Supplementary Material

FigS1, related to Fig1

(A) Localization of CFP-FRB-ER and OMM-FKBP-YFP

Confocal images of RBL-2H3 cells expressing CFP-FRB-ER, OMM-FKBP-YFP and mtDsRed show the co-localization of OMM-FKBP-YFP with the mitochondria. The slight difference between the YFP and DsRed images could have resulted from sequential acquisition.

(B) Visualization of the rapamycin-induced tethering of the OMM to the ER in a z-series

Pseudo-3D projection of z-image stacks of a whole RBL-2H3 cell overexpressing the OMM-ER linkers. Distribution of CFP-FRB-ER and OMM-FKBP-mRFP before and after (10 min) rapamycin (100nM) treatment is shown.

(C) Lateral mobility of the inducible linker components in the ER and OMM

RBL-2H3 cells were co-transfected with OMM-FKBP-YFP and mRFP-FRB-ER. Middle: The confocal images show the distribution of the YFP fluorescence in a cell before (top) and after (bottom) 2-photon photobleaching (740 nm, Millenia V/Tsunami, 70% power) in a region of interest (ROI, 5x5 μm, red dashed lines). Left: time courses of the mean fluorescence changes in the ROI and over the whole cell area (WC). In the case of the ER-targeted mRFP (bottom panel), rapid bleaching in the ROI is followed by a partial recovery and by a decrease at the WC level, indicating high mobility of the construct throughout the ER. By contrast, photobleaching of the OMM-targeted YFP (upper panel) is not followed by rapid fluorescence recovery in the ROI and the whole-cell fluorescence does not change significantly, reflecting that mitochondria consist of discrete structures with discontinuous membrane. Right: time courses of YFP fluorescence in individual mitochondria selected by the yellow masks. Traces 1 and 2 correspond to a mitochondrion located half inside (1) and half outside (2) the ROI. Note the similar bleaching kinetics and intensities, indicating rapid lateral diffusion of the construct in the OMM of a single mitochondrion.

FigS2, related to Fig5

(A) ER-mitochondrial linkage by pericam-tagged linker

Fluorescent images of an RBL-2H3 cell overexpressing OMM-ER linker pairs show the distribution of OMM-pcm (green) and ER-mRFP (red) before and after (3 min) rapamycin (100nM) treatment. Note the rapamycin-induced colocalization of mRFP with pericam appearing in yellow on the green/red overlay images. Lower: Line scan type images were created by selecting a line parallel to the direction of the process, obtaining the corresponding fluorescent signal from every image in the time series and stacking the successive lines horizontally. This presentation shows the simultaneous rapamycin-induced

concentration of the OMM-pcm within mitochondria (see light green bars, left) and the redistribution of ER-mRFP to the same areas (dark green bars, right). Representative of 10 similar measurements.

(B) Lack of an effect of rapamycin treatment on ER and mitochondrial Ca^{2+} handling

Simultaneous measurements of $[Ca^{2+}]_c$ (rhod2) and $[Ca^{2+}]_m$ (compartmentalized furaFF) responses evoked by IP_3 additions in suspensions of permeabilized RBL-2H3 cells. Rapamycin (rapa) was added during cell permeabilization similar to the experiments shown in Fig5. IP_3 (100nM and 8 μM)-induced $[Ca^{2+}]_c$ and $[Ca^{2+}]_m$ increase was monitored in control (black) cells or in cells pretreated with rapamycin (red). Left, analog traces (mean of 3 runs for each condition), right: bar charts (n=6).

FigS3, related to Fig6

(A) Suppression of the Cch-induced $[Ca^{2+}]_{Nuc}$ signal in EGTA/AM loaded cells

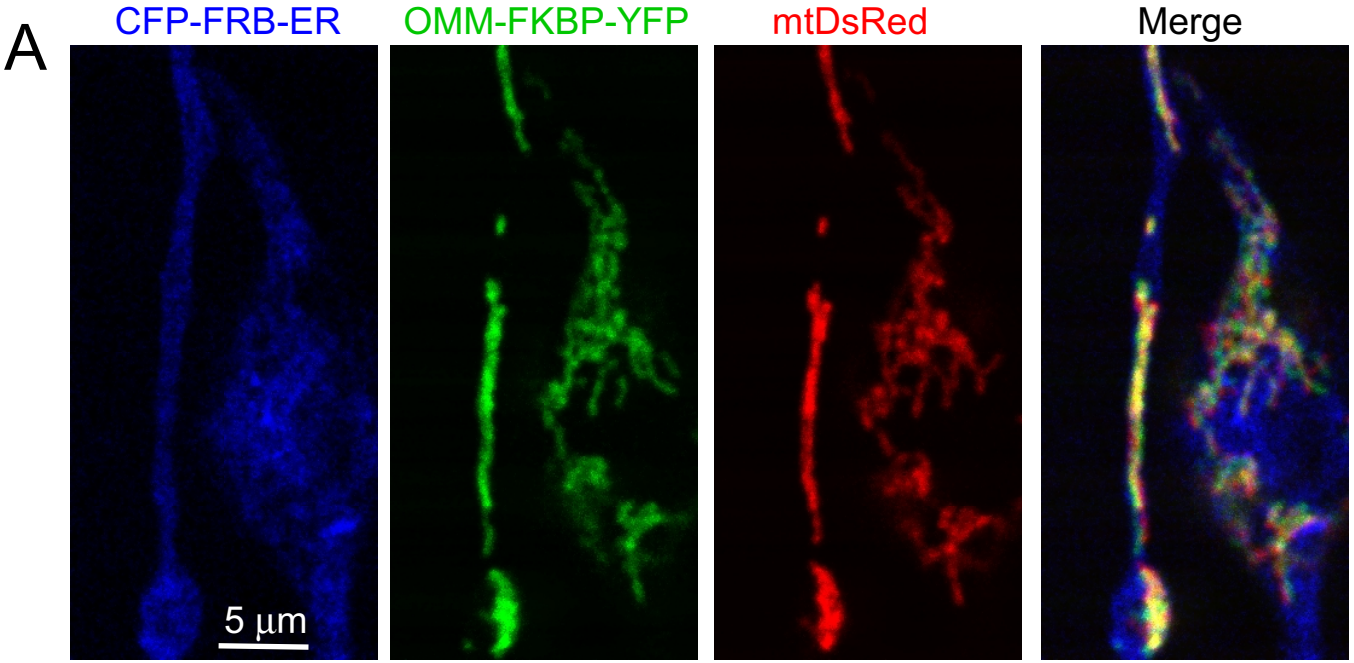
RBL-2H3 cells transiently overexpressing type1 muscarinic receptor and Nuc-pcm were preincubated with (thin line) or without (thick line) EGTA/AM and were stimulated with saturating dose of carbachol (Cch 100 μM) in Ca^{2+}-free extracellular buffer. Each trace represents the mean of 3 separate measurements.

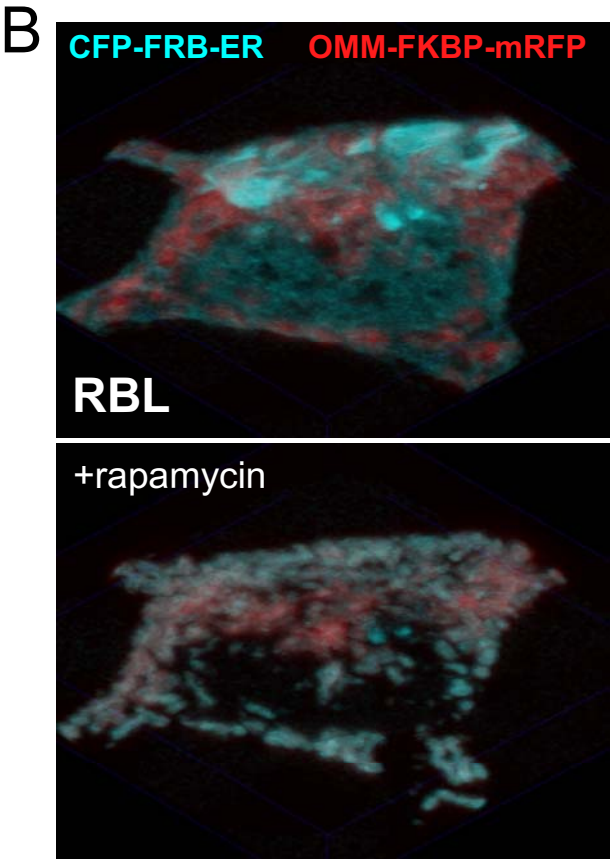
(B) Calibration of the pericam ratio in terms of nM $[Ca^{2+}]$ (related to Fig5 and Fig6)

The gray symbols and trace (sigmoid) show the OMM-pcm-ER ratio values recorded at various $[Ca^{2+}]$ (calibrated from the simultaneously measured rhod2 fluorescence).

When the IP_3-induced $[Ca^{2+}]_{ER\text{-}mt}$ was measured in the EGTA/Ca^{2+} (100μM/40μM) buffer containing medium, a large $CaCl_2$ pulse (60μM) was added as a reference in the end of the recording. The addition of $CaCl_2$ increased $[Ca^{2+}]_{rhod2}$ from 100nM to 3.1μM (lower horizontal dashed arrow). The corresponding R_{pcm} value was used as a reference (ΔR_{Ref}) for the IP_3-induced ΔR (50% in this case). The nM $[Ca^{2+}]$ that belongs to the 50% ΔR increase was then read from the x scale (540nM, see solid arrows). Thus, the $[Ca^{2+}]_{ER\text{-}mt}$ rise evoked by IP_3 addition attained 540nM, while the bulk $[Ca^{2+}]_c$ ($[Ca^{2+}]_{rhod2}$) remained essentially unchanged (100nM) (see Fig5B, thick red traces).

Fig.S1.


A
CFP-FRB-ER
OMM-FKBP-YFP
mtDsRed
Merge
5 µm


B
CFP-FRB-ER
OMM-FKBP-mRFP
RBL
+rapamycin

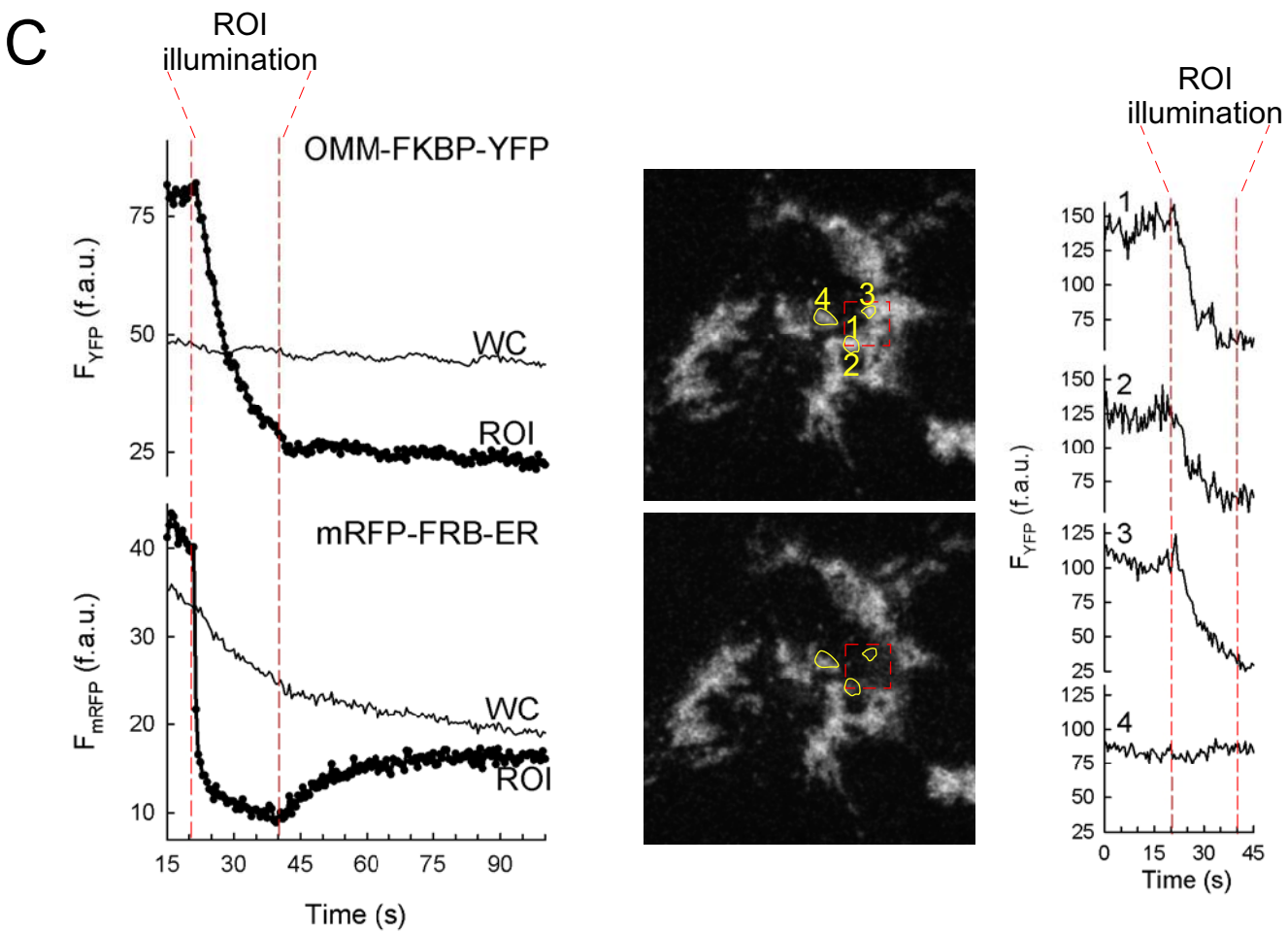

C
ROI illumination
OMM-FKBP-YFP
WC
ROI
mRFP-FRB-ER
WC
ROI
F_YFP (f.a.u.)
F_mRFP (f.a.u.)
Time (s)
1
2
3
4
ROI illumination
Time (s)

Fig.S2.

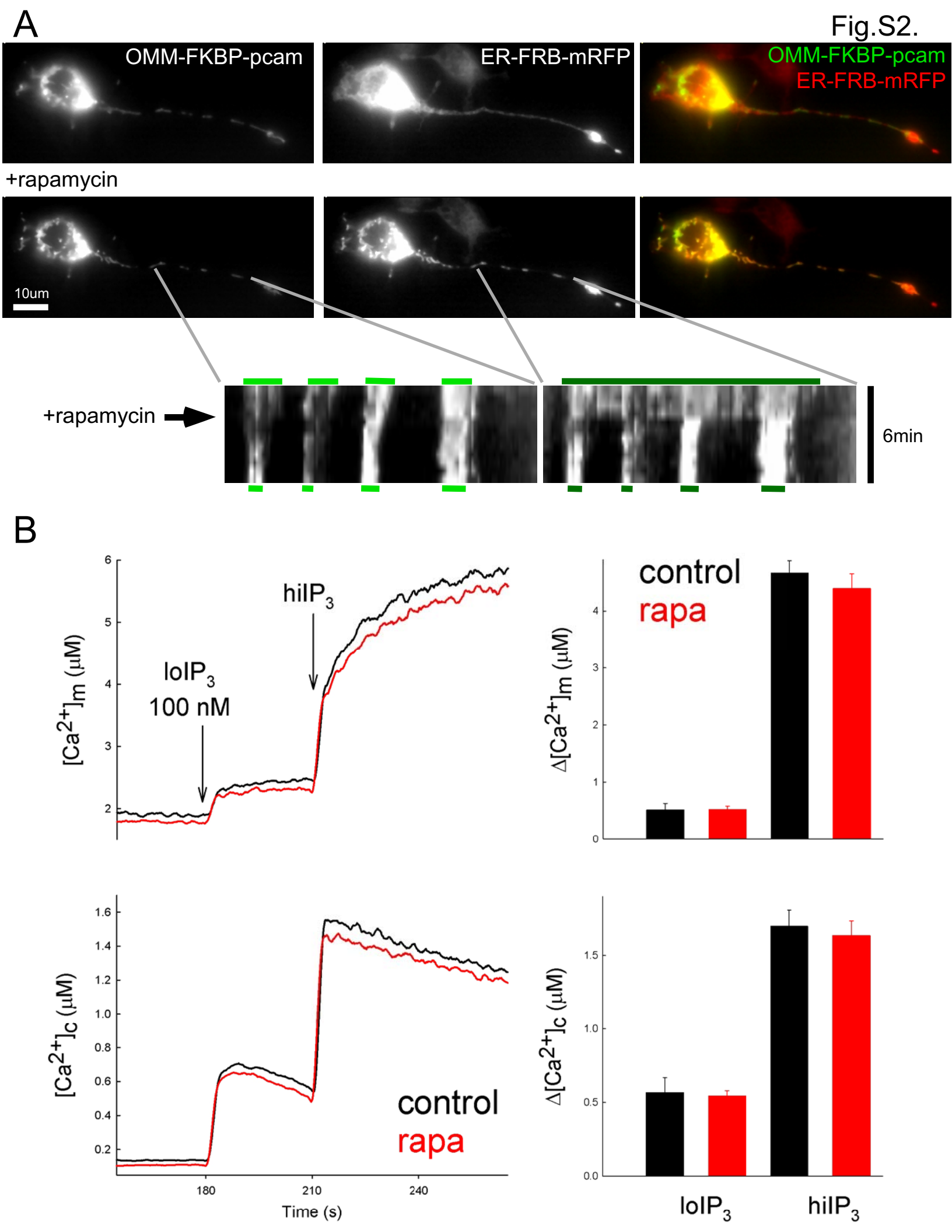
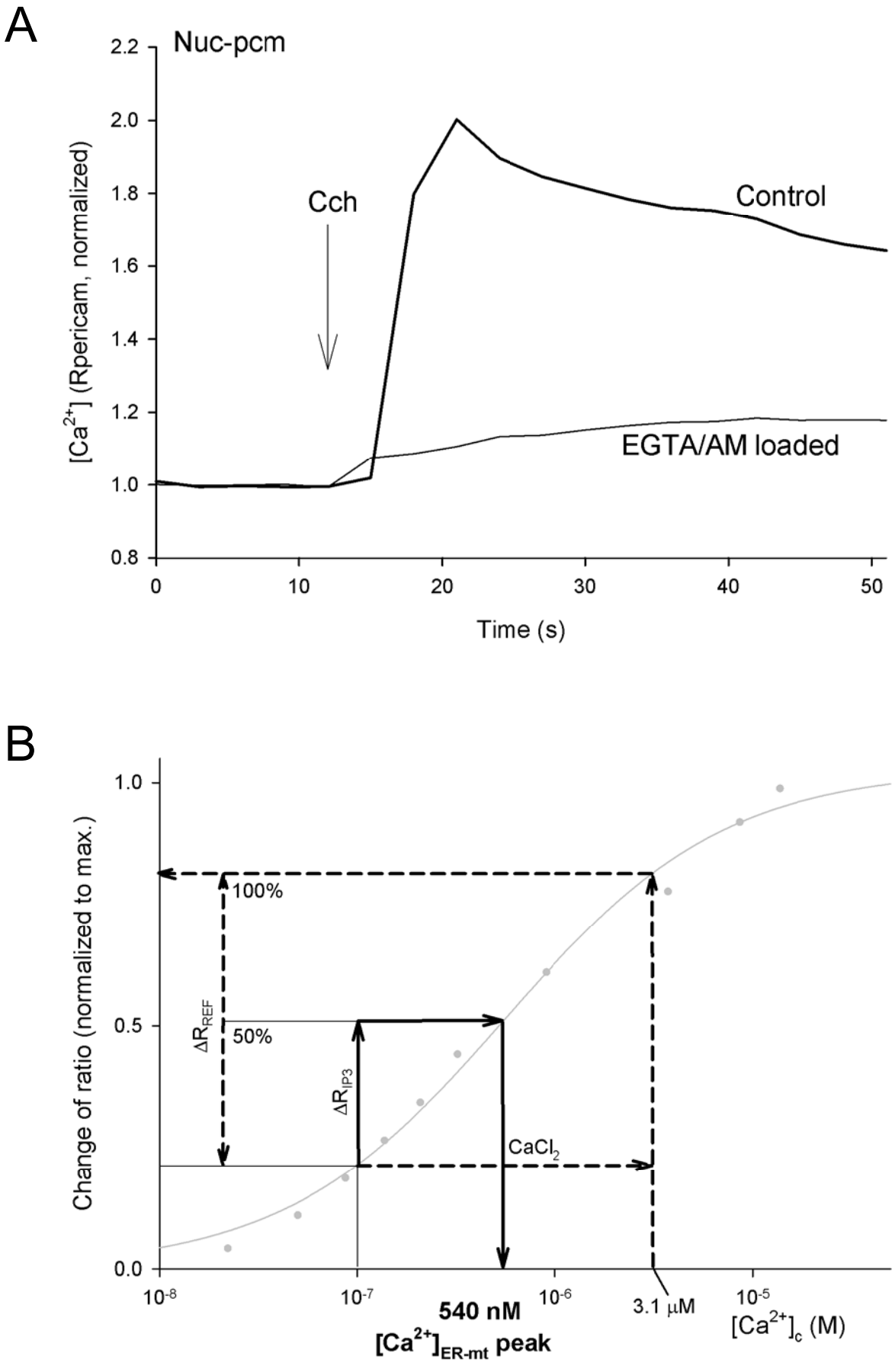

A
OMM-FKBP-pcam
ER-FRB-mRFP
OMM-FKBP-pcam
ER-FRB-mRFP
+rapamycin
10um
+rapamycin
6min
B
$[Ca^{2+}]_m$ (μM)
loIP3
100 nM
hiIP3
control
rapa
Δ$[Ca^{2+}]_m$ (μM)
$[Ca^{2+}]_c$ (μM)
control
rapa
Time (s)
Δ$[Ca^{2+}]_c$ (μM)
loIP3
hiIP3

Fig.S3.


A
Nuc-pcm
Cch
Control
EGTA/AM loaded
[Ca2+] (Rpericam, normalized)
Time (s)
B
Change of ratio (normalized to max.)
100%
50%
ΔRREF
ΔRIP3
CaCl2
540 nM
[Ca2+]ER-mt peak
3.1 μM
[Ca2+]c (M)