Revollo, Körner *et al.*

Supplementary Materials

Experimental Procedures

Detection of intracellular Nampt in tissues

C57BL/6 mice at 3-4 months of age, which were fed ad libitum, were sacrificed by carbon dioxide asphyxiation. Organs were immediately collected, homogenized in 1X Laemmli's SDS sample buffer, and boiled for 5 minutes. Samples were then centrifuged at 20,000g to remove debris, and protein concentrations were measured by the Bradford assay (BIORAD, CA). 22.5 μg of tissue extracts were analyzed by Western blotting with a rabbit polyclonal anti-Nampt antibody as described previously (Revollo et al., 2004).

Differentiation of 3T3-L1, HIB-1B and SGBS preadipocyte cell lines

All mouse 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 preadipocytes were differentiated by adding 5 μg/ml insulin, 0.5 mM isobutylmethylxanthine (IBMX), 1 μM dexamethasone, and 1 nM triiodothyronine (T3, Sigma) for 2 days and then re-feeding the media containing 5 μg/ml insulin and 1 nM T3 every other day for 6 more days. 3T3-L1 white preadipocytes were differentiated similarly, except that T3 was absent in the media through the whole differentiation process.

Human SGBS preadipocyte cell line (Wabitsch et al., 2001), kindly provided by Dr. M. Wabitsch (University of Ulm, Germany), were grown and differentiated into mature adipocytes as described previously (Körner et al., 2005). Briefly, preadipocytes at confluence were exposed to serum-free basal medium supplemented with 10 μg/ml apo-transferrin, 20 nM human recombinant insulin, 10 nM hydrocortisone, and 0.2 nM tri-iodothyroxine. For the first 4 days, this adipocyte culture medium was additionally supplemented with 25 nM dexamethasone, 500 μM IBMX, and 2 μM rosiglitazone.

iNampt and eNampt proteins were detected by Western blotting with whole cell extracts or culture supernatants and anti-Nampt antibodies. A rabbit polyclonal (Revollo et al., 2004) and a mouse monoclonal antibody (OMNI379, ALX-804-717, Axxora, Lörrach, Germany) were used for these experiments.

Expression and immunoprecipitation of intra- and extracellular Nampt-FLAG

A C-terminally FLAG-tagged mouse Nampt (Nampt-FLAG) cDNA was created with the following forward and reverse primers cotaining EcoRI sites:

Forward: TTAGAATTCAGCCCATTTTTCTCCTTGCT; Reverse:

AATGAATTCTACTTATCGTCGTCATCCTTGTAATCTCCTCCATGAGGTGCCACGT

CCTGCTCGATGTT.

The resulting cDNA was confirmed by sequencing. The Nampt-FLAG cDNA was then cloned into the mammalian expression vector pCXN2. To create HIB-1B cell lines

expressing Nampt-FLAG, HIB-1B cells were transfected with pCXN2-Nampt-FLAG or pCXN2 and selected at 500 μg/ml of G418 (Invitrogen, CA) for 2 weeks.

For immunoprecipitation of intracellular Nampt-FLAG, whole cell extracts were prepared with ice-cold immunoprecipitation (IP) buffer (phosphate buffer saline [pH 7.4], 0.5% NP-40, 1mM EDTA, 1mM NaF, 10 μM Trichostatin A, 10 mM nicotinamide, 0.5 mM DTT, protease inhibitor cocktail (Roche)) and mixed with agarose beads conjugated with the mouse monoclonal M2 anti-FLAG antibody (F2426, Sigma) for 3-4 hours at 4 °C. For immunoprecipitation of extracellular Nampt-FLAG, HIB-1B culture supernatants were collected after incubating differentiated HIB-1B cells overnight with DMEM without fetal bovine serum but supplemented with 1 μg/ml insulin and 1 nM triodothyronine, filtered through a 0.22-μm PES membrane, concentrated with Amicon Ultra-15 columns (Millipore, MA), and mixed with anti-FLAG beads for 3-4 hours at 4 °C. Immunoprecipitates were washed twice with the IP buffer and twice with PBS.

Enzymatic reactions

Nampt enzymatic reactions were conducted as previously described (Revollo et al., 2004). Briefly, immunoprecipitates on anti-FLAG beads were incubated in enzymatic reaction buffer (50 mM Tris-HCl (pH8.5), 100 mM NaCl, 0.25 mM nicotinamide, 10 mM $MgSO_4$, 1.5% ethanol, 0.5 mM PRPP, 2.0 mM ATP) for 55 min at 37 °C. After this reaction, mouse recombinant nicotinamide mononucleotide adenylyltransferase and yeast alcohol

dehydrogenase (Sigma) were added at 10 μg/ml as the final concentration for each, and the mixture was incubated for 5 min at 37 °C. Supernatants were then collected by spinning down anti-FLAG beads, and autofluorescence of NADH was measured in a Perkin Elmer LS 50B fluorometer (excitation: 340 nm; emission: 460 nm). Values of NADH autofluorescence were converted to the amounts of NMN using the standard curve drawn with known amounts of NMN. Immunoprecipitates bound on anti-FLAG beads were extracted with Laemmli's sample buffer, boiled for 5 minutes, and analyzed by Western blotting with anti-Nampt antibodies. The amounts of Nampt used for enzymatic reactions were quantitated compared to the standards of mouse recombinant Nampt. k_{cat} values were calculated based on the molar amount of NMN generated per the molar amount of immunoprecipitated Nampt-FLAG protein per unit time.

eNampt secretion assays

C-terminally FLAG-tagged versions of mouse dihydrofolate reductase (Dhfr, a gift from Dr. Wayne Yokoyama, Washington University) and bovine preprolactin (Ppl, a gift from Dr. Christopher Nicchitta, Duke University) were created as intracellular and extracellular protein controls (pCXN2-Dhfr-FLAG and pCXN2-Ppl-FLAG), respectively, and HIB-1B preadipocyte cell lines stably expressing these proteins were generated.

HIB-1B cells expressing Nampt-FLAG, Dhfr-FLAG, and Ppl-FLAG were differentiated as described above. To detect intracellular FLAG-tagged proteins, cell

extracts were collected and immunoprecipitated as described above. To detect extracellular FLAG-tagged proteins, culture supernatant (~8 ml) were collected and immunoprecipated overnight at 4 °C with anti-FLAG antibody-conjugated beads and analyzed by Western blotting with a rabbit polyclonal anti-FLAG antibody (Sigma, MO). For the brefeldin A (BFA) experiment, cells were treated with BFA (0.5 μg/ml, Sigma, MO) for 12 hours.

Chinese hamster ovary (CHO) cells at 50-60% confluency were transfected with pCXN2, pCXN2-Dhfr-FLAG, pCXN2-Ppl-FLAG, and pCXN2-Nampt-FLAG. Cell extracts and culture supernatants were collected 48 hrs after transfection, subjected to immunoprecipation with anti-FLAG antibody-conjugated beads, and analyzed by Western blotting as described above.

Adipogenesis, glucose uptake, insulin signaling assays

Recombinant eNampt/visfatin proteins produced in E. coli (ALX-201-319) or HEK293 cells (ALX-201-336) were obtained from Axxora (Lörrach, Germany) for these experiments. Mouse fibroblast cell lines in which the IGF-I receptor is disrupted (R-) and the human insulin receptor was stably overexpressed (R-IR) (Miura et al., 1995) were kindly provided by Dr. R. Smith (Division of Endocrinology, Rhode Island Hospital, Providence, Rhode Island). R- and R-IR cells were cultured in DMEM supplemented with 10% FBS and G418 (R-, 200 μg/ml) or puromycin (R-IR, 2.5 μg/ml).

For adipogenesis assays, human SGBS adipocytes differentiated with insulin or eNampt/visfatin were washed with PBS for 5 min and subsequently exposed to Sudan III staining solution for 30 min to stain intracellular lipids, followed by washing in 50% ethanol for 3 min before counterstaining with Mayer's hemalum solution (Merck KGaA, Darmstadt, Germany). Quantitation of adiponectin and PPARγ mRNA levels was performed as described previously (Körner et al., 2005). Simultaneous amplification of 18S ribosomal RNA (Applied Biosystems Applera Deutschland GmbH, Darmstadt, Germany) was used as an internal control and the amounts of target gene were normalized to the amount of the internal control of 18S.

For glucose uptake assays, fully differentiated SGBS and 3T3-L1 adipocytes were incubated in 20mM Hepes-Krebs-Ringer/0.1% BSA buffer, pH 7.4 (136 mM NaCl, 4.7 mM KCl, 1.25 mM $MgSO_4$, 1.25 mM $CaCl_2$) for 2h at 37°C and subsequently stimulated with insulin or eNampt/visfatin for 2h at indicated concentrations. Glucose uptake was determined by adding [^{14}C]-2-deoxyglucose to a final concentration of 0.5 μCi/ml for 5 min. The reaction was stopped by adding 10 mM 2-deoxyglucose with subsequent washing in ice-cold PBS, and cell lysates were prepared in 0.1% SDS. Radioactivity was determined after adding scintillation fluid (Opti-Phase-SuperMix, Perkin Elmer) using a WALLACE 1409 DSA Liquid Scintillation counter. Analyses were performed in quadriplicates. Glucose uptake was normalized to the amount of protein in each sample.

For insulin signaling assays, cells were seeded in 6-well plates and serum-starved overnight prior to stimulation with the indicated concentrations of insulin or eNampt/visfatin for 5 or 15 min under serum free conditions. Cells were washed once with ice-cold PBS containing 1 mM Na_3VO_4 and 50 mM NaF and lysed in ice-cold HEPES lysis buffer (50 mM HEPES, pH 7.5, 150 mM NaCl, 10 mM EDTA, 2 mM Na_3VO_4, 20 mM NaF, protease inhibitor cocktail complete (Roche Diagnostics GmbH, Mannheim, Germany), 1 % (v/v) Triton X-100). Western blot analyses of insulin receptor (Tyr 1150/1151) and Akt (Thr308, Ser473) phosphorylation were performed with specific antibodies against these phosphorylated proteins (Cell Signalling Technology, Denvers, USA). Membranes were re-blotted with antibodies against non-phosphorylated proteins. Signal intensities of bands were quantitated using the AIDA image analyzer v3.44 software (Raytest GmbH, Straubenhardt, Germany).

For immunoprecipitation, cells were lysed in ice-cold modified RIPA buffer (Tris-HCl: 50 mM, pH 7.4, NP-40: 1%, Na-deoxycholate: 0.25%, NaCl: 150 mM, EDTA: 1 mM, 2 mM Na_3VO_4, 20 mM NaF, protease inhibitor cocktail complete (Roche Diagnostics GmbH, Mannheim, Germany). 250 μg of cell lysate was incubated with 5 μg of a mouse monoclonal antibody against insulin receptor β subunit (29B4, Santa Cruz Biotechnology (SCBT), Heidelberg, Germany) overnight and precipitated with Protein G agarose beads (SCBT, Germany). Subsequently, immunoprecipitates were blotted with a pan-phospho-tyrosine antibody (p-Tyr 100, Cell Signalling Technology, Denvers, USA).

Blots were re-probed with an insulin receptor antibody (C-19, SCBT, Germany).

Production and characterization of Nampt$^{+/-}$ mice

The original ES cell line, RRT307, was obtained from Bay Genomics (San Francisco, CA). For this RRT307 ES cell line, the information provided by Bay Genomics indicated that the β-geo exon-trap vector was inserted between exon 8 and 9 in the mouse *Nampt/PBEF/visfatin* gene locus. To map the exact location of the exon-trap vector, PCR was performed with tail DNA samples from Nampt$^{+/-}$ mice and specific primer sets. Sequencing of the PCR product located the vector 127 bp downstream of exon 8. The β-geo genomic fragment and the Nampt-β-geo fusion mRNA was also detected by PCR and RT-PCR, respectively. Primer sequences are available upon request.

Nampt$^{+/-}$ mice were backcrossed to the C57BL/6 background at least six times. Homozygous mice were found to be embryonic lethal. Nampt$^{+/-}$ and control littermates at 3-4 months of age were sacrificed by carbon dioxide asphyxiation. Extracts of heart, liver, kidney, and BAT were prepared and analyzed as described above. The Nampt-β-geo fusion protein was detected by a mouse monoclonal anti-β-gal antibody (Roche). Plasma eNampt was detected in 2 μl of mouse plasma by Western blotting with an anti-Nampt antibody. For the measurement of tissue total NAD levels, tissue samples of BAT and liver were extracted in 1M perchloric acid (10 μl and 20 μl per 1 mg of wet tissue for BAT

and liver, respectively). The extracts were neutralized with 3M K_2CO_3, and total NAD levels were measured by HPLC as described previously (Revollo et al., 2004).

Percent islet area and islet morphology

Pancreata were fixed overnight in 4% paraformaldehyde and embedded in paraffin. Consecutive 4 μm sections were made, mounted on slides, and stained with HE by the Washington University Histology Core facility. The percent islet area was quantitated using the ImageJ software (n=3 for each genotype).

Glucose and insulin measurements

Glucose levels were determined using the Accu-Chek II glucometer (Roche Diagnostics) with blood collected from the tail vein. For determining insulin levels, blood was collected from the tail vein into chilled heparinized capillary tubes, and plasma was separated by centifugation and stored at -80°C. Insulin levels were determined on 5-10 μl aliquots using rat insulin ELISA kits with mouse insulin standards (ALPCO) at the Washington University RIA Core Facility.

Intraperitoneal glucose tolerance tests (IPGTTs) and insulin tolerance tests (ITTs)

For IPGTTs, after mice were injected with PBS or NMN (500 mg/kg body weight) and fasted for 14 hrs, dextrose (3 g/kg body weight) was injected intraperitoneally, and blood

glucose levels were measured at 0, 15, 30, 60, and 120 min after injection. Plasma was also collected at 0, 15, and 30 min time points after glucose injection and submitted for insulin measurements to the Washington University RIA Core facility. For ITTs, after female mice were fasted for 4 hrs, human insulin (0.75 U/kg body weight) (Lilly) was injected intraperitoneally to the mice, and blood glucose levels were measured at 0, 15, 30, 45, and 60 min after insulin injection. All animal procedures were approved by the Washington University Animal Studies Committee.

Glucose-stimulated insulin secretion from primary islets

Islets were isolated by collagenase digestion as described previously (Moynihan et al., 2005). Briefly, pancreata were inflated with isolation buffer (10x HBSS, 10 mM HEPES, 1 mM $MgCl_2$, 5 mM glucose [pH 7.4]) containing 0.375 mg/ml collagenase (Sigma) via the pancreatic duct after clamping off its entry site to the duodenum. The inflated pancreas was then removed, incubated at 37°C for 12–15 min, and shaken vigorously. Islets were separated from acinar tissue after a series of washes and passages through a 70 μm nylon BD Falcon Cell Strainer (BD Biosciences). Hand-picked islets were cultured overnight in RPMI media containing 1 μM nicotinamide, 5 mM glucose, 2 mM L-glutamine, penicillin/streptomycin, and 10% fetal bovine serum. The islets were then preincubated in oxygenated Krebs-Ringer bicarbonate buffer (KRB) (1 μM nicotinamide, 119 mM NaCl, 4.7 mM KCl, 25 mM $NaHCO_3$, 2.5 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, and

0.25% radioimmunoassay grade BSA) supplemented with 2 mM glucose for 1 hr at 37°C. Islets of similar size were hand-picked into groups of ten islets in triplicate and incubated with 1 ml KRB buffer containing either 2 mM glucose and 20 mM glucose for 1 hr at 37°C.

For the treatments with NMN, FK866, or the combination of these two compounds, primary islets were pooled from three or four wild-type B6 mice and grouped for each experimental condition. Islets were cultured overnight or for up to 48h in RPMI media containing 50-100 μM NMN and/or 10 nM FK866 prior to insulin secretion experiments.

NAD and NMN measurements

HPLC was performed as described previously (Revollo et al., 2004) with Waters 515 pumps and a 2487 detector (Waters, MA) with a Supelco LC-18-T column (15 cm × 4.6 cm; Supelco, PA). For these measurements, the sensitivity of the detector (AUFS; Absorbance Units Full Scale) was set at 0.01, and 5 and 10 pmole of NAD and NMN, respectively, can be detected reliably with this sensitivity.

For NAD measurements in primary islets, primary islets (~100 per sample) were cultured in 6 cm dishes and harvested 24-48 hrs later in 800 μl of ice-cold PBS. Islets were then spun down and lysed with 100 μl of 1M perchloric acid on ice for 15 min. Lysates were cleared by centrifugation and neutralized by adding 33 μl of 3M K_2CO_3 and incubating on ice for 10 minutes. After centrifugation, 100 μl of the supernatant were

mixed with 300 μl of Buffer A (50 mM K_2PO_4/$KHPO_4$, pH 7.0) and loaded onto the column. The HPLC was run at a flow rate of 1 ml/min with 100% Buffer A from 0 to 5 min, a linear gradient to 95% Buffer A and 5% Buffer B (100% methanol) from 5 to 6 min, 95% Buffer A and 5% Buffer B from 6 to 11 min, a linear gradient to 85% Buffer A and 15% Buffer B from 11 to 13 min, 85% Buffer A and 15% Buffer B from 13 to 23 min, and a linear gradient to 100% Buffer A from 23 to 24 min. NAD eluted as a sharp peak at 15 min. The amounts of NAD were quantitated based on the peak areas compared to a standard curve. The measurements were conducted in triplicates of primary islets pooled from three or four individual mice.

For NMN measurements, the HPLC was run at a flow rate of 0.7 ml/min in an isocratic condition with Buffer A. 10 μl of freshly collected mouse plasma were extracted with 100 μl of 1M perchloric acid, and the extracts were neutralized by adding 33 μl of 3M K_2CO_3 and incubating on ice for 10 minutes. After clearing extracts, 25 μl of the plasma extract were mixed with 275 μl of Buffer A or 175 μl of Buffer A plus 100 μl of water. NMN eluted as a sharp peak at ~3 min. The amounts of NMN were quantitated based on the peak areas compared to a standard curve. The measurements were conducted with three or four individual mice for each sex and genotype.

Ion trap tandem mass spectrometry analysis of the NMN peak fraction

Mass spectrometry was performed using a QTRAP-4000 instrument (Applied Biosystems,

Foster City, CA) fitted with a nanospray II interface. Fractions from the above-described HPLC analysis and standards of NMN were infused at a flow rate of 800 nl per min (Harvard Apparatus, Holliston, MA) in a solution containing 5 mM ammonium formate and methanol (50:50, v/v) as previously described (Yamada et al., 2006). The ion spray voltage was 2.0 keV with an interface heater temperature of 40^{o}C. The instrument was operated in the 'enhanced product ion scan' mode. The parent mass was set to 335.2 for NMN, and the product ion spectrum was acquired over an *m/z* range between 100 to 400 per 0.5 s. The optimum voltage setting for the declustering potential and collision energy were 30 and 10, respectively. The Analyst software (version 1.4) was used for spectral acquisition and data processing

Recombinant Nampt injection into wild-type mice

1000 pmoles of the bacterially produced recombinant protein of mouse His-tagged Nampt diluted in PBS were injected intraperitoneally to ad libitum-fed wild-type B6 males. A similar volume of PBS were injected as a control. Blood glucose levels were monitored with a glucometer at 0, 30, and 60 min time points after injection.

Statistical analysis

All values are expressed as mean ± standard error (SE), and statistical analyses were carried out using an unpaired Student's t test. Differences were considered to be statistically significant when $p \leq 0.05$.

Supplementary References

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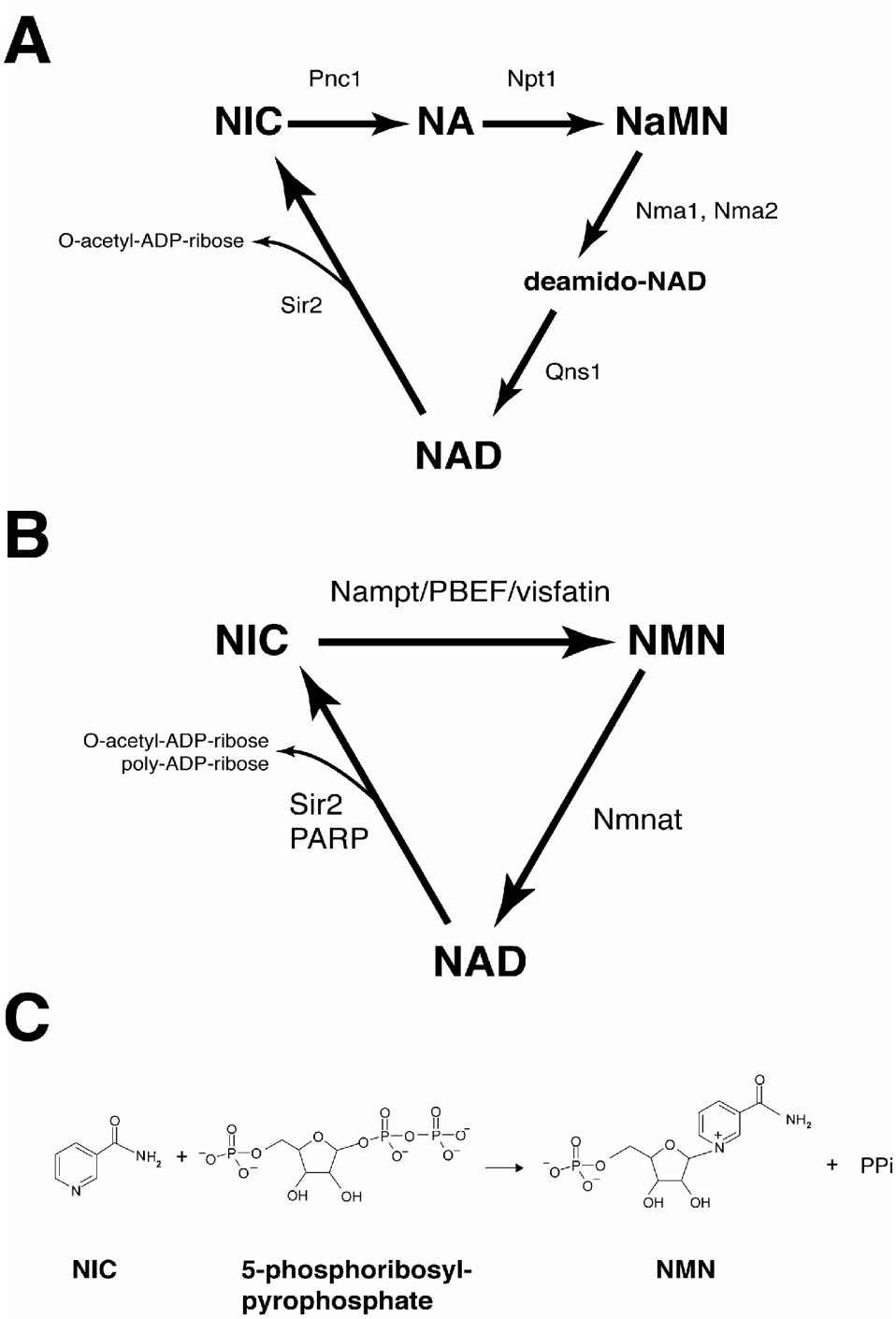
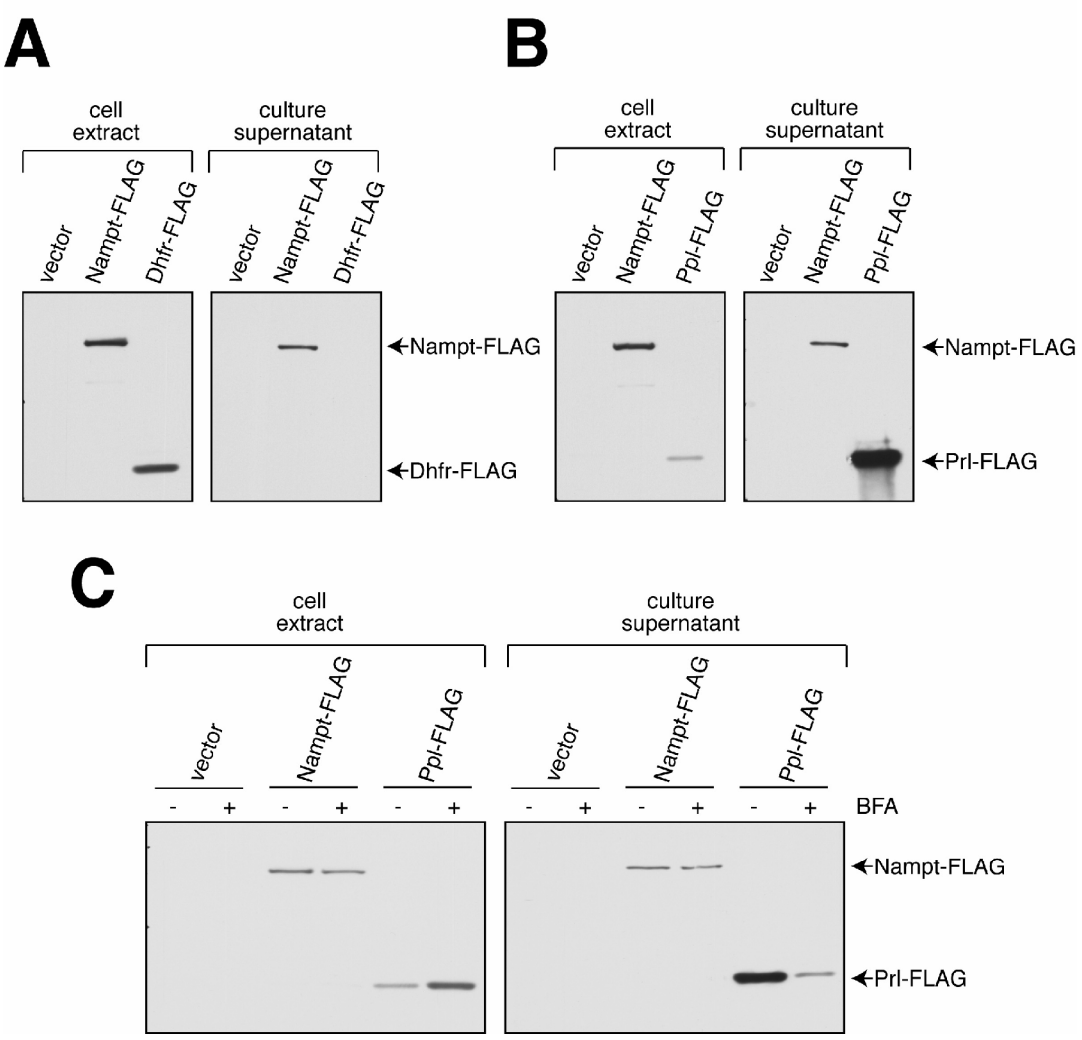
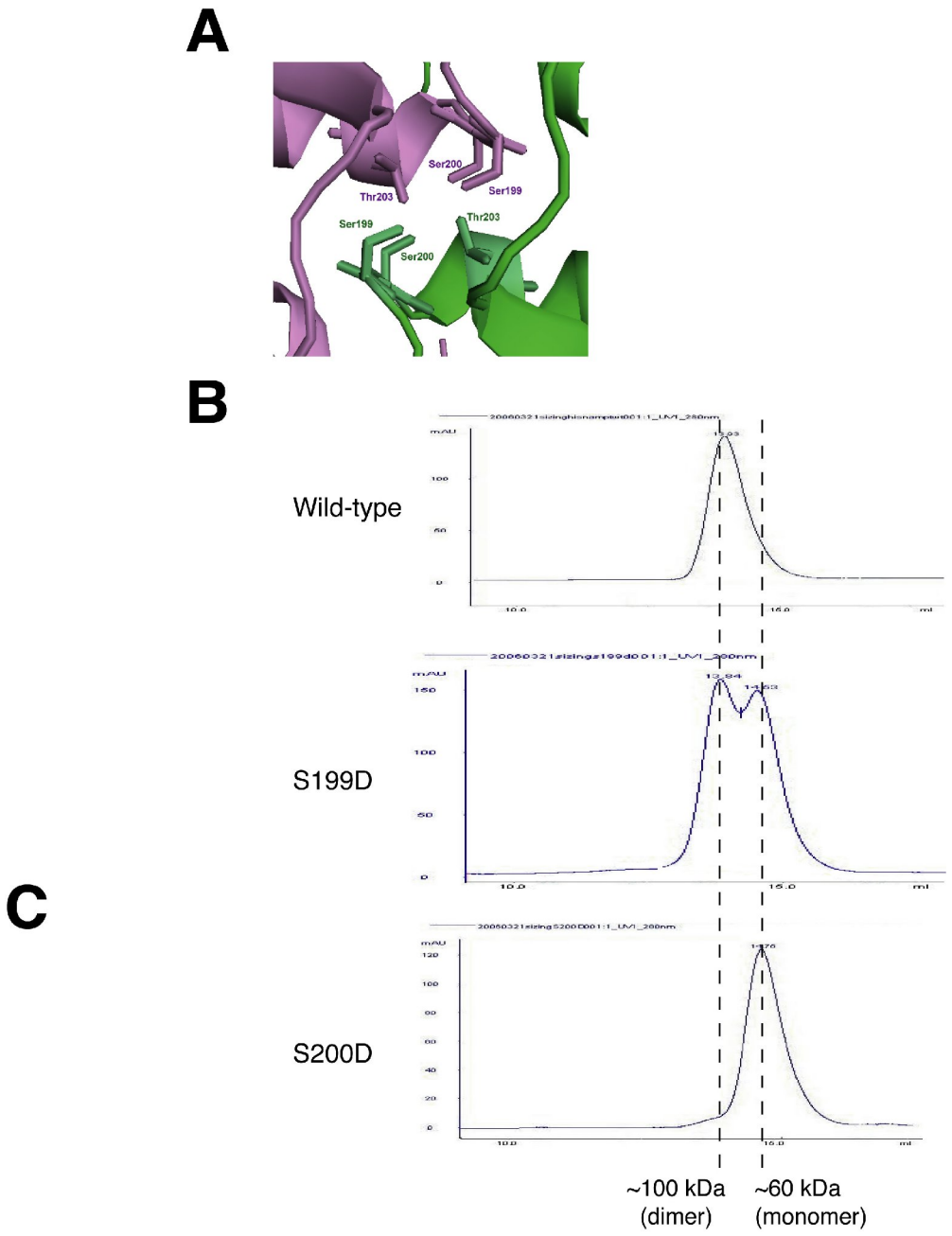


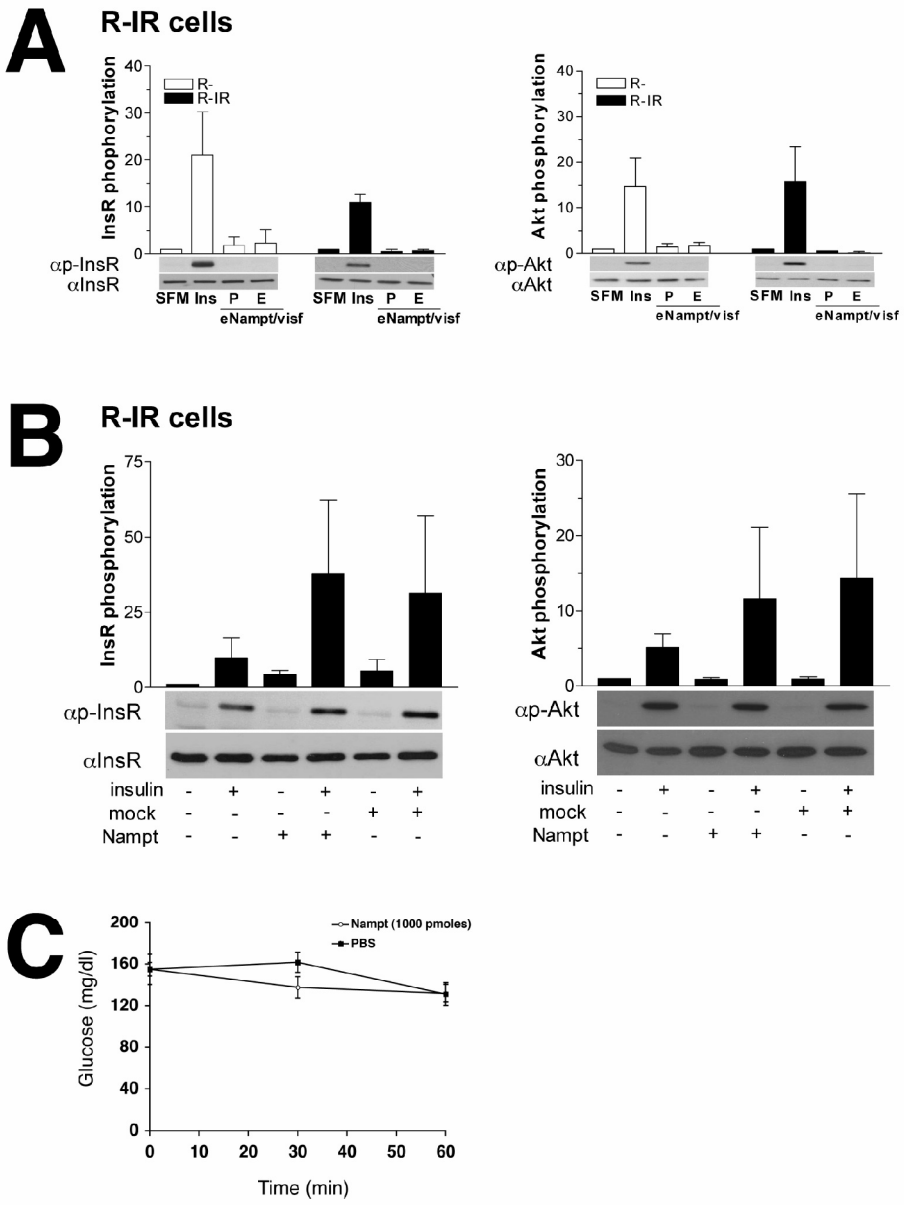
Supplementary Figure 1. Major NAD biosynthetic pathways in yeast and mammals and the enzymatic reaction of nicotinamide phosphoribosyltransferase (Nampt/PBEF/visfatin). **A)** The NAD biosynthetic pathway in the budding yeast *Saccharomyces cerevisiae*. The *de novo* pathway from tryptophan is not shown in this scheme. Pnc1, nicotinamidase; Npt1, nicotinic acid phosphoribosyltransferase; Nma1 and Nma2, nicotinic acid mononucleotide adenylyltransferase 1 and 2; Qns1, NAD synthetase; Sir2, silencing information regulator 2; NIC, nicotinamide; NA, nicotinic acid; NaMN, nicotinic acid mononucleotide. **B)** The NAD biosynthetic pathway from nicotinamide in mammals. The pathways from tryptophan, nicotinic acid, and nicotinamide riboside are not shown in this scheme. Sir2 and PARP are shown as two representative enzymes that catalyze NAD for their enzymatic activities. Nampt, nicotinamide phosphoribosyltransferase; PBEF, pre-B cell colony-enhancing factor; Nmnat, nicotinamide mononucleotide adenylyltransferase; PARP, poly-ADP-ribose polymerase; NMN, nicotinamide mononucleotide. **C)** The reaction catalyzed by Nampt. PPi, inorganic pyrophosphate.



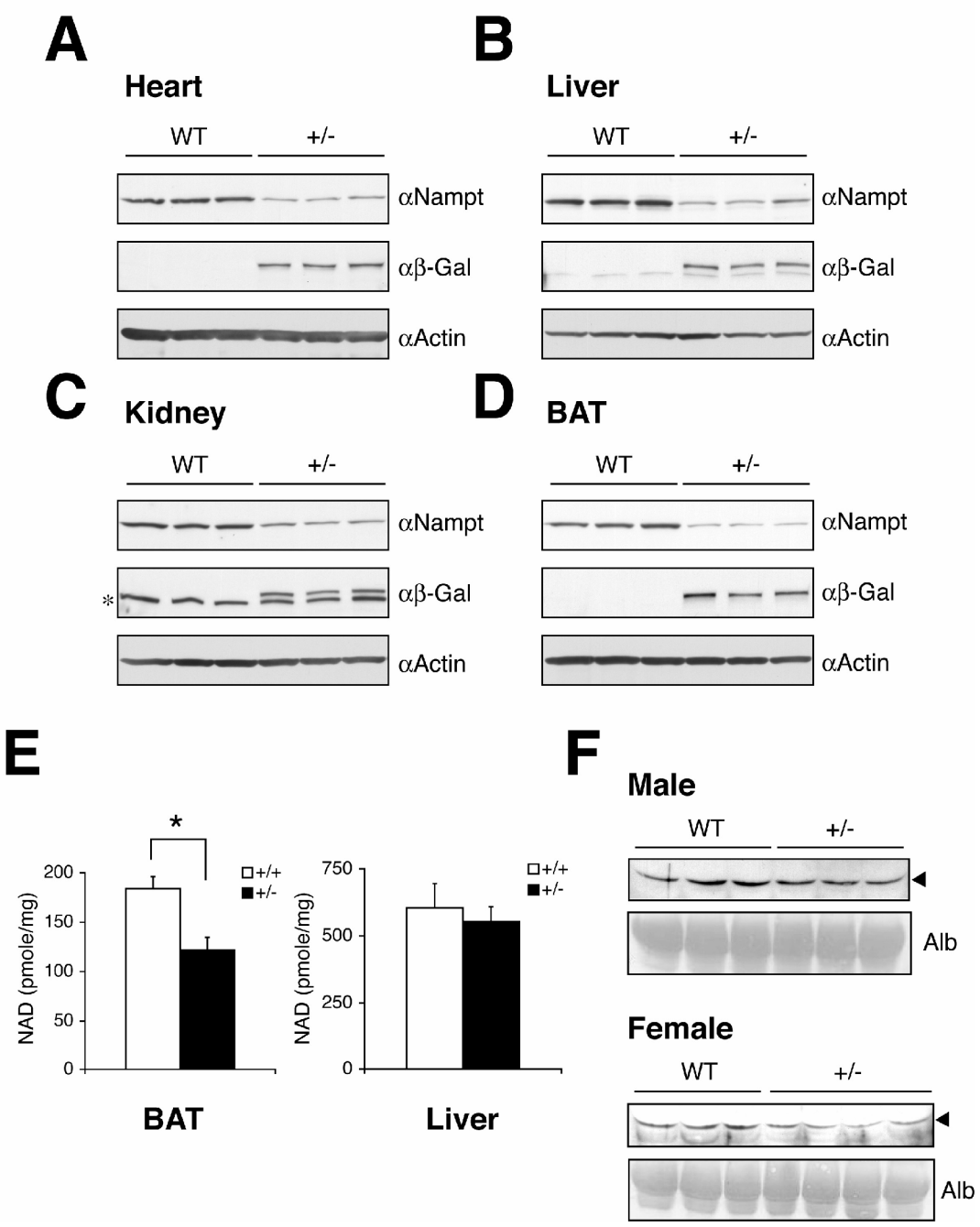
Supplementary Figure 2. eNampt is positively secreted through a non-classical secretory pathway in differentiated HIB-1B brown adipocytes. In addition to the HIB-1B cell line that expresses Nampt-FLAG, two other HIB-1B cell lines that express C-terminally FLAG-tagged mouse dihydrofolate reductase (Dhfr) and bovine preprolactin (Ppl) were established. **A**) The Nampt-FLAG protein was detected in both cell extracts and culture supernatants, while the Dhfr-FLAG protein was detected exclusively in cell extracts. **B**) The Nampt-FLAG and Ppl/Prolactin (Prl)-FLAG proteins were detected in both cell extracts and culture supernatants. **C**) While the Prl-FLAG secretion was inhibited by brefeldin A (BFA), the eNampt secretion was not inhibited by BFA.



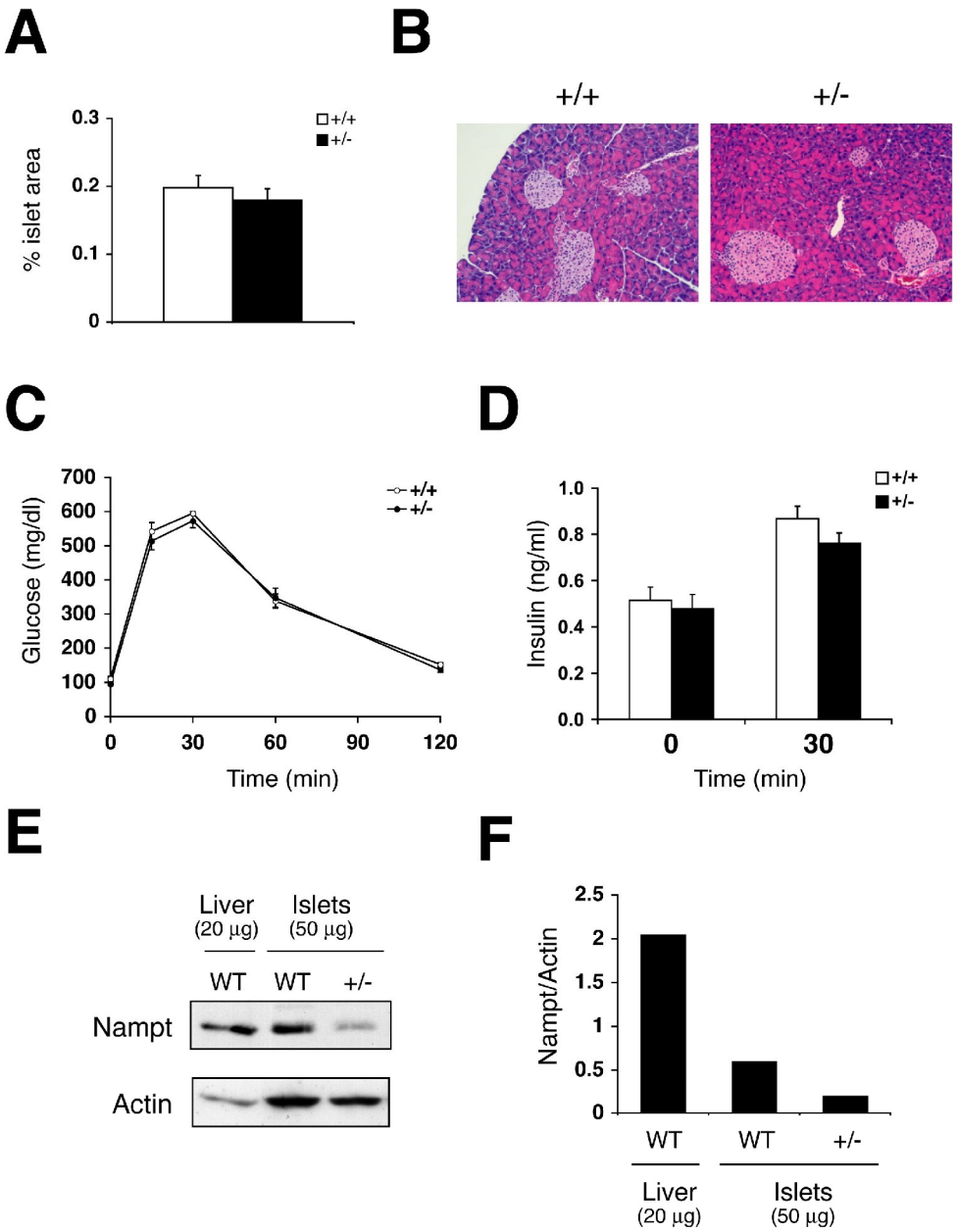
Supplementary Figure 3. Nampt dimerization interface mutants. **A)** The Structure of Nampt dimerization interface. At the center of the interface, Ser 199, Ser200, and Thr203 from one monomer form tight hydrogen bonds with the corresponding residues from the other monomer. **B)** Gel-filtration analysis on the sizes of his-tagged Nampt proteins including wild-type (upper), S199D (middle) and S200D (lower). The wild-type and mutant Nampt recombinant proteins were applied onto SP200 10/300 GL analytical sizing column in elution buffer (20mM Tris (pH 8.0), 100mM NaCl, 5mM DTT, 10% glycerol). The sizes of the proteins were determined based on standard molecular markers eluted under the same conditions. Wild-type Nampt forms a dimer (~100 kDa), S199D forms a mixture of monomer (~60 kDa) and dimer, and S200D forms a monomer.



Supplementary Figure 4. eNampt does not exert insulin-mimetic effects on insulin signaling and blood glucose levels. **A**) The effect of eNampt/visfatin on phsphorylation of the insulin receptor (InsR, upper panels) and Akt/PKB kinase (Akt, lower panels) was examined in an IGF-I receptor-deficient mouse fibroblast line (R-) and its derivative cell line that overexpress human insulin receptor (R-IR). Cells were starved overnight and then exposed to serum free medium (SFM), 10 nM insulin (Ins), or 10 nM eNampt/visfatin produced in bacteria (P) or in mammalian cells (E). Signals of phosphorylated proteins are normalized to those of non-phosphorylated proteins, and values are shown relative to the signal in serum free medium ($n \geq 3$). **B**) R-IR cells were stimulated with serum free media or conditioned media prepared from COS-7 cells transfected with full-length human Nampt cDNA or from mock-transfected COS-7 cells. Both conditioned media were concentrated 10-fold. Insulin (1 nM) was added to serum free media or concentrated conditioned media as a positive control. **C**) The effect of Nampt injection on blood glucose levels. Intraperitoneal injection of the recombinant Nampt protein (1000 pmoles) into wild-type mice does not affect blood glucose levels (PBS, n=3; Nampt, n=3).
All results are expressed as mean ± SEM.



Supplementary Figure 5. The production of Nampt$^{+/-}$ mice. **A-D)** Nampt expression levels in (**A**) heart, (**B**) liver, (**C**) kidney, and (**D**) brown adipose tissue (BAT) of Nampt$^{+/-}$ and control littermates. 45 μg of each tissue extract from males was analyzed by Western blotting with Nampt-, β-gal- and actin-specific antibodies. An asterisk in **C** indicates a cross-reacting band in kidney extracts. **E)** Total NAD levels were measured in BAT and liver from Nampt$^{+/-}$ (n=3) and control (n=3) mice using HPLC. The NAD levels were normalized to wet weights of tissues used. Results are expressed as mean ± SEM. **F)** eNampt was detected in 2 μl of each plasma sample from male and female Nampt$^{+/-}$ and control mice by Western blotting with an Nampt-specific antibody (upper panel). Plasma albumin (Alb) was visualized with Ponceau S staining as a loading control (lower panel).



Supplementary Figure 6. The analysis of Nampt$^{+/-}$ mice. **A)** Percent islet areas were measured in 2 month-old control and Nampt$^{+/-}$ mice (n=3). Results are presented as mean ± SEM. **B)** Representative islets on HE-stained pancreatic sections from 2 month-old control and Nampt$^{+/-}$ mice. **C)** Intraperitoneal glucose tolerance tests (IPGTTs) in Nampt$^{+/-}$ and control male mice. Nampt$^{+/-}$ (n=10) and control (n=6) males were fasted for 12-14 hrs and injected with dextrose (3 g/kg body weight) intraperitoneally, and blood glucose levels were measured. **D)** Plasma insulin levels in Nampt$^{+/-}$ and control male littermates at 0 and 30 min time points in IPGTTs. Nampt$^{+/-}$ mice (n=10), control mice (n=6). Results are presented as mean ± SEM. **E)** Nampt protein levels in primary islets isolated from Nampt$^{+/-}$ and control mice. 50 µg of each primary islet extract collected from three control and three Nampt$^{+/-}$ male mice were blotted with an anti-Nampt polyclonal antibody. 20 µg of a control liver extract was used as a comparison. **F)** Quantitation of the Western blot result shown in **E**.

Supplementary Table 1. Kinetic parameters of Nampt dimerization interface mutants

Enzyme	K_M (μM)	k_{cat} (min^{-1})	k_{cat}/K_M ($\mu M^{-1}min^{-1}$)
wild type	**1.8**	**6.3**	**3.5**
S199D	**0.54**	**0.081**	**0.16**
S200D	**3.9**	**0.21**	**0.052**

The nicotinamide phophoribosyltransferase activity of Nampt was measured as previously described (Revollo et al., 2004; Wang et al., 2006). For kinetics studies on His-tagged Nampt wild type and mutants (at least 95% pure after one-step Histrap purification), each reaction consists of 100 μl of 20 mM Tris, pH 8.0, 50 mM NaCl, 2 mM DTT, and 12.5 mM $MgCl_2$, 2.5 mM ATP, 0.03% BSA, 1.5% ethanol, 13 μg/ml His-tagged Nmnat, 100 μg/ml alcohol dehydrogenase, 500 μM PRPP, and varying concentrations of nicotinamide. Depending on different catalytic abilities, different amounts of His-Nampt were used for the wild type and the mutants. Catalytic constants K_M and k_{cat} were determined for His-tagged mutant and wild type proteins by analyzing plots of initial rate measurements at 25°C under varying nicotinamide concentrations with the program, KaleidaGraph 3.6 (Synergy Software), which calculates constants K_M and k_{cat} automatically.