



The C2H2-type zinc finger protein *SIHair5* acts as a regulatory node coordinating trichome development and leaf morphogenesis in tomato

Seong-Min Kim¹ · Jiyoung Kim² · Jae-In Chun³ · Jang-Kyun Seo^{1,2,3} · Choonkyun Jung^{1,2,3} · Jin-Ho Kang^{1,2,3}

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Abstract

Key message *SIHair5* is a pivotal regulatory node downstream of the *H-H4* module that coordinates epidermal differentiation with leaf morphogenesis and plant architecture through a dosage-sensitive transcriptional network and a reciprocal feedback loop.

Abstract C2H2-type zinc finger proteins (C2H2 ZFPs) are essential regulators of plant development, yet the mechanisms governing their functional diversification and hierarchical organization in tomato (*Solanum lycopersicum*) remain largely elusive. In this study, we identified and characterized *SIHair5* (*H5*), a previously unassigned C2H2 ZFP that operates downstream of the established *H-H4* regulatory module. *H5* localizes to the nucleus, where its expression is indirectly activated by the *H-H4* module. Functional characterization using CRISPR-Cas9-mediated knockout (*h5-sko*) and overexpression (*H5-OX*) lines demonstrated that *H5* is a pivotal developmental regulatory node. At the cellular level, *H5* promotes epidermal differentiation by modulating trichome density and stalk cell elongation in a type-specific manner. Intriguingly, *H5* exhibits dosage-sensitive and non-linear regulatory effects; while trichome traits showed a relatively linear response to *H5* levels, organ-level traits—such as leaf shape and plant height—displayed a threshold effect, with pronounced growth retardation and narrowed leaflets observed in *H5-OX* lines. Transcriptomic profiling identified 569 differentially expressed genes, revealing that *H5* orchestrates a broad network including regulators of trichome development (*JAZ4* and *WRKY57*), leaf morphogenesis (*Tf*, *GH3.15*, *BOPa*, and *CKX2*), and stem elongation (*GT-26* and *ER*). Furthermore, *H5* exerts an indirect feedback control over *H3* and *H4*, thereby fine-tuning the ZFP regulatory hierarchy to maintain developmental homeostasis. Collectively, our findings establish *H5* as a higher-order regulatory hub that coordinates cellular patterning with organismal architecture, providing critical insights into the transcriptional programs governing vegetative development in tomato.

Keywords Leaf morphogenesis · Tomato · Transcriptional regulation · Trichome development · Zinc finger protein

Seong-Min Kim and Jiyoung Kim these authors are equally contributed to this work.

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✉ Jin-Ho Kang
kangjinho@snu.ac.kr

¹ Crop Biotechnology Institute, Institutes of Green-Bio Science and Technology, Seoul National University, Pyeongchang 25354, Republic of Korea

² Department of International Agricultural Technology, Seoul National University, Pyeongchang 25354, Republic of Korea

³ Integrated Major in Global Smart Farm, College of Agriculture and Life Sciences, Seoul National University, Seoul 08826, Republic of Korea

Introduction

C2H2-type zinc finger proteins (C2H2 ZFPs) constitute one of the largest families of transcription factors (TFs) in plants, governing a diverse array of biological processes. These range from seed germination and leaf development to floral induction and fruit ripening (Liu et al. 2022). Beyond their fundamental roles in growth, C2H2 ZFPs are essential mediators of responses to abiotic stresses, including osmotic, thermal, and oxidative challenges (Han et al. 2020). At the molecular level, these TFs primarily operate through the transcriptional regulation of downstream targets or through intricate protein–protein interactions with other TFs and hormonal signaling components, thereby integrating developmental programs with environmental stimuli (Laity et al.

2001). Notably, C2H2 ZFPs have emerged as central regulators of epidermal cell differentiation, particularly in the complex patterning and morphogenesis of trichomes (Han et al. 2021). As specialized epidermal outgrowths, trichomes provide a robust physical and chemical defense against environmental stressors (Kang et al. 2010a, b), while serving as an ideal model for investigating the fundamental mechanisms of multi-scale cell differentiation (Hülkamp 2004; Yang and Ye 2013).

Trichome morphology varies significantly across plant species. In the model plant *Arabidopsis thaliana*, trichomes are typically unicellular, non-glandular structures consisting of a stalk with two to three branches (Hülkamp et al. 1994). Their relatively simple architecture has facilitated the identification of genetic networks controlling cell fate, initiation, and maturation (Hülkamp 2004). In contrast, tomato (*Solanum lycopersicum*) trichomes are multicellular and are classified into seven distinct types (I–VII) based on their morphology, presence of glandular heads, and size (Luckwill 1943). Unlike the unicellular structures in *Arabidopsis*, the morphogenesis of tomato trichomes involves precisely regulated rounds of cell division and expansion, particularly during the formation of multicellular stalks (Glas et al. 2012). Type I and IV trichomes are glandular and possess long and short stalks, respectively; types III and V are non-glandular; and types VI and VII are short glandular trichomes specialized for secondary metabolite biosynthesis. These glandular and non-glandular trichomes contribute to chemical and physical defense against herbivore attack (Kang et al. 2010a, b). This structural diversity renders the tomato trichome a powerful platform for studying not only cellular initiation but also multicellular differentiation and specialized metabolic functions (Li et al. 2025). Recent evidence suggests that tomato trichomes are differentially regulated as either digitate (Types I–V) or peltate (Types VI–VII) forms by distinct molecular pathways (Chang et al. 2024; Wu et al. 2023, 2024).

The regulatory framework of trichome development is well-characterized in *Arabidopsis*, where C2H2 ZFPs such as *GLABROUS INFLORESCENCE STEMS (GIS)*, *GIS2*, *GIS3*, *ZFP5*, *ZFP6*, and *ZFP8* function within a hierarchical network (Gan et al. 2006, 2007; Sun et al. 2015; Zhou et al. 2011, 2013). These proteins modulate the MYB–bHLH–WD40 (MBW) transcriptional complex and regulate *GLABRA2* to specify trichome cell fate (Larkin et al. 2003). These findings establish C2H2 ZFPs as key upstream regulators, integrating developmental and environmental cues to fine-tune trichome development in *Arabidopsis*. While some homologous pathways exist in tomato, the mechanisms are markedly more complex due to the structural variety of multicellular trichomes. Key regulators identified to date include *Hair (H)* and *Hair2 (H2; SLZFP8-like)*, which promote the initiation and elongation

of specific trichome types (Chang et al. 2018; Chun et al. 2021; Zheng et al. 2022). Recently, *Hair3 (H3)* and *Hair4 (H4)* were shown to act upstream of *H*, influencing both trichome patterning and overall vegetative growth (Kim et al. 2025). Furthermore, C2H2 ZFPs like *Lyrate* and *Obscure vein* have been implicated in coordinating leaf morphogenesis with epidermal development (David-Schwartz et al. 2009; Lu et al. 2021). These findings underscore that C2H2 ZFPs involved in trichome development frequently intersect with pathways governing whole-plant architecture and organogenesis.

Despite these advancements, the broader genetic framework governing tomato vegetative development remains incomplete. Only a limited number of downstream transcriptional targets have been functionally validated, and the discovery of additional regulators is essential to fully map the ZFP hierarchy. Our previous transcriptomic analysis of *h3/h4* double-knockout (*h3/h4-dko*) mutants identified several candidate genes potentially linked to trichome development, providing a foundation for further investigation (Kim et al. 2025). In this study, we identify *H5 (Soly01g060420)* as a novel C2H2 ZFP that serves as a developmental hub downstream of the *H3–H4* module and is transcriptionally regulated by *H* and *H2*. Using CRISPR-Cas9-mediated knockout (*h5-sko*) and overexpression (*H5-OX*) lines, we demonstrate that *H5* specifically governs the initiation and stalk-cell-mediated elongation of type I trichomes across various tissues in a dosage-dependent manner. Furthermore, we show that *H5* modulates leaf expansion and vegetative growth, extending its regulatory influence beyond epidermal differentiation. By characterizing a reciprocal but indirect feedback loop between *H5* and its upstream activators (*H3* and *H4*), our findings establish *H5* as a central node in the C2H2 ZFP hierarchy. Transcriptomic profiling further reveals that *H5* orchestrates these diverse developmental processes by coordinating a broad network of hormone-signaling components and transcription factors. Collectively, this study provides new insights into the transcriptional integration of multicellular patterning, organ morphogenesis, and whole-plant architecture in tomato.

Materials and methods

Phylogenetic analysis and sequence characterization

To characterize the 25 C2H2 ZFP genes identified from the differentially expressed genes (DEGs) in *h3/h4-dko* mutant leaves (Kim et al. 2025), a phylogenetic analysis was conducted. The dataset included 114 C2H2 ZFPs, adapted from a previously reported list of 112 members in tomato (*Solanum lycopersicum*) (Ming et al. 2020).

Amino acid sequences were aligned using ClustalW, and a phylogenetic tree was constructed in MEGA7 using the neighbor-joining method with 1,000 bootstrap replications. Sequence similarities were assessed between the putative protein H5 (Soly01g060420) and known trichome regulators, including H (Soly01g078970), H2 (Soly01g078990), H3 (Soly03g058160), and H4 (Soly01g080600). Protein motifs in H5 were predicted using the MEME Suite (<https://meme-suite.org/meme/>) Bailey et al. 2015) and LOCALIZER (<https://localizer.csiro.au/index.html>) (Sperschneider et al. 2017).

Plant materials and growth conditions

Tomato cv. Ailsa Craig (LA2838A) was used as the wild-type (WT) background. Seeds of tomato and tobacco (*Nicotiana benthamiana*) were germinated and grown under controlled conditions as previously described (Chun et al. 2021). Morphological parameters, including plant height and compound leaf number, were recorded from 6-week-old tomato plants; these plants were also used for trichome characterization and gene expression analysis. For leaf morphology studies, 8-week-old plants were analyzed. Five-week-old *N. benthamiana* plants were used for *Agrobacterium*-mediated transient expression assays.

Subcellular localization

To generate the *H5-YFP* fusion construct, the coding sequence (CDS) of *H5* (excluding the stop codon) was amplified from WT leaf cDNA using the H5-local primer set (Table S1). The amplified fragment was cloned into the *Bam*HI and *Xho*I sites of the pENTR3C entry vector (#A10464, Invitrogen, Carlsbad, CA, USA) and subsequently recombined into the pKCo-DC-YFP destination vector using the Gateway LR Clonase II system (#11,791–020, Invitrogen, Carlsbad, USA) to generate pBKO-H5:YFP. A construct expressing CFP-tagged MYC2 (pBKO-MYC2:CFP) served as a nuclear marker (Chun et al. 2021). These constructs were introduced into *Agrobacterium tumefaciens* strain GV3101 and co-infiltrated into *N. benthamiana* leaves. YFP and CFP fluorescence signals were detected as previously described (Chun et al. 2021).

RNA isolation and qRT-PCR analysis

To investigate tissue-specific expression of *H5*, samples were harvested from 6-week-old WT plants, including: the second compound leaves from the shoot apical meristem (SAM), stems (internodes between the second and third compound leaves), stem-derived trichomes, immature floral buds (0.8 cm length), fully opened flowers (2 cm length), hypocotyls (1 cm below the cotyledons), and roots (5 cm

from the root tip). For stem-derived trichome isolation, stems between the second and third compound leaves were excised, flash-frozen in liquid nitrogen, and gently scraped with a pre-chilled flat-end spatula to collect trichomes from the stem surface as previously described (Kim et al. 2025). For analysis of trichome-related genes and candidate TFs, leaf and stem tissues were collected from WT and transgenic plants at the 6-week-old stage. All samples were flash-frozen in liquid nitrogen. Total RNA isolation, cDNA synthesis, and quantitative real-time PCR (qRT-PCR) were conducted following established protocols (Chun et al. 2021). *SIAC7* (Soly03g078400) was used as the internal reference gene. All primers are listed in Table S1.

Generation of transgenic tomato plants

For *H5* single-knockout (*h5-sko*) lines, a single-guide RNA (sgRNA) was designed using CRISPR RGEN Tools (<http://www.rgenome.net/>) (Park et al. 2015) and cloned into the pAGM4723 binary vector (Brooks et al. 2014). The resulting construct was transformed into *A. tumefaciens* strain LBA4404 and subsequently into tomato cotyledon explants, as previously described (Kang et al. 2016). Mutations were verified by Sanger sequencing of PCR amplicons from genomic DNA (gDNA) of T₀ plant using *h5-sko-sel* primers (Table S1). Gene-edited T₀ lines were self-pollinated to obtain T₁ seeds. Transgene-free (*Cas9*-free) homozygous *h5-sko* T₂ transgenic lines were selected using *Cas9-sko-sel* primers (Table S1). For *H5* overexpression (*H5-OX*), the full-length *H5* CDS was amplified using H5-OX primers (Table S1), cloned into the pENTR3C, and transferred into the pKCo-DC destination vector via the Gateway LR reaction. The *H5-OX* vector was transformed into WT tomato using the same *Agrobacterium*-mediated method. T-DNA insertion was verified by PCR using H5-OX-sel primers (Table S1) spanning the *CaMV* 35S promoter and the *H5* CDS. Overexpression levels were confirmed by qRT-PCR using H5-qRT primers (Table S1). Transgenic T₀ plants were grown to obtain T₁ seeds, and homozygous *H5-OX* T₂ lines were identified by T-DNA segregation analysis.

Trichome characterization and leaf morphology analysis

Trichome phenotypes were examined across various tissues of 6-week-old plants. Specifically, observations were performed on the adaxial surface of the primary leaflet of the second compound leaf (counted from the shoot apical meristem), stem internodes between the second and third compound leaves, the hypocotyl (1 cm below the cotyledons), and immature floral buds (0.8 cm in length). Trichomes were visualized using dissecting microscopy (CH-M205A, Leica Microsystems, Wetzlar, Germany) and scanning

electron microscopy (SEM; TM3030 Plus, Hitachi, Tokyo, Japan) following established protocols (Jeong et al. 2017). Trichome density and length were quantified as previously described (Kim et al. 2025). Leaf architecture—comprising leaf length, width, and length-to-width ratio—was assessed in 8-week-old plants using the fifth compound leaf and its terminal leaflet.

RNA-sequencing and transcriptome analysis

Total RNA was extracted from the second compound leaves of 6-week-old *h5*-sko, *H5*-OX, and WT plants using TRIzol reagent (#15596018, Invitrogen, Carlsbad, USA). For each of the three biological replicates, three second compound leaves were pooled to minimize biological variation. RNA quality was verified via agarose gel electrophoresis and a NanoPhotometer (NP80, Implen, Munich, Germany). Library construction and RNA-sequencing (RNA-seq) were performed by SEEDERS Inc. (Seoul, Republic of Korea) according to established protocols (Kim et al. 2025). Differentially expressed genes (DEGs) were identified using DESeq with thresholds of $\log_2\text{Fold Change} \geq 0.5$ and a false discovery rate (FDR) ≤ 0.01 (Anders and Huber 2010). Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses were performed using ShinyGo 0.80 (<http://bioinformatics.sdstate.edu/go/>) (Ge et al. 2020). Venn diagrams were generated using InteractiVenn (www.interactivenn.net) (Heberle et al. 2015), and heatmaps were generated using SRplot (<https://www.bioinformatics.com.cn/en>) (Tang et al. 2023). Transcriptional regulation was further investigated by intersecting DEGs with a curated TF dataset derived from PlantTFDB (<https://planttfdb.gao-lab.org/>) (Jin et al. 2016).

Cis-element prediction and yeast one-hybrid assay

Putative C2H2 ZFP-binding sites in promoter regions were predicted using PlantPAN 4.0 (<https://plantpan.itsp.ncku.edu.tw/plantpan4/>) (Chow et al. 2024). For yeast one-hybrid (Y1H) assays, the *H5* promoter (3,759 bp) was amplified from gDNA using the proH5-Y1H primer set (Table S1) and cloned into the pLacZi bait vector at the *Kpn*I and *Xho*I sites (yielding pLacZi-proH5). Prey constructs (pGADT7-H, -H2, -H3, and -H4) were obtained from a previous study (Kim et al. 2025). Bait constructs were linearized with *Apa*I and co-transformed into the *Saccharomyces cerevisiae* strain YM4271 along with the respective prey vectors. A yeast colony co-transformed with pLacZi-proH5 and the empty pGADT7 vector (pGADT7-EV) served as a negative control. Transformants were selected on SD/-Ura/-Leu medium for 3 days at 30 °C. Interactions were monitored on plates containing 80 mg/L X-gal, as previously described (Kim et al. 2025). Reciprocal Y1H assays were performed using the

promoters of *H3* (2,772 bp) and *H4* (2,997 bp) as bait in the pLacZi vector and *H5* CDS in the pGADT7-H5 prey vector. Promoter fragments were amplified from gDNA using specific primers (Table S1) and cloned into the pLacZi vector at the *Xma*I and *Xho*I sites (pLacZi-proH3 and pLacZi-proH4), following the same cloning strategy used for the *H5* promoter.

Results

RNA-seq and phylogenetic analyses identify *H5* as a downstream target of *H3* and *H4*

Our previous work demonstrated that the C2H2 ZFPs *H3* and *H4* are key regulators of trichome initiation and elongation by modulating the expression of *Woolly* (*Wo*)-dependent TF genes, including *Wo*, *WUSCHEL-related homeobox 3b* (*Wox3b*), and *H* (Kim et al. 2025). To systemically identify novel trichome regulators acting downstream of *H3*–*H4* module, we reanalyzed RNA-seq data comparing leaves of *h3/h4*-dko mutants with those of WT plants. We identified 5,246 DEGs ($\log_2\text{Fold Change} \geq 0.5$, FDR ≤ 0.01 ; Table S2). Among these, 205 TFs were significantly downregulated and 202 were upregulated in the *h3/h4*-dko compared to the WT (Table S3 and Fig. S1).

Given the critical role of the C2H2 ZFPs in trichome development (Kim et al. 2025; Zheng et al. 2022), we focused on the 25 C2H2 ZFP genes present among the DEGs (Table S4). Phylogenetic analysis using these 25 candidates and 114 tomato C2H2 ZFPs (adapted from Ming et al. 2020) showed they were distributed across multiple clades (Fig. 1). Notably, *Solyc01g060420* was downregulated in *h3/h4*-dko and formed a close phylogenetic cluster with established trichome regulators *H*, *H2*, *H3*, and *H4* (Chang et al. 2018; Chun et al. 2021; Kim et al. 2025; Zheng et al. 2022). Consequently, we designated *Solyc01g060420* as *Hair5* (*H5*) and prioritized it for functional characterization.

Molecular characterization and subcellular localization of *H5*

The *H5* gene spans 465 bp and consists of a single exon without introns, encoding a predicted polypeptide of 155 amino acids (ITAG 2.4 annotation; Fig. S2). *H5* contains four conserved motifs: an RLFGV-like motif, a canonical C2H2 ZFP motif, a nuclear localization signal (NLS), and an EAR-like motif (Fig. 2A). *H5* shares 28.2% to 36.0% sequence similarity with its homologs (*H*, *H2*, *H3*, and *H4*), with these functional motifs being highly conserved (Fig. 2B).

To verify the predicted nuclear localization, an *H5*-YFP fusion construct was transiently co-expressed

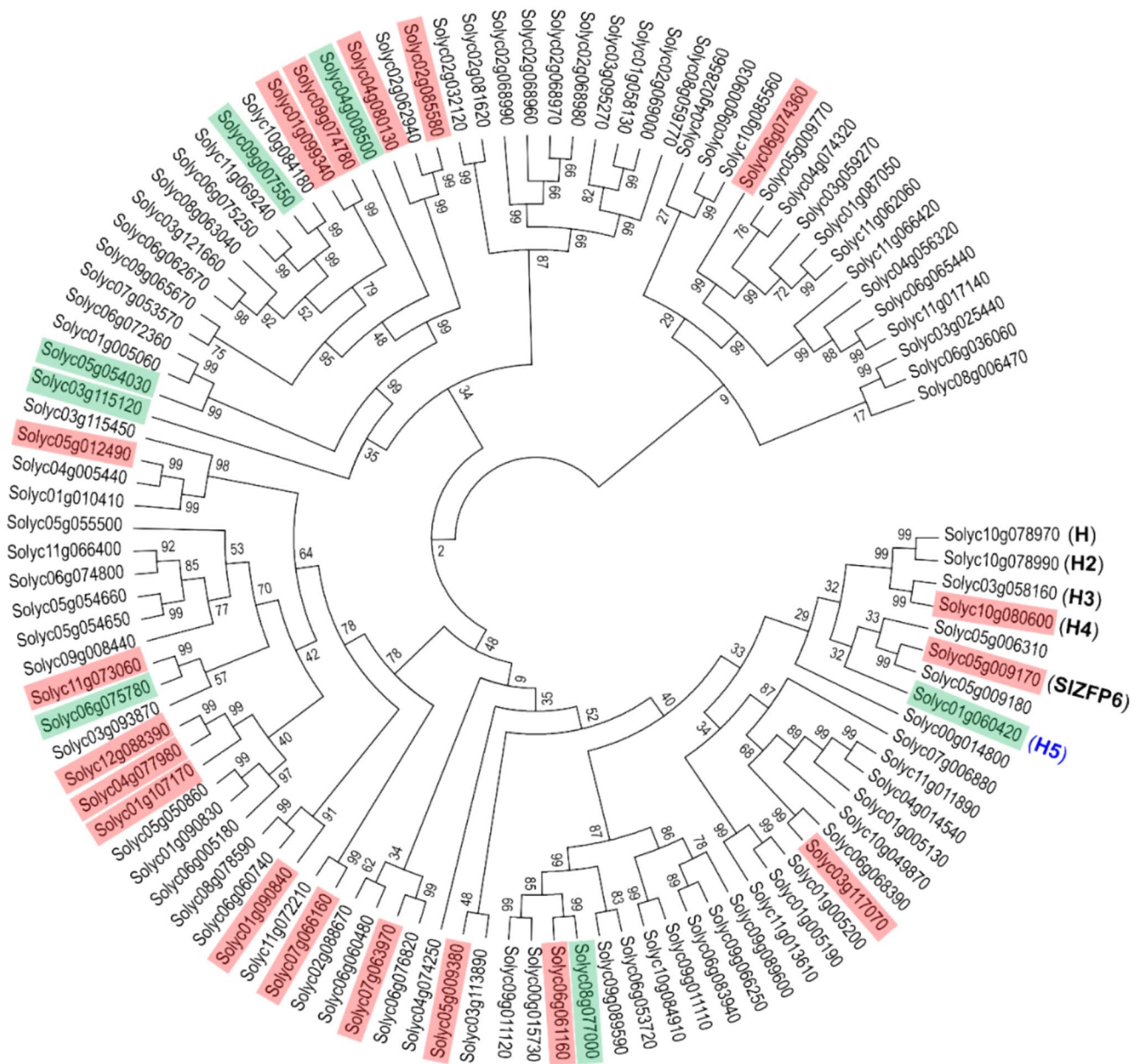
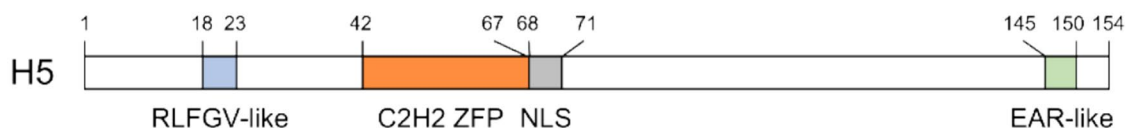


Fig. 1 Phylogenetic analysis of C2H2 ZFPs in tomato. The phylogenetic tree was constructed using the neighbor-joining method in MEGA7 based on the amino acid sequences of 114 C2H2 ZFPs. This dataset was adapted from the 112 C2H2 ZFPs previously reported by Ming et al. (2020). Sequences were aligned using ClustalW, and bootstrap values (1,000 replicates) are shown at each node. The 25

C2H2 ZFP genes identified as DEGs in *h3/h4*-dko plants (Table S4) are highlighted in orange-red (up-regulated in *h3/h4*) and light green (down-regulated in *h3/h4*). Known trichome regulators (H, H2, H3, H4, SIZFP6) are indicated in bold black, while H5 (Solyc01g060420) is highlighted in bold blue

with a nuclear marker (MYC2-CFP) in *N. benthamiana* leaves. Confocal microscopy revealed a complete overlap of H5-YFP and MYC2-CFP signals (Fig. 2C). Furthermore, qRT-PCR analysis showed that *H5* is predominantly expressed in trichome-enriched aerial tissues, including

leaves, stems, flowers, and hypocotyls, as well as in isolated stem trichomes, whereas expression in roots was negligible (Fig. 2D). This expression profile is consistent with the proposed regulatory role of *H5* in trichome-associated epidermal development in tomato.

A**B**

Solyc01g060420 (H5)	-----MGESSVQTNFSSSS---PRQVKLFQGVTECDQ-----	30
Solyc10g078970 (H)	-MEKIGREAVDYMNMKSF-----SQPL--RKKSIRLFGKEFSVGDSNTMSESTDKNPLHH	52
Solyc10g078990 (H2)	-MEKIEREALDFMNVESSSQLPITLPV--NEKPIRLFGKEFGGDSINMTATNMSEFIEN	57
Solyc03g058160 (H3)	-MEKTDTETRDFMNVESFSQLPFIRPVPKEKTGIRLFGKEFVAGTTTTTHHEES--ETI--	55
Solyc10g080600 (H4)	MEKTKDKETQDFMNVESFSQLPFMRPT-KEKAAIRLFGKELLGTTIRHE-DQS--IEI--	54
Solyc01g060420 (H5)	-----TTPPPLVPSEDKRFECQFCHREFANSQALGGHQNAHKKERQRTTRAK	77
Solyc10g078970 (H)	EPKPNTMSISANRIDKTGHVDEISRKYECCYCFRSFPTSQALGGHQNAHKKERQNAKLSH	112
Solyc10g078990 (H2)	NPFYDKPNIV--KENHIKKNVEIMRKYECHYCFRSFPTSQALGGHQNAHKKERKNAKR-H	114
Solyc03g058160 (H3)	-----EAEETKENSNTNRKFECHYCCRNFPSTSQALGGHQNAHKRERQHAQRLH	103
Solyc10g080600 (H4)	-----HDSVGDTEINNRRKFECCYCCRNFPSTSQALGGHQNAHKRERQHAQRAQ	104
Solyc01g060420 (H5)	FIGYQQRFF--RPVPLI-----NAHAVRTGALINNVR-----YYRSQILSGV--	118
Solyc10g078970 (H)	LQSSIVHETNRNRFGEPTAATRLTHYHSTWSNINNINVY-----SP	154
Solyc10g078990 (H2)	DQPSIYGITNRNRLGEATV--STRTHYHSTWTNINNNTPRFDGNH-----NVNVISP	165
Solyc03g058160 (H3)	LQSAMIQGEANNSYGMNNYRL-PNYHHPSTWITNT-SRFYATP-----SHHHQTPP	152
Solyc10g080600 (H4)	YAKYQYNAYFNS-----ALYNTSSTSTTS-GGLYVNNNGHSHYYSQQINRDINE	153
Solyc01g060420 (H5)	----PLR-YQIHLRQQQ-----HMM----	133
Solyc10g078970 (H)	--NYNEAFWQIPPTIHHYQNNINP---PSSFSH---DSFFP-----	187
Solyc10g078990 (H2)	INGNPLTFWQIPPAFNQYISSSSDNNNLFVSN---DDLIRN-----PPMVNIS	211
Solyc03g058160 (H3)	INGSPLALWRIPASY-----NYGRPLVFPAHHQDF---KSSP----	187
Solyc10g080600 (H4)	NHRSLSALWRVPHVHSSISSSSNSYSPNNVSNFVRPIVYNNVNGDLKKINTKSSSISLT	213
Solyc01g060420 (H5)	-----AVQNQDVGTEVDLHLRLAPP	154
Solyc10g078970 (H)	---NDEEKREVQNHVSLDLHL-----	205
Solyc10g078990 (H2)	NNCEYKPKGEVQDHVSVDLHL-----	232
Solyc03g058160 (H3)	---IINTNSATQDHVSVDLHL-----	205
Solyc10g080600 (H4)	RFGYELKEGVHQDHVSVDLHL-----	234

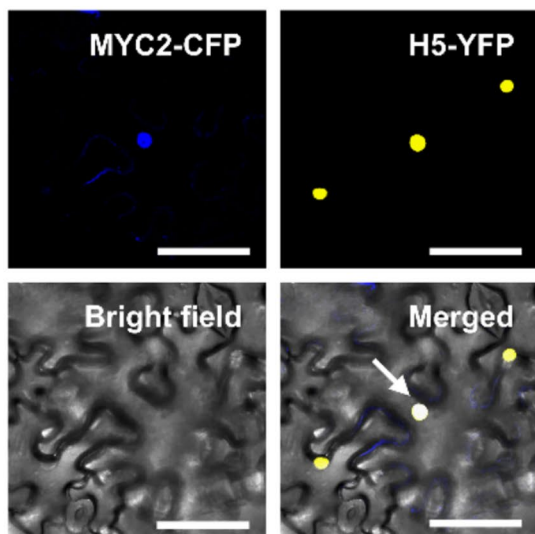
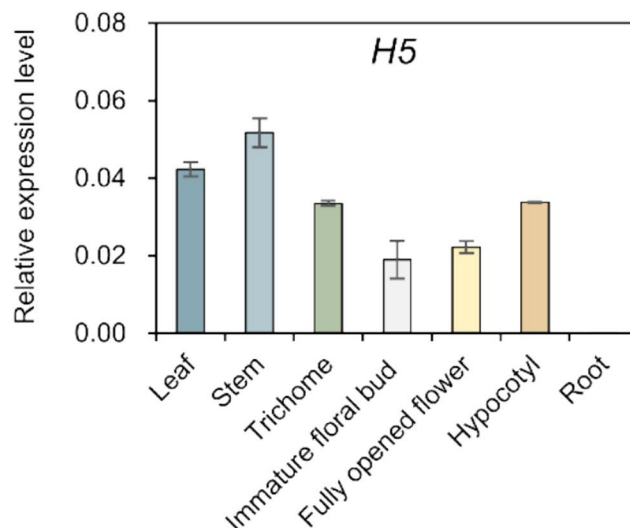
C**D**

Fig. 2 Molecular characterizations of H5. **A** Schematic representation of the H5 protein domain structure. **B** Multiple amino acid sequence alignment of H5 with its closest homologs in tomato (H, H2, H3, and H4). Conserved motifs are highlighted in color: conserved cysteine (C) and histidine (H) residues are indicated by dots above the sequence. **C** Subcellular localization of H5. An *H5-YFP* fusion construct was co-expressed with the nuclear marker *MYC2-CFP* in *Nicotiana benthamiana* leaves via *Agrobacterium*-mediated transient expression. An arrow indicates the colocalization of H5-YFP and MYC2-CFP signals. Scale bar = 20 μ m. **D** qRT-PCR analysis of *H5* expression in various tissues of WT plants. Data represent the mean $\pm$ standard error (SE) of three independent biological replicates, each consisting of pooled tissue from three individual plants

H5 functions as a key regulator of trichome initiation and stalk-cell-mediated elongation in a tissue-specific manner

To elucidate the functional significance of *H5* in trichome development, we first generated loss-of-function and gain-of-function transgenic lines. Using the CRISPR-Cas9 system, we targeted the single exon of *H5* to produce single-knockout (*h5-sko*) mutants (Fig. S3A, B). Among several independent T₀ plants, two representative T₂ lines—*h5-sko-3* and *h5-sko-4*—were selected for further study. These lines carried biallelic mutations leading to premature stop codons and truncated proteins (Fig. S3C). These lines were confirmed to be transgene-free (*Cas9*-free) to exclude potential off-target effects. (Fig. S3D). In parallel, we developed *H5*-OX lines driven by the CaMV 35S promoter (Fig. S4A–C). Two homozygous lines, *H5*-OX-2 and *H5*-OX-14, exhibited a 176- to 187-fold increase in *H5* expression compared to the WT and were selected for detailed phenotypic analysis (Fig. S4D).

Initial phenotypic screening via light microscopy revealed that *h5-sko* mutants exhibited reduced trichome density on leaves and stems, whereas *H5*-OX lines showed the opposite phenotype (Fig. 3A). To investigate these phenotypes at a higher resolution, we employed SEM and quantified density and length for each trichome type across various tissues (Fig. 3B–E and Fig. S5). On leaves, *h5-sko* mutants exhibited a significant reduction in the density of all trichome types. In addition, type I and III trichomes were markedly shorter due to impaired stalk cell elongation, while the lengths of other types remained largely unchanged. In contrast, *H5*-OX lines displayed an increased density of type I but a decreased density of type VI. Furthermore, *H5* overexpression promoted the elongation of type I, V, and VI trichomes, with type I trichomes exhibiting a significant increase in both the number and length of stalk cells (Fig. 3C–E and Fig. S5).

This tissue-specific regulatory pattern was also evident on the stems and hypocotyls. On stems, *h5-sko* plants showed decreased densities of type I, IV, and VII trichomes, along with shorter type I, III, and VI trichomes, resulting from

fewer and shorter stalk cells. Conversely, *H5*-OX lines exhibited increased densities of type I, III & V, and VII trichomes, and increased lengths of type I, V, and VI trichomes, primarily driven by enhanced stalk cell development in type I trichomes (Fig. 3C–E and Fig. S5). On hypocotyls, *h5-sko* plants displayed diminished densities of type I and VI trichomes, with shortened type I trichomes caused by reduced stalk cell number and length. In contrast, *H5*-OX lines showed increased density of type I and decreased density of type VI trichomes. Notably, trichome lengths were increased across all types in *H5*-OX hypocotyls, most prominently in type I (Fig. 3C–E). Interestingly, on sepals, both *h5-sko* and *H5*-OX plants displayed a decrease in the density of type VI trichomes, while the densities and lengths of other types remained comparable to the WT (Fig. 3C–E and Fig. S5). Collectively, these results demonstrate that *H5* acts as a key regulator of trichome initiation and elongation in a tissue- and trichome-type-dependent manner. While *H5* primarily functions as a positive regulator of trichome development, its differential effects on type VI density suggest a distinct regulatory role in specific trichome sub-types. The *h5-sko* loss-of-function phenotype specifically indicates that *H5* is indispensable for maintaining full trichome density and ensuring proper stalk cell-mediated elongation in vegetative organs.

H5 modulates vegetative growth and leaf morphogenesis in a dose-dependent manner

Beyond its established role in trichome development, C2H2 ZFPs frequently function as pivotal regulators of various plant developmental processes (Liu et al. 2022). To investigate whether *H5* influences vegetative growth and leaf development, we compared the overall plant architecture and leaf morphology of transgenic and WT plants. Regarding overall vegetative architecture, *h5-sko* mutants exhibited no significant differences in plant height or the number of compound leaves compared to WT plants. In contrast, *H5*-OX lines showed severe growth retardation and a significant reduction in the total number of compound leaves (Fig. 4A–C).

To further characterize leaf morphology, we analyzed the dimensions and shape indices of the compound leaves. Compared with the WT, *h5-sko* plants produced broader compound leaves characterized by rounder leaflets, whereas *H5*-OX plants exhibited markedly narrower compound leaves with elongated, less expanded leaflets (Fig. 4D). Quantitative morphometric analysis revealed that while compound leaf length was reduced in both *h5-sko* and *H5*-OX plants, compound leaf width was significantly reduced only in the *H5*-OX lines. Consequently, the length-to-width ratios of compound leaf—a key indicator of leaf shape—decreased in *h5-sko* mutants but increased in *H5*-OX lines (Fig. 4E–G). Consistent trends were observed in the terminal leaflets;

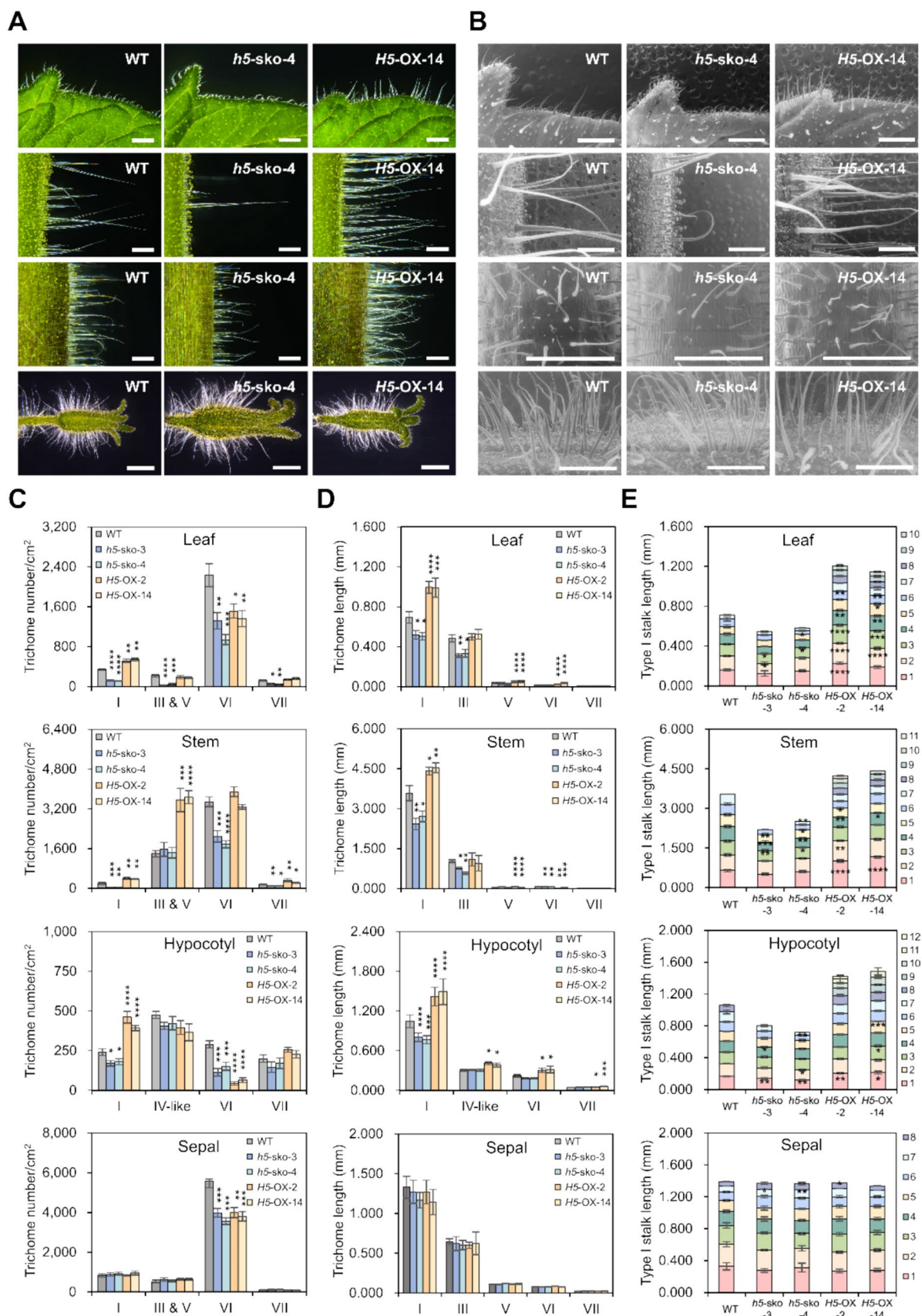


Fig. 3 Trichome phenotypes of *h5-sko* and *H5-OX* plants. **A, B** Dissection microscopy and scanning electron microscopy images of leaves, stems, hypocotyls, and sepals from WT, *h5-sko*, and *H5-OX* plants. Scale bars=1 mm. **C** Trichome density and **D** Trichome length on leaves, stems, hypocotyls, and sepals. **E** Stalk cell number and length of type I trichomes. Numbers on the right indicate the stalk cell order from base (1) to apex (up to 12). All images and data were obtained from six-week-old plants. Data represent the mean $\pm$ SE of eight independent biological replicates per tissue for each genotype. Asterisks indicate significant differences compared to WT (unpaired Student's *t*-test: **P*<0.05, ***P*<0.01, ****P*<0.001, *****P*<0.0001)

while leaflet length was reduced in both genotypes, terminal leaflet width increased in *h5-sko* plants but decreased in *H5-OX* lines. These changes resulted in a reciprocal shift in the length-to-width ratios of terminal leaflet, which were lower in *h5-sko* and higher in *H5-OX* compared to the WT (Fig. 4H–J). Collectively, these findings demonstrate that while the loss of *H5* function has a negligible impact on overall plant height, it promotes lateral leaf expansion. Conversely, the ectopic overexpression of *H5* severely restricts both vegetative growth and leaf lamina expansion. These results suggest that *H5* is a critical regulator of leaf morphogenesis and that maintaining optimal *H5* levels is essential for normal plant architecture, extending its functional role beyond trichome development.

Reciprocal and indirect regulation between *H5* and the *H-H4* module

C2H2 ZFPs are known to modulate trichome development through hierarchical regulation among family members in tomato (Li et al. 2021; Zheng et al. 2022). Our RNA-seq data indicated that *H5* expression is significantly downregulated in *h3/h4-dko* leaves (Table S4). To further dissect this regulatory network, we analyzed *H5* transcript levels via qRT-PCR in various C2H2 ZFP mutants, including *h*, *h2*, *h3*, and *h4* single and double knockouts (Chun et al. 2021; Kim et al. 2025). The transcript levels of *H5* were markedly reduced in the leaves of *h3/h4-dko* and *h2-sko* mutants, and significantly diminished in the stems of all tested knockout lines (Fig. 5A). These results genetically position *H5* downstream of the *H-H4* module.

To determine whether this regulation is direct, we examined the *H5* promoter, which contains several putative ZFP binding motifs (A[AG/CT]CNAC) (Zhou et al. 2011) (Fig. 5B). However, Y1H assays showed that none of these proteins—H, H2, H3, or H4—directly bind to the *H5* promoter fragments (Fig. 5C), indicating that the *H-H4* module regulates *H5* indirectly. Notably, we found that *H5* also exerts regulatory control over its upstream activators. In *H5-OX* lines, the transcript levels of *H3* and *H4* were significantly reduced (Fig. 5D), suggesting that *H5* may function as a feedback repressor of *H3* and *H4*. To test the potential

for a direct interaction in this feedback loop, we performed Y1H assays, which confirmed that *H5* does not bind directly to the promoters of *H3* or *H4* (Fig. 5E–H). Taken together, these findings demonstrate a reciprocal but indirect regulatory relationship between *H5* and the *H-H4* module. In this model, the *H-H4* module is required for the activation of *H5*, while *H5* in turn modulates the expression of *H3* and *H4*, forming a complex, indirect feedback loop that fine-tunes the trichome developmental program.

Transcriptomic profiling identifies *H5* as a central hub in developmental and transcriptional networks

To elucidate the transcriptional mechanisms by which *H5* regulates plant development, we performed RNA-seq analysis using leaves from *h5-sko*, *H5-OX*, and WT leaves. We identified 10,363 DEGs in the *h5-sko* vs. WT comparison and 6,683 DEGs in the *H5-OX* vs. WT comparison. Among these, a core set of 4,080 DEGs overlapped between the two datasets (Fig. 6A), representing the primary transcriptional signature influenced by *H5*. Gene Ontology (GO) enrichment analysis of these overlapping DEGs highlighted the role of *H5* in transcriptional regulation and developmental processes (Fig. 6B). Specifically, molecular function (MF) terms, such as DNA-binding transcription factor activity and sequence-specific DNA binding, were significantly enriched, supporting the role of *H5* as a key transcriptional regulator. Consistent with this, the cellular component (CC) category showed enrichment for nuclear-localized terms, while biological process (BP) terms were significantly associated with developmental processes. Furthermore, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis revealed significant enrichment in plant-pathogen interaction and plant hormone signal transduction (Fig. 6C), suggesting that *H5* integrates defense responses and development through hormone-mediated signaling networks.

To identify putative downstream targets of *H5*, we refined the 4,080 overlapping DEGs to a subset of 569 genes exhibiting an inverse expression pattern—downregulated in *h5-sko* and upregulated in *H5-OX*, or vice versa (Fig. 6D; Table S5). This contrasting regulation strongly implicates these genes as tightly linked downstream targets of *H5*. Based on the phenotypes observed in *H5* transgenic plants, we further screened this subset for genes with established roles in development, identifying nine well-characterized genes as potential effectors (Fig. 6E; Table S6). In the category of trichome development, *JAZ4* and *WRKY57*, both reported as negative regulators of trichome development (Hao et al. 2025; Hua et al. 2021), exhibited expression patterns inversely correlated with *H5*. Regarding leaf morphogenesis, *Trifoliolate (Tf)*, an auxin-mediated MYB transcription factor promoting leaf marginal differentiation (Naz et al. 2013), and *Gretchen Hagen 3.15 (GH3.15)*, an auxin-responsive

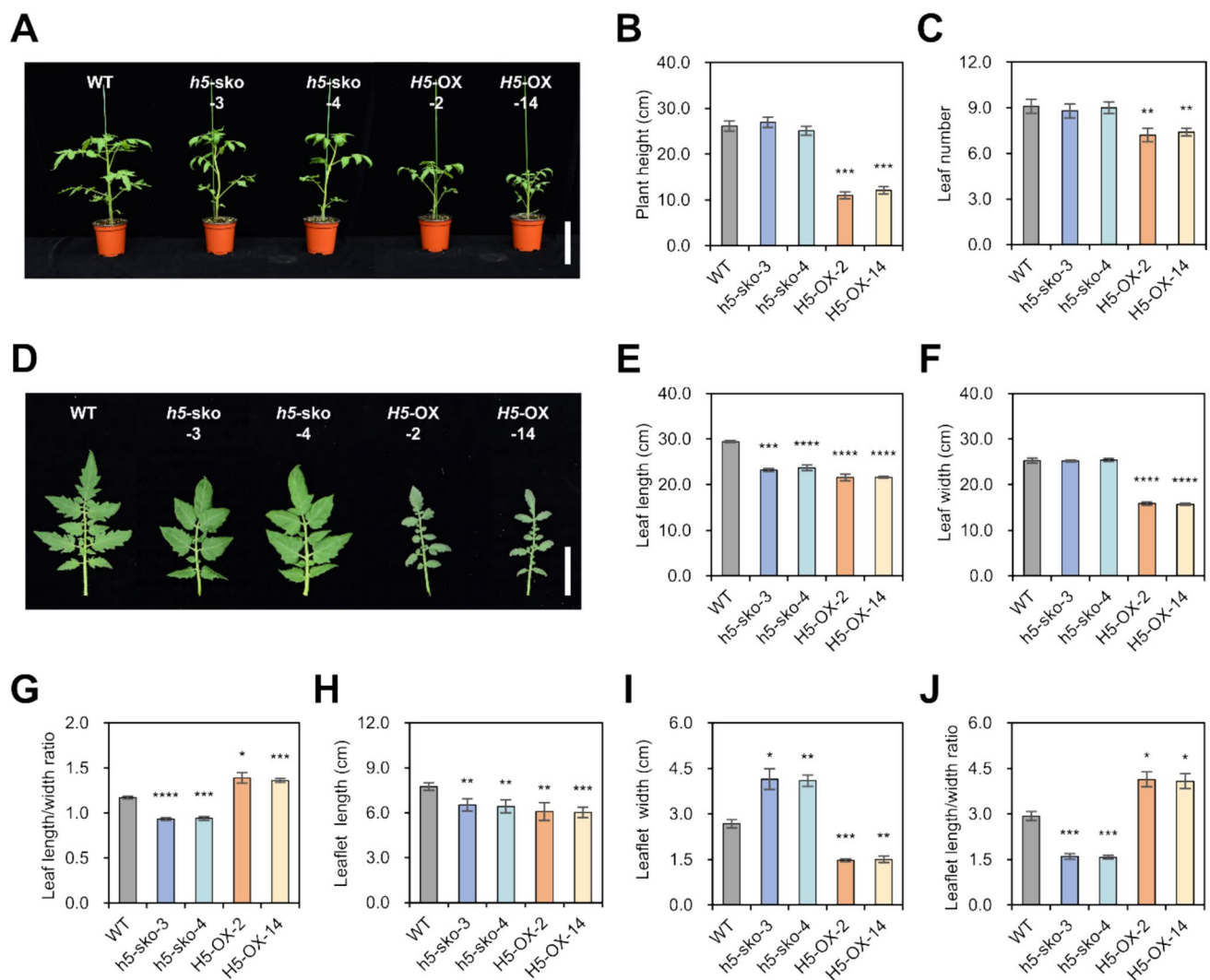


Fig. 4 Plant growth and leaf morphology of WT, *h5-sko*, and *H5-OX* plants. **A** Representative photographs of six-week-old WT, *h5-sko*, and *H5-OX* plants. Scale bar = 10 cm. **B**, **C** Plant height (B) and compound leaf number (C). Data represent the mean $\pm$ SE of eight independent biological replicates for each genotype. **D** Close-up images of the fifth compound leaves from eight-week-old WT, *h5-sko*, and *H5-OX* plants. Scale bar = 10 cm. **E–G** Length (E), width (F), and

length-to-width ratio (G) of the fifth compound leaves. **H–J** Length (H), width (I), and length-to-width ratio (J) of the terminal leaflet of the fifth compound leaves. For (E–J), data represent the mean $\pm$ SE of six independent biological replicates for each genotype. For all measurement data, asterisks indicate significant differences compared to WT (unpaired Student's *t*-test: **P* < 0.05, ***P* < 0.01, ****P* < 0.001, *****P* < 0.0001)

gene suppressing leaf expansion (Ai et al. 2023), both exhibited expression patterns negatively correlated with *H5* expression. In addition, *BLADE-ON-PETIOLE a* (*BOPa*) and *Cytokinin oxidase 2* (*CKX2*), which negatively regulate leaf complexity through cytokinin signaling (Hu et al. 2023; Ichihashi et al. 2014), were also inversely correlated with *H5* expression. Furthermore, genes associated with plant architecture, including *GT factor-26* (*GT-26*) and *Erecta* (*ER*), both of which positively govern stem elongation (Kwon et al. 2020; Li et al. 2023), were negatively regulated by *H5* (Fig. 6E).

Beyond these individual targets, we analyzed the 569 core DEGs to identify broader regulatory networks,

revealing 45 TFs as putative downstream targets of *H5* (Fig. 7A; Table S7). Based on their correlation with *H5* expression, these 45 TFs were categorized into two groups: 16 TFs were downregulated in *h5-sko* and upregulated in *H5-OX*, while 29 TFs were upregulated in *h5-sko* and downregulated in *H5-OX* (Fig. 7B). The 16 TFs showing a positive correlation with *H5* were predominantly members of the MYB, NAC, and MADS families, whereas the 29 TFs exhibiting a negative correlation were mainly enriched in the bHLH, WRKY, and C2H2 ZFP families (Fig. 7C). To validate these transcriptomic findings, we performed qRT-PCR on selected TFs from each group. TFs positively correlated with *H5*,

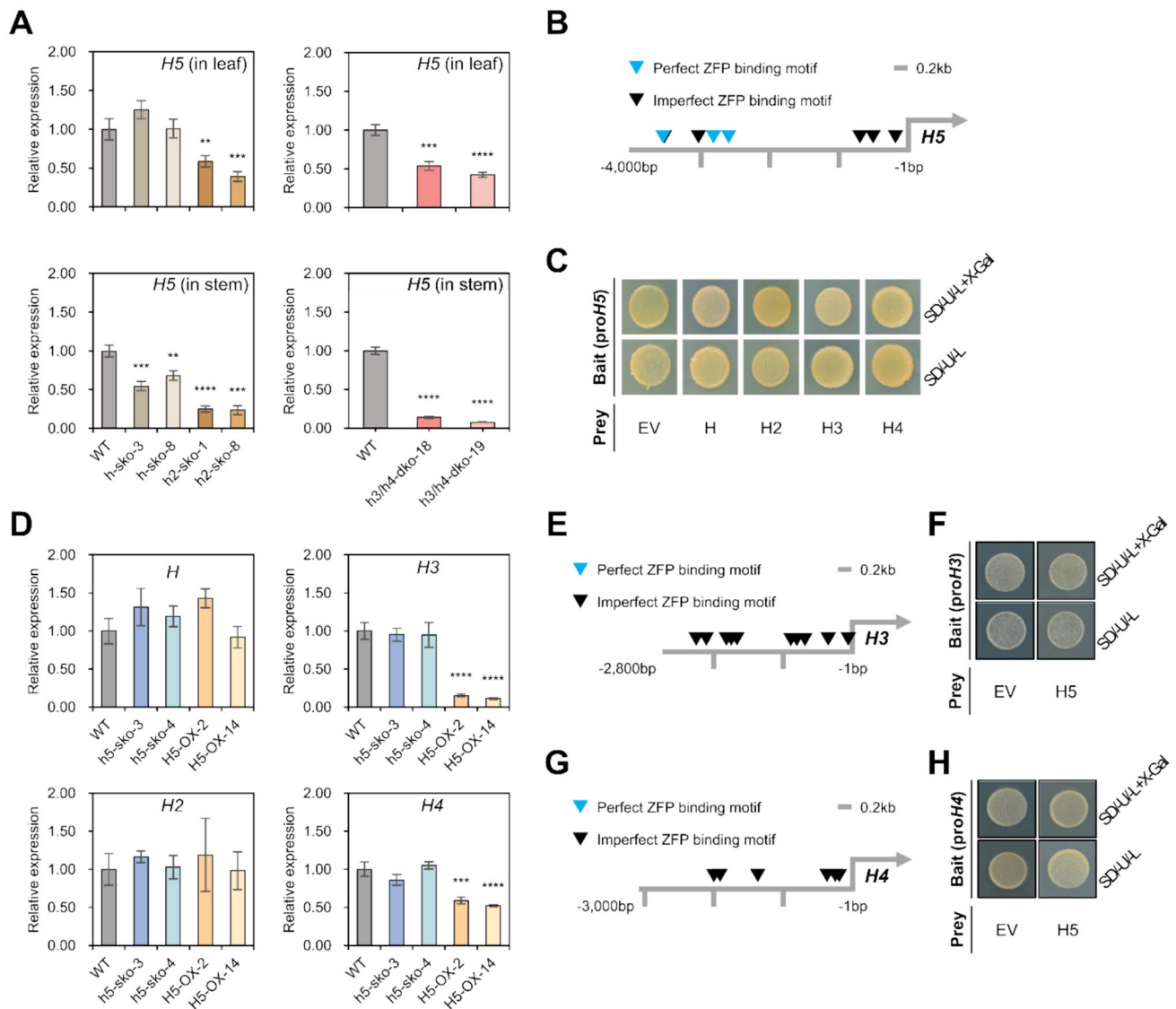


Fig. 5 Transcriptional regulation of *H5* by the *H-H4* genes. **A** qRT-PCR analysis of *H5* expression in the leaves and stems of *h5-sko*, *h2-sko*, and *h3/h4-dko* plants. Values represent the mean $\pm$ SE of three biological replicates (three pooled plants per replicate). Expression levels were normalized to WT. **B** Schematic diagram of the *H5* promoter region (3.8 kb) showing putative ZFP-binding motifs (perfect: blue triangles; imperfect: black triangles). **C** Yeast-one-hybrid (Y1H) assay testing the binding of H, H2, H3, and H4 to the *H5* promoter. The *H5* promoter was fused to the pLacZi bait vector; full-length *H-H4* cDNAs were fused to the GAL4 activation domain (AD) in pGADT7. An empty vector (EV) was used as a negative control. The transformants were selected on SD/-Ura/-Leu medium for 3 days and

then transferred to SD/-Ura/-Leu medium containing X-gal for color development. **D** qRT-PCR analysis of *H*, *H2*, *H3*, and *H4* expression in *h5-sko* and *H5-OX* leaves. Values represent the mean $\pm$ SE of three biological replicates (three pooled leaves per replicate). Expression levels were normalized to WT. **E, G** Schematic diagrams of the *H3* (2.7 kb) and *H4* (3.0 kb) promoter regions. **F, H** Y1H assay testing the binding of H5 to the *H3* or *H4* promoters. Each promoter was fused to the pLacZi bait vector; full-length *H5* cDNA was fused to the GAL4 AD in pGADT7. Assay conditions are identical to (C). For all qRT-PCR data, asterisks indicate significant differences compared to WT (unpaired Student's *t*-test; ***P* < 0.01, ****P* < 0.001, *****P* < 0.0001).

including *GRAS14*, *HDZ4*, and *WRKY48*, were significantly downregulated in *h5-sko* plants and upregulated in *H5-OX* lines. In contrast, negatively correlated TFs, such as *MYB95*, *bHLH19*, *bHLH86*, *bHLH106*, and *ZFP36*, showed the opposite pattern (Fig. 7D). Collectively, these findings establish *H5* as a central transcriptional regulator

that coordinates multiple TF networks to fine-tune complex developmental programs, including trichome formation, leaf morphogenesis, and plant architecture in tomato.

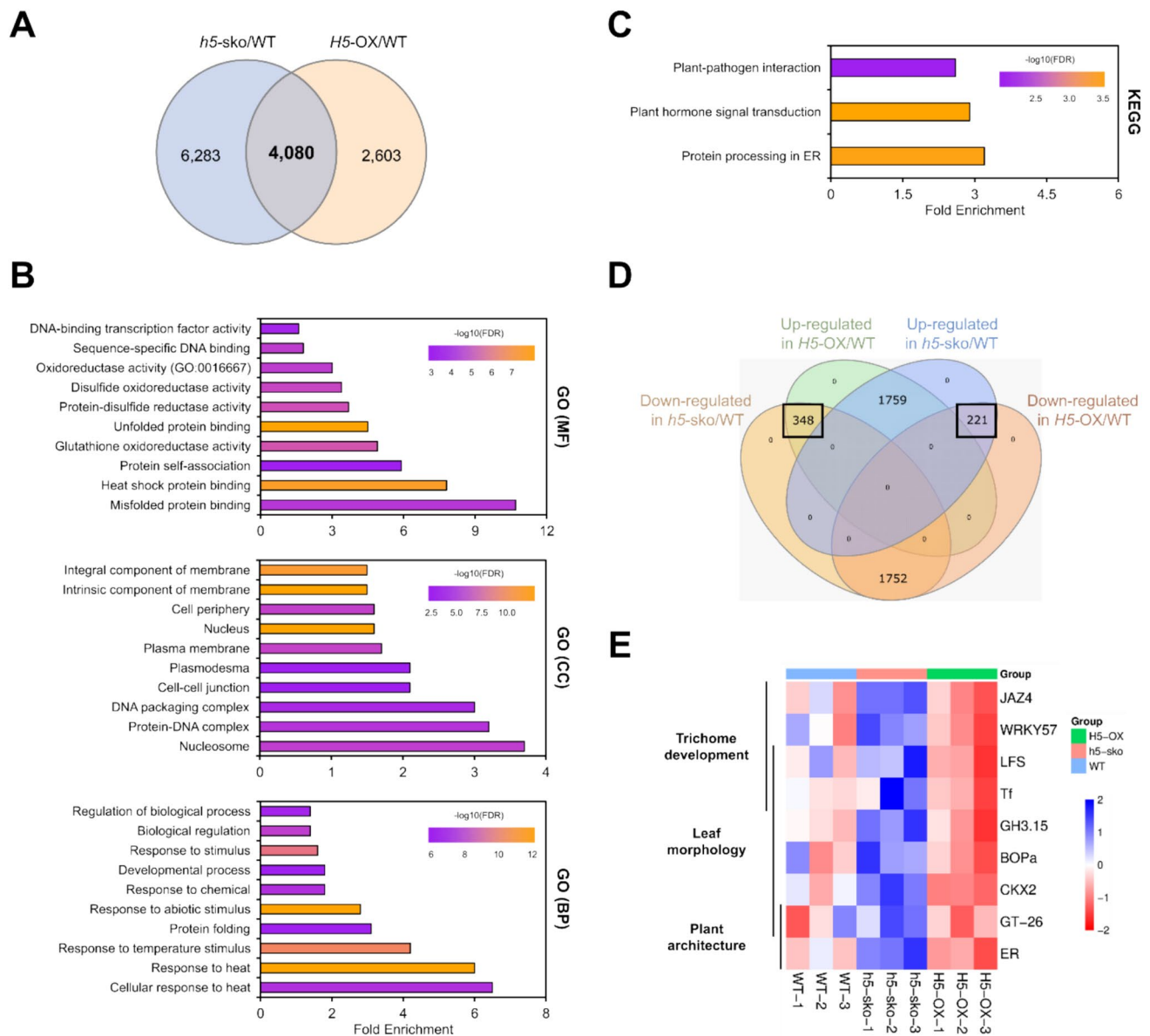


Fig. 6 Comparative transcriptomic analysis of *h5-sko* and *H5-OX* leaves. RNA-seq was performed on leaves from WT, *h5-sko*, and *H5-OX* plants. **A** Venn diagram showing common and exclusive DEGs ($|\log_2\text{FoldChange}| \geq 0.5$, $\text{FDR} \leq 0.01$) identified in *h5-sko* vs. WT and *H5-OX* vs. WT comparison. **B**, **C** GO enrichment (B) and KEGG pathway (C) analyses of the overlapping DEGs. The y-axes indicate GO terms (B) or KEGG pathways (C), and x-axes represent

fold enrichment. **D** Venn diagram illustrating DEGs with inverse expression patterns between *h5-sko*/WT and *H5-OX*/WT comparisons (black boxes, see Table S5). **E** Heatmap showing the expression profiles of known regulators involved in trichome development, leaf morphogenesis, and plant architecture among the DEGs identified in (D, see Table S6)

Discussion

H5 acts as a developmental regulatory node in tomato

C2H2 ZFPs are pivotal regulators of plant development, mediating processes from epidermal cell differentiation to complex organogenesis (Han et al. 2021; Liu et al. 2022). Despite their importance, the functional diversification

within this large family in tomato remains only partially understood. In this study, we characterized *H5*, a previously unassigned C2H2 ZFP, and demonstrated its role as a pivotal developmental regulator. *H5* coordinates developmental signals across three distinct biological scales. At the cellular level, *H5* promotes epidermal differentiation, specifically impacting trichome density and stalk cell-mediated elongation (Fig. 3 and Fig. S5). At the organ level, it regulates leaf morphogenesis, and

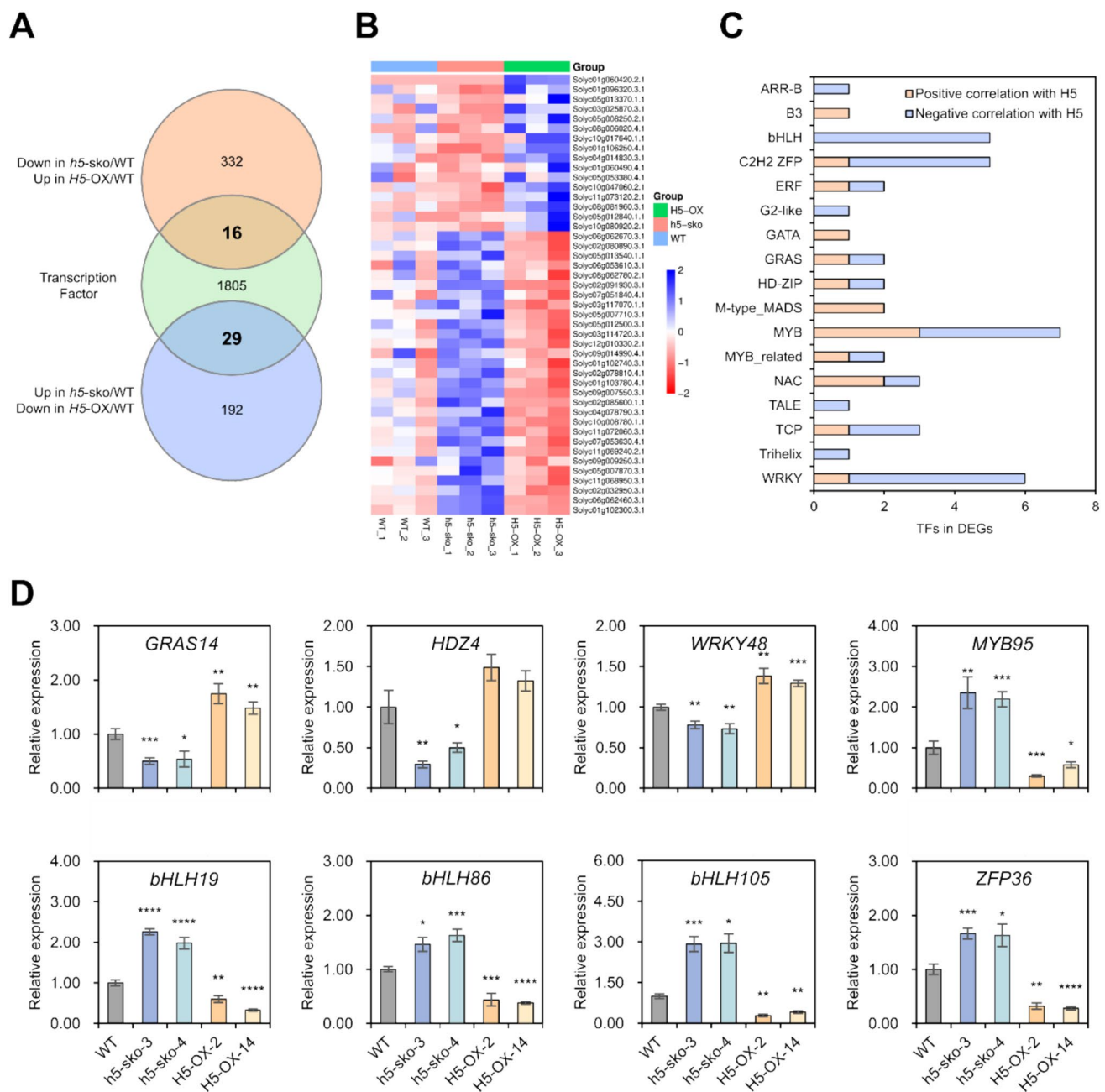


Fig. 7 Identification and validation of downstream transcription factors (TFs) regulated by *H5*. **A** Venn diagram showing the overlap between *H5*-regulated DEGs and tomato TF dataset adapted from Jin et al. (2016). Overlapping TFs are listed in Table S7. **B** Heat-map showing the expression profiles of the overlapping TFs across WT, *h5-sko*, and *H5-OX* plants. **C** Classification of TFs among the *H5*-regulated DEGs, grouped by family and color-coded by their

correlation with *H5* expression (light orange: positive; light blue: negative). **D** qRT-PCR validation of selected TFs. Data represent the mean $\pm$ SE of three biological replicates (each consisting of three pooled plants). Expression levels were normalized to WT. Asterisks indicate significant differences compared to WT (unpaired Student's *t*-test: **P* < 0.05, ***P* < 0.01, ****P* < 0.001, *****P* < 0.0001)

at the whole-plant level, its activity modulates overall architecture by affecting stem internode elongation and plant height (Figs. 3, 4). This capacity to integrate

developmental signals across cellular, organ, and architectural scales distinguishes *H5* from other characterized

tomato ZFPs, highlighting its unique position in the transcriptional regulatory network.

Structural distinctiveness and functional specialization of *H5*

A comparative analysis of *H5* and its closest paralogs (*H*–*H4*) provides essential context for its distinct functional profile. While *H5* retains key conserved domains—the RLFGV-like motif, the C2H2 ZFP motif, the NLS, and the EAR-like motif—it is notably shorter than its closest paralogs, *H*–*H4* (Fig. 2). This structural truncation suggests that *H5* may operate through a distinct regulatory mechanism, potentially involving differential target specificity or unique protein–protein interaction dynamics. Functionally, *H5* appears to bridge the specialized roles of its paralogs. While *H* and *H2* are primarily tissue-specific regulators of type I trichomes (Chang et al. 2018; Chun et al. 2021; Hua et al. 2022), and *H3/H4* exhibit broader roles affecting multiple trichome types and vegetative growth (Kim et al. 2025), *H5* integrates these features by coordinating epidermal patterning with systemic growth programs. This links cellular-level differentiation with organismal-level developmental cues.

Hierarchical positioning and the indirect feedback regulation of *H5*

C2H2 ZFPs often operate within intricate hierarchical transcriptional networks (Han et al. 2022; Sun et al. 2015; Zheng et al. 2022). Our expression analyses place *H5* genetically downstream of the *H*–*H4* regulatory module (Fig. 5A). However, the absence of direct promoter binding in Y1H assays indicates that this activation is indirect, likely requiring intermediate transcription factors. Furthermore, we identified a reciprocal indirect feedback loop where *H5* overexpression significantly reduces *H3* and *H4* transcription levels (Fig. 5D). These findings suggest *H5* as a central node that fine-tunes the ZFP regulatory hierarchy. Such a feedback mechanism is essential for maintaining developmental homeostasis. By constraining the expression of its upstream activators, *H5* may prevent the over-proliferation of epidermal structures at the expense of overall plant vigor. Elucidating the molecular intermediates bridging these indirect interactions will be critical for fully mapping the complex hierarchy of the tomato C2H2 ZFP network.

H5 orchestrates development through complex transcriptional networks

Transcriptome profiling of *h5-sko* and *H5*-OX plants revealed that *H5* acts as a higher-order regulatory hub that integrates hormone signaling with morphological outputs (Fig. 6). Our data suggest that *H5* does not operate through

a simple linear pathway but rather through a sophisticated network of downstream effectors. In the context of trichome development, the inverse correlation between *H5* and negative regulators such as *WRKY57* and *JAZ4* (Hao et al. 2025; Hua et al. 2021) suggests a fine-tuning mechanism for trichome initiation and elongation. Interestingly, the absence of known type I trichome regulators among the core DEGs implies that *H5* may function through yet-to-be-identified effectors or acts downstream of established pathways. A key finding is that type VI trichome density was reduced in both *H5* loss- and gain-of-function lines (Fig. 3C). This suggests that *H5* activity follows a dosage-sensitive model, where a precise transcriptional threshold is required to maintain specific epidermal structures. Any deviation from this homeostatic level—whether through depletion or overabundance—disrupts the delicate balance of the trichome developmental program. This dosage-sensitive regulation also provides insight into the observed discrepancies between knockout and overexpression lines in plant architecture. The fact that architectural phenotypes were predominantly observed in *H5*-OX lines, while *h5-sko* remained relatively similar to the WT, suggests that *H5* functions within a redundant regulatory framework, similar to other C2H2 ZFPs like *H* and *H2* (Zheng et al. 2022). Under this model, the native role of *H5* in regulating plant height may be masked by functional redundancy in knockout mutants, whereas its overexpression triggers supra-physiological outputs that surpass the transcriptional buffering capacity required for normal growth. Furthermore, the observation that trichome phenotypes were not globally antagonistic, but instead varied by tissue type, points to the context-dependent regulation of *H5*. These variations likely arise from differences in developmental timing; for example, epidermal differentiation in hypocotyls occurs under a distinct hormonal and regulatory environment compared to later-developing organs like leaves or sepals. Additionally, the transcriptional output of *H5* is likely shaped by the availability of tissue-specific cofactors or the pre-existing ZFP hierarchy unique to each organ.

A similar pattern of complex integration was observed for leaf and stem morphogenesis. *H5* negatively regulates several auxin- and cytokinin-associated genes involved in leaf morphogenesis, including *Leafless* (*LFS*), *Tf*, *GH3.15*, *BOPa*, and *CKX2* (Ai et al. 2023; Capua and Eshed 2017; Hu et al. 2023; Ichihashi et al. 2014; Naz et al. 2013) (Fig. 6E). While the misregulation of these genes broadly aligns with the altered leaf shapes in *H5* transgenic lines, the specific phenotypes differed from those of individual null mutants. For instance, whereas *lfs* mutants completely fail to initiate leaf development (Capua and Eshed 2017), *H5*-OX plants—despite reduced *LFS* levels—successfully develop leaves and leaflets (Fig. 4D). This discrepancy indicates that *H5* does not act as an absolute "on/off" switch for these pathways. Instead, it functions as a pivotal regulatory node, modulating

the expression of multiple genes to generate a composite, *H5*-specific leaf architecture. In this model, the phenotypic outcome may not be the result of a single downstream gene's failure, but the integrated effect of shifting multiple hormone-responsive modules simultaneously. Similarly, *H5* negatively regulates *ER* and *GT-26*, both of which are positive regulators of stem elongation (Kwon et al. 2020; Li et al. 2023). While *H5*-OX plants exhibit a compact architecture resembling *er* knockout or *gt-26* knockdown mutants, *h5*-sko plants—despite elevated expression of these genes—maintain a near-WT height (Fig. 4A, B). This non-linear relationship suggests that stem elongation is governed by multiple converging pathways that provide functional redundancy or buffering. In the *h5*-sko mutant, the overexpression of growth-promoting factors like *ER* and *GT-26* may be compensated for by other *H5*-regulated constraints within the broader developmental network. Collectively, these results position *H5* as a crucial integrator that determines plant architecture in a context-dependent and non-linear manner, ensuring developmental robustness.

Balancing transcriptional modules underlies *H5*-mediated development

Transcriptome analysis identified 45 TFs among the *H5*-regulated DEGs, confirming that *H5* acts as a hub in a broad regulatory network. To gain mechanistic insight into *H5*-mediated pleiotropy, these TFs were categorized into positively and negatively correlated transcriptional modules relative to *H5* expression. Excluding previously characterized or study-validated TFs, we prioritized 40 uncharacterized TFs exhibiting robust responses to *H5* perturbation (Fig. 7B; Table S7). Among the 15 positively co-expressed TFs, *GRAS14*, *WRKY48*, and *HDZ4* were identified as representative candidates. GRAS family members are established regulators of plant architecture acting downstream of gibberellin signaling (Huang et al. 2015; Zhou et al. 2018), while *WRKY* and *HD-ZIP I* (e.g., *HDZ4*) factors modulate trichome development and defense (Hao et al. 2025; Hong et al. 2021; Zocca et al. 2025). Their strong co-expression suggests they may function as downstream effectors of *H5*-dependent epidermal differentiation and organ growth programs. Conversely, the 25 TFs negatively correlated TFs (e.g., *MYB95*, *bHLHs*, and *ZFP36*) likely represent growth-promoting modules constrained by *H5*. Since these families regulate laminar expansion and stem elongation in *Arabidopsis* or tomato (Chen et al. 2020; Naz et al. 2013; Oppenheimer et al. 1991; Sun et al. 2015), their repression in *H5*-OX lines suggests *H5* prevents over-growth by titrating the activity of these modules. Rather than operating through a singular linear pathway, *H5* appears to orchestrate development by balancing multiple antagonistic transcriptional modules. This network-based framework provides the

necessary plasticity for *H5* to function as a pivotal regulatory node in a dosage- and tissue-dependent manner. While our current assessment is primarily transcriptional, future functional and genetic interaction studies will be essential to fully map the regulatory topography of the *H5* network.

Conclusion

In conclusion, our study establishes *H5* as a pivotal developmental regulatory node in tomato. Functioning as a transcriptional rheostat, *H5* coordinates cellular differentiation, organ morphogenesis, and whole-plant architecture by balancing antagonistic transcriptional modules. This dosage-sensitive regulation, coupled with an indirect feedback loop within the C2H2 ZFP hierarchy, provides a robust mechanistic basis for maintaining developmental homeostasis. These findings significantly expand our understanding of the complex transcriptional networks that coordinate vegetative growth and structural patterning in tomato.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s00299-026-03765-1>.

Author Contributions JHK conceived and designed the research. JKS, CJ, and JHK supervised the project. SMK, JK, and JIC performed the experiments. SMK, JK, JIC, JKS, CJ, and JHK analyzed the data. SMK, JK, and JHK wrote the manuscript. All authors have read and approved the submission of the final draft.

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Data availability All data are incorporated into the article and its supplementary information files.

Declarations

Conflict of interest The authors declare no competing interests.

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