

Supplemental Information

Nucleoporin 160 Regulates Flowering through Anchoring HOS1 for Destabilizing CO in *Arabidopsis*

Chunying Li^{1,2,3}, Lu Liu^{2,3}, Zhi Wei Norman Teo^{1,2}, Lisha Shen² & Hao Yu^{1,2,*}

¹Department of Biological Sciences, Faculty of Science, National University of Singapore, Singapore 117543, Singapore.

²Temasek Life Sciences Laboratory, National University of Singapore, Singapore 117604, Singapore.

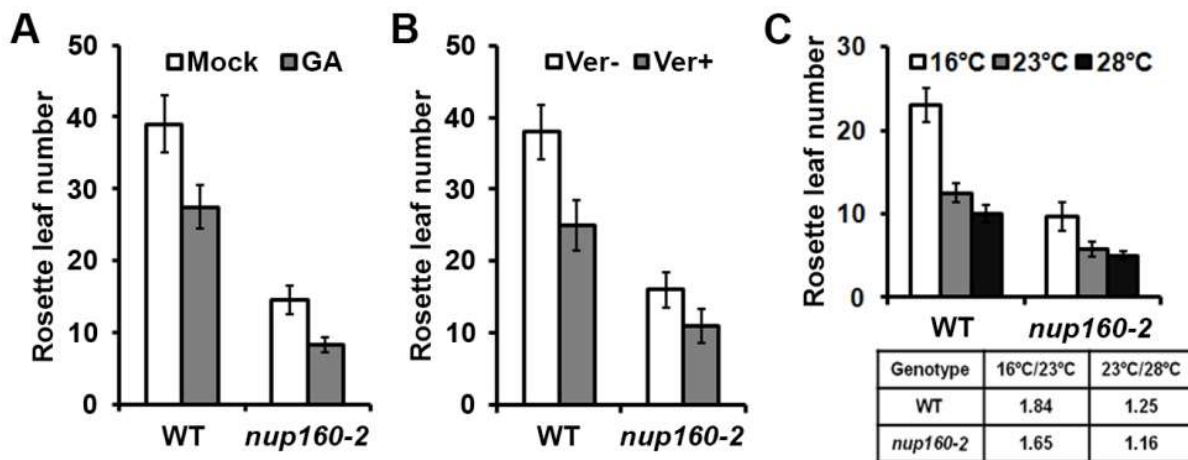
³These authors contributed equally to this article.

*Correspondence: Hao Yu (dbsyuhao@nus.edu.sg)

This PDF file includes:

Supplemental Figures 1-11

Supplemental Table 1

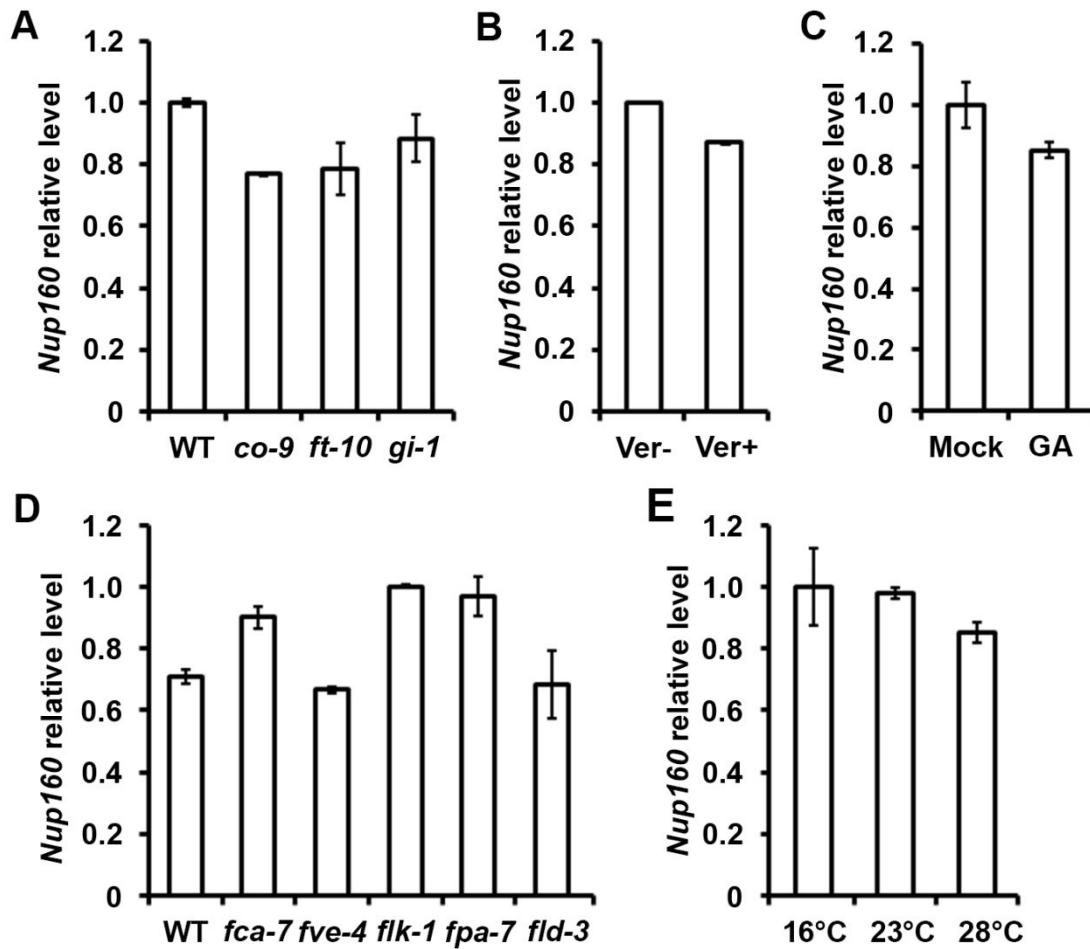


Supplemental Figure 1. Effects of *Nup160* on the Flowering Response to GA, Vernalization, and Ambient Temperature.

(A) Flowering time of wild-type (WT) and *nup160-2* in response to GA treatment under short days. Exogenous GA (100 μ M) or mock treatment was applied weekly after germination ($n \geq 16$, \pm SD).

(B) Flowering time of wild-type (WT) and *nup160-2* in response to vernalization. Seeds were germinated on MS medium and vernalized at 4°C under low light conditions for 6 weeks and then transferred to soil to grow under short days ($n \geq 20$, \pm SD).

(C) Flowering time of wild-type (WT) and *nup160-2* in response to changes in ambient temperature (16°C, 23°C, and 28°C) under long days. The ratios of flowering time between 16°C and 23°C (16°C/23°C) and between 23°C and 28°C (23°C/28°C) for wild type and *nup160-2* are shown in the table ($n \geq 16$, \pm SD).



Supplemental Figure 2. Effects of Various Flowering Pathways on *Nup160* Expression.

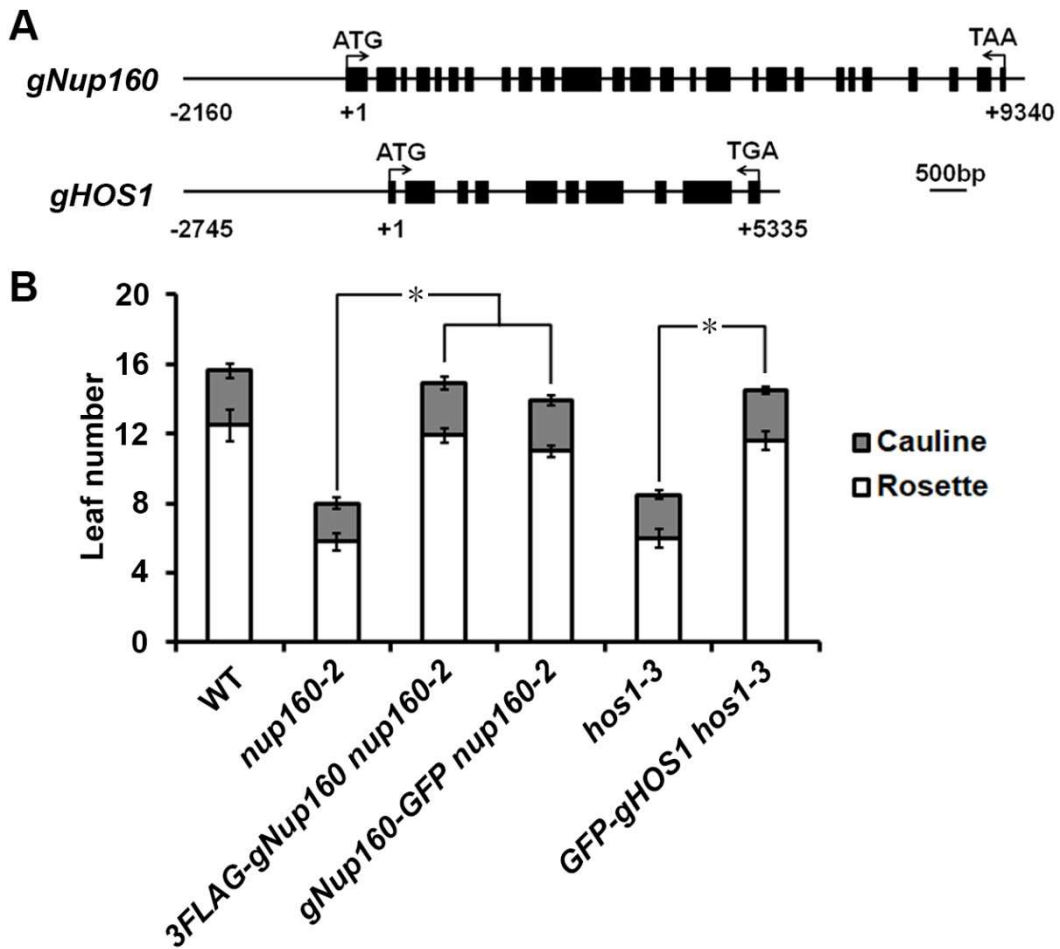
(A) *Nup160* expression in 9-day-old seedlings of photoperiod pathway mutants grown under long days.

(B) Expression of *Nup160* in response to vernalization. Seeds were germinated on MS medium and vernalized at 4°C under low light conditions for 6 weeks before being transferred to soil to grow under long days ($n \geq 20$, \pm SD). 9-day-old seedlings were harvested for expression analysis.

(C) Expression of *Nup160* in 9-day-old wild-type seedlings in response to GA treatment. Samples were harvested 6 h after GA or mock treatment.

(D) *Nup160* expression in 9-day-old seedlings of autonomous pathway mutants grown under long days.

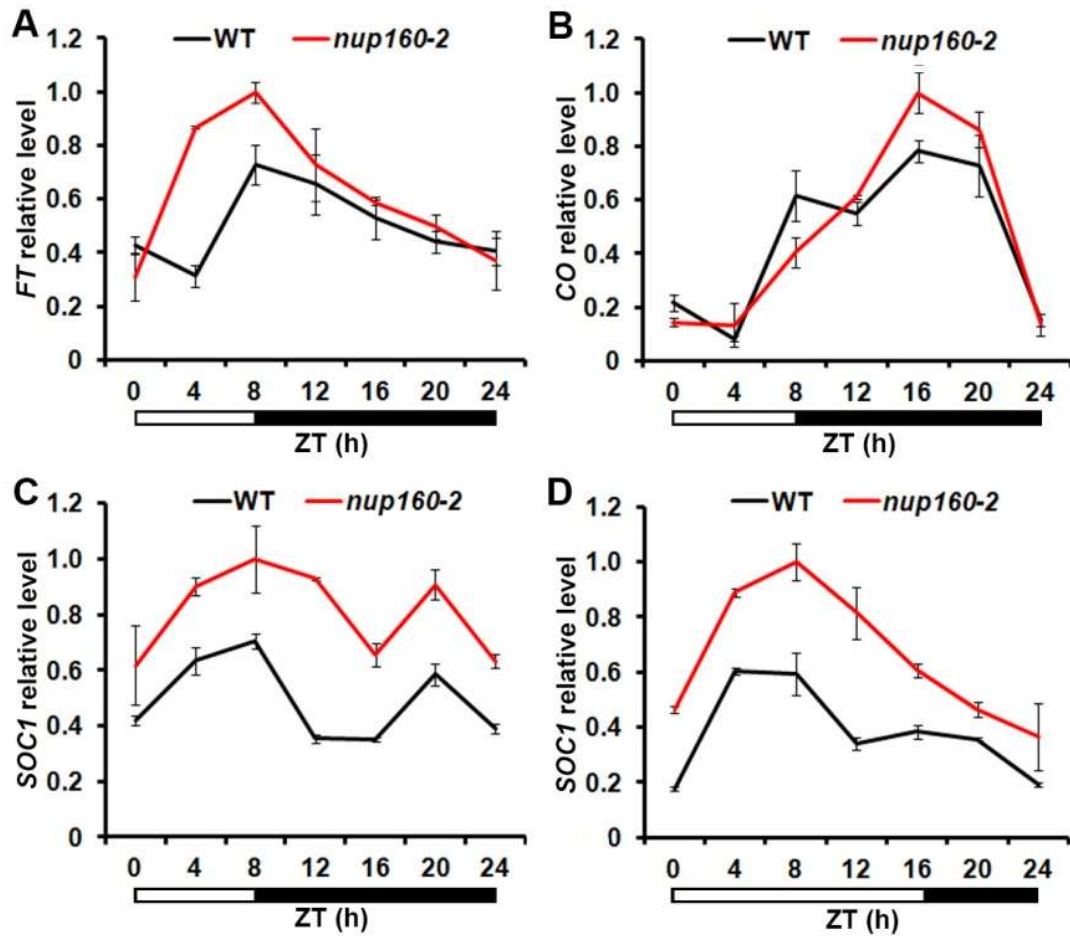
(E) Effects of ambient temperature on *Nup160* expression. 9-day-old wild-type seedlings grown at 16°C, 23°C, and 28°C under long days were harvested for expressions analysis. Gene expression levels in all panels were normalized against the expression of *TUB2* and shown as relative values to the highest level in each panel as 1. Error bars indicate SD.



Supplemental Figure 3. Rescue of *nup160-2* and *hos1-3* Mutants by Various Gene-Tagging Lines.

(A) Schematic diagrams showing the *Nup160* and *HOS1* genomic fragments used for complementation experiments. Exons are represented by black boxes.

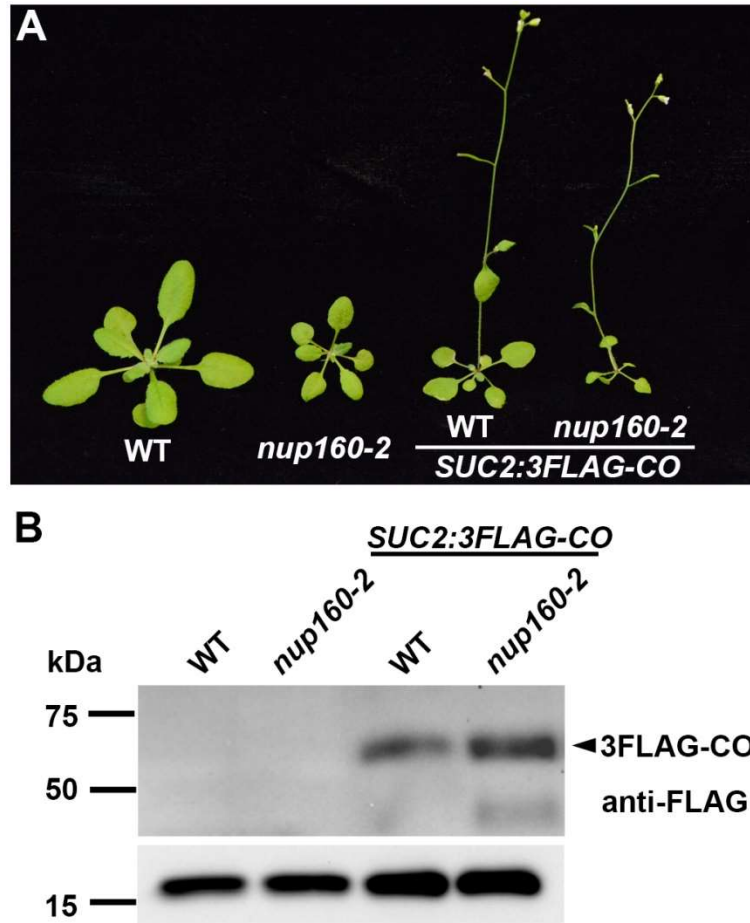
(B) Flowering time of wild-type, *nup160-2* and *hos1-3* as well as their rescued lines grown under long days ($n \geq 16$, \pm SD). Asterisks indicate statistically significant differences in flowering time between specified genotypes (two-tailed paired Student's *t* test, $P < 0.005$).



Supplemental Figure 4. Diurnal Expression of *FT*, *SOC1*, and *CO* in Wild-Type and *nup160-2* Plants under Short Days or Long Days.

(A-C) Diurnal expression of *FT* (A), *CO* (B), and *SOC1* (C) in 2-week-old wild-type and *nup160-2* seedlings grown under short days.

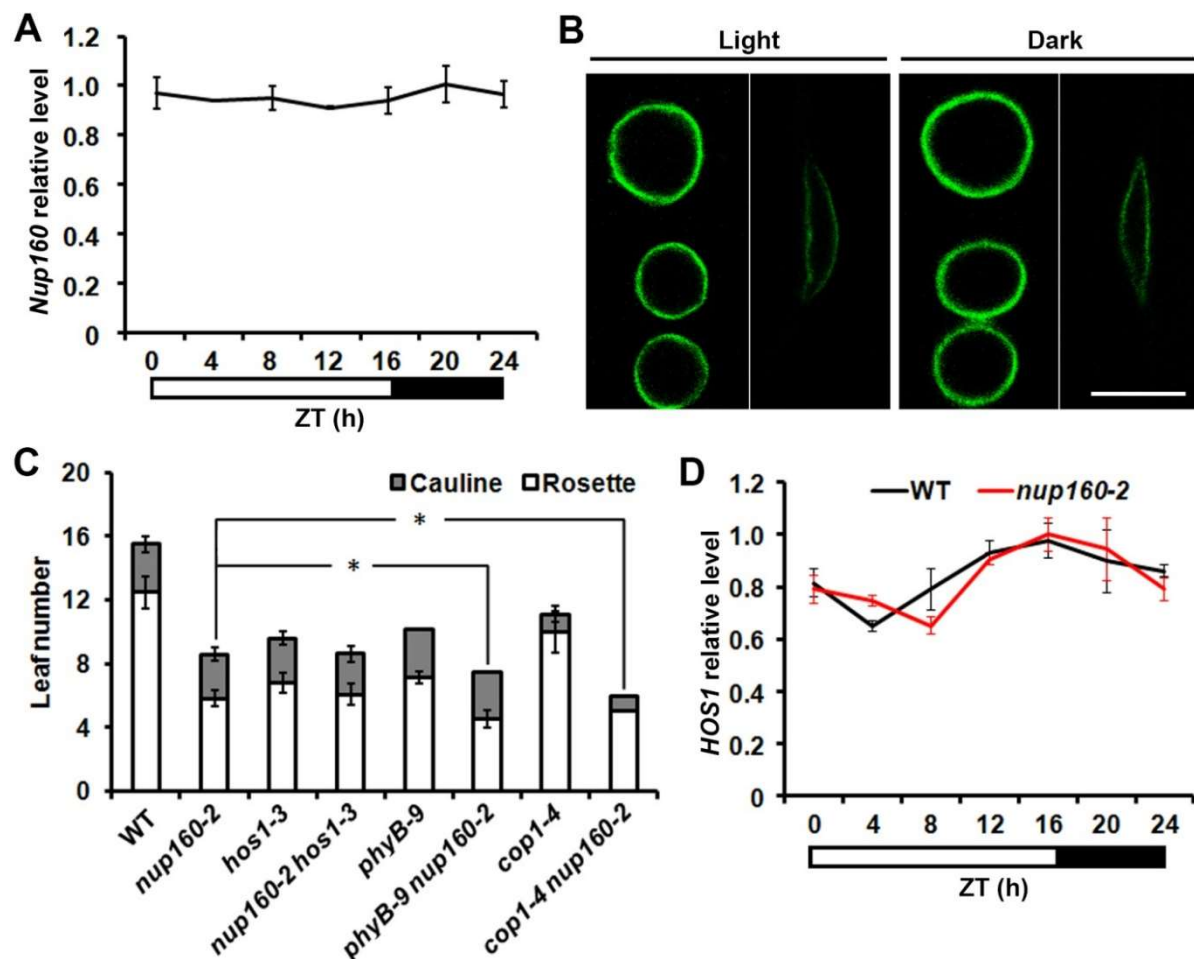
(D) Diurnal expression of *SOC1* in 9-day-old wild-type and *nup160-2* seedlings grown under long days. Gene expression levels were normalized against the expression of *TUB2* and shown as relative values to the highest level in each panel as 1.0. Error bars indicate SD.



Supplemental Figure 5. Characterization of *SUC2:3FLAG-CO* in Wild-Type and *nup160-2* Backgrounds.

(A) Flowering phenotype of wild-type, *nup160-2*, *SUC2:3FLAG-CO*, and *SUC2:3FLAG-CO nup160-2* plants grown under long days.

(B) Immunoblot analysis of nuclear protein extracts using anti-FLAG antibody shows detection of 3FLAG-CO in *SUC2:3FLAG-CO* and *SUC2:3FLAG-CO nup160-2*, but not in wild-type and *nup160-2* seedlings (upper panel). 9-day-old seedlings grown under long days were collected at ZT4. Histone H3 is shown as a loading control (lower panel).



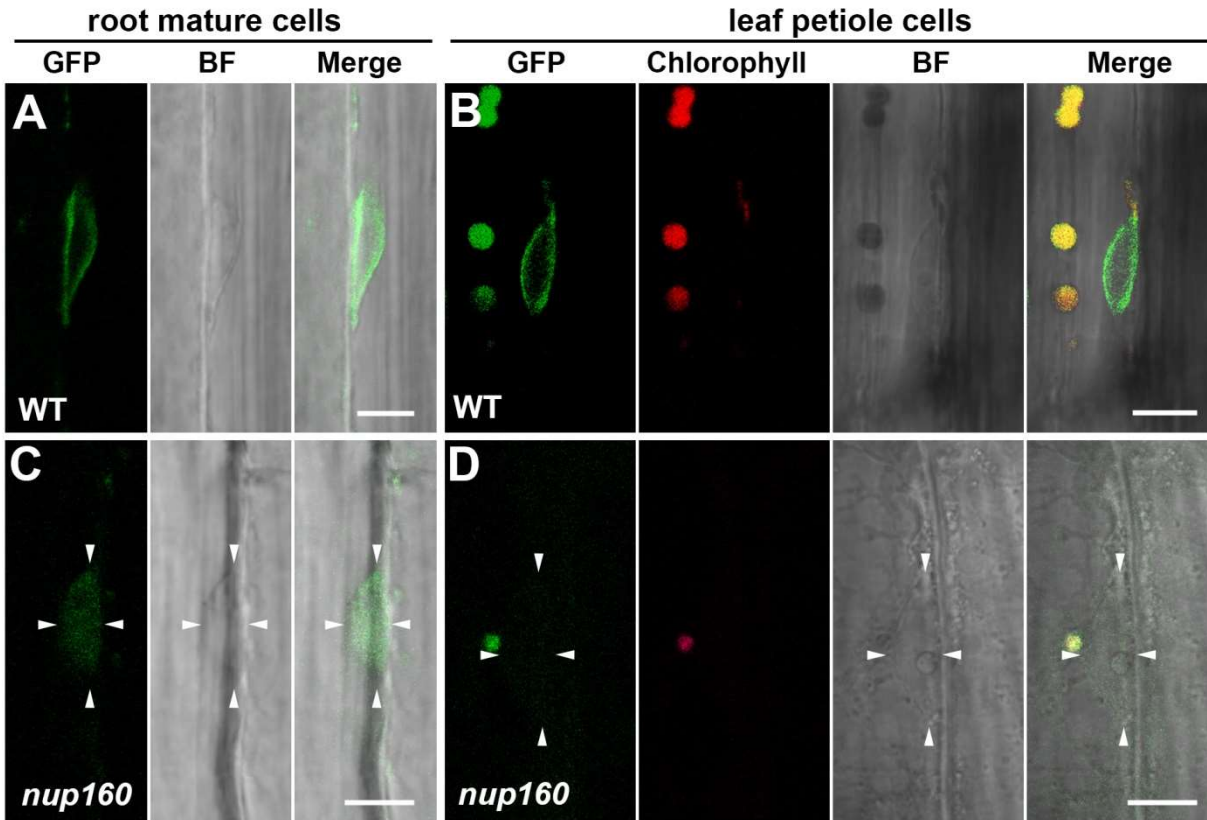
Supplemental Figure 6. Characterization of Genetic or Molecular Interaction between *Nup160* and Regulators of *CO*.

(A) Diurnal expression of *Nup160* in 9-day-old wild-type seedlings grown under long days. Samples were harvested every 4 hours from the onset of illumination, which are shown in hours as Zeitgeber time (ZT). Gene expression levels were normalized against the expression of *TUB2* and shown as relative values to the highest level as 1.0. Error bars indicates SD.

(B) Subcellular localization of Nup160-GFP detected in meristematic root cells (left) and mature root cells (right) of 5-day-old seedlings grown under continuous light or transferred to dark for 4 hours. Scale bar corresponds to 10 μ m.

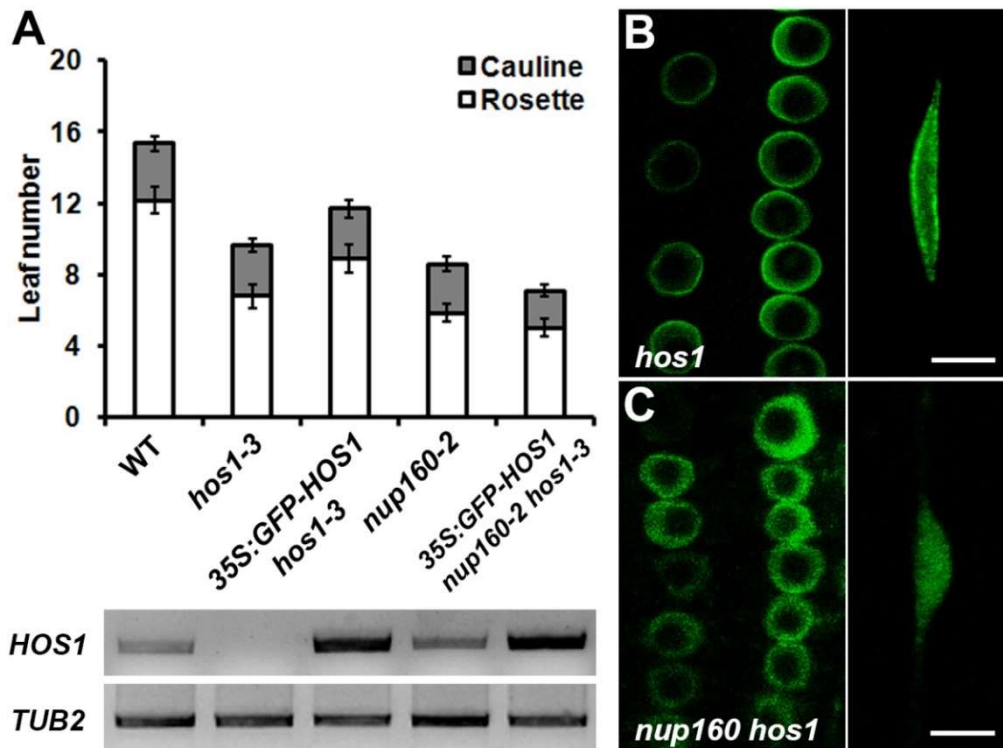
(C) Flowering time of various mutants grown under long days. Asterisks indicate statistically significant differences in flowering time between specified genotypes (two-tailed paired Student's *t* test, $P < 0.005$) ($n \geq 16$, \pm SD).

(D) Diurnal expression of *HOS1* in 9-day-old wild-type and *nup160-2* seedlings grown under long days. Gene expression levels were normalized against the expression of *TUB2* and shown as relative values to the highest level as 1.0. Error bars indicate SD.



Supplemental Figure 7. Nup160 Affects NPC-Localization of HOS1.

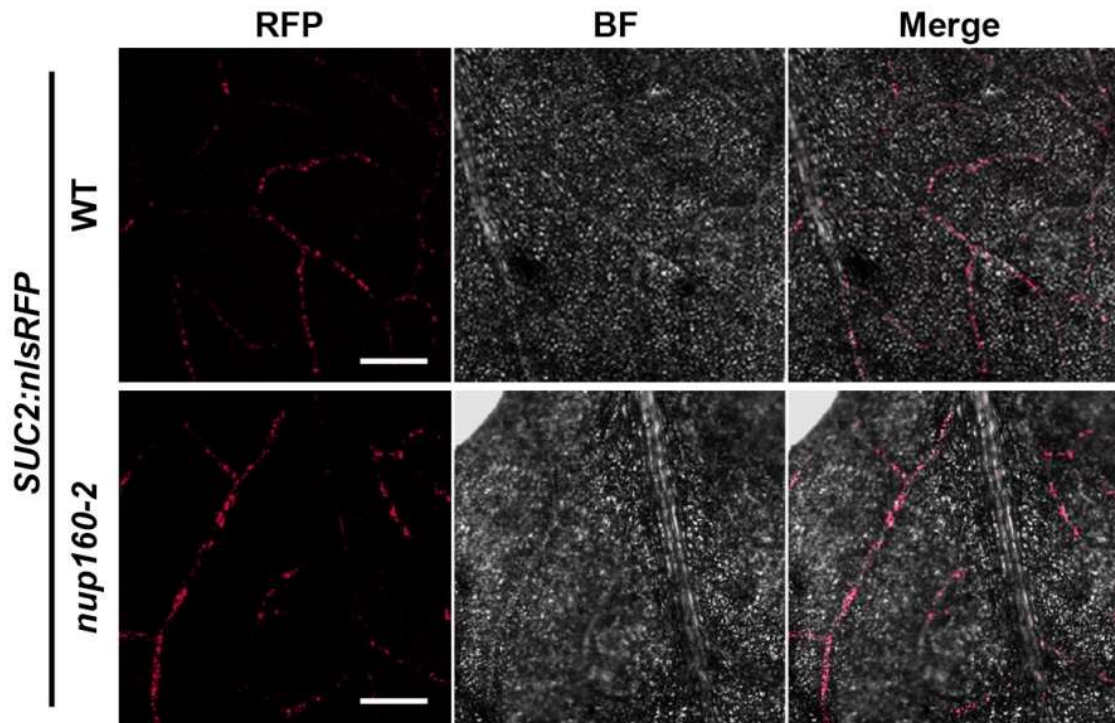
(A and B) Subcellular localization of GFP-HOS1 in a mature root cell (A) and a leaf petiole cell (B) of *GFP-gHOS1 hos1-3* in the wild-type (WT) background. Scale bars correspond to 10 μ m. (C and D) Subcellular localization of GFP-HOS1 in a mature root cell (C) and a leaf petiole cell (D) of *GFP-gHOS1 hos1-3* in the *nup160-2* background. Arrowheads outline the nucleus localization observed in the *nup160-2* background. GFP, GFP fluorescence; BF, bright field; Chlorophyll, autofluorescence of chlorophyll; Merge, merge of GFP and BF with/without Chlorophyll images. Scale bars correspond to 10 μ m.



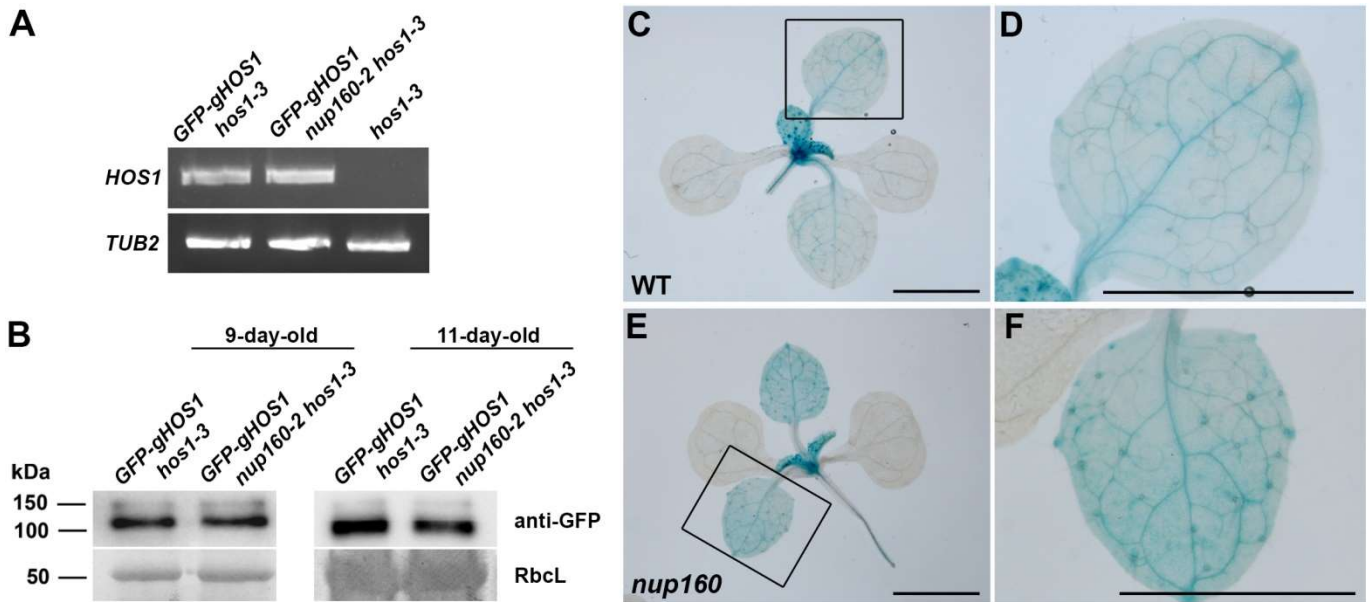
Supplemental Figure 8. Nup160 Affects NPC-Localization of HOS1 When *HOS1* Is Overexpressed.

(A) Flowering time (upper panel; $n \geq 16$, \pm SD) and *HOS1* expression (lower panel) in wild-type, *hos1-3*, *35S:GFP-HOS1 hos1-3*, *nup160-2*, and *35S:GFP-HOS1 nup160-2 hos1-3* seedlings grown under long days. 9-day-old seedlings were collected for semi-quantitative PCR analysis. *TUB2* was amplified as an internal control.

(B and C) Subcellular localization of GFP-HOS1 in meristematic root cells (left panels) and mature root cells (right panels) of 5-day-old *35S:GFP-HOS1 hos1-3* (**B**) and *35S:GFP-HOS1 nup160-2 hos1-3* (**C**) plants. Scale bars correspond to 10 μ m.



Supplemental Figure 9. Detection of nlsRFP in Leaf Vasculature Cells of *SUC2:nlsRFP*. Confocal analysis of nlsRFP expressed in the first true leaf of 9-day-old *SUC2:nlsRFP GFP-gHOS1 hos1-3* (indicated as WT; upper panel) and *SUC2:nlsRFP GFP-gHOS1 nup160-2 hos1-3* (indicated as *nup160-2*; lower panel). RFP, RFP fluorescence; BF, bright field; Merge, merge of RFP and BF images. Scale bars correspond to 200 μ m.

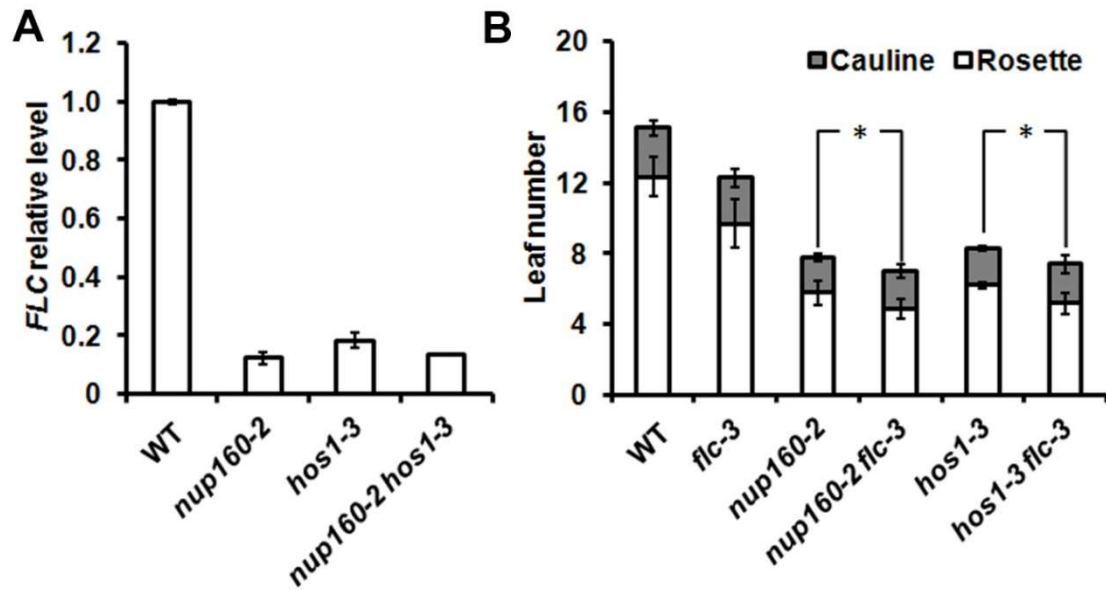


Supplemental Figure 10. NUP160 Does Not Affect *GFP-HOS1* mRNA and Protein Expression.

(A) Semi-quantitative PCR shows comparable *GFP-HOS1* mRNA expression in 9-day-old *GFP-gHOS1 hos1-3* and *GFP-gHOS1 nup160-2 hos1-3* seedlings. The fragment covering a 500-bp *HOS1* coding region was amplified, while *TUB2* was amplified as an internal control.

(B) Immunoblot analysis shows comparable GFP-HOS1 protein expression in *GFP-gHOS1 hos1-3* and *GFP-gHOS1 nup160-2 hos1-3*. Total protein was extracted from 9 and 11-day-old seedlings and analyzed using anti-GFP antibody. The membrane stained with Ponceau Red showing the ribulose biphosphate carboxylase large subunit (RbcL) was included as a loading control.

(C-F) *HOS1-GUS* expression in 9-day-old *gHOS1-GUS* in the wild-type (WT) (C and D) and *nup160-2* (E and F) backgrounds. (D and F) High-magnification images of the black squares shown in (C and E), respectively. Scale bars correspond to 1 mm (C-F).



Supplemental Figure 11. Nup160 and HOS1 Similarly Affect *FLC* Expression.

(A) *FLC* expression in 9-day-old of wild-type (WT), *nup160-2*, *hos1-3*, and *nup160-2 hos1-3* seedlings grown under long days. Gene expression levels were normalized against the expression of *TUB2* and shown as relative values to the highest level as 1. Error bars indicate SD.

(B) Flowering time of various mutants grown under long days ($n \geq 16$, \pm SD). Asterisks indicate statistically significant differences in flowering time between specified genotypes (two-tailed paired Student's *t* test, $P < 0.05$).

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

Primers for Plasmid Construction

Construct	Primer Name	Sequence (5'-3')
<i>miR-Nup160</i>	1-AmiR-Nup160-s	GATTGATACATGTAACCTGGCGCTCTCTCT TTTGTATTCC
	2-AmiR-Nup160-a	GAGCGCCAAGTTACATGTATCAATCAAAG AGAATCAATGA
	3-AmiR-Nup160-*s	GAGCACCAAGTTACAAGTATCATTACACAG GTCGTGATATG
	4-AmiR-Nup160-*a	GAATGATACTTGTAACCTGGTGCTCTACAT ATATATTCTT
<i>gNup160</i>	gNup160-F	CACCTGATTGTAACCTAACACATGACCAC
	gNup160-R	TGTTACTTCTTCTTCTCACACGCAC
<i>3FLAG-gNup160</i>	3FLAG-gNup160-F	GGTTTAGGGTTCAAAGAAGAAATGGATTA CAAGGATCATGATGGA
	3FLAG-gNup160-R	ATCCGACGATTCTCCTCCTTATCGTCATCA TCTTTGTAATC
<i>gNup160-KpnI</i>	gNup160-KpnI-F	CCTCTGCAACCGGTGGTACCAGAAAGTCT TGATTGTTCCGTG
	gNup160-KpnI-R	CAATCAAGACTTTCTGGTACCACCGGTTG CAGAGGATACAG
<i>gHOS1</i>	gHOS1-F	CACCTGGTGTGACCGTCTCATGGAGA
	gHOS1-R	GTGACGGAACGATAGGAAGAGAC
<i>gHOS1-KpnI</i>	gHOS1-KpnI-F	AGCAAGAGGTACCTGAAATGAAACAAAC ACTCGAAAAGAC
	gHOS1-KpnI-R	TTCATTTTCAGGTACCTCTTGCTGCGAATCT ACGTCTC
<i>GUS-gHOS1</i>	GUS(N)-KpnI-F	CGGGGTACCATGTTACGTCCTGTAGAAAC C
	GUS(N)-KpnI-R	CGGGGTACCTCCACCTCCTTGTTCCTCC CTGCTGCG
<i>GFP-gHOS1</i>	GFP-F-KpnI	CGGGGTACCATGAGTAAAGGAGAAGAAGT TTTCAC
	GFP(N)-R-KpnI	CGGGGTACCTCCACCTCCTTGTATAGTTC ATCCATG
<i>gNup160-GUS</i>	GUS(C)-KpnI-F	CGGGGTACCGGAGGTGGAATGTTACGTCC TGTAGAAACC
	GUS(C)-KpnI-R	CGGGGTACCCTATTGTTTGCCTCCCTGCTG CG
<i>35S:GFP-HOS1</i>	GFP-F-XhoI	CCGCTCGAGATGAGTAAAGGAGAAGAAGT TTTCAC
	GFP-R-XhoI	CCGCTCGAGTTTGTATAGTTCATCCATGC
	HOS1-F-XmaI	CCCCCGGGATGGATACGAGAGAAATCAA CGGT

	HOS1-R-XmaI	CCCCCGGGTCATCTTGCTGCGAATCTACG
<i>SUC2:3FLAG-CO</i>	3FLAG-CO-F-XmaI	CCCCGGGATGGATCCCCGGGATGGATTAC AAGGATCATGATGGAGACTACAAGGATCA TGATATTGATTACAAAGATGATGACGATAA GTTGAAACAAGAGAGTAACGACA
	CO-R-XmaI	CCCCGGGTCAGAATGAAGGAACAATCCCA
<i>gNup160-cEYFP</i>	cEYFP-F-KpnI	CGGGGTACCGGCAGCGTGCAGCTCGCC
	cEYFP-R-KpnI	CGGGGTACCTCACTTGTACAGCTCGTCCA
<i>nEYFP-gHOS1</i>	nEYFP-F-KpnI	CGGGGTACCATGGTGAGCAAGGGCGAGG A
	nEYFP-R-KpnI	CGGGGTACCGTCCTCGATGTTGTGGCGGA
<i>SUC2:nlsRFP</i>	nlsRFP-F-XmaI	CCCCGGGATGCCAAAGAAAAAGAGGAAA GTGCCTAAGAAGAAGAGAAAGGTTGCGA AGGCAGATAAGAAAC
	RFP-R-XbaI	GCTCTAGACTAGGCGCCGGTGGAGTGGC

Primers for Quantitative Real-Time PCR

Primer Name	Sequence (5'-3')
TUB2-F	GAGAATGCTGATGAGTGCATGG
TUB2-R	AGAGTTGAGTTGACCAGGGAACC
Nup160-F	AAAGGAACTCTGTGGTCATCGC
Nup160-R	TGATCAGCAGGGCTTCTATCAGG
FT-F	CTTGGCAGGCAAACAGTGTATGCAC
FT-R	GCCACTCTCCCTCTGACAATTGTAGA
CO-F	TCAGGGACTCACTACAACGACAATGG
CO-R	TTGGGTGTGAAGCTGTTGTGACACAT
SOC1-F	AGCTGCAGAAAACGAGAAGCTCTCTG
SOC1-R	GGGCTACTCTTTCATCACCTCTTCC
HOS1-F	GTCATTGCTTTCTTGCTGGA
HOS1-R	TCTAACCACGCTTGCATCTC
FLC-F	CTAGCCAGATGGAGAATAATCATCATG
FLC-R	TTAAGGTGGCTAATTAAGTAGTGGGAG

Primers for Semi-Quantitative PCR

Primer Name	Sequence (5'-3')
TUB2-semi-F	ATCCGTGAAGAGTACCCAGAT
TUB2-semi-R	TCACCTTCTTCATCCGCAGTT
Nup160-semi-F	ATGGAGGAGAATCGTCGGAATC
Nup160-semi-R	GTCGAACATCCAAGTGAATCAAATGAT
HOS1-semi-F	CATAATGGTCTGGTGCATCAGA
HOS1-semi-R	AATCCTACAAGCTTCCTGGAGTG