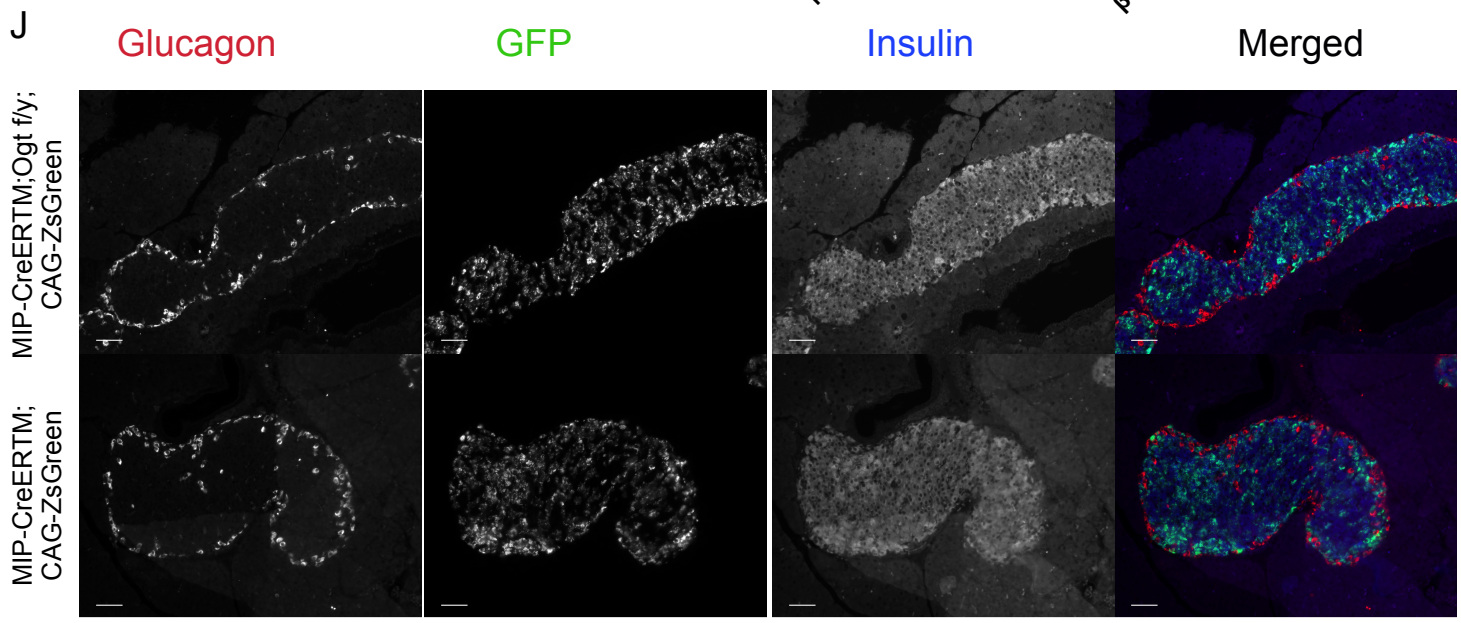


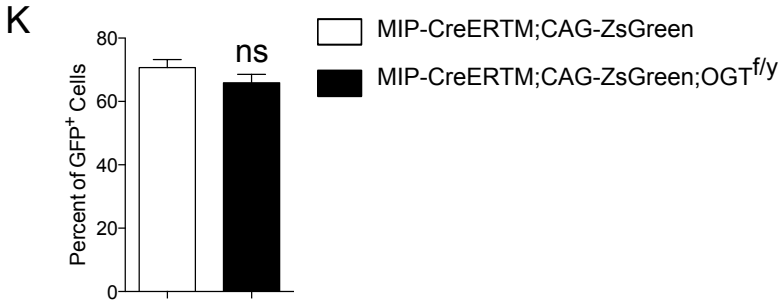
Supplemental Figure 2



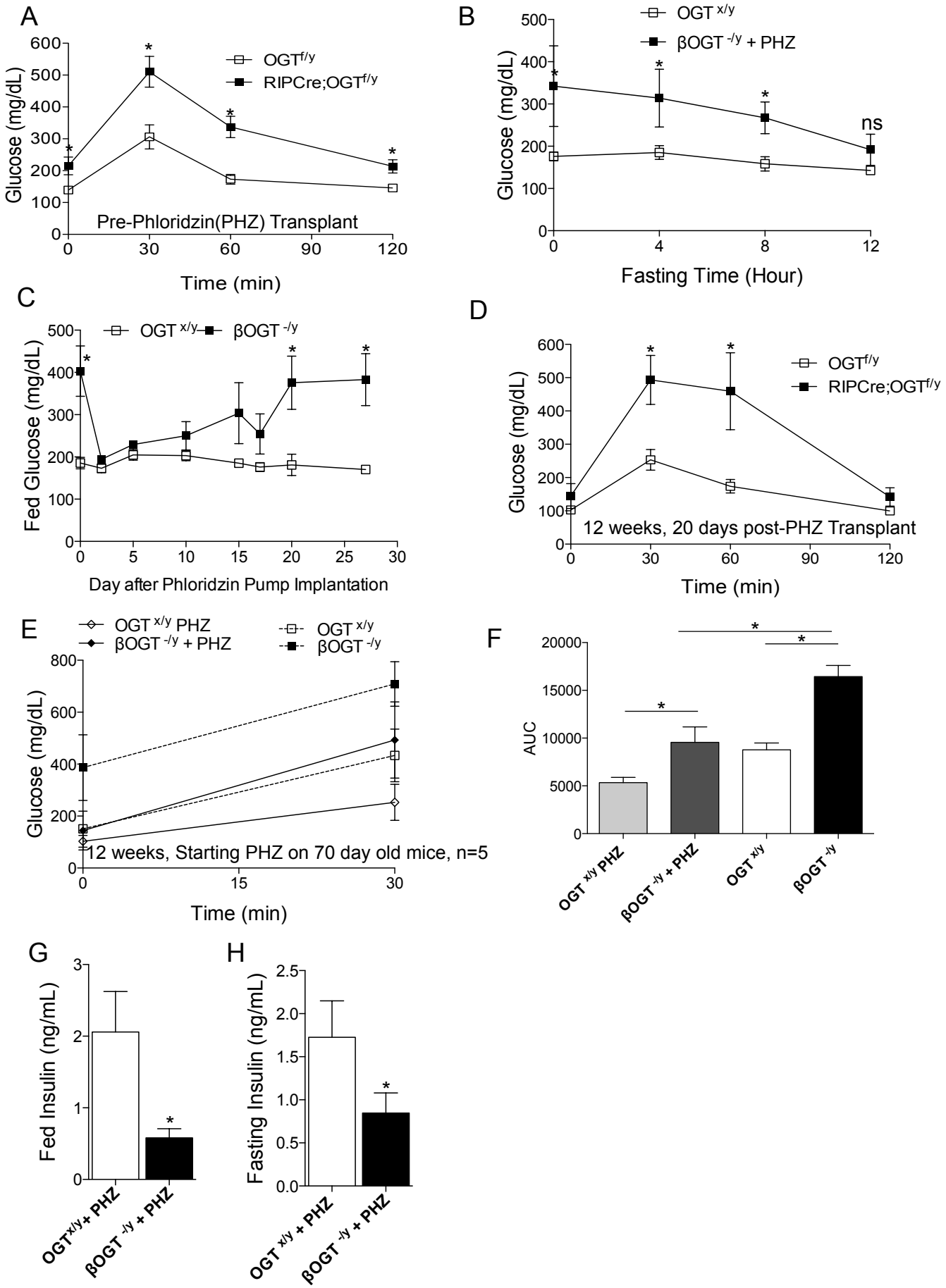
Supplemental Figure 3



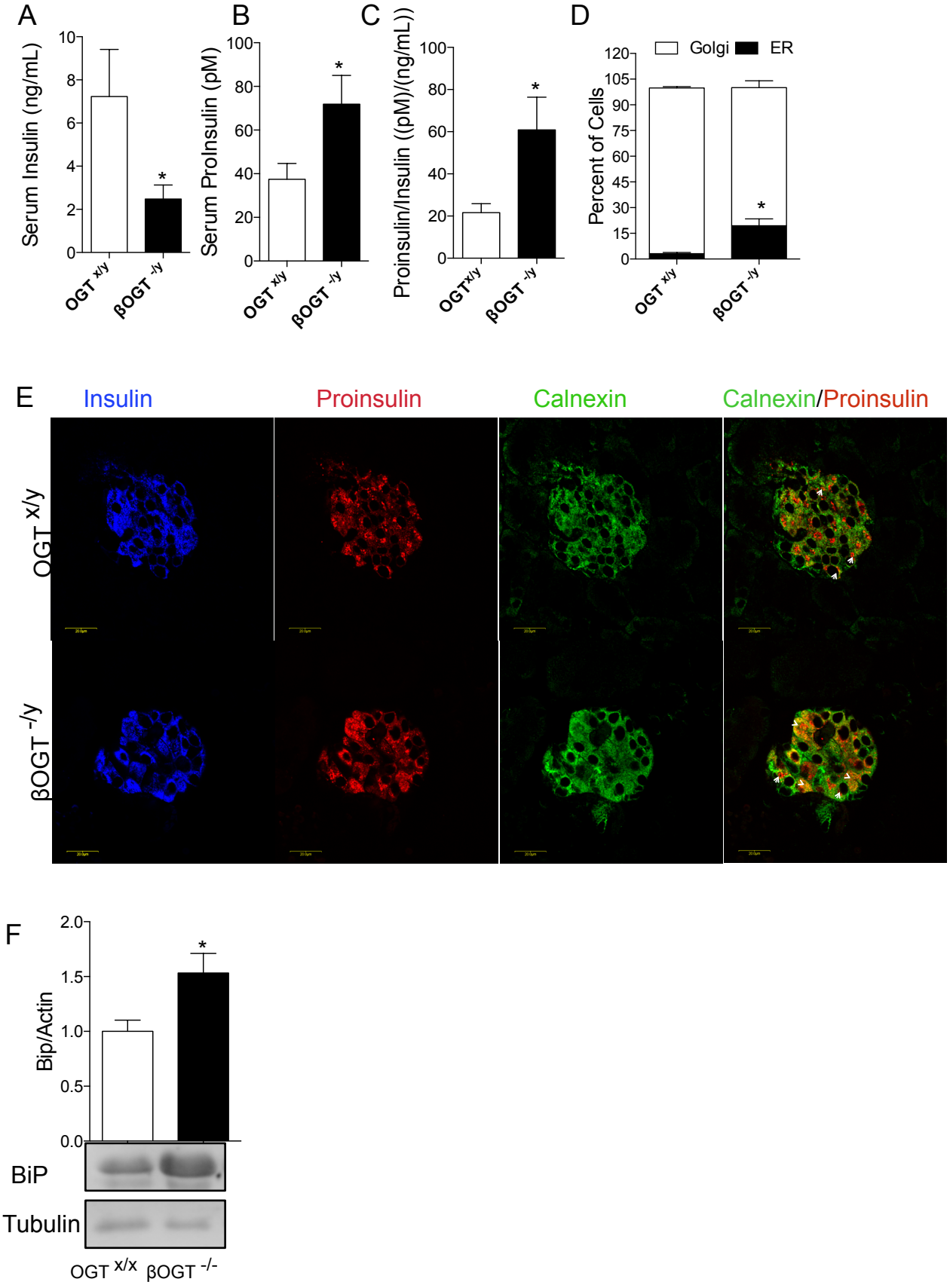




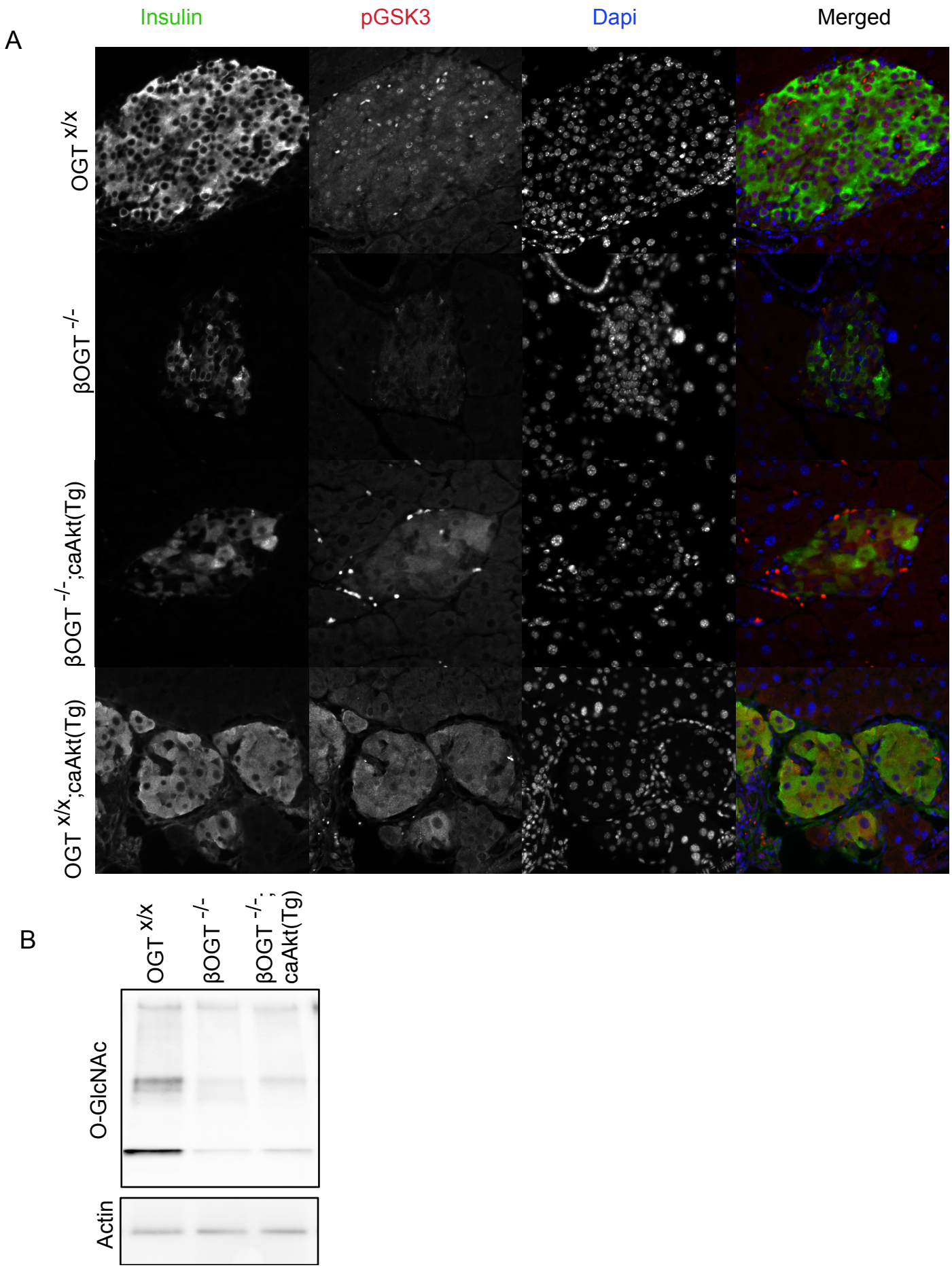
Supplemental Figure 4



Supplemental Figure 5



Supplemental Figure 6



Supplemental Figure 7

A