

SUPPLEMENTARY INFORMATION

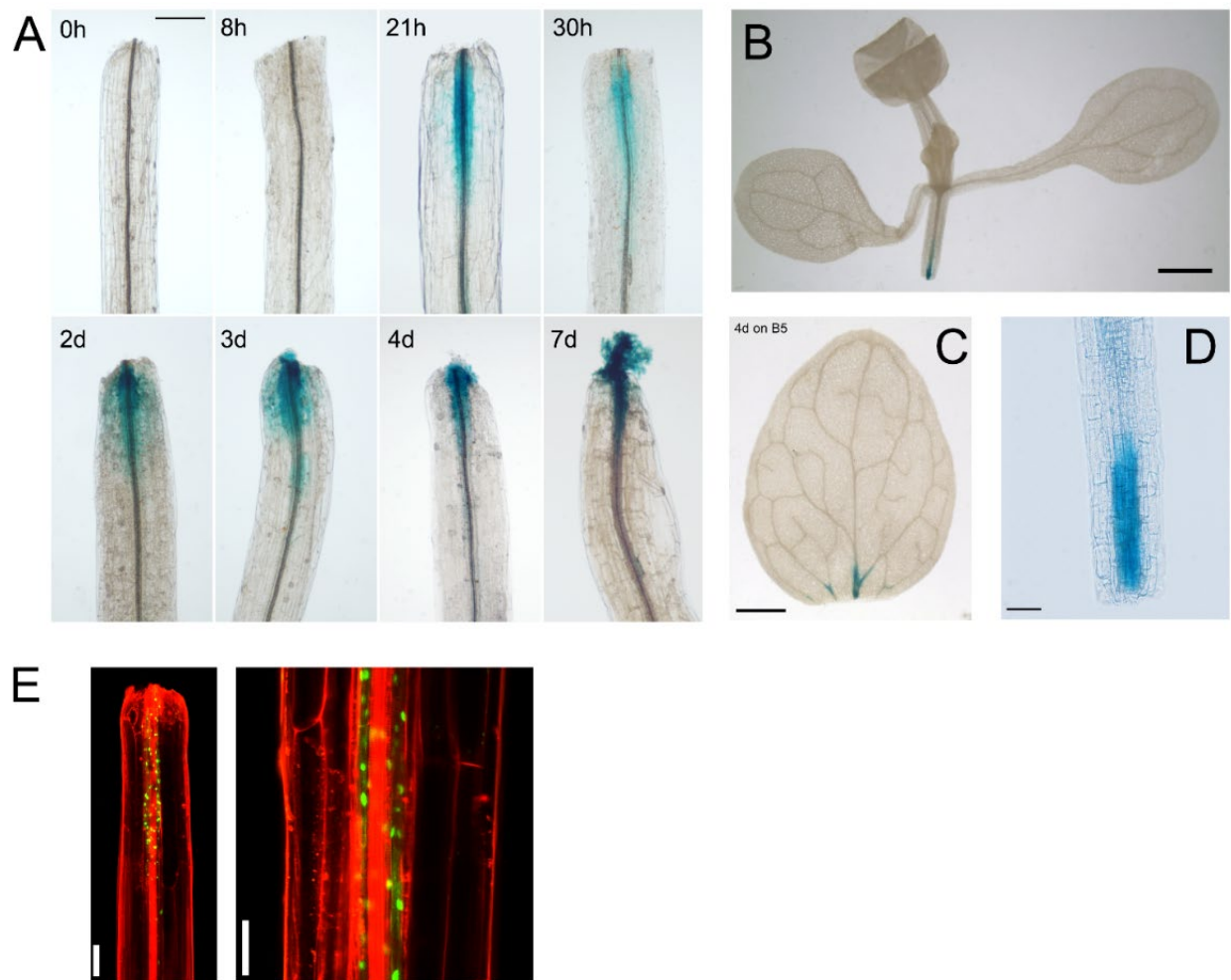


Fig. S1. *HTR15* expression was induced at wound sites in various *Arabidopsis* tissues. (A) GUS staining shows *HTR15* expression at wound sites of hypocotyls. *HTR15* expression is induced within a few hours after wounding. *HTR15* promoter activity was also observed at wound sites of stems (B), leaves (C), and roots (D). (E) Confocal imaging shows *HTR15* expression in pericycle-like cells at wound site of hypocotyl. Scale bars: 200 μm (A); 1 mm (B,C); 50 μm (D,E).

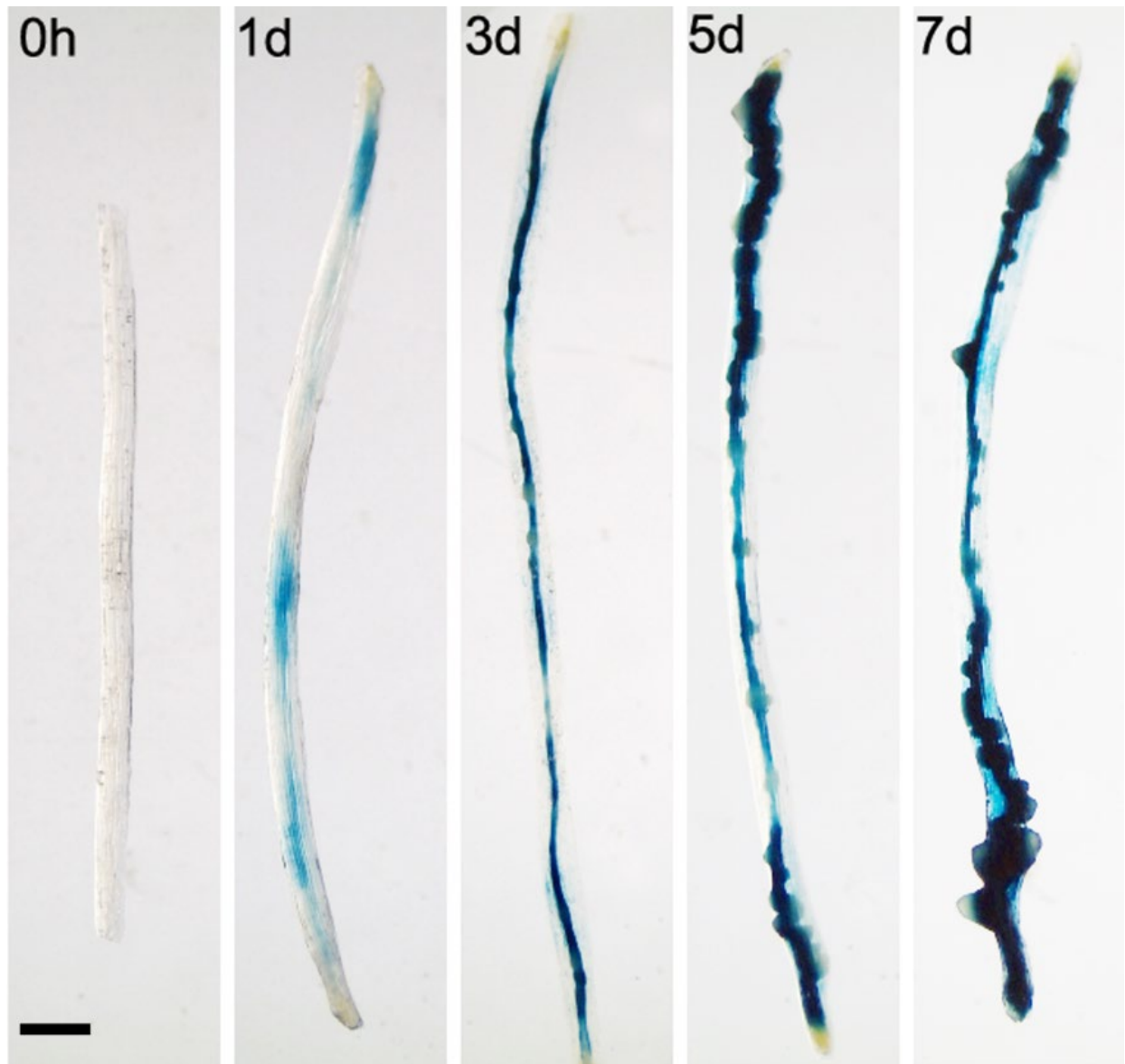


Fig. S2. Expression pattern of *HTR15* during CIM-induced callus formation. GUS staining shows *HTR15* expression during CIM incubation. Hypocotyl explants of 7-day-old *pHTR15::GUS* were incubated on CIM and GUS staining assay was performed at the indicated time points. Scale bar: 200 μm .

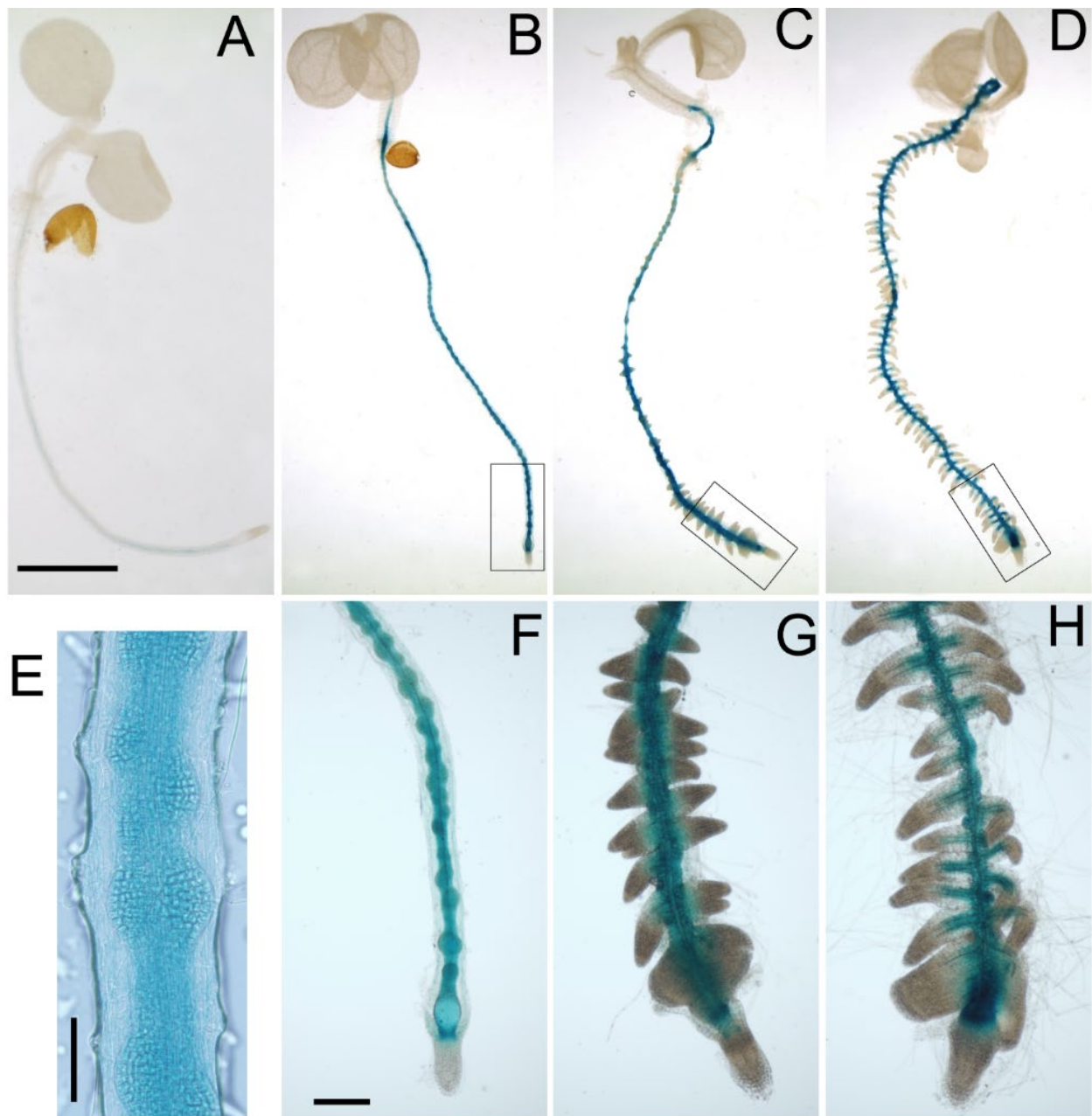


Fig. S3. *HTR15* expression is up-regulated by auxin. (A) GUS staining shows *HTR15* promoter activity before NAA treatment. (B,F) GUS staining shows *HTR15* promoter activity after *pHTR15::GUS* was treated with 10 μM NAA for 2 days. (C,G) GUS staining shows *HTR15* promoter activity after *pHTR15::GUS* was treated with 10 μM NAA for 3 days. (D,H) GUS staining shows *HTR15* promoter activity after *pHTR15::GUS* was treated with 10 μM NAA for 5 days. (E) GUS staining shows *HTR15* promoter activity at lateral root primordium. Scale bars: 1 mm (A-D); 100 μm (F-H); 50 μm (E).

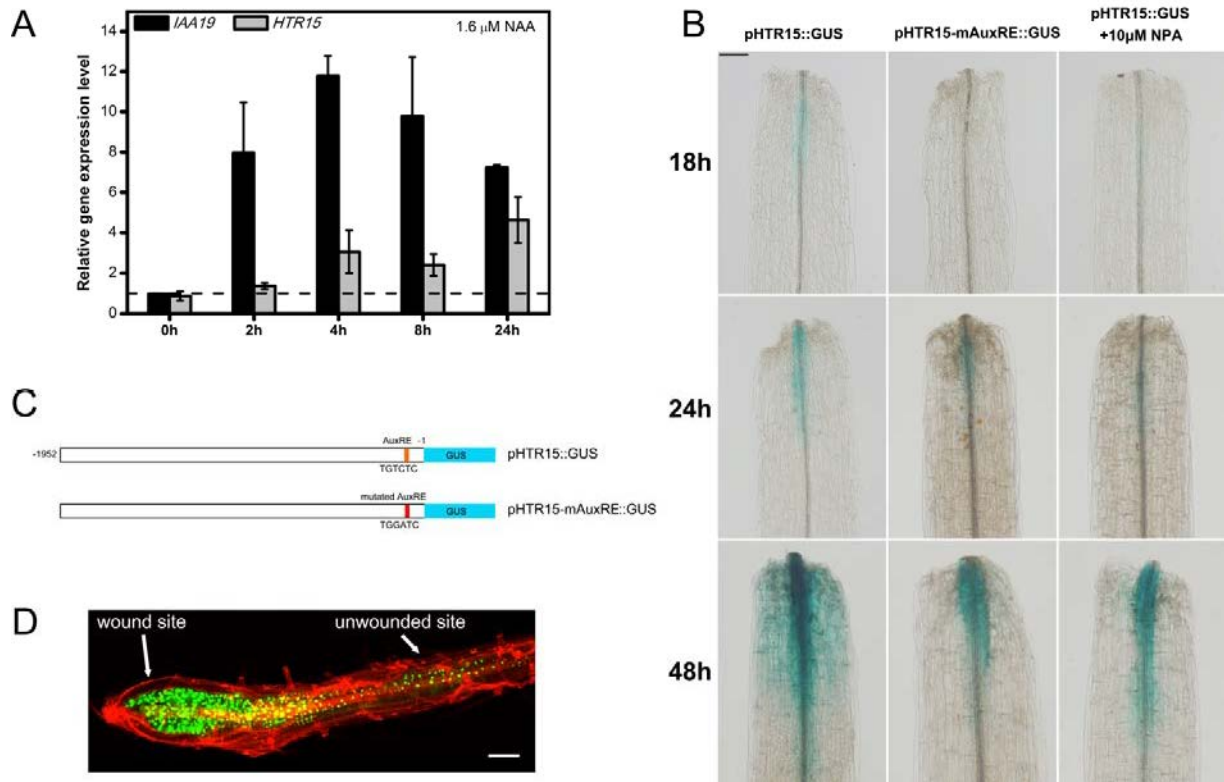


Fig. S4. Auxin is involved in the regulation of *HTR15* expression. (A) qRT-PCR analysis of *HTR15* induction by auxin. 7-day-old seedlings were transferred to ½ MS media supplemented with 1.6 μM NAA for the indicated period. *IAA19* was used as a positive control. Relative expression levels of *IAA19* and *HTR15* are normalized against those of the *PP2AA3* gene and shown relative to their expression levels at 0 h. Expression data are mean±s.d. ($n=3$, biological replicates). (B) GUS staining shows *HTR15* promoter activity at wound sites of *pHTR15::GUS* (left panel), mutated *HTR15* promoter activity at wound site of *pHTR15-mAuxRE::GUS* (middle panel), *HTR15* promoter activity at wound site of *pHTR15::GUS* hypocotyls after 10 μM NPA treatment (right panel). Scale bar: 100 μm. (C) Schematic diagram shows *HTR15* promoter-GUS reporter. (D) Confocal imaging shows *pHTR15::3GFP* expression at wound site and unwound site of root explant incubated on CIM. Scale bar: 100 μm.

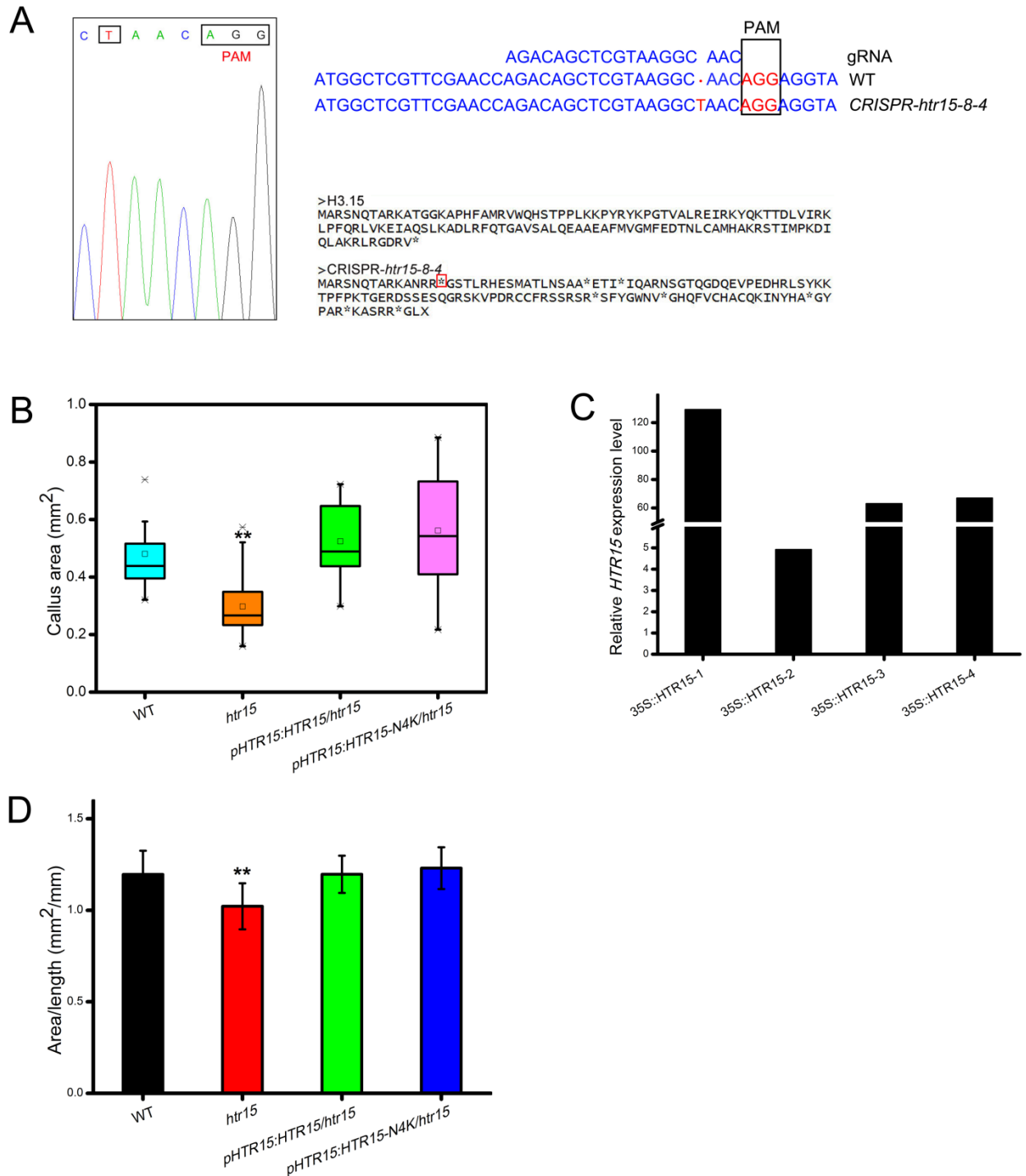


Fig. S5. H3.15 promotes CIM-induced callus formation. (A) Mutation of *HTR15* by CRISPR/Cas9 technology. Sequencing chromatogram indicated a thymine (T) insertion in the front of the *HTR15* coding sequence, which introduces a premature stop codon. (B) Quantitative analysis of callus formation at

wound sites of wild-type, *htr15*, *pHTR15:HTR15/htr15*, and *pHTR15:HTR15-N4K/htr15* hypocotyls. After dissection, explants were cultured on phytohormone-free MS medium for 14 days. Box plots represent the distribution of projected callus area ($n \geq 11$). The statistical significance was determined by Student's *t*-test (** $P < 0.01$). (C) RT-qPCR analysis of *HTR15* overexpression lines. Relative expression levels of *HTR15* are normalized against those of the *PP2AA3* gene and shown relative to their expression levels in wild type. (D) Quantitative analysis of callus formation of WT, *htr15*, *pHTR15:HTR15/htr15*, and *pHTR15:HTR15-N4K/htr15* hypocotyl explants incubated on CIM for 28 days. The length and area of hypocotyl explants were measured with image-Pro plus 6.0 software. Data are mean \pm s.d. ($n \geq 12$, ** $P < 0.01$; Student's *t*-test).

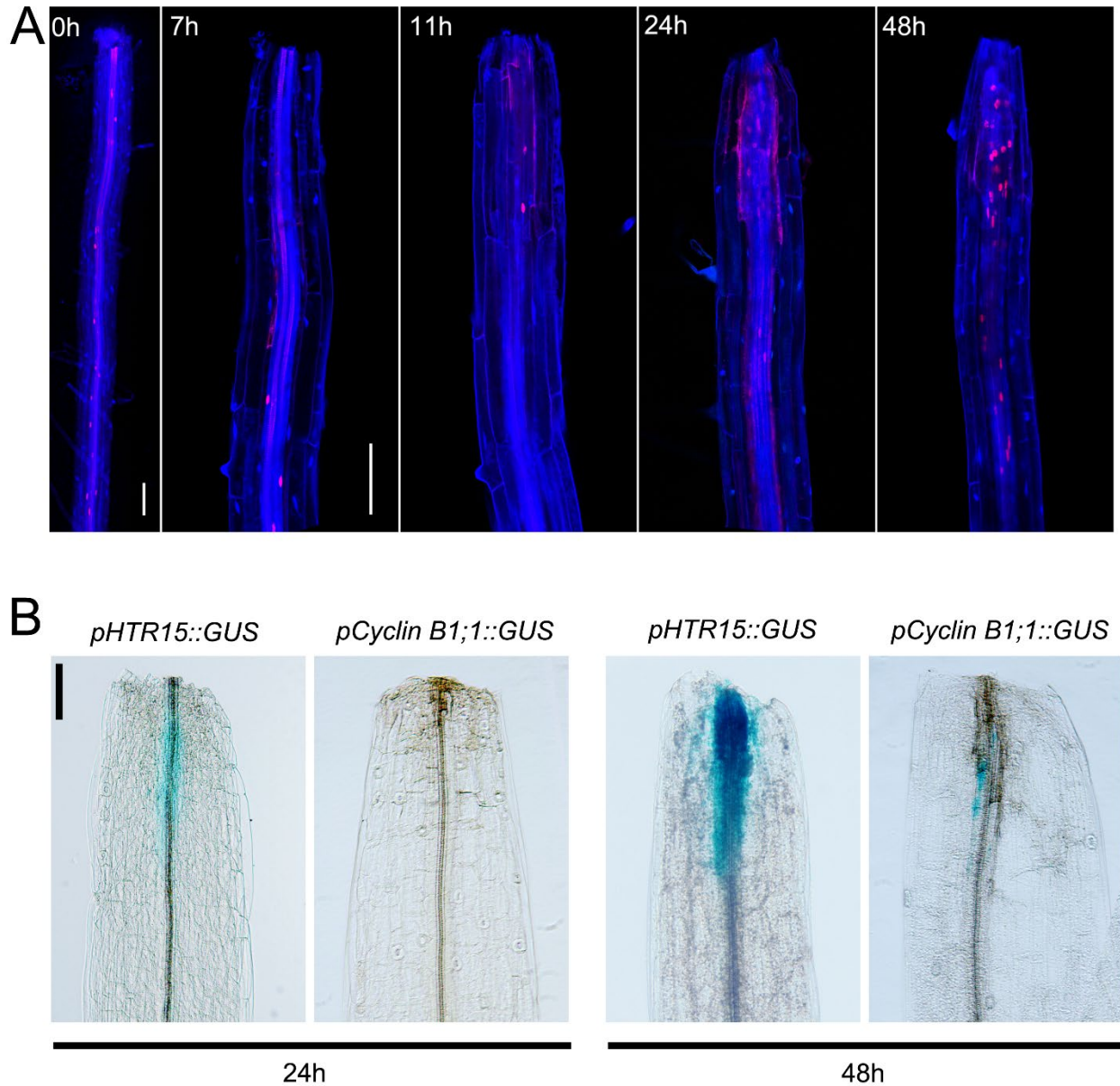


Fig. S6. H3.15 expression and incorporation is independent of cell cycle dynamics. (A) EdU staining shows DNA replication after root wounding. Nuclei were labeled with Hoechst 33342 and visualized as blue dot (425-475 nm), while nuclei undergoing DNA synthesis were labelled by EdU and visualized as red dot (663-738 nm). Scale bars: 100 μ m. (B) GUS staining shows induction of *HTR15* and *Cyclin B1;1* at hypocotyl wound sites. Scale bar: 100 μ m.



Fig. S7. Homologs of H3.10 and H3.15. Sequence alignment of homologs of variants H3.10 and H3.15 in close relatives of *Arabidopsis thaliana* (*At*), *Arabis lyrata* (*AL*), *Arabis halleri* (*Araha*) and *Boechera stricta* (*Bostr*) as well as in other dicots *Brassica oleracea* (*Bol*), *Capsella rubella* (*Carub*), *Capsella grandiflora* (*Cagra*), and *Medicago truncatula* (*Medtr*). The two groups of homologs are highlighted in purple (H3.10) and green (H3.15).

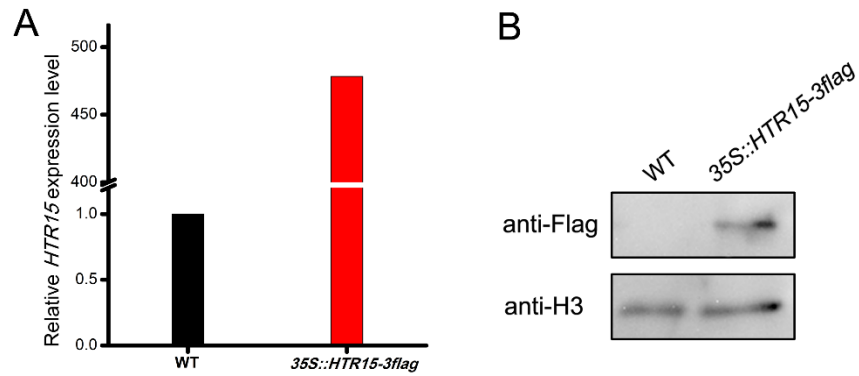


Fig. S8. Verification of 35S::*HTR15-3flag*. (A) RT-qPCR analysis of *HTR15* expression. (B) Western blot assay of H3.15-flag fusion protein. WT and 35S::*HTR15-3flag* hypocotyl explants were incubated on CIM for 30 days. H3 served as a loading control.

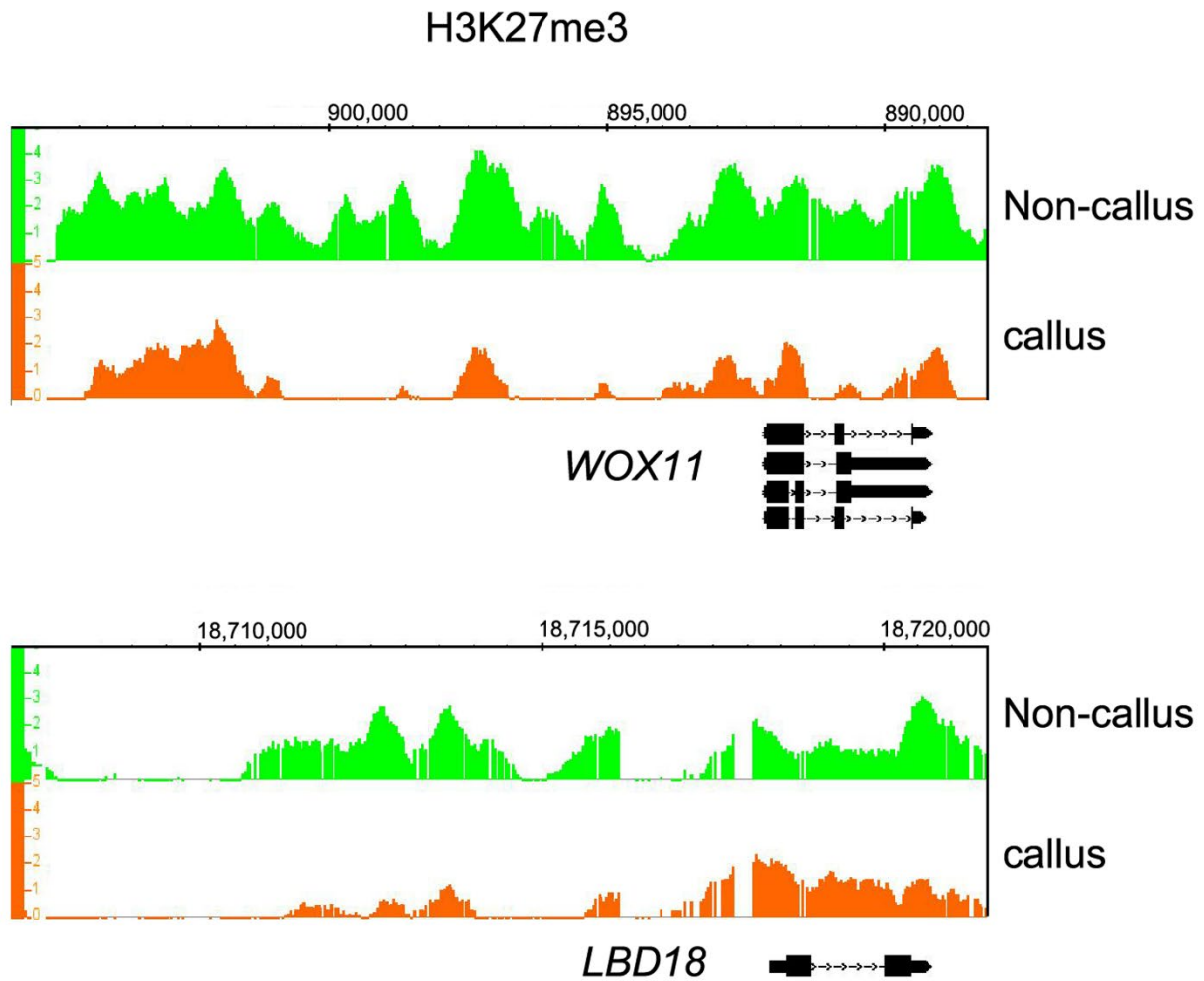


Fig. S9. H3K27me3 levels decreased at *WOX11* and *LBD18* loci during CIM-induced callus formation. Data were retrieved from previously established databases (He et al., 2012).

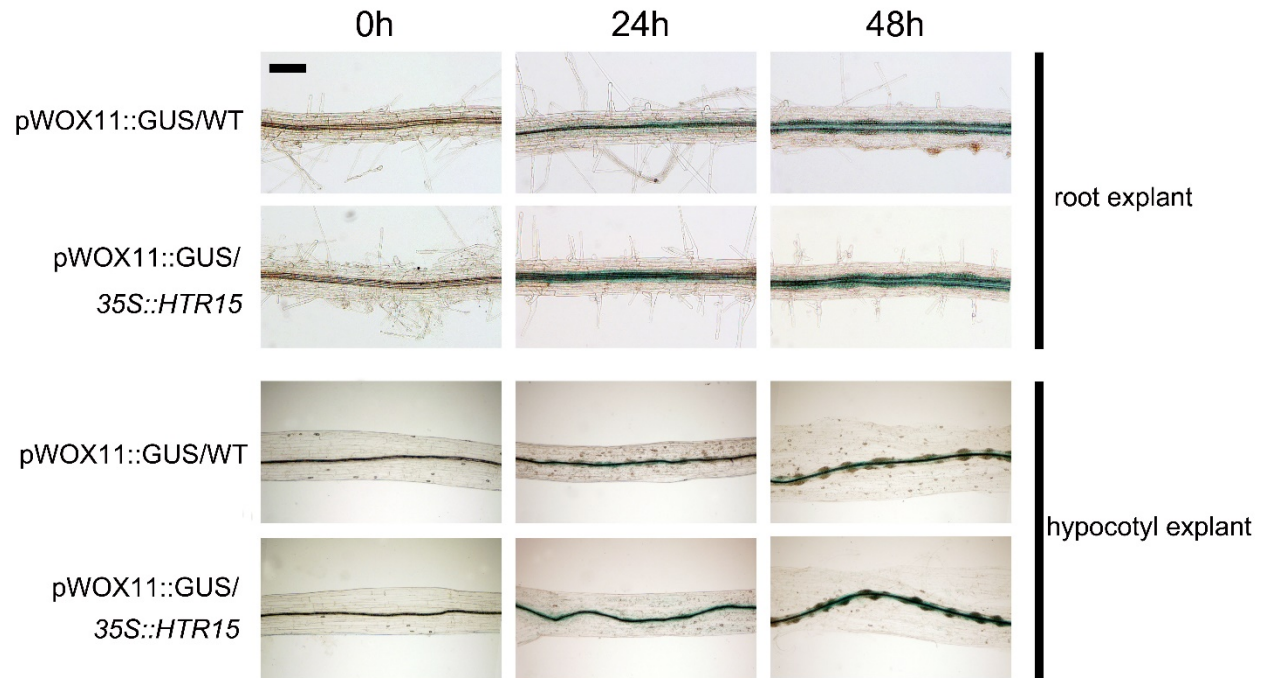


Fig. S10. H3.15 promotes *WOX11* expression during CIM-induced callus formation. GUS staining shows promoter activity of *WOX11* in wild-type and *35S::HTR15* explants during CIM incubation, respectively. Scale bar: 100 μ m.

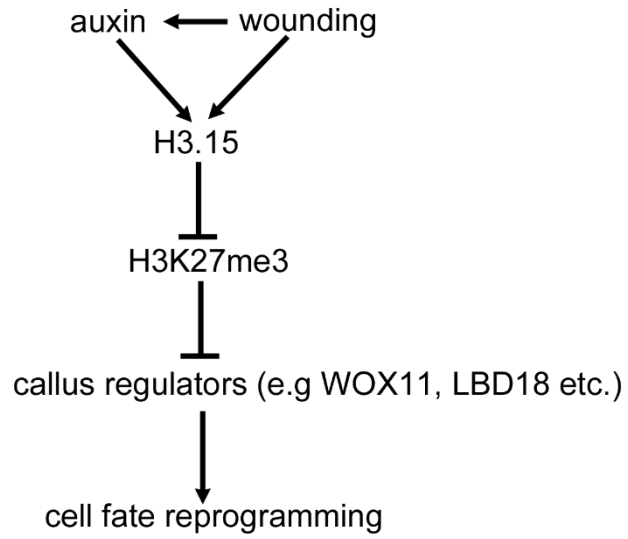


Fig. S11. Proposed model of the role of the H3.15 pathway in plant regeneration.

Table S1. Primers used in this study

A. plasmid construction

<i>HTR15</i> CDS	HTR15cd-F	ccctcgagTGCTCACTATGGCTCGTT
	HTR15cd-R	cgggatccTCAAACCCTATCGCCTCG
<i>HTR15</i> genomic sequence	HTR15pm-F	atgggcccGAGAAATGCTATCCCGTTA
	HTR15cdnos- R	ccctcgagAACCTATCGCCTCGAAG
<i>HTR15</i> promoter	HTR15pm-F	atgggcccGAGAAATGCTATCCCGTTA
	HTR15pm-R	ccctcgagAGTGAGCAAATCAACTTTG
	mAuxRE-F	TAAATGGATCCACCCACAACTCTCAATACATATC
	mAuxRE-R	GGGTGGATCCATTTATTTATTTGTGGAGAATCTAA
H3.15 mutation	N4K-F	ccctcgagATGGCTCGTTCGAAGCAGACAGCT
	H27K-F	TGGCAAAGTCAACTCCGCCGCTTAAGAAACC
	H27K-R	AGTTGACTTTTGCCATACTCTCATGGCGAAGT
	KA-step1-F	TGGCGAAAGTCAGCTCCGCCGCTTAAGAAACC
	KA-step1-R	GCTGACTTTCGCCATACTCTCATGGCGAAGTGTG
	KA-step2-F	GCTCCGGCGACCGGAGGAGTTAAGAAACCATATAGATACAA
	KA-step2-R	GGTCGCCGGAGCTGACTTTCGCCATACTCTC
	KA-step3-F	CCACAAAGGCGGCGCGAAAGTCAGCTCCG
	KA-step3-R	GCCGCCTTTGTGGCGAAGTGTGGAGCCTTA

B. qRT-PCR

PP2AA3-Fwd	GACCAAGTGAACCAGGTTATTGG
PP2AA3-Rev	TACTCTCCAGTGCCTGTCTTCA
IAA19-Fwd	GCCTTTGCTCTTGATAAGCTCTTC
IAA19-Rev	CTCTAGAAACATCCCCCAAGGTAC
HTR1-Fwd	CCGTTCCAGCGTTTGGTTCGT
HTR1-Rev	CGAGGTATGCTTCAGCCGCT
HTR2-Fwd	GCCACACAGATTCAGACCCGGA
HTR2-Rev	CGACGGCACTGCTCTGGAAA
HTR3-Fwd	GCCACACAGATTCGGTCCAGGA
HTR3-Rev	GCTGCTTCTTGAAGAGCTGCGA
HTR4-Fwd	CGGGTTTTGTCCCATTCCAGTT
HTR4-Rev	CGGATTTACGGAGAGCAACAGT
HTR5-Fwd	CTTGCTACAAAGGCTGCACGT
HTR5-Rev	GTCAGTCTTGAAATCCTGGGCA
HTR6-Fwd	CCGTCGCTCTTCGTGAGATTCGT
HTR6-Rev	GGCTTTGGAATCTCAGATCCGT
HTR8-Fwd	CAGGAACCGTCGCTCTTCGTGA
HTR8-Rev	CAGATCCGTCTTGAAGTCCTGAGCT

HTR9-Fwd	CCGCGAGGAAATCCACAGGA
HTR9-Rev	CCTGGACGGAATCTATGCGGCT
HTR10-Fwd	GTCGCTCTTCGTGAGATCCGCAA
HTR10-Rev	GCACCGCATGGCTTTGGAACCT
HTR12-Fwd	CTTCTCAGGCGGCAGGTCCAA
HTR12-Rev	CTTCTTCTGTGAGCCTCGTGGCA
HTR13-Fwd	GCCACACAGATTCCGTCCAGGA
HTR13-Rev	CGGCACTGCTCTGGAACCTCA
HTR14-Fwd	AGCTCCGAGGACTCTGCTCG
HTR14-Rev	GGGACGGTAACGGTGAGGTTT
HTR15-Fwd	GAACCAGACAGCTCGTAAGGCAA
HTR15-Rev	GTGCCACTGTTCTGGCTTGT
WOX11-Fwd	CTTCTTATGGTGGTGGATGT
WOX11-Rev	AGCCCCTTGTGGTATTGA
LBD16-Fwd	CCTCCAACAACAGGTGGCTT
LBD16-Rev	TGGTACTTTCCGAGCTGTGTC
LBD18-Fwd	TCAGCAACAGGTGGTGAATC
LBD18-Rev	TCGGTTATGGCAAGAGGG
LBD29-Fwd	CCAACAACAGGTTGTGAATT
LBD29-Rev	CCTTAGTAGTGTCTCCATAGTA

ERF115-Fwd	TTCCCTTTCTTCTCTGCCCG
ERF115-Rev	CAATAGCCCTTGATCTTGAGTTGG

C. ChIP-qPCR

TUB2-Fwd	ATCCGTGAAGAGTACCCAGAT
TUB2-Rev	AAGAACCATGCACTCATCAGC
WOX11-1-Fwd	GCAAACCTAACGCGTCTCACA
WOX11-1-Rev	GAGACAGATATGCTTTAAATTG
WOX11-2-Fwd	GGGAGATGCAAATGTCTTCT
WOX11-2-Rev	CCAAATCACTCCCCTATGA
WOX11-3-Fwd	TGGTGCATTCTCAGGTGTT
WOX11-3-Rev	CACGAGGAATTCGATCAGCA
WOX11-4-Fwd	CCCTTACTTCACATAATGGGA
WOX11-4-Rev	CATGTCTGTCTTGGAACCTG
LBD18-1-Fwd	CGTGAATGCTCATTTTAACTTA
LBD18-1-Rev	GAAGAAAGACCTTTTTGATAAG
LBD18-2-Fwd	GCCATGTGGTGCTTGTAAGT
LBD18-2-Rev	CTTCGATGAACCGGTATGTG
LBD18-3-Fwd	GGTGAATAACAGGTAATTGCT

LBD18-3-Rev	CGCTTAACGTGATAGCTTAAT
LBD18-4-Fwd	CGTCACCAATACGGTGTATC
LBD18-4-Rev	TAGACATAGTTCGAGACGGC

References

He, C., Chen, X., Huang, H. and Xu, L. (2012). Reprogramming of H3K27me3 is critical for acquisition of pluripotency from cultured *Arabidopsis* tissues. *PLoS Genet.* **8**, e1002911.