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Myeloperoxidase inhibition increases neurogenesis after ischemic stroke

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Running title: MPO inhibition increases neurogenesis after stroke

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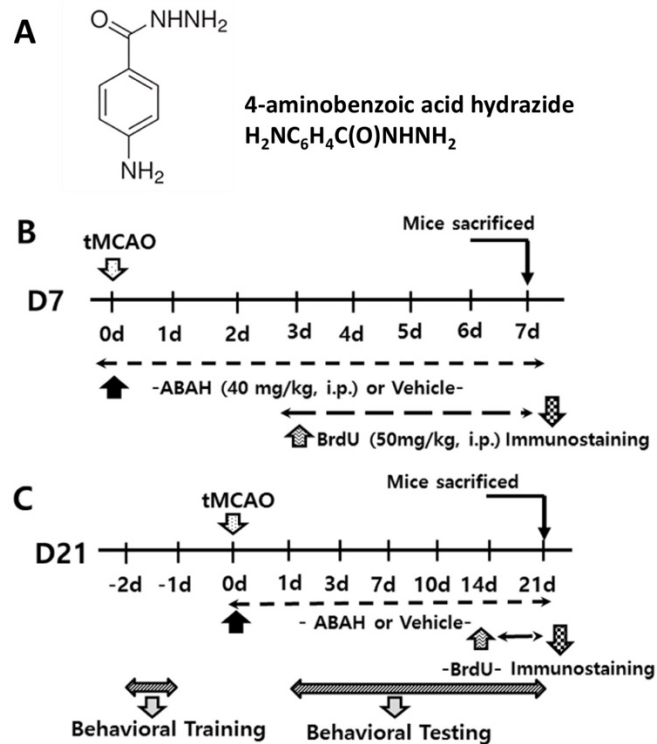
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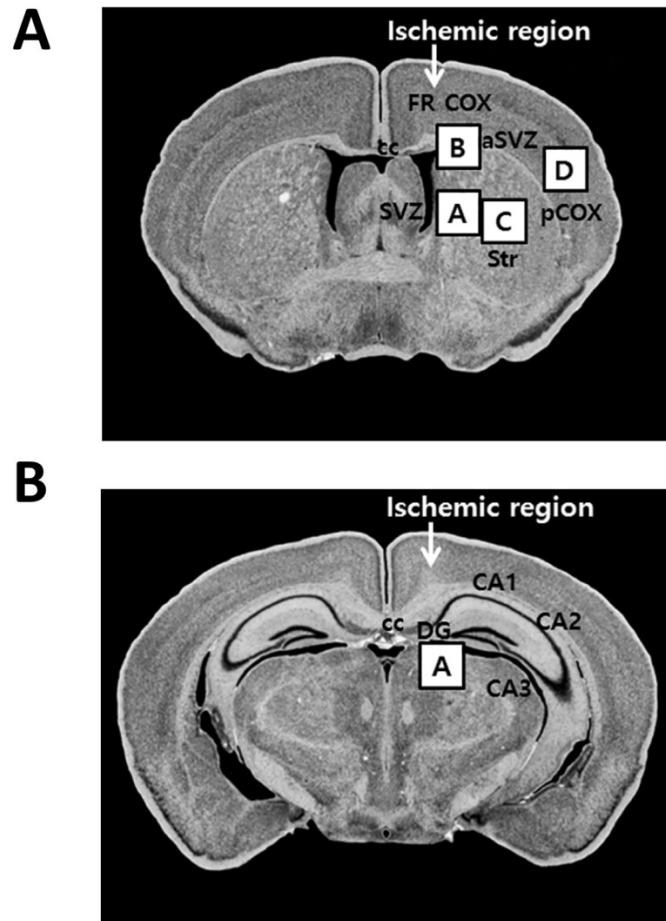
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SUPPLEMENTARY FIGURES:

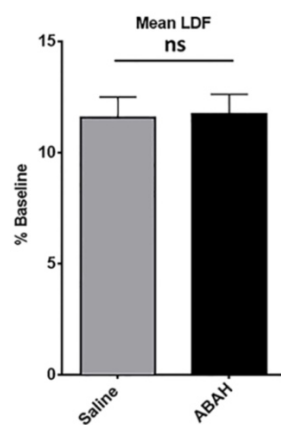


Supplementary Fig. 1. (A) Chemical structure of 4-aminobenzoic acid hydrazide.

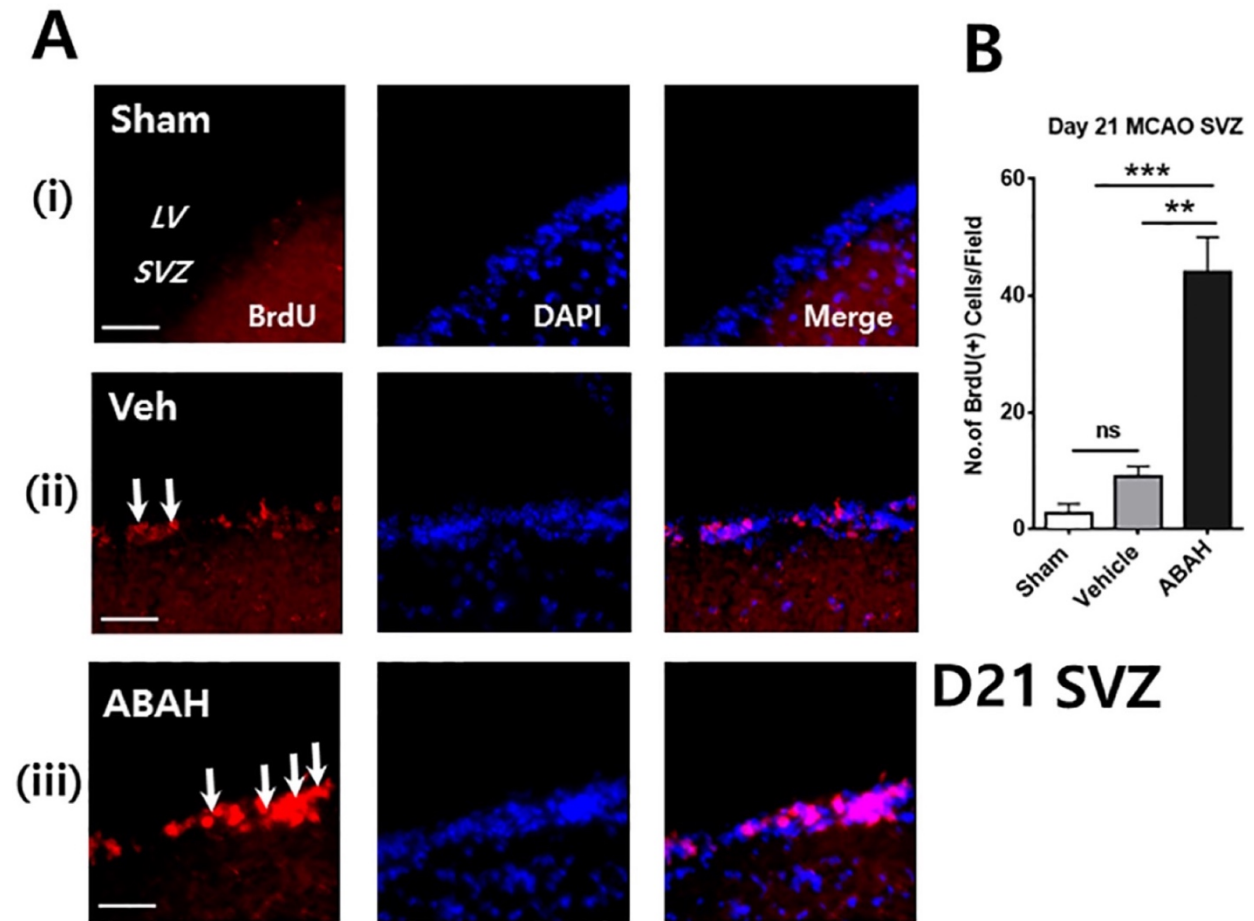
(B) A schematic diagram of experiments on day 7 after stroke: Mice were treated twice daily with intraperitoneal injections of either 4-aminobenzoic acid hydrazide (ABAHA, 40 mg/kg body weight) or vehicle, starting immediately after tMCAO and sacrificed on day 7 after stroke. To label dividing cells, mice were intraperitoneally injected with bromo-2'-deoxyuridine (BrdU) (50 mg/kg, i.p.) twice daily from days 3 to 7 after ischemia. (C). Experimental scheme for day 21 after stroke: Mice were treated with the same conditions in (A) with ABAHA treatment administered to day 21 after stroke. Mice were injected with BrdU from days 14 to 21 after ischemia, and sacrificed on day 21. Behavioral test was performed with pre-training and 8-point neurological test up to day 21 after stroke. Immunostaining sample was collected on day 21 after stroke.



Supplementary Fig. 2. Schematic diagram of the anatomic locations utilized for immunohistochemistry in tMCAO. (A) shows the regions of the SVZ, aSVZ, striatum and parietal cortex used for immunohistochemical examinations (as shown in boxes, A-D) in a brain section after tMCAO. SVZ, subventricular zone; aSVZ, anterior subventricular zone; Str, striatum; FR COX, frontal cortex; CC, corpus callosum; pCOX, parietal cortex. (B) shows the area in the hippocampal dentate gyrus used for immunohistochemical examinations (as shown in box, A). DG, dentate gyrus; CC, corpus callosum.



Supplementary Fig. 3. Laser Doppler Flowmetry (LDF) measurements after stroke. Vehicle (n=30) and ABAH (n=30). ns=not significant.



Supplementary Fig. 4. MPO inhibition increased the number of BrdU⁺ cells in the ischemic SVZ on day 21 after tMCAO. (A) BrdU staining in the ipsilateral SVZ in sham, vehicle-treated, and ABAH-treated tMCAO mice. BrdU⁺ (red), DAPI⁺ (blue). Merge (BrdU⁺/DAPI⁺). LV: lateral ventricle, SVZ: subventricular zone. Arrows identify BrdU-positive cells. (B) Quantification of (A). Sham, vehicle- and ABAH-treated mice (n=3 in each group). Magnification, ×40. Scale bar, 50 μm. Data are mean ± SEM. ANOVA followed by Bonferroni's *post-hoc* test. *** $p < 0.001$.