TRPM7 activity drives human CD4 T-cell activation and differentiation in a Mg^{2+} dependent manner

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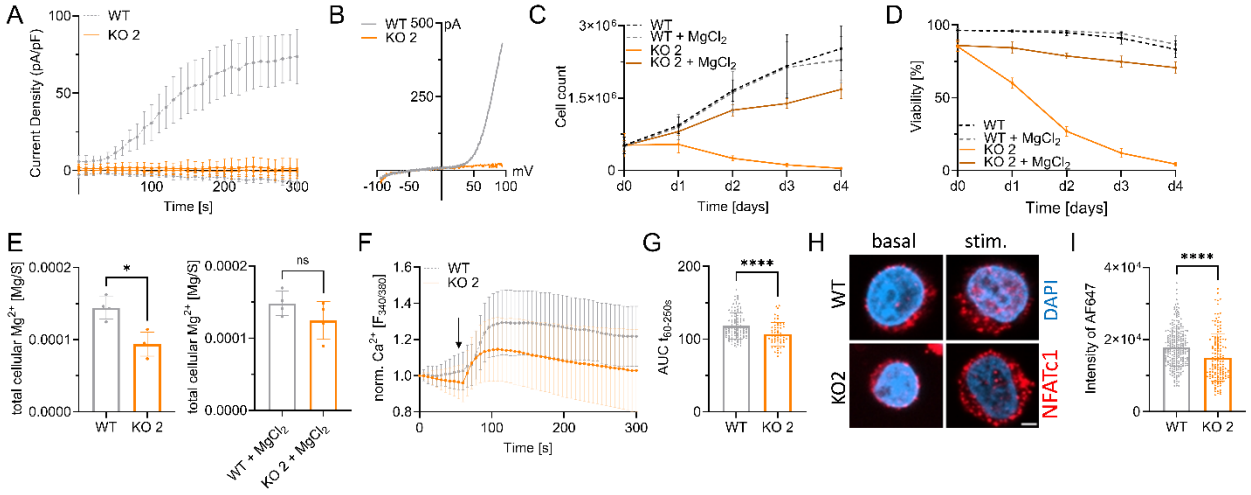
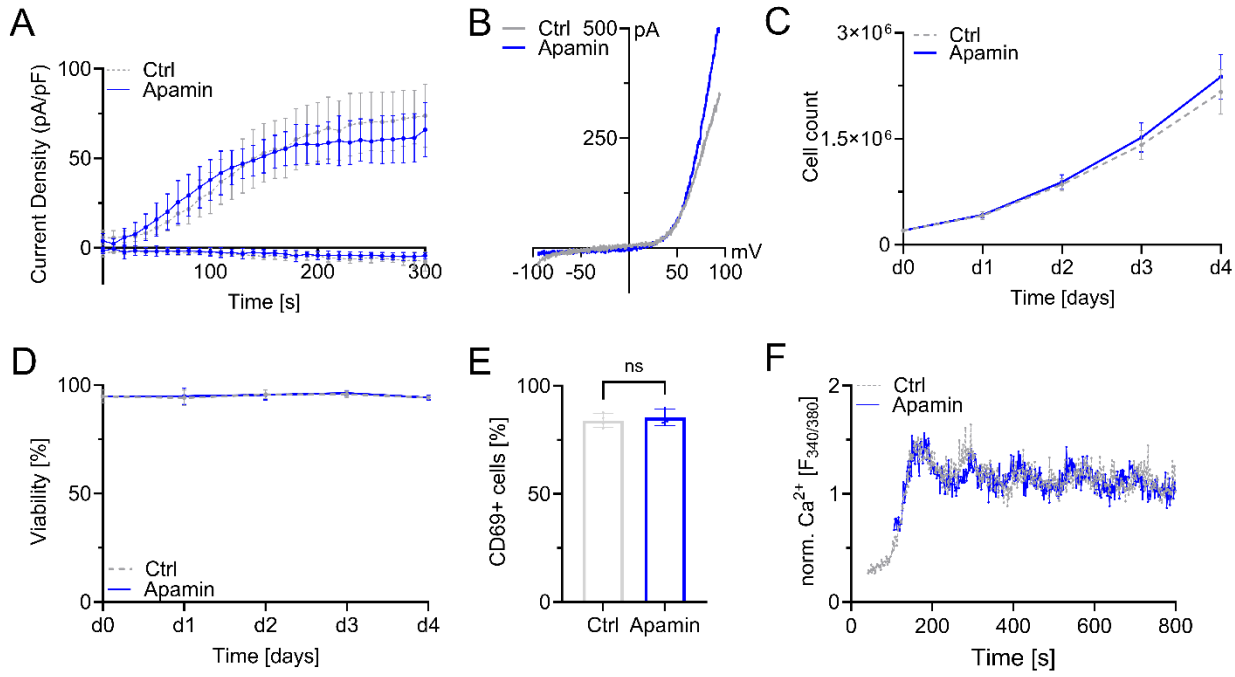


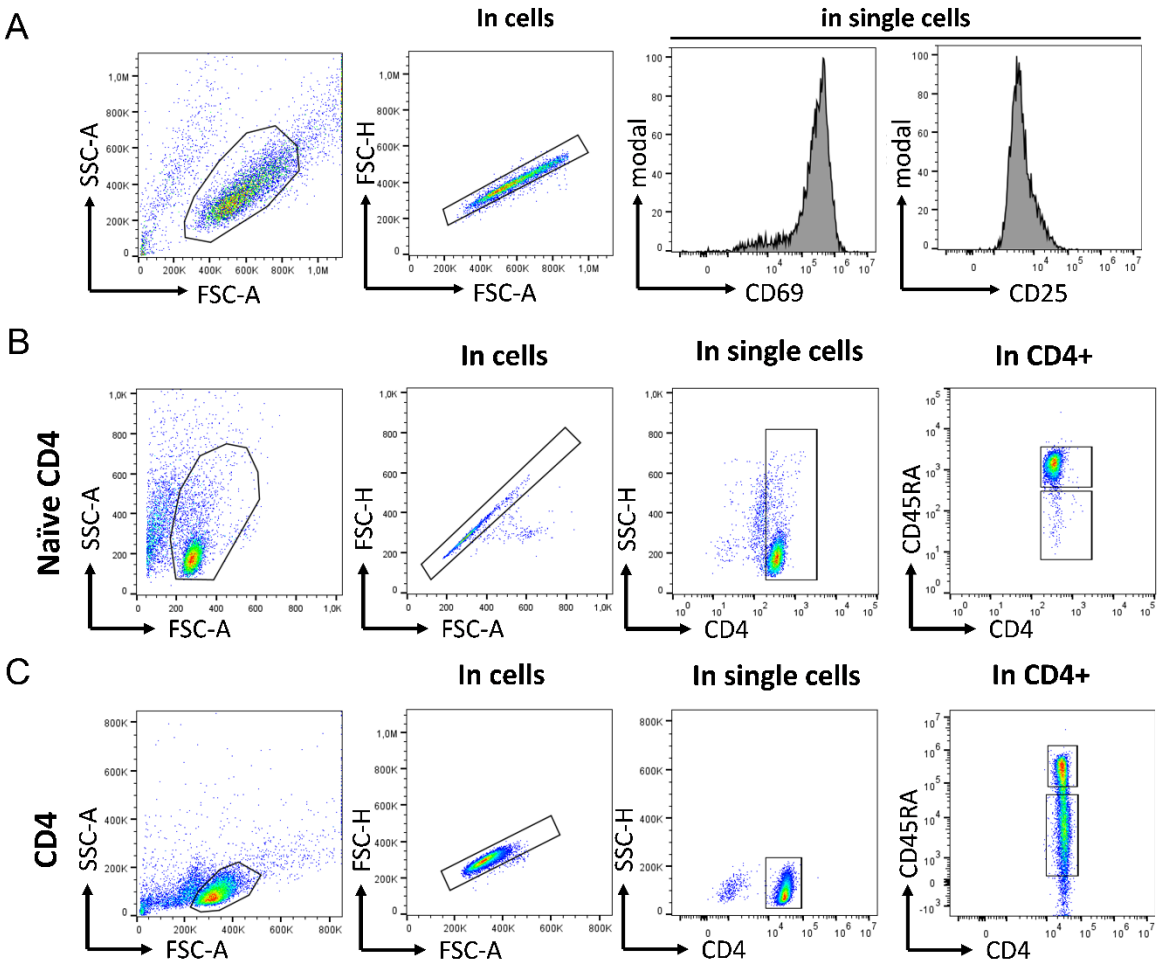
Supplementary Figure 1: Validation of Jurkat TRPM7 KO clone 2 shows reduced proliferation and activation

A) TRPM7 current densities and B) TRPM7 I/V relationship of Jurkat cells during whole-cell patch clamp experiment with Mg^{2+}-free intracellular solution. WT (WT, grey) and TRPM7 KO2 Jurkat clone (KO2, orange). n(WT)=9; n(KO2)=10. C) Cell counts and D) viability of natively proliferating TRPM7 WT and KO2 Jurkat clone in RPMI medium with 10% FBS, with and without supplementation with 6 mM $MgCl_2$. n=3, measured in duplicates. E) Cellular Mg contents quantified by ICP-MS. WT and TRPM7 KO2 Jurkat clone, cultured in regular (WT-)media or in medium supplemented with 6 mM $MgCl_2$ for 18 h ahead of sampling, n=4. F) Fura-2 based imaging of cytosolic Ca^{2+} concentration of Jurkat cells. Stimulation with 5 µM thapsigargin at indicated time point (arrow). WT (WT, grey) and TRPM7 KO2 (KO2, orange) Jurkat clone, n (WT) =111; n (KO2) = 59; G) Quantification of the area under the curve (AUC) of respective curves shown in F. H) Representative immuno-fluorescent images of NFATc1 localization in WT and KO2 clone before (basal) and after 30 min stimulation (stim.) with 5 µM thapsigargin, scale bar = 2 µm. NFATc1 in red, DAPI in blue. I) Quantification of nuclear NFATc1 levels upon stimulation of TRPM7 WT (WT, grey) and KO (KO2, orange) clone. n (WT) = 261; n (KO2) = 149. Statistics: Two-way ANOVA (C, D), one-way ANOVA (E) or Student´s t test (G, I). * P<0.05; and **** P<0.0001. Data are mean ± SD.



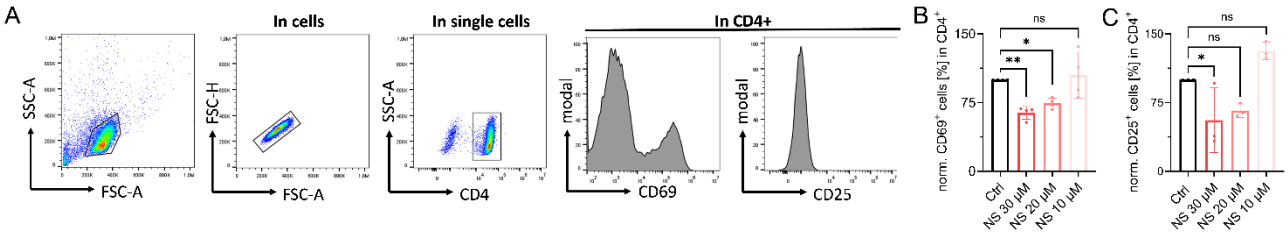
Supplementary Figure 2: Apamin as control substance for potential off target effects of NS8593

A) TRPM7 current densities and B) TRPM7 I/V relationship of Jurkat T cells during whole-cell patch clamp experiment with Mg^{2+}-free intracellular solution. Controls (Ctrl, grey) and cells treated with 1 µM apamin (Apamin, blue), n (Ctrl)=9, n (Apamin)=6. C) Cell counts and D) viability of natively proliferating Jurkat cells in RPMI medium with 10% FBS, with and without 1 µM apamin (Apamin, blue), n=4. E) Flow cytometry of upregulation of activation markers CD69 in primary CD4 T-lymphocytes 48 h after anti-CD3/CD28 stimulation. Cells treated either as control (Ctrl, grey) or with 1 µM apamin (Apamin, blue), n=4. F) Representative trace of CD4 T cells Fura-2 based imaging of cytosolic Ca^{2+} concentrations following anti-CD3/CD28 stimulation. Antibodies bound to microscopy chamber bottom with cells sinking down in saline containing 2 mM Ca^{2+} during running measurement, coming to rest in focus plane with contact to stimulation antibodies. Cells measured as control (Ctrl, grey) or in presence of 1 µM apamin (Apamin, blue). Statistics: Student´s t test (D). n.s.—not significant. Data are mean ± SD.



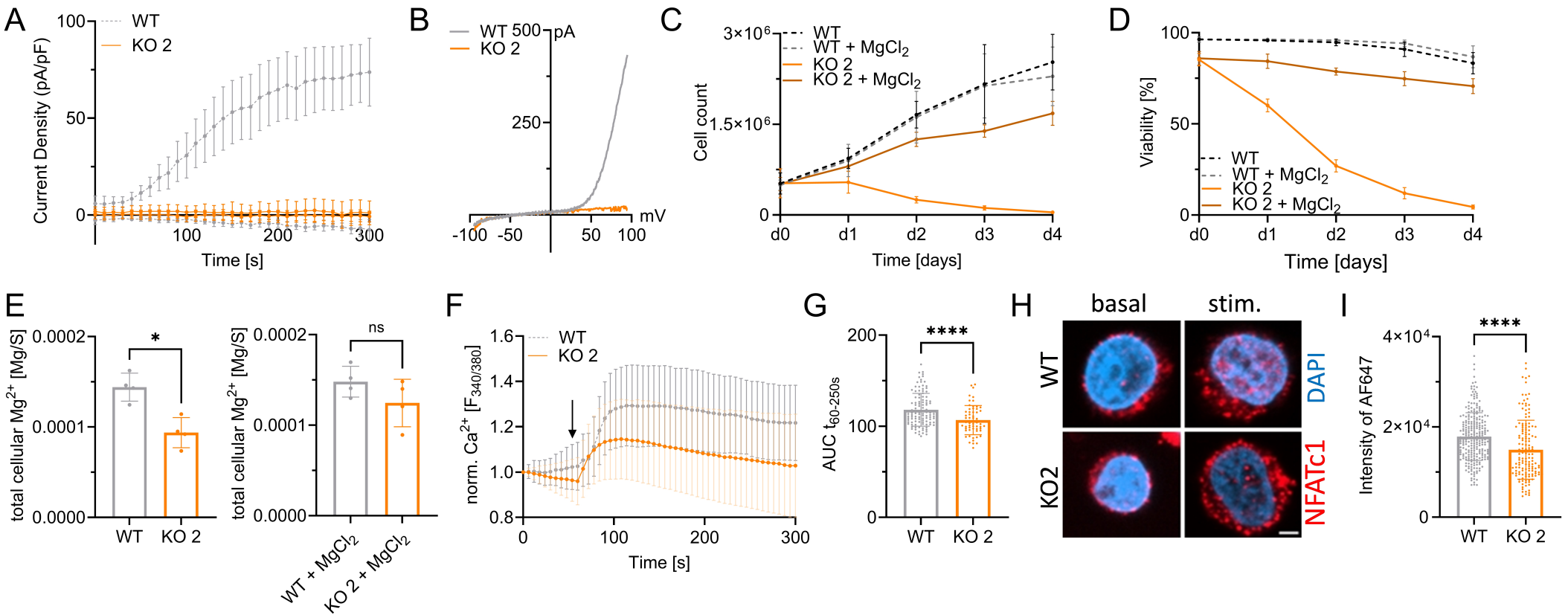
Supplementary Figure 3: T cell isolation controls and additional FACS data

A) Representative FACS plots and gating strategy for CD69 and CD25 visualization, shown for Jurkat WT cells. B) Representative FACS plots and gating strategy to confirm identify of isolated naïve CD4 T cells and C) conventional CD4 T cells.



Supplementary Figure 4: Dose response curve of TRPM7 inhibitor NS8593 on CD4 T-cell activation

A) Representative FACS plots and gating strategy for CD69 and CD25 shown for conventional CD4 T cells. B+C) Quantification of flow cytometry data of NS8593 dose-dependent upregulation of CD69 (B) and CD25 (C) expression on conventional CD4 T cells, 48 h after anti-CD3/CD28 stimulation or PMA/ionomycin stimulation, respectively, n=3-4. Statistics: One-way ANOVA (B, C). * $P<0.05$; ** $P<0.005$ and n.s.—not significant. Data are mean ± SD.



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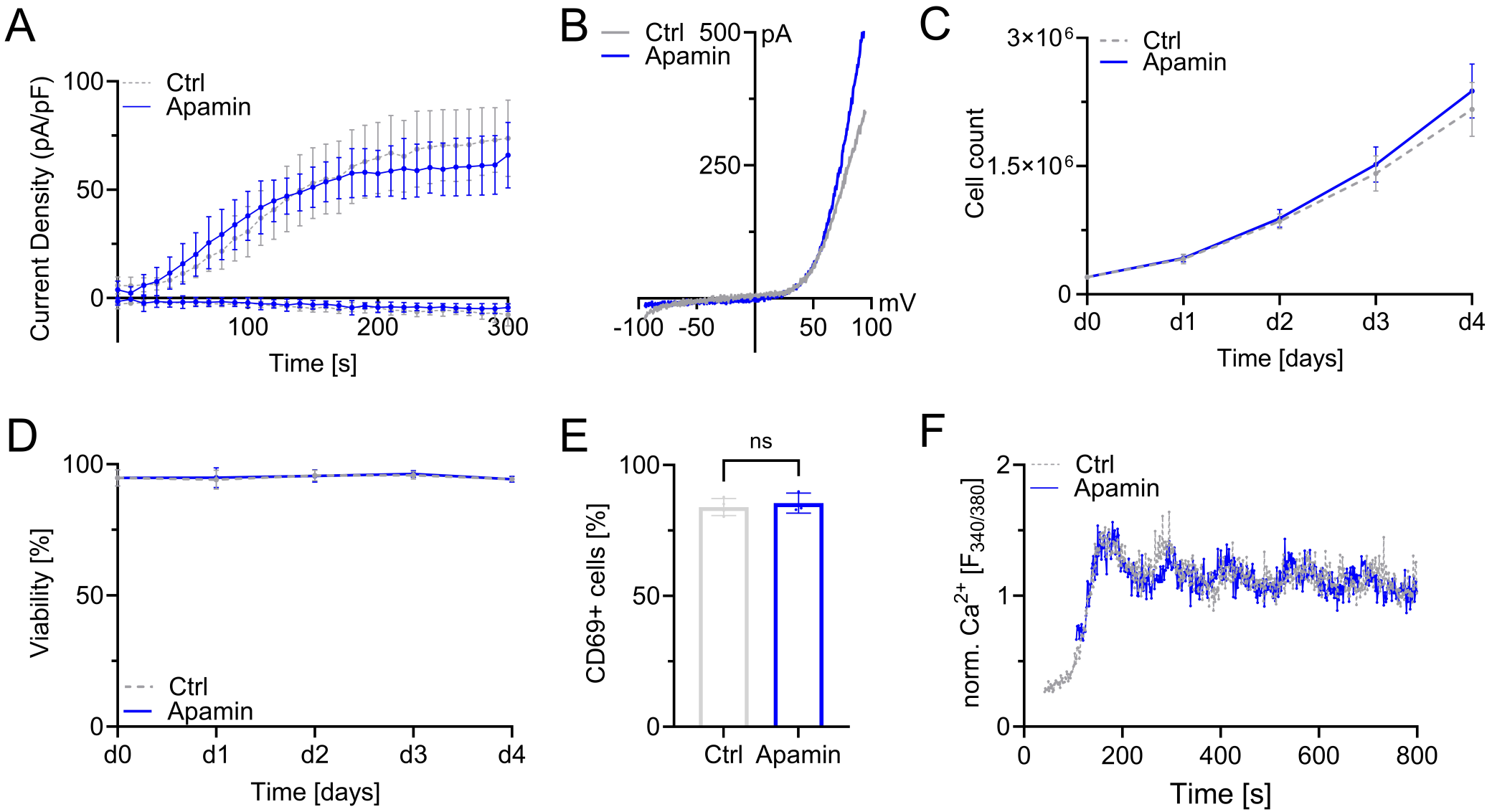
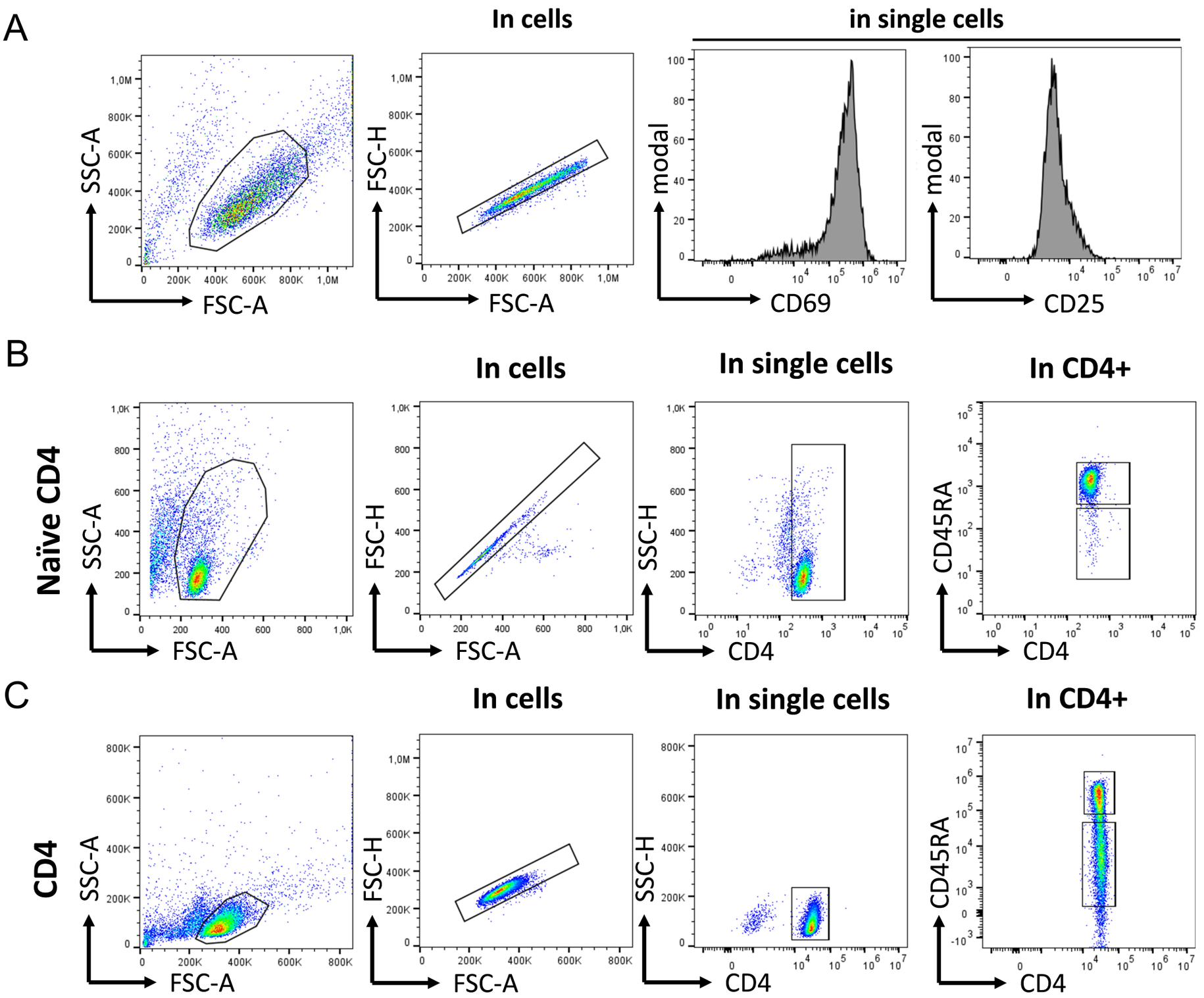
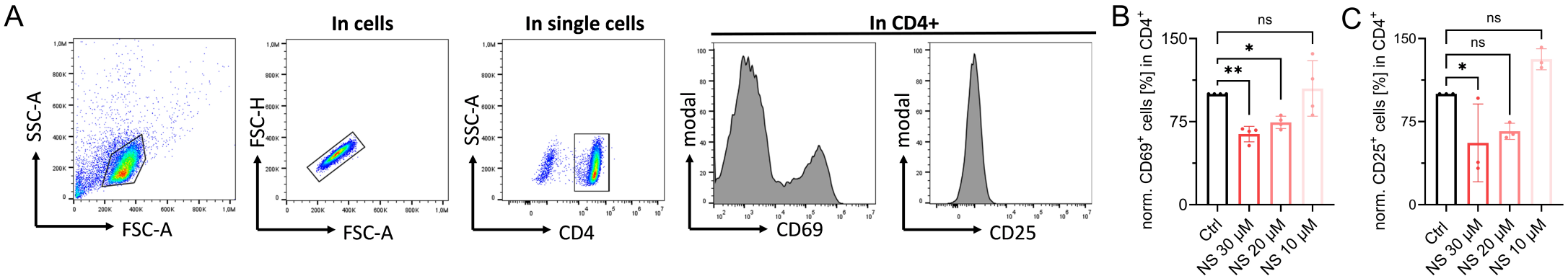


Supplementary Figure 2: Apamin as control substrance for potential off target effects of NS8593 on SK channels



Supplementary Figure 3: Isolation controls and additional FACS data



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