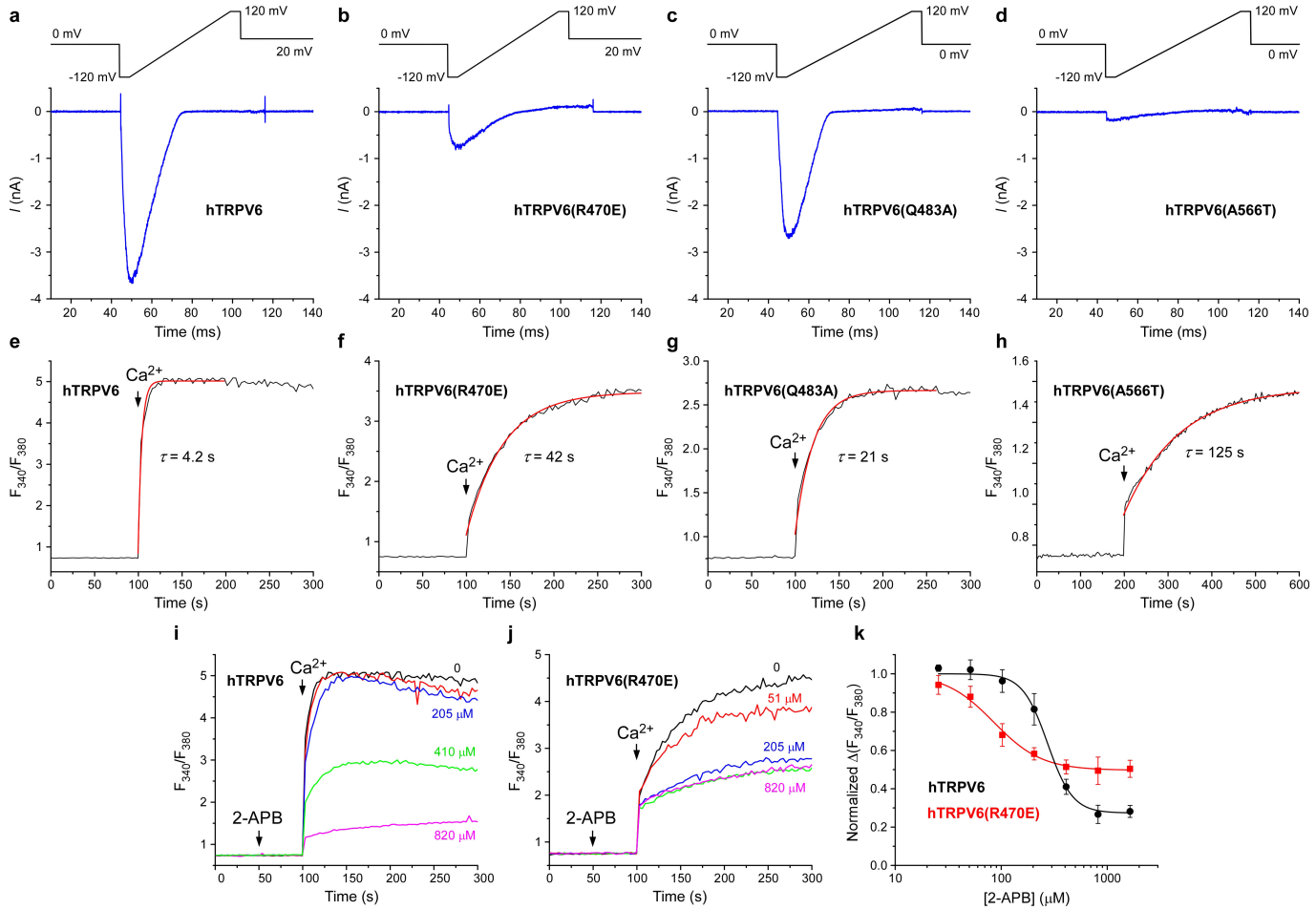

Extended Data Figure 1 | Functional characterization of wild-type and mutant hTRPV6 channels. a–d, Whole-cell patch-clamp recordings from HEK 293 cells expressing wild-type hTRPV6 (**a**), hTRPV6(R470E) (**b**), hTRPV6(Q483A) (**c**) and hTRPV6(A566T) (**d**). Leak-subtracted currents (blue) are shown in response to voltage ramp protocols illustrated above the recordings. Although the shapes of the currents for wild-type and mutant hTRPV6 channels were similar, their amplitudes were different. The average current amplitudes at −60-mV membrane potential (mean ± s.e.m.) were 3,171 ± 767 pA ($n = 11$) for wild-type hTRPV6; 918 ± 267 pA ($n = 9$) for hTRPV6(R470E); 2,239 ± 398 pA ($n = 7$) for hTRPV6(Q483A); and 145 ± 52 pA ($n = 5$) for hTRPV6(A566T). **e–h**, Kinetics of calcium uptake using Fura-2 AM ratiometric fluorescence measurements. Representative fluorescence curves are shown for wild-type hTRPV6 (**e**), hTRPV6(R470E) (**f**), hTRPV6(Q483A) (**g**) and hTRPV6(A566T) (**h**) in response to application of 2 mM Ca^{2+} (arrow). Exponential fits are shown in red, with the time constants indicated. Over five measurements, the time constants (mean ± s.e.m.) were 4.2 ± 0.5 s for hTRPV6; 47 ± 13 s for hTRPV6(R470E); 18.9 ± 0.8 s for hTRPV6(Q483A); and 121 ± 12 s for hTRPV6(A566T). At $n = 5$ and $P = 0.05$, the time constant values for wild-type and mutant channels were statistically different (two-sided *t*-test). **i, j**, Fluorescence curves for wild-type hTRPV6 (**i**) and hTRPV6(R470E) (**j**) in response to application of 2 mM Ca^{2+} after pre-incubation of cells in different concentrations of 2-APB. These experiments were repeated independently three times with similar results. **k**, Dose–response curves for 2-APB inhibition calculated for wild-type hTRPV6 (black) and hTRPV6(R470E) (red) ($n = 3$ for all measurements). The changes in the fluorescence intensity ratio at 340 and 380 nm (F_{340}/F_{380}) evoked by addition of 2 mM Ca^{2+} after pre-incubation with various concentrations of 2-APB were normalized to the maximal change in F_{340}/F_{380} after addition of 2 mM Ca^{2+} in the absence of 2-APB. Curves through the data points are fits with the logistic equation, with the mean ± s.e.m. values of half maximal inhibitory concentration (IC_{50}), 274 ± 27 μM and 85 ± 5 μM, and the maximal inhibition, 72.6 ± 2.7% and 50.3 ± 1.1%, for hTRPV6 and hTRPV6(R470E), respectively. The leftward shift of the 2-APB dose–response curve of hTRPV6(R470E), when compared to the dose–response curve of wild-type hTRPV6, indicates an increased affinity of the channel for 2-APB. This is likely to result from the R470E mutation reducing the affinity of the channel for an activating lipid ligand. On the other hand, the reduced maximum inhibition of hTRPV6(R470E) at high concentrations of 2-APB, when compared to that of wild-type hTRPV6, indicates a reduced efficacy of 2-APB that could be a result of the R470E mutation disrupting the mechanism by which 2-APB binding is allosterically coupled to channel gating.