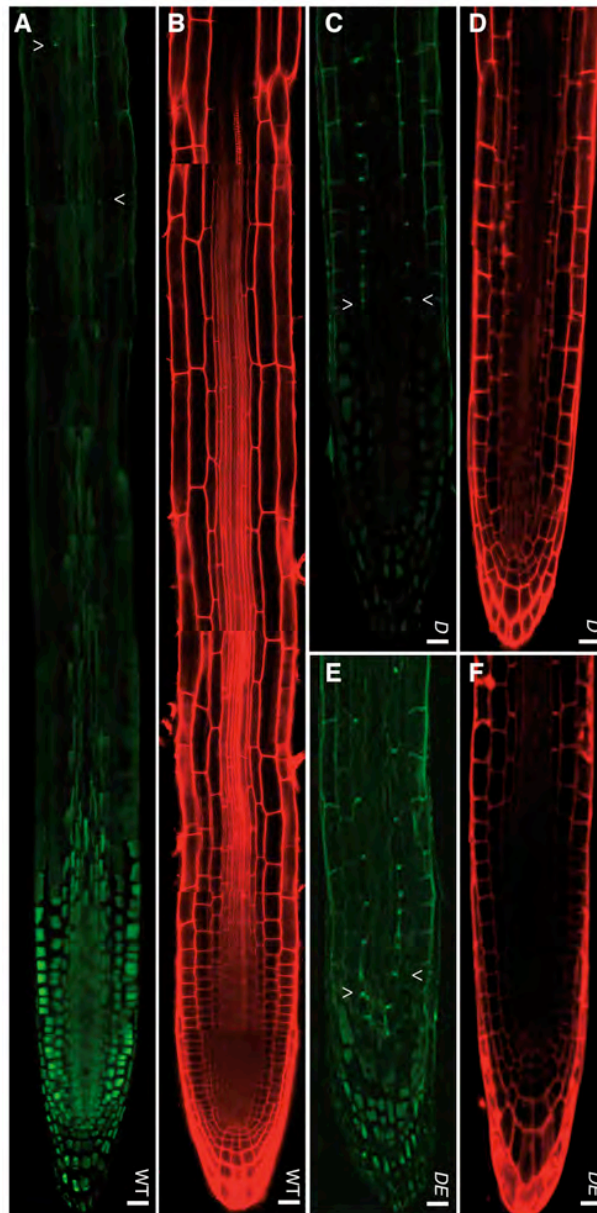
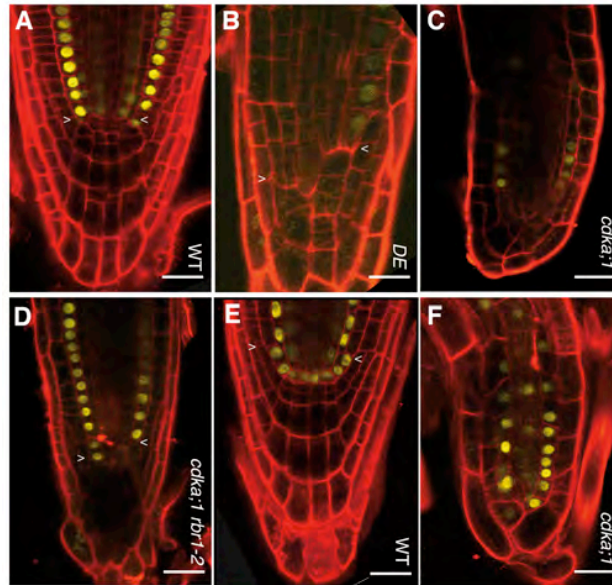


**SUPPLEMENTAL FIGURES AND TABLES**



**Supplemental Figure 1.** Casparian strip formation is not affected in weak *CDKA;1* alleles.

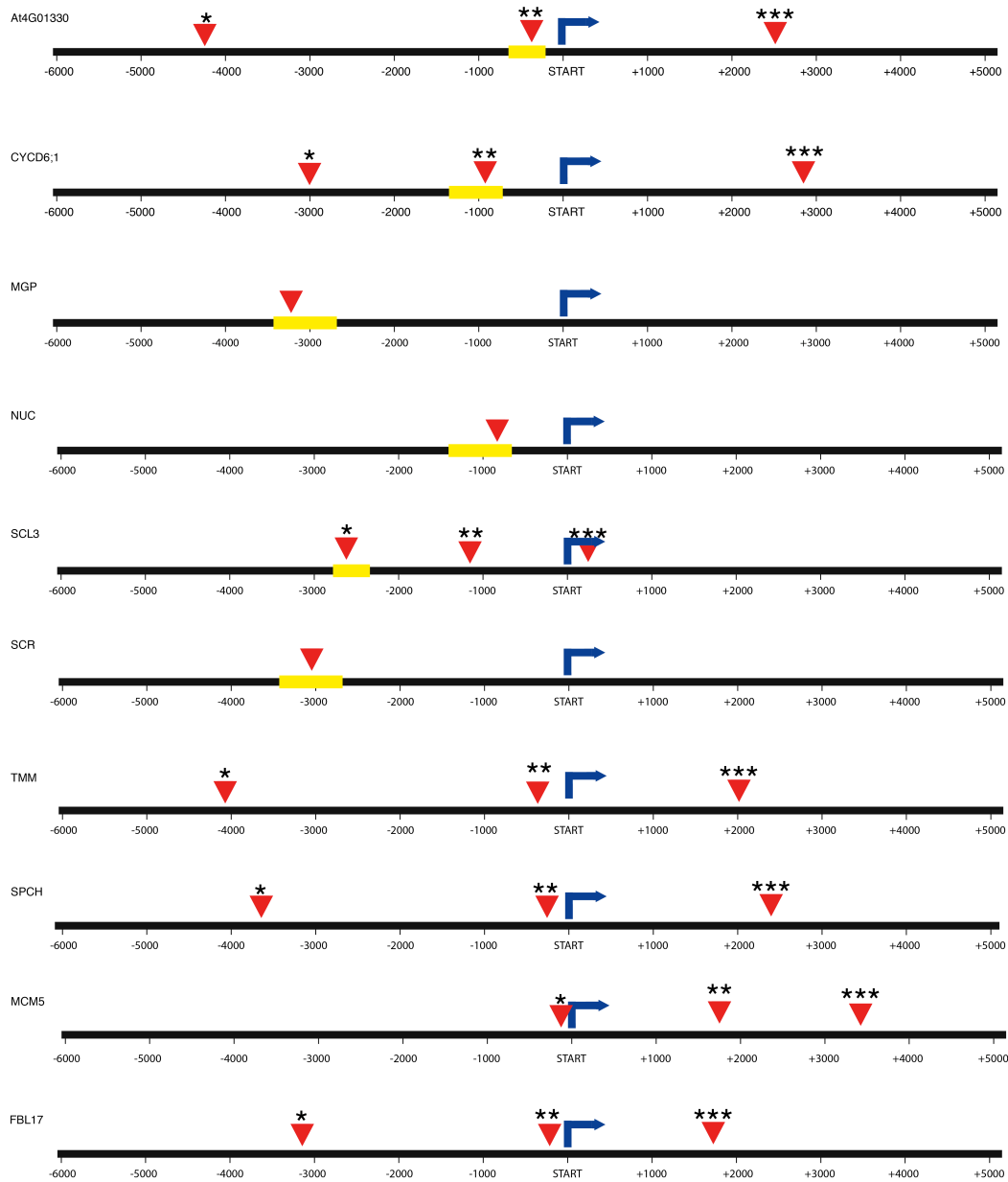
(**A**) Formation of the Casparian strip is the hallmark of endodermis differentiation in the wild type, as seen by autofluorescence (arrowheads). (**B**) After formation of the Casparian strip, propidium iodide (PI) cannot diffuse into the stele of the root. (**C-F**) Casparian strip formation is not affected in the weak loss-of-function *cdka;1* alleles *D* (**C** and **D**) and *DE* (**E** and **F**), as judged by autofluorescence and propidium iodide exclusion of the stele. All scale bars: 20  $\mu$ m.



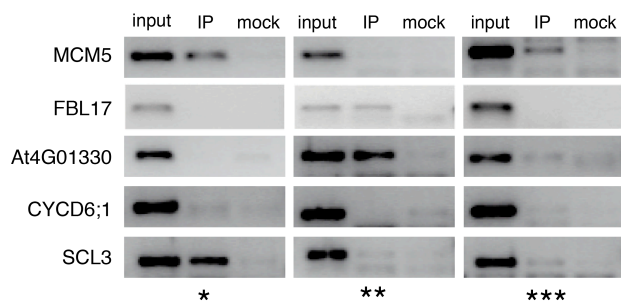
**Supplemental Figure 2.** Cortex and endodermis differentiation.

(A) Expression of the *ProCO2:H2B:YFP* construct labels the cortex of wild-type plants. (B) A weak but correctly positioned CO<sub>2</sub> pattern is found in the weak loss-of-function *cdka;1* mutant *DE*. (C) CO<sub>2</sub> activity is weak in the *cdka;1* null mutants and often appears to label a single file between the epidermis and the stele, i.e., fails to separate the endodermis and cortex cell files. (D) In the double mutant *cdka;1 rbr1-2*, strong activity of the CO<sub>2</sub> promoter is restored and labels a clearly visible cortex layer. (E) *ProSCR:SCR:YFP* marks the initial, initial daughter and the endodermis layer in the wild type. (F) The subepidermal layer that is the cortex in wild-type plants occasionally shows SCR expression in homozygous *cdka;1* null mutants, indicating that the asymmetric division of an initial daughter into an endodermis and cortex file failed. All scale bars: 20 μm.

A

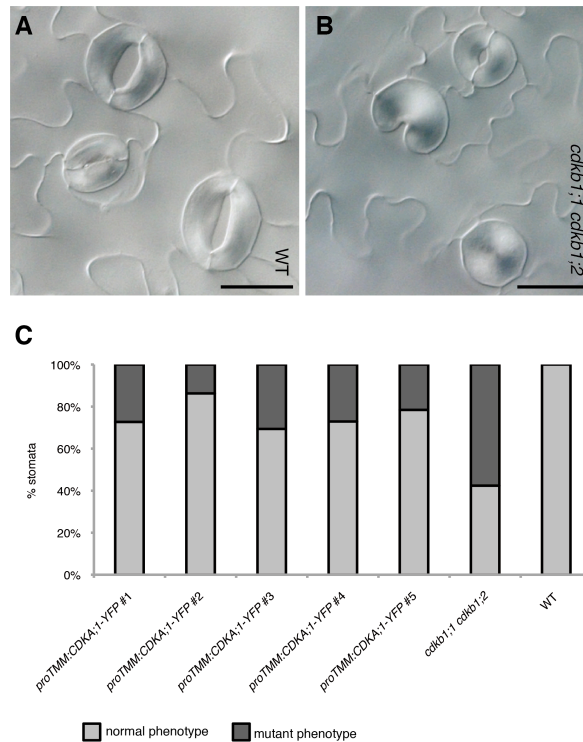


B



**Supplemental Figure 3. RBR ChIP.**

(A) Upper panel, gene structure of genes tested by ChIP with an antibody against RFP in *ProRBRI:RBRI:RFP* plants. Red arrowheads indicate the primer binding sites for PCR (the three fragments are tested indicated with one to three asterisks) and (B) qPCR after ChIP. Yellow boxes mark the SHR binding region as identified by (Sozzani et al., 2010).



**Supplemental Figure 4. *CDKA;1* expression partially rescues *cdkb1;1 cdkb1;2* stomata defects.**

(A) Mature stomata on wild-type leaves are formed by two guard cells. (B) In the *cdkb1;1 cdkb1;2* double mutant, arrested GMCs in the shape of one guard cell (middle), and circular arrested GMCs can be found besides regular stomata comprised of two guard cells (top). (C) Expression of *CDKA;1* under the control of the *TMM* promoter can partially rescue the mutant stomata phenotype in *cdkb1;1 cdkb1;2*. Scale bars: 25  $\mu$ m.

## Supplemental Tables

### Supplemental Table 1: Time frame of cortex-endodermis initial daughter

#### division

	division of cortex-endodermis initial daughter cell in %		N
	immediately	delayed	
WT	93	7	30
<i>rbr1-2</i>	71	29	92
<i>DE</i>	7	93	18
<i>D</i>	33	67	24
<i>DE rbr1-2</i>	18	82	28
<i>cdkb1;1 cdkb1;2</i>	83	17	36
<i>cycd6;1</i>	80	20	20
<i>D cycd6;1</i>	35	65	17
<i>cdkb1;1 cdkb1;2 cycd6;1</i>	74	26	43

### Supplemental Table 2: Stomata density in *cdka;1* mutants

	number of stomata per mm <sup>2</sup>	% of single GC	N
WT	130.4 ± 6.0	0	614
<i>D</i>	45.0 ± 4.5	0	106
<i>DE</i>	25.3 ± 9.6	4	124
<i>cdka;1</i>	0.0 ± 0.0	0	0*
<i>cdka;1 rbr1-2</i>	56.5 ± 31.2	0	266
<i>cdka;1 ProCDKA;1:CDKB1;1</i>	4.5 ± 1.3	0	21

\* 25 leaves analyzed

**Supplemental Table 3: Oligonucleotids**

## PRIMER SEQUENCES FOR GENOTYPING

<b>T-DNA insertion</b>	<b>primer</b>	<b>sequence 5' &gt; 3'</b>
<i>cdka;1-1</i>	A01	GCGTGGACCGCTTGCTGCAACTCTCTCAGG
	A02	CCAGATTCTCCGTGGAATTGCG
<i>rbr1-2</i>	M206	CTTCCACAGCCCGGTCGTTTC
	J504	GCGTGGACCGCTTGCTGCAACTCTCTCAGG
<i>cdkb1;1-1</i>	A67	TGGTTCACGTAGTGGGCCATCGCCCTGATA
	A68	TGTCTTTGAGCAGCCATCTGTGTTG
<i>cdkb1;2-1</i>	A67	TGGTTCACGTAGTGGGCCATCGCCCTGATA
	A70	TTTTTGTACTCAGGGCCGGCTTTAC
<i>cycd6;1</i>	A220	AATTCGACGACCCATCTCTG
	A222	ATA TTG ACC ATC ATA CTC ATT GC
<b>wild type</b>	<b>primer</b>	<b>sequence 5' &gt; 3'</b>
CDKA;1	A03	CAGATCTCTTCCTGGTTATTCACA
	A04	TGTACAAGCGAATAAAGACATTTGA
RBR1-2	M206	CTTCCACAGCCCGGTCGTTTC
	M207	GATTACCGCAGCATTCTAGTTGAACGC
CDKB1;1	A265	GCTTACCAATTGAGAACAACACTGATTC
	A68	TGTCTTTGAGCAGCCATCTGTGTTG
CDKB1;2	A70	TTTTTGTACTCAGGGCCGGCTTTAC
	A71	GGTTCAAAACAAATTATCATCAACTAGG

CYCD6;1            A220    AATTCGACGACCCATCTCTG  
                          A221    CTGCAATCACCGATGGTTTA

## PRIMER SEQUENCES FOR CLONING IN PROTEIN WORK

<b>gene</b>	<b>primer</b>	<b>sequence 5' &gt; 3'</b>
CYCD6;1	dT-AP_M13	GTAAAACGACGGCCAGT
	CYCD6;1_s1	ATGGAGTTTCATCTTGAACATCCTC
	M13-forward	GTAAAACGACGGCCAGT
	CYCD6;1_as2	TTAGTAACGACGAGTACTAGTTTTCCTCC
	attB1Ad-CYCD6;1_s	AAAAAGCAGGCTTCATGGAGTTTCATCTTGA ACATCCTC
	attB2Ad-CYCD6;1_as	AGAAAGCTGGGTCTTAGTAACGACGAGTACT AGTTTTCCTCC
	attB1 adapter	GGGGACAAGTTTGTACAAAAAAGCAGGCT
	attB2 adapter	GGGGACCACTTTGTACAAGAAAGCTGGGT
D/DE in e.coli	ACYCDuetUP1	GGATCTCGACGCTCTCCCT
	ND35	CAGAGAGTAACAACCTCATGATCAAATGTCC TGACAGGGATAC
	ND34	GTATCCCTGTCAGGACATTTGATCATGAGGT TGTTACTCTCTG
	DuetDOWN1	GATTATGCGGCCGTGTACAA
	ND04	ATAAACCACACCCTCATCTCCTTCACCAAT
	ND03	ATTGGTGAAGGAGATGAGGGTGTGGTTTAT

## PRIMER SEQUENCES FOR ChiP qPCR

<b>gene</b>	<b>primer</b>	<b>location of fragment</b>	<b>sequence 5' &gt; 3'</b>
At4g01330	At4g01330_2F	upstream of START	CCCCAACACCGTTATCTCTC
	At4g01330_2R	upstream of START	GAGTGTGTGTGCTTGGGATG
CYCD6;1	CYCD6_2F	upstream of START	TGGACGAGATTCCAAAGTGA
	CYCD6_2R	upstream of START	GGCTGGGGAGATTAAATATGA
MGP	MGP_1F	upstream of START	CGGAAAAGGTAAGGTGGTTG
	MGP_1R	upstream of START	TCGGACTTGACCAATCCAAT
NUC	NUC_2F	upstream of START	GAGGAAAGGGCAACACAAAA
	NUC_2R	upstream of START	CAAATTCGAAGCGAGCTGTT
SCL3	SCL3_2F	upstream of START	CGTACCGGCTCTCTTCGATA
	SCL3_2R	upstream of START	GCATCGGTCATCGTCTCTCT
SCR	SCR_2F	upstream of START	AGTTGGTGCCCCATCTTAGT
	SCR_2R	upstream of START	TCATTATGTGAAATGAATGGGTTT
MCM5	MCM5_4F	upstream of START	TCCCGCCAAAACATCATAGTC
	MCM5_4R	upstream of START	TGACATCGTTGCTTCGTCTC
PCNA1	PCNA1_F	upstream of START	TCTTAAAACGATTGAGGCCG
	PCNA1_R	upstream of START	AATCGTTTGCGGCTATTTTG
RB32,5	LB32,5-F	upstream of START	CGAACACACGGATATGTTGC
	LB32,5-R	upstream of START	TGGTGATGTACTCGCTGTCAA
RB45	RB45-F	upstream of START	GCGGAACCAATTATAGATGAGG
	RB45-R	upstream of START	CGTCAACAGCTCCAAATCAC



SPCH	SPCHF1	upstream of START	ATCCTCCCCCAAATTCATC
	SPCHR1	upstream of START	ATGAGGGACTCGCATTCATC

## PRIMER SEQUENCES FOR ChIP-PCR

<b>gene</b>	<b>primer</b>	<b>location of fragment</b>	<b>sequence 5' &gt; 3'</b>
At4g01330	AT4GUF1	upstream of START	GTTCGTGACTGCAACTAGAG
	AT4GUR1	upstream of START	ACATTGGAGCACTGAAAGAG
	AT4GDF2	downstream of START	TTCTCATCCTTTCGTAACTC
	AT4GDR2	downstream of START	ACATTCAGGAAGGTTAGGAC
CYCD6;1	CYCD6;1UF1	downstream of START	GAGACTTGGTCATGGTATGG
	CYCD6;1UR1	downstream of START	GGGAAATACATCAAACATGG
	CYCD6;1DF1	upstream of START	GGTCATCTTATAGCCACAAG
	CYCD6;1DR1	upstream of START	AGTGATTAGAATCGAGCAAC
SPCH	spchluf1	downstream of START	AGTAAACATGACGATGGCTG
	spchlur1	downstream of START	TACTCACTTTCTCTCCTTAG
	spchldf2	upstream of START	GCAAACACATATAGCGCATC
	spchldr2	upstream of START	AACAGGTGATAACGAACGCTC
MCM5	MCM5UF1	downstream of START	ACATCATCTGGTGTGGCCTC
	MCM5UR1	downstream of START	ACAGTGAGACAACTCGAAGC
	MCM5UF2	downstream of START	GATTTTGCAGTTGATGGGTC
	MCM5UR2	downstream of START	TTCTGTGCACTTTGTATACG
SCL3	SCL3DF1	upstream of START	GTTTGGACGTTTCCTTCTTC

	SCL3DR1	upstream of START	CATGGCATGAGGTGGATTTG
	SCL3DF2	downstream of START	CTCTCACCTCGCTTCTCCTG
	SCL3DR2	downstream of START	GAGTTGCGTTAAGAGCCTTG
TMM	TMMUF2	downstream of START	CGAGGACACATTTACTTGAG
	TMMUR2	downstream of START	CTCAGTAAAGCACAAAGACAG
	TMMDF2	upstream of START	GGAAAAAGGTAACCTGACTC
	TMMDR2	upstream of START	GATTTTGGGTTTGTTGAGAG
	TMMF1	upstream of START	CAGTGCCCAGTTCAAATAC
	TMMF2	upstream of START	AGATATTCCTTCATTCGTC
FBL17	FBL17U1_1	upstream of START	TTCTGATTGCAGTTGGTGGA
	FBL17U1_2	upstream of START	CGGCATCAGAATCAATAGCA
	FBL17_1	upstream of START	GCTAGACCTCACGCTCTTTC
	FBL17_2	upstream of START	GAGATTTGTGAGATTGGGAG
	FBL17D4_1	downstream of START	TGCTCTCGCTAGTTCTTGGA
	FBL17D4_2	downstream of START	ACCTGAGGAAATGGCAGCTA