

## **Supporting Information**

### **A PLC- $\gamma$ 1 Feedback Pathway Regulates Lck Substrate Phosphorylation at the T Cell**

#### **Receptor and SLP-76 Complex**

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## Supplemental Tables

**Table S1:** Complete list of unique quantified phosphopeptides. Corresponding SIC peak areas and q value statistics, protein accession numbers, gene ontologies and KEGG and GO functional annotations are included. The MSMS peptides assignments were performed with MOWSE score > 20 and a logistic spectra score filter to achieve a final estimated 1% FDR by decoy database approach. Only forward database hits are included.

**Table S2:** Complete list of all phosphopeptides detected from each replicate and timepoint of TCR stimulation. The excel file lists all the data from each LC/MS run as a separate worksheet. Included in these tables are all phosphotyrosine containing peptides with MOWSE score > 20 including decoy database reversed sequence hits. Reversed hits, if any, for each replicate and time point are labeled with protein name descriptor ###REV### and an "R" designation in database direction. Listed are the assigned names of the corresponding proteins, the position of the phosphorylation site within the protein sequences, and the assigned peptide sequence. For the peptide sequence, \* represents phosphorylation, and # represents Met oxidation. Every reported peptide includes the Logistic Spectral Validation score, Mascot ion score, and Mascot expectation value reflecting confidence in the sequence assignment as well as the Ascore, which reports the confidence in the assigned location of the phosphorylation site. Also reported is the mass error in ppm, the isolated mass of the peptide, the charge state, SIC peak area, and the scan number.

## Supplemental Figures

**Figure S1:** Validation of PLC- $\gamma$ 1 levels in Jgamma1 and Jgamma1.WT cells.

**Figure S2:** Annotated MSMS spectra for all PTM-containing peptides.

**Figure S3:** Pairwise inter-replicate comparison of phosphopeptide peak areas observed in biological replicates. Each data point represents the peak areas observed in separate replicates of phosphopeptides with the same charge state and sequence sampled at the same timepoint. A) Jgamma1 replicate comparisons. B) Jgamma1.WT replicate comparisons.

**Figure S4:** Validation of equivalent receptor subunit expression in Jgamma1 and Jgamma1.WT. A) For each cell line, three replicates of the 0 and 3 minute timepoints were examined. Antibodies to TCR $\zeta$  and CD3 $\epsilon$  were used, and band intensities were normalized to GAPDH. B) Quantitation of Western blot data. Error bars indicate the standard error of the mean. t test *p* values were thresholded at 0.05 when assessing significance.

**Figure S5:** Validation of Zap-70 phosphoproteomic data. A) For each cell line, three replicates of the 0 and 3 minute timepoints were examined by Western blot. Phospho-specific antibodies to pY319 and pY493 were used, and band intensities were normalized to a non-phospho-specific Zap-70 antibody. B) Quantitation of Western blot data. Error bars indicate the standard error of the mean. \* indicates *p* < 0.05.

**Figure S6:** Validation of equivalent Itk expression in Jgamma1 and Jgamma1.WT. A) For each cell line, three replicates of the 0 and 3 minute timepoints were examined by Western blot. Antibody to Itk was used, and band intensities were normalized to GAPDH. B) Quantitation of Western blot data. Error bars indicate the standard error of the mean. t test *p* values were thresholded at 0.05 when assessing significance.



Figure S1.

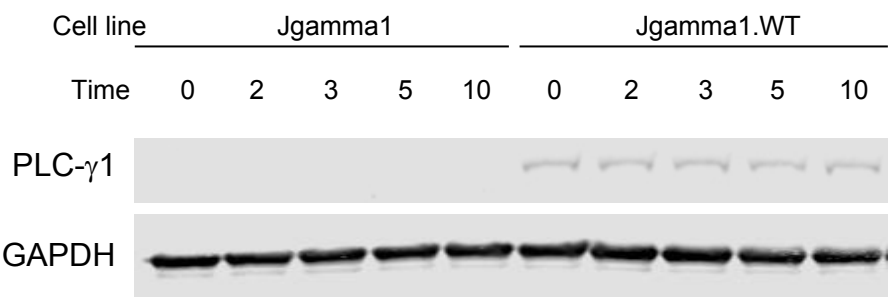
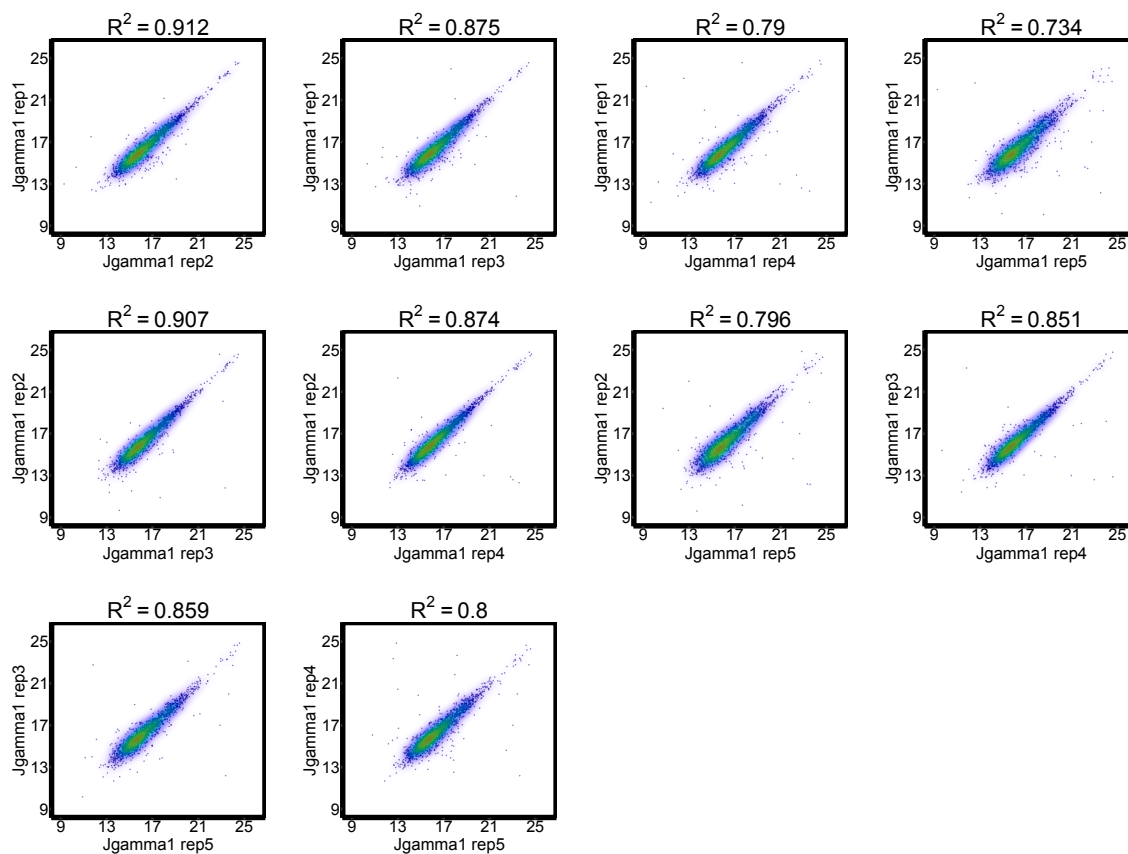


Figure S3.

A



B

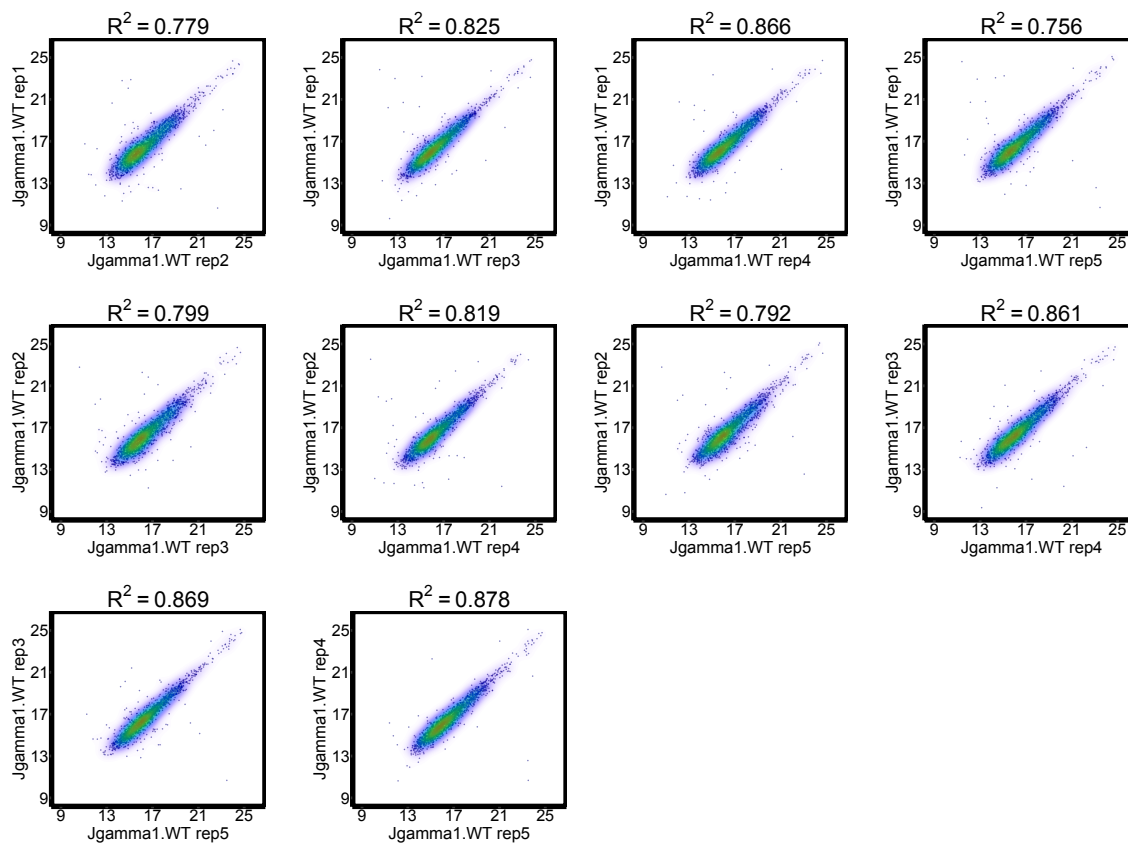
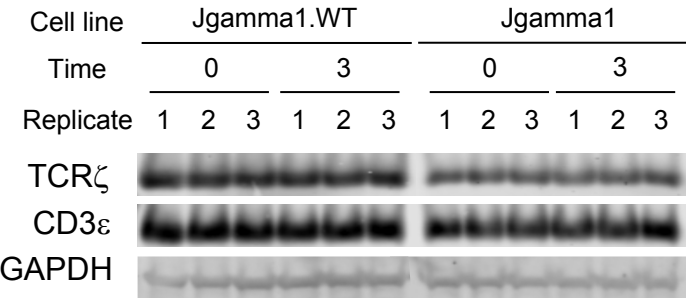


Figure S4.

A



B

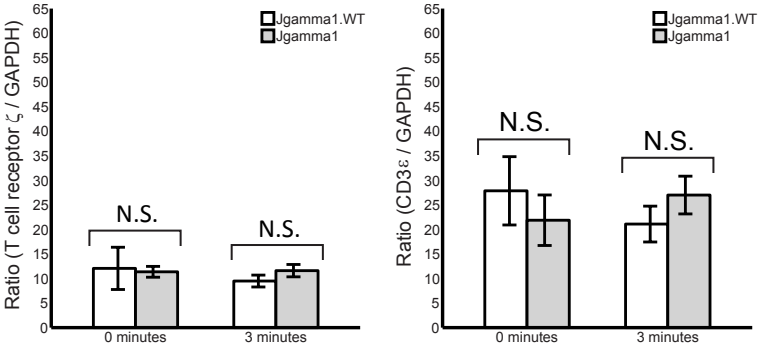
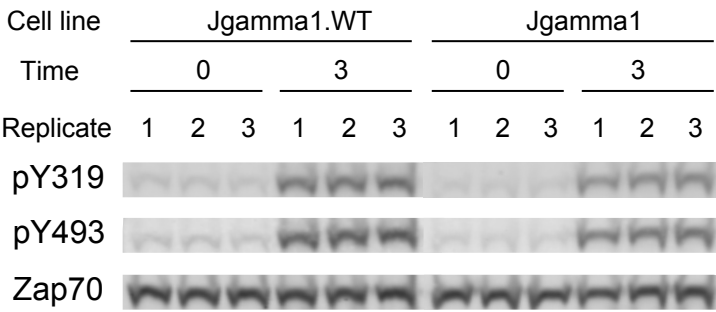


Figure S5.

A



B

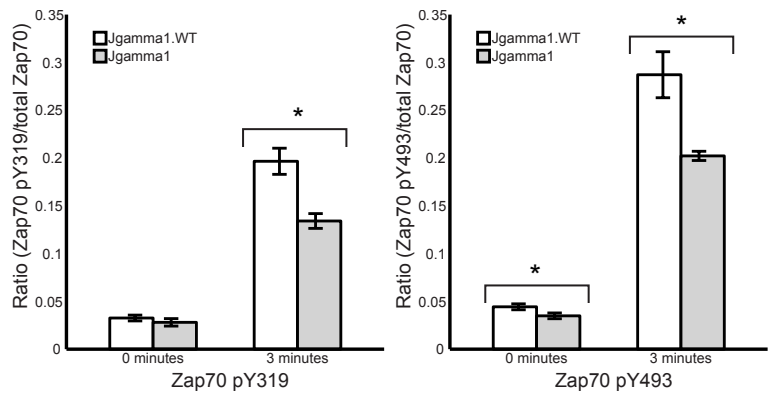
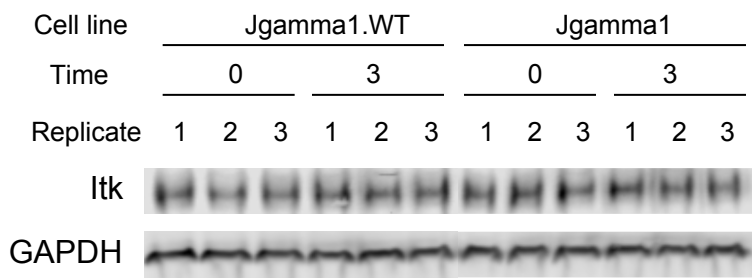


Figure S6.

A



B

