Supplementary Information

Chronic depletion of subcellular NAD pools reveals their interconnectivity and a buffering function of mitochondria

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Supplementary Figure 1

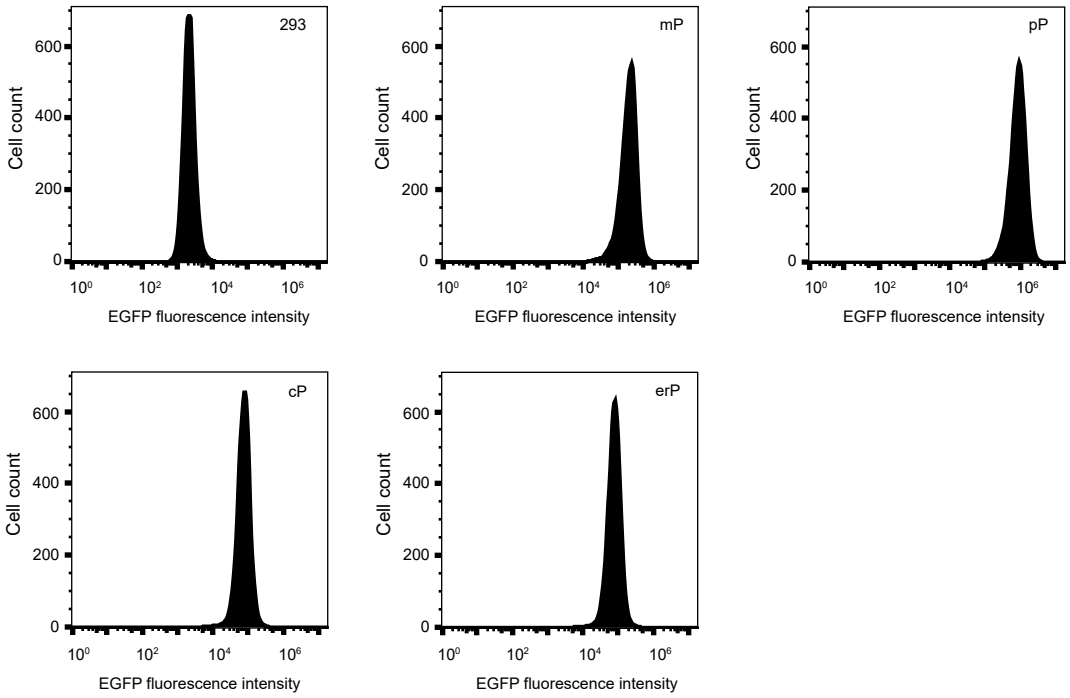
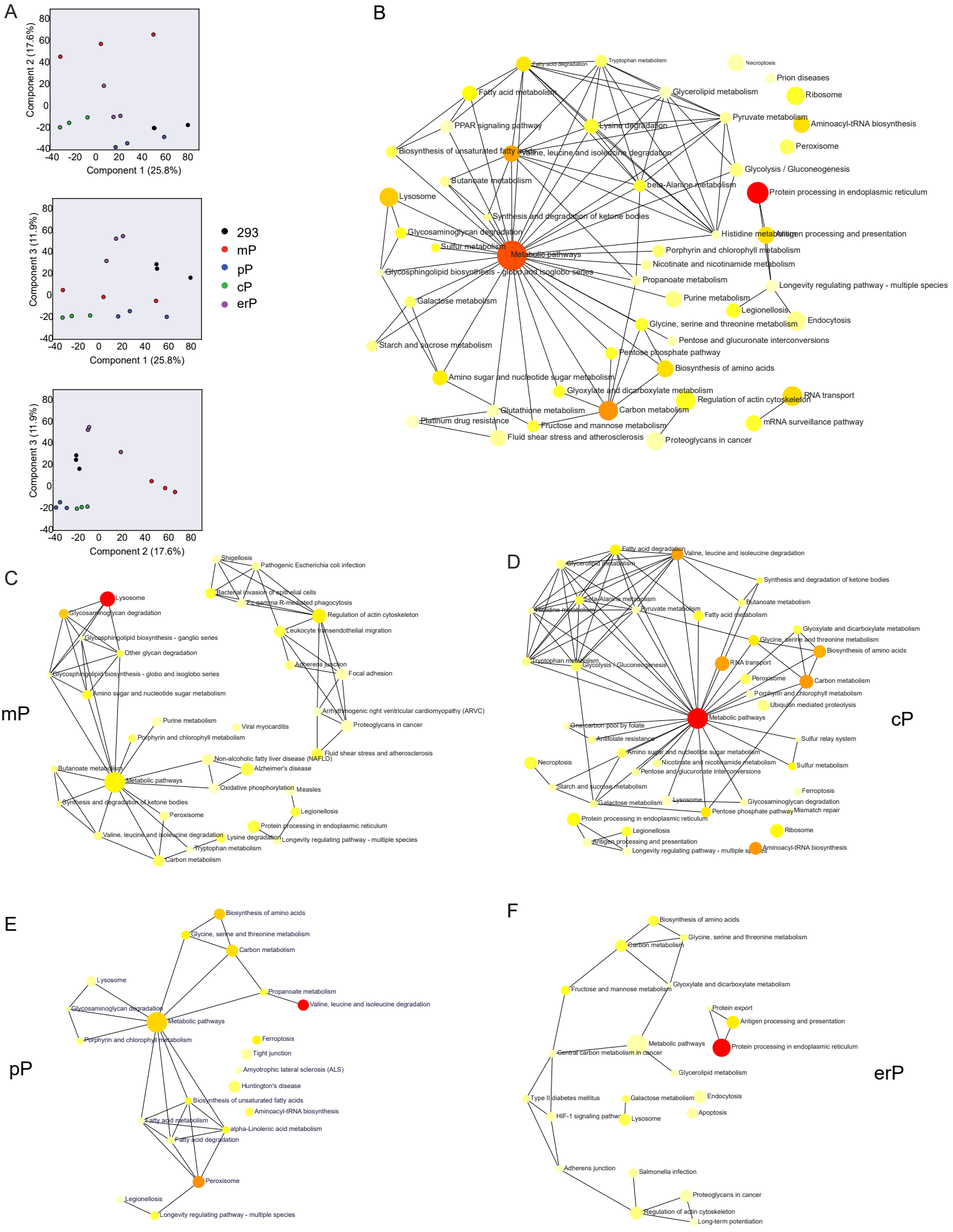


Figure S1

Representative flow cytometric analysis of EGFP fluorescence intensity of parental 293 cells and stably transfected PARP1cd cell lines. Cells were gated to exclude debris, followed by standard doublet exclusion.

mP - 293 mitoPARP1cd, pP – 293 pexPARP1cd, cP – 293 cytoPARP1cd, erP – 293 erPARP1cd.

Supplementary Figure 2



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gene name	mP	cP	erP	pP
ASS1	0.41	0.34	0.43	0.38
SCIN	0.62	0.65	0.50	1.75
ANXA1	0.36	0.85	0.42	2.28
ECH1	0.67	0.84	0.66	1.10
H2AFY2	0.51	0.52	0.58	0.68
NAMPT	1.00*	0.75	0.83*	0.81*
NAPRT	0.50	0.58	0.80*	0.85*

Figure S2

A) PCA analysis of quantitative proteomics measurements. The graph shows the first 3 principal components, which together account for about 55% of the total variation.

B) Network enrichment analysis of proteins differentially expressed in stable PARP1cd cell lines in comparison to parental 293 cells including all proteins that are significantly changed in at least one cell line ($p<0.001$).

(C-F) Cell line specific network enrichment analysis of significant differentially expressed proteins between parental 293 cells and stable PARP1cd cell lines ($p<0.001$).

G) Top: Proteins differentially expressed in all PARP cell lines in comparison to parental 293 cells ($p < 0.001$). Bottom: changes in NAD synthesis and consumption related proteins ($p < 0.001$) in at least one of the PARP1cd overexpressing cell lines. *indicates changes with p-value above 0.001. Colors indicate the strength of the up-(green) and down-(yellow to red)regulation.

mP - 293 mitoPARP1cd, pP – 293 pexPARP1cd, cP – 293 cytoPARP1cd, erP – 293 erPARP1cd.

Supplementary Figure 3

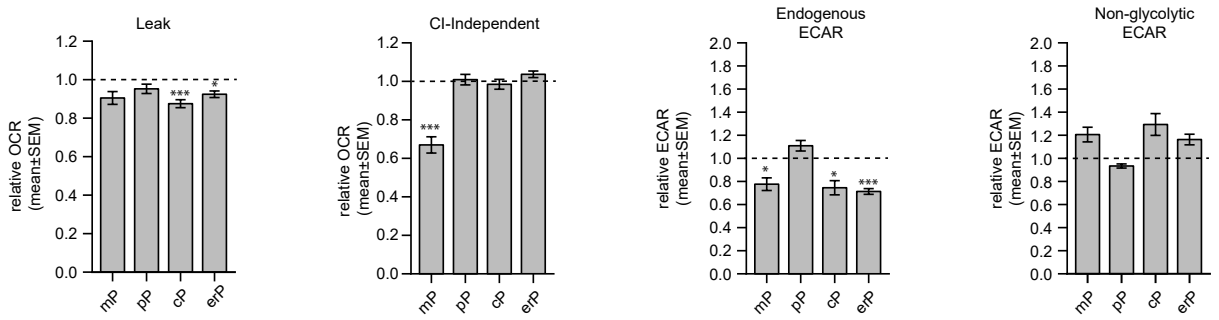
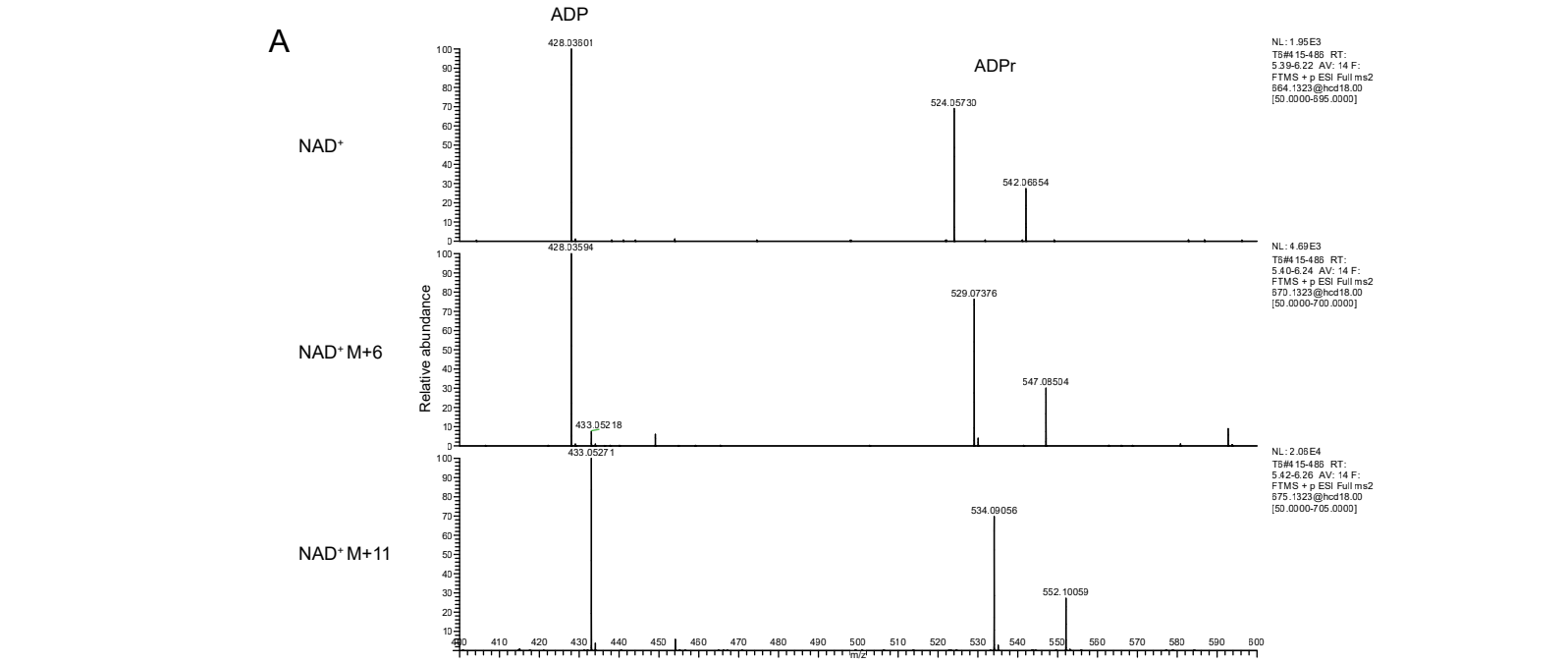


Figure S3

Activity and integrity of mitochondrial respiration in terms of oxygen consumption rate (OCR), and glycolysis in terms of extracellular acidification rate (ECAR) in stably transfected PARP1cd cell lines compared to parental 293 cells as determined by extracellular flux analysis. Data are presented relative to parental 293 cells as mean ± SEM where n = 3. *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001 (Student's t-test).

mP - 293 mitoPARP1cd, pP – 293 pexPARP1cd, cP – 293 cytoPARP1cd, erP – 293 erPARP1cd.

Supplementary Figure 4



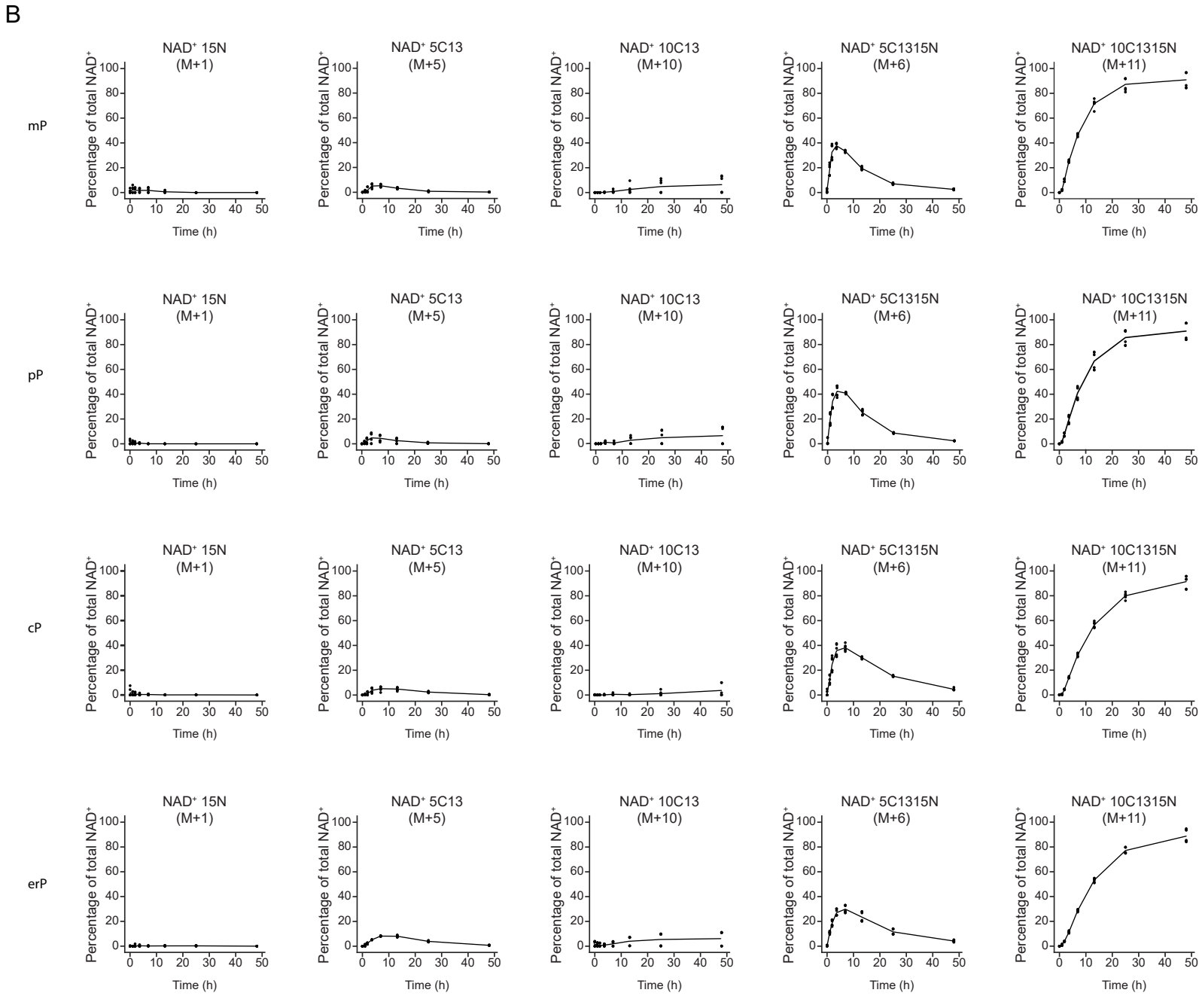


Figure S4

A) Exemplary fragment-ion spectra of NAD^+ and the NAD^+ isotopologs NAD^+ M+6 and NAD^+ M+11 as generated by MS-MS analysis from 293 cell extracts after 13.2 h of incubation with isotopically labelled nicotinamide (15N) and glucose (13C).

B) Time-dependent appearance of labelled isotopologs in stably transfected 293PARP1cd cell lines upon incubation in the presence of isotopically labelled nicotinamide (15N), and glucose (13C) where n = 6.

mP - 293mitoPARP1cd, pP – 293pexPARP1cd, cP – 293cytoPARP1cd, erP – 293erPARP1cd.

Supplementary Figure 5

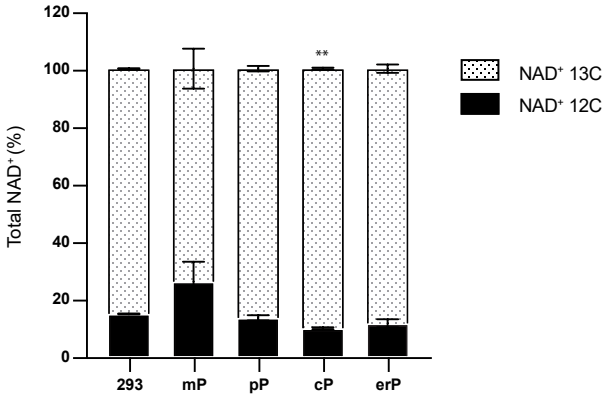


Figure S5

Distribution of NAD labeling following 48 hours incubation of parental 293 cells and stably transfected PARP1cd cell lines in presence of NA 13C and FK866 (2mM).

mP - 293 mitoPARP1cd, pP – 293 pexPARP1cd, cP – 293 cytoPARP1cd, erP – 293 erPARP1cd.

Supplementary Figure 6

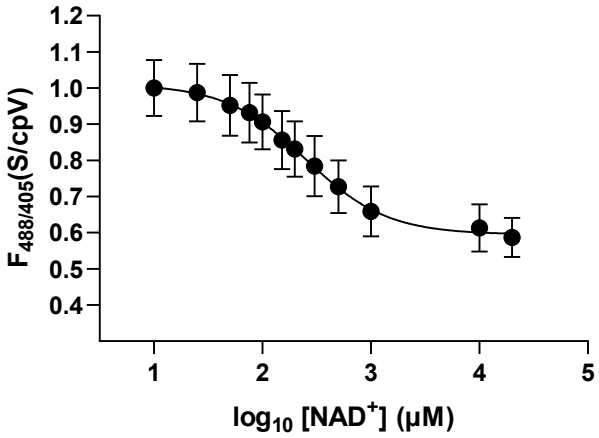


Figure S6

Dose-response curve of the NAD biosensor upon permeabilization by Digitonin. HeLa cells stably expressing the NAD biosensor or the cpVenus control in the cytosol were permeabilized with digitonin and exposed to varying concentrations of NAD^+. The fluorescence ratio (488/405 nm) of the NAD biosensor, as measured by flow cytometry, was normalized to the fluorescence ratio (488/405 nm) of the corresponding cpVenus control and the values were plotted relative to 10 µM NAD^+. Each point represents the mean ± SD, n > 3.