

**Protective effects of duloxetine against cerebral ischemia-reperfusion injury via TRPM2 inhibition**

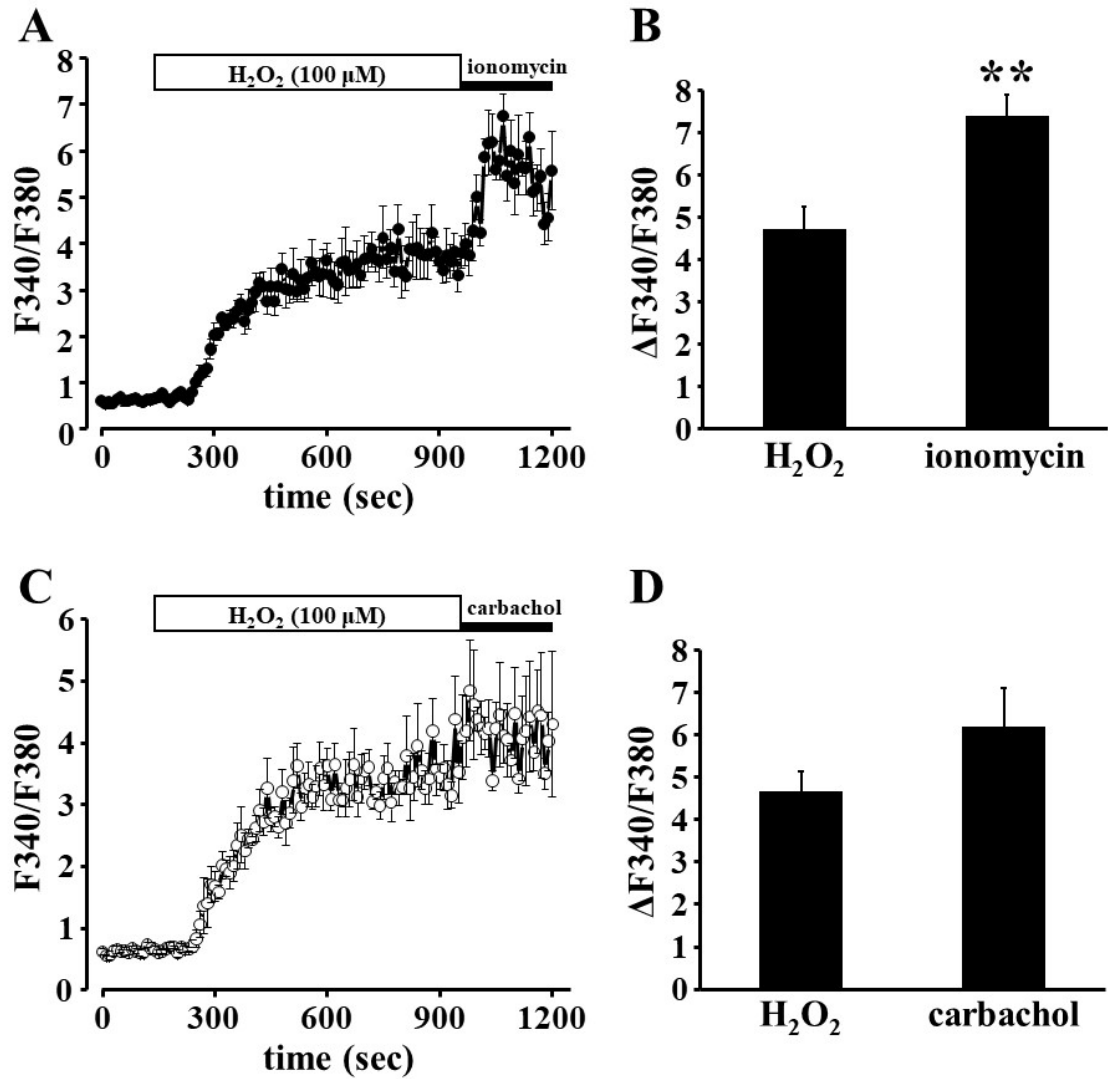
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Supplementary Figure 1

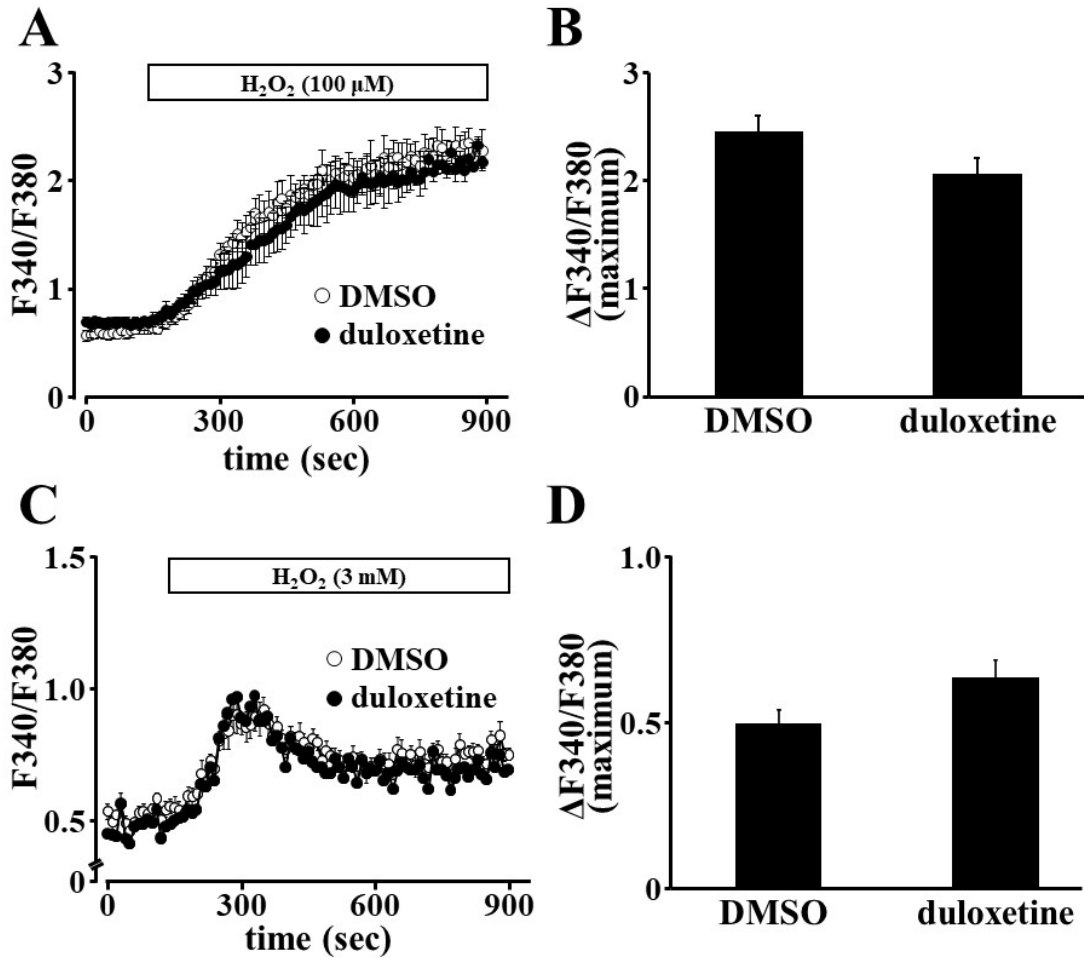


Supplementary Figure 1. Comparison of H<sub>2</sub>O<sub>2</sub>-induced [Ca<sup>2+</sup>]<sub>i</sub> increases with Ca<sup>2+</sup> ionophore- and carbachol-induced responses.

TRPM2/HEK cells were treated with H<sub>2</sub>O<sub>2</sub> (100 μM), and then treated with 2 μM ionomycin (A) or 100 μM carbachol (C). Delta ratios (ΔF340/F380) at maximum responses during H<sub>2</sub>O<sub>2</sub>, ionomycin (B), and carbachol (D) treatments were calculated from A and C, respectively. Results are shown as the mean ± S.E.M. of 4 experiments.

\*\*P < 0.01 vs. H<sub>2</sub>O<sub>2</sub>.

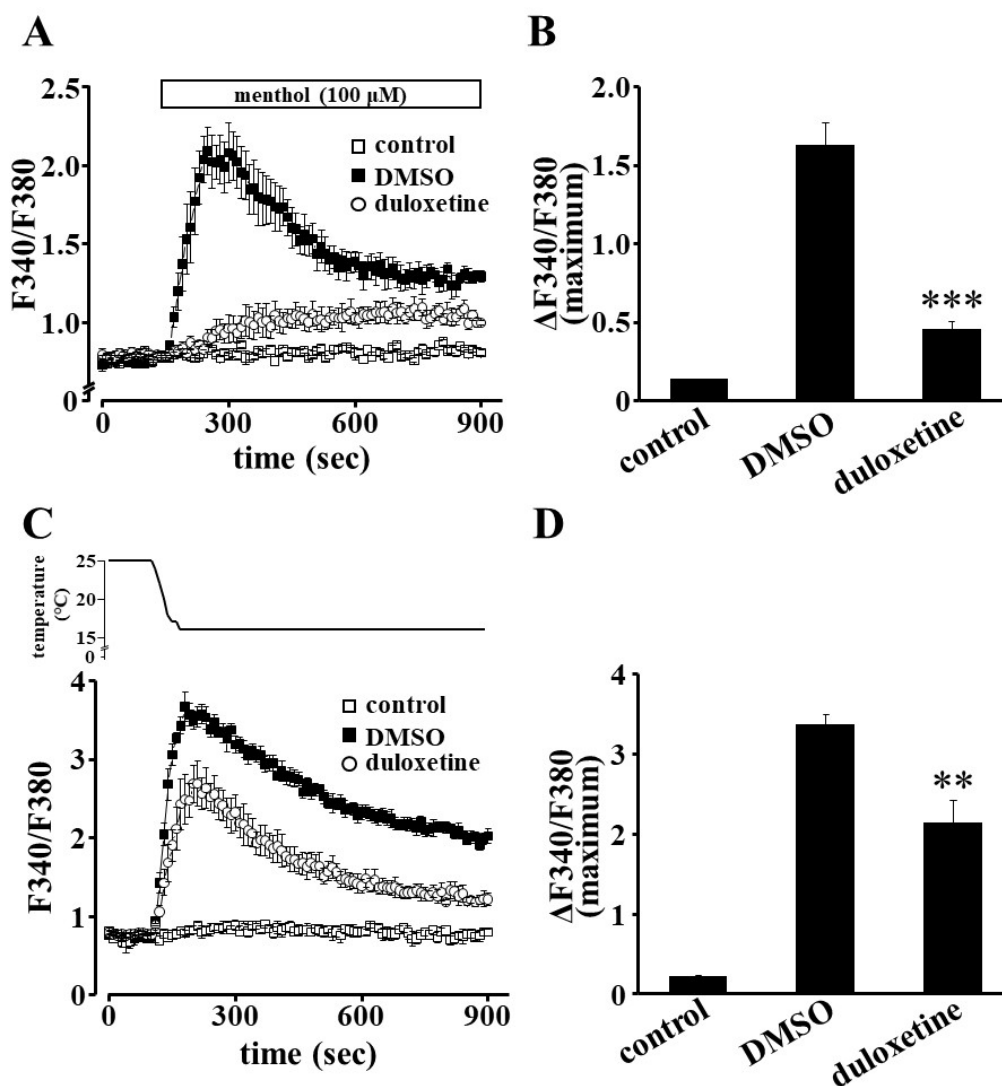
Supplementary Figure 2



Supplementary Figure 2. Effects of duloxetine on H<sub>2</sub>O<sub>2</sub>-induced [Ca<sup>2+</sup>]<sub>i</sub> increases in TRPA1/HEK, TRPV1/HEK.

TRPA1/HEK cells (A) and TRPV1/HEK cells (C) were treated with 0.1% DMSO or 10 μM duloxetine, and then H<sub>2</sub>O<sub>2</sub> (100 μM) was added in the presence of duloxetine. (B and D) Delta ratios (ΔF<sub>340</sub>/F<sub>380</sub>) at maximum responses were calculated from A and C, respectively. Results are shown as the mean ± S.E.M. of 4 experiments.

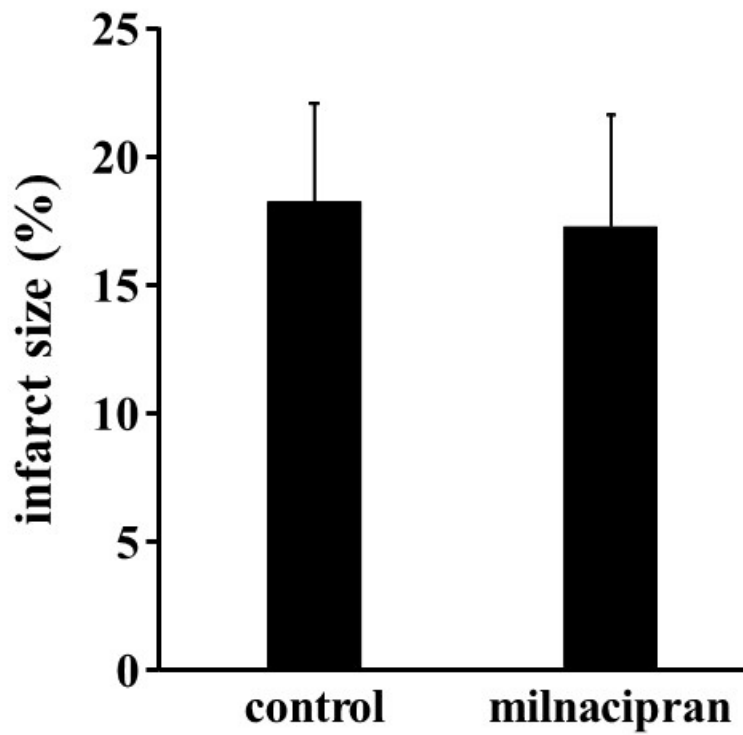
### Supplementary Figure 3



Supplementary Figure 3. Effects of duloxetine on menthol- and cold temperatures-induced  $[Ca^{2+}]_i$  increases in TRPM8/HEK.

(A) HEK cells were pretreated with 0.1% DMSO (control). TRPM8/HEK cells were treated with 0.1% DMSO (DMSO) or 10  $\mu$ M duloxetine, and then menthol (100  $\mu$ M) was added in the presence of duloxetine. (B) Delta ratios ( $\Delta$ F340/F380) were calculated from A. (C) HEK cells were pretreated with 0.1% DMSO (control). TRPM8/HEK cells were pretreated with 0.1% DMSO (DMSO) or 10  $\mu$ M duloxetine at 25 $^{\circ}$ C. The temperature was then decreased to 16  $^{\circ}$ C in the presence of duloxetine. (D) Delta ratios ( $\Delta$ F340/F380) were calculated from C. Results are shown as the mean  $\pm$  S.E.M. of 4 experiments. \*\*P < 0.01, \*\*\*P < 0.001 vs. DMSO group.

**Supplementary Figure 4**



Supplementary Figure 4. Effects of milnacipran on CIR injury.

Vehicle (control) or milnacipran (10 mg/kg, i.p.) were administered 1 h before ischemia, and then infarct size was determined at 21 h after reperfusion. Results are shown as the mean  $\pm$  S.E.M., n = 6.