Supplemental Tables and Figures for:

Extracellular HCO_3^- is sensed by mouse cerebral arteries: regulation of tone by receptor protein tyrosine phosphatase γ

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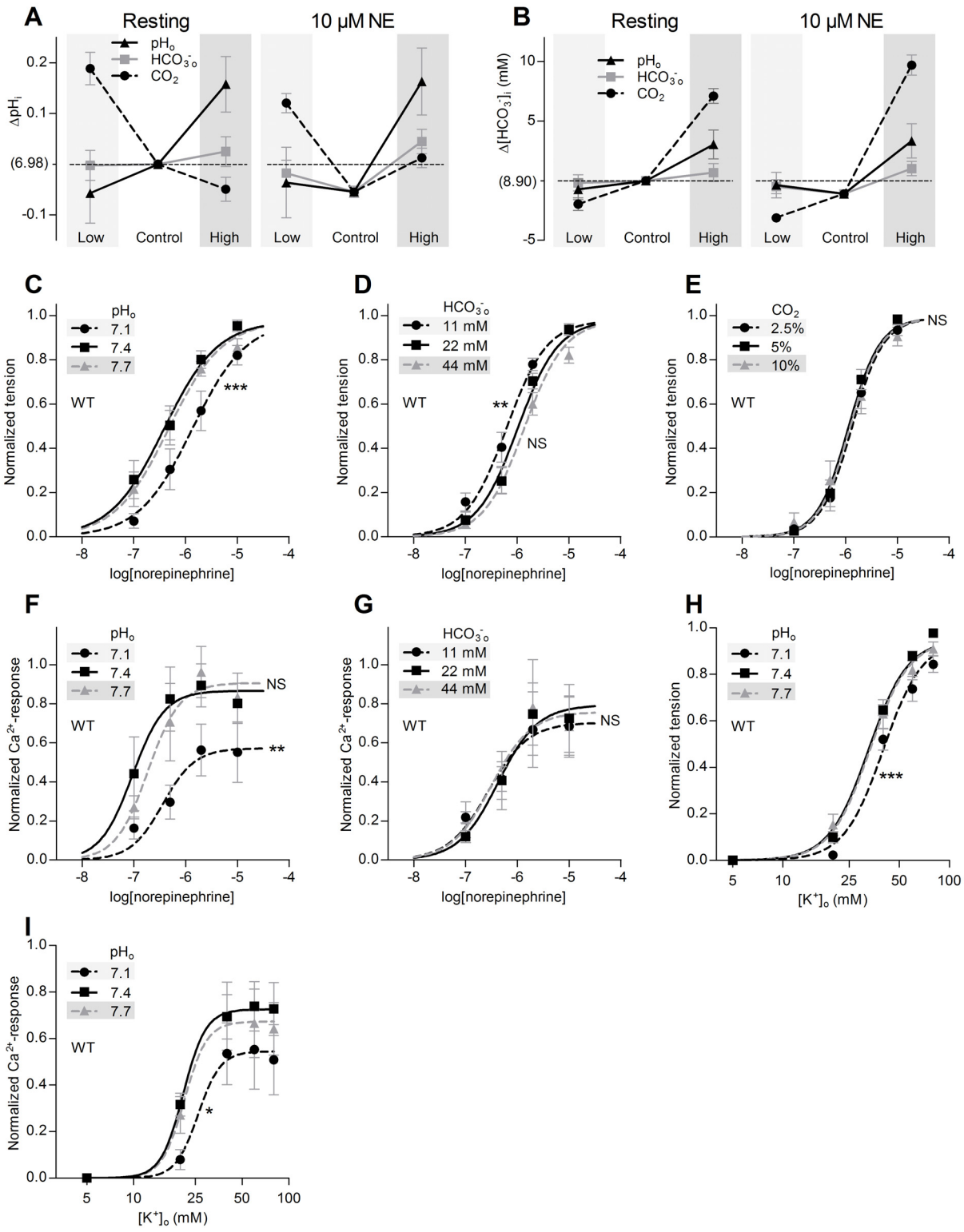
Part 1	**1** pH 7.40 22 mM HCO_3^- 5% CO_2	**2** pH 7.10 11 mM HCO_3^- 5% CO_2	**3** pH 7.10 22 mM HCO_3^- 10% CO_2	**4** OOE pH 7.40 22 mM HCO_3^- 2.5% CO_2			**5** OOE pH 7.40 22 mM HCO_3^- 10% CO_2			**6** OOE pH 7.40 11 mM HCO_3^- 5% CO_2		
				4a	4b	4Mix*	5a	5b	5Mix*	6a	6b	6Mix*
NaCl	101	112	101	113	91	102	113	91	102	135	91	113
KCl	5	5	5	10	0	5	10	0	5	10	0	5
NaH_2PO_4	2	2	2	0	4	2	0	4	2	0	4	2
$CaCl_2$	1.6	1.6	1.6	0	3.2	1.6	3.2	0	1.6	3.2	0	1.6
$MgSO_4$	1.2	1.2	1.2	0	2.4	1.2	0	2.4	1.2	0	2.4	1.2
Glucose	5.5	5.5	5.5	0	11	5.5	0	11	5.5	0	11	5.5
CO_2 (%)	5	5	10	5	0	2.5	20	0	10	10	0	5
$NaHCO_3$	22	11	22	44	0	22	44	0	22	22	0	11
HEPES	32.5	32.5	32.5	0	65	32.5	0	65	32.5	0	65	32.5
EDTA	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
pH	7.40	7.10	7.10	7.70	7.34	7.40	7.10	7.46	7.40	7.10	7.43	7.40

Supplemental Table 1 - Part 1. Compositions of three equilibrated and three out-of-equilibrium solutions. Concentrations are in mM except for CO_2, where concentration is in %CO_2 in a gas cylinder. All solutions were aerated (with a gas mixture containing the indicated %CO_2, balance air) and titrated with NaOH or HCl to the indicated pH at 37°C. For each OOE solution (e.g., "4"), we mix equilibrated solutions "a" and "b" (e.g., "4a" and "4b") to produce the OOE solution.*The columns named "Mix" represent the predicted instantaneous concentrations after mixing solutions "a" and "b". The compositions of the mixed solutions change over time as the system moves closer towards equilibrium. However, due to the continuous flow conditions, concentrations at the site of the artery (located <100 ms from the point of mixing) will be virtually identical to the instantaneous concentrations.

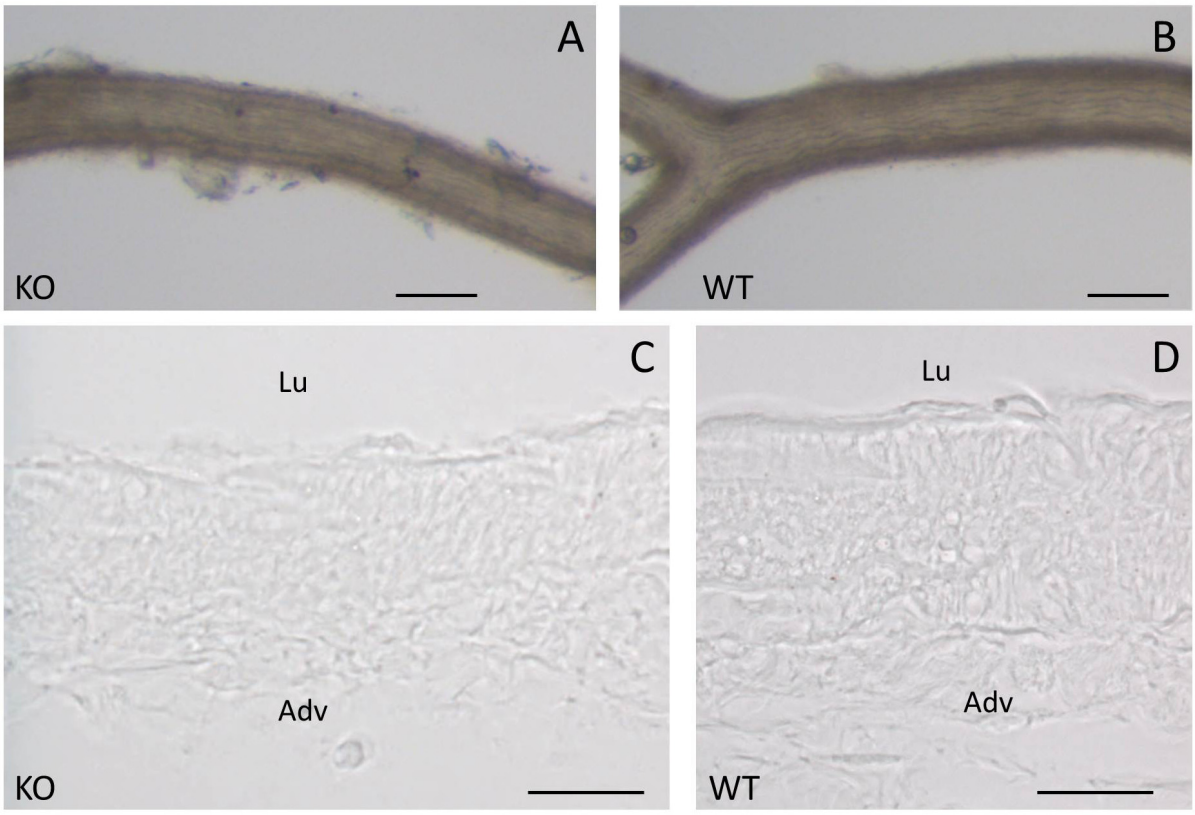
Part 2	**7** OOE pH 7.40 44 mM HCO_3^- 5% CO_2			**8** OOE pH 7.10 22 mM HCO_3^- 5% CO_2			**9** OOE pH 7.70 22 mM HCO_3^- 5% CO_2			**10** OOE pH 7.10 44 mM HCO_3^- 5% CO_2			**11** OOE pH 7.10 22 mM HCO_3^- 2.5% CO_2		
	7a	7b	7Mix*	8a	8b	8Mix*	9a	9b	9Mix*	10a	10b	10Mix*	11a	11b	11Mix*
NaCl	69	91	80	113	91	102	113	91	102	69	91	80	113	91	102
KCl	10	0	5	10	0	5	10	0	5	10	0	5	10	0	5
NaH_2PO_4	0	4	2	0	4	2	0	4	2	0	4	2	0	4	2
$CaCl_2$	0	3.2	1.6	0	3.2	1.6	3.2	0	1.6	0	3.2	1.6	0	3.2	1.6
$MgSO_4$	0	2.4	1.2	0	2.4	1.2	0	2.4	1.2	0	2.4	1.2	0	2.4	1.2
Glucose	0	11	5.5	0	11	5.5	0	11	5.5	0	11	5.5	0	11	5.5
CO_2 (%)	10	0	5	10	0	5	10	0	5	10	0	5	5	0	2.5
$NaHCO_3$	88	0	44	44	0	22	44	0	22	88	0	44	44	0	22
HEPES	0	65	32.5	0	65	32.5	0	65	32.5	0	65	32.5	0	65	32.5
EDTA	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
pH	7.70	7.29	7.40	7.40	7.04	7.10	7.40	7.76	7.70	7.70	6.86	7.10	7.70	6.98	7.10

Supplemental Table 1 - Part 2. Compositions of five additional out-of-equilibrium solutions. See part 1 for details.



Supplemental Figure 1. Selective changes in pH_o and to a smaller degree $[HCO_3^-]_o$—but not pCO_2—directly modulate mesenteric artery tone under OOE conditions. **A.** Effects of selectively varying pH_o, $[HCO_3^-]_o$ or pCO_2 (maintaining other two at control levels) on VSMC pH_i in resting

mesenteric arteries (left panel) or mesenteric arteries contracted by 10 μM norepinephrine (NE, right panel). Under "Control" conditions, CO_2 is 5%, pH_o 7.4, and $[HCO_3^-]_o$ 22 mM. Compared to "Control", "Low" refers to selective changes in CO_2 to 2.5%, $[HCO_3^-]_o$ to 11 mM or pH_o to 7.1. "High" refers to selective changes in CO_2 to 10%, $[HCO_3^-]_o$ to 44 mM or pH_o to 7.7. Arteries are from wild-type mice (n=5-9). **B.** Effects of selectively varying pH_o, $[HCO_3^-]_o$ or pCO_2 (maintaining other two at control levels) on VSMC calculated $[HCO_3^-]_i$ in resting mesenteric arteries (left panel) or mesenteric arteries contracted by 10 μM norepinephrine (right panel). Arteries are from wild-type mice (n=5-9). **C-E.** Effects of selectively varying pH_o, $[HCO_3^-]_o$, or pCO_2 (maintaining other two at control levels) on norepinephrine-induced tension development in mesenteric arteries from wild-type mice (n=8 in panel C, n=14 in panel D and n=6 in panel E). **F-G.** Effects of selectively varying pH_o or $[HCO_3^-]_o$ (maintaining unvaried parameters at control levels) on norepinephrine-induced VSMC Ca^{2+}-responses in mesenteric arteries from wild-type mice (n=6 for both panels). **H.** Effects of selective changes in pH_o ($[HCO_3^-]_o$=22 mM, CO_2=5%) on depolarization-induced tension development of mesenteric arteries from wild-type mice (n=11). We induce depolarization by raising $[K^+]_o$ in the presence of 100 nM prazosin to inhibit post-synaptic α_1-adrenergic effects of norepinephrine released from perivascular sympathetic nerves. **I.** Effects of selective changes in pH_o ($[HCO_3^-]_o$=22 mM, CO_2=5%) on depolarization-induced VSMC Ca^{2+}-responses in mesenteric arteries from wild-type mice (n=8). The curves in panel C through I are the result of least-squares fits to sigmoidal functions, and we compare them using extra sum-of-squares *F*-tests. **P*<0.05, ***P*<0.01, ****P*<0.001, NS: not significantly different *vs.* control conditions (pH_o=7.4, $[HCO_3^-]_o$=22 mM, CO_2=5%).



Supplemental Figure 2. *Ptprg* transcriptional activity is low in mesenteric arteries. **A-B.** Mesenteric arteries from an RPTPγ-knockout (KO, panel A) and a wild-type (WT, panel B) mouse, stained histochemically for β-galactosidase activity. Each image is representative of 5 experiments. **C-D.** Histological sections (8 µm thick) of mesenteric arteries from an RPTPγ-knockout and a wild-type mouse, stained histochemically for β-galactosidase activity. Each image is representative of 3 experiments. The size bars in panel A and B represent 200 µm; in panel C and D 20 µm. "Lu" and "Adv" indicate lumen and adventitia, respectively.