Supporting Information

Tripeptide IRW upregulates NAMPT protein levels in cells, obese C57BL/6J mice and *Drosophila Melanogaster*

Khushwant S. Bhullar[1,2,3], Myoungjin Son[1], Evan Kerek[2], Christopher R. Cromwell[2], Bentley M. Wingert[4], Kaiyu Wu[1], Juan Jovel[5], Carlos J. Camacho[4], Basil P. Hubbard[2] and Jianping Wu[1*]

[1]Department of Agricultural, Food, and Nutritional Science, University of Alberta, Edmonton, AB, Canada; [2]Department of Pharmacology, University of Alberta, Edmonton, AB, Canada; [3]Department of Medical Microbiology and Immunology, University of Alberta, Edmonton, AB, Canada; [4]Department of Computational and Systems Biology, University of Pittsburgh, Pittsburgh, PA 15261, USA; [5]Office of Research, University of Alberta, Edmonton, AB, Canada.

* Corresponding Author: Tel: 780.492.6885; Fax: 780.492.8524; Email: jwu3@ualberta.ca

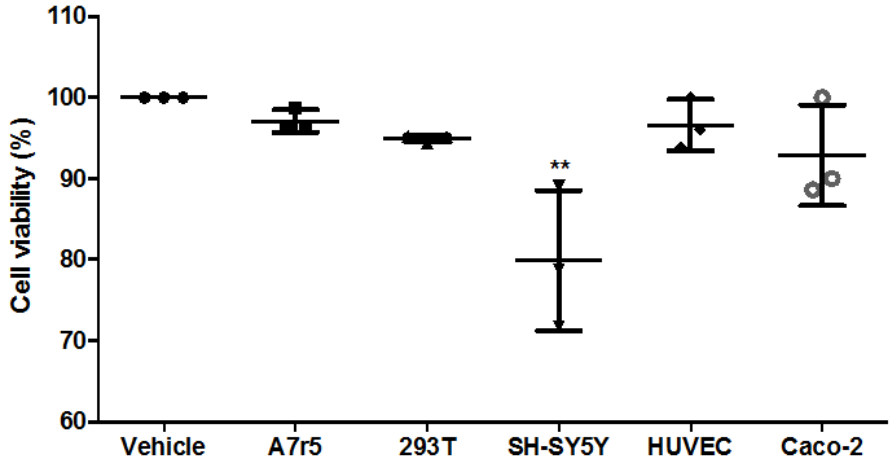

Figure S1. Impact of IRW on cellular viability of different cells. Cell viability of different mammalian cells pre-treated with 50 µM of IRW for 24h using MTT assay. Results are the mean ± S.E.M of experiments performed in triplicates.

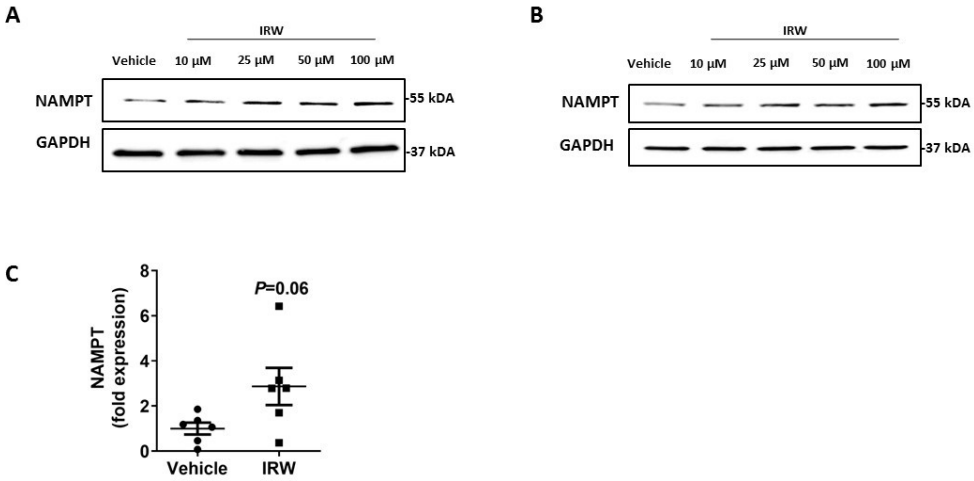

Figure S2. Impact of IRW on NAMPT in different mammalian cell lines. (A, B) Dose-dependent response of IRW-induced NAMPT expression in Hela and MDAMB231 cells. Cells were incubated with indicated concentrations of IRW for 24 h and analyzed by western blot (C) NAMPT mRNA expression in 293T cells was quantified by qPCR and normalized to β-actin

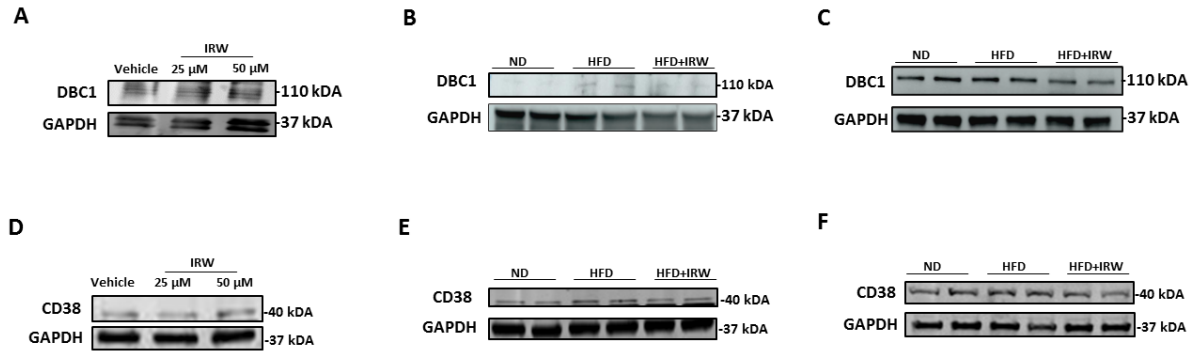

Figure S3. Impact of IRW on DBC1 and CD38. (A) IRW-induced no impact of DBC1 in L6 cells. The cells were incubated with indicated concentrations of IRW for 24 h and analyzed by western blot B) Muscle and C) Liver tissue of C57BL/6J mice exhibited marginal changes in DBC1 levels. IRW had no impact on NDAase CD38 in (D) L6 cells (E) Muscle (F) Liver tissues of C57BL/6J mice fed a high-fat diet. The C57BL/6J mice were divided into three groups (n=6, per group) (1) control group (ND): receiving regular diet (9% fat) for 14 weeks 2) HFD group: receiving HFD (40% fat) for 14 weeks 3) Treatment group (HFD+IRW): receiving HFD (40% fat) for 14 weeks with incorporation of IRW (45mg/kg BW) during the last 8 weeks.

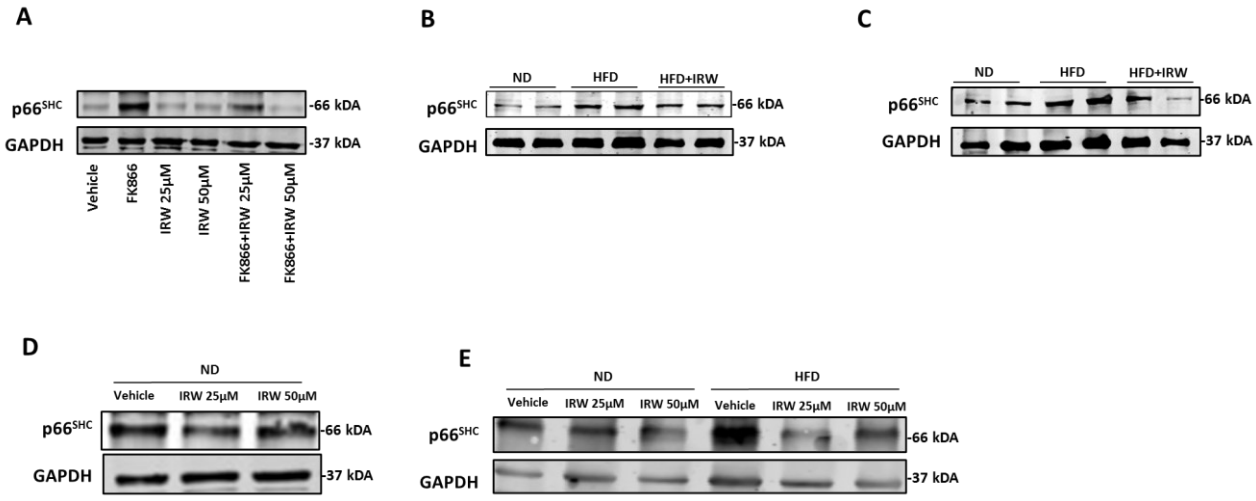

Figure S4. IRW lowers the protein levels of p66^{SHC} in cells, mice tissues, and flies (A) L6 cells (B) Muscle (C) Liver tissues of C57BL/6J mice fed a high-fat diet and (D-E) *yw Drosophila Melanogaster*. The C57BL/6J mice were divided into three groups (n=6) (1) control diet group (ND): receiving regular diet (9% fat) for 14 weeks 2) HFD group: receiving HFD (40% fat) for 14 weeks 3) Treatment group (HFD+IRW): receiving HFD (40% fat) for 14 weeks with the incorporation of IRW (45mg/kg BW) during the last 8 weeks. The *yw* flies were divided into two groups (n=30, per group) 1) Vehicle: receiving regular diet for 10 days 2) IRW group 1: receiving low IRW dose (25 μM) supplemented in the regular diet for 10 days and IRW group 2: receiving high IRW dose (50 μM) supplemented in regular diet for 10 days. Flies were fed with ND or HFD food containing indicated concentrations of IRW for 10 days and whole-body protein and RNA were extracted using RIPA buffer, respectively. ND: Normal diet, HFD: High fat diet.

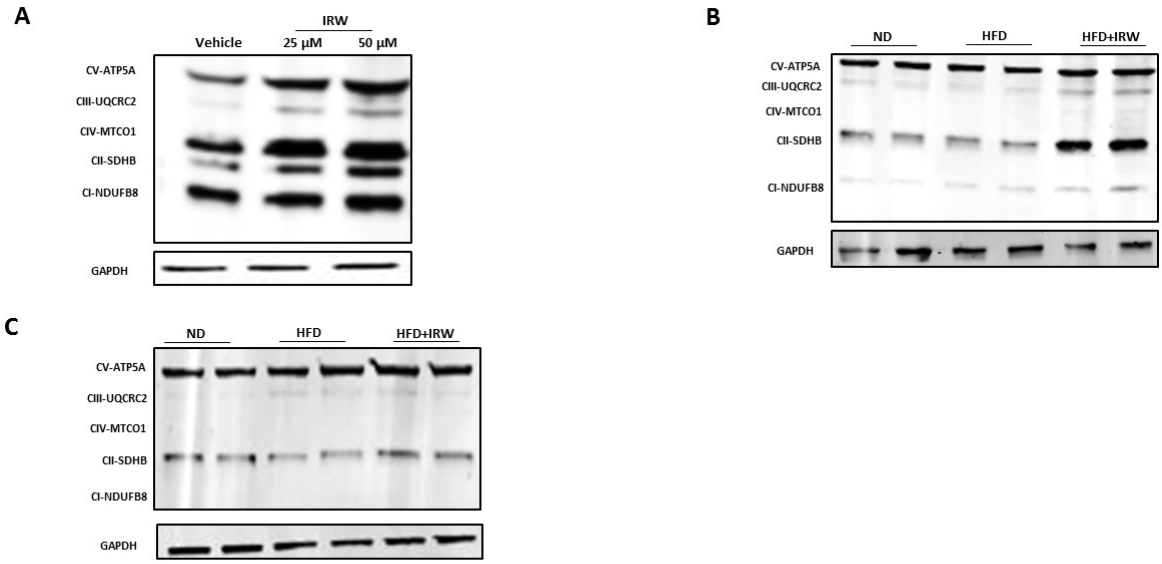

Figure S5. Impact of IRW on OXPHOS complexes in mitochondria. (A) IRW-induced increase in 5 OXPHOS complexes of L6 cells. The cells were incubated with indicated concentrations of IRW for 24 h and analyzed by western blot B) Muscle and C) Liver tissue of C57BL/6J mice exhibited a marginal increase in OXPHOS complexes. The C57BL/6J mice were divided into three groups (n=6, per group) (1) control group (ND): receiving regular diet (9% fat) for 14 weeks 2) HFD group: receiving HFD (40% fat) for 14 weeks 3) Treatment group (HFD+IRW): receiving HFD (40% fat) for 14 weeks with incorporation of IRW (45mg/kg BW) during the last 8 weeks.

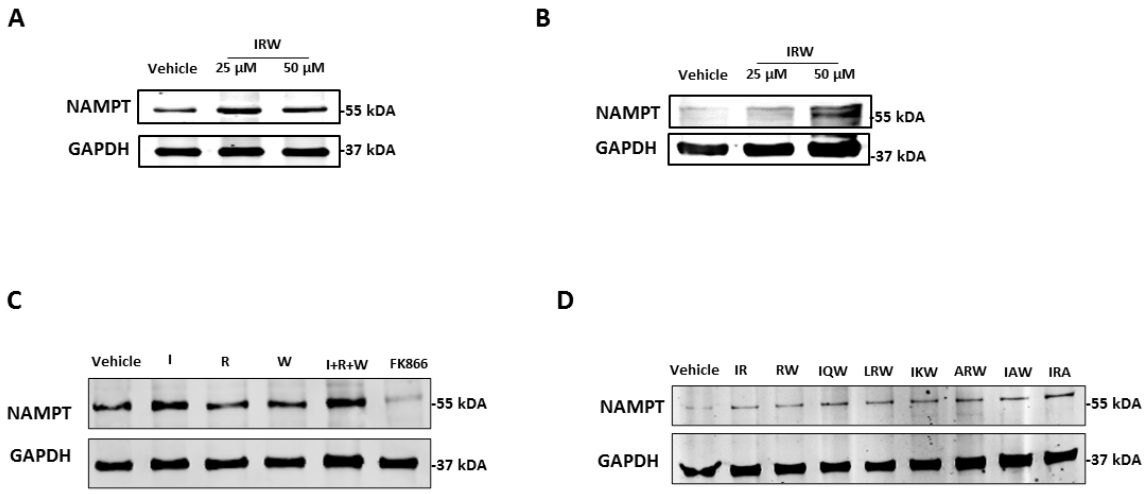

Figure S6. Increase in NAMPT levels in different cellular compartments and structure-function relationship (A) Nucleus and (B) Mitochondrial following treatment with IRW; and the impact of (C) amino acids (D) analogous peptides on NAMPT levels. The cells were incubated with indicated concentrations of IRW for 24 h, the cell compartments were separated and analyzed by western blot. GAPDH was used as the control.

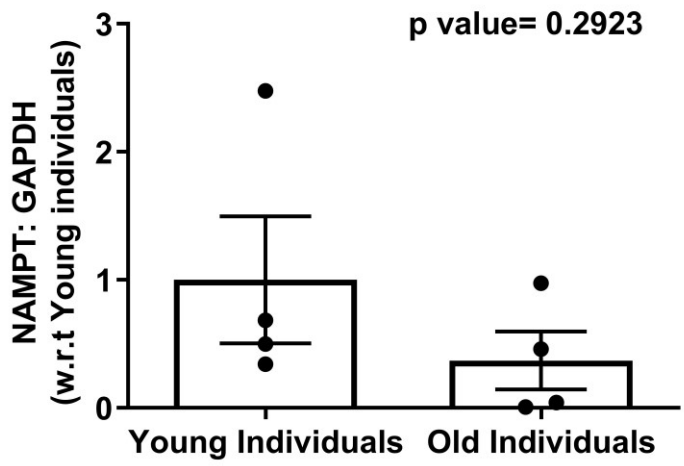

Figure S7. Comparative analysis of NAMPT levels in muscle tissues of younger and older individuals. The values are based on human muscle NAMPT blot shown in Figure 1B.

Table 1: List of the primers used in the study

Gene	Forward primer	Reverse primer
D-NAAM	TTGTCATCGAAGATTCAAACGGA	GGCGGTCATCACTGTCCTTG
Mouse NAMPT	ACCAGCGGGGAACTTTGTTA	ACGTCCTGCTCGATGTTCAG
Rat NAMPT	AGGGGCATCTGCTCATTTGG	CTCTGCCGCTGGAACAGAAT
Human NAMPT	CTTCGGTTCTGGTGGAGGTT	AATCGGCCCTTTTTGGACCT
Mouse GAPDH	AAGAGGGATGCTGCCCTTAC	TACGGCCAAATCCGTTCACA
Rat GAPDH	TGATTCTACCCACGGCAAGTT	TGATGGGTTTCCCATTGATGA
Human β-actin	TCGTGCGTGACATTAAGGAG	GTCAGGCAGCTCGTAGCTCT