Supporting information for

PI3K block restores age-dependent neurovascular coupling defects associated with cerebral small vessel disease

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Supplemental figures

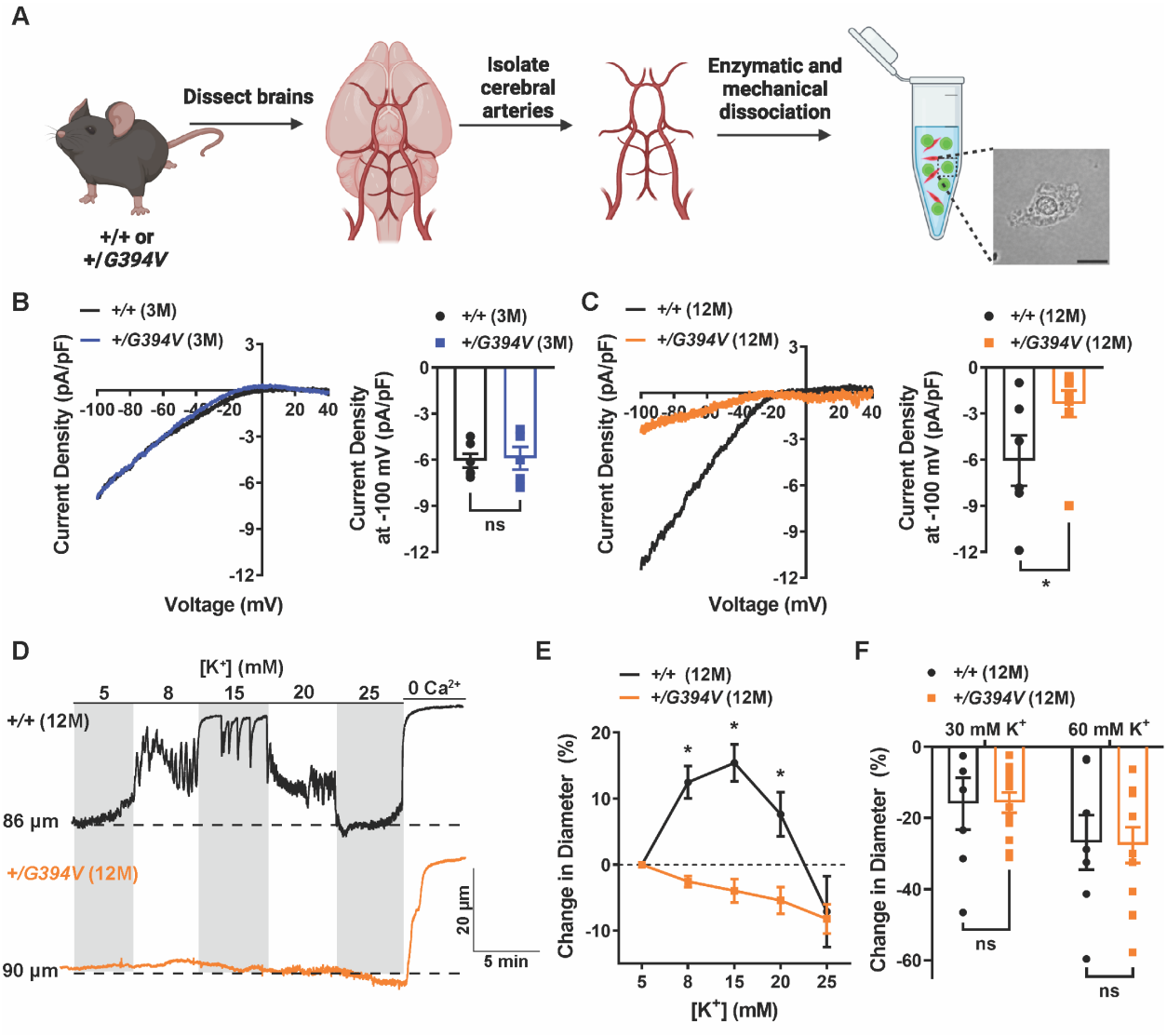
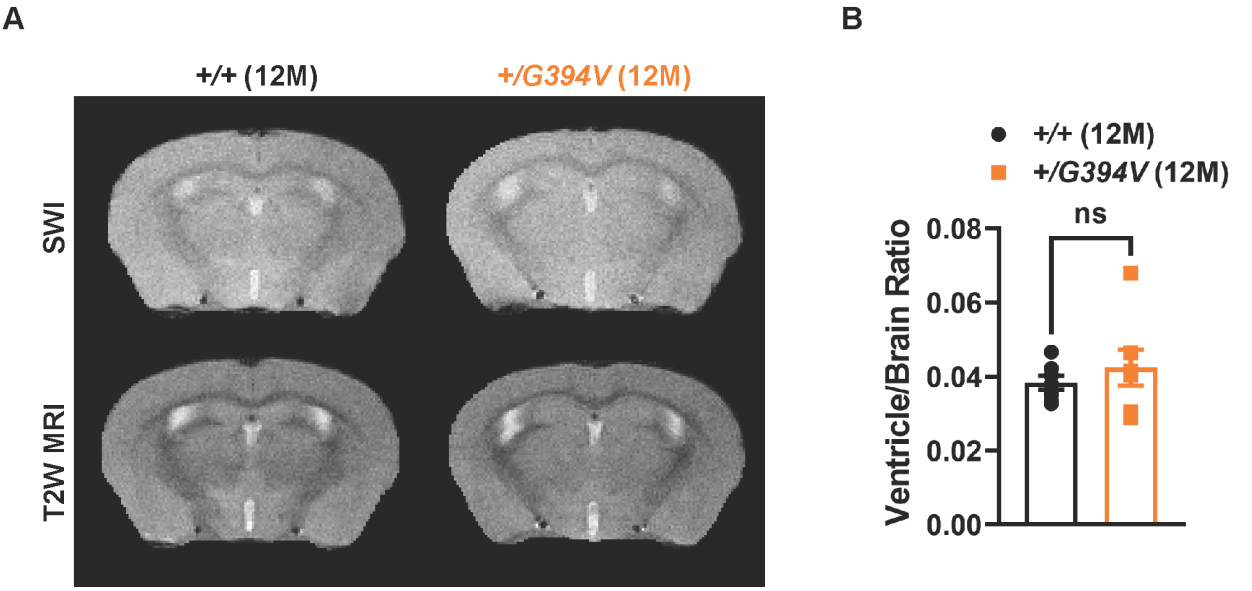


Fig. S1 – supplement 1. Kir2.1 currents in cerebral artery ECs. (A) Illustration of the cerebral artery EC isolation procedure. Scale bar = 10 µm. (B) Representative I-V traces and summary data showing Kir2.1 current densities in freshly isolated cerebral artery ECs from 3 M-old *Col4a1*$^{+/+}$ and *Col4a1*$^{+/G394V}$ mice (n = 5–6 cells from 5 animals per group, ns = not significant, unpaired t-test). (C) Representative I-V traces and summary data showing Kir2.1 current densities in freshly isolated cerebral artery ECs from 12 M-old *Col4a1*$^{+/+}$ and *Col4a1*$^{+/G394V}$ mice (n = 6–10 cells from 3 to 4 animals per

group; *p<0.05, unpaired t-test). (D and E) Representative traces (D) and summary data (E) demonstrating the vasodilator response to raising extracellular [K+] in cerebral arteries from 12 M-old *Col4a1*$^{+/+}$ and *Col4a1*$^{+/G394V}$ mice (n = 9–13 preparations from 6 to 10 animals per group, *p<0.05, non-repeated measures two-way ANOVA). (F) Summary data showing the contractile response to 30 and 60 mM [K+] in cerebral arteries from 12 M-old *Col4a1*$^{+/+}$ and *Col4a1*$^{+/G394V}$ mice (n = 7–12 preparations from 5 to 10 animals per group, ns = not significant, unpaired t-test).



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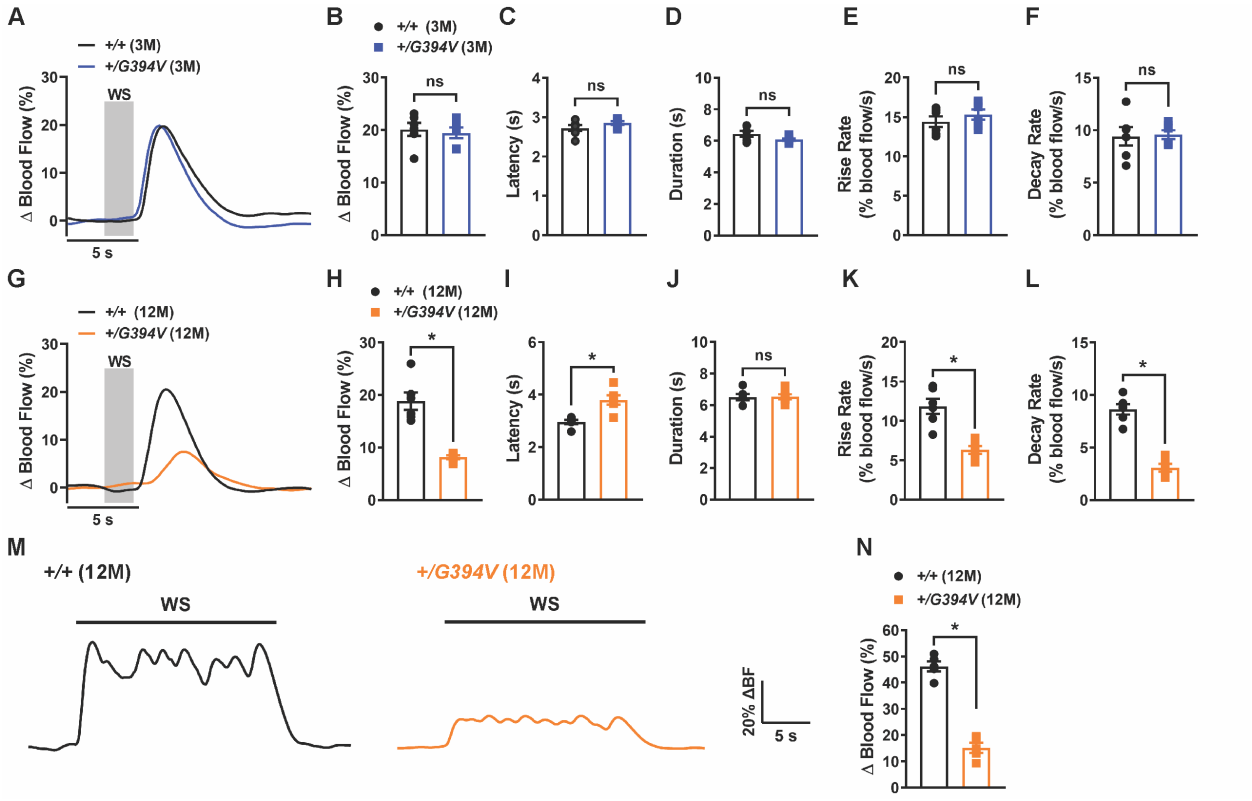
42 ***Fig. S2 – supplement 2. High field magnetic resonance imaging.*** (A) Representative
43 susceptibility weighted (SWI) and T2 weighted (T2W) MRI images from 12 M-old
44 *Col4a1*$^{+/+}$ and *Col4a1*$^{+/G394V}$ mice. (B) Quantification of ventricle/brain ratio for 12 M-old
45 *Col4a1*$^{+/+}$ and *Col4a1*$^{+/G394V}$ mice (n = 7 animals per group, ns = not significant, unpaired
46 t-test).



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48 ***Fig. S3 – supplement 1. Functional hyperemic response following 2 and 20 s***

49 ***whisker stimulation.*** (A and B) Representative traces (A) and summary data (B)

50 showing the increase in blood flow following 2 s contralateral whisker stimulation (WS)

51 in 3 M-old *Col4a1*$^{+/+}$ and *Col4a1*$^{+/G394V}$ mice (n = 6 animals per group, ns = not

52 significant, unpaired t-test). (C to F) Latency (C), duration (D), rise rate (E) and decay

53 rate (F) were also analyzed (n= 6 animals per group, ns = not significant, unpaired t-

54 test). (G and H) Representative traces (G) and summary data (H) showing the increase

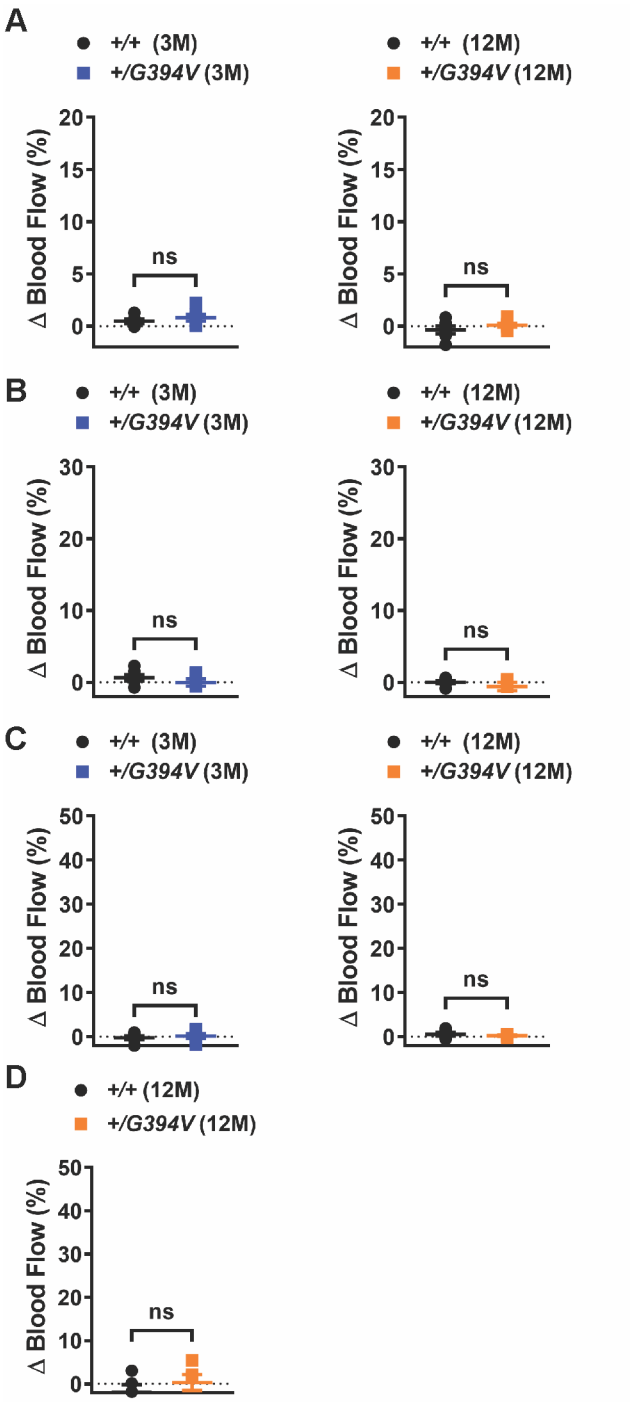
55 in blood flow following 2 s contralateral WS in 12 M-old *Col4a1*$^{+/+}$ and *Col4a1*$^{+/G394V}$ mice

56 (n = 6 animals per group, *p<0.05, unpaired t-test). (I to L) Latency (I), duration (J), rise

57 rate (K) and decay rate (L) were also analyzed (n = 6 animals per group, *p<0.05, ns =

58 not significant, unpaired t-test). (M and N) Representative traces (M) and summary data

(N) the increase in blood flow following 20 s contralateral WS in 12 M-old *Col4a1*$^{+/+}$ and *Col4a1*$^{+/G394V}$ mice (n = 5 animals per group, *p<0.05, unpaired t-test).



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62 ***Fig. S3 – supplement 2. Ipsilateral whisker stimulation.*** (A to C) Summary data
63 showing no change in blood flow following ipsilateral whisker stimulation for 1 s (A), 2 s
64 (B), and 5 s (C) in 3 M- and 12 M-old *Col4a1*$^{+/+}$ and *Col4a1*$^{+/G394V}$ mice (n = 6 animals
65 per group, ns = not significant, unpaired t-test). (D) Summary data showing no change

in blood flow following ipsilateral whisker stimulation for 20 s in 12 M-old *Col4a1*$^{+/+}$ and *Col4a1*$^{+/G394V}$ mice (n = 5 animals per group, ns = not significant, unpaired t-test).

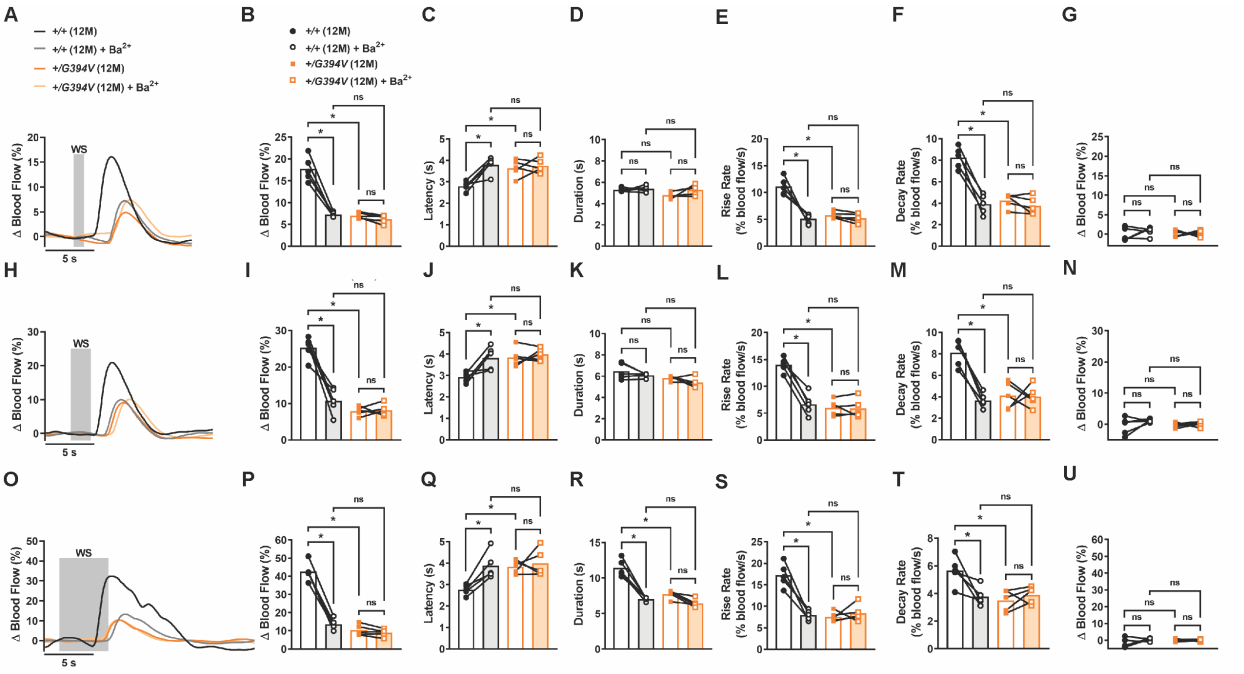


Fig. S3 – supplement 3. Whisker stimulation response in the presence of $BaCl_2$. (A and B) Representative traces (A) and summary data (B) showing the increase in blood flow following 1 s contralateral whisker stimulation (WS) in 12 M-old *Col4a1*$^{+/+}$ and *Col4a1*$^{+/G394V}$ mice before and after topical $BaCl_2$ (100 µM) application to the cranial surface (n = 5 animals per group, *p<0.05, ns = not significant, repeated measures two-way ANOVA). (C to F) Latency (C), duration (D), rise rate (E) and decay rate (F) were also analyzed (n= 5 animals per group, *p<0.05, ns = not significant, repeated measures two-way ANOVA). (G) No change in blood flow was detected following 1 s ipsilateral whisker stimulation (n= 5 animals per group, *p<0.05, ns = not significant, repeated measures two-way ANOVA). (H and I) Representative traces (H) and summary data (I) showing the increase in blood flow following 2 s contralateral whisker stimulation (WS) in 12 M-old *Col4a1*$^{+/+}$ and *Col4a1*$^{+/G394V}$ mice before and after topical $BaCl_2$ (100 µM) application to the cranial surface (n = 5 animals per group, *p<0.05, ns = not significant, repeated measures two-way ANOVA). (J to M) Latency (J), duration

(K), rise rate (L) and decay rate (M) were also analyzed (n= 5 animals per group, *p<0.05, ns = not significant, repeated measures two-way ANOVA). (N) No change in blood flow was detected following 2 s ipsilateral whisker stimulation (n= 5 animals per group, *p<0.05, ns = not significant, repeated measures two-way ANOVA). (O and P) Representative traces (O) and summary data (P) showing the increase in blood flow following 5 s contralateral whisker stimulation (WS) in 12 M-old *Col4a1*$^{+/+}$ and *Col4a1*$^{+/G394V}$ mice before and after topical $BaCl_2$ (100 μM) application to the cranial surface (n = 5 animals per group, *p<0.05, ns = not significant, repeated measures two-way ANOVA). (Q to T) Latency (Q), duration (R), rise rate (S) and decay rate (T) were also analyzed (n= 5 animals per group, *p<0.05, ns = not significant, repeated measures two-way ANOVA). (U) No change in blood flow was detected following 5 s ipsilateral whisker stimulation (n= 5 animals per group, *p<0.05, ns = not significant, repeated measures two-way ANOVA).

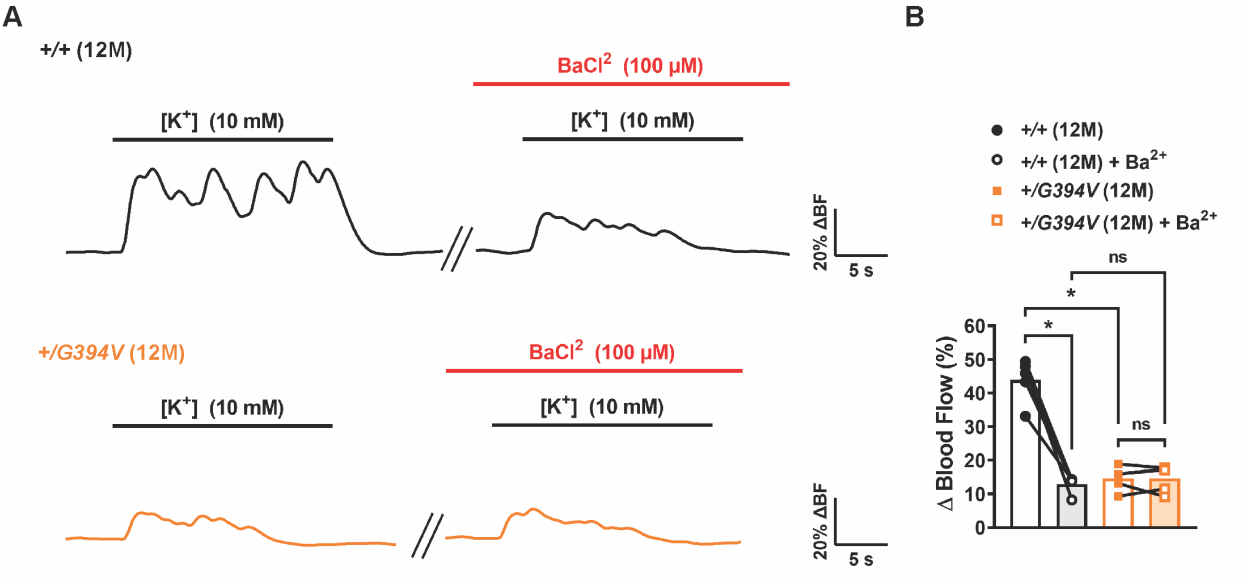
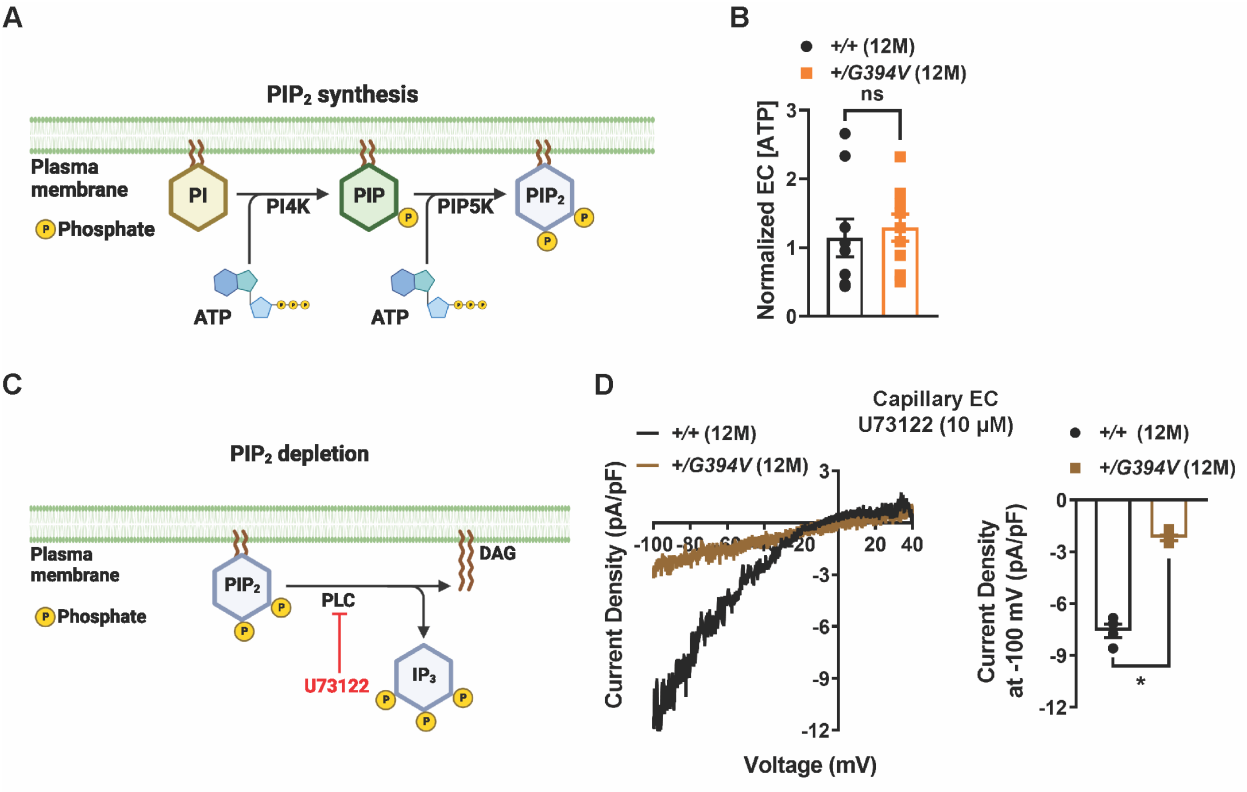


Fig. S3 – supplement 4. Cerebral blood flow response to application of KCl to the cranial surface. (A and B) Representative traces (A) and summary data (B) showing the increase in blood flow in 12 M-old *Col4a1*$^{+/+}$ and *Col4a1*$^{+/G394V}$ mice following topical application of KCl (10 mM) onto the cranial surface, in the absence and presence of $BaCl_2$ (100 µM) (n= 5 animals per group, *p<0.05, ns = not significant, repeated measures two-way ANOVA).



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104 ***Fig. S4 – supplement 1. ATP levels in brain capillary ECs.*** (A) PIP_2 synthesis

105 pathway. (B) Summary data showing normalized [ATP] in brain capillary ECs from 12

106 M-old *Col4a1*$^{+/+}$ and *Col4a1*$^{+/G394V}$ mice. (n = 9 sets of ~500 cells from three mice per

107 group, ns = not significant, unpaired t-test). (C) PIP_2 depletion pathway. (D)

108 Representative I-V traces and summary data showing Kir2.1 current densities in freshly

109 isolated brain capillary ECs treated with the PLC inhibitor U73122 (10 μM) from 12 M-

110 old *Col4a1*$^{+/+}$ and *Col4a1*$^{+/G394V}$ mice (n = 4 cells from 3 animals per group, *p<0.05,

111 unpaired t-test).

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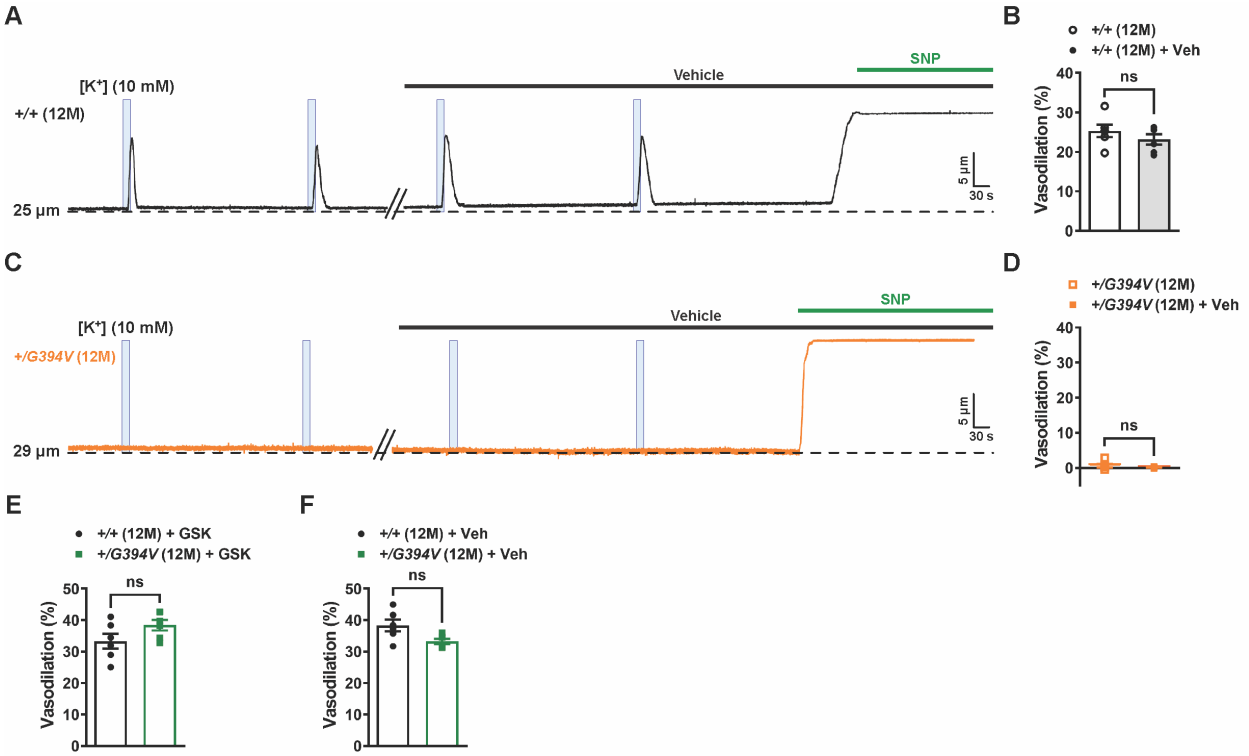


113

114 ***Fig. S4 – supplement 2. GSK1059615 vehicle and SNP response.*** (A and B)

115 Representative trace (A) and summary data (B) showing K^+ (10 mM, blue box)-induced

116 dilation of upstream arterioles in preparations before and after superfusing the vehicle

117 (0.01% v/v DMSO, 30 min) from 12 M-old *Col4a1*$^{+/+}$ mice (n = 6 preparations from 4

118 animals per group, ns = not significant, paired t-test). (C and D) Representative trace

119 (C) and summary data (D) showing K^+ (10 mM, blue box)-induced dilation of upstream

120 arterioles in preparations before and after superfusing the vehicle (0.01% v/v DMSO, 30

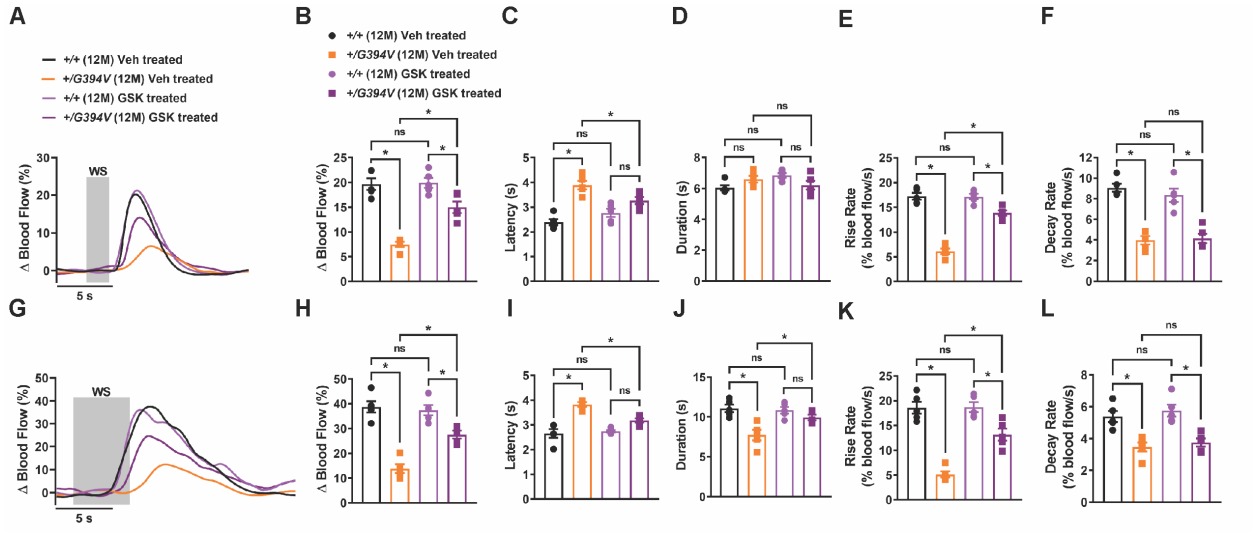
121 min) from 12 M-old *Col4a1*$^{+/G394V}$ mice (n = 6 preparations from 4 animals per group, ns

122 = not significant, paired t-test). (E and F) Summary data showing that the dilation

123 produced by superfusing SNP (10 µM) in the presence of GSK1059615 (10 nM, 30 min)

124 (E), and vehicle (0.01% v/v DMSO, 30 min) (F) in preparations from 12 M-old *Col4a1*$^{+/+}$

and *Col4a1*$^{+/G394V}$ mice (n = 6 preparations from 3 to 5 animals per group, ns = not significant, unpaired t-test).



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128 ***Fig. S5 – supplement 1. Functional hyperemic response following 2 s and 5 s***

129 ***whisker stimulation in GSK1059615-treated animals.*** (A and B) Representative

130 traces (A) and summary data (B) showing the increase in blood flow following 2 s

131 contralateral whisker stimulation (WS) in 12 M-old *Col4a1*$^{+/+}$ and *Col4a1*$^{+/G394V}$ mice

132 treated with vehicle (saline) or GSK1059615 (10 mg/kg) for 28 days, s.c. (n = 5 animals

133 per group, *p<0.05, ns = not significant, non-repeated measures two-way ANOVA). (C

134 to F) Latency (C), duration (D), rise rate (E) and decay rate (F) were also analyzed (n= 5

135 animals per group, *p<0.05, ns = not significant, non-repeated measures two-way

136 ANOVA). (G and H) Representative traces (G) and summary data (H) showing the

137 increase in blood flow following 5 s contralateral WS in 12 M-old *Col4a1*$^{+/+}$ and

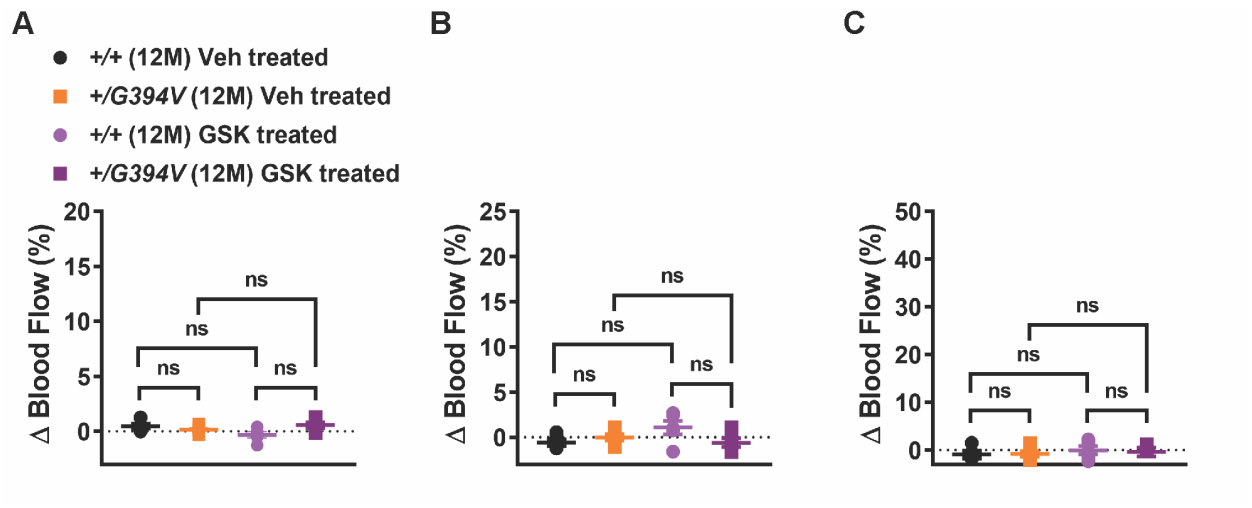
138 *Col4a1*$^{+/G394V}$ mice treated with vehicle (saline) or GSK1059615 (10 mg/kg) for 28 days,

139 s.c. (n = 5 animals per group, *p<0.05, ns = not significant, non-repeated measures two-

140 way ANOVA). (I to L) Latency (I), duration (J), rise rate (K) and decay rate (L) were also

141 analyzed (n= 5 animals per group, *p<0.05, ns = not significant, non-repeated measures

142 two-way ANOVA).



143

144 ***Fig. S5 – supplement 2. Ipsilateral whisker stimulation in GSK1059615 treated***

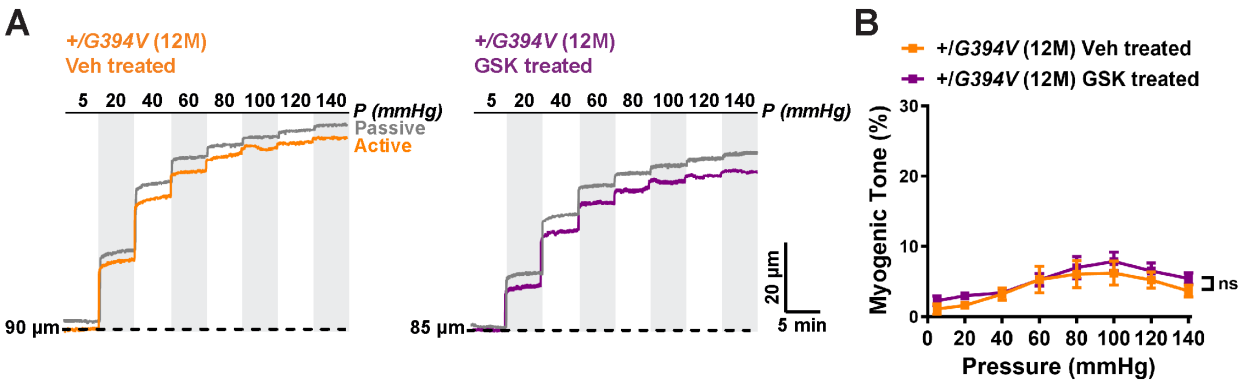
145 ***animals.*** (A to C) Summary data showing no change in blood flow following ipsilateral

146 whisker stimulation for 1 s (A), 2 s (B), and 5 s (C) in 12 M-old *Col4a1*$^{+/+}$ and

147 *Col4a1*$^{+/G394V}$ mice treated with vehicle (saline) or GSK1059615 (10 mg/kg) for 28 days,

148 s.c. (n = 5 animals per group, ns = not significant, non-repeated measures two-way

149 ANOVA).



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151 ***Fig. S5 – supplement 3. Myogenic response in GSK1059615 treated animals.*** (A)
152 Typical recordings of the inner diameter of isolated cerebral arteries from 12 M-old
153 *Col4a1*$^{+/+}$ and *Col4a1*$^{+/G394V}$ mice treated with GSK1059615 (10 mg/kg) for 28 days
154 showing the myogenic response to increases in intraluminal pressure (active) and the
155 dilation of the same arteries when extracellular Ca^{2+} has been removed (passive). (B)
156 Summary data showing the effects of GSK1059615 treatment on myogenic tone (n= 5
157 preparations from 3 animals per group, ns = not significant, repeated measures two-way
158 ANOVA).