Figure S1. Evidence of ionic interactions between R352(KCa3.1) and E84(CaM) derived from charge substitution experiments. (A) Residue R352 is predicted to be in sufficient proximity of CaM E84 for strong electrostatic coupling. Inside-out current recordings illustrating the action of charge substitution at R352C using the positively charged reactive reagent MTSET\(^+\) (1 mM). As seen, the binding at this site of the positively charged MTSET\(^+\) reagent caused a 65 ± 10% \((n = 4)\) decrease in channel activity, an effect not seen when the R352C channel was coexpressed with the E84A CaM mutant. A similar MTSET\(^+\)-induced current inhibition could also be observed with E84C of CaM coexpressed with the R352E mutant. The mutation E84A(CaM) prevented the action of MTSET\(^+\) on the R352C channel, suggesting a MTSET\(^+\)-induced current inhibition involving ionic interactions between MTSET\(^+\) at position 352 of KCa3.1 and E84 of CaM. Label “c” refers to zero current measured in Ca\(^{2+}\)-free conditions (thick line). These observations provide evidence for E84 of CaM being within an electrostatic interaction range with R352 and vice versa. (B) Single-channel inside-out experiments performed on the R352C(KCa3.1)–WT(CaM) or R352C(KCa3.1)–E84A(CaM) systems. The results clearly show an important decrease in the R352C channel activity, with P0max of 0.21 ± 0.07 \((n = 3)\) and 0.03 ± 0.02 \((n = 3)\) before and after MTSET\(^+\) application, respectively. The action of MTSET\(^+\) was essentially caused by an increase of the channel closed dwell time, with little impact on the channel mean open time. Notably, in two additional single-channel experiments, exposure of R352C to MTSET\(^+\) caused a complete loss in channel activity. In contrast, bath application of MTSET\(^+\) to the R352C(KCa3.1)–E84A(CaM) complex failed to modify the channel P0max, an indication that the binding of MTSET\(^+\) to R352C does not, per se, have a detrimental effect on the channel activity, but it requires the presence of a Glu residue at position 84 in CaM.
Figure S2. Effect on the channel activation time $t_{on}$ of mutating residues of the CaMBD2B of KCa3.1 by their KCa2.2 equivalent. Results obtained with the KCa2.2 channel are presented as control. The sequence of the KCa3.1-7AA channel corresponds to R362N-E363D-Q364-V365A-N366-S367T-M368L-V369-D370-I371L-S372A. A decrease in $t_{on}$ was observed with the single S367T mutant but not with M368L. Substituting seven of the KCa3.1 amino acids of the CaMBD2B caused a decrease of the activation time $t_{on}$ from 306 ± 47 ms ($n = 8$) for the WT KCa3.1 to 91 ± 31 ms ($n = 5$) for the 7AA-KCa3.1 channel. This value still remained five times slower than the estimated activation time of KCa2.2 of 17 ± 2 ms ($n = 3$).