Supporting Information

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SI Materials and Methods

Mutant Characterization. Germination assays were performed on filter paper as described (1) using 3-mo-old seeds harvested at the same time. Germination was scored by seed coat rupture after 8-d incubation under constant light at 22 °C. For root and hypocotyl elongation assays, seeds were plated on 0.5× MS media and 1% sucrose ± paclobutrazol (PAC) or gibberellins (GA). After 4 d at 4 °C in the dark, the square plates were placed vertically in continuous light at 22 °C. For assays of the etiolated seedlings, the plates (after 24 h in the light) were incubated in the dark at 22 °C until measurement on day 6. Root and hypocotyl lengths were measured with the aid of ImageJ software (http://rsbweb.nih.gov/ij/). No less than 30 seedlings were measured for each genotype. For phenotype analysis of soil-grown plants in the *ga1-3* background, seeds were imbibed in 100 μM GA_3 for 3 d at 4 °C and then washed thoroughly with water before planting in soil.

Plasmid Construction. Sequences of primers used in this study are listed in Table S1. The PCR-amplified fragments in all constructs were analyzed by DNA sequence analysis to ensure that no mutations were introduced.

Constructs for expression of SCARECROW-LIKE 3 in transgenic Arabidopsis. For making *SCARECROW-LIKE 3 SCL3-OE* [Cauliflower Mosaic Virus (CaMV) 35S promoter: *SCL3* cDNA] and *35S:SCL3-GFP*, a full-length coding region of *SCL3* cDNA was amplified by PCR using primers SCL3 GF1 and SCL3 GR. The amplified DNA was then inserted into pENTR/D-TOPO (Invitrogen) followed by site-directed recombination to enter the binary vectors pGWB2 and pGWB5 for *SCL3-OE* and *35S:SCL3-GFP*, respectively (2). For making P_{SCL3}*:SCL3*, a 5.3-kb genomic DNA fragment carrying the *SCL3* gene, including a promoter region (2,783 bp) and a 3′ downstream region (383 bp), was amplified by PCR with primers SCL3 GWP F1 and SCL3 3′ Rev using a plasmid clone containing a 6.2-kb EcoRI *SCL3* genomic DNA fragment derived from a BAC clone (F11F12; GenBank accession no. AC012561.2) as a template. The amplified DNA fragment was then inserted into pENTR/D-TOPO (Invitrogen) followed by site-directed recombination to enter the binary vector pGWB1 (2). P_{SCL3}*:SCL3-GFP* is a plasmid that contains 4-kb *SCL3* genomic DNA (including ~2.5 kb upstream sequence from the ATG start site of *SCL3* and the full-length *SCL3* coding sequence) fused to *GFP* (3).

Constructs for coimmunoprecipitation. To make *35S:HA-RGA* (pEG201-RGA), the *RGA* cDNA sequence was amplified by PCR using primers 224 and 536 and then inserted into pENTR 1A (Invitrogen) by BamHI/NotI digestion followed by site-directed recombination to enter binary vector pEarleyGate201 (4). To make *35S:cMyc-SCL3* (pEG203-SCL3), the *SCL3* coding sequence was PCR-amplified with primers SCL3-GW-N(C)5 and SCL3-GW-N3 and then introduced into the pCR8/GW/TOPO gateway vector (Invitrogen) by topo cloning followed by site-directed recombination to enter pEarleyGate203 (4). To make *35S:cMyc-GUS-NLS* (pEG203-GUS-NLS, a negative control), the coding sequence of GUS (β-glucuronidase) was PCR-amplified from pBI101 vector using primers GUS-11 and GUS-12 to create GUS-SV40 NLS (5). This GUS-NLS fragment was cloned into pCR8/GW/TOPO vector and then into pEG203 by recombination.

Constructs for bombardment transient expression. Reporter constructs are derived from plasmids pRAB-5 and p35S:Renilla-luciferase (*35S:rLUC*) that have been described before (6, 7). The firefly luciferase (*fLUC*) gene from pRAB-5 was excised using the restriction enzymes NcoI and XbaI and cloned into the same sites in pFGC5941 (http://www.chromdb.org/rnai/pFGC5941.html) to generate a 35S-TMV-Ω-LUC construct that includes the Ω leader sequence of the Tobacco Mosaic Virus (TMV). This construct was designated pRZ500. A DNA fragment of the *SCL3* promoter was amplified from Col-0 genomic DNA by PCR with primers SCL3-prom-RI-Swa-F and SCL3-prom-Xho-R, restriction digested with EcoRI and XhoI, and cloned into the same sites of pRZ500. The resulting plasmid was cut with SwaI and NcoI to recover the 1-kb *SCL3* promoter fragment that includes the TMV-Ω leader sequence and was cloned into the polylinker of pRAB-5 to generate plasmid pRZ506 (SCL3-1kb-LUC). DNA fragments of the promoters of *GA3ox1* and *GA20ox2* were PCR-amplified with primer pairs 3ox1-prom-Swa-F and 3ox1-prom-Xho-R and 20ox2-prom-RI-Swa-F and 20ox2-prom-Xho-R, respectively. The PCR products were digested with SwaI and XhoI and cloned into the same sites in pRZ506, replacing the *SCL3* promoter. The new plasmids were designated pRZ507 (GA20ox2-LUC) and pRZ511 (GA3ox1-LUC). A 2-kb *SCL3* promoter construct was obtained by amplifying a DNA fragment from genomic DNA with primers SCL3-1900prom-H3-F and SCL3-prom-Xho-R. The resulting PCR product was cut with HindIII and XhoI and cloned into the same sites of pRZ506, replacing the 1-kb promoter with the 2-kb PCR product and generating plasmid pRZ535 (SCL3-2kb-LUC). Replacement of the *SCL3* TATA box with the *35S* promoter TATA box was done in two steps. A PCR product was generated using pRZ500 as a template and primers 35S-minimal-BHI-RV and LUC-750-R; the resulting PCR product was cut with EcoRV and SphI and cloned into the same sites in pRAB-5, generating plasmid pRZ516 (35S-minimal-LUC). Subsequently, a *SCL3* promoter fragment lacking the putative TATA box was obtained by PCR using pRZ535 as a template and primers SCL3-1900prom-H3-F and SCL3p-100-BHI-R. The PCR product was cut with HindIII and BamHI and cloned into the same sites of pRZ516, generating the construct pRZ536 (SCL3-2kb+TATA-LUC).

Effector constructs are derived from pRTL2 (35S-Empty) and pRTL2-mGFP that were provided by Jim Carrington (8, 9). The mGFP sequence in pRTL2-mGFP was substituted with the coding sequence of *RGA* as an NcoI and BglII fragment to generate pRG37 (35S:RGA). To create pRZ530 (35S:SCL3), the coding sequence of *SCL3* was PCR-amplified from a cDNA clone using primers SCL3-NcoI-F and SCL3-XbaI-R cloned into pCR2.1 (Invitrogen); it was subsequently cut with XbaI and partially digested with NcoI to recover the full-length coding sequence. The fragment was then ligated into the same sites in pRTL2 (9).

Constructs for yeast two-hybrid. For making SCL3-DB (pSCL3-1), the coding sequence of *SCL3* was amplified from Col-0 genomic DNA by PCR using primers SCL3-1 and SCL3-2, cut with EcoRI and BamHI, and inserted into the DNA-BD vector pLexA-NLS. Sequencing results indicated that there were two silent mutations (^{138}A→^{138}G and ^{1263}C→^{1263}T) in the coding sequence of *SCL3* gene. To make SCL31-AD, the coding sequence of *SCL31* (At1g07520) was PCR-amplified from a BAC clone (F22G5) using primers SCL31-3 and SCL31-2. The PCR fragment was first cloned into pCR8/GW/TOPO cloning vector (Invitrogen), and the *SCL31* coding sequence was then subcloned into NcoI and EcoRI sites of the prey vector pACT II. RGA-AD, GAI-AD, RGL1-AD, RGL2-AD, and RGL3-AD plasmids were made previously (1, 10).

Constructs for expressing recombinant SCL3 proteins. The coding sequence of *SCL3* in pSCL3-1 was excised by EcoRI/BamHI

digestion and subcloned into the same sites of pMAL-c2 (NEB). The construct was designated pMBP-SCL3.

To make the GST-SCL3 fusion construct, the *SCL3* coding sequence was amplified using PCR primers SCL3 CDS-5 (BamHI) and SCL3 CDS-3 (SalI); after digestion with BamHI/SalI, the PCR fragment was cloned into the same sites of pGEX-KG. The final construct was designated pGEX-SCL3, in which the GST-SCL3 fusion gene was placed under the control of the inducible *tac* promoter.

In Vitro Pull-Down Assays. The assay was performed as described before (11) with the following modifications. Both recombinant GST and GST-SCL3 were expressed in *Escherichia coli* TB1 (NEB), and ~8 μg recombinant proteins together with the glutathione Sepharose 4B beads (GE Healthcare) were used for the pull-down assay. Five grams of 10-d-old *sly1-10* and *rga-24 sly1-10* (in the L*er* background) were used as starting material. The final pull-down protein samples were separated by a 6% SDS/PAGE gel and immunoblotted with affinity-purified anti-RGA antibodies (DU176) as described (12). Signal was detected by SuperSignal West Dura chemiluminescent substrate (Thermo Scientific Pierce).

Transient Expression in *Nicotiana benthamiana* by Agro-Infiltration and Coimmunoprecipitation of SCL3 and RGA. The transient expression constructs in pEarleyGate were transformed into agrobacterium GV3101. After overnight growth, agrobacterium cells were incubated in induction media (60 mM K_2HPO_4, 33 mM KH_2PO_4, 7.6 mM $(NH_4)_2SO_4$, 2 mM sodium citrate, 1 mM $MgSO_4$, 0.2% glucose, 0.4% glycerol, 10 mM MES, 50 μg/mL acetosyringone, pH 5.6) with antibiotics (50 μg/mL Kanamycin and 50 μg/mL Gentamicin) for 4 h. Cells were then resuspended in infiltration media (0.5× MS, 10 mM Mes, 150 μg/mL acetosyringone, pH 5.6) without antibiotics. Agrobactium strains carrying different expression constructs were mixed to make final OD_{600} ~ 0.8 for each strain and infiltrated into 5-wk-old *N. benthamiana* leaves by needle-less syringe; 72-h transiently transformed *N. benthamiana* leaves were cross-linked in 1% formaldehyde for 15 min by vacuum infiltration. Coimmunoprecipitation (co-IP) was performed using nuclear proteins extracted from cross-linked *N. benthamiana* leaves. Nuclear protein extracts were first diluted 10-fold in 1× PBS and 0.5% Triton X-100 with plant protease inhibitor mixture (P9599; Sigma) and then, incubated with 10 μL anti-cMyc agarose-conjugated beads (A7470; Sigma) for 90 min at 4 °C. After four washes with 1× PBS with protease inhibitors, proteins were eluted by boiling for 5 min in 2× Laemmli buffer and analyzed by SDS/PAGE and immunoblotting using monoclonal anti-cMyc antibody (MMS-150P; Covance) or monoclonal anti-HA antibody (MMS-101P; Covance) as primary antibodies. HRP-conjugated donkey anti-mouse antibody (Jackson ImmunoResearch) was used as secondary antibody. The blots were incubated in Supersignal Dura Reagent for chemiluminescence detection.

Transient Expression Assays by Particle Bombardment of Arabidopsis Seedlings. Surface-sterilized seeds were placed on 60- × 15-mm Petri dishes containing MS medium and incubated under constant light at 22 °C for 11–14 d. Particle bombardment was carried out using the PDS-1000/He particle gun delivery system (Bio-Rad Laboratories) as described previously (7), except that, instead of detached leaves, whole seedlings were used. Mixtures of plasmids were prepared using DNA molar ratios of 5:3 (reporters:effectors). When an effector construct was omitted, a similar molar amount of pRTL2 (35S-Empty Control) was included in the DNA mixture. Plasmid *35S:rLUC* was included as a control of transformation. Each DNA mixture was bombarded to two different plates, and two samples were collected from each plate. After bombardment, the Petri dishes were sealed again and returned to the growth chamber for 20 h. A dual-luciferase reporter assay (DLRA) system (Promega) was used to test for promoter activity. Firefly and Renilla LUC activities were measured with a fusion α-FP HT universal plate reader (Perkin-Elmer). Relative promoter activity was calculated as the ratio of fLUC to rLUC activities for each sample. The average of three or four replicates was used for each treatment.

ChIP-qPCR. The ChIP was performed as described previously (13, 14) with some modifications. Briefly, 7.5 g 10-d-old seedlings of *scl3-1* (control) and *scl3-1* P_{SCL3}*:SCL3-GFP* were fixed for 15 min in 1% formaldehyde by vacuum infiltration. Nuclei were isolated and followed by sonication using a Digital Sonifier S-250D (Branson Ultrasonics) to obtain DNA fragments around 0.5–1 kb. After preclarification with BSA equilibrated Protein A agarose beads (Upstate), sonicated chromatin was incubated at 4 °C over night with anti-GFP ab290 (Abcam). The GFP antibody, together with associated protein–DNA complex, was then pulled down by incubating with Protein A agarose beads for 6 h at 4 °C with rotation. The beads were then washed as described by Bowler et al. (13). Elution and cross-linking reversal was done by boiling the beads for 10 min in 500 μL freshly made elution buffer (100 mM $NaHCO_3$, 0.1% SDS) followed by 1 h incubation at 45 °C with 200 mM NaCl, 10 mM EDTA, 40 mM Tris-HCl pH 6.5, and 0.1 mg/mL proteinase K. The chromatin was precipitated by ethanol after phenol/chloroform extraction. The purified DNA was resuspended in 200 μL TE (10 mM Tris-HCl pH 8.0, 1 mM EDTA) buffer.

For promoter scanning of the *SCL3*, *GA20ox1*, *GA20ox2*, and *GA20ox3* genes, different regions of each promoter were examined by ChIP-qPCR assay. Primers used for the assay are listed in Table S1. All qPCR reactions were carried out using the LightCycler SYBR Green I Fast Start DNA kit (Roche). Reactions were performed with 1 μL immunoprecipitated DNA as template, and samples from *scl3-1* control line and *scl3-1* P_{SCL3}*:SCL3-GFP* transgenic line were compared. The amount of 18S rRNA gene from the ChIP samples was quantified by qPCR (1) to normalize the results between the control and test samples. The copy numbers of the *SCL3-GFP* transgene and its corresponding promoter regions were determined by real-time qPCR using genomic DNAs of the transgenic line *scl3* P_{SCL3}*:SCL3-GFP* and the *scl3* mutant as template. Student *t* tests were performed using the statistical package SPSS version 17.0.

Generation of Anti-SCL3 Antibodies and Immunoblot Analysis. The purified maltose binding protein (MBP)-SCL3 fusion protein from pMBP-SCL3 was used to raise polyclonal anti-SCL3 antibodies in rat (Cocalico Biologicals). The anti-SCL3 crude antiserum (DU-R24) was affinity-purified by GST-SCL3 fusion proteins using Affi-gel Blue gel followed by Affi-gel 15 (Bio-Rad). The endogenous SCL3 protein from different GA mutants was detected by using affinity-purified DU-R24. The immunoblot analysis procedure was similar to the protocol described before (12), except that 1× TBS buffer (50 mM Tris·HCl, pH 7.5, 150 mM NaCl, 0.05% Tween 20) was used (instead of PBS) in the entire procedure, and 5% milk was omitted when blotting with primary (DU-R24) and secondary antibodies (donkey anti-rat; Jackson ImmunoResearch Laboratory). The DU-R24 will not be available for distribution after publication, because the titer of DU-R24 is extremely low.

Yeast Two-Hybrid Assays. The LexA-based yeast two-hybrid (Y2H) assay using the yeast strain L40 was performed as described (10). For 3-AT assay, various concentrations of 3-AT (0, 1, 2, 5, 10, 30, and 60 mM) were mixed in synthetic complete medium lacking Trp, Leu, and His. For each combination, 2 μL yeast cells with OD_{600} values of 0.25, 0.125, and 0.05, respectively, were spotted on media plates.

1. Tyler L, et al. (2004) Della proteins and gibberellin-regulated seed germination and floral development in Arabidopsis. *Plant Physiol* 135:1008–1019.
2. Nakagawa T, et al. (2007) Development of series of gateway binary vectors, pGWBs, for realizing efficient construction of fusion genes for plant transformation. *J Biosci Bioeng* 104:34–41.
3. Heo J-O, et al. (2010) Funneling of gibberellin signaling by the GRAS transcription factor SCARECROW-LIKE 3 in the Arabidopsis root. *Proc Natl Acad Sci USA*, 10.1073/pnas.1012215108.
4. Earley KW, et al. (2006) Gateway-compatible vectors for plant functional genomics and proteomics. *Plant J* 45:616–629.
5. Kalderon D, Roberts BL, Richardson WD, Smith AE (1984) A short amino acid sequence able to specify nuclear location. *Cell* 39:499–509.
6. Matsuo N, Minami M, Maeda T, Hiratsuka K (2001) Dual luciferase assay for monitoring transient gene expression in higher plants. *Plant Biotechnol* 18:71–75.
7. Yamakawa S, et al. (2004) Systematic transient assays of promoter activities for leaf-specific genes identified by gene-expression profiling with cDNA microarrays in *Arabidopsis thaliana*. *J Biosci Bioeng* 98:140–143.
8. Schaad MC, Jensen PE, Carrington JC (1997) Formation of plant RNA virus replication complexes on membranes: Role of an endoplasmic reticulum-targeted viral protein. *EMBO J* 16:4049–4059.
9. Carrington JC, Freed DD, Oh CS (1990) Expression of potyviral polyproteins in transgenic plants reveals three proteolytic activities required for complete processing. *EMBO J* 9:1347–1353.
10. Dill A, Thomas SG, Hu J, Steber CM, Sun T-p (2004) The Arabidopsis F-box protein SLEEPY1 targets GA signaling repressors for GA-induced degradation. *Plant Cell* 16:1392–1405.
11. Griffiths J, et al. (2006) Genetic characterization and functional analysis of the GID1 gibberellin receptors in Arabidopsis. *Plant Cell* 18:3399–3414.
12. Silverstone AL, et al. (2001) Repressing a repressor: Gibberellin-induced rapid reduction of the RGA protein in Arabidopsis. *Plant Cell* 13:1555–1566.
13. Bowler C, et al. (2004) Chromatin techniques for plant cells. *Plant J* 39:776–789.
14. Cui HC, et al. (2007) An evolutionarily conserved mechanism delimiting SHR movement defines a single layer of endodermis in plants. *Science* 316:421–425.
15. Zentella R, et al. (2007) Global analysis of della direct targets in early gibberellin signaling in Arabidopsis. *Plant Cell* 19:3037–3057.
16. Poullet P, Tamanoi F (1995) Use of yeast two-hybrid system to evaluate Ras interactions with neurofibromin-GTPase-activating protein. *Methods Enzymol* 255:488–497.
17. Bolle C (2004) The role of GRAS proteins in plant signal transduction and development. *Planta* 218:683–692.
18. Dill A, Sun T (2001) Synergistic derepression of gibberellin signaling by removing RGA and GAI function in *Arabidopsis thaliana*. *Genetics* 159:777–785.

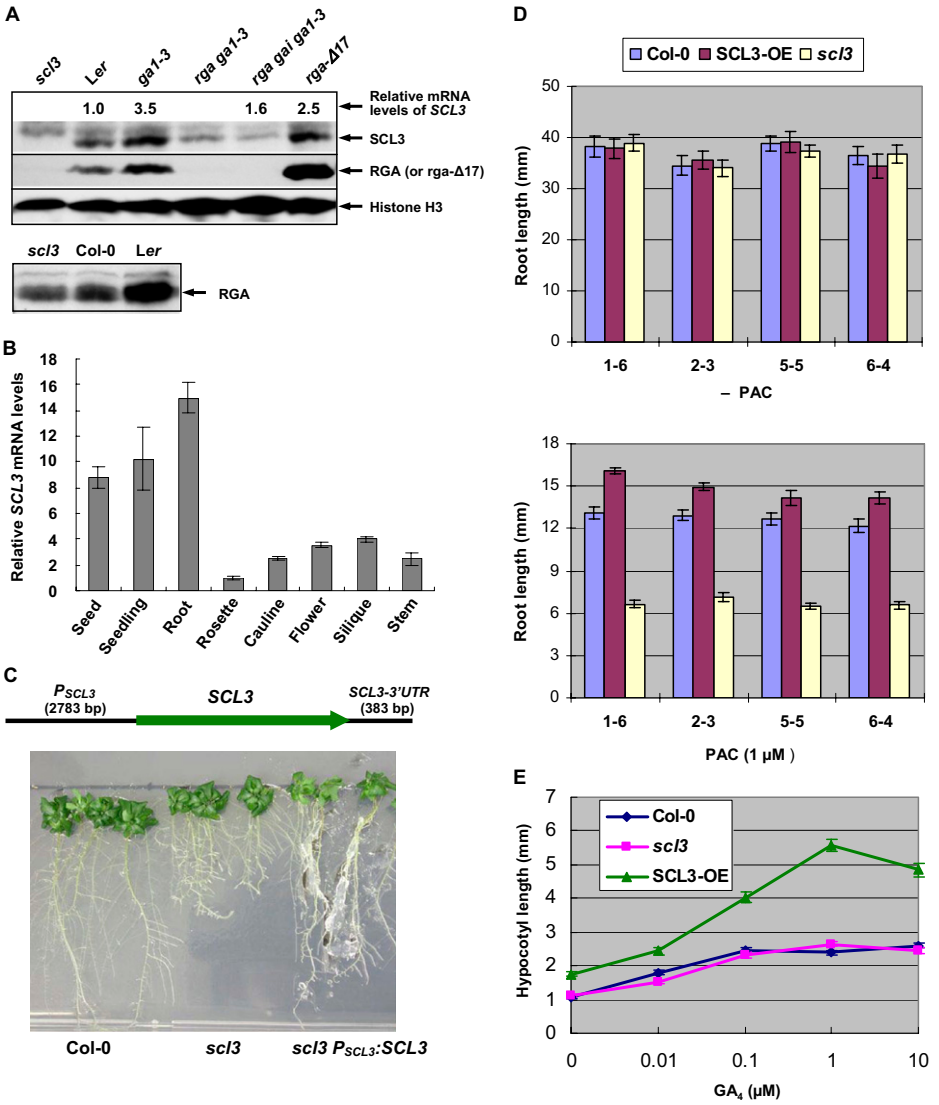
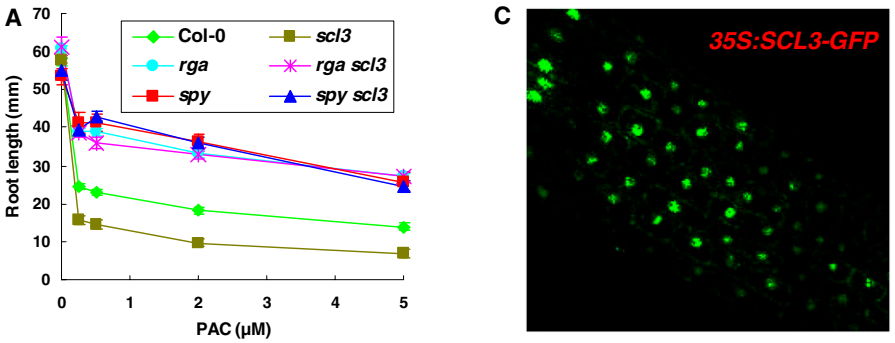


Fig. S1. Expression profile of *SCL3* and phenotypes of *scl3* and *SCL3-OE* lines. (*A*) DELLA induces both SCL3 mRNA and protein accumulation. (*Upper*) Both SCL3 mRNA and protein levels are increased in *ga1-3* and *rga-Δ17* and reduced by *rga-24* and *gai-t6* null mutations. Relative *SCL3* transcript levels in 8-d-old seedlings (15) are listed above the blot. For immunoblot assays, proteins were extracted from roots of 8-d-old WT (L*er*) and different GA mutants. All mutants except *scl3* are in the L*er* background. Immunoblot analyses were carried out using affinity-purified antibodies against SCL3 and RGA, respectively. The histone proteins detected with antihistone H3 antibody (ab1791; Abcam) served as a loading control. (*Lower*) RGA proteins accumulate to higher levels in the L*er* background than in the Col-0 background (WT and *scl3* mutant). This explains why RGA was not detected in *scl3* (in the Col-0 background) in *Upper*. Proteins were extracted from roots of 8-d-old *scl3*, Col-0, and L*er*, respectively. Immunoblot analysis was carried out using affinity-purified anti-RGA antibodies. A longer exposure time (than in *Upper*) was needed to detect the RGA protein in *scl3* and Col-0. (*B*) *SCL3* in WT (Col-0) is expressed highest in germinating seeds and seedlings. Data represent the average of three qRT-PCR measurements ± SE. The housekeeping gene *Actin 11* (At3g12110), whose expression remains similar in different tissues, was used to normalize different samples. The tissue with the lowest *SCL3* expression (rosette leaves) was arbitrarily set to 1. Seed, germinating seeds; seedling, 10-d-old seedlings; root, from 10-d-old seedlings; rosette, rosette leaves of 5-wk-old plants; cauline, cauline leaves; flower, flower clusters; silique, siliques; stem, stem tissue only, with all leaves and inflorescences removed. (*C*) P_{SCL3}*:SCL3* rescued *scl3* root phenotype (14-d-old seedlings). (*Upper*) The P_{SCL3}*:SCL3* genomic DNA construct. (*Lower*) The *scl3* root phenotype was rescued by P_{SCL3}*:SCL3* in the presence of 0.5 μM uniconazole, a GA biosynthesis inhibitor with similar effect as PAC. (*D*) Overexpression of SCL3 caused longer root phenotype. Col-0, *scl3*, and four independent *SCL3-OE* lines were subjected to root-length assay in the absence (*Upper*) or presence (*Lower*) of 1 μM PAC as in Fig. 1*B*. Root lengths were measured on day 7. (*E*) Overexpression of SCL3 caused longer hypocotyl phenotype. Hypocotyl elongation assays in response to 1–10 μM GA_4 were performed using WT, *scl3*, and *SCL3-OE* line (#5) as in Fig. 1*D*. The *x* axis is on a log scale.



B

Line	Rosette diameter (mm)	Days to flower	Plant height (cm, d65)
ga1-3	26.6 ± 0.3[a]	41.5 ± 0.5	ND
ga1-3 scl3	21.3 ± 0.4[a]	46.7 ± 0.6	ND
ga1-3 rga-28	41.7 ± 0.5[b]	30.8 ± 0.3	16.9 ± 0.2
ga1-3 rga-28 scl3	33.1 ± 0.5[b]	33.0 ± 0.3	12.5 ± 0.2

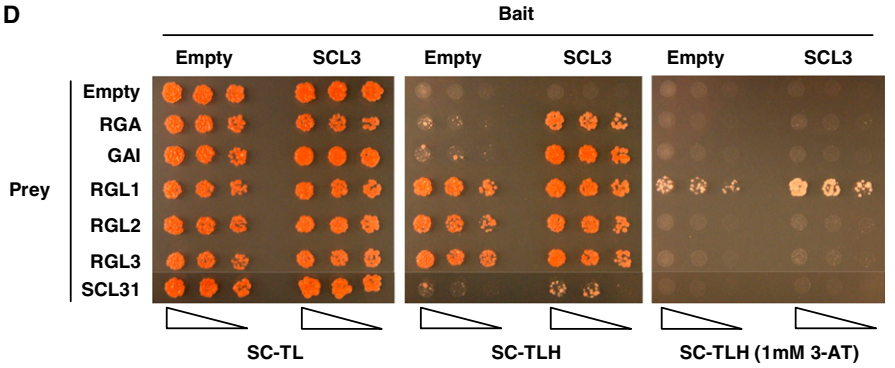


Fig. S2. Genetic and protein–protein interactions between SCL3 and RGA/SPY. (*A*) *rga* and *spy* are epistatic to *scl3* in root elongation assays. Root lengths were measured on day 10. (*B*) *rga* is partially epistatic to *scl3* in regulating rosette leaf expansion, flowering time, and stem elongation. a, the rosette diameters of *ga1-3* and *ga1-3 scl3* plants were measured on day 58; b, the rosette diameters of *ga1-3 rga-28* and *ga1-3 rga-28 scl3* plants were measured on day 37. (*C*) SCL3-GFP fusion proteins are nuclear-localized. A root segment of transgenic plant containing *35S:SCL3-GFP* (in the *rga-24 ga1-3* background) was imaged by confocal microscopy. (*D*) Weak interactions between SCL3 and DELLAs in yeast two-hybrid assays. SC-TL, synthetic complete medium lacking Trp and Leu; SC-TLH, synthetic complete medium lacking Trp, Leu, and His; 3-AT, a competitive inhibitor of His3 enzyme. For each strain, 2 μL yeast cells with OD_{600} values of 0.25, 0.125, and 0.05, respectively, were spotted on media plates. The triangle symbols indicate serial dilutions of yeast cells. Red colonies indicate more robust growth, because the host strain is $ade2^{-}$; a red color intermediate of the adenosine biosynthesis pathway accumulates when the colony grows to saturation (16). SCL3 showed a weak interaction with RGA and GAI in the SC-TLH media (0 mM 3-AT). As shown in the SC-TLH panel, RGL1, RGL2, and RGL3 alone (in the prey vector) self-activated the His3 reporter gene. RGL1 showed a weak interaction with SCL3 in the presence of 1 mM 3-AT. No interaction could be detected between SCL3 and the other two DELLAs (RGL2 and RGL3). SCL31, a GRAS protein that is divergent from both the DELLA subfamily and SCL3 (17), did not show any interaction with SCL3, suggesting that SCL3–DELLA interactions are specific.

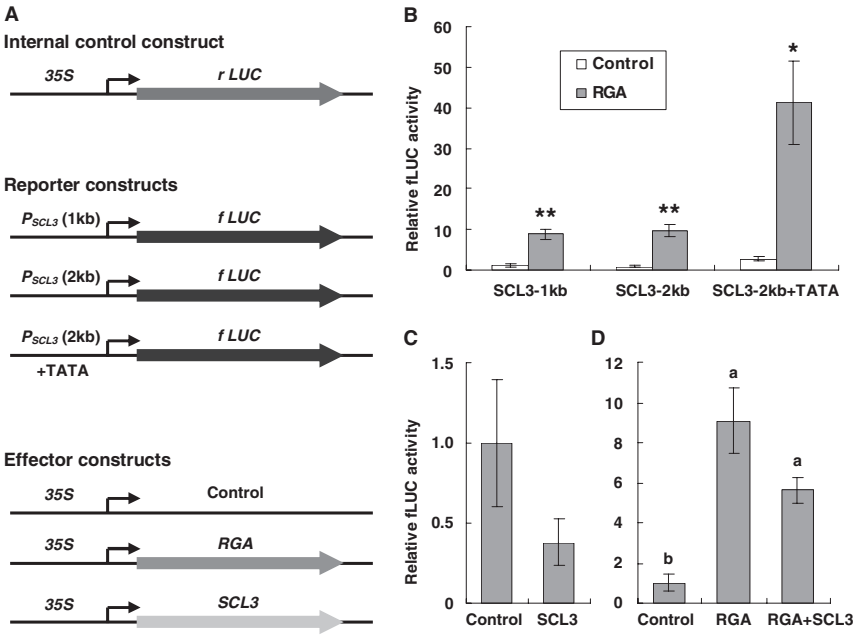


Fig. S3. SCL3 interferes with RGA to regulate *SCL3* promoter in transient expression studies. (*A*) Schematics of the internal control, reporter, and effector constructs. The firefly luciferase gene (*fLUC*) was placed under the control of different versions of *SCL3* promoter (P_{SCL3}:*fLUC*: 1, 2, and 2 kb + TATA) serving as reporter constructs. The 1- and 2-kb *SCL3* promoter constructs also contain 68 bases of the 5′ UTR. The third reporter construct (same as in Fig. 3) has the 2-kb promoter, but its −100 to ∼+1 region was replaced by the CaMV minimal promoter (−45- to ∼+1-bp region that includes the TATA box). *35S:Renilla LUC* (*rLUC*) served as an internal control for normalization of transformation efficiency; *35S:RGA* and *35S:SCL3* served as two effector constructs, respectively. The empty vector was used as a control in the transient coexpression assay. (*B*) The 2-kb *SCL3* promoter with the CaMV TATA sequence showed enhanced expression and remained responsive to RGA. P_{SCL3}:*fLUC* and *35S:rLUC* constructs were cobombarded into 14-d-old *ga1-3 rga-24 gai-t6* seedlings (in the L*er* background) (18) with the empty effector constructs (Control) or *35S:RGA* using the same molar ratios. The relative fLUC activity of *SCL3*-1kb was set to 1. Data represent the average value ± SE of three replicates. (*C* and *D*) The construct P_{SCL3}:*fLUC* (with 1-kb *SCL3* promoter) and the internal control *35S:rLUC* were cobombarded into 12-d-old *ga1 rga gai* seedlings with various effector construct, respectively, using the same molar ratio. The average fLUC activity of the control was set to 1. Data represent the average value ± SE of four replicates. Pair-wise *t* tests were performed. (*B*) $^{**}P < 0.01$; $^{*}P < 0.05$. (*D*) When two samples show different letters (a–b) above the bars, the difference between them is significant ($P < 0.05$).

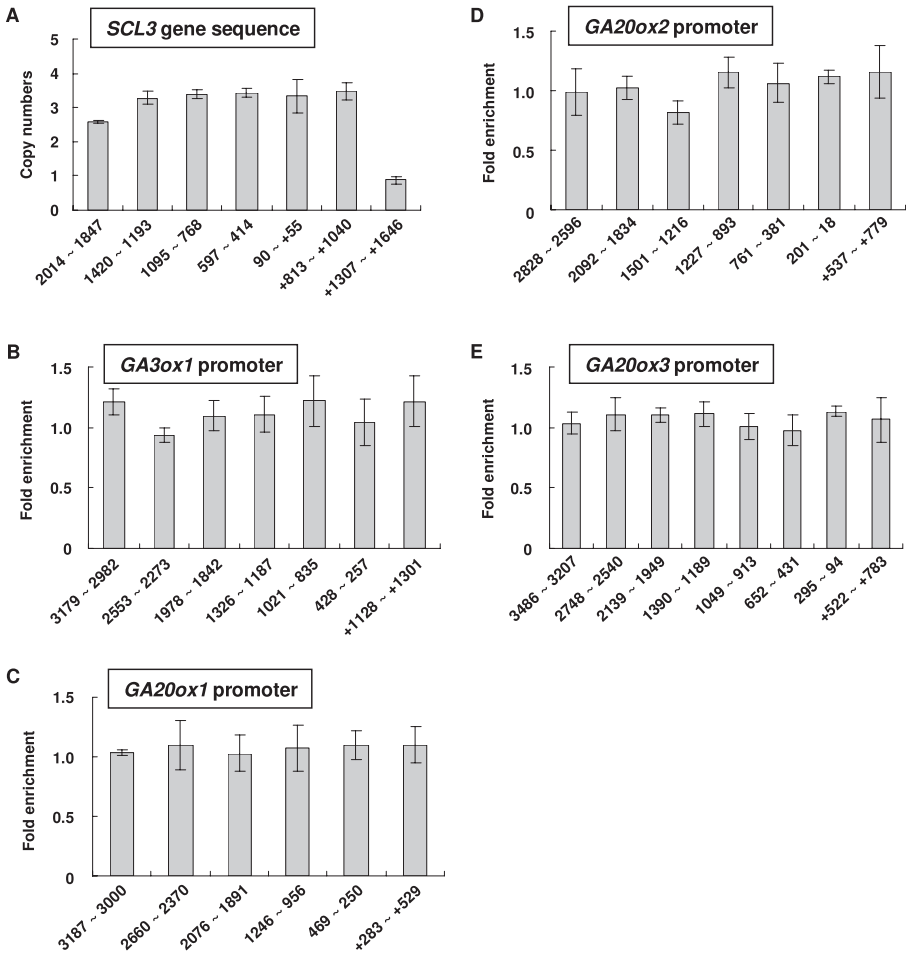


Fig. S4. Copy number of *SCL3* sequence in the *scl3* P_{SCL3}*:SCL3-GFP* line and promoter scanning of GA biosynthetic genes. (*A*) qPCR analysis of the copy numbers of each indicated region in the *scl3* P_{SCL3}*:SCL3-GFP* line (Fig. 4). The genomic DNAs of the transgenic line *scl3* P_{SCL3}*:SCL3-GFP* and the *scl3* mutant were used as templates. A series of primers were used to amplify different regions of the transgene P_{SCL3}*:SCL3-GFP* and the endogenous *SCL3* gene (Fig. 4). The only exception was that a primer pair flanking the stop codon of the native *SCL3* gene can amplify the endogenous *SCL3* gene sequence but not the P_{SCL3}*:SCL3-GFP* transgene (+1,307 to ~+1,646). The fold change of the *SCL3-GFP* line was normalized to the control *scl3*. (*B–E*) Promoter scanning of *GA3ox1* (*B*), *GA20ox1* (C), *GA20ox2* (*D*), and *GA20ox3* (*E*) by ChIP-qPCR. Chromatin preparations of the control *scl3* or the *SCL3-GFP* transgenic line were subjected to ChIP followed by qPCR. Fold enrichment of each region in the *SCL3-GFP* line was calculated by comparing with the control *scl3*. The values of fold enrichment are the average ± SE of three qPCR reactions from two independent ChIP experiments. (*A–E*) The numbers underneath each bar indicate base pairs upstream of the ATG of each gene. A plus sign indicates base pairs downstream of the ATG.

Table S1. List of primers and their uses

Primer	Sequence	Use	Notes
T-DNA analysis			
SALK_002516 LP	TTCCTCTGTTCTTTAACCCCC	Genotyping *SCL3*	WT allele 998-bp PCR product
SALK_002516 RP	AGCGCAGTTCTTTCTCATGAG		
SALK_LBa1	TGGTTCACGTAGTGGGCCATCG	Genotyping *scl3-1*	Mutant allele 844-bp PCR product
SALK_LBb1.3	ATTTTGCCGATTTCGGAAC	Genotyping *scl3-1*; sequencing T-DNA insertion site	Mutant allele 701-bp PCR product
Cloning			
SCL3-1	GTAGGAATTCATGGTGGCTATGTTTCAAGAAG	Cloning of SCL3 coding sequence	Construction of pSCL3-1
SCL3-2	ACTAGGATCCTCACTTCCTGCATCTCCAAGCT		
SCL3 CDS-5 (BamHI)	CGAGGATCCATGGTGGCTATGTTTCAAGAAG	Cloning of SCL3 coding sequence	Construction of pGEX-SCL3
SCL3 CDS-3 (SalI)	ATAGTCGACTCACTTCCTGCATCTCCAAG		
SCL31-3	CGTCCATGGAATCGAATTACTCAGGTG	Cloning of SCL31 coding sequence	Construction of pACT2-SCL31 (SCL31-AD)
SCL31-2	CGAGAATTCCTAAGAAGGGACCCAACAAGAAG		
RGA-224	ACGCGGATCCGAATGAAGAGAGATCATCACC	Cloning of RGA	Construction of pEG201-RGA
RGA-536	GACAGCGGCCGCTCAGTACGCCGCCGTCGAGA		
SCL3-GW-N(C)5	ATGGTGGCTATGTTTCAAGAAGATAATGG	Cloning of SCL3 coding sequence	Construction of pEG203-SCL3
SCL3-GW-N3	TCACTTCCTGCATCTCCAAGCTGATAC		
GUS-11	ATGTTACGTCCTGTAGAAACCCC	Cloning of GUS (+ NLS)	Construction of pEG203-GUS-NLS
GUS-12	TTATACCTTTCTCTTCTTTTTTGGATCTTGTTTG CCTCCCTGCTGCGG		
SCL3-prom-RI-Swa-F	AGAATTCATTTAAATCCCACACCCAAGCCTCAG	Cloning of 1-kb SCL3 promoter	Construction of pRZ506
SCL3-prom-Xho-R	AGCCTCGAGGGTTCTCTCAATCTTTATC		
3ox1-prom-Swa-F	CATTTAAATAACTGGTATTCAAAGATAG	Cloning of 1-kb GA3ox promoter	Construction of pRZ511
3ox1-prom-Xho-R	CCTCGAGAAGTGTGGTGTTTGGTG		
20ox2-prom-RI-Swa-F	AGAATTCATTTAAATTCAAACTATGTAAGACG	Cloning of 1-kb GA20ox2 promoter	Construction of pRZ507
20ox2-prom-Xho-R	AGCCTCGAGTGAGTGTTGTTGAGGAG		
SCL3-1900prom-H3-F	CTTCTTAAAGCTTCAAATAAGTTGATTCATCCATC	Cloning of 2-kb SCL3 promoter	Construction of pRZ535
SCL3-prom-Xho-R	AGCCTCGAGGGTTCTCTCAATCTTTATC		
35S-minimal-BHI-RV	CGGATCCGATATCGCAAGACCCTTCCTCTATA	Cloning of 35S-TATA box and 5′ end of LUC	Construction of pRZ516
LUC-750-R	GTGTAGTAAACATTCCAAAACC		
SCL3-1900prom-H3-F	CTTCTTAAAGCTTCAAATAAGTTGATTCATCCATC	Cloning of 2-kb SCL3 promoter (without TATA)	Construction of pRZ536
SCL3p-100-BHI-R	GGGAGGATCCTTTAAGAAAGAAAACACTA		
SCL3-NcoI-F	ATCCCATGGTGGCTATGTTTCAAG	Cloning of SCL3 coding sequence	Construction of pRZ530
SCL3-XbaI-R	GAGTCTAGAGTCACTTCCTGCATCTCCAAG		
SCL3 GF1	CACCATGGTGGCTATGTTTCAAGAAG	Cloning of SCL3 cDNA	Construction of SCL3-OE and 35S:SCL3-GFP
SCL3 GR	TCACTTCCTGCATCTCCAAG	Cloning of SCL3 cDNA	Construction of SCL3-OE and 35S:SCL3-GFP
SCL3 GWP F1	CACCAAAGGCAAGAGTTCAGGAGG	Cloning of SCL3 genomic DNA	Construction of P_{SCL3}:SCL3
SCL3 3′ Rev	GAGATCTTCTTTCCTTTTGGGATC	Cloning of SCL3 genomic DNA	Construction of P_{SCL3}:SCL3
Quantitative PCR			
Act11 fLC	TACCTCAGCAGAGAGCGT	Act 11 qRT-PCR	184-bp PCR product
Act11 rLC	GAACAGAACCTGGCCC		
GAPC F2	AGCTGCTACCTACGATG	GAPC qRT-PCR	219-bp PCR product
GAPC R2	CACACGGGAACTGTAAC		
At4g33380-5DQ	TAGAAACGTGGCGCAA	EXP-PT qRT-PCR	239-bp PCR product
At4g33380-3DQ	TGGTCTGCCTGCCAATA		
At18SFrt	ATACGTGCAACAAACCC	18S ChIP-qPCR	301-bp PCR product
At18SRrt	CTACCTCCCCGTGTCA		
SCL3 fLC	AACAACAATGGGTATAGCC	qRT-PCR analysis of developmental profile of SCL3	195-bp PCR product
SCL3 rLC	TGCTGCGTAGGTGTAA		
KS F	AACGGAGATTGGACTCAGAA	KS qRT-PCR	237-bp PCR product
KS R	CATGGTTATCAAGTCCCCAAG		
AtKO1 f	TGTCTGCGGAGGAGAAAAGT	KO qRT-PCR	149-bp PCR product
AtKO1 r	GATAGCCTCCGATTTGCGTA		
KAO1fLC	CTGACTCCTTCACTCGC	KAO1 qRT-PCR	224-bp PCR product
KAO1rLC	CCTGAGACGCTTGTGTT		
KAO2fLC	TCCATTTGGACCCTGAAATC	KAO2 qRT-PCR	234-bp PCR product
KAO2rLC	TGTGAGGCAAGAACATCACC		

Table S1. Cont.

Primer	Sequence	Use	Notes
GA20ox1-5DQ	GGGCTAAGTTTAGGCGT	GA20ox1 qRT-PCR	377-bp PCR product
GA20ox1-3DQ	GGTGGCGTCACTACTC		
GA20ox2F	TCCAACGATAATAGTGGCT	GA20ox2 qRT-PCR	234-bp PCR product
GA20ox2R	TTGGCATGGAGGATAATGA		
GA20ox3-5DQ	TCCTAACCCTCACGCT	GA20ox3 qRT-PCR	266-bp PCR product
GA20ox3-3DQ	TGTCTGCCCTATAATGCT		
GA4F	CCATTCACCTCCCACACTCT	GA3ox1 qRT-PCR	401-bp PCR product
GA4R	GCCAGTGATGGTGAAACCTT		
3ox2Frt	TGGTCCGAAGGTTTCAC	GA3ox2 qRT-PCR	298-bp PCR product
3ox2Rrt	GGGTCGAGTCTGTATGG		
GA2ox1FSGT	TGAGGACGAGAGGTTGTACGA	GA2ox1 qRT-PCR	101-bp PCR product
GA2ox1RSGT	TCCTTTCGAATTGTTGAAGCC		
GA2ox2-5DQ	TGGAAGTTGGGTCGCT	GA2ox2 qRT-PCR	199-bp PCR product
GA2ox2-3DQ	GACAAGGCATGGCAAT		
GA2ox4-5DQ	GGTCGAGTATTTGCTGTT	GA2ox4 qRT-PCR	221-bp PCR product
GA2ox4-3DQ	CGGACGGTGGATAATG		
GA2ox6-5DQ	GCGTTAAGTGGCGTTG	GA2ox6 qRT-PCR	212-bp PCR product
GA2ox6-3DQ	CTATGCCTCACGCTAGT		
SCL3CDS5UTR-5RQ (P2 F)	ACACACGCTATTACTCACAA	Detection of endogenous SCL3 transcript	349-bp PCR product
SCL3CDS5UTR-3RQ (P2 R)	CGAGGAGAGTCGGTCTG		
SCL3CDS-5RQ-2 (P1 F)	GAACTGCGCTTTACGG	Detection of tansgenic and endogenous SCL3 transcript	228-bp PCR product
SCL3CDS-3RQ-2 (P1 R)	GTGACCACCATGACCT		
SCL3-scan1A-F	TAAACGAGAGTTGCCTCC	ChIP-qPCR SCL3 promoter scanning (region 2,014 to 1,847)	168-bp PCR product
SCL3-scan1A-R	GCTTGGTACGAAGACCG		
SCL3-1300-F	CACACCCAAGCCTCAG	ChIP-qPCR SCL3 promoter scanning (region 1,420 to 1,139)	228-bp PCR product
SCL3-1300-R	TGGCATGAGGTGGATT		
SCL3-900-F	AATGCCAAATGGGTTCA	ChIP-qPCR SCL3 promoter scanning (region 1,095 to 768)	328-bp PCR product
SCL3-900-R	TGAGTGCTCTTAAAGTGGT		
SCL3-500-F	AACCGACTATCACGCA	ChIP-qPCR SCL3 promoter scanning (region 597 to 414)	184-bp PCR product
SCL3-500-R	GGGAGTGAGAGGGTTC		
SCL3-scan3A-F	TCGTTACGAGTCTCTCAAAG	ChIP-qPCR SCL3 promoter scanning (region 90 to +55)	145-bp PCR product
SCL3-scan3A-R	GTGGTGATGAAGCTACAG		
SCL3CDS-5RQ-2	GAACTGCGCTTTACGG	ChIP-qPCR SCL3 promoter scanning (region +813 to +1,040)	228-bp PCR product
SCL3CDS-3RQ-2	GTGACCACCATGACCT		
SCL3-5RQ	ATTATGCGATGTTGCAGG	ChIP-qPCR SCL3 promoter scanning (region +1,307 to +1,646)	340-bp PCR product
SCL3-3RQ	ATTACACCCACACCAGAC		
3ox1-scan8A-F	TGTTGGACATTGTCGTG	ChIP-qPCR GA3ox1 promoter scanning (region 3,179 to 2,982)	198-bp PCR product
3ox1-scan8A-R	GTGTGACTGTGTGTCAT		
3ox1-scan10A-F	CCCTTTGTTAAGATGCCAG	ChIP-qPCR GA3ox1 promoter scanning (region 2,553 to 2,273)	281-bp PCR product
3ox1-scan10A-R	TGATAATCACTCCCGAATCTA		
3ox1-scan11A-F	ACATATCACGGTCATTACCA	ChIP-qPCR GA3ox1 promoter scanning (region 1,978 to 1,842)	152-bp PCR product
3ox1-scan11A-R	TGCTGCAAAGTAGACG		
3ox1-scan12A-F	AGACTAGCGACCACAT	ChIP-qPCR GA3ox1 promoter scanning (region 1,326 to 1,187)	159-bp PCR product
3ox1-scan12A-R	TTGGTTAGACGATTACGGAG		
GA3ox1-900-F	CGTTCCCTTCCAAAGT	ChIP-qPCR GA3ox1 promoter scanning (region 1,021 to 835)	187-bp PCR product
GA3ox1-900-R	AAGGGCCAATGTTGTC		
3ox1-scan14A-F	ATGGATAGATACGGTTTAACTT	ChIP-qPCR GA3ox1 promoter scanning (region 428 to 257)	172-bp PCR product
3ox1-scan14A-R	GTCCCGCCATTTCCTT		
3ox1-coding2-F	ATACCGACTCCACCCT	ChIP-qPCR GA3ox1 promoter scanning (region +1,128 to +1,301)	174-bp PCR product
3ox1-coding2-R	GGTGCAACACGCTTTTA		
GA20ox1-scan-F4	ATCTTGATCGTACACTTGTT	ChIP-qPCR GA20ox1 promoter scanning (region 3,187 to 3,000)	188-bp PCR product
GA20ox1-scan-R4	TGGACCACTAGAGGTTG		
GA20ox1-scan-F5	GACATGCTTTCAGCGT	ChIP-qPCR GA20ox1 promoter scanning (region 2,660 to 2,370)	291-bp PCR product
GA20ox1-scan-R5	ACCCAACAATGCTGGA		
GA20ox1-scan-F7	GAGCCGGAATCAACCT	ChIP-qPCR GA20ox1 promoter scanning (region 2,076 to 1,891)	186-bp PCR product
GA20ox1-scan-R7	AGACATGAGCATGGTCG		
GA20ox1-scan-F9	AAGCGGTAGCAAGACT	ChIP-qPCR GA20ox1 promoter scanning (region 1,246 to 956)	291-bp PCR product
GA20ox1-scan-R9	GGCTGGGTGATTGAGAA		
GA20ox1-300-F	TGCCACACAACAAACAT	ChIP-qPCR GA20ox1 promoter scanning (region 469 to 250)	220-bp PCR product
GA20ox1-300-R	TTCGCTAAAACAAGTGGT		
GA20ox1-scan-F13	GTGGTCAATCACGGCA	ChIP-qPCR GA20ox1 promoter scanning (region +283 to +529)	247-bp PCR product
GA20ox1-scan-R13	CCAACGCATCGCAGAA		

Table S1. Cont.

Primer	Sequence	Use	Notes
GA20ox2-scan11-F	AACAGTTAGGTTCCTAGTCT	ChIP-qPCR GA20ox2 promoter scanning (region 2,828 to 2,596)	233-bp PCR product
GA20ox2-scan11-R	GGTTTCAGTCATGCCTT		
GA20ox2-scan9-F	GCACTCTCTAAGCAGCG	ChIP-qPCR GA20ox2 promoter scanning (region 2,092 to 1,834)	259-bp PCR product
GA20ox2-scan9-R	AGGTAGGGCCAATAGC		
GA20ox2-scan7-F	ATCATTCGTGGGTGCC	ChIP-qPCR GA20ox2 promoter scanning (region 1,501 to 1,216)	286-bp PCR product
GA20ox2-scan7-R	GGTTGGTTCGACCAGT		
GA20ox2-scan5-F	GTCGAACCAACCATGAAT	ChIP-qPCR GA20ox2 promoter scanning (region 1,227 to 893)	335-bp PCR product
GA20ox2-scan5-R	AGGAGGAAGAGGTTCCC		
GA20ox2-scan3-F	GACCGAAGCCAAAATTGA	ChIP-qPCR GA20ox2 promoter scanning (region 761 to 381)	381-bp PCR product
GA20ox2-scan3-R	CATTCTTATCGGAATCTTGC		
GA20ox2-scan1-F	ATGAAATCCCATGTGGC	ChIP-qPCR GA20ox2 promoter scanning (region 201 to 18)	184-bp PCR product
GA20ox2-scan1-R	TCTTGAGAGTTTTGGGGT		
GA20ox2-coding1-F	GCAGTTTGGGTACGTG	ChIP-qPCR GA20ox2 promoter scanning (region +537 to +779)	243-bp PCR product
GA20ox2-coding1-R	GTCTCGGTTTACGCCT		
GA20ox3-scan-F1	CACGACATTGTTGTGTTATGC	ChIP-qPCR GA20ox3 promoter scanning (region 3,486 to 3,207)	280-bp PCR product
GA20ox3-scan-R1	GGTCATCACGGTAGAGTT		
GA20ox3-scan-F3	CGCTCGTCAGTAAAGCA	ChIP-qPCR GA20ox3 promoter scanning (region 2,748 to 2,540)	209-bp PCR product
GA20ox3-scan-R3	TTGACAGATAGATAGCCTCAC		
GA20ox3-scan-F6	GAATGAACTTGCTCTCTGAT	ChIP-qPCR GA20ox3 promoter scanning (region 2,139 to 1,949)	191-bp PCR product
GA20ox3-scan-R6	GTGATAGGTGGTGCTCT		
GA20ox3-scan-F7	TGTTGTGTTCTTCCACTTT	ChIP-qPCR GA20ox3 promoter scanning (region 1,390 to 1,189)	202-bp PCR product
GA20ox3-scan-R7	GTGAGGCCCATCGTAG		
GA20ox3-1000-F	ACGGACGGATTTAGAGG	ChIP-qPCR GA20ox3 promoter scanning (region 1,049 to 913)	137-bp PCR product
GA20ox3-1000-R	GCTACAACCCCCGTTA		
GA20ox3-500-F	TATTCCGTTTGGATGGGT	ChIP-qPCR GA20ox3 promoter scanning (region 652 to 431)	222-bp PCR product
GA20ox3-500-R	AAATTGCCAGTCGAGTAT		
GA20ox3-scan-F9	GTCGATACAGCCAGTTCA	ChIP-qPCR GA20ox3 promoter scanning (region 295 to 94)	202-bp PCR product
GA20ox3-scan-R9	GGTCGGTAAGTGGGAGA		
GA20ox3-scan-F11	ATGGGCGATGGATACG	ChIP-qPCR GA20ox3 promoter scanning (region +522 to +783)	262-bp PCR product
GA20ox3-scan-R11	ACTGCGGGTAGTAATTCA		