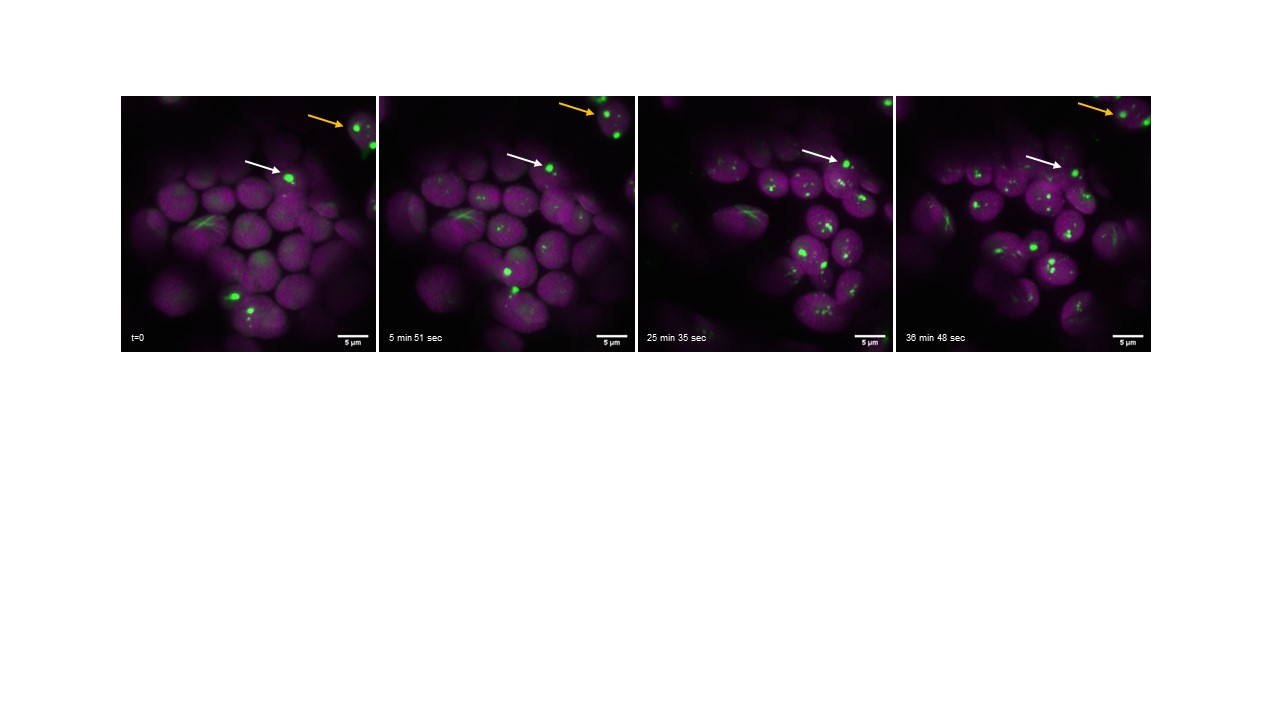
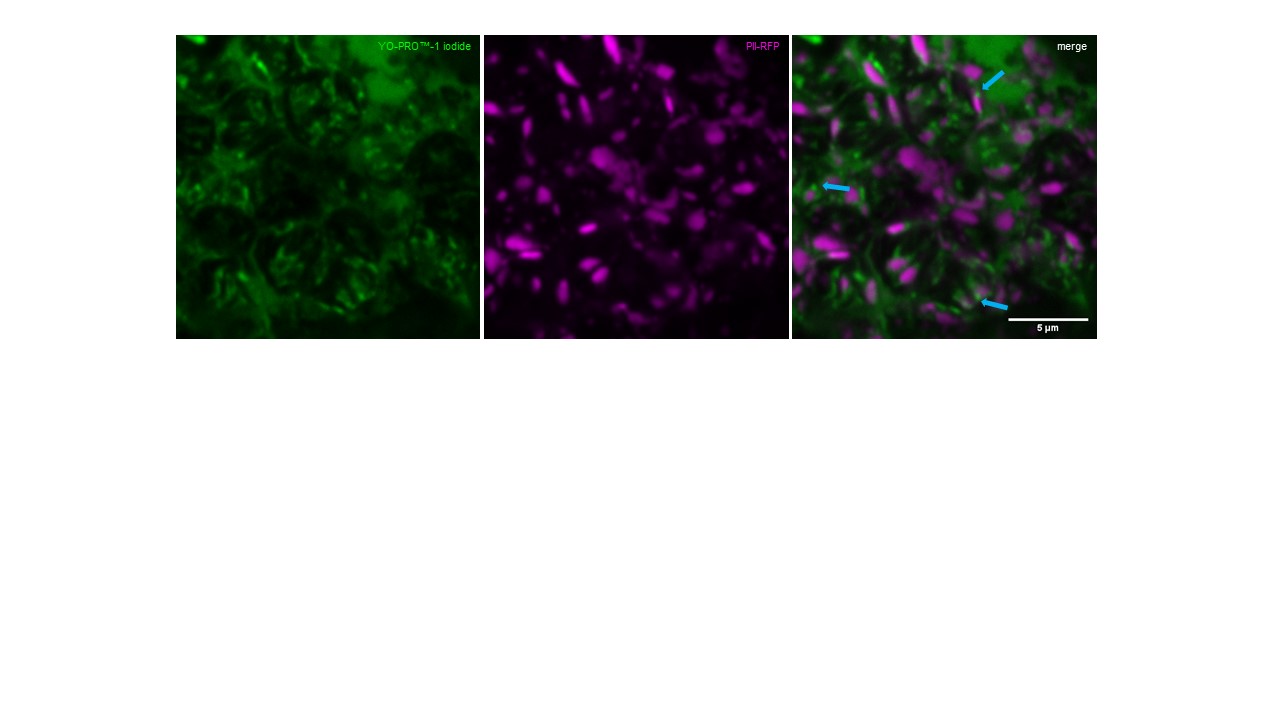
Supplementary Materials： *Arabidopsis* PII proteins form characteristic foci in chloroplasts indicating novel properties in protein interaction and degradation

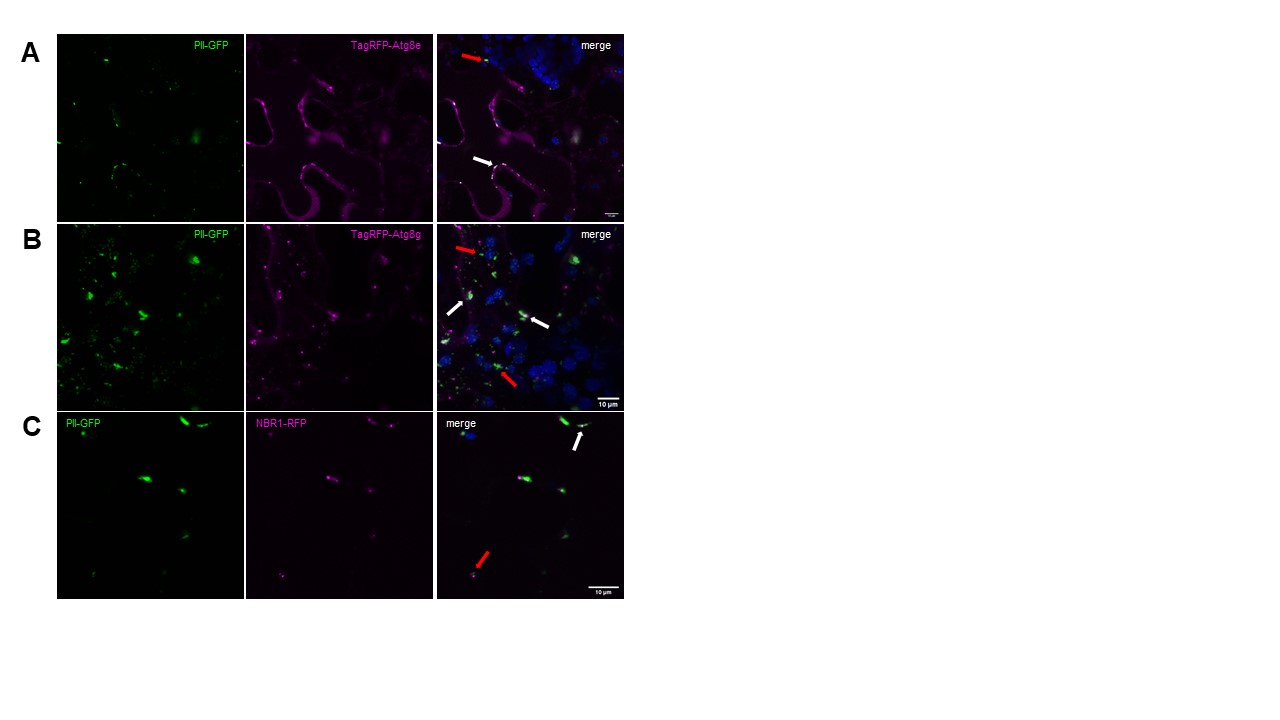
Natalie Krieger, Kai-Florian Pastryk, Karl Forchhammer and Üner Kolukisaoglu

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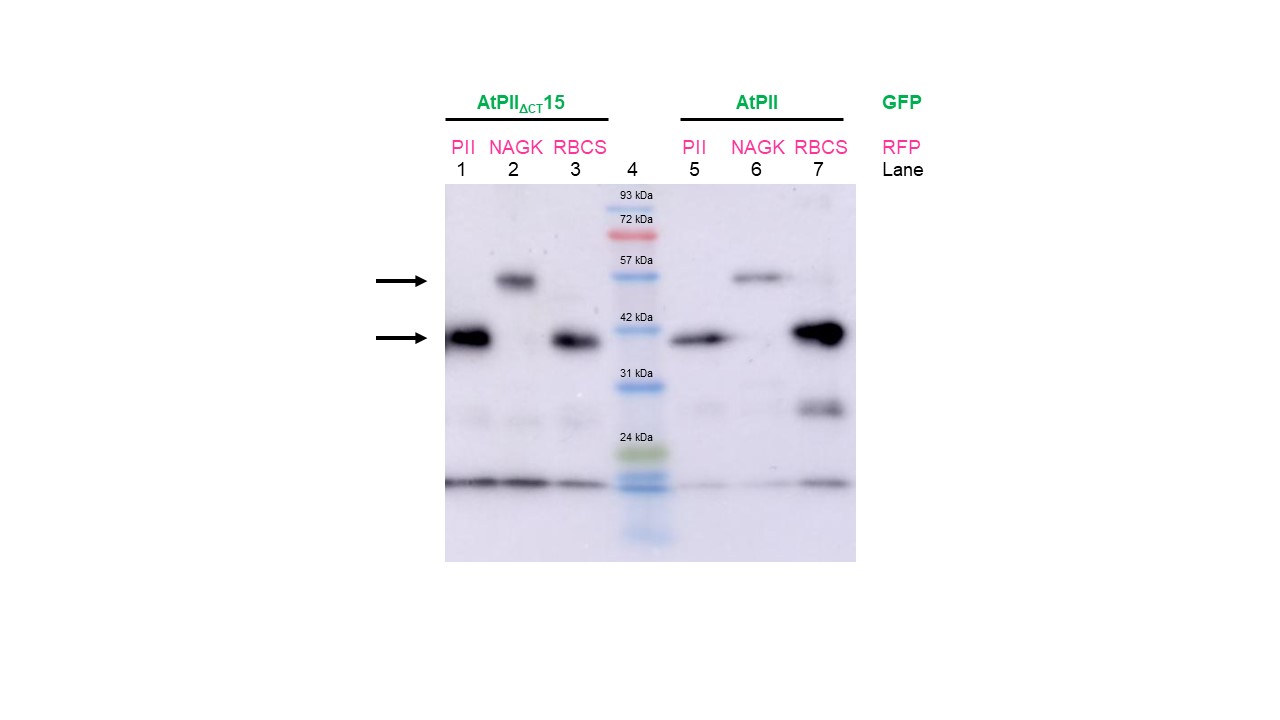
**Figure S1.** PII foci can be stable for more than 30 min. *At*PII-GFP (green) under control of the *p35S* promotor was expressed in transiently transformed *N. benthamiana* and aggregates in PII foci in chloroplasts (magenta). Z-Stacks of time series were acquired 3 days after transient transformation of *N. benthamiana* leaves. Arrows indicate PII foci found over the whole recorded time. Scale bar: 5 µm.

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**Figure S2.** PII foci are not nucleoids. YO-PRO™-1 iodide stained nucleoids (green) in transiently transformed *N. benthamiana* leaves expressing *At*PII-RFP (magenta) under the control of the *p35S* promotor. *N. benthamiana* leaf disks were fixed overnight 3 days after transient transformation followed by staining with YO-PRO™-1 iodide for 15 min. Blue arrows indicate examples of PII foci proximal to nucleoids. Scale bar: 5 µm.



**Figure S3.** PII co-localizes partially with autophagy-related proteins. A)-C) TagRFP-*At*g8e (A), TagRFP-*At*g8g (B), and *At*NBR1-RFP (C), which all localize in the cytoplasm of *N. benthamiana*, were co-expressed with *At*PII-GFP (all genes under control of *p35S)*. Images were taken 2 days after transient transformation in *N. benthamiana*. GFP (green), TagRFP and RFP (magenta), brightfield (grey), autofluorescence (blue). White arrows: co-localization; red arrows: no co-localization. Scale bar= 10 µm



**Figure S4.** *At*PIIΔCT15-GFP can also bind to its interactors. *N. benthamiana* leaves were transiently co-transformed with *At*PII-GFP or *At*PIIΔCT15-GFP together with *At*PII-mCherry, *At*NAGK-mCherry or *At*RBCS3B- mCherry, respectively (all transformed constructs under the control of the *p35S* promotor). After 3 days of transient transformation GFP-fused proteins were immunopurified from protein extracts of transfected leaves. After SDS-PAGE of the Nano-Trap© eluates western blots were treated with anti-RFP antibodies to detect co-purified mCherry-fused proteins. In lanes 1-3 the eluates of extracts from co-expression of *At*PIIΔCT15-GFP with *At*PII-mCherry, **At**NAGK-mCherry or *At*RBCS3B-mCherry, respectively, have been loaded. In lanes 5-7 the same has been done with extracts from co-expressions with *At*PII-GFP. The upper arrow indicates the predicted size for AtNAGK-mCherry (56.6 kDa) and the lower arrow the predicted sizes for *At*PII-mCherry and *At*RBCS3B-mCherry (40.2 kDa and 40.9 kDa, respectively).

**Table S1.** Primers used for the construction of the different plant expression vectors.

|  |  |
| --- | --- |
| AtGLB1-Start | 5’-caccATGGCGGCGTCAATGACGAAAC-3’ |
| AtGLB1-End | 5’-AGACGGTGAAAGCATATCACCAG-3’ |
| AtPII-C2A | 5’-ACGCTCACCTGTCCTAACTC-3’ |
| NK\_proAtPIIstart | 5’-caccTTTTGTTTCACCTTAACCAG-3’ |
| NK\_AtNAGKstart | 5’-caccATGGCCACCGTCACATCCAATGCTTC-3’ |
| NK\_AtNAGKend | 5’-TCCAGTAATCATAGTTCCAGCTCCTTC-3’ |
| NK\_AtBCCP1start-2 | 5’-caccATGGCGTCTTCGTCGTTCTCAGTCAC-3’ |
| NK-BCCP1end | 5’-CGGTTGAACCACAAACAGAGGAGTGTC-3’ |
| NK\_RGCS1A-FP | 5’-caccATGGCTTCCTCTATGCTCTCTTCCG-3’ |
| NK\_RGCS1A-RP | 5’-ACCGGTGAAGCTTGGTGGCTTGTAGG-3’ |
| NK\_attP2P3-PIIstart | 5’-GGGGACAACTTTGTATAATAAAGTTGTAATGGCGGCGTCAATGACG-3’ |
| NK\_attP2P3-PIIend | 5’-GGGGACCACTTTGTACAAGAAAGCTGGGTTAGACGGTGAAAGCATATC-3’ |
| NK\_attP1P4-PIIstart | 5’-GGGGACAAGTTTGTACAAAAAAGCAGGCTTAATGGCGGCGTCAATGACG-3’ |
| NK\_attP1P4-PIIend | 5’-GGGGACAACTTTGTATAGAAAAGTTGGGTGAGACGGTGAAAGCATATC-3’ |
| NK\_attP1P4-NAGKstart | 5’-GGGGACAAGTTTGTACAAAAAAGCAGGCTTAATGGCCACCGTCACATCC-3’ |
| NK\_attP1P4-NAGKend | 5’-GGGGACAACTTTTGTATAGAAAAGTTGGGTGTCCAGTAATCATAGTTCC-3’ |
| NK\_attP1P4-BCCP1start | 5’-GGGGACAAGTTTGTACAAAAAAGCAGGCTTAATGGCGTCTTCGTCGTTC-3’ |
| NK\_attP1P4-BCCP1end | 5’-GGGGACAACTTTGTATAGAAAAGTTGGGTGCGGTTGAACCACAAACAG-3’ |
| NK\_RGCS1A-P1P4-FP | 5’-GGGGACAAGTTTGTACAAAAAAGCAGGCTTAATGGCTTCCTCTATGCTC-3’ |
| NK\_RGCS1A-P1P4-RP | 5’-GGGGACAACTTTGTATAGAAAAGTTGGGTGACCGGTGAAGCTTGGTGG-3’ |
| NK\_attP1-FP-DXS | 5’-GGGGACAAGTTTGTACAAAAAAGCAGGCTTAATGGCTTCTTCTGCATTT-3’ |
| NK\_attP4-RP-DXS | 5’-GGGGACAACTTTGTATAGAAAAGTTGGGTGAAACAGAGCTTCCCTTGG-3’ |
| NK\_attP1-FP-DXR | 5’-GGGGACAAGTTTGTACAAAAAAGCAGGCTTAATGACATTAAACTCACTA-3’ |
| NK\_attP4-RP-DXR | 5’-GGGGACAACTTTGTATAGAAAAGTTGGGTGTGCATGAACTGGCCTAGC-3’ |

**Table S2.** Vectors used for the construction of the different plant expression vectors.

|  |  |
| --- | --- |
| pENTR™/D-TOPO® | Invitrogen Thermo Fisher Scientific (Carlsbad, USA) |
| pUBQ10-Dest | [1] |
| pMDC107 | [2] |
| pH7FWG2,0-Dest | [3] |
| pB7RWG2,0-Dest | [3] |
| pFRETgc-2in1-CC | [4] |
| pBiFCt-2in1-CC | [5] |
| pDONR221-P1P4 | Invitrogen Thermo Fisher Scientific (Carlsbad, USA) |
| pDONR221-P3P2 | Invitrogen Thermo Fisher Scientific (Carlsbad, USA) |
| pENTR-L1-GentR-L4 | [5] |
| pB7FWG2,0-DXS | Gift from Manuel Rodriguez-Concepcion; [6] |
| pB7FWG2,0-DXR | Gift from Manuel Rodriguez-Concepcion; [6] |
| CD3-999 pt-rk (Plastids, mCherry) | [7] |

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