Supplementary Information for

**INTERMEDIATE-M encodes an HvAP2L-H5 ortholog and is required for inflorescence indeterminacy and spikelet determinacy in barley**

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Materials and Methods

Plant materials and growth conditions. Grains of the two original *intermedium-m (int-m)* allelic mutants (*int-m.85* in Bonus and *int-m.1a* in Lamont), the near isogenic lines (NIL) in the common genetic background Bowman (*BW429, int-m.85* *BC7 and BW430, int-m.1a *BC5), *Triticum monococcum* (PI167615, Einkorn) and other wild Triticeae species were obtained from the GRIN USDA seed bank (*SI Appendix* Table S1). Twenty-four *dub1* mutants were ordered from the NordGen seed bank (*SI Appendix* Table S1). *int-m.85* and *int-m.1a* mutants were chemically induced in the Bonus and Lamont background, respectively, and initially characterized as one of a series of spike row-type (defined by the fertility of the two lateral spikelets (LSs) of a spikelet triplet at each node; two LSs are sterile and fertile in two-rowed and six-rowed barley, respectively) mutants, with “enlarged and pointed lateral spikelets” (32). The inflorescences of *int-m* mutants were described as having several “fused or fasciated terminal spikelets” due to limited elongation of rachis internodes (27).

Grains were sowed directly in soil (“Mini Tray”, Einheitserde®) in a 96-well tray, stratified in dark at 4°C for three days before being moved to the long-day Bronson greenhouse for germination and growth (16h light:8h dark cycle; day temperature = 20°C, night temperature = 16°C). The seedlings were repotted into 20-well trays one week after germination.

Complementation test. *double seed1 (dub1)* mutants phenotypically resemble *int-m* mutants having abnormally fused or fasciated terminal spikelets, and seem to be caused by a monofactorial recessive locus that was mapped to the chromosome 5HL (49). Therefore, *dub1* mutants are putatively allelic to the *int-m* mutants. To determine whether *dub1* mutants are allelic to *int-m* mutants, we crossed *dub1 M1* (*dub1.1*, maternal parent) with *int-m.85* (pollen donor) and *int-m.85* (maternal parent) with *dub1 M3* (*dub1.3*, pollen donor). The F1 plants were genotyped using a molecular marker HvAP2L-H5 (identified from SNP analysis) and the restriction enzyme Hpy188I to confirm the success of the crosses and phenotyped for inflorescence termination morphology. We obtained five successful F1 hybrids from the cross of *int-m.85* x *dub1.3*, we further confirmed the allelism of *int-m* and *dub1* using two F2 populations of the *int-m.85* x *dub1.3* hybrids that included a total of 68 (32 + 36) plants.

Phenotyping of *int-m (dub1)* mutants. The developing main shoot apices (MSAs) of the two *int-m* NIL mutants (*BW429* and *BW430*) and the wild-type Bowman, *dub1.1* (Bonus), *int-m.85* (Bonus), *dub1.3* (Foma) and *dub1.7* (Foma) and their respective wild-type Bonus and Foma were weekly examined using a dissecting microscope (Nikon-SMZ18) and imaged using a Nikon Digital camera (Sight DS-Fi2).

To characterize the developmental defects of inflorescences of *int-m/dub1* mutants, developing MSAs of the mutants and the corresponding wildtypes were dissected every 2-3 days, shock-frozen in liquid nitrogen followed by sublimation, and sputter-coated with gold (EMITECH, K1250X). Samples were visualized in a scanning electron microscope (SEM, ZEISS SUPRA 40VP) at the Max Planck Institute for Plant Breeding Research (MPIPZ).
We quantified the effects of \textit{int-m} mutations on inflorescence length and numbers of rachis nodes (i.e. spikelet triplet units) using microscopy and SEM images. We measured the length using the Nikon imaging program or ImageJ (https://imagej.nih.gov/ij/) and counted the number of rachis nodes per inflorescence.

To compare the meristem size, we used SEM micrographs and confocal microscopy images. Specifically, we considered the apex above the latest visible lateral primordium as the presumptive (vegetative, inflorescence or terminal spikelet) (VM, IM or TSM) shoot apical meristem (SAM). We measured their transverse width (i.e. the diameter of the lowest part) and longitudinal length (i.e. between the tip and the lowest part) (Fig. 3D) using ImageJ. For confocal imaging, developing MSAs were treated with ClearSee (50) and stained with 1‰ Renaissance for cell walls, followed by imaging using a confocal microscopy (Leica, SP8). MorphoGraphX (51) was used to reconstruct the 3D structure of the MSAs. However, the resolution using the confocal microscopy was low due to poor penetration of fluorescent dye and deep imaging with large barley MSAs. We thus used only SEM images to compare the meristem size (i.e. length and width) for all lines/species with multiple biological replicates to minimize the potential bias due to orientation/angle.

**Staging of SAM development in Triticeae.** As in Arabidopsis, a shoot apical meristem (SAM) in grasses may be a vegetative meristem (VM) and an inflorescence meristem (IM). In Arabidopsis \textit{tfl1} and snapdragon \textit{cen} mutants, the identity shift of an IM to a floral meristem (FM) leads to meristem determinacy (3). By contrast, in grasses, the acquisition of a terminal spikelet meristem (TSM) identity in inflorescences results in inflorescence meristem determinacy (e.g. wheat, barley \textit{int-m/dub1} mutants). Therefore, on the basis of the developmental progression, an SAM in grasses may be a VM, an IM and a TSM.

Stages of developing inflorescences in Triticeae are largely comparable except that barley produces triple mounds (TMs) after double ridge (DR) stage and the TMs give rise to three spikelet meristems (SMs) of a spikelet triplet (one central spikelet meristem and two lateral spikelet meristems) at each rachis node. In contrast, most Triticeae species produce SMs directly during the late DR stage. Therefore, to properly compare inflorescences over development in Triticeae, we aligned stages using the distinct meristems that are sequentially initiated as developmental landmarks, including vegetative meristem (VM; a.k.a. forming single ridge (leaf ridge) before the emergence of double ridges), double ridge (DR; VM transitions to inflorescence meristems (IM), producing leaf and spikelet ridges), triple mounds (i.e. spikelet triplets initiated) - spikelet meristems (i.e. glume primordium initiation) (TM-SM), floret meristem (FM; i.e. lemma primordium initiation), stamen primordium (StP) and awn primordium (AP) stages.

**Plotting and statistical test.** Phenotyping and qRT-PCR results were all plotted using ggplot2 and statistically analyzed using R3.5.3 (R: The R Project for Statistical Computing). Analysis of covariance was performed to compare the predicted regression lines of rachis node of spikes against sampling time points (Fig. 1I; Days after emergence) using the R library “lsmeans”. We used the Tukey test (R package “multcompView”) for comparison across stages within a genotype/species (Fig. 1I, rachis nodes in \textit{int-m} mutants; Fig. 3E, 4B, meristem length between stages). The
Dunnett’s test (R “DescTools” library) was used for comparisons between the wildtype and mutants (Fig. 3E, between Bowman and int-m mutants), and the Student’s t-test for between species comparison (SI Appendix Fig. S17C; MADS3 expression between Einkorn and Bowman at DR stage).

**Gene identification using RNA-sequencing (RNA-seq).** To determine the causal gene underlying the int-m locus, we used RNA-seq of developing MSAs of the two allelic int-m NIL mutants (BW429 and BW430) in Bowman and the wild-type Bowman and Bonus. With phenotypic alterations in inflorescences, we assumed that the underlying causal gene is expressed in developing inflorescences, and changes in the coding or regulatory regions lead to the mutant phenotype. Therefore, the genome-wide transcriptomes from developing MSAs would thus allow us to (i) detect sequence mutations by comparing the variants and (ii) identify differentially expressed genes between the mutants and wildtypes (however, we collected a different set of samples for this purpose). Specifically, total RNA of the developing MSAs from three successive developmental stages – DR, FM, and carpel primordium stages were sequenced as previously described (52). To identify the candidate gene, we used two mapping strategies by aligning all RNA-seq reads to either a set of high-confidence (HC) annotated genes (gene-based) or the genome reference sequences (genome-based) of Morex (53). We used the short-read aligner bwa-mem (38) with mismatch penalty of 3 (-B 3) for the gene-based alignment and the STAR 2-pass mapping algorithm (55) for the genome-based mapping with recommended settings in “the calling variants in RNAseq of GATK Best Practices” (56). For both mapping strategies, variants were identified using HaplotypeCaller in GATK 3.8 (-stand_call_conf 20.0) followed by filtering using the parameters (-window 35 -cluster 3 -filterName FS -filter \"FS > 30.0\" -filterName QD -filter \"QD < 2.0\" -filterName MQRankSum -filter \"MQRankSum < -12.5\" -filterName ReadPosRankSum -filter \"ReadPosRankSum < -8.0\").

For the genome-based mapping strategy, we first annotated variants against the HC genes using a perl script. However, this annotation-dependent comparison could not identify genes that were not annotated as candidate genes. To include the introgression regions where SNPs were not annotated in the HC genes, we de novo annotated the SNP-containing DNA segments (2000bp in length) using BLAST searches.

To narrow down the candidate genes, we expected that mutations (i) were only present in the focal mutants but not in the reference Morex, the wild-type Bowman or Bonus; (ii) caused non-synonymous amino acid changes for the allelic NIL mutants (BW429 and BW430); and (iii) were likely different in the two allelic mutants as they were derived from independent mutational events in different backgrounds using different mutagens (27). SnpEff (57) was used to annotate and predict the functional effects of the identified variants on genes and proteins (SI Appendix Dataset S1).

The SNPs/INDELs or alleles were plotted along the barley chromosomes using the package karyoploteR (58) in R3.5.3 (R: The R Project for Statistical Computing).

**Segregation analysis of a biparental mapping population.** To confirm whether the candidate gene co-segregates with the int-m phenotype, BW429 (int-m.85 NIL) was crossed with the wild-type Proctor (PI 512036). For co-segregation analysis, an F3
segregating population derived from the heterozygous F2 individuals were phenotyped (208 plants) and genotyped (158 plants) for the int-m.85 allele. The int-m.85 allele was diagnosed by the Hpy188I restriction enzyme.

**Resequencing of HvAP2L-H5 and phenotyping of the allelic dub1 mutants.** To identify the molecular changes in an allelic series of dub1 mutants, we sequenced the genomic regions containing the two AP2 domains, and that spans microRNA172 (miR172) cleavage site. We used PROVEAN (Protein Variation Effect Analyzer) (59) to predict the effects of mutations on the protein function.

We phenotyped all types of dub1 mutants to determine whether dub1 mutations produce a determinate inflorescence (i.e. formation of a terminal spikelet), de-suppressed RSMs and the production of extra florets in central spikelets like int-m mutants.

**Synteny, phylogeny and diversity analysis.** Synteny and phylogenetic analyses were performed to identify orthologous genes of HvAP2L-H5 in other cereals. We applied the MCscan (Python version) pipeline implemented in JCVI utility libraries (60) to perform pairwise synteny and search for multi-species microsynteny. We used 7 publicly available genomes, including barley(53), the wheat A subgenome *Triticum urartu* (61), the wheat D subgenome *Aegilops tauschii* (62), and *Brachypodium distachyon*, rice and maize from the Phytozome12.3 (63) (SI Appendix Table S6).

The INT-M protein sequence was used as a query sequence to blastp (e-value 10^{-5}) against an in-house customized database including genome sequences of 24 species from Phytozome 12.3 (63) and other databases (SI Appendix Table S6). The corresponding nucleotide sequences were extracted and aligned using the translation alignment with MAFFT 7.409 (64) in Geneious 6.1.8 (BioMatter). We manually refined the alignment and performed phylogenetic analysis using RAxML 8.2.3 (65) with the maximum likelihood (GTRCAT) method and 1000 replicates of bootstrap resampling.

To explore natural diversity of HvAP2L-H5, we used a published exome-capture dataset including a panel of cultivated and wild barley (66). The haplotype network of HvAP2L-H5 was visualized with Median-joining Network with epsilon 0 using PopART (Population analysis with Reticulate Trees) (67) (SI Appendix Fig. S19).

The SID1 (*Sister of IDS1*) clade genes were lost in Triticeae species as suggested by the phylogenetic inference (Fig. 2B). To confirm the gene loss in Triticeae genomes, we conducted blastn (e-value 10) search using the genomic region of AP2 domains of BdSID1 as a query sequence against the genomes of barley, *Aegilops tauschii* and *Triticum urartu*. In addition, we used synteny analysis to identify the syntenic block containing the BdSID1 locus (BdSID1 block) and the flanking syntenic blocks of the BdSID1 block (SI Appendix Fig. S14).

**Orthology sorting.** To identify orthologous genes for barley-wheat comparisons, we used OrthoFinder (68) with genome sequences of barley, *Brachypodium distachyon*, wheat, *Triticum urartu*, *Aegilops tauschii*, rice, maize, sorghum and *Setaria viridis* (SI Appendix Table S6). Specifically, DIAMOND (69) was used to substitute blastp to perform all-versus-all protein searches, followed by orthologous groups (orthogroups) sorting using the MCL algorithm (70). Orthogroups (SI Appendix Dataset S3)
containing differentially expressed barley genes in the transcriptomic profiling of int-m mutants were phylogenetically analyzed as previously described (71).

**Comparative transcriptomics of spike development.** To identify genes that were affected by the int-m mutations, we compared genome-wide transcriptomic changes between each of the two NIL mutants with the wild-type Bowman. To identify genes whose expression are altered primarily due to early HvAP2L-H5 activity, we focused on the double rige (DR) (Fig. 1B) before spikelet initiation when a terminal spikelet is emerging and the TM-SM (Fig. 1C) when the terminal spikelet is clearly formed but before the differentiation of floral organs of CSs. The StP stage was added to identify changes associated with the production of extra florets in spikelets in int-m mutants (Fig. 1E) despite expected large secondary effects caused by an altered inflorescence meristem.

Specifically, developing main shoot apices enriched for three stages DR, TM-SM and StP were dissected by removing all visible surrounding leaves. Four biological replicates, each pooled from 15 main shoot apices, were sampled per genotype per stage, yielding a total of 36 samples. Total mRNA was isolated using Trizol (Life Technologies), and cleaned with RNeasy Micro Kit (QIAGEN). Due to RNA degradation verified by agarose gel electrophoresis, two Bowman samples at TM-SM and one int-m.1a at DR stage were discarded. A total of 33 RNA samples were assessed using Agilent 2100 Bioanalyzer system for quality control and sequenced subsequently. The PolyA+ selection method was used for RNA-seq library preparation. The RNA-seq libraries were sequenced using an Illumina Genome Analyzers IIx with 2x100 bp paired-end and 18 million reads per sample at the Cologne Center for Genomics (CCG) in the University of Cologne.

**Differential gene expression analyses.** About 1674 million high-quality reads were obtained. To quantify transcripts, we used the latest Morex genome annotation (72) and estimated read abundance using Salmon (73) with --validdatemapping option and other default parameters. Lowly expressed genes were filtered out and genes that have a minimum of 1 CPM (counts per million) in at least three samples were used for differential gene expression analysis. Pairwise comparisons between each of two int-m mutants and the wild-type Bowman per developmental stage were conducted to identify differentially regulated genes using Limma-Voom packages (74, 75). To minimize the effects of background mutations, we focused our analyses on genes that were differentially regulated in the same direction in the two int-m NIL mutants (SI Appendix Dataset2).

We de novo annotated the reference coding sequences by blastx against nr database from NCBI (accessed on February 10th, 2019) with an e-value of 10^{-5}. OmicsBox (76) was used to map the GO (gene ontology) terms. To identify significantly enriched GO terms, we performed enrichment analysis using AgriGO v2.0 (77) with the customized reference gene set and the misregulated genes from early developmental (DR and TM-SM) stages.

To make barley-wheat comparison, we reanalyzed publicly available wheat transcriptomes using the same abundance estimation strategy as for barley. The latest wheat reference IWGSC RefSeq v1.0 (78) was used as the reference. The wheat transcriptomes were retrieved from a previously published study using
developing spikes sampled from four successive stages of spikes, including DR, FM, StP and tetrads stages (79). The first three stages are largely comparable to our sampling time points of DR, TM-SM and StP for barley.

**Comparative analyses of barley HvAP2L-H5 and the wheat orthologs.** Changes in INT-M alone in barley are sufficient to convert an indeterminate to a wheat-like determinate inflorescence (Fig.1). We thus hypothesize the differences between barley HvAP2L-H5 and its wheat orthologs AP2L-A5 may similarly contribute to the differences in IM determinacy/indeterminacy between the species. Specifically, distinct protein and/or regulatory changes between barley HvAP2L-H5 and the wheat orthologs may explain the architectural differences between their inflorescences. First, we identified the amino acid differences between barley HvAP2L-H5 and the functional wheat ortholog (AP2L-A5) and predicted how the differences may influence the function of the proteins using PROVEAN (59). Second, we used in situ mRNA localization to determine the temporal and spatial expression patterns of barley HvAP2L-H5 and AP2L-A5 during inflorescence development. Third, the evolutionary histories of AP2L-5 (i.e. functional redundancy due to duplication, subsequent paralog retention and sub-functionalization) may influence the effects of HvAP2L-H5 on inflorescence development and hence phenotypical divergence. Finally, changes in orthologs of the downstream targets identified from comparative transcriptomics of the int-m mutants may contribute to the divergence between barley and wheat inflorescences.

**RNA in situ localization.** To determine the expression patterns of genes of interest, in situ mRNA hybridization was performed using the standard protocol (80). To prepare probes, cDNA was used to amplify DNA templates of target genes for in vitro transcription reaction using gene-specific primers appended with promoter sequences of T3 and T7, respectively. Probes, ca. 200–700bp in length from the variable coding and/or UTR regions of the genes, were synthesized using T3/T7 RNA Polymerase (Roche) and labelled with Digoxigenin-11-UTP (Roche). To determine the specificity of the probes, we used the whole-gene deletion mutant dub1.1 as the negative control for barley HvAP2L-H5, and sense probes were used as controls for the remaining focal genes (SI Appendix Fig. 20). We tested at least two different probes from separate parts of the genes to identify the probes that are specific. In some cases, we used probes designed from barley for Einkorn, including TmKN1, TmLOG and TmMADS1 with their high degree of sequence identity (>95%). Color reaction was extended as needed, typically for one, two or three nights.

Results were imaged using a Zeiss Axiolmager M2 microscope equipped with an AxioCam512 color camera. Images were adjusted for brightness and contrast using GIMP2.10 (https://www.gimp.org/) and cropped/assembled in Inkscape 0.92.4 (https://inkscape.org/).

**Quantitative real-time PCR (qRT-PCR).** We harvested developing inflorescences (i.e. shoot apices) at the DR (4 biological replicates) and the spikelet meristem (SM) (7 biological replicates) stages for Einkorn using a dissecting microscopy. Total RNA was extracted as described in the previous section and cDNA was synthesized using SuperScriptTM II RT (Invitrogen). qRT-PCR was used to quantify the expression levels of AP2L-5, MADS3 and MADS58 in Bowman and Einkorn using EvaGreen
(Biotium) on a LightCycler480 (Roche). Common gene-specific primers for qRT-PCR were designed for both barley and Einkorn to amplify the same region for the reference (actin) and all focal genes (SI Appendix Table S5).

**Phylogenetic footprinting of regulatory sequences of HvAP2L-H5 orthologs.** To compare the regulatory divergence of HvAP2L-H5 and the wheat functional ortholog (AP2L-A5), we analyzed their regulatory sequences. We extracted 10Kb genomic sequences flanking each end of the AP2L-5 orthologs for barley, wild barley (http://db.ncgr.ac.cn/wild_barley/) (81), wheat genomes (Aegilops tauschii, Triticum urartu, Triticum turgidum, and Triticum aestivum) and wheatgrass (Thinopyrum elongatum) and a 6709 bp fragment in Hordeum pubiflorum (PRJEB3404) containing the AP2L-5 ortholog due to incomplete genome sequences. Extracted sequences were analyzed using mVISTA (http://genome.lbl.gov/vista/index.shtml) to identify the conserved regions and aligned using MAFFT 7.409 (64) in Geneious 6.1.8 (BioMatter). We identified the accessible chromatin regions using the ATAC-seq of barley Morex leaf (47) and DNase-seq of wheat seedlings (48).

**Evolution of inflorescence determinacy/indeterminacy in Triticeae.** To infer the ancestral state of inflorescence determinacy in Pooideae, we used a simplified phylogeny of Pooideae modified from previous phylogenetic analyses of grasses (82) and Triticeae (83). Character states of inflorescence determinacy were coded using SEM micrographs in this study and from previous studies (5, 84). Ancestral character state inference was conducted using a maximum parsimony criterion implemented in Mesquite 3.61 (85).

The length and width of SAM were quantified as in Bowman and int-m NIL mutants of barley. However, the absolute size of SAM differs among different species, we therefore performed a within-species data normalization to a range of 0 to 1 for cross-species comparison using the following formula.

\[ x' = \frac{(x - \text{min}(x))}{(\text{max}(x) - \text{min}(x))} \]

The timing of IM-TSM transition in each focal species was determined using the developmental series of inflorescences. Specifically, the final spikelet number in species with determinate inflorescences is defined and stable after the initiation of terminal spikelet in different plant individuals/tillers of the same species under the same growth conditions and experimental batch. Thus, the final spikelet number within a species was determined from later stages when the terminal spikelet is clearly formed. We considered that the TSM is initiated when the final spikelet number is reached in the species (SI Appendix Fig. S12).

**Captions of Supplementary Figures**

**Fig. S1.** Phenotyping of the int-m NIL mutants. (A-B) Developmental series of int-m.85 spike (BW429, int-m.85*BC7 in Bowman) showing the formation of a multi-floreted terminal spikelet (TS). The two glumes of the TS are highlighted with green pseudo-color. (B)-(D) int-m NIL mutants form a multi-floreted terminal spikelet. The two basal sterile bracts (marked with green dots), subtending a TS that bears multiple florets (multiple pairs of lemmas/paleae), are morphologically similar to glumes of central spikelets (CSs; pointed with green arrows) that have short awns in the distal ends. Sterile bracts that develop into lemma-like organs in
the TS are labelled with pink asterisks. (D) Dissected parts of multiple TSs from glumes to tip were arranged from left to right or from bottom to top in each panel, and glumes of a CS was included in the leftmost/bottommost in each panel for comparison. The upper non-basal sterile bracts (marked in pink asterisks in B-D) in the TS produce extensively elongated awns in the distal tips and are phenotypically lemma-like. (E) A representative image showing expression patterns of HvMADS1 in lemmas but not in glumes (consecutive sections were used to determine the identity the tissue-specific expression patterns). TMM, triple mound meristem (a.k.a. SPR, spikelet ridge); LR, leaf ridge. (F) Developmental series of the wildtype Bowman showing the RSMs. The single floret per spikelet initiates in the adaxial side, while SMs are suppressed in the abaxial side as residual SMs (RSMs). (G) De-suppression of residual SMs (RSMs) and initiation of extra florets in some CSs (circled with dotted lines) in int-m.1a and int-m.85 NIL mutants. (H) These extra florets may often become sterile or occasionally develop into sessile grains in mature spikes, suggesting that the rachillae (spikelet axis) are not de-suppressed. (I) Two exemplary multi-floreted terminal spikelets showing the phenotypical similarities and perpendicular orientation of glumes of TS and CSs. (J) The length of the whole inflorescences in int-m mutants is reduced compared to that of the wildtype Bowman. Analysis of covariance is used to compare the predicted regression lines of spike length vs days after emergence (DAE) of Bowman, int-m.1a and int-m.85 (N=449). No significant difference ($p = 0.9925$) is found between int-m.1a and int-m.85 mutants, while the spike length in Bowman is significantly longer than that in each int-m mutant ($p < 0.0001$). The images highlighted with fuchsia squares are also shown in Fig. 1.

Fig. S2. Phenotypic and genetic analyses of int-m and dub1 mutants. (A-B) int-m and dub1 mutants are morphologically similar, producing a terminal spikelet (A and B), which seems to emerge no later than the TM-SM stage, and multi-floreted spikelets (B, dashed circles). (C-G) dub1.3 is allelic to int-m.85. All F1 (5) and F2 (68) plants of the crosses of int-m.85 x dub1.3 form a terminal spikelet as the parental donors. Representative SEM images of an F1 (#4) out of five hybrids (C) and three F2 plants (different genotypes at int-m locus) (D) of the crosses of int-m.85 x dub1.3 showing the formation of a TS and de-suppressed RSMs. (E-G) Genotyping of the F1 hybrids (E, F) and F2 plants (G) of the crosses of int-m.85 x dub1.3. The int-m.85 allele was diagnosed by HPY188I restriction enzyme digest that the allele was cleaved into two smaller pieces while other alleles (e.g. dub1.3, Bowman and int-m.1a) were resistant to the digest. (E) The crosses between dub1.1 x int-m.85 were not successful. In addition, the dub1.1 mutant appears to have lost the genomic segment containing the HvAP2L-H5 gene (See the following resequencing section) as we could not amplify the gene using several pairs of primers (SI Appendix Table S5) from different parts of the gene (only showed one example here) and HvLOG and HvKN1 were used as positive controls (E). The two glumes of the terminal spikelet are highlighted with green pseudo-color. Note – dub1.1, M1 mutant of dub1; dub1.3, M3 of dub1; and dub1.7, M7 of dub1.

Fig. S3. Overview of mapping quality of RNA-seq of the two int-m NIL mutants and the wildtype Bowman and Bonus. The barley reference Morex genome was used as the reference and variants were called and filtered using GATK. The tracks
showed the total high-confidence (HC) genes in the reference, passed SNP (PASS SNP) filtered by GATK and the corresponding alleles (PASS Allele).

**Fig. S4.** Introgression mapping using the barley high-confidence coding sequences as the reference (CDS-based variant calling). (A) Schematic view of the introgressed regions across different chromosomes. (B) Common introgressed region of the two int-m mutants on the chromosome 5. (C) Total introgressed genes in each of the two int-m mutants and the number of shared introgressed genes of the two int-m mutants.

**Fig. S5.** Introgression mapping using the barley Morex genome as the reference (genome-based variants) and annotation using barley high-confidence (HC) reference coding sequences. (A) Schematic view of the introgressed regions across different chromosomes. (B) Common introgressed region of the two int-m mutants on the chromosome 5H. (C) Total introgressed genes in each of the two int-m mutants and the number of shared introgressed genes of the two int-m mutants. (D) Only one candidate gene contains (HvAP2L-H5) mutant-specific mutations in the two int-m mutants. (E) Annotation of the identified SNPs using the gene models in the barley high-confidence coding sequences. Using genome-based variant calling method could identify much more SNPs as a large proportion of SNPs were found in the annotated INTRON region with the HC gene models (likely due to alternative splicing, as only one transcript is kept for each gene in the HC CDS sequences), and untranslated regions.

**Fig. S6.** Cosegregation analysis of a segregating F3 population derived from a cross between int-m.85 (NIL) and Proctor. We phenotyped and genotyped 158 plants in an F3 segregating population, and found all and only 36 plants had mutant phenotype and homozygous recessive int-m.85 alleles. Here we only show an exemplary agarose-gel image after HPY188I digest. Abbreviations: D, dominant homozygote; H, heterozygote; r, recessive homozygote; x, no DNA template or PCR not working.

**Fig. S7.** double seed1 (dub1) mutations convert a barley indeterminate inflorescence to a wheat-like determinate inflorescence. All ten types of dub1 mutations (SI Appendix Table S1) have determinate inflorescences terminated by a terminal spikelet (A-J). Some dub1 mutants also showed de-suppression of RSMs that might results in production of extra florets in spikelets. (M) Indeterminate spikes at FM or StP stages were observed for some mutants. This observation suggests that the int-m/dub1 mutant phenotypes (TS formation and RSM de-suppression) seem to be influenced by genetic background and growth conditions. For instance, the timing of TS formation varied between mutants or batches so that some mutants produce a TS after FM/StP stage (M6, M10, M11, M19 and M21). In addition, their effects on RSM de-suppression appear to differ among different mutants, with dub1.3 being most prominent. However, this might result from the sampling bias as only 1-3 spikes were examined by SEM for the other mutants. Furthermore, the floral differentiation in the TS is not so advanced as in int-m NIL mutants. The wildtypes Bonus (K) and Foma (L) maintain active IMs; however, the wildtype Kristina (the parental line for M13, M15, M18a and M18b) was not examined due to the lack of material. Detailed developmental time series of three selected mutants and the corresponding wildtype background are shown in SI Appendix Fig. S2. (N) Mature spikes of several dub1 mutants compared with (O)
Fig. S8. Comparative transcriptomic profiling of developing inflorescences in int-m NIL mutants and Bowman. (A) Number of differentially expressed genes in int-m mutants compared to the wildtype Bowman at each developmental stage (D12 (DR), D16 (TM-SM) and D21(StP)). Particularly, pairwise comparisons between each of the int-m mutants and the wildtype Bowman are shown in black dots and common changes in the two focal int-m NIL mutants are connected with black lines in the lower subfigure. (B) Gene Ontology (GO) Enrichment analysis of misregulated genes at early development stages before floral organ differentiation (DR and TM-SM).

Fig. S9. INT-M/HvAP2L-H5 suppresses spikelet/floral differentiation. (A) HvAP2L-H5 is localized in IMs, SMs (RSMs), lemmas, paleae of inflorescences in the wildtype Bowman. (B) The expression patterns of HvAP2L-H5 and AG-like MADS-box genes HvMADS3 and HvMADS58 are mutually exclusive, with the latter being localized in stamens and pistils in Bowman at the stamen primordium (StP) stage. (C) HvMADS58, similar to HvMADS3 (Fig. 3B), is ectopically and precociously expressed in int-m mutants. Abbreviations: spikelet ridge, SPR; leaf ridge LR. (D) HvMADS1 shows partly overlapping expression patterns as HvAP2L-H5 in lemmas, paleae and RSMs in Bowman (shown in consecutive sections). In the IM, transcripts of HvMADS1 are not detectable before the triple-mound-spikelet meristem (TM-SM) stage (Fig. 3B), but become clearly detectable during the StP stage.

Fig. S10. Expression patterns of putative inflorescence meristem (IM) identity genes. (A, A’) HvLFY is significantly downregulated at the TM-SM stage in int-m mutants and (A’) localized in emerging tillers and vegetative meristems (VMs), IMs, leaf ridges (LRs), SMs and FMs. (B) The expression of an HvLFY co-factor HvAPO1 is not significantly altered at the DR or TM-SM stages in int-m mutants and (B’) restricted in the emerging tillers, VMs, IMs and FMs, but not in TMMs or SMs. (C) A SEP-like MADS-box gene HvMADS34 is significantly downregulated in int-m mutants, and (C’) confined to VMs, IMs, SMs and lemmas. (D-F, D’-F’, E’-F”) Three AP1/FUL-like genes are all expressed in VMs, IMs, TMMs, SMs, FMs and floral organs during inflorescence development in the wildtype Bowman and int-m mutants. We used two different probes, one in the coding region and the other in 3’ UTR for HvMADS15/HvFUL2 (E’, E”) and HvMADS18 (F’, F”). The images highlighted with fuchsia squares are also shown in Fig. 3. The significant changes between the mutants and Bowman were marked with asterisks. The detailed expression levels and statistical tests were also included in the SI Appendix Dataset S3.

Fig. S11. HvAP2L-H5 maintains active shoot apical meristems (SAMs). (A) Expression of putative meristem maintenance genes in the wildtype Bowman and int-m mutants during vegetative meristem (VM), double ridge (DR) and triple mound-spikelet meristem (TM-SM) stages. Meristem genes HvWOX2, HvWOX7, HvERL, HvLOG and HvKN1 are transcribed in various meristems with distinct patterns in Bowman. Specifically, HvERL, HvWOX2 and HvWOX7 are localized in precursors of (i.e. emerging) lateral primordia (pLPs) in VMs, IMs and lateral primordia (LPs, producing leaf ridges (LRs) and triple mound meristems (TMMs)).
Moreover, HvWOX2 transcripts are subsequently detected in LRs and TMMs, HvWOX7 is found in TMMs and HvERL is restricted in LRs. HvLOG is exclusively detected in the L1 epidermal cells of VM, IM, TSM and TMM, whereas HvKN1 is found in the non-L1 epidermal meristem cells. These expression patterns suggest their potential roles in SAM maintenance and/or initiation of lateral organs of the developing shoot apices. Furthermore, all these genes are expressed before and during the initiation of the terminal spikelets (TS) in int-m mutants in VMs/plPs/LPs/LRs, and TSMs or emerging lemmas of the TSs, hinting at their pleiotropic functions in the TS formation. However, unlike MADS-box genes (Fig. S9), no obvious spatial or temporal expression of these genes were detected prior to or concurrent with the TSM initiation in int-m mutants compared to that in Bowman. (B, C) Front view of the SAM in Bowman for staging and determination of tissue types of sections for in situ mRNA hybridization. (A, B, C) Same scale bars are used. (B) Representative SEM micrographs and (C) digital sections using confocal microscopy. (D) The width of the presumptive SAM in Bowman is generally wider than that of int-m mutants after DR. The images highlighted with fuchsia squares are also shown in Fig. 3.

Fig. S12. Evolution of inflorescence (in)determinacy in Triticeae. (A) Reconstruction of ancestral character state of inflorescence determinacy in Triticeae inferred that determinate inflorescence in most Triticeae species is ancestral and indeterminacy in Hordeum is derived. (B-G) Representative SEM micrographs of the developing spikes, showing the identity shift of the shoot apical meristems (SAMs) from an inflorescence meristem (IM) to a terminal spikelet meristem (TSM) between the DR and TM-SM stages in most examined Triticeae species and between the TM-SM and FM stages in Einkorn (Triticum monococcum). Hordeum chilense maintains an active SAM/IM over development and has an indeterminate IM.

Fig. S13. Comparison of expression patterns among AP2L-5 orthologs in Brachypodium distachyon (BdIDS1), Triticum monococcum (Einkorn, TmAP2L-A5) and barley (HvAP2L-H5). Expression of BdIDS1 (A), Einkorn TmAP2L-A5 (B) and barley HvAP2L-H5 (C) in inflorescence meristems (IMs), spikelet meristems (SMs), lemmas, and/or abscission zones (AZs). Abbreviations: TS, terminal spikelet; NTC, non-terminal spikelet; LR, leaf ridge; TMM, triple mound meristem; TSM, terminal spikelet meristem; VM, vegetative shoot apical meristem. (D) Quantification of HvAP2L-H5 and TmAP2L-A5 between barley and Einkorn during DR (6 biological replicates) and TM-SM stages (7 biological replicates). The images in with fuchsia squares are also shown in Fig. 4. No significant (DR, p = 0.5445; TM-SM, p = 0.2839) changes were found between Bowman and Einkorn.

Fig. S14. Analysis of promoter sequences of AP2L-5 orthologs. (A) mVISTA plots of pairwise comparisons of different Triticeae species and barley (Morex). Graph illustrates a 100 bp sliding window comparison and conservation of more than 70% with a minimum width of 50 bp is colored. AP2L-5 orthologs in Triticum urartu, Aegilops tauschii, the hexaploid wheat A subgenome and Thinopyrum elongatum are in the opposite direction (-) relative to those in Hordeum spp, while the directions of AP2L-5 orthologs in wild barley and Hordeum pubiflorum were not determined due to incomplete genome information. (B) Pairwise alignment of AP2L-5 orthologs in barley and wheat showing accessible chromatin regions identified by barley ATAC-seq (47) (C) and wheat DNase-seq (48) (D). All plots
were aligned according to the genomic coding regions of the gene (blue block). Conserved accessible chromatin regions (red) are identified between species and appear to partly overlap with several conserved blocks identified by mVISTA, while green blocks represent putative lineage-specific accessible chromatin regions and more highly variable regions between barley and wheat.

Fig. S15. Synteny analysis of the sister paralog of IDS1 (SID) clade. (A) SID orthologs of rice and Brachypodium are connected with a red line. The SID-containing block in Brachypodium (a shaded pinked rectangle) is evidently syntenic to a region on the rice chromosome 7. (A–C) However, this SID-containing block (shaded in pink) has no syntenic regions in three Triticeae genomes, i.e. Triticum urartu (urartu) (A), barley (B), and Aegilops tauschii (C). Several segments from different chromosomes are found in the non-syntenic region in urartu, suggesting genomic rearrangements in this block and likely loss of the SID in the sequenced Triticeae genomes as also suggested by blastn-based searches.

Fig. S16. Expression patterns of MADS1 correlate with the TSM initiation in Einkorn, Kronos and Dasypyrum villosum. (A) Expression profiles of MADS1 homeologs in hexaploid wheat from a reanalysis of a published RNA-seq study (79). (B) MADS1 transcripts were first detected in spikelet meristem (SM) and lemma primordium between the SM and FM stages, subsequently localized in lemmas and meristems (TSMs) of the emerging terminal spikelet (TS) at FM and StP stages in Einkorn (B). (C–D) MADS1 expression in Kronos wildtype and ap2l-A5 mutants (C) and Dasypyrum villosum (D). (E–G) Expression patterns of meristem genes using in situ hybridization. Specifically, (E) TmLOG, (F) TmWOX2 (consecutive sections of a single sample were marked by a rectangle) and (G) TmKN1 showed comparable expression to their respective orthologs in Bowman/int-m mutants (SI Appendix Fig. S11).

Fig. S17. Expression of AGAMOUS(AG)-like genes. (A–C) Expression of AG-like genes MADS3 and MADS58 in Bowman/int-m mutants (A; RNA-seq), hexaploid wheat (B; RNA-seq), and Einkorn (C; qRT-PCR). MADS3 transcripts are detectable at double-ridge (DR) stage in Bowman/int-m mutants (A), hexaploid wheat (B) and Einkorn (C). However, early expression of AG-like MADS58 is only found in int-m mutants but not in wheat (B) or Einkorn (C). Orthologous relationships of barley and wheat genes are inferred using the program OrthoFinder with eleven published grass genomes. The expression profiles of wheat AG-like genes are from a reanalysis of a published wheat transcriptomic study (79). At least 3 biological replicates are used for each stage of each species/genotype. For HvMADS3, significant differences (***p < 0.001) are found between Bowman and Einkorn using the Student’s t-test and between Bowman and the int-m mutants using the Dunnett’s test. (D) Transcripts of TmMADS3 are detected in spikelet ridges (SPRs) at DR stage, and spikelet meristems (SMs) at the spikelet meristem (SM) stage before the initiation of a terminal spikelet meristem (TSM). TmMADS3 is expressed in SMs and emerging TSM at the floret meristem (FM) stage, and strongly in stamens at the StP stage. (E) MADS3 show early expression in Einkorn and Kronos and ectopic expression in ap2l-A5 mutants (Kronos background). Einkorn, Kronos and ap2l-A5 mutants differ in the gene AP2L-A5. Einkorn has a wildtype AP2L-A5, Kronos has miR172-resistant AP2L-A5 alleles, and ap2l-A5 mutants have null mutations in AP2L-A5. MADS3
shows precocious and ectopic expression in the \textit{ap2l}-A5 mutants as its barley ortholog in \textit{int-m} mutants.

\textbf{Fig. S18.} Significantly misregulated MADS-box genes during the stamen primordium (StP) stage. (A-C) Downregulated MADS-box genes in \textit{int-m} mutants, including SOC-like genes in (A), E-class SEP-like genes in (B) and C-class MADS-box genes in (C). (D-E) Upregulated MADS-box genes in \textit{int-m} mutants, including AGL6-like genes in (D) and SVP-like genes in (E).

\textbf{Fig. S19.} Evolution of \textit{HvAP2L-H5} genes. (A) \textit{HvAP2L-H5} is syntenic to maize \textit{INDETERMINATE SPIKELET1 (IDS1)}, \textit{Brachypodium BdIDS1}, \textit{rice OsIDS1} and wheat \textit{Q}. (B) Diversity analysis of \textit{HvAP2L-H5} using a published diversity panel of 229 barley germplasms (66) revealed additional non-synonymous mutations.

\textbf{Fig. S20.} Specificity test of the probes used in this study. We tested specificity for antisense (AS) probes using negative controls, including a whole-gene deletion mutant \textit{dub1.1} for \textit{HvAP2L-H5} in (A) and sense probes for \textit{TmAP2L-A5 (B)}, \textit{HvMADS1 (C)}, \textit{HvMADS3 (D)}, \textit{HvMADS58 (E)}, \textit{TmMADS3 (F)}, \textit{HvLOG (G)}, \textit{HvCKX (H)}, \textit{HvKN1 (I)}, \textit{HvWOX7 (J)}, \textit{HvWOX2 (K)}, \textit{HvBAM (L)}, \textit{HvERL (M)}, \textit{TmWOX2 (N)} and \textit{HvLFY (O)}, \textit{HvAPO1 (P)}, \textit{HvMADS34 (Q)}, \textit{HvVRN1 (R)}, \textit{HvMADS15 (S)} and \textit{HvMADS18 (T)}. 
Fig. S1. Phenotyping of the int-m NIL mutants. (A-B) Developmental series of int-m.85 spike (BW429, int-m.85*BC7 in Bowman) showing the formation of a multifloreted terminal spikelet (TS). The two glumes of the TS are highlighted with green pseudo-color. (B)-(D) int-m NIL mutants form a multifloreted terminal spikelet. The two basal sterile bracts (marked with green dots), subtending a TS that bears multiple florets (multiple pairs of lemmas/paleae), are morphologically similar to glumes of central spikelets (CSs; pointed with green arrows) that have short awns in the distal ends. Sterile bracts that develop into lemma-like organs in the TS are labeled with pink asterisks. (D) Dissected parts of multiple TSs from glumes to tip were arranged from left to right or from bottom to top in each panel, and glumes of a CS was included in the leftmost/bottommost in each panel for comparison. The upper non-basal sterile bracts (marked in pink asterisks in B-D) in the TS produce extensively elongated awns in the distal tips and are phenotypically lemma-like. (E) A representative image showing expression patterns of HvMADS7 in lemmas but not in glumes (consecutive sections were used to determine the identity the tissue-specific expression patterns). TMM, triple mound meristem (a.k.a. SPR, spikelet ridge); LR, leaf ridge. (F) Developmental series of the wildtype Bowman showing the RSMs. The single floret per spikelet initiates in the adaxial side, while SMs are suppressed in the abaxial side as residual SMs (RSMs). (G) De-suppression of residual SMs (RSMs) and initiation of extra florets in some CSs (circled with dotted lines) in int-m.1a and int-m.85 NIL mutants. (H) These extra florets may often become sterile or occasionally develop into sessile grains in mature spikes, suggesting that the rachillae (spikelet axis) are not de-suppressed. (I) Two exemplary multi-floreted terminal spikelets showing the phenotypical similarities and perpendicular orientation of glumes of TS and CSs. (J) The length of the whole inflorescences in int-m mutants is reduced compared to that of the wildtype Bowman. Analysis of covariance is used to compare the predicted regression lines of spike length vs days after emergence (DAE) of Bowman, int-m.1a and int-m.85 (N=449). No significant difference (p = 0.9925) is found between int-m.1a and int-m.85 mutants, while the spike length in Bowman is significantly longer than that in each int-m mutant (p < 0.0001). The images highlighted with fuchsia squares are also shown in Fig. 1.
Fig. S2. Phenotypic and genetic analyses of int-m and dub1 mutants. (A-B) int-m and dub1 mutants are morphologically similar, producing a terminal spikelet (A and B), which seems to emerge no later than the TM-SM stage, and multi-floreted spikelets (B, dashed circles). (C-G) dub1.3 is allelic to int-m.85. All F1 (5) and F2 (68) plants of the crosses of int-m.85 x dub1.3 form a terminal spikelet as the parental donors. Representative SEM images of an F1 (#4) out of five hybrids (C) and three F2 plants (different genotypes at int-m locus) (D) of the crosses of int-m.85 x dub1.3 showing the formation of a TS and de-suppressed RSMs. (E-G) Genotyping of the F1 hybrids (E, F) and F2 plants (G) of the crosses of int-m.85 x dub1.3. The int-m.85 allele was diagnosed by HPY188I restriction enzyme digest that the allele was cleaved into two smaller pieces while other alleles (e.g. dub1.3, Bowman and int-m.1a) were resistant to the digest. (E) The crosses between dub1.1 x int-m.85 were not successful. In addition, the dub1.1 mutant appears to have lost the genomic segment containing the HvAP2L-H5 gene (See the following resequencing section) as we could not amplify the gene using several pairs of primers (SI Appendix Table S5) from different parts of the gene (only showed one example here) and HvLOG and HvKVN1 were used as positive controls (E). The two glumes of the terminal spikelet are highlighted with green pseudo-color. Note – dub1.1, M1 mutant of dub1; dub1.3, M3 of dub1; and dub1.7, M7 of dub1.
**Fig. S3.** Overview of mapping quality of RNA-seq of the two int-m NIL mutants and the wildtype Bowman and Bonus. The barley reference Morex genome was used as the reference and variants were called and filtered using GATK. The tracks showed the total high-confidence (HC) genes in the reference, passed SNP (PASS SNP) filtered by GATK and the corresponding alleles (PASS Allele).
Fig. S4. Introgression mapping using the barley high-confidence coding sequences as the reference (CDS-based variant calling). (A) Schematic view of the introgressed regions across different chromosomes. (B) Common introgressed region of the two int-m mutants on the chromosome 5. (C) Total introgressed genes in each of the two int-m mutants and the number of shared introgressed genes of the two int-m mutants.
Fig. S5. Introgression mapping using the barley Morex genome as the reference (genome-based variants) and annotation using barley high-confidence (HC) reference coding sequences. (A) Schematic view of the introgressed regions across different chromosomes. (B) Common introgressed region of the two int-m mutants on the chromosome 5H. (C) Total introgressed genes in each of the two int-m mutants and the number of shared introgressed genes of the two int-m mutants. (D) Only one candidate gene contains (HvAP2L-H5) mutant-specific mutations in the two int-m mutants. (E) Annotation of the identified SNPs using the gene models in the barley high-confidence coding sequences. Using genome-based variant calling method could identify much more SNPs as a large proportion of SNPs were found in the annotated INTRON region with the HC gene models (likely due to alternative splicing, as only one transcript is kept for each gene in the HC CDS sequences), and untranslated regions.
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Fig. S8. Comparative transcriptomic profiling of developing inflorescences in int-m NIL mutants and Bowman. (A) Number of differentially expressed genes in int-m mutants compared to the wildtype Bowman at each developmental stage (D12 (DR), D16 (TM-SM) and D21(StP)). Particularly, pairwise comparisons between each of the int-m mutants and the wildtype Bowman are shown in black dots and common changes in the two focal int-m NIL mutants are connected with black lines in the lower subfigure. (B) Gene Ontology (GO) Enrichment analysis of misregulated genes at early development stages before floral organ differentiation (DR and TM-SM).
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Fig. S12. Evolution of inflorescence (in)determinacy in Triticeae. (A) Reconstruction of ancestral character state of inflorescence determinacy in Triticeae inferred that determinate inflorescence in most Triticeae species is ancestral and indeterminacy in *Hordeum* is derived. (B-H) Representative SEM micrographs of the developing spikes, showing the identity shift of the shoot apical meristems (SAMs) from an inflorescence meristem (IM) to a terminal spikelet meristem (TSM) between the DR and TM-SM stages in most examined Triticeae species and between the TM-SM and FM stages in Einkorn (*Triticum monococcum*). *Hordeum chilense* maintains an active SAM/IM over development and has an indeterminate IM.
Fig. S13. Comparison of expression patterns among AP2L-5 orthologs in *Brachypodium distachyon* (*BdIDS1*), *Triticum monococcum* (Einkorn, *TmAP2L-A5*) and barley (*HvAP2L-H5*). Expression of *BdIDS1* (A), Einkorn *TmAP2L-A5* (B) and barley *HvAP2L-H5* (C) in inflorescence meristems (IMs), spikelet meristems (SMs), lemmas, and/or abscission zones (AZs). Abbreviations: TS, terminal spikelet; NTC, non-terminal spikelet; LR, leaf ridge; TMM, triple mound meristem; TSM, terminal spikelet meristem; VM, vegetative shoot apical meristem. (D) Quantification of *HvAP2L-H5* and *TmAP2L-A5* between barley and Einkorn during DR (6 biological replicates) and TM-SM stages (7 biological replicates). The images in with fuchsia squares are also shown in Fig. 4. No significant (DR, $p = 0.5445$; TM-SM, $p = 0.2839$) changes were found between Bowman and Einkorn.
**Fig. S14.** Analysis of promoter sequences of AP2L-5 orthologs. (A) mVISTA plots of pairwise comparisons of different Triticeae species and barley (Morex). Graph illustrates a 100 bp sliding window comparison and conservation of more than 70% with a minimum width of 50 bp is colored. AP2L-5 orthologs in Triticum urartu, Aegilops tauschii, the hexaploid wheat A subgenome and Thinopyrum elongatum are in the opposite direction (-) relative to those in Hordeum spp, while the directions of AP2L-5 orthologs in wild barley and Hordeum pubiflorum were not determined due to incomplete genome information. (B) Pairwise alignment of AP2L-5 orthologs in barley and wheat showing accessible chromatin regions identified by barley ATAC-seq (37) (C) and wheat DNase-seq (38) (D). All plots were aligned according to the genomic coding regions of the gene (blue block). Conserved accessible chromatin regions (red) are identified between species and appear to partly overlap with several conserved blocks identified by mVISTA, while green blocks represent putative lineage-specific accessible chromatin regions and more highly variable regions between barley and wheat.
Fig. S15. Synteny analysis of the *sister paralog of IDS1* (*SID*) clade. (A) *SID* orthologs of rice and *Brachypodium* are connected with a red line. The *SID*-containing block in *Brachypodium* (a shaded pinked rectangle) is evidently syntenic to a region on the rice chromosome 7. (A-C) However, this *SID*-containing block (shaded in pink) has no syntenic regions in three Triticeae genomes, i.e. *Triticum urartu* (*urartu*) (A), barley (B), and *Aegilops tauschii* (C). Several segments from different chromosomes are found in the non-syntenic region in urartu, suggesting great genomic rearrangements in this block and likely loss of the *SID* in the sequenced Triticeae genomes as suggested by blastn-based searches.
**A** *MADS1* in bread wheat

**B** *TmMADS1*

**C** *MADS1*

**D** *DvMADS1*

**E** *TmLOG*

**F** *TmWOX2*

**G** *TmKN1*

**Fig. S16.** Expression patterns of *MADS1* correlate with the TSM initiation in Einkorn, Kronos and *Dasypyrum villosum*. (A) Expression profiles of *MADS1* homeologs in hexaploid wheat from a reanalysis of a published RNA-seq study (79). (B) *MADS1* transcripts were first detected in spikelet meristem (SM) and lemma primordium between the SM and FM stages, subsequently localized in lemmas and meristems (TSMs) of the emerging terminal spikelet (TS) at FM and StP stages in Einkorn (B). (C-D) *MADS1* expression in Kronos wildtype and *ap2l-A5* mutants (C) and *Dasypyrum villosum* (D). (E-G) Expression patterns of meristem genes using *in situ* hybridization. Specifically, (E) *TmLOG*, (F) *TmWOX2* (consecutive sections of a single sample were marked by a rectangle) and (G) *TmKN1* showed comparable expression to their respective orthologs in Bowman/int-m mutants ([SI Appendix](#) Fig. S11).
Fig. S17. Expression of AGAMOUS(AG)-like genes. (A-C) Expression of AG-like genes MADS3 and MADS58 in Bowman/int-m mutants (A; RNA-seq), hexaploid wheat (B; RNA-seq), and Einkorn (C; qRT-PCR). MADS3 transcripts are detectable at double-ridge (DR) stage in Bowman/int-m mutants (A), hexaploid wheat (B) and Einkorn (C). However, early expression of AG-like MADS58 is only found in int-m mutants but not in wheat (B) or Einkorn (C). Orthologous relationships of barley and wheat genes are inferred using the program OrthoFinder with eleven published grass genomes. The expression profiles of wheat AG-like genes are from a reanalysis of a published wheat transcriptomic study (79). At least 3 biological replicates are used for each stage of each species/genotype. For HvMADS3, significant differences (**p < 0.001) are found between Bowman and Einkorn using the Student's t-test and between Bowman and the int-m mutants using the Dunnett's test. (D) Transcripts of TmMADS3 are detected in spikelet ridges (SPRs) at DR stage, and spikelet meristems (SMs) at the spikelet meristem (SM) stage before the initiation of a terminal spikelet meristem (TSM). TmMADS3 is expressed in SMs and emerging TSM at the floret meristem (FM) stage, and strongly in stamens at the StP stage. (E) MADS3 show early expression in Einkorn and Kronos and ectopic expression in ap2l-A5 mutants (Kronos background). Einkorn, Kronos and ap2l-A5 mutants differ in the gene AP2L-A5. Einkorn has a wildtype AP2L-A5, Kronos has miR172-resistant AP2L-A5 alleles, and ap2l-A5 mutants have null mutations in AP2L-A5. MADS3 shows precocious and ectopic expression in the ap2l-A5 mutants as its barley ortholog in int-m mutants.
Fig. S18. Significantly misregulated MADS-box genes during the stamen primordium (StP) stage. (A-C) Downregulated MADS-box genes in int-m mutants, including SOC-like genes in (A), E-class SEP-like genes in (B) and C-class MADS-box genes in (C). (D-E) Upregulated MADS-box genes in int-m mutants, including AGL6-like genes in (D) and SVP-like genes in (E).
Fig. S19. Evolution of *HvAP2L-H5* genes. (A) *HvAP2L-H5* is syntenic to maize 
*INDETERMINATE SPIKELETT1* (*IDS1*), *Brachypodium BdIDS1*, rice *OsIDS1* and 
wheat *Q*. (B) Diversity analysis of *HvAP2L-H5* using a published diversity panel of 
229 barley germplasms (21) revealed additional non-synonymous mutations.
Fig. S20. Specificity test of the probes used in this study. We tested specificity for antisense (AS) probes using negative controls, including a whole-gene deletion mutant *dub1.1* for *HvAP2L-H5* in (A) and sense probes for *TmAP2L-A5* (B), *HvMADS1* (C), *HvMADS3* (D), *HvMADS58* (E), *TmMADS3* (F), *HvLOG* (G), *HvCKX* (H), *HvKN1* (I), *HvWOX7* (J), *HvWOX2* (K), *HvBAM* (L), *HvERL* (M), *TmWOX2* (N) and *HvLFY* (O), *HvAPO1* (P), *HvMADS34* (Q), *HvVRN1* (R), *HvMADS15* (S) and *HvMADS18* (T).
| MUTANT TYPE               | Accession   | Background | Protein change | PROVEAN score | Prediction (cutoff=-2.5) |
|--------------------------|-------------|------------|----------------|---------------|-------------------------|
| *intermedium-m* 1a       | GSHO1773    | Lamont     | Q138L          | -6.489        | Deleterious             |
| *double seed1* M24       | NGB114354   | Bonus      | G108E          | -7.396        | Deleterious             |
| *double seed1* M25       | NGB114355   | Bonus      | G108E          | -7.396        | Deleterious             |
| *double seed1* M3        | NGB114333   | Foma       | Splicing       | NA            | NA                      |
| *double seed1* M4        | NGB114334   | Foma       | Splicing       | NA            | NA                      |
| *double seed1* M5        | NGB114335   | Foma       | Splicing       | NA            | NA                      |
| *double seed1* M19       | NGB114348   | Kristina   | Splicing       | NA            | NA                      |
| *double seed1* M21       | NGB114351   | Bonus      | Splicing       | NA            | NA                      |
| *double seed1* M6        | NGB114336   | Foma       | G142D          | -6.337        | Deleterious             |
| *double seed1* M7        | NGB114337   | Foma       | G142D          | -6.337        | Deleterious             |
| *double seed1* M8        | NGB114338   | Foma       | G142D          | -6.337        | Deleterious             |
| *double seed1* M10       | NGB114340   | Foma       | G142D          | -6.337        | Deleterious             |
| *double seed1* M11       | NGB114341   | Foma       | G142D          | -6.337        | Deleterious             |
| *double seed1* M12       | NGB114342   | Bonus      | G142D          | -6.337        | Deleterious             |
| *intermedium-m*.85       | GSHO 1772   | Bonus      | G142D          | -6.337        | Deleterious             |
| *double seed1* M13       | NGB114343   | Kristina   | A150V          | -3.822        | Deleterious             |
| *double seed1* M15       | NGB114344   | Kristina   | A150V          | -3.822        | Deleterious             |
| *double seed1* M22       | NGB114352   | Bonus      | Splicing       | NA            | NA                      |
| *double seed1* M28       | NGB114358   | Bonus      | Splicing       | NA            | NA                      |
| *double seed1* M26       | NGB114356   | Bonus      | R162W          | -7.926        | Deleterious             |
| *double seed1* M18a      | NGB114345   | Kristina   | R162W          | -7.926        | Deleterious             |
| *double seed1* M18b      | NGB114347   | Kristina   | R162W          | -7.926        | Deleterious             |
| *double seed1* M20       | NGB114350   | Bonus      | Splicing       | NA            | NA                      |
| *double seed1* M1        | NGB114331   | Bonus      | DELETION       | NA            | NA                      |
| *double seed1* M2        | NGB114332   | Bonus      | DELETION       | NA            | NA                      |
| Species       | Accession | Source                  |
|--------------|-----------|-------------------------|
| *Triticum turgidum* | Kronos         | Debernardi et al. 2017  |
| *Triticum turgidum* | T4-2726       | Debernardi et al. 2017  |
| *Triticum turgidum* | T4-2992       | Debernardi et al. 2017  |
| *Triticum turgidum* | T4-3946       | Debernardi et al. 2017  |
| *Triticum turgidum* | JD359 #21-2  | Debernardi et al. 2017  |
| *Triticum turgidum* | JD359 #18-1  | Debernardi et al. 2017  |
| *Triticum turgidum* | JD360#4, 3946 BC3F4 | Debernardi et al. 2017  |
| *Triticum turgidum* | MiM172        | Debernardi et al. 2017  |
| *Triticum turgidum* | JD360#8, MiM172 | Debernardi et al. 2017  |

**Triticeae species**

| Species                  | Accession |
|--------------------------|-----------|
| *Australopyrum retrofractum* | PI53013   |
| *Thinopyrum*             | PI531711  |
| *Dasypyrum brevialistratum* | PI516547  |
| *D. villosum*            | PI470279  |
| *Eremopyrum distans*     | PI193264  |
| *E. triticum*            | PI502364  |
| *Henrardia persica*      | PI577112  |
| *Heterantherium piliferum* | PI401354  |
| *Pseudoroegneria gracillima* | PI440000  |
| *P. stipifolia*          | PI325181  |
| *Triticum monococcum*    | PI167615  |
| *Hordeum chilense*       | NGB90119  |
Table S2. Statistical comparisons of meristem size between barley and *int-m* mutants.

| Stage | Comparison              | diff   | lwr.ci | upr.ci   | pval     | Significance | Method |
|-------|-------------------------|--------|--------|----------|----------|--------------|---------|
|       | Length: *int-m* mutants vs Bowman |        |        |          |          |              |         |
| VM    | int-m.1a-Bowman          | 19.67257 | 10.342185 | 29.00296 | 2.5E-05 *** | Dunnett     |         |
| VM    | int-m.85-Bowman          | 4.32516  | -5.326815 | 13.97714 | 0.5019 na    | Dunnett     |         |
| DR    | int-m.1a-Bowman          | -48.91553 | -58.373 | -39.45806 | 1.1E-15 *** | Dunnett     |         |
| DR    | int-m.85-Bowman          | -47.38025 | -57.57676 | -37.18373 | 3.6E-14 *** | Dunnett     |         |
| TM-SM | int-m.1a-Bowman          | -55.83219 | -65.2759 | -46.38848 | 2E-16 ***   | Dunnett     |         |
| TM-SM | int-m.85-Bowman          | -55.36535 | -63.51514 | -47.21556 | 2.2E-16 *** | Dunnett     |         |
| FM    | int-m.1a-Bowman          | -62.73802 | -71.06998 | -54.40605 | 2E-16 ***   | Dunnett     |         |
| FM    | int-m.85-Bowman          | -59.03387 | -68.99247 | -49.07526 | 2.2E-16 *** | Dunnett     |         |
| StP   | int-m.1a-Bowman          | -58.24494 | -67.11249 | -49.37738 | 2.2E-16 *** | Dunnett     |         |
| StP   | int-m.85-Bowman          | -53.28124 | -61.9502  | -44.61227 | 2.2E-16 *** | Dunnett     |         |
|       | Width: *int-m* mutants vs Bowman |        |        |          |          |              |         |
| VM    | int-m.1a-Bowman          | 22.637966 | 16.475085 | 28.800848 | 4.9E-11 *** | Dunnett     |         |
| VM    | int-m.85-Bowman          | 2.530959  | -3.844338 | 8.906257 | 0.5784 na    | Dunnett     |         |
| DR    | int-m.1a-Bowman          | -8.203963 | -14.68091 | -1.727016 | 0.0109 *    | Dunnett     |         |
| DR    | int-m.85-Bowman          | -10.705263 | -17.68834 | -3.722185 | 0.0019 **   | Dunnett     |         |
| TM-SM | int-m.1a-Bowman          | -22.27824 | -28.42289 | -16.13359 | 8.5E-12 *** | Dunnett     |         |
| TM-SM | int-m.85-Bowman          | -23.32132 | -28.62407 | -18.01857 | 4E-15 ***   | Dunnett     |         |
| FM    | int-m.1a-Bowman          | -25.0618  | -29.60162 | -20.52198 | 8.9E-16 *** | Dunnett     |         |
| FM    | int-m.85-Bowman          | -24.7941  | -30.22022 | -19.36798 | 5.6E-13 *** | Dunnett     |         |
| StP   | int-m.1a-Bowman          | -27.39963 | -33.51091 | -21.28836 | 3.5E-14 *** | Dunnett     |         |
| StP   | int-m.85-Bowman          | -20.70323 | -26.67764 | -14.72882 | 2.2E-10 *** | Dunnett     |         |
|       | Cross stages: Bowman_SAM_length |        |        |          |          |              |         |
| DR-VEG |                      | 31.0479268 | 20.2236776 | 41.872176 | 0 ***      | Tucky       |         |
| SPP-TM-DR |              | -20.4433389 | -31.0699158 | -9.8167621 | 5.9E-06 *** | Tucky       |         |
| FP-SPP-TM |                | -4.3229733 | -15.7262121 | 7.08026540  | 8.293187 na | Tucky       |         |
| StP-FP |                        | -7.1735298 | -19.2330737 | 4.88601410  | 0.4672672 na | Tucky       |         |
| Stage          | Comparison          | diff       | lwr.ci         | upr.ci         | pval         | Significance | Method |
|---------------|---------------------|------------|----------------|----------------|--------------|--------------|---------|
| Cross stages: int-m.1a_SAM_length | DR-VEG              | -37.54017  | -48.62991      | -26.4504299   | 0***         | Tucky        |         |
|               | SPP-TM-DR           | -27.36     | -38.15396      | -16.5660374   | 0***         | Tucky        |         |
|               | FP-SPP-TM           | -11.2288   | -22.02276      | -0.4348374    | 0.0372543**  | Tucky        |         |
|               | StP-FP              | -2.68045   | -13.47441      | 8.1135126     | 0.9580061    | Tucky        |         |
| Cross stages: int-m.85_SAM_length | DR-VEG              | -20.657479 | -34.20002      | -7.1149335    | 0.0004855*** | Tucky        |         |
|               | SPP-TM-DR           | -28.428441 | -39.96245      | -16.894433    | 0***         | Tucky        |         |
|               | FP-SPP-TM           | -7.991492  | -21.4214       | 5.4384196     | 0.466744na   | Tucky        |         |
|               | StP-FP              | -1.4209    | -15.79196      | 12.9501577    | 0.9987255na  | Tucky        |         |
| Cross stages: Bowman_SAM_width    | DR-VEG              | 17.746785  | 11.226019      | 24.267551     | 0***         | Tucky        |         |
|               | SPP-TM-DR           | -9.429623  | -15.831307     | -3.027939     | 0.0008218*** | Tucky        |         |
|               | FP-SPP-TM           | -4.48844   | -11.358002     | 2.381122      | 0.3701427na  | Tucky        |         |
|               | StP-FP              | -1.601516  | -8.86645       | 5.663419      | 0.9727291na  | Tucky        |         |
| Cross stages: Bowman_SAM_width    | DR-VEG              | -13.09514  | -21.06523      | -5.1250615    | 0.000143***  | Tucky        |         |
|               | SPP-TM-DR           | -23.5039   | -31.26141      | -15.7463909   | 0***         | Tucky        |         |
|               | FP-SPP-TM           | -7.272     | -15.02951      | 0.4855091     | 0.0771392na  | Tucky        |         |
|               | StP-FP              | -3.93935   | -11.69686      | 3.818159      | 0.6210918na  | Tucky        |         |
| Cross stages: int-m.85_SAM_width | DR-VEG              | 4.510563   | -4.280433      | 13.301558     | 0.6120481na  | Tucky        |         |
|               | SPP-TM-DR           | -22.045676 | -29.532851     | -14.5585      | 0***         | Tucky        |         |
|               | FP-SPP-TM           | -5.961224  | -14.679104     | 2.756656      | 0.3236508na  | Tucky        |         |
|               | StP-FP              | 2.489355   | -6.83946       | 11.818169     | 0.9459896na  | Tucky        |         |

| Genotype | Stage | Replicate number |
|----------|-------|------------------|
| Bowman   | VM    | 29               |
| int-m.1a | VM    | 24               |
| int-m.85 | VM    | 22               |
| Bowman   | DR    | 19               |
| Genotype   | Stage | Replicate number |
|------------|-------|------------------|
| int-m.1a   | DR    | 20               |
| int-m.85   | DR    | 15               |
| Bowman     | TM    | 34               |
| int-m.1a   | TM    | 26               |
| int-m.85   | TM    | 45               |
| Bowman     | FM    | 15               |
| int-m.1a   | FM    | 20               |
| int-m.85   | FM    | 11               |
| Bowman     | StP   | 24               |
| int-m.1a   | StP   | 26               |
| int-m.85   | StP   | 29               |
Table S3. Amino acid differences in barley \textit{INT-M/HvAP2L-H5} and wheat ortholog

| Position | barley variant | wheat variant | Variant | PROVEAN score | Prediction (cutoff=−2.5) |
|----------|----------------|---------------|---------|---------------|--------------------------|
| 27       | A*            | G             | A27G    | 0.785         | Neutral                  |
| 29       | A*            | G             | A29G    | 0.636         | Neutral                  |
| 29       | A*            | GG            | A29delinsGG | 2.437       | Neutral                  |
| 38       | L*            | P             | L38P    | 1.876         | Neutral                  |
| 47       | L             | P             | L47P    | 0.513         | Neutral                  |
| 50       | A*            | del           | A50del | 0.299         | Neutral                  |
| 51       | A*            | V             | A51V    | -0.534        | Neutral                  |
| 52       | A*            | G             | A52G    | 0.187         | Neutral                  |
| 53       | S             | P             | S53P    | -0.564        | Neutral                  |
| 54       | P*            | S             | P64S    | 0.555         | Neutral                  |
| 66       | P*            | PGHAG         | P66_A67insGHAG | 3.026       | Neutral                  |
| 71       | M             | T             | M71T    | 0.559         | Neutral                  |
| 74       | Q             | QQ            | Q74_Q75insQ | 0.058       | Neutral                  |
| 79       | P*            | A             | P79A    | -0.283        | Neutral                  |
| 81       | T*            | M             | T81M    | 0.074         | Neutral                  |
| 94       | V             | L             | V94L    | -0.561        | Neutral                  |
| 99       | V             | M             | V99M    | 0.418         | Neutral                  |
| 102      | K*            | A             | K102A   | 2.035         | Neutral                  |
| 258      | D*            | E             | D258E   | 2.58          | Neutral†                  |
| 264      | D*            | E             | D264E   | 1.277         | Neutral†                  |
| 273      | T*            | P             | T273P   | -1.037        | Neutral                  |
| 276      | V*            | A             | V276A   | 2.009         | Neutral                  |
| 324      | V             | I             | V324I   | -0.194        | Neutral                  |
| 333      | S*            | SS            | S333_Q334insS | -0.611       | Neutral                  |
| 345      | P             | A             | P345A   | 0.175         | Neutral                  |
| 373      | L*            | M             | L373M   | 0.641         | Neutral                  |
| 375      | P             | A             | P375A   | 0.684         | Neutral                  |
| 379      | E             | EQ            | E379_P380insQ | -0.429       | Neutral                  |
| 389      | H*            | Q             | H389Q   | 2.648         | Neutral                  |
| Position | barley | wheat | Variant       | PROVEAN score | Prediction (cutoff = -2.5) |
|----------|--------|-------|---------------|---------------|----------------------------|
| 393      | V*     | M     | V393M         | -0.061        | Neutral                    |
| 403      | L*     | P     | L403P         | -1.421        | Neutral                    |
| 415      | A      | AA    | A415_G416insA | 0.257         | Neutral                    |
| 419      | P      | L     | P419L         | 0.002         | Neutral                    |
| 424      | S      | P     | S424P         | 0.01          | Neutral                    |
| 428      | H*     | del   | H428del       | 0.357         | Neutral                    |

*Amino acid changes unique in barley but not in other non-*Hordeum* Triticaceae species
†Amino acids in the AP2 domains.
| Species      | Comparison   | diff     | lwr       | upr       | p          |
|--------------|--------------|----------|-----------|-----------|------------|
| Henrardia    | DR-VM        | 12.88545 | -4.177602 | 29.9484951| 0.2188227  |
| Henrardia    | TM-SM-DR     | -23.45038| -39.934846| -6.9659039| 0.0018495  |
| Henrardia    | FM-TM-SM     | -28.00474| -41.702492| 14.3069838| 6.1E-06    |
| Henrardia    | StP-FM       | -17.04356| -33.449348| -0.6377761| 0.0381994  |
| Eremopyrum   | TM-SM-DR     | -28.71774| -43.74607 | -13.689412| 9.94E-05   |
| Eremopyrum   | FM-TM-SM     | -14.10157| -32.03213 | 3.828998  | 0.1619587  |
| Eremopyrum   | StP-FM       | -3.43135 | -20.31242 | 13.449718 | 0.9436178  |
| Heteranthelium| DR-VM      | 16.9315  | -7.500744 | 41.363744 | 0.2790745  |
| Heteranthelium| TM-SM-DR   | -26.185  | -66.082688| 13.712688 | 0.3295265  |
| Heteranthelium| FM-TM-SM   | -23.2782 | -63.175888| 16.619488 | 0.441808   |
| Heteranthelium| StP-FM     | -22.2138 | -41.02175 | 13.449718 | 0.9436178  |
| Monococcum   | DR-VM        | 17.1654085| 2.53034   | 31.800477 | 0.0130431  |
| Monococcum   | TM-SM-DR     | -0.5063529| -13.615368| 12.602663 | 0.9999694  |
| Monococcum   | FM-TM-SM     | -46.6689333| -60.0039831| -33.333884 | 0          |
| Monococcum   | StP-FM       | -14.9840667| -27.0699818| -2.898151 | 0.0073605  |
| Taeniathelium| TM-SM-DR     | -30.8694 | -44.52746 | -17.21134 | 0.0010865  |
| Taeniathelium| FM-TM-SM     | -2.836   | -19.16051 | 13.48851  | 0.8584861  |
| chilense     | DR-VM        | 29.97007143| 13.643069 | 46.2970742| 1.51E-05   |
| chilense     | TM-SM-DR     | -0.02311905| -11.568053| 11.5218153| 1          |
| chilense     | FM-TM-SM     | -9.94408396| -19.042288| -0.8458799| 0.0248183  |
| chilense     | StP-FM       | -15.00173939| -23.099837| -6.9036417| 1.24E-05   |
| Species      | Stage     | Sample_size |
|--------------|-----------|-------------|
| Henrardia    | VM        | 7           |
| Henrardia    | DR        | 8           |
| Henrardia    | TM-SM     | 8           |
| Henrardia    | FM        | 21          |
| Henrardia    | StP       | 5           |
| Eremopyrum   | DR        | 11          |
| Eremopyrum   | TM-SM     | 6           |
| Eremopyrum   | FM        | 5           |
| Eremopyrum   | StP       | 8           |
| Heteranthelium | VM     | 4           |
| Heteranthelium | DR       | 5           |
| Heteranthelium | TM-SM   | 1           |
| Heteranthelium | FM       | 5           |
| Heteranthelium | StP     | 15          |
| Monococcum   | VM        | 9           |
| Monococcum   | DR        | 34          |
| Monococcum   | TM-SM     | 12          |
| Monococcum   | FM        | 30          |
| Monococcum   | StP       | 16          |
| Taeniathelium | DR       | 5           |
| Taeniathelium | TM-SM   | 2           |
| Taeniathelium | FM       | 2           |
| chilense     | VM        | 6           |
| chilense     | DR        | 14          |
| chilense     | TM-SM     | 21          |
| chilense     | FM        | 38          |
| chilense     | StP       | 31          |
| Usage                  | Name              | Sequence (5’-3’)       | Note       |
|------------------------|-------------------|------------------------|------------|
| **Amplification**       |                   |                        |            |
| HvIDS1_F2              |                   | GGCCCCCAAGAAGAAGAC     | Genotyping |
| HvIDS1_F3              |                   | CAGGCTACGGCTTGAACCTCT  |            |
| HvIDS1_R2              |                   | ATTCCTACGCCTAGTCCTCA   | Genotyping |
| HvIDS1_R3              |                   | CAGCTGCCCCTGTCATCTA    |            |
| HvIDS1_F4              |                   | GCTCGGCAAGAAGTGAGAGC   |            |
| HvIDS1_R5              |                   | GACGACTCAGGGGAATCAA    |            |
| HvIDS1_F9              |                   | GGCCTCCTCTCGATCTCATG   |            |
| HvIDS1_R9              |                   | GAGAGGAACCAGCAGTGAC    |            |
| HvIDS1_19F             |                   | GACGTCCTACCTCGGTTCCT   |            |
| HvIDS1_743R            |                   | GTCTGCGGTAGAAGGTCG     |            |
| HvIDS1_1183R           |                   | AAGTTGATGTCGCCCTCAG    |            |
| **in situ mRNA hybridization** |      |                       |            |
| HvIDS1_is_T3_50F       | ATTACCCCTCACAATAGGGATTTTCCCTTTCTCCACTGGC |            |
| HvIDS1_is_T7_333R      | TAATACGAACCTCACTATAGGGGAGATCCAGCACCATCTCCG |            |
| HvIDS1_is_T3_809F      | ATTACCCCTCACTAAAGGATGGAGCCGACATCAACTTC | Hv1         |
| HvIDS1_is_T7_1210R     | TAATACGAACCTCACTATAGGGCGTGGTGGTGCAGATGCTCTACTACA | Hv1         |
| HvIDS1_is_T3_872F      | TAATACCCCTCACTAAAGGGAGGACCAAGGAGGAGTTCCCTG | Hv2         |
| HvIDS1_is_T7_1285R     | TAATACGAACCTCACTATAGGATTGGTTGTTGACGACTCAAGG | Hv2         |
| HvIDS1_is_T3_1083F     | ATTACCCCTCACTAAAGGATGGGAGAGGATGCTGTGACTATA | Hv3†        |
| HvIDS1_is_T7_1409R     | TAATACGAACCTCACTATAGGACGTTGGGTAGTAAGCCATG | Hv3†        |
| HvIDS1_T3_F9           | TAATACCCCTCACAATAGGGACTACCCCAACGTACAGTGGTC | Hv4         |
| HvIDS1_T7_R9           | TAATACGAACCTCACTATAGGGAGAGGAACCAGCAGTGAC | Hv4         |
| TmlIDS1_T3_F1          | ATTACCCCTCACTAAAGGAGAAGTTGAAGCTGCAAGGGGC | Tm1†        |
| TmlIDS1_T7_R1          | TAATACGAACCTCACTATAGGGGTGCCATTTGAGTGCACAGG | Tm1†        |
| TmlIDS1_T3_F2          | TAATACCCCTCACAATAGGGAATAGGCTTCTTACCCGAACTG | Tm2         |
| TmlIDS1_T7_R2          | TAATACGAACCTCACTATAGGGGGAAGTGAAGCAGGCTTGGTG | Tm2         |
| TmlIDS1_T3_F3          | ATTACCCCTCACAATAGGGACGACGTACAGTGCAGGTG | Tm2         |
| TmlIDS1_T7_R3          | TAATACGAACCTCACTATAGGCGAGGGATAGCAGGATCCGAC | Tm2         |
| BdIDS1_T7_R1           | TAATACGAACCTCACTATAGGGAATGTGTTGACGAGGAGGAG | Bd1         |
| Usage          | Name                  | Sequence (5'-3')                          | Note          |
|----------------|-----------------------|------------------------------------------|---------------|
| in situ mRNA hybridization |                       |                                          |               |
| BdIDS1_T3_F2   | ATTAAACCCTCACTAAAGGGA| CAGCCAAATGAGCGCATCTTC                    | Bd2†          |
| BdIDS1_T7_R2   | TAATACGACTCACTATAGGGG | GGTGCGGTAGAGAATACCTTG                    | Bd2†          |
| BdIDS1_T3_F3   | ATTAACCCTCACTAAAGGGA | CAGGATTTCTCTACCGCACC                    | Bd3           |
| BdIDS1_T7_R3   | TAATACGACTCACTATAGGGG | ATCGGCGGTGCTCTTCTTTGG                   | Bd3           |
| mads3_T3_353F  | ATTAACCCCTCACTAAAGGGA | CGGTGCGACAAATGACACTCTTC                | M3.1          |
| mads3_T7_631R  | TAATACGACTCACTATAGGGG | CAGGACACCTTGGCTCTATCTAGG                | M3.1          |
| mads3_T3_612F  | ATTAACCCCTCACTAAAGGGA | ACTGAGGAGCAAGGTGTCTC                    | M3.2          |
| mads3_T7_812R  | TAATACGACTCACTATAGGGG | ATCGGCGTGTCTCTCTTTGG                   | M3.2          |
| mads58_T3_455F | ATTAACCCCTCACTAAAGGGA | TCAATGCCCAGCACTACCAG                    | M58.1         |
| mads58_T7_827R | ATTAACCCCTCACTAAAGGGA | ACTGAGGAGCAAGGTGTCTC                    | M58.1         |
| bmads58_T3_482F| ATTAACCCCTCACTAAAGGGA | ACTGAGGAGCAAGGTGTCTC                    | M58.2†        |
| wmads58_T3_605F| ATTAACCCCTCACTAAAGGGA | TCAATGCCCAGCACTACCAG                    | TmM58.2†      |
| mads58_T3_808F | ATTAACCCCTCACTAAAGGGA | ACTGAGGAGCAAGGTGTCTC                    | TmM58.3       |
| bmads58_T7_1123R| ATTAACCCCTCACTAAAGGGA | ACTGAGGAGCAAGGTGTCTC                    | TmM58.3       |
| wmads58_T7_1109R| ATTAACCCCTCACTAAAGGGA | ACTGAGGAGCAAGGTGTCTC                    | TmM58.3       |
| bdmads3_T3_F1  | ATTAACCCCTCACTAAAGGGA | GCGCCAGCAAATTAGTAGCC                    | BdM3.1        |
| bdmads3_T7_R1  | ATTAACCCCTCACTAAAGGGA | TCTTCTCAGGCGAGGTACATATG                | BdM3.1        |
| bdmads3_T3_F2  | ATTAACCCCTCACTAAAGGGA | GCGCCAGCAAATTAGTAGCC                    | BdM3.2        |
| bdmads3_T7_R2  | ATTAACCCCTCACTAAAGGGA | GCGCCAGCAAATTAGTAGCC                    | BdM3.2        |
| bdmads58_T3_F1 | ATTAACCCCTCACTAAAGGGA | GCGCCAGCAAATTAGTAGCC                    | BdM58.1       |
| bdmads58_T7_R1 | ATTAACCCCTCACTAAAGGGA | GCGCCAGCAAATTAGTAGCC                    | BdM58.1       |
| bdmads58_T3_F2 | ATTAACCCCTCACTAAAGGGA | GCGCCAGCAAATTAGTAGCC                    | BdM58.2       |
| bdmads58_T7_R2 | ATTAACCCCTCACTAAAGGGA | GCGCCAGCAAATTAGTAGCC                    | BdM58.2       |
| bdmads58_T3_F3 | ATTAACCCCTCACTAAAGGGA | GCGCCAGCAAATTAGTAGCC                    | BdM58.3       |
| bdmads58_T7_R3 | ATTAACCCCTCACTAAAGGGA | GCGCCAGCAAATTAGTAGCC                    | BdM58.3       |
| log_T3_201F    | ATTAACCCCTCACTAAAGGGA | GATATGGGATCTGGGTAGGCGC                 | LOG (95.5%)†  |
| log_T7_545R    | ATTAACCCCTCACTAAAGGGA | GATAGGGGAGCCAGGGATCCG                 | genotyping    |
| log_T7_649R    | ATTAACCCCTCACTAAAGGGA | GATAGGGGAGCCAGGGATCCG                 | LOG†          |
| kn1_T3_307F    | ATTAACCCCTCACTAAAGGGA | GATAGGGGAGCCAGGGATCCG                 |               |
| kn1_T7_729R    | ATTAACCCCTCACTAAAGGGA | GATAGGGGAGCCAGGGATCCG                 |               |
| Usage            | Name                  | Sequence (5'-3')                                                                 | Note          |
|------------------|-----------------------|---------------------------------------------------------------------------------|---------------|
| *in situ* mRNA hybridization | kn1_T3_708F          | ATTAAACCCTCACTAAAGGGATGAGATGGATGCCACCGGAG                                      | KN1 (96.8%)*† |
|                  | kn1_T7_1049R          | TAAATCGACTCATACTAGGGATGTAGAAGGCGGCCAAGGGTG                                      | KN1†          |
|                  | mads1_T3_336F         | ATTAAACCCTCACTAAAGGGATGAGATGGATGCCACCGGAG                                      | MADS1 (95.3%)*† |
|                  | mads1_T7_644R         | TAATACGACTCATACTAGGGGAGGTATCATGAGCTCCGAGG                                      |              |
|                  | mads1_T3_132F         | ATTAAACCCTCACTAAAGGGACCTCATGACTCCGAGG                                          |              |
|                  | mads1_T7_581R         | TAATACGACTCATACTAGGGCTACTCTGCCCCAACCGTTCGAG                                    |              |
|                  | ckx3_T3_178F          | ATTAAACCCTCACTAAAGGGATGAGATGGATGCCACCGGAG                                      | CKX†          |
|                  | ckx3_T7_630R          | TAATACGACTCATACTAGGGGACGTACGAGCTCCGAGG                                          | CKX†          |
|                  | ckx3_T3_1002F         | ATTAAACCCTCACTAAAGGGATGAGATGGATGCCACCGGAG                                      |              |
|                  | ckx3_T7_1326R         | TAATACGACTCATACTAGGGCTCTTCTACAGGGGTTGAGG                                       |              |
|                  | wox2_T3_63F           | ATTAAACCCTCACTAAAGGGATGAGATGGATGCCACCGGAG                                      |              |
|                  | wox2_T7_361R          | TAATACGACTCATACTAGGGGAGACGGCTTCCGAGG                                           |              |
|                  | wox2_T3_78F           | ATTAAACCCTCACTAAAGGGATGAGATGGATGCCACCGGAG                                      |              |
|                  | Tmwox2_T7_401R        | TAATACGACTCATACTAGGGGAGACGGCTTCCGAGG                                           | TmWOX2†       |
|                  | Hvwox2_T7_438R        | TAATACGACTCATACTAGGGGAGACGGCTTCCGAGG                                           | TmWOX2†       |
|                  | erl_T3_340F           | ATTAAACCCTCACTAAAGGGATGAGATGGATGCCACCGGAG                                      | ERL†          |
|                  | erl_T7_881R           | TAATACGACTCATACTAGGGGAGACGGCTTCCGAGG                                           | ERL†          |
|                  | erl_T3_1569F          | ATTAAACCCTCACTAAAGGGATGAGATGGATGCCACCGGAG                                      |              |
|                  | erl_T7_2033R          | ATTAAACCCTCACTAAAGGGATGAGATGGATGCCACCGGAG                                      |              |
|                  | bam_T3_360F           | ATTAAACCCTCACTAAAGGGATGAGATGGATGCCACCGGAG                                      |              |
|                  | bam_T7_809R           | TAATACGACTCATACTAGGGGAGACGGCTTCCGAGG                                           | BAM†          |
|                  | bam_T3_1033F          | ATTAAACCCTCACTAAAGGGATGAGATGGATGCCACCGGAG                                      | BAM†          |
|                  | bam_T7_1338R          | TAATACGACTCATACTAGGGGAGACGGCTTCCGAGG                                           |              |
|                  | bam_T7_1607R          | TAATACGACTCATACTAGGGGAGACGGCTTCCGAGG                                           |              |
|                  | wox7_T3_181F          | ATTAAACCCTCACTAAAGGGATGAGATGGATGCCACCGGAG                                      |              |
|                  | wox7_T7_767R          | TAATACGACTCATACTAGGGGAGACGGCTTCCGAGG                                           |              |
|                  | wox7_T3_748F          | ATTAAACCCTCACTAAAGGGATGAGATGGATGCCACCGGAG                                      |              |
|                  | Hvwox7_T7_1250R       | TAATACGACTCATACTAGGGGAGACGGCTTCCGAGG                                           | HvWOX7†/TmWOX7† |
|                  | Tmwox7_T7_1218R       | TAATACGACTCATACTAGGGGAGACGGCTTCCGAGG                                           | TmWOX7†       |
|                  | HvVRN1_T3_9F          | ATTAAACCCTCACTAAAGGGATGAGATGGATGCCACCGGAG                                      | VRN1†         |
|                  | HvVRN1_T7_415R        | TAATACGACTCATACTAGGGGAGACGGCTTCCGAGG                                           | VRN1†         |
### Usage

| Usage          | Name                  | Sequence (5'-3')                                | Note  |
|----------------|-----------------------|-------------------------------------------------|-------|
| **in situ mRNA hybridization** |                       |                                                 |       |
| HvMADS15_T3_30F | ATTAACCCTCACTAAAGGGA| TCCTGGAATCAGCTTGGATGGTGTC                       | FUL2† |
| HvMADS15_T7_530R| TAATACGACTCACTATAGGG | TCTCTCAGTGCCTCTCCTT                             | FUL2† |
| HvMADS18_T3_167F| ATTAACCCCTACTAAAGGGA| AGTACTCCAGCCAGGACAGT                            | MADS18†|
| HvMADS18_T7_652R| TAATACGACTCACTATAGGG | GTGGTGTAGGTGACGGTGAG                            | MADS18†|
| HvMADS34_T3_337F| ATTAACCCCTACTAAAGGGA| ACTAGGCGAGGATTTTGCTCC                           | MADS34†|
| HvMADS34_T7_632R| TAATACGACTCACTATAGGG | TGGGAAGAAGTGCTCTGGCTG                           | MADS34†|
| APO1_T3_579F   | ATTAACCCCTACTAAAGGGA| AGGTGGCTTTGATGGGATG                              | APO1c†|
| APO1_T7_830R   | TAATACGACTCACTATAGGG | ACCACCGATTCCAAACCTGT                            | APO1c†|
| LFY_T3_405F    | ATTAACCCCTACTAAAGGGA| GGTGGCTTTGATGGGATG                              | LFY3† |
| LFY_T7_778R    | TAATACGACTCACTATAGGG | GTCCGGTTGTAGATGCGCTA                            | LFY3† |
| VRN1_T3_782F   | ATTAACCCCTACTAAAGGGA| AGGTGGCTTTGATGGGATG                              | 3'UTR†|
| VRN1_T7_1076R  | TAATACGACTCACTATAGGG | CTCGTACAGCCATCTCCAGGC                           | 3'UTR†|
| HvMADS15_T3_867F| ATTAACCCCTACTAAAGGGA| GGTGGCTTTGATGGGATG                              | 3'UTR†|
| HvMADS15_T7_1212R| TAATACGACTCACTATAGGG | AATGAACTGTCGGTGCATATG                           | 3'UTR†|
| MADS18_T3_945F | ATTAACCCCTACTAAAGGGA| ATCGGGAACAGATGAGGGCT                           | 3'UTR†|
| MADS18_T7_1395R| TAATACGACTCACTATAGGG | ATCGGGAACAGATGAGGGCT                           | 3'UTR†|
|                  |                       |                                                 |       |
| **qRT-PCR**     |                       |                                                 |       |
| IDS1_q_1019F    | AGTGGCCTGTGCCATCAACAT |                                                |       |
| TmlIDS1_q_1109R | ACGTTGGGTAAGCCGATG    |                                                |       |
| HvIDS1_q_1091R  | ACGTTGGGTAAGCCGATG    |                                                |       |
| MADS3_q_403F    | CGCCACGAGTCAGTGCCTT   |                                                |       |
| MADS3_q_519R    | TATGCGTTTCTCCAGCCGC   |                                                |       |
| MADS58_q_718F   | GAGACTGAAAGGGGCAACA   |                                                |       |
| MADS58_q_827R   | AACTGCGAAGGTTCTCGG    |                                                |       |
| Actin_q_145F    | TCAGAACCCTTAGTGCAGAG  |                                                |       |
| Actin_q_254R    | GCTACACCACTACCCAGAGT  |                                                |       |

*indicates probes used for barley and wheat and the sequence identity of the probes between barley and wheat

†Primers were used to generate the probes.
Table S6. Genome sequences used for phylogenetic inference, synteny analysis and orthology sorting.

| Species               | Lineage                        | Source        | Usage                      |
|-----------------------|--------------------------------|---------------|----------------------------|
| Aquilegia coerulea    | "basal" eudicots               | phytozome12.5 | Phylogenetics              |
| Arabidopsis tahliana  | Eudicots - Brassicaceae        | phytozome12.5 | Phylogenetics              |
| Carica papaya         | Eudicots - Caricaceae          | phytozome12.5 | Phylogenetics              |
| Cucumis sativus       | Eudicots - Cucurbitaceae       | phytozome12.5 | Phylogenetics              |
| Ricinus communis      | Eudicots - Euphorbiaceae       | phytozome12.5 | Phylogenetics              |
| Medicago truncatula   | Eudicots - Fabaceae            | phytozome12.5 | Phylogenetics              |
| Phaseolus vulgaris    | Eudicots - Fabaceae            | phytozome12.5 | Phylogenetics              |
| Populus trichocarpa   | Eudicots - Salicaceae          | phytozome12.5 | Phylogenetics              |
| Solanum lycopersicum  | Eudicots - Solanaceae          | phytozome12.5 | Phylogenetics              |
| Solanum lycopersicum  | Eudicots - Solanaceae          | (90)          | Phylogenetics              |
| Vitis vinifera        | Eudicots - Vitaceae            | phytozome12.5 | Phylogenetics              |
| Ananas comosus        | Monocots - Musaceae            | Ensembl Plants| Phylogenetics              |
| Phalaenopsis equestris| Monocots - Orchidaceae         |               | Phylogenetics              |
| Panicum virgatum      | Monocots - Poaceae             | phytozome12.5 | Phylogenetics              |
| Setaria viridis       | Monocots - Poaceae             | phytozome12.5 | Phylogenetics              |
| Oryza sativa          | Monocots - Poaceae             | phytozome12.5 | Phylogenetics              |
| Sorghum bicolor       | Monocots - Poaceae             | phytozome12.5 | Phylogenetics              |
| Setaria italica       | Monocots - Poaceae             | phytozome12.5 | Phylogenetics              |
| Zea mays              | Monocots - Poaceae             | phytozome12.5 | Phylogenetics              |
| Hordeum vulgare       | Monocots - Poaceae             | (57)          | Phylogenetics              |
| Hordeum vulgare       | Monocots - Poaceae             | IBSC, 2012    | Phylogenetics              |
| Triticum aestivum     | Monocots - Poaceae             | IWGSC 2018, version 1.0 | Phylogenetics and orthogroups sorting |
| Species             | Lineage          | Source              | Usage                             |
|---------------------|------------------|---------------------|-----------------------------------|
| *Leersia perrieri*  | Monocots - Poaceae | Ensembl Plants      | phylogenetics and orthogroups sorting |
| *Triticum urartu*   | Monocots - Poaceae | (65)                | phylogenetics and orthogroups sorting |
| *Aegilops tauschii* | Monocots - Poaceae | (66)                | phylogenetics and orthogroups sorting |
| *Brachypodium distachyon* | Monocots - Poaceae | phytozome12.5       | phylogenetics and orthogroups sorting |
| *Hordeum spontaneum*| Monocots - Poaceae | (85)                | Phylogenetic footprinting         |
| *Hordeum pubiflorum*| Monocots - Poaceae | NCBI PRJEB3404      | Phylogenetic footprinting         |
Legends for Datasets

Dataset S1. Annotations and effects of SNPs in the common introgressed region of int-m.85 and int-m.1a NIL mutants.

Dataset S2. Alignment of nucleotides of HvAP2L-H5 in int-m, dub1 and wildtype barley cultivars.

Dataset S3. Lists of differentially expressed genes between Bowman and int-m mutants in DR, TM-SM and StP stages.

Dataset S4. OrthoGroups identified with eleven published grass genomes and OrthoFinder.

SI References

1. U. Lundqvist, A. Lundqvist, Induced intermediate mutants in barley: origin, morphology and inheritance. *Hereditas* **108**, 13–26 (1988).

2. P. Bregitzer, U. Lundqvist, V. Carollo Blake, *Barley Genetics Newsletter* (2007).

3. C. Pozzi, D. di Pietro, G. Halas, C. Roig, F. Salamini, Integration of a barley (*Hordeum vulgare*) molecular linkage map with the position of genetic loci hosting 29 developmental mutants. *Heredity* **90**, 390–396 (2003).

4. D. Kurihara, Y. Mizuta, Y. Sato, T. Higashiyama, ClearSee: a rapid optical clearing reagent for whole-plant fluorescence imaging. *Development* **142**, 4168–4179 (2015).

5. P. Barbier de Reuille, et al., MorphoGraphX: a platform for quantifying morphogenesis in 4D. *eLife* **4**, e05864 (2015).

6. P. Bommert, C. Whipple, Grass inflorescence architecture and meristem determinacy. *Sem Cell Dev Biol* **79**, 37–47 (2018).

7. G. W. van Esse, et al., Six-Rowed Spike3 (VRS3) is a histone demethylase that controls lateral spikelet development in barley. *Plant Physiol* **174**, 2397–2408 (2017).

8. M. Mascher, et al., A chromosome conformation capture ordered sequence of the barley genome. *Nature* **544**, 427–433 (2017).

9. H. Li, Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. *arXiv:1303.3997 [q-bio]* (2013) (September 2, 2019).

10. B. A. Veeneman, S. Shukla, S. M. Dhanasekaran, A. M. Chinnaiyan, A. I. Nesvizhskii, Two-pass alignment improves novel splice junction quantification. *Bioinformatics* **32**, 43–49 (2016).
11. R. Poplin, et al., Scaling accurate genetic variant discovery to tens of thousands of samples. bioRxiv, 201178 (2018).

12. P. Cingolani, et al., A program for annotating and predicting the effects of single nucleotide polymorphisms, SnpEff: SNPs in the genome of Drosophila melanogaster strain w^{1118} ; iso-2; iso-3. Fly 6, 80–92 (2012).

13. B. Gel, E. Serra, karyoploteR: an R/Bioconductor package to plot customizable genomes displaying arbitrary data. Bioinformatics 33, 3088–3090 (2017).

14. Y. Choi, A. P. Chan, PROVEAN web server: a tool to predict the functional effect of amino acid substitutions and indels. Bioinformatics 31, 2745–2747 (2015).

15. H. Tang, V. Krishnakumar, J. Li, jcvi: JCVI utility libraries (Zenodo, 2015) https://doi.org/10.5281/zenodo.31631 (September 2, 2019).

16. H.-Q. Ling, et al., Genome sequence of the progenitor of wheat A subgenome Triticum urartu. Nature 557, 424–428 (2018).

17. M.-C. Luo, et al., Genome sequence of the progenitor of the wheat D genome Aegilops tauschii. Nature 551, 498–502 (2017).

18. D. M. Goodstein, et al., Phytozome: a comparative platform for green plant genomics. Nucleic Acids Res 40, D1178–D1186 (2012).

19. K. Katoh, D. M. Standley, MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol Biol Evol 30, 772–780 (2013).

20. A. Stamatakis, RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30, 1312–1313 (2014).

21. J. Russell, et al., Exome sequencing of geographically diverse barley landraces and wild relatives gives insights into environmental adaptation. Nat Genet 48, 1024–1030 (2016).

22. J. W. Leigh, D. Bryant, popart: full-feature software for haplotype network construction. Methods Ecol Evol 6, 1110–1116 (2015).

23. D. M. Emms, S. Kelly, OrthoFinder: phylogenetic orthology inference for comparative genomics. Genome Biol 20, 238 (2019).

24. B. Buchfink, C. Xie, D. H. Huson, Fast and sensitive protein alignment using DIAMOND. Nat Methods 12, 59–60 (2015).

25. A. J. Enright, S. Van Dongen, C. A. Ouzounis, An efficient algorithm for large-scale detection of protein families. Nucleic Acids Res 30, 1575–1584 (2002).

26. J. Zhong, M. Robbett, A. Poire, J. C. Preston, Successive evolutionary steps drove Pooidaeae grasses from tropical to temperate regions. New Phytol 217, 925–938 (2018).
27. C. Monat, et al., TRITEX: chromosome-scale sequence assembly of Triticeae genomes with open-source tools. Genome Biol 20, 284 (2019).

28. R. Patro, G. Duggal, M. I. Love, R. A. Irizarry, C. Kingsford, Salmon provides fast and bias-aware quantification of transcript expression. Nat Methods 14, 417–419 (2017).

29. C. W. Law, Y. Chen, W. Shi, G. K. Smyth, voom: precision weights unlock linear model analysis tools for RNA-seq read counts. Genome Biol 15, R29 (2014).

30. M. E. Ritchie, et al., limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Res 43, e47–e47 (2015).

31. A. Conesa, et al., Blast2GO: a universal tool for annotation, visualization and analysis in functional genomics research. Bioinformatics 21, 3674–3676 (2005).

32. T. Tian, et al., agrigGO v2.0: a GO analysis toolkit for the agricultural community, 2017 update. Nucleic Acids Res 45, W122–W129 (2017).

33. T. I. W. G. S. IWGSC, et al., Shifting the limits in wheat research and breeding using a fully annotated reference genome. Science 361, eaar7191 (2018).

34. N. Feng, et al., Transcriptome profiling of wheat inflorescence development from spikelet initiation to floral patterning identified stage-specific regulatory genes. Plant Physiol 174, 1779–1794 (2017).

35. Q. Sang, et al., Mutagenesis of a quintuple mutant impaired in environmental responses reveals roles for CHROMATIN REMODELING4 in the Arabidopsis floral transition. The Plant Cell 32, 1479–1500 (2020).

36. M. Liu, et al., The draft genome of a wild barley genotype reveals its enrichment in genes related to biotic and abiotic stresses compared to cultivated barley. Plant Biotechnol J 18, 443–456 (2020).

37. Z. Lu, et al., The prevalence, evolution and chromatin signatures of plant regulatory elements. Nat Plants 5, 1250–1259 (2019).

38. Z. Li, et al., The bread wheat epigenomic map reveals distinct chromatin architectural and evolutionary features of functional genetic elements. Genome Biol 20, 139 (2019).

39. Grass Phylogeny Working Group II, New grass phylogeny resolves deep evolutionary relationships and discovers C4 origins. New Phytol 193, 304–312 (2012).

40. N. Bernhardt, J. Brassac, B. Kilian, F. R. Blattner, Dated tribe-wide whole chloroplast genome phylogeny indicates recurrent hybridizations within Triticeae. BMC Evol Biol 17, 141 (2017).

41. O. T. Bonnett, Inflorescences of maize, wheat, rye, barley, and oats: their initiation and development (University of Illinois, 1966) (September 2, 2019).
42. E. A. Kellogg, et al., Early inflorescence development in the grasses (Poaceae). *Front Plant Sci* 4 (2013).

43. W. P. Maddison, D. R. Maddison, *Mesquite: a modular system for evolutionary analysis*. Version 3.61 [http://www.mesquiteproject.org](http://www.mesquiteproject.org) (2019).