Table 2. *suvh* and *suvr* mutants used in this study

Mutant	ChromDB set domain group (SDG)	AT identifier	Mutant line
suvh1	SDG32	AT5G04940	SALK_003675
suvh2	SDG3	AT2G33290	SALK_079574
suvh3	SDG19	AT1G73100	SAIL_401b_D01
suvh4/kyp	SDG33	AT5G13960	SALK_041474
suvh6	SDG23	AT2G22740	SAIL_1244_F04
suvh7	SDG17	AT1G17770	GABI-Kat 037C06
suvh8	SDG21	AT2G24740	SALK_123140
suvh9	SDG22	AT4G13460	SALK_048033
suvr1	SDG13	AT1G04050	SALK_012786
suvr2	SDG18	AT5G43990	SAIL_832_E07
suvr3	SDG20	AT3G03750	SALK_063174
suvr5	SDG6	AT2G23750	SALK_026224

Table 3. Markers for genotyping mutants described in this study

Lesion	Diagnostic marker	Primer 1	Primer 2	Notes
vrn1-2	dCAPS	AAGAGAGGGAGGAAGCAGAAAA	TCTGTAAGGACAATATCAAAAGG	BstAPI digest. WT, 134 bp; vrn1-2, 114 + 20 bp
vrn1-3	SAIL_1247_D06 T-DNA PCR	TGGTCATTGGTTGTGTTTGG	CTTGCCAACCATCTTGAAAC	Primers amplify WT. Use primer 2 with GARLIC LB to amplify T-DNA.
vin3-5	SALK_004766 T-DNA PCR	TTTCGATCTTGTGTTCTTCATCA	GATCATCACTCCCAATCCCA	Primers amplify WT. Use primer 2 with SALK LB to amplify T-DNA.
kyp-2	dCAPS	GCAGTGAAGATGAGAATGCGCCAGAGTTC	CGCTATCAAGCGCATATCCATAGTCGTAAGTGAGATC	Bgl II digest. *kyp-2*, 270 + 36 bp; WT, 306 bp
fca-1	Phenotype or dCAPS	AACCTCTTCACAGTCCACAGGG	TTGGCCGTAGATTATTGTTCAAAGG	MseI digest. *fca-1*, 158 + 30 bp; WT, 188 bp
vrn2-1	dCAPS	TGCGTTCATTAAGTAGGCAACAGAAAATGG	GAGAAGTAGTTACCTTTGTTTTCTTACAGAAGAGT	XmnI digest. *vrn2-1*, 200 + 20 bp; WT, 220 bp
lhp1-3	Phenotype or CAPS	CTAAGCGGTTCGAGTCTATT	GCCATTGGGTCTTACATTAT	AfI II digest. *lhp1-3*, 49 + 191 bp; WT, 240 bp
clf-50	Phenotype			
swn-3	SALK_050195 T-DNA PCR	GTCTGTCTGGAAAAGAATAGCTGGT	TGGTTTAGTGTGATTGGTTCGTTCAC	Primers amplify WT. Can use either with SALK LB to amplify T-DNA (inverted repeat).
emf2-3	Phenotype or SSLP	CGGACCGGGATAGTGAAGATGAAG	AGTCAGAGAACATGGATGCGTATG	WT, 644 bp; emf2-3, 609 bp

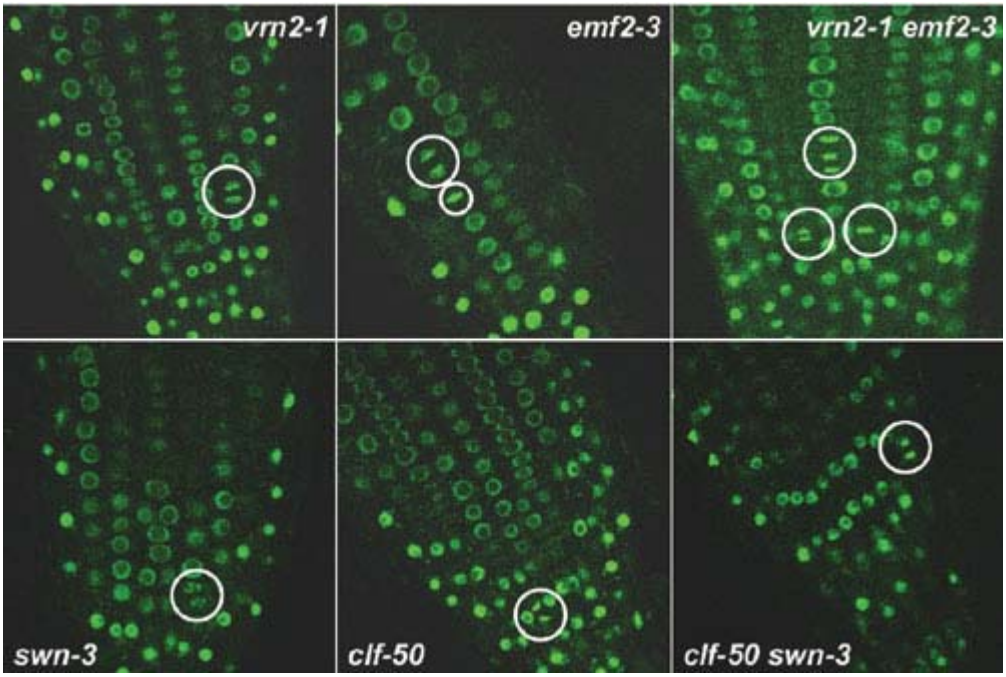

Fig. 5. VRN1/GFP expression pattern in the root tips of single and double Polycomb mutants. Mitotic cells are circled.

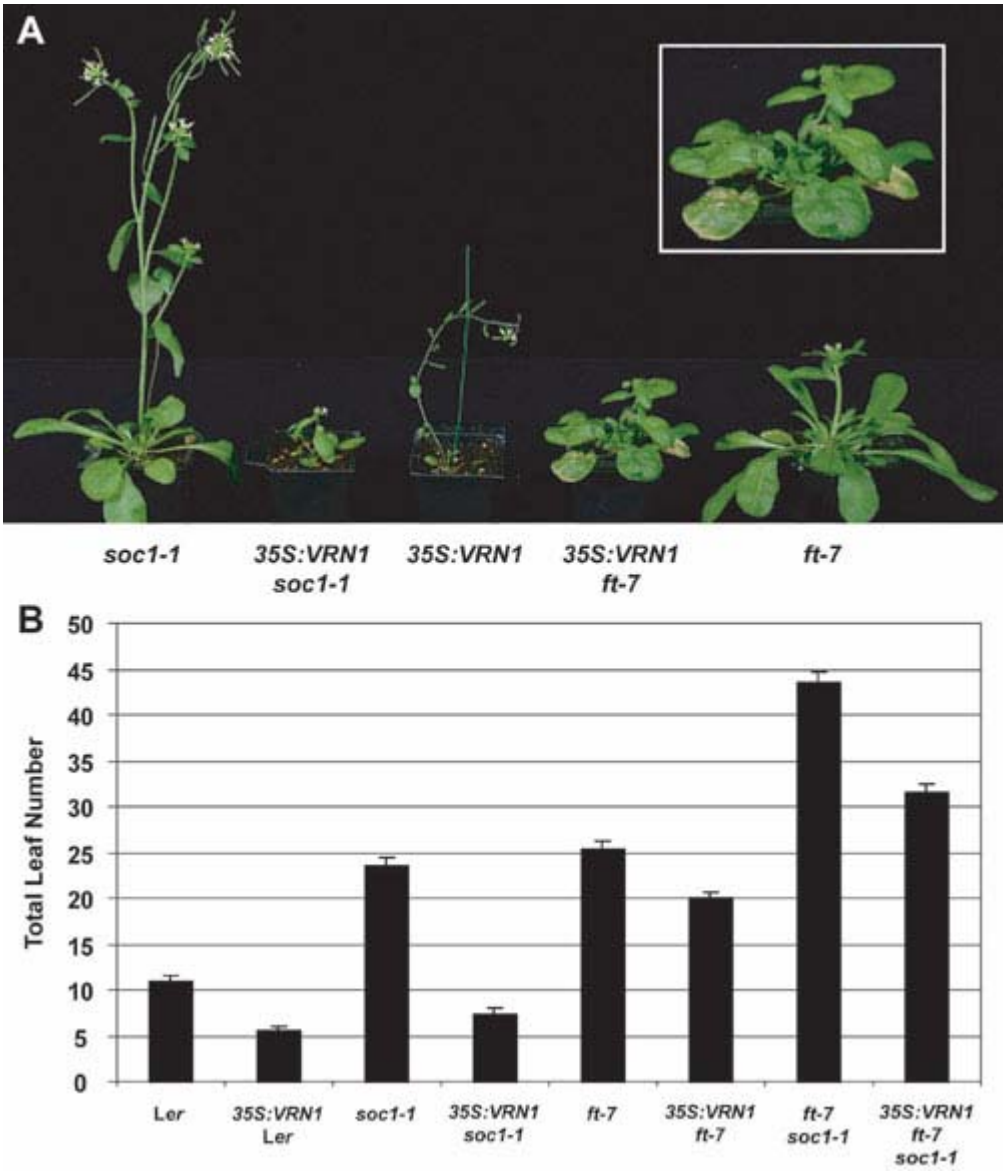

Fig. 6. *ft-7* and *soc1-1* mutations reduce but do not remove the phenotypic effects of a *35S:VRN1* transgene. (*A*) Plants grown for 1 month in extended short days. Left to right are *soc1-1*, *35S:VRN1 soc1-1*, *35S::VRN1 Ler*, *35S::VRN1 ft-7*, and *ft-7* (*Inset*). (*B*) Effect of different genetic backgrounds on the flowering time (in extended short days) conferred by the same *35S:VRN1* transgene, measured as total leaf number ± SE.

Supporting Materials and Methods

Plant Materials and Growth Conditions. For soil experiments, seeds were sown on soil in plastic pots (7 × 7 cm). Plants were vernalized for 4 or 6 weeks immediately after sowing at 4°C with an 8-h photoperiod [photosynthetically active radiation (PAR), 9.5 μmol·m^{-2}·sec^{-1}; red/far red ratio, 3.9]. Nonvernalized plants were stratified for 2 days in the same conditions. Plants were subsequently moved from the cold to extended short days at 20°C in controlled-environment rooms (Sanyo Gallenkamp, Loughborough, U.K.) with a 16-hr photoperiod (PAR, 114 μmol·m^{-2}·sec^{-1}) composed of 10 h from 400-W Wotan metal halide lamps and 100-W tungsten halide lamps (red/far red ratio, 2.4) and a 6-h extension of exclusively tungsten halide lamps (red/far red ratio, 0.66). Young seedlings were transferred to trays with 40 cells of 2 × 2 cm. Flowering time was measured by counting total leaf number, which was scored as the number of rosette leaves plus cauline leaves. Plants grown on plates were sown aseptically in Petri dishes containing GM medium (1× Murashige and Skoog salts, 1% Glc, 0.5 mg/liter pyridoxine, 0.5 mg/liter nicotinic acid, 0.5 mg/liter thymidine, 100 mg/liter inositol, 0.5 g/liter MES, and 0.8% agar, pH 5.7).

Immunolabeling and Detection. Slides were incubated with primary antibodies overnight at room temperature. If the slide was incubated with both the VRN1 antibody and any of the histone modification antibodies mentioned above, VRN1 was detected with the secondary antibody donkey anti-rat FITC (1:100; The Jackson Laboratory), and the histone modification was detected with donkey anti-rabbit Cy3 (1:500; The Jackson Laboratory). If the slide was incubated with only an antibody against histone modification, it was then detected with the secondary antibody goat anti-rabbit Alexa Fluor 488 (1:100, Molecular Probes) and the tertiary antibody donkey anti-goat FITC (1:200, The Jackson Laboratory). Nuclei were counterstained with DAPI (2 μg/ml in Vectashield (Vector Laboratories) before observation.

Luciferase Assays. Plants were sprayed with a 1 mM solution of beetle luciferin (E1603, Promega) and left in the dark for 45 min to 1 hour at room temperature before they were imaged by using a Photek High Resolution Photon Counting System [HRPCS 218, camera model 6045-2/2149-1 (Photek, St. Leonards-on-Sea, East Sussex, U.K.)]. Images were acquired and processed by using the program IFS32 (Photek).

Expression Analysis. RNA was isolated by using a scaled-down protocol described in Etheridge *et al.* (1). Blotted membranes were probed with a region of *FLC* corresponding to bases 297–705 *FLC* cDNA (GenBank accession no. AF116527), a β-*TUBULIN* probe specific for AtIg20010 or an *18S rDNA* probe. RT-PCR for *FLC* was performed as described in Michaels *et al.* (2), and *TUB* control primers are described in Kobayashi *et al.* (3).

1. Etheridge, N., Trusov, Y., Verbelen, J. P. & Botella, J. R. (1999) *Plant Mol. Biol.* **39,** 1113–1126.

2. Michaels, S. D., Ditta, G., Gustafson-Brown, C., Pelaz, S., Yanofsy, M. & Amasino, R. M. (2003) *Plant J.* **33,** 867–874.

3. Kobayaski, Y., Kaya, H., Goto, K., Iwabuchi, M. & Araki, T. (1999) *Science* **286,** 1960–1962.