

Colour key

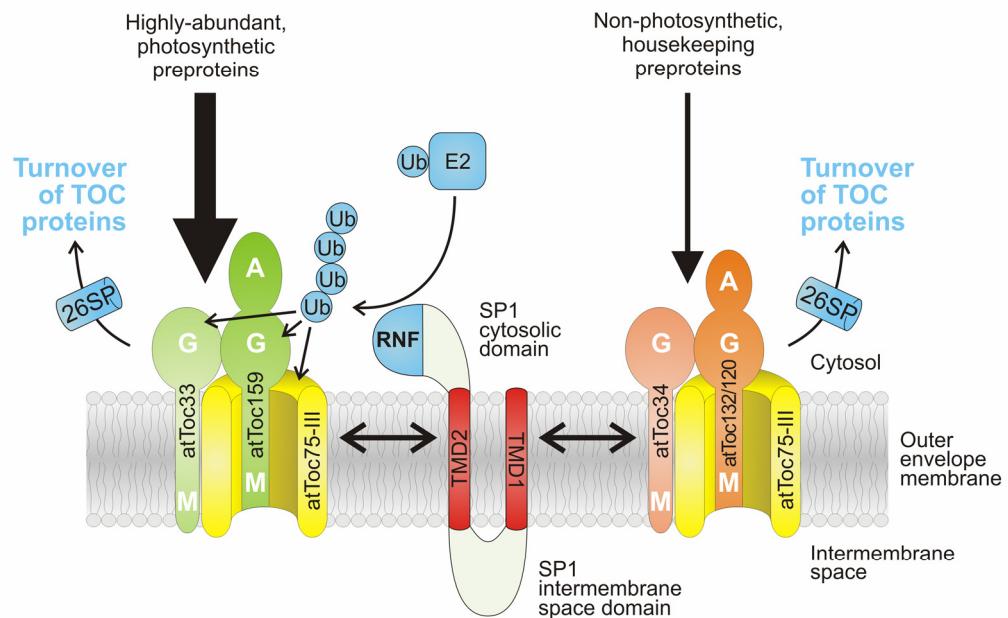
Blue – main figures, 1-4 **(HIGHLIGHTER = FINISHED FIGURE)**

Red – supplementary figures **(HIGHLIGHTER = FINISHED FIGURE)**

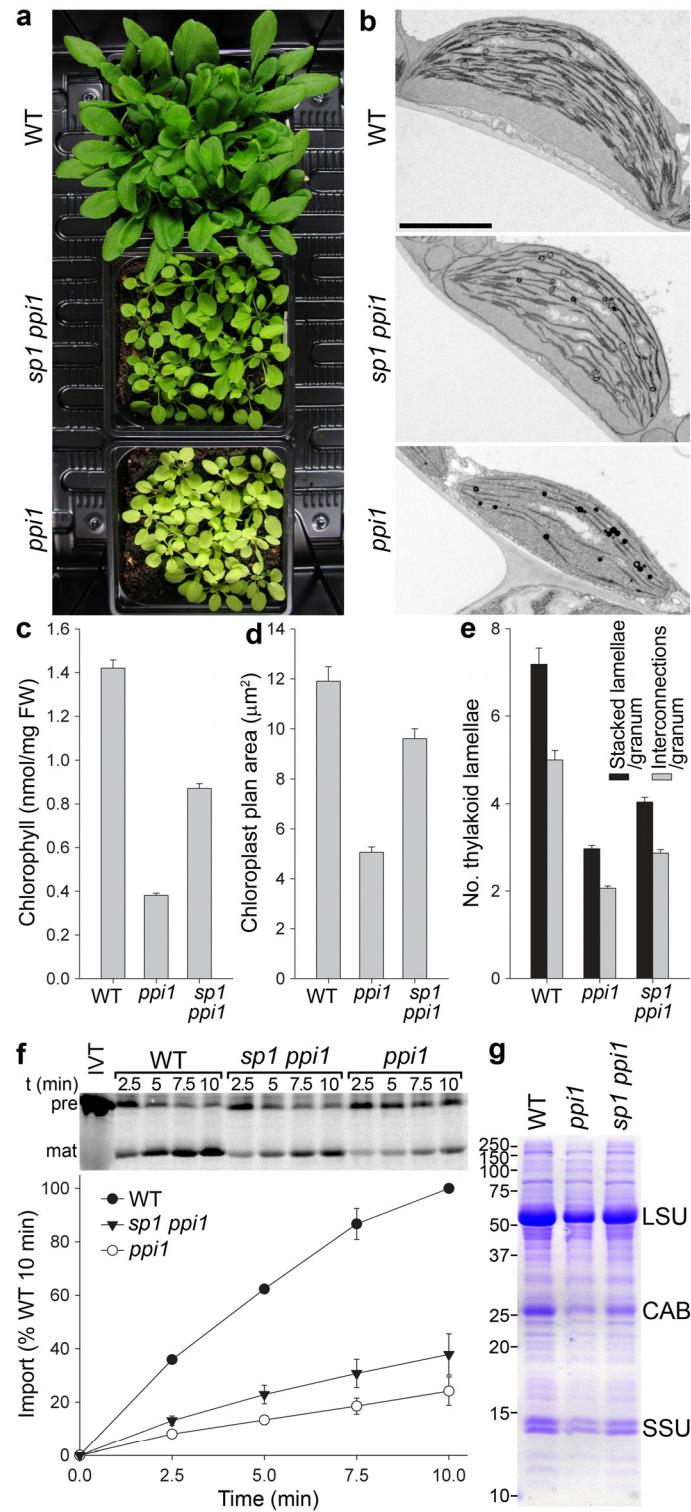
Gray – data not shown?

Black – PJ's comments, suggestions and queries

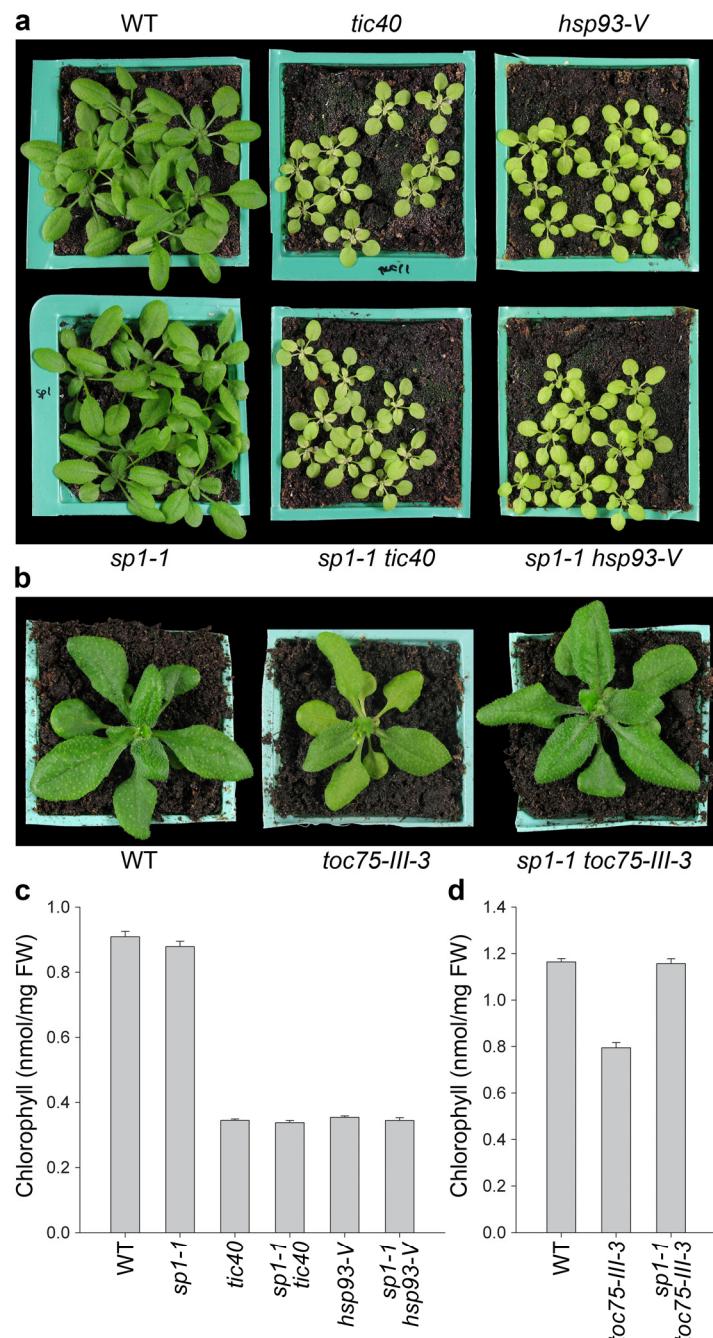
S1. Schematic model.



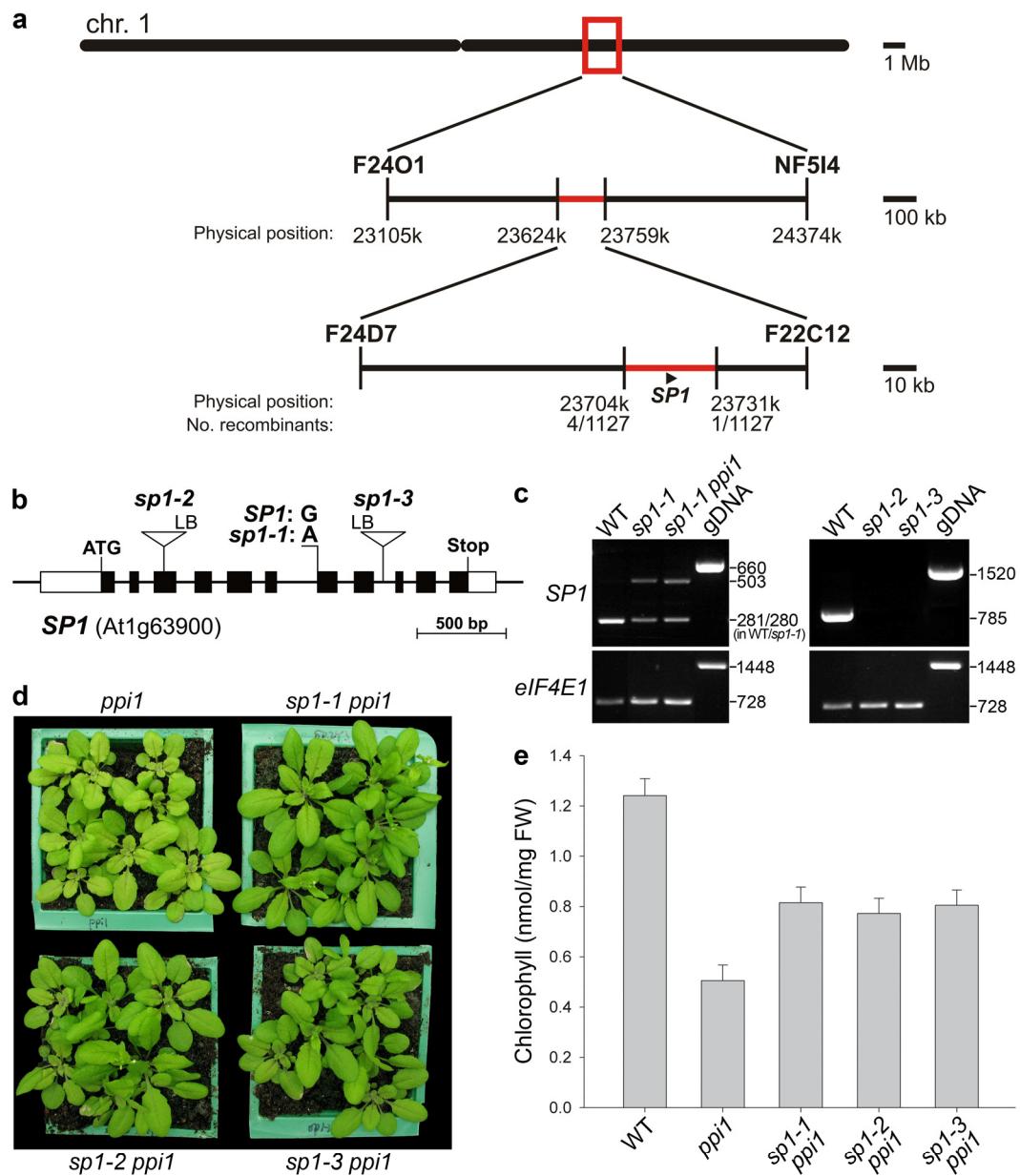
1. SP1 can suppress the defect of *ppi1* in leaf size, colour, chlorophyll content and chloroplast ultrastructure. SP1 can suppress the import defect of *ppi1*



S2. The suppression of *ppi1* by *sp1* is specific



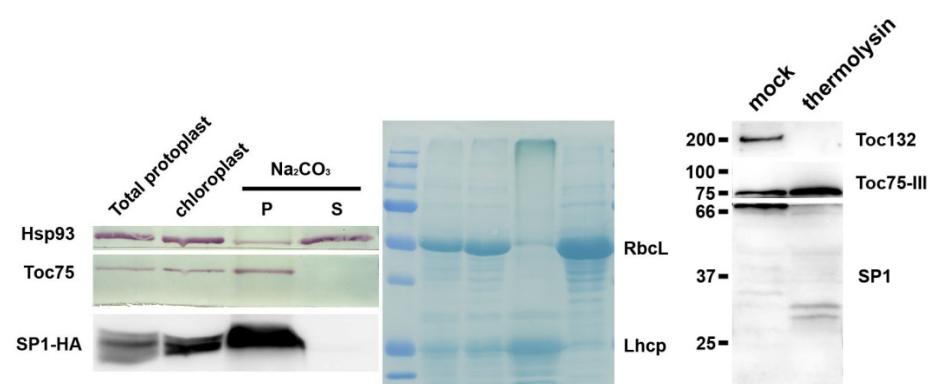
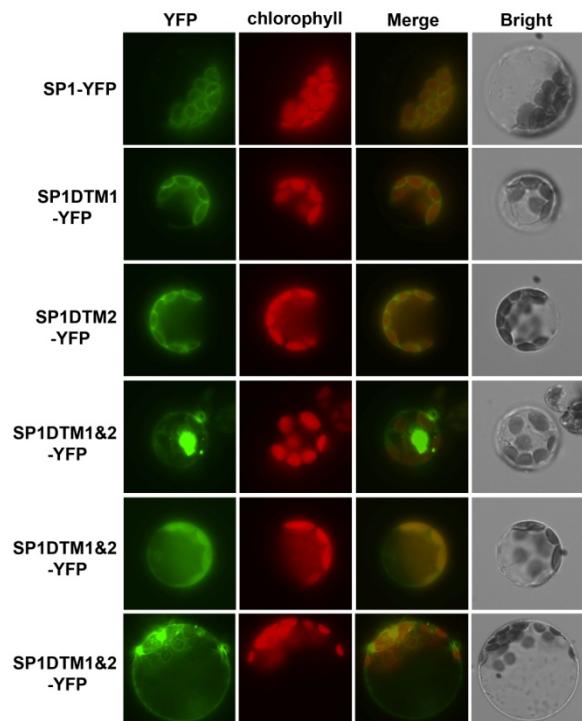
S3 Map-based cloning identify that SP1 encodes a Ring E3 ligase. All three alleles of sp1 mutant show the same suppression on ppi1.



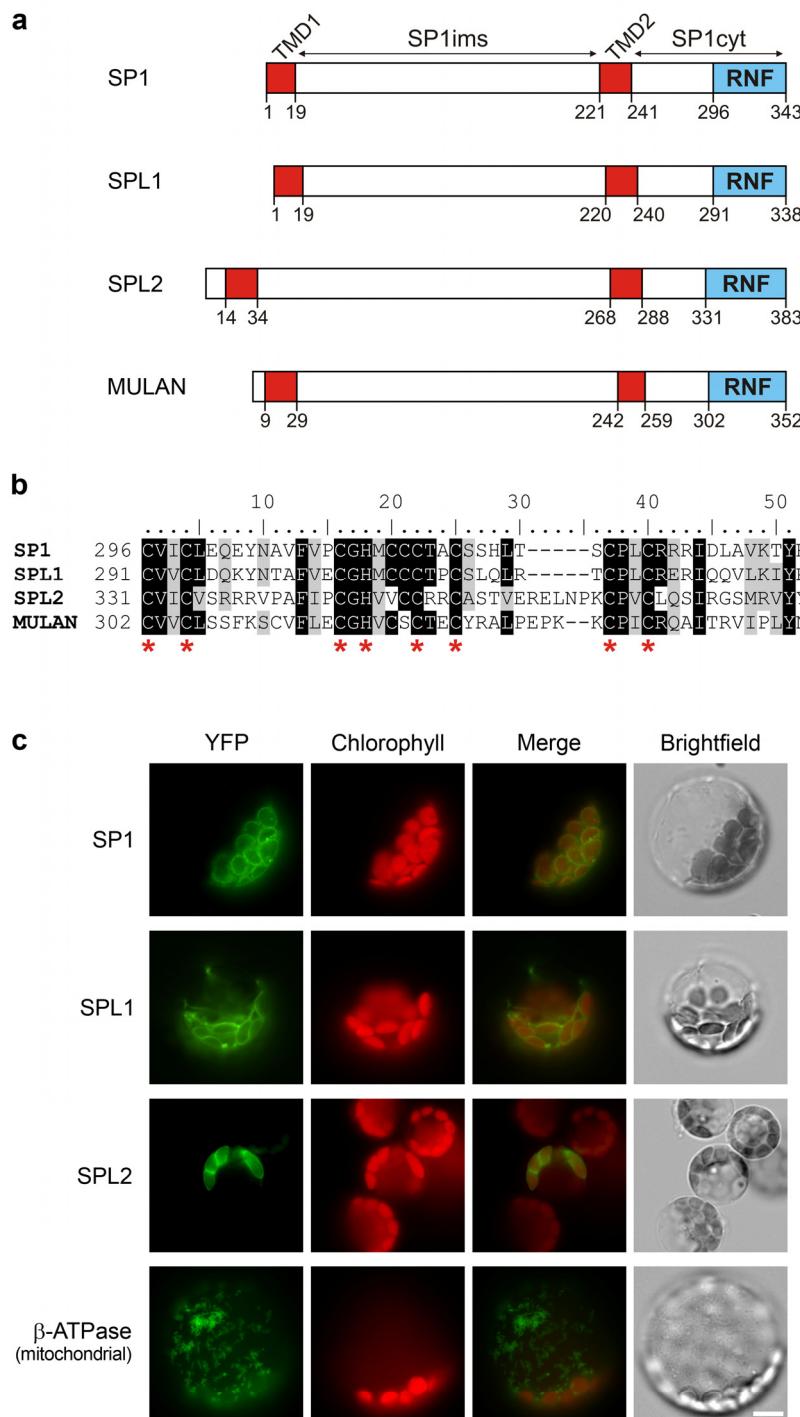
2. SP1 is a chloroplast integral outer membrane protein, showing a c-terminal facing cytosol topology with two transmembrane domains and one IMS domain.

[all the microscopy images lack scale bars – these need to be added. Also, it looks like the magnification level varies between some images – this needs to be checked and, if appropriate, corrected]

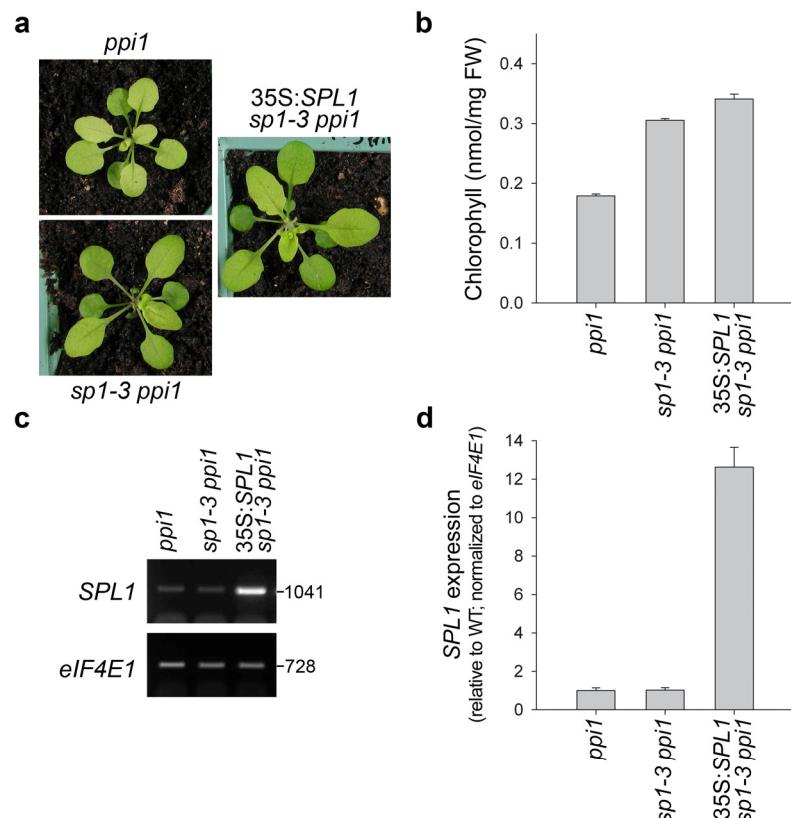
[I think it might be better not to show the data for constructs lacking just a single TMD]



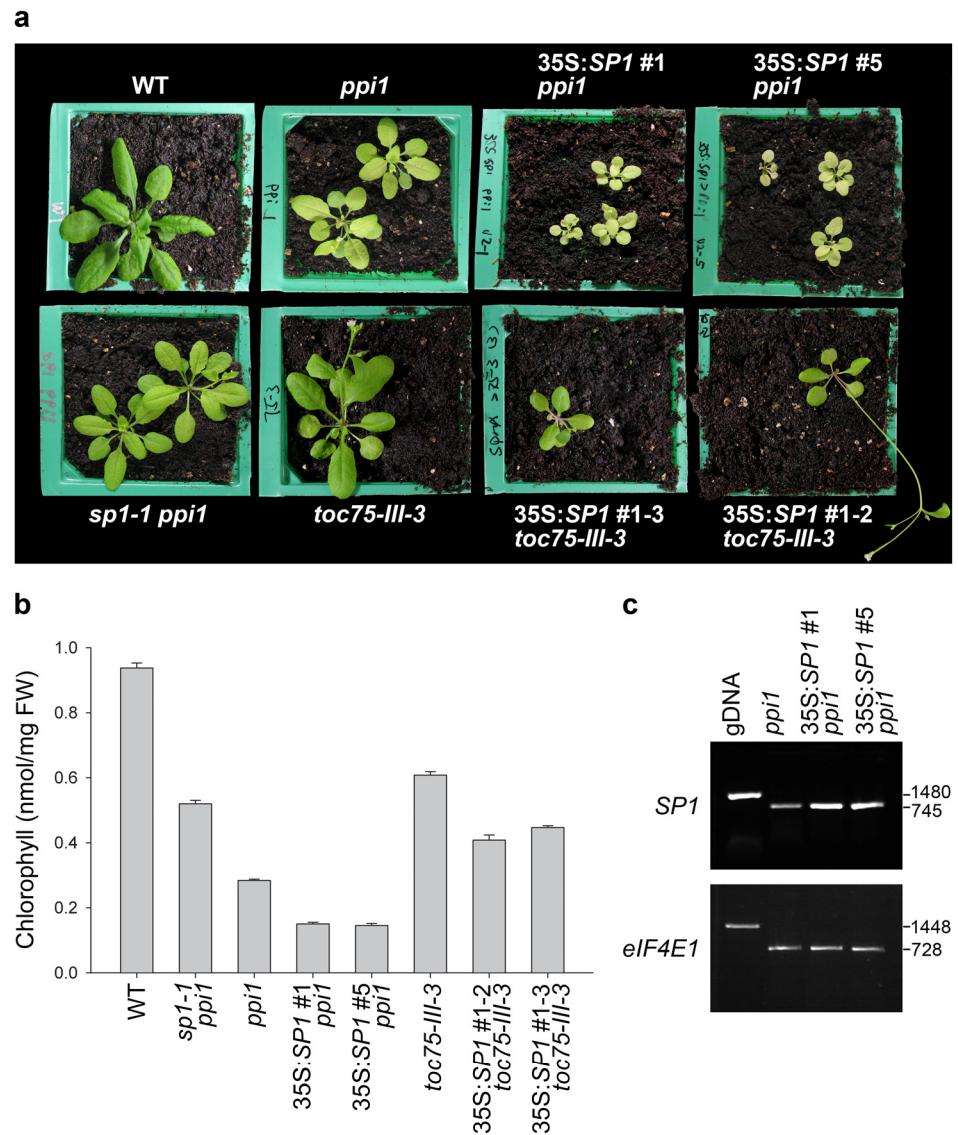
S4. SPL1 and SPL2 have the same domains and localization as SP1.



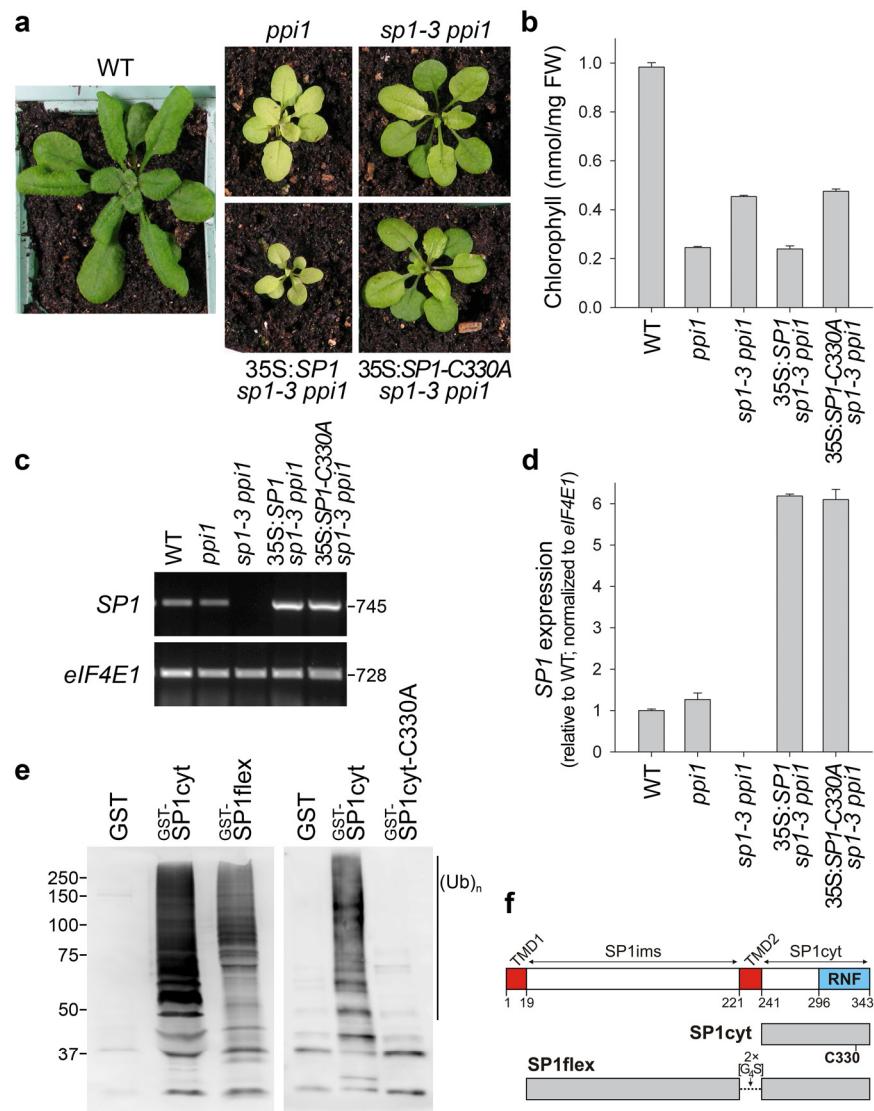
S5. Overexpression of SPL1 cannot complement the sp1 mutation.



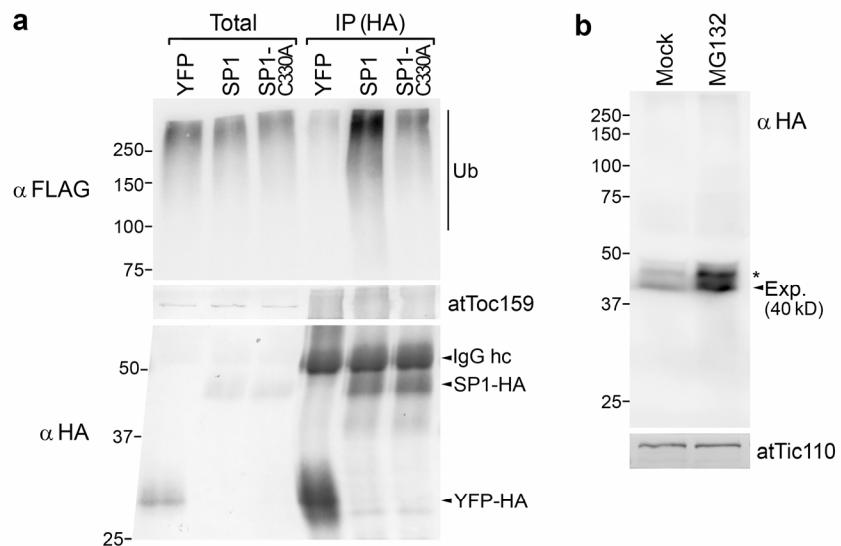
S6. SP1 overexpression enhances the phenotypes of TOC mutants, indicating SP1 is a regulator of the TOC complex.



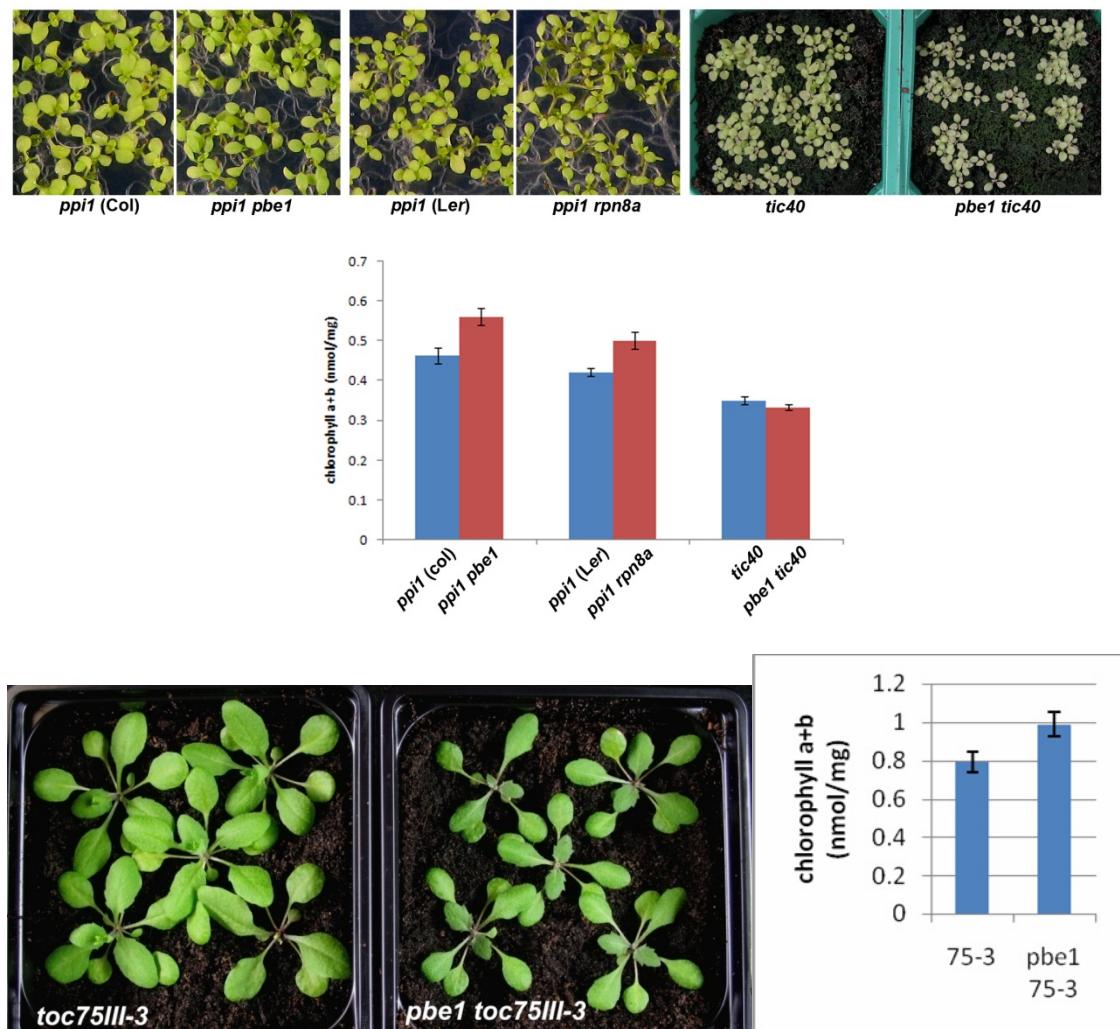
S7. The SP1 RING domain is vital for the protein function in planta, indicating SP1 plays a real role of ubiquitination. SP1 has E3 ligase activity in vitro, and it is RING domain dependent.



S8. SP1 is auto-ubiquitinated in vivo, and it is regulated by the UPS system.



S9. 26S proteasome mutants have the similar suppression effect on TOC mutants, suggesting the role of UPS on TOC protein in planta.



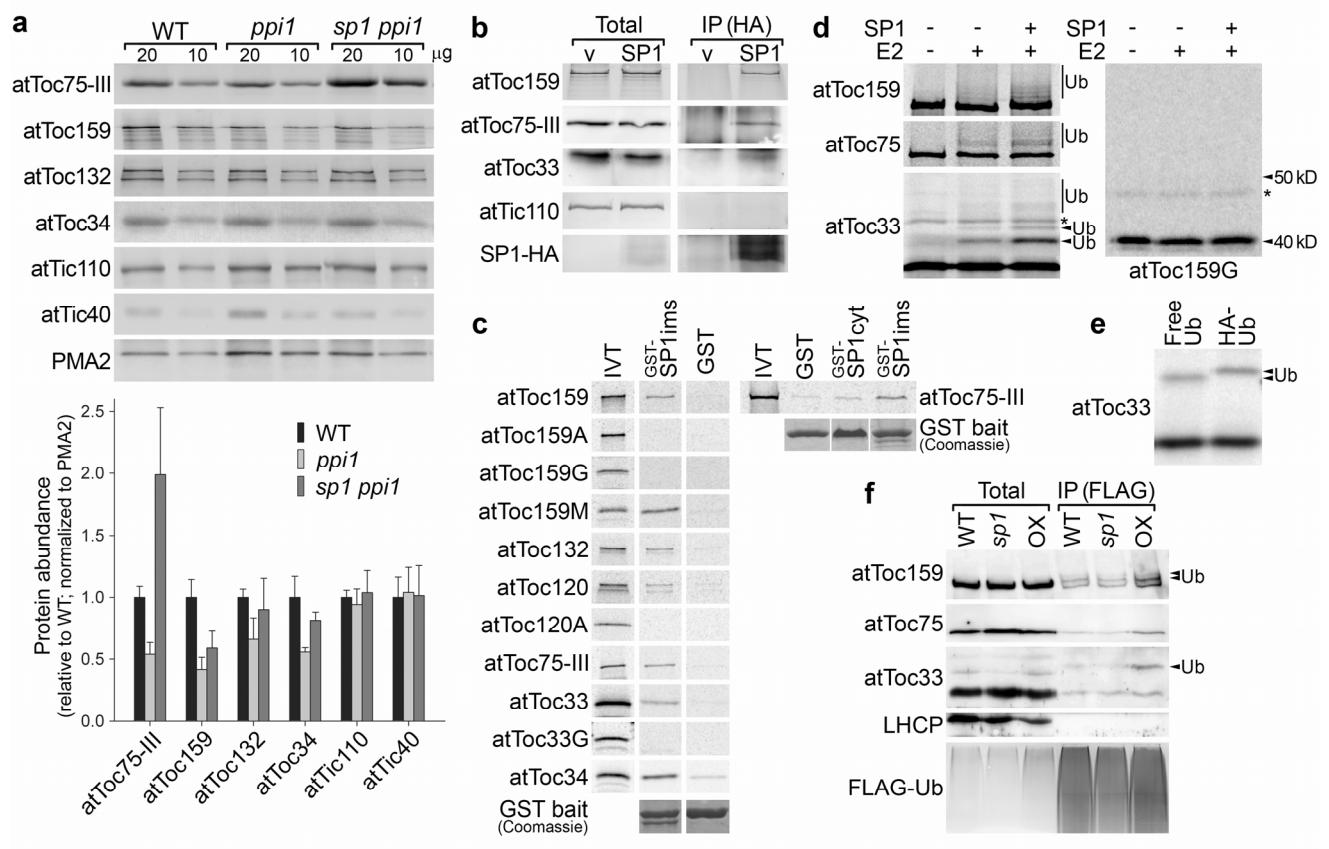
3 (a) *sp1* might suppress *ppi1* by increase the TOC protein level, which indicating SP1 might be responsible for the degradation of TOC proteins.

3 (b) SP1 is associated with TOC complex

3 (c) SP1 can directly interact with the key TOC components. The interaction site is probably the SP1 IMS domain and the transmembrane domains of TOC receptors.

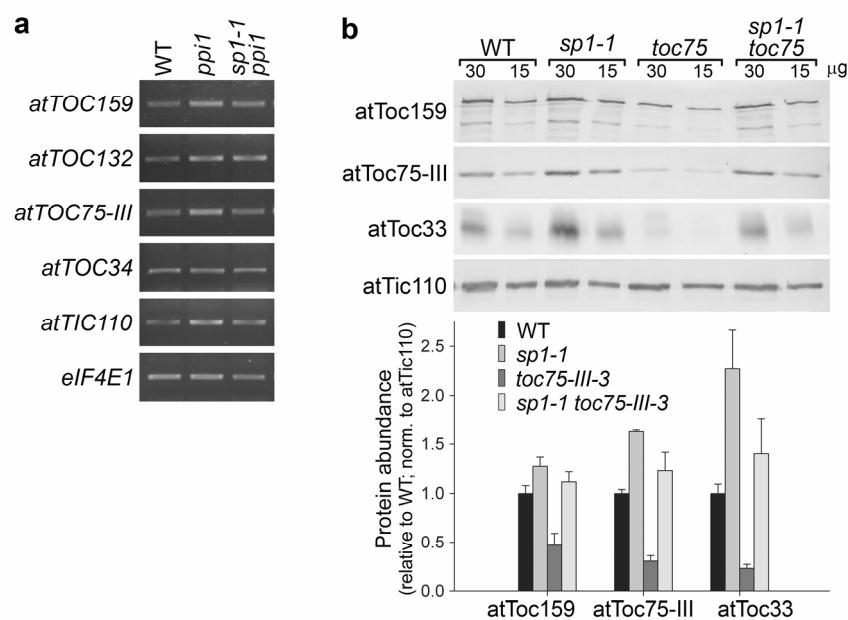
3 (d,e) SP1 can ubiquitinate TOC receptors in vitro

3 (f) SP1 is likely to ubiquitinate TOC receptors in vivo

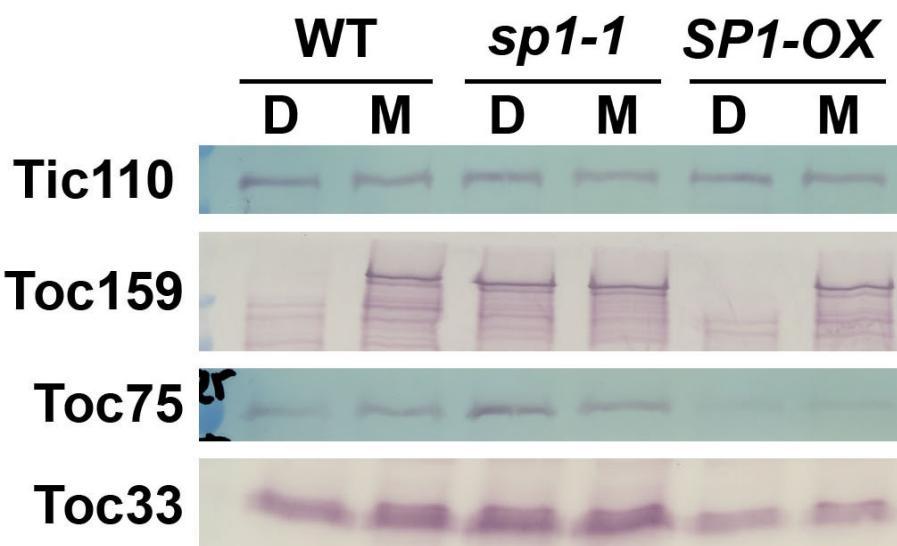


S10. TOC protein level increases in sp1 are not due to the transcriptional level changes.

The suppression of *toc75-III-3* is also associated with increased TOC protein levels.

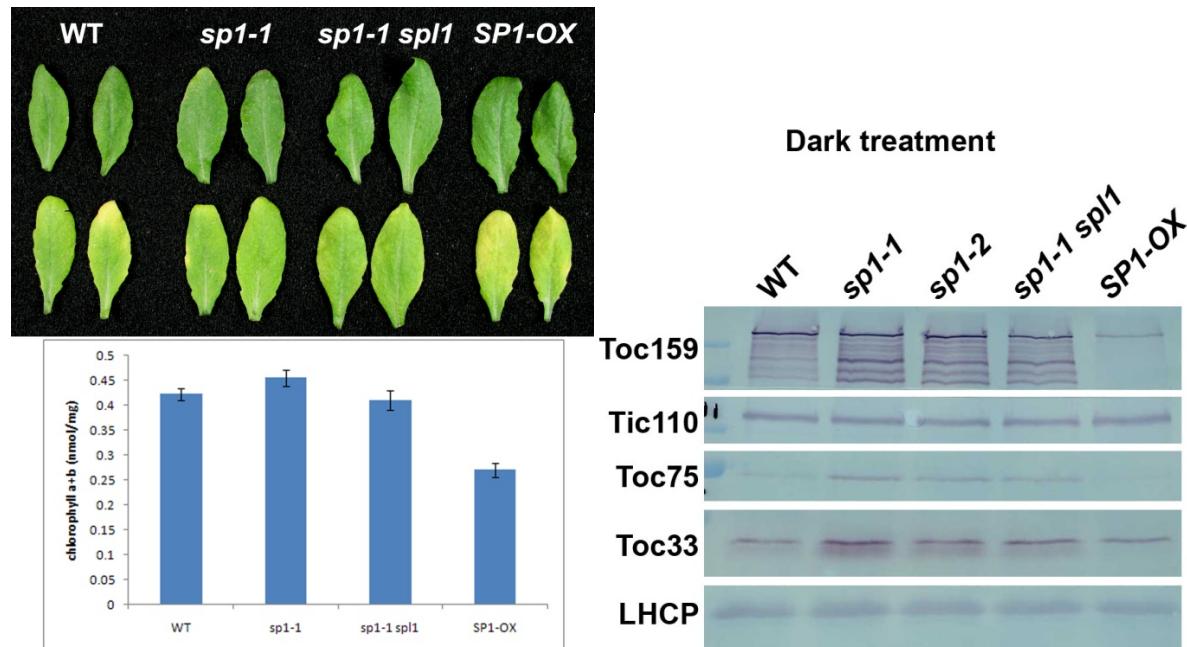


? The protein level of receptors increase in *sp1* protoplasts and decrease in *SP1-OX* protoplasts, and MG132 treatment can lead to the same increasing effect as *sp1* mutation (but have no additive effect on *sp1* mutant), indicating the proteins are regulated by UPS intermediated by SP1.

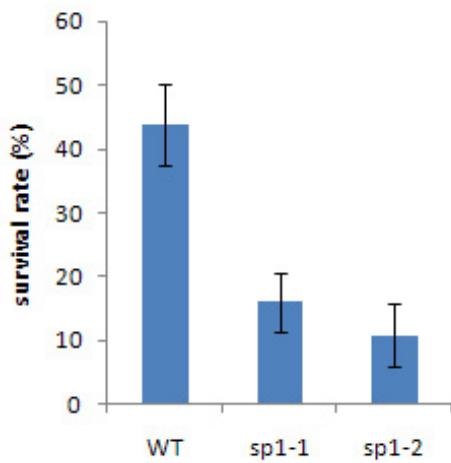


4. SP1 might play an important role in plastid transition by degrading TOC proteins

[I would consider removing the *sp1 spl1* double mutant from this figure]
 [what do the western blots here tell us exactly?]



Deetiolation assay



[As a minimum, the deetiolation figure needs a nice accompanying photograph to show typical phenotypes. It would also be good if this part could be “boosted” somehow, e.g., with TEMs or immunoblots or something – any ideas? You could do chlorophyll measurements as in Mochizuki 1996 perhaps?]

ROOT GREENING DATA TO GO HERE ALSO, HOPEFULLY