

## Supplementary Movies and Figures

**Movie S1:** Live imaging of *pRPSSA::TagRFP:TUB4* tubulin marker during the first and second meiotic division in wild type plant.

**Movie S2:** Live imaging of *pRPSSA::TagRFP:TUB4* tubulin marker during regular meiotic divisions and two postmeiotic spindle reassembly cycles in *smg7-6* mutants.

**Movie S3:** Live imaging of *pRPSSA::TagRFP:TUB4* tubulin marker from the 3<sup>rd</sup> to 6<sup>th</sup> cycle of spindle reassembly in *smg7-6* mutants.

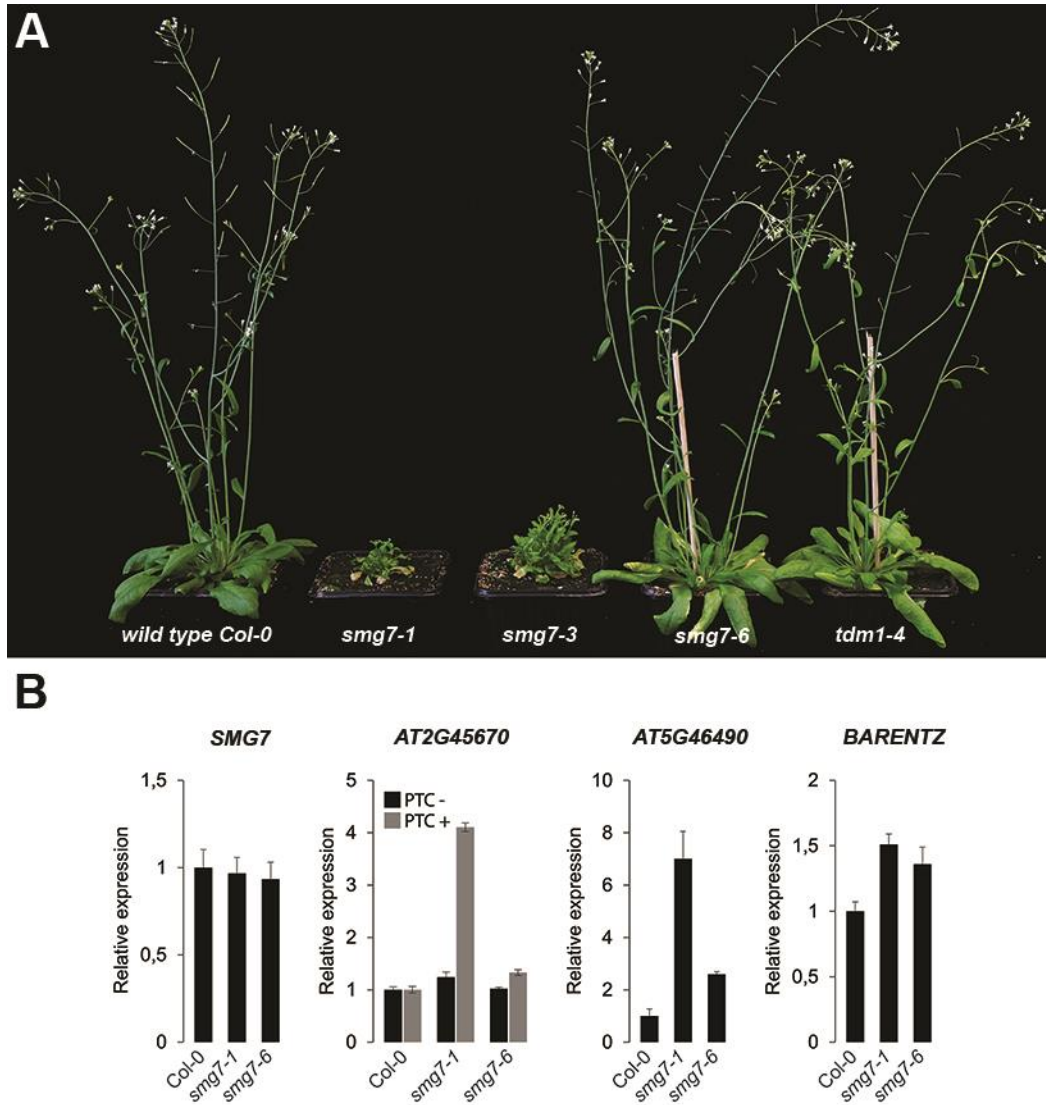
**Movie S4:** Live imaging of *HTA10:RFP* chromatin marker during mitosis in a root tip of a wild type plant.

**Movie S5:** Live imaging of *HTA10:RFP* chromatin marker during mitosis in a root tip of a *cenh3-2* mutant.

**Movie S6:** Live imaging of *HTA10:RFP* chromatin marker during mitosis in a oryzalin treated root tip of a wild type plant.

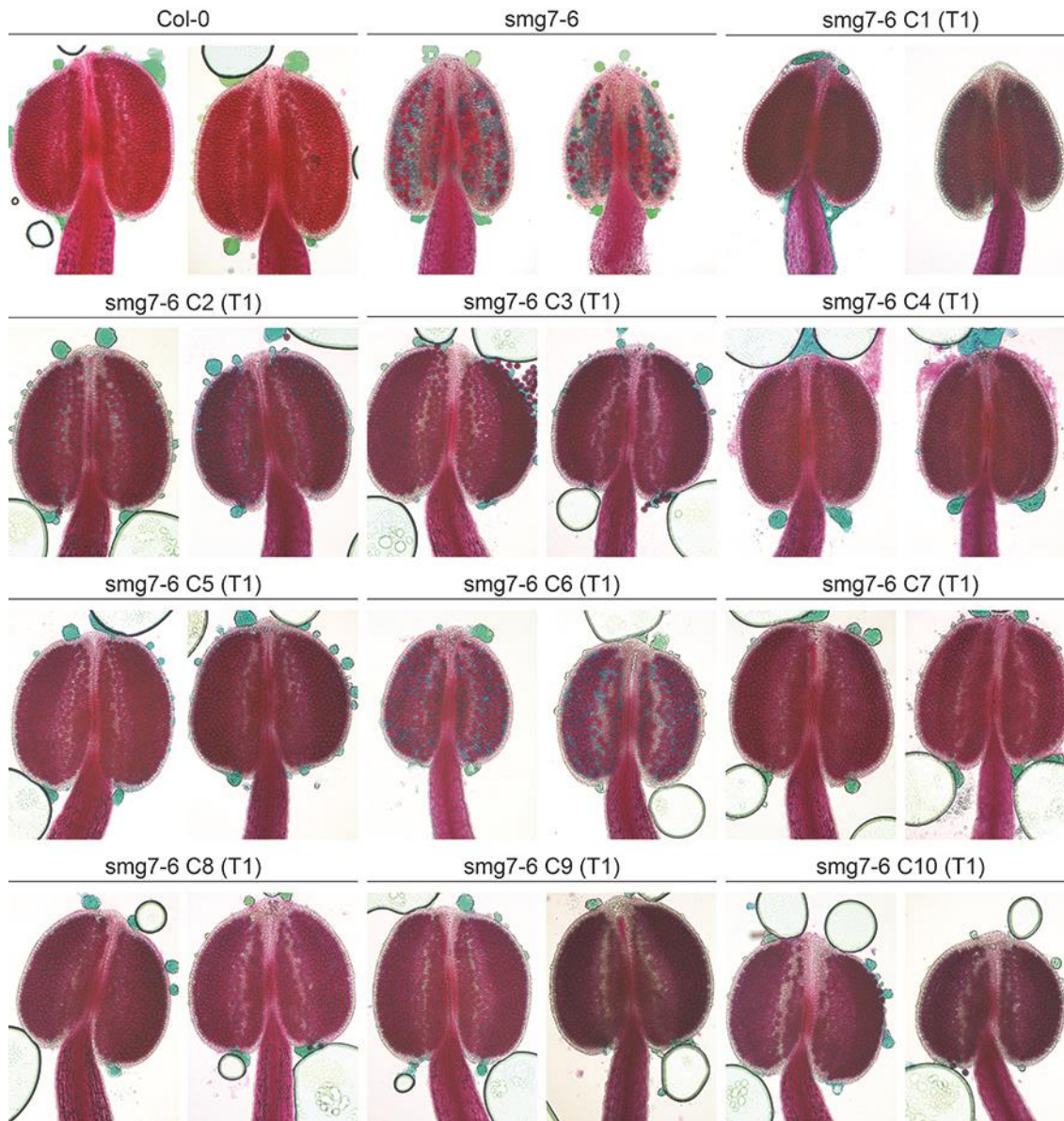
**Movie S7:** Live imaging of *HTA10:RFP* chromatin marker during mitosis in a oryzalin treated root tip of a *cenh3-2* mutant.

Figure S1



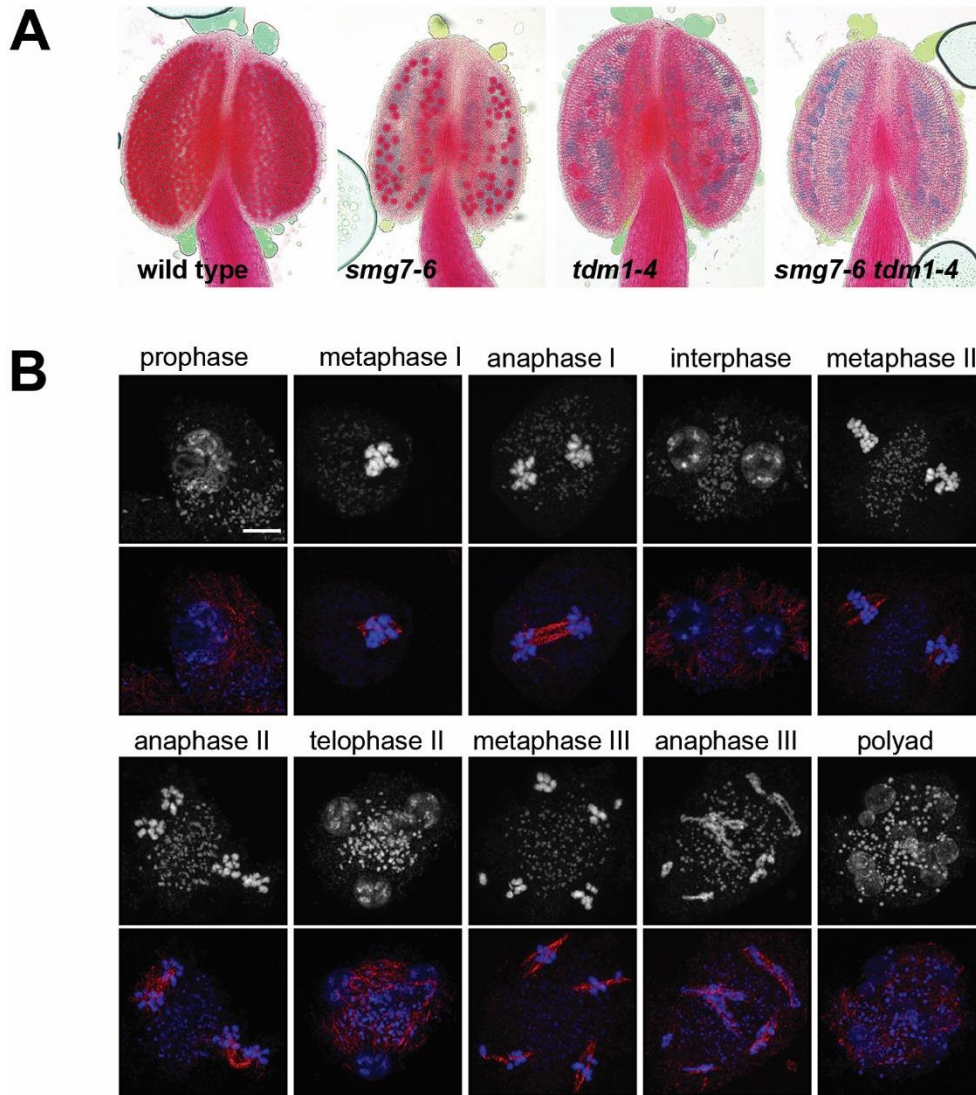
**Figure S1. Allelic series of Arabidopsis *smg7* mutants.** (A) Five week-old Arabidopsis mutants homozygous for the indicated alleles. (B) Expression of *SMG7* mRNA from the region located upstream of the T-DNA insertions in *smg7-1* and *smg7-6*. (C) Quantitative RT-PCR analysis of transcripts targeted by NMD in *smg7-1* and *smg7-6* mutants. Two mRNA splice variants, one containing a premature termination codon (PTC+), were quantified for the *AT2G45670* locus. Error bars indicate standard deviations from three biological replicas.

Figure S2



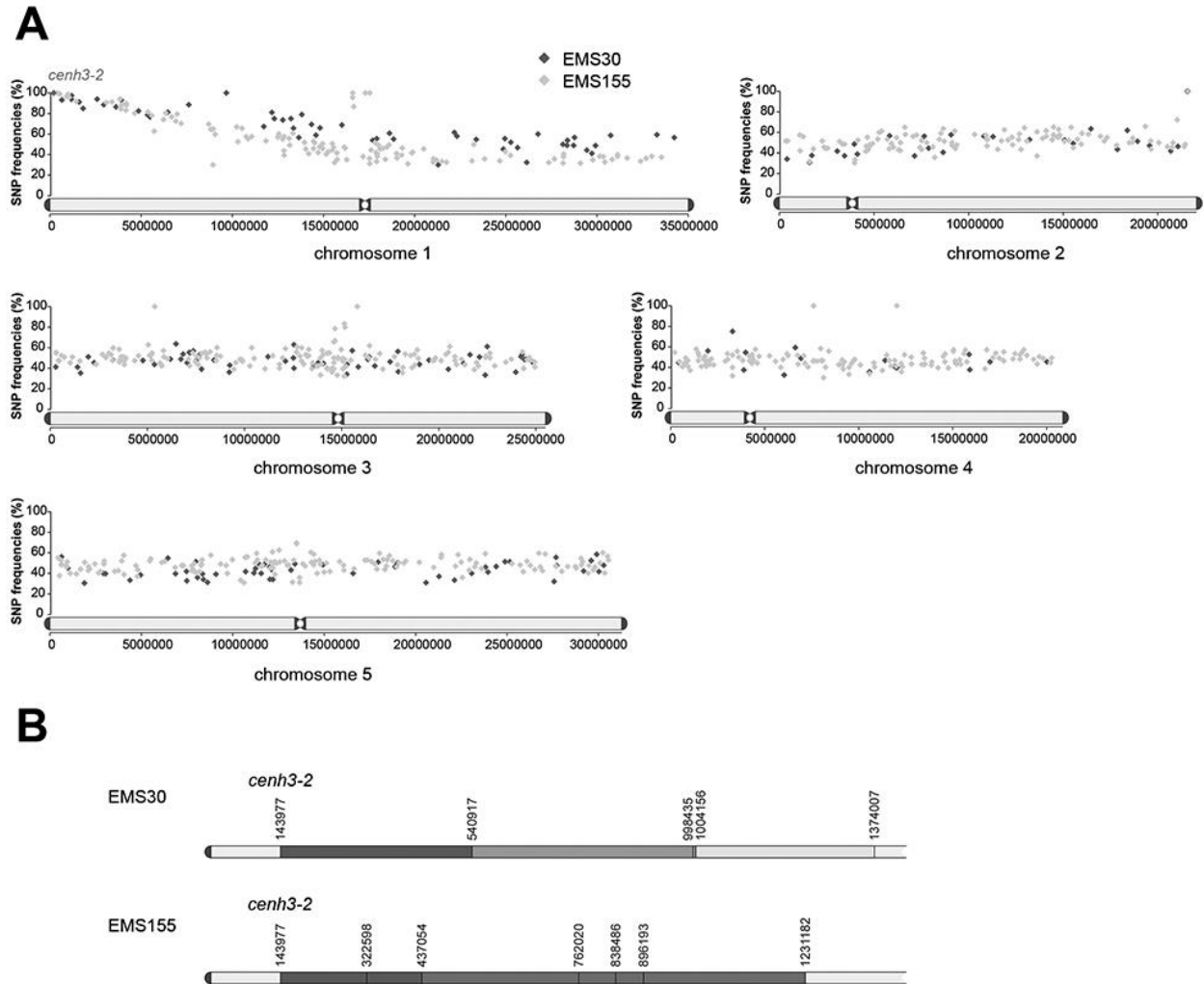
**Figure S2. Genetic complementation of *smg7-6* mutation with the endogenous *pSMG7::SMG7* gene construct.** Anthers from 10 independent T1 *smg7-6* transformants carrying the *pSMG7::SMG7* construct with viable pollen detected by Alexander staining. All T1 lines show a restoration of viable pollen.

Figure S3



**Figure S3. Epistatic analysis of *smg7-6* and *tdm1-4* mutations. (A)** Anthers of the indicated mutants after Alexander staining. Viable pollen stain red. **(B)** Meiotic progression in PMCs of *smg7-6 tdm1-4* double mutants. Spindles are stained with anti- $\alpha$ -tubulin antibody (red), DNA is counterstained with DAPI. Scale bar corresponds to 5  $\mu$ m.

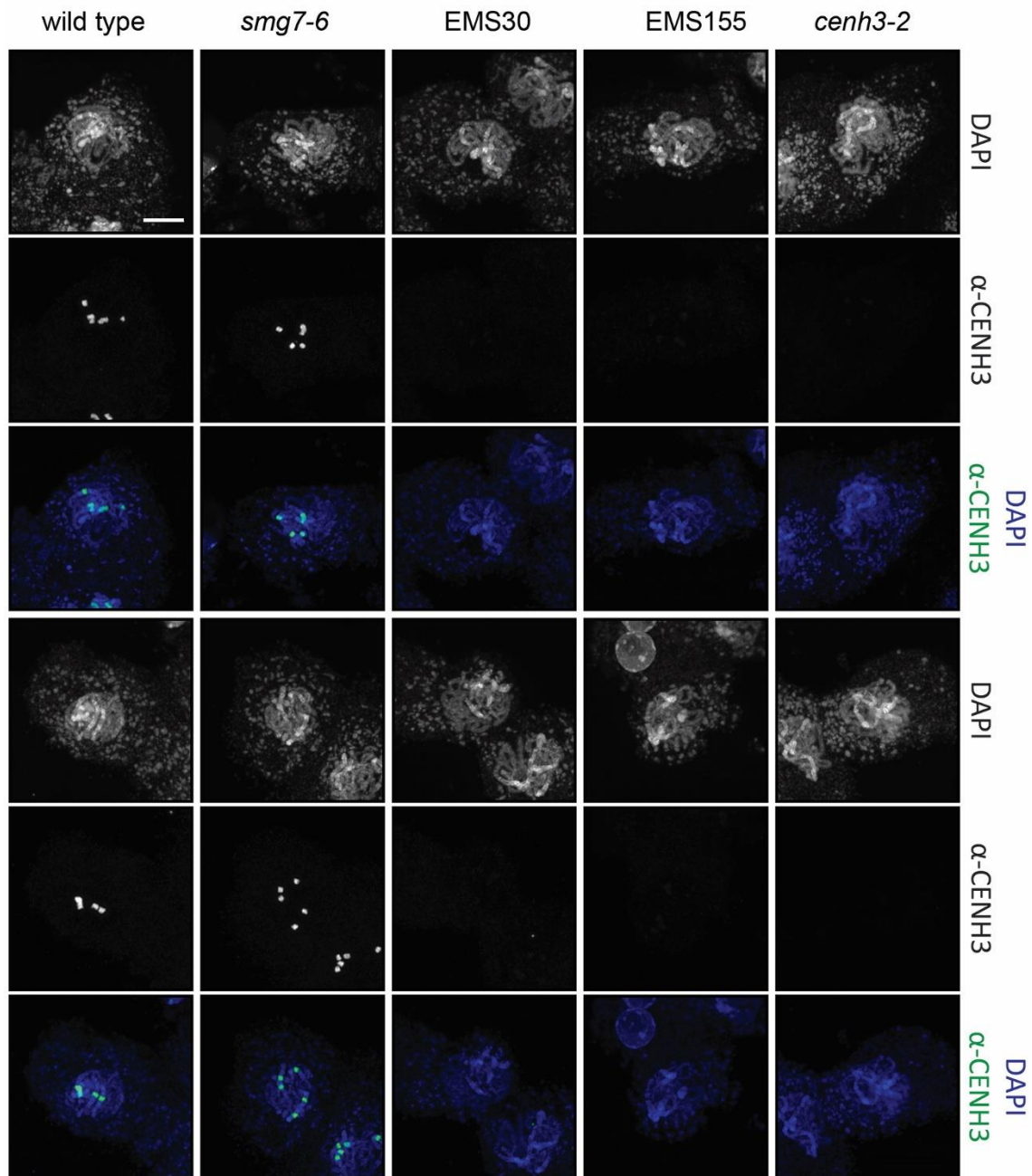
Figure S4



**Figure S4. Association mapping of *de novo* mutations with the *smg7-6* suppressor trait in EMS30 and EMS155 lines. (A) Genome-wide distribution of *de novo* mutations and their frequency in fertile B2plants generated from backcrosses of EMS30 and EMS155 lines with parental *smg7-6* plants. (B) *De novo* mutations at the left arm of chromosome 1 in the EMS30 and EMS155 lines. Coordinates of these mutations in TAIR10 annotation are indicated.**

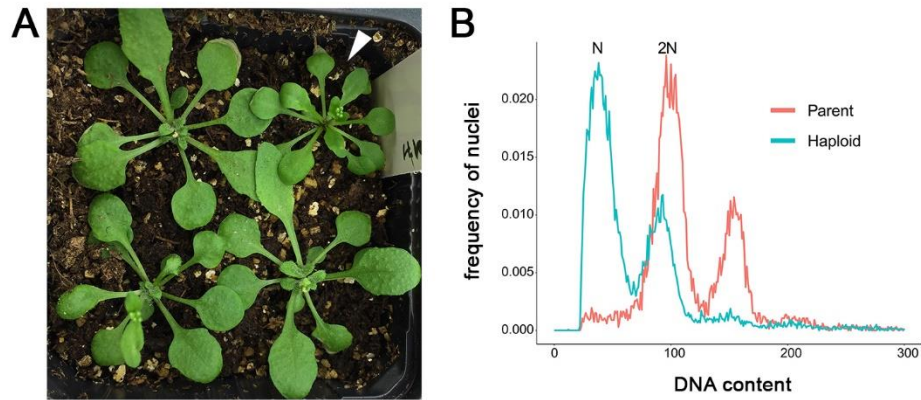


Figure S5



**Figure S5. Immunodetection of CENH3 in prophase I using the same imaging conditions.** CENH3 is visualized with CENH3 antibody; DNA is counterstained with DAPI (blue). Scale bar represents 5  $\mu$ m.

Figure S6



**Figure S6. Haploid plant obtained from the cross between *cenh3-2* and *gl1-1 Col-0*.** (A) The haploid plant (indicated by arrowhead) was recognized based on its trichome-less phenotype. (B) Nuclear content of inflorescence nuclei from the haploid and a parent plant determined by flow cytometry.