SUPPLEMENTARY DATA

Dual Effect of *Raptor* on Neonatal β Cell Proliferation and Identity Maintenance

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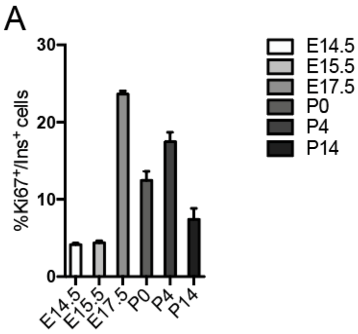
Supplementary Figure 1. Proliferation ratio of pancreatic β cells at different developmental period. (A) Proliferation ratios were counted by percentage of Ki67$^+$Insulin$^+$ double positive cells in insulin$^+$ pancreatic β cells at indicated time. (n=3-5).



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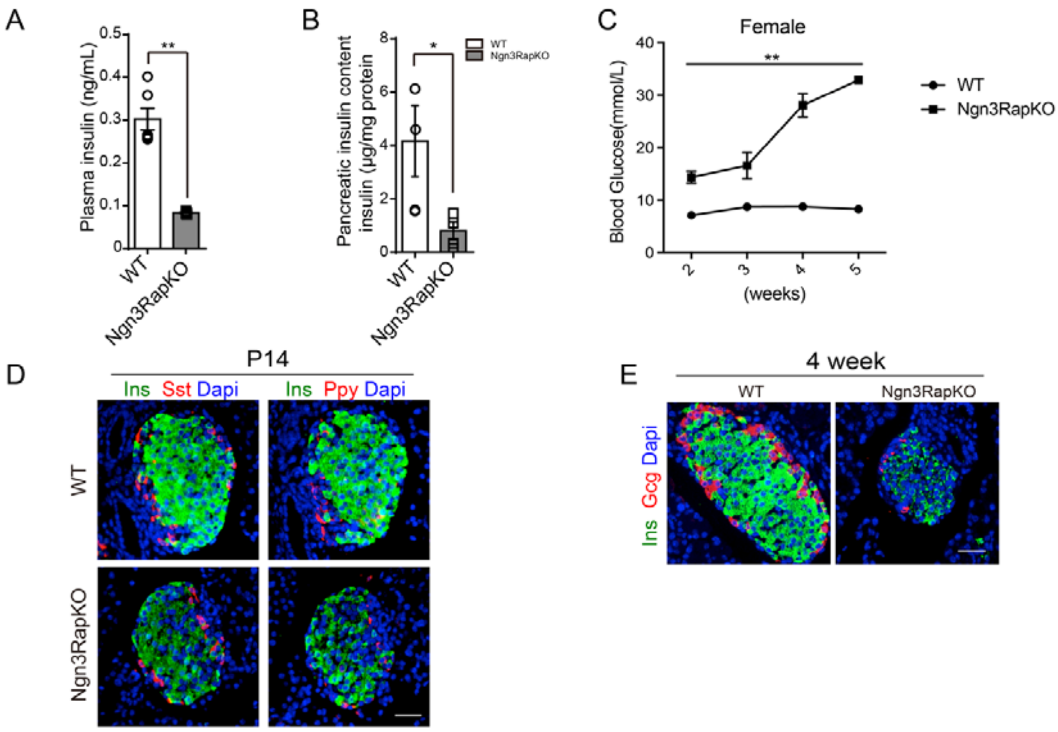
Supplementary Figure 2. Phenotype and islet morphology of Ngn3*Rap*KO mice. (A) Fasting plasma insulin levels of 4-week-old Ngn3*Rap*KO and WT mice (n=6-12). (B) Pancreas insulin content of 4-week-old Ngn3*Rap*KO and WT mice (n=3-4). (C) Random blood glucose levels of female Ngn3*Rap*KO and WT mice (n=4-7). (D) Immunostaining of insulin (Ins) and somatostatin (Sst), insulin (Ins) and pancreatic polypeptide (Ppy) of P14 Ngn3*Rap*KO and WT mice. (E) Immunostaining of insulin (Ins) and glucagon (Gcg) of 4-week-old Ngn3*Rap*KO and WT mice. Data were presented as mean ± SEM of independent experiment indicated as above.*p<0.05. **p<0.01; Student's t test. Nuclei were counterstained with Dapi. Scale bars: 50μm.



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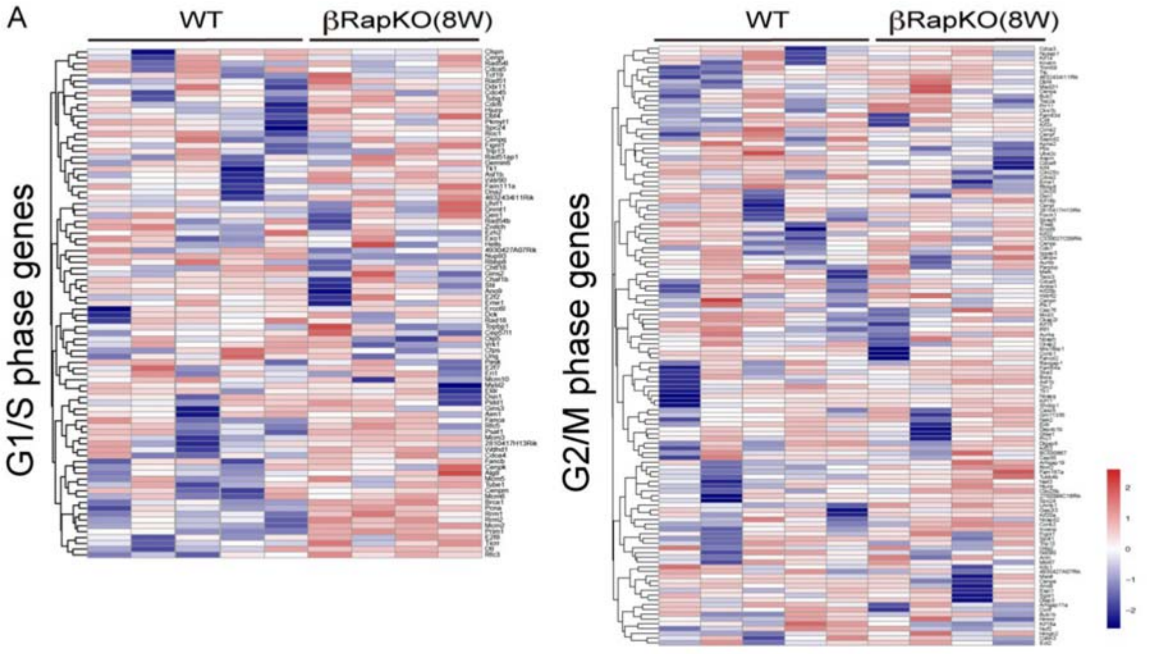
Supplementary Figure 3. Heatmap of cell cycle genes in 8-week-old WT and βRapKO. (A) Heatmap of the genes involved in different cell cycle stages between 8-week-old WT and β*Rap*KO.



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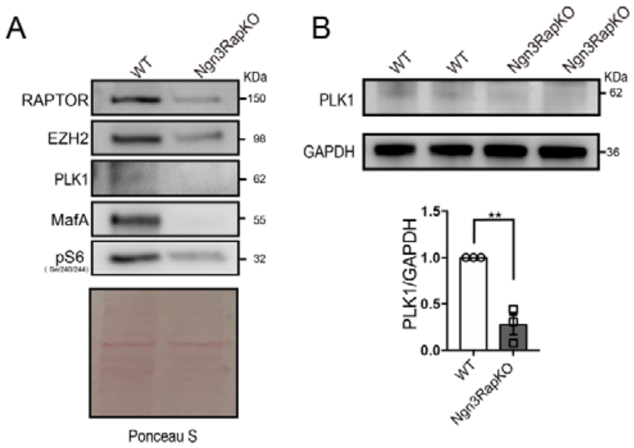
Supplementary Figure 4. Western Blot validated reduced expression of critical genes in P14 Ngn3*Rap*KO islets. (A) Relative RAPTOR/EZH2/PLK1/MafA/pS6 protein expression in P14 Ngn3*Rap*KO islets were assayed by immunoblot. (B) Relative PLK1 protein expression in P14 Ngn3*Rap*KO islets were assayed by immunoblot. PLK1 protein were normalized based on the corresponding GAPDH intensity (n=3).



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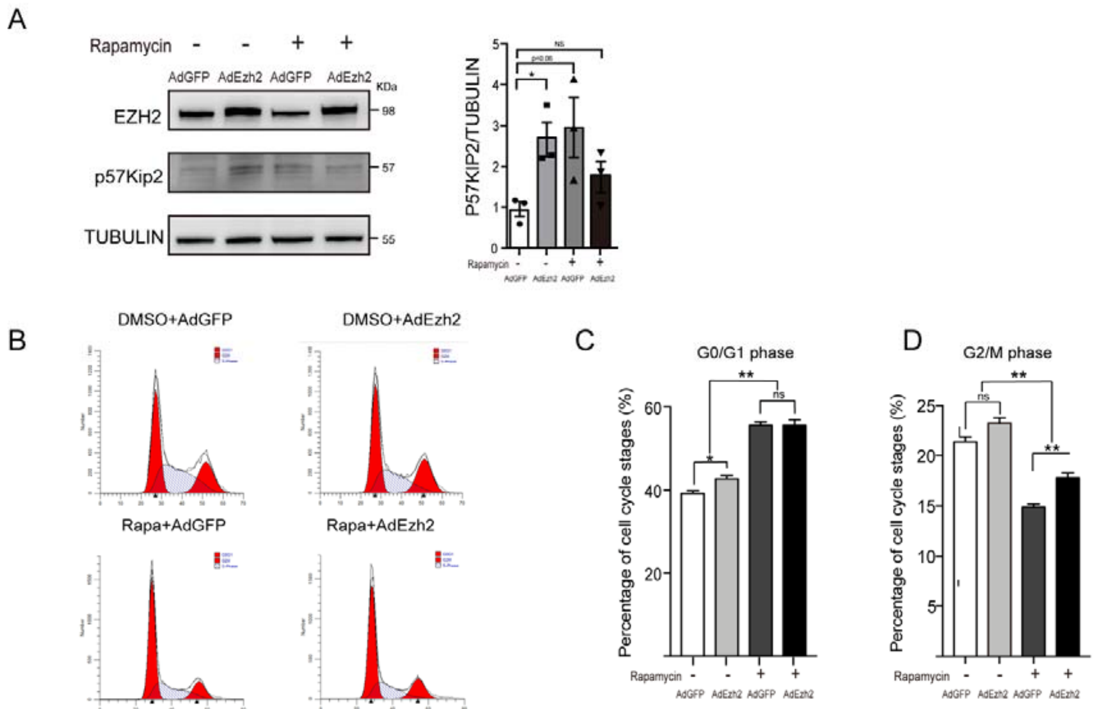
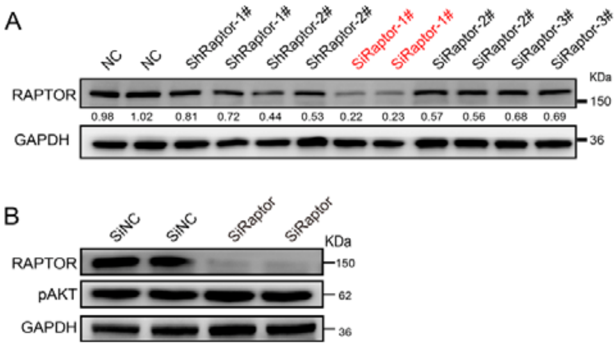
Supplementary Figure 5. Overexpression of Ezh2 reversed mTORC1-inhibition mediated proliferation inhibition. (A-D) MIN6 cells were infected with AdGFP or AdEzh2, in the present or absence of rapamycin: (A) Representative immunoblot pictures of EZH2 and P57KIP2 in MIN6 cells infected with the indicated adenoviruses for 48h were shown. Band intensities of P57KIP2 protein were normalized based on the corresponding TUBULIN intensity (n=3). (B) Representative flow cytometry images of cell cycle distribution. (C, D) The percentage of cells in G0/G1 (C) or G2/M (D) phases related to the total cell population were quantified (n=4). Data were presented as mean ± SEM of independent experiment indicated as above, **p*＜0.05, ***p*＜0.01; *p* values were obtained using a two-way ANOVA.



SUPPLEMENTARY DATA

Supplementary Figure 6. Raptor silence did not lead to compensatory activation of mTORC2. (A) MIN6 cells were transfected with Raptor SiRNA or ShRNA or control for 72h. (A) The knockdown effects of different sequences were verified at protein levels and SiRaptor-1# sequence was chosen for further experiments. (B) MIN6 cells were transfected with SiRaptor-1# or control for 72h. RAPTOR and pAKT473 protein abundance were assayed by immunoblot.



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Supplementary Table 1. Antibodies used in this article.

Antibodies	Source	
Guinea pig anti-Insulin	Dako	CAT# A0564
Mouse anti-Glucagon	Abcam	CAT#ab10988
Rabbit anti-Somatostatin	Millipores	CAT# AB5494
Rabbit anti-Pancreatic polypepetide	Millipores	CAT# AB939
Rabbit anti-Synaptophysin	LsBio	CAT#LS-C174787
Rabbit anti-Ki67	Bethyl	CAT#IHC00375
Mouse anti-Ki-67	BD	CAT# 556003
Mouse anti-NKX2.2	DSHB	CAT# 74.5A5-C
Mouse anti-MafA	Bethyl	CAT# IHC00352
Rabbit anti-Arx	A kind gift from Dr. Kunio Kitamura	
Rabbit anti-Plk1	Cell Signaling Technology	CAT#4513S
Mouse anti-Ngn3	DSHB	CAT# F25A1R3
Rabbit anti-cyclin D1	Cell Signaling Technology	CAT#2978S
Rabbit anti-cyclin D2	Cell Signaling Technology	CAT#3741S
Rabbit anti-Ezh2	Cell Signaling Technology	CAT#5246S
Rabbit anti-p57Kip2	Cell Signaling Technology	CAT#2557S
DAPI Fluoromount-G	SouthernBiotech	CAT#0100-20
Mouse anti-alpha-tubulin	Abcam	CAT# ab7291
Rabbit anti-pS6 ribosomal protein (240/244)	Cell Signaling Technology	CAT# 9205S
Alexa Fluor® 488 AffiniPure Donkey Anti-Guinea Pig IgG (H+L)	Jackson Immunoresearch	CAT# 706-545-148; RRID:AB_234472
Alexa Fluor® 647 AffiniPure Donkey Anti-Guinea PigIgG (H+L)	Jackson Immunoresearch	CAT# 706-605-148; RRID:AB_2340476
Alexa Fluor® 488 AffiniPure Donkey Anti-Rabbit IgG (H+L)	Jackson Immunoresearch	CAT# 711-545-152; RRID:AB_2313584
Alexa Fluor® 594 AffiniPure Goat Anti-Rabbit IgG (H+L)	Jackson Immunoresearch	CAT# 111-585-003
Alexa Fluor 594 AffiniPure Donkey Anti-Mouse	Jackson Immunoresearch	CAT# 715-585-150; RRID:AB_2340584

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Supplementary Table 2. RT-PCR primer sequences of target genes.

Gene	**Species**	**Forward**	**Reverse**
Ngn3-Cre	Mouse	CGATGCAACGAGTGATGAGG	GCATTGCTGTCACTTGGTCGT
Raptor	Mouse	CTCAGTAGTGGTATGTGCTCAG	GGGTACAGTATGTCAGCACAG
Ki67	Mouse	AGCTTCTGTGCTGACCCTGATG	TGCAGAAAGGCCCTTGGCATAC
Foxm1	Mouse	CACTTGGATTGGGACCACTT	GTCGTTTCTGCTGTGATTCC
Cyclin A2	Mouse	CTTGGCTGCACCAACAGTAA	CAAACTCAGTTCTCCCAAAAACA
Cyclin B1	Mouse	TCTTGACAACGGTGAATGGA	TCTTAGCCAGGTGCTGCATA
Cyclin D1	Mouse	CTGACACCAATCTCCTCAACGAC	GCGGCCAGCTTCCACTTAGC
Cyclin D2	Mouse	TCCACTTCAGCTTACCCAACA	CACCGACAACTCTGTGAAGC
Cdk1	Mouse	CATGGACCTCAAGAAGTACCTGG	CAAGTCTCTGTGAAGAACTCGCC
Cdk2	Mouse	CTGCCATTCTCACCGTGTCC	AGCTTGATGGACCCCTCTGC
Cdk4	Mouse	GAGGAATCTGGAGCGCAGTT	CTCTCCTCACTCCGGGTCAC
Mcm3	Mouse	TTCTCTGCGGACGATATAG	GGATTGCCTTCTTGACATAG
Mcm4	Mouse	TTGAACGTTATCCTGACTC	GCTGTGATGTTCTGATGAC
Mcm7	Mouse	ATCTCTGGCTGACTACATC	AGAAGTATAGGTGGCATCC
Ezh2	Mouse	GACAAATACATGTGCAGCTTTCTGT	GCCCTTTCGGGTTGCAT
p57Kip2	Mouse	ATGTAGCAGGAACCGGAGATGGTT	ATGTAGCAGGAACCGGAGATGGTT
Plk1	Mouse	CCATCTTCTGGGTCAGCAAGTG	CCGTCATTGTAGAGAATCAGGCG
MafA	Mouse	GCTTCAGCAAGGAGGAGGTCAT	TCTCGCTCTCCAGAATGTGCCG
Arx	Mouse	CTGCTGAAGCGCAAACAGAGGC	CTCTGTCAGGTCCAGCCTCATG
Nkx2.2	Mouse	AGAGCCCTTTCTACGACAGCAG	GGATTTGGAGCTCGAGTCTTGG

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Supplementary Table 3. Differentially expressed G1/S and G2/M phase genes in P14 Ngn3*Rap*KO islets. (Related to Figure 5C)