Supporting Information

Vaistij et al. 10.1073/pnas.1301647110

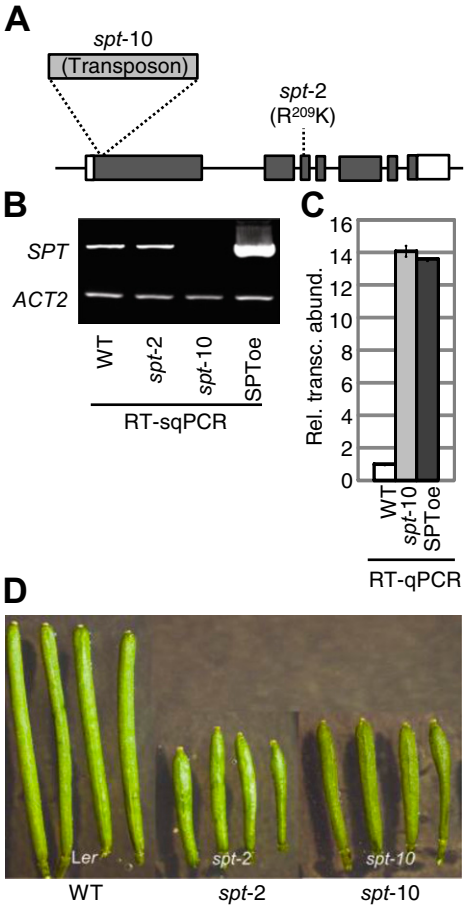

Fig. S1. Characterization of the Landsberg *erecta* (L*er*) *spt*-10 mutant. (*A*) Schematic representation of the *SPATULA* (*SPT*) locus and the *spt*-10 and *spt*-2 alleles: dark gray rectangles, *SPT* exons; white rectangles, predicted 5′ and 3′ UTRs; light gray rectangle, *spt*-10 transposon insertion 290 nucleotides downstream of the ATG start of translation, and location of the point mutation leading to the $R^{209}K$ amino acid substitution in *spt*-2 is indicated. (*B*) Transcript abundance assessed by reverse-transcription followed by semiquantitative PCR (sqPCR) using primers designed to amplify the full-length *SPT* transcript [oligo sequences: SPT-For GCGACGCGTAATTACTACTACCATGATATCACAGAGAGAAGAA and SPT-Rev GCGGGGCCCAGTAATTCGATCTTTTAGGT; RNA samples collected 2-d after imbibition (2-DAI). (*C*) RT-quantitative PCR (qPCR) determination of transcript abundance using primers specific for the *SPT* 3′ region (oligo sequences: qSPT-For CCTTACTTCACCCGTGGAGATG and qSPT-Rev GCGTTGGAATGACCAATGTTC; RNA samples collected 10-DAI). Error bars represent standard error (SE). (*D*) Picture of siliques from WT, *spt*-2, and *spt*-10 showing the typical abnormal silique phenotype of *spt* loss-of-function mutants. We first reported that *spt*-10 was acting as a loss-of-function mutant based on the fact that it lacked the full-length *SPT* transcript (*B*) and exhibited the abnormal fruit development phenotype of other *spt* loss-of-function mutants (*D*). However, we now revise this description of the *spt*-10 phenotype in terms of seed germination on the basis that (*i*) the mutant phenotype is similar to that of SPT overexpressing (SPToe)-L*er* line in that it shows significantly reduced seed dormancy and is opposite to that of the *spt*-2 and L*er* retrogressed *spt*-12 germination phenotypes (1) (see also Fig. 1 *A* and *B*) and (*ii*) spt-10 overaccumulates a truncated transcript (*C*). These results are consistent with SPT acting as a promoter rather than, as previously reported, a repressor of germination and *GA3OX1* and *GA3OX2* expression in L*er* (1). The fact that *spt*-10 acts as a loss-of-function mutant in terms of fruit development suggests that the SPT N-terminal region is necessary for its fruit-related but not germination-related function.

1. Penfield S, et al. (2005) Cold and light control seed germination through the bHLH transcription factor SPATULA. *Curr Biol* 15(22):1998–2006.

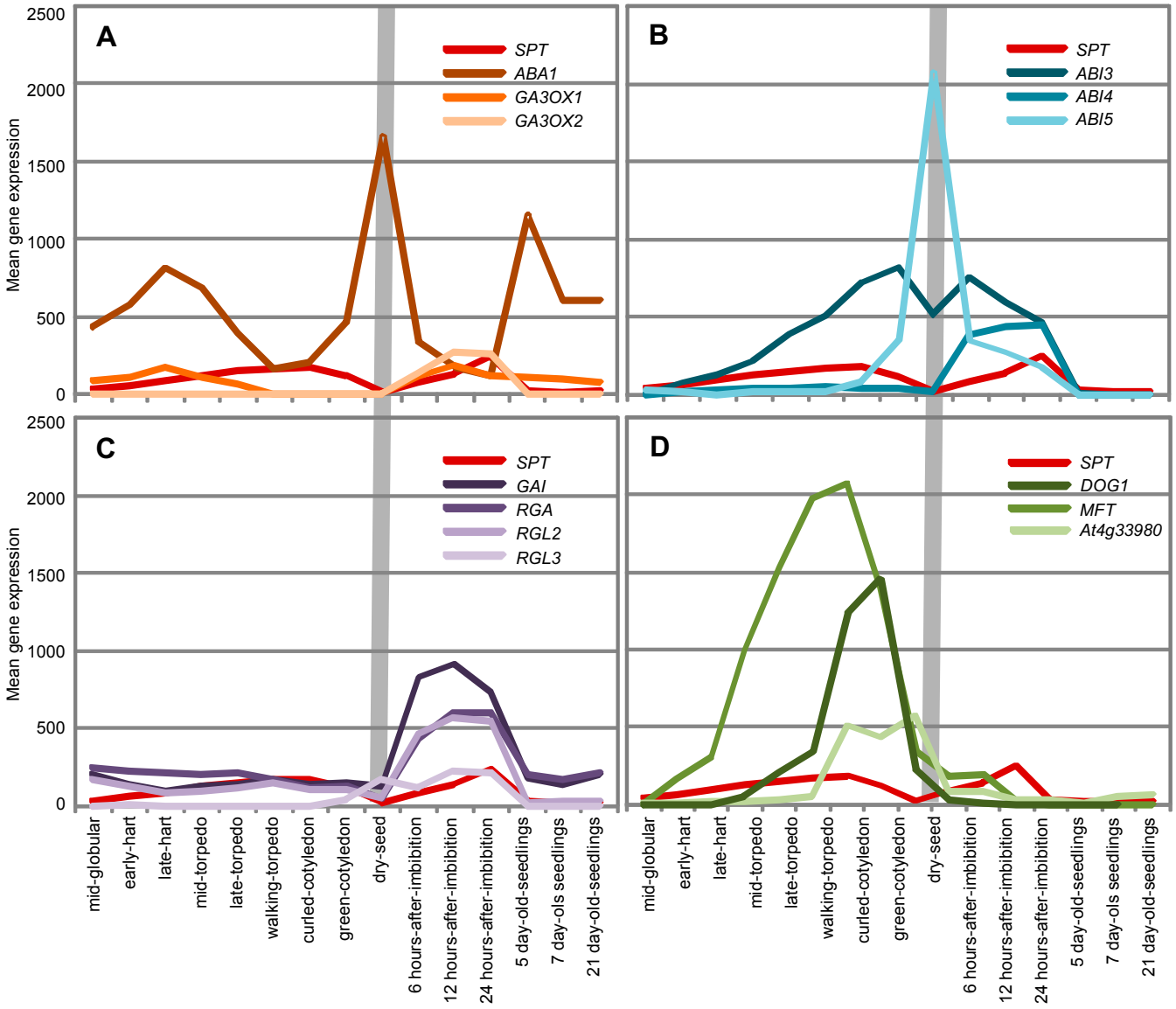

Fig. S2. Expression of *SPT*, selected genes involved in abscisic acid (ABA) and gibberellic acid (GA) biosynthesis [*ABA1*, *GA-3-OXIDASE1* (*GA3OX1*), and *GA3OX2*)] and signaling [*ABA-INSENSITIVE3* (*ABI3*), *ABI4*, *ABI5*, *GA-INSENSITVE* (*GAI*), *REPRESSOR-OF-GA* (*RGA*), *RGA-LIKE2* (*RGL2*), and *RGL3*], *DELAY-OF-GERMINATION* (*DOG1*), and *MOTHER-OF-FLOWERING-LOCUS-AND-TERMINAL-FLOWER1* (*MFT*) and *At4g33980* during seed development and early seedling stages (for clarity, the profiles are shown in four different panels, *A–D*). The data have been derived from publicly available resources. The vertical thick gray line marks the dry-seed stage.

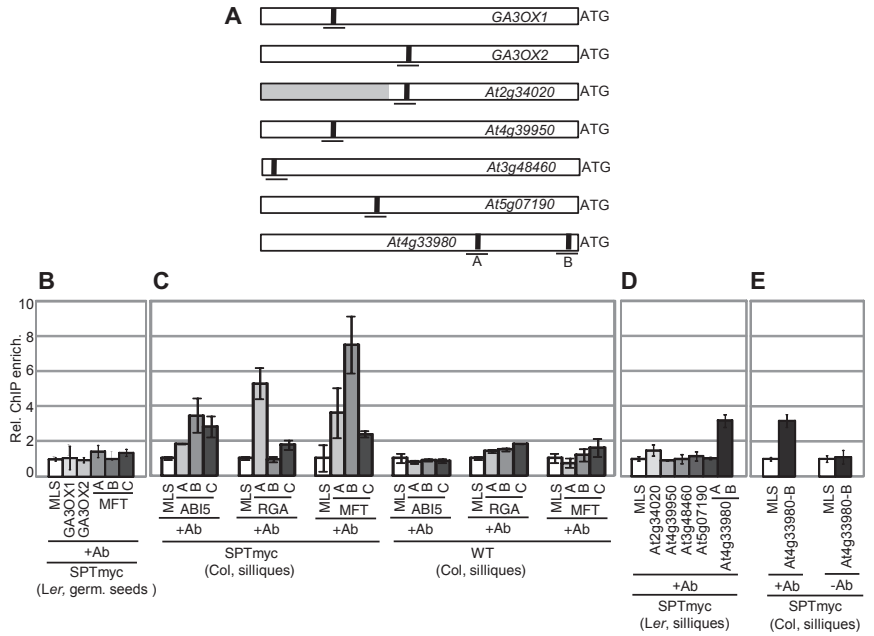

Fig. S3. Analyses of SPT interaction with the promoter regions of putative primary targets. (*A*) Schematic representation of the 2,500-bp region upstream of the ATG start codon of *GA3OX1* and *GA3OX2* and genes harboring G-box motifs listed in Table S2 (apart from *MFT*): Vertical thick black bars represent G-box motifs; light gray rectangle represents the presence of an upstream gene; horizontal thin black lines represent regions assessed for SPT binding. ChIP-qPCR assays were performed on germinating seeds (*B*), or siliques (*C*–*E*). The ecotype (L*er* or Col) and the genetic background (SPTmyc or WT) of the biological material are indicated below panels. Assays were performed with (+Ab) or without (−Ab) anti-MYC also as indicated. Amplicons assessed for enrichment are described in *A* and Figs. 2 and 3. Error bars represent SE.

Table S1. Amounts of ABA and GA (ng/g DW) in freshly matured DS, 1- and 2-DAI

	Fig. 1 *G*–*J*												Fig. 4 *E* and *F*			
	DS				1-DAI				2-DAI				DS			
	WT		*spt*-2/12		WT		*spt*-2/12		WT		*spt*-2/12		WT		*mft*-2	
ABA or GA	Aver	SD	Aver	SD	Aver	SD	Aver	SD	Aver	SD	Aver	SD	Aver	SD	Aver	SD
ABA																
L*er*	19.5	1.0	31.4	3.2	3.5	0.4	7.2	0.9	4.0	1.4	9.2	0.9	23.0	1.5	44.8	9.0
Col	31.4	7.5	22.0	2.9	22.2	5.4	11.4	3.8	16.4	6.9	7.5	3.4	29.1	4.7	64.6	5.3
GA																
L*er*	13.8	1.5	5.4	0.9	2.7	0.7	2.3	0.5	2.6	0.8	2.8	1.1	9.5	0.8	10.9	3.2
Col	7.8	1.2	9.9	1.9	4.1	0.7	4.0	0.7	3.4	0.4	3.1	0.5	9.8	1.5	14.1	1.1

These data were used to calculate relative levels in Figs. 1 *G*–*J* and 4 *E* and *F* (Aver, average; DAI, day-after-imbibition; DS, dry-seed; DW, dry weight). WT and mutant plants of the same ecotype were grown in parallel, but plants of different ecotypes were not. Our experience of measuring endogenous ABA and GA amounts has indicated that even small changes in environmental conditions (such as variation in temperatures or day-length in the glasshouse) lead to differences in phytohormone levels. For this reason we show relative ABA and GA levels in Fig. 1 *G*–*J* and 4 *E* and *F*, and we avoid making direct comparisons between ecotypes. The ABA/GA ratios are higher in Col compared with L*er* in both datasets. We have observed the same trend in multiple experiments in our laboratory and these results suggest different phytohormone sensitivity of the two ecotypes.

Table S2. Position of G-box and E-box motifs

Gene name	G-Box	E-Box
ABA1	None	−1,256; −1,218; −678; −539; −163; −141
ABI4	None	−2,104; −1,845; −1,654; −1,490; −1,067; −681
ABI5	−1,681; −1,205; −1,159; −1,148; −343	Not determined
RGA	−1,874; −1,785; −1,772	Not determined
RGL3	None	−970; −745; −424; −185
MFT	−1,592; −1,548; −705	Not determined
GA3OX1	−1,730	Not determined
GA3OX2	−1,288	Not determined
At2g34020	−1,263	Not determined
At4g39950	−2,051	Not determined
At3g48460	−2,366	Not determined
At5g07190	−1,599	Not determined
At4g33980	−876; −181	Not determined

The indicated positions within the promoter region of candidate genes are relative to the ATG start codon of each gene.

Table S3. Transcriptomic analyses of *spt* mutants

Locus	Description	SPToe	WT	*spt*-2	*aba1*-1	*spt*-2 *aba1*-1	G-box
At2g34020	Similar to calcium ion binding	942 ± 43	254 ± 87	28 ± 2	2897 ± 669	1072 ± 41	1
At4g39950	CYP79B2	1731 ± 276	371 ± 395	63 ± 24	4964 ± 947	2257 ± 205	1
At3g48460	GDSL-motif lipase/hydrolase family protein	1259 ± 76	230 ± 225	71 ± 13	5780 ± 180	2680 ± 202	1
At2g18050	HIS1-3 (HISTONE H1-3)	461 ± 207	1720 ± 938	4541 ± 781	23 ± 8	163 ± 29	0
At1g68240	bHLH transcription factor	845 ± 489	1849 ± 759	5314 ± 551	47 ± 28	287 ± 62	0
At1g18100	MOTHER OF FT AND TFL1 (MFT)	507 ± 72	1463 ± 323	3493 ± 706	38 ± 4	150 ± 43	3
At5g07190	ARABIDOPSIS THALIANA SEED GENE 3	819 ± 351	2158 ± 1315	5288 ± 383	42 ± 3	163 ± 30	1
At5g25180	CYP71B14	381 ± 159	1498 ± 449	3478 ± 689	28 ± 5	87 ± 9	0
At4g15620	Integral membrane family protein	707 ± 68	2374 ± 944	4841 ± 349	70 ± 20	297 ± 88	0
At4g33980	Unknown protein	736 ± 82	1860 ± 949	3620 ± 835	85 ± 31	311 ± 95	2

Transcriptomic assays were performed on SPToe, WT, *spt*-2, *aba1*-1 and *spt*-2 *aba1*-1 double mutant backgrounds of seeds (L*er*) which were freshly matured, stratified and sampled 1 d-after-imbibition in continuous light. Genes shown in the table passed significance tests (false-discovery rate < 0.05) of multiple comparisons: SPToe vs. WT; WT vs. spt-2; and *aba1*-1 vs. *spt*-2 *aba1*-1. Values were MAS5 normalized. SD of three independent experiments are shown. The final column of the table indicates the number of G-boxes found in the promoter region (2,500 base pair) of each gene. To aid visualization dark and light shading indicate higher and lower expression than WT, respectively.

Table S4. Sequence of primers used in this study for RT-qPCR and ChIP-qPCR

Oligo name	Sequence (5′→3′)
RT-qPCR	
ACT2-For (LH39)	TGAGAGATTCAGATGCCCAGA
ACT2-Rev (LH40)	TGGATTCCAGCAGCTTCCAT
ABAI-For	ATGGCTTGCAATTATGGCTTC
ABAI-Rev	TCATCGGCTTTGTCAGTGAG
GA3OX1-For	AAGTGGACCCCTAAAGACGATCT
GA3OX1-Rev	GTCGATGAGAGGGATGTTTTCAC
GA3OX2-For	TGAGTTCCTCACCGGAAGTCTT
GA3OX2-Rev	CGAGCCGCCTTGAGCTT
ABI3-For	TCCATTAGACAGCAGTCAAGGTTT
ABI3-Rev	GGTGTCAAAGAACTCGTTGCTATC
ABI4-For	TCCGCTCAACGCAAACG
ABI4-Rev	TTGTCGAACGCCACGGTA
ABI5-For	CAATAAGAGAGGGATAGCGAACGAG
ABI5-Rev	CGTCCATTGCTGTCTCCTCCA
GAI-For	TGCGGCTGCACATATTGG
GAI-Rev	CCCTCACCGCCGTTGA
RGA-For	CAAGGTTATCGTGTGGAGGAGAGT
RGA-Rev	GGTGGTAATGAGTGGACGAGTGT
RGL2-For	CCGACCCGAATCTGAAACCTTAGTG
RGL2-Rev	AAGCGCTTCGTTGAACCTATCGAG
RGL3-For	CAAACGAAACCTCTAATCGCTGCAT
RGL3-Rev	GGGCGAAATTGTCACAAAACGAAC
DOGI-For	AACCATCGACGGTACGAATC
DOGI-Rev	GCTTGTCGAGAGCTTGATCC
ChIP-qPCR	
MFT-For	ATCACTAACGGCTGCGAGAT
MFT-Rev	CGGGAATATCCACGACAATC
MLS-For (VA86)	TGGATTGAACCCAAAACAGTG
MLS-Rev (VA87)	TCCAATACCTCTGGCTCTGC
ABAI-A-For	GGATCAATGTTTCATGAGCAA
ABAI-A-Rev	AGACCTTCCACTTCTCCGTCT
ABAI-B-For	TTTCTGGATCCCTACTCTTTATGT
ABAI-B-Rev	TCATCATCATAAAATCAAATGGTC
ABAI-C-For	GAAGTGTGTTGCGTTGCTG
ABAI-C-Rev	CAAAACGGAGTTGAACCCA
GA3OX1-A-For	CAAACGATCAATCGTCTACTTTGC
GA3OX1-A-Rev	CACCAACGTTCCCTAAGATTTTCA
GA3OX2-A-For	GTGAAGAAGAAGAAGAACACGTG
GA3OX2-A-Rev	GAGCCAAGCCTTATACCGTTAC
PIL2-3-For	CTAATACACTGCATACGGGTACCC
PIL2-3-Rev	GTGTTGGGGAGGAGAGAGAGAGAG
ABI4-A-For	GCAATGGATCTGCAACACTC
ABI4-A-Rev	AGGGATCGGAGGAAAAACAT
ABI4-B-For	AGGGTTGTTGGTAATGGTAGATT
ABI4-B-Rev	GGGGGCTGATTAGCCATAA
ABI4-C-For	TGCTTGCCAACTTTAAACGA
ABI4-C-Rev	AAGAGAAATAAGCGTGTTCAAAA
ABI5-A-For	TTAGGTCGCTGGTTCGATTC
ABI5-A-Rev	CATGATTCCGAACTTCCATTG
ABI5-B-For	TGTGTAGCCGAAGTCACACGTG
ABI5-B-Rev	CTTTCGACCAATGGAATGCT
ABI5-C-For	CGATGTGGACCGTTCTTCTT
ABI5-C-Rev	TCCCTGTTCAGCTATTCACG
RGA-A-For	CAGACTCGGTCCCTACCGTTT
RGA-A-Rev	GCCGTCATTAACGGCCTCTTTCT
RGA-B-For	TATGTTTTCGATGGCTGAGC
RGA-B-Rev	GATGGAAGAACTGAAGATGCT
RGA-C-For	TTCTGGTATGAATGATGATTGAA
RGA-C-Rev	CAGCTATGAGTTTCGATTAGATTAGG
RGL3-A-For	CCGGCCATTAAATAGTCTCG
RGL3-A-Rev	TGGCTTATTATTTTCCGTTTT

Table S4. Cont.

Oligo name	Sequence (5′→3′)
RGL3-B-For	GAAAGCAACGTAATGTTGTAGACG
RGL3-B-Rev	AATTTGTTTGCCGAAATGGT
RGL3-C-For	AAAACGGGCTGTTACTTTCG
RGL3-C-Rev	CGCTCTGATAAGGGCGTGT
MFT-A-For	CCAATCGATCGAGTACCACA
MFT-A-Rev	GCCATTTGAAACTCCTTTGC
MFT-B-For	CGACCGACCATAAATCATACG
MFT-B-Rev	CACGTGTTGCATGATTAGCC
MFT-C-For	AGGAAATTATCGCCAACGTG
MFT-C-Rev	TTTTGTTTTGTCGCATGTCC
At2g34020-A-For	GAACACGTGGTTTTCGCATA
At2g34020-A-Rev	ATGCTGCAGGATCACATCAA
At4g39950-A-For	GGGTTTTACGTAGTCGTGTCA
At4g39950-A-Rev	CAGAGGGGATTGGTTTGTAA
At3g48460-A-For	TGAGACCTCTCCCTTTGACG
At3g48460-A-Rev	TGGTCGGTTACATTTTGCTG
At5g07190-A-For	GCCCACATACGTAACATTCG
At5gD719D-A-Rev	CACGTGGATCCGGTAAAATC
At4g33980-A-For	CCATGGTTTGTACCATCTGC
At4g33980-A-Rev	CTGAAGATGCGGTCTCAACA
At4g33980-B-For	AACCTCCCTTCTTCTCCTTGAGC
At4g33980-B-Rev	TTCTTCTTCGAAAGAGCCAAA