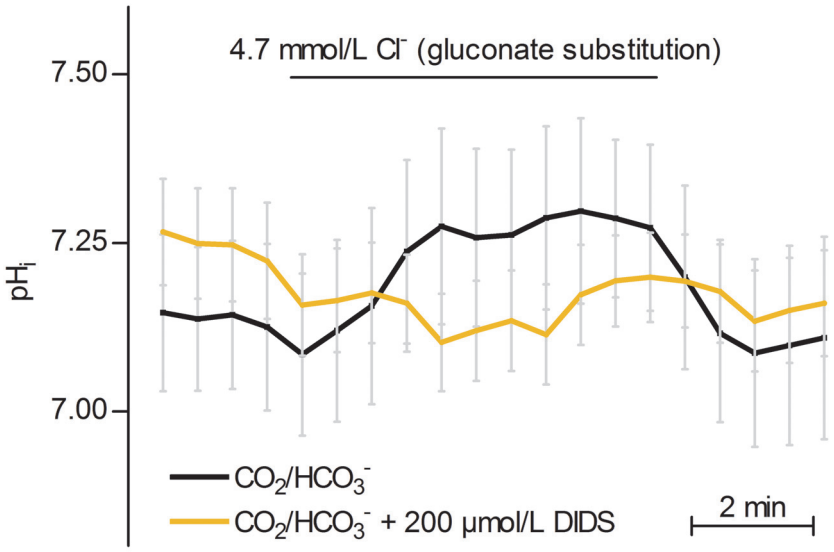
Ebbe Boedtkjer, Sukhan Kim, Christian Aalkjaer

Endothelial alkalinisation inhibits gap junction communication and endothelium-derived hyperpolarisations in mouse mesenteric arteries



Supplementary Figure 1. Reducing the extracellular [Cl^-] from 121.9 mmol/L to 4.7 mmol/L by substitution with gluconate caused endothelial alkalinisation, which could be inhibited by prior application of 200 µmol/L DIDS. Average pH_i responses are shown (n=5); the vertical lines represent SEM.

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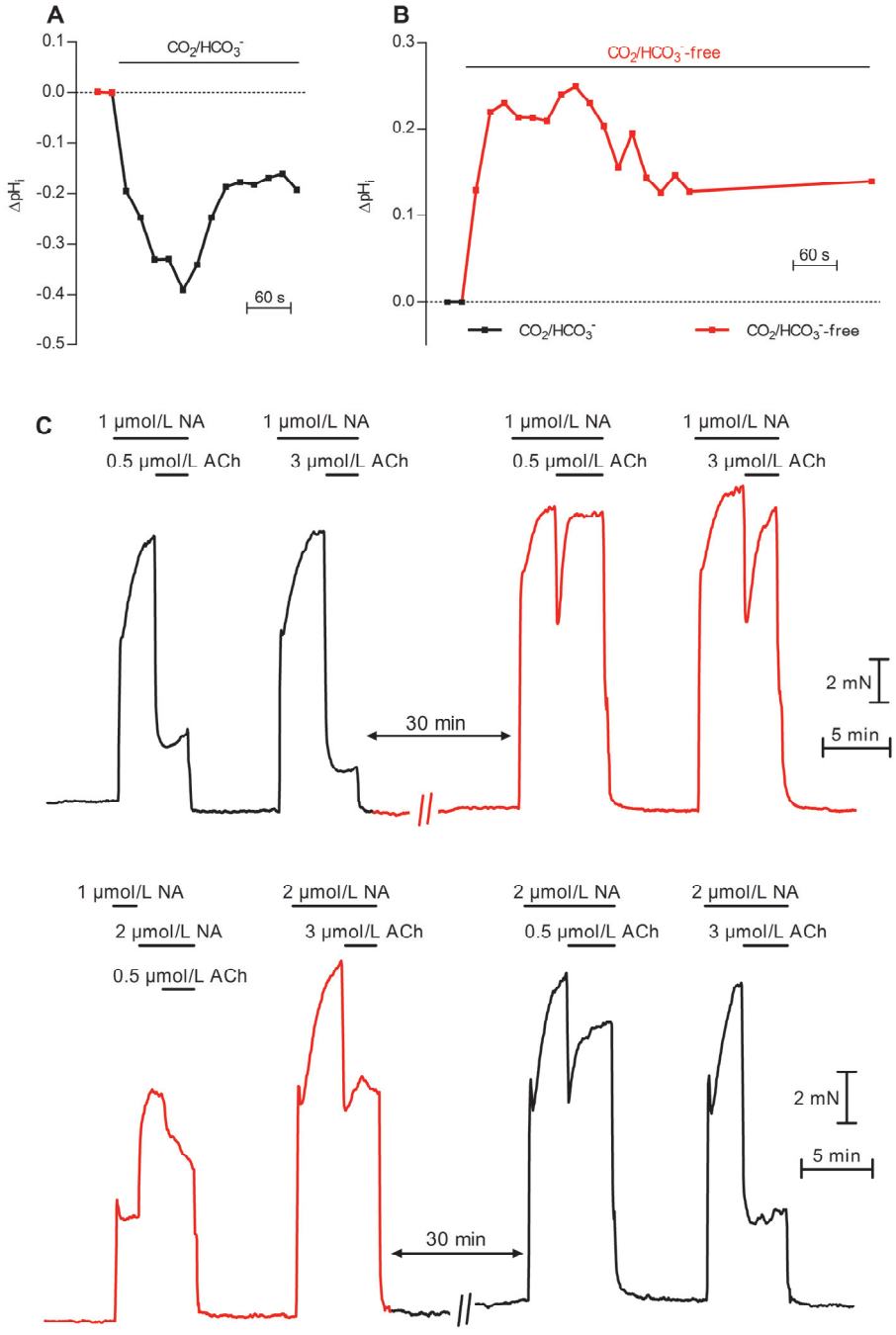
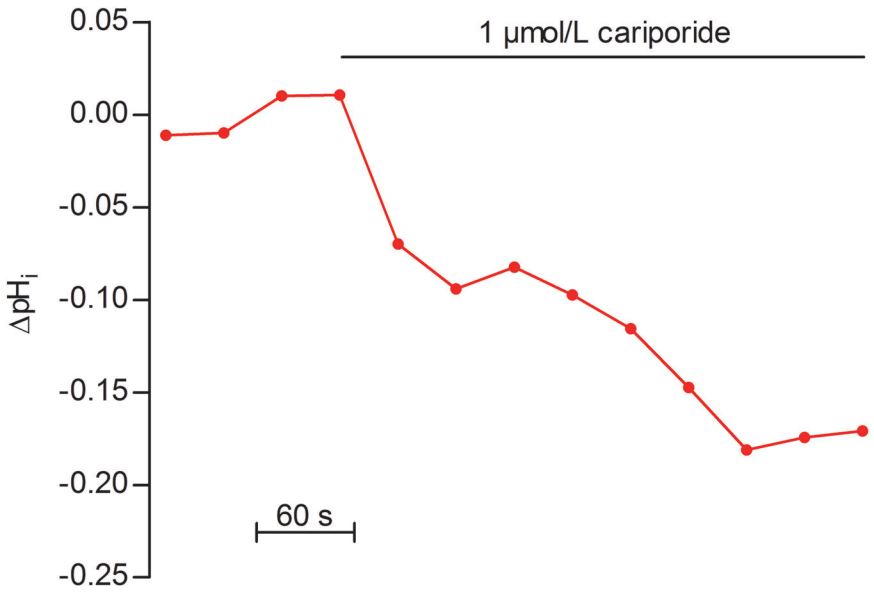
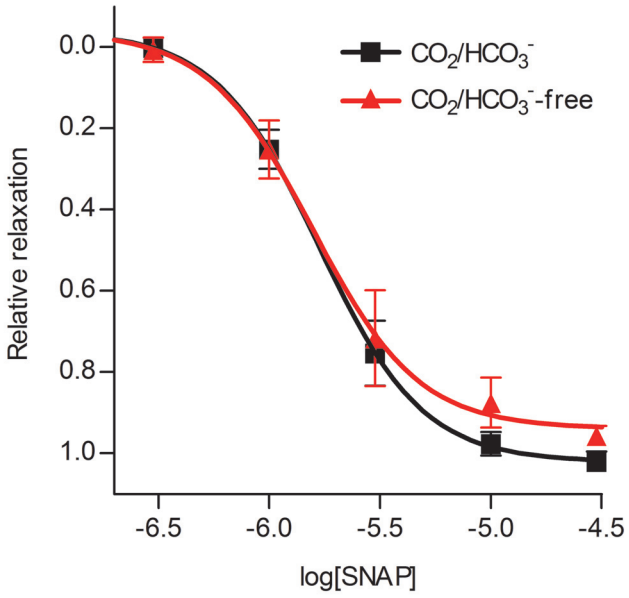


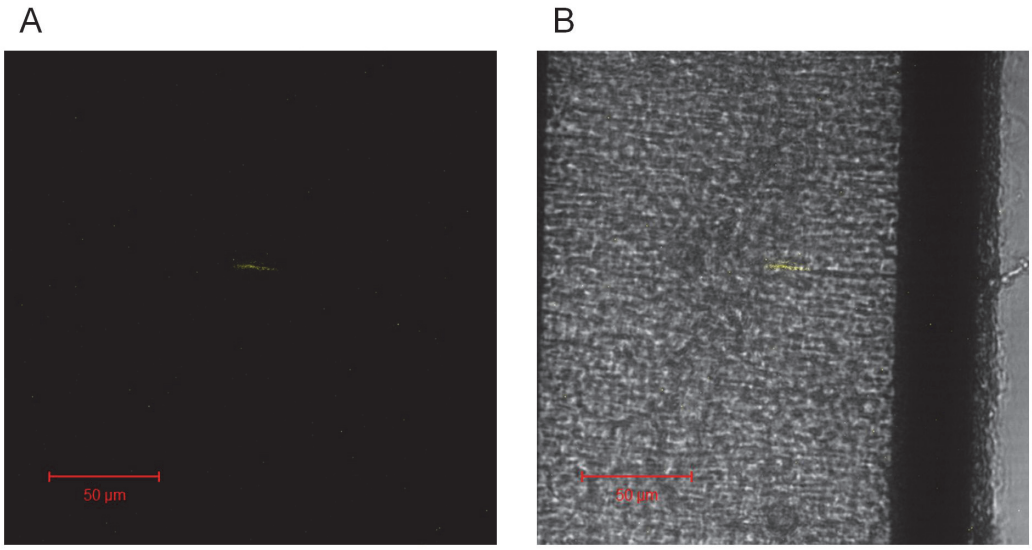
Supplementary Figure 2. Effects of intracellular alkalinisation were studied at steady-state and resulted in reversible inhibition of endothelium-dependent vasorelaxation. **A.+ B.** Original traces showing the pH_i effects of extracellular addition (A) or omission (B) of CO_2/HCO_3^-. **C.** Original traces showing the protocol used to investigate the effect of CO_2/HCO_3^--free conditions on acetylcholine (ACh)-induced endothelium-dependent vasorelaxation of noradrenaline (NA)-precontracted arteries in the presence of 100 µmol/L L-NAME. The effect of omitting or restoring CO_2/HCO_3^- was investigated 30 minutes after the buffer change to allow a new steady-state pH_i level to be reached; the order of the experimental protocol was alternated between preparations. Omission of CO_2/HCO_3^- reversibly inhibited endothelium-dependent vasorelaxation. Black lines indicate CO_2/HCO_3^--containing conditions while red lines indicate CO_2/HCO_3^--free conditions.



Supplementary Figure 3. Time course of the pH_i change following addition of the NHE1-selective inhibitor cariporide in the absence of CO_2/HCO_3^-.



Supplementary Figure 4. The endothelium-independent vasorelaxation to the NO-donor SNAP was unaffected by omission of CO_2/HCO_3^-. Experiments were performed on noradrenaline-precontracted arteries in the presence and absence of CO_2/HCO_3^-. The response to SNAP was investigated at least 30 minutes after the change of bath solution to allow pH_i to reach a new steady-state level. The concentration-response relationships were analysed by sigmoidal curve-fits and the derived $logEC_{50}$-values and maximum-responses compared by extra sum-of-squares *F*-tests. No significant differences between the responses seen in the presence and absence of CO_2/HCO_3^- were observed (n=6). Vertical lines represent SEM.



Supplementary Figure 5. Staining of a VSMC nucleus is seen following impalement from the adventitial side of a mouse mesenteric artery with an electrode containing propidium iodide. The thin nucleus running perpendicular to the axis of the artery is characteristic of a VSMC and clearly distinguishable from the rounder EC nuclei, which are oriented more parallel to the axis of the artery (see Figure 8A,B). **A.** Fluorescence image. **B.** Overlay image of the fluorescence image and a differential interference contrast image showing the axis of the artery.