



Figure S1. Pyk2 FERM-kinase site-specific autophosphorylation. Autophosphorylation was detected by Western blotting using site-specific anti-phosphotyrosine antibodies and quantified by densitometry.

Data Set	FERM	kinase	FERM-kinase
HDX reaction details	150 mM KCl, 50 mM HEPES, 2 mM DTT, pH 7.40, 90% D ₂ O, 24 °C	150 mM KCl, 50 mM HEPES, 2 mM DTT, pH 7.40, 90% D ₂ O, 24 °C	150 mM KCl, 50 mM HEPES, 2 mM DTT, pH 7.40, 90% D ₂ O, 24 °C
HDX time course	0.167, 0.75, 3, 10, 30, 60, 180	0.167, 0.75, 3, 10, 30, 60, 180	0.167, 0.75, 3, 10, 30, 60, 180, O?N
HDX control samples	Maximum-labeling estimated by 18 hour on-exchange time point (FERM-kinase), n=2		
Back-exchange (mean / IQR)	36% / 12%		
# of Peptides	77	52	129
Sequence coverage	92%	81%	88%
Average peptide length / Redundancy	10 (2.20)	11 (2.14)	10 (1.99)
Replicates (biological or technical)	3 (technical)	3 (technical)	3 (technical)
Repeatability	0.083 (average standard deviation)	0.117 (average standard deviation)	0.096 (average standard deviation)
Significance testing	two-tailed, unpaired t test, p<0.005 at time point(s) approximating the middle range of exchange		

Table S1. HDX-MS Data Summary Table.