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Supplementary Material

Activation of the EGFR/p38 /JNK Pathway by Mitochondrial-Derived Hydrogen Peroxide Contributes To Oxygen-induced Contraction Of Ductus Arteriosus

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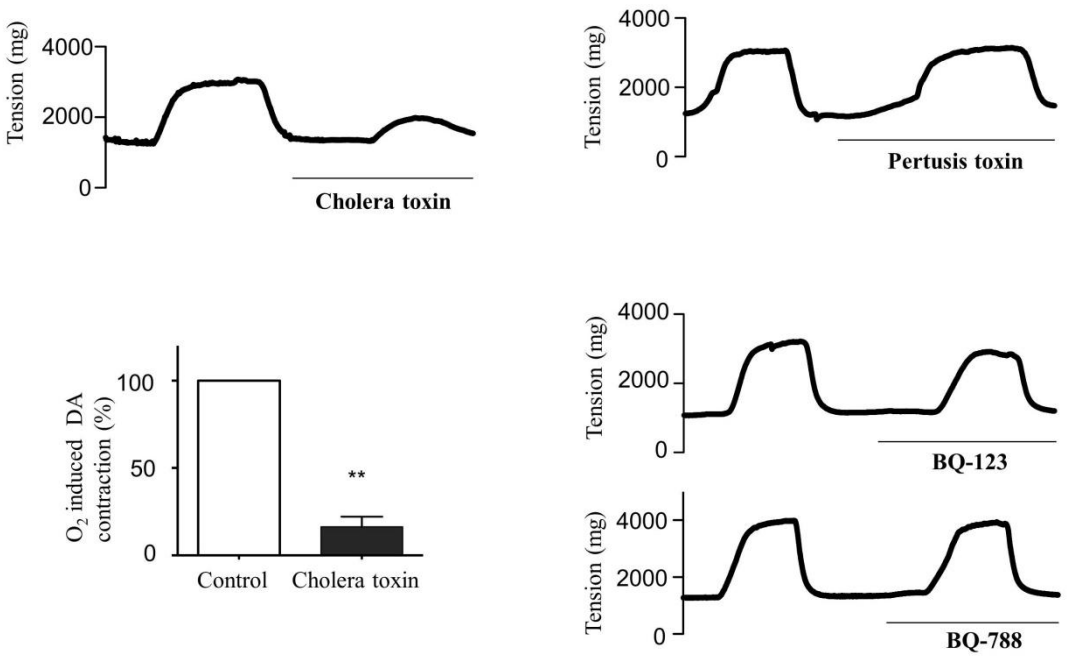
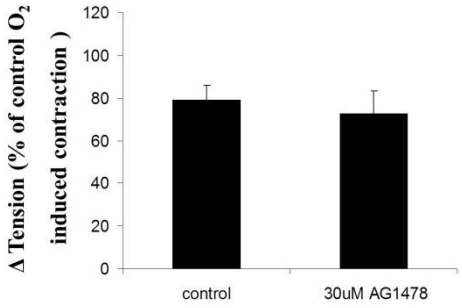
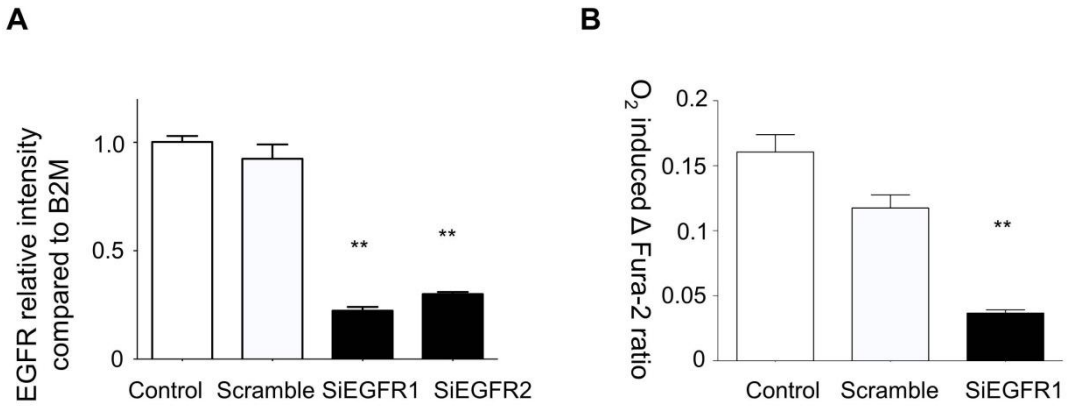
Supplementary figures



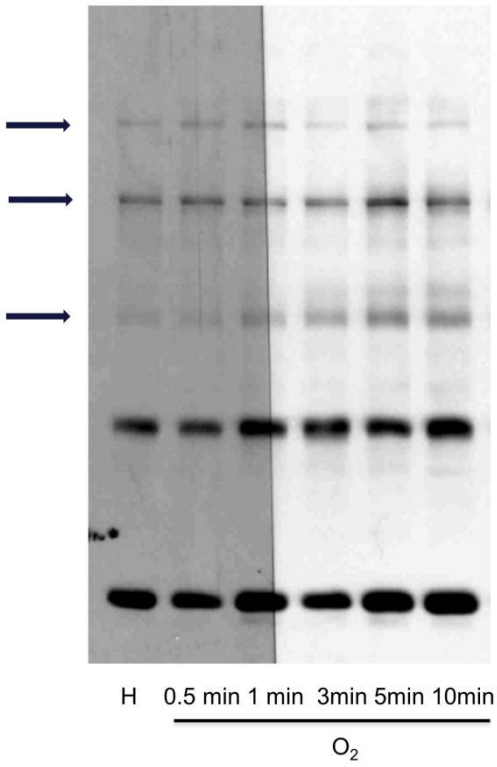
Supplemental figure 1: DA ring studies suggest that G proteins are involved in normoxia-induced DA contraction. Modulators of G proteins such as pertussis toxin (PTX, an inhibitor of Gi) and cholera toxin (CTX, a persistent activator of Gs) were used to detect the possibility of a role for G proteins in DA contraction.
A: shows that CTX (1 mg/ml) can attenuate a normoxic contraction and can subsequently diminish the normoxic response of the DA without affecting the baseline hypoxic tone. **B:** is the summary data for CTX effect on normoxia-induced DA contraction (n=10). This suggests that normoxia-induced DA contraction involves the decrease of Gs activity. **C:** Shows that 20 to 60 minutes PTX pretreatment (1 mg/ml) has no effect on normoxia-induced DA contraction. 3 μM indomethacin and 100 μM l-NAME were present throughout all experiments. These inhibitors of prostaglandin and nitric oxide synthesis, respectively, were used so that a contractile mechanism independent of these mediators could be studied.
D: Representative traces: Both BQ-123 (selective ETA receptor antagonist, 3 μM) and BQ-788 (selective ETB receptor antagonist, 3 μM) minimally reduced O2-induced contraction, consistent with our previous findings.



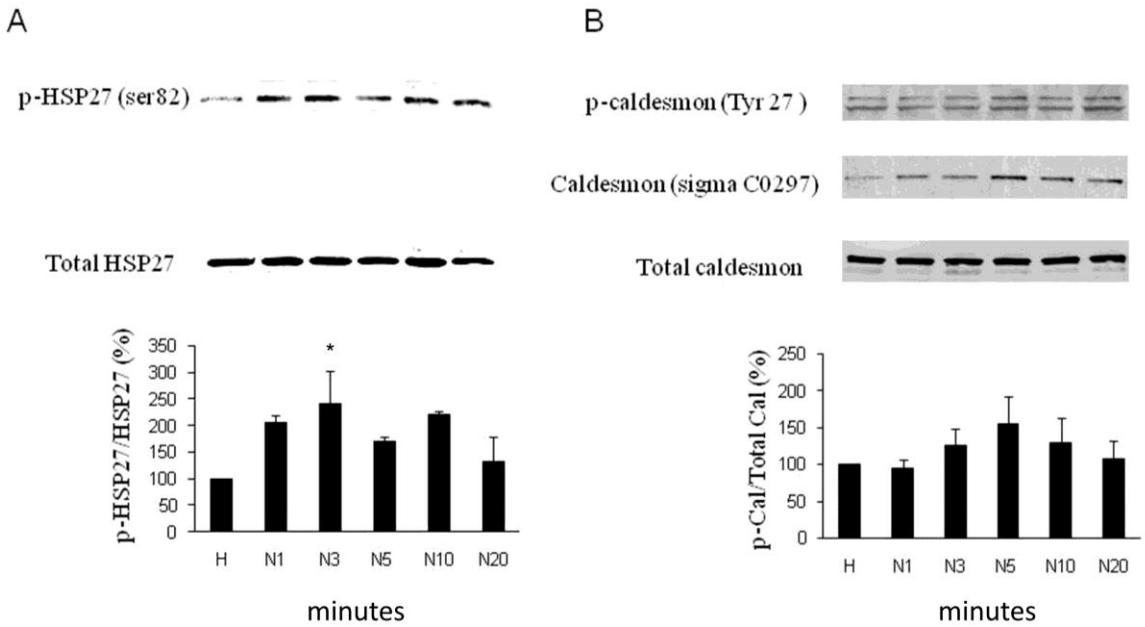
Supplemental fig. 2: The effect of AG1478 on KCl-induced DA contraction (n=8).



Supplemental fig. 3: SiEGFR attenuates the O_2 induced cytosolic calcium change. A: Effect of SiEGFR on EGFR mRNA in cultured human DASMCs (n=4-6). The IDT SiEGER1 was used in the calcium imaging study. ** P<0.01 compared to scrambled siRNA. B: SiEGFR attenuates the O_2 induced cytosolic calcium change (fura-2 ratio) compared to scrambled siRNA. N=51-87, ** P<0.01 compared to Scrambled siRNA.



Supplemental fig. 4: PTP activity is decreased after 5 to 10 min O_2 exposure in cultured human DASMCs by a modified cysteinyl-labeling assay from Tonks NK group.



Supplemental fig. 5: O_2 caused HSP27 and caldesmon phosphorylation in human DASMCs (n=3).