ONLINE INFORMATION

Mechanisms of STIM1 Activation of Store-Independent LeukotrieneC_4-Regulated Ca^{2+} Channels

By

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LEGENDS TO ONLINE FIGURES

Online Figure 1. Inhibition of 5-lipo-oxygenase has no effects on SOCE

A; Representative Ca^{2+} imaging traces showing thrombin-activated Ca^{2+} entry in VSMCs that were pretreated with either the vehicle control or various drugs targeting AA synthesis and metabolism at the concentrations indicated in the figure panel (**A**; see Figure 1A, green checkmarks and Figure 1D for statistics). **B, C**; Representative Ca^{2+} imaging traces of PDGF-activated SOCE in VSMCs that were pretreated with either the vehicle control or the 5-lipo-oxygenase inhibitor Nordihydroguaiaretic Acid (NDGA) shown to alter thrombin-activated Ca^{2+} entry. Statistics are shown in **C**.

Online Figure 2. Thrombin-activated Ca^{2+} currents do not show depotentiation in DVF bath solutions

A, Zoom-in view of a DVF pulse for CRAC currents activated by BAPTA (black trace) and thrombin-activated current (red trace) showing clear differences in current depotentiation. B; Current depotentiation was measured 10 seconds after maximal DVF current is reached and statistical analyses from 8 independent recordings are shown in **B**.

Online Figure 3. STIM1 requirement in thrombin-activated Ca^{2+} entry is independent of EB1 binding

Representative confocal images of VSMCs transfected with 1.5 µg of either eYFP-STIM1 (**A**; **n=10**) or eYFP-STIM1with a mutation in EB1-binding site (642TRIP→TRNN) (**B**; **n=4**) showing differences in STIM1 protein distribution: tubular staining for STIM1 versus ER staining for EB1 mutant. **C**; Representative Ca^{2+} imaging traces from VSMCs subjected to the *"erase and replace"* approach where endogenous STIM1 is knocked down and replaced by either forms of human STIM1, showing rescue of thrombin-activated Ca^{2+} entry with exogenous expression of either eYFP-STIM1 versions. Statistics on the Ca^{2+} imaging data is shown (**D**).

Online Figure 4. Orai3 co-localizes with STIM1 under basal conditions but does not lead to constitutive current activation

Representative confocal images in HEK293 cells expressing different versions of eYFP-STIM1 and CFP-Orais: STIM1/Orai1 (**A**) and STIM1-A376K/Orai1 (**B**), before (panels **1-3**) and after store depletion (panels **4-6**). Store depletion activates a large inwardly rectifying Ca^{2+} current when Orai1 is co-expressed with STIM1 (**C;** black trace). However, co-expression of Orai1 with STIM1-A376K fails to activate the same Ca^{2+} current (**C**; red trace) and I/V relationships are shown in **D**. Representative confocal images in HEK293 cells expressing STIM1/Orai3 (**E**) and STIM1-A376K/Orai3 (**F**), before (panels **1-3**) and after store depletion (panels **4-6**). Store depletion activates a large Ca^{2+} current when Orai3 is co-expressed with STIM1 (**G;** black trace).

However, co-expression of Orai3 with STIM1-A376K fails to activate Ca^{2+} currents (**G**; red trace); I/V relationships are shown in **H**. Representative confocal images of HEK293 cells expressing STIM1/Orai3-Orai1 tandem (**I**) and STIM1-A376K/Orai3-Orai1 tandem (**J**), before (panels **1-3**) and after store depletion (panels **4-6**). Store depletion activates a Ca^{2+} current when the Orai3-Orai1 tandem is co-expressed with STIM1 (**K;** black trace). Co-expression of Orai3-Orai1 tandem with STIM1-A376K fails to activate Ca^{2+} currents (**K**; red trace). Ca^{2+} I/V relationships are shown in **L**.

LEGENDS TO ONLINE MOVIES

Online Movie 1. STIM1 aggregates into sustained puncta in response to thapsigargin

Confocal time-lapse z-sections of VSMCs transfected with 0.75 µg of YFP-STIM1. The first 5 images are taken before addition of thapsigargin (TG; 4µM). Images were acquired every 30 seconds for a period of 10 minutes. Notice the sustained STIM1 puncta that occurs in response to thapsigargin. The experiment is conducted on a heated chamber in HBSS containing 2mM extracellular Ca^{2+}.

Online Movie 2. STIM1 aggregates into sustained puncta in response to PDGF

Confocal time-lapse images of VSMCs transfected with 0.75 µg of YFP-STIM1. The first 3 images are taken before addition of PDGF (100ng/mL). Images were acquired every minute for a period of 12 minutes. Notice the sustained STIM1 puncta upon PDGF stimulation. The experiment is conducted on a heated chamber in HBSS containing 2mM extracellular Ca^{2+}.

Online Movie 3. STIM1 forms transient puncta in response to thrombin

Confocal time-lapse z-sections of VSMCs transfected with 0.75 µg of YFP-STIM1. The first 5 images are taken before addition of thrombin (100nM). Images were acquired every 20 seconds for a period of 10 minutes. Notice the transient nature of the puncta that occurs in response to thrombin stimulation (maximal within 30 seconds of thrombin addition and lasting less than 60 seconds). The experiment is conducted on a heated chamber in HBSS containing 2mM extracellular Ca^{2+}.

Online Movie 4. STIM1 co-localizes with Orai1 at the PM in response to thapsigargin

Confocal time-lapse images of VSMCs co-transfected with eYFP-STIM1 (0.5µg; green channel) and CFP-Orai1 (3 µg; red channel). The first 5 images are taken before addition of thapsigargin (TG; 2µM). Images were acquired every 20 seconds. Notice that upon thapsigargin addition, STIM1 (green) and Orai1 (red) co-localize in proximity of the PM. STIM1/Orai1 interaction occurred upon thapsigargin addition and lasted for the duration of the experiment.

Online Movie 5. STIM1 and Orai1 do not co-localize upon thrombin stimulation

Confocal time-lapse images of VSMCs co-transfected with eYFP-STIM1 (0.5µg; green channel) and CFP-Orai1 (3µg; red channel). The first 5 images were taken before addition of thrombin (100nM). Images were acquired every 20 seconds. Notice that upon thrombin stimulation, STIM1 (green) and Orai1 (red) do not redistribute or co-localize. Also note that, as expected, the VSMC contracts in response to thrombin stimulation.

Online Movie 6. STIM1 does not co-localize with Orai1 under basal conditions

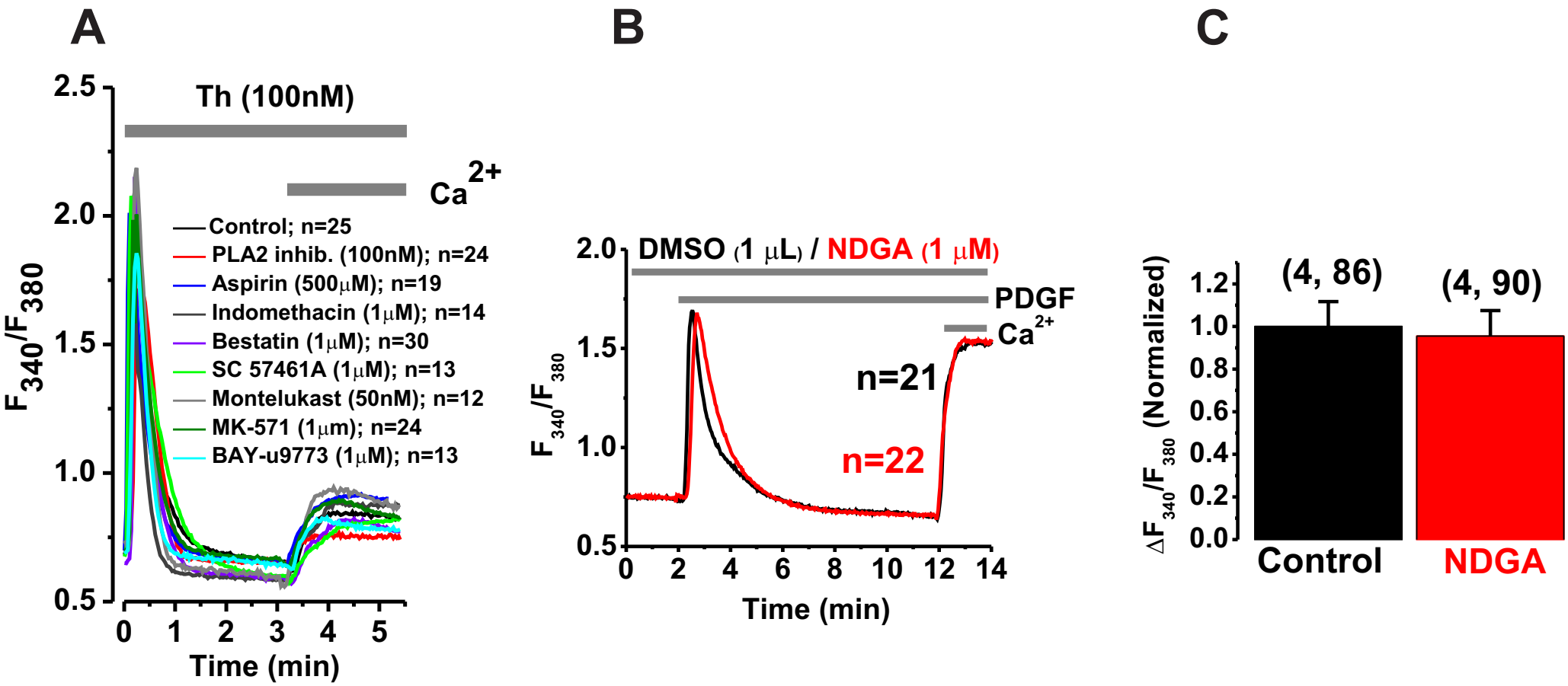
Three dimensional reconstructions of confocal z-stack images of VSMCs expressing eYFP-STIM1 (0.5µg; green channel) and CFP-Orai1 (3µg; red channel) acquired under basal

conditions. The yellow spheres represent the quantification of STIM1/Orai1 three-dimensional surface overlap.

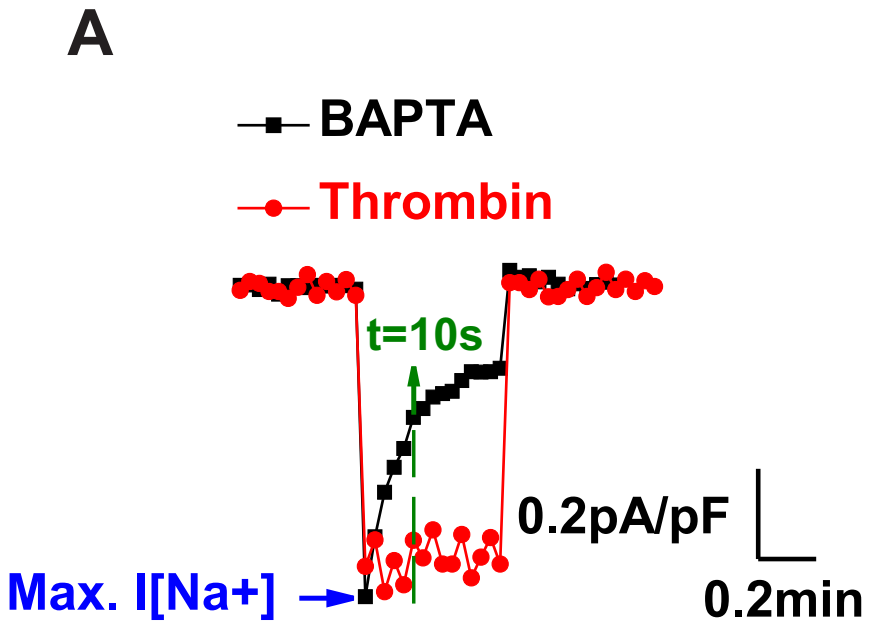
Online Movie 7. STIM1 strongly co-localizes with Orai3 under basal conditions

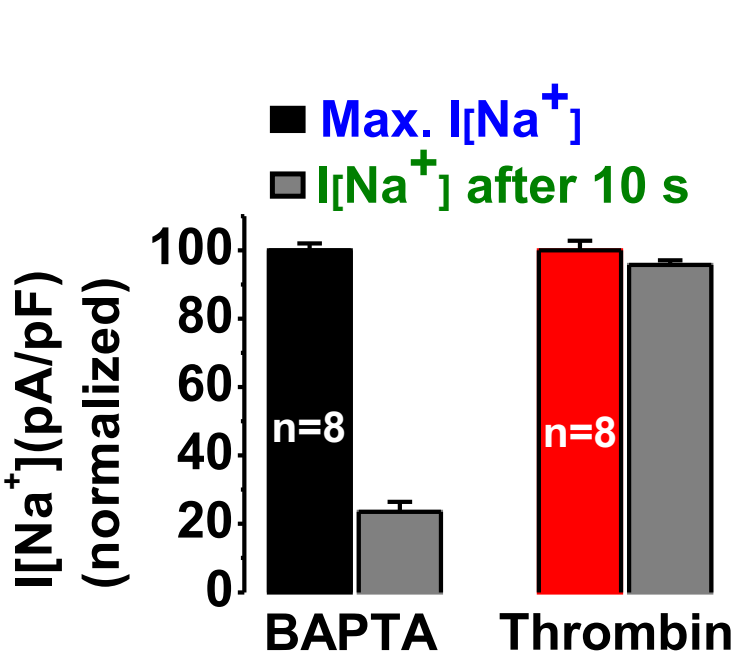
Three dimensional reconstructions of confocal z-stack images of VSMCs expressing eYFP-STIM1 (0.5µg; green channel) and CFP-Orai3 (3µg; red channel) acquired under basal conditions. The yellow spheres represent the quantification of STIM1/Orai3 three-dimensional surface overlap.

Online Figure 1

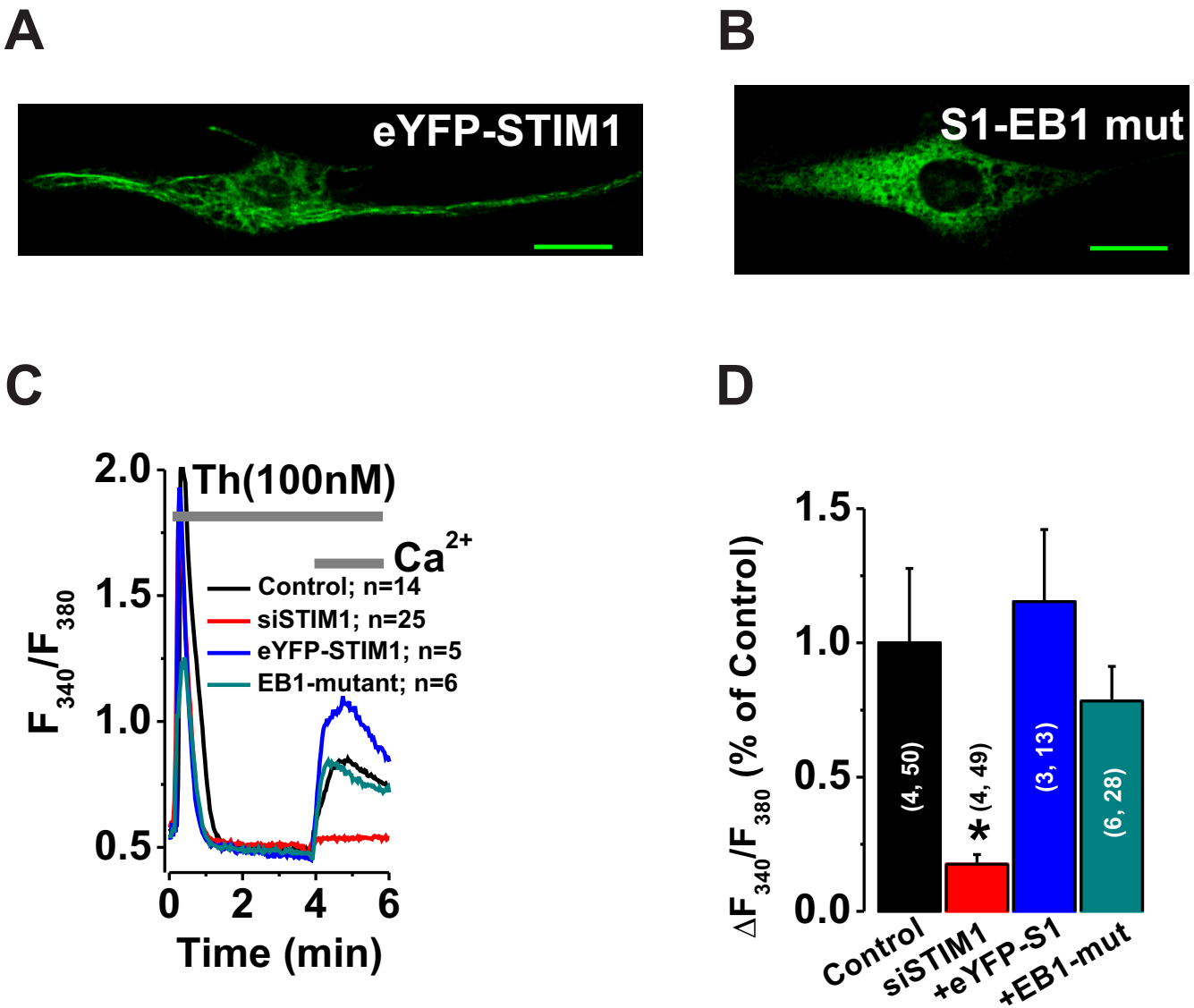


Online Figure 2





Online Figure 3



Online Figure 4