MOLECULAR BIOLOGY

PRC2 recruitment and H3K27me3 deposition at FLC require FCA binding of COOLAIR

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The cold-induced antisense transcript COOLAIR represses FLOWERING LOCUS C (FLC) transcription with increased H3K27me3 and decreased H3K36me3 levels in response to cold temperatures. However, the molecular connection between COOLAIR and histone modification factors in the absence of cold treatment remains unclear. We report that the RNA binding protein FCA interacts with the PRC2 subunit CURLY LEAF (CLF) and binds nascent COOLAIR transcripts to allow deposition of H3K27me3 at FLC. Loss of COOLAIR function results in a reduction in FCA and CLF enrichment, which, in turn, decreases H3K27me3 levels at FLC. The Arabidopsis protein phosphatase SSU72 physically interacts with the RRM1 motif of FCA to antagonize FCA binding with COOLAIR. Mutations in SSU72 caused early flowering, reduced FLC transcription, increased CLF enrichment and H3K27me3, and enhanced affinity between FCA and COOLAIR. Our results suggest that FCA binding of COOLAIR and SSU72 is critical for PRC2 enrichment and H3K27me3 deposition in Arabidopsis.

INTRODUCTION

FLOWERING LOCUS C (FLC) encodes a MADS box transcription regulator that functions as a central floral repressor (1). FLC expression is activated by FRIGIDA and repressed by the prolonged cold of winter through vernalization (1, 2). FLC is also repressed by an autonomous pathway involving several factors, including the RNA binding proteins FLOWERING CONTROL LOCUS A (FCA), FLOWERING LOCUS K (FLK), and FPA, and the additional factors FY, FVE, LUMINIDEPENDENS (LD), and FLOWERING LOCUS D (FLD) (3–5). FCA has a WW domain for protein-protein interaction and two RNA recognition motifs (RRMs); these bind to RNA and prefer sequences rich in U and G (3). FCA interacts with FY, which is involved in mRNA 3' end processing (4). Loss of FCA function results in late flowering, whereas overexpression of FCA leads to early flowering under long-day (LD) and short-day (SD) photoperiods. FCA represses the production of FLC sense and antisense transcripts, notably COOLAIR (6, 7). FCA also binds the FLC locus and maintains the dimethylation of K4 at histone H3 (H3K4me2) through the histone demethylase FLD (6). In addition to flowering time, FCA contributes to RNA-mediated chromatin silencing and DNA methylation in response to a homologous inverted repeat (SUC-PDS) (8, 9).

The activation of FLC requires histone methylation by SEI domain group (SDG) proteins. ARABIDOPSIS TRITHORAX–RELATED 1 (ATX1) is associated with methylation of histone H3 at Lys4 (H3K4) at FLC chromatin and activates FLC expression (10–12). SDG8 delays flowering by promoting FLC transcription and the methylation of histone H3 at Lys36 (H3K36) (13). FLC is repressed by Polycomb repressive complex 2 (PRC2), which catalyzes the methylation of histone H3 at Lys27 (H3K27). PRC2 was first found in Drosophila and is composed of four core proteins: Enhancer of zeste [E(z)], Extra sex combs (ESC), Suppressor of zeste 12 [Su(z)12], and P55 (14). The SET domain protein E(z) is the catalytic subunit, and Esc is crucial for boosting E(z) enzymatic activity. The noncatalytic subunits Su(z)12 and P55 are essential for nucleosome binding (14–16). PRC2 is evolutionarily conserved in Drosophila, mammals, and plants (17). Sequence similarity and genetic experiments have shown that Arabidopsis E(z) homologs, such as CURLY LEAF (CLF), MEDEA (MEA), and SWINGER (SWN), potentially function as H3K27me3 methyltransferases (17–19). CLF and SWN are mainly active in vegetative stages, whereas MEA is mostly active in reproductive stages (17). CLF loss of function results in early flowering due to induced transcription and reduced H3K27me3 methylation of multiple genes, including the floral organ identity gene AGAMOUS (AG), flowering-promoting genes FLOWERING LOCUS T (FT) and AGAMOUS-LIKE 19 (AGL19), and the floral repressor gene FLC (20).

In addition to chromatin modifications, FLC is regulated by multiple long noncoding RNAs (lncRNAs), including COOLAIR, COLD ASSISTED INTRONIC NONCODING RNA (COLDAIR), and COLDWRAP (21–24). These three transcripts are induced by cold treatment and contribute to transcriptional repression of FLC via enrichment of H3K27me3 at FLC (21–23). COOLAIR is an FLC antisense transcript with alternative polyadenylation and multiple splice variants linked to different FLC expression states (21, 25). COOLAIR splicing is also altered by natural intronic polymorphisms that regulate FLC expression (26). Complementation of the flc-2 mutant using genomic FLC DNA and exchanging the FLC terminator/COOLAIR promoters disrupted the synchronized replacement of H3K36me3 with H3K27me3 at the intragenic FLC transcriptional site during cold treatment (27), suggesting that COOLAIR might be involved in the switching of FLC chromatin states. Unlike COOLAIR, COLDAIR and COLDWRAP are sense transcripts (relative to FLC mRNA transcription) and directly associate with PRC2 to suppress FLC expression (22, 23). In addition to environment, the transcription of COOLAIR was induced by FCA, and reduction of COOLAIR levels resulted in late flowering (6, 7, 28).
The C-terminal repeat domain (CTD) of the largest subunit (Rbp1) of RNA polymerase II (Pol II) comprises 25 to 52 tandem copies of the consensus repeat heptad Y₃S₂P₃T₁S₃P₃S₃ from yeast to animals and plants. Each repeat has one phosphorylated tyrosine, one phosphorylated threonine, and three phosphorylated serines: Ser³, Ser⁷, and Ser⁷ (29). Changes in phosphorylation may affect the association of Pol II with different factors. Ssu72 interacts with TFIIB and functions as a component of the cleavage/polyadenylation factor complex. Loss of Ssu72 function leads to impaired transcription and defects in pre-mRNA and small nucleolar RNA 3’ end formation (30, 31). Ssu72 binds phosphorylated Ser¹ and Ser⁷ of the CTD and is highly conserved among eukaryotes (32). In addition, human Ssu72 specifically dephosphorylates Ser5P of the CTD (30).

IncRNAs and PRC2-mediated deposition of H3K27me3 are critical for development, chromatin modification, and transcriptional regulation. COOLAIR represses FLC transcription by inducing H3K27me3 with cold treatment (21). In addition to vernalization, COOLAIR is involved in the autonomous flowering pathway (7, 28). Although these findings hint at the importance of COOLAIR in FLC regulation, the fundamental question of how COOLAIR targets FLC to participate in histone modifications remains to be understood. CLF binds to COOLAIR, and these two factors form a CLF-COOLAIR complex to induce H3K27me3 at FLC (22). By contrast, no known CLF binds COOLAIR. The recruitment of PRC2 to FLC by COOLAIR might depend on its interaction with specific RNA binding proteins; however, the mechanisms that allow COOLAIR to alter histone modifications in the autonomous pathway remain to be identified.

Here, we show that FCA interacts with the PRC2 subunit CLF. FCA directly binds to long and short COOLAIR transcripts, and this allows CLF to target FLC for H3K27me3 deposition. SSU72 interacts with the RRM1 of FCA and antagonizes its binding to COOLAIR. Defects in COOLAIR and FCA result in the reduction of H3K27me3 and decreased CLF enrichment, whereas the loss of SSU72 function leads to increases in H3K27me3 and CLF enrichment at FLC. Together, our results suggest that the binding between COOLAIR and FCA is required for H3K27me3 deposition, and SSU72 antagonizes this binding, thus participating in CLF enrichment and H3K27me3 deposition at FLC.

RESULTS

FCA binds to COOLAIR in vitro and in vivo

The N terminus of FCA (FCAN) contains two RRM, which may have RNA binding ability (Fig. 1A). We therefore investigated whether these two RRM bind the transcripts from the FLC locus, including FLC, COOLAIR, COLDAIR, and COLDWRAP (Fig. 1B). Beads attached to His-RMs were incubated with FLC, COOLAIR, COLDAIR, and COLDWRAP RNAs, and then the amount of RNA pulled down by the beads was quantified by RT-PCR. The two classes of COOLAIR transcripts were highly enriched by pull-down with beads attached to the His-tag fused FCAN (His-FCAN), but COLDAIR, COLDWRAP, and FLC transcripts were not enriched (Fig. 1C). However, the RNA binding protein FPA is not observed to bind to COOLAIR transcripts (Fig. 1D and fig. S1A). These results were confirmed by RNA pull-down, as bead-bound biotin-GU-rich RNA and biotin-COOLAIR RNA bound to His-FCAN, but COLDAIR, COLDWRAP, and reverse-complementary COOLAIR RNA did not (Fig. 1E). To further confirm these results, we incubated biotin-COOLAIR with whole-cell extracts and detected FCA binding with a specific antibody (fig. S1, B to D). The GU-rich RNA and two classes of COOLAIR transcripts were observed to bind to FCA, but COLDAIR, COLDWRAP, and reverse-complementary COOLAIR RNA did not (Fig. 1F), suggesting that FCA specifically bound to COOLAIR.

COOLAIR is required for FCA occupancy

To investigate whether COOLAIR is required for FCA enrichment, we isolated two Arabidopsis thaliana coolair T-DNA insertion mutants. Genotyping showed that the coolair-1 and coolair-2 alleles each contain a T-DNA in the COOLAIR promoter region (Fig. 1B and fig. S2A). RT-PCR analyses revealed that coolair-1 and coolair-2 are knockdown alleles (fig. S2B). coolair-1 was originally named fcl_utr4 (28). The coolair-1 and coolair-2 mutants displayed late flowering under an LD photoperiod (Fig. 1G and fig. S2C), consistent with previous results (28). Transforming the coolair-1 and coolair-2 mutants with a full-length COOLAIR DNA driven by the COOLAIR promoter complemented their late-flowering phenotype (fig. S2, D to F). We then examined FLC transcripts and found higher levels of spliced and unspliced FLC in coolair mutants (Fig. 1H and fig. S2G), suggesting that COOLAIR suppresses FLC transcription.

The FCA-COOLAIR complex was investigated using RNA immunoprecipitation (RIP) with specific FCA antibodies, followed by quantitative RT-PCR analysis to measure the amount of RNA. COOLAIR RNA was present at high levels in the wild type and at lower levels in the coolair mutants (Fig. 1I and fig. S2, H to J), suggesting that COOLAIR is a limiting factor for the FCA-COOLAIR interaction in vivo.

FCA interacts with CLF in vitro and in vivo

Given that COOLAIR modulates FLC transcription and histone methylation and that FCA directly binds COOLAIR, we asked whether FCA interacts with one of the SET domain proteins that are responsible for histone methylation. In a yeast two-hybrid system, FCA fused with a binding domain (BD) bound to CLF fused with an activation domain (AD) (Fig. 2A). This yeast two-hybrid interaction was verified by a protein pull-down assay. Soluble His-tagged FCA was observed to bind to beads attached to glutathione S-transferase (GST)-CLF, but not GST alone (Fig. 2B and fig. S3A). In complementary experiments, soluble GST-tagged CLF bound to beads attached to His-FCA, but not to the His control (Fig. 2C and fig. S3B).

We also tested this interaction by bimolecular fluorescence complementation (BiFC) and observed signal from reconstitution of yellow fluorescent protein (YFP) in the nucleus in cells coexpressing CLF fused to the YFP N terminus (CLF-YFPN) and FCA fused to the YFP C terminus (FCA-YFPC) (Fig. 2D), thus supporting our finding that FCA binds directly to CLF. We further tested the FCA-CLF interaction by coimmunoprecipitation (Co-IP). To this end, we expressed hemagglutinin (HA)–tagged FCA and FLAG-tagged CLF in Arabidopsis thaliana protoplasts and performed anti-FLAG immunoprecipitation. CLF, but not the controls, bound to FCA (Fig. 2E). This interaction was confirmed with immunoprecipitation using anti-HA, followed by detection of CLF using anti-FLAG (fig. S4A).

To investigate whether CLF could bind FCA in cells, we generated a construct containing the CLF native promoter driving CLF tagged with HA (ProCLF:HA-CLF) and transformed this construct into the clf-29 mutant. ProCLF:HA-CLF rescued the early-flowering phenotype of clf-29, indicating that the fusion protein retains function (fig. S5, A and B). Cell extracts from 10-day-old seedlings were
Fig. 1. FCA binds COOLAIR in vitro and in vivo. (A) Diagram of FCA showing its different domains. (B) Gene structure of FLC, COOLAIR, COLDWRAP, and COLDAIR, indicating exons (boxes), introns (lines), and T-DNA insertions (triangles). The primers used for genotyping are marked with arrows. The locations of the gene regions analyzed by RIP reverse transcription polymerase chain reaction (RT-PCR) are marked. bp, base pair. (C) Beads containing a His tag (His) or His-fused FCAN were tested for their binding of COOLAIR, COLDAIR, COLDWRAP, and FLC RNAs, followed by RT-PCR. (D) Beads containing a His tag (His) or His-fused N terminus of FPA (FPAN) were tested for their binding of COOLAIR RNAs, followed by RT-PCR. (E) Beads containing biotin-fused COOLAIR (+), complementary COOLAIR (−), COLDAIR, COLDWRAP, and FLC RNAs were tested for their binding of FCAN. The GU-rich RNA (GUUGUUUUGUUU) was used as a positive control, and FPA was used as a negative control. (F) Beads containing biotin-fused COOLAIR (+), complementary COOLAIR (−), COLDAIR, COLDWRAP, and FLC RNAs were tested for their FCA binding in vivo. The GU-rich RNA (GUUGUUUUGUUU) was used as a positive control. (G) Thirty-day-old Col-0 and coolair plants under an LD photoperiod. (Photo credit: Y.T., University of Science and Technology of China.) (H) FLC transcripts were tested by RT-PCR in the coolair mutants. Experiments were repeated three or more times, and data from a representative experiment are shown. Data are shown as means ± SE (n = 3 replicates). UBIQUITIN 10 was used as an internal control. (I) The FCA-COOLAIR complex was examined by RIP RT–quantitative PCR in wild-type and coolair mutants. RNA was immunoprecipitated with anti-FCA or anti-immunoglobulin G (IgG), followed by RT-PCR using region 1 primers. The amount of COOLAIR RNA immunoprecipitated with anti-FCA was measured relative to the amount of COOLAIR immunoprecipitated with anti-IgG. Experiments were repeated at least three times, and data from the representative experiments are presented as means ± SE (n = 3 replicates). Data that differ significantly from Col-0 (based on Student’s t test, P < 0.01) are marked with asterisks.
immunoprecipitated using anti-HA antibody and then detected with anti-FCA antibody. CLF, but not IgG control, bound to FCA (Fig. 3F). This result was confirmed with immunoprecipitation using anti-FCA antibody and then with immunoblotting using anti-HA antibody (fig. S4B). In addition, this interaction is mostly independent of RNA binding. Together, these results suggest that CLF interacts with FCA in vitro and in vivo.

**CLF enrichment at FLC requires FCA function**

We next examined the FCA-CLF genetic interaction by crossing *fca-9* into the *clf-29* mutant background. The *fca-9* *clf-29* double mutant displays late flowering under LD conditions (fig. S6, A and B). FCA represses *FLC* and activates other flowering regulators such as *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1* (*SOC1*); moreover, CLF represses both *FLC* and *FT*. Therefore, the late-flowering
phenotype of the fca-9 clf-29 double mutant might be due to the misregulation of FT, SOC1, and FLC. To test this, we investigated the transcripts of FT and SOC1 in the fca-9 clf-29 double mutant. The activation of FT in clf-29 is reversed in the fca-9 clf-29 double mutant, and SOC1 is down-regulated in the fca-9 clf-29 double mutant, as in fca-9 (fig. S6, C and D). Moreover, FLC is slightly up-regulated in the fca-9 clf-29 double mutant compared with the clf-29 and fca-9 single mutants (fig. S6E), suggesting that FCA and CLF function as repressors of FLC.

Loss of FCA function induces expression of FLC. We next investigated whether loss of FCA function caused changes in the level of H3K27me3. The deposition of H3K27me3 was measured using chromatin immunoprecipitation (ChIP) with anti-H3K27me3, followed by quantitative PCR to measure the amount of DNA. This analysis showed lower levels of H3K27me3 in the fca-9 mutant compared with wild type (Fig. 3A). These results were consistent with previous results (33), suggesting that FCA is required for H3K27me3 deposition at FLC.

We next investigated whether the loss of FCA function leads to reduced CLF enrichment, in turn affecting H3K27me3 at FLC. We crossed clf-29 ProCLF:HA-CLF into the fca-9 background to generate ProCLF:HA-CLF clf-29 fca-9 plants. The ChIP-PCR results revealed that CLF is highly enriched on FLC in ProCLF:HA-CLF clf-29 but less enriched on FLC in ProCLF:HA-CLF clf-29 fca-9 plants (Fig. 3B). We then examined whether loss of FCA function affects CLF and found that the CLF transcripts and CLF protein levels are not different between wild-type and fca-9 plants (fig. S7, A and B). These results suggested that FCA is required for CLF enrichment on FLC.

COOLAIR is required for CLF enrichment at FLC

Because loss of COOLAIR function reduces FCA enrichment and loss of FCA function reduces CLF enrichment at FLC, we therefore investigated whether loss of COOLAIR function caused changes in CLF occupancy. The ProCLF:HA-CLF clf-29 coolair-1 line was generated by crossing ProCLF:HA-CLF clf-29 into the coolair-1 background. The ChIP-PCR results revealed that CLF enrichment was reduced in ProCLF:HA-CLF clf-29 coolair-1 plants (Fig. 3C), suggesting that COOLAIR is required for CLF enrichment. To confirm these results, we measured the H3K27me3 level and found that it was reduced in coolair mutants (Fig. 3D). In addition, the CLF transcript and CLF protein levels were not different between wild-type and coolair plants (fig. S7, A and B). These observations suggest that COOLAIR is required for CLF enrichment and H3K27me3 deposition at FLC.

Next, we investigated whether the reduction of the FCA-COOLAIR complex in coolair mutants caused any changes in H3K4me2 levels at FLC. The deposition of H3K4me2 was measured using ChIP with a
specific anti-H3K4me2 antibody, followed by quantitative PCR to measure the amount of immunoprecipitated DNA. High levels of H3K4me2 were observed in the coolair and fca-9 mutants compared with wild type (fig. S7C). These observations were consistent with results that down-regulation of COOLAIR led to reduced enrichment of FCA.

**SSU72 interacts with FCA in vitro and in vivo**

Our results show that binding COOLAIR allows enrichment of CLF at FLC, so we next asked whether any negative effectors regulate the binding between FCA and COOLAIR. We used BD-fused FCA and screened for potential interacting proteins with a yeast two-hybrid system. AD-fused SSU72 (AD-SSU72) was observed to interact with FCA (Fig. 4A). This yeast two-hybrid interaction was confirmed by an in vitro protein pull-down assay. His-FCA bound to GST-SSU72 beads, but not to GST beads (Fig. 4B and fig. S3C). Reciprocally, soluble GST-tagged SSU72 bound only to His-FCA beads (Fig. 4C and fig. S3D).

We then confirmed the FCA-SSU72 interaction with BiFC and observed fluorescence from reconstituted YFP in the nucleus in cells coexpressing SSU72 fused to the YFP N-terminus (SSU72-YFP) and FCA fused to the YFP C-terminus (FCA-YFP). Moreover, the controls failed to show a YFP fluorescent signal. This BiFC supports our finding that SSU72 binds directly to FCA (Fig. 4D). To further validate this interaction, we immunoprecipitated FLAG-tagged SSU72 (using anti-FLAG antibody) from Arabidopsis protoplasts coexpressing HA-tagged FCA. SSU72 bound to FCA, but the control did not (Fig. 4E). Immunoprecipitation using anti-HA antibody showed that FCA bound to SSU72, but not control (fig. S8A). These results indicate that SSU72 interacts with FCA in vitro and in vivo.

We next asked which domain is critical for the FCA-SSU72 interaction. FCA was divided into N- and C-terminal fragments, which were tested for SSU72 interaction. The FCAN bound to SSU72, but the C terminus did not. Within the N terminus, the RRM1 motif is sufficient to interact with SSU72 (Figs. 1A and 4F). This interaction was confirmed by Co-IP of FLAG-tagged SSU72 and HA-tagged RRM1, which showed that RRM1, but not the controls, bound to SSU72 (fig. S8, B and C).

**SSU72 is involved in FLC-dependent regulation of flowering time**

To characterize the function of Arabidopsis SSU72, we identified two ssu72 alleles carrying T-DNA insertions (a T-DNA insertion in exon 1 in ssu72-1 and in intron 1 in ssu72-2; Fig. 5A). RT-PCR failed to detect full-length SSU72 mRNA in ssu72-1 and ssu72-2, which revealed that ssu72-1 and ssu72-2 are bona fide knockout mutants (Fig. 5, B and C). The loss of SSU72 function produced an early-flowering phenotype during LD and SD photoperiods (Fig. 5, D and E), suggesting that the photoperiod response does not require SSU72 function. The ssu72 mutants showed normal vernalization and gibberellin (GA) responses (Fig. 5, D and E), indicating that SSU72 might function in the FLC-dependent regulation of flowering time.

To test these results, we introduced ssu72-1 and ssu72-2 into lines carrying a dominant FRIGIDA (FRI) allele (34, 35). The FRI phenotype of late flowering was reversed by the ssu72 mutants (Fig. 5, F and G), and mutations in SSU72 did not change the FRI transcript levels (fig. S7D), suggesting that SSU72 delays flowering time via FLC. To further confirm the SSU72-FLC genetic interaction, we produced ssu72-1 fca-3 double mutants and found that the ssu72-1 fca-3 double mutant and fca-3 single mutants flowered at similar times (Fig. 5, H and I). We then examined transcripts of FLC and COOLAIR, which showed that FLC, but not COOLAIR, was down-regulated in the ssu72 mutants (Fig. 5) and fig. S7E). These results indicate that SSU72 is involved in FLC-dependent regulation of flowering time.

To confirm that the ssu72 mutation causes the early-flowering phenotype, we produced a construct containing a native promoter-driven full-length SSU72 complementary DNA fused with an HA tag (ProSSU72:HA-SSU72) and transformed it into the ssu72-1 mutant plants. The early-flowering phenotype of ssu72-1 was completely rescued by the ProSSU72:HA-SSU72 construct (fig. S9, A and B). The possibility of a genetic interaction between SSU72 and FCA was tested by crossing ssu72-1 into fca-9. The ssu72-1 fca-9 double mutant exhibited late flowering (fig. S9, C and D), indicating that FCA is epistatic to SSU72 as the loss of FCA function suppressed the early flowering of ssu72.

**SSU72 antagonizes the COOLAIR-binding ability of FCA**

Because RRMs are essential for RNA binding, we therefore asked whether SSU72 antagonizes FCA’s ability to bind COOLAIR RNA. COOLAIR accumulation decreased with an increased dosage of SSU72 (Fig. 6A), and the efficiency with which FCAN could pull down SSU72 was independent of the SSU72 dosage (fig. S8D), indicating that SSU72 antagonizes FCA binding of COOLAIR. To confirm this result, we used biotinylated COOLAIR RNA to assess its binding affinity with cell extracts from ssu72 plants. FCA binding to COOLAIR RNA was increased in ssu72 mutants compared with wild-type plants, suggesting that SSU72 negatively regulates the binding between COOLAIR and FCA (Fig. 6, B to D). These results were further confirmed by RIP, followed by quantitative RT-PCR. In this assay, COOLAIR RNA was highly enriched in ssu72 mutants compared with wild type (Fig. 6E and fig. S9, E to G). These observations indicated that SSU72 negatively affects the binding between COOLAIR and FCA.

**SSU72 antagonizes the occupancy of CLF at FLC**

On the basis of the results presented above, we concluded that FCA binds COOLAIR and allows CLF to add H3K27me3 at FLC. In addition, we found that SSU72 decreases FCA-COOLAIR binding. We propose that CLF enrichment and H3K27me3 deposition at FLC might be induced by the loss of SSU72 function. To examine this, we first tested H3K27me3 in ssu72 mutants with ChIP, followed by quantitative PCR to measure the immunoprecipitated DNA. H3K27me3 was highly enriched in ssu72 mutants (Fig. 6F), suggesting that SSU72 is responsible for H3K27me3 deposition. We then examined the H3K4me3 level and found that H3K4me3 was reduced in ssu72 plants (Fig. 6G), suggesting that SSU72 is involved in chromatin regulation at FLC.

We next investigated whether increased FCA induced CLF occupancy at FLC in ssu72 mutants. To test this, ssu72-1 was crossed into ProCLF:HA-CLF clf-29 to generate ProCLF:HA-CLF clf-29 ssu72-1 plants. CLF enrichment was measured using ChIP-PCR. CLF enrichment was increased in ProCLF:HA-CLF clf-29 ssu72-1 plants, compared with ProCLF:HA-CLF clf-29 plants (Fig. 6H), suggesting that SSU72 antagonizes CLF occupancy and H3K27me3 deposition at FLC.
In this study, we determined that FCA physically interacts with CLF and directly binds COOLAIR; these interactions allow the PRC2 subunit CLF to target the FLC (COOLAIR) locus and increase levels of H3K27me3. Our study provides new insights into the mechanisms by which the antisense transcript COOLAIR triggers CLF to induce H3K27me3 at FLC and shows that SSU72 antagonizes CLF enrichment and H3K27me3 deposition at FLC. FCA physically binds COOLAIR in vitro and in vivo. Loss of COOLAIR function leads to a reduction in FCA enrichment at FLC. FCA interacts with CLF to allow it to increase the levels of H3K27me3 at FLC. Loss of FCA function results in the induction of FLC transcription and the reduction of H3K27me3 partially due to reduced CLF enrichment at FLC. SSU72 interacts with the RRM1 motif of FCA to antagonize the binding of FCA and COOLAIR. Loss of SSU72 function led to early flowering with a reduction in FLC expression. Mutations in SSU72...
Fig. 5. Loss of SSU72 function results in early flowering. (A) Gene structure of SSU72, indicating exons (boxes), introns (lines), and T-DNA insertions (triangles). (B) Full-length SSU72 transcripts were examined with RT-PCR in the ssu72 mutants. (C) The SSU72 transcripts were quantified by RT-PCR in the ssu72 mutants. UBIQUITIN 10 was used as an internal control. Experiments were repeated three or more times, and data from a representative experiment are shown. Data are shown as means ± SE (n = 3 replicates). (D) Twenty-five–day–old Col-0 and ssu72 mutant plants under an LD photoperiod. (Photo credit: Y.T., University of Science and Technology of China.) (E) Total leaf numbers of Col-0 and ssu72 mutant plants under an LD photoperiod and vernalization (LD + Ver), an SD photoperiod, and GA treatment (SD + GA). Flowering time was measured using the number of leaves (rosette, shown in dark blue; cauline, shown in light blue) at bolting as a proxy. Tests were conducted under LD, LD + Ver, SD, and SD + GA. More than 40 plants were scored for each line. Asterisks indicate a significant difference from total leaf numbers using Student’s t test (P < 0.05 or P < 0.01). (F) Forty-day-old FRI and FRI ssu72 mutant plants grown under an LD photoperiod. (Photo credit: Y.T., University of Science and Technology of China.) (G) Total leaf numbers of FRI and FRI ssu72 mutant plants grown under an LD photoperiod. Flowering time was measured using the number of leaves (rosette, shown in dark blue; cauline, shown in light blue) at bolting as a proxy. More than 40 plants were scored for each line. Asterisks indicate a significant difference from total leaf numbers using Student’s t test (P < 0.01). (H) Total leaf numbers of Col-0, ssu72-1, flc-3, and ssu72-1 flc-3 mutant plants grown under an LD photoperiod. (Photo credit: Y.T., University of Science and Technology of China.) (I) Total leaf numbers of Col-0, ssu72-1, ssu72-2, flc-3, ssu72-1 flc-3, and ssu72-2 flc-3 mutant plants grown under an LD photoperiod. Dark blue indicates rosette leaves, and light blue indicates cauline leaves; more than 40 plants were scored for each line. Asterisks indicate a significant difference from total leaf numbers using Student’s t test (P < 0.05). (J) FLC transcripts were quantified by RT-PCR in ssu72 mutants. UBIQUITIN 10 was used as an internal control. Experiments were repeated three or more times, and data from a representative experiment are shown. Data are shown as means ± SE (n = 3 replicates). Data that differ significantly from Col-0 (based on Student’s t test, P < 0.01) are marked with asterisks.
Fig. 6. SSU72 antagonizes FCA binding of COOLAIR. (A) Beads containing the His-fused FCAN were tested for binding of COOLAIR RNA with increasing dosage of SSU72, followed by RT-PCR. (B to D) Beads containing biotin-fused COOLAIR (class I) (B), COOLAIR [class II (i)] (C), and COOLAIR [class II (ii)] RNAs (D) were tested for binding of FCA in wild-type and ssu72 plants. (E) The FCA-COOLAIR complex was determined by RIP RT-PCR in wild-type and ssu72 mutants. RNA was immunoprecipitated with anti-FCA and anti-IgG, followed by RT-PCR, using region 1 primers. The amount of COOLAIR RNA immunoprecipitated with anti-FCA was measured relative to the amount of COOLAIR immunoprecipitated with anti-IgG. Experiments were repeated three or more times, and data from a representative experiment are shown. Data are shown as means ± SE (n = 3 replicates). Fca-9 was used as a negative control. Significant difference using Student’s t test, **P < 0.01. The regions analyzed by RIP RT-PCR are indicated in Fig. 1B. (F and G) The deposition of H3K27me3 (F) and H3K4me3 (G) in different regions of FLC was determined by ChIP-PCR in wild-type and ssu72 mutants. (H) The occupancy of CLF in different regions of FLC was determined by ChiP-PCR in T10 and ProCLF:HA-CLF clf-29 ssu72-1 plants. T10 was clf-29 complemented with ProCLF:HA-CLF, as indicated in fig. S5. From (F) to (H), experiments were repeated three or more times, and data from a representative experiment are shown. Data are shown as means ± SE (n = 3 replicates). ACTIN2 and PP2A were used as internal controls. IgG, fca-9, or ssu72-1 was used as a negative control. Significant difference using Student’s t test, *P < 0.05; **P < 0.01. The regions analyzed by ChiP-PCR are indicated in fig. S1C.
increased the binding between FCA and COOLAIR, which, in turn, enhanced CLF recruitment and H3K27me3 at FLC. Together, our results indicate that the H3K27me3 deposited by PRC2 at FLC is required for the binding between FCA, COOLAIR, and SSU72.

COOLAIR transcripts were induced by cold treatment (27), causing vernalization-induced repression of FLC by recruiting plant homeo-domain (PHD)-PRC2 (21). The following results suggested that disruption of COOLAIR by T-DNA insertion did not produce notable changes in H3K27me3 after cold treatment (36). In addition to the vernalization pathway, COOLAIR participates in the autonomous pathway (7, 28). Our study showed that loss of COOLAIR resulted in late flowering under LD conditions. FCA, but not FPA, binds to COOLAIR and CLF, suggesting that FCA evolved divergent functions in chromatin regulation. FCA alters the polyadenylation site of the COOLAIR (class I) antisense transcript and induces its transcripts in silencing FLC (6, 7), suggesting that FCA has dual functions. The long COOLAIR transcript of class II (i), but not class II (ii), was induced in the fca mutant (7). FCA also binds COOLAIR RNA, and this complex might form a positive regulatory loop affecting FLC transcription, suggesting that FCA might be crucial for FLC regulation and the basis of FLC repression. Therefore, we proposed a model that FCA stabilizes class I of COOLAIR transcripts, and FCA binds to the two classes of COOLAIR transcripts to add H3K27me3, resulting in silencing of FLC (fig. S10).

In mammalian cells, RNA binding proteins interact with PRC2 to mediate PRC2-targeted deposition of H3K27me3 genome-wide (37). However, little is known about this process in plants. Our study shows that the RNA binding protein FCA physically interacts with CLF and allows it to add H3K27me3 at FLC. Unlike COLDAIR and COLDWRAP, COOLAIR is not observed to directly bind to PRC2. Our results show that the interaction between COOLAIR and PRC2 is indirect and depends on the RNA binding protein FCA. FCA physically binds COOLAIR and CLF, functioning as a link; moreover, FCA might be only one of several factors that allow CLF to target FLC. Although there are multiple GU-rich regions in COOLAIR (including class I and class II), whether FCA binds these specific sequences or a higher-order structure of COOLAIR remains to be tested. IncRNAs regulate various aspects of genome activity, affecting DNA modifications, histone modifications, and chromatin structure (38). COOLAIR might also affect chromatin structure, which, in turn, induces FLC H3K27me3 deposition and represses FLC mRNA production. Therefore, the role of COOLAIR in H3K27me3 deposition needs further study. The interaction between PRC2 and RNA in mammals is unresolved, and FCA homologs might have critical functions in this interaction.

Yeast Ssu72 is associated with transcription and specifically dephosphorylates the Ser5p of the CTD of Pol II (30). Our study shows that SSU72 physically interacts with FCA at its RRM1 motif and antagonizes its binding with COOLAIR. The affinity between FCA and COOLAIR decreases with increasing dosage of SSU72, and mutations in SSU72 increase the binding between FCA and COOLAIR. SSU72 is involved in the FLC-dependent flowering-regulatory pathway, and loss of SSU72 function leads to early flowering with a reduction in FLC expression and increased H3K27me3. In addition, CLF was highly enriched in ssu72 mutants because the enhanced binding between FCA and COOLAIR promotes CLF recruitment. Collectively, our study provides compelling evidence that FCA functions as a bridge, binding COOLAIR, CLF, and SSU72, and that these four factors coordinate to modulate H3K27me3 levels and FLC expression.

**METHODS**

**Plant materials**

The *A. thaliana* Columbia were grown at 22°C under either an LD photoperiod (16-hour light/8-hour dark cycles) or an SD photoperiod (8-hour light/16-hour dark cycles) with a light intensity of 160 μmol m⁻² s⁻¹. The mutants procured from the SALK collection were as follows: coolair-1, SALK_131491; coolair-2, SALKseq_125494; fca-9, an fca allele in the Columbia background; clf-29, SALK_021003; ssu72-1, SALKseq_120634; and ssu72-2, SALK_059245. Vernalization for flowering time measurement was performed as previously described (35, 39).

**Plasmid constructs**

The oligonucleotide primers and methods used to produce the constructs were described in Data file S1. All cloned DNA was confirmed by DNA sequencing.

**Protein expression and purification**

The plasmids were transformed into Rosetta (DE3) *Escherichia coli* cells. The single clones were cultured in 2YT medium containing anti-biotic at 37°C to an optical density of 0.6 nm and then induced with 0.3 mM Isopropyl-β-D-1-thiogalactopyranoside (IPTG) for 12 hours at 16°C. After the cells were harvested and sonicated, the supernatant was incubated with glutathione resin (GenScript) or Ni-charged resin (GenScript) at 4°C for 2 hours. The resins were washed with washing buffer [50 mM tris (pH 7.5), 200 mM NaCl, and 0.1% Tween 20] and then eluted with washing buffer containing 20 mM reduced glutathione for GST fusion proteins or 100 mM imidazole for His fusion proteins.

**Pull-down assays, Co-IP, and immunoblotting**

The pull-down assays were performed as previously described (39): 3 μg of fusion protein was bound to beads. These beads were incubated overnight with soluble protein at 4°C and then washed. SDS-polyacrylamide gel electrophoresis (PAGE) was used to resolve the proteins, which were detected by immunoblotting with anti-GST (A08866-100; lot: 13D000626; GenScript) or anti-His (M301111; lot: 273884; Abmart).

The Co-IP assays were also conducted as previously described (39, 40). The proteins of interest—SSU72, FCA, and CLF—were tagged with FLAG or HA, and the fusion constructs were cloned into pUC19. The constructs were transformed into protoplasts from *Arabidopsis*; the protoplasts were then incubated overnight at 22°C. Protein extracts from lysed protoplasts were immunoprecipitated with anti-FLAG (H9608; lot: SLBQ7119V; Sigma-Aldrich) or anti-HA (H9658; lot: 095M4778V; Sigma-Aldrich). After washing the beads, the immunoprecipitated proteins were resolved by SDS-PAGE and detected by immunoblotting with anti-HA or anti-FLAG.

**Protein binding RNA assay**

For protein binding of RNA, 2 μg of the fusion protein was bound to beads, which were washed and incubated with 2 to 3 μg of RNA in binding buffer [50 mM tris (pH 7.5), 100 mM NaCl, and 0.6% Triton X-100] for 30 min at 4°C. After washing with washing buffer [50 mM tris (pH 7.5), 150 mM NaCl, 1 mM MgCl₂, 1 mM dithiothreitol (DTT), 0.05% NP-40, 0.5 M urea, and RNAsin (40 U/ml)], the RNA was recovered by phenol/chloroform extraction and ethanol precipitation. The purified RNA was resuspended in water and amplified by RT-PCR. Data file S1 shows the specific primers used.
RNA pull-down assay and RNA Co-IP

The RNA synthesis was conducted following the protocol from the manufacturer (MAXiScript Kit, part number AM1308–AM1326; Thermo Fisher Scientific). Briefly, the transcripts of COOLAIR, COLDAIR, and COLDWRAP were amplified with RT-PCR and driven by the T7 promoter, and then RNAs were generated with biotin–uridine triphosphate using an in vitro Transcription Kit (AM1312, Thermo Fisher Scientific). The primers used for RNA pull-down are described in Data file S1.

The pull-down assays were conducted by incubating beads with 3 µg of RNA. The beads were washed and mixed gently with soluble protein (3 µg) for 30 min at 4°C. The beads were washed five to eight times with 50 mM tris (pH 7.5), 150 mM NaCl, 1 mM DTT, 0.05% NP-40, and RNasin (40 U/ml). Beads were resolved by SDS-PAGE and immunoblotted with anti-His (M30111 M, lot: 273884; Abmart).

For the RNA Co-IP, 0.5 g of 10-day-old seedlings was ground in a mortar and pestle in buffer [50 mM tris (pH 7.5), 150 mM NaCl, 20 mM KCl, 1 mM MgCl₂, 1 mM DTT, 0.05% NP-40, bovine serum albumin (0.125 mg/ml), and RNasin (40 U/ml)]. Beads with 3 µg of RNA were incubated with cell extracts for 30 min at 4°C. After being washed five to eight times with 50 mM tris (pH 7.5), 150 mM NaCl, 1 mM DTT, 0.05% NP-40, and RNasin (40 U/ml), the samples were separated by SDS-PAGE and immunoblotted with anti-FCA (R3399-1, lot: 15088; Abicodie).

RT and RT-PCR

The RT and RT-PCR were performed as described previously (39). Leaves of seedlings at 10 days old were used for RNA isolation, and oligo(dt) primers (Promega) were used for RT. The CFX real-time PCR machine (Bio-Rad) and SYBR Green mixture (Roche) were used. The antibody-protein complexes were isolated by binding to Protein A beads. The RNA was then extracted with TRIzol. After protein A beads. The RNA was then extracted with TRIzol. After being precipitated in ethanol, RNA was resuspended in water. The purified RNA was analyzed by RT-PCR with the gene-specific primers shown in Data file S1.

RNA immunoprecipitation

For the RIP, 3 g of 10-day-old seedlings was homogenized with a mortar and pestle. After sonication, the sample was diluted with RIP buffer [1% Triton X-100, 1.2 mM EDTA, 16.7 mM tris-HCl (pH 8.0), and 167 mM NaCl] and precleared with protein A magnetic beads. Specific anti-FCA antibodies (R3399-1, lot: 15088; Abicodie) were used. The antibody-protein complexes were isolated by binding to protein A beads. The RNA was then extracted with Trizol. After being precipitated in ethanol, RNA was resuspended in water. The purified RNA was analyzed by RT-PCR with the gene-specific primers shown in Data file S1.

SUPPLEMENTARY MATERIALS

Supplementary material for this article is available at http://advances.sciencemag.org/cgi/content/full/5/4/eaau7246/DC1

REFERENCES AND NOTES

1. S. D. Michaelsen, R. M. Amasino, FLOWERING LOCUS C encodes a novel MADS domain protein that acts as a repressor of flowering. Plant Cell 11, 949–956 (1999).
2. D.-H. Kim, M. R. Doyle, S. Sung, R. M. Amasino, Vernalization: Winter and the timing of flowering in plants. Annu. Rev. Cell. Dev. Biol. 25, 277–299 (2009).
3. R. Macknight, I. Bancroft, T. Page, C. Lister, R. Schmidt, K. Love, L. Westphal, G. Murphy, S. Sherston, C. Cobbett, FCA, a gene controlling flowering time in Arabidopsis, encodes a protein containing RNA-binding domains. Cell 89, 737–745 (1997).
4. G. G. Simpson, P. P. Dijkwel, V. Quesada, I. Henderson, C. Dean, FY is an RNA 3′ end-processing factor that interacts with FCA to control the Arabidopsis floral transition. Cell 113, 777–787 (2003).
5. P. Crevillen, C. Dean, Regulation of the floral repressor gene FLC: The complexity of transcription in a chromatin context. Curr. Opin. Plant Biol. 14, 38–44 (2011).
6. F. Liu, V. Quesada, P. Crevillen, I. Baure, S. Swieziwski, C. Dean, The Arabidopsis RNA-binding protein FCA requires a lysine-specific demethylase 1 homolog to downregulate FLC. Mol. Cell 28, 398–407 (2007).
7. F. Liu, S. Marquardt, C. Lister, S. Swieziwski, C. Dean, Targeted 3′ processing of antisense transcripts triggers Arabidopsis FLC chromatin silencing. Science 327, 94–97 (2010).
8. I. Baure, L. Smith, D. C. Baulcombe, C. Dean, Widespread role for the flowering-time regulators FCA and FPA in RNA-mediated chromatin silencing. Science 318, 109–112 (2007).
9. C. Sonmez, I. Baure, A. Maguisin, R. Drees, S. Laubinger, D. Weigel, C. Dean, RNA 3′ processing functions of Arabidopsis FCA and FPA limit intergenic transcription. Proc. Natl. Acad. Sci. U.S.A. 108, 8508–8513 (2011).
10. Y. Ding, Z. Avramova, M. Fromm, Two distinct roles of ARABIDOPSIS HOMOLOG OF TRITHORAX (ATX1) at promoters and within transcribed regions of ATX1-regulated genes. Plant Cell 23, 350–363 (2011).
11. Y. Ding, I. Ndamkong, Z. Xu, H. Lapko, M. Fromm, Z. Avramova, ATX1-generated H3K4me3 is required for efficient elongation of transcription, not initiation, at ATX1-regulated genes. PLOS Genet. 8, e1003111 (2012).
12. S. Pien, D. Fleury, J. S. Mylene, P. Crevillen, D. Inze, Z. Avramova, C. Dean, U. Grossniklaus, ARABIDOPSIS TRITHORAX1 dynamically regulates FLOWERING LOCUS C activation via histone 3 lysine 4 trimethylation. Plant Cell 20, 580–588 (2008).
13. Z. Zhao, Y. Yu, D. Meyer, C. Wu, W. H. Shen, Prevention of early flowering by expression of FLOWERING LOCUS C requires methylation of histone H3 K36. Nat. Cell Biol. 7, 1256–1260 (2005).
14. J. Müller, J. A. Kassi, Polycrome response elements and targets of Polycrome group proteins in Drosophila. Curr. Opin. Genet. Dev. 16, 476–484 (2006).
15. M. Nekrasov, B. Wild, J. Müller, Nuclosome binding and histone methylation transferase activity of Drosophila PRC2. EMBO Rep. 6, 348–353 (2005).
16. C. S. Ketel, E. F. Andersen, M. L. Vargas, J. Suh, S. Strome, J. A. Simon, Subunit contributions to histone methylation transferase activities of fly and worm polycrome group complexes. Mol. Cell. Biol. 25, 6857–6868 (2005).
17. S. Pien, U. Grossniklaus, Polycrome group and trithorax group proteins in Arabidopsis. Biochim. Biophys. Acta 1769, 375–382 (2007).
18. C. Liu, I. Lu, X. Cui, X. Cao, Histone methylation in higher plants. Annu. Rev. Plant Biol. 61, 395–420 (2010).
19. J. Goodrich, P. Puangsomsie, M. Martin, D. Long, E. M. Meyerowitz, G. Coupland, A Polycrome-group gene regulates homeotic gene expression in Arabidopsis. Nature 386, 44–51 (1997).
20. M. Lopez-Vernaza, S. Yang, R. Müller, F. Thorpe, E. de Leau, J. Goodrich, Antagonistic roles of SEPALATA3, FT and FLC genes as targets of the polycrome group gene CURLY LEAF. PLOS ONE 7, e30715 (2012).
21. S. Swieziwki, F. Liu, A. Maguisin, C. Dean, Cold-induced silencing by long antisense transcripts of an Arabidopsis Polycrome target. Nature 462, 799–802 (2009).
22. J. B. Heo, S. Sung, Vernalization-mediated epigenetic silencing by a long intronic noncoding RNA. Science 311, 76–79 (2011).
23. D.-H. Kim, S. Sung, Vernalization-triggered intragenic chromatin loop formation by long noncoding RNAs. Dev. Cell 40, 302–312.e4 (2017).

24. D.-H. Kim, S. Sung, Environmentally coordinated epigenetic silencing of FLC by protein and long noncoding RNA components. Curr. Opin. Plant Biol. 15, 51–56 (2012).

25. S. Marquardt, O. Raitskin, Z. Wu, F. Liu, Q. Sun, C. Dean, Functional consequences of splicing of the antisense transcript COOLAIR on FLC transcription. Mol. Cell 54, 156–165 (2014).

26. P. Li, Z. Tao, C. Dean, Phenotypic evolution through variation in splicing of the noncoding RNA COOLAIR. Genes Dev. 29, 696–701 (2015).

27. T. Csorba, J. I. Questa, Q. Sun, C. Dean, Antisense COOLAIR mediates the coordinated switching of chromatin states at FLC during vernalization. Proc. Natl. Acad. Sci. U.S.A. 111, 16160–16165 (2014).

28. S. Swiezewski, P. Crevillen, F. Liu, J. R. Ecker, A. Jerzmanowski, C. Dean, Small RNA-mediated chromatin silencing directed to the 3′ region of the Arabidopsis gene encoding the developmental regulator, FLC. Proc. Natl. Acad. Sci. U.S.A. 104, 3633–3638 (2007).

29. C. Jeronimo, A. R. Bataille, F. Robert, The writers, readers, and functions of the RNA polymerase II C-terminal domain code. Chem. Rev. 113, 8491–8522 (2013).

30. S. Krishnamurthy, X. He, M. Reyes-Reyes, C. Moore, M. Hampsey, Ssu72 is a RNA polymerase II CTD phosphatase. Mol. Cell 14, 387–394 (2004).

31. C. Ganem, F. Devaux, C. Torchet, C. Jacq, S. Quevillon-Cheruel, G. Labesse, C. Facca, G. Faye, Ssu72 is a phosphatase essential for transcription termination of snRNAs and specific mRNAs in yeast. EMBO J. 22, 1588–1598 (2003).

32. K. Xiang, J. L. Manley, L. Tong, An unexpected binding mode for a Pol II CTD peptide phosphorylated at Ser7 in the active site of the CTD phosphatase Ssu72. Genes Dev. 26, 2265–2270 (2012).

33. X. Yu, S. D. Michaela, The Arabidopsis Paf1c complex component CDC73 participates in the modification of FLC chromatin. Plant Physiol. 153, 1074–1084 (2010).

34. X. Hu, X. Kong, C. Wang, L. Ma, J. Zhao, J. Wei, X. Zhang, G. J. Loake, T. Zhang, J. Huang. Y. Yang, Proteasome-mediated degradation of FRIGIDA modulates flowering time in Arabidopsis during vernalization. Plant Cell 26, 4763–4781 (2014).

35. C. Lu, Y. Tian, S. Wang, Y. Su, T. Mao, T. Huang, Q. Chen, Z. Xu, Y. Ding, Phosphorylation of SPTS by CDKD:2 is required for VPS recruitment and normal flowering in Arabidopsis thaliana. Plant Cell 29, 277–291 (2017).

36. C. A. Helliwell, M. Robertson, E. J. Finnegan, D. M. Buzas, E. S. Dennis, Vernalization-repression of Arabidopsis FLC requires promoter sequences but not antisense transcripts. PLOS ONE 6, e21513 (2011).

37. C. Wei, R. Xiao, L. Chen, H. Cui, Y. Zhou, Y. Xue, J. Hu, B. Zhou, T. Tsutsui, J. Qiu, H. Li, L. Tang, X. D. Fu, RBFox2 binds nascent RNA to globally regulate polycomb complex 2 targeting in mammalian genomes. Mol. Cell 62, 875–889 (2016).

38. G. Böhmddorfer, A. T. Wierzbicki, Control of chromatin structure by long noncoding RNA. Trends Cell Biol. 25, 623–632 (2015).

39. Y. Su, S. Wang, F. Zhang, H. Zheng, Y. Liu, T. Huang, Y. Ding, Phosphorylation of histone H2A at Serine 95: A plant-specific mark involved in flowering time regulation and H2A.Z deposition. Plant Cell 29, 2197–2213 (2017).

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Competing interests: The authors declare that they have no competing interests.

Data and materials availability: All data needed to evaluate the conclusions in the paper are present in the paper and/or the Supplementary Materials. Additional data related to this paper may be requested from the authors.

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