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A conserved serine residue of Hd3a/RFT1 is imperative for its interaction with 14-3-3 proteins and function in plant floral transition in rice

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Abstract

Florigen is a pivotal signaling molecule that governs the initiation of plant floral transition. In rice, florigen is encoded by *Hd3a* and *RFT1*. Hd3a and RFT1 are synthesized in leaves and subsequently transported into shoot apical meristem (SAM). It was proposed that in the SAM, the incoming Hd3a and RFT1 are recognized by 14-3-3 proteins in the cytoplasm, then translocated into the nucleus to form the florigen activation complex (FAC), thereby inducing floral transition. However, the effect of 14-3-3 proteins on the nuclear translocation of Hd3a and RFT1 remains to be elucidated. In this study, more than fifty *hd3a rft1* double mutants were generated. Genotyping analysis of the no flowering mutant plants, indicated that a critical serine residue Ser48 (numbering as per Hd3a) of Hd3a/RFT1 is required for its function in promoting flowering. The Hd3a/RFT1 mutants lacking the serine residue were still able to localize in the nucleus. However, they exhibited a loss of the binding affinity to the 14-3-3 proteins, and a failure to form the FAC to activate the expression of floral identical genes, thus failing to promote floral transition. Moreover, a reported Hd3a mutant lacking the Thr68 residue was found to be unable to bind to 14-3-3 proteins and exhibited a subcellular localization similar to that of the Hd3a△S48 and RFT1△S47 mutants. Taken together, these data uncovered an essential serine residue of Hd3a/RFT1 for its function in floral transition, and suggested that the 14-3-3 proteins are not necessary for Hd3a/RFT1 nuclear translocation, thereby providing new insights into the FAC assembly process.

Keywords

Rice, Heading date, Florigen, Hd3a, RFT1, 14-3-3 protein

Background

Flowering time, also known as the heading date, is a critical agronomic trait that governs grain yield and the adaptability of rice to diverse geographical regions and seasonal conditions. Heading date is coordinately regulated by both endogenous genetic composition and environmental factors, such as photoperiod, temperature, and biotic and abiotic stresses (Zhou *et al.*, 2021). These exogenous signals are perceived by the leaves, thereby stimulating the synthesis of florigen, a mobile protein signal that is transported from the leaves to the shoot apical meristem (SAM), where it initiates flowering (Taoka *et al.*, 2011, Peng *et al.*, 2021). FLOWERING LOCUS T (FT) and its orthologs are small globular proteins belonging to the phosphatidylethanolamine-binding protein (PEBP) family that function as florigen in plants (Gao *et al.*, 2026). Rice

is a facultative short-day (SD) plant that flowers earlier under SD conditions than under long-day (LD) conditions (Zhou *et al.*, 2021). Rice florigen is encoded by two homologous genes, *Heading date 3a* (*Hd3a*) and *RICE FLOWERING LOCUS T 1* (*RFT1*) (Tamaki *et al.*, 2007, Komiya *et al.*, 2008). The *Hd3a* gene primarily functions under SD conditions, whereas *RFT1* predominantly functions under LD conditions in rice (Kojima *et al.*, 2002, Komiya *et al.*, 2008). Null mutations in both *Hd3a* and *RFT1* resulted in a defect in floral transition under all photoperiods (Kojima *et al.*, 2002, Komiya *et al.*, 2008, Mineri *et al.*, 2023).

Heading date is orchestrated by a sophisticated regulatory network in rice. It integrates multiple interconnected pathways that respond to various environmental and endogenous signals (Zhou *et al.*, 2021). At the core of this network is the florigen encoding genes *Hd3a* and *RFT1*, which function as the final integrators of multiple upstream pathways (Gao *et al.*, 2026). HEADING DATE 1 (Hd1) and Early heading date 1 (Ehd1) are the core transcriptional factors in regulating the expression of *Hd3a* and *RFT1* (Yano *et al.*, 2000, Doi *et al.*, 2004). Both Hd1 and Ehd1 act as activators by direct binding to the promoters of *Hd3a* and *RFT1* and promoting their expression under SD conditions. Under LD conditions, Ehd1 also acts as an activator, whereas, Hd1 interacts with GRAIN NUMBER, PLANT HEIGHT, AND HEADING DATE 7 (Ghd7) and Days to heading on chromosome 8 (DTH8) to form repressive complexes, thereby suppressing Ehd1 transcription (Zong *et al.*, 2021). *Hd1* and *Ehd1* are themselves subject to complex regulation by multiple upstream factors (Zhou *et al.*, 2021). In addition to the aforementioned mechanisms, the expression of *Hd3a* and *RFT1* is also subject to regulation by epigenetic regulation (Li *et al.*, 2026). This exquisite regulatory network ensures that florigen production is precisely tuned when the internal developmental status and external environmental conditions are both optimal for reproduction.

The movement of florigen from leaf companion cells through the phloem to the sink meristem is a highly regulated process that involves specific transport machinery, membrane trafficking, and potential sequestration mechanisms (Song *et al.*, 2017,

Susila *et al.*, 2021, Zhang *et al.*, 2022). OsFTIP1 and OsFTIP9 are ER-localized membrane proteins, that interact with RFT1 and Hd3a, facilitating its export from companion cells to sieve elements under LD and SD conditions, respectively (Song *et al.*, 2017, Zhang *et al.*, 2022). OsTPR075 strengthens the interactions with Hd3a and RFT1, thereby enhancing the efficiency of florigen transport (Zhang *et al.*, 2022). Given these traffics are achieved through plasmodesmata, the closure of plasmodesmata is also vital for florigen movement (Pandey *et al.*, 2026). Additionally, phosphatidylglycerol (PG), a major lipid component of cellular membranes, has been demonstrated to sequester florigen proteins within leaf companion cells (Susila *et al.*, 2021).

In SAM, the arrived florigen together with 14-3-3 proteins and FD transcription factors form hetero-hexametric florigen activation complex (FAC) (Taoka *et al.*, 2011). Crystallographic studies have revealed that 14-3-3 proteins act as molecular scaffolds, simultaneously binding Hd3a and phosphorylated OsFD1 through distinct grooves in their structure (Taoka *et al.*, 2011). Take Hd3a–GF14c interface for an example. The structure is comprised of two principal regions: a hydrophobic cavity and an acidic lobe. The hydrophobic cavity interacts with Hd3a residues M63, F103, P96, T98 and T99, while the acidic lobe interacts with Hd3a residues D62, R64 and R132. The Hd3a proteins bearing either of the R64A, P96I, F103A or R132K mutations showed a loss of binding activity with OsGF14b and OsGF14c. The disruption of the interaction between Hd3a and 14-3-3 proteins has been demonstrated to impede the formation of FAC. The Hd3a-GF14b complex was mainly detected in the protoplast cytoplasm, whereas in the presence of OsFD1, the complex was evidently concentrated into the nucleus. It was thus proposed that Hd3a binds to 14-3-3 proteins in the cytoplasm, and that the resultant Hd3a–14-3-3 complex enters into the nucleus to form FAC with phosphorylated OsFD1. This, in turn, activates the expression of floral identical genes expression, thereby promoting floral transition (Taoka *et al.*, 2011). In this model, the Hd3a–14-3-3 complex is first assembled in the cytoplasm and subsequently transported into the nucleus, where it undergoes further assembly with OsFD1 to form

FAC. Nevertheless, there is an absence of direct evidence supporting this FAC assemble model.

In the present study, more than fifty *hd3a rft1* double mutants were generated. Through a combination of phenotypic and genetic analysis, an unreported key serine residue of Hd3a/RFT1 was identified. Hd3a/RFT1 mutants lacking the serine residue, were unable to interact with 14-3-3 proteins, and thus failed to form the FAC to promote floral transition. Surprisingly, these Hd3a/RFT1 mutants were still able to localize in the nucleus, suggesting that 14-3-3 proteins are unnecessary for the translocation of Hd3a/RFT from the cytoplasm to the nucleus. Consequently, it is concluded that the serine residue of Hd3a/RFT1 is essential for its function in initiating flowering, and a new model for the assembly of FAC in rice is proposed.

Results

The phenotypes of the *hd3a rft1* double mutants

To investigate the function of florigen in the mechanism underlying plant floral transition, a CRISPR-Cas9 genome editing assay was employed and a specific guide RNA was designed to target the conserved region of *Hd3a* and *RFT1*, with the aim of generating knockout mutants of *Hd3a* and *RFT1* (Fig. 1a). 51 independent transgenic positive T_0 plants were obtained. Among them, 49 plants exhibited no flowering up to 250 days in paddy field under natural SD conditions (Table S1). Genotyping analysis revealed that 20 plants in which both the *Hd3a* and *RFT1* had been knocked out exhibited a non-flowering phenotype (Fig. 1b, c, Table S1), consistent with previous reports (Komiya *et al.*, 2008, Mineri *et al.*, 2023). Surprisingly, 15 of the non-flowering T_0 plants exhibited a null mutation in Hd3a and a loss of the Ser47 residue of RFT1 (RFT1△S47), or a null mutation in RFT1 and a loss of the Ser48 residue of Hd3a (Hd3a△S48) (Fig. 1b, c, Table S1). These findings suggested that the serine residue is crucial for Hd3a/ RFT1 function in plant floral transition.

FT acts as a mobile floral activator, while its homolog TERMINAL FLOWER 1 (TFL1)

represses flowering (Khosa *et al.*, 2021). It has been demonstrated that they compete for 14-3-3 binding, thereby playing an antagonistic role in the regulation of plant flowering (Kaneko-Suzuki *et al.*, 2018). Multiple sequence analyses of the characterized plant FT homologs were conducted, which revealed that the Ser48 residue (numbering as per Hd3a) is highly conserved in these proteins across flowering plants (Fig. S1).

The Ser48/47 residue affects the subcellular localization of Hd3a/RFT1 in rice

As protein subcellular localization is co-related to its biological function, we first analyzed the localization of the mutated proteins Hd3a△S48 and RFT1△S47 in rice protoplasts. The distribution of both Hd3a-GFP and RFT1-GFP was found to be uniform within the cell, with equal presence in both the cytosol and nucleus (Fig. 2a, b). The distribution of Hd3a△S48-GFP and RFT1△S47-GFP in the cell nucleus was found to be similar to that of Hd3a-GFP and RFT1-GFP (Fig. 2a, b). However, in contrast to Hd3a-GFP and RFT1-GFP, Hd3a△S48-GFP and RFT1△S47-GFP exhibited a punctate distribution in the cell cytosol (Fig. 2a, b).

Since the serine residue is essential for the function and subcellular localization of Hd3a/RFT1, we next tested whether this residue affects the formation of the FAC complex. A typical FAC complex is constituted by a hetero-hexamer comprising two molecules of Hd3a, GF14c and OsFD1 (Taoka *et al.*, 2011). Bimolecular fluorescence complementation (BiFC) experiments were conducted, and the results showed that both Hd3a-YN and RFT1-YN interacted with YC-OsFD1 in the cell nucleus. In contrast, Hd3a△S48-YN and RFT1△S47-YN exhibited no interaction with YC-OsFD1 (Fig. 2c). The interaction between Hd3a-YN and YC-Hd3a was observed in both the cell cytosol and nucleus (Fig. 2d). In the presence of OsFD1, this interaction was exclusively detected in the cell nucleus. However, Hd3a△S48-YN and YC-Hd3a△S48 exhibited no interaction either with or without in the presence of OsFD1 (Fig. 2d). These results suggested that loss of the serine residue of Hd3a/RFT1 led to a deficiency in the formation of FAC in rice.

The Ser48/47 residue of Hd3a/RFT1 is required for Hd3a/RFT1 interacting with 14-3-3 proteins

In the FAC complex, a central 14-3-3 protein dimer acts as a molecular scaffold to physically bridge Hd3a/RFT1 to FD transcriptional factor (Taoka *et al.*, 2011, Peng *et al.*, 2021). Given that the interaction between Hd3a△S48/RFT1△S47 and OsFD1 was not observed in the BiFC assay, it was hypothesized that the Ser48/47 residue of Hd3a/RFT1 may be essential for their interaction with 14-3-3 proteins. To address this hypothesis, the interactions between Hd3a△S48/RFT1△S47 and 14-3-3 proteins were investigated. In rice, 14-3-3 proteins are encoded by eight homologous genes, designated *GF14a-f* (Chen *et al.*, 2006). Among them, GF14b, GF14c, GF14e and GF14f were observed interacting with Hd3a (Taoka *et al.*, 2011). As FAC functions in floral transition in shoot tips, the expression of *GF14a-f* in rice shoot apical tips was first analyzed by RT-qPCR. The results showed that *GF14a*, *GF14b*, *GF14c*, *GF14d*, *GF14e* and *GF14f* were expressed in the tissue, while the transcripts of *GF14g* and *GF14h* were barely detected (Fig. S2). Given that GF14a exhibited no binding affinity to Hd3a (Taoka *et al.*, 2011), the following 14-3-3 proteins were selected for further analysis: GF14b, GF14c, GF14d, GF14e and GF14f. All the tested 14-3-3 proteins exhibited interactions with Hd3a and RFT1 by the BiFC assay (Fig. 3a, c). Indeed, Hd3a and RFT1 interacted with GF14c, GF14d, GF14e and GF14f both in the cell cytosol and nucleus, except GF14b, which interacted only in the cell cytosol. In accordance with the hypothesis, Hd3a△S48 and RFT1△S47 exhibited no interactions with the 14-3-3 proteins (Fig. 3b, d). Subsequently, GF14b, GF14c and GF14e were selected as representative 14-3-3 proteins to further assess the interactions by yeast two hybrid (Y2H) and pull-down assay, which yielded similar results (Fig. 4). Taken together, these findings indicated that the Ser48/47 residue of Hd3a/RFT1 is indispensable for Hd3a/RFT1 to interact with 14-3-3 proteins in rice.

Given that the serine residue is a potential site for phosphorylation, the serine residue of Hd3a/RFT1 was mutated to glutamic acid and alanine, thus mimicking the phosphorylated and unphosphorylated states, respectively. The subcellular location of

these mutated Hd3a/RFT1 proteins was similar to that of Hd3a/RFT1 in rice protoplasts (Fig. 2a). Next, the mutants Hd3a(S48E) and Hd3a(S48A) were selected as representative for the purpose of evaluating the effect of the serine residue on binding affinity to 14-3-3 proteins. The results showed that Hd3a(S48E) and Hd3a(S48A) exhibited comparable binding affinity to 14-3-3 proteins in comparison with Hd3a (Fig. S3).

The application of AlphaFold protein structure modelling to the Hd3a/RFT1 proteins revealed that Ser48 is situated within an α-helix (Fig. S4). It is noteworthy that the Hd3a△S48 and RFT1△S47 mutants exhibited a loss of the α-helix (Fig. S4). These findings suggested that the Ser48 residue may primarily serve to maintain Hd3a structure or support the interface conformation in the interaction of Hd3a and 14-3-3 proteins.

RICE CENTRORADIALIS (RCN) proteins have been shown to belong to the TFL1-like protein family (Zhao *et al.*, 2022). It has been demonstrated that these proteins compete with Hd3a for 14-3-3 binding, thereby antagonizing florigen activity (Kaneko-Suzuki *et al.*, 2018). Given that the Ser48 residue is conserved across RCN proteins (Fig. S1), we mutated RCN3 by deleting this residue, with a view to investigating the interactions between these RCN mutations and 14-3-3 proteins. The interactions between RCN3 and GF14b/c/d/e/f were observed in rice protoplasts by BiFC experiments (Fig. S5a). However, no interactions between RCN3△S46 and GF14b/c/d/e/f were detected (Fig. S5b). Similar results were obtained by Y2H experiments (Fig. S5c). These results indicated that the serine residue is also essential for the interaction between RCN proteins and 14-3-3 proteins.

Loss of the Ser48/47 residue of Hd3a/RFT1 disrupts FAC activity in floral transition

In the shoot apical meristem, FAC activates the expression of floral organ identity genes, including *OsMADS14*, *OsMADS15* and so on (Taoka *et al.*, 2011). It was reported that transient expression of Hd3a and OsFD1 in rice protoplasts has been demonstrated to promote FAC formation, thereby activating florigen downstream gene

expression, such as *OsMADS15* (Taoka *et al.*, 2011). So, we employed this method to assess the effects of the Ser48/47 residue of Hd3a/RFT1 on FAC transcriptional activity. To coordinate expression of OsFD1 and the corresponding Hd3a/RFT1 or their mutants, IntF2A-based polyprotein expression cassettes were designed (Zhang *et al.*, 2017). The resulting constructs were transiently expressed in rice protoplasts. The relative expression of the representative FAC downstream genes were analyzed. In instances which OsFD1 and Hd3a/RFT1 were co-expressed, a marked increase in the expression levels of *OsMADS14* was observed, along with the observation of *OsMADS15* (Fig. 5). In contrast, when OsFD1 and Hd3a△S48/RFT1△S47 were co-expressed, the expression levels of *OsMADS14* and *OsMADS15* were comparable to those observed in the controls (Fig. 5). Previously, *OsMADS62*, *OsPP2C1*, *Os01g0968100*, *Os02g0219800*, *Os08g0299400* and *Os06g0110000* were identified as the candidate downstream targets of OsFD1 mediated FAC complex (Cerise *et al.*, 2021). The expression patterns of these genes were similar to that of *OsMADS14* and *OsMADS15* in this experiment (Fig. 5). These data suggested that the conserved Ser48/47 residue of Hd3a/RFT1 is vital for FAC transcriptional activity.

The transcriptional activity of FAC is a prerequisite for plant floral transition (Taoka *et al.*, 2011, Ho and Weigel, 2014). Overexpression of Hd3a or RFT1 has been demonstrated to induce extreme early flowering (Kojima *et al.*, 2002, Pasriga *et al.*, 2019). To further assess the biological function of the Ser48/47 residue of Hd3a/RFT1, Hd3a CDS and the mutant CDS lacking the codon of Ser48 were infused with an HA tag and driven by Ubiquitin promoter were transferred into rice by Agrobacteria mediated plant transformation, respectively. A total of 28 independent Hd3a overexpressing transgenic T_0 plants were obtained, of which 20 produced panicles during the seedling stage (Fig. 6a). However, all the obtained 21 independent Hd3a△S48 overexpressing plants appeared normal during the seedling stage (Fig. 6b). To evaluate the consequence of Hd3a△S48 overexpressing, three independent transgenic lines of Hd3a△S48 overexpressing plants were selected for further analysis, and three independent Hd3a overexpressing lines that did not exhibited heading

phenotypes during the seedling stage were selected as a control. HA-tagged Hd3a or Hd3a△S48 proteins were detected in all the T_0 transgenic plants (Fig. S6). The T_1 generation of Hd3a△S48 overexpressing line 2 segregated into two categories: transgenic positive and negative plants. Although, the transgenic positive plants showed marked higher gene expression level of *Hd3a*, its heading date was comparable to that of the transgenic negative plants (Fig. S7). As previously reported, the *Hd3a* overexpressing plants exhibited earlier heading (Fig. 6c, g), reduced plant height and panicle length in comparison to the wild type (Fig. 6e, h, i). Intriguingly, the Hd3a△S48 overexpressing plants showed similar phenotypes to the wild type (Fig. 6d, f-i). These findings implied that loss of the serine residue resulted in the disfunction of Hd3a in regulating plant floral transition.

The function of 14-3-3 proteins in Hd3a nuclear translocation

The prior data revealed that Hd3a△S48 and RFT1△S47 were unable to interact with 14-3-3 proteins, the localization of them were also observed in both the cell cytosol and nucleus. This observation led to the hypothesis that 14-3-3 proteins do not appear to be a prerequisite for Hd3a /RFT1 translocation from the cytoplasm to the nucleus. It has been established that the amino acid residues R64, T68, P96, F103 and R132 of Hd3a are critical for Hd3a interacting with 14-3-3 proteins (Taoka *et al.*, 2011). In order to provide further evidence to support this hypothesis, these residues of Hd3a were analyzed. The results showed that Hd3a interacted with all the test 14-3-3 proteins by the Y2H experiments (Fig. S8). The Hd3a△T68 mutant exhibited a complete absence of interaction with 14-3-3 proteins, while all other Hd3a mutant proteins showed an interaction with GF14g (Fig. S8). Interestingly, Hd3a△T68-GFP exhibited punctate structures in the cytoplasm and a uniform distribution in the nucleus, which was similar to that observed in Hd3a△S48-GFP (Fig. 7). No discernible alterations in the subcellular localization of proteins were observed between the other GFP-tagged Hd3a mutants and the Hd3a-GFP (Fig. 7). In summary, these data confirmed that the interaction between Hd3a and 14-3-3 proteins is not a prerequisite for Hd3a entering into the nucleus.

Discussion

The Ser48 residue is vital for FT- and TFL1-like proteins interacting to 14-3-3 proteins

FT belongs to the phosphatidylethanolamine-binding protein (PEBP) family, which was grouped into three subfamilies, MOTHER OF FT AND TFL1 (MFT)-like proteins, FT-like proteins and TFL1-like proteins (Zhu *et al.*, 2021). FT-like proteins and TFL1-like proteins play opposite roles in regulating flowering time and inflorescence architecture. FT-like proteins including rice Hd3a and RFT1, function as florigen, which are synthesized in leaves in response to exogenous and endogenous cues (Gao *et al.*, 2026). These proteins are subsequently transported through the phloem into the SAM and ultimately activate floral transition. The overall structure of FT consists of a central anti-parallel β-sheet flanked by short α-helices on one side and a smaller β-sheet on the other side (Taoka *et al.*, 2011). In the FAC-DNA complex, two interfaces were found in FT. The interface 1 located in the C-terminal tail of FT, has been demonstrated to interact directly with DNA to facilitating FT recruitment to the DNA−FD−14-3-3 complex (Gao *et al.*, 2025). The interface 2, which interacts to 14-3-3 proteins, is constituted by a hydrophobic cavity and an acidic lobe (Taoka *et al.*, 2011). Besides these, a conserved region designated as segments B, is suggested to be required for FT to recruit transcriptional coactivators (Ahn *et al.*, 2006). Structure-function studies have progressively delineated the molecular determinants governing FT activity. The mutation of all the interacting amino acid residues (FT R166A/R173A/R174A) in the FT interface 1 resulted in the abolition of FT recruitment to the DNA−FD−14-3-3 complex, and the failure to promote flowering. Overexpressing Hd3a promoted plant flowering (Gao *et al.*, 2025). However, the plants overexpressing the Hd3a mutants with reduced Hd3a binding affinity to 14-3-3 proteins exhibited a loss in accelerated flowering (Taoka *et al.*, 2011). Point mutations of specific surface-exposed residues that may interact with the transcriptional activator converted FT from an activator to a repressor of flowering (Ho and Weigel, 2014). In this study, a conserved serine residue

(Ser48) of Hd3a/RFT1 was identified as being essential for plant floral transition. The absence of the aforementioned residue in Hd3a/RFT1 resulted in an inability to interact with 14-3-3 proteins (Fig. 3, 4), thus hindering the assembly of FAC and the promotion of flowering (Fig. 2c-d; 5; 6b, g). AlphaFold predicted that the serine residue is required for the formation of an α-helix of Hd3a (Fig. S4). This α-helix of Hd3a is not located on the previously reported interaction interface between Hd3a and 14-3-3 proteins. These data imply that the Ser48 residue may serve to maintain the Hd3a structure or support the interface conformation for the Hd3a−14-3-3 complex.

The TFL1-like protein subfamily includes four members in rice, which are designated RCN1-4. These RCN proteins function in flowering as repressors by competing with Hd3a for binding to 14-3-3 proteins (Kaneko-Suzuki *et al.*, 2018). The Ser48 residue is highly conserved in these RCN proteins (Fig. S1). Loss of this serine residue of the RCN proteins also blocked its binding affinity to 14-3-3 proteins (Fig. S5). Consequently, the conserved serine residue is necessary for the functions of both FT-like and TFL1-like proteins.

The FAC-DNA complex may be assembled *de novo* in the nucleus

In the classic florigen signaling model, FT-like protein is synthesized in leaves, and is subsequently transported into SAM cells, in where FT-like protein binds to 14-3-3 proteins in the cytoplasm, and that the resultant Hd3a–14-3-3 complex enters into the nucleus to form FAC with phosphorylated OsFD1 (Taoka *et al.*, 2011, Peng *et al.*, 2021). In the nucleus, FAC activates transcription of floral organ identity genes, thereby initiating floral transition. Nevertheless, the location of FAC assembly remains a subject of considerable controversy. The hypothesis that Hd3a interacts with GF14b in the cell cytoplasm, subsequently entering the nucleus to form FAC with FD1, is supported by numerous experimental findings (Taoka *et al.*, 2011). Subcellular localization analysis demonstrated that Hd3a-mCherry was distributed in both the cytoplasm and the nucleus, while GFP-GF14b was predominantly located in the cytoplasm and exhibited a minimal nuclear presence. BiFC analysis revealed that there was an interaction between Hd3a and GF14b in the cytoplasm. In contrast, the Hd3a–GF14b interaction

was exclusively observed in the nucleus in the presence of OsFD1. Nevertheless, the findings are insufficient to exclude the possibility that Hd3a and GF14b can be translocated into the nucleus independently and subsequently form FAC with OsFD1 in the nucleus. Previous researches revealed that 14-3-3 proteins could retain its interacting protein BZR1 orthologs in the cytoplasm in Arabidopsis and rice (Bai *et al.*, 2007, Gampala *et al.*, 2007). OsGF14c has been found to be predominantly localized in the cytoplasm and weakly in the nucleus. The interaction of Hd3a and OsGF14c was observed within the cytoplasm primally. Plants overexpressing *OsGF14c* exhibited delayed heading (Purwestri *et al.*, 2009). In accordance with this observation, it was reported that the bamboo 14-3-3 protein PvGF14c localized and interacted with PvFT in the cytoplasm, and heterologous overexpression of *PvGF14c* in Arabidopsis resulted in a substantial delay in flowering time under long-day conditions (Li *et al.*, 2018). In contrast, the mango 14-3-3 proteins MiGF6A and MiGF6B are localized in the nucleus. Overexpression of either *MiGF6A* or *MiGF6B* in Arabidopsis led to earlier flowering (Xia *et al.*, 2023). These data suggest that cytoplasmic 14-3-3 proteins may bind to FT-like proteins, thereby preventing them from entering into the nucleus. In this study, it was observed that the Hd3a△S48 and RFT1△S47 mutants, which exhibited a deficiency in binding affinity to 14-3-3 proteins, were also found to localize in the cytoplasm and the nucleus (Fig. 2a, b). Furthermore, the mutation of the amino acid residue T68 of Hd3a, which has been previously reported to be involved in interacting with 14-3-3 proteins, exhibited a subcellular localization of the mutated Hd3a (Hd3a△T68) was similar to that of Hd3a△S48 (Fig. 7). These findings imply that the translocation of Hd3a/RFT1 from the cytoplasm to the nucleus is independent to its interaction with 14-3-3 proteins. Recently, the blue-light receptor FLAVIN-BINDING, KELCH REPEAT, F-BOX 1 (FKF1) was identified as an FT1 interactor, which protects FT1 from degradation and promotes its entry into the nucleus in *Brachypodium distachyon* (Lyu *et al.*, 2026). Notably, in addition to the FT-14-3-3 protein interaction, the interaction between the C-terminal tail of FT and the DNA of the DNA-FD-14-3-3 complex was confirmed to be a prerequisite for FT recruitment to this complex in Arabidopsis (Gao *et al.*, 2025). Taken together, a revised FAC assembly model is

proposed that, in the SAM, the incoming Hd3a and RFT1 are translocated into the nucleus, where they form a FAC-DNA complex with 14-3-3 proteins, FD transcriptional factors and the corresponding DNA in rice. However, the underlying mechanism by which Hd3a/RFT1 enter into the nucleus requires further study.

Materials and Methods

Plant materials and growth conditions

The *japonica* rice variety Wuyungeng 7 (WYG) was used as the wild type in the present study. The plants were cultivated in a patty field, with regular maintenance under natural short-day conditions in Shenzhen (22.73° N, 113.95° E).

Vector construction and rice transformation

For constructing the gene editing vector, a specific gRNA was designed to target the conserved region of *Hd3a* and *RFT1* was designed (Table S) and cloned into the *pYLCRISPRCas9* vector (Ma *et al.*, 2015). In the gene overexpressing vector, the coding sequences of the wild-type *Hd3a* and the mutant *Hd3a⊿S48* were fused with a 3×HA tag and cloned into the *pCambia1300-proZmUBI* vector, respectively. The resulting plasmids were then introduced into Agrobacterium tumefaciens strain AG10 via electroporation. Transgenic plants were subsequently generated using Agrobacterium-mediated rice transformation.

Phenotypic analysis

For the agronomic phenotypic analysis, more than twenty plants from each plant line were selected and the heading date, plant height and panicle length were measured. The statistical significance of the difference among samples was determined using a one-way analysis of variance (ANOVA) followed by a Tukey's post hoc test ($P < 0.05$).

Protein sequence alignments

FT homologs including FT- like and TFL1-like proteins, for which the function has been confirmed, were selected for phylogenetic analysis. All sequences were retrieved from the plant genomic database, and the corresponding accession numbers for the

proteins are listed in Table S2. Sequence alignments were performed by MUSCLE v5.3 (Edgar, 2022). The result was visualized using a custom Python script.

Protein structure analysis

The 3D protein structures of Hd3a/RFT1, both with and without the conserved serine residue, were predicted using AlphaFold3 (Abramson *et al.*, 2024). The spatial structural alignments of the predicted models were subsequently analyzed and visualized using PyMOL.

Protein subcellular localization

The coding sequences of *Hd3a* and *RFT1*, with or without lacking the serine codon, or with the serine codon substituted for glutamic acid or alanine were cloned into the *PAN580-EGFP* vector. The resulting constructs were then introduced into rice protoplasts together with a nuclear marker construct *PAN580-mCherry-2NLS*, via the PEG-mediated method. Following a 12–16 h incubation period at 26°C, the fluorescence signals were examined using a laser scanning confocal microscope (Zeiss LSM800).

Bimolecular fluorescence complementation analysis

For the BiFC analyses, the coding sequences of *Hd3a*, *RFT1* and *RCN3*, with or without lacking the serine codon were cloned into the *PUC-YN* vector, respectively. The coding sequences of *GF14b/c/d/e/f* and *OsFD1* were then cloned into the *PUC-YC* vector, respectively. The resulting YN and YC plasmids were co-introduced with the nuclear marker plasmid into rice protoplasts according to certain combination. After 12–16 hours of incubation at 26°C, the fluorescence signals were examined using a laser scanning confocal microscope.

Yeast two-hybrid assay

The coding sequences of *Hd3a*, *RFT1* and *RCN3*, with or without lacking the serine codon, were cloned into the *pGBKT7* vector. The coding sequences of *GF14b/c/e* were cloned into the *pGADT7* vector, respectively. The corresponding plasmids were co-introduced into Y2H Gold yeast competent cells. The final yeast cells were incubated

on double dropout (DDO) synthetic defined medium (SD/-Leu/-Trp; Clontech). Positive transformants were subsequently transferred onto quadruple dropout (QDO) medium (SD/-Leu/-Trp/-His/-Ade; Clontech) and incubated at 30°C for 2–3 days in order to evaluate protein-protein interactions.

Pull-down assay

The coding sequences of *Hd3a* and *RFT1*, with or without lacking the serine codon, were cloned into the *pGEX-3X* vector to generate GST-Hd3a, GST-Hd3a△S48, GST-RFT1, and GST-RFT1△S47 expression constructs, respectively. The coding sequences of *GF14b/c/e* were cloned into the *pET-30a* vector to generate His-GF14b/c/e expression constructs. The resultant constructs were then transformed into *Escherichia coli* BL21(DE3) competent cells (TransGen, Cat#CD601) and protein expressions were induced by 0.4 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) at 16°C for 14–16 h, respectively. The recombinational proteins were purified using GST-resin (TransGen, Cat# DP201) for GST-tagged proteins or Mag-MK His-Tag Protein Purification Beads (Sangon Biotech, Cat# C650033) for His-tagged proteins according to the product manuals.

The purified GST, GST-Hd3a, GST-Hd3a△S48, GST-RFT1, and GST-RFT1△S47 proteins were adjusted to equivalent concentrations. The GST-tagged proteins were then incubated with the corresponding His-GF14b/c/e proteins and pre-equilibrated Mag-MK His-Tag magnetic beads at 4°C for 2 hours, respectively. Following a decontamination process involving ten cycles of washing, the proteins that had been retained were subjected to SDS-PAGE for analysis. This was then followed by Western blot analysis using an anti-GST antibody (TransGen, Cat# HT601) and an HRP-conjugated anti-mouse IgG antibody (TransGen, Cat# HS002).

Gene expression analysis

To analyze the expression levels of the *14-3-3* genes, shoot tips were collected from 40-day-old wild-type plants. To examine the expression level of Hd3a△S48 in its overexpressing plants, mature leaves were collected.

To investigate the impact of the conserved Ser48/47 residue of Hd3a/RFT1 on FAC transcriptional activity, IntF2A-based polyprotein expression cassettes were constructed (Zhang *et al.*, 2017). The CDS of Hd3a/RFT1 or their mutants, IntF2A and OsFD1, were fused in order, and driven by the 35S promoter. The resulting constructs were transformed into protoplasts. The protoplasts were harvested after 16 hours of incubation at 26°C.

Total RNA was isolated from these materials using an RNA Extraction Kit (Magen, Cat# MD024). First-strand cDNA was synthesized via reverse transcription (TransGen, Cat# AU341), followed by RT-qPCR analysis (TransGen, Cat# AQ601). The rice *OsUbq (LOC_Os03g13170*) gene was used as an internal control. Three biological replicates were performed. The primers used in this study are listed in Table S3.

Supplementary Information

Fig.S1 Sequence alignments of FT homologs.

Fig.S2 RT-qPCR analysis of the expression of *GF14a-h* in the shoot tips from 40-day-old rice plants. *OsUbq* was used as an internal control. Three biological replicates were performed.

Fig.S3 Y2H assay for analyzing the interaction between GF14b/c/e and the Hd3a(S48E)/Hd3a(S48A) mutants.

Fig.S4 Comparison of the 3D structures of Hd3a and Hd3a△S48 (a), and RFT1 and RFT1△S47 (b), respectively. The protein structures were predicted using AlphaFold3.

Fig.S5 BiFC and Y2H assays for analyzing the interaction between 14-3-3 proteins and RCN3△S46 mutant. (a) BiFC assay for GF14b-f and RCN3. (b) BiFC assay for GF14b-f and RCN3△S46. (c) Y2H assay for GF14b/c/e and RCN3/ RCN3△S46.

Fig.S6 Protein expression analysis of the HA-Hd3a and HA-Hd3a △ S48 overexpressing plants by western blot. The Coomassie brilliant blue (CBB) stained proteins were used as protein loading controls.

Fig.S7 Characterization of the T_1 progenies of *Hd3a⊿S48* overexpressing plants. (a) Plants at heading stage. (b) The relative gene expression level of the total of *Hd3a⊿S48* and *Hd3a* in the transgenic positive and negative plants. (c) The protein expression level of HA-Hd3a in the transgenic positive and negative plants. HA-Hd3a level was examined by Western blot. The Actin expression levels were used as a protein loading control. (d) Comparison of the heading date of the wild type plants, and the transgenic positive and negative plants.

Fig.S8 Y2H assays for analyzing the interactions between 14-3-3 proteins and Hd3a mutants.

Fig.S9 The negative controls of the BiFC experiments in this study.

Table S1 The genotypes of the *hd3a rft1* double mutants.

Table S2 The FT homologs used for protein sequence alignments.

Table S3 The primers used in this study.

Acknowledgements

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Author Contributions

JW and XT conceived the study and supervised the project. HN, YZ and CW were responsible for vector construction and gene knockout. CW and PZ performed the Y2H analysis. CW conducted gene expression analysis, GST pull-down, BiFC and phenotypic analysis. CW, XT and JW designed the experiments, analyzed the data, and wrote the manuscript. JW obtained funding. All authors read and approved the manuscript.

Data Availability

All data generated or analyzed during this study are included in this published article and its supplementary information files.

Declarations

Ethics Approval and Consent to Participate

Not applicable.

Consent for Publication

Not applicable.

Competing Interests

The authors declare no competing interests.

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Figure legends

Fig. 1 Characterization of the *hd3a rft1* mutants. (a) Gene structures of *Hd3a* and *RFT1*, along with the location of the gRNA targeted DNA sequence on these genes. (b) The genotype of the *hd3a rft1* mutants. (c) >150 day-old *hd3a rft1* mutants.

Fig.2 Subcellular localization of Hd3a△S48 and RFT1△S47 and their interaction with OsFD1. (a) Images of the protoplasts expressing Hd3a-GFP, Hd3a△S48-GFP, Hd3a(S48E)-GFP or Hd3a(S48A)-GFP. (b) Subcellular localization of RFT1-GFP, RFT1△S47-GFP, Hd3a(S47E)-GFP and Hd3a(S47A)-GFP. (c) BiFC assay showing the interactions of Hd3a-OsFD1, Hd3a△S48-OsFD1, RFT1-OsFD1 and RFT1△S47-OsFD1. (d) BiFC assay for the interactions of Hd3a-Hd3a and Hd3a△S48-Hd3a△S48. Scale bars = 5 μm.

Fig.3 BiFC assay showing interactions between the Hd3a/RFT1 mutants and the 14-3-3 proteins. Interaction between Hd3a and GF14b-f (a); Hd3a△S48 and GF14b-f (b); RFT1 and GF14b-f (c); and RFT1△S47 and GF14b-f (d). The negative controls are shown in Fig.S8. Scale bars = 5 μm.

Fig.4 Y2H and pull-down assays for the interactions between the Hd3a/RFT1 mutants and the 14-3-3 proteins. Y2H assay for the interaction between Hd3a△S48 and GF14b/c/e (a), and RFT1△S47 and GF14b/c/e (b). Pull-down assay for the interaction between Hd3a/Hd3a△S48/RFT1/RFT1 and GF14b (c), GF14c (d) and GF14e (e). –Leu-Trp and -Leu-Trp-His-Ade represent the media lacking the corresponding amino acids.

Fig. 5 The effects of the Hd3a/RFT1 mutations on the activation of FAC downstream genes. The corresponding constructs were transiently expressed in rice protoplasts for 16 hours. RT-qPCR was then performed. *OsUbq* was used as an internal control. Three biological replicates were performed.

Fig. 6 Comparison of the phenotypes of the *Hd3a* and *Hd3a⊿S48* overexpressing plants. (a-b) Seedling stage. (c-d) Heading stage. (e-f) Panicles. Statistical analyses of heading date (g), plant height (h) and panicle length (i). Data represent means ± SD ($n > 20$), and significant differences ($p < 0.05$) are indicated by different letters (one-way ANOVA with Tukey's post hoc test).

Fig. 7 Subcellular localization of the Hd3a mutants. Hd3a mutants lacking the residues R64, T68, P96, F103 or R132 were infused with GFP. The resulting constructs together with nuclear marker were transiently expressed in rice protoplasts. Free GFP was used as a control. Scale bars = 5 μm.

Figures and legends

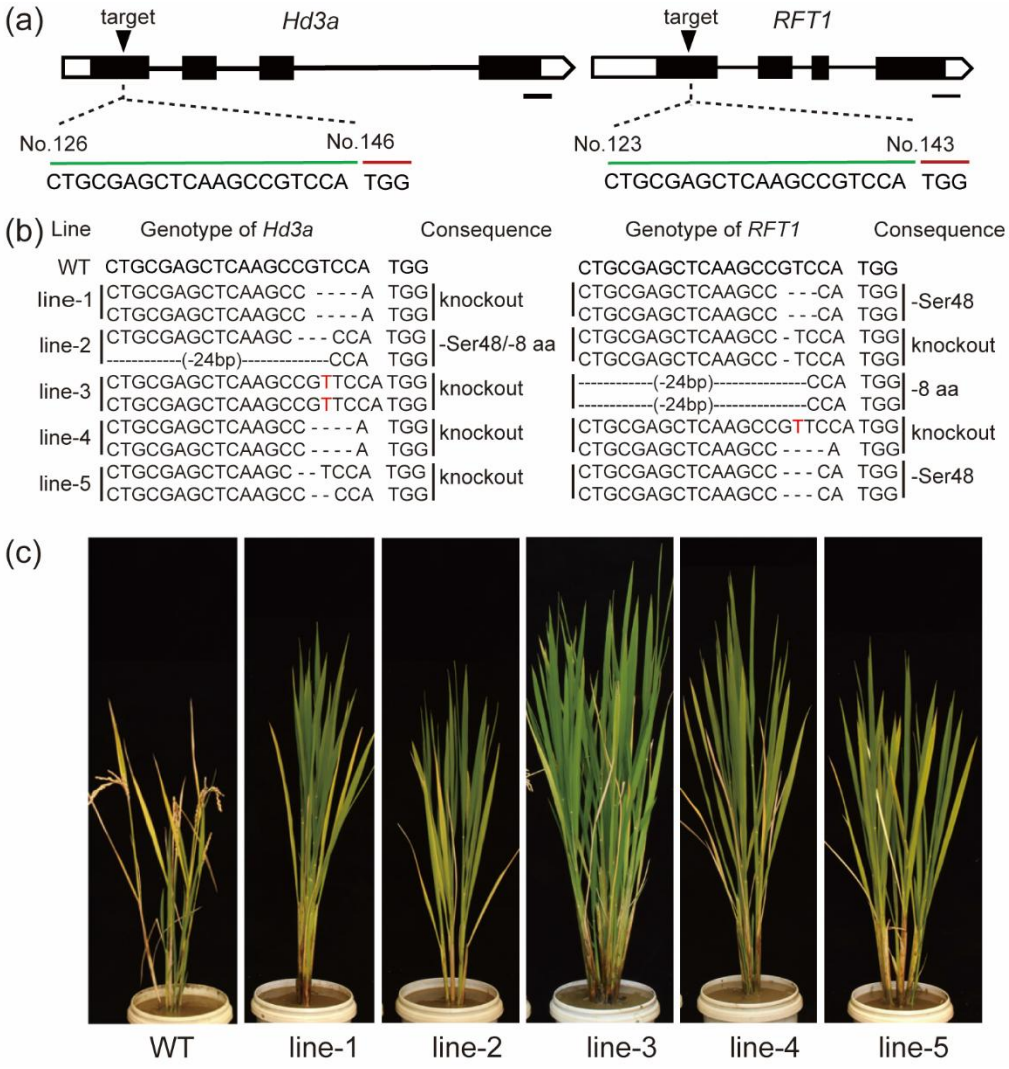


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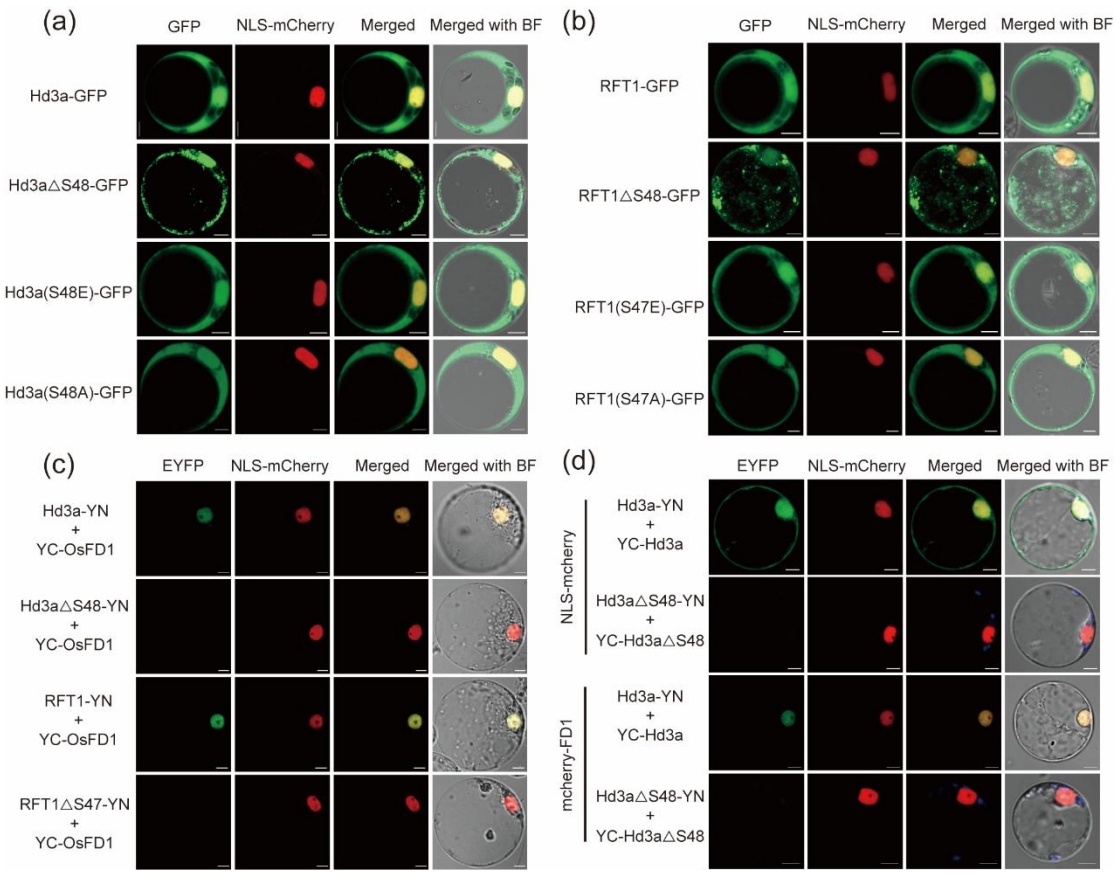


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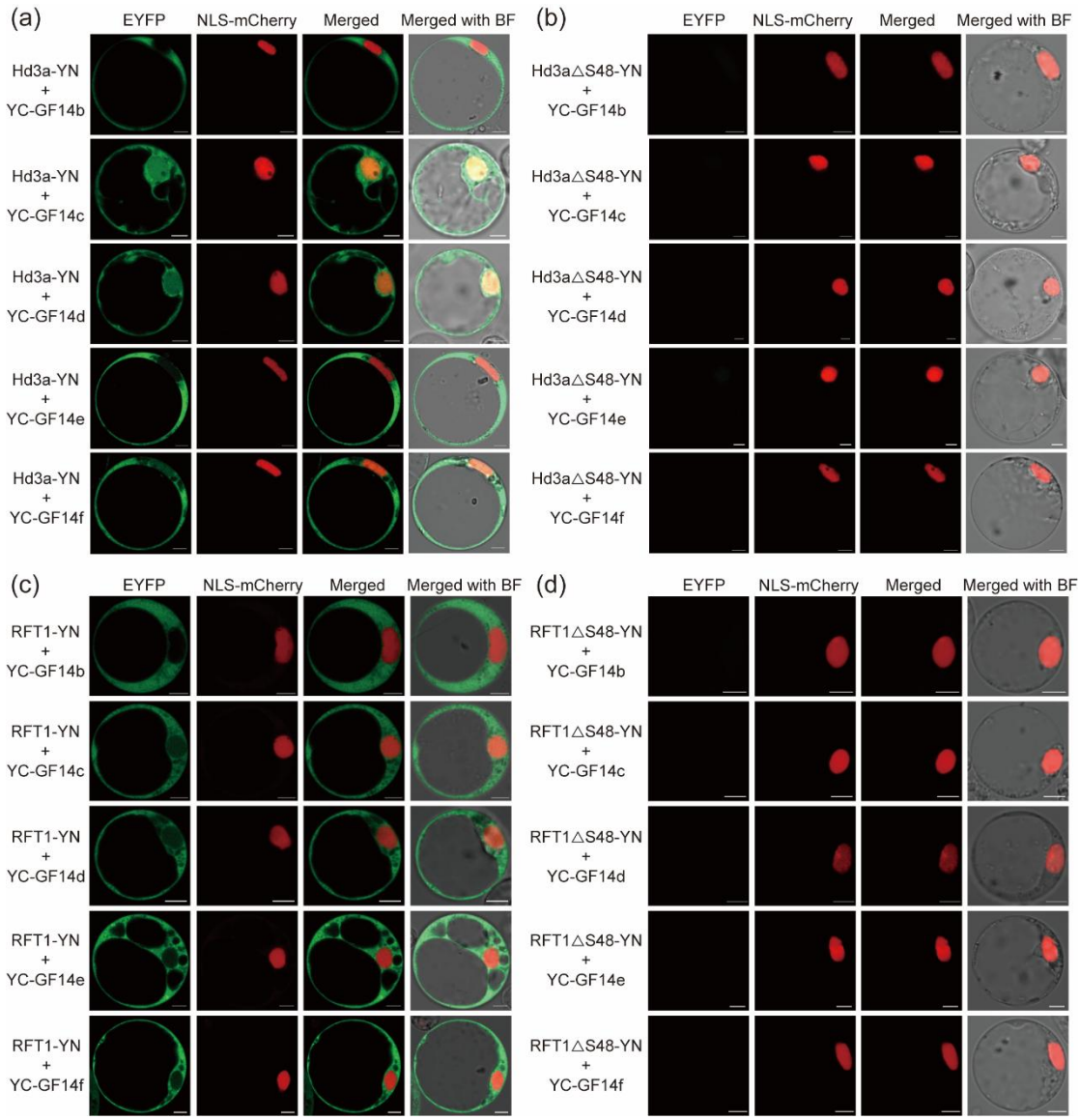
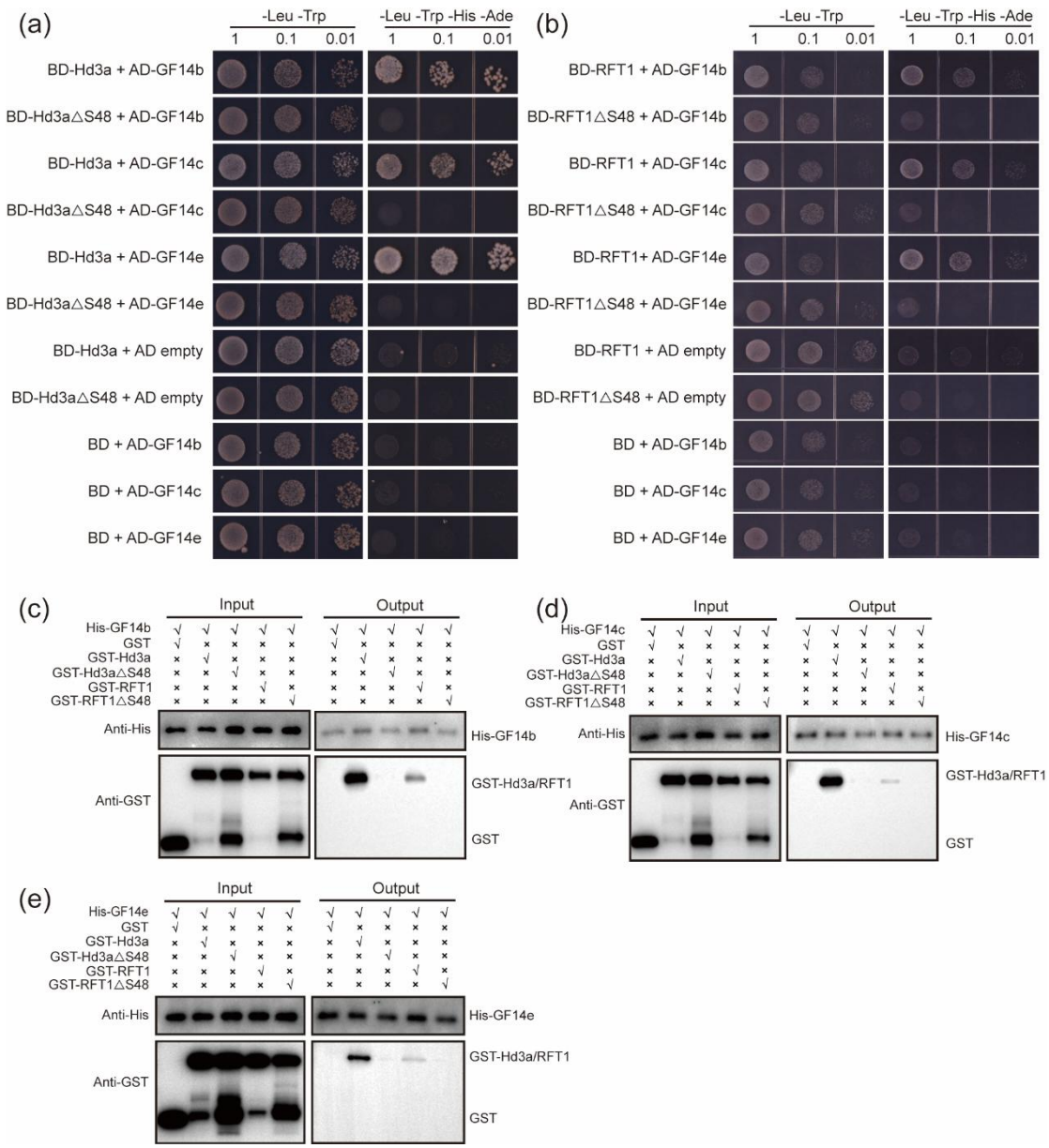


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707 Fig.4 Y2H and pull-down assays for the interactions between the Hd3a/RFT1 mutants
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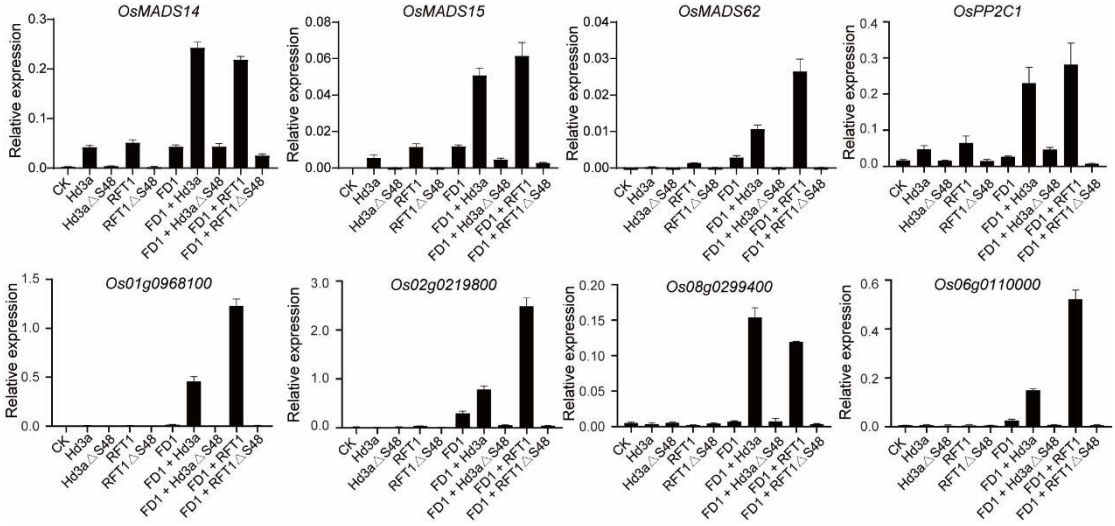


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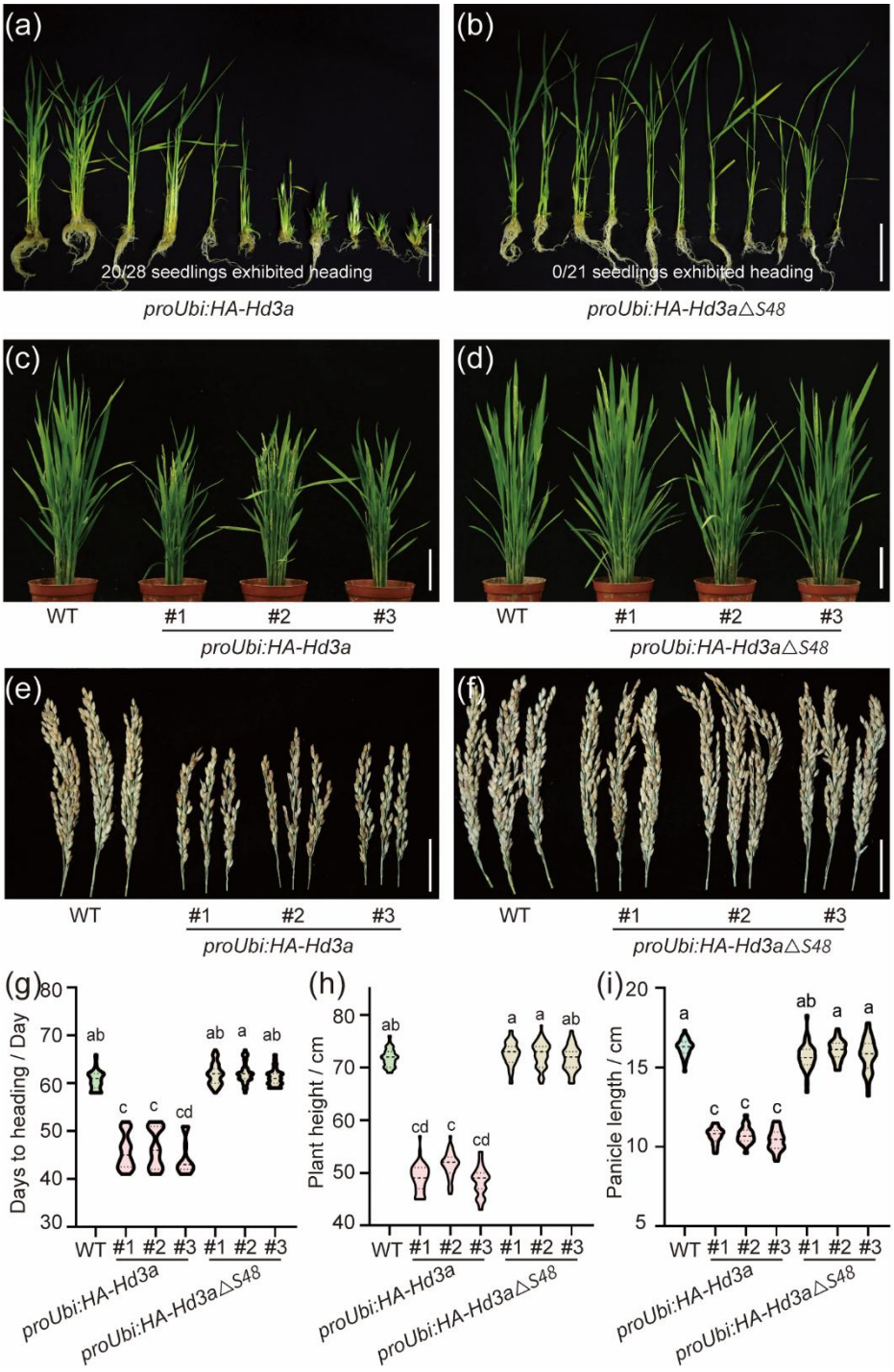


Fig. 6 Comparison of the phenotypes of the *Hd3a* and *Hd3a⊿S48* overexpressing plants. (a-b) Seedling stage. (c-d) Heading stage. (e-f) Panicles. Statistical analyses of heading date (g), plant height (h) and panicle length (i). Data represent means ± SD (n > 20), and significant differences ($p < 0.05$) are indicated by different letters (one-way ANOVA with Tukey's post hoc test).

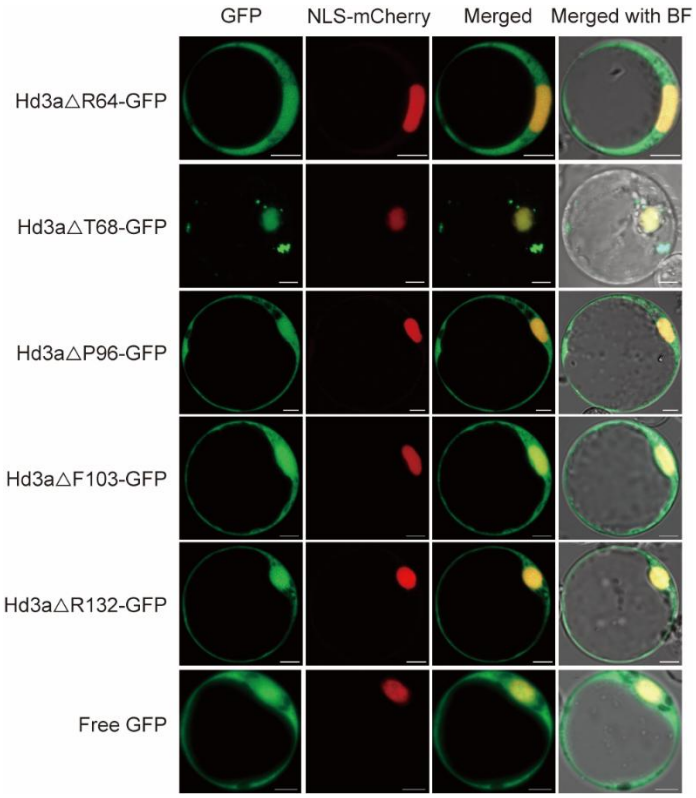


Fig. 7 Subcellular localization of the Hd3a mutants. Hd3a mutants lacking the residues R64, T68, P96, F103 or R132 were infused with GFP. The resulting constructs together with nuclear marker were transiently expressed in rice protoplasts. Free GFP was used as a control. Scale bars = 5 μm.

Supplementary Information

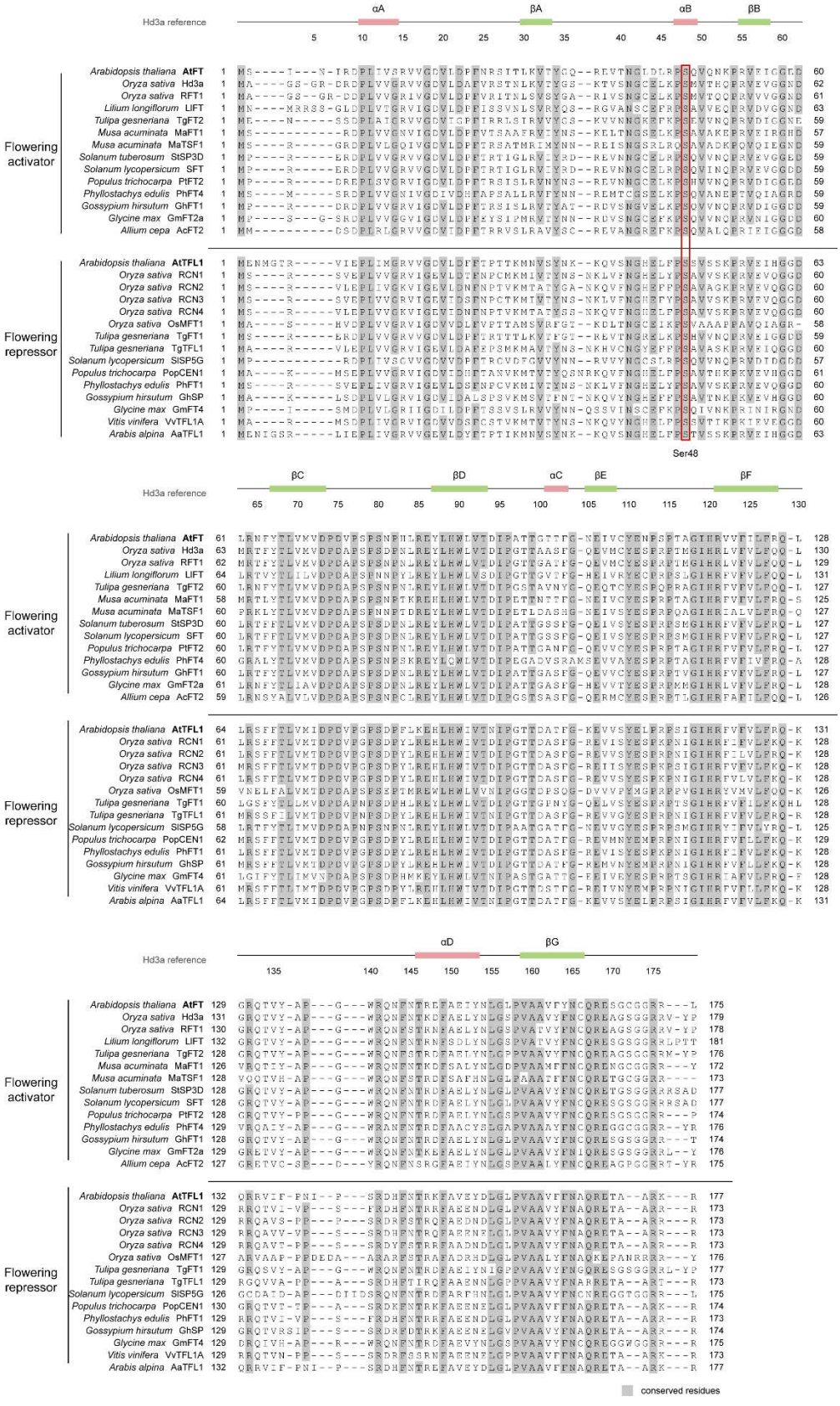
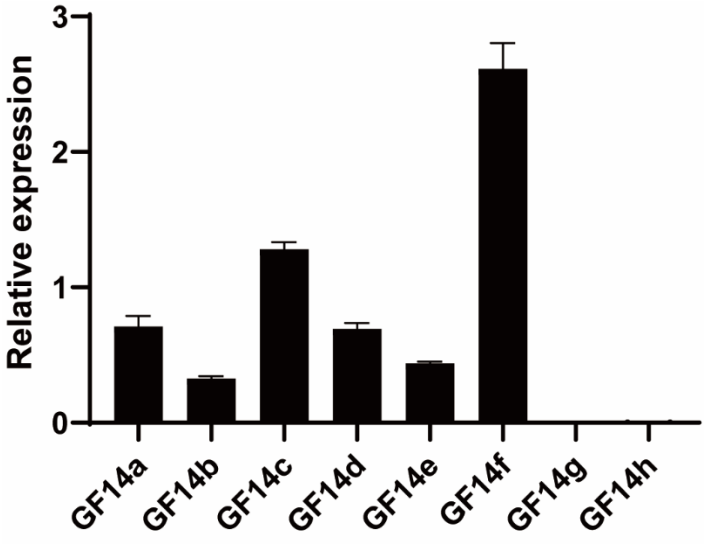
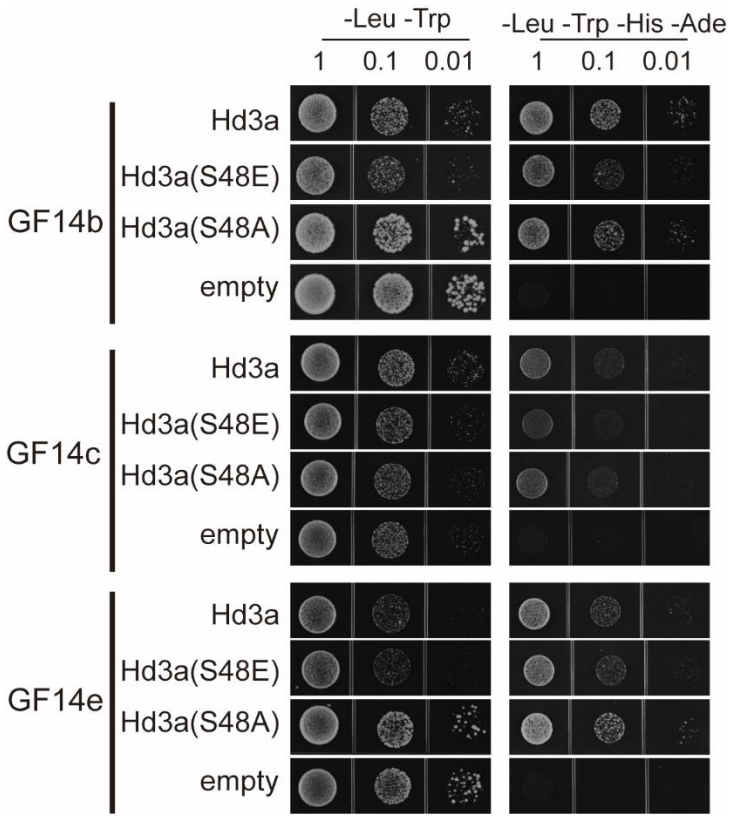


Fig.S1 Sequence alignments of FT homologs.



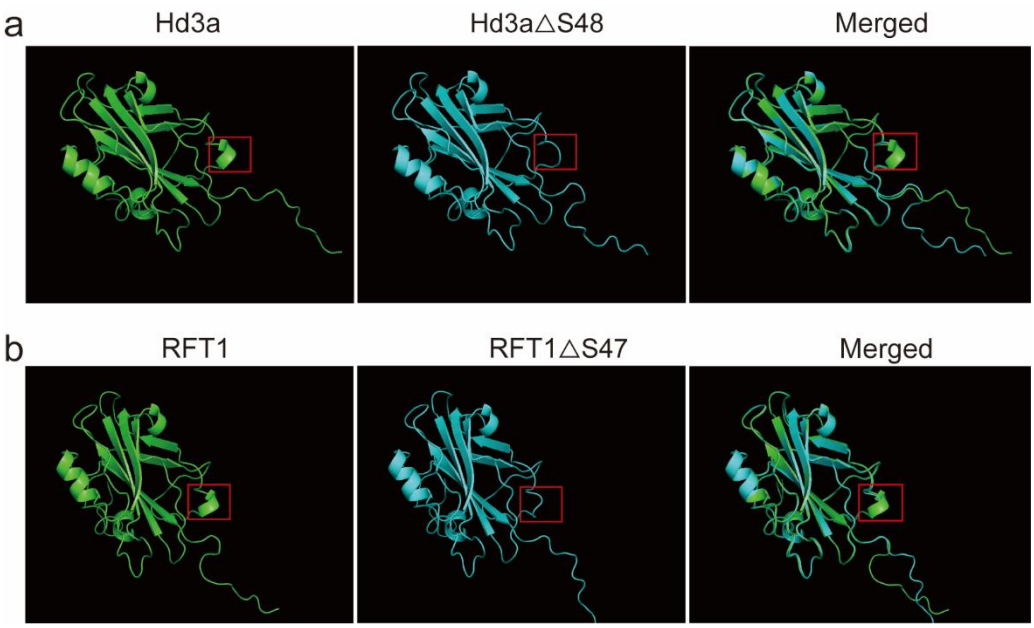
737

738 Fig.S2 RT-qPCR analysis of the expression of *GF14a-h* in the shoot tips from 40-day-
739 old rice plants. *OsUbq* was used as an internal control. Three biological replicates were
740 performed.



741

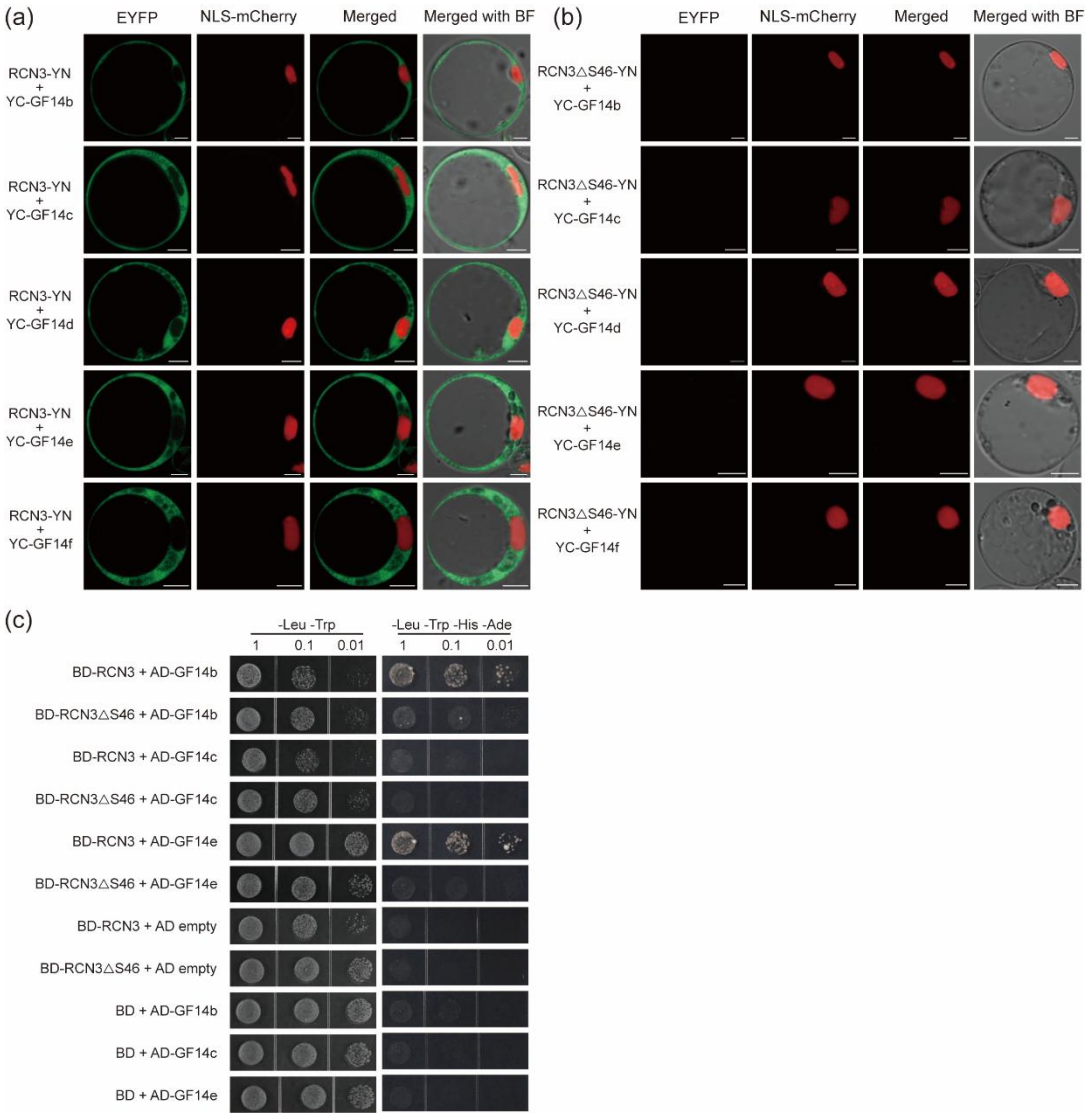
742 Fig.S3 Y2H assay for analyzing the interaction between GF14b/c/e and the
743 Hd3a(S48E)/Hd3a(S48A) mutants.



744

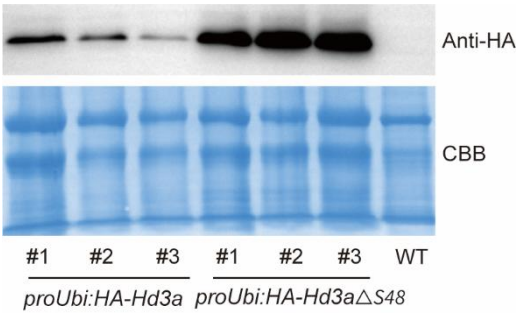
745 Fig.S4 Comparison of the 3D structures of Hd3a and Hd3a△S48 (a), and RFT1 and

746 RFT1△S47 (b), respectively. The protein structures were predicted using AlphaFold3.



747

748 Fig.S5 BiFC and Y2H assays for analyzing the interaction between 14-3-3 proteins and
749 RCN3△S46 mutant. (a) BiFC assay for GF14b-f and RCN3. (b) BiFC assay for GF14b-
750 f and RCN3△S46. (c) Y2H assay for GF14b/c/e and RCN3/ RCN3△S46.



751

752 Fig.S6 Protein expression analysis of the HA-Hd3a and HA-Hd3a △ S48
753 overexpressing plants by western blot. The Coomassie brilliant blue (CBB) stained

proteins were used as protein loading controls.

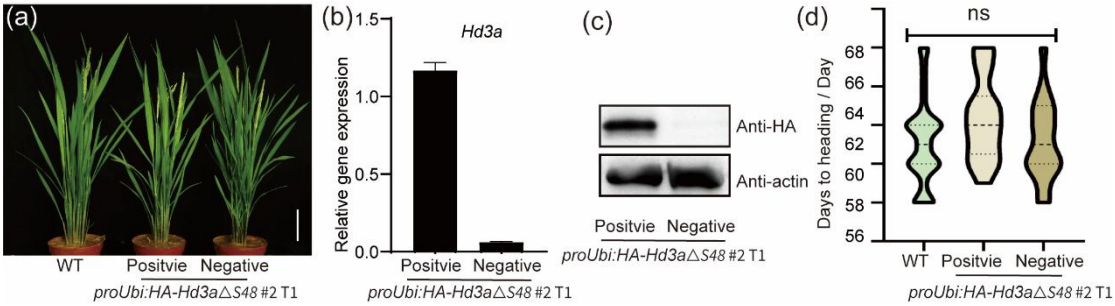
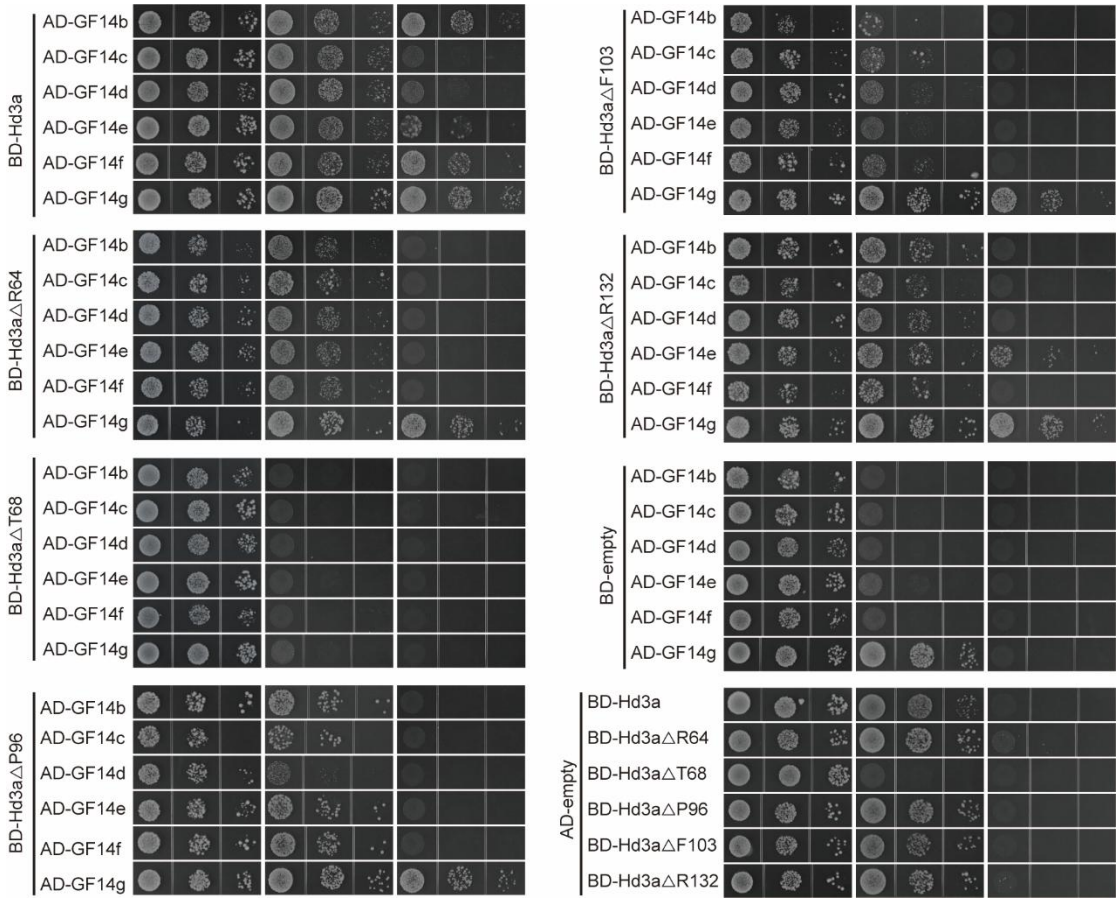


Fig.S7 Characterization of the T_1 progenies of *Hd3a⊿S48* overexpressing plants. (a) Plants at heading stage. (b) The relative gene expression level of the total of *Hd3a⊿S48* and *Hd3a* in the transgenic positive and negative plants. (c) The protein expression level of HA-Hd3a in the transgenic positive and negative plants. HA-Hd3a level was examined by Western blot. The Actin expression levels were used as a protein loading control. (d) Comparison of the heading date of the wild type plants, and the transgenic positive and negative plants.



764

765 Fig.S8 Y2H assay for analyzing the interactions between 14-3-3 proteins and Hd3a

766 mutants.

767

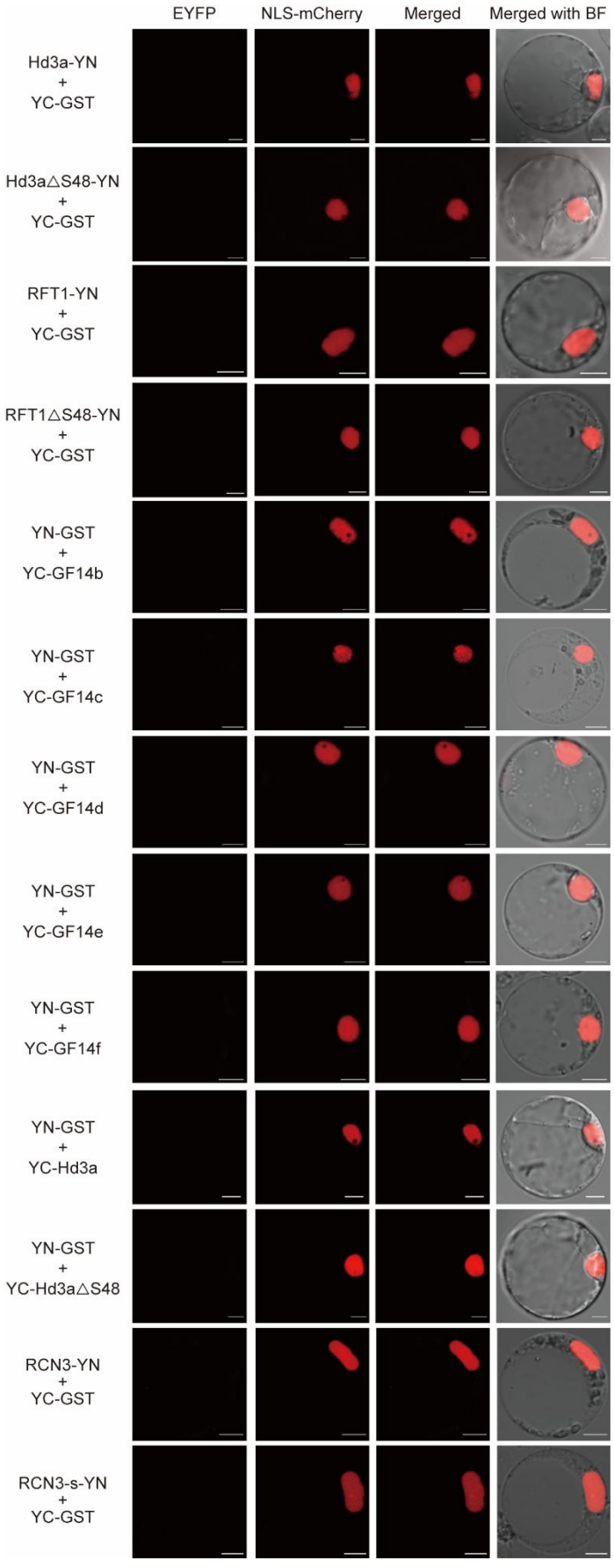


Fig.S9 The negative controls of the BiFC experiments in this study.

Table S1 The genotypes of the *hd3a rft1* double mutants.

Line NO.	*Hd3a* genotype	*RFT1* genotype	Consequences
line-1	-GTCC/-GTCC	-GTC/-GTC(-Ser)	Frame shift
line-2	-GTC(-Ser)/-24BP	-G/-G	Frame shift
line-3	+T/+T	-24BP/-24BP	Frame shift
line-4	-GTCC/-GTCC	+T/-GTCC	Knock out
line-5	-CG/-GT	-GTC/-GTC(-Ser)	Frame shift
line-6	-CG/-G	-T/+T	Knock out
line-7	-GTCC	-GTC/-GTC(-Ser)	Frame shift
line-8	-10BP/-10BP	-24BP/-24BP	Frame shift
line-9	-CG/+T	-CT/-T	Knock out
line-10	-GT/-GT	+T/+T	Knock out
line-11	-24BP/-24BP	-G/-G	Frame shift
line-12	-24BP/-CGT(-Ser)	-G/-G	Frame shift
line-13	-CG/+T	-11BP/-11BP	Knock out
line-14	-24BP/-24BP	-G/-G	Frame shift
line-15	+T/+T	-CCG/-CCGT	Frame shift
line-16	-T/-T	-G/-G	Knock out
line-18	+T/+T	-CCG/-CCGT	Frame shift
line-19	+T/+T	-24BP/-24BP	Frame shift
line-20	+T/+T	-CCG/-CCGT	Frame shift
line-21	-8bp/+T	+T/+T	Knock out
line-22	-9bp/+t	-CG/+T	Frame shift
line-23	-G/+T	+T/+T	Knock out
line-24	-GTCC	-GTC/-GTC(-Ser)	Frame shift
line-25	+A/+T	-G/+6bp	Frame shift
line-26	-CG/-CG	-GTC(-Ser)/+T	Frame shift
line-27	-14BP/-14BP	+T/+T	Knock out
line-28	WT/-12bp	WT/-16BP	Heterozygous mutation
line-29	-4bp/-11bp	WT/-CTCC	Heterozygous mutation
line-30	-GT/-GT	+T/+T	Knock out
line-31	+T/+T	-CCG/-CCGT	Knock out
line-32	-GT/-GT	+T/+T	Knock out
line-33	-T/-T	+C/+T	Knock out
line-34	-T/-T	+C/+T	Knock out
line-35	-T/-T	+C/+T	Knock out
line-36	-T/-T	+C/+T	Knock out
line-37	+T/+T	-G/+T	Knock out
line-38	-GTC(-Ser)/+T	+T/+T	Frame shift
line-39	-8BP/+T	-G/+T	Knock out
line-40	-8BP/+T	-23BP/+T	Knock out
line-41	-GTC(-Ser)/+T	+C/+T	Frame shift
line-42	-18bp/-GT	-G/+T	Frame shift
line-43	-18bp/-GT	-G/+T	Frame shift
line-44	-GTC/-GTC(-Ser)	+C/+T	Frame shift
line-45	-8BP/+T	-23BP/+T	Knock out
line-46	-18BP/-GT	-G/+T	Frame shift
line-47	-GTC/-GTC(-Ser)	+C/+T	Frame shift
line-48	-GTC/-GTC(-Ser)	+C/+T	Frame shift
line-49	-GTC/-GTC(-Ser)	+C/+T	Frame shift
line-50	-GTC/-GTC(-Ser)	+C/+T	Frame shift
line-51	-GTC/-GTC(-Ser)	+C/+T	Frame shift

772 Table S2 The FT homologs used for protein sequence alignments.

Gene name	Gene ID	Database	Species	Function
AtFT	NP_176726.1	NCBI RefSeq	*Arabidopsis thaliana*	flowering activator
Hd3a	BAB61030.1	NCBI Protein	*Oryza sativa*	flowering activator
RFT1	LOC_Os06g06300	RGAP	*Oryza sativa*	flowering activator
LlFT	ATU06687.1	NCBI Protein	*Lilium longiflorum*	flowering activator
TgFT2	ATU06685.1	NCBI Protein	*Tulipa gesneriana*	flowering activator
MaFT1	AGX25183.1	NCBI Protein	*Musa acuminata*	flowering activator
MaTSF1	AIU38043.1	NCBI Protein	*Musa acuminata*	flowering activator
StSP3D	BAV67096.1	NCBI Protein	*Solanum tuberosum*	flowering activator
SFT/SP3D	AAO31792.1	NCBI Protein	*Solanum lycopersicum*	flowering activator
PtFT2	Potri.010G179700.1	Phytozome	*Populus trichocarpa*	flowering activator
PhFT4	Ped12Dg04440.1	BambooBase	*Phyllostachys edulis*	flowering activator
GhFT1	ADK95113.1	NCBI GenBank	*Gossypium hirsutum*	flowering activator
GmFT2a	BAJ33491.1	SoyBase	*Glycine max*	flowering activator
AcFT2	AGZ20208.1	NCBI Protein	*Allium cepa*	flowering activator
AtTFL1	NP_196004.1	NCBI RefSeq	*Arabidopsis thaliana*	flowering repressor
RCN1	LOC_Os11g05470	RGAP	*Oryza sativa*	flowering repressor
RCN2	LOC_Os02g32950	RGAP	*Oryza sativa*	flowering repressor
RCN3	LOC_Os12g05590	RGAP	*Oryza sativa*	flowering repressor
RCN4	LOC_Os04g33570	RGAP	*Oryza sativa*	flowering repressor
OsMFT1	LOC_Os06g30370	RGAP	*Oryza sativa*	flowering repressor
TgFT1	ATU06684.1	NCBI Protein	*Tulipa gesneriana*	flowering repressor
TgTFL1	AQR58151.1	NCBI Protein	*Tulipa gesneriana*	flowering repressor
SlSP5G	Solyc05g053850.2.1	Sol Genomics Network	*Solanum lycopersicum*	flowering repressor
PopCEN1	Potri.004G203900.1	Phytozome	*Populus trichocarpa*	flowering repressor
PhFT1	Ped12Dg02320.1	BambooBase	*Phyllostachys edulis*	flowering repressor
GhSP	Gohir.D07G113500.1.p	CottonGen	*Gossypium hirsutum*	flowering repressor
GmFT4	Glyma08g47810.1	SoyBase	*Glycine max*	flowering repressor
VvTFL1A	AAM46142.1	NCBI Protein	*Vitis vinifera*	flowering repressor
AaTFL1	AEH43348.1	NCBI Protein	*Arabis alpina*	flowering repressor

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774

Table S3 The primers used in this study.

Primer name	Primer sequence	Purpose
OsU6aT1-H/R	TGGACGGCTTGAGCTCGCAGCGGCAGCCAAGCCAGCA	Gene editing
gRT1-H/R	CTGCGAGCTCAAGCCGTCCAGTTTTAGAGCTAGAAAT	
UBI-HA-Hd3a-F	TTTGGTGTTACTTCTGCAGGAGCTCATGGGCTACCCATACGATGTT	Gene overexpression
UBI-HA-Hd3a-R	CCAAATGTTTGAACGATCTCAGGTACCCTAGGGGTAGACCCTCCTGC	
F2A-HA-H/R-F	CAACCCAGGGCCCGTCGACATGGGCTACCCATACGATGTTCCTGACTATG	Gene transiently expression in rice protoplasts
F2A-HA-H/R-R	GACTGGTGATTTTTGCGGACTCTAGACTAGGGGTAGACCCTCCTGCC	
F2A-HA-RFT1-R	GACTGGTGATTTTTGCGGACTCTAGACTAGGGGTAGACCCTCCTGCC	
PUC-HA-H/R-F	TGGCGCGCCACTAGTGGATCCATGGGCTACCCATACGATGTTC	
PUC-HA-H/R-R	CGATCGGGGAAATTCGAGCTCCTAGGGGTAGACCCTCCTGCC	
PUC-4myc-FD1-F	TGGCGCGCCACTAGTGGATCCATGGAACAAAAACTAATATCAGAGGAAG	
PUC-4myc-FD1-R	CGATCGGGGAAATTCGAGCTCTCAGAATGGCGCGGAGAGCAC	
puc-Hd3a-YN-F	CACGGGGGACTCTAGAATGGCCGGAAGTGGCAGG	BiFC assay
puc-Hd3a-YN-R	TCCATCCCGGGAGCGGTACCGGGGTAGACCCTCCTGCC	
PUC-RFT1-YN-F	GGAGAGAACACGGGGGACTCTAGAATGGCCGGCAGCGGCAGG	
PUC-RFT1-YN-R	TGCTCCATCCCGGGAGCGGTACCGGGGTAGACCCTCCTGCCG	
PUC-RCN3-YN-F	GAGAACACGGGGGACTCTAGAATGTCTAGGTCTGTGGAGCCTCTT	
PUC-RCN3-YN-R	CTCCATCCCGGGAGCGGTACCGCGTCTCCTGGCAGCAGTC	
PUC-YN-GST-F	GTCTATATCATGGCCGAGCTCATGTCCCCTATACTAGGTTATTGG	
PUC-YN-GST-R	CGATCGGGGAAATTCGAGCTCTCATTTTGGAGGATGGTCGCCA	
PUC-YC-GST-F	GACGAGCTGTACAAGGAGCTCATGTCCCCTATACTAGGTTATTGG	
PUC-YC-GST-R	CGATCGGGGAAATTCGAGCTCTCATTTTGGAGGATGGTCGCCA	
PUC-YC-GF14b-F	TCGGCATGGACGAGCTGTACAAGGAGCTCATGTCGGCACAGGCGGAG	
PUC-YC-GF14b-R	CGATCGGGGAAATTCGAGCTCTTACTGCCCCTCGCTGGAG	
PUC-YC-GF14C-F	GACGAGCTGTACAAGGAGCTCATGTCTCGGGAGGAGAATGTCTAC	
PUC-YC-GF14C-R2	CGATCGGGGAAATTCGAGCTCTCAGTCGACCTGGCCCTCGCA	
PUC-YC-GF14D-F	GACGAGCTGTACAAGGAGCTCATGTCGCCGGCGGAGCCG	
PUC-YC-GF14D-R	CGATCGGGGAAATTCGAGCTCTCAGTCGACCTGATCCCCAGGC	
PUC-YC-GF14e-F	GACGAGCTGTACAAGGAGCTCATGTCGCAGCCTGCTGAGC	
PUC-YC-GF14e-R	CGATCGGGGAAATTCGAGCTCTCACTGTCCATCTCCTGATTCGCCCT	
PUC-YC-GF14f-F	GACGAGCTGTACAAGGAGCTCATGTCGCCTGCTGAGGCA	
PUC-YC-GF14f-R	GACGAGCTGTACAAGGAGCTCATGTCGCAGCCTGCTGAGC	
PUC-YC-FD1-F	GCATGGACGAGCTGTACAAGGAGCTCATGGCGATGGAGGACGACG	
PUC-YC-FD1-R	CGATCGGGGAAATTCGAGCTCTCAGAATGGCGCGGAGAG	
BD-Hd3a-F	ATGGCCATGGAGGCCGAATTCATGGCCGGAAGTGGCAGG	Y2H assay
BD-Hd3a-R	ATGCGGCCGCTGCAGGTCGACCTAGGGGTAGACCCTCCTGCC	
BD-RFT1-F	ATGGCCATGGAGGCCGAATTCATGGCCGGCAGCGGCAGG	
BD-RFT1-R	ATGCGGCCGCTGCAGGTCGACCTAGGGGTAGACCCTCCTGCC	
BD-RCN3-F	ATGGCCATGGAGGCCGAATTCATGTCTAGGTCTGTGGAGCCTCTT	
BD-RCN3-R	CCGCTGCAGGTCGACGGATCCTTAGCGTCTCCTGGCAGCA	
AD-GF14b-F	GCCATGGAGGCCAGTGAATTCATGTCGGCACAGGCGGAG	
AD-GF14b-R	CAGCTCGAGCTCGATGGATCCTTACTGCCCCTCGCTGGAG	
AD-GF14C-F	GCCATGGAGGCCAGTGAATTCATGTCTCGGGAGGAGAATGTCTAC	
AD-GF14C-R	CAGCTCGAGCTCGATGGATCCTTACTGGCCCTCGCAGGC	
GST-Hd3a-F	TGATCGAAGGTCGTGGGATCCCCATGGCCGGAAGTGGCAGG	Protein expression in E.coli.
GST-Hd3a-R	TCAGTCAGTCACGATGAATTCCTAGGGGTAGACCCTCCTGCC	
GST-RFT1-F	TGATCGAAGGTCGTGGGATCCCCATGGCCGGCAGCGGCAGG	
GST-RFT1-R	TCAGTCAGTCACGATGAATTCCTAGGGGTAGACCCTCCTGCC	
His-GF14b-F	CAGCACATGGACAGCCCAGATCTGATGTCGGCACAGGCGGA	
His-GF14b-R	GGGGTTATGCTAGTTATTGCTCAGCTTACTGCCCTCGCTGGAG	
His-GF14o-F	CAGCACATGGACAGCCCAGATCTGATGTCTCGGGAGGAGAATGT	
His-GF14o-R	GGGGTTATGCTAGTTATTGCTCAGCTTACTGGCCCTCGCAGGCGT	
His-GF14e-F	CAGCACATGGACAGCCCAGATCTGATGTCGCAGCCTGCTGA	
His-GF14e-R	GGGGTTATGCTAGTTATTGCTCAGCTCACTGTCCATCTCCTGAT	
Hd3a-EGFP-F	GATAGTACTGTCGACCTCGAGATGGCCGGAAGTGGCAGG	Protein subcellular localization
RFT1-EGFP-F	ATCGATAGTACTGTCGACCTCGAGATGGCCGGCAGCGGCAG	
H/R-EGFP-R	CTCGCCCTTGCTCACGGATCCGGGGTAGACCCTCCTGCCG	
H-R64-F	TCGGCGGCAATGACATGACATTCTACAC	
H-R64-R	AATGTCATGTCATTGCCGCCGACC	
H-T68-F	CATGAGGACATTCTACCTTGTGATG	
H-T68-R	AAGGTAGAATGTCCTCATGTCA	
H-P96-F	GTTGGTCACTGATATTGGTACTACTGCA	
H-P96-R	TACCAATATCAGTGACCAACCAA	
H-F103-F	ACTACTGCAGCGTCAGGGCAAGAGGTGA	
H-F103-R	TTGCCCTGACGCTGCAGTAGTAC	
H-R132-F	TTCCAGCAGCTGGGGCAGACAGTGTAC	
H-R132-R	TGTCTGCCCCAGCTGCTGGAACA	
RT-Hd3a-F-1	CCTATGGCTCCAAGACCGTG	Gene expression analysis
RT-Hd3a-R-1	GCTTGGTGCATCTGGGTCTA	
RT-RFT1-F	ATGGTGCAAGGATCGTCTCC	
RT-RFT1-R	GCATCCGGGTCTACCATCAC	
RT-OsFD1-F	CAACAACCTGGAGACCGAGG	
RT-OsFD1-R	TCAGCTGCTCGTACTTGACG	
RT-UBI-F	GCCCAAGAAGAAGATCAAGAAC	
RT-UBI-R	AGATAACAACGGAAGCATAAAAGTC	
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RT-OsMADS14-R	GAAAGACGGTGCTGGACGAA	
RT-OsMADS15-F	CGTCGTCGGCCAAACAG	
RT-OsMADS15-R	TGACTTCAATTCATTCAAGGTTGCT	
RT-MADS62-F	AGGACCGCCTTAGGATGTTCG	
RT-MADS62-R	GACTCGGGTCAGCAATTCCAT	
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RT-PP2C1-R	TGTGCTCCACTGAACTTGGT	
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RT-Os02g0219800-R	TCAGTGACGAACACCGACAA	
RT-GF14a-F1	TACGAGATCCTCAACTCGCC	
RT-GF14a-R1	GGCTTGGTTGCATCCCTCAT	
RT-GF14b-F1	ATCCTCAACTCGCCTGATCG	
RT-GF14b-R1	TCGGAGCTTCCCTGATCTCT	
RT-GF14o-F1	TGCCGAGAGAAAGGAAGCTG	
RT-GF14o-R1	GAGATGGCTTCGTCAAACGC	
RT-GF14d-F1	TCTTGAACTCCCCTGACCGT	
RT-GF14d-R1	ATCCCCAGGCTCTTTTGGAG	
RT-GF14e-F1	CTCCCACCTTGTGCCTTCAT	
RT-GF14e-R1	GCCAGCCCAAGTCTGATAGG	
RT-GF14f-F1	GGCTTGGACTTGCACTGAAC	
RT-GF14f-R1	TCACCACCATCCTCCGCATT	
RT-GF14g-F1	CCGGTACCTTGCAGAGTTCA	
RT-GF14g-R1	CAGAGCAGGCAAGGAATGAGA	
RT-GF14h-F1	GCCTTGCGCTCAACTTTTCA	
RT-GF14h-R1	TCCACAATGCCAGGTTCTCC	