Inventory of Supplemental Information:

Supplemental Figures and Legends

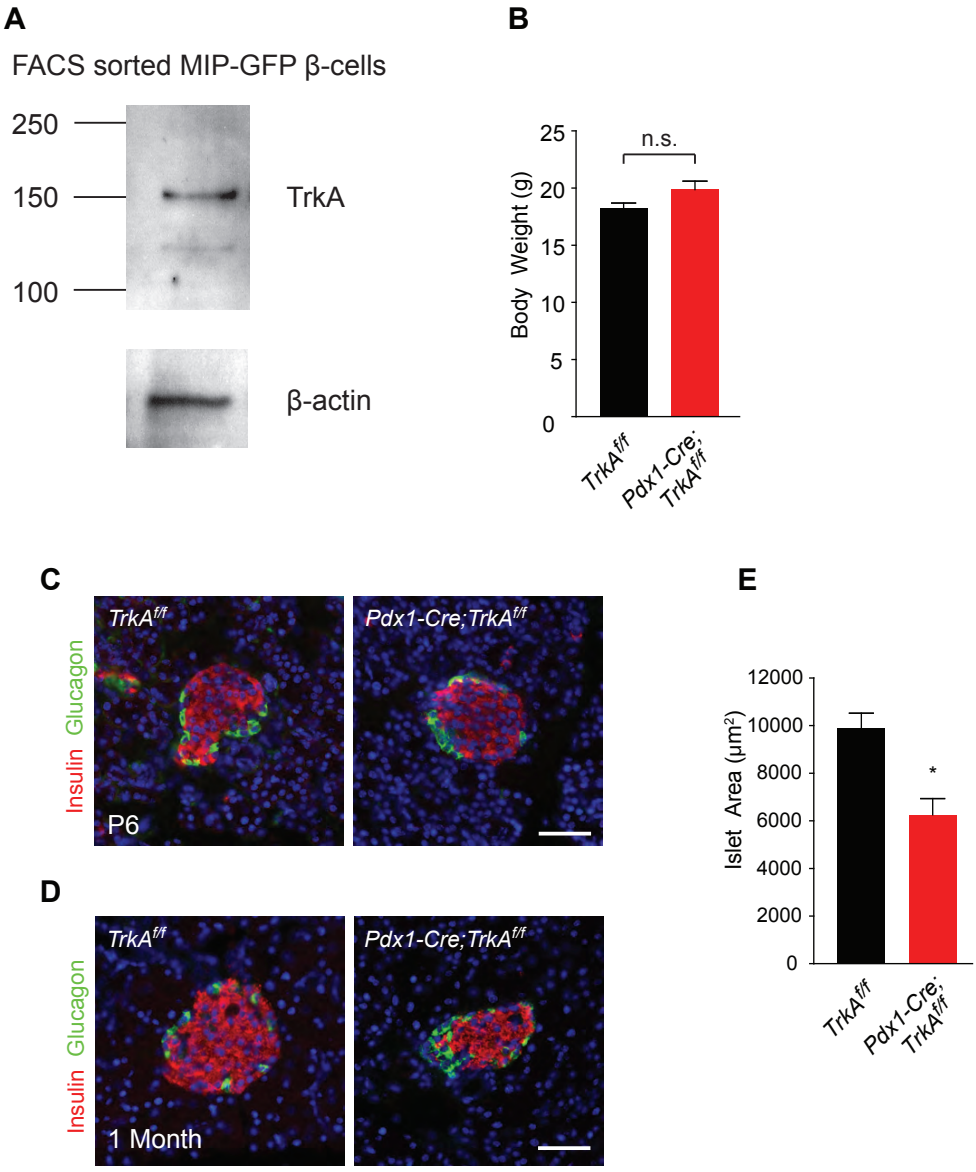

Figure S1, related to Figure 1. *Pdx1-Cre;TrkA$^{f/f}$* mice have normal body weight and islet morphology but exhibit a modest decrease in islet size

(A) Western blot analysis of TrkA expression in FAC-sorted β-cells from *MIP-GFP* mice. The TrkA immunoblot was stripped and reprobed for β-actin. **(B)** Body weights of 1.5 month old *TrkA$^{f/f}$* and *Pdx1-Cre;TrkA$^{f/f}$* mice. Values are mean ± SEM from n=33 control, 20 mutant mice. **(C-E)** Immunostaining for insulin (red) and glucagon (green) shows that islet morphology and cyto-architecture are normal in *Pdx1-Cre;TrkA$^{f/f}$* mice at postnatal day 6 (P6), although islet sizes are slightly reduced in 1 month old mutant mice. Representative images are shown from at least 3 animals per genotype that were analyzed. Scale bars, 50 μm. Islet size values are mean ± SEM from 5 control and 3 mutant animals, *p<0.05, *t*-test.

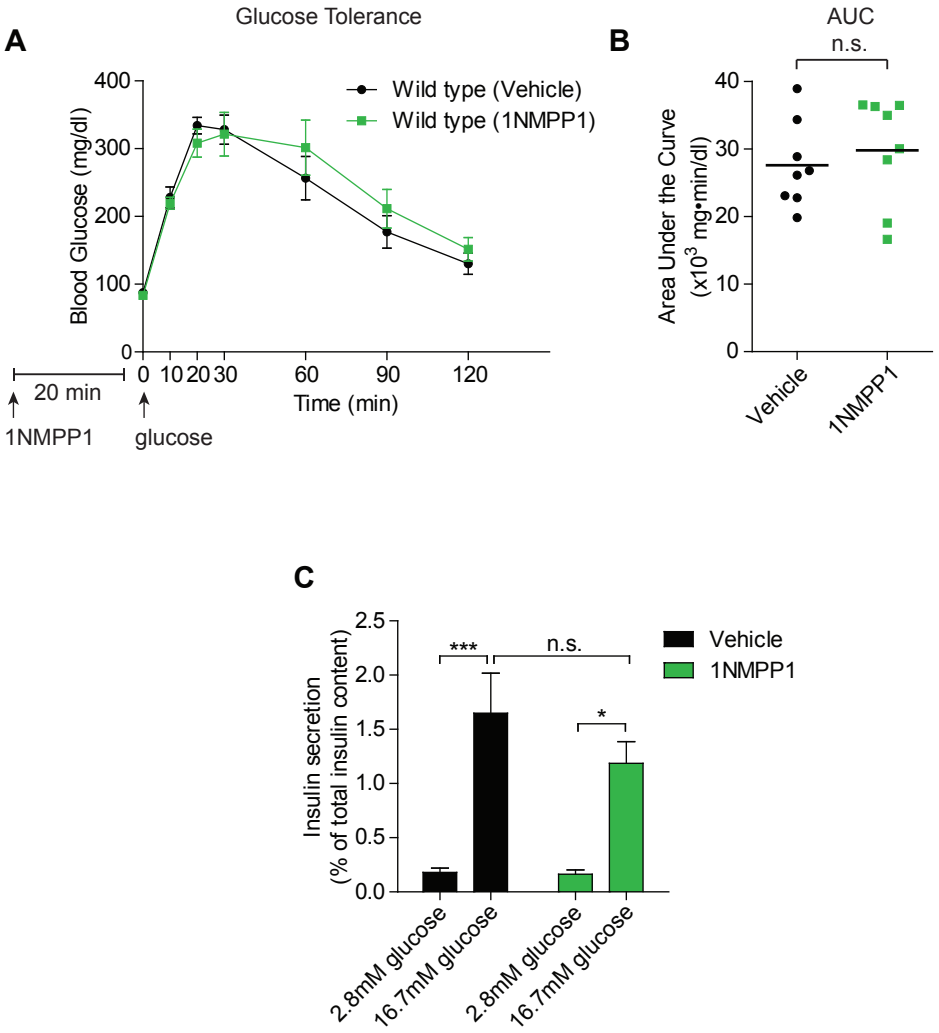

Figure S2, related to Figure 2. 1NMPP1 treatment does not influence glucose tolerance or insulin secretion in wild-type mice

(A) Glucose tolerance tests for wild-type mice injected with 1NMPP1 or vehicle (DMSO) 20 minutes prior to an intra-peritoneal glucose challenge. **(B)** Area under the curve (AUC) analysis for glucose tolerance tests in **(A)**. Values are means ± SEM for n=8 animals each for vehicle and 1NMPP1 injections. **(C)** Normal glucose-stimulated insulin secretion in wild-type islets treated with 1NMPP1. Values are means ± SEM from n=4 independent experiments, ∗p<0.05, ∗∗∗p<0.001, n.s. not significant, two-way ANOVA with Bonferroni post-test.

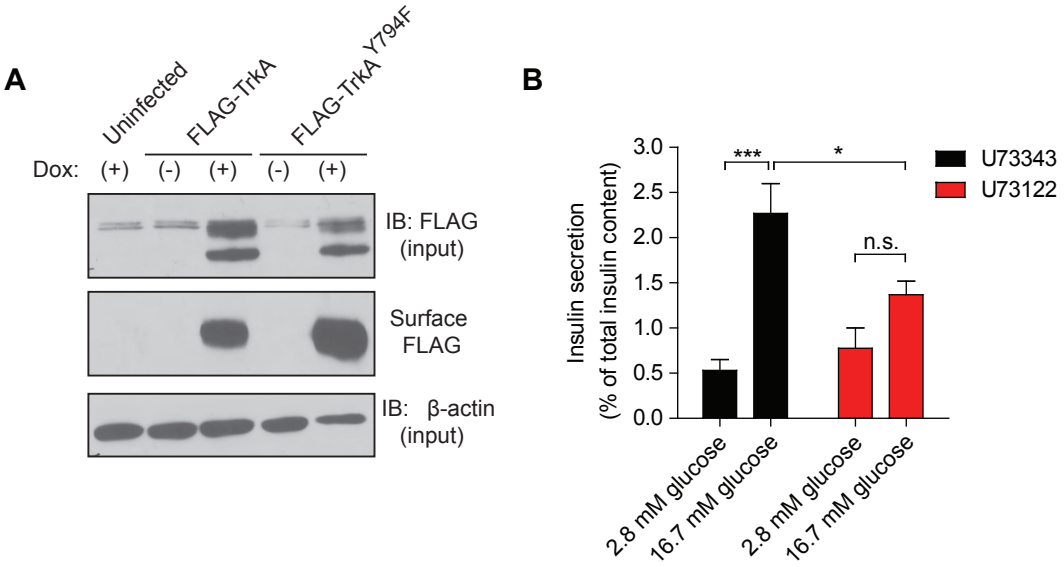

Figure S3, related to Figure 4. Adenoviral expression of FLAG-TrkA receptors, and role of PLCγ activity in insulin secretion

(A) Doxycycline-induced expression of ectopic TrkA receptors in MIN6 cells infected with adenoviruses carrying FLAG-TrkA^{Y794F} or control FLAG-TrkA receptors. FLAG-tagged TrkA receptors appear on the cell surface as determined by a surface biotinylation assay. The FLAG immunoblot (input) was stripped and reprobed for β-actin for normalization. **(B)** Glucose-stimulated insulin secretion is impaired in islets treated with a PLCγ inhibitor, U73122, compared to islets treated with an inactive analog, U73343. Values are means ± SEM from n=8 independent experiments, *p<0.05, ***p<0.001, n.s. not significant, two-way ANOVA with Bonferroni post-test.

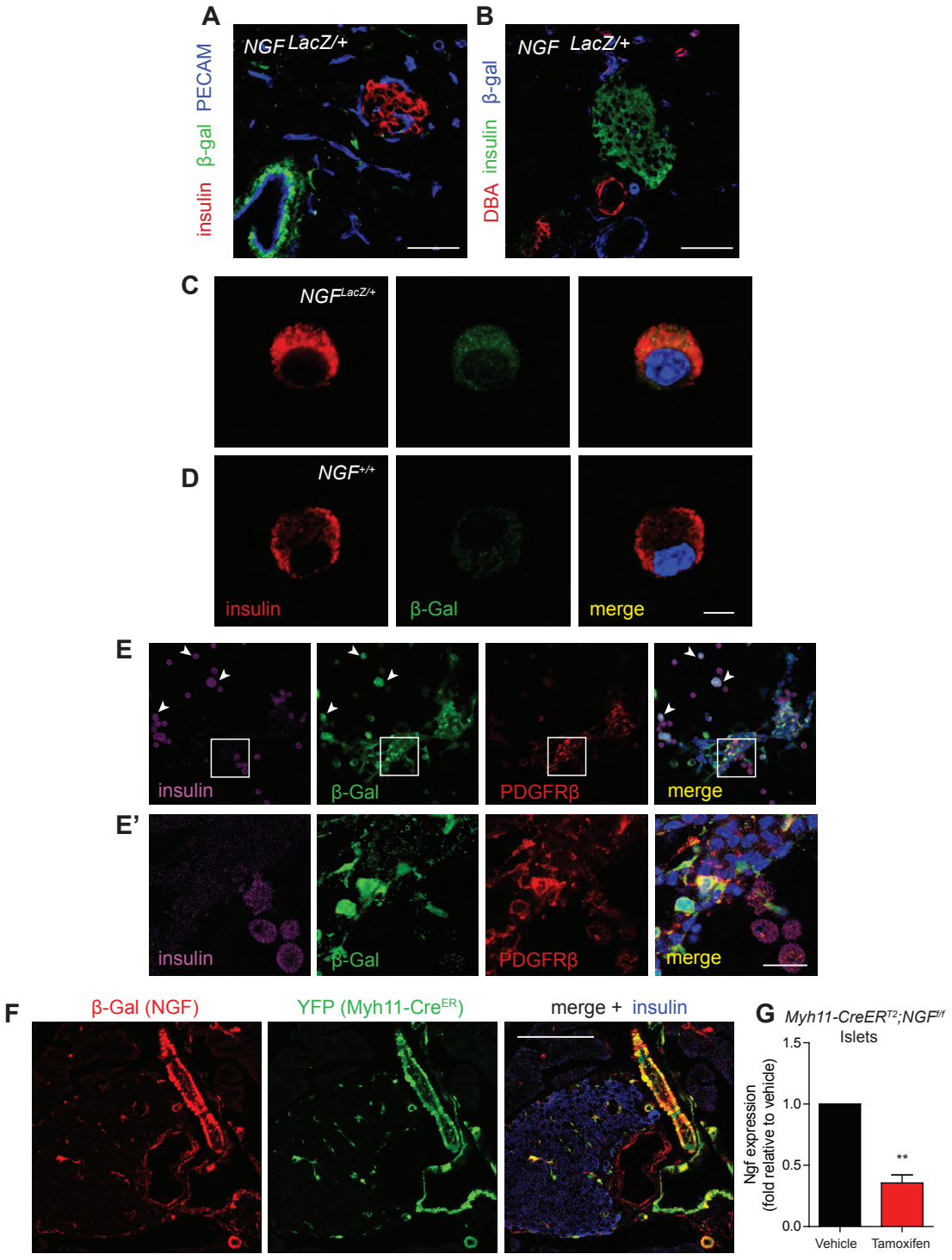

Figure S4, related to Figure 5. Genetic labeling shows NGF expression in vascular contractile cells, and low expression in cultured β-cells

(A,B) Immunostaining for β-galactosidase that reports NGF expression shows little co-localization with endothelial cells stained with PECAM, or pancreatic ducts labeled with DBA, in $NGF^{LacZ/+}$ tissue sections. Islets are marked by insulin immunostaining. Scale bar, 50 μm. **(C,D)** Single dissociated $NGF^{LacZ/+}$ β-cells in culture show low expression of

β-galactosidase compared to control $NGF^{+/+}$ β-cells. β-cells were identified by co-labeling with insulin. Representative images are shown from at least 3 animals per genotype that were analyzed. Scale bar, 5 μm. **(E)** In dissociated islet cultures, β-galactosidase immunostaining shows NGF expression predominantly in pericytes (inset). Arrowheads indicate NGF expression in a few isolated β-cells. The pericytes and β-cells are marked by PDGFRβ and insulin immunostaining respectively. **(E')** shows a higher magnification of boxed region in **(E)**. Scale bars, 50 μm for **(E)** and 20 μm for **(E')**. **(F)** Genetic labeling shows tamoxifen-induced YFP fluorescence in NGF-expressing large-diameter blood vessels outside islets and intra-islet pericytes in $NGF^{LacZ/+}$;*Myh11-CreER*^{T2};*R26-YFP* mice. Islets are marked by insulin immunostaining. Scale bars, 100 μm. **(G)** qPCR analysis reveals decreased NGF expression in islets from tamoxifen-injected *Myh11-CreER;*$NGF^{f/f}$ mice, compared to vehicle. Values are the mean ± SEM from n=4 independent experiments. **$p<0.01, t-test.

Supplemental Experimental Procedures

Mouse Lines

Mice	Source	Stock Number
TrkA$^{f/f}$ (*TrkA ^{F592A}*)	Jackson Laboratory	022362
Myh11-cre/ER^{T2}	Jackson Laboratory	019079
R26-EYFP	Jackson Laboratory	006148
MIP-GFP	Jackson Laboratory	006864
Pdx1-Cre^{Tuv}	NCI Frederick mouse repository	01XL5

Antibodies

Primary antibodies for immunofluorescence include guinea pig anti-insulin (1:300, abcam ab7842), mouse anti-glucagon (1:400, abcam ab10988), rabbit anti-p75 (1:200, Promega G323A), chicken anti-beta-galactosidase (1:100, Millipore AB3403-I), rat anti-PECAM (CD31) (1:200, BD Biosciences 550274), FITC-mouse-anti-SMA (1:500, Sigma F3777), rabbit anti-PDGFRβ (1:100, abcam ab32570), mouse anti-EEA1 (1:500, BD Biosciences 610457) and rabbit anti-β-actin (1:1000, Cell Signaling, 4970). Rabbit anti-FLAG (1:100, Sigma Aldrich F7425) and mouse M2 anti-FLAG (1:100, Sigma Aldrich F3165) were used for live-cell antibody feeding assays. Fluorescent secondary antibodies were Alexafluor-350, -488,-546, or -647-conjugated and used at 1:200 (Invitrogen)

Primary antibodies for western blots include rabbit anti-Phospho-TrkA (Tyr785)/TrkB (Tyr816) (1:1000, Cell Signaling C67C8), rabbit anti-TrkA (1:1000, Millipore 06-574), and rabbit anti-p85 (1:3000, Upstate Biotechnology 06-195).

Plasmids and adenoviral vectors

FLAG-TrkA^{Y794F} and FLAG-TrkA^{Y499F} constructs were generated by point mutations in a parent FLAG-TrkA vector (Bodmer et al., 2011), using QuikChange II XL site-directed mutagenesis kit (Agilent, #200521). Mutant plasmids were verified by DNA sequencing. Recombinant adenoviruses expressing FLAG-TrkA or FLAG-TrkA^{Y794F} were generated by sub-cloning FLAG-TrkA or FLAG-TrkA^{Y794F} from PCDNA3.1 into pAdenoX-Tet3G using the Adeno-X^{TM} Adenoviral System 3 kit (Clontech). Recombinant adenoviral backbones were packaged into infectious adenoviral particles by transfection into HEK 293 cells (ATCC) using Lipofectamine (Invitrogen). Generation of adenovirus expressing chimeric FLAG-TrkB:TrkA receptors have been described previously (Ascano et al., 2009). High-titer viral stocks were purified using a CsCl gradient.

Tamoxifen injections

Beginning at 4-5 weeks of age, *Myh11-CreER^{T2};R26-YFP;NGF$^{LacZ/+}$* or *Myh11-CreER^{T2};NGF$^{f/f}$* mice were injected subcutaneously with 180 mg/kg tamoxifen (Sigma) in corn oil or corn oil alone, every day for 5 days. 2-3 weeks after the last injection, vehicle- and tamoxifen-injected mice were subjected to glucose tolerance, insulin secretion tests, or used to establish dissociated islet cultures.

Tissue processing, immunohistochemistry, and islet morphology

For X-gal staining, pancreata from *NGF$^{LacZ/+}$* mice were fixed in 0.2% glutaraldehyde and 2mM $MgCl_2$ in PBS overnight at 4°C. Tissues were equilibrated in 30% sucrose and embedded in OCT. 10 μm-thick sections were collected and stained with a solution containing X-gal (1 mg/ml, Invitrogen), 5mM $K_3Fe(CN)_6$, 5mM $K_4Fe(CN)_6$, 2mM $MgCl_2$, 0.1M NaPO4, 0.01% sodium deoxycholate, and 0.02% NP40.

For immunofluorescence, tissues were fixed overnight in 4% paraformaldehyde (PFA) (Sigma) at 4°C, cryoprotected in 30% sucrose overnight, frozen in OCT, and 10 μm thick sections were collected. Sections were permeabilized in 1% Triton X-100 in PBS, blocked in 5% goat serum with 0.1% Triton X-100 in PBS, and incubated in blocking solution containing primary antibodies overnight at 4ºC. Sections were then washed with PBS and incubated with secondary antibodies diluted in blocking solution for 1 hour at room temperature. Ductal epithelium was detected using rhodamine-labeled Dolichos Biflorus Agglutinin (DBA) (1:200, Vector Laboratories RL-1032) applied during secondary antibody incubation. Sections were washed in PBS and mounted in Fluoromount Aqueous Mounting Medium (Sigma Aldrich, F4680) containing 100μg/ml DAPI. Images representing 1μm optical slices were taken using a Zeiss LSM 700 confocal microscope equipped with 405, 488, 555, and 633 nm lasers.

For morphometric analyses of islets, 10μm thick sections of pancreata were collected every 200μm covering the entire pancreas, immunostained for insulin and glucagon, and imaged on a Zeiss AxioImager. Islet areas were determined using the ImageJ software (NIH).

Mouse islet isolations

Islets were isolated as previously described (Wollheim et al., 1990). Briefly, pancreata collected from 1-2-month old mice were distended using collagenase (Collagenase P, 0.375 mg/ml, Roche) dissolved in Hank's Balanced Salt Solution (HBSS, Mediatech), and digested at 37°C. Digested pancreata were washed with HBSS, and subjected to discontinuous density gradient centrifugation using varying histopaque densities (Sigma-Aldrich). The islet layer was collected, washed with HBSS, and islets handpicked under an inverted microscope for subsequent analyses.

Fluorescence-activated cell sorting (FACS)

Isolated islets from 3 adult *MIP-GFP* animals were pooled and dissociated in HBSS containing 2% FBS and 5mM EDTA. Dissociated cells were sorted using a Sony Biotechnology SH 800 cell sorter and GFP-positive cells were selected based on detection using the FL1 channel (absorption 530 nm).

Immunoblotting

Islets isolated from mature *TrkA$^{f/f}$* or *Pdx1-Cre;TrkA$^{f/f}$* mice were lysed in boiling laemmli sample buffer, and subjected to SDS-PAGE. Following transfer, membranes were probed with rabbit anti-TrkA. Immunoblots were stripped and re-probed with rabbit anti-p85 for protein normalization. For detection of phosphorylated TrkA, isolated islets were allowed to recover overnight in RPMI 1640 medium containing 5% fetal bovine serum (FBS) and 5 U/l penicillin/streptomycin. Islets were incubated with 1NMPP1 (20 mM) or vehicle (DMSO) dissolved in RPMI 1640 medium for 20 min, and then lysed in

boiling laemmli buffer. For assessing glucose effects on TrkA phosphorylation, islets were pre-incubated in Krebs-Ringer HEPES buffer (KRHB) containing low (2.8 mM) glucose for 1 hour, and then either left in 2.8 mM glucose or stimulated with 16.7 mM glucose in KRHB buffer for 15 minutes, and lysates prepared in boiling laemmli buffer. Lysates were subjected to immunoblotting with rabbit anti-Phospho-TrkA (Tyr785)/TrkB (Tyr816), and membranes were later re-probed for rabbit anti-TrkA. All immunoblots were visualized with ECL Plus Detection Reagent (Thermo Scientific, 32132) and scanned with a Typhoon 9410 Variable Mode Imager (GE Healthcare). Densitometric analysis of bands was performed using ImageJ software.

Electron microscopy

Pancreata were fixed in buffer containing 3% formaldehyde, 1.5% glutaraldehyde, 5mM $CaCl_2$, 2.5% sucrose, and 0.1M sodium cacodylate for 1hr at room temperature. Tissues were post-fixed in 1% Palade's OsO_4 for 1hr on ice, followed by incubation in Kellenberger's uranyl acetate overnight at room temperature. After dehydration through graded alcohols, tissues were embedded in Epon, and ultrathin (~90 nm) sections were collected onto EM grids. Grids were then imaged using an FEI Tecnai-12 TWIN transmission electron microscope operating at 100 kV.

Phalloidin staining

Islets were isolated from adult 1-2 month old $TrkA^{F592A}$ mice and dissociated into single cells by gentle trituration in HBSS containing 4 mM EDTA. Cells were resuspended in RPMI 1640 media containing 5% FBS and 5 U/l penicillin/streptomycin, and plated on glass coverslips coated with poly-D lysine. After 24 hours, islet cultures were treated with 1NMPP1 (20 mM) or DMSO for 20 minutes, followed by incubation in low (2.8 mM) or high (16.7 mM) glucose for another 30 minutes. Cells were fixed in 4% PFA and stained with Alexa546–conjugated phalloidin (Invitrogen). β-cells were identified by immunostaining for insulin. Images representing 1μm optical sections were acquired using a Zeiss LSM 510 confocal microscope. Average fluorescence intensities of Alexa-546 per μm^2 in individual β-cells were measured using ImageJ software (NIH). 20-25 cells were analyzed per condition per experiment.

To assess the effects of endocytosis-defective TrkA receptors on F-actin, isolated β-cells from $TrkA^{F592A}$ mice were incubated with adenoviruses expressing either FLAG-$TrkA^{Y794F}$ or control FLAG-TrkA receptors, and treated with doxycycline (100 ng/ml) in RPMI 1640 media containing 5% FBS and 5 U/l penicillin/streptomycin for 24 hours. b-cells were then incubated in 1NMPP1 (20μM) for 20 minutes to silence endogenous TrkA receptors, and then fixed and labeled with Alexa546–phalloidin as described above.

Rac1 activity

GTP-bound Rac1 levels in islet lysates were measured using Rac1 G-protein linked immunosorbent assay (G-LISA) (Cytoskeleton Inc., Cat. # BK126). Islets were treated with 1NMPP1 (20 mM) or DMSO for 20 minutes, followed by incubation in low (2.8 mM) or high (16.7 mM) glucose for another 15 minutes. Islet protein concentrations were measured using Precision Red Advanced Protein Assay (Cytoskeleton Inc., Cat. # ADV02-A), and normalized prior to processing the samples for the Rac1 G-LISA assays.

NGF secretion from vascular smooth muscle cells

Primary aortic vascular smooth muscle cell cultures were established as previously described (Metz et al., 2012). *C57BL/6 mice*, 1-2 months of age, were euthanized and the aorta was dissected, de-endothelialized, and cut into small (~1mm x 1mm) squares. The squares were pressed lumen side down on a collagen-coated dish and allowed to adhere for 15-30 minutes before the addition of fresh media (DMEM media plus 20% FBS, 5 U/L penicillin/5µg/L streptomycin, 2mM GlutaMAX, and 1mM sodium pyruvate). Smooth muscle cells were allowed to migrate out of the explants and proliferate for 5 days before passaging. After the third passage in culture, smooth muscle cells were plated in 6 cm dishes and allowed to grow to confluency for 3 days. Cells were equilibrated in Krebs-Ringer HEPES buffer (KRHB) containing low (2.8 mM) glucose for 40 minutes, and then 3mL of fresh Krebs-Ringer HEPES buffer with 2.8 mM glucose was added for 15 minutes and collected before the addition of 3mL Krebs-Ringer HEPES buffer with 16.7 mM glucose for 15 minutes. Supernatants (2.8 and 16.7mM glucose Krebs-Ringer HEPES buffer) were concentrated to 100µl using Amicon Ultracel 3K filter devices (Millipore) and cells were lysed with RIPA before being subjected to quantification with NGF ELISA (Millipore).

TrkA receptor internalization assays

Cell surface biotinylation assays were performed in MIN6 cells as previously described (Bodmer et al., 2011). Briefly, MIN6 cells were biotinylated at 4ºC with a reversible membrane-impermeable form of biotin (EZ-Link™ NHS-SS-Biotin, 1.5mg/ml in PBS, ThermoFisher Scientific, Cat# 21441) for 25 min. For PLCg inhibition, MIN6 cells were pre-treated for 30 minutes with U73122 (10 µM, Sigma Aldrich). Cells were washed briefly with PBS containing 50mM glycine (Sigma) to remove remaining unconjugated biotin, and then moved to 37ºC in DMEM containing 10% FBS and 5 U/l penicillin/streptomycin ± NGF (100ng/ml) for 30 minutes to promote internalization. Cells were returned to 4ºC, the remaining biotinylated surface receptors were stripped of their biotin tag with 50mM glutathione (Sigma), followed by two washes with 50mM iodoacetamide (Sigma) to quench excess glutathione. Cells were lysed with 500µl of RIPA buffer (50mM Tris-HCl, 150mM NaCl, 1mM EDTA, 1% NP-40, 0.25% deoxycholate), and supernatants subjected to precipitation with 40µl-immobilized neutravidin agarose beads (ThermoFisher Scientific) and immunoblotted for TrkA.

Live cell antibody feeding assays were performed in dissociated β-cells and MIN6 cells as previously described (Ascano et al., 2009). Dissociated β-cells plated on poly-D lysine-coated coverslips, were transduced with an adenovirus expressing FLAG-TrkB:A chimeric receptors and infected cells identified by GFP expression. Cells were rinsed briefly with PBS and incubated with rabbit anti-FLAG antibody (1:100) for 30 minutes at 4ºC, followed by a brief wash with cold PBS, and then stimulated with or without BDNF (100 ng/ml) in DMEM for 10 minutes at 37ºC. Cells were quickly washed with PBS, fixed in 4%PFA/PBS for 30 minutes at room temperature, and permeabilized in 0.1% Triton X-100/1% BSA/PBS for 30 minutes at room temperature. Immunostaining for insulin and EEA1 were performed overnight at 4ºC. Cells were washed in PBS and fluorescently conjugated secondary antibodies were applied for 1 hour at room temperature, washed again in PBS, and mounted on slides with Fluoromount. Images representing 1 µm optical slices were acquired using a Carl Zeiss LSM 510 confocal

equipped with Confocor 3 Fluorescence Correlation Spectroscopy module, and argon (458–488 nm) and helium/neon (543–633) lasers. The same confocal acquisition settings were applied to all images taken from a single experiment. Threshold settings for blue and red scans were determined, and internalization was quantified as the ratio of anti-FLAG immunofluorescence (red) that colocalized with EEA1 (blue) relative to the total anti-FLAG immunofluorescence. Weighted coefficients of co-localization between the anti-FLAG and EEA1 fluorescence were determined by ImageJ software. 10-20 cells were analyzed per condition per experiment. For antibody feeding assays in MIN6 cells, cells were first transfected with plasmids carrying FLAG-TrkA, FLAG-TrkA^{Y794F}, or FLAG-TrkA^{Y499F}. GFP was co-transfected in all cases. Cells were stimulated with NGF for 30 minutes or left untreated in media alone. Internalization was quantified as the ratio of anti-FLAG immunofluorescence (red) that co-localized with cytoplasm (GFP signal) relative to the total anti-FLAG immunofluorescence for 10-15 cells per experiment.

Statistical Analyses

Sample sizes, number of experiments and animals were based on previous publications (Bodmer et al., 2011; Borden et al., 2013; Song et al., 2011; Veluthakal et al., 2015). Data were collected randomly. Analyses were done in a semi-blinded manner such that the investigator was aware of the genotype/treatment during sample collection, but did not know the group allocation during data collection and analyses. For practical reasons, the investigator was not blinded to the genotype/treatment during *in vivo* analyses.

Serum NGF levels were analyzed using a paired *t*-test, since each value (fasted and glucose stimulated) was collected from an individual mouse. In analyses in which the hypothetical mean was set to a value of 1, a one-sample *t*-test was used.

Supplemental References

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