Supplemental Material

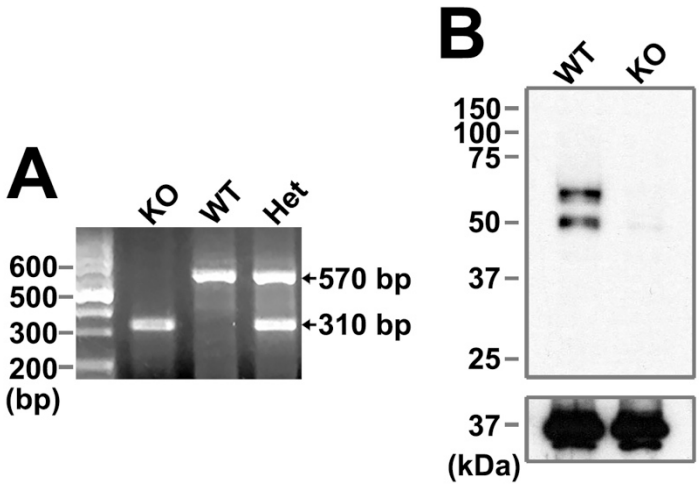

Figure S1. Characterization of the MFG-E8-knockout (KO) mice. (A) Genotyping of MFG-E8-KO mice. (B) Western blotting of MFG-E8 expression in the common carotid arteries (CCAs) of wild-type (WT) and MFG-E8-KO mice.

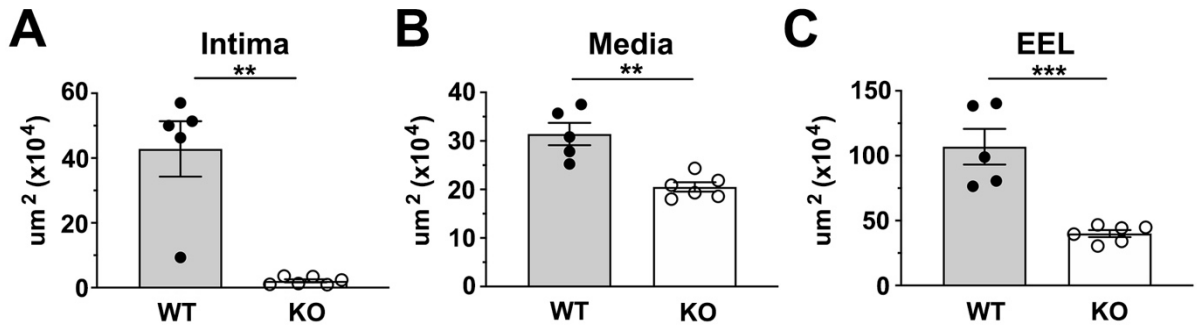

Figure S2. MFG-E8-knockout (KO) mice exhibit reduced neointimal hyperplasia.

Paraffin sections of the ligated common carotid arteries (CCAs) of wild-type (WT) and MFG-E8-KO mice were subjected to Verhoeff–van Gieson staining 21 days after ligation. Morphometric analyses of the intima (A), media (B), and area bounded by the external elastic lamina compartment (EEL; C) were conducted. Results are presented as mean ± SEM. Each point is derived from an assessment of three sections of an individual animal. (A) Intima (WT: $n_{mice} = 5$, MFG-E8-KO: $n_{mice} = 6$), $^{**}P < .01$, as obtained using the nonparametric Mann–Whitney *U*test. (B) Media (WT: $n_{mice} = 5$, MFG-E8-KO: $n_{mice} = 6$), $^{**}P < .01$, as obtained using the *t* test. (C) EEL (WT: $n_{mice} = 5$, MFG-E8-KO: $n_{mice} = 6$), $^{***}P < .001$, as obtained using the *t* test.

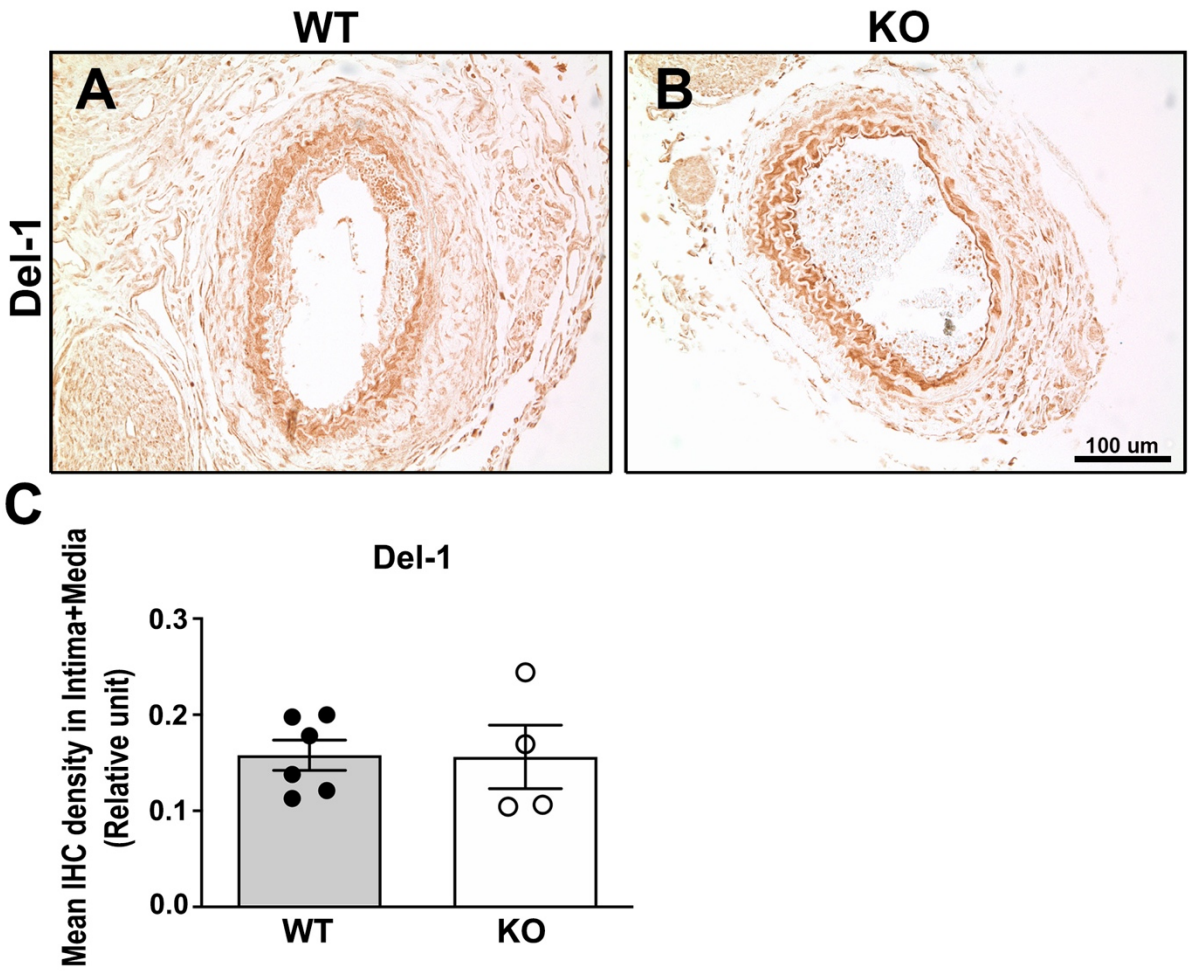

Figure S3. Expression of developmental endothelial locus-1 (Del-1) is unaffected by the genetic depletion of MFG-E8. (A) and (B) Immunohistochemistry (IHC) analysis of Del-1 in ligated common carotid arteries (CCAs) at 10 days after surgery. Bar, 100 μm. (C) Quantification of IHC intensity of Del-1 in the intima–media area 10 days after ligation (wild-type (WT): n_{mice} = 6, MFG-E8-KO: n_{mice} = 4). Results are presented as mean ± SEM. Each point is derived from an assessment of three sections of an individual animal. *P* = .9560.

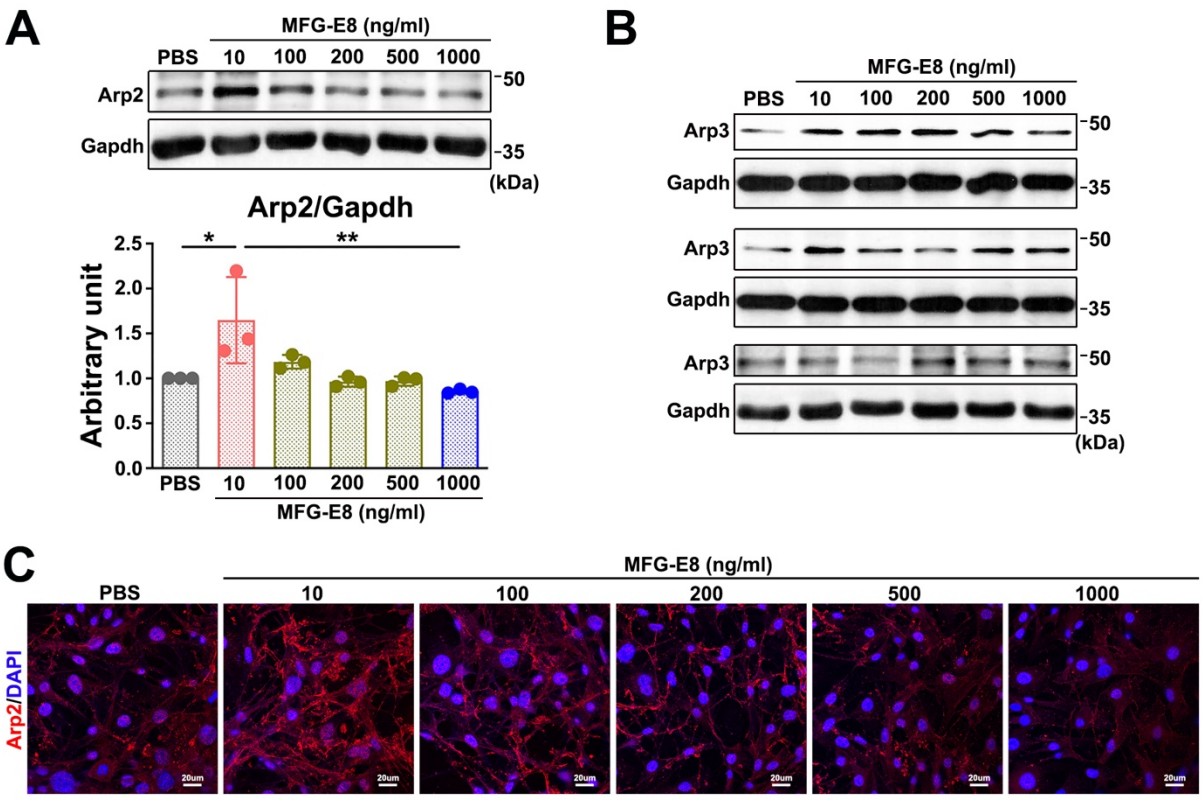

Figure S4. Biphasic regulation of Arp2 and Arp3 expression in vascular smooth muscle cells (VSMCs) by exogenous MFG-E8. A10 cells were treated with various doses of recombinant MFG-E8 (rMFG-E8) from 10 to 1000 ng/mL for 16 h. Immunoblotting was conducted to evaluate the protein expression of Arp2 (A) and Arp3 (B) in A10 cells treated with various doses of MFG-E8 for 16 h. Quantitative analyses of Arp2 (A) levels normalized to those of GAPDH were conducted (n = 3). Data are presented as mean ± SD. Three independent experiments were performed. Each point is derived from each of the three repeated experiments. *$P < .05$ and **$P < .01$, as obtained using one-way ANOVA followed by Tukey's multiple comparisons test. (C) VSMCs isolated from the aortas of wild-type (WT) mice were immunostained with antibodies against Arp2. Bar, 20 μm.

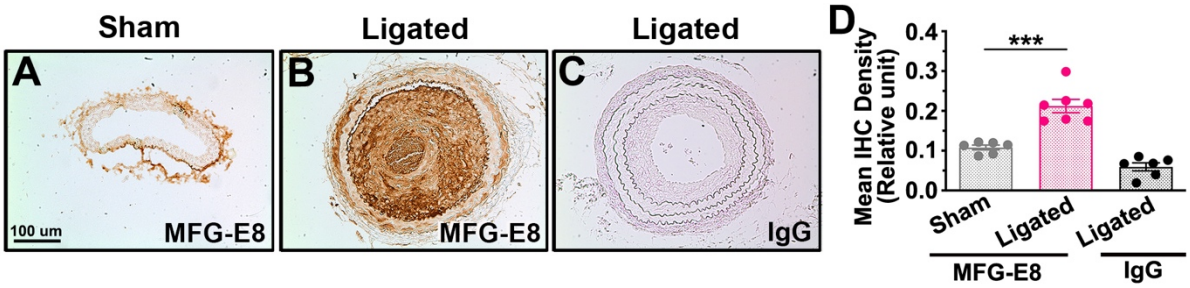

Figure S5. Ligation injury induces the expression of MFG-E8 in the arterial wall.

Representative immunohistochemistry (IHC) images of MFG-E8 in the sham-operated (A) and ligated (B) common carotid arteries (CCAs) of wild-type (WT) mice 21 days after ligation. Negative control (IgG) illustrating the absence of immunoreactivity (C). Bar, 100 μm. (D) Immunostaining intensities of MFG-E8 in the intima–media area were assessed 21 days after ligation (sham: $n_{mice} = 6$, ligated: $n_{mice} = 7$, IgG: $n_{mice} = 6$). Data are presented as mean ± SEM. Each point is derived from an assessment of three sections of an individual animal. ***$P < .001$, as obtained using the *t* test.

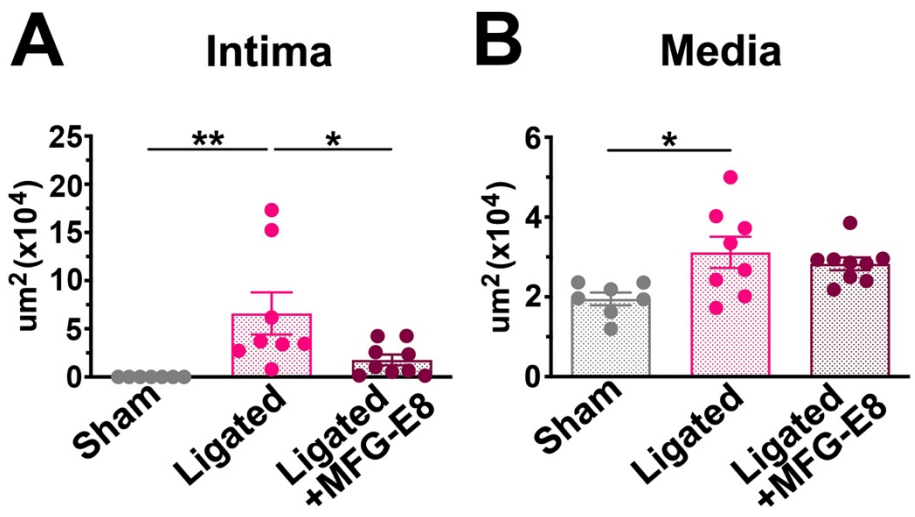

Figure S6. High-dose MFG-E8 treatment alleviates ligation-induced neointimal hyperplasia. Paraffin sections of the sham-operated common carotid arteries (CCAs), ligated CCAs, and ligated CCAs with MFG-E8 treatment (2 μg/mL) 21 days after ligation were subjected to Verhoeff–van Gieson staining. Morphometric analyses of the intima (A) and media (B) were conducted (sham: n = 7, ligated: n = 8, IgG: n = 9). Data are presented as mean ± SEM. **P* < .05 and ***P* <.01, as obtained using one-way ANOVA followed by Tukey's multiple comparisons test.

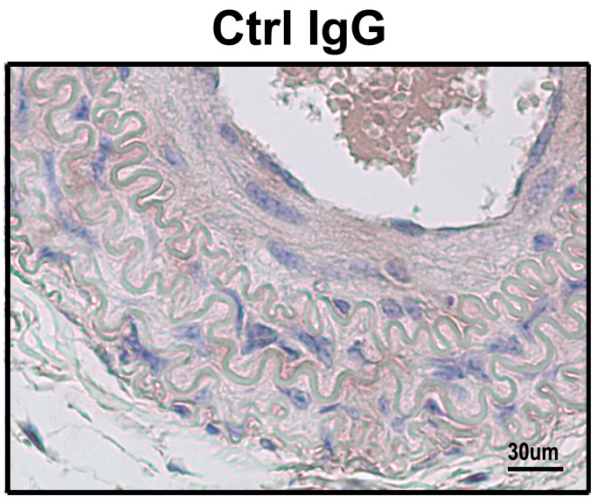

Figure S7. Negative control for anti-Ki-67 antibody in the intima–media of the vessel 10 days after ligation. Bar, 30 μm.