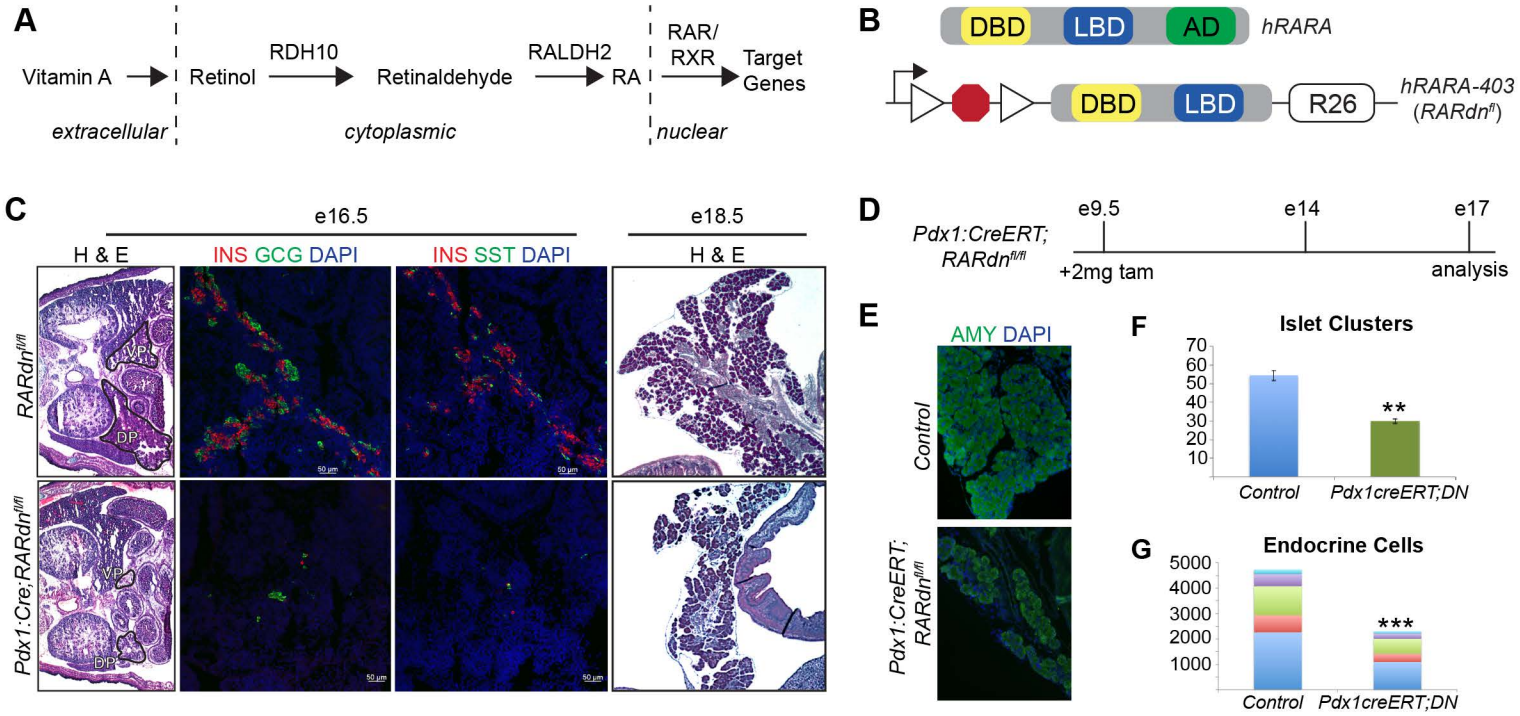

Figure S1. The *RAR* dominant negative efficiently inhibits RA signaling during pancreas development. (A) Summary of the retinoic acid signaling pathway. (B) Diagram of the *RAR* dominant negative (*RARdn*^{flox}), a truncated version human *RARA* gene lacking an activation domain and placed in the *Rosa26* locus. (C) Combined H & E and immunofluorescence analysis of *RARdn*$^{flox/flox}$*; Pdx1:cre* mice examining pancreas morphology and hormone+ endocrine cells at e16.5 and e18.5. DP is dorsal pancreas, VP is ventral pancreas in the H & E (n=3). (D) Summary of tamoxifen injection and analysis schedule of experiments described in S1E-G. (E) Immunofluorescence analysis of *RARdn*$^{flox/flox}$*;Pdx1:creERT* AMYLASE in the pancreas at e17.0. (F-G) Number of islet clusters and endocrine cells, respectively, in tamoxifen treated *RARdn*$^{flox/flox}$*;Pdx1:creERT* pancreata compared to either cre-alone or *RARdn*$^{flox/flox}$ alone controls at e17.0. In G, blue is INS, red is GCG, green is PPY, purple is SST, and light blue is GHRL (n=5).

Table S1 – Differentially expressed genes in e16.5 RA mutants Genes significantly changed in *RARdn*$^{flox/flox}$*; Neurog3:cre* mutants compared to *Neurog3:cre* only controls (padj < 0.05). To filter low reads, all genes with a raw read count of 0 in any one of columns H-M are not reported. DESeq2 was used to generate differentially expressed genes.

Click here to Download Table S1

Table S2 – Binding site identification within significantly differentially expressed genes at e16.5 Putative RARα binding sites within 500bp of the transcriptional start site of each of the genes from Table S1.

Click here to Download Table S2

Table S3 – Antibodies and Probes

Antibody	SOURCE	IDENTIFIER
DAPI (4',6-diamidino-2-phenylindole) (1:1000)	Thermo Fisher	Cat# D1306 RRID:AB_2629482
Rabbit anti-Glucagon (1:250)	Cell Signaling Technologies	Cat# 2760S RRID:AB_659831
Guinea Pig anti-Insulin (1:5)	Dako/Agilent	Cat# IR00261-2
Rabbit anti-Somatostatin (1:500)	Phoenix	Cat# H-060-03 RRID:AB_2687415
Rabbit anti-amylase (1:500)	Sigma	Cat# A8273 RRID: AB_258380
Goat anti-Ghrelin (1:500)	Santa Cruz	Cat# sc-10368, RRID:AB_2232479
Rabbit anti-Ki67 (1:500)	Abcam	Cat# Ab15580 RRID:AB_443209
488 goat anti-guinea pig (1:500)	Thermo Fisher/Invitrogen	Cat# A11073 RRID:AB_2534117
555 goat anti-guinea pig (1:500)	Thermo Fisher/Invitrogen	Cat# A21435 RRID:AB_1500610
488 donkey anti-rabbit (1:500)	Thermo Fisher/Invitrogen	Cat# A21206 RRID:AB_141708
594 donkey ant-rabbit (1:500)	Thermo Fisher/Invitrogen	Cat# A21207 RRID:AB_141637
647 goat anti-rabbit (1:500)	Thermo Fisher/Invitrogen	Cat# A21244 RRID:AB_141663
488 donkey anti-goat (1:500)	Thermo Fisher/Invitrogen	Cat# A11055 RRID:AB_2534102
555 donkey anti-goat (1:500)	Thermo Fisher/Invitrogen	Cat# A21432 RRID:AB_2535853
Biotinylated goat anti PDX-1/IPF1 (1:50)	R&D Systems	Cat# BAF2419 RRID:AB_416757
Mouse IgG1 anti-NKX6.1(1:250)	DSHB	Cat# F55A10 RRID:AB_532378
Rat anti-Somatostatin (1:100)	Santa Cruz	Cat# sc-47706 RRID:AB_628268
Mouse IgG1 anti-Glucagon (1:2000)	Sigma-Aldrich	Cat# G2654 RRID:AB_259852
Rabbit anti-C-Peptide (1:100)	Cell Signaling	Cat# 4593S RRID:AB_10691857
Goat anti-mouse IgG1-488 (1:400)	Jackson Immunoresearch	Cat# 115-545-205 RRID:AB_2338854
Goat anti-mouse IgG1-PE (1:400)	Jackson Immunoresearch	Cat# 115-115-205 RRID:AB_2338620
Goat anti-mouse IgG1-647 (1:400)	Jackson Immunoresearch	Cat# 115-605-205 RRID:AB_2338916
Goat anti-rabbit alexa 647 (1:400)	Invitrogen	Cat# A21245 RRID:AB_2535813
Goat anti-rabbit IgG-PE (1:400)	Jackson Immunoresearch	Cat# 111-116-144 RRID:AB_2337985
Donkey anti-mouse IgG alexa647 (1:400)	Jackson Immunoresearch	Cat# 715-605-150 RRID:AB_2340862
Donkey anti-rabbit IgG-PE (1:400)	Jackson Immunoresearch	Cat# 711-116-152 RRID:AB_2340599

Streptavidin, Pacific Blue conjugate (1:400)	Thermo Fisher Scientific	Cat# S11222
Goat anti Rat alexa 647 (1:400)	Thermo Fisher scientific	Cat# A21247 RRID:AB_141778
Mm Ins2-01 (pre-diluted)	ACD Bio	Cat# 497811
Mm Sst C3 (1:150)	ACD Bio	Cat# 404631-C3
Mm Gcg C2 (1:150)	ACD Bio	Cat# 400601-C2
Opal 520 Reagent (1:1500)	Akoya Biosciences	Cat# FP1487A
Opal 570 Reagent (1:1000)	Akoya Biosciences	Cat# FP1488A
Opal 620 Reagent (1:1000)	Akoya Biosciences	Cat# FP1495A
Opal 650 Reagent (1:1000)	Akoya Biosciences	Cat# FP1496A
Taqman Probe Ins1	ThermoFisher	Cat# Mm01950294
Taqman Probe Ins2	ThermoFisher	Cat# Mm00731595
Taqman Probe Gcg	ThermoFisher	Cat# Mm00801714
Taqman Probe Sst	ThermoFisher	Cat# Mm00436671
Taqman Probe Ghrl	ThermoFisher	Cat# Mm00445450
Taqman Probe Actb	ThermoFisher	Cat# Mm00607939

Table S4 – Quantitative real time PCR and genotyping primers

Assay	Animal	Primer Name	Sequence
PCR	Mouse	RARdn_F	ATG GTG TAC ACG TGT CAC C
PCR	Mouse	RARdn_R	CAC CTT CTC AAT GAG CTC C
PCR	Mouse	General Cre_F	CTG CCA CGA CCA AGT GAC AGC
PCR	Mouse	General Cre_R	CTT CTC TAC ACC TGC GGT GCT
PCR	Mouse	Neurog3cre_F	CGT GCA GTG ACC TCT AAG TCA G
PCR	Mouse	Neurog3cre_R	GTG AAA CAG CAT TGC TGT CAC TT
PCR	Mouse	Pdx1cre_F	CTG GAC TAC ATC TTG AGT TGC
PCR	Mouse	Pdx1cre_R	GGT GTA CGG TCA GTA AAT TTG
PCR	Mouse	RosaWT_F	AAG GGA GCT GCA GTG GAG TA
PCR	Mouse	RosaWT_R	CCG AAA ATC TGT GGG AAG TC
PCR	Mouse	Pdx1Cre ER_F	AGC AGT GGA GAA CTG TCA AAG CGA
PCR	Mouse	Pdx1Cre ER_R	TGG ATG TGG TCC TTC TCT TCC AGA
qPCR	Human	INS_F	TTT GTG AAC CAA CAC CTG TGC GG
qPCR	Human	INS_R	GCG GGT CTT GGG TGT GTA GAA GAA
qPCR	Human	GCG_F	TTC CCA GAA GAG GTC GCC ATT GTT
qPCR	Human	GCG_R	CAA CCA GTT TAT AAA GTC CCT GGC GG
qPCR	Human	SST_F	GAG AAT GAT GCC CTG GAA CCT GAA GA
qPCR	Human	SST_R	ATT CTT GCA GCC AGC TTT GCG T
qPCR	Human	TBP_F	TTG CTG AGA AGA GTG TGC TGG AGA TG
qPCR	Human	TBP_R	CGT AAG GTG GCA GGC TGT TGT T
qPCR	Human	CHGB_F	TTG CTG AGA AGA GTG TGC TGG AGA TG
qPCR	Human	CHGB_R	CGT AAG GTG GCA GGC TGT TGT T