Supplementary Figures and Tables

Supplementary Tables

Supplementary Table 1: Primer used for RT-PCR

Gene (gene identifier)	**Name**	**Sequence**
PP2A (AT1G69960)	PP2a Fw	CAGCAACGAATTGTGTTTGG
	PP2a Rv	AAATACGCCCAACGAACAAA
Actin	Actin Fw	GGTAACATTGTGCTCAGTGGTGG
	Actin Rv	AACGACCTTAATCTTCATGCTGC
LUC	LUC Fw	AAGCGGTTGCCAAGAGGTTCC
	LUC Rv	CGCGCCCGGTTTATCATC
CO 5´UTR Fw (AT5G15840)	CO UTR Fw	CAGATACCAGCTCCCACACC
SUC2 5 ´UTR Fw (AT1G22710)	SUC2 UTR Fw	GCGAGCTTAAATGGCATACC
3x HA Tag Rv	HA Rv	ATAGTCCGGGACGTCATAGG
FT (AT1G65480)	FT Fw	CGAGTAACGAACGGTGATGA
	FT Rv	CGCATCACACACTATATAAGTAAAACA
AtCO (AT5G15840)	CO Fw	TAAGGATGCCAAGGAGGTTG
	CO Rv	CCCTGAGGAGCCATATTTGA
AtCOL1 (AT5G15850)	COL1 Fw	CACCTTACCCTCCAGCTCAG
	COL1 Rv	GTGGAGAAAGCTTGGTTTGC
AtCOL2 (AT3G02380)	COL2 Fw	CCAGGGAAAAACATCGGTAA
	COL2 Rv	TTTTGTTGGCTTTGTTGCAG
ThCOL	ThCOL Fw	CGCTAACCCACTCGCTAGAC
	ThCOL Rv	CGTCCTCATCTTCAGCTTCC
AeCO	AeCO Fw	AAGGCAGACAACGCATCTCT
	AeCO Rv	TCTGTTGTCGCTCTTTGCTG
AeCOL2	AeCOL2 Fw	GCCTGTGATGCAGAGATTCA
	AeCOL2 Rv	TCCATTATCTGCATCCGTGA

Supplementary Table 2: Accession numbers for protein sequences used for phylogenetic comparison (Fig 3B)

Protein	Reference Number	Database
CO	NP_197088.1	NCBI
COL1	NP_197089.1	NCBI
COL2	NP_186887.1	NCBI
BrCO	XP_009121540.1	NCBI
BrCOL1	XP_009126071.1	NCBI
BrCOL1	CDX69493.1	GenBank
BrCOL2	XP_009117976.1	NCBI
BrCOL2	XP_009134666.1	NCBI
BsCO	Bostr.2902s0319.1	Phytozome 10
BsCOL1	Bostr.2902s0320.1	Phytozome 10
BsCOL2	Bostr.1460s0153.1	Phytozome 10
EsCO	EUTSA_v10015486mg	NCBI
EsCOL1	EUTSA_v10013895mg	NCBI
CrCO	CARUB_v10001192mg	NCBI
CrCOL1	CARUB_v10001279mg	NCBI
CrCOL2	CARUB_v10015501mg	NCBI
AeCO	KE151199.1 (725253 to 726038 + 726241 to 726582)	GenBank (DNA)
AeCOL2	KE150775.1 (459607 to 460401 + 459171 to 459533)	GenBank (DNA)
AaCO	CM002876.1 (10849443 to 10850180 + 10848784 to 10849146)	GenBank (DNA)
AaCOL1	CM002876.1 (10853658 to 10854716)	GenBank (DNA)
AaCOL2	CM002871.1 (740879 to 741850)	GenBank (DNA)
ThCOL	ABD96940.1	GenBank
CpCO	DS981590.1 (73183..73953,74563..74934)	Genbank (DNA)

Supplementary Table 3: Accession numbers for Brassicaceae *CO* Promoter sequences (Fig. 4A, suppl. Fig. 4A)

Species	Length [bp]	Reference number	Location /position
Aethionema arabicum	[3179]	KE151199.1	722079 to 725255
Arabidopsis thaliana	[3603]	CP002688.1	5172695 to 5176297
Arabidopsis lyrata	[3558]	NW_003302550.1	6468343 to 6471900
Arabis alpina	[3477]	CM002876.1	10850178 to 10853654
Brassica napus	[2872]	CM002768.1	16635884 to 16638739
Brassica oleracea	[4827]	AM295778.1	1835 to 6661
Capsella rubella	[3756]	KP347978	
Nasturtium officinale	[2937]	KP347979	
Sisymbrium irio	[3366]	AC240101.1	52211 to 55576
Sisymbrium officinale	[3322]	KP347980	

Supplementary Table 4: Accession numbers of putative promoter sequences used for *cis*-element comparison (Fig. 4B-D, suppl. Fig. 4B-D) (length: 4000bp)

Promoter Sequence	Species	Reference number	Position
pAeCO	*Aethionema arabicum*	KE151199.1	721256 to 725255
pAeCOL2		KE150775.1	460399 to 464400
pCO	*Arabidopsis thaliana*	NC_003076.8	5172695 to 5176694
pCOL1		NC_003076.8	5177471 to 5181470
pCOL2		NC_003074.8	488622 to 492621
pAaCO	*Arabis alpina*	CM002876.1	10850178 to 10854177
pAaCOL1		CM002876.1	10854714 to 10858713
pAaCOL2		CM002871.1	741848 to 745873
pBrCO	*Brassica rapa*	NC_024804.1	13313898 to 13317897
pBrCOL1		NC_024804.1	13309689 to 13313688
pBrCOL1		NC_024796.1	3860236 to 3864235
pBrCOL2		NC_024795.1	25142738 to 25146737
pBrCOL2		NC_024797.1	14514058 to 14518057
pEsCO	*Eutrema parvulum*	CM001192.1	13405655 to 13409654
pEsCOL1		CM001192.1	13401161 to 13405160
pEsCOL2		CM001189.1	463764 to 467763
pMtCOLa	*Medicago truncatula*	NC_016413.1	5837084 to 5841083
pPtCO1	*Populus trichocarpa*	NC_008483.2	14471166 to 14475165
pPtCO2		NC_008470.2	9352675 to 9356674
pStCO1	*Solanum tuberosum*	NW_006238930.1	968883 to 972882
pStCO2	*Solanum tuberosum*	NW_006238930.1	958533 to 962532
pThCOL	*Tarenaya hassleriana*	KE706297.1	4355966 to 4360215
pVvCO	*Vitis vinifera*	NW_003724108.1	776848 to 780847

Supplementary Table 5: Accession numbers of *COL* loci used for phylogenetic analysis (Fig. 6C):

Locus [length bp]	Species	Reference number	Position
AeCO [4900]	*Aethionema arabicum*	KE151199.1	723739 to 728638
AeCOL2 [5284]		KE150775.1	456692 to 461975
CO [4794]	*Arabidopsis thaliana*	NC_003076.8	5169362 to 5174155
COL1 [4897]		NC_003076.8	5174156 to 5179052
COL2 [4532]		NC_003074.8	485615 to 490146
ThCOL [5378]	*Tarenaya hassleriana*	KE706297.1	4352092 to 4357468

Supplementary Figure Legends

Supplementary Figure 1

A) Phylogenetic relationships of species from the order Brassicales. The Brassicaceae family can be divided in two major lineages, the core Brassicaceae and the *Aethionema* lineage, here represented by *Aethionema arabicum*. *Tarenaya hassleriana* (formerly *Cleome hassleriana*) belongs to the Cleomaceae family, which is the sister family of the Brassicaceae. Independent whole genome polyploidization (WGD) events (At-α and Th-α, asterisks) occurred in both families. *At*, *Arabidopsis thaliana*, *Th*, *Tarenaya hassleriana* (Bailey et al. 2006; Franzke et al. 2011; Cheng et al. 2013; Haudry et al. 2013).

Supplementary Figure 2

A. thaliana co-10 null mutant was complemented with cDNA of *COL1*, *COL2*, *AeCO*, *AeCOL2* and *ThCOL* expressed from *pSUC2* (A) or *pCO* (B). Flowering time (total leaf number +/- SD) was examined using independent transgenic plants (T_1) grown in LD conditions. *wt*, *co-10* and homozygous *pCO::HA:CO* (#1) and *pSUC2::HA:CO* (#3) single lines are shown as controls (n=12). Asterisks (**) indicate statistically significantly earlier flowering (p<0.001) compared to Col-0 (wt).

C-F) Expression levels of the transgene (5´UTR-3xHA, C,E) and *FT* (D,F) in homozygous lines expressing subgroup Ia *COL* genes from *pSUC2* (C,D) or *pCO* (E,F) as used for flowering determination in Fig. 3C,D. Mean +/- SD of two biological experiments after normalization to *PP2A* levels except *pCO::3xHA:ThCOL* #9 which is single experiment.

Supplementary Figure 3

A) Sequence alignment (amino acids) of B-Box1, B-Box2 and CCT domain from subgroup Ia COL proteins used in Fig. 3B. Amino acids that are conserved in all COL orthologs but different in CO orthologs are indicated (v + AA number in CO). Asterisks indicate conserved amino acids. Gene identifier or protein prediction is indicated (see Material and Methods, suppl. Table 2).

B) Synonymous (d_s) and non-synonymous (d_n) substitution rates and ratio (d_n/d_s) corrected by Jukes-Cantor method in pairwise comparison of subgroup Ia *COL* genes from *A. thaliana*, *A. arabicum* and *T. hassleriana*.

C) Model for the evolutionary history of *CO*, *COL1* and *COL2* in the Brassicaceae and *T. hassleriana COL*.

D) Spatial expression (GUS signal) conferred by *pCO*, *pCOL1* and *pCOL2* in 14 day old seedlings grown in LD conditions. Bar, 2mm.

Supplementary Figure 4

A) VISTA plot as displayed in Fig. 4A but with *A. thaliana CO* promoter as the base sequence.

B-D) Mean abundance of three putative *cis*-elements in *CO* promoters as analysed in Fig. 4B-D: LUX binding sites (LBS, B, GATWCG), G-Boxes (C, CACGTG) and CCAAT Boxes (D, CCAAT).

Supplementary Figure 5

A) Diurnal expression of *LUC* driven in the empty vector control (*pnos::LUC* #4, #5) in LD conditions. Data was obtained as described in Fig. 5 and serves as negative control.

B) Diurnal expression pattern of *A. thaliana CO* promoter *LUC* fusion (*pCO::LUC*) compared to *CPM:pnos::LUC #7 CMM:pnos::LUC #1* and *CDM:pnos::LUC #2* and in LD conditions. Mean ± SD after normalization to *PP2A* for technical triplicates and a biological replicate gave similar results.

C) Flowering time of *pCO::GFP:CO* constructs carrying 2.6kb or 2.1kb of *CO* promoter sequence introduced into the *co-2* mutant in the *A. thaliana* L*er* accession. Flowering time as total leaf number was examined in homozygous T_3 transgenic lines. Asterisks ($**p<0.001$) indicate significantly later flowering of 2.1kb lines compared to 2.6kb lines.

D) Flowering time of *pCO::GFP:CO* constructs introduced into co-2 mutant as shown in C. Two independent transgenic lines of 0.6kb are displayed and asterisks ($**p<0.001$) indicate significantly earlier flowering compared to *co-2* mutant control.

E) Chemiluminescence of LUCIFERASE in 10 day old LD grown seedlings transformed with *CPM:pnos::LUC* #4, *CMM:pnos::LUC* #2and *CDM:pnos::LUC* #4 compared to empty vector control (*pnos::LUC* #5).

Supplementary Figure 6

Selected sequence alignments of homologous regions of *CO* promoter as displayed in Fig 4A in the indicated species. *Conserved Proximal Motif* (*CPM*), *Conserved Middle Motif* (*CMM*) and *Conserved Distal Motif* (*CDM*) were deduced from this alignment (Fig. 4A). Length and position of the Motifs is indicated. Identified *cis*-elements in *A. thaliana* are represented

(CCAAT-Box, DOF, G-Box, LUX binding site: LBS, TCP). Asterisks indicate 100% base pair conservation between all 10 Brassicaceae *CO* promoters

Supplementary Figure 7

mRNA levels in LD (A, C, E) or SD (B, D, F) grown *A. thaliana* seedlings comparing Col-0 and *cdf1235* mutant for *CO* (A, B), *COL1* (C, D) and *COL2* (E, F). Mean +/- SD of two biological experiments after normalization to *PP2A* levels.

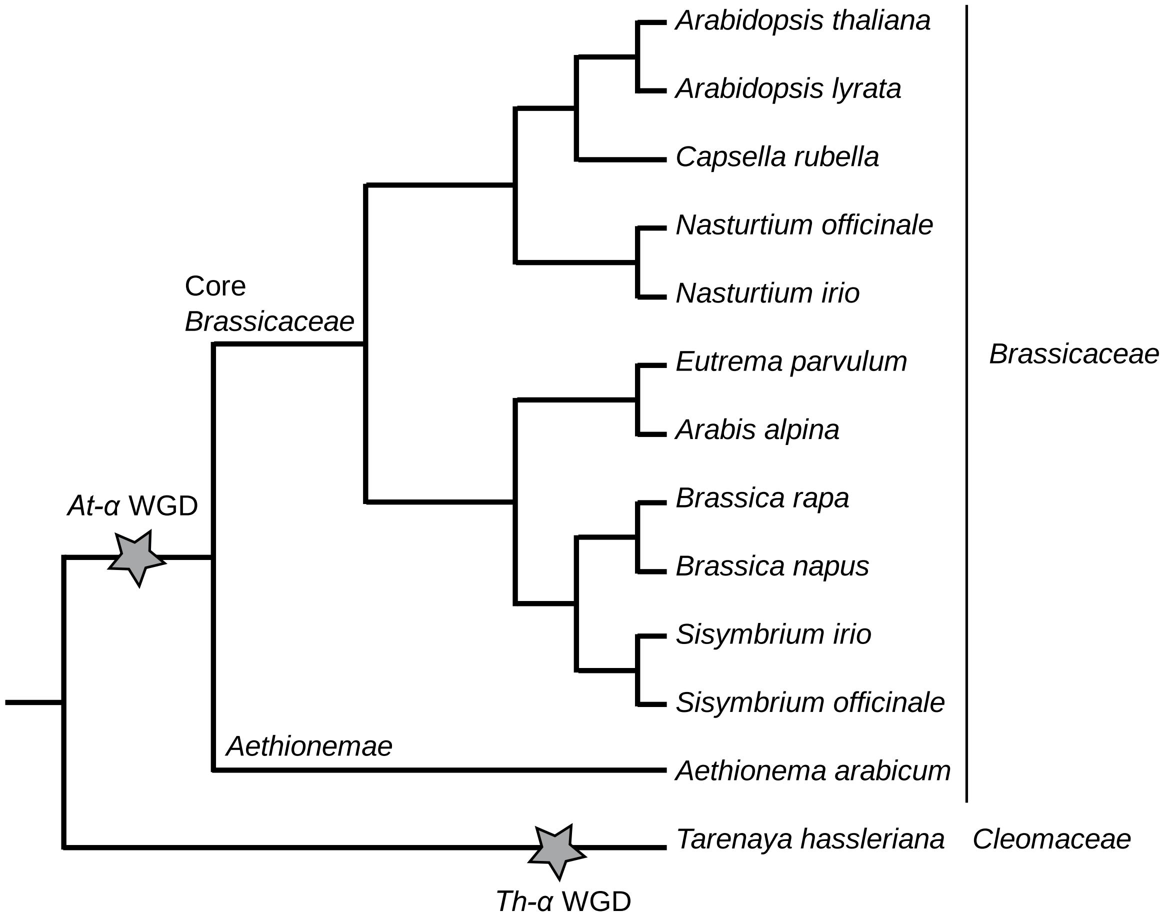
Supplementary Figure 8

CO (A), *COL1* (B) or *COL2* (C) mRNA levels of LD grown *A. thaliana* seedlings either kept in LD (Light) or shifted to dark before light on (Dark). Mean +/- SD after normalization to *PP2A* for technical triplicates and a biological replicate gave similar results

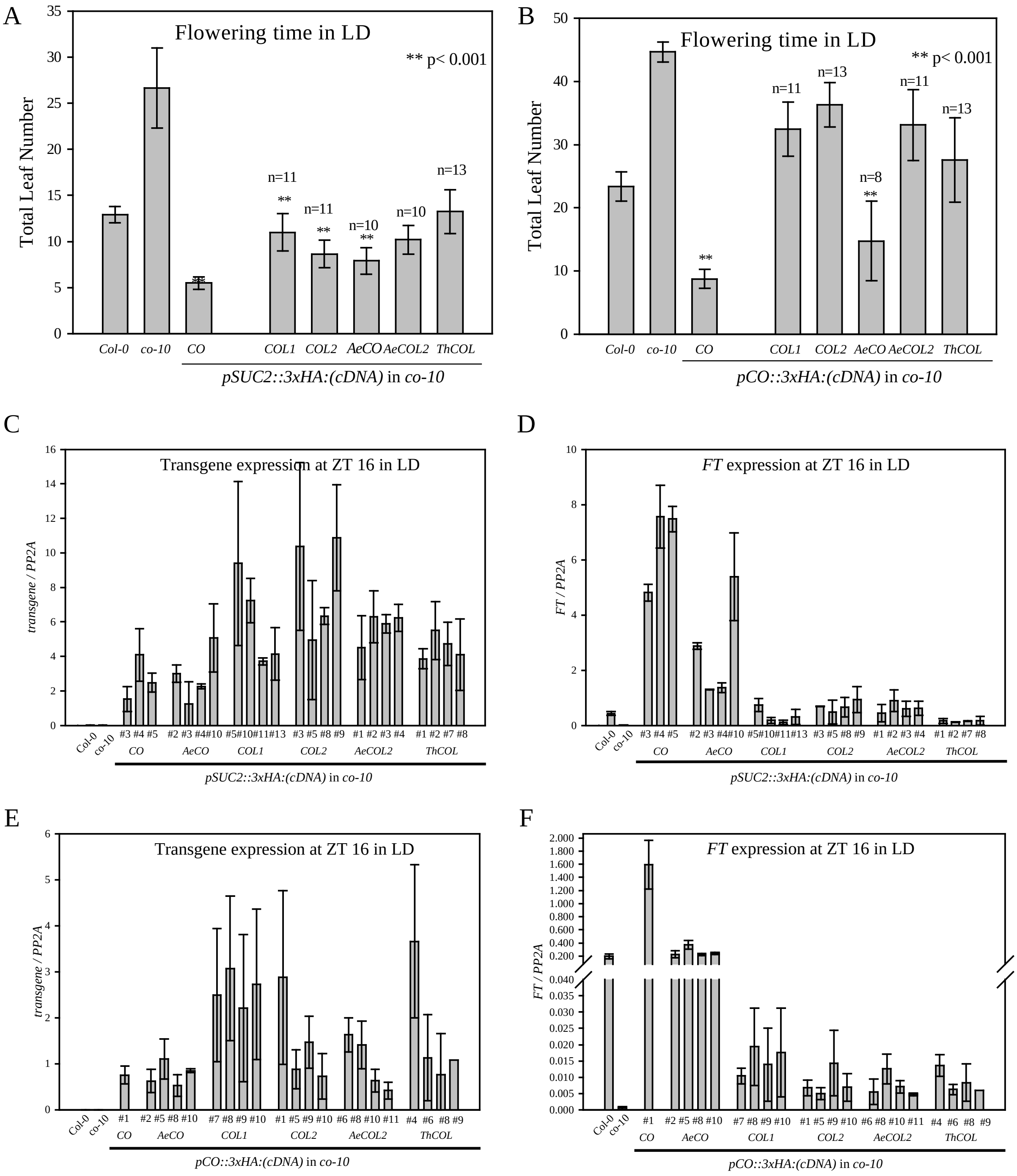
Supplementary Figures

Supplementary Figure 1

A

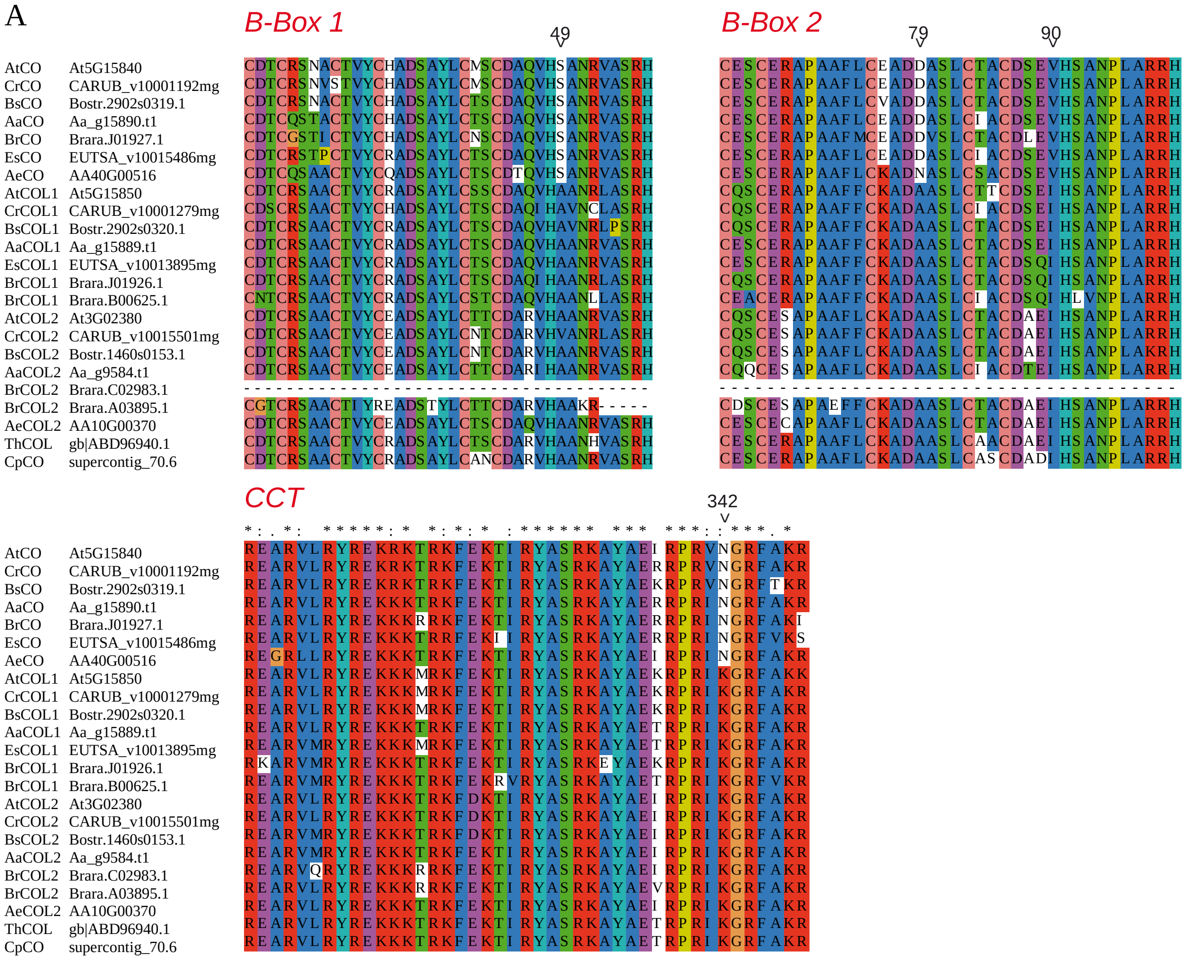


Supplementary Figure 2



Supplementary Figure 3

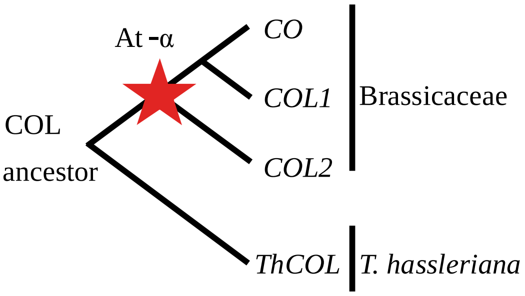
A



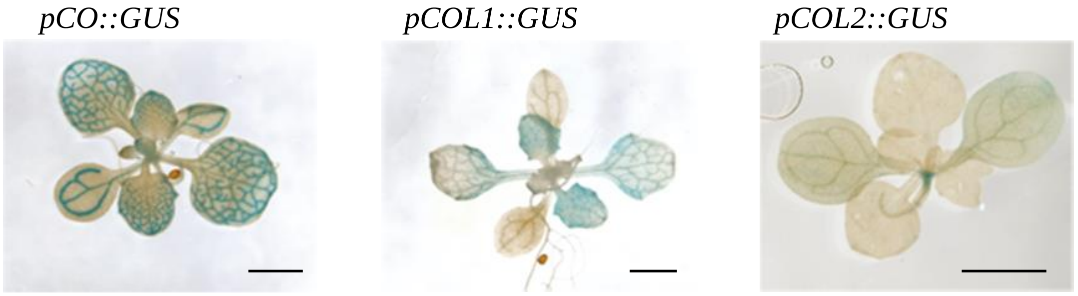
B

Gene name		d_s	d_n	d_n/d_s
AtCO	*ThCOL*	0.726	0.194	0.267
AeCO	*ThCOL*	0.855	0.183	0.214
AtCOL1	*ThCOL*	0.772	0.196	0.254
AtCOL2	*ThCOL*	0.774	0.162	0.210
AeCOL2	*ThCOL*	0.714	0.137	0.192
AtCO	*AtCOL1*	0.457	0.198	0.433
AtCO	*AtCOL2*	0.679	0.191	0.281
AtCOL1	*AtCOL2*	0.808	0.193	0.239

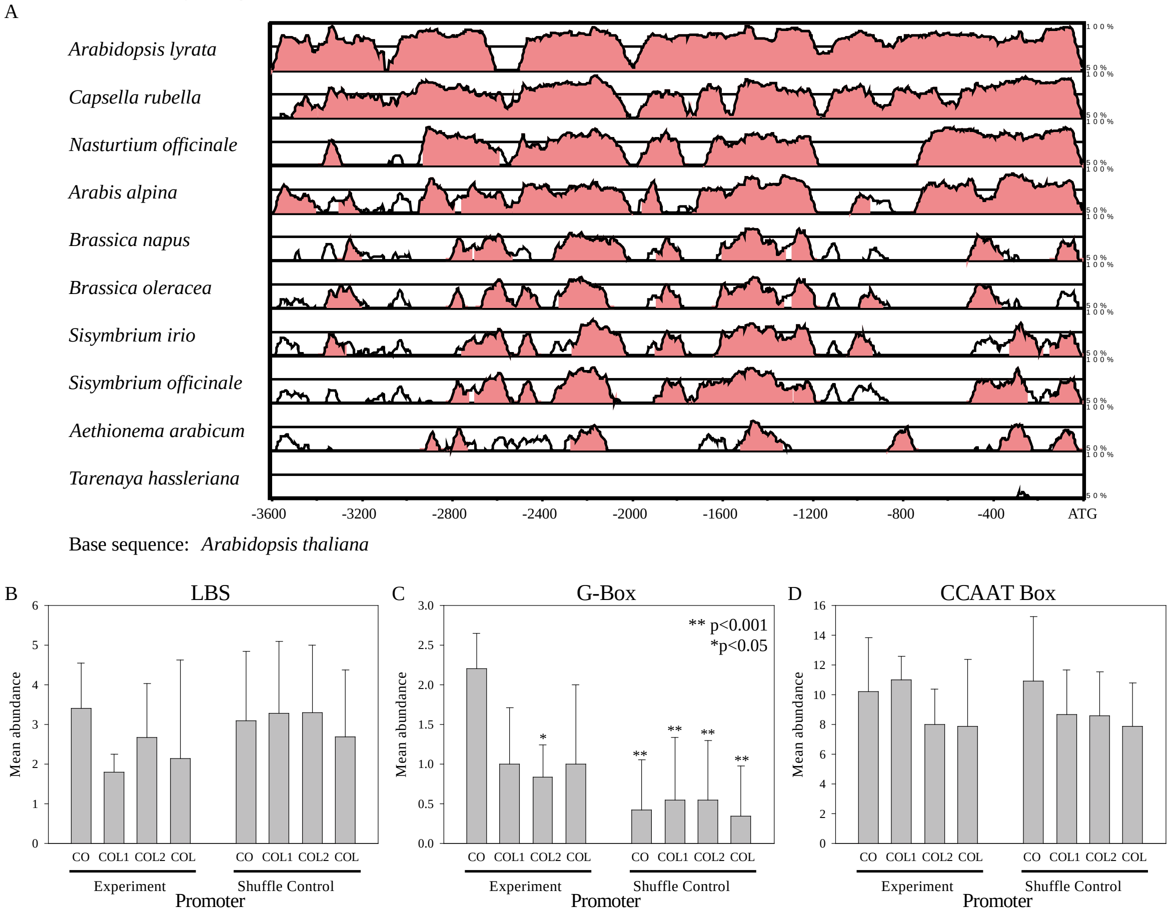
C



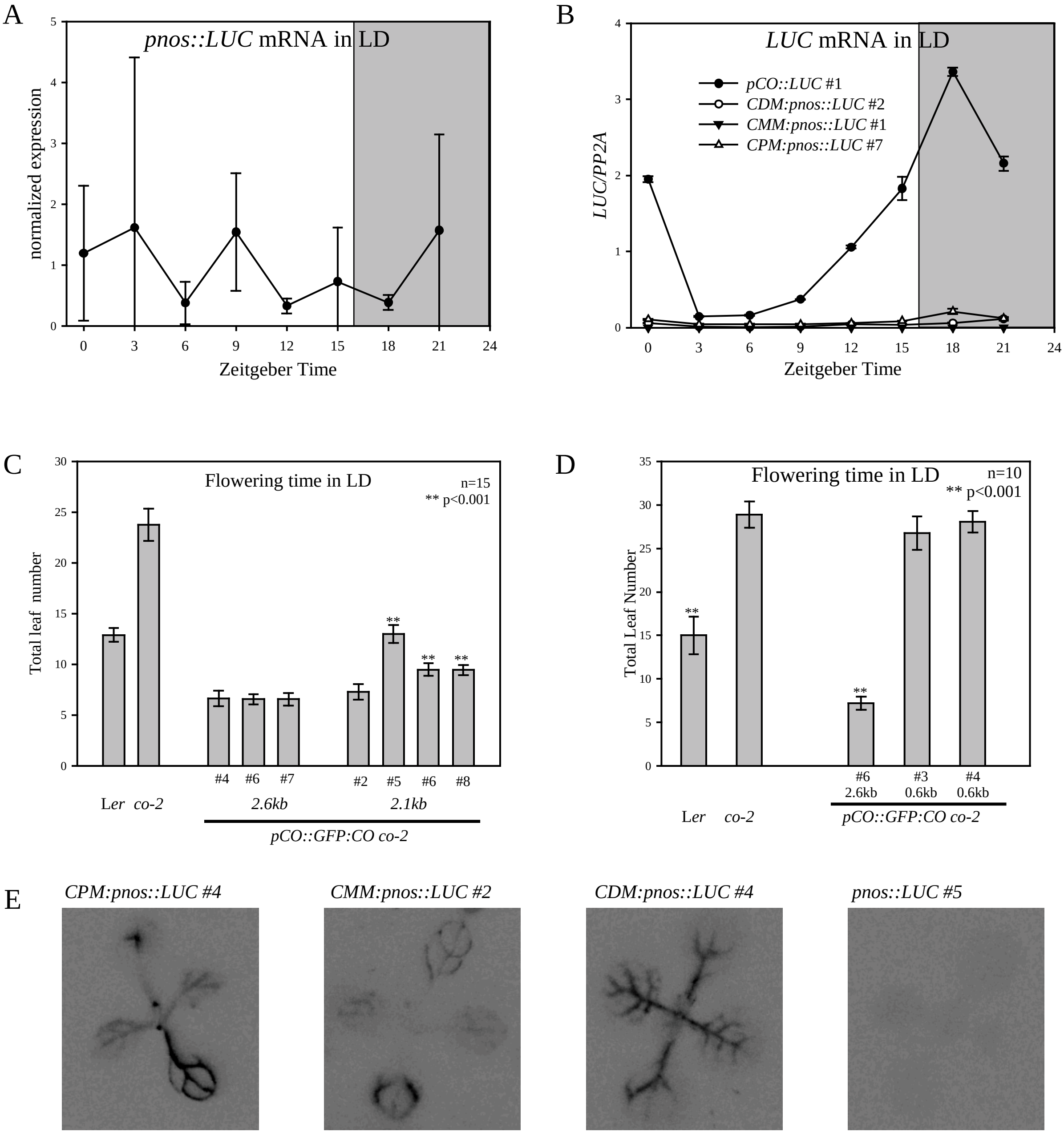
D



Supplementary Figure 4

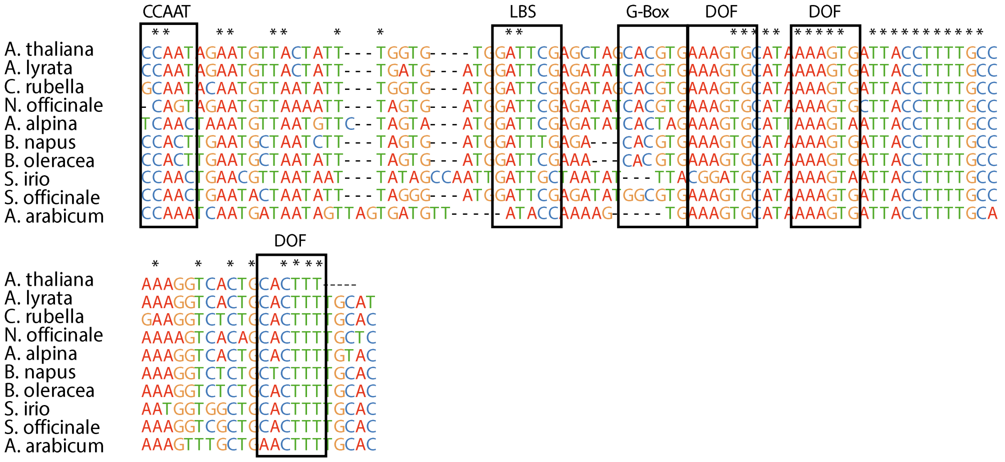


Supplementary Figure 5

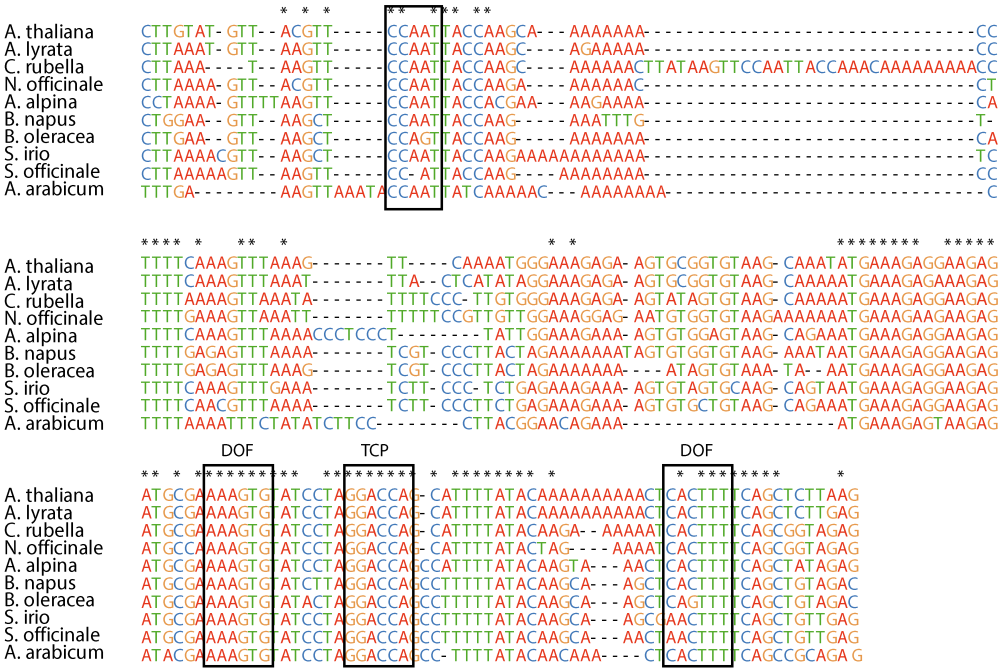


Supplementary Figure 6

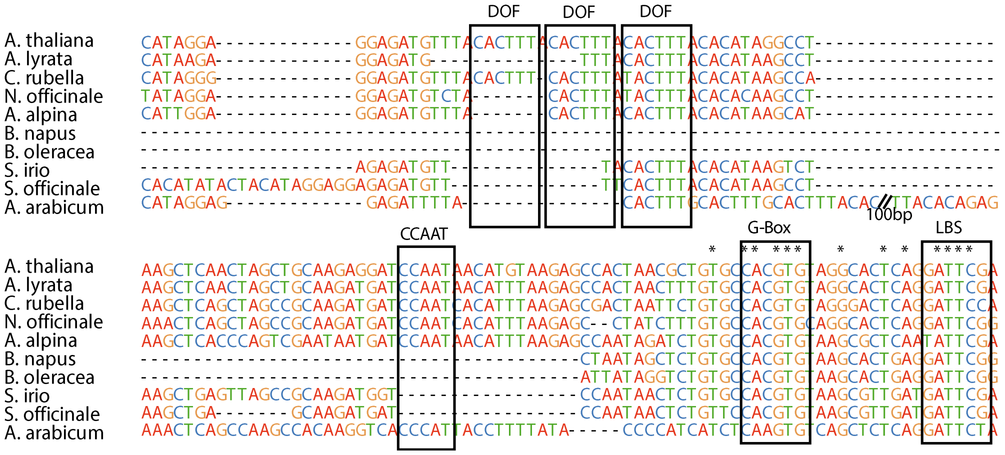
A Conserved Distal Motif 93bp (-2308 to -2214): *CDM:pnos::LUC*



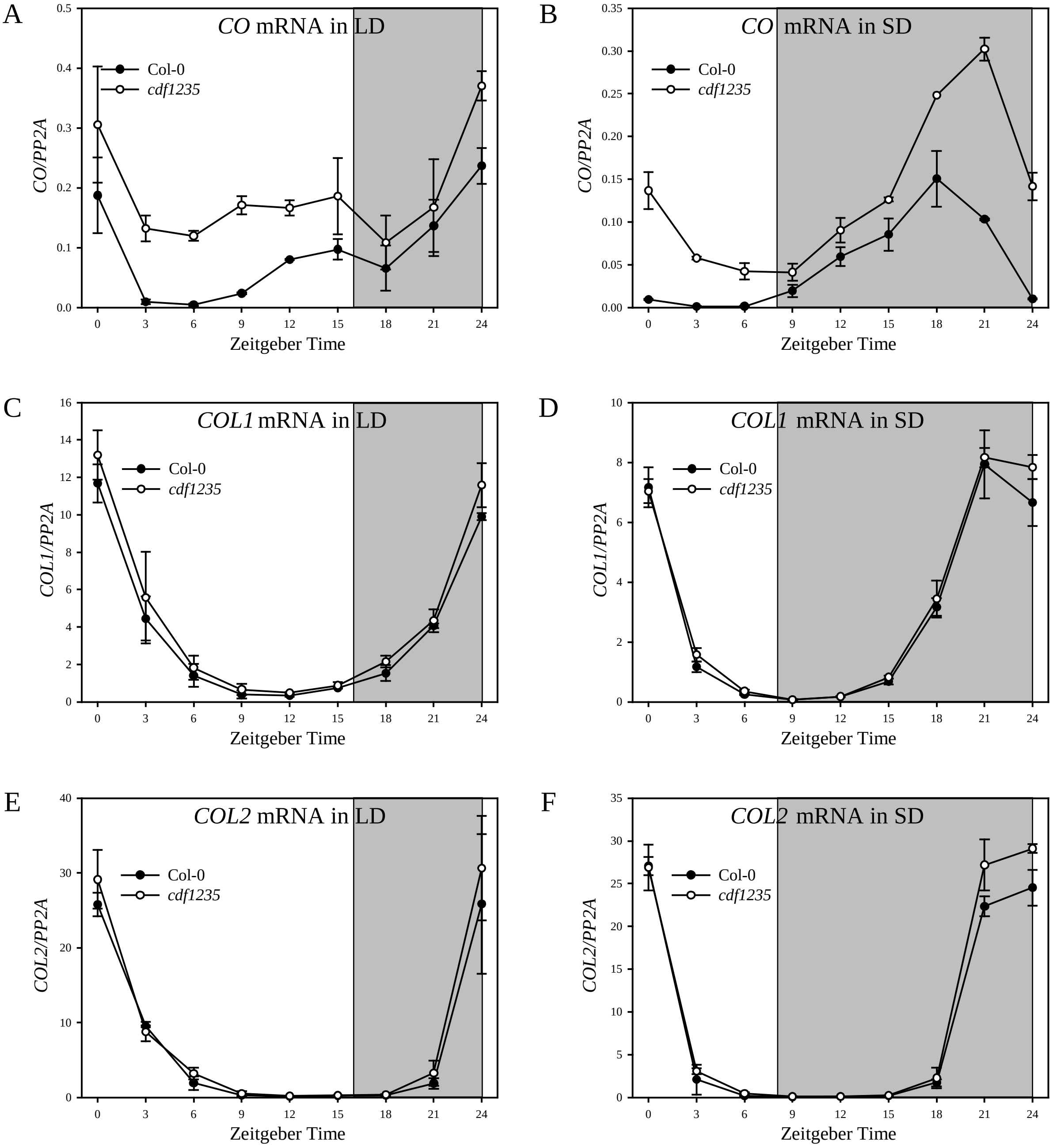
B Conserved Middle Motif 171bp (-1663 to -1491): *CMM:pnos::LUC*



C Conserved Proximal Motif 135bp (-237 to -102): *CPM:pnos::LUC*



Supplementary Figure 7



Supplementary Figure 8