

Supplemental Information

Phylogeny of SK channels and functional characterization of the conserved Phe in the S3–S4 loop

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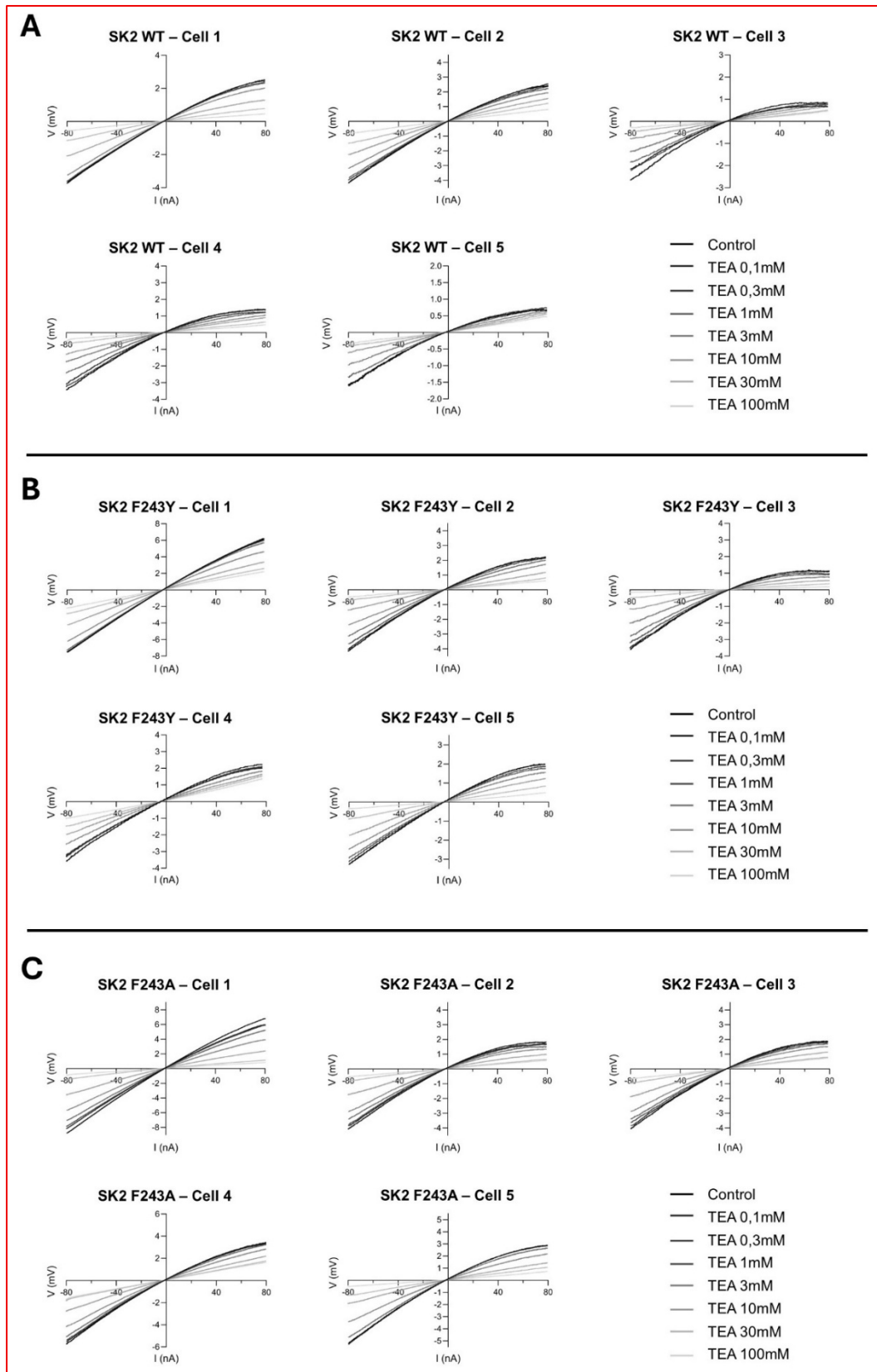


Figure S1. Whole-cell patch-clamp recordings of SK2 WT and mutant channel currents in response to increasing TEA concentrations. Raw current traces recorded from HEK293 cells expressing SK2 WT (A), SK2 F243Y (B), or SK2 F243A (C). For each condition, currents were measured in absence and in the presence of increasing concentrations of TEA. Each panel shows recordings from five independent cells.

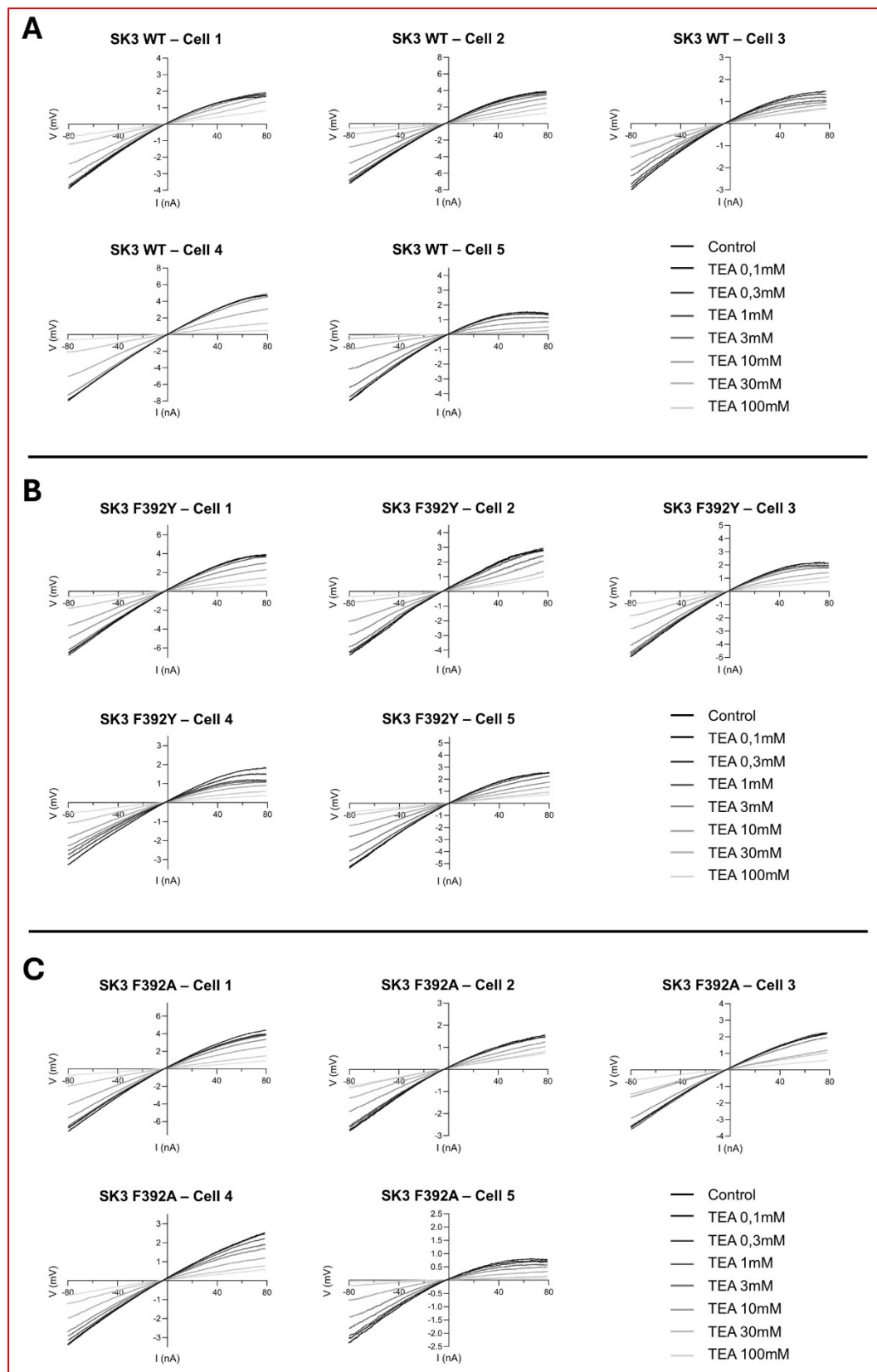


Figure S2. Whole-cell patch-clamp recordings of SK3 WT and mutant channel currents in response to increasing TEA concentrations. Raw current traces recorded from HEK293 cells expressing SK3 WT (A), SK3 F392Y (B), or SK3 F392A (C). For each condition, currents were measured in absence and in the presence of increasing concentrations of TEA. Each panel shows recordings from five independent cells.

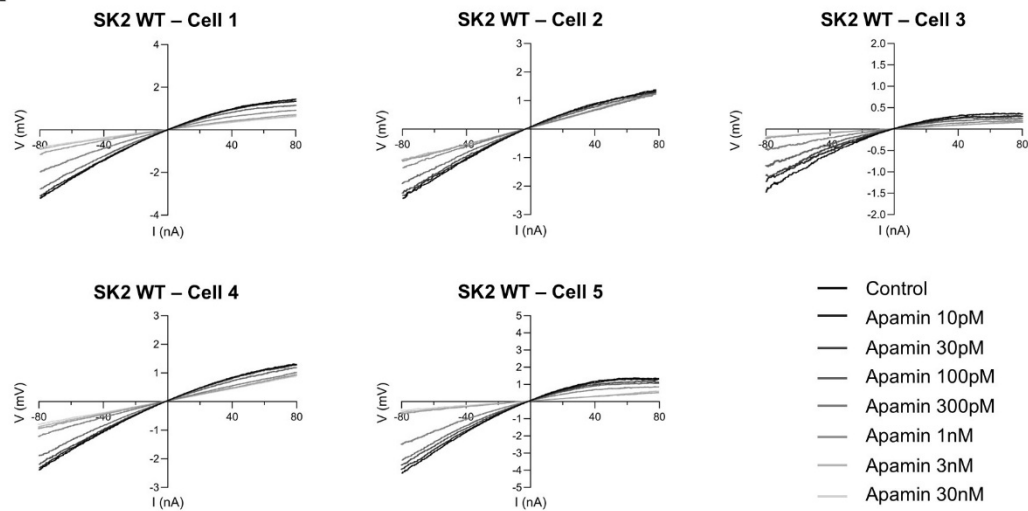
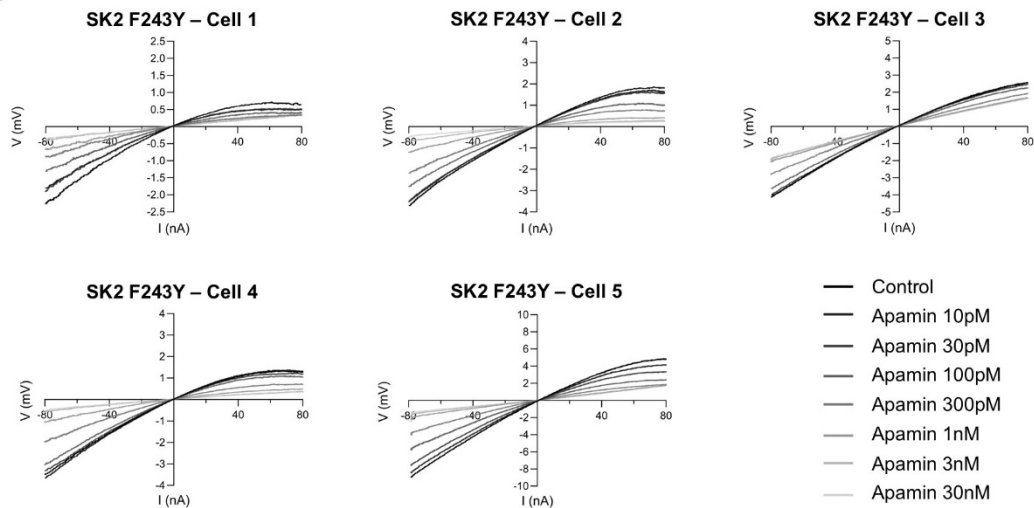
A**B**

Figure S3. Whole-cell patch-clamp recordings of SK2 WT and tyrosine mutant channel currents in response to increasing apamin concentrations. Raw current traces recorded from HEK293 cells expressing SK2 WT (A) or SK2 F243Y (B). For each condition, currents were measured in absence and in the presence of increasing concentrations of apamin. Each panel shows recordings from five independent cells.

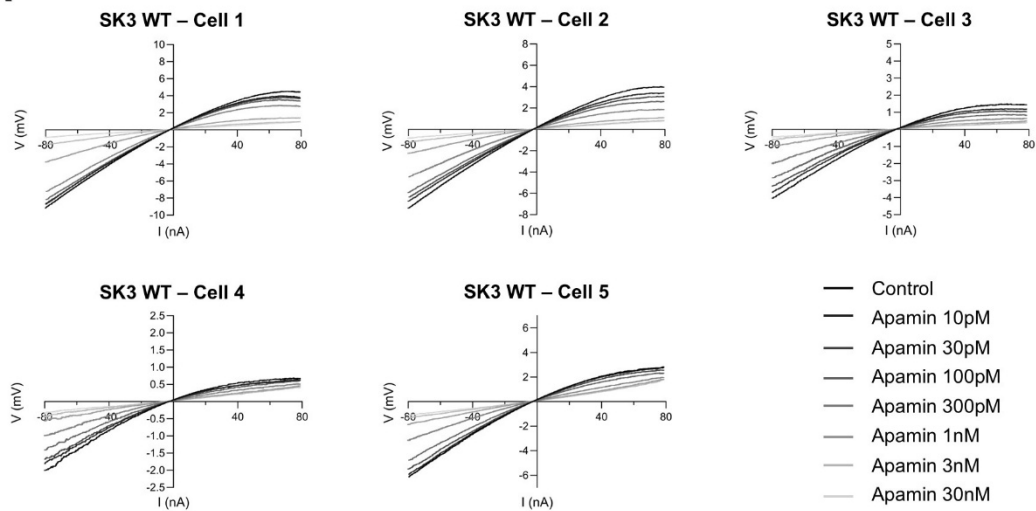
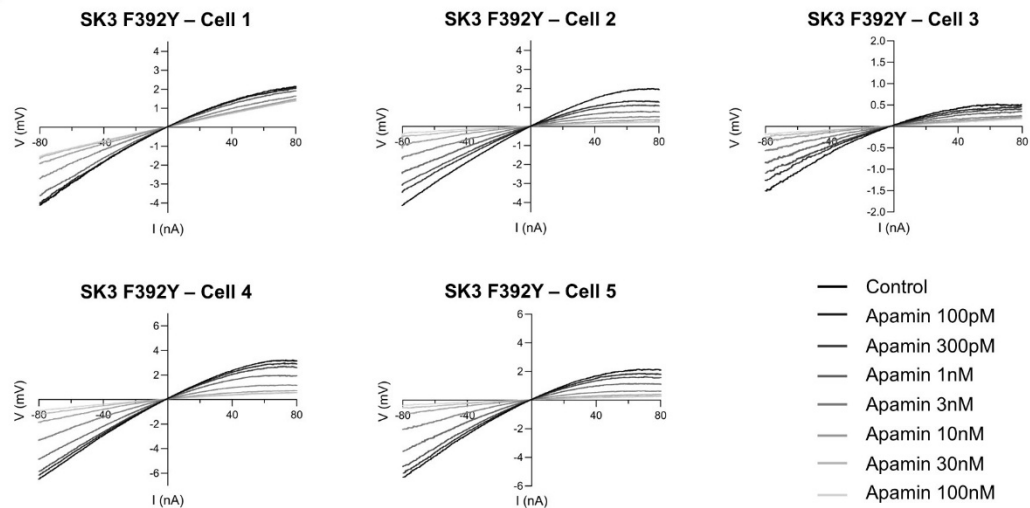
A**B**

Figure S4. Whole-cell patch-clamp recordings of SK3 WT and tyrosine mutant channel currents in response to increasing apamin concentrations. Raw current traces recorded from HEK293 cells expressing SK3 WT (A) or SK3 F392Y (B). For each condition, currents were measured in absence and in the presence of increasing concentrations of apamin. Each panel shows recordings from five independent cells.

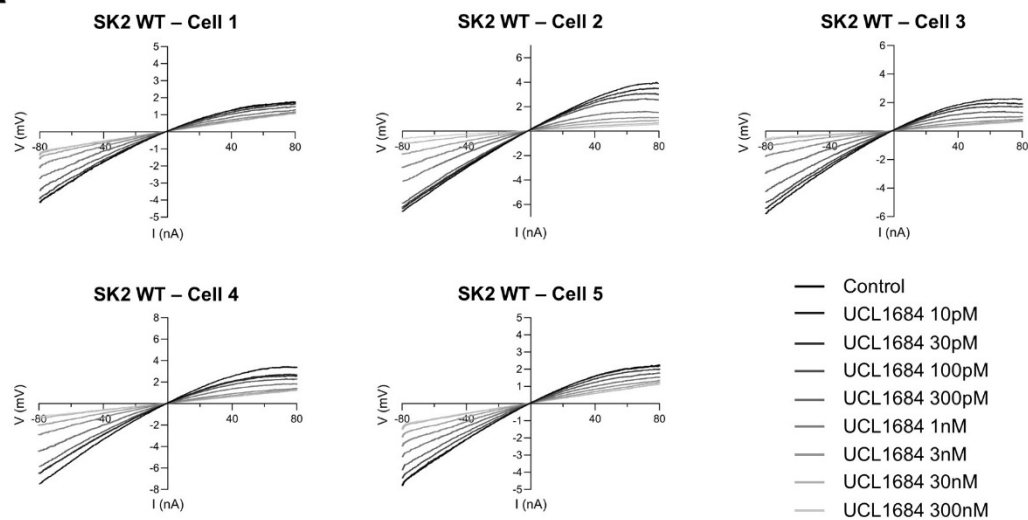
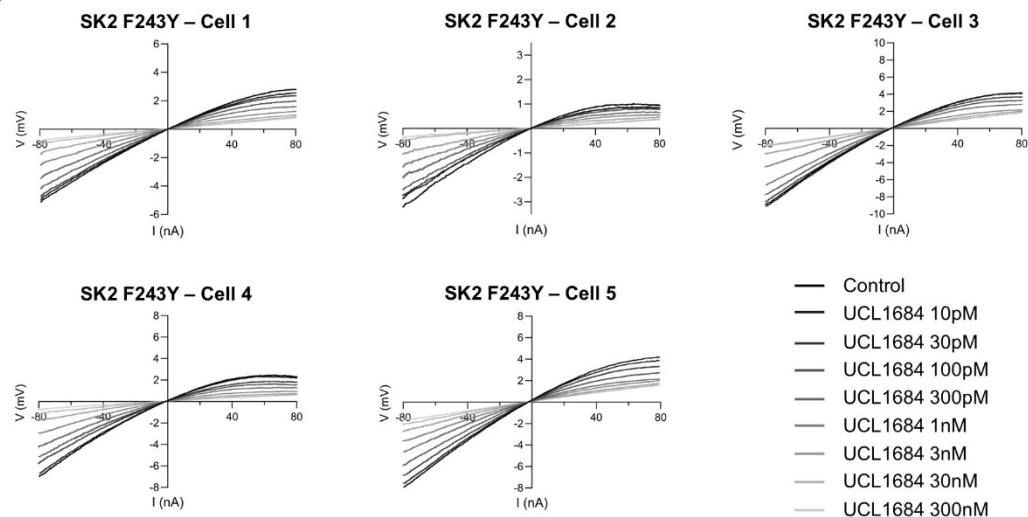
A**B**

Figure S5. Whole-cell patch-clamp recordings of SK2 WT and tyrosine mutant channel currents in response to increasing UCL1684 concentrations. Raw current traces recorded from HEK293 cells expressing SK2 WT (A) or SK2 F243Y (B). For each condition, currents were measured in absence and in the presence of increasing concentrations of UCL1684. Each panel shows recordings from five independent cells.

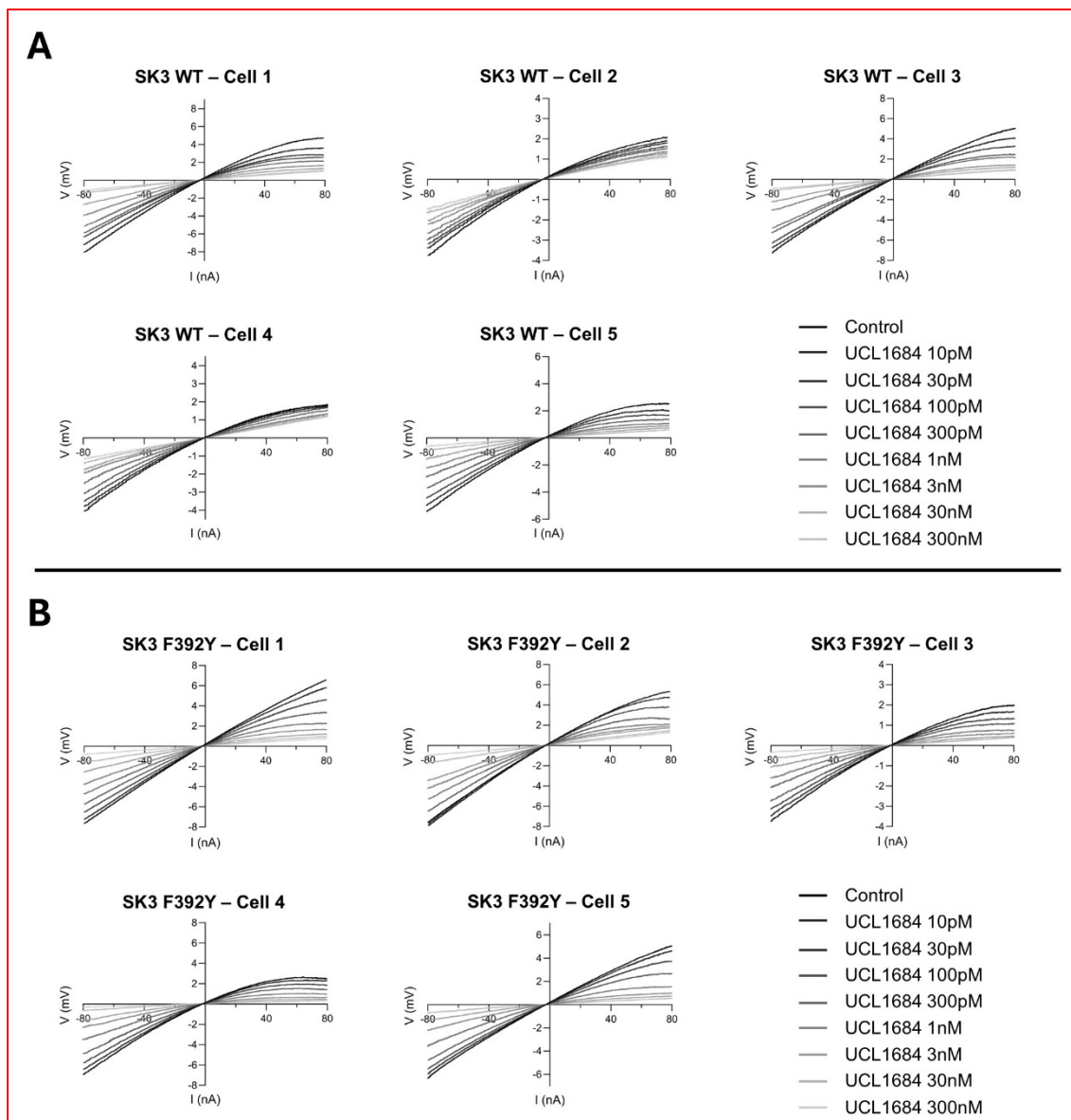


Figure S6. Whole-cell patch-clamp recordings of SK3 WT and tyrosine mutant channel currents in response to increasing UCL1684 concentrations. Raw current traces recorded from HEK293 cells expressing SK3 WT (A) or SK3 F392Y (B). For each condition, currents were measured in absence and in the presence of increasing concentrations of UCL1684. Each panel shows recordings from five independent cells.

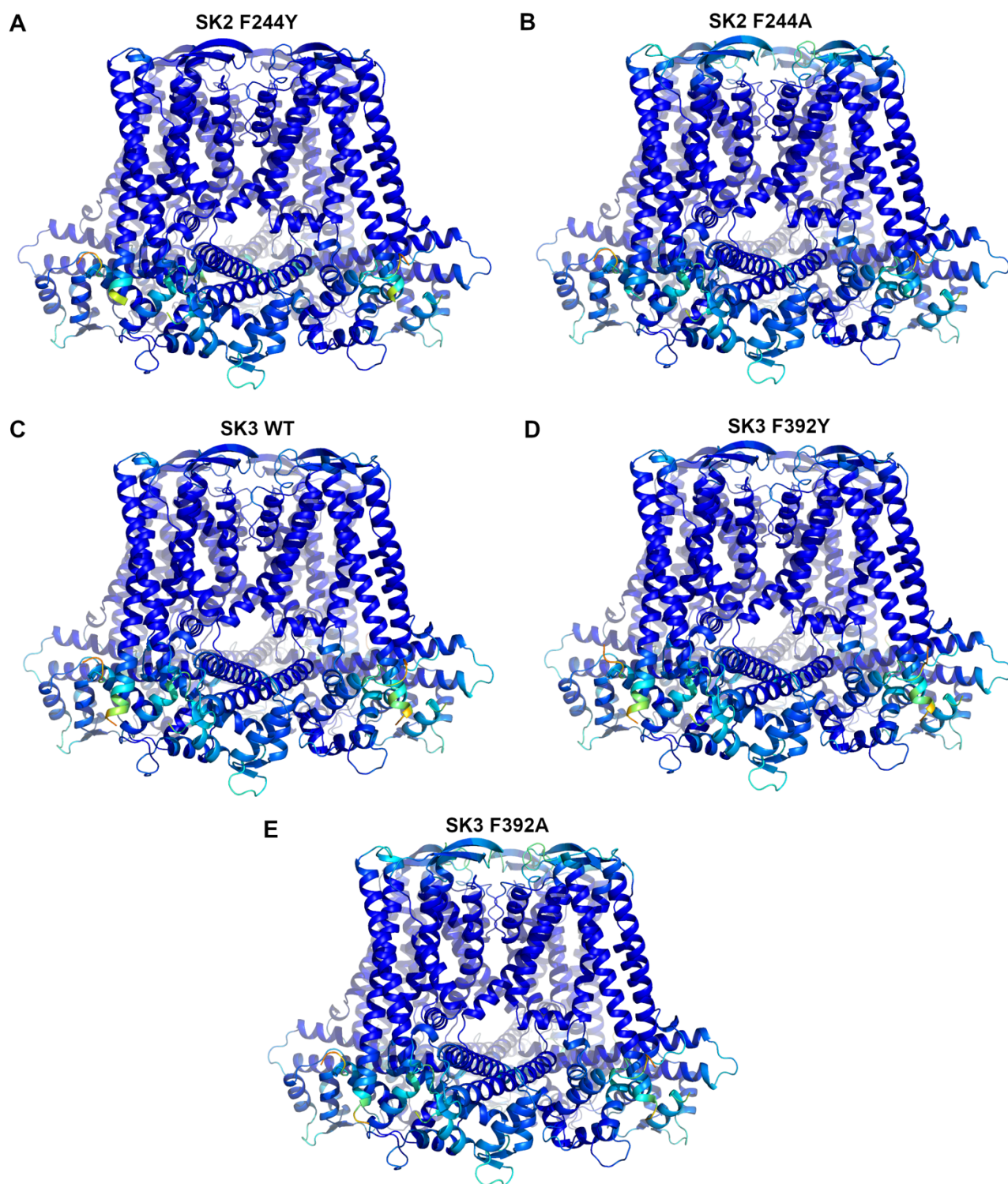


Figure S7. Quality of AlphaFold3 models of SK2 and SK3 channels. (A) Model of the SK2 F244Y mutant represented as cartoon colored according to the pLDDT of the C α of each residue, color gradient going from blue (pLDDT >90) to cyan, yellow and orange (pLDDT < 50). (B) same as (A) for the SK2 F244A mutant). (C) same as A for the SK3 WT. (D) same as A for the SK3 F392Y mutant. (E) same as A for the SK3 F392A mutant.

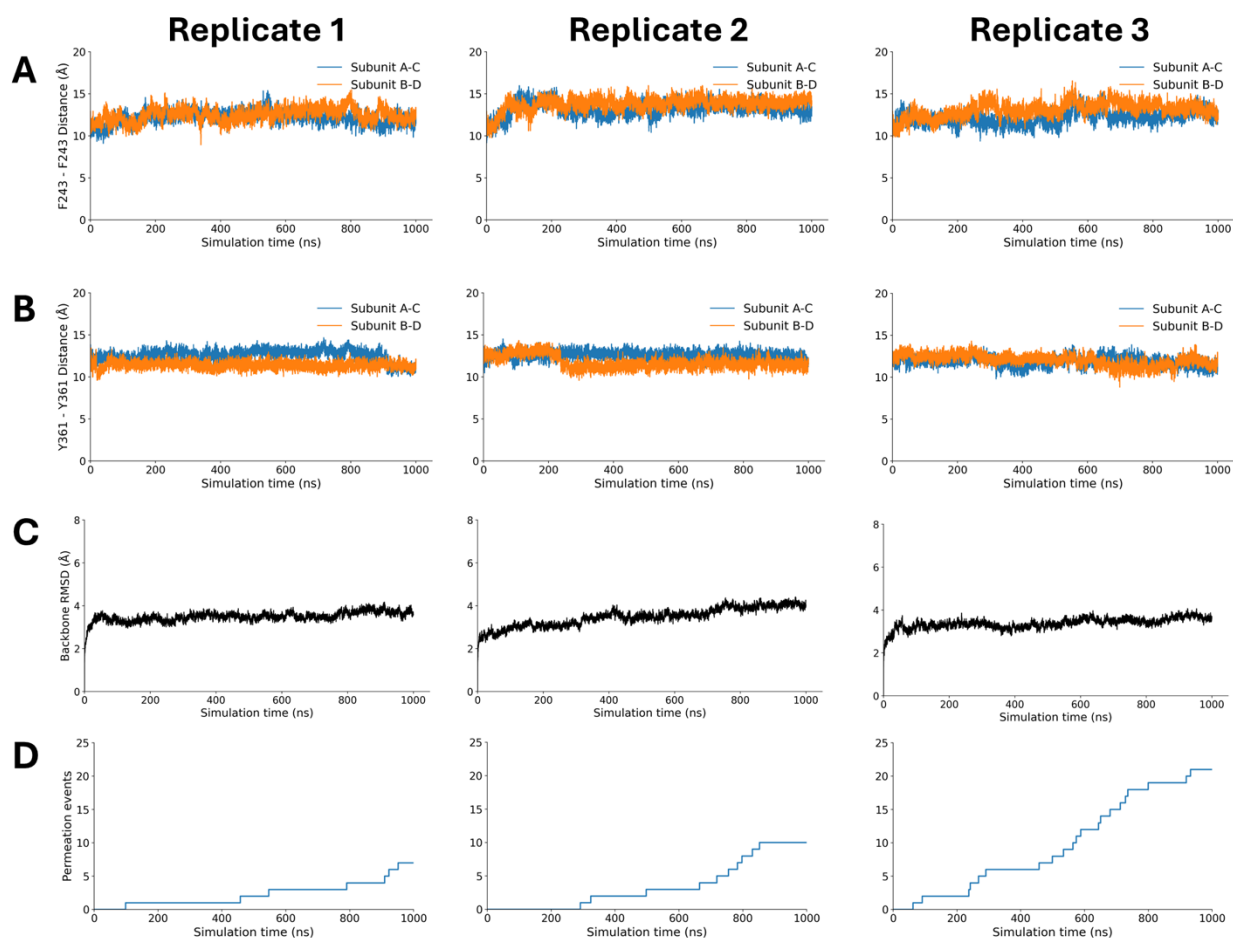


Figure S8. MD simulations of the SK2 WT structure. (A) Variation of the distance between the S3-S4 F243 C α of two opposite SK molecules for the three replicates. (B) Same as (A) for the selectivity filter Y361 C α . (C) Root Mean Square Deviation (RMSD) plots for the three SK2 WT simulations. (D) Ion permeation events count throughout the 1 μ s trajectories.

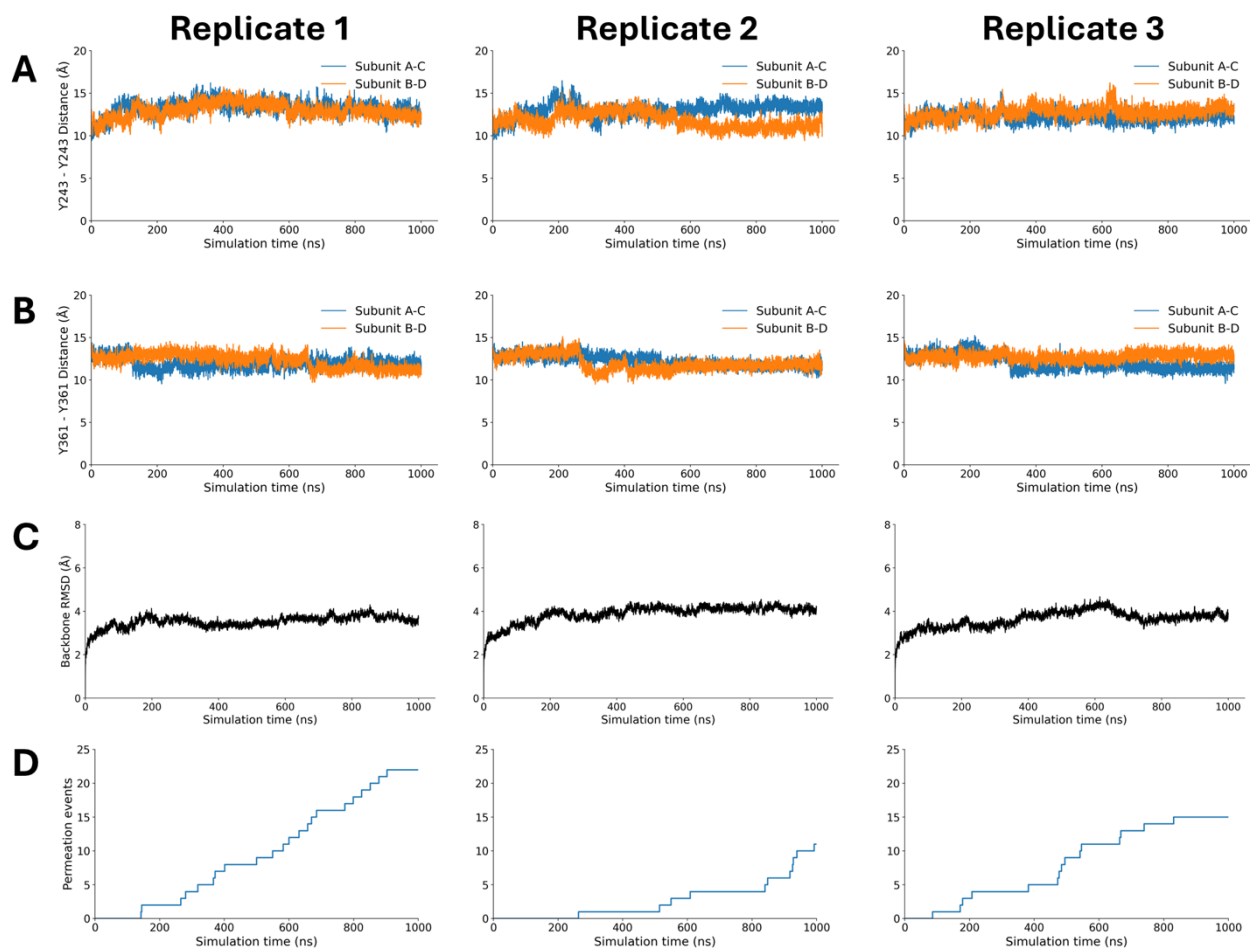


Figure S9. MD simulations of the SK2 F243Y model. (A) Variation of the distance between the S3-S4 Y243 C α of two opposite SK molecules for the three replicates. (B) Same as (A) for the selectivity filter Y361 C α . (C) Root Mean Square Deviation (RMSD) plots for the three SK 2 F243Y simulations. (D) Ion permeation events count throughout the 1 μ s trajectories.

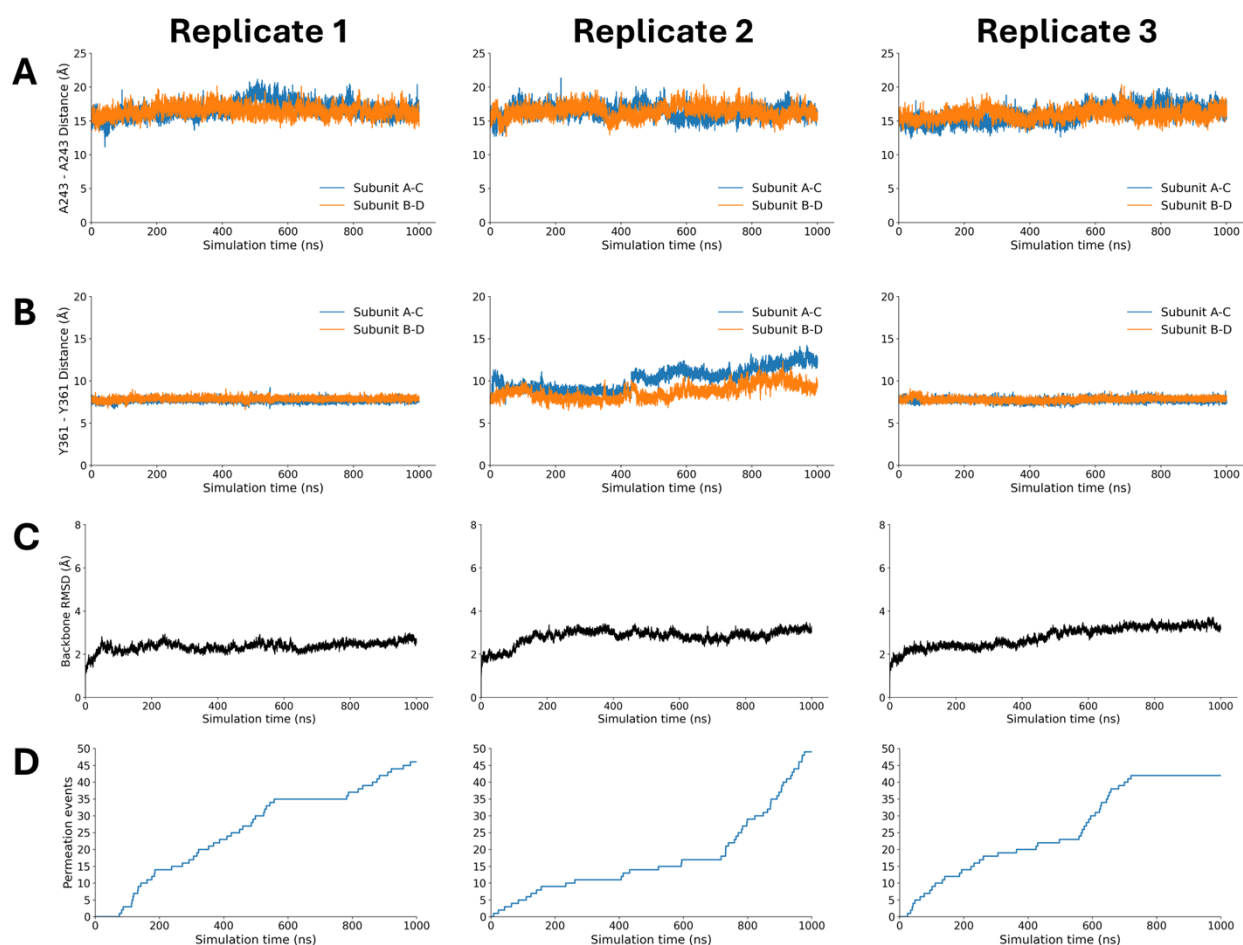


Figure S10. MD simulations of the SK2 F243A model. (A) Variation of the distance between the S3-S4 A243 C α of two opposite SK molecules for the three replicates. (B) Same as (A) for the selectivity filter Y361 C α . (C) Root Mean Square Deviation (RMSD) plots for the three SK2 F243A simulations. (D) Ion permeation events count throughout the 1 μ s trajectories.

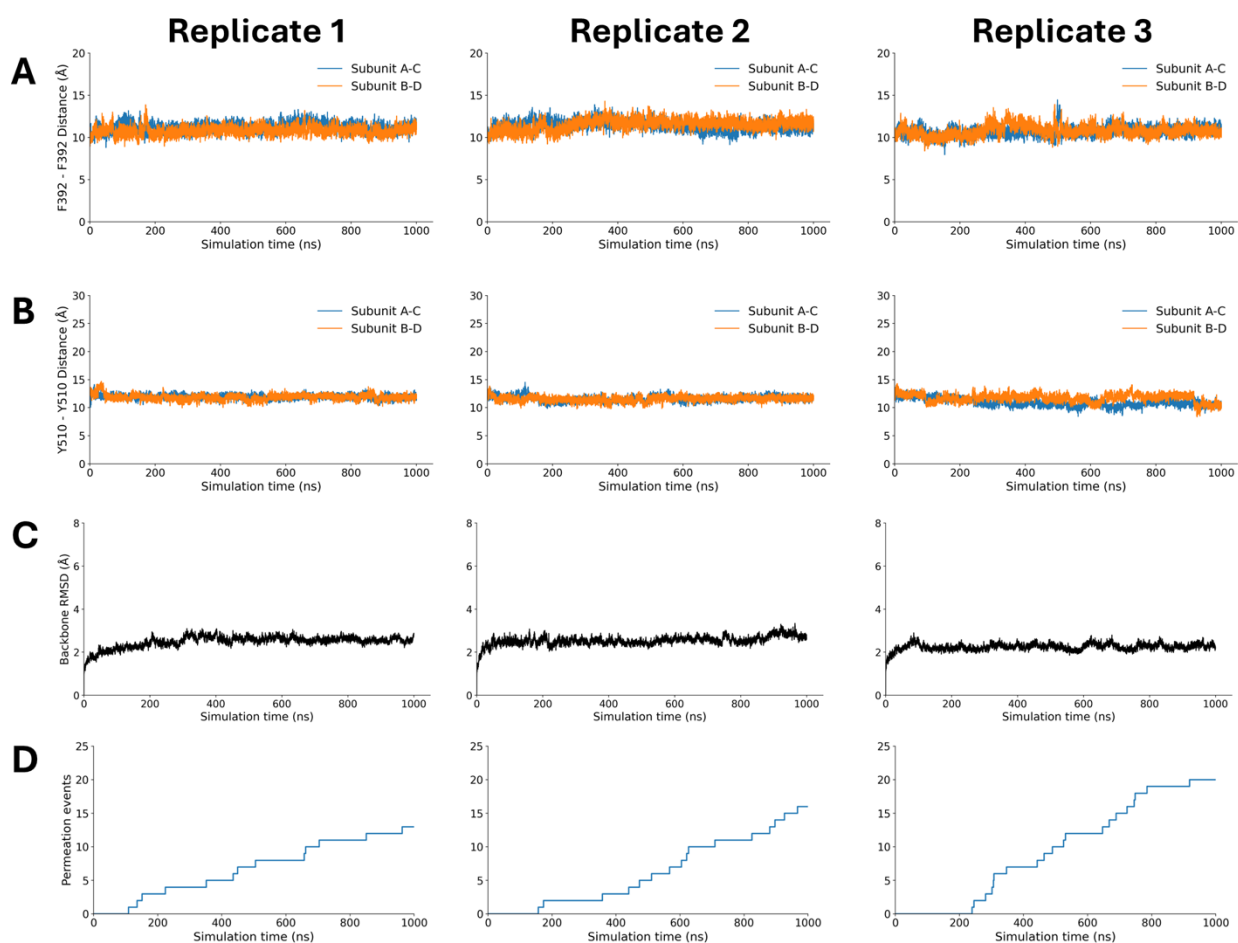


Figure S11. MD simulations of the SK3 WT model. (A) Variation of the distance between the S3-S4 F392 C α of two opposite SK molecules for the three replicates. (B) Same as (A) for the selectivity filter Y510 C α . (C) Root Mean Square Deviation (RMSD) plots for the three SK3 WT simulations. (D) Ion permeation events count throughout the 1 μ s trajectories.

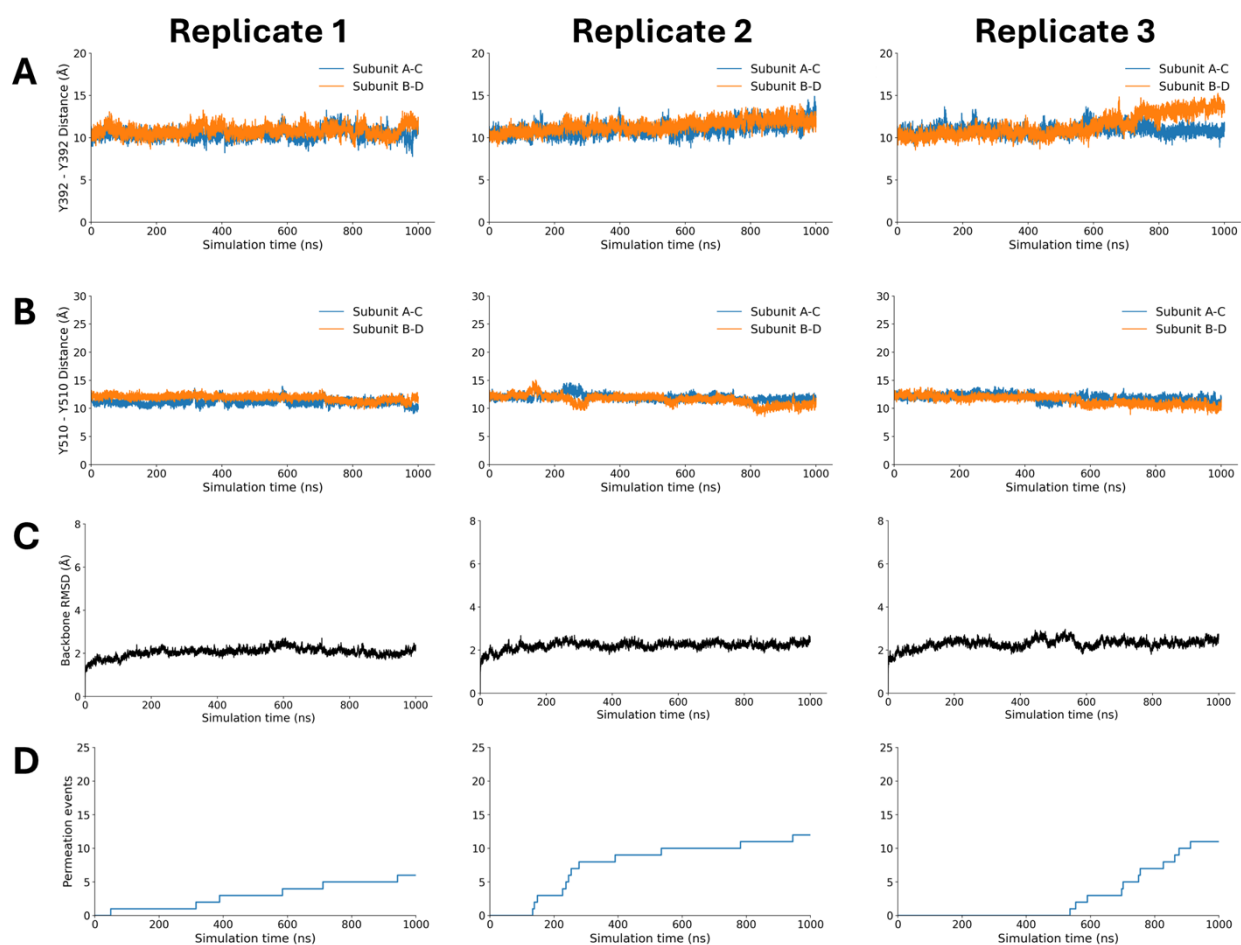


Figure S12. MD simulations of the SK3 F392Y model. (A) Variation of the distance between the S3-S4 Y392 C α of two opposite SK molecules for the three replicates. **(B)** Same as (A) for the selectivity filter Y510 C α . **(C)** Root Mean Square Deviation (RMSD) plots for the three SK F392Y simulations. **(D)** Ion permeation events count throughout the 1 μ s trajectories.

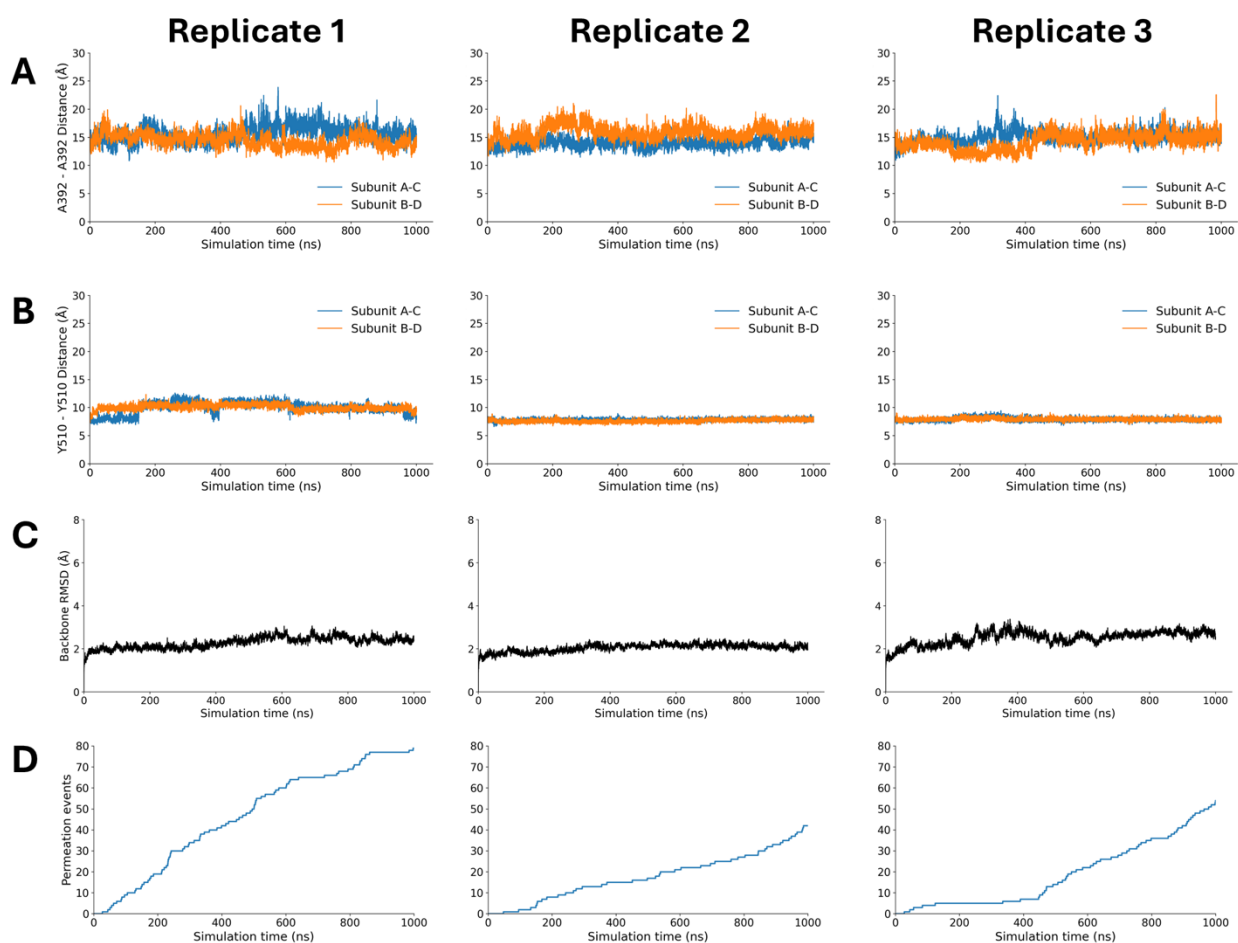


Figure S13. MD simulations of the SK3 F392A model. (A) Variation of the distance between the S3-S4 A392 C α of two opposite SK molecules for the three replicates. (B) Same as (A) for the selectivity filter Y510 C α . (C) Root Mean Square Deviation (RMSD) plots for the three SK3 F392A simulations. (D) Ion permeation events count throughout the 1 μ s trajectories.

Supplemental tables

Table S1. Summary of the IC₅₀ values and Hill coefficients obtained in whole cell patch clamp experiments for SK2 WT, SK2 F243Y, SK2F243A, SK3 WT, SK3 F392Y and SK3 F392A channels with TEA, apamin and UCL1684.

	TEA		Apamin		UCL1684	
	IC ₅₀ (mM)	Hill coeff (<i>h</i>)	IC ₅₀ (pM)	Hill coeff (<i>h</i>)	IC ₅₀ (pM)	Hill coeff (<i>h</i>)
SK2 WT	5.91 ± 1.72	1.05 ± 0.08	220.5 ± 41.3	1.68 ± 0.11	365.9 ± 55.9	0.89 ± 0.06
SK2 F243Y	6.94 ± 1.46	0.98 ± 0.09	226 ± 37.7	1.20 ± 0.09	457.5 ± 81.6	0.73 ± 0.06
SK2 F243A	5.86 ± 0.48	1.12 ± 0.05	/	/	/	/
SK3 WT	7.83 ± 2.09	1.16 ± 0.12	377.6 ± 94.7	1.27 ± 0.10	411.2 ± 98.3	0.60 ± 0.04
SK3 F392Y	9.89 ± 2.37	0.90 ± 0.05	2033 ± 245	1.05 ± 0.12	380.4 ± 51.6	0.60 ± 0.07
SK3 F392A	8.42 ± 1.67	1.08 ± 0.06	/	/	/	/