Supporting Information

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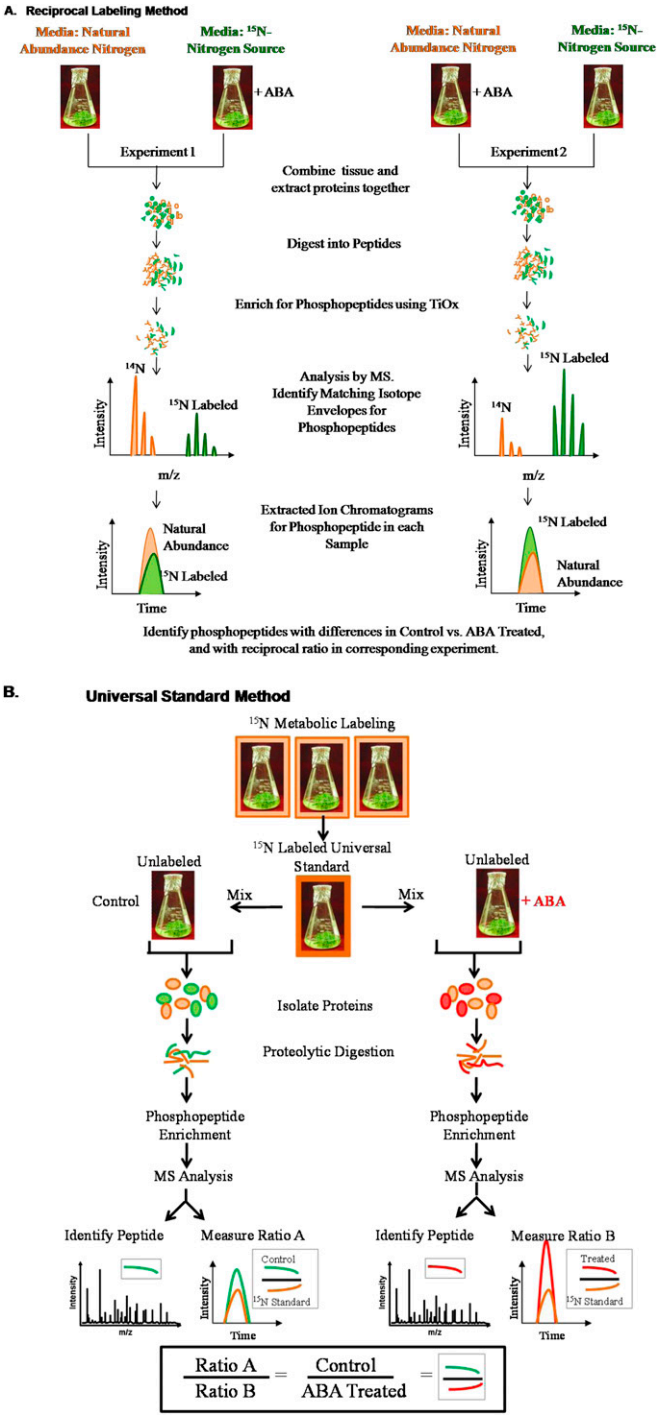

Fig. S1. Overview of the two *Arabadopsis* metabolic-labeling methods used. (*A*) Reciprocal-labeling method. In experiment one, the control plant is grown in natural-abundance medium, and the plant grown in ^{15}N isotope-enriched medium is treated with abscisic acid (ABA). In experiment two, the treatment and isotopic label are exchanged to create the reciprocal experimental set. Tissue samples are combined, and, after protein extraction, digestion, phosphopeptide enrichment, and MS analysis, the relative phosphopeptide abundances can be determined from the ratio of the extracted ion chromatograms of the natural-abundance peptide and ^{15}N-labeled peptide. Phosphorylation changes resulting from experimental treatment show reciprocal ratios in experiment one and experiment two, whereas changes caused by isotope effects show constant ratios in the two experimental groups. (*B*) Universal standard method. Experimental plants are grown in natural-abundance medium, and universal standard plants are grown in ^{15}N isotope-enriched medium. All unlabeled experimental tissue samples are combined at a 1:1 (weight:weight) ratio with the pooled ^{15}N-labeled universal standard tissue. After protein extraction, digestion, phosphopeptide enrichment, and MS analysis, the relative phosphopeptide abundances can be determined by the fractional change between the control and ABA-treated samples, as calculated by the ratio of two ratios. With this method data are acquired for one biological replicate per time point.

Table S1. Significantly altered phosphopeptides in response to ABA treatment (50 μM for 5, 15, or 30 min)

ATG accession	Protein	Phosphopeptide	Average phosphorylation change with ABA		
			5 min	15 min	30 min
Other targets					
AT1G43910	AAA-type ATPase family protein	K.GED**S***SVEEEGEIEDAETK.E	N/a	N/a	Decrease (284%)
AT5G60980	Nuclear transport factor 2 (NTF2)	R.VVVSHEPEVEPEPVASIEEEDLDNVAEVYDP**S***DK.D	N/a	N/a	Decrease (57%)
AT5G22010	Replication factor C 1 (ATRFC1)	R.DEDGEPLADNEEGNG**S***DAEEDSEEATDGEK.L	N/a	N/a	Decrease (68%)
AT1G14170	KM domain-containing protein	K.VHDMVVADADQDDG**T***DDDNDLGEK.Q	Decrease (37%)	Decrease (24%)	Decrease (72%)
AT3G60240	Eukaryotic translation initiation factor 4G (EIF4G)	R.RPAGGLNGFG**S***HRPASPVTHGR.S	N/a	N/a	Decrease (75%)
AT4G28080	Binding protein	K.LVKPEATVHEDDD**S***DEGWQEAVPK.N	Decrease (75%)	No change	Decrease (78%)
AT2G38280	Embryonic factor1 (FAC1)	R.SH**S***VSGDLHGVQPDPIAADILR.K	N/a	N/a	Decrease (99%)
AT2G26110	Unknown protein	K.**S***INFSSFTSPDK.S	N/a	N/a	Decrease (36%) EDT
AT4G27500	Proton pump interactor 1 (PPI1)	K.KTGGNTETETEEVPEA**S***EEEIEAPVQEEKPQK.E	Decrease (59%)	No change	N/a
AT1G06190	ATP-binding ATPase	K.KAELVELLGSD**S***S.-	Decrease (96%)	No change	No change
AT1G06190	ATP-binding ATPase	K.AELVELLGSD**S***S.-	Decrease (108%)	No change	N/a
AT5G46750	ARF-GAP domain 9 (AGD9)	K.ESSLKQEAAVVS**S***PK.A	N/a	N/a	Increase (28%)
AT3G18390	Embryo defective-1865 (EMB1865)	R.NLGLG**S***DDEDDVEDDEGGGINGGDVKPVTGEER.L	Decrease (117%)	No change	Increase (28%)
AT3G06550	Unknown: CAS1P-like	K.ALLIEDGGGLQSA**S***PR.A	Decrease (26%)	No change	Increase (29%)
AT3G22850	Unknown protein	R.VD*S*SGEVCGVTFK.V	Increase (8%)	Increase (18%)	Increase (52%)
AT1G59610	Arabidopsis dynamin-like 3 (ADL3)	R.AAAASSWSDNSGTES**S***PR.T	Increase (129%)	Increase (32%)	Increase (80%)
AT5G20650	Copper ion transmembrane transporter(COPT5)	R.SS**S***GVSAPLIPK.S	Increase (38%)	No change	Increase (81%)
AT2G32240	Unknown protein	R.DIDLSFS**S***PTK.R	Increase (60%)	Increase (77%)	Increase (98%)
AT5G42950	GYF domain-containing protein	K.DIQGSDNAIPL**S***PQWLLSKPGENK.T	Increase (83%)	No change	N/a
AT1G03060	WD-40 repeat family protein	K.HINDADD*S*G*S*QG*S*LPHDQDQSTK.T	N/a	Increase (104%)	N/a
AT1G73980	Phosphoribulokinase	K.LSLDDDTVS**S***PK.E	Increase (49%)	Increase (74%)	N/a
AT5G35980	Protein kinase family protein	K.TVYS**Y***IQSR.Y	Increase (481%)	N/a	N/a
AT1G59870	Penetration-3 ATPase (PEN3)	R.NIEDIFSSG**S***R.R	Increase (51%)	N/a	N/a
AT2G14835	Zinc finger family protein	R.QN**S***ETLYYADDEDGNR.K	Increase 36%)	N/a	N/a
AT4G13510	Ammonium transporter 1–1 (AMT1-1)	R.**S***PSPSGANTTPTPV.-	Increase (80%)	N/a	N/a
AT4G37040	Methionine aminopeptidase 1D (MAP1D)	R.TF**S***GLTDLLFNR.R	N/a	Increase (155%)	N/a
AT4G24520	Arabidopsis P450 reductase 1 (ATR1)	K.**S***MESNVANGNTTIDIHHPCR.V	Increase (40%)	Increase (53%)	No change
AT2G46820	Photosystem I P subunit (PSI-P)	R.A*TT*EVGEAPATTTEAETTELPEIVK.T	Increase (48%)	Increase (34%)	No change

Phosphorylated amino acids are in bold followed by (*) as determined from manual inspection and validation of MS^2 spectra. When assignment could not be determined conclusively from the MS^2 spectra, all possible sites of phosphorylation are denoted by italics. Phosphorylation changes are presented as the average percent change of the treated sample relative to control with three technical replicates each, with significance ($P < 0.05$) as determined by two-tailed t test. Unless otherwise denoted, identifications were made by collision-induced dissociation fragmentation. ETD, electron transfer dissociation; N/a, not applicable.

Table S2. Proteins whose level of phosphorylation was significantly altered in response to calyculin-A treatment (100 nM, 30 min)

ATG accession	Protein	Phosphopeptide	Average phosphorylation change with calyculin (30 min)
AT1G59870	Penetration 3 (PEN3)	R.NIEDIFSSG**S***R.R	Increase (1,270%)
AT4G30160	*Arabidopsis thaliana* villin 4 (VLN4)	R.NL**S***TPPPVVR.K	Increase (386%)
AT4G39260	GR-RBP8	R.EGGGYGGGDGG**S***YGGGGGGW.-	Increase (348%)
AT4G35230	BR-signaling kinase 1 (BSK1)	K.**S***YSTNLAYTPPEYLR.N	Increase (194%)
AT1G22300	General regulatory factor 10 (GRF10)	R.DNLTLWTSDLNEEGDER**T***KGADEPQDEN.-	Increase (158%)
AT3G57530	Calcium-dependent protein kinase 32 (CPK32)	R.EGFQIMDT**S***QR.G	Increase (126%)
AT5G08080	Syntaxin of plants 132 (SYP132)	R.GQ**S***SREGDVELGEQQGGDQGLEDFFK.K	Increase (120%)
AT2G38940	*Arabidopsis thaliana* phosphate transporter 2 (ATPT2)	K.SLEEM#SGENEDNEN**S***NNDSR.T	Increase (59%)
AT2G32240	Unknown protein	R.DIDLSFS**S***PTK.R	Increase (50%)
AT3G05000	Transport protein particle (TRAPP)	R.VSIDPSSENETQDPS**T***PGESK.A	Increase (48%)
AT5G42950	GYF domain-containing protein	K.DIQGSDNAIPL**S***PQWLLSKPGENK.T	Increase (43%)
AT5G61210	Soluble n-ethylmaleimide-sensitive factor adaptor protein 33 (SNAP33)	R.T**T***SEPSLADMTNPFGGER.V	Increase (42%)
AT1G59610	Arabidopsis dynamin-like 3 (ADL3)	R.AAAASSWSDNSGTES**S***PR.T	Increase (39%)
AT3G53180	Catalytic/glutamate-ammonia ligase	K.LNIDTDSS**S***PQNIISPK.L	Increase (28%)
AT2G38280	Embryonic factor1 (FAC1)	R.SH**S***VSGDLHGVQPDPIAADILR.K	Increase (26%)
AT5G57330	Aldose 1-epimerase family protein	K.GRLELSAVPSSYS**S***GQLDPK.K	Increase (23%)
AT3G53180	Catalytic/glutamate-ammonia ligase	K.LNIDTDSSSPQNII**S***PK.L	Increase (20%)
AT2G45200	Golgi snare 12 (GOS12)	R.FTQGGYVD**T***GSPTVGSGR.S	Increase (14%)
AT3G02880	Leucine-rich repeat transmembrane protein kinase	R.LIEEVSHSSG**S***PNPVSD.-	Increase (6%)

Phosphorylated amino acids are in bold followed by (*) as determined from manual inspection and validation of MS^2 spectra. When assignment could not be conclusively determined from the MS^2 spectra, all possible sites of phosphorylation are denoted by italics. Oxidation of methionine is indicated by (#). Phosphorylation changes are presented as the average percent change of the treated sample relative to control in the two reciprocal experiments with three technical replicates each.