Cargo proteins for adaptor protein complexes AP-3 and AP-4 identified by differential (phospho-)protein distribution in sucrose gradients

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Supplementary Figures

Supplementary Figure 1: Assignment of ^{15}N-labeled partners to unlabelled peptide species identified by MaxQuant. (**A**) Workflow of linking data from "evidence.txt" and "matched features.txt". (**B**) Mass spectrum of an unlabelled and ^{15}N-labeled peptide ion with highlighted sampling window to identify the ^{15}N-form within a 1:1 mixture of labeled and unlabeled membrane protein. Sampling window and sampled ^{15}N ion peak is indicated in red. Sampled ^{14}N peak is indicated in blue.

Supplementary Figure 2: K-means clustering of wild type and mutant protein abundance profiles. (**A**) overview of the six clusters. (**B**) Distribution of wild type and mutant proteins across the different clusters. (**C**) Overlap of the identified candidates based on individual altered distribution profiles or the clustering method ("clustering").

Supplementary Figure 3: Localization of GFP-PIP2A in transiently transformed mesophyll protoplasts. (**A**) Single sections (top row) and maximum projections (bottom row) of representative protoplasts for each genotype. GFP fluorescence is shown in green, autofluorescence of chlorophyll is shown in red. Scale bars represent 10 µm. (**B**) Distribution of the GFP-PIP2A locations to different subcellular compartments in individual transformation events to plasma membrane (PM, as represented by col-0 in panel A) or "other" cell internal compartments (e.g. as represented by *ap-4ß* in panel A). The numbers of images scored with at least 10 fluorescent protoplasts eachwere 18 for *ap-3ß*, 22 for col-0 and 24 for *ap-4ß*. Mean values indicate the location counts per image and are shown with standard error.

Supplementary Figure 4: Distribution across fractions for proteins with known mis-targeting in the ap3-ß mutant. (**A**) PIN7, (**B**) PIN3, (**C**) BRI1 (**D**) VSP1. Values describe the mean with standard deviation of four independent gradient preparations for col-0 and two gradients for *ap-3ß* and *ap-4ß* mutants.

Supplementary Figure 5: Spectra of identified phosphopeptides. Spectra were plotted based on the MaxQuant results.

Supplementary Tables

Supplementary Table 1: Reproducibility of the step gradient preparations regarding sucrose concentration at the interphase, protein concentration and the numbers of identified peptide sequences and proteins.

Supplementary Table 2: List of marker proteins and their predicted and confirmed sub-cellular localizations used across the gradient to assign the interphases to organelle-enriched fractions.

Supplementary Table 3: List of proteins with altered distribution profiles in replicated experiments.

Supplementary Table 4: List of phosphopeptides and pairwise comparison between wild type and AP-complex mutants.

Supplementary Table 5: List of all identified protein groups including numbers of peptides, proteins per protein group and sequence coverage of each protein.