



Figure S4. Vacuolin-induced enlargement of lysosomes has no significant effect on NAADP-induced Ca^{2+} release.

A-C WT MEFs were either treated with vacuolin-1 (1 μM for 4 h) or were left untreated (Control) and fura-2 Ca^{2+} signals measured after addition of 10 μM NAADP/AM in Ca^{2+} -containing medium. Vacuolin-induced enlargement of lysosomes had no significant effect on NAADP-induced Ca^{2+} release, neither in terms of amplitudes nor kinetics.

A,B (A) Maximum Ca^{2+} amplitude and the mean Ca^{2+} response over a 10-min period post-addition of NAADP/AM. (B) Lag time (delay between NAADP/AM addition and commencement of the Ca^{2+} response) and the time to peak (time period from first Ca^{2+} increase to maximum Ca^{2+} peak). $n = 102-105$; ns, $p > 0.05$ (ns) relative to control using the ANOVA-Tukey test.

C Enlargement of lysosomes was confirmed by LysoTracker-Red DND99 staining (100 nM for 3 min) in WT MEFs.