

**Supporting Material**

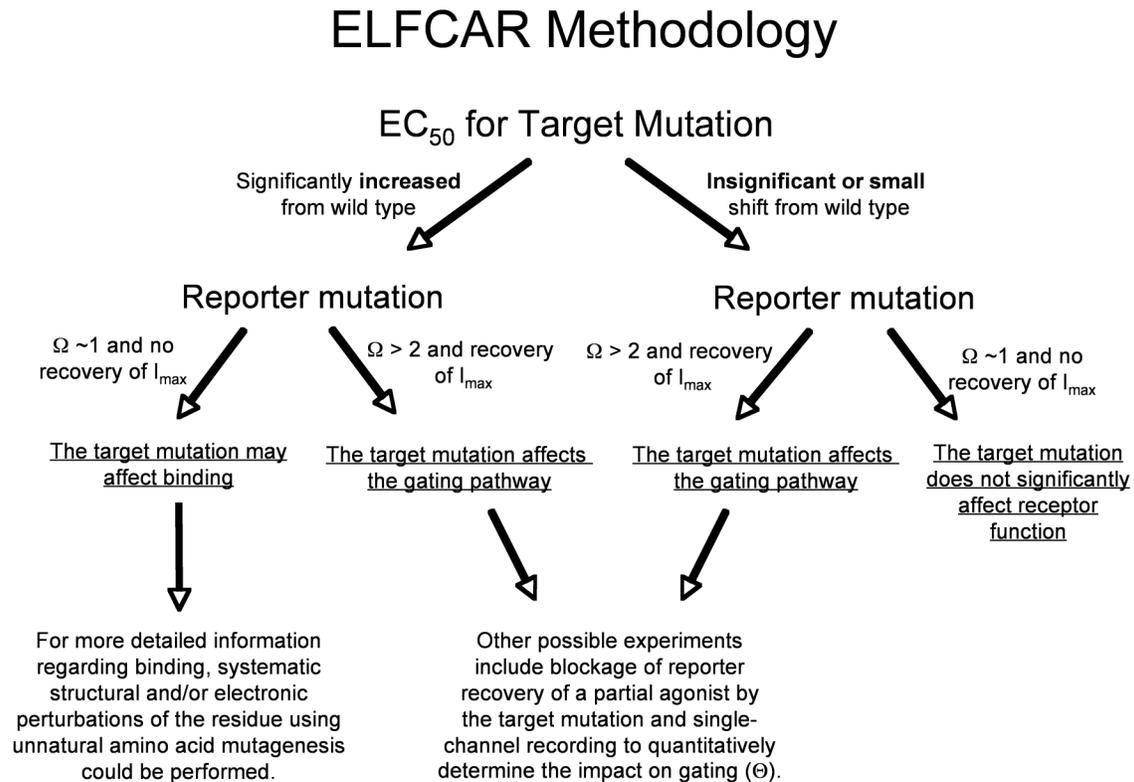
**Long-Range Coupling in an Allosteric Receptor Revealed by Mutant Cycle Analysis**

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**Figure S1**

Experimental outline for applying the ELFCAR methodology introduced here to identify mutations that affect the gating pathway.



## *Supporting Text*

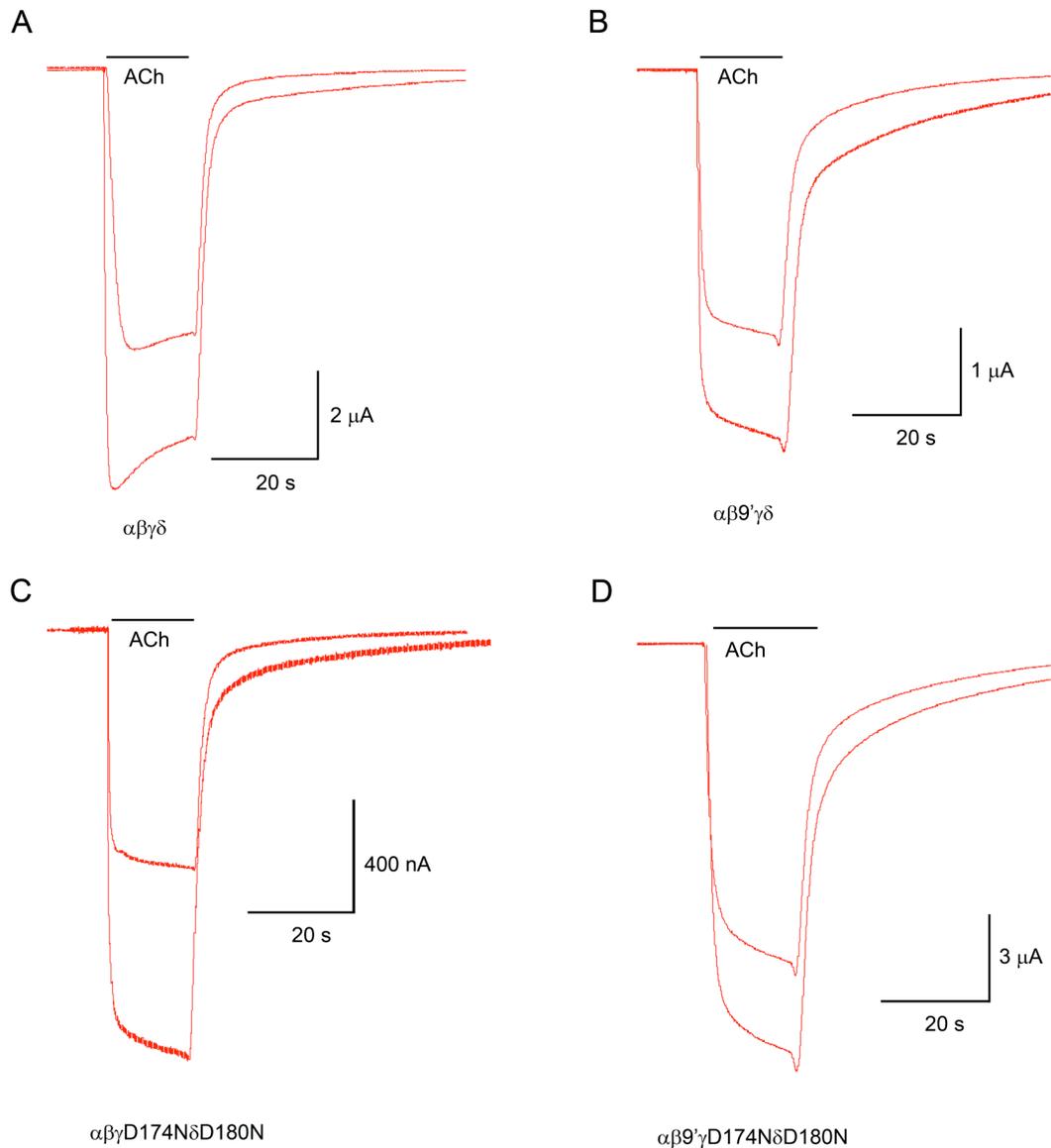
### **The relationship between $\Omega$ , $I_{\max}$ , and $NP_{\text{open}}$**

In < 10% (2 of > 20) of patches obtained for single-channel recordings of the three target mutations shown in Table 2, a higher  $P_{\text{open}}$  mode was seen as part of the channel activity. Since this mode accounted for << 50% of events even when it was present, it accounts for a small minority (<<10%) of total events. Moreover, its rare occurrence indicates that this  $P_{\text{open}}$  is clearly not representative of the functional behavior of these mutations. Rather, it appears to be an infrequently visited mode of the channel. We thus report  $NP_{\text{open}}$  values ignoring this rare mode.

The macroscopic and microscopic data display correlating shifts for the target mutations versus target + reporter mutations. However, there are apparent quantitative differences between the following comparisons (in the background of a target mutation):  $I_{\max}^9/I_{\max}$  wild type (11-17) and  $NP_{\text{open}}^9/NP_{\text{open}}$  wild type (14-116, reflecting a similar shift in  $\Theta$  over the range of our measured  $NP_{\text{open}}$  values). These differences are accounted for by our observation of a high  $P_{\text{open}}$  mode. If channels are in this mode ~1% of time, then that effectively limits the range over which  $I_{\max}$  can shift—adding a fixed ~0.01 to each  $NP_{\text{open}}$  value would limit the maximal possible  $I_{\max}$  shift to 50 since  $P_{\text{open,max}}$  at  $EC_{50}$  is 0.5. The present results for  $I_{\max}$  shifts accord with this observation well: the drastic gating pathway mutations that ELFCAR identifies display a near-maximal 11-17-fold increase in  $I_{\max}$  when a reporter mutation is added. On the other hand,  $EC_{50}$  increases are 4-26-fold, corresponding to  $\Theta$  decreases of 15-680-fold which are quantitatively similar to the microscopic  $NP_{\text{open}}$  results. Since the high  $P_{\text{open}}$  mode would account for, at most, a small minority of the total open conductance, it is not reflected in the  $EC_{50}$  shifts. Thus, the macroscopic ( $I_{\max}$  and  $EC_{50}$ ) data are in accordance with the drastic change in function obvious in microscopic data (Fig. 5).

**Figure S2**

Agonist-induced voltage-clamp currents. Each panel shows the inward macroscopic current elicited near  $EC_{50}$  (Table 1) and at a saturating concentration as downward deflections for one of 4 receptors: A)  $\alpha\beta\gamma\delta$ , B)  $\alpha\beta9'\gamma\delta$ , C)  $\alpha\beta\gamma D174N\delta D180N$ , D)  $\alpha\beta9'\gamma D174N\delta D180N$ . Note that  $I_{max}$  is similar for  $\alpha\beta\gamma\delta$  and  $\alpha\beta9'\gamma\delta$ , but that  $I_{max}$  increases significantly when the L9'S mutation is introduced in the background of the target mutation ( $\gamma D174N\delta D180N$ ).



**Figure S3**

For extracellular domain mutations with  $\Omega > 2$ , the normal recovery of SuCh towards full agonism in the presence of an L9'S reporter mutation is blocked. The gray bars represent receptors with a wild type pore domain, while the white bars represent receptors with a  $\beta$ L9'S mutation. The wild type receptor and four mutations to the right all show the characteristic increase in efficacy in the presence of the L9'S pore mutation, while the three mutations on the far left do not.

