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The CELL NUMBER REGULATOR FW2.2 protein regulates cell-to-cell communication in tomato by modulating callose deposition at plasmodesmata

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1 **The CELL NUMBER REGULATOR FW2.2 protein regulates cell-to-cell**
2 **communication in tomato by modulating callose deposition at plasmodesmata**

3
4 **Short Title:** FW2.2/CNR regulates cell-to-cell communication

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30

31 **Abstract**

32 *FW2.2* (standing for *FRUIT WEIGHT 2.2*), the founding member of the *CELL*
33 *NUMBER REGULATOR (CNR)* gene family, was the first cloned gene underlying a
34 quantitative trait locus (QTL) governing fruit size and weight in tomato (*Solanum*
35 *lycopersicum*). However, despite this discovery over 20 years ago, the molecular
36 mechanisms by which *FW2.2* negatively regulates cell division during fruit growth
37 remain undeciphered. In the present study, we confirmed that *FW2.2* is a membrane-
38 anchored protein whose N- and C-terminal ends face the apoplast. We unexpectedly
39 found that *FW2.2* is located at plasmodesmata (PD). *FW2.2* participates in the
40 spatiotemporal regulation of callose deposition at PD and belongs to a protein
41 complex which encompasses callose synthases. These results suggest that *FW2.2*
42 has a regulatory role in cell-to-cell communication by modulating PD transport
43 capacity and trafficking of signaling molecules during fruit development.

44

45 **INTRODUCTION**

46 The tight coordination of developmental processes such as cell division, cell
47 expansion and cell differentiation, is pivotal for proper plant growth at the whole
48 organismal, organ
49 and tissue level. Unravelling the genes that contribute to impact plant yield and
50 biomass, and improve agronomic quality traits, is thus a major goal of plant biology
51 and agronomy. In the particular case of tomato (*Solanum lycopersicum*) fruit size
52 determination, nearly 30 Quantitative Trait Loci (QTL) governing fruit size/weight
53 have been identified (Grandillo et al., 1999; Lippman and Tanksley, 2001; van der
54 Knaap and Tanksley, 2003). However, the molecular basis governing these QTLs
55 remains mostly undeciphered, and only three major genes underlying such QTLs in
56 tomato have been identified and cloned so far (Frary et al., 2000; Chakrabarti et al.,
57 2013; Mu et al., 2017).

58 *FW2.2* (standing for Fruit Weight QTL on chromosome 2, number 2;
59 Solyc02g090730) was the first cloned gene underlying a QTL related to fruit size in
60 tomato (Alpert et al., 1995; Frary et al., 2000). The encoded protein *FW2.2* was
61 defined as a major negative regulator of cell divisions in young developing fruit, thus
62 impacting fruit size (Frary et al., 2000; Cong et al., 2002; Liu et al., 2003; Nesbitt and

63 Tanksley, 2001; Baldet et al., 2006). FW2.2 was the founding member of the CELL
64 NUMBER REGULATOR/FW2.2-Like (CNR/FWL) protein family (Guo et al., 2010),
65 whose function in organ size control seems to be conserved in both monocotyledon
66 and dicotyledon plants (for a review, see Beauchet et al., 2021). Members of this
67 protein family possess a conserved PLAC8 (Placenta-specific gene 8 protein) domain
68 (Galaviz-Hernandez et al., 2003), which is composed of one or two hydrophobic
69 segments, predicted to form transmembrane (TM) helices (Song et al., 2004). The
70 hydrophobic segments are characterized by the presence of conserved Cys-rich
71 motifs of the type CLXXXXCPC or CCXXXXCPC, separated by a variable region and
72 located at the N-terminal part of a first TM domain (Beauchet et al., 2021). A
73 localization at the plasma membrane (PM) was indeed demonstrated for the tomato
74 FW2.2 protein (Cong and Tanksley, 2006), as well as for CNR/FWL homologous
75 proteins in various fruit species such as eggplant (*Solanum melongena*), pepper
76 (*Capsicum annuum*), Physalis (*Physalis floridana*), avocado (*Persea americana*),
77 cherry (*Prunus cerasus*) (Dahan et al., 2010; De Franceschi et al., 2013; Doganlar et
78 al., 2002; Li and He, 2015), but also in Arabidopsis (*Arabidopsis thaliana*), cereal and
79 leguminous species (Libault et al., 2010; Guo et al., 2010; Song et al., 2010; Xu et
80 al., 2013). In soybean (*Glycine max*), the CNR/FWL protein GmFWL1 (*Glycine max*
81 FW2.2-Like 1) was shown to display a punctate localization in plasma membrane
82 nanodomains, which supported its ability to interact with membrane nanodomain-
83 associated proteins such as flotillins, prohibitins, remorins, proton- and vacuolar-
84 ATPases, receptor kinases, leucine-rich repeat proteins (Qiao et al., 2017).

85 Despite the seemingly conserved roles in cell division and organ size control
86 (Beauchet et al., 2021), the precise physiological and biochemical function of FW2.2
87 or its CNR/FWL homologues remains unknown so far. The conceptual question in
88 studying the functional role of FW2.2 and CNR/FWL is thus how to conciliate a
89 localization at the plasma membrane and nanodomains with a spatial and temporal
90 control of cell divisions in order to regulate plant organ growth.

91 In plants, important biological functions are associated to membrane
92 nanodomains, such as cell-to-cell communication occurring at plasmodesmata (PD).
93 PD are cell wall- and membrane-spanning channels, which provide direct cytosolic
94 continuity to mediate symplastic communication between cells (Maule et al., 2011;
95 Petit et al., 2020). PD control cell-to-cell movements of different mobile signalling
96 molecules (Van Norman et al., 2011; Gallagher et al., 2014), and thus regulate the

97 connection between cells ensuring both local and systemic responses to biotic and
98 abiotic stresses, the exchange of nutrients and organs, regulating symbiotic
99 interactions and supporting the coordination of developmental processes (Gaudioso-
100 Pedraza et al., 2018; Grison et al., 2019; Han et al., 2014a; O'Leary et al., 2018; Yan
101 et al., 2019). Hormones, metabolites, non-cell autonomous proteins, including
102 transcription factors (TFs), and small RNAs represent such mobile signalling
103 molecules, trafficking from cell-to-cell via PD. The symplastic communication via PD
104 is finely tuned by developmental or environmental factors, which exert a control on
105 the size exclusion limit of PD. Among these factors, the deposition of callose, a (1,3)-
106 β -glucan polymer, regulated by the antagonistic action of callose synthases and β -
107 glucanases, is a major process that constricts the PD channel, and thus decreases
108 the aperture of PD (Amsbury et al., 2018). Consequently, the balance between
109 callose deposition and degradation at the neck region of PD plays a major role in the
110 regulation of cell-to-cell communication.

111 In an effort to unravel the cellular and molecular mechanisms sustaining the mode
112 of action of FW2.2 in tomato, we re-investigated its subcellular localization *in planta*.
113 We unexpectedly found that FW2.2 protein not only associates with bulk PM but also
114 clusters at PD in the different tissues we examined. We further show that FW2.2
115 modulates the functionality of PD by modifying callose levels. FW2.2-induced
116 regulation of callose most likely occurs through an interaction with PD-associated
117 Callose Synthases. Our data shed light on an unforeseen function of FW2.2 in
118 modulating cell-to-cell communication in tomato.

119

120 RESULTS

121 FW2.2 localizes at the plasma membrane with the N- and C-terminal parts 122 facing the apoplast.

123 The first and only demonstration that FW2.2 addresses the PM was provided by
124 transient expression analysis using onion (*Allium cepa*) epidermal cells and tomato
125 young leaf cells (Cong and Tanksley, 2006). This PM localization was described at
126 the time as being conferred by two predicted transmembrane domains (TMD)
127 contained in the PLAC8 domain, but the exact topology of the FW2.2 protein at PM is
128 still uncharacterized.

129 First, we confirmed the PM localization of FW2.2, using transient expression in
130 *Nicotiana benthamiana* leaves. FW2.2 fused to GFP either at its C-terminus or N-
131 terminus was indeed addressed to the PM (**Figure 1A**). The localization at the PM
132 was corroborated after plasmolysis using a 0.4 M mannitol treatment (**Supplemental**
133 **Figure S1A**). We then investigated the topology of FW2.2 at PM by using a Bi-
134 molecular Fluorescent Complementation (BiFC) approach that had been validated for
135 PM-located proteins (Thomas et al., 2008). The FW2.2 protein was fused at its N- or
136 C-terminus to the truncated version of GFP, namely GFP11, which contains the last
137 and eleventh β -sheet. The GFP11-FW2.2 or FW2.2-GFP11 construct was then co-
138 expressed with the cytosolic truncated version of GFP, namely GFP1-10 containing
139 the first ten β -sheets. Alternatively, the GFP11-FW2.2 or FW2.2-GFP11 construct
140 was co-expressed with a secreted apoplastic version of GFP1-10, namely SP-GFP1-
141 10 (SP for Signal Peptide of the Arabidopsis PR1 protein; At2g14610). As a positive
142 control for a cytosolic interaction, we fused GFP11 to the C-terminal part of the PM
143 located protein Lti6b (Low-temperature induced 6b protein; At3g05890) that faces the
144 cytosol (Martinière et al., 2012), and co-infiltrated this construct with GFP1-10. The
145 Lti6b-GFP11 construct was thus expected to be unable to interact with the apoplastic
146 SP-GFP1-10.

147 A strong GFP signal was observed when the Lti6b-GFP11 was co-expressed with
148 the cytosolic GFP1-10, and no signal was observed when co-expressed with the
149 apoplastic SP-GFP1-10 (**Figure 1B**). The co-expression of FW2.2 fused to GFP11 at
150 both its C- and N-terminus with the cytosolic GFP1-10, did not result in any visible
151 fluorescence signal. On the contrary, the co-expression of FW2.2 fused to GFP11
152 with the apoplastic SP-GFP1-10 resulted in a strong GFP signal at the PM (**Figure**
153 **1B**). Therefore, FW2.2 is associated to PM as previously reported (Cong and
154 Tanksley, 2006), and we here provided evidence that the N- and C-terminus are
155 facing the apoplast.

156 To confirm this topology, we performed a second transient expression assay,
157 using a system of apoplastic and cytoplasmic pH sensors described by Martinière et
158 al. (2018) (**Figure 1C**). This system takes advantage of the pH-sensitive ratiometric
159 behavior of the protein pHluorin (pHGFP), whose emitted fluorescence differs
160 according to its location in the cytosol or the apoplast, depending on their respective
161 pH value of ~ 7.5 or ~ 6.0 . Following agro-infiltration of *N. benthamiana* leaves, the
162 fluorescence emitted by pHGFP was recorded after an excitation wavelength of 405

163 nm and 488 nm, to establish a 405/488 fluorescence intensity ratio, indicative of pH
164 differences. The discrimination between the apoplastic and cytosolic 405/488 ratio
165 was made possible by the use of the following constructs. The apoplastic membrane
166 pH sensor pHGFP-PM-Apo resulted from the fusion of pHGFP with the TMD of the
167 PM-localized protein TM23 (Brandizzi et al., 2002), and the cytosolic membrane pH
168 sensor pHGFP-PM-Cyto corresponded to the fusion of pHGFP with the C-terminal
169 farnesylation sequence of Ras which is anchored to the PM (Martinière et al., 2018).

170 As expected, the 405/488 nm fluorescence ratio measured in *N. benthamiana* cells
171 was higher for the pHGFP-PM-Cyto (median=2.2) when compared to that for
172 pHGFP-PM-Apo (median=1.3), revealing the higher pH of the cytosolic compartment
173 than that of apoplast (**Figure 1D**). The 405/488 nm fluorescence ratio was then
174 measured in cells transformed with FW2.2 fused with the pHGFP either at its N-
175 terminal or C-terminal end. It was shown to be very close to the fluorescence ratio
176 measured with the pHGFP-PM-Apo (median=1.3), thus demonstrating unequivocally
177 that the N- and C-terminal parts of FW2.2 are facing the apoplast (**Figure 1C, D**).

178 Interestingly, a 3D model predicting the structure of FW2.2 using the AlphaFold
179 Protein Structure Database (Q9LKV7) confirmed that the N- and C-terminal parts of
180 FW2.2 are folded on the same side of the protein (**Supplemental Figure S1B**). In
181 addition, the use of currently available tools for transmembrane topology prediction,
182 such as DeepTMHMM and the PPM web server, indicated that (i) FW2.2 does not
183 cross the plasma membrane as no transmembrane domain can be predicted
184 (**Supplemental Figure S1C**), but rather (ii) FW2.2 is anchored in the outer leaflet of
185 the plasma membrane via its hydrophobic domain encompassing the PLAC8 domain,
186 thus exposing N- and C-terminal termini to the apoplast (**Supplemental Figure S1D**).

187

188 **FW2.2 is enriched at plasmodesmata**

189 To go deeper into the study of the FW2.2 subcellular localization, we generated
190 stable transgenic lines expressing FW2.2 fused to YFP at its C-terminal end under
191 the control of the Cauliflower Mosaic Virus (CaMV) 35S promoter (referred to as
192 *35S::FW2.2-YFP* plants), in the cultivated tomato variety Ailsa Craig (AC). In these
193 plants, the emitted fluorescence associated to YFP was highly detectable in roots
194 and leaves, and in reproductive organs, namely flowers and fruits (**Supplemental**
195 **Figure S2A**). The localization of FW2.2-YFP at the PM was confirmed in all tissues
196 investigated, namely in roots and fruit pericarp (**Figure 2A**), according to a pattern of

197 punctate spots at the cell periphery, suggesting that FW2.2-YFP was enriched at
198 nanodomains as observed previously for the soybean homolog GmFWL1 (Qiao et al.,
199 2017). The same tissue preparations were then stained with aniline blue (AB) to
200 reveal callose deposition, as a marker of PD. The fluorescent dots revealing FW2.2-
201 YFP co-localised with AB staining, at pit field junctions, as shown by the overlapping
202 signal intensity plots (**Figure 2A**), thus indicating a localization at PD. It is noteworthy
203 that the localization of FW2.2 at PD was independent from the position of YFP at the
204 C-terminal or N-terminal end of the protein, since we obtained similar results using a
205 *35S::YFP-FW2.2* construct (**Supplemental Figure S2B**). The enrichment of FW2.2
206 at PD was quantified by measuring the plasmodesmata enrichment ratio, named 'PD
207 index', corresponding to the FW2.2-YFP fluorescence intensity at PD vs that at the
208 cell periphery, as previously described (Brault et al., 2019; Grison et al., 2019). To
209 measure the PD index in control plants, root and fruit pericarp tissues from WT plants
210 were stained with AB together with FM4.64, a membrane-specific dye (Bolte et al.,
211 2004), as illustrated in **Supplemental Figure S2C**. While the PD index in controls
212 was equal to 1 regardless of the tissue tested, a high PD-index ranging from 1.7 to
213 1.9 was measured in root and pericarp cells of *35S::FW2.2-YFP* plants, (**Figure 2B**),
214 thus demonstrating that FW2.2 was enriched at PD.

215

216 **The overexpression of FW2.2 in leaves enhances cell-to-cell diffusion capacity**

217 Since FW2.2 localizes at PD, we hypothesized that it could contribute to a function
218 associated to cell-to-cell communication. To test this hypothesis, a new set of gain-of-
219 function plants were generated in the tomato cultivar AC, as to overexpress *FW2.2*
220 constitutively and ectopically, under the control of the 35S promoter (referred to as
221 *35S::FW2.2*). Three lines were selected with medium- (2-fold more) to very high
222 levels (50-fold more) of *FW2.2* overexpression in 5 days-post-anthesis (DPA) fruits, a
223 stage when the endogenous *FW2.2* expression is at its maximum (**Supplemental**
224 **Figure S3A**). In parallel, loss-of-function plants were generated using the
225 CRISPR/Cas9 technology. To knock out *FW2.2*, two single-guide RNAs (sgRNAs)
226 were designed as close as possible to the start codon of the coding sequence to
227 create a frameshift or an early stop codon resulting in a dysfunctional FW2.2 protein
228 in which the PLAC8 domain is missing (**Supplemental Figure S4**). We selected
229 three different homozygous lines, referred to as *CR-fw2.2* hereafter.

230 In all three independent *35S::FW2.2* overexpressing lines, a significant reduction
231 in mean leaf surface was observed, from 33% to 42% compared to that in WT
232 (**Figure 3A**). This reduction in leaf surface was not due to any alteration in cell size,
233 as the leaf epidermal cell density, used as a proxy for cell size, was unaffected
234 (**Figure 3B**). No growth-related phenotype was observed in leaves of *CR-fw2.2*
235 plants, which was expected as *FW2.2* is not naturally expressed in leaves
236 (**Supplemental Figure S3B**).

237 We next investigated whether the overexpression of *FW2.2* in leaves could affect
238 the permeability of PD, and consequently the cell-to-cell communication. The PD
239 permeability in WT, *35S::FW2.2* and *CR-fw2.2* lines was compared by performing
240 “Drop-AND-See” (DANS) quantitative assays (Cui et al., 2015), using the membrane-
241 permeable, non-fluorescent dye Carboxy-Fluorescein DiAcetate (CFDA). DANS
242 assays are based on the ability of cells to uptake CFDA rapidly; intracellular
243 esterases then cleave CFDA into fluorescent but membrane-impermeable Carboxy-
244 Fluorescein (CF), and CF diffuses symplastically into the neighbouring cells only via
245 PD. To our knowledge, the use of this technique has never been reported in tomato.
246 We first checked that DANS assays are functional in tomato using leaflets of 4
247 weeks-old plants (**Supplemental Figure S5A**).

248 In Arabidopsis, a pre-treatment with 10 mM H₂O₂ alters PD permeability through
249 an increase in callose deposition (Cui and Lee, 2016). Such an effect was also
250 observed in tomato WT leaves, as revealed by the reduction in CF-foci area
251 compared to mock-treated leaves, thus indicating a decrease in PD permeability
252 affecting the cell-to-cell movement of CF in tomato leaves (**Figure 3C-D**). We then
253 examined whether gain- or loss-of-function of *FW2.2* alters cell-to-cell
254 communication. The CF-foci area was increased (from 20 to 30%) in all
255 overexpressing *35S::FW2.2* lines compared to that in WT, suggesting an increased
256 PD permeability (**Figure 3C-D**). Interestingly, the H₂O₂ treatment which increases
257 callose deposition in WT and thereby decreases PD permeability, had no effect on
258 the *35S::FW2.2* lines, compared to the mock treatment. Hence, not only the
259 overexpression of *FW2.2* in leaves increased PD permeability, but it also inhibited the
260 negative effects of H₂O₂ on it. On the contrary, the CF-foci area in *CR-fw2.2* lines was
261 similar to that in WT (**Figure 3C-D**), showing no difference in CF diffusion, which
262 suggests that the PD permeability was not affected. This absence of effects on PD
263 permeability in *CR-fw2.2* lines can be explained by the absence of endogenous

264 *FW2.2* expression in leaves, as mentioned above. It also corroborates with the
265 absence of any alteration in epidermal cell size in *35S::FW2.2* and *CR-fw2.2* lines
266 (**Supplemental Figure S5B**). Therefore, the observed difference in CF diffusion was
267 the result of the overexpression of *FW2.2* in tomato leaves, which induced a
268 modification in the cell-to-cell communication status, as revealed by the altered PD
269 permeability.

270

271 **FW2.2 affects the callose deposition at PD in leaves**

272 A key mechanism for the regulation of PD aperture, and therefore for intercellular flux
273 of signalling molecules, involves the accumulation of the cell wall polysaccharide
274 callose at the neck regions of PD (Amsbury et al., 2018). To verify whether the
275 increase in cell-to-cell diffusion mediated by the overexpression of *FW2.2* was due to
276 a modified level of callose accumulation, the levels of callose at PD were measured
277 in leaves from WT, *35S::FW2.2* and *CR-fw2.2* plants, following a pre-treatment with
278 or without H₂O₂. The levels of callose were quantified by immunofluorescence
279 labelling using a callose-specific antibody as illustrated for WT in **Figure 4A**, and the
280 signal intensity was subsequently quantified as a proxy of callose deposition at PD
281 (**Figure 4B** and **Supplemental Figure S6**), as commonly described (Grison et al.,
282 2019; Platre et al., 2022; Wang et al., 2023). Compared to control conditions (mock
283 treatment), the signal intensity for callose in WT leaves treated with H₂O₂ was
284 increased, in agreement with DANS assays showing decreased cell-cell
285 communication. The immunofluorescence intensity in the *35S::FW2.2* leaves was
286 decreased when compared to that in WT, indicating that less callose was deposited,
287 in the absence of any alteration in cell size and leaf thickness as verified before
288 (**Figure 3B** and **Supplemental Figure S5B**). In response to H₂O₂, the levels of
289 callose deposition in *35S::FW2.2* leaves also increased, but to a much lower extent
290 than in WT (**Figure 4B**). On the contrary, the levels of callose deposition in *CR-fw2.2*
291 leaves with or without H₂O₂ were highly similar to that in WT, in accordance with the
292 absence of phenotype when *FW2.2* is mutated (**Figure 3**). These results clearly
293 indicated that *FW2.2* alters the process of callose deposition at PD.

294

295 **FW2.2 regulates negatively callose deposition at PD in fruit pericarp**

296 Since *FW2.2* was found as a major regulator of fruit weight, we next examined
297 whether the misexpression of *FW2.2* would affect the level of callose deposition at
298 PD in fruit pericarp tissue.

299 At a macroscopic level, among the three selected overexpressing lines, a
300 significant reduction in mean fruit weight was observed for the *35S::FW2.2-1* and
301 *35S::FW2.2-3* lines (according to an average decrease of 19.6% and 11.3%
302 respectively) (**Figure 5A**). The mean fruit weight in the three *CR-fw2.2* loss-of
303 function plants was higher than that of the WT (7,2%, 7,1% et 6,3% respectively).
304 However, these differences were not statistically significant, because of a high
305 variability in fruit weight values. In addition, there was no modification in pericarp
306 thickness in mature fruits from the three *35S::FW2.2* lines compared to WT fruits,
307 while pericarp from *CR-fw2.2* fruits appeared thinner (**Figure 5B**). Related to fruit
308 structure, fruits from gain- and loss-of-function plants were all affected for the number
309 of locules to various degrees (**Figure 5C**). More fruits with less than 3 locules were
310 encountered in the overexpressing *35S::FW2.2* lines, while fruits with 4 and even
311 more locules were observed in *CR-fw2.2* lines, compared to WT fruits from the AC
312 cultivar which usually contain 3 locules. This converse impact on the number of fruit
313 locules in the gain- and loss-of-function plants suggests that cell divisions have been
314 impacted in the floral meristem (FM) termination process, through the increased or
315 repressed negative regulatory effect in *35S::FW2.2* or *CR-fw2.2* lines respectively.

316 The level of callose deposition was then investigated on pericarp sections of fruits
317 from the *35S::FW2.2* and *CR-fw2.2* plants harvested at 5 and 15 DPA. These two
318 different developmental stages were chosen because *FW2.2* is highly expressed in
319 the pericarp of 5 DPA fruit and much less at 15 DPA (**Supplemental Figure S3B**). At
320 both 5 and 15 DPA, the immunofluorescence signal intensity in the pericarp of
321 *35S::FW2.2* fruits was decreased when compared to that in WT, indicating that the
322 level of callose deposition was reduced (**Figure 5E-F** and **Supplemental Figure S7**).
323 On the contrary, the immunofluorescence signal intensity in the pericarp of *CR-fw2.2*
324 fruits at 5 DPA was increased significantly when compared to that in WT, thus
325 revealing a higher level of callose deposition. Interestingly, except for a slight
326 significant increase in the *CR-fw2.2-3* line, no increase in callose deposition was
327 observed at 15 DPA in pericarp sections from *CR-fw2.2* fruits compared to WT. This
328 can be explained by the very low expression of *FW2.2* in 15 DPA fruits

329 **(Supplemental Figure S3B)**, and thus the absence of any loss-of-function effect
330 from the CRISPR-Cas9 construct on *FW2.2* at this developmental stage.

331 Cell perimeters were measured for all genotypes in all the different cell layers
332 composing the fruit pericarp at 5 DPA, and in the mesocarp at 15 DPA, to ascertain
333 that these differences in callose deposition was not due to any heterogeneity in cell
334 size, and thus in the density of cell walls. The cell perimeter was comparable in all
335 WT, *35S::FW2.2* and *CR-fw2.2* lines, with only slightly smaller values in some cases,
336 especially in the internal part of the mesocarp (**Supplemental Figure S8**). Hence,
337 the observed differences in callose deposition did originate from the effects of *FW2.2*
338 gain- and loss-of-function, demonstrating that *FW2.2* regulates negatively the
339 process of callose deposition at PD within fruit pericarp.

340

341 **FW2.2 pull-down reveals plasmodesmata-related proteins**

342 To go deeper into the functional and biochemical characterization of *FW2.2*, an *in*
343 *vivo* approach using immunoprecipitation followed by tandem-mass spectrometry (IP-
344 MS/MS) was performed to identify interacting protein partners of *FW2.2* inside the
345 pericarp from *35S::FW2.2-YFP* fruits harvested at 10 DPA. Since *FW2.2* is still
346 expressed endogenously at this developmental stage, it was therefore expected that
347 its natural interacting proteins would be present in the protein extracts. The IP-
348 MS/MS experiment resulted in the identification of 662 proteins that co-
349 immunoprecipitated with *FW2.2*, which were enriched in the *35S::FW2.2-YFP* sample
350 when compared to WT (**Figure 6A, Supplemental Data Set 1**). To identify potential
351 PD-localized candidates in relation with *FW2.2* function, we compared this list with a
352 tentative PD proteome from tomato made of a total of 400 proteins corresponding to
353 the deduced orthologs of the 115 proteins constituting the refined PD proteome from
354 *Arabidopsis* published by Brault *et al.* (2019). Seventeen proteins were found
355 overlapping between the two proteomes (**Figure 6B**). Three distinct classes of
356 proteins, all key regulators of cell-to-cell signalling in plants, represented almost two
357 thirds of the identified proteins (**Figure 6C**): i) two proteins of the C2 calcium/lipid-
358 binding phosphoribosyl transferase family (Solyc01g080430 and Solyc01g094410),
359 belonging to the large family of multiple C2 domains and transmembrane region
360 proteins (MCTP) (Brault *et al.*, 2019); ii) three proteins of Leucine-Rich Repeat
361 Receptor-Like kinases (LRR-RLKs) family (Solyc03g111670, Solyc06g082610 and
362 Solyc05g052350) (Wei *et al.*, 2015); iii) six different Callose Synthases (CalS), which

363 were identified based on their phylogenetic proximity to Arabidopsis counterparts,
364 namely SICaLS1 (Solyc01g006350), SICaLS3a (Solyc01g006370), SICaLS3b
365 (Solyc01g073750), SICaLS9 (Solyc01g006360), SICaLS10a (Solyc03g111570) and
366 SICaLS12 (Solyc07g053980) (**Supplemental Figure S9A**). The co-
367 immunoprecipitation of FW2.2 with Callose synthases in 10 DPA fruits was thus fully
368 relevant with its aforementioned role in regulating callose deposition at PD in the
369 pericarp. RT-qPCR analyses confirmed that these 6 *Ca/S* genes were expressed in
370 WT fruit pericarp at 10 DPA (**Supplemental Figure S9B**), and no significant change
371 in their expression level occurred in the *FW2.2* loss- and gain-of-function plants
372 (**Supplemental Figure S10**).

373 These results indicate that *FW2.2* belongs to a protein complex at PD which
374 includes Callose Synthases, and thus support the functional role of *FW2.2* on PD
375 permeability and cell-to-cell communication.

376

377 **DISCUSSION**

378 *FW2.2* was the first gene underlying a QTL related to fruit size to be cloned in tomato
379 (Frary et al., 2000). It is by far the major QTL of such type, as it accounts for as much
380 as a 30% difference in fruit fresh weight between domesticated (large-fruited)
381 tomatoes and their wild (small-fruited) relatives (Frary et al., 2000; Grandillo et al.,
382 1999). Most wild -small fruited- tomatoes (if not all) possess 'small-fruit' alleles;
383 conversely all domesticated/cultivated -large fruited- tomatoes possess 'large-fruit'
384 alleles (Bianca et al., 2015). Comparative sequence analysis of *FW2.2* from the
385 large- and small-fruited alleles indicated that the *FW2.2* effects on fruit size do not
386 originate from differences in the sequence and structure of the protein, but rather
387 from the timing of its transcription (heterochronic changes) and the overall quantity of
388 transcripts in the fruit (Cong et al., 2002). The 'large-fruit' allele is rapidly transcribed
389 to reach a peak of expression around 5 DPA, whereas the 'small-fruit' allele is
390 transcribed more slowly and displays its maximum of expression nearly a week later
391 (12 to 15 DPA), reaching almost twice the mRNA level observed in large-fruit allele
392 (Cong et al., 2002). Since this difference in timing of expression was found inversely
393 associated to the mitotic activity, *FW2.2* was defined as a negative regulator of cell
394 divisions in pre-anthesis ovary and developing fruit, thus modulating final fruit size
395 (Frary et al., 2000; Cong et al., 2002). Such a function in regulating organ size by

396 modulating cell number was found conserved for many other plant homologs of
397 FW2.2 (Beauchet et al., 2021), which led to the attribution of the CELL NUMBER
398 REGULATOR (CNR) protein family name (Guo et al., 2010). Members of the CNR
399 protein family are targeted to the PM, due to the presence of the PLAC8 domain
400 (Beauchet et al., 2021). However, the precise biological function and mechanism of
401 action of membrane-embedded FW2.2 and CNRs in controlling organ size via the
402 regulation of cell divisions remained totally elusive so far.

403

404 **FW2.2 regulates cell-to-cell diffusion by modulating callose deposition at** 405 **plasmodesmata**

406 It was long known that FW2.2 is a plasma membrane-located protein (Cong and
407 Tanksley, 2006). Using transient expression in *N. benthamiana* leaves and stable
408 transformants in the tomato AC cultivar, we confirmed this PM localization for FW2.2
409 (**Figures 1-2**). The topology of FW2.2 within the PM was established and revealed
410 that the N- and C-terminal regions are extracellular, thus facing the apoplast (**Figure**
411 **1**). This is in agreement with a topological model predicted for PfCNR1, the FW2.2
412 putative orthologue from *Physalis floridana*, which displays a high degree of identity
413 (80%) with FW2.2 (Li and He, 2015). However, our study provides information about
414 the FW2.2 3-D structure and its PM localization. FW2.2 is not a transmembrane
415 protein *per se*, as no transmembrane domains can be predicted using the current
416 prediction tools, but it is most likely anchored in the outer leaflet of the PM, via the
417 hydrophobic portion of the protein encompassing the PLAC8 domain (**Supplemental**
418 **Figure S1**). More importantly, we demonstrated unequivocally that FW2.2 is enriched
419 at PD (**Figure 2**) and participates in cell-to-cell communication mechanisms via the
420 regulation of PD permeability (**Figure 3**).

421 This localization at PD is most probably functionally conserved with other
422 members of the CNR family. Indeed, the localization of the soybean GmFWL1 protein
423 was described as associated to membrane microdomains (Qiao et al., 2017),
424 according to a punctate pattern very similar to what we observed for FW2.2 in tomato
425 (**Figure 2**). It is thus highly probable that GmFWL1 also localizes at PD. The homolog
426 of FW2.2 in Arabidopsis, namely AtPCR2 sharing 44% of identity with FW2.2,
427 belongs to the PD proteome established by Brault et al. (2019), together with well-
428 established PD proteins, and presents a ~50- to 100-fold enrichment at PD compared
429 to the PM, total protein, microsomal or cell wall fraction.

430 PD make the connection between adjacent cells to enable the diffusion of mobile
431 signalling molecules (Wu and Gallagher, 2011). Using DANS assays, we
432 demonstrated that FW2.2 is involved in cell-to-cell diffusion mechanisms and
433 contributes to increase PD permeability (**Figure 3**). The permeability and thus the
434 aperture of PD are mechanically regulated by the extent of deposited callose at the
435 neck of PD (Amsbury et al., 2018). The increase in PD permeability mediated by
436 FW2.2 occurs via a modification in the level of callose deposition, as FW2.2 regulates
437 negatively its accumulation (**Figures 4-5**). The level of callose deposition is a highly
438 regulated process involving two antagonistic enzymes, Callose Synthases and β -1,3-
439 glucanases (Chen and Kim, 2009). Callose deposition is enhanced according to two
440 main signalling pathways, one Reactive Oxygen Species (ROS)-dependent and the
441 other one salicylic acid (SA)-dependent, which both induce the expression of receptor
442 proteins such as PDLP5 that participate with Callose Synthase proteins in the
443 regulation of PD permeability (Cui and Lee, 2016; Amsbury et al., 2018; Tee et al.,
444 2022). The expected decrease in PD permeability under H₂O₂ stress was not
445 observed when FW2.2 is overexpressed, suggesting that FW2.2 play a role in the
446 ROS-dependent pathway. Whether FW2.2 also plays a role in the SA-dependent
447 pathway to regulate PD permeability remains to be determined.

448

449 **FW2.2 is part of a protein complex involved in plasmodesmata function, which** 450 **includes Callose Synthases**

451 A proteomics approach using IP-MS/MS revealed that FW2.2 belongs to a protein
452 complex that includes different Callose Synthases: SICaIS1, SICaIS3a, SICaIS3b,
453 SICaIS9, SICaIS10 and SICaIS12 (**Figure 6**). Interestingly, all these tomato proteins
454 are the putative orthologs of Arabidopsis CalS known to contribute to callose
455 homeostasis at PD, thereby regulating the permeability of PD and consequently the
456 symplastic molecular exchanges between neighboring cells (Saatian et al., 2023;
457 Usak et al., 2023). It is noteworthy that among the 178 proteins found to interact with
458 GmFWL1, three distinct callose synthases, namely CalS5 (Glyma13g31310), CalS8
459 (Glyma04g36710) and CalS10 (Glyma10g44150) were also identified following the
460 co-immunoprecipitation assays (Qiao et al., 2017). This observation suggests not
461 only that GmFWL1 is probably located at PD as well, but also that the interaction
462 between FW2.2 and CNRs with proteins involved in the metabolic process of callose
463 deposition at PD seems to be a conserved feature for the balance between synthesis

464 and degradation of callose at PD. Hence, we can hypothesize that CNRs regulate
465 negatively the activity of Callose Synthases.

466 The activity of PD-associated Callose Synthases is of prime importance in
467 numerous developmental processes, such as in the response to biotic and abiotic
468 stress, organ and tissue patterning, cell differentiation, phloem transport, and cell
469 division via the formation of the cell plate at cytokinesis (Amsbury et al. 2018; Wu et
470 al., 2018; Usak et al., 2023). In Arabidopsis, AtCalS1 and AtCalS10 localize at the
471 nascent cell plate where they synthesize callose as the first and fundamental
472 polysaccharide component of the nascent cell plate, and AtCalS9 is essential for the
473 proper commitment to mitosis during male gametogenesis (Usak et al., 2023). Again,
474 putative orthologs for these three CalS were found to co-immunoprecipitate with
475 FW2.2 in tomato. Interestingly, the CRR1 protein from rice encodes a CalS which is
476 essential for ovary growth following fertilization (Song et al., 2016). The loss-of-
477 function of *CRR1* induces a disordered patterning of vascular cells in the ovaries of
478 the mutant, with aberrant cell wall formation and reduced callose deposition at PD.
479 Furthermore, the cell number inside the *crr1* ovaries is reduced when compared to
480 the WT, establishing a link with callose synthesis and deposition, symplastic pathway
481 via PD and control of cell division during ovary development.

482

483 **How to reconcile a function of FW2.2 in cell-to-cell communication, cell cycle-** 484 **and fruit growth regulation?**

485 As FW2.2 was described as a negative regulator of cell division during early fruit
486 development, which ultimately impacts fruit growth (Cong et al., 2002), it would have
487 been expected that a loss-function of FW2.2 results in increased cell divisions and
488 possibly larger organs (including fruits), and conversely that the ectopic
489 overexpression of FW2.2 reduces mitotic activities and results in smaller organs. This
490 latter effect could be observed at least in leaves from *35S::FW2.2* overexpressing
491 lines (**Figure 3**), *i.e.* in organs where FW2.2 is not naturally expressed
492 (**Supplemental Figure S3B**). Since the reduction in leaf growth was unrelated to any
493 modification in cell size, this suggests that cell divisions were reduced under the
494 effects of FW2.2 overexpression. In two out of three gain-of-function lines, we could
495 also observe such a phenotype of reduced size for fruits although limited in extent
496 (**Figure 5**).

497 These results are puzzling since genetics studies showed that the *fw2.2* QTL
498 accounts for 22% to 47% of fruit mass variation when cultivated tomato cultivars are
499 crossed with the wild species *Solanum pimpinellifolium* or *Solanum pennellii* (Alpert
500 et al., 1995; Lippman and Tanksley, 2001; van der Knaap and Tanksley, 2003).
501 Nevertheless, the literature is still devoid of any functional characterization of *FW2.2*
502 in cultivated tomato plants, albeit the gene was discovered and cloned more than 20
503 years ago. This is most probably the result of a lack of phenotypes when *FW2.2* is
504 artificially deregulated in transgenic fruits. For instance, Zsögön et al. (2018) aimed at
505 introducing by CRISPR-Cas9 engineering, yield and productivity traits from modern
506 ('large-fruited') tomato cultivars into the wild ('small-fruited') tomato *Solanum*
507 *pimpinellifolium*. Among the six traits studied, these authors selected the *FW2.2* locus
508 for fruit weight, and produced several mutants with deletions disrupting *FW2.2*.
509 However, none of them induced any change in fruit size in T2 lines compared to *S.*
510 *pimpinellifolium* WT, despite the mutations (Zsögön et al., 2018). These results
511 corroborate the functional analysis reported herein in *S. lycopersicum* cv AC, when
512 *FW2.2* was mutated in the *CR-fw2.2* loss-of-function plants (**Figure 5**). Hence, the
513 ectopic and constitutive expression of *FW2.2* driven by the 35S promoter, definitely
514 outside its natural timeframe and territorial regulation, and its loss of function did not
515 impact fruit development significantly, which probably obeys to precise changes in
516 *FW2.2* spatio-temporal expression, according to the heterochronic regulation of
517 expression described for the original *fw2.2* mutation (Cong et al., 2002). To cope with
518 this difficulty, we developed an 'allele swapping' complementation strategy
519 (**Supplemental Figure S11**). This strategy aimed at generating transgenic plants in
520 which the 'large-fruit'-allele promoter from *S. lycopersicum* cv. AC is used to govern
521 the expression of *FW2.2* in a 'small-fruit' background, namely the wild tomato *S.*
522 *pimpinellifolium* (Pi). Conversely, we used the 'small-fruit'-allele promoter from *S.*
523 *pimpinellifolium* to govern the expression of *FW2.2* in the 'large-fruit' AC background.
524 Although we succeeded in the expected allele expression swapping according to the
525 right spatio-temporal expression governed by each of the promoters, we failed to
526 produce any fruit weight phenotypes in the complemented *S. pimpinellifolium* and *S.*
527 *lycopersicum* cv. AC transgenic lines compared to WT plants. Therefore, the effects
528 of *FW2.2* on fruit size obeys probably to a subtler regulation than the sole quantity of
529 transcripts and availability of the protein. In addition, we cannot exclude that this lack
530 of tangible phenotype may be related to gene redundancy within the *CNR/FWL*

531 family, as 11 genes homologous to *FW2.2* have been reported (Beauchet et al.,
532 2021).

533 Despite the lack of consistent phenotypes when *FW2.2* is misexpressed, the
534 functionality of the protein itself within its cellular and protein environment may be of
535 prime importance. The discovery of the *FW2.2* function in cell-to-cell communication
536 via PD thus raises the question of its link with the regulation of cell division, and
537 subsequent fruit size control. By impairing callose deposition and thus maintaining
538 PD aperture, *FW2.2* may contribute to facilitate the diffusion of signalling molecules
539 whose nature is still unknown. As reviewed by Han et al. (2014b), TFs are well
540 characterized examples of such signalling molecules that could play an important
541 part in the determination of fruit size. So far, direct evidences for the symplastic
542 movements via PD of cell cycle regulators have not been reported. However, Weinl
543 et al., (2005) showed that Cyclin-Dependent Kinase (CDK)-specific inhibitors called
544 Kip-Related Proteins (KRPs) can act non-cell-autonomously, as to regulate cell
545 division and growth pattern in leaf epidermis. During tomato fruit development, KRPs
546 are key players in the regulation of cell cycle, and the commitment to
547 endoreduplication, which drives ploidy-dependent fruit growth (Bisbis et al., 2006;
548 Nafati et al., 2011; Tourdot et al., 2023). Whether the negative regulation on cell
549 division exerted by *FW2.2* in fruit growth goes through the inactivation of CDK/Cyclin
550 activities via the traffic of KRPs from cell to cell across the pericarp remains an
551 exciting matter of investigation. Recently, Ruan et al. (2020) reported that *OscNR1*,
552 encoded by the underlying gene of a major QTL for grain width and weight in rice, is
553 able to interact with *OskRP1* in the cell membrane. Therefore, this remarkable
554 finding provided evidence of a direct link between a CNR protein controlling organ
555 size and a well-established cell cycle regulator inhibiting cell division. Whether this
556 applies to *FW2.2* for the regulation of cell cycle during early fruit development is a
557 challenge for future research as to unravel definitely the function of *FW2.2* in the
558 control of fruit size/weight in tomato. Then, the lack of phenotypes observed in our in
559 planta functional analysis may not be only related to the proper spatio-temporal
560 expression of *FW2.2*, but also to the protein environment itself and the spatio-
561 temporal availability of these putative signaling molecules.

562 How PD-mediated symplastic signalling affects fruit growth is still poorly
563 understood. By demonstrating that *FW2.2* contributes to the spatiotemporal
564 regulation of callose deposition dynamics via regulating the CalS activity, we here

565 provide an important breakthrough for the identification of the molecular and cellular
566 mode of action of FW2.2. Based on our data, we propose a model integrating FW2.2
567 in the regulation of PD aperture via the dynamics of callose deposition (**Figure 7**).
568 We propose that FW2.2 regulates callose deposition, most likely in interaction with a
569 protein complex encompassing Callose synthases, which may modulate negatively
570 their activity, thus ultimately impacting PD permeability and facilitating the cell-to-cell
571 movement of mobile signalling molecules. A future challenge will be to identify the
572 nature of such signalling molecules, which will provide a valuable insight into the
573 molecular mechanisms underlying the complex regulation of organ size, especially
574 fruits.

575

576 **MATERIALS AND METHODS**

577 **Plant materials and growth conditions**

578 Tomato (*Solanum lycopersicum* cv. AC) and *N. benthamiana* plants were grown in
579 soil in a greenhouse under the following conditions: 16 h day/8 h night cycle, using a
580 set of 100 W warm white LED projectors providing an irradiance of 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$
581 at the level of canopy. The light spectrum was constituted by equivalent levels of blue
582 irradiation (range 430–450 nm) and red irradiation (640–660 nm). For *in vitro* culture,
583 tomato seeds were sterilized for 10 min under agitation in a solution of 3.2% (v/v)
584 sodium hypochlorite. Seeds were then washed three times with sterile water and
585 dried under a laminar flow hood. Seeds were sowed in Murashige and Skoog
586 medium (1/4 MS) and transferred in a growth chamber under the following conditions:
587 16 h day/8 h night cycle, 22°C/20°C day/night, using white light (Osram L36 W/77
588 Fluora 1400 lm) providing 80 to 100 $\mu\text{E m}^{-2} \text{s}^{-1}$ intensity light at the stirring plate.

589

590 **Vector constructs and plant transformation**

591 Vectors for the overexpression of *FW2.2* in plants were generated using the
592 Gateway® cloning system (Invitrogen, Carlsbad, CA, USA), following manufacturer's
593 instruction. The *FW2.2* full-length coding sequence was amplified from cDNAs
594 prepared from tomato (cv. AC) fruits at 5 DPA using PrimeSTAR MAX DNA
595 polymerase (TAKARA BIO Inc., Kusatsu, Japan) and primers including the attB sites
596 (**Supplemental Table S1**). The resulting PCR products were cloned into the
597 corresponding Gateway vectors described in **Supplemental Table S2**. For
598 CRISPR/Cas9 mutagenesis, constructs were assembled using the Golden Gate

599 cloning method (Weber et al., 2011). Two sgRNAs were designed at the 5' end of the
600 coding sequence of *FW2.2* using CRISPOR (Concordet and Haeussler, 2018) to
601 generate a premature stop codon (**Supplemental Table S1**). Primers for creating the
602 sgRNA were designed as follows: tgtggtctcaATTG-NNNNNNNN-
603 gtttagagctagaaatagcaag as a forward primer containing the sgRNA, and
604 tgtggtctCAAGCGTAATGCCAACTTTGTAC as a reverse primer. The sequences
605 corresponding to the sgRNA were then PCR amplified using the two aforementioned
606 primers, and cloned into the pSLQ1651-sgTelomere plasmid (Addgene #51024).
607 *fw2.2*-sgRNA-1 and *fw2.2*-sgRNA-2 were fused to the Arabidopsis AtU6-26 promoter
608 (Addgene #46968) by digestion-ligation reaction in pICH47751 (Addgene #48002)
609 and pICH47761 (Addgene #48003) respectively. These two level 1 vectors were
610 assembled with the Kanamycin resistance gene (pNOS::NPTII-OCST; Addgene
611 #51144), the AtCas9 (2x35S::AtCAS9-OCST; Addgene #112079) and the linker
612 pICH41780 (Addgene #48019) into the level 2 vector pICSL4723 (Kind gift from Dr
613 Mark Youles, The Sainsbury Laboratory, Norwich, UK). Transgenic plants were
614 generated by *Agrobacterium tumefaciens* (strain C58C1) mediated transformation
615 using explants of tomato cotyledons as described (Swinnen et al., 2022).

616

617 **RNA extraction and RT-qPCR analysis**

618 Total RNA was isolated from cotyledons, hypocotyls, shoot apical meristems, leaves,
619 roots, flowers and pericarp tissues from fruits harvested at different developmental
620 stages (5, 10, and 15 DPA), using TRIzol reagent (Invitrogen) in combination with
621 RNeasy Plant Mini Kit (Qiagen) following the manufacturers' instructions. RNase-free
622 DNase (Qiagen) treatment was performed on each sample. Reverse transcription
623 was performed using the iScriptTM cDNA Synthesis Kit (Bio-Rad, Hercules, CA). RT-
624 qPCR was performed using Gotaq® qPCR mastermix (Promega, Madison, WI) and a
625 CFX 96 real-time system (Bio-Rad). RT-qPCR primers were designed with
626 PerlPrimer software (Marshall, 2004) to overlap 2 exons in order to limit genomic
627 DNA amplification (**Supplemental Table S1**) and amplify an 80 to 200 bp-long
628 amplicon, with a T_m of 60°C. The transcript levels of the expressed genes were
629 normalized to that of the housekeeping genes: *SITUBULIN* (Solyc04g081490) in
630 combination with *SINUDK* (Solyc01g089970) for fruit samples, or with *SIEIF4 α*
631 (Solyc12g095990) for other tissue samples, using the $\Delta\Delta CT$ normalization. Data are
632 presented as mean and SD of biological replicates. Statistical significance was

633 evaluated by the Kruskal-Wallis test and p-values are indicated. All primers used for
634 expression analyses are listed in Supplemental table S1.

635

636 **Phenotypic characterization**

637 Plants were cultivated randomly side-by-side with WT plants. Flowers were vibrated
638 every day to ensure optimal self-pollination. Seven flowers per inflorescence were
639 maintained to ensure proper development of fruit per inflorescence. Fruits from four
640 to six plants of each genotype of two biological replicates were used to determine
641 fruit weight, fruit size, locule number and pericarp thickness at the breaker stage of
642 fruit development. Fruits were weighted and measured using a caliper. Then, pictures
643 of equatorial transverse sections of fruits were taken to count the locule number and
644 measure the pericarp thickness, using a Nikon D5300 camera. Image analysis was
645 performed using the ImageJ software (<https://imagej.nih.gov/ij/>). The number of
646 measurements ranged from n=50 to n=200 depending on the number of fruits
647 produced by the different transgenic plants. For leaf surface phenotyping, pictures of
648 full-grown leaves were taken using a Nikon D5300, and analysed by intensity
649 threshold filtering. To measure the leaf thickness, images of leaf sections acquired for
650 immuno-labelling experiments were used with three measurement for each picture
651 (n=70 to 100).

652

653 **PD index determination**

654 The localization of FW2.2-YFP at PM and PD was observed using confocal imaging
655 performed on a Zeiss LSM 880 confocal laser scanning microscope equipped with
656 fast AiryScan, using a Zeiss C PL APO x63 oil-immersion objective (numerical
657 aperture 1.4). To ascertain the PM localization of FW2.2, *N. benthamiana* leaf cells
658 agro-infiltrated with 35S::FW2.2-YFP and fruit pericarp cells from 35S::FW2.2-YFP
659 tomato plants were plasmolyzed using 0.4 M Mannitol for 15min before observation.
660 Staining with FM4.64 at a final concentration of 4 μ M was used as a control for PM
661 localization (Bolte et al., 2004). For FM4.64 imaging, excitation was performed at 561
662 nm and fluorescence emission was collected at 630-690 nm. For YFP imaging,
663 excitation was performed at 514 nm and fluorescence emission collected at 520-580
664 nm. Staining with aniline blue (AB; Biosupplies, Victoria, Australia) was performed by
665 infiltration of a 0.0125% (w/v) solution; excitation was performed at 405 nm and
666 fluorescence emission collected at 420-480 nm. The calculation of PD index was

667 determined by calculating the fluorescence intensity of FW2.2-YFP at
668 plasmodesmata and at PM as described (Grison et al., 2019). Images were all
669 acquired with the same parameters (zoom, gain, laser intensity, etc.), and YFP and
670 AB channels were acquired sequentially. Ten to twenty images were acquired with a
671 minimum of three biological replicates. Individual images were processed using
672 ImageJ. A minimum of ten regions of interest (ROI) at PD (using AB as a marker) and
673 in the surrounding PM were manually outlined, and the signal intensity was
674 calculated as the mean gray value (sum of gray values of all the pixels in the selected
675 area divided by the ROI surface) for each ROI.

676

677 **Immuno-labelling of callose**

678 The level of callose deposition was determined in leaves and in the pericarp of fruits
679 harvested at 5 and 15 DPA. Leaf fragments were fixed with a 4% (v/v) formaldehyde
680 solution in 1X PBS for 30 min, using vacuum infiltration (~100 kPa). They were then
681 embedded in 6% (w/v) SeaKem® LE agarose (Lonza, Basel, Switzerland), and
682 sections of 100 µm were realized using a vibrating blade microtome (Microm 650V;
683 Thermo Fischer Scientific, Walldorf, Germany). Equatorial pericarp fragments were
684 fixed using the same protocol. Pericarp sections of 80 or 150 µm were prepared, and
685 fixed once more in fresh formaldehyde solution for 30 min, rinsed and kept in 1X PBS
686 until use. The leaf and pericarp sections were then processed using the same
687 protocol. The sections were deposited into a small basket containing MTSB buffer
688 (50 mM PIPES, 5 mM EGTA, 5 mM MgSO₄, pH=7) to perform the immuno-labelling
689 of callose using the In situ Pro VSi automated immunohistochemistry device from
690 Intavis (Köln, Germany). Leaf and pericarp sections were rinsed 4 times for 10 min
691 with 700 µL of MTSB. The sections were then incubated for 1 h with 700 µL of a 10%
692 (v/v) DMSO/3% (v/v) IGEPAL® CA-630 (Merck, Darmstadt, Germany) in MTSB. After
693 rinsing, pericarp sections were incubated for 2 h in a 5% (v/v) Normal Donkey serum
694 (NDS; Merck) blocking solution in MTSB, and 4 h with 700 µL of a 1/250 dilution of
695 Anti-callose primary antibody (Biosupplies) in MTSB supplemented with 5% (v/v)
696 NDS. The sections were then washed 6 times with 700 µL of MTSB, and incubated
697 for 2 h with 700 µL of a 1/250 dilution of anti-mouse IgG Alexa Fluor™ 555 secondary
698 antibody (ab150106; Abcam, Cambridge, UK) in MTSB + 5% (v/v) NDS. Sections
699 were rinsed 6 times in MTSB and incubated with 1 µg/mL Calcofluor white
700 (Fluorescent Brightener 28 disodium salt solution, Merck, in MTSB). After rinsing, the

701 sections were mounted on glass slides with citifluor (AF1-25) (EMS Acquisition Corp.,
702 PA, USA) and the slides sealed with nail polish.

703 Imaging was performed using a Zeiss LSM 880 confocal microscope equipped
704 with a Zeiss x20 dry objective (numerical aperture 0.8). For Alexa 555, excitation was
705 performed at 561 nm with an argon laser (0.3% intensity) and fluorescence emission
706 was collected at 570-630 nm by a GaAsP detector with 700V gain. For Calcofluor
707 imaging, excitation was performed at 405 nm (0.2% intensity) and fluorescence
708 emission collected at 430-490 nm by a PMT with 700V gain. Identical confocal
709 microscope acquisition parameters were used for all the samples. Because of the
710 highly heterogeneous cellular structure of pericarp and leaf, the total signal intensity
711 of each tissue was quantified, and signal intensity values were measured by
712 integrating the grey value of all the pixels above the same threshold. A minimum of
713 six measurements was performed at least on 5 sections from at least three different
714 fruits or leaves from different plants, and the experiment was repeated twice.

715 During the callose immuno-labelling experiments, leaf thickness, cell perimeter in
716 leaves or fruits have been manually measured following staining with Calcofluor on
717 pictures acquired from confocal microscopy using ImageJ.

718

719 **DANS assays**

720 Before proceeding the DANS assay, 4-week-old tomato plants were pre-treated by
721 spraying water (mock) or 10 mM H₂O₂, followed by a 2 h incubation. Then eight
722 separated droplets (corresponding to 1µL each) of 1mM CFDA (Merck, Darmstadt,
723 Germany) were loaded on the upper (adaxial) surface of a leaf. Then, the diffusion of
724 the dye was monitored on the lower (abaxial) surface of the leaf, 5 min after loading
725 CFDA, using an Axiozoom stereomicroscope V16 (Carl Zeiss Microscopy) equipped
726 with a Zeiss Plan-Neofluar 0.5x (NA 0.19) objective lens, a fluorescence lamp
727 (Lumencor Sola LED) and a GFP-BP filter cube (Excitation 450/490 and Emission
728 500/550). Several leaves with the same size were used from at least 4-5 plants
729 (n=100). Imaging was performed at the same magnification (28x), fluorescence lamp
730 power (70%) and exposure time (750ms). Images were acquired using a CMOS
731 Axiocam 105 color camera. The CF signal intensity was measured on ImageJ by
732 integrating the signal intensity to the pixel surface.

733

734 **Co-immunoprecipitation and mass-spectrometry analysis**

735 Total protein extracts from 100 mg of 35S::FW2.2-YFP fruit pericarp tissue were
736 prepared using the following buffer: 1X PBS, cOmplete Protease Inhibitor Cocktail
737 tablets (Roche, Mannheim, Germany) and 1% (v/v) Triton X-100. Samples were
738 incubated in the extraction buffer at 4°C for 30 min with agitation, and then
739 centrifuged (16000g, 10 min, 4°C). Prior to co-immunoprecipitation, western-blotting
740 was used to check the presence of the expressed tagged-FW2.2 protein in the
741 supernatant (**Supplemental Figure S12**). The supernatant containing the
742 resuspended proteins was then used for immunoprecipitation assay using anti-GFP
743 microbeads provided in the μ MACS Epitope Tag Protein Isolation Kit according to the
744 manufacturer's protocol (Miltenyi Biotec, Bergisch Gladbach, Germany).
745 Approximately, 500 μ g of soluble proteins were loaded for each co-IP assay.

746 Fifty μ L of the resulting eluate was loaded on a 10% (w/v) SDS-PAGE acrylamide
747 gel; gel bands were manually cut and transferred to 1.5 mL Eppendorf tubes. Bands
748 were first washed with 500 μ L of water and then 500 μ L of 25 mM NH_4HCO_3 .
749 Destaining was performed twice in the presence of 500 μ L of 50% (v/v) acetonitrile
750 (ACN) in 25 mM NH_4HCO_3 . Gel bands were dehydrated twice by 500 μ L of 100%
751 (v/v) ACN, and finally dried at room temperature. Following destaining, proteins were
752 reduced with 500 μ L of 10 mM DTT at 56°C for 45 min. The supernatant was then
753 removed and proteins were alkylated with 500 μ L of 55 mM iodoacetamide for 30
754 min. Gel bands were washed twice with 500 μ L of 50% (v/v) ACN in 25 mM
755 NH_4HCO_3 , then dehydrated by 500 μ L of 100% (v/v) CH_3CN , and finally dried at
756 room temperature. Twenty μ L of a trypsin solution (Sequencing Grade Modified
757 Trypsin, Promega, Madison, USA), at a concentration of 0.0125 μ g/ μ L in 25 mM
758 NH_4HCO_3 , was added to every gel region and gel bands were kept for 10 min on ice.
759 Fifty μ L of 25 mM NH_4HCO_3 were added, and the samples were kept for another 10
760 min at room temperature. The digestion was performed overnight at 37°C; then
761 peptides were extracted by addition 100 μ L of 2% (v/v) formic acid (FA). Gel bands
762 were extracted twice by addition of 200 μ L of 80% (v/v) ACN and 2% FA. After
763 solvent evaporation in a Speed-vac, peptides were resuspended in 10 μ L of 2% (v/v)
764 FA, then purified with a micro tip C18 (Zip-Tip C18 Millipore Corporation Billerica MA,
765 USA). Peptides were eluted with a solution containing 2% (v/v) FA and 80% (v/v)
766 ACN and dried until total evaporation. Peptides were resuspended in 7 μ L 2% (v/v)
767 FA before LC-MS/MS analysis.

768 The LC-MS/MS were performed using the Ultimate 3000 RSLC nano system
769 (Thermo Fisher Scientific Inc, Waltham, MA, USA) interfaced online with a nano easy
770 ion source and the Exploris 240 Plus Orbitrap mass spectrometer (Thermo Fisher
771 Scientific Inc, Waltham, MA, USA). The samples were analysed in Data Dependent
772 Acquisition (DDA). The raw files were analysed with MaxQuant version 2.0.3 using
773 default settings. The files were searched against the *Solanum lycopersicum* genome
774 (ITAG4.1_release January 2022
775 https://solgenomics.net/organism/solanum_lycopersicum/genome 34689 entries)
776 added with the FW2.2-YFP. Identified proteins were filtered according to the following
777 criteria: at least two different trypsin peptides with at least one unique peptide, an E
778 value below 0.01 and a protein E value smaller than 0.01 were required. Using the
779 above criteria, the rate of false peptide sequence assignment and false protein
780 identification were lower than 1%. Proteins were quantified by label-free method with
781 MaxQuant software using unique and razor peptides intensities (Cox et al., 2014).
782 Statistical analyses were carried out using RStudio package software. The protein
783 intensity ratio and statistical tests were applied to identify the significant differences in
784 the protein abundance. Hits were retained if they were quantified in at least four of
785 the five replicates in at least one experiment. Proteins with a significant quantitative
786 ratio ($P < 0.05$ or 0.01 with or without Benjamini-Hochberg correction) were
787 considered as significantly up-regulated and down-regulated respectively.

788 The mass spectrometry proteomics data have been deposited to the
789 ProteomeXchange Consortium via the PRIDE (Perez-Riverol et al., 2022) partner
790 repository with the dataset identifier PXD045350.

791

792 **Tools for the prediction of the FW2.2 structure and topology**

793 The three-dimensional structure of the full-length FW2.2 (Q9LKV7) was obtained
794 from the AlphaFold protein structure database (<https://alphafold.ebi.ac.uk/>) (Jumper et
795 al., 2021; Varadi et al., 2022). DeepTMHMM (<https://dtu.biolib.com/DeepTMHMM>)
796 (Hallgren et al., 2022) was used to predict the presence of transmembrane helix in
797 FW2.2. The PPM 3.0 Web Server (https://opm.phar.umich.edu/ppm_server3_cgopm)
798 (Lomize et al., 2022) was used with default parameters and plasma membrane
799 (plants) type to predict the topology and insertion of FW2.2 in the plasma membrane.

800

801 **Phylogenetic analyses**

802 The SICaLS protein sequences were first retrieved from NCBI Blast using Arabidopsis
803 CaLS sequences, and analysed at NGphylogeny.fr (Lemoine et al., 2019) using the
804 following parameters: Muscle alignment, BMGE alignment curation, Maximum
805 likelihood analysis PhyML). Bootstrap values are located at each node and were
806 calculated from 1000 replicates.

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850

851 **Author contributions**

852 N.B., F.G., E.B., N.G. and C.C. conceived the project and designed the research.
853 A.B. and N.B. performed the research. V.R. performed the IP-MS-MS proteomics
854 experiments. M.G. helped in the callose immuno-labelling experiments using the
855 InsituPro VSi automate. All authors analyzed and discussed the results. A.B., N.B.,
856 N.G. and C.C. wrote the manuscript with input from the other authors.

857

858 **Conflict of interest**

859 The authors declare that they have no conflicts of interest.

860

861 **Figure legends**

862 **Figure 1. Topological analysis of FW2.2 at the plasma membrane.**

863 **(A)** Subcellular localization of FW2.2 fused to GFP in *N. benthamiana* leaf epidermal
864 cells. Scale bar = 50 μ m.

865 **(B)** BiFC assays deciphering the topology of FW2.2 at the plasma membrane.
866 Transient expressions of FW2.2 or Lti6b fused to GFP11 and with a cytosolic GFP
867 (GFP1-10) or an apoplastic GFP (SP-GFP1-10) were performed in *N. benthamiana*
868 leaves, followed by observation using confocal microscopy Scale bar = 50 μ m.

869 **(C)** Confocal imaging of pHGFP-PM-Apo, pHGFP-PM-Cyto and pHGFP fused to
870 FW2.2 at the N- and C-terminus in *N. benthamiana* leaf epidermal cells. The four
871 images were taken using the same confocal settings. Scale bar = 10µm.

872 **(D)** 405/488 intensity ratio at plasma membrane. Boxplot (whiskers extend from
873 minimum to maximum, box extends from the 25th to 75th percentiles, the line in the
874 middle is the median) 405/488 intensity ratio at plasma membrane. $n > 15$ different
875 images. ANOVA followed by Tukey's test; $P < 0.05$ between a and b groups.

876

877 **Figure 2. FW2.2 is enriched at PD.**

878 **(A)** Confocal microscope observations of FW2.2-YFP localization in roots, pericarp
879 and pit field junctions in pericarp cells from *35S::FW2.2-YFP* plants. Scale bar = 10
880 µm (root and pericarp); = 5µm (pit field). Intensity plots delineated by the two white
881 arrowheads are shown for each co-localisation pattern. A.U. = Arbitrary unit.

882 **(B)** PD index for FW2.2 in roots and pericarp tissue of *35S::FW2.2-YFP* plants
883 compared to WT. Boxplot : whiskers extend from minimum to maximum, box extends
884 from the 25th to 75th percentiles, the line in the middle is the median. $n > 20$ ROIs
885 from 5 images. Statistical analysis: Student's t-test. **** $P < 0.0001$.

886

887 **Figure 3. The overexpression of FW2.2 enhances cell-to-cell diffusion in leaves.**

888 **(A)** Determination of the mean mature leaf surface in WT, *35S::FW2.2* and *CR-fw2.2*
889 lines. $n > 15$ leaves from 5 plants per genotype (each dot represents one leaf surface
890 measurement).

891 **(B)** Determination of the cell density in leaves from WT, *35S::FW2.2* and *CR-fw2.2*
892 lines. $n > 24$ images from 3 plants per genotype (each dot represents one
893 measurement of the number cells per mm²).

894 **(C)** DANS assays using leaves from WT, *35S::FW2.2* and *CR-fw2.2* lines with or
895 without H₂O₂ treatment. Scale bar = 500 µm.

896 **(D)** Quantification of the CF-foci area in WT, *35S::FW2.2* and *CR-fw2.2* lines with or
897 without H₂O₂ treatment. Statistical analysis: Kruskal–Wallis test with post hoc Dunn
898 multiple comparison test. * $P < 0.05$; *** $P < 0.001$; **** $P < 0.0001$. $n > 100$ CF-foci from
899 > 20 different leaflets from ≥ 6 plants per genotype (each dot represents the
900 measurement of individual foci area).

901

902 **Figure 4. The overexpression of FW2.2 alters callose deposition in leaves.**

903 **(A)** Immuno-labelling of callose in leaves of WT plants. Close-up images correspond
904 to the white square location. Scale bar = 100 μm and = 50 μm (close-up).

905 **(B)** Quantification of callose deposition in WT, *35S::FW2.2* and *CR-fw2.2* lines. The
906 signal intensity for callose deposition is integrated to the pixel surface measured.
907 Statistical analysis: Kruskal–Wallis test with post hoc Dunn multiple comparison test.
908 * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$. $n > 20$ measurements on 2-3 leaflets
909 from 2-3 plants.

910

911 **Figure 5. Callose deposition is altered at 5 and 15 DPA in fruit pericarp of**
912 ***35S::FW2.2* and *CR-fw2.2* plants.**

913 **(A-C)** Phenotypic analysis of fruits (at breaker stage) from *35S::FW2.2* and *CR-fw2.2*
914 plants compared to that of WT: Determination of the mean fruit weight **(A)**, $n > 40$ fruits
915 from 4 plants per line; Determination of the pericarp thickness **(B)**; Determination of
916 the number of fruit locules **(C)**, $n > 25$ fruits from 4 plants per lines.

917 **(D)** Immunolabelling of callose in 5 DPA (top) and 15 DPA (bottom) pericarp from WT
918 fruits. Scale bar = 100 μm (5 DPA); = 10 μm (5 DPA close-up); = 500 μm (15 DPA); =
919 25 μm (15 DPA close-up).

920 **(E-F)** Level of callose deposition in WT, *35S::FW2.2* and *CR-fw2.2* lines at 5 **(E)** and
921 15 DPA **(F)**. The signal intensity for callose deposition is integrated to the pixel
922 surface measured.

923 Statistical analysis applied to all panels **(A-F)** was as follows: Kruskal–Wallis test with
924 post hoc Dunn multiple comparison test. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** P
925 < 0.0001 . $n > 80$ images measurement from 4-5 pericarp slices of 4-5 fruits for each
926 line.

927

928 **Figure 6. FW2.2 co-immunoprecipitates with several PD-localized proteins**
929 **including callose synthases.**

930 **(A)** Dot plots showing enriched proteins in *35S::FW2.2-YFP* IP-MS/MS experiments
931 in 10 DPA pericarp. Red dot indicates significantly enriched protein (based on a
932 Student's t-test with Benjamini-Hochberg correction $P < 0.05$ and an enrichment ratio
933 > 1.15). Blue dots indicate proteins found in the PD proteome.

934 **(B)** Venn diagram showing the overlap between the IP-MS/MS proteome and the PD
935 proteome from Brault et al. (2019). Statistical analysis: Hypergeometric test $P =$
936 0.0021.

937 **(C)** List of plasmodesmata associated proteins detected in the IP-MS/MS proteome.

938

939 **Figure 7. Model illustrating the function of FW2.2 in regulating callose**
940 **synthesis at PD.**

941 **(A)** Regulation of PD aperture by callose deposition at the neck region of PD. A high
942 callose deposition level restricts the aperture of PD and the size of signalling
943 molecules passing through.

944 **(B)** Molecular and cellular model for the regulation of Callose synthase activity by
945 FW2.2 at PD.

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948 **References**

949 Alpert, K. B., Grandillo, S. and Tanksley, S. D. (1995). *fw2.2*: a major QTL controlling
950 fruit weight is common to both red- and green-fruited tomato species. *Theor. Appl.*
951 *Genet.* 91: 994–1000.

952 Amsbury, S., Kirk, P. and Benitez-Alfonso, Y. (2018). Emerging models on the
953 regulation of intercellular transport by plasmodesmata-associated callose. *J. Exp.*
954 *Bot.* 69: 105–115.

955 Baldet, P., Hernould, M., Laporte, F., Mounet, F., Just, D., Mouras, A., Chevalier, C.
956 and Rothan C. (2006). The expression of cell proliferation-related genes in early
957 developing flower is affected by fruit load reduction in tomato plants. *J. Exp. Bot.*
958 57: 961-970

959 Beauchet, A., Gévaudant, F., Gonzalez, N. and Chevalier, C. (2021). In search of the
960 still unknown function of FW2.2/CELL NUMBER REGULATOR, a major regulator
961 of fruit size in tomato. *J. Exp. Bot.* 72: 5300–5311.

962 Bisbis, B., Delmas, F., Joubès, J., Sicard, A., Hernould, M., Inzé, D., Mouras, A. and
963 Chevalier, C. (2006). Cyclin-Dependent Kinase Inhibitors are involved in
964 endoreduplication during tomato fruit development. *J. Biol. Chem.* 281: 7374-7383.

965 Blanca, J., et al. (2015). Genomic variation in tomato, from wild ancestors to
966 contemporary breeding accessions. *BMC Genomics* 16: 257.

967 Bolte, S. et al. (2004). FM-dyes as experimental probes for dissecting vesicle
968 trafficking in living plant cells. *J. Microsc.* 214: 159–173.

969 Brandizzi F., et al. (2002). The destination for single-pass membrane proteins is
970 influenced markedly by the length of the hydrophobic domain. *Plant Cell* 14: 1077–
971 1092.

972 Brault, M.L. et al. (2019). Multiple C2 domains and transmembrane region proteins
973 (MCTPs) tether membranes at plasmodesmata. *EMBO Rep.* 20: e47182.

974 Chakrabarti, M. et al. (2013). A cytochrome P450 regulates a domestication trait in
975 cultivated tomato. *Proc. Natl. Acad. Sci. USA* 110: 17125–17130.

976 Chen, X.Y. and Kim, J.Y. (2009). Callose synthesis in higher plants. *Plant Signal.*
977 *Behav.* 4: 489–492.

978 Concordet, J.-P. and Haeussler, M. (2018). CRISPOR: intuitive guide selection for
979 CRISPR/Cas9 genome editing experiments and screens. *Nucleic Acids Res.* 46:
980 W242–W245.

981 Cong, B., Liu, J. and Tanksley, S. D. (2002). Natural alleles at a tomato fruit size
982 quantitative trait locus differ by heterochronic regulatory mutations. *Proc. Natl.*
983 *Acad. Sci. USA* 99: 13606–13611.

984 Cong, B. and Tanksley, S. D. (2006). FW2.2 and cell cycle control in developing
985 tomato fruit: a possible example of gene co-option in the evolution of a novel
986 organ. *Plant Mol. Biol.* 62: 867–880.

987 Cox, J., Hein, M.Y., Lubner, C.A., Paron, I., Nagaraj, N. and Mann, M. (2014).
988 Accurate proteome-wide label-free quantification by delayed normalization and
989 maximal peptide ratio extraction, termed MaxLFQ. *Mol Cell Proteomics* 13: 2513–
990 2526.

991 Cui, W. and Lee, J.-Y. (2016). Arabidopsis callose synthases CalS1/8 regulate
992 plasmodesmal permeability during stress. *Nat. Plants* 2: 16034.

993 Cui, W., Wang, X. and Lee, J.-Y. (2015). Drop-ANd-See: A Simple, Real-Time, and
994 Noninvasive Technique for Assaying Plasmodesmal Permeability. In
995 Plasmodesmata: Methods and Protocols, M. Heinlein, ed, Methods in Molecular
996 Biology (Springer: New York, NY), pp. 149–156.

997 Dahan, Y., Rosenfeld, R., Zadiranov, V. and Irihimovitch, V. (2010). A proposed
998 conserved role for an avocado FW2.2-like gene as a negative regulator of fruit cell
999 division. *Planta* 232: 663–676 .

1000 De Franceschi, P. et al. (2013). Cell number regulator genes in Prunus provide
1001 candidate genes for the control of fruit size in sweet and sour cherry. *Molecular*
1002 *Breeding* 32: 311–326.

- 1003 Doganlar, S., Frary, A., Daunay, M.-C., Lester, R.N. and Tanksley, S.D. (2002).
1004 Conservation of gene function in the Solanaceae as revealed by comparative
1005 mapping of domestication traits in Eggplant. *Genetics* 161: 1713–1726.
- 1006 Frary, A. et al. (2000) .*fw2.2*: A Quantitative Trait Locus Key to the Evolution of
1007 Tomato Fruit Size. *Science* 289: 85–88.
- 1008 Galaviz-Hernandez, C. et al. (2003). Plac8 and Plac9, novel placental-enriched
1009 genes identified through microarray analysis. *Gene* 309: 81–89.
- 1010 Gallagher, K. L., Sozzani, R. and Lee, C.-M. (2014). Intercellular Protein
1011 Movement: Deciphering the Language of Development. *Annu. Rev. Cell Dev. Biol.*
1012 30: 207–233.
- 1013 Gaudio-Pedraza, R. et al. (2018). Callose-Regulated Symplastic Communication
1014 Coordinates Symbiotic Root Nodule Development. *Curr. Biol.* 28: 3562-3577.
- 1015 Grandillo, S., Ku, H. M. and Tanksley, S. D. (1999). Identifying the loci responsible for
1016 natural variation in fruit size and shape in tomato. *Theor. Appl. Genet.* 99: 978–
1017 987.
- 1018 Grison, M. S. et al. (2019). Plasma Membrane-Associated Receptor-like Kinases
1019 Relocalize to Plasmodesmata in Response to Osmotic Stress. *Plant Physiol.* 181:
1020 142–160.
- 1021 Guo, M. et al. (2010). *Cell Number Regulator1* Affects Plant and Organ Size in
1022 Maize: Implications for Crop Yield Enhancement and Heterosis. *Plant Cell* 22:
1023 1057–1073.
- 1024 Hallgren, J., et al. (2022). DeepTMHMM predicts alpha and beta transmembrane
1025 proteins using deep neural networks. *BioRxiv.* 2022-04.
- 1026 Han, X. et al. (2014a). Auxin-Callose-Mediated Plasmodesmal Gating Is Essential for
1027 Tropic Auxin Gradient Formation and Signaling. *Dev. Cell* 28: 132–146.
- 1028 Han, X. et al. (2014b). Transcription factor-mediated cell-to-cell signalling in plants. *J.*
1029 *Exp. Bot.* 65: 1737–1749.
- 1030 Jumper, J., et al. Highly accurate protein structure prediction with AlphaFold (2021).
1031 *Nature* 596: 583–589.
- 1032 Lemoine, F., Correia, D., Lefort, V., Doppelt-Azeroual, O. Mareuil, F., Cohen-
1033 Boulakia, S. and Gascuel, O. (2019). NGPhylogeny.fr: new generation
1034 phylogenetic services for non-specialists. *Nucleic Acids Res.* 47: W260-W265.

- 1035 Li, Z. and He, C. (2015). *Physalis floridana* Cell Number Regulator1 encodes a cell
1036 membrane-anchored modulator of cell cycle and negatively controls fruit size. *J.*
1037 *Exp. Bot.* 66: 257–270.
- 1038 Libault, M. et al. (2010). A member of the highly conserved FWL (tomato FW2.2-like)
1039 gene family is essential for soybean nodule organogenesis: A soybean FWL
1040 essential for nodulation. *Plant J.* 62: 852–864.
- 1041 Lippman, Z. B. and Tanksley, S.D. (2001). Dissecting the Genetic Pathway to
1042 Extreme Fruit Size in Tomato Using a Cross Between the Small-Fruited Wild
1043 Species *Lycopersicon pimpinellifolium* and *L. esculentum* var. Giant Heirloom.
1044 *Genetics* 158: 413–422.
- 1045 Liu, J., Cong, B. and Tanksley, S.D. (2003). Generation and analysis of an artificial
1046 gene dosage series in tomato to study the mechanisms by which the cloned
1047 quantitative trait locus fw2.2 controls fruit size. *Plant Physiol.* 132: 292–299.
- 1048 Lomize, A., L., Todd, S., C., Pogozheva, I., D. (2022) Spatial arrangement of proteins
1049 in planar and curved membranes by PPM 3.0. *Protein Sci.* 31: 209-220.
- 1050 Marshall, O.J. (2004). PerlPrimer: cross-platform, graphical primer design for
1051 standard, bisulphite and real-time PCR. *Bioinformatics* 20: 2471–2472.
- 1052 Martiniere, A. et al. (2012). Cell wall constrains lateral diffusion of plant plasma-
1053 membrane proteins. *Proc. Natl. Acad. Sci. USA* 109: 12805–12810.
- 1054 Martinière, A., Gibrat, R., Sentenac, H., Dumont, X., Gaillard, I., and Paris, N. (2018).
1055 Uncovering pH at both sides of the root plasma membrane interface using
1056 noninvasive imaging. *Proc. Natl. Acad. Sci. USA* 115: 6488–6493.
- 1057 Maule, A. J., Benitez-Alfonso, Y. and Faulkner, C. (2011). Plasmodesmata –
1058 membrane tunnels with attitude. *Curr. Opin. Plant Biol.* 14: 683–690.
- 1059 Mu, Q. et al. (2017). Fruit weight is controlled by Cell Size Regulator encoding a
1060 novel protein that is expressed in maturing tomato fruits. *PLOS Genet.* 13:
1061 e1006930.
- Nafati, M., Cheniclet, C., Hernould, M., Do, P.T., Fernie, A., Chevalier, C. and
Gévaudant F. (2011). The specific overexpression of a Cyclin Dependent Kinase
Inhibitor in tomato fruit mesocarp cells uncouples endoreduplication and cell
growth. *Plant J.* 65: 543–556.
- 1062 Nesbitt, T.C. and Tanksley, S.D. (2001). fw2.2 Directly Affects the Size of Developing
1063 Tomato Fruit, with Secondary Effects on Fruit Number and Photosynthate
1064 Distribution. *Plant Physiol.* 127: 575–583.

- 1065 O'Leary, R. et al. (2018). Exposure to heavy metal stress triggers changes in
1066 plasmodesmatal permeability via deposition and breakdown of callose. *J. Exp.*
1067 *Bot.* 69, 3715–3728.
- 1068 Perez-Riverol, Y., et al. (2022). The PRIDE database resources in 2022: A Hub for
1069 mass spectrometry-based proteomics evidences. *Nucleic Acids Res.* 50: D543-
1070 D552.
- 1071 Petit, J. D., Li, Z. P., Nicolas, W. J., Grison, M. S. and Bayer, E. M. (2020). Dare to
1072 change, the dynamics behind plasmodesmata-mediated cell-to-cell
1073 communication. *Curr. Opin. Plant Biol.* 53: 80–89.
- 1074 Platre, M.P., et al. (2022). The receptor kinase SRF3 coordinates iron-level and
1075 flagellin dependent defense and growth responses in plants. *Nat Commun.* 13:
1076 4445.
- 1077 Qiao, Z. et al. (2017). The Gm *FWL1* (*FW2-2-like*) nodulation gene encodes a
1078 plasma membrane microdomain-associated protein: A FW2-2-like protein is
1079 located in plasma membrane microdomains. *Plant Cell Environ.* 40: 1442–1455.
- 1080 Ruan, B. et al. (2020). Natural variation in the promoter of *TGW2* determines grain
1081 width and weight in rice. *New Phytol.* 227: 629-640.
- 1082 Saatian, B., Kolhalmi S.E. and Cui, Y. (2023). Localization of Arabidopsis Glucan
1083 Synthase-Like 5, 8, and 12 to plasmodesmata and the GSL8-dependent role of
1084 PDLP5 in regulating plasmodesmal permeability. *Plant Signaling Behav.* 18:
1085 e2164670.
- 1086 Song, W.-Y. et al. (2004). A Novel Family of Cys-Rich Membrane Proteins Mediates
1087 Cadmium Resistance in Arabidopsis. *Plant Physiol.* 135: 1027–1039.
- 1088 Song, W.-Y. et al. (2010). Arabidopsis PCR2 Is a Zinc Exporter Involved in Both Zinc
1089 Extrusion and Long-Distance Zinc Transport. *Plant Cell* 22: 2237–2252.
- 1090 Song L., Wang, R., Zhang, L., Wang, Y. and Yao, S. (2016). CRR1 encoding callose
1091 synthase functions in ovary expansion by affecting vascular cell patterning in rice.
1092 *Plant J.* 88: 620–632.
- 1093 Swinnen, G., et al. (2022) SIKIX8 and SIKIX9 are negative regulators of leaf and fruit
1094 growth in tomato. *Plant Physiol.* 188: 382-396.
- 1095 Tee, E. E., Johnston, M. G., Papp, D. and Faulkner, C. (2022). A PDLP-NHL3
1096 complex integrates plasmodesmal immune signaling cascades. *Proc. Natl. Acad.*
1097 *Sci. USA* 120: e2216397120.

- 1098 Thomas, C.L., Bayer, E.M., Ritzenthaler, C., Fernandez-Calvino, L., and Maule, A.J.
1099 (2008). Specific targeting of a plasmodesmal protein affecting cell-to-cell
1100 communication. *PLoS Biol.* 6: e7.
- 1101 Tourdot, E., Mauxion, J.-P., Gonzalez, N. and Chevalier C. (2023). Endoreduplication
1102 in plant organogenesis: a means to boost fruit growth. *J. Exp. Bot.* erad235,
1103 <https://doi.org/10.1093/jxb/erad235>.
- 1104 Usak, D., Haluska, S. and Pleskot, R. (2023). Callose synthesis at the center point of
1105 plant development - An evolutionary insight. *Plant Physiol.* kiad274,
1106 <https://doi.org/10.1093/plphys/kiad274>
- 1107 van der Knaap, E. and Tanksley, S. D. (2003). The making of a bell pepper-shaped
1108 tomato fruit: identification of loci controlling fruit morphology in Yellow Stuffer
1109 tomato. *Theor. Appl. Genet.* 107: 139–147.
- 1110 Van Norman, J. M., Breakfield, N. W. and Benfey, P. N. (2011). Intercellular
1111 Communication during Plant Development. *Plant Cell* 23: 855–864.
- 1112 Varadi, M., et al. (2022). AlphaFold Protein Structure Database: massively expanding
1113 the structural coverage of protein-sequence space with high-accuracy models."
1114 *Nucl. Acids Res.* 50: 439-444.
- 1115 Wang, Y., et al. (2023). Plasmodesmata mediate cell-to-cell transport of
1116 brassinosteroid hormones. *Nat. Chem. Biol.* In press.
- 1117 Weber, E., Gruetzner, R., Werner, S., Engler, C. and Marillonnet, S. (2011).
1118 Assembly of Designer TAL Effectors by Golden Gate Cloning. *PLoS ONE* 6:
1119 e19722.
- 1120 Wei, Z., Wang, J., Yang, S. and Song, Y. (2015). Identification and expression
1121 analysis of the LRR-RLK gene family in tomato (*Solanum lycopersicum*) Heinz
1122 1706. *Genome* 58: 121–134.
- 1123 Weinl, C. et al. (2005). Novel Functions of Plant Cyclin-Dependent Kinase Inhibitors,
1124 ICK1/KRP1, Can Act Non-Cell-Autonomously and Inhibit Entry into Mitosis. *Plant*
1125 *Cell* 17: 1704–1722.
- 1126 Wu, J. et al. (2023). Cold stress induces malformed tomato fruits by breaking the
1127 feedback loops of stem cell regulation in floral meristem. *New Phytol.* 237: 2268–
1128 2283.
- 1129 Wu, S. and Gallagher, K.L. (2011). Mobile protein signals in plant development. *Curr.*
1130 *Opin. Plant Biol.* 14: 563–570

- 1131 Wu, S.-W., Kumar, R., Iswanto, A.B.B. and Kim, J.-Y. (2018). Callose balancing at
1132 plasmodesmata. *J. Exp. Bot.* 69: 5325–5339.
- 1133 Xu, J. et al. (2013). Molecular characterization and functional analysis of “fruit-
1134 weight2.2-like” gene family in rice. *Planta* 238: 643–655.
- 1135 Yan, D. et al. (2019). Sphingolipid biosynthesis modulates plasmodesmal
1136 ultrastructure and phloem unloading. *Nat. Plants* 5: 604–615.
- 1137 Zsögön, A. et al. (2018). De novo domestication of wild tomato using genome editing.
1138 *Nat. Biotechnol.* 36: 1211–1216.
- 1139
- 1140

ACCEPTED MANUSCRIPT

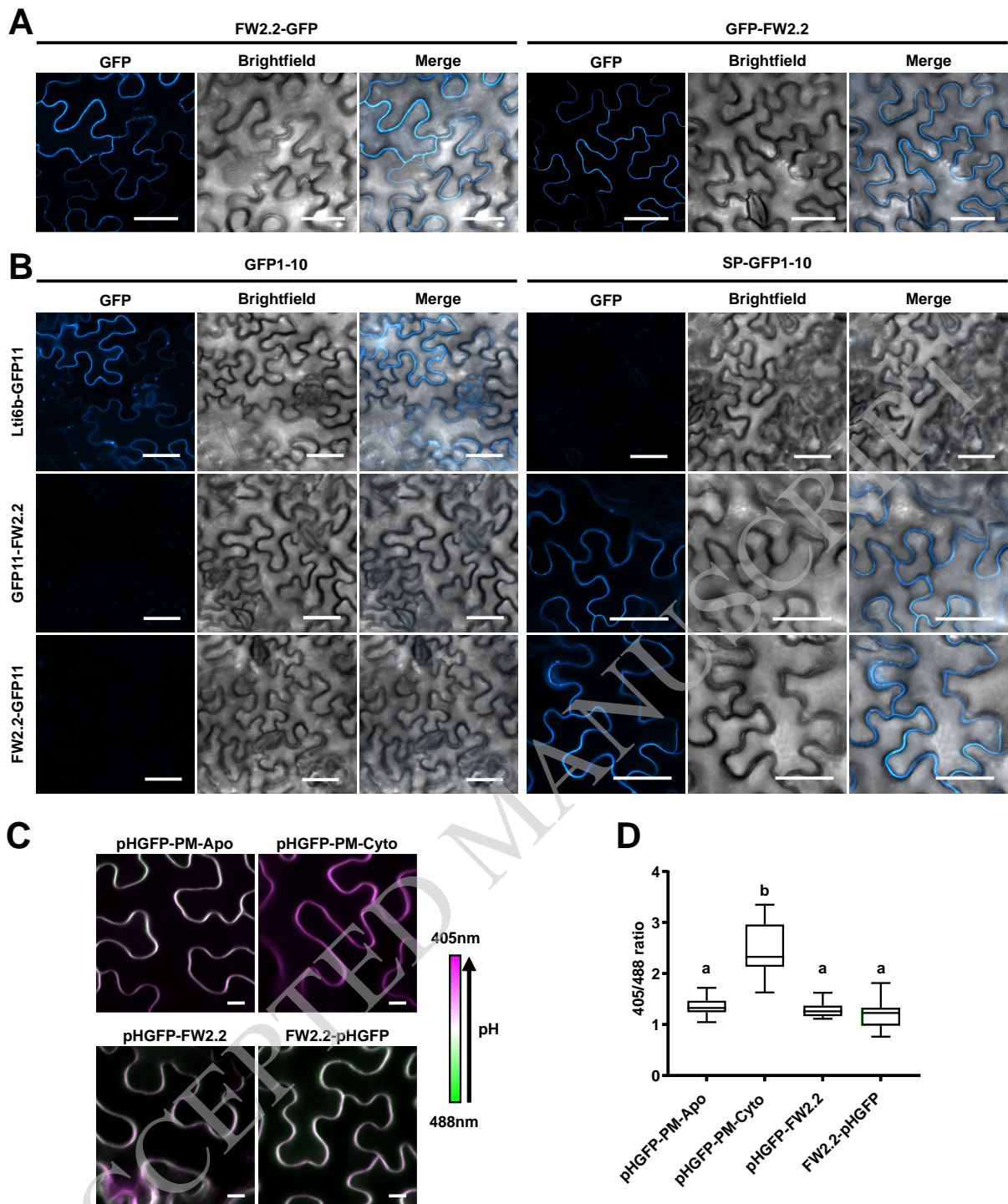


Figure 1. Topological analysis of FW2.2 at the plasma membrane.

(A) Subcellular localization of FW2.2 fused to GFP in *N. benthamiana* leaf epidermal cells. Scale bar= 50 μ m.

(B) BiFC assays deciphering the topology of FW2.2 at the plasma membrane. Transient expressions of FW2.2 or Lti6b fused to GFP11 and with a cytosolic GFP (GFP1-10) or an apoplastic GFP (SP-GFP1-10) were performed in *N. benthamiana* leaves, followed by observation using confocal microscopy. Scale bar= 50 μ m.

(C) Confocal imaging of pHGFP-PM-Apo, pHGFP-PM-Cyto and pHGFP fused to FW2.2 at the N- and C-terminus in *N. benthamiana* leaf epidermal cells. The four images were taken using the same confocal settings. Scale bar= 10 μ m.

(D) 405/488 intensity ratio at plasma membrane. Boxplots : whiskers extend from minimum to maximum, box extends from the 25th to 75th percentiles, the line in the middle is the median. $n > 15$ different images. ANOVA followed by Tukey's test; $P < 0.05$ between a and b groups.

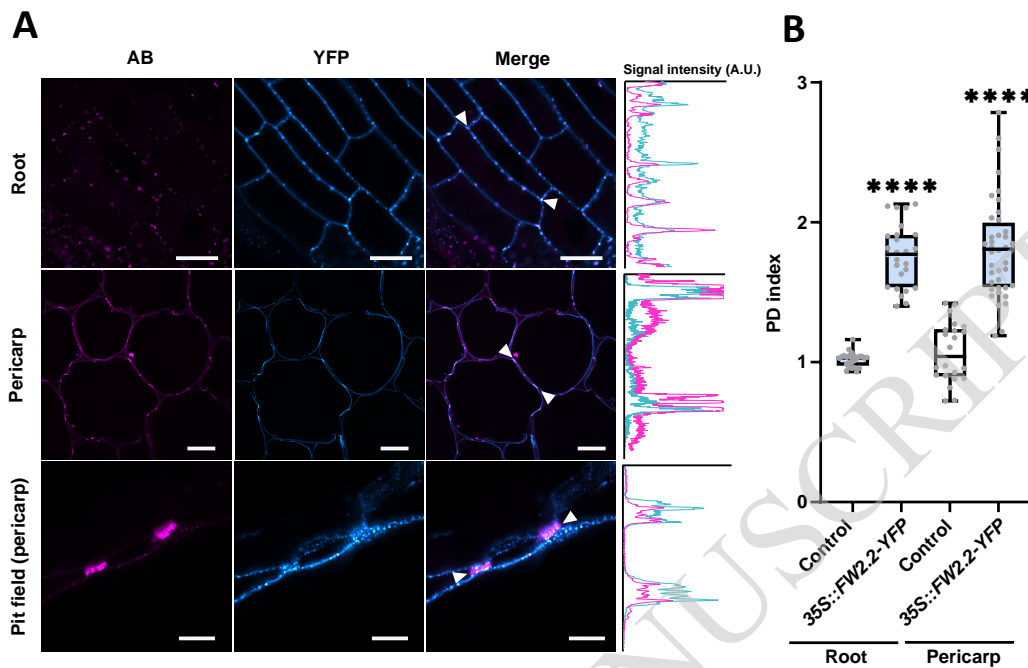


Figure 2. FW2.2 is enriched at PD.

(A) Confocal microscope observations of FW2.2-YFP localization in roots, pericarp and pit field junctions in pericarp cells from 35S::FW2.2-YFP plants. Scale bar = 10 μ m (root and pericarp); = 5 μ m (pit field). Intensity plots delineated by the two white arrowheads are shown for each co-localisation pattern. A.U. = Arbitrary unit.

(B) PD index for FW2.2 in roots and pericarp tissue of 35S::FW2.2-YFP plants compared to WT. Boxplot : whiskers extend from minimum to maximum, box extends from the 25th to 75th percentiles, the line in the middle is the median. n>20 ROIs from 5 images. Statistical analysis: Student's t-test. ****P < 0.0001.

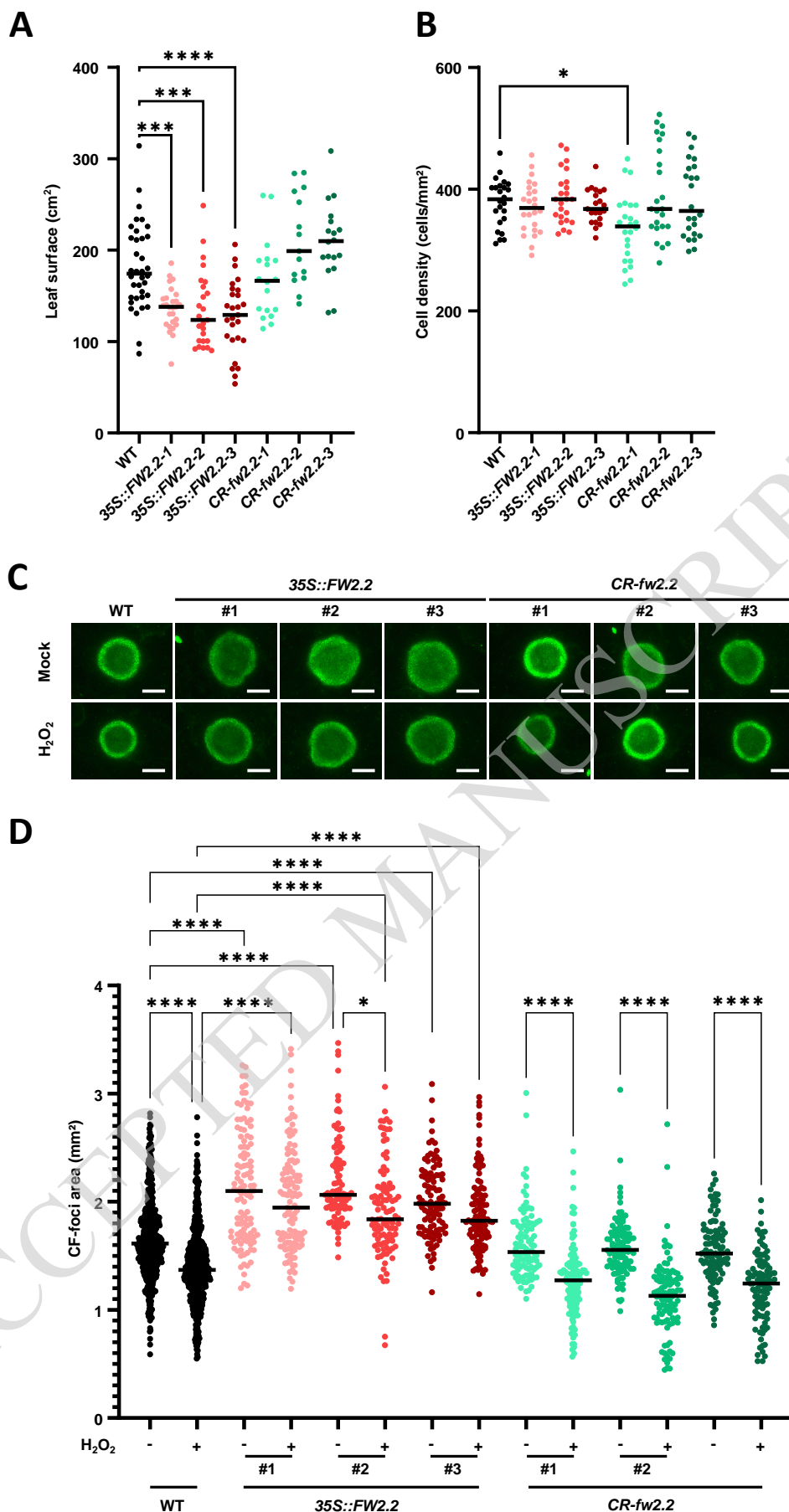


Figure 3. The overexpression of FW2.2 enhances cell-to-cell diffusion in leaves.

(A) Determination of the mean mature leaf surface in WT, 35S::FW2.2 and CR-fw2.2 lines. $n > 15$ leaves from 5 plants per genotype (each dot represents one leaf surface measurement).

(B) Determination of the cell density in leaves from WT, 35S::FW2.2 and CR-fw2.2 lines. $n > 24$ images from 3 plants per genotype (each dot represents one measurement of the number of cells per mm²).

(C) DANS assays using leaves from WT, 35S::FW2.2 and CR-fw2.2 lines with or without H₂O₂

(D) Quantification of the CF-foci area in WT, 35S::FW2.2 and CR-fw2.2 lines with or without H₂O₂ treatment. Statistical analysis: Kruskal–Wallis test with post hoc Dunn multiple comparison test. * $P < 0.05$; *** $P < 0.001$, **** $P < 0.0001$. $n > 100$ CF-foci from > 20 different leaflets from ≥ 6 plants per genotype (each dot represents the measurement of individual foci area).

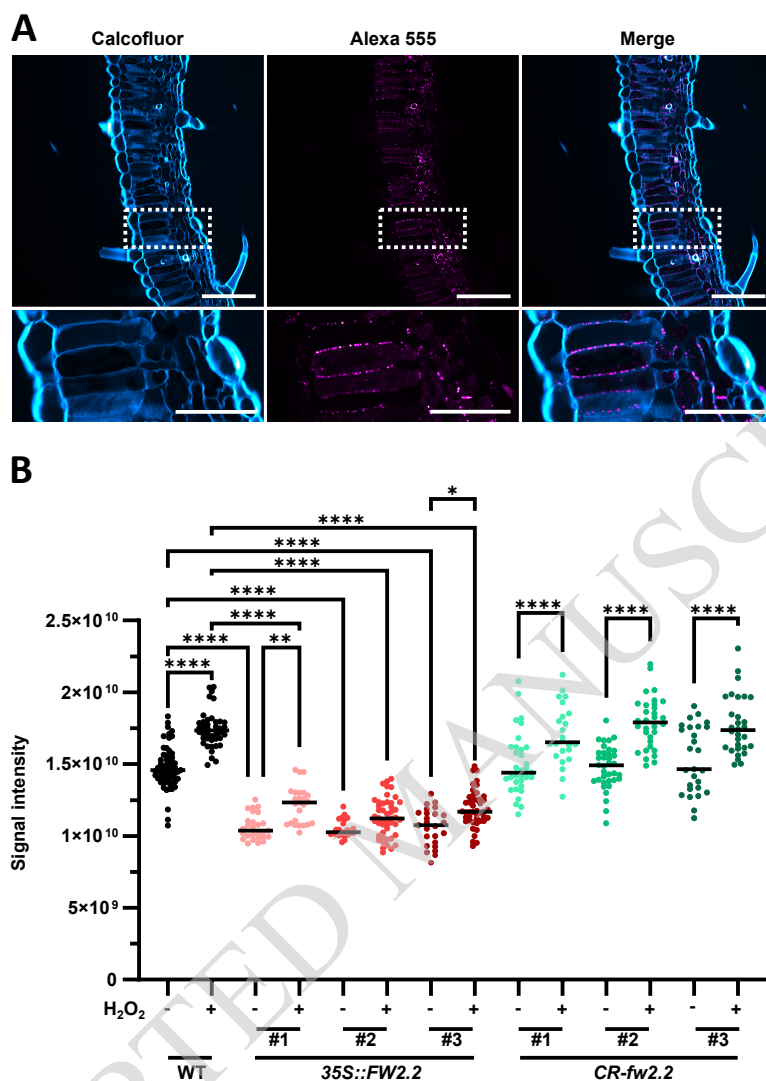


Figure 4. The overexpression of FW2.2 alters callose deposition in leaves.

(A) Immuno-labeling of callose in leaves of WT plants. Close-up images correspond to the white square location. Scale bar = 100 μm and 50 μm (close-up).

(B) Quantification of callose deposition in WT, 35S::FW2.2 and CR-fw2.2 lines. The signal intensity for callose deposition is integrated to the pixel surface measured. Statistical analysis: Kruskal–Wallis test with post hoc Dunn multiple comparison test. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$. $n > 20$ measurements on 2–3 leaflets from 2–3 plants.

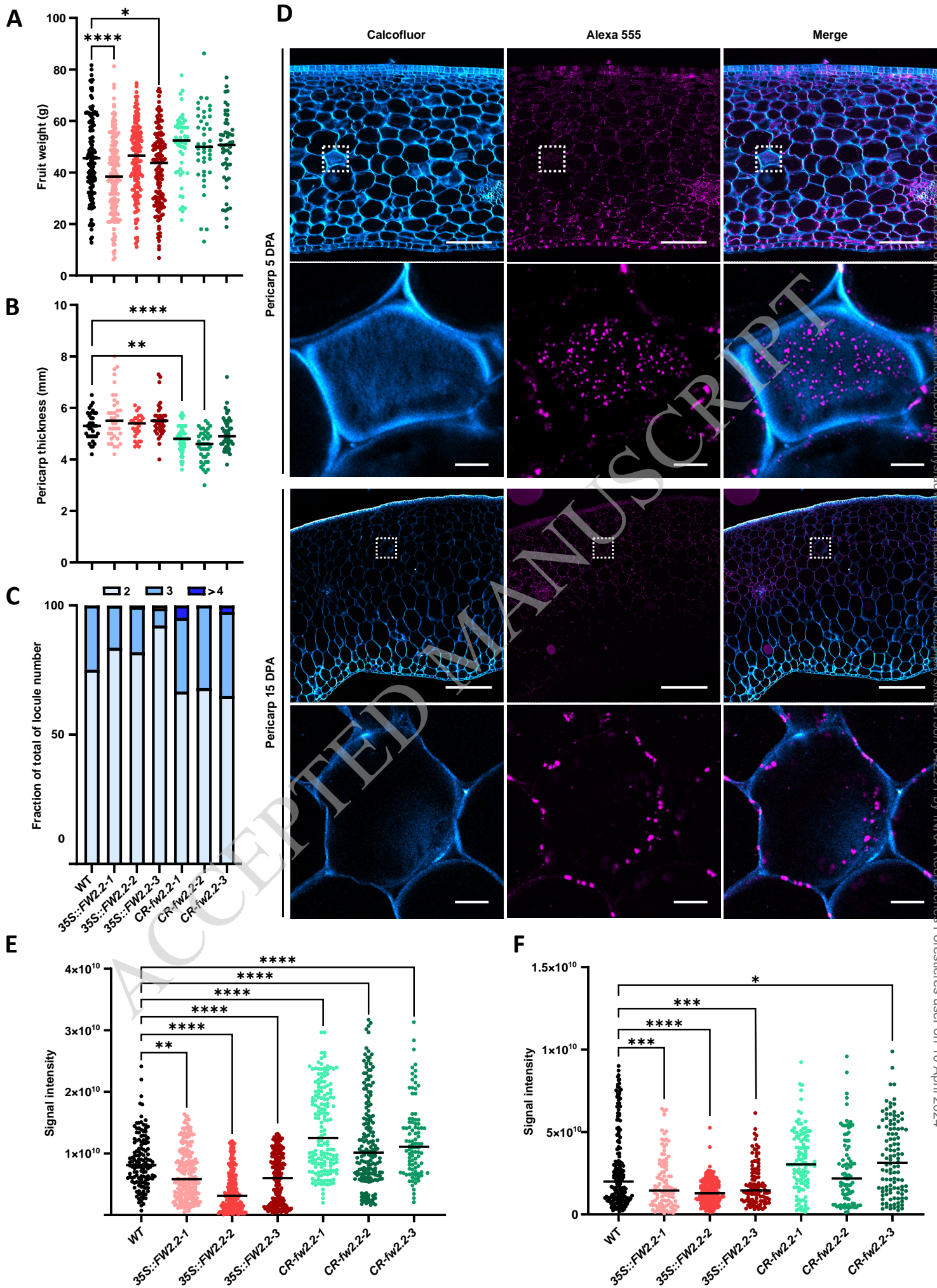


Figure 5. Callose deposition is altered at 5 and 15 DPA in fruit pericarp of 35S::*FW2.2* and *CR-fw2.2* plants.

(A-C) Phenotypic analysis of fruits (at breaker stage) from 35S::*FW2.2 fw2.2*

n>40 fruits from 4 plants per line; Determination of the pericarp thickness (**B**); Determination of the number of fruit locules (**C**), n>25 fruits from 4 plants per lines.

(D) Immunolabelling of callose in 5 DPA (top) and 15 DPA (bottom) pericarp from WT fruits. Scale bar = 100 μm (5 DPA); = 10 μm (5 DPA close-up); = 500 μm (15 DPA); =25 μm (15DPA close-up).

(E-F) Level of callose deposition in WT, 35S::*FW2.2* and *CR-fw2.2* lines at 5 (**E**) and 15 DPA (**F**). The signal intensity for callose deposition is integrated to the pixel surface measured.

(A-F) Statistical analysis: Kruskal–Wallis test with post hoc Dunn multiple comparison test. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$. n>80 images measurement from 4-5 pericarp slices of 4-5 fruits for each line.

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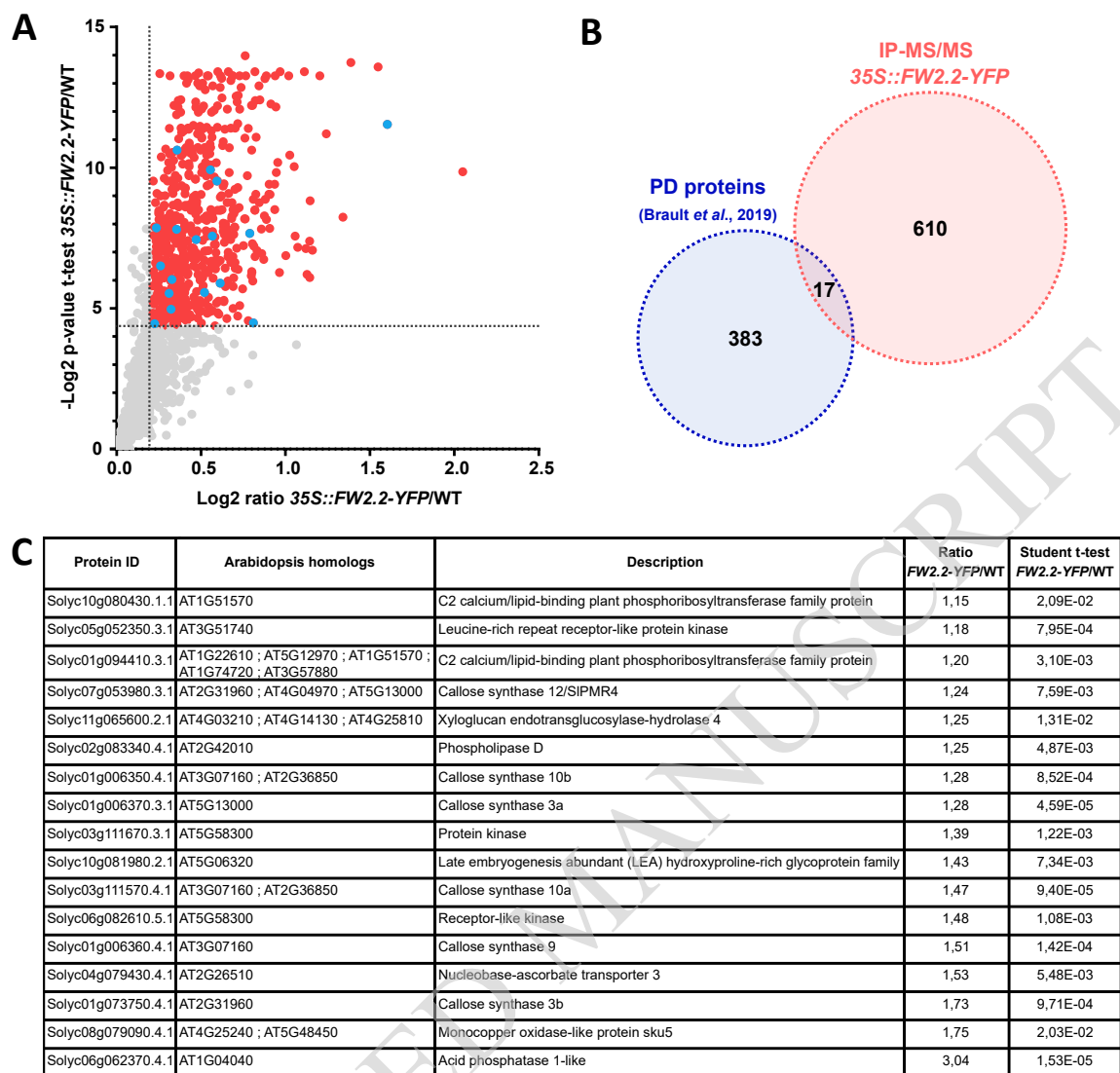


Figure 6. FW2.2 co-immunoprecipitate with several PD localized proteins including callose synthases.

(A) Dot plots showing enriched proteins in 35S::FW2.2-YFP IP-MS/MS experiments in 10 DPA pericarp. Red dot indicates significantly enriched protein (based on a Student's t-test with Benjamini-Hochberg correction $P < 0.05$ and an enrichment ratio > 1.15). Blue dots indicate proteins found in the PD proteome.

(B) Venn diagram showing the overlap between the IP-MS/MS proteome and the PD proteome from Brault et al. (2019). Statistical analysis: Hypergeometric test $P = 0.0021$.

(C) List of plasmodesmata associated proteins detected in the IP-MS/MS proteome.

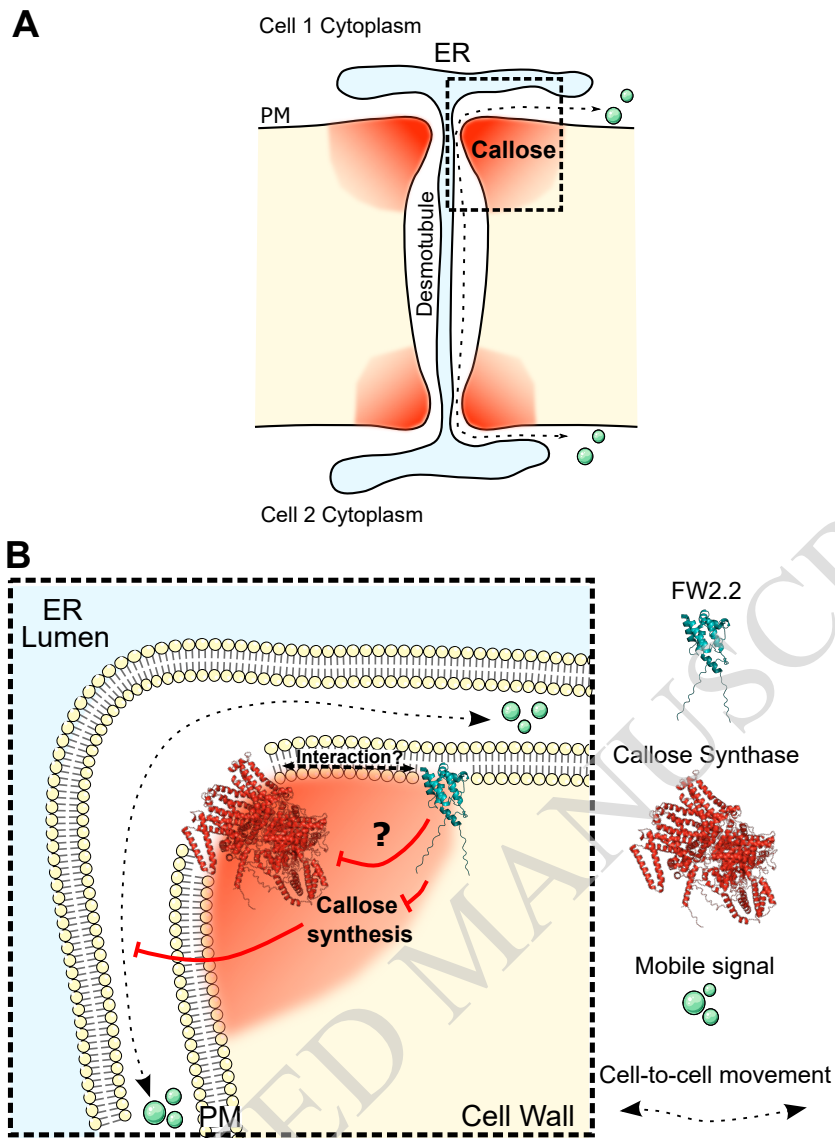


Figure 7. Model illustrating the function of FW2.2 in regulating callose synthesis at PD.

(A) Regulation of PD aperture by callose deposition at the neck region of PD. A high callose deposition level restricts the aperture of PD and the size of signalling molecules passing through.

(B) Molecular and cellular model for the regulation of callose synthase activity by FW2.2 at PD.

Parsed Citations

- Alpert, K. B., Grandillo, S. and Tanksley, S. D. (1995).** fw2.2: a major QTL controlling fruit weight is common to both red- and green-fruited tomato species. *Theor. Appl. Genet.* 91: 994–1000.
Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Amsbury, S., Kirk, P. and Benitez-Alfonso, Y. (2018).** Emerging models on the regulation of intercellular transport by plasmodesmata-associated callose. *J. Exp. Bot.* 69: 105–115.
Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Baldet, P., Hernould, M., Laporte, F., Mounet, F., Just, D., Mouras, A., Chevalier, C. and Rothan C. (2006).** The expression of cell proliferation-related genes in early developing flower is affected by fruit load reduction in tomato plants. *J. Exp. Bot.* 57: 961-970
Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Beauchet, A., Gévaudant, F., Gonzalez, N. and Chevalier, C. (2021).** In search of the still unknown function of FW2.2/CELL NUMBER REGULATOR, a major regulator of fruit size in tomato. *J. Exp. Bot.* 72: 5300–5311.
Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Bisbis, B., Delmas, F., Joubès, J., Sicard, A., Hernould, M., Inzé, D., Mouras, A. and Chevalier, C. (2006).** Cyclin-Dependent Kinase Inhibitors are involved in endoreduplication during tomato fruit development. *J. Biol. Chem.* 281: 7374-7383.
Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Blanca, J., et al. (2015).** Genomic variation in tomato, from wild ancestors to contemporary breeding accessions. *BMC Genomics* 16: 257.
Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Bolte, S. et al. (2004).** FM-dyes as experimental probes for dissecting vesicle trafficking in living plant cells. *J. Microsc.* 214: 159–173.
Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Brandizzi F., et al. (2002).** The destination for single-pass membrane proteins is influenced markedly by the length of the hydrophobic domain. *Plant Cell* 14: 1077–1092.
Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Braut, M.L. et al. (2019).** Multiple C2 domains and transmembrane region proteins (MCTPs) tether membranes at plasmodesmata. *EMBO Rep.* 20: e47182.
Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Chakrabarti, M. et al. (2013).** A cytochrome P450 regulates a domestication trait in cultivated tomato. *Proc. Natl. Acad. Sci. USA* 110: 17125–17130.
Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Chen, X.Y. and Kim, J.Y. (2009).** Callose synthesis in higher plants. *Plant Signal. Behav.* 4: 489–492.
Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Concordet, J.-P. and Haeussler, M. (2018).** CRISPOR: intuitive guide selection for CRISPR/Cas9 genome editing experiments and screens. *Nucleic Acids Res.* 46: W242–W245.
Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Cong, B., Liu, J. and Tanksley, S. D. (2002).** Natural alleles at a tomato fruit size quantitative trait locus differ by heterochronic regulatory mutations. *Proc. Natl. Acad. Sci. USA* 99: 13606–13611.
Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Cong, B. and Tanksley, S. D. (2006).** FW2.2 and cell cycle control in developing tomato fruit: a possible example of gene co-option in the evolution of a novel organ. *Plant Mol. Biol.* 62: 867–880.
Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Cox, J., Hein, M.Y., Luber, C.A., Paron, I., Nagaraj, N. and Mann, M. (2014).** Accurate proteome-wide label-free quantification by delayed normalization and maximal peptide ratio extraction, termed MaxLFQ. *Mol Cell Proteomics* 13: 2513-2526.
Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Cui, W. and Lee, J.-Y. (2016).** Arabidopsis callose synthases CalS1/8 regulate plasmodesmal permeability during stress. *Nat. Plants* 2: 16034.
Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Cui, W., Wang, X. and Lee, J.-Y. (2015).** Drop-ANd-See: A Simple, Real-Time, and Noninvasive Technique for Assaying Plasmodesmal Permeability. In *Plasmodesmata: Methods and Protocols*, M. Heinlein, ed, *Methods in Molecular Biology* (Springer: New York, NY), pp. 149–156.
Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Dahan, Y., Rosenfeld, R., Zadiranov, V. and Irihimovitch, V. (2010). A proposed conserved role for an avocado FW2.2-like gene as a negative regulator of fruit cell division. *Planta* 232: 663–676 .

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

De Franceschi, P. et al. (2013). Cell number regulator genes in *Prunus* provide candidate genes for the control of fruit size in sweet and sour cherry. *Molecular Breeding* 32: 311–326.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Doganlar, S., Frary, A., Daunay, M.-C., Lester, R.N. and Tanksley, S.D. (2002). Conservation of gene function in the Solanaceae as revealed by comparative mapping of domestication traits in Eggplant. *Genetics* 161: 1713–1726.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Frary, A. et al. (2000). fw2.2: A Quantitative Trait Locus Key to the Evolution of Tomato Fruit Size. *Science* 289: 85–88.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Galaviz-Hernandez, C. et al. (2003). Plac8 and Plac9, novel placental-enriched genes identified through microarray analysis. *Gene* 309: 81–89.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Gallagher, K. L., Sozzani, R. and Lee, C.-M. (2014). Intercellular Protein Movement: Deciphering the Language of Development. *Annu. Rev. Cell Dev. Biol.* 30: 207–233.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Gaudio-Pedraza, R. et al. (2018). Callose-Regulated Symplastic Communication Coordinates Symbiotic Root Nodule Development. *Curr. Biol.* 28: 3562–3577.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Grandillo, S., Ku, H. M. and Tanksley, S. D. (1999). Identifying the loci responsible for natural variation in fruit size and shape in tomato. *Theor. Appl. Genet.* 99: 978–987.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Grison, M. S. et al. (2019). Plasma Membrane-Associated Receptor-like Kinases Relocalize to Plasmodesmata in Response to Osmotic Stress. *Plant Physiol.* 181: 142–160.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Guo, M. et al. (2010). Cell Number Regulator1 Affects Plant and Organ Size in Maize: Implications for Crop Yield Enhancement and Heterosis. *Plant Cell* 22: 1057–1073.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Hallgren, J., et al. (2022). DeepTMHMM predicts alpha and beta transmembrane proteins using deep neural networks. *BioRxiv*. 2022-04.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Han, X. et al. (2014a). Auxin-Callose-Mediated Plasmodesmal Gating Is Essential for Tropic Auxin Gradient Formation and Signaling. *Dev. Cell* 28: 132–146.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Han, X. et al. (2014b). Transcription factor-mediated cell-to-cell signalling in plants. *J. Exp. Bot.* 65: 1737–1749.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Jumper, J., et al. Highly accurate protein structure prediction with AlphaFold (2021). *Nature* 596: 583–589.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Lemoine, F., Correia, D., Lefort, V., Doppelt-Azeroual, O. Mareuil, F., Cohen-Boulakia, S. and Gascuel, O. (2019). NGPhylogeny.fr: new generation phylogenetic services for non-specialists. *Nucleic Acids Res.* 47: W260–W265.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Li, Z. and He, C. (2015). *Physalis floridana* Cell Number Regulator1 encodes a cell membrane-anchored modulator of cell cycle and negatively controls fruit size. *J. Exp. Bot.* 66: 257–270.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Libault, M. et al. (2010). A member of the highly conserved FWL (tomato FW2.2-like) gene family is essential for soybean nodule organogenesis: A soybean FWL essential for nodulation. *Plant J.* 62: 852–864.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Lippman, Z. B. and Tanksley, S.D. (2001). Dissecting the Genetic Pathway to Extreme Fruit Size in Tomato Using a Cross Between the Small-Fruited Wild Species *Lycopersicon pimpinellifolium* and *L. esculentum* var. Giant Heirloom. *Genetics* 158: 413–422.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Liu, J., Cong, B. and Tanksley, S.D. (2003). Generation and analysis of an artificial gene dosage series in tomato to study the mechanisms by which the cloned quantitative trait locus fw2.2 controls fruit size. *Plant Physiol.* 132: 292–299.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Lomize, A. L., Todd, S., C., Pogozheva, I., D. (2022) Spatial arrangement of proteins in planar and curved membranes by PPM 3.0. *Protein Sci.* 31: 209-220.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Marshall, O.J. (2004). PerlPrimer: cross-platform, graphical primer design for standard, bisulphite and real-time PCR. *Bioinformatics* 20: 2471–2472.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Martiniere, A. et al. (2012). Cell wall constrains lateral diffusion of plant plasma-membrane proteins. *Proc. Natl. Acad. Sci. USA* 109: 12805–12810.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Martinière, A., Gibrat, R., Sentenac, H., Dumont, X., Gaillard, I., and Paris, N. (2018). Uncovering pH at both sides of the root plasma membrane interface using noninvasive imaging. *Proc. Natl. Acad. Sci. USA* 115: 6488–6493.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Maule, A. J., Benitez-Afonso, Y. and Faulkner, C. (2011). Plasmodesmata – membrane tunnels with attitude. *Curr. Opin. Plant Biol.* 14: 683–690.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Mu, Q. et al. (2017). Fruit weight is controlled by Cell Size Regulator encoding a novel protein that is expressed in maturing tomato fruits. *PLOS Genet.* 13: e1006930.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Nafati, M., Cheniclet, C., Hernould, M., Do, P.T., Fernie, A., Chevalier, C. and Gévaudant F. (2011). The specific overexpression of a Cyclin Dependent Kinase Inhibitor in tomato fruit mesocarp cells uncouples endoreduplication and cell growth. *Plant J.* 65: 543–556.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Nesbitt, T.C. and Tanksley, S.D. (2001). fw2.2 Directly Affects the Size of Developing Tomato Fruit, with Secondary Effects on Fruit Number and Photosynthate Distribution. *Plant Physiol.* 127: 575–583.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

O'Lexy, R. et al. (2018). Exposure to heavy metal stress triggers changes in plasmodesmatal permeability via deposition and breakdown of callose. *J. Exp. Bot.* 69, 3715–3728.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Perez-Riverol, Y., et al. (2022). The PRIDE database resources in 2022: A Hub for mass spectrometry-based proteomics evidences. *Nucleic Acids Res.* 50: D543-D552.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Petit, J. D., Li, Z. P., Nicolas, W. J., Grison, M. S. and Bayer, E. M. (2020). Dare to change, the dynamics behind plasmodesmata-mediated cell-to-cell communication. *Curr. Opin. Plant Biol.* 53: 80–89.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Platre, M.P., et al. (2022). The receptor kinase SRF3 coordinates iron-level and flagellin dependent defense and growth responses in plants. *Nat Commun.* 13: 4445.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Qiao, Z. et al. (2017). The Gm FWL1 (FW2-2-like) nodulation gene encodes a plasma membrane microdomain-associated protein: A FW2-2-like protein is located in plasma membrane microdomains. *Plant Cell Environ.* 40: 1442–1455.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Ruan, B. et al. (2020). Natural variation in the promoter of TGW2 determines grain width and weight in rice. *New Phytol.* 227: 629–640.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Saatián, B., Kolhalmi S.E. and Cui, Y. (2023). Localization of Arabidopsis Glucan Synthase-Like 5, 8, and 12 to plasmodesmata and the GSL8-dependent role of PDL5 in regulating plasmodesmal permeability. *Plant Signaling Behav.* 18: e2164670.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Song, W.-Y. et al. (2004). A Novel Family of Cys-Rich Membrane Proteins Mediates Cadmium Resistance in Arabidopsis. *Plant Physiol.* 135: 1027–1039.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Song, W.-Y. et al. (2010). Arabidopsis PCR2 Is a Zinc Exporter Involved in Both Zinc Extrusion and Long-Distance Zinc Transport. *Plant Cell* 22: 2237–2252.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

- Song L., Wang, R., Zhang, L., Wang, Y. and Yao, S. (2016). CRR1 encoding callose synthase functions in ovary expansion by affecting vascular cell patterning in rice. *Plant J.* 88: 620–632.
Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Swinnen, G., et al. (2022) SIKIX8 and SIKIX9 are negative regulators of leaf and fruit growth in tomato. *Plant Physiol.* 188: 382-396.
Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Tee, E. E., Johnston, M. G., Papp, D. and Faulkner, C. (2022). APDLP-NHL3 complex integrates plasmodesmal immune signaling cascades. *Proc. Natl. Acad. Sci. USA* 120: e2216397120.
Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Thomas, C.L., Bayer, E.M., Ritzenthaler, C., Fernandez-Calvino, L., and Maule, A.J. (2008). Specific targeting of a plasmodesmal protein affecting cell-to-cell communication. *PLoS Biol.* 6: e7.
Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Tourdot, E., Mauxion, J.-P., Gonzalez, N. and Chevalier C. (2023). Endoreduplication in plant organogenesis: a means to boost fruit growth. *J. Exp. Bot.* erad235, <https://doi.org/10.1093/jxb/erad235>.
Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Usak, D., Haluska, S. and Pleskot, R. (2023). Callose synthesis at the center point of plant development - An evolutionary insight. *Plant Physiol.* kiad274, <https://doi.org/10.1093/plphys/kiad274>
Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- van der Knaap, E. and Tanksley, S. D. (2003). The making of a bell pepper-shaped tomato fruit: identification of loci controlling fruit morphology in Yellow Stuffer tomato. *Theor. Appl. Genet.* 107: 139–147.
Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Van Norman, J. M., Breakfield, N. W. and Benfey, P. N. (2011). Intercellular Communication during Plant Development. *Plant Cell* 23: 855–864.
Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Varadi, M., et al. (2022). AlphaFold Protein Structure Database: massively expanding the structural coverage of protein-sequence space with high-accuracy models." *Nucl. Acids Res.* 50: 439-444.
Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Wang, Y., et al. (2023). Plasmodesmata mediate cell-to-cell transport of brassinosteroid hormones. *Nat. Chem. Biol.* In press.
Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Weber, E., Gruetzner, R., Werner, S., Engler, C. and Marillonnet, S. (2011). Assembly of Designer TAL Effectors by Golden Gate Cloning. *PLoS ONE* 6: e19722.
Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Wei, Z, Wang, J., Yang, S. and Song, Y. (2015). Identification and expression analysis of the LRR-RLK gene family in tomato (*Solanum lycopersicum*) Heinz 1706. *Genome* 58: 121–134.
Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Weinl, C. et al. (2005). Novel Functions of Plant Cyclin-Dependent Kinase Inhibitors, ICK1/KRP1, Can Act Non-Cell-Autonomously and Inhibit Entry into Mitosis. *Plant Cell* 17: 1704–1722.
Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Wu, J. et al. (2023). Cold stress induces malformed tomato fruits by breaking the feedback loops of stem cell regulation in floral meristem. *New Phytol.* 237: 2268–2283.
Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Wu, S. and Gallagher, K.L. (2011). Mobile protein signals in plant development. *Curr. Opin. Plant Biol.* 14: 563–570
Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Wu, S.-W., Kumar, R., Iswanto, A.B.B. and Kim, J.-Y. (2018). Callose balancing at plasmodesmata. *J. Exp. Bot.* 69: 5325–5339.
Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Xu, J. et al. (2013). Molecular characterization and functional analysis of "fruit-weight2.2-like" gene family in rice. *Planta* 238: 643–655.
Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Yan, D. et al. (2019). Sphingolipid biosynthesis modulates plasmodesmal ultrastructure and phloem unloading. *Nat. Plants* 5: 604–615.
Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Zsögön, A. et al. (2018). De novo domestication of wild tomato using genome editing. *Nat. Biotechnol.* 36: 1211–1216.
Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)