Quantitation of the external peptide standard, LIEDAEpYTAK, for the 9 time points used to normalize mast cell-derived phosphopeptide peak areas.[a] *The standard accompanied the true cellular phosphopeptides through peptide IP, desalt, IMAC, and reversed-phase elution and detection in the mass spectrometer.*

	Peak Area
0 s	2.2×10^7
10 s	3.2×10^7
30 s	2.3×10^7
1 min	2.1×10^7
1.5 min	2.6×10^7
2 min	3.7×10^7
3 min	2.7×10^7
5 min	1.4×10^7
10 min	1.3×10^7

[a] A 10 pmol portion of LIEDAEpYTAK was added to all time points prior to peptide immunoprecipitation, and automated IMAC enrichment.

Consistency of phosphopeptide retention times amongst time points. *Standard deviation (Stdev) of retention times of each phosphopeptide plotted against its average half maximal peak width amongst the nine time points. Each dot represents a unique, manually validated phosphopeptide. The magnitude of the variation of retention time amongst the time points for each detected phosphopeptide was well within the width of the peaks on average, indicating coelution of phosphopeptides in the separate time point analyses (average standard deviation of 6.3 s for peptide retention time amongst the nine time points, average maximal variation in retention time of 19 s amongst the nine time points, average half-maximal SIC peak width of 22 s).*