Supplemental Data

Nutrient-sensitive Mitochondrial NAD^+ Levels Dictate Cell Survival

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Supplementary Figures

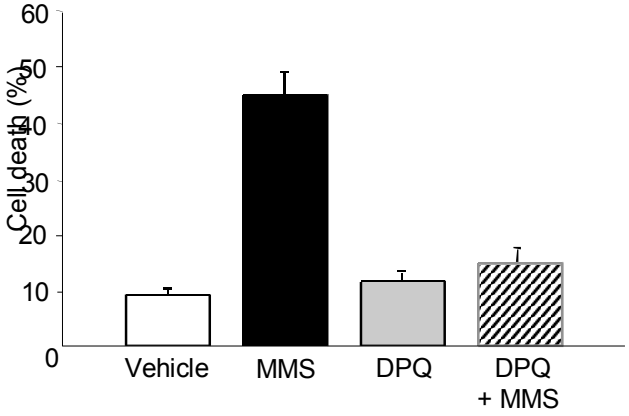

Figure S1. MMS-Induced Cell Death Is Attenuated by Inhibiting PARP-1.

Survival of HEK293 cells after MMS treatment in presence or absence of 30 μM of the PARP-1 inhibitor DPQ.

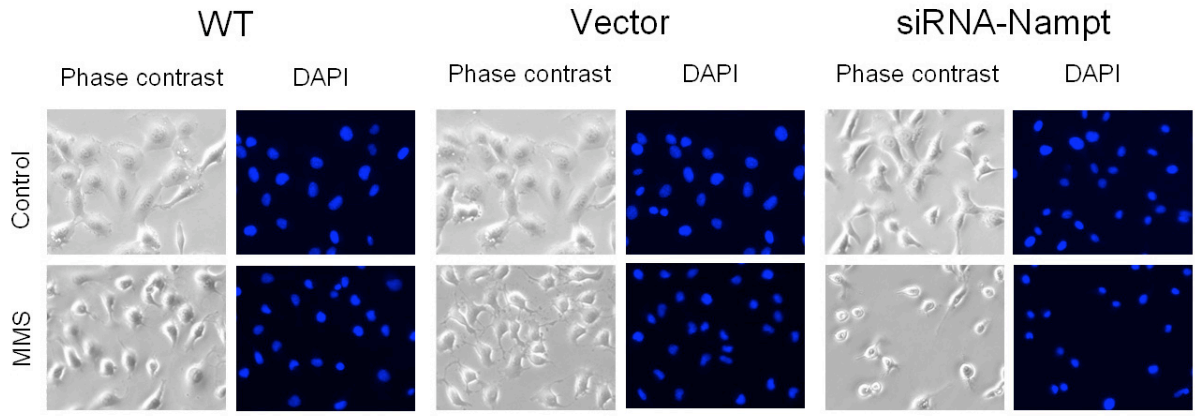

Figure S2. Knockdown of Nampt Sensitizes HT1080 Cells to MMS-Induced Cell Death.

Phase-contrast images of HT1080 control cells or Nampt stable knockdown cells treated with MMS for 4 h. Cells with a rounded-up morphology are dying cells, and are more abundant in the cultures of Nampt knockdown cells (see Figure 2A for quantitation).

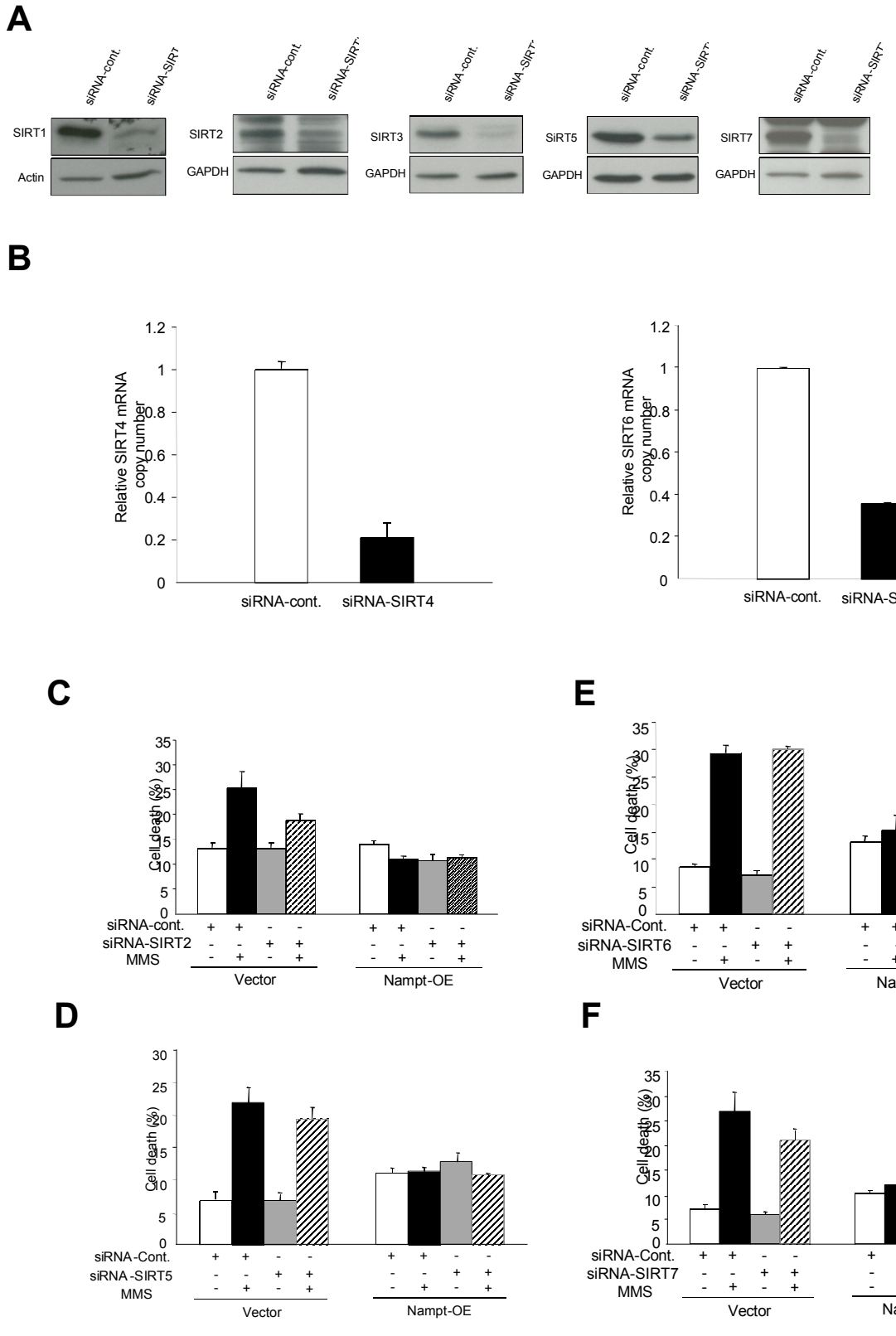
A
siRNA-cont.
siRNA-SIRT1
siRNA-SIRT2
siRNA-SIRT3
siRNA-SIRT5
siRNA-SIRT7
SIRT1
Actin
SIRT2
GAPDH
SIRT3
SiRT5
SIRT7
B
Relative SIRT4 mRNA copy number
siRNA-cont.
siRNA-SIRT4
Relative SIRT6 mRNA copy number
siRNA-SIRT6
C
Cell death (%)
siRNA-cont.
siRNA-SIRT2
MMS
Vector
Nampt-OE
D
siRNA-SIRT5
E
siRNA-Cont.
siRNA-SIRT6
F
siRNA-SIRT7

Figure S3. Knockdown of SIRT2 or SIRT5-7 by siRNA Does Not Significantly Affect Survival of Nampt Overexpressing HEK293 Cells after MMS Treatment.

(A-B) Effectiveness of sirtuin knockdown by pools of four siRNA oligos (60 nM) targeted against endogenous SIRT1, 2, 3, 5, 7, as assessed by Western Blotting (A) or SIRT4, SIRT6 mRNA (B), as assessed by quantitative RT-PCR. Relative mRNA copy number was determined in comparison to β-actin.

(C-F) Survival after treatment with MMS of HEK293 empty vector or Nampt-overexpressing cells transiently transfected with sirtuin siRNA or scrambled siRNA oligos.

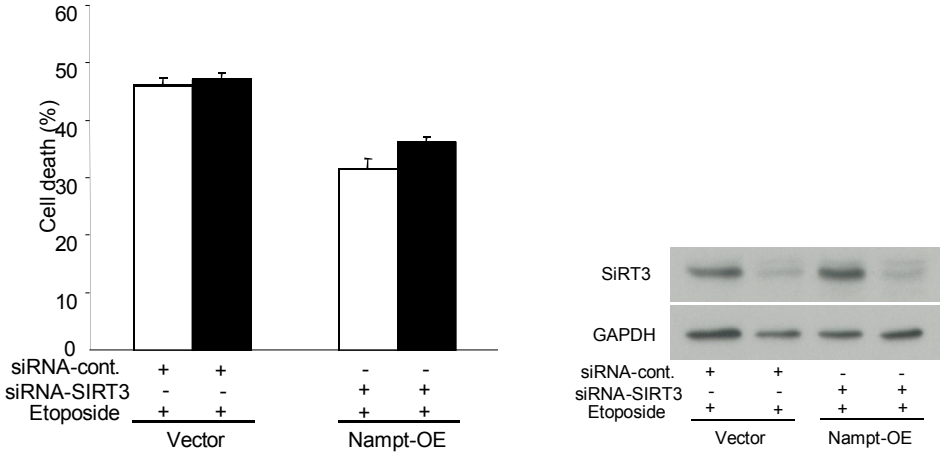

Figure S4. Effect of SIRT3 Knockdown on Nampt Protection Against Etoposide.

Survival of HEK293 cells after etoposide treatment in cells transfected with siRNA-cont or siRNA-SIRT3 oligos (60 nM). The efficacy of knockdown of SIRT3 by siRNA oligos was assessed by Western blotting.

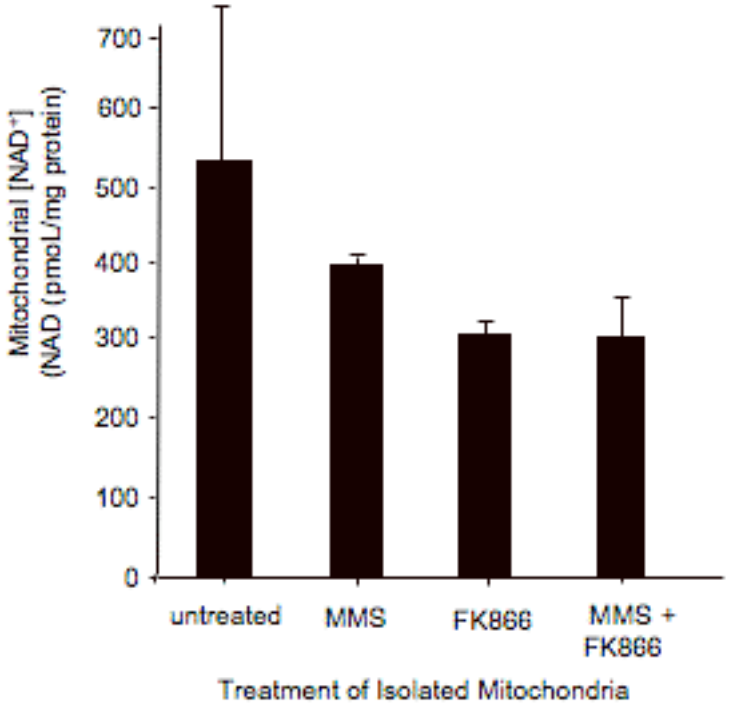

Figure S5. Effect of the Nampt Inhibitor FK866 on NAD^+ Levels in Isolated Mitochondria (Protocol 2).

Mitochondria were isolated using the differential centrifugation protocol #2 (see Materials and Methods) and incubated for 30 min with methylmethane sulfonate (MMS), FK866, or both. Suspensions were spun-down and analyzed for NAD+ content by HPLC-MALDI-MS, using ^{18}O-NAD^+ as a reference.

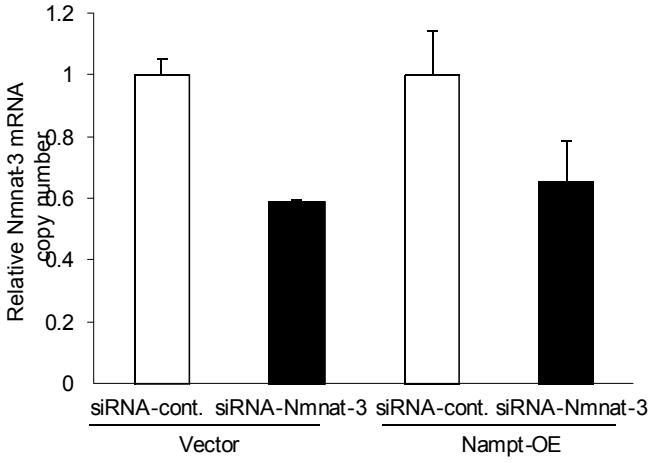

Figure S6. Knockdown of Nmnat-3 by siRNA Oligos.

Quantitative RT-PCR of Nmnat-3 in siRNA-treated cells corresponding to Figure 5F. Relative mRNA copy number was determined relative to β-actin.

A

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yndt1  82   LSGAFAGFLSGVAVCPLDVAKTRLQ    -AQGLQTRFENPYYRGIMGTLSTIVRDEGPRGLYK  140
             ++G   G LS +A+ PLD+ K R   + GL+ R   P Y GI+  L+TI +      +G RGLY+
hMFT   27   IAGVSGGVLSNLALHPLDLVKIRFAVSDGLELR    --- PKYNGILHCLTTIWKLDGLRGLYQ  83

yndt1  141  GLVPIVLGYFPTWMIYFSVYEFSKKF    -FHGIFPQFDFVAQSCAAITAGAASTTLTNPIWV  199
              G+ P + G   +W +YF  Y   K +   G   + +      +A  AGA +       +TNP+WV
hMFT   84   GVTPNIWGAGLSWGLYFFFYNAIKSYKTEGRAERLEATEYLVSAAEAGAMTLCITNPLW      V  143

yndt1  200  VKTRLMLQSNLGEHPTH   --YKGTFDAFRKLFYQEGFKALYAGLVPSLLGLFHVAIHFPIY  257
              KTRLMLQ +   +  H  YKG FD   K++  EG + LY G VP L G  H      A+ F  Y
hMFT   144  TKTRLMLQYDAVVNSPHRQYKGMFDTLVKIYKYEGVRGLYKGFVPGLFGTSHGALQFMA      Y  203

yndt1  258  EDLKVRFHCYSRENNTNSINLQRLIMASSVSKMIASAVTYPHEILRTRMQLK      SDIPDSIQ  317
             E LK++++ +        ++    I  +++SK+ A A TYP++++R R+Q +           +
hMFT   204  ELLKLKYNQHINRLPEAQLSTVEYISVAALSKIFAVAATYPYQVVRARLQDQHMFYSGV      -  262

tndt1  318  RRLFPLIKATYAQEGLKGFYSGFTTNLVRTIPASAITLVSFE  359
                   +I  T+ +EG+ GFY G   NL+R  PA  IT V +E
hMFT   263   --- IDVITKTWRKEGVGGFYKGIAPNLIRVTPACCITFVVYE  301
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B

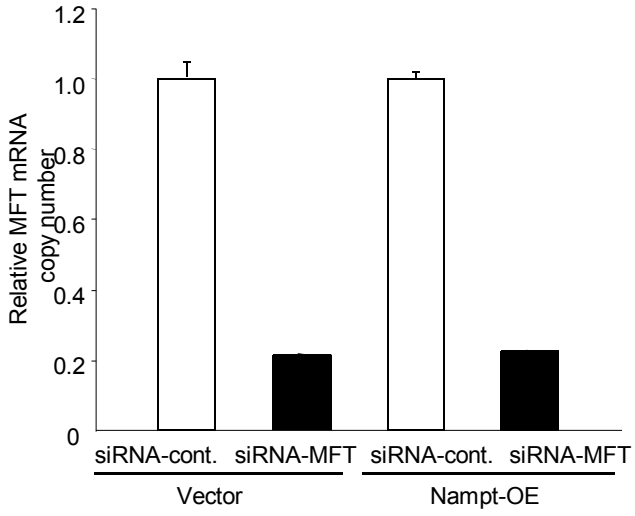

Figure S7. Knockdown of a Putative Human Mitochondrial NAD$^+$ Transporter hMFT.

(A) Sequence alignment of yeast Ndt1 and the putative folate transporter hMFT.

(B) Quantitative RT-PCR of hMFT in siRNA-treated cells. Relative mRNA copy number was determined in comparison to β-actin.

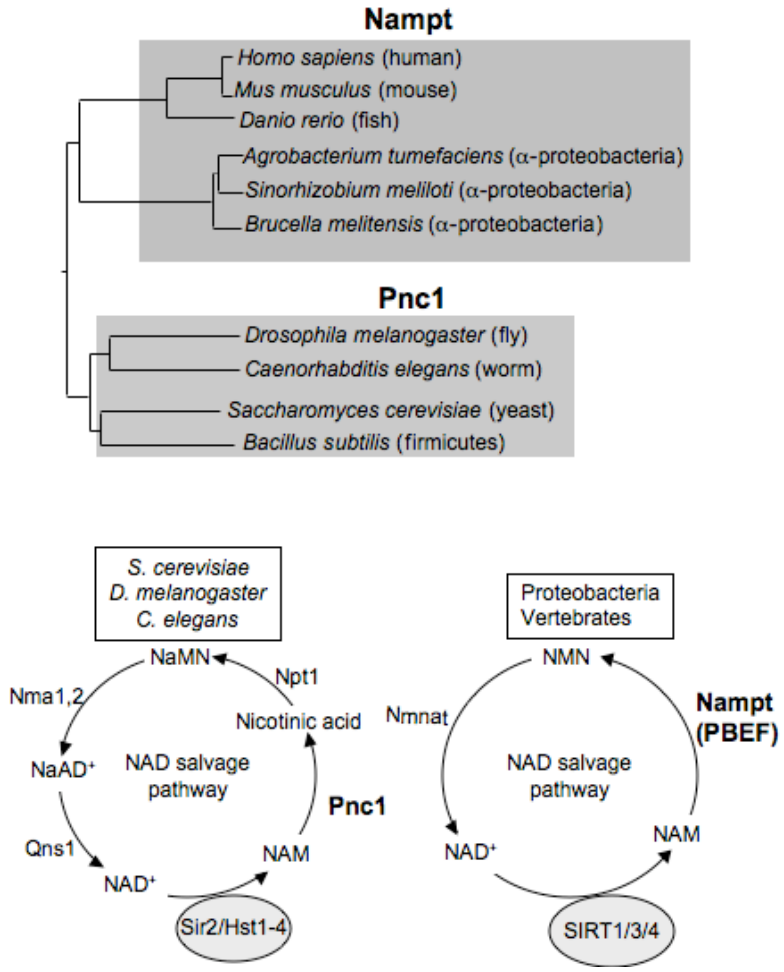

Figure S8. Mammalian Nampt is Similar to Bacterial Relatives of Mitochondria and May be a Functional Equivalent of the yeast *PNC1* Longevity Gene.

(A) Phylogenetic comparison of the enzyme that recycles NAD^+ from NAM in various species. Nampt sequences of vertebrates share a higher degree of homology with those of α-proteobacteria (relatives of the first mitochondria) than with yeast, worms and flies, which utilize Pnc1, a nicotinamidase that is similar to that of *Bacillus subtilis*.

(B) NAD^+ salvage pathways fall into two classes: those catalyzed by NAMases (e.g. Pnc1) and those catalyzed by NAM phosphoribosyltransferases (e.g. Nampt). Its responsiveness to stress and nutrient restriction and an ability to regulate sirtuins makes NAMPT a plausible functional equivalent of the yeast *PNC1* longevity gene.