

Supporting Information

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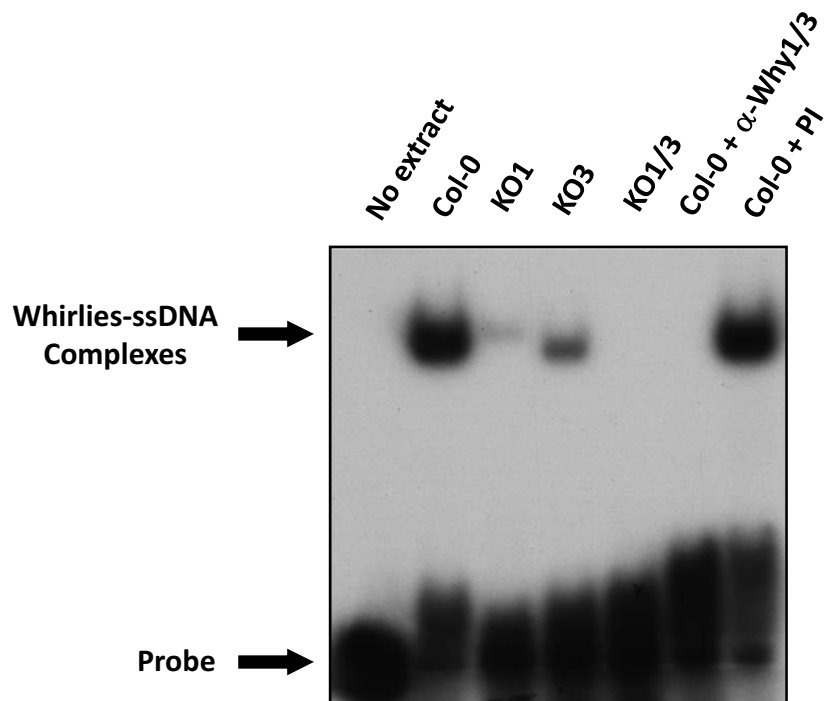


Fig. S1. ssDNA-binding activity of AtWhy1/3 in *Arabidopsis* ptWhirlies mutants. EMSA was performed by using 20 μ g of crude plastid proteins isolated from plants of the indicated genotypes and a radiolabeled probe of 32 nt (5'-TGTCATTTTGTGTCATTTTGTGTCATTTTGTGCA-3'). As a control, an anti-AtWhy1/3 antibody (α -Why1/3) or preimmune serum (PI) was preincubated with Col-0 extracts before adding the probe. Addition of the antibody eliminated the signal in the Col-0 extract, confirming that this signal corresponds to a complex between Whirlies and ssDNA.

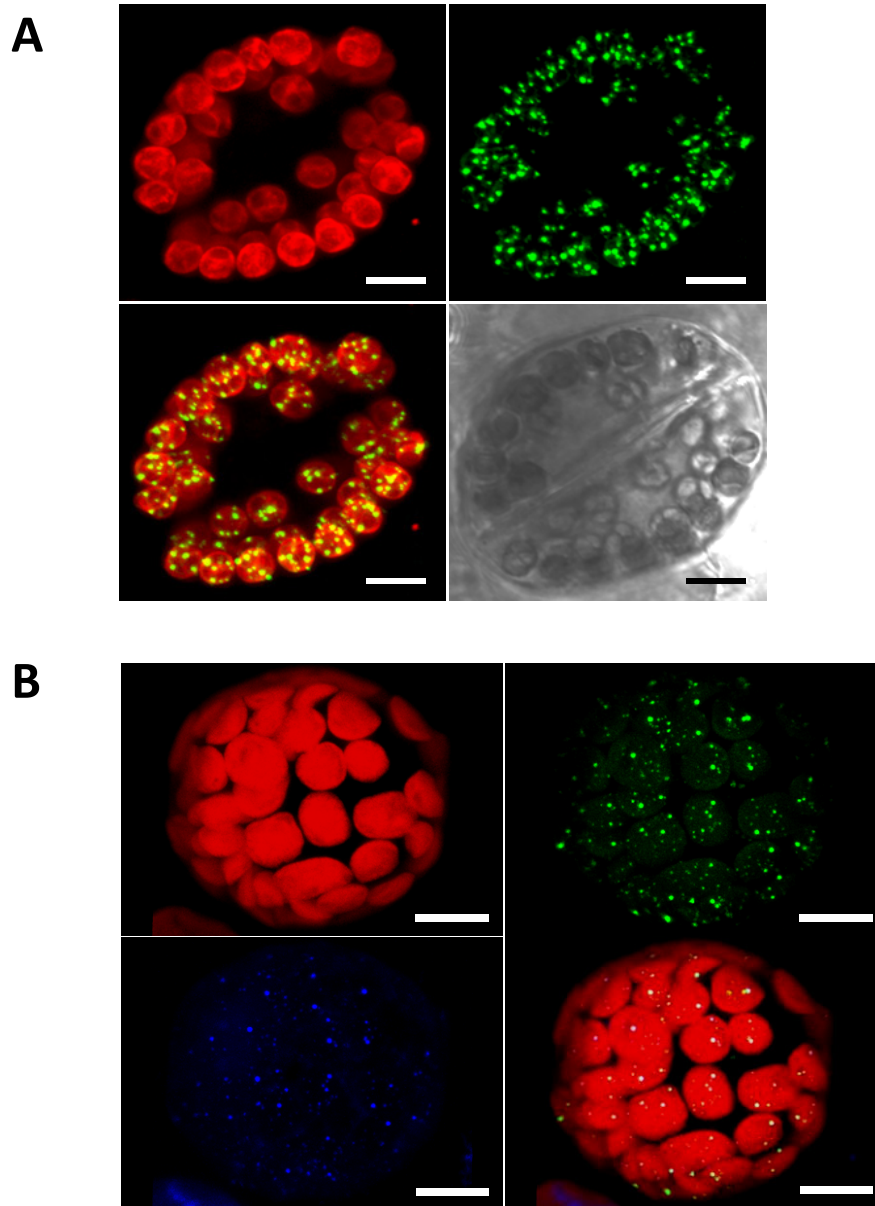


Fig. S2. Colocalization of StWhy1 with chloroplast DNA. (A) Laser scanning confocal microscopy of StWhy1-GFP tobacco leaf guard cells. (Upper Left) Chlorophyll autofluorescence pseudocolored in red. (Upper Right) GFP fluorescence pseudocolored in green. (Lower Left) Overlay of chlorophyll and GFP fluorescence. (Lower Right) Corresponding phase-contrast image. (B) Laser scanning confocal microscopy of a tobacco leaf mesophyll protoplast transiently expressing StWhy1-GFP and stained with the DNA dye Syto85. (Upper Left) Chlorophyll autofluorescence pseudocolored in red. (Upper Right) GFP fluorescence pseudocolored in green. (Lower Left) Syto85 fluorescence pseudocolored in blue. (Lower Right) Overlay of all 3 images. Maximum projections are shown. (Scale bars: 8 μm .)

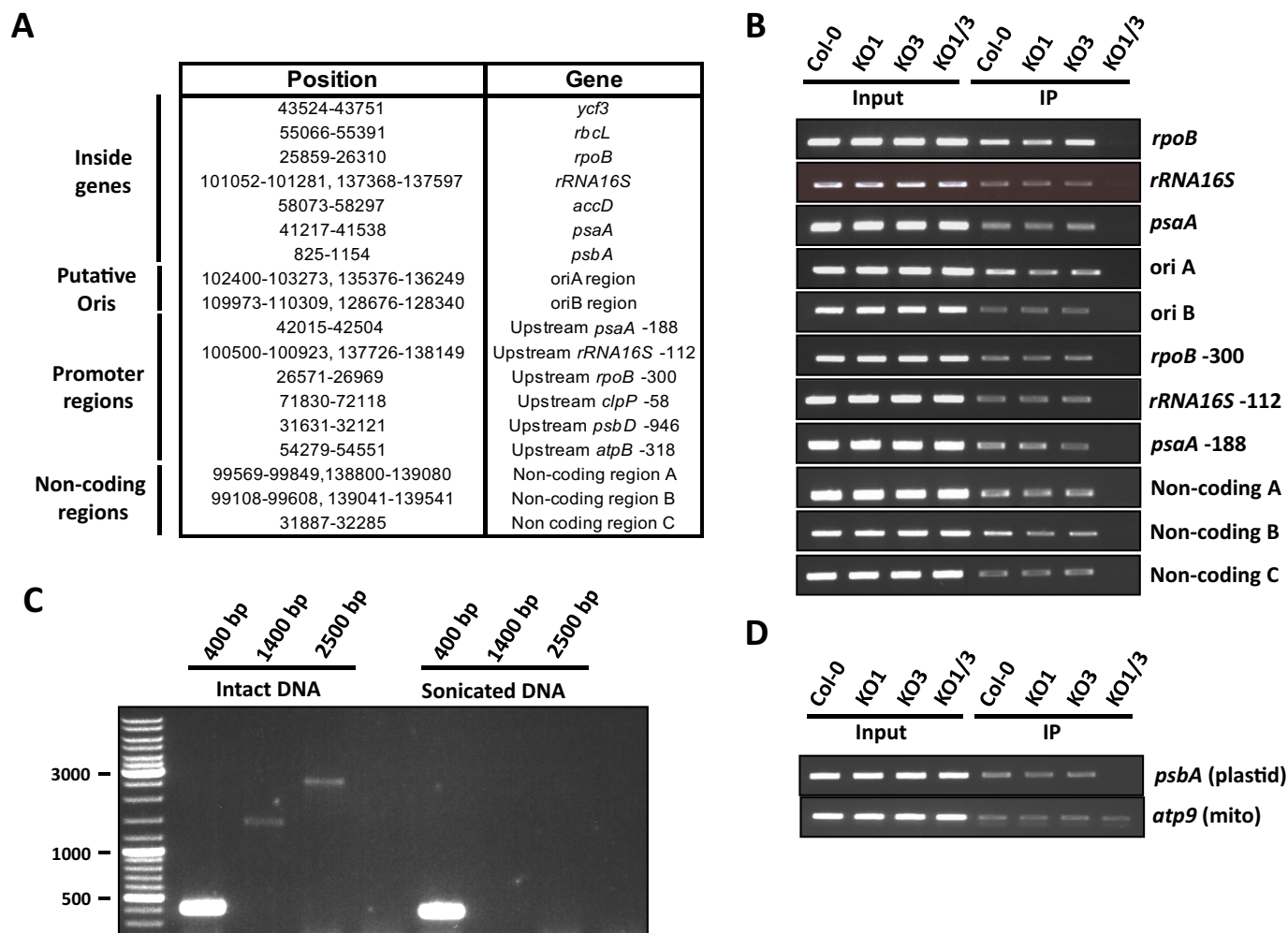


Fig. S3. AtWhy1 and AtWhy3 interact with chloroplast DNA. (A) Position of the amplified regions on the plastid genome. (B) PCR amplification of ptDNA regions after immunoprecipitation on crude plastid extracts of the indicated genotypes. Inputs represent 20% of the total DNA used in the immunoprecipitation. Representative regions are shown indicating specific interaction with AtWhy1/3. (C) Total ptDNA after sonication was purified and used as a template for PCR. Oligonucleotides designed to amplify regions of increasing length were used to verify the efficiency of the sonication regimen. (D) Immunoprecipitation on total crude organelles was performed and plastid or mitochondrial DNA was assessed by PCR using oligonucleotides designed to amplify part of the plastid *psbA* or mitochondrial *atp9* genes.

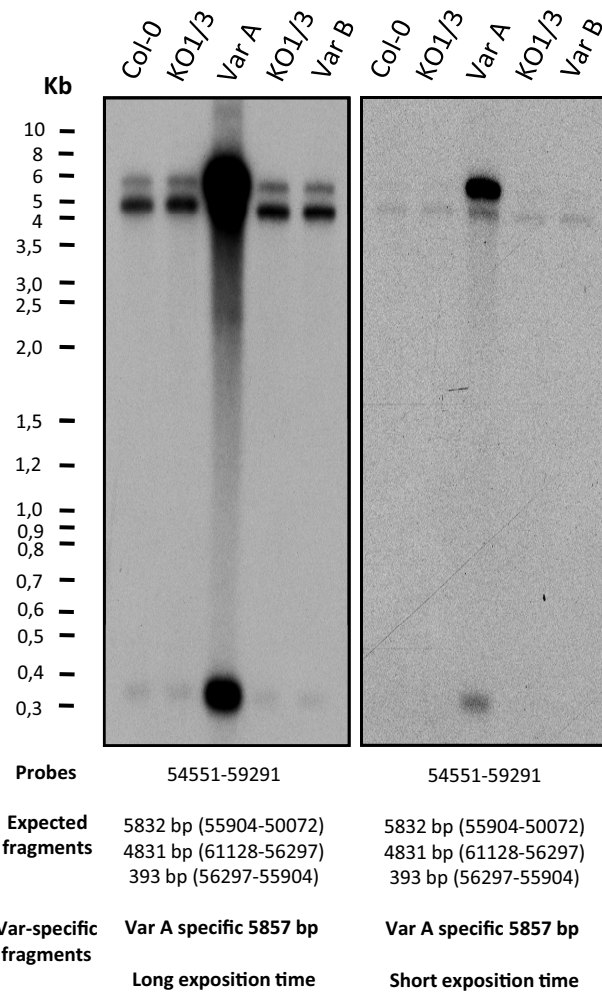


Fig. S5. Long (16 h) and short (4 h) exposure from Fig. 2A. DNA gel blot (10 μ g per lane) of total leaf DNA digested with HindIII and hybridized with the probes indicated below the gel. The probe numbers refer to the nucleotides of the published *Arabidopsis* chloroplast genome (8). Expected fragments from restriction analysis of Col-0 ptDNA and the size of new fragments observed in variegated lines are presented below the probes.

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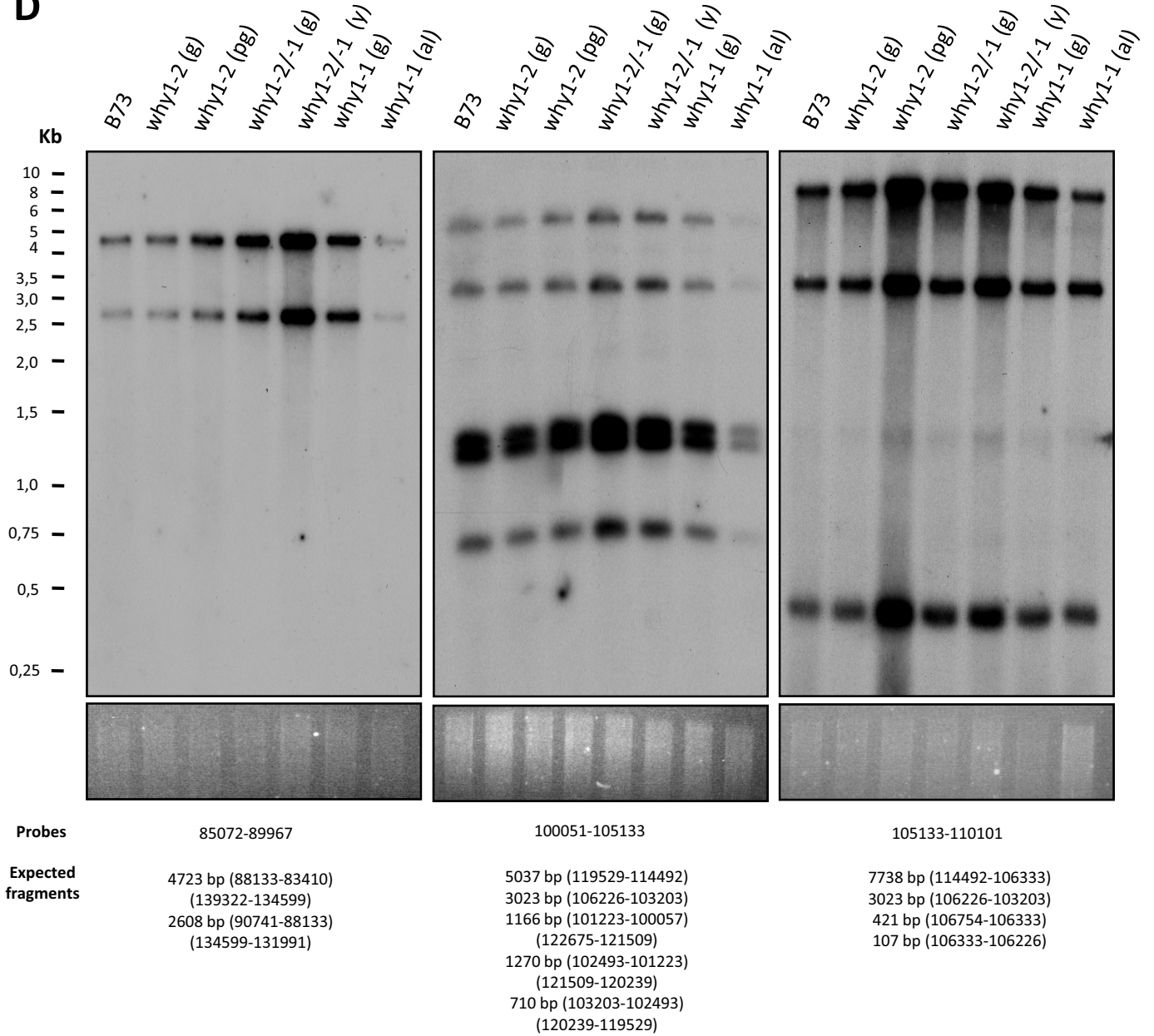


Fig. S6. Continued.

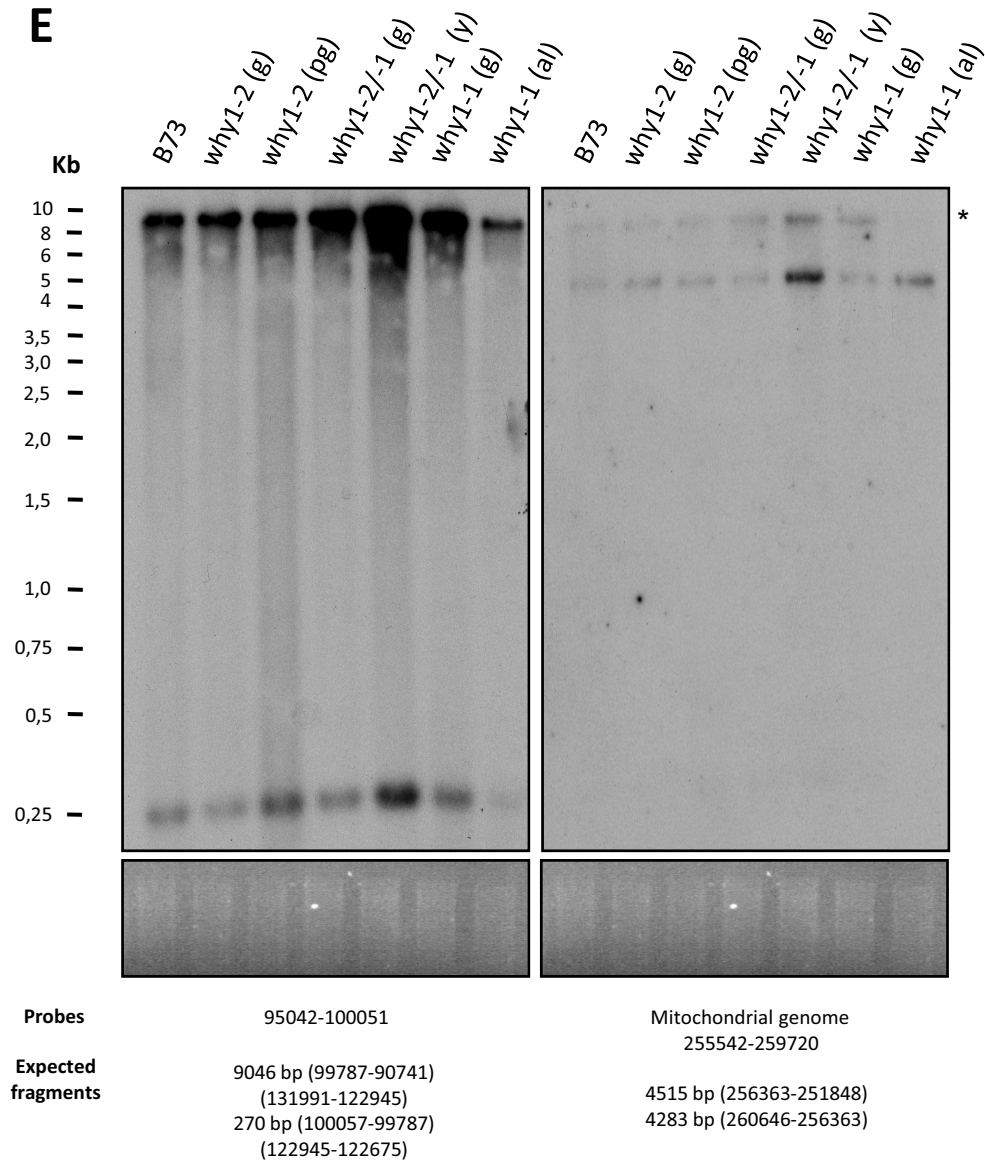


Fig. S6 Continued.

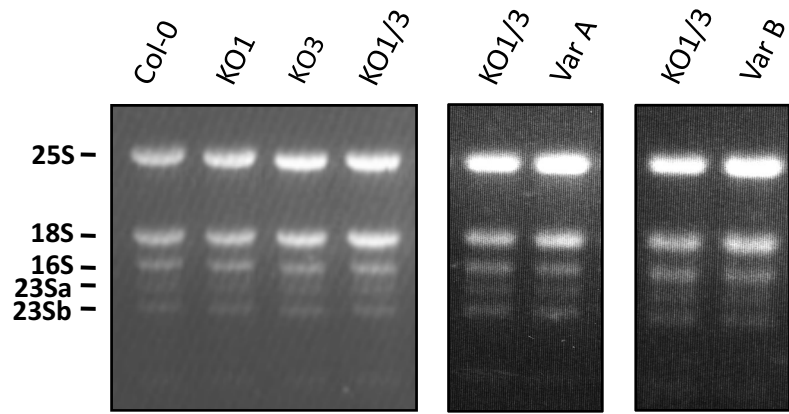


Fig. S8. Levels of plastid 23S and 16S rRNA in *Arabidopsis* ptWhirlies mutants. Total RNA was extracted from 100 mg of mature leaf tissue from the indicated plants. Ten micrograms of purified RNA was migrated on a 1.2% agarose gel and stained with ethidium bromide.

Table 2. Representative recombination events between short direct repeats in *Arabidopsis* plants lacking ptWhirlies

Reaction	Band #	Plant lines	DR positions	Short direct repeat sequences	DR length and (mismatches)
A	1	KO1, KO3 KO1/3	20366 recombinant 31005	aatatatataCAAAAATTTTCTTTTTTTacacttctta gttaagttatCAAAAATTTGCTTTTTTTacacttctta gttaagttatCAAAAATTTGCTTTTTTTtttttagaa	(1) 18 bp (0)
A	2*	KO1/3	30300 recombinant 30827	gtcatttatgCAAAAAAAAAAAtggttatgta attggctctaaCATAAAAAAAAAAAtggttatgta attggctctaaCATAAAAAAAAAAagaaaaaag	(1) 14 bp (0)
B	1	Col-0 KO1, KO3 KO1/3	50427 recombinant 69424	aattccaatcGGAGTGGATTGGGcaagggata tctaaaaaatGGAGTTGATTGGGcaagggata tctaaaaaatGGAGTTGATTGGattgacca	(1) 12 bp (0)
B	2	KO1 KO1/3	49181 recombinant 68999	tttttatttcCCCCACCTTTTTTatataaaatt ctctaaccttCCCCACCTTTTTTatataaaatt ctctaaccttCCCCACCACTATTTTTtgctaggta	(0) 15 bp (3)
B	3	KO3	49972 recombinant 70402	gttttttttaCTTTTTTTTTTTTAttattgtatc cccaaagtgctTTTTTTTTTTTTTAttattgtatc cccaaagtgctTTTTTTTTTTTTTAcggtgtgaaa	(0) 15 bp (0)
B	4	KO1/3	48553 recombinant 68998	tagaattgtaTCCCCCCTTCATTATTGCTttccgatctt actctaacctTCCCCCCTTCATTATTGCTttccgatctt actctaacctTCCCCACCCTATTTTTTGCTaggatatttc	(0) 21 bp (5)
C	1	KO3 KO1/3	59043 recombinant 69807	ttttgtaccTATTTTTTTATTctatttctat ttctcttcaaTATTTTTTTATTctatttctat ttctcttcaaTATTTTTTTATTtttataattga	(0) 12 bp (0)
C	2	KO1/3	59287 Recombinant 70228	acttacctcTATTTTTTGTCCTTtagtaggcct cgttacaggcTTTTTTTGTCCTTtagtaggcct cgttacaggcTTTTTTTGTCATTgcatagcct	(1) 14 bp (1)
C	3	KO1/3	59028 recombinant 70225	tatttagtttGGCTTTTTTTGTacctattttt taaccgtacaGGCTTTTTTTGTacctattttt taaccgtacaGGCTTTTTTTGTgattgcata	(0) 12 bp (0)
C	4	KO1/3	58721 recombinant 70186	attagactagACAAACAAAAAAttcattttc agacgggataACAAACAAAAAAttcattttc agacgggataACAAACAAAAAAtagataaat	(0) 14 bp (1)

2*: Non-specific annealing of primer 20481REV at positions 30367-30346 yielded this product.

Table S3. Short direct repeat-mediated recombination frequency in various *Arabidopsis* genotypes

Plant line	PCRs, <i>n</i>	Total events, <i>n</i>
Col-0	30	2
KO1	30	7
KO3	30	6
KO1/3	30	40

Other Supporting Information Files

[SI Appendix \(PDF\)](#)

[Table S4 \(PDF\)](#)

[Table S5 \(PDF\)](#)

[Table S6 \(PDF\)](#)