Dear Editor,

Strokes cause 5.8 million deaths each year. Among these victims, ~30% are from China. Acute ischemic stroke (AIS) is the most prevalent subtype of strokes. Although drugs can alleviate the symptoms, the recoveries of functional vessels within ischemic areas are the critical factor determining the prognosis of patients suffering from AIS. Nevertheless, the mechanisms involved in cerebral revascularization remain largely unknown. Myeloid cells are among the first cells arriving around and within the injured areas after the ischemic assault. A specific subgroup of Tie2-expressing monocytes (TEMs) has demonstrated vessel-repairing properties in tumors and ischemic limbs. But, it is not clear whether TEMs participate in revascularization and neurological recovery in AIS. Hence, we explore the impacts of TEMs on the prognosis of AIS and the potential mechanism beneath with clinical samples and mouse models.

Pre-therapeutic blood samples from patients within 24 h after onset of AIS and from age-matched controls (AMCs) were collected and analyzed to determine whether TEMs are upregulated in response to ischemic brain injury. The demographics of the enrolled participants are listed in Supplementary Table 1. Figure 1a shows representative magnetic resonance imaging (MRI) from an enrolled patient taken before emergency treatment at the onset day. By flow cytometric analysis, we found the proportion of circulating monocytes relative to the total number of white blood cells in patients with AIS was higher than in the AMCs (Supplementary Figs. S1, 2). Similarly, the proportion and level of Tie2 expressing in CD14+ monocytes were higher in the AIS group than in AMC (Fig. 1b). Within 24 h before the patient was discharged, the modified Rankin Scale (mRS) was used to estimate stroke prognosis (scores ≤2 consider better clinical outcome). Although the Tie2 expression was not correlated with the accurate mRS score of AIS patients (Supplementary Fig. S2d), patients with mRS scores ≤2 presented higher Tie2 expression in CD14+ monocytes than scores >2 at baseline evaluation (Fig. 1b). Overall, our clinical study demonstrated the frequency of TEMs (CD45+/CD14+/Tie2−) was increased in brain tissue after tMCAO surgery. Of note, the blood-brain barrier (BBB) formed by continuous endothelial cell (EC) membrane acts as a regulating interface with adherens and tight junctions to protect the brain compartment as an immunoprivileged site. In this scenario, questioning whether myeloid-derived TEMs can penetrate the BBB into the brain parenchyma is reasonable. Here, we collected brain sections at 24 h post tMCAO induction, and immunofluorescence staining showed severely damaged blood vessels (CD31+) accompanied by rapidly accumulating microglia and monocytes/macrophages (CD11b+) in the occlusion areas of tMCAO mice (Fig. 1e, f). Notably, emerging TEMs (CD11b+/Tie2−) were identified around ruptured blood vessels, and they were not colocalized with the fractalkine receptor CX3CR1, a highly expressed marker on mature microglia (Supplementary Fig. S5). Our data suggest TEMs infiltrate the injured brain tissue from the peripheral blood after tMCAO induction.

Next, we generated a transgenic mouse model of Tie2<sup>lox<sup>-/</sup></sup>-/Lyz<sup>Cre<i>−</i></sup>- mice (M<sup>Tie2−</sup>) and used the littermates with a genotype Tie2<sup>lox<sup>-/</sup></sup>-Lyz<sup>Cre<i>−</i></sup> as the control (CTR; Supplementary Fig. S6) to investigate the effects of TEM deficiency in the ischemic brain. As shown in Fig. 1g, Tie2 deficiency in monocytes/macrophages was associated with markedly increased infarction areas by MRI performance 24 h post tMCAO. Statistical analysis of the data from MRI screening indicated the infarct volumes were enlarged mainly in mice lacking Tie2+ myeloid cells (Fig. 1g), which further confirms the effects of TEMs on infarction in vivo. Consistent with brain injury levels, the stepping speed and cadence were decreased in M<sup>Tie2−</sup> mice compared with the CTR counterparts at 7 days after surgery (Fig. 1h, i), indicating that Tie2 deficiency delayed functional improvement. In the meantime, bodyweight loss was more frequently observed in M<sup>Tie2−</sup> mice after tMCAO.
induction (Fig. 1j). Taken together, these results indicate the myeloid deficiency of Tie2 promotes the development of infarction and impairs the recovery of motor function in tMCAO-treated mice.

Considering the pro-angiogenesis function of TEMs, we wondered if TEMs support recovery after AIS via revascularization. We generated lentivirus-transduced macrophages overexpressing Tie2 and performed the tube formation assay. In comparison with the vector-transduced counterparts, Tie2-overexpressing macrophages significantly enhanced tube formation of bEnd.3 mouse cerebral endothelial cells (Supplementary Fig. S7), indicating the infiltrating TEMs may initiate the reconstruction of cerebral microvessels after ischemia. We next analyze the gene expression of Tie2 agonist angiopoietin-1 and -2 in brain lysates 24 h after tMCAO. We observed the proangiogenic gene Angpt2 was 12 times increased in the ischemic cortex versus the contralateral area, but not Angpt1 (Supplementary Fig. S8). The magnetic resonance angiography suggested the blood supply through the right cerebral artery occluded more severely after ischemia/reperfusion in M^{Tie2−} mice in contrast to CTR in vivo (Fig. 1k). In agreement, the cerebrovascular in peri-infarct regions of M^{Tie2−} mice appeared less perfused (Lectin ^{+}) than in the CTR (Fig. 1l),
together with the lower ratio of Lectin+/PDGFRβ+ area, strongly suggested worse vessel recovery and function in M1Δ2Δ mice. Overall, these data imply the TEMs may reinforce the resilience of the peri-infarct regions to mount and activate vessel repair processes for survival quickly.

In conclusion, we demonstrate here bone marrow-derived TEMs promote endogenous revascularization in the mouse brain after ischemic injury. More notably, in patients with AIS, the relative changes in peripheral TEM counts were significantly related to better outcomes. Our findings highlight the necessity to better understand the mechanisms of the endogenous and protective responses in the brain after ischemia/reperfusion. As such, the identified functions of TEMs may offer new therapeutic avenues for augmenting the degree of spontaneous recovery after AIS.

DATA AVAILABILITY
Data are available from the corresponding author upon reasonable request.

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