

Supplemental Figure Legends

Figure S1. Parallel experimental pipelines used to increase phosphoproteome coverage. A represents the standard pipeline, in which trypsin, SCX, and CID fragmentation are used, and B represents the complementary pipeline, in which endopeptidase-LysC, one chromatographic platform, and ETD fragmentation are used.

Figure S2. Full data for motif enrichment search. A window of 11, centered on S, with an occurrence threshold of 6 was used against the Arabidopsis proteome. A cutoff significance threshold of 0.005 was used.

Figure S3. A comparison of untargeted and targeted MS analyses. Whereas untargeted experiments rely upon data-dependent peak picking for fragmentation and analysis, targeted experiments are designed with sequence knowledge to begin with. Untargeted experiments in this report used metabolic labeling to quantify phosphopeptide changes, whereas targeted experiments used a calculated ratio of areas under elution chromatograms for endogenous fragment ions/spiked internal standard fragment ions to quantify phosphopeptide changes.

Figure S4. Comparison of fold changes induced by ABA in wildtype using untargeted mass spec/metabolic labeling and targeted mass spec/spiked internal standards. Shown are all the peptides for which data was obtained in both experiments. Statistical significance ($p \leq 0.05$) is indicated (*).

Figure S5. Variability was tested to determine what magnitude fold changes can be accurately quantified using SRM. A, Design of experiment. B, Box plot demonstrating resulting coefficient of variation spread.

Figure S1.

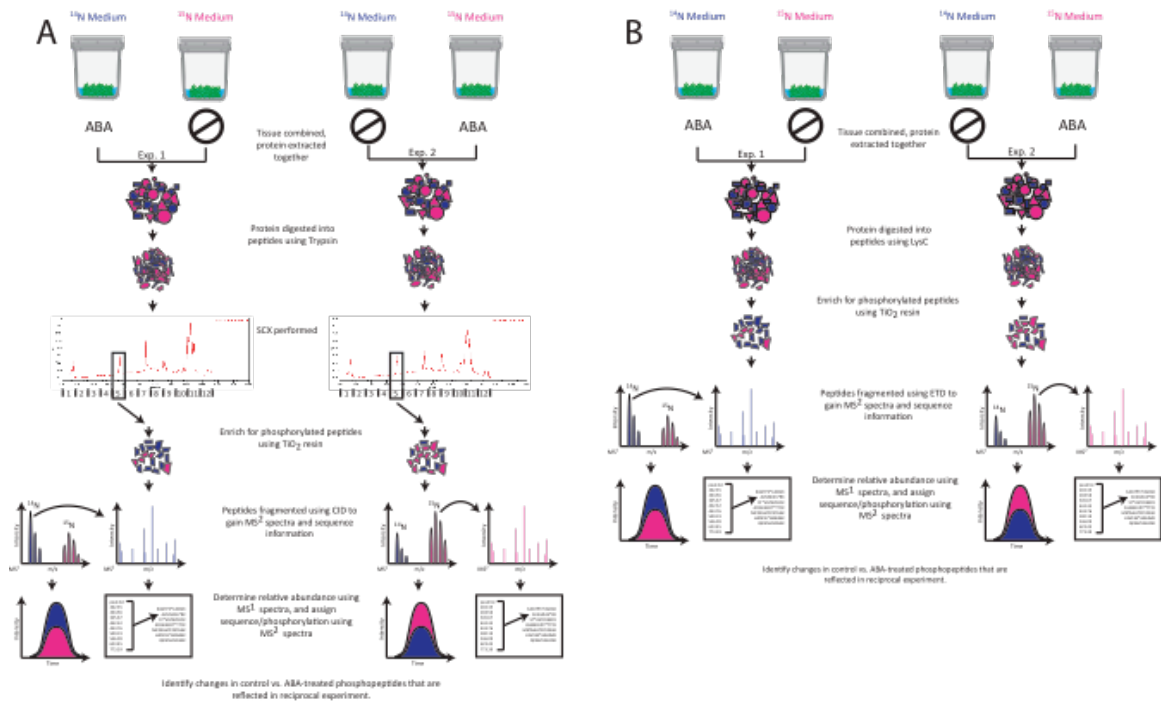


Figure S2.

#	Motif	Motif Score	Foreground Matches	Foreground Size	Background Matches	Background Size	Fold Increase
1.S..R..	2.95	15	112	54448	1002460	2.47
2.	L.R..S.....	5.49	6	97	5592	948012	10.49
3.S..S..	2.69	21	91	112134	942420	1.94
4.SP....	3.04	11	70	42940	830286	3.04
5.SR....	2.38	9	59	42458	787346	2.83

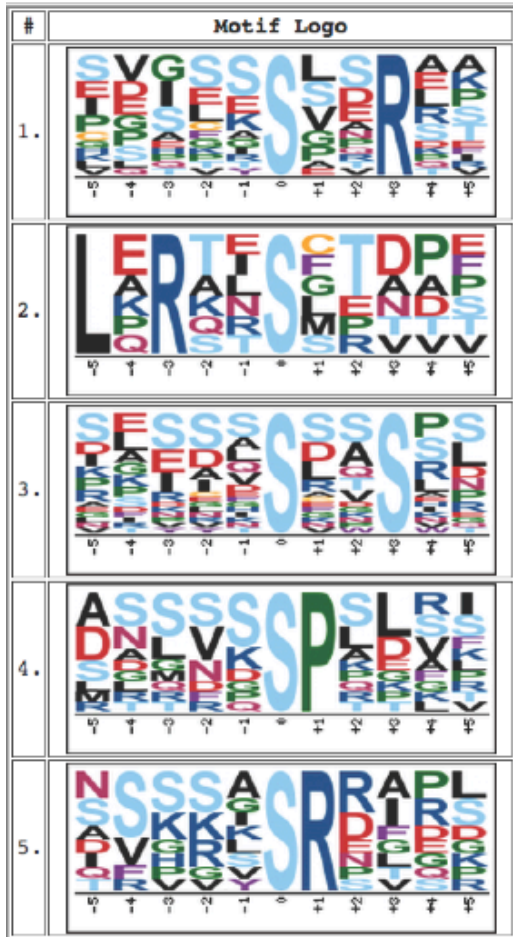


Figure S3.

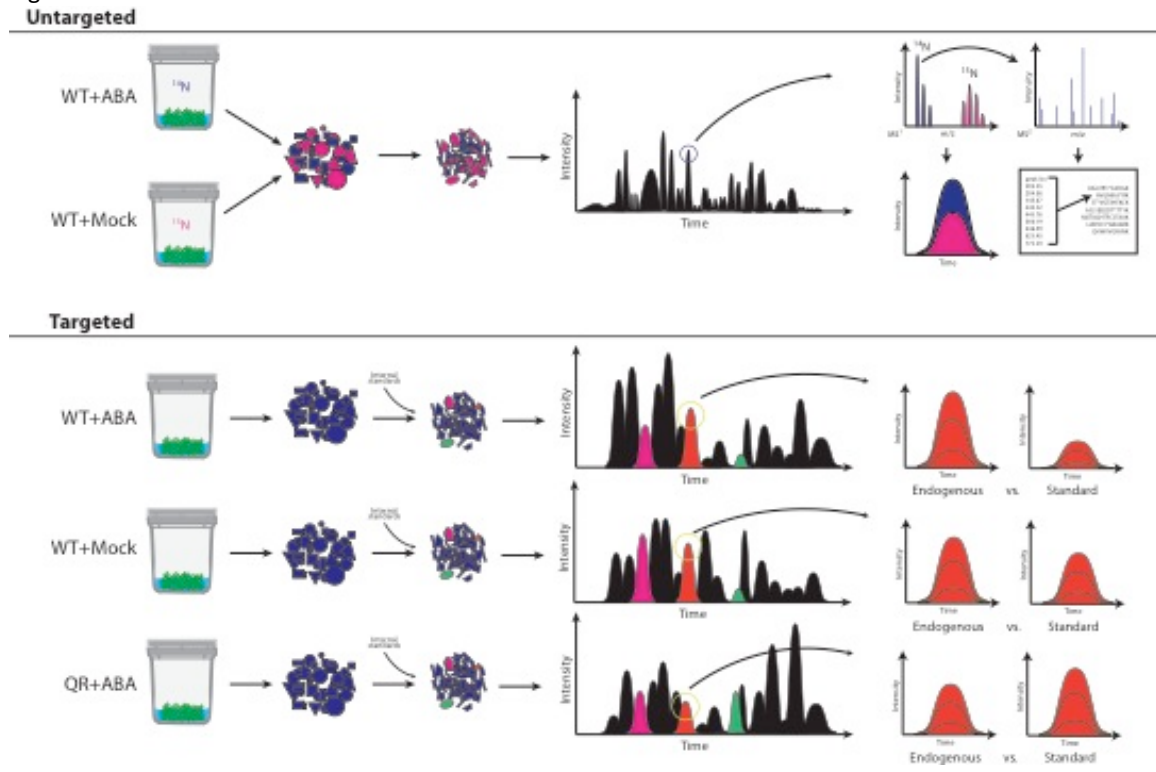


Figure S4.

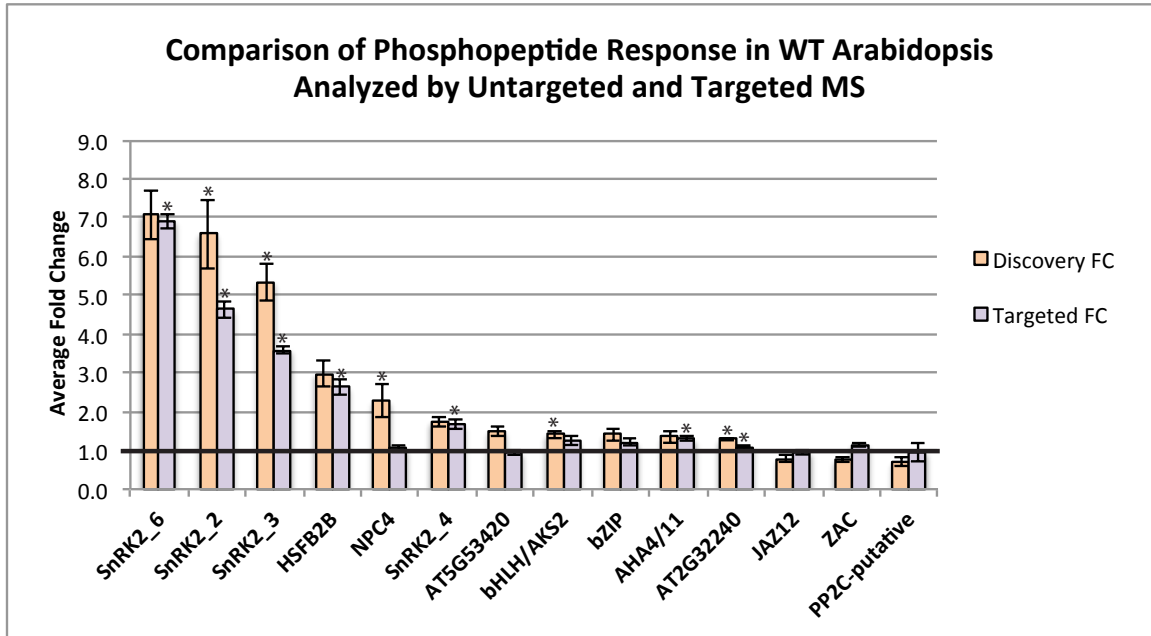
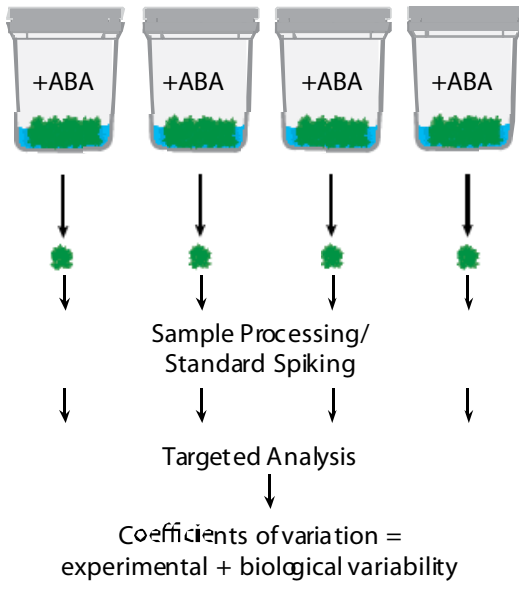


Figure S5.

A



B

