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SUPPLEMENTARY ONLINE DATA

InsP_3 receptors and Orai channels in pancreatic acinar cells: co-localization and its consequences

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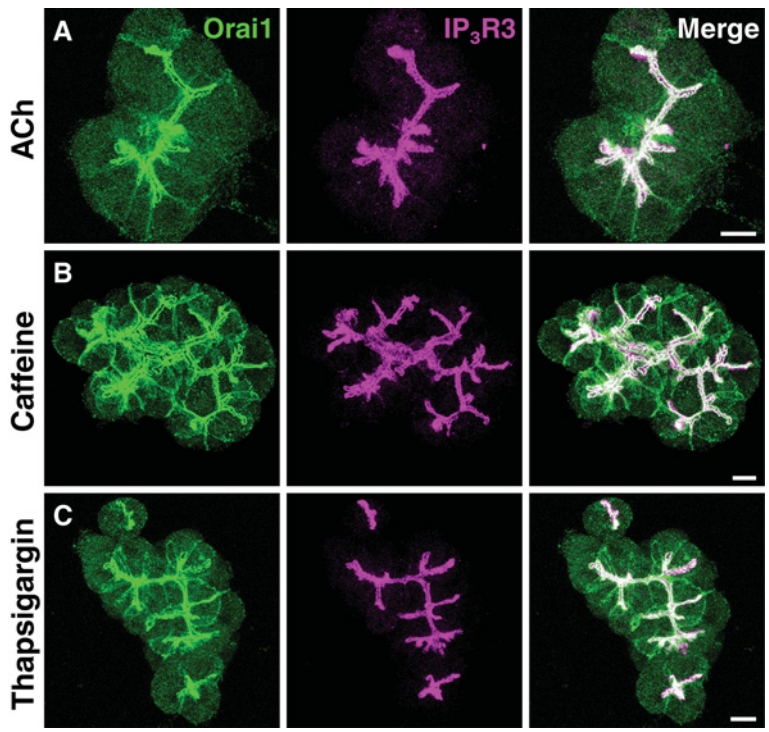

Figure S1 Orai1 and IP_3R3 staining in acinar cells following various stimuli

All images show maximum projections of confocal optical slices from pancreatic acinar cell clusters. The distribution of Orai1 (green) in pancreatic acinar cells in the presence of (**A**) ACh (300 nM, $n = 3$), (**B**) caffeine (10 mM, $n = 3$) and (**C**) TG (2 μM, $n = 3$). In the apical pole, Orai1 was co-localized with type 3 IP_3Rs (magenta) in every condition. Note that we have not observed clearance of Orai1 or IP_3R3 from the apical region following the ACh stimulation, which should trigger significant exocytosis of zymogen granules and therefore additional membrane turnover in the apical region of the cells. The distribution of Orai1 and IP_3R3 was similar to that in control (unstimulated) cells. Some lateral and basal Orai was observed (**A**–**C** right panels, green colour on the merged images). Scale bars correspond to 10 μm.

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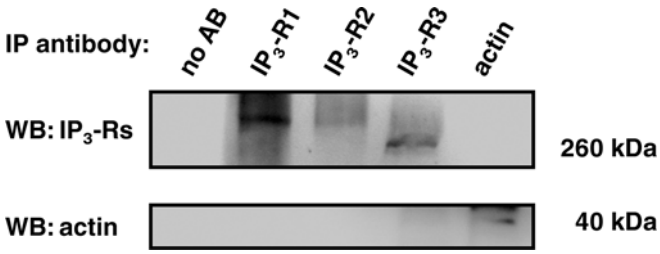

Figure S2 IP_3Rs do not co-immunoprecipitate actin from pancreatic acinar cells

Western blots show immunoprecipitates from pancreatic acinar cell lysates. Protein G–Sepharose beads without antibodies (no AB) do not precipitate significant amounts of protein from acinar cell lysates (lane 1). Antibodies against all three subtypes of IP_3Rs precipitate the corresponding IP_3R (lanes 2–4, upper panel) but not actin (same lanes on the lower panel), while an anti-actin antibody precipitates actin from the lysate but none of the three IP_3Rs (lane 5). Hence it is unlikely that the observed IP_3R–Orai1 co-immunoprecipitation (reported in the main paper and illustrated in Figure 1 of the main paper) is mediated by actin.

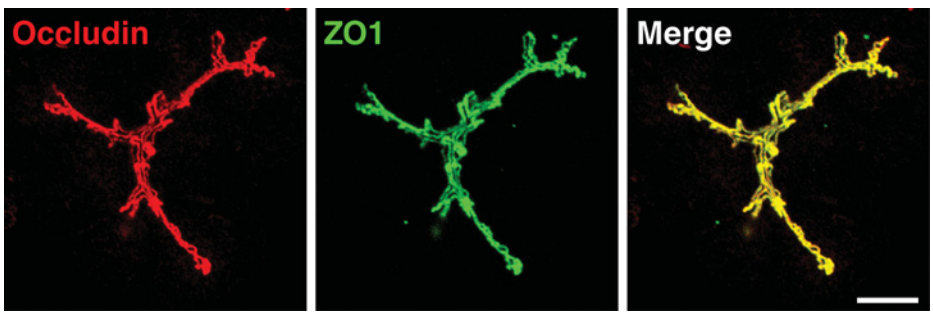

Figure S3 Occludin and ZO1 co-localize in pancreatic acinar cells

Antibodies against occludin or ZO1 were used (see Figure 3 and accompanying text in the main paper) to probe the localization of tight junctions and to define the apical membrane regions of the cells. Anti-ZO1 antibody [1] was a gift from Dr M. Furuse from Kobe University. The images demonstrate the co-localization of the two proteins ($n = 4$) which both can therefore be used to label tight junctions. Scale bar corresponds to 10 μm.

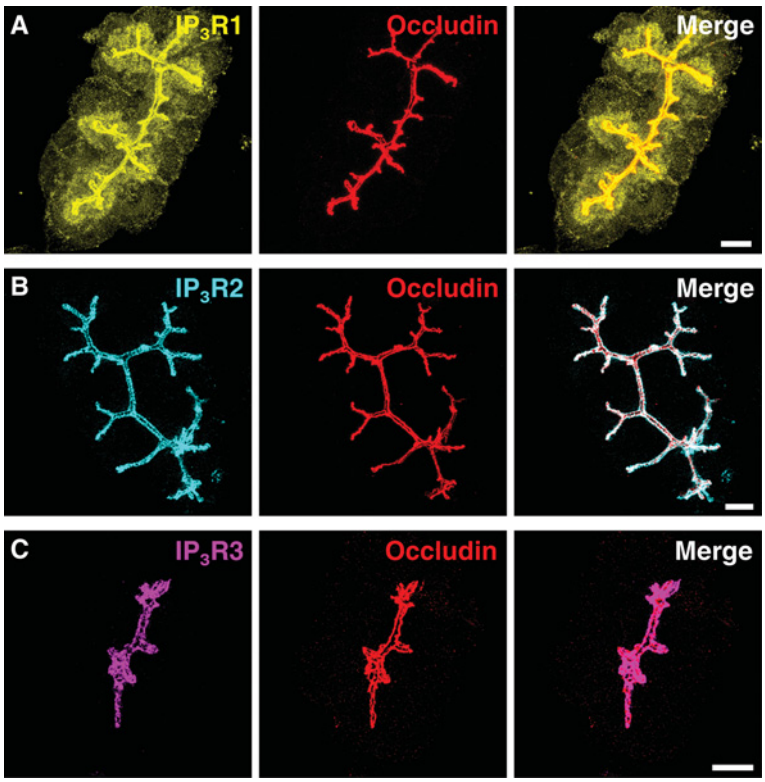

Figure S4 Apically localized IP_3Rs co-localize with tight junction marker occludin

Images show the maximum projections of optical sections from pancreatic acinar cell clusters. IP_3R1 (yellow, **A**), IP_3R2 (cyan, **B**) and IP_3R3 (magenta, **C**) co-localize with occludin (red, central panels) in the apex of acinar cells ($n = 3$, 4 and 3 respectively). Merged images are shown in the right-hand panels. Scale bars correspond to 10 μm.

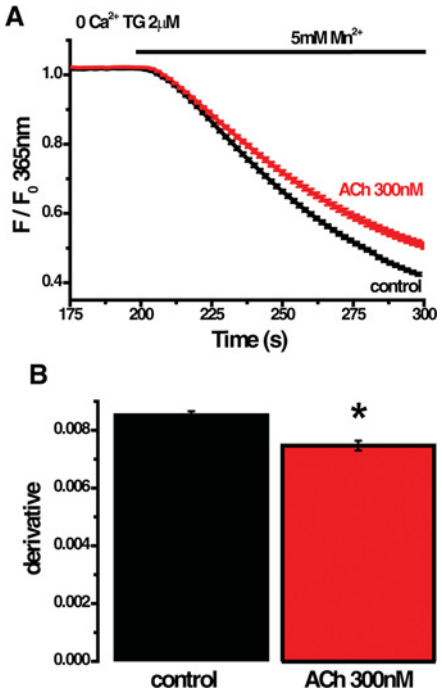

Figure S5 Effect of ACh on Mn^{2+} influx in pancreatic ainar cells

(**A**) Intracellular Ca^{2+} stores of fura 2-loaded acinar cells were depleted by a 15 min pre-incubation with nominally Ca^{2+}-free extracellular solution containing 2 μM TG . To assess the effect of ACh on Mn^{2+} entry, store-depleted cells were treated for an additional 5 min with Ca^{2+}-free extracellular solution containing 2 μM TG (control, black trace, $n = 139$) or 2 μM TG and 300 nM ACh (red trace, $n = 145$). Subsequently 5 mM Mn^{2+} was added to the solutions. (**A**) Shows average traces $\pm$S.E.M. (**B**) Quantification (averaged maximal amplitude of the derivatives of individual traces) of Mn^{2+} quench in control (black bar) and in ACh (300 nM, red bar)-treated acinar cells. The rate of fura 2 quench was measured by differentiating the declining part (caused by the addition of 5 mM Mn^{2+}) of the curve and determining the maximal amplitude of the derivative.

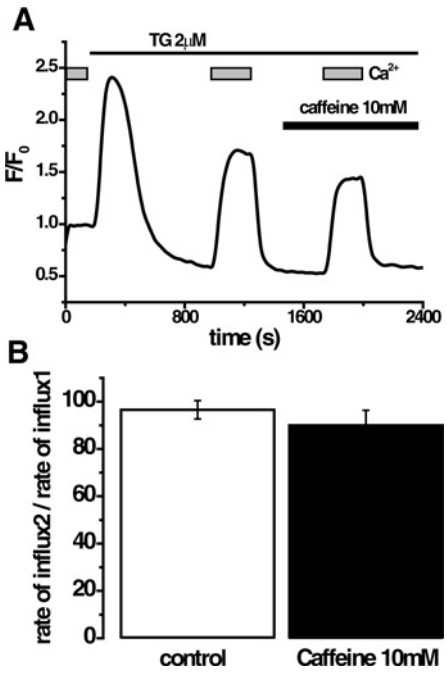

Figure S6 Inhibition of IP_3Rs by caffeine does not affect the rate of SOCE in acinar cells

Fluo-4 was used as the cytosolic Ca^{2+} indicator in experiments involving caffeine because of the strong effect of caffeine on fluorescence of fura 2 [2]. Caffeine was shown to efficiently inhibit InsP_3-induced Ca^{2+} responses in pancreatic acinar cells [3] as well as responses to InsP_3-producing secretagogues [2]. (**A**) Example trace illustrates an experiment designed to test the effect of caffeine using the double pulse protocol (for details of the procedure see Figure 6 and the accompanying text in the main paper). Acinar cells were placed in nominally Ca^{2+}-free extracellular solution and internal Ca^{2+} stores were depleted by the addition of 2 μM TG to the bath. Following store depletion two calcium pulses (2 mM) were applied to measure the effect of caffeine on store operated Ca^{2+} entry. The curves were differentiated and the maximal derivative attained during external Ca^{2+} pulses determined. (**B**) SOCE rate (averaged and normalized maximal derivative) in control conditions (white bar) and in the presence of caffeine (black bar). Caffeine had no statistically significant effect on the maximal SOCE rate (probed using Student's *t*-test).

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