Supporting Information

Hayakawa et al. 10.1073/pnas.1121146109

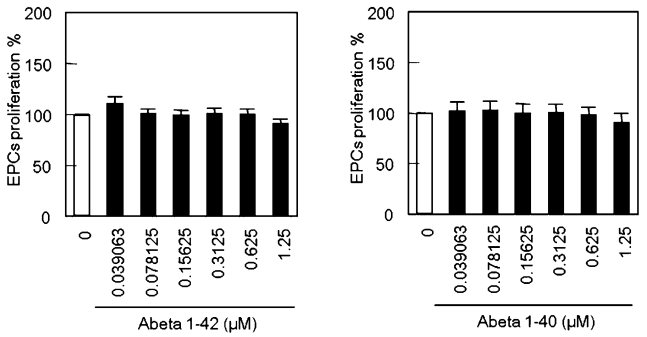

Fig. S1. Because receptor for advanced glycation endproducts (RAGE) can bind other ligands besides high-mobility-group-box-1 (HMGB1), we tested the effects of amyloid-β-1-42 and amyloid-β-1-40 as an alternative stimulus. Rat endothelial progenitor cell (EPC) cultures were grown as previously described. Amyloid-β were added from 0.039- to 1.25-μM concentrations. There was no detectable cytotoxicity, and no effects on EPC proliferation were observed. These data suggest that at least one other major RAGE ligand did not influence EPCs in our model system.

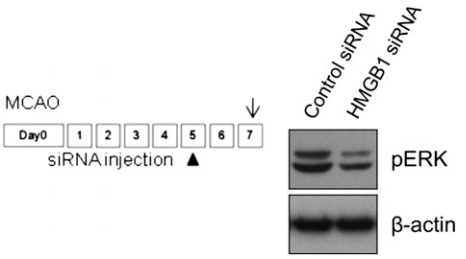

Fig. S2. In our hypothesis, HMGB1 acts as the signal that mediates cell–cell crosstalk between reactive astrocytes and EPCs. However, what are the downstream intracellular mechanisms involved? Because HMGB1-RAGE signaling is known to trigger MAPKs, we assessed ERK MAPK as a representative signaling pathway. Using our in vivo mouse focal cerebral ischemia model, we applied Western blotting to check brain homogenates of the peri-infarct cortex. In stroke-only brains, phospho-ERK levels were elevated. However, in animals treated with HMGB1-siRNA, phospho-ERK appeared to suppresed. These data suggest that ERK MAPK cascades may also operate downstream as one of the intracellular signals after HMGB1 signaling that connects astrocytes to EPCs. Of course, ERK may just be one representative pathway. Other signaling pathways may also be important in this multicellular phenomenon during stroke recovery.

Table S1. Lectin-positive cells from the peri-infarct cortex comprise a very large proportion of endothelial cells, but there is also a persistent and reproducible subset that are EPCs

	Mature ECs + EPCs lectin$^+$ population (% of total events)	Mature ECs Flk1$^-$/CD34$^-$ cells (% of total lectin$^+$ cells)	EPCs Flk1$^+$/CD34$^+$ cells (% of total lectin$^+$ cells)
Sham (n = 4)	26.3 ± 1.1	96.9 ± 1.2	0.25 ± 0.14
Day 3 (n = 5)	27.6 ± 2.8	94.2 ± 2.4	1.33 ± 0.29
Day 7 (n = 5)	22.6 ± 2.7	92.1 ± 4.9	1.87 ± 0.43
Day 14 (n = 5)	21.4 ± 2.8	93.7 ± 2.0	2.78 ± 0.88
Day 14 (control siRNA) (n = 5)	21.9 ± 2.1	94.4 ± 3.1	2.34 ± 0.71
Day 14 (HMGB1 siRNA) (n = 5)	18.7 ± 1.7	95.8 ± 1.1	1.15 ± 0.24

In our primary cell cultures, we can be sure of our EPC purity. However, how can we be sure about the responses we see in our in vivo stroke models? We used FACS analysis to check the distribution of cells extracted from the remodeling peri-infarct cortex. First, we pulled out all lectin-positive cells. Then we checked for Flk1$^-$/CD34$^-$ cells vs. Flk1$^+$/CD34$^+$ cells. We acknowledge that there are no 100% perfect markers for EPCs. However, as described in the main text, we operationally define Flk1$^+$/CD34$^+$ cells as being EPCs in our model system, and Flk1$^-$/CD34$^-$ cells as representing mature cells with endothelial phenotypes. In this table, we show that although the lectin-positive cells from the peri-infarct cortex comprise a very large proportion of endothelial cells, there is also a persistent and reproducible subset that are EPCs. The actual numbers are low. However, as discussed in the main text, these small cell numbers are known to significantly impact on neurovascular remodeling.