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Supplemental information

Deceleration of the cell cycle underpins a switch from proliferative to terminal divisions in plant stomatal lineage

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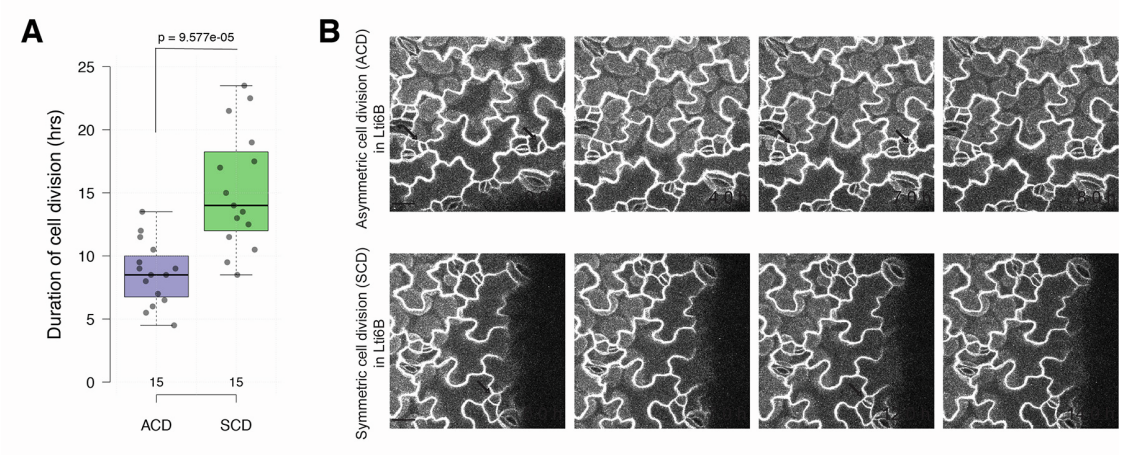

Figure S1. Cell division time of amplifying ACD is faster than that of the terminal SCD, Related to Figure 1

(A) Duration of the division time of stomatal precursors undergoing ACD and SCD measured by plasma membrane marker Lti6B line. n=15 for each cell division mode. Two-tailed Student t-test was performed. p=9.577 e-05.

(B) Still images series of representative ACD and SCD duration in plasma membrane marker Lti6B line. Black arrows indicate the meristemoid in amplifying ACD (top) or GMC undergoing SCD (bottom), respectively. Scale Bar: 20 μm

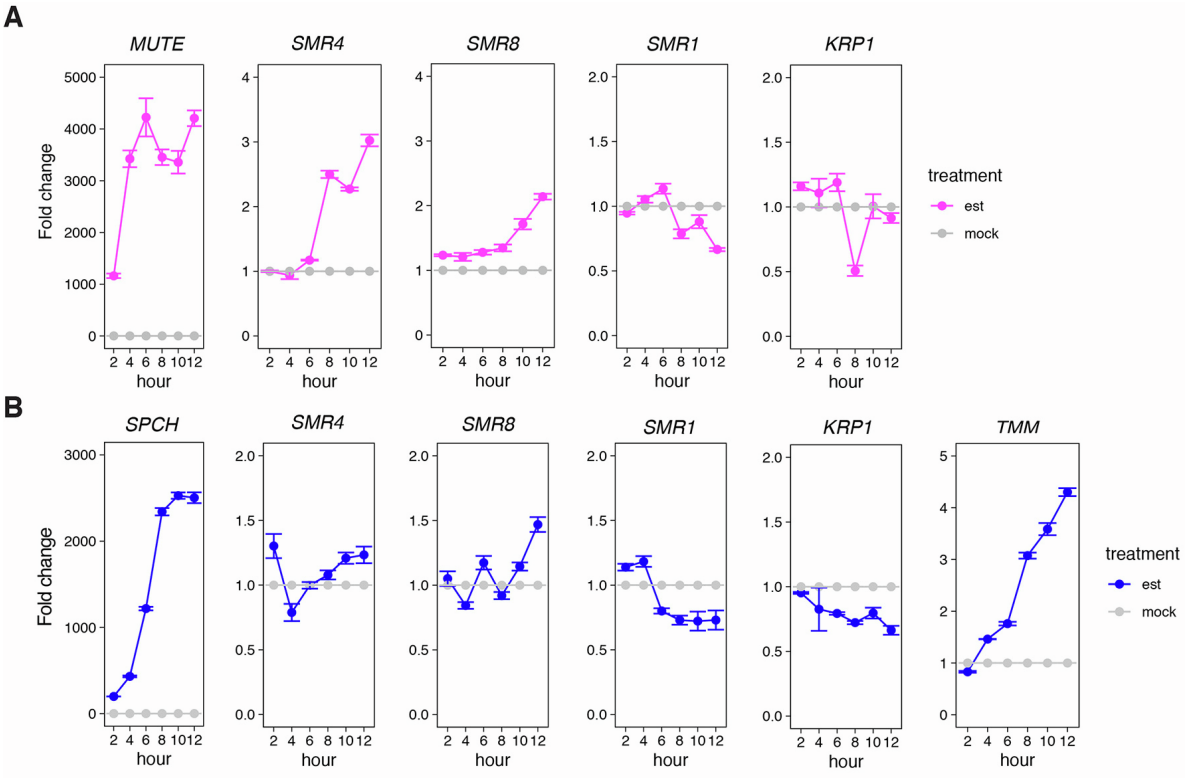

Figure S2. Time course analysis of *SMRs* and *KRP1* expression fold change upon induction of MUTE and SPCH, Related to Figure 2

(A, B) Representative qRT-PCR analysis of SMRs and KRP1 expression fold change upon MUTE induction (A) and SPCH induction (B) by estradiol application. In both cases, qRT-PCR was normalized against *ACT2,* and then expression fold change upon MUTE/SPCH induction was normalized against mock control at each time point. Bars, mean of three technical replicates. Error bars, mean ± s.e.m. Mock: DMSO only, est: 10 μM estradiol

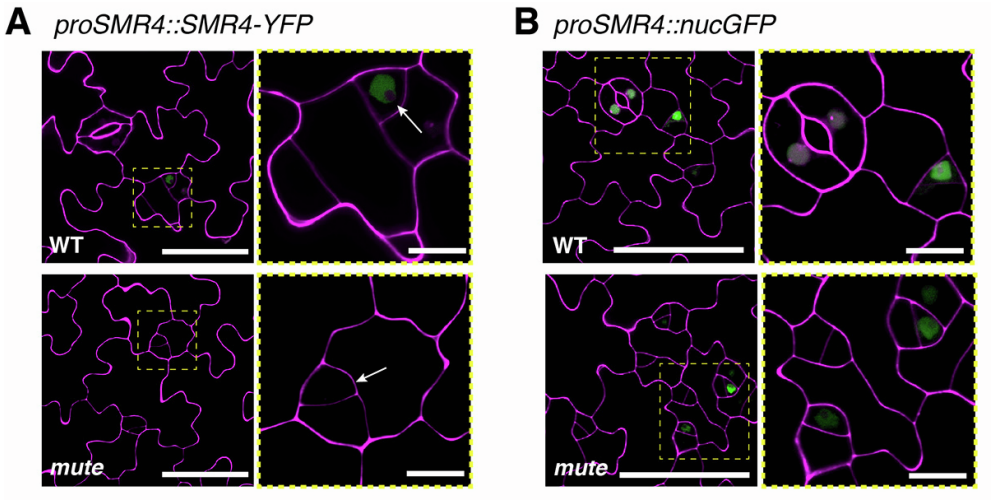

Figure S3. SMR4 transcriptional and translational reporter expressions in *mute*, Related to Figure 2

(A) Confocal microscopy images of cotyledon abaxial epidermis from 5-day-old Arabidopsis wild-type (top) and *mute-2* (bottom) seedlings expressing *proSMR4::SMR4-YFP*. In wild type, SMR4-YFP signal (green) is visible in the nucleus of a late meristemoid (arrow). No SMR4-YFP signal is observed in *mute-2* late meristemoid (arrow). Yellow dotted areas in the left panels are enlarged in the right panels. Scale bars, 50 μm (left panels), 10 μm (right panels).

(B) Confocal microscopy images of cotyledon abaxial epidermis from 5-day-old Arabidopsis wild-type (top) and *mute-2* (bottom) seedlings expressing *proSMR4::nucGFP*. In wild type, nucGFP signal (green) is visible in a late meristemoid to immature guard cells (some residual GFP signals are visible in guard cells). In *mute-2,* weak, background GFP signals can be detected in some meristemoids and SLGCs, implying general expression of SMR4. Yellow dotted areas in the left panels are enlarged in the right panels. Scale bars, 50 μm (left panels), 10 μm (right panels).

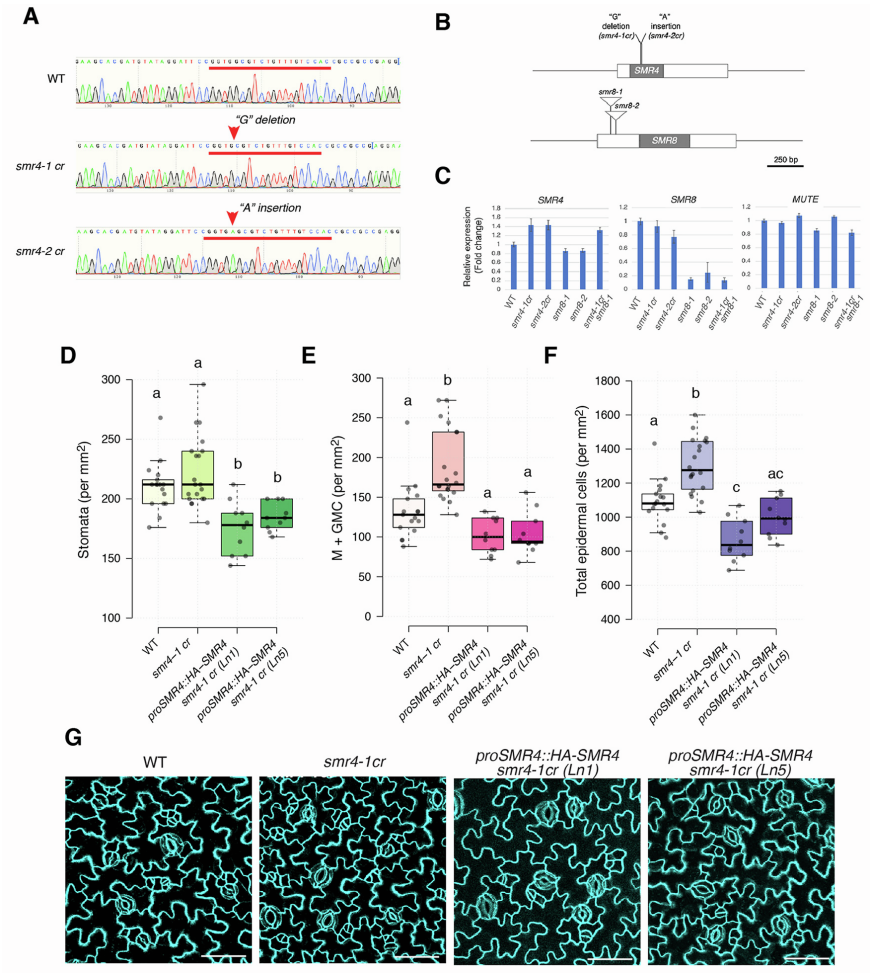

Figure S4. Characterization of *smr4 and smr8* mutants and complementation test, Related to Figure 3

(A) Comparison of Sanger sequencing chromatograms of WT and two homozygous CRISPR mutant alleles of *SMR4*, *smr4-1cr* and *smr4-2cr*, indicating the exact location of 1-base deletion (G deletion) and insertion (A insertion), respectively. (B) Location of mutation introduced at *SMR4 CRISPR* and *SMR8 T-DNA* loci. Two *smr8* mutants (*smr8-1*: SALK_126253 and *smr8-2*: SALK_074523) have T-DNA inserted at 5' UTR. Gray, exon; white, 5' and 3' UTR.

(C) qRT-PCR analysis of relative *SMR4* and *SMR8* expression in each single mutant and a double mutant. The transcripts were normalized against *ACT2* first, then plotted relative to transcript levels in wild-type seedlings. *MUTE* was used as a negative control. Error bars, mean ± s.e.m from three technical replicates. Ridges inside GCs can be seen due to z-stack images.

(D-G) Complementation analysis. *proSMR4::HA-SMR4* is introduced into *smr-4-1cr* mutant plants. Cotyledons from two independent lines harboring homozygous transgenes were imaged at the 5 -day post germination and the number of epidermal cells were counted. Number of stomata (D), number of stomatal precursor cells (E), and number of epidermal cells (F) per 1.0 mm^2. One-way ANOVA followed by Tukey's HSD analysis indicates that *scr4-1cr* epidermal/stomatal phenotypes are fully rescued. WT: n=17, *smr4-1cr*: n=20, Complementation Ln1: n=10, and Ln5: n=10 (G) Confocal microscopy images of 5-day-old cotyledon abaxial epidermis from wild-type (WT), *smr4-1cr*, *proSMR4::HA-SMR4 smr4-1cr* Line 1, and Line 5, showing full complementation.

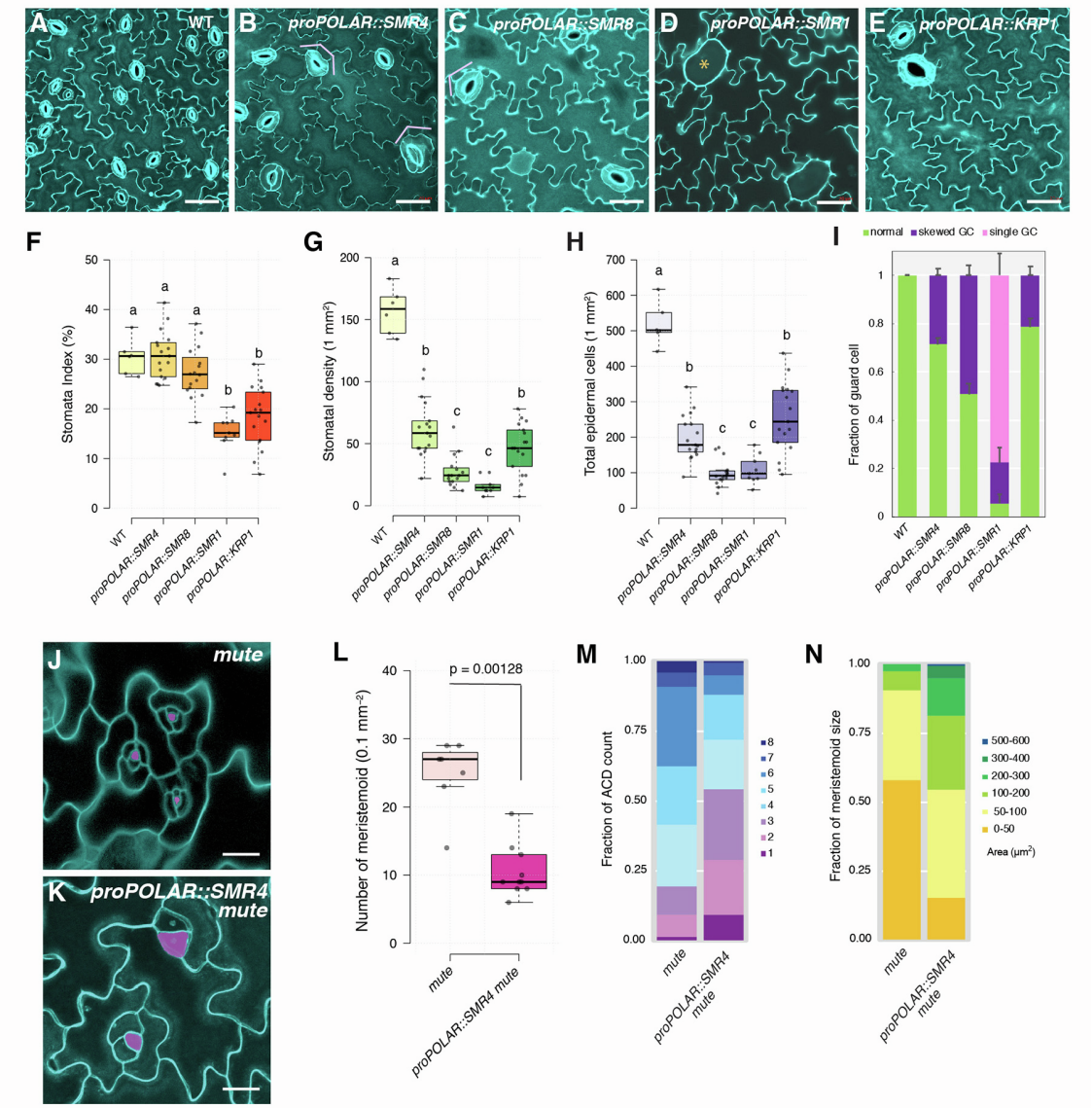

Figure S5. SMRs and KRP1 display distinct activity in epidermal cell development, Related to Figure 4

(A-E) Epidermal phenotype of 8 dpg abaxial cotyledon from WT (A), *SMR4* (B), *SMR8* (C), *SMR1* (D) and *KRP1* (E) driven by the *POLAR* promoter. Scale bar: 50 μm. (F-I) Stomatal index (F), stomatal density (G), total epidermal number (H), and fraction of normal, skewed and single-celled stomata (I) in the unit area per mm^2. Error bars, SD

(J) *mute* mutant, (K) *proPOLAR::SMR4* introduced into *mute*, images were taken at 2-week old cotyledons. Arrested meristemoids are false colored in magenta. (L) The number of meristemoids per 0.1 mm^2 area (*mute*: n=7, *proPOLAR::SMR4 mute*: n= 9). (M) The fraction of the ACD count, (N) Size distribution of meristemoid, 86 and 204 meristemoids were used for *mute* and *proPOLAR::SMR4 mute*, respectively.

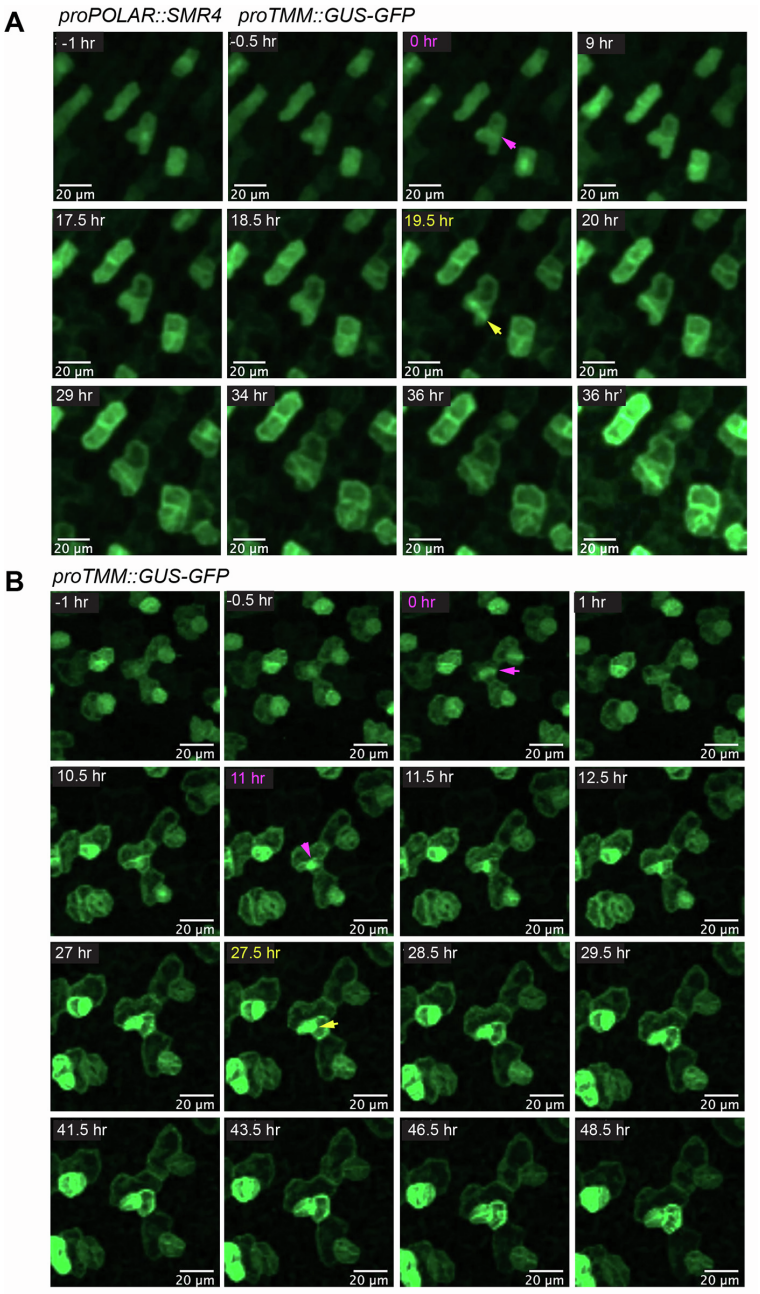

Figure S6: Time-lapse imaging of *proTMM::GUS-GFP* epidermis highlights the effects of *proPOLAR::SMR4* on delayed and aberrant ACDs, Related to Figure 5, Movies S7 and S8
Shown are still images from time-lapse live imaging of *proTMM::GUS-GFP* in *proPOLAR::SMR4* (A) and wild type (B). A magenta and yellow arrow indicate ACD and SCD division plane, respectively. Hours are displayed relative to the time when ACD plane is first visible and indicated at the left top corner. Scale bars, 20 µm

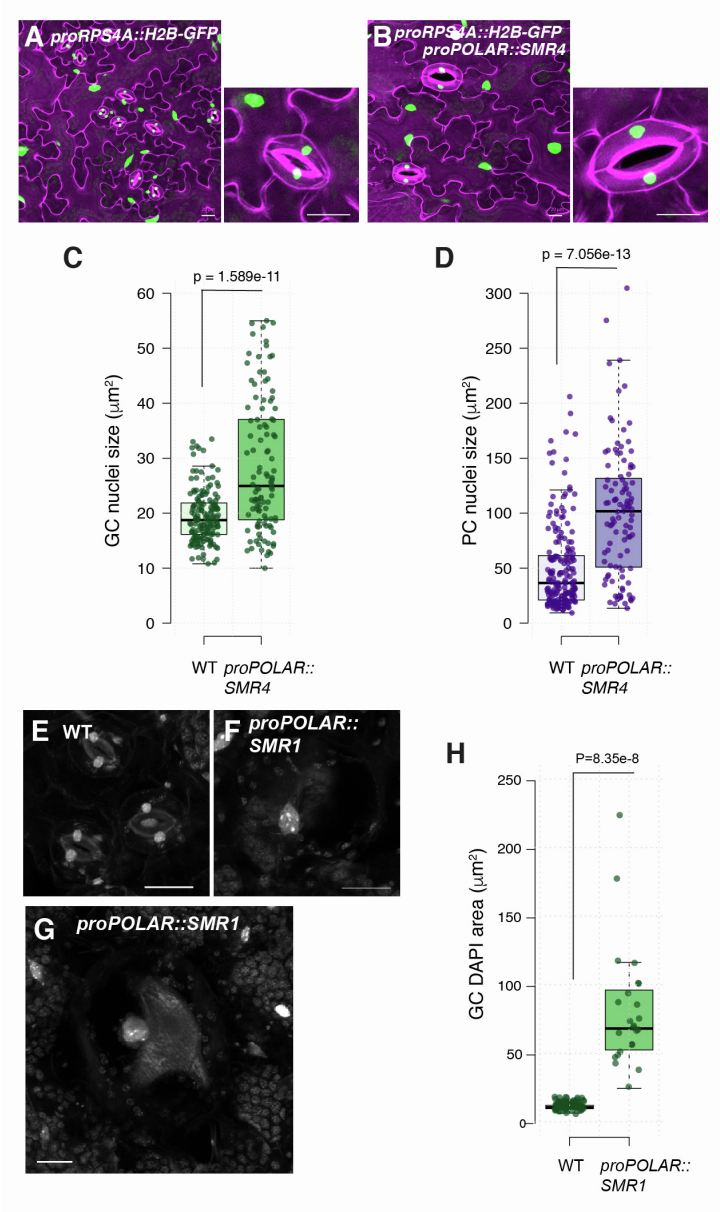

Figure S7. SMR4 does not trigger endoreduplication unlike SMR1, Related to Figure 5
(A-B) Orthogonal projection image from z-stack images covering whole nuclei from epidermis (A) *proRPS5A::H2B-GFP*, (B) *proRPS5A::H2B-GFP proPOLAR::SMR4,* Insets: mature guard cells, Scale bars: 20 µm. (C) Measurement of guard cell (GC) nuclei size, The area of 155 and 108 GC nuclei from wild type and *proPOLAR::SMR4*, respectively were measured. Student t-test was performed ($P<1.58911e^{-11}$). (D) Measurement of pavement cell (PC) nuclei size. The area of PC was measured. n= 191 (wild type), n=103 (*proPOLAR::SMR4*). Student t-test was performed ($P< 7.05614e^{-13}$). (E-G) DAPI stained nuclei in wild type (E) and single-celled stomata from *proPOLAR::SMR1* (F,G), Scale bar, 20 µm. (H) Quantitative analysis of DAPI-stained nuclear area in wild-type mature GC (n=102) and *proPOLAR::SMR1* (n=24) single-celled GCs. Two-tailed Student t-test was performed. p=8.35e-8.