

# **Liquid–liquid phase separation of florigen activation complex induces flowering**

Ken-ichiro Taoka<sup>1†\*</sup>, Hiroyuki Tsuji<sup>1†\*</sup>, Suai Anzawa<sup>2</sup>, Mayu Enomoto<sup>2</sup>, Yuka Koizumi<sup>2</sup>, Juri Nakamura<sup>1</sup>, Mari Tanaka<sup>1</sup>, Akiko Fujita<sup>3</sup>, Kyoko Furuita<sup>4</sup>, Takashi S. Kodama<sup>4</sup>, Masaru Fujimoto<sup>5</sup>, Kazuo Kurokawa<sup>6</sup>, Takashi Okamoto<sup>7</sup>, Toshimichi Fujiwara<sup>4</sup>, Akihiko Nakano<sup>6</sup>, Chojiro Kojima<sup>2, 4\*</sup>

<sup>1</sup>Kihara Institute for Biological Research, Yokohama City University, Yokohama, Japan

<sup>2</sup>Graduate School of Engineering Science, Yokohama National University, Yokohama, Japan

<sup>3</sup>Graduate School of Biological Sciences, Nara Institute of Science and Technology, Nara, Japan

<sup>4</sup>Institute for Protein Research, Osaka University, Suita, Osaka, Japan

<sup>5</sup>Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo, Japan

<sup>6</sup>Live Cell Super-Resolution Imaging Research Team, RIKEN Center for Advanced Photonics, Wako, Saitama, Japan

<sup>7</sup>Department of Biological Sciences, Tokyo Metropolitan University, Minami-osawa, Hachioji, Tokyo, Japan

<sup>†</sup>These authors contributed equally to this work.

\*Corresponding authors: ktaoka@yokohama-cu.ac.jp, tsujih@yokohama-cu.ac.jp, kojima-chojiro-xk@ynu.ac.jp

## 25 Abstract

26 Floral transition, regulated by the systemic action of the mobile florigen protein  
 27 FLOWERING LOCUS T (FT), is essential for successful plant reproduction<sup>1</sup>. How FT  
 28 controls downstream gene expression remains incompletely understood, although it relies on  
 29 the florigen activation complex (FAC), a core component of FT function<sup>2-4</sup>. The FAC is a  
 30 nucleus-localized transcriptional activator of genes encoding MADS-box transcription factors  
 31 critical to reproductive development and consists of florigen FT; a scaffold 14-3-3 protein  
 32 that is a key component for complex assembly; and FD, a basic leucine-zipper protein that  
 33 recruits the FAC to DNA. Here we report that the FAC exhibits phase separation. In rice  
 34 shoot apical meristem cells, rice florigen Heading date 3a (Hd3a) fused to the green  
 35 fluorescent protein formed speckles in the nucleus. The FAC speckle is formed in a FAC-  
 36 dependent manner in tobacco cells. Recombinant Hd3a, but not OsFD1, phase-separated in  
 37 vitro, and this effect was enhanced in the presence of 14-3-3 protein. Furthermore, mutations  
 38 affecting functionally important residues in the pocket region or C-terminal disordered region  
 39 of Hd3a affected FAC phase separation, providing a biochemical framework for the protein's  
 40 effect on flowering. The ability to form condensates via phase transition represents a  
 41 previously unknown mechanism for gene activation by the FAC.

## 43 Main

44 Florigen is a systemic signal that induces flowering<sup>5,6</sup> whose molecular identity was  
 45 elucidated with the cloning of Arabidopsis (*Arabidopsis thaliana*) FLOWERING LOCUS T  
 46 (FT) and orthologues from other plant species, such as HEADING DATE 3A (Hd3a) in rice  
 47 (*Oryza sativa*)<sup>1,7,8</sup>. FT, Hd3a and orthologues are expressed and translated in leaves and then  
 48 transported to the shoot apical meristem (SAM) responsible for the production of all above-  
 49 ground tissue<sup>7,9,10</sup>. Next, the SAM is converted from a leaf-forming vegetative meristem to a

flower-forming reproductive meristem through the formation of the florigen activation complex (FAC), comprising the florigen itself, the florigen receptor 14-3-3 protein and the basic leucine zipper (bZIP) transcription factor FD<sup>2-4,11,12</sup>. Although great strides have been made in understanding florigen function, the biochemical mechanisms by which the FAC activates downstream gene expression are not well understood. As a first step toward elucidating these, we determined the subcellular localization of the FAC. We performed super-resolution imaging microscopy by stimulated emission depletion (STED)<sup>13,14</sup> of cells in the rice SAM. Because Hd3a is expressed in the phloem of vascular bundles<sup>7,15,16</sup>, we generated rice plants expressing *green fluorescent protein (GFP)* or *Hd3a-GFP* driven by the *rolC* promoter from *Agrobacterium rhizogenes*, which is highly active in the phloem<sup>7,17,18</sup>. The resulting translated GFP and Hd3a-GFP were transported to the SAM, where they accumulated in cell nuclei (Fig. 1a,b). As a control for STED, we determined the localization of histone H2B fused to GFP (H2B-GFP)<sup>19</sup> in the nucleus of SAM cells, revealing punctate signals and exclusion from the nucleolus indicative of the nucleosome (Fig. 1c). Free GFP showed a faint signal in the cytoplasm and was uniformly distributed in the nucleus, whereas Hd3a-GFP formed speckle-like structures in the nucleus (Fig. 1d,e). To confirm the formation of Hd3a-containing speckles, we visualized GFP fluorescence from the nuclei of SAM cells of transgenic plants harbouring the *Hd3apro:Hd3a-GFP* transgene<sup>7</sup> by super-resolution confocal live imaging microscopy (SCLIM)<sup>20,21</sup>, a super-resolution imaging technique based on principles distinct from those underlying STED. However, as observed by STED, Hd3a-GFP defined a speckle-like structure in the nuclei of rice SAM cells (Fig. 1f,g,h).

Cellular speckle-like structures have recently been proposed to constitute biomolecular condensates, i.e., phase separation<sup>22-27</sup>. To assess whether the observed speckle-like Hd3a

75 structures are caused by liquid condensates, we purified recombinant Hd3a and its paralogue  
 76 rice FT1 (RFT1) and examined their ability to form condensates in vitro. Indeed, both  
 77 recombinant Hd3a and RFT1 proteins appeared to form condensates, with a diameter of  
 78 about 10  $\mu\text{m}$ , in vitro in solutions containing polyethylene glycol (PEG) (Fig. 1i,j). To  
 79 characterize these condensates, we measured their absorbance in the range of 330–800 nm, as  
 80 absorbance over this range indicates the degree of condensation and reflects the size of the  
 81 dispersed condensates in solution: larger condensates show a constant absorbance, as  
 82 illustrated with glass beads of different diameters in solution (Extended Data Fig. 1). Hd3a  
 83 showed a constant absorbance over the measurement range (Fig. 1k) that was reminiscent of  
 84 the pattern seen for large-size glass beads, suggesting the presence of large condensates  
 85 containing Hd3a and RFT1 (Extended Data Fig. 2). This result was consistent with  
 86 microscopic observations of the larger condensates with roughly 10- $\mu\text{m}$  condensates.  
 87 Furthermore, recombinant Hd3a and RFT1 formed spherical condensates rather than  
 88 aggregated precipitates (Fig. 1i,j), indicating that Hd3a and RFT1 undergo liquid phase  
 89 separation in vitro.

90

91 Transient expression of *Hd3a* and *OsFD1* in rice protoplasts reconstituted the FAC by  
 92 complexing with endogenous 14-3-3 proteins, leading to the activation of one of its  
 93 transcriptional targets, *OsMADS15*, which encodes a MADS-box transcription factor<sup>2,28</sup> (Fig.  
 94 2a). FAC reconstitution has also been reported from the transient expression of Arabidopsis  
 95 *FT* and *FD* or rice *FD4* and *RFT1* in *N. benthamiana* leaves<sup>11,29</sup>. To investigate whether  
 96 nuclear speckles form upon reconstitution of the FAC, we transiently expressed *Hd3a-GFP*  
 97 and *OsFD1* in *N. benthamiana* leaves and determined the green fluorescence pattern  
 98 detectable in their nuclei. The mean number of speckles per nucleus was small (less than 0.5)  
 99 when *Hd3a* alone or *OsFD1* alone was expressed, while nuclei contained an average of three

100 speckles upon co-expression of *Hd3a* and *OsFD1* (Fig. 2b,c). This observation suggests that  
101 FAC formation entails the localization of Hd3a into nuclear speckles.

102

103 To characterize whether these nuclear speckles formation is explained by condensate  
104 formation in vitro, we measured the absorbance of recombinant Hd3a, 14-3-3 protein GF14c<sup>2</sup>  
105 and OsFD1 alone or in mixtures over the range of 330–800 nm. We were unable to prepare a  
106 large amount of full-length OsFD1, but we succeeded in preparing a C-terminal truncated  
107 version of OsFD1 that retains the coiled-coil region. Hd3a showed constant absorbance over  
108 the measurement range, whereas 14-3-3 and OsFD1 showed little absorbance on their own  
109 (Fig. 2d,e). To determine the effect of 14-3-3 protein on condensate formation by Hd3a or  
110 OsFD1, we added 14-3-3 protein to recombinant Hd3a or OsFD1 and measured the  
111 absorbance of the resulting mixtures. The absorbance was higher when 14-3-3 was included  
112 than with recombinant Hd3a or OsFD1 protein alone (Fig. 2d,e). We next added recombinant  
113 14-3-3 protein to a mixture of recombinant Hd3a and OsFD1 proteins and measured the  
114 absorbance of the resulting solution (Fig. 2e), finding that the mixture displayed a constant  
115 absorbance of about 0.2. The absorbance increased considerably when recombinant 14-3-3  
116 protein was added to the mixture (Fig. 2e). Increasing ratio of Hd3a against 14-3-3 resulted in  
117 higher absorbance (Extended Data Fig.3). These results suggest that the formation of the  
118 three-protein FAC promotes the appearance of condensates in vitro.

119

120 To better understand FAC function, we examined whether the interaction between Hd3a and  
121 14-3-3 is required to form nuclear speckles. Accordingly, we introduced mutations in Hd3a  
122 that abolish its interaction with 14-3-3 proteins. The phenylalanine 103 (F103), arginine 64  
123 (R64) and arginine 132 (R132) residues of Hd3a form a hydrogen bond that is essential for  
124 interaction with 14-3-3 proteins, and their mutation to create Hd3a<sup>F103A</sup> or Hd3a<sup>R64K,R132K</sup>

125 mutants abolished the interaction between Hd3a and 14-3-3 interaction and resulted in a loss  
126 of FAC transcriptional activity<sup>2</sup> (Fig. 2a). Neither *Hd3a*<sup>F103A</sup>-GFP nor *Hd3a*<sup>R64K,R132K</sup>-GFP  
127 led to the formation of nuclear speckles when transiently expressed in *N. benthamiana* leaves  
128 (Fig. 3a). To investigate whether the interaction between Hd3a and 14-3-3 is necessary for  
129 condensate formation, we performed an in vitro assay in which we added recombinant 14-3-3  
130 protein to mutant recombinant Hd3a and measured the absorbance of the solutions. The  
131 absorbance of recombinant Hd3a<sup>F103A</sup> and Hd3a<sup>R64K,R132K</sup> in the presence of recombinant 14-  
132 3-3 protein was lower than that of intact Hd3a with 14-3-3 (Fig. 3b,c). These results indicate  
133 that the interaction between Hd3a and 14-3-3 is a prerequisite for condensate formation.

134

135 The family of phosphatidylethanolamine-binding proteins (PEBP), to which florigen belongs,  
136 is characterized by an anion-binding pocket that has been suggested to play an important role  
137 in the function of florigen<sup>30,31</sup>. The tyrosine 87 (Y87) residue is located deep in this potential  
138 ligand-binding pocket on the surface of florigen and is conserved across all FT orthologues,  
139 but is replaced by histidine in TERMINAL FLOWER 1 (TFL1) and its orthologues, floral  
140 repressors that are closely related to FT<sup>32</sup>. To explore the potential link between the pocket  
141 and condensate formation, we characterized a mutant version of Hd3a harbouring histidine  
142 instead of tyrosine at residue 87 (Y87H). Hd3a<sup>Y87H</sup> failed to activate *OsMADS15* expression  
143 (Fig. 2a) but retained the ability to form nuclear speckles (Fig. 4a,b) and condensates in vitro  
144 (Fig. 4c). These results indicate that the Y87H mutation in the pocket region causes loss of  
145 transcriptional activation by the FAC without affecting nuclear condensate formation.

146

147 We also examined the link between FAC function and condensate formation for other known  
148 loss-of-function mutations, namely those corresponding to the *Arabidopsis* *ft-1* and *ft-3*  
149 alleles. The FT protein in the *ft-1* mutant contains two consecutive glycine residues (G) in the

150 C-terminal disordered region, one of which is mutated to glutamic acid (E)<sup>15,16,33,34</sup>. A  
 151 transient expression assay revealed that the equivalent mutant in rice Hd3a is G173E, as it  
 152 failed to activate *OsMADS15* transcription (Fig. 2a). We next examined whether Hd3a<sup>G173E</sup>  
 153 can form nuclear speckles. We discovered that Hd3a<sup>G173E</sup> could not form nuclear speckles  
 154 when transiently expressed in *N. benthamiana* leaf cells (Fig. 4a,b). Likewise, recombinant  
 155 Hd3a<sup>G173E</sup> suppressed condensate formation when incubated with recombinant 14-3-3 protein  
 156 in vitro (Fig. 4d). Since the G173 residue is located in the C-terminal disordered region of  
 157 Hd3a<sup>2,34</sup>, this substitution does not affect the overall structure. Thus, the behaviour of the  
 158 Hd3a<sup>G173E</sup> mutant underscores the importance of the C-terminal disordered region following  
 159 FAC formation.

160

161 The Arabidopsis *ft-3* allele is equivalent to a mutant of Hd3a in which arginine 121 is  
 162 replaced by histidine (R121H)<sup>15,16,33</sup>. The R121 residue is located internally within the Hd3a  
 163 structure. Hd3a<sup>R121H</sup> can interact with 14-3-3 proteins<sup>2</sup> but appeared to have partially lost its  
 164 transcriptional activation potential (Fig. 2a). When we transiently expressed Hd3a<sup>R121H</sup> in *N.*  
 165 *benthamiana* leaf cells, we observed that it could not form nuclear speckles (Fig. 4a), and it  
 166 inhibited condensate formation in the presence of 14-3-3 protein to a greater extent than  
 167 intact Hd3a in vitro (Fig. 4e). Since R121 is embedded in the Hd3a molecule<sup>2,34</sup>, we  
 168 investigated whether R121H disrupted the structure of Hd3a. Indeed, the structure of  
 169 Hd3a<sup>R121H</sup> appeared to be different from WT, since the nuclear magnetic resonance (NMR)  
 170 spectrum of Hd3a<sup>R121H</sup> was different from WT (Extended Data Fig. 4). These results suggest  
 171 that R121H is a mutation that prevents condensate formation by changing the protein  
 172 structure of Hd3a while maintaining its interaction surface with 14-3-3 proteins.

173

174 Collectively, our results suggest that the FAC exerts its transcriptional activation through a

175 mechanism that involves nuclear speckles and condensation and that depends on the  
176 conserved R121 residue and the C-terminal disordered region of Hd3a (to which the G173  
177 residue maps).

178

179 Although the mechanism of condensate formation by florigen is currently unknown, we  
180 hypothesize that it involves a multivalent interaction such as charge interactions between  
181 FAC components. For example, the surface of florigen is basic, while that of 14-3-3 is  
182 acidic<sup>2,35</sup>. The electrostatic interactions based on these differences may induce a multivalent  
183 interaction between florigen and 14-3-3 to promote condensate formation. In addition, the  
184 phosphorylated SAP motif of FD may help bridge multiple 14-3-3 dimers to facilitate  
185 condensate formation through multivalent interactions.

186

187 The results of this study suggest that condensate formation serves as a mechanism by which  
188 the FAC activates downstream gene expression (Fig. 4f). Interaction between Hd3a and FD  
189 mediated by 14-3-3 protein is essential for condensate formation, and the Hd3a C-terminal  
190 disordered region containing the G173 residue promotes this process after FAC assembly.  
191 This multi-layered regulation along with condensate formation contributes to FAC-mediated  
192 transcriptional activation.

193

194 Phase-separated condensation of FAC may provide a cooperative switching mechanism for  
195 gene expression related to flowering. The phase-separated condensation of FAC occurs when  
196 all required proteins are simultaneously present in high concentrations in a cell: for FAC,  
197 Hd3a, 14-3-3 and OsFD1. In rice SAMs, 14-3-3 and OsFD1 accumulate in high  
198 concentrations in the cells during the vegetative phase<sup>36</sup>. Therefore, the phase-separated  
199 condensation of FAC may provide a cooperative switching mechanism whereby Hd3a is

transported into the SAM and its concentration is increased, and the phase-separated condensation of FAC and transcriptional activation occur only when the concentrations of Hd3a, 14-3-3 and OsFD1 are sufficiently high.

## Methods

### *Plant materials and growth conditions*

Rice (*Oryza sativa* L. subspecies *japonica*) variety Nipponbare was used as wild type. *Hd3apro:Hd3a-GFP*, *rolCpro:Hd3a-GFP*, *rolCpro:GFP* and *Ubqpro:H2B-GFP* transgenic rice plants were described previously<sup>7,19</sup>. Transgenic rice cell lines derived from the *OsMADS15-nanoLuc* gene targeting line (*M15NL-KI*) were described previously<sup>28</sup>. Transgenic rice plants were generated using *Agrobacterium* (*Agrobacterium tumefaciens*)-mediated transformation of rice calli, as previously described, and hygromycin-resistant plants were regenerated from the transformed calli<sup>7</sup>. Plants were grown in growth chambers at 70% humidity, under short-day conditions with daily cycles of 10 h of light at 28 °C and 14 h of darkness at 25 °C. Light was provided by white fluorescent tubes (400–700 nm, 100  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ). Cells from rice suspension cultures were maintained as described previously. *Nicotiana benthamiana* plants were grown under long-day conditions with daily cycles of 16 h of light at 22 °C and 8 h of darkness at 20 °C. Light was provided by white fluorescent tubes (400–700 nm, 100  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ).

### *Plasmid construction*

To make an expression vector in rice protoplast, a 2005-bp fragment of maize ubiquitin promoter and a 252-bp fragment of NOS terminator were amplified by PCR. A 1784-bp of Gateway recombination site were obtained by digestion of pGWB26 vector<sup>36</sup> with restriction enzymes, XbaI and SacI. These three fragments were inserted into pGreen II plasmid<sup>37</sup> to

make the expression vector, pGIIpUbqGWT7Ct. Mutations in Hd3a were introduced by PCR mutagenesis with a designed primer set. The resultant Hd3a mutants were cloned into pENTR-D-TOPO (Thermo Fisher Scientific) and introduced into pGIIpUbqGWT7Ct with LR clonase II (Thermo Fisher Scientific). For OsFD1 expression, pUbq-OsFD1<sup>2</sup> was used. For Agroinfiltration in tobacco, pGWB602 or pGWB605<sup>38</sup> were used for the destination vectors.

### ***Agroinfiltration***

Agroinfiltration was performed as described previously<sup>39</sup>. The Agrobacterium strains EHA105 and MP90 were used for transformation of pGWB602 or pGWB605 derivatives<sup>38</sup> and pBIC p19<sup>40</sup>, respectively. pBIC p19 harbours the silencing suppressor p19 from tomato bushy stunt virus (TBSV). After overnight growth in LB medium, agrobacteria were collected by centrifugation and resuspended to an OD<sub>600</sub> = ~0.6 in infiltration buffer (2 mg ml<sup>-1</sup> MgCl<sub>2</sub>·6H<sub>2</sub>O, 150 μM acetosyringone, 10 mg ml<sup>-1</sup> MES-KOH, pH 5.6). The appropriate combinations of cell suspensions were mixed in equal volumes and infiltrated into *N. benthamiana* leaves 4–5 weeks after germination with a 1-mL syringe. The cells were fixed with 4 % paraformaldehyde for 5-6 days after infiltration and observed within a month.

### ***Imaging by confocal laser scanning microscopy***

The shoot apical meristems (SAMs) from transgenic rice plants were dissected under a microscope<sup>41</sup>. For imaging by stimulated emission depletion (STED), SAMs were fixed by 4% paraformaldehyde and cleared with ClearSee, stained by SCRI SR2200 for cell wall staining and visualized using a confocal laser-scanning microscope (TCS SP8; Leica Microsystems, Tokyo, Japan) equipped with 592-nm STED laser, a 405-nm laser and a pulsed white-light laser (WLL) line and 93· oil-immersion objective lens. Samples were

excited with the 488-nm wavelength of the WLL (80 MHz) and depleted with the 592-nm STED laser. Collected images were deconvolved using the default settings of the STED module in Huygens Professional Deconvolution software. For imaging by super-resolution confocal live imaging microscopy (SCLIM), SAMs were dissected and visualized without fixation. GFP was excited with the 473-nm laser. The fluorescence emission spectra were separated with the custom-made dichroic mirror and filtered through a 490-545 bandpass filter. Images were acquired with an ImagEM EM-CCD camera (Hamamatsu Photonics). High-resolution images were constructed via deconvolution analysis performed with Volocity (PerkinElmer). A three-dimensional SCLIM image was constructed from 40 images taken at 0.1- $\mu$ m vertical intervals using a theoretical point-spread function optimized for CSU10 confocal microscopes (Yokogawa Electric). *N. benthamiana* epidermal leaf cells were visualized with a confocal laser-scanning microscope (LSM 880; Zeiss, Tokyo, Japan) equipped with a 488-nm source and a 63 $\times$  glycerol-immersion objective lens. For GFP fluorescence, images were captured at 500–600 nm after excitation at 488 nm. After image acquisition, the images were processed using Zen software (Zeiss).

265

### 266 ***Recombinant protein production and purification***

The coding sequence of *Hd3a* and RFT1 were cloned into the pCold-GST vector<sup>42</sup> and produced as a glutathione S-transferase (GST) fusion protein in *Escherichia coli* BL21 Rosetta (DE3) (Novagen). Cells were grown in LB medium or minimal medium containing 0.5 g l<sup>-1</sup> of <sup>15</sup>N-ammonium chloride. Recombinant GST fusion protein was purified with glutathione Sepharose 4B resin (Cytiva). After removal of the GST tag using GST-HRV 3C protease, *Hd3a* was purified by gel filtration chromatography using a Superdex75 column (Cytiva) with 50 mM potassium phosphate buffer (pH 6.8) containing 50 mM KCl and 1 mM dithiothreitol (DTT). To prepare *Hd3a* point mutants, plasmids were constructed by PCR

using KOD plus DNA polymerase (TOYOBO) and the resulting recombinant proteins were purified essentially as described above.

The coding sequence of the 14-3-3 gene *GF14c*<sup>43</sup> was cloned into the pCold-GST vector. GF14c was produced as a GST fusion protein in *E. coli* BL21 Rosetta (DE3) and purified with Glutathione Sepharose 4B resin and a Superdex 200 column (Cytiva), followed by removal of the GST tag.

The coding sequence of *OsFD1*<sup>2</sup> was cloned into the pCold-GST vector. We could not purify the large amount of recombinant full-length protein. We therefore turned to a N-terminal-truncated version of OsFD1 (147–195) (lacking amino acids 1–146) with the S192E mutation, which was produced as a GST fusion protein in *E. coli* BL21 Rosetta (DE3) and purified as above.

### ***In vitro protein phase separation***

Polyethylene glycol 8,000 (BioUltra grade, Sigma-Aldrich) was added to protein samples to a final concentration of 15% (w/v) in 25 mM potassium phosphate buffer (pH 6.8) containing 25 mM KCl and 0.5 mM DTT. The solutions were incubated for 24 h at 4 °C for protein phase separation. Phase separation was then assessed by measuring protein solution turbidity from 200 µl of protein solution (absorbance scanned between 330 and 800 nm) in 96-well plates (Stem, Tokyo) with a spectral scanning multimode reader (VarioSkan Flash 2.4, Thermo Fisher). Total protein concentration was 100 µM for all samples except for R121H mutant where 50 µM was used due to the difficulty in concentrating the protein solution. For phase-contrast image analyses, a Leica DMI300B microscope was used. The droplets of condensed Hd3a and RFT1 proteins were spherical and were hard to merge with each other even over a long lifetime, much like aging Maxwell glass.<sup>44</sup>.

## 299 ***Nuclear magnetic resonance (NMR) experiments***

300 All NMR spectra were acquired on an AVANCE III HD 800 spectrometer (Bruker) at 303 K,  
301 processed and analysed with TopSpin 4.11 software.  $^1\text{H}$ - $^{15}\text{N}$  HSQC experiments on  
302 0.057 mM  $^{15}\text{N}$ -labelled Hd3a mutants were performed in 50 mM potassium phosphate buffer  
303 (pH 6.8) containing 50 mM KCl and 1 mM DTT.

## 305 ***Protoplast transformation***

306 Transformation of rice protoplasts was performed as described previously<sup>2,28</sup>. Five  
307 micrograms of *Hd3a* and *OsFD1* effector constructs and 0.5  $\mu\text{g}$  of pUbqFluc reporter  
308 plasmid<sup>2</sup> were transfected into  $0.3\text{--}1.0 \times 10^7$  protoplasts per ml by the PEG-mediated  
309 transformation method. After a 24-h incubation at 30 °C, the protoplast suspension was  
310 centrifuged and the cell pellet was frozen at  $-80$  °C for measurement of luciferase activity.

## 312 ***Measurement of luciferase activity in protoplast lysates***

313 Luciferase activity was measured as described previously<sup>28</sup>. The activities derived from  
314 NanoLuc (Nluc) and firefly luciferase (Fluc) in the lysates were measured separately and  
315 their ratio calculated. The Nano-Glo Luciferase Assay System (Promega) and Dual  
316 Luciferase Reporter Assay System (Promega) were used according to the manufacturer's  
317 instructions for Nluc and Fluc activity, respectively. Transfected protoplasts were lysed with  
318 25  $\mu\text{l}$  of Passive lysis buffer (Promega). Nluc luminescence was measured with a TriStar  
319 LB941 microplate reader (Berthold Technologies) immediately after mixing of 10  $\mu\text{l}$  of  
320 lysate and 10  $\mu\text{l}$  of Nano-Glo Luciferase Assay Reagent in a 96-well plate. Fluc  
321 luminescence was measured immediately after mixing 2  $\mu\text{l}$  of lysate and 10  $\mu\text{l}$  of LARII  
322 Reagent in a 96-well microtiter plate.

## Data availability

The plasmids for transient expressions, plant transformations and protein purifications are available upon requests. Any other relevant data are available from the corresponding authors upon reasonable request.

## Figure legends

### Fig. 1 | Hd3a forms nuclear speckles in the shoot apical meristem.

**a,b**, Representative GFP fluorescence pattern from *GFP* (**a**) or *Hd3a-GFP* (**b**) constructs driven by the phloem-specific *rolC* promoter in the rice shoot apical meristem. Scale bars, 10  $\mu$ m. **c–e**, Stimulated emission depletion (STED) imaging of cells within the rice shoot apical meristem harbouring the transgenes *Ubqpro:H2B-GFP* (**c**), *rolCpro:GFP* (**d**) and *rolCpro:Hd3a-GFP* (**e**). **f**, Super-resolution confocal live imaging microscopy (SCLIM) imaging of cells in the rice shoot apical meristem harbouring the transgene *Hd3apro:Hd3a-GFP*. Scale bars, 5  $\mu$ m. **g**, Enlarged view of the region highlighted by a dashed box in (**f**). **h**, Enlarged view of a nucleus in the shoot apical meristem from a *Hd3apro:Hd3a-GFP* transgenic rice line. **i,j**, Spherical condensates of Hd3a (**i**) and RFT1. Scale bars, 10  $\mu$ m. (**j**). **k**, Absorbance of recombinant Hd3a in a PEG-containing solution.

### Fig. 2 | Formation of the florigen activation complex (FAC) facilitates nuclear speckle formation and in vitro condensation.

**a**, Effects of Hd3a mutations on *OsMADS15* transcriptional activation. **b**, Hd3a-GFP localization in the nucleus of *N. benthamiana* leaf epidermal cells. Scale bars, 10  $\mu$ m. **c**, Effects of Hd3a mutations on the mean number of nuclear speckles. **d,e**, Absorbance of recombinant Hd3a alone (red), GF14c alone (blue) and Hd3a with GF14c (purple) (**d**) and of

348 OsFD1 alone (light green), Hd3a with OsFD1 (brown) and Hd3a with OsFD1 and GF14c  
349 (black) (e).

350

351 **Fig. 3 | Interaction with 14-3-3 protein facilitates nuclear speckle formation and in vitro**  
352 **condensation of Hd3a.**

353 **a**, Localization pattern of Hd3a<sup>R64K,R132K</sup>-GFP and Hd3a<sup>F103A</sup>-GFP when co-expressed with  
354 *OsFD1* in the nucleus of *N. benthamiana* leaf cells. Scale bars, 10 µm. **b,c**, Absorbance of  
355 recombinant Hd3a with GF14c (green) and Hd3a<sup>R64K,R132K</sup> with GF14c (black) (**b**) and of  
356 Hd3a with GF14c (green) and Hd3a<sup>F103</sup> with GF14c (black) (**c**). Scale bars, 10 µm.

357

358 **Fig. 4 | Substitutions in the potential ligand-binding pocket and C-terminal disordered**  
359 **region of Hd3a modulate nuclear speckle formation and in vitro condensation.**

360 **a**, Localization pattern of Hd3a<sup>Y87H</sup>-GFP, Hd3a<sup>R121H</sup>-GFP and Hd3a<sup>G173E</sup>-GFP when co-  
361 expressed with *OsFD1* in the nucleus of *N. benthamiana* leaf cells. Scale bars, 10 µm. **b**,  
362 Effects of Hd3a mutations on the number of nuclear speckles. **c-e**, Absorbance of  
363 recombinant Hd3a with GF14c (green throughout) and of Hd3a<sup>Y87H</sup> with GF14c (black, **c**),  
364 Hd3a<sup>R121H</sup> with GF14c (black, **d**) and Hd3a<sup>G173E</sup> with GF14c (black, **e**), mixed 50:50 in each  
365 case. **f**, Proposed model of FAC activity through condensate formation by condensate  
366 formation in the nucleus. FAC formation induces condensate formation, which requires the  
367 interaction of Hd3a with 14-3-3 via the Hd3a residues R64, F103 and R132. G173 in the C-  
368 terminal disordered region and the internal R121 are essential for condensate formation after  
369 FAC formation. The potential anion-binding pocket of Hd3a includes Y87, which is essential  
370 for FAC function but is dispensable for condensate formation, suggesting that it acts  
371 downstream of condensate formation.

372

373

374 **Extended Data Fig. 1 | Changes in the absorbance of suspensions consisting of glass**  
375 **beads of different sizes.**

376 Absorbance between 330 and 800 nm of glass bead solutions with diameters of 3  $\mu\text{m}$ , 200 nm  
377 and 70 nm.

378

379 **Extended Data Fig. 2 | Absorbance of RFT1 with or without the 14-3-3 protein GF14c.**

380 Absorbance between 330 and 800 nm of recombinant RFT1 protein with (purple) or without  
381 (magenta) GF14c.

382

383 **Extended Data Fig. 3 | Ratio dependence of absorbance in Hd3a and GF14c.**

384 Absorbance between 330 and 800 nm of recombinant Hd3a with GF14c mixed in ratios of  
385 50:50 (blue) or 25:75 (purple).

386

387 **Extended Data Fig. 4 | NMR spectra of Hd3a WT and R121H.**

388  $^1\text{H}$ - $^{15}\text{N}$  heteronuclear single quantum coherence (HSQC) spectra of  $^{15}\text{N}$ -labelled recombinant  
389 Hd3a proteins for wild-type (WT, blue) and R121H mutant (red). p.p.m., parts per million.

390

391

392

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#### **Author contributions**

K.T., H.T. and C.K. designed the research, interpreted data and wrote the manuscript with the inputs from all authors.. K.T. performed transient expression analysis. S.A., M.E., Y.K. performed phase-separation assays. K.F. performed NMR measurements. T.K. and T.F. supervised protein analysis. M.T. performed microscopic observations of transient assays and plant transformation. J.N., A.F., M.F. and K.K. performed super-resolution imaging under the supervision of H.T. and A.N.. T.O. generated H2B-GFP transgenic rice.

#### **Competing Interests statement**

The authors declare no competing financial interests.

#### **Supplementary materials**

421 Extended Data Figs. 1–4

422

## 423 References

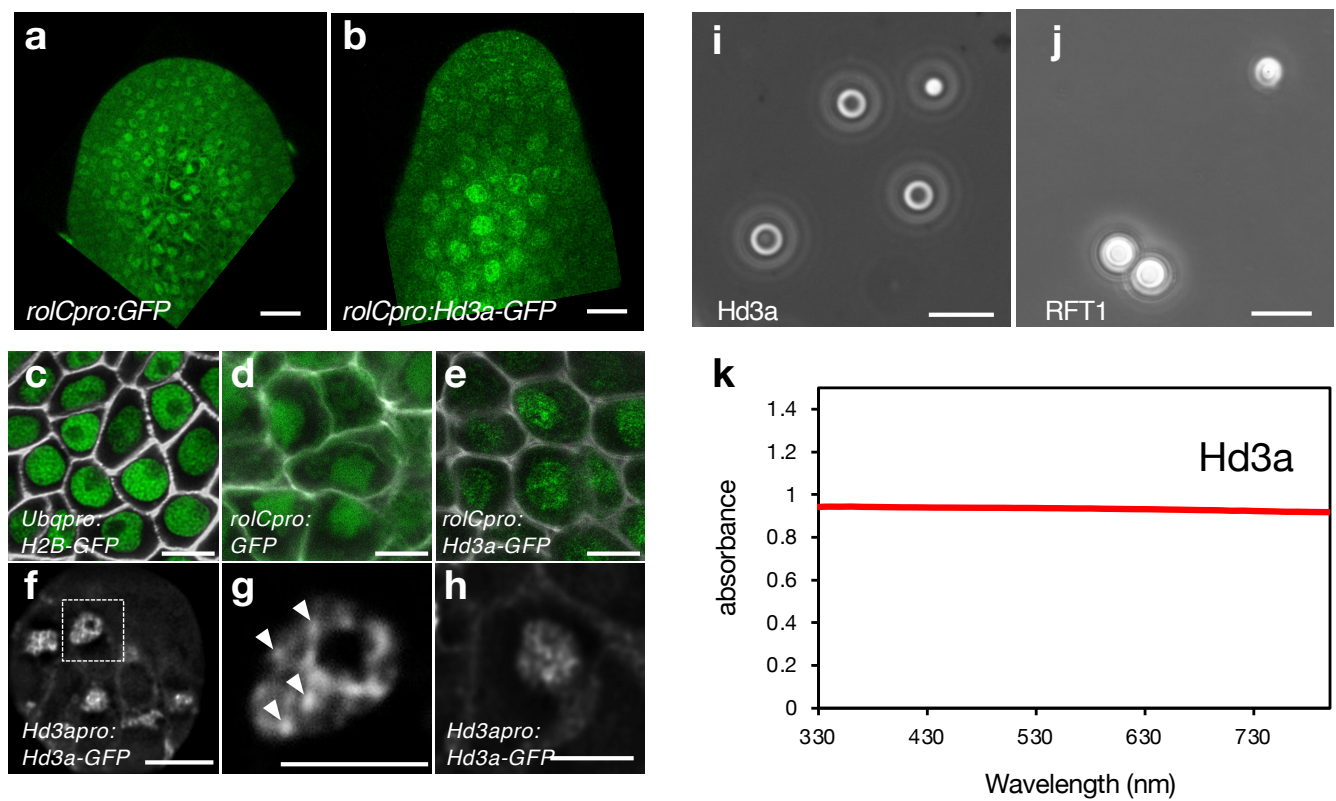
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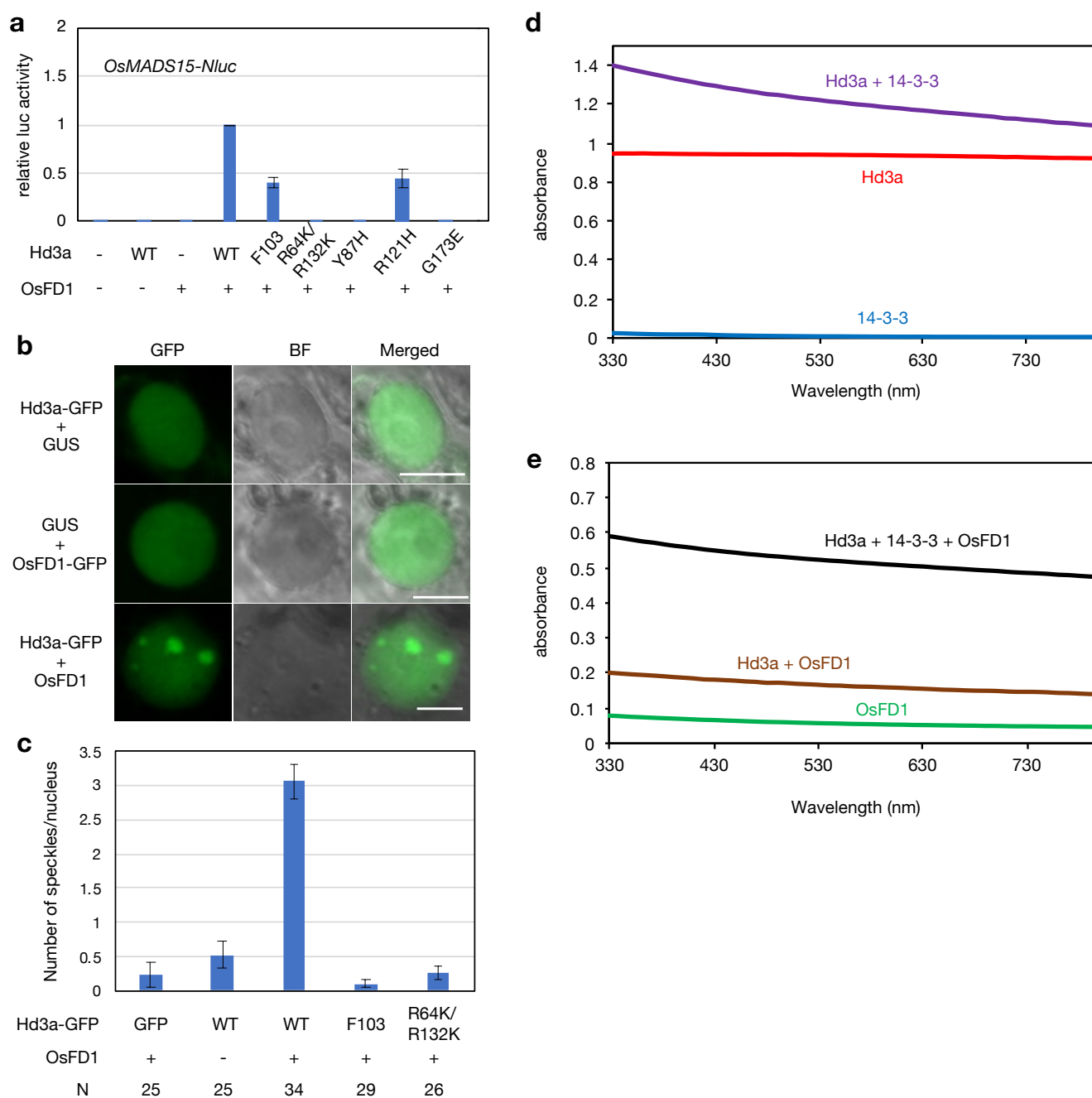
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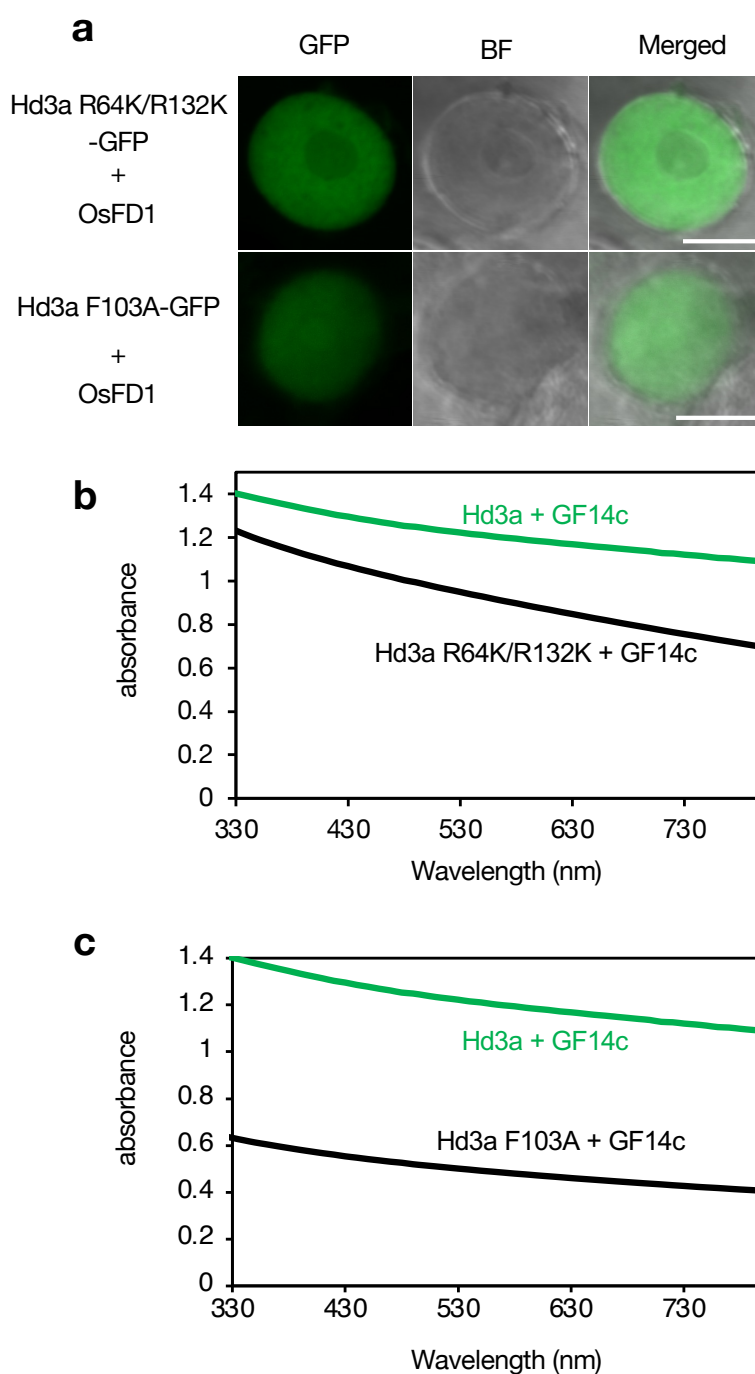
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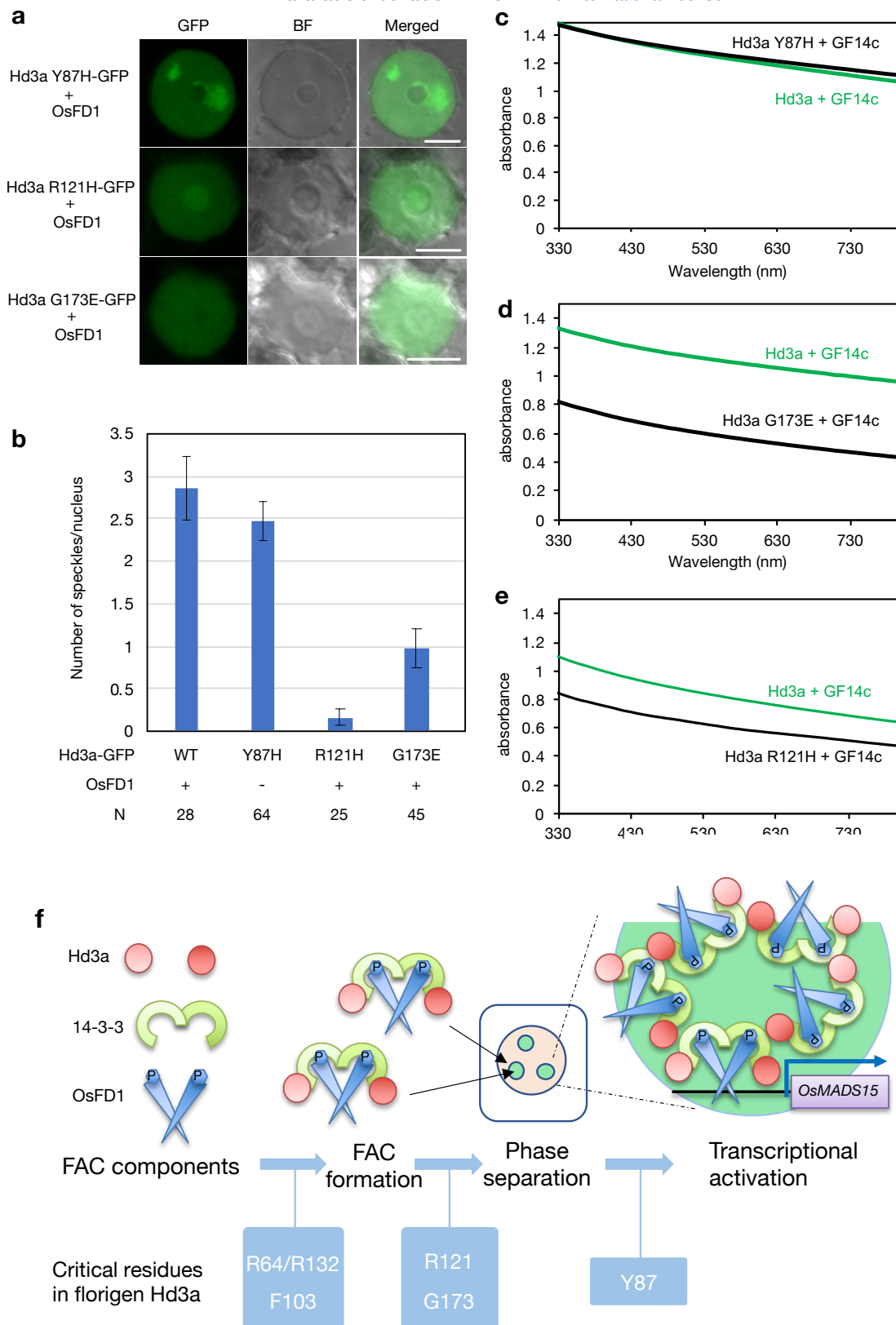
**Fig. 1**



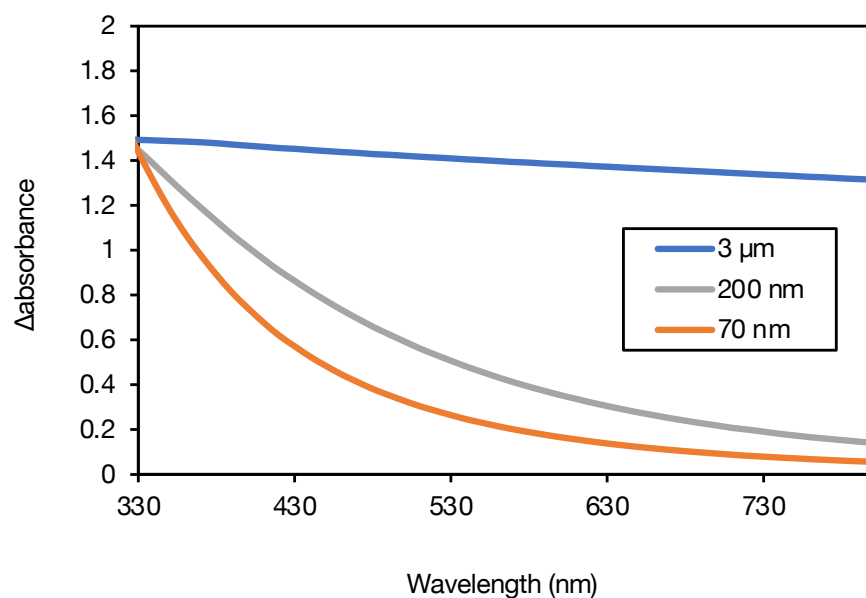
**Fig. 2**



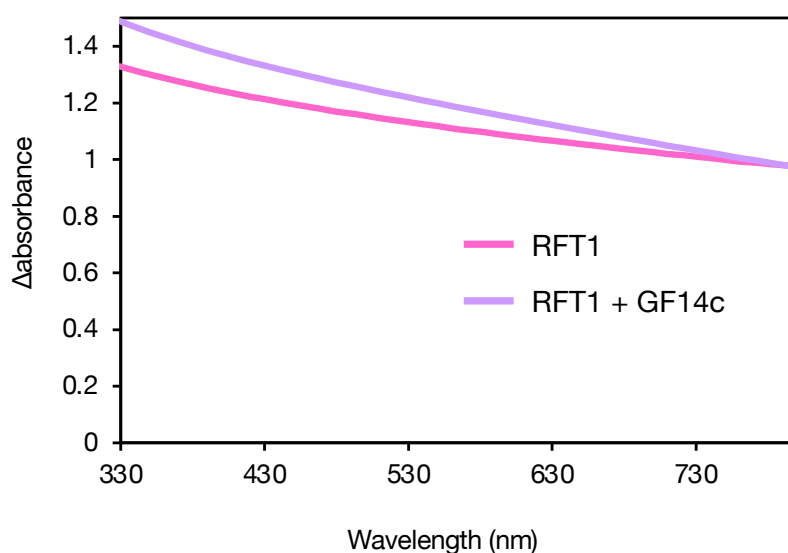
**Fig. 3**



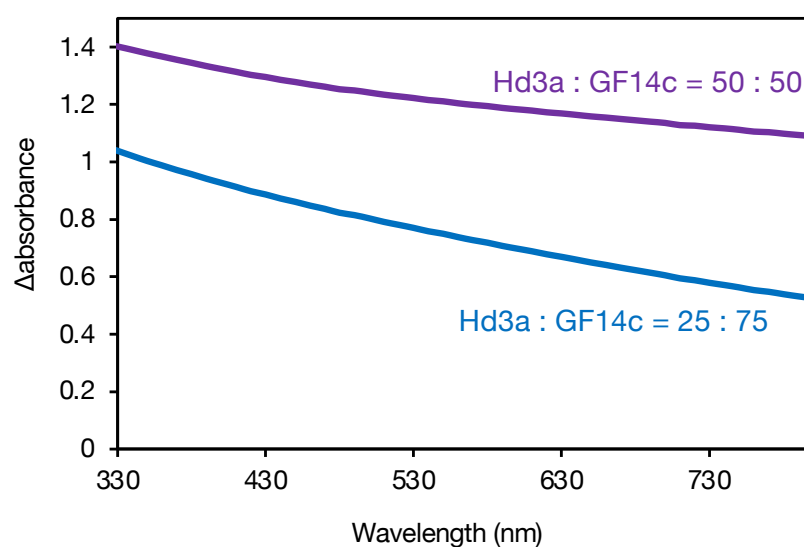
**Fig. 4**



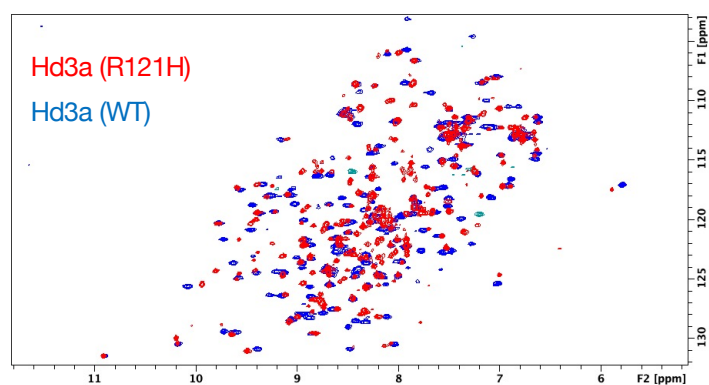
**Extended Data Fig. 1**



**Extended Data Fig. 2**



**Extended Data Fig. 3**



**Extended Data Fig. 4**