SUPPLEMENTAL TABLE AND FIGURES

Zhang *et al.* (2009) - "Enzymes in the NAD^+ Salvage Pathway Regulate SIRT1 Activity at Target Gene Promoters"

This document contains supplemental data (1 table and 6 figures) as follows:

Table S1. Gene ontology analysis of NAMPT-, NMNAT-1-, and SIRT1-regulated genes.

A. NAMPT-Regulated Genes

Category	*Count*	*Percent of Total*	*p-value*	*Example*
Cell Cycle Regulation [1]	19	8.3%	< 0.0304	*CCNE2, PLK4, MTSS1*
Negative Regulation of Biological Process [2]	21	9.2%	< 0.0367	*RDX, AMBP, NSD1*
Tumor Suppressor [3]	3	1.3%	< 0.0206	*RB1, FHIT, NF1*
Transporter [4]	8	3.5%	< 0.0305	*SLC4A7, SLC7A8, SLC24A3*

B. NMNAT-1-Regulated Genes

Category	*Count*	*Percent of Total*	*p-value*	*Example*
Development and Morphogenesis [5]	38	21%	< 0.0142	*STX2, GJA1, MID1*
Cell Organization and Biogenesis	30	16%	< 0.0031	*TRAK1, LIMCH1, RAB14*
Neuron Differentiation [6]	6	3.3%	< 0.0357	*AGRN, NRCAM, RTN1*
Cell Signaling [7]	30	16%	< 0.0491	*SNF1LK2, SMAD5, GRB10*
Ion Homeostasis [8]	26	14%	< 0.0466	*SLC9A2, CALR, ANXA1*
Cell Proliferation [9]	13	7.1%	< 0.0498	*EPS15, COL4A3, LAMB1*
Cell Adhesion [10]	17	9.2%	< 0.0486	*SGCE, PCDH7, PLEKHC1*
Cytoskeletal Protein Binding	10	5.5%	< 0.0196	*DIAPH2, MAPRE3, TRIM2*
Enzyme Inhibitor Activity	8	4.4%	< 0.0178	*TFPI, CSTA, THBS1*

C. NAMPT and NMNAT-1 Commonly Regulated Genes

Category	*Count*	*Percent of Total*	*p-value*	*Example*
Neuron Differentiation [6]	3	8.1%	< 0.0254	*SOCS2, RTN1, TGFB2*
Cell Signaling [7]	16	43%	< 0.0461	*SMAD5, CXCR7, PLCL1*
Cellular Membrane [11]	9	24%	< 0.0473	*SCNN1A, CAV1, ST3GAL5*

D. SIRT1-Regulated Genes

Category	*Count*	*Percent of Total*	*p-value*	*Example*
Cell Signaling [7]	17	8.6%	< 0.0484	*DUSP8, PPP2R1B, PIK3R1*
Metabolism [12]	4	2.2%	< 0.0448	*GMDS, PGM3, GNE*

(Table S1 continues on the next page)

Table S1 ***(continued)***

E. NAMPT and SIRT1 Commonly Regulated Genes

Category	*Count*	*Percent of Total*	*p-value*	*Example*
Cellular Membrane [11]	6	15%	< 0.0450	*ITPR1, CAV1, ATP11B*
Metabolism [12]	3	7.5%	< 0.0438	*SLC35A3, PGM3, GNE*

F. NMNAT-1 and SIRT1 Commonly Regulated Genes

Category	*Count*	*Percent of Total*	*p-value*	*Example*
Cell Adhesion [10]	5	16%	< 0.0350	*LAMB1, THBS1, NELL2*
Cellular Membrane [11]	4	13%	< 0.0321	*CAV2, CAV1, ST3GAL5*
Metabolism [12]	2	6.3%	< 0.0472	*SLC35A3, PGM3*
Response to Wounding	4	13%	< 0.0482	*TRGV9, LGALS3BP, TFPI*

G. NAMPT, NMNAT-1 and SIRT1 Commonly Regulated Genes

Category	*Count*	*Percent of Total*	*p-value*	*Example*
Cellular Membrane [11]	4	18%	< 0.0368	*CAV2, CAV1, ST3GAL5*
Metabolism [12]	2	9.1%	< 0.0220	*SLC35A3, PGM3*

Associated GO terms:

[1] Cell cycle, regulation of cell cycle, negative regulation of progression through cell cycle
[2] Negative regulation of biological process, negative regulation of physiological process, negative regulation of cellular process, negative regulation of progression through cell cycle
[3] Tumor suppressor, negative regulation of progression through cell cycle
[4] Porter activity, electrochemical potential-driven transporter activity
[5] Morphogenesis, development, organ morphogenesis, organ development, cellular morphogenesis
[6] Neuron differentiation, positive regulation of neuron differentiation, neurogenesis, regulation of cell differentiation
[7] Receptor binding, response to external stimulus, cell communication, signal transducer activity, transmembrane receptor protein tyrosine kinase signaling pathway, MAPKKK cascade, protein serine/threonine phosphatase activity, negative regulation of signal transduction, regulation of protein kinase activity, negative regulation of MAPK activity
[8] Ion homeostasis, metal ion homeostasis, calcium ion binding, clustering of voltage-gated sodium channels, homeostasis
[9] Cell proliferation, regulation of cell proliferation,
[10] Cell adhesion, basement membrane, basal lamina, extracellular matrix
[11] Integral to plasma membrane, intrinsic to plasma membrane, endomembrane system, Golgi membrane, organelle membrane, lipid raft, caveola, caveolar membrane
[12] Glucosamine metabolism, UDP-N-acetylglucosamine metabolism, nucleotide-sugar metabolism, amino sugar metabolism

(Table S1 continues on the next page)

Table S1 ***(continued)***

Methods

The gene list for each factor was generated based on a two-tailed Student's t-test, p-value <0.05, and filtered using a fold change cutoff of $\log_2 < -0.5$ or > 0.5. Commonly regulated genes were identified by comparing individual gene sets. The gene sets were analyzed for enrichment of GO terms using the Functional Annotation Clustering and Functional Annotation Chart tools from the DAVID Bioinformatics Resources website (http://david.abcc.ncifcrf.gov/home.jsp).

FIGURES

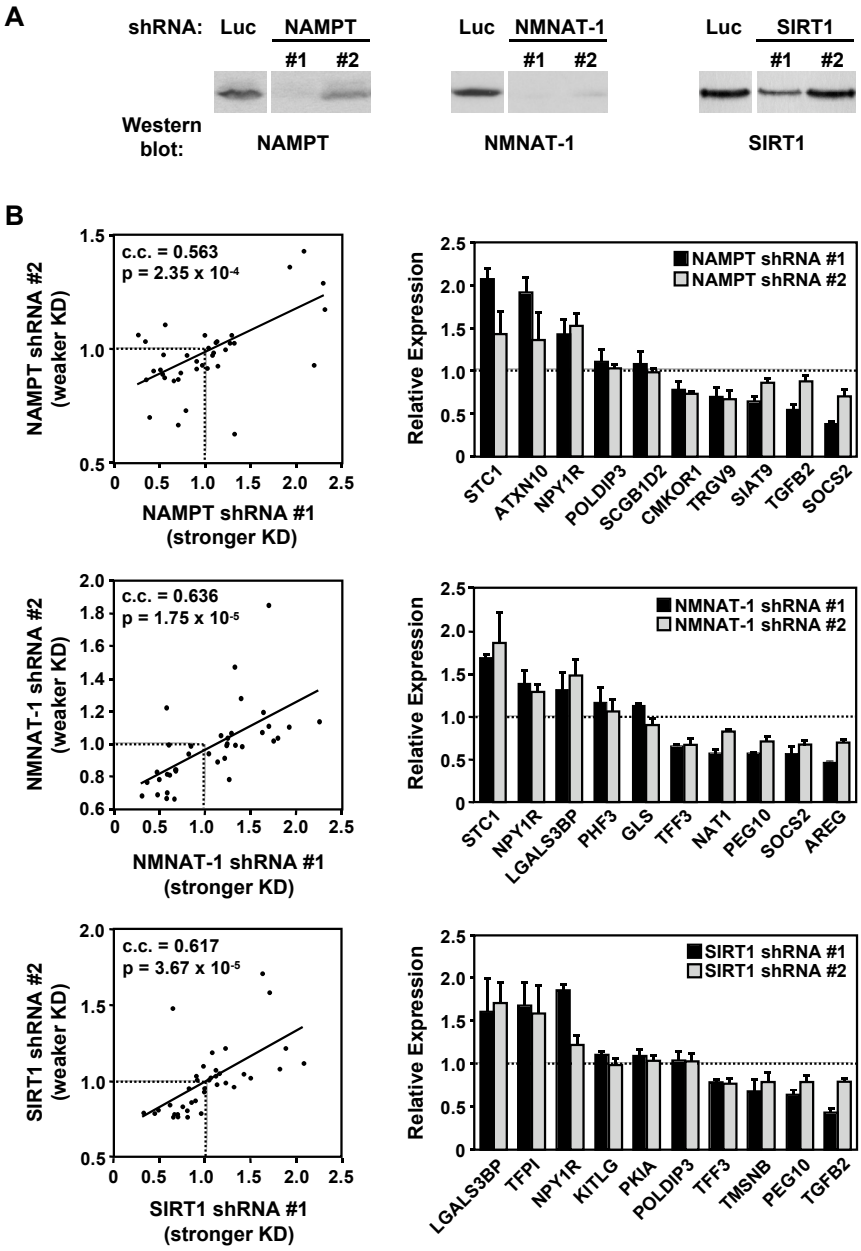

Figure S1. Comparison of different shRNA constructs in target protein knockdown and regulation of gene expression. For each factor studied (NAMPT, NMNAT-1, and SIRT1), two distinct shRNA sequences were examined for target protein knockdown **(A)** and gene expression regulation **(B)**. The expression levels of 38 genes were determined by RT-qRCR and presented as fold change relative to the luciferase knockdown control cells. For each target protein, the scatter plot *(left)* shows the expression fold changes of the 38 genes in response to the two different shRNAs. The Pearson's correlation coefficient (c.c.) and p-value are indicated. Similarly, the bar graph *(right)* highlights the expression responses of a subset of the genes. Error bars, SEM; $n \geq 3$ independent biological replicates.

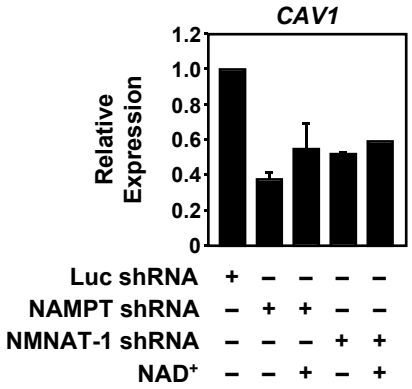

Figure S2. NAD$^+$-independent regulation of gene expression by NAMPT and NMNAT-1. Effect of NAMPT or NMNAT-1 knockdown with or without exogenously added NAD$^+$ (1 mM) on the expression of an NAMPT- and NMNAT-1-regulated gene, *CAV1*. Gene expression levels were determined by RT-qRCR using β-actin as a reference gene. The data are normalized to *CAV1* expression levels in the Luciferase (Luc) control cells. Error bars, SEM; n ≥ 3 independent biological replicates.

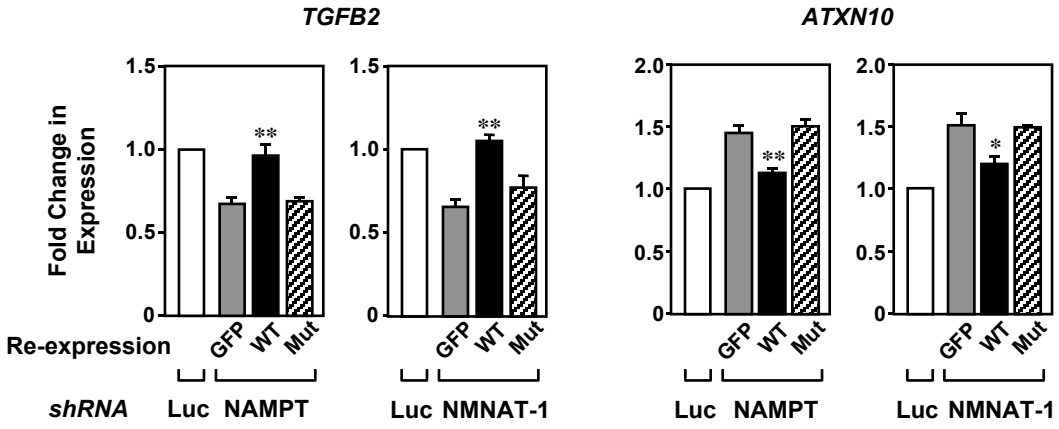

Figure S3. Regulation of gene expression by the NAD$^+$-producing enzymes requires their enzymatic activity. In the NAMPT and NMNAT-1 knockdown MCF-7 cells, expression levels of *TGFB2* and *ATXN10* were rescued by re-expression of the respective NAD$^+$-producing enzyme. The expression constructs for NAMPT and NMNAT-1 used in this experiment are RNAi-resistant through introduction of silent mutations in the shRNA target sequence. The catalytic mutants used are: NMNAT-1 W169A (1,2) and NAMPT H247A (3). GFP was used as a control for re-expression. The effect of wild type enzymes was compared to that of GFP control using two-tailed Student's t-test: * $p<0.05$, ** $p<0.01$. Error bars represent S.E.M. of at least three independent experiments.

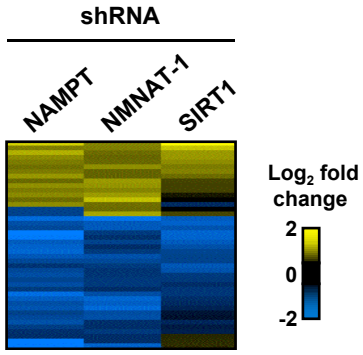

Figure S4. Similar regulation of NAMPT- and NMNAT-1-responsive genes by SIRT1. Microarray expression analyses reveal similar expression profiles for 37 commonly regulated NAMPT and NMNAT-1 target genes upon knockdown of SIRT1 in MCF-7 cells. The genes were selected for significant regulation by both NAMPT and NMNAT-1 knockdown ($p < 0.05$, Student's t-test) with a fold change cutoff of $\log_2 < -0.5$ or > 0.5.

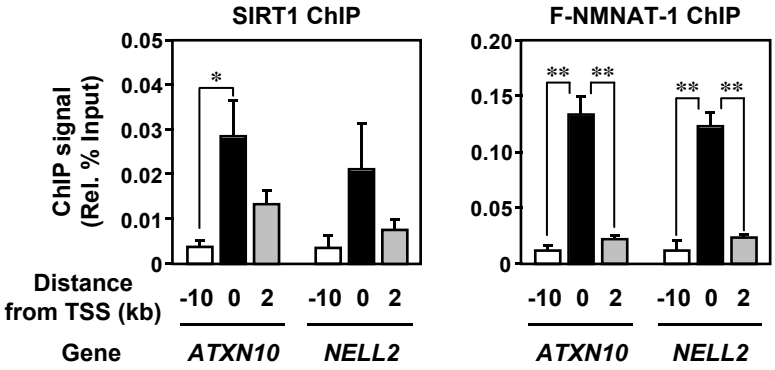

Figure S5. Recruitment of SIRT1 and NMNAT-1 to *ATXN10* and *NELL2* genes. ChIP-qPCR analysis of SIRT1 and FLAG-NMNAT-1 localization at upstream (approx. -10 kb), promoter and downstream (approx. +2 kb) regions of *ATXN10* and *NELL2* genes in MCF-7 cells. Error bars, SEM; n ≥ 3 independent biological replicates. Statistical significance was determined by two-tailed Student's t-test (* $p < 0.05$, ** $p < 0.01$).

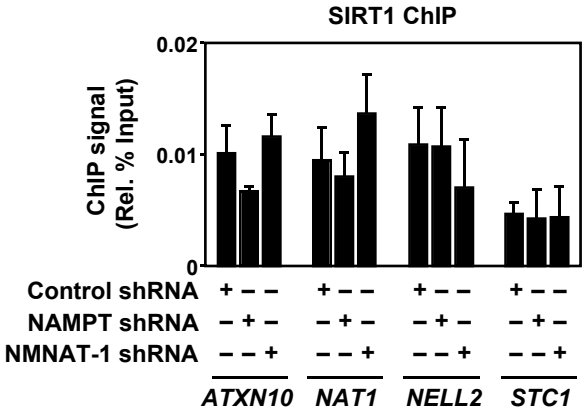

Figure S6. Knockdown of NAMPT and NMNAT-1 did not change SIRT1 recruitment to target gene promoters. ChIP-qPCR analysis of SIRT1 localization at promoter regions of target genes in MCF-7 cells. Error bars, SEM; n ≥ 3 independent biological replicates.

REFERENCES

1. Zhou, T., Kurnasov, O., Tomchick, D. R., Binns, D. D., Grishin, N. V., Marquez, V. E., Osterman, A. L., and Zhang, H. (2002) *J Biol Chem* **277**(15), 13148-13154
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3. Wang, T., Zhang, X., Bheda, P., Revollo, J. R., Imai, S., and Wolberger, C. (2006) *Nat Struct Mol Biol* **13**(7), 661-662