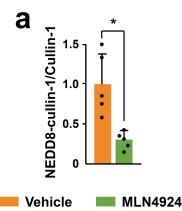
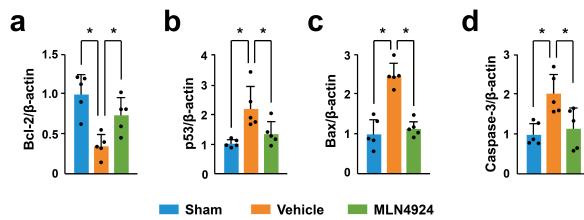


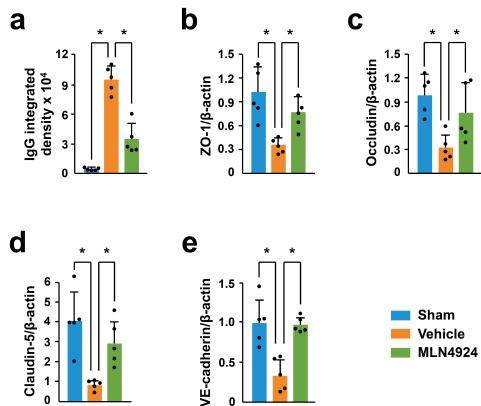
Supplementary Figures



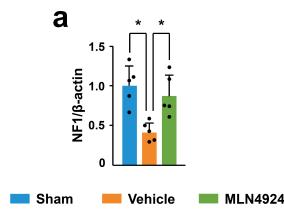
Supplementary Figure 1. MLN4924 reduces cullin-1 neddylation after ischemic stroke. **a** Quantification of cullin-1 neddylation in the ischemic cortex at 24 hours after ischemic stroke in mice treated with vehicle or MLN4924 (n = 5). Data were analyzed using unpaired Student's t test. Values are mean \pm SD. * P < 0.05.



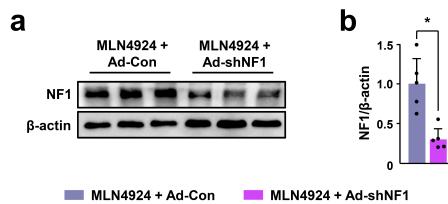
Supplementary Figure 2. MLN4924 decreases apoptotic responses after ischemic stroke. **a-d** Quantitative determinations of antiapoptotic proteins Bcl-2 (a) and proapoptotic proteins p53 (b), Bax (c), and caspase-3(d) in the ipsilateral hemisphere in ischemic mice treated with vehicle or MLN4924 (n = 5). Data were analyzed using one-way ANOVA followed by Bonferroni multiple comparison test. Values are mean \pm SD. * P < 0.05.



Supplementary Figure 3. MLN4924 reduces BBB breakdown and preserves BBB integrity after ischemic stroke. **a** Quantitation of IgG perivascular accumulation in the ischemic brain in sham-operated mice and ischemic mice treated with vehicle or MLN4924 (n = 6). **b-e** Quantification of tight junction protein ZO-1 (**b**), occludin (**c**), claudin-5 (**d**) and adherens junction protein VE-cadherin (**e**) in isolated brain microvessels of sham-operated mice and ischemic mice treated with vehicle or MLN4924 (n = 5). Data were analyzed using one-way ANOVA followed by Bonferroni multiple comparison test. Values are mean \pm SD. * P < 0.05.

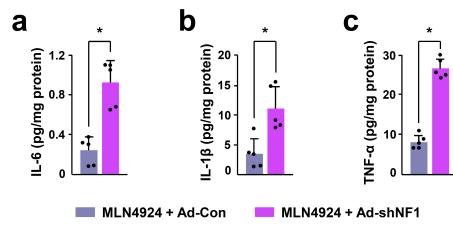


Supplementary Figure 4. Effects of MLN4924 on stroke-induced NF1 expression. **a** Quantification of NF1 in the ipsilateral hemisphere in sham-operated mice and ischemic mice treated with vehicle or MLN4924 (n = 5). Data were analyzed using one-way ANOVA followed by Bonferroni multiple comparison test. Values are mean \pm SD. * P < 0.05.



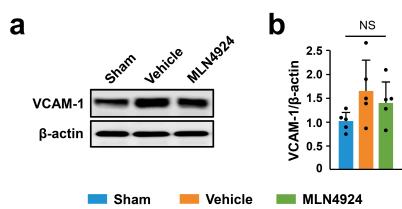
Supplementary Figure 5. Efficiency of NF1 silencing on NF1 expression. a, b

Representative immunoblots (a) and quantification (b) of NF1 in the ischemic hemisphere in mice treated with MLN4924 and control adenovirus (Ad-Con) or MLN4924 and NF1 shRNA (Ad-shNF1) ($n = 6$). Data were analyzed using unpaired Student's t test. Values are mean \pm SD. * $P < 0.05$.



Supplementary Figure 6. NF1 silencing blocks MLN4924-mediated reduction in

proinflammatory cytokines in the ischemic brains. a-c Quantification of proinflammatory cytokines IL-6 (a), IL-1 β (b) and TNF- α (c) by ELISA in ischemic mice treated with MLN4924 and control adenovirus (Ad-Con) or MLN4924 and NF1 shRNA (Ad-shNF1) at 24 hours after stroke ($n = 5$). Data were analyzed using unpaired Student's t test. Values are mean \pm SD. * $P < 0.05$.



Supplementary Figure 7. Effects of MLN4924 on VCAM-1 expression after ischemic stroke. **a, b** Representative immunoblots (**a**) and quantification (**b**) of VCAM-1 in isolated brain microvessels in sham-operated mice and ischemic mice treated with vehicle or MLN4924 (n = 5). Data were analyzed using one-way ANOVA followed by Bonferroni multiple comparison test. Values are mean ± SD. NS, not significant.