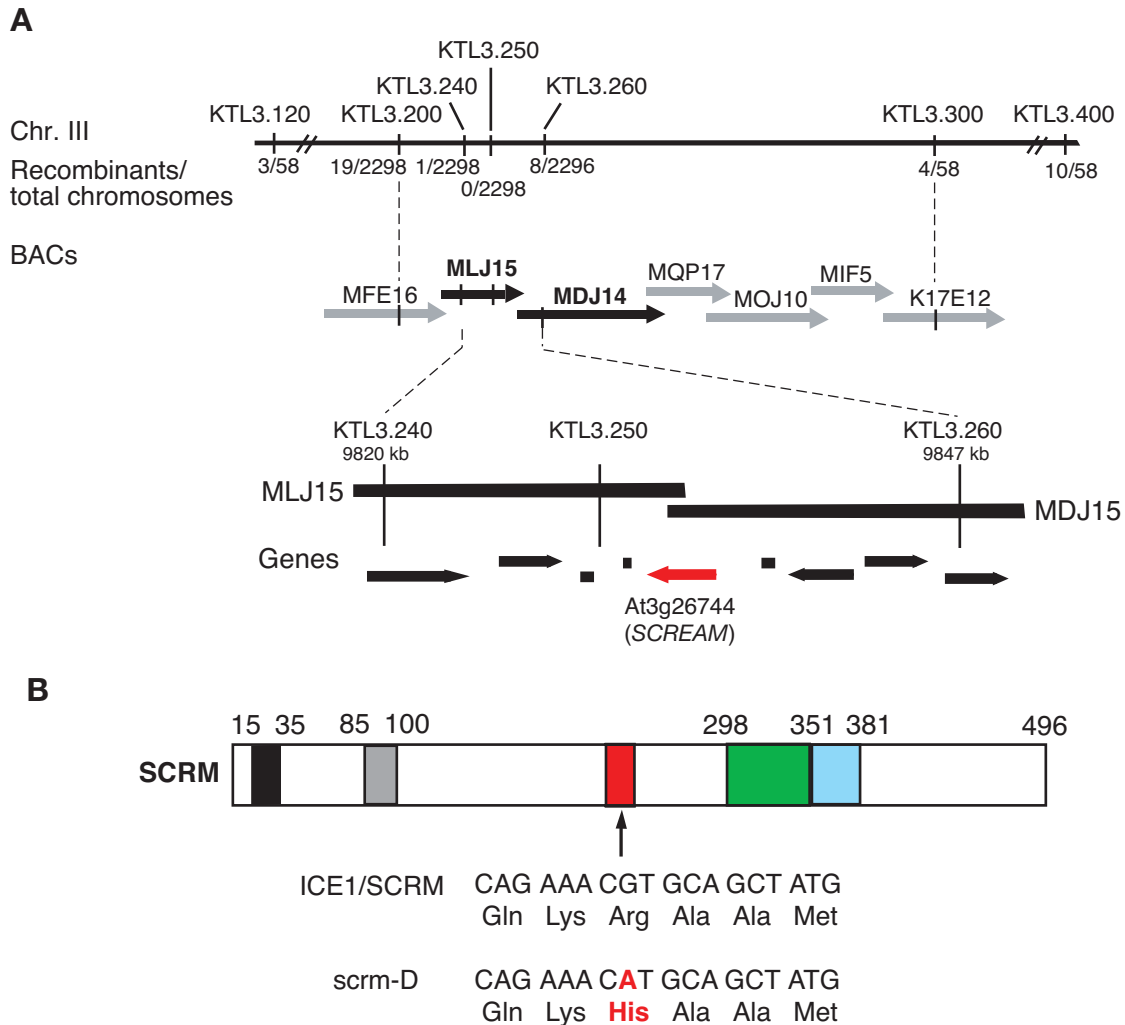


Kanaoka, Pillitteri, Fujii, Yoshida, Bogenschutz, Takabayashi, Zhu, and Torii (2008)  
SCRM/ICE1 and SCRM2 specify three cell-state transitional steps leading to Arabidopsis stomatal differentiation

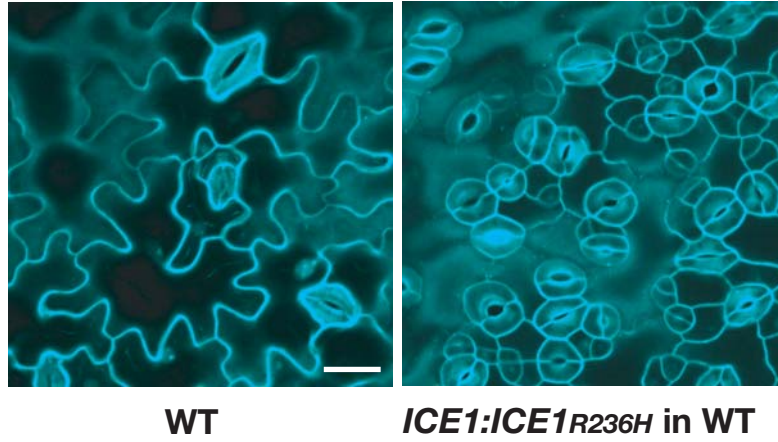


**Supplemental Fig. 1.** Seedling phenotypes of *scrm-D/+* and *scrm-D*. Shown are ten-day-old seedlings of wild type (WT: left), *scrm-D/+* (middle), and *scrm-D* (right). Compared to the wild type, cotyledons and rosette leaves of *scrm-D/+* and *scrm-D* are wrinkled and disintegrated. *scrm-D/+* rosette leaves produce reduced numbers of trichomes, and *scrm-D* does not produce any trichomes: excessive stomatal differentiation occurs in the expense of trichome development. Images are taken under the same magnifications. Scale bar, 2 mm.



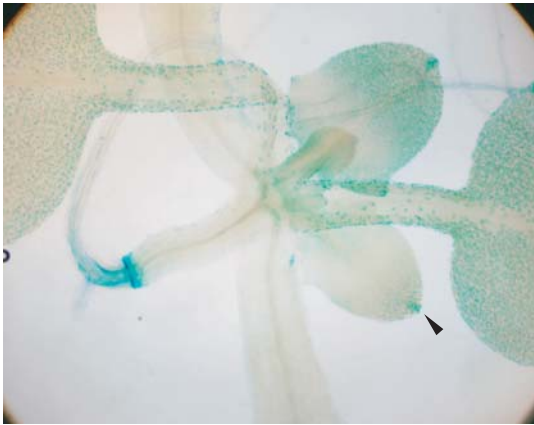
### Supplemental Figure 2. Map-based cloning of *SCRM*

(A) Physical mapping. The locations of molecular markers (SSLP markers, KTL3.120, KTL3.200, KTL3.250, KTL3.300 and KTL3.400; CAPS markers, KTL3.230 and 3.260), the number of recombinants and chromosomes, and the corresponding BAC clone are indicated. Open reading frames within the 27 kb interval are shown at the bottom. *SCRM* encodes At3g26744 (red). (B) Domain structure of *SCRM* protein and the point mutation in *scrm-D*. An acidic domain (black), a serine-rich domain (gray), a KRAAM motif (red), a bHLH domain (green) and a zipper region (light blue) are shown. The arginine at amino-acid 236 is replaced with histidine in *scrm-D* mutant (arrow).

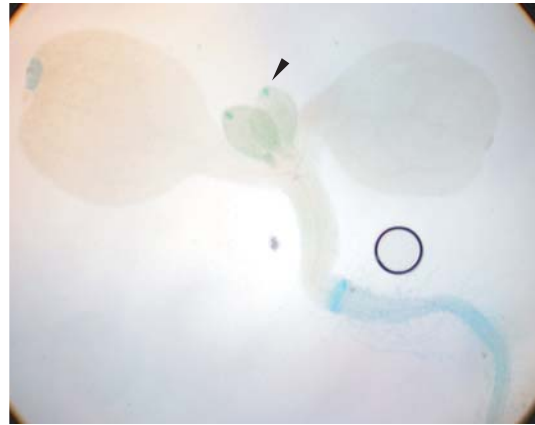


**Supplemental Figure 3.** Recapitulation of *scrm-D* phenotype by introduction of *ICE1:CE1<sub>R236H</sub>* to wild-type plants.

(Left) Abaxial rosette leaf epidermis of wild-type (WT). (Right) Abaxial rosette leaf epidermis of a transgenic plant expressing *ICE1:ICE1<sub>R236H</sub>*. Images are taken under the same magnification. Scale bar, 20  $\mu\text{m}$ .

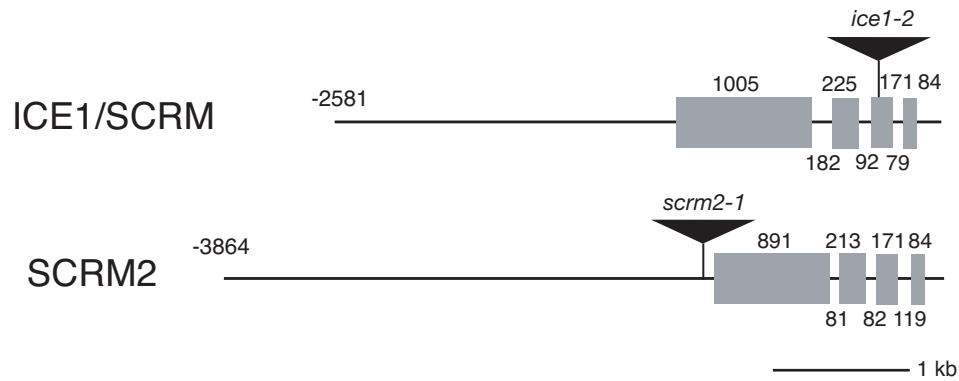


***ICE1:GUS* in WT**



***ICE1:GUS* in *spch***

**Supplemental Figure 4** Effects of *spch* on *ICE1:GUS* expression in non-stomatal cells/tissues. (Left) *ICE1:GUS* in wild-type seedling. (Right) *ICE1:GUS* in *spch* seedling. While *ICE1:GUS* activity in leaf epidermis is abolished, it is detected in roots and hydathodes (arrowhead) in the *spch* mutant seedling.

**A**

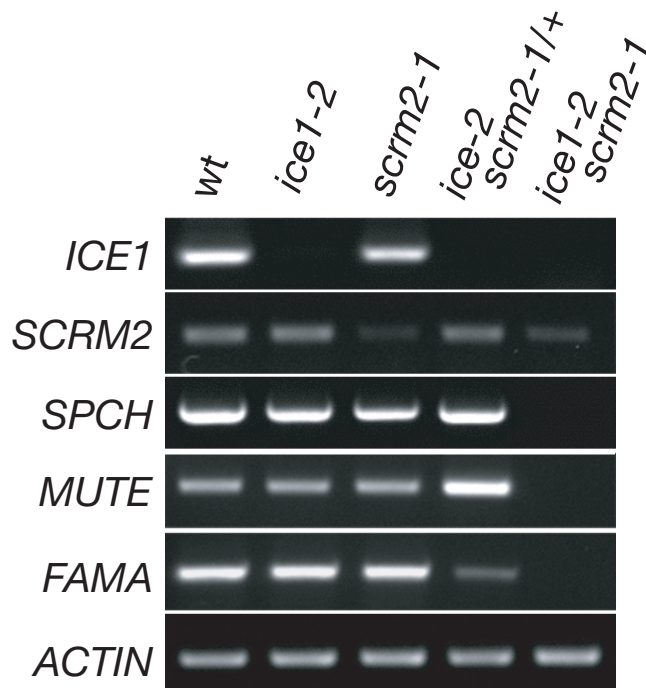
**Supplemental Figure 5.** Structure of *ICE1* and *SCRM2* genes, and *SCRM2* mRNA and amino-acid sequence. (A) Structure of *ICE1* and *SCRM2* genes. Lines, non-coding regions (5' promoter region and introns); Grey boxes, exons. The size of each exon and intron is indicated. The locations of T-DNA insertions in *ice1-2* and *scrm2-1* are indicated. Next page: (B) Full-length cDNA and amino-acid sequence of *SCRM2*. The major (black) and minor (grey) transcripts start at 71 and 135 bp upstream of a translation initiation site (bold), respectively. The 5' end of *SCRM2* mRNA was determined by 5' RACE PCR. (C) Nucleotide sequence of the 5' end of *scrm2-1* mRNA and a potential open reading frame. The *scrm2-1* mRNA contains a chimeric 5' untranslated region (UTR), with an upstream, 136 bp sequence derived from T-DNA (red) preceding the 49 bp *SCRM2* 5' UTR (black). The T-DNA-derived transcripts codes for a short open-reading frame (red).

# B

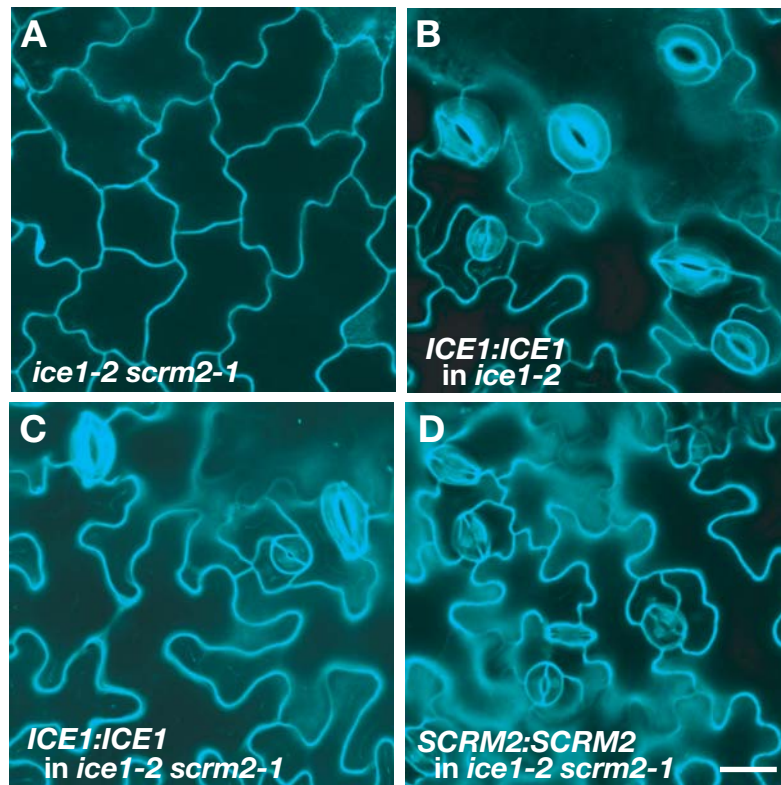
-135 ACGCCTCCTTTCAAG  
AAAAATCTCTCTCTATATTATCTACGTTTCTCTCGCCTTGTCTCCTTTT **GAGGTTCCATT** (-71)  
TCTACTTCTCTCCAAAATTCGAAACTTTTCCACTCTCTCTCTCTCTACTAGTTTGAC  
+1 **ATGAACAGCGACGGTGTGGCTTGACGGCTCCGGTGAATCTCCGGAAGTTAATAACGGT** +20  
M N S D G V W L D G S G E S P E V N N G  
GAAGCTGCGTCTTGGGTGAGAAACCCAGATGAAGACTGGTTCAATAACCCACCACCACCA  
E A A S W V R N P D E D W F N N P P P  
+121 CAACACACTAATCAAAAACGACTTCAGATTCAATGGTGGCTTTCCTTTAAACCCCTCAGAG +60  
Q H T N Q N D F R F N G G F P L N P S E  
AATCTGCTTCTTCTTCTCAGCAATCGATTGATTCTTCTTCTTCTTCTTCTCCGTTATTA  
N L L L L L Q Q S I D S S S S S P L L  
+241 CATCCTTTACACTCGACGCTGCTTCACAGCAACAACAACAACAACAACAACAGGAA +100  
H P F T L D A A S Q Q Q Q Q Q Q Q Q E  
CAGTCTTTCTTAGCTACGAAAGCTTGTATAGTTTCTTCTCAACGTCCCAACCATCAAT  
Q S F L A T K A C I V S L L N V P T I N  
+361 AACAACACTTTTCGATGACTTCGGCTTTGACTCTGGTTTCTTAGGACAACAATCCATGGA +140  
N N T F D D F G F D S G F L G Q Q F H G  
AATCATCAATCTCCGAACTCGATGAATTTCACTGGCTTAAACCACTCAGTACCGGATTTT  
N H Q S P N S M N F T G L N H S V P D F  
+481 CTTCCAGCTCCGAAAAACAGCTCAGGATCATGTGGATTGAGTCTCTGTTCTCAAACAGA +180  
L P A P E N S S G S C G L S P L F S N R  
GCAAAGGTTTTAAAACCGTTACAGGTAATGGCTTCATCTGGCTCGCAGCCAACCTCTGTTT  
A K V L K P L Q V M A S S G S Q P T L F  
+601 CAGAAACGAGCTGCAATGCGTTCAGAGCTCGAGTAGCAAAATGTGCAATTCTGAGAGTTCT +220  
Q K R A A M R Q S S S S K M C N S E S S  
TCTGAAATGAGGAAATCGAGCTACGAGAGAGATTGACGATACTAGTACCGGAATCATC  
S E M R K S S Y E R E I D D T S T G I I  
+721 GATATCTCTGGATTGAATTACGAATCTGATGACCATAATACTAATAACAACAAGGTAAG +260  
D I S G L N Y E S D D H N T N N N K G K  
AAGAAAGGAATGCCTGCAAAGAATCTTATGGCTGAGAGAAGAAGAAGGAAGAAGCTTAAT  
K K G M P A K N L M A E R R R R K K L N  
+841 GATAGGCTTTACATGCTTAGATCAGTTGTTCCCAAGATCAGCAAAATGGATAGAGCATCA +300  
D R L Y M L R S V V P K I S K M D R A S  
ATACTGGAGATGCTATTGATTACCTCAAAGAGCTTTTCAAAGAATCAACGATCTTCAC  
I L T G D A I D Y L K E L L Q R I N D L H  
+961 ACCGAACTTGAATCTACTCCACCGAGTTCTTCAAGCTTGCATCCGTTAACACCGACTCCA +340  
T E L E S T P P S S S S L H P L T P T P  
CAAACGCTGTCTTACCGTGTTAAGGAAGAGTTGTGTCCATCTTCTCCTTGCCAAGTCTCT  
Q T L S Y R V K E E L C P S S L P S P  
+1081 AAAGCCAAACAACCAAGAGTTGAGGTTAGATTAAGAGAAGGAAAGGCAGTGAACATACAC +380  
K G Q Q P R V E V R L R E G K A V N I H  
ATGTTCTGTGGACGTAGACCAGGCTTTTACTTTCCACCATGAGAGCTTTGGATAACTTA  
M F C G R R P G L L L S T M R A L D N L  
+1201 GGATTGGATGTTCAACAAGCGGTGATTAGCTGTTTCAATGGTTTTGCTTTGGATGTTTTTC +420  
G L D V Q Q A V I S C F N G F A L D V F  
CGCGCTGAGCAATGTCAAGAAGACCATGACGTGTACCTGAACAAATCAAAGCAGTGCTT  
R A E Q C Q E D H D V L P E Q I K A V L  
+1321 TTAGATACAGCAGGTTACGCTGGTTTTGGTTTTGA +450  
L D T A G Y A G L V \*

# C

ACCAA  
TACATTACACTAGCATCTGAATTTTATAACCAATCTCGATACACCAAATCGAATTC AATT  
CGGCGTTAATTCAGTACATTA AAAACGTC CGCAATGTGTTATTAAGTTGTCTAAGCGTCA  
-49 M C Y \*  
+1 **ATTTGTTTAC** ACCAAAATTCGAAACTTTTCCACTCTCTCTCTCTCTACTAGTTTGAC  
**ATGAACAGCGACGGTGTGGCTTGACGGCTCCGGTGAATCTCCGGAAGTTAATAACGGT**  
M N S D G V W L D G S G E S P E V N N G



**Supplemental Figure 6.** RT-PCR analysis of *ICE1*, *SCRM2*, *SPCH*, *MUTE*, and *FAMA* transcript accumulation in wild-type (wt), *ice1-2*, *scrm2-1*, *ice1-2 scrm2-1/+*, and *ice1-2 scrm2-1* seedlings. Actin serves as a control. No *SPCH*, *MUTE*, or *FAMA* transcripts were detected in *ice1-2 scrm2-1* double mutant plants, consistent with the complete absence of stomatal cell lineage. In *ice1-2 scrm2-1/+*, *FAMA* transcripts were negligible in accordance with the absence of GMC formation. *MUTE* transcripts accumulated to higher levels in *ice1-2 scrm2-1/+*, which may reflect the increased numbers of meristemoids due to developmental arrest. *ice1-2* single mutation did not lead to significant change in transcript accumulation of other stomatal bHLH genes, consistent with the fact that only a small fraction of stomatal precursors form abnormal tumors.



**Supplemental Figure 7.** Complementation of *ice1 scrm2* loss-of-function mutants by *ICE1* and *SCR2*. Shown are abaxial epidermis of rosette leaves. (A) *ice1-2 scrm2-1*. The complete loss of *ICE1* and *SCR2* results in the epidermis solely composed of pavement cells. (B) Transgenic *ice1-2* plant expressing pMK157, a full-length *ICE1* cDNA driven by its native promoter (*ICE1:ICE1*). *ICE1* is sufficient to rescue the ‘caterpillar-like’ tumors occasionally formed in *ice1-2*. (C) Transgenic *ice1-2 scrm2-1* plant expressing pMK157. Introduction of *ICE1* rescues the pavement cell-only phenotype in *ice1-2 scrm2-1*. (D) Transgenic *ice1-2 scrm2-1* plant expressing pMK181, a full-length *SCR2* cDNA driven by its native promoter (*SCR2:SCR2*). Introduction of *SCR2* rescues the pavement cell-only phenotype in *ice1-2 scrm2-1*. Scale bar, 20  $\mu$ m.



**Supplemental Table 1: List of primers and their DNA sequence used for genotype analysis**

Mutant name	Type	Restriction en	Primer name	Primer sequence
tmm	dCAPS	Apo1 cuts mutant	TMM 960 dCAPS	AACGCGTTCAAAGGGCTCAAGAAATT
			TMM 1254rc	GAACCGAATGCATCATCCAAGTCACT
mute	dCAPS	BslI cuts WT	MUTE dCAPs F	TTCGTTCTTTGACTCCTTGTTTCTACCTCAAAG
			MUTE dCAPs R	CTTCGAGAAAATAATTAGGATTGTGAATTGAG
spch	T-DNA PCR		SAIL_LB3	TAGCATCTGAATTTTCATAACCAATCTCGATACAC
fama	T-DNA PCR		53210 1281.rc	AACCTGAAGAATCTCAAGAGCC
			SALK_LBa1	TGGTTCACGTAGTGGGCCATCG
scrm-D	CAPS	Nla3	FAMA -609	ATGTGTACCATTACACCC
			SCRMg429F	CAAATCCATGCTCCTATTTTCGATGG
ice1-2	T-DNA PCR		Chr3-9835011F	GTAAGTGTTTACTTTGCTGATCTTGG
			SALK_003155RP	TGAGGAAGAGGCTCGTGATAG
scrm2-1	T-DNA PCR		SALK_LBa1	TGGTTCACGTAGTGGGCCATCG
			SAIL808B10RP	TAACTTCCGGAGATTCACCG
			SAIL_LB1	GCCTTTTCAGAAATGGATAAATAGCCTTGCTTCC

**Supplemental Table 2: List of primers and their DNA sequence used for map-based cloning of *SCRM***

Marker name	Chrc	Location (k	Type	Restrictic Col bands (t	Ler bands (t	Forward primer	Reverse primer
KTL1.100	1	3200	SSLP		630	460 AAGACTCCATCTTGACACTGTTATGC	CTCAGAAAGGAAAAACGCACAGTAGC
KTL1.120	1	11000	SSLP		820	600 CTTTACCCCTCCACGATTTTTAAGCAATGG	GAGCAAAAATTAACATCAGTGACATGACC
KTL1.130	1	23000	SSLP		780	500 GACAACAAAACCCCTGTTGTTCTGAGC	TCCTCTAGTCATTTCTCATAAGATCCAC
MSAT2.36	2	8827	SSLP		158	200 GATCTGCCTCTTGATCAGC	CCAAGAAGCTCAAAACCGTT
MSAT2.9	2	18295	SSLP		173	135 TAAAAGAGTCCCTCGTAAAG	GTTGTTGTTGTTGGCATT
KTL4.100	4	7200	SSLP		700	440 GACCAAGCTTCGTTATCGAAGATAACC	AAAGAGAAGCTCACCGGCATACC
KTL4.120	4	16800	SSLP		795	550 TAATTTGTCTCCCTGTGTTAACTTGC	GTAGGTAACGAGATCCAGATCTTCC
KTL5.100	5	7600	SSLP		815	435 GTTTTTATTGGTGGTGTGAGAGGAATGG	TATCTCTTGTGTTGTGAGTGTGTTGG
KTL5.120	5	16800	SSLP		830	430 TTTGGTGGACCATAGAGATTGATTGG	CTTTGTACTTTTACTCGGTTGATGACG
KTL5.130	5	25000	SSLP		750	530 ATACTGTGCGACTTCAGGTTCTACTCC	GCAAACCTATAATCTCAGCTTGGTTCAGG
KTL3.110	3	2400	SSLP		780	460 ACGTGCTCTCTGGTTCATCTATATATCC	AGTATTGAATCCGTCAAAAGCTGTGC
KTL3.120	3	9100	SSLP		670	510 ATGAGTTTAGATCATCAAGATCGGAGG	CCAACCGAAATGAACCAAAAACCATCG
KTL3.130	3	9250	SSLP		247	220 ATAGTTCTGGTTCACCTTACAGAACCC	CGCAACAATTACAGGTCATAAACC
KTL3.140	3	9400	SSLP		1197	409 GCTTTTTATGTTTCATAAGACTTGAAGC	GACTCAACGCAATTTGAGGTTTTCG
KTL3.150	3	9540	SSLP		240	199 TGGAAGGAGAAACATCTTTCTAGG	CCAGATGTGACCTTTTGAAGCTTTGC
KTL3.160	3	9570	SSLP		213	191 AGATTACACTTATTGTTGCCTTATCG	GTCAAAATAGCCAAAATATTCAAAAATAGG
KTL3.170	3	9620	SSLP		213	240 GGACAAAAGATTTCAGCAACCTTCG	TTAGCAGCATACGGAATTAGTTACG
KTL3.180	3	9640	SSLP		272	234 AACACAGAGCTTCTCAACTCTTTTCC	TCGTAGTTTCTCTCGGATACAGC
KTL3.190	3	9680	CAPS	Pst1	738	738 TCAGACTCACGAGAATACAAAAGTGC	AATTCGGATTTGGGAGTTCCTCTGC
KTL3.200	3	9705	SSLP		171	155 TTACAAAATCTACGCAACGCAATGG	AGGAGCAACTAGCGATGGTGG
KTL3.210	3	9776	CAPS	Mbo1	460 226, 234	TGTAAGTGTGAGCGATTAATGCTACC	GCAATCCCAAGCCATTTAAGTTTCC
KTL3.220	3	9786	SSLP		140	155 GACAGAGGTGATCAGCTGAACC	CACTTTGCCAATTCGTTATCTTTGG
KTL3.230	3	9791	CAPS	Xho1	933 590, 343	CTCTGCATTTTCGTTCCGATTGC	GAACCCGTTTGATCGATATGTATAGC
KTL3.240	3	9820	CAPS	Mfe1	598, 375	973 GCAACTTCTTACTGATACCAACG	ATGTCCATTTGTCTTCATGGTGG
KTL3.250	3	9830	SSLP		1102	414 TCTACAAGTCTTTGTAACCACTCC	ACAAAACGAACATTGCCAAAACG
KTL3.260	3	9847	CAPS	Nco1	179, 314	493 CTTGAATGAGTTATGGCATGGTCAGG	ACCTAGCCGGAATCATAAACCCTAGC
KTL3.270	3	9862	SSLP		1032	771 GTCACACTGGTAATTTGACAAAATCC	CAGCACTAAGAACTGAAAGAAGTGC
KTL3.280	3	9960	SSLP		158	144 CGAATGATGCTATATTGGTTAATTCTGG	TTATTAATGTCTCTTTCTATAGGTCTAAG
KTL3.290	3	10010	CAPS	Mbo1	240, 491	731 GCTGCATCTTCACGAAGAGTTTTGG	GTATTGGGAGGAGTCTAAACGTTGC
KTL3.300	3	10030	SSLP		243	221 GTTCCACTATATAACCACGCTCTCG	ACGAGATCATGATACGTGTCAGTG
KTL3.310	3	10160	SSLP		157	143 GTAGTTAATGAGCTTTAGGAGTGTG	AAGCTTTGATTAAGTAAATATGGTTTCATAC
KTL3.320	3	10270	CAPS	Xba1	1083 715, 368	TCATCTCTATGGATGAAACAATCC	GCATTGCATATTTGTACTTCTCTACC
KTL3.330	3	10300	SSLP		200	176 AATGTGGTGATGTAGCAACCACTGG	CTTTCTCTCACTCTCTTCTAAATGG
KTL3.340	3	10400	SSLP		251	232 AGCTGGAGGATTAGTCCGATGC	CGTTTACTGTTTACCTTTATCGTTTGC
KTL3.350	3	10700	SSLP		230	201 GACCATGAGATTGAAATAACTTACAGC	TTGGAATAATCTTGACAGTAAACAAGG
KTL3.360	3	11000	SSLP		885	343 AGATTTCCAAGCTGAGGTGACG	ACGATTGACTTGTACGTGTCACG
KTL3.370	3	11400	SSLP		872	573 CAGAGATAGGCCATTGTGACG	TTTTCGTTTTGTCGGGAGTTGAGG
KTL3.380	3	11800	SSLP		1564	422 GGATGAAGATTTGTTGACTCAAGG	GGTTTAACCAACGAGCTGAAAGC
KTL3.390	3	12300	SSLP		1280	415 TAACATTAGCAGCAACTGGACTTGG	AGCCGGTACAACAACCTAGAAAACG
KTL3.035	3	12560	SSLP		257	200 TGGTTGTTACAATTTGCACTC	GTGGAGAAGAACAAGAGTGTG
KTL3.400	3	17000	SSLP		930	530 CACCGAGTTTTGCTTTGTTTCTTTTGC	GTGAGATCCGACGAAGAGTTTACC

Supplemental Table S3: List of plasmids constructed in this study and their description

	plasmid ID	description	insert	vector	process	PCR primer forward1	PCR primer reverse1	PCR primer for PCR primer re	restriction enzymes
Yeast Two Hybrid	pMK151	ICE1-cDNA in pCR17	ICE1 cDNA	pGBKT7	PCR, ligation	SCR1M1-EcoRI/F	SCR1MendBanH1.c		EcoRI, BanH1
	pMK152	ICE1-cDNA in pGADT7	ICE1 cDNA	pGADT7	PCR, ligation	SCR1M1-EcoRI/F	SCR1MendBanH1.c		EcoRI, BanH1
	pMK153	scrm-D-cDNA in pGBKT7	scrm-D cDNA	pGBKT7	PCR, ligation	SCR1M1-EcoRI/F	SCR1MendBanH1.c		EcoRI, BanH1
	pMK154	scrm-D-cDNA in pGADT7	scrm-D cDNA	pGADT7	PCR, ligation	SCR1M1-EcoRI/F	SCR1MendBanH1.c		EcoRI, BanH1
	pNLB101	MUTE cDNA in pCR1-TOPO	MUTE cDNA	pCR1-TOPO	PCR, TOPO cloning	MUTE1-EcoRI linker	MUTE09 BanH1.c		EcoRI, BanH1
	pNLB103	SPCH cDNA in pCR1-TOPO	SPCH cDNA	pCR1-TOPO	PCR, TOPO cloning	53210_1-EcoRI linker	53210_1099 BanH1.c		EcoRI, BanH1
	pNLB104	FAMA cDNA in pCR1-TOPO	FAMA cDNA	pCR1-TOPO	PCR, TOPO cloning	FAMA1-EcoRI linker	FAMA_1244 BanH1.c		EcoRI, BanH1
	pNLB105	MUTE cDNA in pGBKT7	MUTE cDNA	pGBKT7	PCR, ligation				EcoRI, BanH1
	pNLB106	SPCH cDNA in pGBKT7	SPCH cDNA	pGBKT7	PCR, ligation				EcoRI, BanH1
	pNLB107	FAMA cDNA in pGBKT7	FAMA cDNA	pGBKT7	PCR, ligation				EcoRI, BanH1
	pNLB108	MUTE cDNA in pGADT7	MUTE cDNA	pGADT7	PCR, ligation				EcoRI, BanH1
pNLB109	SPCH cDNA in pGADT7	SPCH cDNA	pGADT7	PCR, ligation				EcoRI, BanH1	
pNLB110	FAMA cDNA in pGADT7	FAMA cDNA	pGADT7	PCR, ligation				EcoRI, BanH1	
ICE1	pMK142	ICE1 genomic in pENTR	ICE1 genomic	pENTR-TOPO	PCR, TOPO cloning	SCR1MendGW	SCR1MendSTOP.c		
	pMK143	ICE1 <sub>1-1000</sub> genomic in pENTR	ICE1 <sub>1-1000</sub> genomic	pENTR-TOPO	PCR, TOPO cloning	SCR1MendGW	SCR1MendSTOP.c		
	pMK144	ICE1 promoter in pCR2.1	ICE1 promoter	pCR2.1	PCR, TA cloning	SCR1MendGW	SCR1MendSTOP.c		
	pMK145	ICE1 <sub>1-1000</sub> -NotI in pMDC99	ICE1 <sub>1-1000</sub> -NotI	pMDC99	ligation	SCR1Mprom-25815B1F	SCR1Mprom-1Kp1R.c		
	pMK155	GFP-ICE1 in pENTR	GFP-ICE1	pMDC99	PCR, ligation	NotI/GFP.F	GFPProsto-NotI.R.c		Sbf1, NotI
	pMK156	GFP-ICE1 <sub>1-1000</sub> in pENTR	GFP-ICE1 <sub>1-1000</sub>	pMDC99	PCR, ligation	NotI/GFP.F	GFPProsto-NotI.R.c		NotI
	pMK157	ICE1 <sub>1-1000</sub> -NotI in pMDC99	ICE1 <sub>1-1000</sub> -NotI	pMDC99	GATEWAY LR reaction				
	pMK158	ICE1 <sub>1-1000</sub> -NotI in pMDC99	ICE1 <sub>1-1000</sub> -NotI	pMDC99	GATEWAY LR reaction				
	pMK162	ICE1-GFP-ICE1 <sub>1-1000</sub> -NotI in pMDC99	ICE1-GFP-ICE1 <sub>1-1000</sub> -NotI	pMDC99	GATEWAY LR reaction				
	pMK163	ICE1-GFP-ICE1-NotI in pMDC99	ICE1-GFP-ICE1-NotI	pMDC99	GATEWAY LR reaction				
	pMK171	ICE1 <sub>1-1000</sub> in pENTR	ICE1 <sub>1-1000</sub>	pENTR-TOPO	PCR, TOPO cloning	SCR1MendGW	SCR1MendSTOP.c		
pMK172	ICE1 <sub>1-1000</sub> genomic E3120 mutation in pENTR	ICE1 <sub>1-1000</sub> genomic E3120 mutation	pENTR-TOPO	PCR, TOPO cloning	SCR1MendGW	SCR1MendSTOP.c			
pMK173	ICE1 <sub>1-1000</sub> -NotI in pMDC99	ICE1 <sub>1-1000</sub> -NotI	pMDC99	GATEWAY LR reaction					
pMK174	ICE1 <sub>1-1000</sub> -NotI in pMDC99	ICE1 <sub>1-1000</sub> -NotI	pMDC99	GATEWAY LR reaction					
SCR2	pMK147	SCR2 <sub>2300</sub> genomic in pENTR	SCR2 <sub>2300</sub> genomic	pENTR-TOPO	PCR, TOPO cloning	At1g12860gGenGW	At1g12860gGenSTOP.c		
	pMK178	SCR2 <sub>2300</sub> genomic in pENTR	SCR2 <sub>2300</sub> genomic	pENTR-TOPO	PCR, TOPO cloning	At1g12860gGenGW	At1g12860gGenSTOP.c		
	pMK176	SCR2 <sub>2300</sub> genomic 3600 to 2300 in pCR2.1	SCR2 <sub>2300</sub> genomic 3600 to 2300	pCR2.1	PCR, TA cloning	ICE2prom-13283B1F	ICE2prom-1Kp1R.c		
	pMK180	SCR2 <sub>2300</sub> genomic 3600 to 2300-NotI in pMDC99	SCR2 <sub>2300</sub> genomic 3600 to 2300-NotI	pMDC99	ligation	SCR1Mprom-25815B1F	SCR1Mprom-1Kp1R.c		
	pMK181	SCR2 <sub>2300</sub> genomic 3600 to 2300-NotI in pMDC99	SCR2 <sub>2300</sub> genomic 3600 to 2300-NotI	pMDC99	ligation	SCR1Mprom-25815B1F	SCR1Mprom-1Kp1R.c		
pMK185	SCR2 <sub>2300</sub> genomic 3600 to 2300-NotI in pMDC99	SCR2 <sub>2300</sub> genomic 3600 to 2300-NotI	pMDC99	GATEWAY LR reaction					
SpiriYFP	pMK128	ICE1-cDNA	ICE1-cDNA	pENTR-TOPO	PCR, TOPO cloning	SCR1MendGW	SCR1MendSTOP.c		
	pMK130	scrm-D cDNA in pENTR	scrm-D cDNA	pENTR-TOPO	PCR, TOPO cloning	SCR1MendGW	SCR1MendSTOP.c		
	pMK132	ICE1-cDNA (pMK128)	ICE1-cDNA (pMK128)	pENTR-TOPO	PCR, TOPO cloning	SCR1MendGW	SCR1MendSTOP.c		
	pMK133	dual 35S <sub>prom</sub> :-(17S-end)YFP-ICE1 in pE3130	dual 35S <sub>prom</sub> :-(17S-end)YFP-ICE1	pE3130	GATEWAY LR reaction				
	pMK136	dual 35S <sub>prom</sub> :-(17S-end)YFP-scrm-D in pE3130	dual 35S <sub>prom</sub> :-(17S-end)YFP-scrm-D	pE3130	GATEWAY LR reaction				
	pMK137	dual 35S <sub>prom</sub> :-(17S-end)YFP-scrm-D in pE3132	dual 35S <sub>prom</sub> :-(17S-end)YFP-scrm-D	pE3132	GATEWAY LR reaction				
	pMK217	SCR2-cDNA in pENTR	SCR2-cDNA	pENTR-TOPO	PCR, TOPO cloning	SCR1MendGW	SCR1MendSTOP.c		
	pMK213	dual 35S <sub>prom</sub> :-(17S-end)YFP-SCR2 in pE3136	dual 35S <sub>prom</sub> :-(17S-end)YFP-SCR2	pE3136	GATEWAY LR reaction				
	pMK214	dual 35S <sub>prom</sub> :-(17S-end)YFP-SCR2 in pE3130	dual 35S <sub>prom</sub> :-(17S-end)YFP-SCR2	pE3130	GATEWAY LR reaction				
	pLJP173	dual 35S <sub>prom</sub> :-(17S-end)YFP-MUTE in pE3136	dual 35S <sub>prom</sub> :-(17S-end)YFP-MUTE	pE3136	GATEWAY LR reaction				
	pLJP174	dual 35S <sub>prom</sub> :-(17S-end)YFP-MUTE in pE3130	dual 35S <sub>prom</sub> :-(17S-end)YFP-MUTE	pE3130	GATEWAY LR reaction				
	pLJP175	dual 35S <sub>prom</sub> :-(17S-end)YFP-FAMA in pE3136	dual 35S <sub>prom</sub> :-(17S-end)YFP-FAMA	pE3136	GATEWAY LR reaction				
	pLJP176	dual 35S <sub>prom</sub> :-(17S-end)YFP-FAMA in pE3130	dual 35S <sub>prom</sub> :-(17S-end)YFP-FAMA	pE3130	GATEWAY LR reaction				
	pLJP224	SCR2 <sub>2300</sub> genomic 3600 to 2300-NotI in pGWB4	SCR2 <sub>2300</sub> genomic 3600 to 2300-NotI	pGWB4	GATEWAY LR reaction				
	pLJP226	dual 35S <sub>prom</sub> :-(17S-end)YFP-SPCH in pE3136	dual 35S <sub>prom</sub> :-(17S-end)YFP-SPCH	pE3136	GATEWAY LR reaction				
	pLJP227	dual 35S <sub>prom</sub> :-(17S-end)YFP-SPCH in pE3130	dual 35S <sub>prom</sub> :-(17S-end)YFP-SPCH	pE3130	GATEWAY LR reaction				

**Supplemental Table 4: List of primers and their DNA sequence used for plasmid construction**

SCRM1-EcoR1F	TGTCAGAATTCGCGATGGGTCTTGACG
SCRM3endBamH1.rc	AGTCAGGATCCGATCAGATCATACC
SCRM5endGW	CACCATGGGTCTTGACGGAAACAATGG
SCRM3endSTOP.rc	TCAGATCATACCAGCATAACCCTGC
SCRMprom-2581Sbf1F	AAGCCTGCAGGACCACCGTCAATAACATCG
SCRMprom-1Kpn1R.rc	AGAGGTACCGCCAAAGTTGACACC
Not1GFP-F	GCTAGCGGCCGCCACCATGGTGAGC
GFPnostop-Not1R.rc	AGCGGCAGCGGCCGCAGCTCC
SCRM-DBDmutationsF	CCTCTGATGGCTGGGAGGAGAAGGGGAAGAAG
SCRM-DBDmutationsR.rc	CCCTTCTCCTCCCAGCCATCAGAGGCTTAGCAG
At1g12860g5endGW	CACCATGGAGAGTAGAGAGGATTCATTC
At1g12860g3endSTOP.rc	TCAAACCAAACCAGCGTAACCTGC
ICE2prom-1326Sbf1F	GGTCCTGCAGGTTTTCATGAGCTTCC
ICE2prom-1Kpn1.rc	TACTCTGGTACCCACTTTTATAAAATACTAG
ICE2R581HmutationF	CAACTCTGTTTCAGAAACATGCTGCAATGCGTCAG
ICE2R581HmutationR.rc	CTGACGCATTGCAGCATGTTTCTGAAACAGAGTTG
ICE2g5endEcoR1GWF	CACCGAATTCATGAACAGCGACGGTGTTTGG
MUTE1-EcoRI linker	CGGAATTCATGTCTCACATCGCTGTTGAA
MUTE609 BamH1.rc	CGGGATCCTTAATTGGTAGAGACGATCAC
53210_1-EcoRI linker	CGGAATTCATGCAGGAGATAATACCGGAT
53210_1095 BamH1.rc	CGGGATCCCTAGCAGAATGTTTGCTGAAT
FAMA1-EcoRI linker	CGGAATTCATGGATAAAGATTACTCGGCA
FAMA 1244 BamH1.rc	CGGGATCCTCAAGTAAACACAATATTTCCC

**Supplemental Table 5: List of primers and their DNA sequence used for RT-PCR and 5' RACE PCR analysis**

Gene	Primer name	Sequence
ICE1	SCRM 1209F	GGAGATGCAATTGATTATCTGAAGG
	SCRM 3'END BAMH1.RC	AGTCAGGATCCGATCAGATCATACC
SCRM2	SCRM2 2784	CACACTCGACGCTGCTTCAC
	SCRM2 3110.rc	GAAGCCATTACCTGTAACGG
SPCH	53210 1.GW	CACCATGCAGGAGATAATACCG
	53210 886.rc	CTAGCAGAATGTTTGCTG
MUTE	MUTE 1.GW	CACCATGTCTCACATCGCTGTTG
	MUTE 1486.rc	TTAATTGGTAGAGACGATC
FAMA	FAMA 1.GW	CACCATGGATAAAGATTACTCGG
	FAMA 799.rc	AGGCATGAGAGATCTAAGG
ACTIN	ACT2-1	GCCATCCAAGCTGTTCTCTC
	ACT2-2	GTCGTAGTCAACAGCAACAA

**5' RACE PCR of SCRM2**

reverse primer	ACCCAAGACGCAGCTTCACCGTTA
reverse nested primer	AGCCGTCAAGCCAAACAC