Expanded View Figures

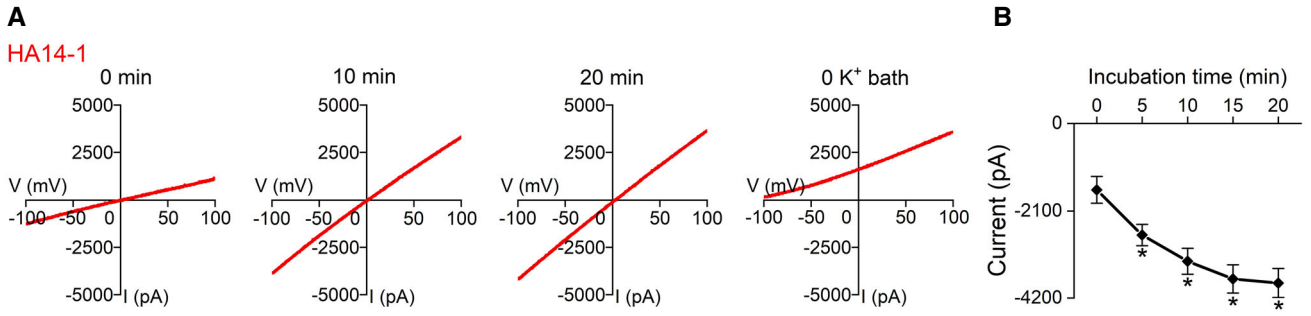

Figure EV1. HA14-1 activates nontagged TMEM175 current.

A Representative whole-cell currents recorded in HEK293T cells expressing nontagged TMEM175.
B Current amplitudes measured at −100 mV in the ramp protocol used in (A). $n = 6$.

Data information: The data are presented as the mean ± SEM. Statistical significance was analyzed with two-sided Student's *t*-tests, and is indicated with * for $P < 0.05$. *n* value means the number of biological replicates made for each data point.

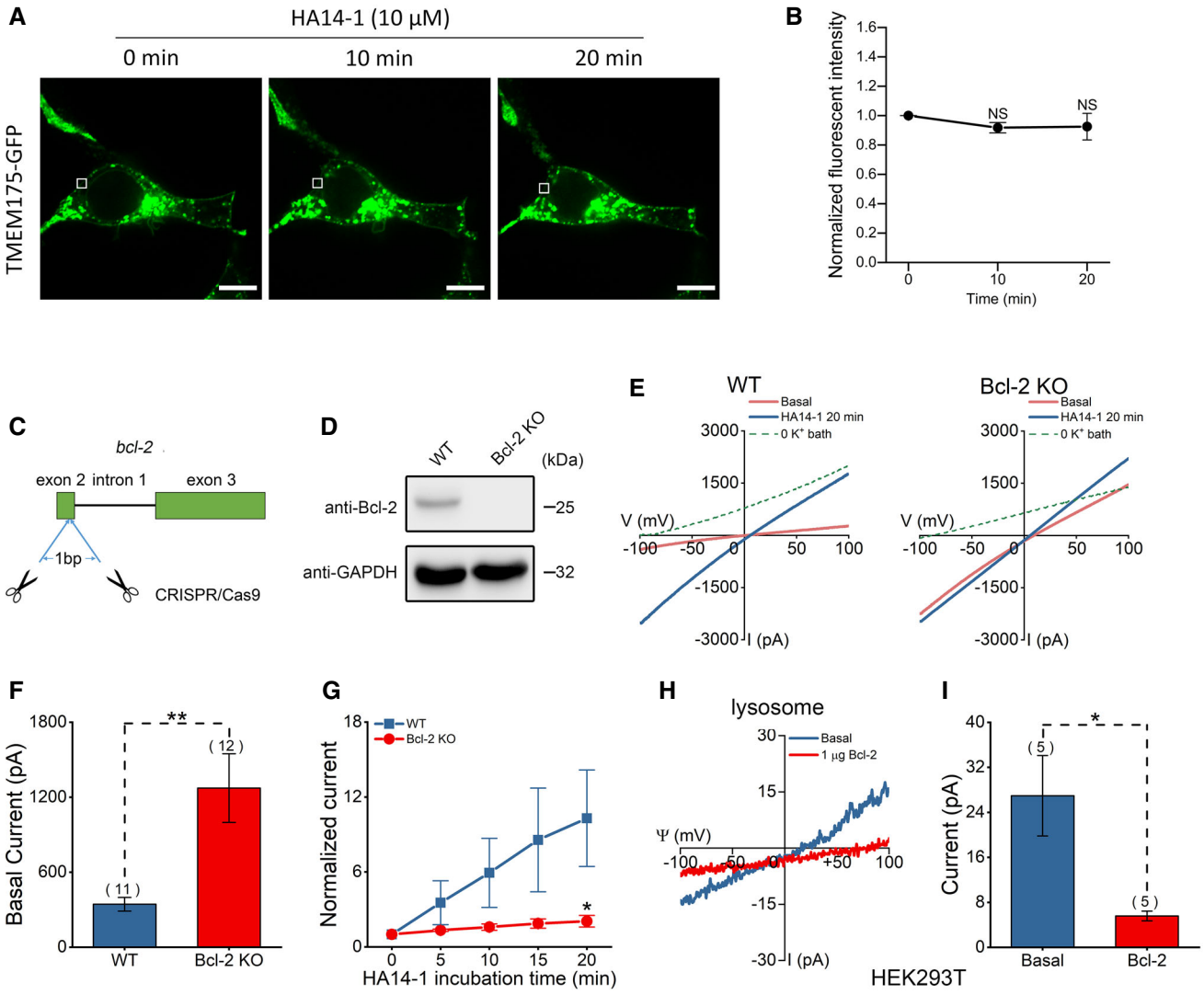

Figure EV2. HA14-1 activates TMEM175 through Bcl-2 without inducing translocation of TMEM175 to the plasma membrane (PM).

A Localization of C-terminally green fluorescent protein (GFP)-tagged TMEM175 before (0 min) and after incubation with HA14-1; 10 and 20 min are the incubation times. The white boxes indicate TMEM175-expressing PM regions. Scale bars = 10 μm.

B Fluorescence intensities of selected PM regions indicating the expression of TMEM175. $n = 4$ (four PM regions selected from different cells in the same experiment).

C Schematic diagram showing the knockout (KO) strategy of Bcl-2 in HEK293T cells.

D The knockout (KO) of Bcl-2 protein was detected by Western blotting.

E Representative whole-cell currents recorded in wild-type (WT) (left) and Bcl-2 KO (right) HEK293T cells transfected with TMEM175 at 0 min (basal) and 20 min after bath application of 10 μM HA14-1. The currents were recorded with ramp protocols (−100 to +100 mV in 1 s, every 10 s, Vh = 0 mV).

F Amplitudes of basal currents measured at −100 mV in the ramp protocol used in (E).

G Current amplitudes measured at different time points after the application of HA14-1 normalized to the basal values (0 min).

H Representative endogenous TMEM175 lysosomal currents recorded in HEK293T cells with or without 1 μg Bcl-2 application in bath solution.

I Statistics of the currents in (H).

Data information: The data are presented as the mean ± SEM. Statistical significance was analyzed with two-sided Student's *t*-tests, and is indicated with NS for not significant ($P > 0.05$), and * for $P < 0.05$, ** for $P < 0.01$. In panel (B), *n* value means the number of technical replicates. In other panels, *n* value means the number of biological replicates made for each data point.

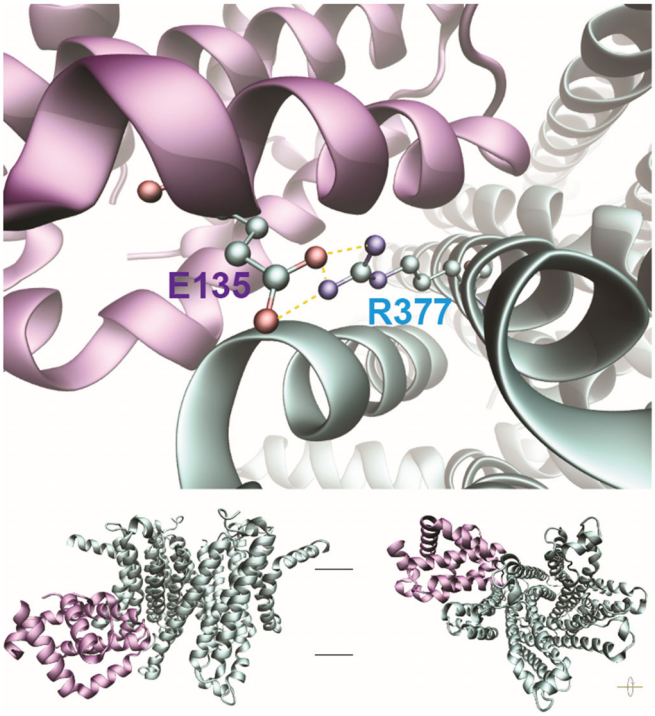

Figure EV3. Predicted binding mode I for Bcl-2 bound with open-state TMEM175.

The protein backbone is shown as cartoon and colored in cyan for TMEM175 and purple for Bcl-2. Pairs of residues forming hydrogen bond are displayed in sticks and colored by atom type. Hydrogen bonds are displayed in yellow dashed lines. Lower: the overhead and lateral views of the bound complexes.

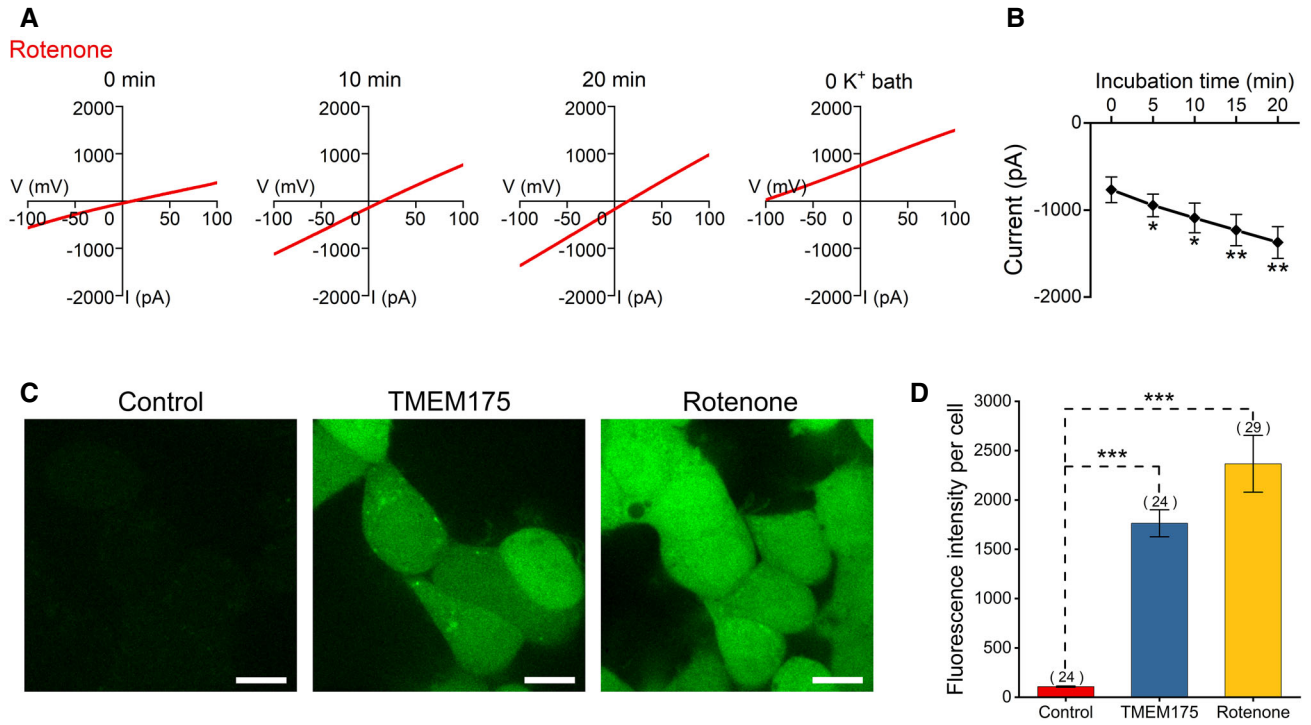

Figure EV4. Activation of TMEM175 by rotenone.

A Representative current traces showing the effects of 10 μM rotenone in the bath solution.

B Quantification of the current amplitudes measured at −100 mV in the ramp protocol used in (A). $n = 8$.

C CM-H2DCFDA (5-(and-6)-chloromethyl-2'-7'-dichlorodihydrofluorescein diacetate) (green) indicating cellular reactive oxygen species (ROS) levels of HEK293T cells over-expressing TMEM175 or pretreatment of 10 μM rotenone for 1 h. Scale bars = 10 μm.

D Statistics of CM-H2DCFDA fluorescence intensity of (C). The n values represent the cell number counted within the same experiment.

Data information: The data are presented as the mean ± SEM. Statistical significance was analyzed with two-sided Student's *t*-tests, and is indicated with * for $P < 0.05$, ** for $P < 0.01$, and *** for $P < 0.001$. In panel (B), *n* value means the number of biological replicates made for each data point. In panel (D), *n* value means the number of technical replicates.

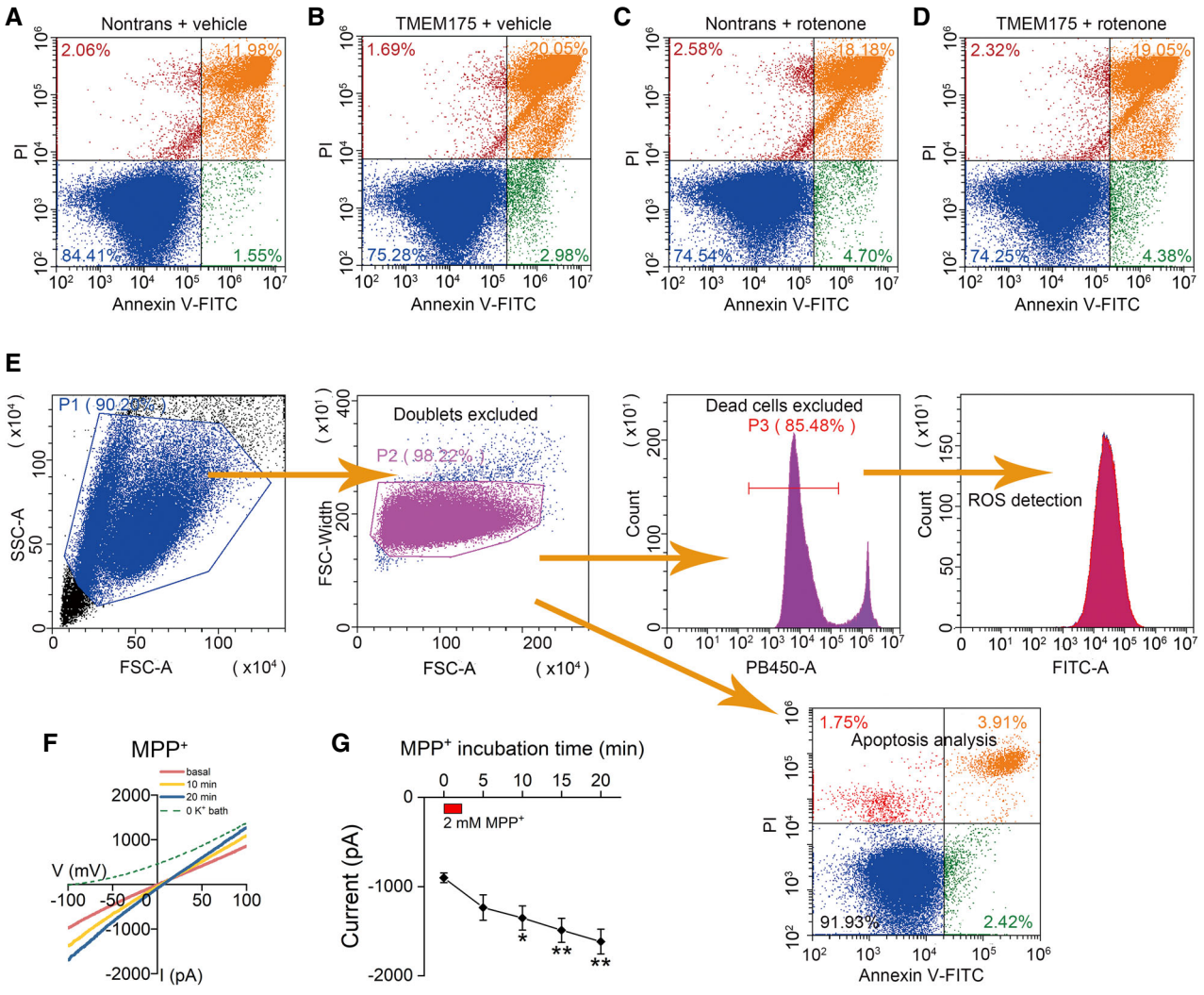

Figure EV5. Rotenone and MPP$^+$ (1-methyl-4-phenylpyridinium) regulate TMEM175 and apoptosis.

A–D Nontransfected (A and C) and TMEM175 transfected (B and D) HEK293T cells were treated with vehicle (A and B) or 10 μM rotenone (C and D) for 24 h. The apoptotic cell death was detected by Annexin V–fluorescein isothiocyanate (FITC)/propidium iodide (PI) double staining followed by flow cytometric analysis.

E Gating strategy of (A–D) and flow cytometry of Figs 4I, 5A, C and D, and 6A, C and E. The initial cell population gating (forward scatter (FSC)-Area vs. side scatter (SSC)-Area) was adopted to remove cell debris. Secondary cell population gating (FSC-Area vs. FSC-Width) was applied to exclude doublets. For the detection of intracellular reactive oxygen species (ROS), 4',6-diamidino-2-phenylindole (DAPI) staining was used to exclude dead cells. The same gating strategy was applied to all samples analyzed simultaneously.

F Representative current traces recorded at different time points after transient bath application (2 min) of 2 mM MPP$^+$.

G Quantification of the current amplitudes in (F) (measured at −100 mV). The red rectangle indicates the time frame for MPP$^+$ application. $n = 7$.

Data information: The data are presented as the mean ± SEM. Statistical significance was analyzed with two-sided Student's *t*-tests, and is indicated with * for $P < 0.05$, and ** for $P < 0.01$. *n* value means the number of biological replicates made for each data point.