Wozniak et al., http://www.jcb.org/cgi/content/full/jcb.200608035/DC1

Supplemental materials and methods

Mitochondrial calcium imaging

The mitochondrial-targeted, ratiometric, calcium-sensitive, circularly permutated YFP construct (mt-ratiometric pericam) has been previously described (Nagai et al., 2001) and was a gift from A. Miyawaki (RIKEN, Saitama, Japan). Jurkat cells were transfected with Lipofectamine 2000 (Invitrogen) and allowed to express ratiometric pericam for 48 h. Intramitochondrial calcium was monitored by alternating excitation wavelengths between 380 and 495 nm, and monitoring emission at 535 nm. The calcium-bound excitation wavelength is 495 nm, therefore the data was collected and expressed as the 495:380 ratio. The excitation filters used were a D380/30x and a HQ495/15x, the beamsplitter used was a 505DCXR, and the emission filter used was a HQ535/50m (Chroma Technology Corp.). Rapid excitation filter changes were accomplished by a 10–2 filter wheel (Lambda; Sutter) and controller. Data acquisition and analysis was accomplished using MetaFluor software (Molecular Devices), and ratio images are presented in the Intensity Modulated Display mode solely for display purposes.

Reference

Nagai, T., A. Sawano, E.S. Park, and A. Miyawaki. 2001. Circularly permuted green fluorescent proteins engineered to sense Ca^{2+}. *Proc. Natl. Acad. Sci. USA.* 98:3197–3202.