**Supplemental Data**

# Altered flavonoid and lignin profiles in rice mutants deficient in *CHS*, *CHI*, and *CHIL* genes

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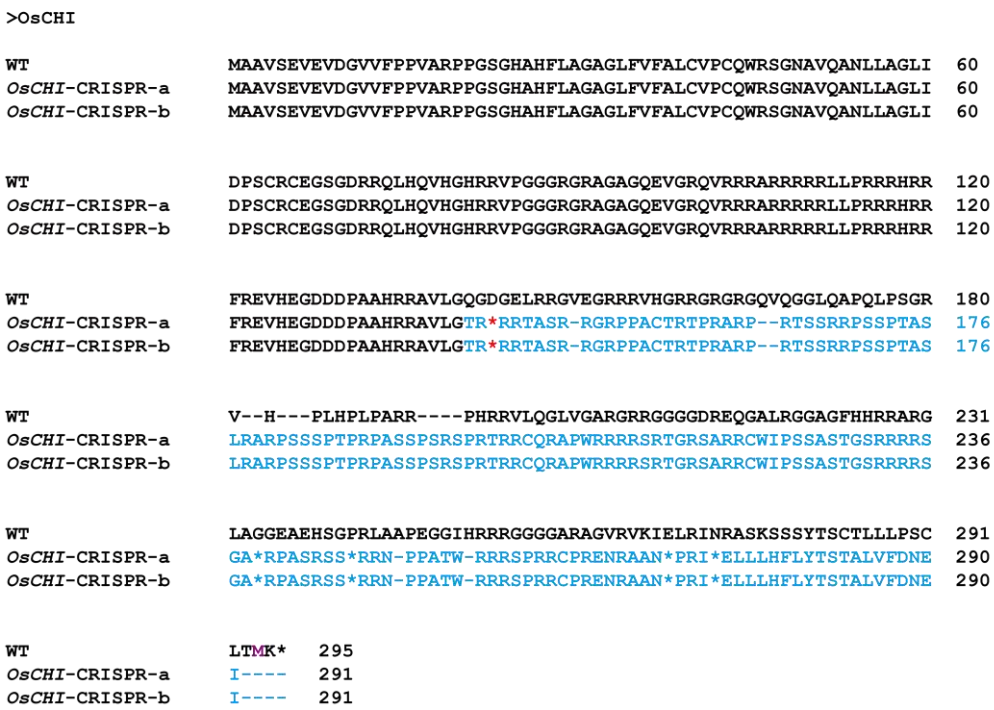
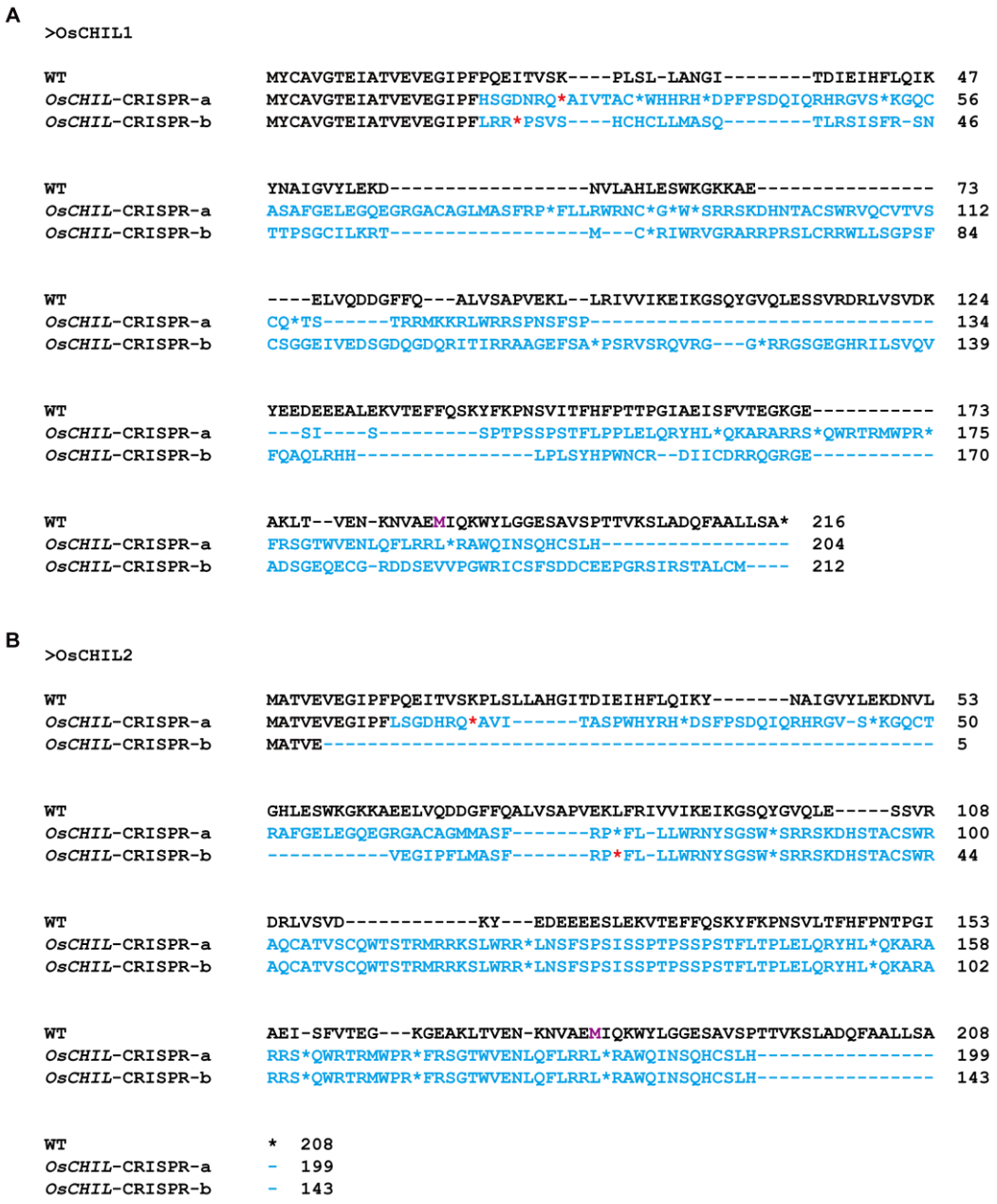
## List of Materials




**Supplemental Fig. S1.** Predicted effects of mutations on OsCHI in *OsCHI*-CRISPR mutant lines.

Protein sequence alignment of wild-type OsCHI and the mutated OsCHI in *OsCHI*-CRISPR-a and *OsCHI*-CRISPR-b. If translation starts using the native start codon, frame shift mutation occurs in *OsCHI*-CRISPR-a and *OsCHI*-CRISPR-b, leading to the formation of a premature stop codon. If translation starts using the in frame start codon after the mutation site, the region before the start codon is missing.

Asterisks, stop codons. In red, the first premature stop codon. In blue, protein sequence different with wild-type OsCHI if translation starts using the native start codon. In purple, in frame start codon after the mutation site.



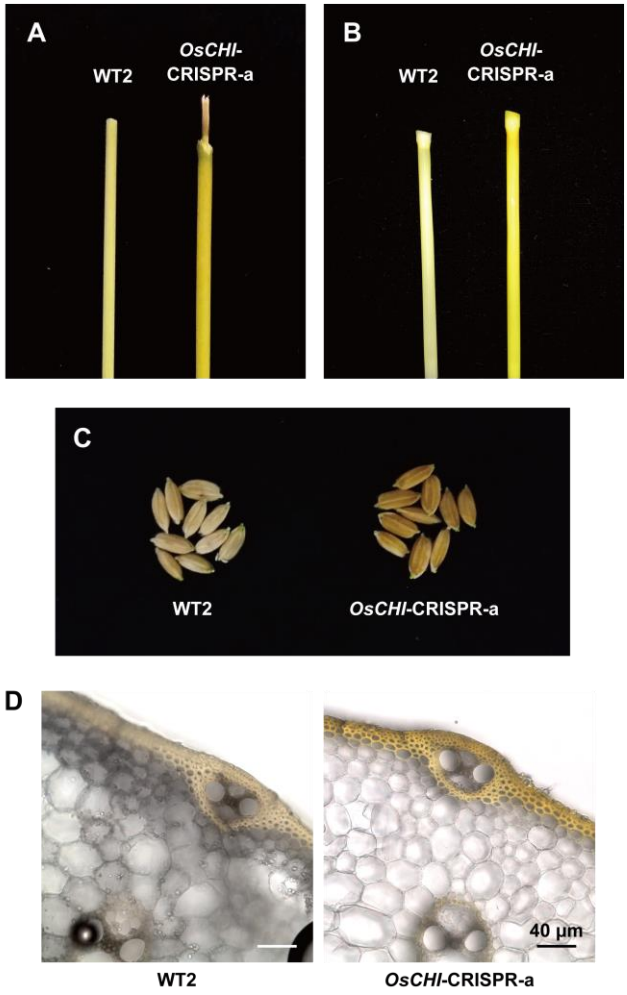

**Supplemental Fig. S2.** Predicted effects of mutations on OsCHIL1 and OsCHIL2 in *OsCHIL*-CRISPR mutant lines.

A, Protein sequence alignment of wild-type OsCHIL1 and the mutated OsCHIL1 in *OsCHIL*-CRISPR-a and *OsCHIL*-CRISPR-b.

B, Protein sequence alignment of wild-type OsCHIL2 and the mutated OsCHIL2 in *OsCHIL*-CRISPR-a and *OsCHIL*-CRISPR-b.

If translation starts using the native start codon, frame shift mutation occurs in *OsCHIL*-CRISPR-a and *OsCHIL*-CRISPR-b, leading to the formation of a premature stop codon. If translation starts using the in frame start codon after the mutation site, the region before the start codon is missing.

Asterisks, stop codons. In blue, protein sequence different with wild-type OsCHIL1 (A) or OsCHIL2 (B) if translation starts using the native start codon. In red, the first premature stop codon. In purple, in frame start codon after the mutation site.

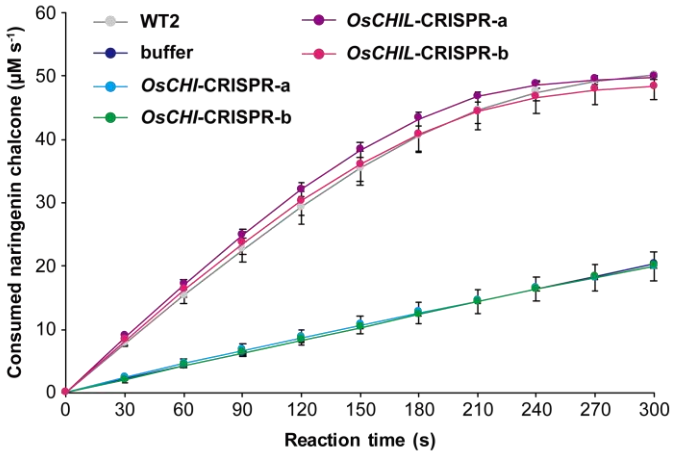



**Supplemental Fig. S3.** Phenotypes of *OsCHI*-CRISPR and wild-type control lines.

A and B, Mature culm tissues of WT2 and *OsCHI*-CRISPR-a without any treatment (A), and after fixation and solvent extraction (B). Culm of *OsCHI*-CRISPR-a appears to be yellow.

C, Rice grains of WT2 and *OsCHI*-CRISPR-a. Spikelets of *OsCHI*-CRISPR-a appear to be yellow.

D, Transverse sections of culm tissues of WT2 and *OsCHI*-CRISPR-a after fixation and solvent extraction and before subjecting to any staining. The secondary cell walls of the vascular bundles and cortical sclerenchyma fibers in *OsCHI*-CRISPR-a appear to be yellow.




**Supplemental Fig. S4.** CHI catalytic activities of crude proteins of *OsCHI*-CRISPR, *OsCHIL*-CRISPR and their isogenic wild type.

Crude proteins extracted from wild-type control (WT2), *OsCHI*-CRISPR and *OsCHIL*-CRISPR mutants were used to perform CHI enzyme assay with naringenin chalcone as a substrate. Crude proteins of WT2 and *OsCHIL*-CRISPR mutant lines could rapidly consume naringenin chalcone. Crude proteins of *OsCHI*-CRISPR mutant lines harbored similar CHI activities with that of spontaneous isomerization occurred in reaction mixtures that only contained extraction buffer. These results suggest that OsCHILs unlikely harbor CHI catalytic activities nor serve as enhancers of CHI.

Values refer to means ± standard deviations from biologically independent plants ($n$ = 3).

**Supplemental Table S1.** Off target analysis of $T_3$ generation of *OsCHI*-CRISPR and *OsCHIL*-CRISPR mutant lines.

| sgRNA name | Off-target site name | Sequence (5′ to 3′)[a] | CRISPR-P off-target score | Location (Chromosome:start) | Gene locus | Mutation |
|---|---|---|---|---|---|---|
| sgRNA-a | Off-target-a1 | CGGC**AG**GCAGTACTCGGAC**G**TGG | 0.8 | Chr3:+19289 | Exon (LOC_Os03g01018) | n.d. |
| | Off-target-a2 | CG**T**CG**C**G**G**AG**C**ACTCGGACACGG | 0.5 | Chr3:+8809743 | Exon (LOC_Os03g15980) | n.d. |
| | Off-target-a3 | CGG**G**G**C**G**G**AGTACTCG**T**ACATGG | 0.4 | Chr3:+31598624 | Exon (LOC_Os03g55530) | n.d. |
| sgRNA-b | Off-target-b1 | G**A**A**CTC**CATCCCATTTCCTCAAG | 0.8 | Chr6:+15503611 | Exon (LOC_Os06g27380) | n.d. |
| | Off-target-b2 | G**C**AGGG**A**ATCCCATTTC**AG**CAGG | 0.1 | Chr1:+4971979 | Exon (LOC_Os01g09649) | n.d. |
| | Off-target-b3 | **A**G**G**GGGCATCCCATT**G**CCT**G**AAG | 0.1 | Chr3:−28010595 | Exon (LOC_Os03g49170) | n.d. |
| sgRNA-c | Off-target-c1 | G**CT**G**T**GCTT**T**TGCAGGATGAGGG | 1.3 | Chr3:−563747 | Exon (LOC_Os03g01915) | n.d. |
| | Off-target-c2 | GAG**C**AGCT**A**GTG**G**AGGATGAAAG | 0.6 | Chr4:+218051 | Exon (LOC_Os04g01280) | n.d. |
| | Off-target-c3 | GAGG**C**GCTTGTG**G**AGG**T**TGATGG | 0.4 | Chr6:−12412691 | Exon (LOC_Os06g21480) | n.d. |

Top 3 off target sites predicted by CRISPR-P 2.0 (Liu et al., 2017) were sequenced.

[a]Bold: mismatch nucleotides compared with the sequence of sgRNA. Underlined: PAM sites.

n.d.: not detected.

**Reference**

**Liu H, Ding Y, Zhou Y, Jun W, Xie K, Chen L-L** (2017) CRISPR-P 2.0: an improved CRISPR-Cas9 tool for genome editing in plants. Mol Plant **10:** 530-532

**Supplemental Table S2**. Klason lignin assay of *OsCHI*-CRISPR-b, *OsCHIL*-CRISPR-b and wild-type control line.

| | WT2 | *OsCHI*-CRISPR-b | *OsCHIL*-CRISPR-b |
|---|---|---|---|
| Klason lignin (mg/g CWR) | 120.4 ± 6.3 | 112.0 ± 9.8 | 126.0 ± 3.2 |

Values refer to means ± standard deviations from biologically independent plants (Student's *t*-test, *n* = 3).

**Supplemental Table S3.** Cell wall polysaccharide analysis of *OsCHS1*-TDNA, *OsCHI*-CRISPR-a, *OsCHIL*-CRISPR-a and their isogenic wild types.

| | WT1 (cv. Zhonghua 11) | *OsCHS1*-TDNA (cv. Zhonghua 11) | WT2 (cv. Nipponbare) | *OsCHI*-CRISPR-a (cv. Nipponbare) | *OsCHIL*-CRISPR-a (cv. Nipponbare) |
|---|---|---|---|---|---|
| Crystalline glucan (mg/g CWR) | 391.2 ± 5.6 | 381.3 ± 3.9 | 376.9 ± 2.7 | 342.0 ± 11.7** | 381.1 ± 6.5 |
| Amorphous glucan (mg/g CWR) | 44.8 ± 6.0 | 53.8 ± 5.2 | 46.7 ± 1.1 | 52.9 ± 4.6 | 48.9 ± 2.6 |
| Arabinan (mg/g CWR) | 38.1 ± 2.3 | 36.3 ± 2.5 | 36.0 ± 2.3 | 39.0 ± 0.3 | 32.8 ± 1.6 |
| Xylan (mg/g CWR) | 127.6 ± 15.6 | 129.5 ± 15.5 | 108.2 ± 3.4 | 124.0 ± 6.9* | 112.6 ± 3.2 |
| Mannan (mg/g CWR) | 2.4 ± 0.2 | 2.3 ± 0.1 | 3.0 ± 0.2 | 3.3 ± 0.1 | 2.5 ± 0.1** |
| Galactan (mg/g CWR) | 18.2 ± 2.2 | 17.0 ± 1.4 | 15.3 ± 1.6 | 16.5 ± 1.0 | 14.1 ± 0.9 |

Values refer to means ± standard deviations from biologically independent plants. Asterisks indicate significant differences between mutant line and its isogenic wild type (Student's *t*-test, $n = 3$, *: $p < 0.05$, **: $p < 0.01$). CWR, cell wall residue.

**Supplemental Table S4.** Primers used in this study.

| Primer name | Sequence (5′ to 3′) | Target gene(s) | Purpose |
|---|---|---|---|
| LL302-a-F | GGCAGGAGGGCATCCCATTTCCTC | *OsCHIL1* & *OsCHIL2* | Construction of sgRNA-b in CRISPR/Cas9 binary vector used in the generation of *OsCHIL*-CRISPR |
| LL302-a-R | AAACGAGGAAATGGGATGCCCTCC | | |
| LL302-b-F | GCCGGAGGAGCTTGTGCAGGATGA | | Construction of sgRNA-c in CRISPR/Cas9 binary vector used in the generation of *OsCHIL*-CRISPR |
| LL302-b-R | AAACTCATCCTGCACAAGCTCCTC | | |
| CL2223 | ATGGTCGAGCTCAAGGAGAA | *OsCHS1* | Left primer. Genotyping of *OsCHS1*-TDNA |
| CL2224 | CAAACCGTTGTCCTCTTTCC | | Right primer. Genotyping of *OsCHS1*-TDNA |
| CL2227 | AATCCAGATCCCCCGAATTA | T-DNA | Border primer. Genotyping of *OsCHS1*-TDNA |
| CL3988 | CGGAGTAATCGGCAACAACG | *OsCHI* | Genotyping of *OsCHI*-CRISPR |
| CL3989 | CATCAAGTTCACGGCCATCG | | |
| CL4569 | TGATTGTTGTAAGTGCAGTGGG | *OsCHIL1* | Genotyping of *OsCHIL*-CRISPR |
| CL4570 | CCACACGTAAACATCATCAATCA | | |
| CL4571 | TGGTAGTAGCTGCACACACA | *OsCHIL2* | Genotyping of *OsCHIL*-CRISPR |
| CL4572 | GAGCGGCAGACTGAACCTTA | | |

**Supplemental Table S4.** Primers used in this study (continued).

| Primer name | Sequence (5′ to 3′) | Target gene(s) | Purpose |
|---|---|---|---|
| OsUBQ5-F | ACCACTTCGACCGCCACTACT | *OsUBQ5* | RT-qPCR of *OsUBQ5* |
| OsUBQ5-R | ACGCCTAAGCCTGCTGGTT | | |
| OsCHS1-F | ACCCGGACTACTACTTCAGGAT | *OsCHS1* | RT-qPCR of *OsCHS1* |
| OsCHS1-R | TCTTCCTGATCTGCGACTTGT | | |
| OsCHS2-F | CACCAACAGCGAGCACCTAA | *OsCHS2* | RT-qPCR of *OsCHS2* |
| OsCHS2-R | ACACGCACATGCTAGGGTTC | | |
| OsCHI-F | CGCAGGTTTGTTTGTGTTTGC | *OsCHI* | RT-qPCR of *OsCHI* |
| OsCHI-R | CGGATCGATCAAACCAGCAAG | | |
| OsCHIL1-F | GAGGATAGTGGTGATCAAGGAG | *OsCHIL1* | RT-qPCR of *OsCHIL1* |
| OsCHIL1-R | CATCCTCCTCGTACTTGTCTAC | | |
| OsCHIL2-F | CCATGGCATTACAGACATTGAG | *OsCHIL2* | RT-qPCR of *OsCHIL2* |
| OsCHIL2-R | GATCACCACGATCCTGAATAGT | | |