

1 **Systematic mining and genetic characterization of regulatory factors**
2 **for wheat spike development**

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19
20 **Abstract**

21 Spike architecture largely affects grain number embraced, which is a key factor
22 influencing wheat grain yield. Here, we systematically explore the genetic regulation
23 network governing wheat spike development by integration of multi-omic data with
24 population genetics. Transcriptome and epigenome profile of shoot apex at eight
25 developmental stages were generated. Gain-of-chromatin accessibility and changes of
26 H3K27me3 coordinately associate with the progressive transcriptome alteration for
27 flowering. A core transcriptional regulation network (TRN) that likely drives various
28 meristematic cell identities transition to form spike was constructed. Integration of the

29 TRN and genome-wide association analysis (GWAS), 260 transcription factors (TFs)
30 were identified, including 52 characterized factors in crops, but mostly unstudied.
31 Multi-layer regulatory module among *TaSPL6*, *TaMADS34* and *TaMADS14* suggested
32 by TRN was further validated experimentally. About 85 novel TFs contain high impact
33 mutant lines in KN9204 TILLING library. Of them, 44 TFs with homozygous mutation,
34 including NAC, bHLH, MYB, and WRKY, show altered spike architecture. In
35 particular, *TaMYB4-A* positively regulates fertile spikelet number likely via regulating
36 hormones homeostasis or signaling, while acting downstream of- and repressed by
37 WFZP. The elite allele of *TaMYB4-A*, with higher expression and more fertile spikelet,
38 was selected during breeding process in China. Collectively, we present invaluable
39 resource of high-confidence regulators and novel strategy for understanding the genetic
40 regulation of wheat spike development systematically.

41

42 **Key words:** Spike architecture, multi-omics, GWAS, TRN, MYB

43

44 **Introduction**

45 Wheat (*Triticum aestivum*) is a major crop worldwide and provides about 20% of the
46 daily calories and proteins consumed by human population. Yield is a polygenic
47 complex quantitative trait composed of multiple elements including fertile tiller per
48 area, grain number per spike (GNPS) and grain weight (Xiao et al., 2022). GNPS is
49 determinate by the architecture of spike/inflorescence, which involves the number and
50 arrangement of spikelet and fertile florets within spikelet. The spike architecture is
51 shaped by endogenous developmental programs as well as environmental conditions
52 (Gao et al., 2019; Lee et al., 2019; Qi et al., 2019).

53

54 During seedling stage, shoot apex meristem (SAM) constitutively produce primordium
55 of leaves, nodes and internodes. The bract leaf primordia first appeared on the growth
56 cone to form a single edge (SR) structure, and then the spikelet meristem (SM) was
57 formed in the upper part of the bract to enter into double ridge (DR) stage, which is also

58 a symbol for flowering transition. SM gradually differentiates and enlarges from the
59 middle of inflorescence to both ends. After formation of apical SM, the maximum
60 number of spikelets is reached. Following the completion of spikelet differentiation, the
61 lemma primordium (floral primordia differentiation stage) is formed, then it
62 differentiates to produce pistil primordium and pollen sacs (floral organ primordia
63 differentiation stage), and finally enters the booting stage (Bonnett, 1936). Throughout
64 wheat spike development, SM differentiation during and after DR stage determines the
65 spikelet number per spike (SNS) (Dobrovolskaya et al., 2015; Li et al., 2019; Li et al.,
66 2021).

67

68 Much progresses have been made in the molecular regulation of cereal inflorescence
69 architecture, including rice, maize and wheat (Gao et al., 2019; Yuan et al., 2020). In
70 general, several steps are critical for the inflorescence structure, such as the duration of
71 inflorescence development, the initiation, arrangement and terminal of spikelets, as
72 well as the fertility of spikelet and floret (Yuan et al., 2020). In wheat, flowering time
73 regulator such as *Photoperiod-1 (Ppd-1)*, *FLOWERING LOCUS T1 (FT1)*, *FT2*
74 coordinately regulate the transition of SAM to inflorescence meristem (IM) and the
75 duration of spike development, which in turn affects the paired spikelet formation
76 (Boden et al., 2015; Finnegan et al., 2018). *TEOSINTE BRANCHED1 (TB1)* encoding
77 a TCP transcription factor, controls lateral branching in maize (Doebley et al., 1997).
78 In wheat, TaTB1 interacts with FT1 to control the production of paired spikelets via
79 regulating *VRN1* expression (Dixon et al., 2018). *VERNALIZATION1 (VRN1)* and the
80 other orthologs of AP1/FUL-like factor, *FRUITFULL2 (FUL2)*, and *FUL3* are required
81 synergistically for initiation and maintenance of the SM identity (Li et al., 2019).
82 Overexpression of *PANICLE PHYTOMER2 (TaPAP2)*, a MADS box TF coding gene
83 in wheat, inhibits SM formation and reduces SNS (Wang et al., 2017). While *WHEAT*
84 *ORTHOLOG OF APO1 (WAPO1)* affects SNS by regulating the timing of terminal
85 spikelet formation (Kuzay et al., 2022; Kuzay et al., 2019). *Q (AP2L5)*, which encodes
86 an APETALA 2 (AP2) TF, can significantly reduce the SNS when loss-of-function,
87 possibly due to the premature transition of the spike meristem to terminal spikelets

88 (Debernardi et al., 2020). Overexpression of *TERMINAL FLOWER 1 (TaTFL1)-2D*
89 delayed the terminal spikelet formation and increased SNS, while *Grain Number*
90 *Increase 1 (GNII)* played the opposite role on determining SNS (Sakuma et al., 2019;
91 Wang et al., 2017).(Wang et al., 2017; Sakuma et al., 2019). *WFZP*, a homologous of
92 rice *FRIZZY PANICLE (FZP)*, determines the SNS and the floret meristem fate partially
93 by *VRN1* and *HOMEBOX4 (TaHOX4)* (Li et al., 2021), as well as by inhibiting the
94 repressor of spikelet formation gene *TaBA1* through epigenetic modification factors
95 TaHDA1 and TaLHP1 (Du et al., 2021). Several SQUAMOSA promoter-binding
96 protein-like (SPL) and MADS-box TFs are involved in the floral organ formation and
97 regulate floret fertility, such as *TaSPL13-B* (Li et al., 2020), *TaSPL14* (Cao et al., 2021),
98 *TaAGL6-A* (Kong et al., 2022). Besides of the key TFs that mediating the meristem
99 identity switch, factors in auxin and cytokinin (CK) hemostasis also play important role
100 in regulation of spike development in wheat, such as *TaCYP78A5* and *TaCKX2.1*,
101 *TaCKX2.2* (Guo et al., 2022; Jablonski et al., 2020; Jablonski et al., 2021; Qi et al.,
102 2019).

103
104 The factors identified above are mainly through homologous cloning or forward genetic
105 mapping from mutants or bi-parental population. Recently, population genetics has
106 flourished owing to the sequencing technology innovation (Sehgal and Dreisigacker,
107 2022). Large-scale GWAS analysis was performed among landmark cultivars with
108 single nucleotide polymorphism (SNP) generated with genotyping-by-sequencing
109 (GBS) (Pang et al., 2020), whole genome resequencing (Hao et al., 2020), or exome
110 capture sequencing (Li et al., 2022a). Various genetic loci were associated with spike
111 developmental related traits, such as spike length (SL), SNS, GNPS, grain setting and
112 spike compactness (Pang et al., 2020; Sun et al., 2017). However, wheat is an
113 allohexaploid of large genome plant with generally triple genes number, large
114 proportion of intergenic regions, more complex sub-genome interaction, and long LD
115 intervals (Hao et al., 2020; Li et al., 2022a; Pang et al., 2020), which greatly reduced
116 the accuracy of association study (Huang and Han, 2014). Other types of information,
117 such as genes expression network provides different filter for identification of key

118 regulators, as proved by previous studies (Li et al., 2018; VanGessel et al., 2022; Wang
119 et al., 2017).

120

121 Here, we carried out a time serial profiling of transcriptome and epigenome at shoot
122 apex in elite wheat cultivar Kenong 9204 (KN9204), to understand how chromatin
123 landscape is coordinated with transcriptome dynamics during the process of spike
124 formation. More importantly, we integrate genes co-expression and regulatory network
125 with GWAS/QTL to identify key factors shaping wheat spike architecture, and further
126 validate and do in-depth analysis to uncover the potential regulators' function.

127

128 **Results**

129 **A transcriptome and chromatin landscape atlas for wheat spike formation**

130 To understand the transcriptional regulation for wheat spike development, shoot tips of
131 eight distinct stages of cultivar KN9204 were sampled, including SAM, elongation
132 stage (EL), SR, DR, spikelet meristem initiation stage (SMI), glume primordium
133 differentiation stage (GPD), floral meristem initiation stage (FMI), floral organ
134 primordia differentiation stage (FOP) (Figure 1A). RNA-seq and the Assay for
135 Transposase Accessible Chromatin with high-throughput sequencing (ATAC-seq)
136 were used to profile global gene expression, chromatin accessibility with two or three
137 bio-replicates (Figure 1A and Supplemental Figure 1A) (Buenrostro et al., 2015; Kaya-
138 Okur et al., 2019; Zhao et al., 2022).

139

140 In total, 58,875 high-confidence genes were found to express at least in one stage
141 (TPM > 0.5) (Supplemental Table 1). The principal component analysis (PCA) showed
142 that the eight stages could be subdivided into two major categories, as vegetative group
143 including SAM, EL, SR, DR and reproductive group including SMI, GPD, FMI and
144 FOP (Figure 1B). More differential expressed genes (DEGs) between adjacent stages
145 occurs at morphological transition points, such as DR to SMI, SMI to GPD, FMI to
146 FOP, when spikelet meristem, glume primordial and floral organ primordial initiate

147 respectively (Figure 1C and Supplemental Table 2). Stage-specific expressed genes are
148 identified via clustering (Figure 1D). Known factors involved in flowering time
149 regulation and inflorescence development is highlighted from specific cluster. For
150 instance, flowering time gene *TaPPD1* in cluster2 is expressed before floral transition
151 from SAM to DR (Boden et al., 2015; Perez-Gianmarco et al., 2020); *WAP01*, required
152 for the maintenance of SM activity, is highly expressed at SMI (Kuzay et al., 2022);
153 *TaTFL1* and *WFZP*, involved in terminal spikelet formation and floret fate, are
154 expressed at GPD and FMI (Li et al., 2021; Wang et al., 2017); floral organ regulator
155 *TaAGL6* is highly expressed at FOP (Kong et al., 2022) (Figure 1D). Besides, various
156 members of SPL family TF (*TaSPL9/12*, *TaSPL1*, *TaSPL14*, and *TaSPL8/16*) are
157 highly expressed at different stages (Figure 1D), consistent with recent report (Li et al.,
158 2022b). Such transcriptome pattern correlates with the morphological characteristic of
159 individual stage, indicating our sampling could capture the dynamic gene expression
160 profile during wheat spike development.

161

162 As our previous report (Zhao et al., 2022), accessible chromatin is mainly located in
163 the distal intergenic regions and promoters (Figure 1E). ATAC-seq peak intensity at
164 genic region, in particular around TSS, is positively correlated with gene expression
165 (Supplemental Figure 1B). Unsupervised PCA revealed a continuous trajectory of
166 chromatin accessibility dynamics during wheat spike development (Figure 1F). Eight-
167 stages could partition into five sub-clusters, as vegetative cluster before flowering
168 including SAM, EL, SR, flowering transition stage (DR), inflorescence initiation
169 (SMI), spikelet meristem formation (FMI, GPD) and floret meristem formation (FOP)
170 (Supplemental Figure 1C). A globally increasing of chromatin accessibility at genic
171 regions was observed from vegetative cluster to flowering transition and inflorescence
172 initiation, whereas, declined during spikelet and floret formation (Figure 1G and
173 Supplemental Figure 1D).

174

175 Based on the chromatin accessibility dynamics, SAM, DR, SMI, GPD and FOP stages
176 were chosen for histone modification profiling via Cleavage Under Targets and

177 Tagmentation (CUT&Tag) (Figure 1A) (Zhao et al., 2022). In general, H3K27ac,
178 H3K4me3 and H3K36me3 intensity around TSS are positively correlated with gene
179 expression, while H3K27me3 is enriched at gene body of no/low expressed genes
180 (Figure 1E and Supplemental Figure 1B). H3K27me3 and H3K36me3 was mutually
181 exclusive for each other, H3K4me3 and H3K27ac was positively correlated, whereas,
182 histone variants H2A.Z was overlapped with both active H3K27ac and H3K4me3 and
183 repressive H3K27me3, but depleted with H3K36me3 (Supplemental Figure 1E and
184 Supplemental Table 3). The PCA and cluster analyses showed that various histone
185 modifications had stage-specific transitions during different developmental stages
186 (Figures 1H and 1I and Supplemental Figures 1F-1H). Of note, H3K27me3 presents
187 continuous trajectory while others are not, suggesting higher correlation with
188 transcriptional profile dynamics (Figure 1H). Indeed, H3K27me3 abundance and
189 chromatin accessibility jointly affect different genes expression pattern during spike
190 development (Figure 1J).

191

192 Taken together, we have generated transcriptomic and epigenomic profile of shoot apex
193 at important stages during wheat spike formation, which can facilitate elucidating the
194 transcriptional regulatory insights for shaping wheat inflorescence architecture.

195

- 206 stages during spike development. Three bio-replicates were sequenced for each
207 stage.
- 208 (C) Number of differential expressed genes (DEGs) between adjacent developmental
209 stages. DEGs were defined by the threshold $|\log_2(\text{Fold Change})| \geq 1$ and $\text{FDR} \leq$
210 0.05 by DESeq2.
- 211 (D) Heatmap of expressed genes sorted by k-mean clustering across the samples
212 collected at different developmental stages. The representative genes of each
213 cluster are listed on the right.
- 214 (E) Peak distribution of ATAC-seq and various histone modifications relative to genes.
- 215 (F) PCA of ATAC-seq samples during spike development. Each dot represents one
216 sample. Color represents different stages. Two bio-replicates were sequenced for
217 each stage.
- 218 (G) Chromatin accessibility dynamics in proximal regions (promoter and genic regions)
219 during wheat spike development.
- 220 (H-I) PCA of H3K27me3 (H) and H3K27ac (I) samples during spike development.
221 Each dot represents one sample. Two bio-replicates were sequenced for each stage.
- 222 (J) Gene expression, chromatin accessibility and H3K27me3 changes at representative
223 genes during spike development. The y-axis indicates relative values of Z-scaled
224 gene expression, chromatin accessibility and H3K27me3 levels.

225

226 **A permissive chromatin environment facilitates vegetative-to-reproductive** 227 **transition**

228 As indicated, chromatin accessibility increased globally during vegetative-to-
229 reproductive transition at shoot apex (Figure 1G). We further look at differential
230 accessible promoter regions (DAPR) from SAM to EL, SR, DR till SMI stages (Figure
231 2A and Supplemental Table 4). In total, 49,153 DAPR was identified, categorized into
232 6 clusters. Of them, majority of DAPR showed increased accessibility at either DR or
233 SMI stages, including clusters 2,3,4,6 (Figure 2A and Supplemental Table 5).
234 Interestingly, genes of those four clusters show higher positive correlation between
235 chromatin accessibility and transcriptional dynamics ($R > 0.5$) (Figure 2B). Indeed,
236 genes with increased chromatin accessibility at DR or SMI stages is significantly
237 overlapped with genes of elevated expression at DR or SMI as compared to SAM (1,920
238 genes within gene set I; Figure 2C, Supplemental Figure 2A and Supplemental Table
239 6). The degree of increased chromatin accessibility is highly correlated ($R = 0.85$) with
240 fold change of elevated expression level (Figure 2D). Gene Ontology (GO) term
241 analysis suggests that hormone biosynthesis and signaling, inflorescence meristem

242 identity, asymmetric cell division are enriched in gene set I (Figure 2E). This highlights
243 the synchronous between gain-of chromatin accessibility and elevated gene expression
244 during vegetative apical meristem to inflorescence meristem transition (Figure 2F).

245

246 Besides, open chromatin could set a ‘primed’ status for gene activation later on (Bonifer
247 et al., 2017; He and Li, 2018). There are 3,435 genes with gained chromatin
248 accessibility at DR or SMI but elevated mRNA level at stages after SMI (Gene set II;
249 Figure 2C, Supplemental Figure 2B and Supplemental Table 6). Those genes are mainly
250 involved in hormones metabolism, floral organ identity, meristem development
251 (Supplemental Figure 2C), which are important for spike formation similar as gene set
252 I. By contrast, large amount of genes with gain-of chromatin accessibility but no change
253 of mRNA level at tested spike developmental stages (Gene set III; Figure 2C and
254 Supplemental Table 6), such as genes participated in polarity specification, cell size
255 regulation, regulation of translation or protein transport (Supplemental Figure 2D).
256 Thus, other chromatin features may also involve in the gene expression regulation of
257 different gene sets.

258

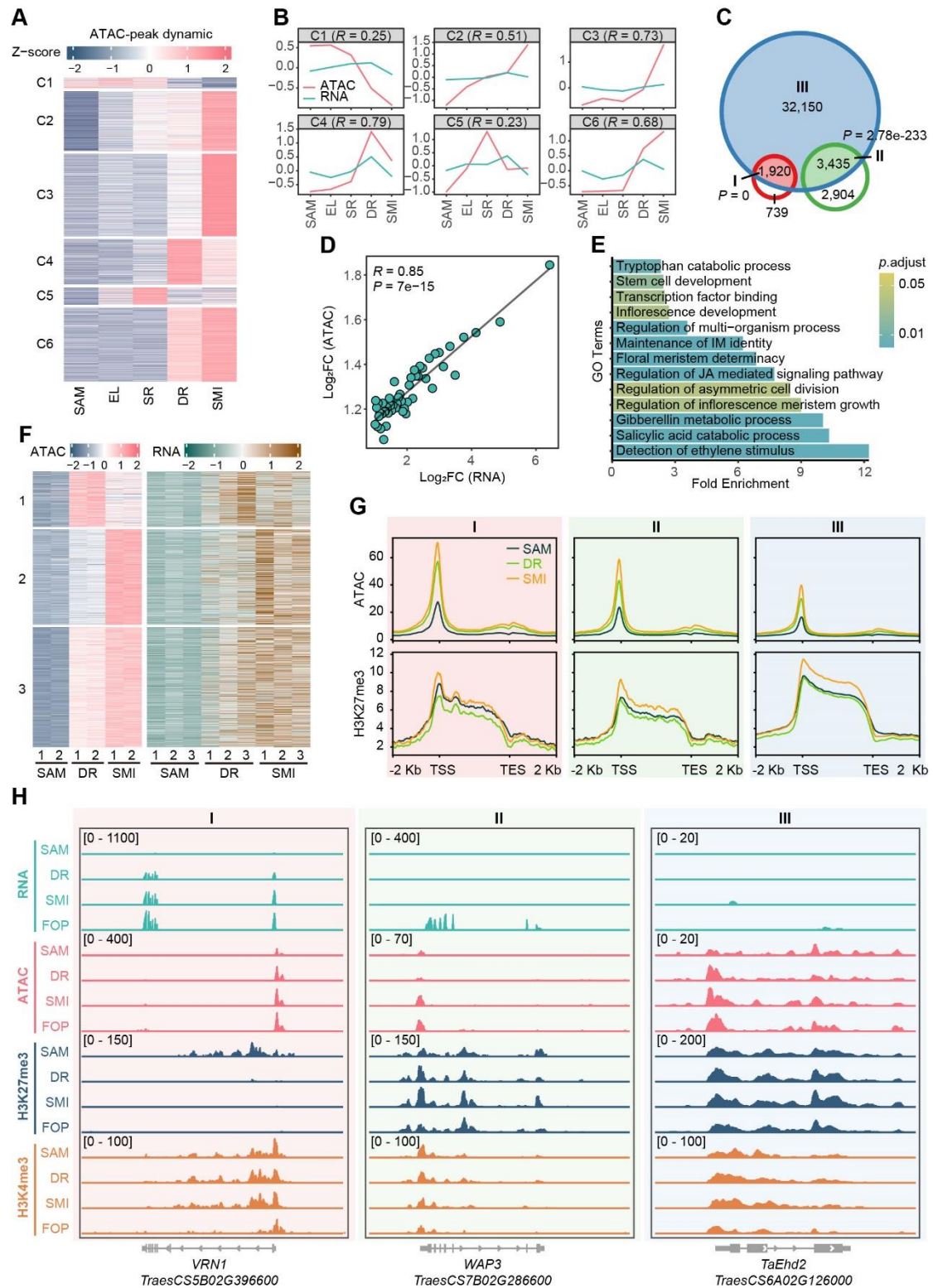
259 Indeed, H3K27me3 was closely related to transcriptional pattern of gene set I, II, III,
260 while other histone modifications such as H3K27ac, H3K4me3, H2A.Z were not
261 specifically correlated (Figure 2G and Supplemental Figure 2E). In details, genes in set
262 I have relative higher chromatin accessibility at SAM stage, and sharply increased
263 openness at DR and SMI stages, while reduced H3K27me3 level at DR as compared to
264 SAM (Figure 2G). As example, well known flowering promoting gene *TaVRN-B1*
265 shows increased chromatin accessibility and decreased H3K27me3 in genic region,
266 while no change for H3K4me3 from SAM to DR and SMI stages (Figure 2H). Whereas
267 for gene set II, though chromatin accessibility is elevated at SMI and GPD stages, but
268 the relative openness is low. Meanwhile, H3K27me3 level doesn’t change from SAM
269 to DR, even increased at SMI stage. The combined effect may result in the ‘primed’
270 transcriptional status, which gene expression level increased later with the reduction of
271 H3K27me3, such as *WAP3* (Figures 2G and 2H). For gene set III, lowest chromatin

272 accessibility gained and highest H3K27me3 coverage is observed, which can restrict
273 gene activation (Figure 2G), as the case for *TaEhd2* gene (Figure 2H).

274

275 In summary, from vegetative to reproductive transition, the genome-wide chromatin
276 accessibility increased and H3K27me3 reduced at selective part of genome that create
277 a permissive environment for activation of inflorescence meristem genes and initial the
278 reproductive development of shoot apex.

279



280

281 **Figure 2. Chromatin accessibility and H3K27me3 dynamics facilitate vegetative-**
 282 **to-reproductive transition of shoot apex.**

283 (A) K-means clustering of differential accessible promoter regions (DAPR) across the
 284 samples collected at stage from SAM to SMI.

285 (B) The chromatin accessibility and gene expression tendency for each cluster in A. R

286 value stands for the Pearson correlation coefficients.
287 (C) Overlap between genes with gained-chromatin accessibility of cluster 2, 3, 4, 6 in
288 (A) (blue circle), elevated expression at DR and SMI stage versus SAM stage (red
289 circle) and up-regulated at GPD, FMI, FOP stage versus former stages (green circle).
290 See also Supplemental Figures 2A and 2B. Geneset I showing overlap between red
291 and blue circle genes; geneset II showing overlap between red and green circle; while
292 rest genes from red circle belong to geneset III. Hypergeometric test was used to
293 calculate P-value for the enrichment of geneset I and II.
294 (D) Correlation of gene expression elevation (x-axis) and chromatin accessibility gain
295 (y-axis) from SAM to DR or SMI based on 1920 genes in geneset I in (C). Genes
296 were ranked by fold change of elevated expression and separated into 50 bins.
297 (E) GO enrichment analysis of 1920 genes in gene set I.
298 (F) Synchronous pattern of gene set I between the gain of chromatin accessibility and
299 elevation of gene expression from SAM to DR or SMI stage. Individual bio-replicate
300 are shown separately.
301 (G) Chromatin accessibility (top) and H3K27me3 (bottom) levels of genes in gene set
302 I, II, III at SAM, DR and SMI stages.
303 (H) IGV browser showing expression, chromatin accessibility and histone
304 modifications change at representative genes of *VRNI* (gene set I-left), *WAP3* (gene
305 set II-middle) and *TaEhd2* (gene set III-right).

306

307 **Gene co-expression and transcriptional regulatory network for spike architecture** 308 **formation**

309 Following the initiation of reproductive growth (DR), inflorescence undergoes several
310 processes, including SMI, GPD, FMI, and FOP to form the spike architecture (Figure
311 1A and Figure 3A). This is critical for determining SNS, contributing to grain yield.
312 We thus analyzed the genes co-expression and regulatory network to highlight the
313 potential factors that may govern the process.

314

315 We calculated the pseudo time of stages covering SMI to FOP based on PCA distance
316 (Figure 3A). GPD and FMI stages are close to each other, while distinct from SMI and
317 FOP. A total of 8,200 DEG was identified in the process of spike architecture formation
318 (any DEG among SMI, GPD, FMI, FOP), which could be clustered into 6 categories
319 (Figure 3B and Supplemental Table 7). Floral meristem determinacy and various
320 hormone metabolic genes are highly expressed at SMI; hormone signaling, polarity
321 establishment genes are active at GPD and FMI; while floral organ identity, polarity

322 specification genes are elevated at FOP (Figures 3B and 3C). Among those stage
323 specific DEGs, we looked for enriched TFs. Within clusters 3 and 6, ERF and WRKY
324 TFs are enriched and highly expressed at SMI stage. For GPD and FMI stages, NF-Y
325 and SBP TFs are abundantly expressed within clusters 2 and 4. Whereas, MADS box
326 TFs are outstanding at FOP stage of cluster 5 (Figures 3B and 3C). Such expression
327 pattern fits well with the spatiotemporal switch of meristem identities in the context of
328 hormone regulation (Kellogg, 2022; Koppolu et al., 2022), and indicates potential
329 importance of individual TFs family in driving the transcriptional network that
330 governing spike formation.

331

332 Accessible chromatin regions provide docking sites for TF binding. Binding motifs of
333 AP2/ERF, bHLH and SPL showed greatest accessibility variability during SMI to FOP
334 (Figure 3D), suggesting the potential importance of those TFs. Based on the TF-motif
335 recognition pairs, in combination with the co-expression pattern of TFs and
336 downstream targets, we are able to construct a hierarchy TRN by integrating the multi-
337 stages ATAC-seq and RNA-seq data (Supplemental Figure 3A). Totally, 36,908 pairs
338 of TFs-target genes interactions were identified, covering a total of 5,106 genes, of
339 which 4,916 pairs were regulated between TFs (Supplemental Figure 3B). A clear
340 sequential regulatory relation among time-serial expressed gene clusters from SMI to
341 FMI is observed as C6-C3-C2-C4 (Figure 3E). Whereas, FOP specific expressed genes
342 in C1 and C5 are separated and regulated each other (Figure 3E), indicating different
343 transcriptional regulatory networks between spikelet and floret development. TFs of
344 ERF, B3, TCP, DOF and MIKC-MADS may play core regulatory role in this network
345 as more targets being identified (Figure 3F and Supplemental Figure 3C). Some
346 functionally characterized factors that involved in wheat spike development can be
347 found in the network, such as *TaTb1* (Dixon et al., 2018), *VRN1* (Li et al., 2019),
348 *TaFUL3* (Li et al., 2019) (Figure 3G), indicating the capability of our TRN in capturing
349 important regulators.

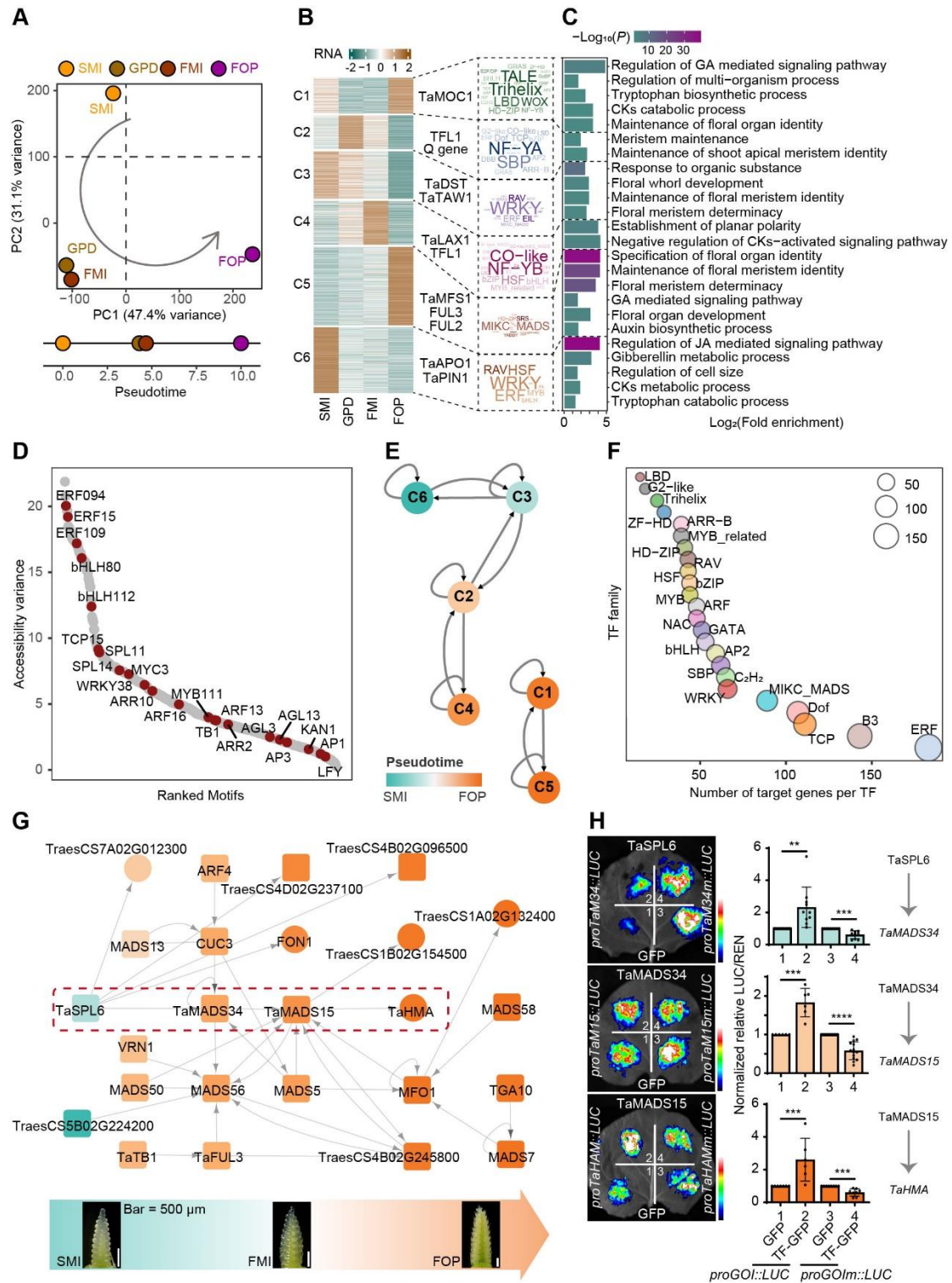
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351 To validate the prediction power of the hierarchy of transcriptional regulation among
352 TFs, we extract a small module that contains factors being functionally studied
353 individually in other crops but without known of regulatory relations, such as *SPL6*
354 (Wang et al., 2018), *MADS34* (Lin et al., 2014; Meng et al., 2017; Zhu et al., 2022),
355 *MADS15* (Wang et al., 2010; Wu et al., 2017) (Figure 3G). From their temporally
356 expression pattern (Supplemental Figure 3D) and the presence of specific motifs in the
357 open chromatin of each gene (Supplemental Figure 3E), we speculate a positive
358 transcriptional regulation hierarchy following *SPL6*-*MADS34*-*MADS15*-*HMA*
359 module. Indeed, we observed a positive transcriptional regulatory circuit among them
360 by using luciferase reporter assay in tobacco leaves (Figure 3H and Supplemental
361 Figure 3F). As well, such regulation circuit is dependent on the TF-motif specific
362 recognition (Figure 3H and Supplemental Figure 3F).

363

364 Thus, the TRN constructed could well predict the potential important regulatory factors
365 and their transcriptional regulatory relationships during the process of spike
366 development.

367



368

369 **Figure 3. Transcriptional regulatory network (TRN) governing spike architecture**
 370 **formation.**

371 (A) Principal-components plots of gene expression from SMI to FOP stages. The
 372 developmental time units (DTU) values calculated based on scaled straight
 373 distance between two adjacent stages was shown in the lower panel.

374 (B) K-mean clustering of DEGs from SMI to FOP stage and representative genes in

- 375 each cluster.
- 376 (C) TF family enrichment and GO enrichment analysis within each cluster in B.
- 377 (D) Motif accessibility variance from SMI to FOP stage.
- 378 (E) Hierarchical transcriptional regulations of sequentially expressed gene clusters
379 from (B). *P*-value was determined by the hypergeometric test.
- 380 (F) Average target genes number of each TF family in the TRN. The size of dot
381 represents number of target genes.
- 382 (G) TRN for represented key TFs participated in spike development, from SMI to FOP
383 stages. Genes were roughly ranked by the expression timing from left to right as
384 indicated by different colors gradients. TFs and non-TF coding target genes were
385 in solid rectangle or circle, respectively. The dashed red rectangle frame indicates
386 a four-layer regulation module tested in (H).
- 387 (H) Luciferase reporter assays validation of transcriptional regulation among
388 representative TF-target pairs as indicated. Mutations of the TF binding sites were
389 introduced in the promoter region of target genes separately. Student's *t* test was
390 used for the statistical significance (**, $p \leq 0.01$; ***, $p \leq 0.001$).

391

392 **Integration of multi-data to systematically screen regulators shaping spike** 393 **development**

394 The open region of chromatin is crucial for the establishment of transcriptional
395 regulatory relationships (Klemm et al., 2019). Indeed, more binding motifs of TFs are
396 significantly present in the open region of chromatin (Figure 4A), with lower DNA
397 variation at the TFs binding footprints (Figure 4B) (Zhou et al., 2020). Furthermore,
398 the open chromatin regions contain significantly higher GWAS signals with related to
399 spike morphology traits from previous publication (Figure 4C and Supplemental Table
400 8). The QTL frequency of open chromatin regions quintupling that of the whole genome
401 totally, with analogical results obtained for concrete spike related traits, such as spike
402 length (SL), SNS, GNPS (Figure 4D). Take the fertile spikelet number per spike (FSPS)
403 as an example, a total of 153 SNPs passed the significance threshold from GWAS
404 analysis and considered as significant associated loci (Figure 4F and Supplemental
405 Table 9). We enlarged the candidate region to 1 Mb centered on the GWAS signal peak,
406 2,916 genes were identified as candidate genes. Among the 2,916 genes, 1762 genes
407 (60.43%, C1 and C4) present the highest openness of chromatin accessibility at DR or
408 SMI, when spikelet primordia were undergoing initiation/ differentiation and
409 considered to be critical for determination of final SNS (Figure 4E and Supplemental

410 Figure 4A). In addition, 438 genes (15.02%, C6) exhibit to most open at FMI, which is
411 crucial to the floret development and seed setting (Supplemental Table 9). Analogical
412 results were observed for SL, spikelet density (SD), and SNS (Supplemental Figure
413 4B). This result indicated that large proportion of GWAS candidate genes possess high
414 chromatin openness at key developmental stage relating to the corresponding trait.

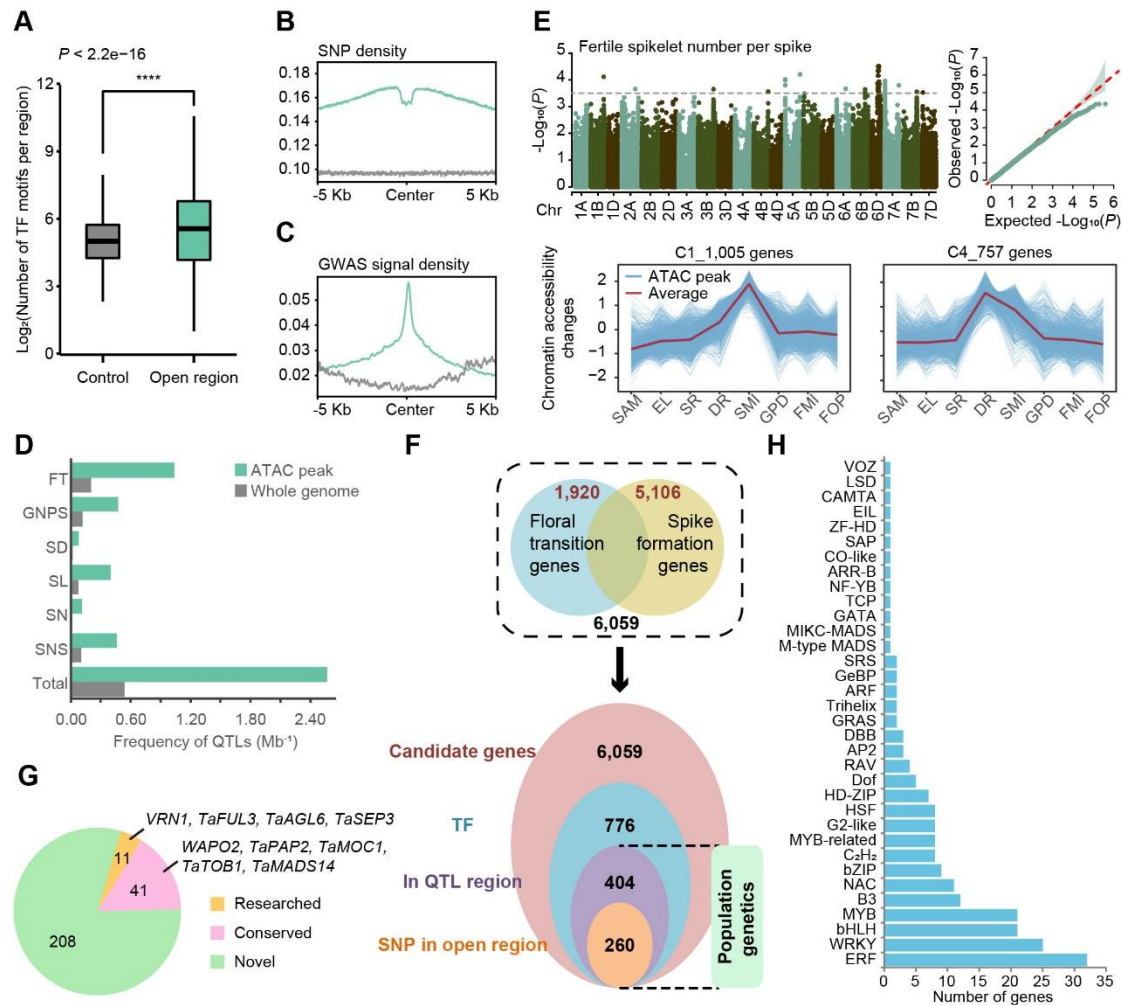
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416 We further integrate the spike traits-associated genetic regions from population genetics
417 study (Supplemental Table 8) with key regulators identified for floral transition, giving
418 the importance of regulating duration of inflorescence meristem development (Boden
419 *et al.*, 2015; Finnegan *et al.*, 2018), as well as the regulators in the TRN network that
420 potentially governing the formation of spike (Figure 4F). With focusing on TFs coding
421 genes, we got 776 candidates for further screening (Figure 4F and Supplemental Table
422 10). Among them, more than half (404 TFs) are within the spike morphological traits-
423 associated genetic regions (Figure 4F and Supplemental Table 10). We further narrow-
424 down the candidates to 260 TFs by looking for presence of SNP in the open chromatin
425 region of those TFs (Figure 4F and Supplemental Table 10), which is likely to affect
426 the transcriptional regulatory circuit and rewire the TRN of spike development.

427

428 Of the candidate TFs, we categorize them into three classes, termed as ‘researched’ for
429 functional studied in wheat and ‘conserved’ as orthologue being studied in other species
430 as well as ‘novel’ for functional unknown for wheat and other crops (Figure 4G).
431 Among the 260 TFs, 11 factors are ‘researched’ in wheat, including *VRN1* (Li *et al.*,
432 2019), *TaFUL3* (Li *et al.*, 2019), *TaAGL6* (Kong *et al.*, 2022), *TaSEP3* (Zhang *et al.*,
433 2021). Another 41 factors are ‘conserved’, including *MOC1* (Zhang *et al.*, 2015), *PAP2*
434 (Kobayashi *et al.*, 2010; Kobayashi *et al.*, 2012), *MADS14* (Wu *et al.*, 2017), etc. (Figure
435 4G and Supplemental Table 11). Of note, majority of candidates identified are ‘novel’
436 TFs, as many as 208, without study in wheat or other crops. These TFs are enriched in
437 ERF, WRKY, bHLH, MYB, B3 families (Figure 4H and Supplemental Table 11). Thus,
438 by integrating of multi-dimensional data, we have identified potential key regulators
439 for spike development.

440



441

442 **Figure 4. Screening for key TFs in spike development by integration of multi-**
 443 **dimensional data.**

444 (A-C) Enrichment of TF binding motifs (A), SNP density (B) and GWAS signals of
 445 multi-traits (C) in open chromatin regions. Green and gray refer to open chromatin
 446 regions and control regions, respectively.

447 (D) The QTL frequency of spike morphology related traits in open chromatin regions
 448 (light green bar) and whole genome (gray bar). The QTL frequency of each trait
 449 were determined with QTL numbers divided by physical length of the open
 450 chromatin regions and whole genome, respectively.

451 (E) The chromatin openness patterns of fertile spikelet number per spike (FSPS) trait
 452 correlated candidate genes. Manhattan plot (up-left) and Quantile-Quantile plot
 453 (up-right) showing the significant associated signals of FSPS using a mixed linear
 454 model (MLM). The chromatin accessibility change showed GWAS signal
 455 associated genes specifically open at DR and SMI (low), with average trends
 456 highlighted in red. The y-axis represents z-scaled chromatin accessibility across
 457 developmental stages.

458 (F) Schematic of the strategy used for key factors identification for shaping spike

459 architecture.

460 (G) Pie chart summary of candidate factors for spike development in each category as
461 indicated. Represented genes' name were listed.

462 (H) Gene number of corresponding TF families for the 208 potential novel TFs
463 governing spike development.

464

465 **Validation of the genetic regulatory hierarchy of 'conserved' factors for spike** 466 **development**

467 Among the 'conserved' factors, we again found *TaSPL6*, *TaMADS34*, *TaMADS15*
468 (Figure 4G), as showing up in the TRN governing spike development (Figures 3G and
469 3H). Thus, we wonder whether these factors are indeed involved in shaping spike
470 architecture in wheat.

471

472 *In situ* hybridization finely characterize the spatial-temporal expression profile of these
473 factors (Figure 5A). *TaSPL6* was weakly expressed in the SAM, fixedly expressed in
474 the IM tip, spikelet primordia and floret meristem. *TaMADS34* and *TaMADS15* were
475 both expressed throughout the SAM, SMI and FOD stages and highly expressed in the
476 spikelet and floral primordia. *TaHMA*, a downstream target of *TaMADS15*, also
477 expressed in the spikelet and floral primordia. Similar spatiotemporal expression
478 pattern supports their transcriptional regulatory hierarchy and potential role in spikelet
479 or floret development. Furthermore, we generate RNAi transgenic wheat of *TaSPL6*,
480 *TaMADS34* and *TaMADS15*, respectively (Supplemental Figure 5A). As expected,
481 *TaMADS34-RNAi* and *TaMADS15-RNAi* plants exhibit similar phenotypes, with
482 shorter SL, decreased SNS and GNPS (Figures 5B-5D and Supplemental Figure 5B).
483 *TaSPL6-RNAi* plants also show altered spike developmental phenotype, but with longer
484 SL, increased SNS and GNPS (Figures 5B-5D and Supplemental Figure 5B). This is
485 likely because that *TaSPL6* is on the top layer of hierarchy TRN, it can regulate multi-
486 targets in addition to *TaMADS34* (Figure 3G). Consistent with the LUC reporter assay
487 *in vitro* (Figure 3H), in *TaSPL6-RNAi* transgenic wheat, we observed significant
488 reduced expression of *TaMADS34* (Figure 5E), further indicating that *TaSPL6*
489 positively regulate *TaMADS34*. Similar expression pattern was observed for supporting

490 that TaMADS34 activated *TaMADS15*, and TaMADS15 promoted *TaHMA* (Figure
491 5E). Therefore, the spike developmental defects of TF-RNAi lines, similar
492 spatiotemporal expression pattern and relative genes expression level in transgenic
493 wheat prove that TaSPL6-TaMADS34-TaMADS15-*TaHMA* functions as a regulatory
494 module during wheat spike development.

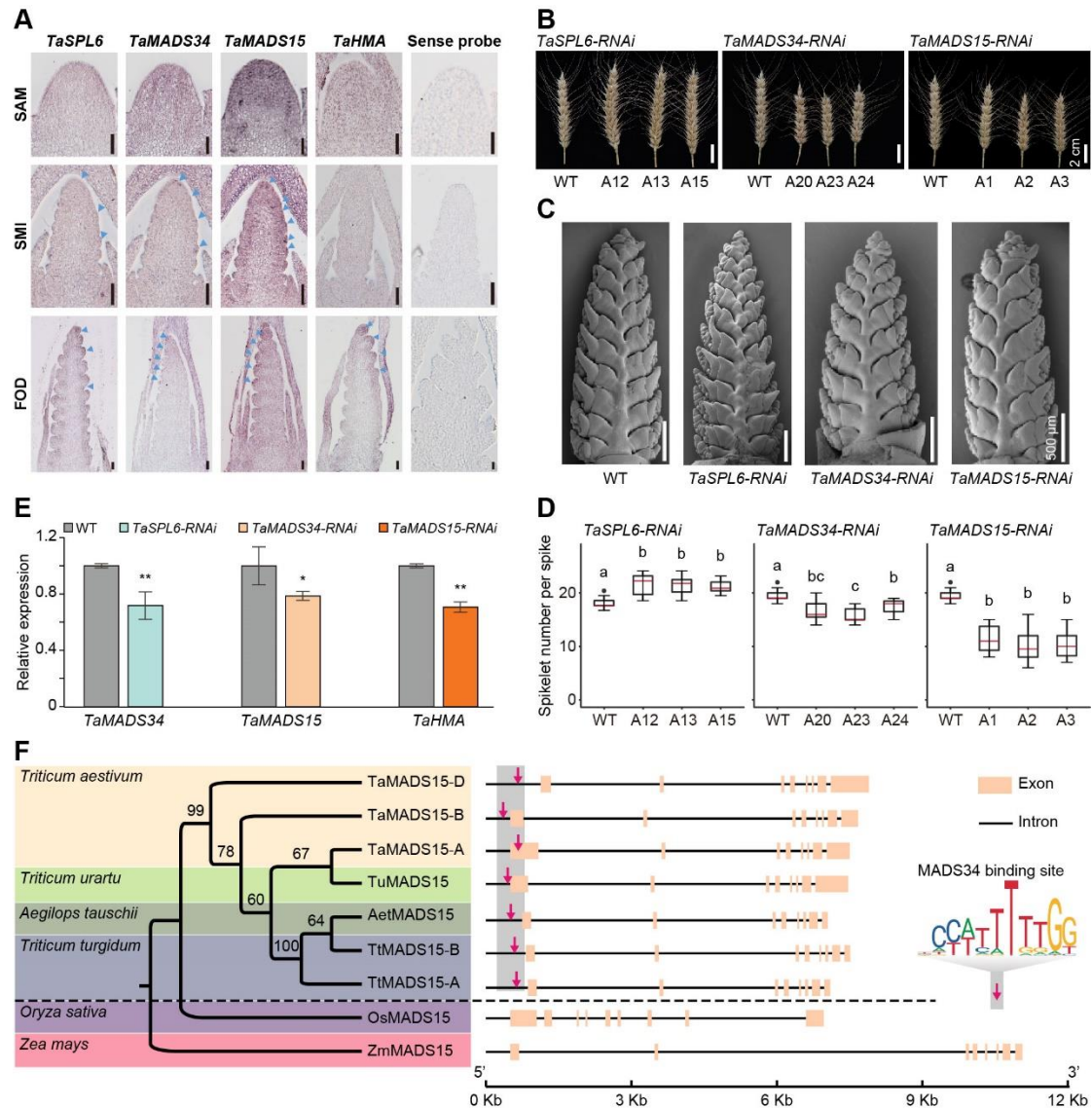
495

496 We further analyzed the conservation of this hierarchy regulatory module among
497 TaSPL6-TaMADS34-TaMADS15-*TaHMA* in *Triticum* and *Gramineae* from an
498 evolutionary perspective (Figure 5F). In diploid wheat AA, DD, tetraploid wheat
499 AABB, and hexaploid wheat AABBDD, there is a MADS34 binding motif
500 (CCATTTTGG) in the similar promoter region of the *MADS15* homologous genes
501 (Figure 5F). Similarly, in the promoter region of *MADS34* homologous genes, the
502 binding motif of SPL6 (CCGTACGG) exists (Supplemental Figure 5C). This indicates
503 that this regulatory module may be conserved within *Triticum*. However, in rice (*Oryza*
504 *sativa*) or Maize (*Zea mays*), the promoter region of *MADS15* lacks the MADS34
505 binding motif, the promoter region of *MADS34* lacks the SPL6 binding motif,
506 indicating that this regulatory module may be differentiated in grasses (Figure 5F and
507 Supplemental Figure 5C).

508

509 Thus, the ‘conserved’ factors we identified were indeed involved in regulating spike
510 development in wheat, and the hierarchy transcriptional regulatory circuit is likely
511 maintained within *Triticum* tribe.

512



513

514 **Figure 5. SPL6-MADS34-MADS15-HMA module regulates spike development in**
515 **wheat.**

516 (A) Spatiotemporal expression patterns of *TaSPL6*, *TaMADS34*, *TaMADS15* and
517 *TaHMA* at different spike developmental stages, as indicated by *in situ*
518 hybridization. Sense probe is used as negative control. Scale bars = 100 μ m.

519 (B) Spike developmental defects of T2 RNAi transgenic plants of *TaSPL6*, *TaMADS34*,
520 *TaMADS15* as compared to wild-type (WT) Fielder. Scale bars=2 cm.

521 (C) Scanning electron micrographs (SEM) of young spikes from WT and RNAi
522 transgenic plants of *TaSPL6*, *TaMADS34*, *TaMADS15* at DR, GPD and FOP stages.
523 Scale bars = 500 μ m.

524 (D) Quantification of spikelet number per spike (SNS) between WT, *TaSPL6-RNAi*,
525 *TaMADS34-RNAi* and *TaMADS15-RNAi* transgenic plants. Two-tailed Student's t-
526 tests. In box plots, the box limits indicate the 25th and 75th percentiles, the
527 whiskers indicate the full range of the data, and the centre line indicates the
528 median. Different letters mean significant difference at $p < 0.01$.

529 (E) The expression level of *TaMADS34*, *TaMADS14*, *TaHMA* in WT or *TaSPL6-RNAi*,
530 *TaMADS34-RNAi*, *TaMADS15-RNAi* transgenic plants. Expression level of genes
531 in WT is set as 1.0, the relative expression of each gene in RNAi plants is shown
532 as average \pm SD of three replicates. Student's *t* test was used for the statistical
533 significance (*, $p \leq 0.05$; **, $p \leq 0.01$).

534 (F) The conservation of TaMADS34 binding motif (CCATTTTGG) at chromatin
535 accessible region of *MADS15* orthologs in different *Triticum*, but not *Oryza sativa*
536 or *Zea mays*. Phylogenetic tree of corresponding species is indicated on the left.
537 Schematic diagram of gene structure and presence or absence of MADS34 binding
538 CARG motif sites is shown on the right.

539

540 **Verification of novel regulators for spike development**

541 To evaluate our strategy of identifying novel regulators for wheat spike development,
542 we investigated the spike developmental defects of meta TILLING mutant lines in
543 Kronos (Krasileva et al., 2017; Uauy et al., 2009), Cadenza (Krasileva et al., 2017) and
544 KN9204 (we generated and did exome sequenced, unpublished), of which the mutated
545 sites were identified by whole exome sequencing.

546

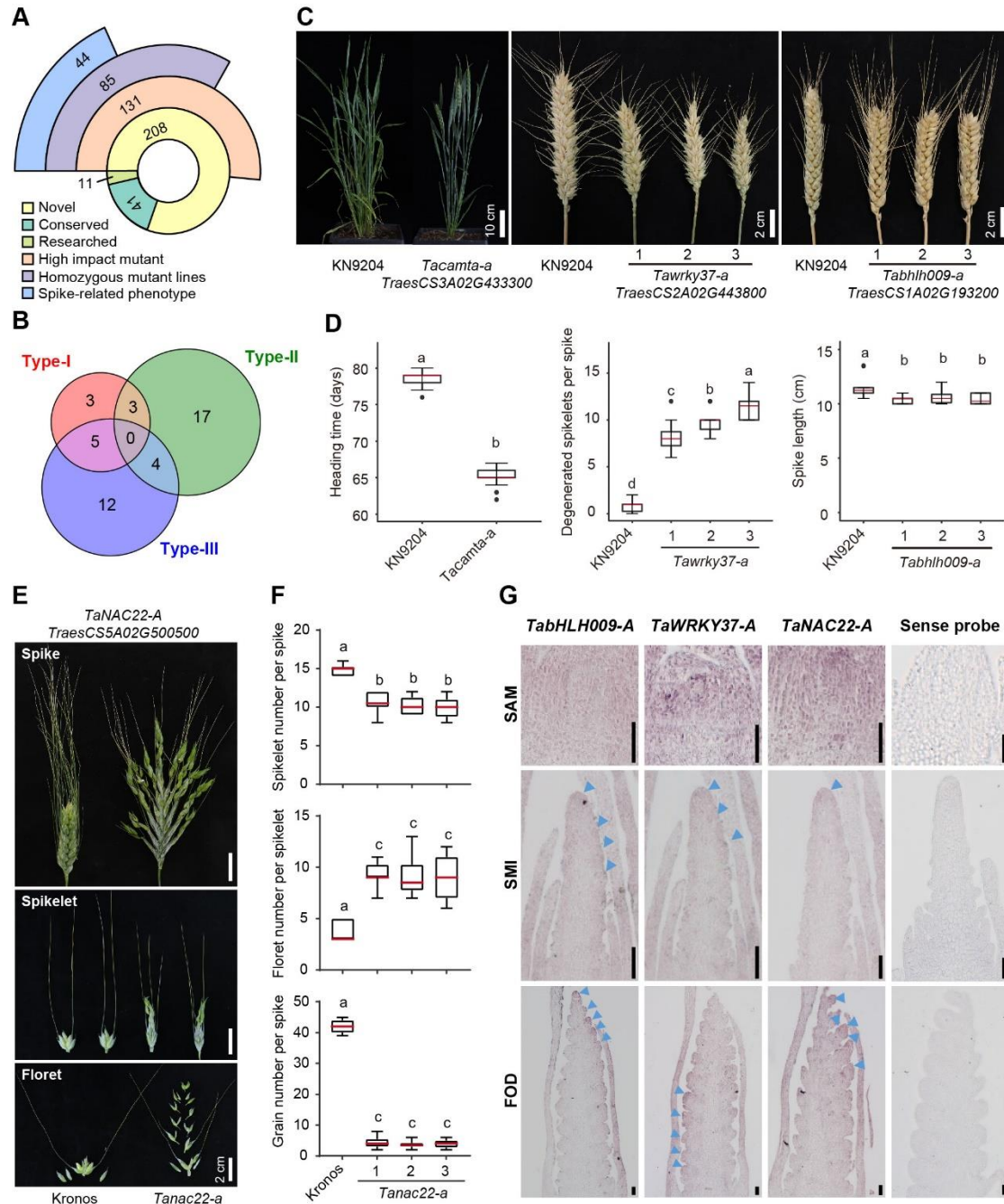
547 Of the 208 novel TFs, 131 TFs were found to have at least one mutant line that
548 containing loss-of-function mutation (Figure 6A and Supplemental Table 11). Among
549 the 85 TFs with homozygous TILLING mutant lines, 44 TFs (51.76%) exhibited spike
550 developmental defects within three major types, including flowering time difference
551 (Type I, n=11), degeneration of spikelet or floret (Type II, n=24), and altered SL or
552 SNS (Type III, n=21) (Figure 6B and Supplemental Table 11). Representative mutant
553 lines of different types were shown (Figures 6C and 6E and Supplemental Figure 6).
554 For instance, *TraesCS3A02G433300* (*TaCAMTA-A*) mutant showed an early flowering
555 phenotype with about 2 weeks earlier than the control line; *TraesCS2A02G443800*
556 (*TaWRKY37-A*) mutant showed more degenerated spikelet number per spike (DSNS)
557 at basal part of inflorescence, resulted in decreased SNS/GNPS; *TraesCS1A02G193200*
558 (*TabHLH009-A*) mutant showed reduced spike length and increased spikelet density
559 (Figures 6B-6D and Supplemental Figure 6A). The similar phenotype of *TaWRKY37-*
560 *A* and *TabHLH009-A* genes also exhibit in the Cadenza mutant (Supplemental Figures
561 6B and 6C). The loss-of-function mutant of *TraesCS5A02G500500* (*TaNAC22-A*) in

562 the tetraploid wheat Kronos (*Triticum turgidum*) exhibits increased floret number per
563 spikelet (about 10 florets in a spikelet) due to non-terminated floral primordium
564 differentiation, and most of the florets were abortive which result in decreased GNPS
565 (Figures 6E and 6F). We further confirmed the spatiotemporal expression pattern of
566 those genes by *in situ* hybridization (Figure 6G). Consistent with their morphologic
567 defects, *TaWRKY37-A*, *TabHLH009-A* and *TaNAC22-A* genes are all expressed at the
568 spikelet initiation region and the spikelet/floral primordia; while *TaWRKY37-A* is
569 highly expressed in the IM tip and the spikelet and floral meristem at the base of spike,
570 *TabHLH009-A* is highly expressed in the spikelet and floral meristem at the upper
571 region of spike, and *TaNAC22-A* has significant expression in the SAM and floral
572 primordia (Figure 6G).

573

574 Taking the advantage of available TILLING mutant lines, we have proved that novel
575 TFs identified by our integration strategy played important role in regulating spike
576 development. The strategy used for screening novel factors is efficient and the
577 identified factors were worth for in-depth study.

578



579

580 **Figure 6. Novel factors identified regulate spike development.**

581 (A) Summary of novel TFs with different categories of KN9204 TILLING mutant lines.

582 (B) Venn-diagram of different type of spike-related phenotype presented in KN9204
583 TILLING mutant lines containing homozygous mutation at novel TFs coding
584 genes.

585 (C) Represented KN9204 TILLING mutant lines of type I/II/III spike developmental
586 defects as compared to control KN9204. Type I, CAMTA (*TraesCS3A02G433300*,
587 *Tacamta-a*), Scale bar = 10 cm; type II, WRKY (*TraesCS2A02G443800*,
588 *Tawrky37-a*), Scale bar = 2 cm; type III, bHLH (*TraesCS1A02G193200*,
589 *Tabhlh009-a*), Scale bars = 2 cm.

- 590 (D) Quantification of different types of spike developmental defects in represented
591 KN9204 TILLING mutant lines, as heading date of *Tacamta-a*, degenerated
592 spikelet number per spike (DSNP) of *Tawrky37-a*, spikelet density of *Tabhllh009-*
593 *a*, the Wild-type is KN9204. Two-tailed Student's *t*-tests. Different letters mean
594 significant difference at $p < 0.01$.
- 595 (E) The spike developmental defect of *TraesCS5A02G500500* (*TaNAC22-A*) mutant
596 lines as compared to control Kronos. Scale bars =2 cm.
- 597 (F) Quantification of floret number per spikelet (FNPS), gain number per spike
598 (GNPS), spikelet number per spike (SNS) for represented Kronos TILLING
599 mutant lines containing mutation of *TraesCS5A02G500500* (*Tanac22-a*). Two-
600 tailed Student's *t*-tests. In box plots, the box limits indicate the 25th and 75th
601 percentiles, the whiskers indicate the full range of the data, and the centre line
602 indicates the median. Different letters mean significant difference at $p < 0.01$.
- 603 (G) Spatiotemporal expression patterns of *TaWRKY37-A*, *TabHLH009-A* and
604 *TaNAC22-A* at different spike developmental stages, as indicated by *in situ*
605 hybridization. Sense probe is used as negative control. Scale bars = 100 μm .

606

607 **Regulation of wheat spike architecture by novel factor TaMYB4-A**

608 To further explore the potential application of integrated TRN and GWAS analysis in
609 dissecting molecular function of individual gene in wheat spike development, we took
610 a novel regulator TaMYB4-A for in-depth study.

611

612 *TraesCS6A02G224200* (*TaMYB4-A*) was one of the TFs from the TRN (Supplemental
613 Figure 3B). It also located within a genetic region that was significantly associated with
614 FSPS by GWAS analysis (Figure 7A and Figure 4F). Two haplotypes (C-type Vs T-
615 type) of *TaMYB4-A* could separate a natural population of 214 wheat varieties with
616 significant differences in FSPS (Figure 7B). Consistently, we find *TaMYB4-A* is
617 initially expressed in the spikelet initiation region at DR stage, and highly expressed in
618 the spikelet and floral primordia during SMI and FMI stages by *in situ* hybridization
619 (Figure 7C), which fits its role in determining spikelet number and. Further, we
620 obtained loss-of-function mutant lines of *TaMYB4-A* from KN9204 and Cadenza
621 TILLING libraries (Figure 7D and Supplemental Figure 7A). Mutation of *TaMYB4-A*
622 significantly reduced SL, SNS, GNPS (Figures 7D and 7E and Supplemental Figures
623 7B and 7C), which further confirmed its function in regulating spikelet development.

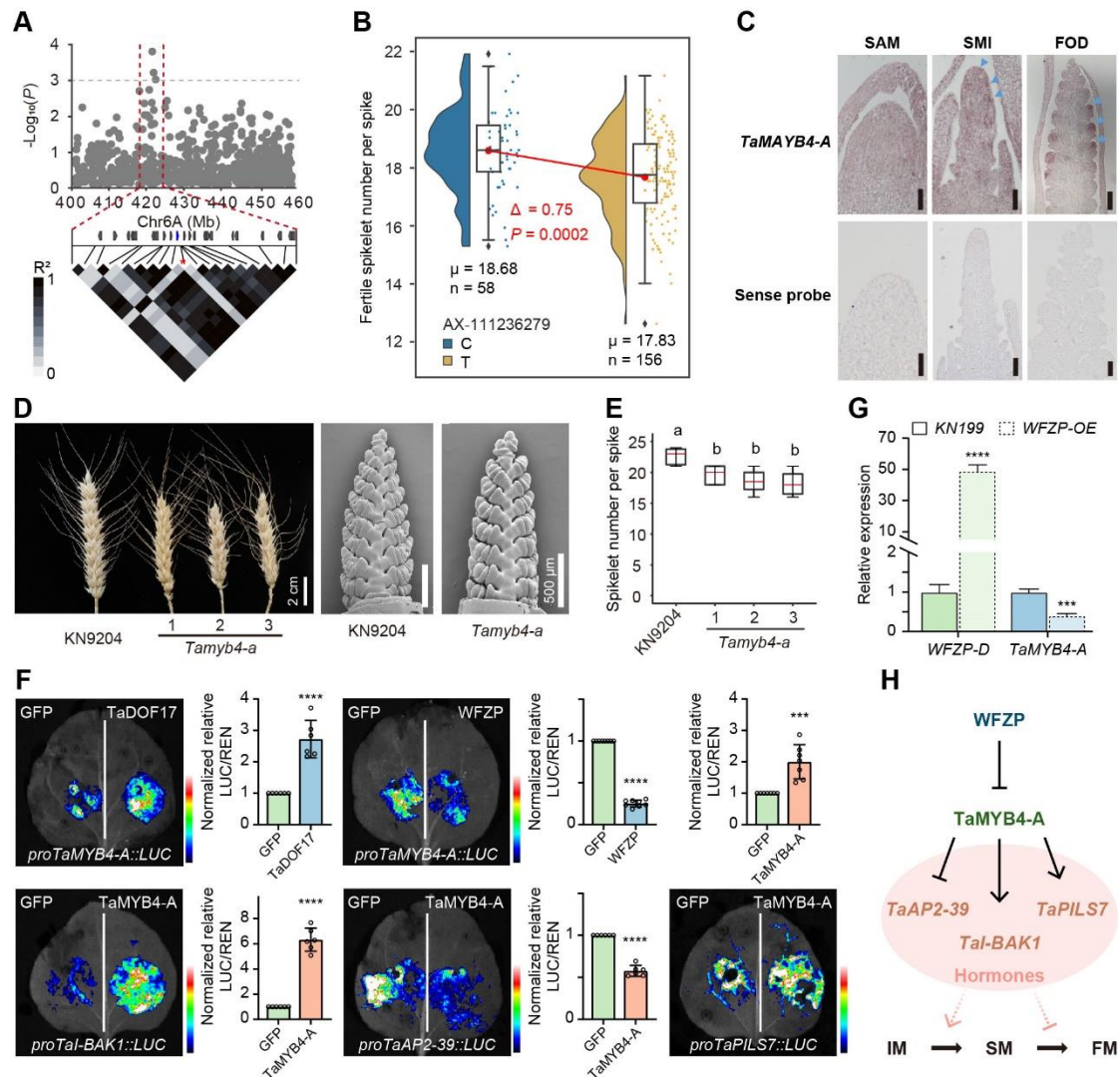
624

625 We further study the molecular regulation network of *TaMYB4-A* in mediating spike
626 development. Based on the TRN generated, we extract the hierarchy transcription
627 regulatory module containing *TaMYB4-A* (Supplemental Figure 7D). Among the
628 potential upstream regulators of *TaMYB4-A*, we further confirmed that WFZP and a
629 DOF type TF *TaDOF17* (TraesCS2B02G592700) could repress or active *TaMYB4-A*
630 in the reporter assay in tobacco leaves, respectively (Figure 7F and Supplemental Figure
631 7E). This also fits with the temporal expression pattern of *WFZP*, *TaDOF17* and
632 *TaMYB4-A* during spike formation process. *TaDOF17* and *TaMYB4-A* showed similar
633 pattern but *TaDOF17* changed ahead of *TaMYB4-A*, whereas *WFZP* elevated after SMI
634 with *TaMYB4-A* reduction after SMI stage (Supplemental Figure 7F). *WFZP* repression
635 of *TaMYB4-A* is further evidenced by down regulation of *TaMYB4-A* in *WFZP* gain-of
636 function transgenic wheat (Figure 7G) (Li et al., 2021). Among the numerous potential
637 downstream targets of *TaMYB4-A* (Supplemental Figure 7D), several genes were
638 confirmed to be regulated by *TaMYB4-A* in reporter assay in tobacco leaves (Figure
639 7F). This includes *TaAP2-39* (TraesCS2D02G425700), of which homologous being
640 proved to regulate the development of tiller and panicle/inflorescence by controlling
641 the balance of ABA/GA in rice (Yaish et al., 2010), and *TaI-BAK1*
642 (TraesCS7D02G416900), with homologous encoding a Brassinosteroid insensitive 1-
643 associated kinase 1, reported to increase panicle length and grain number per panicle
644 when overexpressed in rice (Khew et al., 2015), as well as *TaPILS7*
645 (TraesCS5A02G354300), encoding an auxin efflux carrier component (Figure 7F).
646 Consistently, *TaAP2-39*, *TaI-BAK1* and *TaPILS7* showed a synchronized temporal
647 expression pattern as *TaMYB4-A* during spike formation process (Supplemental Figure
648 7F).

649

650 Thus, *TaMYB4-A* positively regulates fertile spikelet likely through regulating
651 hormones homeostasis and/or signaling, acting downstream of and repressed by *WFZP*
652 (Figure 7H).

653



654

655 **Figure 7. The novel factor TaMYB4-A regulates spike architecture.**

656 (A) *TaMYB4-A* located in a GWAS signal associated with FSPS. A manhattan
657 locuszoom were plot with gene models and linkage disequilibrium plot of SNPs
658 shown below, in 5-Mb physical scale centered on the peak SNP.

659 (B) FSPS distribution between haplotypes (C and T) defined by the peak SNP. The bars
660 within raincloud box plots represent 25th percentiles (haplotype T), medians, and
661 75th percentiles (haplotype C). Mean values of two haplotypes were linked by a
662 red line, and one-way ANOVA was used to determine significant differences.

663 (C) Spatiotemporal expression pattern of *TaMYB4-A* is indicated by *in situ*
664 hybridization. Scale bars = 100 μ m.

665 (D) The spike phenotype and scanning electron micrographs (SEM) of *TaMYB4-A*
666 (*TraesCS6A02G224200*) mutant from the KN9204 TILLING mutant library. Scale
667 bars =2 cm (left), 500 μ m (right).

668 (E) Statistics comparison of spikelet number per spike (SNS) between KN9204 and
669 *Tamyb4-a* mutant lines. Two-tailed Student's *t*-tests. In box plots, the box limits
670 indicate the 25th and 75th percentiles, the whiskers indicate the full range of the
671 data, and the centre line indicates the median. Different letters mean significant
672 difference at $p < 0.01$.

- 673 (F) Luciferase reporter assays of *TaMYB4-A* regulatory network. Schematic diagram in
674 the left part showing the vectors used in this array. Student's *t*-test was used for
675 the statistical significance. **, $p < 0.001$; ****, $p < 0.0001$.
676 (G) The expression level of *WFZP* and *TaMYB4-A* in KN199, *WFZP*-OE transgenic
677 plants by RT-qPCR. The error bars denote \pm SD. ***, $p \leq 0.001$; ****, $p \leq 0.0001$.
678 (H) The working model representing the possible function and genetic network of
679 *TaMYB4-A* in wheat.

680

681 **SNP at WFZP binding site within *TaMYB4-A* promoter mediates spike** 682 **architecture selection during breeding process in China**

683 Next, we wonder how the different haplotypes of *TaMYB4-A* could affect its function
684 and regulation of spike architecture. Interestingly, the DNA variation SNP-939 (C/T)
685 is located in the promoter region of *TaMYB4-A*, right under the *WFZP* core binding
686 motif (Figure 8A). This suggested that the C/T SNP may contribute to *TaMYB4-A*
687 expression difference via affecting *WFZP* binding. Indeed, we confirmed the *WFZP*
688 repressed *TaMYB4-A* via the recognition of the conserved motif, and such repression is
689 abolished with C-to-T mutation in reporter assay in tobacco leaves (Figure 8B). We
690 further selected 33 different wheat varieties of the two types (C-type and T-type) to
691 measure the *TaMYB4-A* expression level and spike morphology. Wheat varieties of C-
692 type showed elite agronomic traits with longer SL with more FSPS and a significant
693 higher level of *TaMYB4-A* expression, while the T-type containing wheat varieties
694 showed shorter SL, less FSPS and lower level of *TaMYB4-A* expression (Figure 8C).
695 This result provides genetic evidence that the C/T SNP could cause expression level
696 difference of *TaMYB4-A* and in turn affects SL and FSPS.

697

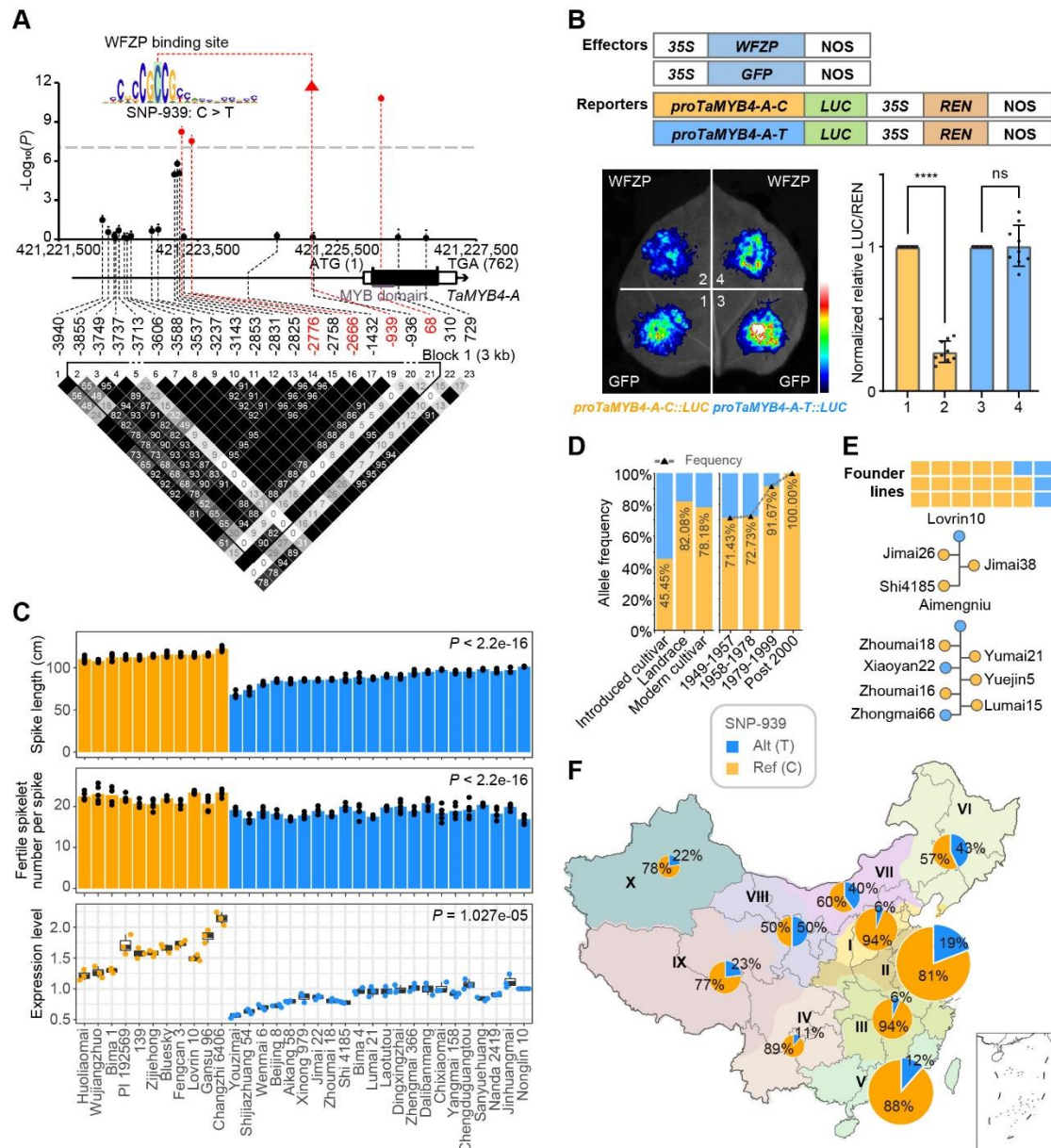
698 Furthermore, we wonder how this C/T SNP site within *TaMYB4-A* promoter being
699 selected during the breeding process in China. Based on the exome capture sequencing
700 data of the Chinese wheat mini-core collection (MCC) (Li et al., 2022a), percentages
701 of accessions carrying the reference allele (C-type) was considerably higher in
702 landraces (82.08%) and modern cultivar (78.18%) compared with introduced cultivar
703 (45.45%) (Figure 8D). Of note, the frequency of C-type was significantly elevated
704 along with the progress of breeding, indicating that the elite C-type of *TaMYB4-A* has

705 been widely used in the past breeding process of China, especially after year 1978
706 (Figure 8D). Founder parents have extensively promoted the improvement of wheat
707 varieties in China since the 1950s, including Zhoumai16, St2422/464, Nanda2419,
708 Nanda2419, St2422/464, Funo, Xiaoyan6, Aimengniu, Zhou8425B, Abbondanza and
709 Lovrin10 (Yang et al., 2022). 17 out of 21 founder lines were C-type at SNP-93, expect
710 for Aimengniu (T/T) and Lovrin10 (T/T) (Figure 8E). Importantly, the most widely
711 grown derived varieties from Aimengniu or Lovrin10 (for instance, Jimai26, Jimai38
712 and Shi4185 derived from Lovrin10, and Yumai21, Yuejin5, Lumai15, Zhoumai16 and
713 Zhoumai18 from Aimengniu) are apt to retain the C-type at SNP-939 from another
714 parent (Figure 8E). Interestingly, the alleles showed distinct distribution characteristics
715 in the major Chinese agro-ecological zones. The inferior T-type was relatively frequent
716 ($\geq 40\%$) in spring wheat regions (VI, VII, VIII), followed by winter-spring mixed
717 regions (IX and X, 22.22% and 23.08% respectively), and appears to be with low
718 frequency ($< 20\%$) in the winter wheat regions (I, II, III, IV, V). (Figure 8F).

719

720 Thus, the elite C-type allele of *TaMYB4-A* is likely originated from Chinese local
721 germplasm and widely used during the breeding process but still hold the potential to
722 be used for cultivar improvement for certain wheat production region in China.

723



724

725 **Figure 8. *TaMYB4-A* has a key SNP at WFZP binding site associate with spike**
 726 **architecture selection.**

727 (A) Association analysis between genetic variations in *TaMYB4-A*. Dots connected with
 728 the blue dashed lines indicate the variants that are significantly associated with
 729 fertile spikelet number per spike (FSPS). The schematic diagram of the ~6-kb
 730 genomics region of *TaMYB4-A* is shown, followed by a LD plot with white to
 731 black represents $r^2 = 0-1$. The SNP-939 was located in the core WFZP DNA-
 732 binding motif. Haplotype of selected 33 varieties for expression level and spike
 733 morphology determination were shown below.

734 (B) Luciferase reporter assays of *TaMYB4-A* promoter activity with the WFZP binding
 735 site C/T. Schematic diagram in the left part showing the vectors used in this array.
 736 Student's *t*-test was used for the statistical significance; ****, $P < 0.0001$; ns, no
 737 significant difference.

738 (C) Spike length and FSPS of cultivars with different haplotypes of *TaMYB4-A* based
739 on five-point data from two years and their expression level of *TaMYB4-A*. Dots
740 show data distribution (n=3 or 5 biologically independent samples), *p* values
741 calculated using two-tailed *t*-test. (D) The percentages of accessions with reference
742 and alternative allele of SNP-939 in different category (I, left) and breeding
743 periods (I, right). The line chart shows the allele frequency of reference C-type.
744 (E) The genotype of wheat founder lines and their derived cultivars. The derived
745 cultivars of Aimengniu and Lovrin10 were shown in the tree, and cultivars with
746 reference C-type were in yellow and the T-type in blue.
747 (F) The percentages of accessions with reference and alternative allele of SNP-939 in
748 different ecological zones of China. The size of pie charts in the geographical map
749 showing the number of cultivars, with percentage of the two SNP haplotypes in
750 different color (C-type, yellow; T-type, blue).
751

752 **Discussion**

753 Wheat is one of the first domesticated crops and domestication is linked with the
754 modification of inflorescence architecture to ease harvesting and improve grain yield
755 (Gauley and Boden, 2019). Increasing yields is also one of the primary goals for
756 breeding. For cereal crops, inflorescence architecture largely determines the grain
757 productivity via affecting the spikelet and floret development. Better understanding of
758 the molecular mechanism that governs inflorescence architecture would facilitate the
759 trait-designed breeding process. As compared to rice, maize and barley (Gao et al.,
760 2019; Wang et al., 2021; Yuan et al., 2020; Zhang and Yuan, 2014), the genetic and
761 molecular regulation of spike/inflorescence development in wheat is largely delayed.
762 Here, we have generated a time-serial epigenomic landscapes consisting of various
763 types of histone modifications, accessible chromatin, and transcriptomes of wheat shoot
764 apex from vegetative development to spike architecture formation. This would be a
765 valuable data resource for systematic study of molecular insights for wheat
766 inflorescence/spike development and mining of key regulators for shaping spike
767 architecture (Figure 1).

768

769 **Epigenetic layer regulation of vegetative-to-reproductive transition in wheat**

770 The meristem of shoot apex generates different primordium cells for initiation of
771 various tissues, such as leaf primordia from SAM at vegetative stage, and spikelet

772 primordia from IM during flowering transition. This transition is tightly regulated by
773 various factors, such as TaTB1, VRN1, TaFT1, Ppd1 via mediating endogenous and
774 environmental signals (Figure 1D). Here, we found during the transition from SAM to
775 DR/SMI (vegetative to reproductive growth), the chromatin accessibility is generally
776 increased (Figure 1G), showing a synchronous pattern with gene activated during floral
777 induction such as inflorescence meristem identity genes, hormone biosynthesis and
778 signaling (Figures 2B and 2F). However, open chromatin is not sufficient to active
779 genes expression, especially when H3K27me3 is covered at the genic region (Figures
780 2G and 2H). But gain-of chromatin accessibility does set a ‘primed status’ for later
781 activation of genes when the H3K27me3 is removed at a late developmental stage
782 (Figures 2G and 2H). Such chromatin status affected genes including well-known
783 flowering time gene *VRN1*, and genes involved in spikelet meristem formation, such as
784 *WAP3*, *TaFUL3*. Thus, chromatin layer regulation is associated with the transcriptional
785 status of key regulators during vegetative-to-reproductive transition in wheat.

786

787 **Integration of TRN with GWAS enables systematic and efficient identification of** 788 **key factors in determine inflorescence architecture**

789 The inflorescence of wheat is made up with spikelets and florets harbored within
790 spikelet. Wheat inflorescence is determined at the time of terminal spikelet formation,
791 which in turn affects the SNS. Whereas the arrangement of spikelet could influence the
792 floret development inside. Thus, the initiation, distribution and termination of spikelet
793 largely shape the inflorescence architecture and grain yield (Gao et al., 2019; Wang et
794 al., 2021). This is likely driven by identity transition of different primordia cells in the
795 context of hormone signaling and transcriptional regulatory network (Feng et al., 2017;
796 Qi et al., 2019). Understanding the main regulatory network and identification of key
797 factors that driving such network would give us potential candidates for shaping
798 inflorescence structure. In addition, one could expect that genetic variation on such key
799 factors within the regulatory network *per se* or variations that changing the regulatory
800 circuit could generate influence on the outcome of inflorescence architecture.

801

802 Following this logic, by taking advantage of our time-serial profiling of transcriptome
803 and epigenome dataset, in combination with TF-motif binding information in model
804 plant (Castro-Mondragon et al., 2022), we build-up a transcriptional regulatory
805 networks that likely governs the spike formation after floral transition (Figure 3).
806 Numerous TFs are identified to take part in the TRN, including functional studied
807 factors such as *VRN1*, *TaTBI*, *TaFUL3* in wheat and TFs from MADS-box, ARF, SPL
808 families that being reported to regulate inflorescence formation in other crops (Figure
809 3) (Liu et al., 2022a; Ram et al., 2020; Rong et al., 2018; Wang et al., 2022; Xu et al.,
810 2016). On top of this, we combined public available GWAS or QTL analysis with focus
811 on the traits related to spike development to filter for those TFs located within the
812 GWAS associated genetic regions and have SNP in the regulatory open chromatin
813 regions that likely affects the transcriptional regulation circuit (Figure 4). This strategy
814 identified 260 TFs, including 52 functionally analyzed in wheat or other crops. Through
815 TILLING mutant lines screening, we confirmed mutant lines with 44 novel TFs
816 showing spike development defects, including initiation, distribution and termination
817 or degeneration of spikelet or even floret (Figure 6). Thus, such strategy sets a good
818 example for batch screening factors for agronomic traits in crops.

819

820 **TRN facilitates gene functional study and elite allele discovery in breeding** 821 **application**

822 TRN generated not only concentrate the attention for identification of key factors
823 involved in spike development, but also provide guidance for gene functional study.
824 This is evidenced by revealing the hierarchy regulation module of known individual
825 factors such as *SPL6-MADS34-MADS15-HMA* (Figure 5). Importantly, such
826 regulation module is relatively conserved within *Triticum* tribe, indicating a broad
827 application for TRN generated in wheat to be used in other species. What is more, the
828 regulatory circuit suggested by TRN could promote functional study of novel factor,
829 for instance *TaMYB4-A* (Figure 7). In addition to the genes regulatory relation, TRN
830 could enable the identification of critical region with high resolution that mediating
831 transcriptional regulation. For the case of *TaMYB4-A*, we found the SNP presence in

832 the binding motif of upstream regulator WFZP serves as selection site during the
833 breeding process in China (Figure 8). The elite allele (C-type) within promoter of
834 *TaMYB4-A* is likely originated from Chinese local germplasm. The frequency of this
835 elite allele is sharply increased during the later breeding process might because of the
836 founder effect.

837

838 In summary, we integrated multi-omics data to reveal transcriptional regulatory
839 network and epigenetic dynamic during wheat spike formation. With combination of
840 GWAS analysis, we have identified dozens of novel factors that shape spike
841 architecture and revealed that SNP under the WFZP binding site within promoter of
842 *TaMYB4-A* is critical during the wheat breeding process in China.

843

844 **Materials and Methods**

845 **Plant materials, growth condition and sampling**

846 The winter wheat cultivar KN9204 was used in this study. The germinated seeds were
847 treated at 4 °C for 30 days. The seedlings were transplanted into soil and grown in the
848 greenhouse at 22°C/20°C day/night, under long day conditions (16 h light/8 h dark).
849 The stage-specific shoot apex of wheat was dissected under the stereomicroscope based
850 on the anatomic and morphological features, and immediately frozen in liquid nitrogen
851 and stored at -80°C. About 10 to 50 spikes were pooled for each of biological replicate
852 for RNA-seq (three replicates), ATAC-seq and CUT&Tag (two replicates) analysis at
853 eight or five development stages.

854

855 **Transgenic wheat plant generation and spike related morphological trait 856 observation**

857 The winter wheat cultivar KN9204 was used to amplify gene sequences and the spring
858 wheat cultivar Fielder was used to generate transgenic wheat plants. To obtain RNAi
859 transgenic wheat plants, the specific fragment of *TaSPL6*, *TaMADS34* and *TaMADS15*
860 was separately amplified and inserted into pc336 (*Ubi:GWRNAi:NOS*) vector using

861 gateway cloning method. All constructed vectors were transformed into callus to
862 generate the transgenic plants as described previously (Liu et al., 2022b). The pc336
863 vector was kindly provided by Dr. Daolin Fu at College of Agronomy, Shandong
864 Agricultural University, Tai'an, Shandong, China.

865

866 The transgenic lines and mutant lines were grown at the Experimental Station of
867 Institute of Genetics and Developmental Biology, Chinese Academy of Sciences,
868 Changping, Beijing for 2 consecutive years (planted in September in 2020 and 2021).
869 Primers for genotyping are listed in Supplemental Table 12.

870

871 The phenotypic traits of mutant lines, T2 generation transgenic and control plants (lines
872 transformed with an empty vector) which were planted under natural conditions were
873 recorded for 20 to 40 randomly selected transgenic plants 30 days after flowering.

874

875 **RNA extraction, sequencing, quantitative PCR and *in situ* hybridization**

876 Total RNA was extracted using HiPure Plant RNA Mini Kit according to the
877 manufacturer's instructions (Magen, R4111-02). RNA-seq libraries construction and
878 sequencing platform were the same as previous description (Zhao et al., 2022), by
879 Annoroad Gene Technology.

880

881 First-strand cDNA was synthesized from 2 µg of DNase I-treated total RNA using the
882 TransScript First Strand cDNA Synthesis SuperMix Kit (TransGen, AT301-02).
883 Quantitative PCR was performed using the ChamQ Universal SYBR qPCR Master Mix
884 (Vazyme, Q711-02) by QuantStudio5 (Applied biosystems). Expression of genes of
885 interest were normalized to Tubulin for calibration, relative expression level is
886 calculated via the $2^{-\Delta\Delta Ct}$ analysis method (Livak and Schmittgen, 2001). Primers used
887 for qPCR are listed in Supplemental Table 12.

888

889 RNA *in situ* hybridization was carried out as described previously (Cui et al., 2010).
890 Fresh young spikes were fixed in formalin-acetic acid-alcohol overnight at 4°C,

891 dehydrated through a standard ethanol series, embedded in Paraplast Plus tissue-
892 embedding medium (Sigma-Aldrich, P3683), and sectioned at 8 μ m width using a
893 microtome (Leica Microsystems, RM2235). Digoxigenin-labeled RNA probes were
894 synthesized using a DIG northern Starter Kit (Roche, 11277073910), according to the
895 manufacturer's instructions. Primer sequences used for probe synthesis are listed in
896 Supplemental Table 12.

897

898 **Data Preprocessing and reads alignment**

899 Raw reads were filtered by fastp v0.20.1 with parameter "--detect_adapter_for_pe" for
900 adapters removing, low-quality bases trimming, and reads filtering (Chen et al., 2018).
901 Furthermore, FastQC v0.11.8 ([http://www.bioinformatics.babraham.ac.uk/projects](http://www.bioinformatics.babraham.ac.uk/projects/fastqc/)
902 [/fastqc/](http://www.bioinformatics.babraham.ac.uk/projects/fastqc/)) was performed to ensure the high quality of reads.

903

904 Reads were aligned using either BWA-MEM v0.7.17 with parameter "-M" (for ATAC-
905 seq and CUT&Tag seq) or hisat2 v2.1.0 with default parameters (for RNA-seq) to the
906 *Triticum aestivum* (Chinese Spring) reference genome (IWGSC RefSeq v1.0,
907 https://urgi.versailles.inra.fr/download/iwgsc/IWGSC_RefSeq_Assemblies/v1.0/)
908 (Appels et al., 2018; Kim et al., 2019; Li and Durbin, 2009). Gene models from the
909 IWGSC Annotation v1.1 was used as the reference and high-confidence genes were
910 used throughout this study. The resulting SAM files were converted to BAM format,
911 sorted, and indexed using Samtools v1.4 (Danecek et al., 2021). Sam files of RNA-seq
912 generated from hisat2 were converted to bam files without deduplication. For ATAC-
913 seq and CUT&Tag, SAM files were further filtered with "samtools view -bS -F 1,804 -
914 f 2 -q 30" to filter out the low-quality mapped reads. Duplicates in the high-quality
915 mapped reads were removed using Picard v2.23.3. Two replicates bam files were
916 merged by samtools. To normalize and visualize the individual and merged replicate
917 datasets, the BAM files were converted to bigwig files using bamCoverage provided
918 by deepTools v3.3.0 with 10 bp bin size and normalized by RPKM (Reads Per Kilobase
919 per Million mapped reads) with parameters "-bs 10 --effectiveGenomeSize
920 14,600,000,000 --normalizeUsing RPKM --smoothLength 50" (Ramirez et al., 2014).

921

922 **RNA-seq data analyses**

923 The number of paired reads that mapping to each gene was counted using feature
924 Counts v2.0.1 with the parameter "-p" (Liao et al., 2014). The counts files were then
925 used as inputs for DEGs (differentially expressed genes) analysis by DESeq2 v1.26.0
926 with a threshold "absolute value of Log2 Fold Change ≥ 1 and FDR ≤ 0.05 " (Love
927 et al., 2014). The raw counts were further normalized to TPM (Transcripts Per Kilobase
928 Million) for gene expression quantification. For subsequent clustering and
929 visualization, we obtained mean counts by merging three biological replicates. TPM
930 values of genes were Z-scaled and clustered by k-means method and displayed using R
931 package ComplexHeatmap (v2.4.3) (Gu et al., 2016). Functional enrichment was
932 performed using an R package clusterProfiler v3.18.1, and GO annotation files were
933 generated from IWGSC Annotation v1.1 (Yu et al., 2012).

934

935 **Cut&Tag and ATAC-seq experiment and data analyses**

936 ATAC-seq and CUT&Tag experiment were done follow the previous described method
937 (Zhao et al., 2022). Tn5 transposase used and tagmentation assay is done following the
938 manual (Vazyme, TD501-01). Libraries were purified with AMPure beads (Beckman,
939 A63881) and sequenced using the Illumina Novaseq platform at Annoroad Gene
940 Technology. Antibodies used for histone modifications are listed in Supplemental Table
941 13.

942

943 Data processing and reads alignment were performed as previously described (Zhao et
944 al., 2022). MACS2 v2.1.4 was used to call peaks. Parameters "-p 1e-3" was used for
945 H3K27ac, H3K4me3 and H2A.Z; parameters "--broad -broad-cutoff 0.05" were used
946 for H3K27me3 and H3K36me3 (Zhang et al., 2008). For ATAC-seq data, MACS2 was
947 used with parameters "--cutoff-analysis --nomodel --shift -100 --extsize 200". The
948 peaks were annotated by R package ChIPseeker v1.26.2 with "annotatePeak" function
949 (Yu et al., 2015). The gene promoters are defined as 3.0 kb upstream of gene TSS.

950

951 For the identification of transcription factor footprints in ATAC-seq peaks, we used the
952 HINT tool v0.13.2 of the Regulatory Genomics Toolbox (RGT) (Gusmao et al., 2014).
953 Custom wheat genome was configured using IWGSC refseq v1.1 Chinese Spring
954 genome based on the introduction of HINT software. TF motifs were downloaded from
955 JASPAR Plantae database (<https://jaspar.genereg.net/>) (Castro-Mondragon et al.,
956 2022).

957

958 **Differential chromatin modification enriched regions detection**

959 For Cut&Tag and ATAC-seq, reads count and CPM normalized value of peaks were
960 calculated by R package DiffBind v2.16.2 with the setting
961 "DBA_SCORE_TMM_READS_EFFECTIVE_CPM". DiffBind was also used to
962 identify differentially accessible regions and histone modification enriched regions with
963 parameters "method = DBA_DESEQ2" and a threshold "absolute value of Log2 Fold
964 Change ≥ 1 and FDR ≤ 0.05 ".

965

966 **Pseudotime indexing and gene regulatory network construction**

967 We used the pseudotime indexing method to analyze gene expression as described in
968 previous studies with some modifications (Hao et al., 2021; Leiboff and Hake, 2019;
969 Zhao et al., 2022). All of the expressed genes during spike reproductive development
970 (From SMI to FOP) were used to separate samples on a PCA plot. Then, each
971 developmental stage was assigned a location and the Euclidean distance between
972 adjacent stages was calculated and scaled from 0.0 to 10.0. For each gene, we calculated
973 the fitted curve and interpolated the curve into 500 points based on gene expression
974 using the "loess" function in R. We further performed PCA for each gene based on the
975 standardized expression data and used atan2 function in R to order genes based on the
976 time of expression.

977

978 For GRNs construction, we only focused on DEGs with TPM values higher than 0.5 in

979 any stages from SMI to FOP. For one gene, its potential upstream regulatory TFs was
980 predicted based on the motif present at gene regulatory region. Here we firstly used
981 HINT tool v0.13.2 to identify footprints within ATAC-seq peaks and motifs within the
982 footprints. Then matched the motifs to TFs based on JASPAR Plantae database. TFs in
983 wheat were mapped to TFs of JASPAR Plantae database (Castro-Mondragon et al.,
984 2022) used blastp (v 2.10.1) (Camacho et al., 2009) with criteria “ $e\text{-value} < 1e-10$ and
985 $\text{identity} > 40\%$ ”. In this way, we obtained the regulatory relationship between TFs and
986 target genes. We further filtered the obtained TF-target regulation according to the
987 following criteria: Firstly, the TPM values of TFs and target genes must be higher than
988 0.5 at any stage from SMI to FOP simultaneously. Secondly, we overlapped these TF-
989 target regulations with WGCNA network constructed based on transcriptome of 8 stage
990 from SAM to FOP, and only retained TF-target regulations also supported by WGCNA
991 network. In this way we got the final TF-target gene regulatory network. We used k-
992 means function in R to cluster genes into 6 categories and performed hypergeometric
993 test to calculate P -value of regulation among gene categories.

994

995 **Phylogenetic tree construction**

996 Wheat and maize orthologs were identified by reciprocal BLAST of rice MADS34 and
997 MADS15 protein sequences. Sequence alignment was performed using MUSCLE
998 v3.8.1551 (Multiple Protein Sequence Alignment, <http://www.drive5.com/muscle>) with
999 default settings (Edgar, 2004). We only retained amino acid positions that were present
1000 in at least 50% of sequences using trimAl v1.4.rev15 (<http://trimal.cgenomics.org>) with
1001 parameters “-gt 0.5” (Capella-Gutierrez et al., 2009). We used RAxML v8.2.12 to create
1002 maximum likelihood phylogenetic trees using model PROTGAMMAGTR and 100
1003 rapid bootstraps (Stamatakis, 2014).

1004

1005 **GWAS analysis**

1006 The 319, 558 SNPs (missing rates ≤ 0.1 , MAF ≥ 0.05) from wheat 660K SNP array
1007 screening of 214 samples were performed to do association analysis with phenotypic
1008 data, implemented in Tassel v5.2 using the mixed linear model. The threshold for

1009 genome-wide significance was determined by the value $1/\text{independent number of SNPs}$
1010 (SNPs of weak LD with other SNPs ($r^2 < 0.5$) were regarded as independent SNPs).
1011 The genome-wide significant marker-trait associations were identified using a threshold
1012 cutoff of $3.16E-04$. Manhattan plots and quantile-quantile plots were generated using
1013 R package “CMplot” (<https://github.com/YinLiLin/R-CMplot>).

1014

1015 **Gene based association analysis**

1016 The nucleotide polymorphisms in 6 kb genomic region of *TaMYB4-A*, including exons,
1017 intron regions, 4 kb promoter regions and 0.5 kb 3'-UTR regions, were identified using
1018 the whole genome exon capture sequencing data of 287 Chinese wheat mini-core
1019 collection samples (Li et al., 2022a). Tassel v5.2 was used to establish the association
1020 of polymorphisms with the FSS, and Haploview 4.2 was used to calculate the pairwise
1021 linkage disequilibrium and draw the LD plot.

1022

1023 **Spike morphology observation by scanning electron microscopy (SEM)**

1024 Photomicrographs of young spikes were taken using a stereomicroscope (S8 APO,
1025 Leica Microsystems) equipped with a digital camera (Canon, A640). For SEM, young
1026 spikes from each stage were fixed in 2.5% glutaraldehyde at 4°C. After dehydration in
1027 a series of ethanol solutions and substitution with 3-methylbutyl acetate, the samples
1028 were subjected to critical point drying, coated with platinum, and observed using a
1029 variable pressure scanning electron microscope (Hitachi S-3000N).

1030

1031 **Luciferase (LUC) reporter assay**

1032 For LUC analyses, full-length coding sequences of *TaSPL6*, *TaMADS34*, and
1033 *TaMADS15*, *TaMYB4-A*, *WFZP*, *TaDOF17* were cloned into PTF101 vector to generate
1034 the effector construct *35Spro: TF-GFP*, and about 3 Kb promoter fragment of
1035 *TaMADS34*, *TaMADS15*, *TaHMA*, *TaMYB4-A*, *TaAP2-39*, *TaI-BAK1* and *TaPILS7*
1036 were amplified and fused in-frame with the CP461-LUC vector to generate the reporter
1037 construct *target-pro: LUC* (see Supplemental Table 12 for primers). The plasmids were
1038 transformed into *Agrobacterium* GV3101. The mixture of bacterial solution

1039 *35Spro*:TF-GFP (OD=0.5), target-pro:LUC (OD=0.5) and P19 (OD=0.3) in activation
1040 buffer (10 mM MES, 150 μ M AS, 10 mM MgCl₂) was injected to tobacco (*Nicotiana*
1041 *benthamiana*). pSUPER-GFP, target-pro:LUC and P19 as control. Firefly luciferase
1042 (LUC) and Renilla luciferase (REN) activities were measured using dual luciferase
1043 assay reagent (Promega, VPE1910) after 1 day' co-cultivation in dark and 2 days in
1044 light, the relative value of LUC/REN is indicated as average with standard error of
1045 multiple replicates.

1046

1047 **Statistics and data visualization**

1048 R (<https://cran.r-project.org/>;version 4.0.2) was used to compute statistics and generate
1049 plots if not specified. For two groups' comparison of data, the student's t-test was used,
1050 such as Figure 3H, 5D, 5E, 7F, 7G, 8B, 8C, and Supplemental Figure 5A. For
1051 enrichment analysis, Fisher's exact test was used, such as Figure 2C, 2E, Figure3B, 3C,
1052 4H, and Supplemental Figure 2C, 2D. For three or more independent groups
1053 comparison of data, Fisher's Least Significant Difference (LSD) was used, such as
1054 Figure 5D, 6D, 7E, and Supplemental Figure 5B, 6A, 6C, 6D, 7B, 7C.

1055

1056 **Data availability**

1057 The raw sequence data reported in this paper have been deposited in the Genome
1058 Sequence Archive (Chen et al., 2021) in National Genomics Data Center (CNCB-
1059 NGDC Members and Partners, 2022), China National Center for Bioinformation /
1060 Beijing Institute of Genomics, Chinese Academy of Sciences (PRJCA013096) that are
1061 publicly accessible at <https://ngdc.cncb.ac.cn/gsa>

1062

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1070

1071 **Author contributions**

1072 J.X. designed and supervised the research, J.X., X.-L. L., Y.-X.X., D.-Z.W. wrote the
1073 manuscript. X.-L. L. did the sample collection and *in situ* hybridization; X.-L. L. and
1074 X.-Y.Z. did plasmid construction and qRT-PCR. X.-M.B. did wheat transformation;
1075 X.-L. L. and Y.-M.Y. performed CUT&Tag, ATAC-seq and RNA-seq experiments;
1076 X.-Y. Z. and H.-Z. W. did the reporter assay; Y.-L.D., X.-Y.Z., F.L., X.-S.Z. and X.-
1077 D.F. provide some raw data or plant materials; X.-Y. X., D.-Z. W. performed bio-
1078 informatics analysis; J.-F. J., X.-Y.Z., F.L., X.-S. Z. and X.-D.F. polished the
1079 manuscript; X.-L. L., Y.-X. X., D.-Z.W., Y.-M.Y. and J.X. prepared all the figures. All
1080 authors discussed the results and commented on the manuscript.

1081

1082 **Competing interests**

1083 The authors declare no competing interests

1084

1085 **References**

1086

- 1087 **Appels, R., Eversole, K., Feuillet, C., Keller, B., Rogers, J., Stein, N., Pozniak, C.J.,**
1088 **Choulet, F., Distelfeld, A., Poland, J., et al.** (2018). Shifting the limits in wheat research and
1089 breeding using a fully annotated reference genome. *Science* **361**:661. eaar7191
1090 10.1126/science.aar7191.
- 1091 **Boden, S.A., Cavanagh, C., Cullis, B.R., Ramm, K., Greenwood, J., Finnegan, E.J.,**
1092 **Trevaskis, B., and Swain, S.M.** (2015). Ppd-1 is a key regulator of inflorescence architecture
1093 and paired spikelet development in wheat. *Nature Plants* **1**:1-6. 10.1038/Nplants.2014.16.
- 1094 **Bonnett, O.T.** (1936). The development of the wheat spike. *J Agric Res* **53**:0445-0451.
- 1095 **Buenrostro, J.D., Wu, B., Chang, H.Y., and Greenleaf, W.J.** (2015). ATAC-seq: A Method
1096 for Assaying Chromatin Accessibility Genome-Wide. *Curr Protoc Mol Biol* **109**:21.29.21-
1097 21.29.29. 10.1002/0471142727.mb2129s109.
- 1098 **Camacho, C., Coulouris, G., Avagyan, V., Ma, N., Papadopoulos, J., Bealer, K., and**
1099 **Madden, T.L.** (2009). BLAST+: architecture and applications. *BMC Bioinformatics* **10**:421.
1100 10.1186/1471-2105-10-421.

- 1101 **Cao, J., Liu, K., Song, W., Zhang, J., Yao, Y., Xin, M., Hu, Z., Peng, H., Ni, Z., Sun, Q., et**
1102 **al.** (2021). Pleiotropic function of the SQUAMOSA PROMOTER-BINDING PROTEIN-LIKE
1103 gene TaSPL14 in wheat plant architecture. *Planta* **253**:44. 10.1007/s00425-020-03531-x.
- 1104 **Capella-Gutierrez, S., Silla-Martinez, J.M., and Gabaldon, T.** (2009). trimAl: a tool for
1105 automated alignment trimming in large-scale phylogenetic analyses. *Bioinformatics* **25**:1972-
1106 1973. 10.1093/bioinformatics/btp348.
- 1107 **Castro-Mondragon, J.A., Riudavets-Puig, R., Rauluseviciute, I., Lemma, R.B., Turchi, L.,**
1108 **Blanc-Mathieu, R., Lucas, J., Boddie, P., Khan, A., Manosalva Perez, N., et al.** (2022).
1109 JASPAR 2022: the 9th release of the open-access database of transcription factor binding
1110 profiles. *Nucleic Acids Res* **50**:D165-D173. 10.1093/nar/gkab1113.
- 1111 **Chen, S., Zhou, Y., Chen, Y., and Gu, J.** (2018). fastp: an ultra-fast all-in-one FASTQ
1112 preprocessor. *Bioinformatics* **34**:i884-i890. 10.1093/bioinformatics/bty560.
- 1113 **Chen, T., Chen, X., Zhang, S., Zhu, J., Tang, B., Wang, A., Dong, L., Zhang, Z., Yu, C.,**
1114 **Sun, Y., et al.** (2021). The Genome Sequence Archive Family: Toward Explosive Data Growth
1115 and Diverse Data Types. *Genomics Proteomics Bioinformatics* **19**:578-583.
1116 10.1016/j.gpb.2021.08.001.
- 1117 **Cui, R., Han, J., Zhao, S., Su, K., Wu, F., Du, X., Xu, Q., Chong, K., Theissen, G., and**
1118 **Meng, Z.** (2010). Functional conservation and diversification of class E floral homeotic genes
1119 in rice (*Oryza sativa*). *Plant J* **61**:767-781. 10.1111/j.1365-313X.2009.04101.x.
- 1120 **Danecek, P., Bonfield, J.K., Liddle, J., Marshall, J., Ohan, V., Pollard, M.O., Whitwham,**
1121 **A., Keane, T., McCarthy, S.A., Davies, R.M., et al.** (2021). Twelve years of SAMtools and
1122 BCFtools. *Gigascience* **10**10.1093/gigascience/giab008.
- 1123 **Debernardi, J.M., Greenwood, J.R., Jean Finnegan, E., Jernstedt, J., and Dubcovsky, J.**
1124 (2020). APETALA 2-like genes AP2L2 and Q specify lemma identity and axillary floral
1125 meristem development in wheat. *Plant J* **101**:171-187. 10.1111/tpj.14528.
- 1126 **Dixon, L.E., Greenwood, J.R., Bencivenga, S., Zhang, P., Cockram, J., Mellers, G., Ramm,**
1127 **K., Cavanagh, C., Swain, S.M., and Boden, S.A.** (2018). TEOSINTE BRANCHED1
1128 Regulates Inflorescence Architecture and Development in Bread Wheat (*Triticum aestivum*).
1129 *Plant Cell* **30**:563-581. 10.1105/tpc.17.00961.
- 1130 **Dobrovolskaya, O., Pont, C., Sibout, R., Martinek, P., Badaeva, E., Murat, F., Chosson,**
1131 **A., Watanabe, N., Prat, E., Gautier, N., et al.** (2015). FRIZZY PANICLE drives
1132 supernumerary spikelets in bread wheat. *Plant Physiol* **167**:189-199. 10.1104/pp.114.250043.
- 1133 **Doebley, J., Stec, A., and Hubbard, L.** (1997). The evolution of apical dominance in maize.
1134 *Nature* **386**:485-488. 10.1038/386485a0.
- 1135 **Du, D., Zhang, D., Yuan, J., Feng, M., Li, Z., Wang, Z., Zhang, Z., Li, X., Ke, W., Li, R.,**
1136 **et al.** (2021). FRIZZY PANICLE defines a regulatory hub for simultaneously controlling
1137 spikelet formation and awn elongation in bread wheat. *New Phytol* **231**:814-833.
1138 10.1111/nph.17388.
- 1139 **Edgar, R.C.** (2004). MUSCLE: a multiple sequence alignment method with reduced time and
1140 space complexity. *BMC Bioinformatics* **5**:113. 10.1186/1471-2105-5-113.
- 1141 **Feng, N., Song, G.Y., Guan, J.T., Chen, K., Jia, M.L., Huang, D.H., Wu, J.J., Zhang, L.C.,**
1142 **Kong, X.Y., Geng, S.F., et al.** (2017). Transcriptome Profiling of Wheat Inflorescence
1143 Development from Spikelet Initiation to Floral Patterning Identified Stage-Specific Regulatory
1144 Genes. *Plant Physiol* **174**:1779-1794. 10.1104/pp.17.00310.

- 1145 **Finnegan, E.J., Ford, B., Wallace, X., Pettolino, F., Griffin, P.T., Schmitz, R.J., Zhang, P.,**
1146 **Barrero, J.M., Hayden, M.J., Boden, S.A., et al.** (2018). Zebularine treatment is associated
1147 with deletion of FT-B1 leading to an increase in spikelet number in bread wheat. *Plant Cell*
1148 *Environ* **41**:1346-1360. 10.1111/pce.13164.
- 1149 **Gao, X.Q., Wang, N., Wang, X.L., and Zhang, X.S.** (2019). Architecture of Wheat
1150 Inflorescence: Insights from Rice. *Trends Plant Sci* 10.1016/j.tplants.2019.06.002.
- 1151 **Gauley, A., and Boden, S.A.** (2019). Genetic pathways controlling inflorescence architecture
1152 and development in wheat and barley. *J Integr Plant Biol* **61**:296-309. 10.1111/jipb.12732.
- 1153 **Gu, Z., Eils, R., and Schlesner, M.** (2016). Complex heatmaps reveal patterns and correlations
1154 in multidimensional genomic data. *Bioinformatics* **32**:2847-2849.
1155 10.1093/bioinformatics/btw313.
- 1156 **Guo, L.J., Ma, M., Wu, L.N., Zhou, M.D., Li, M.Y., Wu, B.W., Li, L., Liu, X.L., Jing, R.L.,**
1157 **Chen, W., et al.** (2022). Modified expression of TaCYP78A5 enhances grain weight with yield
1158 potential by accumulating auxin in wheat (*Triticum aestivum* L.). *Plant Biotechnology Journal*
1159 **20**:168-182. 10.1111/pbi.13704.
- 1160 **Gusmao, E.G., Dieterich, C., Zenke, M., and Costa, I.G.** (2014). Detection of active
1161 transcription factor binding sites with the combination of DNase hypersensitivity and histone
1162 modifications. *Bioinformatics* **30**:3143-3151. 10.1093/bioinformatics/btu519.
- 1163 **Hao, C.Y., Jiao, C.Z., Hou, J., Li, T., Liu, H.X., Wang, Y.Q., Zheng, J., Liu, H., Bi, Z.H.,**
1164 **Xu, F.F., et al.** (2020). Resequencing of 145 Landmark Cultivars Reveals Asymmetric Sub-
1165 genome Selection and Strong Founder Genotype Effects on Wheat Breeding in China. *Mol*
1166 *Plant* **13**:1733-1751. 10.1016/j.molp.2020.09.001.
- 1167 **Hao, Z., Zhang, Z., Xiang, D., Venglat, P., Chen, J., Gao, P., Datla, R., and Weijers, D.**
1168 (2021). Conserved, divergent and heterochronic gene expression during Brachypodium and
1169 *Arabidopsis* embryo development. *Plant Reprod* **34**:207-224. 10.1007/s00497-021-00413-4.
- 1170 **Jablonski, B., Ogonowska, H., Szala, K., Bajguz, A., Orczyk, W., and Nadolska-Orczyk,**
1171 **A.** (2020). Silencing of TaCKX1 Mediates Expression of Other TaCKX Genes to Increase Yield
1172 Parameters in Wheat. *Int J Mol Sci* **21**10.3390/ijms21134809.
- 1173 **Jablonski, B., Szala, K., Przyborowski, M., Bajguz, A., Chmur, M., Gasparis, S., Orczyk,**
1174 **W., and Nadolska-Orczyk, A.** (2021). TaCKX2.2 Genes Coordinate Expression of Other
1175 TaCKX Family Members, Regulate Phytohormone Content and Yield-Related Traits of Wheat.
1176 *Int J Mol Sci* **22**10.3390/ijms22084142.
- 1177 **Kaya-Okur, H.S., Wu, S.J., Codomo, C.A., Pledger, E.S., Bryson, T.D., Henikoff, J.G.,**
1178 **Ahmad, K., and Henikoff, S.** (2019). CUT&Tag for efficient epigenomic profiling of small
1179 samples and single cells. *Nat Commun* **10**:1930. 10.1038/s41467-019-09982-5.
- 1180 **Kellogg, E.A.** (2022). Genetic control of branching patterns in grass inflorescences. *Plant Cell*
1181 **34**:2518-2533. 10.1093/plcell/koac080.
- 1182 **Khew, C.Y., Teo, C.J., Chan, W.S., Wong, H.L., Namasivayam, P., and Ho, C.L.** (2015).
1183 Brassinosteroid insensitive 1-associated kinase 1 (OsI-BAK1) is associated with grain filling
1184 and leaf development in rice. *J Plant Physiol* **182**:23-32. 10.1016/j.jplph.2015.05.003.
- 1185 **Kim, D., Paggi, J.M., Park, C., Bennett, C., and Salzberg, S.L.** (2019). Graph-based genome
1186 alignment and genotyping with HISAT2 and HISAT-genotype. *Nat Biotechnol* **37**:907-915.
1187 10.1038/s41587-019-0201-4.
- 1188 **Klemm, S.L., Shipony, Z., and Greenleaf, W.J.** (2019). Chromatin accessibility and the

1189 regulatory epigenome. *Nat Rev Genet* **20**:207-220. 10.1038/s41576-018-0089-8.

1190 **Kobayashi, K., Maekawa, M., Miyao, A., Hirochika, H., and Kyojuka, J.** (2010). PANICLE
1191 PHYTOMER2 (PAP2), encoding a SEPALLATA subfamily MADS-box protein, positively
1192 controls spikelet meristem identity in rice. *Plant and Cell Physiology* **51**:47-57.
1193 10.1093/pcp/pcp166.

1194 **Kobayashi, K., Yasuno, N., Sato, Y., Yoda, M., Yamazaki, R., Kimizu, M., Yoshida, H.,**
1195 **Nagamura, Y., and Kyojuka, J.** (2012). Inflorescence Meristem Identity in Rice Is Specified
1196 by Overlapping Functions of Three AP1/FUL-Like MADS Box Genes and PAP2, a
1197 SEPALLATA MADS Box Gene. *Plant Cell* **24**:1848-1859. 10.1105/tpc.112.097105.

1198 **Kong, X.C., Wang, F., Geng, S.F., Guan, J.T., Tao, S., Jia, M.L., Sun, G.L., Wang, Z.Y.,**
1199 **Wang, K., Ye, X.G., et al.** (2022). The wheat AGL6-like MADS-box gene is a master regulator
1200 for floral organ identity and a target for spikelet meristem development manipulation. *Plant*
1201 *Biotechnology Journal* **20**:75-88. 10.1111/pbi.13696.

1202 **Koppolu, R., Chen, S., and Schnurbusch, T.** (2022). Evolution of inflorescence branch
1203 modifications in cereal crops. *Curr Opin Plant Biol* **65**:102168. 10.1016/j.pbi.2021.102168.

1204 **Krasileva, K.V., Vasquez-Gross, H.A., Howell, T., Bailey, P., Paraiso, F., Clissold, L.,**
1205 **Simmonds, J., Ramirez-Gonzalez, R.H., Wang, X.D., Borrill, P., et al.** (2017). Uncovering
1206 hidden variation in polyploid wheat. *P Natl Acad Sci USA* **114**:E913-E921.
1207 10.1073/pnas.1619268114.

1208 **Kuzay, S., Lin, H., Li, C., Chen, S., Woods, D.P., Zhang, J., Lan, T., von Korff, M., and**
1209 **Dubcovsky, J.** (2022). WAPO-A1 is the causal gene of the 7AL QTL for spikelet number per
1210 spike in wheat. *PLoS Genet* **18**:e1009747. 10.1371/journal.pgen.1009747.

1211 **Kuzay, S., Xu, Y., Zhang, J., Katz, A., Pearce, S., Su, Z., Fraser, M., Anderson, J.A.,**
1212 **Brown-Guedira, G., DeWitt, N., et al.** (2019). Identification of a candidate gene for a QTL
1213 for spikelet number per spike on wheat chromosome arm 7AL by high-resolution genetic
1214 mapping. *Theor Appl Genet* **132**:2689-2705. 10.1007/s00122-019-03382-5.

1215 **Lee, Z.H., Hirakawa, T., Yamaguchi, N., and Ito, T.** (2019). The Roles of Plant Hormones
1216 and Their Interactions with Regulatory Genes in Determining Meristem Activity. *International*
1217 *Journal of Molecular Sciences* **20**ARTN 4065 10.3390/ijms20164065.

1218 **Leiboff, S., and Hake, S.** (2019). Reconstructing the Transcriptional Ontogeny of Maize and
1219 Sorghum Supports an Inverse Hourglass Model of Inflorescence Development. *Curr Biol*
1220 **29**:3410-3419 e3413. 10.1016/j.cub.2019.08.044.

1221 **Li, A., Hao, C., Wang, Z., Geng, S., Jia, M., Wang, F., Han, X., Kong, X., Yin, L., Tao, S.,**
1222 **et al.** (2022a). Wheat breeding history reveals synergistic selection of pleiotropic genomic sites
1223 for plant architecture and grain yield. *Mol Plant* **15**:504-519. 10.1016/j.molp.2022.01.004.

1224 **Li, C., Lin, H., Chen, A., Lau, M., Jernstedt, J., and Dubcovsky, J.** (2019). Wheat VRN1,
1225 FUL2 and FUL3 play critical and redundant roles in spikelet development and spike
1226 determinacy. *Development* **146**10.1242/dev.175398.

1227 **Li, H., and Durbin, R.** (2009). Fast and accurate short read alignment with Burrows-Wheeler
1228 transform. *Bioinformatics* **25**:1754-1760. 10.1093/bioinformatics/btp324.

1229 **Li, L., Shi, F., Wang, G.L., Guan, Y.B., Zhang, Y.F., Chen, M.J., Chang, J.L., Yang, G.X.,**
1230 **He, G.Y., Wang, Y.S., et al.** (2022b). Conservation and Divergence of SQUAMOSA-
1231 PROMOTER BINDING PROTEIN-LIKE (SPL) Gene Family between Wheat and Rice.
1232 *International Journal of Molecular Sciences* **23**ARTN 2099 10.3390/ijms23042099.

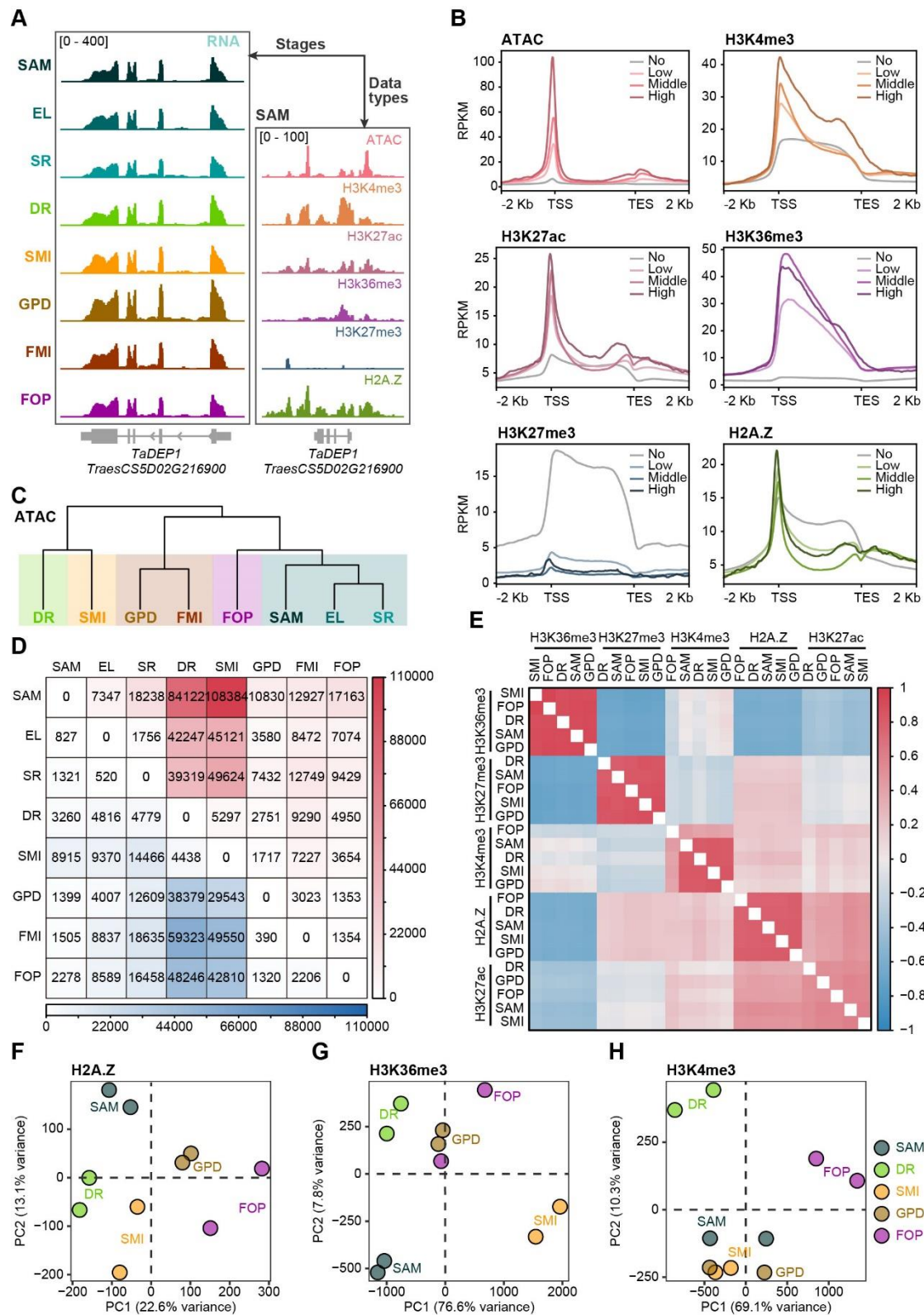
- 1233 **Li, L., Shi, F., Wang, Y., Yu, X., Zhi, J., Guan, Y., Zhao, H., Chang, J., Chen, M., Yang, G.,**
1234 **et al.** (2020). TaSPL13 regulates inflorescence architecture and development in transgenic
1235 wheat (*Triticum aestivum* L.). *Plant Sci* **296**:110516. 10.1016/j.plantsci.2020.110516.
- 1236 **Li, Y., Fu, X., Zhao, M., Zhang, W., Li, B., An, D., Li, J., Zhang, A., Liu, R., and Liu, X.**
1237 (2018). A Genome-wide View of Transcriptome Dynamics During Early Spike Development
1238 in Bread Wheat. *Sci Rep* **8**:15338. 10.1038/s41598-018-33718-y.
- 1239 **Li, Y., Li, L., Zhao, M., Guo, L., Guo, X., Zhao, D., Batool, A., Dong, B., Xu, H., Cui, S.,**
1240 **et al.** (2021). Wheat FRIZZY PANICLE activates VERNALIZATION1-A and HOMEBOX4-
1241 A to regulate spike development in wheat. *Plant Biotechnol J* 10.1111/pbi.13535.
- 1242 **Liao, Y., Smyth, G.K., and Shi, W.** (2014). featureCounts: an efficient general purpose
1243 program for assigning sequence reads to genomic features. *Bioinformatics* **30**:923-930.
1244 10.1093/bioinformatics/btt656.
- 1245 **Lin, X.L., Wu, F., Du, X.Q., Shi, X.W., Liu, Y., Liu, S.J., Hu, Y.X., Theissen, G., and Meng,**
1246 **Z.** (2014). The pleiotropic SEPALLATA-like gene OsMADS34 reveals that the 'empty glumes'
1247 of rice (*Oryza sativa*) spikelets are in fact rudimentary lemmas. *New Phytologist* **202**:689-702.
1248 10.1111/nph.12657.
- 1249 **Liu, J., Shi, X., Chang, Z., Ding, Y., and Ding, C.** (2022a). Auxin Efflux Transporters
1250 OsPIN1c and OsPIN1d Function Redundantly in Regulating Rice (*Oryza sativa* L.) Panicle
1251 Development. *Plant Cell Physiol* **63**:305-316. 10.1093/pcp/pcab172.
- 1252 **Liu, X., Bie, X., Lin, X., Li, M., Wang, H., Zhang, X., Yang, Y., Zhang, C., Zhang, X., and**
1253 **Xiao, J.** (2022b). Uncovering transcriptional regulatory network during regeneration for
1254 boosting wheat transformation. *bioRxiv* <https://doi.org/10.1101/2022.10.21.513305>.
- 1255 **Livak, K.J., and Schmittgen, T.D.** (2001). Analysis of relative gene expression data using
1256 real-time quantitative PCR and the 2(T)(-Delta Delta C) method. *Methods* **25**:402-408.
1257 10.1006/meth.2001.1262.
- 1258 **Love, M.I., Huber, W., and Anders, S.** (2014). Moderated estimation of fold change and
1259 dispersion for RNA-seq data with DESeq2. *Genome Biol* **15**:550. 10.1186/s13059-014-0550-
1260 8.
- 1261 **Meng, Q., Li, X., Zhu, W., Yang, L., Liang, W., Dreni, L., and Zhang, D.** (2017). Regulatory
1262 network and genetic interactions established by OsMADS34 in rice inflorescence and spikelet
1263 morphogenesis. *J Integr Plant Biol* **59**:693-707. 10.1111/jipb.12594.
- 1264 **Pang, Y., Liu, C., Wang, D., St Amand, P., Bernardo, A., Li, W., He, F., Li, L., Wang, L.,**
1265 **Yuan, X., et al.** (2020). High-Resolution Genome-wide Association Study Identifies Genomic
1266 Regions and Candidate Genes for Important Agronomic Traits in Wheat. *Mol Plant* **13**:1311-
1267 1327. 10.1016/j.molp.2020.07.008.
- 1268 **Perez-Gianmarco, T.I., Severini, A.D., and Gonzalez, F.G.** (2020). Photoperiod-sensitivity
1269 genes (Ppd-1): quantifying their effect on the photoperiod response model in wheat. *Journal of*
1270 *Experimental Botany* **71**:1185-1198. 10.1093/jxb/erz483.
- 1271 **Qi, P.F., Jiang, Y.F., Guo, Z.R., Chen, Q., Ouellet, T., Zong, L.J., Wei, Z.Z., Wang, Y.,**
1272 **Zhang, Y.Z., Xu, B.J., et al.** (2019). Transcriptional reference map of hormone responses in
1273 wheat spikes. *BMC Genomics* **20**:390. 10.1186/s12864-019-5726-x.
- 1274 **Ram, H., Sahadevan, S., Gale, N., Caggiano, M.P., Yu, X.L., Ohno, C., and Heisler, M.G.**
1275 (2020). An integrated analysis of cell-type specific gene expression reveals genes regulated by
1276 REVOLUTA and KANADII in the Arabidopsis shoot apical meristem. *Plos Genetics* **16**ARTN

- 1277 e1008661 10.1371/journal.pgen.1008661.
- 1278 **Ramirez, F., Dundar, F., Diehl, S., Gruning, B.A., and Manke, T.** (2014). deepTools: a
1279 flexible platform for exploring deep-sequencing data. *Nucleic Acids Res* **42**:W187-191.
1280 10.1093/nar/gku365.
- 1281 **Rong, X.F., Sang, Y.L., Wang, L., Meng, W.J., Zou, C.H., Dong, Y.X., Bie, X.M., Cheng,**
1282 **Z.J., and Zhang, X.S.** (2018). Type-B ARR_s Control Carpel Regeneration Through Mediating
1283 AGAMOUS Expression in Arabidopsis. *Plant and Cell Physiology* **59**:761-769.
1284 10.1093/pcp/pcx187.
- 1285 **Sakuma, S., Golan, G., Guo, Z., Ogawa, T., Tagiri, A., Sugimoto, K., Bernhardt, N.,**
1286 **Brassac, J., Mascher, M., Hensel, G., et al.** (2019). Unleashing floret fertility in wheat through
1287 the mutation of a homeobox gene. *Proc Natl Acad Sci U S A* **116**:5182-5187.
1288 10.1073/pnas.1815465116.
- 1289 **Sehgal, D., and Dreisigacker, S.** (2022). GWAS Case Studies in Wheat. *Methods Mol Biol*
1290 **2481**:341-351. 10.1007/978-1-0716-2237-7_19.
- 1291 **Stamatakis, A.** (2014). RAxML version 8: a tool for phylogenetic analysis and post-analysis
1292 of large phylogenies. *Bioinformatics* **30**:1312-1313. 10.1093/bioinformatics/btu033.
- 1293 **Sun, C.W., Zhang, F.Y., Yan, X.F., Zhang, X.F., Dong, Z.D., Cui, D.Q., and Chen, F.** (2017).
1294 Genome-wide association study for 13 agronomic traits reveals distribution of superior alleles
1295 in bread wheat from the Yellow and Huai Valley of China. *Plant Biotechnology Journal* **15**:953-
1296 969. 10.1111/pbi.12690.
- 1297 **Uauy, C., Paraiso, F., Colasuonno, P., Tran, R.K., Tsai, H., Berardi, S., Comai, L., and**
1298 **Dubcovsky, J.** (2009). A modified TILLING approach to detect induced mutations in tetraploid
1299 and hexaploid wheat. *BMC Plant Biol* **9**:115. 10.1186/1471-2229-9-115.
- 1300 **VanGessel, C., Hamilton, J., Tabbita, F., Dubcovsky, J., and Pearce, S.** (2022).
1301 Transcriptional signatures of wheat inflorescence development. *Sci Rep* **12**:17224.
1302 10.1038/s41598-022-21571-z.
- 1303 **Wang, C., Yang, X., and Li, G.** (2021). Molecular Insights into Inflorescence Meristem
1304 Specification for Yield Potential in Cereal Crops. *Int J Mol Sci* **22**10.3390/ijms22073508.
- 1305 **Wang, K., Tang, D., Hong, L., Xu, W., Huang, J., Li, M., Gu, M., Xue, Y., and Cheng, Z.**
1306 (2010). DEP and AFO regulate reproductive habit in rice. *PLoS Genet* **6**:e1000818.
1307 10.1371/journal.pgen.1000818.
- 1308 **Wang, Q.L., Sun, A.Z., Chen, S.T., Chen, L.S., and Guo, F.Q.** (2018). SPL6 represses
1309 signalling outputs of ER stress in control of panicle cell death in rice. *Nat Plants* **4**:280-288.
1310 10.1038/s41477-018-0131-z.
- 1311 **Wang, Y., Yu, H., Tian, C., Sajjad, M., Gao, C., Tong, Y., Wang, X., and Jiao, Y.** (2017).
1312 Transcriptome Association Identifies Regulators of Wheat Spike Architecture. *Plant Physiol*
1313 **175**:746-757. 10.1104/pp.17.00694.
- 1314 **Wang, Y., Du, F., Wang, J., Wang, K., Tian, C., Qi, X., Lu, F., Liu, X., Ye, X., and Jiao, Y.**
1315 (2022). Improving bread wheat yield through modulating an unselected AP2/ERF gene. *Nat*
1316 *Plants* 10.1038/s41477-022-01197-9.
- 1317 **Wu, F., Shi, X., Lin, X., Liu, Y., Chong, K., Theissen, G., and Meng, Z.** (2017). The ABCs
1318 of flower development: mutational analysis of AP1/FUL-like genes in rice provides evidence
1319 for a homeotic (A)-function in grasses. *Plant J* **89**:310-324. 10.1111/tpj.13386.
- 1320 **Xiao, J., Liu, B., Yao, Y.Y., Guo, Z.F., Jia, H.Y., Kong, L.R., Zhang, A.M., Ma, W.J., Ni,**

- 1321 **Z.F., Xu, S.B., et al.** (2022). Wheat genomic study for genetic improvement of traits in China.
1322 *Science China-Life Sciences* **65**:1718-1775. 10.1007/s11427-022-2178-7.
- 1323 **Xu, M., Hu, T., Zhao, J., Park, M.Y., Earley, K.W., Wu, G., Yang, L., and Poethig, R.S.**
1324 (2016). Developmental Functions of miR156-Regulated SQUAMOSA PROMOTER
1325 BINDING PROTEIN-LIKE (SPL) Genes in *Arabidopsis thaliana*. *PLoS Genet* **12**:e1006263.
1326 10.1371/journal.pgen.1006263.
- 1327 **Yaish, M.W., El-kereamy, A., Zhu, T., Beatty, P.H., Good, A.G., Bi, Y.M., and Rothstein,**
1328 **S.J.** (2010). The APETALA-2-Like Transcription Factor OsAP2-39 Controls Key Interactions
1329 between Abscisic Acid and Gibberellin in Rice. *Plos Genetics* **6**ARTN e1001098
1330 10.1371/journal.pgen.1001098.
- 1331 **Yang, Z., Wang, Z., Wang, W., Xie, X., Chai, L., Wang, X., Feng, X., Li, J., Peng, H., Su,**
1332 **Z., et al.** (2022). ggComp enables dissection of germplasm resources and construction of a
1333 multiscale germplasm network in wheat. *Plant Physiol* **188**:1950-1965.
1334 10.1093/plphys/kiac029.
- 1335 **Yu, G., Wang, L.G., and He, Q.Y.** (2015). ChIPseeker: an R/Bioconductor package for ChIP
1336 peak annotation, comparison and visualization. *Bioinformatics* **31**:2382-2383.
1337 10.1093/bioinformatics/btv145.
- 1338 **Yu, G.C., Wang, L.G., Han, Y.Y., and He, Q.Y.** (2012). clusterProfiler: an R Package for
1339 Comparing Biological Themes Among Gene Clusters. *Omics* **16**:284-287.
1340 10.1089/omi.2011.0118.
- 1341 **Yuan, Z., Persson, S., and Zhang, D.** (2020). Molecular and genetic pathways for optimizing
1342 spikelet development and grain yield. *aBIOTECH* **1**:276-292. 10.1007/s42994-020-00026-x.
- 1343 **Zhang, B., Liu, X., Xu, W., Chang, J., Li, A., Mao, X., Zhang, X., and Jing, R.** (2015).
1344 Novel function of a putative MOC1 ortholog associated with spikelet number per spike in
1345 common wheat. *Sci Rep* **5**:12211. 10.1038/srep12211.
- 1346 **Zhang, D.B., and Yuan, Z.** (2014). Molecular Control of Grass Inflorescence Development.
1347 *Annu Rev Plant Biol* **65**:553-578. 10.1146/annurev-arplant-050213-040104.
- 1348 **Zhang, L., Zhang, H., Qiao, L.Y., Miao, L.F., Yan, D., Liu, P., Zhao, G.Y., Jia, J.Z., and**
1349 **Gao, L.F.** (2021). Wheat MADS-box gene TaSEP3-D1 negatively regulates heading date. *Crop*
1350 *J* **9**:1115-1123. 10.1016/j.cj.2020.12.007.
- 1351 **Zhang, Y., Liu, T., Meyer, C.A., Eeckhoute, J., Johnson, D.S., Bernstein, B.E., Nusbaum,**
1352 **C., Myers, R.M., Brown, M., Li, W., et al.** (2008). Model-based analysis of ChIP-Seq
1353 (MACS). *Genome Biol* **9**:R137. 10.1186/gb-2008-9-9-r137.
- 1354 **Zhao, L., Lin, X., Yang, Y., Bie, X., Zhang, H., Chen, J., Liu, X., Wang, H., Jiang, J., Fu,**
1355 **X., et al.** (2022). Chromatin reprogramming and transcriptional regulation orchestrate
1356 embryogenesis in hexaploid wheat. *bioRxiv* <https://doi.org/10.1101/2022.01.21.477188>.
- 1357 **Zhou, Y., Zhao, X.B., Li, Y.W., Xu, J., Bi, A.Y., Kang, L.P., Xu, D.X., Chen, H.F., Wang,**
1358 **Y., Wang, Y.G., et al.** (2020). Triticum population sequencing provides insights into wheat
1359 adaptation. *Nature Genetics* **52**:1412-1422. 10.1038/s41588-020-00722-w.
- 1360 **Zhu, W.W., Yang, L., Wu, D., Meng, Q.C., Deng, X., Huang, G.Q., Zhang, J., Chen, X.F.,**
1361 **Ferrandiz, C., Liang, W.Q., et al.** (2022). Rice SEPALLATA genes OsMADS5 and
1362 OsMADS34 cooperate to limit inflorescence branching by repressing the TERMINAL
1363 FLOWER1-like gene RCN4. *New Phytologist* **233**:1682-1700. 10.1111/nph.17855.
- 1364

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Supplemental Figures



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Supplemental Figure 1. Features of various histone modifications.

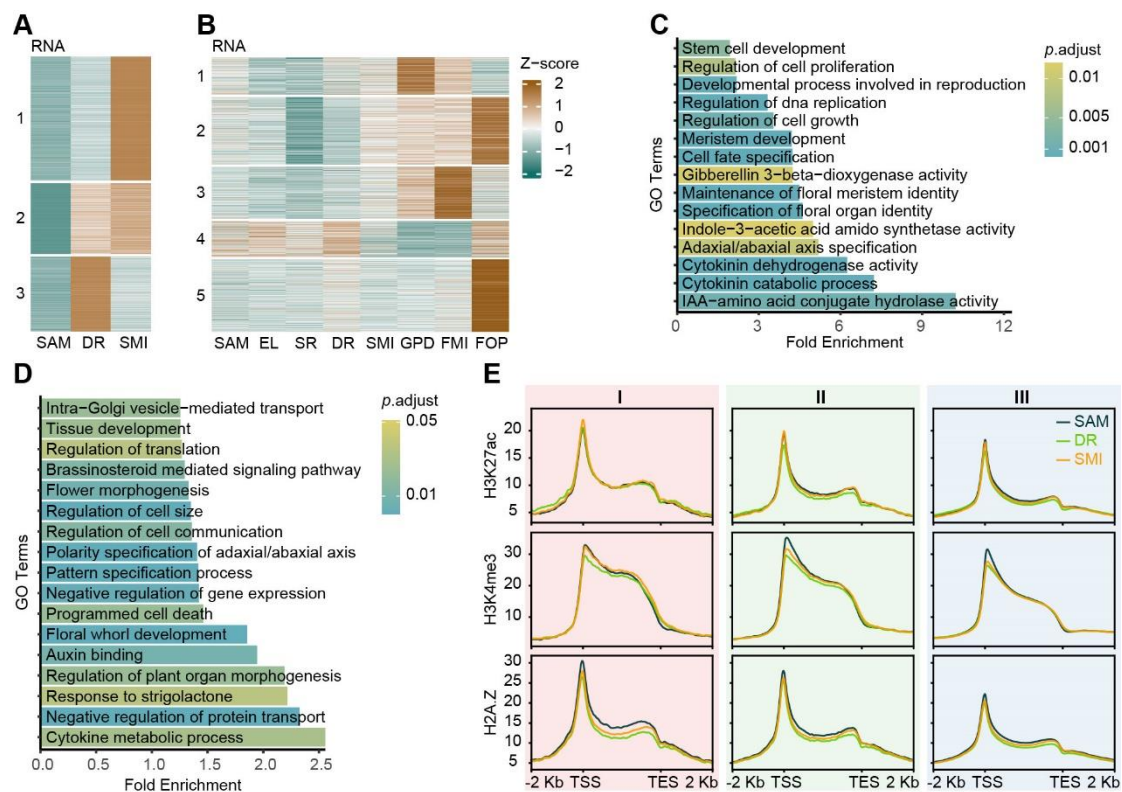
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(A) IGV browser view at *TaDEP1* locus showing various epigenomic data types at

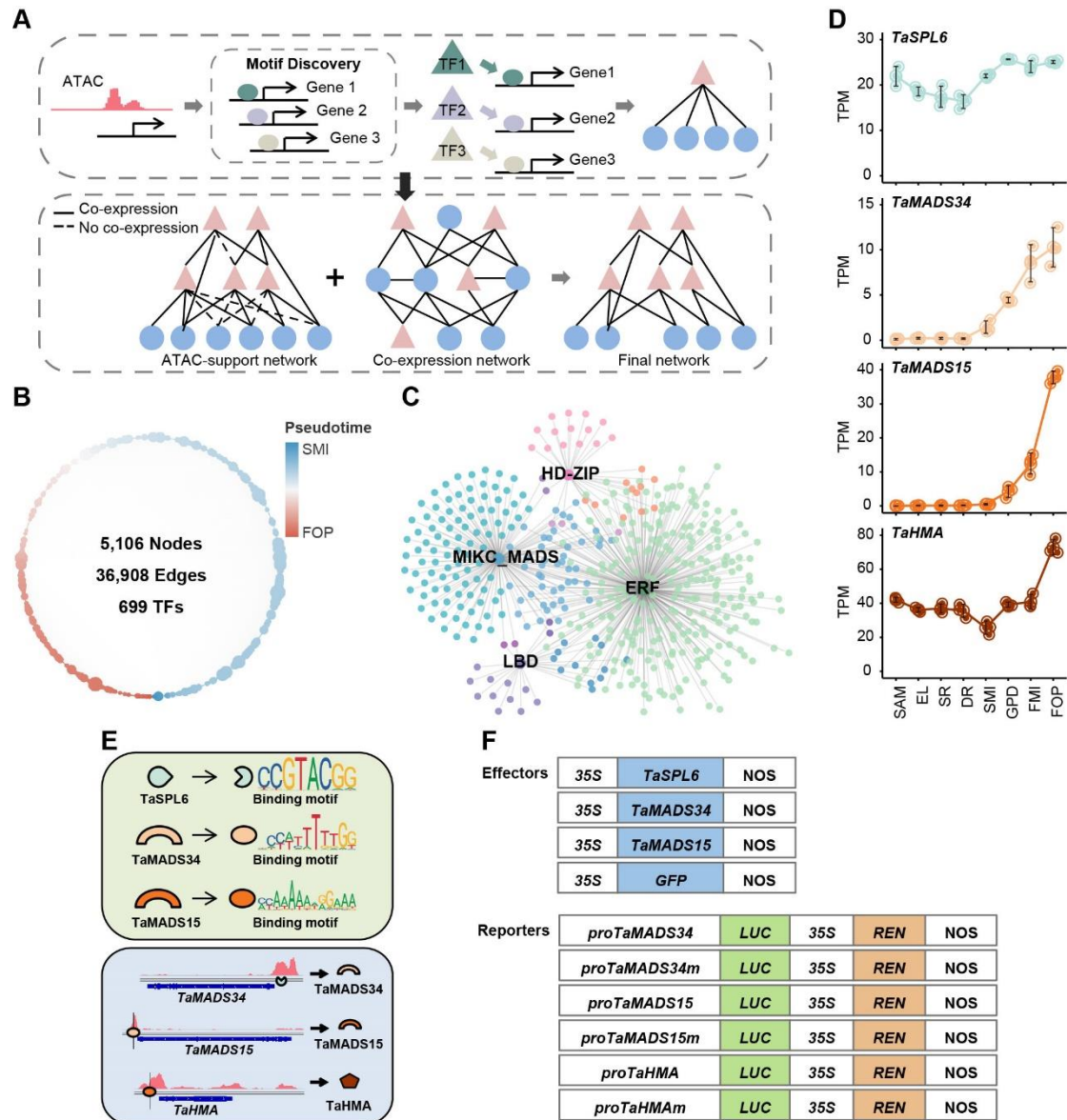
1369

SAM stage and transcriptome levels of different sampling stages.

- 1370 (B) Correlation between different types of histone modification profiles and gene
 1371 expression levels.
- 1372 (C) Cluster dendrogram of ATAC-seq data showing five distinct development clusters:
 1373 vegetative cluster (SAM, EL, SR), flowering transition stage (DR), inflorescence
 1374 initiation (SMI), spikelet meristem formation (GPD, FMI) and floret meristem
 1375 formation (FOP).
- 1376 (D) The matrix of differentially accessible regions (DARs) numbers among
 1377 developmental stages. The number of decreased and increased chromatin
 1378 accessibility compared with former stages were represented in the lower-triangle
 1379 (number in light blue) and upper-triangle panel (number in light red), respectively.
 1380 A region with $|\log_2(\text{Fold Change})| \geq 1$ and $\text{FDR} \leq 0.05$ by DiffBind between any
 1381 two stages was considered as DAR.
- 1382 (E) Pair-wise correlation map among different histone modification profiles. Jaccard
 1383 index was calculated based on the peaks overlap, and then Pearson correlation scores
 1384 were generated.
- 1385 (F-H) PCA of H2A.Z (F), H3K36me3 (G) and H3K4me3 (H) samples during spike
 1386 development. Each dot represents one sample; two bio-replicates are sequenced for
 1387 each stage.
- 1388



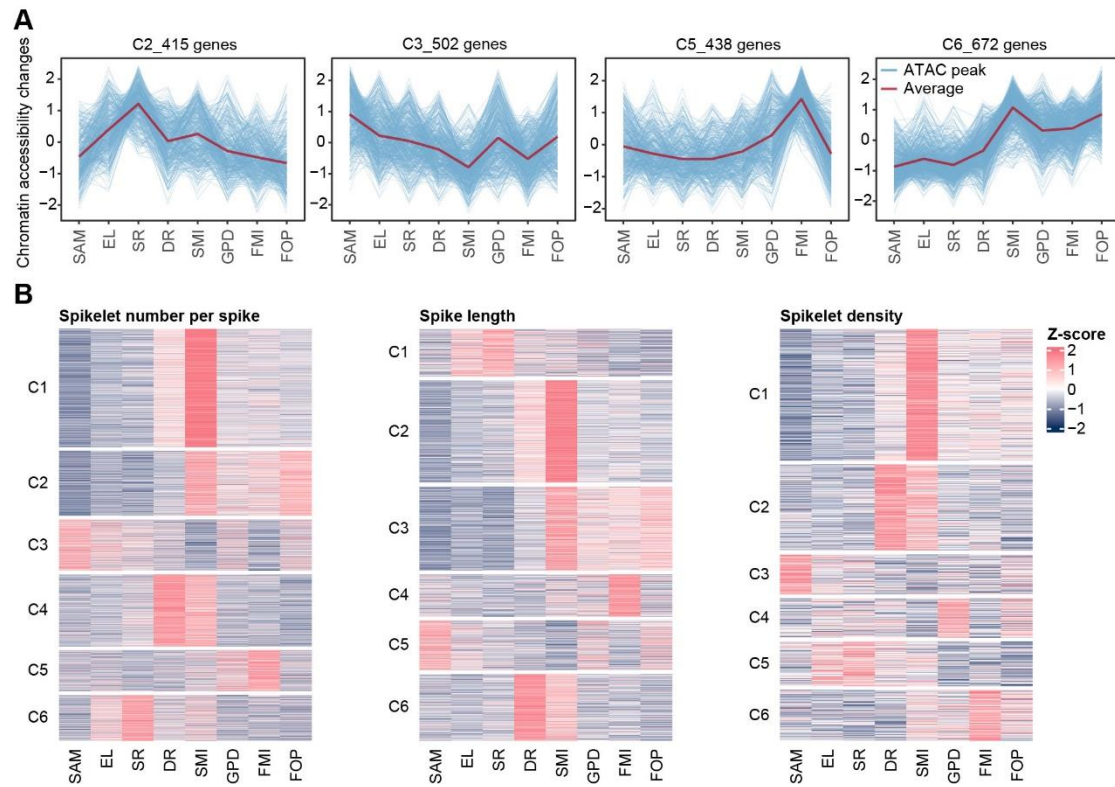
1395 (C and D) GO enrichment analysis of genes in gene set II (C), III (D) in Figure 2C.
 1396 (E) H3K27ac (top), H3K4me3 (middle) and H2A.Z (bottom) levels of genes in gene
 1397 set I, II, III in Figure 2C at SAM, DR and SMI stages.
 1398



1399

1400 **Supplemental Figure 3. Construction of transcription regulatory network (TRN).**

1401 (A) Schematic of the strategy for TRNs building (see method for detail).
 1402 (B) Total TRNs characterized for governing spike architecture formation.
 1403 (C) Map view of TF-targets for different TF families.
 1404 (D) Dynamic expression profile of TaSPL6, TaMADS34, TaMADS15 and TaHMA at
 1405 different stages during spike development.
 1406 (E) Presence of different TF binding motifs in the target gene's open chromatin regions
 1407 from the SPL6-MADS34-MADS15-HMA regulatory module.
 1408 (F) Schematic diagram showing the vectors used in the Luciferase reporter assays of
 1409 Figure 3H.



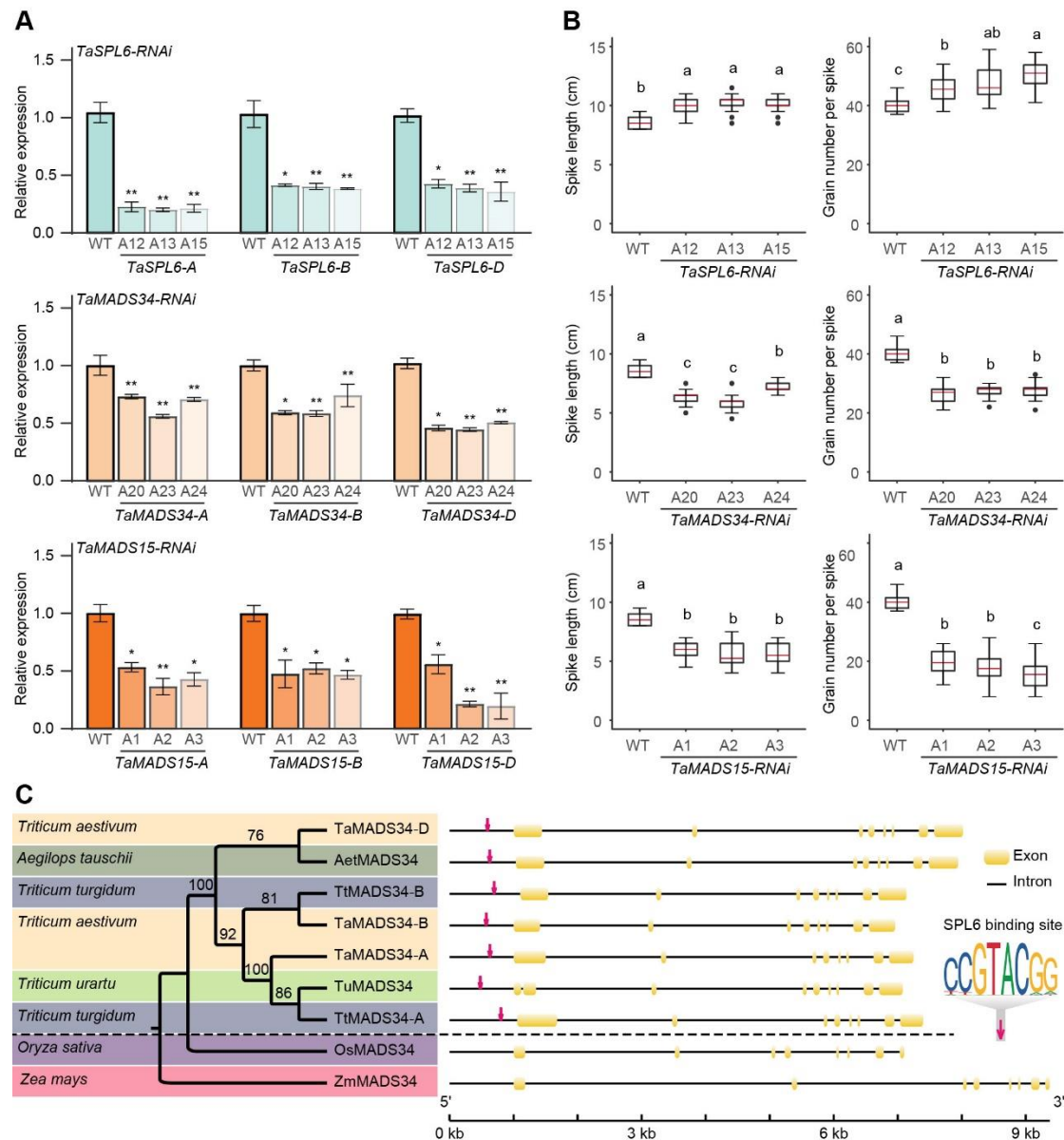
1410

1411 **Supplemental Figure 4. The chromatin accessibility dynamics of GWAS signal**

1412 **associated genes.**

1413 (A and B) K-means clustering of chromatin accessibility of fertile spikelet number per
1414 spike (FSPS) (A), spikelet number per spike (SNS) (left-B), spike length(SL)
1415 (middle-B), spikelet density (SD) (right-B) related genes during spike
1416 development.

1417



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1419 **Supplemental Figure 5. SPL6-MADS34-MADS15-HMA module affects spike**

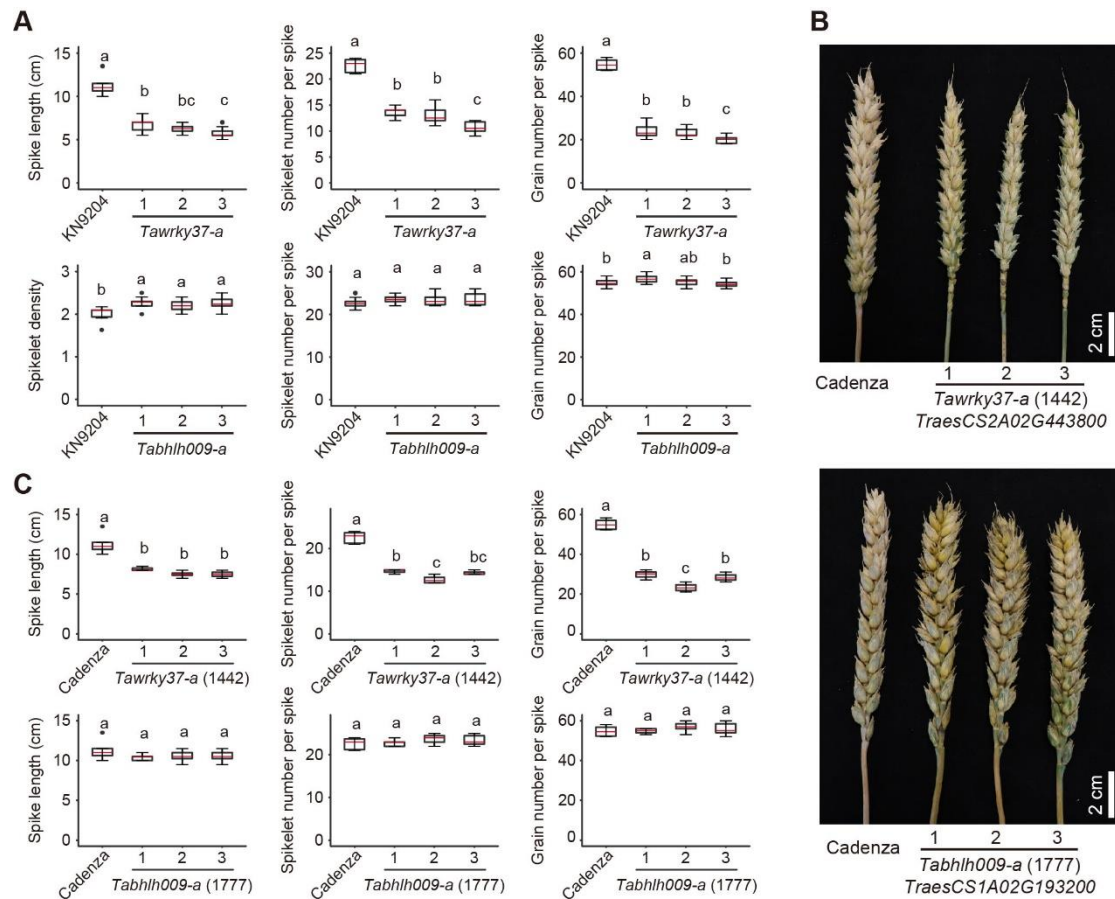
1420 **development in wheat.**

1421 (A) The knock-down efficiency of target genes in different *TaSPL6-RNAi*, *TaMADS34-*
 1422 *RNAi*, *TaMADS15-RNAi* transgenic plants as measured by qPCR. Fielder served
 1423 as wild-type (WT). Expression level of genes in WT is set as 1.0, the relative
 1424 expression of each gene in RNAi plants is shown as average \pm SD of three
 1425 replicates. Student's *t* test was used for the statistical significance (*, $p \leq 0.05$; **, $p \leq 0.01$).

1427 (B) Quantification of spike length, grain number per spike (GNPS) between WT,
 1428 *TaSPL6-RNAi*, *TaMADS34-RNAi* and *TaMADS15-RNAi* transgenic plants. Two-
 1429 tailed Student's *t*-tests. In box plots, the box limits indicate the 25th and 75th
 1430 percentiles, the whiskers indicate the full range of the data, and the centre line
 1431 indicates the median. Different letters mean significant difference at $p < 0.01$.

1432 (C) TaSPL6 binding motif (CCGTACGG) at chromatin accessible region of *MADS34*
 1433 orthologs in different *Triticum* was conserved, but not in *Oryza sativa* or *Zea*
 1434 *mays*. Phylogenetic tree of corresponding species is indicated on the left.
 1435 Schematic diagram of gene structure and presence or absence of SPL6 binding
 1436 motif sites is shown on the right.

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1439 **Supplemental Figure 6. The quantification data of spike phenotype associated**

1440 **with novel factors mutations.**

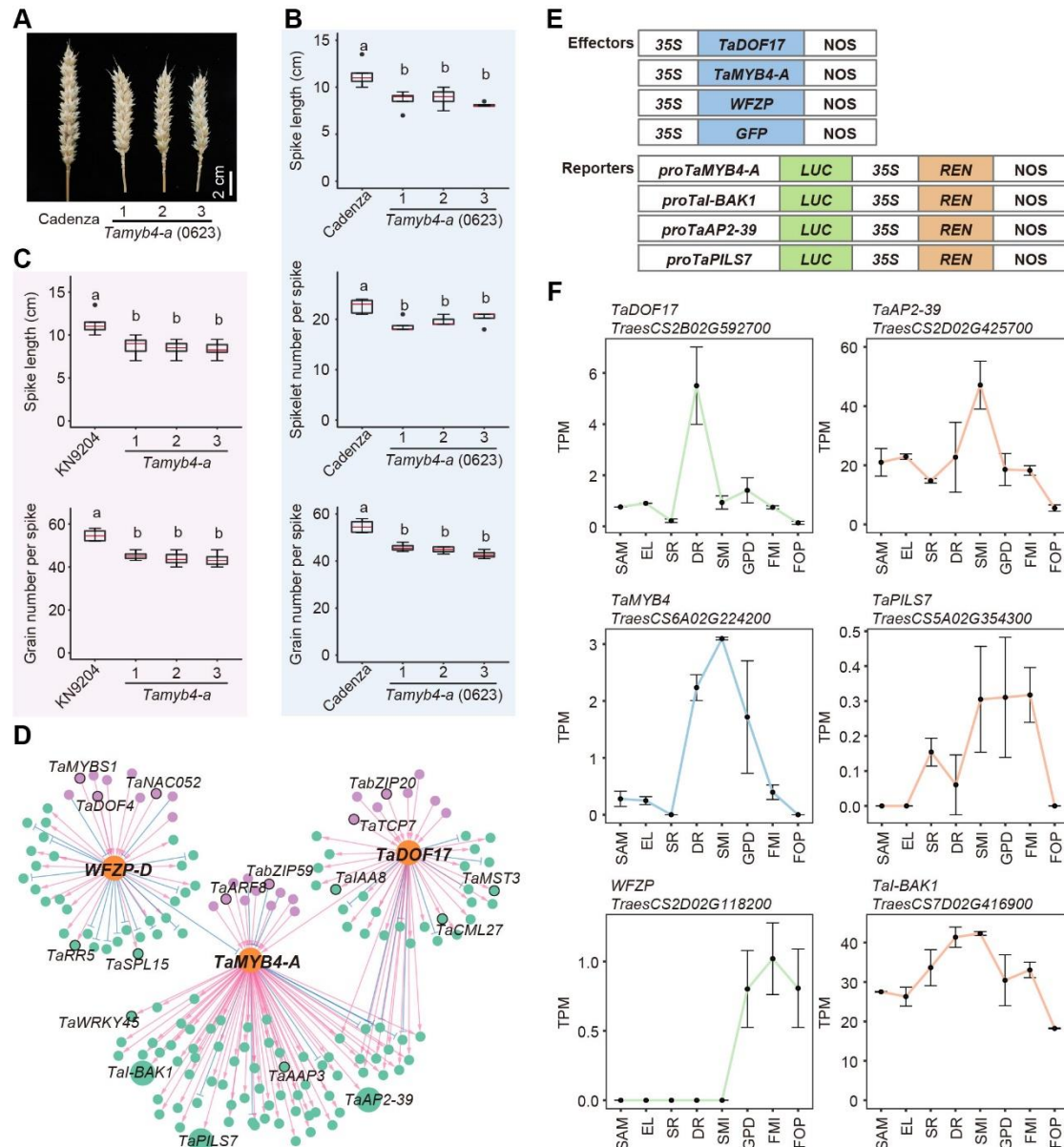
1441 (A) Quantification of spike length, spikelet number per spike (SNS), gain number per
 1442 spike (GNPS) for represented KN9204 TILLING lines containing mutation of
 1443 *Tabh09-a* or *Tawrky37-a*. Two-tailed Student's *t*-tests. In box plots, the box
 1444 limits indicate the 25th and 75th percentiles, the whiskers indicate the full range
 1445 of the data, and the centre line indicates the median. Different letters mean
 1446 significant difference at $p < 0.01$.

1447 (B) The spike developmental defect of *TraesCS2A02G443800* (*TaWRKY37-A*, line
 1448 1442) and *TraesCS1A02G193200* (*TabHLH009-A*, line 1777) mutant lines as
 1449 compared to control Cadenza. Scale bars =2 cm.

1450 (C) Quantification of spike length, spikelet number per spike (SNS), gain number per
 1451 spike (GNPS) for represented Cadenza TILLING lines containing mutation of
 1452 1442 (*TaWRKY37-A*, *TraesCS2A02G443800*) and 1777 (*TabHLH009-A*,
 1453 *TraesCS1A02G193200*). Two-tailed Student's *t*-tests. In box plots, the box limits

1454 indicate the 25th and 75th percentiles, the whiskers indicate the full range of the
 1455 data, and the centre line indicates the median. Different letters mean significant
 1456 difference at $p < 0.01$.

1457



1458

1459 **Supplemental Figure 7. The regulation network of TaMYB4-A of wheat**
 1460 **architecture.**

1461 (A) Comparison of the spike phenotype of Wild-type (Cadenza) and mutant lines from
 1462 Cadenza mutant library. 0623 (*TaWRKY37-A*, *TraesCS2A02G443800*). Scale bars
 1463 =2 cm.

1464 (B) Statistics comparison of spike length and spikelet number per spike (SNS) between
 1465 Wild-type (Cadenza) and 0623 (*TaWRKY37-A*, *TraesCS2A02G443800*) mutant
 1466 lines. Two-tailed Student's *t*-tests. In box plots, the box limits indicate the 25th
 1467 and 75th percentiles, the whiskers indicate the full range of the data, and the centre
 1468 line indicates the median. Different letters mean significant difference at $p < 0.01$.

- 1469 (C) Statistics comparison of spike length and grain number per spike (GNPS) between
1470 KN9204 and *Tamyb4-a* mutant lines. Two-tailed Student's *t*-tests. In box plots, the
1471 box limits indicate the 25th and 75th percentiles, the whiskers indicate the full
1472 range of the data, and the centre line indicates the median. Different letters mean
1473 significant difference at $p < 0.01$.
- 1474 (D) The transcription regulatory network (TRN) of *TaMYB4-A*.
- 1475 (E) Schematic diagram showing the vectors used in the Luciferase reporter assays of
1476 *TaMYB4-A* regulatory network of Figure 7F.
- 1477 (F) The expression level of *TaDOF17*, *TaMYB4-A*, *WFZP*, *TaAP2-39*, *TaI-BAK1*,
1478 *TaPILS7*.