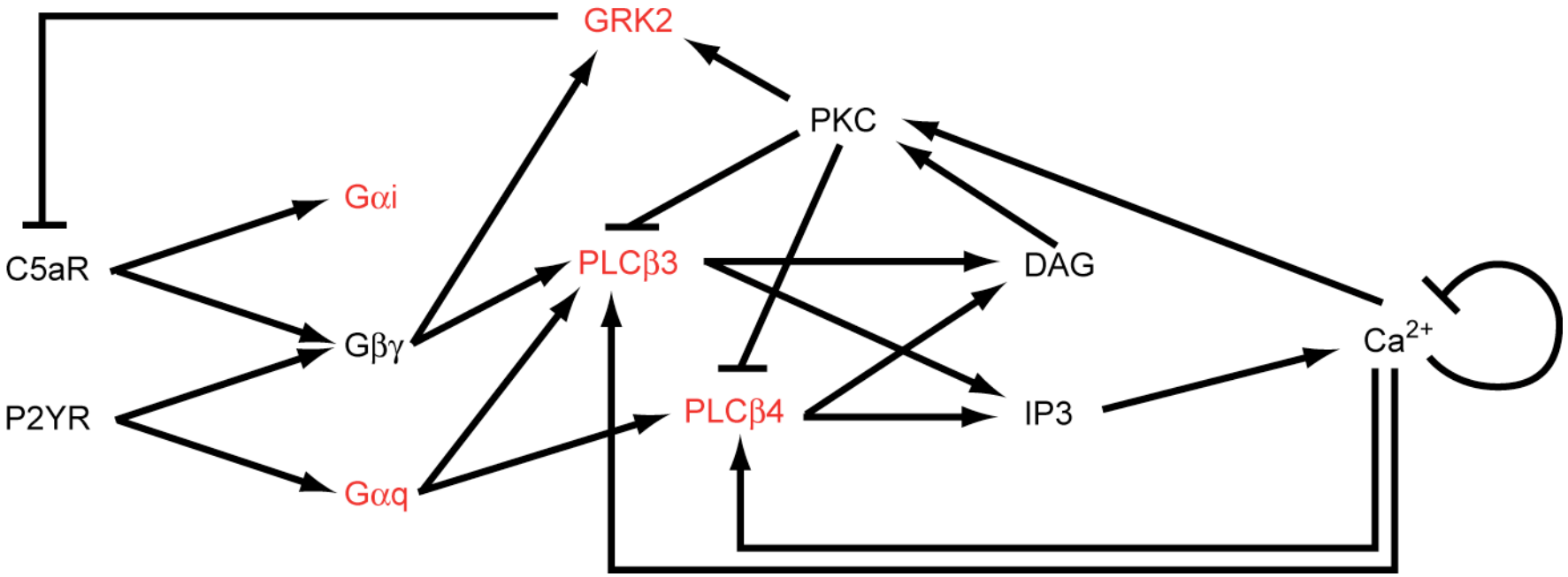

Figure 1: The model for crosstalk between the Gαi and Gαq pathways depends on both differential specificity and activity for Gαi, Gαq and Gβγ interactions with PLCβ3 and PLCβ4 to catalyze PIP2 hydrolysis and calcium dependent feedback control mediated by GRK and PKC. Selected model parameters are informed by calcium measurements taken for various ligand doses on wild-type and cell lines with shRNAi knockdowns on the proteins shown in red.

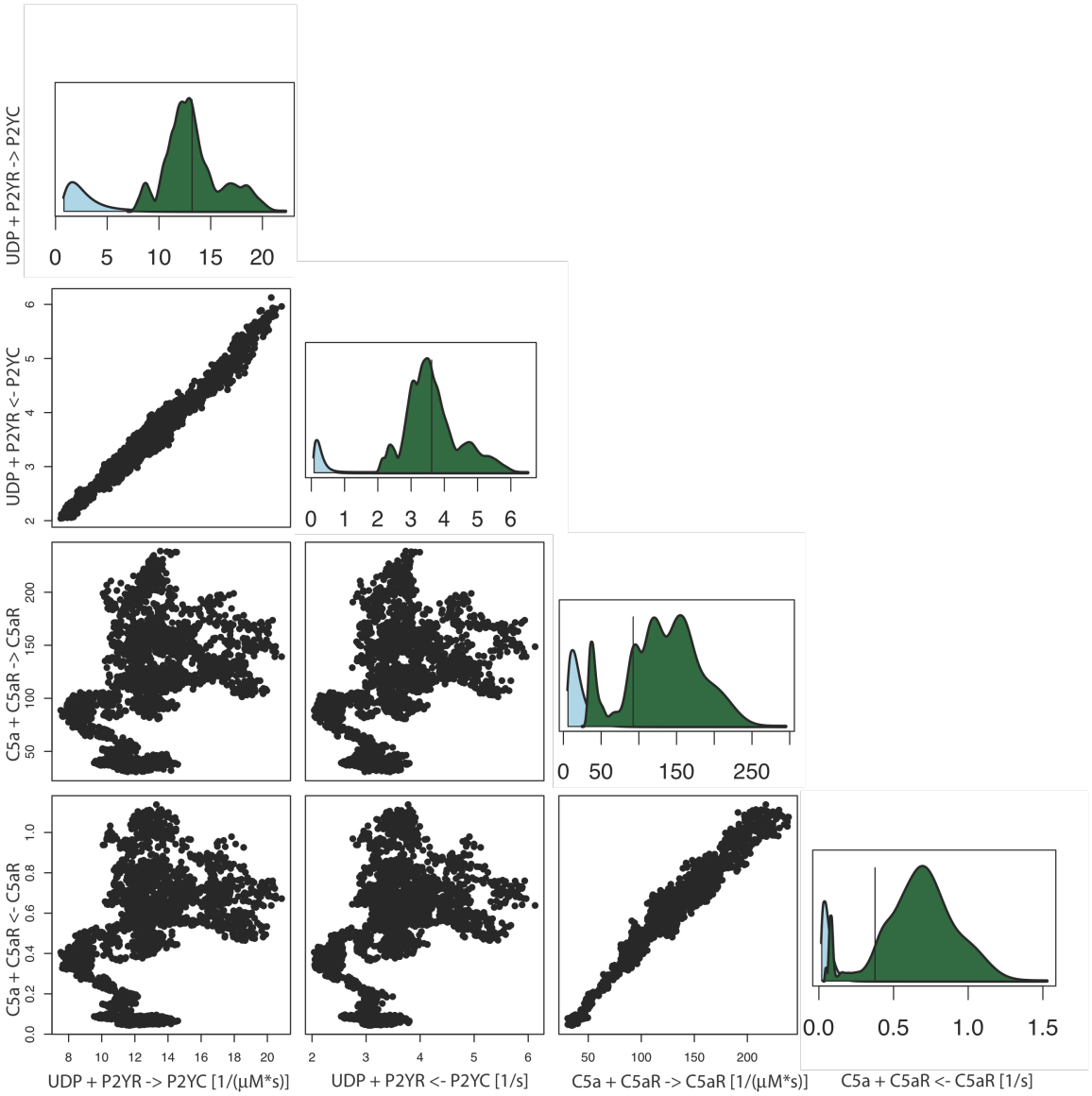

Figure 2: This figure shows that the single and pairwise marginal posterior distributions for the ligand binding reactions for the P2YR and C5aR receptors. The vertical line in the single marginal posterior distributions shows the point estimate that were selected. The posterior distributions show the dissociation constants for the reactions are tightly constrained by the data, while the values of the forward and reverse rates that make up the ratio are not as well constrained by the data. Additionally, as expected the UDP binding rates are not correlated with the C5a binding rates. Marginal posterior distributions for all parameters and a discussion of the point estimate selection can be found in Figure S2 (supplementary information).

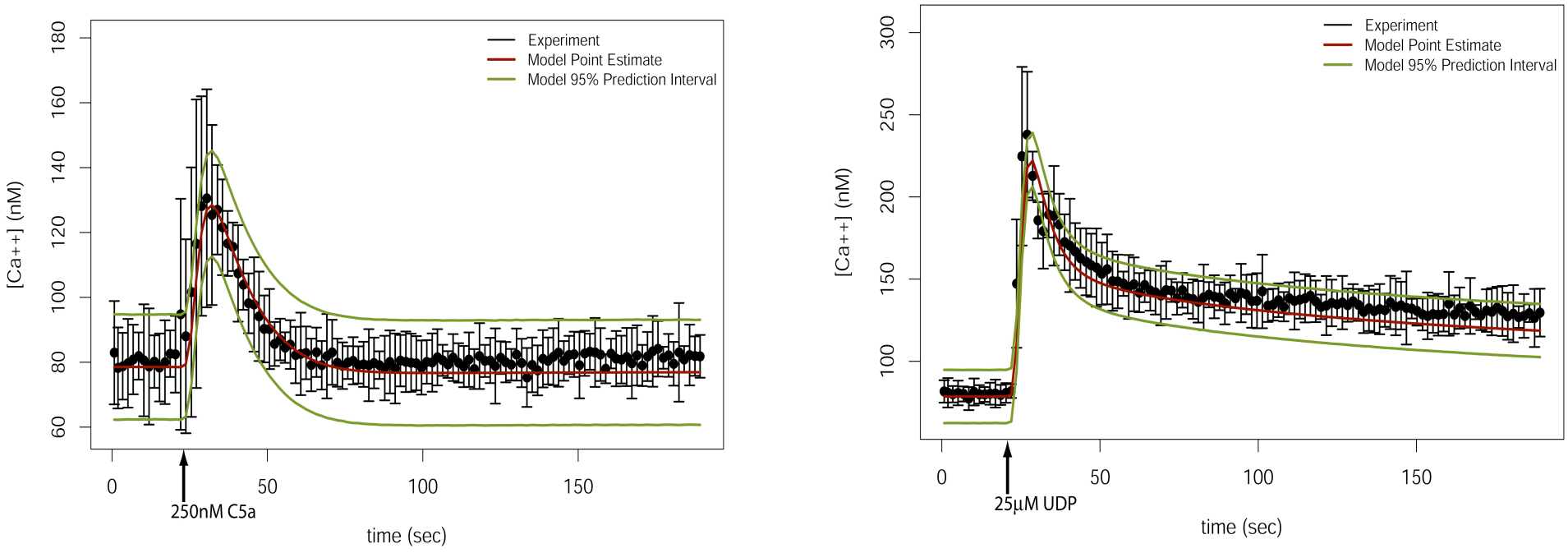

Figure 3: Model simulations are compared to experimental data. The point estimate is computed using the posterior distribution of the parameter as estimated by Markov chain Monte Carlo given the data from 96 experiments on C5a and UDP at various doses in combination with 5 different shRNAi knockdown cell lines. The 95% posterior predictive intervals are estimated by Monte Carlo simulations including both parameter and measurement uncertainty. The measured mean and approximate 95% confidence intervals of four replicates is shown by a black dot and error bar. (left) C5a at 250nM was introduced at 20s and the experimentally observed pulse in cytosolic calcium concentration is shown. (right) The qualitative shape of the calcium pulse for 25µM UDP is different than for 250nM C5a. The pulse does not completely adapt and return to the prestimulated level. For both ligands, the model prediction confidence intervals overlap the data error bars that indicate the model fit is consistent with the data within the measurement uncertainty.

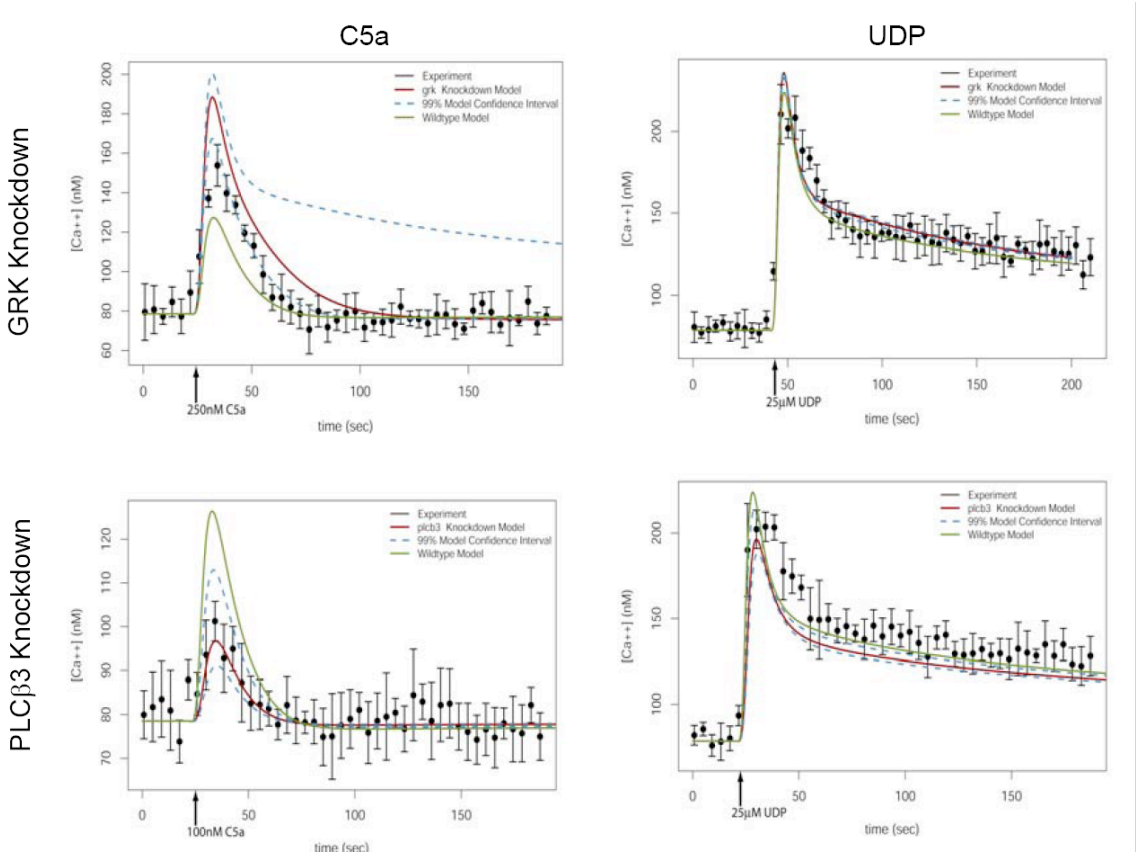

Figure 4: The model simulation results for GRK and PLCβ3 knockdown cell lines stimulated with C5a and UDP are shown. The experimental mean ± 1s.d. of 3-4 replicates within one experimental run is shown in black. The knockdown simulation result with nominal knockdown fraction and parameters is shown in red and the wild-type simulation result is shown in green for comparison. Upper and lower model 99% confidence intervals (shown as blue dashed lines) are simulated using the upper and lower knockdown fraction values from Table 1. As expected the Ca^{2+} response to C5a in the GRK knockdown line (upper-left panel) was increased compared to wild-type. The quantitative deviation between the model and data is possibly due to the availability of multiple redundant GRK isoforms. The upper-right panel shows that the expected effect of the GRK knockdown on the UDP response is an increase in the cytosolic calcium levels. Because GRK2 does not directly desensitize the P2Y receptor in this model, the effect is likely due to a reduction of sequestration of Gβγ by GRK. The lower-left panel shows that the signal transduction of the C5a response is predominantly through the PLCβ3 isoform. The effect of the PLCβ3 knockdown is much greater for C5a than for UDP (shown in the lower-right panel).

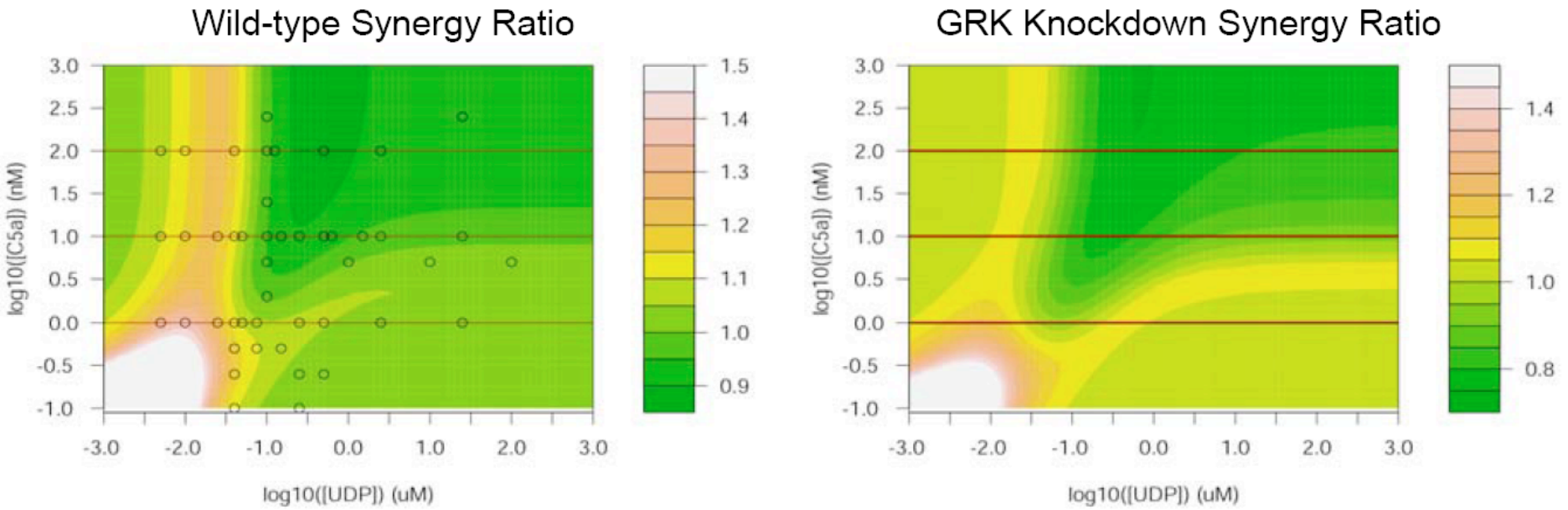

Figure 5: The model is used as a predictive tool to infer the effect of stimulating the cell simultaneously with UDP and C5a that signal through the Gαq and Gαi pathways respectively. Synergy was measured as the ratio of peak height offset from baseline attained from simultaneous stimulation to the peak height offset calculated by the sum of the responses to each ligand individually. The left panel shows the expected synergy ratio as a function of UDP and C5a dose (truncated at 1.5). The simulations show a ridge of synergy at a moderate UDP dose for most C5a doses. The black circles indicate dose combinations points of experiments that were conducted to test the model. The right panel shows the expected synergy ratio as a function of UDP and C5a dose for a simulated GRK2 knockdown cell line. Without the GRK-mediated negative feedback to keep the IP3 generation from the C5a receptor within the non-linear range of calcium release the ridge in the synergy dose response is diminished. The synergy in the GRK knockdown simulation is not entirely eliminated because the shRNAi knockdown of GRK does not constitute a complete loss-of-function and low concentrations of ligand are still able to synergize. Furthermore, the asymmetric synergy dose response surface is more symmetric in the GRK knockdown simulation because the asymmetric calcium-dependent feedback mechanism is reduced.