

Identifying Interacting Proteins of Arabidopsis Cyclophilin38 (AtCYP38), a Key Factor for PSII Assembly/Repair, via Multiple Screening Approaches

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Abstract

Background

AtCYP38, a thylakoid lumen localized immunophilin, is essential for photosystem II (PSII) assembly and maintenance, but how AtCYP38 functions in chloroplast remains unknown. Based on previous functional studies and its crystal structure, we hypothesize that AtCYP38 should function via binding its targets or cofactors in the thylakoid lumen to influence PSII performance. Therefore, identifying its target proteins and cofactors would be a key step to understand the working mechanism of AtCYP38.

Results

To identify potential interacting proteins of AtCYP38, we first adopted two web-based tools, ATTED-II and STRING, and found 15 proteins functionally related to AtCYP38. We then screened a yeast two-hybrid library including an Arabidopsis genome wide cDNA with the N-terminal domain, the C-terminal domain, and the full-length mature protein of AtCYP38. 25 positive targets were identified, but a very limited number of target proteins were localized in the thylakoid lumen. In order to specifically search interacting proteins of AtCYP38 in the thylakoid lumen, we created a yeast two-hybrid mini library including the thylakoid luminal proteins and lumen fractions of thylakoid membrane proteins. After screening the mini library with 3 different forms of AtCYP38, we obtained 6 thylakoid membrane proteins and 9 thylakoid luminal proteins as interacting proteins of AtCYP38. We further confirmed the localization of several identified proteins and their interaction between AtCYP38.

Conclusions

After analysis with two web-based tools and yeast two-hybrid screenings against two different libraries, we identified a couple of potential interacting proteins, which could be functionally related to AtCYP38. We believe that the results will lay a foundation for unveiling the working mechanism of AtCYP38 in photosynthesis.

Background

In photosynthetic organisms, photosystem II (PSII) is a large multiprotein complex, which is composed of more than 20 protein subunits, many cofactors and pigment molecules on the thylakoid membrane [1, 2]. It catalyzes the light-driven oxidoreduction of water and plastoquinone, and pumps electrons to the photosynthetic electron transfer chain [3, 4]. Proper biogenesis and maintenance of PSII is essential for photosynthesis in chloroplasts. A large number of auxiliary proteins, including immunophilins in the thylakoid lumen, have been demonstrated to be required for the assembly and/or repair of PSII [5, 6, 7].

Immunophilins include two groups of proteins without significant sequence homology: cyclophilins (CYPs) and FK506-binding proteins (FKBPs), which are cellular receptors for potent immunosuppressive drugs: cyclosporin A and FK506, respectively [8, 9]. CYPs and FKBPs share a common feature of peptidyl-

prolyl cis/trans isomerase (PPlase) activity, which is essential for trafficking and protein folding in cells. These proteins are widely present in prokaryotic and eukaryotic organisms with diverse cellular functions, including mediating protein folding, chaperone activity, scaffold facilitating [10, 11]. In Arabidopsis, a large fraction of immunophilins (17 out of 52 members) are localized in chloroplasts, and interestingly, 16 of the 17 chloroplast immunophilins are presumably present in the thylakoid lumen [11, 12, 13]. Given a small number (~100) of proteins in the thylakoid lumen proteome and relatively a large number (16) of immunophilins in this compartment [12, 13, 14], it is reasonable to assume that immunophilins could be functionally related to the assembly and/or maintenance of thylakoid protein complexes. The recent findings that AtCYP38 and AtFKBP20-2 play key roles in PSII assembly and repair confirm the critical physiological roles of immunophilins in the thylakoid lumen [15, 16, 17].

Arabidopsis cyclophilin38 (AtCYP38) is a greatly divergent immunophilin located in the thylakoid lumen of eukaryotic photosynthetic organisms [18]. Its ortholog in spinach, also named as TLP40, was originally identified as a cofactor of a chloroplast phosphatase [19], and was proposed to function as a negative regulator of a putative thylakoid protein phosphatase [20, 21]. In Arabidopsis, AtCYP38 is indispensable for the correct assembly and rapid repair of PSII [16, 17]. Arabidopsis mutant plants with a T-DNA disrupted *AtCYP38* gene show a stunt growth phenotype and are very sensitive to high light stress [16, 17]. This protein may be required for the proper folding of PSII core proteins D1 and CP43, and the successful assembly of PSII oxygen evolving complex (OEC) [17]. Recently, a study showed that AtCYP38 may negatively regulate PsbO2 activity, and subsequently facilitate the degradation of photo-damaged D1 protein [22]. By far, how AtCYP38 plays its roles in PSII assembly and maintenance is not clearly understood.

Crystal structure analysis demonstrated that AtCYP38 contains two distinct major domains: an N-terminal α -helical bundle and a C-terminal cyclophilin β -sheet barrel, with a short acidic loop as a linker in the middle [23]. The two domains are packed together to form a strong intramolecular interaction. This particular structure of AtCYP38 indicates a possible autoinhibition mechanism for its function via the N- and C-terminal interaction. Enzyme activity assay and structure analysis showed that AtCYP38 is not an active PPlase [23]. It is consistent with previous reports that PPlase activity in the thylakoid lumen was strictly attributed to only two immunophilins, AtCYP20-2 and AtFKBP13 [24, 25, 26]. It is likely that AtCYP38 plays important roles in maintaining normal configuration of its substrates with a chaperone-like activity via protein-protein interaction. According to the above comprehensive analysis, searching for partner proteins of AtCYP38 would be of great significance for understanding its working mechanism.

To identify its substrate proteins and potential cofactors, we first carried out the co-expression network ATTED-II [27] and protein-protein interaction network STRING [28] analysis to find potential partner proteins of AtCYP38. Then we performed yeast two-hybrid (YTH) assays to screen against an Arabidopsis genome wide cDNA library with three different forms of AtCYP38. Since AtCYP38 protein is located in the thylakoid lumen, we further constructed an YTH mini library including previously identified luminal proteins and membrane proteins with luminal fractions [12, 13, 14], and screened it with three different forms of AtCYP38. At the end, we confirmed their chloroplast localization of several identified

proteins and their interactions with AtCYP38. We believe that we obtained certain interacting proteins functionally related to AtCYP38, and it will lay a foundation to understand the possible mechanism on how AtCYP38 plays its roles in the thylakoid lumen.

Results

Functionally related proteins of AtCYP38 revealed by web-based tools

In order to search potential interacting proteins of AtCYP38, we first adopted the co-expression network program ATTED-II to search for proteins whose expression pattern is related to AtCYP38. The program revealed 20 proteins are tightly co-expressed with AtCYP38 (Fig. 1A and Table 1). Among these proteins, 8 proteins are localized in the thylakoid lumen, including FKBP18 (At1g20810), PPL (At1g76450), FKPB16-3 (At2g43560), PRP (At2g44920), PPD6 (At3g56650), TAPX4 (At4g09010), TLP15 (At5g52970), and TLP17.4 (At5g53490); and 1 protein, HHL1 (At1g67700), resides on the thylakoid membrane. Considering the thylakoid lumen localization of AtCYP38, we assumed that these 9 proteins might be functionally related to AtCYP38. Meanwhile, the other 10 co-expression proteins, which are localized in the chloroplast stroma (Fig. 1A and Table 1), are not likely to physically interact with AtCYP38.

In order to evaluate the ATTED-II co-expression results, we also applied the functional protein association tool, STRING, to screen potential proteins related to AtCYP38. The results showed that there are 10 chloroplast proteins functionally related to AtCYP38 at the highest confidence value. Out of these proteins, 4 are localized in the chloroplast thylakoid lumen, including TLP18.3 (At1g54780), PRXQ (At3g26060), MPH2 (At4g02530), and FKBP13 (At5g45680), and 2 proteins, LQY1 (At1g75690) and ATPD (At4g09650), reside on the thylakoid membrane (Fig. 1B and Table 2). All the other proteins are not localized in the chloroplast thylakoid lumen. They might not physically interact with AtCYP38, though they could be functionally related.

After network analysis with these 2 different programs, we found that no protein was co-detected by both software. We assumed that the different results from these two web-based detections could be partially caused by their unique algorithms. Considering their localization and their functions in photosynthesis, we presumed that all these 15 lumen or membrane localized proteins might be functionally related to AtCYP38.

Target proteins screening against a library containing genome wide cDNA

Yeast two-hybrid (YTH) screening is a powerful and well-established tool to identify interested proteins which could be physically associated with a specific target protein. To identify potential targets and cofactors of AtCYP38, we launched a yeast two-hybrid screening with AtCYP38 as the bait protein. X-ray crystal structural analysis showed that the mature full-length AtCYP38 is composed of an N-terminal α -helical domain and a C-terminal cyclophilin β -sheet domain, and these two domains could possess different functions [23]. Therefore, we utilized three baits representing different forms of AtCYP38, including the mature full-length protein (CYP38F, without signal peptide), the N-terminal α -helical domain

(CYP38N), and the C-terminal β -sheet domain (CYP38C) to perform yeast two-hybrid screening against an Arabidopsis genome wide cDNA library (Fig. 2A).

We screened approximately a total of 2.0×10^7 yeast transformants with these 3 bait proteins, respectively. When CYP38F is used as the bait, we obtained about 70 confirmed positive colonies. After data filtering and interaction confirmation, we found that 8 chloroplast proteins could be interacting partners of AtCYP38 (Fig. 2B, 3A and Table 3). Only 3 proteins were localized in the chloroplast thylakoid lumen, including PSBP-1 (At1g06880), TSP9 (At3g47070) and DEG8 (At5g39830). After screening with CYP38N as the bait, we obtained approximately 50 confirmed positive clones, and further narrowed down to 4 candidate proteins according to their sequence results. However, none of 4 candidate protein was localized in chloroplast (Fig. 2B, 3B and Table 4). Notably, there are a very limited number of positive colonies obtained from screening with CYP38F and CYP38N as baits, indicating that those two baits display a low activity in the YTH screening assay.

When screening the same library with the CYP38C domain, we then obtained about 200 confirmed positive colonies, which were much more than those obtained from screening with CYP38F or CYP38N. After data cleaning up and re-validation, we obtained 15 chloroplast proteins to be interacting candidates of AtCYP38 (Fig. 2B, 3C and Table 5). Interestingly, several chlorophyll binding proteins were identified in this screening, including LHCB1.1, LHCB1.2, LHCB1.5 and LHCB4.1. Other 3 thylakoid membrane proteins, the PSI subunit PASG, the Cytb6f complex subunit PETC, and ROD1[29], an enzyme involved in lipid metabolism, were also found to interact with AtCYP38. Three interacting proteins are thylakoid lumen localized proteins, including PSBP-1, PETE2, and AtCYP38. Notably, PSBP-1 is also a partner of CYP38F. In addition, we also found some proteins are not localized in the thylakoid lumen but still can be identified by yeast two-hybrid assay (Table 5). However, it is unlikely that these proteins physically interact with AtCYP38, considering the different compartmental locations of these proteins and AtCYP38.

It was suggested that the C-terminal domain plays its function to bind other proteins, and the N-terminal bundle domain acts as the regulatory domain, which suppressing AtCYP38 to bind its targets [23]. To test this hypothesis, we cross examined the identified proteins via cDNA library screening with different baits individually. We found that all target proteins screened by CYP38F and CYP38N could interact with CYP38C, but proteins identified from screening with CYP38C could not interact with CYP38F or CYP38N (Fig. 4), which is in agreement with the above assumption.

Altogether, after screening 2.0×10^7 colonies with three different forms of AtCYP38, we obtained a very limited number of potential interacting partners of AtCYP38. Only a few thylakoid lumen proteins were identified in the YTH screening, including PSBP-1, TSP9, DEG8, PETE2 and AtCYP38. We initially expected that a larger number of interacting proteins of AtCYP38 could be identified, given its the chaperone-like activity. The unexpected YTH screening results prompted us to think that screening against a genome wide cDNA library may not be an efficient way to identify the interested interacting proteins. Because proteins in the thylakoid lumen only occupy a very small fraction of the plant cell proteome, it is not surprising that cDNA for thylakoid lumen proteins are relatively rare in this library. Therefore, there exist

too much noise background due to an overwhelming amount of unrelated proteins in this screening, and consequently, the chance would be very low for AtCYP38 to fish out target proteins which are located in the chloroplast thylakoid lumen.

Yeast two-hybrid screening against a thylakoid lumen protein mini library

Given that screening the genome wide cDNA library was not a very successful experience, we decided to launch an alternative screening strategy. Since AtCYP38 is a thylakoid luminal protein, it would be a better choice to screen a library only harboring thylakoid lumen proteins and thylakoid membrane protein with luminal domains, therefore massive unrelated proteins outside of this compartment would not interfere this screening process.

In order to achieve that, we constructed a yeast two-hybrid mini library harboring a majority of the previously identified luminal proteins and luminal domains of various thylakoid membrane proteins. The library was screened against baits including CYP38F, CYP38N and CYP38C, respectively. Interestingly, when we used CYP38F or CYP38N domain to perform the screening, we failed to find any positive potential interacting proteins.

When we used CYP38C to screen the YTH luminal protein mini library, we obtained 15 potential interacting proteins, including 9 thylakoid lumen soluble proteins, and lumen fragments of 6 thylakoid membrane proteins (Fig. 5, Table 6). In detail, these soluble luminal proteins are, At1g21500, At2g23670, At2g26340, CTPB (Carboxyl terminal peptidase, At3g57680), CTPA (At4g17740), TLP17.9 (At4g24930), DEG14 (At5g27660), CTPC (At5g46930), and TLP15 (At5g52970). Interestingly, 3 proteases responsible for process the C-terminal extension of D1 precursor (pD1), including CTPA, CTPB, and CTPC were all found to be interacting proteins of AtCYP38. And another protease, DEG14, was also found to interact with AtCYP38. 6 thylakoid membrane proteins whose lumen fragments could be bind to AtCYP38 included PSBM, CYTF, ALB3, CP43, CP47, and PSAF. We assumed that the membrane proteins and those proteases might be substrates of AtCYP38. The result from this screening is consistent with the *in vivo* function of AtCYP38 in the assembly of PSII supercomplex [16, 17].

Validating localization and *in vitro* pull-down confirmation assay.

AtCYP38 is known to be present in the chloroplast thylakoid lumen, and interacting proteins should be in this subcellular compartment as well. In order to verify the localization of candidate proteins, we selected three predicted luminal proteins, At1g21500, TLP17.9 (At4g24930) and TLP15 (At5g52970), and fused the green fluorescent protein (GFP) tag to their C-terminus. The GFP-fused proteins were transiently expressed in leaf epidermal cells from *Nicotiana benthamiana* and analyzed by confocal microscopy. As we expected, At1g21500, TLP17.9 and TLP15 are localized in the chloroplast (Fig. 6A).

In order to further confirm the direct interaction between identified proteins with AtCYP38, we selected PSBP-1 and FKBP13 to perform the GST pull-down assays. As shown in Fig. 6B, GST-PSBP-1 and GST-FKBP13 were specifically pulled down by CYP38C-HIS but not GST, confirming the interaction between

those two proteins and CYP38C *in vitro*. At the meantime, we also included two proteins with unknown function, At1g21500 and TLP17.9, to confirm their interaction with AtCYP38 by the same method. GST-21500 or GST-24930 also can be observed an obvious interaction with CYP38C in the pull-down assay (Fig. 6B). Overall, these results indicated that the interactions between AtCYP38 and our candidate proteins are valid, and our screening strategies are reliable and efficient.

Discussion

As a key protein functioning in PSII repair and assembly, AtCYP38 should physically interact with its substrate proteins and cofactor proteins to play its roles in the chloroplast thylakoid lumen. Finding its luminal interacting proteins is a key initial step to understand how AtCYP38 perform its functions. In this study, we applied multiple approaches to identify luminal proteins potentially interacting with AtCYP38.

From the web-based programs, we obtained 12 thylakoid luminal proteins and 3 membrane proteins (Table 1 and 2). Screening against a genome wide cDNA library revealed that 5 luminal proteins (PSBP-1 detected twice) and 8 thylakoid membrane proteins could potentially interact with AtCYP38 (Table 3, 4 and 5). From the screening against thylakoid lumen mini library, we obtained 9 soluble thylakoid luminal proteins and 6 membrane proteins (Fig. 5, Table 6). We consider that all of these proteins identified by different approaches could be potential interacting proteins of AtCYP38, because they meet functional profiles in assembly and/or maintenance of photosynthetic apparatus.

Possible functional roles of AtCYP38 based on interacting proteins

Identified interacting proteins of AtCYP38 could be classified into two major groups: thylakoid membrane proteins and thylakoid lumen soluble proteins. Membrane proteins included light harvesting complex proteins (LHCBs), subunit proteins in PSII, PSI, Cytb6f, and ATPase complexes. The identification of PSII components strengthens the conception that AtCYP38 is functionally tightly related to PSII's function. The finding of components in other photosynthetic complexes implied that AtCYP38 might have additional functions other than a restricted role in PSII. The other membrane proteins such as HHL1 (At1g67700), LQY1 (At1g75690), and ALB3B (At2g28800) also probably suggested the multiple roles of AtCYP38 in chloroplast.

Remarkably, several thylakoid lumen proteases, including CTPA, CTPB, CTPC, DEG8, and DEG14 are identified as interacting proteins of AtCYP38. CTPs are responsible for the pD1 C-terminal extension processing, which is an essential step for PSII assembly and repair [30, 31]. DEG8 and DEG14 protein are involved in damaged protein degradation in the chloroplast thylakoid [32, 33], indicating that AtCYP38 is very likely to be involved in the photosynthetic apparatus repair process. TAPX4 and PRXQ are essential proteins protecting thylakoid membrane complexes from attack of reactive oxygen species (ROS) [34, 35], showing that AtCYP38 might play a role to response against ROS stress. We also found two thylakoid lumen immunophilins, including FKBP18 and FKBP16-3, to be interacting proteins of AtCYP38, indicating that these proteins could be the working partners of AtCYP38. A number of functionally unknown proteins could interact with AtCYP38, including At1g21500, At2g23670, At2g26340, TLP17.9 (At4g24930), TLP15

(At5g52970), and the phosphatase protein, TLP18.3 (At1g54780) [36, 37]. Especially, TLP15 and TLP18.3 were identified both by the web-based program and the YTH screening. There should be a strong functional relationship between these two proteins and AtCYP38. The TLP15 protein is also predicted to harbor phosphatase domain, implying a possible phosphatase function in thylakoid lumen. It indicated that AtCYP38 could be involved in protein de-phosphorylation, in agreement with earlier reports that AtCYP38 homolog in spinach is identified in a process of chloroplast phosphatase purification [19, 20, 21].

The possible working mechanism of AtCYP38

AtCYP38 protein contains two distinct domains: an N-terminal domain and a C-terminal domain, and these two domains were closely packed to form a strong intramolecular interaction [23]. The N-terminal domain was proposed to be a leucine zipper domain and responsible for the protein-protein interaction [19]. However, in our YTH screening, the N-terminal domain showed a very low affinity to interact with other proteins in YTH screenings. Similarly, the full-length mature AtCYP38 protein also showed a low affinity to interact with other proteins. On the contrary, the C-terminal domain could interact with a substantial number of thylakoid lumen proteins, which could be functionally related to AtCYP38. Therefore, we consider that the C-terminal domain is responsible for binding its functional targets, and the N-terminal domain serves as an inhibitory regulatory domain. The dynamic intramolecular interaction is required for AtCYP38 *in vivo* function under different energy status in chloroplast. We postulated that thylakoid membrane proteins and proteases in the thylakoid lumen might be substrates of AtCYP38, while the other small lumen soluble proteins would be its cofactors.

In summary, several thylakoid proteins were identified to be functionally related to AtCYP38. It is a good initial step to uncover the working mechanism of AtCYP38 in the thylakoid lumen. We understand this research only lay a foundation for future study to characterize how AtCYP38 works in thylakoid lumen. Next step, we will apply molecular biology, genetics and biochemistry tools to study the functional relationship between AtCYP38 and its partners. For those uncharacterized proteins, we plan to use CRISPR-Cas9 technology to create protein deficiency mutants to explore their physiological function and their relationship with AtCYP38. We anticipated that future research on the partner proteins of AtCYP38 will help us to understand its molecular mechanism.

Methods

Potential interacting targets prediction

Co-expression network analysis of AtCYP38 was performed by using the analytical tool of ATTED-II database: <http://atted.jp> [27], from which the associated gene was analyzed. In this study, genes with lower mutual rank value (MR) were considered as the co-expression network modules. AtCYP38 protein interaction network was analyzed through the STRING database on the website: <http://string-db.org> [28]. Based on biological functions and localizations, we selected interactors that connected with AtCYP38 using the highest confidence interaction score for further network construction.

Yeast two-hybrid library screening against genome wide cDNA library

The mature AtCYP38 protein has two domains: an N-terminal α -helical domain and a C-terminal cyclophilin β -sheet barrel domain, which linked by an intermediate acidic loop [23]. DNA fragments encoding mature full-length AtCYP38 (residues 92-437), N-terminal domain (residues 92-232), and C-terminal domain (residues 236-437), were cloned into a pGBKT7 vector to generate pGBKT7-CYP38F, pGBKT7-CYP38N and pGBKT7-CYP38C, respectively. They were transformed into host strain AH109 using the modified lithium acetate/PEG method according to the protocol described previously by Fields and Ausubel et al [38, 39], and assayed auto-activation tests on a series of nutrient deficient selective plates. The Arabidopsis cDNA library (prey) were transformed into yeast AH109 harboring BD-bait and plated on selective medium for target colonies. Meanwhile, dilution yeast cells spread onto control SD/-Leu/Trp plates to make sure the transfer efficiency, the estimate counting results indicated that we have more than 2×10^7 transformants in our three individual screenings. The colonies showing potential interaction on SD/-Leu/Trp/His plates were further assayed on SD/-Leu/Trp/His/Ade selective medium until new colonies were grown up.

The plasmid DNA of candidate positive yeast colonies was extracted according to manufacturer's instructions of TIANprep Yeast Plasmid DNA kit (TIANGEN Biotech, Beijing), and then retransformed back to *Escherichia coli* to amplify the cDNA inserts. The colonies were then sequenced with T7 primer: TAATACGACTCACTATAGGGCG.

Yeast two-hybrid analysis against lumen protein library

To identify potential interacting partners for AtCYP38 in the chloroplast thylakoid lumen and on the thylakoid membrane, we constructed a yeast two-hybrid mini library harboring chloroplast luminal proteins/domains. DNA fragments encoding proteins experimentally identified or predicted to present in the thylakoid lumen [12, 13], and domains in the lumen of PSII subunits [40, 41], PSI subunits [42], and Cytb(6)f complex subunits [43] were cloned into the Gal4 AD vector pGADGH. Each AD vector in this library was screened with BD vectors pGBKT7-CYP38F, pGBKT7-CYP38N and pGBKT7-CYP38C individually as described above.

Subcellular localization analysis

For subcellular localization studies, At1g21500, TLP17.9 and TLP15 coding sequences were recombined into modified pMDC83 destination vector, respectively. The constructs were transfected into *Agrobacteria* GV3101, and the strains were infiltrated into tobacco (*Nicotiana benthamiana*) epidermal cell using a needle-less syringe [44]. The GFP fluorescent signal was detected after 48 hours infiltration by using confocal laser scanning microscope. The PCR primers used in this study are listed in supplementary Table 1.

In vitro pull-down assay

The *in vitro* pull-down assay was performed according to the protocol as detailed by previous described [45]. Recombinant GST-21500F (mature protein, without signal peptide), GST-TLP17.9F (mature protein, without signal peptide), GST-FKBP13, GST-PSBP-1 or CYP38C-HIS was transformed into *E. coli* strain BL-21 and purified using glutathione or Ni sepharose beads, respectively. Reaction components were mixed and incubated in PH 7.4 phosphate buffer solution at 4°C for 2 hours, the precipitated beads were then washed thoroughly with PBST buffer (PBS buffer with 0.1% Tween 20). Subsequently, washed beads were eluted and analyzed by SDS-PAGE. The PCR primers used in this study are listed in supplementary Table 1.

Abbreviations

PSI: photosystem I

PSII: photosystem II

Cytb(6)f: cytochrome b6f complex

CYPs: cyclophilins

FKBPs: FK506-binding proteins

PPIase: peptidyl-prolyl cis/trans isomerase (PPIase)

OEC: oxygen evolving complex

LHCBs: light harvesting complex proteins

ROS: reactive oxygen species

ATTED-II: Arabidopsis thaliana trans-factor and cis-element prediction database

STRING: Search Tool for the Retrieval of Interacting Gene

MR: mutual rank

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

All the data on the present study has been included in the tables or supplementary tables in this manuscript. The datasets used and/or analyzed in this study are available from the corresponding author on reasonable request.

Competing interests

Aigen Fu is a member of the BMC plant biology editor board, we declare that we have no other conflicts of interest.

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Authors' contributions:

A.F. and Y.H. designed research; Y.H. J.C. L.S. L.H. C.M. and X.C. performed research; Y.H. J.C. Y.W. M.X. and A.F. analyzed data; Y.H. M.X. and A.F. wrote the paper with contribution and approval from all authors.

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References

1. Umena Y, Kawakami K, Shen JR, Kamyra N. Crystal structure of oxygen-evolving photosystem II at a resolution of 1.9 Å. *Nature*. 2011; 473: 55-60.<https://doi.org/10.1038/nature09913>

2. Wei X, Su X, Cao P, Liu X, Chang W, Li M, Zhang X, Liu Z. Structure of spinach photosystem II-LHCII supercomplex at 3.2 Å resolution. *Nature*. 2016; 534: 69-74. <https://doi.org/10.1038/nature18020>
3. Nelson N, Yocum CF. Structure and function of photosystems I and II. *Annu Rev Plant Biol*. 2006; 57: 521-65. <https://doi.org/10.1146/annurev.arplant.57.032905.105350>
4. Barber J. Photosystem II: an enzyme of global significance. *Biochem Soc T*. 2006; 34: 619-31. <https://doi.org/10.1042/BST0340619>
5. Nixon PJ, Michoux F, Yu J, Boehm M, Komenda J. Recent advances in understanding the assembly and repair of photosystem II. *Ann Bot*. 2010; 106: 1-16. <https://doi.org/10.1093/aob/mcq059>
6. Nickelsen J, Rengstl B. Photosystem II assembly: from cyanobacteria to plants. *Annu. Rev. Plant Biol*. 2013; 64: 609-35. <https://doi.org/10.1146/annurev-arplant-050312-120124>
7. Lu Y. Identification and roles of photosystem II assembly, stability, and repair factors in *Arabidopsis*. *Front Plant Sci*. 2016; 7:168-95. <https://doi.org/10.3389/fpls.2016.00168>
8. Schreiber SL. Chemistry and biology of the immunophilins and their immunosuppressive ligands. *Science*. 1991; 251: 283-7. <https://doi.org/10.1126/science.1702904>
9. Luan S, Kudla J, Gruissem W, Schreiber SL. Molecular characterization of a FKBP-type immunophilin from higher plants. *Proc Natl Acad Sci USA*. 1996; 93: 6964-9. <https://doi.org/10.1073/pnas.93.14.6964>
10. Romano P, Gray J, Horton P, Luan S. Plant immunophilins: functional versatility beyond protein maturation. *New Phytol*. 2005; 166: 753-69. <https://doi.org/10.1111/j.1469-8137.2005.01373.x>
11. He Z, Li L, Luan S. Immunophilins and parvulins. superfamily of peptidyl prolyl isomerases in *Arabidopsis*. *Plant Physiol*. 2004; 134: 1248-67. <https://doi.org/10.1104/pp.103.031005>
12. Peltier JB, Emanuelsson O, Kalume DE, Ytterberg J, Friso G, Rudella A, Liberles DA, Söderberg L, Roepstorff P, von Heijne G, van Wijk KJ. Central functions of the lumenal and peripheral thylakoid proteome of *Arabidopsis* determined by experimentation and genome-wide prediction. *Plant Cell*. 2002; 14: 211-36. <https://doi.org/10.1105/tpc.010304>
13. Schubert M, Petersson UA, Hass BJ, Funk C, Schröder WP, Kieselbach T. Proteome map of the chloroplast lumen of *Arabidopsis thaliana*. *J Biol Chem*. 2002; 277: 8354-65. <https://doi.org/10.1074/jbc.M108575200>
14. Zybailov B, Rutschow H, Friso G, Rudella A, Emanuelsson O, Sun Q, van Wijk KJ. Sorting signals, N-terminal modifications and abundance of the chloroplast proteome. *PLoS One*. 2008; 3: e1994. <https://doi.org/10.1371/journal.pone.0001994>

15. Lima A, Lima S, Wong JH, Phillips RS, Buchanan BB, Luan S. A redox-active FKBP-type immunophilin functions in accumulation of the photosystem II supercomplex in *Arabidopsis thaliana*. Proc Natl Acad Sci USA. 2006; 103: 12631-6.<https://doi.org/10.1073/pnas.0605452103>
16. Fu A, He Z, Cho HS, Lima A, Buchanan BB, Luan S. A chloroplast cyclophilin functions in the assembly and maintenance of photosystem II in *Arabidopsis thaliana*. Proc Natl Acad Sci USA. 2007; 104: 15947-52.<https://doi.org/10.1073/pnas.0707851104>
17. Sirpiö S, Khrouchtchova A, Allahverdiyeva Y, Hansson M, Fristedt R, Vener AV, Scheller HV, Jensen PE, Haldrup A, Aro EV. AtCYP38 ensures early biogenesis, correct assembly and sustenance of photosystem II. Plant J. 2008; 55: 639-51.<https://doi.org/10.1111/j.1365-313X.2008.03532.x>
18. He Z, Li L, Luan S. Immunophilins and parvulins. Superfamily of peptidyl prolyl isomerases in *Arabidopsis*. Plant Physiol. 2004; 134: 1248–1267.<http://doi.org/10.1104/pp.103.031005>
19. Fulgosi H, Vener AV, Altschmied L, Herrmann RG, Andersson B. A novel multi-functional chloroplast protein: identification of a 40 kDa immunophilin-like protein located in the thylakoid lumen. EMBO J. 1998; 17: 1577-87.<https://doi.org/10.1093/emboj/17.6.1577>
20. Rokka, A, Aro EM, Herrmann RG, Andersson B, Vener AV. Dephosphorylation of photosystem II reaction center proteins in plant photosynthetic membranes as an immediate response to abrupt elevation of temperature. Plant Physiol. 2000; 123: 1525-36.<https://doi.org/10.1104/pp.123.4.1525>
21. Vener AV, Rokka A, Fulgosi H, Andersson B, Herrmann RG. A cyclophilin-regulated PP2A-like protein phosphatase in thylakoid membranes of plant chloroplasts. Biochemistry. 1999; 38: 14955-65.<https://doi.org/10.1021/bi990971v>
22. Wang Y, Zeng L, Xing D. ROS-mediated enhanced transcription of CYP38 promotes the plant tolerance to high light stress by suppressing GTPase activation of PsbO2. Front Plant Sci. 2015; 6: 777.<https://doi.org/10.3389/fpls.2015.00777>
23. Vasudevan D, Fu A, Luan S, Swaminathana K. Crystal structure of *Arabidopsis* cyclophilin38 reveals a previously uncharacterized immunophilin fold and a possible autoinhibitory mechanism. Plant Cell. 2012; 24: 2666-74.<https://doi.org/10.1105/tpc.111.093781>
24. Shapiguzov A, Edvardsson A, Vener AV. Profound redox sensitivity of peptidyl-prolyl isomerase activity in *Arabidopsis* thylakoid lumen. FEBS Lett. 2006; 580: 3671-6.<https://doi.org/10.1016/j.febslet.2006.05.054>
25. Edvardsson A, Shapiguzov A, Petersson UA, Schröder WP, Vener AV. Immunophilin AtFKBP13 sustains all peptidyl-prolyl isomerase activity in the thylakoid lumen from *Arabidopsis thaliana* deficient in AtCYP20-2. Biochemistry. 2007; 46: 9432-42.<https://doi.org/10.1021/bi700426q>

26. Ingelsson B, Shapiguzov A, Kieselbach T, Vener AV. Peptidyl-prolyl isomerase activity in chloroplast thylakoid lumen is a dispensable function of immunophilins in *Arabidopsis thaliana*. *Plant Cell Physiol.* 2009; 50: 1801-14.<https://doi.org/10.1093/pcp/pcp122>
27. Obayashi T, Aoki Y, Tadaka S, Kagaya Y, Kinoshita K. ATTED-II in 2018: A plant coexpression database based on investigation of the statistical property of the mutual rank index. *Plant Cell Physiol.* 2018; 59: e3.<https://doi.org/10.1093/pcp/pcx191>
28. Szklarczyk, D, Gable AL, Lyon D, Junge A, Wyder S, Huerta-Cepas J, Simonovic M, Doncheva NT, Morris JH, Bork P, Jensen LJ, von Mering C. STRING v11: protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. *Nucleic Acids Res.* 2019; 47: D607-13.<https://doi.org/10.1093/nar/gky1131>
29. Lu C, Xin Z, Ren Z, Miquel M, Browse J. An enzyme regulating triacylglycerol composition is encoded by the ROD1 gene of *Arabidopsis*. *Proc Natl Acad Sci USA.* 2009; 106: 18837-42.<https://doi.org/10.1073/pnas.0908848106>
30. Satoh K, Yamamoto Y. The carboxyl-terminal processing of precursor D1 protein of the photosystem II reaction center. *Photosynth Res.* 2007; 94: 203-15.<https://doi.org/10.1007/s11120-007-9191-z>
31. Che Y, Fu A, Hou X, McDonald K, Buchanan BB, Huang W, Luan S. C-terminal processing of reaction center protein D1 is essential for the function and assembly of photosystem II in *Arabidopsis*. *Proc Natl Acad Sci USA.* 2013; 110: 16247-52.<https://doi.org/10.1073/pnas.1313894110>
32. Sun X, Peng L, Guo J, Chi W, Ma J, Lu C, Zhang L. Formation of DEG5 and DEG8 complexes and their involvement in the degradation of photodamaged photosystem II reaction center D1 protein in *Arabidopsis*. *Plant Cell.* 2007; 19: 1347-61.<https://doi.org/10.1105/tpc.106.049510>
33. Basak I, Pal R, Patil KS, Dunne A, Ho HP, Lee S, Diluka P, Maple-Grødem J, Odell M, Chang EJ, Larsen JP, Møller SG. *Arabidopsis* AtPARK13, which confers thermotolerance, targets misfolded proteins. *J Biol Chem.* 2014; 289: 14458-69.<https://doi.org/10.1074/jbc.m114.548156>
34. Van Buer J, Cvetkovic J, Baier M. Cold regulation of plastid ascorbate peroxidases serves as a priming hub controlling ROS signaling in *Arabidopsis thaliana*. *BMC Plant Bio.* 2016; 16(1): 163.<https://doi.org/10.1186/s12870-016-0856-7>
35. Wang YY, Hecker AG, Hauser BA. The APX4 locus regulates seed vigor and seedling growth in *Arabidopsis thaliana*. *Planta.* 2014; 239: 909-19.<https://doi.org/10.1007/s00425-014-2025-2>
36. Sirpiö S, Allahverdiyeva Y, Suorsa M, Paakkarinen V, Vainonen J, Battchikova N, Aro EM. TLP18.3, a novel thylakoid lumen protein regulating photosystem II repair cycle. *Biochem J.* 2007; 406: 415-25.<https://doi.org/10.1042/bj20070460>

37. Wu HY, Liu MS, Lin TP, Cheng YS. Structural and functional assays of AtTLP18.3 identify its novel acid phosphatase activity in thylakoid lumen. *Plant Physiol.* 2011; 157: 1015-25.<https://doi.org/10.1104/pp.111.184739>
38. Fields S, Song O. A novel genetic system to detect protein-protein interactions. *Nature.* 1989; 340: 245-6.<https://doi.org/10.1038/340245a0>
39. Ausubel FM, Brent R, Kingston RE, Moore DD, Seidman JG, Smith JA, Struhl K. *Current Protocols in Molecular Biology.* John Wiley, New York. 1990.
40. Shi LX, Schröder WP. The low molecular mass subunits of the photosynthetic supracomplex, photosystem II. *Biochim Biophys Acta.* 2004; 1608: 75-96.<https://doi.org/10.1016/j.bbabi.2003.12.004>
41. Loll B, Kern J, Saenger W, Zouni A, Biesiadka J. Towards complete cofactor arrangement in the 3.0 Å resolution structure of photosystem II. *Nature.* 2005; 438: 1040-4.<https://doi.org/10.1038/nature04224>
42. Amunts A, Drory O, Nelson N. The structure of a plant photosystem I supercomplex at 3.4 Å resolution. *Nature.* 2007; 447: 58-63.<https://doi.org/10.1038/nature05687>
43. Stroebel D, Choquet Y, Popot JL, Picot D. An atypical haem in the cytochrome b6f complex. *Nature.* 2003; 426, 413-8.<https://doi.org/10.1038/nature02155>
44. Sparkes IA, Runions J, Kearns, A, Hawes C. Rapid, transient expression of fluorescent fusion proteins in tobacco plants and generation of stably transformed plants. *Nat Protoc.* 2006; 1: 2019-25.<https://doi.org/10.1038/nprot.2006.286>
45. Hao Y, Oh E, Choi G, Liang Z, Wang ZY. Interactions between HLH and bHLH factors modulate light-regulated plant development. *Mol Plant.* 2012; 5: 688-97.<https://doi.org/10.1093/mp/sss011>

Tables

Table 1. Proteins co-expressed with AtCYP38 identified by ATTED-II.

Locus	Protein	Description	Location
At1g20810	FKBP18	FKBP-like peptidyl-prolyl cis-trans isomerase family protein	Thylakoid Lumen
At1g76450	PPL	Photosystem II OEC PsbP family protein	Thylakoid Lumen
At2g43560	FKBP16-3	FKBP-like peptidyl-prolyl cis-trans isomerase family protein	Thylakoid Lumen
At2g44920	PRP	Pentapeptide-repeat protein in thylakoid lumen	Thylakoid Lumen
At3g56650	PPD6	PSBP-domain protein 6	Thylakoid Lumen
At4g09010	TAPX4	Thylakoid ascorbate peroxidase 4, thylakoid lumen 29	Thylakoid Lumen
At5g52970	TLP15	Thylakoid lumen 15.0 kDa protein	Thylakoid Lumen
At5g53490	TLP17.4	Thylakoid lumen 17.4 kDa protein, putative pentapeptide protein	Thylakoid Lumen
At1g67700	HHL1	Involved in protection of photosystem II from photodamage	Thylakoid Membrane
At1g08520	CHLD	Magnesium chelatase D subunit, CHLD	Chloroplast Stroma
At1g80380	GLYK	P-loop containing nucleoside triphosphate hydrolases superfamily protein	Chloroplast Stroma
At2g47940	DEG2	DEGP protease 2	Chloroplast Stroma
At2g36990	SIGF	RNA polymerase sigma-subunit F	Chloroplast Stroma
At3g23700	SRRP1	S1 RNA-binding ribosomal Protein 1	Chloroplast Stroma
At4g01800	AGY1	Albino or grassy yellow 1, has ATPase activity	Chloroplast Stroma
At5g03940	CPSRP54	Chloroplast signal recognition particle 54 kDa subunit	Chloroplast Stroma
At4g15110	CYP97B3	Cytochrome P450, family 97, subfamily B, polypeptide 3	Chloroplast Stroma
At4g01690	PPO1	Protoporphyrinogen oxidase	Chloroplast Stroma
At4g32590	FDL	2Fe-2S ferredoxin-like superfamily protein	Chloroplast Stroma

At4g39970	HAD	Haloacid dehalogenase-like hydrolase (HAD) superfamily protein	Chloroplast Stroma
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Table 2. Proteins functionally related to AtCYP38 identified by STRING.

Locus	Protein	Description	Location
At1g54780	TLP18.3	Thylakoid lumen protein 18.3	Thylakoid Lumen
At3g26060	PRXQ	Thioredoxin superfamily protein	Thylakoid Lumen
At4g02530	MPH2	Maintenance of photosystem II under high light 2	Thylakoid Lumen
At5g45680	FKBP13	FKBP-type Peptidyl-prolyl <i>cis-trans</i> isomerase	Thylakoid Lumen
At1g75690	LQY1	Low quantum yield of photosystem II 1	Thylakoid Membrane
At4g09650	ATPD	ATP synthase subunit delta	Thylakoid Membrane
At1g35680	RPL21C	Chloroplast ribosomal protein L21	Chloroplast Stroma
At3g26900	SKL1	Probable inactive shikimate kinase like 1	Chloroplast Stroma
At3g63140	CSP41A	Chloroplast stem-loop binding protein of 41 kDa	Chloroplast Stroma
At5g47190	PRPL19	Plastid ribosomal proteins of the 50S subunit 19	Chloroplast Stroma

Table 3. Interacting proteins of the mature full-length AtCYP38 identified by genome wide c-DNA library screening.

Locus	Name	Description	Location
At1g06680	PSBP-1	Regulation of oxygen evolution	Thylakoid Lumen
At3g47070	TSP9	Thylakoid soluble phosphoprotein TSP9	Thylakoid Lumen
At5g39830	DEG8	Cleavage of photodamaged D2 protein of photosystem II	Thylakoid Lumen
At5g02160	FIP	FTSH5 interacting protein	Thylakoid Membrane
At3g54900	GRXCP	Arabidopsis PICOT protein. It activates CAX1 gene calcium transport activity	Chloroplast Stroma
At4g25100	FSD1	Fe superoxide dismutase	Chloroplast Stroma
At4g26530	FBA5	Fructose-bisphosphate aldolase activity	Chloroplast Stroma
At5g20720	CPN21	Chloroplast co-chaperonin with similarity to <i>E.coli</i> GroES	Chloroplast Stroma

Table 4. Interacting proteins of the AtCYP38 N-terminal domain identified by genome wide c-DNA library screening.

Locus	Name	Description	Location
At4g26530	FBA5	Fructose-bisphosphate aldolase activity	Chloroplast Stroma
At1g72370	AP40	Acidic protein associated to 40S ribosomal subunit of ribosomes	Cytoplasm
At1g68560	XYL1	Bifunctional alpha-l-arabinofuranosidase/beta-d-xylosidase	Cell Wall
At5g15090	VDAC3	Involved in metabolite exchange between the organelle and the cytosol	Cell Wall

Table 5. Interacting proteins of the AtCYP38 C-terminal domain identified by genome wide c-DNA library screening.

Locus	Name	Description	Location
At1g06680	PSBP-1	Photosystem II extrinsic protein and participates in the regulation of oxygen evolution	Thylakoid Lumen
At1g20340	PETE2	Plastocyanin 2	Thylakoid Lumen
At3g01480	AtCYP38	Assembly and maintenance of photosystem II supercomplexes	Thylakoid Lumen
At1g29920	LHCB1.1/CAB2	Chlorophyll binding	Thylakoid Membrane
At1g29910	LHCB1.2/CAB3	Chlorophyll binding	Thylakoid Membrane
At1g55670	PSAG	Photosystem I subunit G	Thylakoid Membrane
At2g34420	LHCB1.5/LHB1B2	Chlorophyll binding	Thylakoid Membrane
At3g15820	ROD1	Diacylglycerol choline phosphotransferase activity	Thylakoid Membrane
At4g03280	PETC	Cytochrome b6f complex	Thylakoid Membrane
At5g01530	LHCB4.1	Chlorophyll binding	Thylakoid Membrane
At1g58380	XW6	Structural constituent of ribosome	Chloroplast Stroma
At1g67090	RBCA1A	Encodes a member of the Rubisco small subunit (RBCS) multigene family	Chloroplast Stroma
At1g68010	HPR	Hydroxypyruvate reductase	Chloroplast Stroma
At5g38410	RBCS3B	Monooxygenase activity, ribulose-bisphosphate carboxylase activity	Chloroplast Stroma
At5g38420	RBCS2B	Monooxygenase activity, ribulose-bisphosphate carboxylase activity	Chloroplast Stroma

Table 6. Interacting proteins of the AtCYP38 C-terminal domain identified by lumen protein library screening.

Locus	Name	Description	Location
ArthCP016	PSBM	Photosystem II intrinsic M subunit	Thylakoid Membrane
ArthCP035	CYTF	Cytb6f complex Cytf subunit	Thylakoid Membrane
At2g28800	ALB3	Protein translocation across thylakoid membrane	Thylakoid Membrane
ArthCP018	CP43	E loop of PSII CP43 subunit	Thylakoid Membrane
ArthCP049	CP47	E loop of PSII CP47 subunit	Thylakoid Membrane
At1g31330	PSAF	Lumen loop of PSI PSAF subunit	Thylakoid Membrane
At1g21500	Unknown protein	Thylakoid lumen protein with unknown function	Thylakoid Lumen
At2g23670	Unknown protein	Homolog of synechocystis YCF37	Thylakoid Lumen
At2g26340	Unknown protein	A Thylakoid lumen protein	Thylakoid Lumen
At3g57680	CTPB	D1 Carboxyl-terminal protease	Thylakoid Lumen
At4g17740	CTPA	D1 Carboxyl-terminal protease	Thylakoid Lumen
At4g24930	TLP17.9	Thylakoid lumen 17.9 kDa protein,	Thylakoid Lumen
At5g27660	DEG14	Arabidopsis PARK13 homolog	Thylakoid Lumen
At5g46390	CTPC	D1 Carboxyl-terminal protease	Thylakoid Lumen
At5g52970	TLP15	Thylakoid lumen 15.0 kDa protein	Thylakoid Lumen

Figures

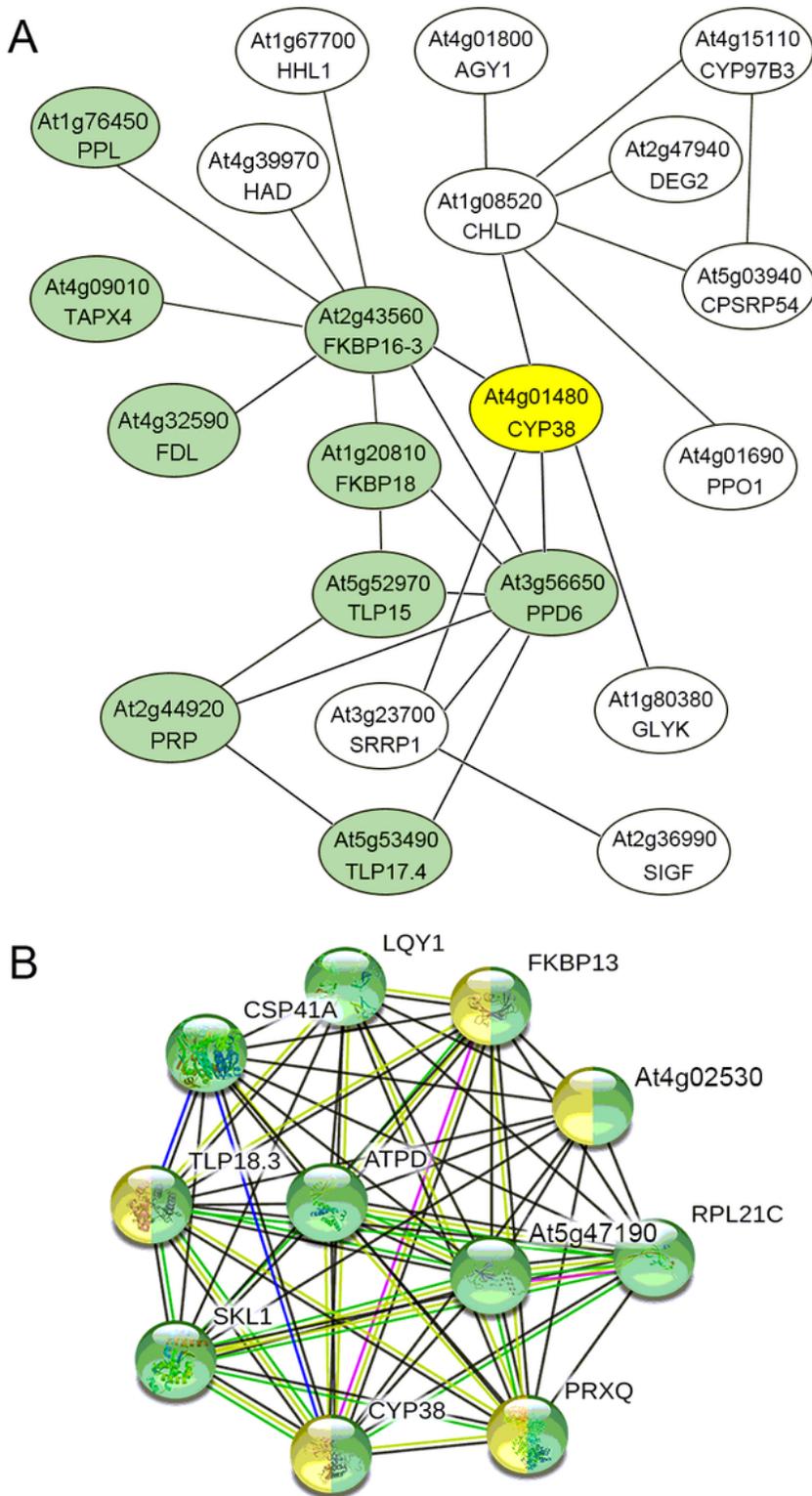


Figure 1

The correlation networks between AtCYP38 and its potential interacting proteins. A. The co-expression gene network using the AtCYP38 as query gene on the ATTED-II (<http://atted.jp>). AtCYP38, shown in yellow module, is co-expressed with multiple photosynthesis related genes. Thylakoid lumen localized proteins are colored in green. B. Protein-protein interaction network is generated by STRING search tool for CYP38 regulatory factors at highest confident score (>0.970). Yellow module indicate the proteins

domain (amino acid residues from 92 to 232) and C-terminal cyclophilin β -sheet domain (amino acid residues from 236 to 437). B. Venn diagram showing the 25 potential AtCYP38-interacting partners are identified by YTH screening using 3 different domains.

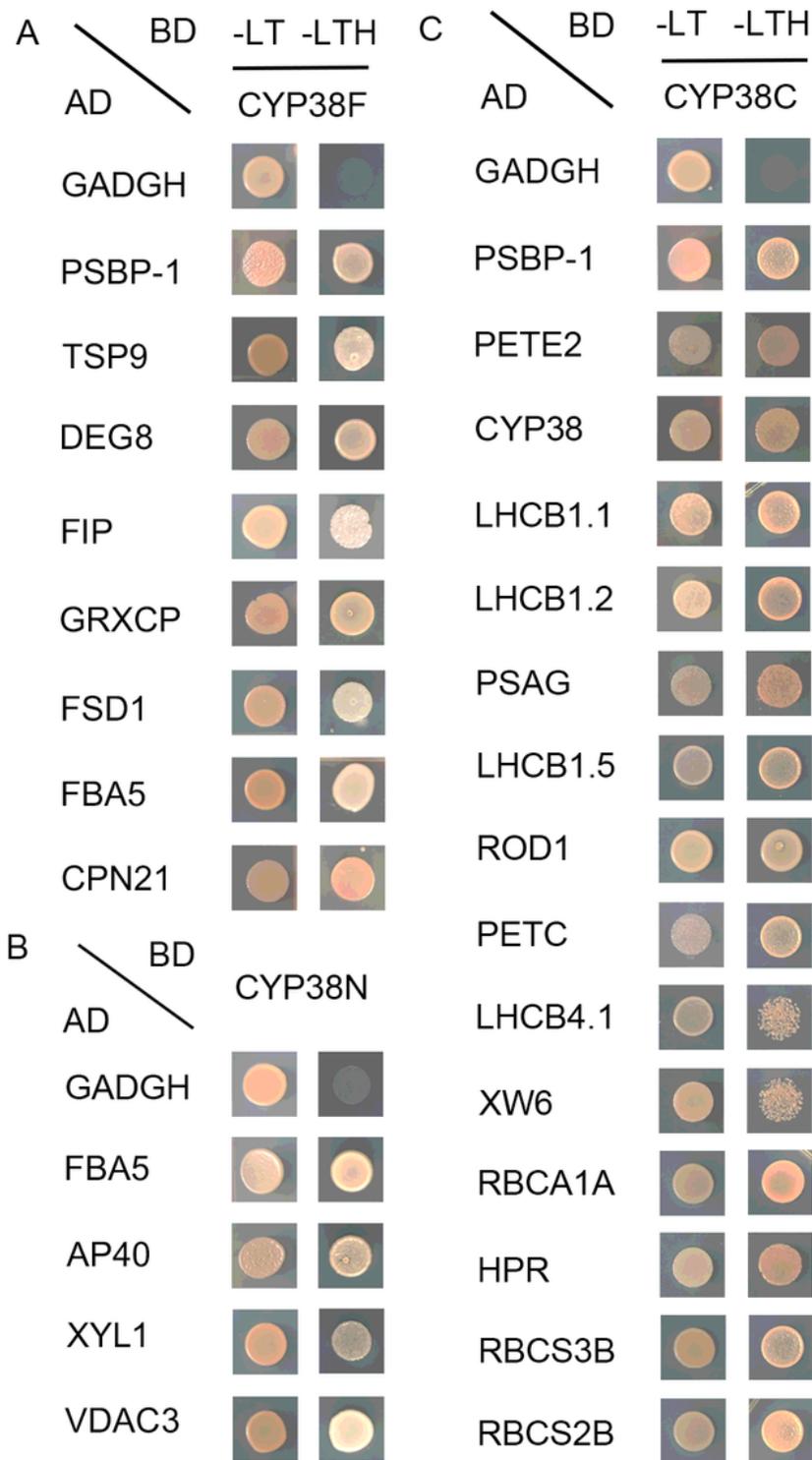


Figure 3

Confirmation of AtCYP38 candidate partners by yeast two-hybrid system. To test interaction results by yeast two-hybrid screening, both prey and bait vectors were co-transformed into yeast AH109 strain and

inoculated onto SD/-Leu/Trp and SD/-Leu/Trp/His plates. A shows the confirmation of interaction between CYP38F domain and its screening results. B. Confirmation of interaction between CYP38N domain and its screening results. C. Confirmation of interaction between CYP38C domain and its screening results.

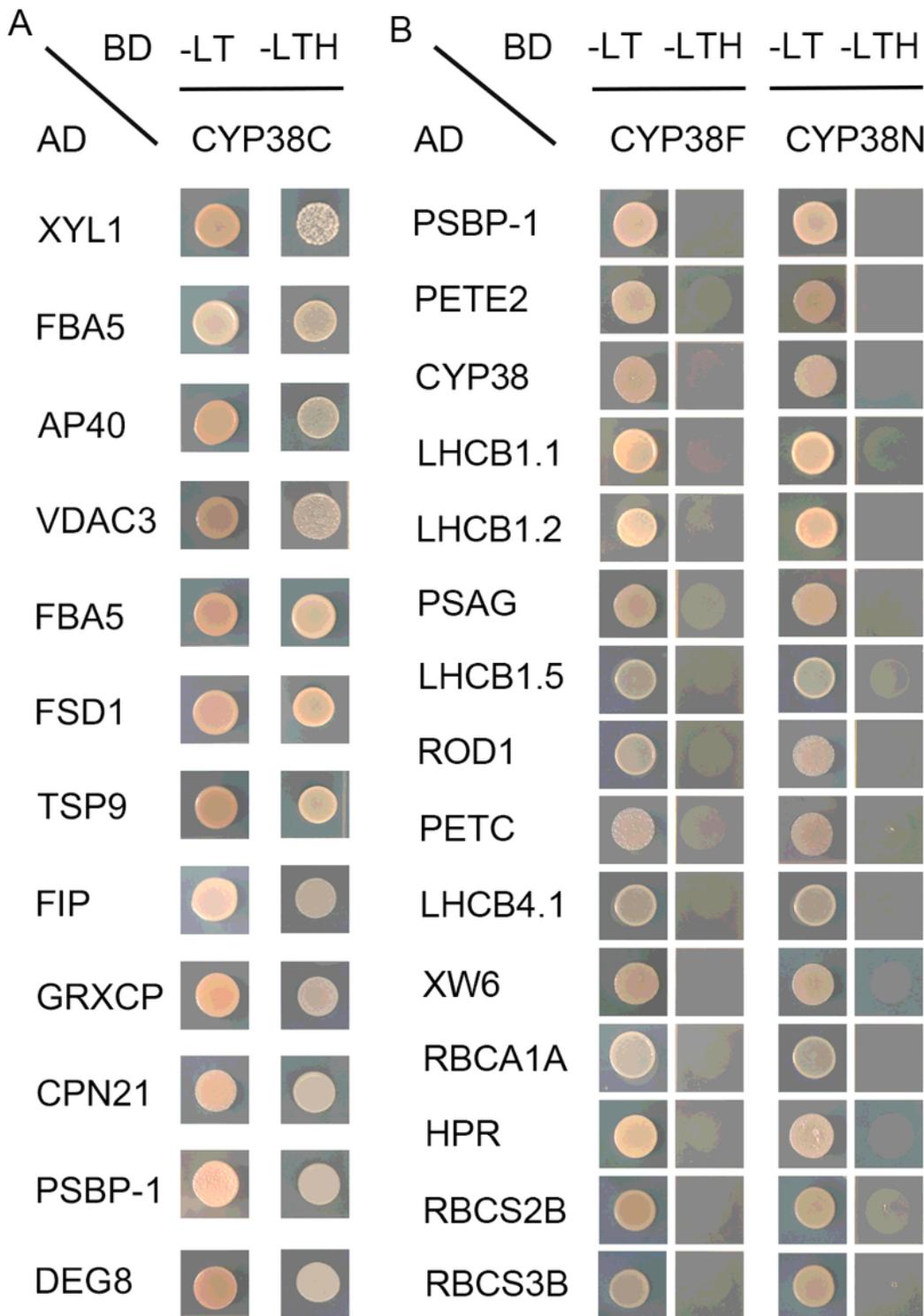


Figure 4

Cross examination of interaction of potential targets with AtCYP38 different domains. A. Confirmation of interaction between CYP38C domain and the screening results which identified by CYP38F and CYP38N domains. B. Confirmation of interaction between CYP38F or CYP38N domain and screening results by using CYP38C protein as a bait.

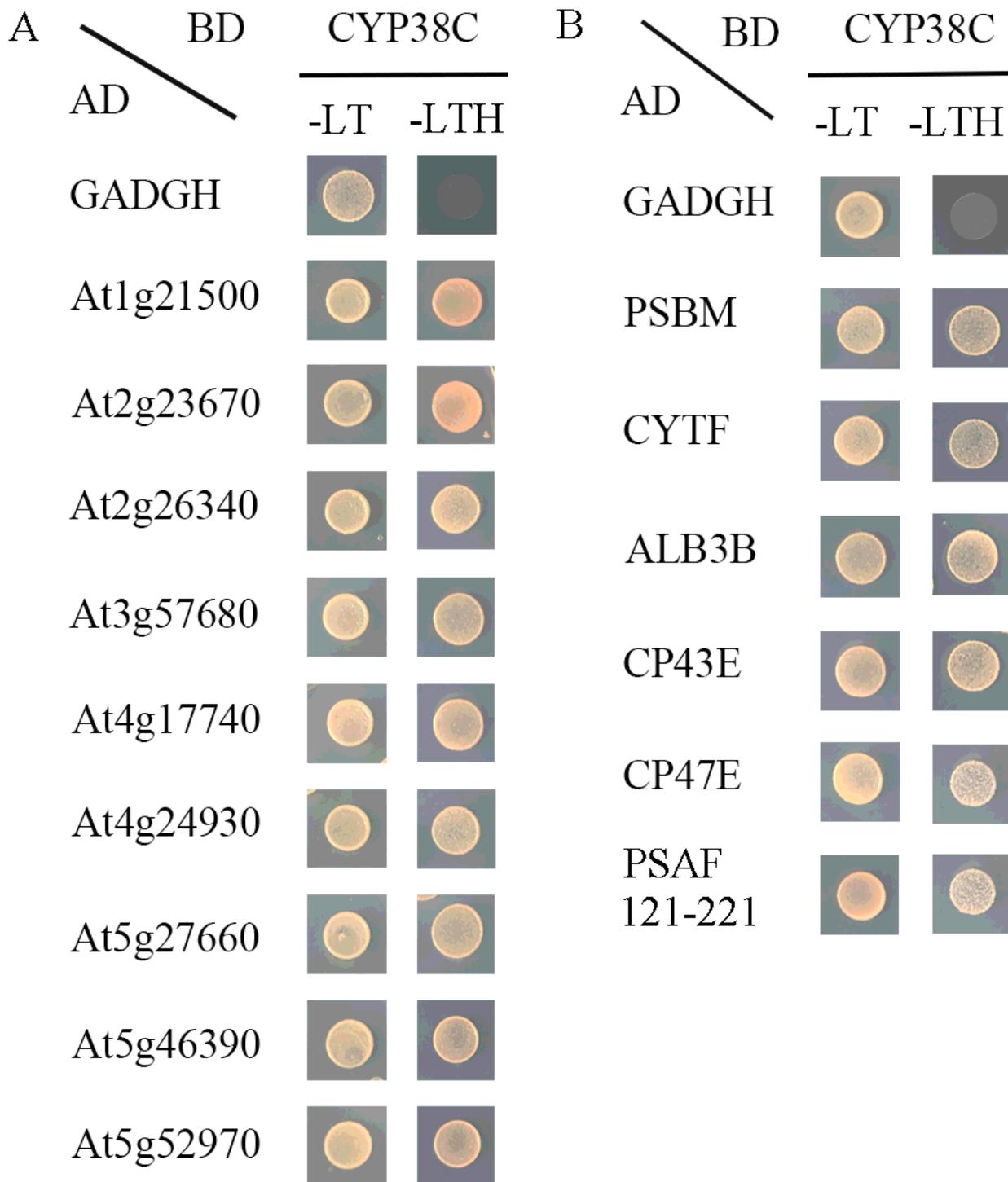


Figure 5

Interacting proteins of the AtCYP38 C terminal domain by screening a thylakoid lumen mini-library. A. Identification of potential interaction proteins in thylakoid lumen. B. Identification of potential interacting proteins on thylakoid membrane with luminal fragments.

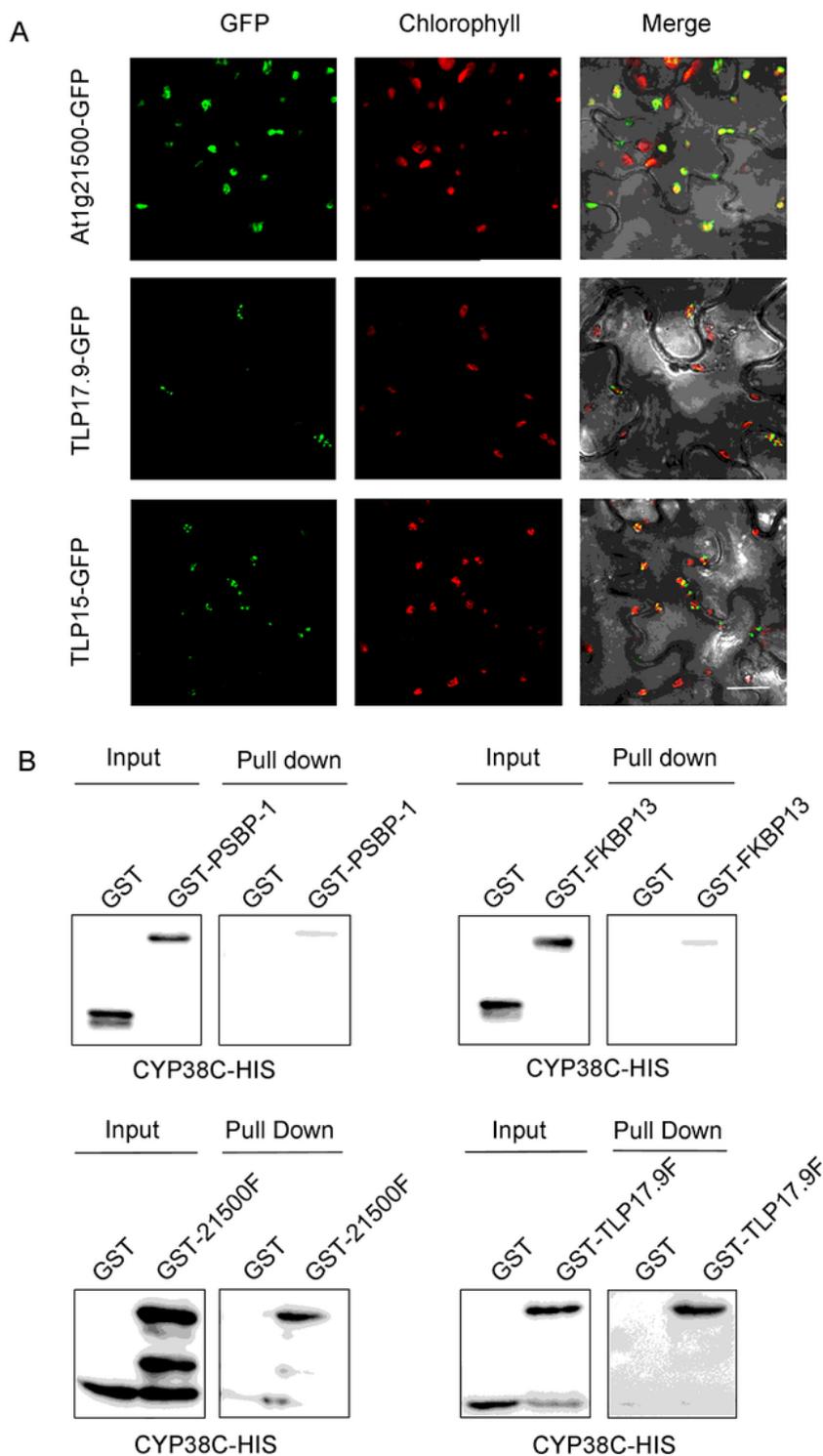


Figure 6

Subcellular localization of candidate proteins and the in vitro pull-down confirmation. A. At1g21500-GFP, At1g24930-GFP or At5g52970-GFP driven by 35S promoter and transiently expressed in the Nicotiana

benthamiana epidermal cells (Scale bars:10 μm). B. In vitro pull down assays showing interaction between CYP38C-HIS and GST-21500F, GST-TLP17.9F, GST-PSBP-1 or GST-FKBP13 proteins, respectively. GST, GST-21500F, GST-TLP17.9F, GST-PSBP-1 or GST-FKBP13 was pulled down by CYP38C-HIS immobilized on beads. Protein were detected by immunoblotting using GST antibody, the interaction of CYP38C and GST were used as a negative control.

Supplementary Files

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