Supplementary information

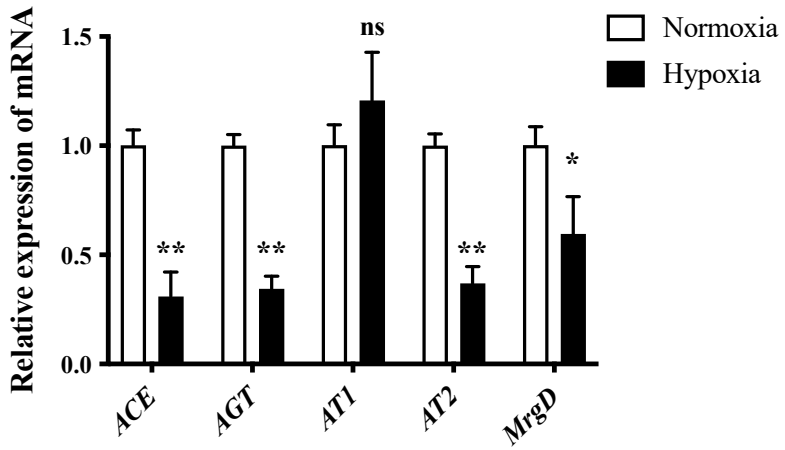

Fig. S1 Angiotensin converting enzyme (ACE), angiotensinogen (AGT), angiotensin II type 1 (AT1), and angiotensin II type 2 (AT2) messenger RNA (mRNA) expression levels were lower in hypoxia-induced human retinal microvascular endothelial cells (HRMECs) versus control. The mRNA expression levels of ACE, AGT, AT1, AT2, and Mas-related G protein-coupled receptor D (MrgD) in HRMECs from different groups were determined by the quantitative real-time polymerase chain reaction (qRT-PCR) method. All mRNA expression was normalized to β-actin. All the results are expressed as mean±standard error of mean (SEM) (*n*=6 per group; ns indicates not significant, * *P*<0.05, ** *P*<0.01 compared to normoxia group).

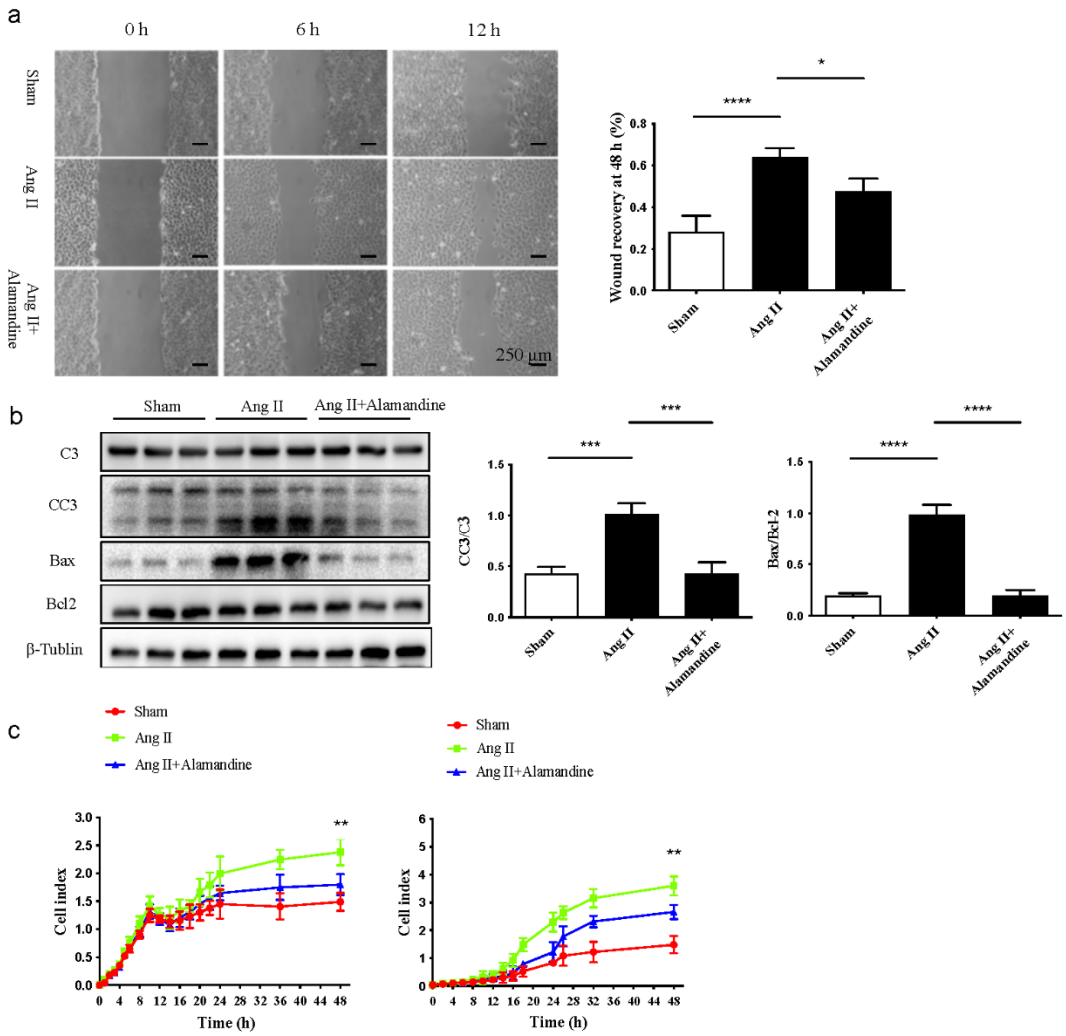

Fig. S2 Alamandine ameliorated Angiotensin II (Ang II)-induced endothelial cell (EC) apoptosis, migration, and proliferation. (a) The wound healing assay was performed to examine the role of Alamandine in Ang II-induced angiogenesis in human retinal microvascular endothelial cells (HRMECs). (b) The protein expression levels of caspase-3 (C3), cleaved caspase-3 (CC3), B cell lymphoma-2 (Bcl-2)-associated X (Bax), Bcl-2, and β-Tublin in HRMECs from different groups were determined by western blot (left) and the corresponding densitometric analysis (right). β-Tublin was detected as the loading control. (c) The results of real-time cellular analysis (RTCA) migration and proliferation assays performed to determine the effects of Alamandine on Ang II-induced proliferation and migration of HRMECs. All the results are expressed as mean±standard error of mean (SEM) (*n*=5 per group; * *P*<0.05, ** *P*<0.01, * *P*<0.001, **** *P*<0.0001, compared to sham or Ang II group).**

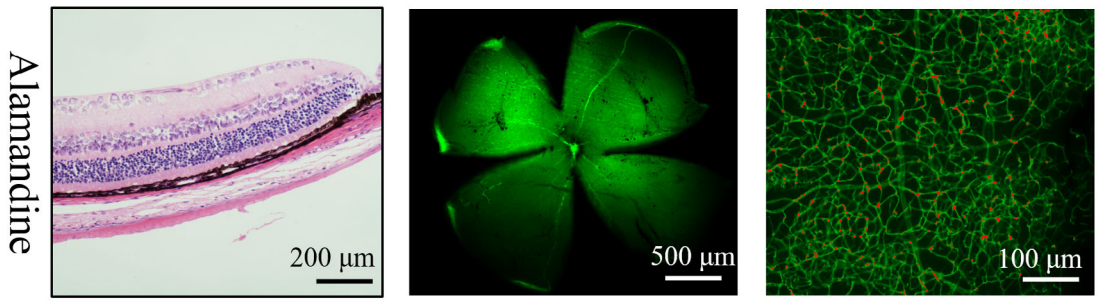

Fig. S3 Alamandine alone has no significant adverse effects on retinal vessel development in a normal retina. Representative images of staining retinal paraffin sections with hematoxylin & eosin (H&E) (left; scale bar=200 μm), and retinal whole-mount immunostaining with isolectin B4 (IB4) (middle: scale bars=500 μm; right: scale bar=100 μm) from the given group (blood vessels: green; neovascularized areas: red).

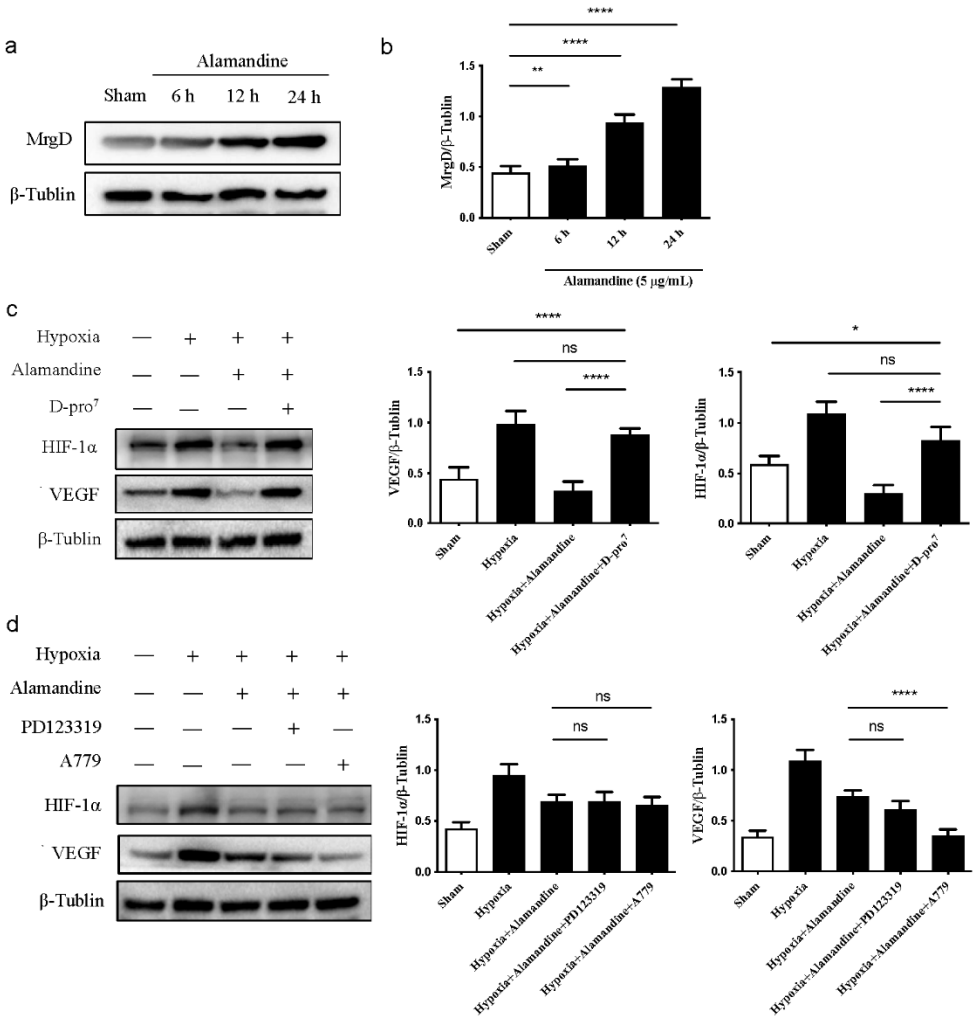

Fig. S4 Role of Mas-related G protein-coupled receptor D (MrgD) in the protective effects of Alamandine against vascular endothelial growth factor (VEGF)-induced angiogenesis in human retinal microvascular endothelial cells (HRMECs). (a–b) The protein expression levels of MrgD and β-Tublin were determined by western blot in HRMECs treated with different inductive times of Alamandine (10 μg/mL) (a), and the corresponding densitometric analysis (b). (c–d) The protein expression levels of hypoxia-inducible factor-1α (HIF-1α), VEGF, and β-Tublin were determined by western blot in HRMECs from the different groups (left) and the corresponding densitometric analysis (right). β-Tublin was detected as the loading control. All the results are expressed as mean±standard error of mean (SEM) (*n*=5 per group; ns indicates not significant, * *P*<0.05, ** *P*<0.01, *** *P*<0.001, **** *P*<0.0001).

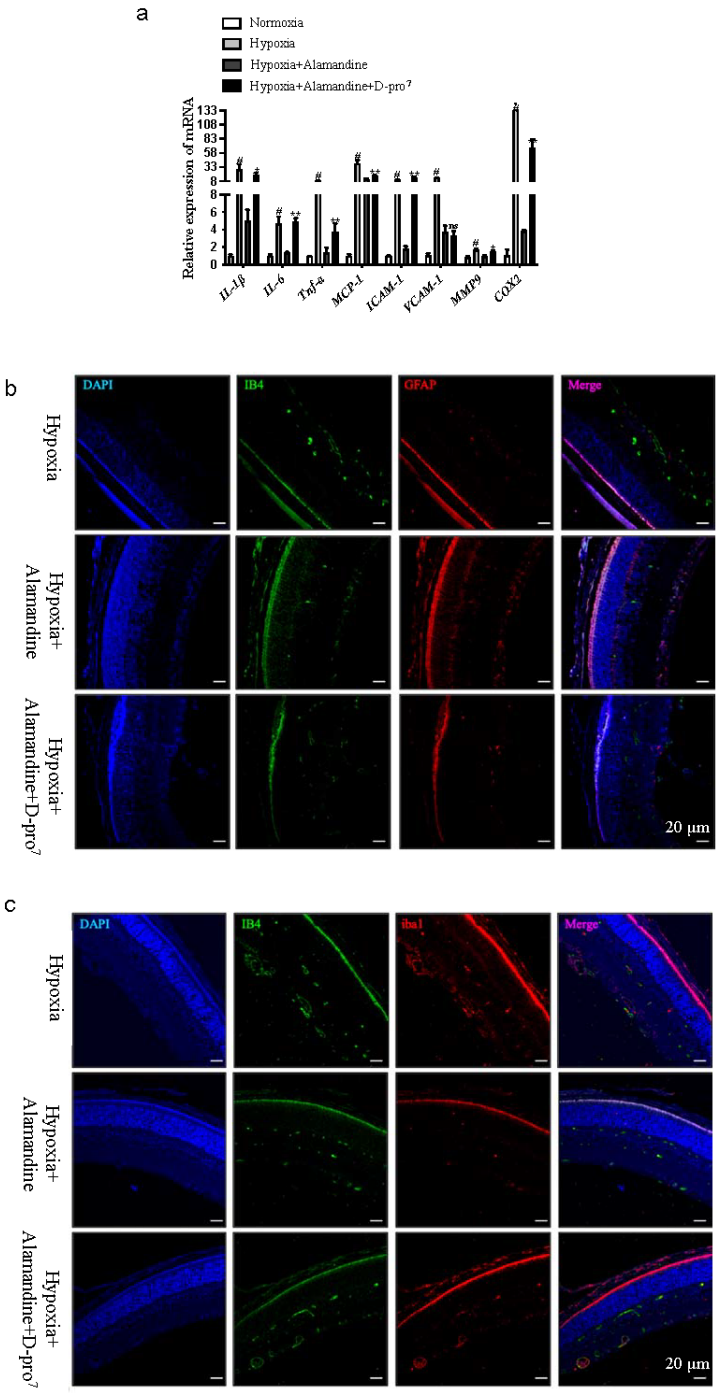

Fig. S5 Role of Mas-related G protein-coupled receptor D (MrgD) in the protective effects of Alamandine against glial cell dysfunction and inflammation in the retinas of oxygen-induced retinopathy (OIR) mice. Mouse retinas were harvested from the hypoxia group, hypoxia+Alamandine group, and hypoxia+Alamandine+D-Pro^7 group. (a) The messenger RNA (mRNA) expression levels of interleukin-1β (*IL-1β*), *IL-6*, tumor necrosis factor-α (*TNF-α*), monocyte chemoattractant protein-1 (*MCP-1*), matrix metalloproteinase-9 (*MMP-9*), and cyclooxygenase-2 (*COX-2*), as well as intercellular adhesion molecule-1 (*ICAM-1*) and vascular cell adhesion molecule-1 (*VCAM-1*) in retinal tissues from the given groups determined by quantitative real-time polymerase chain reaction (qRT-PCR) method. All mRNA expression was normalized to β-actin. (b) Representative immunofluorescence images of retinal paraffin sections stained with isolectin B4 (IB4), glial fibrillary acidic protein (GFAP), and 4',6-diamidino-2-phenylindole (DAPI) from the given groups (IB4: green; GFAP: red; DAPI: blue). (c): Representative immunofluorescence images of retinal paraffin sections stained with IB4, ionized calcium-binding adaptor molecule 1 (iba1), and DAPI from the given groups (IB4: green; iba1: red; DAPI: blue). All the results are expressed as mean±standard error of mean (SEM) (*n*=5 per group; ns indicates not significant, * *P*<0.05, ** *P*<0.01, *** *P*<0.001, **** *P*<0.0001).