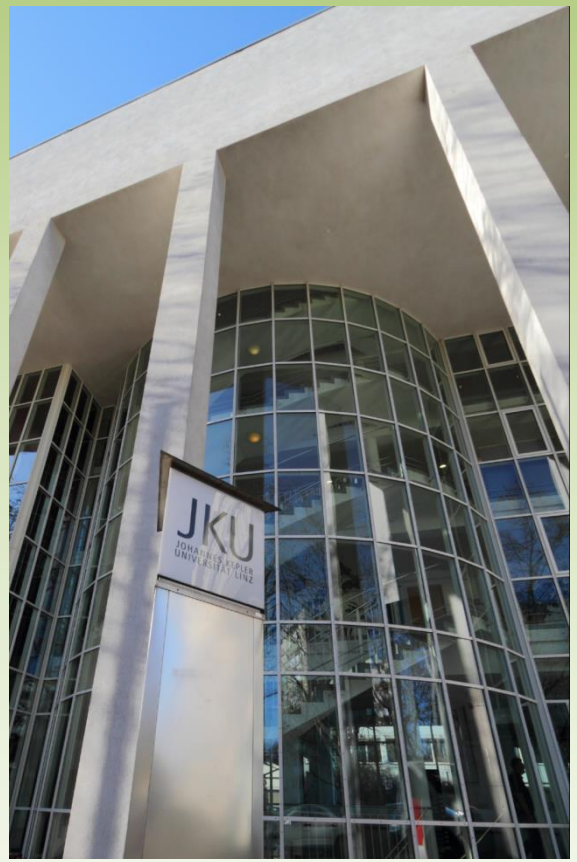
# The multiple roles of Orai N-terminus in CRAC channel function



**Life Science Center**
**Johannes Kepler University**

**RBC, 2018**

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Isabella Derler
University of Linz, Austria

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- Introduction
- Techniques
- Results
- Summary

STIM1 and Orai - the main players in store-operated $Ca^{2+}$ channels

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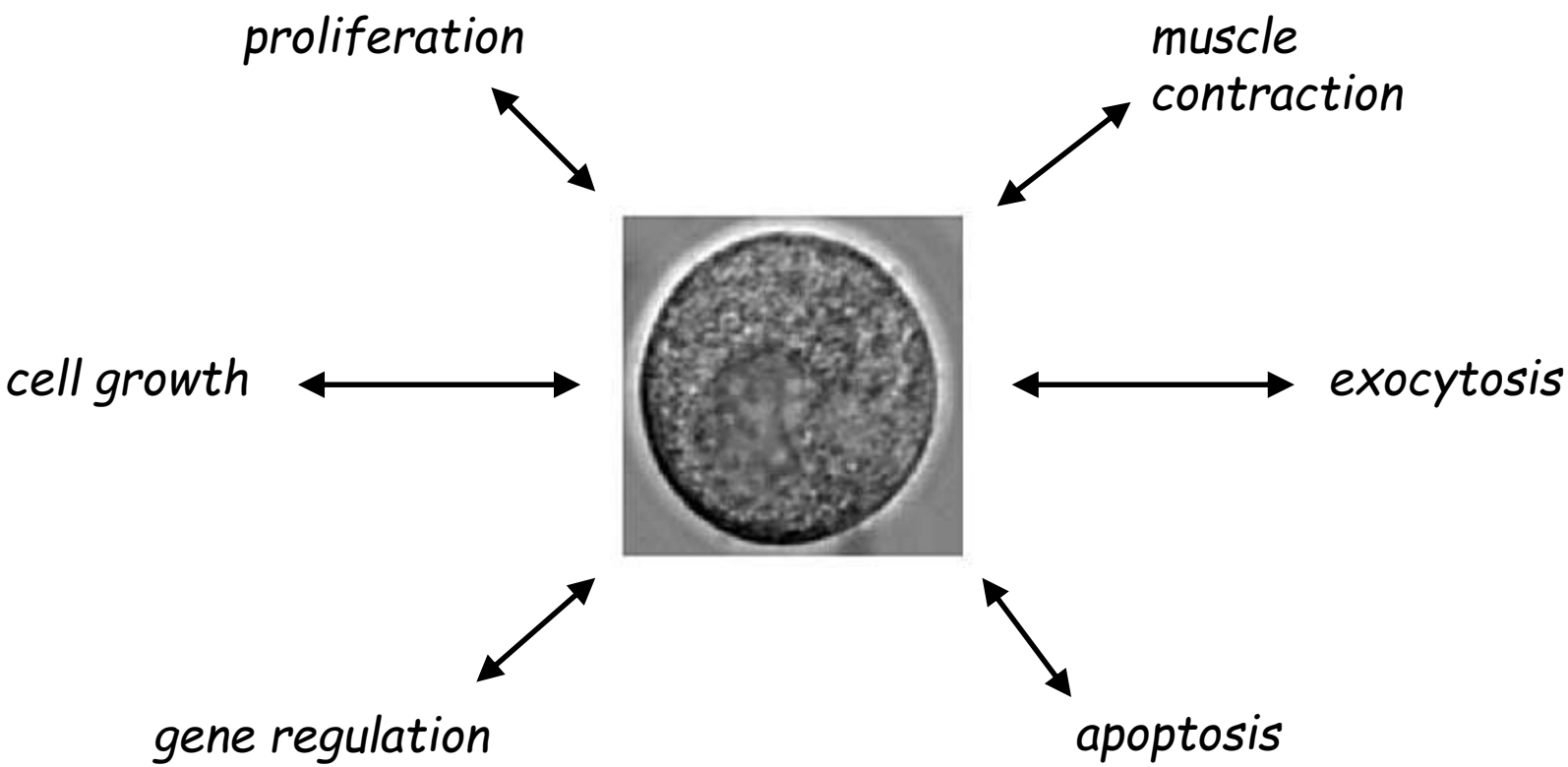
# $Ca^{2+}$ is a major factor regulating cellular processes




**major $Ca^{2+}$ entry pathway mediated by store-operated $Ca^{2+}$ channels**

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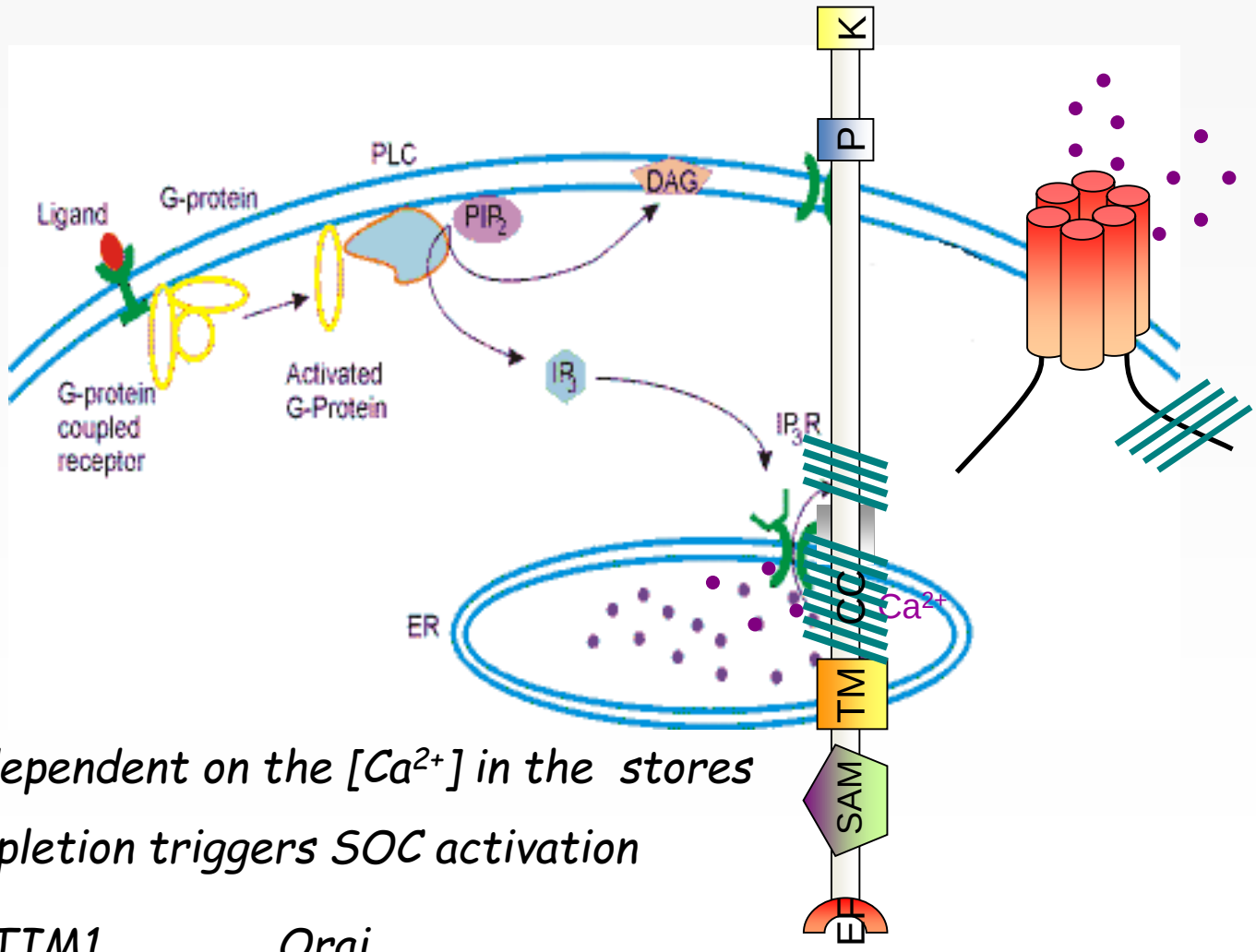
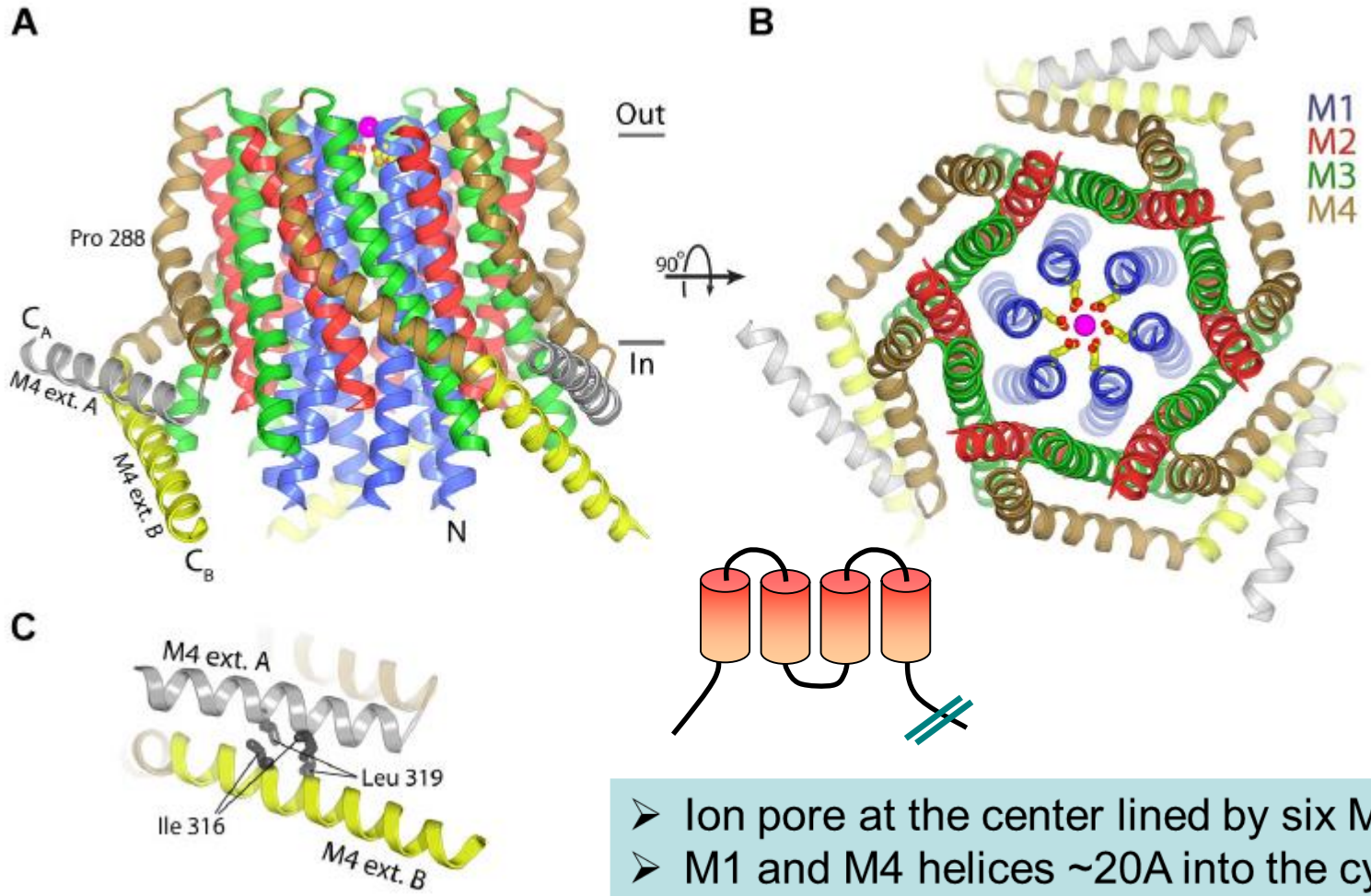
# Store-Operated-Channel (SOC)




- *SOC is dependent on the [$Ca^{2+}$] in the stores*
- *store depletion triggers SOC activation*

*STIM1* *Orai*

# CRAC channel – STIM1 – Orai1




- Ion pore at the center lined by six M1
- M1 and M4 helices ~20A into the cytosol
- M4 extension of Orai1A/B pack into pairs involving L316 (L273) and L319 (L276)

Hou et al., Science 2012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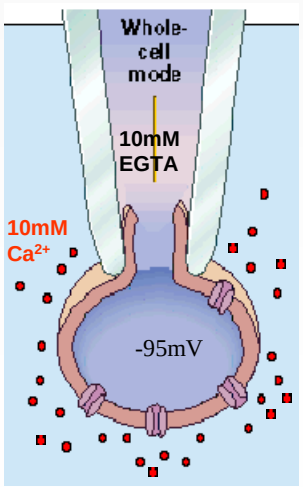
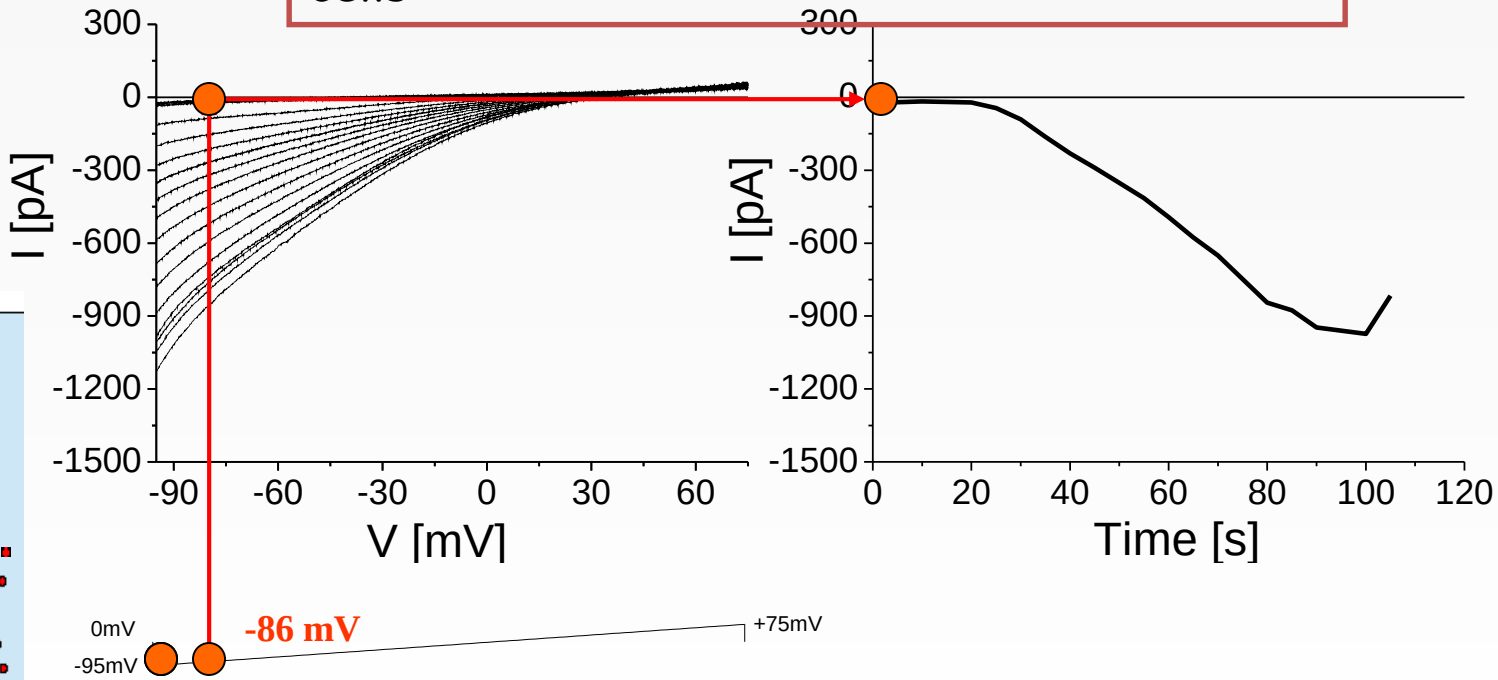
- Fluorescence Resonance Energy Transfer (FRET) Microscopy

# Activation of store-operated Orai1 channels

STIM1 and Orai1 over-expression in HEK cells




-> Store depletion in HEK cells co-expressing STIM1 and Orai1 activates store-operated $Ca^{2+}$ currents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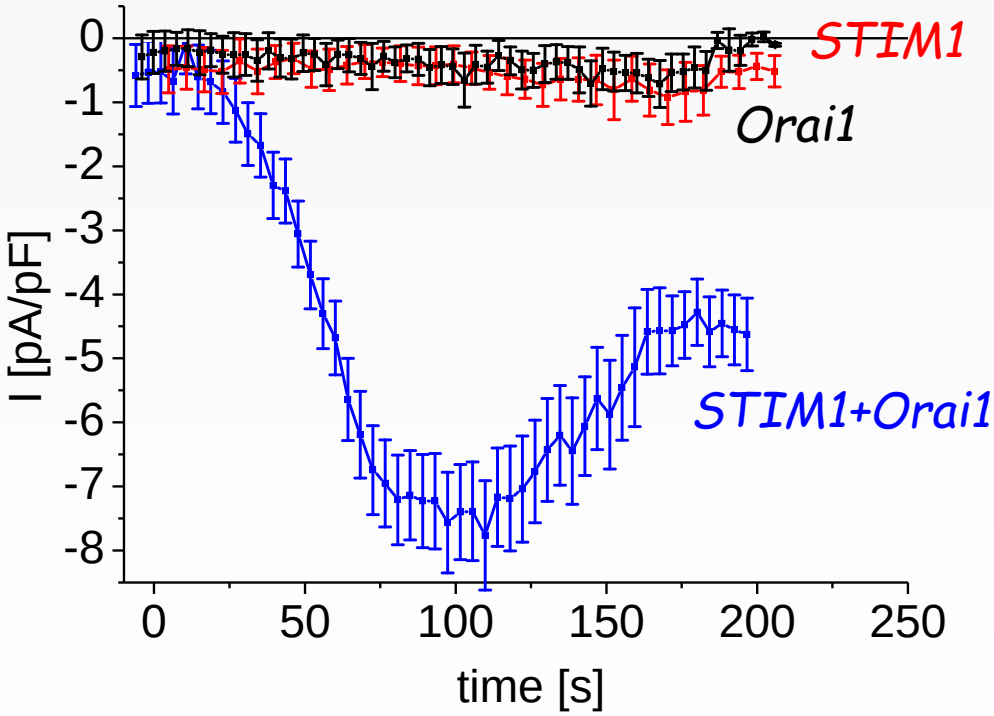
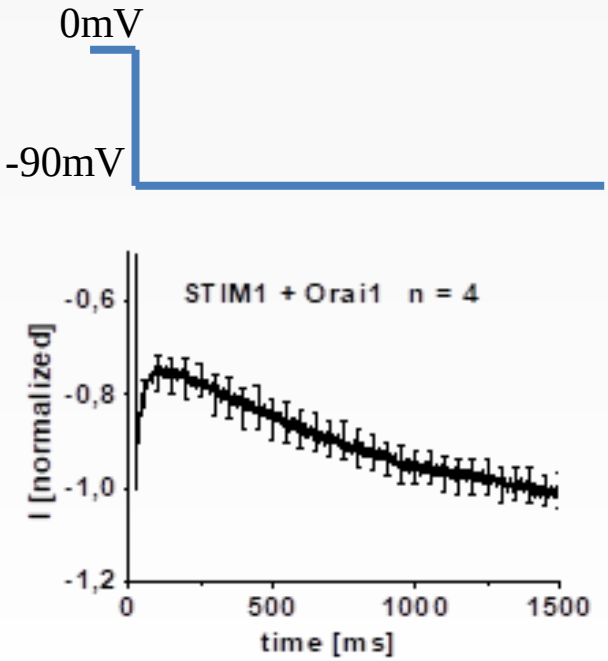
# STIM1 and Orai1 required for channel activity




-> *Co-expression of STIM1 and Orai1 activates of store-operated $Ca^{2+}$ currents*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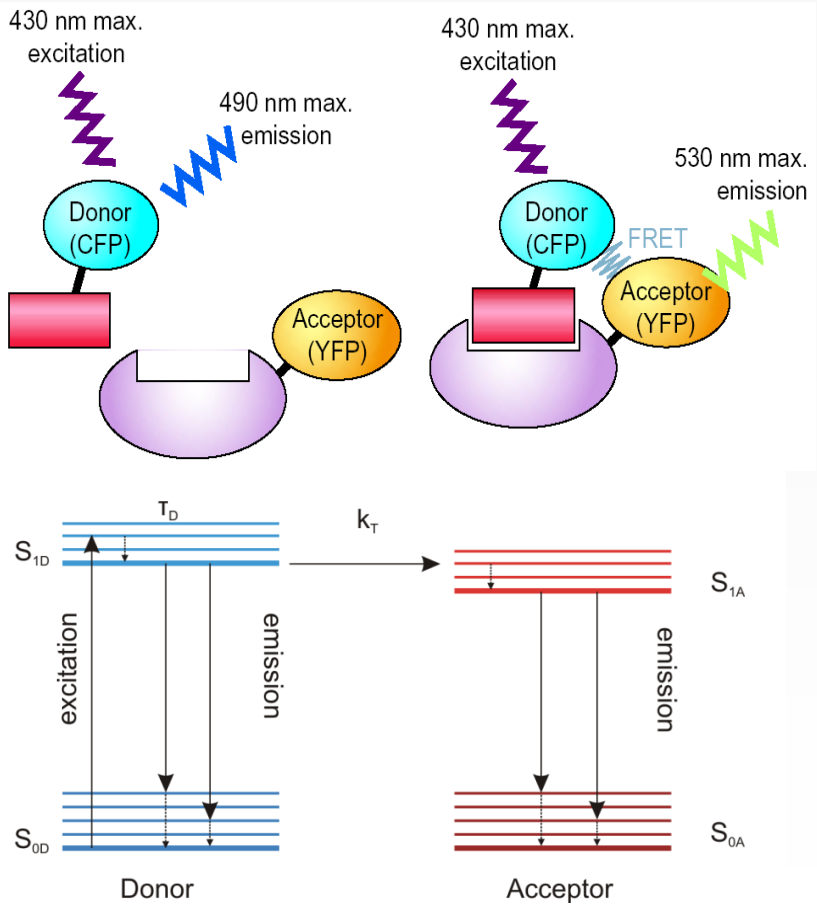
# Förster Resonance Energy Transfer (FRET)




- FRET is the radiationless transfer of emission energy from a donor to an acceptor that occurs beyond a distance of 10 nm.

  **Nice technique to detect protein-protein interactions**

- Cyan and yellow fluorophores are used from *Aequorea victoria* – keeper of GFP

- A pixel per pixel readout allows a time-dependent measurement of FRET within a single cell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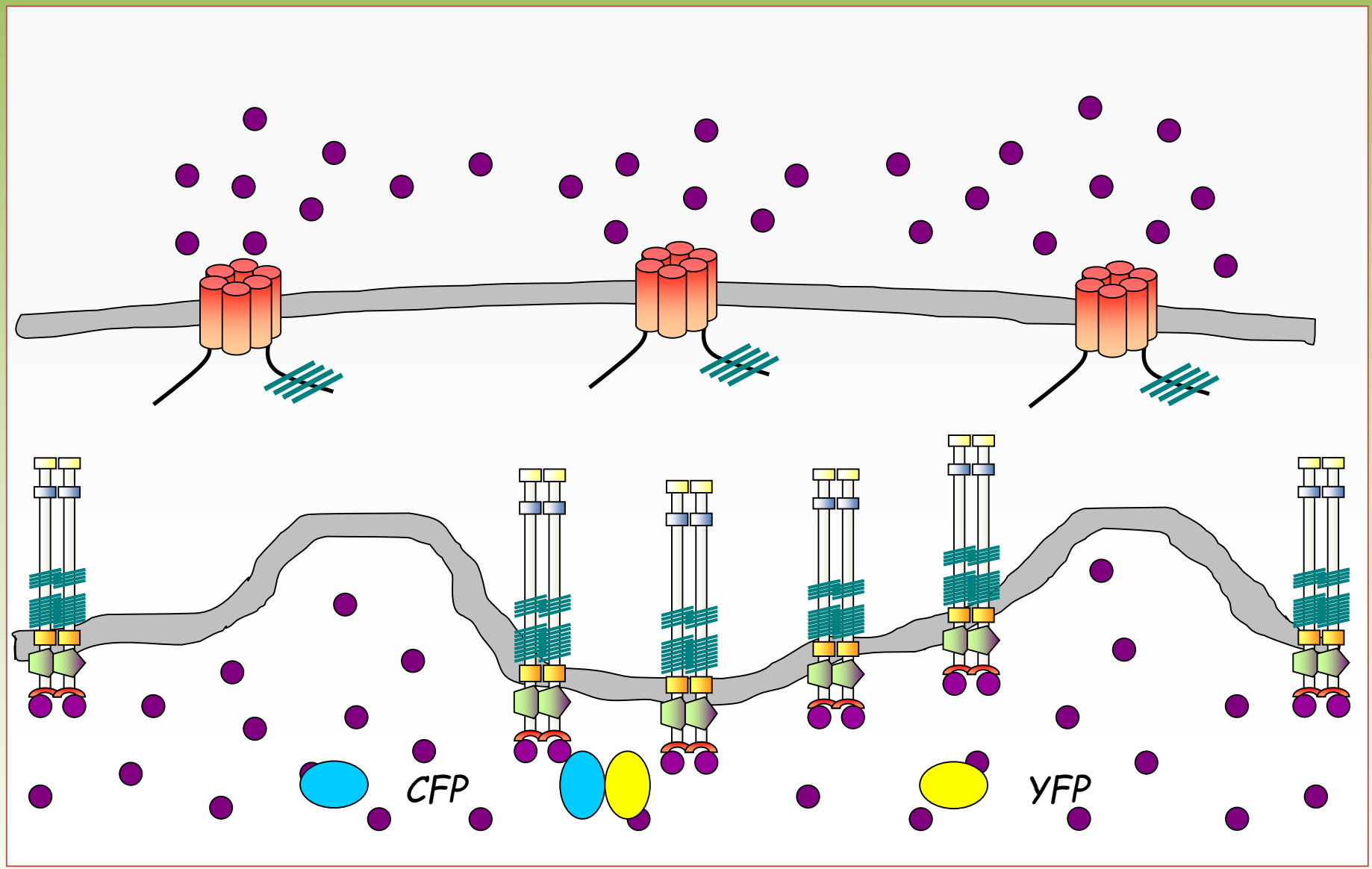
Direct coupling of STIM1 to Orai1 results in $Ca^{2+}$ channel activation

# STIM1 oligomerisation

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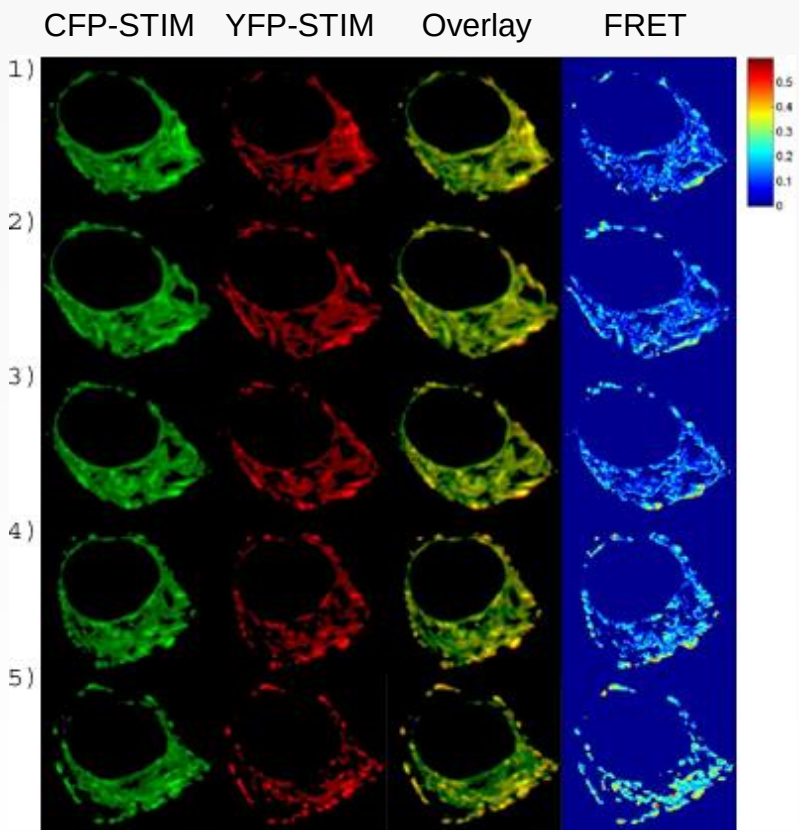
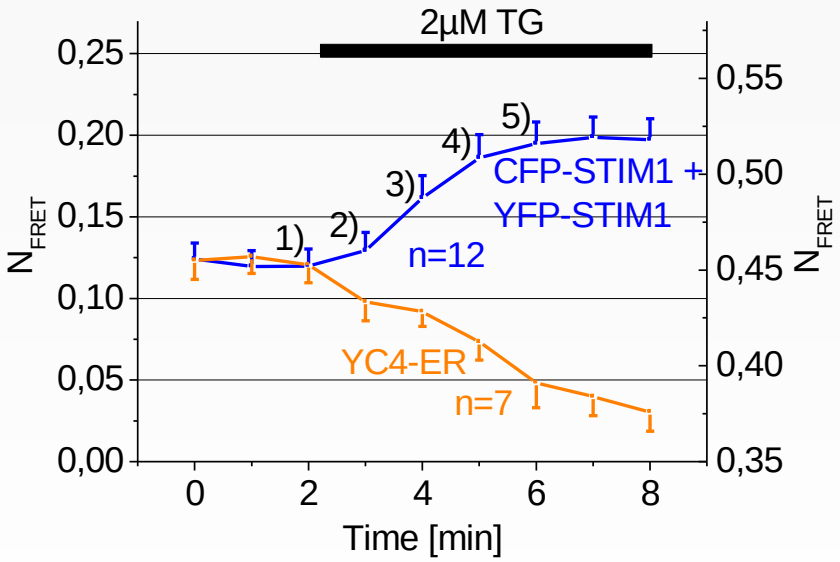
# STIM1 oligomerisation

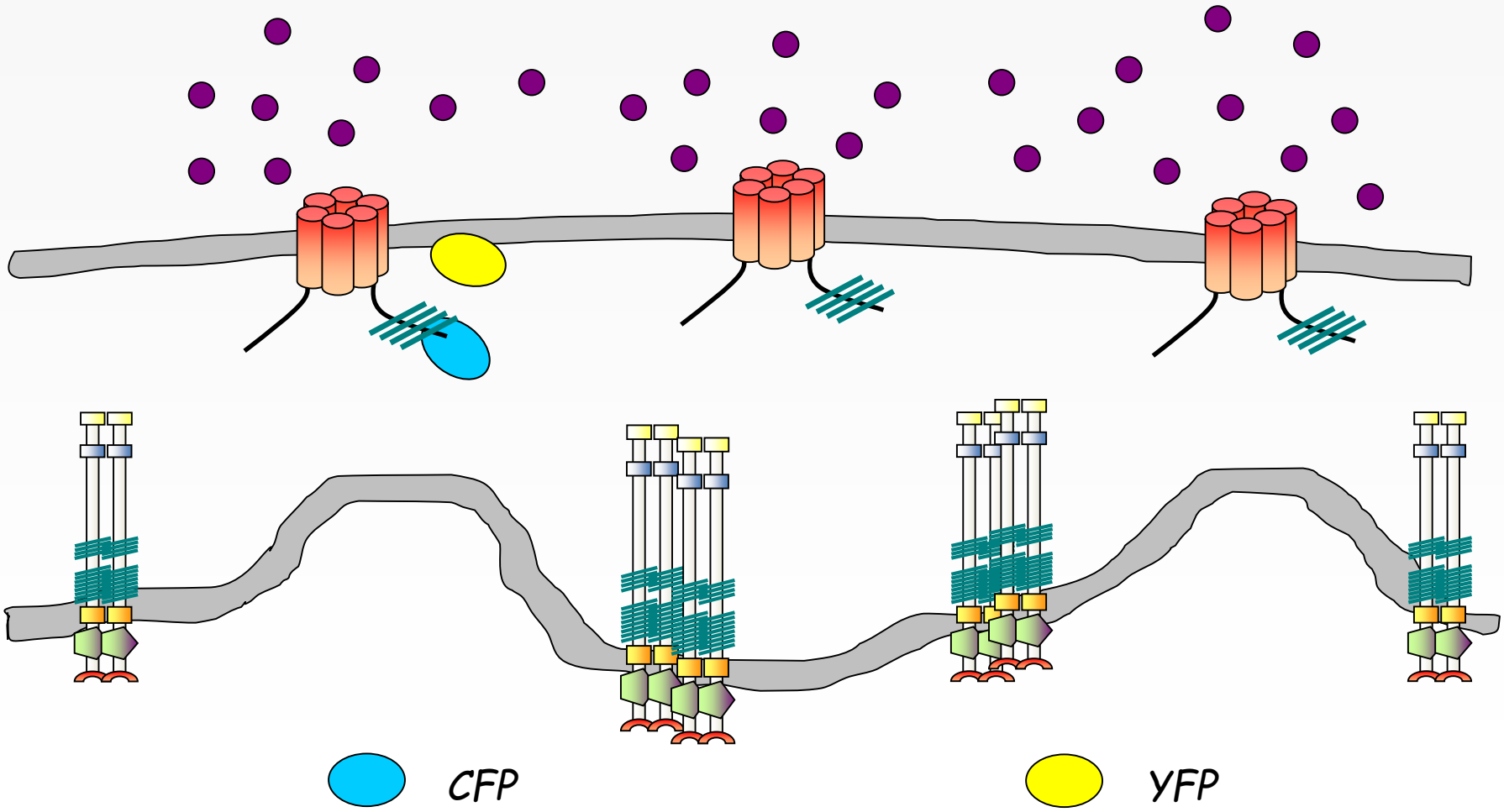



**STIM1 forms stable homomers after store store depletion with Thapsigargin (TG)**

Muik et al., JBC 2008

# STIM1 to Orai1 coupling




Muik et al., JBC 2008

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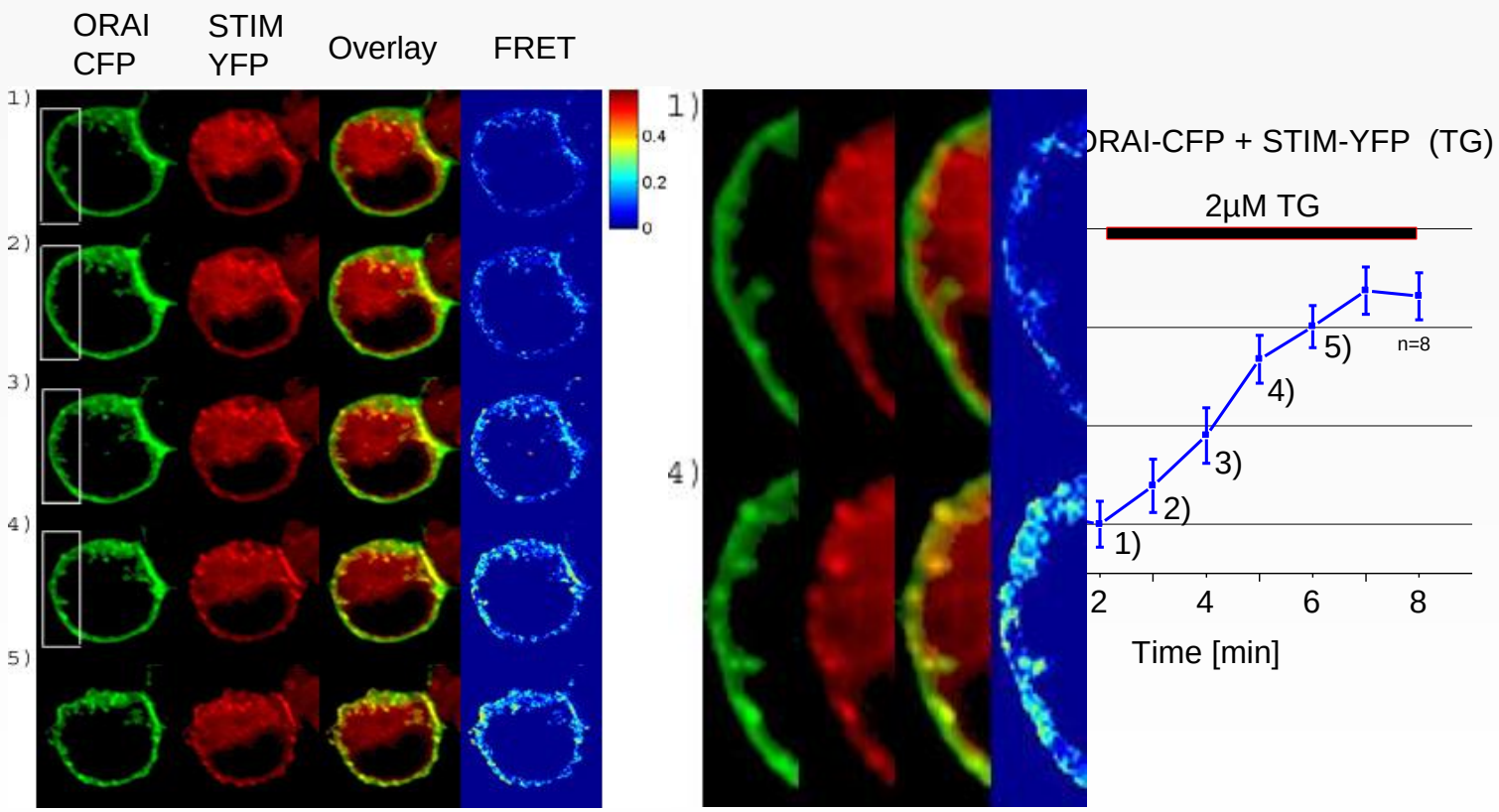
# STIM1 to ORAI1 coupling




**STIM1 to Orai1 coupling is temporally as well as spatially correlated with an increase in FRET**

Muik et al., JBC 2008

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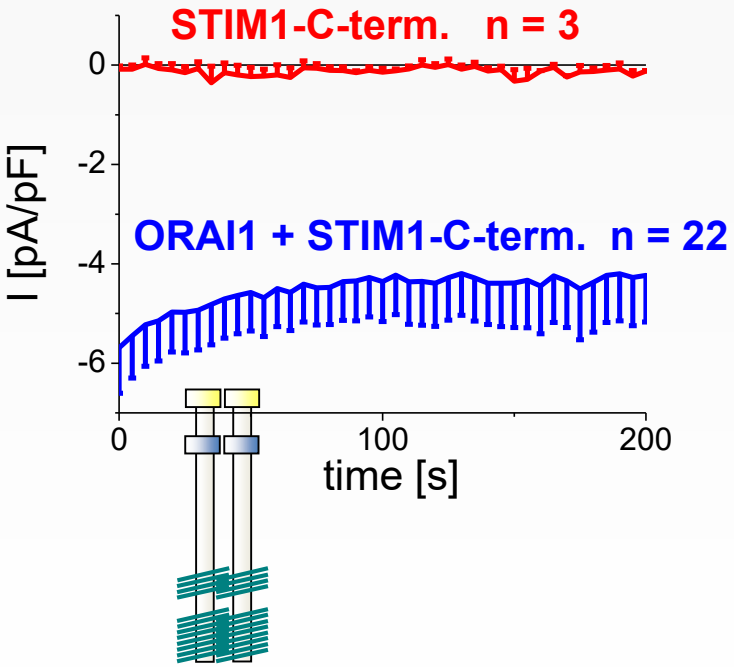
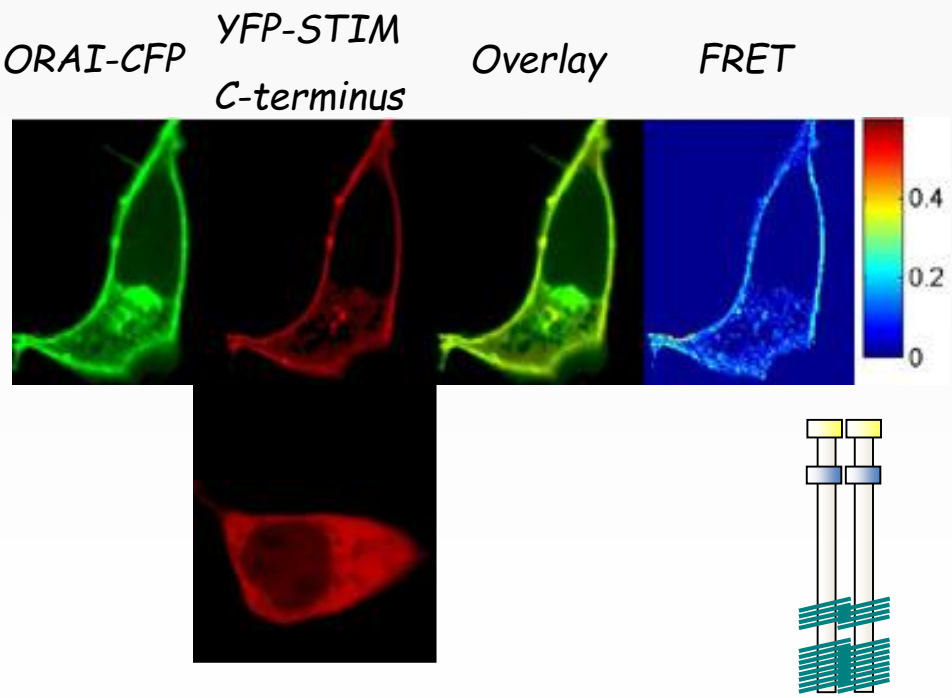
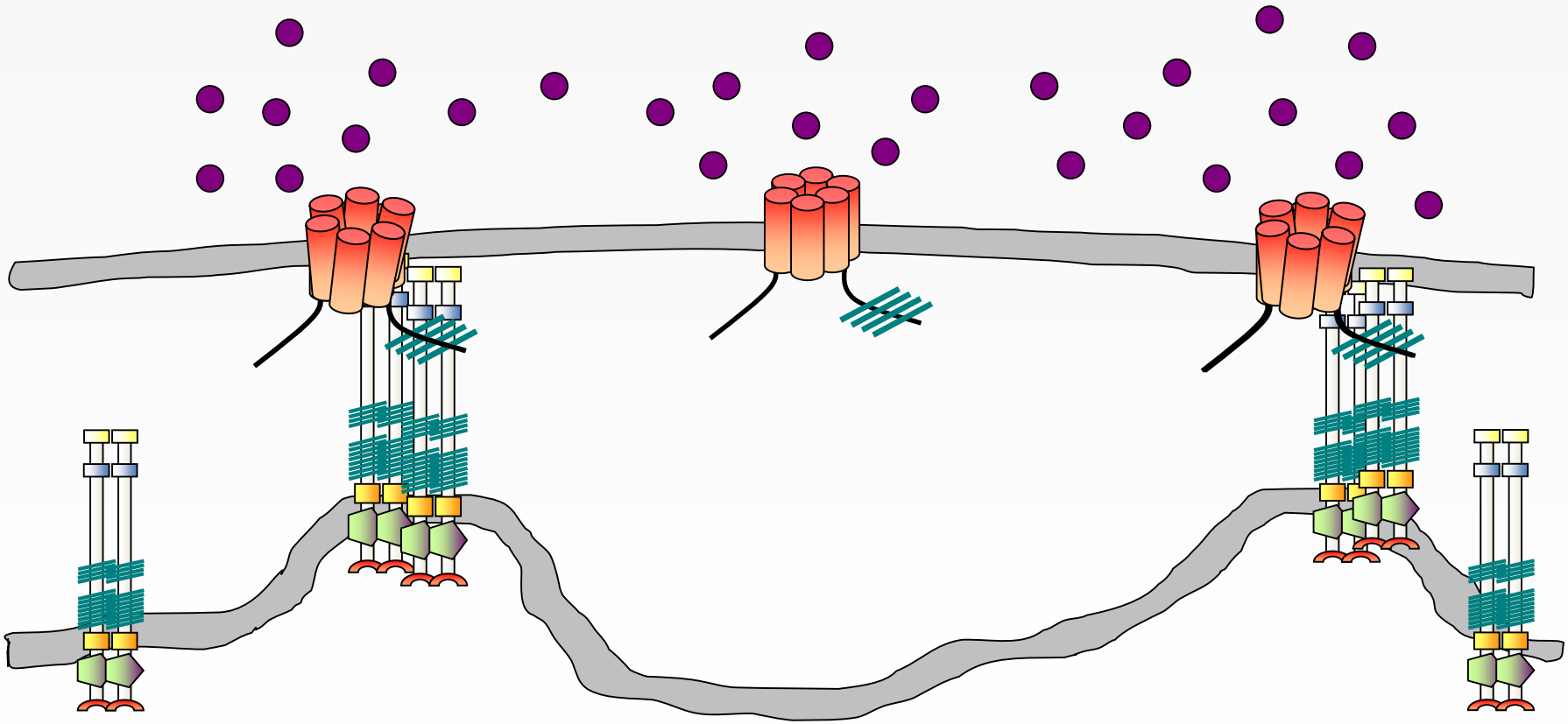
# STIM1 C-terminus is sufficient for ORAI1 activation




Muik et al., JBC 2008

# STIM1-mediated ORAI1 conformational change



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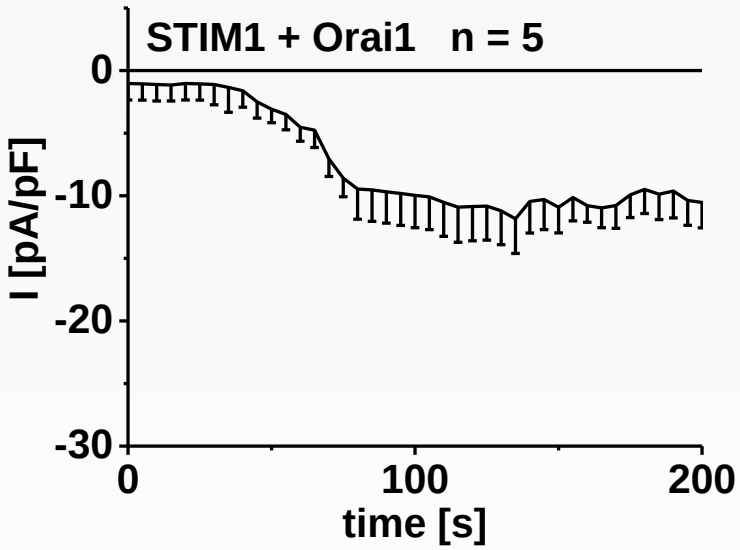
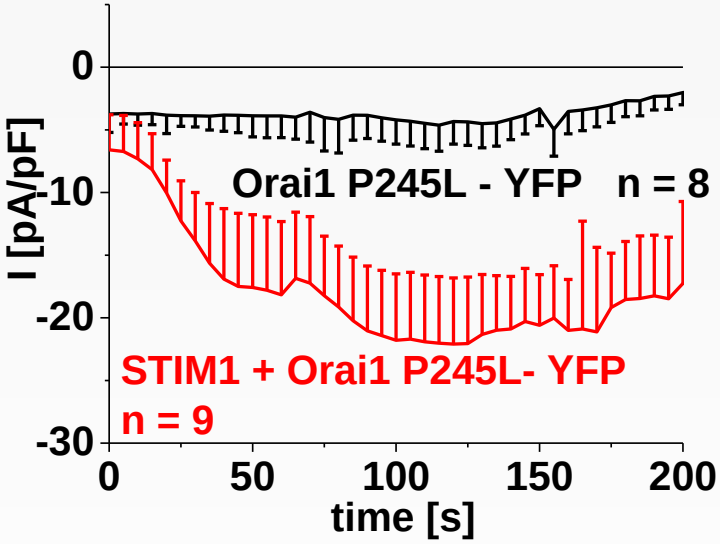
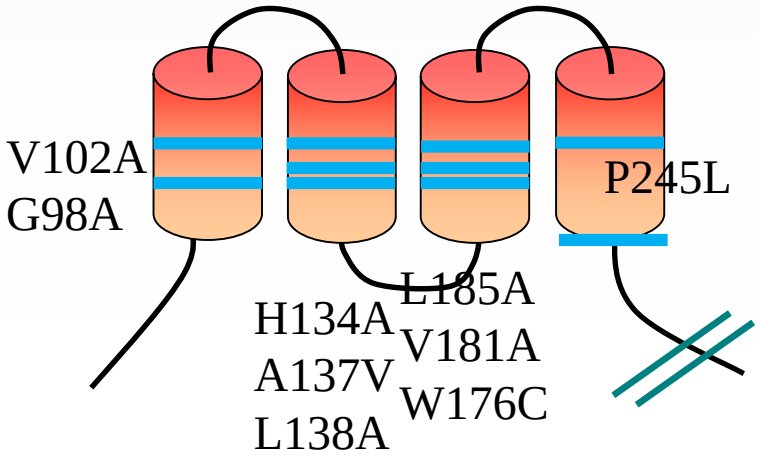
# STIM1-mediated ORAI1 conformational change



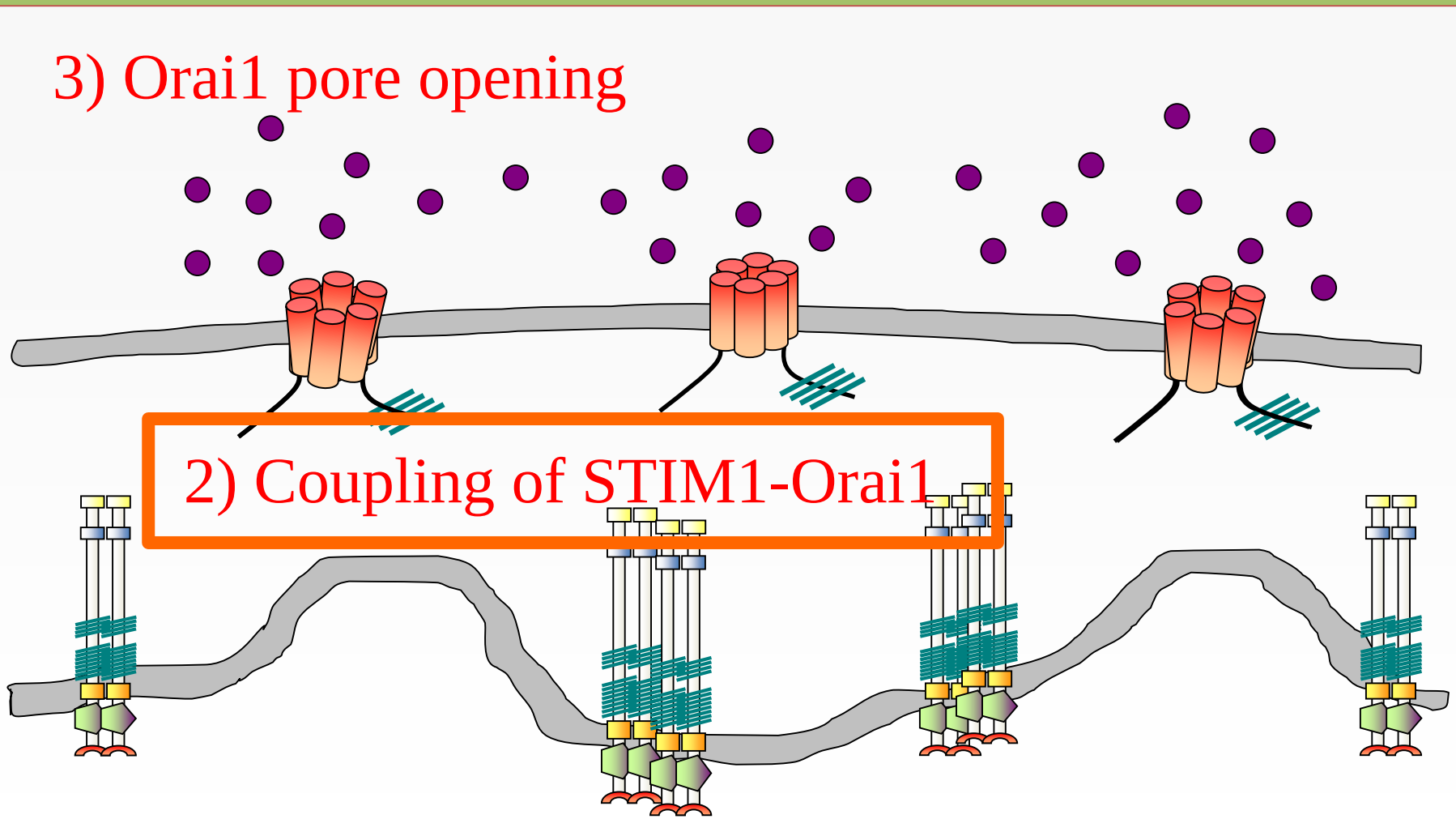




Global conformational change of Orai channel upon activation

McNally et al, Nature, 2012,
Derler et al., JBC, 2013
Palty et al., Cell Res, 2015
Frischauf et al., Sci Signal, 2018

# STIM1-mediated ORAI1 conformational change

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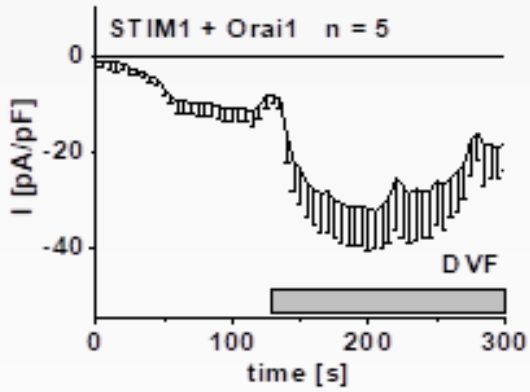
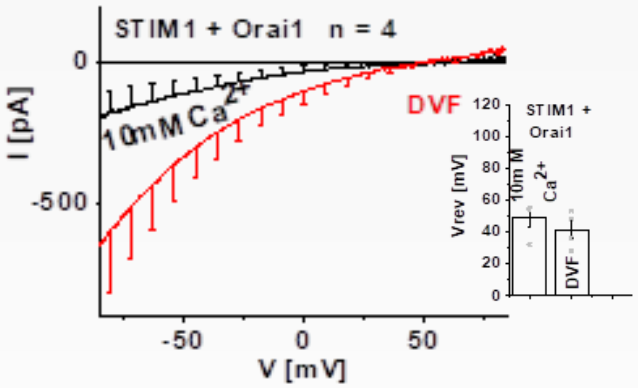
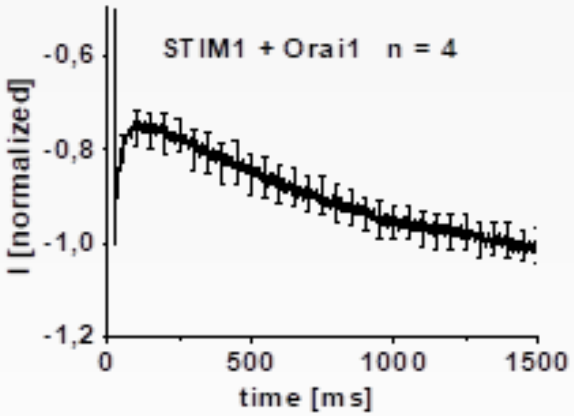
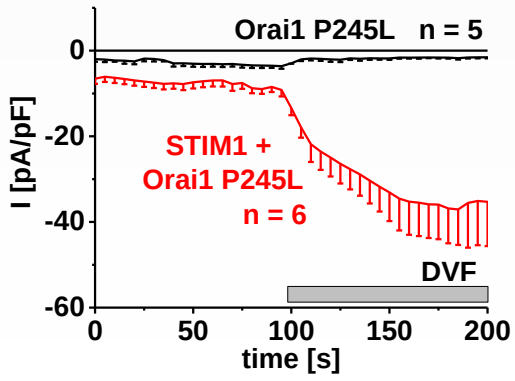
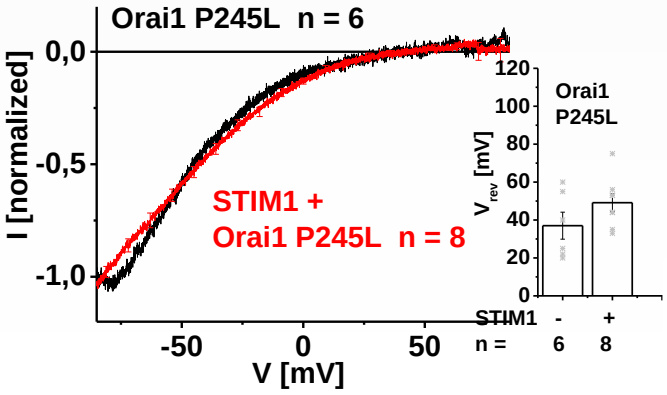
# STIM1 adjusts authentic CRAC channel hallmarks

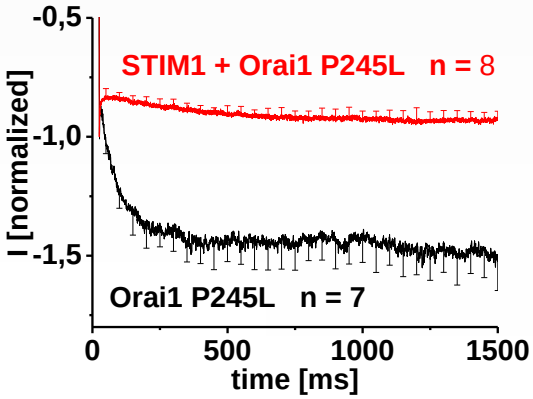


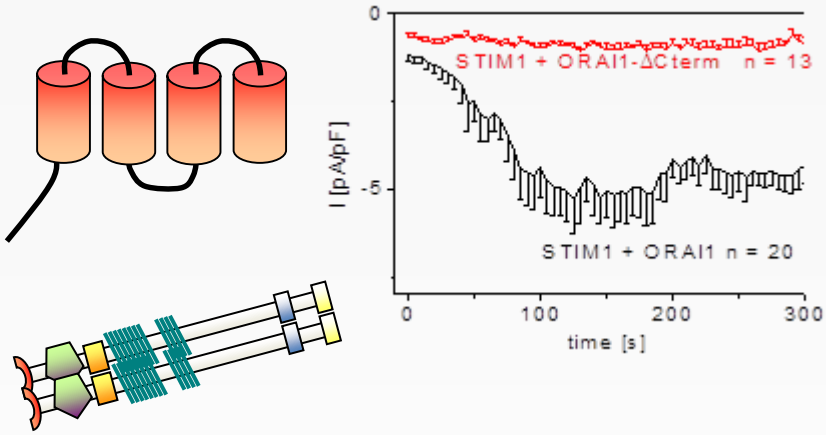



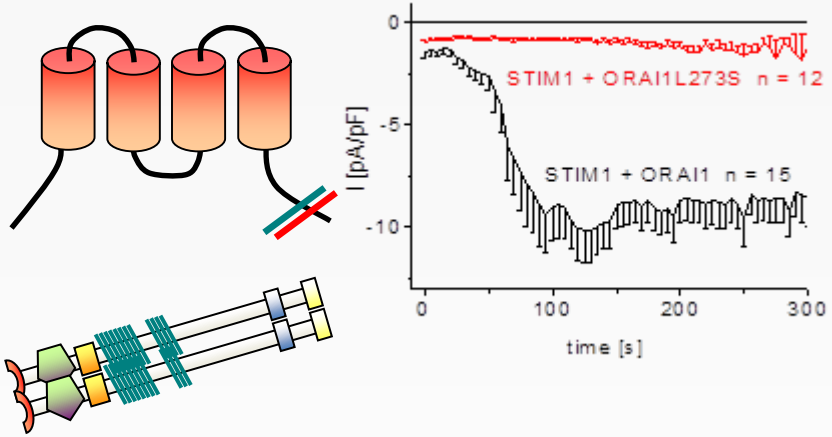



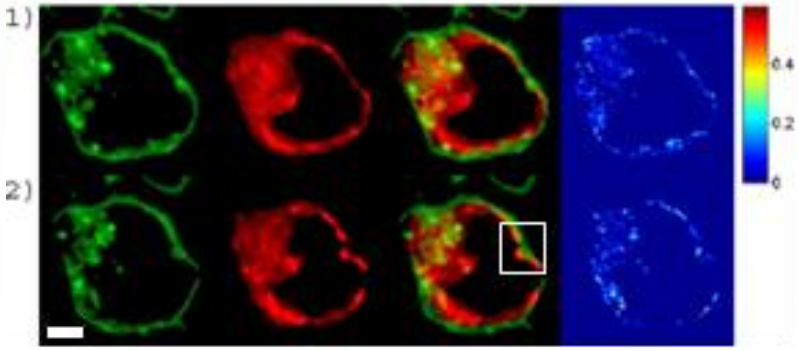

Derler et al., JBC; 2018

# Orai1 C-terminus indispensable for binding to STIM1

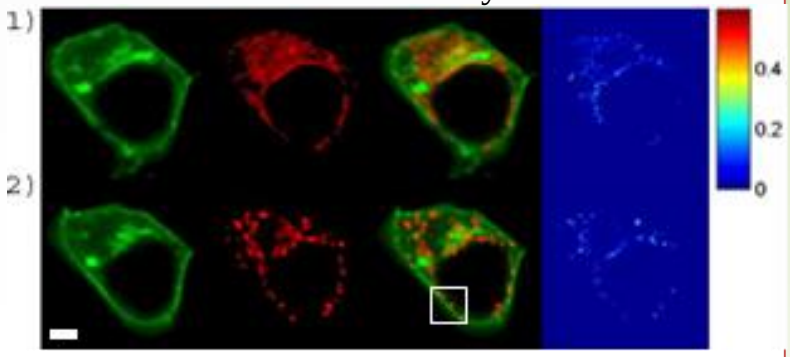



CFP-ORAI1 -ΔCterm | STIM1 -YFP | Overlay | FRET

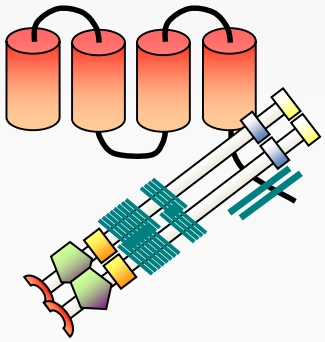


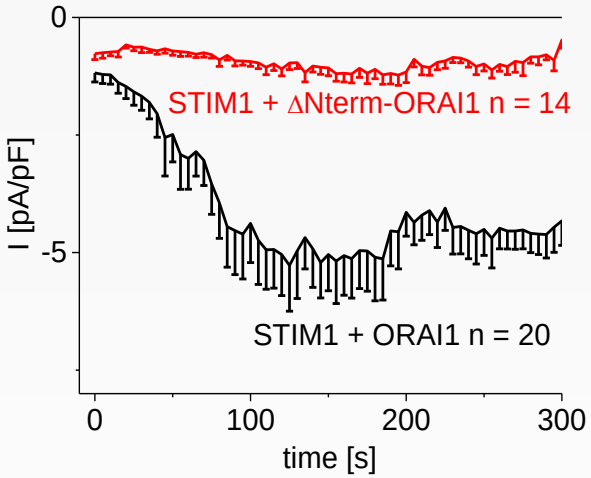

CFP-ORAI1 -L273S | STIM1 -YFP | Overlay | FRET



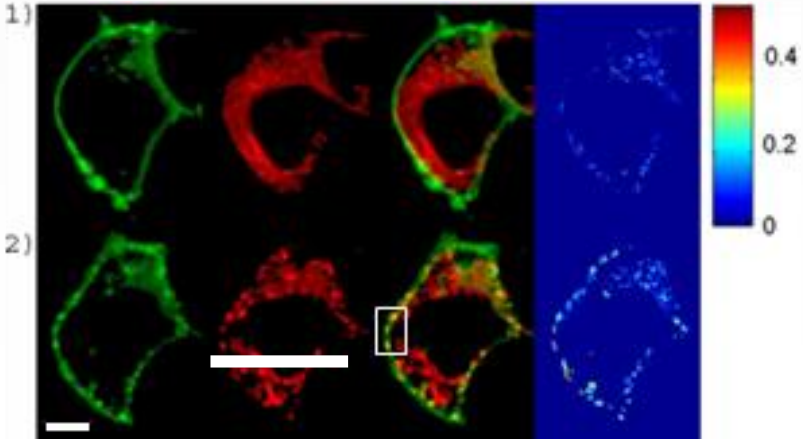

Muik et al., JBC; 2008

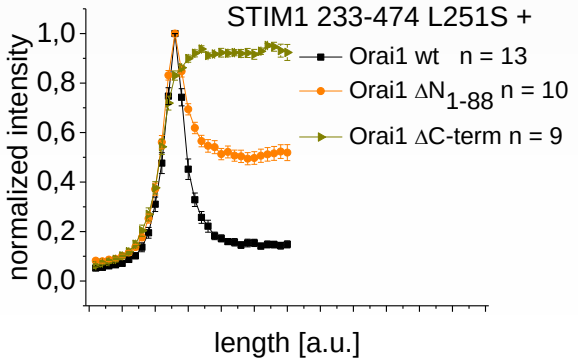
# Orai1 N-terminus essential for STIM1-mediated Orai1 gating





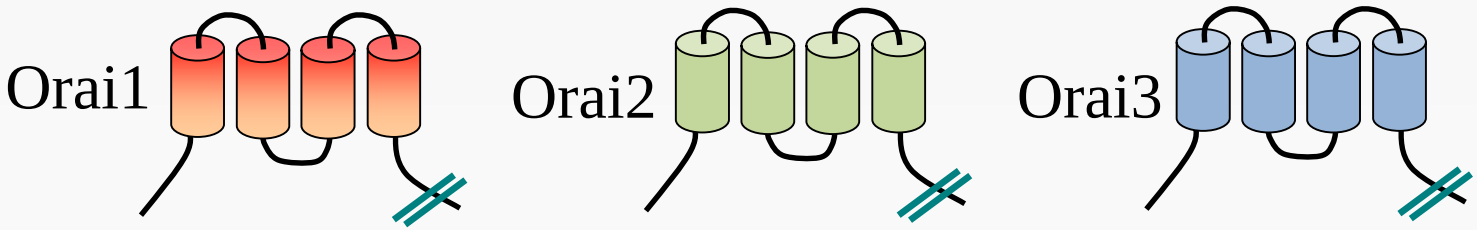

CFP-ORAI1 -ΔNterm | STIM1 -YFP | Overlay | FRET




Muik et al., JBC; 2008

# Last 15aa of Orai1 N-terminus are indispensable for STIM1 mediated Orai1 gating

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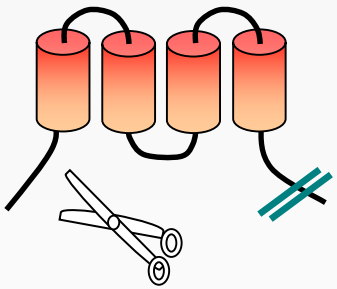
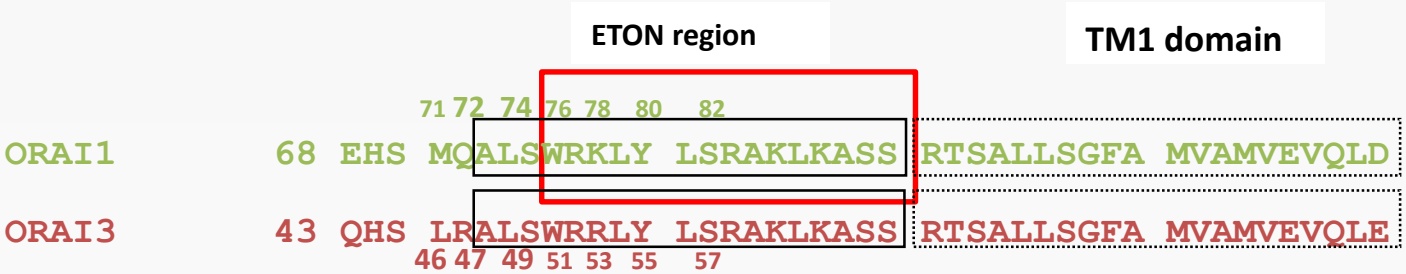
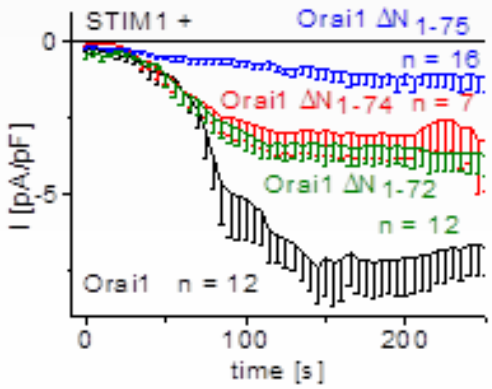
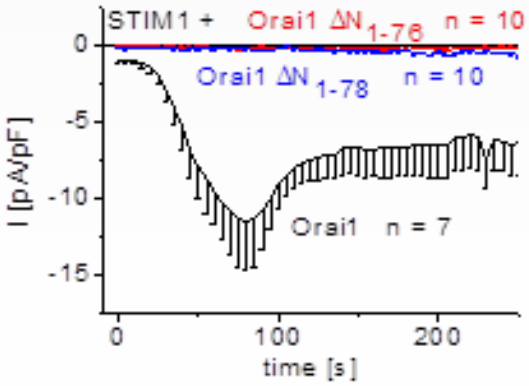
# Last 15aa of Orai1 N-terminus are indispensable for STIM1 mediated Orai1 gating

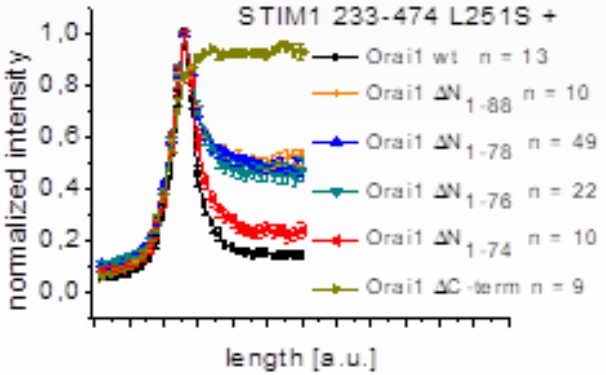





Store-operated activation

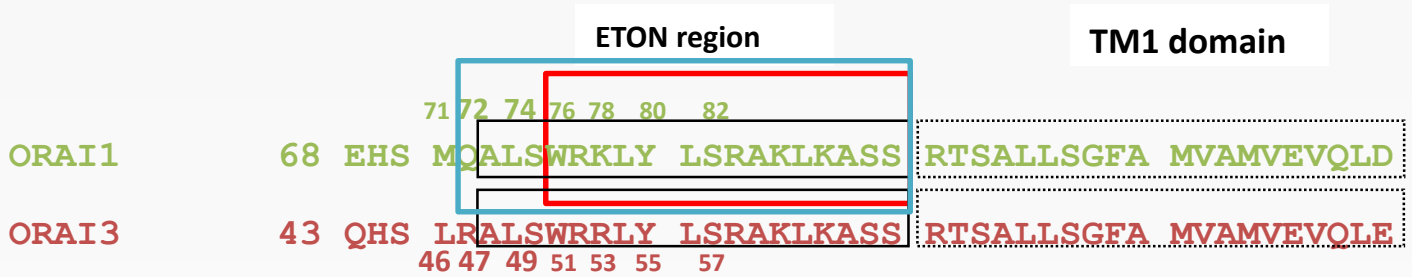


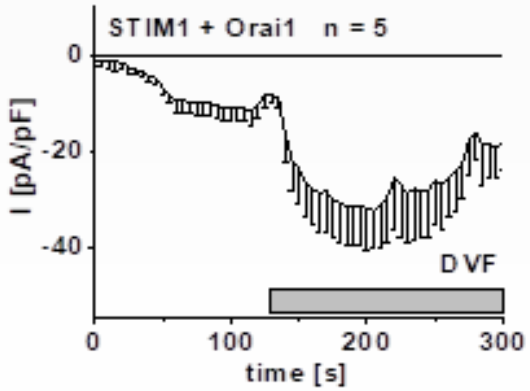

Derler et al., JBC; 2013

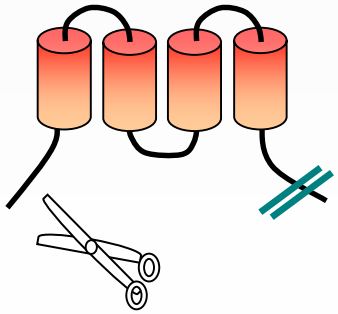
# Orai1 N-terminus mediates authentic CRAC channel characteristics

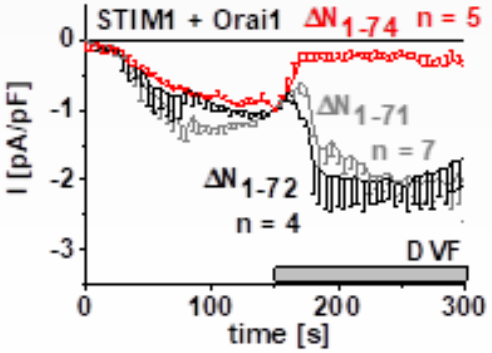



Store-operated activation

CRAC channel hallmarks






Derler et al., JBC; 2018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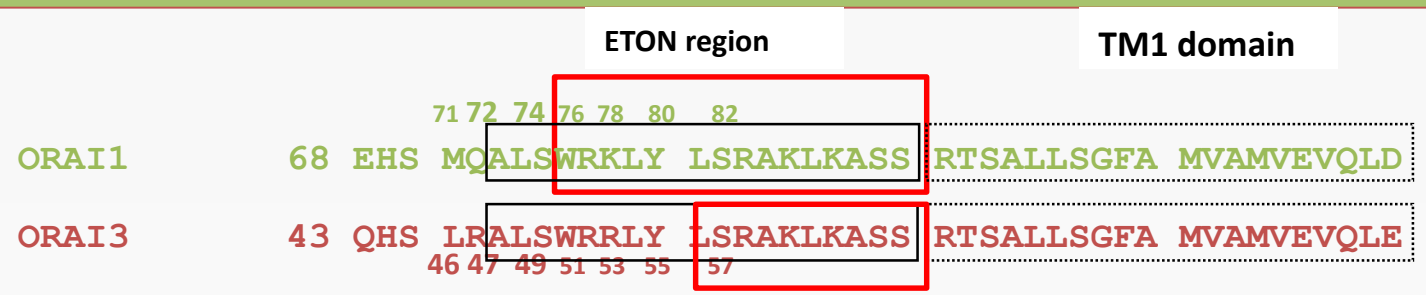
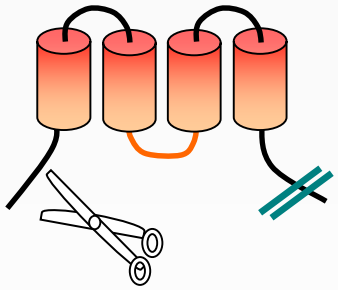
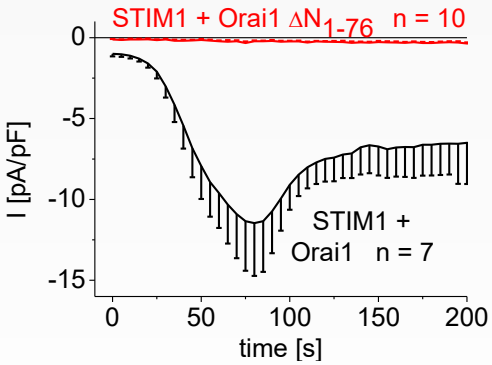
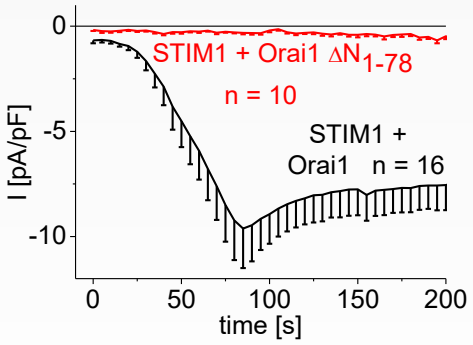
# Isoform specific Orai channel activation

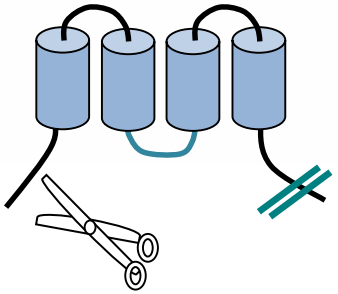


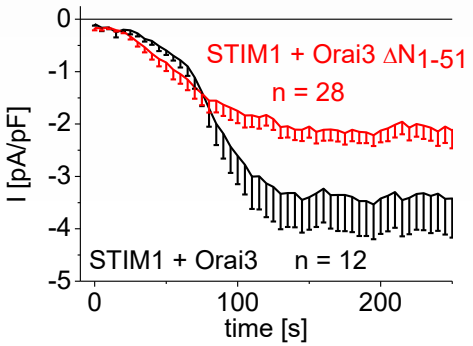

Store-operated activation

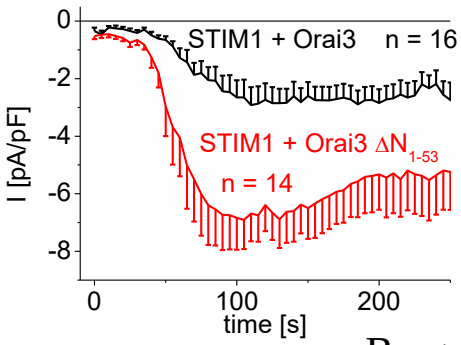







Bergsmann et al., JBC, 2011;
Fahrner et al., JBC; 2018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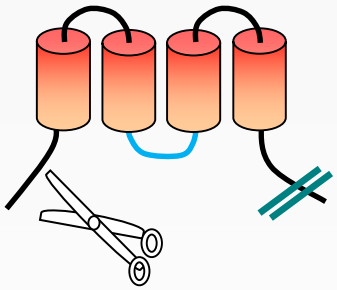
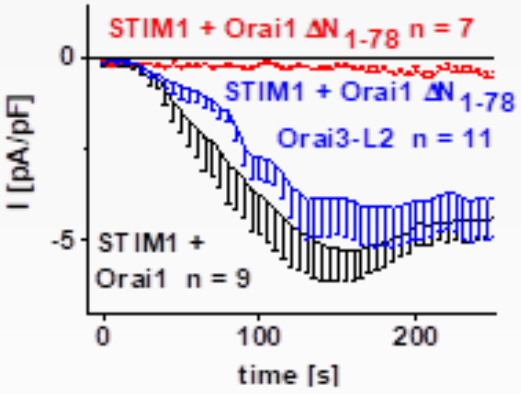
# Orai1 N-terminus communicates with loop2 to mediate Orai channel gating

**Orai1 ΔN1-78**

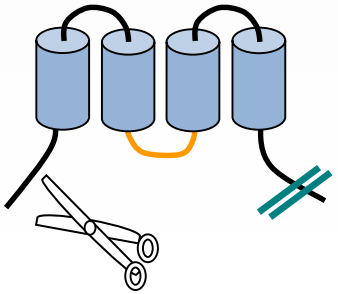
**Orai3-L2**

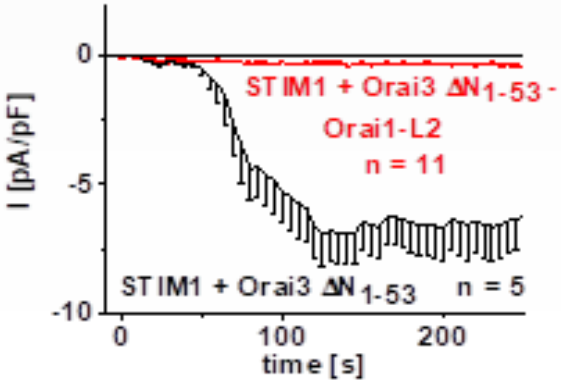


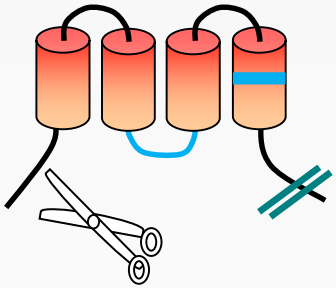


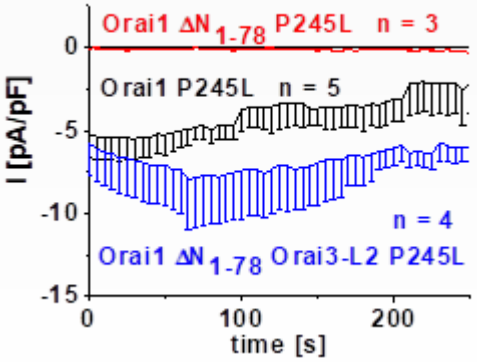

**Orai3 ΔN1-53**

**Orai1-L2**



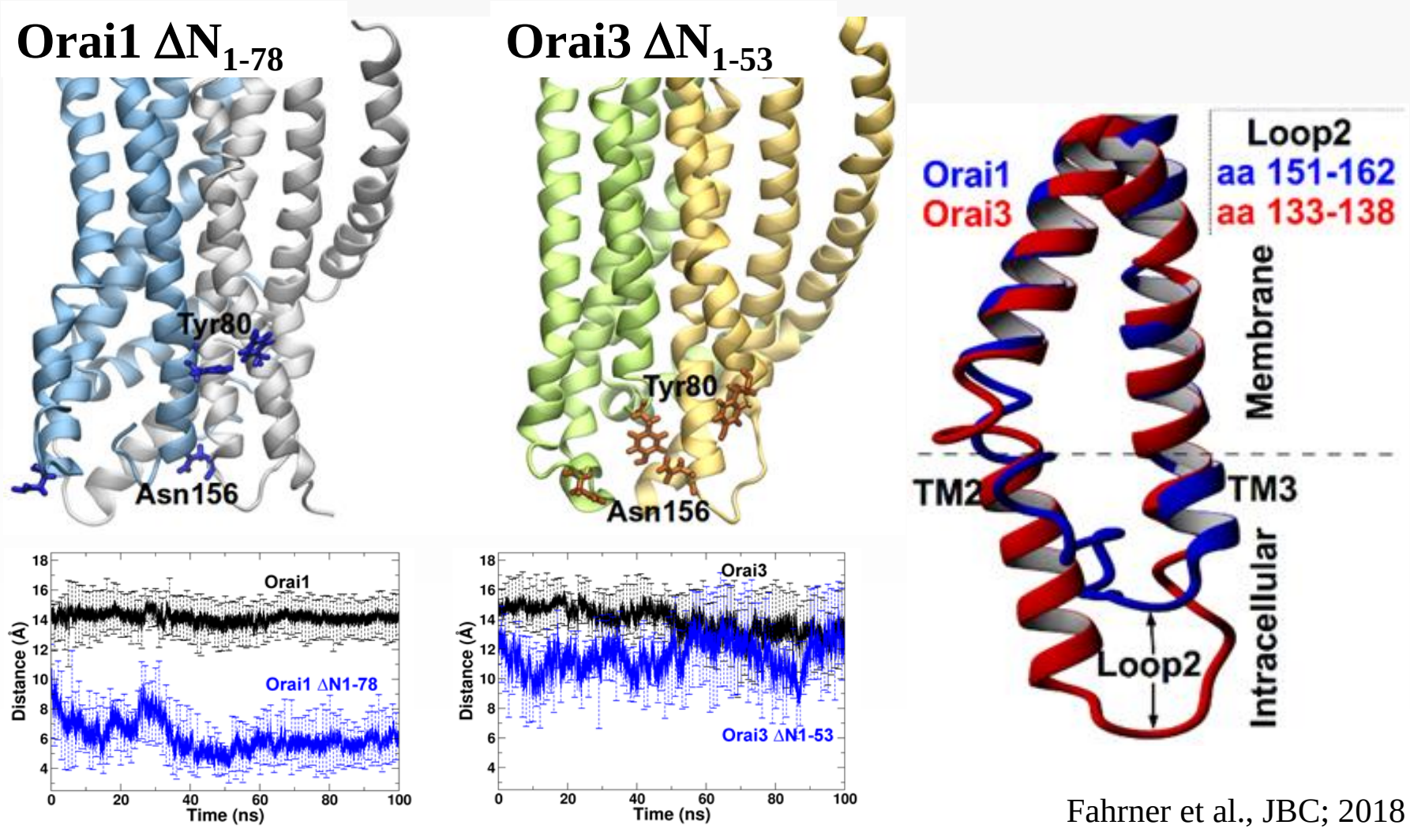





Fahrner et al., JBC; 2018

# Orai1 N-terminus communicates with loop2 to mediate Orai channel gating




Fahrner et al., JBC; 2018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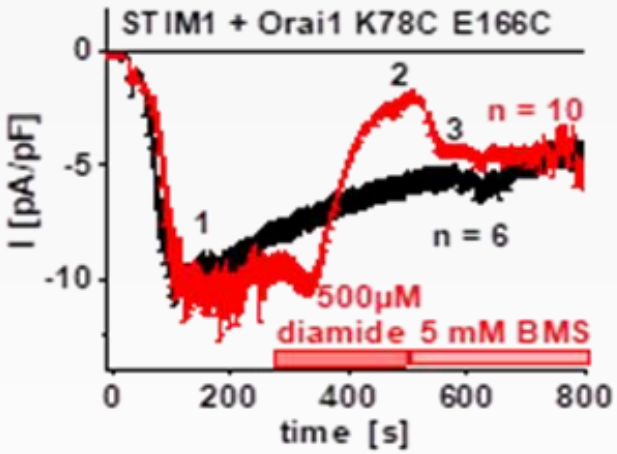
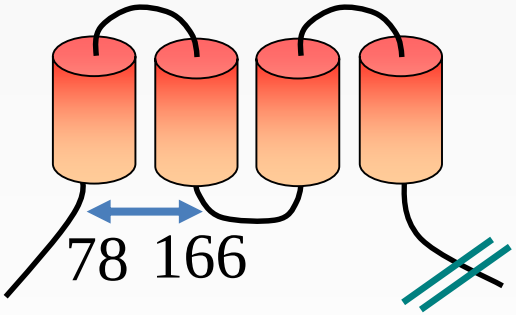
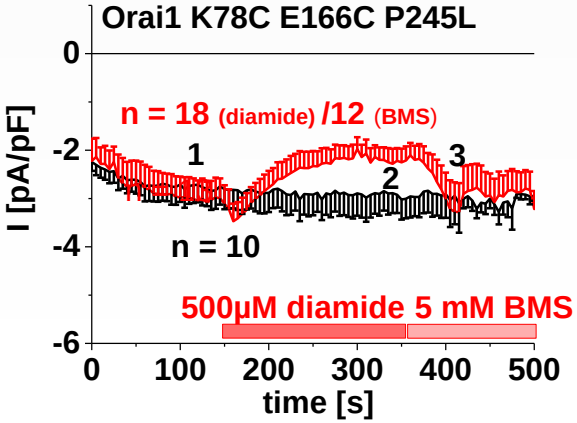
# Orai1 N-terminus communicates with loop2 to mediate Orai channel gating

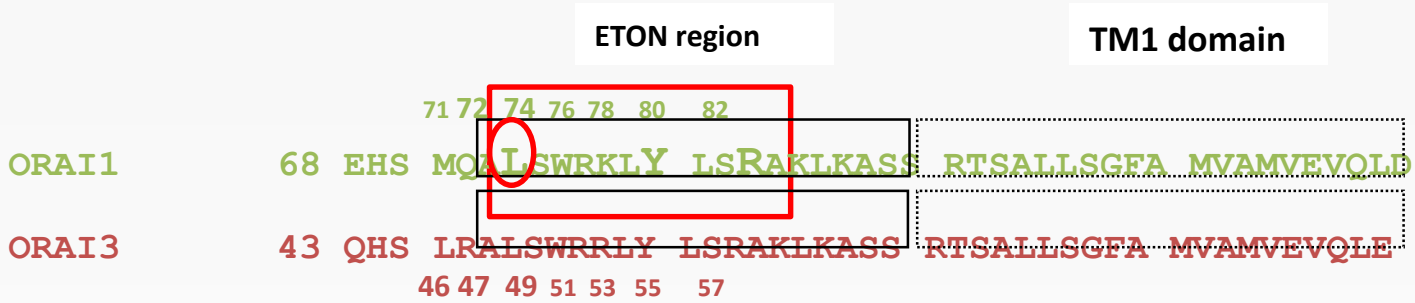


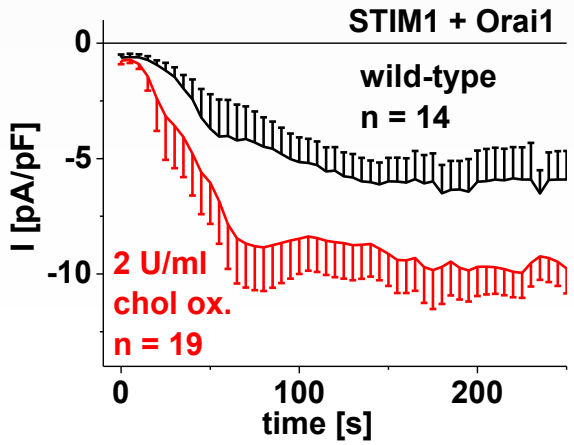



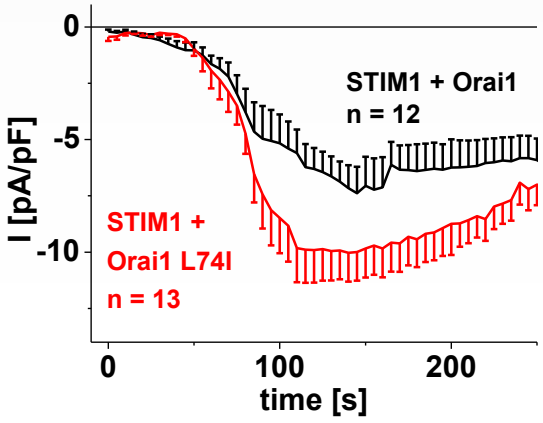

Fahrner et al., JBC; 2018

# Gating of Orai1 is further controlled via a communication of N-terminus with cholesterol



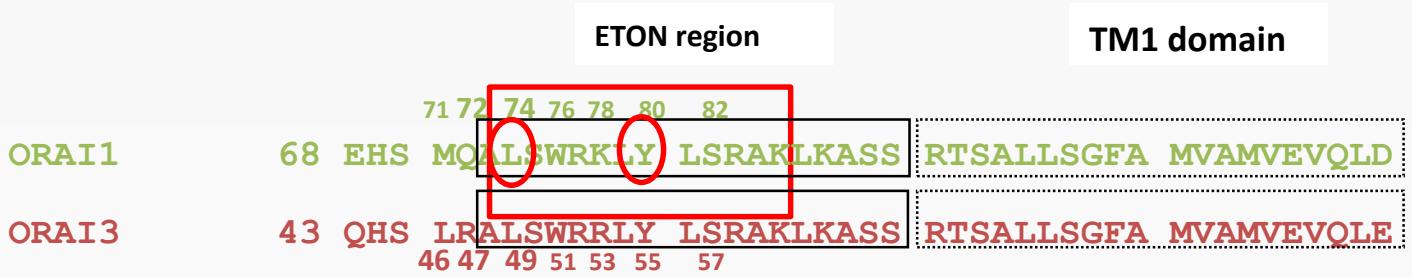

(L/V)-$X_{1-5}$-(Y)-$X_{1-5}$-(K/R) – consenus motif of cholesterol binding motif



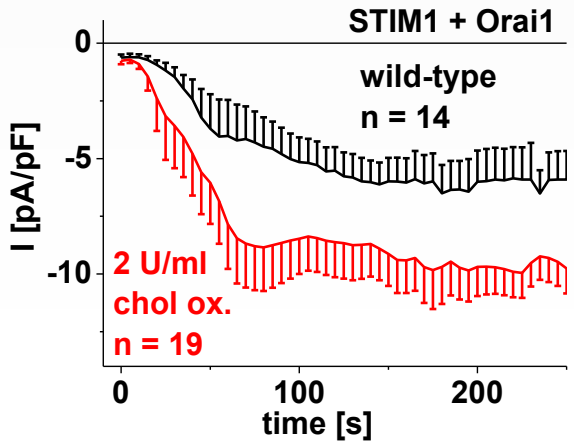



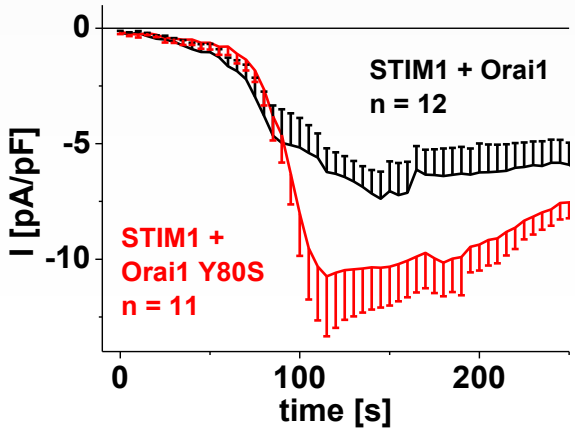

Derler et al., Sci Signal; 2016

# Gating of Orai1 is further controlled via a communcation of N-terminus with cholesterol



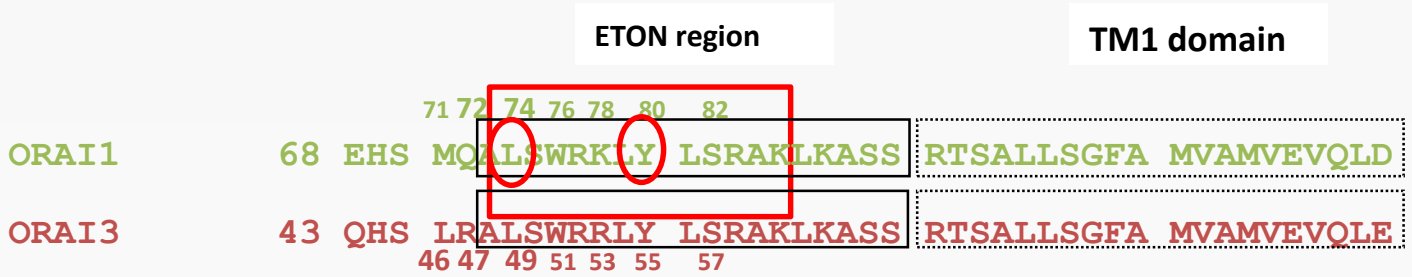

(L/V)-$X_{1-5}$-(Y)-$X_{1-5}$-(K/R) – consenus motif of cholesterol binding motif

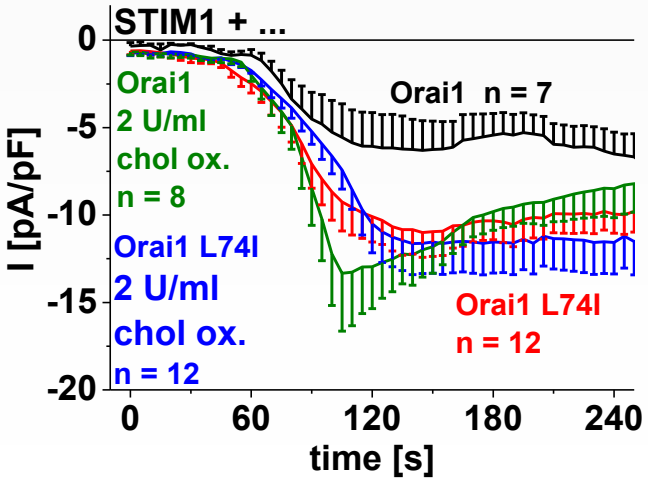


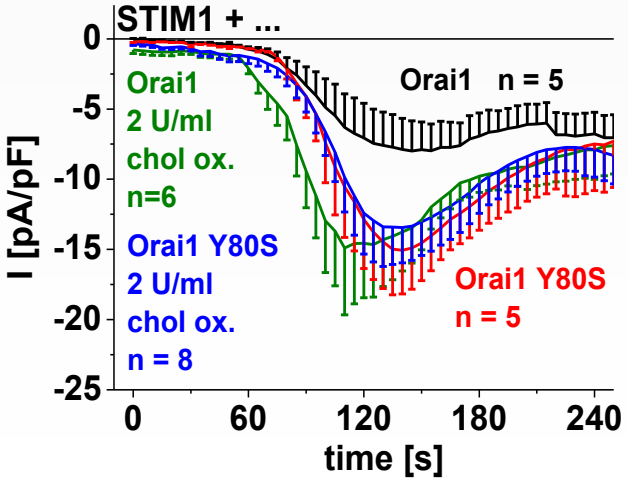




Derler et al., Sci Signal; 2016

# Gating of Orai1 is further controlled via a communcation of N-terminus with cholesterol

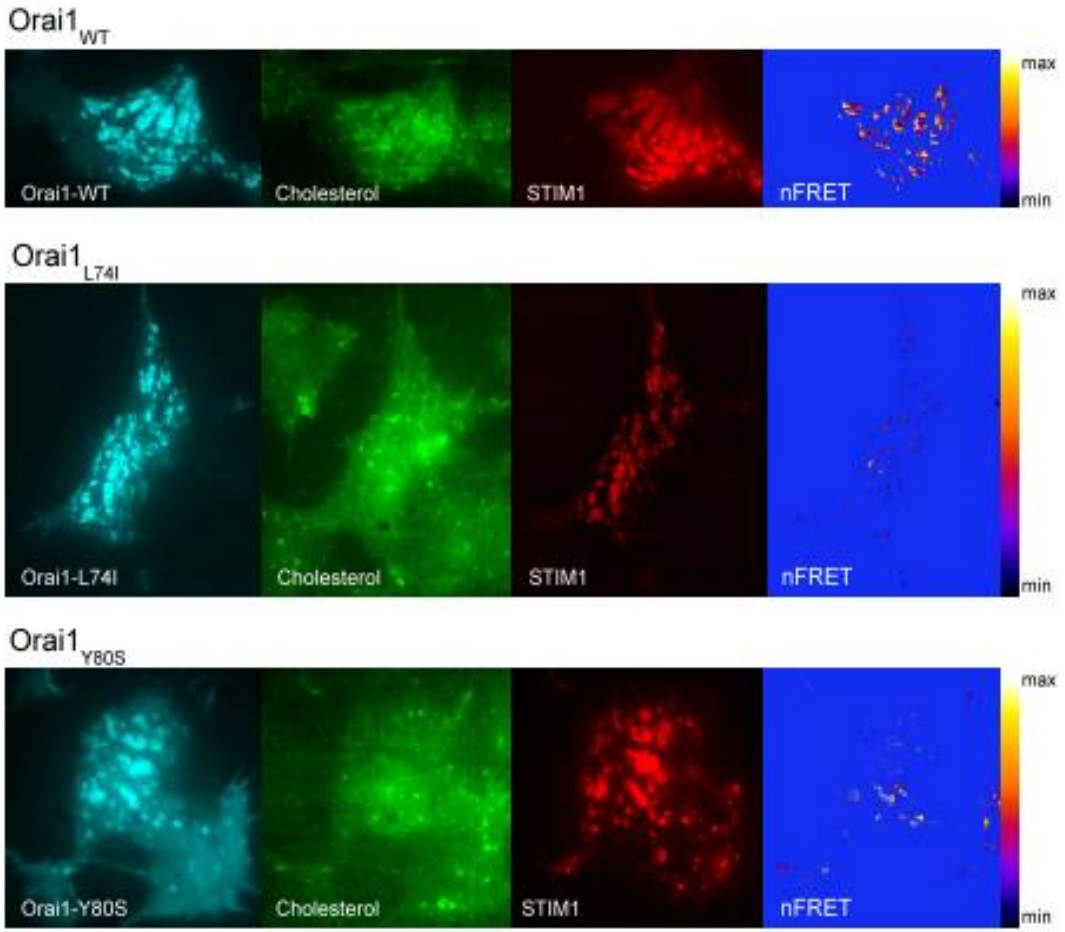


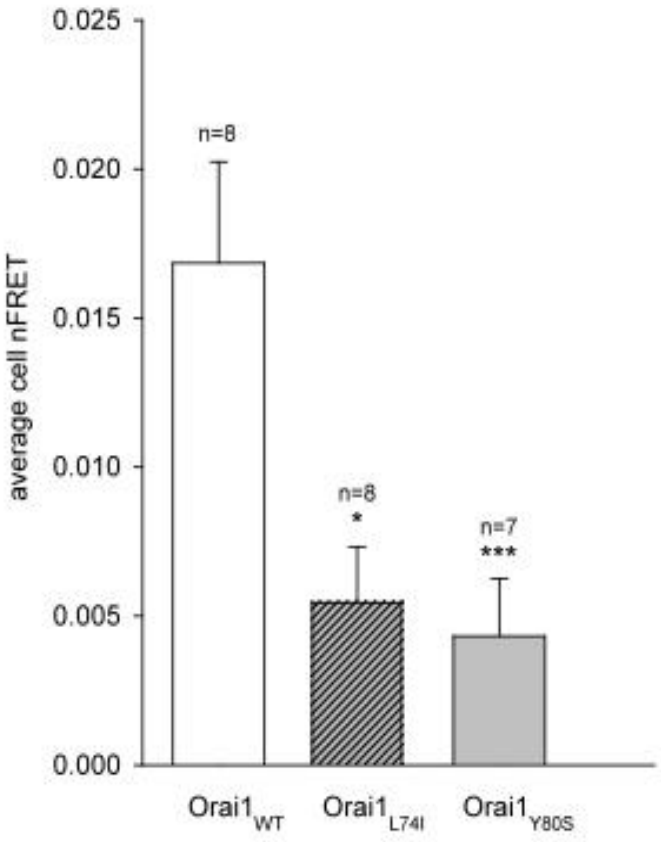




Derler et al., Sci Signal; 2016

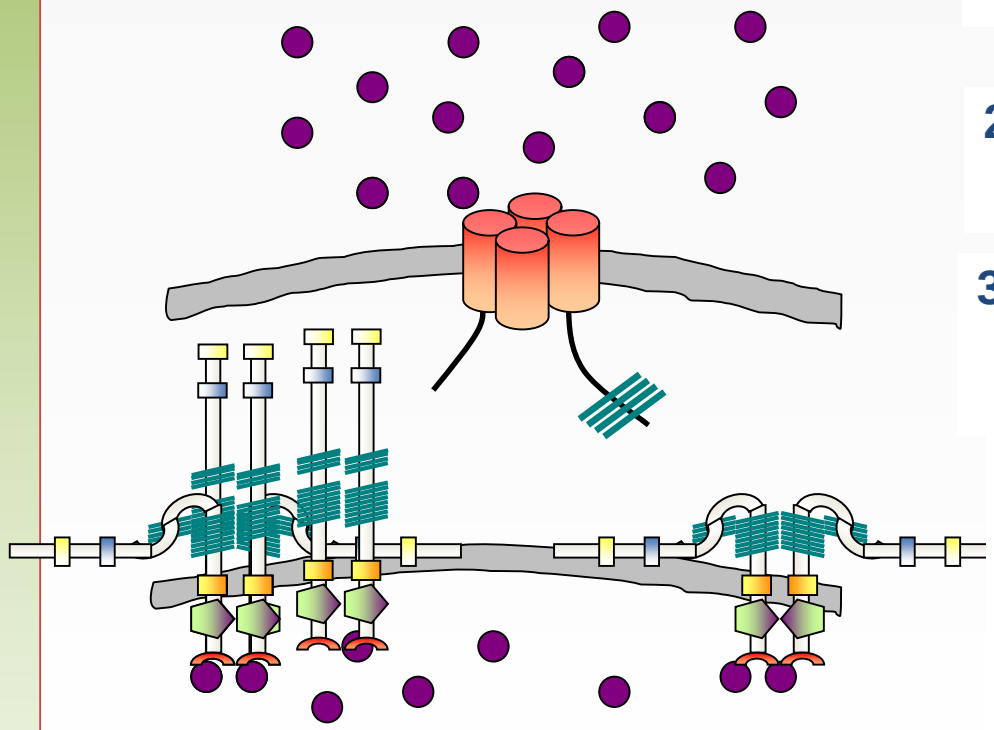
# Cholesterol binding is affected by a mutated Orai1 N-terminus




Derler et al., Sci Signal; 2016

# Conclusion



1. Upon STIM1 multimerisation, it couples directly to Orai1 via coiled-coil domains in punctae strutures.

2. Binding results in Calcium influx via Orai1 channels

3. Orai1 C-terminus is main binding site for STIM1

4. Orai1 N-terminus:
   - Control STIM1 coupling partly
   - Essential for store-operated activation
   - Controls CRAC channel hallmarks
   - Controls distance to Orai1 loop2
   - Is involved in cholesterol mediated Orai regulation.

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## Thanks to:

Christoph Romanin

Martin Muik

Irene Frischauf

Victoria Lunz

Marc Fahrner

Adéla Krizova

Carmen Butrac

Romana Schober

Lena Maltan

Sonja Lindinger

## Cooperation:

### Groschner Lab

### Ikura Lab, especially Peter Stathopulos

### Ettrich Lab

Supported by:

FWF Der Wissenschaftsfonds.

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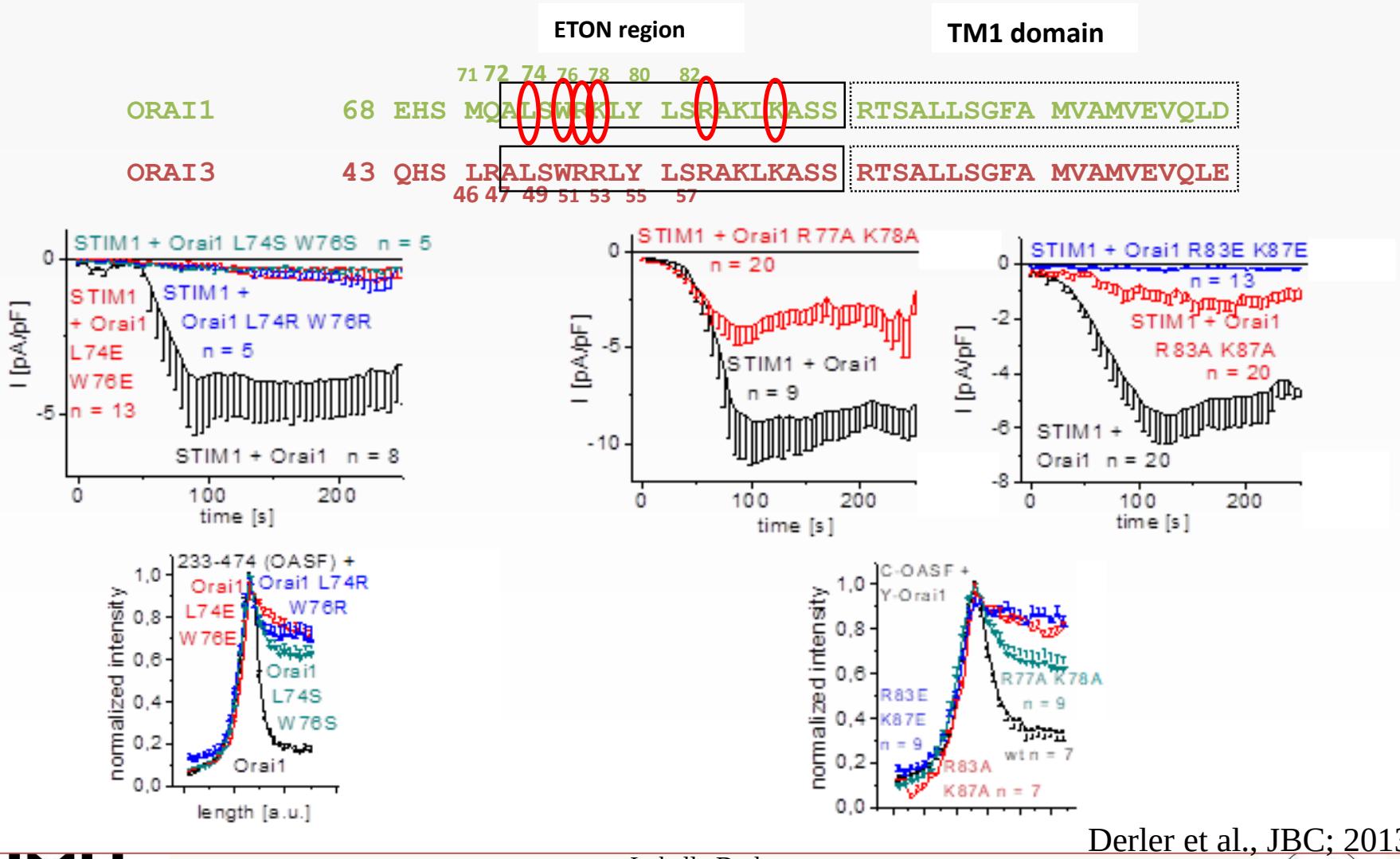
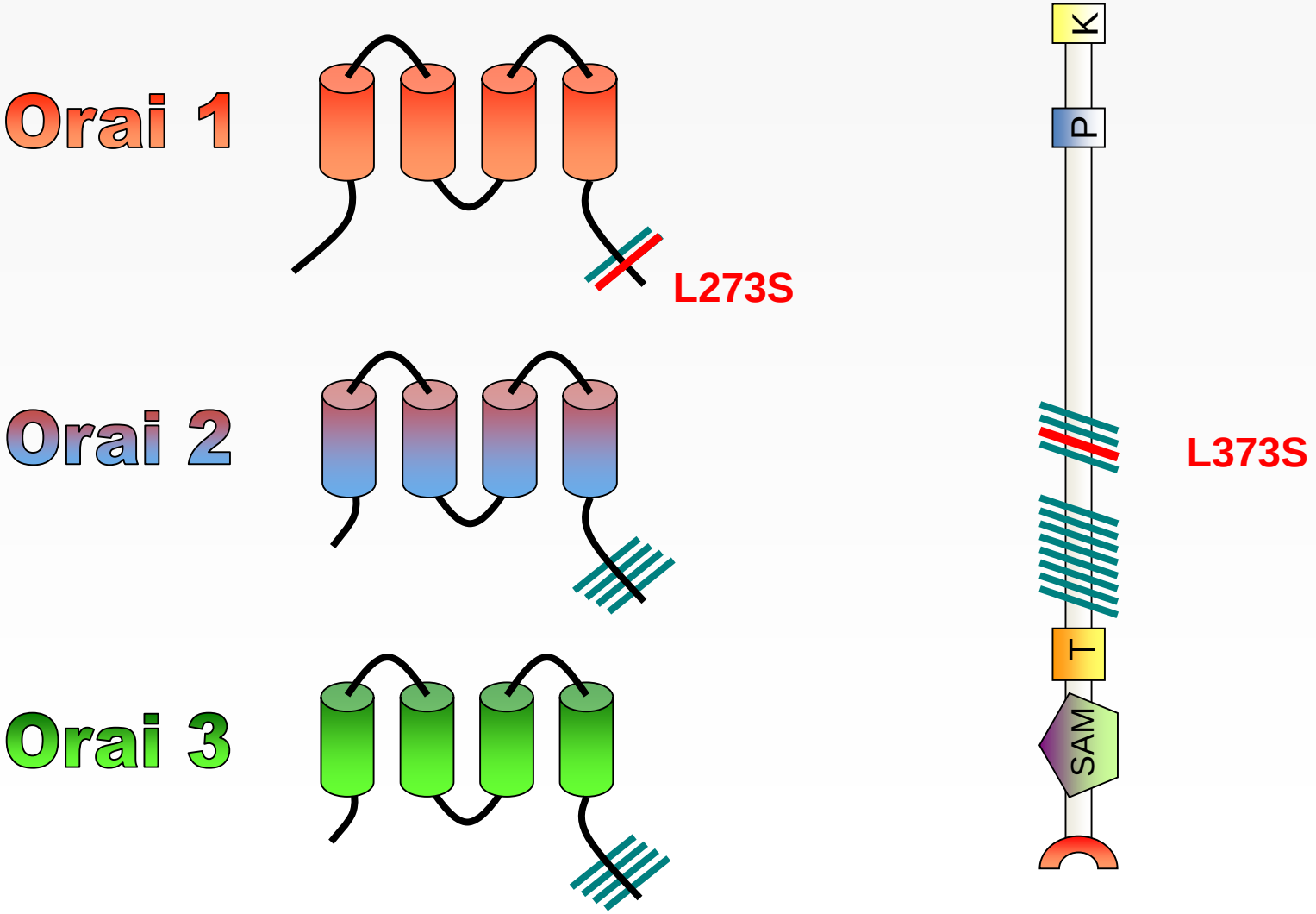
# Several hot spots within Orai1 N-terminus control Orai1 gating




Derler et al., JBC; 2013

# STIM1 to Orai coupling via coiled-coil domains

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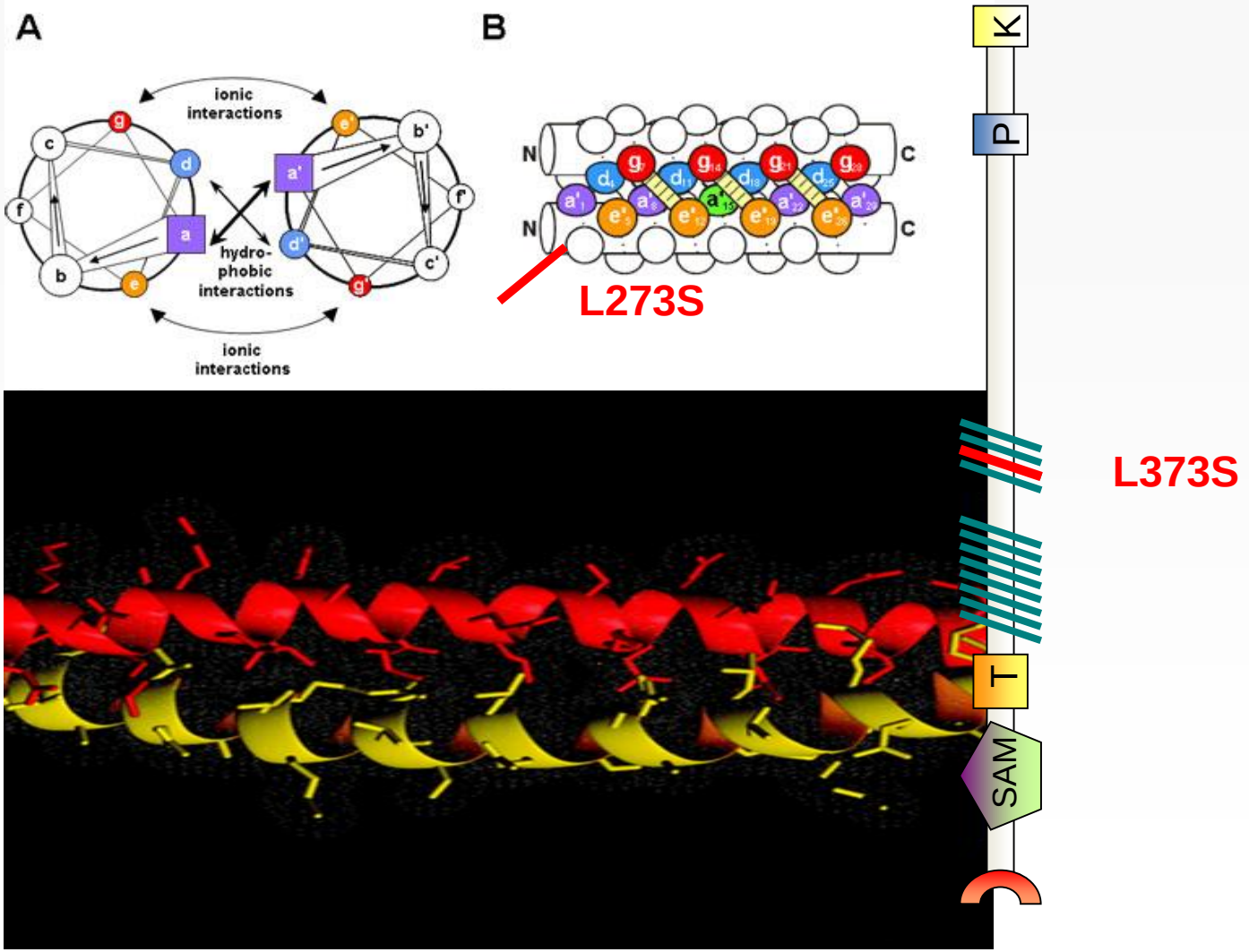
# STIM1 to Orai coupling via coiled-coil domains





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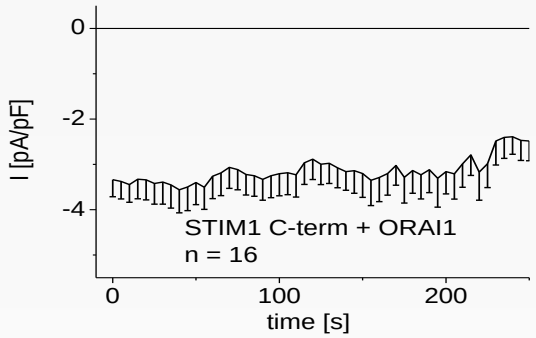
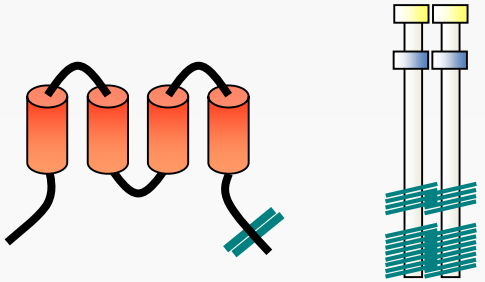
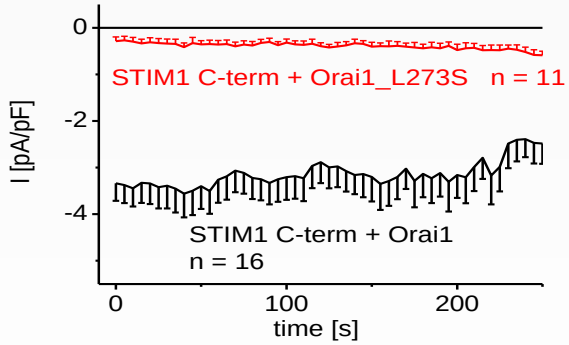
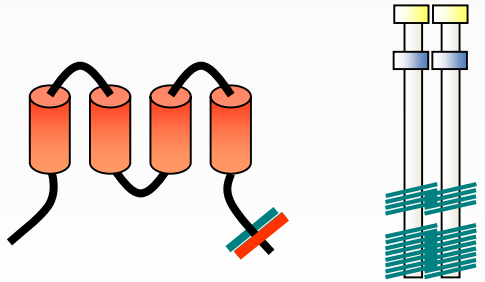
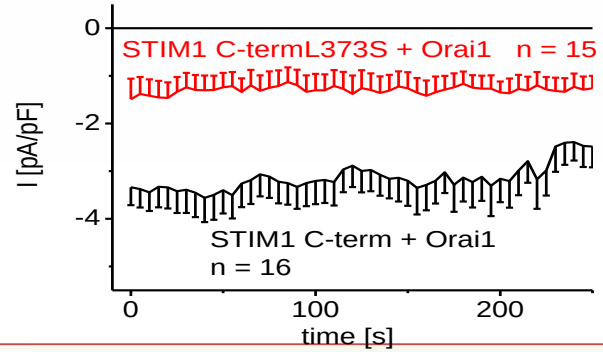
# STIM1 to Orai coupling via coiled-coil domains

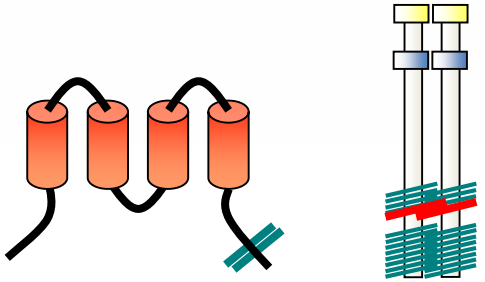




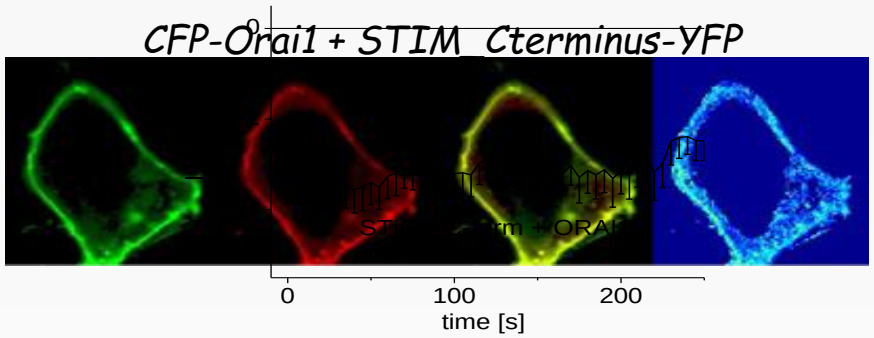



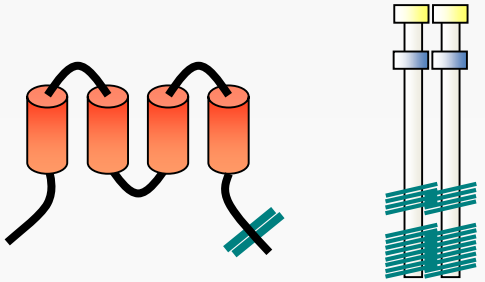


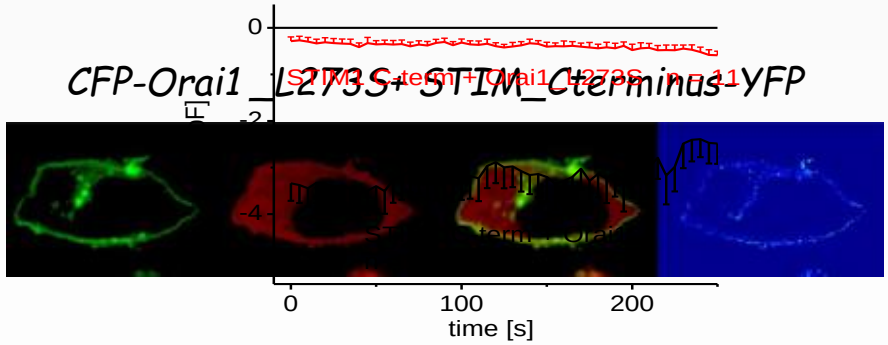



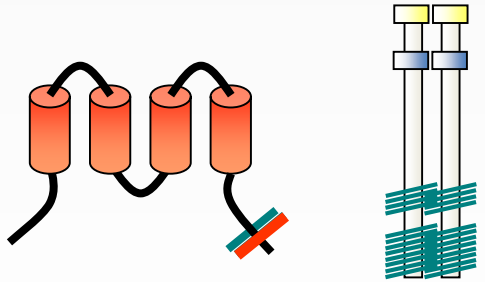
Frischauf et al., JBC 2009

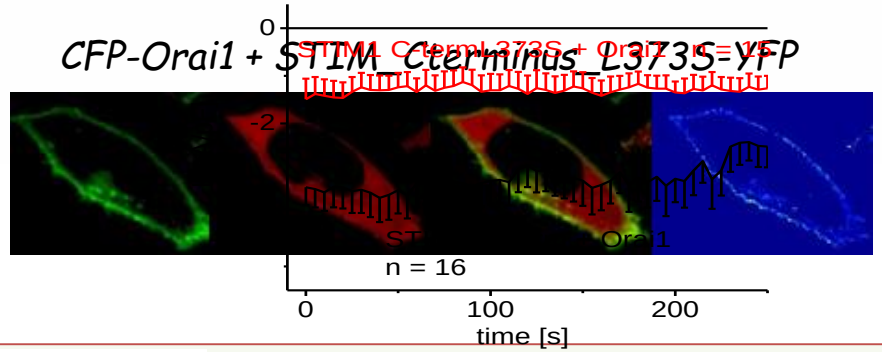
# STIM1 to Orai coupling via coiled-coil domains

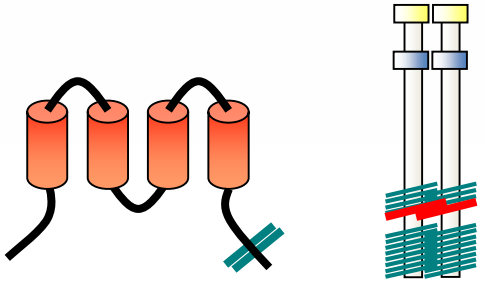




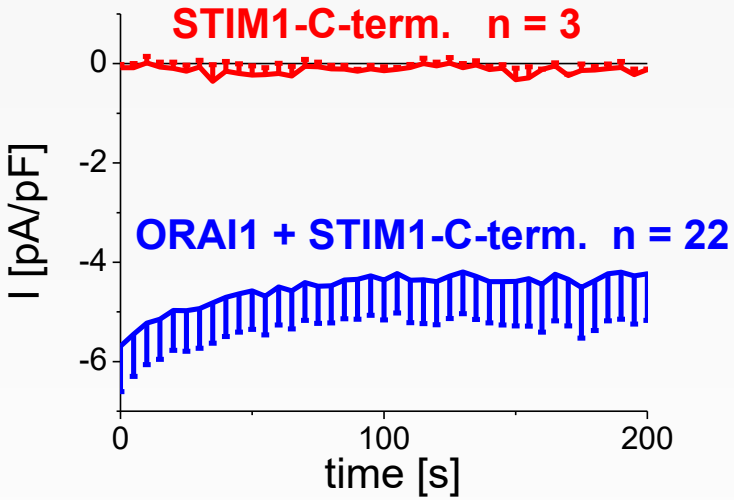



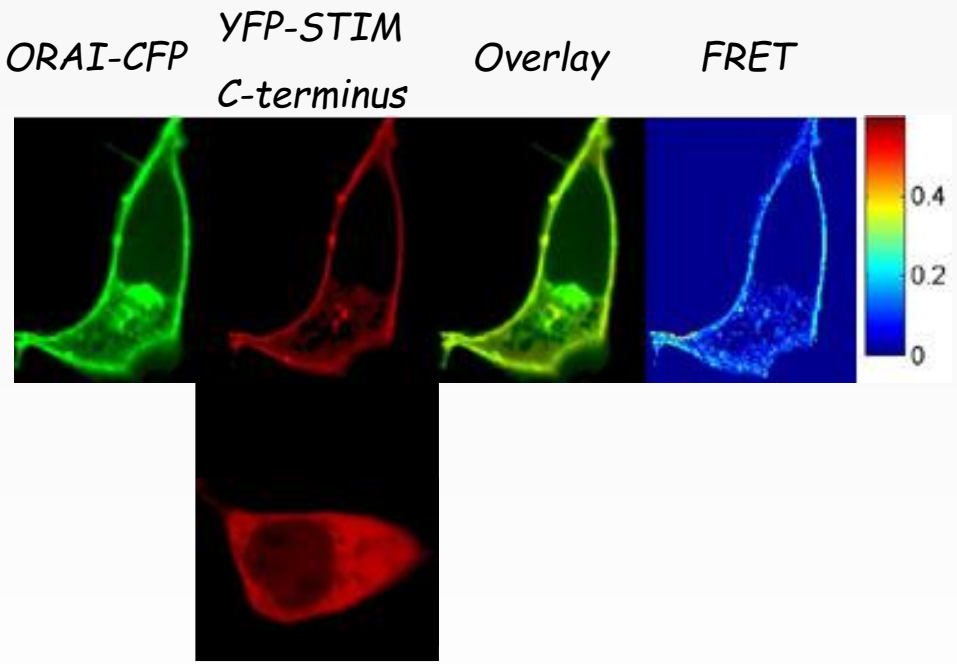


Frischauf et al., JBC 2009

# Regulation of STIM1 C-terminal coupling to Orai



**Which domains within STIM1 C-terminus are required to activate Orai channels?**

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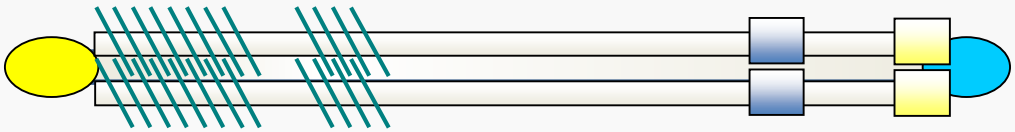
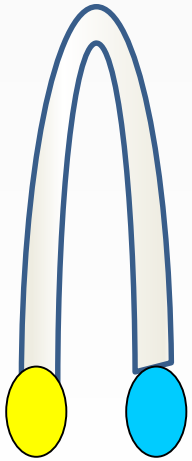
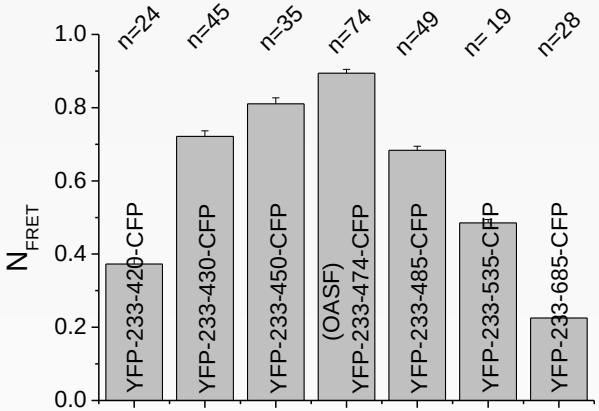
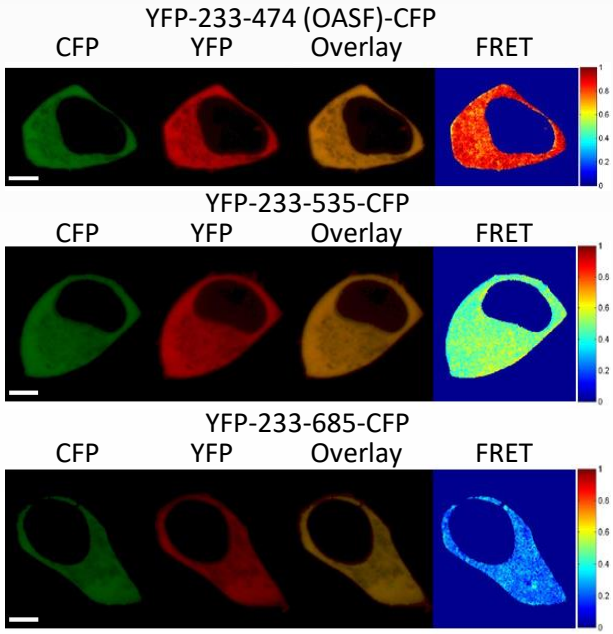
# Conformational rearrangement of STIM1 C-terminus








Muik et al., EMBO J 2011

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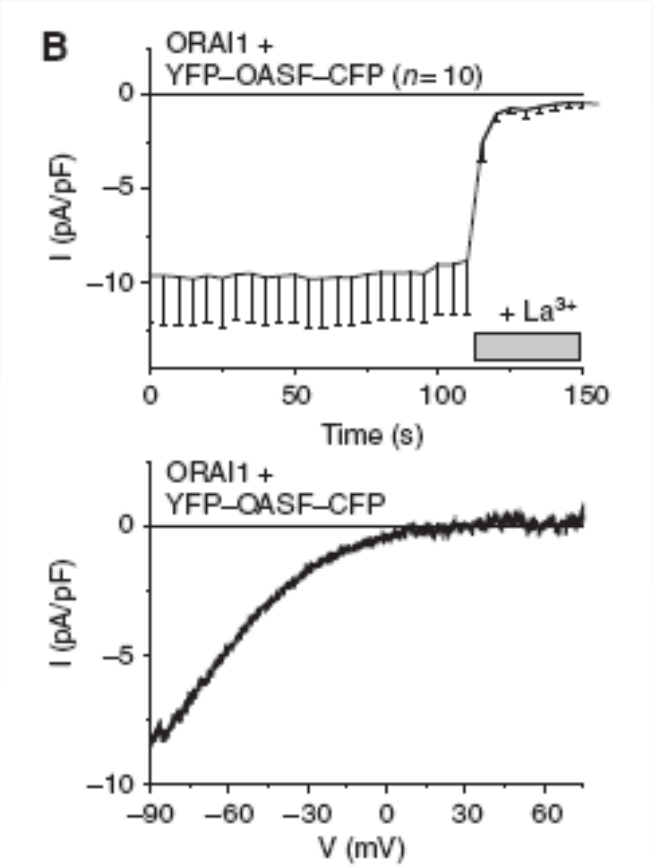
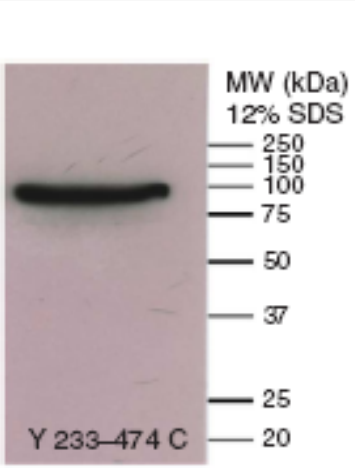
ION CHANNEL GROUP

# Double labeled STIM1 C-terminus is functional




**Double labeled STIM1 C-terminus constitutively activates Orai1 currents**