

Temperature-dependent increase in the calcium sensitivity and acceleration of activation of ANO6 chloride channel variants

Haiyue Lin^{1,#}, Ikhyun Jun^{2,3,#}, Joo Han Woo^{4,5}, Min Goo Lee³, Sung Joon Kim^{1,*} & Joo Hyun Nam^{4,5,*}

Supplementary Information

Figure S1. Figure showing uncropped western blots from Fig. 1a.

Surface biotinylation (upper) and whole cell lysate western blotting (middle-ANO6 blotting, bottom-aldolase blotting) uncropped membrane images.

Figure S2. $I_{\text{ANO6-V1}}$ with 300 nM $[\text{Ca}^{2+}]_i$ at 42°C.

(a) Representative current trace of I_{ANO6} generated from V1-expressed HEK293T cells with 300 nM $[\text{Ca}^{2+}]_i$ at 42°C. The relative I-V relation curve for initial current (1) is presented in the right upper panel. (b) and (c) Mean current (I)-voltage (V) relationship curves obtained from initial (1) and peak (2) currents of the whole-cell patch clamp with 300 nM $[\text{Ca}^{2+}]_i$ at 42°C, respectively (n = 5).

Figure S3. I_{ANO6} of variants 1, 2 and 5 detected under calcium-free condition at 42°C

(a) Representative trace of the I_{ANO6} of V1 (left-panel), V2 (middle-panel), and V5 (right-panel), in the presence of 0 $[\text{Ca}^{2+}]_i$, expressed in HEK293T cells. The pulse protocol is the same as that described in Fig. 2. (b) I-V relationship curves obtained from V1 (square), V2 (circle), and V5 (up-triangle) (n = 3 for all variants).

Figure S4. PLA2 effects on I_{ANO6} of variants 1, 2, and 5 under symmetrical Cl^- condition.

(a, c) Representative currents trace of I_{ANO6} generated from the three variants (V1, left panel; V2, middle panel; and V5, right panel) at 27°C with 1 μM free calcium under (a) 0.5 unit/ml PLA2 in pipette solution and (c) NEM (50 μM) treatment conditions in

ANO6-overexpressing HEK293T cells. **(b, d)** Current (I) - voltage (V) relation curve obtained from each variant detected at PLA2 in pipette **(b)** and NEM-treated conditions **(d)**, respectively (V1- square, V2 – circle, and V5 - up-triangle). Data represent the means $\pm$ SEM (n = 4 for V1, V2, and V5 for all conditions). **(e, f)** Representative currents trace of I_{ANO6} generated from the three variants (V1, left panel; V2, middle panel; and V5, right panel) at 37°C with 1 μ M free calcium under ACA (20 μ M) treatment **(e)** and MAFP (5 μ M) treatment **(f)** conditions, respectively, in ANO6-transfected HEK293T cells. **(g)** Summary bar graph of $t_{1/2, peak}$ of I_{ANO6} of each variant estimated from the trace chart of the control (Figure 2b) under ACA and MAFP treatment conditions. Data represent the means $\pm$ SEM (n = 5 for V1, V2, and V5 at all conditions). NS indicates not significant, compared to the control.

Figure S5. Intracellular calcium levels induced by ionomycin

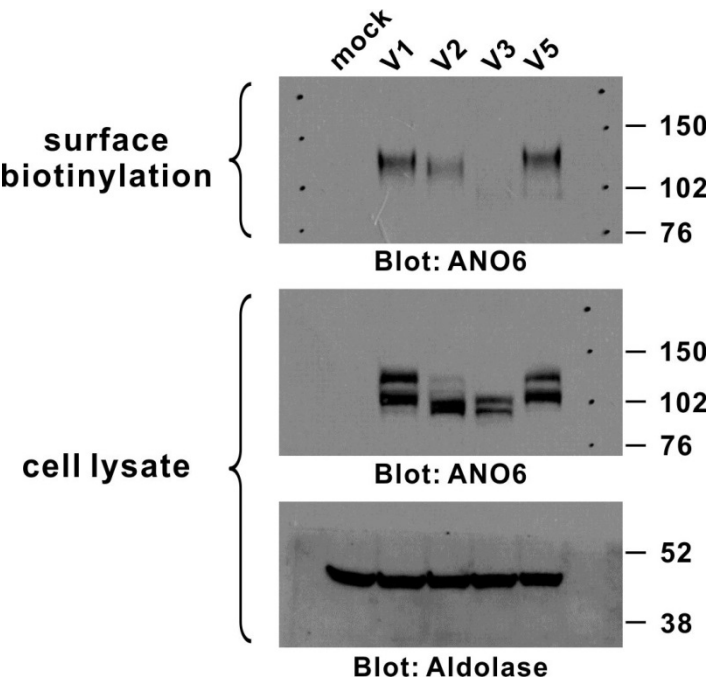
(a) Representative trace of the intracellular calcium concentration induced in ANO6 (V1, V2, and V5)-expressing HEK293T cells with 10 μ M ionomycin. The intracellular calcium levels were measured in NT solution containing free calcium concentrations of 100 μ M and 200 μ M at 27°C (right), and 30 μ M (middle) and 100 μ M (left) at 37°C using Fura 2-AM probe. At 27°C, free calcium concentration of NT was changed from 100 μ M to 200 μ M by adding an appropriate amount of $CaCl_2$ at the time point where the intracellular calcium concentration reached steady state. **(b)** Summary bar graph of the intracellular calcium concentration at 27°C (left) and 37°C (right) (n = 3~4).

Figure S6. PS exposure comparison of V1, V2, and V5.

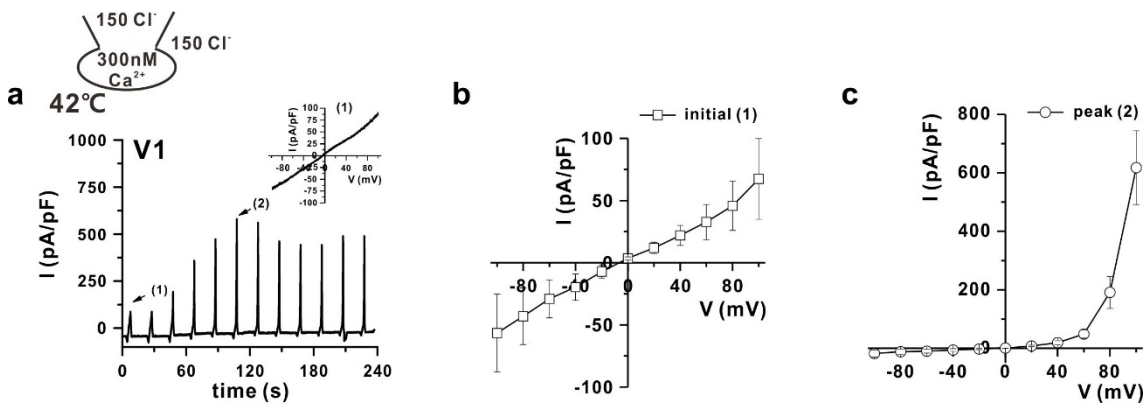
(a) Annexin V-binding (X-axis) and PI-binding (Y-axis) cell counts in ANO6-V1-transfected HEK293T cells from flow cytometry. The correct vertical and horizontal lines for the 4-quadrant windows of Annexin V-/PI-binding (right) were based on the Annexin V (left) and PI (middle) single staining, respectively. **(b)** Summary bar graph of the response of Annexin V-positive cells [% , percentage of total cell counts (Q4-region)] to ionomycin treatment for each variant at 27°C (left) and 37°C (right), estimated from data presented in Figure 9. Data are presented as the means $\pm$ SEM (n =

5~10). NS means not significant compared to V1.

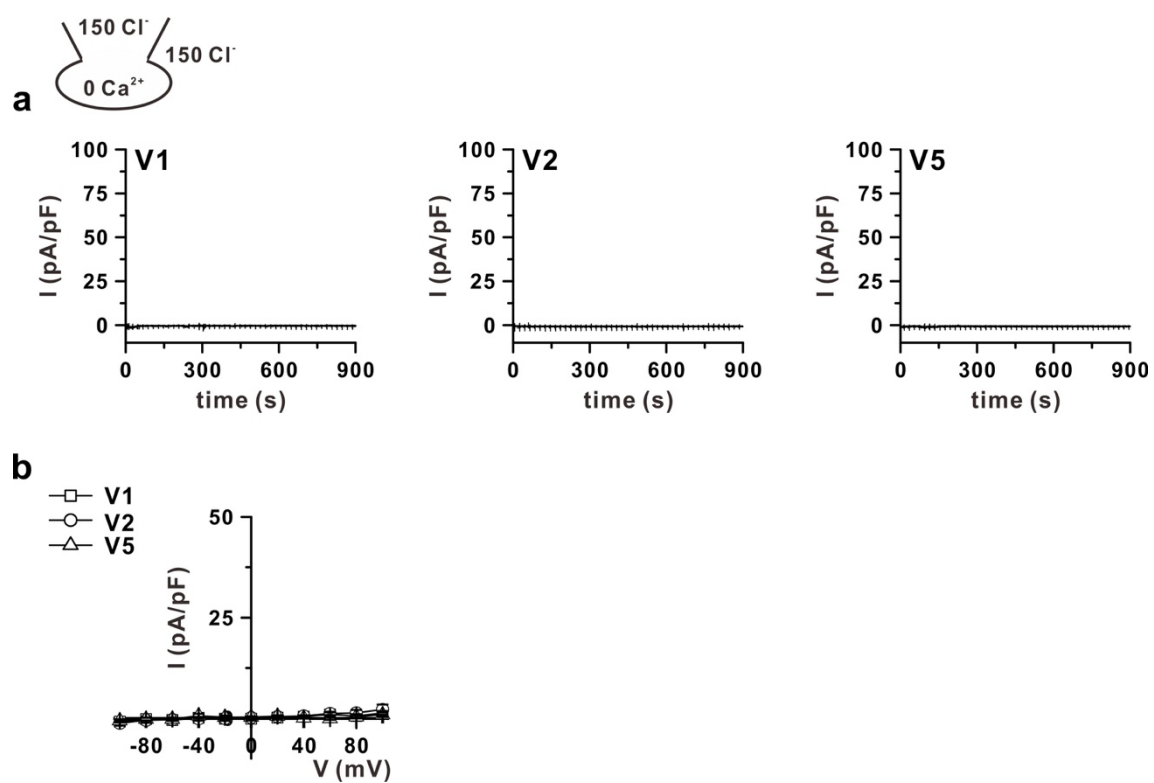
Supplementary Figure 1.



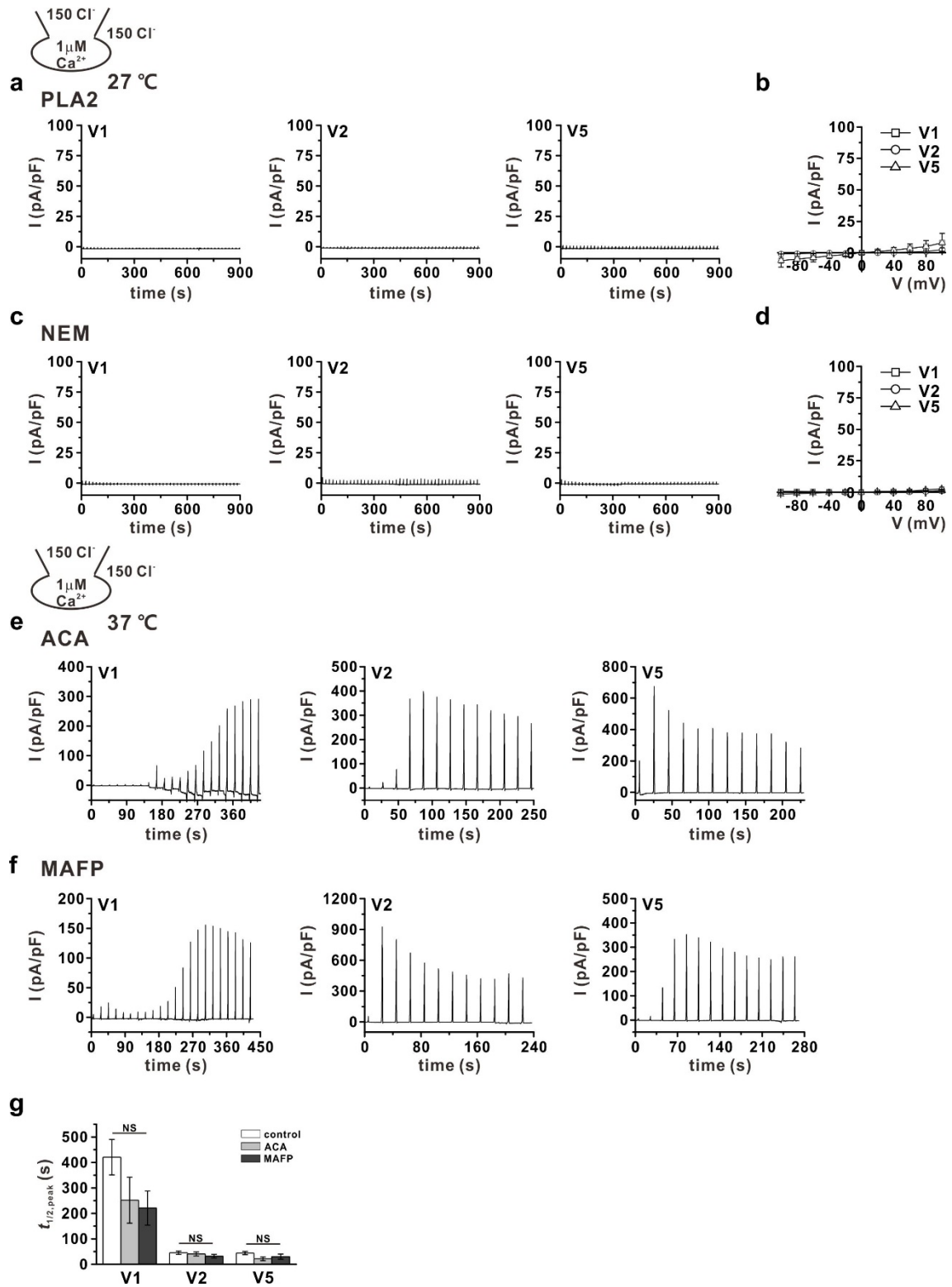
Supplementary Figure 2.



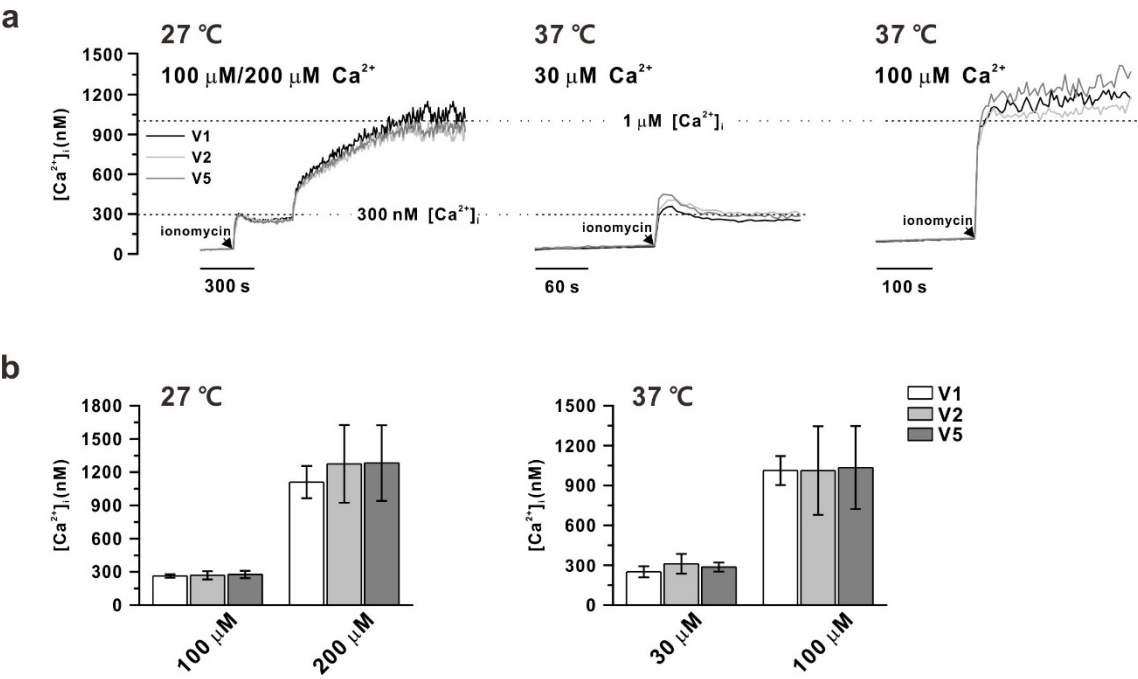
Supplementary Figure 3.



Supplementary Figure 4.

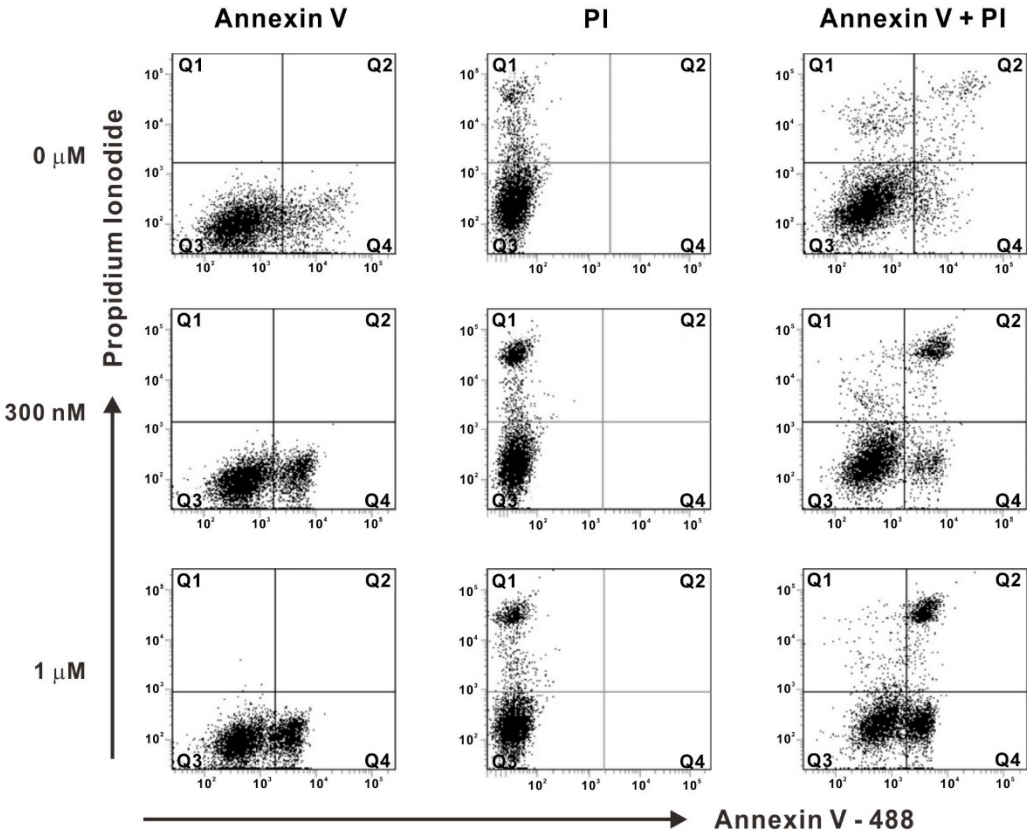


Supplementary Figure 5.



Supplementary Figure 6.

a



b

