Supplemental Methods

T cell isolation and staining: Negative immunofluorescence controls were performed on cells that do not express BK channels. Whole blood was fixed in 16% formaldehyde and centrifuged. The supernatant was removed and the sample was re-suspended in 50% MeOH in PBS. Small aliquots of the sample were then dried to slides, permeabilized, and blocked in donkey serum. Samples were then treated with anti-CD3 (1:100); a T-cell marker, BKα (1:100) or BKβ1 (1:100). Primary antibodies were detected with fluorescently labeled secondary antibodies and nuclei were labeled with Hoechst 33342. All images were collected on confocal microscope on a 63x oil immersion objective.

Electron microscopy of rat aortic endothelial cells: To validate that the methyl-β-cycodextrin (MBCD) concentration (100 μM) chosen to mildly deplete membrane cholesterol did not drastically impact caveolar structure, electron microscopic images were obtained in control tissue and tissue subjected to cholesterol chelation. Aortic sections were fixed for 2 hours at room temperature in 2% formaldehyde + 3% glutaraldehyde in 0.1 M Na cacodylate, pH 7.4. Tissues were washed in 0.1 M cacodylate and treated for 1 hour at room temperature in 1% osmium-potassium ferrocyanide in 0.1 M cacodylate, followed by washes in 0.1M cacodylate and then in Barnstead H_2O. Tissues were then treated *en bloc* with 1% uranyl acetate (aq), for 1 hour at room temperature and washed with Barnstead H_2O, dehydrated through a graded series of ethanol to 100%, infiltrated and embedded in Epon-Araldite resin (cured at 65ºC). Sections were cut to 70-90 nm thick, mounted on Cu grids, stained with uranyl acetate and lead citrate, and examined on a Hitachi H7500 TEM at 80kV. Digital images were taken on a CCD camera (AMT XR60).

Effect of Nitric Oxide on BK_{Ca} Channel Activity in Isolated Endothelial Cells. As further confirmation of the activity of endothelial BK channels following CH, whole cell patch clamp experiments were performed in the presence and absence of a donor of nitric oxide (NO), a known activator of the channel (1). After cell dialysis, recordings were taken before and after 5 min of superfusion with the NO donor S-nitroso-N-acetyl-D,L-penicillamine (SNAP) (100 μM) in aortic endothelial cells from control and CH rats.

Supplemental Results

Validation of Specificity of BK Channel Immunofluorescence. Additional experiments validated the specificity of immunofluorescence for BKα and BKβ1. Supplemental Figure 1a shows the lack of staining in the presence of only the secondary (no primary) antibody in gracilis arterioles. Furthermore, blocking peptides for each primary antibody prevented staining (Supplemental Figure 1 b,c). In addition, the endothelial cell localization of BKα was confirmed in sections of gracilis arterioles stained for both BKα and the endothelial cell specific marker PECAM-1 (Supplemental Figure 2) as described in the Methods section of the paper. As a final determination of specificity of the BKα and BKβ1 primary antibodies, we examined staining in isolated rat T-cells that do not express the channel. Supplemental Figure 3 shows the lack of BKα or BKβ1 immunofluorescence in these cells, whereas they stain positive for the T-cell marker CD3. The same secondary antibody was used for all images.

Lack of Effect of MBCD on Gross Structure of Caveolae. A dose of MBCD (100 μM) was chosen that unmasked endothelial BK channels activity in control cells, but was much lower than the 10 mM concentration used by others in vascular preparations (2; 3). Following administration of this lower dose of MBCD, no apparent differences were

47 detectable in caveolae number or structure from untreated controls. CH treatment
48 additionally did not result in an apparent reduction in caveolae number or structure
49 (Supplemental Figure 4). However, 10 mM MBCD eliminated EC caveolae
50 (Supplemental Figure 4).

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52 Administration of the NO donor SNAP elicited a further increase in outward current in
53 aortic endothelial cells from CH but not control rats (Supplemental Figure 5). NO has
54 been identified as an activator of BK channels (1) and thus this observation confirms their
55 presence in endothelial cells from CH rats. Our other data showing that MBCD treatment
56 unmasks channel activity in cells from control animals suggests that the channels are
57 normally inhibited by likely association with caveolin-1 (4) that is inhibition is removed
58 following CH, thus permitting activation by NO.
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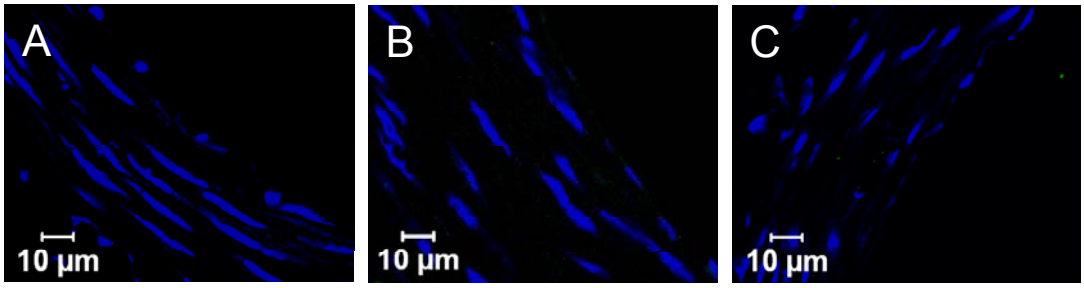
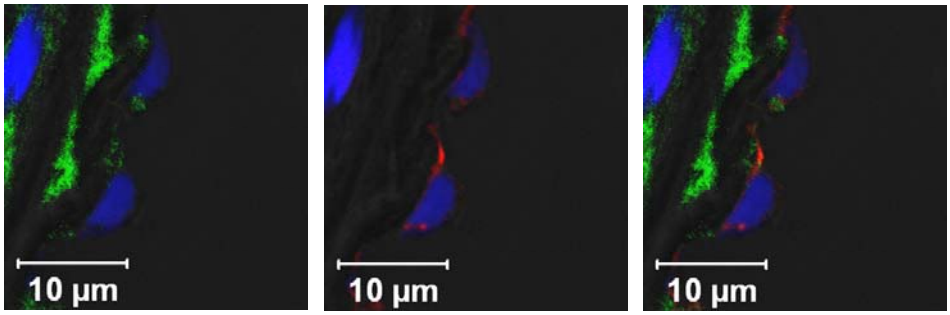
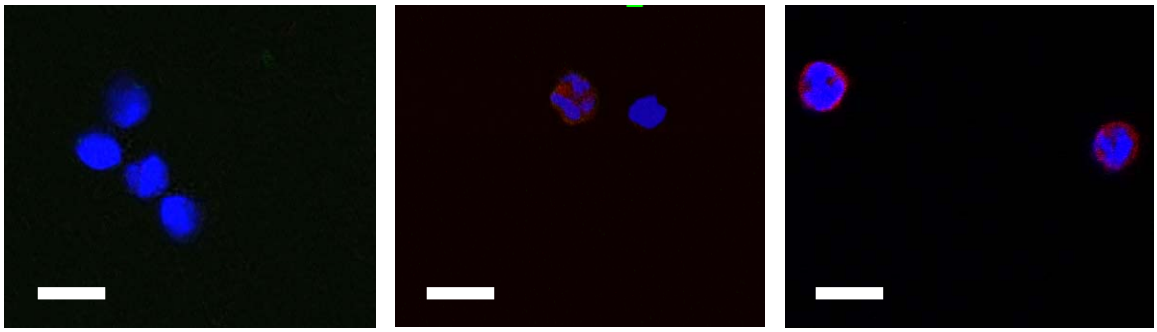


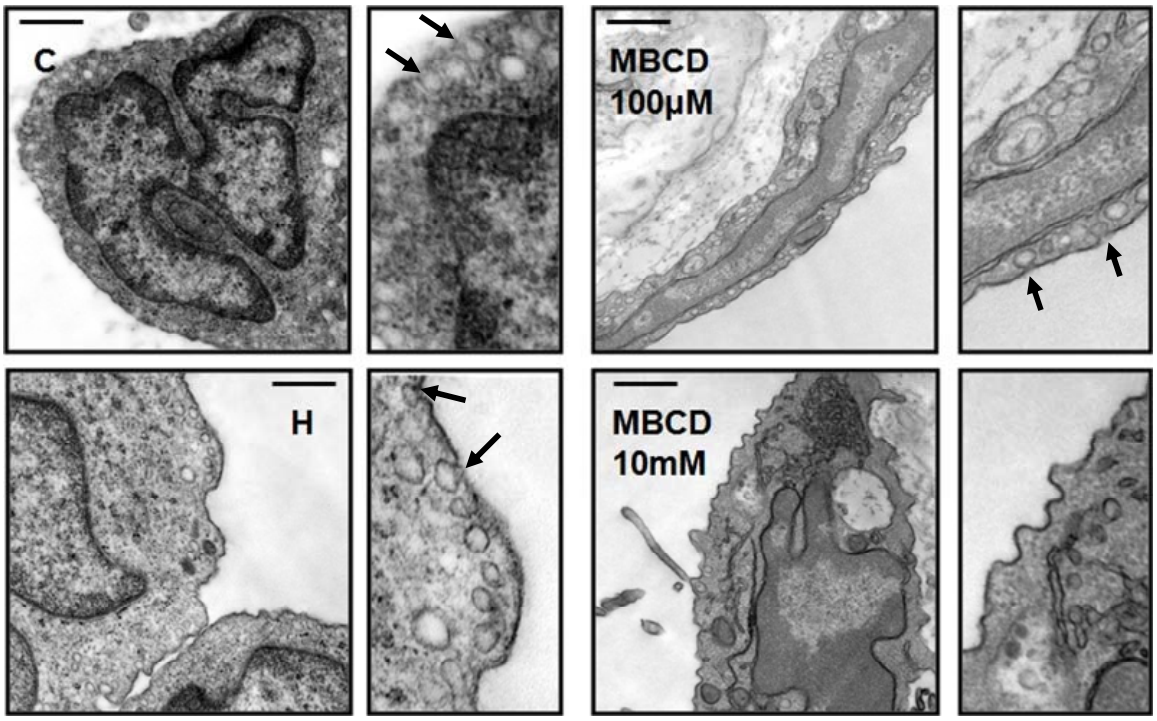
Supplemental Figure 1. Gracilis arteriole cross sections were treated with secondary antibodies and no primary antibodies to confirm lack of staining in the absence of primary antibody. (panel A). Additional cross sections were treated with BKα (panel B) or BKβ1 (panel C) primary antibodies plus respective blocking peptides to confirm primary antibody specificity.

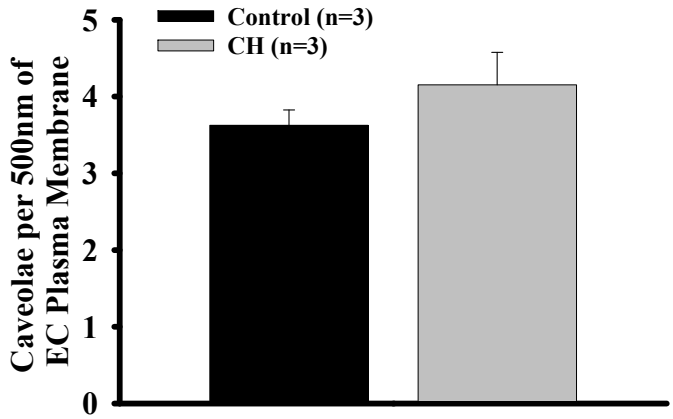


Supplemental Figure 2. BKα and PECAM-1 primary antibodies demonstrated endothelium specific staining. Cross sections were stained for BKα (green) and the endothelial marker PECAM-1 (1:100) (red). PECAM-1 staining appeared only in endothelial cells (middle and right panels), while BKα staining appeared in vascular smooth muscle cells and endothelial cells in the cross sections (left and right panels). Nuclei are blue.



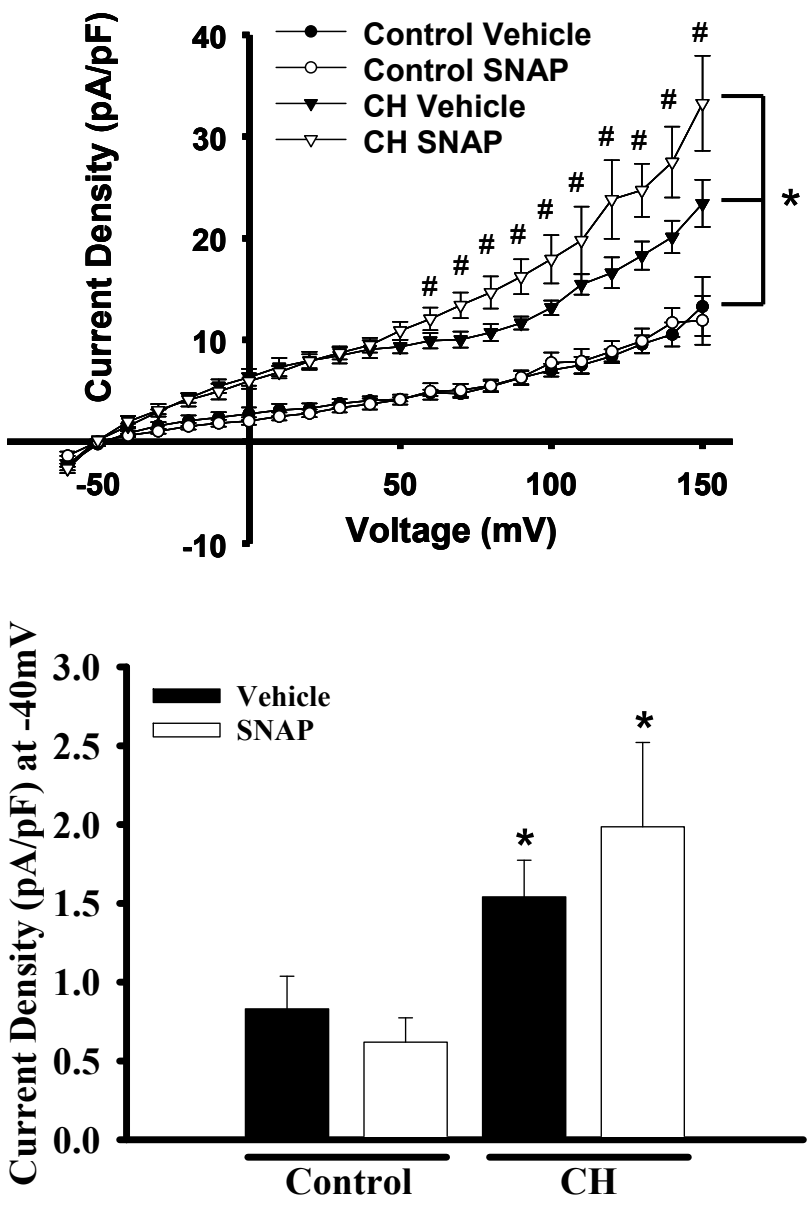
Supplemental Figure 3. Isolated leukocytes (T cells) from control rats were treated with primary antibodies to BKα as a negative control, since these cells do not express BK channels. Slides were stained for BKα (middle panel) or BKβ1 1:100 (right panel), the T-cell marker CD3 (red), and a nuclear stain (blue). No BKα or BKβ1 staining appeared in leukocytes, in contrast to positive staining in vascular smooth muscle cells and endothelial cells in figure 8 and supplemental figure 2. Scale bar = 10 μm.





Supplemental Figure 4. Electron microscopy images of endothelial cells from control (labeled C in top left) gracilis arterioles treated with vehicle, 100 μM or 10mM methyl β-cylcodextrin (MBCD) or arteries from CH rats (labeled H in lower left panels). Enlargements illustrating membrane structure are shown for each condition and caveolae are indicated by arrows. Treatment with MBCD did not eliminate caveolar structures at the 100 μM concentration (top right, arrows), however the 10 mM MBCD concentration eliminated caveolae (bottom right). CH treatment did not alter caveolar presence or apparent structure compared to normoxic controls (bottom panel). The 100 μM MBCD treatment elicited increased outward K^+ currents from control ECs (Fig. 11a of the main paper) with no significant effect on outward currents in ECs from CH animals (Fig. 11b). Interestingly, putative reduction in cellular cholesterol by MBCD treatment without

ablation of caveolae enabled EC BK_{Ca} channel activity in controls to a level similar to that found in CH animals (Figure 12a: iberiotoxin sensitive currents) Scale bar = 500 nM, images at 15000x. The bar graph shows that the number of membrane caveolae counted in images of gracilis arterioles did not differ between control and CH groups.



Supplemental Figure 5. Exogenous nitric oxide (NO) increased outward K^+ currents in ECs from CH animals (n=6 cells) but had no effect on currents in controls (n=4 cells) (top panel). Current density at -40mV was significantly larger from ECs from CH animals (bottom panel) and exogenous NO from the NO donor S-nitroso-N-acetyl-D,L-penicillamine (SNAP) 100μM increased outward current only in ECs from CH animals. *p<0.05 CH vs. control vehicle, #p<0.05 CH SNAP vs. CH vehicle (+70mV through +150mV).

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