Supplemental Information (Bozadjieva *et al.*)

Supplemental Table 1. Antibodies

Antibody	Specie	Source	Application
Glucagon	Mouse	Abcam (ab10988)	IFC
Glucagon	Rabbit	Chemicon/Millipore (AB932)	IFC
Glucagon-BV421	Human/Mouse	BD Biosciences (565891)	FC
Insulin	Guinea Pig	Dako (A0564)	IFC
Insulin-APC	Human/Mouse/Bovine	R&D Systems (IC1417A)	FC
pS6 (S240)	Rabbit	Cell Signaling (5364)	IFC
pS6 (S240)-PE	Mouse	BD Biosciences (560430)	FC
FoxA2	Rabbit	Cell Signaling (3143)	IFC
FoxA2	Mouse	Abcam (ab60721)	WB
FoxA2-Dylight488 (conjugated in house)	Mouse	Abcam (ab60721) and DyLight488 Fast Conjugation Kit (ab201799)	FC
KIR6.2	Rabbit	Alomone Labs (APC-020)	WB
SUR1	Rabbit	Show-Ling Shyng Lab	WB
Somatostatin	Rabbit	Santa Cruz (SC-13099)	IFC
Pancreatic Polypeptide	Guinea Pig	Linco/Millipore (4041-01)	IFC
Actin	Mouse	Sigma (A5441)	WB
Ghost Dye Red 780		Tonbo (13-0865)	FC
Ki67	Rabbit	Thermo Fisher (PA5-19462)	IFC
Ki67-BV786	Mouse	BD Biosciences (563756)	FC

AnnexinV-PE		BD Biosciences (556421)	FC
Glucagon	Guinea Pig	Linco (4031-01F)	IFC for patched cells
DAPI	VECTASHIELD Antifade Mounting Medium with DAPI	Vector (H-1200)	IFC
GFP	Chicken	Abcam (ab13970)	IFC

WB = Western Blot

IFC = Immunofluorescent Chemistry

FC=Flow Cytometry

Supplemental Table 2. Primer Sequences

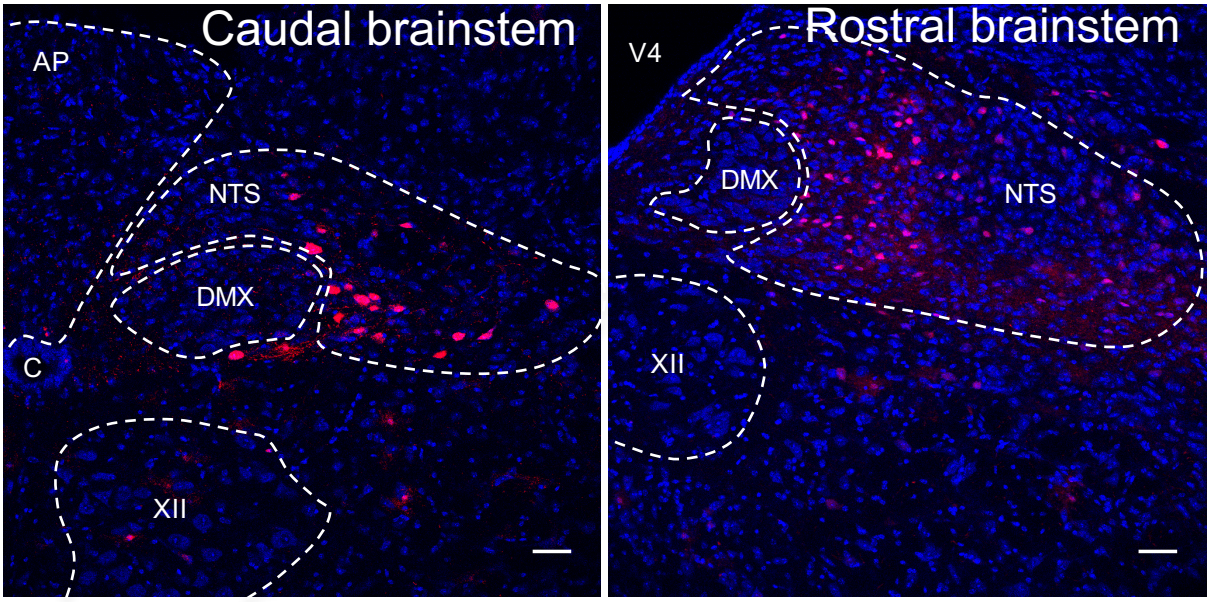
Gene	Forward	Reverse
FoxA2	CAGCTACTACGCGGAGCC	GCTCATTCCAGCGCCCAC
SUR1	CTGGTGGCCATCGACACAA	TGTACAGGAGCCAGCAGAAT
KIR6.2	GCTGCATCTTCATGAAAACG	TTGGAGTCGATGACGTGGTA
MafB	GAACGAGAAGACGCAGCTCT	CGAGTTTCTCGCACTTGACCT
Nkx2.2	TCGCTCTCCCCTTTGAACTTT	GTTAACGTTGGGATGGTTTGG
Pou3f4	TTCCTCAAGTGTCCCAAGCC	TAA ACCTCGTGTGGCTGCTG
FoxP1	CGAATGTTTGCTTACTTCCGA	GCCAGGCTGTGA AAGCATATGTGA
FoxP2	GCCAGGCTGTGAAAGCATATGTGA	CATTTGCACTCGACATTGGGCAGT
FoxP4	GTCAGCCTGCAGCCCAAGCCAAGCCTC	GGAGCTGTCTCTCCGAGATGTGAGCAC
18S	GCAATTATTCCCCATGAACG	GGGACTTAATCAACGCAAGC

Single Cell	Forward	Reverse
SUR1 (inside)	CTGGTG GCCATCGACACAA	TGTACAGGAGCCAGCAGAAT
SUR1 (outside)	GTCTACTTCCAACCTGTCAAT	ACCCTCGAGCAGAAGATGTT
Raptor (inside; exon 6)	CCTCTGTCCATATACGACCT	CTCTGCAGTGCAAACTGT
Raptor (outside; exons 5&7)	CCTACTGTGGATGAAGTCAAG	CACTCCCATCAAGATTGCTC

Supplemental Table 3. Fluidigm Gene Targets Included in Analysis

CCNB1	*NOTCH1*	*ABCC8*	*CCND1*	*FOXO1*	*LAMP1*	*PAX6*
EIF4E	*SNAP25*	*HSP90AB1*	*CCND2*	*FOXP1*	*LAMTOR2*	*PCSK2*
CHRM3	*ULK2*	*CACNA1S*	*CDKN1A*	*FOXP4*	*LAMTOR3*	*PTF1A*
NEUROG3	*MAFB*	*XBP1*	*CDKN1B*	*GCG*	*MAFA*	*RFX6*
LAMTOR1	*FOXA2*	*ARX*	*CPE*	*GIPR*	*NKX6-1*	*TM4SF4*
NKX2-2	*POU3F4*	*CACNA1A*	*CRYBA2*	*GLP1R*	*NOTCH2*	*UCP2*
ULK1	*KCNJ11*	*CASP3*	*DDIT3*	*HSPA5*	*OGT*	*VAMP2*
GATA4	*RBPJ*	*CCNA2*	*ERN1*	*IRS2*	*PAX4*	

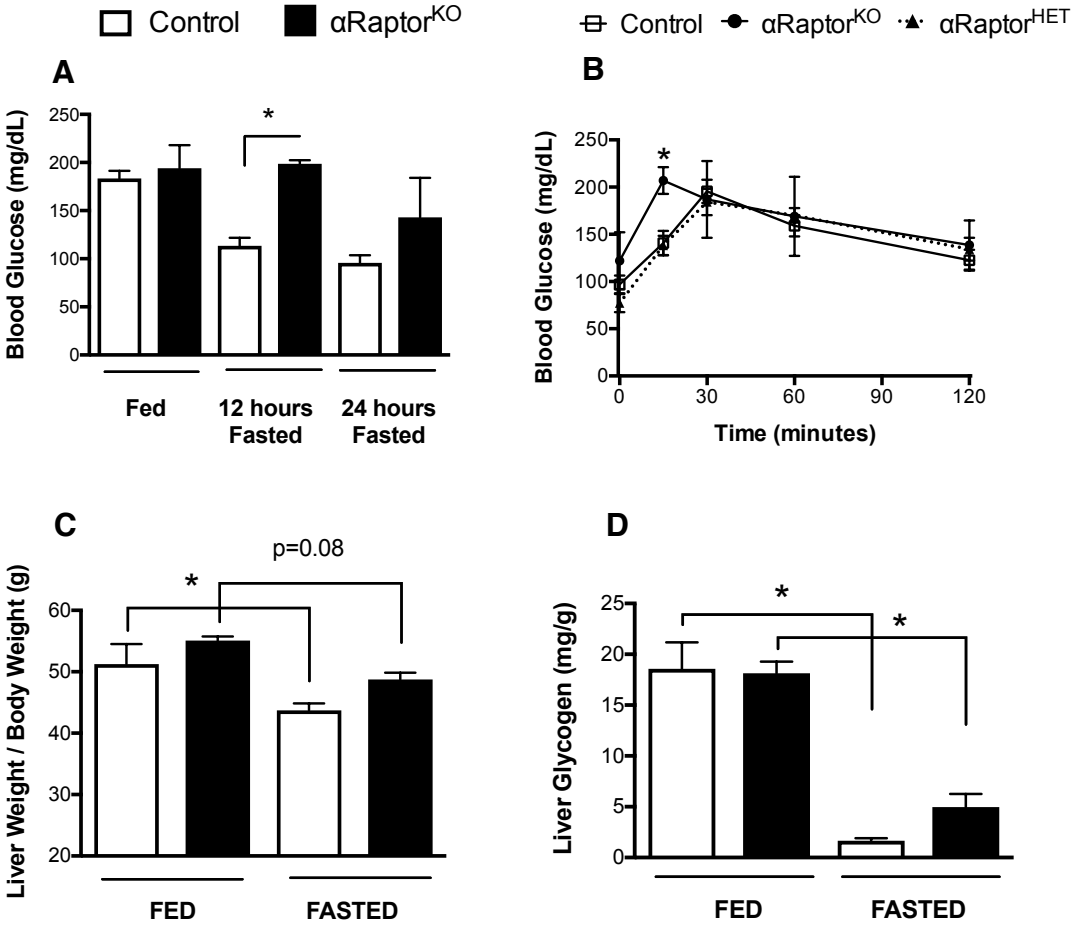
Supplemental Figure 1



AP – Area Postrema
NTS – Nucleus of the Solitary Tract
C – Central Canal
DMX – Dorsal Motor Nucleus of Vagus
XII – Hypoglossal Nucleus
V4 – Fourth Ventricle

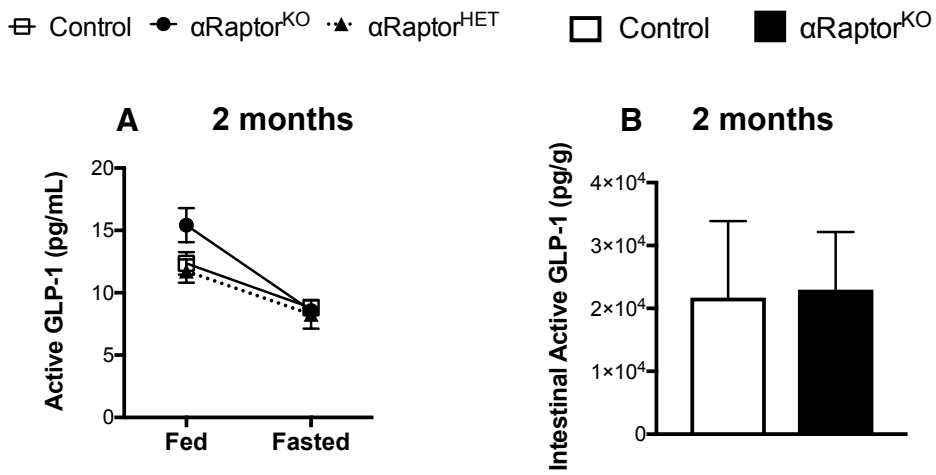
Supplemental Figure 1. *Glucagon-Cre* recombination in the Nucleus of Solitary Tract (NTS). Immunofluorescent images (scale=50 μm) of *Glucagon-Cre* recombination in the Nucleus of Solitary Tract (NTS) using a reporter transgenic mouse *CAG-tdTomato*.

Supplemental Figure 2



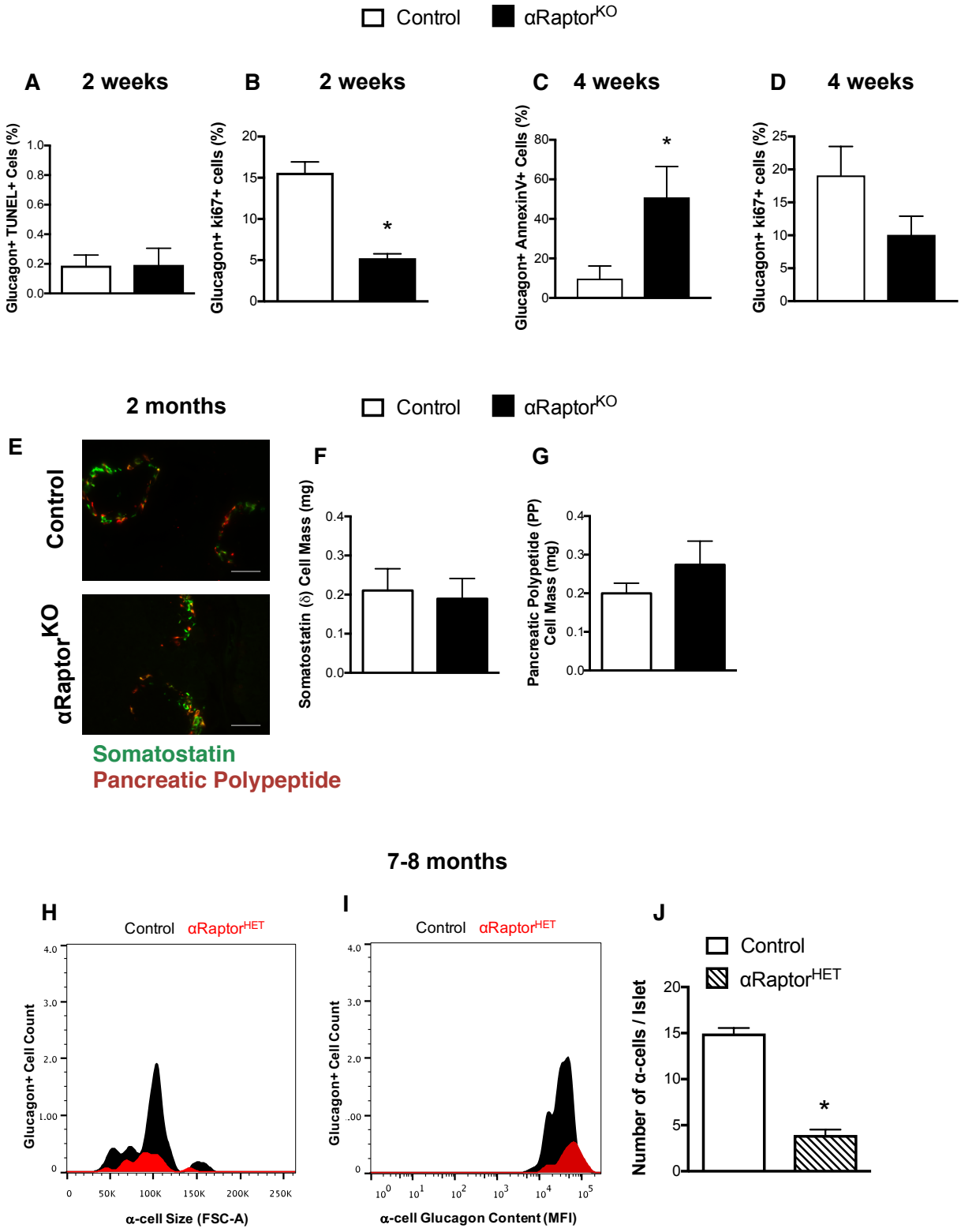
Supplemental Figure 2. αRaptor^{KO} mice have increased gluconeogenesis. A. Fasting blood glucose (n=3-4). **B.** Pyruvate tolerance test (2 g/kg) in 2-months old mice (n=3-6). **C.** Fed and fasted liver weight (n=3-6) and **D.** Liver glycogen content (n=3-4) in 8 months-old control and αRaptor^{KO} mice. Data for (A) shown as means ± S.E.M. *p≤0.05; (Student's 2-tailed *t* test). Data for (B-D) are shown as means ± S.E.M. *p≤0.05; (1-Way ANOVA with Dunnett's post-test).

Supplemental Figure 3



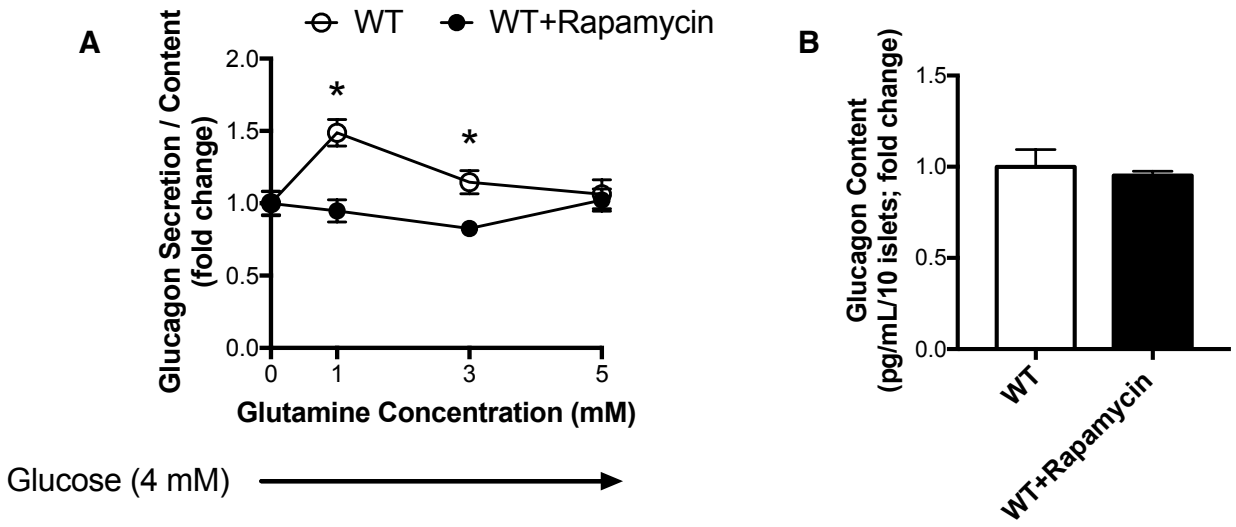
Supplemental Figure 3. Circulating and intestinal active GLP-1 levels. A. Fed and fasted active GLP-1 levels in 2 month-old mice (n=4-5). Data shown as means ± S.E.M. *p≤0.05; (1-Way ANOVA with Dunnett's post-test). **B.** Intestinal active GLP-1 levels in 2 month-old mice (n=4-5). Data are shown as means ± S.E.M. *p≤0.05; (Student's 2-tailed *t* test).

Supplemental Figure 4



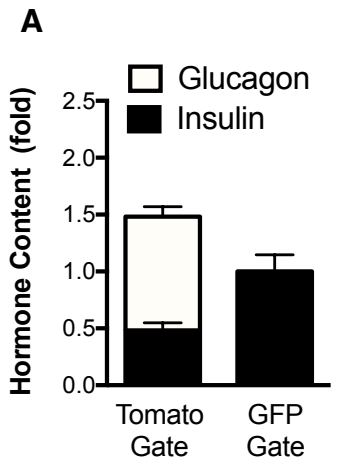
Supplemental Figure 4. Loss of mTORC1 signaling in α-cells leads to decreased proliferation and increased apoptosis in αRaptor^{KO} and decreased cell number in older αRaptor^{HET} mice. A. Quantifications of α-cell apoptosis by TUNEL stain (%; n=4) and **B.** Proliferation by ki67 stain at 2 weeks of age (%; n=3) in paraffin sections. **C.** Flow cytometry analysis of α-cell apoptosis by AnnexinV staining (%; n=4-5) and **D.** Proliferation by ki67 staining (%; n=6-9). **E.** Immunofluorescent images (scale=50 μm) and quantification of **F.** Somatostatin (δ) and **G.** PP (pancreatic polypeptide) cell mass in 2 month-old mice (n=3-4). **H.** Flow cytometry analysis of α-cell size and **I.** Glucagon content in older αRaptor^{HET} mice (n=3). **J.** Quantifications of α-cell number per islet of older αRaptor^{HET} mice (n=4). Data are shown as means ± S.E.M. *$p \leq 0.05$; (Student's 2-tailed *t* test).

Supplemental Figure 5



Supplemental Figure 5. Increase in glucagon secretion by glutamine is dependent on mTORC1 activation. A. Glucagon secretion and **B.** Islet glucagon content from isolated wild type islets treated with vehicle control or rapamycin (30nM) at increasing concentrations of glutamine (0, 1, 3, 5 mM) in 4 mM glucose KRBB (n= 5). Data are presented as fold change from 0 mM glutamine. Data are shown as means ± S.E.M. *p≤0.05; (Student's 2-tailed *t* test).

Supplemental Figure 6



Supplemental Figure 6. Glucagon and insulin levels in FACS-enriched α-cell population from Control and αRaptor^{HET} mice. A. Hormone quantification of Tomato (glucagon-enriched) and GFP (insulin) gates obtained through FACS using isolated and dispersed islets from 2 month-old Glucagon-Cre$^{Ins1GFP;\ tdTomato}$ mice (n=4). Glucagon data is presented as fold change from glucagon content in Tomato Gate. Insulin data is presented as fold change from insulin content in GFP Gate. Cells sorted from Tomato Gate (glucagon-enriched population) were used for experiments.