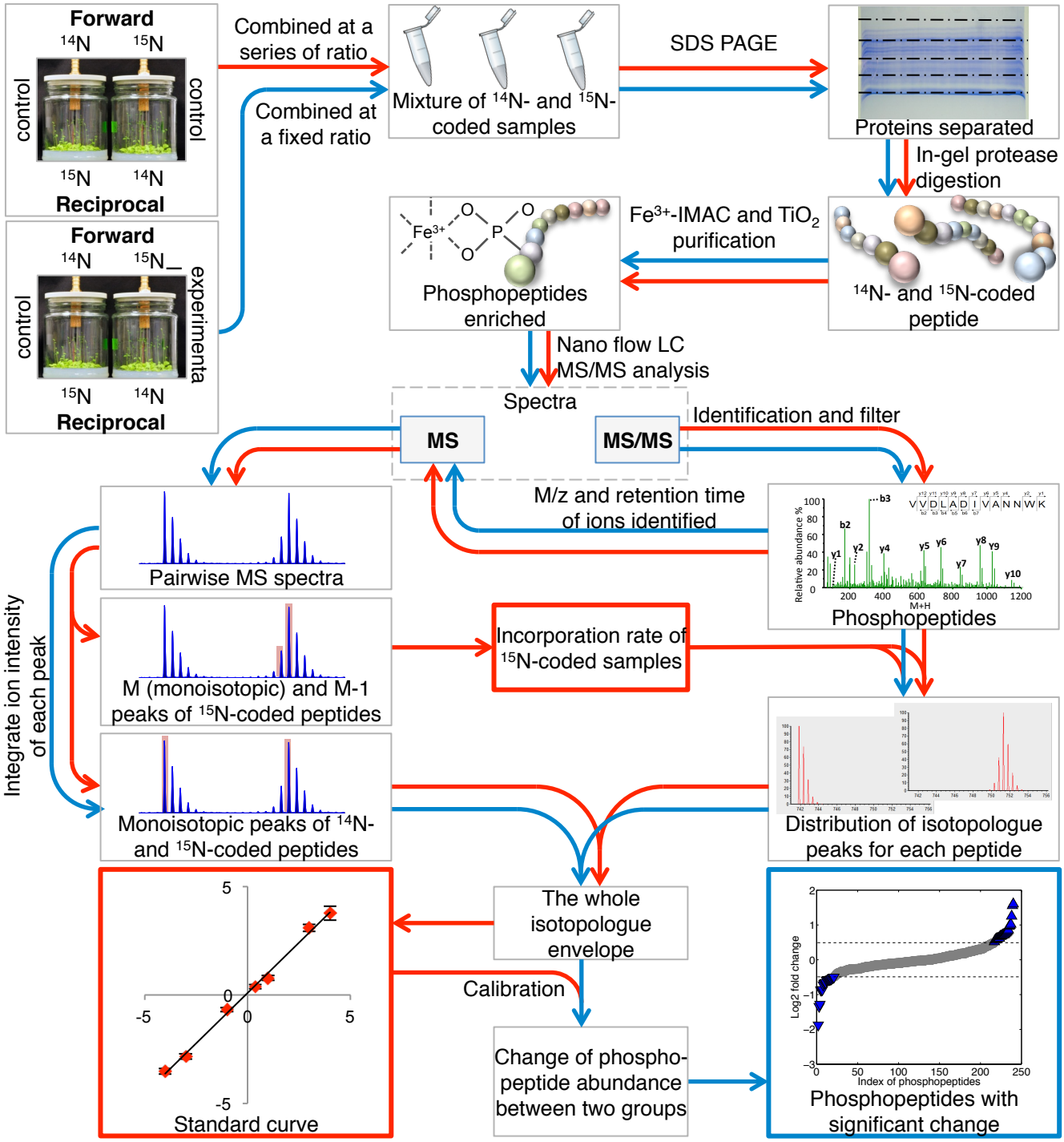
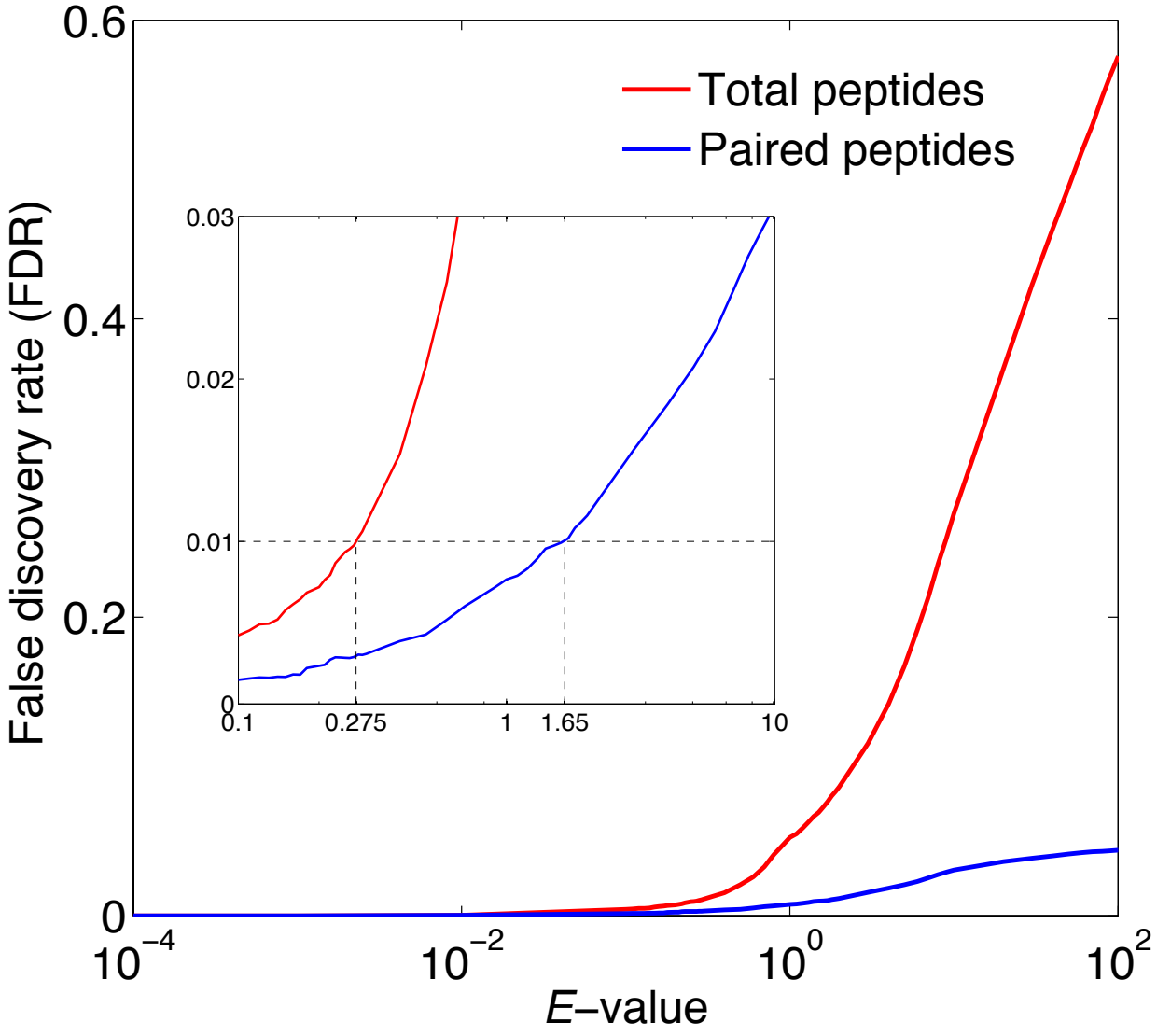


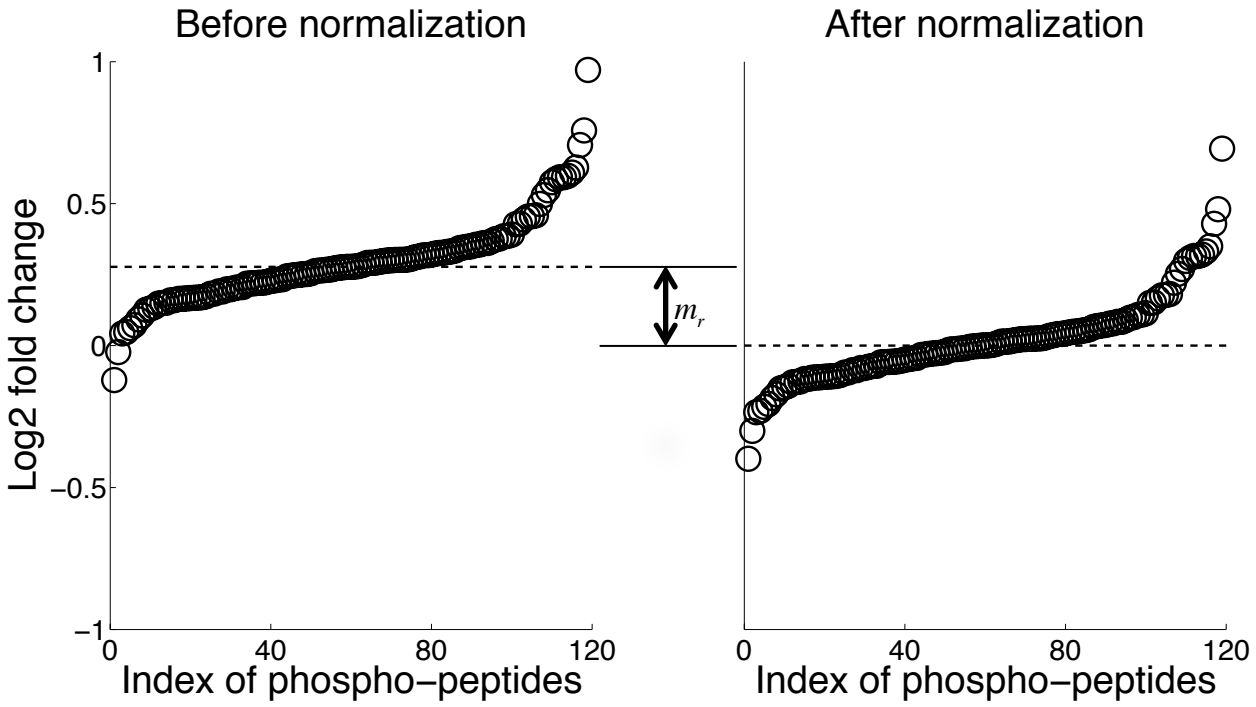
Supplementary Figure S1. Overview of *SILIA*-based phosphoproteomics.

The red and blue arrows show the workflow of two steps of our work. First, two sets of same seeds grown under identical conditions but labeled by distinct isotope of nitrogen is used to estimate incorporation rate of heavy nitrogen and construct standard curve (the red boxes), which will be used in the following calculation, following the red arrows. And the blue arrows show the SILIA approach to study the differentials between experimental set and control set quantitatively and the significantly changed phosphopeptides are determined (the blue box). A pair of both Forward and Reciprocal heavy nitrogen isotope labeling is always performed. For example, in the Forward experiment of a short-term ethylene-treated *rcn1-1* , the ethylene- and air-treated *rcn1-1* was labeled with ^{15}N and ^{14}N stable isotope-coded salt, respectively. Conversely, in the case of Reciprocal experiment, the two groups of plants were subjected to the nitrogen stable isotope labeling in a reverse order *i.e.* ^{14}N for ethylene-treated and ^{15}N for air-treated *rcn1-1*. This pair of labeling protocol is to eliminate the stable isotope labeling-resulting difference in ion intensity between $^{14}N/^{15}N$-coded phosphopeptides (Kline *et al.*, 2010).



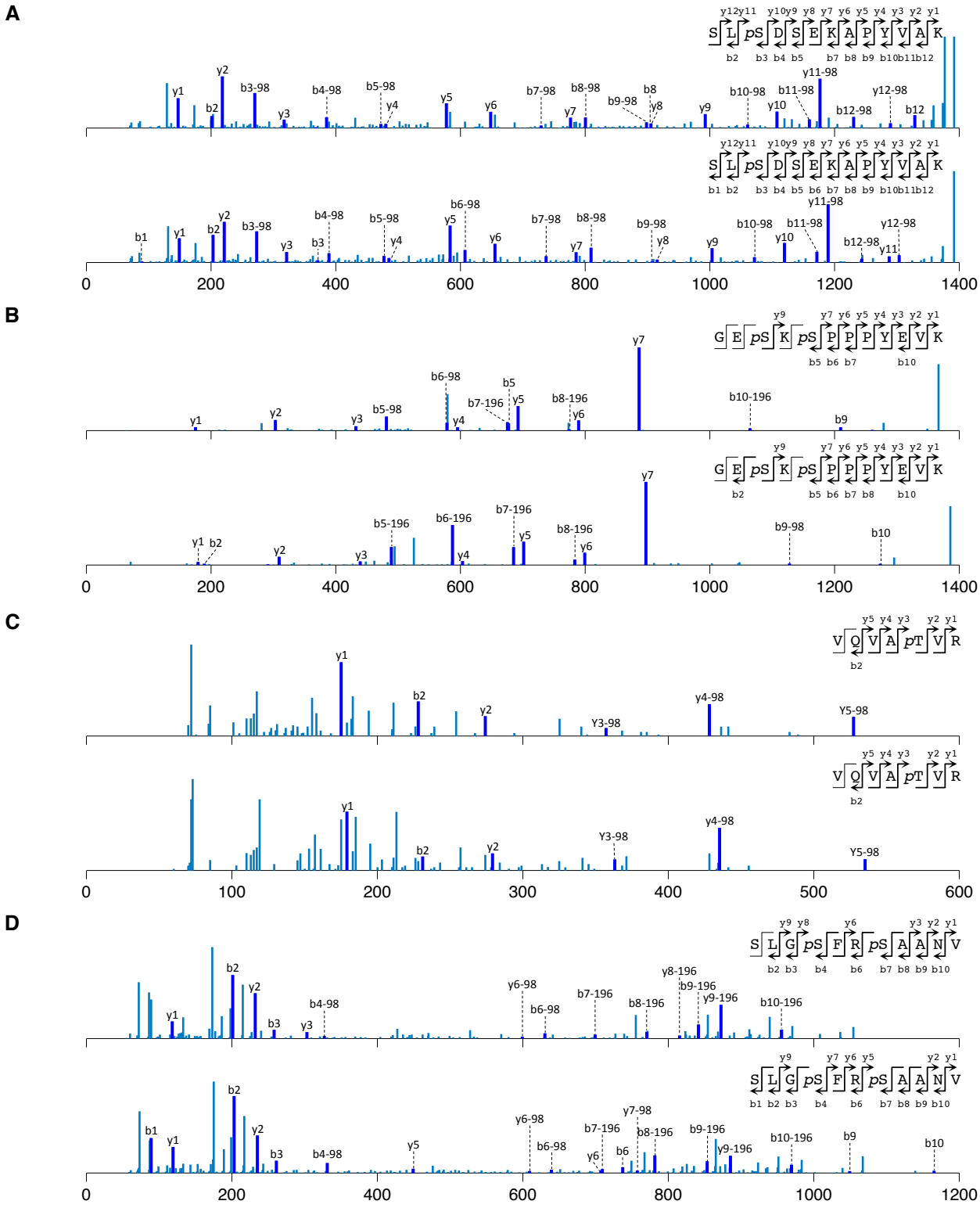
Supplementary Figure S2. False discovery rate (FDR) calculated using target-decoy searching.

Varying *E*-value (expectation value) is employed as the composite score for quality of peptide identification offered by the Mascot search engine. Red and blue curves show FDR estimated for identification of either ^{14}N- or ^{15}N-coded peptides and that for peptides discovered in pairs, *i.e.* both the ^{14}N- and ^{15}N-coded identical peptides varying with increase of *E*-value. The range around FDR =1% is shown enlarged in the insect figure.

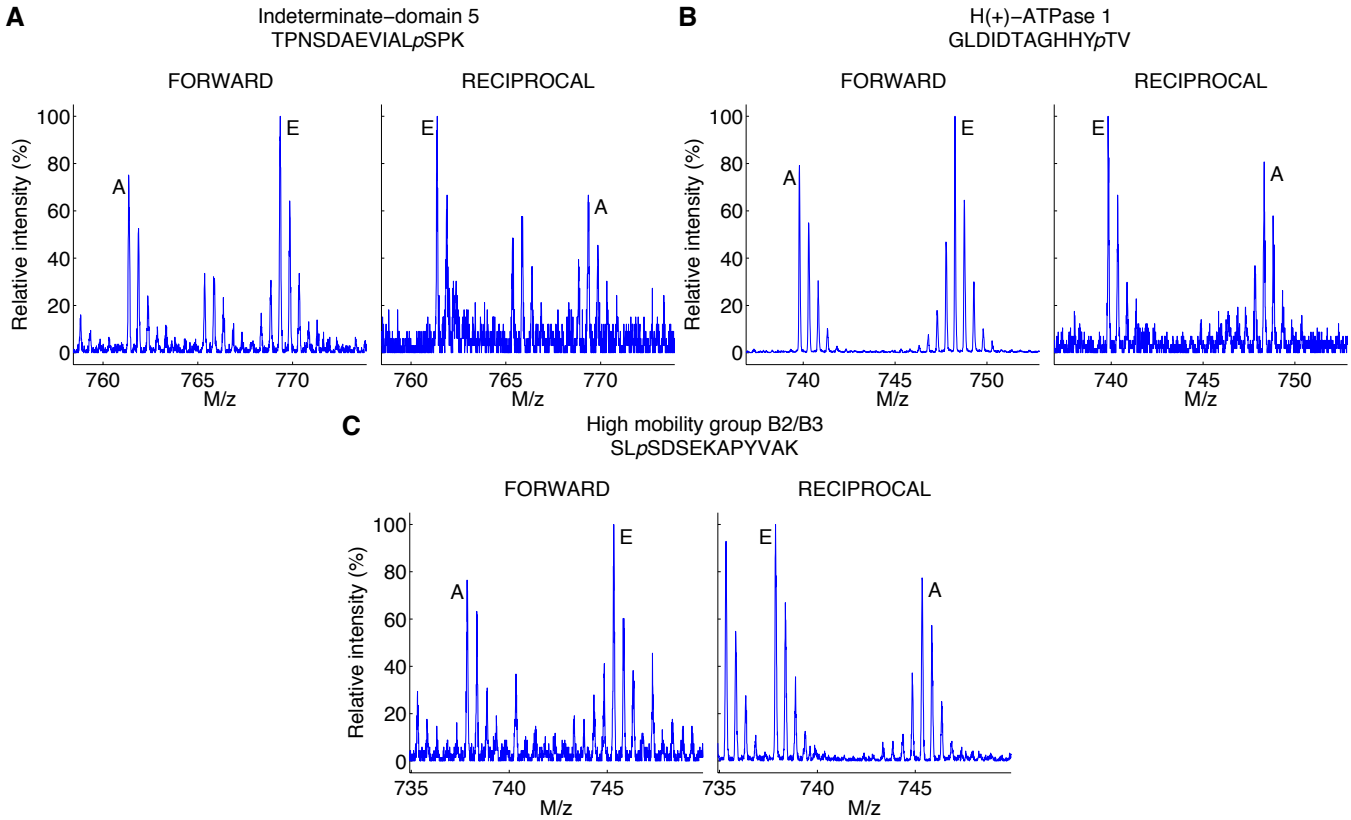


Supplementary Figure S3. Normalization of log2 ratio of phosphopeptides.

Here m_r is the median value of all log2 ratio, which is used to estimated actual mix ratio q using $m_r=\log_2(1/q)$. The media value m_r are subtracted from the log2 ratio of each pair of phosphopeptide. All phosphopeptides detected in each biological replicate (either Forward or Reciprocal one) were quantified. The median values of log2 ratios of all phosphopeptides from four mixtures of 1-min ethylene- and air-treated *rcn1-1* plants were found as 0.057, 0.183, -0.086 and 0.277 as well as that from four mixtures of wild type and *ctr1-1* mutants were found as 0.538, 0.287, 0.015 and 0.489. The ratio of each phosphopeptide quantified in each replicates were normalized based on the m_r value of the replicate.

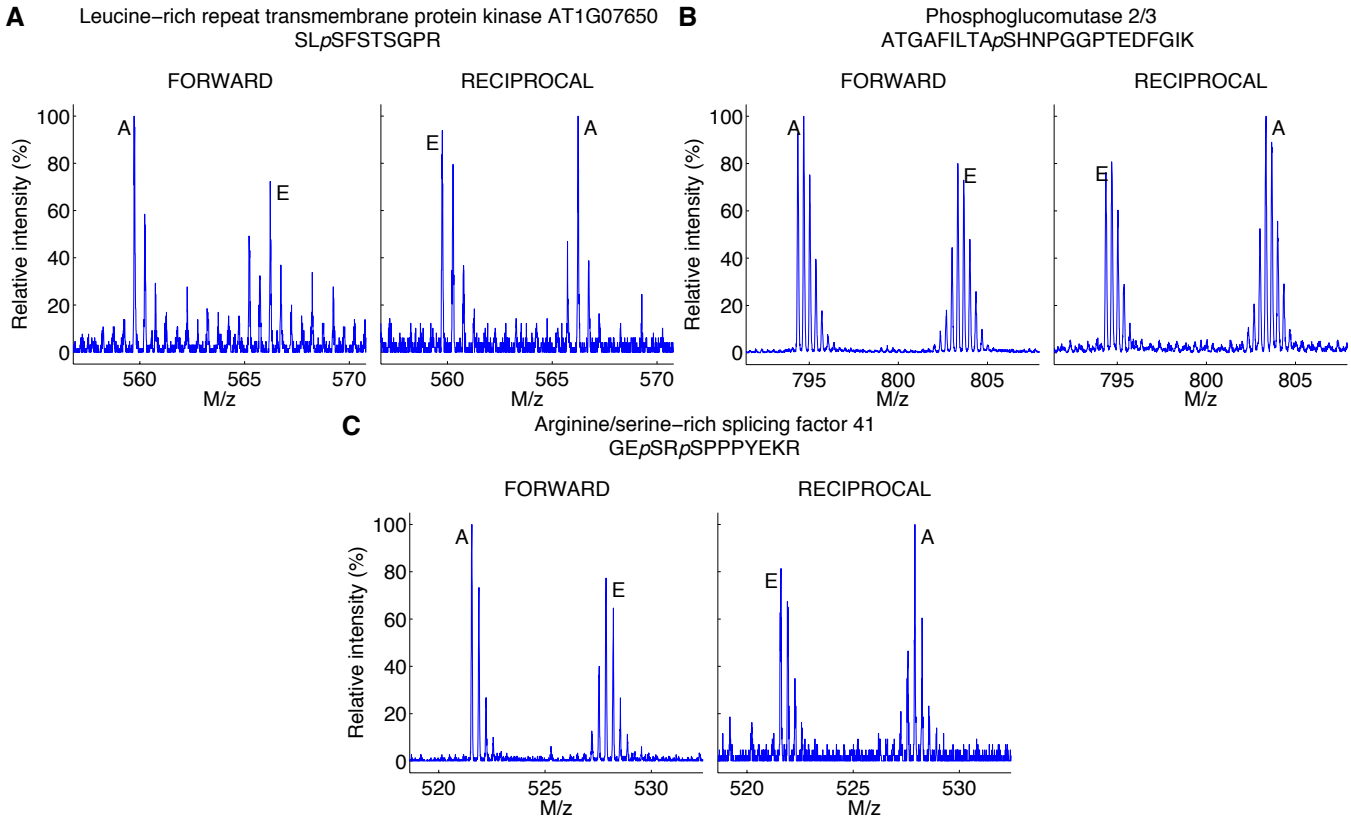


Supplementary Figure S4. MS/MS spectrum of significantly altered phosphopeptides.
(A) and (B) MS/MS spectra of one-minute ethylene up- and down-regulated phosphopeptides, respectively.
(C) and (D) MS/MS spectrum of CTR1 up- and down-regulated phosphopeptides, respectively.
Both b-type ions and y-type ions are labeled to determine the peptide sequence and to localize the sites of phosphorylation. The singly and doubly H_3PO_4 neutral loss peptide ions are indicated as -98 and -196 in MS/MS spectrogram, respectively.



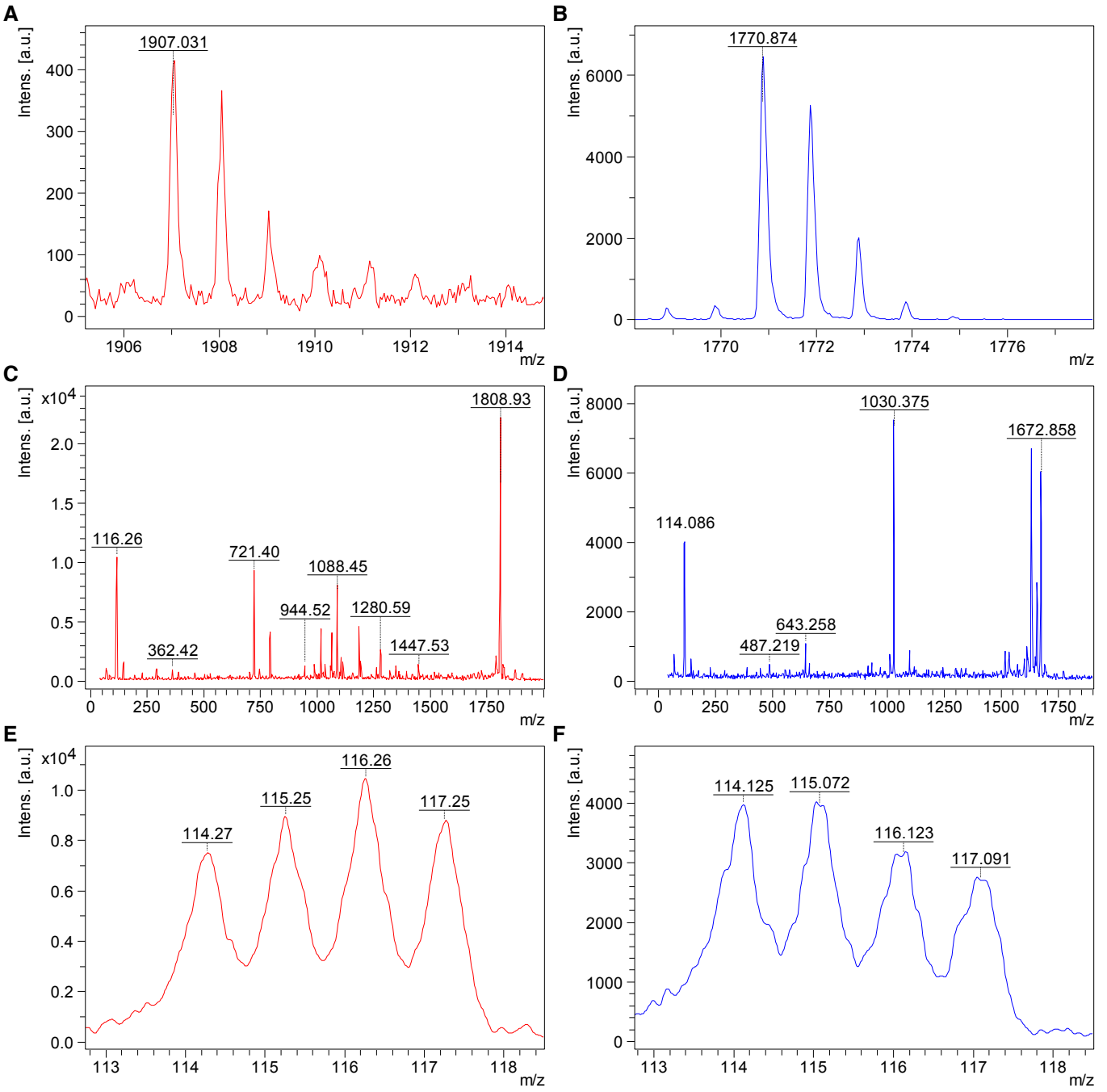
Supplementary Figure S5. Mass spectra of phosphopeptides enhanced by ethylene 1-min treatment in *rcn1-1*.

(A) TPNSDAEVIAL*p***S**PK from Indeterminate-Domain 5 (IDD5, AT2G02070);
(B) GLDIDTAGHHY*p***T**V from H^+-ATPase 1 (HA1, AT2G18960);
(C) SL*p***S**DSEKAPYVAK from High Mobility Group B2 (HMGB2, AT1G20693), and/or High Mobility Group B3 (HMGB3, AT1G20696).
The phosphorylated residues are marked by *p*. Ethylene- and air-treatment are annotated by "E" and "A", respectively.



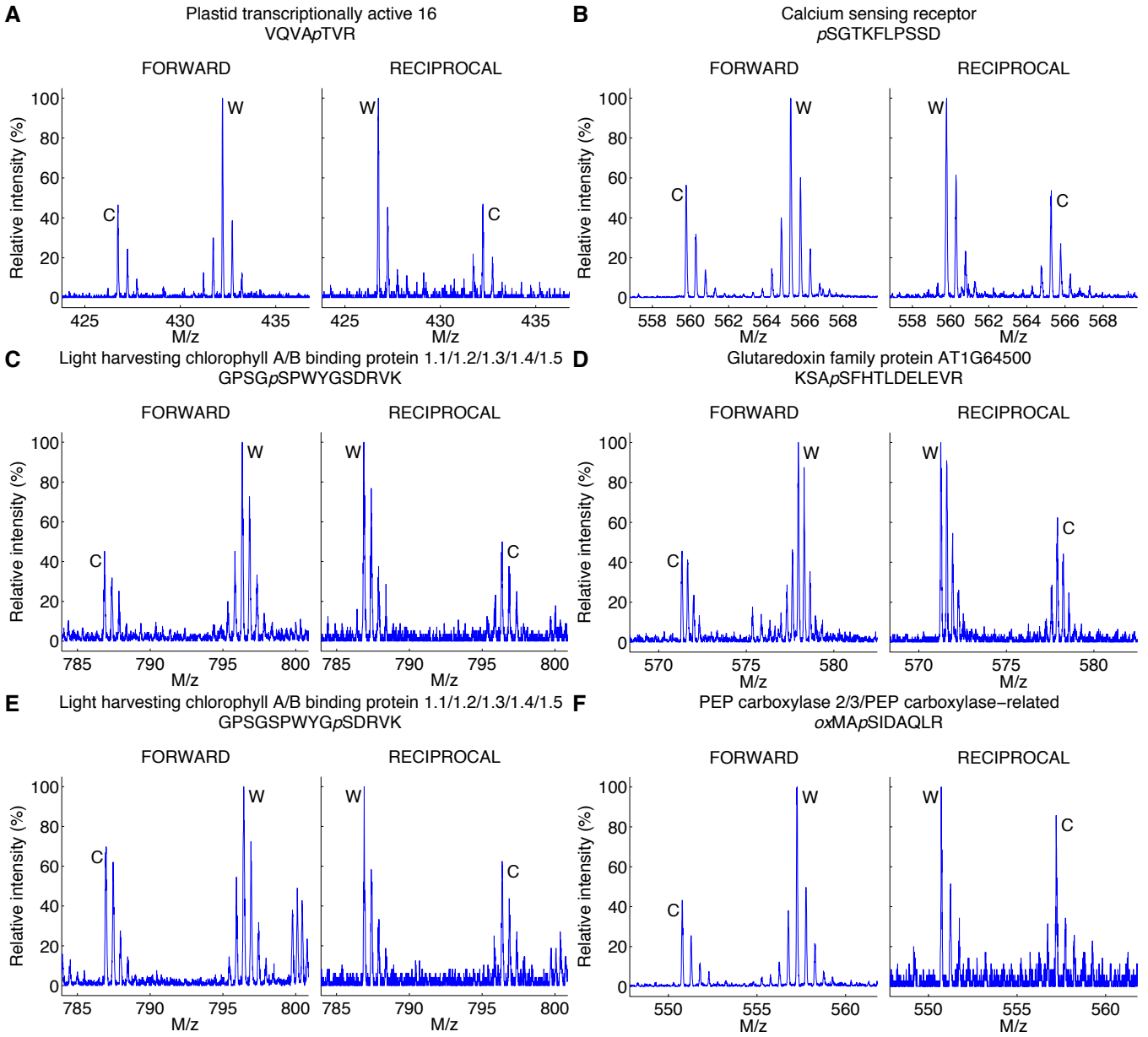
Supplementary Figure S6. Mass spectra of phosphopeptides repressed by ethylene 1-min treatment in *rcn1-1*.

(A) SL*p***S**FSTSGPR from the leucine-rich repeat transmembrane protein kinase AT1G07650;
(B) ATGAFILTA*p***S**HNPGGPTEDFGIK from Phosphoglucomutase 2 (PGM2, AT1G70730), and/or Phosphoglucomutase 3 (PGM3, AT1G23190);
(C) GE*p***S**R*p***S**PPPYEKR from Arginine/Serine-rich Splicing Factor 41 (ATRSP41, AT5G52040).
The phosphorylated residues are marked by *p*. Ethylene- and air-treatment are annotated by "E" and "A", respectively.



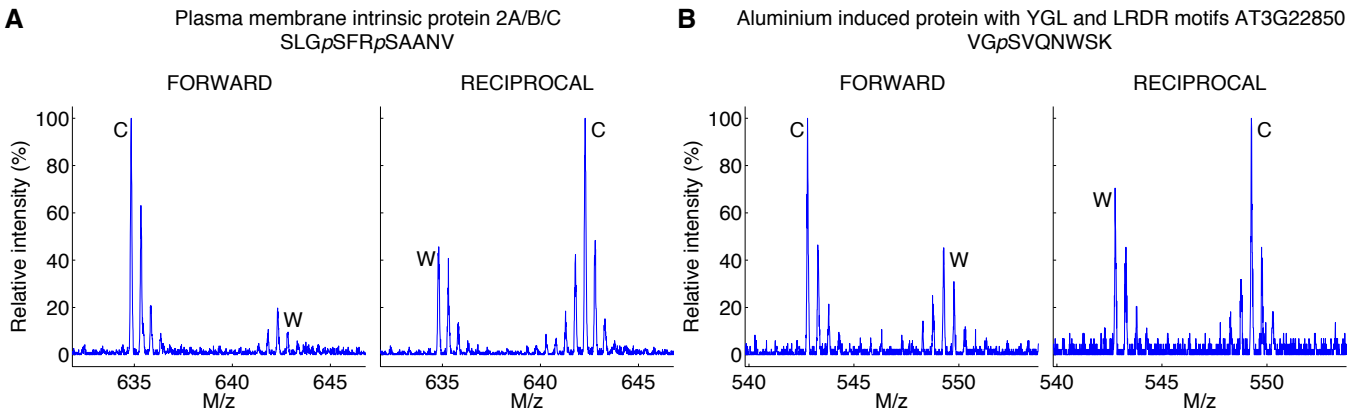
Supplementary Figure S7. iTRAQ validations of significantly altered phosphorylation.

(A) and **(B)** MS spectra of iTRAQ-labeled phosphopeptides SL*p***S**DSEKAPYVAK (derived from HMGB2/B3, AT1G20693/AT1G20696) **(A)** and GE*p***S**R*p***S**PPPYEKR (derived from ATRSP41, AT5G52040) **(B)**.
(C) and **(D)** MS/MS spectra of iTRAQ-labeled phosphopeptides SL*p***S**DSEKAPYVAK **(C)** and GE*p***S**R*p***S**PPPYEKR **(D)**.
(E) and **(F)** Spectra of iTRAQ reporter ions detected for SL*p***S**DSEKAPYVAK **(E)** and GE*p***S**R*p***S**PPPYEKR **(F)** phosphorylated by kinase extrats from 0- (M/z 114), 1- (M/z 115), 5- (M/z 116) and 15-min (M/z 117) ethylene-treated plants.



Supplementary Figure S8. The MS spectrum of CTR1 up-regulated phosphopeptides.

(A) VQVA*p***T**VR from Plastid Transcriptionally Active 16 (PTAC16, AT3G46780);
(B) *p***S**GTKFLPSSD from Calcium Sensing Receptor, (CaS, AT5G23060);
(C) GPSG*p***S**PWYGSDRVK from Light Harvesting Chlorophyll A/B Binding Protein 1.1 (LHCB1.1, AT1G29920), Light Harvesting Chlorophyll A/B Binding Protein 1.2 (LHCB1.2, AT1G29910), Light Harvesting Chlorophyll A/B Binding Protein 1.3 (LHCB1.3, AT1G29930), Light Harvesting Chlorophyll A/B Binding Protein 1.4 (LHCB1.4, AT2G34430), and/or Light Harvesting Chlorophyll A/B Binding Protein 1.5 (LHCB1.5. AT2G34420);
(D) KSA*p***S**FHTLDELEVR from the glutaredoxin family protein AT1G64500;
(E) GPSGSPWYG*p***S**DRVK from Light Harvesting Chlorophyll A/B Binding Protein 1.1 (LHCB1.1, AT1G29920), Light Harvesting Chlorophyll A/B Binding Protein 1.2 (LHCB1.2, AT1G29910), Light Harvesting Chlorophyll A/B Binding Protein 1.3 (LHCB1.3, AT1G29930), Light Harvesting Chlorophyll A/B Binding Protein 1.4 (LHCB1.4, AT2G34430), and/or Light Harvesting Chlorophyll A/B Binding Protein 1.5 (LHCB1.5. AT2G34420);
(F) *ox*MA*p***S**IDAQLR from Phosphoenolpyruvate (PEP) carboxylase 2 (PPC2, AT2G42600), PEP carboxylase 3 (PPC3, AT3G14940), and/or PEP carboxylase-related protein AT3G42628.
The phosphorylated residues are marked by *p* and the oxidized Met is marked by *ox*. Wild type and *ctr1-1* mutant are annotated by "W" and "C", respectively.



Supplementary Figure S9. The MS spectrum of CTR1 down-regulated phosphopeptides.

(A) SLG*p***S**FR*p***S**AANV from Plasma Membrane Intrinsic Protein 2A (PIP2A, AT3G53420), Plasma Membrane Intrinsic Protein 2B (PIP2B, AT2G37170), and/or Plasma Membrane Intrinsic Protein 2C (PIP2C, AT2G37180); (B) VG*p***S**VQNWSK from the aluminium induced protein with YGL and LRDR motifs AT4G27450.
The phosphorylated residues are marked by *p*. Wild type and *ctr1-1* mutant are annotated by "W" and "C", respectively.