Supplementary Information

Supplemental Tables 2 and ten figures

**TITLE:** WSL5, a pentatricopeptide repeat protein, is essential for chloroplast biogenesis in rice under cold stress.

X. Liu1 • J. Lan1 • Y.S. Huang1 • P.H. Cao1 • C.L. Zhou1 • Y.K. Ren1 • N.Q. He1 • S.J. Liu1 • Y.L. Tian1 • T.L. Nguyen1 • L. Jiang1 (✉) • J.M. Wan1,2 (✉)

1 State Key Laboratory for Crop Genetics and Germplasm Enhancement, Jiangsu Plant Gene Engineering Research Center, Nanjing Agricultural University, Nanjing 210095, China.

2 National Key Facility for Crop Gene Resources and Genetic Improvement, Institute of Crop Science, Chinese Academy of Agricultural Sciences, Beijing 100081, China

* Corresponding authors:

Ling Jiang

Telephone: +86-25-84399061

Fax: +86-25-84399061

E-mail: jiangling@njau.edu.cn

Wan Jianmin

Telephone: +86-25-84396516

Fax: +86-25-84396516

E-mail: wanjm@njau.edu.cn
Supporting information

Additional Supporting Information may be found online for this article:

**Data S1** Genes differentially expressed in wild type and *wsl5*.

**Data S2** Genes differentially expressed in wild type and *wsl5* under different temperature condition.

**Figure S1.** Comparison of pigment contents from the second (L2), third (L3), fourth (L4) and fifth (L5) leaves of five-leaf-stage plants between WT and the *wsl5* mutant. (Student’s *t* test, **P < 0.01, *P < 0.05**).

**Figure S2.** Transmission electron microscopy images of cells from WT and the *wsl5* mutant grown under different temperature conditions. Cells and chloroplast structures from WT (A-F) and *wsl5* (G-M) at 30°C, 25°C and 20°C. Scale bar, 1 μm in A, C, E, G, I, and H; 500 nm in B, D, F, H, J, and K). Note the disorganized structures in *wsl5* at C20 and C25 images relative to WT and C30.

**Figure S3.** Alignment of WSL5 orthologs in maize and *Arabidopsis*. The RRM domain is red underlined. PPR repeats are indicated below the sequences by black-headed arrows.

**Figure S4.** *WSL5* was expressed in all tissues, especially during leaf development according to Rice eFP Browser.

**Figure S5.** GO analysis of genes differentially expressed between wild type and *wsl5*.

**Figure S6.** Pathway analysis of genes differentially expressed between wild type and *wsl5*.

**Figure S7.** Expression levels of chlorophyll synthesis genes in wild type and *wsl5*. (Student’s *t*-test, **P < 0.01**).

**Figure S8.** Editing efficiencies of *rpl2* and *atpA* genes in WT and the *wsl5* mutant. RT-PCR products of *rpl2* and *atpA* transcripts from WT and *wsl5* plants grown in a growth chamber were sequenced. In *wsl5* mutant, the editing efficiency of *rpl2* at C1 and *atpA* at C383 were significantly decreased compared to WT and complementation plants (Com). The panel corresponds to the genomic nucleotide sequence.

**Figure S9.** Quantitative RT-PCR analyses of *rpl2*, and *rps12* transcripts in WT and the *wsl5* mutant. Histograms show log₂ ratios of spliced to unspliced RNA in *wsl5* plants compared to WT. Values are means ± SD of three replicates.
**Figure S1.** qRT-PCR analysis of genes differently expressed in RNA-seq. W3 and W2 represent wild type plants grown at 30 and 20°C, respectively. M3 and M2 represent wsl5 plants grown at 30 and 20°C, respectively (Student’s t-test, **P < 0.01).**

**Table S1.** Comparison of agronomic traits between WT and wsl5 under field conditions.

**Table S2.** Primers used in this study.

![Figure S1](image1.png)

**Figure S1.** Comparison of pigment contents from the second (L2), third (L3), fourth (L4) and fifth (L5) leaves of five-leaf-stage plants between WT and the wsl5 mutant.(Student’s t-test, **P < 0.01, *P < 0.05**).

![Figure S2](image2.png)

**Figure S2.** Transmission electron microscopy images of cells from WT and the wsl5 mutant grown under different temperature conditions. Cells and chloroplast structures from WT (A-F) and...
wsil5 (G-M) at 30°C, 25°C and 20°C. Scale bar, 1 μm in A, C, E, G, I, and H); 500 nm in B, D, F, H, J, and K). Note the disorganized structures in wsil5 at C20 and C25 images relative to WT and C30.

Figure S3. Alignment of WSL5 orthologs in maize and Arabidopsis. The RRM domain is red underlined. PPR repeats are indicated below the sequences by black-headed arrows.

Figure S4. WSL5 was expressed in all tissues, especially during leaf development according to
Rice eFP Browser.

**Figure S5.** GO analysis of genes differentially expressed between wild type and wsl5.

**Figure S6.** Pathway analysis of genes differentially expressed between wild type and wsl5.
**Figure S7.** Expression levels of chlorophyll synthesis genes in wild type and *wsl5*. (Student’s *t*-test, **P < 0.01).  

**Figure S8.** Editing efficiencies of *rpl2* and *atpA* in WT and the *wsl5* mutant. RT-PCR products of *rpl2* and *atpA* transcripts from WT and *wsl5* plants grown in a growth chamber were sequenced. In *wsl5* mutant, the editing efficiency of *rpl2* at C1 and *atpA* at C383 were significantly decreased compared to WT and complementation plants(Com). The panel corresponds to the genomic nucleotide sequence.
**Figure S9.** Quantitative RT-PCR analyses of *rpl2*, and *rps12* transcripts in WT and the *wsl5* mutant. Histograms show log₂ ratios of spliced to unspliced RNA in *wsl5* plants compared to WT. Values are means ± SD of three replicates.

**Figure S10.** qRT-PCR analysis of genes differently expressed in RNA-seq. W3 and W2 represent wild type plants grown at 30 and 20°C, respectively. M3 and M2 represent *wsl5* plants grown at 30 and 20°C, respectively (Student’s *t*-test, **P < 0.01).
### Supplemental Table 1 Comparison of agronomic traits between WT and wsl5 under field condition

| Trait                        | WT          | wsl5         |
|------------------------------|-------------|--------------|
| Plant height (cm)            | 103.32±2.61 | 101.76±4.85  |
| Panicle length (cm)          | 26.54±1.49  | 26.44±1.09   |
| Days to flowering (d)        | 80.3±1.23   | 79.8±1.32    |
| 1000 grain weight (g)        | 28.21±1.21  | 27.85±0.56   |
| Number of tillers            | 14.4±3.05   | 13.9±1.52    |
| Flag leaf length (cm)        | 25.81±3.73  | 26.02±2.64   |
| Flag leaf width (cm)         | 1.84±0.15   | 1.82±0.11    |

### Supplemental Table 2 Primers used in this study

| F primer                  | R primer                  |
|---------------------------|---------------------------|
| RM8217                    | ACTAGCGATGTCTGAGTTGAC     |
| RM559                     | TATTCACATGCTTTGCTCATC     |
| RM3466                    | GGTACACTTGGCCCTATGC       |
| RM559                     | ATGGGCTGTCAGTTGCTTCC      |
| ID4-14                    | GTCTCCCTCCACCTCTTC       |
| Y4                        | AAGGACGAGATCCGAAGCCAAG    |
| Y4                        | AGGACGAGATCCGAAGCCAAG     |
| Y11                       | TGGCTCTGAGTAGTAGTAGTTCG   |
| Y16                       | ACAGCTACGGGTCAATTACTATGT |
| Y17                       | TGGCTCTGAGTAGTAGTAGTTCG   |
| Y18                       | ACAGCTACGGGTCAATTACTATGT |
| Y47                       | TGGCTCTGAGTAGTAGTAGTTCG   |
| WSL5cds                   | TGGCTCTGAGTAGTAGTAGTTCG   |
| pAN580WSL5                | TGGCTCTGAGTAGTAGTAGTTCG   |
| WSL5-RT                   | TGGCTCTGAGTAGTAGTAGTTCG   |
| rpl2cds                   | ACCGTGCCCTCCACCTGCTCCTCC |
| rpl2-R                     | TGGCTCTGAGTAGTAGTAGTTCG   |
| rps12cds                  | ACCGTGCCCTCCACCTGCTCCTCC |
| rps12-RT                  | TGGCTCTGAGTAGTAGTAGTTCG   |
| OsLFRN2-RT                | ACCGTGCCCTCCACCTGCTCCTCC |
| PORA-RT                   | GATGGAGCGCAGGAGTAGTGA     |
| OS08G0276100-RT           | ACCGTGCCCTCCACCTGCTCCTCC |
| OS03G0333400-RT           | ACCGTGCCCTCCACCTGCTCCTCC |
| Gene         | Forward Primer            | Reverse Primer            |
|--------------|---------------------------|---------------------------|
| OS04G0457000-RT | CAAGCTCGCCGAGATCAAG       | CCGGCCCTCGAAGTAGAAGAT     |
| OsHSP24.1-RT  | CAAGGCCGAGATGAAGAAGC      | CTCGACGTTGACCTGGAGAAGA    |
| RAD51-RT     | GAAAGCTGTGGAGCATCTTTG     | GGACGGTGCTGCTTTCTTTTG     |
| ClpB3-RT     | GGTGACTGATGTCGTTGAGT     | TGGCTCAGATCCAGAAACGCA     |
| St1-RT       | GTCGACATCGAGAGGGAGTT      | GCGACGAACCTCGATGTACTG     |
| OsFAD7-RT    | TCAAAGCAACGCCAAGGTGAA     | TTGCCAGGAACCTGCTTTTCT     |
| OsFAH2-RT    | GTCCTGCTGCGTTGCTGAGA     | ATGTTGCTGTTTGGCCCAAT      |
| OsFAH1-RT    | GCCCTTCGACTTGTCTTTCC      | AAACACGCGGCTAGGTGTAG      |
| OsTrxz-RT    | GACATCGAGATGCTTTGAGA     | GGGCGCTTCTTTGCTTTGATCT    |
| PHO1-RT      | TTCTTCTTCCTCGCCACCT      | CTCTGCCCTTGACAAAGCTTCC    |
| AGPS2b-RT    | GGTGGTGCAAGGGAGCTAGATT   | TTAGCCAGTGACGGTGTCAGA     |
| OsTrxm-RT    | AAGGAGTACGTCGAGCAAGAT    | CGTAGTTTGCGGCGATGTTT      |
| V3-RT        | CTGTCAGCAAGGGCATTTCTC    | TGGAAAGCAGTACGGCATTGGA    |
| OS12G0194800-RT | GGTGACCTGATGTCGTTGTG   | TGGCTCAGATCCAGAAAGCA     |
| OS10G0320100-RT | CCCTCTTAGCTAACGTCTTG   | ATCAGCACCTTGGACATCCA     |