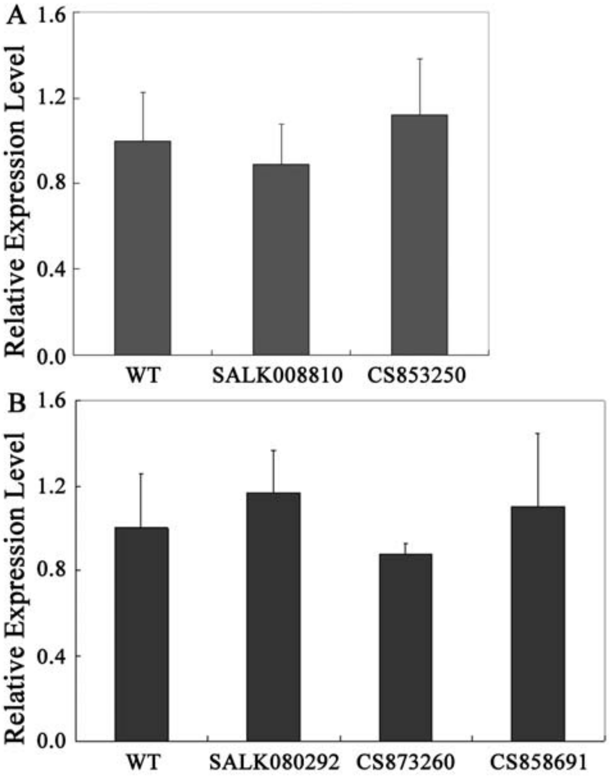
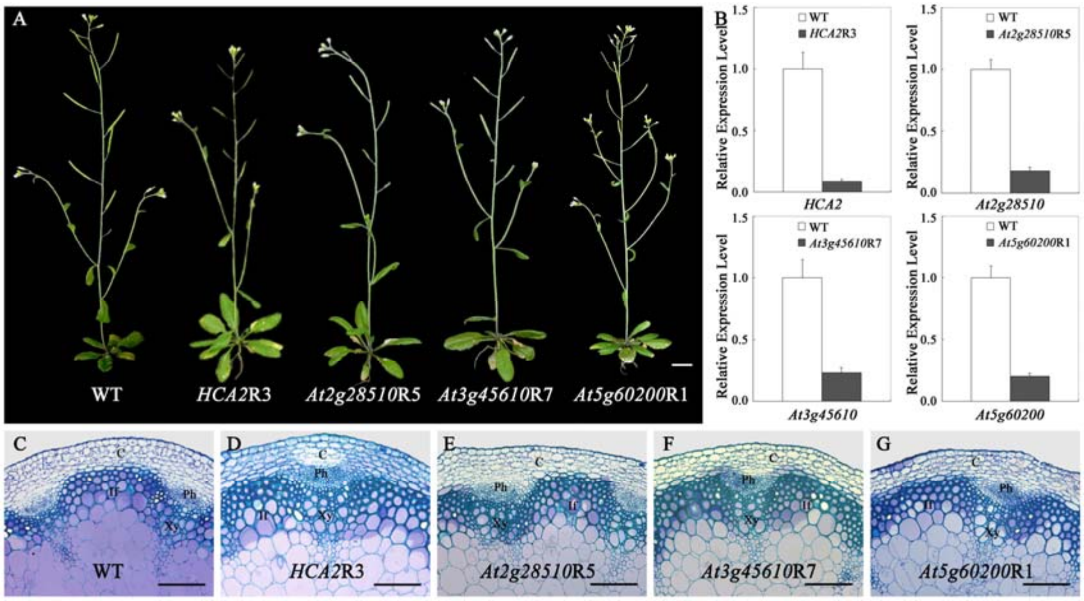
Supplemental Data. Guo et al., (2009) *HCA2*, a Dof transcription factor gene, regulates interfascicular cambium formation and vascular tissue development in *Arabidopsis*



Supplemental Figure 1. The expression level of *HCA2* and *At5g60200* was not altered in their corresponding SALK mutants

Expression level of *HCA2* in WT, SALK008810 and CS853250 (A) or that of *At5g60200* in WT, SALK080292, CS873260 and CS858691 (B) was examined by quantitative RT-PCR analysis. The expression levels of each gene in WT are set to 1.0 and error bars represent SD of three biological replicates.



Supplemental Figure 2. RNAi transgenic lines of *HCA2* and its homologous genes did not display obvious vascular tissue-associated phenotypes.

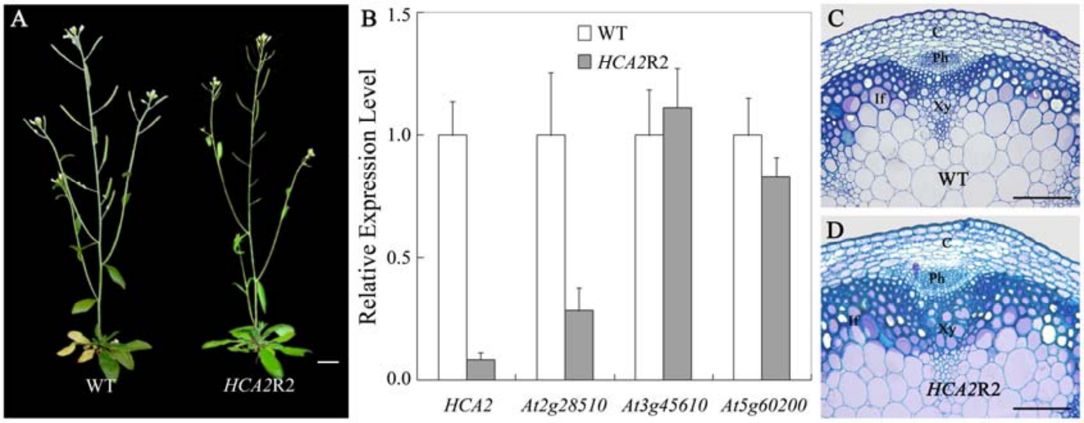
(A) Six-week-old RNAi transgenic plants of *HCA2* or its homologous genes are shown.

(B) Corresponding genes in WT and their respective RNAi lines were examined by quantitative RT-PCR analysis. The expression levels of each gene in WT are set to 1.0 and error bars represent SD of three biological replicates.

(C-G) Resin-embedded transverse sections of the basal part of inflorescence stems from WT (C), HCA2R3 (D), At2g28510R5 (E), At3g45610R7 (F) and At5g60200R1 (G) plants.

C, cortex; If, interfascicular fibers; Ph, phloem; Xy, xylem.

Scale bars represent 1 cm (A) or 100μm (C-G).



Supplemental Figure 3. The expression of *HCA2* and one of its homologous genes was reduced in one *HCA2* RNAi transgenic line.

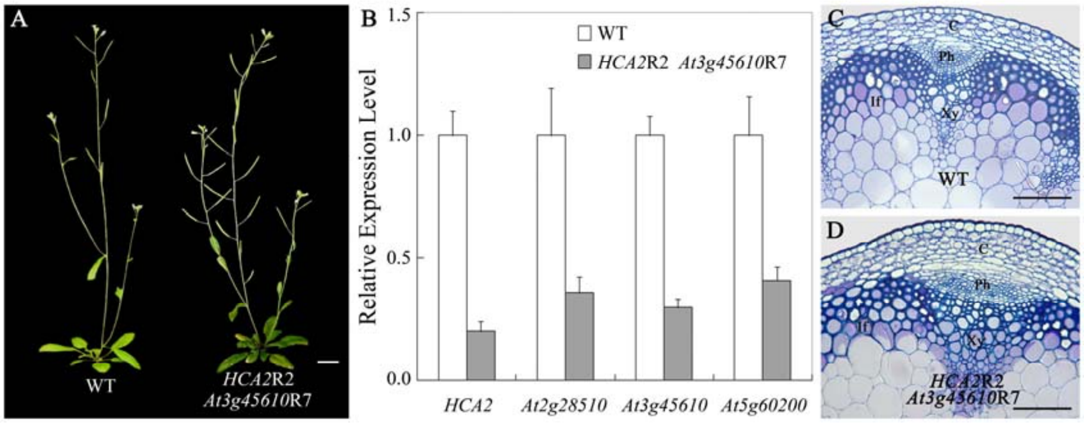
(A) Six-week-old WT and *HCA2* RNAi transgenic plants are shown.

(B) Expression of *HCA2* and its homologous genes in WT and one of the *HCA2* RNAi lines was examined by quantitative RT-PCR analysis. The expression levels of each gene in WT are set to 1.0 and error bars represent SD of three biological replicates.

(C-D) Resin-embedded transverse sections of the basal part of inflorescence stems from WT (C) and HCA2R2 (D) plants.

C, cortex; If, interfascicular fibers; Ph, phloem; Xy, xylem.

Scale bars represent 1 cm (A) or 100 μm (C-D).



Supplemental Figure 4. ***HCA2*** **and its homologous genes were all down-regulated in the double RNAi transgenic line and led to no obvious phenotype.**

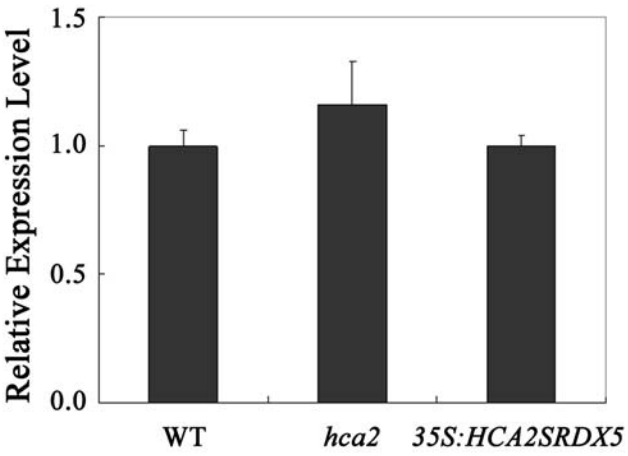
(A) Six-week-old WT and double RNAi transgenic plants are shown.

(B) Expression of *HCA2* and its homologous genes in WT and double RNAi lines was examined by quantitative RT-PCR analysis. The expression levels of each gene in WT are set to 1.0 and error bars represent SD of three biological replicates.

(C-D) Resin-embedded transverse sections of the basal part of inflorescence stems from WT (C) and double RNAi transgenic plant (D).

C, cortex; If, interfascicular fibers; Ph, phloem; Xy, xylem.

Scale bars represent 1 cm (A) or 100 μm (C-D).



Supplemental Figure 5. ***COV1*** **is not regulated by** ***HCA2***

Expression of *COV1* in WT, *hca2* and *35S:HCA2SRDX5* was examined by quantitative RT-PCR analysis. The expression levels of *COV1* in WT are set to 1.0 and error bars represent SD of three biological replicates.

Supplemental Table 1. Primer information for genes used in this study

HCA2-1	5'-ATG GGT CTC ACT TCT CTT CAA -3'
HCA2-2	5'-TCA AAC CAA GGA GTT TGT TTT -3'
HCA2NS	5'-AAC CAA GGA GTT TGT TTT AGT G-3'
At5g62950-1	5'-ATG AAA ATA GTC AAG GCA AAT GCA G -3'
At5g62950-2	5'-TCA CGA CTG TTC ACC ATT CTC TGT -3'
ProHCA2-1	5'-TCG TCC TCC ACT TTC CAA TA-3'
ProHCA2-2	5'-GAG ACC CAT TTT GTG TTC TGT AT-3'
HCA2RNAi-1	5'-GGG AGT GAA CAA TGA CAA CCT G-3'
HCA2RNAi-2	5'-ATT CCA CGA CGA ACC TAA ACC-3'
HCA2a-2	5'-GGA GGA ATT AGA GGA AGA TGG-3'
HCA2b-1	5'-TCC TCC ACT TCT TCC GGC A-3'
HCA2b-2	5'-GAA ATC AAT AGG TCT AGG GCT CT-3'
HCA2c-1	5'-TTG GAC AGT AAG TTT GAT CTC A-3'
YFP-1	5'-ATG GTG AGC AAG GGC GAG GAG C-3'
YFP-2	5'-GCT TTA CTT GTA CAG CTC GTC CAT GC-3'
YFPNS	5'-CTT GTA CAG CTC GTC CAT GCC GA-3'
ProATHB8-1	5'-CAT GTG CCC GAT ACT CAA TTC TA-3'
ProATHB8-2	5'-GTT GTC CAT ATT GTG ACT ATT ATT GCT-3'
ProSUC2-1	5'-ACG CAA ACT AAC TAC AAC CTT CTT-3'
ProSUC2-2	5'-CTC CAT TGG ATG GCT GAC C-3'
APL-1	5'-ACC AAG TCC TCG ACC ATC ACA-3'
APL-2	5'-CTC CGA CAA AGA ATC CAA ATC C-3'
RTM1-1	5'-ATT GTA AAC TGG GAC GAA GGA T-3'
RTM1-2	5'-CGT ACT CGC TAG TGT TGG TAT TG-3'
AHA3-1	5'-GCT GGT ATG GAT GTT CTG TGC-3'
AHA3-2	5'-GGT TCG CTT ATC AAC TGG ATT-3'
ANT-1	5'-GAT GTA GCA GCA ATT AAG TTC CG-3'

ANT-2	5'-GAG CGG TTT GGT CTT CAG TAT T-3'
EXPA9-1	5'-TGA TCT CGC TAT GCC TAT GTT T-3'
EXPA9-2	5'-TGA CCA GCA CCA AGT TGA AGT A-3'
IRX1-1	5'-CAT CCC AAC GCT ATC AAA CCT A-3'
IRX1-2	5'-GCT GAG ACA CCT CCA ATA ACC C-3'
IRX5-1	5'-TCT GGG TGA TTG GCG GTG-3'
IRX5-2	5'-GTC GGA GGG ATG AGA AGG GT-3'
At2g28510-1	5'-AAG ATG GCT TCT ACA TTG GGG-3'
At2g28510-2	5'-CCA GAA CAC CTT GCT AGG ATT C-3'
At3g45610-1	5'-GGT ATG CGT TTG GAA ATG GTG-3'
At3g45610-2	5'-CTC TCC CTG AAT CAA TCT GAT CAA-3'
At5g60200-1	5'-GGT TTC ATT CAG AAC TCG GTA-3'
At5g60200-2	5'-TGG AAA TCC CCA TAA GAC TC-3'
At2g28510RNAi-1	5'-CTC CAA AAG CAT CAT TTA TCC T-3'
At2g28510RNAi-2	5'-CAA GAT GAA ACC ATC CCA TTC-3'
At3g45610RNAi-1	5'-GAT GGA GCT TGG GTT AGC ATA-3'
At3g45610RNAi-2	5'-GCA CCA GTA TTA ATG TAG TTG ACA GT-3'
At5g60200RNAi-1	5'-ATC TGA GCT TAG CCT TTG CCT-3'
At5g60200RNAi-2	5'-CAA TCT GAT CTA CAT GAC CAC CA-3'
COV1-1	5'-CCG AGG AGG AAC TTT GCT G-3'
COV1-2	5'-CAC CCG AAA CAA CAA TCT CAA-3'
SRDX-1	5'-CGG ACT AGT CTG GAT CTG GAT CTA GAA CTC CGT TTG GGT TTC GCT TAG CTA GTG TTA ACG CTA GCG AG-3'
SRDX-2	5'-CTC GCT AGC GTT AAC ACT AGC TAA GCG AAA CCC AAA CGG AGT TCT AGA TCC AGA TCC AGA CTA GTC CG-3'