GIF1 controls ear inflorescence architecture and floral development by regulating key genes in hormone biosynthesis and meristem determinacy in maize

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Abstract

Background: Inflorescence architecture and floral development in flowering plants are determined by genetic control of meristem identity, determinacy, and maintenance. The ear inflorescence meristem in maize (Zea mays) initiates short branch meristems called spikelet pair meristems, thus unlike the tassel inflorescence, the ears lack long branches. Maize growth-regulating factor (GRF)-interacting factor1 (GIF1) regulates branching and size of meristems in the tassel inflorescence by binding to Unbranched3. However, the regulatory pathway of gif1 in ear meristems is relatively unknown.

Result: In this study, we found that loss-of-function gif1 mutants had highly branched ears, and these extra branches repeatedly produce more branches and florets with unfused carpels and an indeterminate floral apex. In addition, GIF1 interacted in vivo with nine GRFs, subunits of the SWI/SNF chromatin-remodeling complex, and hormone biosynthesis-related proteins. Furthermore, key meristem-determinacy gene RAMOSA2 (RA2) and CLAVATA signaling-related gene CLV3/ENDOSPERM SURROUNDING REGION (ESR) 4a (CLE4a) were directly bound and regulated by GIF1 in the ear inflorescence.

Conclusions: Our findings suggest that GIF1 working together with GRFs recruits SWI/SNF chromatin-remodeling ATPases to influence DNA accessibility in the regions that contain genes involved in hormone biosynthesis, meristem identity and determinacy, thus driving the fate of axillary meristems and floral organ primordia in the ear-inflorescence of maize.

Keywords: Spikelet pair meristem, Branch meristem, Carpel, Clavata3/esr-related gene, Inflorescence

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to hundreds of kernels, is an important reproductive and agronomic tissue. In normal development of the ear, the inflorescence meristem (IM) first initiates indeterminate spikelet pair meristems (SPMs), which are short branches [1, 2]. Thus, unlike tassels, ears lack long branches. The ear florets initiate a palea, a lemma, two lodicules, three stamens, and three carpels. After initiation, the stamens abort in female flowers, but the carpels develop into a single pistil by fusing congenitally along their edges; two indeterminate abaxial carpels fuse to form the silk, and the third elongates to cover the ovule forming the ovary wall [3, 4]. After double fertilization, ovules enclosed by carpels develop into kernels (caryopsis). Therefore, branching and gynoecium development are both critically important for inflorescence architecture and floral fertility, as well as for grain yield.

Previous studies revealed a complex functional hierarchy of genes involved in inflorescence branching in maize. Mutations in three classical RAMOSA (RA) genes produce highly branched male and female inflorescences, in which the SPMs are converted into branch meristems (BMs) [5]. The RA3 encodes a trehalose-6-phosphate phosphatase [6], which removes the phosphate from trehalose 6-phosphate (T6P) to produce free trehalose, suggesting that sugar signaling triggered by polysaccharides may be involved in inflorescence architecture [7–9]. Mutation of RA3 leads to reduced expression of the zinc-finger domain protein-encoding gene RA1 [10], suggesting that RA3 may regulate RA1 directly or indirectly. The RA2 encodes a lateral organ boundary (LOB) domain transcription factor (TF) required for initiation of axillary meristems in both inflorescences. In ra2 mutants, expression of RA1 is also down-regulated, suggesting that both RA2 and RA3 positively regulate expression of RA1 [11]. Genes in the CLAVATA-WUSCHEL (CLV-WUS) feedback loop, such as Fasciated ear2 (FEA2) [12], FEA3 [13] and Thick tassel dwarf1 (TD1) [14] also regulate inflorescence branching.

Growth-regulating factor (GRF)-interacting factor1 (GIF1), which is one of the transcriptional coactivators of plant-specific growth-regulating factors (GRFs), has been characterized as a major regulator of plant vegetative and reproductive development [15–20]. In Arabidopsis (Arabidopsis thaliana), GIF1/AN3 is required for maintenance of the shoot apical meristem; establishment of the carpel margin meristem; development of flower organs, cotyledons, and leaves; cell proliferation and expansion; and lateral organ growth [17, 21–25]. In rice, OsGIF1 positively regulates plant height, leaf size, stem internodes, grain size, number of grains per panicle, and branches per panicle [26, 27], playing a major role in regulating the size of vegetative and reproductive organs and the number of inflorescence branches.

Recently, we found that maize gif1 mutants have fewer branches in the tassels than wild-type plants but extra branches in the ears [28], showing opposite effects of GIF1 on the branches in the tassel and the ear. Here, we compared the detailed morphological and anatomical differences in the ear inflorescence between the gif1-1 mutant and the wild type. We further identified GIF1-interacting proteins using immunoprecipitation-mass spectrometry (IP-MS) and GIF1 target genes through chromatin immunoprecipitation sequencing (ChIP-seq), integrating these data with transcriptome data. We propose that GIF1 regulates axillary meristems and floral organ primordia in the ear-inflorescence by targeting genes in hormone biosynthesis, genes in meristem identity and determinacy involving RAMOSA and CLV-WUS pathway.

Materials and methods

Plant materials and phenotypic characterization

The maize gif1-1 mutant was from our lab, which was originally found in the BS238 family line. The gif1-1 mutant and GIF1-GFP overexpressing lines were planted at Wuhan (30°N, 114°E), China. The phenotypes of traits including plant height, ear height and leaf length. The sample size for each phenotypic value was more than 30 individuals. All methods, including plant experimental research, were performed in accordance with the relevant institutional, national, and international guidelines and legislation.

GIF1-GFP fusion construct and genetic transformation

The GIF1 coding sequence was amplified with primers GIF1-F and GIF1-R (Supplemental Table 2) and fused to the green fluorescent protein (GFP) coding sequence. The GIF1-GFP fusion construct (Supplemental Fig. 4) was cloned into the pZZ01523 vector (Life Science and technology Center, China National Seed Group CO., LTD, China. http://www.chinaseeds-lstc.com), and the resulting vector was transformed into the maize ZZC01 line by Agrobacterium-mediated transformation [29]. Transgenic genotypes were determined using Trans-F/R primers, with the Trans-F primer designed against the GIF1 sequence and the Trans-R primer designed against the vector sequence. The expression level of gif1 was measured using Q-gif1-F/R primers designed against the 3’-UTR of gif1 (Supplemental Table 3).

Genetic complementation

To analyze biological functions, phenotypes of GIF1-GFP overexpressing lines were evaluated. Additionally, the gif1 allele of the gif1-1 mutant was introduced into ZZC01 genetic background through two cycles of backcrossing using marker-assisted selection. Those +/gif1
heterozygotes were crossed to line OE2. Individuals with gif1gif1 genotype and overexpressing GIf1-GFP (referred to as complemented plants) were selected through genotyping using gene-specific primers (Supplemental Table 3) and evaluated for phenotypic rescue of plant characteristics and inflorescence architecture.

**Microscopy observation**
For stereomicroscope observation, immature ears were collected from the gif1-1 mutant and its wild-type siblings, and the complemented plants. The ear inflorescence images were acquired using a Nikon SMZ25 microscope (Nikon, Japan) and merged by NIS (Nikon Imaging Software) elements. Immature ear inflorescences (5 mm) from the gif1-1 mutant and wild type were sampled according to Li et al. [30]. A sequential sampling procedure was performed from the beginning of the ninth leaf stage to observe the time course of inflorescence development. Inflorescence samples were fixed in a glutaraldehyde solution (2.5% v/v glutaraldehyde in 0.08 M phosphoric acid buffer) for 24 h at 4 °C and then dehydrated through a graded series of ethanol from 30 to 90%. Tissue samples were dried using a critical point dryer, sputter coated with gold palladium for roughly 45 s, and observed on a Hitachi S-4700 scanning electron microscope (Hitachi, Japan) at an accelerating voltage of 5 kV [28].

**Immunoprecipitation-massspectrometry (IP-MS)**
Developing ears (~5 mm in length) were collected from line OE2 and ground in a mortar using liquid nitrogen. The frozen powder was mixed with ice-cold extraction buffer (50 mM Tris–HCl, 150 mM NaCl, 1 mM phenylmethanesulfonyl fluoride, and 1% Triton X-100). The mixture was centrifuged at 3,000 g for 15 min. The supernatant was used as total protein for immunoblotting. Total proteins were separated using 30% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE). The gel was stained with 0.2% (w/v) silver nitrate. For immunoblotting, proteins were electrotransferred onto polyvinylidene difluoride membranes under 100 V and incubation with a 1:3,000 horseradish peroxidase (HRP)-goat anti-rabbit monoclonal antibody (M048-3, MBL, China) was used as the primary antiserum, followed by incubation with a 1:2,000 dilution of anti-GFP mouse monoclonal antibody (M048-3, MBL, China) was used as the primary antiserum, followed by incubation with a 1:3,000 horseradish peroxidase (HRP)-goat anti-rabbit secondary antibody (ab6721, Abcam).

For co-immunoprecipitation assay, total proteins were placed on ice for 30 min and centrifuged at 10,000 g for 10 min at 4 °C. GFP-Trap™_MA beads (ChromoTek, Planegg-Martinsried, Germany) were washed twice with 500 µL of extraction buffer. Each sample was mixed with 25 µL of clean beads, and the mixture was tumbled end-over-end for 3 h at 4 °C. The beads were magnetically separated, washed twice, and then heated in 100 µL of 2 × SDS sample buffer at 95 °C. Proteins from corresponding non-transgenic line of OE2 were used as a negative control. Immunocomplexes were analyzed by mass spectrometry. The IP-MS experiment was performed with three biological replicates. Proteins identified in at least two IP-MS experiments were referred to as GIf1-interacting proteins.

**Firefly luciferase complementation imaging (LCI) assay**
The open reading frames (ORFs) of GIf1 and the four genes encoding GIf1-interacting proteins were separately cloned into both JW771 (NLUC) and JW772 (CLUC) [31] using a CloneExpress II One Step Cloning Kit (Vazyme Biotech, Nanjing, China). The constructs were transformed into Agrobacterium tumefaciens strain GV3101. The transformed Agrobacterium cells were grown to OD (optical density)_{600} = 0.8, pelleted, and resuspended in infiltration buffer (10 mM methylester sulfonate, 10 mM MgCl₂, and 150 mM acetylsyringone, pH 5.7) and then infiltrated in different combinations into 3-week-old N. benthamiana leaves using a needleless syringe. After 48 h under 16 h of light and 8 h of dark, leaves were injected with 1 mM luciferin (Promega, Madison, WI, USA). The resulting luciferase signals were observed using a Tanon-5200 image system (Tanon Science, Shanghaï, China). The LCI assay was performed three times independently.

**Transcriptome libraryconstruction and sequencing**
The transcriptome library construction and RNA-seq analysis were done as Zhang et al. [28]. Ten immature ears (~5 mm) from the gif1-1 and wild-type sibling were collected and pooled, respectively, with three biological replicates. Fresh immature ears were immediately frozen in liquid nitrogen. Total RNA was extracted from each pool using Trizol (Life Technologies, Invitrogen, USA). After removing DNA with RQ1DNase (Promega, USA), 10 mg of total RNA was used for RNA-seq library preparation. Polyadenylated mRNAs were purified and concentrated with oligo (dT)-conjugated magnetic beads (Life Technologies, USA). Purified mRNAs were fragmented at 95 °C for 1 min, followed by end repair and 5′-adaptor ligation. Reverse transcription was then performed with a specific primer harboring a 3′-adaptor sequence and randomized hexamer. The cDNA was purified and amplified using random hexamers, and PCR products of 200 to 500 bp were collected, purified, quantified, and subjected to paired-end sequencing on an Illumina HiSeq 2000 system (Illumina Inc., San Diego, CA, USA) at the Beijing Genomics Institute (BGI).

For quantifying gene expression level, clean reads were mapped to the maize reference genome (B73 RefGen_v4) using SOAPaligner/SOAP2 [32] with no more than five
mismatches allowed in the alignment. Gene expression level was calculated using the FPKM method (fragments per kilobase transcriptome per million mapped). Differentially expressed genes (DEGs) between the gif1-1 mutant and the wild type were identified using \( p < 10^{-5} \) and a two-fold difference. The DEGs are listed in Supplemental Data Set 2. Gene Ontology (GO) analysis (by agriGO, http://systemsbiology.cau.edu.cn/agriGOv2/) was used to identify the enrichment of the DEGs.

**Chromatin immunoprecipitation (ChIP)-sequencing and data analysis**

The ChIP-Seq was done as Zhang et al. [28]. Approximately 1 g of ear inflorescences (~5 mm) was harvested from \( p3SS::GIF1-GFP \) line OE2 grown in a greenhouse with three biological replicates. Expression of the fused GIF1-GFP was verified by protein gel blotting using anti-GFP antibody (Abcam, AB290) at a dilution of 1:1,000 (v/v) in Tris-buffered saline buffer containing 5% nonfat milk powder. The inflorescences were immediately cross-linked in buffer containing 1% (v/v) formaldehyde for 15 min under vacuum, followed by addition of glycine to a concentration of 0.1 M and infiltration for 5 min. After three washes with distilled water (4 °C), the cross-linked tissues were dried with paper towels and flash-frozen in liquid nitrogen. Frozen tissues were ground thoroughly to a fine powder, which was then transferred to a precooled 50-mL tube with 20 mL of cold complete extraction buffer 1 (0.4 M sucrose, 10 mM Tris–HCl, pH 8.0, 10 mM MgCl2, 2.5 mM \( \beta \)-mercaptoethanol, and Plant Protease Inhibitor Cocktail (P9599, Sigma-Aldrich)). Homogenized tissues were centrifuged for 20 min at 1,000 g at 4 °C. The pellets were washed five times with 5 mL of complete extraction buffer 2 (0.25 M sucrose, 10 mM Tris–HCl, pH 8.0, 10 mM MgCl2, 1% (v/v) Triton X-100, 5 mM \( \beta \)-mercaptoethanol, and Plant Protease Inhibitor Cocktail) and once with extraction buffer 3 (1.7 M sucrose, 10 mM Tris–HCl, pH 8.0, 2 mM MgCl2, 0.15% (v/v) Triton X-100, 5 mM \( \beta \)-mercaptoethanol, and Plant Protease Inhibitor Cocktail). Washed pellets were resuspended in 300 mL of sonication buffer (50 mM Tris–HCl, pH 8.0, 10 mM EDTA, 1% SDS, and Plant Protease Inhibitor Cocktail), and the suspension was treated with a Bioruptor (Diagenode, Belgium) for 8 to 10 cycles with settings 30 s ON/30 s OFF at 4 °C. The sonicated sample was centrifuged for 10 min at 12,000 g at 4 °C, and the supernatant was collected and used for chromatin isolation. The extracted chromatin was immunoprecipitated with anti-GFP antibody (Invitrogen, A11122) with a Plant ChIP-seq kit (Diagenode, Belgium) according to the manufacturer’s instructions. Following de-cross-linking, isolation, and purification of the immunoprecipitated DNA, libraries were constructed using an Ovation Low Input DR kit (NuGEN Technologies, San Carlos, CA, USA). Two input and two IP libraries were subjected to sequencing on an Illumina HiSeq 2000 sequencer (Illumina Inc., USA).

ChIP-seq reads were aligned to the maize reference genome (AGPv4) using Hisat2 v.2.0.5 [33]. Only uniquely mapped reads were considered for further processing. PCR duplicates were removed using Picard Mark Duplicates (v.2.9.0; http://picard.sourceforge.net/). Peak calling was performed with MACS (v.1.4.2) [34]. Peaks were identified as significantly enriched (\( p < 10^{-5} \)) in each of the ChIP-seq libraries compared with input DNA. The FGS (Functional Gene Set) gene model within 10 kb of the peak summit was considered as a putative target of GIF1. ChIP tracks showing GIF1-GFP fusion protein binding sites were visualized using integrative genomics viewer [35].

**ChIP-qPCR**

The ChIP-qPCR was done as Zhang et al. [28]. To detect specific DNA targets, ChIP-qPCR was performed to quantify DNA targets immunoprecipitated by anti-GFP antibody relative to input DNA using SYBR Green qPCR Master Mix (Bio-Rad, Hercules, CA, USA) with three biological replicates, each with three technical replicates. The DNA target-specific primers used for the ChIP-qPCR assay are listed in Supplemental Table 3. The abundance of a target was normalized to that of nonspecific genomic regions, and fold enrichment of the DNA target relative to the input sample was then calculated. Significant differences were estimated by a Student’s t-test.

**Reverse-transcription quantitativePCR (RT-qPCR)**

To analyze gene expression, immature ears (~5 mm) were collected from the \( gif1-1 \) and wild-type plants. Total RNA was extracted from plant tissues using Ambion Pure Link Plant RNA Reagent (Life Technologies, USA) and reverse-transcribed with M-MLV reverse transcriptase (Life Technologies, USA) according to the manufacturer’s instructions. RT-qPCR was performed using a SYBR Green qRT-PCR kit (Bio-Rad, USA) according to the manufacturer’s instructions with three biological replicates; each replicate contained 10 individuals. Fold changes in RNA transcripts were calculated by the \( 2^{-\Delta\Delta Ct} \) method with maize \( Actin \) gene (Zm00001d010159) as an internal control. All reactions were performed on a CFX96 real-time system (Bio-Rad). All primers used for RT-qPCR are listed in Supplemental Table 3.

**Results**

The \( gif1 \) mutant has highly branched ears with unfused carpels

To uncover the roles of GIF1 in the ear-inflorescence development, we observed the initiation and differentiation of meristems during inflorescence development. We
found that wild-type maize ears produced paired spikelets with no long branches (Fig. 1A and C). By contrast, the ears of gif1-1 mutants frequently displayed highly short branched inflorescences (Fig. 1B and D). Spikelet meristems (SMs) on the branched inflorescences produced variable numbers of BMs or SPMs (Fig. 1B and D) and floral meristems (FMs) on the branched inflorescence also convert into BMs (Fig. 1E and F), indicating that GIF1 regulates determinacy of BMs and SMs or FMs identity.

In wild-type florets, development of the three stamen primordia is arrested and carpel primordia fuse to make a functional silk, and the ovule is well enclosed by the carpel (Fig. 1G). In gif1-1 florets, development of stamen primordia was also arrested; however, carpel primordia frequently failed to be initiated, or were initiated but failed to fuse, or were well developed in only one of the two florets (Fig. 1H). The ovule primordia were enlarged and naked (Fig. 1H), and the floral apex was indeterminate and frequently initiated extra ovule-like protrusions in gif1-1 florets, showing similarities to the ifa1 mutant [36], such as an expanded nucellus. These results indicated that GIF1 regulates fate and determinacy of meristems on the ear inflorescence.

Overexpression of GIF1 rescues the defective phenotypes of the gif1 mutant
To uncover the function of GIF1 in the ear inflorescence, we created five transgenic lines by introducing the Ubi promoter-driven GIF1-GFP constructs into the maize
ZZC01 inbred line. The gif1-1 mutant appeared as a dwarf plant (Fig. 2A), while transgenic lines overexpressing GIF1-GFP (OE) showed normal characteristics with greater plant height and ear height, and longer leaves than the gif1-1 mutant (Fig. 2C, G, H), but displayed non-significant difference from its non-transgenic sibling (NT2) (Fig. 2G). The ears from overexpression line (OE2) generated orderly and fertile florets without long branches (Fig. 2D). As contrast, gif1 ears produced long branches at the base of the ear inflorescence (Fig. 1D and Fig. 2B). To reveal the roles of GIF1 in the ear development, we first introduced gif1 into ZZC01 genetic background through two generations of backcrossing, and then crossed transgenic line OE2 to +/gif1 heterozygotes under ZZC01 genetic background followed by one generation of selfing. Those plants with homozygous gif1/gif1 and overexpressing GIF1-GFP which are referred to as complementation individuals were then selected by genotyping. All of these complementation individuals had normal plant characteristics and inflorescence architectures, both female and male florets were well developed and fertile (Fig. 2E, F). In addition, plant height, ear height and leaf length of complementation individuals were slightly greater than that of wild-type individuals with +/gif1 or +/- genotype although the difference is not statistically significant (Fig. 2H), but were significantly different from in the defective phenotypes of those gif1/gif1 individuals (n = 30). The results show that overexpression of GIF1-GFP can rescue the defective phenotypes on the ear inflorescence of the gif1 mutant.

GIF1 interacting proteins are involved in diverse biological processes

To identify GIF1-interacting proteins, we performed an IP assay using anti-GFP antibody in 5-mm ears from transgenic line OE2 with three biological replicates (Supplemental Fig. 1). We identified 56 GIF1-interacting proteins substantially enriched in at least two biological replicates (Table 1). Some of these proteins might indirectly interact with GIF1, given that GIF1 is a coactivator of GRFs; regardless, they are likely to be components of a GIF1-GRF recruiting complex. Consistent with proteins identified previously in Arabidopsis [37] and maize, nine GRFs, one SWI3D, two SNF12s, two Agenet domain proteins, two helicases, two actin-related proteins (ARP4 and ARP7), and one ATPase were identified as GIF1-interacting proteins in developing ears (Table 1 and Supplemental Table 1). These proteins were identified as subunits of

![Fig. 2 Overexpressing GIF1-GFP rescues the defective phenotypes on the ear inflorescence of the gif1 mutant under ZZC01 background.](image)

A and B Whole plant (A) and ear inflorescence (B) of the gif1-1 mutant. The gif1-1 mutant produced highly branched inflorescence (B). C and D Whole plant (C) and ear inflorescence (D) of transgenic line OE2 overexpressing GIF1-GFP. E and F Whole plant (E) and ear inflorescence (F) of the complemented line. Gene-specific molecular markers were used to genotype the gif1/gif1/OE2 individuals in the progeny families derived from +/gif1 (ZZC01) x GIF1-GFP-OE2 (ZZC01), which are homozygous genotype at the gif1 locus and are expressing the GIF1-GFP. G Plant height, ear height and leaf length of transgenic line OE2 overexpressing GIF1-GFP and corresponding non-transgenic line (NT) under ZZC01 background. H Plant height, ear height and leaf length of complementation individuals with gif1/gif1, wild-type individuals with GIF1/gif1 or GIF1/GIF1 genotype, and gif1/gif1 individuals identified from the progeny families derived from +/gif1(ZZC01) x GIF1-GFP-OE2 (ZZC01). The scale bars = 500 μm in (B), (D) and (F), and 10 cm in (A), (C) and (E). Sample size = 30 in (G) and (H). The column shows mean ± standard deviation (s.d.), the statistical significance was estimated using a Student's t-test. ** P < 0.01. ns: not significant statistically
### Table 1 GIP1-interacting proteins identified in maize ears by Co-IP

| Gene ID               | Function or domain               | No. of unique peptides | Annotation                                      | Expression at the ear (FPKM) |
|----------------------|----------------------------------|------------------------|-------------------------------------------------|-----------------------------|
| Zm00001d027326       | ARP                              | 8 4 9                  | Actin related protein 4                         | 58.73                       |
| Zm00001d013410       |                                  | 10 10 3                | Actin related protein 1                         | 133.24                      |
| Zm00001d053177       |                                  | 11 10 2                | Actin related protein 7                         | 155.76                      |
| Zm00001d011087       |                                  | 13 10 4                | Actin related protein 7                         | 195.84                      |
| Zm00001d047079       | Agenet domain                    | 18 10 3                | G2428-1                                         | 8.58                        |
| Zm00001d029889       |                                  | 22 14 2                | G2428-1                                         | 12.25                       |
| Zm00001d051840       | Helicase                         | 4 4 0                  | RNA helicase 2                                  | 48.5                        |
| Zm00001d044540       |                                  | 6 3 9                  | DNA replication licensing factor MCM4           | 42.79                       |
| Zm00001d017742       | GRF                              | 1 1 1                  | GRF1                                            | 174.02                      |
| Zm00001d021362       |                                  | 1 1 1                  | GRF10                                           | 57.97                       |
| Zm00001d000238       |                                  | 1 2 1                  | GRF11                                           | 235.59                      |
| Zm00001d045533       |                                  | 1 1 1                  | GRF12                                           | 207.44                      |
| Zm00001d033876       |                                  | 1 1 1                  | GRF15                                           | 152.36                      |
| Zm00001d051456       |                                  | 2 2 1                  | GRF17                                           | 110.11                      |
| Zm00001d006348       |                                  | 1 1 1                  | GRF4                                            | 61.03                       |
| Zm00001d037117       |                                  | 1 1 1                  | GRF7                                            | 29.60                       |
| Zm00001d017602       |                                  | 1 1 1                  | GRF18                                           | 172.67                      |
| Zm00001d022322       | SWI/SNF subunit                  | 4 3 0                  | SWI/SNF component SNF12 homolog               | 17.40                       |
| Zm00001d007039       |                                  | 5 5 4                  | SWI/SNF component SNF12 homolog               | 25.78                       |
| Zm00001d013391       |                                  | 3 3 0                  | SWI/SNF complex subunit SWI3D                 | 9.16                        |
| Zm00001d012052       | RRM/RBD/RNP                      | 2 2 0                  | RRM/RBD/RNP motifs protein                     | 372.4                       |
| Zm00001d002244       |                                  | 3 3 2                  | RNA recognition motif containing protein       | 82.37                       |
| Zm00001d011336       | hnRNP                            | 5 4 2                  | Heterogeneous nuclear ribonucleoprotein A3     | 80.57                       |
| Zm00001d041214       | ATPase                           | 9 3 0                  | ATPase 4 plasma membrane type                  | 57.64                       |
| Zm00001d038923       | Small GTPase                     | 6 6 1                  | Guanine nucleotide-binding protein β subunit   | 327.87                      |
| Zm00001d034590       |                                  | 3 5 0                  | Rho GTPase 1 mitochondrial                     | 34.43                       |
| Zm00001d017239       |                                  | 4 3 0                  | Ran GTPase-activating protein 2                | 36.21                       |
| Zm00001d037004       |                                  | 4 4 2                  | Ras-related protein RABA1d                     | 77.91                       |
| Zm00001d009572       |                                  | 3 4 1                  | Ras-related protein RABA1f                     | 48.05                       |
| Zm00001d011855       |                                  | 3 5 2                  | Arf-GTPase-activating protein AGD11             | 67.93                       |
| Zm00001d022316       | Protein biosynthesis and         | 4 2 0                  | Proteasome subunit alpha type                  | 135.42                      |
| Zm00001d027896       | metabolism                       | 4 4 3                  | Proteasome subunit alpha type                  | 137.84                      |
| Zm00001d049230       |                                  | 2 3 3                  | SKP1-like protein 1A                           | 880.84                      |
| Zm00001d046449       |                                  | 13 8 0                 | Elongation factor 1-alpha9                    | 615.43                      |
| Zm00001d034856       |                                  | 6 6 2                  | Elongation factor TU                           | 89.24                       |
| Zm00001d053196       |                                  | 3 5 5                  | Proline-tRNA ligase cytoplasmic                | 33.85                       |
| Zm00001d048021       | Hormone-related                  | 3 3 0                  | Allene oxide synthase 1 (AOS1)                 | 44.48                       |
| Zm00001d014887       |                                  | 4 3 3                  | Nana Plant2 (NA2)                              | 215.56                      |
| Zm00001d041711       |                                  | 0 1 1                  | Auxin-binding protein 1(Abp1)                  | 325.99                      |
| Zm00001d037700       | Chaperone                         | 9 17 11                | Heat shock protein 4                           | 41.36                       |
| Zm00001d017401       |                                  | 4 7 3                  | Hsp70-Hsp90 organizing protein 3               | 32.23                       |
| Zm00001d032789       |                                  | 9 15 2                 | Chaperonin2                                    | 41.26                       |
| Zm00001d050375       | Regulatory molecule              | 14 13 4                | 14–3–3-like protein                            | 410.94                      |
| Zm00001d052698       |                                  | 8 9 0                  | 14–3–3-like protein Gf14 nu                    | 37.90                       |
| Zm00001d003401       |                                  | 9 12 2                 | 14–3–3-like protein Gf14-6                     | 550.72                      |
| Zm00001d030968       | Nucleus                           | 0 1 4                  | Flowering locus K homology domain              | 96.52                       |
| Zm00001d042091       | Developmental process            | 1 0 4                  | Flowering time control protein FY             | 13.25                       |
| Zm00001d024523       |                                  | 2 2 0                  | Ramosa1 enhancer locus2                        | 87.75                       |
the SWI/SNF complex that regulates chromatin structure by altering nucleosome composition and interactions [38]. And these proteins have been repeatedly identified to interact with GIF1 in different plant species, suggesting a reliable interaction between GIF1 and SWI/SNF subunits.

In addition to subunits of the SWI/SNF complex, Importin subunit alpha, a homologous protein of (Importin β4) that regulates Arabidopsis ovule development mediating nuclear import of GRF-interacting factors [21], was found to interact with GIF1 (Table 1). Auxin-binding protein1 (ABP1), one of putative auxin receptors, was also found to interact with GIF1 (Table 1). Notably, a brassinosteroid (BR) biosynthesis-related protein NANA PLANT2 (NA2) [39], an enzyme in jasmonic acid (JA) biosynthesis AOS1 [40, 41], and a key inflorescence factor RAMOSA1 ENHANCER LOCUS2 (REL2) [42] were found to interact in vivo with GIF1 (Table 1). Subsequently, several interaction pairs including NA2-GIF1 and ABP1-GIF1 were verified in vitro by firefly luciferase complementation imaging (LCI) assays in Nicotiana benthamiana leaves (Fig. 3A-D). These data suggest that GIF1-interacting complexes are directly and indirectly involved in diverse biological processes, including chromatin remodeling, hormone biosynthesis, and protein transport. GIF1 contains an N-terminal, a C-terminal, and a SSXT domain. Furthermore, to understand the domain mediating protein interaction, we created three GIF1 constructs containing N-terminal and C-terminal truncations and performed the yeast-two-hybridization (Y2H) experiments with NA2 and REL2 proteins. We found both N-terminal and SSXT domain are required for mediating interaction with NA2 and REL2 (Fig. 3E). Similarly, a set of NA2 constructs and REL2 constructs were created as illustrated in Fig. 3E. Y2H experiments revealed that GIF1 can interact with the REL2 C-terminal domain (REL2-C), but not the N-terminal domain, the REL2 C-terminal domain lacking the WD40-2 motif can interacts with GIF1 protein as well (Fig. 3F). NA2 has a signal peptide (SP), a transmembrane region (TM), a FAD_lactone_oxidase domain (FAD_lactone_ox), and C-terminal. Of them, the FAD_lactone_ox domain was required for mediating GIF1-NA2 interaction (Fig. 3F).

**Hormone and inflorescence development-related genes are regulated by GIF1**

To identify genes regulated by GIF1, we performed RNA sequencing (RNA-seq) and found that 2,145 down-regulated and 3,401 up-regulated genes were differentially expressed in 5-mm ears of the gif1 mutant compared to that of wild-type sibling under \( p < 10^{-5} \) and ~two-fold expression difference (Fig. 4A, Supplemental Data Set 1). Those down-regulated genes were primarily enriched in several biological processes, including 16.0% in the single-organism metabolic process (GO:0,044,710, \( p = 3.8e-5 \)), 9.7% in the regulation of biosynthetic process (GO:0,009,889, \( p = 2.9e-5 \)), 5.6% in response to chemical (GO:0,042,221, \( p = 3.8e-5 \)), and 6.6% of up-regulated genes were enriched in the transporter activity (GO:0,005,634, \( p = 5.2e-14 \)) molecular function (Supplemental Data Set 2). These up-regulated genes were primarily enriched in several biological processes, including 16.0% in the single-organism metabolic process (GO:0,044,710, \( p = 3.8e-5 \)), 9.7% in the regulation of biosynthetic process (GO:0,009,889, \( p = 2.9e-5 \)), 5.6% in response to chemical (GO:0,042,221, \( p = 1.4e-5 \)), and 6.6% of up-regulated genes were enriched in the transporter activity (GO:0,005,215, \( p = 1.8e-5 \)) molecular function (Supplemental Data Set 3). Because of the interaction between GIF1 and NA2, we detected expression of hormone-related genes, and found that genes in BR metabolism and signaling (GO:0,016,131 and GO:0,009,742), including nana plant1 (Zm00001d042843, NA1),

| Gene ID          | Function or domain                      | No. of unique peptides | Annotation                              | Expression at the ear (FPKM) |
|------------------|----------------------------------------|------------------------|------------------------------------------|----------------------------|
| Zm00001d009850   | Intracellular transport                 | 5                      | Importin subunit alpha                   | 86.48                     |
| Zm00001d033734   |                                        | 6                      | Coatomer subunit alpha-1                | 10.78                     |
| Zm00001d007758   |                                        | 8                      | Coatomer subunit gamma                  | 19.90                     |
| Zm00001d049155   |                                        | 2                      | Sec23/Sec24 protein                      | 28.91                     |
| Zm00001d028143   |                                        | 1                      | COP9 signalosome complex subunit 4       | 62.60                     |
| Zm00001d032859   | Cell division                          | 13                     | Cell division control protein 48 homolog | 36.74                     |
| Zm00001d014124   |                                       | 13                     | Cell division cycle protein 4B           | 50.72                     |
| Zm00001d040429   | Argonaute                              | 2                      | Argonaute105                            | 74.68                     |

EXP Experiment
brassinosteroid-deficient dwarf1 (Zm00001d033180, BRD1), and BR-signaling kinases (Zm00001d030021 and Zm00001d047053), were drastically down-regulated, while response to auxin (GO:0,009,733) and response to jasmonic acid (GO:0,009,753) including JA-related genes silkless1 (Zm00001d002970, SK1), tasselseed1 (Zm00001d003533, TS1) were up-regulated, suggesting disturbed homeostasis of hormones in the gif1-1 ear (Fig. 4B, Supplemental Data Sets 2 and 3). Furthermore, down-regulated genes were significantly enriched in those terms for development of floret, inflorescence and meristem (Fig. 4B). In particular, genes in the CLV-WUS feedback loop were differentially expressed in ears of the gif1-1 and wild-type plants: two clv3/endosperm surrounding region-related genes (CLE4a and CLE23) were down-regulated, while four WUS-related homeobox genes (WOX2b, WOX4, WOX5b, and WOX13b) were up-regulated in gif1-1 ears (Fig. 4C, Supplemental Data Set 3). Importantly, a subset of well-characterized genes for inflorescence architecture including RA1 [6, 10], RA3 [11] Unbranched2 (UB2), UB3, Tassel sheath1 (TSH1) and TSH4 [43, 44], and genes for floral development including Silky1 (SI1) and Silkless 1 (SK1) [45, 46] were down-regulated in
gif1-1 ears (Supplemental Data Set 4). Expression levels of 20 representative DEGs, which are candidates to be associated with defective phenotypes in the gif1-1 mutant, was verified by reverse transcription-quantitative PCR (RT-qPCR) (Fig. 4D, E).

To determine the occupancy of GIF1, we performed ChIP-seq to detect GIF1-bound DNA regions in immature ears (~5 mm) of transgenic line OE2 over-expressing GIF1-GFP using anti-GFP antibody. A total of 10,460 high-confidence peaks were identified.
by comparing significantly GIF1-enriched peaks with the input control \((p < 10^{-5})\), of which, 1,308 peaks were shared in at least two replicates, respectively (Supplementary Fig. 2). GIF1 bound to various genomic contexts, with a high proportion (45.0\%) of binding within intergenic regions which agree with the interaction between GIF1 and SWI/SNF subunits, and 12.9\% and 11.9\% binding within 1.0 kb downstream of the terminal site and within exons, respectively (Supplementary Fig. 2). Within 10 kb of high-confidence peaks, we identified 540 genes as putative targets of GIF1 in at least two replicates (Supplementary Fig. 2). Furthermore, 79 DEGs including 47 down-regulated (59.5\%) and 32 up-regulated (40.5\%) genes in gif1 ears were bound by GIF1 (Fig. 5A, Supplementary Table 2), suggesting that these genes are direct targets of GIF1, which acts as a repressor and an activator of gene expression in developing ears. The function of GIF1 in repression of gene expression can be partially explained by its interactors (Table 1), such as subunits of SWI/SNF chromatin remodeling complex, and RAMOSA1 ENHANCER LOCUS2 (REL2) which is a transcriptional co-repressor functioning in vegetative and reproductive architecture [42]. Notably, these targets of GIF1 were significantly enriched in 5 GO terms including cell periphery (GO: 0,071,944) and response to hormone (GO: 0,009,725) (Fig. 5B). In addition, several meristem identify, determinacy and maintenance-related genes were also bound by GIF1 (Supplementary Table 2). For example, GIF1 bound to the promoter and 3′-untranslated region (UTR) of CLE4a, the promoter region of TPS2, gene body of AGO108. As expression of CLE4a and AGO108 was significantly down-regulated in gif1 ears (Fig. 5C-E-I-K), the data suggest that CLE4a and AGO108 are two positively regulated targets of GIF1. The expression of TPS2 was significantly up-regulated in gif1 ears (Fig. 5F-H), indicating that TPS2 is a negatively regulated target of GIF1. Moreover, PIN8, RA2, OFP21 (Supplementary Fig. 3) and several TF-encoding genes (Supplementary Table 2) were also strong candidates for key targets of GIF1.

Discussion

To explore the role of GIF1 regulation in ear meristems, we created GIF1-overexpressing lines to complement gif1 and identified GIF1-interacting proteins and GIF1 target genes. We found that a set of GRFs and some subunits of the SWI/SNF complex interact with GIF1 in vivo, the finding is consistent with those results from early investigations on GIF1-interacting protein in vegetative and reproductive development of Arabidopsis [16, 18], rice [26, 27], and maize [35]. SWI/SNF are high molecular weight complexes that could change interactions between histone octamers and the DNA [38]. GIF1 working together with GRFs recruits SWI/SNF chromatin-remodeling ATPases to influence DNA accessibility and might expose the cis-element of target genes to GRFs. GRFs could stimulate or inhibit the transcription of target genes (Fig. 6). In addition to GRFs and SWI/SNF factors, we found that GIF1 interacts with proteins that involve in cell division, molecular signaling, etc. Thus, we suggest that GIF1 is involved in a wide spectrum of biological processes by selectively interacting with diverse proteins during ear development.

GIF1 regulates the fate of ear axillary meristems and floral organ primordia in ear inflorescence of maize. We found that GIF1 could regulate identity and determinacy of reproductive axillary meristems by hormone biosynthesis process (Fig. 6). We found that BR biosynthesis enzyme NA2 interacts with GIF1, and 10 genes related with BR metabolism and signaling pathway including BRD1 and NA1 were significantly down-regulated in the gif1 mutant. BR is found to affect plant height, branching, and sexual organ (stamen and pistil) development in maize [39, 47, 48]. The severely reduced plant height and ear with anthers in na2 mutants was similar to dwarf plant and highly frequent branches in gif1 mutants. We also found that JA-related genes SK1, TS1 and TS2 were up-regulated in gif1 ears. Both TS1 and TS2 are required for JA-mediated elimination of pistils in the staminate [49, 50]. Conversely, SK1 could protect pistils in the ear florets from JA-mediated elimination [46]. The up-regulated JA-related genes might participate in the identity of SMs and FMs in the gif1 mutant. Therefore, we propose that BR-related and JA-related pathway for floral organ development and meristem identity are regulated by GIF1 (Fig. 6).

GIF1 also regulates BM determinacy by targeting RAMOSA and CLV-WUS pathway. The gif1 mutant produced ear with long branches similar to that of ramosa mutants. The well characterized RAMOSA genes, RA1, RA2, RA3 and REL2 are involved in AM formation and BM determinacy [6, 10, 11, 51]. We found that GIF1 is directly interacted with REL2, an enhancer of RA1. The GIF1-REL2 complex might regulate the BM determinacy by RA1. GIF1 is a positive regulator of RA2 but a negative regulator of TPS2. TPS2 encodes a trehalose-6-phosphate (T6P) synthase. RA3 controls long branches in the ear and the tassel by catalyzing dephosphorylation of T6P [6]. Thus, we infer that GIF1 regulates AM determinacy with RAMOSA pathway genes. The CLAVATA genes encode CLV ligands and CLV receptors. CLV3, a small peptide ligand secreted...
Fig. 5 Putative targets of GIF1 detected by chromatin immunoprecipitation sequencing (ChIP-seq). A Genes both detected by ChIP-seq and showing differential expression detected by RNA-seq. DEG: differentially expressed gene. B Gene Ontology (GO) enrichment of 79 GIF1 targets. Gene num. = gene number. C, F, I Peak distribution of three representative targets including CLE4a, TPS2 and AGO108. D, G, J Fold enrichment of three representative targets detected by ChIP-qPCR. Gene specific primers were used to quantify DNA targets including CLE4a, TPS2 and AGO108 immunoprecipitated by anti-GFP antibody relative to input DNA, respectively. The columns are the mean value of fold enrichment detected in three separate experiments, each with three technical replicates. Error bars show the standard deviation. The statistical significance was estimated using a Student's t-test. ** P < 0.01. E, H, K Relative expression levels of CLE4a, TPS2 and AGO108 in ears of the gif1 and the wild type detected by RNA-seq and qPCR. qPCR is performed with three biological replicates, each with three technical replicates. Error bars show the standard deviation. CLE4a: CLAVATA3 (CLV3)/ENDOSPERM SURROUNDING REGION (ESR) 4a. TPS2: Trehalose-6-phosphate synthase 2. AGO108: Argonaute108
from cells of the central zone, is perceived by CLV1 and CLV2 to repress WUS transcription to regulate the meristem size [52]. Two maize CLV3 orthologs secreted peptides, ZmCLE7 and ZmFCP1, interact with CLV2 ortholog FEA2 to transmit signals and regulate inflorescence meristem size [53]. We found that GIF1 binds to the promoter of CLE4a and down-regulated the expression of CLE4a in the gif1 mutant, suggesting that GIF1 is a positive regulator of CLE4a and the IM activity of gif1 ears might result from transcriptional repression of CLE4a.

Conclusions
The transcription coactivator Growth-regulating factor (GRF)-interacting factor 1 (GIF1) interacts with given GRFs dependent upon the developmental context, and also recruit additional protein factors, for example SWI/SNF chromatin remodeling complexes, Ramosa1 Enhancer Locus 2 (REL2), to establish a multi-factor transcription complex. The transcription complex specifically bind to its target genes to repress Brassinolide (BR) biosynthesis and metabolism genes (BRD1 and NA1); meristem maintenance gene CLE4a and auxillary meristem determinacy gene TPS, but activate Jasmonic acid (JA) biosynthesis genes (SK1, TS1 and TS2). Consequently, the fine transcription control of these target genes determines the identity and determinacy of reproductive axillary meristems in the ear inflorescence (Fig. 6).

Abbreviations
BM: Branch meristem; BR: Brassinolide; CHIP: Chromatin immunoprecipitation; DEG: Differentially expressed gene; FEA: Fasciated ear; FM: Floral meristem; GFP: Green fluorescent protein; GIF: Growth-regulating factor-interacting factor; GO: Gene ontology; GRF: Growth-regulating factor; HRP: Horseradish peroxidase; IM: Inflorescence meristem; IP-MS: Immunoprecipitation-mass spectrometry; JA: Jasmonic acid; LCI: Firefly luciferase complementation imaging; LOB: Lateral organ boundary; NT: Non-transgenic sibling; OE: Overexpression line; ORF: Open reading frame; SDS-PAGE: Sodium dodecyl sulfate–polyacrylamide gel electrophoresis; SM: Spikelet meristem; SPM: Spikelet pair meristem; T6P: Trehalose 6-phosphate; TF: Transcription factor; UTR: 3′-Untranslated region; Y2H: Yeast-two-hybridization.

Supplementary Information
The online version contains supplementary material available at https://doi.org/10.1186/s12870-022-03517-9.

Additional file 1: Supplemental Figure 1. Identification of total proteins and immunoprecipitated proteins by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting. Supplemental Figure 2. Summary of chromatin immunoprecipitation sequencing (ChIP-seq). Supplemental Figure 3. Targets of GIF1 detected by chromatin immunoprecipitation sequencing (ChIP-seq). Supplemental Figure 4. Schematic diagram of the gif1 over-expression construct. The construct components include the T-DNA right border, RB, and left border, LB; CaMV35S promoter, CaMV35S; terminator of nopaline synthase gene, tnos; enhanced green fluorescent protein gene, eGFP; the phosphonothricin acetyltransferase cassette, bar.

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Authors' contributions
Conceived and designed the experiments: ZZ. Performed the experiments: YZ, YD, WS, ML and DZ. Analyzed the data: ZZ and ML. Revised the manuscript: ML and HD. Wrote the manuscript: ZZ. All authors have read and approved the manuscript.
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Availability of data and materials
Sequence data from this article can be found in the NCBI SRA dataset under the following accession numbers: PRJNA790036.

Declarations

Ethics approval and consent to participate
Not applicable in this study.

Consent for publication
Not applicable in this study.

Competing interests
The authors have declared that no competing interests exist.

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