Supporting Information

SI Experimental Procedures

Mutagenesis, mutant screening and cloning

EMS Mutagenesis, screening for *lhp1-3* enhancers and cloning of causal mutations was performed as previously described (1). Briefly, M2 mutant families were grown in soil in a green house at 22 °C in SDs (8 h light/16 h dark) and scored visually for an enhancement of the early flowering and reduced size *lhp1-3* mutant phenotype. For confirmation of the phenotype, M3 seeds of mutants were grown in growth cabinets (60% humidity, 12 h;16°C light/12h dark;14°C cycles). Confirmed M3 mutants were backcrossed twice to *lhp1-3* to generate a BC2F2 population of 1000 individuals. Leaf material from approximately 100 plants with the *eol* phenotype was pooled for DNA preparation (DNeasy Plant Maxi Kit, Qiagen according manufacturer's instructions) and NGS library preparation and sequenced to 35-fold genome coverage (Illumina platform 35bp reads).

Plant materials and cultivation conditions

The EMS-induced *lhp1-3* allele in the Col-0 background has been described previously as *terminal flower 2-1* (*tfl2-1*) (2) but has also been referred to as *tfl2-2*. The *eol1-2* allele is a T-DNA insertion line from NASC (GABI_382A06). The *clf-28* or *swn-7* mutants are T-DNA insertion lines previously described (3, 4).

For RNA-seq, seeds of Col-0 and mutants were sterilized in 75% ethanol and sowed on GM medium. Material was collected from 10-day-old seedlings grown in Percival growth cabinets at 22°C (LD, 16h light/8h dark). For phenotypic analysis, seeds were sowed on soil and transferred to LDs or MDs after stratification (4°C, 3 days). Flowering time was determined by the number of rosette and cauline leaves of the main shoot, and plant size measured as the longest diameter when plants flowered. Test for statistical significance was performed by one-way ANOVA followed by a Tukey HSD correction for multiple comparisons and by a Student's t-test for comparisons of two groups.

Protein phylogenetic analysis and in vivo modelling

The SWISS-Model server was used to perform in silico structural modeling of partial EOL1 lacking the amino-terminal WD40 domain based on the template of Ctf4 and the resulting structures were visualized with the Swiss-PdbViewer (5). For phylogenetic

tree construction the 6-β-prop domain of EOL1 was used to blast the NCBI protein database and top hits for selected species across three eukaryotic kingdoms were aligned using CLUSTAL-W. A bootstrap consensus tree was calculated from 500 independent neighbor-joining trees as implemented in MEGA6 (6). The PROMALS server was used to align full length Ctf4 homologs from mouse, *D. melanogaster*, yeast and Arabidopsis guided by structure prediction (7).

Plasmid construction, generation of transgenic plants and histochemical GUS staining

For Complementation, a 8 Kb fragment (from 2 Kb upstream of the ATG to 1.5 Kb downstream from the TAA stop codon) of *EOL1* was PCR-amplified from genomic DNA from Col-0 Gateway (GW) compatible primers (Table S2 for primer information) and cloned into *pGD2B* vecor; for localization analysis, 6498bp fragment (without TAA) of *EOL1* and a 4031bp fragment (without TAA) of *LHP1* were PCR-amplified from genomic DNA of Col-0 with GW compatible primers (Table S2 for primer information) and cloned into *CZN656-GW-RFP* and *pXCQ-YFP*, respectively; for promoter reporter analysis, 2 Kb sequence upstream to the ATG of *EOL1* was PCR-amplified from genomic DNA of Col-0 with GW compatible primers (Table S2 for primer information) and cloned into a previously described binary vector *GW-GUS-pGREEN* (8). Transgenic plants were generated by Agrobacterium-mediated gene transfer using the floral dip method (9). The cellular localizations of EOL1 and LHP1 were examined under a LSM 700 confocal laser-scanning microscope (Carl Zeiss). Histochemical GUS detection was performed as previously described (8). For 2,4-D treatment, seeds were grown on MS plates for 5 days under continuous light, then half of the seeds were transferred to MS plates supplemented with 750 nM 2,4-dichlorophenoxyacetic acid (2,4-D). Controls and treated plants were grown for 3 additional days.

BiFC Assay and Co-localization

Coding sequence of *EOL1*, *LHP1*, *CLF, SWN* and *ATJ3* were PCR-amplified with GW-compatible specific primers (Table S2) and recombined into the split YFP-binary vectors p112-sYFPn and p113-sYFPc or RfA-sYFPn-pBatTL-B and RfA-sYFPc-pBatTL-B or expression vectors *pAM-GFP-GW* or *CZN656-GW-RFP* (10). *Agrobacterium tumefaciens* strains carrying plasmids for BiFC and the p19 silencing suppressor were grown overnight at 28°C in 10 mL YEP medium plus selective antibiotics, collected by centrifugation, and resuspended in infiltration medium (1 mM

$MgCl_2$, 150 μg/mL acetosyringone, and 1 mM MES-KOH, pH 5.6). After incubation at 28°C for 3 h in darkness, cells were infiltrated into the abaxial surface of 3-week-old *Nicotiana benthamiana* plants. The fluorescence signal of YFP was observed and recorded using an LSM 700 confocal laser-scanning microscope. The cellular localization of EOL1 and LHP1 was also examined under a LSM 700 confocal laser-scanning microscope (Carl Zeiss).

***In vitro* pull-down assays**

For recombinant protein expression, full-length coding sequences of *SLD5*, *PSF1*, *LHP1* and *EOL1* were introduced into *pET28b-His* (see Addgene for information) or *pGEX* (GE Healthcare). pGEX-5p-1 or pDEST-MC2-YFP were used as negative control for GST or MBP expression, respectively. The resultant plasmids were transformed into *E. coli* BL21-DE3 cells and expression was induced at 16°C with 1 mM isopropyl β-D-1 thiogalactopyranoside (IPTG) overnight. Sonication was used to break the *E. coli* cells and release recombinant proteins.*CLF-pNT74i* or *SWN-pNT74i* were used to produce CLF or SWN protein in insect cells (Sf21) using the baculovirus system as previously described (11). Lysed cell extracts were obtained by pipetting insect cells into RIPA buffer (150 mM NaCl, 0.1% SDS and 50 mM Tris, pH 8.0). Extracts containing His tagged proteins (His-SLD5, His-PSF1, His-LHP1, His-CLF or His-SWN) were cleared by centrifugation for 10 min at 4°C, and the supernatants were incubated with GST or GST-EOL1 proteins bound to Glutathione Sepharose 4B (GE Healthcare) for 2 hours at 4°C. Washing and elution were performed according to the Glutathione Sepharose 4B (GE healthcare) manufacturer's instructions. For immunoblot analyses, anti-HIS (Millipore, 05-949) or anti-MBP (NEB, E8032S) antibodies were used.

Transient expression and Co-IP

Full-length coding sequences for *EOL1*, *SLD5*, *PSF1*, *CLF, SWN* and *LHP1* were fused with amino-terminal *GFP* or *RFP* (for *EOL1*), the HA-epitope (*SLD5*, *PSF1*, *CLF* or *SWN*) and carboxy-terminal GFP (for *LHP1*) in the pAM Vector or *pER8* vectors for expression under the *CaMV 35S* promoter or β-estradiol inducible promoter, respectively. The constructs were cotransformed into Arabidopsis protoplasts by the polyethylene glycol method as described (12). For *pER8-CLF* or *pER8-SWN*, 5 μM beta-estradiol (sigma) was added to the W5 solution. The protoplasts were harvested 12 h after transformation and lysed in lysis buffer (50 mM Tris-HCl, pH 7.5, 150 mM

KCl, 2 mM EDTA, 0.1% Triton-X 100, 10% glycerol and 5 mM DTT) with freshly added proteinase inhibitor cocktail (Sigma, P9599). The lysate was centrifuged at 13,000g, 4°C for 10 min, and the supernatant incubated with GFP-trap beads (ChromoTek) for 2 h at 4°C while rotating on a bohemian wheel. The beads were washed with lysis buffer five times, diluted in 2 X SDS loading buffer, and boiled for 5 min before separation on SDS-PAGE. Immunoblots were probed according to standard procedures with anti-GFP (Abcam, ab290), anti-HA (Abcam, ab9110) and anti-RFP (Clontech, 632496) antibodies.

RNA Isolation, quantitative RT-PCR and RNA-seq

Total RNA extracted with the RNeasy mini kit (Qiagen) according manufacturer's instructions. Total RNA (5µg) was treated with DNaseI (DNA-free kit, Ambion). For RT-PCR, cDNA was generated at 42 °C for 2 h using Superscript II reverse transcriptase and T18 oligonucleotide for priming (Life Technologies). Expression of *EOL1* in the T-DNA line was measured by PCR using *PP2A* as control. Quantitative RT-PCR measurements were performed in a BioRad iQ5 apparatus using a home-made Eva-GREEN amplification cocktail for detection as described (13). Quantification was performed using the relative -ΔΔCT method using PP2A as for normalization. Oligonucleotide primers are indicated in Table S2. For RNA-seq, material was collected from 2 and 3 independent biological replicates respectively for single *eol1* mutants to Col-0 and double *eol1 lhp1* to *lhp1* mutants and Col-0 analysis. DNA-free total RNA was generated as described above for Illumina True-seq library preparation, which was carried out from DNA-free total RNA (3µg) by the Max Planck Genome Centre in Cologne. Reads were mapped to the annotated Arabidopsis genome of version TAIR10. Differential expression was evaluated using the edgeR (14) implementation in the CLC genomic workbench using a false discovery (FDR) corrected p-Value of <0.05 and a fold-change cut-off of >|4|.

ChIP

ChIP experiments were performed as previously described (13) using anti-H3K27me3 (Millipore, 07-449) or anti-H3 (Abcam, ab1791) antibodies. Briefly, 10-day-old seedlings were fixed in PBS buffer with 1% formaldehyde under vacuum for twice 10 minutes, after which the fixed seedlings were homogenized in liquid nitrogen. The chromatin was extracted and sonicated to produce DNA fragments of 200~500 bp.

H3K27me3 and H3 were detected by corresponding antibodies and bound by protein A sepharose (GE). Primers used for ChIP-qPCR are listed in Table S2.

Drug Treatments

The seeds were sterilized, evenly spread on GM medium with or without zebularine (Sigma-Aldrich) and bleocin (Calbiochem) in concentrations specified in the text and grown at LD condition. For root elongation assays, 7-day-old Col-0, *eol1-1* and *eol1-2* seedlings grown continuously on mock and drug containing GM media were used. For RT-qPCR, 10-day-old Col-0, *eol1-1* and *eol1-2* seedlings grown under the same

conditions and transferred to mock and drug containing GM media for 24 hours, then seedlings were collected and followed the procedures of RT-qPCR. Primers used for RT-qPCR are listed in Table S2

Supplementary Tables and Figures

Table S1. Summary of mapping by sequencing results

Chr	Pos	Ref Base	SNP	Support	Concordance	Quality	Annotation	AGI	Isoform	Codon	Codon Pos	AAchange	Ref AA	SNP AA
3	12425773	C	T	40	0.98	40	intergenic							
3	13431009	G	A	40	0.95	40	intergenic							
3	**14751354**	**G**	**A**	**31**	**1**	**40**	**CDS**	**AT3G42660**	**1**	**75**	**3**	**Non-syn**	**W**	**STOP**
3	15619720	G	A	40	0.95	40	intergenic							
3	15866733	G	A	40	0.89	38	intronic/noncoding	AT3G44100	1					
3	16343365	G	A	59	0.94	40	CDS	AT3G44800	1	33	3	Syn	K	K
3	16637669	G	A	43	0.9	32	intergenic							
3	17829427	G	A	43	0.86	36	intronic/noncoding	AT3G48195	1					
3	17967474	G	A	41	0.91	40	three_prime_UTR	AT3G48510	1					
3	**22294351**	**G**	**A**	**29**	**0.74**	**25**	**CDS**	**AT3G60320**	**1**	**878**	**2**	**Non-syn**	**S**	**F**
3	22888463	G	A	36	0.72	25	intronic/noncoding	AT3G61830	1					

Table S2 List of oligonucleotide primers used in this study

List of oligonucleotides used as primers			
Purpose	**Primers**	**Gene**	**Sequence (5' to 3')**
Genotype	EOL1 cutWT F	*EOL1*	TGAAGTGACGAAATGTTCGGCTTTGGGATC
Genotype	EOL1 cutWT R	*EOL1*	GAAACTGGCGCCAAAATCGAGG
Genotype	EOL1 LP	*EOL1*	ATGATGGAACCAAAGATTCCC
Genotype	EOL1 RP	*EOL1*	TGTCCATGCTGGAAAGAAAAC
Genotype	CLF LP	*CLF*	GAAGGGAGCTCTCTGCTTGAT
Genotype	CLF RP	*CLF*	CTGCCAGTTCAGGAATGGTT
Genotype	LHP1 allR	*LHP1*	GTTGTCCACCAATGCTTCCT
Genotype	LHP1 WTF	*LHP1*	TAACGGTGCAGGATGTTCAC
Genotype	LHP1 MF	*LHP1*	TAACGGTGCAGGATGTTCAT
Genotype	SWN LP	*SWN*	GTCTTTTAGAATTGGGACCTCACGC
Genotype	SWN RP	*SWN*	GGATAAGCAGAATACCGAGGAATTTTC
GUS	EOL1 F3	*EOL1*	GGGGACAAGTTTGTACAAAAAAGCAGGCTTAAATCAAGGAACCTCCAAA
GUS	EOL1 R3	*EOL1*	GGGGACCACTTTGTACAAGAAAGCTGGGTACGGAGATGTTGATTGTTTT
Complementation	EOL1 R4	*EOL1*	GGGGACCACTTTGTACAAGAAAGCTGGGTCATTCAAAGTTTGCTCATAGAAAG
Localization	EOL1 R5	*EOL1*	GGGGACCACTTTGTACAAGAAAGCTGGGTAAACAGTTGATTTGAGGAAC
Localization	LHP1 gF	*LHP1*	GGGGACAAGTTTGTACAAAAAAGCAGGCTTATAACCACTAAATTTGTTTGAAC
Localization	LHP1 gR	*LHP1*	GGGGACCACTTTGTACAAGAAAGCTGGGTCAGGCGTTCGATTGTACTTGAG
Interaction	EOL1 F1	*EOL1*	GGGGACAAGTTTGTACAAAAAAGCAGGCTTAATGAAATCACGATCTCTAAAG
Interaction	EOL1 R1	*EOL1*	GGGGACCACTTTGTACAAGAAAGCTGGGTATTAAACAGTTGATTTGAGGAAC
Interaction	LHP1 F1	*LHP1*	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGAAAGGGGCAAGTGGTGCTG
Interaction	LHP1 nostop	*LHP1*	GGGGACCACTTTGTACAAGAAAGCTGGGTCAGGCGTTCGATTGTACTTGAGATG
Interaction	CLF F	*CLF*	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGTCTGTCAAGAATAATAG
Interaction	CLF R	*CLF*	GGGGACCACTTTGTACAAGAAAGCTGGGTCCTAAGCAAGCTTCTTGGGTC
Interaction	SWN F	*SWN*	GGGGACAAGTTTGTACAAAAAAGCAGGCTTAATGGTGACGGACGATAGCAAC
Interaction	SWN R	*SWN*	GGGGACCACTTTGTACAAGAAAGCTGGGTCTCAATGAGATTGGTGCTTTC
Interaction	ATJ3 F	*ATJ3*	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGTTCGGTAGAGGACCCTCG
Interaction	ATJ3 R	*ATJ3*	GGGGACCACTTTGTACAAGAAAGCTGGGTCCTGCTGGGCACATTGCACCC
Interaction	LHP1 stop	*LHP1*	GGGGACCACTTTGTACAAGAAAGCTGGGTCTTAAGGCGTTCGATTGTACTTG
Interaction	SLD5 F	*SLD5*	GGGGACAAGTTTGTACAAAAAAGCAGGCTTAATGGCGTCGAATTCGGAAGC
Interaction	SLD5 R	*SLD5*	GGGGACCACTTTGTACAAGAAAGCTGGGTCTCAGATCAAGTCAATTTGGCC
Interaction	PSF1 F	*PSF1*	GGGGACAAGTTTGTACAAAAAAGCAGGCTTAATGTACGGGAGAAAAGGGTATC

Interaction	PSF1 R	*PSF1*	GGGGACCACTTTGTACAAGAAAGCTGGGTCTCATCCTGT CAGCTCCTCC
RT-PCR	EOL1 F2	*EOL1*	GACGGCGTCACCTCCTTGGC
RT-PCR	EOL1 R2	*EOL1*	ATGATGGAACCAAAGATTCCC
RT-PCR	PP2A F	*PP2A*	AAATACGCCCAACGAACAAA
RT-PCR	PP2A R	*PP2A*	CAGCAACGAATTGTGTTTGG
RT-PCR	AG F1	*AGAMOUS*	ACCACCTCAAACGCAATCTC
RT-PCR	AG R1	*AGAMOUS*	GGCCATTTCCTTCAGCCTAT
RT-PCR	SEP3 F1	*SEP3*	GAAAGCTGTACGAGTTTTGCAG
RT-PCR	SEP3 R1	*SEP3*	TCTGAAGATCGTTGAGCTGGT
RT-PCR	FT F1	*FT*	GGTGGAGAAGACCTCAGGAA
RT-PCR	FT R1	*FT*	ACCCTGGTGCATACACTGTT
RT-PCR	BRCA1qF	*BRCA1*	CCTAAAGAACCACTGCACTCTC
RT-PCR	BRCA1qR	*BRCA1*	GTGAGCCCTGAGCAAGATAAG
RT-PCR	RAD51qF	*RAD51*	CCGCTCTCTACAGAACAGATTTC
RT-PCR	RAD51qR	*RAD51*	CAGCCACACCAAACTCATCT
RT-PCR	PARP2qF	*PARP2*	TCTGCGAGGTTGCTTTGG
RT-PCR	PARP2qR	*PARP2*	TTGAGCCTCTGATGGGTTTG
ChIP-PCR	AG F2	*AGAMOUS*	CGTTGTGATGTTACTCGGACA
ChIP-PCR	AG R2	*AGAMOUS*	GCCATGCTGGCAAAATTAGA
ChIP-PCR	SEP3 F2	*SEP3*	TCTGCCAAGAAAGTTTGATGCT
ChIP-PCR	SEP3 R2	*SEP3*	CGCCATCTCCACCTTCCATT
ChIP-PCR	FT F2	*FT*	CAAAAGTTTATATTTAGGAGCAGTCAA
ChIP-PCR	FT R2	*FT*	TCAATTCATCATCTCTTCTTTGGA
ChIP-PCR	UBQ10 F	*UBQ10*	TCCAGGACAAGGAGGTATTCCTCCG
ChIP-PCR	UBQ10 R	*UBQ10*	CCACCAAAGTTTTACATGAAACGAA

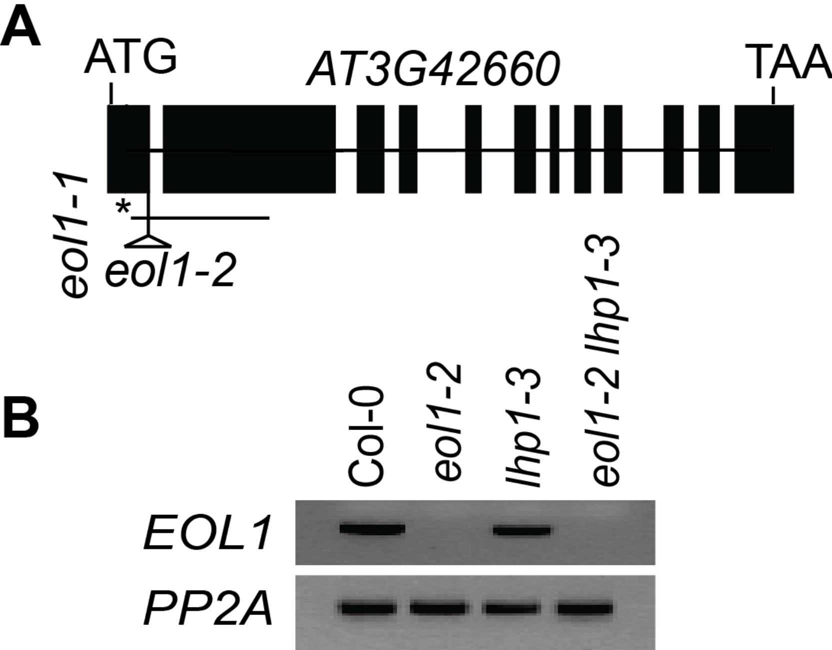

Fig S1. A. Schematic representation of the *At3G42660* locus. Exons are indicated by black boxes, introns by lines. Position of induced point mutations are indicated by asterisks, position of T-DNA insertion by triangles, position of PCR fragment for semi-quantitative RT-PCR indicated by line. **B.** Expression of *EOL1* measured by semi-quantitative RT-PCR. *PP2A* was used as reference gene.

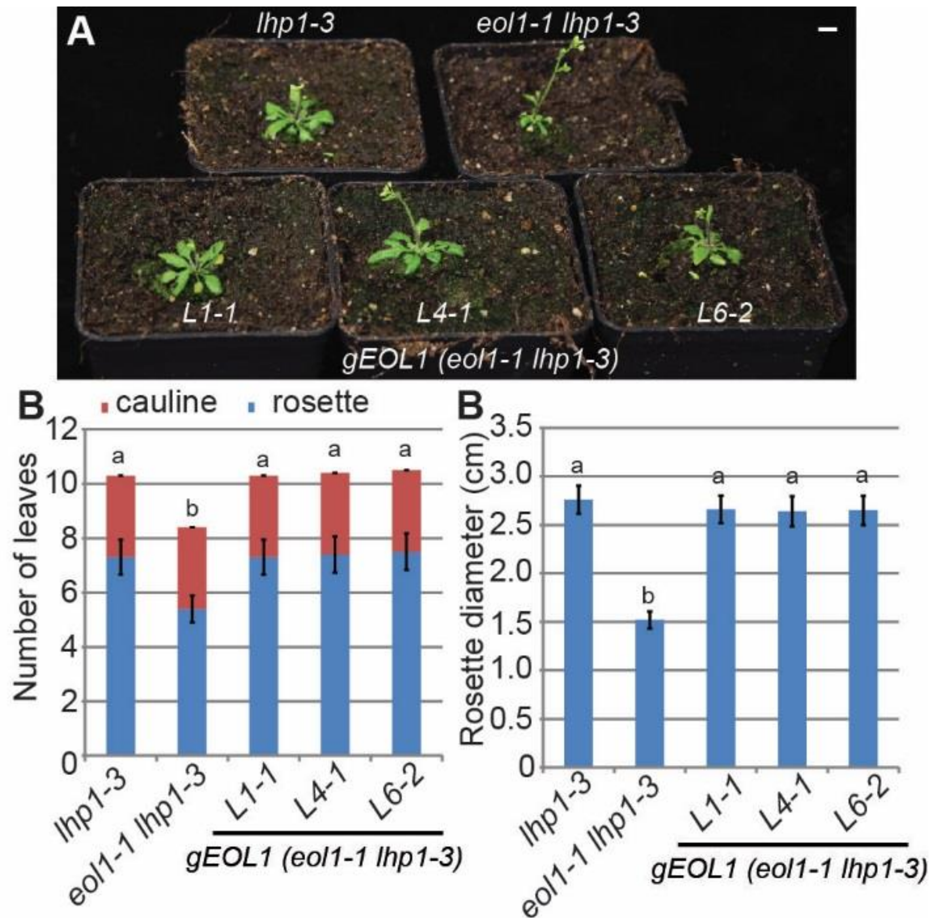

Fig. S2. **Confirmation of mutant mapping results.** **A.** Phenotype of *lhp1-3*, *eol1-1 lhp1-3* and 3 independent lines of *gEOL1* in *eol1-1 lhp1-3* background at day 20. Plants were grown at 22°C in LDs (scale bar: 1 cm). **B.** Flowering time of genotypes grown as in (A) as number of leaves before flowering. Error bars indicate mean ± SE (n=9). Statistical significance was determined by one-way ANOVA with multiple comparison correction by Tukey HSD. Different letters indicate significance groups (p<0.001). **C.** Rosette size of plants as in (A), statistical significance tested as above.

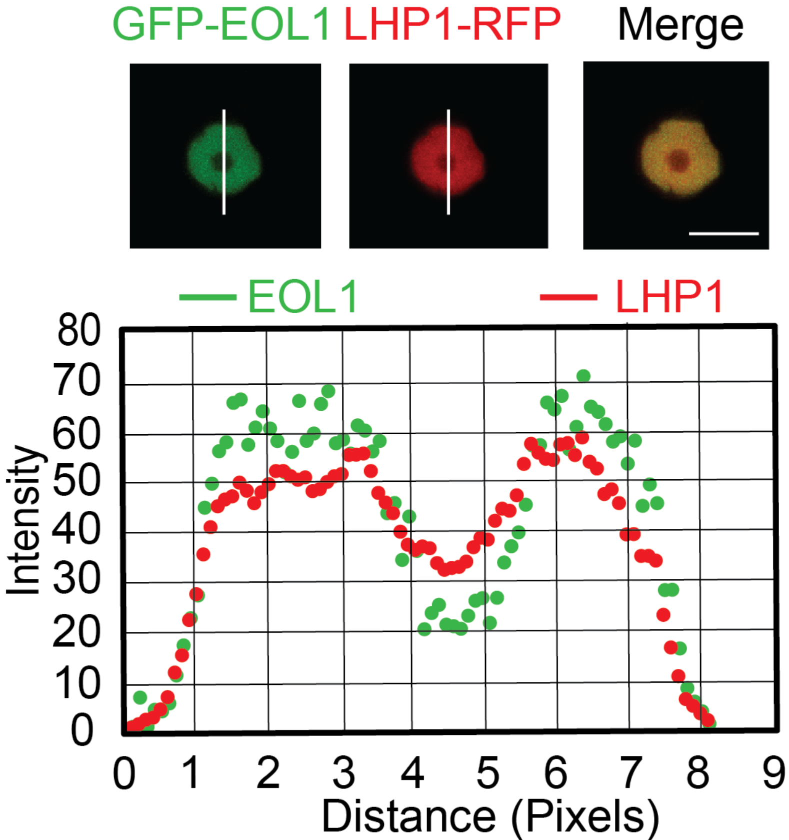

Fig. S3. Nuclear co-localization of fluorescent GFP-EOL1 (green) and LHP1-RFP (red) transiently expressed in *N. benthamiana* leaf epidermis (scale bar: 10 μM). Lower panel shows intensity scans for red and green fluorescence along the white line indicated in the upper panel.

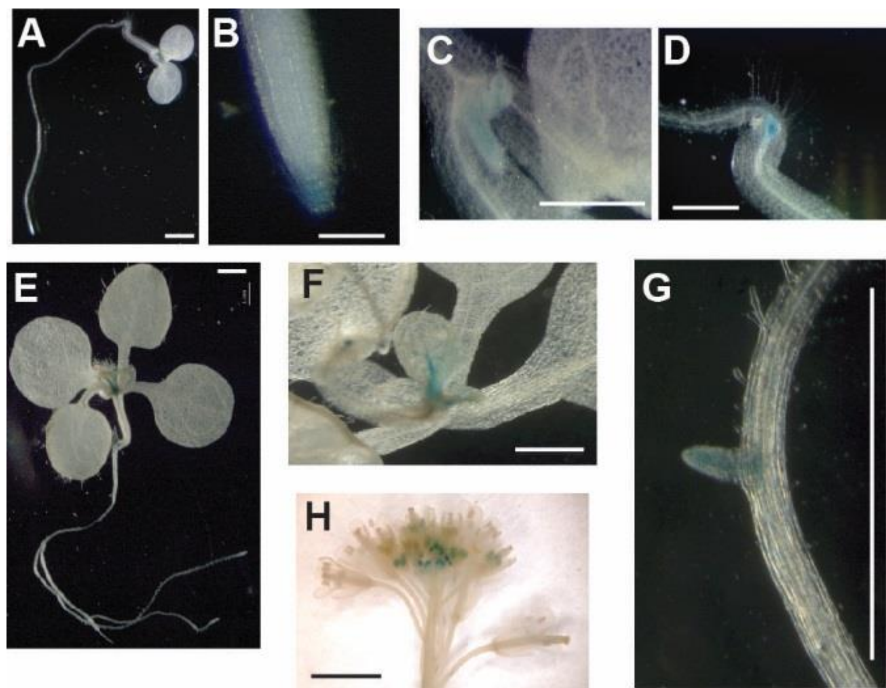

Fig. S4. (A) to **(H)** Histochemical detection of *pEOL1-GUS* expression in Col-0 background in 5-day-old seedlings ([A] to [D]), 10-day-old seedlings ([E] to [G]), root tips (B), new leaves ([C] and [F]), emerging lateral roots (D and G) and flowers (H) from 35-day-old plants. Scale bar = 1000 μm in (A), (E) and (H) and 200 mm in (B) to (D) and (F) to (G).

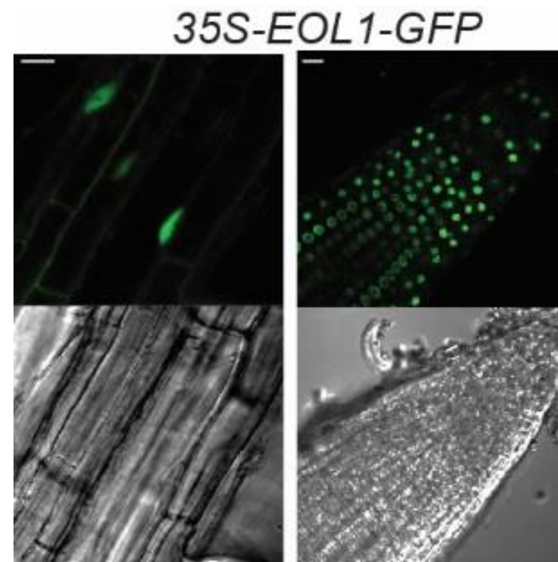

Fig. S5. EOL1-GFP fusion protein is detected in non-dividing tissues if expressed under the control of a constitutive promoter. Roots of transgenic *A. thaliana* plants expressing *EOL1-GFP* fusion under the control of the *CaMV 35S* promoter. Green fluorescence detected in the differentiation zone (left) and the meristematic zone (right). Scale bars: 5μm left panels, 10μm right panels.

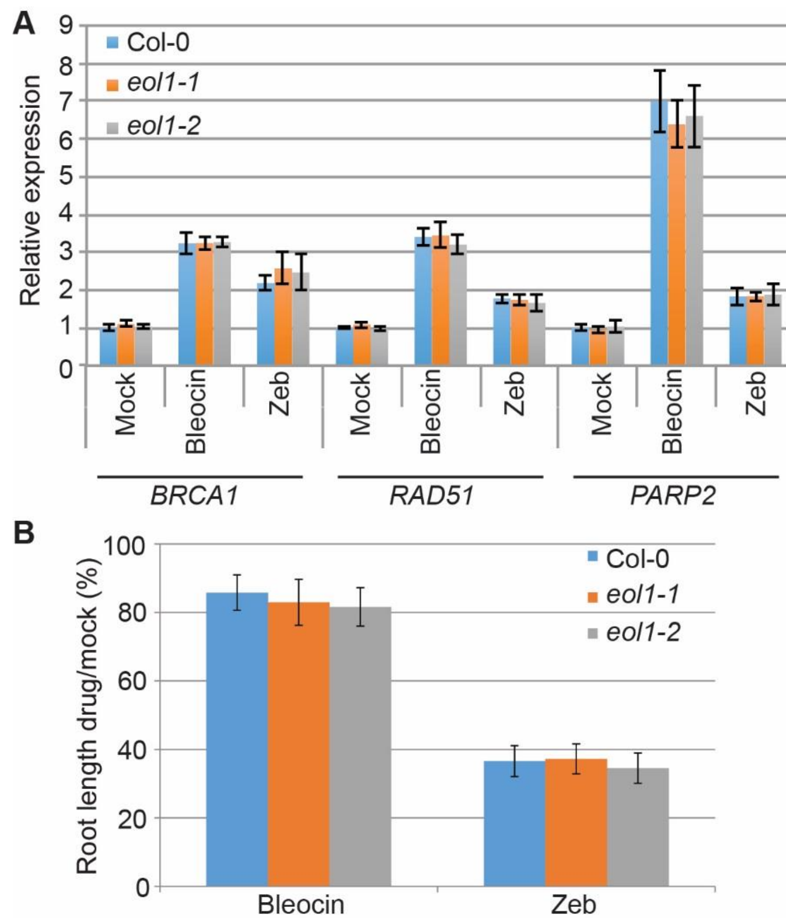

Fig. S6. *Eol1* mutants are not affected in their response to DNA damaging drugs.

A. RT-qPCR analysis of DNA damage marker genes *BRCA1*, *RAD51* and *PARP2* in 10-day-old Col-0, *eol1-1* and *eol1-2* seedlings after 24 hours of treatment with 100 nM Bleocin and 20 mM zebularine (Zeb). **B.** Relative root length of 7-day-old *eol1-1* and *eol1-2* seedlings in response to 20 mM Zeb or 50 nM Bleocin treatment relative to Col-0 controls.

Fig. S7. Alignment of Ctf4 homologs. A. Alignment of the Mouse, Yeast, Drosophila CTF4 and Arabidopsis EOL1. Numbers indicate position of amino-acids, amino-acid consensus and secondary structure indicated below (e: β-sheet, h:α-helix) for each sequence without gaps. Predicted secondary structure is indicated below.

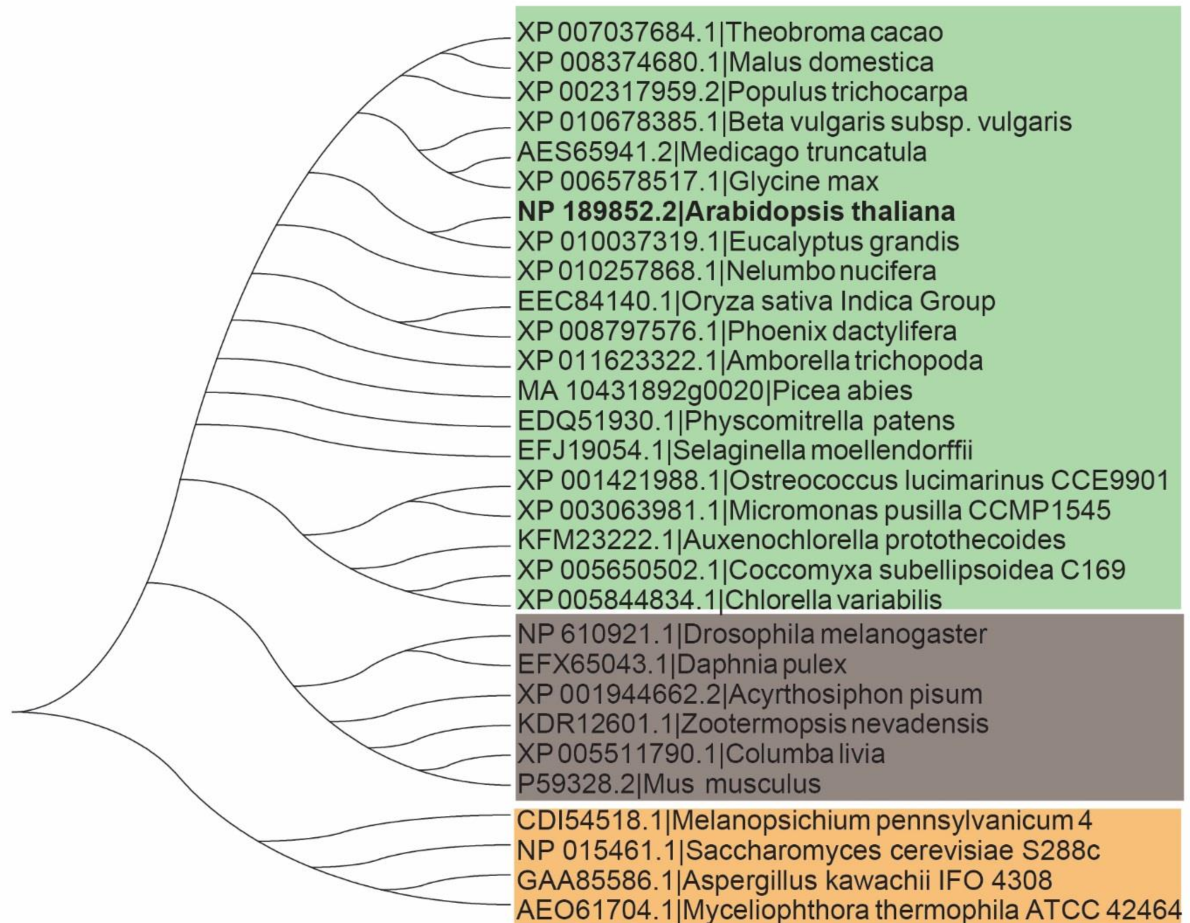

Fig. S8. Phylogenetic tree of Ctf4 homologs from fungi, animals and plants. Sequences corresponding to the 6-bladed propeller domain of Ctf4 were compared to homologs in animals (brown box), fungi (orange box) and plants (green box). A bootstrap consensus tree was calculated from 100 independently generated trees.

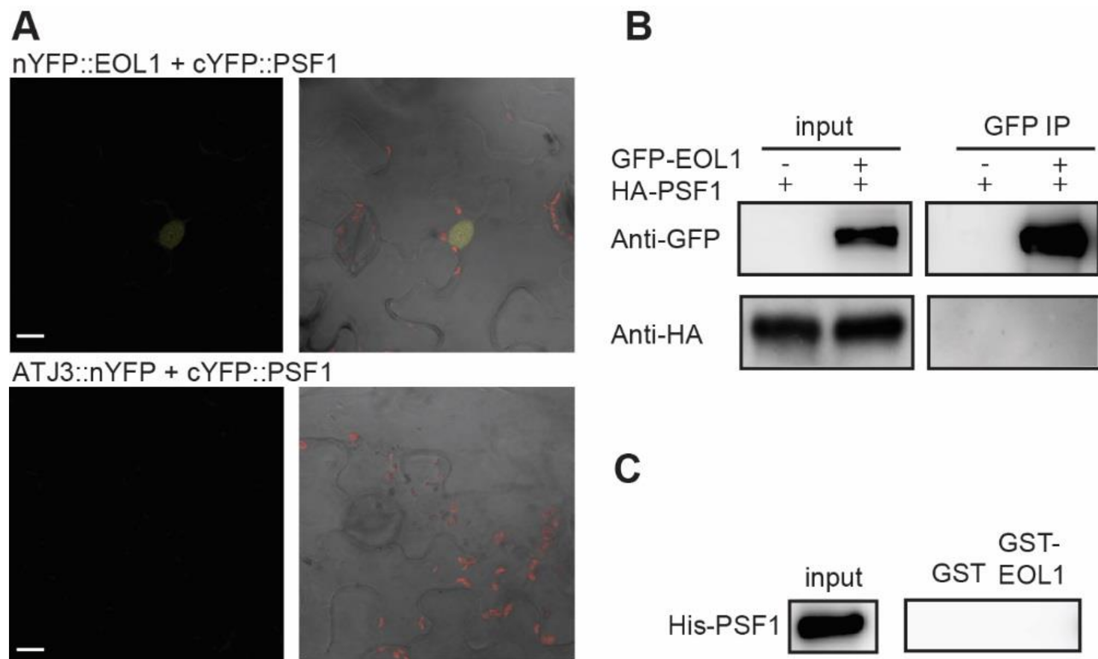

Fig. S9. Interaction between EOL1 and PSF1

A. BiFC analysis of the physical association of EOL1 with PSF1. Plasmid pairs, as indicated, were co-infiltrated into *N. benthamiana* leaves using *Agrobacterium tumefaciens.* ATJ3 was used as a negative control. Scale bars, 10 μm. **B.** CoIP assay of EOL1 with SLD5 in Arabidopsis mesophyll protoplasts. GFP-EOL1 was immunoprecipitated with anti-GFP trap beads from protoplasts co-transfected with HA-PSF1 as indicated, the precipitates were analyzed by Western blotting with anti-HA or anti-GFP antibodies. **C.** Protein pull down assay with GST-EOL1 or GST as bait. Total protein extracts of bacteria expressing His-PSF1 was incubated with bait proteins bound to glutathione-linked resins. Proteins associated with the resins were analyzed by Western blotting with anti-His antibodies.

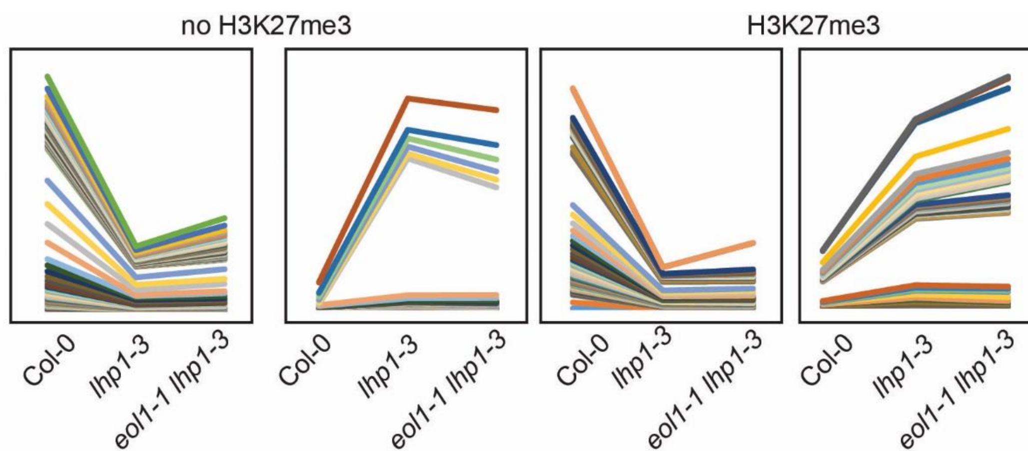

Fig. S10. Expression patterns of genes differentially expressed in *lhp1* or *eol1 lhp1* mutants classified by H3K27me3 coverage as indicated. Note that representation as stacked line charts visualizes patterns but not absolute levels.

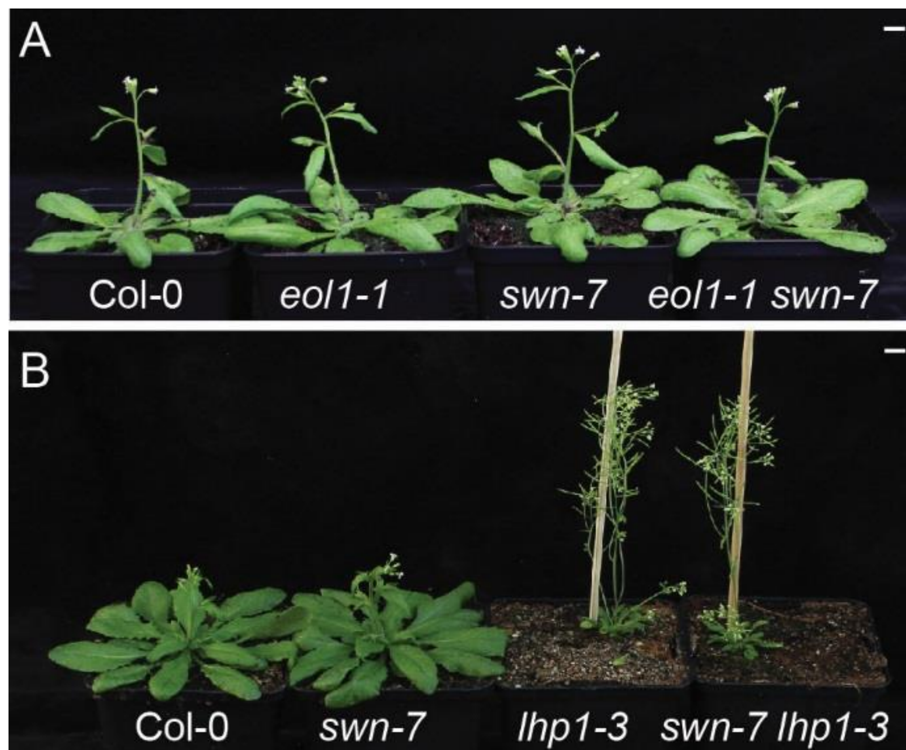

Fig. S11. **Genetic relation between *EOL1*, *SWN* and *LHP1*. A.** Phenotype of Col-0, *eol1-1*, *swn-7* and *eol1-1 swn-7* plants at day 30. Plants were grown at 22°C in LDs (scale bar: 1 cm). **B.** Phenotype of Col-0, *swn-7*, *lhp1-3* and *swn-7 lhp1-3* plants at day 30. Plants were grown at 22°C in LDs (scale bar: 1 cm).

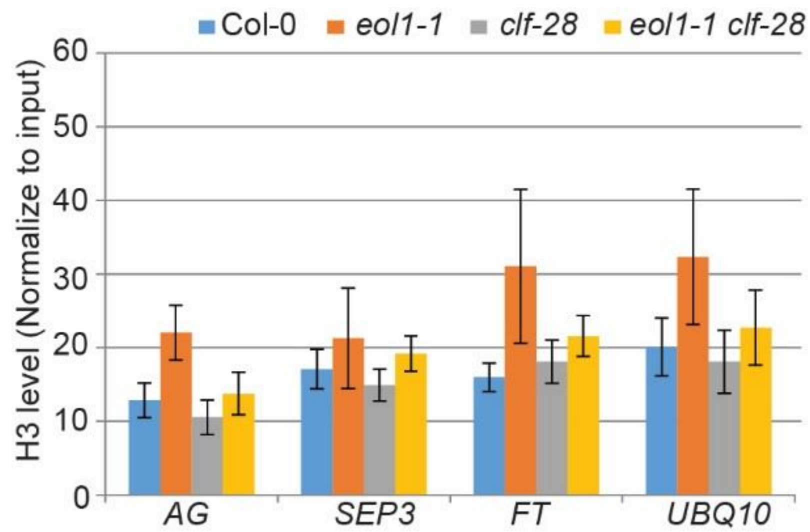

Fig. S12. Control ChIP using histone H3 specific antibodies.

ChIP analysis of H3 levels at *AG*, *SEP3*, *FT* and *UBQ10* in 10-day-old *Col-0*, *eol1-1*, *clf-28* and *eol1-1 clf-28* seedlings. Error bars indicate mean ± SD calculated from three biological replicates.

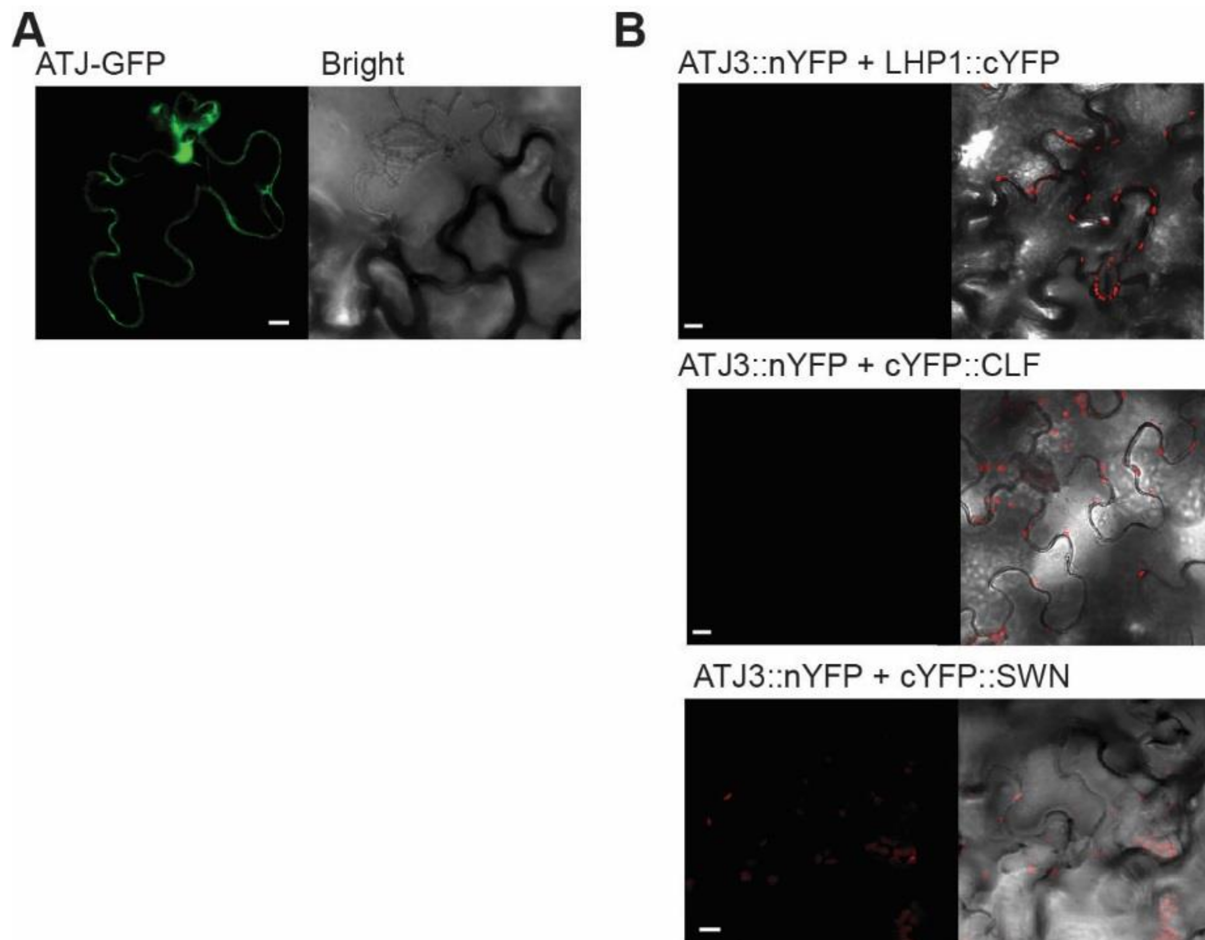

Fig. S13. Interactions using ATJ3 as control.

A. ATJ3-GFP fusion proteins are detected in nuclei of *N. benthamiana* epidermis cells after infiltration with *A. tumefaciens*. **B.** BiFC analysis shows absence of physical association between ATJ3-nYFP and cYFP fused to LHP1, CLF and SWN. Plasmid pairs, as indicated, were co-infiltrated into *N. benthamiana* leaves using *Agrobacterium tumefaciens*. Scale bars: 10 µm.

Supplementary References

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