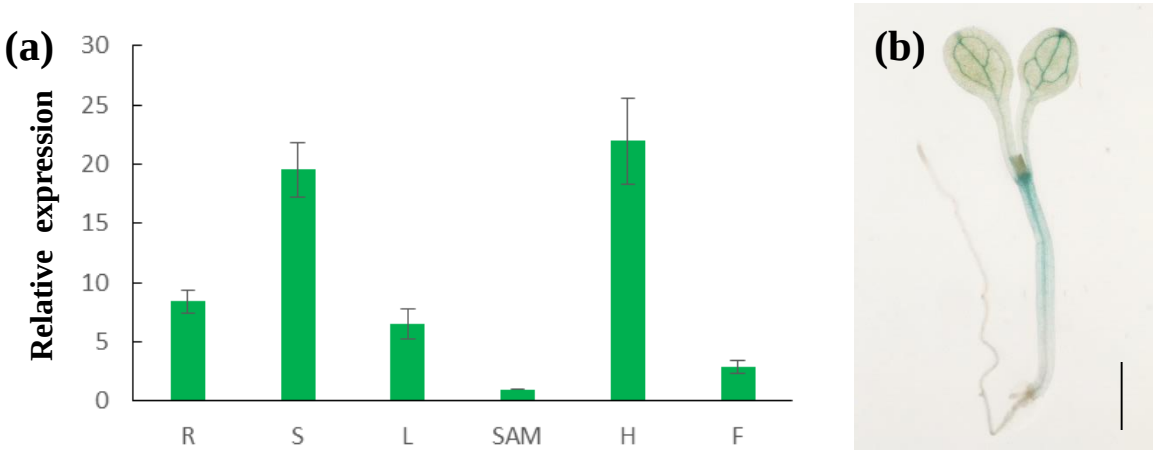


Fig.1 COL13 RNA accumulates to high levels in hypocotyl. (a) Quantitative real time-PCR analysis of *AtCOL13* transcript abundance in different tissues. R=Root, S=Stem, L=Leaf, SAM=Shoot apical meristem, H=Hypocotyl, F=Flower. (b) Activity of COL13 promoter revealed by β-glucuronidase (GUS) staining in Arabidopsis seedlings. Bar=100 mm.

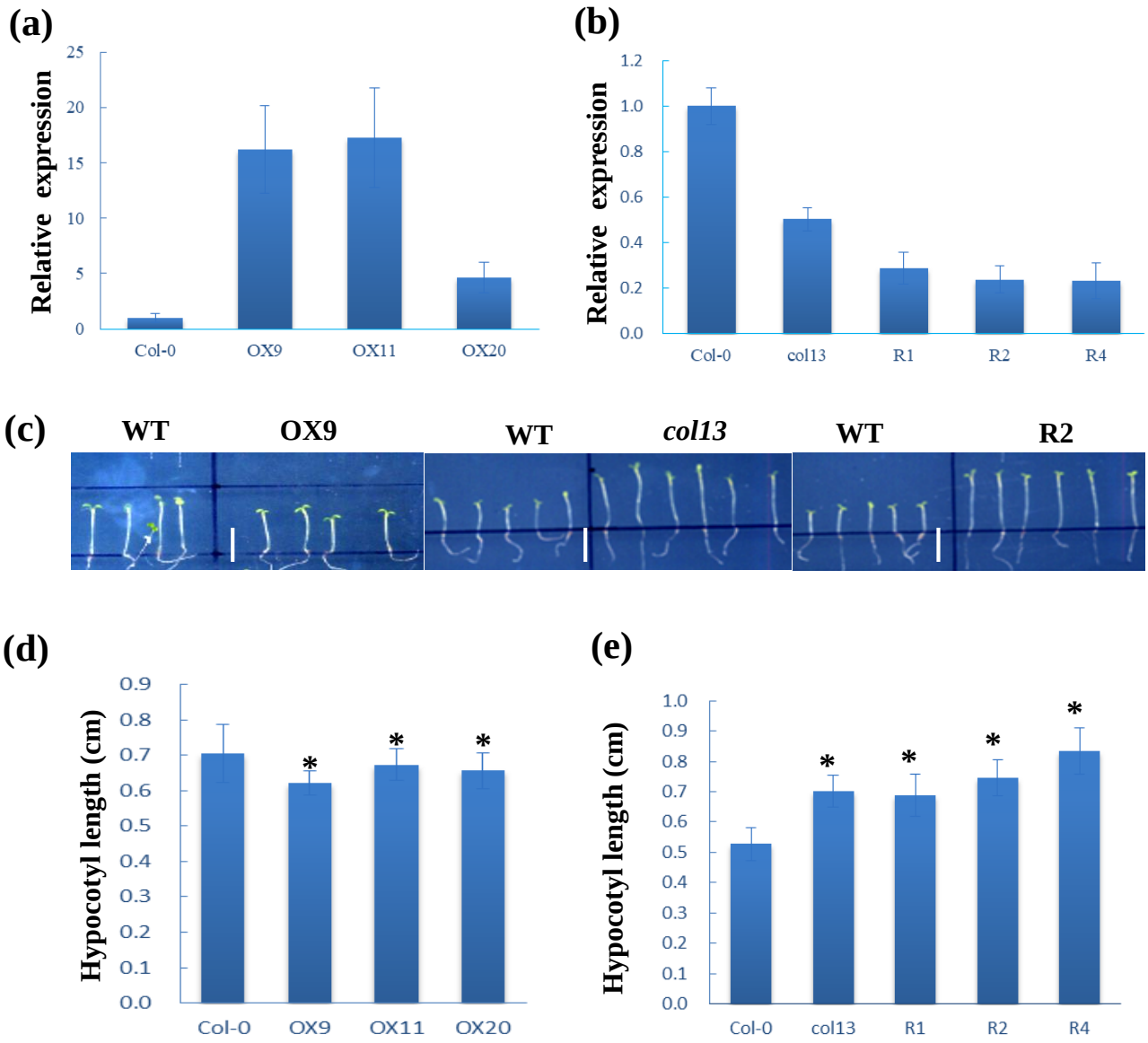


Fig.2 COL13 regulates hypocotyl elongation under red-light conditions. (a) Relative expression of *COL13* in Col-0 and overexpression (OX) lines. (b) Relative expression of *COL13* in Col-0, T-DNA mutant (*col13*) and RNAi lines (R1-1 etc.). (c)-(e) Phenotypic analysis seedlings of the indicated genotypes were grown in the presence of red light. Images of representative seedlings are shown in (c), white bar=0.5cm. The hypocotyl lengths of the indicated genotypes were measured at the 5th or 3rd day, and are shown in (d) and (e), respectively. Error bars indicate SD (n >15). Asterisks indicate that hypocotyl lengths in OX9 and col13, COL13 RNAi are significantly different with WT under red light ($P < 0.05$).

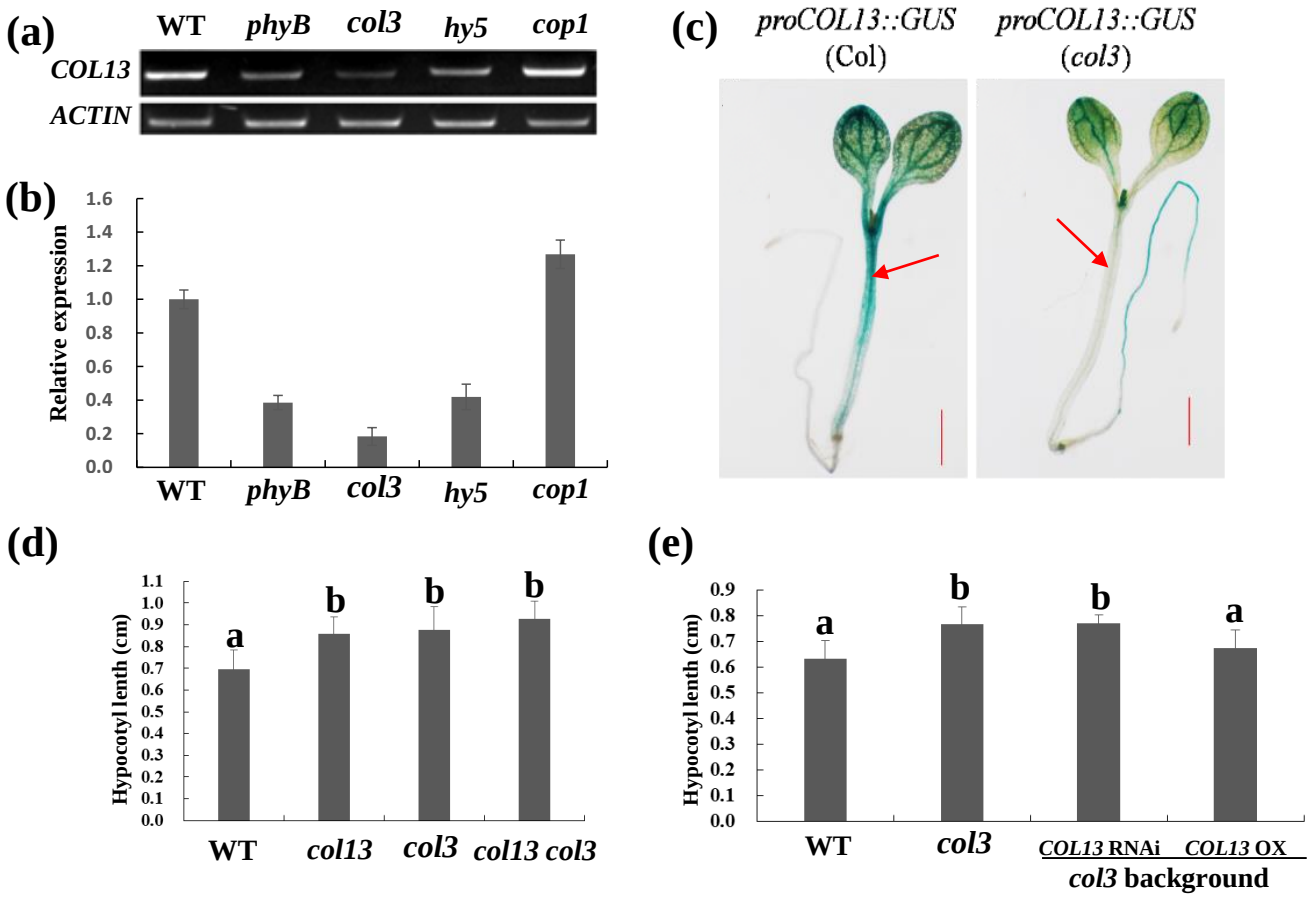
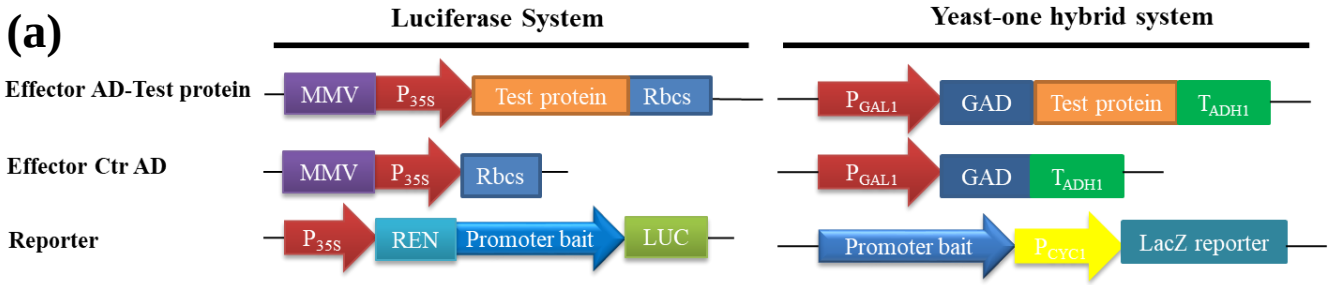
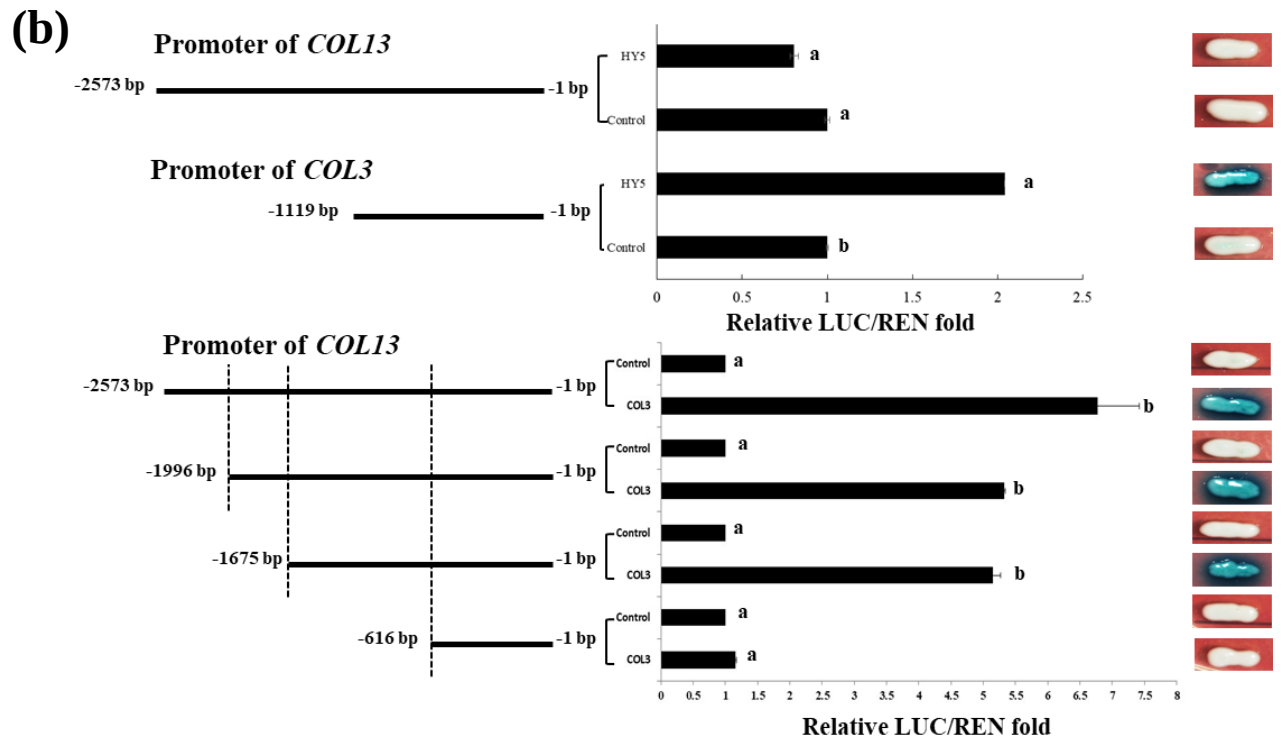
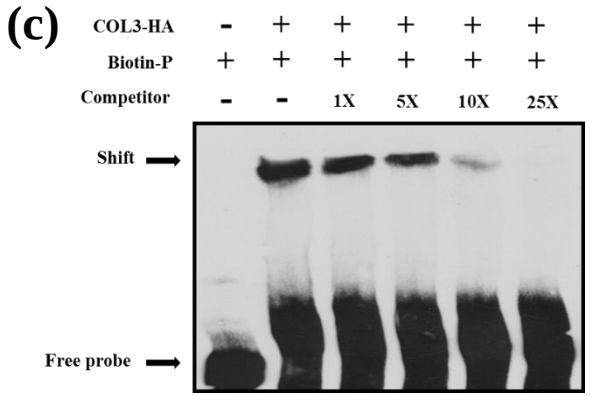


Fig. 3 Genetic interaction and physiological characterization of hypocotyl elongation. (a) Semi-quantitative RT-PCR analyses of *COL13* expression in *phyB*, *col3*, *hy5* and *cop1* mutants. (**b**) qRT-PCR analyses of *COL13* expression in *phyB*, *col3*, *hy5* and *cop1* mutants. (**c**) Activity of the *COL13* promoter revealed by β-glucuronidase (GUS) staining in WT and *col3* mutant backgrounds. (**d**) Hypocotyl length in WT, single- and double-mutant plants. Here we use the F1 hybrid of Col-0×WS as WT. (**e**) Hypocotyl length in WT and *col3* plants compared to transgenic plants with *COL13* RNAi or *COL13* overexpression (OX) in the *col3* background. Here we use WS as WT. Error bars indicate SD (n>15). Lower-case letters indicate significantly different data groups (hypocotyl length) of the indicated seedlings grown in red light.


(a)
Luciferase System
Yeast-one hybrid system
Effector AD-Test protein
MMV
P35S
Test protein
Rbcs
PGAL1
GAD
Test protein
TADH1
Effector Ctr AD
MMV
P35S
Rbcs
PGAL1
GAD
TADH1
Reporter
P35S
REN
Promoter bait
LUC
Promoter bait
PCYC1
LacZ reporter


(b)
Promoter of COL13
-2573 bp
-1 bp
HY5
Control
a
a
Promoter of COL3
-1119 bp
-1 bp
HY5
Control
a
b
0
0.5
1
1.5
2
2.5
Relative LUC/REN fold
Promoter of COL13
-2573 bp
-1996 bp
-1675 bp
-616 bp
-1 bp
Control
COL3
a
b
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
5.5
6
6.5
7
7.5
8
Relative LUC/REN fold


(c)
COL3-HA
Biotin-P
Competitor
-
+
1X
5X
10X
25X
Shift
Free probe

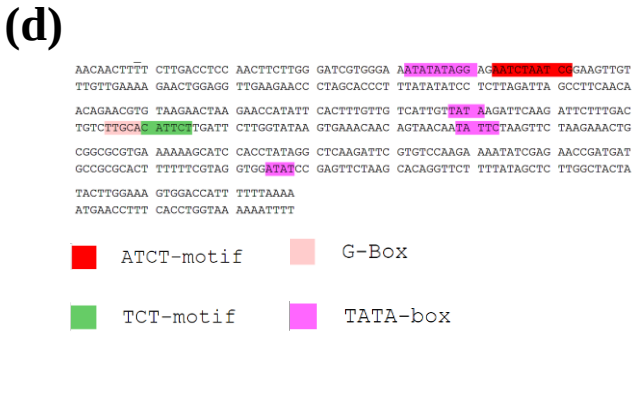

(d)
AACAACTTTT CTTGACCTCC AACTTCTTGG GATCGTGGGA AATATATAGG AGAATCTAAT CGGAAGTTGT
TTGTTGAAAA GAACTGGAGG TTGAAGAACC CTAGCACCCT TTATATATCC TCTTAGATTA GCCTTCAACA
ACAGAACGTG TAAGAACTAA GAACCATATT CACTTTGTTG TCATTGTTAT AAGATTCAAG ATTCTTTGAC
TGTCTTGCAC ATTCTTGATT CTTGGTATAA GTGAAACAAC AGTAACAATA TTGTAAGTTC TAAGAAACTG
CGGCGCGTGA AAAAAGCATC CACCTATAGG CTCAAGATTC GTGTCCAAGA AAATATCGAG AACCGATGAT
GCCGCGCACT TTTTTCGTAG GTGGATATCC GAGTTCTAAG CACAGGTTCT TTTATAGCTC TTGGCTACTA
TACTTGGAAA GTGGACCATT TTTTAAAA
ATGAACCTTT CACCTGGTAA AAAATTTT
ATCT-motif
G-Box
TCT-motif
TATA-box

Fig.4 Analysis of the binding of HY5 to *COL3* promoter, and COL3 to *COL13* promoter truncations. (a) Diagram of constructs used in this study. For luciferase system, The AD-HY5 or AD-COL3 fusion gene driven by the 35S promoter produces a potential effector protein, while the AD protein alone represents a negative control for basal activity of *COL3* promoter or each *COL13* promoter truncation. The *LUC* gene driven by the series of *COL3* promoter or *COL13* promoter truncations tests the ability of the AD-HY5 or AD-COL3 fusion protein to bind to each promoter truncation. For yeast-one hybrid system, the GAD-HY5 or GAD-COL3 fusion genes driven by the GAL1 (P_{GAL1}) promoter serves as effectors. The GAD protein served as negative control to see if there exits the self activity of COL3 or COL13 promoters. The *LacZ* gene driven by COL3 or COL13 promoter truncations served as the reporter to test the binding activity of the GAD-HY5 or GAD-COL3 fusion protein to individual promoter truncations. (**b**) For luciferase assay, the fusion protein AD-HY5 can up-regulate *LUC* expression from the *COL3* promoter, but not from COL13 promoter; and the fusion protein AD-COL3, but not AD alone, can up-regulate *LUC* expression from some of the *COL13* promoter truncations. For Yeast-one hybrid assay, the fusion protein GAD-HY5 can strongly bind to the promoter of *COL3*, but not *COL13* to direct LacZ expression in yeast cells that turns 5-bromo-4-chloro-3-indolyl-b-D-galactopyranoside to blue compound; and the fusion protein AD-COL3, but not AD alone, can bind to some of the *COL13* promoter truncations. (**c**) Electrophoretic mobility shift assay (EMSA) analysis showing the binding of COL3 on the -1421 to -1184 bp promoter of *COL13* in vitro. The black arrow indicates binding of COL3 to the biotin-labeled *COL13* promoter. The + and – represent the presence and absence of corresponding components, respectively. Competition experiments were carried out by adding 5-, 10- and 25-fold excessive competitor. (d) In probe 2, there were three light responsive elements (ATCT-motif, G-Box and TCT-motif) and one core promoter element for transcription start (TATA-box).

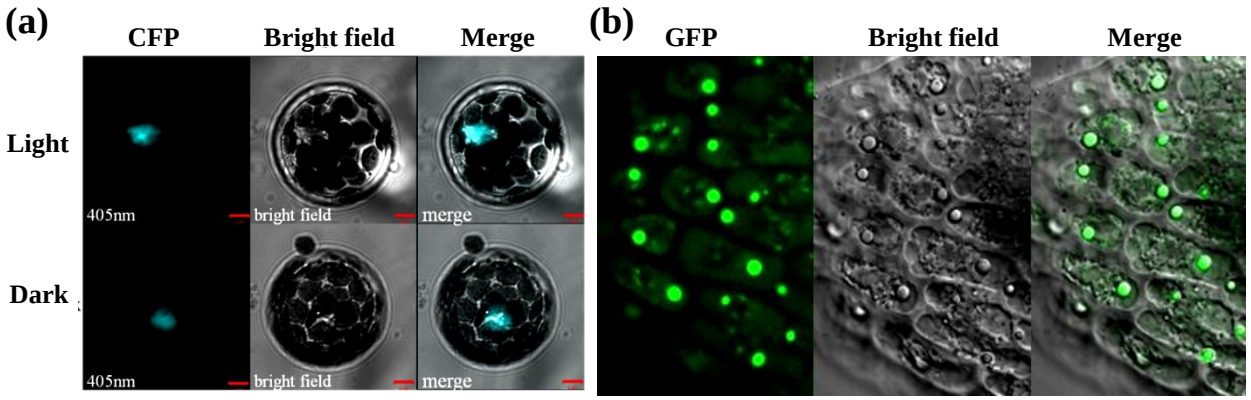


Fig.5 Subcellular localization of COL13. (a) COL13-CFP localizes to the nucleus in protoplasts. (**d**) COL13-GFP localizes to the nucleus in root tip cells.

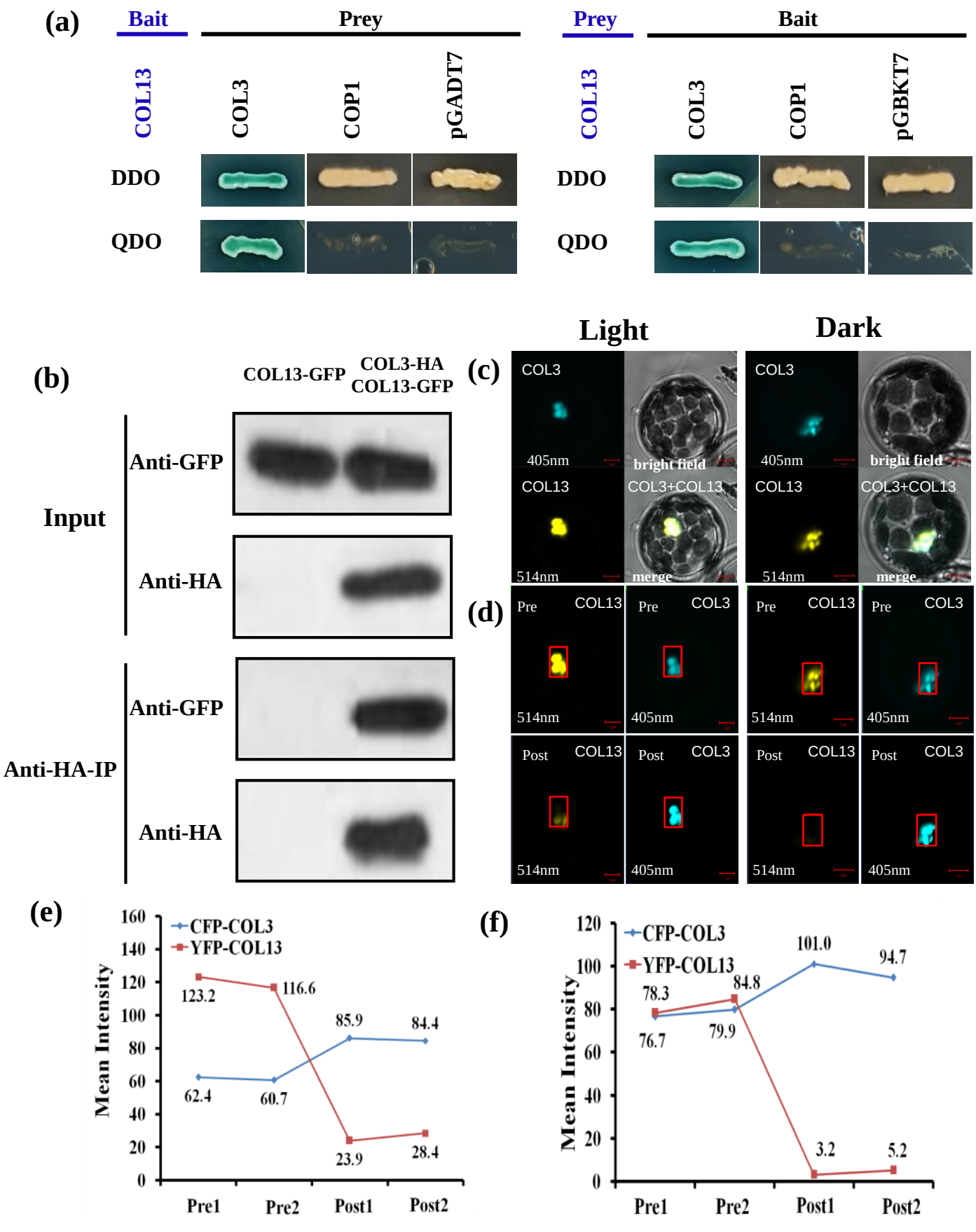
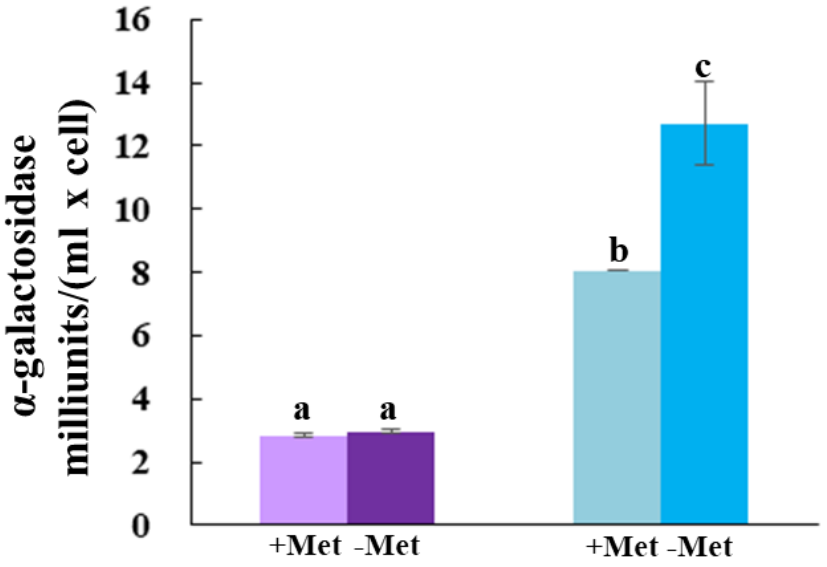

(a)
Bait
Prey
COL13
COL3
COP1
pGADT7
DDO
QDO
Prey
Bait
COL13
COL3
COP1
pGBKT7
DDO
QDO
(b)
COL13-GFP
COL3-HA
COL13-GFP
Input
Anti-GFP
Anti-HA
Anti-HA-IP
Anti-GFP
Anti-HA
(c)
Light
Dark
COL3
405nm
bright field
COL13
514nm
COL3+COL13
merge
(d)
Pre
Post
COL13
COL3
514nm
405nm
(e)
CFP-COL3
YFP-COL13
Mean Intensity
123.2
116.6
85.9
84.4
62.4
60.7
23.9
28.4
Pre1
Pre2
Post1
Post2
(f)
CFP-COL3
YFP-COL13
Mean Intensity
78.3
84.8
101.0
94.7
76.7
79.9
3.2
5.2
Pre1
Pre2
Post1
Post2

Fig.6 COL13 interacts with COL3. (a) Yeast Two-Hybrid assay between COL13 and COL3. DDO, Double Dropout; QDO, Quadruple Dropout; pGADT7, prey plasmid; pGBKT7, bait plasmid. (**b**) Co-immunoprecipitation (Co-IP) in *Arabidopsis*. Immunoprecipitations (IPs) were performed on protein extracted from 10-d-old *Arabidopsis* seedlings grown under long-day illumination (16L: 8D) at 22˚C. Leaf tissues were harvested 1 h after the light cycle commenced. IP was performed using anti-HA antibody and COL13 was co-immunoprecipitated with anti-GFP antibody. A 5% input was used. Western blots were performed on 10% (wt/vol) precast gels (Bio-Rad). (**c**) COL3-CFP and COL13-YFP colocalize to the nucleus in protoplasts in light and dark. (**d-f**) FRET between CFP-COL3 and YFP-COL13 analyzed by acceptor bleaching in the nucleus. The top panels in (**d)** show a representative pre-bleach nucleus coexpressing YFP-COL13 and CFP-COL3 excited with either a 514- or a 405-nm laser in light and dark, resulting in emission from YFP (yellow) or CFP (blue), respectively. The bottom panels in (**d)** show the same nucleus post-bleaching after excitation with a 514- or a 405-nm laser. The relative intensities of both YFP and CFP were measured before and after bleaching, as indicated in (**e)** and **(f)**.

(a)



pGADT7	+	-
pGADT7-COP1	-	+
COL3-COL13-pBridge	+	+

(b)

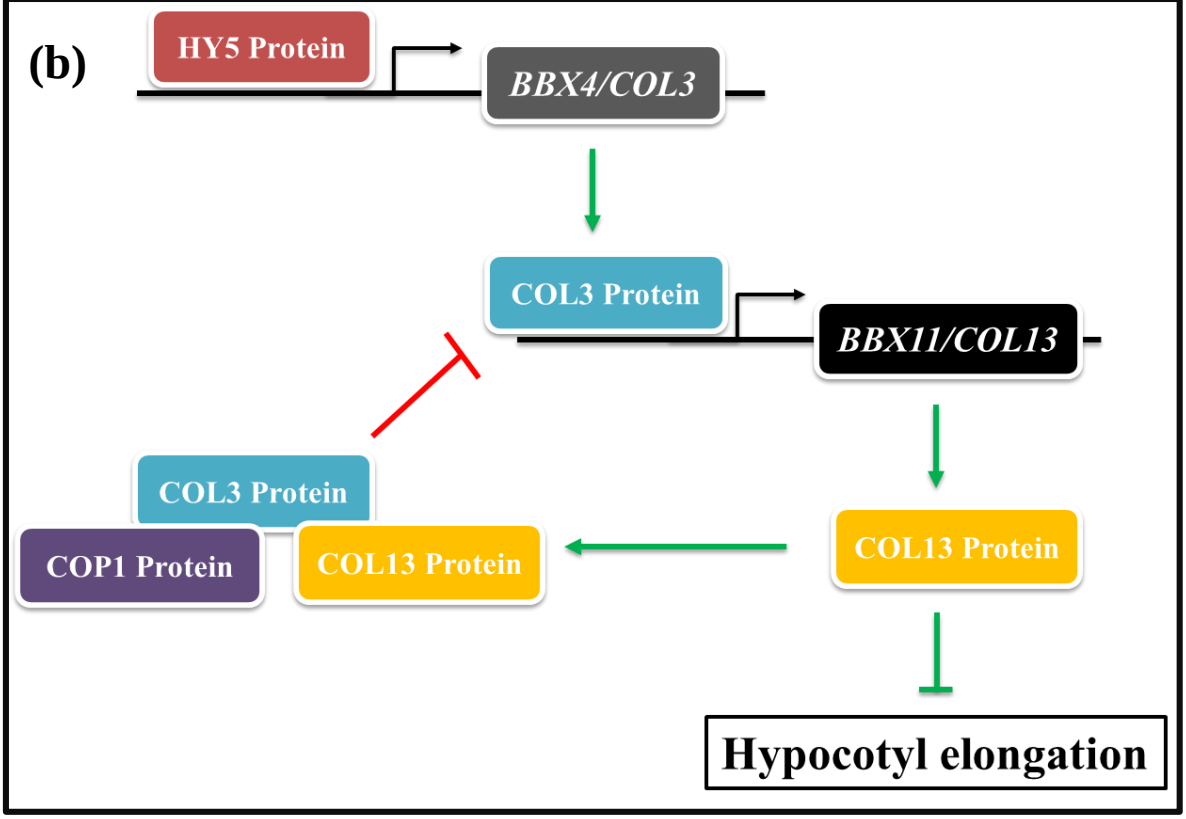


Fig.7 COL13 promotes the interaction between COL3 and COP1. (a) Yeast three-hybrid analysis of the COP1-COL3 interaction in the presence of COL13. Normalized Miller Units were calculated as a ratio of α-galactosidase activity in yeast. Additionally, normalized Miller Units here are reported separately for yeast grown on media without or with 1 mM methionine (Met), corresponding to induction (-Met) or repression (+Met) of *Met25* promoter-driven *COL13* expression, respectively. Means and SEM for three biological repetitions are shown. Lower-case letters indicate significant difference of α-galactosidase. **(b)** A model representing the HY5-COL3-COL13 regulatory chain and COP1-dependent COL3-COL13 feedback pathway in regulation of hypocotyl elongation.