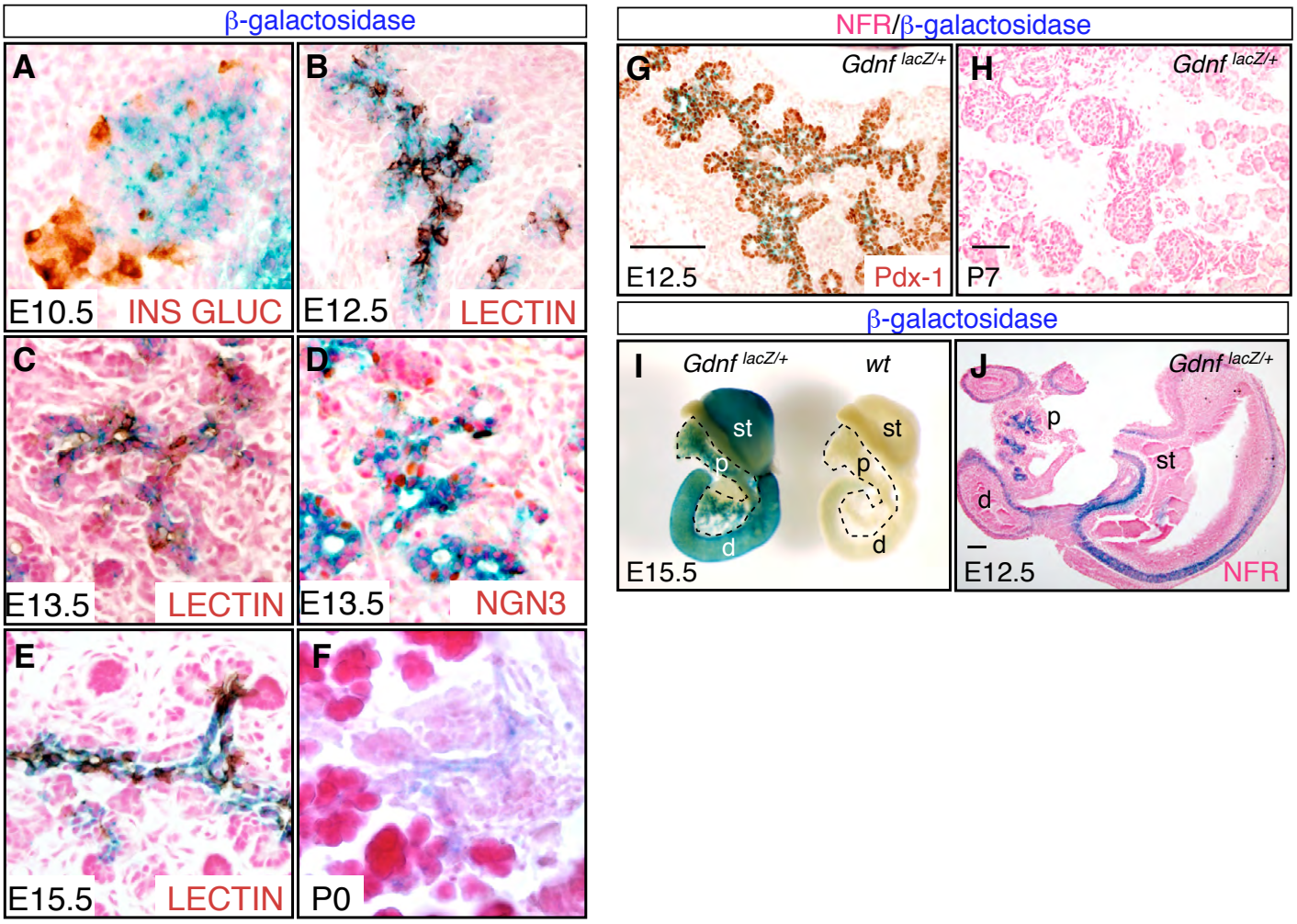

Fig. S1. *Gdnf* expression in the embryonic pancreas. (**A-F**) High-magnification of images shown in Fig. 1. (**G**) β-galactosidase is broadly expressed in PDX1^{+} pancreatic epithelium in *Gdnf*$^{lacZ/+}$ mice at E12.5. (**H**) No β-galactosidase activity is observed in *Gdnf*$^{lacZ/+}$ pancreas at P7. (**I**) Whole-mount detection of β-galactosidase activity in the gastrointestinal tract of *Gdnf*$^{lacZ/+}$ (left) and control (right) E15.5 embryos. The pancreas is outlined for better visualization. (**J**) Paraffin section of E12.5 *Gdnf*$^{lacZ/+}$ gut counterstained with Nuclear Fast Red. p, pancreas; st, stomach; d, duodenum. Scale bars: 100 μm.

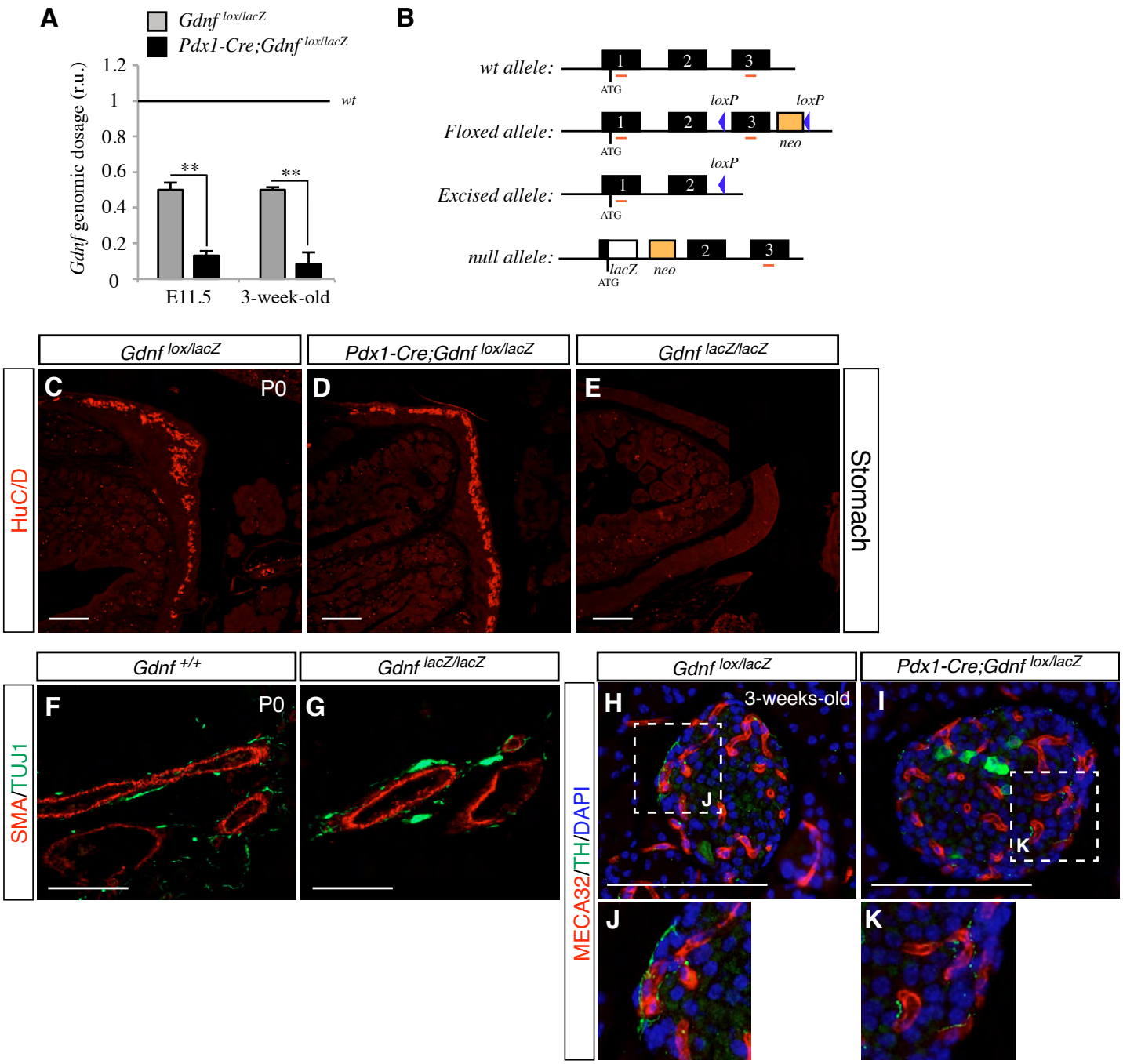

Fig. S2. Genomic loss of the *Gdnf*^{lox} allele and innervation of *Gdnf* mutant mice. (**A**) Relative genomic loss of the *Gdnf*^{lox} allele in E11.5 and 3-week-old *Pdx1-Cre; Gdnf*$^{lox/lacZ}$ pancreata measured by quantitative PCR. The quantification of the excision levels of the *Gdnf*^{lox} allele was performed as previously described (Pascual et al., 2008). Data are mean ± s.e.m. ***P*<0.01 (Student's *t*-test). (**B**) Scheme of wild-type, floxed, excised and *lacZ Gdnf* alleles. The positions of the primers used for semi-quantitative RT-PCR in the different alleles are indicated in red. (**C-E**) HuC/D^{+} neuronal cells are found in the stomach of P0 control (C) and *Pdx1-Cre; Gdnf*$^{lox/lacZ}$ (D) mice. (E) No neurons are observed in the stomach of P0 *Gdnf*$^{lacZ/lacZ}$ mice. (**F**,**G**) Most of the remaining nerve fibers in *Gdnf*$^{lacZ/lacZ}$ pancreata appeared to be associated with blood vessels, as assessed by smooth muscle immunostaining. (**H-K**) TH^{+} nerve fibers are associated with blood vessels, as assessed by panendothelial cell antigen (Meca 32 clone) staining in control (H,J) and *Pdx1-Cre; Gdnf*$^{lox/lacZ}$ (I,K) islets. J and K are higher magnification views of the boxed areas in H and I, respectively. Scale bars: 100 µm.

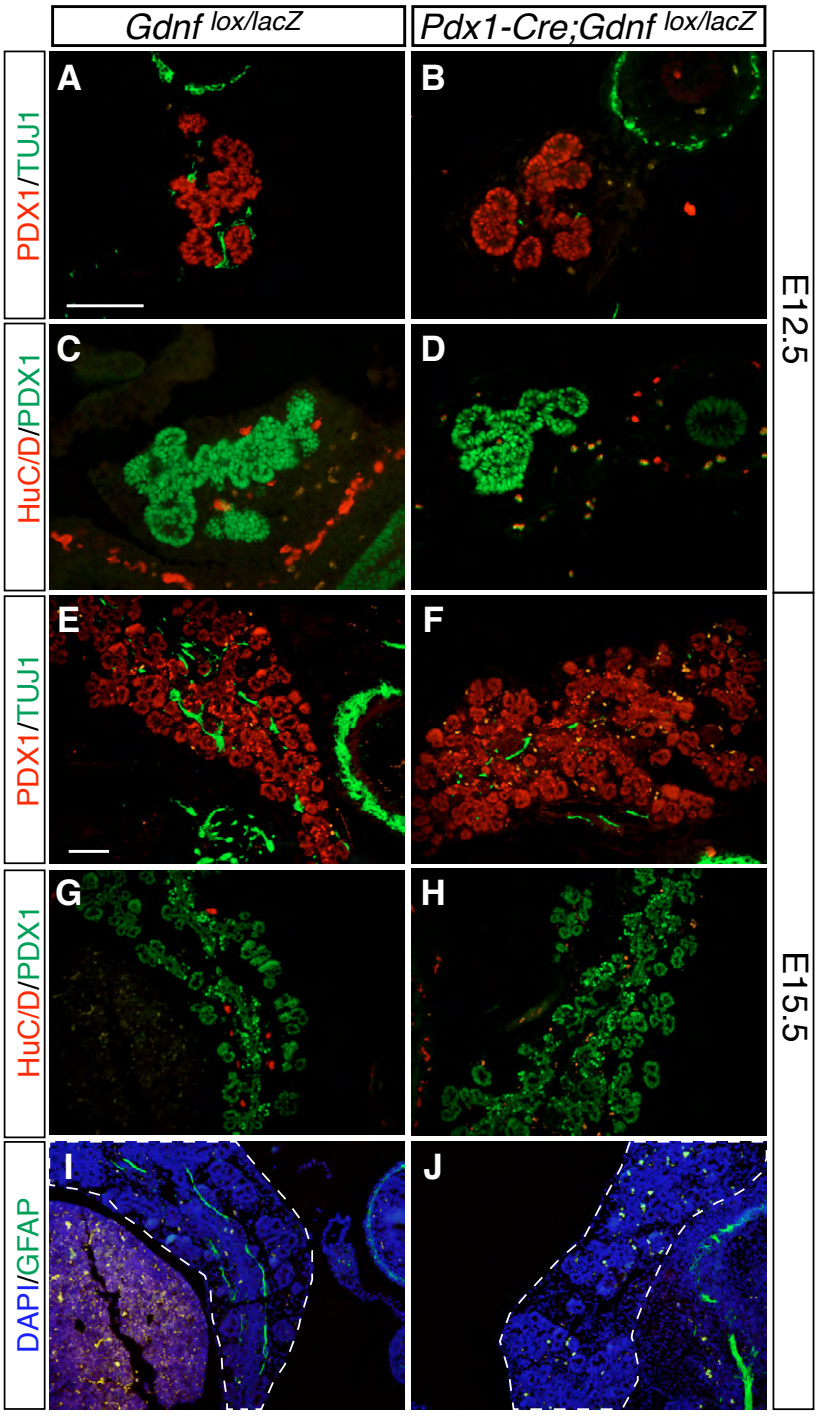

Fig. S3. Decreased number of NC derivatives in *Gdnf* mutant mouse embryos. (**A-H**) E12.5 (A-D) and E15.5 (E-H) paraffin sections of *Pdx1-Cre; Gdnf*$^{lox/lacZ}$ and control pancreata immunostained for neural markers TUJ1 (A,B,E,F) and HuC/D (C,D,G,H). (**I**,**J**) E15.5 paraffin sections of *Pdx1-Cre; Gdnf*$^{lox/lacZ}$ and control pancreata immunostained for the glial marker GFAP. Scale bars: 100 µm (in A for A-D; in E for E-J).

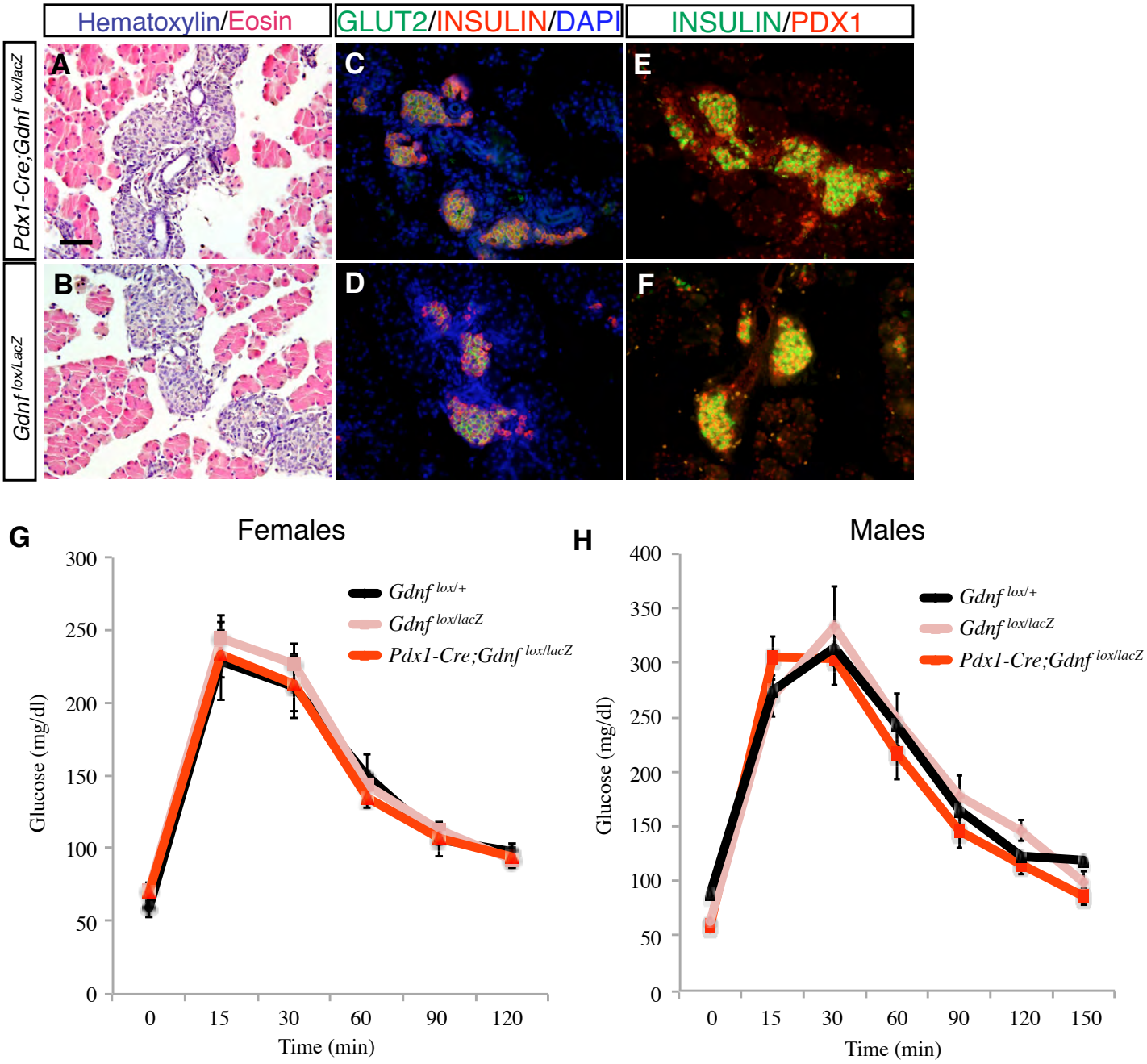

Fig. S4. Islet formation and β-cell function in adult *Gdnf* mutant mice. (**A**,**B**) Hematoxylin and Eosin staining of control (A) and *Pdx1-Cre; Gdnf*$^{lox/lacZ}$ (B) sections of P0 pancreata. (**C-F**) Immunostaining for mature markers GLUT2 (SLC2A2) (C,D) and PDX1 (E,F) reveals that islet differentiation is not affected in the absence of GDNF. (**G**,**H**) Intraperitoneal glucose tolerance tests performed in 3-month-old female (G) and male (H) mice shows that β-cell function is not affected in *Pdx1-Cre; Gdnf*$^{lox/lacZ}$ mice. Scale bars: 50 µm (in A for A-F).

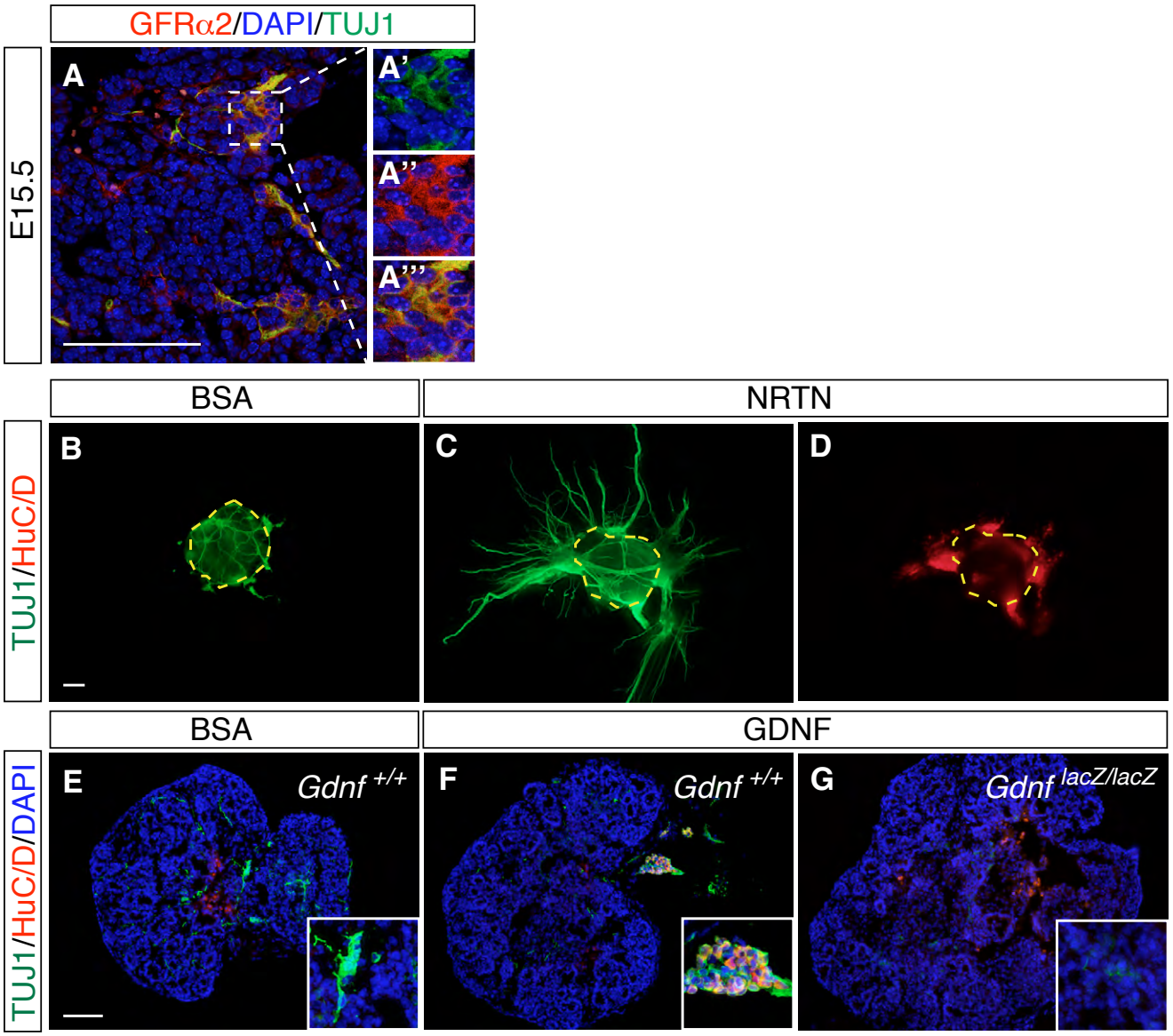

Fig. S5. Effects of GDNF and NRTN in pancreas explants. (**A-A‴**) NRTN co-receptor GFRα2 is expressed in pancreatic neuronal progenitors (marked by TUJ1 reactivity) but not in PDX1^{+} epithelial cells. (**B-D**) E12.5 wild-type pancreatic explants treated with 100 ng/ml NRTN exhibit increased neurite outgrowth (C) and neuronal cells (D) compared with BSA-treated explants (B). (**E-G**) Sections of pancreatic explants of GDNF-treated control (F) and *Gdnf*$^{lacZ/lacZ}$ (G) and BSA-treated control pancreatic explants (E) after 4 days of culture. Note that GDNF does not induce increased neurite outgrowth and neuronal cell differentiation in *Gdnf*$^{lacZ/lacZ}$ pancreatic explants. GDNF-treated *Gdnf*$^{lacZ/lacZ}$ pancreatic explants display reduced innervation compared with BSA-treated controls. Insets show high-magnification images. HUC/D^{+} TUJ1^{-} cells are autofluorescent red blood cells.

Table S1. Primer sequences

Primer	Sequence	Source
CycloA-Fwd	TCACAGAATTATTCCAGGATTCATG	Puri et al., 2009
CycloA-Rv	TGCCGCCAGTGCCATT	
GDNF_lox-Fwd	ACTCCAATATGCCTGAAGATTATCCT	Pascual et al., 2008
GDNF_lox-Rv	TCAGTCTTTTAATGGTGGCTTGAA	
GDNF_Ex-Rv	GAACTCCAGGTAAATAATCC	
Gusb-Fwd	CATTCAGTTCTGGATCAGAAACGTA	Pascual et al., 2008
Gusb-Rv	CATGAAGTCGGCGAAATTCC	
Neo-Fwd	GGATGGAAGCCGGTCTTGT	Pascual et al., 2008
Neo-Rv	CCTGATGCTCTTCGTCCAGATC	
Ngn3-Fwd	TTTGAGTCGGGAGAACTAGGATGG	Mwangi et al., 2010
Ngn3-Rv	TTGGAACTGAGCACTTCGTGGT	
Nkx2.2-Fwd	CGGGCGGAGAAAGCATT	Carrasco et al., 2012
Nkx2.2-Rv	TCCACCTTGCGGACACTATG	
Pdx1-Fwd	CTTAACCTAGGCGTCGCACAA	Carrasco et al., 2012
Pdx1-Rv	GAAGCTCAGGGCTGTTTTTCC	
E1-E3 GDNF-Fwd	CGGGCCACTTGGAGTTAATG	This work
E1-E3 GDNF-Rv	ATGACGTCATCAAACTGGTC	

Table S2. Primary antibodies

Antibody	Species	Dilution	Staining	Source (catalog number)
α-amylase	Rabbit	1:300	IF	Sigma (A8273)
CPA1	Rabbit	1:1000	IF, P	AbD (1810-0006)
RET	Rabbit	1:25	IF*	IBL (18121)
GFAP	Rabbit	1:500	IF	DAKO (70334)
GFRα1	Goat	1:50	IF, P‡	R&D Systems (AF560)
GFRα2	Goat	1:100	IF, P‡	R&D Systems (AF429)
Glucagon	Mouse	1:8000	IF, P	Sigma (G2654)
GLUT2	Rabbit	1:200	IF	Millipore (07-1402)
HuC/D	Mouse	1:100	IF*	Molecular Probes (16A11)
Insulin	Mouse	1:200	IF	Sigma (I2018)
Insulin	Guinea pig	1:2000	IF, P	Sigma (I8510)
KI67	Mouse	1:100	IF	BD Pharmingen (550609)
KI67	Rabbit	1:200	IF	Thermo Scientific (RM9106-S0)
Panendothelial cell antigen	Rat	1:300	IF‡	BD Pharmingen (BD 553849)
NGN3	Mouse	1:200	P	Developmental Studies Hybridoma Bank (F25A1B3)
PDX1	Guinea pig	1:200	IF	Abcam (ab7308)
		1:500	P	
PDX1	Mouse	1:100	IF	Developmental Studies Hybridoma Bank (F6A11)
pHH3	Rabbit	1:500	IF	Millipore (06-570)
PHOX2B	Rabbit	1:1000	IF	Gift of J. F. Brunet (Pattyn et al., 1997)
SMA	Mouse	1:500	IF	Sigma (A2547)
SOX10	Guinea pig	1:500	IF	Gift of M. Wegner, Erlangen, Germany (Stolt et al., 2003)
SOX10	Goat	1:200	IF	R&D Systems (AF2864)
TH	Rabbit	1:1000	IF	Novus (NB300-109)

TUJ1	Rabbit	1:1000	IF	Abcam (ab18207)
VAChT	Guinea pig	1:100	IF	Millipore (AB1588)

P, peroxidase; IF, immunofluorescence.

*Requires tyramide amplification.

‡Requires biotin-avidin amplification.