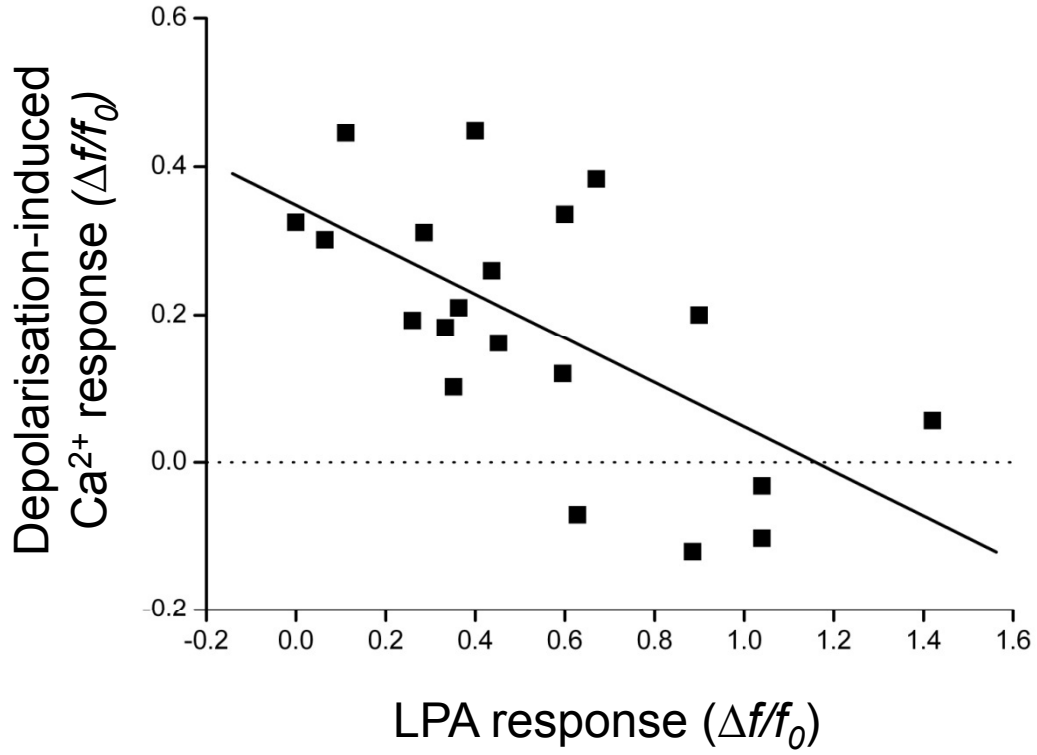
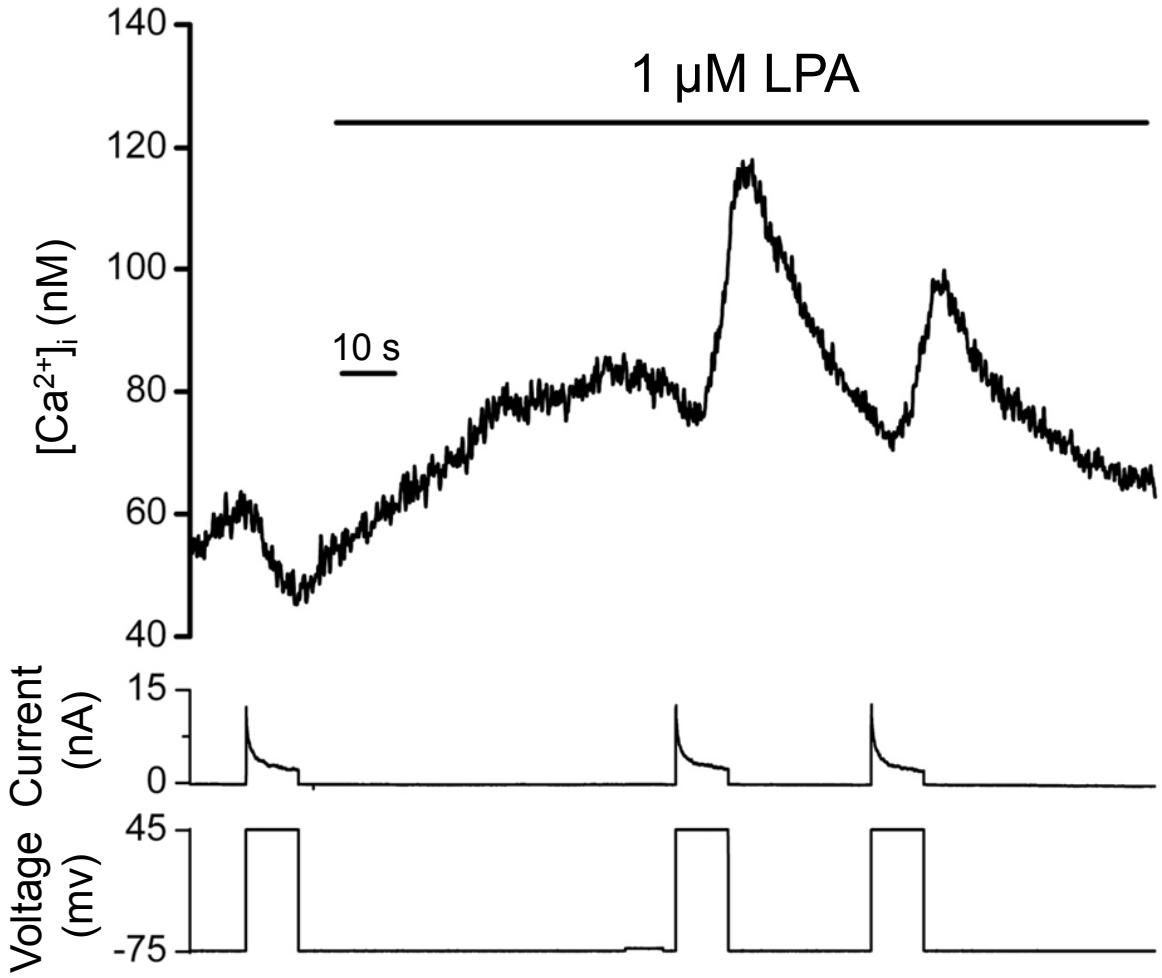
Supplemental figure 1



Supplemental figure 1. Inverse correlation between the response to LPA and the effect of a depolarisation. Relationship between the LPA-evoked peak response (10 nM, $\Delta f/f_0$) versus the depolarisation-induced $[Ca^{2+}]_i$ response ($\Delta f/f_0$) for a voltage step of +70 mV (from -80 to -10 mV) for a duration of 60 seconds in a Ca^{2+}-containing Ringer. The data were best fit with a linear regression (solid line): $Y = A + B\,X$, where A is the intercept and B is the slope. The correlation coefficient (R) had a value of -0.63 ± 0.03 (n = 20, p = 0.0027). The dotted line indicates the lack of effect of the depolarisation ($\Delta f/f_0 = 0$). The points below the dotted line ($\Delta f/f_0 < 0$) correspond to cells in which the depolarisation induced a $[Ca^{2+}]_i$ decrease, while the points above the dotted line ($\Delta f/f_0 > 0$) correspond to cells in which the depolarisation induced a $[Ca^{2+}]_i$ increase. Each point represents a different cell.

Supplementary figure 2



Response to depolarisation in a HEK-293 cell before and during stimulation of endogenous LPA receptors. Fura-2 fluorescence recording of intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) from a HEK-293 cell under whole-cell patch clamp. All depolarisations were from a holding potential of -75mV to +45mV for a duration of 10 seconds. Depolarisation prior to application of LPA caused a modest reduction in basal $[Ca^{2+}]_i$. Application of LPA induced a small, gradual increase in $[Ca^{2+}]_i$ and two depolarisations in the continued presence of LPA each stimulated an additional $[Ca^{2+}]_i$ increase. This result indicates that mammalian LPA receptors signaling is also amplified by depolarisation, although the full characteristics of this response in terms of dependence on amplitude of the voltage change and agonist concentration are currently under investigation.

Experimental procedures:
Conventional whole-cell patch clamp recordings were carried out in voltage clamp mode using an Axopatch 200B amplifier (Axon Instruments, CA) with 70–75% series resistance compensation. External solution: 145 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM Hepes, 10 mM d-glucose, pH 7.35 (NaOH). Internal saline: 150 mM KCl, 2 mM $MgCl_2$, 0.1 mM EGTA, 0.05 mM K_5fura2, pH 7.2 (KOH). Fura-2 fluorescence measurements of $[Ca^{2+}]_i$ were made using a Cairn Spectrophotometer System (Cairn Research Ltd, Kent, UK) coupled to a Nikon Diaphot inverted microscope (Nikon, Japan). Excitation wavelengths were 340 and 380 nm with emission bandwidths of ~480–600. Fluorescence emission and electrophysiological data were simultaneously recorded to computer using Cairn acquisition hardware and software. Fluorescence data were sampled at 60 Hz, averaged to give a final acquisition rate of 15 Hz, and exported for analysis within Microcal Origin (Microcal Software Inc., Northampton, MA). For calibration of fura-2, the maximum and minimum 340/380 nm fluorescence ratios were measured extracellularly, and background-corrected ratios were converted to $[Ca^{2+}]_i$ after application of a viscosity correction factor of 0.85. A K_d of 258 nm was derived using a calibration kit from Molecular Probes. Recordings were made at room temperature (20–24°C).