Supporting information

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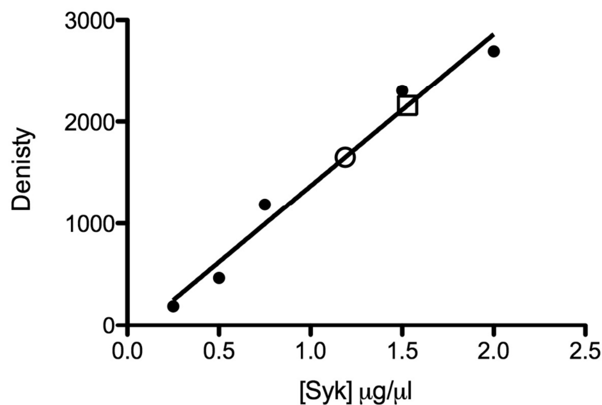
S1. To characterize the amount of Syk-EGFP obtained from each immunoprecipitation and verify the amount of kinase used in each assay, we performed a dot-blot of recombinant Syk kinase (Millipore) at a range of concentrations and blotted with the anti-Syk antibody N-19 (Santa Cruz), using IR-dye-labeled secondary antibody. The blot was analyzed using a LiCor Odyssey scanner and density of each spot was determined to generate a standard curve. 1 μl of each IP-Syk-EGFP preparation (as described in the Materials and Methods) was spotted and blotted/analyzed as for the standard curve. Density values were obtained and interpolated to quantify the amount of Syk-EGFP per μl in each preparation.



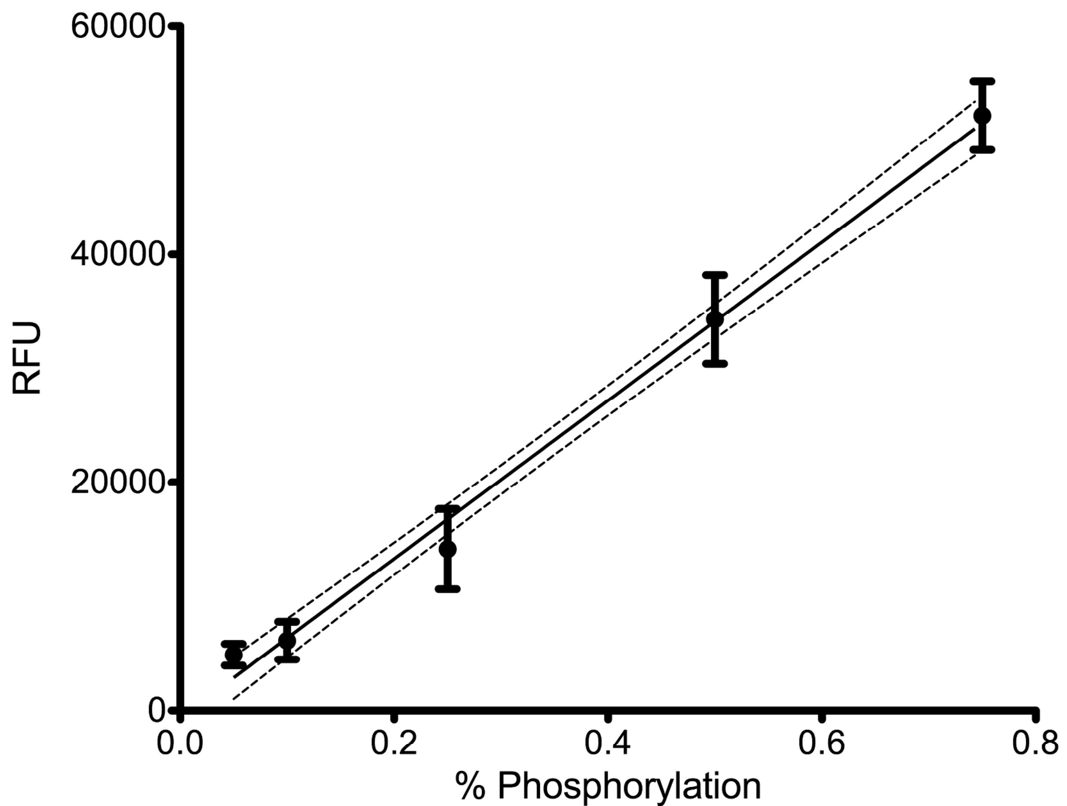
□ = 1.5 μg/μl (used in substrate comparison experiments)

○ = 1.2 μg/μl (used in kinetics experiments)

Table S1. Additional experimental details for characterization of SAStide kinetic parameters

Concentration of SAStide (μM)	Reaction Volume (μL)	Volume of reaction mixture quenched per time point (μL)	Total pmol SAStide starting material in reaction
100	35	4	3500
32	35	4	1120
16	35	4	560
8	35	4	280
4	50	5	200
1	170	20	170

S2. To calibrate the ELISA assay to interpret the amount of phosphopeptide represented by a given level of signal (RFU), we incubated wells of a Neutravidin plate (blocked as described for the ELISA assay in the Materials and Methods) with calibration standards of total peptide amounts of 20, 32, 64, 128 and 400 pmol (to mimic the assay analysis conditions for each substrate concentration) containing increasing fractions of phosphorylated SAStide (diluted into ELISA wash buffer) in the presence of SAStide and analyzed the amount of phosphotyrosine signal using the ELISA conditions described in the Materials and Methods. Amounts above 0.5 pmol phospho-SAStide produced saturated Amplex Red® signal, but between 0.025-0.5 pmol phospho-SAStide a linear increase in RFU was observed. Error bars represent SD, dashed line represents 95% confidence interval. This standard curve was used to calculate the amount of product produced in subsequent *in vitro* experiments.



S3. Cell lysates from triplicate experiments for the Syk-EGFP reconstituted cell-based assay were diluted 1:10 and 1 μl was spotted on nitrocellulose membrane. Membranes were blocked as described in the Materials and Methods and incubated with streptavidin-DyLight-680 (1:10,000) and incubated for 2h. Blot imaging and densitometry were performed as described above in S1. Shown are a representative blot and the graphical results from analysis of triplicate experiments (error bars indicating SEM).