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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  $\pm$  SD of one of this triplicate, while the reported fitted values c50, r50... are the mean  $\pm$  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

Titration data (Fig. 2 and Supplementary Fig. 1) are represented as mean  $\pm$  s.d. of the emission ratio (TMR/SiR) from technical triplicates. The calculated fitting parameters ( $c_{50}$ ,  $r_{50}$ ,  $KD'$ ,  $K_{50}$ ,  $R_{min}$ ,  $R_{max}$ ) used for the quantification of NAD<sup>+</sup> and NADPH/NADP<sup>+</sup> by ratio imaging, FLIM and flow cytometry (estimations) were determined as mean  $\pm$  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  $\alpha = 0.05$  and two-tailed p-values were reported (\* $p < 0.05$ ; n.s.  $p \geq 0.05$ ).

## Group allocation

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## Additional data files ("source data")

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