Supplementary Material

Protective effects and mechanisms of auricular vagus nerve stimulation in the alleviation of acute ischemic cerebral injury in mice

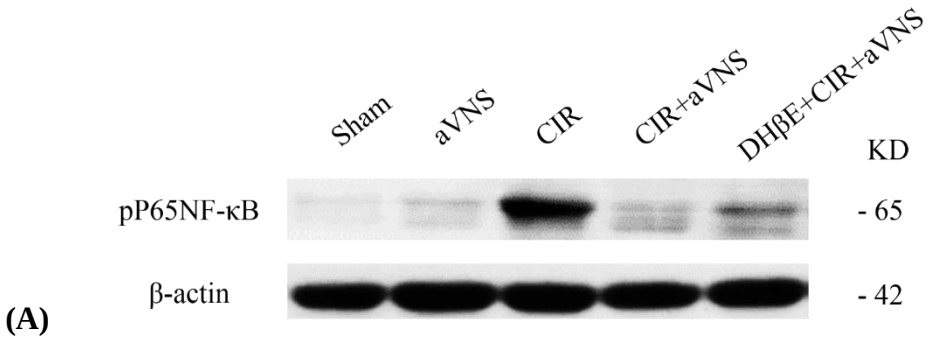
Cher-Chia Chang, Kuo-Tong Liou, Yea-Hwey Wang, Chih-hung Hsu, Yuh-Chiang Shen*, and Jiin-Cherng Yen*

Supplementary Table

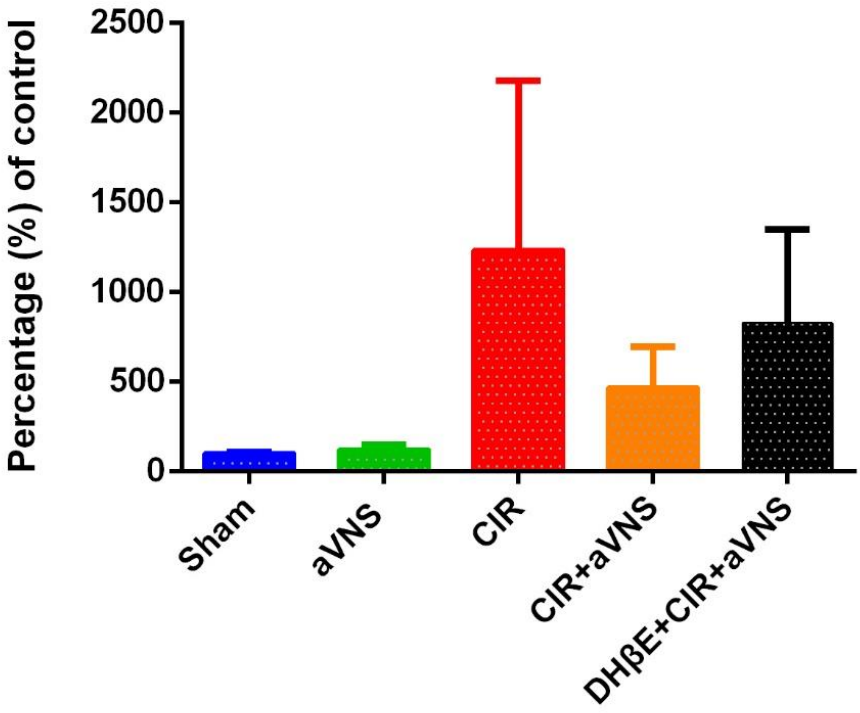
Supplementary Table 1 The effect of aVNS on survival rate after ischemic stroke injury.

	Day 0 (N)	Day 1 (N)	Survival rate
CIR	34	14	41%
CIR+aVNS	20	12	60%
DHβE+CIR+aVNS	12	5	42%

Supplementary Figures

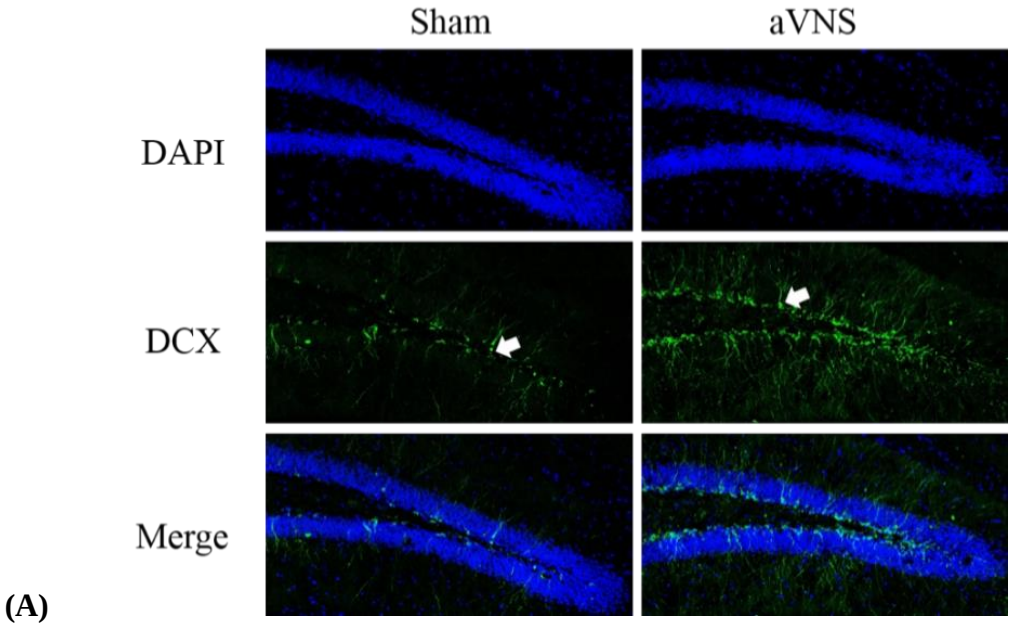


(A)

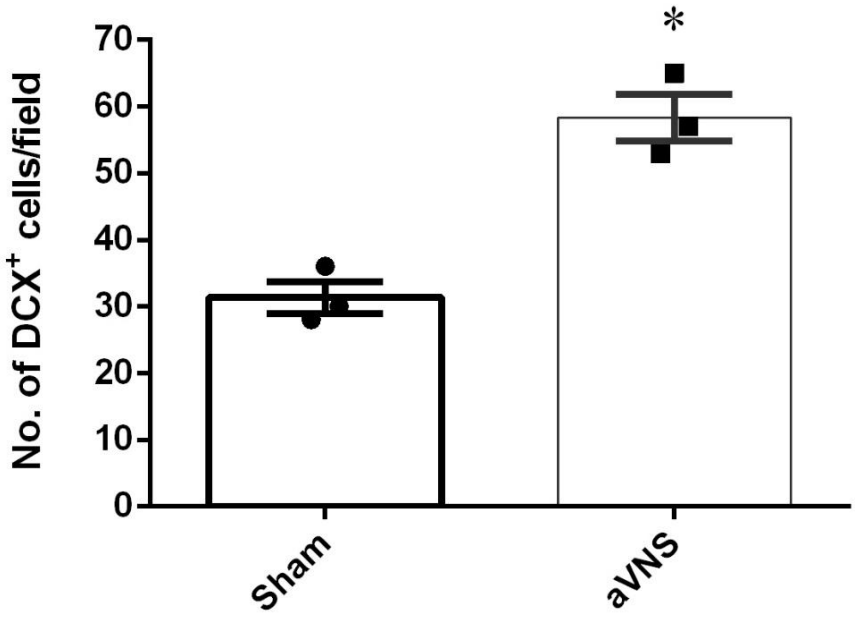


(B)

Supplementary Fig. 2 Protein expression of aVNS on inflammatory injury factors after ischemic stroke injury. (A) Protein expression of pP65NF-κB after ischemic stroke injury using Western blotting. (B) Statistical results of the Western blotting. Data are mean ± SEM (N=3 for each group).

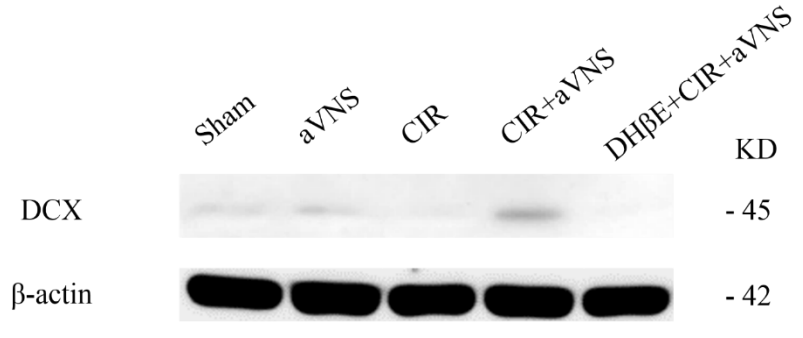


(A)

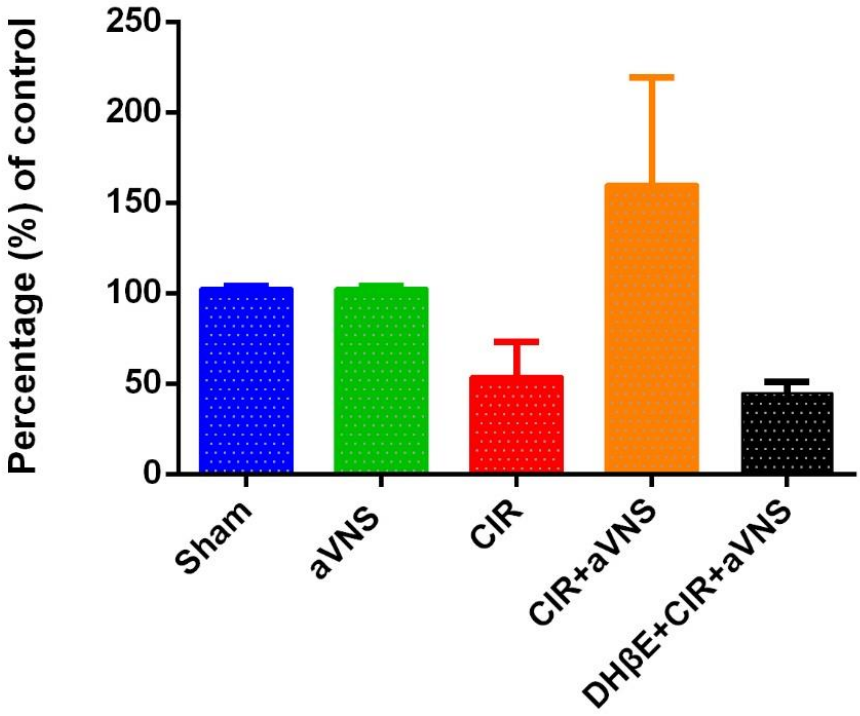


(B)

Supplementary Fig. 2 The effect of aVNS on the number of doublecortin (DCX)-positive cells in the hippocampal dentate gyrus. **(A)** Representative images showing comparison of the number of DCX positive cells (green, a marker of newborn neuroblasts)) and DAPI (blue, a marker of nucleus) in the Sham group and aVNS group in the hippocampal dentate gyrus **(B)** Statistics of the number of DCX positive cells in the hippocampal dentate gyrus. Data are mean ± SEM (N=3 for each group). * $p < 0.05$ versus Sham group.

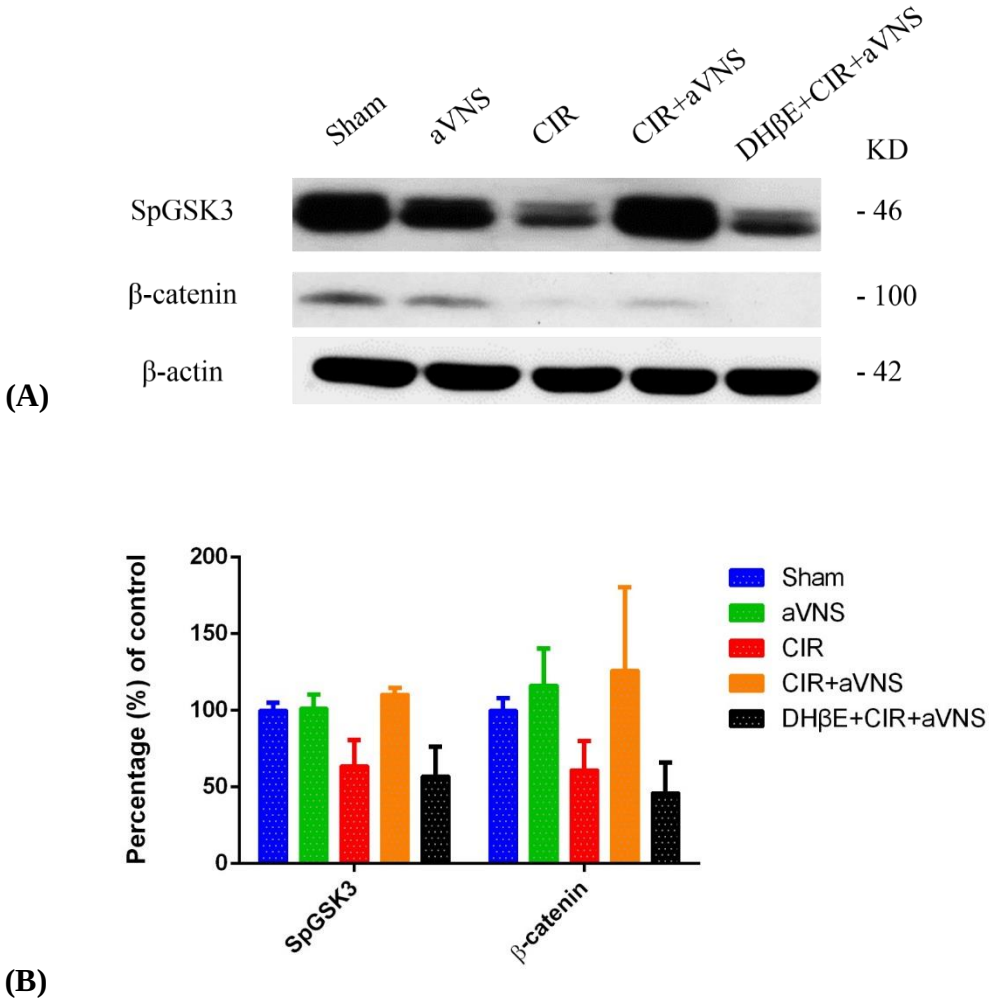


(A)

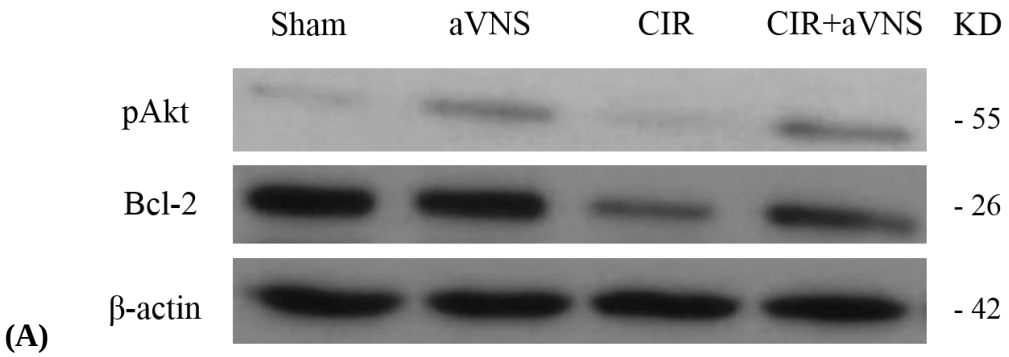


(B)

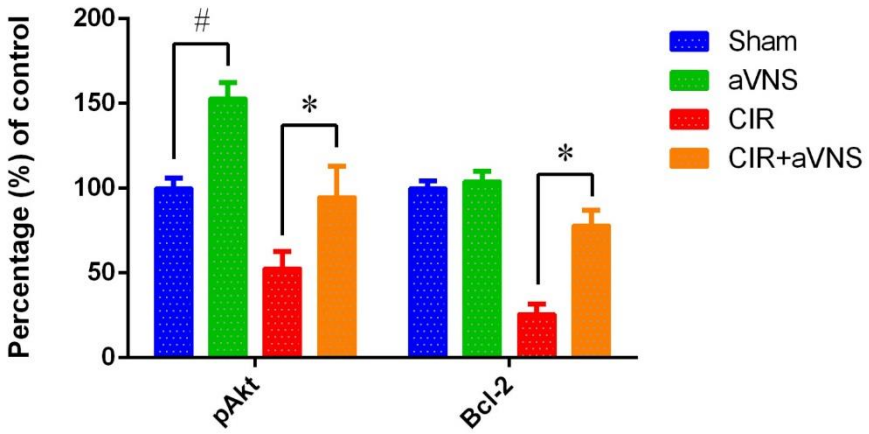
Supplementary Fig. 3 Protein expression of aVNS on doublecortin (DCX) after ischemic stroke injury. **(A)** Protein expression of DCX after ischemic stroke injury using Western blotting. **(B)** Statistical results of the Western blotting. Data are mean ± SEM (N=3 for each group).



Supplementary Fig. 4 The effect of aVNS on SpGSK3 and β-catenin after ischemic stroke injury. **(A)** Protein expression of SpGSK3 and β-catenin after ischemic stroke injury using Western blotting. **(B)** Statistical results of the Western blotting. Data are mean ± SEM (N=3 for each group).

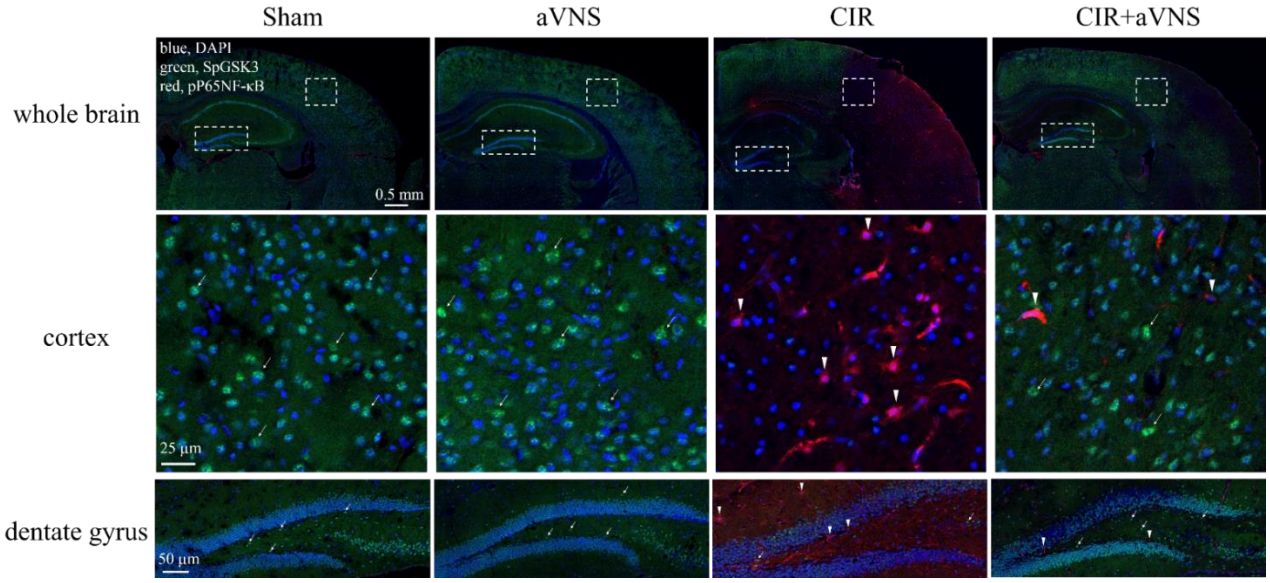


(A)

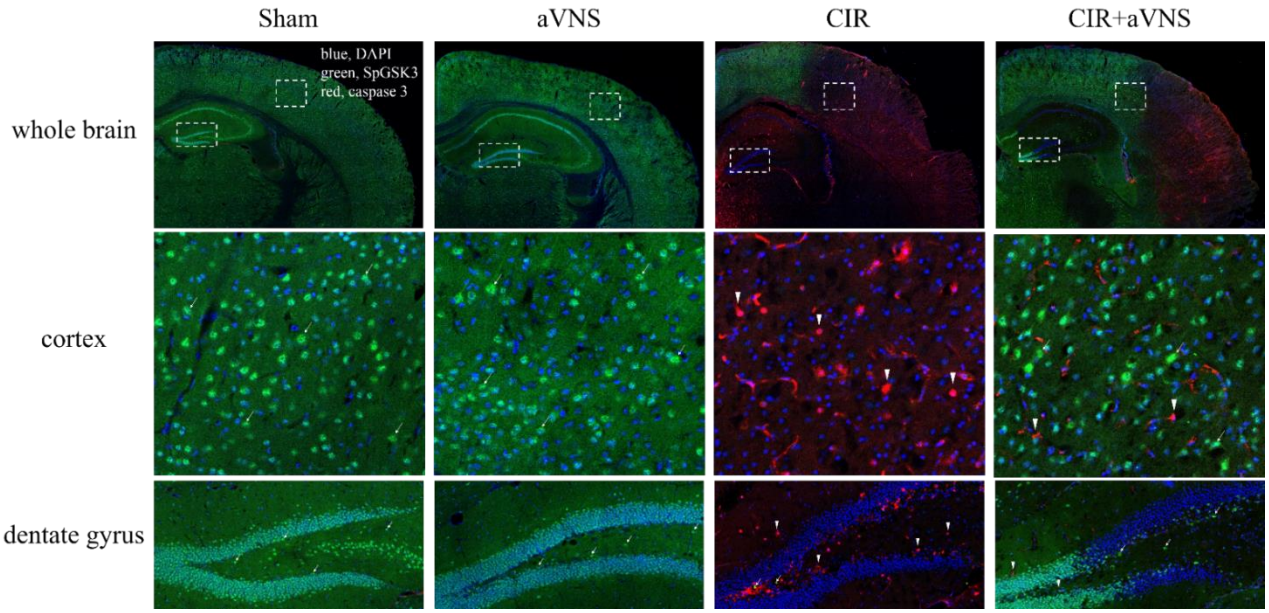


(B)

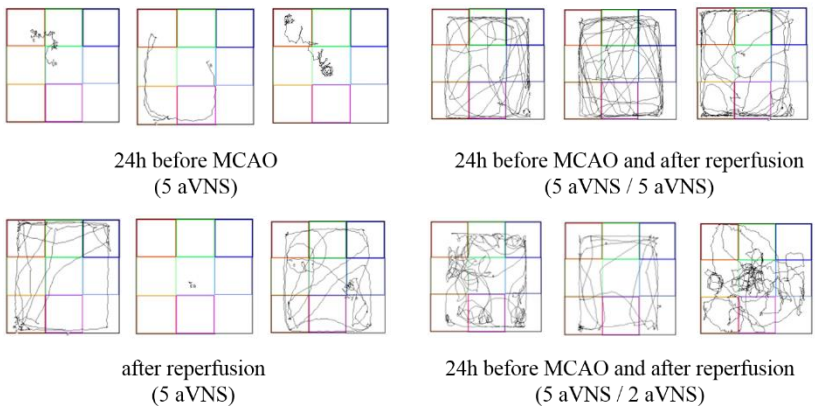
Supplementary Fig. 5 The effect of aVNS on pAkt and Bcl-2 after ischemic stroke injury. **(A)** Protein expression of pAkt and Bcl-2 after ischemic stroke injury using Western blotting. **(B)** Statistical results of the Western blotting. Data are mean ± SEM (N=3 for aVNS group of pAkt and Bcl-2 , N=4 for other groups). * $p < 0.05$ versus CIR group. # $p < 0.05$ versus Sham group.



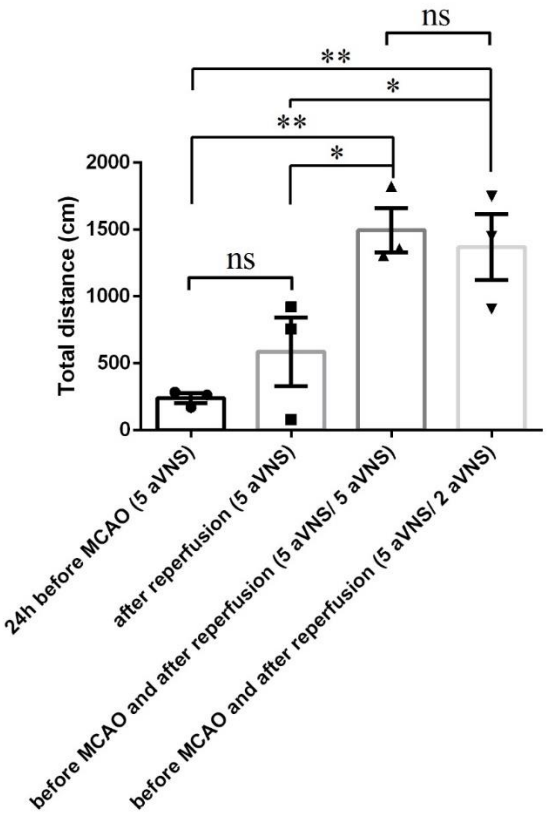
Supplementary Fig. 6 The relationship between SpGSK3 and inflammatory factors after aVNS on ischemic stroke injury. Representative images showing comparison of SpGSK3 (green), pP65NF-κB (red, a marker of inflammation) and DAPI (blue, marker of nucleus) in the whole brain, cortex and hippocampal dentate gyrus of each group. The white arrows indicate the distribution of cells showing positive staining for SpGSK3 (green). The white arrowheads indicate the distribution of cells showing positive staining for p65NF-κB (red). (N=3 for each group).



Supplementary Fig. 7 The relationship between SpGSK3 and apoptotic proteins after aVNS on ischemic stroke injury. Representative images showing comparison of SpGSK3 (green), active form caspase 3 (red, marker of apoptosis) and DAPI (blue, marker of nucleus) in the whole brain, cortex and hippocampal dentate gyrus of each group. The white arrows indicate the distribution of cells showing positive staining for SpGSK3 (green). The white arrowheads indicate the distribution of cells showing positive staining for active form caspase 3 (red, marker of apoptosis). (N=3 for each group).



(A)



(B)

Supplementary Fig. 8 The effect of various aVNS conditions on the neurological deficits after ischemic stroke injury. **(A)** A three-minute behavior recording of the animal in the open field test was used to assess neurological deficits. **(B)** Statistical results of the neurological deficits were analyzed by measuring the tracking distance for 3 minutes. Data are mean ± SEM (N=3 for each group). * $p < 0.05$