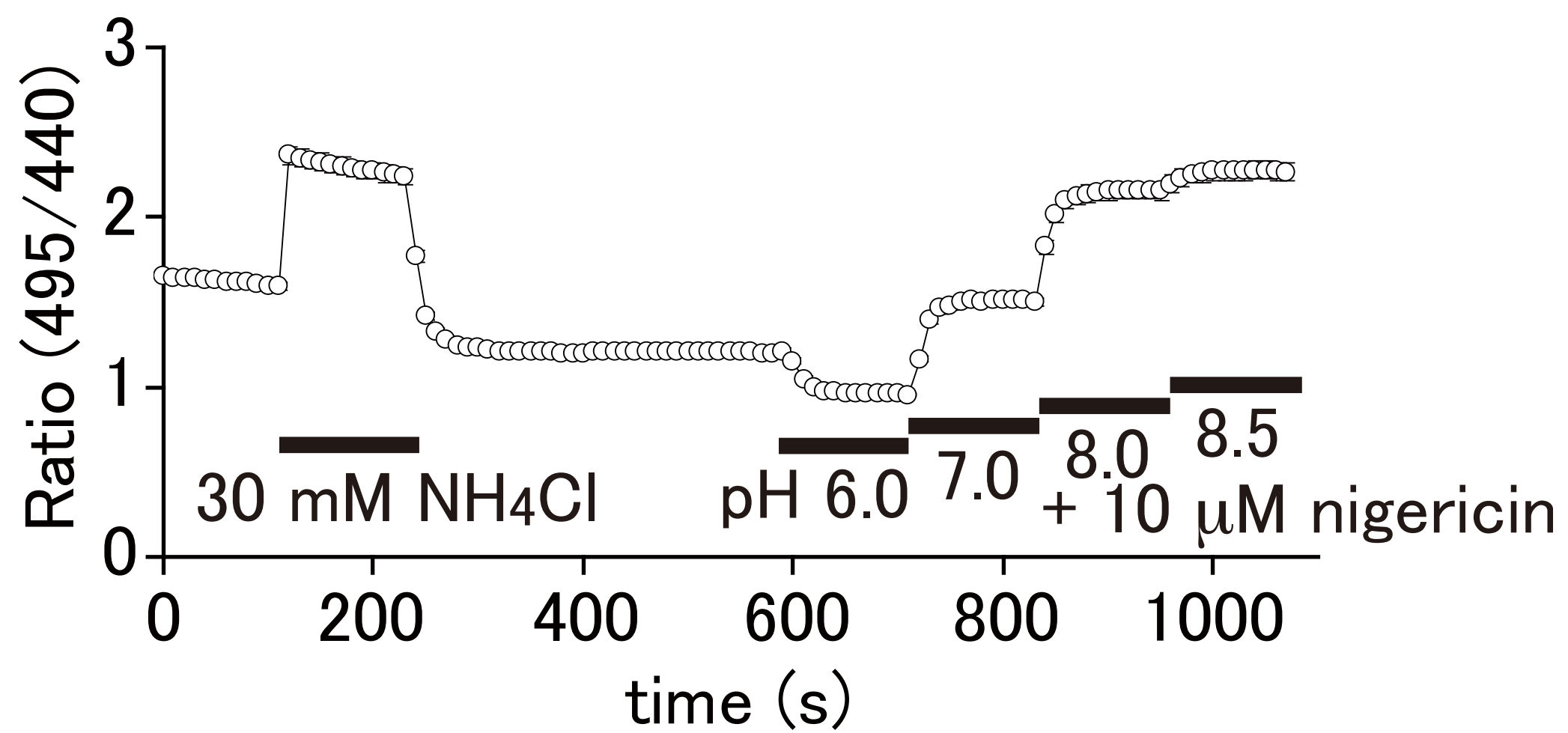


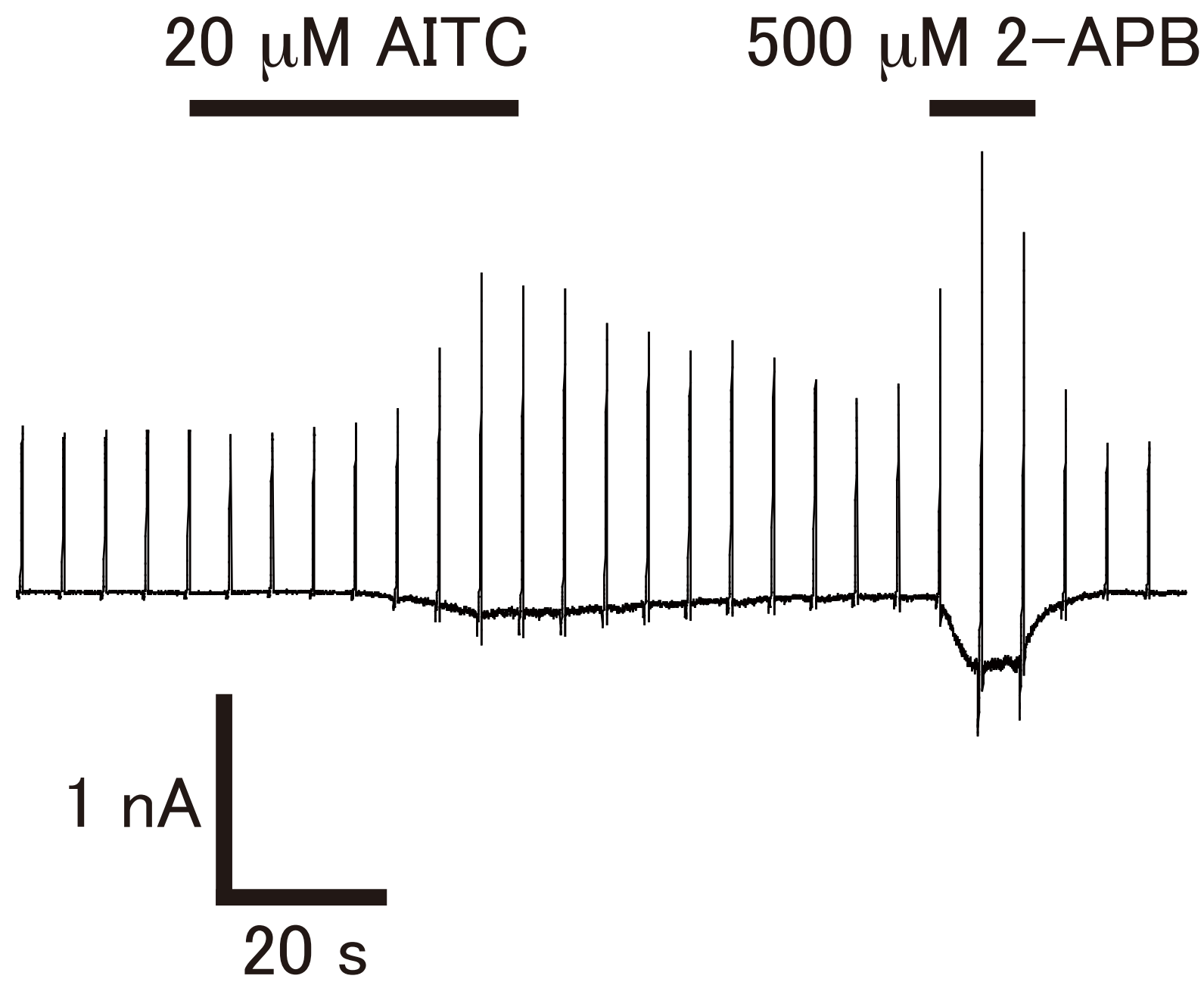
Suppl. Fig. 1

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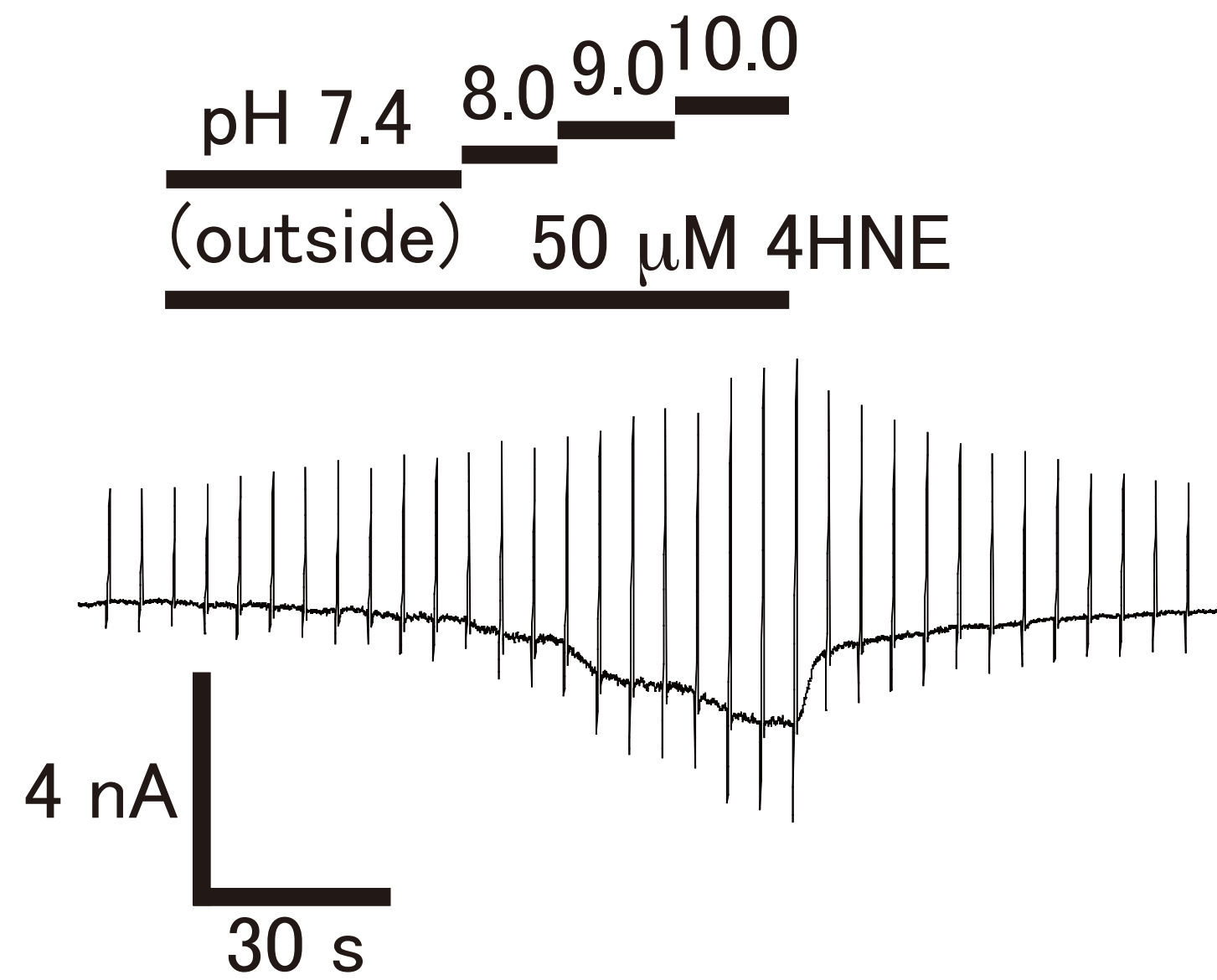
Sup. 2

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Suppl. Figure 3

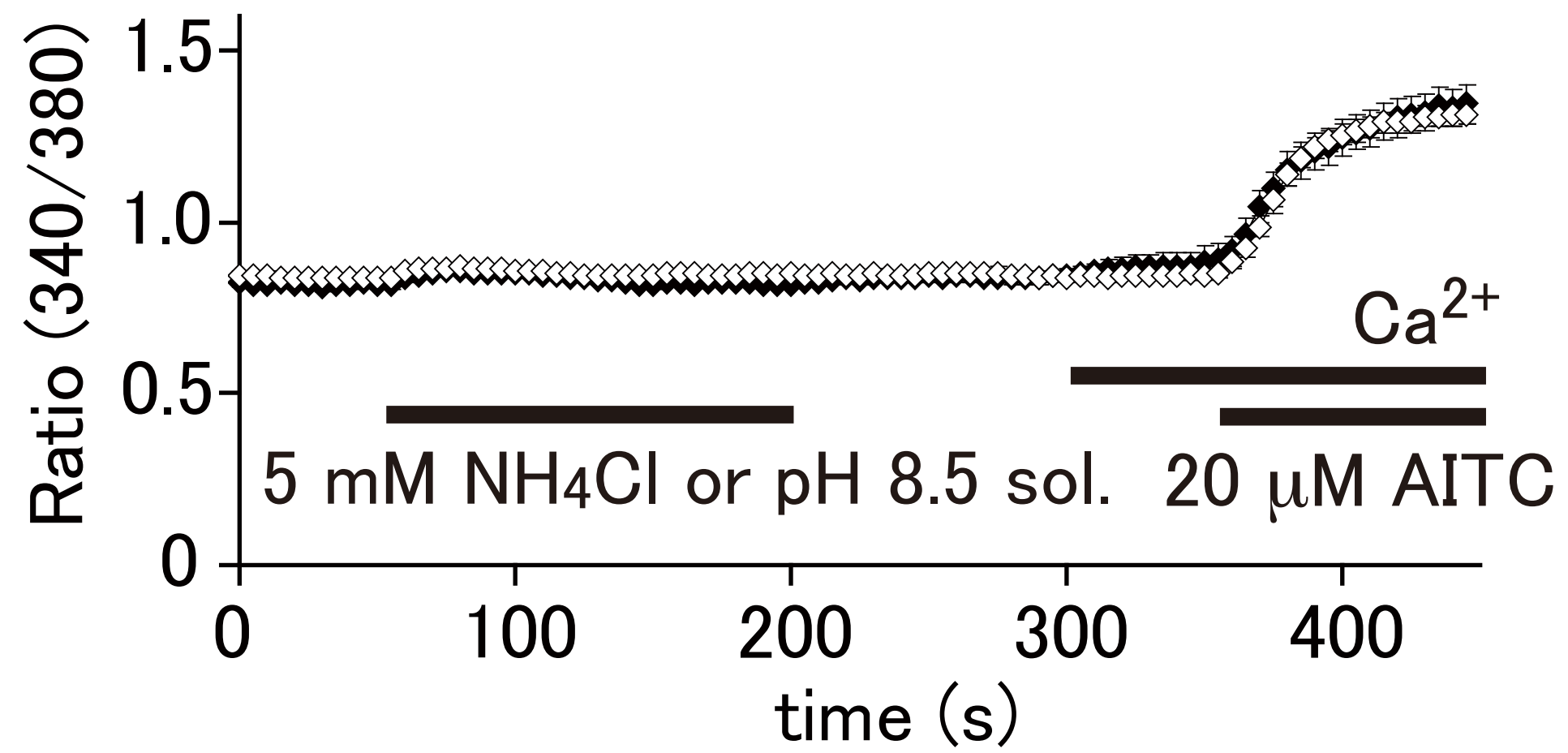
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Sup. 4 (1 column)

Suppl. Figure 4

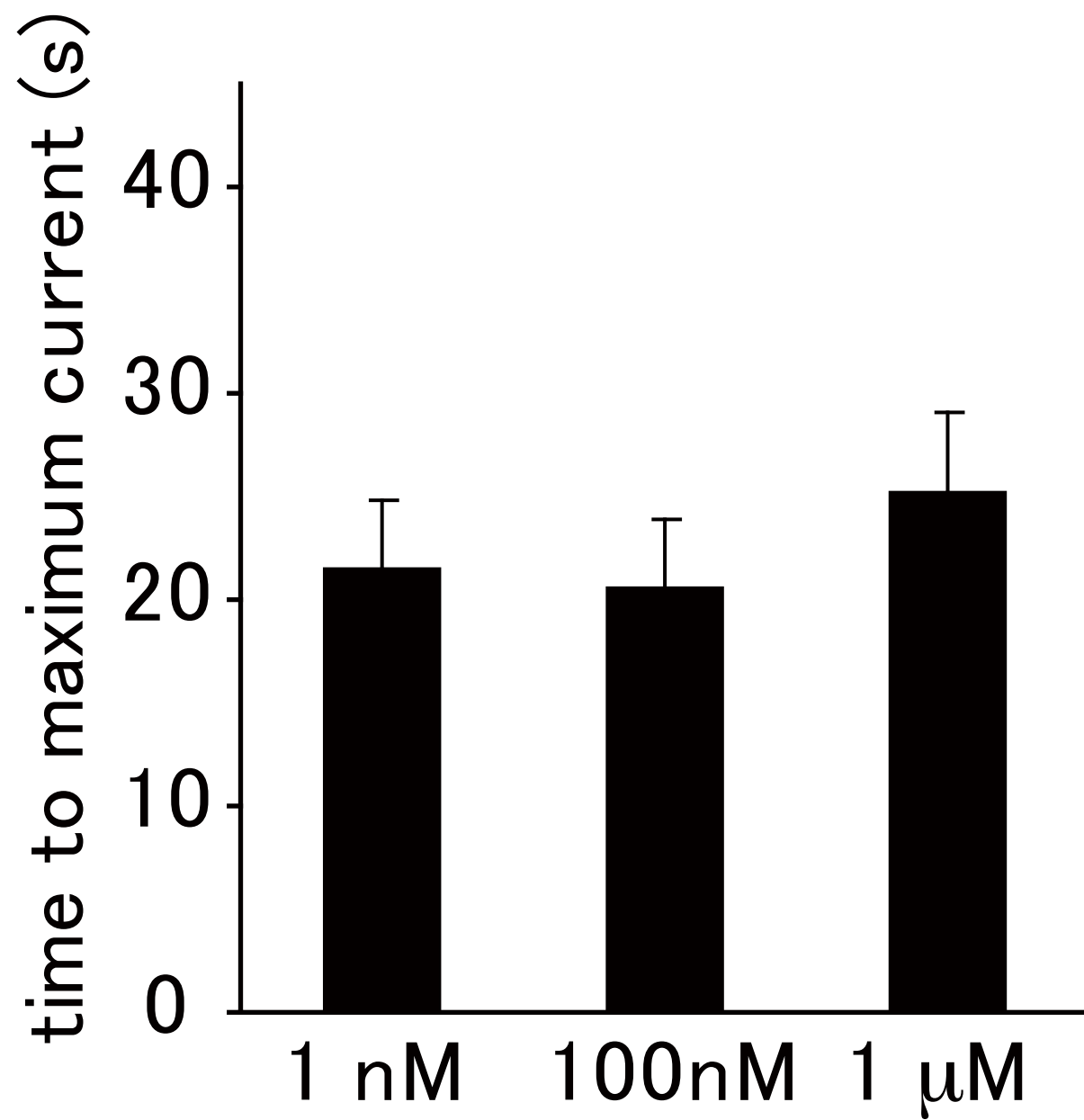
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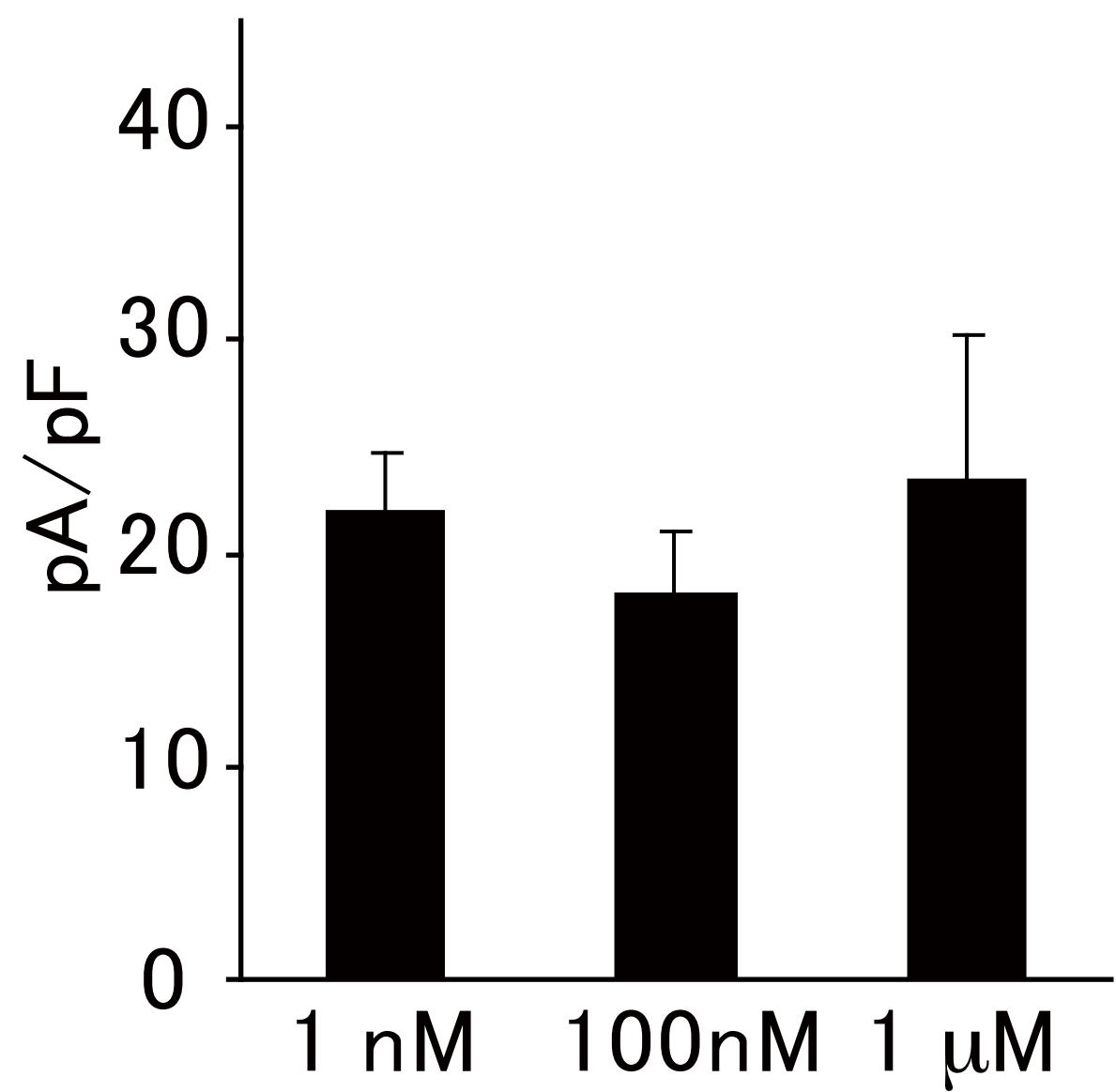
Suppl. Figure 5

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A

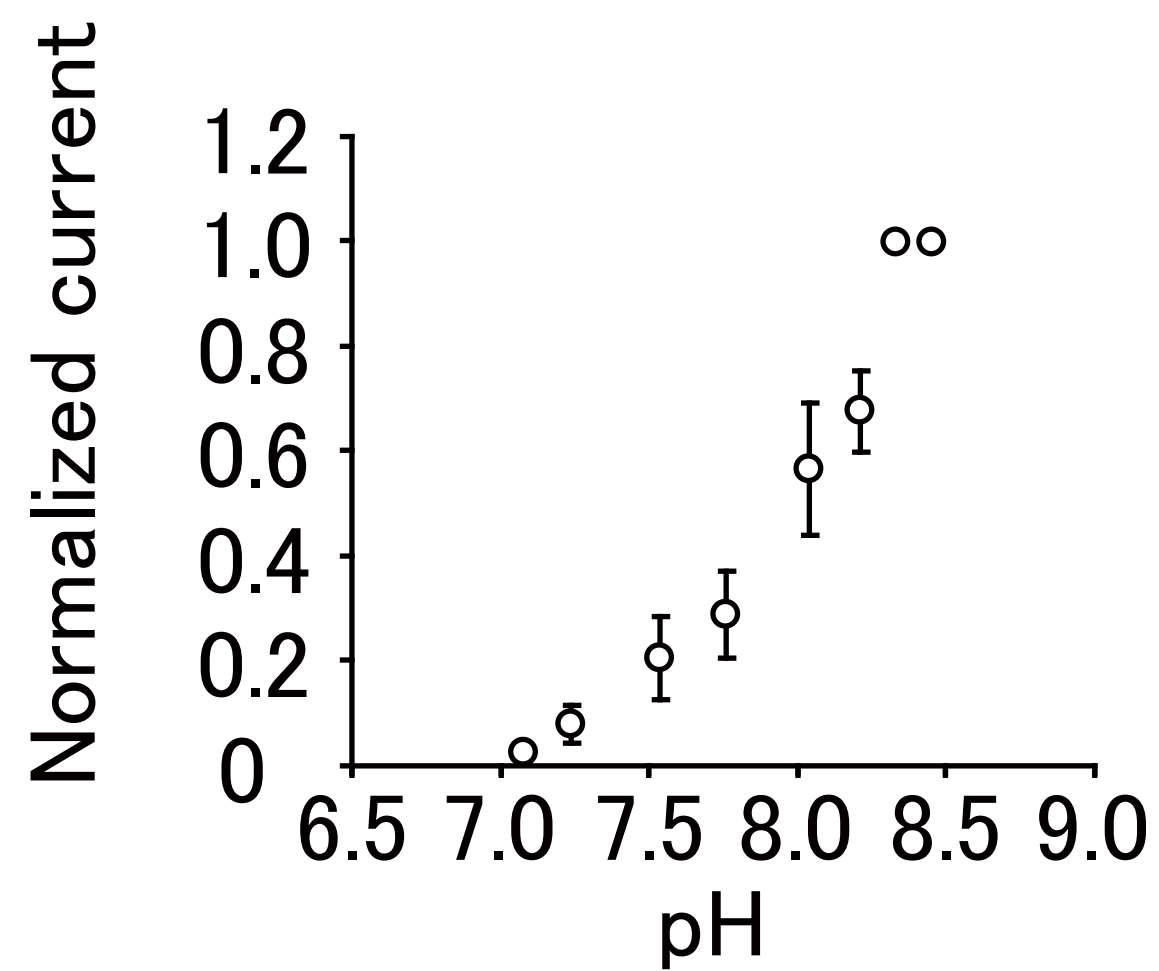


B



Suppl. Figure 6

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Supplementary Figure 1. Ammonium chloride (30 mM) or alkaline pH solutions (n = 27) causes an increase in intracellular pH (indicated by the ratio (488/440) of BCECF) in HEK293 cells expressing mouse TRPA1 in the presence of extracellular Ca^{2+} . Each standard solution contained 10 μM nigericin. Horizontal bars indicate the duration of applied stimulus.

Supplementary Figure 2. A representative whole cell current trace in response to AITC (20 μM) or 2-aminoethoxydiphenyl borate (2-APB, 500 μM) in the cell expressing mutant TRPA1-2C in the presence of extracellular Ca^{2+} . $V_h = -60$ mV. Bars indicate duration of the compound application.

Supplementary Figure 3. A representative current trace activated by 4-hydroxy-2-nonenal (50 μM) in the bath solution with different pH values.

Supplementary Figure 4. Tris buffer (pH 8.5) (filled squares, n = 34) or 5 mM ammonium chloride (opened squares, n = 34) did not cause an increase in cytosolic Ca^{2+} concentration in HEK293 cells expressing mouse TRPA1 in the absence of extracellular Ca^{2+} . TRPA1 response was confirmed by application of 20 μM AITC in the presence of 2 mM extracellular Ca^{2+} . Horizontal bars indicate the duration of applied stimulus.

Supplementary Figure 5. A, Comparison of times to maximum current responses evoked by 30 mM ammonium chloride using the pipette solutions containing different

Ca²⁺ concentrations (1 nM, 100 nM, 1 μ M) in HEK293 cells expressing TRPA1. **B**, Comparison of current densities of the responses evoked by 30 mM ammonium chloride using the pipette solutions containing different Ca²⁺ concentrations (1 nM, 100 nM, 1 μ M) in HEK293 cells expressing TRPA1.

Supplementary Figure 6. A pH-response profile of intracellular pH-activated TRPA1-mediated inward currents in HEK293 cells exposed to NH₄Cl.

The pH-response profile was generated from the data in Figures 1C and 3C.