

Supporting information

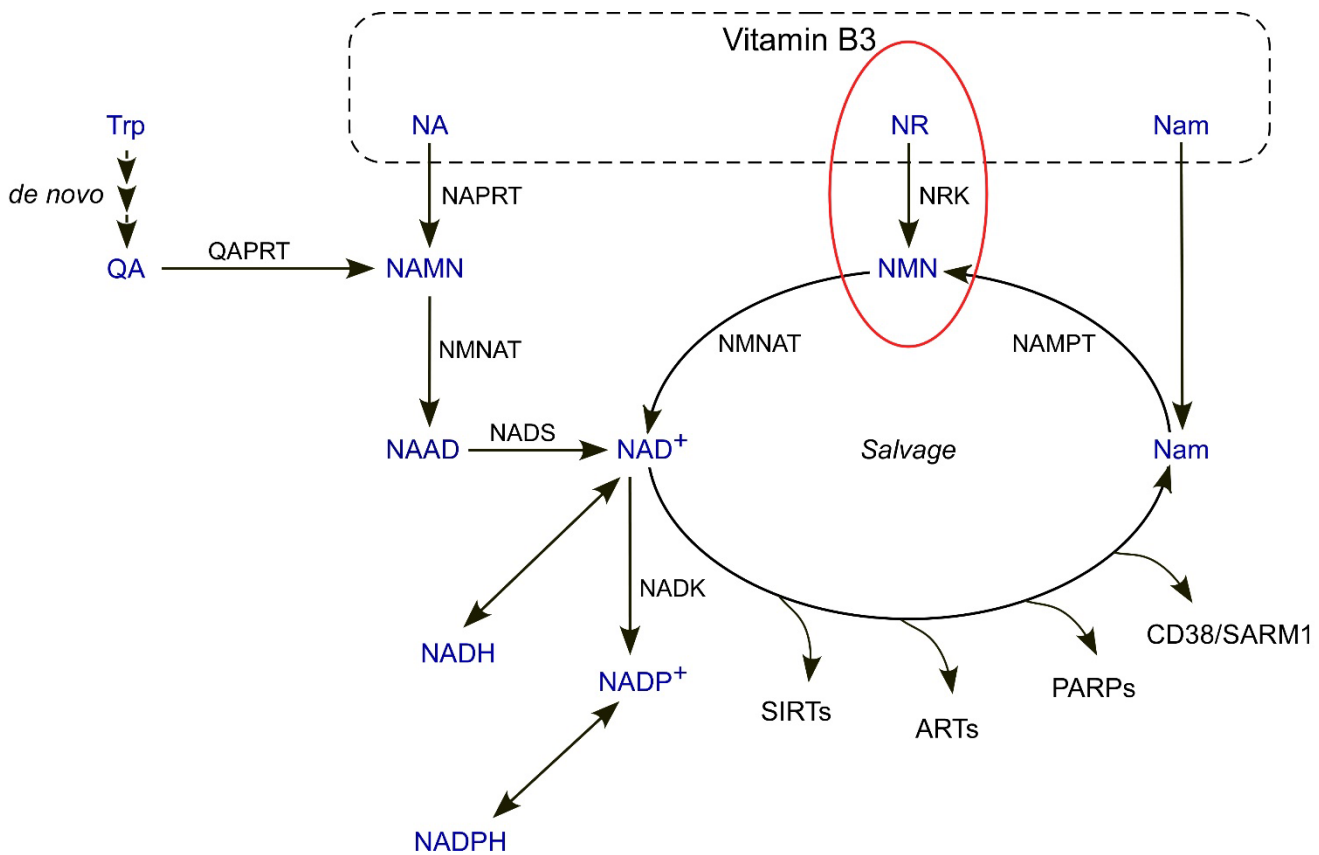
Purine nucleoside phosphorylase controls nicotinamide riboside metabolism in mammalian cells

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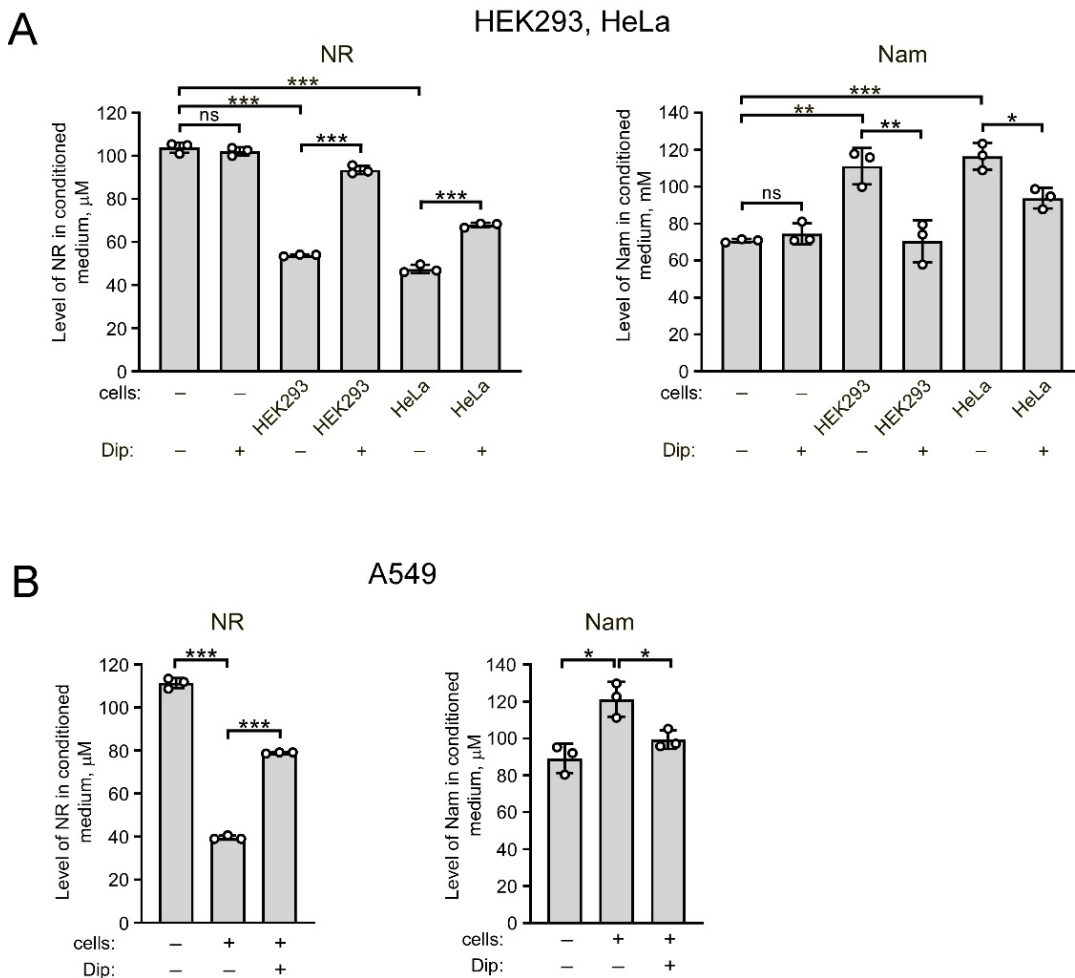
The Supporting information includes: Figures S1-S6

Figure S1



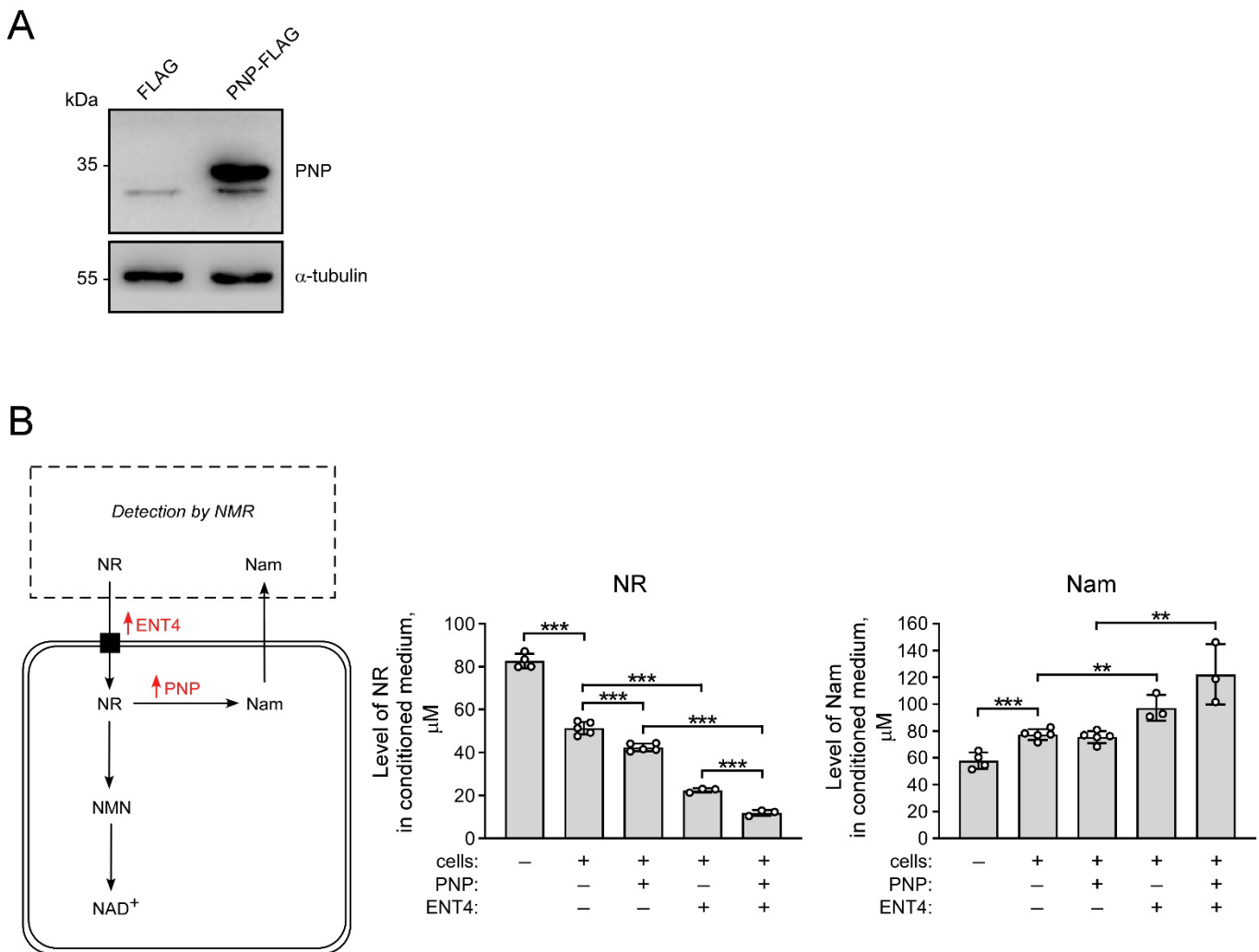
Supplementary Figure 1. NAD metabolism in human and animal cells. NAD can be synthesized *de novo* from tryptophan (Trp) or from various forms of vitamin B3: the pyridine bases nicotinamide (Nam) and nicotinic acid (NA) or the nucleoside Nam riboside (NR). Quinolinic acid (QA), a Trp degradation product, is transformed to NA mononucleotide (NAMN) by quinolinic acid phosphoribosyltransferase (QAPRT). Nam and NA are converted to the corresponding mononucleotides (NMN and NAMN) by nicotinamide phosphoribosyltransferase (NAMPT) and nicotinic acid phosphoribosyltransferase (NAPRT), respectively. NMN is also generated through phosphorylation of NR by nicotinamide riboside kinases (NRK). NAMN and NMN are converted to the corresponding dinucleotide (NAAD or NAD⁺) by NMN adenylyltransferases (NMNAT). NAD synthetase (NADS) amidates NAAD to NAD⁺. Phosphorylation by NAD kinase (NADK) converts NAD⁺ to NADP⁺. The oxidized and reduced forms of the dinucleotides, NAD(P)⁺ and NAD(P)H, serve as reversible hydrogen carriers in redox reactions. NAD⁺ is used as a substrate by various regulatory proteins: protein deacetylases sirtuins (SIRT6), mono-ADP-ribosyltransferases (ARTs), poly (ADP-ribose) polymerases (PARPs) or NAD glycohydrolases CD38 and SARM1. As a result of these signaling reactions, Nam is cleaved from NAD⁺. Released Nam is used for NAD⁺ resynthesis via *salvage* pathway.

Figure S2



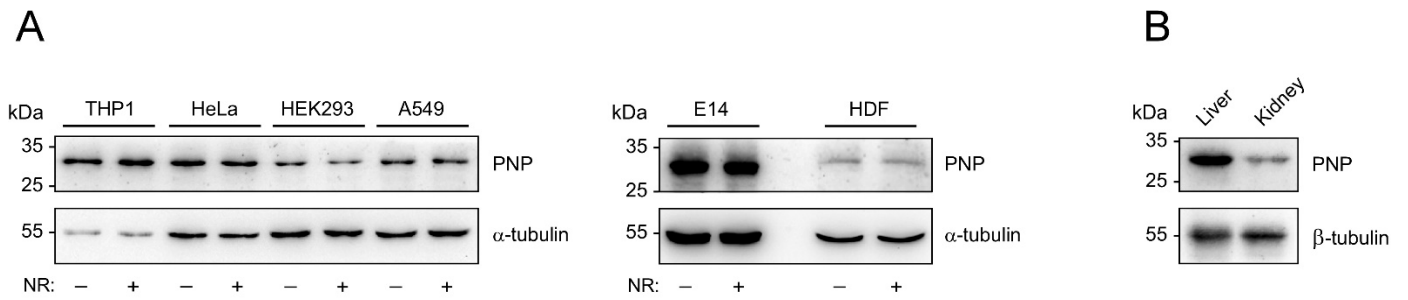
Supplementary Figure 2. NR is metabolized in mammalian cells predominantly by conversion to Nam. HEK293, HeLa (A) and A549 (B) cells were treated with 150 μM NR in the presence or absence of inhibitor of equilibrative nucleoside transporters, dipyridamole (Dip), as indicated. Twenty-four hours after the treatment, cell culture media were analyzed by NMR spectroscopy. Concentrations of NR and Nam in the medium are presented as mean \pm S.D. ($n = 3$). Statistical analysis of differences between the groups was carried out by one-way ANOVA with post hoc comparisons using the Tukey test. * indicates statistical significance at $p < 0.05$, ** indicates statistical significance at $p < 0.01$, *** indicates statistical significance at $p < 0.001$.

Figure S3



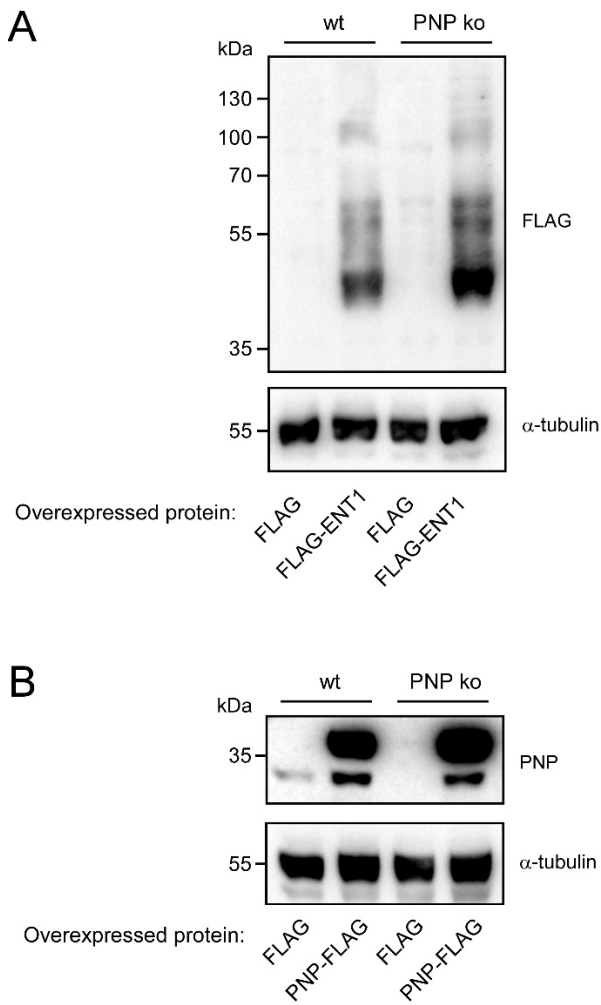
Supplementary Figure 3. Overexpression of purine nucleoside phosphorylase (PNP) increases NR conversion to Nam in HEK293 cells. *A*, HEK293 cells were transiently transfected with vectors encoding FLAG peptide or FLAG-tagged PNP. Twenty-four hours after transfection, the expression of endogenous and FLAG-tagged PNP proteins was detected by immunoblotting using antibody to PNP. α -tubulin served as a loading control. *B*, HEK293 cells were transiently transfected with vectors encoding FLAG-tagged PNP or/and ENT4. Twenty-four hours after transfection, cells were treated with 150 μ M NR. Twenty-four hours after the treatment, culture medium was analyzed by NMR spectroscopy. Concentrations of NR and Nam in culture medium are presented as mean \pm S.D. ($n = 3$ -5). Statistical analysis of differences between the groups was carried out by one-way ANOVA with post hoc comparisons using Tukey test. ** indicates statistical significance at $p < 0.01$, *** indicates statistical significance at $p < 0.001$.

Figure S4



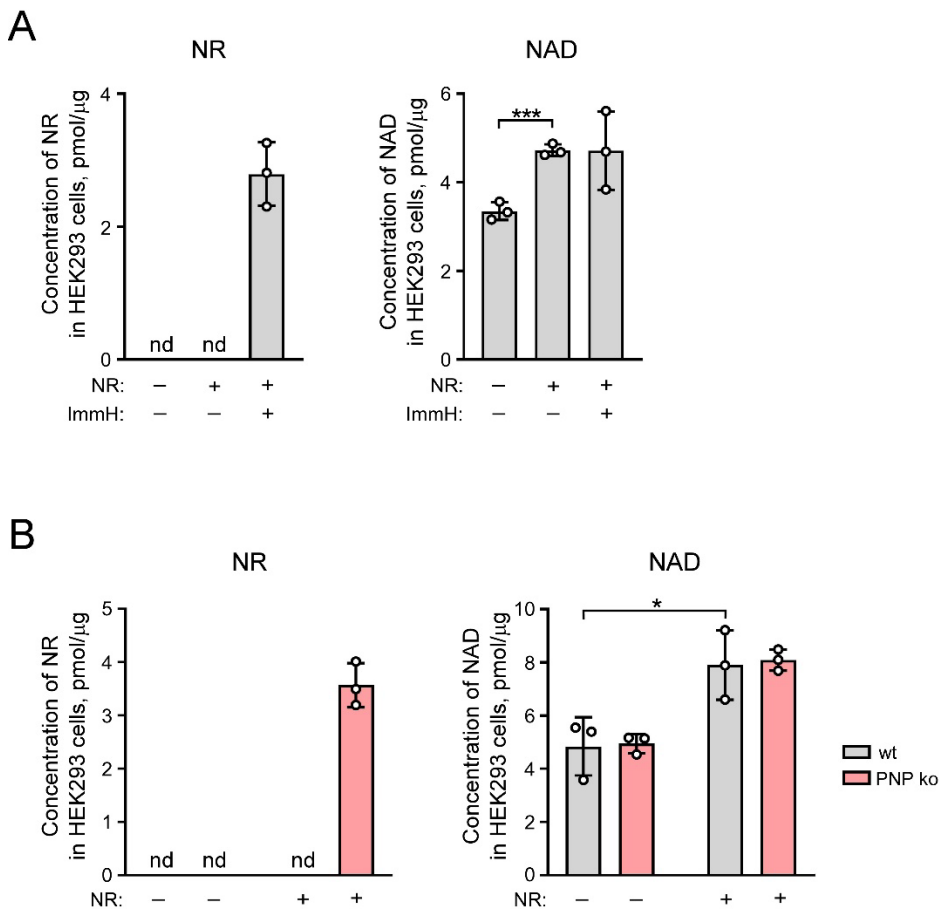
Supplementary Figure 4. PNP is expressed in various types of mammalian cells and mouse tissues. *A*, THP1, HeLa, HEK293, A549 cells, mouse embryonic stem cells E14 (E14) or human dermal fibroblasts (HDF) were treated with 150 μ M NR. Twenty-four hours after the treatment, the expression of PNP in control and NR-treated cells was detected by immunoblotting using antibody to PNP. *B*, Liver and kidney of C57BL/6 mice were collected and analyzed by immunoblotting using antibody to PNP. β -tubulin served as a loading control.

Figure S5



Supplementary Figure 5. Overexpression of FLAG-tagged ENT1 and PNP in wild type and PNP ko HEK293 cells. Wild type (wt) or PNP knockout (ko) HEK293 cells were transiently transfected with vectors encoding FLAG peptide, FLAG-tagged ENT1 (*A*) or FLAG-tagged PNP (*B*). Twenty-four hours after transfection, the expression of FLAG-tagged proteins was detected by immunoblotting using antibody to FLAG peptide (*A*) or PNP (*B*). α -tubulin served as a loading control.

Figure S6



Supplementary Figure 6. Pharmacological inhibition of PNP leads to NR accumulation in wild type or PNP knockout HEK293 cells. *A*, HEK293 cells were treated with 150 μ M NR and PNP inhibitor, Immucillin H (ImmH). *B*, wild type (wt) or PNP knockout (ko) HEK293 cells were treated with 150 μ M NR. Twenty-four hours after the treatment, cell extracts were prepared and analyzed by NMR spectroscopy. Concentrations of NR and NAD in cell extract are presented as mean \pm S.D. ($n = 3$). nd, not detected. Statistical analysis of differences between the groups was carried out by one-way ANOVA with post hoc comparisons using the Tukey test. * indicates statistical significance at $p < 0.05$, *** indicates statistical significance at $p < 0.001$.