Supplementary Methods

Flow cytometric analysis of circulating monocyte-platelet aggregates

Citrated whole blood was drawn from the retro-orbital plexus 24 hours after cerebral ischemia induction in wild type and *Cd39*$^{-/-}$ mice. The blood was mixed with Fc recptor as well as CD62p blocking antibodies (BD) and immediately fixed with 5% buffered formalin. Following fixation, the red blood cells were lysed using water. The cells were stained with antibodies against the platelet marker GPIIb conjugated to FITC (BD) and monocytes were gated based on their characteristic forward and side scatter properties (1). Monocytes were examined for coexpression of the platelet marker GPIIb.

Supplemental References

1. Michelson, A., Barnard, M., Krueger, L., Valeri, C., and Furman, M. 2001. Circulating monocyte-platelet aggregates are a more sensitive marker of in vivo platelet acivation than platelet surface P-selectin: studies in baboons, human coronary intervention, and human acute myocardial infarction. *Circulation* 104:1533-1537.

Supplementary Figure Legends

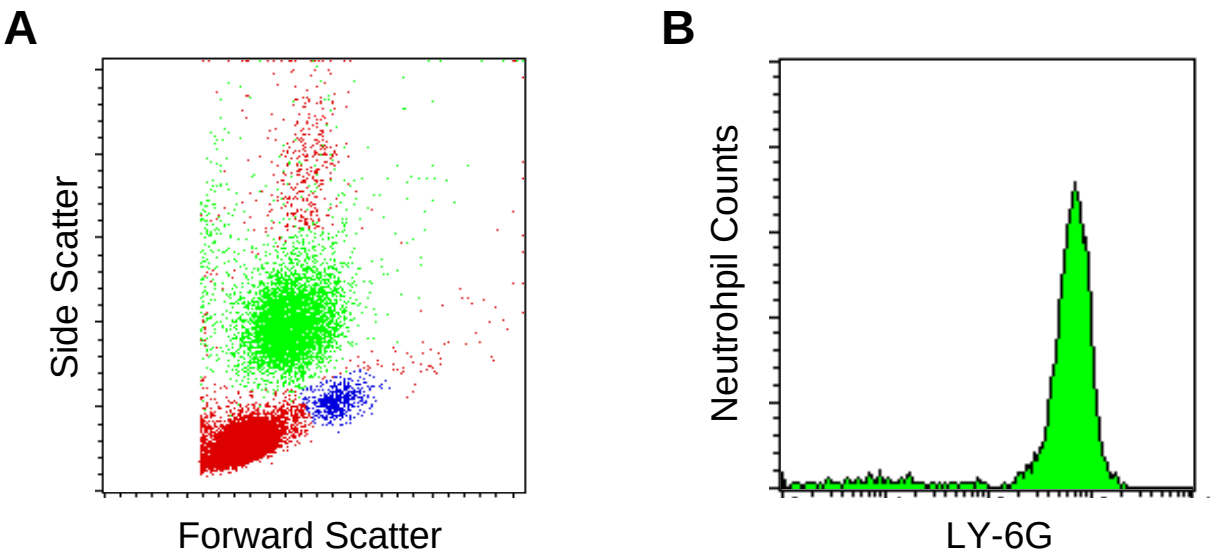
Supplement Figure 1

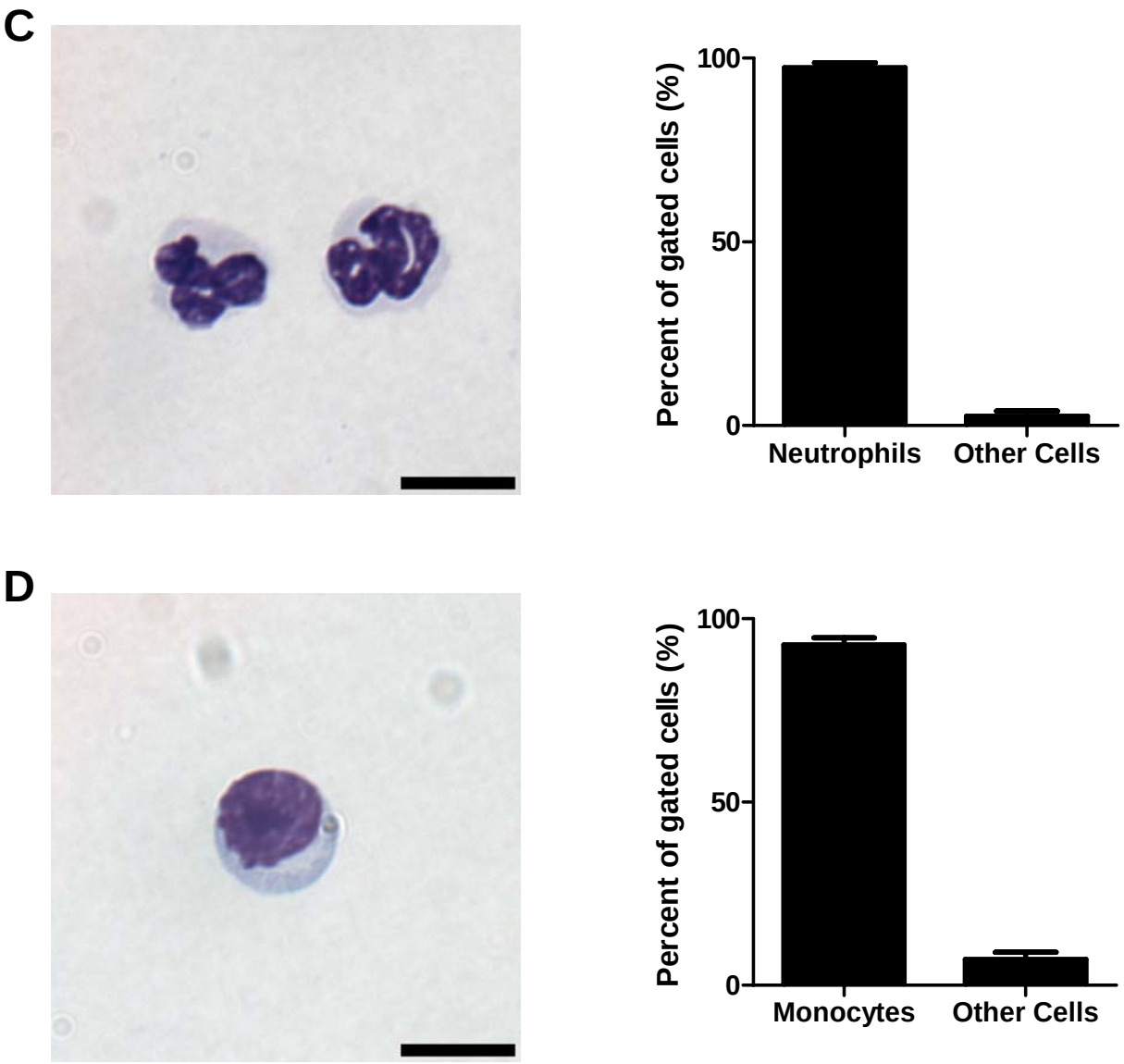
Cytopspin evaluation of flow cytometry cell sorting of peripheral blood. (A) Representative forward and side scatter dot plot of leukocytes purified from whole blood showing neutrophils (green) and monocytes (blue). (B) Representative histogram showing LY-6G staining in the forward and side scatter gated neutrophil population. (C) Neutrophils were identified by flow cytometry using a combination of forward scatter, side scatter, and LY-6G positivity and collected. The cells were then stained with HEMA-3 to assess the purity of the population and imaged with a 60x objective. (D) Monocytes were identified based on their characteristic forward and side scatter properties and collected. The cells were stained with HEMA-3 to assess the purity of the population and imaged with a 60x objective. n=3 per group.

Supplement Figure 2

CD39 deficiency does not affect post-ischemic circulating monocyte-platelet aggregates. (A) Following cerebral ischemia, circulating monocyte platelet aggregates were measured in wild type and *Cd39*$^{-/-}$ mice by flow cytometry. (B) Aggregate data showing the percentage of circulating monocytes that were bound to platelets in post-ischemic mice. n = 8 per group.

Supplement 1





Supplement 1 (continued)

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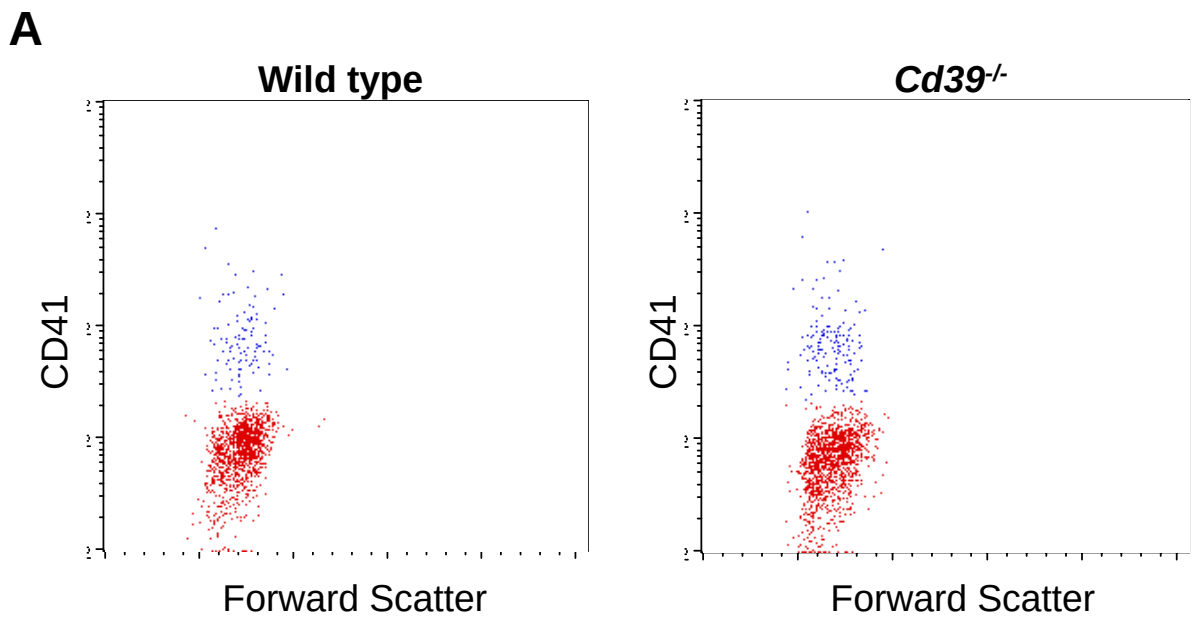
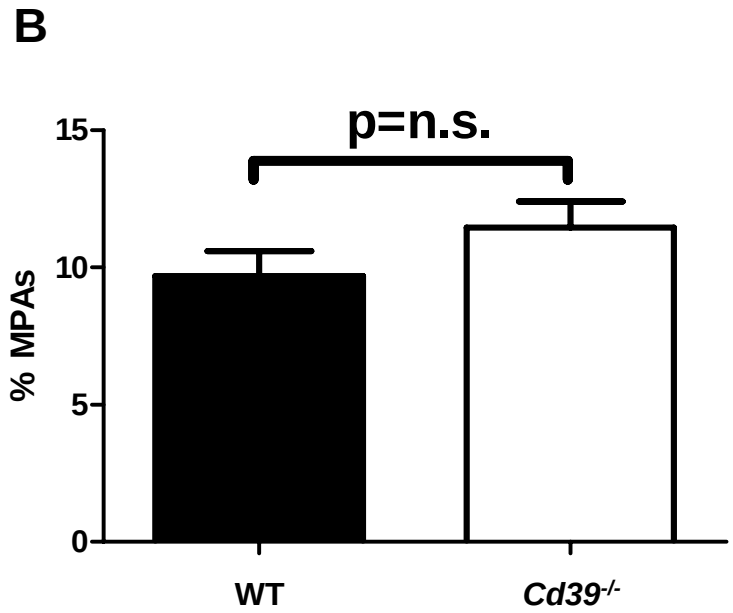
Supplement 2





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