

Supplemental data

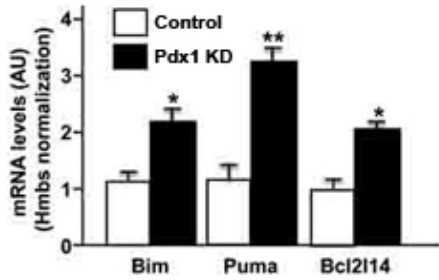


Figure S1

Pdx1 deficiency increases Bim, Puma and Bcl2l14 mRNA expressions in MIN6 cells. Real-time PCR of Bim, Puma and Bcl2l14 mRNAs in Pdx1 knock down (KD) MIN6 cells. ** $p < 0.01$; * $p < 0.05$. Mean \pm SEM; $n = 4-6$ per group.

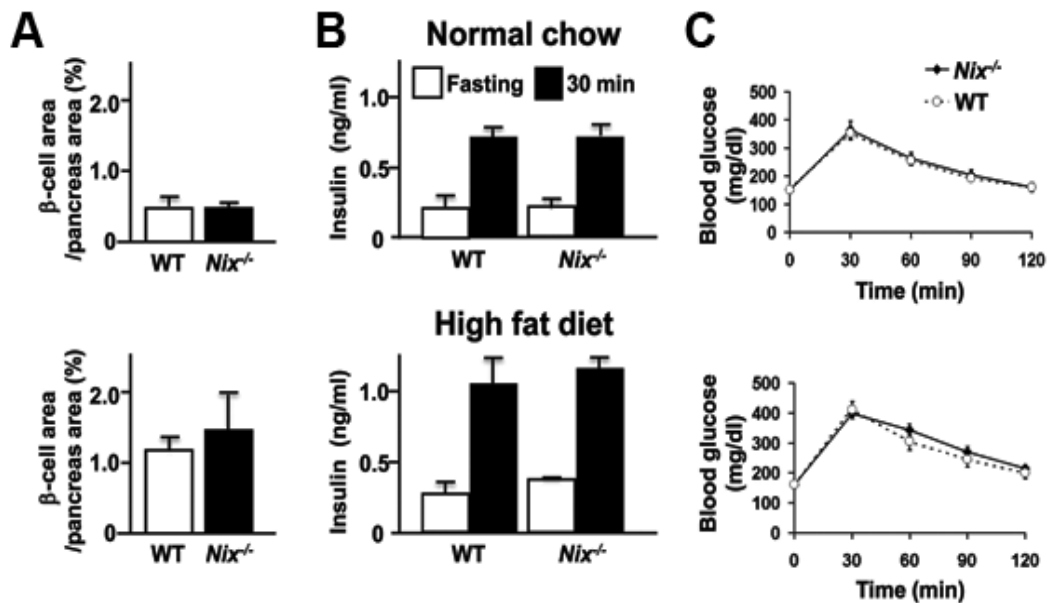


Figure S2

β -cell mass, insulin secretion and glucose tolerance test in normal chow and high fat diets treated $Nix^{-/-}$ mice. (A) Quantitation of β -cell per pancreas area on normal chow (upper panel) and high fat diets (lower panel) treated $Nix^{-/-}$ and wild-type (WT) mice ($n = 5$, each). (B) Insulin levels measured fasting and 30 min after intraperitoneal dextrose in normal chow (upper panel) and high fat diets (lower panel) treated $Nix^{-/-}$ or WT mice ($n = 8-19$, each). (C) Glucose levels following intraperitoneal injection of dextrose (1 g/kg) on normal chow (upper panel) and high fat diets (lower panel) treated mice. $Nix^{-/-}$, triangle; WT, circles ($n = 8-19$, each). All group data are mean \pm SEM of 8-10 weeks of age.

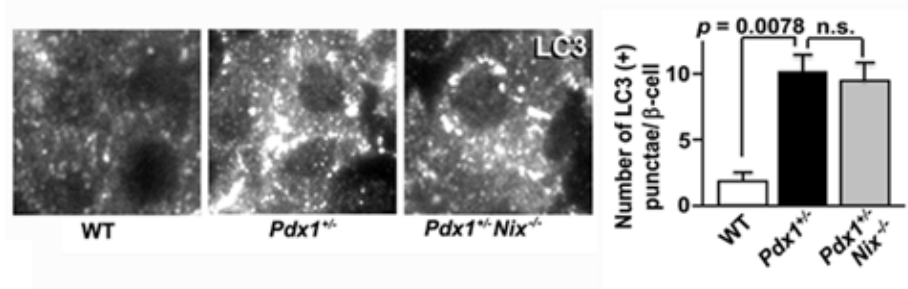


Figure S3

No change in autophagy in islets from *Pdx1*^{+/-}*Nix*^{-/-} mice. LC3 staining in wild-type (WT), *Pdx1*^{+/-}, and *Pdx1*^{+/-}*Nix*^{-/-} mice. Quantitation of group data for LC3-positive punctae per β-cell are shown on the right ($n = 4-6$, each group). Original magnification, x400. Data represent mean ± SEM.

Supplemental Table S1

Gene symbol	Gene name	Accession	p-value	Fold-change	Gene ontology
Upregulated					
Ddit3	DNA-damage inducible transcript 3	NM_007837	0.00068	1.90	Apoptosis
Ambp	alpha 1 microglobulin/bikunin	NM_007443	0.0094	1.89	Transport
Tcl1	T-cell lymphoma breakpoint 1	NM_009337	0.0044	1.86	Nucleus
Upp1	uridine phosphorylase 1	NM_001159401	0.0013	1.86	Glucose metabolic process
Sufu	suppressor of fused homolog	NM_001025391	0.0061	1.75	Nucleus
Ppap2b	phosphatidic acid phosphatase type 2B	NM_080555	0.0072	1.75	Lipid metabolic process
Puma	p53-upregulated modulator of apoptosis	NM_133234	0.0023	1.75	Apoptosis
Ephx1	epoxide hydrolase 1, microsomal	NM_010145	0.0032	1.73	Response to stress
Nix	BCL2/adenovirus E1B interacting protein 3-like	NM_009761	0.0027	1.73	Apoptosis
Aldh2	aldehyde dehydrogenase 2, mitochondrial	NM_009656	0.0055	1.73	Mitochondrion
Il7	interleukin 7	NM_008371	0.0092	1.71	Apoptosis
Paox	polyamine oxidase	NM_153783	0.0084	1.70	Apoptosis
Mmel1	membrane metallo-endopeptidase-like 1	NM_013783	0.0082	1.68	Proteolysis
Nras	neuroblastoma ras oncogene	NM_010937	0.0083	1.68	Intracellular signaling cascade
Fkbp11	FK506 binding protein 11	NM_024169	0.002	1.68	Apoptosis
Mmab	methylmalonic aciduria type B homolog	NM_029956	0.0072	1.67	Mitochondrion
Apof	apolipoprotein F	NM_133997	0.0059	1.65	Transport
Syt7	synaptotagmin VII	NM_018801	0.0092	1.56	Transport
Bim	BCL2-interacting protein	NM_009754	0.0011	1.54	Apoptosis
Kiss1r	KISS1 receptor	NM_053244	0.0073	1.52	Cell surface receptor linked signal transduction
Sgk1	serum/glucocorticoid regulated kinase 1	NM_001161845	0.0004	1.51	Transport
Frzb	frizzled-related protein	NM_011356	0.00025	1.48	Cell surface receptor linked signal transduction
Bcl2l14	BCL2-like 14	NM_025778	0.0069	1.48	Apoptosis
Dcxr	dicarbonyl L-xylulose reductase	NM_026428	0.0021	1.46	Glucose metabolic process
Gpx2	glutathione peroxidase 2	NM_030677	0.0092	1.46	Response to stress
Arhgef3	Rho guanine nucleotide exchange factor (GEF) 3	NM_027871	0.0072	1.45	Intracellular signaling cascade
Jag2	jagged 2	NM_010588	0.0091	1.43	Apoptosis
Nde1	nuclear distribution gene E homolog 1	NM_001114085	0.0098	1.43	Cytoskeleton
Mdm2	transformed mouse 3T3 cell double minute 2	NM_010786	0.0037	1.43	Nucleus
Ctsh	cathepsin H	NM_007801	0.0038	1.42	Proteolysis
Cartpt	CART prepropeptide	NM_001081493	0.0095	1.42	Intracellular signaling cascade
Fastk	Fas-activated serine/threonine kinase	NM_023229	0.0094	1.41	Apoptosis
Epn2	epsin 2	NM_010148	0.0048	1.40	Lipid metabolic process
Sirt3	sirtuin 3	NM_001127351	0.0088	1.40	Regulation of transcription
Olf159	olfactory receptor 159	NM_019476	0.0078	1.39	Cell surface receptor linked signal transduction
Aifm2	apoptosis-inducing factor, mitochondrion-associated 2	NM_001039194	0.0023	1.39	Apoptosis
Nme5	non-metastatic cells 5, protein expressed in	NM_080637	0.0014	1.39	Apoptosis
Irf6	interferon regulatory factor 6	NM_016851	0.0085	1.38	Regulation of transcription
Nudt8	nudix (nucleoside diphosphate linked moiety X)-type motif 8	NM_025529	0.0086	1.38	Mitochondrion
Smc1a	structural maintenance of chromosomes 1A	NM_019710	0.002	1.37	Response to stress

Bag3	BCL2-associated athanogene 3	NM_013863	0.0031	1.37	Apoptosis
Hadh	hydroxyacyl-Coenzyme A dehydrogenase	NM_008212	0.0051	1.34	Lipid metabolic process
Acaa2	acetyl-Coenzyme A acyltransferase 2	NM_177470	0.0084	1.34	Lipid metabolic process
Tst	thiosulfate sulfurtransferase, mitochondrial	NM_009437	0.0026	1.34	Transport
Hyal2	hyaluronoglucosaminidase 2	NM_010489	0.0044	1.34	Glucose metabolic process
Kcnp3	Kv channel interacting protein 3, calsenilin	NM_001111331	0.0047	1.33	Apoptosis
Htr1d	5-hydroxytryptamine (serotonin) receptor 1D	NM_008309	0.0028	1.33	Cell surface receptor linked signal transduction
Agt	angiotensinogen	NM_007428	0.0061	1.32	Apoptosis
Rhobtb2	Rho-related BTB domain containing 2	NM_153514	0.0033	1.32	Intracellular signaling cascade
Hgfac	hepatocyte growth factor activator	NM_019447	0.0019	1.32	Proteolysis
Trappc6b	trafficking protein particle complex 6B	NM_030057	0.0031	1.32	Transport
Ddc	dopa decarboxylase	NM_016672	0.0023	1.31	Response to stress
Klf3	Kruppel-like factor 3	NM_008453	0.0029	1.30	Nucleus
Hip1r	huntingtin interacting protein 1 related	NM_145070	0.0047	1.21	Cytoskeleton
Gpx3	glutathione peroxidase 3	NM_001083929	0.0072	1.18	Response to stress
Fgf10	fibroblast growth factor 10	NM_008002	0.0068	1.08	Intracellular signaling cascade
Downregulated					
Pdx1	pancreatic and duodenal homeobox 1	NM_008814	0.0085	-1.99	Glucose metabolic process
Rbm3	RNA binding motif protein 3	NM_001166409	0.0019	-1.76	Transport
Fdps	farnesyl diphosphate synthetase	NM_134469	0.0047	-1.71	Lipid metabolic process
Incenp	inner centromere protein	NM_016692	0.0045	-1.64	Nucleus
Ece1	endothelin converting enzyme 1	NM_199307	0.0058	-1.62	Proteolysis
Rab6	RAB6, member RAS oncogene family	NM_001163663	0.0082	-1.54	Transport
CD47	CD47 antigen	NM_010581	0.0079	-1.53	Response to stress
Cdk2	cyclin-dependent kinase 2	NM_016756	0.0051	-1.48	Transport
Chst8	carbohydrate sulfotransferase 8	NM_175140	0.0092	-1.44	Glucose metabolic process
Gemin6	gem (nuclear organelle) associated protein 6	NM_026053	0.0057	-1.44	Nucleus
Caml	calcium modulating ligand	NM_007596	0.008	-1.39	Cell surface receptor linked signal transduction
Dok5	docking protein 5	NM_001163686	0.0094	-1.37	Intracellular signaling cascade
Phox2a	paired-like homeobox 2a	NM_008887	0.0072	-1.34	Regulation of transcription
Hk1	hexokinase 1	NM_001146100	0.0011	-1.23	Glucose metabolic process

Regulated mRNAs in Pdx1 knock down MIN6 cells. The fold change of mRNA transcripts in Pdx1 knock down MIN6 cells ($n = 3$) are shown in comparison to control ($n = 3$). Transcripts ($p < 0.01$) are presented in order of p -value, lowest to highest. 56 transcripts are upregulated, 14 are downregulated.

Supplemental methods

Quantification of mRNA levels. RNA isolation, first strand cDNA synthesis, and TaqMan gene expression assays were performed as previously described (1, 2). Applied Biosystems TaqMan assay numbers were: Hmbs, Mm00660262_g1; Bim, Mm01333921_m1; Puma, Mm00519268_m1; Bcl2l14, Mm01261010_m1.

In vivo characterization of mice. The *Pdx1*^{+/-} mice have been previously described (3), and were kindly provided by Dr. Helena Edlund (University of Umea, Umea Sweden). *Nix*^{-/-} mice have been described (4). Male mice were fed a normal chow or high fat diet containing 42% fat

(Harlan Laboratories, Inc.) from 3 weeks of age, and provided with water ad libitum. Intraperitoneal glucose tolerance tests (IPGTTs) were performed after a 4 hour fast (1 g dextrose/kg body weight). Insulin levels were measured at fasting and 30 minutes after glucose challenge. β -cell area was quantified from anti-insulin stained pancreas sections counterstained with hemotoxylin using the intensity thresholding function of the integrated morphometry package in MetaMorph. All experiments in this study using animal protocols were approved by the Washington University Animal Studies Committee.

Detection of autophagy by immunostaining. The presence of autophagosomes in islets was evaluated using immunostaining as previously described (1). For immunostaining, the mice were anesthetized and perfused through the left ventricle with 3.7% formaldehyde. The pancreas was isolated and then fixed at room temperature overnight. To quantitate the extent of autophagy, the number of LC3 punctae in insulin-positive cells was counted in five independent visual fields from at least two independent experiments.

Statistical analysis. Multiple experimental groups were compared using one-way ANOVA and Tukey's post hoc test. The 2-tailed unpaired Student's *t* test was used to assess the statistical significance of differences between 2 sets of data. Differences were considered significant when $p < 0.05$. Results are presented as mean \pm SEM.

Supplemental references

1. Fujimoto K, et al. Autophagy regulates pancreatic beta cell death in response to Pdx1 deficiency and nutrient deprivation. *J Biol Chem.* 2009;284(40):27664-27673.
2. Fujimoto K, Chen Y, Polonsky KS, Dorn GW 2nd. Targeting cyclophilin D and the mitochondrial permeability transition enhances β -cell survival and prevents diabetes in Pdx1 deficiency. *Proc Natl Acad Sci USA.* 2010;107(22):10214-10219.
3. Jonsson J, Carlsson L, Edlund T, Edlund H. Insulin-promoter-factor 1 is required for pancreas development in mice. *Nature.* 1994;371(6498):606-609.
4. Diwan A, et al. Unrestrained erythroblast development in *Nix*^{-/-} mice reveals a mechanism for apoptotic modulation of erythropoiesis. *Proc Natl Acad Sci USA.* 2007;104(16):6794-6799.