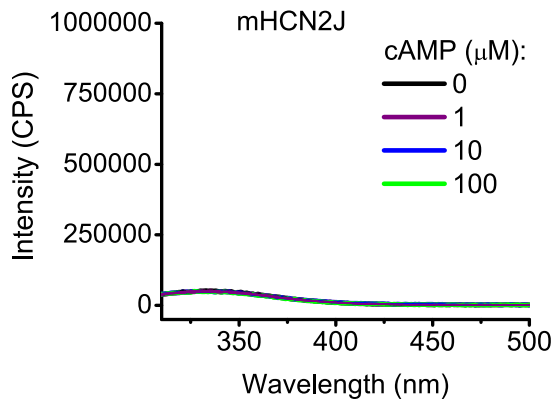
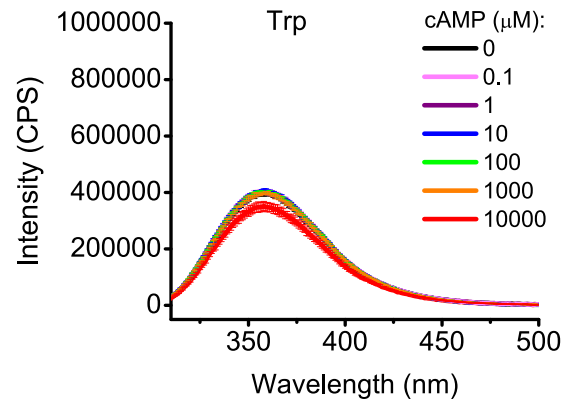
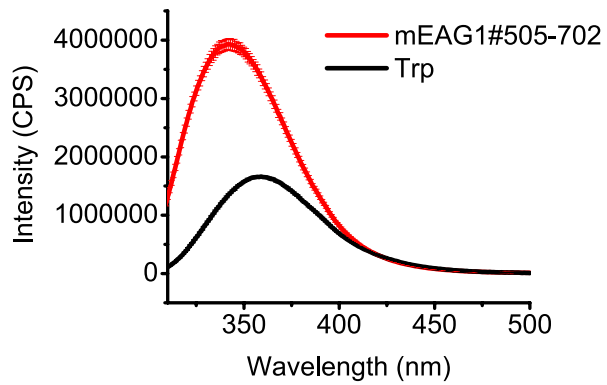
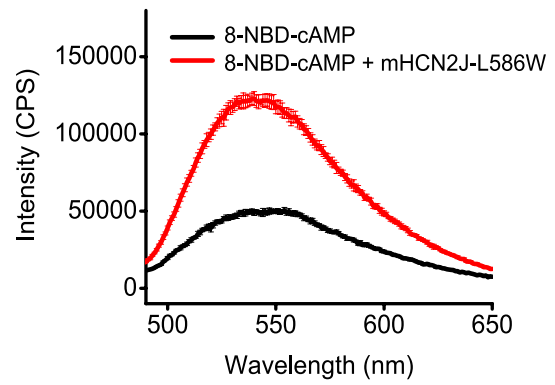


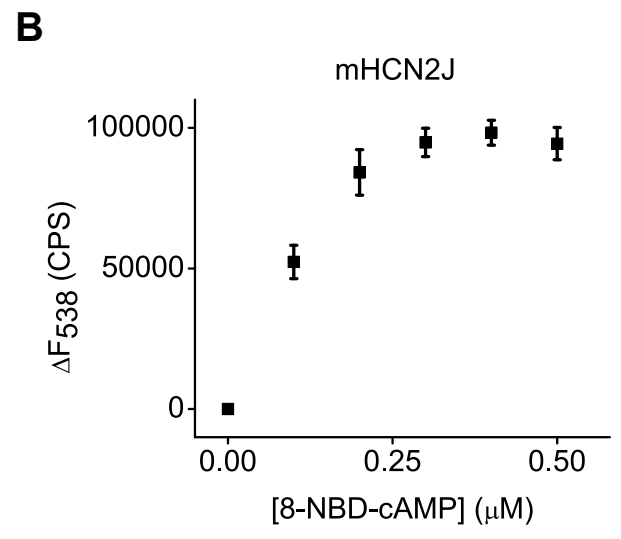
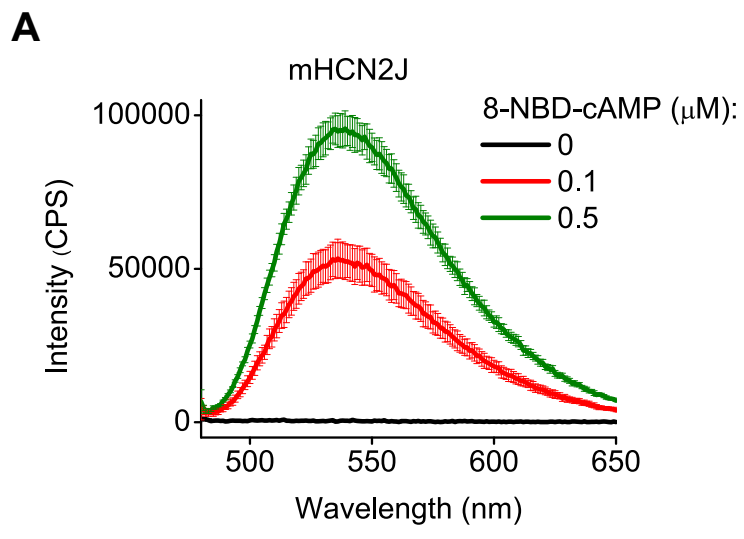
SUPPLEMENTARY FIGURE 1. Cyclic nucleotide concentration-dependent fluorescence changes in mHCN2J-L586W and mEAG1#505-702 are specific to the Trp586 and Trp649 residues, respectively. (A) Emission spectra of wild-type mHCN2J with and without cAMP. mHCN2J shows little fluorescence upon excitation at 295 nm with or without cAMP. (B) and (C) Emission spectra of a free Trp in solution acquired at the same time as the data in Fig. 3B and 3D, respectively. Addition of cAMP had little effect on the fluorescence of the free Trp in solution. Trp was used at the same concentration as the protein in Fig. 3 (4 μ M). The spectrophotometer lamp was changed between the experiments. As a result the fluorescence intensity of the Trp is different in (B) and (C). (D) Emission spectra of 3 μ M 8-NBD-cAMP recorded in the absence (black) and presence (red) of 1 μ M mHCN2J-L586W. The fluorescence intensity of 8-NBD-cAMP increases dramatically upon binding to mHCN2J-L586W.

SUPPLEMENTARY FIGURE 2. Fluorescence of 8-NBD-cAMP increases upon interaction with mHCN2J. (A) Emission spectra of wild-type mHCN2J with and without indicated concentrations of 8-NBD-cAMP. mHCN2J shows little fluorescence upon excitation at 470 nm however the fluorescence intensity increases dramatically in the presence of 8-NBD-cAMP. (B) Plot of the change in the peak fluorescence intensity versus total 8-NBD-cAMP concentration for mHCN2J. Protein concentration was 0.5 μ M. Due to the low concentrations of the protein and 8-NBD-cAMP used the inner filter effect is expected to be negligible and therefore no inner filter correction was applied.

SUPPLEMENTARY FIGURE 3. Cyclic nucleotides do not modulate currents from wild-type hERG1 channels. Currents and conductance-voltage relations for hERG1 channels recorded in the inside-out patch configuration with cAMP (red), cGMP (green) and without the cyclic nucleotides (black). cAMP was applied at 4mM and cGMP at 16 mM. The conductance-voltage relations were obtained from tail currents.

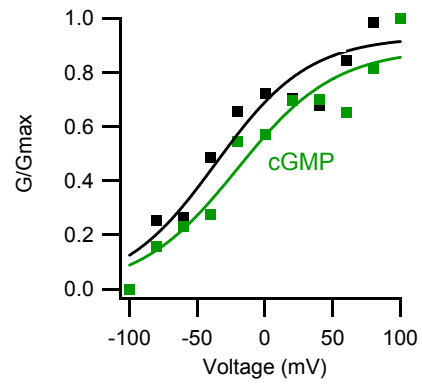
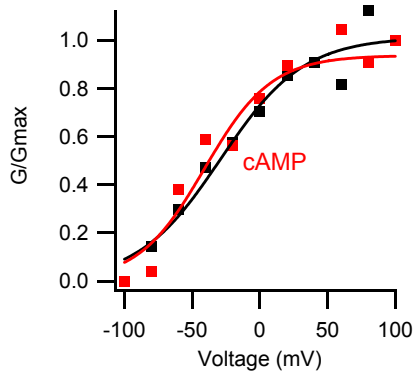
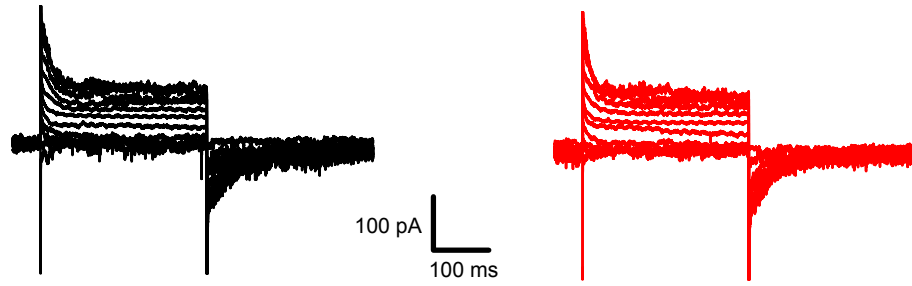
A**B****C****D**

SUPPLEMENTARY FIGURE 1



SUPPLEMENTARY FIGURE 2

hERG1



SUPPLEMENTARY FIGURE 3