ASSOCIATED CONTENT

Supporting Information

Table S1, complete list of all phosphopeptides detected from each replicate and time point of TCR stimulation. Table S2, complete list of quantified unique phosphopeptides Figure S1, volcano plots for q value versus fold change. Figure S2, annotated MSMS spectra for all PTM containing peptides. Figure S3**,** unpaired replicate comparison of selected ion chromatogram peak areas for all identified peptides <1% FDR. Figure S4, histograms of the frequency versus average J.Vav1.WT peak areas. Figure S5**,** temporal heatmaps representing the change in phosphorylation of J.Vav1.WT cells through the time course of TCR stimulation. Figure S6, histogram of frequency versus average J.Vav1 to J.Vav1.WT peptide peak ratios. This material is available free of charge via the Internet at http://pubs.acs.org.

SUPPORTING INFORMATION

Supplemental Tables

Table S1: Complete list of quantified unique phosphopeptides. Corresponding SIC peak areas and q value statistics, protein accession numbers, gene ontologies and KEGG functional annotations are included. The MSMS peptides assignments were performed with >20 Mowse score 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 time point of TCR stimulation. The excel file lists all the data from each LC/MS run as a separate sheet. 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 a "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 and Mascot Mowse score reflecting confidence in the sequence assignment and the Ascore, which reports the confidence in the localization of the phosphorylation site. Also reported is the mass error in ppm, the isolated mass of the peptide, the charge state, and the scan number**.**

Supplemental Figures

Figure S1: Volcano plots for q value versus fold change for the 3 minute time point illustrating our selected significance threshold. Black dots are defined as significant, and have q-values below 0.05 and fold changes >1.5 or <0.67.

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

Figure S3: Unpaired replicate comparison of selected ion chromatograph peak areas for all phosphopeptides identified across all time points from A) J.Vav1 cells and B) J.Vav1.WT cells. Each dot in the plots represents the comparison from a single time point of TCR stimulation of the peak area for a single phosphopeptide measured in the 2 replicates indicated on the axes of the plot.

Figure S4: Histogram of frequency versus average J.Vav1.WT peak areas was plotted for all phosphopeptides identified at 1% FDR, as well as for the KEGG T cell receptor subset, for four time points of TCR stimulation. Peak areas were calculated from the average of five biological replicate experiments.

Figure S5: Tyrosine phosphorylation of TCR signaling proteins in J.Vav1.WT cells display expected kinetics. Temporal heatmaps representing the change in phosphorylation of J.Vav1.WT cells through the time course of TCR stimulation were generated for known sites of phosphorylation upon TCR stimulation. Heatmaps were calculated from the averages of five biological replicate experiments. In the heatmaps, black color represents peptide abundance equal to the geometric mean for that peptide across all time points. Blue color represents peptide abundance less than the mean, whereas yellow corresponds to peptide abundance more than the mean. A white dot within a heatmap square indicates a statistically significant difference (q value < 0.05) in the fold change in peptide abundance for that time point relative to the time point with the minimal average peak area for that phosphopeptide.

Figure S6: Histogram of frequency versus average J.Vav1 to J.Vav1.WT peptide peak ratios was plotted for all phosphopeptides identified at 1% FDR for four time points of TCR stimulation. Peptide peak ratios were calculated from the average of five biological replicate experiments.

Figure S1

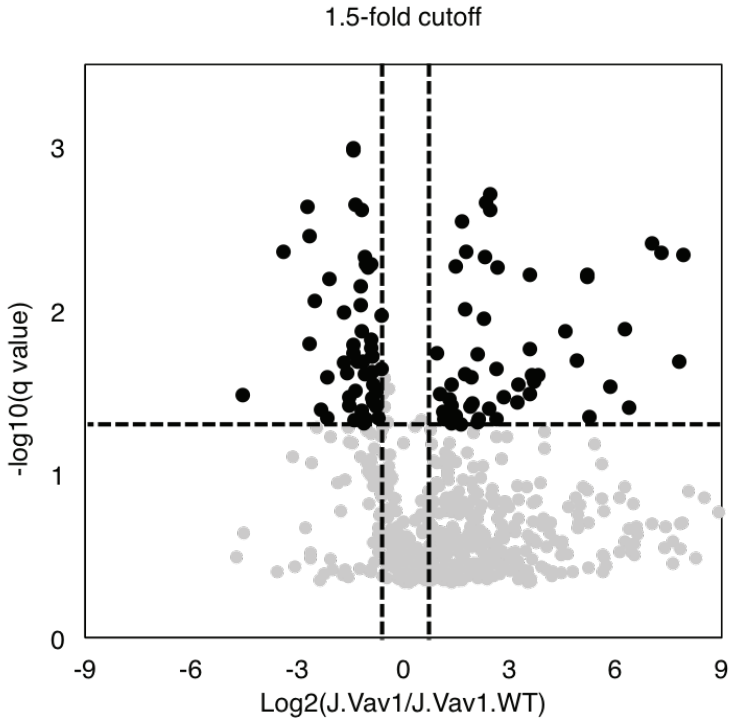

1.5-fold cutoff
-log10(q value)
3
2
1
0
-9
-6
-3
0
3
6
9
Log2(J.Vav1/J.Vav1.WT)

Figure S3

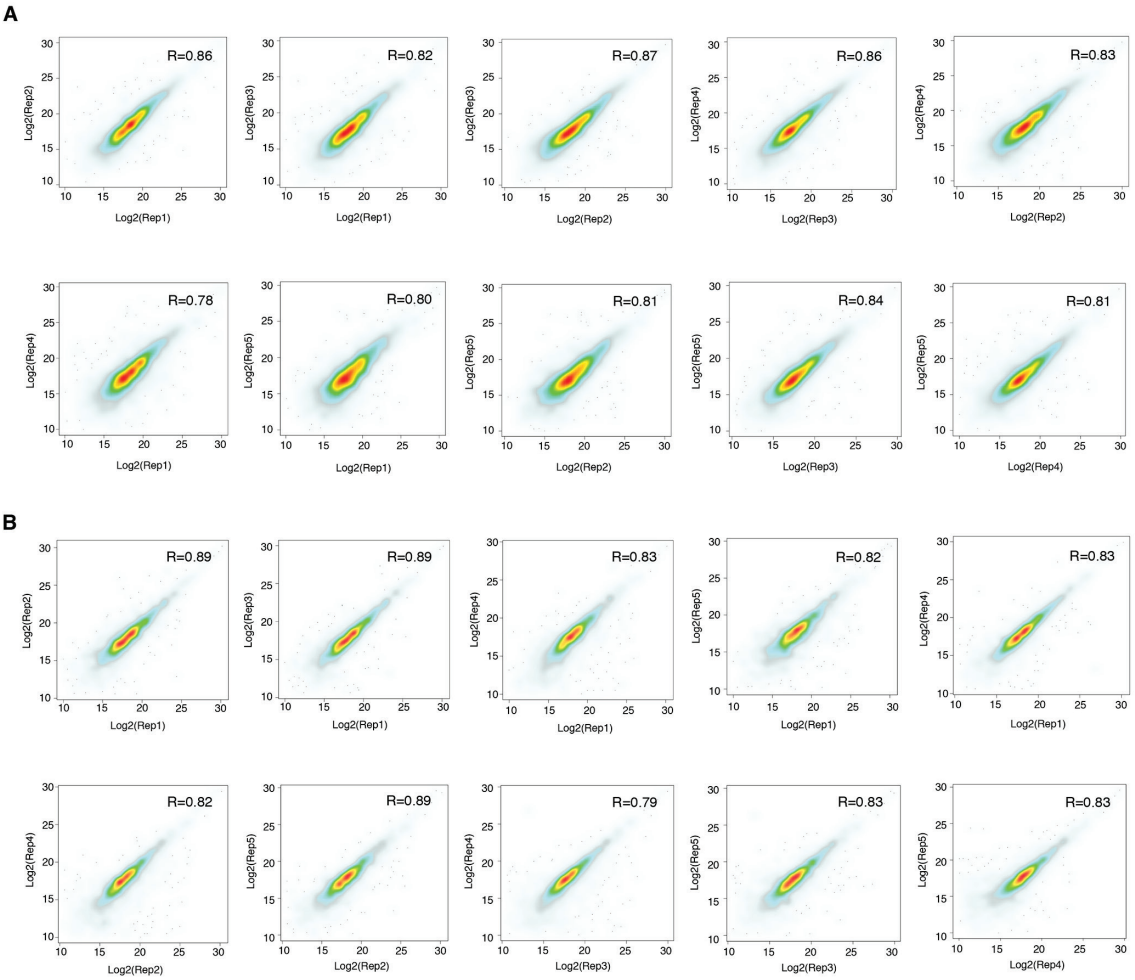

A
R=0.86
Log2(Rep2)
Log2(Rep1)
R=0.82
Log2(Rep3)
Log2(Rep1)
R=0.87
Log2(Rep3)
Log2(Rep2)
R=0.86
Log2(Rep4)
Log2(Rep3)
R=0.83
Log2(Rep4)
Log2(Rep2)
R=0.78
Log2(Rep4)
Log2(Rep1)
R=0.80
Log2(Rep5)
Log2(Rep1)
R=0.81
Log2(Rep5)
Log2(Rep2)
R=0.84
Log2(Rep5)
Log2(Rep3)
R=0.81
Log2(Rep5)
Log2(Rep4)
B
R=0.89
Log2(Rep2)
Log2(Rep1)
R=0.89
Log2(Rep3)
Log2(Rep1)
R=0.83
Log2(Rep4)
Log2(Rep1)
R=0.82
Log2(Rep5)
Log2(Rep1)
R=0.83
Log2(Rep4)
Log2(Rep1)
R=0.82
Log2(Rep4)
Log2(Rep2)
R=0.89
Log2(Rep5)
Log2(Rep2)
R=0.79
Log2(Rep4)
Log2(Rep3)
R=0.83
Log2(Rep5)
Log2(Rep3)
R=0.83
Log2(Rep5)
Log2(Rep4)

Figure S4

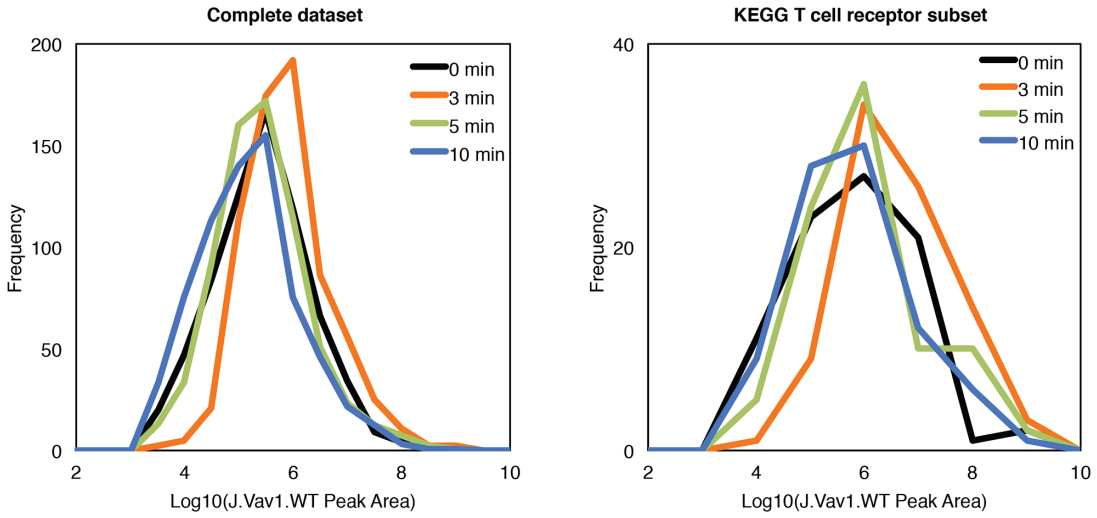
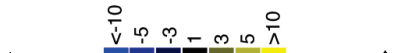


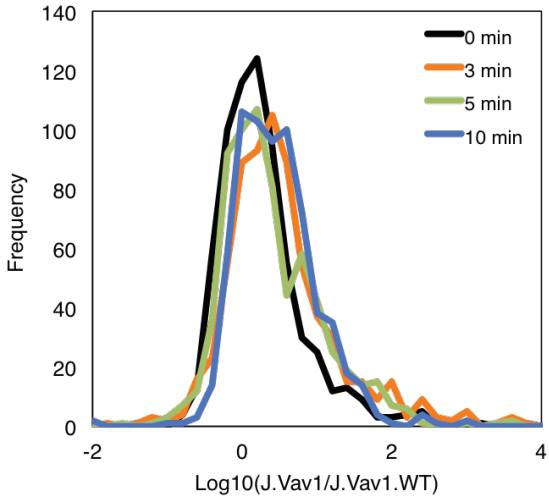
Figure S5



Temporal Heatmap Fold Change Color Key

Protein	Phosphosite	min 0 3 5 10
CD3 ε	Y188	
CD3 ε	Y199S200	
CD3 γ	Y160	
CD3 γ	Y171	
ζ chain	Y64	
ζ chain	Y72	
ζ chain	Y83	
ζ chain	Y111	
ζ chain	Y142	
ERK1	T202Y204	
ERK2	T185Y187	
Lck	Y470	
SHP-1	Y536	
ZAP-70	Y292	
ZAP-70	Y492	
ZAP-70	Y492Y493	

Figure S6


140
120
100
80
60
40
20
0
Frequency
-2
0
2
4
Log10(J.Vav1/J.Vav1.WT)
0 min
3 min
5 min
10 min