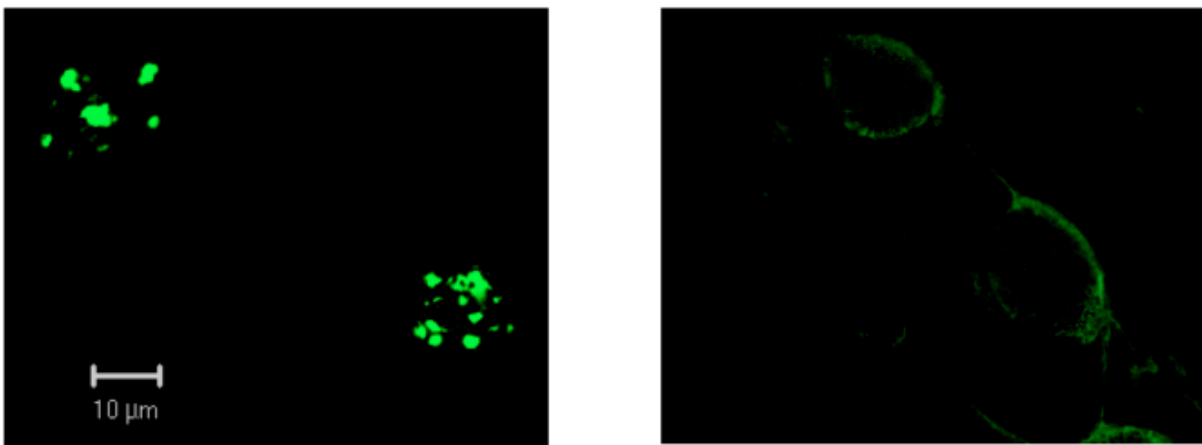
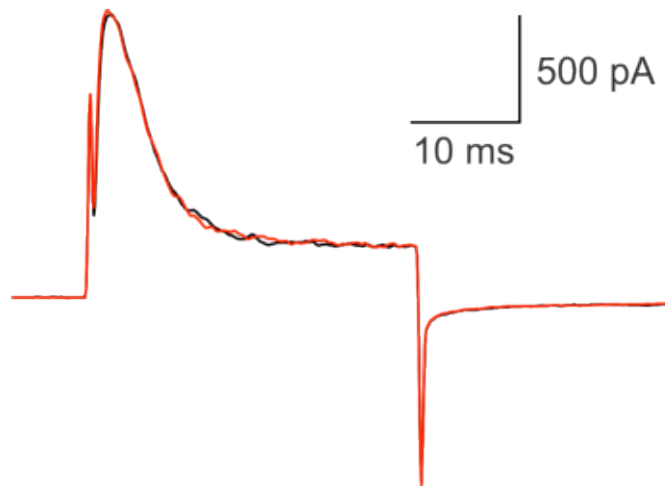


**b**



**Supplementary Figure S1** Functional properties of double cysteine mutants. (a) Current traces in response to 30 ms depolarizations to +60 mV from a holding potential of -80 mV. In each case, the two consecutive traces were recorded ~1 min after patch excision. (b) Pictures of HEK cells expressing the intracellular cysteine less WT Shaker fused at the C-terminus with GFP (right) and the 2C-470C channel fusion construct (left). Similar results were observed in four independent parallel transfections with these two constructs.



**Supplementary Figure S2** Functional properties of A2C mutant channels. The current traces shown were acquired in response to 30 ms depolarizations to +60 mV from a holding potential of -80 mV. Both current traces were recorded ~1 min after patch excision.