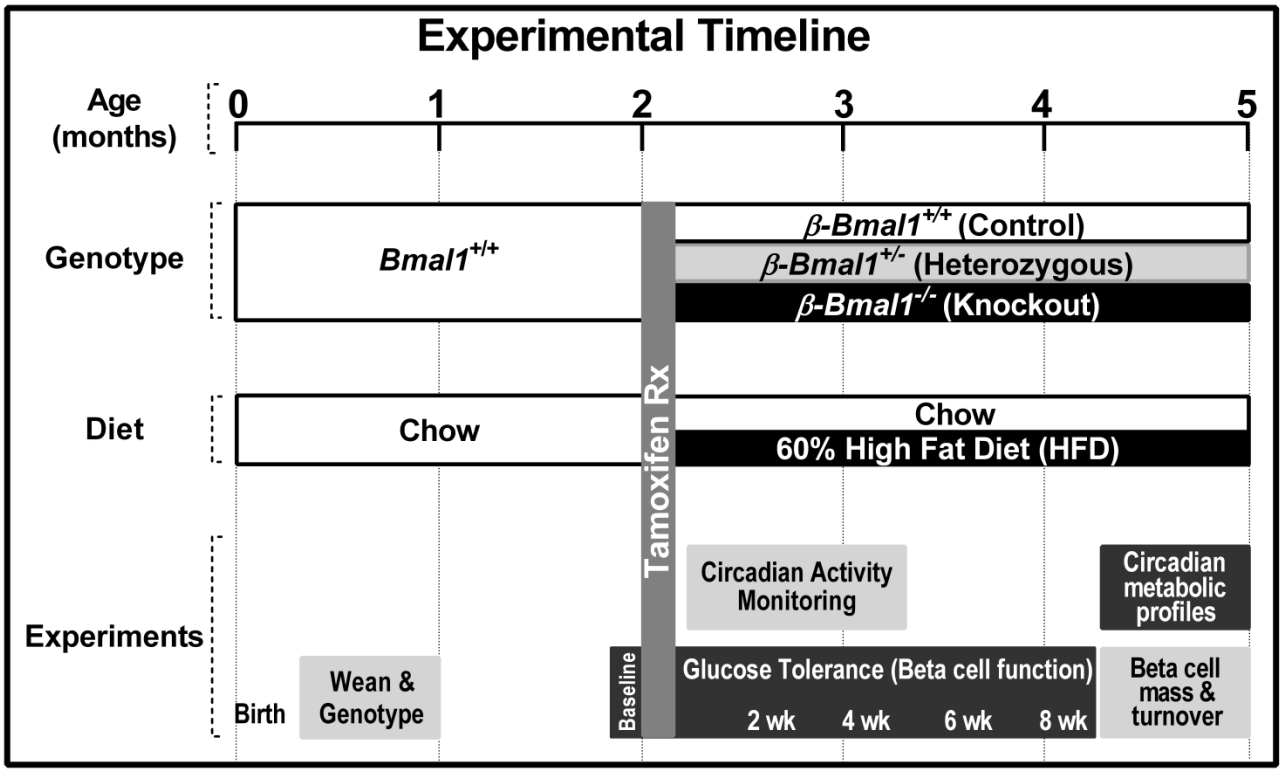
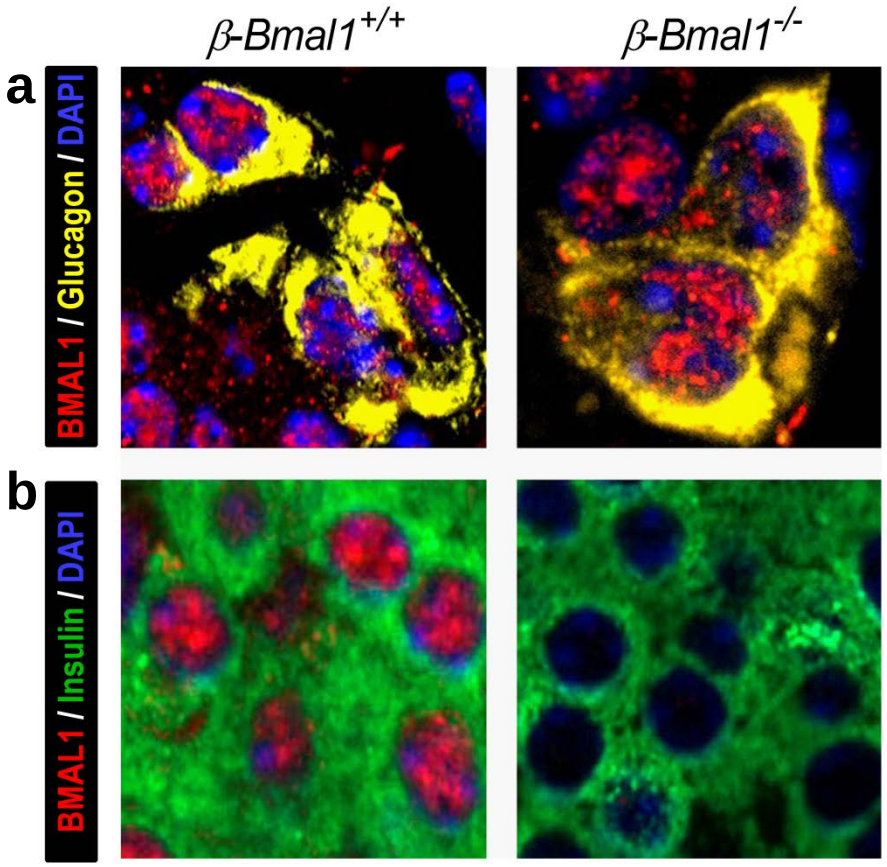
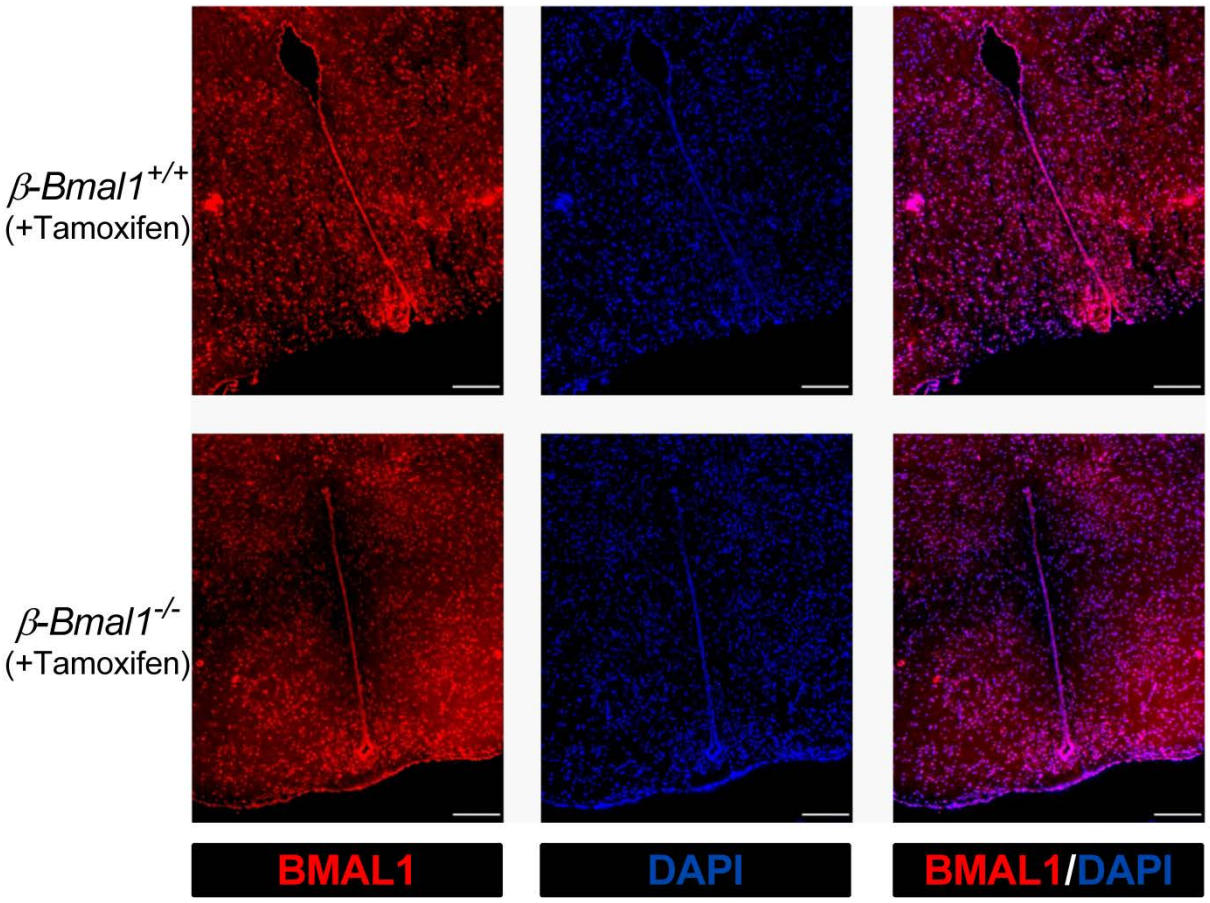


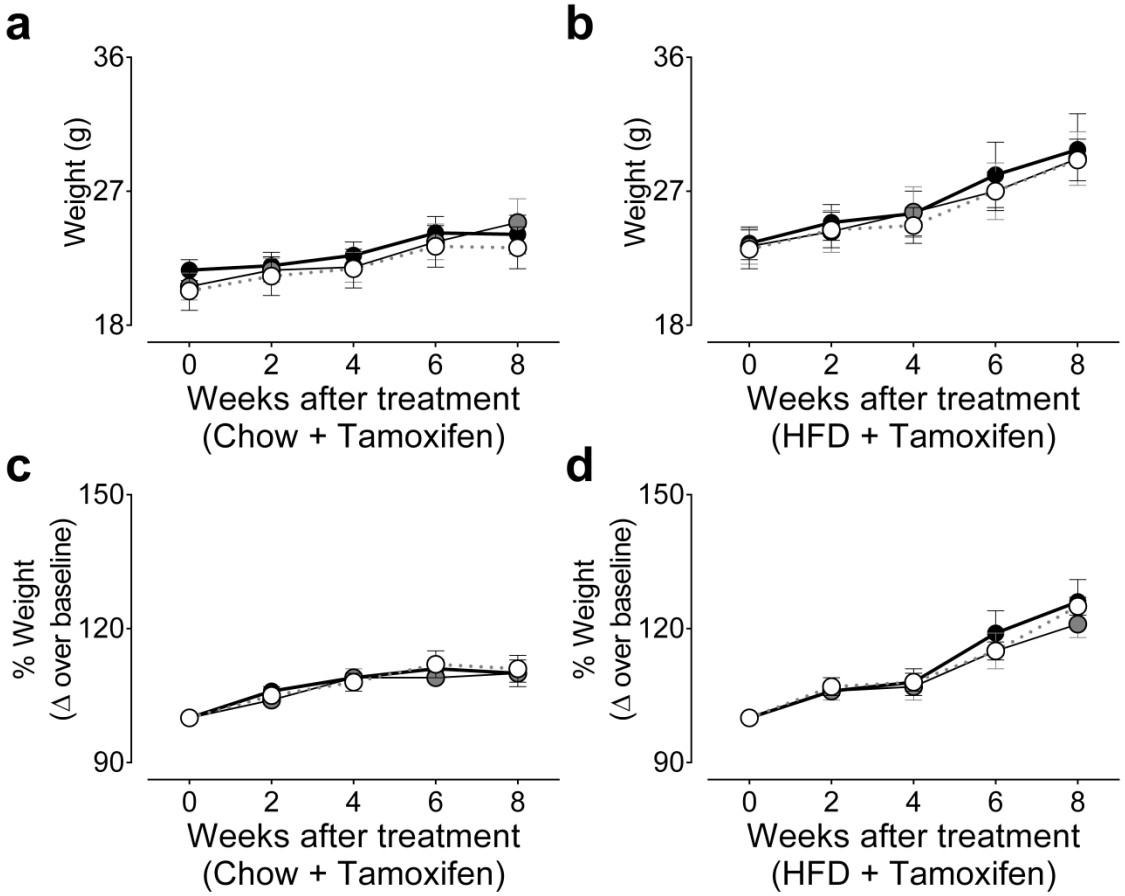
ESM Fig. 1 Diagrammatic depiction of the experimental design. Mice were fed chow diet throughout or switched to 60% high fat diet at the age of 2 months. All mice received x3 i.p. injections of tamoxifen for conditional deletion of *Bmal1* gene in beta cells. Behavioral circadian activity was monitored for a period of 3 weeks after tamoxifen dose. Glucose tolerance tests were serially conducted starting at 2 months to establish baseline before tamoxifen and chow/HFD, and subsequently 2, 4, 6, and 8 weeks after tamoxifen and chow/HFD administration. Circadian metabolic profiles, circadian mRNA expression, and beta cell turnover were assessed 8 weeks after tamoxifen and chow/HFD administration



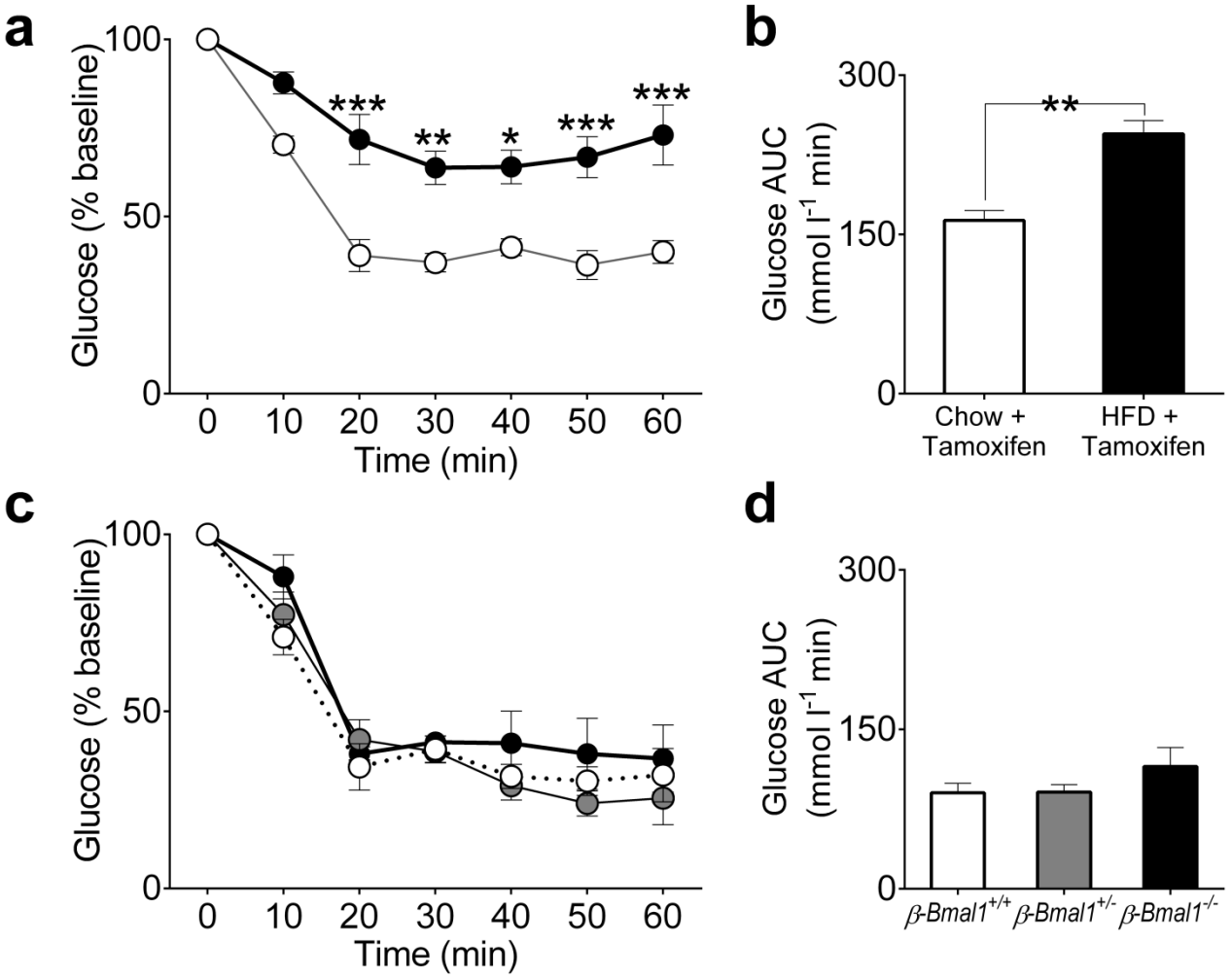
ESM Fig. 2 Beta cell-specific *Bmal1* knockout mice display BMAL1 in alpha cells. Representative images of pancreas sections taken at x63 magnification and stained by immunofluorescence for BMAL1 in red (**a–b**), glucagon in yellow (**a**), and insulin in green (**b**) in *β-Bmal1*$^{+/+}$ and *β-Bmal1*$^{-/-}$ mice 8 weeks after tamoxifen administration



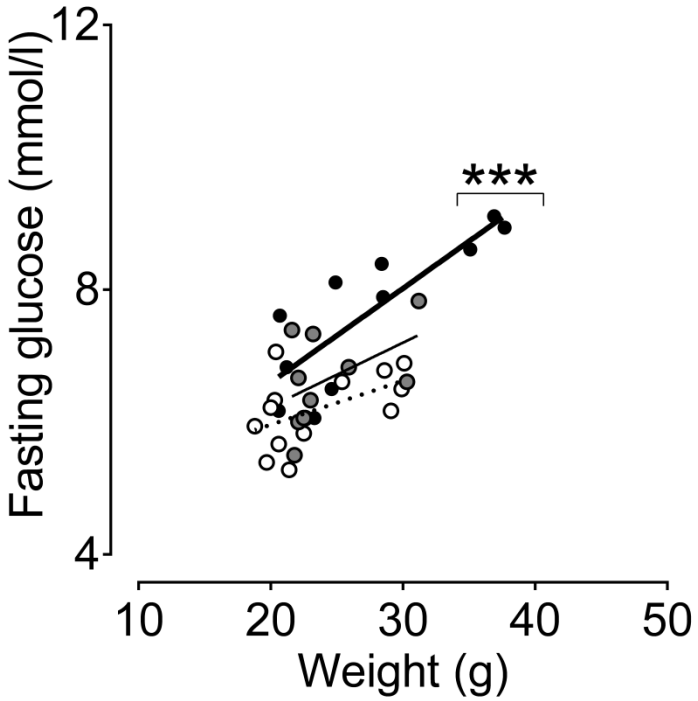
ESM Fig. 3 Beta cell-specific *Bmal1* knockout mice display normal BMAL1 expression in the hypothalamus. Representative images of frozen hypothalamic sections in the suprachiasmatic nucleus region stained by immunofluorescence for BMAL1 in red from *β-Bmal1*$^{+/+}$ and *β-Bmal1*$^{-/-}$ mice after tamoxifen administration (scale bars, 200 µm)



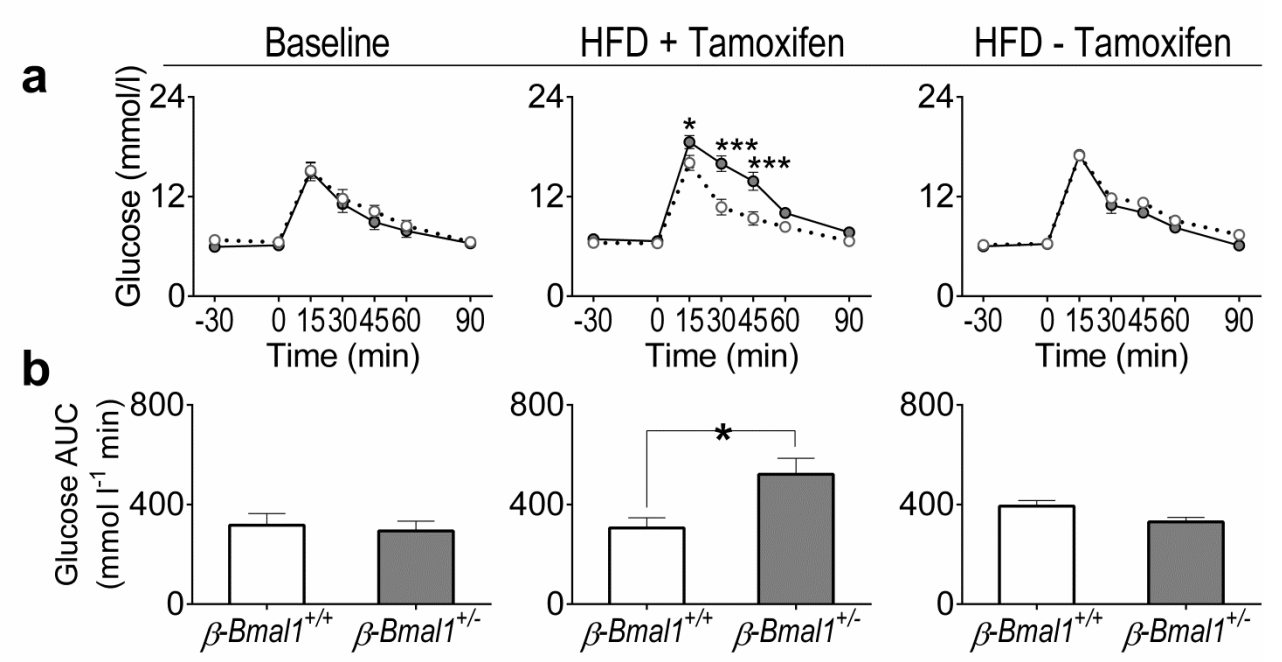
ESM Fig. 4 Beta cell-specific *Bmal1* knockout mice display similar weight gain in response to HFD as controls. Graphs showing body weight and % increase in weight of mice over 8 weeks of either (**a-b**) chow or (**c-d**) HFD treatment. (**c-d**) Baseline weight (0 week) is set at 100% for each group. (**a-d**) Data expressed as mean ± S.E.M (*n*=7-9 per group). *β-Bmal1*$^{+/+}$, white circles; *β-Bmal1*$^{+/-}$, grey circles; *β-Bmal1*$^{-/-}$, black circles



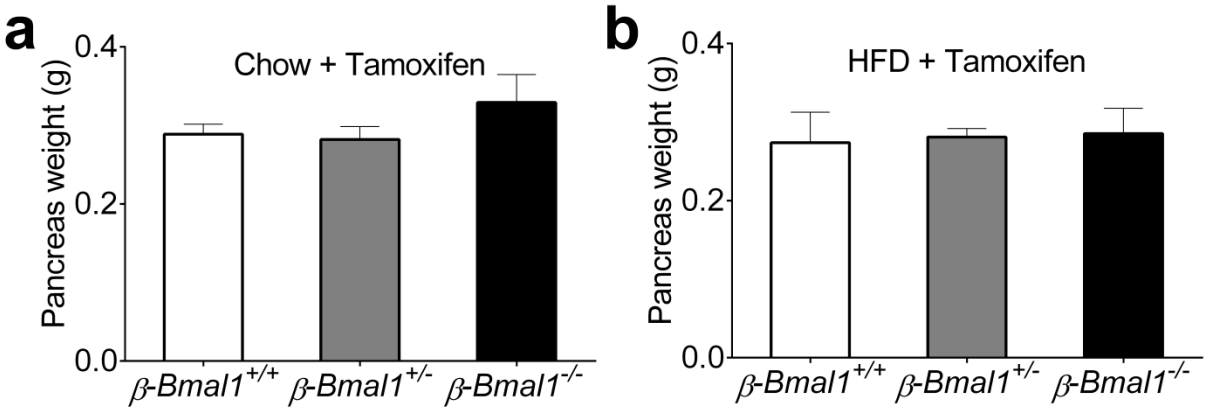
ESM Fig. 5 Beta cell-specific *Bmal1* knockout mice display similar levels of insulin tolerance in response to HFD as controls. (**a**) Change in glucose levels (% baseline glucose) and (**b**) mean glucose area under curve during an insulin tolerance test in control *β-Bmal1*$^{+/+}$ mice following 8 week exposure to chow (white circles/bars) or HFD (black circles/bars). Statistical significance is denoted by *=p<0.05, **=p<0.01 and **=p<0.001. (**c**) Change in glucose levels (% baseline glucose) and (**d**) mean glucose area under curve during an insulin tolerance test in control *β-Bmal1*$^{+/+}$ (white circles/bars) heterozygous *β-Bmal1*$^{+/-}$ (grey circles/bars), or knockout *β-Bmal1*$^{-/-}$ (black circles/bars) mice following 8 week exposure to HFD. (**a-d**) Data expressed as mean ± S.E.M (n=3-7 per group)



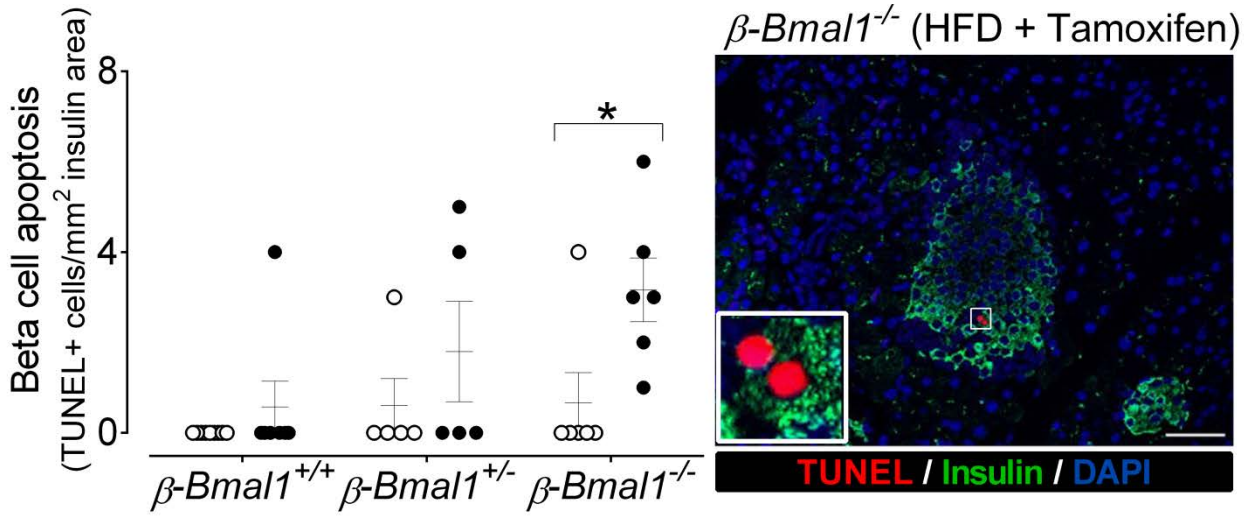
ESM Fig. 6 Beta cell-specific *Bmal1* deletion leads to significant increase in fasting glucose levels in response to increase in body weight. Graph depicting the correlation of body weight with fasting glucose levels in *β-Bmal1*$^{-/-}$ (black circles, thick line, r=0.83, p<0.001) heterozygous *β-Bmal1*$^{+/-}$ (grey circles, thin line, r=0.48, p>0.05), or control *β-Bmal1*$^{+/+}$ mice (white circles, dotted line, r=0.51, p>0.05). Data expressed as mean ± S.E.M (n=5-9 per group)



ESM Fig. 7 Beta cell-specific *Bmal1* knockout mice display metabolic phenotype only after tamoxifen administration. Graphs depicting (**a**) sampled blood glucose concentrations and (**b**) corresponding glucose AUC during 90 min i.p. glucose tolerance tests conducted at baseline and 4 weeks after tamoxifen/vehicle administration in *β-Bmal1*$^{+/+}$ (white circles/bars) and *β-Bmal1*$^{+/-}$ (grey circles/bars) mice exposed to 4 weeks of HFD. Data expressed as mean ± S.E.M. (n=3-7 per group). Statistical significance is denoted by *=p<0.05, **=p<0.01 and **=p<0.001



ESM Fig. 8 Beta cell-specific *Bmal1* deletion does not alter pancreatic weight. Mean pancreatic weight in mice following 8 week exposure to (**a**) chow and (**b**) HFD treatment in *β-Bmal1*$^{-/-}$, heterozygous *β-Bmal1*$^{+/-}$, or control *β-Bmal1*$^{+/+}$ mice. Data expressed as mean ± S.E.M (*n*=5-9 per group)



ESM Fig. 9 Beta cell-specific *Bmal1* knockout mice display significantly increased beta cell apoptosis on exposure to HFD. Graph indicating the number of TUNEL+ cells/mm^2 insulin area in control *β-Bmal1*$^{+/+}$, heterozygous *β-Bmal1*$^{+/-}$, and knockout *β-Bmal1*$^{-/-}$ mice exposed to 8 weeks of either chow (white circles) or HFD (black circles). Statistical significance is denoted by *=p<0.05. Representative image of pancreas section stained by immunofluorescence for TUNEL in red, and insulin in green in *β-Bmal1*$^{-/-}$ mice exposed to 8 weeks of HFD (scale bar, 50 μm)

ESM Methods:

Metabolic *in vivo* studies: To assess circadian metabolic profiles, blood was sampled from the saphenous vein at 1200 h (ZT 6, day) and 2400 h (ZT 18, night) and immediately stored at -80 ºC for subsequent glucose and insulin measurements. To assess glucose tolerance, mice received 1 g/kg body weight of 50% dextrose solution via i.p. injection performed at ZT 8 following brief food depravation (5 h), and blood was serially sampled from the saphenous vein at 0, 15, 30, 60, and 90 min post dextrose injection. To assess insulin tolerance, mice received 0.75 $mU.g^{-1}$ body weight of insulin solution via i.p. injection performed at ZT 8, and blood glucose was serially measured at 0, 10, 20, 30, 40, 50, and 60 min post insulin injection.

Immunofluorescence and immunohistochemistry: Mice were euthanized and pancreas immediately harvested and fixed in 4% paraformaldehyde overnight. To quantify beta/alpha cell mass, beta cell area and islet density, paraffin-embedded pancreatic sections were stained for hematoxylin, insulin (Guinea pig polyclonal, 1:100, ab7842, Abcam, Cambridge, MA, USA), and glucagon (Mouse monoclonal, 1:1000, G2654, Sigma-Aldrich, St. Louis, MO, USA). Images were acquired at 20x magnification by the Aperio ScanScope AT (Leica Microsystems, Bannockburn, IL, USA) and analyzed using ImageScope software (Leica Microsystems). Beta/alpha cell mass was measured by first quantifying the fraction of pancreatic cross-sectional area positive for insulin/glucagon and then multiplying this by the pancreatic weight. Pancreatic sections were also co-stained by immunofluorescence for insulin, glucagon, apoptosis marker TUNEL (12156792910; Roche, Nutley, NJ, USA), replication marker Ki67 (Mouse monoclonal, 1:40, 550609, BD Pharmingen, Franklin Lakes, NJ, USA), BMAL1 (Rabbit polyclonal, 1:1000, ab3350, Abcam), and hGH (Mouse monoclonal, 1:500, ab15317, Abcam). Slides were viewed using Leica DM6000 (Leica Microsystems) and Zeiss (Carl Zeiss Microscopy, LLC,

Thornwood, NY, USA) microscopes and images acquired using OpenLab 5 (PerkinElmer, Waltham, MA, USA) and ZEN Pro (Carl Zeiss Microscopy) software. Beta cell replication and apoptosis were quantified as the number of Ki67 and TUNEL positive beta cells per unit of insulin positive area. For hypothalamic BMAL1 staining, brains were perfused with 4% paraformaldehyde, frozen immediately in Optimal Cutting Temperature compound and cut using a cryostat.

Measurements of beta cell function and proliferative potential in isolated islets: Mice aged 3.5 months were euthanized and islets isolated using standard collagenase method. To assess glucose-stimulated insulin secretion *in vitro,* islet perifusion experiments were conducted using the ACUSYST-S system (Cellex Biosciences, Inc., Minneapolis, MN, USA) as previously described [1]. To assess beta cell proliferation potential, isolated islets were (100 islets per animal) were recovered overnight in standard RPMI media (11 mmol/l glucose) followed by 24 h incubation in RPMI medial supplemented with 20 mmol/l glucose containing 100 nM Exendin-4 (Sigma-Aldrich, St. Louis, MO, USA) designed to promote beta cell proliferation [2]. Total RNA was extracted from the islets using RNeasy Mini Kit (Qiagen, Valencia, CA, USA), cDNA was extracted using RT^2 PreAMP cDNA Synthesis Kit (Qiagen) and subject to Mouse Cell Cycle RT^2 Profiler^{TM} PCR Array (Qiagen) in the ABI StepOnePlus qPCR machine (Applied Biosystems, Carlsbad, CA, USA). PCR array data were analyzed by the $\Delta(\Delta C_t)$ method using the online SABiosciences platform (Qiagen).

References

[1] Song SH, Kjems L, Ritzel R, et al. (2002) Pulsatile insulin secretion by human pancreatic islets. J Clin Endocrinol Metab 87: 213-221

[2] Liu Z, Habener JF (2008) Glucagon-like peptide-1 activation of TCF7L2-dependent Wnt signaling enhances pancreatic beta cell proliferation. The Journal of biological chemistry 283: 8723-8735