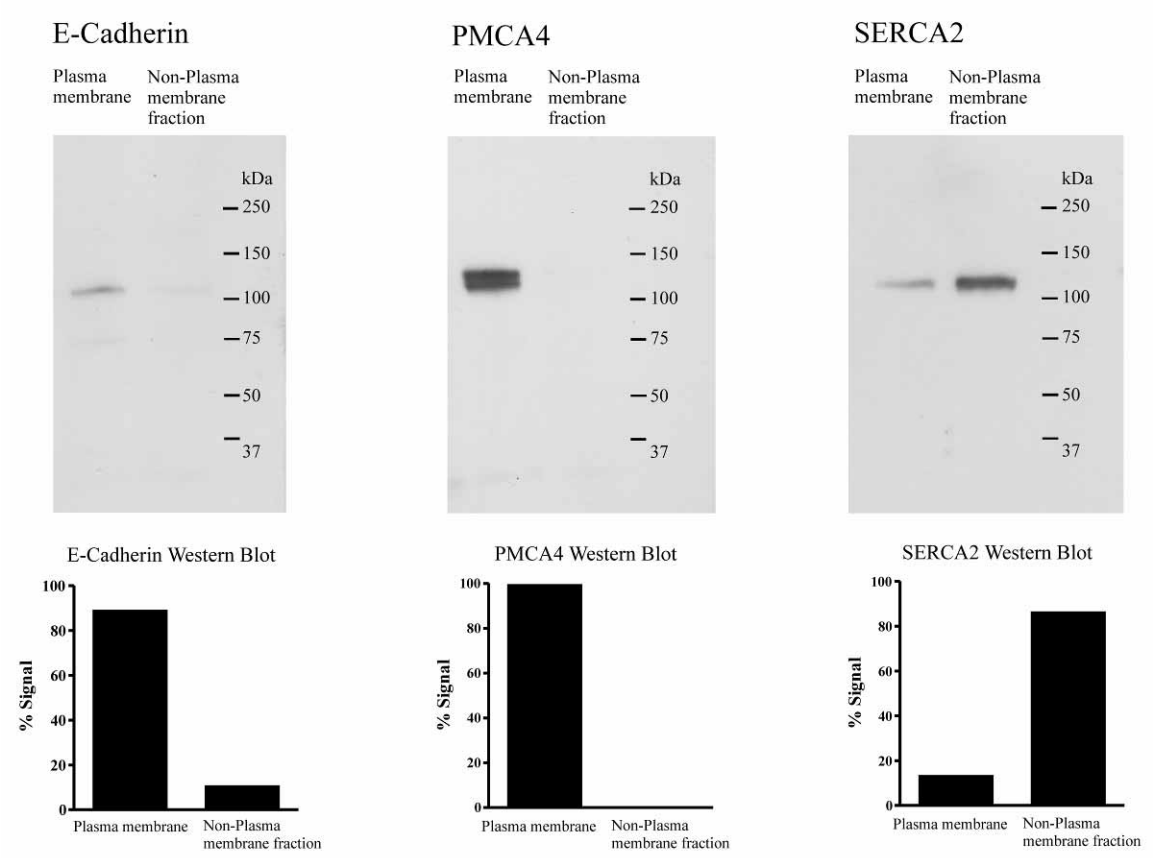
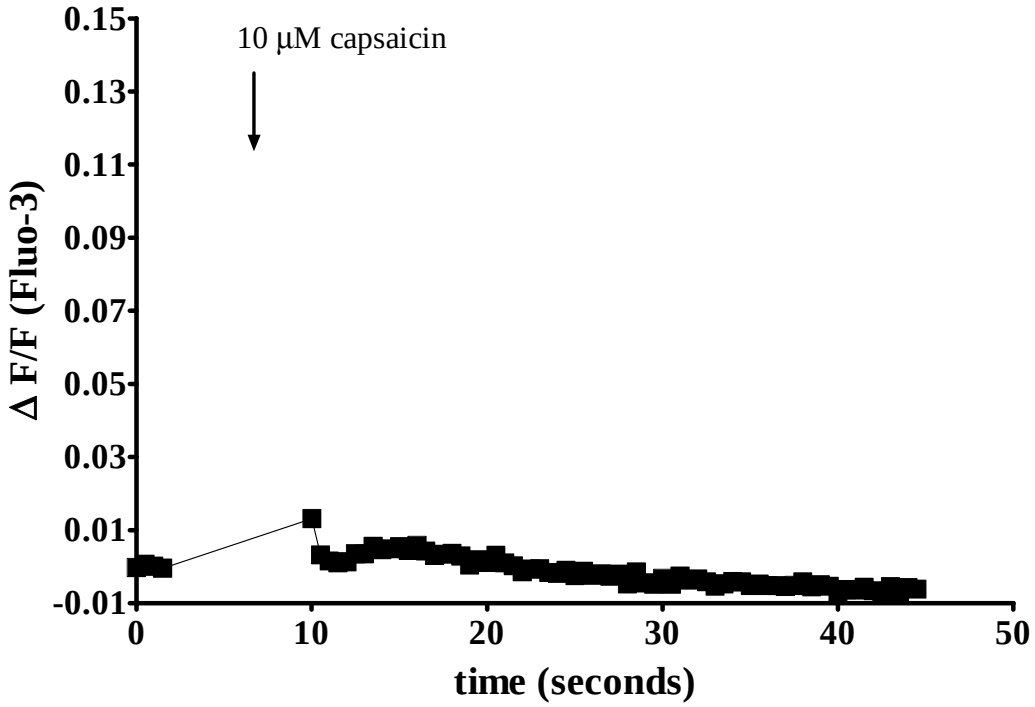
Supplemental Data



Supplementary Figure 1

Analysis of purity of plasma membrane and non-plasma membrane fractions.

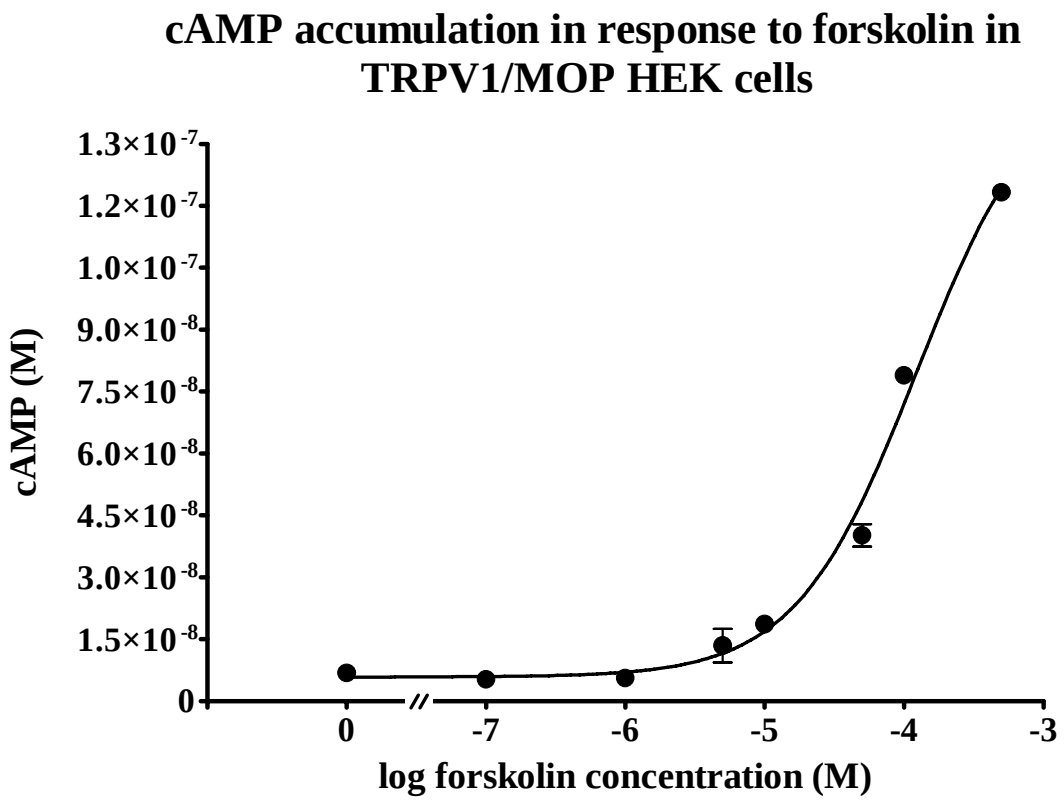
Plasma membrane and remaining non-plasma membrane fractions were prepared from HEK cells using the plasma membrane isolation kit (MBL International Corp) according to manufacturer's instructions. Purity of both fractions was analysed by assessing expression of plasma membrane proteins E-Cadherin (rabbit E-Cadherin antibody, 1:3000, a kind gift from Alpha Yap, Institute of Molecular Biosciences, Queensland, Australia) and PMCA4 (mouse anti-PMCA4 antibody JA9, 1:1000, Abcam) as well as the endoplasmic protein SERCA2 (mouse anti-SERCA2 antibody MA3-910, 1:1000, Affinity Bioreagents). Optical density of bands was determined using GraphPad Prism and expressed as % of the combined signal from the plasma membrane and remaining non-plasma membrane fractions.



Supplementary Figure 2

Capsaicin-induced Ca^{2+} responses in double stable TRPV1/MOP HEK cells are mediated through TRPV1.

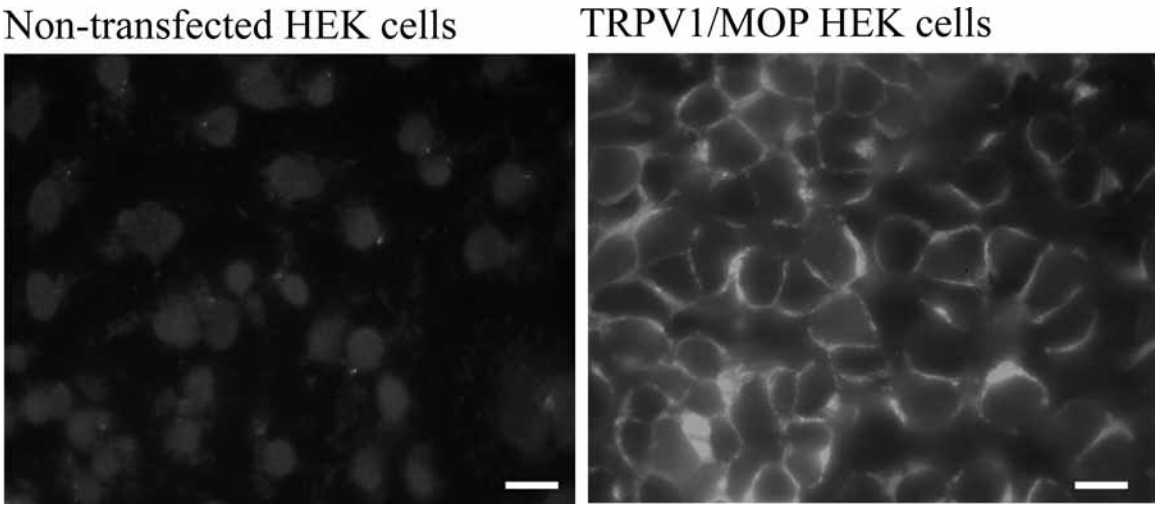
Non-transfected HEK cells were loaded with the fluorescent Ca^{2+} dye Fluo-3 and responses to addition of 10 μM capsaicin monitored using the fluorescent plate reader NOVOstar. Non-transfected HEK cells did not respond with an increase in intracellular Ca^{2+} to capsaicin, even when very high (10 μM) capsaicin concentrations were applied.



Supplementary Figure 3

Treatment with forskolin dose-dependently increases cAMP accumulation in TRPV1/MOP HEK cells.

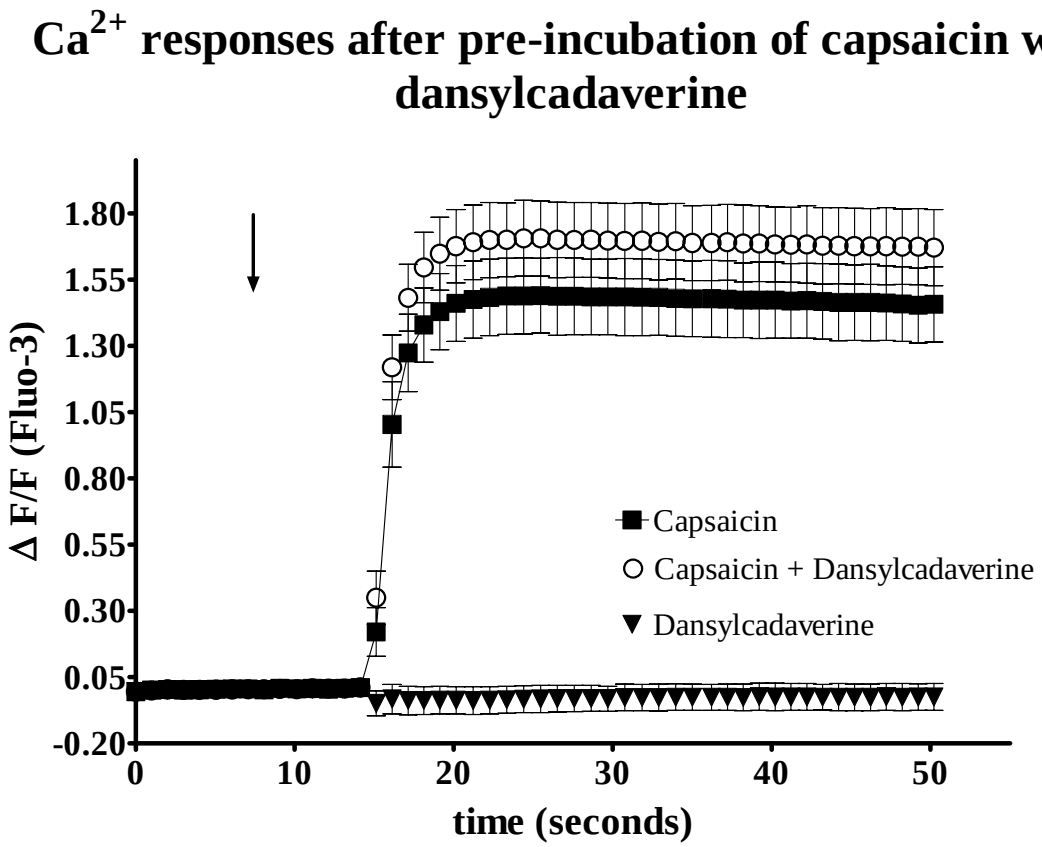
cAMP accumulation in response to incubation with varying concentrations of forskolin and constant concentrations of IBMX (100 μM) was assessed in TRPV1/MOP HEK cells using an AlphaScreen cAMP accumulation assay. Data are presented as mean ± SEM with n = 3.



Supplementary Figure 4

TRPV1 immunofluorescence and DAPI stain in untransfected HEK cells.

Non-transfected HEK cells and TRPV1/MOP HEK cells were plated on PDL-coated glass coverslips, fixed with methanol-acetone (1:1) and incubated with anti-TRPV1 antibody (1:100, Santa Cruz) followed by anti-rabbit FITC conjugated secondary antibody (1:300, Zymed) and DAPI (300 nM) for visualisation of nuclei. Minimal non-specific immunofluorescence is visible in non-transfected HEK cells. Scale bar, 20 μm.



Supplementary Figure 5

Ca^{2+} responses in TRPV1/MOP HEK cells after pre-incubation of capsaicin with dansylcadaverine

TRPV1/MOP HEK cells were loaded with the fluorescent Ca^{2+} dye Fluo-3 and responses to addition of 300 nM capsaicin (Capsaicin, ■) were monitored using the fluorescent plate reader FLIPR. Pre-incubation of capsaicin with dansylcadaverine (1 mM; Capsaicin+Dansylcadaverine, ○) for 10 minutes did not decrease capsaicin-induced Ca^{2+} responses. Addition of dansylcadaverine alone did not affect Ca^{2+} responses (Dansylcadaverine, ▼). Data are presented as mean ± SD from n = 28 wells.