Supplementary Figure and Table Legends

Supplementary Figure1. Auxin induces synchronously lateral root initiation

(a) Expression of pPLT3::GUS is detected at lateral root primordia, (b) expression of pPLT3::eCFP is detected at lateral root primordia, (e) lateral root initiation and PLT3 expression after 3 days 10 µM auxin efflux inhibitor naphthylphthalamic acid (NPA), with 8 hours mock treatment on ½ GM; no GUS expression is detected. (f) After 3 days NPA inhibition, 8 hours 10µM auxin Naphthaleneacetic acid (NAA) treatment synchronously induces lateral root initiation indicated by GUS expression, (c) magnification of pPLT3::GUS expression in pericycle of figure 1f. (g) After 3 days NPA inhibition, 20 hours 10µM auxin (NAA) synchronously induces cell division indicated by eCFP expression in pericycle; (d) magnification reveals the pPLT3::eCFP expression in pericycle of 1g. Cell walls stained with propidium iodide appear in red.

Supplementary Figure 2. High incorporation rate of ^{15}N achieved after removing vitamins from MS medium.

(a) Removing vitamins does not influence Arabidopsis seedling growth. Seedlings on ½ MS medium with (top) or without (bottom) vitamins at 7 days after germination. (b) MS spectrum of a peptide isolated from a complete mixed lysate of ^{14}N root sample treated with NAA and ^{15}N root mock treated. MS spectra correlate to the peptide YGAGIGPGVYDIHSPR from the protein 5-methyltetrahydropteroyltriglutamate-homocysteine methyltransferase (At5g17920).

Supplementary Figure 3. Induction of MAP kinase phosphorylation by NAA and inactive auxin analogues

Root tissue was treated with 10 µM auxin (NAA) or inactive auxin analogues (PCIB, 1-NOA) for indicated time. MAPK phosphorylation levels and MPK6 proteins levels were analysed by Western blot probed with antibodies against the phosphorylated TEY motif in the activation loop (α-p42/44) and MPK6 specific antibodies (α-MPK6) (Menke et al., 2004). hpi, hours post induction

Supplementary Figure 4. Primary root growth, lateral root density of SNX1-GFP, SNX1^{S16A}-GFP and SNX1^{S16E}-GFP

Growth of primary roots at day 3, 6 and 9 (n>10), averages of six independent lines continuously expressing SNX1-GFP (a), SNX1^{S16A}-GFP (c) and SNX1^{S16E}-GFP (e), error bar represents standard errors , P value of three time points of each line to Col-0 are indicated (day 3, top; day 6, middle and day 9, bottom). Lateral root densities of day 6 and day 9 are shown. Significant difference of SNX1-GFP (b), SNX1^{S16A}-GFP (d) and SNX1^{S16E}-GFP (f) comparing to Col-0 marked with*. (* 0.01<P<0.05,** 0.001<P<0.01, *** P<0.001,* lower than Col-0 * higher that Col-0).

Supplementary Figure 5. SNX1^{S16E}-GFP lines display normal growth and lateral root formation without induction.

Ten-day post germination seedlings of wild type SNX1-GFP-S3/S5 and SNX1^{S16E}-GFP-E5/E6, with 3 days on ½ GM medium post germination and transferred to fresh medium for another 7 days. SNX1^{S16E}-GFP-E6 line with estradiol induction showed growth inhibition.

Supplementary Figure 6. SNX1-GFP localization

Localization of SNX1-GFP-S3 or SNX1^{S16E}-GFP-E5 in epidermal root cells in meristematic zone (top) or differentiation zone (bottom), different localization pattern of SNX1^{S16E}-GFP in epidermal cells of differentiation zone.

Supplementary Figure 7. Induction of mutant SNX1^{S16S19}-GFP had a suppressive effect on growth

Seedlings of Col-0, SNX1-GFP or mutants as indicated, with 3 days on estradiol induction medium post germination and transferred to fresh estradiol medium for another 7 days.

Legends to the Supplementary tables

Supplementary Table I Quantified proteins

The auxin to control ratio (column B, C) for each protein (AG code column A) is log2-transformed (column F, G). The average of auxin to control ratio and standard deviation for each protein is listed (column D, E) and log2-transformed (column H, I), the unique peptides number (column J, K) and the description of the proteins are listed (column L).

The differentially regulated auxin related proteins discussed in paper are highlighted in green.

Supplementary Table II Quantified regulated phosphopeptides

Peptides sequences are listed in column A, capital letters in the peptide sequence column indicate amino acids and lower case p indicates phosphorylation of the Serine, Threonine or Tyrosine residue that follows and (ox) indicates oxidation of the Methionine that follows. The mass to charge ratio, precursor charge and peptide identification score for each phosphopeptide are list in (column B-G). The auxin to control ratio, average and standard deviation (column H, I, J, K) for each phosphopeptide (column A) is log2-transformed (column L, M, N, O). The AG code and description of the proteins are in (column P, Q).

Supplementary Table III Quantified phosphopeptides normalized by proteins

In the sheet of Normalization, peptides sequences are listed in column A, capital letters in the peptide sequence column indicate amino acids and lower case p indicates phosphorylation of the Serine, Threonine or Tyrosine residues that follow and (ox) indicate oxidation of the Methionine that follows. The auxin to control ratios for each phosphopeptide are shown in column B. The ratios in the column B are log2-transformed and are shown in column C. The protein ID and description are shown in columns D and E. The log2-transformed ratios of proteins are shown in column F. The numbers of non-modified peptides used for quantification of protein are shown in column G. The log2-transformed ratios of normalized phosphopeptides are shown in column H (column C minus column F). The normalized phosphopeptides ratios (auxin to control) are showed in column I. The distribution of log2-transformed normalized

phosphopeptide ratio of auxin to control ranked from low to high is shown in the sheet distribution plot.

Supplementary Table IV Quantified phosphopeptides normalized by non phospho proteins in both experiments.

Phosohopeptides sequences are listed in column A, capital letters in the peptide sequence column indicate amino acids and lower case p indicates phosphorylation of the Serine, Threonine or Tyrosine residue that follows and (ox) indicates oxidation of the Methionine that follows. The two auxin to control ratios, average and standard deviation (column B, C, D, E) for each phosphopeptide (column A) is log2-transformed (column F, G, H, I). The total intensity and p value for each phosphopeptides are listed (column J, K), the AG code and description of the corresponding proteins are in (column L, M).

Supplementary Table V Comparisons to other proteomic data sets

Comparison to Pep2Pro. Column A, Proteins identifiers for all unique proteins in supplementary table 1. Column C, Proteins identifiers for all unique proteins in the Pep2Pro Database for root samples. Overlap between datasets shown in Column A and C. **Phospho comparisons** Protein identifiers and phosphopeptide sequences for different datasets are shown in columns. Modifications other than phosphorylation where filtered out and ignored for comparisons. Column A, Unique phosphopeptides from supplementary Table 2. Column C, Unique phosphopeptides from PhosPhAt 4.0 database. Column E, Unique phosphopeptides from Lan et al., (2012). Column G, overlap between Column A and Column C. Column I, overlap between Column A and Column E. Column K, overlap between Column A , Column C and Column E. Numbers between brackets show overlap in columns G, I and K.

Supplementary Table VI Primers used for cloning and mutagenesis