SUPPLEMENTAL INFORMATION

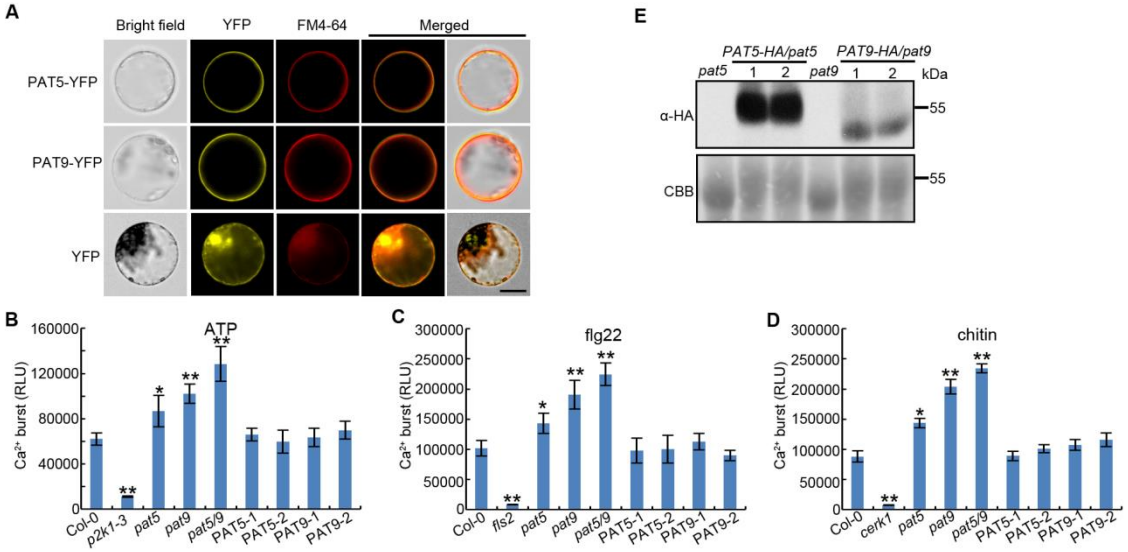


Figure S1. Subcellular localization of PAT5 and PAT9, Related to Figure 1

(A) PAT5-YFP and PAT9-YFP were transiently expressed in the *Arabidopsis* protoplasts, and merged well with the plasma membrane marker FM-64. Free YFP was used a control. Bar = 20 μm.

(B-D) Ligand-induced calcium influx. 5-day-old seedlings were treated with 100 μM ATP, 1 μM flg22 or 50 μg/ml chitin. RLU, relative luminescence units.

(E) Relative expression protein levels of *NP::ATPAT5-HA/Atpat5* and *NP::ATPAT9-HA/Atpat9* complemented transgenic lines using their own native promoters. CBB was used as a loading control.

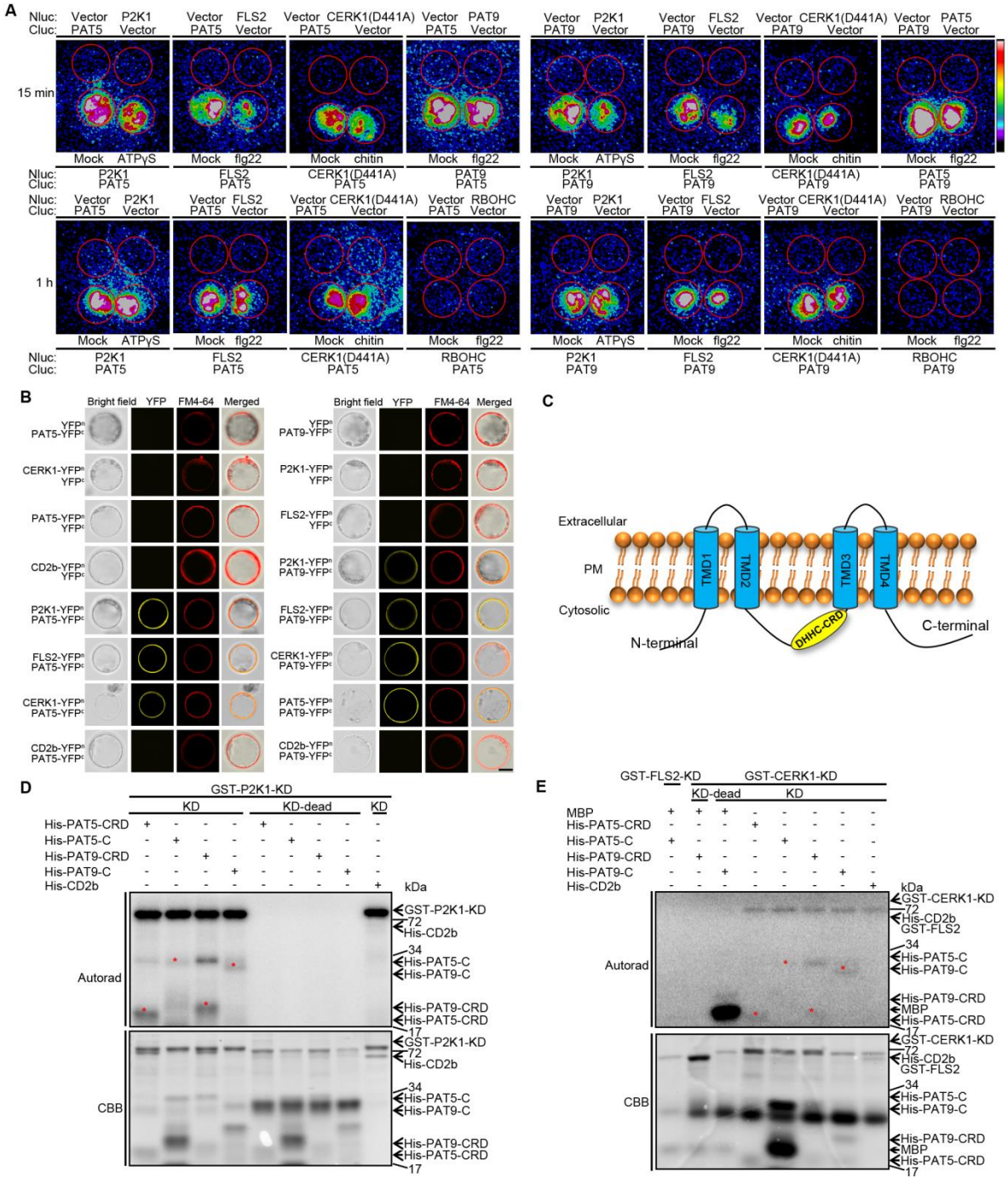


Figure S2. PAT5 and PAT9 interact with and are phosphorylated by receptors, Related to Figure 2

(A) PAT5 and PAT9 interact with P2K1, FLS2 and CERK1 in *N. benthamiana* in an elicitor-dependent manner. The indicated constructs were transiently expressed in *N. benthamia* leaves with, where indicated in red circles, the addition of 500 μM ATPγS, 2 μM flg22 or 100 μg/ml chitin. Nluc, N-terminal fragment of firefly luciferase; Cluc, C-terminal fragment of firefly luciferase; Vector, empty vector. The color bar represents the color code

for fluorescence intensities.

(B) Interactions of PATs and receptors at *Arabidopsis* protoplast plasma membrane. FM4-64 was used to stain the plasma membrane. Bar = 20 μm.

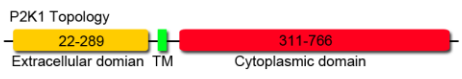
(C) Protein topology of PATs on plasma membrane. PM, plasma membrane. TMD, transmembrane domains. DHHC, Asp-His-His-Cys. CRD, a stretch of DHHC within a Cys-rich domain.

(D and E) Receptors directly phosphorylate PAT5 and PAT9. Purified P2K1, FLS2 and CERK1 kinase domain recombinant proteins were incubated with PAT5/PAT9-CRD and -C domains in an *in vitro* kinase assay. Autophosphorylation and trans-phosphorylation were measured by incorporation of γ-[32P]-ATP. MBP and GST-CD2b were used as positive and negative controls, respectively. The protein loading was measured by CBB staining. Red stars represent the trans-phosphorylated proteins.

A

ID	Position	Peptide	Score	Cutoff	Cluster
FLS2	61	IIGSLRHCNWTGITC	0.195	0.0	S-Palmitoylation: Cluster C
FLS2	68	CNWTGITCDSTGHVV	0.293	0.0	S-Palmitoylation: Cluster B
FLS2	165	GDVPEEICKTSSLVL	0.835	0.0	S-Palmitoylation: Cluster B
FLS2	187	LTGKIPECLGDLVHL	0.499	0.0	S-Palmitoylation: Cluster A
FLS2	263	IPAEIGNCSSLVQLE	0.018	0.0	S-Palmitoylation: Cluster A
FLS2	407	IPSSISNCTGLKLLD	0.0	0.0	S-Palmitoylation: Cluster C
FLS2	454	IPDDIFNCSNLETLS	0.244	0.0	S-Palmitoylation: Cluster A
FLS2	672	IPRSLQACKNVFTLD	0.447	0.0	S-Palmitoylation: Cluster C
FLS2	783	LMGNTDLCGSKKPLK	0.783	0.0	S-Palmitoylation: Cluster C
FLS2	792	SKKPLKPCTIKQKSS	0.0	0.0	S-Palmitoylation: Cluster B
FLS2	830	LLVLILTCCKKKEKK	9.137	0.903	S-Palmitoylation: Cluster C
FLS2	831	LVLILTCCKKKEKKI	8.015	0.903	S-Palmitoylation: Cluster C
FLS2	975	LLEKIDLCVHIASGI	2.121	1.991	S-Palmitoylation: Cluster B
FLS2	996	YGFPIVHCDLKPANI	0.257	0.0	S-Palmitoylation: Cluster C
FLS2	1132	IEDFLKLCLFCTSSR	0.795	0.0	S-Palmitoylation: Cluster C
FLS2	1135	FLKLCLFCTSSRPED	0.329	0.0	S-Palmitoylation: Cluster C

B



ID	Position	Peptide	Score	Cutoff	Cluster
P2K1	87	SFSTHFVCALVPKPG	0.447	0.0	S-Palmitoylation: Cluster B
P2K1	296	LIILLPVCLAILVLA	0.502	0.0	S-Palmitoylation: Cluster A
P2K1	394	AEVVSMRCLKHRNLV	2.301	1.991	S-Palmitoylation: Cluster B
P2K1	407	LVPLFGYCRRKRELL	1.966	0.903	S-Palmitoylation: Cluster C
P2K1	538	VFMLEVTCGRRPVEP	0.461	0.0	S-Palmitoylation: Cluster C
P2K1	559	RHMIKWVCECWKKDS	1.598	0.0	S-Palmitoylation: Cluster B
P2K1	561	MIKWVCECWKKDSLL	0.638	0.0	S-Palmitoylation: Cluster C
P2K1	594	VMKLGLLCSNIVPES	0.355	0.0	S-Palmitoylation: Cluster A

C

ID	Position	Peptide	Score	Cutoff	Cluster
CERK1	25	FFAVESKCRTSCPLA	3.048	1.991	S-Palmitoylation: Cluster B
CERK1	29	ESKCRTSCPLALASY	1.817	0.893	S-Palmitoylation: Cluster A
CERK1	91	RVLVPFPCECQPGDF	0.701	0.0	S-Palmitoylation: Cluster C
CERK1	93	LVPFPCECQPGDFLG	0.684	0.0	S-Palmitoylation: Cluster C
CERK1	153	TLNVLVNCSCGDESV	0.038	0.0	S-Palmitoylation: Cluster C
CERK1	155	NVLVNCSCGDESVSK	0.136	0.0	S-Palmitoylation: Cluster C
CERK1	381	LVRLIGYCVEGSLFL	0.328	0.0	S-Palmitoylation: Cluster C
CERK1	572	MAELGKACTQENAQL	0.943	0.0	S-Palmitoylation: Cluster B

Figure S3. FLS2, P2K1 and CERK1 protein domains and *S*-acylation sites prediction, Related to Figure 3

(A-C) Schematic representation of FLS2, P2K1 and CERK1 protein structures contain an extracellular domain, a transmembrane domain (TM) and a cytoplasmic domain. The numbers in the topology and position indicate amino acids. The receptor protein sequences were analyzed by GPS-Lipid 1.0 software, the *S*-acylation residues were shown in red font.

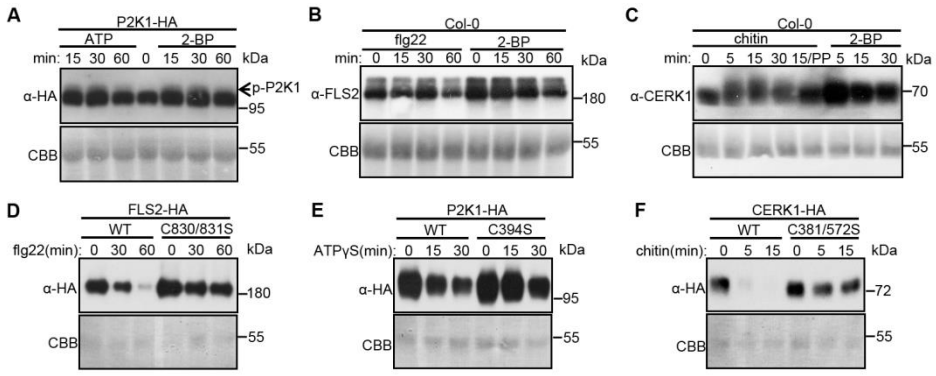


Figure S4. PAT5 and PAT9 regulate P2K1, FLS2 and CERK1 phosphorylation and degradation, Related to Figure 4

(A) P2K1-HA phosphorylation was analyzed by immunoblot in wild type plant upon addition of 200 μM ATPγS or 50 μM 2-BP (*S*-acyltransferase inhibitor). p-P2K1, phosphorylation of P2K1.

(B) 2-BP reduced endogenous FLS2 degradation. Leaf discs of Col-0 treated with 20 μM flg22 and 50 μM 2-BP were used for FLS2 protein detection.

(C) 2-BP enhances endogenous CERK1 phosphorylation and reduces CERK1 degradation. Leaves of Col-0 infiltrated with 100 μg/ml chitin and 50 μM 2-BP were used for CERK1 protein detection. PP, lambda protein phosphatase.

(D-F) Analysis of the rate of turnover of P2K1, FLS2 and CERK1 proteins modified (C→S) at the site of *S*-acylation. The indicated constructs were transformed into *Arabidopsis* wild-type Col-0 protoplasts treated with 200 μM ATPγS, 10 μM flg22 or 100 μg/ml chitin.

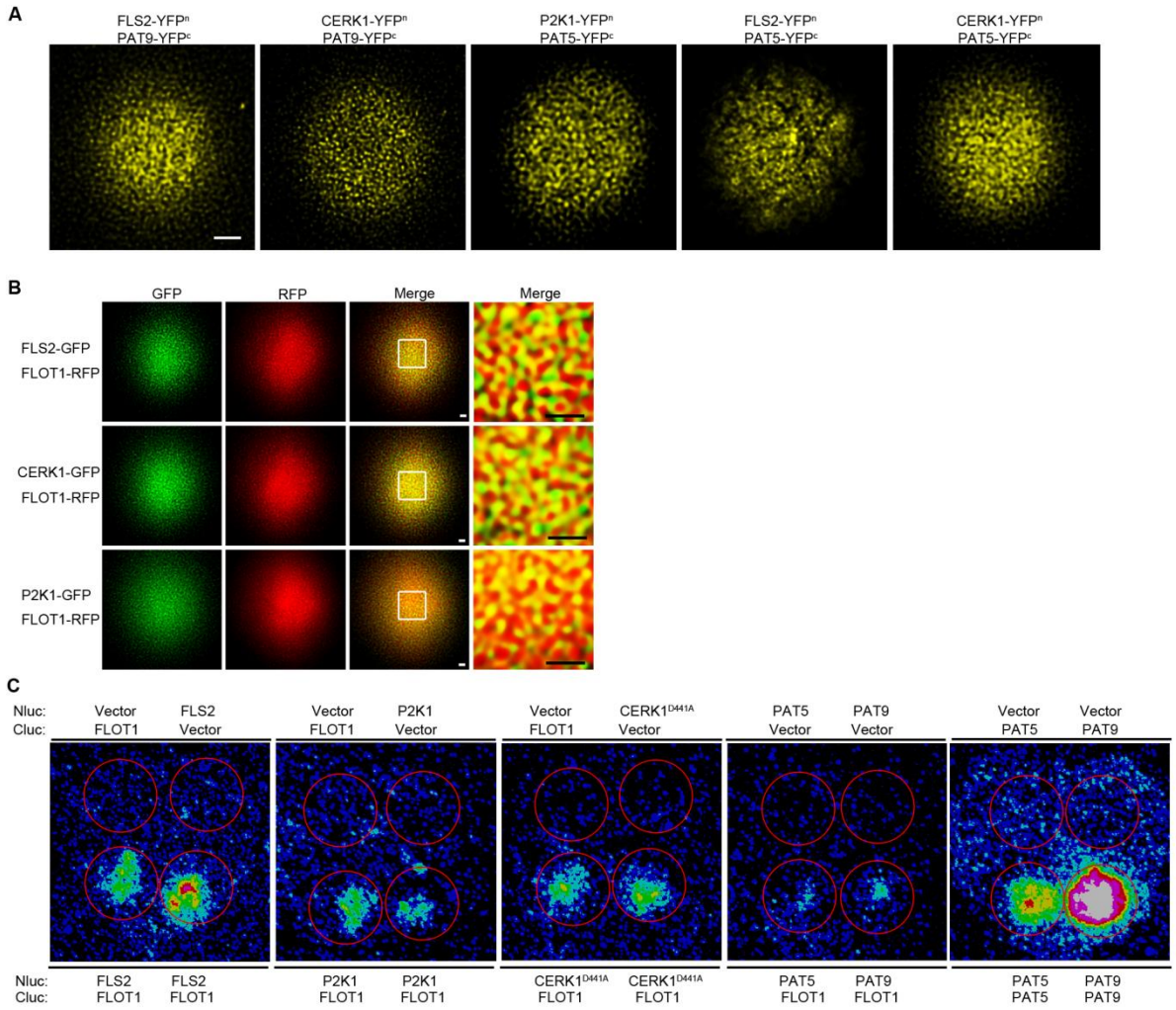


Figure S5. PAT5, PAT9 and FLOT1 interact with FLS2, CERK1 and P2K1 in PM nanodomains, Related to Figure 5

(A) PAT9 and PAT5 interact with receptors in PM nanodomains. The indicated constructs were transiently co-expressed in *Arabidopsis* protoplasts, the dispersed punctate fluorescence signaling was observed and analyzed using confocal microscopy. Bar = 5 μM.

(B) FLS2, CERK1 and P2K1 co-localize with the nanodomain marker flotilin (FLOT1). Confocal micrographs of FLS2-, CERK1- and P2K1-GFP plasma membrane localization after transient co-expression with FLOT1-RFP in *Arabidopsis* protoplasts. The dashed squares represent the areas magnified within the far right image. Bar = 1 μM.

(C) FLOT1 interacts with FLS2, CERK1, P2K1, PAT5 and PAT9 by the firefly LCI assays in tobacco leaves in the dark. PAT5 and PAT9 form homodimers and show stronger LCI signaling.

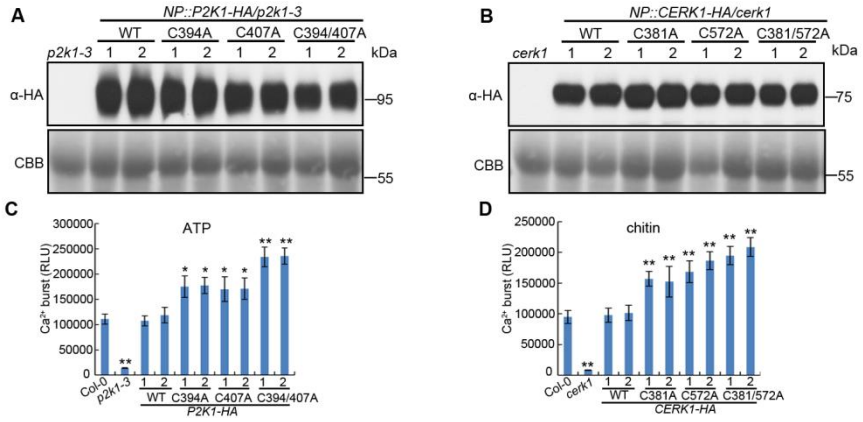


Figure S6. *S*-Acylation of FLS2, CERK1 and P2K1 negatively mediate PTI, Related to Figure 6

(A and B) Total P2K1-HA and CERK1-HA proteins were detected by anti-HA immunoblot for the stable transgenic plants. CBB, Coomassie brilliant blue staining.

(C and D) ATP and chitin-induced calcium influx were significantly increased in plants expressing CERK1 or P2K1 proteins lacking critical cysteine residues for *S*-acylation. RLU, relative luminescence units; Error bars indicate ± SEM; n = 12 (biological replicates, one-sided ANOVA). *P < 0.05, **P < 0.01, Student's t test.