## **Supporting Information**

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Fig. S1. Oxygen saturation of arterial blood from matrix Gla protein  $(Mgp)^{+/+}$  and  $Mgp^{-/-}$  mice (n = 6).



Fig. S2. Localization of phosphorylated (p)SMAD1/5/8 in the brain of  $Mgp^{-/-}$  mice. PSMAD1/5/8 colocalized with smooth muscle protein 22- $\alpha$  (SM22 $\alpha$ ) and  $\alpha$ -smooth muscle actin ( $\alpha$ -SMA). (Scale bar, 100  $\mu$ m.)



Fig. S3. Expression of Notch and target genes in the brains of  $Mgp^{-/-}$  mice. The expression of Notch1–4, Dll 1, 3, and 4, Jagged (Jag) 1 and 2, and Hey1 and -2 in the brains of  $Mgp^{-/-}$  mice was examined by real-time PCR (n = 3).  $Mgp^{+/+}$  mice were used as controls. \*\*\*P < 0.001.



Fig. S4. Depletion of matrix Gla protein (MGP) in human brain microvascular endothelial cells (HBMECs) Expression of MGP in HBMECs after transfection of scrambled siRNA and siRNA to MGP, as determined by immunostaining. (Scale bar, 100 μm.)



Ephrin B2 DAPI

**Fig. S5.** Expression of Ephrin B2 in HBMECs. Expression of Ephron B2, as determined by immunostaining, after transfection of siRNA to MGP alone or together with siRNA to Jagged 1 (Jag1), Jagged 2 (Jag2), or Jag1 and Jag2, in combination with (+) or without (–) BMP-9 treatment. Scrambled siRNA (SCR) was used as control. (Scale bar, 100 μm.)



**Fig. S6.** Relative density of retained fluorescence in lungs and kidneys of  $Mgp^{+/+}$ ,  $Jag1^{+/-}$ ,  $Jag2^{+/-}$ ,  $Mgp^{-/-}Jag1^{+/-}$ , and  $Mgp^{-/-}Jag2^{+/-}$  mice after UV-fluorescent microsphere injection (n = 8).



**Movie S1.** *Mgp*<sup>+/+</sup> brain vasculature. Skull bone fragments are visible.



**Movie S2.** Mgp<sup>+/-</sup> brain vasculature. Skull bone fragments are visible.



Movie S3.  $Mgp^{-/-}$  brain vasculature.

Movie S2

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Movie S4. Mgp<sup>+/+</sup> brain vasculature.

Movie S4



Movie S5.  $Mgp^{+\prime+}Jag1^{+\prime-}$  brain vasculature.



Movie S6. Mgp<sup>+/+</sup>Jag2<sup>+/-</sup> brain vasculature.

Movie S5



Movie S7.  $Mgp^{-\prime-}$  brain vasculature. Skull bone fragments are visible.

Movie S7



Movie S8.  $Mgp^{-\prime-}Jag1^{+\prime-}$  brain vasculature. Skull bone fragments are visible.



Movie S9. Mgp<sup>-/-</sup>Jag2<sup>+/-</sup> brain vasculature.

Movie S8

DNAS