

Supplementary data

A COP1-PIF-HEC regulatory module fine-tunes photomorphogenesis in *Arabidopsis*

Praveen Kumar Kathare, Xiaosa Xu, Andrew Nguyen and Enamul Huq

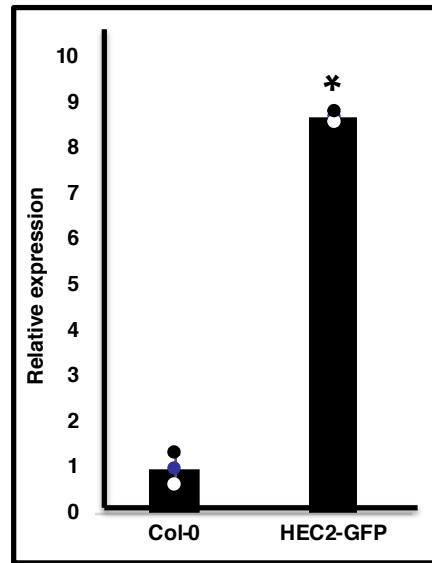


Figure S1: *HEC2* mRNA level in *HEC2-GFP* overexpression seedlings.

Bar graph showing the expression levels of *HEC2* in wild type and *HEC2-GFP* seedlings. Four-day-old dark-grown seedlings were harvested for RNA isolation. *PP2A* was used as an internal control for RT-qPCR. Error bars indicate standard deviation, * indicates statistically significant difference compared to wild type based on student's t-test ($p < 0.05$, $n = 3$ independent biological repeats).

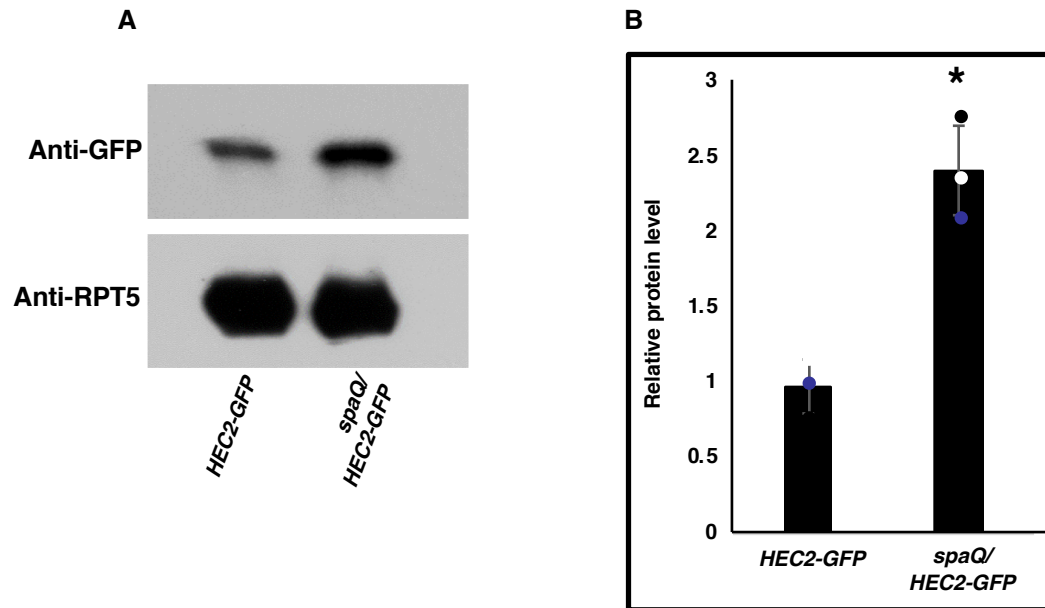


Figure S2: HEC2-GFP is more abundant in *spaQ* compared to Col-0 background.

(A) Immunoblots show the HEC2-GFP protein level in the 4-day old dark grown seedlings of *HEC2-GFP* transgenic seedlings in either Col-0 or *spaQ* background. (B) Quantification of relative HEC2-GFP protein level using western blot results from three independent experiments. RPT5 protein level was used for normalization. Error bars indicate SEM (n=3 biological repeats). Asterisk indicates significant difference between the two genotypes. *P<0.01 by student's t-test.

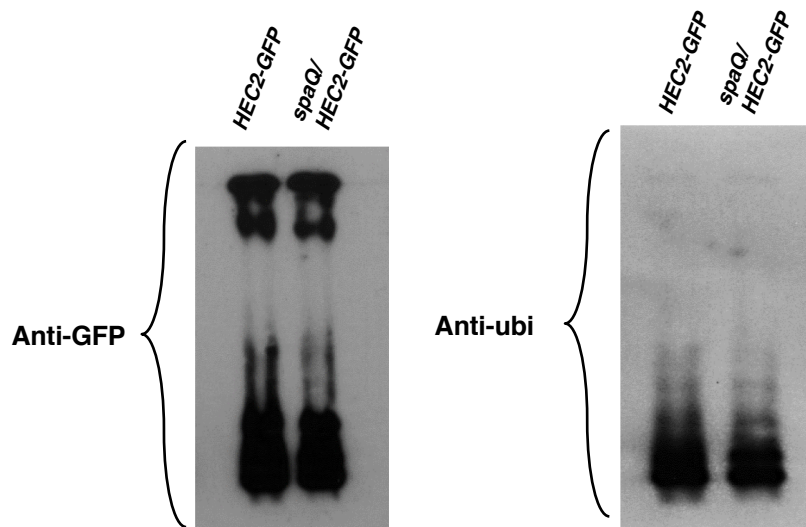


Figure S3: SPA proteins promote HEC2 polyubiquitylation *in vivo*.

The ubiquitylation level of HEC2-GFP is lower in the *spaQ* compared with Col-0 background in darkness. Total protein was extracted from 4 day-old dark grown seedlings and HEC2-GFP was immunoprecipitated using anti-GFP antibody (Rabbit). The immunoprecipitated samples were then separated on 10% SDS-PAGE gels and probed with anti-GFP (Mouse) (Left blot) or anti-Ub antibodies (Right blot).

Supplementary Table S1: Primer sequences used in experiments described in the text.

Gene	Forward	Reverse
<u>qRT-PCR</u>		
<i>PP2A</i>	TATCGGATGACGATTCTTCGTGCAG	GCTTGGTCGACTATCGGAATGAGAG
<i>GFP</i>	AAGCTGACCCTGAAGTTCATCTGC	CTGTAGTTGCCGTCGTCCTTGAA
<u>BiFC Cloning</u>		
HEC2	CACCATGGATAACTCCGACATTCTAATGA	TCTAAGAATCTGTGCATTTCCAAGC
SPA1	GGGGACCACTTTGTACAAGAAAGCTGGGT- GATGCCTGTTATGGAAAGAGTAG	GGGGACCACTTTGTACAAGAAAGCT- GGGTGAACAAGTTTTAGTAGCTTCATG
COP1	CACCATGGAAGAGATTTTCGACGGATCCG	CGCAGCGAGTACCAGAACTTTGATG