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Supporting Information for

Faulty TRPM4 channels underlie age-dependent cerebral vascular dysfunction in Gould syndrome

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Supplemental Figures:

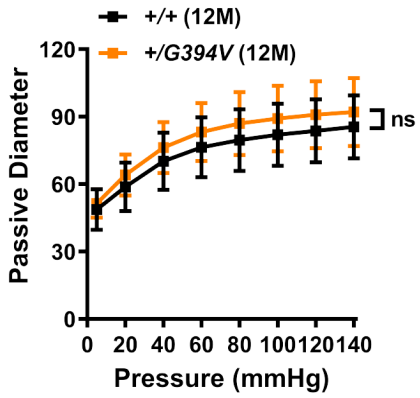

Figure 1 – figure supplement 1 – Passive diameter of cerebral arteries from 12 M-old Col4a1$^{+/G394V}$ mice. Summary data of the passive diameter of cerebral arteries expressed as a function of intraluminal pressure. n = 8 arteries from 5 or 6 animals per group. ns = not significant, two-way ANOVA.

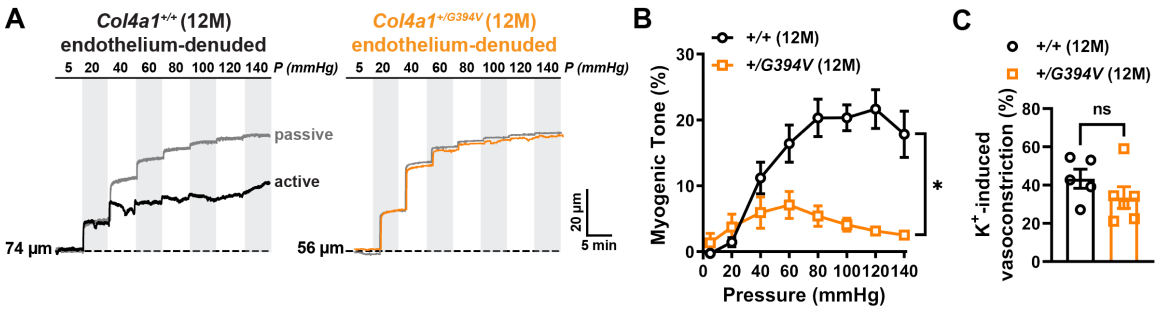

Figure 1 – figure supplement 2 – Endothelium-denuded cerebral arteries from 12 M-old Col4a1$^{+/G394V}$ mice fail to develop myogenic tone. (A) Typical recordings of the inner diameter of endothelium-denuded cerebral arteries from 12 M-old *Col4a1$^{+/+}$ and Col4a1$^{+/G394V}$* mice in response to step-wise increases in intraluminal pressure when Ca^{2+} is present (active) and when extracellular Ca^{2+} has been removed (passive). (B) Summary of myogenic tone expressed as mean ± SEM as a function of intraluminal pressure. n = 5 or 6 arteries from 3 animals per group. *P<0.05, two-way ANOVA. (C) Summary data showing vasoconstriction of endothelium-denuded isolated cerebral arteries from 12 M-old *Col4a1$^{+/+}$ and Col4a1$^{+/G394V}$* mice in response to KCl (60 mM). n = 5 or 6 arteries from 3 animals per group. ns = not significant, unpaired t-test.

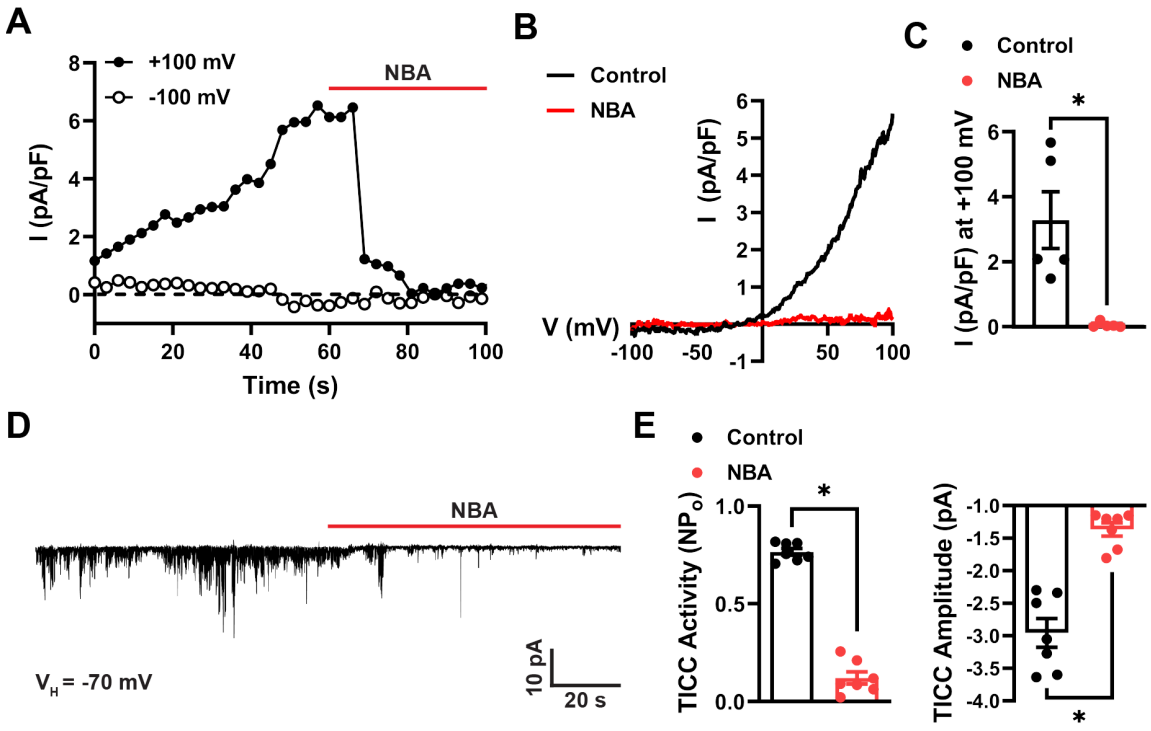

Figure 3 – figure supplement 1 – TRPM4 inhibitor NBA blocks whole-cell Ca^{2+}-activated currents and TICCs. (A) Representative time course of a conventional whole-cell patch-clamp recording of a SMC from a 3 M-old *Col4a1*$^{+/+}$ mouse showing an outwardly rectifying cation current activated by 200 μM free Ca^{2+} in the intracellular solution as voltage ramps (-100 to +100 mV) were applied. This current was abolished by the selective TRPM4 inhibitor 4-chloro-2-(2-(naphthalene-1-yloxy) acetamido) benzoic acid (NBA; 3 μM). (B) I-V plots for experiment shown in (A) in the presence and absence of NBA. Currents were obtained after peak current stabilized (~60s after break-in). (C) Summary of current amplitude at +100 mV normalized to cell capacitance. n = 5 cells from 4 animals. *P<0.05, unpaired t-test. (D) Typical recordings of whole-cell TICC in a SMCs a 3 M-old *Col4a1*$^{+/+}$ mouse. V_H = -70 mV. TICCs were inhibited by NBA (3 μM). (E) Summary of TICC activity and amplitude in the presence and absence of NBA. n = 7 cells from 3 animals. *P<0.05, unpaired t-test.

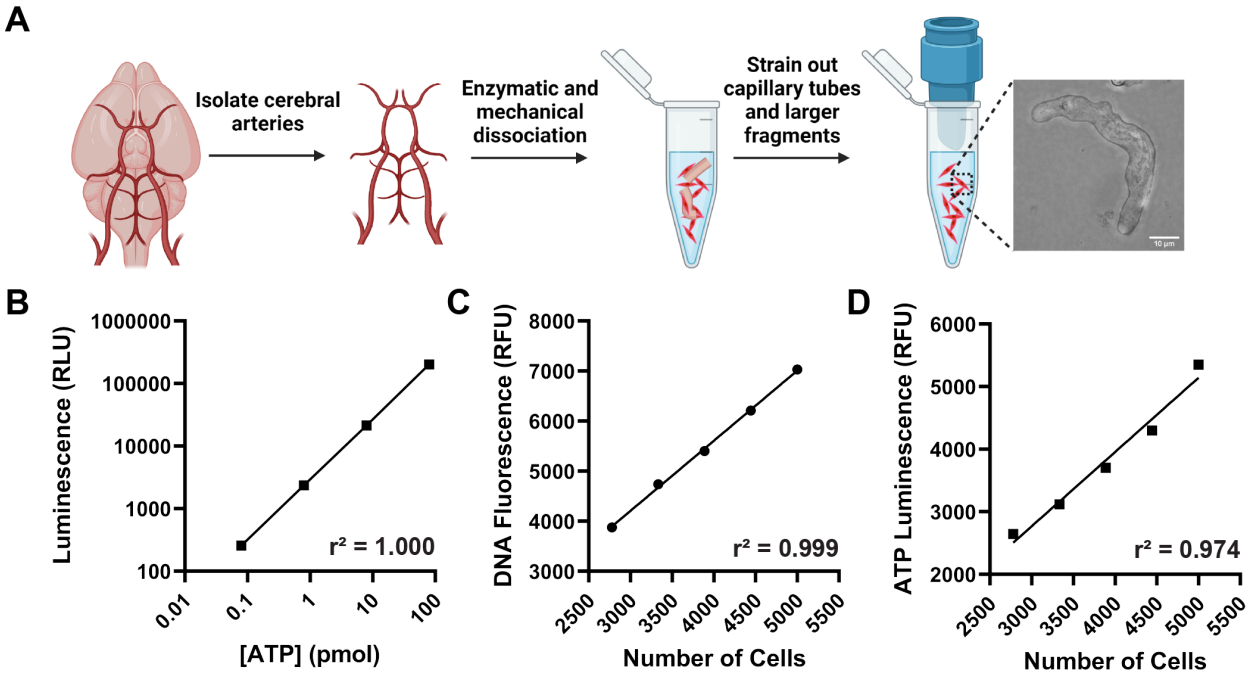

Figure 5 – figure supplement 1 – ATP assay validation. (A) Preparation of SMCs for ATP assay. (B) Relative luminescence units (RLU) are directly proportional to the [ATP] over four orders of magnitude (0.08 to 80 pmol) (r^2 = 1.00). (C) DNA fluorescence (relative fluorescence units, RFU) as a function of cell number (r^2 = 0.99). (D) ATP luminescence (RLU) as a function of cell number (r^2 = 0.97).

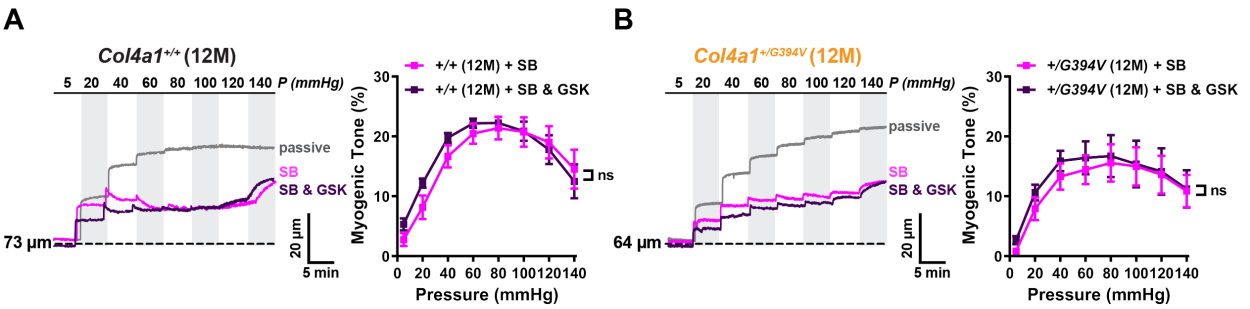

Figure 5 – figure supplement 2 – Myogenic tone with SB-431542 and GSK1059615.

(A and B) Representative traces and summary data of the myogenic response of cerebral arteries from 12 M-old *Col4a1*$^{+/+}$ (A) and *Col4a1*$^{+/G394V}$ (B) mice after first blocking TGF-β receptors with SB-431542 (1 μM, 30 min) and then blocking both TGF-β receptors and PI3K with the addition of GSK1059615 (10 nM, 30 min). Combined blockade did not have an additive effect. n = 5 or 6 arteries from 3 animals per group. *P<0.05, ns = not significant, 2-way ANOVA.

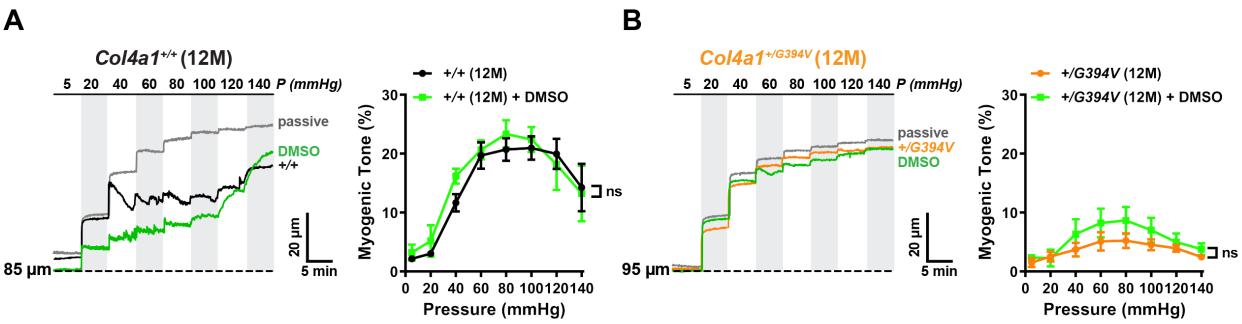

Figure 5 – figure supplement 3 – Vehicle control experiments. (A and B) Representative traces and summary data of the myogenic response of cerebral pial arteries from 12 M-old *Col4a1*$^{+/+}$ (A) and *Col4a1*$^{+/G394V}$ (B) mice before and after treating arteries with DMSO (0.01%), the vehicle for GSK1059615 and SB-431542 (30 min). n = 4-6 arteries from 4 or 5 animals per group. ns = not significant, 2-way ANOVA.