

## **SUPPLEMENTAL MATERIAL**

### **Ectopic expression of *WRI1* affects fatty acid homeostasis in *Brachypodium distachyon* vegetative tissues<sup>‡</sup>**

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**Supplemental Figure S1.** Alignment of *WRI1* amino acid sequences from *Brachypodium* and *Arabidopsis*.

**Supplemental Figure S2.** Lipid droplets are abundant in embryo and endosperm of *UBI::BdWRI1* line 5A dry grains.

**Supplemental Figure S3.** Lipid droplets in the internodes of *UBI::BdWRI1* plants.

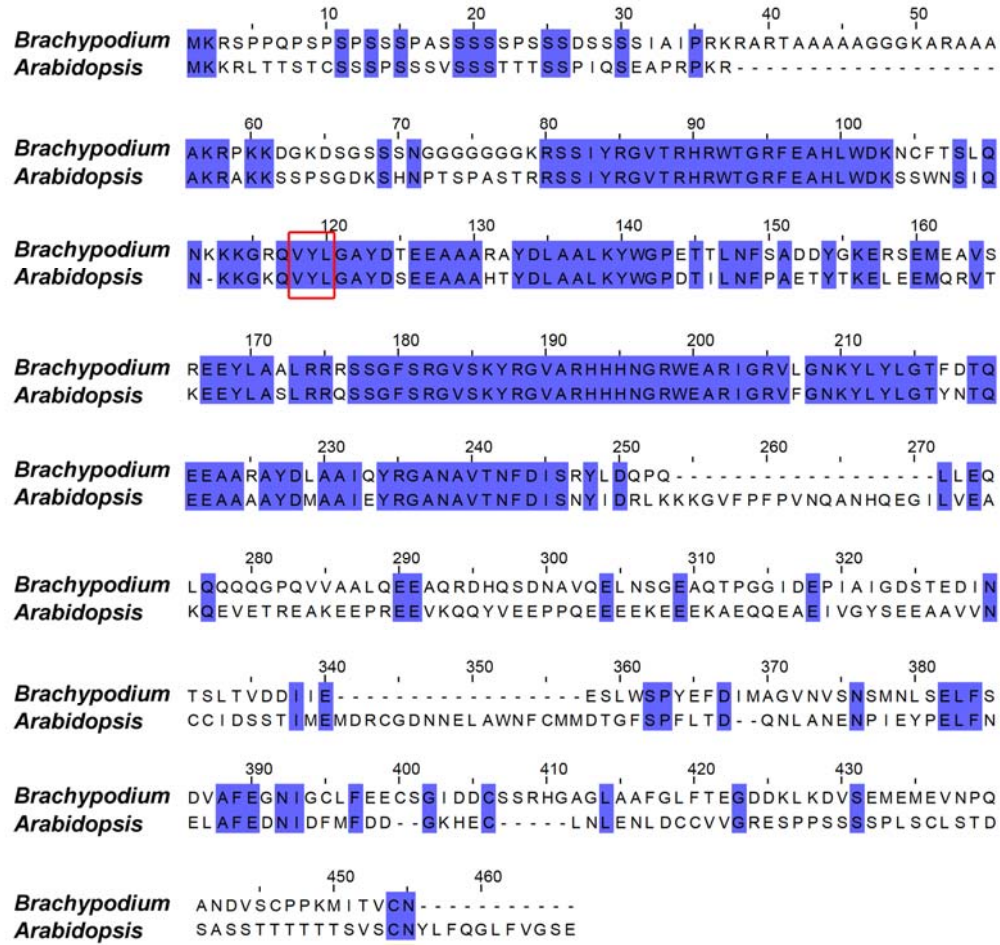
**Supplemental Figure S4.** Ectopic expression of *AtWRI1* also led to cell death in *Brachypodium*.

**Supplemental Figure S5.** Ectopic expression of *AtWRI1* in *Arabidopsis* did not lead to cell death.

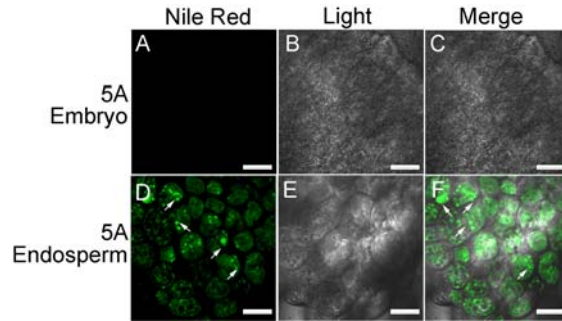
**Supplemental Figure S6.** No induction of TAG turnover in *35S::AtWRI1* vegetative tissues of *Arabidopsis*.

**Supplemental Figure S7.** Palmitic acid (16:0) treatment of *Brachypodium* leaf blade results in cell death.

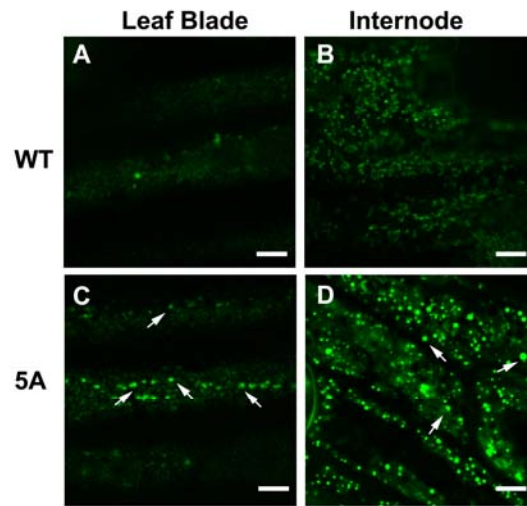
**Supplemental Table S1.** PCR primers used in real-time RT-PCR.



**Figure S1.** Alignment of WR11 amino acid sequences from *Brachypodium* and *Arabidopsis*. The alignment was analyzed by Jalview software. The blue boxes indicate highly conserved amino acids. The red box delineates the "VYL" motif, which is important for AtWR11 function.



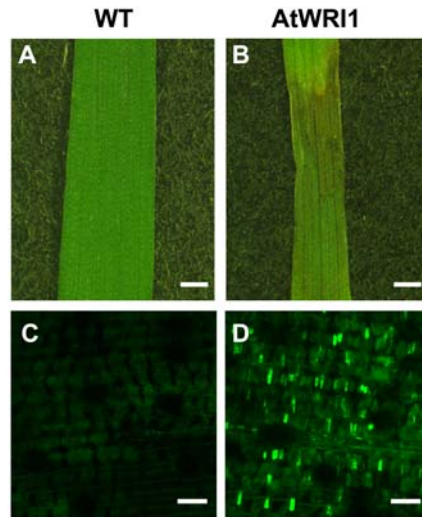
**Figure S2.** Lipid droplets are abundant in embryo and endosperm of *UBI::BdWR11* line 5A dry grains. A-C, confocal fluorescence micrographs of *UBI::BdWR11* line 5A embryo. D-F, confocal fluorescence micrographs of *UBI::BdWR11* line 5A endosperm.. Bars=20  $\mu$ m.



**Figure S3.** Lipid droplets in the internodes of *UBI::BdWR11* plants.

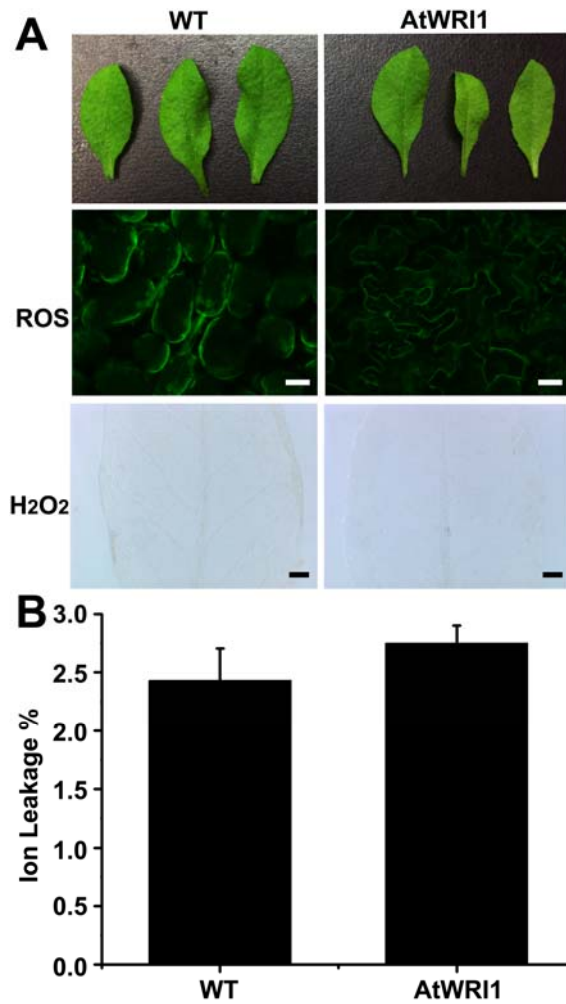
A and B, Nile Red staining of 8-week-old Bd21-3 leaf blade (A) and internode (B).

Bar=5  $\mu$ m. C and D, Nile Red staining of 8-week-old *Ub::BdWR11* leaf blade (C) and internode (D). Bar=5  $\mu$ m.



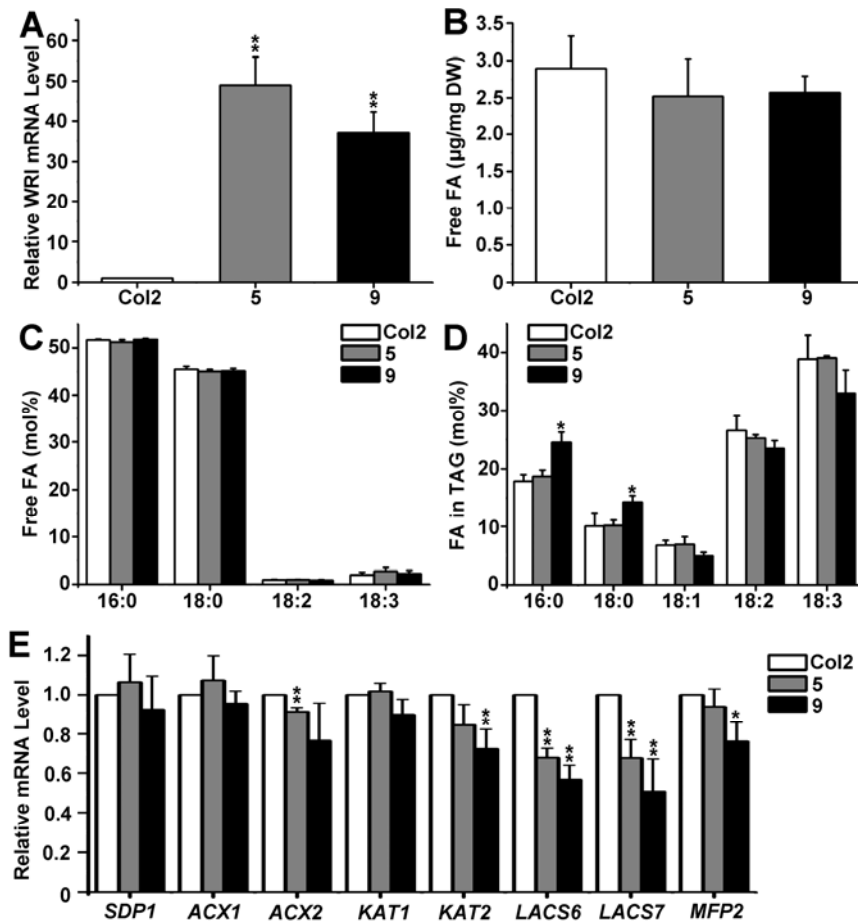
**Figure S4.** Ectopic expression of *AtWR11* also led to cell death in Brachypodium.

A and B, Phenotypes of 8-week-old leaf blades from Bd21-3 (A) and *UBI::AtWR11* plants (B). Bars=1 mm. C and D, H<sub>2</sub>DCF-DA staining of 8-week-old leaf blades from Bd21-3 (C) and *UBI::AtWR11* plants (D). Bars=20  $\mu$ m.

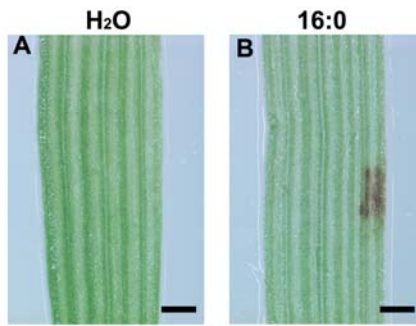


**Figure S5.** Ectopic expression of *AtWR11* in Arabidopsis did not lead to cell death.

A, phenotypes of 4-week-old *35S::AtWR11* leaves (top panels). General ROS accumulation was detected by H<sub>2</sub>DCF-DA staining (middle panels, Bars=20 μm). H<sub>2</sub>O<sub>2</sub> accumulation was detected by DAB staining (lower panels, Bars=0.5 mm). B, quantification of cell death by electrolyte leakage assay. 4-week-old detached leaves were inoculated with water for 3 hours and then excised to measure conductivity. Each value represents the mean and SD of three replicates.



**Figure S6.** No induction of TAG turnover in *35S::AtWRII* vegetative tissues of Arabidopsis. A and E, expression levels of *AtWRII* (A) and key genes predicted to encode enzymes involved in TAG degradation (E) in 4-week-old leaf tissues from *35S::AtWRII* plants. Total RNA was isolated and relative mRNA levels were determined by quantitative real-time RT-PCR using *AtACTIN1* as an internal control. Data represent three independent experiments, and the error bars represent SD. \* indicates significant difference ( $p < 0.05$ , student's t-test), and \*\* at  $p \leq 0.01$ . B, C and D, free FA contents (B), composition of free FA (C), and FA composition of TAG (D) from 4-week-old tissues of *35S::AtWRII*. Total lipids were extracted and TAGs were separated by TLC. Three independent measurements were averaged, and the error bars represent SD. \* indicates significant difference ( $p < 0.05$ , student's t-test), and \*\* at  $p \leq 0.01$ .



**Figure S7.** Palmitic acid (16:0) treatment of *Brachypodium* leaf blade results in cell death. Phenotypes of wild-type Bd21-3 detached leaf blades treated with 200  $\mu$ m 16:0 for 48 hours. Bars=0.5 mm.



**Supplemental Table S1. PCR primers used in real-time RT-PCR**

Species	Gene	Gene ID	Primer sequence
Brachypodium	<i>BdWRI1</i>	<i>Brai4g43877</i>	F: 5'-CACCTCCCTTACGGTTGATGACA-3' R: 5'-GCATTCTTCAAACAGGCACCCA-3'
	<i>BdPKP-β1</i>	<i>Bradi2g45620</i>	F: 5'-TACCAAGGCGTTTGGCCAGTT-3' R: 5'-TGAATATTGTGGGTGGATTGCGAC-3'
	<i>BdKASI</i>	<i>Bradi1g46610</i>	F: ATCCGAAGAAGCGGGTGG R: GCCCAGCCGCTCTCTC
	<i>BdACP1</i>	<i>Bradi1g01000</i>	F: CCTTCTTCCCGTCGGTTCAGC R: CGTGTCAAGTGAATCAGCCCCA
	<i>BdSDP1-1</i>	<i>Bradi2g50610</i>	F: GGGATTGCTGGACCATCTAACA R: CAGTATCACTTCGGCTTTCCT
	<i>BdSDP1-2</i>	<i>Bradi1g04310</i>	F: AACAAGGTCCTGGAGCATCAAA R: GGAGTGGAGAGATGTGAGGATTAG
	<i>BdACX1</i>	<i>Bradi1g52320</i>	F: TTCCAAGGCTTTGTCCCGT R: CTCACCCACAAATCTAAATGCATAA
	<i>BdACX2</i>	<i>Bradi4g14090</i>	F: CCTACTTGTCCCAGCCTAATCCC R: TGCAAGTGTGAGCAGATGGTTT
	<i>BdLACS6</i>	<i>Bradi4g26610</i>	F: GAGAAATTTGTGTCCGGGGACC R: TCCAGGCAGCCACAAACC
	<i>BdLACS7</i>	<i>Bradi4g42950</i>	F: GGTGGACGGGTGAGGCTTAT R: AGGGTTTGGAGATCCAACATGAC
	<i>BdMFP2-1</i>	<i>Bradi2g43020</i>	F: AACACAGTTCGGAATGCCAATG R: CATCGCTCAGGAAAACCTTGGTAAT
	<i>BdMFP2-2</i>	<i>Bradi4g28310</i>	F: TAAGGGTGAGGAGGCATACCAA R: TCCAAAGCCCAACGACGAG
	<i>BdMFP2-3</i>	<i>Bradi4g28310</i>	F: TGGTTTTGGTGTGCTGTGGC R: GTGGCTTCACCTGCCCTTTT
	<i>BdKAT1</i>	<i>Bradi3g27960</i>	F: GGGCTTCAGGCAGTCGCAGAT R: AGGCAATCACGTGCTTGAGAAAATA
	<i>BdKAT2</i>	<i>Bradi3g55420</i>	F: GATGGTGCTGGAGCCGTTCT R: CTACCGCAGGACCAACGCC
	<i>BdUBC18</i>	<i>Bradi4g00660</i>	F: GGAGGCACCTCAGGTCATTT R: ATAGCGGTCATTGTCTTGCG
Arabidopsis	<i>AtWRI1</i>	<i>At3g54230</i>	F: 5'-AAAGGTGTTTTCCCGTTCCTGTGA-3' R: 5'-TAGGCTCTTCCTTCGCTTCTCTCGT-3'
	<i>AtSPD1</i>	<i>At5g04040</i>	F: 5'-AAATGGCTTACCGGAGGAAGTT-3' R: 5'-TGAGCCCATTCTCATAAGTCA-3'
	<i>AtACX1</i>	<i>At4g16760</i>	F: 5'-ACGGATGTAAGTCAAAGACTGGC-3' R: 5'-GTGGTGGTGAGAGACTTCAATCCTG-3'
	<i>AtACX2</i>	<i>At5g65110</i>	F: 5'-TGTTACGCCTTCATTGTTCCG-3' R: 5'-TCAACGCACCATTATCCACTCCATT-3'

**Supplemental Table S1 continued**

<b>Species</b>	<b>Gene</b>	<b>Gene ID</b>	<b>Primer sequence</b>
Arabidopsis	<i>AtLACS6</i>	<i>At3g05970</i>	F: CTA CTGGATGATTTGGCTGCTC R: CTTTCCATTCAAGAGAGCCTGC
	<i>AtLACS7</i>	<i>At5g27600</i>	F: GGTTACCTGGTGGTCCGGCTC R: TGAAACACTGCGAAACGAATCTA
	<i>AtKAT1</i>	<i>At1g04710</i>	F: CCAGCCGTTGCCATTCCT R: ATAAACAAACTGAGATGCAAATGCC
	<i>AtKAT2</i>	<i>At2g33150</i>	F: CGAGATGAAACGCCGTGGTA R: GACCTTGCCTTCAACTTTCCTT
	<i>AtMFP2</i>	<i>At3g06860</i>	F: GAGGAAGGTCATTCTTTGGGTCT R: AACGTCAGTATCTCCCTTGCCTCT
	<i>AtACTIN1</i>	<i>At2g37620</i>	F: 5'-CTCTGTCTGGATTGGAGGGTC-3' R: 5'-GCTTGAGAAATGGTCCGAAA-3'