Supplementary data by Xin Li et al

Figure 1 H₂O₂-induced concentration- and duration-dependent hippocampal neuronal death. Representative fluorescence images showing PI and Hoechst staining of WT neurons under control (CTL) or treatment with 30-300 μ M H₂O₂ for 2, 8 and 24 hr. Scale bar is 100 μ m. The mean data are shown in Fig. 1a.

Figure 2 Lack of an inhibition of PJ34 on H_2O_2 -induced neuronal death in TRPM2-KO neurons. Mean percentage of PI positive TRPM2-KO hippocampal neurons under control (CTL) or neurons exposed to 300 μ M H_2O_2 for 24 hr or treated with 1 μ M PJ34, 30 min prior to and during exposure to 300 μ M H_2O_2 , from 3 independent experiments with each independent experiment examining 450-600 neurons. *** p < 0.005 indicates difference from CTL. NS, no significant difference. The black bar shows H_2O_2 -induced neuronal death in WT neurons as shown in Fig. 1c.

Figure 3 No effect of PJ34 and 2-APB on the $[Zn^{2+}]_c$ in H₂O₂-exposed TRPM2-KO neurons. (a) Representative fluorescence images showing FluoZin3 fluorescence in TRPM2-KO hippocampal neurons under control (CTL) or exposed to 300 μ M H₂O₂ for 30 min or treated with 1 μ M PJ34 or 10 μ M 2-APB, 30 min prior to and during exposure to H₂O₂. The scale bar is 10 μ m. (b) Mean FluoZin3 fluorescence intensity under indicated conditions from 3 independent experiments with a total of 40-60 neurons examined. All values are normalized to the basal fluorescence level in control neurons in matched experiments. NS, no significant difference.

Figure 4 Effect of exposure to H_2O_2 on TRPM2 protein expression. Representative immunofluorescent images showing no increase in the TRPM2 expression in WT neurons under control (CTL) conditions or after exposure to 100 μ M or 300 μ M H_2O_2 for 30 min. Similar observations were made in two independent experiments. Scale bar is 20 μ m.







