

**Supplemental Fig. S1.** TRPC5 immunostaining detection, with a monoclonal antibody (NeuroMab, UC Davis/NIH, Davis CA, USA). Hoechst-stained non-transfected HEK-293 cells visible in transmitted (A) and confocal microscopy (blue) (B). TRPC5-GFP transfected HEK cells visible in transmitted (C) and confocal microscopy showing the GFP labelling (green) (D) and TRPC5 immunoreactivity (red) (E). (F) shows the overlaying of (D) and (E) indicating co-localization of TRPC5-GFP labelling and TRPC5 immunoreactivity. Scale bar: 10  $\mu$ M.

**Supplemental Fig. S2.** Measurements of relative cell volume of TRPC5-expressing cells in response to hypoosmotic stimulus in the same experiment. The relative cell volume was calculated from Fura-2 fluorescence at the isosbestic point (see text). The thick trace corresponds to the fluorescence change and the thin line to the cell volume change. The values of volume change were scaled to facilitate the comparison with its fluorescence increase. The hypoosmotic stimulus was applied at the time indicated by the bar.