Supplementary data



Figure S1. Loss of WTG1 reduces the yield trait of Arabidopsis. (A) The length of siliques from wild-type, wtg1, and complementary lines. (B) Seed setting ability of wild type, wtg1, and complementary lines. Data are presented as mean \pm SD of triplicates.



Figure S2. WTG1 is expressed ubiquitously in *Arabidopsis* and targeted to chloroplasts. (A) Reverse-transcriptase PCR (upper) and real-time PCR (bottom) assays of *WTG1*. *UBQ5* served as the loading control; data are presented as mean \pm SD of triplicates. (B) Subcellular localization of WTG1-GFP in transformed mesophyll cells. Chlorophyll autofluorescence is shown in red. The image appears yellow where the signals from GFP and chloroplasts overlap. Bars = 10 µm.



Figure S3. RNA-seq analysis of wild-type and wtg1 leaves (A) Read numbers of sample sequences. (B) Frequencies of detected genes sorted according to their expression levels. (C) Number of differentially expressed transcripts.



Figure S4. Transcript analysis of chloroplast-associated genes in 18-day-old *wtg1* **by RNA-seq.** The relative expression levels are represented by the RNA-seq fold change values with *wtg1* in black bars and WT in white bars (fold change value = 1). (A) The expression levels of plastid genes transcribed by nuclear-encoded polymerase (NEP), plastid-encoded polymerase (PEP), both nuclear-encoded and plastid-encoded polymerase (NEP + PEP) and nuclear-encoded chloroplast genes (NC). (B) The expression levels of chlorophyll biosynthesis genes. (C) The expression levels of genes involved in retrograde signaling.



Figure S5. Controls for the Bimolecular fluorescence complementation assay. (A) The negative control showed no interactions between WTG1 and MORF2. (B) The interaction between MORF2 and MORF9 was used as the positive control (Takenaka *et al.*, 2012). Chlorophyll autofluorescence is shown in red in (A) and (B). Bars = 5

μm.

Primer name	Sequences (5'-3')	Purpose
53080cds_L1	CGCGGATCCATGGTGTTCTCTAACTCTC	
53080link_R1	GACTAGACCTCCGCCGTAGCTAAGACTCTGCTTG	
linkGFP_L1	GCCGGCGGAGGTCTAGTCATGGTGAGCAAGGGCGAG	
linkGFP_R1	CGCGTCGACTTACTTGTACAGCTCGTCC	
BamHI-53080-F	CACGGATCC ATGGTGTTCTCTAACTCTCTCC	
53080- EcoR1-R	CCCGAATTC CTAGTAGCTAAGACTCTGCTTG	
L1	TGGCAGTGTGTTTGTTTACG	
R1	TTGAGCAGAAGTTTACCGAG	
L2	TGTCCTTGTGCCTTCTCG	
R2	GAAGGAAGTTGGAAATGGAT	
071495-LP	CCTCAAAAGACGACAAACCTG	
071495-RP	GAAGCCGTTATTGAGGAGGAG	
006120-LP	TGCTTCAGCAGCTTTACCTTC	
006120-RP	TTTCAGGGCAAATCAATCAAG	
015164-LP	TCAGGATATAGCTGGTGGTGG	RT-PCR
015164-RP	GAGGTGGAAAAACTCTGGCTC	
LBa1	TGGTTCACGTAGTGGGCCATCG	RT-PCR
UBQ5F	AAGGTTCAGCGTTTGAGGAAGG	
JBQ5R	TCTTTCTGGTAAACGTAGGTGAGTC	RT-PCR
Y2H-EcoRI-53080F	GCGAATTCATGGTGTTCTCTAACTCTCT	
Y2H-53080-SalIR	GCGTCGACCTAGTAGCTAAGACTCTGCT	
Y2H-EcoRI-otp82F	GCGAATTCATGATGCTCT CGTGTTCTCC	
Y2H-otp82-BamHIR	GCGGATCCCTACCAGTAGTCATTGCAGG	
Y2H-EcoRI-morf2F	GCGAATTCATGGCTTTGC CTTTGTCTGG	
Y2H-morf2-SacIR	GCGAGCTCTCATCTTGTGTTTTCTCTGC	
Y2H-EcoRI-morf8F	GCGAATTCATGGCGACGC ATACCATTTC	
Y2H-morf8-BamHIR	GCGGATCCTTAACCCTGGTAGGGGTTGC	
Y2H-EcoRI-morf9F	GC GAATTCATGGCTTCCT TCACAACAAC	
Y2H-morf9-BamHIR	GCGGATCCTTAAGAGGAATCAGAGGCTG	
Y2H-EcoRI-orrm1F	GCGAATTCATGGAAGCTC TTATTGCTTC	
Y2H-orrm1-BamHIR	GCGGATCCCTAGAGCCCGAAACTTGGTT	
Y2H-NdeI-OZ1F	GGCATATGATGAACAACT CCACCAGACT	
Y2H-OZ1-BamHIR	GCGGATCCTCATTTATCTCCTTTACCAG	
Y2H-EcoRI-ppo1-F	GCGAATTCATGGAGTTAT CTCTTCTCCG	
Y2H-ppo1-BamHI-R	GCGGATCCTTACTTGTAAGCGTACCGTG	
BiFC-BamHI-53080F	CGCCACTAGTGGATCCATGGTGTTCTCTAACTCTCT	

Table S1 List of primers used in this study.

BiFC-53080-SalIR	TACCCTCGAGGTCGACGTAGCTAAGACTCTGCTTGT
BiFC-BamHI-morf2F	CGCCACTAGTATGGCTTTGC CTTTGTCTGG
BiFC-morf2-SalIR	TACCCTCGAGTCATCTTGTGTTTTTCTCTGC
BiFC-BamHI-morf8F	CGCCACTAGTGGATCCATGGCGACGCATACCATTTC
BiFC-morf8-SalIR	TACCCTCGAGGTCGACACCCTGGTAGGGGTTGCCAC
BiFC-BamHI-morf9F	CGCCACTAGTGGATCCATGGCTTCCTTCACAACAAC
BiFC-morf9-SalIR	TACCCTCGAG GTCGACAGAGGAATCAGAGGCTGCTG
petL-pyro-F	AACATATTTTATTGAGTCCCTTC
petL-pyro-R	TTOCTTACACCAATAAACACAA
(biotinylated)	IIGCIIAGACCAAIAAACAGAAC
ndhG-pyro-F	GATTTTCTTTTAGTTTTTCTGGG
ndhG-pyro-R	
biotinylated)	IACAAACAAUAACAAAICCIAAG
petLq-F	ATGCCTACTATAACTAGTTATTTC
petLq-R	TCAAATAAGTCGTATTTTGCTTAG
ndhGq-F	TGGTACGGGGTTATTTGGACTAC
ndhGq-R	CACCTATCAAAGCAACTAAAAGAATT
18srRNA-F	AAACGGCTACCACATCCAAG
18srRNA-R	ACTCGAAAGAGCCCGGTATT
psaB-F	GGACCCCACTACTCGTCGTA
psaB-R	ATTGCTAATTGCCCGAAATG
psaD-F	GATCTTCAACTCCGCCATAA
psaD-R	ATCCCACGGGAGCTTCTTTC
Lhca1-F	CAGGAATGGGCAGCACTA
Lhca1-R	GCAATGGCTAAGAACTCAAT
Lhcb2-F	CTCCCCAAAGCATCTGGTAT
Lhcb2-R	CCCATCTGCTGTGGATTACTT
psbA-F	CCTATGGGGTCGCTTCTGTA
psbA-R	GAGCAGCAATGAATGCGATA
psbB-F	CGTGCGACTTTGAAATCTGA
psbB-R	TAGCACCATGCCAAATGTGT
atpC1-F	CGTGGATTATGTGGTGGA
atpC1-R	GCTTCTTTAGCCGTAGGT
atpB-F	GAGCTCGTATGAGAGTTGGT
atpB-R	ACCCAATAAGGCGGATACCT
atpE-F	TCCACAAGAAGCTCAGCAAA
atpE-R	GTGTCCGAGCTCGTCTGAG
petC-F	CAAGTGGGTTGTTTGTGA

petC-R	AAGCATGTAGCCAGTAGGT
petD-F	TCCTTTTGCAACTCCTTTGG
petD-R	CCGCTGGTACTGAAACCATT
rbcS-F	CTTCCTGACCTTACCGATTC
rbcS-R	CGGGTGAGTTACCGTGCT
rbcL-F	GTGTTGGGTTCAAAGCTGGT
rbcL-R	CATCGGTCCACACAGTTGTC
ndhB-F	GGCCTATATTTCTTGGTTTCAATAG
ndhB-R	AATCGGGTTCATTGATATTCCTG
ndhI-F	GTCAACAAACCCTACGAG
ndhI-R	ACATACTTCACAAGCAAT
rpoA-F	CAAGCCGACA CAATAGGCAT
rpoA-R	AGCGCGTTGCGCGTTCCATA
rpoB-F	GCAGGTTAGAATTAGAGATTGATA
rpoB-R	GGGTAGCAAACATTCTCTAGAAT
rpoC1-F	GCCTAGTATACTGCGATTTTTC
rpoC1-R	CTCGATTTCGAAATATATCGAAAC
rrn16-F	CGGTATCTGGGGAATAAGCA
rrn16-R	GATTTGACGGCGGACTTAAA
rrn23-F	GGGCGACTGTTTACCAAAAA
rrn23-R	TTACCCGACAAGGAATTTCG
rpl24-F	TTGTCGGTGCTACTTTGG
rpl24-R	CTCGTCCTTGGTCTTCTTGATT
rpl16-F	AACACTTGAACCCGCTTGG
rpl16-R	GCGTTTCCGCAGGTCTTA
rps17-F	CCCGAACCACAGAATGCC
rps17-R	TCCACCGCCACAGTCTTG
petL-A-F	AGTACTTTAAGAAACATATGTA
petL-A-R	TCAAATAAGTCGTATTTTGCT
ndhG-B-F	AAACAGCCTAATACTAATAA
ndhG-B-R	ΤΑΤΑΤΤΑΤΤΑΑΑΑΤΑΑGAAAAAATCG
ndhG-C-F	TAATATAATGGATTTGCCTGGACCAATAC
ndhG-C-R	GGGAAGTAATACCACTCCCAG
ndhG-D-F	AACCCAATATTTTCAGCCTTTTC
ndhG-D-R	GAAAAGGCTGAAAATATTGGGTT
petB-nc-F	TTCATCGATGGTCGGCAAG
petB-nc-R	TGCGGTCAATACACCCAGAA

qPCR

Table S2. Plastid RNA editing sites affected in the *wtg1* mutant plants.

All 34 reported editing sites in *Arabidopsis* plastid were detected by bulk sequencing. The affected sites, *petL-50* and *ndhG-5*, are shown in bold.

Editing site	Locus	Editing extent in wild-type (%)	Editing extent in wtg1 (%)	Related editing factors	References
matK-640	2931	90	90	QED1 (PPR), MORF2, MORF9, ORRM1, OZ1	(Tillich <i>et al.</i> , 2005; Takenaka <i>et al.</i> , 2012; Sun <i>et al.</i> , 2013; Sun <i>et al.</i> , 2015; Wagoner <i>et al.</i> , 2015)
atpF-92	12707	100	100	AEF1 (PPR), MORF2, MORF9	(Tillich <i>et al.</i> , 2005; Chateigner-Boutin & Small, 2007; Takenaka <i>et al.</i> , 2012)
rpoC1-488	21806	30	30	DOT4 (PPR), MORF2, MORF8, MORF9, OZ1, PPO1	(Tillich <i>et al.</i> , 2005; Bentolila <i>et al.</i> , 2012; Takenaka <i>et al.</i> , 2012; Hayes <i>et al.</i> , 2013; Zhang <i>et al.</i> , 2014; Sun <i>et al.</i> , 2015)
rpoB-2432	23898	90	90	QED1 (PPR), MORF2, MORF8, MORF9, ORRM1, OZ1	(Tillich <i>et al.</i> , 2005; Bentolila <i>et al.</i> , 2012; Takenaka <i>et al.</i> , 2012; Sun <i>et al.</i> , 2013; Sun <i>et al.</i> , 2015; Wagoner <i>et al.</i> , 2015)
rpoB-551	25779	90	90	CRR22 (PPR), MORF2, MORF9, ORRM1, OZ1	(Tillich <i>et al.</i> , 2005; Okuda <i>et al.</i> , 2009; Takenaka <i>et al.</i> , 2012; Zhang <i>et al.</i> , 2014; Sun <i>et al.</i> , 2015)
<i>rpoB</i> -338	25992	90	90	YS1 (PPR), MORF2, MORF9, OZ1, PPO1	(Tillich <i>et al.</i> , 2005; Zhou <i>et al.</i> , 2009; Takenaka <i>et al.</i> , 2012; Zhang <i>et al.</i> , 2014; Sun <i>et al.</i> , 2015)

psbZ-50	35800	100	100	OTP84 (PPR), MORF2, MORF9, OZ1, PPO1	(Chateigner-Boutin & Small, 2007; Hammani <i>et al.</i> , 2009; Takenaka <i>et al.</i> , 2012; Zhang <i>et al.</i> , 2014; Sun <i>et al.</i> , 2015)
rps14-80	37161	100	100	MORF2, MORF9	(Tillich et al., 2005; Takenaka et al., 2012)
rps14-149	37092	100	100	OTP86 (PPR), MORF2, MORF9	(Tillich <i>et al.</i> , 2005; Hammani <i>et al.</i> , 2009; Takenaka <i>et al.</i> , 2012)
accD-794	57868	100	100	RARE1/AtECB2 (PPR), MORF2, MORF8, MORF9, OZ1	(Tillich <i>et al.</i> , 2005; Robbins <i>et al.</i> , 2009; Yu <i>et al.</i> , 2009; Tseng <i>et al.</i> , 2010; Bentolila <i>et al.</i> , 2012; Takenaka <i>et al.</i> , 2012; Sun <i>et al.</i> , 2015; Wagoner <i>et al.</i> , 2015)
accD-3'UT R	58642	80	80	QED1/AtECB2 (PPR), MORF2, MORF9, ORRM1, OZ1	(Chateigner-Boutin & Small, 2007; Robbins <i>et al.</i> , 2009; Tseng <i>et al.</i> , 2010; Takenaka <i>et al.</i> , 2012; Sun <i>et al.</i> , 2013; Sun <i>et al.</i> , 2015; Wagoner <i>et al.</i> , 2015)
psbF-77	63985	100	100	LPA66 (PPR), MORF2, MORF9, OZ1	(Tillich <i>et al.</i> , 2005; Cai <i>et al.</i> , 2009; Takenaka <i>et al.</i> , 2012; Sun <i>et al.</i> , 2015)
<i>psbE</i> -214	64109	100	100	CREF3 (PPR), MORF2	(Tillich <i>et al.</i> , 2005; Takenaka <i>et al.</i> , 2012; Yagi <i>et al.</i> , 2013)
petL-5	65716	100	40	MORF2, MORF8, MORF9, OZ1, PPO1	(Tillich <i>et al.</i> , 2005; Bentolila <i>et al.</i> , 2012; Takenaka <i>et al.</i> , 2012; Zhang <i>et al.</i> , 2014; Sun <i>et al.</i> , 2015)
<i>rps12-</i> i58	69553	30	30	OTP81/QED1 (PPR), MORF2, MORF8, MORF9, ORRM1, OZ1	(Chateigner-Boutin & Small, 2007; Hammani <i>et al.</i> , 2009; Bentolila <i>et al.</i> , 2012; Takenaka <i>et al.</i> , 2012; Sun <i>et al.</i> , 2013; Sun <i>et al.</i> , 2015; Wagoner <i>et al.</i> , 2015)

<i>clpP</i> -559	69942	80	80	CLB19 (PPR), MORF2, MORF9, ORRM1, OZ1	(Tillich <i>et al.</i> , 2005; Chateigner-Boutin <i>et al.</i> , 2008; Takenaka <i>et al.</i> , 2012; Sun <i>et al.</i> , 2013; Sun <i>et al.</i> , 2015)
rpoA-200	78691	80	80	CLB19 (PPR), MORF2, MORF9, OZ1	(Chateigner-Boutin & Small, 2007; Chateigner-Boutin <i>et al.</i> , 2008; Takenaka <i>et al.</i> , 2012; Sun <i>et al.</i> , 2015)
rpl23-89	86055	80	80	OTP80 (PPR), MORF2, MORF9, OZ1, PPO1	(Chateigner-Boutin & Small, 2007; Hammani <i>et al.</i> , 2009; Takenaka <i>et al.</i> , 2012; Zhang <i>et al.</i> , 2014; Sun <i>et al.</i> , 2015)
ndhB-1481	94999	90	90	OTP84 (PPR), MORF2, MORF8, MORF9, OZ1, PPO1	(Tillich <i>et al.</i> , 2005; Hammani <i>et al.</i> , 2009; Bentolila <i>et al.</i> , 2012; Takenaka <i>et al.</i> , 2012; Zhang <i>et al.</i> , 2014; Sun <i>et al.</i> , 2015)
ndhB-1255	95225	100	100	CREF7 (PPR), MORF2, MORF9, ORRM1, OZ1, PPO1	(Tillich <i>et al.</i> , 2005; Takenaka <i>et al.</i> , 2012; Sun <i>et al.</i> , 2013; Yagi <i>et al.</i> , 2013; Zhang <i>et al.</i> , 2014; Sun <i>et al.</i> , 2015)
ndhB-872	95608	90	90	QED1 (PPR), MORF2, MORF9, ORRM1, OZ1, PPO1	(Tillich <i>et al.</i> , 2005; Takenaka <i>et al.</i> , 2012; Sun <i>et al.</i> , 2013; Zhang <i>et al.</i> , 2014; Sun <i>et al.</i> , 2015; Wagoner <i>et al.</i> , 2015)
ndhB-836	95644	100	100	OTP82 (PPR), MORF2, MORF8, MORF9, ORRM1, OZ1, PPO1	(Tillich <i>et al.</i> , 2005; Bentolila <i>et al.</i> , 2012; Takenaka <i>et al.</i> , 2012; Hayes <i>et al.</i> , 2013; Sun <i>et al.</i> , 2013; Zhang <i>et al.</i> , 2014; Sun <i>et al.</i> , 2015)
ndhB-830	95650	100	100	ELI1 (PPR), MORF2, MORF8, MORF9, ORRM1, OZ1, PPO1	(Tillich <i>et al.</i> , 2005; Okuda <i>et al.</i> , 2009; Bentolila <i>et al.</i> , 2012; Takenaka <i>et al.</i> , 2012; Sun <i>et al.</i> , 2013; Zhang <i>et al.</i> , 2014; Sun <i>et al.</i> , 2015)

ndhB-746	96419	100	100	CRR22 (PPR), MORF2, MORF8, MORF9, OZ1	(Tillich <i>et al.</i> , 2005; Bentolila <i>et al.</i> , 2012; Takenaka <i>et al.</i> , 2012; Zhang <i>et al.</i> , 2014; Sun <i>et al.</i> , 2015)
ndhB-586	96579	100	100	MORF2, MORF8, MORF9, ORRM1, OZ1, PPO1	(Tillich <i>et al.</i> , 2005; Bentolila <i>et al.</i> , 2012; Takenaka <i>et al.</i> , 2012; Sun <i>et al.</i> , 2013; Zhang <i>et al.</i> , 2014; Sun <i>et al.</i> , 2015)
ndhB-467	96698	90	90	CRR28 (PPR), MORF2, MORF8, MORF9, ORRM1, OZ1, PPO1	(Tillich <i>et al.</i> , 2005; Okuda <i>et al.</i> , 2009; Bentolila <i>et al.</i> , 2012; Takenaka <i>et al.</i> , 2012; Sun <i>et al.</i> , 2013; Zhang <i>et al.</i> , 2014; Sun <i>et al.</i> , 2015)
ndhB-149	97016	100	100	MORF2, MORF9, PPO1	(Tillich <i>et al.</i> , 2005; Takenaka <i>et al.</i> , 2012; Zhang <i>et al.</i> , 2014)
ndhF-290	112349	100	100	OTP84/AtECB2 (PPR), MORF2, MORF8, MORF9, OZ1, PPO1	(Tillich <i>et al.</i> , 2005; Hammani <i>et al.</i> , 2009; Tseng <i>et al.</i> , 2010; Bentolila <i>et al.</i> , 2012; Takenaka <i>et al.</i> , 2012; Zhang <i>et al.</i> , 2014; Sun <i>et al.</i> , 2015)
ndhD-887	116281	90	90	CRR22 (PPR), MORF2, MORF9, ORRM1, OZ1, PPO1	(Tillich <i>et al.</i> , 2005; Okuda <i>et al.</i> , 2009; Takenaka <i>et al.</i> , 2012; Sun <i>et al.</i> , 2013; Zhang <i>et al.</i> , 2014; Sun <i>et al.</i> , 2015)
ndhD-878	116290	80	80	CRR28 (PPR), MORF2, MORF9, ORRM1, OZ1, PPO1	(Tillich <i>et al.</i> , 2005; Okuda <i>et al.</i> , 2009; Takenaka <i>et al.</i> , 2012; Sun <i>et al.</i> , 2013; Zhang <i>et al.</i> , 2014; Sun <i>et al.</i> , 2015)
ndhD-674	116494	100	100	OTP85 (PPR), MORF2, MORF9, ORRM1, OZ1, PPO1	(Tillich <i>et al.</i> , 2005; Hammani <i>et al.</i> , 2009; Takenaka <i>et al.</i> , 2012; Sun <i>et al.</i> , 2013; Zhang <i>et al.</i> , 2014; Sun <i>et al.</i> , 2015)

ndhD-383	116785	100	100	CRR21 (PPR), MORF2, MORF8, MORF9, PPO1	(Tillich <i>et al.</i> , 2005; Okuda <i>et al.</i> , 2007; Bentolila <i>et al.</i> , 2012; Takenaka <i>et al.</i> , 2012; Zhang <i>et al.</i> , 2014)
ndhD-2	117166	50	50	CRR4 (PPR), MORF2, MORF8, MORF9, OZ1, PPO1	(Kotera <i>et al.</i> , 2005; Tillich <i>et al.</i> , 2005; Okuda <i>et al.</i> , 2006; Bentolila <i>et al.</i> , 2012; Takenaka <i>et al.</i> , 2012; Zhang <i>et al.</i> , 2014; Sun <i>et al.</i> , 2015)
ndhG-50	118858	100	50	OTP82 (PPR), MORF2, MORF9, ORRM1, OZ1, PPO1	(Tillich <i>et al.</i> , 2005; Okuda <i>et al.</i> , 2010; Takenaka <i>et al.</i> , 2012; Sun <i>et al.</i> , 2013; Zhang <i>et al.</i> , 2014; Sun <i>et al.</i> , 2015)

Trans- Gene		Gene family	Editing e muta	extent in the ant (%)	Editin change 1 W	g level nutant :: 'T	Reference
lactor	ID		petL	ndhG	petL	ndhG	-
			-5	-50	-5	-50	
							(Hammani et
	AT1						al., 2009;
OTP82	G080	PPR	n/a	1	n/a	-99%	Okuda et al.,
01102	70			-		2270	2010;
							Kindgren et
							al., 2015)
	AT2						(Takenaka et
MORF2	G334	MORF	65	40	-35%	-60%	al., 2012)
	30						
	AT3						(Bentolila et
MORF8	G150	MORF	35	72	-60%	-6%	al., 2012)
	00						
MODEO	ATT	MODE	0	0	1000/	1000/	(Takenaka et
MORF9	G114	MORF	0	0	-100%	-100%	al., 2012)
	30 AT2						
ODDM1	A15	ODDM	66	o	+ 1.0/	0.40/	(Sun et al.,
OKKIVII	30	UKKIVI	00	0	+1%	-94%	2013)
	50 AT4						
	G016	Protoporphyrinogen	65	85	-19%	_15%	(Zhang et
PPUI	90	oxidase	05	05	-1970	-1370	al., 2014)
	AT5						
OZ 1	G177	RanBP2-type	45	0	-42%	-100%	(Sun et al.,
021	90	zinc finger	10	Ū	1270	10070	2015)

Table 55 Trans factors involved in peril 5 and/or nano 50 carting	Tε	able	S 3	Trans	-factors	invo	lved	in	petL-5	and/or	ndhC	G- 50	editing
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+ indicates increased editing extent compared to that of wild-type plants; - indicates decreased editing extent compared to that of wild-type plants. n/a, not assayed.

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