Kopljar et al., http://www.jgp.org/cgi/content/full/jgp.201210890/DC1

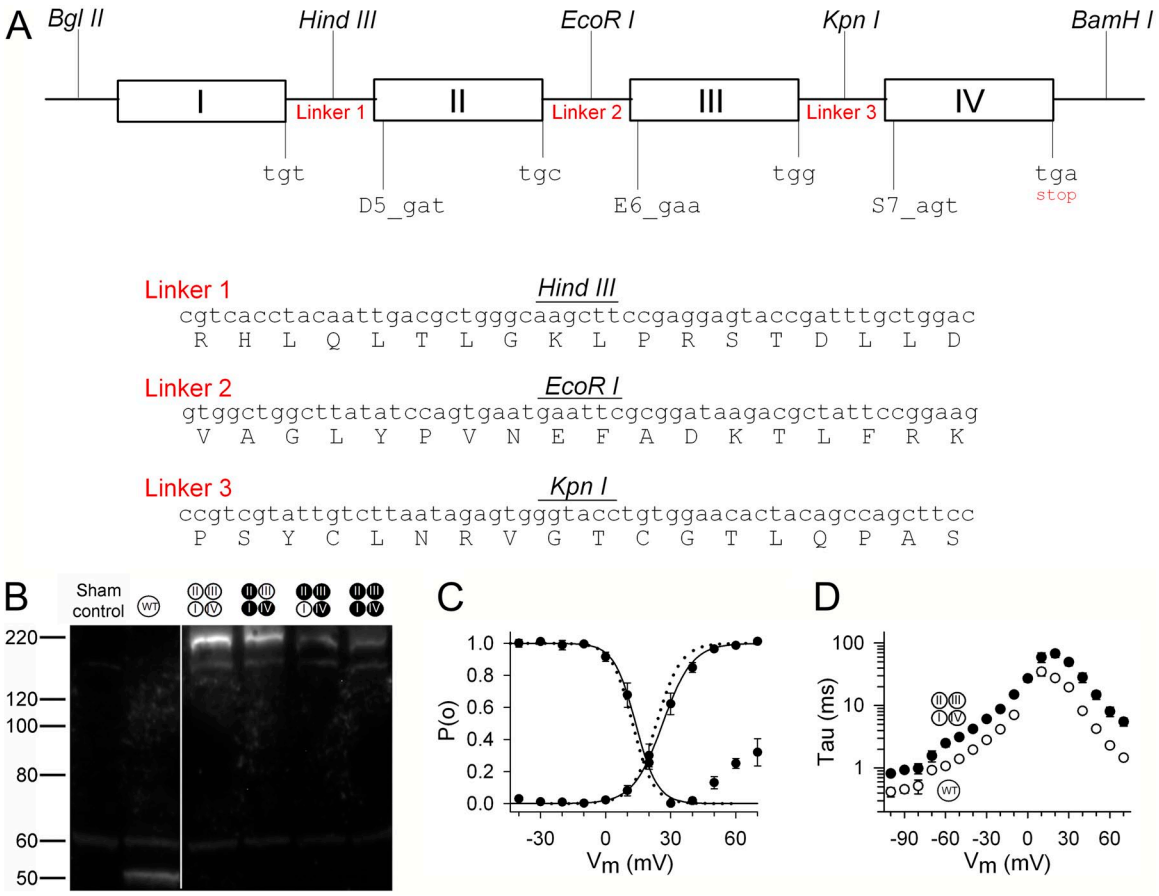

Figure S1. Biophysical properties and Western blot analysis of the WT concatemer. (A) cDNA design of concatemers where the four subunits are covalently linked with unique linker sequences (shown below). Each subunit has its own recognition mark at the 5′ (silent mutation) and 3′ end (mutation of the stop codon sequence) to identify the correct subunit during sequencing. Neighboring subunits have a common, unique restriction enzyme (RE) site enabling the linkage of subunits using specific RE digests. The nucleotide and amino acid sequences are shown in lower-case and upper-case characters, respectively. The white line indicates that intervening lanes have been spliced out. (B) Western blot analysis showed that all concatemers were detected as a single polypeptide of four subunits with an approximate mol wt of 230 kD. WT monomers expressed with an expected mol wt of 57 kD. The leftmost lane shows sham-transfected ltk$^-$ cells (negative control). Molecular mass standards (in kilodaltons) are indicated to the left of the gel blot. (C) Voltage dependence of activation and inactivation, obtained by plotting the tail currents and test-pulse currents, respectively, as a function of the prepulse potential. Data points are shown for the WT concatemer. The fit with the Boltzmann equation is represented by a solid line for the WT concatemer and with a dotted line for the WT Kv3.1 channels assembled from monomers. The WT concatemer had a $V_{1/2\,act}$ = 26.3 ± 2.2 mV (n = 5) and a $V_{1/2\,inact}$ = 13.8 ± 1.5 mV (n = 3). The corresponding values for WT channels assembled from monomers were $V_{1/2\,act}$ = 23.5 ± 1.0 mV (n = 5) and $V_{1/2\,inact}$ = 12.5 ± 1.0 mV (n = 6). (D) Activation and deactivation kinetics for the WT concatemer (closed circles) and WT channels assembled from monomers (open circles). Time constants were determined from fitting a single exponential function to the recorded currents.

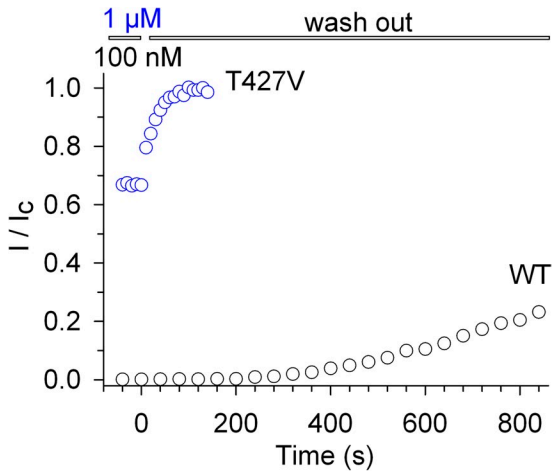

Figure S2. Washout of gambierol in the T427V mutant. Recovery kinetics for the T427V mutant (blue) and WT channels (black) after washing out gambierol are shown. Concentrations of gambierol used were 1 μM for T427V mutant and 100 nM for WT channels. Test pulses to +40 mV from a holding potential of −80 mV were elicited every 10 s for T427V and every 40 s for WT channels. Recovery for the T427V mutant was mono-exponential, with a $\tau = 26 \pm 3$ s ($n = 4$).