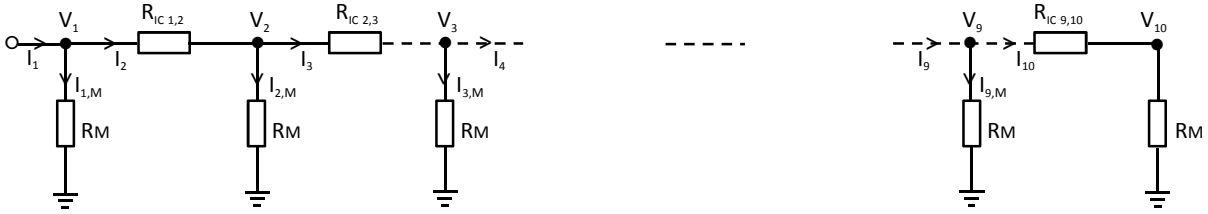
Electronic Supplement, Figures

a

b



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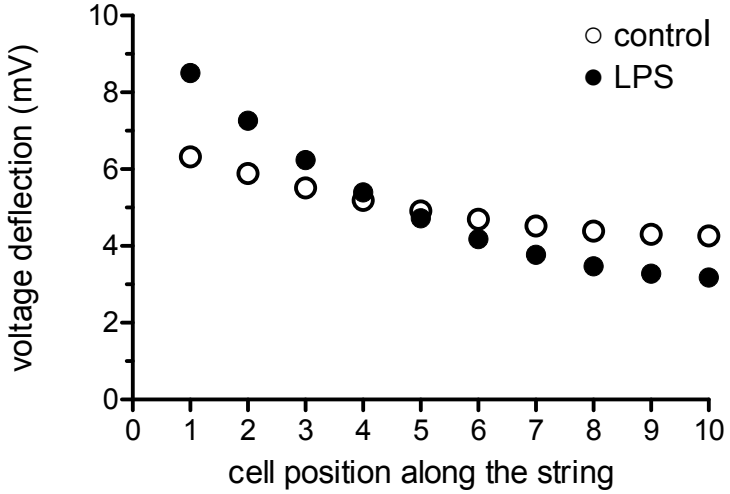

Figure S1

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Figure S1. Model of constant-current injection and its spread along a string of 10 cells. *Panel a:* Physical arrangement of 10 cells, the site of current injection, and the sites of voltage deflection determinations in cells (an example is shown for the site of V_1 deflection determination in cell #1). *Panel b:* Electrical circuit equivalent of the string of cells. R_{IC} is intercellular resistance between adjacent cells (R_{IC} is assumed the same for all cell pairs), R_M is transmembrane resistance (also the same for all cells) connecting the cell interior to the outside cell culture medium, which is electrically grounded. The cytosolic intracellular resistance and the resistance of the medium are assumed to be negligible. I_1 is the injected current, I's are currents in the various circuit branches, and V_1, V_2 … V_{10} are the voltage deflections determined for cells 1, 2, … 10. To compute V_1, the total resistance of the network, R_{TOT}, is computed first by adding appropriate resistances in series and in parallel. The injected current I_1 then flows through this total resistance, forming a voltage drop V_1 across R_{TOT}. Using the fact that $V_1 - V_2 = I_2 \times R_{IC\,1,2}$, and that $I_2 = I_1 - V_1/R_M$, the voltage V_2 can be computed. Similarly, V_3, V_4 …V_{10} are computed. *Panel c:* Voltages V_1, V_2 …V_{10} for control (open symbols) and LPS-treated cells (closed symbols). These realistic values were used: $I_1 = 0.5$ nA, $R_{IC\,CONTROL} = 1$ MΩ, $R_{M\,CONTROL} = 100$ MΩ, $R_{IC\,LPS} = 3$ MΩ, $R_{M\,LPS} = 100$ MΩ. Then $R_{TOT\ CONTROL} = 12.6$ MΩ and $V_{1\,CONTROL} = 6.3$ mV, $R_{TOT\,LPS} = 17.0$ MΩ and $V_{1\ LPS} = 8.5$ mV. The plot of voltage deflections along the string shows two features (i) the steepness of the decay along the string is larger for the larger R_{IC} value (i.e., , $R_{IC\,LPS} > R_{IC\,CONTROL}$) and (ii) the largest difference in deflection between the LPS and control conditions is at the site of the first cell. Providing that R_M remains unchanged after LPS (unchanged transmembrane resistivity was observed in the present study), $R_M >> R_{IC}$ (this condition ensures conduction along blood vessels), and $R_{IC\,LPS} > R_{IC\,control}$, these two features are observed for other realistic choices of $R_{IC\,control}$, $R_{IC\,LPS}$, R_M, and I_1.

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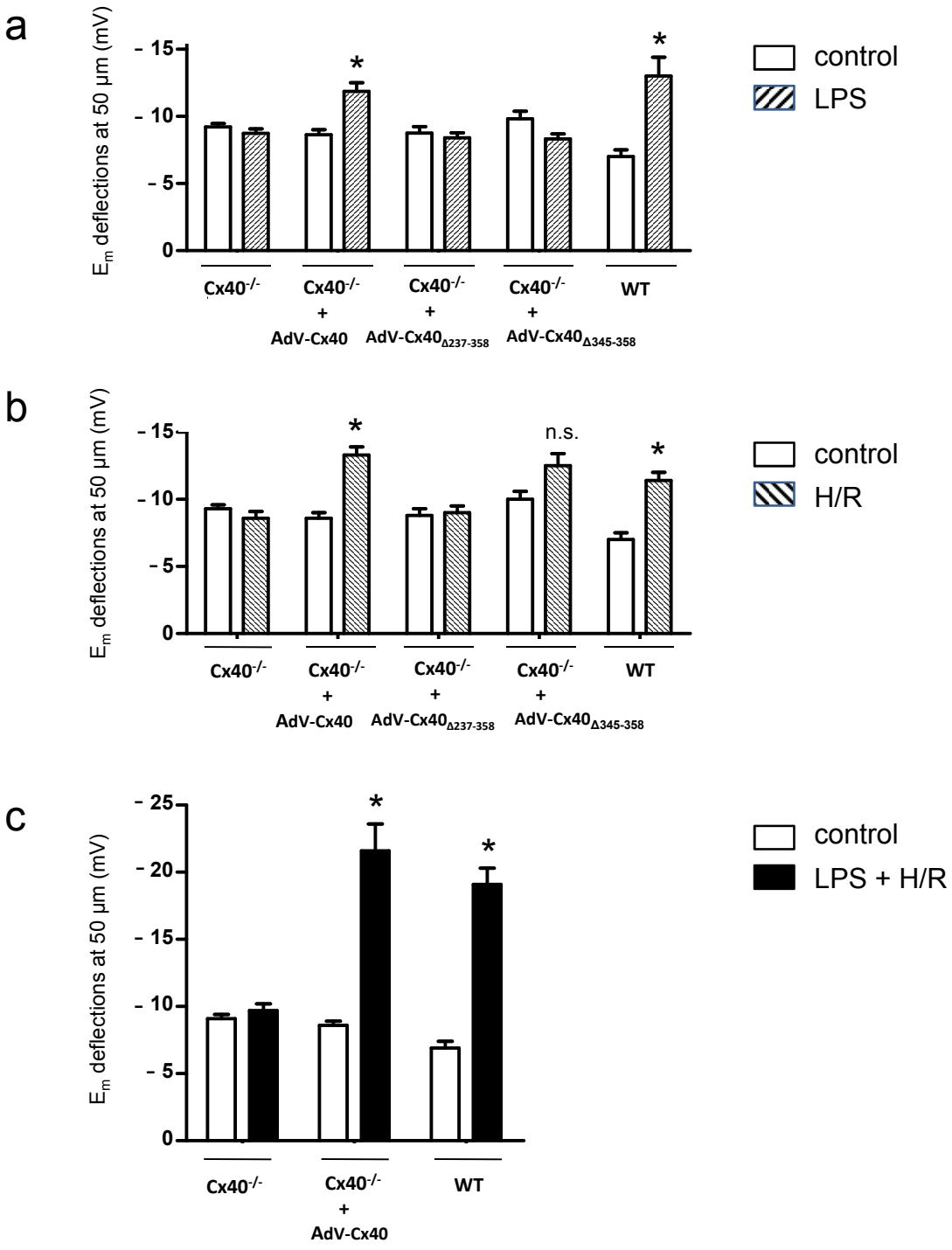

Figure S2. E_m deflections at the 50 µm interelectrode distance caused by constant-current injection into monolayers of $Cx40^{-/-}$, $Cx40^{-/-}$ + AdV-Cx40, $Cx40^{-/-}$ + AdV-$Cx40_{\Delta 237-358}$, $Cx40^{-/-}$ + AdV-$Cx40_{\Delta 345-358}$, and wild type (WT) cells, under control, LPS (panel a), H/R (panel b) and LPS+H/R treatments (panel c). Treatments increased deflections only in Cx40-/- + AdV-Cx40 and WT cells. n.s. (not significant), *P < 0.05 compared to control, n= 6 - 36 per group in panel a, 7 - 36 per group in panel b, and 5 - 36 per group in panel c.

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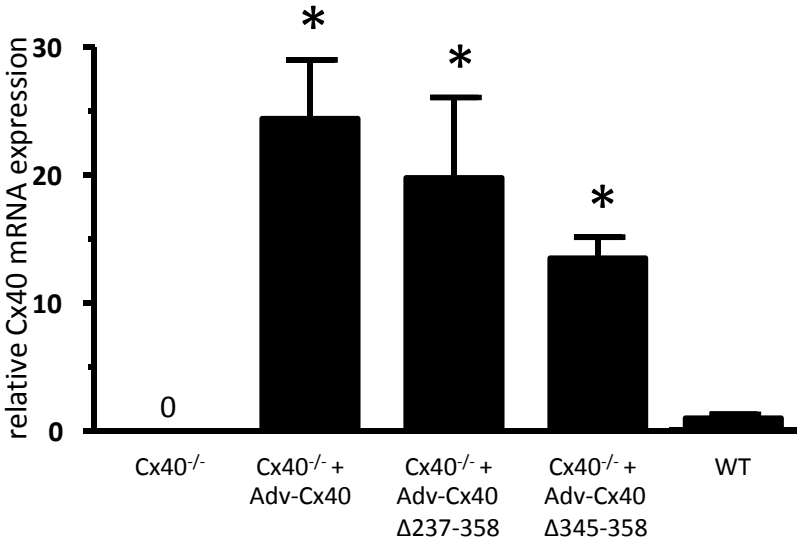

Figure S3. Cx40 mRNA expression in $Cx40^{-/-}$, $Cx40^{-/-}$ + AdV-Cx40, $Cx40^{-/-}$ + AdV-$Cx40_{\Delta 237\text{-}358}$, $Cx40^{-/-}$ + AdV-$Cx40_{\Delta 345\text{-}358}$, and WT cells. Cx40 mRNA expression in infected $Cx40^{-/-}$ cells was normalized to Cx40 mRNA in WT cells. No Cx40 mRNA was detected in non-infected $Cx40^{-/-}$ cells. *$P < 0.05$ compared to WT group, n= 3 monolayers per group.

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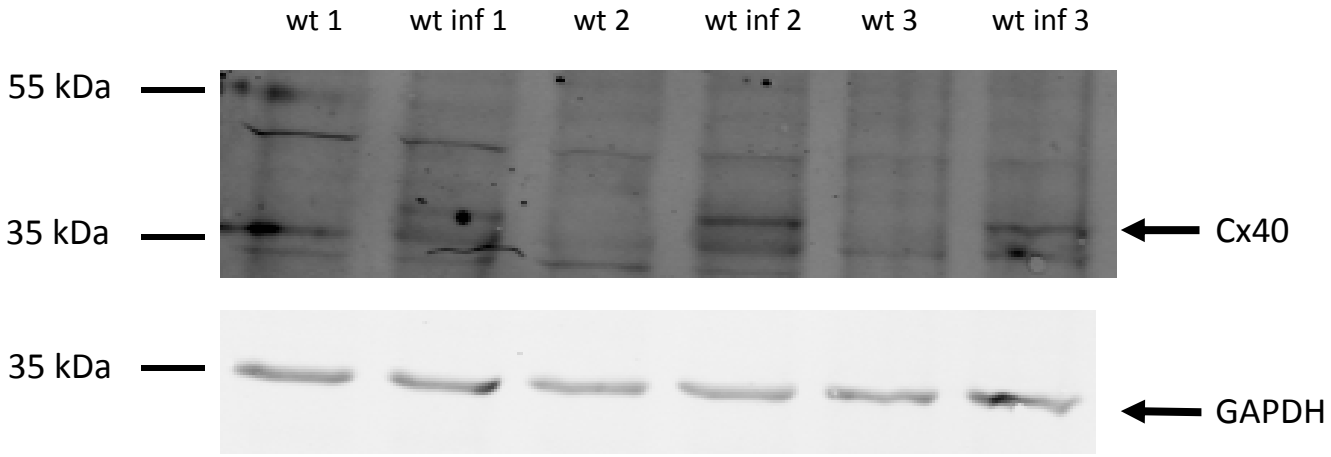

Figure S4. Microvascular endothelial cells from hindlimb skeletal muscle were harvested from 3 wild type (wt) mice. Cells were cultured and subjected to western blot analysis of Cx40 protein (Zymed antibody) and GAPDH protein expression as described in Electronic Supplement, Methods. Prior to this analysis, cells were subjected to control condition or to infection with AdV-Cx40. Only infected cells showed noticeable Cx40 protein expression (lanes wt inf 1, wt inf 2, and wt inf 3). GAPDH protein expression was not affected by infection.

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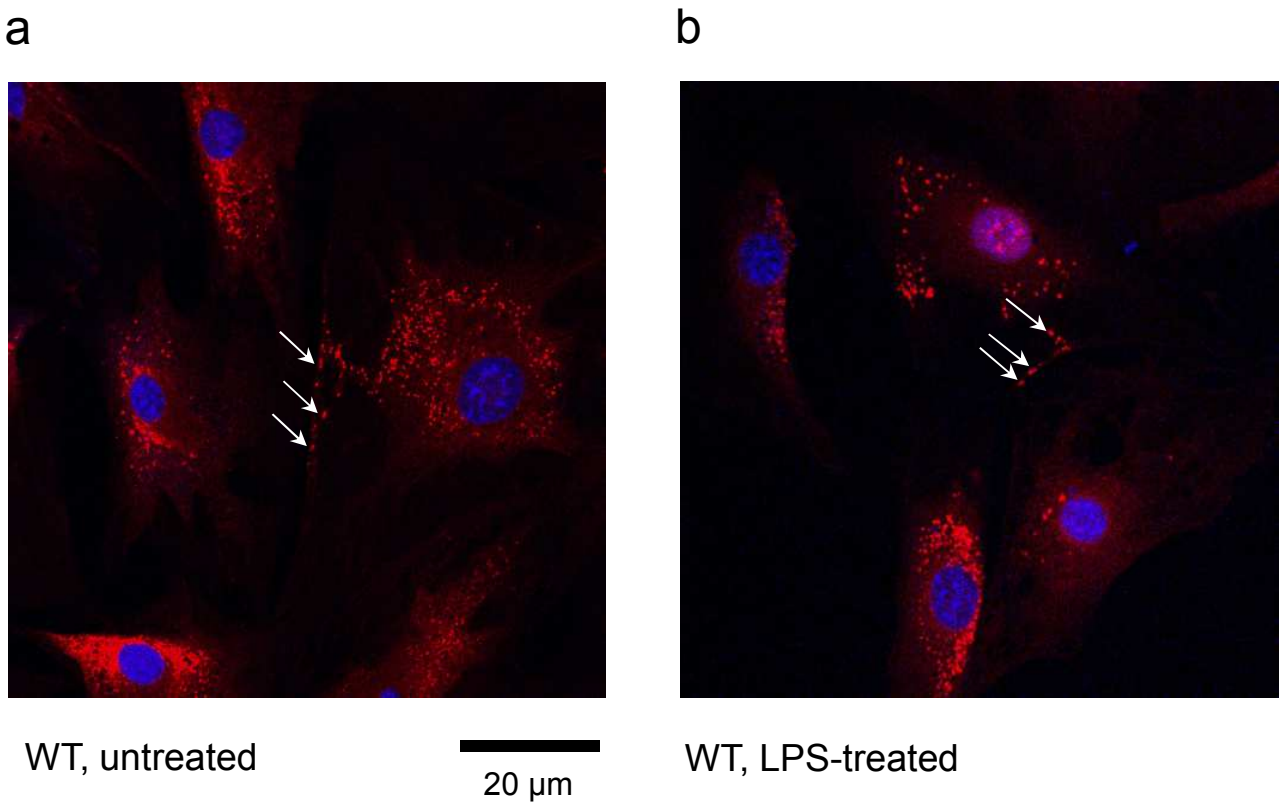

Figure S5. Cx40 protein distribution in WT microvascular endothelial cells under control (panel a) and LPS treatment conditions (panel b). Control and LPS-treated cells were probed with an anti-Cx40 antibody (Zymed or Chemicon) targeting the carboxyl terminal of Cx40. Among 17 control monolayers examined in 40 fields of view, and among 17 LPS-treated monolayers examined in 73 fields of view, there was only rare occurrence of punctate staining denoting putative Cx40 gap junction plaques (i.e., 1 - 2 fields per monolayer in both control and LPS-treated cells). Examples of this rare occurrence are seen in panels a and b (arrows) where cells were probed with the Chemicon antibody. The superimposed blue staining represents cell nuclei identified with Hoechst 33342 stain. Based on the examination of all control and LPS monolayers, there was no difference in the apparent Cx40 protein distribution between the control and LPS-treated cells. Bar = 20 µm.