## Supplemental Figures and Legends

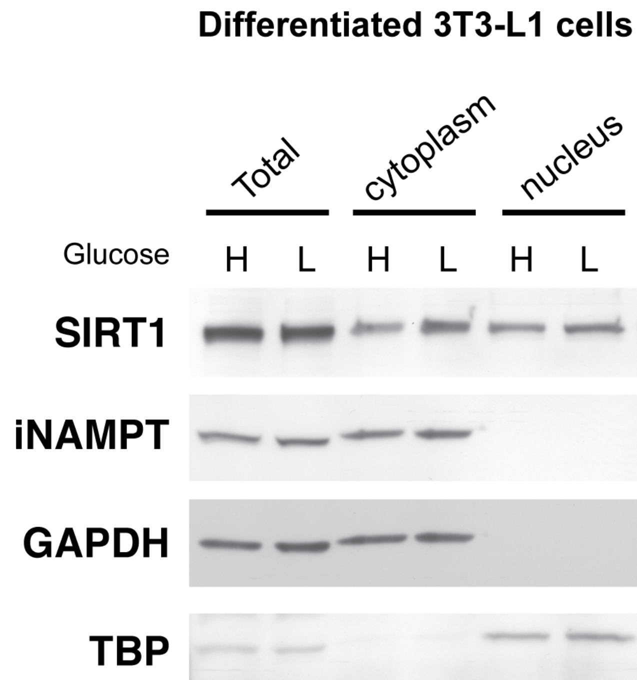



**Figure S1, related to Figure 3.** Subcellular localization of SIRT1 and iNAMPT in differentiated 3T3-L1 cells. GAPDH and TBP were examined as representative cytoplasmic and nuclear proteins, respectively. Cells were incubated with media containing high glucose (H, 25mM) or low glucose (L, 5 mM) for 3 hr.

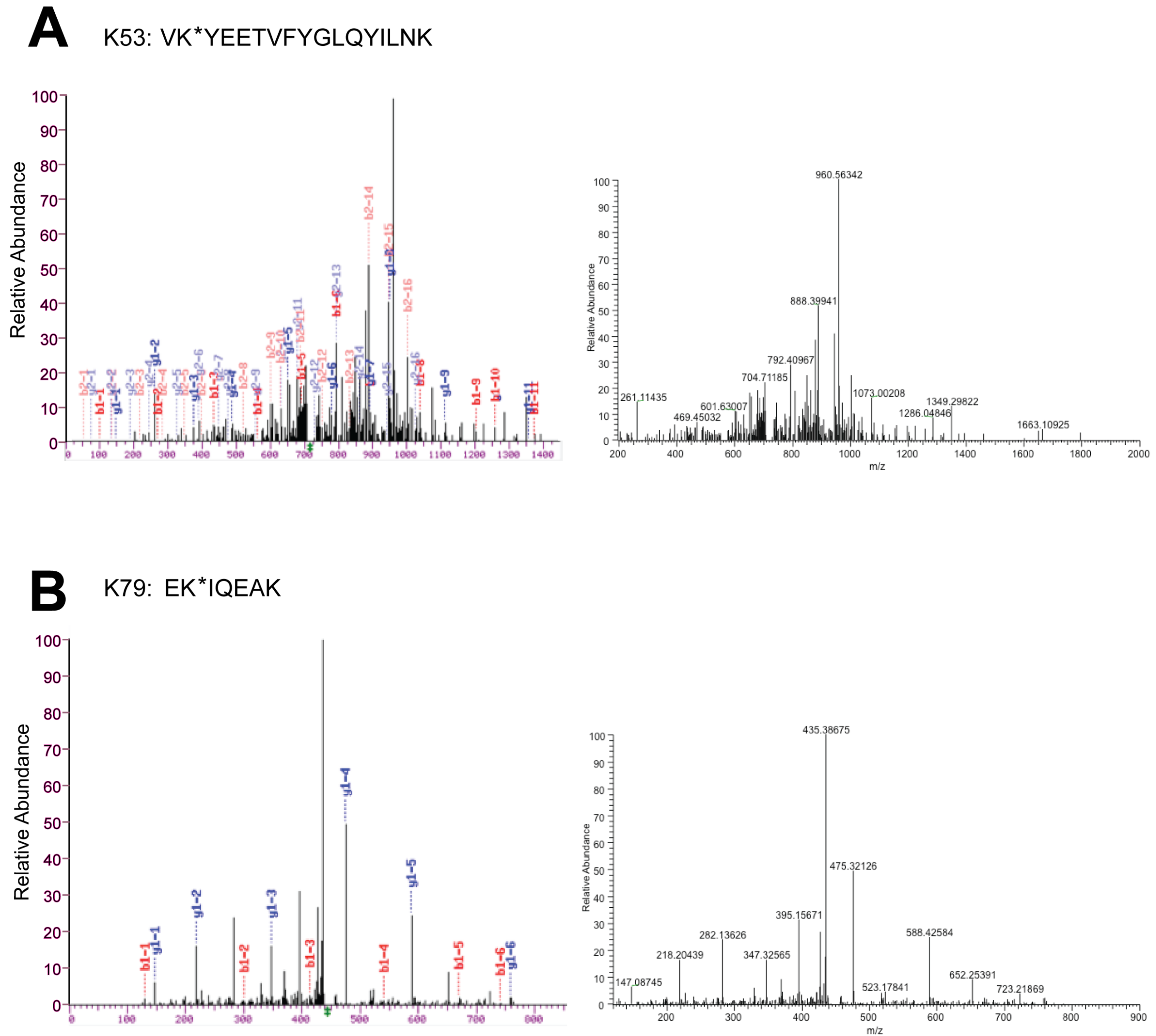



**Figure S2, related to Figure 4.** Detection of acetylated lysines 53 (K53, **A**) and 79 (K79, **B**) on mouse iNAMPT by MS/MS analysis. $K^*$ indicates acetylated lysine. Left panels display results from the search algorithm for N- and C-terminal fragments produced by breakage at the peptide bond in the mass spectrometer (b and y, respectively). The numbers represent N- or C-terminal residues present in the peptide fragment. Right panels show the raw data of the spectra.

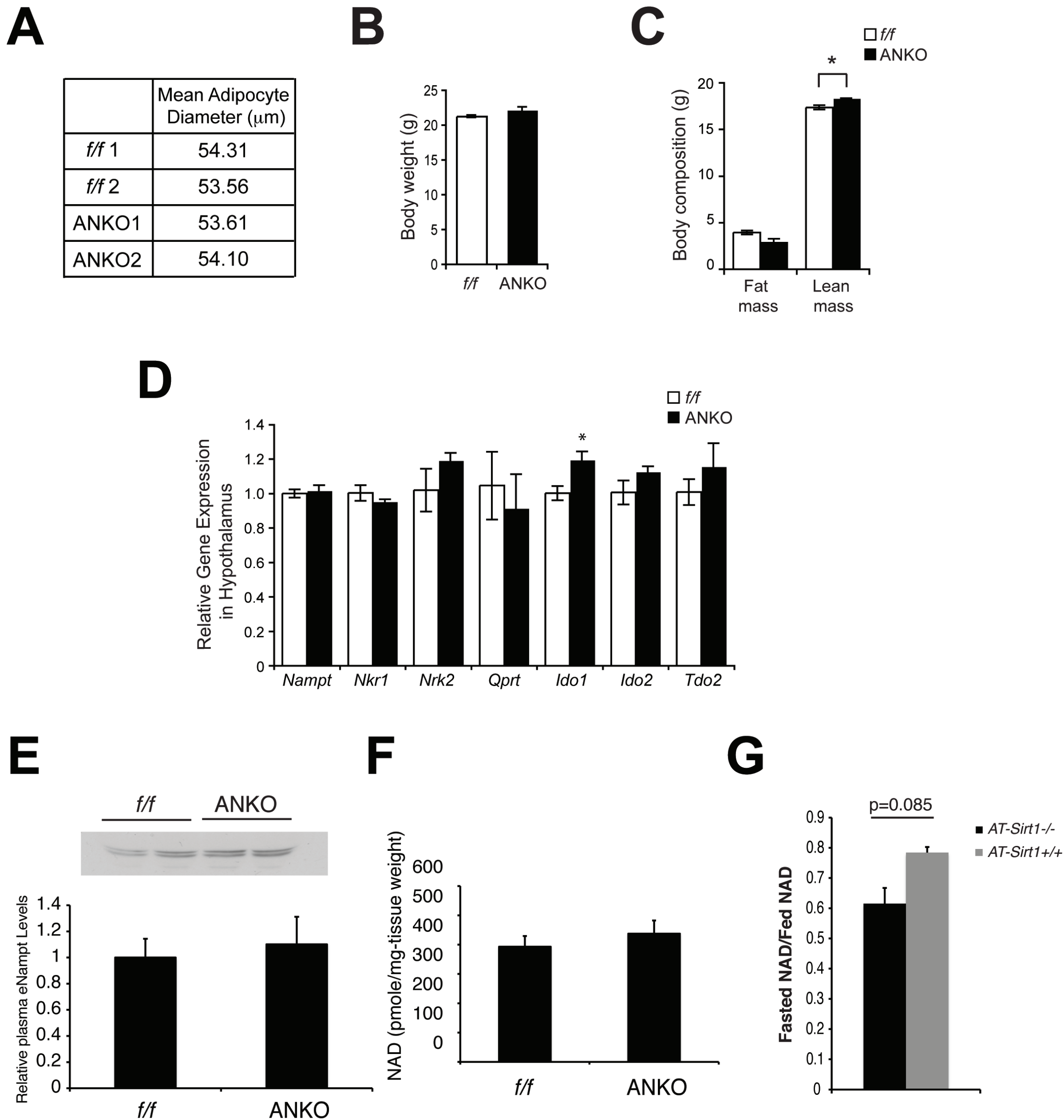
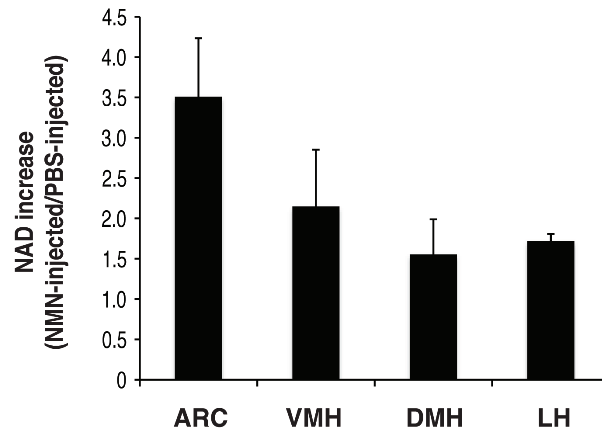


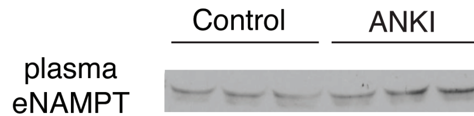

**Figure S3, related to Figure 5.** Adipose, hypothalamic, and plasma eNAMPT features of ANKO and *Nampt*$^{flox/flox}$ (*f/f*) mice, and hypothalamic NAD$^+$ ratio between fasted and fed conditions in *AT-Sirt1*$^{+/+}$ and *AT-Sirt1*$^{-/-}$ mice. **(A)** Mean adipocyte diameter values from two independent 3-4-month-old females each for control (*f/f*) and ANKO mice. **(B)** Body weights of 4-month-old control and ANKO female mice (n=9-11). **(C)** Body composition analysis of 4-month-old control and ANKO female mice by Echo-MRI (n=2-4). **(D)** Quantitative RT-PCR results for NAD$^+$ biosynthetic genes in the hypothalami of 3-4-month-old control and ANKO female mice (n=4). *Nrk*, nicotinamide ribose kinase; *Qpt*, quinolinic acid phosphoribosyltransferase; *Ido*, Indoleamine 2, 3-dioxygenase; *Tdo*, Tryptophan 2, 3-dioxygenase. (**E**) Plasma eNAMPT levels in 3-month-old control and ANKO male mice after 48-hr fasting (n=5). Representative Western blot results are shown in the upper panel. (**F**) Hypothalamic NAD$^+$ levels in 3-month-old control and ANKO male mice. NAD$^+$ levels were measured by HPLC (n=3). (**G**) Hypothalamic NAD$^+$ ratio between fasted and fed conditions in *AT-Sirt1*$^{+/+}$ and *AT-Sirt1*$^{-/-}$ female mice at 6 months of age. Hypothalami were collected from ad libitum-fed and 48 hr-fasted *AT-Sirt1*$^{+/+}$ and *AT-Sirt1*$^{-/-}$ mice, and hypothalamic NAD$^+$ levels were measured by HPLC (n=5). Data were analyzed by the Student's *t* test. All values are presented as mean ± SEM. $^*p < 0.05$.

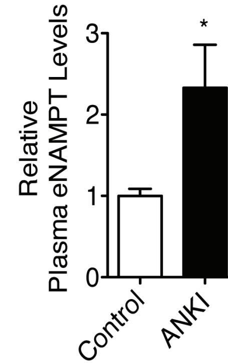
**A**



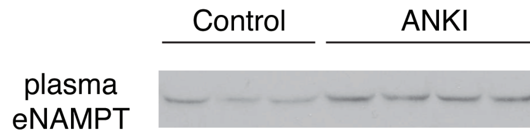

**B**

**Males**




**Females**



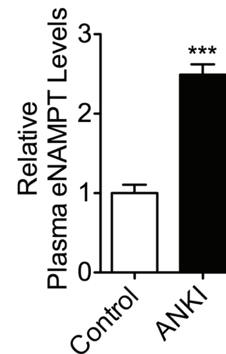




**Figure S4, related to Figure 6.** $NAD^+$ levels in individual hypothalamic nuclei 1 hr after systemic NMN administration, and plasma eNAMPT levels in ANKI and control mice. (**A**) The ARC, VMH, DMH, and LH were collected by laser-captured microdissection 1hr after NMN (500 mg/kg) or PBS (equal volume) injection to 4 month-old female mice. $NAD^+$ levels in each hypothalamic nucleus were measured by HPLC, and the fold $NAD^+$ increases were calculated (n=3). (**B**) Plasma eNAMPT levels from 48 hr-fasted control and ANKI male and female mice at 4-6 months of age. The right panels show relative plasma eNAMPT levels in ANKI mice normalized to those in control mice (n=3-4). Data were analyzed by the Student's *t* test. ***$p \leq 0.001$.

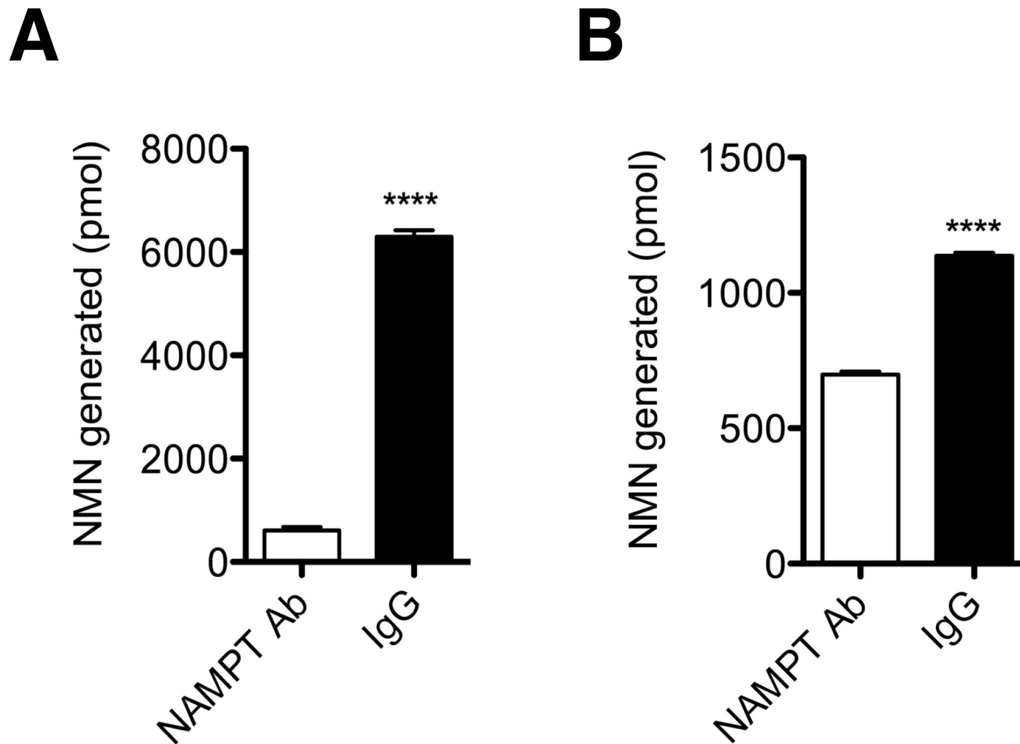



**Figure S5, related to Figure 7.** The NAMPT-neutralizing effects of an anti-NAMPT rabbit polyclonal antibody *in vitro* and *ex vivo*. (**A**) 2 μg of recombinant NAMPT produced in bacteria was incubated with 20μg of the anti-NAMPT antibody (Bethyl Laboratory, TX) or control rabbit IgG (Sigma, MO) in enzymatic reaction buffer [50 mM Tris-HCl, pH 8.5, 100 mM NaCl, 0.25 mM nicotinamide, 10 mM MgSO4, 1.5% ethanol, 0.5 mM phosphoribosyl phyrophosphate (PRPP), 2 mM ATP] for 1 hour at 37°C. (**B**) 103μl of plasma freshly collected from 6 month-old mice was incubated with 5μg of the anti-NAMPT antibody or control rabbit IgG in the enzymatic reaction buffer for 3 hours at 37°C. The amounts of NMN generated were measured using HPLC (n=3 in each experiment). Data were analyzed by the Student's *t* test. All values are presented as mean ± SEM. ****$p \leq 0.0001$.

## Supplemental Experimental Procedures

### RNA isolation and analysis

Total RNA from WAT and BAT was isolated using the RNeasy lipid tissue mini-kit (Qiagen, CA). Total RNA samples from all other tissues were isolated using the RNeasy mini-kit (Qiagen, CA). cDNA was synthesized using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, CA) with random primers. Quantitative real-time RT-PCR was performed using the 7500 Fast Real-Time PCR system (Applied Biosystems, CA). Relative expression levels were determined based on Ct values and normalized to *Gapdh*.

### Differentiation of 3T3L1 and HIB-1B preadipocyte cell lines

All cell lines were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum and antibiotics. Confluent cultures of HIB-1B brown pre-adipocytes were differentiated by adding 0.5 mM isobutylmethylxanthine (IBMX), 1 mM dexamethasone, 5 mg/ml insulin, and 1 nM triiodothyronine (T3) for 2 days and then changing to the media containing 5 mg/ml insulin and 1 nM T3 every other day for 6 more days. 3T3-L1 white pre-adipocytes were differentiated similarly, except that T3 was absent in the media through the whole differentiation process. All chemicals were purchased from Sigma.

**MS analysis of acetylated lysine sites on NAMPT**

Recombinant mouse NAMPT was expressed and prepared as described previously (Revollo et al., 2004). iNAMPT and eNAMPT were immunoprecipitated from HEK293 cells and from media of differentiated HIB-1B cells expressing NAMPT-FLAG, respectively, as described above. Each protein was resolved by 10% SDS/PAGE and visualized by Coomassie blue staining. The bands of these proteins were excised and subjected to MS analysis provided by the Taplin Biological Mass Spectrometry Facility at Harvard Medical School.

**Subcellular protein fractionation**

Cytoplasmic and nuclear protein fractions were separated and prepared using the Subcellular Protein Fractionation Kit (Thermo Scientific, IL) according to the manufacturer's protocol.

**$NAD^+$ measurements**

Frozen tissues were extracted in perchloric acid and neutralized in $K_2CO_3$ on ice as described previously (Ramsey et al., 2009). $NAD^+$ levels were determined using an HPLC system (Shimadzu, Japan) with a Supelco LC-18-T column (15 cm x 4.6 cm; Sigma, MO). The HPLC was run at a flow rate of 1 ml/min with 100% buffer A (50 mM phosphate

buffer, pH 7.4) from 0-5 min, a linear gradient to 95% buffer A /5% buffer B (100% methanol) from 5-6 min, 95% buffer A /5% buffer B from 6-11 min, a linear gradient to 85% buffer A /15% buffer B from 11-13 min, 85% buffer A /15% buffer B from 13-23 min, and a linear gradient to 100% buffer A from 23-24 min. $NAD^+$ is eluted at 11 min. $NAD^+$ levels were quantitated based on the peak area compared to a standard curve and normalized to the weight of the frozen tissue.

**Generation of eNAMPT-containing conditioned media**

HEK293 cells were transfected with pCXN2-Nampt-FLAG and selected by incubating with 500 μg/ml of G418 (Invitrogen, CA) for 2 weeks. Subsequently selected cells were transduced with FM1-SIRT1 lentivirus to promote eNAMPT secretion. NAMPT/SIRT1-overexpressing or control HEK293 cells were plated and grown to confluency in 12-well plate. Supernatants were collected after incubating cells for 72 hr with serum-free neurobasal media. Collected conditioned media were centrifuged at 3000 rpm for 2 min at 4°C, concentrated 10-fold with Amicon Ultra-30 columns (Millipore, MA). The concentration of eNAMPT was approximately 160 ng/ml in eNAMPT-containing conditioned media.

## Supplemental References

Ramsey, K.M., Yoshino, J., Brace, C.S., Abrassart, D., Kobayashi, Y., Marcheva, B., Hong, H.K., Chong, J.L., Buhr, E.D., Lee, C., et al. (2009). Circadian clock feedback cycle through NAMPT-mediated $NAD^+$ biosynthesis. Science *324*, 651-654.

Revollo, J.R., Grimm, A.A., and Imai, S. (2004). The NAD biosynthesis pathway mediated by nicotinamide phosphoribosyltransferase regulates Sir2 activity in mammalian cells. J. Biol. Chem. *279*, 50754-50763.