SUPPLEMENTARY MATERIALS AND METHODS

Plant growth condition and plasmid construction

*Arabidopsis thaliana* (L.) Heynh. (ecotype Columbia-0) plants were grown under long-day conditions (16 h of light and 8 h of darkness) at 22°C on 0.5x Murashige and Skoog (MS) agar plate (Valvekens *et al.*, 1988). The *etg1-1* (SALK_071046) and *etg1-2* (SALK_145460) alleles were retrieved from the Salk Institute Genomic Analysis Laboratory engine (http://signal.salk.edu/cgi-bin/tdnaexpress) and the seeds were acquired from the Arabidopsis Biological Research Center. To screen for homozygous insertion alleles, the following primer pairs were designed: 5'-AGACCAAGATGGTCAGAGGATC-3' and 5'-ACTGGAACACAGTAAAGCAAGC-3' for *etg1-1*, and 5'-AAATTAACCGGAATGGGTTTG-3' and 5'-ATGACTCAGATTTGATGCCTGG-3' for *etg1-2*. The full-length open reading frames of *ETG1* and *MCM5* with or without a stop codon were amplified by PCR with F-ETG1 (5'-GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGGGAGGACCAGCTTACGATT-3') and R-ETG1 (5'-GGGGACCACTTTGTACAAGGAAGCAGGCTTCATGTCAGGATGGGACGGAG-3') primers, and cloned into the pDONR201 ENTRY vector by BP recombination reaction according to the manufacturer's instructions (Invitrogen). The *ETG1* promoter sequence was amplified from *Arabidopsis* genomic DNA by PCR with the F-ETG1 (5'-GGGGACAAGTTTGTACAAGGAAGCAGGCTTCATGTCAGGATGGGACGGAG-3') and R-MCM5 (5'-GGGGACCACTTTGTACAAGGAAGCAGGCTTCATGTCAGGATGGGACGGAG-3') primers, respectively, and cloned into the pDONR201 ENTRY vector by BP recombination reaction according to the manufacturer's instructions (Invitrogen). The *ETG1* promoter sequence was amplified from *Arabidopsis* genomic DNA by PCR with the
CTG-3') and RP-ETG1 (5'-GGGGACCACTTTGTACAAGAAAGCTGGGTCGGTCAGACAATCGTAAGCTG GT-3') primers. Each E2F element in the ETG1 promoter was exactly deleted by PCR with FP-ETG1, RP-ETG1, FPΔI-ETG1 (5'-ATGGATAATGAACCTAGGAGATATG-3') and RPΔI-ETG1 (5'-CTCCTAGGTTTCATTATCCATGCCCATTCC-3') for ΔI-ETG1 promoter, and FP-ETG1, RP-ETG1, FPΔII-ETG1 (5'-AGGAGATATGGGCCCAACTATACACTTG-3') and RPΔII-ETG1 (5'-TAGTTGGGCCCATATCTCCTAGGTT-3') for ΔII-ETG1 promoter, respectively. Both E2F elements in the ETG1 promoter were deleted by PCR with fragments of the ΔI-ETG1 promoter with FP-ETG1, RP-ETG1, FPΔII-ETG1, and RPΔII-ETG1 primers. Each PCR fragment was cloned into the pDONR201 entry vector by BP recombination reaction and subsequently transferred into the pKGWFS7 destination vector (Karimi et al., 2002) by LR recombination reaction, resulting in a transcriptional fusion between the ETG1 promoter and the eGFP-GUS fusion gene. All constructs were transferred into the Agrobacterium tumefaciens C58C1Rif’ strain harboring plasmid pMP90. The obtained Agrobacterium strains were used to generate stably transformed Arabidopsis with the floral dip transformation method (Clough and Bent, 1998). Transgenic plants were obtained on kanamycin-containing medium and later transferred to soil. The atr-2 and wee1-1 mutants and E2Fa-DPa-, and E2Fc-overexpressing plants have been described previously (De Veylder et al., 2002; del Pozo et al., 2002; Culligan et al., 2004; De Schutter et al., 2007).

**Kinematic growth analyses**

Growth kinetics were analyzed as described by Boudolf et al. (2004). Briefly, wild-type and etg1-1 plants were germinated and grown in round 12-cm Petri dishes filled with 100 ml of 0.5x MS medium (Duchefa, Haarlem, The Netherlands) and 0.8% plant tissue culture agar (Lab M, Bury, UK) at 22°C and 65 μE m⁻² sec⁻¹ radiation in a 16-h-light/8-h-dark photoperiod. From day 5 until day 22 after sowing, plants were harvested, cleared
overnight in methanol, and subsequently stored in lactic acid for microscopy. The youngest plants were mounted on a slide and covered. The leaf primordia were observed under a microscope fitted with differential interference contrast optics (DMLB; Leica, Wetzlar, Germany). The total (blade) area of first leaves of each seedling were first determined from drawing-tube images with the public domain image analysis program ImageJ (version 1.30v; http://rsb.info.nih.gov/ij/). At older stages, the primordia were digitized directly with a charge-coupled device camera mounted on a binocular (Stemi SV11; Zeiss, Jena, Germany), which was connected to a personal computer fitted with a frame-grabber board LG3 (Scion Corp., Frederick, MD) running the image analysis program Scion Image (version 3b for Windows NT). Cell density was determined from scanned drawing-tube images of outlines of at least 20 cells of the abaxial epidermis located 25 and 75% from the distance between the tip and the base of the leaf primordium (or blade once the petiole was present), halfway between the midrib and the leaf margin. In the youngest primordia (up to day 6), a single group of cells was drawn. The following parameters were determined: total area of all cells in the drawing, total number of cells, and number of guard cells. From these data, we calculated the average cell area and estimated the total number of cells per leaf by dividing the leaf area by the average cell area (averaged between the apical and basal positions). Average cell division rates were calculated as described by Fiorani and Beemster (2006). Cell cycle duration times were calculated as the inverse of the average of the cell division rates between days 5 to 9, when leaves are still mitotically active. Raw data are available upon request.

**Quantitative PCR analysis**

RNA was extracted from *Arabidopsis* tissues with RNeasy Plant Mini Kit (Qiagen). First-stranded cDNAs was prepared from total RNA with the Superscript™ III First-Strand Synthesis System (Invitrogen) according to the manufacturer's instructions. For quantitative PCR, a LightCycler® 480 SYBR Green I Master (Roche) was used with 100 nM primers
and 0.1 µg of RT reaction product. Reactions were run and analyzed on the LightCycler® 480 Real-Time PCR System (Roche) according to the manufacturer's instructions. Quantitative reactions were done in triplicate and averaged. Primers used were 5'-GGCTCCTCTTTAACCAAAGGC-3' and 5'-CACACCATCACCAGAATCCAGC-3' for \textit{ACTIN2}, 5'-TTGCAACCAGGCACCTTGAA-3' and 5'-CAAATCGGCGGGACATTATGT-3' for \textit{ETG1}, 5'-ACCAAATTGCGTGTGTTTTCCATTG-3' and 5'-ATGTCTGGTGGAAAGGAGGAG-3' for \textit{HISTONE H4}, 5'-GACGGTTTACAAGGGTTTGCACAAG-3' and 5'-GCAGCCACAGTAGACCGGAAGAAG-3' for \textit{CYCA3;1}, 5'-CTCGAGATGGGACGAAGAAGG-3' and 5'-CGACGCAGAGTAATCGAACA-3' for \textit{CDKB1;1}, 5'-TGATGCAGCACAAGGAGATTG-3' and 5'-CGATCTCGTCCATCTGTTCA-3' for \textit{KNOLLE}, 5'-CTCAAAATCCCAACGCTTCTGTTG-3' and 5'-CACGTCTACTACCTTTGGTTTCCC-3' for \textit{CYCB1;1}, 5'-TGTTGCTGGACATTTCAGTCGG-3' and 5'-CAAGAGCTTGGACATTTCAGTCGG-3' for \textit{WEE1}, and 5'-CGAGGAAGGATCTCTTGCAG-3' and 5'-GCACTAGTGAACCCCAGAGG-3' for \textit{RAD51}.

\textbf{ChIP analysis}

ChIP experiments were according to Bowler \textit{et al}, (2004) with a few modifications. Briefly, 1 g of 8-day-old seedlings was harvested, rinsed in ddH$_2$O, and crosslinked in 1% formaldehyde for 10 min. Crosslinking was stopped by addition of glycine to a final concentration of 0.125 M. Tissue was ground and chromatin extracted. The chromatin solution was sonicated with a Branson 1200 sonifier. After preclearing, 10 µL of the appropriate antibodies was added to the chromatin solution and incubated overnight at 4°C.
After collection of the immunoprecipitate with protein A agarose beads, beads were washed and immunocomplexes eluted. Crosslinking was reversed by incubation at 65°C overnight. Proteinase K digestion was followed by phenol/chloroform extraction and ethanol precipitation. Recovered DNA was used in 25 cycles of PCRs. Primers used were 5'-CTTAAGACCAAGATGGTCAGAGGATC-3' and 5'-GAGTCTTTGCTCAACACGAATTAAGG-3'.

**CDK assay**
p9\(^{\text{CksHs1}}\) was purified from an overproducing *Escherichia coli* strain and linked to CNBr-Sepharose 4B (GE-Healthcare) according to Azzi *et al.* (1992). The beads were washed three times with homogenization buffer (HB) containing 25 mM Tris-HCl (pH 7.6), 60 mM β-glycerophosphate, 15 mM nitrophenyl phosphate, 15 mM EGTA (pH 8), 15 mM MgCl\(_2\), 85 mM NaCl, 1 mM dithiothreitol, 0.1 mM vanadate, 1 mM NaF, 0.1 mM benzamidine, 1 mM phenylmethylsulfonylfluoride, 0.1% NP-40, and 1 tablet/10 ml protease inhibitor cocktail (Roche). Protein extracts were prepared from 12-day-old seedlings of *A. thaliana* ecotype Columbia-0 (Col-0) and *etg1* in HB. In a total volume of 125 µl of HB, 180 µg of protein extract was loaded on 50 µl of 50% (v/v) p9\(^{\text{CksHs1}}\)-Sepharose beads and incubated on a rotating wheel for 2 h at 4°C. After a brief centrifugation at 110 g and removal of the supernatant, the beads were carefully washed three times with bead buffer containing 50 mM Tris-HCl (pH 7.5), 5 mM NaF, 250 mM NaCl, 5 mM EGTA, 5 mM EDTA, 0.1% NP-40, 0.1 mM benzamidine, 0.1 mM vanadate and 1 tablet/10 ml protease inhibitor cocktail (Roche). The beads were washed once with kinase buffer (50 mM Tris-HCl (pH 7.8), 15 mM MgCl\(_2\), 5 mM EGTA and 2 mM dithiothreitol), and the supernatant was removed carefully. The histone H1 kinase reactions were initiated by resuspending the pellets of p9\(^{\text{CksHs1}}\)-Sepharose beads with 35 µl of the reaction mixture containing 5 µCi \([γ-33\text{P}]ATP\) (3000 Ci/mmol), 0.5 mg/ml histone H1, 50 mM Tris-HCl (pH 7.8), 15 mM MgCl\(_2\), 5 mM EGTA, 1 mM dithiothreitol,
60 μg/mL cAMP-dependent kinase inhibitor, 10 μM ATP. After 20 min incubation at 30°C, the kinase reactions were stopped by the addition of 10× SDS/PAGE loading buffer. Aliquots were boiled, loaded on a 12% (w/v) acrylamide gel, and stained by Coomassie Blue. The gel was dried overnight and incorporation of [γ-32P] ATP into histone H1 was detected by autoradiography.

**Histochemical GUS measurements**

Histochemical GUS assays were carried out according to standard protocols (Beeckman and Engler, 1994). The young seedlings were incubated in 90% acetone for 2 h at 4°C. After the material had been washed in phosphate buffer, it was immersed in the enzymatic reaction mixture (1 mg/mL of 5-bromo-4-chloro-3-indolyl β-D-glucuronide, 2 mM ferricyanide, and 0.5 mM of ferrocyanide in 100 mM phosphate buffer, pH 7.4). The reaction was carried out at 37°C in the dark for 4 h to overnight, the material was cleared with chlorolactophenol (chloral hydrate/phenol/lactic acid 2:1:1), and observed under light microscope or stereoscope.

**Localization analysis and BiFC assay**

Full-length open reading frames of ETG1 and MCM5 were transferred into the pH7FWG2 destination vector (Karimi et al., 2002) by LR recombination reaction, resulting in ETG1-eGFP and MCM5-YFP fusion proteins, respectively. BiFC assay was done as described by Walter et al., (2004). The coding region of ETG1 was amplified with 5'-GCCACTAGTGGATCCATGGGAGGACCAGCTTACGATT-3' and 5'-AGCGGTACCCTCGAGGTACTTGAGCCTCTCTTCTTA-3' primers, and cloned via BamHI-XhoI into the plasmid pUC-SPYNE (Walter et al., 2004), resulting in the ETG1:YFPN fusion protein; the coding region of MCM5 was also obtained with 5'-TGGCGCGCCACTAGTATGTCAGGATGGGACGAAGGAG-3' and 5'-ACCCTCGAGGTGTCGAGTAAGCTTTGCGGACAATAGAA-3' primers, and cloned
via SpeI-SalI into the plasmid pUC-SPYCE (Walter et al, 2004) to give rise to the MCM5-YFPC fusion protein.

**Yeast two-hybrid experiments**

The *ETG1* and *MCM5* open reading frames were recombined into the pDEST22 and pDEST32 vectors (Invitrogen) by an LR reaction, resulting in translational fusions between the open reading frames and the GAL4 transcriptional activation and GAL4 DNA-binding domains, respectively. Plasmids encoding the baits and preys were transformed into the yeast strain PJ69-4alfa (*MATalpha; trp1-901, leu2-3,112, ura3-52, his3-200, gal4Δ, gal80Δ, LYS2::GAL1-HIS3, GAL2-ADE2, met2::GAL7-lacZ*) and PJ69-4a (*MATa; trp1-901, leu2-3,112, ura3-52, his3-200, gal4Δ, gal80Δ, LYS2TGAL1-HIS3, GAL2-ADE2, met2TGAL7-lacZ*) by the LiAc method (Gietz et al, 1992), and plated on synthetically defined plates without Leu and without Trp for 2 days at 30°C, respectively. Interactions between fusion proteins were assayed by the mating method.

**Root growth analysis**

For root growth experiments, seedlings were grown in square plates in vertical position in 0.5x MS medium containing 10 g/L plant tissue culture agar. Root growth was marked every 24 h on plates. The plates were photographed, and the root growth was measured with ImageJ software by calculating the distance between successive marks along the root axis. For DNA replication inhibitory experiments, 1 mM HU (Sigma) or 3 mg/L bleomycin was added to 0.5x MS plates (as above).

**References**


Figure S1. Increased endoreduplication in *etg1* roots. Ploidy level distribution of roots of 8-day-old wild-type (WT) and *etg1-1* plants as measured by flow cytometry. Data represent average ± SD (n=4). Asterisks indicate significant statistical differences by t-test (p < 0.05) between wild-type and *etg1-1*.
Figure S2. Faster onset of endoreduplication in *etg1* mutant leaves. The DNA ploidy level distribution of the first leaf pair of wild-type (A) and *etg1-1* mutant (B) plants was measured during development. Leaves were harvested at the indicated time points. Data represent average ± SD (n=4).
Figure S3. Root growth inhibition in *etg1* mutant plants. Kinematic analysis of the root elongation rate of wild-type (Col-0) and *etg1-1* plants. Plants were grown on MS agar plates. Data represent average ± SD (n=8 to 10).
Figure S4. Reduced CDKA;1 activity in the etg1 mutant. Kinase activity assays of purified CDKA;1 complexes of wild-type (WT) and etg1-1 plants. Relative CDKA;1 activity was measured with histone H1 as substrate. For quantification, the control was arbitrary set at 100%. The indicated values represent average ± SD (n=2).
Figure S5. Noninduction of ETG1 in E2Fc-overexpressing plants. ETG1 expression levels were determined by real-time PCR with cDNA prepared from wild-type (WT) and E2Fc-overexpressing (E2Fc OE) 8-day-old seedlings. All values were normalized to the expression level of the ACT2 housekeeping gene. The normalized value of the wild-type sample was arbitrary set to 1. Data represent average ± SD (n=3).
Figure S6. Conservation of the ETG1 protein in eukaryotes. Alignment of ETG1 and its orthologous proteins: Os01g0166800 (rice), C10orf119 (human), 1110007A13Rik (mouse), CAJ81286 (frog), CG3430 (fruitfly), and SPAC1687.04 (fission yeast).
**Figure S7. ETG1 TAP-tag analysis.** Analysis of the TAP protein eluates from the untransformed control cell line PSB-D (left) and a transgenic cell suspension culture expressing TAP-tagged *ETG1* (right). TAP-tagged protein complexes were purified, precipitated with trichloroacetic acid (TCA) (25% v/v), separated by 4-12% Nu-PAGE, and visualized with colloidal Coomassie G-250. Protein bands were cut from the gel and sequenced by tandem MS. The protein band corresponding with the bait is indicated with an asterisk and those corresponding to co-purified MCM proteins by a number (2 to 7 for MCM2 to MCM7, respectively). MCM6 was detected on the gel at a position corresponding to its molecular mass and in a protein band running higher at approximately 140 kDa.
Figure S8. Unsensitivity to hydroxyurea or bleomycin of etg1 mutants. Wild-type (A, C, and E) and etg1-1 (B, D, and F) seeds were germinated on 0.5x MS plates, grown for 5 days, and transferred to control medium (A and B), medium containing 1 mM HU (C and D), or medium containing 3 mg/l bleomycin. (E and F). Arrowheads indicate the positions of root tips when plants were transferred to each media. (G) Relative root growth of untreated (control) versus treated seedlings shown in (A) to (F) is presented for wild-type (WT) and etg1-1 samples. Error bars indicate SE (n=20).