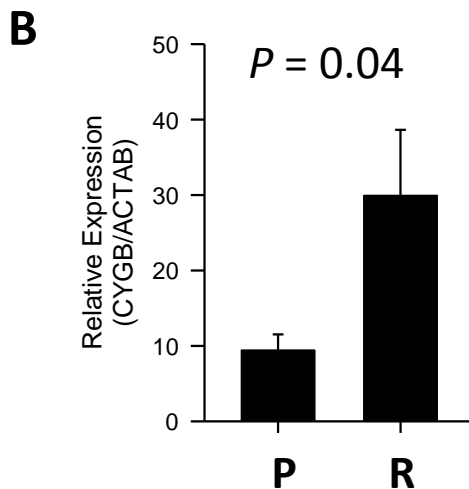
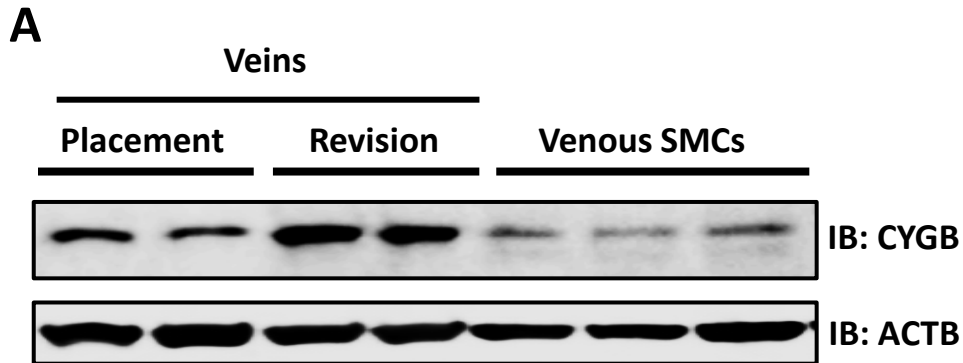
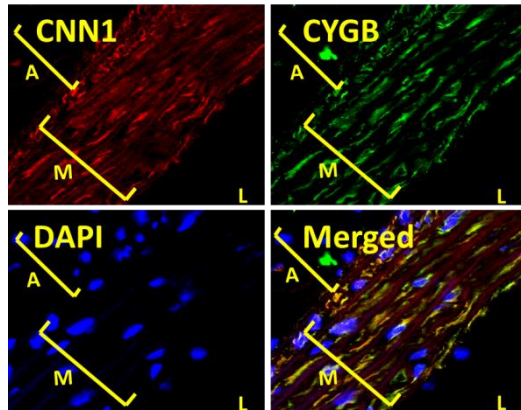


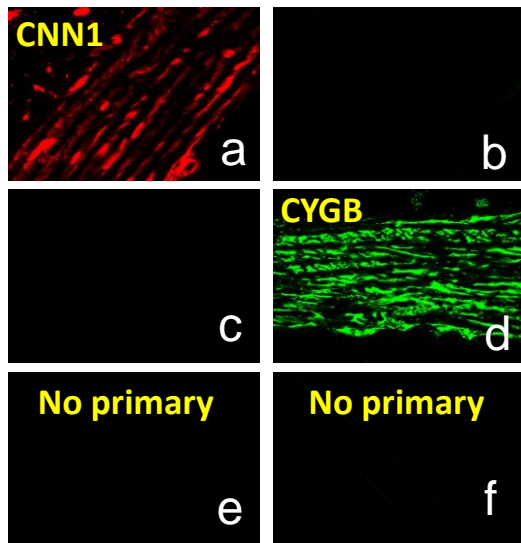
Online-only figure supplement



Supplemental Figure I. Human veins and human venous smooth muscle cells express cytoglobin. **A**, Western blot of representative human veins derived from vessels trimmings obtained from patients undergoing arteriovenous fistula (AVF) placement (P) or at revision (R) of failed AVFs. The WB also included lysates derived from three different dispersions of VSM cells derived from placement veins. **B**, Densitometry analysis of results shown in A for placement and revision vessels. Values represent the mean \pm SEM, n=7.

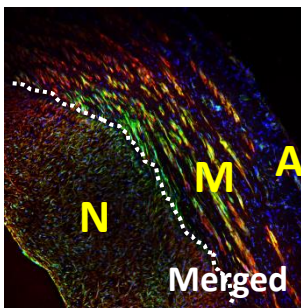
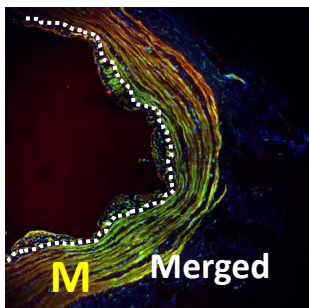
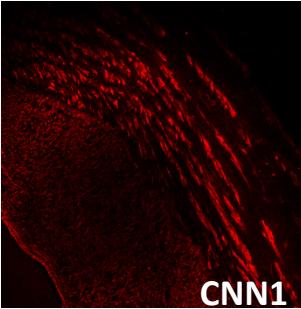
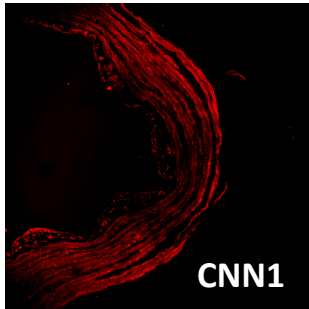
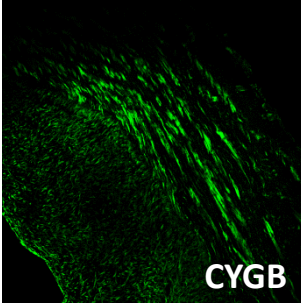
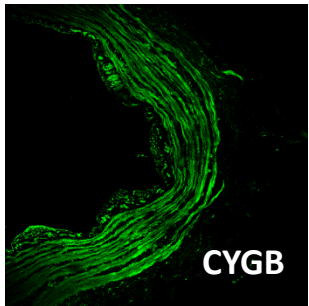
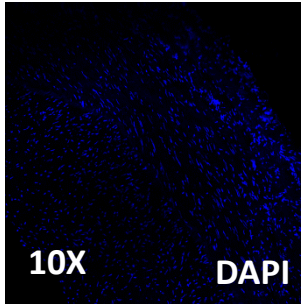
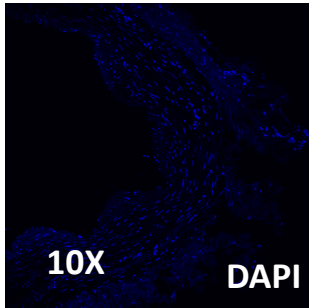
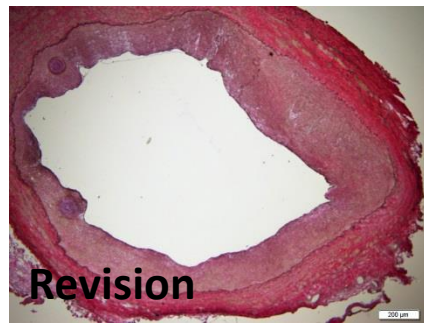


Rat Aorta

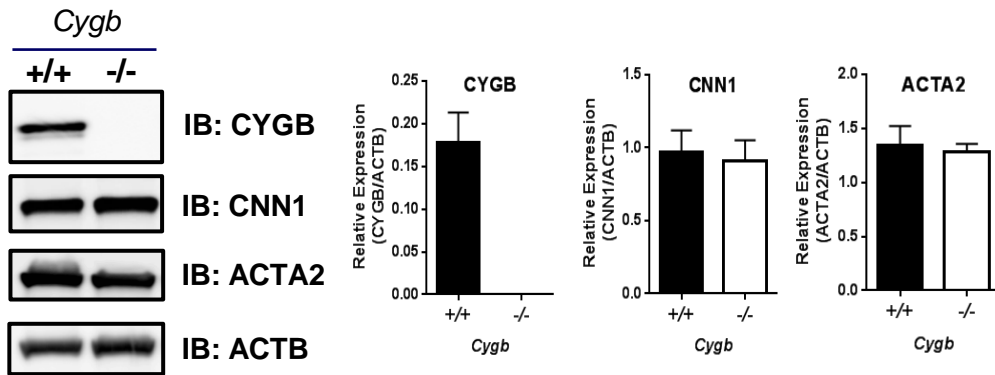


**Negative Controls
(Rat Aorta)**

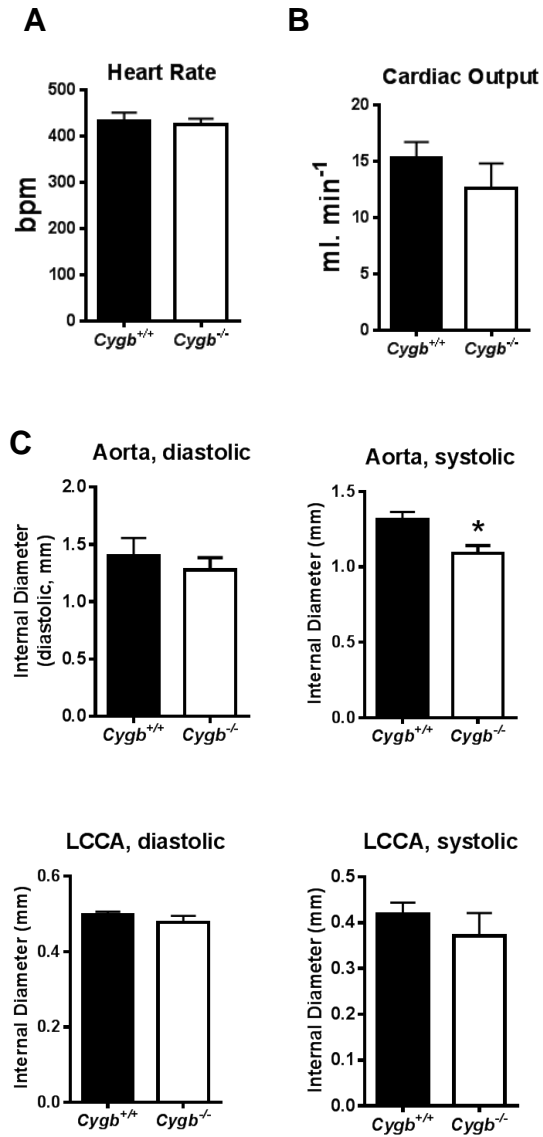
Supplemental Figure II. Cytoglobin is expressed in medial rat aortic VSM cells. Double immunofluorescence studies of rat aortas. We used antibodies against CYGB (green) and the SMC contractile marker calponin (CNN1). Blue staining is DAPI. A = adventitia, M = media, L = lumen; representative of at least three independent experiments. Negative controls were obtained with single immunostaining for either CNN1 or CYGB alone and detection in both red and green channels (a, b, c, and d) or in the presence of secondary antibodies alone (e and f).



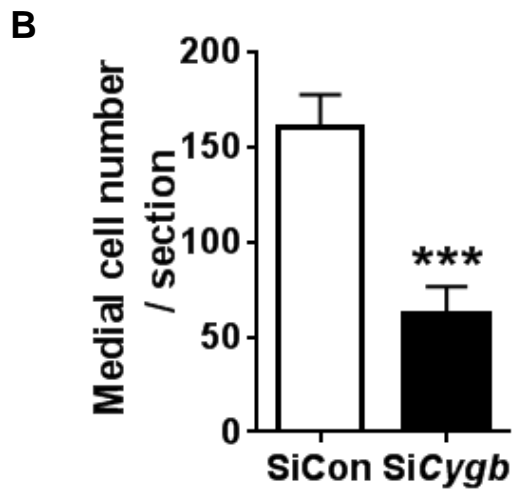
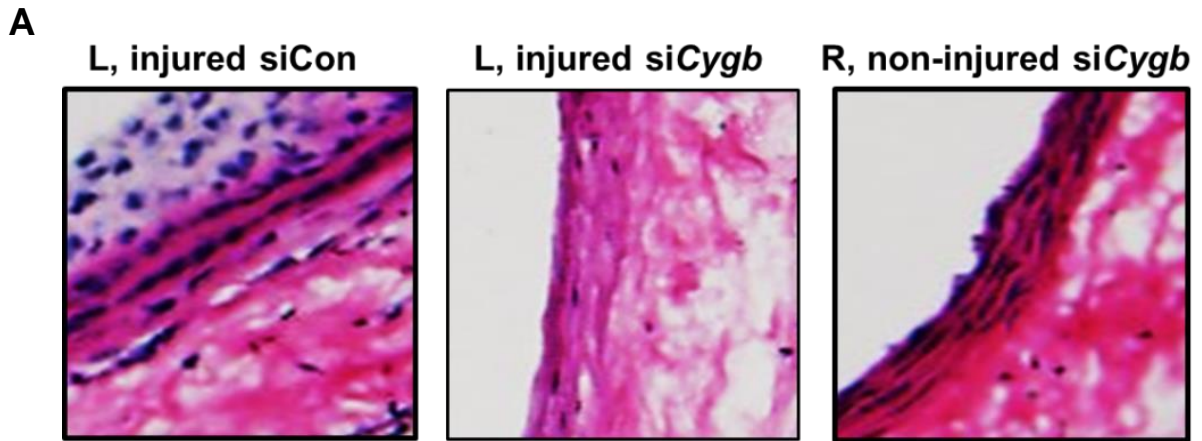
Supplemental Figure III. Cytoglobin is expressed in medial human VSM cells. Representative Double immunofluorescence studies of human veins obtained from patients undergoing arteriovenous fistula placement or revision. We used antibodies against CYGB (green) and the SMC contractile marker calponin (CNN1). Blue staining is DAPI. A = adventitia, M = media, N = neointima, and L = lumen. Panels on top represent H&E staining of placement and revision vessels. Pictures are representative of 4 placement and 4 revision vessels obtained from different patients.



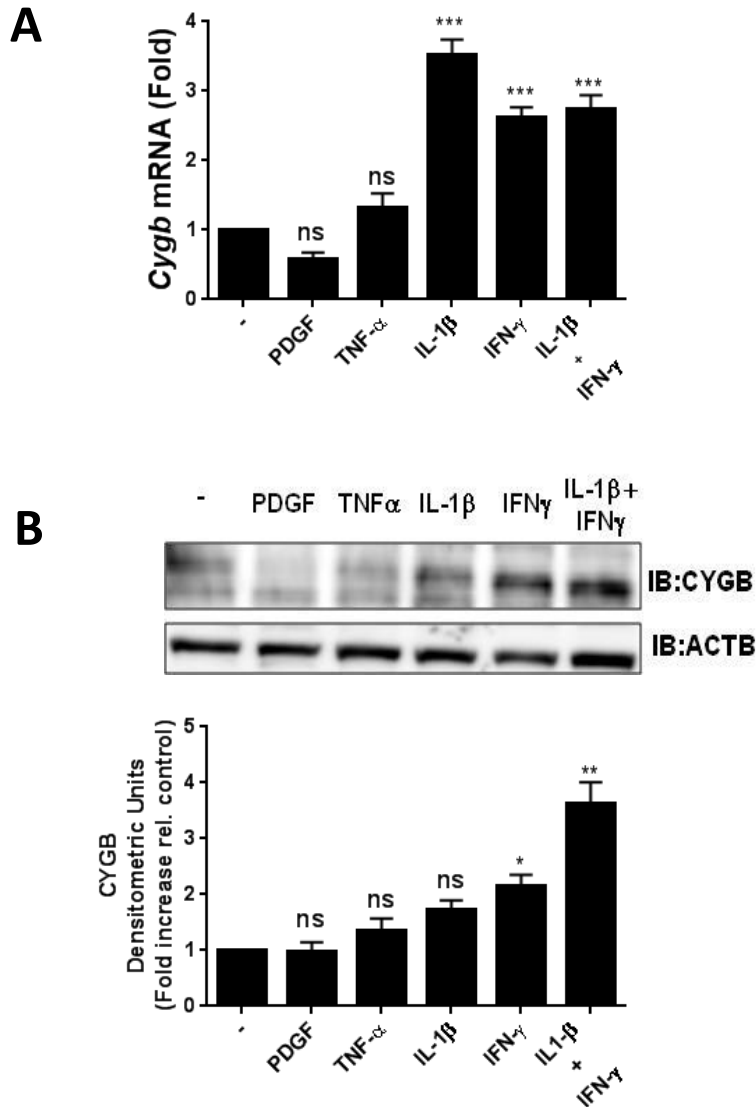
Supplemental Figure IV. Characterization of *Cygb*^{+/+} and *Cygb*^{-/-} mice. WB analysis from mouse aortas from *Cygb*^{+/+} and *Cygb*^{-/-} littermates. Primary antibodies against CYGB, CNN1, and ACTA2 were used. Right panels, densitometry analysis of results shown in left panel. Mean ± SEM (n=4).



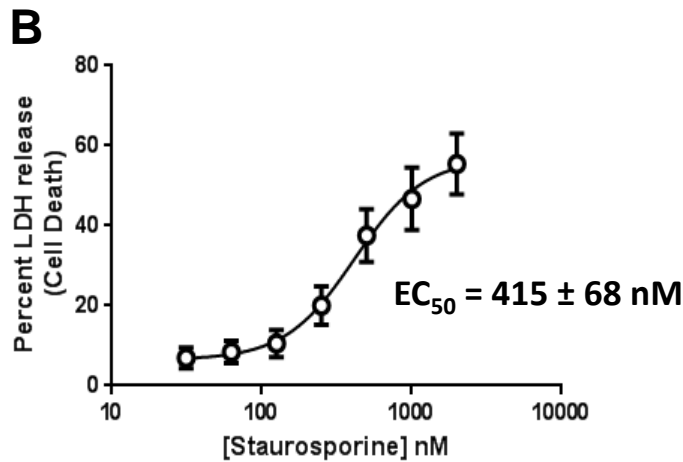
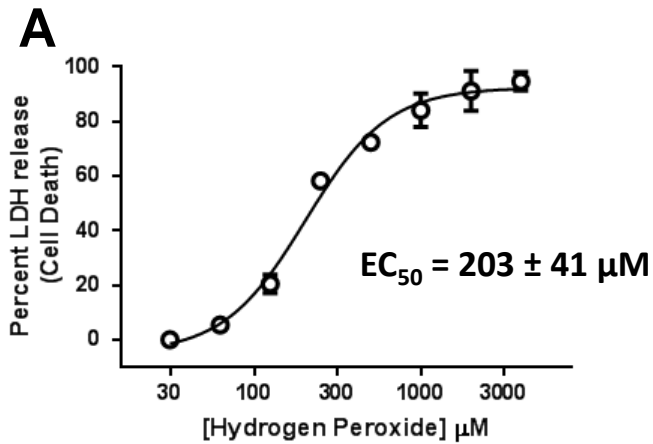
Supplemental Figure V. High resolution ultrasound characterization of aortas and carotids from *Cygb*^{+/+} and *Cygb*^{-/-} mice. Summary for heart rate (A), cardiac output (B), and aorta and LCCA internal diameter (C). Results are expressed as mean \pm SEM (n = 5-6 mice/group). *p<0.05 compared to *Cygb*^{+/+}.



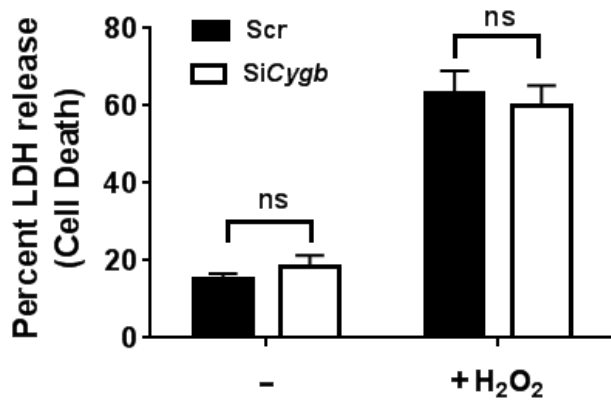
Supplemental Figure VI. Silencing of CYGB reduces medial cell count, 7 days after balloon carotid injury in the rat. B, medial cell nuclear enumeration 7 days after balloon-injury; values shown represent the mean \pm SEM (n = 6).



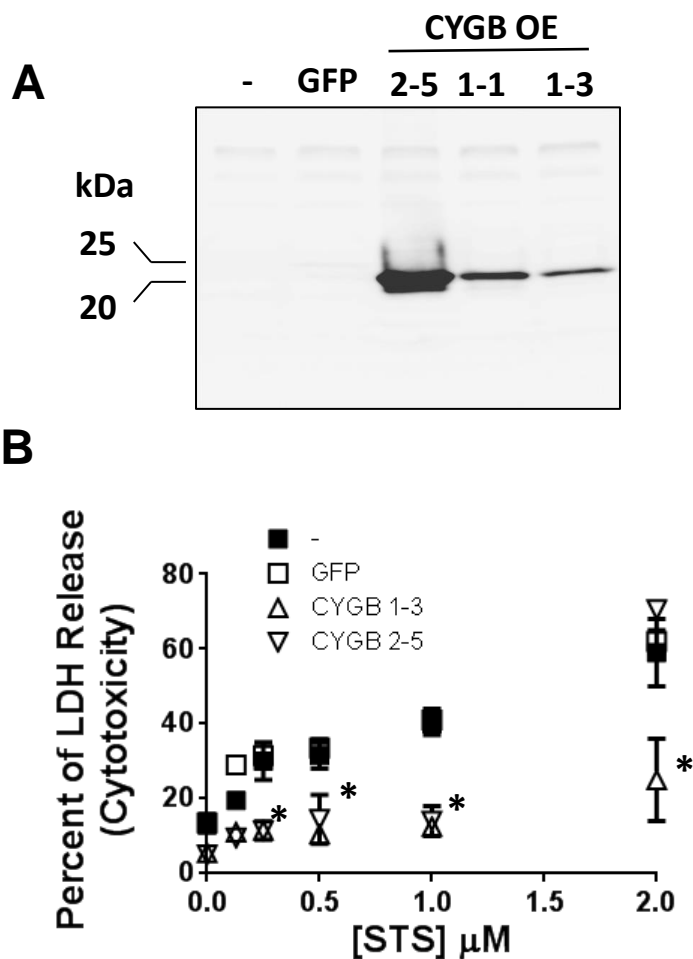
Supplemental Figure VI. Expression of CYGB with cytokines in RAVSMs. **A)** qRT-PCR results showing mRNA levels of CYGB in sub-cultured rat aortic vascular smooth muscle (RAVSM) cells. The cells were stimulated for 48 hours with the indicated cytokine. Mean \pm SEM, $n \geq 3$. **B)** WB results showing protein levels of CYGB in sub-cultured RAVSM using the same conditions described in (A). Densitometric analysis are shown below representative blots. Mean \pm SEM, $n \geq 5$; for (A) and (B) * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$, as determined by single or paired sample t-test with Bonferroni correction.



Supplemental Figure VII. Cytotoxicity dose response of Human Aortic Vascular Smooth Muscle (HAoVSM) cells to hydrogen peroxide (Panel A) and staurosporine (Panel B). LDH release was determined 24 hours after exposure to the cytotoxic agent. Results are presented as Mean \pm SEM, n = 4. Solid lines represent non-linear regression fitted to saturation curves.



Supplemental Figure VIII. Loss of CYGB does not change the sensitivity of HAoVSM to 250 μ M hydrogen peroxide (H_2O_2). LDH release was determined 24 hours after exposure to the cytotoxic agent. Results are presented as Mean \pm SEM, n = 4.



Supplemental Figure IX. Overexpression of CYGB is sufficient to inhibit staurosporine mediated cell death. **A)** Protein expression of CYGB in HEK293 cells stably expressing empty vector (-), GFP or CYGB (CYGB OE). The numbers for each lane specify different clones expressing different levels of CYGB. Equal amounts of protein were loaded. Representative of 3 experiments. **B)** Cytotoxicity was determined by measuring LDH release. Cells were incubated with staurosporine (STS) for 24 hours. Each value represents the mean \pm SEM, $n = 4$. * $P < 0.05$ compared to empty vectors (closed squares). There was no difference between empty vector (-) and GFP cells (GFP).