Supporting Information

Kumpf et al. 10.1073/pnas.1210835110
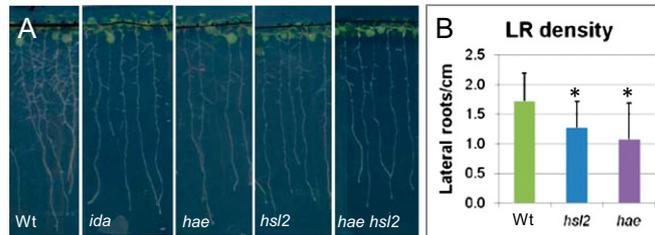

Fig. S1. *Inflorescences deficient in abscission* (*ida*), *haesa* (*hae*), *haesa-like2* (*hsl2*), and *hae hsl2* mutants show reduced lateral root density. (*A*) Wild type (WT) and mutant seedlings as indicated 9 d after stratification. (*B*) Lateral root (LR) density (number of LRs per cm root) for *hsl2* (n = 50), *hae* (n = 48), and WT plants (n = 50) 9 d after stratification. *Significant deviation from WT, Student *t* test.
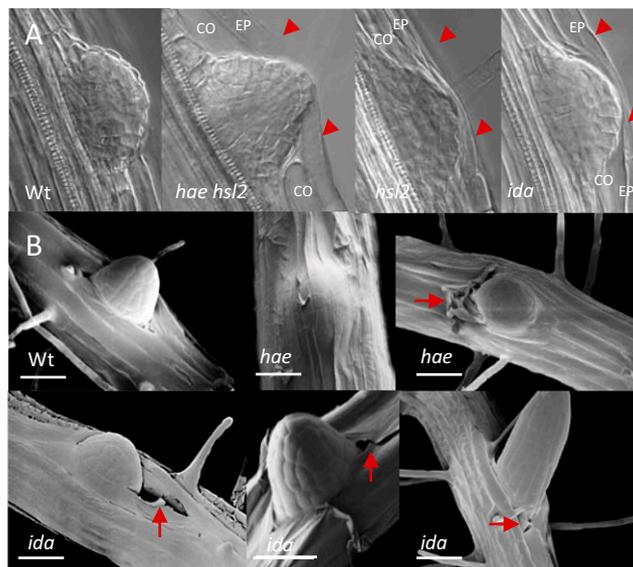

Fig. S2. Mutation in *HAE* and *IDA* results in delayed emergence and ruptured epidermal cells. (*A*) Representative WT lateral root primordia (LRP) at emergence (stage VIII) and *hae hsl2, hsl2*, and *ida* LRP at a similar developmental stage still covered with an epidermal (EP) cell. Arrowheads, EP cell walls. (*B*) Representative cryo scanning electron microscopy (SEM) images of WT, *hae*, and *ida* LRs at emergence. Note *hae* LR still covered with overlaying tissue. Arrows, rifts in flattened and ruptured EP cells, not seen for WT roots (7–10 roots inspected per genotype). (Scale bars: 30 μM.)

Fig. S3. *IDA* has a unique expression pattern at the LR compared with the closely related *IDA-LIKE* (*IDL*) genes. (*A*) Lateral root expression pattern of the promoter:*β-glucuronidase* (*GUS*) transcriptional fusion constructs for *IDL1-IDL5*. Note that *pIDL5:GUS* is not expressed. (*B*) *pIDA:GUS* expression in cells overlaying LR in WT and *like aux1-3* (*lax3*) background at stages II and IV, as indicated. The outlines of LRP are shown in gray. (*C*) *pIDA:GUS* expression in WT and *lax3* mutant background at LR with 1 μM indole-3-acetic acid (IAA) and in WT with 1 μM napthaleneacetic acid (NAA).

Fig. S4. *HSL2* is expressed in the LRP. *(A–C)* Confocal images of *pHSL2:YFP* expression at stages II, IV, and VI, as indicated. Note in (*C*) lower YFP intensity in the cortex (CO) cell the LRP is passing (arrowhead) than in neighboring CO cell (arrow). (*D*) Confocal images of *HSL2:YFP* expression after emergence (stage VIII). Note low expression in the EP cell closest to the LRP. Note that propidium iodide (PI) does not penetrate the EN or the LRP and therefore does not stain the inner endnodermal (EN) wall and LR cell walls. (*Left*) PI-stained root; (*Center*) YFP signal; (*Right*) merge. (Scale bars: 50 μM.) (*E* and *F*) Confocal images of *pHSL2:YFP* expression in the tip of lateral and primary roots stained with PI.
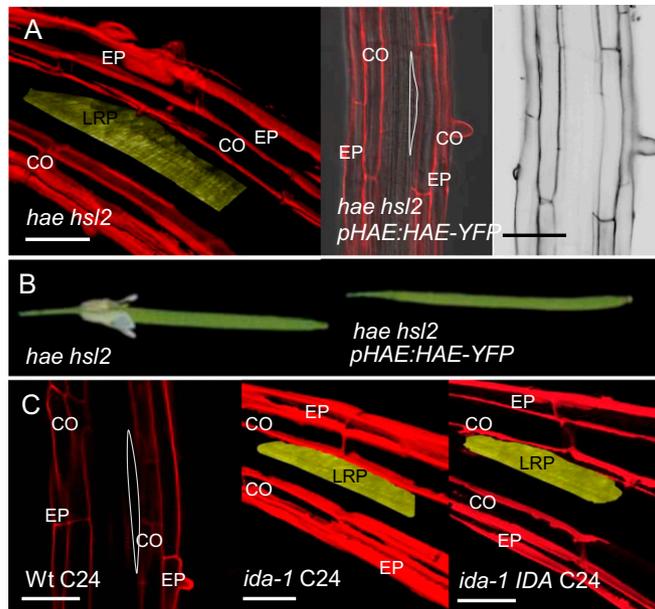
Fig. S5. Complementation of mutant phenotypes demonstrates *IDA* and *HAE* involvement in cell wall degradation. (*A*) PI-stained roots of *hae hsl2* (3D, LRP colored yellow), and *hae hsl2* transformed with *pHAE:HAE-YFP* showing rescue of the *hae hsl2* pectin breakdown deficiency in LRP overlaying cells. (*Left*) 2D confocal image of PI stain merged with image of root; (*Right*) inverted PI stain. (*B*) Rescue of the *hae hsl2* abscission deficiency by the translational fusion construct *pHAE:HAE-YFP*. (*C*) PI-stained roots at the site of stage I LRP (C24 ecotype). (*Left*) 2D confocal image of WT; (*Center*) 3D confocal image of PI stained *ida-1 mutant* merged with image of root (LRP colored yellow); (*Right*) 3D confocal image of PI stained *ida-1 mutant* complemented with the WT IDA gene (C24) merged with image of root (LRP colored yellow). Note that PI does not penetrate the EN or the LRP and therefore does not stain the inner EN wall and LRP cells walls. (Scale bars: 30 μM in 3D images, 20 μM in 2D images.)
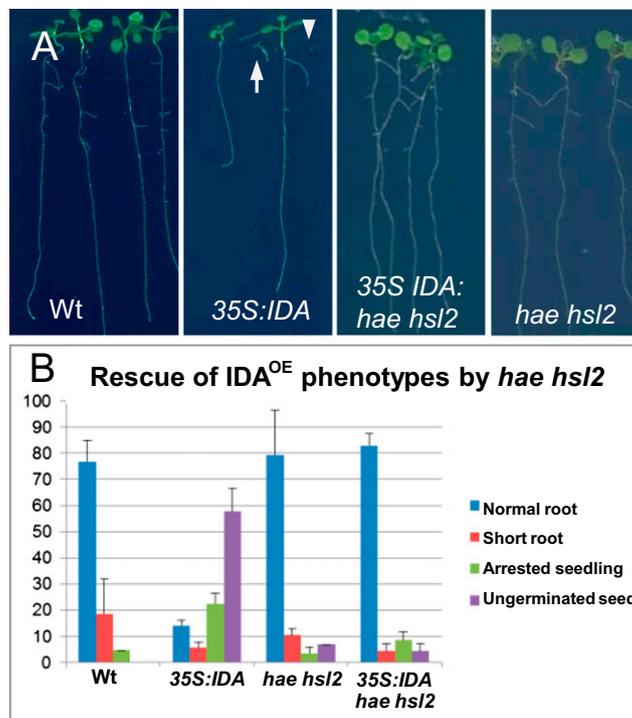
Fig. S6. *IDA* and *HAE/HSL2* regulate the expression of cell wall remodeling genes. (*A*) Expression levels of *XTR6* and *EXP17* in mutant 7-d-old roots relative to WT determined by quantitative RT-PCR. Bars indicate SD. *Significant deviation from WT ($P < 0.05$). (*B*) Expression pattern of *pXTR6:GUS* at sites of LR development and in floral organ abscission zones in WT, *ida*, and *hae hsl2* background, as indicated. Note EP cell (arrows) covering the mutants at stage VII LRPs. (*C*) Expression pattern of *pPGLR:GUS* at sites of LR development and in floral organ abscission zones in WT, *ida*, and *hae hsl2* background, as indicated. (*D*) *pPGAZAT:GUS* expression pattern in WT floral organ abscission zones in WT and *hae hsl2* mutant inflorescences.

Fig. S7. IDA overexpression phenotype is rescued by receptor gene mutations. (*A*) Root phenotypes of WT and *hae hsl2* plants confirmed to overexpress *IDA* [*35S:IDA*, (1)] as indicated, compared with *35S:IDA* and *hae hsl2*. Note four different phenotypes for *35S:IDA*, including arrested seedlings (arrow) and un-germinated seeds (arrowhead). (*B*) Percentage of the different phenotypes seen in the indicated genotypes. *n* = 24–42.

1. Stenvik G-E, et al. (2008) The EPIP peptide of INFLORESCENCE DEFICIENT IN ABSCISSION is sufficient to induce abscission in *Arabidopsis* through the receptor-like kinases HAESA and HAESA-LIKE2. *Plant Cell* 20(7):1805–1817.

Table S1. Primers

Primer name	Primer from 5′ to 3′	Note*
pHAE attB1	TAACAGAAAGGATTAAAACTAAAACTC	attB1
pHAE attB2	TTTTTTTTTGGAAAAGGAATCGTTATT	attB2
pHSL2 attB1	ACTAAATTCATATGAGATTCAATAT	attB1
pHSL2 attB2	CTACGTGTTGGGAAGAGAGGTATGAAAC	attB2
pIDA attB1	TTTTCAATTTTGTTATTGCATC	attB1
pIDA attB2	TTGGTAGTCAATGTTTTTTTTC	attB2
IDA attB1	GTAGCGGCTGCAAGAATTGGAGCCACCAT	attB1
IDA attB2	TCAATGAGGAAGAGAGTTAACAAAA	attB2
At1g04850 F	AGTGGAGAGGCTGCAGAAGA	
At1g04850 R	CTCGGGTAGCACGAGCTTTA	
At5g18800 F	GAAGTGTCTCGACAAAGGTCGT	
At5g18800 R	CCTTTTGGCACTTCTGGTG	
XTR6 F	GTCTCGTCAAAACCGACTGG	
XTR6 R	ACGCAAGCTTCTTCGTTGA	
PGAZAT F	GCAATTCTATTCTCACCGTTCA	
PGAZAT R	CGCCTCCATCGACTGATAG	
EXP17 F	CACCGAGACCTCATTTCGAC	
EXP17 R	GGGACGATTCCAGCTTTGTA	
PGLR F	CATCGATGGACGAGGATCA	
PGLR R	CCTCAAAGCTGTTGGTTTGG	
qIDA F	GGTTACTTACCTAAAGGCGTTCC	
qIDA R	AACAAAAGAGTTGTGTCTCTTAGAAGG	
HAE F	CCCGACAACTTGAATCTGTTAAA	
HAE R	TCTCCGGTAAAGGACCTT	
HSL2 F	AAAACCGAAACGGACCAAC	
HSL2 R	TTGCGGGTATATGTCTTCCTC	

*Primers used for Gateway cloning have additionally the given attB1 or attB2 sequences in the 5′ end.