SUPPLEMENTARY INFORMATION

Supplementary Methods

Cell culture and transfection

Drosophila S2 cells (Invitrogen) were propagated in Schneider's medium (Invitrogen) supplemented with 10% FBS at 24^{o}C. Cells were seeded at a density of 10^6 cells / ml and passaged when the cells achieved a density of ~6 x 10^6 cells / ml. S2 cells were transfected (see clones described in Methods) using a Nucleofector (Amaxa) following the manufacturer's protocol. Forty-eight hours post-transfection, cells were used for patch-clamp, biochemistry or processed for RT-PCR analysis.

Data analysis

Data were analyzed by Pulse (Heka Electronics) and Origin (OriginLab Corp.). Five *I-V* curves were averaged for display. Unless otherwise noted the displayed *I-V* curves are leak-subtracted. Since almost all S2 cells at the moment of break-in display an outward current that disappears during perfusion with internal solution (but before the inward CRAC current starts to develop), the following procedure for leak subtraction was employed. The first three ramp currents after the outward current subsided were averaged (I_{mid}). Then, another set of three ramps after maximal inward CRAC current had developed were averaged (I_{max}). The difference between I_{max} and I_{mid} represents an isolated CRAC current but with amplitude less than the actual one. To correct this the

difference was scaled such that the value of current at –130 mV is equal to maximal inward current minus initial inward current. Then the scaled difference was subtracted from I_{max} followed by fitting with a polynomial function. This fit was considered as the basal leak current. In a few cases (e.g. figure 4b) the initial outward current did not run down until the maximal inward current had developed. In this case a linear I-V curve reversing at 0 mV with current magnitude at +90 mV equal to the sustained outward current was assigned as leak current. For E180D *Orai*-induced CRAC current, the initial current was considered as leak.

RNA isolation and RT-PCR

RNA was isolated using TRIZOL (Invitrogen) following the manufacturer's protocols. The methods for RT-PCR were the same as described[6].

Supplementary Table 1. Solutions for whole-cell recording

Name	Na^+	Divalent / concentration	Ca^{2+} chelator / concentration	Cl^-	Sucrose
External (**Ca2**)	160	Ca^{2+} / 2	–	164	–
Choline external (**Chol**)	1.1	Ca^{2+} / 2	–	164	10
High-Ca^{2+} external (**Ca20**)	124	Ca^{2+} / 20	–	164	10
High-Mg^{2+} external (**Mg20**)	124	Mg^{2+} / 20	–	164	10
High-Sr^{2+} external (**Sr20**)	124	Sr^{2+} / 20	–	164	10
High-Ba^{2+} external (**Ba20**)	124	Ba^{2+} / 20	–	164	10
200 μM Ca^{2+} (**Ca 200μM**)	160	Ca^{2+} / 0.2	–	160	–
20 μM Ca^{2+} (**Ca 20μM**)	160	Ca^{2+} / 0.825	HEDTA / 2	162	–
2 μM Ca^{2+} (**Ca 2μM**)	160	Ca^{2+} / 0.85	EGTA / 2	162	–
Divalent-free Na^+ (**Na**)	152	–	HEDTA / 10	152	–
Divalent-free Cs^+ (**Cs**)	–	–	HEDTA / 10	160	–

Name	Cs^+ aspartate	Cs^+ BAPTA	CsCl	HEPES
Ca^{2+} - free internal	133	12	2	15
High-Ca^{2+} internal	152	–	–	10

Solution names used in figures are indicated in bold. Concentrations of ions and chemicals in the table are indicated in mM. External solutions contained 10 mM D-

glucose and 10 mM HEPES. Choline solution contained 160 mM of choline as choline chloride. Divalent-free Cs^+ solution contained 160 mM of Cs^+ as CsCl. Ca^{2+} - free internal solution contained 8 mM Mg gluconate. High-Ca^{2+} internal solution contained 1 mM $CaCl_2$, 6 mM $Ca(OH)_2$ and 10 mM EGTA. pH of external and internal solutions was 6.6 and 7.2 respectively and was adjusted by appropriate hydroxide. pH of choline solution was adjusted by NaOH. Osmolality was adjusted to 324 mOsm ± 1% by sucrose. Low Ca^{2+} solutions (Ca 20μM and Ca 2μM) were composed using estimates of free Ca^{2+} concentration provided by MaxChelator v. 2.50 (http://maxchelator.stanford.edu/) using tables cmc0204e.tmc and the following parameter settings: t = 23^0C, pH = 6.6, I = 179 (for Ca 20 μM) or I = 184 (for Ca 2 μM). Free Ca^{2+} concentration of high-Ca^{2+} internal solution estimated by MaxChelator was 450 nM. Gd^{3+} was added as $GdCl_3$. 2-APB was diluted from DMSO stock solution. IP_3 stock solution was prepared in water.

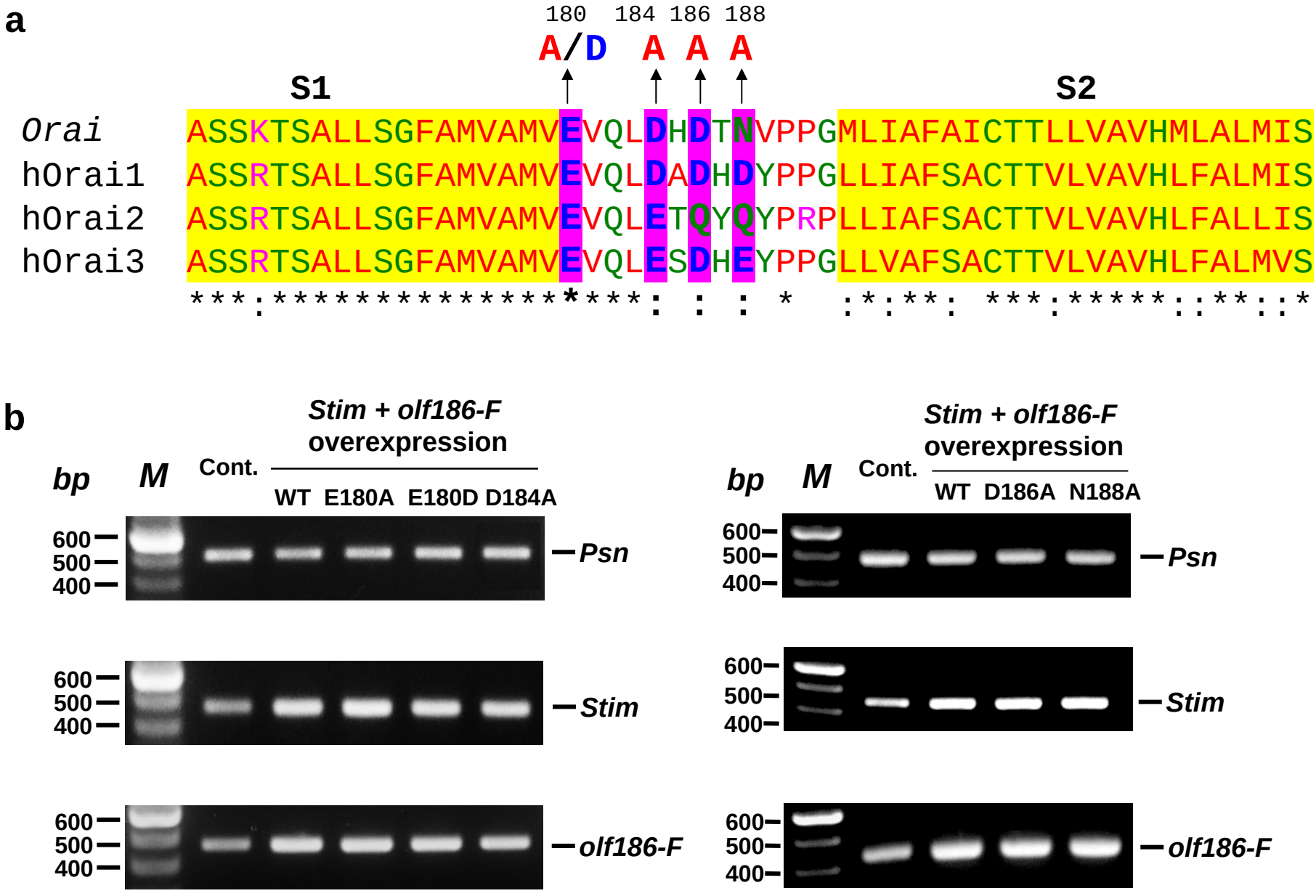
Supplementary Figure Legends

Supplementary Figure 1 Orai sequence, mutants, and expression. a, Partial protein sequence comparison shows an overall 67% identity (*) and 89% similarity (* and :) within the S1 - S2 region of *Drosophila Orai* and its human homologs. Putative transmembrane regions and mutation sites are indicated. **b**, Validation of effective mRNA overexpression. RT-PCR analysis was performed as described in Supplementary Methods using gene-specific primers. Overexpression of *Stim* with WT *Orai*, E180A, E180D, or D184A (left) and with WT *Orai*, D186A or N188A (right).

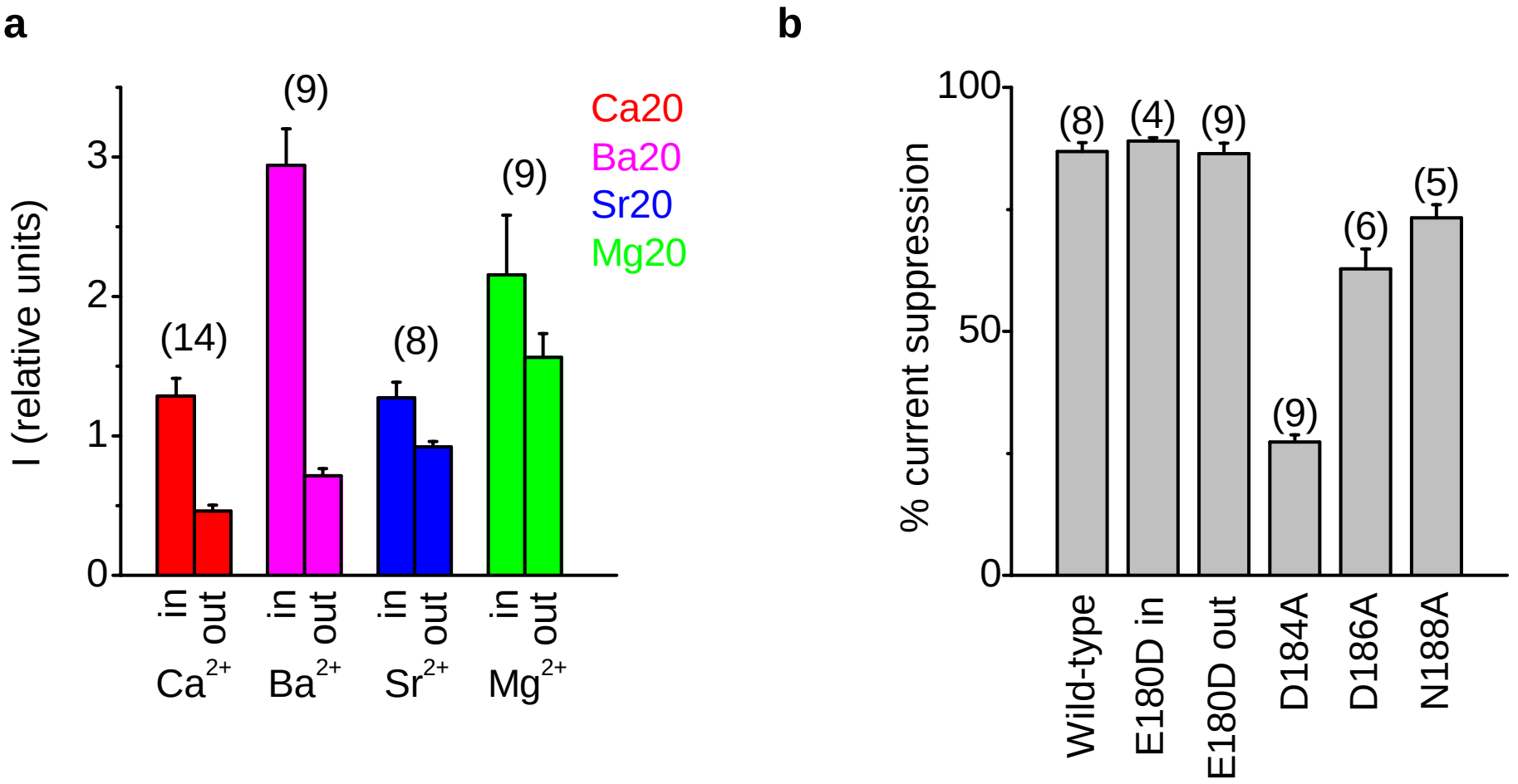
Supplementary Figure 2 Block of inward and outward CRAC current by divalent cations and gadolinium. a, Effect of divalent cations (20 mM) on E180D *Orai*-induced inward current (at –130 mV) and outward current (at 90 mV), normalized to currents in 2 mM Ca^{2+}. Mean ± SEM values are reported; the number of cells for each test divalent cation is indicated above the bars. Ca^{2+} ($P < 5x10^{-6}$ for outward current relative to 2 mM Ca^{2+} control); Ba^{2+} ($P = 6x10^{-4}$ for outward current and $7x10^{-5}$ for inward current); Sr^{2+} (NS); Mg^{2+} ($P = 0.01$ for outward current and 0.03 for inward current). The change in *I-V* shape with 20 mM external Ba^{2+} and increased inward current may result from incomplete block of inward Na^{+} current at negative potentials. **b**, Suppression of CRAC current by 5 nM Gd^{3+} in WT *Orai*; E180D; D184A ($P < 5x10^{-6}$ compared to WT); D186A ($P = 8x10^{-5}$); and N188A ($P = 1.5x10^{-3}$).

Supplementary Figure 3 Effect of 2-APB on CRAC current. a, WT *Orai*-induced CRAC current. Bars indicate time of 2-APB application at indicated concentrations. **b**, Same as **a** for the outward E180D *Orai*-induced CRAC current. Similar results were obtained in three separate experiments.

Cahalan 2006-06 Supplementary Figure 1



Cahalan 2006-06 Supplementary Figure 2



Cahalan 2006-06 Supplementary Figure 3