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SUPPLEMENTARY MATERIAL

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Fine structural detection of calcium ions by photoconversion

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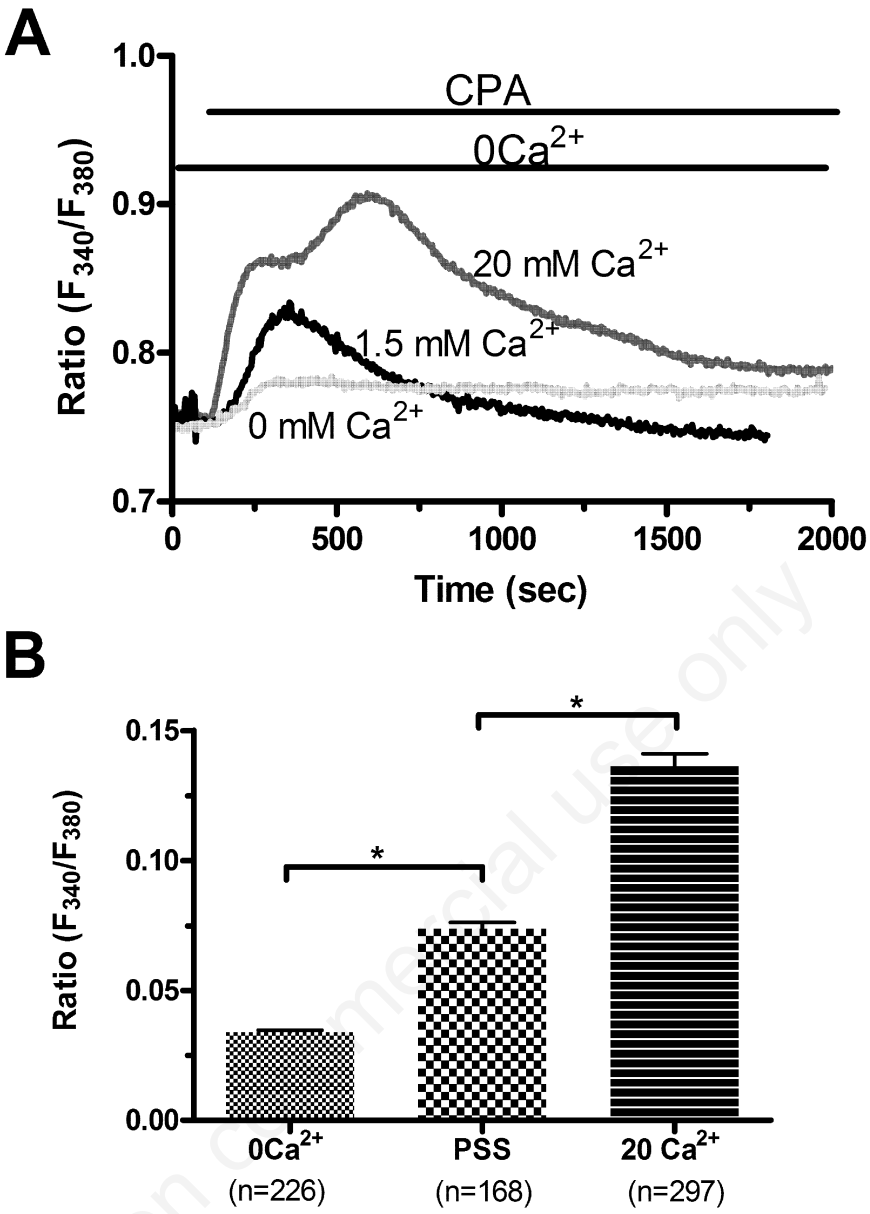
$[Ca^{2+}]_i$ measurements

HeLa cells were loaded with 2 μM Fura-2 acetoxymethyl ester (Fura-2/AM; 1 mM stock in dimethyl sulfoxide) in PSS for 30 min at room temperature. After washing in PSS, the coverslip was fixed to the bottom of a Petri dish and the cells observed by an upright epifluorescence Axiolab microscope (Carl Zeiss, Oberkochen, Germany), usually equipped with a Zeiss ×40 Achroplan objective (water-immersion, 2.0 mm working distance, 0.9 numerical aperture). Cells were excited alternately at 340 and 380 nm, and the emitted light was detected at 510 nm. A first neutral density filter (1 or 0.3 optical density) reduced the overall intensity of the excitation light and a second neutral density filter (optical density=0.3) was coupled to the 380 nm filter to approach the intensity of the 340 nm light. A round diaphragm was used to increase the contrast. The excitation filters were mounted on a filter wheel (Lambda 10, Sutter Instrument, Novato, CA, USA). Custom software, working in the LINUX environment, was used to drive the camera (Extended-ISIS Camera, Photonic Science, Millham, UK) and the filter wheel, and to measure and plot on-line the fluorescence from 10-15 rectangular 'regions of interest' (ROI) enclosing 10-15 single cells. Each ROI was identified by a number. Adjacent ROIs never superimposed. $[Ca^{2+}]_i$ was monitored by measuring, for each ROI, the ratio of the mean fluorescence emitted at 510 nm when exciting alternatively at 340 and 380 nm (shortly termed 'ratio'). An increase in $[Ca^{2+}]_i$ causes an increase in the ratio.[1] Ratio measurements were performed and plotted on-line every 3 s. The experiments were performed at room temperature (22°C). The amplitude of the peak Ca^{2+} response to cyclopiazonic acid (CPA) was measured as the difference between the ratio at the peak and the mean ratio of 1 min baseline before the peak.[1] Pooled data are given as mean±SE and statistical significance ($P<0.05$) was evaluated by the Student's *t*-test for unpaired observations.

References

1. Lodola F, Laforenza U, Bonetti E, Lim D, Dragoni S, Bottino C, et al. Store-operated Ca2+ entry is remodelled and controls in vitro angiogenesis in endothelial progenitor cells isolated from tumoral patients. PloS One 2012;7:e42541.



Supplementary Figure 1.

Endoplasmic reticulum Ca^{2+} levels change as a function of the external Ca^{2+} concentration. A, ER Ca^{2+} levels were assessed by pre-incubating the cells for 1 h in the presence of $0Ca^{2+}$, 1.5 mM Ca^{2+} (PSS) and 20 mM Ca^{2+} in the Fura-2/AM-loading solution. After the washout, the cells were then transferred in the recording chamber. To monitor ER Ca^{2+} levels, we employed the protocol described in Lodola *et al.*[1] HeLa cells were exposed for 100 sec to a 0 Ca^{2+} external solution and challenged with CPA to block SERCA activity and deplete the ER: the following increase in $[Ca^{2+}]_i$ reflects ER Ca^{2+} content. B, mean±SE of the peak amplitude of CPA-induced ER Ca^{2+} mobilization under the designated treatments. The asterisk indicates $P<0.05$.