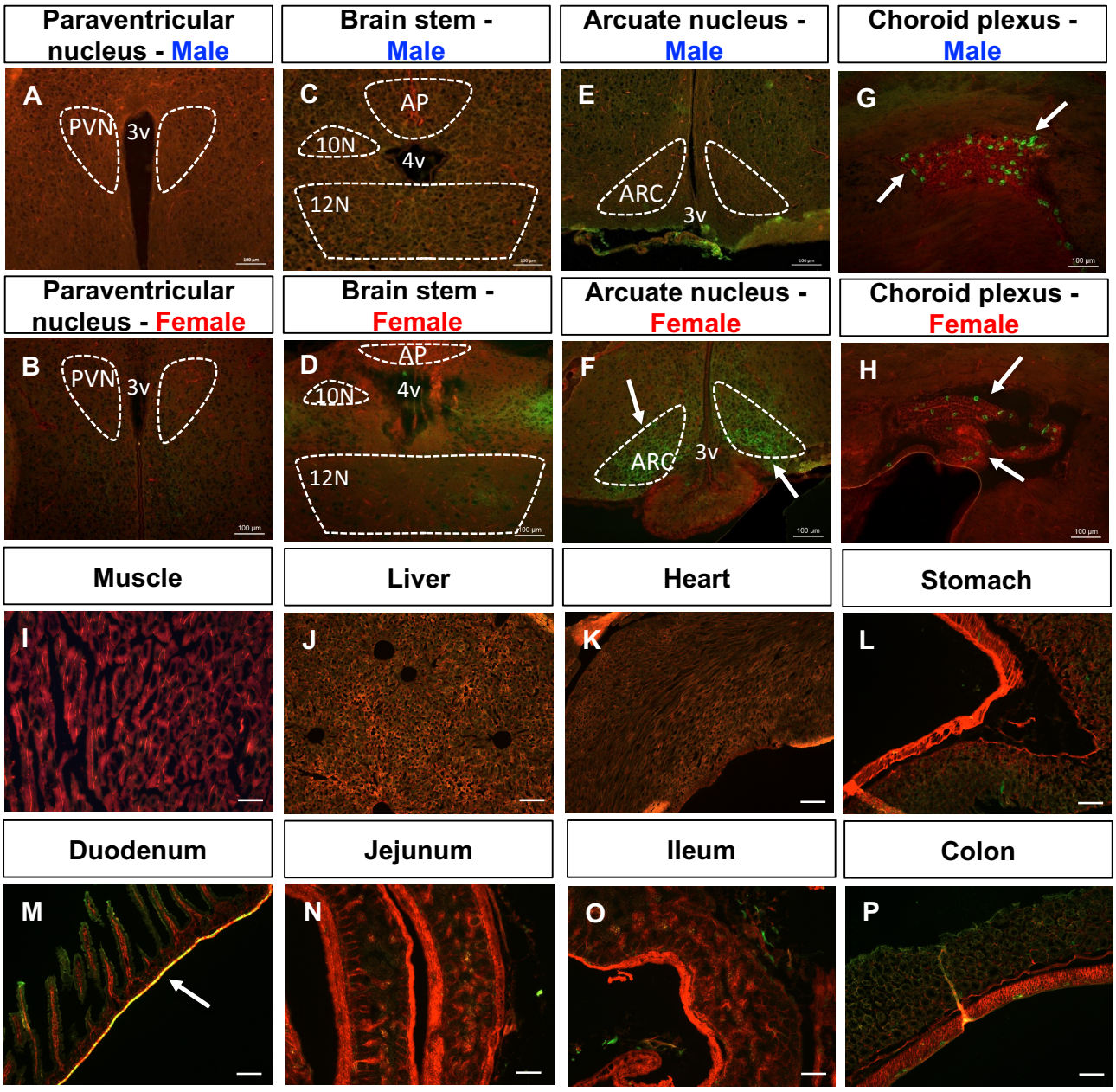
**Supplemental Table 1: Antibodies List**

| Target | Company | Catalog number | Dilution |
|---|---|---|---|
| Insulin | Dako | A056401 | 1:50 |
| Glucagon | Sigma | SAB4501137 | 1:250 |
| Alexa Fluor 488 Anti-Guinea Pig | Jackson ImmunoResearch | 706-545-148 | 1:100 |
| Alexa Fluor 647 Anti-Guinea Pig | Jackson ImmunoResearch | 706-605-148 | 1:100 |
| Alexa Fluor 647 Anti-Rabbit | Jackson ImmunoResearch | 711-605-152 | 1:100 |
| Cy 3 Anti-Rabbit | Jackson ImmunoResearch | 711-165-152 | 1:100 |

## Supplemental Table 2: Primers List

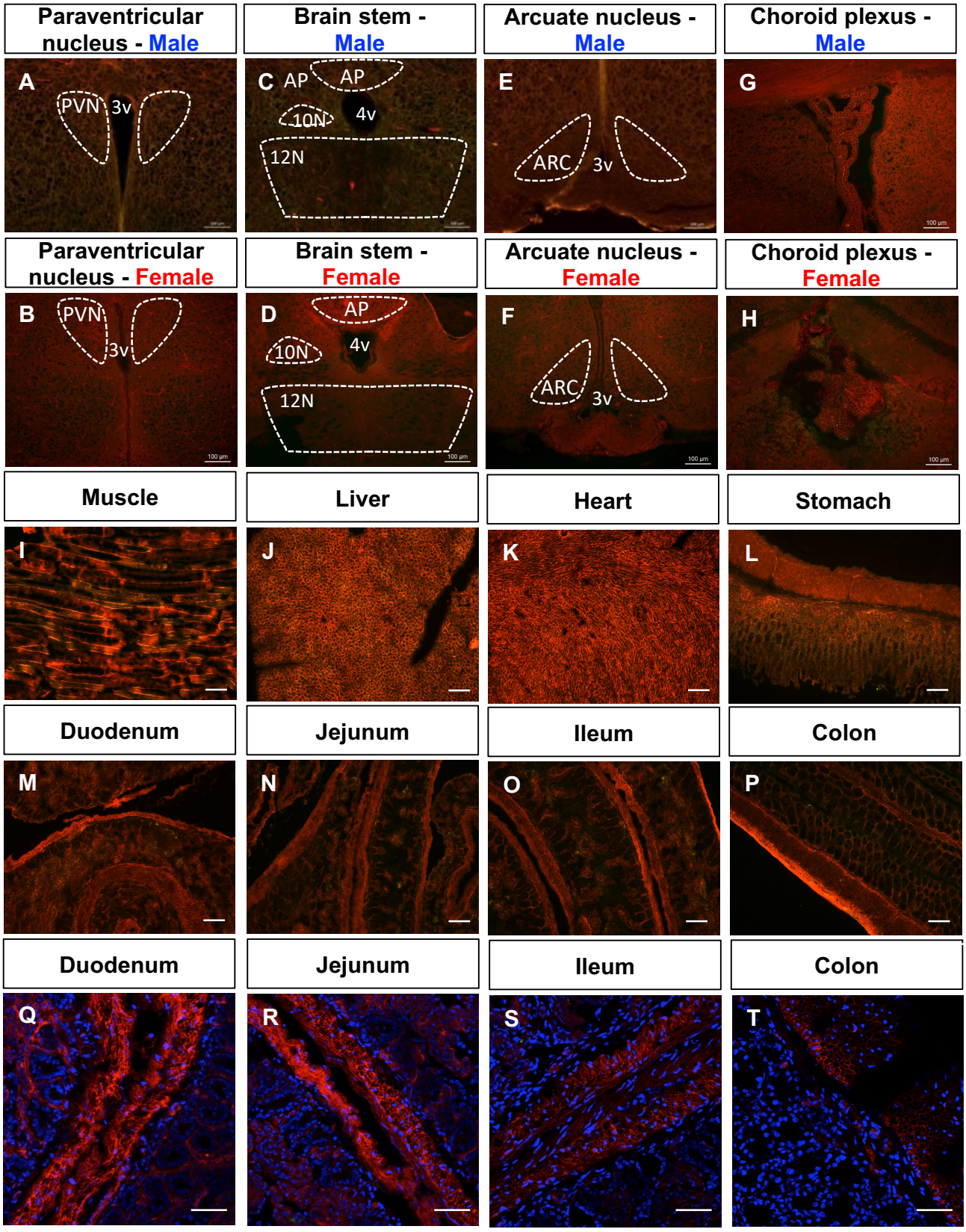
| GENOTYPING | | | |
|---|---|---|---|
| Gene | Gene Name | Forward Primer (5'-3') | Reverse Primer (5'-3') |
| mTmG transgene | | CTC TGC TGC CTC CTG GCT TCT | CGA GGC GGA TCA CAA GCA ATA |
| | | | TCA ATG GGC GGG GGT CGT T |
| TetO-Cre transgene | | TTA GTG AAC CGT CAG ATC GCC TGG | GTG AAA CAG CAT TGC TGT CAC TT |
| itTA transgene | | AGA GCA AAG TCA TCA ACT CTG CC | GTG AGA GCC AGA CTC ACA TTT CA |
| **PCR** | | | |
| Gene | Gene Name | Forward Primer (5'-3') | Reverse Primer (5'-3') |
| IL-2 (Control) | Interleukin 2 | CTA GGC CAC AGA ATT GAA AGA TCT | GTA GGT GGA AAT TCT AGC ATC ATC C |
| hGH | human Growth Hormone | CCT AGC TGC AAT GGC TAC AG | GCA CTG GAG TGG CAA CTT CC |
| **qPCR** | | | |
| Gene | Gene Name | Forward Primer (5'-3') | Reverse Primer (5'-3') |
| *tTA* | Transactivator | GAT GTC AGC CTG GGG GAC GA | CCC CCA ACA TGT CCA GAT CG |
| *itTA* | Improved Transactivator | CAG AAG CTG GGT GTG GAG CAG | GGT GTG GTG CCT GTC CAA C |
| *rtTA* | Reverse Transactivator | CTC AAT GGA GTC GGT ATC G | CTT GTT CTT CAC GTG CCA G |
| *PAC* | Puromycin *N*-acetyl-transferase | GGG TCA CCG AGC TGC AAG AA | GCC TTC CAT CTG TTG CTG CG |
| *mrTbp* | mouse/rat TATA Binding Protein | CCC TAT CAC TCC TGC CAC ACC | GTG CAA TGG TCT TTA GGT CAA |
| *mPdx1* | mouse Pancreatic and Duodenal Homeobox 1 | CTT AAC CTA GGC GTC GCA CAA | GAA GCT CAG GGC TGT TTT TCC |
| *rPdx1* | rat Pancreatic and Duodenal Homeobox 1 | TCA TCT CCC TTT CCC GTG GAT GAA | AGG CTG TAC GGG TCC TCT TAT TCT |
| *mIns 1* | mouse Insulin 1 | GAA GTG GAG GAC CCA CAA GTG | ATC CAC AAT GCC ACG CTT CT |
| *rIns 1* | rat Insulin 1 | ATC TGC TCC CTC TAC CAA CT | GGT GCT CAT TCA AAG GCT TTA T |
| *mIns 2* | mouse Insulin 2 | GAA GTG GAG GAC CCA CAA GTG | GAT CTA CAA TGC CAC GCT TCT G |
| *rIns 2* | rat Insulin 2 | CAG GTG ACC TTC AGA CCT TG | CAG TTG GTA GAG AGA GCA GAT G |
| *mKcnj11* | mouse Potassium Inwardly Rectifying Channel Subfamily J Member 11 | TGT GCA GAA TAT CGT CGG GCT GAT | GCA TGC TTG CTG AAG ATG AGG GTT |
| *rKcnj11* | rat Potassium Inwardly Rectifying Channel Subfamily J Member 11 | TCG TAG GGC TAA TGA TCA ACG CCA | GCA TGC TTG CTG AAG ATG AGG GTT |
| *mrSlc2a2* | mouse/rat Solute Carrier Family 2 Member 2 | AGG TCC AAT CCC TTG GTT CAT GGT | AAT GTA CTG GAA GCA GAG GGC GAT |

# Supplemental Fig. 1



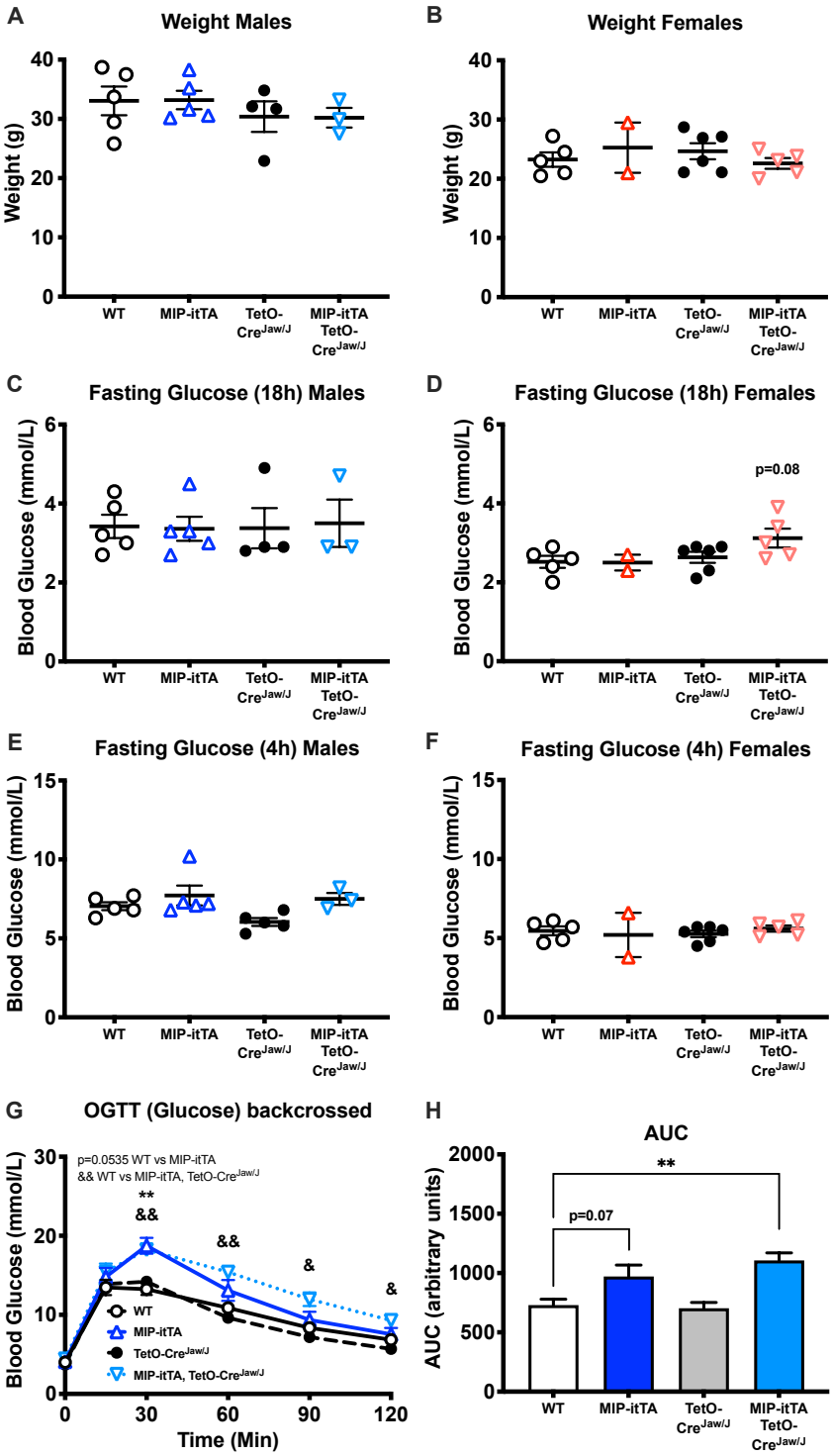

**Supplemental Figure 1: Cre-mediated recombination detected in the arcuate nucleus of females MIP-itTA:TetO-Cre$^{Jaw/J}$:mTmG mice and in the choroid plexus and duodenum of all MIP-itTA:TetO-Cre$^{Jaw/J}$:mTmG mice.** Coronal sections of brain from 18-20 weeks old MIP-itTA:TetO-Cre$^{Jaw/J}$:mTmG mice 4 weeks after doxycycline removal. *A-B*: Paraventricular nucleus (PVN) *C-D*: brain stem *E-F*: arcuate nucleus (ARC), *G-H*: choroid plexus. 3v: third ventricle, 4v: fourth ventricle, AP: area postrema, 10N: dorsal motor nucleus of the vagus, 12N: hypoglossal nucleus from male or female (n=3 mice, per sex). Scale bars, 100 μm. Sections of the *I*: muscle, *J*: liver, *K*: heart, *L*: stomach, *M*: duodenum, *N*: jejunum, *O*: ileum and the *P*: colon from 18-20 weeks old MIP-itTA:TetO-Cre$^{Jaw/J}$:mTmG mice 4 weeks after doxycycline removal. (representative images of *n* = 4 male or female mice). Scale bars, 120 μm.

# Supplemental Fig. 2



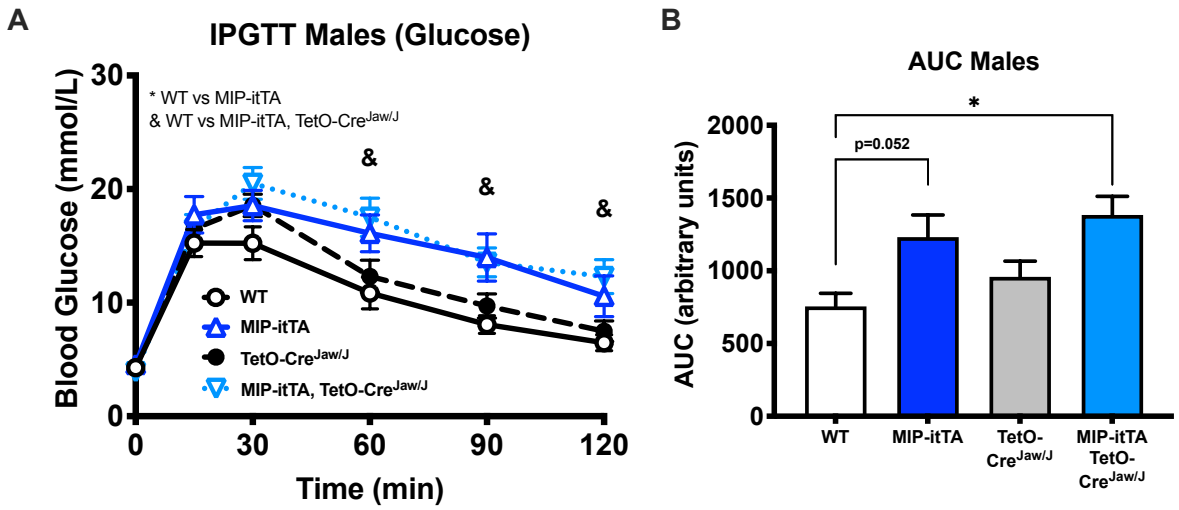
**Supplemental Figure 2: Cre-mediated recombination is not detected in mTmG littermate control mice.** Coronal sections of brain from 18-20 weeks old mTmG mice 4 weeks after doxycycline removal. *A-B*: Paraventricular nucleus (PVN) *C-D*: brain stem *E-F*: arcuate nucleus (ARC), *G-H*: choroid plexus. 3v: third ventricle, 4v: fourth ventricle, AP: area postrema, 10N: dorsal motor nucleus of the vagus, 12N: hypoglossal nucleus from male or female (n=3 mice, per sex). Scale bars, 100 μm. Sections of the *I*: muscle, *J*: liver, *K*: heart, *L*: stomach, *M*: duodenum, *N*: jejunum, *O*: ileum and the *P*: colon from 18-20 weeks old mTmG mice 4 weeks after doxycycline removal. Scale bars, 120 μm. Higher magnification, confocal images of *Q*: duodenum, *R*: jejunum, *S*: ileum, *T*: colon (representative images of $n = 4$ male or female mice). Scale bars, 50 μm.

# Supplemental Fig. 3



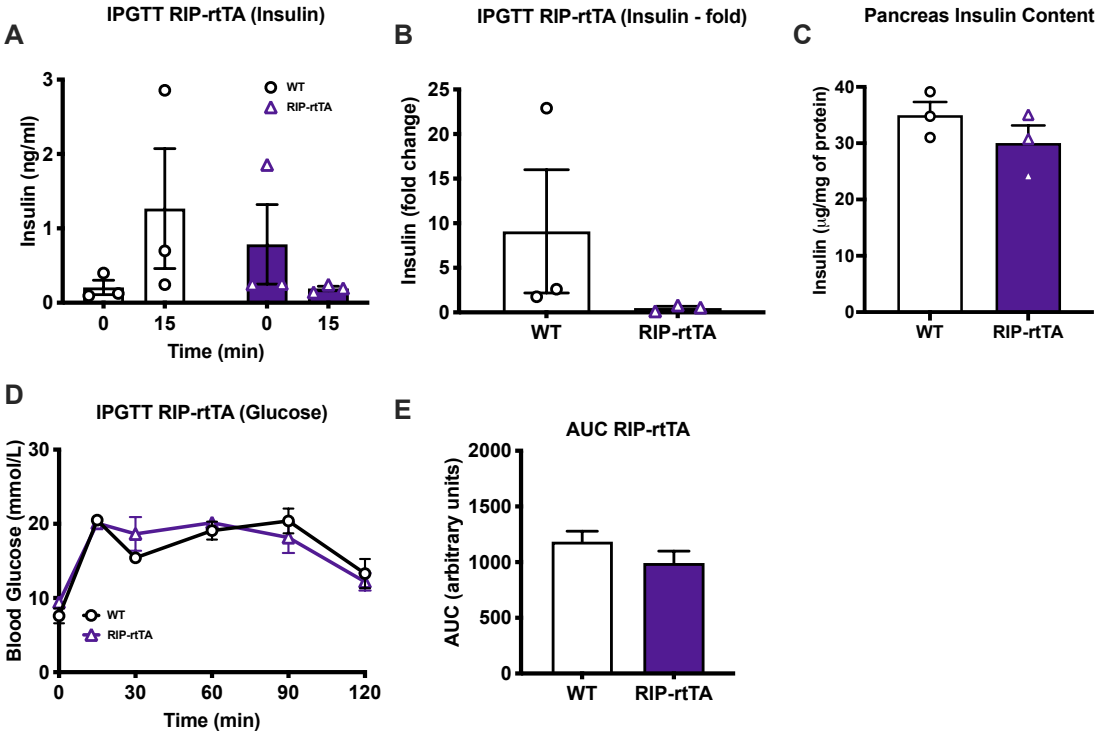

**Supplemental Figure 3: Transgene expression has no effect on weight or fasting blood glucose, while glucose tolerance is decreased by MIP-itTA.** Body weights of 13-15 weeks old *A*: males (*n* = 4-6) and *B*: females (*n* = 2-5). *C,D*: 18 hour or *E,F*: 8 hour fasting blood glucose levels in males or female mice, as indicated. *G*: Blood glucose following oral glucose challenge (1.5g/kg) in 20 weeks old males, never exposed to doxycycline, on a pure C57Bl/6N background (*n* = 4-6). *H*: Area under the curve for groups in *G*. WT: white circle/solid line, MIP-itTA: open, dark blue triangle/solid line, TetO-Cre$^{Jaw/J}$: black circle/dashed line, MIP-itTA, TetO-Cre$^{Jaw/J}$: open, inverted light blue triangle/dash line. Two-way ANOVA and post-hoc comparisons are shown for WT vs MIP-itTA (**p<0.01), and WT vs MIP-itTA:TetO-Cre$^{Jaw/J}$ ($^{\&}$p<0.05, $^{\&\&}$p<0.01) in G.

# Supplemental Fig. 4



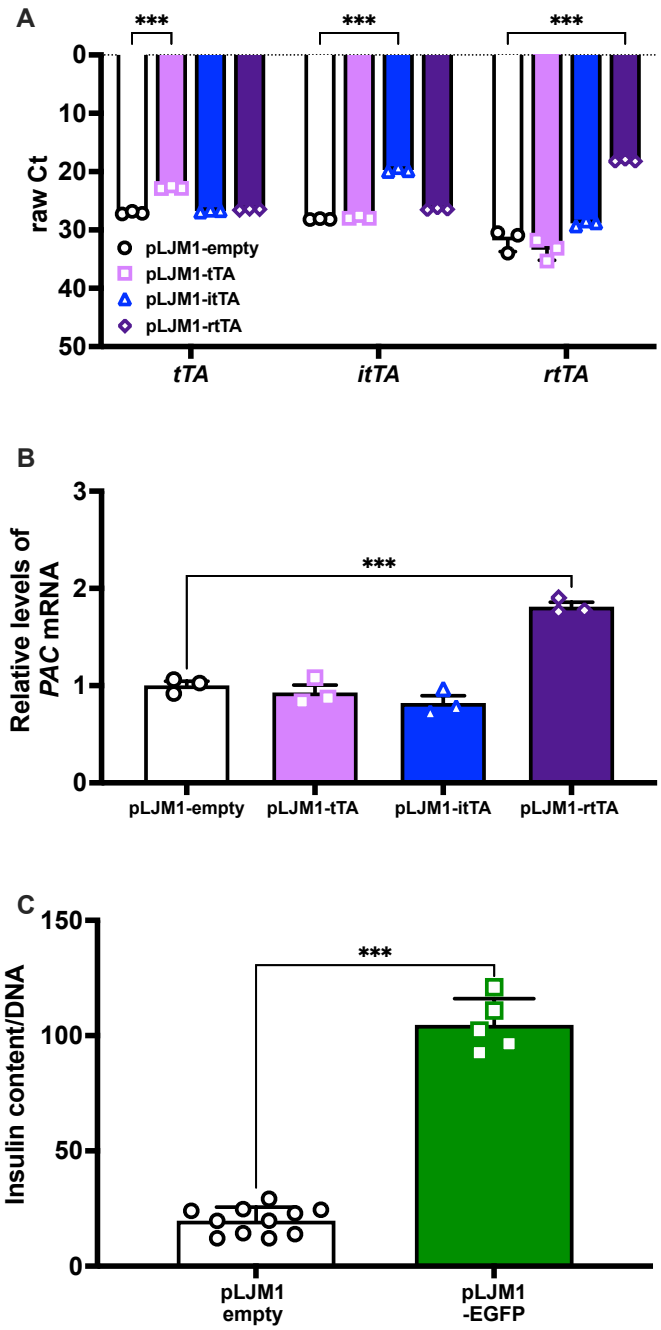

**Supplemental Figure 4: The incretin effect does not play a role in MIP-itTA-dependent impairment of glucose tolerance.** *A*: Blood glucose levels following intraperitoneal injection of glucose (1.5g/kg) in 20 weeks old males (C57Bl/6N, never exposed to doxycycline). *B*: Area under the curve (AUC) of groups in *A*. Values are means ± SEM. (*n* = 4-6 per genotype), two-way ANOVA results and post-hoc comparisons are shown for WT vs MIP-itTA, TetO-Cre$^{Jaw/J}$ $^{\&}p<0.05$).

# Supplemental Fig. 5




**Supplemental Figure 5: Insulin secretion defect in response to glucose in RIP-rtTA mice.** *A*: Insulin levels and *B*: insulin fold change per mouse at 0 and 15 min post-glucose injection following intraperitoneal injection of glucose (2g/kg) from 6-8 weeks old animals (males, C57Bl/6J, never exposed to doxycycline). *C*: Total pancreatic insulin content normalized to protein. *D*: Blood glucose levels during the IPGTT in *A*. *E*: Area under the curve (AUC) of groups in *D*. Values are means ± SEM, *n* = 3 per genotype.

# Supplemental Fig. 6




**Supplemental Figure 6: Relative expression levels of the tetracycline-controlled transactivators stably expressed in INS-1 cells and insulin content of INS-1 cells stably expressing empty vector or EGFP.** *A*: Raw Ct values for each transactivator measured by pPCR from mRNA isolated from each stable cell line. Given poor sequences similarity between cDNAs, individual primer sets were required for each construct**.** *B*: Relative mRNA expression of the *PAC* (puromycin *N*-acetyl-transferase) gene, common to the expression vector used to make the stable cell lines. *C*: Insulin content of INS-1 cells stably overexpressing empty vector or EGFP. Values are means ± SD of biological triplicates, with statistical comparisons made to pLJM1 empty vector (***p<0.001).