SUPPLEMENTAL INFORMATION

Supplemental Figure Legends

Figure S1: Extracellular nicotinic acid, but not tryptophan, is sufficient to maintain mitochondrial NAD$^+$.

A) Extracellular tryptophan, even at 500 µM, is insufficient to maintain cell viability.

B) Time course of NA-dependent regeneration of the mitochondrial NAD^+ pool. 293mitoPARP cells were pre-treated with FK866 in absence or presence of NA for 24 h, as indicated. Then, the cells were further incubated with NA and FK866 for the indicated time period, lysed and subjected to PAR-immunoblot analysis. AIF served as loading control.

Figure S2: Extracelluar degradation of nucleotide derivatives of NAD$^+$ to NR or NAR is essential to support cellular NAD$^+$ synthesis and cell viability in HeLa S3 and 293mitoPARP cells.

A) NPP inhibition by the competitor Ap4A reduces utilization of extracellular NAD^+. 293mitoPARP or HeLa S3 cells were treated with FK866, NAD^+ and Ap4A as indicated. Cell viability was measured by MTT assay.

B) NMN must be dephosphorylated to NR to serve as extracellular NAD^+ precursor. HeLa S3 cells were treated with FK866, NMN and inhibitors for 5'-nucleotidase (CMP) or nucleoside transporters (dipyridamole (DIP), NBTI) and cell viability was measured.

C) CMP, NBTI and DIP do not affect mitochondrial NAD^+ content and cell viability when Nam can be utilized as NAD^+ precursor (no FK866 is added leaving NamPRT uninhibited). 293mitoPARP cells were incubated with the indicated compounds and then either lysed and subjected to PAR immunoblot analysis (top) or analyzed for cell viability (bottom).

D) Inhibition of NMN utilization by CMP is dose-dependent. 293mitoPARP cells were incubated with FK866, NMN and different concentrations of CMP as indicated. Subsequently, cells were either lysed and immunoblotted for PAR detection (top) or subjected to cell viability

measurements (middle). Identical treatment of HeLa S3 cells (bottom) resulted in similar cell viability.

E) Extracellular NAD^+ has to be degraded to NR to support intracellular NAD^+ synthesis.

HeLa S3 cells were treated with FK866, NAD^+ and the indicated inhibitors and assayed for cell viability.

F) Utilization of extracellular NAAD for mitochondrial NAD^+ formation is less sensitive to CMP, NBTI and dipyridamole (DIP) than that of extracellular NAD^+. 293mitoPARP cells were treated with FK866, NAAD and inhibitors as indicated. Subsequently, cells were lysed and immunoblotted for PAR detection (top) or subjected to cell viability measurements (bottom). Note that NA is a degradation product of NAAD which bypasses the inhibition of NamPRT by FK866.

Figure S3: Analysis of potential contributors to mitochondrial NAD^+ synthesis.

A) Subcellular localization of a putative alternatively translated NAPRT. The open reading frame of NAPRT contains a Met at position 25 which might potentially serve as alternative translational start and generate a mitochondrial isoform. HeLa S3 cells transiently expressing the corresponding N-terminally truncated NAPRT-FLAG protein were stained with MitoTracker (MT) 24 h after transfection and subjected to FLAG immunocytochemistry. As shown, the use of the putative alternative translational start site would not alter the cytosolic/nuclear distribution observed for the full-length protein (Fig, 4A). Bar 20 µm.

B) Thymidine kinase 2 (TK2) colocalizes with mitochondria. HeLa S3 cells were subjected to FLAG-immunocytochemistry 24h after transient transfection with a vector encoding C-terminally FLAG-tagged human TK2. Nuclei were stained with DAPI and mitochondria with Mitotracker (MT). Bar 20 µm. The mitochondrial association of TK2 confirms its matrix localization and thereby the conclusions from Fig. 5.

Figure S4: Immunoprecipitation and catalytic activity of human NADS.

A) 293 cells were transiently transfected with a vector encoding FLAG-tagged human NADS. After cell lysis, NADS was immunoprecipitated using M2 anti-FLAG matrix. The immunoblot shows the detection of NADS in whole cell extract (WCE, left), in the immunoprecipitate (IP) and in WCE after immunoprecipitation.

B) Immunoprecipitated NADS is active using NAAD as substrate. Purified FLAG-tagged NADS was incubated with NAAD, glutamine and ATP for 2 h (+NADS). Generated NAD^+ was then converted to NADH by alcohol dehydrogenase (ADH) (scheme, left panel). Nucleotides were separated by HPLC and detected at 259 and 340 nm as indicated (middle and right panel). The control did not contain NADS protein (-NADS).

Figure S1

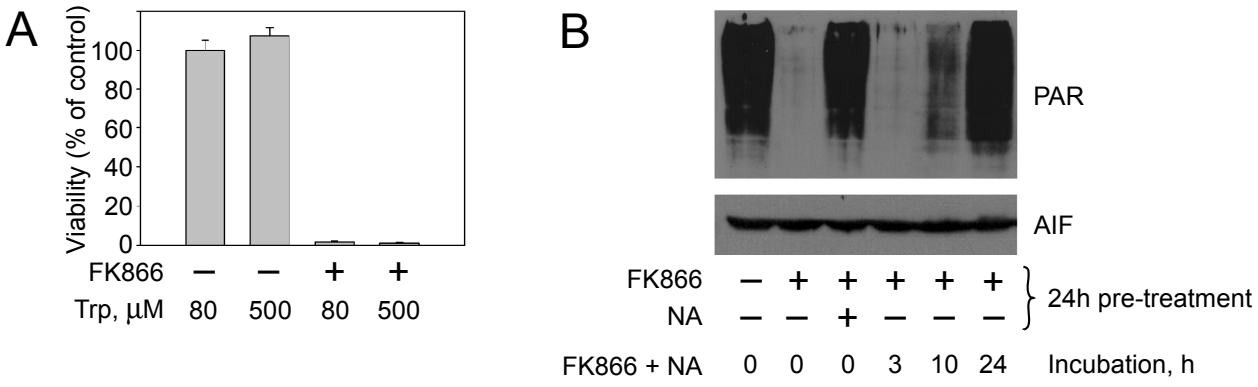

A
Viability (% of control)
100
80
60
40
20
0
FK866 – – + +
Trp, µM 80 500 80 500
B
PAR
AIF
FK866 – + + + + +
NA – – + – – –
24h pre-treatment
FK866 + NA 0 0 0 3 10 24
Incubation, h

Figure S2

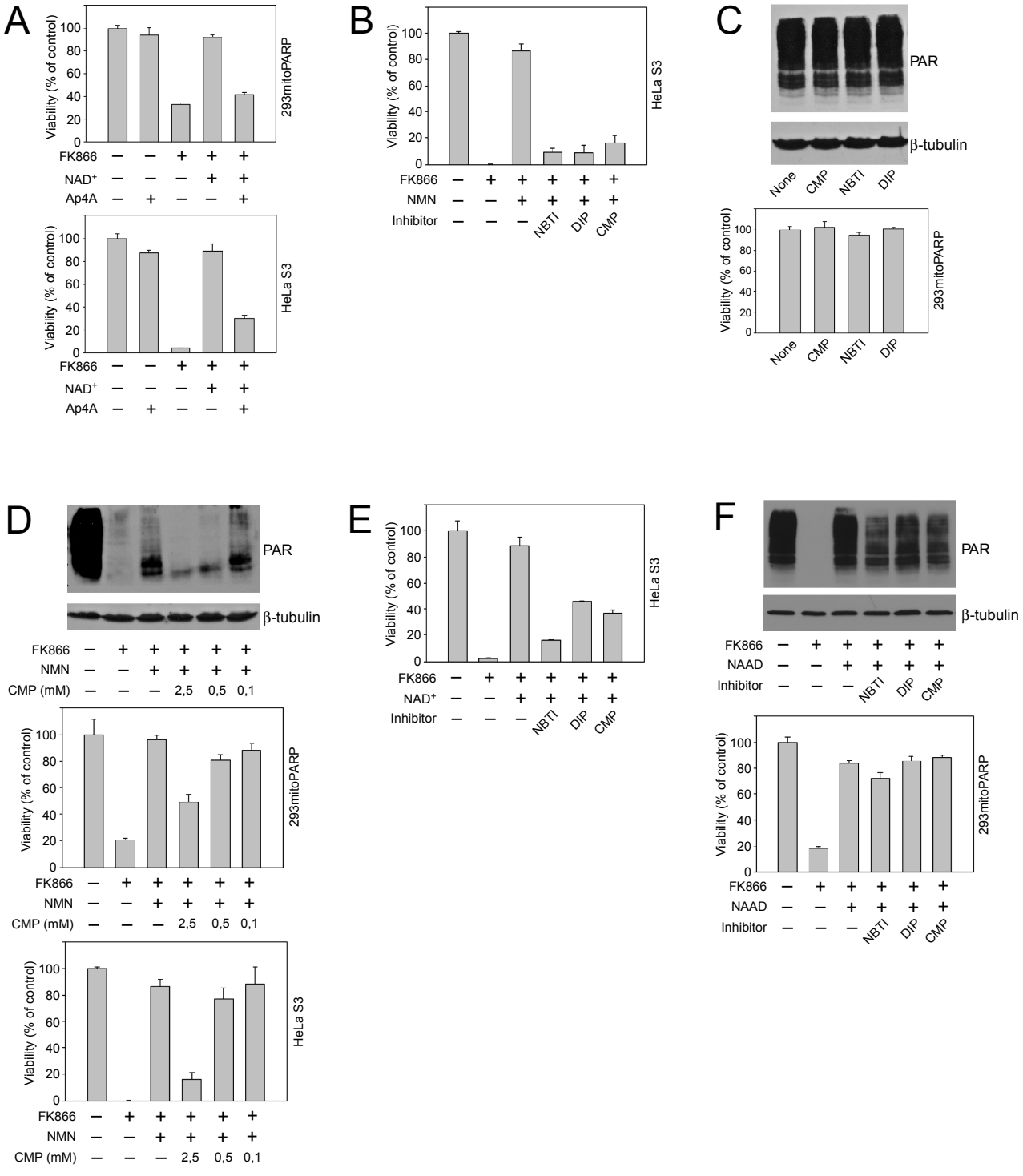

A
Viability (% of control)
293mitoPARP
HeLa S3
FK866
NAD+
Ap4A
B
NMN
Inhibitor
NBTI
DIP
CMP
C
PAR
β-tubulin
None
D
CMP (mM)
2,5
0,5
0,1
E
F
NAAD

Figure S3

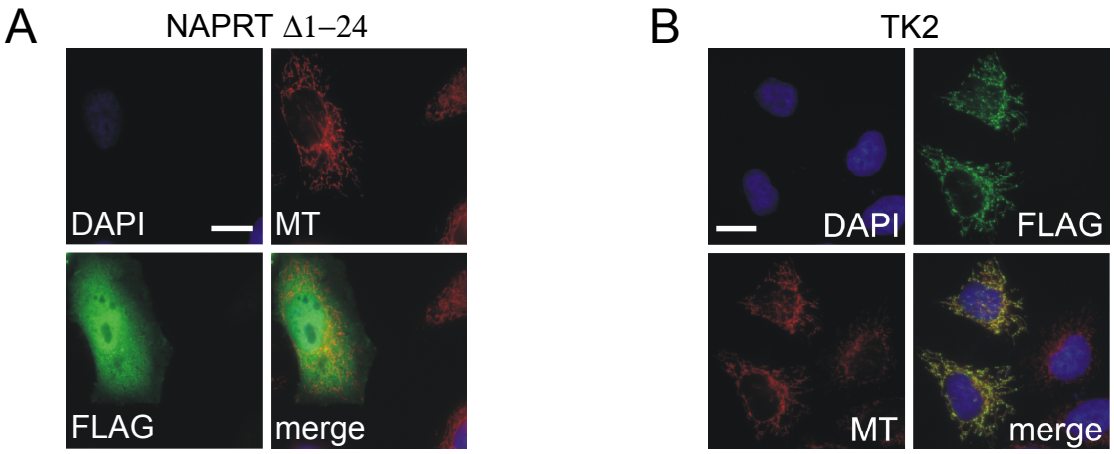
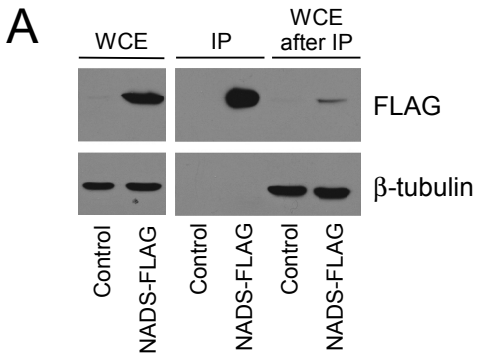
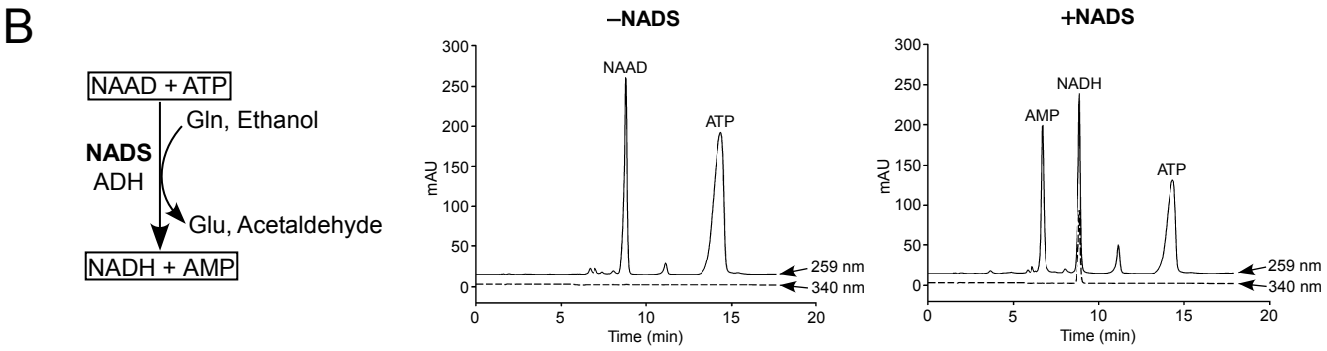


Figure S4


A
WCE
IP
WCE after IP
FLAG
β-tubulin
Control
NADS-FLAG
Control
NADS-FLAG
Control
NADS-FLAG


B
NAAD + ATP
Gln, Ethanol
NADS
ADH
Glu, Acetaldehyde
NADH + AMP
–NADS
+NADS
NAAD
ATP
AMP
NADH
mAU
Time (min)
259 nm
340 nm