Supplementary Material

Protein S-Glutathionylation enhances Ca^{2+}-induced Ca^{2+} release via the IP_3 Receptor in Cultured Aortic Endothelial Cells

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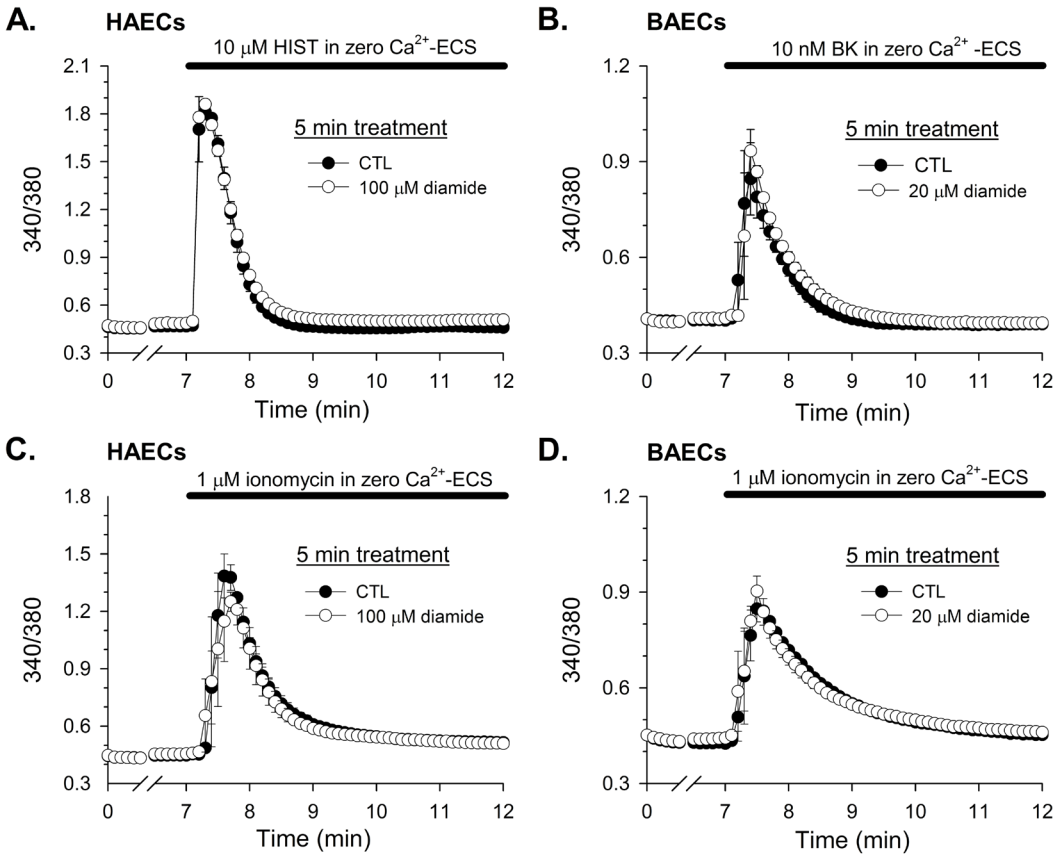

FIGURE S1: Diamide has no effect on the ionomycin- or maximal agonist-releasable Ca^{2+} stores in HAECs and BAECs

Fura-2-loaded HAECs (**A**, **C**) and BAECs (**B**, **D**) bathed in zero Ca^{2+}-ECS were challenged with 100 μM diamide (HAECs) or 20 μM diamide (BAECs) for 5 min immediately prior to stimulation with a maximal conc. of receptor agonist (**A**, **B**) or 1 μM ionomycin (**C**, **D**) for an additional 5 min in the continued presence of diamide. **A-D**) For clarity 4 minutes of the treatment period is omitted by the breaks. Values represent the mean ± SE of 3 experiments for each experimental condition. Where error bars are absent the SE is smaller than the symbol. **A-B**) The total number of cells analyzed for each condition is given in the legends of Figs. 2 and 4. **C-D**) A total of 103 (untreated) and 104 (diamide) HAECs, and 228 (untreated) and 254 (diamide) BAECs were analyzed for each condition.

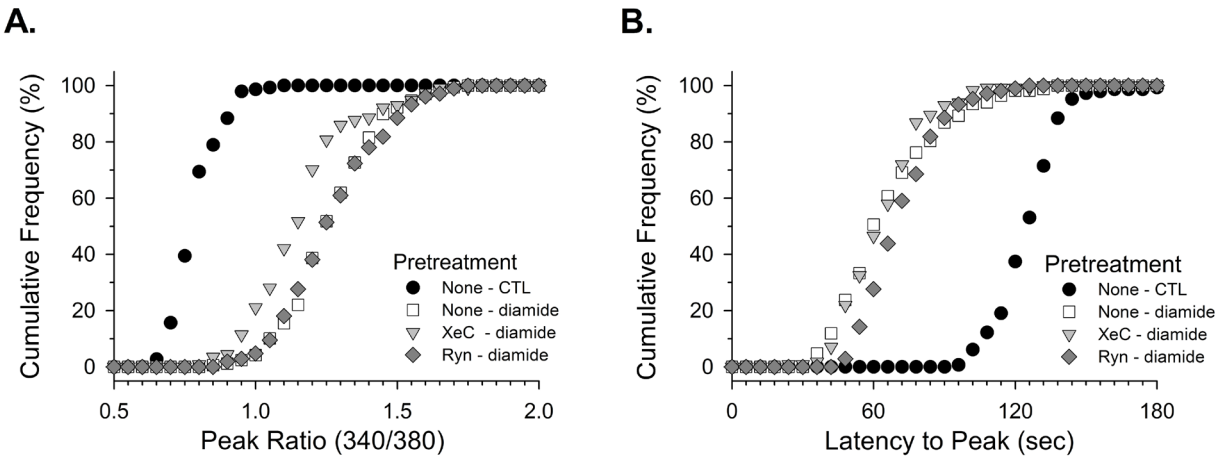

FIGURE S2: Effect of xestospongin C and ryanodine on the diamide-enhanced TG response in HAECs

Cumulative frequency analysis of the *Peak Ratio* (**A**) and *Latency to Peak* (**B**) in response to TG of fura-2-loaded HAECs in zero Ca^{2+}-ECS. The experimental protocol was the same as in Fig 5, except cells were left untreated (*open squares*) or pretreated with either 10 μM xestospongin C (XeC) for 30 min (*light shaded triangles*) or 20 μM ryanodine (Ryn) for 10 min (*dark shaded diamonds*) immediately prior to sequential challenge with 100 μM diamide for 5 min followed by 300 nM TG for an additional 5 min in the continued presence of diamide. Where indicated all recordings were made in the continued presence of the inhibitor. Control cells (*solid circles*) were left untreated and were not challenged with diamide prior to TG exposure. A total of 147 (control), 168 (diamide), 114 (XeC pretreated), 105 (Ryn pretreated) cells were analyzed from 3-4 experiments for each experimental condition. **A-B**) The *Peak Ratio* and *Latency to Peak* of untreated cells or cells pretreated with XeC or Ryn prior to sequential challenge with diamide and TG were significantly different from untreated controls challenged with only TG; $P < 0.001$. The *Peak Ratio* of XeC pretreated cells was significantly different from untreated cells sequentially challenged with diamide and TG; $P < 0.001$.

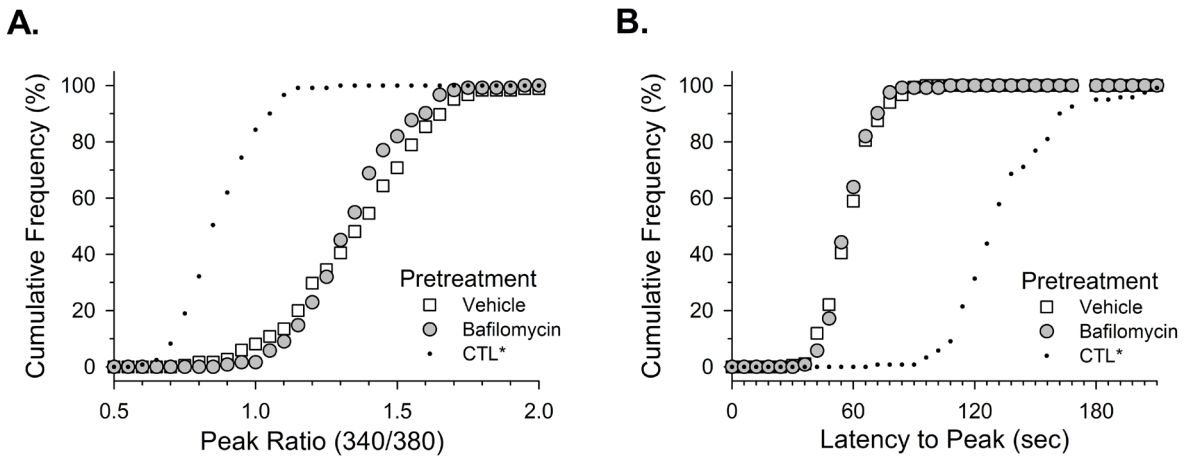

FIGURE S3: Bafilomycin had no effect on the diamide-enhanced TG response in HAECs

Cumulative frequency of the *Peak Ratio* (**A**) and the *Latency to Peak* (**B**) in response to TG of fura-2-loaded HAECs in zero Ca^{2+}-ECS. Experimental protocol was the same as in Fig 5, except HAECs were pretreated with vehicle (*open square*) or 100 nM bafilomycin for 1 hr (*shaded circles*) immediately prior to sequential challenge with 100 μM diamide for 5 min followed by 300 nM TG for an additional 5 min in the continued presence of diamide. **A-B**) For reference, the dotted lines illustrate the cumulative frequency analysis of the TG response in controls cells (data set from Fig 5). A total of 185 (vehicle), and 122 (bafilomycin) cells were analyzed from 3-5 experiments for each condition. The diamide-enhanced *Peak Ratio* and *Latency to Peak* in response to TG of bafilomycin-treated cells was not significantly different from vehicle-treated controls.