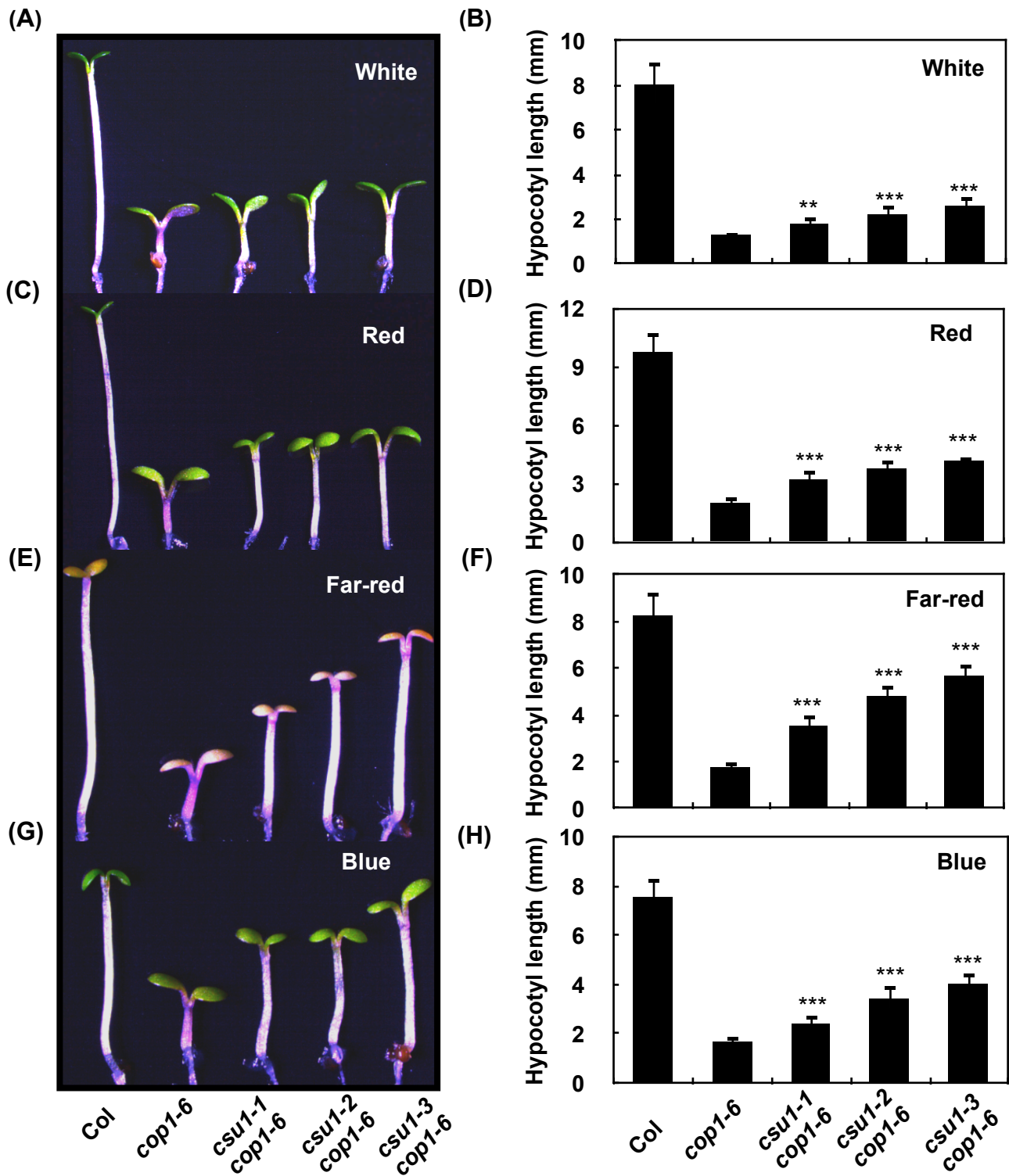


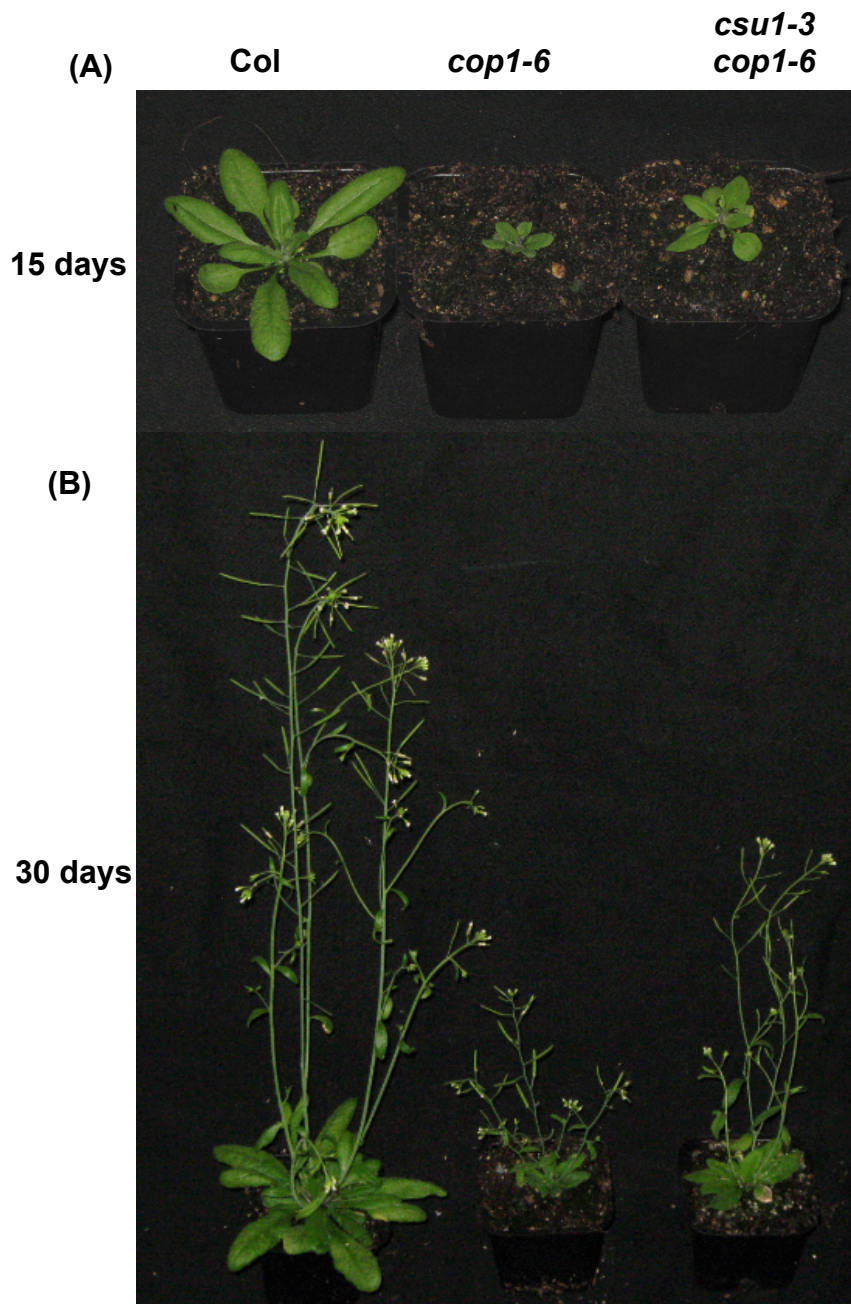
Supplemental Figure 1. *csu1* has no effect on the splicing pattern of *COP1-6* mRNA.

The *cop1-6* mutation led to four cryptically spliced products at intron 4. PCR products generated from wild-type (WT), *cop1-6* and *csu1 cop1-6* mutant seedlings using primers corresponding to the adjacent exons were examined on a 4% agarose gel. M, molecular size markers in base pairs.



Supplemental Figure 2. *csu1* partially suppresses the *cop1* in the light.

Hypocotyl phenotypes and lengths of 5-d-old wild-type, *cop1-6*, and *csu1 cop1-6* mutant seedlings grown under white (A and B), red (C and D) far-red (E and F), blue (G and H) light conditions. Error bars represent SE ($n \geq 20$). ** $P < 0.01$ and *** $P < 0.001$ (Student's *t* test) for the differences between *csu1 cop1-6* and the *cop1-6* mutants.



Supplemental Figure 3. The morphology of adult wild-type and mutant plants.

Plants were grown for 15 (A) or 30 (B) days in soil under long day conditions (16h Light /8h dark).

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At CSU1      1  MPQRHSKNNNDLAFETYEKKK...IGYGTQRERLGRDSIRKFDACCLCLAPFIDPMCCCHGHVECECTILECFLAOKK
Os NP_001049735 1  MPQRHSKNNNDLAFETYEKKK...IGYGTQRERLGRDSIRKFDACCLCLAPLIHPLCCPKGHTFCKECCIIECLLACKK
Ce NP_501214  1  M.TRHKGNSTAAASVYTYEERRRDAKASGYGTLHARLGGADSIKGFHCCSLTLPGRNFWISPTGYIFDRBAILENLAOKK
Dm NP_573288  1  M.TRHARNCTAGAVVTYEKKRDAEESGYGTNAQRLGKDSVRSFDCCSLTLPGRNFWITKGYLFDHBAILOQYIVTKN
Dr NP_001007435 1  M.TRHKGNCTAGAVVTYEKKRDTAAASGYGTQSVRLGKDAIKDFDCCSLSLAPGRDFVLTEDGYLYEBAILOYILHCKT
NOSIP/RUL   1  M.TRHKGNCTAGAVVTYEKKKDTAAASGYGTQNIIRLSRDAVKDFDCCSLSLAPCHDFVVTTPGYLEEBAILEYILHCKK
Mm NP_079809  1  M.TRHKGNCTAGAVVTYEKKKDTAAASGYGTQNIIRLSRDAVKDFDCCSLSLAPCHDFVVTTPGYLEEBAILEYILHCKR

At CSU1      77  DIQRRLAAHSSCKKQDKDEEERLMLCKARELDEFDQNHSAFPRNSDKNH...ED.....K.....NG
Os NP_001049735 77  DIKRRKLAHEAACKKLEKEEEDKRMCLKSKELDAFDQNHGAVPCYHDRSGS...ED.....K.....NG
Ce NP_501214  80  AYAKKLEKEYEKQVAEESAANKIAEG...QAETFTKRTQFS.AIESTPSRTGAVATPRPEVGLKRRQGGVMSTEIAAKV
Dm NP_573288  80  EYSRRLEKEYERLRRAEEDKLSQEAANSQQARMERFVNAEKPMAT...PAHSSAAASEKPKST.....SSAAAAAS
Dr NP_001007435 80  EIAKMKAYEKCKQALKSEGQLESKSEERERAEKFKQRENNIVSKPINPFTSGKSKDEGNQ.....NG
NOSIP/RUL   80  EIAKQMKAYEKCRGTRREEQKELQRAASQDQVHRGFLKESAIIVSRPLNPFATAKALSGTSPD.....DV
Mm NP_079809  80  EIAKQMKAYEKCRGARREEQKELQRAAAQDQVRGFLKKEAAIIVSRPLNPFMPKATLPNTE.....GE

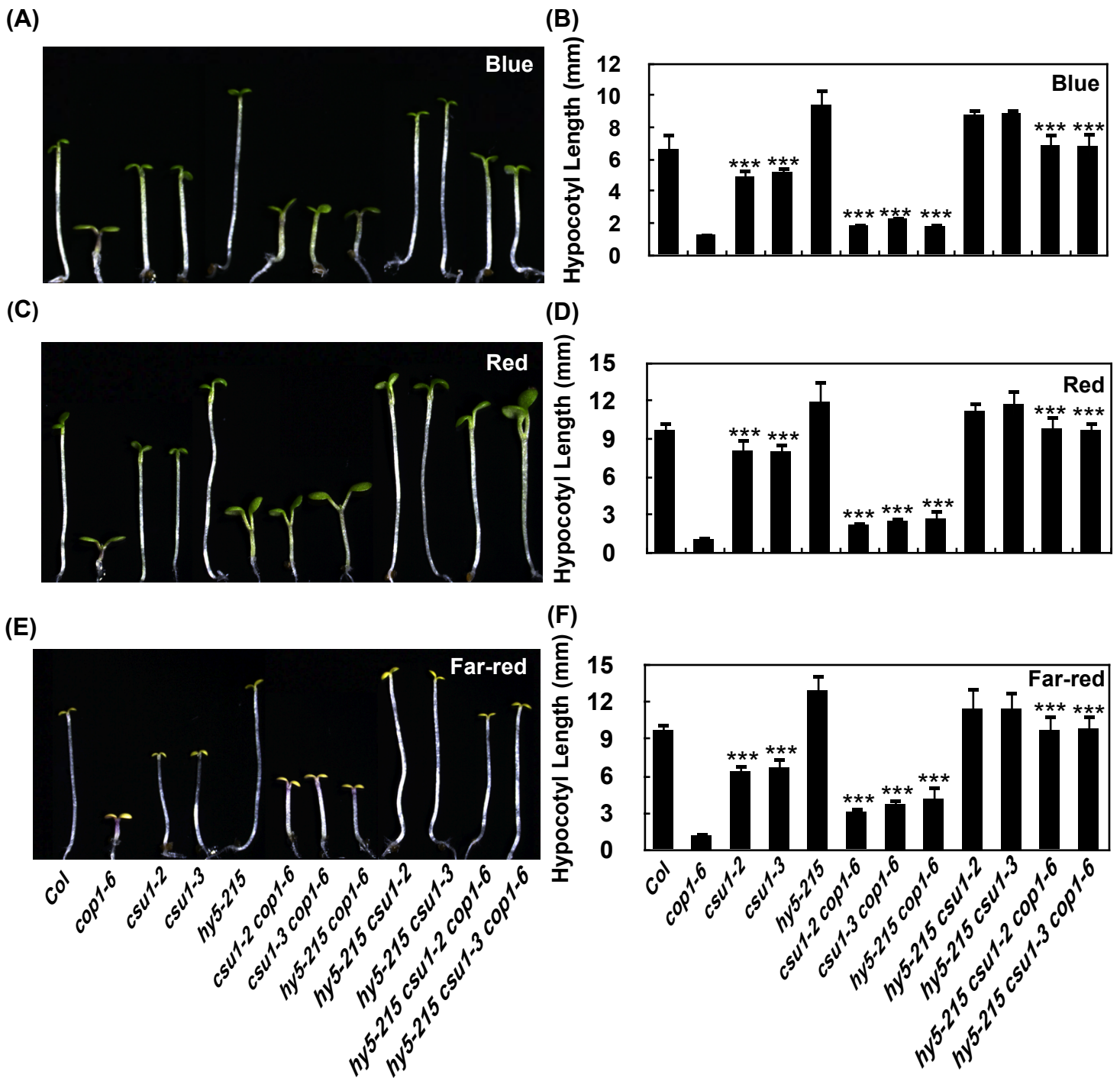
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Os NP_001049735 134 FHGANSVKVTSFEEEEALRNMKAFWLPSATPEAT.AKVEAPSTDTICPEGQEKLEKLSLFPFISFTEENTDQKN.....K
Ce NP_501214  154 KAHGEEGVMSNMKGDKSTSLPSFWLPELNPTAVATKLEKPSKVLCPVSGKPIKLEKLELVKFTPMGTET.....
Dm NP_573288  146 SESSSASSISNMTNGHEKKLPSFWLPSCEPNAGLAKAQKPDATICYVPSQKPLRVKLLIDVRFTLLKDGDTKR....SL
Dr NP_001007435 143 STSSSTDTSSGESSSSALPSFWLPSLTPEAKPTLLKPKSKTVSCPMSSGRPLKMSLLITVRETPLDPSLDRV....AL
NOSIP/RUL   143 QP...GPSVGGPKDKDKVLPFWLPSLTPEAKATKLEKPSRTVTICPMSSGKPLRMSLLTVPVFTPLDSSVDRV....GL
Mm NP_079809  143 QP...GPSVGGPVGKDKKALPSFWLPSLTPEAKATKLEKPSRTVTICPMSSGKPLRMSLLTVPVFTPLDSSVDRV....GL

At CSU1      213 SSYDKSYIICPSCKVTLQNTMSLVALSSCGHVFCKKCAEKFMVVDKVLVCDKPKCDRNLVGLKGGTGFAEHDHLEKME
Os NP_001049735 208 KSVEKSYMOPSCCKSTLQNTMSLVTITSTCGHVFCKKCSDKFLVTDKVLVVCNKPKCKERNFVPLEKGGTGFAAHERLEARD
Ce NP_501214  225 .AAHRKELCPVTRDELQNTTRCAYLKKSKSVKYDVVEKLIKGDGIDPINGSPEMSEDDIELCRGGTGMSATNET.KAKL
Dm NP_573288  221 IAKEARYMCPITHDVLQNAVPCAVLRPTGDVVTMECVERLIRKDMIHPLTDRKLEKDIIPICRGGTGATATNDHLQAME
Dr NP_001007435 218 LTRQDRYVCAVTKDITLQNSVPCAVLRPSGVVVTMECVEKLIKDMVDPITGDKLKEKDIIPICRGGTGAGSGVDLQAME
NOSIP/RUL   215 ITRSERVCAVTRDLSNATPCAVLRPSGAVVTLCEVEKLIKDMVDPVTDKLTDRDIIVLCRGGTGAGSGVKLQAEK
Mm NP_079809  215 ITRSERVCAVTRDLSNATPCAVLRPSGAVVTLCEVEKLIKDMVDPVNGDTLTERDIIIVLCRGGTGAGSGVKLQREM

At CSU1      293 YKHLGSGSGLGLVLPVKT..
Os NP_001049735 286 FMHLGSGSGLGLVKPAPKNY
Ce NP_501214  303 IRPQLELQ.....
Dm NP_573288  301 KRPMQLCA.....
Dr NP_001007435 298 ARFVMQA.....
NOSIP/RUL   295 SRPVMQA.....
Mm NP_079809  295 SRPVMQA.....

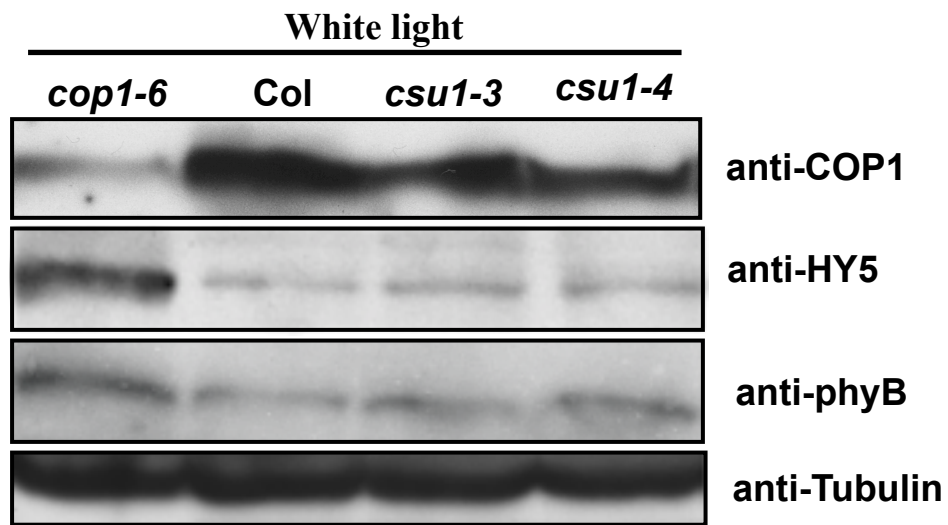
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Supplemental Figure 4. Alignment of CSU1 with its homologs from other species. *Oryza sativa* (NP_001049735), *Caenorhabditis elegans* (NP_501214), *Drosophila melanogaster* (NP_573288), *Danio rerio* (NP_001007435), *Mus musculus* (NP_079809), and *Homo sapiens* (NP_057037). Black boxes are identical residues; dots indicate gaps.

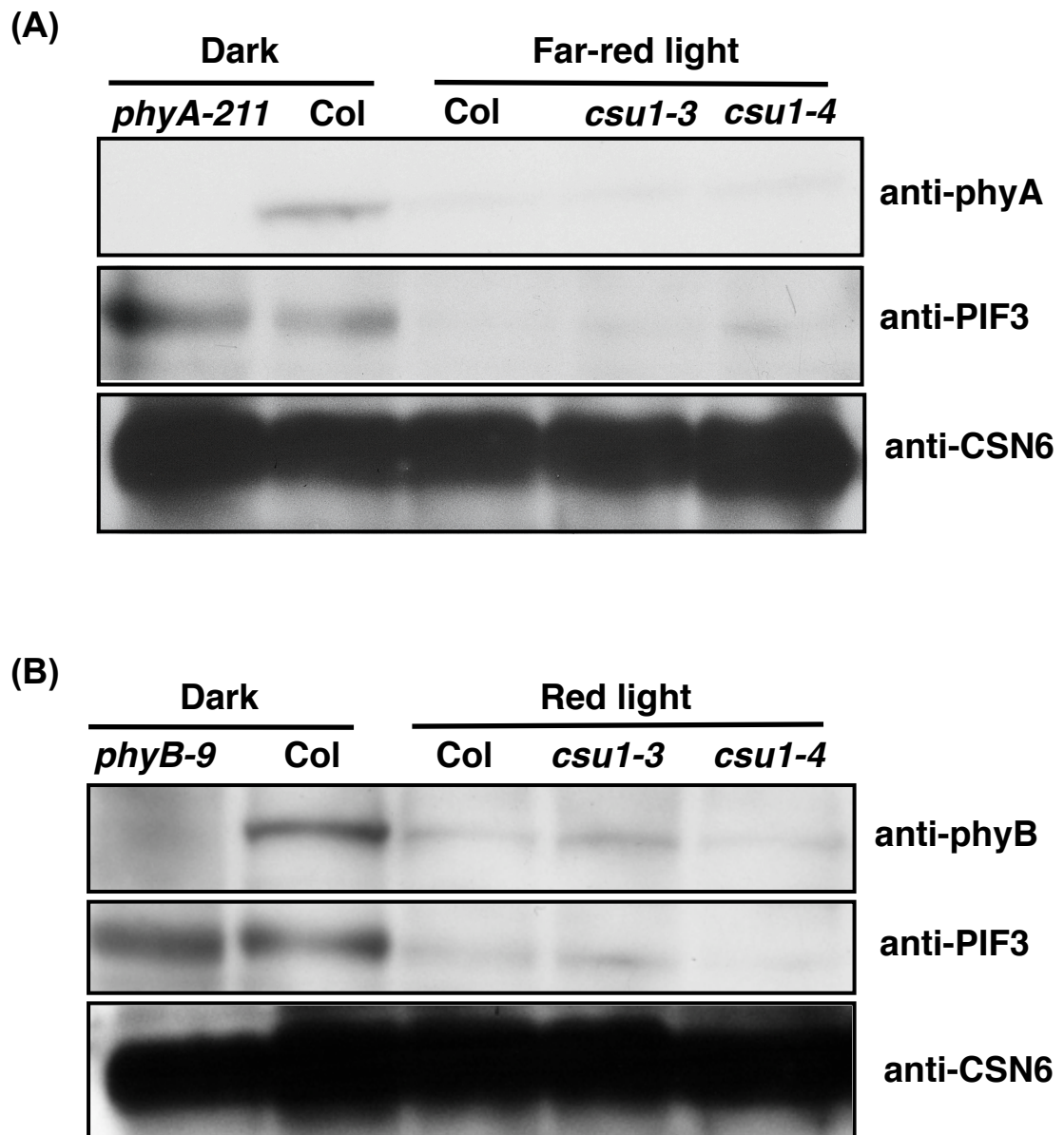


Supplemental Figure 5. *csu1* and *hy5* co-suppress *cop1* in B, R and FR light.

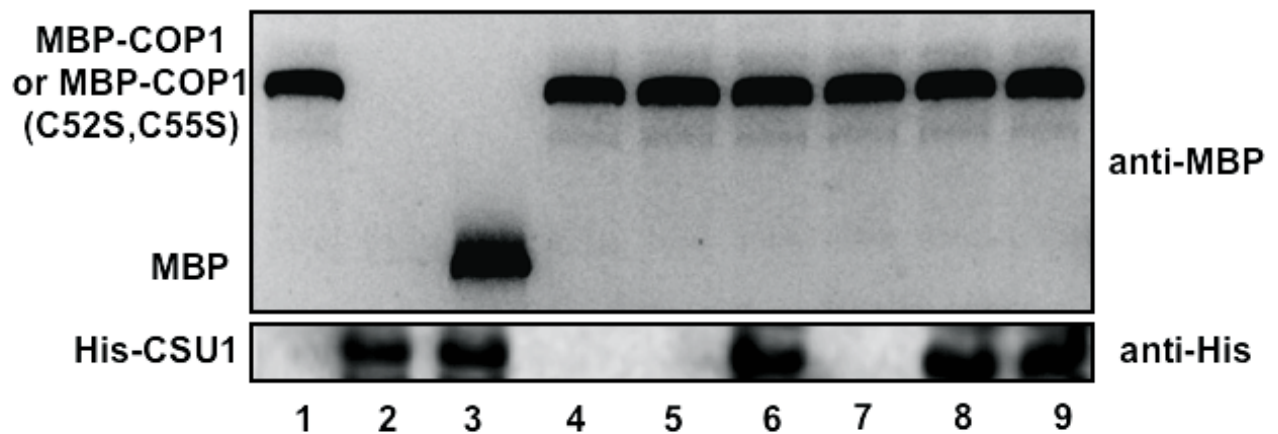
Hypocotyl length and phenotypes of 5-d-old wild-type, *cop1-6*, *csu1*, *hy5-215*, *csu1 cop1-6*, *hy5-215 csu1*, and *hy5-215 csu1 cop1-6* mutant seedlings grown under blue (5 $\mu\text{mol m}^{-2} \text{s}^{-1}$) (A and B), red (20 $\mu\text{mol m}^{-2} \text{s}^{-1}$) (C and D), and far-red (1 $\mu\text{mol m}^{-2} \text{s}^{-1}$) (E and F) light conditions. Error bars represent SE ($n \geq 20$). *** $P < 0.001$ (Student's *t* test) for the differences between WT and the *csu1*, *csu1 cop1-6* and *cop1-6*, or *hy5-215 csu1 cop1-6* and *csu1 cop1-6* seedlings, respectively.



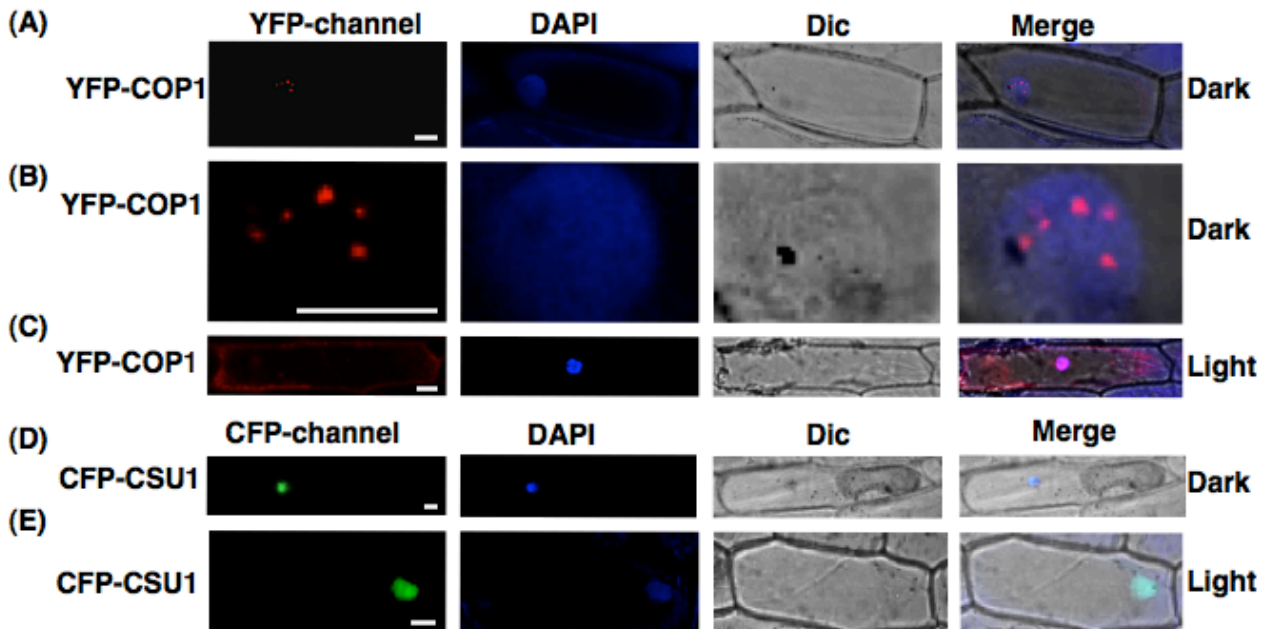
Supplemental Figure 6. COP1 protein levels in the white light-grown *csu1* mutant seedlings. COP1, HY5, and phyB protein levels in *cop1-6*, *csu1*, and Col grown under white light. Plant total protein were extracted from 5-day-old continuous white light grown seedlings. Tubulin levels were used as loading controls.



Supplemental Figure 7. phyA , phyB and PIF3 protein levels in the far-red or red light-grown *csu1* mutant seedlings. (A) PHYA, and PIF3 protein levels in *csu1*, and Col grown in the far-red. (B) phyB, and PIF3 protein levels in *csu1*, and Col grown under red light. Plant total protein were extracted from 5-day-old continuous far-red or red light grown seedlings. Dark-grown *phyA-211*, *phyB-9* and Col were used as negative or positive controls, respectively. CSN6 levels were used as loading controls.



Supplemental Figure 8. 5% input used *in vitro* ubiquitination assays. Related to Figure 8A. 25 ng of MBP or MBP-COP1 and 10 ng of 6×His-CSU1 (1% input) were loaded onto a 10% SDS-PAGE gel and immunoblotted by antibodies against MBP or His.



Supplemental Figure 9. CSU1 localizes to nucleus both in the dark and light.

Onion epidermal cells were bombarded with YFP-COP1 plasmids with a helium gun incubated in the dark for 24 hours (Dark) (A) or in the dark for 12 hours, then treated with white light for 12 hours (Light) (C). (B) are closeups of the nuclei as shown in (A). Onion epidermal cells were bombarded with CFP-CSU1 plasmids with a helium gun, then incubated in the dark for 24 hours (Dark) (D) or in the dark for 12 hours, then treated with white light for 12 hours (Light) (E). YFP channel, YFP channel image; CFP channel, CFP channel image; DAPI, 4',6-diamidino-2-phenylindole staining image; Dic, differential interference contrast in light microscope mode; Merge, merged image between DAPI and YFP or CFP. The scale bar in all panels represents 50 μm .

Supplemental Table 1: List of Primers Used in This Study.

Primer name	Primer sequences (5'→3') (Note: The underlined nucleotides indicate the restriction sites for cloning.)	
Genotyping		
CSU1LP	CCTCTTCGCTGTTGTCTTCTG	
CSU1RP	TTGTTCTCTCTGCTTGAAGCC	
SALK_LBb1.3	ATTTTGCCGATTTTCGGAAC	
Quantitative Real-time RT-PCR		
CSU1-real(F)	CCATAAGGGTCATGTCTTTTGC	
CSU1-real(R)	CTTGCTTCTTTTGAGACGAATG	
PP2A(F)	TATCGGATGACGATTCTTCGTGCAG	
PP2A(R)	GCTTGGTCGACTATCGGAATGAGAG	
RT-PCR		
CSU1-rt(F)	CCTTTGATGCTTGTTCTCTCTG	
CSU1-rt(R)	CCTCCTTTCTTTAACCCAACC	
UBQ10(F)	GATCTTTGCCGAAAACAATTGGAGGATGGT	
UBQ10(R)	CGACTTGTCATTAGAAAGAAAGAGATAACAGG	
Splicing test		
cop1-6-S(F)	GTCAACTGTCTCAATGGCTAGAAA	
cop1-6-S(R)	CTATAGCCTTCCCTCCGTAACA	
Protein expression		Plasmid Constructs
CSU1-NdeI(F)	GGGAATTCC <u>CATATGATGCCGCAAAGACACTCGAA</u>	<i>pET28b-CSU1</i>
CSU1-XhoI(R)	CCG <u>CTCGAG</u> TTATCATGTCTTAACCGGCCTCAC	
Colocalization assay		<i>pAM-PAT-35SS-CFP-CSU1</i>
CSU1-NdeI(F)	GGGAATTCC <u>CATATGATGCCGCAAAGACACTCGAA</u>	
CSU1-XhoI(R)	CCG <u>CTCGAG</u> TTATCATGTCTTAACCGGCCTCAC	
Complementation test		<i>pFP100-CSU1</i>
CSU1-KpnI(F)	CGGG <u>GTACCTCAGATGTGTATAGCTTTGGAGTG</u>	
CSU1-PstI(R)	AAA <u>ACTGCAGTTATCATGTCTTAACCGGCCTCAC</u>	