Supporting Information to:

Puerarin Inhibits Acid-Sensing Ion Channels and Protects Against Neuron Death Induced by Acidosis

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Detection of apoptosis and necrosis by confocal microscopy

The Vybrant Apoptosis Assay Kit (Invitrogen) was used for the detection of apoptotic and necrotic cells according to vendor's protocol. This kit uses a dual-staining protocol in which the apoptotic cells are stained with annexin-V (green fluorescence) and the necrotic cells are stained with propidium iodide (PI; red fluorescence). The fluorescence was measured by a Zeiss 510 confocal microscope. Confocal images of green annexin-FITC fluorescence were collected using 488-nm excitation light from an argon/krypton laser and a 500−550-nm bandpass barrier filter. Images of red PI fluorescence were collected using a 543-nm excitation light from the helium/Ne laser and a 580-nm-long pass filter.

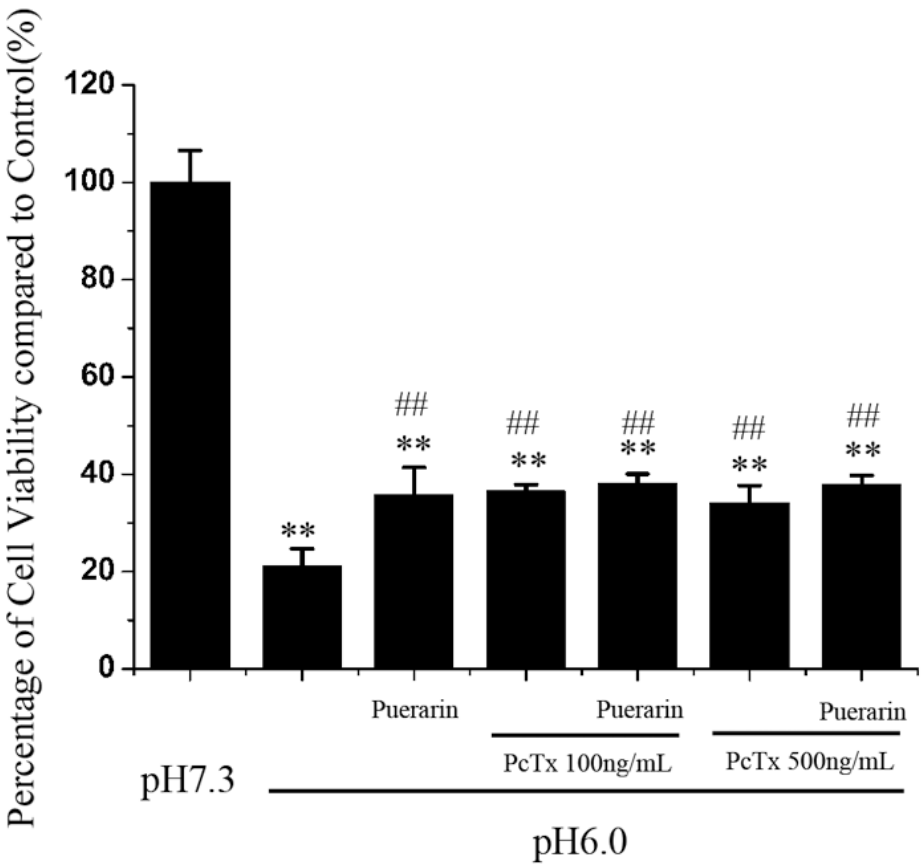
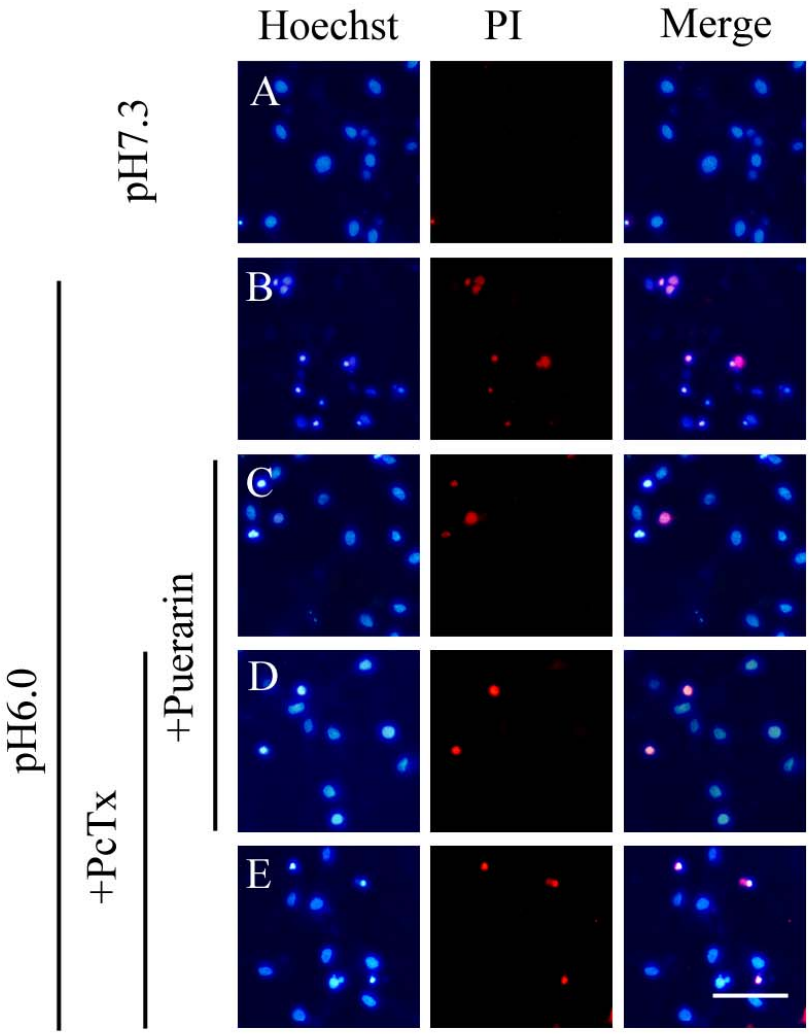
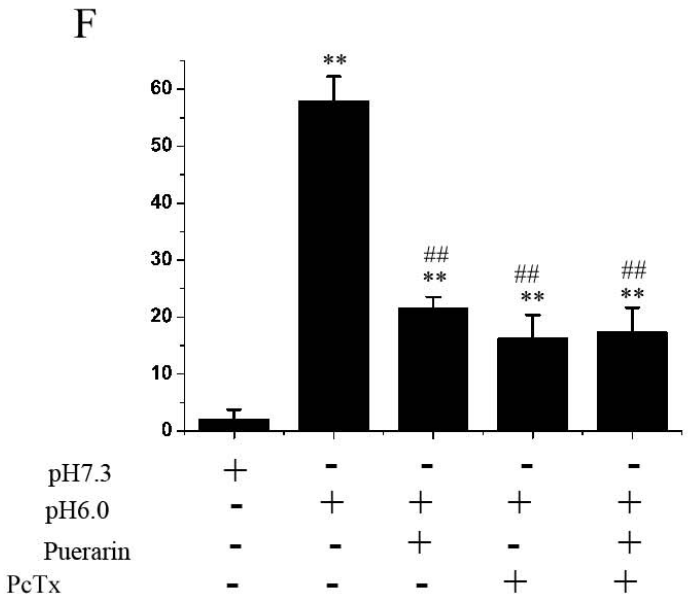


Fig. 1S MTT cleavage treated with puerarin or PcTx venom after acidosis. Cells were treated

with puerarin 100 μM, PcTx venom 100 ng/mL or 500 ng/mL, or both puerarin and PcTx venom with acidotic solution of pH 6.0 for 4 h. Percentage cell viability was determined relative to control group (100%, $n = 5$ for all groups). $^{**}P < 0.01$ vs. control group; $^{\#\#}P < 0.01$ vs. treatment with pH 6.0.


Hoechst
PI
Merge
A
B
C
D
E
pH7.3
pH6.0
+PcTx
+Puerarin


F
60
50
40
30
20
10
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##
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pH7.3
pH6.0
Puerarin
PcTx

Fig. 2S Puerarin protected hippocampal neurons from acidosis-induced death. Cells were stained by Hoechst 33342 and PI. Panel **A**: Neurons treated with standard solution for 4 h and examined by fluorescent microscopy with blue filter and red filter, respectively. Panel **B**: Neurons treated with acidotic solution of pH 6.0 for 4 h. Panel **C**: Neurons treated with puerarin 100 μM at pH 6.0 for 4 h. Panel **D**: Neurons treated with both puerarin 100 μM and PcTx venom 100 ng/mL at pH 6.0 for 4 h. Panel **E**: Neurons treated with PcTx venom 100 ng/mL at pH 6.0 for 4 h. Panel **F**: Percentage of death (n = 5). $^{**}P < 0.01$ vs. control group; $^{\#\#}P < 0.01$ vs. treatment with pH 6.0. Bar: 20 μm.

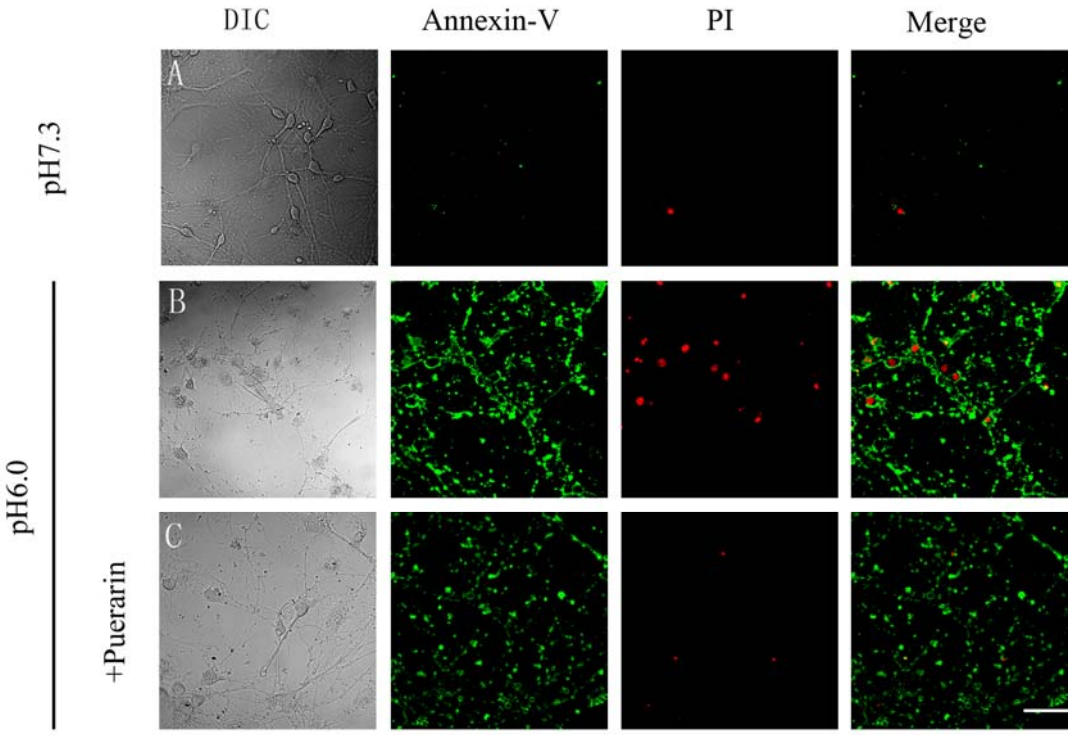

Fig. 3S Puerarin protected hippocampal neurons from acidosis-induced death. Cells were stained by annexin-V and PI. Panel **A**: Neurons treated with standard solution for 4 h and examined by confocal microscopy. Panel **B**: Neurons treated with acidotic solution of pH 6.0 for 4 h. Panel **C**: Neurons treated with puerarin 100 μM at pH 6.0 for 4 h. Bar: 50 μm.

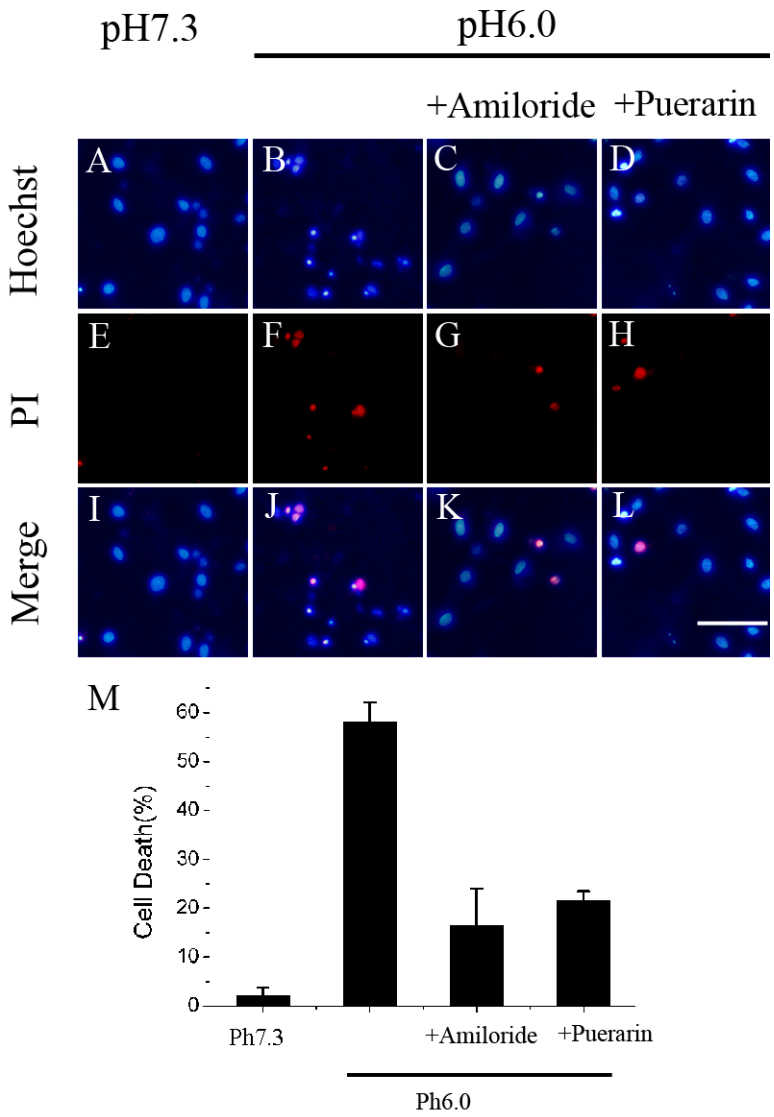

Fig. 4S Puerarin protected hippocampal neurons from acidosis-induced death. Cells were stained by Hoechst 33342 and PI. Panels **A**, **E**, and **I**: Neurons treated with standard solution for 4 h and examined by fluorescent microscopy with blue filter and red filter, respectively. Panels **B**, **F**, and **J**: Neurons treated with acidotic solution of pH 6.0 for 4 h. Panels **C**, **G**, and **K**: Neurons treated with amiloride 10 μM at pH 6.0 for 4 h. Panels **D**, **H**, and **L**: Neurons treated with puerarin 100 μM at pH 6.0 for 4 h. Panel **M**: Percentage of death (n = 5). *P < 0.05 vs. control group; **P < 0.01 vs. control group; $^{\#\#}P$ < 0.01 vs. treatment with pH 6.0 **{Authors: These symbols do not appear in this figure.}**. Bar: 20 μm.