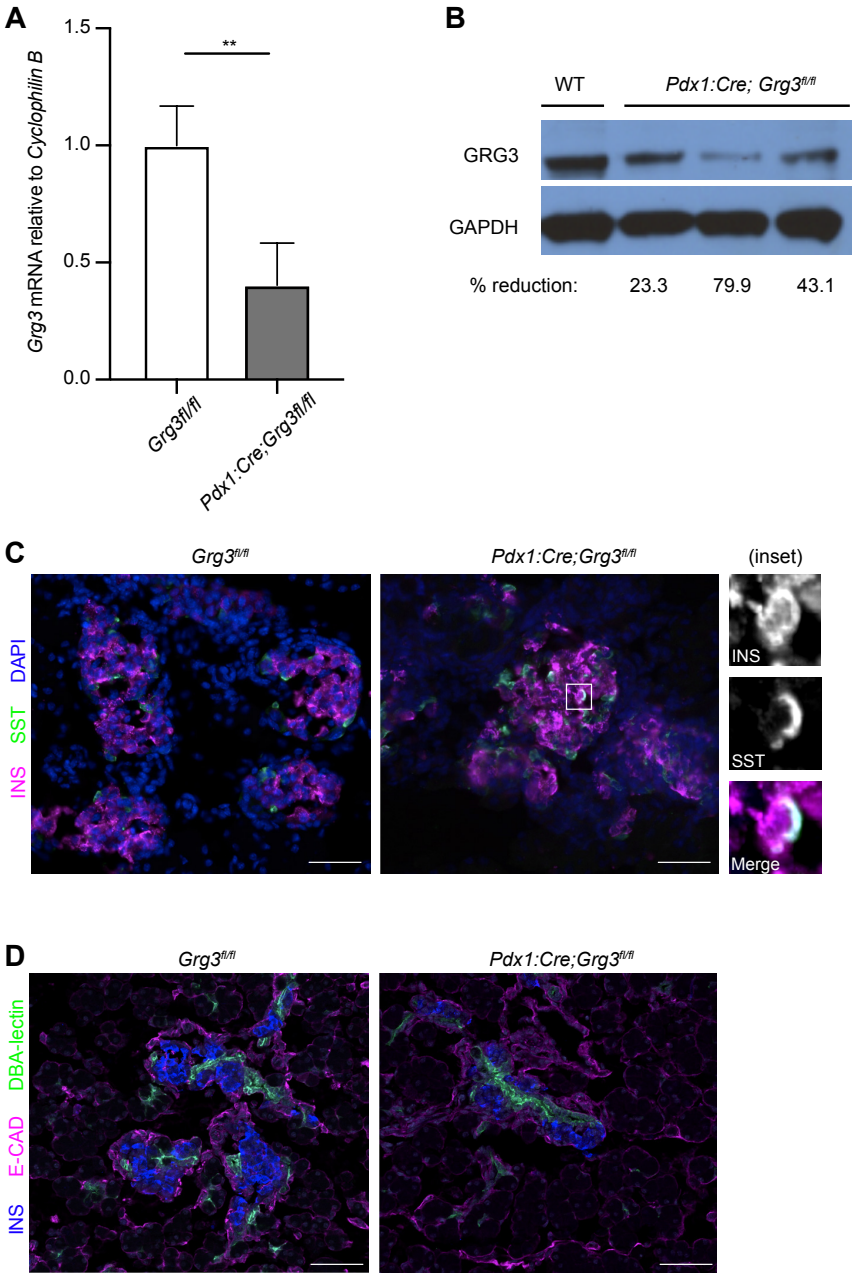


Figure S1. Validation of pancreas-specific *Grg3* knockout.

(A) *Grg3* mRNA levels are decreased in 8 week isolated islets from *Pdx1:Cre; Grg3*$^{fl/fl}$ mutants as measured by qPCR. n = 3 for each genotype. **P≤0.01; two-tailed student's t-test. (B) Western blot of protein from P0 pancreata showing reduction of GRG3 protein in *Pdx1:Cre; Grg3*$^{fl/fl}$ animals. (C) Immunofluorescent staining of 6 week *Pdx1:Cre; Grg3*$^{fl/fl}$

islets hormones. Inset shows example of bihormonal INS+/SST+ cell in *Pdx1:Cre; Grg3*$^{fl/fl}$ animal. Scale bars = 50 μm. (D) Immunofluorescent staining of E-cadherin and DBA-lectin of P0 *Grg3* single knockouts showing no visible defects in delamination of the endocrine population. Scale bars = 50 μm.

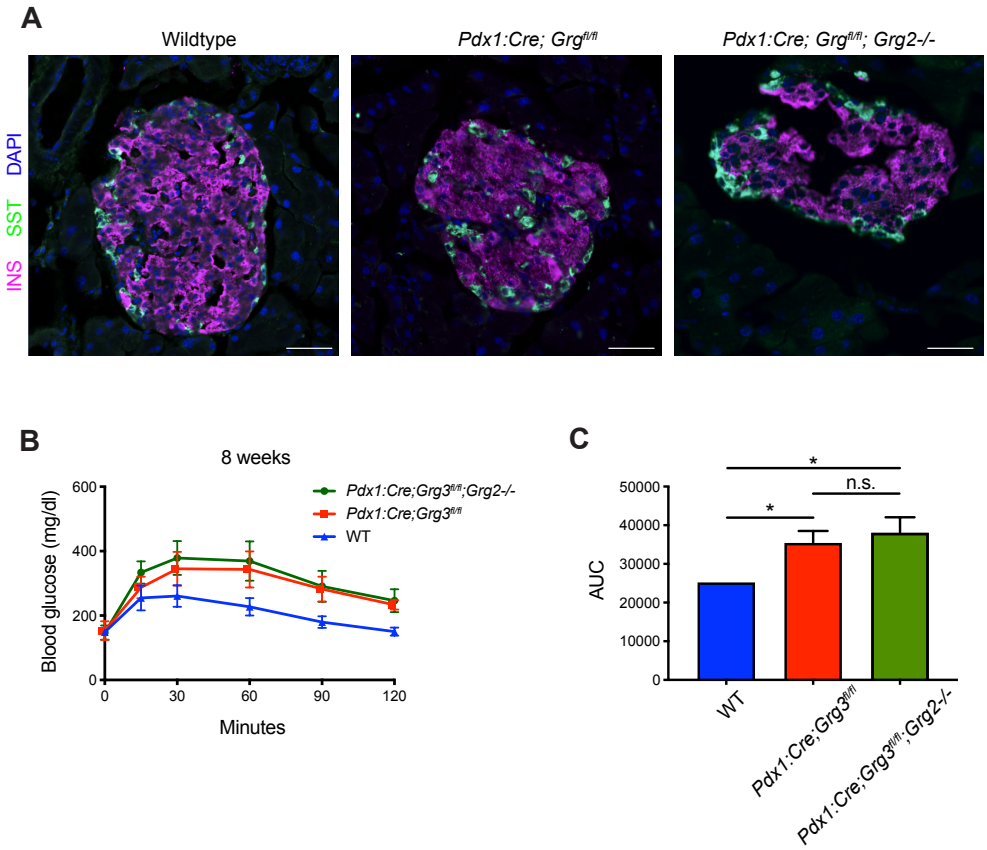


Figure S2. Loss of *Grg2* does not exacerbate *Grg3* knockout phenotype. (A) Immunofluorescent analysis of islets from 7 month control *Grg3* and *Grg2/3* knockout mice showing no visible worsening of the *Grg3* islet phenotype with additional loss of *Grg2*. Scale bars = 50 μm. (B) Glucose tolerance tests of 8 week *Grg3* and *Grg2/3* mutants. (C) AUC calculation of (B) showing no difference between *Grg3* and *Grg2/3* mutants. n = 4-6 for all genotypes. *P≤0.05; two-tailed student's t-test.

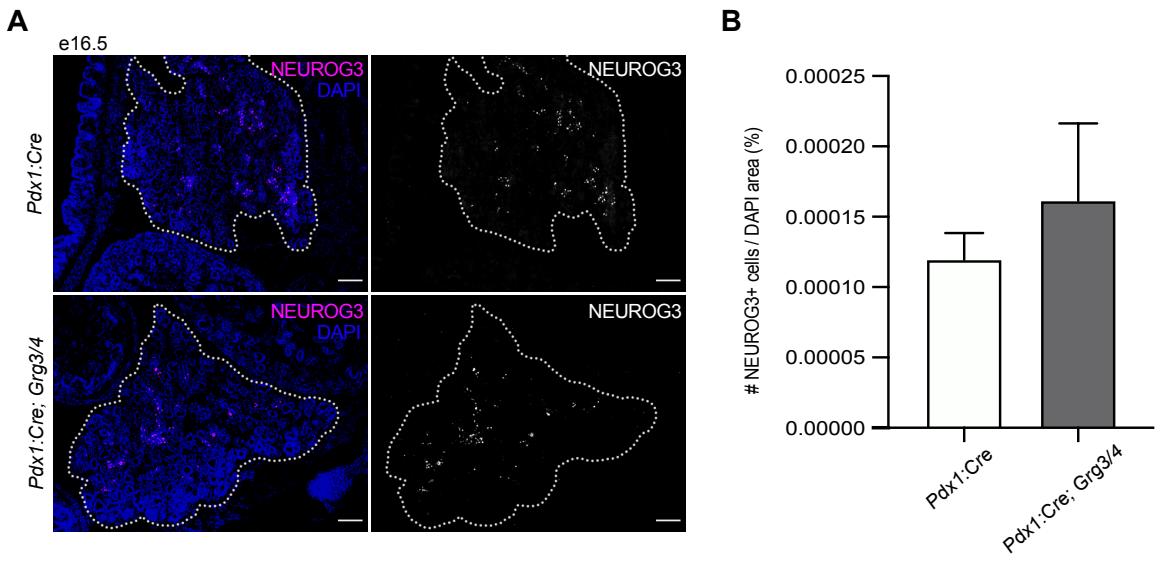
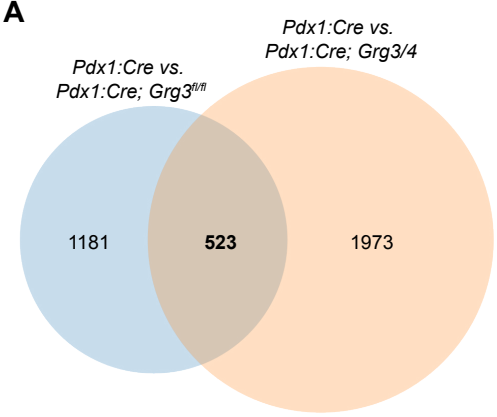


Figure S3. NEUROG3+ endocrine progenitors are unchanged in e16.5 *Pdx1:Cre; Grg3/4* mice.

(A) Immunofluorescent staining of e16.5 embryos for endocrine progenitor (NEUROG3+) population. Scale bars = 50 μm. (B) Quantification of NEUROG3+ cells in (A). n = 3 for each genotype.

A



B

Downregulated genes from Fig 5C

	Pdx1:Cre vs. Pdx1:Cre; Grg3$^{fl/fl}$		*Pdx1:Cre vs. Pdx1:Cre; Grg3/4*	
Gene	**log2FC2**	**padj**	**log2FC**	**padj**
Npy	-0.93	0.1188	-2.56	1.62E-12
Ins2	-0.58	0.0216	-1.52	0.0015
Cpa1	-0.58	0.0692	-0.88	1.77E-21
Neurod1	-0.52	0.0165	-0.88	0.0027
Ins1	-0.47	0.1351	-1.43	0.0081
Mafa	-0.43	0.2477	-1.35	2.75E-14
Foxa2	-0.25	0.2985	-0.57	0.0009
Pdx1	-0.24	0.1980	-0.6	0.0002
Pax6	-0.18	0.5613	-0.52	0.0446
Rbpj	-0.08	0.8964	-1.09	1.53E-07
Pyy	0.25	0.3166	-0.71	3.27E-05
Ppy	0.30	0.3904	-0.55	0.0283

C

Upregulated genes from Fig 5D

	Pdx1:Cre vs. Pdx1:Cre; Grg3$^{fl/fl}$		*Pdx1:Cre vs. Pdx1:Cre; Grg3/4*	
Gene	**log2FC2**	**padj**	**log2FC**	**padj**
Fga	10.19	8.87E-08	10.44	3.19E-17
Fgg	9.38	8.91E-07	9.55	6.13E-15
Apoh	9.35	3.10E-05	9.5	4.09E-11
Foxa1	8.81	1.32E-28	8.77	2.11E-29
Hc	6.00	0.4511	8.42	7.31E-08
Bhmt	8.49	0.0049	8.25	0.0023
F11	7.26	0.3179	8.05	4.45E-07
Kng1	7.68	1.39E-06	7.98	2.84E-22
Ahsg	7.68	0.0081	7.83	0.0012
Kng2	5.71	0.0016	7.77	0.0003
Hsd3b3	5.73	0.4813	7.71	6.40E-06

Figure S4. Comparison of differentially expressed genes in *Pdx1:Cre; Grg3$^{fl/fl}$* vs. *Pdx1:Cre; Grg3/4* e18.5 pancreata.

(A) Venn diagram showing *Pdx1:Cre; Grg3/4* e18.5 pancreata have more differentially expressed genes than *Pdx1:Cre; Grg3$^{fl/fl}$* when compared to *Pdx1:Cre* controls. 523 genes are differentially expressed in both groups. (B) Select list of downregulated genes related to pancreas development and hormone expression from Fig. 5C including *Pdx1:Cre* vs *Pdx1:Cre; Grg3$^{fl/fl}$* comparison. Gray text indicates padj > 0.05 in the *Pdx1:Cre; Grg3$^{fl/fl}$* comparison. (C) Top 10 upregulated genes sorted by fold change including *Pdx1:Cre* vs *Pdx1:Cre; Grg3$^{fl/fl}$* comparison. Gray text indicates padj > 0.05 in the *Pdx1:Cre; Grg3$^{fl/fl}$* comparison.

A

	Gene	log2FC	padj
Acinar	*Cel*	-0.77	7.88E-15
	Cela1	-0.69	7.45E-11
	Cela2a	-0.43	0.0005
	Cela3b	-0.33	0.0120
	Cpa1	-0.88	1.77E-21
	Cpa2	-0.47	5.10E-06
	Cpb1	-0.94	3.61E-13
	Ctrb1	-0.51	5.53E-06
	Ctrc	*-0.05*	*0.9143*
	Ctrl	-2.69	1.01E-141
	Klk1	-1.23	0.0039
	Klk1b3	*-0.08*	*0.8825*
	Klk1b4	*-0.51*	*0.4180*
	Klk1b5	-1.04	2.35E-05
	Pnlip	*-0.14*	*0.8879*
	Pnliprp1	-0.44	7.37E-05
	Pnliprp2	-1.25	5.29E-33
	Prss1	-3.03	1.33E-103
	Prss2	*-0.22*	*0.2787*
	Prss3	-2.44	8.86E-104
	Rnase1	-1.05	3.10E-25
	Serpinb1a	-0.47	9.92E-05
	Serpini2	-1.08	5.28E-10
	Try10	-0.82	5.31E-14
	Try4	-1.16	4.28E-54
	Try5	-0.43	0.0317
Duct	*Muc1*	-0.46	6.56E-06
	Ptf1a	0.02	0.9292
	Hnf1b	0.43	0.4888
	Sox9	-0.20	0.4124
	Krt19	-0.07	0.8622

B

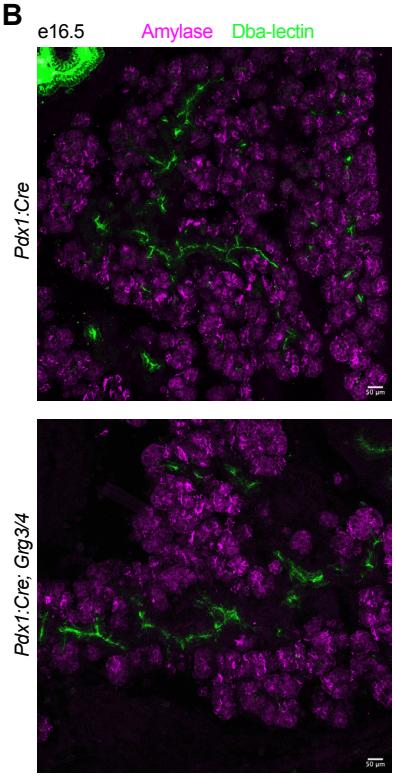


Figure S5. Overall pancreas morphology appears unaffected by loss of *Grg3/4*.

(A) List of acinar and duct genes from e18.5 RNA-seq analysis of *Pdx1:Cre* vs *Pdx1:Cre; Grg3/4* pancreata. Gray text indicates padj > 0.05 in the *Pdx1:Cre; Grg3*$^{fl/fl}$ comparison. (B) Immunofluorescent analysis of acinar (amylase) and duct (Dba-lectin) tissue e16.5 pancreata from control and *Pdx1:Cre; Grg3/4* mice showing no visual differences between genotypes. Scale bars = 100 μm.

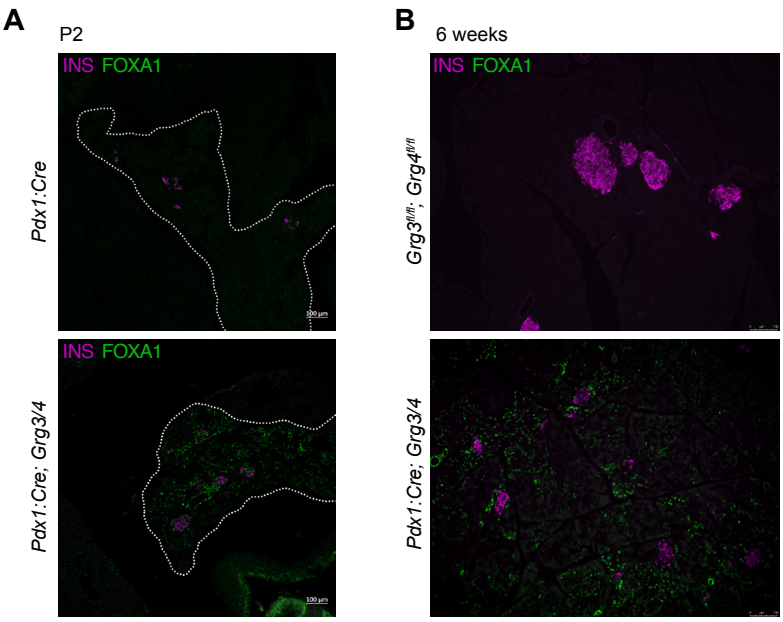


Figure S6. FOXA1 protein is maintained through 6 weeks of age in *Pdx1:Cre; Grg3/4* mutants.

(A) Immunofluorescent staining of P2 pancreata for INS and FOXA1 showing ectopic expression of FOXA1 in the pancreas of *Pdx1:Cre; Grg3/4* mutants. Scale bars = 100 μm. (B) Immunofluorescent staining of 6 week pancreata as in (A), showing ectopic FOXA1 protein maintained through 6 weeks of age. Scale bars = 100 μm.

Table S1. Differentially expressed *Foxa1* targets in e18.5 *Pdx1:Cre; Grg3/4* mutant pancreata.

Click here to Download Table S1

Table S2. Primers used for mouse genotyping.

Primer name	Sequence
Pdx1Cre_FWD	5'-CTG GAC TAC ATC TTG AGT TGC-3'
Pdx1Cre_REV	5'-GGT GTA CGG TCA GTA AAT TTG-3'
GenCre_FWD	5' CTG CCA CGA CCA AGT GAC AGC 3'
GenCre_REV	5' CTT CTC TAC ACC TGC GGT GCT 3'
Grg2WT_FWD	5' GGG ATT CTA GGA TTC TAG GCA GGG C 3'
Grg2WT_REV	5' TTG AGG CAT GGT CTT GCT TTG TAG C 3'
Grg2KO_FWD	5' GCA GCC TCT GTT CCA CAT ACA CTT CA 3'
Grg2KO_REV	5' AGA GCC AGG AAG ATG GTT CAG TTG G 3'
Grg3flox_FWD	5' GCT CCC TTC TTC AGC TTC CT 3'
Grg3flox_REV	5' GCT CCA AGA GGG ATT TTT AT 3'
Grg4flox_FWD	5' AGA AAT GCA GCC CAG AGT AA 3'
Grg4flox_REV	5' GGA GAC TTG GAA AAC GCT GA 3'

Table S3. Antibodies used for immunofluorescence and western blotting.

Target	Company/Source	Catalog #	Host species	Concentration
Alpha1 antitrypsin (AAT)	Thermo Fisher	PA5-16661	Rabbit	1:250
DAPI	Thermo Fisher	D1306	NA	1:1000
DBA-lectin, fluorescein	Vector	FL-1031	NA	1:100
E-cadherin	BD Biosciences	610182	Mouse	1:100
Foxa1	Sigma	HPA050505	Rabbit	1:200
GAPDH	Abcam	ab9485	Rabbit	1:1000 (WB)
Glucagon	Cell Signaling Technologies	2760S	Rabbit	1:500
Insulin	Dako/Agilent	IR00261-2	Guinea pig	1:1000
Ki67	Abcam	Ab15580	Rabbit	1:500
Neurod1	Sigma	HPA003278	Rabbit	1:200
Neurogenin3	From C. Wright	NA	Rabbit	1:200
Pdx1	Abcam	ab47308	Guinea pig	1:500
Somatostatin	Santa Cruz	sc-47706	Rat	1:500
TLE3 (Grg3)	Santa Cruz	sc-9124	Rabbit	1:100 (WB)
488 anti-guinea pig	Thermo Fisher/Invitrogen	A11073	Goat	1:500
555 anti-guinea pig	Thermo Fisher/Invitrogen	A21435	Goat	1:500
488 anti-rabbit	Thermo Fisher/Invitrogen	A21206	Donkey	1:500
594 anti-rabbit	Thermo Fisher/Invitrogen	A21207	Donkey	1:500
647 anti-rabbit	Thermo Fisher/Invitrogen	A21244	Goat	1:500
647 anti-rat	Thermo Fisher/Invitrogen	A21247	Goat	1:500

Table S4. Taqman probes used for RT-qPCR

Gene name	Company	Catalog number
Chga	ThermoFisher	Mm00514341_m1
Cyclophilin B	ThermoFisher	Mm00478295_m1
Ecad	ThermoFisher	Mm01247357_m1
Gcg	ThermoFisher	Mm00514341_m1
Grg2	ThermoFisher	Mm00498094_m1
Grg3	ThermoFisher	Mm00437097_m1
Grg4	ThermoFisher	Mm01195172_m1
Ins1	ThermoFisher	Mm00801712_m1
Ins2	ThermoFisher	Mm00801712_m1
PP	ThermoFisher	Mm00435889_m1
Sst	ThermoFisher	Mm00436671_m1