Supporting Information

Collision Induced Unfolding Differentiates Functional Variants of the KCNQ1 Voltage Sensor Domain

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| Masses of KCNQ1 VSD (Da)		
	Expected	Observed
	Monomer	
KCNQ1 VSD WT	18158	18208 ± 9
KCNQ1 VSD R231C	18086	18152 ± 22
KCNQ1 VSD E115G	18105	18131 ± 16
KCNQ1 VSD H126L	18134	18181 ± 11

Table S1. Measured masses of KCNQ1 VSD variants. Expected and observed masses for KCNQ1 VSD WT, R231C, E115G, and H126L. As peak splitting related to single amino acid truncations was observed (~175 Da), these masses are calculated from the peak without truncation, which is also the most intense peak.
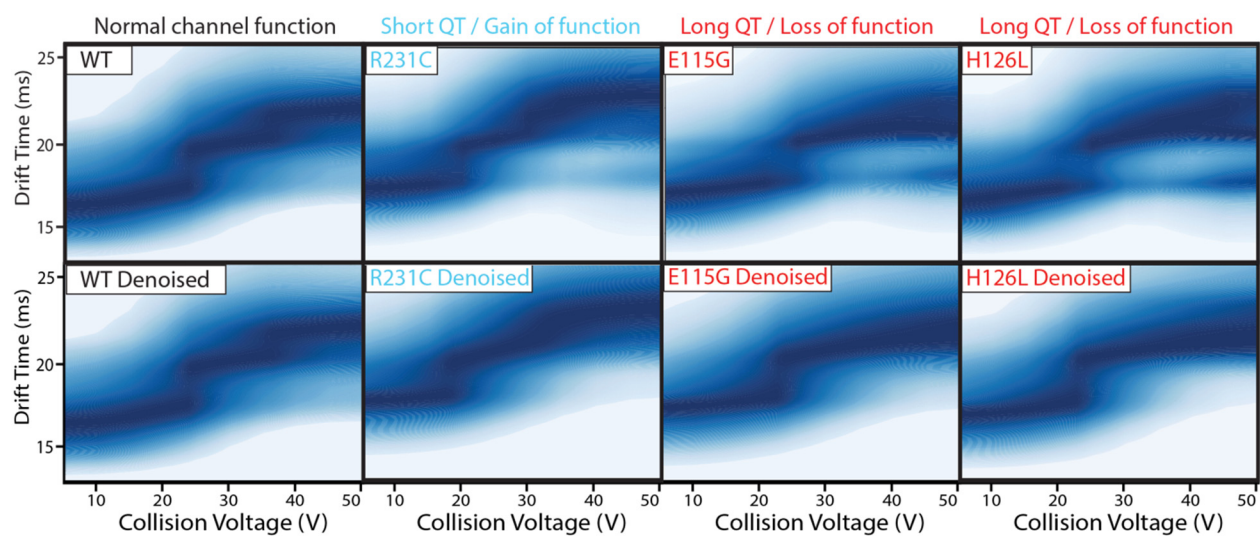
Figure S1. Denoising of KCNQ1 fingerprints. Noise was noted in the low mass to charge and high voltage space, especially for mutant KCNQ1 forms which started at relatively lower concentrations before buffer and detergent exchange (top, averaged fingerprints N = 3). This noise was manually removed from each replicate's text file to yield de-noised fingerprints, for which averaged examples are shown (bottom, N=3).
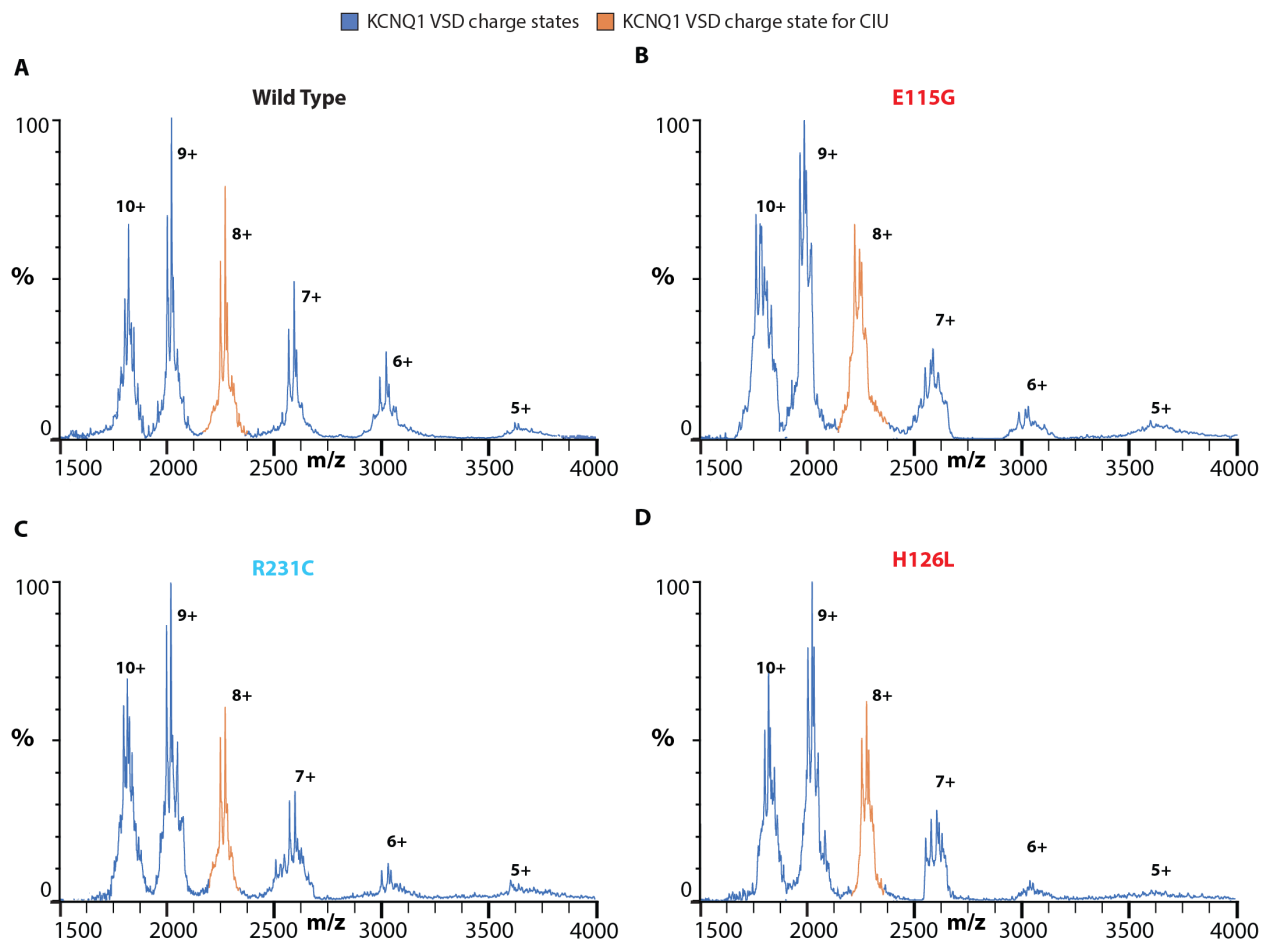
Figure S2. Mass spectra of KCNQ1 variants. **A-D**. Mass spectra for KCNQ1 VSD WT, R231C, E115G, and H126L show the same charge state distribution of 5-10+ monomeric protein. Peak splitting of each charge state indicates a single amino acid truncation (~180 Da) for WT KCNQ1 VSD and possible additional amino acid truncations in the mutant species. CIU fingerprints generated with small extraction windows only encompassing individual truncations exhibited no significant differences, so the entire peak width was extracted for the CIU fingerprints shown in this work. These spectra are post processed through background subtraction to add in visualization.
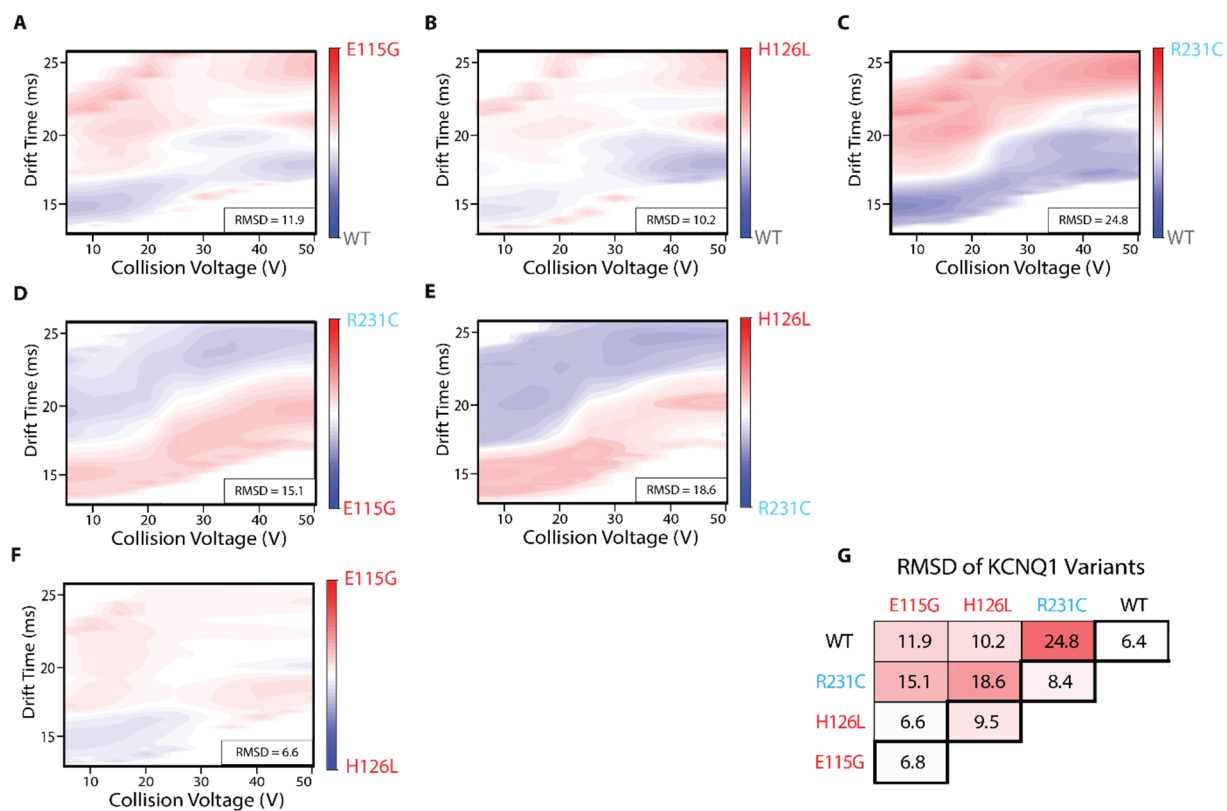
Figure S3. Comprehensive difference plots for all variants. **A-F**. De-noised fingerprints were averaged, N = 3, for each KCNQ1 VSD form and comprehensive difference analysis was performed using CIUSuite 2.1 to yield an RMSD for each pairwise comparison. **G**. Summary of all RMSDs and baseline replicate RMSDs (thick border).
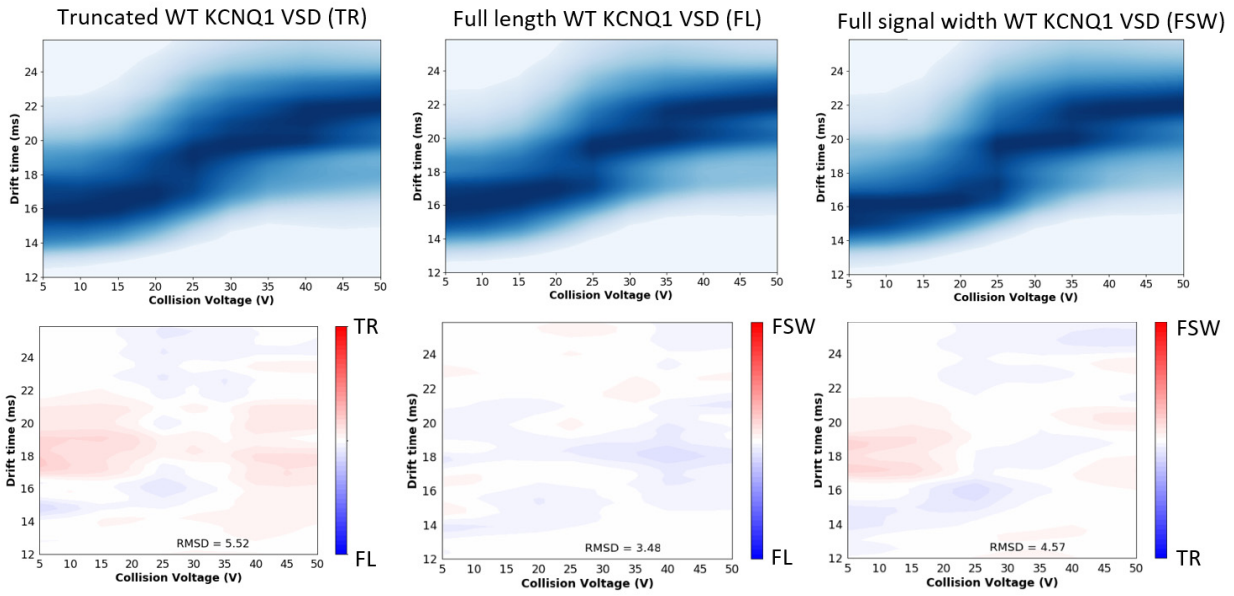
Figure S4. Example CIU fingerprints for truncated, full length, and full signal width KCNQ1 VSD and RMSD values. CIU fingerprints generated from the 8+ monomeric charge state of KCNQ1 using only the peak corresponding to the one amino acid truncated protein form (left), the full length (center) or the full signal width (right) show minimal differences related to signal intensity, but no major trajectory differences. RMSD comparisons show differences between fingerprints between 3.5% and 5.5%.