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9   **Endothelium-targeted overexpression of krüppel-like factor 11 protects blood-brain**
10   **barrier function after ischemic brain injury**
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26 **Running Head**: Endothelial KLF11 protects blood-brain barrier.

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39   **Abstract**

40   Microvascular endothelial cell (EC) injury and the subsequent blood-brain barrier (BBB)

41   breakdown are frequently seen in many neurological disorders, including stroke. We have

42   previously documented that peroxisome proliferator-activated receptor gamma (PPARγ)-

43   mediated cerebral protection during ischemic insults needs Krüppel-like factor 11 (KLF11) as a

44   critical coactivator. However, the role of endothelial KLF11 in cerebrovascular function and

45   stroke outcome is unclear. This study is aimed at investigating the regulatory role of endothelial

46   KLF11 in BBB preservation and neurovascular protection after ischemic stroke. EC-targeted

47   overexpression of KLF11 significantly mitigated BBB leakage in ischemic brains, evidenced by

48   significantly reduced extravasation of BBB tracers and infiltration of peripheral immune cells,

49   and less brain water content. Endothelial cell-selective KLF11 transgenic (EC-KLF11 Tg) mice

50   also exhibited smaller brain infarct and improved neurological function in response to ischemic

51   insults. Furthermore, EC-targeted transgenic overexpression of KLF11 preserved cerebral tight

52   junction (TJ) levels and attenuated the expression of pro-inflammatory factors in mice after

53   ischemic stroke. Mechanistically, we demonstrated that KLF11 directly binds to the promoter of

54   major endothelial TJ proteins including occludin and ZO-1 to promote their activities. Our data

55   indicate that KLF11 functions at the EC level to preserve BBB structural and functional integrity,

56   and therefore confers brain protection in ischemic stroke. KLF11 may be a novel therapeutic

57   target for the treatment of ischemic stroke and other neurological conditions involving BBB

58   breakdown and neuroinflammation.

59

60   **Introduction**

61   Stroke ranks as the fifth leading cause of death, and accounts for 1 out of every 20 deaths in the

62   United States (7, 58). Ischemic stroke leads to cerebrovascular and neuronal damage, both of

63   which contribute to ischemic injury and dictate stroke outcome (24, 66). Although studies have

64   implied the effectiveness of neuroprotectants in animal stroke models (24, 32), there has been

65   very little progress made in the past three decades concerning the successful translation of

66   neuroprotective strategies to the clinical setting. The lack of clinical translation implies that

67   focusing on neuroprotection only is insufficient. Non-neuronal cells and the local

68   microenvironment of the surviving neurons could also serve as therapeutic targets to alleviate

69   ischemic brain injury (61, 65). Cerebral vascular endothelial cells (ECs) are major components

70 of brain microvasculature and play critical roles in maintaining the integrity of the blood-brain

71 barrier (BBB, a brain-specific microvascular structure) and cerebral homeostasis under

72 physiological conditions (1, 5, 50). Cerebral ECs are tightly connected by adherens junctions

73 (AJs) and tight junctions (TJs), including occludin, claudins, and junctional adhesion molecule

74 (JAM), which are anchored to the actin cytoskeleton by scaffold proteins such as zonula

75 occludens (ZO)-1, AF6, and cingulin. Together they form a diffusion barrier that selectively

76 blocks the passage of most compounds through blood to brain compartments (4, 16, 39). When

77 BBB integrity is compromised, proinflammatory factors may pass through the BBB to attract

78 circulating immune cells to the injured brain, leading to secondary ischemic brain parenchymal

79 injuries (15, 33-35, 46, 55, 60). Therefore, inhibition of BBB disruption through stabilizing TJs

80 and protecting brain endothelium has become promising therapeutic strategies for ischemic

81 stroke (18, 22, 45).

82 Krüppel-like factors (KLFs) are a large family of zinc finger transcription factors that are known

83 to trans-activate or trans-repress gene expression in various organisms (63). Recent studies have

84 reported the involvement of KLF members in various developmental and pathological vascular

85 processes (2, 52). A recent RNA sequencing study showed that in human endothelium, KLF11

86 has modest mRNA expression (20). KLF11 has been reported to play functional roles in the

87 regulation of cell growth and differentiation, cholesterol metabolism, and cell death (21, 25, 42,

88 52). Moreover, population genetics studies have demonstrated that mutations in the KLF11 gene

89 are strongly associated with type 2 diabetes (21, 42). Previous studies indicated that KLF11

90 inhibits endothelial activation in response to inflammatory stimuli (19). In addition, vascular

91 smooth muscle cell-selective deletion of *KLF11* aggravates arterial thrombosis in mice (36).

92 However, the role of KLF11 in cerebrovascular biology remains largely undetermined.

93 A previous study by our group reported that KLF11 functions as a peroxisome proliferator-

94 activated receptor-γ coregulator to attenuate middle cerebral artery occlusion (MCAO)-induced

95 ischemic brain injury (62). In conventional *KLF11* knockout mice, we observed larger brain

96 infarct, increased sensorimotor loss, aggravated BBB leakage, higher brain edema, and lower

97 cerebral blood flow (CBF) following cerebral ischemic insult (53). These studies suggest KLF11

98 plays a protective role in the pathogenesis of ischemic stroke. However, the molecular events and

99 regulatory roles of endothelial KLF11 itself in stroke-induced cerebral EC injury and BBB

100 disruption remain virtually unclear. Thus, the present study sought to test the hypothesis that

101   KLF11 stabilizes the cerebrovascular endothelial structure while simultaneously reducing stroke-

102   induced inflammatory events through preserving the integrity of TJs in cerebral ECs.

103

104   **Results**

105   **Generation and characterization of EC-selective KLF11 transgenic mice.** ECs are major

106   components of the BBB and are very sensitive to oxidative stress and ischemic insults (23).

107   Previously, KLF11 expression was found to be significantly decreased in cultured mouse

108   primary brain microvascular endothelial cells (BMECs) after 4 h or 16 h oxygen-glucose

109   deprivation (OGD) treatment (Fig. S1A) as well as in isolated cerebral microvessels from

110   C57BL/6J mice that were subjected to 1 h MCAO followed by 24 h reperfusion (Fig. S1B). We

111   also reported that genetic deletion of KLF11 in mice significantly augmented MCAO-induced

112   BBB disruption through exaggerating cerebrovascular permeability and edema (53). However,

113   whether KLF11 maintains the integrity of the BBB at the very early stages (1-3 h) after ischemic

114   insults, and whether endothelial KLF11 plays distinct roles in the pathogenesis of ischemic brain

115   injury remain unknown. To explore the specific role of KLF11 in endothelial cells after ischemic

116   injury, we generated transgenic mice with vascular endothelial cell-selective overexpression of

117   KLF11 on a C57BL/6J background. The EC-specific KLF11 transgenic construct contains the

118   Tie 2 promoter and Tie 2 enhancer (47, 64) to drive expression of the full-length KLF11 cDNA

119   sequence (Fig. 1A). The Tie 2 promoter allows selective targeting of KLF11 to vascular ECs.

120   The transgenic founder mice with KLF11 overexpression (EC-KLF11 Tg) were identified by

121   genomic PCR genotyping (Fig. 1B). To confirm the overexpression of KLF11 in the brain

122   microvasculature, we isolated cerebral microvessels from EC-KLF11 Tg and WT mouse brains.

123   We found a significant upregulation of cerebrovascular KLF11 protein level in EC-KLF11 Tg

124   mice than that of WT controls (Fig. 1C left). Moreover, KLF11 was not overexpressed in the

125   cortical protein extracts from EC-KLF11 Tg mouse brain in comparison with WT controls (Fig.

126   1C right). Of note, in sham-operated mice, no significant differences were observed in CD31-

127   labeled microvasculature of EC-KLF11 Tg mice compared to WT controls, as indicated by

128   branch points, capillary numbers, and branch length (Fig. 1 D-E). We also noticed that regional

129   CBF did not differ by transgenic overexpression of KLF11 in endothelium 15 minutes before

130   ischemia, 15 minutes after ischemia, or 15 minutes after reperfusion (Fig. 3 D-E), suggesting that

131   transgenic manipulation of KLF11 in endothelium does not cause possible cerebrovascular

132   structural (collateral) changes in the brains of EC-KLF11 Tg mice. Thus, EC-KLF11 Tg mice

133   can be used as a powerful tool to investigate the functional role of KLF11 in vascular endothelial

134   biology and BBB pathologies *in vivo*.

135

136   **EC-selective KLF11 transgenic overexpression ameliorates post-ischemic cerebrovascular**

137   **permeability.** Next, we performed Evans Blue and TMR-Dextran extravasation assays to

138   observe and quantify changes in BBB permeability. EC-KLF11 Tg and WT mice were subjected

139   to 1 h MCAO followed by 1 h, 3 h, or 24 h reperfusion. The results showed that EC-targeted

140   transgenic overexpression of KFL11 markedly reduced, but not completely blocked the leakage

141   of both Evans blue dye and TMR-dextran into ischemic brain after 24 h reperfusion (Fig. 2 A-D).

142   To assess BBB permeability at earlier stages (1-3 h) after ischemic insult, we also analyzed the

143   extravasation of a small fluorescent tracer, Alexa 555 cadaverine (0.95 kDa), which was injected

144   through the tail vein, and the relatively larger endogenous plasma IgG (150 kDa) into the

145   ischemic brain parenchyma (48, 49). The extravasation of the small molecule cadaverine in both

146   the ipsilateral cortex and striatum was detected as early as 1 h reperfusion, whereas endogenous

147   IgG was detected in the same area at 3 h reperfusion (Fig. 2E). Compared with WT controls, EC-

148   KLF11 Tg mice consistently showed significantly reduced extravasation of cadaverine (at 1h, 3h,

149   and 24h reperfusion; Fig. 2F) and IgG (at 3h and 24h reperfusion; Fig. 2G). These results suggest

150   that early-onset BBB impairment occurred following ischemic insult and endothelial-specific

151   KLF11 transgenic overexpression protects against MCAO-induced early-onset and progressive

152   (late-onset) BBB impairments. Moreover, anti-MAP2 immunostaining indicated that the brain

153   regions which present signs of BBB breakdown at earlier stages after MCAO progressed into

154   infarct zones at 24 h after MCAO (Fig. 2E). Quantification analysis of anti-microtubule-

155   associated protein 2 (MAP2) immunostaining showed that EC-KLF11 Tg mice had significantly

156   reduced brain infarction compared with WT controls (Fig. 2H). We further measured and

157   quantified the water content in ipsilateral and contralateral hemispheres of EC-KLF11 Tg and

158   WT mice 72 h after MCAO. The results demonstrated that brain water content in the ipsilateral

159   hemisphere of EC-KLF11 Tg mice was significantly less than that in the WT group (Fig. 2I),

160   whereas water content in the contralateral hemisphere from both groups showed no significant

161   differences (Fig. 2I). It is known that blood neutrophils can migrate through an impaired BBB

162   and carry proinflammatory mediators into the injured brain, thereby causing more serious BBB

163 damage and secondary expansion of ischemic brain parenchymal injury (49). We used

164 immunohistochemistry methods to detect neutrophils with a labeled Ly-6B antibody (Fig. 2J).

165 Quantification analysis of Ly-6B immunostaining confirmed that in comparison with WT

166 controls, MCAO-induced increases in brain infiltration of Ly-6B$^+$ neutrophils were significantly

167 reduced in EC-KLF11 Tg mouse brains (Fig. 2K). Taken together, these results suggest that

168 endothelial-selective KLF11 transgenic overexpression not only protects against ischemia-

169 induced BBB damage but also provides further neurovascular protection against ischemic stroke.

170

171 **EC-targeted KLF11 overexpression reduces ischemic brain injury and improves short-**

172 **term neurological function.** The protective role of EC-KLF11 overexpression against ischemic

173 insult was further confirmed by 2% 2,3,5-triphenyltetrazolium chloride (TTC) staining in another

174 cohort of mice, in which neurological deficits were also scored. Compared with WT controls,

175 EC-KLF11 Tg mice presented much smaller cerebral infarcts (Fig. 3 A-B) and significantly

176 reduced neurological scores (Fig. 3C), indicating better neurological outcome after stroke. Of

177 note, regional CBF showed similar changes between EC-KLF11 Tg and WT mice 15 minutes

178 before ischemia, 15 minutes after ischemia, or 15 minutes after reperfusion (Fig. 3 D-E). In

179 humans, cerebral ischemia always leads to sensorimotor dysfunction. We then investigated

180 whether EC-targeted KLF11 overexpression affects post-stroke functional recovery by a battery

181 of three different behavioral tests: adhesive tape removal, foot fault, and rotarod tests before and

182 up to 7 days following MCAO (Fig. 3 F-I). Compared with WT control mice, EC-KLF11 Tg

183 mice showed better recovery of sensorimotor function: longer staying latency in the rotarod test

184 (Fig. 3F), lower rate of fault steps in the foot fault test (Fig. 3G), and shorter touching or

185 removing time in the adhesive tape removal test (Fig. 3 H-I). These cumulative findings suggest

186 that endothelial-specific overexpression of KLF11 facilitates spontaneous sensorimotor recovery

187 after ischemic brain insult.

188

189 **EC-selective KLF11 overexpression preserves the integrity of junctional proteins after**

190 **ischemic brain injury.** TJ proteins are essential for maintaining the integrity of the BBB (28).

191 Therefore, we further examined the underlying mechanism of endothelial KLF11-mediated BBB

192 protection *in vivo*. EC-KLF11 Tg and WT mice were subjected to 1 h MCAO followed by one or

193 three days of reperfusion. Mouse brain tissue was harvested at the indicated time points after

194 MCAO and subjected to total RNA isolation and qPCR analysis (Fig. 4 A-B) or western blotting

195 analysis (Fig. 4 C-E). Endothelial-targeted KLF11 transgenic overexpression significantly

196 preserved the expression of TJ proteins, ZO-1 and occludin in ischemic brain tissue at both the

197 mRNA (Fig. 4 A-B) and protein (Fig.4 C-E) levels, consistent with the protective role of EC-

198 KLF11 overexpression in MCAO-induced BBB damage. Reduction in ZO-1 and occludin

199 expression in vascular endothelial cells (labeled by CD31) following ischemic brain insults were

200 further confirmed by immunofluorescent staining (Fig. 4F). As expected, EC-KLF11

201 overexpression markedly retained their *in situ* expressions (Fig. 4F). These results indicate that

202 endothelial-targeted KLF11 overexpression robustly protected the structural integrity of the BBB.

203

204 **EC-targeted KLF11 transgenic overexpression mitigates inflammatory activity in ischemic**

205 **brain regions.** Neuroinflammation following ischemic stroke contributes to BBB damage (57).

206 To test whether EC-targeted KLF11 overexpression alleviates MCAO-induced inflammatory

207 activities in the ischemic brain, we measured a panel of inflammatory mediators in the cortex of

208 the ipsilateral brain hemisphere at one or three days after MCAO. The mRNA expression levels

209 of six pro-inflammatory mediators: *TNF-α*, *IL-1β*, *MCP-1*, *IL-6*, *ICAM-1*, and *P-selectin*

210 markedly elevated following MCAO, and EC-targeted KLF11 overexpression attenuated this

211 elevation (Fig. 5 A-F). We then measured the expression of the abovementioned inflammatory

212 factors using Quantikine ELISA kits. Consistent with our previous observations, the six

213 inflammatory mediators all reduced in EC-KLF11 Tg mice when compared with WT controls

214 following MCAO (Fig. 5 G-L). Taken together, these results demonstrate that KLF11 transgenic

215 overexpression in endothelium mitigates post-stroke inflammatory responses.

216

217 **KLF11 alleviates OGD-induced BBB leakage in an *in vitro* BBB model.** To further

218 investigate endothelial KLF11-mediated BBB protection against ischemic insults, we employed

219 a two-cell based *in vitro* BBB model. The co-culture system consists of mouse astrocytes that are

220 seeded on the bottom of a 12-well plate and adenovirus-transduced mouse BMECs that are

221 seeded on the polyester membrane of transwell inserts (Fig. 6A). The co-culture system was then

222 subjected to ischemic-like insult of OGD for 16 h, followed by 2 h reoxygenation.

223 Transepithelial/transendothelial electrical resistance (TEER) was measured to monitor the

224 functional integrity of the cellular barrier (51). When KLF11 was overexpressed in mBMECs by

225   adenovirus-mediated infection before being subjected to OGD, the barrier functional disruption
226   induced by OGD was attenuated in the Ad. KLF11group when compared with Ad. LacZ control
227   group (Fig. 6B). On the contrary, OGD-induced barrier functional disruption was worsened in
228   the adenoviral KLF11 knockdown group (Ad. shKLF11) compared with Ad. shLacZ control
229   group (Fig. 6C). Next, luminal-to-abluminal barrier permeability (paracellular permeability) of
230   the small fluorescent tracer (3 kDa Dextran) was measured at indicated time points after OGD.
231   When KLF11 was upregulated by adenovirus in mBMECs prior to OGD, leakage of the 3 kDa
232   tracer was significantly reduced, but not completely blocked, at 2-8 h post-OGD reoxygenation
233   (Fig. 6D). In contrast, when KLF11 was downregulated in mBMECs by adenovirus-mediated
234   infection (Ad. shKLF11) prior to OGD, leakage of the 3 kDa tracer was significantly increased at
235   2-24 h post-OGD reoxygenation compared to the Ad. shLacZ group (Fig. 6E). Based on the
236   above observations, we conclude that KLF11 overexpression attenuates OGD-induced
237   endothelial barrier functional disruption *in vitro,* while KLF11 knockdown reverses this effect.
238

239   **KLF11 inhibits OGD-induced disruption of junctional proteins in cultured brain**
240   **microvascular endothelial cells.** To dissect functional roles and molecular mechanisms of
241   KLF11 in stabilizing BBB integrity in response to ischemic stimuli, mouse primary BMECs
242   were subjected to ischemic-like insult of OGD for 16 h, followed by 24 or 48 h reoxygenation.
243   We found adenovirus-mediated overexpression of KLF11 blocked OGD-induced upregulation of
244   pro-inflammatory cytokines IL-6 and MCP-1 at both the mRNA (Fig. S2 A-B) and protein levels
245   (Fig. S2 C-D). Moreover, OGD stimuli significantly reduced the expression of TJ proteins ZO-1
246   and occludin in mouse BMECs (Fig. 7 A-D). As expected, adenovirus-mediated KLF11
247   overexpression successfully preserved the expression of both TJ proteins at the mRNA (Fig. 7 A-
248   B) and protein (Fig. 7 C-D) levels, consistent with previous observations in EC-KLF11 Tg mice
249   (Fig. 4). Immunocytochemical studies also confirmed decreased ZO-1 and occludin expression
250   after 4h OGD treatment (Fig. 7E). This effect was markedly attenuated in mouse BMECs
251   overexpressing KLF11 (Fig. 7E), indicating KLF11 overexpression preserves the integrity of
252   tight junction proteins after OGD.
253

254   **KLF11 regulates the expression of tight junction proteins ZO-1 and Occludin at the**
255   **transcription level.**   As a member of the KLF family of transcription factors, KLF11 is known

256   to bind to GC-rich target sequences on promoters (38). We analyzed mouse *ZO-1* and *occludin*

257   promoters and identified three potential KLF11 binding sequences on each respective promoter

258   (Fig. 8A), suggesting KLF11 may transcriptionally regulate ZO-1 and occludin. To confirm that

259   KLF11 regulates *ZO-1* and *occludin* through direct interaction with predicted binding sequences

260   located in the promoter regions of these two genes, we cloned the promoter region of mouse ZO-

261   1 and occludin, respectively into a dual-luciferase reporter vector (pGL4.10 [*luc2*]). We also

262   clone the deleted promoters of mouse *ZO-1* and *occludin* (without the three potential KLF11

263   binding sites) into the pGL4.10[luc2] vector (Fig. 8B, 8E). We then co-transfected mouse bEnd.3

264   cells with these luciferase reporter constructs and a *Renilla* luciferase control reporter vector

265   (pRL-TK). We found adenovirus-mediated KLF11 overexpression significantly increased

266   luciferase activity of the reporter constructs containing wild type *ZO-1* (pGL4_mZO-1) and

267   *occludin* (pGL4_mOccludin) promoter sequences (Fig. 8C, 8F), whereas adenovirus-mediated

268   KLF11 knockdown significantly decreased the luciferase activity of these two promoter

269   constructs (Fig. 8D, 8G). In addition, neither adenovirus-mediated KLF11 overexpression nor

270   KLF11 knockdown affects the luciferase activities of the two deleted promoter constructs

271   (pGL4_Δ mZO-1, pGL4_Δ mOccludin). Taken together, these results confirm that KLF11

272   transactivates *ZO-1* and *occludin* by directly binding to their promoter region.

273

274   **Discussion**

275   This study investigated the protective function of endothelial KLF11 against BBB damage in a

276   mouse ischemic stroke model. The results indicate that KLF11 transgenic overexpression in

277   endothelium attenuates post-ischemic cerebrovascular permeability, brain water accumulation,

278   and brain infarction, presumably through blocking the disruption of the junctional proteins, ZO-1

279   and occludin. By preserving the integrity of the BBB after ischemic insult, EC-targeted KLF11

280   overexpression effectively reduces the infiltration of neutrophils into brain parenchyma and the

281   production of proinflammatory factors in the injured brain, thereby eliciting neurovascular

282   protection against neurological deficits. Our results also demonstrate that KLF11 transactivates

283   ZO-1 and occludin through direct binding to their promoters. Taken together, our findings

284   suggest EC-targeted KLF11 transgenic overexpression is effective in mitigating BBB disruption

285   and improving overall stroke outcome.

286   The functional involvement of KLF family members has been reported in various developmental
287   and pathological vascular processes (2, 52). KLF2 has been demonstrated to regulate endothelial
288   proliferation, migration, and angiogenesis (8, 9, 13). KLF2 has also been reported to regulate
289   vasoreactivity and vascular tone (13, 14, 43). Another KLF family member, KLF4, has been
290   reported as an endothelial regulator in response to pro-inflammatory stimuli (27) and shear stress
291   (40). A study using EC-targeted KLF4 transgenic and conditional knockout mouse models
292   demonstrated vascular anti-inflammatory and anti-atherothrombotic functions of KLF4 (68).
293   KLF4 was also reported to activate VE-cadherin at the transcriptional level, and thereby maintain
294   normal endothelial barrier function (11). In addition, KLF6 has been demonstrated to play
295   functional roles in regulating vascular development, remodeling, and responses to injury (10, 12).
296   KLF14 was reported to mitigate atherosclerosis through modulating hepatic ApoA-I production
297   in mice (26). Moreover, KLF14 was demonstrated to reduce endothelial inflammation by
298   inhibiting the NF-κB signaling cascade (30). However, KLFs are less studied in the context of
299   cerebrovascular biology and diseases such as cerebral ischemia.
300   We have previously performed genome-wide screening for PPARγ coregulators, and identified
301   KLF11 as a novel PPARγ coregulator (62). We further confirmed a physical interaction between
302   PPARγ and KLF11, and the regulatory effects of KLF11 on PPARγ-mediated cerebrovascular
303   protection in primary BMEC cultures and mouse brain after *in vitro* (OGD) and *in vivo* (MCAO)
304   ischemic insults. Later (53), we provided direct evidence of KLF11 itself in the regulation of
305   ischemic brain injury. We found that conventional KLF11 KO mice had larger brain infarcts,
306   along with worsened neurobehavioral performance, increased edema, and greater BBB
307   disruption compared with WT mice (53). Our studies suggest KLF11 as an endogenous
308   protective mediator of ischemic stroke. However, the regulatory role of vascular KLF11 and the
309   underlying mechanisms of KLF11 itself in mediating protection against BBB leakage, especially
310   at the early onset (1-3 h) following ischemic insult, remain unknown.
311   In this manuscript, we found that KLF11 expression was significantly downregulated in cultured
312   BMECs and mouse cerebral microvessels after *in vitro* (OGD) and *in vivo* (MCAO) ischemic
313   insults (Fig. S1). Brain microvascular ECs are the major components of the BBB, and along with
314   pericytes, astrocytes, perivascular microglia, and the basal lamina, they form the neurovascular
315   unit (29, 31). Given the importance of brain microvascular ECs in the formation of the BBB as
316   well as in the pathogenesis of stroke, we therefore generated transgenic mice with vascular EC-

317   targeted transgenic overexpression of KLF11 driven by the Tie-2 promoter. Our results have

318   shown that KLF11 overexpression in ECs not only reduces MCAO-induced BBB permeability

319   and disruption (Fig. 2) but also leads to better neurological outcomes (Fig. 3). Moreover, in our

320   two-cell based *in vitro* BBB model, we determined that KLF11 overexpression effectively

321   preserved endothelial barrier integrity following ischemic-like conditions (Fig. 6). These findings

322   suggest that endothelial KLF11 contributes to its overall protective effects against ischemic

323   insults, therefore opening the possibility of potential therapeutic applications for KLF11.

324   There are numerous well studied mechanisms that contribute to ischemic-induced BBB

325   disruption, such as increased oxidative stress, vascular inflammation, activation of matrix

326   metalloproteinases (MMPs), loss of BBB cellular components, and abnormal pathologies of

327   endothelial tight junctions (33, 37, 44, 46, 49, 55, 60). A recent study demonstrated that MCAO-

328   induced early structural disruption in brain microvascular ECs, including actin stress fiber

329   formation and redistribution of junctional proteins, resulted in impaired BBB integrity (49). This

330   early BBB disruption further facilitates MMP-mediated (especially gelatinase B/MMP-9)

331   degradation of EC tight junctions/basal lamina and the consequent infiltration of circulating

332   immune cells, leading to further BBB breakdown and secondary expansion of ischemic injury

333   (41, 49). In our current study, we found that EC-targeted KLF11 transgenic overexpression

334   ameliorates MCAO-induced early (1-3 h) and late (24 h) onset of BBB impairment (Fig. 2E),

335   through preserving the integrity of tight junction proteins, ZO-1 and occludin (Fig. 5). It remains

336   to be determined whether and how endothelial KLF11 affects MMP-mediated late-stage BBB

337   disruption.

338   Brain ECs express TJ proteins at relatively high levels, thereby maintaining low permeability

339   between blood components and the cerebrospinal fluid (6). The three primary TJ proteins

340   expressed in brain ECs, occludin, claudins, and JAM, are tethered to the actin cytoskeleton by

341   scaffold proteins such as ZO-1, AF6, and cingulin (28). As a member of the zinc finger family of

342   transcription factors, KLF11 binds to GC-rich regions on gene promoters (38). We analyzed the

343   promoter regions of all TJ proteins, and discovered three KFL11 binding motifs on mouse ZO-1

344   and occludin promoters. Further, we confirmed the direct binding of KLF11 to these promoters

345   and transactivation activities on ZO-1 and occludin by dual-luciferase assays (Fig. 8). Although

346   in the current study we did not take into consideration that TJs are known substrates of MMPs

347   (37), whether EC-KFL11 mediated transactivation and upregulation of ZO-1 and occludin are

348 further regulated by MMPs need further investigation. Moreover, increased transcytosis in CNS
349 endothelial cells may also lead to BBB disruption (3). Ben-Zvi et al. reported that genetic
350 deletion of major facilitator superfamily domain containing 2a that is specifically expressed in
351 CNS ECs results in a leaky BBB due to increased transcytotic vesicles within ECs, without
352 affecting tight junctions (6). In our studies, EC-KLF11 Tg mice preserved relatively intact TJs
353 but still developed BBB breakdown to some extent after MCAO, suggesting the possible
354 involvement of transcellular pathway. Whether KLF11 plays a role in transcytosis needs further
355 investigation.

356 Loosening of EC junctions due to ischemic insult facilitates the infiltration of circulating
357 immune cells and inflammatory factors, thereby producing serious clinical consequences other
358 than ischemic injuries, such as vasogenic edema and hemorrhagic transformation (49). We
359 previously observed that KLF11 KO mice show aggravated brain edema after ischemic insult
360 when compared with WT controls (53), whereas in the current study, EC-targeted KLF11
361 transgenic overexpression alleviates edema in the ischemic brain (Fig. 2I). We also observed less
362 infiltration of neutrophils into the ischemic brain of EC-KLF11 Tg mice (Fig. 2K). Moreover,
363 the expression of six inflammatory factors (TNF-α, IL-1β, MCP-1, IL-6, ICAM-1, p-selectin)
364 that are frequently upregulated after ischemic insult were found to be significantly reduced in the
365 ischemic brain of EC-KLF11 Tg mice compared with WT controls (Fig. 5). This is consistent
366 with our previous report that genetic deletion of KLF11 in mice further aggravated MCAO-
367 induced upregulation of IL-6 (53), a major stroke-related pro-inflammatory cytokine. Our
368 observations are also consistent with one previous publication that demonstrated
369 lipopolysaccharide (LPS)-induced endothelial inflammation can be inhibited by KLF11 (19).
370 They reported that compared with WT mice, LPS-induced leukocyte rolling and adhesion on
371 postcapillary venular endothelium were further augmented in KLF11 KO mice. In addition, the
372 expression levels of VCAM-1 and E-selectin were significantly increased in the aortas of KLF11
373 KO mice (19). Therefore, KLF11-triggered anti-inflammation mechanisms may contribute to its
374 overall protective functions against BBB damage and ischemic neurovascular injuries. Taken
375 together, our results demonstrate that endothelial KLF11-mediated improvements in BBB
376 tightness or stabilization limited the infiltration of blood neutrophils and reduced the release of
377 inflammatory mediators into the ischemic brain, ultimately reducing secondary brain injuries

378 caused by neuroinflammation. Also, the regulatory effects of vascular KLF11 on other blood

379 immune cells need further attention.

380 In conclusion, our study demonstrates that EC-targeted KLF11 transgenic overexpression

381 mitigates both early and late BBB disruption through preserving the integrity of TJ proteins and

382 hindering the progression of neuroinflammation, leading to an overall functional improvement

383 after ischemic stroke. Our results may have potential clinical applications because cerebral

384 endothelial barrier disruption is important contributor to several pathological processes in the

385 brain, especially those associated with inflammatory brain disorders. Therefore, the findings of

386 this study may be applied to other disease processes including but not limiting to ischemic stroke.

387

388 **Materials and methods**

389 All procedures using laboratory animals were approved by University of Pittsburgh Institutional

390 Animal Care and Use Committee, and performed in accordance with the National Institutes of

391 Health Guide for the Care and Use of Laboratory Animals. All mice were randomly assigned to

392 various experimental groups using a lottery box. All surgical preparation, stroke outcome

393 assessments, neurobehavioral tests and data analysis were performed in blinded manner.

394 EC-KLF11 Tg and littermate wild-type control (WT) mice (male, 8-10 weeks-old, body weight

395 23-25g) were housed in a temperature- and humidity-controlled animal facility with a 12 h

396 light/dark cycle, and with unlimited access to food and water. Animals that did not show a more

397 than 75% CBF reduction or a less than 60% reperfusion over baseline levels or died after

398 ischemic induction (~10% of stroke animals) were excluded from further experimentation. In

399 this study, we used 138 MCAO-operated mice (WT, n=69; EC-KLF11 Tg, n=69). Sham-

400 operated mice were used as controls (WT, n=28; EC-KLF11 Tg, n=28).

401 **Generation of EC-selective KLF11 transgenic (EC-KLF11 Tg) mice.** Transgenic mice with

402 endothelial cell-selective KLF11 overexpression were generated as described (64). A 1539-bp

403 DNA fragment containing a KLF11 coding sequence was PCR amplified from human genomic

404 DNA using the primers 5'-GATATCATGCACACGCCGGACTTCGCAGGCC-3' (Forward)

405 and 5'-GATATCTCAGGCAGAGGCTGGCATGCTCACC-3' (Reverse). The DNA fragment

406 was then cloned into the EcoRV site of the pBluescript II SK (+) vector to generate a pBlue-

407 KLF11 plasmid. To generate the Tie-2 promoter-driven pBlue-KLF11 construct, we then

408 inserted the Tie-2 promoter (2,089 bp) (a generous gift from Dr. Sato, (47)) into the HindIII site

409 and the polyA plus full Tie-2 enhancer (10,367 bp) (a generous gift from Dr. Sato, (47)) into the

410 Xba I/Not I cloning site on pBlue-KLF11 plasmid, respectively. The construct was injected into

411 fertilized C57 mouse oocytes and implanted into pseudo-pregnant female mice. Transgenic

412 founder mice were identified by PCR genotyping using primers 5'-

413 CTGTGCTCAGACAGAAATGAGAC-3' (forward) and 5'-ATCATCTGGCAAAGGACAGG-

414 3' (reverse). The PCR amplification conditions were 94 °C x 5 min, 40 cycles of 94 °C x 30 sec,

415 55 °C x 30 sec, 72 °C x 80 sec, followed by 72 °C x 5 min. This produced a 1.2 kb DNA

416 fragment that contains both plasmid and Tie-2 promoter sequences. EC-KLF11 Tg mice are

417 viable, fertile, normal in size, and do not display any gross physical or behavioral abnormalities.

418 **Mouse model of transient focal cerebral ischemia.** Focal cerebral ischemia was induced in

419 adult male mice (8-10-weeks-old, 25-30 g) by intraluminal middle cerebral artery occlusion

420 (MCAO) as described previously (53, 59, 61, 67). Briefly, mice were anesthetized with 1.5-3%

421 isoflurane (Henry Schein Animal Health). A 2-cm length of a 7-0 rounded tip nylon suture was

422 gently advanced from the internal carotid artery up to the origin of the middle cerebral artery

423 (MCA) until regional cerebral blood flow (rCBF) was reduced to less than 25% of baseline.

424 After 60 minutes of MCAO, blood flow was restored by removing the suture, and mice were

425 allowed to recover for 1-7 days. In sham-operated mice, the same surgical procedure was

426 performed except for suture insertion. Changes in rCBF, arterial blood gases, mean arterial

427 pressure, and heart rate were monitored in animals 15 min before, during, and 15 min after

428 MCAO. Animals that did not show a CBF reduction of at least 75% over baseline levels were

429 excluded from further experimentation. Approximately 90% survival rate was observed in EC-

430 KLF11 Tg or WT mice at 1-7 d after MCAO. Animals that died after ischemic induction were

431 also excluded. The rectal temperature was controlled at 37.0 ± 0.5°C during surgery.

432 **Measurement of infarct volume, neurological deficits, and sensorimotor function.** 2,3,5-

433 triphenyltetrazolium (TTC) staining was performed to measure brain infarct after MCAO in

434 some mice. These mice were sacrificed, and the brains were harvested 24 h after MCAO. The

435 forebrain was sliced into seven coronal sections, each 1 mm thick. Sections were stained with

436 2% TTC in 0.9% NaCl for 20 min, followed by fixation with 4% paraformaldehyde in PBS.

437 Infarct volume was determined with ImageJ (National Institute of Health) as the volume of the

438 contralateral hemisphere minus the non-infarcted volume of the ipsilateral hemisphere.

439   Neurobehavioral deficits were determined by the adhesive removal test, foot fault test, and

440   rotarod test 1-3 days before MCAO and also at 3, 5 and 7 days of reperfusion after MCAO (53,

441   54, 59, 67). Following cerebral ischemia, mice were also tested for neurological deficits and

442   scored on a 5-point scale: 0, no observable neurological deficits (normal); 1, failure to extend

443   right forepaw (mild); 2, circling to the contralateral side (moderate); 3, falling to the right

444   (severe); 4, mice could not walk spontaneously; 5, depressed level of consciousness (very

445   severe).

446   **Immunohistochemistry and image analysis.** At different time points following MCAO, EC-

447   KLF11 Tg and WT mice were anesthetized with carbon dioxide and transcardially perfused with

448   0.9% NaCl followed by 4% paraformaldehyde in PBS. Brains were harvested and cryoprotected

449   in 30% sucrose in PBS, and frozen serial coronal brain sections (30 μm thick) were prepared on

450   a cryostat (CM1900, Leica). Brain sections were blocked with 5% normal goat serum in PBS for

451   1 h, followed by overnight incubation (4 °C) with the following primary antibodies: mouse anti-

452   MAP2 (1:200; EMD Millipore, Billerica, MA), rabbit anti-NeuN (1:500; EMD Millipore), rat

453   anti-CD31 (1:200; BD Biosciences, San Jose, CA), mouse anti-ZO-1 (1:100; Invitrogen,

454   Carlsbad, CA), rat anti-Ly-6B (1:100; Abcam, Cambridge, UK), rabbit anti-Occludin (1:100;

455   Thermo Fisher Scientific, Waltham, MA) (Supplementary Table S1). Secondary antibodies:

456   Cy3-conjugated goat anti-rat IgG, Alexa Fluor 488-conjugated goat anti-rabbit IgG, and goat

457   anti-mouse IgG (all at 1:400; The Jackson ImmunoResearch Lab). Images were collected by

458   confocal microscopy (FV1000-II; Olympus; Center for Biological Imaging, University of

459   Pittsburgh Medical School) and processed in Adobe Photoshop for compositions (17). Five

460   different sections from each animal were photographed, and six ROIs were randomly selected

461   from the infarct core and inner infarct border, respectively, on each section. Infarct volume was

462   measured on seven equally spaced MAP2-stained sections encompassing the MCA territory

463   using ImageJ. Capillary densities were examined by counting the number of capillaries stained

464   with the anti-CD31 antibody as previously described (56).

465   **Quantification of BBB permeability/leakage after MCAO.** For analysis of cerebrovascular

466   permeability by Evans Blue extravasation, mice were injected with 100 μl of 4% Evans Blue

467   (EB) (Sigma-Aldrich, St. Louis, MO) through tail vein 23 h after MCAO. After 1 h, mice were

468   perfused with 0.9% NaCl, mouse brains were then removed and separated into ipsilateral and

469   contralateral hemispheres. Each hemisphere was homogenized in *N, N*-dimethylformamide

470   (Sigma-Aldrich) and centrifuged for 45 min at 25,000 rcf. The supernatants were collected, and

471   EB levels in each hemisphere were calculated using the formula: $(A_{620\ nm} - (A_{500\ nm} + A_{740\ nm}) /$

472    $2) /$ mg wet weight. Background EB levels in the nonischemic hemisphere were subtracted from

473   the ischemic hemisphere ipsilateral to the MCAO.

474   For analysis of cerebrovascular permeability by intravenous injection and detection of

475   fluorescent-labeled Dextran and fluorescent tracer, mice were subjected to tail vein injection of

476   either Tetramethylrhodamine-Dextran (TMR-Dextran, 70 kDa; 0.1 mg/g body weight,

477   Invitrogen) or Alexa Fluor 555-conjugated cadaverine (0.95 kDa; Invitrogen) 30 min before

478   sacrifice. Mice were anesthetized and transcardially perfused with 0.9% NaCl followed by 4%

479   paraformaldehyde in PBS. Mouse brains were collected and cryoprotected in 30% sucrose in

480   PBS, and frozen serial coronal brain sections (30 μm thick) were prepared on a cryostat. Brain

481   sections were visualized directly under a fluorescent microscope. In parallel, brain hemispheres

482   were homogenized in 1% Triton X-100 (Sigma-Aldrich) in PBS and fluorescent intensity was

483   quantified by a SpectraMax i3x plate reader (Molecular Devices, San Jose, CA) using 555-nm

484   excitation and 580-nm emission.

485   To measure the extravasation of endogenous IgG, sections were blocked in avidin and biotin

486   solution (two drops of avidin/biotin solution into 10 mL of PBS; Vector Laboratories,

487   Burlingame, CA) for 15 min each, followed by blocking in 5% NDS for 1 h. Sections were then

488   incubated with biotinylated anti-mouse IgG (1:500; Vector Laboratories) at 4 °C overnight.

489   Sections were then incubated with Alexa 488 Streptavidin (1:1,000; The Jackson

490   ImmunoResearch Labs). Whole-section images were acquired using an inverted Nikon

491   fluorescence microscope. Six equally spaced sections encompassing the MCA territory were

492   quantified for cross-sectional area of fluorescence. These areas were summed and multiplied by

493   the distance between sections (1 mm) to yield a volume of leakage (in mm^3).

494   **Adenovirus-mediated gain- or loss- of- KLF11 expression in BMECs.** To generate adenoviral

495   vectors overexpressing KLF11 (19), the coding sequences of human KLF11 was PCR-amplified

496   with primers 5'-ATGCACACGCCGGACTTCGCAGG-3' (Forward) and 5'-TCAGGCA

497   GAGGCTGGCATGCTCA-3' (Reverse), and subcloned into the pCR8⁄GW⁄TOPO entry vector

498   (Invitrogen). To generate adenoviral vectors for overexpressing LacZ, the coding sequences of

499   *Escherichia coli* LacZ were PCR amplified with primers 5'-

500   ATGTCGTTTACTTTGACCAACA-3' (Forward) and 5'-

501 TTATTTTTGACACCAGACCAACT-3' (Reverse), and subcloned into the pCR8⁄GW⁄TOPO

502 entry vector (Invitrogen). After sequencing, the LR recombination reactions were carried out

503 between the entry clone pCR8⁄GW⁄TOPO/KLF11 and the destination vector pAd/CMV/V5-

504 DEST according to the manufacturer's protocol (Invitrogen). For knockdown experiments, an

505 siRNA oligo, which targets a region 100% conserved between human and mouse, was purchased

506 from Invitrogen. To prepare adenovirus-containing shRNA for KLF11 or LacZ, synthesized

507 oligos were annealed and inserted into the BLOCK-iT U6 entry vector. The U6 promoter and

508 shRNA were cloned into the adenoviral plasmid pAd/BLOCK-iT-DEST according to the

509 manufacturer's instructions. The sequences for shRNA were as follows:

510 shKLF11, 5'-CACCGGGTAGACTTTTCCCGAAGGCGAACCTTCGGGAAAAGTCTACC-3',

511   5'-AAAAGGTAGACTTTTCCCGAAGGTTCG CCTTCGGGAAAAGTCTACCC-3'.

512 shLacZ,   5'-CACCGCTACACAAATCAGCGATTTCGAAAAATCGCTGATTTGTGTAG-3',

513   5'-AAAACTACACAAATCAGCGATTTTTCGAAATCGCTGATTTGTGTAGC-3'.

514 To package the adenoviruses, adenoviral vectors were linearized with *PacI* and transfected into

515 HEK293 AD cells using Lipofectamine 2000. The recombinant adenoviruses were purified by

516 CsCl2 density gradient ultracentrifugation. Adenovirus genomic DNA was purified with

517 NucleoSpin Virus Kit (Macherey Nagel), the adenovirus titration was determined using the

518 Adeno-X™ qPCR Titration Kit (Clontech). The generated adenovirus was used to infect BMECs

519 for 48-72 h.

520 **Two cell based *in vitro* BBB model.** Mouse astrocytes were purchased from Sciencell research

521 laboratories (M1800-57). Astrocytes (2-8 passages) were grown in astrocyte medium (AM-a

522 1831; Sciencell) and were seeded in a regular 12-well plate at 37 ºC in a humidified incubator

523 until reaching confluence. Transwell chamber inserts with PET membranes (0.4 μm pore;

524 Corning) were coated with collagen type IV (0.3 mg/ml) and fibronectin (0.5 mg/ml). Mouse

525 BMECs were seeded onto the membrane at a density of 1 x 10^5 cells per insert. The inserts were

526 then placed into the 12-well plates containing confluent astrocytes (Figure 7A) and co-cultures

527 were maintained in endothelial growth medium (M1168; Cell Biologics) at 37 ºC in a humidified

528 incubator overnight. The inserts seeded with mBMECs were then separated from the co-culture

529 system and mBMECs were infected with either Ad. LacZ/Ad. KLF11 or Ad. shLacZ/Ad.

530 shKLF11 in endothelial cell infection medium without phenol red and antibiotics (M1168PF;

531 Cell Biologics) for 6 hours. The inserts were placed back to the 12-well plate containing

532 confluent astrocytes and co-cultures were maintained in endothelial growth medium to reach

533 confluence.

534 Changes in transendothelial electrical resistance (TEER) at designated time points or conditions

535 were used to detect permeability changes in the BBB. The reading of total resistance (R_{Total}) was

536 measured with an Epithelial Volt/Ohm Meter (WPI, FL) at room temperature. The value of each

537 sample (R_{TEER}) was corrected by the reading of an empty coated-insert cell (R_{Blank}) and

538 calculated with the polyester membrane area ($S_{Membrane}$) using the following formula and TEER

539 values were reported in units of Ω-cm^2:

540 $$R_{TEER} = (R_{Total} - R_{Blank}) \text{ x } S_{Membrane}$$

541 To assess paracellular permeability, Dextran Alexa Fluor 488 (3,000 MW) was added to the

542 luminal chamber at a concentration of 1μg per 1ml medium. At 0.5h, 1h, 2h, 4h, 8h and 24h after

543 OGD treatment, 50 μl medium was collected from the abluminal chamber. Fluorescence

544 intensity was measured with a SpectraMax i3x Multi-Mode Detection Platform. The

545 accumulated fluorescence intensity of abluminal Dextran Alexa Fluor 488 was measured at each

546 time point and corrected by the respective blank (same treatment without adding Dextran in the

547 luminal). Relative fluorescence unit (RFU) was recorded.

548 **Cerebral microvessel isolation.** Cerebral microvessels from mouse brain were isolated for

549 determining the expression of KLF11, as previously described with modification (61). Briefly,

550 EC-KLF11 Tg and littermate WT control mice were anesthetized with carbon dioxide and

551 transcardially perfused with ice cold 0.9% NaCl. The brain was immediately removed from the

552 skull and immersed in ice cold PBS. Brainstem, meninges and pia vessels were quickly removed,

553 and the brain was cut into 1mm block and transfer to a 15 mL Dounce Tissue Grinder Tube

554 (Kimble Chase, TN) with 5 mL buffer TE (a mixture of 0.25% Trypsin-EDTA and DMEM

555 (Invitrogen, CA) at 1:1). The brain was homogenized by 5 strokes with a small clearance pestle.

556 The homogenate was mixed with another 5 mL of buffer TE and incubate at 37 °C for 1 h with

557 occasional agitation. After triturating 10-20 times with a 5 mL pipette, the creamy texture was

558 centrifuged at 500 x g for 10 min at room temperature. The pellet was collected and dissolved in

559 8 mL HBSS (Invitrogen, CA) by mixing twice with a 5 mL pipette, and the homogenate was

560 suspended in 11 mL HBSS dissolved 25% BSA by gently triturate 5 times with a 10 mL pipette.

561 The homogenate was centrifuged at 3,000 x g for 15 min at 4 °C to separate the lipid and the

562 capillary fraction. The pellet was collected and rinsed with 20 mL PBS once followed by another

563   centrifuge at 1,800 x g for 10 min. The pellet was rinsed with 1 mL PBS, transferred to a 1.5 ml

564   centrifuge tube and spun down at 16,000 x g for 1 min. The final microvessel pellet was stored at

565   -80 °C freezer until use.

566   **Brain water content.** Brain water content was measured by the dry-wet method as described

567   previously (53). Following MCAO, mice were sacrificed by exposure to CO_2. The weights of the

568   ipsilateral and contralateral hemispheres were recorded separately as wet weights. The dry

569   weights of the ipsilateral and contralateral hemispheres were obtained after being heated at 100

570   °C in an oven for 24 h. Brain content was calculated by the following formula: brain content =

571   (wet weight - dry weight)/wet weight x 100%.

572   **Cell cultures and oxygen-glucose deprivation (OGD).** Mouse primary brain microvascular

573   endothelial cells (mBMECs) were purchased from Cell Biologics (C57-6023). Mouse brain

574   microvascular endothelial cells, bEnd.3 were purchased from American Type Culture Collection

575   (CRL-2299, ATCC, Manassas, VA). Mouse BMECs and bEnd.3 cells (2-8 passages) were

576   grown to 85-95% confluency before using for studies. To mimic ischemia-like conditions *in*

577   *vitro*, mouse BMEC cultures were exposed to OGD for up to 16 h. Briefly, culture medium was

578   replaced with deoxygenated glucose-free Dulbecco's Modified Eagle Media (DMEM; Gibco,

579   Grand Island, NY), and cultures were flushed with 95% N_2 and 5% CO_2 for 5 min in a Billups-

580   Rothenberg modular incubator chamber (Del Mar, San Diego, CA). The modular incubator

581   chamber was then sealed and placed in a water-jacketed incubator (Forma, thermo Fisher

582   Scientific, Waltham, MA) at 37 ˚C for the indicated period of time in each experiment before

583   returning to humidified 95% air and 5% CO_2 and glucose-containing medium (62, 67).

584   **Immunocytochemistry.** Mouse BMEC cells were seeded in 4-chamber polystyrene vessel tissue

585   culture treated glass slides (Corning, NY) and infected with Ad. LacZ or Ad. KLF11. Seventy-

586   two hours after infection, BMEC cultures were subjected to OGD treatment and reperfusion for

587   indicated time points. The cells were then fixed in 4% paraformaldehyde followed by blocking

588   with 5% normal goat serum in PBST for 1 h at room temperature. The cells were then incubated

589   with the following primary antibodies overnight at 4 °C: mouse anti-ZO-1 (1:100; Invitrogen,

590   Carlsbad, CA), rabbit anti-Occludin (1:100; Thermo Fisher Scientific, Waltham, MA). After

591   rinse in PBS, cells were incubated with secondary antibodies: Alexa Fluor 488-conjugated goat

592   anti-rabbit IgG, and goat anti-mouse IgG (all at 1:400; The Jackson ImmunoResearch

593   Laboratories). After PBS rinses, cells were counterstained with DAPI for nuclear labeling and

594   mounted with antifade Vecta-Shield solution (Vector Laboratories). Images were collected on an

595   Olympus FV1000-II confocal microscope and processed in Adobe Photoshop for compositions

596   (17). Immunofluorescence intensities of ZO-1 and occludin were quantified by ImageJ. Three

597   ROIs were randomly selected from each chamber, and 2-3 chambers were quantified for each

598   experimental condition in each independent culture.

599   **Molecular cloning.** The pGL4.10[*luc2*] vector was purchased from Promega. To make

600   pGL4_mZO-1 and pGL4_mOccludin promoter reporter constructs, a 1,013 bp fragment of the

601   promoter region of mouse ZO-1 or a 1,014 bp fragment of mouse occludin promoter region

602   containing the putative KLF11 binding sequence was PCR-amplified from mouse genomic DNA

603   using   primers   5-CGAGACGCTAGCCTTGACTTTGAAACCTTAATTGATG   and   5-

604   CTGGACCTCGAGGCAAAACCTGCCGGACCGGGCCACT,   or   5-

605   CGAGACGCTAGCAGATAGTTAACTAACAAGAACTAAAATCTC   and   5-

606   CTGGACCTCGAGCCTACCCCCGGGCATGCGCACCAATT. Both promoter fragments were

607   then subcloned into the pGL4.10[luc2] vector, respectively. Mutant promoter constructs where

608   all three putative KLF11 binding sequences were deleted were generated by QuikChange XL

609   Site-directed Mutagenesis kit (Stratagene, Santa Clara, CA) (64). All constructs were validated

610   by DNA sequencing (Genewiz, South Plainfield, NJ). NCBI RefSeq ID: ZO-1 (mouse):

611   NP_001157046.1, occludin (mouse): NP_001347465.1. Supplementary Table S2.

612   **Dual-luciferase reporter assays.** Mouse bEnd.3 cells (ATCC, Manassas, VA) were seeded at

613   0.5 x 10^5 cells/well in 24-well plates. After overnight incubation, cells were infected with

614   different adenoviruses aimed at achieving KLF11 overexpression or knockdown. The cells were

615   also co-transfected with pGL4_mZO-1/ΔmZO-1 or pGL4_mOccludin/ΔmOccludin luciferase

616   reporter vector and a *Renilla* luciferase control reporter vector (pRL-TK; Promega), along with

617   Lipofectamine 2000 (Invitrogen) for 5 h. Luciferase activity was measured 48 h after transfection

618   by Dual-Luciferase assay kits (Promega) using a SpectraMax i3x Multi-Mode Detection

619   Platform. Individual luciferase activity was normalized to the corresponding *Renilla*-luciferase

620   activity (62, 64).

621   **Quantitative real time PCR.** Total RNA was isolated from BMEC cultures or cerebral cortex

622   by using RNeasy Mini Kit (Qiagen, Valencia, CA) or Trizol (Invitrogen, Carlsbad, CA).

623   Quantitative real-time reverse-transcriptase polymerase chain reaction (RT-PCR) was carried out

624   with a Bio-Rad CFX Connect thermocycler, iScript cDNA synthesis kit and iTaq Universal

625 SYBR green supermix (Bio-Rad, Hercules, CA). Specific primers used for the reactions are ZO-
626 1 Forward, 5'-gccgctaagagcacagcaa-3'; ZO-1 Reverse, 5'-tccccactctgaaaatgagga-3'. Occludin
627 Forward, 5'-tgaaagtccacctccttacaga-3'; Occludin Reverse, 5'-ccgcataaaaagagtacgctgg-3'. TNF-α
628 Forward, 5'-ctcctcacccacaccgtcagc-3'; TNF-α Reverse, 5'-aacacccattcccttcacagagca-3'. IL-1β
629 Forward, 5'-aggagaaccaagcaacgacaaaatac-3'; IL-1β Reverse, 5'-tggggaactctgcagactcaaact-3'.
630 MCP-1 Forward, 5'-gcaccagcaccagccaactctcact-3'; MCP-1 Reverse, 5'-cattccttcttggggtcagcacag-
631 3'. IL-6 Forward, 5'-agttgccttcttgggactga-3'; IL-6 Reverse, 5'-tccacgatttcccagagaac-3'. Icam-1
632 Forward, 5'-ttcacactgaatgccagctc-3'; Icam-2 Reverse, 5'-gtctgctgagacccctcttg-3'. P-selectin
633 Forward, 5'-gtccacggagagtttggtgt-3'; P-selectin Reverse, 5'-aagtggtgttcggaccaaag-3'.
634 Cyclophilin Forward, 5'-actcctcatttagatgggcatca-3'; Cyclophilin Reverse, 5'-
635 gagtatccgtacctccgcaaa-3' (Supplementary Table S3). The relative mRNA expression was
636 normalized to cyclophilin RNA levels. PCR experiments were repeated 3 times, each using
637 separate sets of cultures (53, 59, 67).

638 **Western blot analysis.** Samples from the 0.9% NaCl perfused mouse cerebral cortex or cultured
639 mBMECs were homogenized in T-PER Tissue Protein Extraction Reagent (Pierce, IL)
640 containing Mini Protease Inhibitor Cocktail (cOmplete™, Sigma) by Dounce Tissue Grinder or
641 sonication for 30 seconds with 30% pulse on ice respectively. The tissue or cell lysates were
642 centrifuged at 10,000 x g for 5 min to pellet tissue or cell debris. Then supernatants were
643 collected to measure protein concentrations by using the Bio-Rad Protein Assay (Bradford, Bio-
644 Rad). As described previously (53, 59, 67), equal amounts of protein were loaded into 4-15%
645 precast gels (Bio-Rad) and transferred to polyvinylidene difluoride (PVDF, Bio-Rad)
646 membranes. Membranes were blocked for 1 h with TBS/0.1%-Tween buffer plus 5% (w/v) non-
647 fat dried milk and incubated overnight at 4 °C with primary antibodies diluted in the same
648 blocking buffer. Membranes were then incubated with secondary antibodies diluted in blocking
649 buffer for 1 h and developed using a Pierce® ECL Western blotting detection kit (Thermo
650 Scientific) and Amersham High-Performance Chemiluminescence Films (GE Healthcare).
651 Various primary antibodies were used and are listed in Supplementary Table S1, including
652 mouse anti-KLF11 (1:500; NovusBio), rabbit anti-occludin (1:1,000; Invitrogen), rabbit anti-ZO-
653 1 (1:250; Abcam), and mouse anti-β-actin (1:2000; Sigma-Aldrich).

654 **Enzyme-linked immunosorbent assay (ELISA).** Cerebral cortices of mice were collected and
655 sonicated by an ultrasound homogenizer. After centrifugation, supernatants were collected and

656   concentrations of a series of pro-inflammatory chemokines, cytokines, and adhesive molecules in

657   the supernatants were determined by Quantikine ELISA kits (R&D Systems, Minneapolis, MN)

658   according to the manufacturer's instructions (67). All assays were performed in triplicates.

659   **Statistical analysis.** Quantitative data are expressed as mean ±SEM or ±SD. Differences among

660   three or more groups were statistically analyzed by one or two-way ANOVA followed by

661   Bonferroni's post-hoc test. Comparisons between two experimental groups were conducted by

662   the two-tailed Student's t-test. A *p-value* less than 0.05 was considered significant. Statistical

663   analyses and graphic representations were obtained with GraphPad Prism 7.0 software.

664

665   **Data availability statement**

666   The authors confirm that the data supporting the findings of this study are available within the

667   article and from the corresponding author upon reasonable request.

668

669   **Abbreviation:** Endothelial cell = EC; Krüppel-like factor = KLF; Blood-brain barrier = BBB;

670   Peroxisome proliferator-activated receptor gamma = PPARγ; Tight junction = TJ; Brain

671   microvascular endothelial cells = BMECs; Oxygen-Glucose Deprivation = OGD;

672   Transepithelial/transendothelial electrical resistance (TEER); Middle cerebral artery occlusion =

673   MCAO; 2,3,5-triphenyltetrazolium chloride = TTC; Cerebral blood flow = CBF; Microtubule-

674   associated protein 2 = MAP2.**Reference**

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878

Funding disclosure

882

Author contribution statement

884 X.Z., X.T., Y.E.C., and K.-J.Y. designed the research; X.Z., X.T., F.M., Y.F., T.Z., and J.Z.

885 performed research; X.Z., X.T., F.M., and K.-J.Y. analyzed data; and X.Z., M.H.H, and K.-J.Y.

886 wrote the paper.

887

Competing interests

889 None.

Figure Legends

891 **Figure 1.** Generation of endothelial cell (EC)-selective KLF11 transgenic mice. (A) Schematic

892 diagram of EC-selective KLF11 transgenic structure. The transgene cassette is composed of a

893 ~2.1-kb Tie-2 promoter, a ~10.4-kb Tie-2 enhancer, and a 1539-bp DNA fragment containing the

894 human KLF11 coding sequence. (B) Genomic PCR for genotyping EC-selective KLF11

895 transgenic mice (EC-KLF11 Tg). A 960 bp band is expected from EC-KLF11 transgenics (Lanes

896 1, 3, and 16). (C) Representative western blotting images indicated enhanced protein levels of

897 KLF11 in the cerebral microvessels (cerebral vessels, left) but not in brain parenchyma (cerebral

898 cortex, right) of EC-KLF11 Tg mice than WT controls. n=5 mice per group. (D) Representative

899 immunofluorescent images show CD31-positive capillaries in the cortex and striatum of both

900 WT and EC-KLF11 Tg mice. n=5 mice per group. Scale bar: 50 μm. (E) Quantification of

901 CD31-labeled branch points, capillary numbers, and branch length are shown. In comparison
902 with WT controls, EC-targeted transgenic overexpression of KLF11 does not affect cerebral
903 vascular density and structure in mice. Data are expressed as mean ±SEM.

904 **Figure 2.** Targeted overexpression of KLF11 in endothelium ameliorates both early- and late-
905 onset BBB impairments, edema, and neutrophil infiltration. EC-KLF11 Tg mice and WT
906 controls were subjected to 1 h MCA occlusion followed by 1, 3, or 24 h reperfusion. (A)
907 Extravasation of Evans blue dye and (B) 70 kDa TMR-Dextran 24 h after MCAO in whole
908 brains or coronal sections was shown. Scale bar, 50 μm. (C-D) Quantification of Evans blue in
909 panel A and TMR-Dextran in panel B. n=6 mice per group. (E) Representative images
910 demonstrate the extravasation of Alexa Fluor 555 (0.95 kDa, red) or endogenous plasma IgG
911 (~150 kDa, green) into brain parenchyma 1, 3, or 24 h after MCAO. At 24 h after MCAO, the
912 area with loss of microtubule-associated protein 2 (MAP2) immunofluorescence illustrates the
913 infarct zone on adjacent sections from the same brains. (Scale bar: 1 mm). (F-G) Quantitative
914 analysis of the volume of the brain with leakage of cadaverine and IgG at indicated times of
915 reperfusion after MCAO. n=5 mice per group. (H) Brain infarct volume at 24 h after MCAO
916 was quantitatively measured on MAP2-stained coronal sections. n=5 mice per group. (I) Brain
917 water content was measured by the wet/dry weight protocol as described in the "Material and
918 Methods". n=10 per group, ** $p < 0.01$ vs. WT contralateral hemisphere, # $p < 0.05$ vs. WT
919 ipsilateral hemisphere. (J) Representative images taken from the ipsilateral periinfarct cortex
920 after MCAO or the corresponding region after sham operation; markers used: Ly-6B (neutrophil),
921 and NeuN (neuron) (Scale bar: 50 μm). Rectangle: the region enlarged in high-power images
922 (third column). (Scale bar: 10 μm.) (K) Ly-6B+ cells were counted in the areas described in (J)
923 and data are expressed as the number of cells per mm2. After MCAO, the number of infiltrated
924 neutrophils is significantly less in EC-KLF11 Tg mouse brains compared with WT controls. n=5
925 mice per group. Data are expressed as mean ± SEM. * $p < 0.05$ vs. WT + sham group, # $p < 0.05$
926 vs. WT + MCAO group.

927 **Figure 3.** Transgenic overexpression of KLF11 in endothelium improves short-term histological
928 and functional outcomes after focal cerebral ischemia. (A) EC-KLF11 Tg mice and WT controls
929 were subjected to 1 h MCAO and 72 h reperfusion. Two percent TTC-stained coronal sections
930 were shown at different brain levels from the frontal to the posterior pole. (B) Quantitative
931 analysis was performed on infarct volume and (C) neurological deficits in these mice after

932   ischemic stroke. n= 8 mice per group. (D) Regional CBF was measured by using a laser speckle
933   imager. EC-KLF11 Tg and WT mice were subjected to 1 h MCAO followed by 72 h reperfusion.
934   Representative CBF images were shown at 15 min before MCAO (baseline), 15 min after the
935   onset of MCAO (ischemia), and 15 min after the onset of reperfusion (reperfusion). (E) Two
936   identical elliptical ROIs were selected as indicated on the same brain region of the ipsilateral and
937   contralateral hemispheres. The relative CBF was first determined as the ratio of ischemic to
938   nonischemic cerebral blood flow, and then as the percentage value normalized to the presurgical
939   baseline for each animal. n=6 each group. (F-I) Sensorimotor deficits were assessed before and
940   up to 7 days after MCAO by a battery of behavioral tests, including Rotarod test (F), Foot fault
941   (G), and Adhesive tape removal test (H-I). Compared with WT controls, mice with endothelial-
942   specific overexpression of KLF11 showed dramatically improved sensorimotor function
943   (increased staying latency in the rotarod test, lower rate of fault steps in the foot fault test, and
944   shorter touching or removing time in the adhesive tape removal test. Data are represented as
945   mean ± SD. n=12 per group. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ EC-KLF11 Tg MCAO vs.
946   WT MCAO by one-way ANOVA (individual time point) or two-way ANOVA (bracket).

947   **Figure 4.** EC-targeted KLF11 overexpression preserves the integrity of tight junctions after focal
948   cerebral ischemia. EC-KLF11 Tg mice and WT controls were subjected to 1 h MCA occlusion
949   and 1d or 3d reperfusion. (A-B) Total RNA was extracted from the ipsilateral cortex of EC-
950   KLF11 Tg and WT mice 1d and 3d after MCAO. ZO-1 and occludin mRNA expression levels
951   were determined by qPCR and normalized to cyclophilin (n=6 per group). (C) Total protein was
952   extracted and subjected to gel electrophoresis. The protein levels of ZO-1 and occludin were
953   determined with β-actin as the loading control. (D-E) Quantification of ZO-1 and occludin
954   protein. Experiments were repeated three times and representative blots are displayed. n=6 per
955   group. (F) Representative images and quantification of ZO-1 (green) and occludin (green) on
956   CD31+ microvessels (red) in the cortex of the ischemic hemisphere at 1d after MCAO. (Scale
957   bar: 25 μm.) Endothelial KLF11 overexpression suppressed MCAO-induced disruption of
958   junctional proteins ZO-1 and occludin. n=6 mice per group. * $p < 0.05$ vs. WT + sham group, # p
959   < 0.05 vs. WT + MCAO group.

960   **Figure 5.** Effects of EC-targeted overexpression of KLF11 on inflammatory mediators in mouse
961   cortex after focal cerebral ischemia. EC-KLF11 Tg and WT mice were subjected to sham
962   operation or 1h of MCAO followed by 1d or 3d of reperfusion. (A-F) mRNA expression levels

963 of different inflammatory markers were measured in the ipsilateral cortex by qPCR and
964 normalized to cyclophilin. (G-L) Protein levels of different inflammatory markers were analyzed
965 by ELISA. EC-targeted overexpression of KLF11 inhibited MCAO-induced upregulation of
966 TNFα, IL-1β, MCP-1, IL-6, ICAM-1, and P-selectin expression. n=6 mice per group. * $p < 0.05$
967 vs. WT + sham group, # $p < 0.05$ vs. WT + MCAO group.

968 **Figure 6.** Adenoviral gain- or loss-of-KLF11 expression in mouse BMECs affects endothelial
969 barrier function after OGD *in vitro*. (A) Illustration of the transwell co-culture system. Mouse
970 astrocytes were seeded at the bottom of a 12-well plate with transwell insert. Cultured mBMECs
971 were seeded on top of a collagen- and fibronectin-coated membrane of the insert and grown to
972 form a confluent monolayer. The mBMECs were infected for 48 h with either an empty
973 adenovirus (Ad. LacZ), adenoviral vector carrying mouse KLF11 (Ad. KLF11), adenoviral
974 vector shLacZ (Ad. shLacZ), or adenoviral vector carrying short hairpin sequences targeting
975 mouse KLF11 (Ad. shKLF11), and then subjected to OGD for 16 h before being placed back to
976 the plate with astrocytes. (B-C) Transepithelial electrical resistance (TEER) in the co-culture
977 model was measured at 2 h after OGD and in non-OGD conditions. n=3 per group, * $p < 0.05$ vs.
978 Ad. LacZ/Ad. shLacZ + non-OGD group, # $p < 0.05$ vs. Ad LacZ/Ad. shLacZ + 16h OGD-2h
979 Reoxygenation. (D-E) Paracellular permeability was quantified at 0–24 h after OGD by
980 measuring the fluorescence intensity of abluminal Dextran Alexa Fluor 488 (3,000 MW).
981 Adenovirus-mediated KLF11 overexpression improved endothelial barrier function after OGD
982 treatment, whereas KLF11 knockdown worsened endothelial barrier function. Data are expressed
983 as mean ± SEM of three independent experiments with triplicate wells (n=3 per group). * P <
984 0.05 vs. Ad. LacZ/Ad. shLacZ + 16h OGD group.

985 **Figure 7.** Adenovirus-mediated KLF11 overexpression in mouse BMEC cultures preserves the
986 integrity of tight junction proteins after OGD. Cultured mBMECs were infected with Ad. LacZ
987 or Ad. KLF11 for 48 h prior to 16 h or 4 h OGD followed by 24 h or 48 h reperfusion. (A-B) The
988 mRNA expression levels of ZO-1 and occludin were determined by qPCR and normalized to
989 cyclophilin (n=3 per group). (C-D) Expression levels of tight junction proteins, ZO-1 and
990 occludin were evaluated by western blotting. β-Actin was used as an internal loading control.
991 Data represent three independent experiments and representative blots are displayed. n=3 per
992 group. * P < 0.05 vs. Ad. LacZ + non-OGD group, # P < 0.05 vs. Ad. LacZ + OGD group. (E)
993 Representative images and quantification of ZO-1 (green) and occludin (green) in cultured

994   mBMECs after 4 h OGD, followed by 24 h reoxygenation. (Scale bar: 25 μm.) Adenoviral

995   KLF11 overexpression suppressed OGD-induced disruption of junctional proteins, ZO-1, and

996   occludin. n=3 per group. * P < 0.05 vs. Ad. LacZ + non-OGD group, # P < 0.05 vs. Ad. LacZ +

997   OGD group. H16R0 = 16h OGD + 0h reperfusion, H16R24 = 16h OGD + 24h reperfusion,

998   H16R48 = 16h OGD + 48h reperfusion, H4R0 = 4h OGD + 0h reperfusion, H4R24 = 4h OGD +

999   24h reperfusion.

1000   **Figure 8.** KLF11 transcriptionally activates ZO-1 and occludin. (A) Potential KLF11 binding

1001   site in the promoter region of mouse *ZO-1* and *occludin*. TSS: transcription start site. The green

1002   triangles mark the binding sites of KLF11. The numbers indicate the relative distance to TSS,

1003   which is labeled as +1. (B) 1kb of the mouse *ZO-1* promoter or a mutated *ZO-1* promoter

1004   (deleted all three KLF11 binding sequences, Δ mZO-1) was cloned into the pGL4.10[*luc2*]

1005   vector. The pGL4 promoter constructs were then transfected into bEnd.3 cultures. bEnd.3 cells

1006   were also co-transduced with an empty adenovirus (Ad. LacZ) or adenoviral vectors carrying

1007   mouse KLF11 (Ad. KLF11) for 48-72 h prior to performing luciferase reporter activity assays.

1008   (C) KLF11 overexpression in bEnd.3 cells significantly increased luciferase activity of

1009   pGL4_mZO-1, which contains all three KLF11 binding sequences (n=4 per group). (D) On the

1010   contrary, the luciferase activity was significantly reduced in the pGL4_mZO-1 group co-

1011   transfected with Ad. shKLF11 (n=4 per group). (E) The occludin promoter or a mutated occludin

1012   promoter (deleted all three KLF11 binding sequences, Δ mOccludin) was cloned into the

1013   pGL4.10[*luc2*] vector. (F) KLF11 overexpression significantly increased luciferase activity of

1014   pGL4_mOccludin, but not pGL4_Δ mOccludin (n=4 per group). (G) On the contrary, luciferase

1015   activity was significantly reduced in the pGL4_mOccludin group co-transfected with Ad.

1016   shKLF11 (n=4 per group). Results shown are representative of three separate experiments with

1017   similar results. Data are expressed as mean ± SEM. * P < 0.05 vs. Ad. LacZ group or Ad.

1018   shLacZ group.

A WT   EC-KLF11 Tg

B

% of infarct volume

30
25
20
15
10
5
0

WT   EC-KLF11 Tg

**

C

Neurological scores

4
3
2
1

WT   EC-KLF11 Tg

**

D   Baseline   Ischemia   Reperfusion

WT

EC-KLF11Tg

E

■ WT
□ EC-KLF11 Tg

150

100

50

Baseline   Ischemia   Reperfusion

F   Rota-rod

Latency to fall(s)

200
150
100
50

-2 -1 0 1 2 3 4 5 6 7 8
Days

*   **

***

H   Adhesive tape removal

Time to touch(s)

50
40
30
20
10

-2 -1 0 1 2 3 4 5 6 7 8
Days

*   *   *

***

G   Footfault

Fault steps/All steps

0.25
0.20
0.15
0.10
0.05

-2 -1 0 1 2 3 4 5 6 7 8
Days

*   ***   ***

***

I   Adhesive tape removal

□·· WT Sham   ▽— EC-KLF11 Tg Sham
○·· WT MCAO   ▲— EC-KLF11 Tg MCAO

Time to remove(s)

125
100
75
50
25

-2 -1 0 1 2 3 4 5 6 7 8
Days

*   **   *

***

qPCR   ELISA

A

STX2
Fluorescent tracer
Transwell insert
Luminal
BMECs
Polyester membrane
Abluminal
Astrocytes

B

■ Ad.Lacz
□ Ad.KLF11

EVOM measurement (Ω)

non-OGD   16h OGD-2h Reoxygenation

C

■ Ad.shLacz
□ Ad.shKLF11

EVOM measurement (Ω)

non-OGD   16h OGD-2h Reoxygenation

D

AlexaFluor488 3KD_RFU
Thousands

Reoxygenation time (h)

— Ad.KLF11 16hOGD   — Ad.LacZ 16hOGD
--- Ad.KLF11 nonOGD   --- Ad.LacZ nonOGD

E

AlexaFluor 488 3KD_RFU
Thousands

Reoxygenation time (h)

--- Ad.shLacz nonOGD   — Ad.shLacz 16hOGD
--- Ad.shKLF11 nonOGD   — Ad.shKLF11 16hOGD

A

+1

(-211/-195) (-73/-57)(-55/-39)

TSS
ZO-1

+1

(-299/-284)(-269/-253)(-201/-185)

TSS
Occludin

B

KLF11 3'-…gagccCCCAcccccac   accgggGCGGggagcg   ccgggGCGGgggcggc
                | | | | | | | | | | | |
pGL4_mZO-1 5'-…tctcggGGGTgggggtg ……tggcccCGCCcctcgcggaggcccCGCCcccgccggctggg…

pGL4_ΔmZO-1 5'-…tctcgg------- gggggtg …… tggcccc ------ cctcgcggaggccc ------- cccgccggctggg…

C — Relative Luc. Activity

Ad lacZ   Ad KLF11   Ad lacZ   Ad KLF11
pGL4_mZO-1   pGL4_ Δ mZO-1

D — Relative Luc. Activity

Ad sh lacZ   Ad sh KLF11   Ad sh lacZ   Ad sh KLF11
pGL4_mZO-1   pGL4_ Δ mZO-1

E

KLF11 3'-… gcggggGCGGggagag   gacaagGAAAttgagcc   agggagGGGGagggta
                      | | | | | | | | | | | |
pGL4_mOccludin 5'-…acgccccCGCCcctctcc……gtgctgttcCTTTaactcggc……ctccctCCCCctccat…

pGL4_ΔmOccludin 5'-…acgcccc------- cctctcc…… gtgctgttc------- aactcggc……ctccct------- ctcccat…

F — Relative Luc. Activity

Ad lacZ   Ad KLF11   Ad lacZ   Ad KLF11
pGL4_mOccludin   pGL4_ Δ mOccludin

G — Relative Luc. Activity

Ad sh lacZ   Ad sh KLF11   Ad sh lacZ   Ad sh KLF11
pGL4_mOccludin   pGL4_ Δ mOccludin