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Supplemental information

Deletion of pancreas-specific miR-216a reduces beta-cell mass and inhibits pancreatic cancer progression in mice

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Figure S1

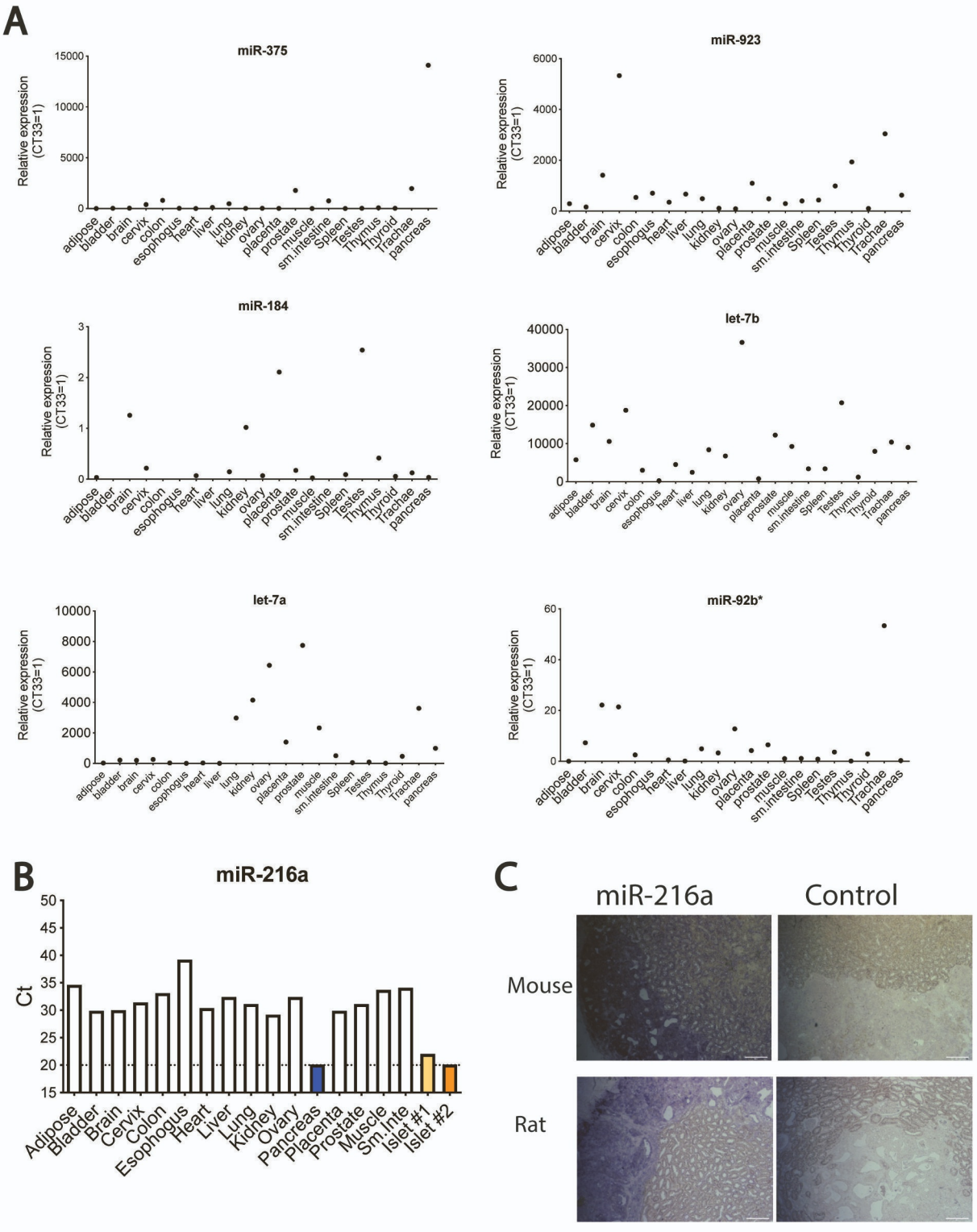

Figure S1. miR-216a is expressed in the endocrine grafts. (A) Expression of the indicated miRNAs in various human tissues. Equal amounts of RNA from human tissues (each a pool of 3 tissue donors) was reverse-transcribed and expression of indicated miRNAs was determined by qRT-PCR. Threshold cycle 33 (Ct = 33) was arbitrarily set as 1. **(B)** qRT-PCR analysis from human tissues as in Figure 1B with added human islets. Ct values are shown. **(C)** miR-216a is expressed in pancreatic tissue differentiated from hESCs. Representative in situ hydridization images of differentiated hESC-derived grafts at 22 weeks post implant in a mouse and a rat. Grafts harvested from mice and rats were probed with DIG-labeled miR-216a and scrambled miRNA control probes. Scale bars = 100 μm. Related to Figure 1.

Figure S2

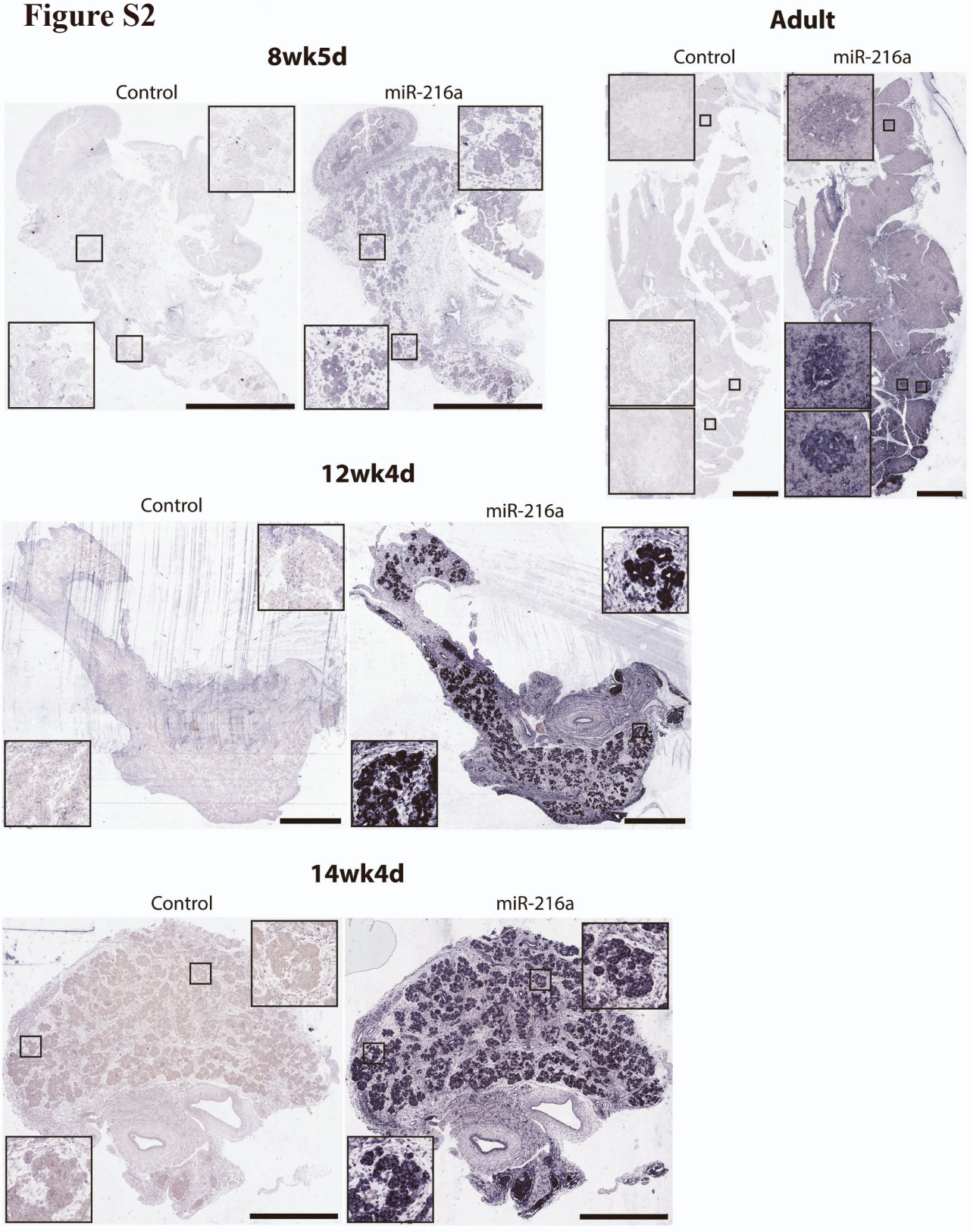

Figure S2. miR-216a expression in human fetal pancreas. Whole fetal pancreatic sections are shown. Adult human pancreata were probed with DIG-labeled miR-216a and scrambled control miRNA probes at the indicated gestational weeks. Purple color indicates presence of miRNA expression. Insets are enlarged 20x. Scale bar = 1 mm. Related to Figure 1.

Figure S3

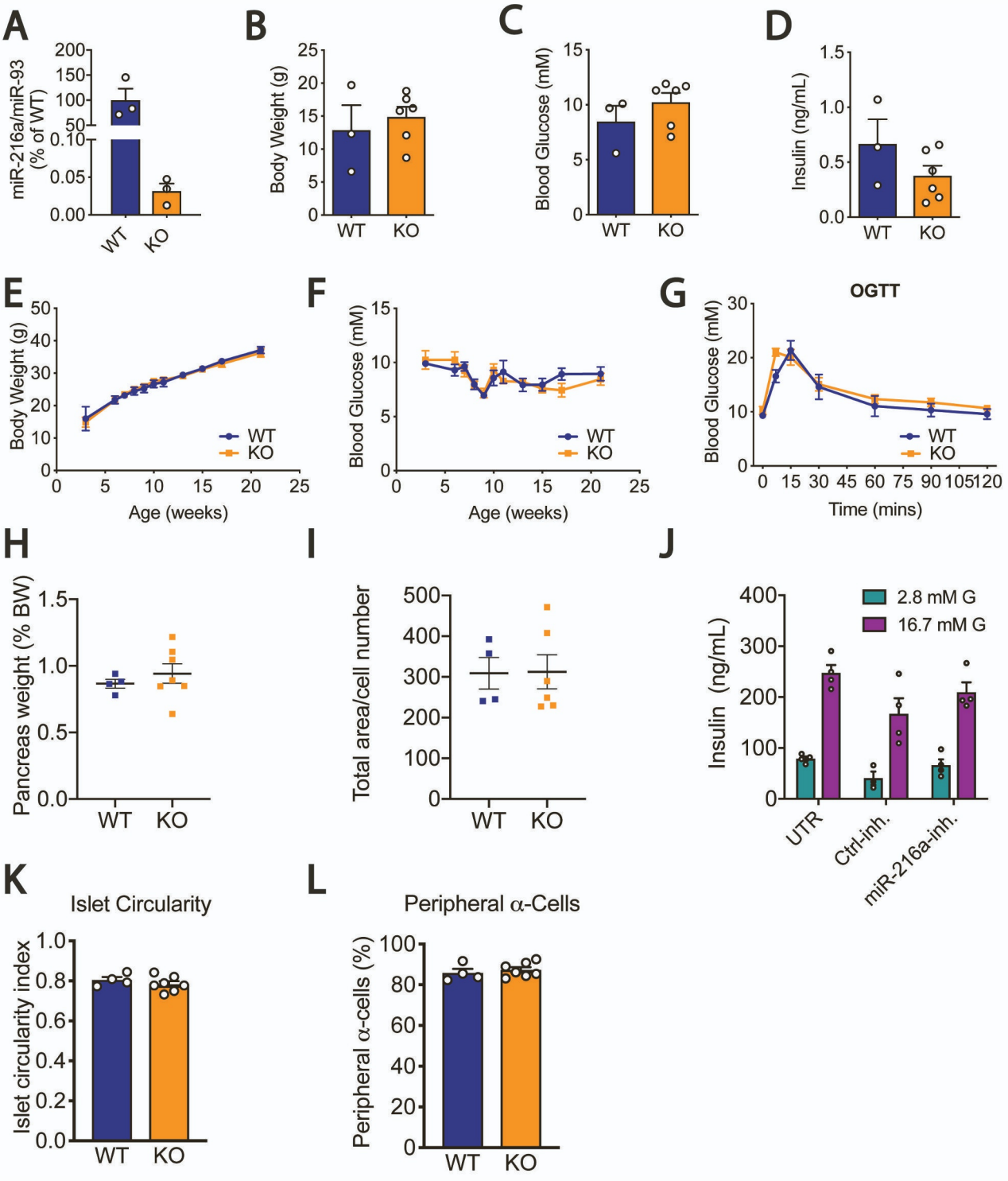

Figure S3. miR-216a KO mice do not display alterations in glucose homeostasis. (A) Islets from 10-week old male WT and miR-216a KO mice were isolated and miRNA expression was quantified with qRT-PCR. n = 3 mice. **(B-D)** Body weight **(B)**, fasting blood glucose **(C)**, and insulin measurements **(D)** from 3-4 week old male mice. n = 3-6 mice. **(E-G)** WT and miR-216a KO male mice on regular chow diet were monitored for body weight **(E)** and blood glucose **(F)** for 21 weeks. n = 4-7 mice. Oral glucose tolerance test (OGTT) performed in 10-week old male mice, with measurement of blood glucose levels **(G)**. **(H)** Pancreata from 21-week old WT and miR-216a KO mice were weighed and normalized to body weight. **(I)** Pancreatic cell size was assessed by analyzing dapi staining from the pancreata of 21-week old male mice. **(J)** INS-1E cells were transfected with the indicated inhibitors and 48 hours post-transfection cells were incubated serially with 2.8 mM and 16.7 mM glucose containing media. Insulin secretion was assessed by insulin ELISA. n=4 mice. **(K)** Pancreata from adult male mice (n=4-7 mice) were immunostained for synaptophysin, insulin and glucagon, and islet circularity, peripheral α-cell percentage were calculated. Individual data points are shown in (A-D, H-L). Data represent mean ± SEM. Related to Figure 2.

Figure S4

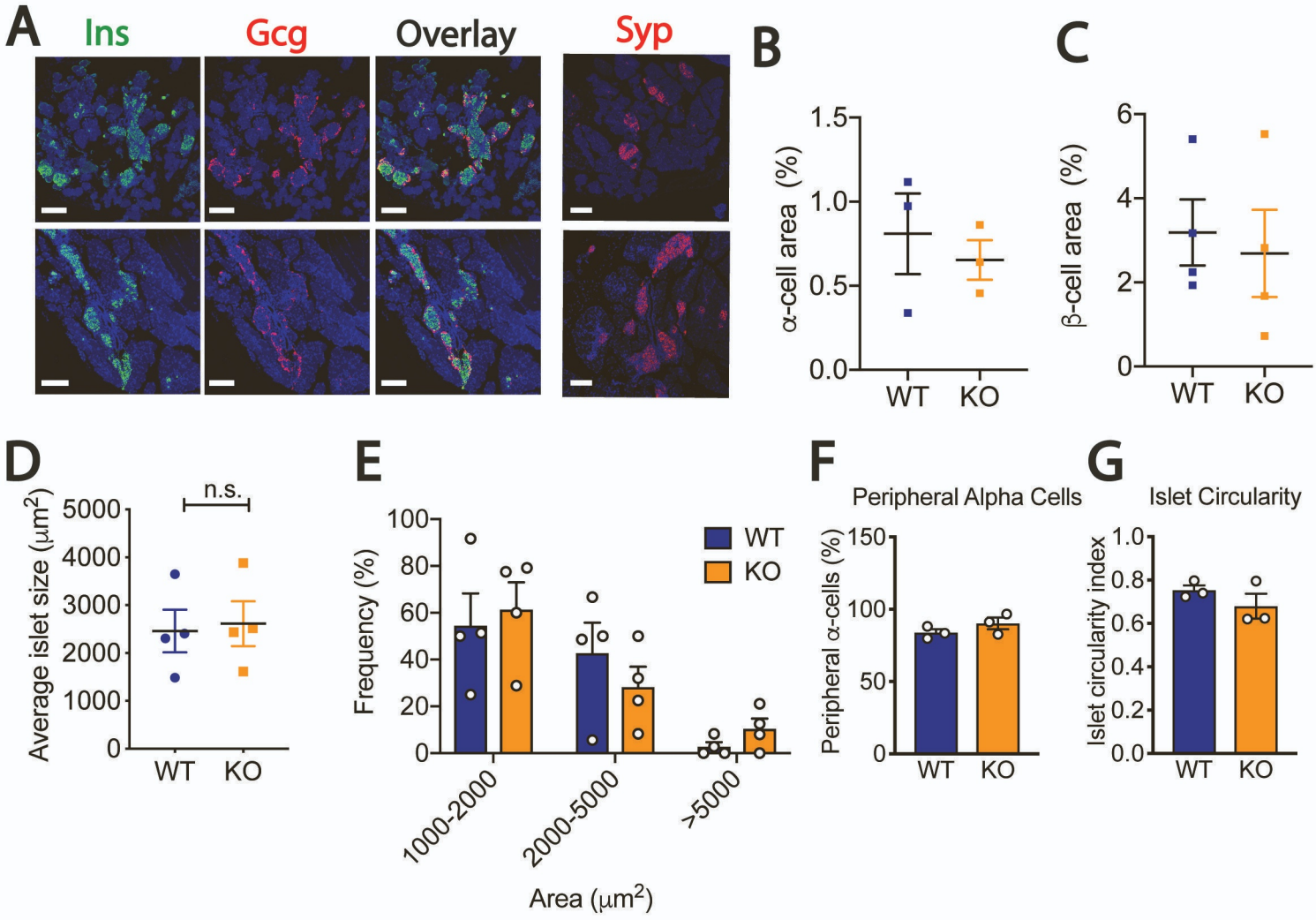

Figure S4. β-cell mass and islet size mass is unchanged in one-day old miR-216a KO mice. **(A)** Pancreata from one-day old male neonatal mice were immunostained for synaptophysin, insulin and glucagon. Nuclei were identified with dapi (blue). Scale bar = 100 μm. **(B)** α-cell area, **(C)** β-cell area, **(D)** average islet size, and **(E)** islet size distribution, **(F)** peripheral α-cells **(G)** islet circularity were calculated. Individual data points are shown. n=3-4 mice. Data represent mean ± SEM. A two-tailed Student`s t-test was performed to assess significance. n.s. not significant. Related to Figure 2.

Figure S5

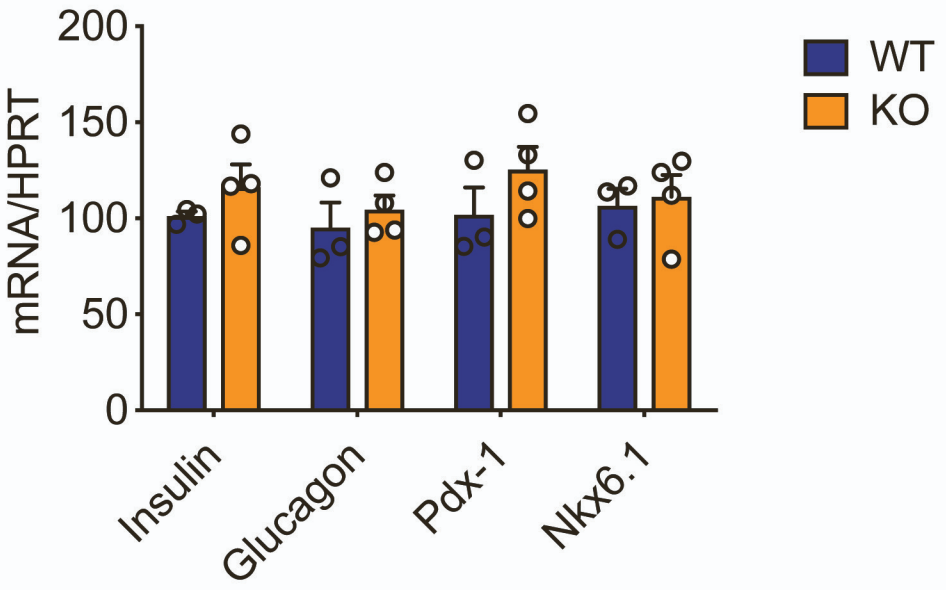

Figure S5. Gene expression analysis using islets isolated from 10-week old male WT and miR-216a KO mice. RNA was isolated, reverse-transcribed and expression of the indicated genes was determined by qRT-PCR. WT levels arbitrarily set as 100. n = 3-4 mice. Individual data points are shown. Data represent mean ± SEM. Related to Figure 3.

Figure S6

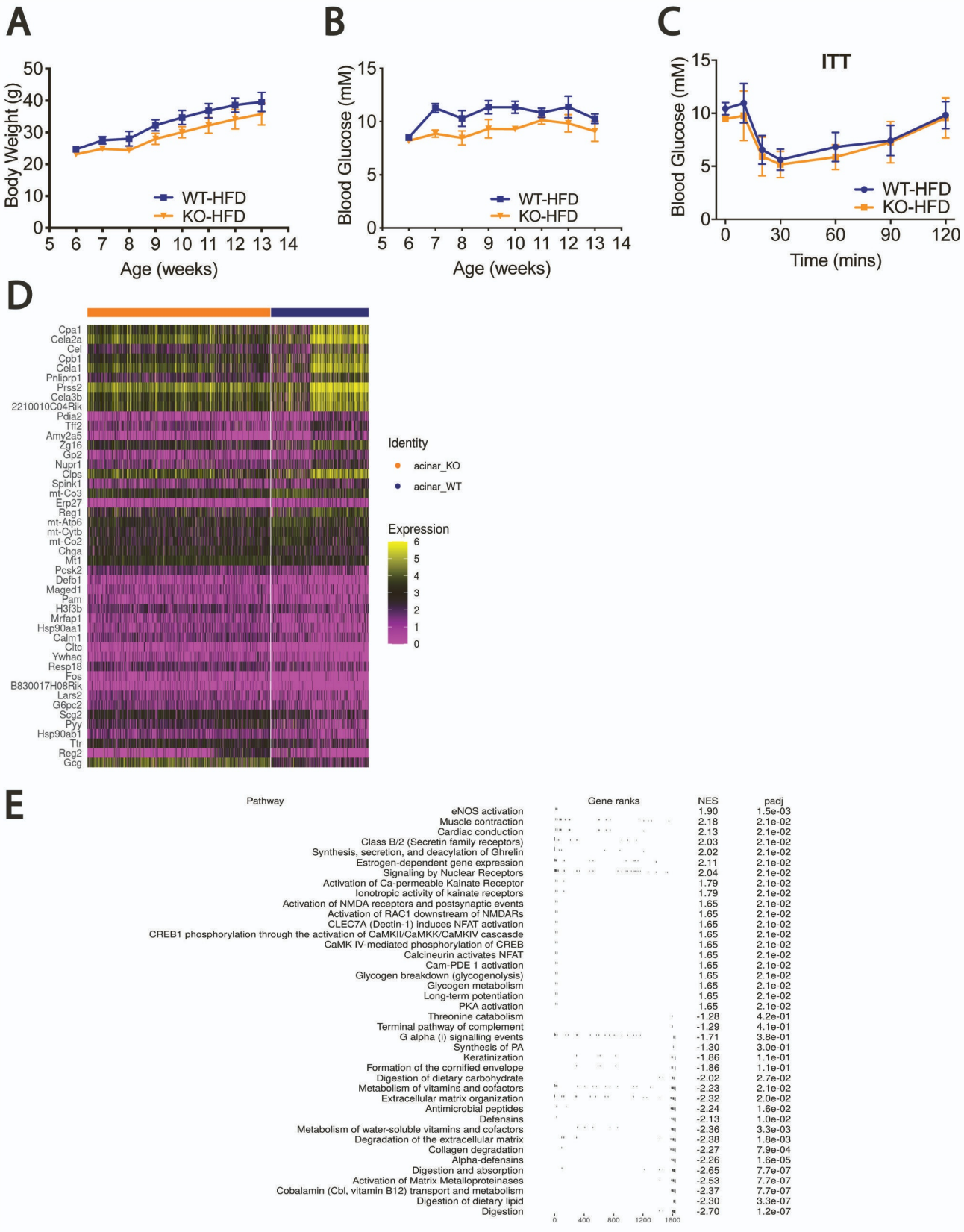

Figure S6. Insulin tolerance test during high-fat-diet (HFD) feeding. WT and miR-216a KO male mice were fed with a 60% HFD for 8-weeks and weekly **(A)** body weight **(B)** and fasted blood glucose levels were measured. **(C)** Blood glucose levels during insulin tolerance tests (ITT) performed 8 weeks post HFD. Data represent mean ± SEM. **(D)** Single-cell RNA-seq was carried out on mouse pancreatic islets. A heatmap showing normalized gene expression for the statistically significantly up- or downregulated genes within the acinar cell cluster comparing between genotypes, further analyzed in **(E)** showing gene set enrichment analysis on all differentially expressed genes. Related to Figure 4.

Figure S7

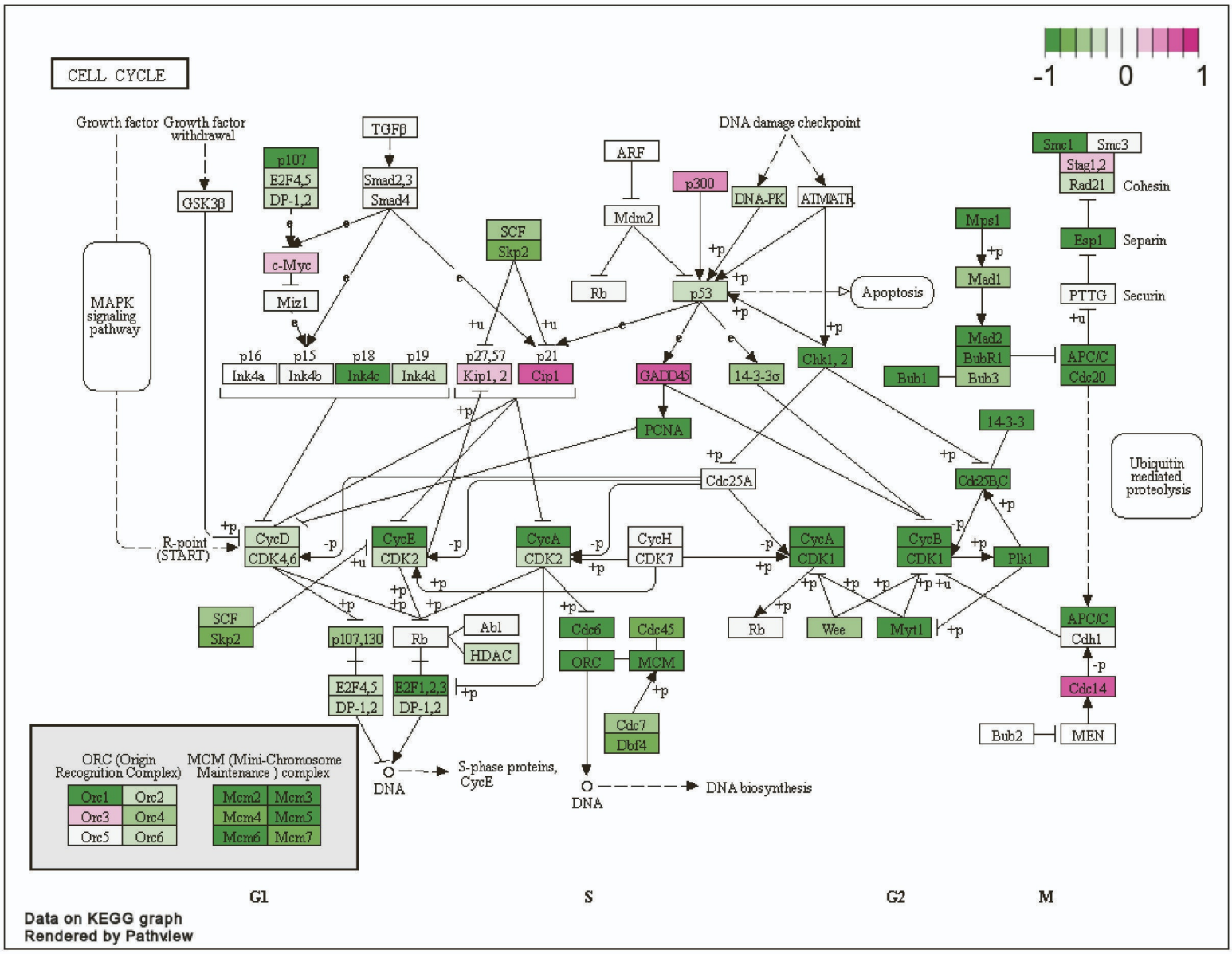

Figure S7. A cell cycle pathway map. Genes significantly upregulated in miR-216a KO mice compared to WT mice are shown in purple, and genes significantly downregulated in the KO mice compared to WT mice are shown in green. Cell cycle is a significantly enriched GO "Biological Process" and KEGG term, with statistical significance defined as a q value < 0.1 (therefore allowing a 10% FDR). Related to Figure 5.

Table S1

Mouse #	Genotype	Body Weight (g)	Blood Glucose (mM)	Cell Count (cells/µL)	Viability (%)
1	KO	39.3	12.7	627	84
2	WT	37.3	11.8	636	80
3	KO	39.1	11.4	473	75
4	WT	42.5	14.5	704	75

Table S1: Characteristics of Donors and Cell Preparations for scRNAseq. Related to Figure 4.

Table S2

Cell Type	Canonical Gene(s)
Alpha	Gcg
Beta	Ins1, Ins2, and Iapp
Delta	Sst
Epsilon	Ghrl
PPY	Ppy
Ductal	Krt19
Acinar	Cpa1
Endothelial	Pecam1
Macrophage	Cd68
Stellate	Rgs5 or Pdgfrb

Table S2: Canonical Genes Used to Identify Cell Clusters. Related to Figure 4.

Table S6

Subject #	Control		T2D		CP		PDAC		mPDAC	
	Age	Gender	Age	Gender	Age	Gender	Age	Gender	Age	Gender
1	66	female	51	female	40	female	56	male	67	male
2	64	female	73	female	86	male	54	male	73	female
3	70	female	44	male	49	male	52	female	69	male
4	75	female	20	female	29	female	47	female	51	male
5	57	male	35	male	54	male	44	male	76	female
6	41	female	46	female	61	female	52	female	58	male
7	51	male	34	female	78	female	70	male	71	male
8	66	female	42	male	55	female	45	female	64	male
9	68	female	31	female	51	male	71	female	70	female
10	57	female					58	male	67	female
11	21	female					63	male	76	male
12	65	female					74	male	59	male
13	64	female					76	female	64	male
14	28	male					62	male	71	male
15	36	female					68	female	74	female
16	48	female					65	male	63	male
17	44	female					71	male	78	male
18	43	male					74	female	65	male
19	30	female					69	male	67	female
20	46	male					58	male		

Table S6: Age and gender of the donors. Related to Figure 6.