Cytosolic Ca^{2+} prevents the subplasmalemmal clustering of STIM1: an intrinsic mechanism to avoid Ca^{2+} overload

JCS034496 Supplementary Material

Files in this Data Supplement:

- **Supplemental Figure S1** -

  Fig. S1. Comparison on the kinetics of STIM1 oligomerization and clustering in the superficial (ROI 1) and deeper ER (ROI 2) domains upon store depletion with histamine/BHQ (100 and 15 μM). (A) Images illustrate the location of the two regions of interest (ROI) measured. (B) Representative tracings of the effect of 100 μM histamine and 15 μM BHQ on STIM1 oligomerization (lines, left graph), STIM clustering (dotted lines, middle graph) and overlay in ROI 1 (red) and ROI 2 (blue). (C) Schematic illustration of the kinetics of STIM1 oligomerization and redistribution upon ER depletion in both ROIs.

- **Supplemental Figure S2** -

  Fig. S2. Determination of the effective correlation concentrations (ECC_{50}) of STIM1. Oligomerization upon ER Ca^{2+} depletion ($ECC_{50/oligomerization}$) and of the disassembly of STIM1 oligomers upon ER Ca^{2+} refilling ($ECC_{50/disassambly}$). (A) Representative tracings of the effect of 100 μM histamine and 15 μM BHQ on $Ca^{2+}{}_{ER}$ (upper panel) and STIM1 oligomerization (lower panel), in the presence of 2 mM extracellular Ca^{2+} followed by the removal of extracellular Ca^{2+} (i.e. EGTA containing solution), and a subsequent re-addition of extracellular Ca^{2+} in the absence of histamine and BHQ. (B) The correlation between ER Ca^{2+} depletion and STIM1 oligomerization (continuous line, black circles, n=9 for STIM1 oligomerization; n=10 for $Ca^{2+}{}_{ER}$) is compared with the correlation between ER Ca^{2+} refilling and the disassembly of STIM1 oligomers (dotted line open squares, n=9 for STIM1 oligomerization; n=10 for $Ca^{2+}{}_{ER}$). $Ca^{2+}{}_{ER}$ was recorded in single endothelial cells that transiently expressed $D1_{ER}$ and STIM1-YFP, respectively. STIM1 oligomerization was measured by following FRET between STIM1-CFP and STIM1-YFP. For STIM1 oligomerization, the ratios $(F_{535}/F_{480})/F_0$ were normalized (Δ_{max}=100%). Curves were fitted using Prism 4.0 for Mac (GraphPad Software, San Diego, CA).

- **Supplemental Figure S3** -

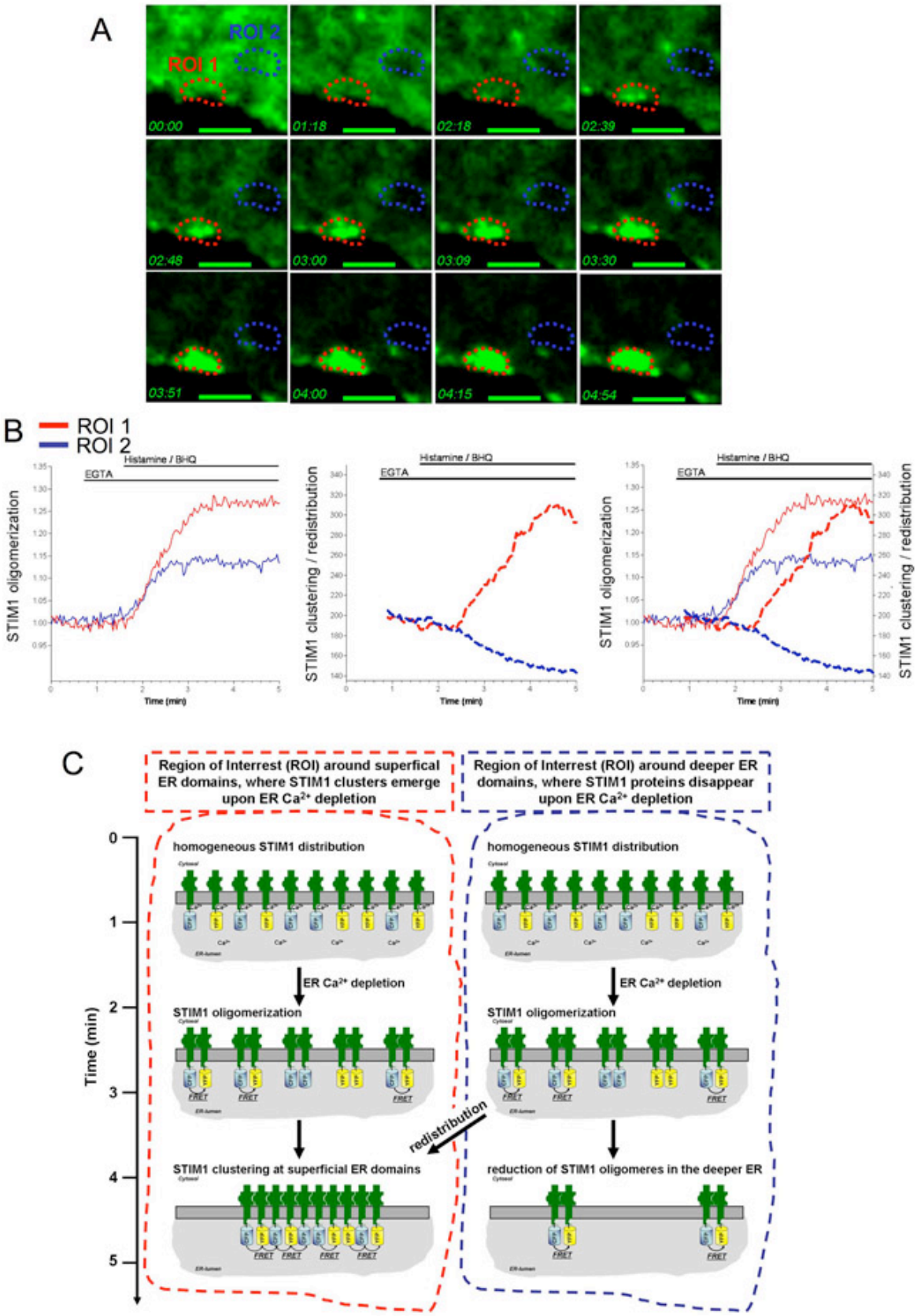
  Fig. S3. Simultaneous recordings of the disassembly/reformation of STIM1 clusters (red traces) with $Ca^{2+}{}_{cyto}$ (black traces). $Ca^{2+}{}_{cyto}$ was recorded simultaneously with STIM1 dynamics in cells that transiently expressed YFP-STIM1 and loaded with fura-2-am. Images for subplasmalemmal STIM1 clustering were captured using an array confocal laser scanning microsope and for fura-2 measurements a conventional high-resolution imaging at the same device was used.

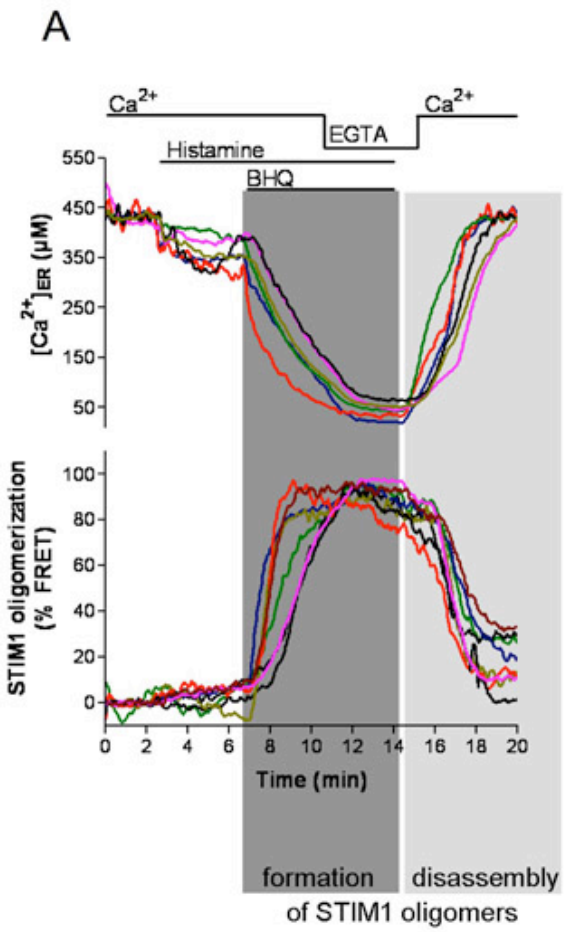
- **Movie 1** -

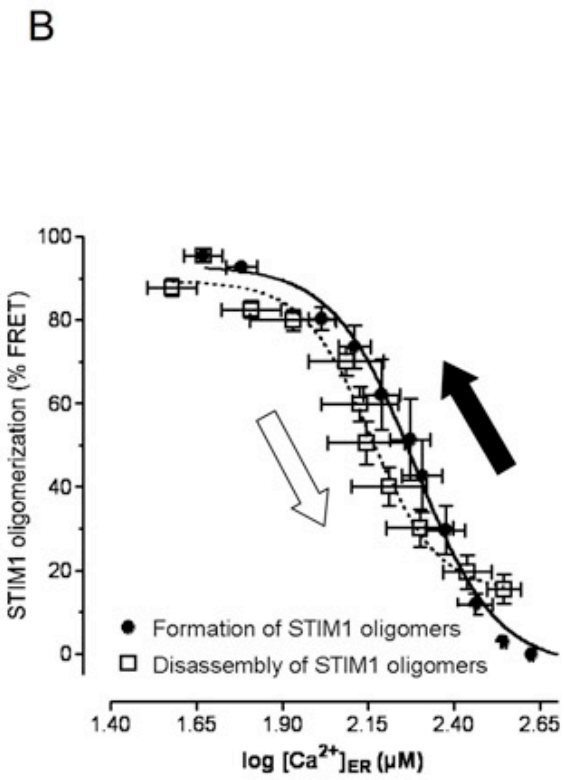
  Movie 1. Histamine-nduced degradation of basal STIM1 clusters. The cellular dynamics of STIM1-YFP is shown upon cell stimulation with the cytosolic-Ca^{2+}-elevating agonist histamine (100 μM) in the presence of 2 mM extracellular Ca^{2+}.

- **Movie 2** -

  Movie 2. STIM1 clustering upon moderate and strong ER Ca^{2+} depletion in the presence and absence of extracellular Ca^{2+}. The cellular dynamics of STIM1-YFP is shown upon moderate ER Ca^{2+} depletion using 100 μM histamine in the presence of 2mM extracellular Ca^{2+} followed by strong ER Ca^{2+} depletion using the combination of 100 μM histamine and 15 μM BHQ first in the presence of 2mM extracellular Ca^{2+} and subsequently in the absence of extracellular Ca^{2+} (i.e. EGTA-containing solution).


A
ROI 1
ROI 2
00:00
01:18
02:18
02:39
02:48
03:00
03:09
03:30
03:51
04:00
04:15
04:54
B
ROI 1
ROI 2
Histamine / BHQ
EGTA
STIM1 oligomerization
STIM1 clustering / redistribution
Time (min)
C
Region of Interrest (ROI) around superfical ER domains, where STIM1 clusters emerge upon ER Ca2+ depletion
Region of Interrest (ROI) around deeper ER domains, where STIM1 proteins disappear upon ER Ca2+ depletion
Time (min)
homogeneous STIM1 distribution
ER Ca2+ depletion
STIM1 oligomerization
redistribution
STIM1 clustering at superficial ER domains
reduction of STIM1 oligomeres in the deeper ER
FRET


A
Ca2+
EGTA
Ca2+
Histamine
BHQ
[Ca2+]ER (μM)
STIM1 oligomerization (% FRET)
Time (min)
formation
disassembly
of STIM1 oligomers


B
STIM1 oligomerization (% FRET)
Formation of STIM1 oligomers
Disassembly of STIM1 oligomers
log [Ca2+]ER (μM)

$[Ca^{2+}]_{cyto}$ o

YFP-STIM1-signal of a basal STIM1-cluster