Supplemental Material for "Comprehensive analysis of *CLE* gene expression and over-expression activity in Arabidopsis"

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Supplemental Methods

Construction of Transgenic Constructs

For *CLE1~CLE8, CLE16~CLE18, CLE22*, and *CLE25~27*, the PCR fragment was cloned into pENTR™/D-TOPO (Invitrogen), then recombined into pBGWFS7,0 using Gateway LR recombination (Invitrogen). For *CLE9* and *CLE10*, each 5' upstream region was cloned into pCR Blunt II TOPO, excised using HindIII and BamHI, and cloned into pBI101. For *CLE11~CLE13*, each 5' upstream region was ligated into pCR2.1 and cloned into the NotI site of binary vector pMLBART (Gleave, 1992). For *CLE21* the 5' upstream region was amplified by PCR, and a second PCR was run with attB adapter primers to complete the attB recombination site on the PCR product. The PCR product was recombined into the GUS-GFP vector pDONR201 using BP recombinase and then recombined into pBGWFS7 using LR recombinase (Invitrogen). To generate the *CLE14* and *CLE20* promoter::GFP translational fusion constructs, the 5' upstream region and coding sequence was PCR-amplified and cloned into SacI and BamHI restriction sites of pEZS-NL (Carnegie Institution). Then a SacI and NotI fragment containing the promoter and coding sequence fused to GFP and *OCS 3'* terminator was excised and cloned into binary vector pART27 (Gleave, 1992). Primer sequences are listed in Table S5.

The *CLE* over-expression constructs were created by cloning each full-length *CLE* cDNA into a binary vector for plant transformation. The *CLE8* coding sequence was PCR amplified and cloned into pENTR™/D-TOPO and then recombined into the binary vector pMDC32 (Curtis and Grossniklaus, 2003). The *CLE12* cDNA was inserted into the EcoRI site of pBJ36/35S behind the CaMV 35S promoter, and this cassette then excised and cloned into pMLBART at the NotI site. The *CLE16* and *CLE17* cDNAs were cloned into the EcoRI and SpeI sites of pCD223 (kindly provided by Chris Day), flanked 5′ by a double CaMV 35S promoter and 3′ by a nopaline synthase transcription termination signal. The *CLE22* and *CLE27* cDNAs were cloned into pCR2.1 using the EcoRI restriction site, and a NotI cassette containing the CaMV 35S promoter and cDNA was excised and cloned into the NotI site of pART27 (Gleave, 1992).

CLE10 Reporter Expression Analysis

In contrast to all other A-type *CLE* promoters, the *CLE10* promoter drove two distinct patterns of reporter expression in independent T1 transformants. The first pattern, observed in 38% of T1 plants, showed promoter activity in the rosette and cauline leaf margins, the abscission zone of

floral organs, the pedicel branch points, and the septum, style, ovary and ovules of the gynoecium in young developing flowers (see main text). The second pattern, observed in 27% of T1 plants, consisted of promoter activity in the root tip, stem, stomata on the stem epidermis, abscission zones and leaf margins, stronger promoter activity at the pedicel branch points, and faint promoter activity in the leaf vasculature including the hydathodes. These two patterns share reporter expression in the pedicel branch points, abscission zones, and leaf margins. A further 27% of plants showed weak reporter gene expression in only the pedicel branch points and floral organ abscission zones. The plants maintained their reporter expression patterns in subsequent generations, suggesting that the differences are not due to transient variability.

We produced two independent *CLE10* reporter constructs to verify that the distinct GUS expression patterns were not an artifact of the original construct. For the first construct, the *CLE10* upstream region was amplified using primers oAR106 and oAR107 and cloned into pCR-Blunt TOPO (Invitrogen). The promoter was excised with BamH1 and HindIII and cloned into pBI101 to create pAR77 (*CLE10::GUS*). We observed that 5 T1 plants carrying pAR77 showed expression pattern 1, 7 showed expression pattern 2, and 4 showed no expression. For the second construct, the *CLE10* upstream region up to the preceding gene was amplified using primers oAR374 (CACCGATGATTGATCGGCGAGAGAA) and oAR431 (CGTTGTGGAGAGAGAGAGAGAGATCTTCGA). The promoter was cloned into pENTR d TOPO (Invitrogen) and LR recombined into pBWGSF7 to create pAR184 (*CLE10::GFP-GUS*). We observed that 20 T1 plants carrying pAR184 showed expression pattern 1, 11 showed expression pattern 2, 18 showed the weak expression pattern, and 1 showed no expression. Thus the two GUS expression patterns are intrinsic to the *CLE10* upstream region and not due to vector-specific artifacts. These data suggest that the *CLE10* upstream region is insufficient to dictate a consistent GUS reporter pattern and that additional influences, potentially including downstream regulatory elements and/or local chromosome structure, play an important role in determining the expression pattern.

Supplemental References

Curtis MD, Grossniklaus, U (2003) A gateway cloning vector set for high-throughput functional analysis of genes *in planta*. Plant Physiol. **133:** 462-469

Gleave AP (1992) A versatile binary vector system with a T-DNA organizational structure conducive to efficient integration of cloned DNA into the plant genome. Plant Mol. Biol. **20:** 1203-1207

Supplemental Table 1. *CLE* over-expression meristem phenotypes.

CLE Gene	Dodecapeptide Sequence	AGI/ Accession No.	Size (aa)	p*35S*:*CLE* Phenotype	
				SAM	RAM
CLE8	RRVPTGPNPLHH	At1g67775	86	-	-
CLE12	RRVPSGPNPLHH	At1g68795	118	+	+
CLE16	RLVHTGPNPLHN	At2g01505	103	-	+
CLE17	RVVHTGPNPLHN	At1g70895	99	-	+
CLE22	RRVFTGPNPLHN	At5g12235	103	+	+
CLE27	RIVPSCPDPLHN	At3g25905	91	-	+

Each color in the CLE dodecapeptide sequence represents a different amino acid residue. SAM (shoot apical meristem) or RAM (root apical meristem) termination is denoted by a plus sign (+), whereas a minus sign (–) indicates no meristem termination phenotype.

Supplemental Table 2. *CLE* insertion alleles.

CLE Gene	Allele Name	Insertion Site Relative to ATG	Insertion Line ID Number	Affect on Transcript Levels
CLE1 (At1g73165)	*cle1-1*	+210 bp (CDS)	RATM12-0322-1_H	Absent
CLE2 (At4g18510)	*cle2-1*	+360 bp	SALK_109358	None
	cle2-2	+256 bp (3'UTR)	GK_471C05	None
CLE3 (At1g06225)	*cle3-1*	-36 bp (5' UTR)	RATM15-0143-1_G	Slightly reduced
CLE4 (At2g31081)	*cle4-1*	+505 bp	SALK_048896	None
CLE6 (At2g31085)	*cle6-1*	-289 bp	GK_521E09	Slightly increased
CLE7 (At2g31082)	*cle7-1*	-56 bp (5' UTR)	SAIL 108_G05	Absent
CLE9 (At1g26600)	*cle9-1*	-62 bp (5' UTR)	WisDsLox297300_04B	Slightly increased
CLE10 (At1g69320)	*cle10-1*	+72 bp (CDS)	GARLIC_1147_A10	Nearly absent
CLE13 (At1g73965)	*cle13-1*	+405 bp (3' UTR)	GK_549B08	Reduced
CLE16 (At2g01505)	*cle16-1*	+37 bp (CDS)	RATM11-1864-1_G	Absent
CLE17 (At1g70895)	*cle17-1*	+412 bp (3'UTR)	SALK_094989	Reduced
	cle17-2	-689 bp	SALK_103714	Slightly increased
CLE18 (At1g66145)	*cle18-1*	+188 bp (CDS)	GK_479A03	Absent
CLE19 (At3g24225)	*cle19-1*	-49 bp (5' UTR)	GK_232C03	Reduced
CLE21 (At5g64800)	*cle21-1*	-248 bp	SALK_088408	None
	cle21-2	-108 bp	GK_203E06	None

The ATG site is denoted as +1 bp. bp, base pairs; CDS, coding sequence; UTR, untranslated region.

Supplemental Table 3. Oligonucleotides used in this study.

Purpose	Gene	Name	Sequence
Cloning			
p*CLE*:*Reporter*	*CLE1*	CLE1p5FG	CACCTTTTATTTGTTCATACATTGTTAC
		CLE1p5RG	GTGTTATGTGACAATGGTTTCA
	CLE2	CLE2p5'FG	CACC TGATAAAGGCAAAACCCAAATCAG
		CLE2p5'RG	TACAGATACAATTAATTAGTTTCTG
	CLE3	CLE3p5'FG	CACCTGATCTCTGCACTTTTTTCATAT
		CLE3p5'RG	ATATATGTAACTTCCTCTTAGTTC
	CLE4	CLE4p5FG	CACCGAAAATCATTAAACAACGTAGTAC
		pCLE4-R1	ACCCATAACTTGAAACTTGCCATA
	CLE5	pCLE5(83)RG	AATAGAGGAAGAAATTAAAGGTGAATTTGG
		pCLE5-F1	CACC ATTGCACTACATTTAATTCGCAGT
	CLE6	CLE6p5FG	CACCATGATCGATACCTAAATAAATACT
		CLE6p5RG	TAAAGGTGATTAAGAAGAAGAAA
	CLE7	pCLE7-F1	CACCAATGATGAGTAGTTTTATAACCTTTT
		pCLE7-R3 (cds)	AGGTGAGCATAACAAATAACAATA
	CLE8	CLE8_FW	CACCAAGAAGCATAAACTACAAGAAAGG
		CLE8_RV	TTTCTCTCCTTTTTTTTTTTTTACTTTTAGTTTCTCATGTAC TTCTTTTTAGC
	CLE9	oAR102	GAAGCTTATCAGAACCTGAGAACTATAG
		oAR103	GGGATCCTGTTTTGGTTTCCAAGAGAGAG
	CLE10	oAR106	GAAGCTTTGCACGGATTAAAACGAGACAC
		oAR107	GGGATCCCGTTGTGGAGAGAGAGAGAGAGATC
	CLE11	5'_11-F	GGACATTTGTATATGCGCATG
		5'_11-R	GGCCTTGTAGCTAGAGAAGA
	CLE12	5'_12-F2	AGACTTTCACTGCAGCTCGT
		5'_12-R	GGAAGATGATGAAGAAATTCTAAGC
	CLE13	5'_13-F	GGAGATTAAAGATCATAGGACC
		5'_13-R	TGTTAGCCGGAGATGTAACC
	CLE16	p16-F	CACC CTTCACTTACTGGTGCTCTT
		p16-R1 cds	TTCTGGAACAAGCTTCCA
	CLE17	p17-F	CACC GTAAGGAATTTAGGAGATGA
		p17-R	CTCACAAAACCTTGTTCCG
	CLE18	CLE18_FW	CACCATAGCTTGAAGAAACAGAGGATTG
		CLE18_RV	CTTTCTGATCCTTATTTTTTGCTTTTTG
	CLE21	oAR210	AAAAAGCAGGCTGCAATTTGGAGGGATTTATTCGACAC
		oAR211	AGAAAGCTGGGTAGATATCGAAAAAAGTATGTTTGAATC
	CLE22	CLE22pFOR	CACCAACAAATTACAGTCAGCCCT
		CLE22pREV	CTCTTAGGAATCTTCAAGA
	CLE25	P25FG2	CACCAGCCACAGTGATTGTCGACAGTTA
		CLE25p5RG	ACTGAAACCTATGTCCCTACAGC
	CLE26	CLE26p5'FG	CACCAAGAACCATTTCTCGGTATGTAAA
		CLE26p5'RG	GGTTTCTAGCCTTTGTGGATATG
	CLE27	pCLE27F35	CACCTCCAGGAGGCTAAACCTGCAATAAGTA
		CLE27p5RG	ATCCCAAACCTAAACCCTAACATAA
p*CLE*:*CLE*:*Reporter*	*CLE14*	SacpCLE14 5F	CGAGCTCAATAATAACATCATTGATCTC
		BamCLE14 3R	GCGGATCCCG TTTGTTGTGAAGCGGGTTAGGAC
	CLE20	Pcle20-5F-1	AACATGCGAAGAAGCTCGAGA
		BamCLE20 3R	GCGGATCCCGTCGTTTGTTGTGCAAAGG
35S:*CLE*	*CLE8*	CLE8_ox_FW	CACCATGAAAGTGTTGAAGAGAGATTC
		CLE8_ox_RV	TCAGTTCCTAGCATAGTTTAGACTTC
	CLE12	CLE12A-F	ATGGCCTTGAAATTTTCTCAAATTC
		CLE12-R	CAATGGTGCAACGGATTGGG
	CLE16	16ox-F	GGAATTCCATGGAAGCTTGTTCCAGAA
		16ox-R	GACTAGTTCAGTTGTGAAGAGGATTTGGA
	CLE17	17ox-F	GGAATTCATGACTCACGTGTTGGTACG
		17ox-R	GACTAGTGTTAGTTGTGGAGAGGATTG

	CLE22	CLE22F	ATGGGAAATTACTACTCT
		CLE22R	TCTATTGTGCAATGGATT
	CLE27	CLE27F-TIGR	ATGACTCATGCTCGAGAATGGAG
		CLE27R-TIGR	CTAGTTATGCAAAGGATCCGGAC
Expression analysis			
RT-PCR	*CLE1*	1-mi-F	GAAATTCTTGCTGTGCTTG
		CLE1-t-R	TTCTAAACCGGTTCTCGATCA
	CLE7	7ox-F(g)	ATGGCTTCTAAAGCGTTATTGT
		7ox-R(g)	TCAAATTCTAGGTTTTGAAGACA
	CLE16	16ox-F	CATGGAAGCTTGTTCCAGAA
		16ox-R	TCAGTTGTGAAGAGGATTTGGA
	CLE17	17ox-F	ATGACTCACGTGTTGGTACG
		17ox-R	GTTAGTTGTGGAGAGGATTG
	CLE18	cle18ATG f	ATGCATTTGTTAAAAGGTGGTG
		cle18-2 rv	GTTTTGGTGAAGGCTGAGGA
	CLE27	27-F(g)	ATGACTCATGCTCGAGAATG
		27-R(g)	CTAGTTATGCAAAGGATCCG
	CLE8	CLE8 RT_FW	ATGAAAGTGTTGAAGAGAGATTC
		CLE8 RT_RV	TCAGTTCCTAGCATAGTTTAGACTTC
	CLE12	CLE12 RT_FW	GGATCCATGGCCTTGAAATTTTCT
		CLE12 RT_RV	TCGGCTGTTATCTCGGCTAT
	CLE19	CLE19_FW	ATGAAGATAAAGGGTTTGATGA
		CLE19_RV	ATCCACTCCACAACAGGTAA
	CLE2	CLE2F	GGATCCATGGCTAAGTTAAGCTTC
		CLE2R	GGATCCCTAGTGATGTTGTGGGTC
	CLE3	3-mi-F	GACTAGTGCTTCTAGTACTCGAATTGA
		3ox-R(g)	TCAGTGATGCCTCGGGTC
	CLE4	CLE4F	GGATCCATGGCAAGTTTCAAGTTA
		CLE4R	GGATCCGTGATGTCTAGGGTCCGG
	CLE6	6-mi-F	AACTGCAG GCAATCTCTAATCATACTC
		6-mi-R3’	AACTGCAGACAAAAGTGTAGGGAAGATT
	CLE9	oAR441	TCTGATCTTGATCTCTCTTCTCTTCGTCTC
		oAR442	AGTTGTGAAGCGGGTTTGGACCAGAGGGGA
	CLE10	oAR403	CAAGAAACTGGACCAACCGAACTC
		oAR126	CGTACCTTTGATCAATCTCCGTCG
Mutant screening			
Genotyping	*CLE1*	CLE1-ko(1)	ACATTACTCAGATCCTTTGCT
		CLE1-ko(2)	ACACGAGCCATTAGAGACT
	CLE7	7-ko(1)	AGGTCAAGTAAATCTAGTGGA
		7-ko(2)	TGGTGAACAAGTGAGGAGGT
	CLE16	p16-F	CACCCTTCACTTACTGGTGCTCTT
		16ox-R	GACTAGTTCAGTTGTGAAGAGGATTTGGA
	CLE17	17ox-F	GGAATTCATGACTCACGTGTTGGTACG
		17-ko(2)	AATTATGAGGGCAGATGCTG
		CLE17RNAi-R	TCTGCTCAATGGCCCACGAAA
		17-ko(1)	ACATGGGTTCATTTGCGTGA
	CLE18	cle18ATG f	ATGCATTTGTTAAAAGGTGGT
		cle18r-stop	GGATCCCTACAGTCCAATCAAATG
	CLE19	cle19_ko_FW	AAATGTGTCCACAGGCCACT
		cle19_ko_RV	GATCGTTCCCATGGAGTCAT
		cle19_ko_RV1	ATCCACTCCACAACAGGTAA
	CLE2	2-ko(1)	GTAAAACAGAATCATGTGTACGT
		2-ko(2)	GACGGTCGAGTTATAAATGGT
		C2-ISf	GGAATTCTACAGAAACTAATTAATTGTATCTGT
	CLE3	CLE3pseq591	CATGCTTCCATAATCAGCTT
		3ox-R(g)	TCAGTGATGCCTCGGGTC

	CLE4	4-ko(1)	CTTAGTGCACACATGAGTAT

		CLE4-s(5)	GAGTACGTACTTCTACTATT
	CLE6	CLE6p5FG	CACCATGATCGATACCTAAATAAATACT
		CLE6p5RG	TAAAGGTGATTAAGAAGAAGAAA
	CLE9	oAR230	TTCCTTTTTCGAGCTTTCACC
		oAR231	AGGAGGAGAAAGAAGTGGCTG
	CLE10	oAR205	GTCACCTCTAGTATTTGGCGGCGA
		oAR126	CGTACCTTTGATCAATCTCCGTCG
	T-DNA	Ds3'-2a	CGATTACCGTATTTATCCCGTTC
		SAIL_LB3	AATTTCATAACCAATCTCGATACAC
		Ds5'-2a	TCCGTTCCGTTTTCGTTTTTTAC
		SALK-LB(b1)	GCGTGGACCGCTTGCTGCAACT
		gabi-kat PCR	GGATCCCTACAGTCCAATCAAATG
		SAIL_LB3	AATTTCATAACCAATCTCGATACAC
		WiscDsLox p745	AACGTCCGCAATGTGTTATTAAGTTGTC

Supplemental Figure Legends

Supplemental Figure 1. *CLE14* and *CLE20* promoter activity. (A) *CLE20* is not expressed in the shoot apex or leaves of a 10-day-old seedling. (B) *CLE20* in the root vascular protoxylem and metaxylem. (C, D) *CLE20* in lateral root primordia. (E) *CLE14* in the trichomes of a 10-day-old seedling. (F) *CLE14* in root epidermal cells. (G) *CLE14* in the trichomes of a rosette leaf. (H) *CLE14* in the sepal trichomes on the inflorescence apex. Arrows in (C, D) and arrowhead in (D) indicate lateral root initiation point and lateral root tip, respectively. Arrow in (F) indicates the root epidermal layer.

Supplemental Figure 2. Examples of *CLE* promoter activity in primary root vasculature. (A) *CLE25* in the metaxylem cells. (B) *CLE22* in the vascular parenchyma. (C) *CLE4* in the pericycle. (D) *CLE7* in the pericycle and endodermis. (E) *CLE1* in the stele and endodermis. Arrows in (A, B) indicate the phloem. Arrowheads indicate protoxylem vessels.

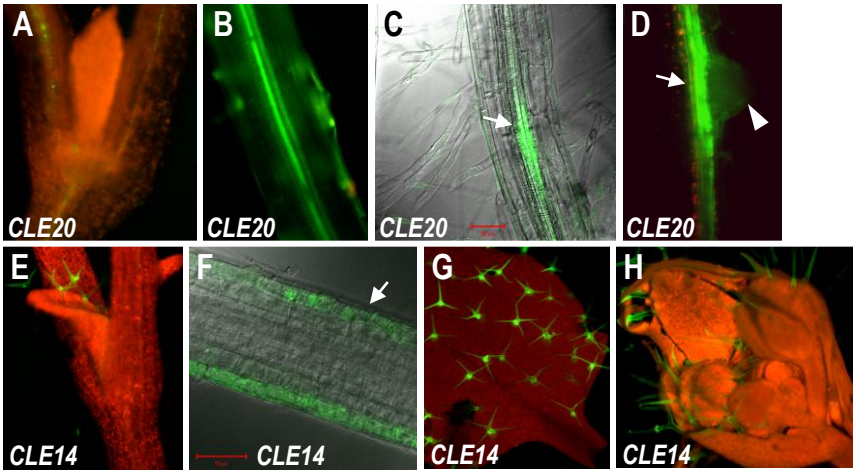
Supplemental Figure 3. *CLE* promoter activity during lateral root development. (A-C) *CLE27* in an initiating lateral root. (D-E) *CLE27* in an elongating lateral root. (F-G) *CLE27* in a mature lateral root. (H-I) *CLE2* in an initiating lateral root. (J) *CLE2* at the junction between the primary and lateral root. (K) *CLE11* in an elongating lateral root. (L) *CLE11*, (M) *CLE5* and (N) *CLE6* at the junction between the primary and lateral root. (O, P) *CLE22*, (Q) *CLE25* and (R) *CLE26* in initiating or elongating lateral roots. (S) *CLE4*, (T) *CLE12* and (U) *CLE7* in mature lateral roots.

Supplemental Figure 4. *CLE* promoter activity in lateral root tips of 11-day-old seedlings. (A-D) *CLE16* and (E-H) *CLE17* throughout the root cap and apical meristem. (I, J) *CLE11*, (K, L) *CLE13,* (M, N) *CLE18* and (O, P) *CLE1* in the root cap. (Q) *CLE22* in newly differentiating vascular tissue. (R) *CLE25* and (S) *CLE26* in the vascular parenchyma.

Supplemental Figure 5. *CLE* promoter activity in the shoot apex region. Longitudinal sections of 10-day-old (A) p*CLE21*:*GUS,* (B) *pCLE10:GUS*, (C) p*CLE4:GUS* and (D) p*CLE26:GUS* seedlings. Bars: 100 μm.

Supplemental Figure 6. *CLE16*, *CLE17* and *CLE27* promoter activity in the shoot apex.
(A, B) Serial sections of 10-day-old p*CLE16*:*GUS* seedlings. (C, D) Serial sections of 10-day-old p*CLE17*:*GUS* seedlings. (E, F) Serial sections of 10-day-old p*CLE27*:*GUS* seedlings. The numbers at the left column indicate independently transformed lines. Bar: 100 μm.

Supplemental Figure 7. *CLE8*, *CLE12* and *CLE22* over-expression phenotypes.
(A) A 13-day-old wild-type seedling. (B) A 13-day-old *p35S*:*CLE8* seedling. (C) A 13-day-old *p35S*:*CLE12* seedling. (D) A 13-day-old *p35S*:*CLE22* seedling. (E) Comparison of root development between a 13-day-old wild-type (left) and a *p35S*:*CLE12* (right) seedling. (F) Comparison of root development between a 13-day-old wild-type (left) and a *p35S*:*CLE22* (right) seedling. Arrows indicate the locations of the root tips. Bars: A-D 2.5 mm; E-F 5 mm.



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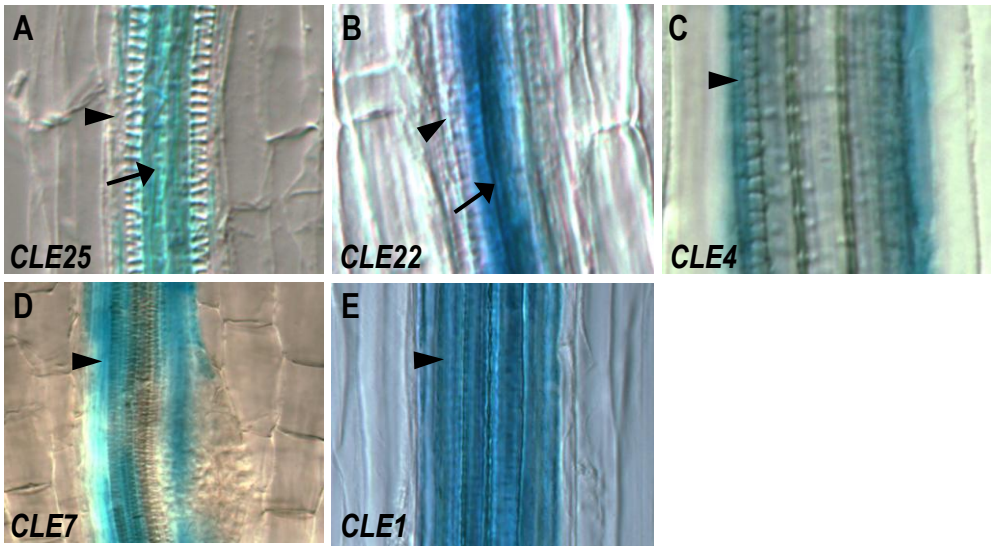

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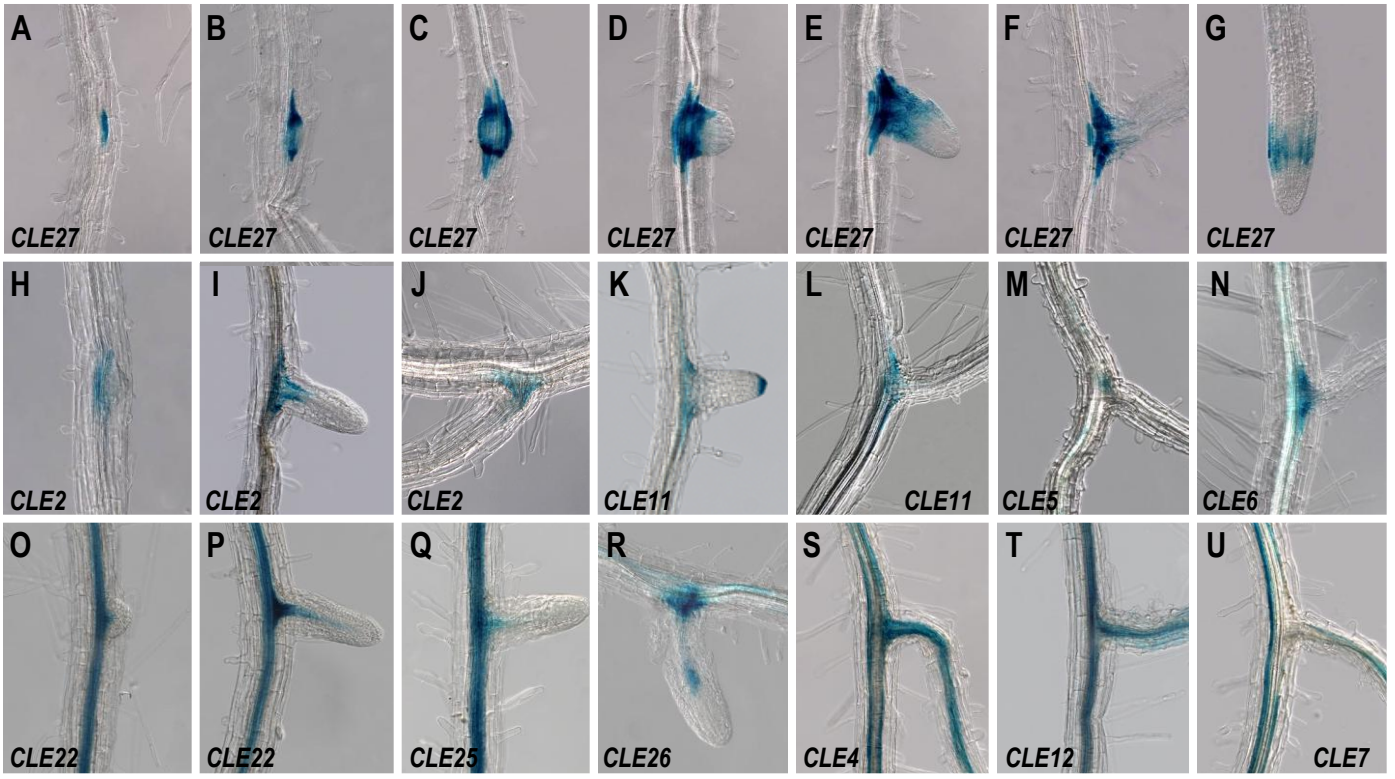
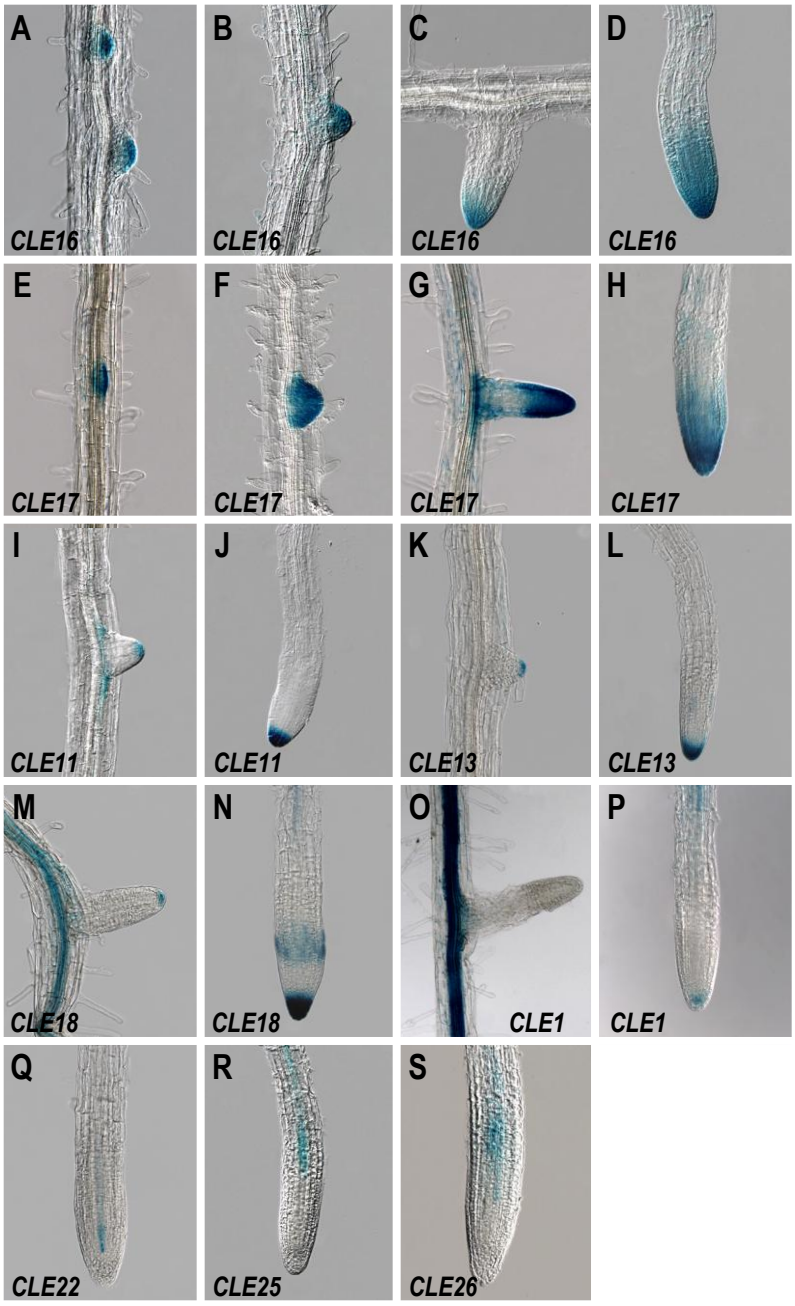
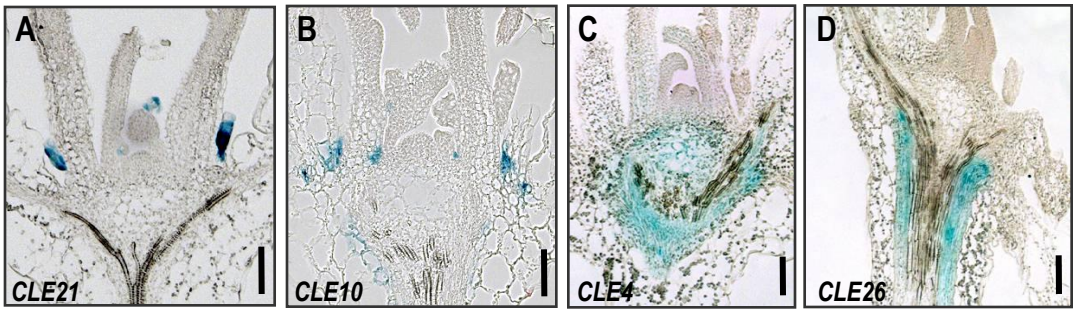


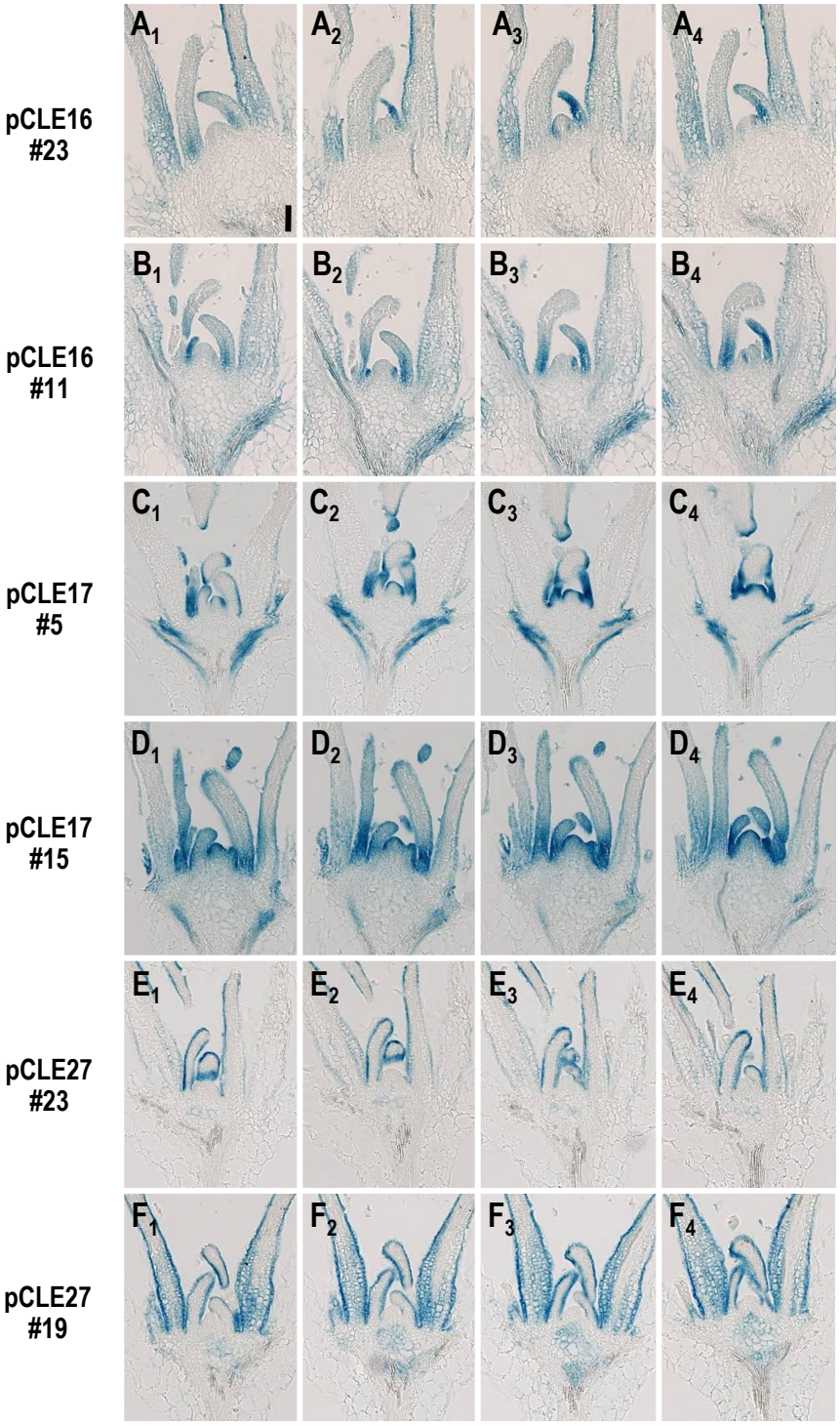
Supplemental Figure 3. *CLE* promoter activity during lateral root development. (A-C) *CLE27* in an initiating lateral root. (D-E) *CLE27* in an elongating lateral root. (F-G) *CLE27* in a mature lateral root. (H-I) *CLE2* in an initiating lateral root. (J) *CLE2* at the junction between the primary and lateral root. (K) *CLE11* in an elongating lateral root. (L) *CLE11*, (M) *CLE5* and (N) *CLE6* at the junction between the primary and lateral root. (O, P) *CLE22*, (Q) *CLE25* and (R) *CLE26* in initiating or elongating lateral roots. (S) *CLE4*, (T) *CLE12* and (U) *CLE7* in mature lateral roots.



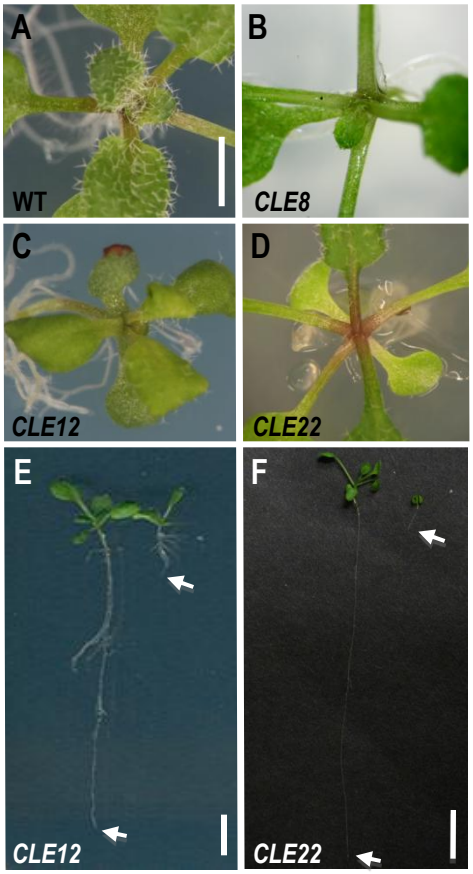
Supplemental Figure 4. *CLE* promoter activity in lateral root tips of 11-day-old seedlings. (A-D) *CLE16* and (E-H) *CLE17* throughout the root cap and apical meristem. (I, J) *CLE11*, (K, L) *CLE13,* (M, N) *CLE18* and (O, P) *CLE1* in the root cap. (Q) *CLE22* in newly differentiating vascular tissue. (R) *CLE25* and (S) *CLE26* in the vascular parenchyma.



Supplemental Figure 5. *CLE* promoter activity in the shoot apex region. Longitudinal sections of 10-day-old (A) p*CLE21*:*GUS*, (B) *pCLE10:GUS*, (C) p*CLE4:GUS* and (D) p*CLE26:GUS* seedlings. Bars: 100 µm.



Supplemental Figure 6. *CLE16*, *CLE17* and *CLE27* promoter activity in the shoot apex. (A, B) Serial sections of 10-day-old p*CLE16*:*GUS* seedlings. (C, D) Serial sections of 10-day-old p*CLE17*:*GUS* seedlings. (E, F) Serial sections of 10-day-old p*CLE27*:*GUS* seedlings. The numbers at the left column indicate independently transformed lines. Bar: 100 µm.



Supplemental Figure 7. *CLE8*, *CLE12* and *CLE22* over-expression phenotypes. (A) A 13-day-old wild-type seedling. (B) A 13-day-old *p35S*:*CLE8* seedling. (C) A 13-day-old *p35S*:*CLE12* seedling. (D) A 13-day-old *p35S*:*CLE22* seedling. (E) Comparison of root development between a 13-day-old wild-type (left) and a *p35S*:*CLE12* (right) seedling. (F) Comparison of root development between a 13-day-old wild-type (left) and a *p35S*:*CLE22* (right) seedling. Arrows indicate the locations of the root tips. Bars: A-D 2.5 mm; E-F 5 mm.