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## The Actin Depolymerizing Factor StADF2 Alters StREM1.3 Plasma Membrane Nanodomains to Inhibit the Virus X

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► **To cite this version:**

Marie-Dominique Jolivet, Paul Gouguet, Anthony Legrand, Kaltra Xhelilaj, Natalie Faiss, et al.. The Actin Depolymerizing Factor StADF2 Alters StREM1.3 Plasma Membrane Nanodomains to Inhibit the Virus X. 2025. hal-04833207

**HAL Id: hal-04833207**

**<https://cnrs.hal.science/hal-04833207v1>**

Preprint submitted on 17 Jan 2025

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1 **TITLE: The actin depolymerizing factor StADF2 alters StREM1.3 plasma membrane**  
2 **nanodomains to inhibit the *Potato Virus X***

3

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32

## 33 **ABSTRACT**

34 The dynamic regulation of the plasma membrane (PM) organization at the nanoscale  
35 emerged as a key element shaping the outcome of host-microbe interactions. Protein  
36 organization into nanodomains (ND) is often assumed to be linked to the activation of cellular  
37 processes. In contrast, we have previously shown that the phosphorylation of the *Solanum*  
38 *tuberosum* REM1.3 (StREM1.3) N-terminal domain disperses its native ND organization and  
39 promotes its inhibitory effect on *Potato Virus X* (PVX) cell-to-cell movement. Here, we show  
40 that the phosphorylation of StREM1.3 modify the chemical environment of numerous  
41 residues in its intrinsically-disordered N-terminal domain. We leveraged exploratory screens  
42 to identify potential phosphorylation-dependent interactors of StREM1.3. Herewith, we  
43 uncovered uncharacterized regulators of PVX cell-to-cell movement, linking StREM1.3 to  
44 autophagy, water channels and the actin cytoskeleton. We show that the *Solanum tuberosum*  
45 actin depolymerizing factors 2 (StADF2) alters StREM1.3 NDs and limits PVX cell-to-cell  
46 movement in a REMORIN-dependent manner. Mutating a conserved single residue reported  
47 to affect ADFs affinity to actin inhibits StADF2 effect on StREM1.3 ND organization and PVX  
48 cell-to-cell movement. These observations provide functional links between the organization  
49 of plant PM and the actin cytoskeleton and suggests that the alteration of StREM1.3 ND  
50 organization promotes plant anti-viral responses. We envision that analogous PM re-  
51 organization applies for additional signaling pathways in plants and in other organisms.

52

## 53 **INTRODUCTION**

54 The plasma membrane (PM) actively hosts, modulates and coordinates a myriad of signaling  
55 events essential to the development and survival of all living organisms. Across the tree of  
56 life, PM lipids and proteins have been found to be dynamically organized into diverse  
57 nanoscopic environments termed nanodomains (NDs) (Gronnier et al., 2018; Jacobson et al.,  
58 2019; Lopez & Koch, 2017; Malinsky et al., 2013). NDs have been proposed to provide  
59 dedicated biochemical and biophysical environments to ensure acute, specific and robust  
60 signaling events (Kusumi et al., 2012; Sezgin et al., 2017). In plants, emerging evidence suggest  
61 that ND formation and integrity rely on the intimate interplay between lipids, proteins, the  
62 cell wall and the cytoskeleton (Galindo-Trigo et al., 2020; Gronnier et al., 2018, 2019; Jaillais  
63 & Ott, 2020). The molecular events underlying context-dependent PM re-organization and  
64 their functional consequences remain largely unknown.

65 REMORINs are structural components of the PM playing regulatory functions in plant-microbe  
66 interactions and hormone signaling (reviewed in Gouguet et al., 2021). REMORINs of different  
67 groups (Raffaele et al., 2007) tend to form distinct and coexisting NDs proposed to regulate  
68 specific signaling pathways (Bücherl et al., 2017; Jarsch et al., 2014). The molecular bases for  
69 REMORINs ND organization are best characterized for group 1 REMORINs (Gronnier et al.,  
70 2018; Jaillais & Ott, 2020). Group 1 REMORINs NDs are proposed to be liquid-ordered and  
71 enriched in sterols and saturated lipids (Demir et al., 2013; Gronnier et al., 2017; Mamode  
72 Cassim et al., 2019; Raffaele et al., 2009). Their native organization is dictated by direct  
73 interactions with anionic phospholipids (Gronnier et al., 2017; Legrand et al., 2019; Legrand  
74 et al., 2022; Perraki et al., 2012), and can be modulated by post-translational modifications  
75 such as S-acylation (Fu et al., 2018; Konrad et al., 2014) and phosphorylation (Perraki et al.,  
76 2018). Group 1 REMORINs regulate cell-to-cell movement of viruses belonging to diverse  
77 genus (Cheng et al., 2020; Fu et al., 2018; Huang et al., 2019; Raffaele et al., 2009; Rocher et  
78 al., 2022). Notably, their function in hindering *Potexviruses*, to which the *Potato Virus X* (PVX)  
79 belongs, is conserved in *Nicotiana benthamiana*, *Solanum lycopersicum* and *Arabidopsis*  
80 *thaliana* (Abel et al., 2021; Perraki et al., 2018; Perraki et al., 2014; Raffaele et al., 2009),  
81 suggesting an ancestral function conserved across at least 100 million years of evolution.  
82 Most REMORINs bear a predicted intrinsically-disordered N-terminal domain found to be  
83 phosphorylated in multiple plant species and across a wide range of physiological conditions  
84 (Gouguet et al., 2021). Phosphorylation affects the chemistry, structure, and conformational  
85 ensemble of intrinsically-disordered domains (IDD), and consequently their association with  
86 protein partners (Mukhopadhyay et al., 2022; Wright & Dyson, 2014). In view of their PM ND  
87 organization, their ability to homo-oligomerize, and their putative IDD, REMORINs have been  
88 proposed to act as scaffolds. Here, we show that the N-terminal domain of StREM1.3 is  
89 intrinsically disordered and that its phosphorylation modifies the chemical environment of  
90 numerous residues. Leveraging yeast two-hybrid screens, we identified interactors of a  
91 phosphomimic variant of StREM1.3 and uncovered uncharacterized regulators of PVX  
92 infection. We show that the *Solanum tuberosum* actin depolymerizing factors 2 (StADF2)  
93 alters StREM1.3 NDs and limits PVX propagation in a REMORIN-dependent manner. We  
94 observed that StADF2 activity is affected by mutating a conserved residue regulating ADFs  
95 affinity to actin. Altogether, these observations suggest that active modulation of REMORIN  
96 ND organization promotes plant anti-viral responses.

97 **RESULTS**

98 ***StREM1.3 N-terminal domain is intrinsically disordered and its phosphorylation leads to***  
99 ***changes in the chemical environment of numerous residues.***

100 We previously showed that phosphorylation of the *Solanum tuberosum* REMORIN1.3  
101 (StREM1.3) N-terminal domain regulates its ND organization and function (Perraki et al.,  
102 2018). Notably, using photoactivated localization microscopy, we observed that a  
103 phosphomimic form of StREM1.3, StREM1.3<sup>S74D T86D S91D</sup> (further referred to as StREM1.3<sup>DDD</sup>)  
104 which restricts PVX cell-to-cell movement, presented a dispersed organization (Perraki et al.,  
105 2018). We confirmed this observation using laser scanning confocal microscopy (Figure 1A).  
106 However, the molecular bases underlying StREM1.3 ND dispersion remain unknown.  
107 StREM1.3 N-terminal domain is predicted to be intrinsically disordered (Marín et al., 2012;  
108 Perraki et al., 2018). We used liquid-state NMR spectroscopy to characterize the properties  
109 of StREM1.3 N-terminal domain. <sup>1</sup>H-<sup>15</sup>N HMQC spectra of untagged StREM1.3<sup>1-116</sup> purified in  
110 native and denaturing conditions (Figure S1A) both displayed a narrow  $\delta(1H)$  distribution  
111 (between  $\sim 7.5$ - $8.5$  ppm, Figure S1B), demonstrating that StREM1.3 N-terminal domain is  
112 intrinsically disordered.

113 We then asked how phosphorylation modulates StREM1.3 N-terminal domain. The  
114 *Arabidopsis thaliana* calcium-dependent protein kinase 3 (AtCPK3) was previously shown to  
115 phosphorylate group 1 REMORINs (Mehlmer et al., 2010; Perraki et al., 2018) and to restrict  
116 PVX cell-to-cell movement in a REMORIN-dependent manner (Perraki et al., 2018). Liquid-  
117 state NMR spectroscopy showed that the addition of AtCPK3, which phosphorylates  
118 StREM1.3 N-terminal domain *in vitro* (Legrand et al., 2022; Perraki et al., 2018) (Figure S2),  
119 induced chemical shift perturbations on several residues without altering the intrinsically  
120 disordered nature of StREM1.3<sup>1-116</sup> (Figures 1B and 1C). The chemical shifts of the amide  
121 groups are very sensitive to the local chemical environment of the concerned residues. These  
122 observations indicate the modification of the chemical environment for multiple residues  
123 induced by phosphorylation which may modulate StREM1.3 intra- and intermolecular  
124 interactions. We therefore hypothesized that the phosphorylation of StREM1.3 intrinsically  
125 disordered N-terminal domain may modulate its association with specific protein partners to  
126 inhibit PVX cell-to-cell movement.

127

128 **A StREM1.3 phosphomimic-based yeast two hybrid exploratory screen identifies**  
129 **uncharacterized regulators of PVX cell-to-cell movement.**

130 To identify proteins which may associate with StREM1.3 in a phosphorylation-dependent  
131 manner, we used exploratory split-ubiquitin yeast two-hybrid (SUY2H) screens comparing WT  
132 and phosphomimic variant of StREM1.3, StREM1.3<sup>DDD</sup>. First, we tested whether StREM1.3 was  
133 a functional bait in SUY2H. We observed that expression of Cub-StREM1.3<sup>WT</sup> or Cub-  
134 StREM1.3<sup>DDD</sup> did not lead to autoactivation and that StREM1.3 self-association (Bariola et al.,  
135 2004.; Martinez et al., 2019; Perraki et al., 2014) could be resolved in this system, indicating  
136 that both Cub-StREM1.3 or Cub-StREM1.3<sup>DDD</sup> were functional in SUY2H assays (Figure S3A and  
137 S3B). Further, we observed that GFP-tagged StREM1.3 and StREM1.3<sup>DDD</sup> localized to the PM  
138 when expressed in yeast (Figure S3C). Interestingly, both proteins formed PM domains in  
139 yeast (Figure S3C), suggesting that StREM1.3<sup>DDD</sup> dispersed organization as observed in *N.*  
140 *benthamiana* (Figure 1A) is conditioned by plant factors.

141 To enrich for relevant factors during viral infection, we generated a cDNA library using mRNA  
142 extracted from both PVX-infected and healthy *N. benthamiana* leaf epidermis (Figure S4A and  
143 S4B). We used peeled epidermis to limit the occurrence of chloroplastic proteins in our screen  
144 as previously reported (Bernard et al., 2012). We confirmed the presence of PVX:GFP in the  
145 infected epidermis by confocal microscopy (Figure S4C). Side-by-side screens of this library by  
146 SUY2H revealed an approximate 6-fold increase in the number of positive clones obtained  
147 with StREM1.3<sup>DDD</sup> compared to StREM1.3 (Figures 2A, 2B and 2C), suggesting that the  
148 phosphomimic form of StREM1.3 has an increased ability to generate protein-protein  
149 interactions. Sequencing of the clones presenting reliable interaction identified 37 and 140  
150 distinct proteins for StREM1.3 and StREM1.3<sup>DDD</sup> respectively (Figure 2D and supplemental  
151 table 1). Among them, only 6 proteins were identified in both screens (Figure 2D) suggesting  
152 that StREM1.3 and StREM1.3<sup>DDD</sup> have largely distinct interactomes. Among identified proteins  
153 are orthologs of Arabidopsis 14-3-3 and HYPERSENSITIVE INDUCED REACTION proteins which  
154 have been previously shown to associate with group 1 REMORINs (Huang et al., 2019; Lv et  
155 al., 2017), pointing toward the functional relevance of the identified proteins. To further  
156 explore the role of REM interactors in PVX infection, we prioritized candidates based on the  
157 number of clones identified in SUY2H screen, their co-purification and/or co-expression with  
158 Arabidopsis REMs in omics studies and cloned them as translational fusions with the HA  
159 epitope-tag for functional studies. Among the 10 candidates tested in PVX:GFP cell-to-cell

160 propagation assays (Figures 3A and 3B), the expression of 7 candidates impaired PVX  
161 propagation: the Plasma membrane intrinsic protein 1C (NbPIP1C), Delta tonoplast integral  
162 protein (NbTIP2;1), Thioredoxin superfamily protein 4 (NbTRX4), Metallothionein 2B  
163 (NbMT2B), Calreticulin 3 (NbCRT3), Autophagy 8i (NbATG8i) and Actin Depolymerizing Factor  
164 3 (NbADF3) (Figure 3B, Figure S5). Altogether, these observations both suggest that the  
165 phosphorylation of StREM1.3 promotes its association with specific proteins to limit PVX cell-  
166 to-cell movement and link plant response to PVX with the autophagy pathway and water  
167 channels notably.

168

### 169 **StADF2 affects StREM1.3 nanodomains and inhibits PVX cell-to-cell movement in a** 170 **REMORIN-dependent manner.**

171 Among the genes tested, the *N. benthamiana* actin depolymerizing factor (ADF) 3 (NbADF3,  
172 NbS00025994g0001.1) was one of the most potent in limiting PVX cell-to-cell movement  
173 (Figure 3B). To corroborate the potential implication of an ADF-REMORIN module involved in  
174 response to PVX, we first tested the ability of an ortholog of NbADF3 from *Solanum tuberosum*  
175 (StADF2, Soltu.DM.04G007350) to associate with StREM1.3. Co-immunoprecipitation  
176 experiments, using transient expression in *N. benthamiana* leaves, showed that StADF2 and  
177 StREM1.3 associate *in planta* (Figure 4A). We next tested whether StADF2 limits PVX infection.  
178 Using group 1 REMORINs knock-down stable transgenic *N. benthamiana* lines (hpREM #1.4  
179 and #10.2; Perraki et al., 2018), we observed that the transient overexpression of StADF2  
180 limited PVX cell-to-cell movement in a REMORIN-dependent manner (Figure 4B, 4C). These  
181 results indicate that ADFs and REMORINs cooperate to limit PVX infection. Because the ND  
182 organization of the *Arabidopsis thaliana* REM1.2 and of the *Medicago truncatula* SYMBIOTIC  
183 REMORIN (SYMREM; MtREM2.2) rely on the integrity of the actin cytoskeleton (Liang et al.,  
184 2018; Szymanski et al., 2015) and that the active form of StREM1.3 correlates with a disperse  
185 PM organization (Figure 1A; Perraki et al., 2018), we wondered whether StADF2 could  
186 regulate StREM1.3 ND organization to inhibit PVX. We observed that overexpression of  
187 StADF2 affected YFP-StREM1.3 ND organization (Figures S6A and S6B), suggesting that  
188 StREM1.3 ND organization relies on actin cytoskeleton integrity. In good agreement,  
189 cytochalasin D treatment, which inhibits actin polymerization, was sufficient to affect YFP-  
190 StREM1.3 ND organization (Figures S6C and S6D). The affinity of ADFs for actin is decreased  
191 by their phosphorylation on a conserved N-terminally located Serine (Figure S7; Augustine et



192 al., 2008; Dong & Hong, 2013; Y. J. Lu et al., 2020; Porter et al., 2012; Ressad et al., 1998).  
193 Interestingly, we observed that replacing the corresponding Serine residue by an Aspartic acid  
194 in StADF2 (StADF<sup>S6D</sup>) altered its ability to modify StREM1.3 ND organization (Figures 5A and  
195 5B) and to restrict PVX cell-to-cell movement (Figure 5C and 5D). Altogether, these data  
196 indicate that StADF2 restricts PVX cell-to-cell movement by actively modulating actin  
197 cytoskeleton and REMORINs ND organization.

198

## 199 **DISCUSSION**

200 How processes are regulated in space and time within the plasma membrane (PM) remains  
201 largely obscure. In plants, REMORINs are structural elements of the PM emerging as versatile  
202 regulatory components of various signaling pathways in plant development and plant-  
203 microbe interactions (Gui et al., 2016; Kohorn et al., 2016; Liang et al., 2018; Perraki et al.,  
204 2014). This functional versatility is suspected to be encoded in their variable and intrinsically  
205 disordered N-terminal domain (Gouguet et al., 2021). REMORINs IDD have been found to be  
206 poly-phosphorylated in many plant species and across a wide range of physiological  
207 conditions (Gouguet et al., 2021). Here we show that the phosphorylation of StREM1.3 IDD  
208 modifies the chemical environment at the level of the protein backbone which may operate  
209 as a molecular switch in regulating its association with specific partners. In good agreement,  
210 we observed that a phosphomimic variant of StREM1.3 has both an increased ability to  
211 generate protein-protein interaction and a distinct interactome from the wild-type protein in  
212 yeast. These observations suggest the existence of context-dependent REMORINs phospho-  
213 codes and open way toward their identification and the study of their implication in the  
214 regulation of REMORINs-associated molecular complexes and signaling pathways.

215 Functional analysis of selected StREM1.3 interactors unveiled uncharacterized host regulators  
216 of PVX infection (Figure 3). These observations suggest that plant immune response to PVX  
217 involves the autophagy machinery (NbATG8i), water permeable channels (NbPIP1;3,  
218 NbTIP2;1), regulators of cellular redox status (NbTRX4) and iron-binding proteins (NbMT2B).  
219 Several orthologs of these proteins were previously linked to immunity in various plants  
220 species against pathogens of several kingdoms. For instance, the autophagy machinery has  
221 been found to play a central role in plant immunity against bacteria, fungi and viruses and can  
222 as well be manipulated by pathogens for their own benefits (Fu et al., 2018; Hafrén et al.,  
223 2018; Leary et al., 2018; Leong et al., 2022; F. Li et al., 2020; MacHaria et al., 2019; Niu et al.,



224 2021; Üstün et al., 2018; Yang et al., 2018). Altogether, these observations suggest that  
225 REMORINs play a central role in regulating key cellular events in plant immunity, the definition  
226 of the precise mechanisms will require further investigation.

227 NbADF3 and StADF2 belong to the subclass 1 of the ADF protein family (Inada, 2017), which  
228 has been reported to be involved in multiple plant-pathogen interactions (Lu et al., 2015; Lu  
229 et al., 2020; Porter et al., 2012; Porter & Day, 2013; Tian et al., 2009). Subclass 1 ADFs have  
230 been identified in *Arabidopsis thaliana* plasmodesmata (PD) proteome (Brault et al., 2019),  
231 which may imply that their role in restricting of PVX propagation (Figure 3B and 4C) relies on  
232 a direct modulation of plasmodesmata. Further, actin disruption has been recently proposed  
233 as an immune response leading to callose deposition (Leontovyčová et al., 2020) and both  
234 AtADF4 and REMORINs are required for callose deposition in response to bacterial elicitors  
235 (Li et al., 2012; Ma et al., 2022). Since StREM1.3 regulates callose deposition at PD (Perraki et  
236 al., 2018) and that StADF2 limits PVX in a REMORIN-dependent manner (Figure 4C),  
237 modulation of actin cytoskeleton and of StREM1.3 ND organization by StADF2 may underly  
238 REMORIN-regulated callose accumulation at PD. Recently, evidences indicate that REMORINs  
239 and the actin cytoskeleton are working hand in hand during plant-microbe interactions.  
240 *Arabidopsis* REM1.2 promotes type-I Formin ND organization to foster actin nucleation in  
241 response to bacterial flagellin (Ma et al., 2022) while SYMREM1-induced membrane topology  
242 modifications during symbiosis depends on the actin cytoskeleton (Su et al., 2023). Our  
243 observations further reinforce the tight functional interplay between REMORINs and the actin  
244 cytoskeleton. Interestingly, two subclass 1 *Arabidopsis* ADFs (AtADF1 and AtADF4) as well as  
245 group 1 REM were shown to be phosphorylated by AtCPK3 (Dong & Hong, 2013a; Lu et al.,  
246 2020). Moreover, it was observed that CPK3 modulated actin cytoskeleton in response to  
247 bacterial elicitors (Lu et al., 2020) and limited PVX cell-to-cell movement in a REMORIN-  
248 dependent manner (Mehlmer et al., 2010; Perraki et al., 2018), which hints that CPK-ADF-  
249 REMs may correspond to a conserved immune signaling module against invading microbes.  
250 Stimuli-dependent organization of proteins in NDs is often presumed to be associated with  
251 initiation and activation of cellular processes. In good agreement, the stabilization of the small  
252 GTPase Rho-of-plants 6 (ROP6) into NDs upon auxin perception supports its function in  
253 regulating gravitropism (Platre et al., 2019). In addition, ROP6 forms specific domains  
254 promoting the production of reactive oxygen species upon osmotic stress (Smokvarska et al.,  
255 2020). However, recent reports suggest that the functional interplay between PM lateral

256 organization and the functional status of its constituents is far more complex. While osmotic  
257 stimulation induces ND organization of the NADPH oxidase RBOHD and of ROP6 (Smokvarska  
258 et al., 2020), it concomitantly leads to an increased mobility of the aquaporin PIP2;1 and of  
259 the proton pump ATPase AHA2 (Martinière et al., 2019). In yeast, the arginine permease Can1  
260 accumulates into so-called membrane compartment occupied by Can1 (MCC) in its inactive,  
261 substrate-free form, while its active form shows a dispersed organization within the PM  
262 (Gournas et al., 2018). Similarly, substrate perception by the yeast methionine permease  
263 Mup1 induces its reorganization from NDs to a disperse plasma membrane network (Busto et  
264 al., 2018). We previously showed that native ND organization of StREM1.3 is required to  
265 support its function in limiting PVX cell-to-cell movement (Gronnier et al., 2017). Yet, active  
266 phosphomimic form of StREM1.3 presents a dispersed organization (Perraki et al., 2018;  
267 Figure 1A) suggesting that StREM1.3 is activated within NDs and subsequently dispersed.  
268 Here, we show that StADF2 alters StREM1.3 ND organization and limits PVX cell-to-cell  
269 movement in a REMORIN-dependent manner (Figure 4). Mutating a conserved single residue  
270 reported to affect ADFs affinity to actin inhibits StADF2 effect on StREM1.3 ND organization  
271 and PVX cell-to-cell movement (Figure 5). Altogether, these observations suggest that active  
272 modulation of actin cytoskeleton by StADF2 changes StREM1.3 nanoscale organization to  
273 limit PVX cell-to-cell movement. In mammals, actin depolymerization have been shown to  
274 promote B cell activation by enabling B cell antigen receptor clustering in the immune synapse  
275 (Droubi et al., 2022; Mattila et al., 2016; Wang & Huse, 2022). Since REMORINs have been  
276 proposed to regulate various cell-surface receptor signaling pathways (Bücherl et al., 2017;  
277 Gui et al., 2016; Liang et al., 2018), a similar mechanism could apply in response to PVX and  
278 in other contexts. Our study shades light on the activation of a PM-based immune response  
279 by membrane scaffold dispersion. We envision that analogous mechanisms may be conserved  
280 across organisms.

281

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600  
601

602 **FIGURE LEGENDS**

603 **Figure 1 | Phosphorylation modulates StREM1.3 intrinsically disordered domain.**

604 **A.** Representative confocal micrograph showing the surface of *N. benthamiana* leaf epidermal  
605 cells expressing YFP-StREM1.3<sup>WT</sup> and YFP-StREM1.3<sup>DDD</sup> (left) and corresponding quantification  
606 of YFP-StREM1.3<sup>WT</sup> and YFP-StREM1.3<sup>DDD</sup> spatial clustering index (right). Graphs are notched  
607 box plots, scattered data points show measurements, colors indicate independent  
608 experiments, n=38 cells for YFP-StREM1.3 and n=39 cells for YFP-StREM1.3<sup>DDD</sup>. P values report  
609 two-tailed non-parametric Mann-Whitney test. Scale bar indicates 5  $\mu$ m.

610 **B.** <sup>1</sup>H-<sup>15</sup>N HMQC spectra of StREM<sup>1-116</sup> before (black) and after (blue) addition of AtCPK3. Peaks  
611 labelled 1,2 and 3 appeared over time. Each spectrum was acquired using 2 scans for an  
612 experimental time of 1 min 42 s each. Temperature: 20°C.

613 **C.** NMR intensities of peaks 1, 2 and 3 (Figure 1B) over time after the addition of AtCPK3.

614

615 **Figure 2 | Comparative analysis of StREM1.3 and StREM1.3<sup>DDD</sup> interactomes in SUY2H.**

616 **A-B.** Drop-tests of SUY2H assays performed with yeast clones retrieved from two independent  
617 side-by-side screens performed using Cub-StREM1.3 (**A**) or Cub-StREM1.3<sup>DDD</sup> (**B**) as bait and  
618 constructs from the *N. benthamiana* leaf epidermis cDNA library.

619 **C.** Quantification of the number of positive clones retrieved from Cub-StREM1.3 and Cub-  
620 StREM1.3<sup>DDD</sup> screens, observed on -AHWL plates 4 days after transformations normalized by  
621 transformation efficiency for each screen (expressed in ‰ of theoretically tested clones).  
622 Data points show values obtained for individual -AHWL plates. P values report two-tailed non-  
623 parametric Mann-Whitney test.

624 **D.** Venn diagram depicting the number of proteins identified with Cub-StREM1.3 and Cub-  
625 StREM1.3<sup>DDD</sup>.

626

627 **Figure 3 | Functional analysis of StREM1.3's putative interactors upon PVX infection.**

628 **A.** Representative images of PVX:GFP infection foci of *N. benthamiana* leaves expressing  
629 empty vector (control condition) and 2 selected HA-tagged candidates. Scale bar = 500  $\mu$ m.

630 **B.** Quantification of PVX infection assays. The table is color coded based on the mean PVX:GFP  
631 infection foci area observed upon the overexpression of individual candidates normalized to  
632 the mean PVX:GFP infection foci area of the corresponding empty vector condition. Each  
633 candidate was N-terminally-fused to an HA-tag and expressed under the control of a 35S

634 promoter. Number of infection foci n=182 for HA-NbMT2B, n=219 for HA-NbPIP1;3, n=177  
635 for HA-NbCRT3, n=249 for HA-NbTRX4, n=208 for HA-NbDRM1, n=223 for HA-NbGST8, n=114  
636 for HA-ATG8i, n=228 for HA-TIP2;1, n=209 for HA-NbADF3, n=175 for HA-RabA4A. Stars report  
637 by Dunn's comparisons test, p<0.05: \* p<0.01: \*\*, p<0.001: \*\*\*, non-significant (n.s): p>0.05.

638

639 **Figure 4 | StADF2 limits PVX cell-to-cell movement in a REMORIN-dependent manner.**

640 **A.** Immunoprecipitation of GFP-StREM1.3 transiently co-expressed with either RFP or RFP-  
641 StADF2 in *N. benthamiana*. Blot stained with Ponceau is presented to show loading. Western  
642 blots were probed with  $\alpha$ -GFP or  $\alpha$ -RFP antibodies.

643 **B-C.** PVX infection assays. Representative micrograph of PVX:GFP infection foci upon  
644 expression of RFP-StADF2 in WT and stable *REMORINs* knock-down transgenic *N.*  
645 *benthamiana* independent lines #1.4 and #10.2 (**B**) and corresponding quantification (**C**).  
646 PVX:GFP foci are normalized based on the average area observed in the absence of RFP-  
647 StADF2 overexpression for each genotype. Graphs are notched box plots, scattered data  
648 points show measurements, colors indicate independent experiments, number of infections  
649 foci n=114 for WT, n=113 for #1.4 and n=104 for #10.2. Conditions which do not share a letter  
650 are significantly different in Holm-Bonferroni's multiple comparison test (p< 0.0001). Scale  
651 bar indicates 500  $\mu$ m.

652

653 **Figure 5 | StADF2 alters StREM1.3 nanodomains to inhibit PVX cell-to-cell movement.**

654 **A-B.** Plasma membrane organization of YFP-StREM1.3 with or without co-expression of HA-  
655 StADF2 and HA-StADF2<sup>S6D</sup>. Representative confocal micrograph (**A**) and corresponding  
656 quantification of YFP-StREM1.3 spatial clustering index (**B**). Graphs are notched box plots,  
657 scattered data points show measurements, colors indicate independent experiments, n=38  
658 cells for YFP-StREM1.3, n=38 cells for YFP-StREM1.3 + HA-StADF2 and n=38 cells for YFP-  
659 StREM1.3 + HA-StADF2<sup>S6D</sup>. Conditions which do not share a letter are significantly different in  
660 Holm-Bonferroni's multiple comparison test (p< 0.0001). Scale bar indicates 2  $\mu$ m.

661 **C-D.** PVX infection assays. Representative micrograph of PVX:GFP infection foci upon  
662 expression of HA-StADF2 or HA-StADF2<sup>S6D</sup> (**C**) and corresponding quantification (**D**). Graphs  
663 are notched box plots, scattered data points show measurements, colors indicate  
664 independent experiments, number of infections foci n=39 for mock, n=38 foci for HA-StADF2

665 and n=31 foci for HA-StADF2<sup>S6D</sup>. Conditions which do not share a letter are significantly  
666 different in Dunn's multiple comparison test ( $p < 0.0001$ ). Scale bar indicates 500  $\mu\text{m}$ .

667

668

669 **Figure supplemental 1 | StREM1.3 N-terminal domain is intrinsically disordered.**

670 **A.** SDS-PAGE of purified untagged StREM1.3<sup>1-116</sup> under native and denaturing conditions.

671 **B.** <sup>1</sup>H-<sup>15</sup>N HMQC spectra of REM<sup>1-116</sup> purified under native (black) or denaturing conditions  
672 using 7M of urea (red). Temperature: 10°C.

673

674 **Figure supplemental 2 | AtCPK3 phosphorylates StREM1.3<sup>1-116</sup> under NMR conditions.**

675 **B.** *In vitro* kinase assay to confirm phosphorylation of StREM1.3<sup>1-116</sup> under liquid-state NMR  
676 conditions. StREM1.3<sup>1-116</sup> incubated for either 30 min or 2 h with wild-type AtCPK3-GST  
677 untreated (WT) or heat-inactivated (D).

678

679 **Figure supplemental 3 | Cub-StREM1.3 and Cub-StREM1.3<sup>DDD</sup> are functional baits in SUY2H.**

680 **A-B.** Drop-tests of SUY2H assays testing binary interactions between Cub-StREM1.3 (**A**) with  
681 NubG-StREM1.3 or StREM1.3-NubG, or Cub-StREM1.3<sup>DDD</sup> (**B**) with NubG-StREM1.3 or  
682 StREM1.3-NubG.

683 **C.** Representative micrograph of GFP-StREM1.3 and GFP-StREM1.3<sup>DDD</sup> imaged in proliferating  
684 *S. cerevisiae*. Scale bar indicates 2  $\mu\text{m}$ .

685

686 **Figure supplemental 4 | Rational design of the SUY2H screens.**

687 **A.** Schematics depicting the experimental design. Cub-StREM1.3 and Cub-StREM1.3<sup>DDD</sup> were  
688 used as baits against a cDNA library obtained from PVX-infected *N. benthamiana* epidermis  
689 in side-by-side screens.

690 **B.** Picture of a *N. benthamiana* leaf peeled. Scale bar indicates 5 cm.

691 **C.** Confocal micrograph of peeled *N. benthamiana* epidermis infected with PVX:GFP. Scale bar  
692 indicates 5  $\mu\text{m}$ .

693

694 **Figure supplemental 5 | Western blots analyses of the expression of protein candidates in**  
695 **PVX cell-to-cell movement assays.**

696 Protein extracts from *N. benthamiana* leaves expressing HA-tagged candidates were analyzed  
697 by western blots with  $\alpha$ -HA antibody. In case of multiple bands, a white star points out the  
698 band corresponding to the expected molecular weight. Abbreviations are as followed:  
699 Dormancy-associated Protein-like 1 (NbDRM1), Calreticulin 3 (NbCRT3), Glutathione S-  
700 transferase 8 (NbGST8), Plasma membrane intrinsic protein 1C (NbPIP1C), Actin  
701 Depolymerizing Factor 3 (NbADF3), Metallothionein 2B (NbMT2B), Delta tonoplast integral  
702 protein (NbTIP2;1), Thioredoxin superfamily protein 4 (NbTRX4), Autophagy 8I (NbATG8i),  
703 GTPase protein (NbRabA4A).

704

705 **Figure supplemental 6 | StADF2 affects YFP-StREM1.3 nanodomain organization and YFP-**  
706 **StREM1.3 nanodomain relies on the actin cytoskeleton integrity.**

707 **A-B.** Plasma membrane organization of YFP-StREM1.3 with or without co-expression of RFP-  
708 StADF2. Representative confocal micrograph (**A**) and corresponding quantification of YFP-  
709 StREM1.3 spatial clustering index (**B**) are shown. Graphs are notched box plots, scattered data  
710 points show measurements, colors indicate independent experiments, n=39 cells for YFP-  
711 StREM1.3, n=41 cells for YFP-StREM1.3 + RFP-StADF2. P values report two-tailed non-  
712 parametric Mann-Whitney test. Scale bar indicates 5 $\mu$ m.

713 **C-D.** Plasma membrane organization of YFP-StREM1.3 co-expressed with LifeAct-mCherry to  
714 label actin upon 80  $\mu$ M Cytochalasin D treatment for 24h or corresponding mock control  
715 (DMSO). Representative confocal micrograph (**C**) and corresponding quantification of YFP-  
716 StREM1.3 spatial clustering index (**D**) are shown. Graphs are notched box plots, scattered data  
717 points show measurements, colors indicate independent experiments, n=34 cells for mock  
718 treatment, n=34 cells for Cytochalasin D treatment. P values report two-tailed non-parametric  
719 Mann-Whitney test. Scale bar indicates 5 $\mu$ m.

720

721 **Figure supplemental 7 | Serine 6 is conserved among ADFs.**

722 Sequence logo of ADFs N-termini generated from 459 protein sequences retrieved from 101  
723 plant species using BLASTp and StADF2 as query.

724

725



## 726 MATERIALS AND METHODS

### 727 Plant materials, culture and transformation

728 *N. benthamiana* plants were cultivated in controlled conditions (16 h photoperiod, 25 °C). *N.*  
729 *benthamiana* plants were transiently transformed using the *Agrobacterium tumefaciens*  
730 strain GV3101 as previously described (Gronnier et al., 2017). For subcellular localization  
731 study and western blotting analyses, plants were analyzed 2 days after infiltration (dai). For  
732 PVX:GFP spreading assays, plants were observed 5 dai. The *N. benthamiana* transgenic RNAi  
733 knock-down lines hpREM #1.4 and hpREM #10.2 were previously described (Perraki et al.,  
734 2018).

735

### 736 PVX cell-to-cell movement

737 The assays were conducted as previously described (Perraki et al., 2018). *Agrobacteria*  
738 solution ( $OD^{600nm} = 0.001$ ) carrying PVX:GFP and the helper plasmid pSoup was mixed to equal  
739 volume with *agrobacteria* carrying a binary plasmid encoding for tested proteins ( $OD^{600nm} =$   
740 0.2) and co-infiltrated in leaves of 3 weeks old *N. benthamiana*. Plants were observed at 5 dai  
741 using Zeiss Axiozoom V16 equipped with a UV lamp, an excitation filter (450-490 nm) and an  
742 emission filter (500-550 nm) to detect GFP signal. Foci were automatically analyzed using the  
743 Fiji software (Schindelin et al., 2012) via a homemade macro.

744

### 745 Membrane-based split-ubiquitin Yeast two-hybrid

746 Split ubiquitin assays were performed using the yeast two-hybrid system from DUAL  
747 membrane system (Dual systems Biotech AG). StREM1.3<sup>WT</sup> and StREM1.3<sup>S74D T86D S91D</sup> coding  
748 sequences were amplified by PCR using Sfil restriction site-containing primers (Supplemental  
749 Table 2) and subsequently cloned in pBT3N bait and pPR3N prey plasmids. To test the  
750 functionality of StREM1.3 in this system, THY.AP4 cells were sequentially transformed with  
751 pBT3N:StREM1.3 and pPR3N:StREM1.3 or pPR3N empty vector as a negative control. The  
752 cDNA library was constructed following the manufacturer's instructions (Evrogen) using  
753 approximately 700 ng total RNA of epidermis peeled from healthy and PVX-infected *N.*  
754 *benthamiana* leaves, harvested three days after agroinfiltration. For screening, NubG-cDNA  
755 library was used for transformation of THY.AP4 yeast strain *MATa*, *ura3-*, *leu2-*, *lexA-*  
756 *lacZ::TRP1*, *lexA::HIS3*, *lexA::ADE2*) previously transformed with pBT3N:StREM1.3 or  
757 pBT3N:StREM1.3<sup>DDD</sup>. Positive clones were selected on synthetic medium (SD) lacking adenine,



758 histidine, tryptophan and leucine (-AHLW) and subsequently tested for  $\beta$ -galactosidase  
759 activity. To measure  $\beta$ -galactosidase activity, yeasts were grown on SD-TL for two days at  
760 28°C. Plates were then covered with a X-Gal-agarose buffer (0.5% agarose, 0.5 M phosphate  
761 buffer, pH 7.0, 0.002% X-Gal) and incubated at 37°C for 10 to 20 min. To identify the proteins  
762 expressed in Yeast positive clones obtained from the SUY2H exploratory screens, the  
763 corresponding plasmids were isolated from Yeast, subsequently propagated in *E. coli*, isolated  
764 and analyzed by Sanger sequencing. Individual coding sequences were BLAST against *N.*  
765 *benthamiana* Genome v1.0.1 predicted cDNA (<https://solgenomics.net/>).

766

### 767 ***In silico* analyses**

768 During SUY2H exploratory screens, identification of proteins expressed in yeast positive  
769 clones was retrieved using BLASTn algorithm against *N. benthamiana* Genome v1.0.1  
770 predicted cDNA. Closest orthologs of the identified StREM1.3-interacting proteins in *A.*  
771 *thaliana* were retrieved on The Arabidopsis Information Resource TAIR using BLASTp  
772 algorithm. The 500 hundreds closest homologs of StADF2 were retrieved from 101 plant  
773 species in Phytozome (Goodstein et al., 2012) using BLASTp. Protein alignment was computed  
774 using MULTiple Sequence Comparison by Log-Expectation (MUSCLE; (Edgar, 2004)) using  
775 BLOSUM62 matrix, an -sv profile scoring method with following parameters: Anchor  
776 spacing:32, diagonal break:1, diagonal length:24, diagonal margin:5, gap extension penalty:-  
777 1, gap open penalty:-12, hydro:5 and hydro factor1.2, through the JABAWS server (Troshin et  
778 al., 2011). Among 500 ADF protein sequences, 41 are predicted splice variants lacking the first  
779 7 amino acids and were excluded for sequence logo analysis, generated using WebLogo  
780 (Crooks et al., 2004).

781

### 782 **Molecular cloning**

783 Candidate genes selected from the screen were cloned from the cDNA bank generated for  
784 the SUY2H screen using primers designed to amplify full length coding sequences. All vector  
785 constructs were generated using classical Gateway cloning strategies  
786 ([www.lifetechnologies.com](http://www.lifetechnologies.com)), using pDONR211 and pDONR207 as entry vectors and  
787 pK7WGY2, pK7YWG2, pK7WGR2, pK7RWG2 (Karimi et al., 2002), pGWB14, pGWB15  
788 (Nakagawa et al., 2007) and pSite BiFC as destination vectors (Martin et al., 2009). StADF2  
789 and StADF2<sup>S6D</sup> bearing attL sequences were synthesized (<https://www.genscript.com/>) in a

790 pUC57 vector to be cloned in aforementioned destination vectors. The StREM1.3<sup>S74D T86D S91D</sup>  
791 was previously described (Perraki et al., 2018). For GFP-StREM1.3 expression in  
792 *Saccharomyces cerevisiae*, StREM1.3<sup>WT</sup> and StREM1.3<sup>DDD</sup> coding sequences were PCR  
793 amplified with oligonucleotides listed in supplemental table S2, digested by BamHI–NsiI and  
794 cloned at BamHI–PstI sites of the plasmids p2717 (pCM189 modified by the introduction of a  
795 myc epitope tag downstream of the tet promoter (Escusa et al., 2006)). The resulting plasmids  
796 (respectively pMC101 and pMC104) were transformed into a wild type strain BY4741 (*MAT $\alpha$* ,  
797 *his3 $\Delta$ 1*, *leu2 $\Delta$ 0*, *met15 $\Delta$ 0*, *ura3 $\Delta$ 0*). All plasmids were propagated using the NEB10 *E. coli*  
798 strain (New England Biolabs).

799

### 800 **Protein expression in *E. coli* and purification**

801 StREM1.3<sup>1-116</sup> was obtained as previously described (Legrand et al., 2022). Shortly, StREM1.3<sup>1-</sup>  
802 <sup>116</sup> was expressed in BL21-DE3 cells in minimal medium with <sup>13</sup>C-labelled glucose and <sup>15</sup>NH<sup>4</sup>Cl,  
803 by addition of 1 mM IPTG at OD<sup>600</sup> = 0.6-0.8 and incubation at 37°C for 3h. Cells were lysed by  
804 sonication and the supernatant was loaded onto a HisTrap column (GE Healthcare)  
805 equilibrated in 20 mM HEPES 150 mM NaCl, 20 mM imidazole, 0.02% NaN<sub>3</sub>, pH=7.4 and eluted  
806 with 20 mM HEPES, 150 mM NaCl, 500 mM imidazole, 0.02% NaN<sub>3</sub>, pH=7.4. For TEV cleavage,  
807 eluted StREM1.3<sup>1-116</sup> was adjusted to 1 mM DTT and 0.5 mM EDTA, the TEV protease was  
808 added in a ~1/200 TEV/REM<sup>1-116</sup> mass ratio and the reaction was incubated for 3h at room  
809 temperature then desalted against 10 mM HEPES, 50 mM NaCl, 0.02% NaN<sub>3</sub>, pH=7.5 with a  
810 HiPrep column (GE Healthcare). Under native conditions, StREM1.3<sup>1-116</sup> was loaded onto a  
811 HisTrap column equilibrated with 20 mM HEPES, 150 mM NaCl, 0.02% NaN<sub>3</sub>, pH=7.4 and  
812 eluted with the same elution buffer as above. Under denaturing conditions, this step was  
813 performed in buffers containing 7M urea. Finally, StREM1.3<sup>1-116</sup> was desalted again against 10  
814 mM HEPES, 50 mM NaCl 0.02%, NaN<sub>3</sub> pH=7.5. AtCPK3-GST recombinant protein was  
815 produced in BL21-DE3-pLys and purified as previously reported (Legrand, et al., 2022). As a  
816 negative control, AtCPK3-GST was inactivated by heating at 95°C for 10 min.

817

### 818 ***In vitro* phosphorylation**

819 *In vitro* phosphorylation of 0.5 mM of StREM1.3<sup>1-116</sup> by AtCPK3 was done as previously  
820 published (Legrand et al., 2022). For NMR analysis, the StREM1.3<sup>1-116</sup> sample was adjusted to

821 10 mM MgCl<sub>2</sub>, 1 mM CaCl<sub>2</sub>, 1 mM DTT and 3 mM ATP. The reaction was initiated by the  
822 addition of 88 μM of AtCPK3 and incubation at 20°C.

823

### 824 **Liquid-state Nuclear magnetic resonance (NMR) spectroscopy**

825 NMR spectroscopy was performed on a Bruker Advance NEO spectrometer operating at 700  
826 MHz for proton with a TXI 5 mm probe. The samples were adjusted to 9/1 H<sub>2</sub>O/D<sub>2</sub>O (v/v) to  
827 lock the magnetic field. Monitoring of phosphorylation kinetics was performed using a <sup>1</sup>H-<sup>15</sup>N  
828 HMQC pulse sequence (Schanda & Brutscher, 2005), with 2 scans per timepoint (*i.e* 1 min 42  
829 s to acquire one spectrum). The spectra were processed and analyzed using TopSpin (Bruker).

830

### 831 **Confocal laser scanning microscopy and image analysis**

832 Live imaging was performed using a Zeiss LSM 880 confocal laser scanning microscopy system  
833 using a 68x objective and the AiryScan detector. YFP fluorescence was observed using an  
834 excitation wavelength of 488 nm and an emission wavelength of 453nm. RFP and mCherry  
835 fluorescence were observed using an excitation wavelength of 561 nm and detected at 579  
836 nm. Acquisition parameters were kept identical across experiments. The SCI was calculated  
837 as previously described (Gronnier et al., 2017). Briefly, 10 μm lines were plotted across the  
838 samples and the SCI was calculated by dividing the mean of the 5 % highest values by the  
839 mean of 5 % lowest values. Three lines were randomly plotted per cell.

840 To disrupt actin cytoskeleton integrity, Cytochalasin D (40 μg/mL) dissolved in DMSO was  
841 infiltrated 24 hours before observation, infiltration of DMSO for 24 hours was used as  
842 corresponding mock control.

843 Yeast cells were observed on a fully-automated Zeiss 200M inverted microscope (Carl Zeiss,  
844 Thornwood, NY, USA) equipped with an MS-2000 stage (Applied Scientific Instrumentation,  
845 Eugene, OR, USA), a Lambda LS 300-Watt xenon light source (Sutter, Novato, CA, USA), and a  
846 100 × 1.4 NA Plan-Apochromat objective. GFP imaging was done using a FITC filter (excitation,  
847 HQ487/25; emission, HQ535/40; beam splitter, Q505lp). Images were acquired using a Prime  
848 sCMOS 95B camera (Photometrics, Tucson, AZ, USA). The microscope, camera, and shutters  
849 (Uniblitz, Rochester, NY, USA) were controlled by SlideBook software 5. 0. (Intelligent Imaging  
850 Innovations, Denver, CO, USA).

851

## 852 **Western-blotting**

853 Proteins were extracted from *N. benthamiana* leaf tissue transiently expressing HA-tagged  
854 proteins in 100 mM Tris (pH 7.5) containing 3% SDS, 5mM EDTA and 2% Protease inhibitor  
855 (Roche, Complete) boiled for 10 min at 70°C in SDS loading buffer, and cleared by  
856 centrifugation. The protein extracts were then separated by SDS-PAGE, transferred to  
857 polyvinylidene difluoride (PVDF) membranes (BioRad), blocked with 5% skimmed milk in TBS-  
858 Tween 0.05%, and incubated with an anti-HA antibody coupled to horse radish peroxidase  
859 (Roche). The resulting western-blotting were developed using an ECL Prime Kit (GE Healthcare)  
860 and detected with an ImageQuant 800 (Amersham).

861

## 862 **Co-immunoprecipitation**

863 Immunoprecipitation assays were performed as previously described in (Dagdaz et al., 2016)  
864 with minor modifications. Approximately 3 g of *N. benthamiana* leaves were ground with a  
865 mortar and pestle in liquid nitrogen and homogenized in 6 mL of extraction buffer (50 mM  
866 Tris- HCl, pH 7.5, 150 mM NaCl, 10% glycerol, 5 mM DTT, 1 mM EDTA, 2% (w/v)  
867 polyvinylpyrrolidone, 1% (v/v) Complete protease inhibitor cocktail [Roche], 0.1% (v/v)  
868 IGEPAL). Samples were then centrifuged for 20 min with 2000 x g at 4°C. Immunoprecipitation  
869 was performed by adding 30 µl of GFP-Trap coupled to agarose beads (ChromoTek) and  
870 samples were continuously agitated for 2 hours at 4°C. Beads were subsequently washed five  
871 times with extraction buffer and eluted with 30 µl of 2X Laemmli buffer at 70 °C for 10  
872 minutes.

873

## 874 **Statistical analyses**

875 Statistical analyses were carried out using Prism 6.0 software (GraphPad). As mentioned in  
876 the figure legends, statistical significances were assessed using non-parametric Kruskal-Wallis  
877 bilateral tests combined with post-hoc Dunn's multiple pairwise comparisons, or using a two-  
878 way non-parametric Mann-Whitney test.

879

## 880 **Accession numbers**

881 NbADF3 (NbS00025994g0001.1), NbCRT3 (NbS00018258g0010.1), NbMT2B  
882 (NbC25904295g0003.1), NbATG8I (NbS00005942g0011.1), NbPIP1C (NbS00006841g0003.1),  
883 NbTIP2;1, (NbS00006781g0007.1), NbDRM1 (NbS00004204g0005.1), NbGST8

884 (NbS00007668g0012.1), NbTRXH4 (NbS00049748g0003.1), NbRabA4A  
885 (NbS00057294g0003.1), StREM1.3 (NP\_001274989), StADF2 (Soltu.DM.04G007350).

886

## 887 **ACKNOWLEDGEMENTS**

888 We thank the Bordeaux Imaging Center, part of the National Infrastructure France-BioImaging  
889 supported by the French National Research Agency (ANR-10-INBS-04). This work was  
890 supported by the French National Research Agency (grant no. ANR-19-CE13-0021 to SGR, SM,  
891 VG and doctoral fellowships to MDJ, PG and JG), and the German Research Foundation (DFG)  
892 grant CRC1101-A09 to JG, the IPS2 benefits from the support of the LabEx Saclay Plant  
893 Sciences-SPS (ANR- 10-LABX-0040-SPS). We thank Isabel Monte and members of the  
894 NanoSignaling Lab for discussions and critical reading of the manuscript.

895

896 The authors declare no conflict of interest

897

## 898 **AUTHORS CONTRIBUTION**

899 ALe purified labelled StREM1.3<sup>1-116</sup> and performed NMR experiments

900 ALe, BH and AL analyzed NMR results

901 JG and VG built the cDNA libraries

902 AML and IS localized fluorescent tagged StREM1.3 in yeast

903 JG performed the split-ubiquitin assays

904 PG, MDJ and JG performed and analyzed virus propagation

905 MDJ performed confocal microscopy

906 MB performed in vitro kinase assay

907 JG, MD, BH, AL, VG, SM designed the project

908 MDJ and JG assembled the figures

909 MDJ and JG wrote the manuscript with input from all authors.

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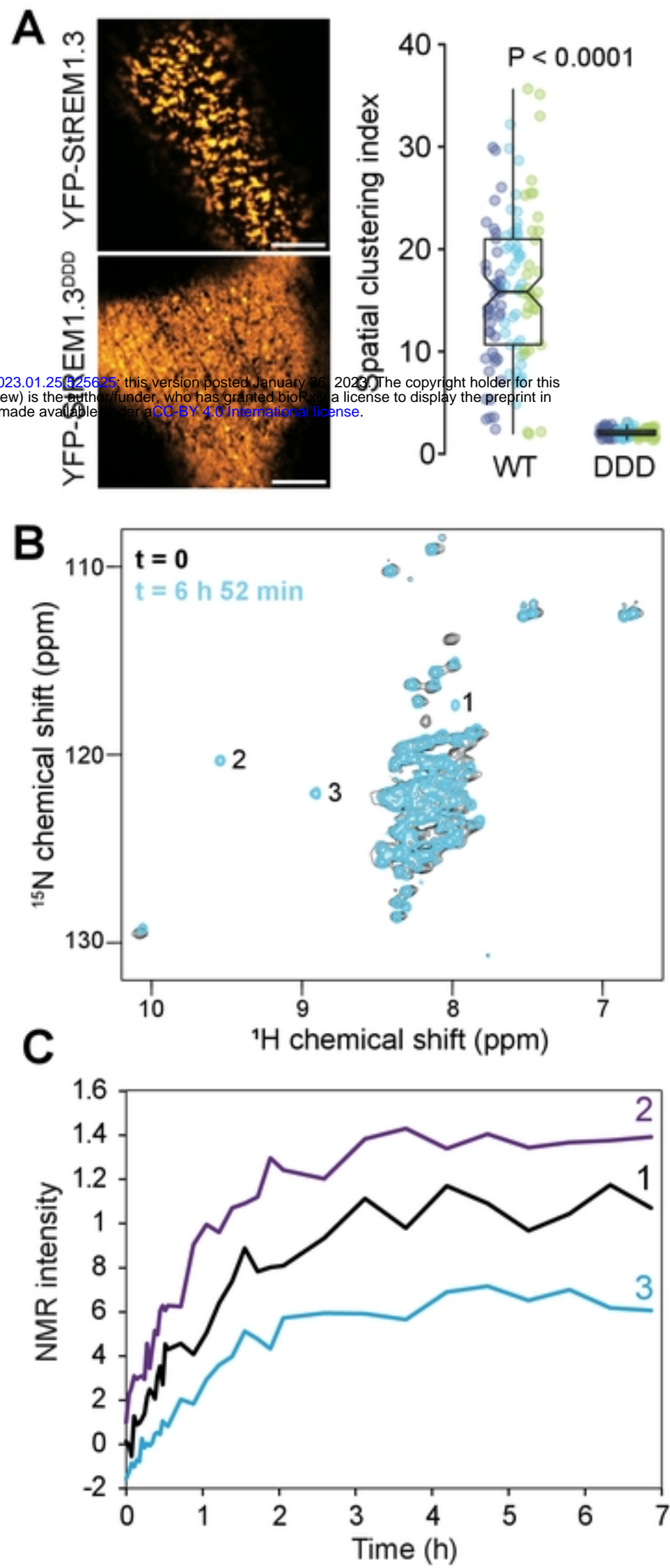


Figure 1 | Phosphorylation modulates StREM1.3 intrinsically disordered domain.



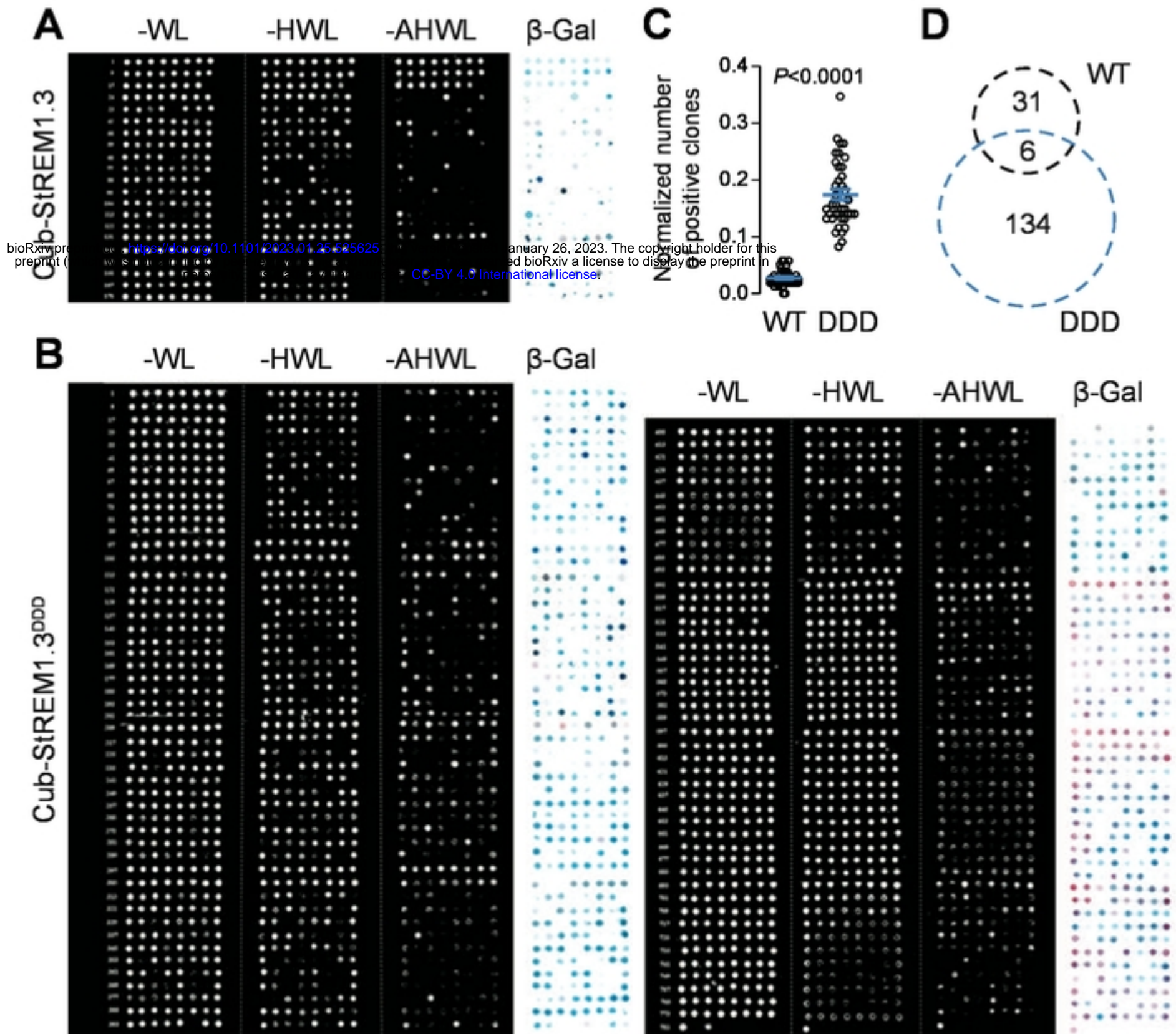
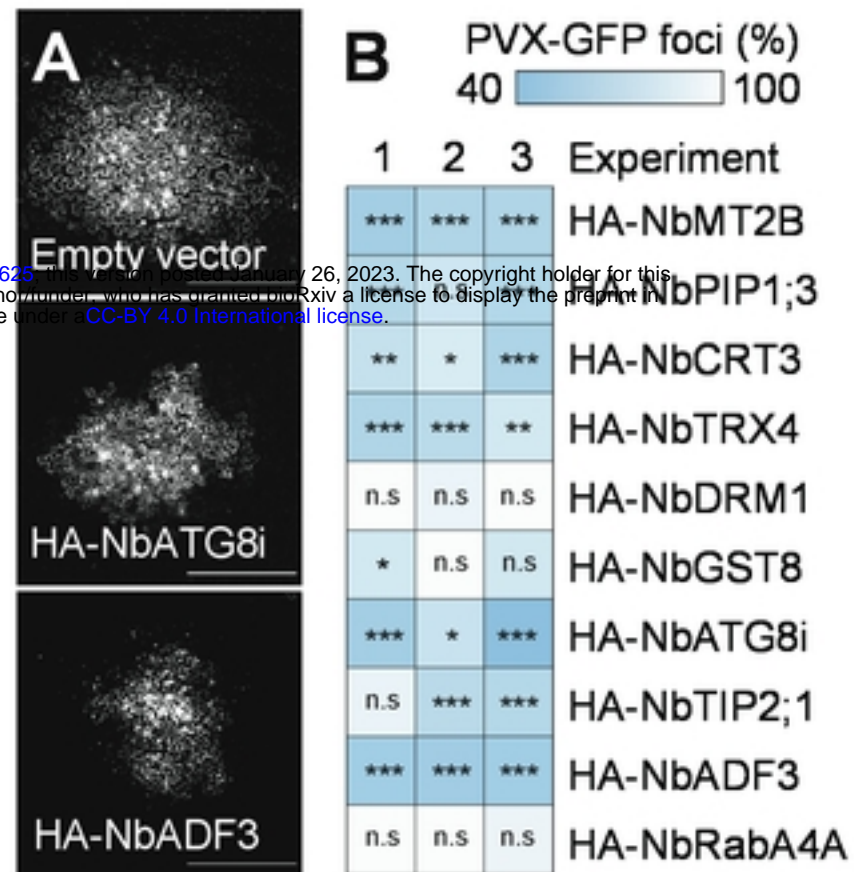


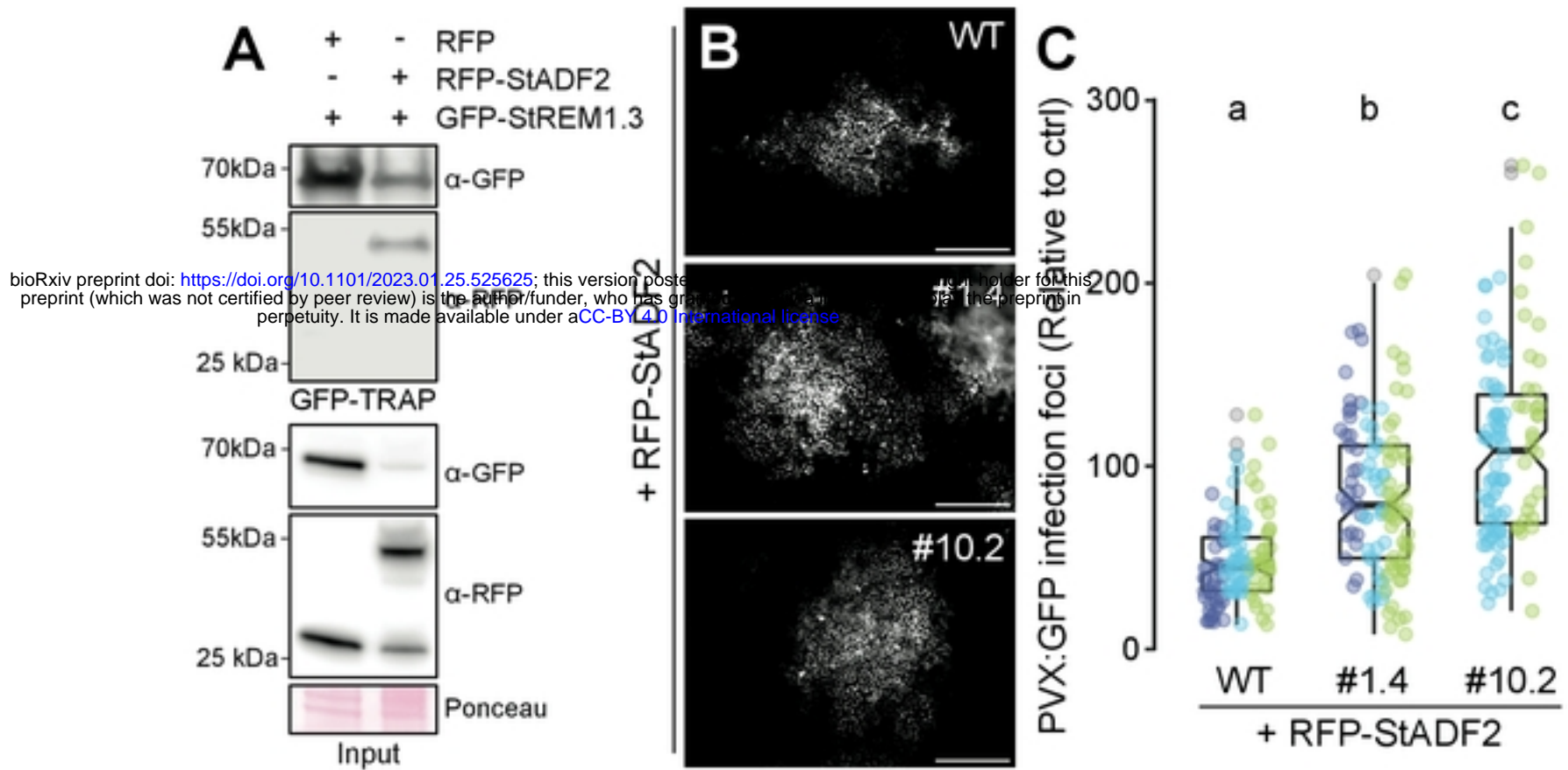
Figure 2 | Comparative analysis of StREM1.3 and StREM1.3<sup>DDD</sup> interactomes in SUY2H.



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**Figure 3 | Functional analysis of StREM1.3's putative interactors upon PVX infection.**



**Figure 4 | StADF2 limits PVX cell-to-cell movement in a REMORIN-dependent manner.**

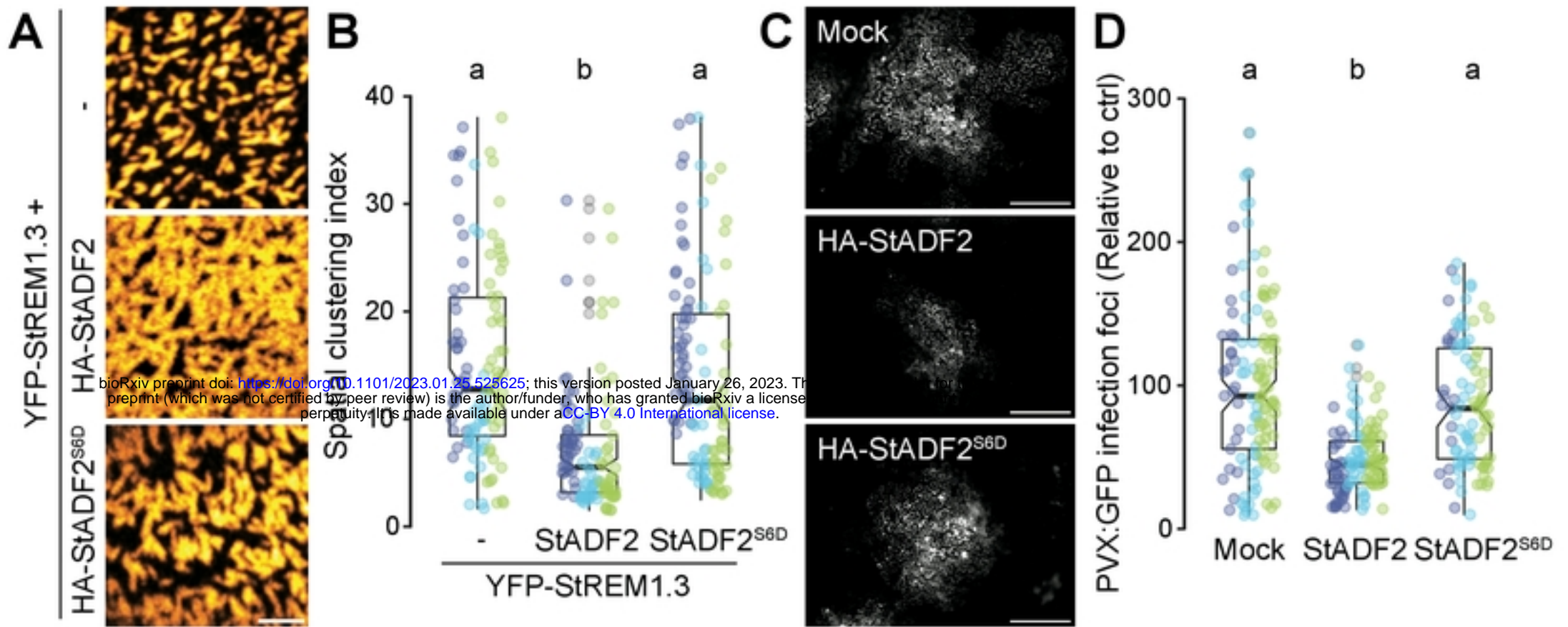
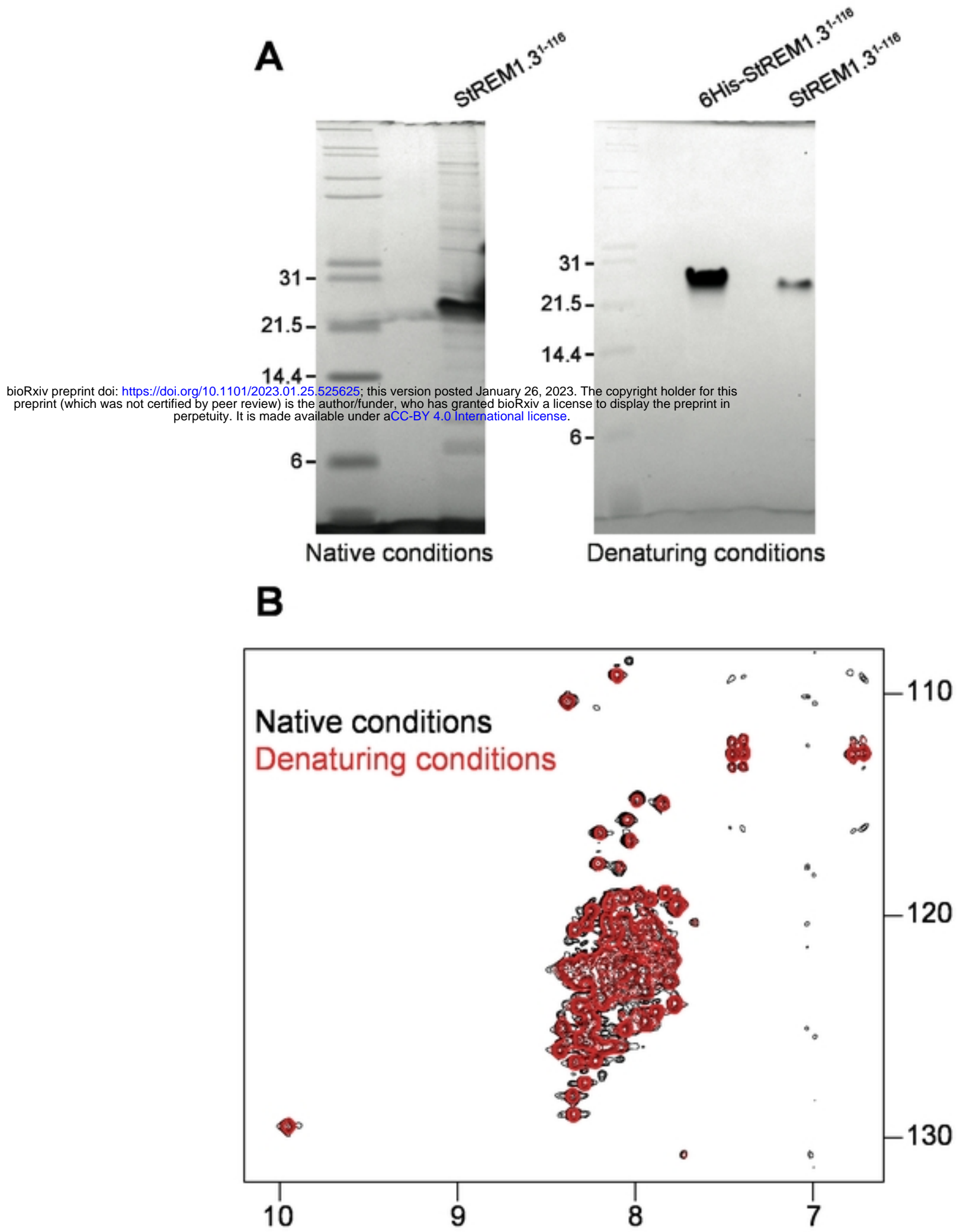
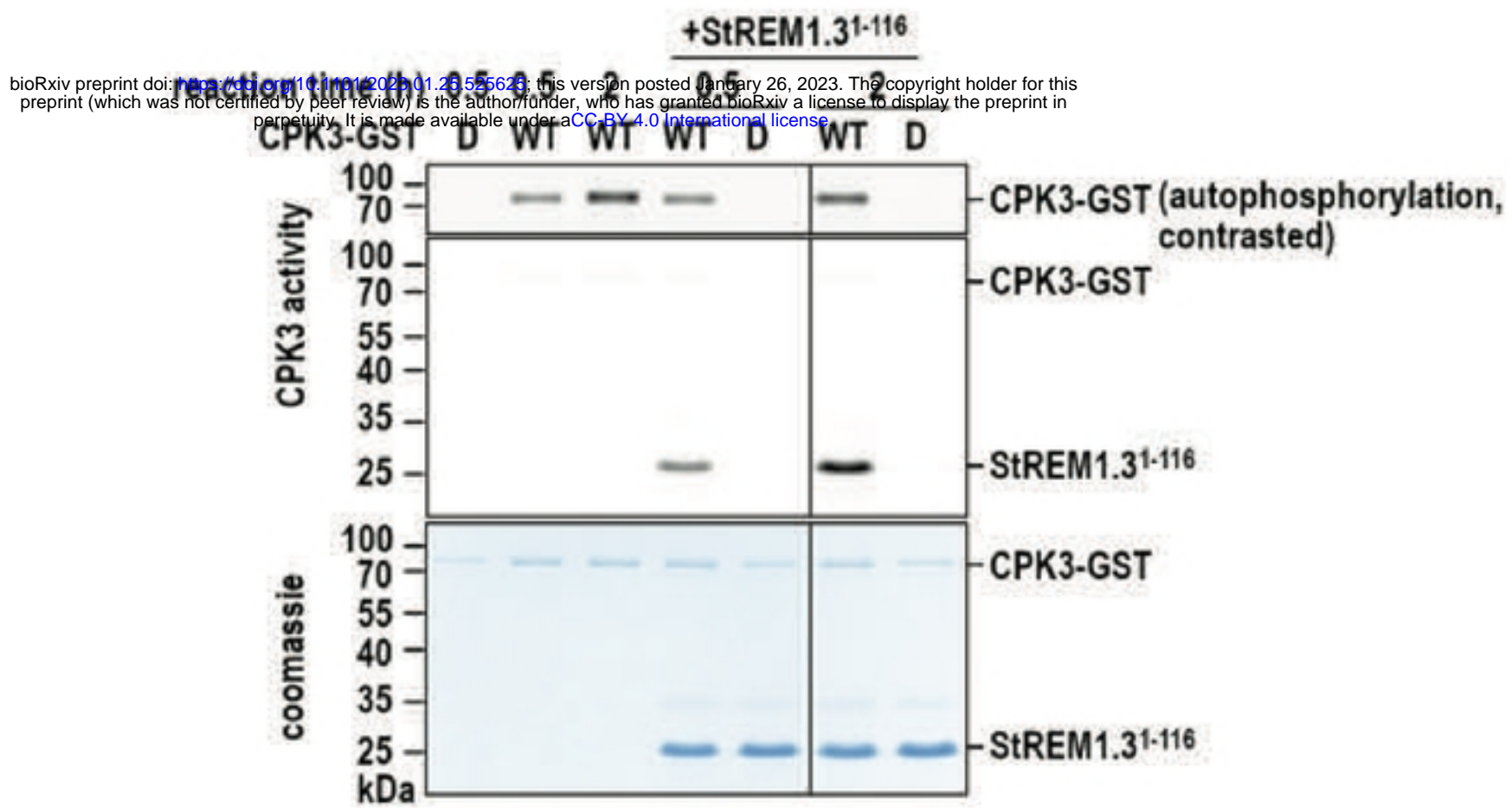


Figure 5 | StADF2 alters StREM1.3 nanodomains to inhibit PVX cell-to-cell movement.



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Figure supplemental 1 | StREM1.3 N-terminal domain is intrinsically disordered



**Figure supplemental 2 | AtCPK3 phosphorylates StREM1.3<sup>1-116</sup> under NMR conditions.**



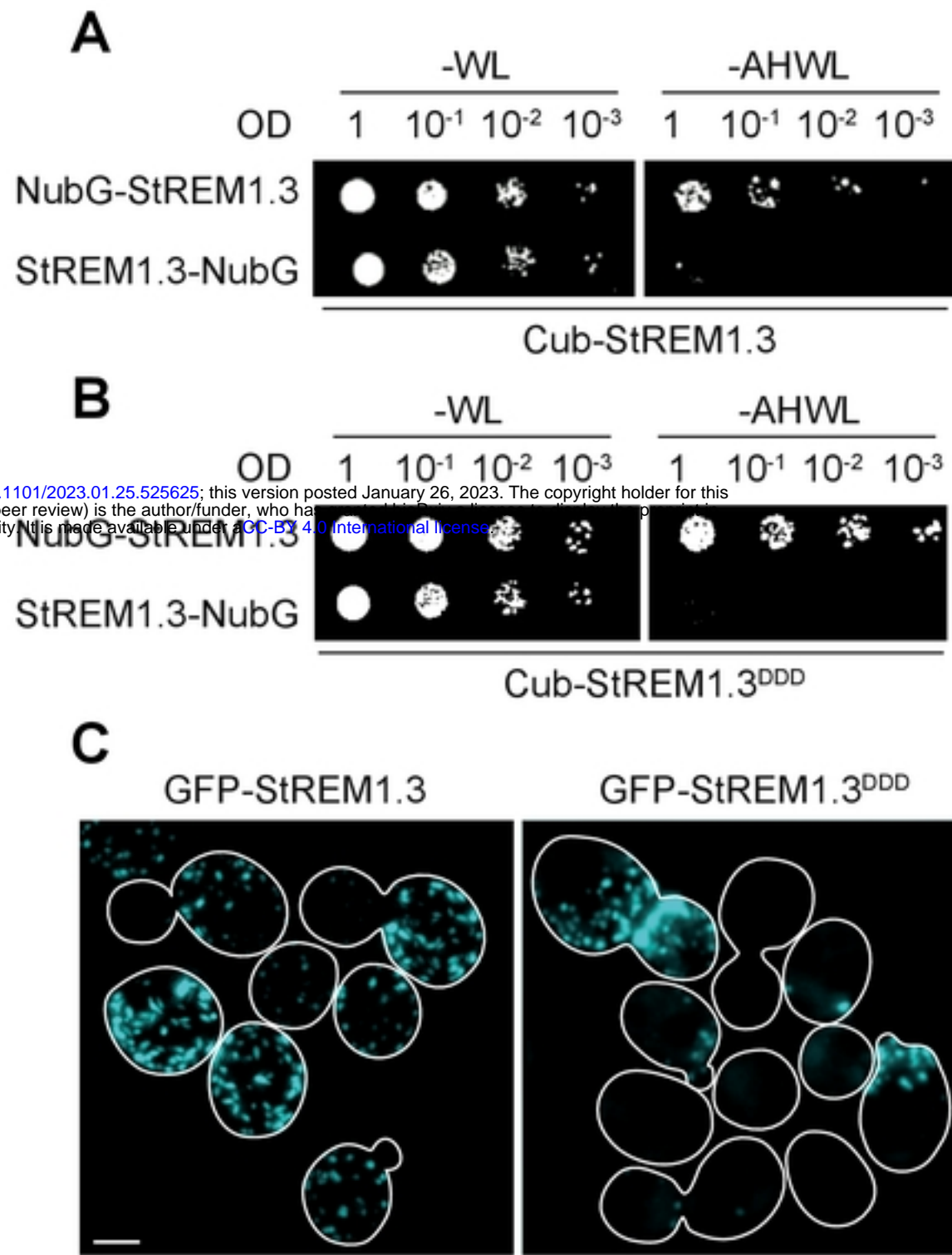
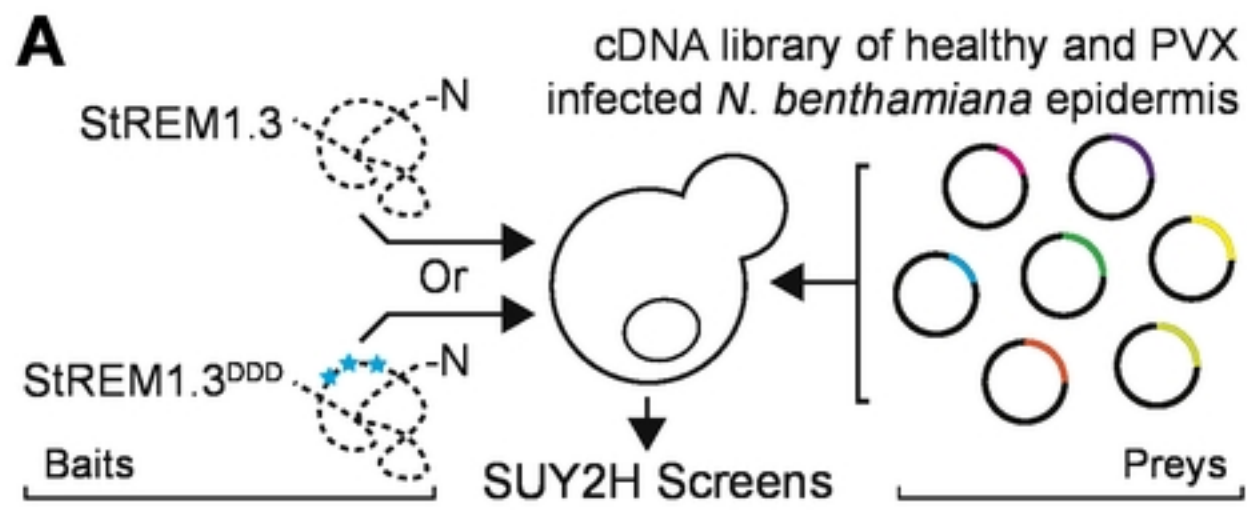


Figure supplemental 3 | Cub-StREM1.3 and Cub-StREM1.3<sup>DDD</sup> are functional baits in SUY2H.



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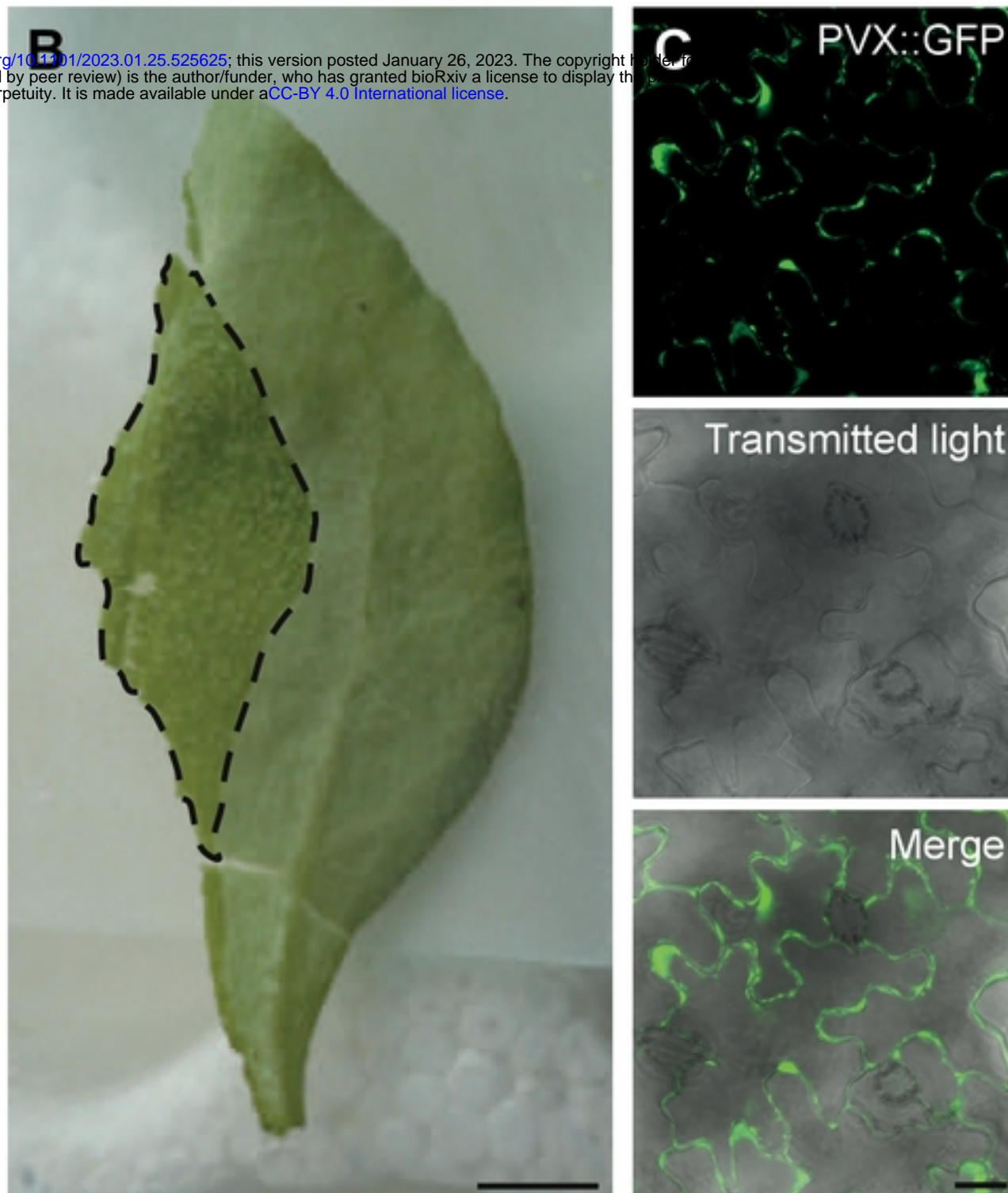
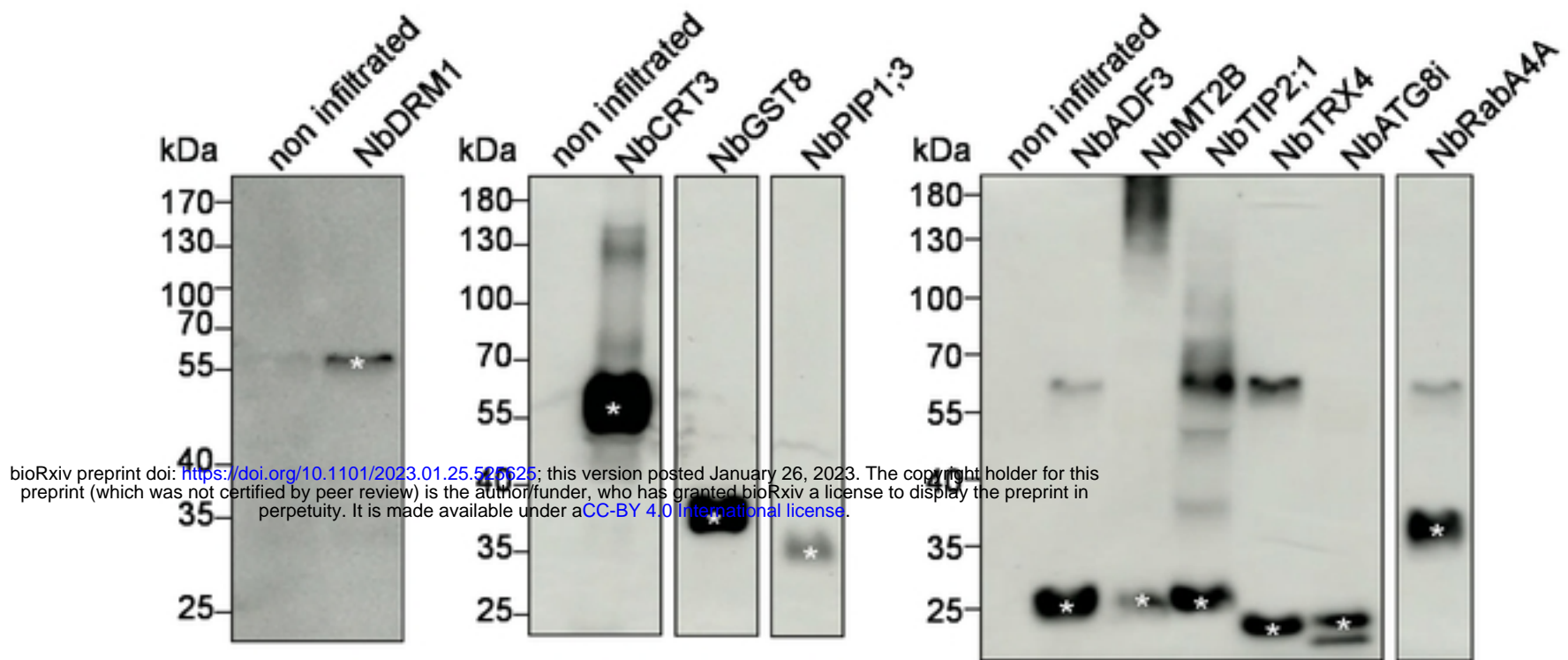


Figure supplemental 4 | Rational design of the SUY2H screens.





**Figure supplemental 5 | Western blots analyses of the expression of protein candidates in PVX cell-to-cell movement assays.**

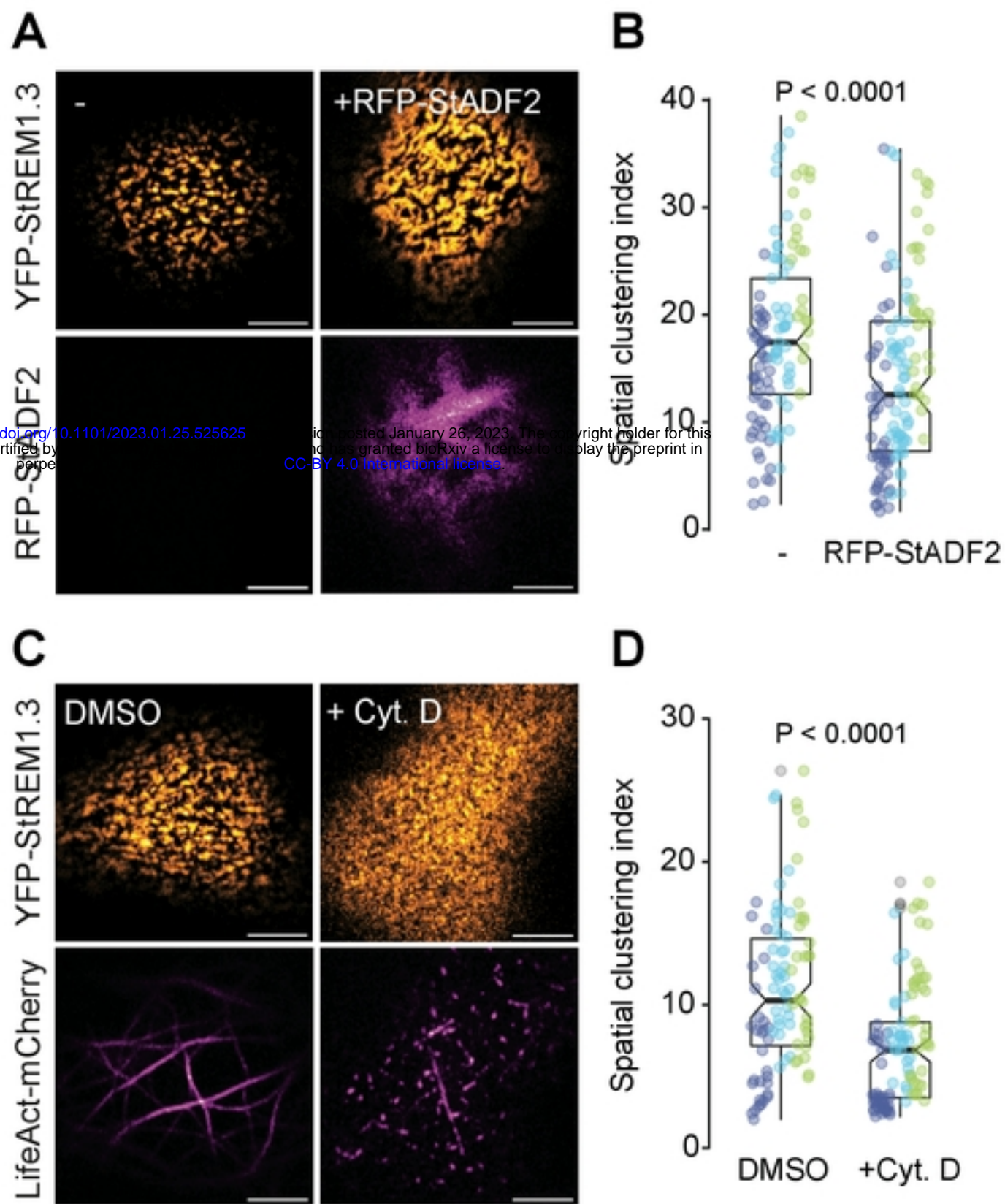


Figure supplemental 6 | StADF2 affects YFP-StREM1.3 nanodomain organization and YFP-StREM1.3 nanodomain rely on the actin cytoskeleton integrity.

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Figure supplemental 7 | Serine 6 is conserved among ADFs