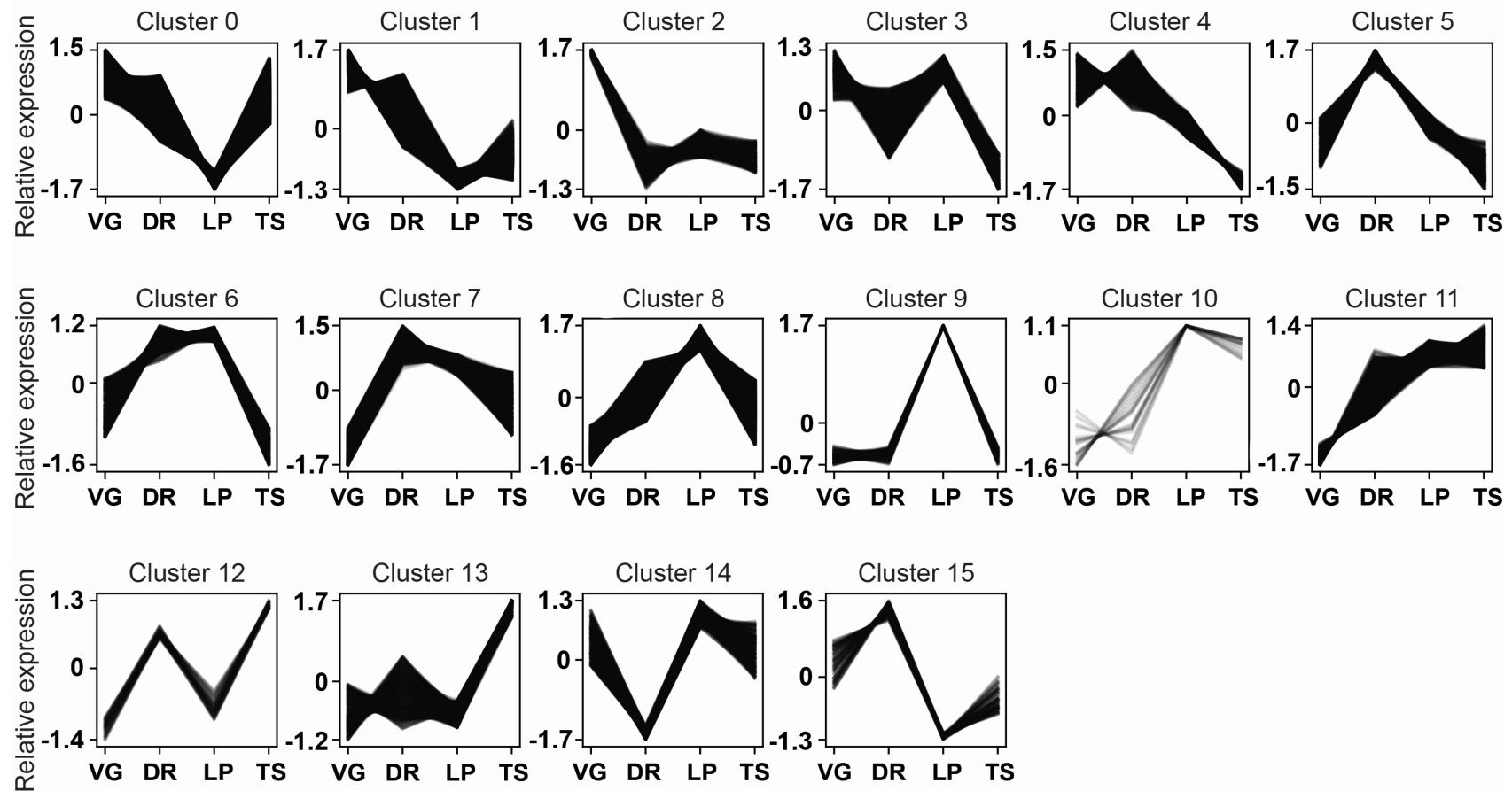


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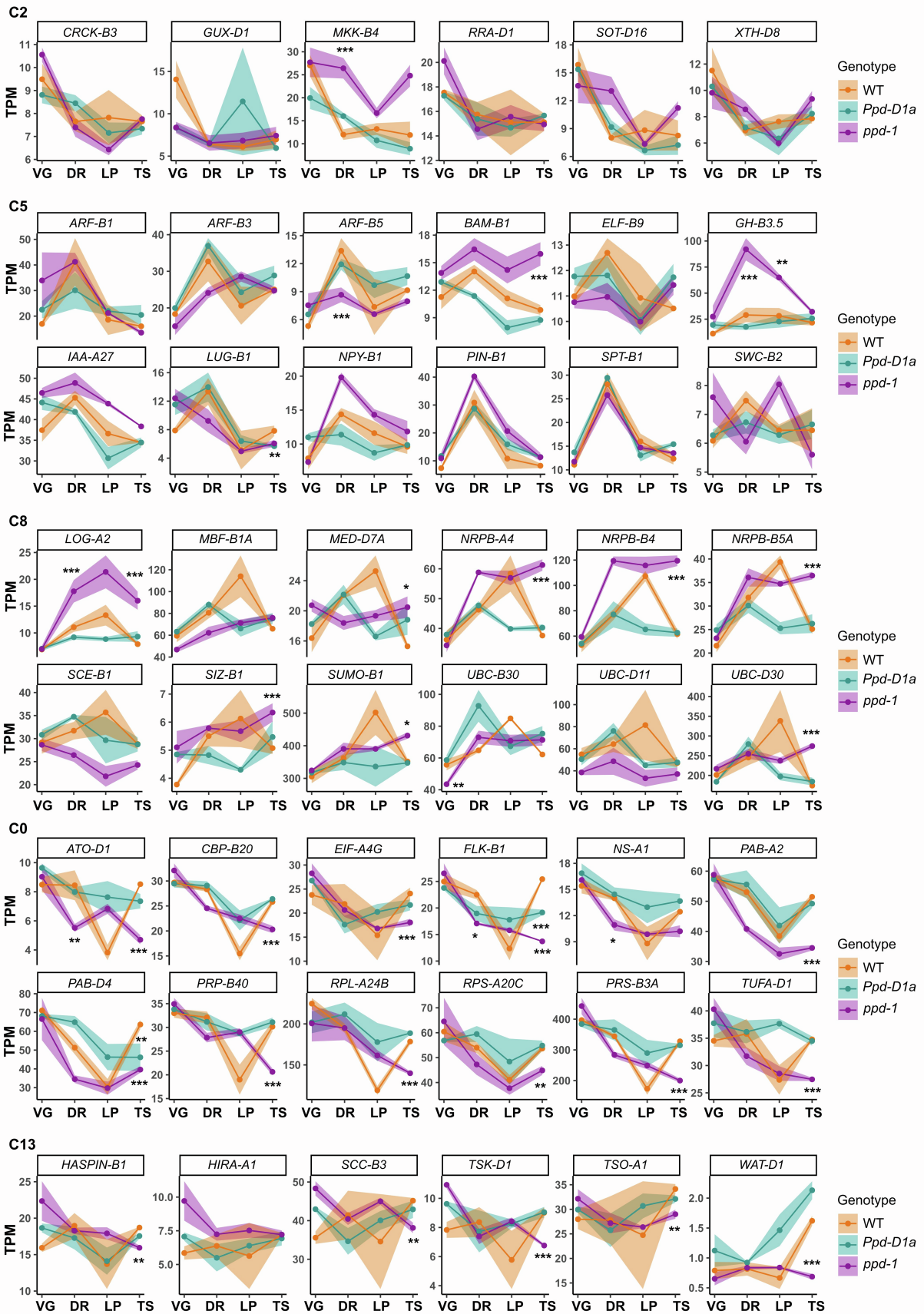
## Supplemental Information

***Photoperiod-1* regulates the wheat inflorescence  
transcriptome to influence spikelet  
architecture and flowering time**

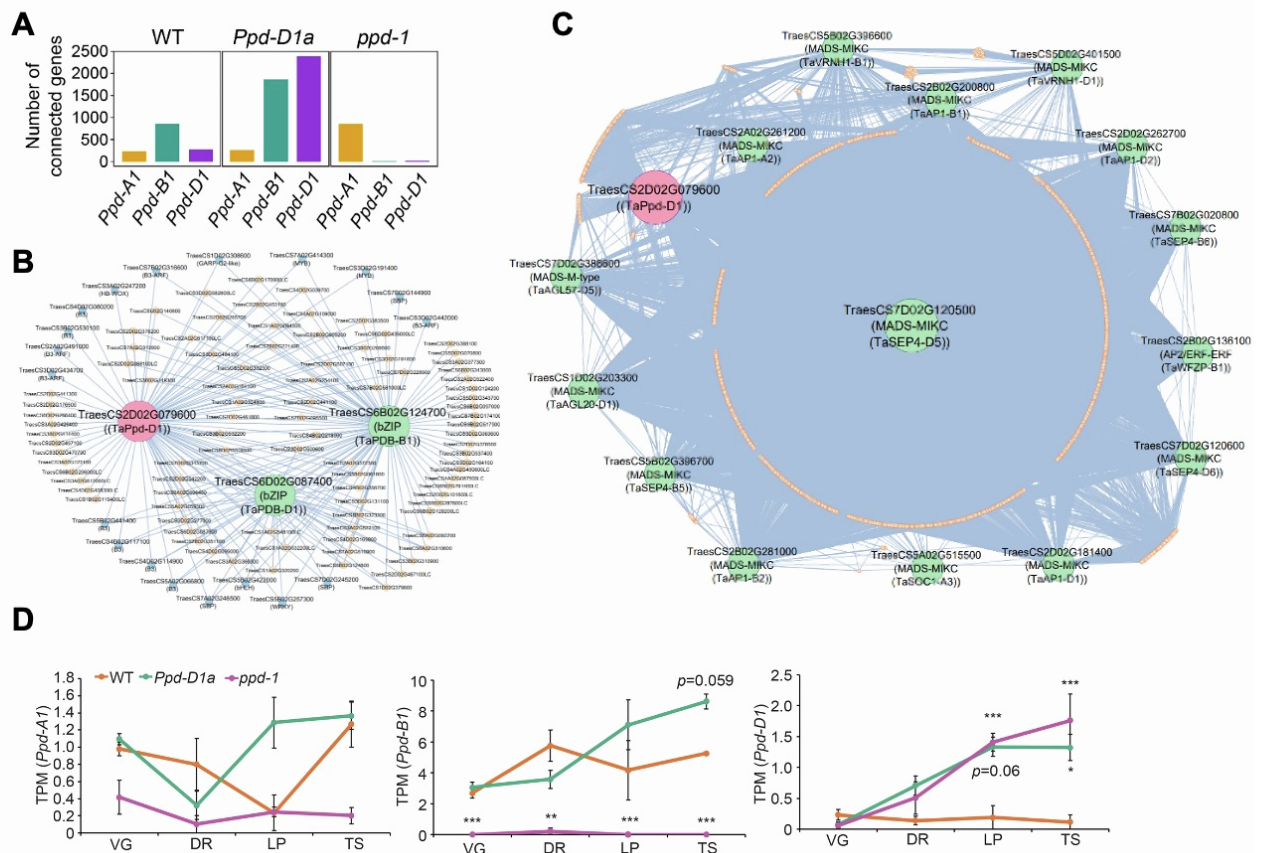
**Adam Gauley, Marianna Pasquariello, Guilherme V. Yoshikawa, Abdul Kader Alabdullah, Sadiye Hayta, Mark A. Smedley, Laura E. Dixon, and Scott A. Boden**



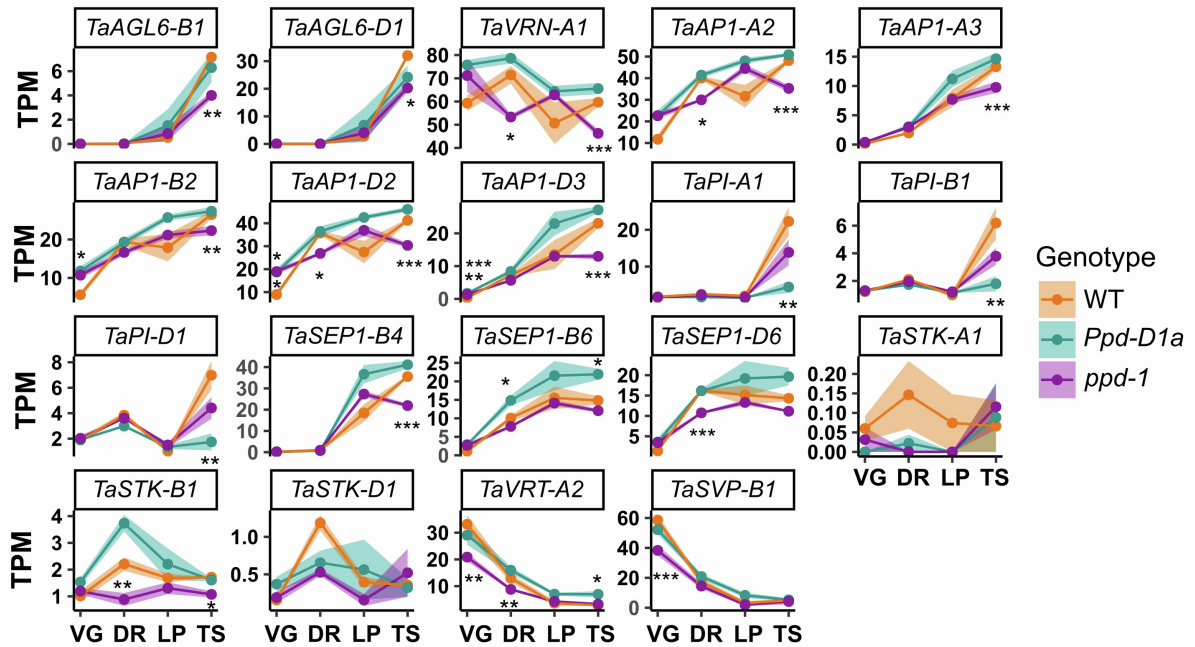
**Figure S1: Cluster analysis of transcripts expressed during early wheat inflorescence development, related to Figure 1.** Analysis of the 30,000 most abundant transcripts during early inflorescence development of wild-type plants identified 15 clusters that show unique stage-specific profiles between the vegetative (VG), double ridge (DR), lemma primordium (LP) and terminal spikelet (TS) stages.



**Figure S2: Expression profiles of transcripts identified from each of the stage-specific clusters, related to Figures 1 and 2.** Example expression profiles of transcripts from the identified GO-terms of C2 (VG), C5 (DR), C0 (LP), C8 (LP) and C13 (TS) of the developing inflorescence transcriptome analysis from wild-type plants. Data are presented as ribbon plots that show transcript levels (TPM, solid line with data points)  $\pm$  s.e.m. (shaded region) of three biological replicates from the wild-type (WT, orange), photoperiod insensitive (*Ppd-D1a*, cyan) and null (*ppd-1*, magenta) genotypes. Stages include vegetative (VG), double ridge (DR), lemma primordium (LP) and terminal spikelet (TS) stages. \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ .



**Figure S3: Co-expression gene network analysis and analysis of *Ppd-1* expression during early inflorescence development, related to Figures 2, 3 and 5. (A)** A summary of genes connected to each of the *Ppd-1* homeologs in the co-expression networks constructed for the photoperiod insensitive (*Ppd-D1a*) and null (*ppd-1*), and wild type (WT). **(B-C)** A sub-network showing genes connected to *Ppd-D1* in the co-expression network of developing inflorescences from **(B)** wild-type (WT) and **(C)** photoperiod insensitive NIL. Guide genes shown in green circles are those shown to perform roles during inflorescence development in wheat and other plant species and *Ppd-D1* gene is shown as a red circle. Blue circles represent transcription factor genes. Edge weight = 1 was used as threshold to visualise genes in the subnetwork using Cytoscape 3.10.1 software. **(D)** Transcript values for *Ppd-A1*, *Ppd-B1* and *Ppd-D1* in wild-type (WT, orange), photoperiod insensitive (cyan, *Ppd-D1a*) and null (*ppd-1*, pink) NILs during the vegetative (VG), double ridge (DR), lemma primordium (LP) and terminal spikelet (TS) stages. Data are TPM  $\pm$  s.e.m. of three biological replicates; \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ .



**Figure S4: Stage-specific expression profiles of transcripts encoding MADS-box transcription factors, related to Figure 4.** Expression profiles of transcripts encoding MADS-box transcription factors in the developing inflorescence, which show a modified developmental expression pattern in either the *Ppd-D1a* (cyan) or *ppd-1* (magenta) NILs, relative to wild-type (orange). Data are presented as ribbon plots that show transcript levels (TPM, solid line with data points)  $\pm$  s.e.m. (shaded region) of three biological replicates. Stages include vegetative (VG), double ridge (DR), lemma primordium (LP) and terminal spikelet (TS) stages. \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ .

*pdb1\_m1*

*PDB-B1*

GCGCAAGCAGCG CCACCTCGACGACCTCGCCGCGCAGGCGGGCGACCTCCGCCGCGAGAACGCGCACGTC  
GCGCA--GCGGGCGCACCTCCGCCGCGAGAACGCGCACGTC

*PDB-D1*

GCGCAAGCAGCG CCACCTCGACGACCTCGCCGCGCAGGCGGGCGACCTGCGCCGCGAGAACGCGCACGTC  
GCGCAAGCAGCGCCACCT--GCGGGCGCGAGAACGCGCACGTC

*pdb1\_m2*

*PDB-A1*

GCGCAAGCAGCG CCACCTCGACGACCTCGCCGCGCAGGCGGGCGACCTGCGCCGCGAGAACGCGCACGTC  
GCGCAAGCAGCGCCACCT--GCGGGCGCGAGAACGCGCACGTC

*PDB-D1*

GCGCAAGCAGCG CCACCTCGACGACCTCGCCGCGCAGGCGGGCGACCTGCGCCGCGAGAACGCGCACGTC  
GCGCAAGCAGCGCCACCT--GCGGGCGCGAGAACGCGCACGTC

*alog1\_m1*

*ALOG-A1*

GGCAGCGCGTTCGTCGGTGTGGGAGCGCCGCGGCCGA GCAGGTACGAGTCGCAGAAGCGGCGGGACTGGCAGACGTTTCGGGCAGTACCTACGGAACCACC GCCCCCGCTGGAGCTGGCGCGGTGC  
GGCAGCGCGTTCGTCGGTGTGGGAGCGCCGCGGCCGAGCAGGTACGAGTCGCA--AGCGGGCGGACTGGCAGACGTTTCGGGCAGTACCTACGGAACCACC GCCCCCGCTGGAGCTGGCGCGG--GCGCGGTGC

*ALOG-B1*

GGCAGCGCGTTCGTCGGGCTGGGAGCGCCGCGGCCGA GCAGGTACGAGTCGCAGAAGCGGCGGGACTGGCAGACGTTTCGGACAGTACCTACGGAACCACC GCCCCCGCTGGAGCTGGCGCGGTGC  
GGCAGCGCGTTCGTCGGGCTGGGAGCGCCGCGGCCGAGCAGGTACGAGTCGCAGAAGCGGGCGGACTGGCAGACGTTTCGGACAGTACCTACGGAACCACC GCCCCCGCTGGAGCTGGCGCGG--GCGCGGTGC

*ALOG-D1*

GGCAGCGCGTTCGTCGGGCTGGGAGCGCCGCGGCCGA GCAGGTACGAGTCGCAGAAGCGGCGGGACTGGCAGACGTTTCGGGCAGTACCTACGGAACCACC GCCCCCGCTGGAGCTGGCGCGGTGC  
GGCA--GGGACTGGCAGACGTTTCGGGCAGTACCTACGGAACCACC GCCCCCGCTGGAGCTGGCGCGG

*alog1\_m2*

*ALOG-A1*

GGCAGCGCGTTCGTCGGTGTGGGAGCGCCGCGGCCGA GCAGGTACGAGTCGCAGAAGCGGCGGGACTGGCAGACGTTTCGGGCAGTACCTACGGAACCACC GCCCCCGCTGGAGCTGGCGCGGTGC  
GGCAGCGCGTTCGTCGGTGTGGGAGCGCCGCGGCCGAGCAGGTACGAGTCGCA--AGCGGGCGGACTGGCAGACGTTTCGGGCAGTACCTACGGAACCACC GCCCCCGCTGGAGCTGGCGCGGTGC

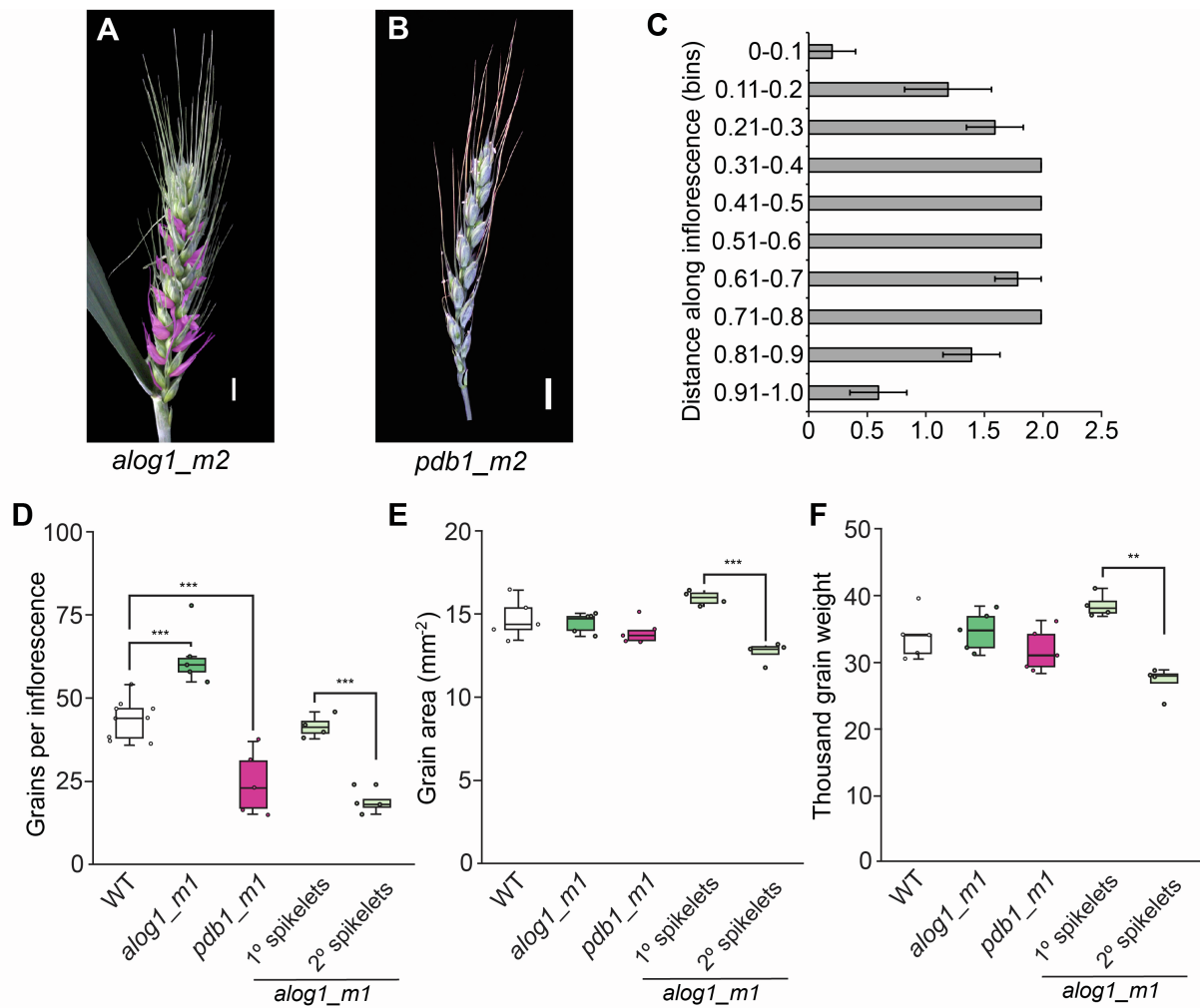
*ALOG-B1*

GGCAGCGCGTTCGTCGGGCTGGGAGCGCCGCGGCCGA GCAGGTACGAGTCGCAGAAGCGGCGGGACTGGCAGACGTTTCGGACAGTACCTACGGAACCACC GCCCCCGCTGGAGCTGGCGCGGTGC  
GGCAGCGCGTTCGTCGGGCTGGGAGCGCCGCGGCCGAGCAGGTACGAGTCGCAGAAGCGGGCGGACTGGCAGACGTTTCGGACAGTACCTACGGAACCACC GCCCCCGCTGGAGCTGGCGCGG--GCGCGGTGC

*ALOG-D1*

GGCAGCGCGTTCGTCGGGCTGGGAGCGCCGCGGCCGA GCAGGTACGAGTCGCAGAAGCGGCGGGACTGGCAGACGTTTCGGGCAGTACCTACGGAACCACC GCCCCCGCTGGAGCTGGCGCGGTGC  
GGCAGCGCGTTCGTCGGGCTGGGAGCGCCGCGGCCGAGCAGGTACGAGTCGCAGAAGCGGGCGGACTGGCAGACGTTTCGGGCAGTACCTACGGAACCACC GCCCCCGCTGGAGCTGGCGCGGTGC

**Figure S5: A summary of the gene edits generated in the *pdb1* and *alog1*, related to Figure 6. The reference sequence for *PDB-A1*, *PDB-B1* and *PDB-D1*, and *ALOG-A1*, *ALOG-B1* and *ALOG-D1*, is provided in black text, with the guideRNA sequence highlighted in red (PAM is underlined). The gene edited sequence is shown underneath the reference (blue text), with deleted base pairs shown as dashes.**



**Figure S6: Inflorescence architecture and grain phenotypes of the *ALOG1* and *PDB1* gene-edited lines, related to Figure 6. (A-B)** Representative inflorescences of the *alog1\_m2* line (A) and the *pdb1\_m2* line (B), with the secondary spikelets highlighted in pink. (C) The rachis nodes of the *alog1\_m1* lines that form paired spikelets are located predominantly in the central region of the inflorescence. (D-F) Grain number per inflorescence, grain area and thousand grain weight phenotypes for the *alog1\_m1* (green) and *pdb1\_m1* (pink) gene edited lines, relative to wild-type (WT, cv. Fielder). Data is also shown for grain isolated from the primary (1°) and secondary (2°) spikelets of nodes that formed paired spikelets in the *alog1\_m1* lines (light green). In the boxplots (D-F), each box is bound by the lower and upper quartiles, the central bar represents the median, and the whiskers indicate the minimum and maximum values of 4-5 biological replicates. \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ .