SUPPLEMENTAL MATERIAL

Supplemental Methods

Glucose and Insulin Tolerance Tests: For glucose tolerance tests, mice at five weeks of age were fasted overnight (20 hours) before the administration of 2g/kg glucose (ip) and the monitoring of blood glucose levels, as above. For insulin tolerance tests, mice were fasted for five hours before the administration of 4U/kg insulin (Novolin) (ip) and blood glucose monitoring.

β-*cell mass measurements*: Mouse pancreases were fixed by formaldehyde and embedded in paraffin prior to sectioning (5 μm). Immunofluorescent staining for insulin was performed using guinea pig anti-human insulin antibody (Linco) and a secondary donkey anti-guinea pig Texas Red-conjugated antibody. Images were captured using Olympus BX60 fluorescence microscope at both 4x and 20x magnification. Images were analyzed using Image J software (http://rsb.info.nih.gov/ij/, NIH, 1997-2009) and the ratio of β-cells to the total pancreatic area was calculated.

Immunohistochemistry. Briefly, mice were deeply anesthetized with an overdose of intraperitoneal pentobarbital (150 mg/kg) and transcardially perfused with sterile phosphate buffered saline (PBS) followed by 10% formalin. Brains were removed, postfixed and cryoprotected before sectioning into 30 μm coronal slices, which were collected into 4 representative series and stored at -20°C until further use. For IHC, sections were pretreated in ice-cold methanol, 0.3% glycine and 0.3% SDS, and then blocked and incubated in the primary antibodies (rabbit anti-c-Fos (1:40,000) and/or goat anti-β-Gal (1:3000)). Anti-phospho-STAT3 was from Cell Signaling and αpS6 was the generous gift of Diane Fingar, PhD (University of Michigan).

Detection of primary antibodies was done either by immunofluorescence (antirabbit-Alexa 488 and anti-goat-biotin followed by streptavidin-Alexa 564 conjugate (all 1:200 dilution)) or using the avidin-biotin/diaminobenzidine-method. Rabbit anti-cFos was from Oncogene Scinces (Uniondale, NY), goat anti-(β-Gal) from Biogenesis (UK), and donkey serum, biotinylated donkey anti-goat and donkey anti-rabbit were from Jackson ImmunoResearch Laboratories, Inc. (West Grove, PA). Alexa-488 conjugated donkey anti-rabbit and Alexa-564 conjugated streptavidin were purchased from Molecular Probes, Inc (Eugene, OR). ABC Vectastain Elite kit was purchased from Vector Laboratories (Burlingame, CA). All other immunohistochemical supplies were purchased from SIGMA (St. Louis, MO).

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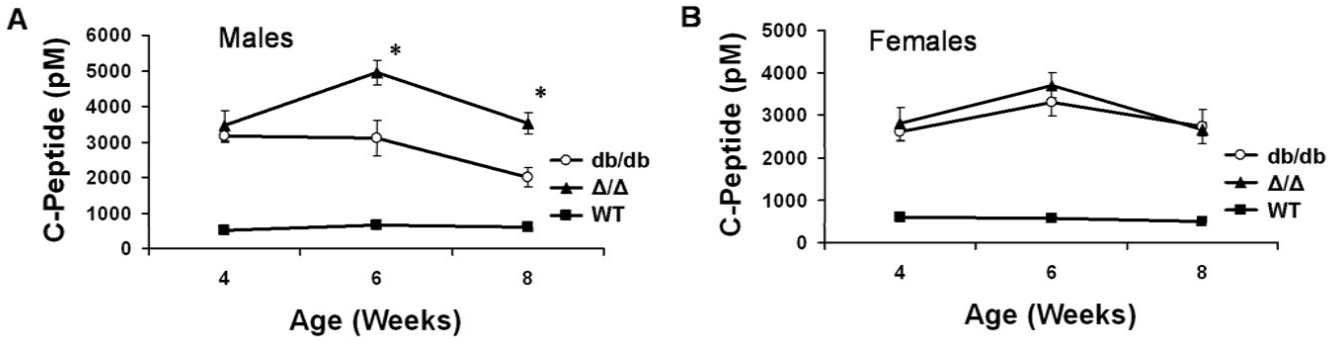
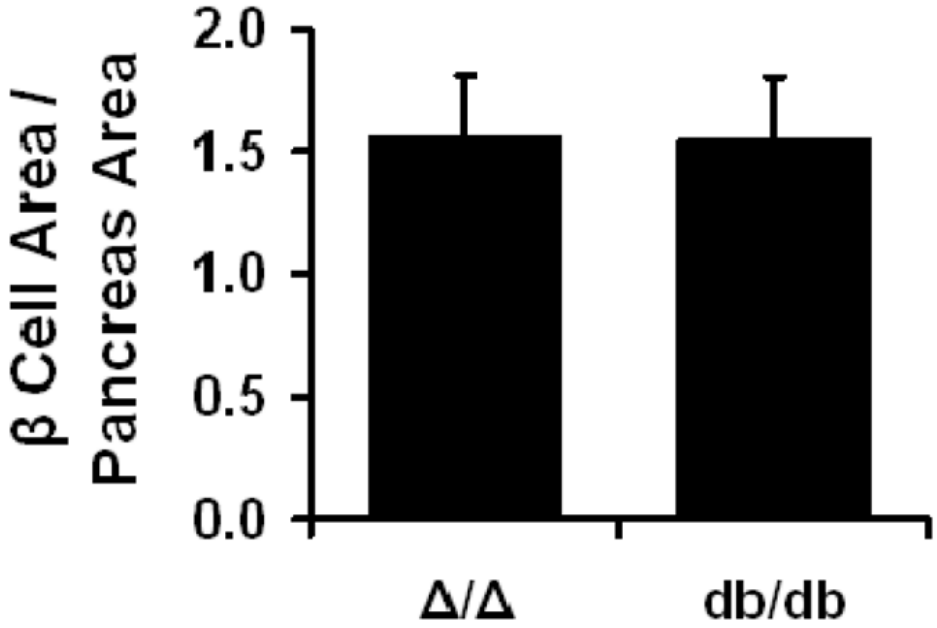
Supplemental Figure 1. Serum C-peptide levels in WT, *db/db* and *Δ/Δ* mice. A-B) Serum was collected from *WT* (black squares), *db/db* (white circles) and *Δ/Δ* (black triangles) mice of the indicated genotype and sex at 4, 6 and 8 weeks of age and Cpeptide content was determined by ELISA as described in the main text. Data are plotted as mean +/-SEM; *db/db* *p<0.05 compared to *Δ/Δ* at the indicated time-points by oneway ANOVA and Tukey's post test. (n = 8-10 for *Δ/Δ* and db/db and n = 3 for *WT*)



Supplemental Figure 2. β cell mass in *db/db* and *Δ/Δ* mice. Pancreata were collected from male *db/db* and *Δ/Δ* mice at the time of sacrifice. Pancreata were fixed and processed for the determination of β-cell mass, as described (20). Data are plotted as mean +/-SEM; p=NS by Student's upaired t-test (n = 8-10 pergenotype).



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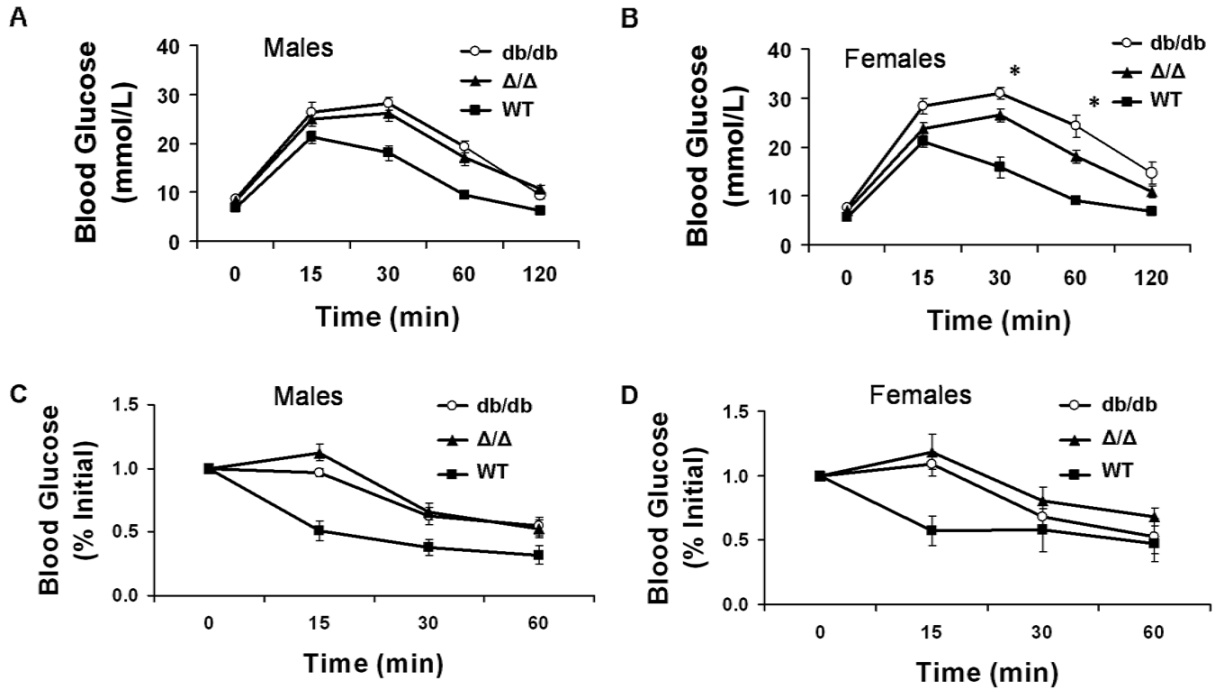
Supplemental Figure 3. Glucose and insulin tolerance in WT, *db/db* and *Δ/Δ* mice. **A-B)** *WT* (black squares), *db/db* (white circles) and *Δ/Δ* (black triangles) mice of the indicated sex were (A-B) fasted overnight at 5 weeks of age and injected with 2g/kg glucose. Blood glucose was monitored prior to injection and post-injection at 15, 30, 60 and 120 min. **C-D)** *WT* (black squares), *db/db* (white circles) and *Δ/Δ* (black triangles) mice of the indicated sex were fasted for 5 hours at 6 weeks of age and injected with 4U/kg insulin. Blood glucose was monitored prior to injection and post-injection at 15, 30, and 60 min. All panels: Data are plotted as mean +/-SEM; *db/db* *p<0.05 compared to *Δ/Δ* at the indicated time-points by one-way ANOVA and Tukey's post test. (n = 8-12 per genotype)



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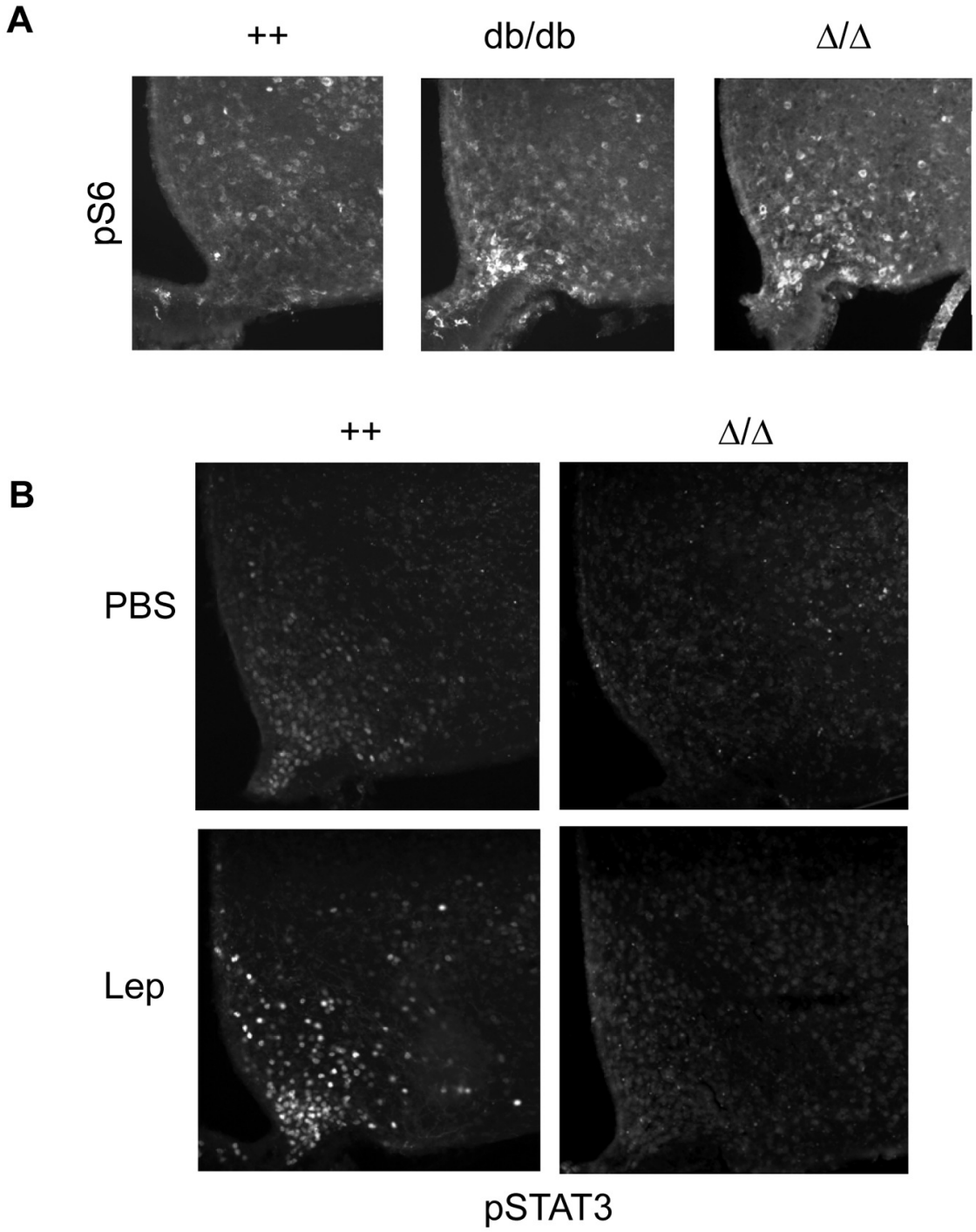
Supplemental Figure 4. Analysis of hypothalamic mTOR activity and STAT3 phosphorylation in Δ/Δ mice. (A) pS6-IR in the ARC of ad-libitum fed *WT*, *db/db*, and *Δ/Δ* mice. (B) Ad libitum-fed WT and Δ/Δ mice were treated with leptin (5 mg/kg, IP) or vehicle (PBS) 1 hour prior to perfusion and processing for immunofluorescent analysis of pSTAT3-IR.



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