Supplemenatry Methods

PCR conditions and sequencing. PCR reactions were carried out with a commercially available Taq polymerase (Go-Taq) from Promega (Promega: http://www.promega.com) with the recommended buffer. Denaturing was at 95°C for 2 min. This was followed by 40 cycles as follows: 95°C 30 sec, annealing for 30 sec as indicated above, and 72°C for 1 min 20 sec. The PCR products were then elongated for 5 min at 72°C. Primers were at a final concentration of 1µM, dNTPs 200 µM and $MgCl_2$ 2mM. Primers used are listed in Table S1. Sequencing was outsourced to MWG Biotech *(*www.Eurofinsdna.com*).*

Analysis of transcription by RT-PCR. RNA was prepared with the TRItidy G reagent from AppliChem GmbH (www.applichem.com) according to the manufacturers' instructions. We used the influorescences of green house grown plants for wild-type Columiba. For the *trs120* alleles, we pooled 50 -100 mutant five day old seedlings germinated on MS sucrose plates and used similarly grown *massue* seedlings as an additional control. For cDNA first-strand synthesis, we used the Moloney Murine Leukemia Virus Reverse Transcriptase, RNase H Minus (M-MLV RT [H–]), from Promega (www.promega.com) together with an Oligo(dT)15 Primer and RNasin® Plus RNase Inhibitor, also from Promega, according to the manufacturer's instructions. Primers used for RT-PCR are: *AtTRS120 (fwd:* CTGCACAGTTCACTGCTTTGGTG, rev: GACAATGGCCCTGCGTAGTG)*; BET5 (fwd:* GGATCCAGTCAATGCGGATAAAGG*, rev:* CTAAGATAATCTTAATACCAGAGGG*).* The region amplified with the *AtTRS120* RT-PCR primers is depicted by a line above the gene model in Figure 4.

allele	polymorphsim	Fwd primer Sequence 5' -> 3'	Rev primer Sequence 5' -> 3'	LB primer	Annealing Temp. [°C]
bet3	GABI_318C08	TCT CCG GGA GTA AAA ACA AC*	CAC GAT TTG AGA CAT TGT GAT AC	Lo8409	55.0
bet5-1	SALK_099482	AAGATAATCTGGAACCTGGCTGATTG*	TCGCGTAAATCTCCGGTCTTTG	LBa1	60.3
bet5-2	SAIL_634_G07	AGAAGAAAATGAGAACCAGGGAGATG*	TGGTCAAGATGCAACAGTGGAAG*	LB3	60.6
trs31	FLAG_488E06	TCGATTTCGAGCATCTGCTGATTGG	CAACAAACGCTCCGCAGTTGAATG*	LB4	62.0
trs33-1	SALK_109244	GCAAACAGAAGCCTGCAATGG*	TGAGGCATGTTTTGTTGCTTCTG	LBb1.3	55.8
trs33-2	SALK_109724	GCAAACAGAAGCCTGCAATGG*	TGAGGCATGTTTTGTTGCTTCTG	LBb1.3	55.8
trs120-1	SALK_125246	CTTTGCCACTGTCCCTCGTC*	TGAGCATTGGCATCAACAGG*	LBb1.3	55.8
trs120-2	SALK_021904	CTTTGCCACTGTCCCTCGTC*	TGAGCATTGGCATCAACAGG*	LBb1.3	55.8
trs120-3	SALK_111574	GGGCATCCATGTCAAAAGTGTC*	TTTTGCCAGAGTCAGCTAAGAACC	LBa1	60.3
trs120-4	SAIL_1285_D07	TGATTGAGCATGGTTTTCTGGAG	TGTCCACTTGGGAGGAATGG*	LB3	58.9
vps53-1	SALK_047230	TCCAATGTACCAGCTCCTTC	ACCCAAACCAGCTCATTGTC	LBb1.3	55.8
vps52-2	SALK_055433	TCATGATCCAGCCTTTTG*	TCAACTCACCTTCAGTACAGC	LBb1.3	51.4
vps53-2	SAIL_87_D06	GTGCAAAATTCATCACATGACACAC	AGAACATAACCACGACAATCACTGC	LB3	59.3
vps53-3	GABI_400C01	CGCGTATCAAAATATTTCTTC*	TGTTGCTTGGTTTACGTAGG	Lo8409	52.0
vps53-4	SAIL_117_D11	CAACCACCTTATCTAAGACCGTGAAG	CGCTTCACGAGATTATTTTGTTCC	LB3	59.3
vps53-5	GABI_463D10	GGAAACAAAGGTGGGAATTG	CCTGATGCCTAAACCCTTTG*	Lo8409	55.3
vps54-1	SALK_036485	GATCCGACTTCCATGGCTAC*	TGGCGGAAGCAGTATAGACC*	LBb1.3	55.8
vps54-2	SALK_062261	TTCCCGGTCTTTATTGTTGG	TTTGCTCGCGAGAGATAAGC*	LBb1.3	55.3

Table S1. Primers used for characterizing or sequencing polymorphisms. a: The primer denoted by an asterisk was used together with the T-DNA left border LB primer to amplify the DNA insertion and flanking genomic sequences, as described at http://signal.salk.edu. Annealing times were 30sec for all primer pairs. T-DNA primers were: LBa1: TGGTTCACGTAGTGGGCCATCG; Lb3: TAGCATCTGAATTTCATAACCAATCTCGATACAC; LbB1.3: ATTTTGCCGATTTCGGAAC; LBo8409: ATATTGACCATCATACTCATTGC; LB4: CGTGTGCCAGGTGCCCACGGAATAGT.

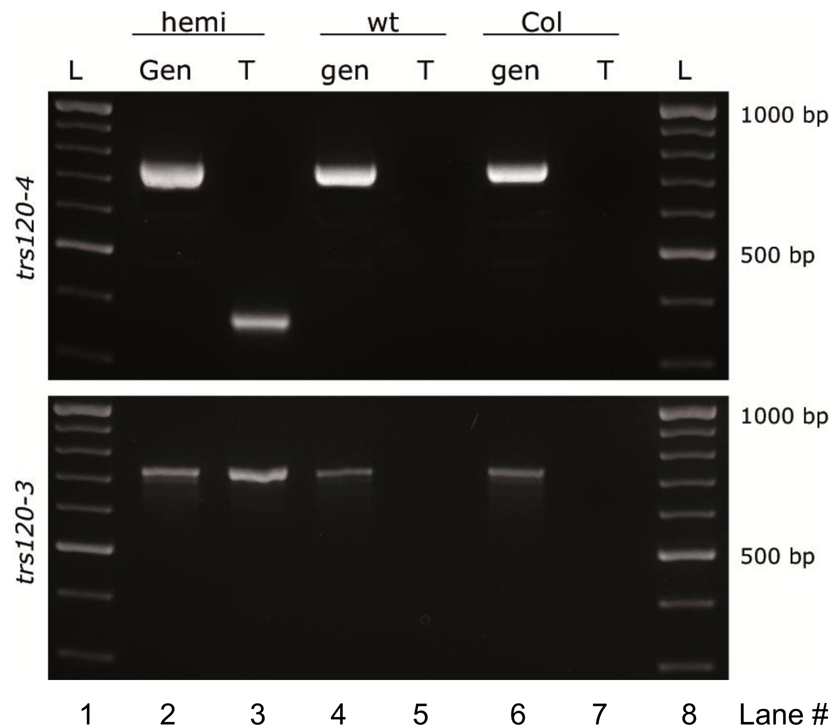

Figure S1. Segregation analysis of the *trs120-3* and *trs120-4* T-DNA insertion

Hemizygote T-DNA insertion lines were planted and single progeny analyzed. Hemi: hemizygote segregant, wt: homozygote wild-type segregant; Col: Columbia wild-type control; L: DNA 100 bp ladder (MBI). Gen: genomic primers used to amplify genomic DNA. T: T-DNA LB (left border) primer used with one genomic primer to amplify sequences flanking the insertion. In the hemizygous segregant, an upper band at c. 700bp with the genomic primers (Gen) is in both alleles of *trs120* (lane 2). Because the T-DNA would disrupt the gene, this band indicates the presence of at least one wild-type copy of the gene. The hemizygous lines can also be seen to harbour a T-DNA insertion (lane 3). In both the wild-type segregants and the Columbia control, a genomic band is present (lanes 4 and 6) but no T-DNA insertion was detected (absence of a band in lanes 5 and 7). In brief, the T-DNA can only be found in the hemizygous (hemi) individuals, which also invariably segregate the cytokinesis-defective phenotype.

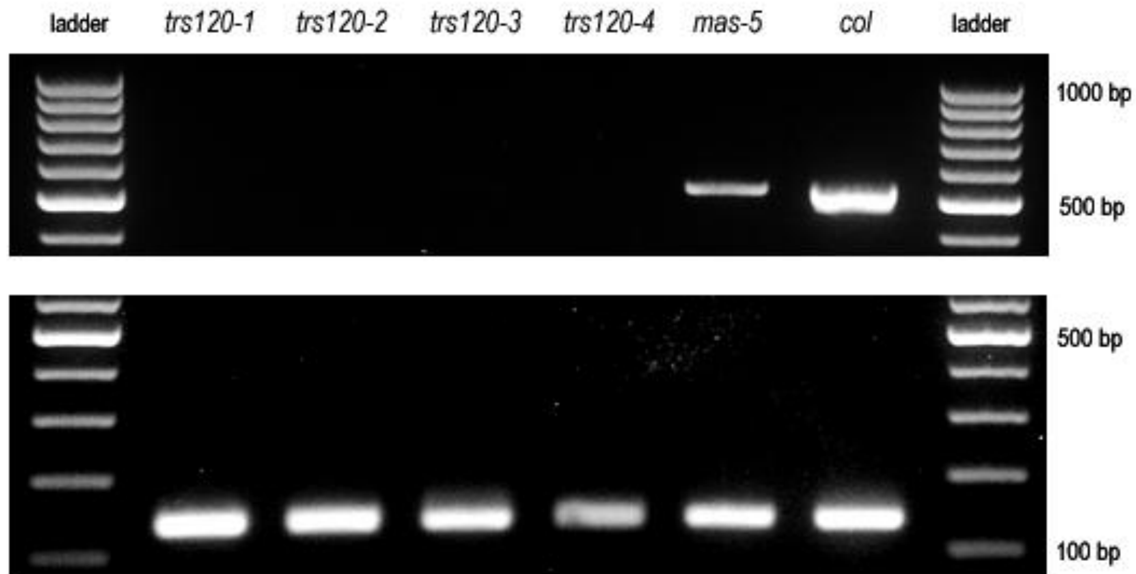

Figure S2. RT-PCR showing the absence of transcripts downstream of the insertion sites (upper panel; see Fig. 4). *BET5* primers were used as a positive control for the RNA isolation and reverse transcription (lower panel).