



Figure S6. Co-localization of TPCs with organelle-marker proteins.

A *Tpcn1/2^{-/-}* MEFs expressing mCherry-tagged TPC1 or TPC2 were immunostained with antibodies against RFP (red signal) and organelle-marker proteins (Lamp1: late endosomes/lysosomes; TfR: recycling endosomes; EEA1: early endosomes; PDI: endoplasmic reticulum; green signal). Representative images correspond to cells with a co-localization value close to the mean value for its group (B).

B Pearson's co-localization coefficient was calculated for individual cells ($n = 26 - 32$). Values represent mean \pm SEM.

Despite the partial ER retention of TPC2.mCh in *Tpcn1/2^{-/-}* MEFs, the Ca^{2+} signals evoked by NAADP in these cells retain the expected acidic Ca^{2+} store pharmacology (Fig 5G).