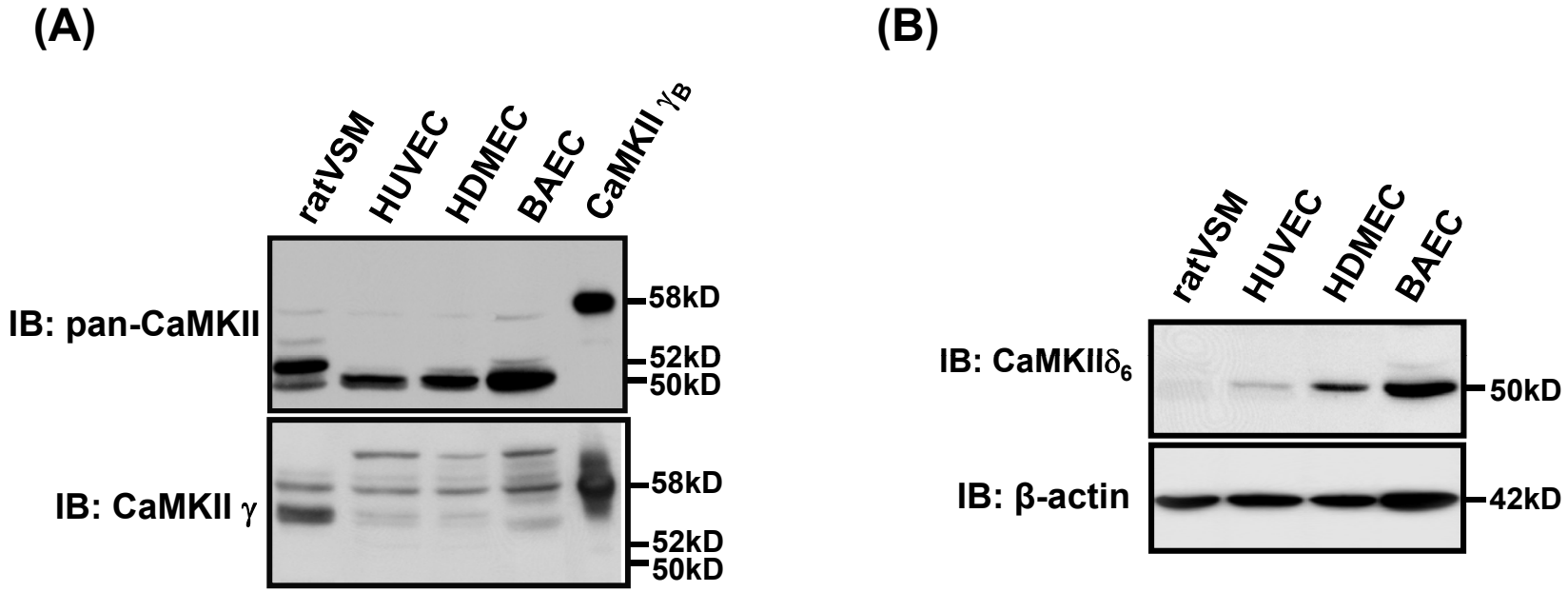
Supplementary data 1: *Western blotting confirms CaMKII δ as the predominant isoform in ECs.* **A.** *Exclusion of CaMKII γ as predominant isoform expressed in ECs.* Confluent endothelial monolayers from human umbilical vein (HUVEC), human dermal microvasculature (HDMEC), bovine aorta (BAEC) or control rat aortic vascular smooth muscle (rat VSM),were lysed and immune-blotted with antibodies recognizing all CaMKII isoforms (IB: pan-CaMKII), or CaMKIIγ only (IB: CaMKIIγ). Same total amount of protein lysates were loaded. Purified $CaMKII\gamma_B$ protein was used as positive control for CaMKIIγ antibody (22). **B.** *Identification of CaMKII δ as predominant endogenous isoform expressed in ECs.* HUVEC, HDMEC, BAEC and rat VSM were lysed and immune-blotted with a custom anti-peptide antibody (or custom CaMKIIδ antibody) supposed to recognize all δ-isoforms (epitope $aa^{465\sim477}$: IHFHRSGSPTVPI based on rat δ_2 AA sequence L13406 or BC107562 in Genebank). This new custom antibody preferred to recognize δ isoform in ECs that is lack of the 21 AA δ unique C-terminus (confirmed by Figure 1B & C), but not recognize the δ_2 isoform in rat VSM containing the 21 AA δ unique C-terminus. β-actin was taken as loading control.

Supplementary data 2: *Thrombin induces HUVEC hyperpermeability at a concentration-dependent manner.* **A.** *Thrombin concentration-dependency of HUVEC permeability.* HUVEC monolayer permeability was measured using Electric Cell-substrate Impedance Sensing (ECIS), as described in Methods. Trans-endothelial electric resistance (TEER) was recorded every 3 min for 4 hr following serum starvation. After approximately 1 hr, thrombin was added to separate wells (indicated by the arrow) at final concentrations of 0, 1, 2.5, 5, 10 and 50 nM. Decreased resistance reflects increases in permeability. Resistance values were normalized to the baseline before thrombin addition, and are plotted as relative resistance units compared to the baseline value set at 1.0. Preliminary data were shown as representative of $n \geq 2$ independent experiments. **B.** *TEER of HUVEC monolayers stimulated by 2.5 nM and 50 nM concentrations of thrombin.* Thrombin was added to separate wells (indicated by the arrow) at final concentrations of 0, 2.5 and 50 nM. Symbol and tickles indicate the mean and SEM of relative resistances from 6 independent experiments, respectively. **C.** Quantification of maximal changes in monolayer resistance in experiments shown in B. Peak % changes are relative to baselines prior to addition of thrombin. Data values are means ± SEM from 6 independent experiments. * $p \leq 0.05$ compared with no thrombin treatment; # $p \leq 0.05$ between different concentrations of thrombin treatment.

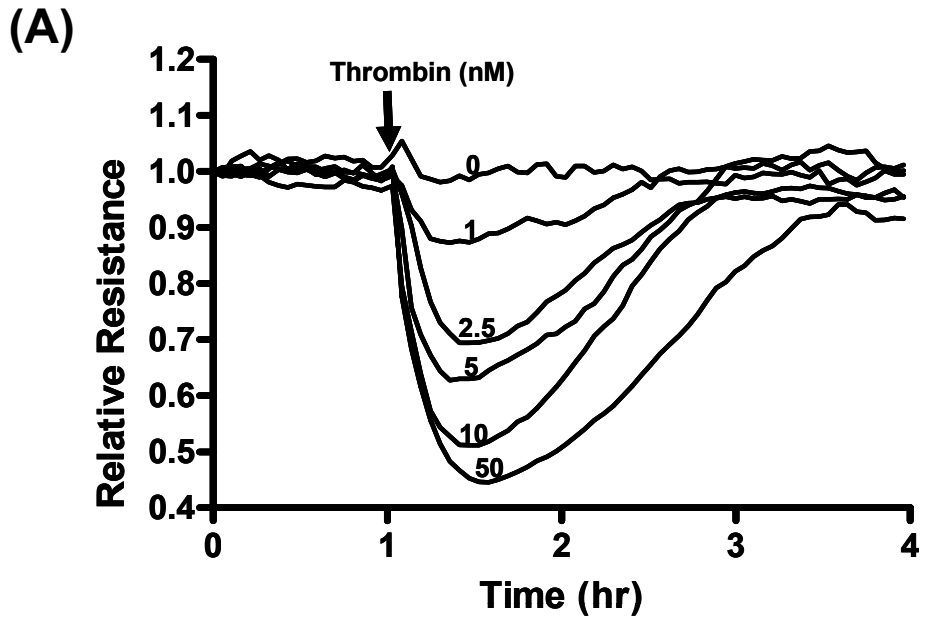
Supplementary data 3: *Thrombin stimulated intracellular Ca^{2+} signals at a concentration-dependent manner in confluent HUVEC monolayer.* Ca^{2+} transients of 2 individual representative cells are shown in **A** and **B** that represent two different cell populations from same monolayer that responded differently upon 2.5 nM thrombin incubation. Ca^{2+} transient from quiescent HUVEC monolayer was not detected (data not shown). 2.5nM thrombin triggered Ca^{2+} oscillations in type **B** cell, but failed to induce Ca^{2+} transient in type **A** cell; while 50nM thrombin induced significant Ca^{2+} rise in both types of cells from same monolayer. **C.** Different concentrations of thrombin induced Ca^{2+} signals in different proportion of cells from one HUVEC monolayer. Data was shown as "# cells with Ca^{2+} response per total # cells analyzed from same monolayer ". Cells from 2 individual monolayers in separate dishes were analyzed and shown in the table. 2.5nM thrombin only induced Ca^{2+} oscillations in 24% and 36% cells from monolayer 1 and 2, respectively; while 50nM thrombin induced Ca^{2+} plateau in essentially all (100% and 94%) cells from both monolayers. n=2

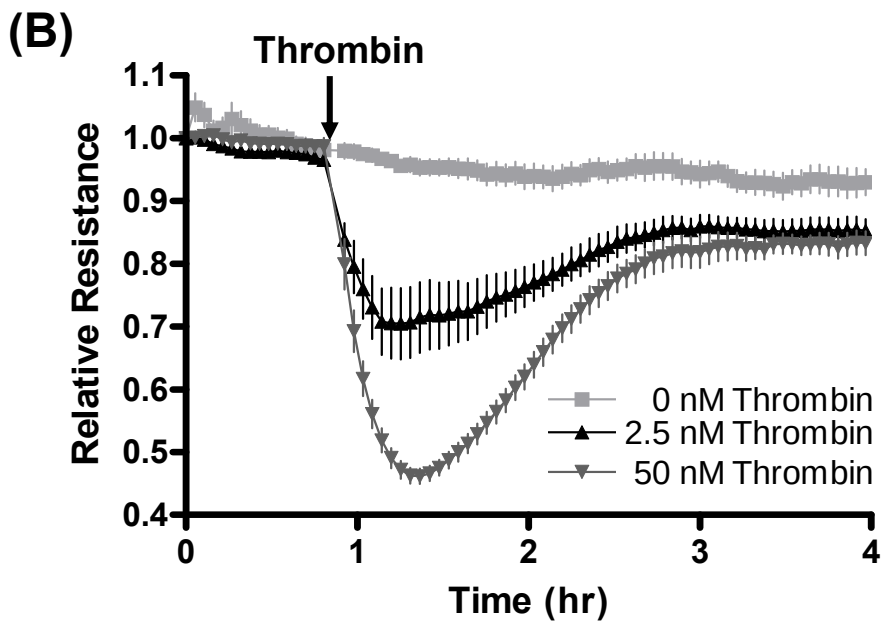
Supplementary data 4: *Rho kinase mediates thrombin-induced HUVEC hyperpermeability.* **A.** Effects of 1 hr pretreatment with the Rho kinase inhibitor Y27632 (0-5 μM) on HUVEC monolayer electric resistance changes in response to 2.5 and 50 nM thrombin. Changes in electric resistance are normalized to resistance values prior to addition of thrombin. Symbols and tickles

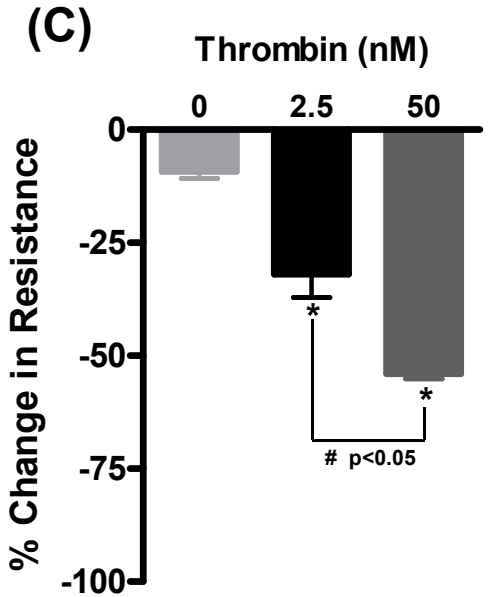
indicate the mean and SEM of relative resistances from 3 independent experiments, respectively. **B.** Quantification of maximal changes in monolayer resistance from experiments shown in A. Peak % changes is relative to baselines prior to addition of thrombin. Data values are means ± SEM from 3 independent experiments. # $p \leq 0.05$ effect of Y27632 addition compared to vehicle (DMSO) control.



Supplementary data 1

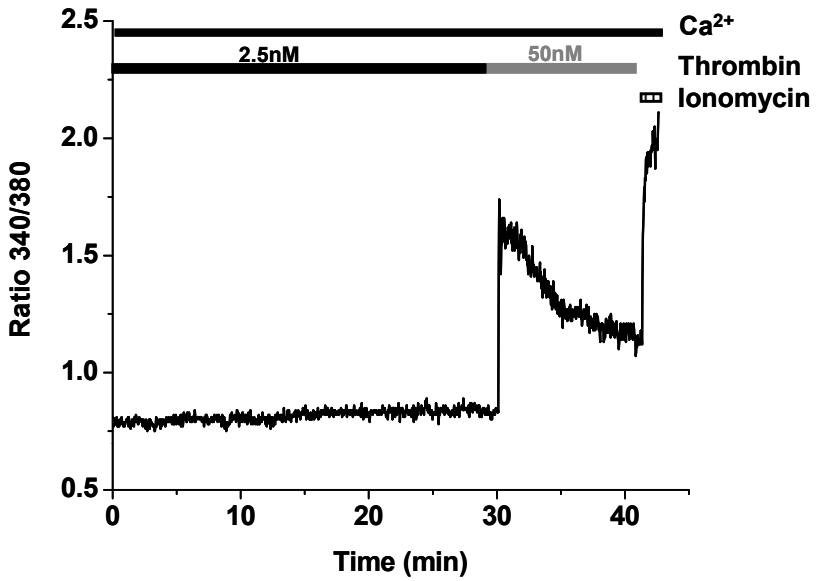






Supplementary data 2

(A)



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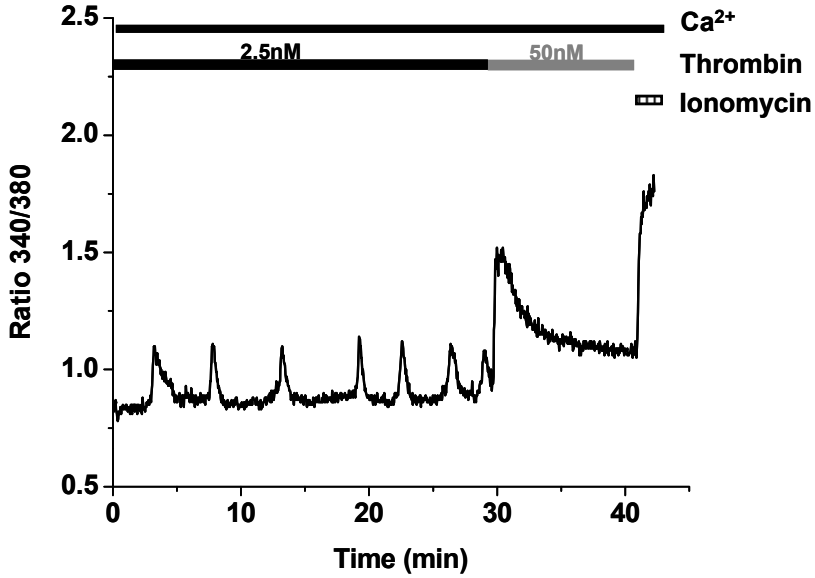
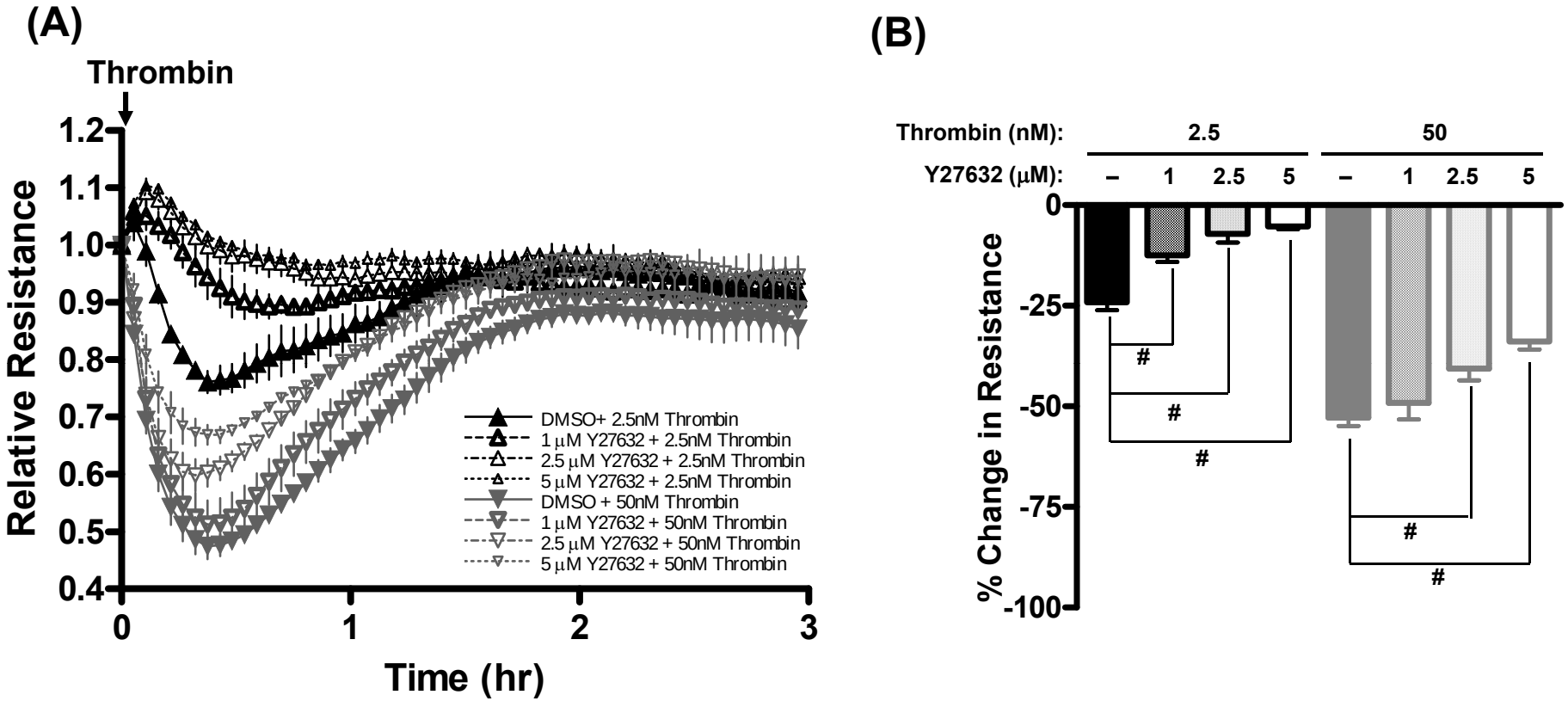


(C)

Thrombin stimulated Ca^{2+} signals in mature HUVEC monolayer
(# cells with responses / total # cells analyzed from indicated monolayer)

Thrombin Concentration	2.5 nM	50 nM
Monolayer 1	9/37	37/37
Monolayer 2	13/36	34/36

Supplementary data 3



Supplementary data 4