

PRX62 and PRX69 regulate RH growth at low-temperature

Apoplasmic class III peroxidases PRX62 and PRX69 regulate ROS-homeostasis and cell wall associated extensins linked to root hair growth at low-temperature in *Arabidopsis thaliana*

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ABSTRACT

Root Hairs (RHs) growth is highly influenced by endogenous as well as by external environmental signals that coordinately regulate its final cell size. RHs actively expand the root surface responsible for nutrient uptake and water absorption. We have recently determined that RH growth was unexpectedly boosted when *Arabidopsis thaliana* seedlings are cultivated at low temperatures. It was proposed that RH growth plasticity in response to low temperature was linked to a reduced nutrient availability in the media. Here, we explored the molecular basis of this strong RH growth response by using the Genome Wide Association Studies (GWAS) approach on *Arabidopsis thaliana* natural accessions. We identified the poorly characterized PEROXIDASE 62 (PRX62) as a key protein triggering this conditional growth under a moderate low-temperature stress. In addition, we identified the related protein PRX69 as an important factor in this developmental process. The *prx62 prx69* double mutant and the *PRX62* and *PRX69* over-expressing lines showed contrasting RH phenotypes, peroxidase activities and cyt/apoReactive Oxygen Species (ROS) levels. Strikingly, a cell wall protein extensin (EXT) reporter revealed the effect of peroxidase activity on the EXT cell wall association at 10°C in the RH apical zone. EXT cell wall insolubilization was enhanced at 10°C, which was completely abolished under the PRX inhibitor salicylhydroxamic acid (SHAM) treatment. Finally, we demonstrated that the *Root Hair defective 6-like 4* (RSL4) bHLH family transcription factor directly controls the expression of PRX69. Collectively, our results indicate that both PRX62 and PRX69 are key apoplastic PRXs that modulate ROS-homeostasis and cell wall EXT-insolubilization linked to RH elongation at low-temperature.

Key words: Arabidopsis, Class-III Peroxidases, Extensins, Genome Wide Association Studies, Low-temperature, RSL4, ROS-homeostasis.

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1 INTRODUCTION

2
3 Root hairs (RH) have emerged as an excellent model system for studying cell size regulation since
4 they can elongate several hundred-fold their original dimensions. The rate at which cells grow is
5 determined both by cell-intrinsic factors as well as by external environment signals. RHs represent an
6 important proportion of the surface root area, crucial for nutrient uptake and water absorption. RH
7 growth is controlled by the interaction of several proteins, including the bHLH transcription factor
8 (TF) RSL4 (*Root Hair Defective Six-like 4*), which defines the final RH length (Datta et al. 2015;
9 Mangano et al. 2017) as well as the related TF RSL2 (*Root Hair Defective 6 Six-like 2*; Bhosale et al.
10 2018; Mangano et al. 2018). Together with the developmental and genetic pathways, several
11 hormones are important modulators of RH cell growth (Lee & Cho 2013; Velasquez et al. 2016; Zhang
12 et al 2016; Mangano et al. 2017). In addition, abnormal Reactive Oxygen Species (ROS) accumulation
13 in RHs triggers either exacerbated growth or cell bursting. Exogenous H₂O₂ inhibited RH polar
14 expansion, while treatment with ROS scavengers (e.g., ascorbic acid) caused RH bursting, reinforcing
15 the notion that a balanced ROS-homeostasis is required to modulate cell elongation by affecting cell
16 wall properties. Accordingly, apoplastic ROS (_{apo}ROS) produced in the apoplast (specifically _{apo}H₂O₂)
17 coupled to apoplastic Class III peroxidase (PRX) activity directly affect the degree of cell wall
18 crosslinking (Passardi et al. 2004) by oxidizing cell wall compounds and leading to the stiffening of
19 the wall in peroxidative cycles (PC) (Orman-Ligeza et al. 2016). In addition, _{apo}ROS coupled to PRX
20 activity enhances non-enzymatic wall loosening by producing oxygen radical species (e.g., •OH) and
21 promoting polar-growth in hydroxylic cycles (HC) (Dunand et al. 2007). Finally, PRXs also contribute
22 to the production of superoxide radical (O₂^{•-}) pool together with NADPH oxidase/respiratory burst
23 oxidase homolog (RBOH) proteins by oxidizing singlet oxygen in the oxidative cycle (OC), thereby
24 affecting _{apo}H₂O₂ levels. Given their multiple enzymatic activities *in vivo*, apoplastic PRXs emerge as
25 versatile regulators of rapid cell elongation. Assigning specific functions to each of the numerous PRXs
26 (73 encoded in Arabidopsis; Valerio et al. 2004; and even more in other plant types, e.g. 138 encoded
27 in Rice; Passardi et al. 2004a) has been challenging. Recently, three PRXs possibly linked to Tyr-
28 crosslinking of cell wall extensins (EXTs), PRX01, PRX44 and PRX73, were characterized as important
29 regulators of RH growth under low-nutrient conditions (Marzol et al. 2021). These RH specific PRXs
30 are under the direct control of the TF RSL4, a master regulator of RH cell size (Yi et al. 2010; Datta et
31 al. 2015; Mangano et al. 2017). In addition, other PRXs were postulated to crosslink EXTs in aerial
32 plant tissues. PRX09 and PRX40 were proposed to crosslink EXTs during tapetum development, and
33 both, PRXs were able to crosslink EXT23 in transient expression experiments (Jacobowitz et al. 2019).
34
35 Although there is a fairly well-known mechanistic view of how RH cell expands, the environmental
36 signals that trigger the cell elongation process remain currently unknown. Due to its important role
37 in root physiology, it has been anticipated that RH would be highly susceptible to environmental
38 stresses such as heat or moderate temperature increase, which trigger extensive DNA methylation,

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39 transcriptomic and proteomic changes (Valdés-López et al. 2016; Quint et al. 2016; Hossain et al.
40 2017). Although RH development during cold acclimation remains largely unexplored, it has been
41 observed that many RH-related genes respond to cold in the whole plant or seedlings (Maruyama et
42 al. 2004; Hannah, Heyer & Hinch 2005; Barah et al. 2013). It is known that plants may perceive cold
43 by a putative receptor at the cell membrane and initiate a signal to activate the cold-responsive genes
44 and transcription factors for mediating stress tolerance (Thomashow 1999; Penfield 2008; Ding et al.,
45 2019; Nurhasanah Ritonga and Chen, 2020; Leuendorf et al., 2020). Previously, we have shown that
46 the plant long noncoding RNA (lncRNA) *AUXIN REGULATED PROMOTER LOOP (APOLO)* recognizes the
47 locus encoding the RH (RH) master regulator *RHD6 (Root Hair Defective 6)* and controls *RHD6*
48 transcriptional activity leading to cold-enhanced RH elongation through the consequent activation of
49 *RSL4* (Moison et al. 2021). In addition, *APOLO* is able to bind and positively control the expression of
50 several cell wall EXTENSIN (EXT) encoding genes, including *EXT3*, a key regulator for RH growth
51 (Martinez-Pacheco et al. 2021). Unexpectedly, our previous results indicate that the low-
52 temperatures (10°C) are able to trigger an exacerbated RH growth compared with cell expansion at
53 room temperature (Moison et al. 2021; Martinez-Pacheco et al. 2021). To explore the molecular basis
54 of this strong growth response, we conducted Genome Wide Association Studies (GWAS) on
55 *Arabidopsis thaliana* natural accessions and identified the uncharacterized PEROXIDASE 62 (PRX62)
56 as a key protein that regulates the conditional growth under a moderate low temperature stress. In
57 addition, we also identified a second PRX, i.e. PRX69, as an important player in this developmental
58 response. Both, PRX62 and PRX69 are key enzymes to trigger RH growth, likely by participating in a
59 ROS-mediated mechanism of polar cell growth at low-temperatures. The expression of both PRX
60 encoding genes could be under the regulation of *RSL4*, which has a direct binding to *PRX69* promoter
61 specific regions. Transcriptomic analyses revealed that upon *PRX62* and *PRX69* knockout, several
62 other PRXs and cell wall EXTs encoding genes were differentially expressed, hinting at a
63 compensatory mechanism.

64
65

66 RESULTS AND DISCUSSION

67

68 PRX62 and PRX69 emerged as positive regulators of RH growth at low-temperatures.

69 In order to identify the natural genetic components involved in RH growth under low-temperature
70 conditions (at 10°C), we analyzed natural *A. thaliana* accessions originated from contrasting
71 environments (Europe, Asia, Africa and North America, **Figure S1**). We assessed RH growth for each
72 seedling accession grown under 22°C for 5 days, and then transferred them to 10°C for 3 days. RH
73 length was the phenotypic trait recorded for each accession, and compared to seedlings grown at
74 22°C for 8 days, taken as a control. We observed 15-folds range of natural variation for average RH
75 length (148-2218 µm) in the accessions grown at 10°C (**Figure S2A; Table S1**) in contrast with a lower
76 variability (~7-folds) and significantly shorter overall RH cells when seedlings were grown at 22°C

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77 (136-1034 μm). There is a strong correlation ($R^2=0.981$) for RH length from accessions grown at
78 $22^\circ\text{C}\rightarrow 10^\circ\text{C}$ compared to plants growth at 22°C (**Figure S2B**), indicating that accessions respond in
79 the same manner to a temperature decrease but varying in intensity. Only the most contrasting
80 accession are shown as examples (**Figure 1A**). Thus, moderate low-temperature triggers RH polar-
81 growth across *Arabidopsis* ecotypes by a yet unknown molecular mechanism. To identify candidate
82 genes involved in RH growth response at moderate low-temperature, we performed a GWAS
83 (GWAPP web tool, Seren et al, 2012) using as input data the RH length recorded only at 22°C or at
84 $22^\circ\text{C}\rightarrow 10^\circ\text{C}$ for each accession (**Table S1**). When GWAS was performed measuring RH length
85 obtained at 22°C , no significant associations were identified (**Figure 1B**). On the other hand, after
86 filtering SNPs for a 10% minor allele frequency in the $22^\circ\text{C}\rightarrow 10^\circ\text{C}$ RH length GWAS, a leader SNP
87 m190905 (TAIR10 position 15847854) was significantly associated with RH length (LOD [for log of the
88 odds] =6.01, FDR=0.06) with RH length. This SNP is located in the intron of *PEROXIDASE62* (*PRX62*,
89 AT5G39580). Three additional SNPs located in *PRX62* exons, in high linkage disequilibrium with
90 m190905 ($r^2 > 0.7$, $p < 0.001$), showed relatively high LOD score of association (m190904/15847644,
91 m190907/15848071, m190909/15848704, LOD ~ 4.99 -4.24, **Figure 1C**). These four SNPs formed
92 seven haplotypes, with two major allele-opposite haplotypes (CTGT, $n=79$; TGAA, $n=18$), two
93 haplotypes with very low frequency (CTGA, $n=5$; TGGA, $n=5$) and three unique haplotypes (**Figure**
94 **S3A**). Analysis of variance between the average trait values for all non-unique haplotypes showed
95 that RH length varies among them, having the first and second most frequent haplotypes significantly
96 different values for RH length (**Figure S3B**). In addition, *PRX62* presents two splice variants, differing
97 in the sequence length of the last exon. We then analyzed if these two *PRX62* isoforms can be equally
98 detected and if low-temperature treatment induces a differential expression of any of them.
99 According to **Figure 1D**, only the full-length transcript of *PRX62* (AT5G39580.1) is detectable in the
100 Col-0 that increased up to 2.54 $\log_2\text{FC}$ in roots under low-temperature (RNA-seq). This was further
101 confirmed by RT-qPCR (**Figure S4**). Altogether, our results hinted at *PRX62* as a potential key factor
102 in the regulation of RH growth under low-temperature. Interestingly, according to publicly available
103 datasets of whole seedlings (Schlaen et al. 2015), six PRX genes appeared as induced at 10°C ; notably
104 *PRX62* and *PEROXIDASE69* (*PRX69*, AT5G64100) were predicted to be highly expressed in RHs (**Table**
105 **S2**). *PRX69* also has two predicted variants, the full length AT5G64100.1 and a shorter one
106 AT5G64100.2. By RNA-seq we also confirmed that only the full-length variant of *PRX69* is the most
107 expressed one with an small upregulation (by 0.21 $\log_2\text{FC}$) by low temperature although with similar
108 overall transcriptional levels to *PRX62* (**Figure 2D**). This was also confirmed by RT-qPCR (**Figure S4**).
109 Therefore, we decided to characterize in depth both PRXs, *PRX62* and *PRX69* and their roles in RH
110 growth at 10°C .

111
112 In agreement with GWAS results, low-temperature-mediated growth requires peroxidase activity
113 since the treatment with salicylhydroxamic acid (SHAM), a peroxidase inhibitor (Kim et al. 2012; Kwon
114 et al. 2015) at inhibitory concentration 50% ($\text{IC}_{50}=65\mu\text{M}$) at 22°C was able to repress up to 90% of this

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115 growth response at low temperature (**Figure 2A-B**). Accordingly, peroxidase activity in whole roots
116 was significantly lower under the SHAM treatments at both temperatures (**Figure 2C**). We then tested
117 if *PRX62* and *PRX69* expression levels were different between contrasting accessions based on the
118 RH phenotype at 10°C (**Figure 2D; Figure S4**). Transcript levels of *PRX62* (after 3 days at 10°C) were
119 positively correlated with the RH length of the given accession, i.e. that the higher the expression of
120 *PRX62* at 10°C, the longer the RHs. This implies that high levels of *PRX62* in Wc-1 and very low levels
121 in Bu-2 accessions might be linked to the differential RH phenotype detected at low-temperature and
122 suggests that the causal variation for RH length is dependent on *PRX62* higher expression (**Figure 2D;**
123 **Figure S4**). On the contrary, *PRX69* transcript levels are higher at 10°C, but they did not show any
124 significant variation across accessions. Altogether, these results suggest that upregulation of *PRX62*
125 transcript levels together with high levels of *PRX69* play an important role in RH growth at low-
126 temperature.

127

128 **PRX62 and PRX69 regulate RH growth under low-temperature.**

129 The *in silico* analysis of *PRX62* and *PRX69* expression using Tissue Specific Root eFP
130 (<http://bar.utoronto.ca/eplant/>) showed that both PRX encoding genes were confined to
131 differentiated RH cells with expression in the elongation phase at similar levels than the RH marker
132 *EXPANSIN 7* (**Figure 3A**). Accordingly, the corresponding reporter lines of *PRX62_{pro}GFP* as well as
133 *PRX69_{pro}GFP* showed high levels of signal in RH cells when grown at 10°C while lower expression was
134 detected at 22°C (**Figure 3B**). When *PRX62* and *PRX69* tagged constructs (*35S_{pro}PRX62-TagRFP* and
135 *35S_{pro}PRX69-TagRFP*) are transiently coexpressed in *Nicotiana benthamiana* leaves with a plasma
136 membrane marker, both PRXs showed an apoplastic localization (**Figure S5**). Overall, these results
137 confirm that *PRX62* and *PRX69* are both cold-responsive specific RH class III PRXs that are secreted to
138 the apoplastic space in the cell wall. In order to test if the absence of *PRX62* and *PRX69* is able to
139 modify growth response at 10°C, we assessed two T-DNA mutants for *PRX62* in the Col-0 background
140 (*prx62-1* and *prx62-2*), being a knock-out (*prx62-1*) and a knock-down (*prx62-2*) allele, respectively
141 (Jemmat et al. 2020). In addition, we also characterized two previously reported T-DNA mutants for
142 *PRX69* (*prx69-1* and *prx69-2*) (Jemmat et al. 2020). By RNA-seq, we confirm they were absence of
143 transcripts for both *PRX62* and *PRX69* in these mutants (**Figure S6A**). Only in *prx69-1* when grown at
144 10°C we found a truncated transcript of *PRX69* (**Figure S6B**). The RH phenotype in both *prx62* and
145 *prx69* single mutants were similar to Col-0 at 22°C and at 10°C (**Figure 3C**) while the double mutant
146 *prx62-1 prx69-1* showed significantly shorter RHs than Col-0 and any of the single mutants *prx62* and
147 *prx69* at 10°C. The double *prx62-1 prx69-1* mutant showed no detectable transcript levels of both
148 PRXs (**Figure S6C**). The overall peroxidase activity was also partially impaired in single mutants *prx62-*
149 *1* and *prx69-1* and double mutant *prx62-1 prx69-1* at both growth temperatures, 22°C and 10°C
150 (**Figure 3D**). We then tested RH growth complementation of the *prx62-1 prx69-1* double mutant by
151 expressing either *PRX62* or *PRX69* coding sequences under 35S promoter (*35S_{pro}PRX62*, *35S_{pro}PRX69*).
152 The RH growth was restored comparable to Col-0 levels at 10°C only for *PRX62* but not for *PRX69*

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153 **(Figure S7)**. This suggests that high levels of *PRX62* but not of *PRX69* are able to compensate the
154 absence of both PRXs in *prx62 prx69* double mutant. To determine whether higher expression of the
155 *PRX62* and *PRX69*-encoding genes are sufficient to trigger changes in RH cell length, we generated a
156 constitutive *35S_{pro}PRX62* overexpression lines in the Col-0 background that expressed up to 13-52
157 folds of transcripts levels of PRX62 as well as the corresponding *35S_{pro}PRX69* overexpression lines
158 with 9-11 folds **(Figure S6C)**. As expected, PRX62 overexpression resulted in significantly longer RH
159 cells than their respective Col-0 while PRX69 overexpression failed to trigger enhanced growth
160 **(Figure 3E)**. This may indicate that PRX62 and PRX69 do not have equal functions in RH growth
161 although both PRXs are required for this enhanced low-temperature growth process. Taken together,
162 these results indicate that the amount of PRX62 and PRX69 proteins linked to their peroxidase
163 activities control RH growth at 10°C.

164
165 **The absence of PRX62 and PRX69 induced a deregulation of several PRXs and cell wall EXTs at low-
166 temperature.**

167 To better understand the transcriptional changes produced at low temperature in a PRX62- and
168 PRX69-dependent manner, we performed an RNA-seq analysis comparing Col-0 and the double
169 *prx62-1 prx69-1* mutant at 10°C or 22°C. We found a central core of 1544 differentially expressed
170 genes (DEG) at low-temperature grouped into 10 clusters that were misregulated in the double
171 *prx62-1 prx69-1* mutant compared to Col-0 **(Figure 4A)**. 1022 genes were upregulated (clusters 1-6)
172 and 522 were downregulated (clusters 7-10) in Col-0 compared to the double *prx62 prx69* mutant.
173 We focused on the largest clusters 1, 2 and cluster 4 (comprised by 873 genes) where the genes
174 upregulated in Col-0 were deregulated in double *prx62 prx69* mutant in response to cold. In these
175 gene clusters, overrepresented GO terms were linked to plant cell walls, extracellular domains, and
176 secretory pathway **(Figure 4B)**. We identified several over-represented PRXs (15 genes) and EXTs-
177 related proteins (7 encoding genes) suggesting a global change in ROS-homeostasis and EXTs cell wall
178 remodeling in the double *prx62-1 prx69-1* mutant at low-temperature **(Figure 4A-B)**. Some of these
179 genes (e.g. *EXT6* and *PRP1*) showed a gene dose-dependent expression at transcript level linked to
180 the RH growth phenotype at 10°C **(Figure 4C)**. This indicated that low-temperature induces global
181 gene expression changes linked to the cell wall remodeling and ROS-homeostasis that positively
182 enhance RH growth. The analysis highlights that the absence of PRX62 and PRX69 proteins triggers
183 major changes in the transcriptional program of other PRXs and EXTs genes at low-temperature. This
184 implies the existence of a feedback regulatory loop from the apoplast-cell wall compartments that
185 triggers major changes at the transcriptional level of cell wall proteins and apoplastic PRXs.

186
187 **PRX62 and PRX69 affect ROS-homeostasis in RH cells under low-temperature.**

188 To get a deeper insight into PRX62 and PRX69 functions in growing RHs at moderate low-
189 temperature, we explored the effect of these PRXs on Reactive Oxygen Species (ROS)-homeostasis.
190 Overall PRX functions are linked to ROS, which are one of the key factors regulating polar growth in

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191 RHs (Mangano et al. 2017; Mangano et al. 2018; Marzol et al. 2018). Then, we measured total
192 cytoplasmic ROS ($_{\text{cyt}}\text{ROS}$) using the cell-permeable fluorogenic probe 2',7'-dichlorodihydrofluorescein
193 diacetate ($\text{H}_2\text{DCF-DA}$) and apoplastic ROS ($_{\text{apo}}\text{ROS}$) levels with cell-impermeable Amplex™ UltraRed
194 Reagent in RH tips at 22°C and 10°C (**Figure 5A-B**). The double mutant *prx62-1 prx69-1* showed higher
195 levels of $_{\text{cyt}}\text{ROS}$ at 10°C in actively growing RH tips compared to Col-0 (**Figure 5A**) while this enhance
196 in ROS level is less evident at 22°C between the double mutant *prx62-1 prx69-1* and Col-0. In
197 agreement, in the plants overexpressing PRX62 or PRX69, $_{\text{cyt}}\text{ROS}$ were reduced at both 22°C and 10°C.
198 On the other hand, the $_{\text{apo}}\text{ROS}$ in the RH tip were enhanced in Col-0 at 10°C compared to the levels
199 at 22°C while they were lower in the double mutant *prx62-1 prx69-1* at both temperatures. In the
200 lines overexpressing PRX62 or PRX69, $_{\text{cyt}}\text{ROS}$ were enhanced at both 22°C and 10°C (**Figure 5B**). The
201 increased level of $_{\text{apo}}\text{ROS}$ in Col-0 under low-temperature is in agreement with a two-fold increase in
202 the transcript levels for *NOXC (RBOHC)*, a key enzyme-encoding gene for ROS production (**Figure S8**).
203 Collectively, these results suggest that ROS-homeostasis is drastically modified in an antagonistic
204 manner by the absence or overexpression of these two PRXs when RH grow at 10°C, affecting RH cell
205 elongation.

206
207 **Low-temperature enhances EXTENSIN cell wall insolubility in RH cells.**
208 EXT-crosslinking can provide architectural stabilization for normal wall reinforcement during cell
209 elongation (Srivastava, 2002; Cannon *et al.*, 2008; Bashline *et al.*, 2014; Bidhendi and Geitmann, 2016;
210 Yaqoob *et al.*, 2020). Since changes in ROS-homeostasis could lead to abnormal cell wall secretion
211 and structure, we wondered whether PRX62 and PRX69 might participate in the cell wall glycoprotein
212 EXTs crosslinking during RH growth at low-temperature. Then, we tested if low-temperature could
213 induce a change in the targeting of EXTs secreted and insolubilized in the wall by the activity of these
214 two PRXs. To this end, we used an EXT-reporter carrying a tdTomato tag (SS-TOM-Long-EXT) that is
215 resistant to acidic pH, a condition usually found in the cell wall- apoplast compartments, and a
216 secreted tdTomato tag (SS-TOM) was used as a control (Marzol et al. 2021). The signal coming from
217 the cell surface in the apical zones of RHs cells under plasmolysis conditions were determined for SS-
218 TOM-Long-EXT and SS-TOM constructs at 22°C/10°C temperatures and SHAM-treated/non-treated
219 roots (**Figure 5C**). Plasmolysis allowed us to retract the plasma membrane and define the EXT-signal
220 coming specifically from the cell walls. Interestingly, of cell wall stabilization/insolubility of SS-TOM-
221 Long-EXT in the RH tip was drastically augmented at 10°C compared to 22°C. Furthermore, the signal
222 increment at 10°C was completely abolished when roots were treated with the peroxidase inhibitor
223 SHAM (**Figure 5C**). Thus, the SS-TOM-Long-EXT reporter tested in the apical zone of the RHs is
224 modified by low-temperature and by the peroxidase activity, at least partially possibly exerted by
225 PRX62 and PRX69 in the apoplast. This result suggests that changes in ROS-homeostasis produced by
226 altered levels of these PRXs in the apoplast might affect the secretion, targeting and, possibly the
227 crosslinking of cell wall components including EXTs, affecting RH cell elongation (**Figure 6**).

228

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229 **RSL4 transcription factor binds to the *PRX69* promoter E -boxes.**

230 It was previously shown that RSL4 controls RH growth at low-nutrient conditions (Mangano et al.
231 2017; Mangano et al. 2018) and at low-temperature (Moison et al. 2021). Moreover, it was also
232 shown that RSL4 directly controls the expression of *PRX01*, *PRX44* and *PRX73*, three PRX-encoding
233 genes important for RH growth at low-nutrient condition at room temperature (Mangano et al. 2017;
234 Marzol et al. 2021). Thus, we wondered whether RSL4 was able to regulate directly the expression of
235 *PRX62* and/or *PRX69*. To this end, we first measured *PRX62* and *PRX69* transcript levels in *rsI4* and
236 *rsI2 rsI4* mutants, which is impaired in RH growth enhancement, and in the RSL4 overexpressing line
237 (*35S_{pro}RSL4*) that develops extra-long RHs regardless the media and temperature conditions (Moison
238 et al. 2021). *PRX62* expression might be positively regulated (indirectly or directly) by RSL2 but not
239 by RSL4 based on the expression profiles in the double mutant *rsI2 rsI4* versus *rsI4* and *35S_{pro}RSL4*.
240 *PRX62* expression enhancement at low temperature is only repressed when *rsI2* mutation is present
241 (**Figure S9A**). On the other hand, an increase of 4.3-folds of *PRX69* transcripts were detected when
242 compared *35S_{pro}RSL4/rsI4* (**Figure S9A**). To test if any of these genes was directly regulated by RSL4,
243 we tested by CHIP-qPCR the binding of RSL4-GFP in the predicted sites (E-boxes) of *PRX62* and *PRX69*
244 promoters using the positive control of *EXPANSIN7* and *LRX1*, two previously reported direct targets
245 of RSL4 (Hwang et al. 2017) and *PP2A* as a negative control (**Figure S9B**). We detected a mild binding
246 of RSL4 protein to one of the predicted E-box sequences in the promoter region *PRX69*. Altogether,
247 our results indicate that RSL4 positively controls the expression of *PRX69* in a direct manner while
248 the regulation of *PRX62* might be related to RSL2. Further research will be required to determine if
249 auxin-ARFs or yet unknown TFs independent of auxin pathway (e.g. RHD6) regulates the expression
250 of *RSL4* under this low-temperature condition. Overall, this work uncovers the key roles of two
251 previously poorly described PRXs, *PRX62* and *PRX69*, in the regulation of low temperature ROS
252 homeostasis and EXT insolubilization in the cell walls that determines an enhanced RH growth. It is
253 hypothesized that these two PRXs might modulate the cell wall EXT-mediated assembly during this
254 fast cell elongation process (**Figure 6**).

255

256

257 **CONCLUSIONS**

258

259 Despite the putative high overall genetic redundant functions of apoplastic Class-III PRXs, in the last
260 years several individual PRXs were characterized to be involved in the oxidative polymerization of
261 monolignols in the apoplast of the lignifying cells in xylem (e.g. *PRX17*; Cossio et al 2017), in the root
262 endodermis (e.g. *PRX64*; Lee et al. 2013) or in petal detachment (Lee et al 2018). Moreover, PRXs are
263 also able to polymerize other components of the plant cell wall, including suberin, pectins and EXTs
264 (Schnabelrauch *et al.*, 1996; Bernards et al., 1999; Jackson et al., 2001; Francoz et al. 2019). While
265 several candidates of PRXs have been described in divers plants to be associated specifically to EXTs
266 crosslinking (EXT-PRXs) by *in vitro* studies (*LEP1*, *GvEP1* and *FBP1*) or immunolocalization evidences

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267 linked them to transient activity measurements (PRX08 and PRX34) (Schnabelrauch et al., 1996;
268 Wojtaszek et al., 1997; Jackson et al., 2001; Price et al., 2003; Pereira et al. 2011; Dong et al., 2015;
269 Jacobowitz et al. 2019), their role *in vivo* remains largely unexplored. Previously it was demonstrated
270 that three PRXs (PRX01, PRX44 and PRX73) directly contribute to ROS-homeostasis and RH growth at
271 room temperature (22°C) under low-nutrient condition (Mangano et al. 2017; Marzol et al. 2021). By
272 using a GWAS-RNAseq approach, we identified here two previously poorly characterized apoplastic
273 peroxidases, PRX62 and PRX69 (Jemmat et al. 2020), as positive regulators of RH growth at low-
274 temperature (10°C). One of the key results of this work is that PRX62 was found using GWAS while
275 PRX69 was identify on the transcriptomic profile. This point out to a different evolutionary history for
276 both proteins. PRX62 has evolved to give a dose response according to the allele encoded in the
277 genome, while PRX69 have a constitutive response at low temperature. These features of PRX62 and
278 PRX69 can be useful in crop improvement, to select varieties with differential responses; better
279 adapted to the environment they are exposed. The evidences shown here indicate that PRX62 and
280 PRX69 are involved in the ROS-homeostasis linked to the association of EXTs to the cell wall during
281 RH cell elongation at low-temperature (**Figure 6**). We speculate that cell wall
282 insolubilization/association of EXTs triggered by low-temperature might not only involve Tyr-covalent
283 crosslinks mediated by these two PRXs identified here but also by EXT hydrophobic associations non-
284 dependent on Tyr as suggested before for Leucine-Rich Extensins 1 (LRX1; Ringli 2010). Further
285 analyses might shed light on these complex processes.

286
287 Previously, our group as well as others have documented that changes in any of the several
288 posttranslational modifications in EXTs and related-EXTs like LRXs (e.g. proline hydroxylation, O-
289 glycosylation, and Tyr-crosslinking), all affected RH growth (Baumberge et al. 2001, 2003; Velasquez
290 et al. 2011; Velasquez et al. 2015; Marzol 2018, 2021) as well as pollen tube growth (Fabrice et al.
291 2017; Sede et al. 2017; Wang et al. 2017). In addition, auxin-dependent ROS-homeostasis controlled
292 by three apoplastic PRXs (e.g. PRX01, PRX44, PRX73) and plasmamembrane RBOHC protein (also
293 known as RHD2, for RH Defective 2) was shown to be determinant for a proper RH growth under low
294 nutrient condition (Mangano et al 2017; Marzol et al. 2021) or under low temperature (Martinez-
295 Pacheco et al. 2021). Collectively, these evidences highlight the predominant role of ROS-
296 homeostasis partially regulated by specific PRXs as a key component in polar RH elongation. The
297 molecular mechanism by which low-temperature-associated nutrient availability in the media
298 (Moison et al. 2021) triggers the expression of these two specific PRXs remains unclear, although
299 RSL4 could play a central role in the regulation of this mechanism. Previously, we have shown that
300 the lncRNA *APOLO* binds to the locus of *RHD6* and controls *RHD6* transcriptional activity leading to
301 cold-enhanced RH elongation through the consequent activation of *RSL4* (Moison et al. 2021) and of
302 several cell wall EXTENSIN (EXT) encoding genes (Martinez-Pacheco et al. 2021). Unexpectedly, our
303 previous results indicate that the low-temperatures (10°C) are able to trigger an exacerbated RH
304 growth compared with cell expansion at room temperature (Moison et al. 2021; Martinez-Pacheco

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305 et al. 2021). Moreover, further research will be needed to uncover the nutritional signal perceived at
306 the RH cell surface to trigger PRX62 and PRX69 low temperature mediated growth response. The
307 expression levels of PRX62 and PRX69 orthologs in other Brassicaceae could be used as biomarkers
308 for crop improvement in the selection of genotypes with longer RHs at moderate low-temperatures
309 in order to boost nutrients uptake in deficient soils.

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310 EXPERIMENTAL PROCEDURES

311
312 **Plant genotyping and growth conditions.** *Arabidopsis thaliana* Columbia-0 (Col-0) was used as the
313 wild type (Wt) genotype in all experiments unless stated otherwise. Seedlings were surface sterilized
314 and stratified in darkness at 4°C for 3 days before been germinated on ½ strength MS agar plates
315 supplemented with MES (Duchefa, Netherlands), in a plant growth chamber in continuous light (120
316 $\mu\text{mol}\cdot\text{sec}^{-1}\cdot\text{m}^{-2}$). Plants were transferred to soil for growth under the same conditions as previously
317 described at 22°C. Mutants and transgenic lines developed and used in this study are listed in **Table**
318 **S3**. For identification of T-DNA knockout lines, genomic DNA was extracted from rosette leaves.
319 Confirmation by PCR of a single and multiple T-DNA insertions in the genes were performed using an
320 insertion-specific LBb1 or LBb1.3 (for SAIL or SALK lines, respectively) or 8474 (for GABI line) primer
321 in addition to one gene-specific primer. In this way, we isolated homozygous for all the genes.
322 *Arabidopsis* T-DNA insertions lines (*prx62-1* [GK_287E07], *prx62-2* [SALK_151762], *prx69-1*
323 [SAIL_691_G12], *prx69-2* [SALK_137991]) were obtained from the European Arabidopsis Stock Centre
324 (<http://arabidopsis.info/>). Using standard procedures homozygous mutant plants were identified by
325 PCR genotyping with the gene-specific primers listed in **Table S4**. T-DNA insertion sites were
326 confirmed by sequencing using the same primers. Plants were routinely grown in Jiffy peat pellets
327 (continuous light, 120 μmol photons/m/s, 22°C, 67% relative humidity). For *in vitro* experiments,
328 seeds were surface-sterilized and sown in Petri dishes on agar-solidified half-MS medium without
329 sucrose, and grown in a culture room with continuous light (120 μmol photons/m/s, 22°C).

330
331 **Root hair phenotype.** Seeds were surface sterilized and stratified in darkness for 3 days at 4°C. Then
332 grew on ½ strength MS agar plates supplemented with MES (Duchefa, Netherlands), in a plant growth
333 chamber at 22°C in continuous light (120 $\mu\text{mol}\cdot\text{sec}^{-1}\cdot\text{m}^{-2}$) for 5 days at 22°C + 3 days at 10°C or for 8
334 days at 22°C as *control*. For quantitative analysis of root hair cell length phenotypes, 10 fully
335 elongated RHs from the elongation root zone were measured from 15-20 roots. Measurements were
336 made after 8 days. Images were captured with an Olympus SZX7 Zoom Stereo Microscope (Olympus,
337 Japan) equipped with a Q-Colors digital camera and QCapture Pro 7 software (Olympus, Japan) and
338 digitally processed with ImageJ software. RH length values were reported as the mean of three
339 replicates \pm SD using the GraphPad Prism 8.0.1 (USA) statistical analysis software.

340
341 **GWAS analysis and haplotype analysis.** To perform Genome Wide Association Analysis (GWAS), 106
342 *Arabidopsis thaliana* natural accessions were phenotyped for RH length in a shift- temperature
343 experiment as described above (**Table S1**). The population was previously genotyped using 214,051
344 Single Nucleotide Polymorphisms (SNPs) and this information is publicly available (Horton et al.,
345 2012). These set of phenotypes and genotypes were used to performed GWAS on the GWAPP web
346 application from the GWA-Portal (Seren et al, 2012, <https://gwas.gmi.oeaw.ac.at/#/home>,
347 Experiment code: 3b316208-0b5d-11e7-b6b1-005056990049) applying the accelerated mixed model,

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348 AMM (Kang et al., 2008; Zhang et al., 2010, Kang et al. 2010). A total of 139,425 SNPs with minor
349 allele frequency (MAF) $\geq 10\%$ were retained for further analysis. P-values of association were log-
350 transformed to LOD values ($-\log_{10}(\text{p-value})$) and corrected for multiple comparisons using FDR
351 procedure (Benjamini and Hochberg, 1995). The threshold for significant associations was set to p-
352 value $\leq 1/N$ (where N is the number of SNPs= 139,425) as described previously (Wen et al. 2014).
353 Manhattan plots were obtained using the qqman package (Turner, 2017) in R (2013), filtering out the
354 SNPs with p-value > 0.4 , to minimize overrepresentation of non-significant SNPs. Linkage
355 disequilibrium, i.e. the degree to which an allele of one SNP co-occurs with an allele of another SNP
356 within a population, was calculated as square coefficient of correlation (r^2) and visualized using the
357 LDheatmap package (Shin et al., 20006) in R. Three additional SNPs in the *PRX62* genomic region
358 (m190904, m190907, m190909) in high linkage disequilibrium ($r^2 > 0.7$, $p \ll 0.001$) with the lead SNP
359 m190905 were used in the haplotype analysis. Mean trait values for each non-unique haplotype were
360 analyzed using ANOVA followed by Tukey test implemented in Infostat (Di Rienzo et al. 2011).

361
362 **Peroxidase activity.** Soluble proteins were extracted from roots grown on vertical plates for 10 days
363 at 22°C or 10°C by grinding in 20mM HEPES, pH 7.0, containing 1 mM EGTA, 10mM ascorbic acid, and
364 PVP PolyclarAT (100mg/g fresh material)(Sigma, Buchs, Switzerland). The extract was centrifuged
365 twice for 10 min at 10,000 g. Each extract was assayed for protein levels with the Bio-Rad protein
366 assay (Bio-Rad, USA). Enzyme activity (expressed in nkatal/mg protein) was determined at 25°C by
367 following the oxidation of 8 mM guaiacol (Fluka™, Honeywell International, USA) at 470 nm in the
368 presence of 2 mM H₂O₂ (Carlo Erba, Italy) in a phosphate buffer (200 mM, pH6.0). Values are the
369 mean of three replicates \pm SD. P-value of one-way ANOVA, (**) $P < 0.01$.

370
371 **Gene transcript analysis by Reverse Transcription followed by quantitative PCR (RT-qPCR).** Total
372 RNA was prepared from 10 days old *in vitro*-grown plantlets using the TRI™ Reagent Solution (Sigma-
373 Aldrich). After quantification by spectrophotometry and verification by electrophoresis, RNA was
374 treated with the RQ1 RNase-free DNase I (Promega). One microgram of total RNA was reverse
375 transcribed using an oligo(dT)₁₅ and the MMLV-RT (Promega) according to the manufacturer's
376 instructions. cDNA was diluted 20-fold before PCR. RT-qPCR were performed on a QuantStudio 6 Flex
377 Real-Time PCR System (Thermo Fisher) using 5 μ L Power SYBR Green PCR Mix (Applied Biosystems),
378 2 μ L of cDNA, and 0.3 μ M of each primer in a total volume of 10 μ L per reaction. Primers used are
379 listed in **Table S4**. ACT2 (AT3G18780) and UBQ1 (AT3G52590) genes were used as references for
380 normalization of gene expression levels. The cycling conditions were 95°C for 10 min., 40 cycles of
381 95°C for 15 sec., 60°C for 1 min. and finally a melting curve from 60°C to 95°C (0.05°/sec). Under these
382 conditions primers efficiency was found to be between 97.0 and 99.7%. No amplification occurred in
383 the no-template controls. Data were analyzed using the Standard curve method (Pfaffl, 2001) and
384 Qiagen REST© 2009 software (Pfaffl et al., 2002). Three independent experiments (and two technical
385 replicates per experiment) were performed.

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386
387 **PRXs-tagged reporter lines.** For the PRX62proGFP and PRX69proGFP reporter lines, a 1.5 kb genomic
388 region upstream of the ATG start codon of each PRX62 (AT5G39580) and PRX69 (AT5G64100) genes
389 was selected using ThaleMine (<https://bar.utoronto.ca/thalemine/begin.do>) synthesized and cloned
390 into the pUC57 vector by GenScript Biotech(USA). Through Gateway cloning Technology (Invitrogen)
391 the 1.5 kb upstream regions were recombined first in pDONR™207 vector and subcloned into
392 pMDC111 destination vector (Invitrogen; (Karimi et al., 2002)) for PRX69 promoter region and into
393 pGWB4 vector (Invitrogen; (Nakagawa et al 2007)) for PRX62 promoter region. These constructs were
394 checked by restriction analysis. Both vectors contain a cassette with a C- terminal GFP tag. For the
395 PRXs-TagRFP lines, the PRX62 and PRX69 coding sequence was amplified by PCR from *A. thaliana* 10-
396 day old plantlets cDNAs using specific primers (**Table S4**). The PCR product was digested with *Hind*III
397 and *Bam*HI (PRX62) or with *Eco*RI and *Sma*I (PRX69), and cloned into Gateway® TagRFP-AS-N entry
398 clone (Evrogen). The PRX62-TagRFP fusion was subcloned (Gateway Technology, Invitrogen) into the
399 pB7WG2 destination vector (Karimi et al., 2002) containing a 35S promoter. This construct was
400 checked by restriction analysis and sequencing. The same procedure was use to generate 35S-PRX69-
401 TagRFP construct. All the constructs were used to transform *A. thaliana* plants and obtain
402 homozygous stable fluorescent lines.

403
404 **Confocal Microscopy:** Confocal laser scanning microscopy for the lines *PRX62pro-GFP* and
405 *PRX69proGFP*, was performed using Zeiss LSM5 Pascal (Zeiss, Germany) (Excitation: 488 nm argon
406 laser; Emission: 490-525 nm, 10X objective N/A 0.30 or 40X water-immersion objective, N/A 1.2,
407 according to experiment purpose). Z stacks were done with an optical slice of 1µm, and fluorescence
408 intensity was measured at the RH tip. For the lines *SS-TOMATO* and *SS-TOMATO-EXT LONG*, roots
409 were plasmolyzed with a mannitol 8% solution and the scanning was performed using Zeiss LSM5
410 Pascal (Zeiss, Germany)(Excitation: 543 nm argon laser; Emission: 560-600 nm, 63X water-immersion
411 objective N/A 1.2) Three replicates for each of ten roots and between 10 to 15 hairs per root were
412 observed. GFP signal and tdTOMATO cell wall signal at RH tip were quantified using the ImageJ
413 software. Fluorescence AU were reported as the mean of three replicates ± SD using the GraphPad
414 Prism 8.0.1 (USA) statistical analysis software.

415 **Apoplasmic and Cytoplasmic ROS measurement in RH Tip:** To measure ROS levels in root hairs cells,
416 8 days-old Arabidopsis seedlings grown at 22°C (control) and 10°C in continuous light were used. For
417 cytoplasmic ROS, the seedlings were incubated in darkness for 10 min with 50 µM H2DCF-DA (Thermo
418 Fisher) at room temperature then washed with liquid 0.5X MS media (Duchefa, Netherlands) and
419 observed with Zeiss Imager A2 Epifluorescence Microscope(Zeiss, Germany) (40X objective, N/A 1.2,
420 exposure time 25 ms). Images were analyzed using ImageJ software. To measure ROS levels, a circular
421 region of interest was chosen in the zone of the root hair tip cytoplasm. Approximately, 20 healthy
422 and alive root hairs of ten plants per treatment were analyzed. To measure apoplasmic ROS, the

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423 seedlings were incubated with 50 μ M Amplex™ UltraRed Reagent (AUR) (Molecular Probes,
424 Invitrogen) for 15 min in darkness and rinsed with liquid 0.5X MS media (Duchefa, Netherlands). Root
425 hairs were imaged with a Zeiss LSM5 Pascal (Zeiss, Germany)) laser scanning confocal microscope
426 (Excitation: 543 nm argon laser; Emission: 560-610 nm, 40X water-immersion objective, N/A 1.2). The
427 intensity of fluorescence was quantified on digital images using ImageJ software. Quantification of
428 the AUR probing fluorescence signal was restricted to apoplastic spaces at the root hair tip. At least
429 10-15 hairs per plant and ten plants per treatment with three replicates were analyzed. Fluorescence
430 AU were reported as the mean of three replicates \pm SD using the GraphPad Prism 8.0.1 (USA)
431 statistical analysis software.

432
433 ***In silico* analysis.** The *in silico* analysis of *PRX62* and *PRX69* expression in the roots were performed
434 using ePlant browser of Araport, Tissue Specific Root eFP (<http://bar.utoronto.ca/eplant/>) (Waese et
435 al. 2017). EXPANSIN7 (*EXP7*) as a RH specific gene was included for comparison.

436
437 **RNA-seq Analyses.** This section is adapted from the 3D RNA-seq package output “Results” (Guo et
438 al., 2020; Calixto et al., 2018) as this was the selected tool to analyze differential expression in our
439 datasets. For the RNA-seq datasets we analyzed 2 datasets, one with 16 factor groups (Col.X10,
440 Col.X22, Bu.X10, Bu.X22, Sf.X10, Sf.X22, Te.X10, Te.X22, Wc.X10, Wc.X22, P62.X10, P62.X22, P69.X10,
441 P69.X22, P6269.X10, P6269.X22) each with 2 biological replicates (32 samples in total). Quantification
442 of transcripts using salmon quant (Patro et al., 2017) from Galaxy.org or salmon-1.5.1_linux_x86_64
443 version in a personal computer. The index of the transcriptome was built using The Arabidopsis
444 Thaliana Reference Transcript Dataset 2 (AtRTD2, Zhang R. et al., 2016) obtained from
445 <https://ics.hutton.ac.uk/atRTD/>. For the data pre-processing, read counts and transcript per million
446 reads (TPMs) were generated using tximport R package version 1.10.0 and lengthScaledTPM method
447 (Soneson et al., 2016) with inputs of transcript quantifications from tool salmon (Patro et al., 2017).
448 Low expressed transcripts and genes were filtered based on analyzing the data mean-variance trend.
449 The expected decreasing trend between data mean and variance was observed when expressed
450 transcripts were determined as which had ≥ 1 of the 32 samples with count per million reads (CPM)
451 ≥ 1 , which provided an optimal filter of low expression. A gene was expressed if any of its transcripts
452 with the above criteria was expressed. The TMM method was used to normalize the gene and
453 transcript read counts to \log_2 -CPM (Bullard et al., 2010). The principal component analysis (PCA) plot
454 showed the RNA-seq data did not have distinct batch effects. For the DE, DAS and DTU analysis, the
455 voom pipeline of limma R package was used for 3D expression comparison (Ritchie et al., 2015; Law
456 et al., 2014). To compare the expression changes between conditions of experimental design, the
457 contrast groups were initially set as Col.X10-Col.X22, Bu.X10-Bu.X22, Sf.X10-Sf.X22, Te.X10-Te.X22,
458 Wc.X10-Wc.X22, P62.X10-P62.X22, P69.X10-P69.X22, P6269.X10-P6269.X22. For DE
459 genes/transcripts, the \log_2 fold change (L_2FC) of gene/transcript abundance were calculated based
460 on contrast groups and significance of expression changes were determined using t-test. P-values of

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461 multiple testing were adjusted with BH to correct false discovery rate (FDR) (Benjamini and Yekutieli,
462 2001). A gene/transcript was significantly DE in a contrast group if it had adjusted p-value < 0.01 and
463 $L_2FC \geq 1.5$. Heatmap: Hierarchical clustering was used to partition the DE genes into 10 clusters with
464 euclidean distance and ward.D clustering algorithm (Saracli et al., 2013). ComplexHeatmap R package
465 version 1.20.0 was used to make the heat-map.

466
467 **Alternative Splicing analysis.** At the alternative splicing level, DTU transcripts were determined by
468 comparing the L_2FC of a transcript to the weighted average of L_2FCs (weights were based on their
469 standard deviation) of all remaining transcripts in the same gene. A transcript was determined as
470 significant DTU if it had adjusted p-value < 0.01 and $\Delta PS \geq 0.15$. For DAS genes, each individual
471 transcript L_2FC were compared to gene level L_2FC , which was calculated as the weighted average
472 of L_2FCs of all transcripts of the gene. Then p-values of individual transcript comparison were
473 summarized to a single gene level p-value with F-test. A gene was significantly DAS in a contrast group
474 if it had an adjusted p-value < 0.01 and any of its transcript had a Δ Percent Spliced (ΔPS) ratio \geq
475 0.15.

476
477 **Gene Ontology Analysis.** Gene ontology (GO) terms assignment for the DE genes datasets were
478 obtained using the PantherDB tool (<http://go.pantherdb.org/index.jsp>). An enrichment test was
479 performed for the following categories: BP (biological process), MF (molecular function), and CC
480 (cellular component). p-values were obtained using the Fisher exact test and corrected for multiple
481 testing using FDR. The enrichment factor (EF) was estimated as the ratio between the proportions of
482 genes associated with a particular GO category present in the dataset under analysis, relative to the
483 number of genes in this category in the whole transcriptome analyzed. We considered the whole
484 transcriptome as those genes that are expressed at least in one of the evaluated conditions. Bubble
485 plots were generated, using a custom script written in Python language ([https://github.com/Lucas-](https://github.com/Lucas-Servi/makeGO)
486 [Servi/makeGO](https://github.com/Lucas-Servi/makeGO)), for all those categories for which the adjusted p-value (FDR) was lower than 0.01.

487
488 **Chromatin immunoprecipitation (CHIP) assays.** ChIP assays were performed on seedlings expressing
489 RSL4-GFP under the native RSL4 promoter, using anti-GFP (Abcam ab290) and anti-IgG (Abcam
490 ab6702) antibodies, mainly as described by Ariel et al. (2020). Seedlings were grown in continuous
491 light at 22°C for 10 days, harvested after 24 h treatment at 10°C, ground in liquid nitrogen, and
492 resuspended in 25 mL of nuclear isolation buffer (Bourbousse et al. 2018). Chromatin was cross-
493 linked first with 1.5 mM ethylene glycol bis(succinimidyl succinate) (Thermo Fisher Scientific 21565)
494 for 20 min at room temperature, and then with formaldehyde at a final concentration of 1% for 10
495 min at room temperature. Cross-linking was stopped by adding 1.7 mL of 2 M glycine and incubating
496 for 10 min at room temperature. Crosslinked chromatin was extracted by cell resuspension,
497 centrifugation, cell membrane lysis, and sucrose gradient as previously described (Ariel et al., 2020).
498 Nuclei were resuspended in Nuclei Lysis Buffer and chromatin was sonicated using a water bath

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499 Bioruptor Pico (Diagenode; 30 s on / 30 s off pulses, at high intensity for 10 cycles). Chromatin
500 samples were incubated for 12 h at 4 °C with Protein A Dynabeads (Invitrogen) precoated with the
501 corresponding antibodies. Immunoprecipitated DNA was recovered using
502 Phenol:Chloroform:Isoamlic Acid (25:24:1; Sigma) and analyzed by qPCR. Untreated sonicated
503 chromatin was processed in parallel and considered the Input sample.

504
505 **SHAM Treatment.** Seeds were germinated on agar plates at 22°C in a growth chamber in continuous
506 light. After 4 days plants were transferred to agar plates with or without 65 µM of SHAM
507 (Salicylhydroxamic acid; Sigma Aldrich, USA), then grown 3 days at 22°C followed by 3 days at 10°C
508 or 6 days at 22°C (control). Root hair phenotype was measured and confocal microscopy analysis was
509 performed.

510
511 **SS-TOM and SS-TOM-Long-EXT constructs.** The binary vector pART27, encoding tdTomato secreted
512 with the secretory signal sequence from tomato polygalacturonase and expressed by the constitutive
513 CaMV 35S promoter (pART-SS-TOM), was a kind gift of Dr. Jocelyn Rose, Cornell University. The entire
514 reporter protein construct was excised from pART-SS-TOM by digesting with *NotI*. The resulting
515 fragments were gel-purified with the QIAquick Gel Extraction Kit and ligated using T4 DNA Ligase
516 (New England Biolabs) into dephosphorylated pBlueScript KS+, also digested with *NotI* and gel-
517 purified, to make pBS-SS-TOM. The plasmid was confirmed by sequencing with primers 35S-FP (5'-
518 CCTTCGCAAGACCCTCCTC-3') and OCS-RP (5'-CGTGCACAACAGAATTGAAAGC-3'). The sequence of
519 the EXT domain from *SIPEX1* (NCBI accession AF159296) was synthesized and cloned by GenScript
520 into pUC57 (pUC57-EXT). The plasmid pBS-SS-TOM-Long-EXT was obtained by digesting pUC57-EXT
521 and pBS-SS-TOM with *NdeI* and *SgrAI*, followed by gel purification of the 2243 bp band from pUC57-
522 EXT and the 5545 bp band from pBS-SS-TOM, and ligation of the two gel-purified fragments. The pBS-
523 SS-TOM-Long-EXT plasmid was confirmed by sequencing with 35S-FP, OCS-RP, and tdt-seq-FP (5'-
524 CCCGTTCAATTGCCTGGT-3'). Both pBS plasmids were also confirmed by digestion. The binary vector
525 pART-SS-TOM-Long-EXT was made by gel purifying the *NotI* insert fragment from the pBS-SS-TOM-
526 Long EXT plasmid and ligating it with pART-SS-TOM backbone that had been digested with *NotI*, gel
527 purified, and dephosphorylated. This plasmid was confirmed by sequencing. The construct SS-TOM
528 and SS-TOM-Long-EXT were transformed into Arabidopsis plants. The secretory sequence (SS) from
529 tomato polygalacturonase is MVIQRNSILLIIIFASSISTCRSGT (2.8kDa) and the EXT-Long domain
530 sequence is

531 BAAAAAACTLPSLKNFTFSKNIFESMDETCRPSESKQVKIDGNENCLGGRSEQRTEKECFVVSFKPVDCSKGHCG
532 VSREGQSPKDPKVTTPPKPSTPTTPKPNPSPPPKTLPPPKTSPPPVHSPPPPVASPPPPVHSPPPPVASPPPP
533 VHSPPPPVASPPPPVHSPPPPVASPPPPVHSPPPPVHSPPPPVASPPPPVHSPPPPVHSPPPPVHSPPPVHSP
534 PPVHSPPPPVASPPPPVHSPPPPVHSPPPPVHSPPPPVASPPPPVHSPPPPVASPPPPVHSPPPPVASPPPPVH
535 SPPPPVASPPPPVHSPPPPVHSPPPPVHSPPPPVASPPALVFSPPPPVHSPPPAPVMSPPPTFEDALPPTLGLSL

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536 YASPPPIFQGY* 395-(39.9kDa). The predicted molecular size for SS-TOM protein is 54.2 kDa and for
537 SS-TOM-EXT-Long Mw is 97.4 kDa.

538

539

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548

549 **Author Contribution**

550 J.M.P. performed most of the experiments and analysed the data. P.R. measured the peroxidase
551 activity and PRXs expression in single *prx* mutants and selected accessions, analysed the subcellular
552 localization of PRXs, cloned the PRXs, generated 35SproPRXs constructs, produced the RNA-seq data.
553 L.K performed GWAS measurements. C.M.F. performed GWAS and haplotype analysis. L.S., R.T, C.M,
554 and E.P. performed the bioinformatics analysis. V.B.G., J.M.P., C.B., E.M., D.R.R.G, Y.R., M.C., analysed
555 part of the data. J.B and M.Y. provided the accessions and analysed the data. L.S, P.T., E.P., C.M.
556 analysed the RNA-seq data. In addition, L.S and E.P. analysed the alternative splicing. L.F. and F.A.
557 performed the CHIP assay. C.D. produced the RNA-seq data and analysed the results. J.M.E. designed
558 research, analysed the data, supervised the project, and wrote the paper. All authors commented on
559 the results and the manuscript. This manuscript has not been published and is not under
560 consideration for publication elsewhere. All the authors have read the manuscript and have approved
561 this submission.

562

563 **Competing financial interest**

564 The authors declare no competing financial interests. Correspondence and requests for materials
565 should be addressed to J.M.E. (Email: jestevez@leloir.org.ar).

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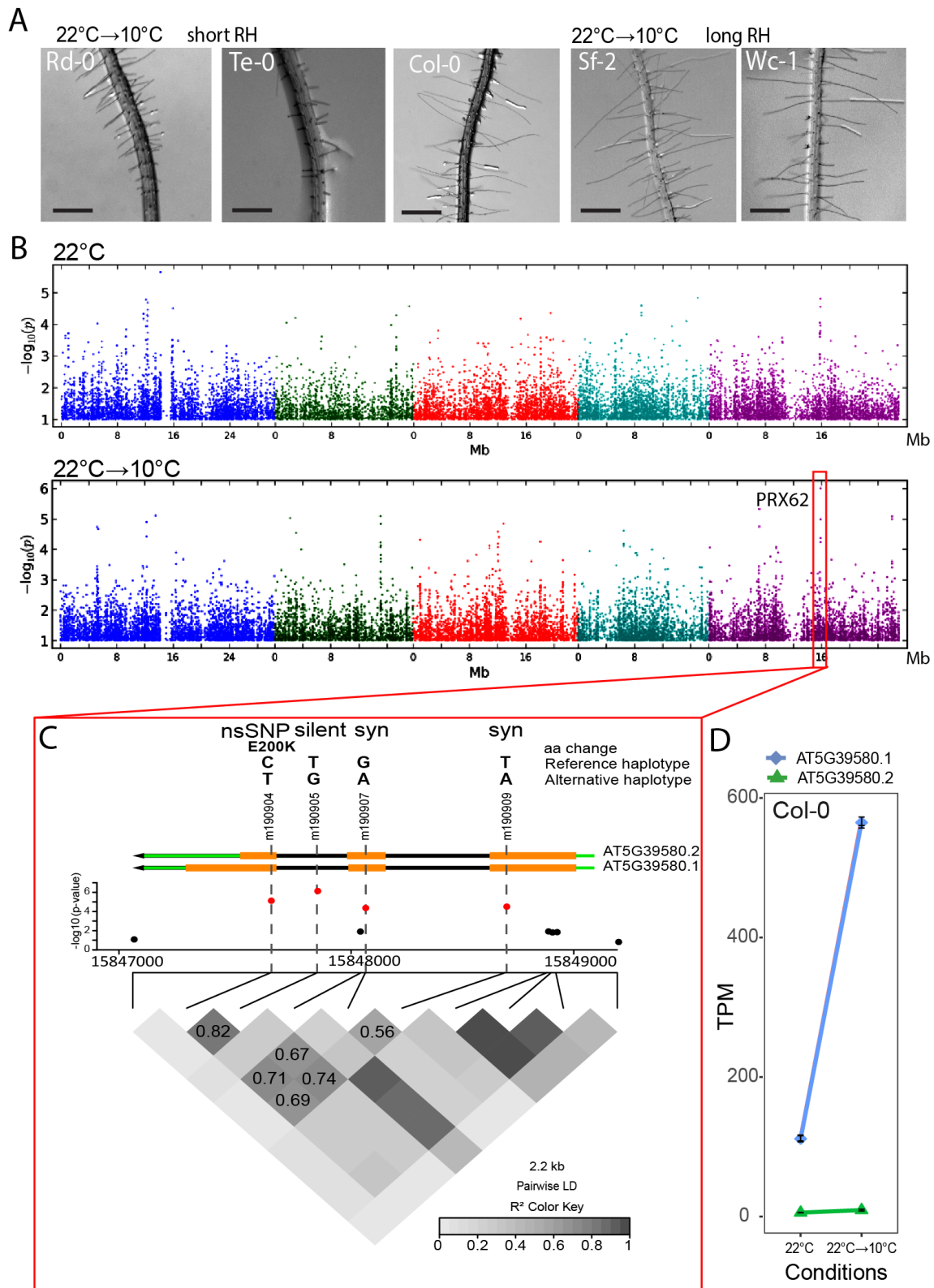


Figure 1. *PRX62* associates with enhanced RH growth under low-temperature condition.

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(A) Representative accessions of *A. thaliana* showing short (Rd-0 and Te-0) and long root hair (RH) phenotypes (Col-0, Sf-2 and Wc-1) when grown at low temperature (10°C).

(B) Manhattan plots for RH length at 22°C (top plot) and at 10°C (bottom plot). Coarse analysis was performed using GWAPP (<https://gwapp.gmi.oeaw.ac.at/>). Arabidopsis chromosomes are depicted in different colors. The red box in the bottom plot indicates the genomic region significantly associated with root hair length at 10°C.

(C) Zoomed-in of the genomic region red-boxed in **(B)**. The lead SNP (m190905) and three additional SNPs highly associated with RH length localize within *PRX62* (AT5G39580). *PRX62* splice variants (AT5G39580.1, AT5G39580.2) are depicted in green-orange-black arrows. The four associated SNPs (in red) are in high linkage disequilibrium (LD) with each other, and they are combined into two major and opposite haplotypes in the population (CTGT and TGAA). LD plot is shown as heat-map at the bottom. Haplotypes and type of mutation for these SNPs are indicated at the top of the figure. The SNP m190904 is a non-synonymous SNP for AT5G39580.2 causing a change from Glutamic Acid to Lysine at position 200 (E200K) in the amino acid sequence.

(D) The full-length variant of *PRX62* (AT5G39580.1) is upregulated at low-temperature (10°C) while the shorter variant (AT5G39580.2) is almost not detected. Expression measured by RNA-seq of *PRX62*. TPM = Transcripts Per Kilobase Million.

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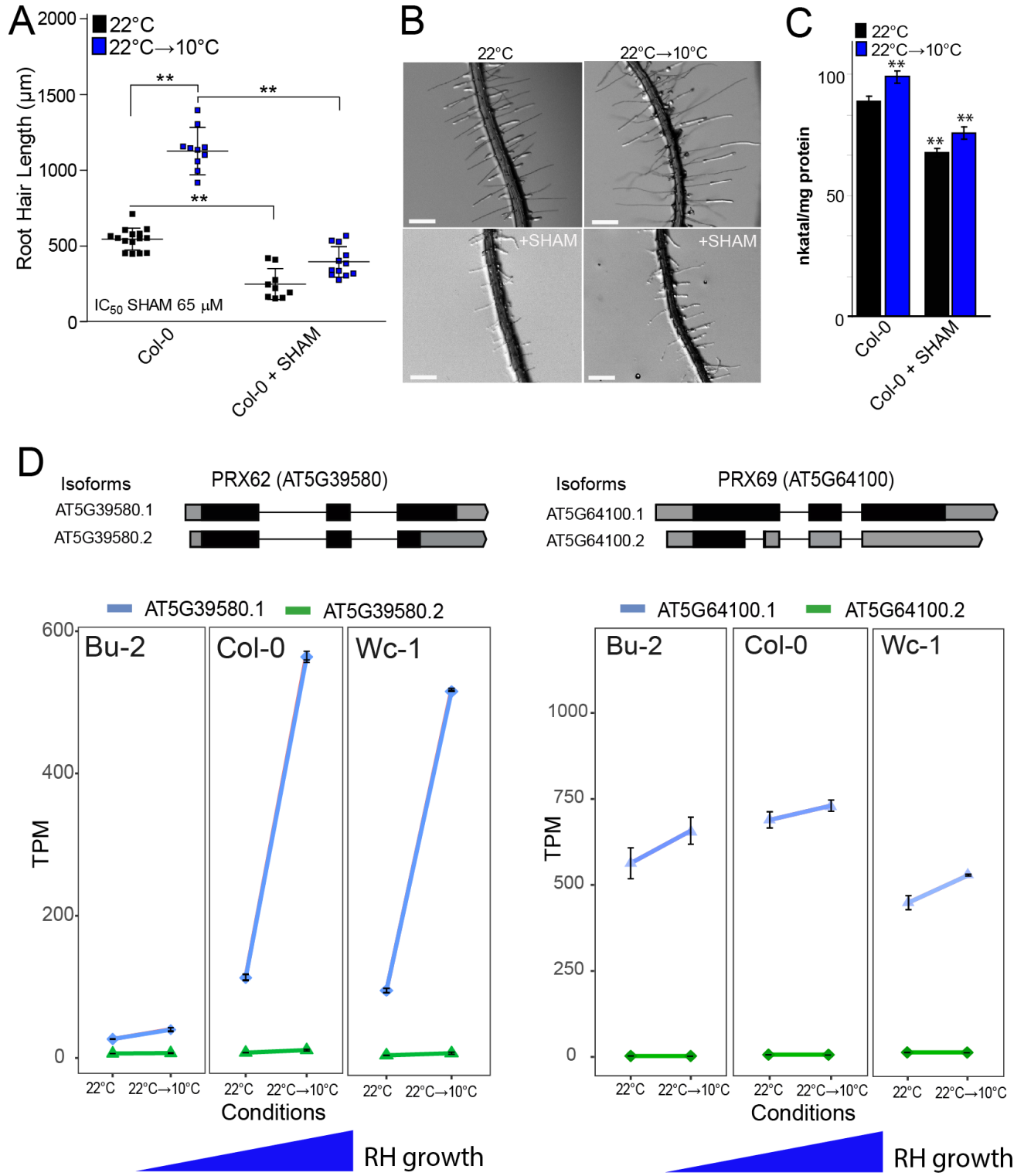


Figure 2. Low-temperature enhanced RH growth requires peroxidase activity and upregulates *PEROXIDASE 62 (PRX62)* expression.

PRX62 and PRX69 regulate RH growth at low-temperature

(A) RH length phenotype of Col-0 at 22°C or 10°C, with or without the addition of the PRX inhibitor SHAM. Inhibitory Concentration 50 (IC₅₀) of RH grown at 22°C was used (65 μM). RH length values are the mean of three replicates ± SD. P-value of one-way ANOVA, (**) P<0.01.

(B) Representative images of RH phenotype of Col-0 quantified in **(A)**. Scale bars= 0.5 mm.

(C) Total root peroxidase activity. Peroxidase activity was assayed using guaiacol/hydrogen peroxide as substrate in root tissues grown with or without 65 μM SHAM, either for 5 days at 22°C or for 5 days at 22°C plus 3 days at 10°C. Enzyme activity values (expressed as nkatal/mg protein) are the mean of three replicates ± SD. P-value of one-way ANNOVA, (**) P<0.01.

(D) In contrast to *PRX69*, *PRX62* is differentially expressed at low temperature (10°C) in *Arabidopsis* accessions with contrasting RH phenotypes. Expression measured by RNA-seq of *PRX62* and *PRX69* in three contrasting *Arabidopsis* accessions based on the RH phenotype (short RH in Bu-2 and extra-long RH in Col-0 and Wc-1) detected at 10°C. Isoforms' schemes were adapted from boxify (<https://boxify.boku.ac.at/>). TPM = Transcripts Per Kilobase Million.

PRX62 and PRX69 regulate RH growth at low-temperature

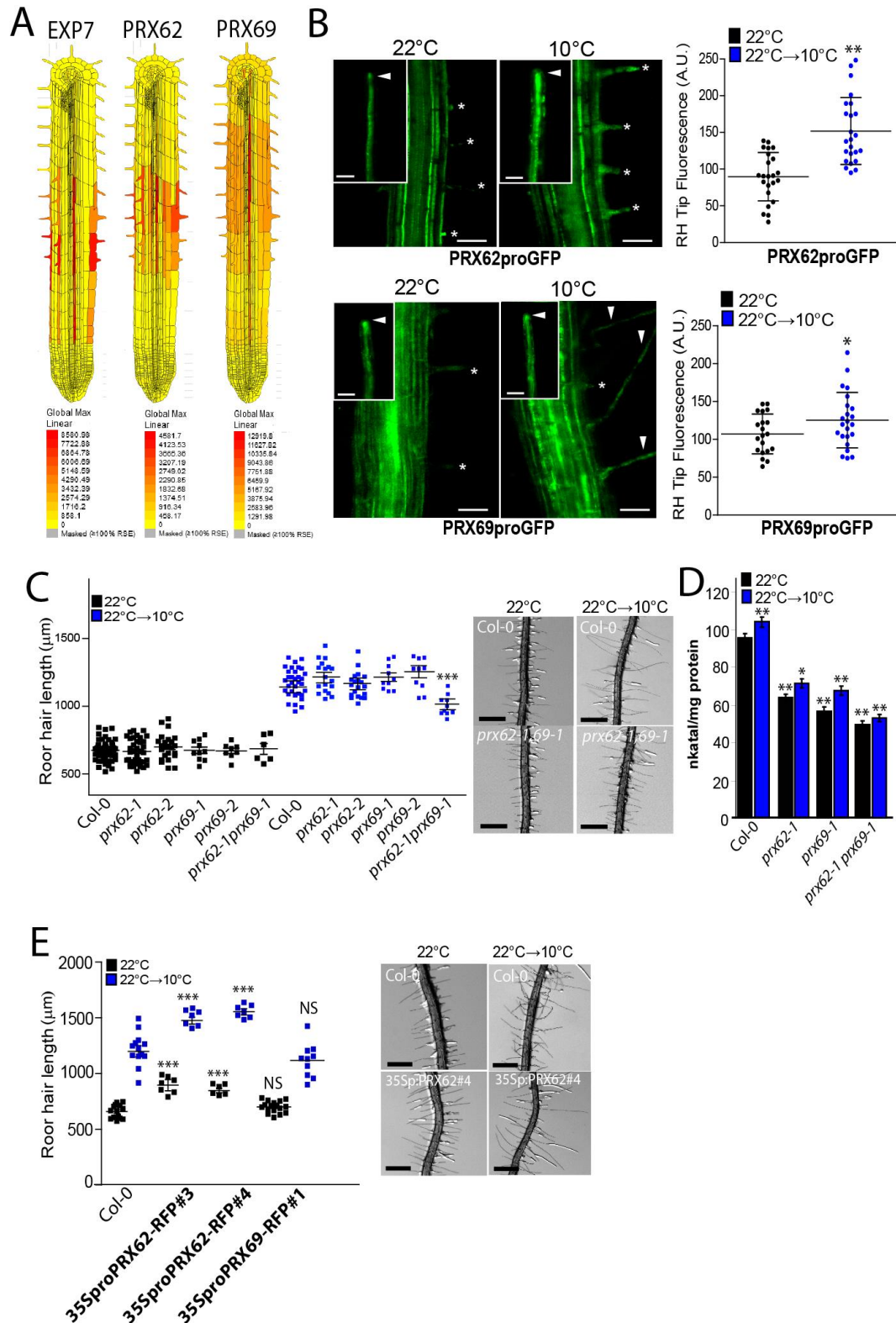


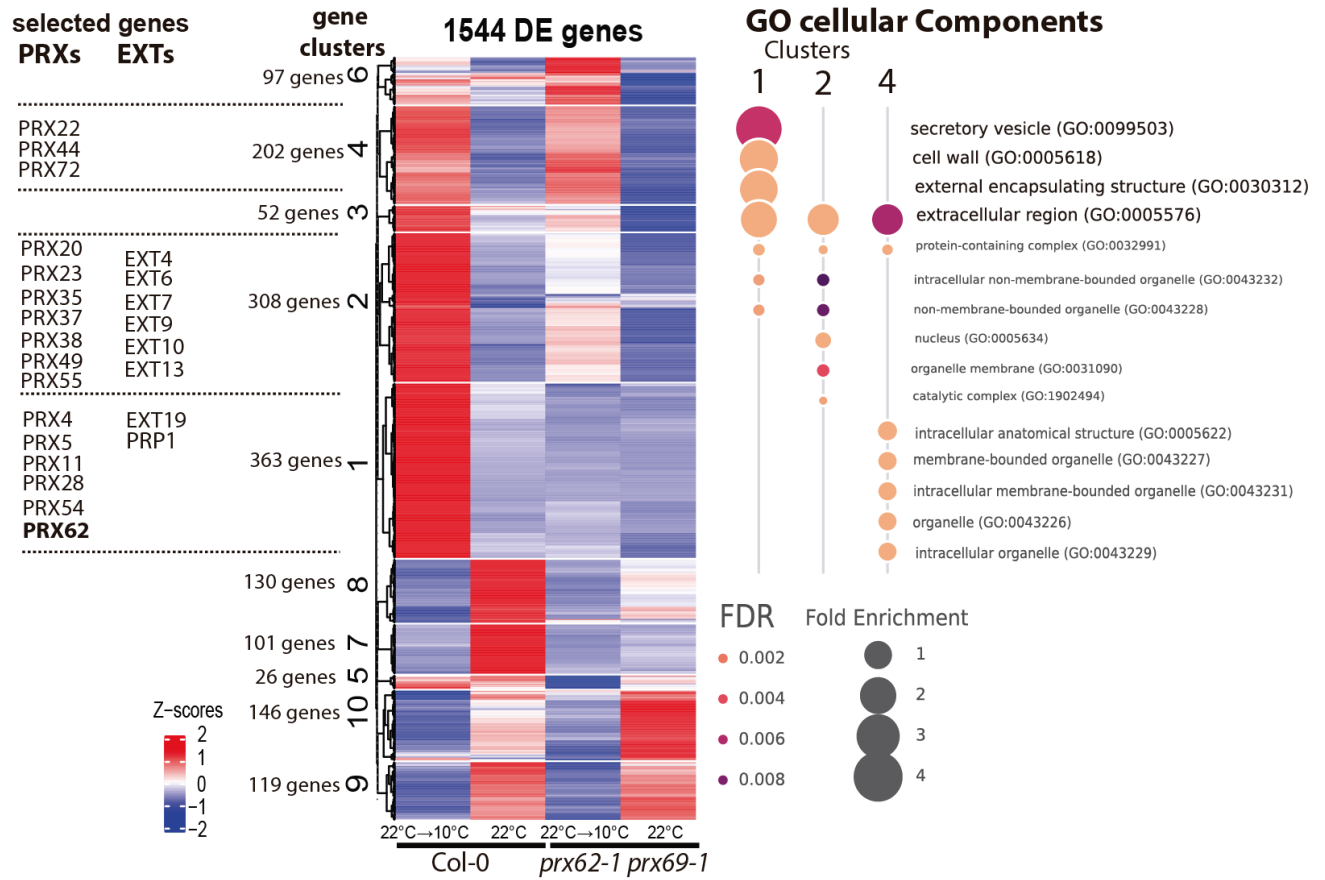
Figure 3. *PEROXIDASE 62* (*PRX62*) and *PEROXIDASE 69* (*PRX69*) regulate RH growth and peroxidase activity under low-temperature conditions.

PRX62 and PRX69 regulate RH growth at low-temperature

- (A)** The *in silico* analysis of *PRX62* and *PRX69* gene expression using Tissue Specific Root eFP (<http://bar.utoronto.ca/eplant/>). The RH marker *EXPANSIN 7* was included for comparison.
- (B)** Transcriptional reporters of *PRX62* (*PRX62_{pro}GFP*) and *PRX69* (*PRX69_{pro}GFP*) in the root elongation zone and specifically in RHs (RH) grown at 22°C or 10°C. Scale bar = 200 μm. Growing RHs are indicated with asterisks while already grown RHs with arrowheads. On the right, GFP signal is quantified. Fluorescence AU were reported as the mean of three replicates ± SD. P-value of one-way ANOVA, (**) P<0.01, (*) P<0.05.
- (C)** Scatter-plot of RH length of Col-0, *PRX62* mutants (*prx62-1* and *prx62-2*) and *PRX69* mutants (*prx69-1* and *prx69-2*) and double mutant *prx62-1 prx69-1* grown at 22°C or at 10°C. RH length values are the mean of three replicates ± SD. P-value of one-way ANOVA, (***) P<0.001.
- (D)** Peroxidase activity was assayed using guaiacol/hydrogen peroxide as substrate in root tissues from Col-0, *prx62-1* and *prx69-1* seedlings grown either at 22°C or 10°C. Enzyme activity values (expressed as nkatal/mg protein) are the mean of three replicates ± SD. P-value of one-way ANOVA, (**) P<0.001, (*) P<0.05.
- (E)** Scatter-plot of RH length of Col-0, *35S_{pro}PRX62/Col-0* and *35S_{pro}PRX69/Col-0* lines. RH length values are the mean of three replicates ± SD. P-value of one-way ANOVA, (***) P<0.001. NS= non-significant differences.

PRX62 and PRX69 regulate RH growth at low-temperature

A



B

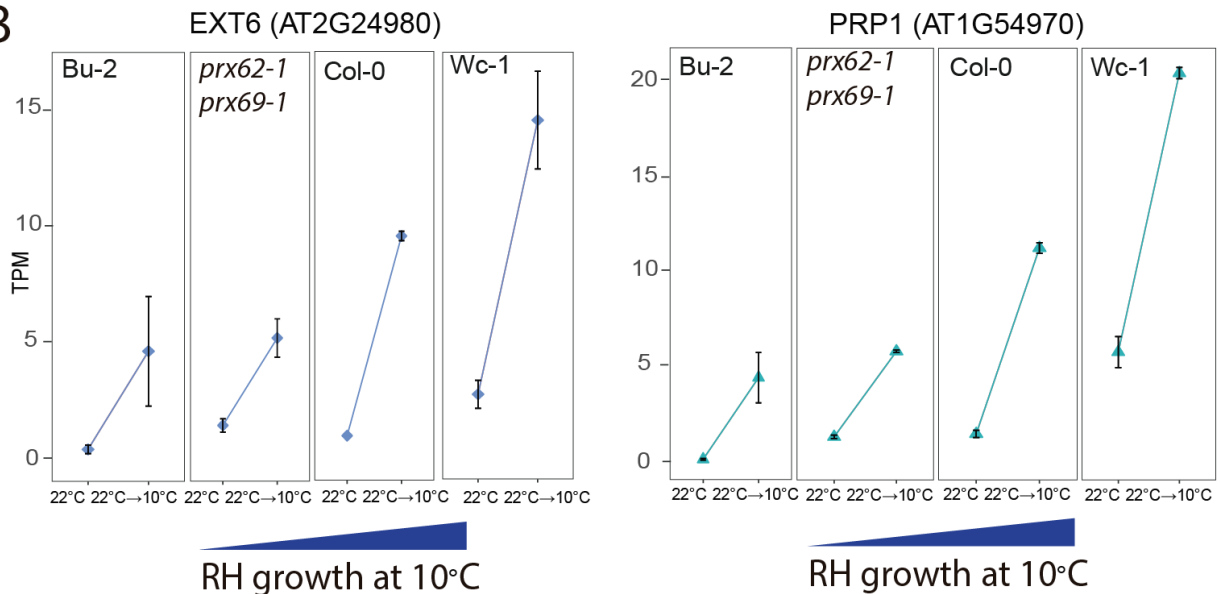


Figure 4. Global transcriptomic changes induced by low-temperature and misregulated in the *prx62 prx69* mutant are associated with PRXs and cell associated-EXTs.

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(A) Heat-map showing the hierarchical gene clustering for 1,544 *A. thaliana* genes differentially expressed (DE) between room temperature growth (22°C) and low-temperature (10°C) growth in wild type Col-0 and in double mutant *prx62-1 prx69-1* roots. Gene Ontology analysis results depicting the top 7 most significantly enriched GO terms are shown as bubble plots on the right for the clusters of interest. DE genes in clusters 1, 2 and 4 were contrasted against all the expressed genes for GO analysis. The size of the points reflects the amount of gene numbers enriched in the GO term. The color of the points means the p value. Relevant gene examples of specific clusters (1, 2 and 4) are listed on the left.

(B) Expression of *EXT6* and *PRP1* is gradually upregulated at low temperature (10°C) in 4 genotypes from very short RHs (Bu-2) to very long RHs (Wc-1) (RNA-seq data). TPM = Transcripts Per Kilobase Million.

PRX62 and PRX69 regulate RH growth at low-temperature

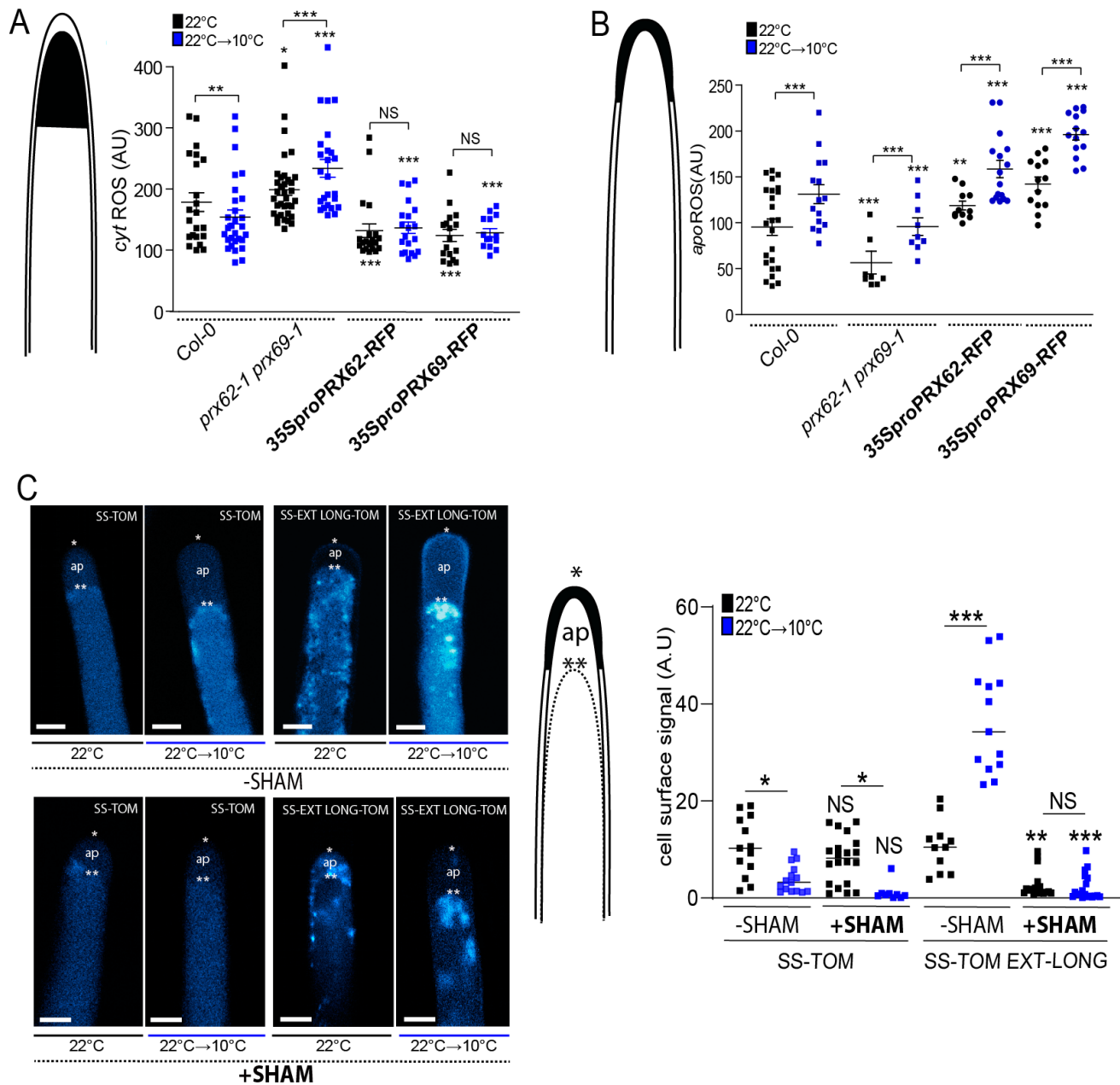


Figure 5. ROS-homeostasis and EXT-stabilization in RH apical cell wall depend on PRX62 and PRX69.

(A) Cytoplasmic ROS ($_{cyt}ROS$) levels were measured using 2',7'-dichlorodihydrofluorescein diacetate ($H_2DCF-DA$) in apical areas of RHs in wild-type (Columbia Col-0), in the double mutant *prx62-1 prx69-1* and in the 35S $_{pro}PRX62/Col-0$ and 35S $_{pro}PRX69/Col-0$ lines grown at 22°C or 10°C. Measurements are the mean of three replicates \pm SD. P-value of one-way ANOVA, (*) P<0.1, (**) P<0.01, (***) P<0.001. NS= non-significant differences.

(B) Apoplastic ROS ($_{apo}ROS$) levels were measured with Amplex[™] UltraRed in apical areas of RHs in wild-type (Columbia Col-0), in the double mutant *prx62-1 prx69-1* and in the 35S $_{pro}PRX62/Col-0$ and

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35S_{pro}PRX69/Col-0 lines grown at 22°C or 10°C. Measurements are the mean of three replicates ± SD. P-value of one-way ANOVA, (**) P<0.01, (***) P<0.001.

(C) Signal of SS-TOM and SS-EXT LONG-TOM in the apical zone of RHs grown at 22°C or 10°C with or without SHAM treatment in Col-0. Cells were plasmolyzed with mannitol 8%. In the images: (*) indicates cell surface including the plant cell walls, (**) indicates the retraction of the plasma membrane, (ap) apoplastic space delimited between the plant cell wall and the retracted plasma membrane. (A.U.) = Arbitrary Units. Fluorescence AU were reported as the mean of three replicates ± SD. P-value of one-way ANOVA, (*) P<0.05, (**) P<0.01, (***) P<0.001. NS= non-significant differences.

PRX62 and PRX69 regulate RH growth at low-temperature

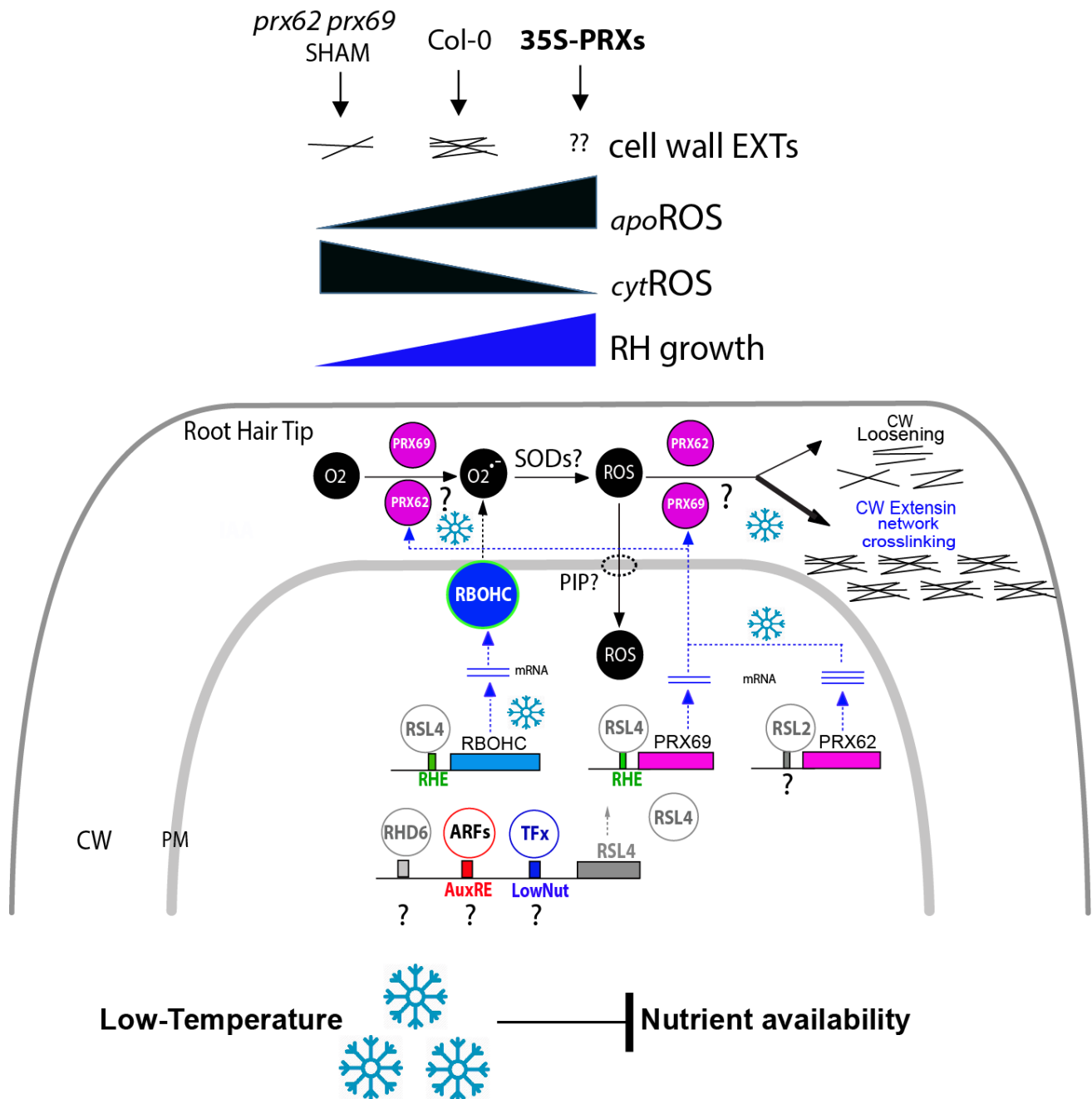


Figure 6. Proposed Model of PRX62 and PRX69 functions in ROS-homeostasis and EXT-cell wall association linked to RH growth at low-temperature. This model is based on the results presented in this study and in previous works (Mangano et al. 2017; Marzol et al. 2021; Moison et al. 2021). ROS-homeostasis ($apoROS + cytROS$) in the RH tip. Higher $apoROS$ /low $cytROS$ than *Col-0* present in $35S_{pro}PRXs$ promotes RH growth while lower $apoROS$ /high $cytROS$ in *prx62-1 prx69-1* represses RH growth at low-temperature. Changes in $apoROS$ triggered by PRX62 and PRX69 might control changes in the EXT-mediated cell wall expansion/crosslinking in the apical RH zone. Part of the $apoROS$ might

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be translocated to the cytoplasm to contribute to the $_{\text{cyt}}\text{ROS}$ helped by Plasma membrane Intrinsic Proteins (PIPs). Finally, at the transcriptional level, RSL4 directly regulates *PRX69* expression and indirectly *PRX62* at low-temperature. How RSL4 is regulated under low-temperature remains to be established. Auxin, RHD6, and/or an unknown TF are the most probable regulators of RSL4 expression at low temperature in RHs. AuxRE=Auxin response element. Low-Nut= low nutrient putative *cis*-element; RHE= Root Hair E-box. TFx= unknown TF.

PRX62 and PRX69 regulate RH growth at low-temperature

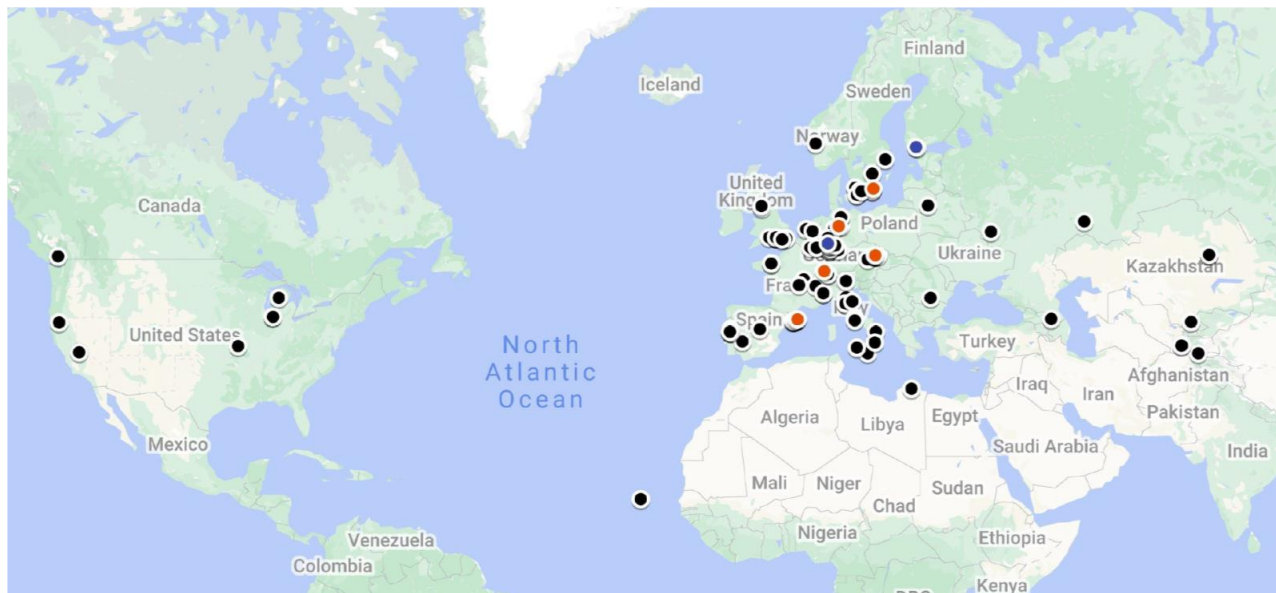


Figure S1. Geographic location of the 106 *Arabidopsis thaliana* accessions used in this study.

Each dot represents the sampling original site of individual accession used for this study. In red color, 5 accessions with the longest RHs at 10°C, and in blue those with the shortest RHs at 10°C.

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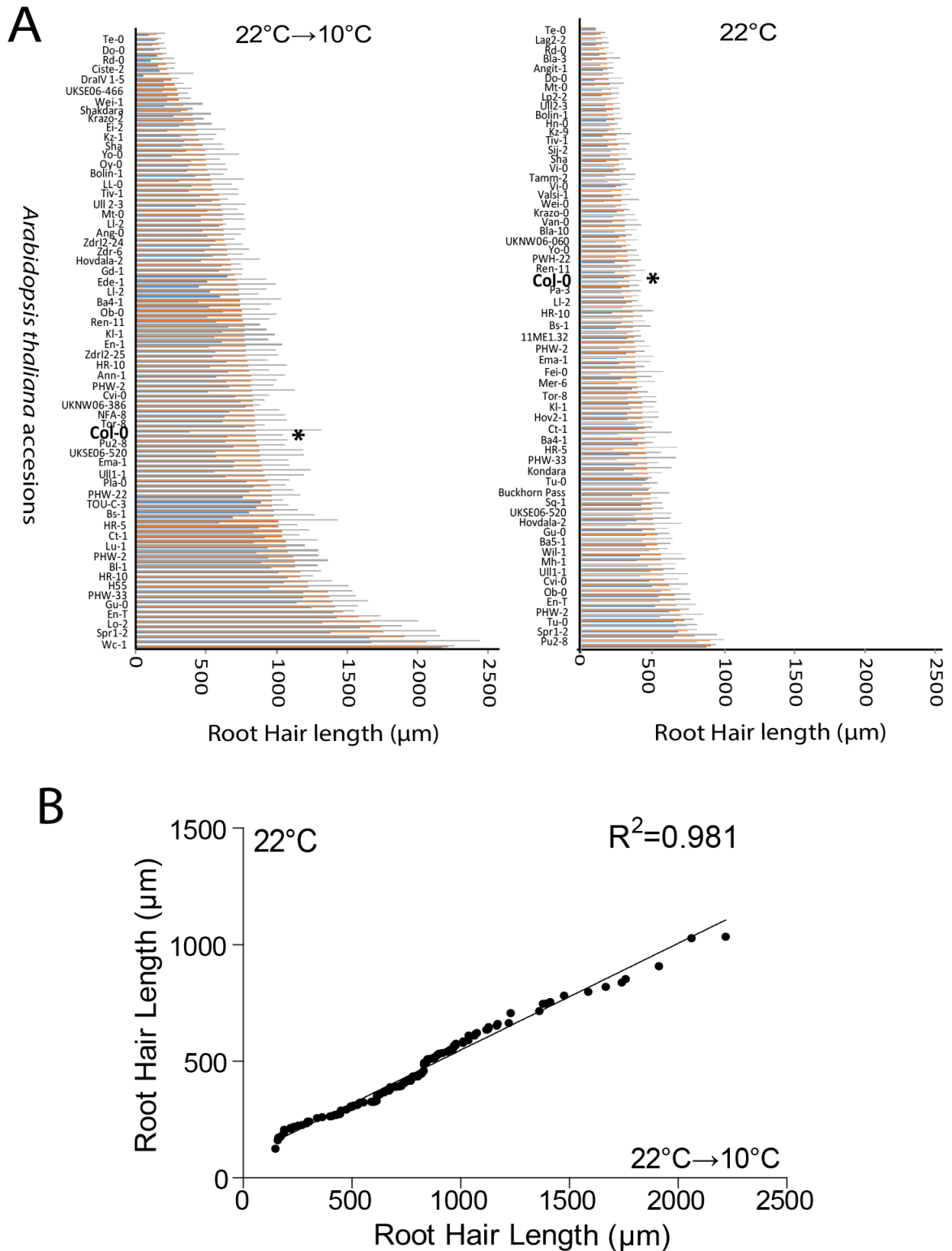


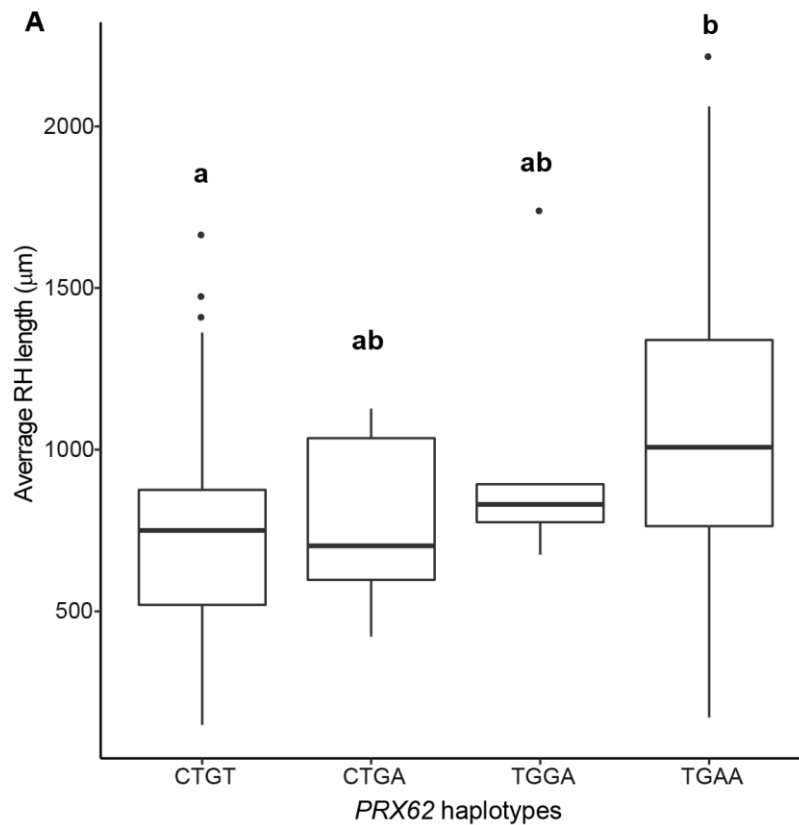
Figure S2. RH cell growth phenotype in *Arabidopsis* accessions at 22°C → 10°C versus at 22°C.

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(A) RH length phenotype in the *Arabidopsis* accessions at 22°C→10°C versus at 22°C. Average cell length on 50-300 fully elongated RHs is indicated (\pm SD) (N=root 5-30). Col-0 is indicated with an asterisk (*). Only 61 accessions are indicated in the edited graphs to improve readability. Average cell length (in orange), highest (in grey) and lowest (blue) values are shown.

(B) Pearson correlation ($R^2=0.981$) between RH growth of *Arabidopsis* accessions grown at 22°C versus the same accessions grown at 22°C→10°C.

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B ANOVA Table (Partial SS)

S.V.	SS	df	MS	F	p-value
Model.	2380630.12	3	793543.37	6.09	0.00074
Haplotype	2380630.12	3	793543.37	6.09	0.00074
Error	13411706.11	103	130210.74		
Total	15792336.23	106			

Test Tukey Alpha=0.05 LSD=359.64070
 Error: 130210.74 df:103

PRX62 haplotype	Means	n	S.E.	
CTGT	725.33	79	40.6	a
CTGA	776.96	5	161.38	a b
TGGA	983.32	5	161.38	a b
TGAA	1113.79	18	85.05	b

Figure S3. Haplotype analysis on PRX62 SNPs.

(A) Average RH length at 10°C was calculated for each informative haplotype (number of accessions carrying the haplotype ≥ 5) obtained with the four highly associated SNPs identified by GWAS and localized in PRX62. Significant differences are indicated by different letters above each haplotype.

(B) Model details and contrast for one-way ANOVA. Haplotype contrasts were identified in a post-hoc Tukey HSD test ($p \leq 0.05$). Significant differences are indicated by different letters.

PRX62 and PRX69 regulate RH growth at low-temperature

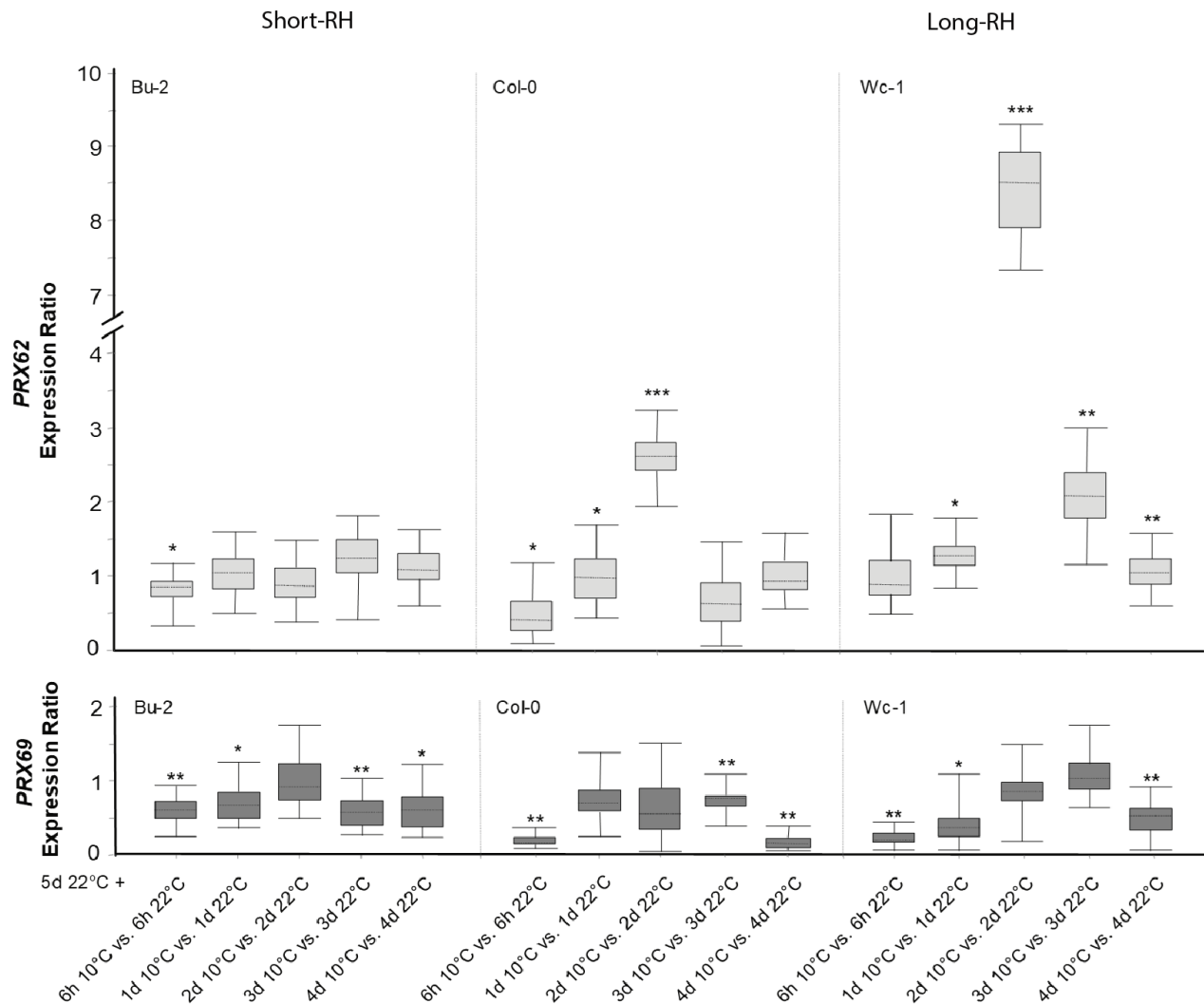


Figure S4. *PRX62*, but not *PRX69*, is differentially expressed at low-temperature (10°C) in contrasting *Arabidopsis* accessions with contrasting RH phenotypes.

Expression measured by qPCR of *PRX62* and *PRX69* in three contrasting *Arabidopsis* accessions based on the RH phenotype detected at 10°C. Total RNA was extracted from roots of *in vitro* plantlets (grown for 5 days at 22°C plus 6h, 1 day, 2 days, 3 days or 4 days either at 22°C or 10°C). *PRX62* and *PRX69* transcript levels determined by RT-qPCR were normalized to *ACT2* and *UBQ1* as internal controls. Boxes represent the interquartile range. The dotted line symbolizes the median gene expression. Whiskers correspond to the minimum and maximum observations (n = 6). Asterisks indicate statistically significant differences between cold-treated and non-cold-treated groups (***) P<0.001, (**)P<0.01, (*)P<0.05.

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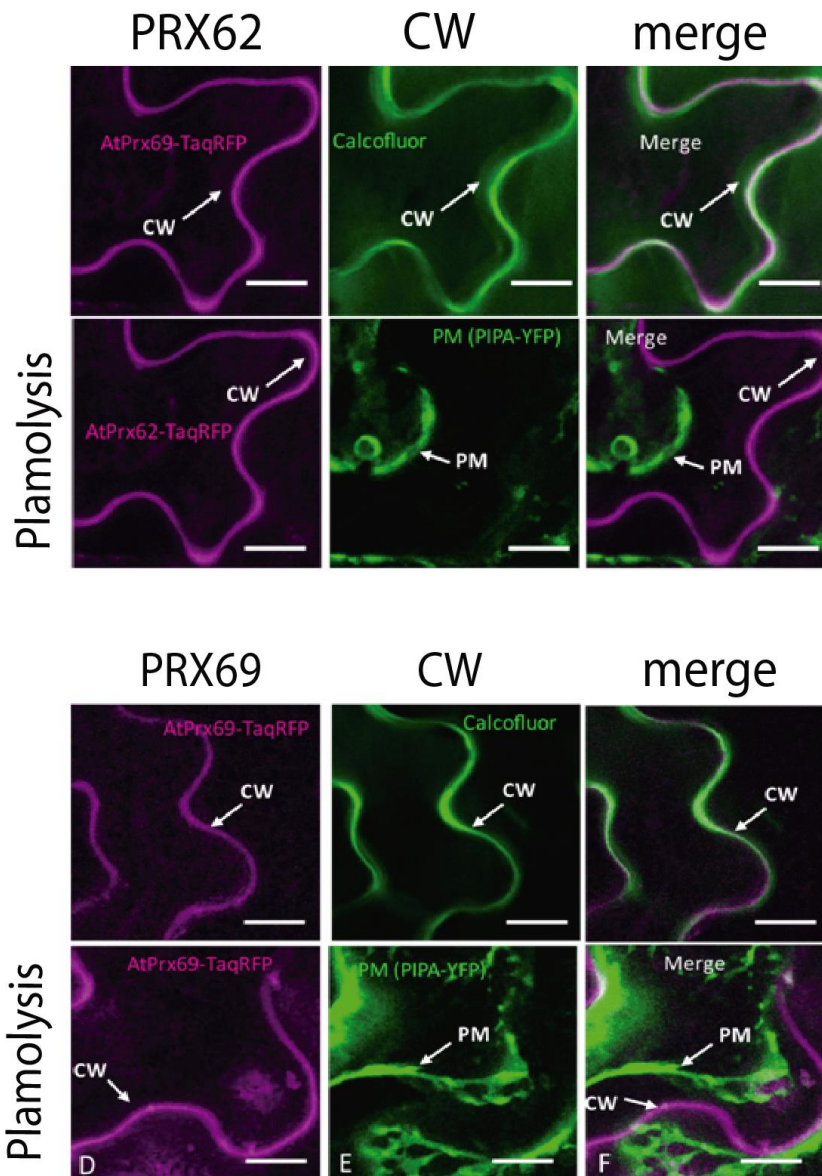


Figure S5. Apoplastic localization of PRX62 and PRX69.

Confocal laser scanning fluorescence signals from *Nicotiana benthamiana* plasmolyzed leaf epidermal cells co-expressing 35SproPRX62-TagRFP or 35SproPRX69-TagRFP (magenta channel, left panels) together with the plasma membrane marker Yellow fluorescent protein (YFP)-tagged Plasma Membrane Aquaporin (PIP2A-YFP) (green channel, central panels). Both channels merged (white signal, right panels). The top line corresponds to a single confocal section whereas the bottom line corresponds to the maximum intensity z projection of six confocal sections. Scale bars= 50 μm. CW= cell wall; PM = plasma membrane.

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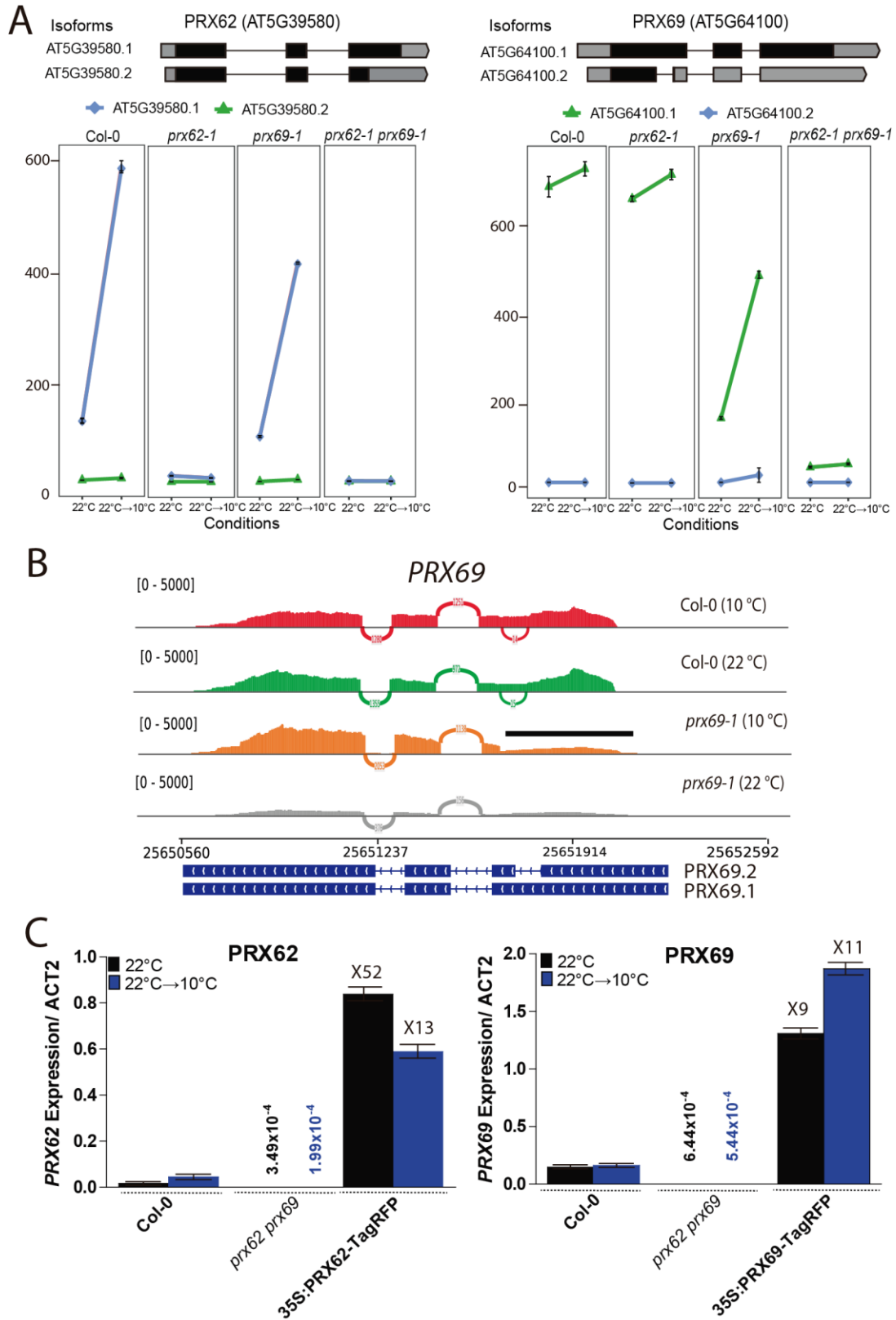


Figure S6. Expression analysis by RNA-seq and Real Time PCR of *PRX62* and *PRX69*.

PRX62 and PRX69 regulate RH growth at low-temperature

(A) Isoforms expression of *PRX62* and *PRX69* in Col-0, *prx62-1*, *prx69-1* and *prx62-1 prx69-1* double mutant determined by RNA-seq. Reads in *PRX69* gene in *prx69-1* mutant backgrounds showed a truncated version being expressed. Isoforms' schemes were adapted from boxify (<https://boxify.boku.ac.at/>). TPM = Transcripts Per Kilobase Million.

(B) Sashimi plots of *PRX69* indicate the expression of a truncated RNA in the *prx69-1* mutant. Sashimi plots (adapted from IGV) show the coverage for each alignment track (Col-0 and the *prx69-1* mutant) plotted as a histogram; arcs represent splice junctions connecting exons. Alternative splicing isoforms for *PRX69* are displayed below. The line on top of the graph highlights the region of the RNA that shows low coverage or low expression.

(C) Levels of *PRX62* and *PRX69* expression in Col-0 roots, *prx62-1 prx69-1* double mutant and over-expressor *PRX62* and *PRX69* lines. *ACT2* was use as a housekeeping gene. Three biological replicates and three technical replicates per experiment were performed.

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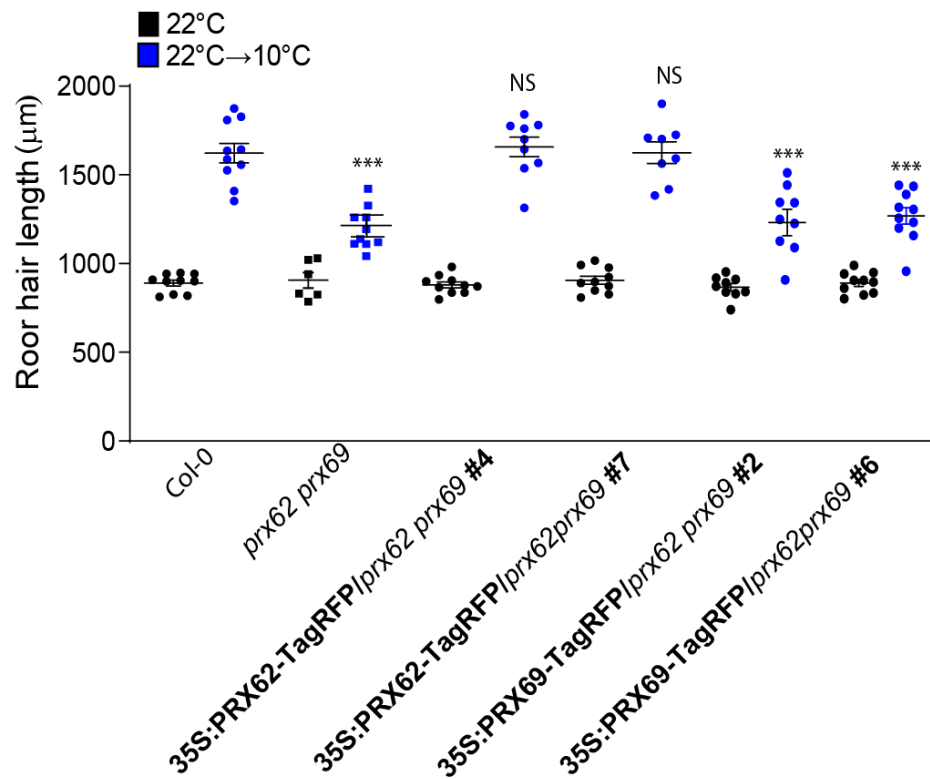


Figure S7. Phenotypic rescue of *prx62 prx69* by overexpression of PRX62 or PRX69.

35SproPRX62 is able to rescue RH growth of *prx62-1 prx69-1* double mutant while 35SproPRX69 failed to rescue of *prx62-1 prx69-1* double mutant. RH length values are the mean of three replicates \pm SD. P-value of one-way ANOVA, (***) $P < 0.001$. NS= no significant differences.

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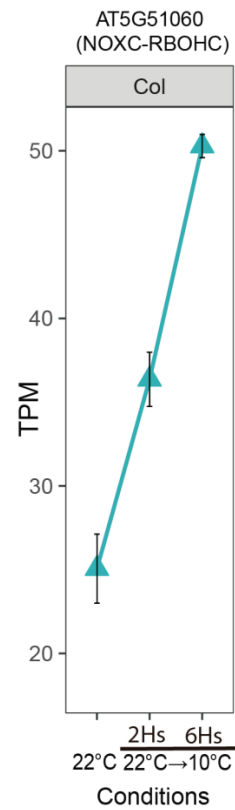


Figure S8. Expression of *NOXC* (*RBOHC/RHD2*) under low-temperature assessed by RNA-seq. TPM = Transcripts Per Kilobase Million.

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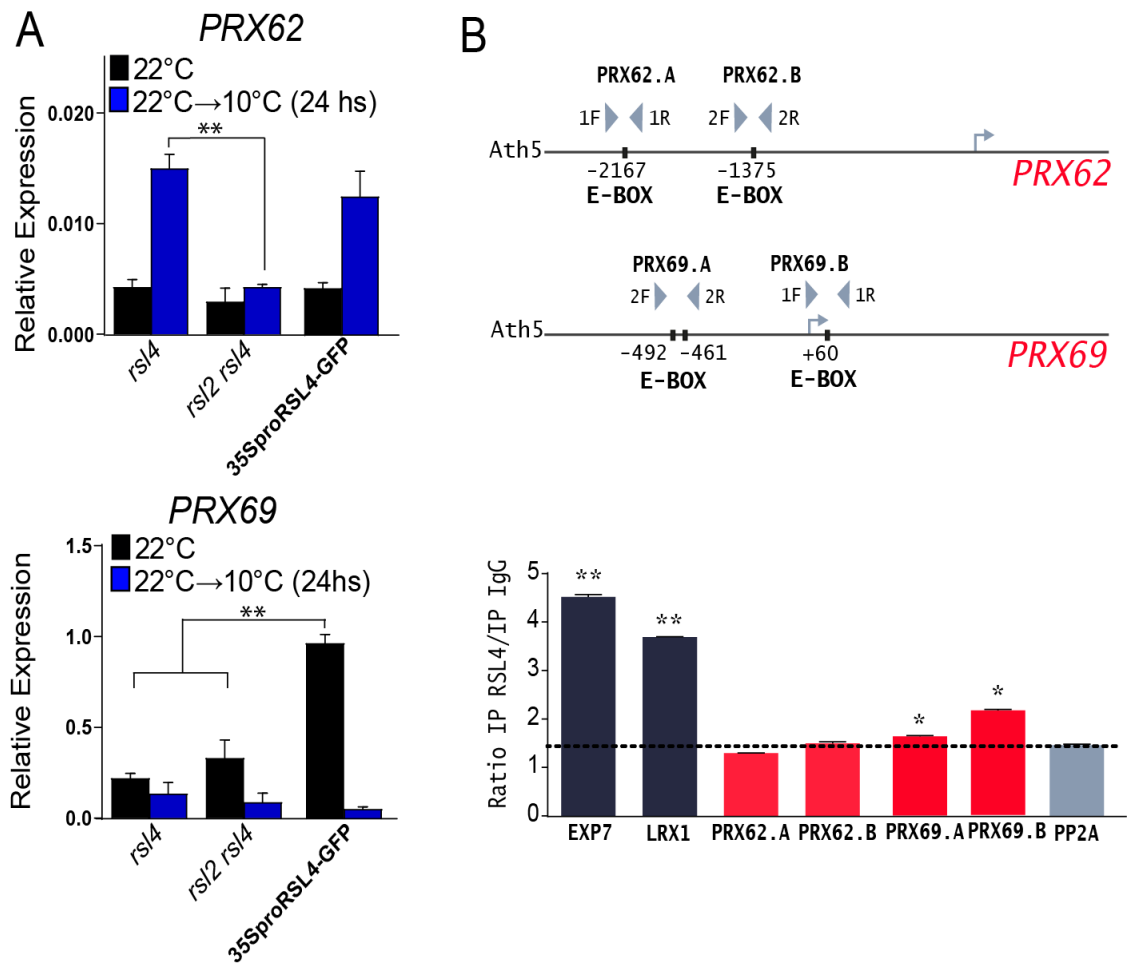


Figure S9. The transcription factor RSL4 controls the expression of PRX69.

(A) Expression analysis of *PRX62* and *PRX69* by qPCR in Col-0, 35S_{pro}RSL4, and in the mutants *rs/4*, and *rs/2rs/4* grown at 22°C and then transferred for 24hs at low-temperature treatment (10°C). Three biological replicates and three technical replicates per experiment were performed. P-value of one-way ANOVA, (***) P<0.01.

(B) ChIP analysis showing RSL4 binding to RHE-boxes elements (E-boxes) on the *PRX62* and *PRX69* promoter regions. The *PRX62* and *PRX69* promoter regions (pPRXs), the relative positions of RHE-boxes (E-boxes) and ChIP-PCR regions are indicated. The enrichment fold of RSL4-GFP in ChIP-PCR is shown for each region as a ratio with IgG IP taken as a negative control. The enrichment of each region was compared to *PP2A* taken as a negative control and determining the background, indicated as a dotted line. *EXP7* and *LRX1* were taken as positive controls according to Hwang et al. (2017). Significant differences are indicated when appropriate (**P < 0.001, *P < 0.01; t test). The experiment was performed three independent times showing the same trend. The graph represents two technical replicates of one of the independent experiments. F= forward and R=reverse primers.

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Table S2. *PRX62* and *PRX69* are transcriptionally induced under low temperature.

gene name	Log2 (WT 24h/WT 0h)	Gene FDR	Tissue expression
<i>PRX05</i>	4,68	4,7E-21	endodermis-vascular
<i>PRX04</i>	3,31	1,5E-43	endodermis-vascular
<i>PRX37</i>	2,10	3,2E-15	endodermis-vascular
<i>PRX62</i>	2,04	6,2E-22	RH
<i>PRX14</i>	1,86	1,1E-05	endodermis-vascular
<i>PRX69</i>	1,76	1,4E-35	RH

FDR= False Discovery Rate. Extracted from Schlaen et al. (2015) where seedlings were grown at 22°C for 9 days and then transferred to 10°C for 24 hs. RH = root hair.

PRX62 and PRX69 regulate RH growth at low-temperature

Table S3. Mutants and transgenic lines generated and used in this study.

Transgenic line name	Gene construct/ mutant lines code	References
<i>prx62-1</i>	GK_287E07	Jemmat et al. 2020
<i>prx62-2</i>	SALK_151762	Jemmat et al. 2020
<i>prx69-1</i>	SAIL_691_G12	Jemmat et al. 2020
<i>prx69-2</i>	SALK_137991	Jemmat et al. 2020
<i>prx62-1prx69-1</i>	<i>prx62-1</i> / GK_287E07 <i>prx69-1</i> / SAIL_691_G12	This study
35SproPRX62-TagRFP/Col-0 #3	35Spromoter::PRX62-TagRFP	This study
35SproPRX62-TagRFP/Col-0 #4	35Spromoter::PRX62-TagRFP	This study
35SproPRX69-TagRFP/Col-0 #1	35Spromoter::PRX69-TagRFP	This study
35SproPRX69-TagRFP/Col-0 #5	35Spromoter::PRX69-TagRFP	This study
35SproPRX62-TagRFP #1/ <i>prx62-1prx69-1</i> #4	35Spromoter::PRX62-TagRFP / <i>prx62-1prx69-1</i>	This study
35SproPRX69-TagRFP #1/ <i>prx62-1prx69-1</i> #7	35Spromoter::PRX69-TagRFP / <i>prx62-1prx69-1</i>	This study
35SproPRX69-TagRFP/ <i>prx62-1prx69-1</i> #2	35Spromoter::PRX69-TagRFP #1 / <i>prx62-1prx69-1</i>	This study
35SproPRX69-TagRFP/ <i>prx62-1prx69-1</i> #6	35Spromoter::PRX69-TagRFP #1 / <i>prx62-1prx69-1</i>	This study
PRX62proGFP	PRX69promoter::GFP	This study
PRX69proGFP	PRX62promoter::GFP	This study
35SproSS-TOMATO/Col-0	35Spromoter::SS-TOMATO / <i>prx62-1prx69-1</i>	This study
35SproSS-TOMATO-EXT-LONG/Col-0	35Spromoter::SS-TOMATO-EXT Long / <i>prx62-1prx69-1</i>	This study
<i>rsl4</i>	<i>rsl4-1</i>	Yi et al. 2010
RSL4proGFP-RSL4	RSL4promoter::GFP-RSL4	Mangano et al. 2017
<i>rsl2rsl4</i>	<i>rsl2-1</i> / SAIL_514C04 <i>rsl4-1</i>	Yi et al. 2010
35SproRSL4	35Spromoter::RSL4	Yi et al. 2010

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Table S4. List of Primers used in this study.

Gene or Line	Primers Sequence (5'->3')	References
genotyping by PCR		
<i>prx62-1</i>	F= GATTACACACTATTAATTAGGAATTAGTTTG R= GAGAGAAACCGAATCACGAG	Jemmat et al. 2020
<i>prx62-2</i>	F= GAGGAGGACACACGATC R= AACGAAATTGAACTTTATTTATTCC	Jemmat et al. 2020
<i>prx69-1</i>	F= ATGGGTCGTGGTTACAATTTG R= CTTGACGTCACCTTCCTTAGG	Jemmat et al. 2020
<i>prx69-2</i>	F= ATGGGTCGTGGTTACAATTTG R= CTTGACGTCACCTTCCTTAGG	Jemmat et al. 2020
RT-qPCR		
<i>PRX62</i>	F= TCGGACCACTGTGGCATCTCA R= GAGTTAGGTCCCATAAAAGCAC	This study
<i>PRX69</i>	F= CTGCTGGCTGCGGTCTAGTAA R= ACTTCCCTCGTCTAACTCCACT	This study
<i>ACTIN 2</i>	F= GGTAACATTGTGCTCAGTGGTGG R= CTCGGCCTTGAGATCCACATC	Fulton and Cobbett, 2003
35SproPRXs-TagRFP	PRX62 F= CCCAAGCTTATGGGCTTGGTCCGAT R= CGCGGATCCGCATTAACCGCAGAGC PRX69 F=CCGGAATTCATGGGTCGTGGTTACA R=CCGCCCGGGAGTTGATGGCGGAACA	This study
CHIP		
<i>PRX62.1 F</i>	AAGAAAATAAAGAGACGTTTTTGAACAG	This study
<i>PRX62.1 R</i>	gggtattcggcttaaatacatctttg	This study
<i>PRX62.2 F</i>	ccaaactcgttcaggttatctagc	This study
<i>PRX62.2 R</i>	atattggcgtcgaagcttaaaga	This study
<i>PRX69.1 F</i>	aaattcccataatttctgcgtcgtgtg	This study
<i>PRX69.1 R</i>	GTTGTGTGTTTTGAGTTTGATGTTAAAGGG	This study
<i>PRX69.2 F</i>	tatcgccacgtaactcattgatct	This study
<i>PRX69.2 R</i>	tgtgattttgaaaaatataaacgcaaa	This study
ChIP LRX1 F	TTTTTGTGACAGACATGCGTCC	This study
ChIP LRX1 R	tcagccgtcaacgttaaactc	This study
ChIP EXP7 F	aaatgtctgctgttcaattaactaatc	This study
ChIP EXP7 R	TGTTGTTTAAAGTGAGGTTTTTGAATATAG	This study

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