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Sample-size estimation

For the microscopy, the number of cells analyzed are specified in Figures 2 and 3 (and are from 3 independent experiments). For the flow cytometry the medians are reported for three independent biological measurements (all performed in technical triplicates, each of them contain 2000 - 7000 cells; the process was automatized the sample size varied from the start of the 96-w plate and the end where some cells already at the time to re-adhere) with the statistical analysis.

Replicates

All experiments were performed with at least three independent replicate (i.e. not the same day, not with the same cells/labeled sensor) and for each experiment technical triplicates were performed. The titration data show the mean +/- SD of one of this triplicate, while the reported fitted values c50, r50... are the mean +/- SD of the three independent experiments. For the microscopy, the number of cells analyzed are specified (and are from 3 independent experiments). For the flow cytometry experiments, the medians are reported for three independent biological measurements (all performed in technical triplicates, each of them contain 2000 - 7000 cells; the process was automatized the sample size varied from the start of the 96-w plate and the end where some cells already at the time to re-adhere) with the statistical analysis.
**Statistical reporting**

Titrations data (Fig. 2 and Supplementary Fig. 1) are represented as mean ± s.d. of the emission ratio (TMR/SiR) from technical triplicates. The calculated fitting parameters (c50, r50, KD', K50, Rmin, Rmax) used for the quantification of NAD+ and NADPH/NADP+ by ratio imaging, FLIM and flow cytometry (estimations) were determined as mean ± s.d. of three independent titrations (each performed in triplicates) (Table 1). Flow cytometry data (Fig. 4 and Supplementary Fig. 5) were characterized by non-normal distributions. In essence, the sample distributions showed a positive kurtosis and skewness, and were heteroscedastic. The statistical analysis (Supplementary Fig. 5) was then performed in R by a Kruskal-Wallis test with post-hoc Dunn’s test using the Benjamini-Hochberg method (FDR) for multiple comparison correction with respect to control conditions. The significance level was set to α = 0.05 and two-tailed p-values were reported (*p < 0.05; n.s. p ≥ 0.05).

**Group allocation**

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**Additional data files (“source data”)**

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