Enhancing Network Activation in Natural Killer Cells: Predictions from *In Silico* Modeling

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Supplementary Figures

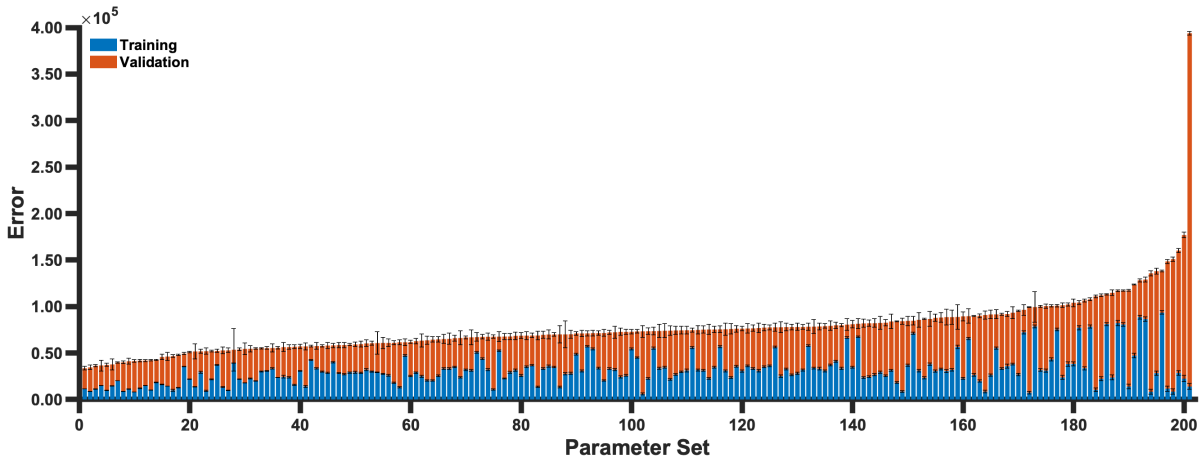

Figure S1. Total error from parameter estimation. The Metropolis-Hastings algorithm was simulated 200 times. For each run, we used the last 1,000 iterations to simulate the model and compare the model predictions to training (blue) and validation (red) data. Bars represent mean value and error bars represent one standard deviation. The total error was sorted in ascending order. The first 14 parameter sets were used to analyze the model predictions.

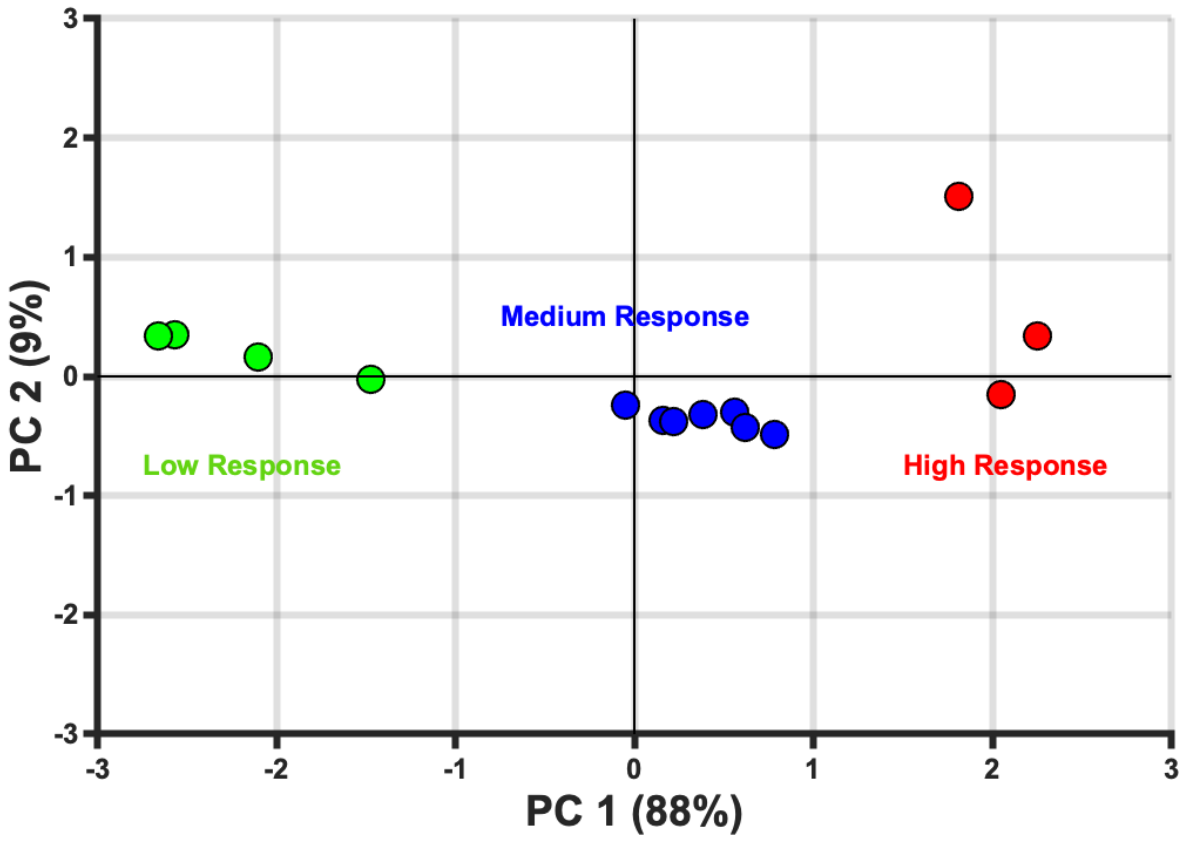

Figure S2. Cluster analysis of model predictions. We analyzed the model predictions using the 14 parameter sets. We clustered the model predictions using kmeans in MATLAB and found three clusters based on the magnitude of network activation (low, medium and high; green, blue and red, respectively). The model predictions were also analyzed using pca in MATLAB, where principal component 1 (PC 1) explained about 88% of the total variance in the model predictions. For both the kmeans and pca functions, the input was a 14 × 3 matrix corresponding to the mean model prediction from the 14 parameter sets using mono-stimulation of the three pathways.

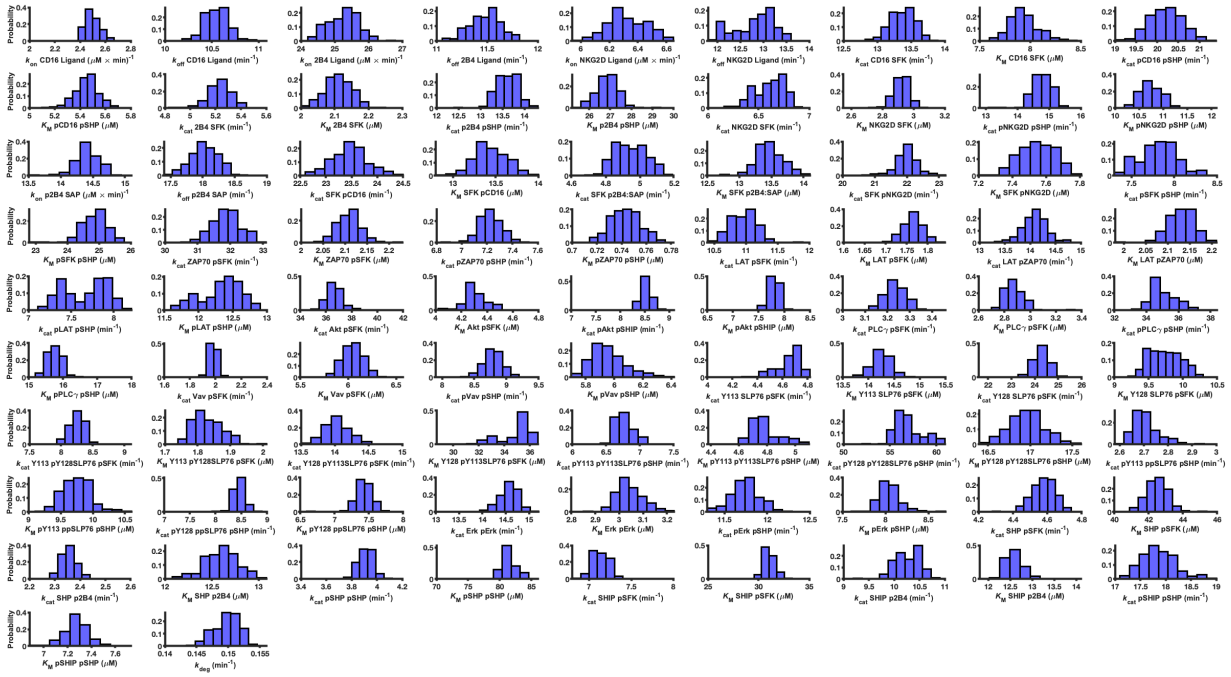

Figure S3. Parameter estimation using the best parameter set. The parameter distribution for each of the 83 estimated parameters are shown. We used the final 1,000 iterations from parameter estimation to plot the histograms for each parameter.

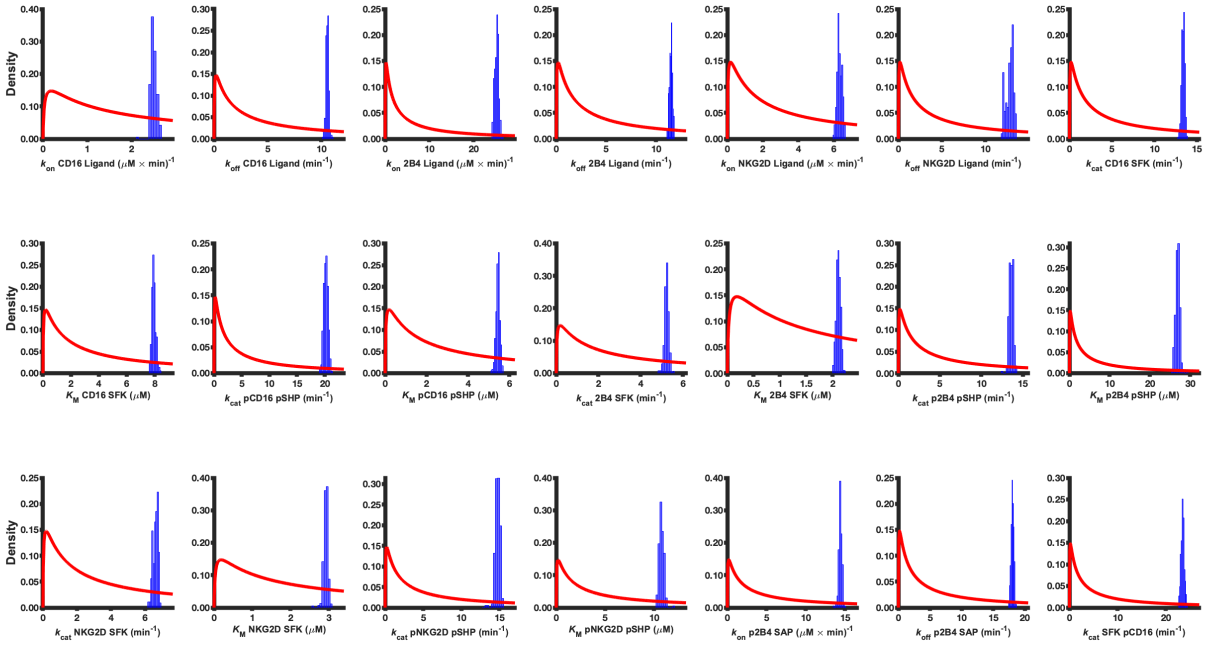

Figure S4. Parameter estimation using the best parameter set. The distribution of the posterior (blue histogram) and the prior (red curve) for parameters 1 – 21 are shown. We used the final 1,000 iterations from parameter estimation to plot the histograms for each parameter.

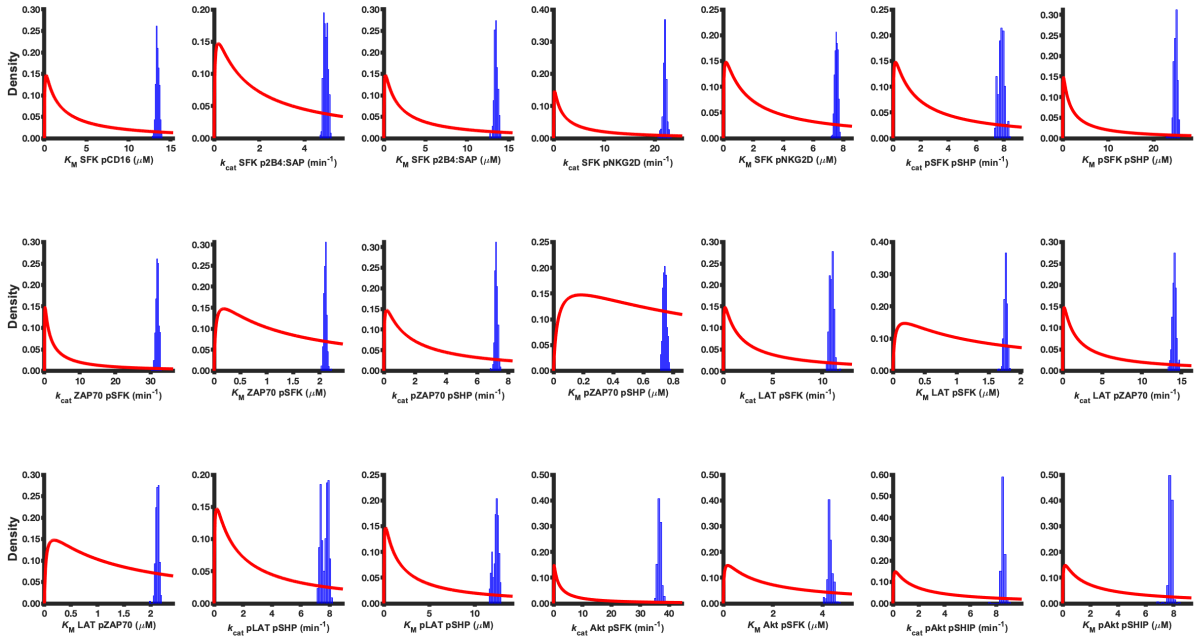

Figure S5. Parameter estimation using the best parameter set. The distribution of the posterior (blue histogram) and the prior (red curve) for parameters 22 – 42 are shown. We used the final 1,000 iterations from parameter estimation to plot the histograms for each parameter.

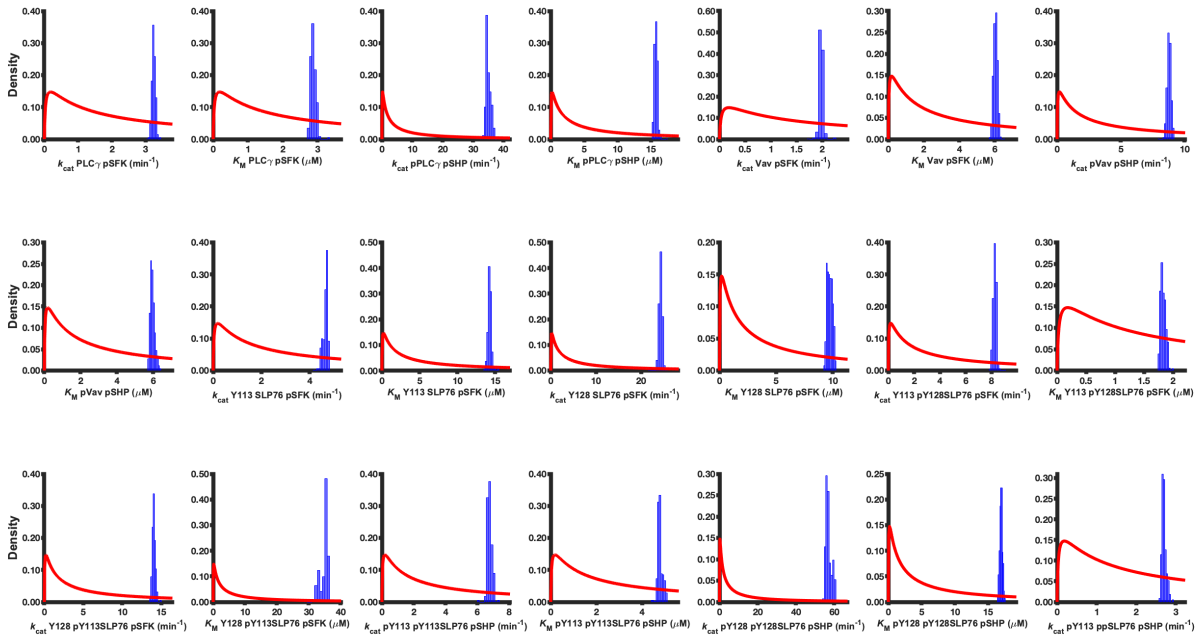

Figure S6. Parameter estimation using the best parameter set. The distribution of the posterior (blue histogram) and the prior (red curve) for parameters 43 – 63 are shown. We used the final 1,000 iterations from parameter estimation to plot the histograms for each parameter.

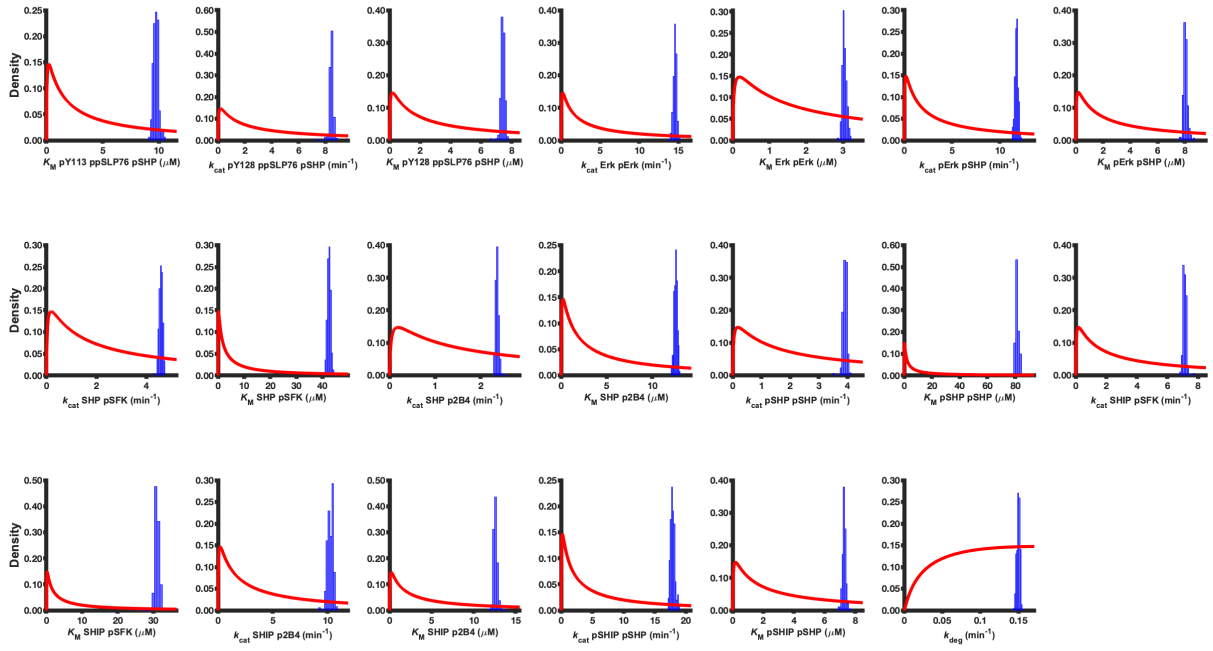

Figure S7. Parameter estimation using the best parameter set. The distribution of the posterior (blue histogram) and the prior (red curve) for parameters 64 – 83 are shown. We used the final 1,000 iterations from parameter estimation to plot the histograms for each parameter.

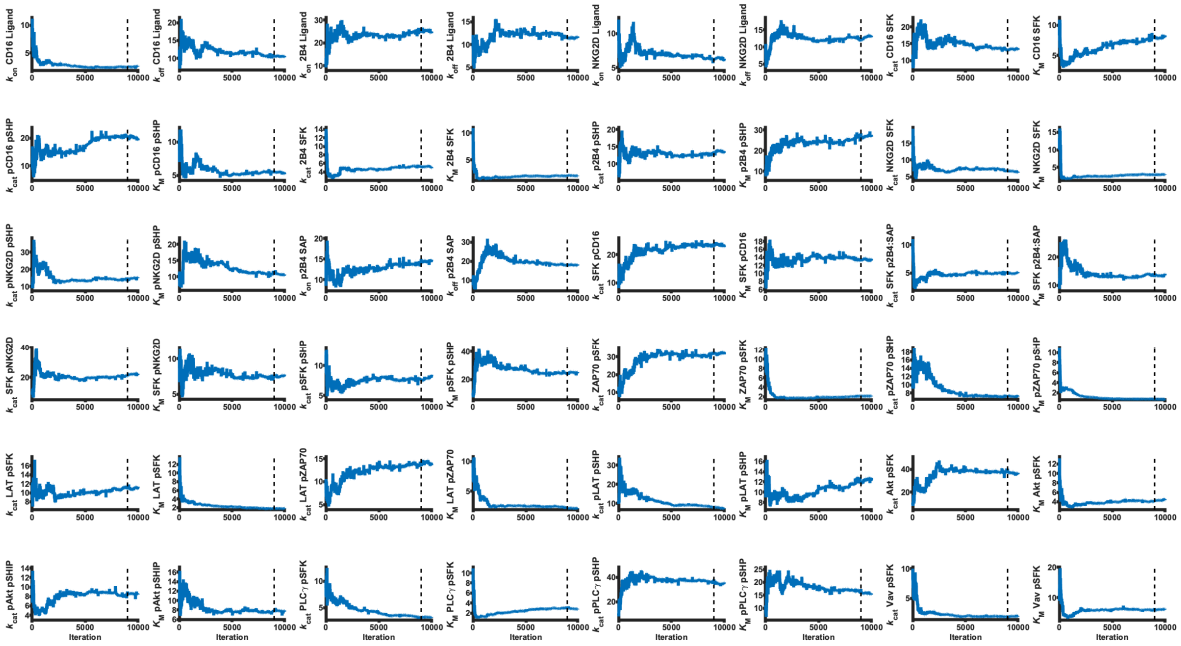

Figure S8. Trace plots for best parameter set. The value of the parameters 1 – 48 are shown as a function of the iteration of the Metropolis-Hastings algorithm. The dashed line (at the 9,000th iteration) for each subplot shows where we designated the cut-off. The left side of the dashed line is the burning-in phase of the algorithm. We discarded the parameter values that are to the left of the dashed line and kept those on the right (iteration 9,001 – 10,000) for model simulation.

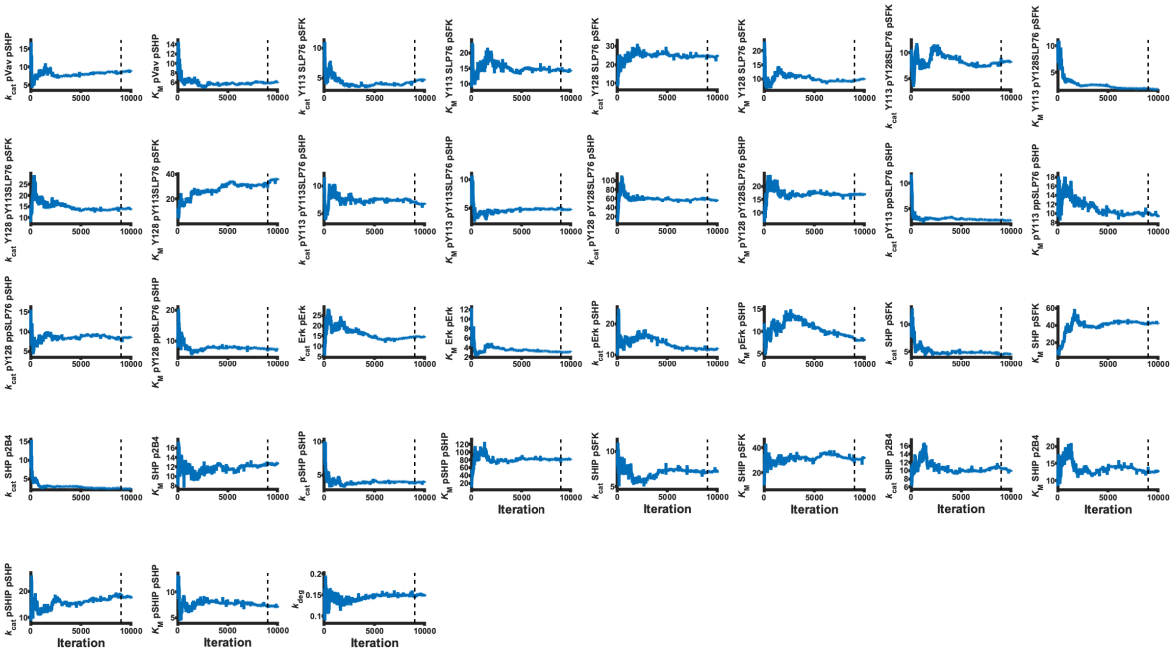

Figure S9. Trace plots for best parameter set. The value of the parameters 49 – 83 are shown as a function of the iteration of the Metropolis-Hastings algorithm. The dashed line (at the 9,000th iteration) for each subplot shows where we designated the cut-off. The left side of the dashed line is the burning-in phase of the algorithm. We discarded the parameter values that are to the left of the dashed line and kept those on the right (iteration 9,001 – 10,000) for model simulation.

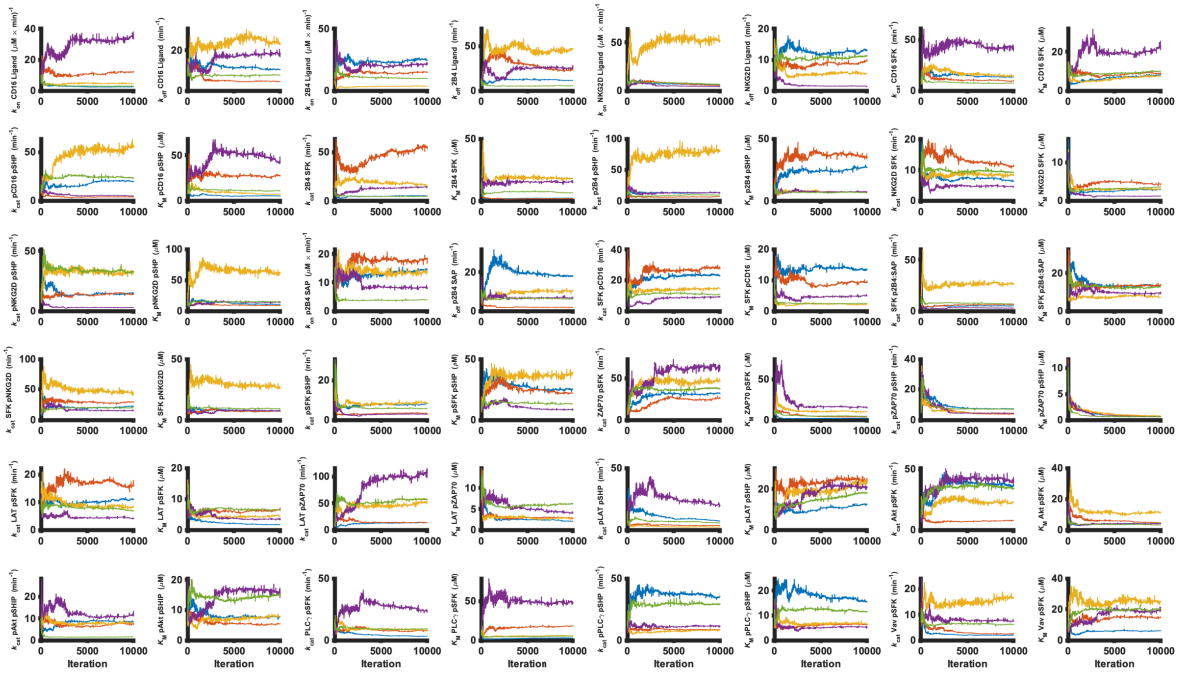

Figure S10. Trace plots for the five parameter sets in the refined medium response cluster. The value of the parameters 1 – 48 are shown as a function of the iteration of the Metropolis-Hastings algorithm. The blue trace plot corresponds to the best parameter set, which is represented in **Figures S8 – S9**. The red, orange, green and purple trace plots correspond to parameter sets 5, 6, 12 and 13, respectively.

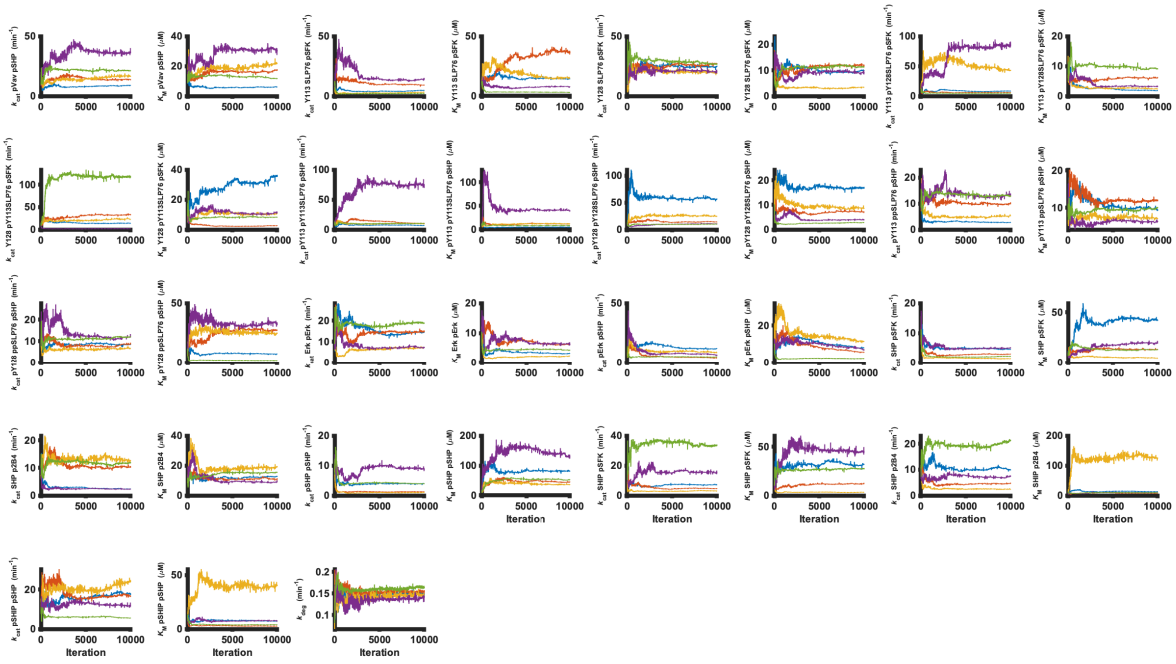

Figure S11. Trace plots for the five parameter sets in the refined medium response cluster. The value of the parameters 49 – 83 are shown as a function of the iteration of the Metropolis-Hastings algorithm. The blue trace plot corresponds to the best parameter set, which is represented in **Figures S8 – S9**. The red, orange, green and purple trace plots correspond to parameter sets 5, 6, 12 and 13, respectively.

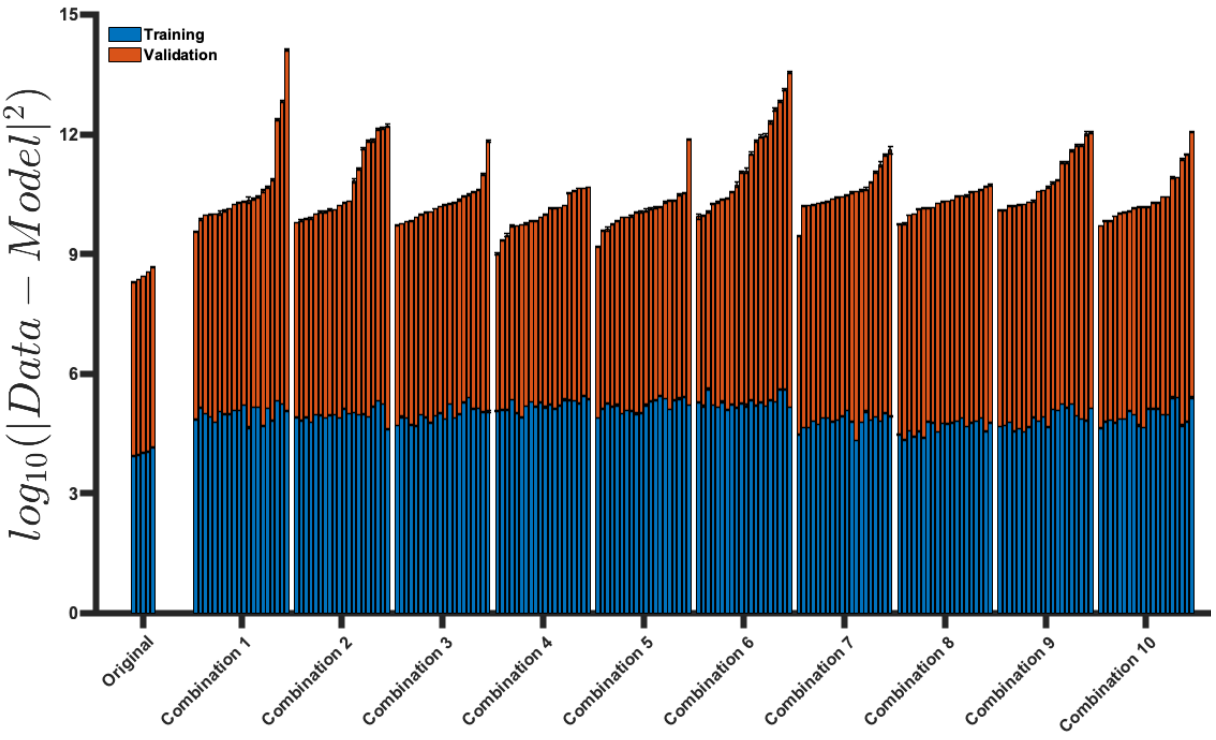

Figure S12. Total error from parameter estimation using different combinations. The Metropolis-Hastings algorithm was simulated 20 times per combination. For each run, we used the last 1,000 iterations to simulate the model and compare the model predictions to training (blue) and validation (red) data. Bars represent mean value and error bars represent one standard deviation. The total error was sorted in ascending order. The 5 sets in the original group come from the **Figure S1**, and correspond to parameter sets 3, 5, 6, 8 and 13 of the best 14 sets. Parameter set 3 was used to simulate the model, which is the left most set in the original group.

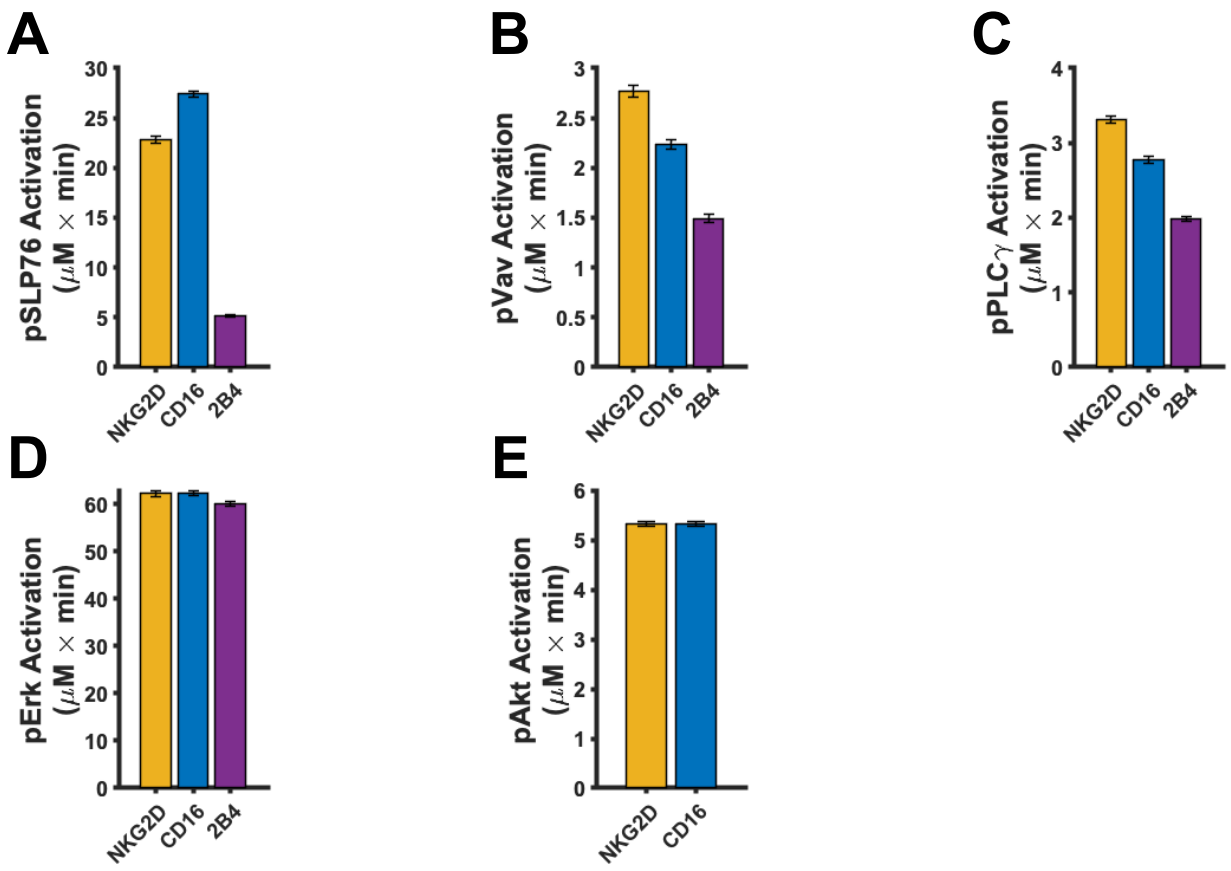

Figure S13. Magnitude of species activation. The magnitude of activation of the phospho-species (**A**) pSLP76, (**B**) pVav, (**C**) pPLCγ, (**D**) pErk and (**E**) pAkt is shown based on mono-stimulation of NKG2D (orange), CD16 (blue) and 2B4 (purple) using the 1,000 iterations from the best parameter set. The bar represents the mean value and the error bars represent one standard deviation.

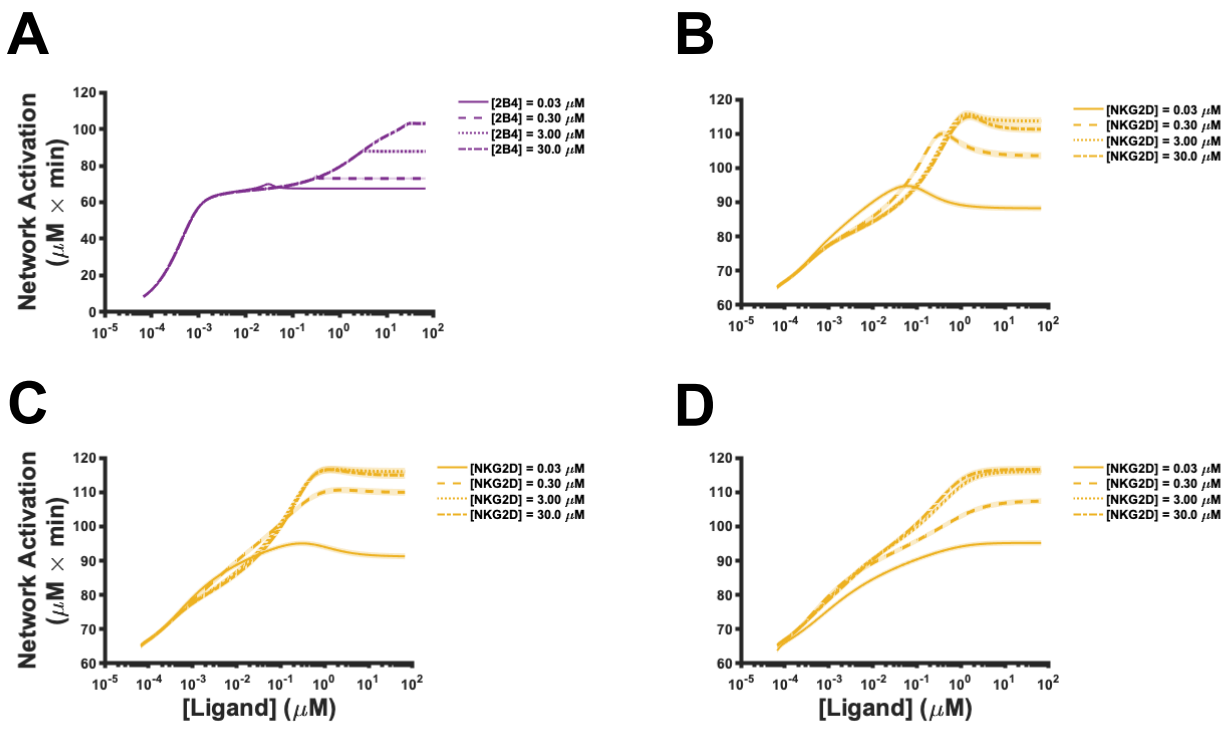

Figure S14. Effect of receptor concentrations on network activation. The (**A**) 2B4 and (**B – D**) NKG2D pathways were stimulated with ligands *in silico*. For all panels, the line represents the mean model prediction using the final 1,000 iterations from parameter estimation using the best set and the shaded area is one standard deviation. The solid, dash, dot and dash-dot lines correspond to 0.03, 0.3, 3 and 30 μM of the receptor, respectively.