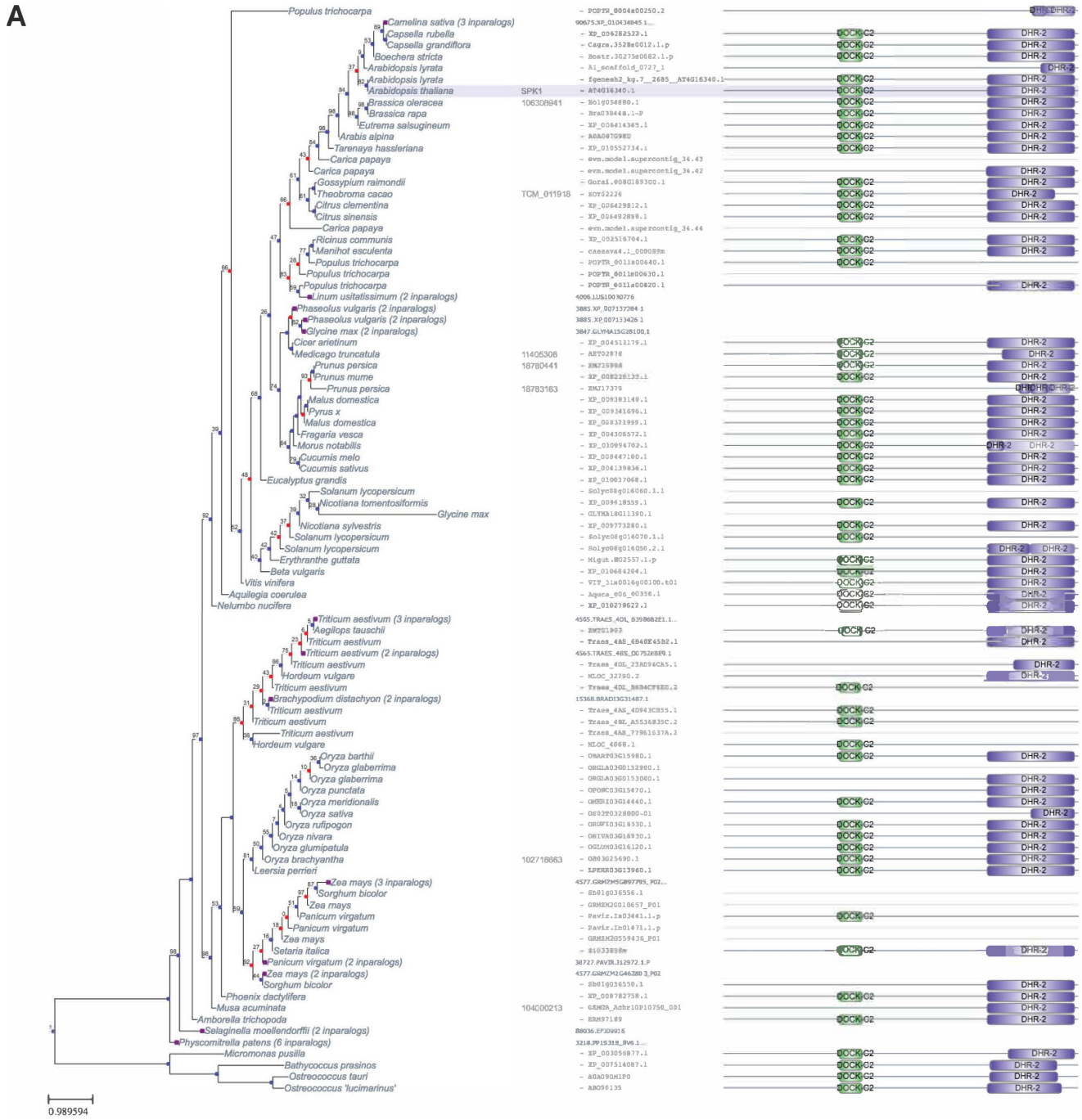


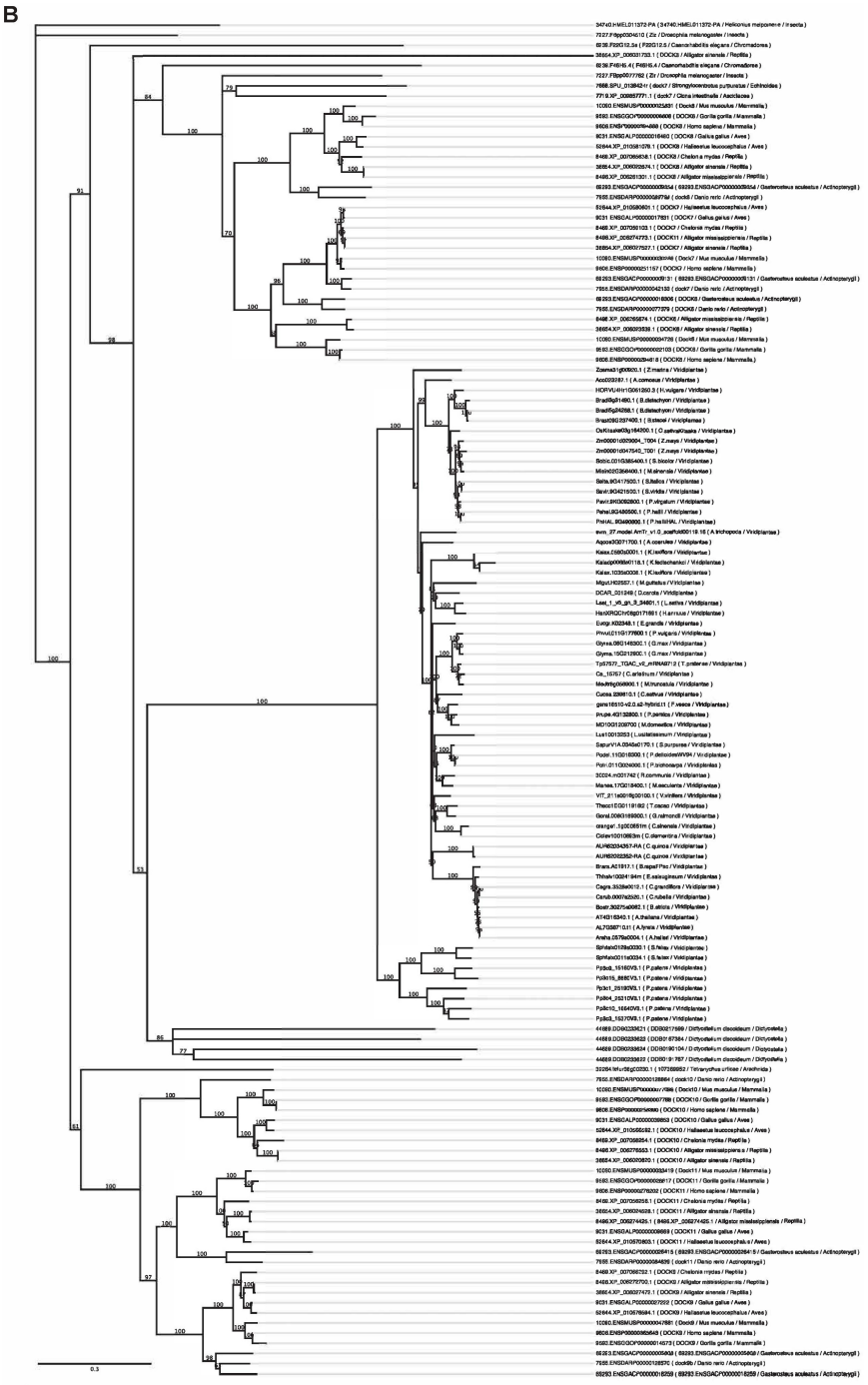


Fig S1. Phylogeny of SPK1 orthologs in the plant and animal kingdoms.

(A) Phylogeny and domain organization of SPK1 orthologs. Plant orthologs to Arabidopsis SPK1 were obtained from EggNOG (http://eggnog5.embl.de) database and searched against Pfam database (http://pfam.xfam.org). Locus IDs of SPK1 orthologs are provided in the tree. Pfam domain organizations are shown next to the phylogenetic tree.

(B) Phylogenetic analysis of plant and metazoan orthologs to Arabidopsis SPK1. Sequences from plant and animal kingdoms were obtained from Phytozome 13 (https://phytozome-next.jgi.doe.gov) and EggNOG (http://eggnog5.embl.de) databases, respectively. Geneious tree builder on Geneious Prime software (version 2021.2.2) was utilized to build the phylogenetic tree based on the multiple sequences alignment result from Cluster Omega. The numbers on the tree are the bootstrap values for clades. The scale bar indicates patristic distances.

(C) YFP:SPK rescue constructs with deletions of the DHR3 or DHR1 domains do not rescue spk1-666 trichome phenotypes. All panels except Col are in spk1-666 background. Whole plant images have scale bars of 5 mm and magntified views of representative trichomes have scale bars of 0.5 mm.

(D) SPK1 mutant proteins of the predicted size accumulate in transgenic lines. Western blots of leaf extracts probed with the anti-SPK1 antibody (upper) or PEP-C (lower) as a loading control. Col indicates wild type plants; all other extracts are from *spk1* plants, and the identity of the SPK1 construct present in *spk1* plants is indicated above each lane. The locations of the molecular weight standards are labeled to the left of the blots. 3xYPET:SPIKE1, 290 kDa; ΔDHR3, 273 kDa; ΔDHR1, 262 kDa; ΔDHR3_ΔDHR1, 245 kDa; ΔDHR2, 271 kDa.

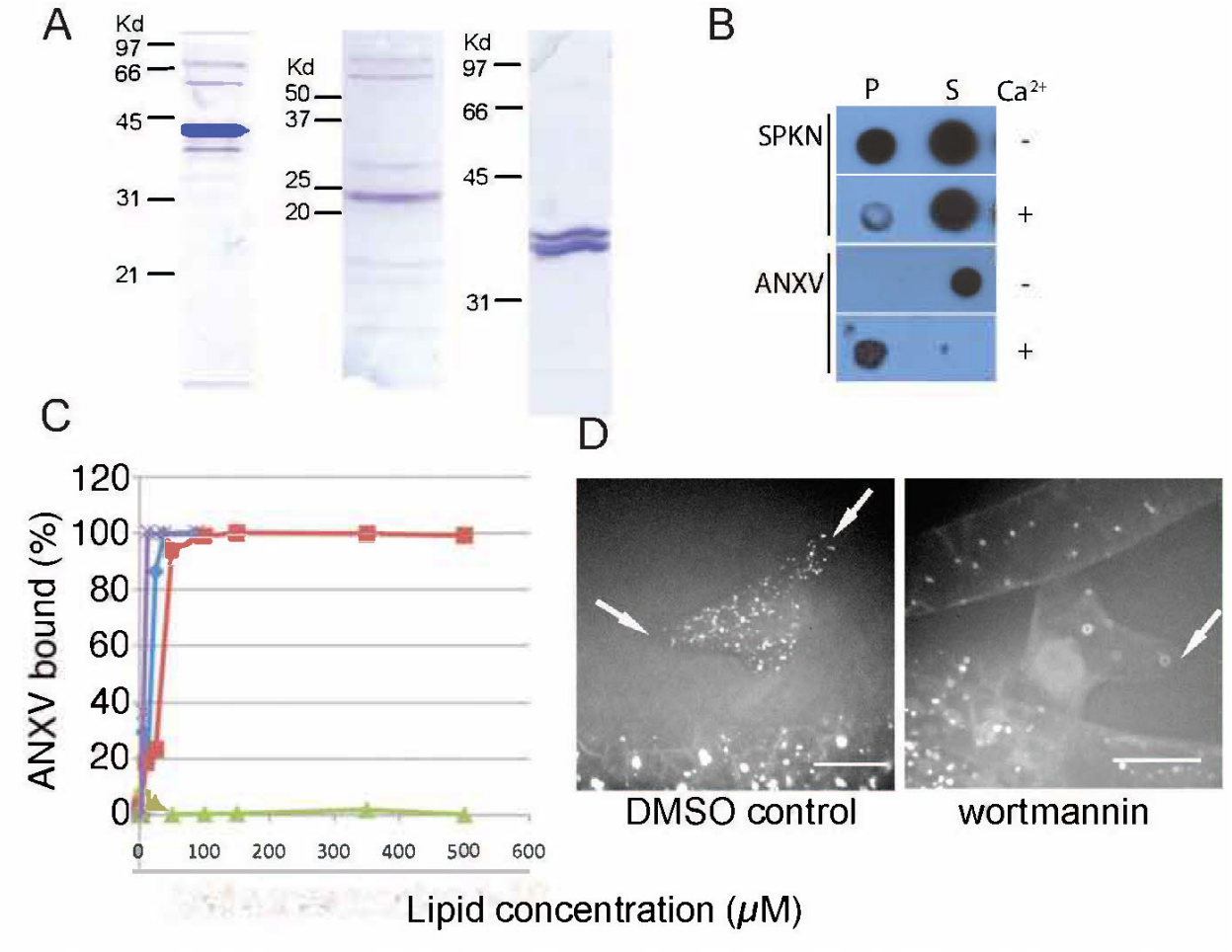


Fig. S2. Supplemental data characterizing the lipid binding selectivity of SPK1 and its wortmannin-dependence.

(A) SDS-PAGE gels of partially purified proteins. SPK N1:6xHis, 37 kDa; 6xHis:SPKN3, 24.8 kDa; human AnnexinV:6xHis, 36.8 kDa.

(B) ANX5, but not SPK1N binds to DPPS in a Ca^{2+}-dependent manner. Lipid overlay binding experiments were conducted in the presence or absence of Ca^{++}. P, pellet; S, supernatant.

(C) AnxV binds to PS without selectivity for fatty acid chain saturation. Liposome binding assays with different lipid compositions. All binding reactions conducted with the ANXV: blue, DPPS/ NTPC; purple, NTPI/NTPC; brick red, NTPS/NTPC; green, NTPC.

(D) Wortmannin reduces the number and increases the size of 2XFYVE-YFP labeled compartments in stage 4 trichomes compared to DMSO controls; arrows label trichome branch tips.

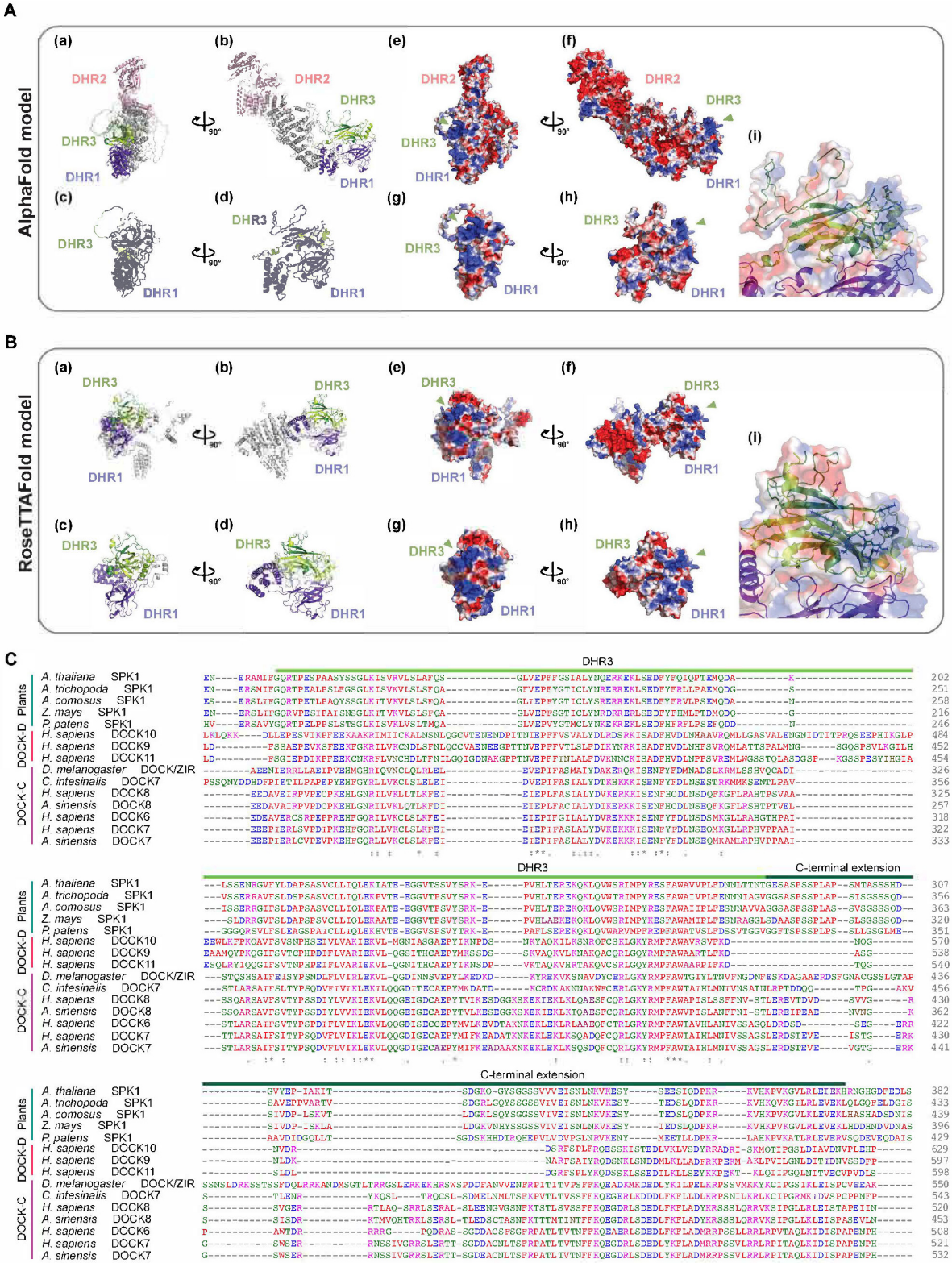
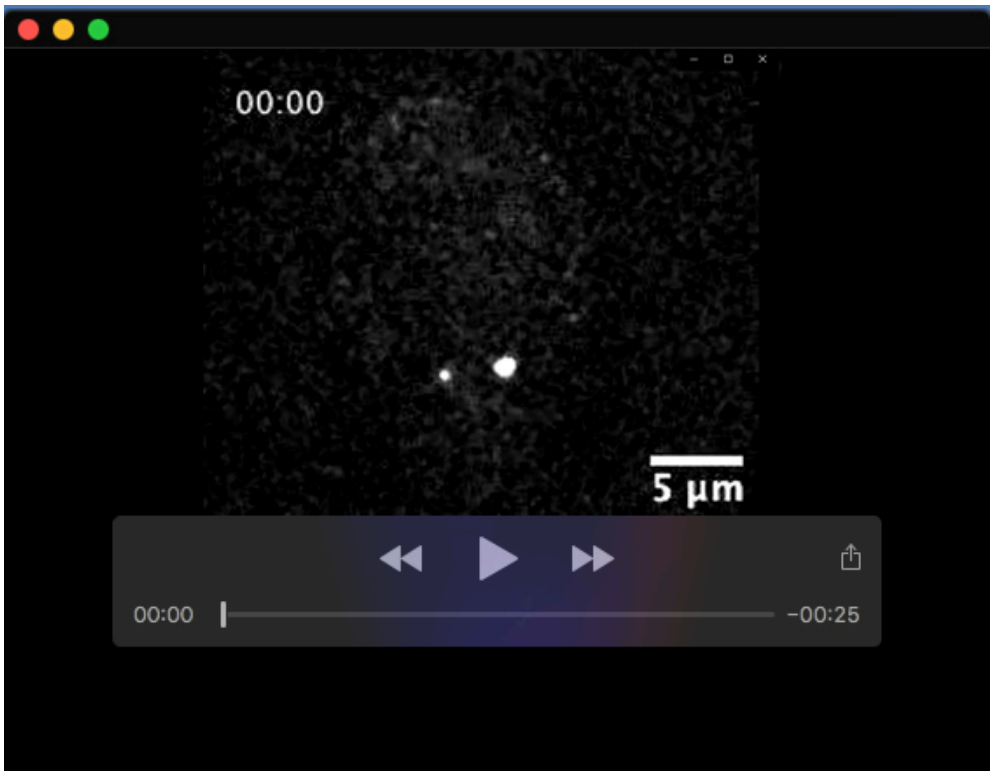


Fig. S3. Similarities of the SPK1 structure predictions using AlphaFold and RoseTTAFold.

(A & B) Overall modeled structures and domain organizations of the SPK1 predicted by AlphaFold (A) and RoseTTAFold (B). Due to length limits, the SPK1 sequence (residues 1 to 1,117) was uploaded onto the Robetta website (https://robetta.bakerlab.org/) to predict protein folding using the RoseTTAFold method on September 4, 2021. The RoseTTAFold-predicted models had a confidence level of 0.48. Two views of the full-length (a & b) and DHR3/C2 and DHR1 (c & d). The electrostatic potentials of the SPK1 models at the same orientation as (a-d) are visualized (e-h). The positively charged patch on DHR3/C2 domain is indicated with light green triangles. The zoom-in views show the basic amino acid residues on DHR3/C2 domain and with the same orientation presented in (h).

(C) The DHR3/C2 domain is conserved among SPK1 and metazoan DOCK-C and DOCK-D orthologs. The DHR3/C2 domain includes a C-terminal extension beyond the core DHR3 element defined in Fig. 1 B, as described in the results section. Clustal Omega was performed at EMBL-EBI website (https://www.ebi.ac.uk/Tools/msa/) to align SPK1 sequence to DOCKs classified as DOCK-C or DOCK-D. DHR-3 domain or the C-terminal extension were marked with the light green or the dark green bars over the sequences. Estimated conservation of amino acids was reported using "*" (a single, fully conserved residue), ":" (scoring > 0.5 in the Gonnet PAM 250 matrix), "." (≤ 0.5 and > 0) symbols.



Movie 1. Time lapsed imaging of the YFP:SPK1 DHR3 deletion mutant reveals motile cytoplasmic organelles. A single image plane was acquired every 10 sec. Size bar and time stamps are labeled.