Supplementary Information

Suppl. Fig. 1. Orai1 but not Orai2 and Orai3 efficiently reconstitutes store-operated Ca^{2+} entry in I_{CRAC}-deficient SCID fibroblasts. *(A)* Ca^{2+} influx was examined by single-cell video imaging in SCID fibroblasts transduced with Orai1, Orai2 or Orai3 using a bicistronic IRES-GFP retroviral vector. Cells were stimulated with thapsigargin (TG, 1 μM). *Left*, Orai1 restores Ca^{2+} influx in GFP^+ cells whereas Orai2 and Orai3 are only marginally effective. *Right*, the Ca^{2+} response of Orai1-reconstituted cells, and the residual Ca^{2+} response of cells reconstituted with Orai2 or Orai3, is inhibited by 2 μM La^{3+} and 75 μM 2-APB respectively. *(B)* SCID fibroblasts were left untransduced (none) or retrovirally transduced with Stim1 alone, Orai1 alone or Stim1 together with either Orai1, Orai2 or Orai3. Ca^{2+} influx was assessed in GFP+ cells. *(C)* Graph of average peak $[Ca^{2+}]_i$ levels and influx rates in GFP^+ SCID fibroblasts. Data are derived from experiments similar to those shown in *(B)*. Error bars represent S.E.M.

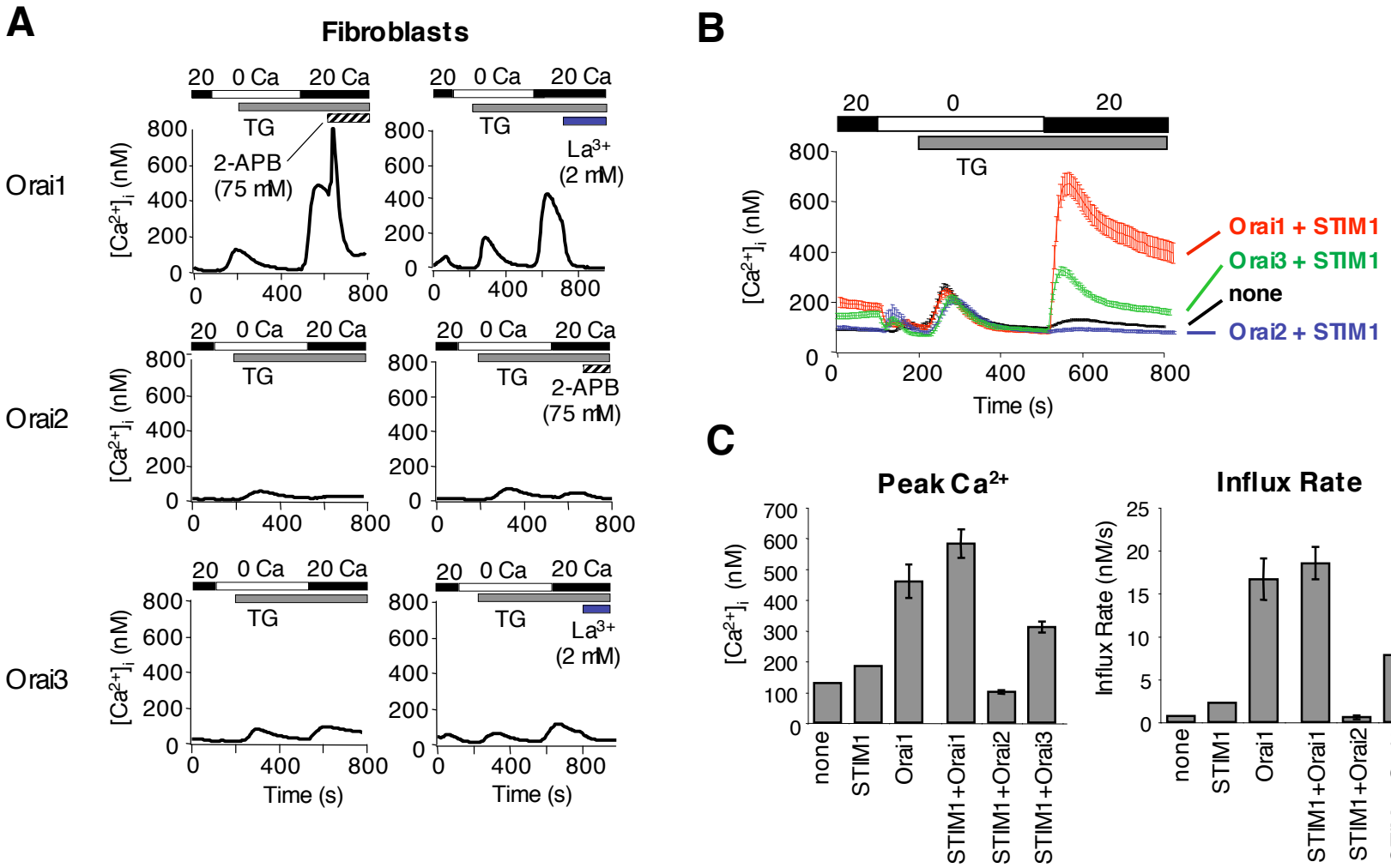
Suppl. Fig. 2. Alignment of protein sequences of *Drosophila* Orai and its three mammalian homologues Orai1, Orai2 and Orai3. The alignment was made using ClustalW. The transmembrane segments predicted by the Kyte-Doolittle hydropathicity score are indicated in blue, two of the conserved glutamate residues (E106 and E190) are shown in red and the N-glycosylation site (N223) in Orai1 is shown in green. This figure was also used as a supplementary figure in (22).

Suppl. Fig. 3. **Colocalisation of Orai1 with Stim1 after thapsigargin treatment**.

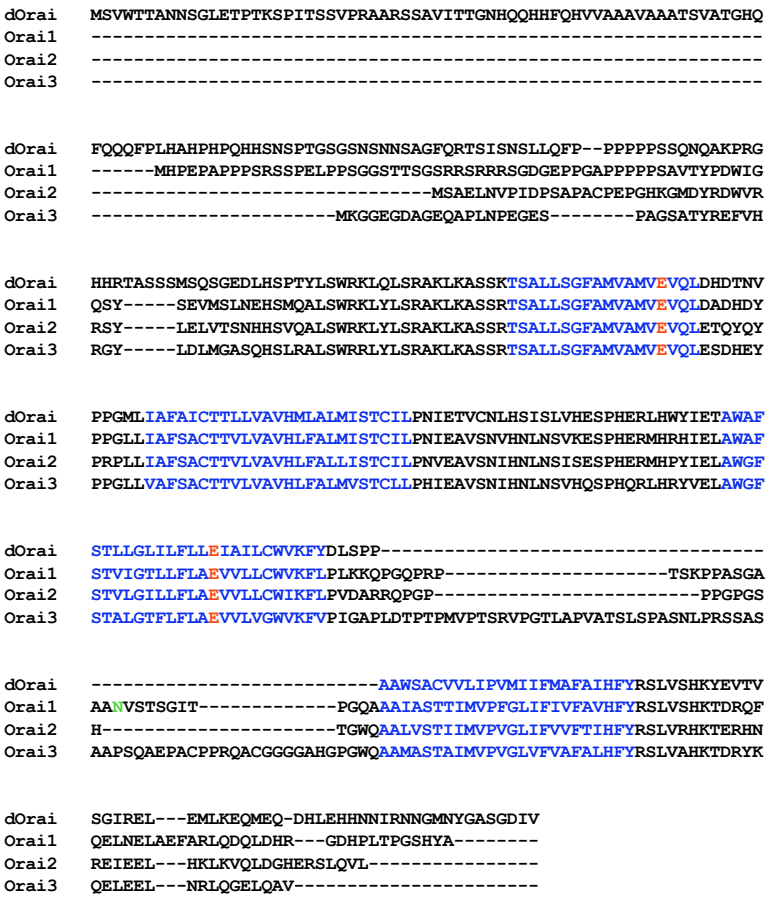
Orai1 colocalises with Stim1 in Jurkat T cells following thapsigargin treatment. Jurkat T cells were transiently transfected with FLAG-tagged Orai1 and Myc- tagged Stim1. After 48 hrs cells were either left untreated or treated with 1 μM thapsigargin for 10 min, fixed, permeabilised and stained with anti-Stim1 and anti-FLAG antibodies.

Suppl. Fig. 4. Expression and subcellular localisation of the E106Q and E190Q mutants of Orai1.

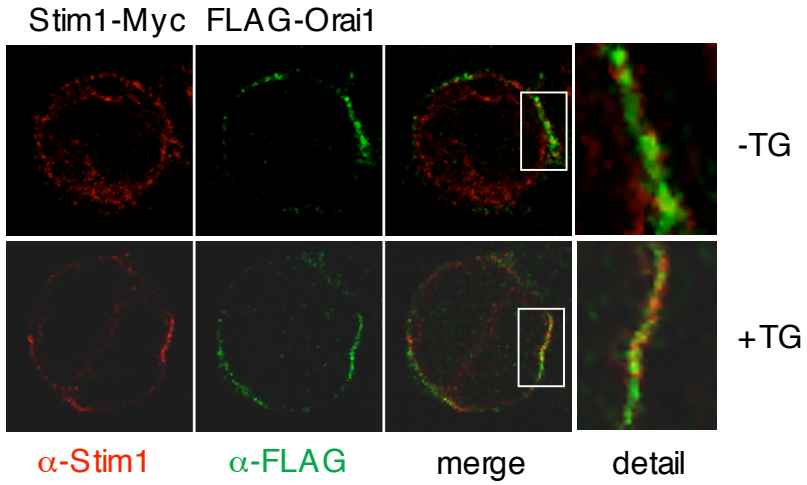
(A) Schematic representation of the topology of human Orai1. The positions of conserved glutamate residues in or near the transmembrane domains are indicated.(B) Expression of mutant E106Q and E190Q Orai1 proteins in murine $CD4^+$ T cells. Purified $CD4^+$ T cells cultured under Th1 conditions were transduced with C-terminally FLAG-tagged wild-type or E>Q mutant Orai1 using a bicistronic IRES-GFP retroviral vector. Expression levels were evaluated by immunoblotting with an anti-FLAG antibody, with GFP and actin as loading controls.(C) Human fibroblasts stably transduced with C-terminally FLAG-tagged wildtype or mutant Orai1 constructs were fixed, permeabilised and stained with anti-FLAG antibodies. GFP expression is from an IRES site in the bicistronic Orai1 expression vector.



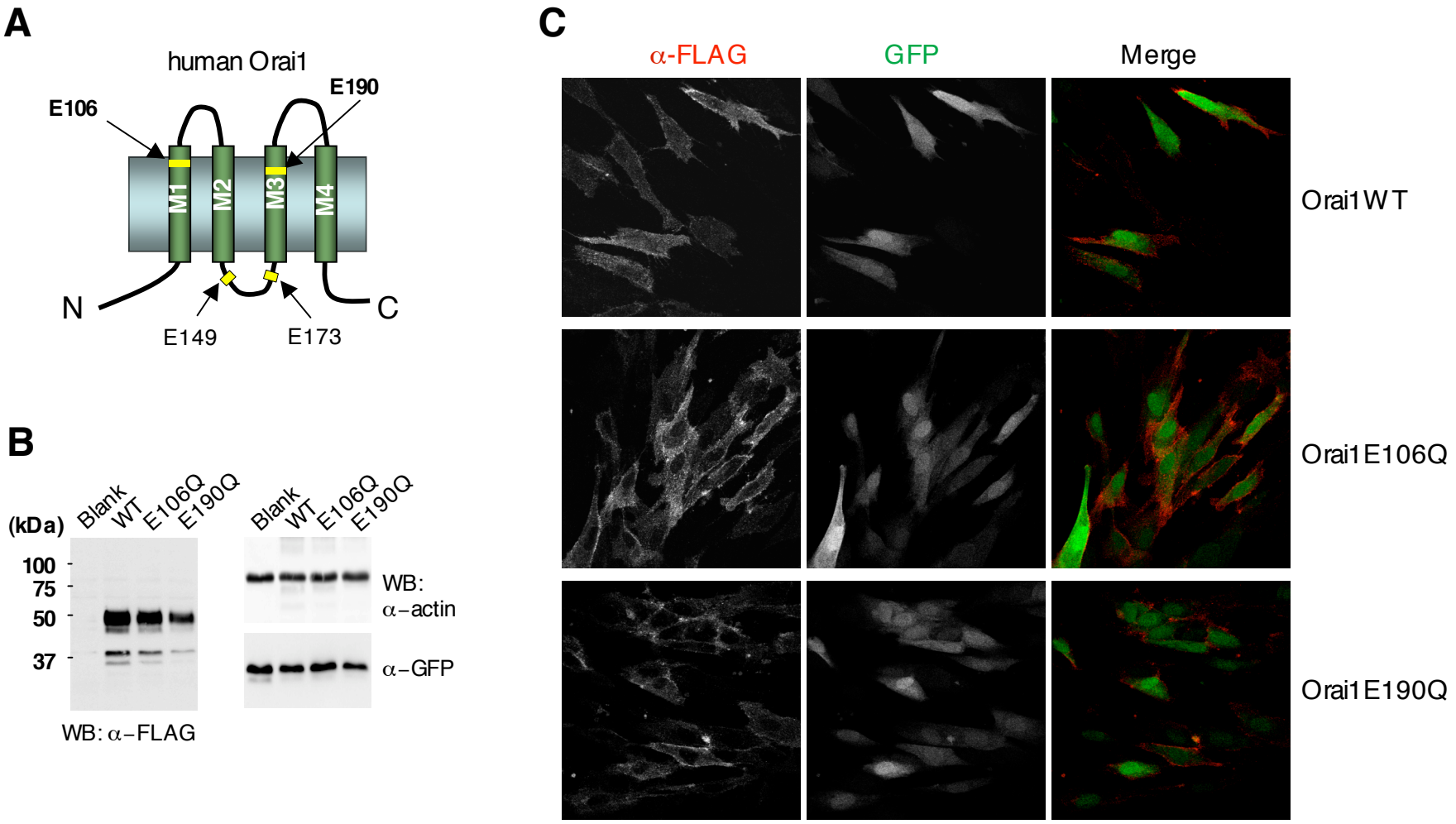
Gwack et al. Suppl Fig. 1



Gwack et al. Suppl Fig. 2



Gwack et al. Suppl Fig. 3



Gwack et al. Suppl Fig. 4