Supplementary Materials for

Transmembrane helix connectivity in Orai1 controls two gates for calcium-dependent transcription

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Other Supplementary Material for this manuscript includes the following:
Movie S1 (.avi format). Ca^{2+} permeation through the Orai1 channel.
Fig. S1. Constitutive activity and cellular localization of cancer database and myopathy Orai1 mutants.

(A) HEK cells expressing wt-Orai1 or Orai1-A137V were cultured for 20 min in a Ca\textsuperscript{2+} free medium. Time course of Ca\textsuperscript{2+} concentrations as assessed by Fura-2 imaging of wt-Orai1 and Orai1-A137V expressing HEK cells upon re-addition of 2mM Ca\textsuperscript{2+} (n = 31 cells each, from three individual transfections) (B) Representative time course of Ca\textsuperscript{2+} concentrations as assessed by Fura-2 imaging of mock-transfected human colon carcinoma cell line HCT 116 cells or cells expressing wild-type Orai1 or Orai1-A137V compared to thapsigargin (TG) triggered SOC entry. (C) Average resting cytosolic Ca\textsuperscript{2+} values (black) and peak values upon TG treatment (red) of mock-transfected HCT 116 cells or cells expressing wild-type Orai1 or Orai1-A137V, n = 23 - 40 cells from 3 independent transfections. Average Ca\textsuperscript{2+} values that are significantly different (p < 0.05) by t-test are indicated with a star (*). (D) Representative images of HEK cells over-expressing YFP-tagged wild-type Orai1 or Orai1 Gly\textsuperscript{183} mutants. Scale bar, 10 µm. n= 37 – 53 cells from 3 - 4 independent transfections. (E) Time course of whole cell patch-clamp experiment of HEK cells co-expressing STIM1 and Orai1 (black), Orai1-L138F (blue), Orai1-L138A (green), or Orai1-L138F alone (red) in a 10mM extracellular Ca\textsuperscript{2+} (n = 7 – 10 cells, from at least 2 individual transfections). Store-operated currents were activated by 20mM EGTA in the patch pipette. (F, G) Representative current-voltage relationships for Orai1-L138F expressing cells (E), or Orai1-L138F co-expressed with STIM1 (F) in a 10mM Ca\textsuperscript{2+} (black) or Na\textsuperscript{+} based divalent free (Na-DVF, red) solution.
Fig. S2. Interaction between TM2 and pore helix residues. (A) Details of the central TM2 segment in Orai1, including cancer- and myopathy-associated mutations (A137V, L138F, and M139V) which are close to TM1 and TM3 (Gly183). A hydrogen bond is observed between His<sup>134</sup> and Ser<sup>97</sup>. (B,C) Time-course determines number of hydrogen bonds between His<sup>134</sup> and Ser<sup>93</sup> (left trace) as well as His<sup>134</sup> and Ser<sup>97</sup> (right trace) in (B) Orai1-L138F and (C) Orai1-A137V molecular dynamics simulations.
**Fig. S3.** **STIM1 and Orai1-His^{134} mutants mediate Ca^{2+}-selective currents.** (A) Average expression of Orai1 His^{134} mutants for cells evaluated in Figure 3F as quantified by YFP fluorescence intensity normalized to wild-type Orai1 fluorescence intensity (n = 20 - 31 cells from two individual transfections). (B, C) Representative current-voltage relationships are shown for maximum currents measured in STIM1 and Orai1-His^{134} mutant expressing cells summarized in Figure 3B and 3C. (D) Representative image of a HEK cell showing the plasma-membrane localization of YFP-Orai1-L138A (n = 13 cells, from at least 2 individual transfections). Scale bar, 10 µm.
Fig. S4. Starvation induces nuclear translocation of the transcription factors MITF and TFEB. (A) Average number of cytotoxic HEK cells in % after 24 hours upon overexpression of wild-type Orai1 and indicated Orai1 mutants (n = 20 – 32 cells, from 2 individual transfections each). (B) Time course of transcription factor activation by starvation (HBSS media) as measured by cytosol-to-nuclear translocation of NFAT (red), TFEB (blue) and MITF (black) in HEK cells (n = 3 individual transfections for each transcription factor).

Fig. S5. Pharmacological regulation and fast inactivation of the Orai1-H134A mutant. (A) Time-course of Orai1-H134A average currents were blocked by 2APB (75µM) and by La³⁺ (10µM) (n = 5 cells, from at least 2 individual transfections). (B) Normalized average currents from voltage step to -80 mV are shown for a coexpression of STIM1 with Orai1 (black), STIM1 with Orai1-H134A (blue) or Orai1-H134A overexpression alone (red) for 100 ms (n = 5 – 9 cells, from at least 2 individual transfections each).
Fig. S6. Pore profile of human and Drosophila Orai. (A,C) Pore profile of (A) human Orai1 used for molecular dynamics simulations is compared to the (C) crystal structure of Drosophila Orai1 (dOrai). Two subunits and pore-lining amino acids of human Orai1 and dOrai are shown. (B) Pore radius for human Orai1 and dOrai are compared along the pore for residues Glu106 to Trp76. Structural information on the Ca\(^{2+}\) accumulation region located extracellularly of Glu106 in dOrai is unavailable.
Fig. S7. Regulation of the Arg⁹¹ gate in closed and constitutively open Orai1. (A) Time course of Orai1-H134A and Orai1-R91W-H134A and treatment of La³⁺ (10µM) after 250s (n = 6 – 8 cells, from at least 2 individual transfections). (B, left) Distances between wild-type Orai1 Arg⁹¹ (CZ atom) side-chains for the last 50ns of 200ns trajectory for the wild-type Orai1. Every color shows distance between side-chain CZ atom of Arg⁹¹ one monomer separately to each Arg⁹¹ (CZ atom) of other five monomers. (B, right) Similar distance measurements for Orai1-H134A mutant. Here, the distance between side-chains of Arg⁹¹ is large, which corresponds to the widened pore diameter in the mutant.
Figure S8

A: WT starting position

B: H134A starting position

C: WT after crossing Arg^{91} ring

D: H134A after crossing Arg^{91} ring
Figure S8. Pulled Ca\textsuperscript{2+} ion through the Orai1 pore extends the Arg\textsuperscript{91} gate. (A,B) Snapshots extracted from the steered molecular dynamics simulation. Panels illustrate the initial position of Arg\textsuperscript{91} side chains in wild-type Orai1 (A) and mutant Orai1-H134A (B) before a Ca\textsuperscript{2+} ion is pulled through the pore. (C,D) Panels shows the orientation of Arg\textsuperscript{91} side chains after Ca\textsuperscript{2+} transition in the wild type (C) and mutant (D). TM1 is depicted in red, TM2 in green, TM3 in blue. Only the side chains of Arg\textsuperscript{91} are shown. The pulled calcium ion is depicted in yellow. (E) The force required to pull a calcium ion through the ring of Arg\textsuperscript{91} side chains in wild-type Orai1 (upper plot) and Orai1-H134A mutant (lower). Force is calculated for the two repetitions of the pulling experiment for wild-type (red and orange) and mutant H134A (blue and green). A time of approximately 3.5 ns is required to cross Arg\textsuperscript{91} ring in both cases (wild-type and mutant).
Fig. S9. The hydrophobic gate forms a barrier for water molecules in Orai1 channels. (A) Representative snap shots of the start of wild-type Orai1 and the end of a 200ns long molecular dynamics simulations for Orai1-H134A showing the position of Phe99 residues in the pore helix. (B,D) Representative snap shots of the equilibrated part of 200ns long molecular dynamics simulations for (B) wild-type Orai1 or (D) Orai1-H134A showing the pore forming TM1 helices (4 out of 6 TM1 helices shown in red) and pore lining residues from Glu106 to Trp76. Ca$^{2+}$ (yellow ball), Na$^{+}$ (orange ball) and Cl$^{-}$ (green ball) are shown in the respective pores. (C) Average number of ions in the respective pores of wild-type Orai1, Orai1-H134A, Orai1-A137V, Orai1-L138F, and Orai1-R91G are shown.
Fig. S10. Cysteine cross-linking of constitutively active Orai1 mutants. Dimerization efficiency (%) of cysteine crosslinking for engineered cysteines in the (A) wild-type Orai1 or Orai1-A137V pore as well as (B) wild-type Orai1 and Orai1-V102A pore. For each cysteine position in (A) or (B) experiments (for each cysteine position n = 3 individual transfections) were performed on the same day, and significant difference (t-test, p < 0.05) is indicated by a star (*).
Fig. S11. Dynamics of water molecules in closed and constitutively active Orai1 channels. Survival probability for the water molecules within a cylinder of 10 Å radius from the center of the pore of the channel. The survival probability is estimated for windows of 10 Å from the given z position. The distance z in each graph is taken with reference to the center of mass of the protein. Residues targeted in the window are given below the z position. Results for the wildtype Orai1, Orai1-H134A, Orai1-R91G, Orai1-A137V and Orai1-L138F are given in black, red, pink, green and blue respectively. The survival probability for an equivalent window in bulk water is given in a black dashed line.
**Table S1. Selectivity of Orai1 mutants.** Reversal potential for maximum currents of wild-type Orai1 and Orai1 mutants with or without co-expressed STIM1 in HEK cells. n, number of cells.

<table>
<thead>
<tr>
<th>Orai1</th>
<th>- STIM1 reversal potential [mV]</th>
<th>+ STIM1 reversal potential [mV]</th>
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<tbody>
<tr>
<td>WT</td>
<td>---</td>
<td>+58 ± 3 (n = 10)</td>
</tr>
<tr>
<td>A137V</td>
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<tr>
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<tr>
<td>H134N</td>
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<td>+60 ± 6 (n = 5)</td>
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**Movie S1. Ca$^{2+}$ permeation through the Orai1 channel.** Steered molecular dynamics simulation of wild-type Orai1 shows a Ca$^{2+}$ ion in purple that is pulled through the pore. Other Ca$^{2+}$ ions are depicted in yellow, Cl$^{-}$ ions in green and two TM1 helices are highlighted in red including pore lining side-chains.