Sir et al., http://www.jcb.org/cgi/content/full/jcb.201309038/DC1

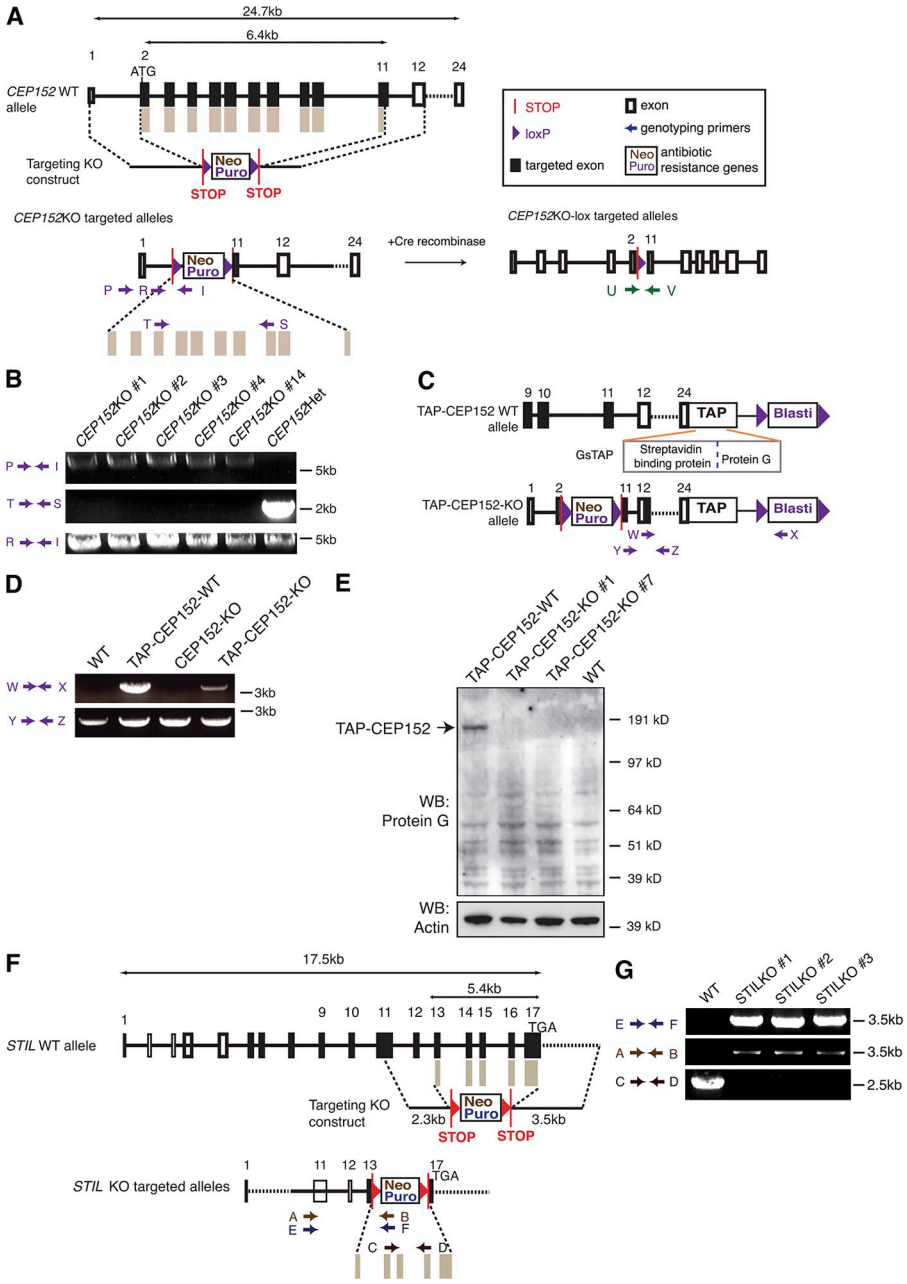


Figure S1. **Genetic manipulations of *CEP152* and *STIL* in DT40 cells.** (A) Design of homologous gene targeting of *CEP152* alleles in DT40 cells. To generate *CEP152*-KO alleles, all of exons 2–10 and part of exon 11 were replaced sequentially by neomycin (Neo)- and then puromycin (Puro)-resistance genes that are flanked by in-frame STOP codons. Note that the translation START site resides in exon 2 of *CEP152*, and this ATG codon is removed by the KO strategy. Antibiotic resistance genes were removed by transient Cre expression to produce *CEP152*-KO-lox alleles. Specific primer positions correspond to diagnostic PCR reactions used to identify CEP152-KO clones as shown in A. (B) PCRs of genomic DNA extracted from cells of the indicated genotypes are shown. The location of primers is marked in A. Primers T and S map within the knocked out region. This region is absent in five independent homozygous KO clones (CEP152-KO No. 1–5) but present in the heterozygous clone (CEP152Het). PCR product amplified by primers P and I confirms the presence of the puromycin-targeting construct at the correct genomic locus. Primers R and I confirm the presence of the neomycin-targeting construct. Note that CEP152Het cells carry only one allele of *CEP152* correctly targeted with the neomycin construct. (C) WT and CEP152-KO cells were tagged with a GsTAP tag (encoding streptavidin-binding protein and protein G) at the endogenous *CEP152* locus to generate TAP-CEP152-WT and TAP-CEP152-KO cells, respectively. A GsTAP tag was introduced in frame into the last exon (exon 24) with the concomitant removal of the STOP codon. Only one allele was targeted in both instances. Blasti, blasticidin. (D) PCRs of genomic DNA extracted from cells of the indicated genotypes are shown. Genomic locations of primers are marked in C. In brief, primer pair W-X was used to check for the targeted integration of the construct. (E) Western blots of cytoplasmic cell extracts from cell lines with the indicated genotypes. Blots were probed with anti–protein G (Western blot [WB]: protein G) or anti-actin (Western blot: actin) antibodies. Unlike WT and CEP152-KO cells, TAP-CEP152-WT and TAP-CEP152-KO cell lines contain an in-frame GsTAP tag in one *CEP152* allele. TAP-CEP152-WT cells express a specific product detected by anti–protein G antibodies. The absence of a protein product in TAP-CEP152-KO cells indicates that no truncated protein is produced downstream of the antibiotic resistance cassette from the disrupted allele of *CEP152*, and thus, the CEP152-KO line is a protein null. Actin serves as a loading control. (F) Design of homologous gene targeting of *STIL* alleles in DT40 cells. In brief, parts of exons 13–17 and all the flanking exons 14–16 in both alleles of *STIL* were sequentially replaced by neomycin- and puromycin-resistance genes that are flanked by in-frame STOP codons. (G) PCRs of genomic DNA extracted from cells of the indicated genotypes are shown. Location of primers is marked as in F. In brief, primer pairs A-B and E-F were used to check for the targeted integration of the *STIL* homologous gene-targeting construct containing neomycin and puromycin, respectively. Primer pair C-D maps within the KO region, and the lack of PCR product confirms the absence of this region in both alleles of STIL-KO cells.

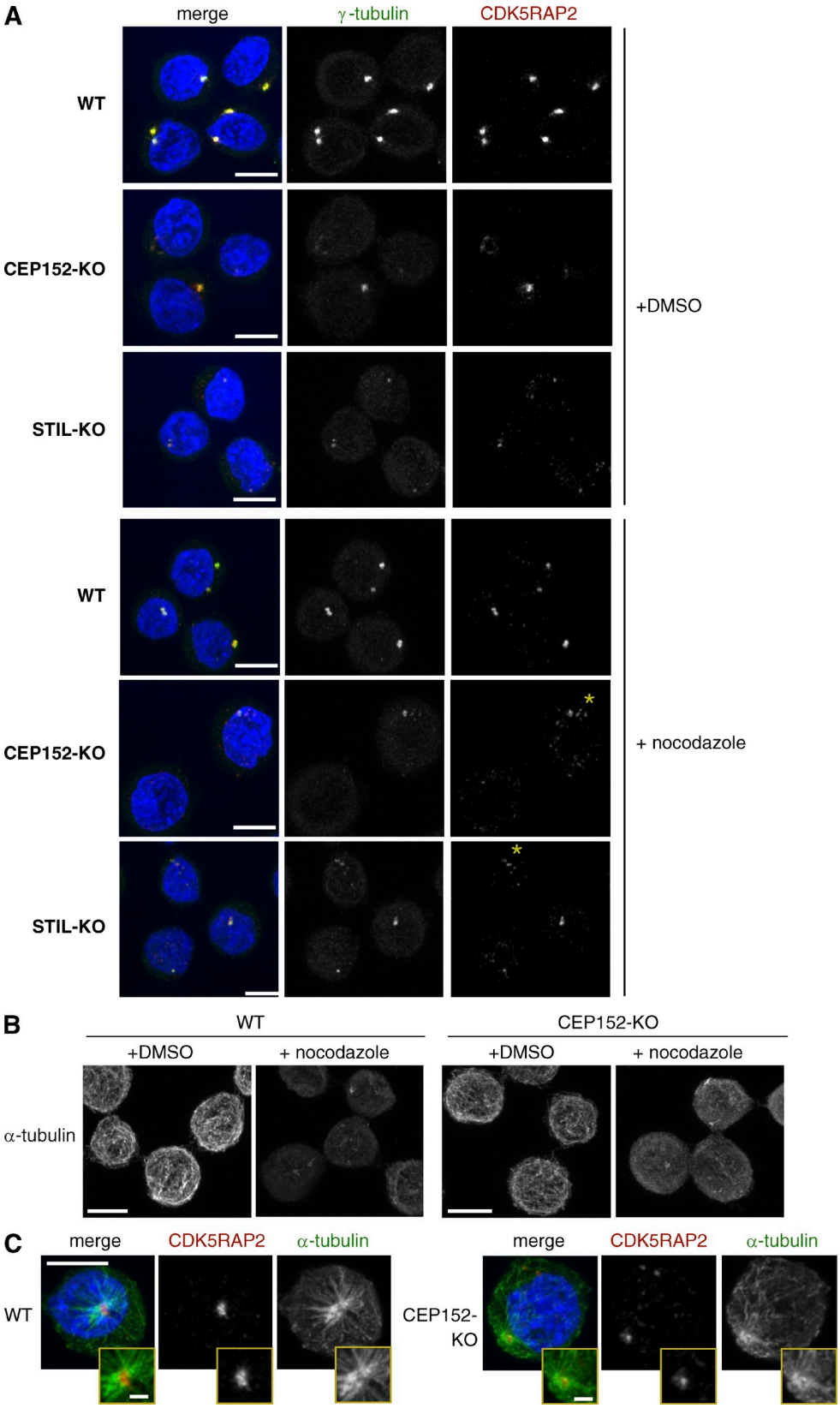


Figure S2. **aMTOCs persist in the absence of MTs in CEP152-KO and STIL-KO cells.** (A) PCM is visualized by antibody stainings against γ-tubulin and CDK5RAP2. Note that a prominent PCM signal persists in KO cells even when MTs are depolymerized by a 5-h treatment with nocodazole. Asterisks show examples for small cytoplasmic CDK5RAP2 and γ-tubulin foci after prolonged nocodazole treatment. These are detectable both in WT and KO cells but much more prominent in the latter. (B) Anti–α-tubulin antibody staining confirms that MTs are depolymerized by the nocodazole treatment. Images in A and B correspond to same experiment. (C) Images depict MT regrowth after 4-h nocodazole and cold treatment. Cells are stained with anti–α-tubulin and anti-CDK5RAP2 antibodies. Insets show higher magnifications of MT asters. Bars: (main images) 5 µm; (insets) 1 µm.

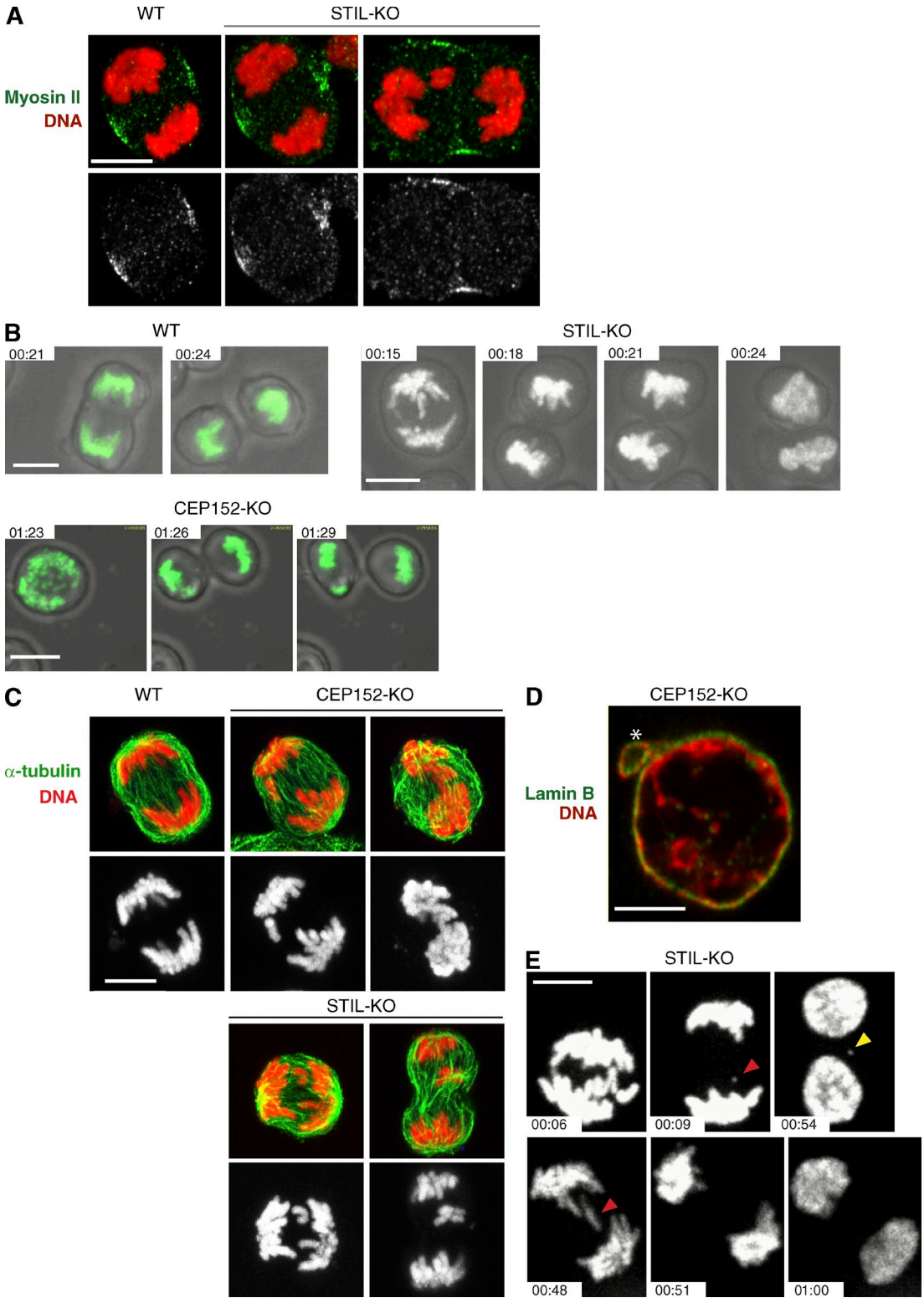
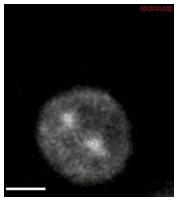
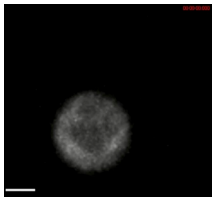


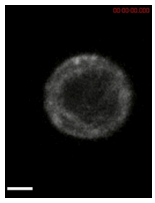
Figure S3. **Cells lacking intact centrosomes exhibit chromosome segregation defects and micronuclei.** (A) Cleavage furrow positioning in WT and STIL-KO anaphase cells. Cells are stained with anti–myosin II antibody. (B) Still frames from time-lapse series to illustrate progression from anaphase onset through telophase and cytokinesis in CEP152-KO and STIL-KO cells. Cells expressing histone H2B-GFP were imaged at a frame rate of 3 min (green or grayscale). Images are fluorescent and bright-field overlays. (C) Lagging chromosomes are present in fixed CEP152-KO and STIL-KO cells in anaphase. Cells are stained with anti–α-tubulin antibody. (D) Micronuclei are present in CEP152-KO cells. A single micronucleus is marked with an asterisk. Nuclear envelope is stained with antibodies against Lamin B. (E) Still frames from time-lapse experiments show STIL-KO cells in mitosis. Cells expressing histone H2B-GFP were imaged at a frame rate of 3 min. Lagging chromatids are evident during anaphase (red arrowheads). The yellow arrowhead marks the micronucleus. Times are given in hours and minutes. Bars: (A–C and E) 5 µm; (D) 2 µm.



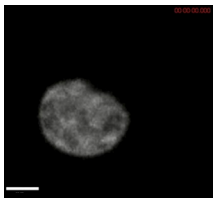
Video 1. **Mitosis in a WT DT40 cell transfected with GFP-tubulin.** Images were analyzed by time-lapse confocal microscopy using a spinning-disc confocal system (PerkinElmer). Frames were acquired every 3 min. Time is given in hours, minutes, seconds, and milliseconds. Bar, 5 µm.



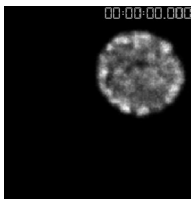
Video 2. **Mitosis in a CEP152-KO DT40 cell transfected with GFP-tubulin.** Images were analyzed by time-lapse confocal microscopy using a spinning-disc confocal system (PerkinElmer). Frames were acquired every 3 min. Time is given in hours, minutes, seconds, and milliseconds. Bar, 5 µm.



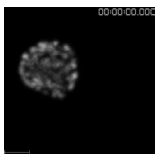
Video 3. **Mitosis in a STIL-KO DT40 cell transfected with GFP-tubulin.** Images were analyzed by time-lapse confocal microscopy using a spinning-disc confocal system (PerkinElmer). Frames were acquired every 3 min. Time is given in hours, minutes, seconds, and milliseconds. Bar, 5 µm.



Video 4. **Mitosis in a WT DT40 cell transfected with histone H2B-GFP.** Images were analyzed by time-lapse confocal microscopy using a spinning-disc confocal system (PerkinElmer). Frames were acquired every 3 min. Time is given in hours, minutes, seconds, and milliseconds. Bar, 2 µm.



Video 5. **Mitosis in a CEP152-KO DT40 cell transfected with histone H2B-GFP.** Images were analyzed by time-lapse confocal microscopy using a spinning-disc confocal system (PerkinElmer). Frames were acquired every 3 min. Time is given in hours, minutes, seconds, and milliseconds. Bar, 2 µm.



Video 6. **Mitosis in a STIL-KO DT40 cell transfected with histone H2B-GFP.** Images were analyzed by time-lapse confocal microscopy using a spinning-disc confocal system (PerkinElmer). Frames were acquired every 3 min. Time is given in hours, minutes, seconds, and milliseconds. Bar, 2 µm.

Table S1. **List of primers used in study**

Primer	Sequence
CEP152-KO	
CEP152-KO LA F (XhoI)	5′-ctcgagGTGAGAACCAAAGACCGTATA-3′
CEP152-KO LA R (SalI)	5′-gtcgacTGTGCTCCAACAGTATTTTCC-3′
CEP152-KO RA F (XbaI)	5′-tctagaTGAGCAAACTTACTGCAAACA-3′
CEP152-KO RA R (NotI)	5′-gcggccgcGCTTATCGTGTGTGCGGACTG-3′
Primer P in Fig. S1	5′-AGCAGAATCAGCCTAGTTCT-3′
Primer R in Fig. S1	5′-CTCGAGGTGAGAACCAAAGACCGTATA-3′
Primer I in Fig. S1	5′-ACGACCCCATGGCTCCGACCGAAG-3′
Primer T in Fig. S1	5′-GATATGCTGGAGGACAGTGGAG-3′
Primer S in Fig. S1	5′-AATACCTAATCTGCTGCGCACTC-3′
Primer Z in Fig. S1	5′-GCGGCCGCCCTGCAGTAGCAGGTGGCAAGG-3′
Primer K in Fig. S1	5′-GCGGCCGCGCAGGACTGAATAGAGTAGGC-3′
STIL-KO	
STIL-KO LA F (SalI)	5′-gtcgacACAAGCACTACATCAATCTGT-3′
STIL-KO LA R (BamHI)	5′-ggatccTCACTGTGCTTCCAATAAGCGTTG-3′
STIL-KO RA F (BamHI)	5′-ggatccTAAAAGAGCATGGTGGGTCTTAG-3′
Primer A in Fig. S2	5′-CATACAGAGGCAGAACAGAC-3′
Primer B in Fig. S2	5′-GCTTTGGTGACAGAAGGCATC-3′
Primer C in Fig. S2	5′-CATACAGAGGCAGAACAGAC-3′
Primer D in Fig. S2	5′-ACGACCCCATGGCTCCGACCGAAG-3′
Primer E in Fig. S2	5′-GCTGAACAAGATGCAAGTAACAC-3′
Primer F in Fig. S2	5′-CCATCATCGTTAACAAACCCTGC-3′
TAP-CEP152	
TAP-CEP152 LA F (SalI) (also Primer Y in Fig. S1)	5′-gtcgacCACTGAGATGAAGGCTAGAC-3′
TAP-CEP152 LA R (XbaI) (also primer Z in Fig. S1)	5′-tctagaGATTGGCAGTGTATTAGTTCT-3′
TAP-CEP152 RA F (BamHI)	5′-ggatccAGTCTCCTTAGCAAGGCACTG-3′
TAP-CEP152 RA R (NotI)	5′-gcggccgcGCATATAGCAGTCTGTTGTGG-3′
Primer W in Fig. S1	5′-GGCGCAGGTCATTGTTTATT-3′
Primer X in Fig. S1	5′-CCCCCTGAACCTGAAACATA-3′

Sequences corresponding to restriction enzyme sites are in lowercase. F, forward; R, reverse; LA, left arm; RA, right arm.