

Supplemental file

Figure S1: Anti-Atphot2 antibody specificity.

Total protein fraction was isolated from leaves of *Arabidopsis thaliana* (WT) and *Nicotiana benthamiana* and subjected to western blotting using Atphot2 antibody. Phot2 protein band is visible only in Arabidopsis. Plants were either dark-adapted for 12 h or kept under white light in growth chambers for 3 h before the protein extraction. 100 µg protein sample was loaded in each lane.

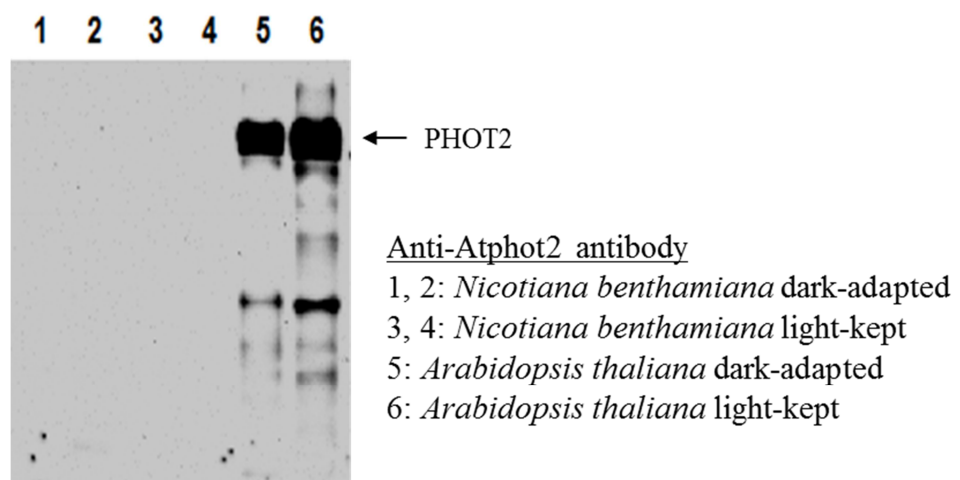


Figure S2: 35S-GFP-phot2 transiently expressed in *N. benthamiana* epidermal cells. Arrows are showing clumping of phot2 in the cytoplasm.

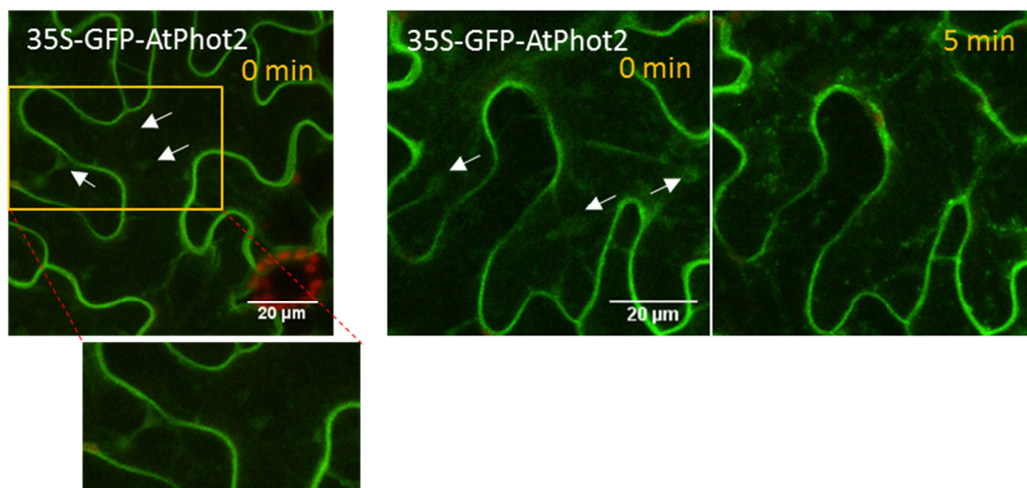


Figure S3: Plasma membrane fluorescence intensity before and after blue-light irradiation.

The corrected total fluorescence is calculated by subtracting the background fluorescence.

Ten different cells were examined in each case.

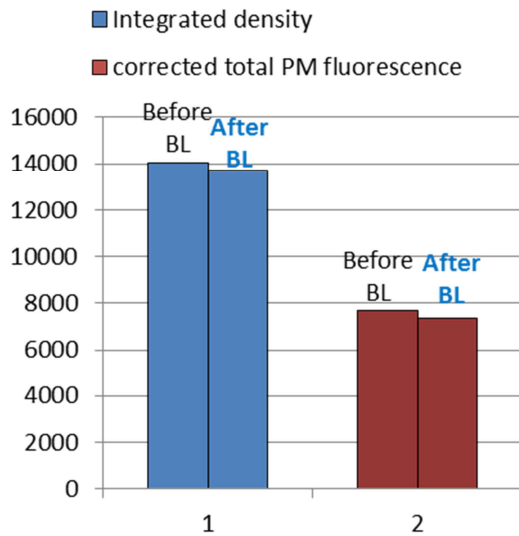


Figure S4: Manders coefficient for co-localization studies (calculated using ImageJ plugin JACop).

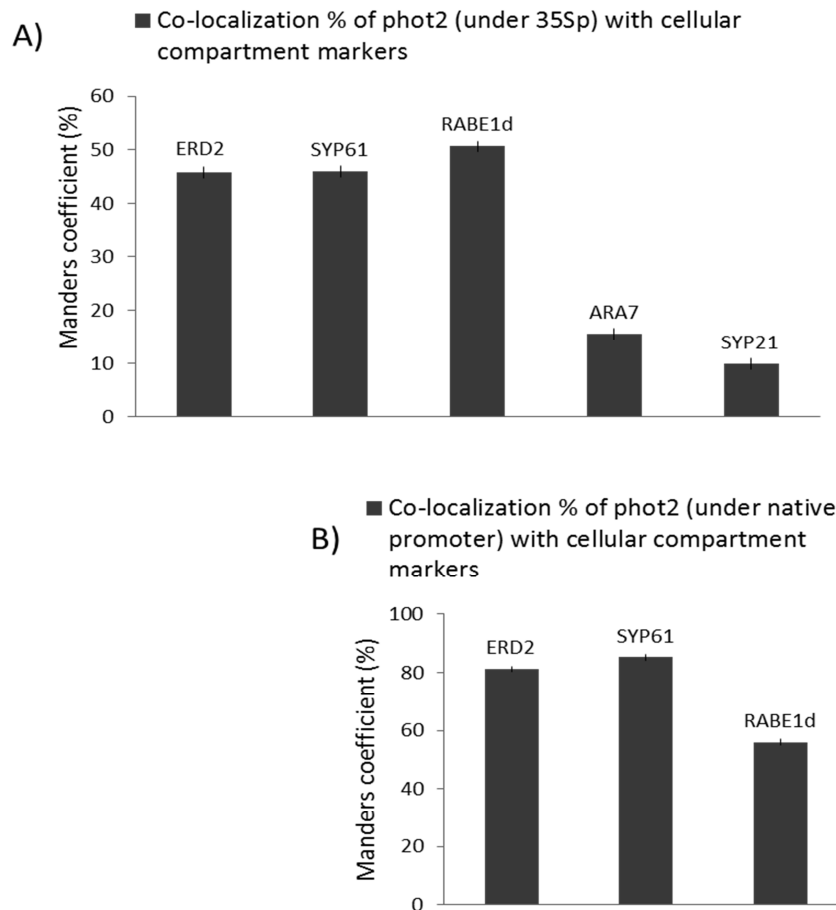


Figure S5: Controls of BiFC technique.

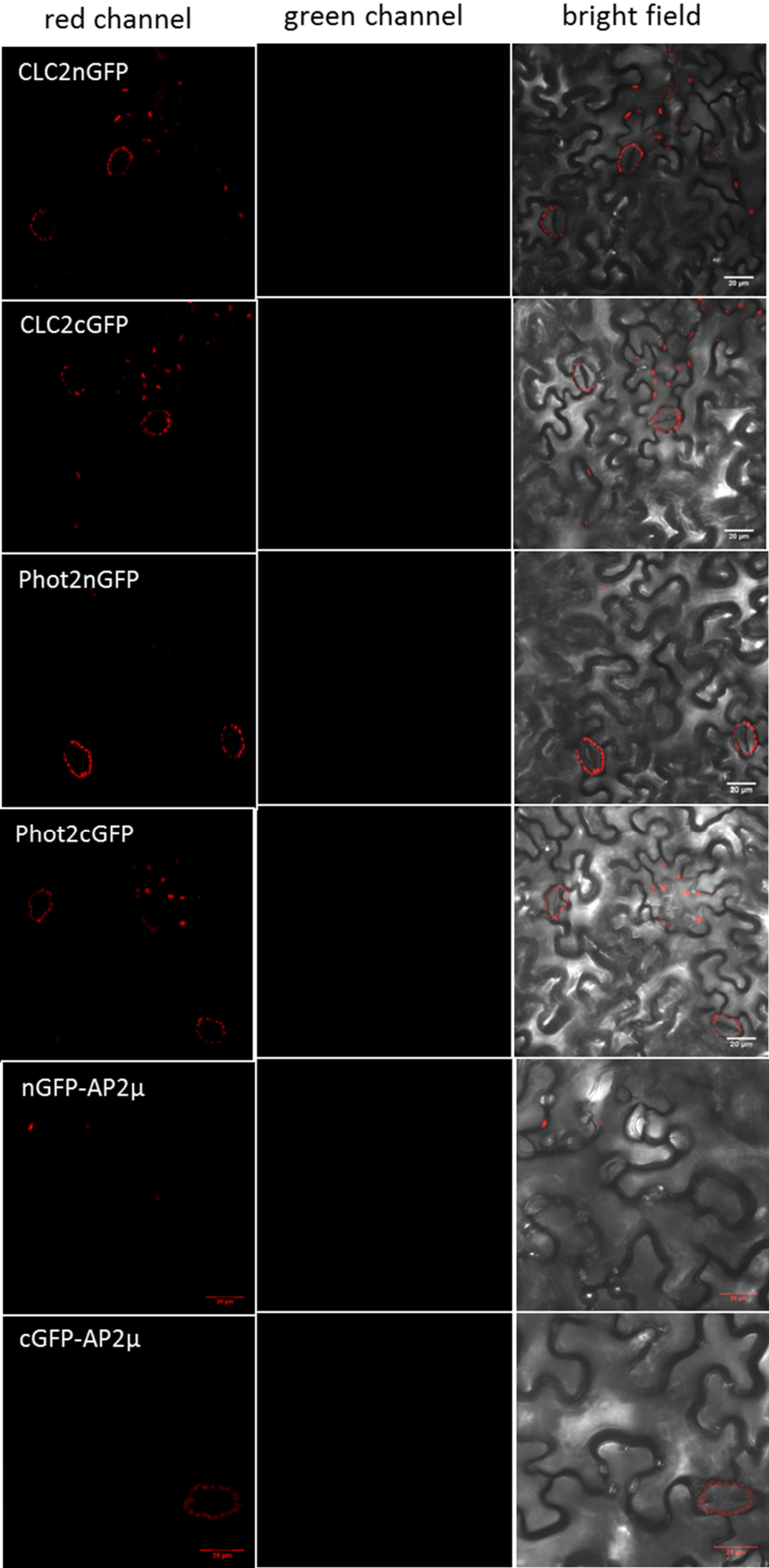


Table S1: Plasmids and primers used in the study

| PLASMIDS | |
|-----------|---|
| pK7FWG2 | Gateway destination vector for expression of the gene under 35S promoter with C-terminal GFP fusion |
| pK7WGF2 | Gateway destination vector for expression of the gene under 35S promoter with N-terminal GFP fusion |
| pH7m34GW | Gateway destination vector for expression of the gene under 35S promoter with N-terminal part of GFP at C-terminal of protein |
| pK7m34GW | Gateway destination vector for expression of the gene under 35S promoter with C-terminal part of GFP at C-terminal of protein |
| pK7m24GW2 | Gateway destination vector for expression of the gene under 35S promoter with C-terminal part of GFP at N-terminal of protein |
| pH7m24GW2 | Gateway destination vector for expression of the gene under 35S promoter with N-terminal part of GFP at N-terminal of protein |
| pMDC7 | Gateway destination vector for expression of the gene under β -estradiol inducible promoter. |

| GATEWAY TECHNOLOGY (PRIMERS) | |
|-------------------------------------|---|
| ARA7FPg | GGGGACAAGTTTGTACAAAAAAGCAGGCTCCATGTTGAGCAAGGGCGAGG |
| ARA7RPg | GGGGACCACTTTGTACAAGAAAGCTGGGTCCCTAAGCACACAAGATGAGCTC |
| CLC2FPg | GGGGACAAGTTTGTACAAAAAAGCAGGCTCCATGTCTGCCTTTGAAGACGATTC |
| CLC2RPg | GGGGACCACTTTGTACAAGAAAGCTGGGTCAGCAGCAGTAACTGCCTCAG |
| ERD2FPg | GGGGACAAGTTTGTACAAAAAAGCAGGCTCCATGAATATCTTTAGATTGCTGGCG |
| ERD2RPg | CATACCTCCACCTCCACCAGCCGGAAGCTTAAGTTTGG |
| mCherryCFPg | GGTGGAGGTGGAGGTATGGTGAGCAAGGGCGAG |
| mCherryCRPg | GGGGACCACTTTGTACAAGAAAGCTGGGTCCCTACTTGTACAGCTCGTCCATG |
| mCherryNFPg | GGGGACAAGTTTGTACAAAAAAGCAGGCTCCATGGTGAGCAAGGGCGAG |
| mCherryNRPg | CTTACCTCCACCTCCACCCTTGTACAGCTCGTCCATG |
| Phot2FPg | GGGGACAAGTTTGTACAAAAAAGCAGGCTCCATGGAGAGGCCAAGAGCCC |
| Phot2RPg | GGGGACCACTTTGTACAAGAAAGCTGGGTCTGAGAGGTCAATGTCCAAGTCC |
| GPhot2FPg | GGGGACAAGTTTGTACAAAAAAGCAGGCTCCATGGAGAGGCCAAGAGCCC |
| GPhot2RPg | GGGGACCACTTTGTACAAGAAAGCTGGGTCTGAGGTCAATGTCCAAGTCC |
| Phot2stopRPg | GGGGACCACTTTGTACAAGAAAGCTGGGTCTTAGAAGAGGTCAATGTCCAAGTCC |
| Phot2CheckFP | CCATTCCTCTACTCTCTACG |
| Phot2CheckRP | CAATCAATAGCACTTGTATGACCAG |
| RABE1dFPg | GGGGACAAGTTTGTACAAAAAAGCAGGCTCCATGGCGGTTGCGCCGG |
| RABE1dRPg | GGGGACCACTTTGTACAAGAAAGCTGGGTCCCTAACGTAACCTACAGCAAGCTG |
| SYP21FPg | GGTGGAGGTATGAGTTTCCAAGATCTCGAAGC |
| SYP21RPg | GGGGACCACTTTGTACAAGAAAGCTGGGTCTTAGACCAAGACAACGATGATGAC |
| SYP61FPg | GGTGGAGGTATGCTTCAGCTCAAGATCCATTC |
| SYP61RPg | GGGGACCACTTTGTACAAGAAAGCTGGGTCTTAGGTCAAGAAGACAAGAACGAATAG |
| SITE DIRECTED MUTAGENESIS (PRIMERS) | |
| Phot2D720NFP | GGACACATAGTATTGGCTAACTTTG |
| Phot2D720NRP | CAAAGTTAGCCAATACTATGTGTCC |

Movie S1: Blue-light-induced phot2 translocation and movement of phot2-labeled punctuate structures in *N. benthamiana* epidermal cell. Two z-scans per minute were performed for a total of 15 min. Images were scanned at a thickness of 1 μm . (Attached avi file)