Warm temperature suppresses plant systemic acquired resistance by intercepting *N*-hydroxypipecolic acid biosynthesis

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Abstract

Climate warming influences disease development by targeting critical components of the plant immune system, including pattern-triggered immunity (PTI), effector-triggered immunity (ETI) and production of the central defence hormone salicylic acid (SA) at the primary pathogen infection site. However, it is not clear if and/or how temperature impacts systemic immunity. Here we show that pathogen-triggered systemic acquired resistance (SAR) in *Arabidopsis thaliana* is suppressed at elevated temperature. This was accompanied by global downregulation of SAR-induced genes at elevated temperature. Abolished SAR under warmer conditions was associated with reduced biosynthesis of the SAR metabolite *N*-hydroxypipecolic acid (NHP) in *Arabidopsis* and other plant species (such as tomato and rapeseed), as demonstrated by downregulation of NHP biosynthetic genes (*ALD1* and *FMO1*) and reduced NHP and pipecolic acid (Pip) levels. Although multiple SAR signals have been shown previously, exogenous NHP or Pip was sufficient to restore disease protection at elevated temperature, indicating that heat-mediated SAR suppression is due to downregulation of the NHP pathway. Along with *ALD1* and *FMO1*, local and systemic expression of the SA biosynthetic gene *ICS1* were also suppressed at warm temperature. Finally, we define a transcriptional network controlling thermosensitive NHP pathway via the master transcription factors CBP60g and SARD1. Our findings demonstrate that warm temperatures impact not only local but also systemic immunity by impinging on

NHP biosynthesis, providing a roadmap towards engineering climate-resilient plant immune systems.

Introduction

Warming global temperatures due to climate change pose serious threats to the natural environment and human civilization (Altizer et al., 2013; Deutsch et al., 2018; Cavicchioli et al., 2019; Delgado-Baquerizo et al., 2020). In agricultural systems, increasing average temperatures are predicted to significantly reduce yields of the world's major crops (Zhao et al., 2017; Chaloner et al., 2021; Singh et al., 2023). As many crop varieties are bred to be grown outside the native growth ranges of their wild relatives, it is important to understand the impact of elevated temperatures on plant growth, development, immunity and phenology. Together with other environmental factors, temperature profoundly affects various aspects of plant physiology, ranging from growth and development (Quint et al., 2016; Lippman et al., 2019; Hayes et al., 2020; Vu et al., 2021; Castroverde and Dina, 2021) to stress responses and immunity (Velasquez et al., 2018; Kim et al., 2021; Castroverde and Dina, 2021; Singh et al., 2023). Environmentally compromised immune signaling is associated with increased plant disease development, as postulated as the plant disease triangle (Stevens, 1960; Colhoun, 1973; Roussin-Léveillée et al., 2024).

Elevated temperatures target various components of the plant immune system (Velasquez et al., 2018; Cheng et al., 2019; Cohen and Leach, 2020; Kim et al., 2021; Castroverde et al., 2024), which encompass both cell surface (transmembrane) and intracellular immune receptors (Jones and Dangl, 2006; Zhou and Zhang, 2020; Kim and Castroverde, 2020; Ngou et al., 2022). Cell surface immune receptors recognize apoplastic immunogenic molecules, typically conserved pathogen-associated molecular patterns (PAMPs; Bigeard et al., 2015; Gust et al., 2017; DeFalco and Zipfel, 2021; Shu et al., 2023), while intracellular nucleotide-binding leucine-rich receptors (NLRs) perceive pathogen effectors and/or effector-induced host modifications (Cui et al., 2015; Jones et al., 2016; Saur et al., 2021; Contreras et al., 2023). There is evidence that warm temperatures lead to reduced abundance, membrane localization or signaling of cell surface receptors (De Jong et al., 2002; Janda et al., 2019; Hilleary et al., 2020) and perturbed nuclear

localization or activation of certain NLRs (Zhu et al., 2010; Mang et al., 2012; Cheng et al., 2013; Venkatesh and Kang, 2019).

Recent studies show that both PRR signaling and NLR signaling activate convergent downstream signaling, including increased cytoplasmic calcium influx, oxidative burst, phosphorylation cascades and production of defence hormone salicylic acid (SA), leading to overlapping defence gene expression and metabolism at the primary infection site (Pieterse et al., 2009; Ngou et al., 2021; Yuan et al., 2021). Previous research has shown that elevated temperature suppresses pathogen-induced biosynthesis of the central defence hormone salicylic acid (SA) (Malamy et al., 1992; Huot et al., 2017; Li et al., 2020; Kim et al., 2022; Rossi et al., 2023), which aligns with temperature modulation of other plant hormones (Franklin et al., 2011; Sun et al., 2012; Ibañez et al., 2018; Martínez et al., 2018; Ferrero et al., 2019; Bruessow et al., 2021; Castroverde and Dina, 2021). Apart from resistance at the local infection site, SA is also involved in systemic immunity known as systemic acquired resistance (SAR) (Fu and Dong, 2013; Zhang and Li, 2019; Kachroo and Kachroo, 2020; Lim et al., 2020; Peng et al., 2021). Primary infection and immune activation lead to SAR, which primes non-infected systemic tissues against secondary infections (Cameron et al., 1994; Maldonado et al., 2002; Fu and Dong, 2013; Yu et al., 2013; Vlot et al., 2021; Zeier, 2021). SAR is widely conserved across the plant kingdom, leading to broad-spectrum host defences by rapidly generating mobile signals at the infection site and transporting them throughout the plant (Carella et al., 2016; Chen et al., 2018; Hartmann et al., 2018; Kachroo and Kachroo, 2020; Shine et al., 2022; Cao et al., 2024).

A major SAR signal is the immune-activating metabolite *N*-hydroxypipecolic acid (NHP) (Chen et al., 2018; Hartmann and Zeier, 2018; Hartmann et al., 2018; Yildiz et al., 2021; Löwe et al., 2023). NHP has been shown to induce SAR, and blocking its biosynthesis results in SAR abolishment (Song et al., 2004; Mishina and Zeier, 2006; Jing et al., 2011; Návarová et al., 2012; Huang et al., 2020; Brambilla et al., 2023). NHP biosynthesis follows three consecutive enzymatic reactions (Hartmann and Zeier, 2018), involving the aminotransferase AGD2-LIKE DEFENSE RESPONSE PROTEIN 1 (ALD1; Zeier, 2013; Bernsdorff et al., 2016), the reductase SAR-DEFICIENT 4 (SARD4; Ding et al., 2016; Hartmann et al., 2017) and *N*-hydroxylase FLAVIN-DEPENDENT MONOOXYGENASE 1 (FMO1), which converts pipecolic acid (Pip) to *N*-hydroxypipecolic acid (NHP; Chen et al., 2018; Hartmann et al., 2018). *ALD1*, *SARD4* and *FMO1* gene expression and NHP levels are induced systemically

127 following pathogenic attack at a primary site (Song et al., 2004; Mishina and Zeier,
128 2006; Ding et al., 2016; Bernsdorff et al., 2016; Hartmann et al., 2017; Yildiz et al.,
129 2021).
130
131 Currently, the impact of elevated temperature on plant systemic immunity is
132 underexplored. Although previous studies have shown that increased temperatures
133 have a negative effect on several aspects of the plant immune system that occur in
134 the local/primary sites of infection (like PTI, ETI and SA production), it is unknown if
135 and how elevated temperature affect NHP-mediated plant immunity and the SAR
136 response. In our study, we show that elevated temperature suppresses SAR-
137 associated NHP biosynthetic gene expression and production of SAR signals Pip
138 and NHP in *Arabidopsis* and other plant species. Because it has been shown that
139 the NHP immune pathway is redundantly controlled by the master transcription
140 factors CALMODULIN-BINDING PROTEIN 60-LIKE G (CBP60g) and SAR-DEFICIENT
141 1 (SARD1) (Sun et al., 2015; Sun et al., 2018; Huang et al., 2020), we further found
142 that warm temperature-suppressed plant systemic immunity is restored by
143 constitutive *CBP60g/SARD1* gene expression or exogenous Pip or NHP application.
144 Collectively, our study indicates that CBP60g and SARD1 control the temperature-
145 vulnerability of *Arabidopsis* systemic immunity by regulating Pip/NHP biosynthesis.
146
147
148 **Results**
149
150 **Warm temperature affects systemic acquired resistance in *Arabidopsis* plants**
151 To determine if SAR is affected by temperature, lower leaves of *Arabidopsis* Col-0
152 plants were inoculated with the virulent bacterial pathogen *Pst* DC3000 as the
153 primary challenge. Two days after primary infection, upper systemic leaves were
154 inoculated with the pathogen *Pst* DC3000 as the secondary challenge. As shown in
155 Figure 1A, primary pathogen infection expectedly lowered the systemic bacterial
156 counts after secondary pathogen challenge at 23°C but not at 28°C in Col-0 plants.
157 This indicates that *Arabidopsis* SAR due to local *Pst* DC3000 infection is effective at
158 normal but not at elevated temperature, suggesting that temperature regulates plant
159 systemic immunity.
160
161 In addition to virulent pathogens, avirulent pathogens that induce local ETI can also
162 induce SAR (Cameron et al., 1994; Zeier, 2021). We therefore tested whether SAR
163 activated by the avirulent strain *Pst* DC3000/AvrRpt2, which activates RPS2-

164 dependent immunity in Col-0 plants (Kunkel et al., 1993; Bent et al., 1994), is also
165 impacted by warm conditions. As expected, SAR was induced at normal temperature
166 (23°C) after primary *Pst* DC3000/AvrRpt2 infection (Figure 1B). Strikingly, systemic
167 *Pst* DC3000 levels remained similar compared to mock treatment at elevated
168 temperature (28°C), indicating that even ETI-induced SAR protection is also
169 negatively affected in *Arabidopsis* Col-0 plants at higher temperature.
170
171 To further understand global SAR immune signaling, we analyzed a previously
172 generated *Arabidopsis* SAR transcriptome after virulent *P. syringae* infection
173 (Hartmann et al., 2018). We interfaced the SAR-induced (SAR+) genes from that study
174 with our previously published temperature-regulated transcriptome (Kim et al., 2022)
175 and categorized *Arabidopsis* SAR+ genes into downregulated or upregulated genes
176 at elevated temperature. As shown in Figure 1C and Supplementary Data File 1, 1037
177 SAR+ genes were downregulated, while 151 SAR+ genes were upregulated at
178 elevated temperatures. Gene ontology enrichment analyses (Figure 1D and
179 Supplementary Data File 2) revealed that SAR genes that were upregulated at
180 elevated temperature are associated with toxin catabolism, glutathione metabolism,
181 tryptophan biosynthesis and responses to stress (e.g. hypoxia). In contrast, warm
182 temperature-downregulated SAR genes were predominantly related to defence
183 responses and plant immunity (Figure 1D).
184
185 As shown in Figure 1E, these heat-suppressed genes include central components of
186 pattern-triggered immunity (e.g. *FRK1, BIR1, SOBIR1, SERK4*, various PRR genes),
187 effector-triggered immunity (e.g. *EDS1, PAD4, SAG101, NDR1, NSL1, NRG1.2,*
188 *NRG1.3, ADR1-L1*, various NLR genes), SA pathway (e.g. *ICS1, EDS5, PBS3, NPR3,*
189 *NPR4, TGA3, PR* genes), NHP pathway (e.g. *ALD1, SARD4, UGT76B1*), calcium
190 signaling (e.g. *ACD6, CNGCs, GLRs, CAMs, CMLs, CPKs*), immune-associated
191 kinases (e.g. MAP kinase and RLCK genes) and defence-related transcription factors
192 (e.g. *WRKYs, NACs, CBP60g, SARD1*). Our transcriptome meta-analyses
193 demonstrated that a significant majority of temperature-regulated SAR genes exhibit
194 downregulated expression at elevated temperature, consistent with loss of systemic
195 protection against secondary infection at 28°C (Figures 1A-B). Overall, our collective

196 results indicate that *Arabidopsis* SAR induced by both virulent and avirulent
197 pathogens are negatively impacted by warm temperatures.
198
199 **Temperature regulation of SAR-associated signals**
200 To determine the mechanism of how temperature regulates systemic immunity, we
201 re-examined our temperature-regulated *Arabidopsis* immune transcriptome dataset
202 (Kim et al., 2022) for differential expression of genes involved in various SAR-
203 activating signals. As shown in Figure S1, these included genes important for NHP
204 biosynthesis, nitric oxide generation, reactive oxygen species burst, azelaic acid
205 production, AzA signaling, glycerol-3-phosphate biosynthesis, $NADP^+$ generation and
206 SAR-associated auxin response factors (*ARFs*) (Yu et al., 2013; Chen et al., 2018;
207 Hartmann et al., 2018; Shine et al., 2022; Li et al., 2023; Cao et al., 2024).
208
209 We found SAR metabolite-associated genes that were either insensitive to
210 temperature change or differentially regulated by elevated temperature.
211 Temperature-insensitive SAR genes included those associated with ROS production
212 (*RBOHD, RBOHF*), $NADP^+$ biosynthesis (*FIN4*), auxin response (*ARF1, ARF3, ARF4*),
213 NO generation (*NIA2*, *NOA1*), AzA biosynthesis (*MGD1, DGD1*) and an AzA signaling
214 gene (*DIR1-LIKE*) (Figure S1). On the other hand, warm temperature-downregulated
215 genes included all Pip and NHP biosynthetic genes (*ALD1, SARD4, FMO1*), two AzA
216 signaling gene (*AZI1, EARLI1*) and a G3P gene (*GLI1*). Finally, a second G3P gene
217 (*GLY1*) was actually upregulated at elevated temperature (Figure S1). Collectively,
218 transcriptome analyses show that warming temperature conditions differentially
219 regulate the expression of diverse SAR-associated genes.
220
221 **The *N*-hydroxypipecolic acid pathway is downregulated by elevated temperature**
222 Because the Pip-NHP pathway acts upstream of NO-ROS amplification and AzA-G3P
223 signalling during SAR (Wang et al., 2018a), we ultimately focused on the NHP
224 biosynthetic pathway (Figure 2A) as the potentially rate-limiting node in temperature-
225 sensitive systemic immunity. To validate the transcriptome results for the NHP
226 pathway at a narrow temperature range (23-28°C), we measured expression levels of
227 NHP biosynthetic genes (Hartmann et al., 2018; Chen et al., 2018) using RT-qPCR
228 analyses. As shown in Figure 2B-C, transcript levels of *ALD1* and *FMO1* in local
229 (primary) leaves of *Arabidopsis* Col-0 plants at 1 day post-infection with *Pst* DC3000
230 were lower at 28°C than at 23°C. In agreement, pathogen-induced levels of NHP and
231 its precursor metabolite Pip were also lower at the warmer temperature (Figure 2D-
232 E). These results indicate that elevated temperature negatively impacts the NHP

233 biosynthetic pathway, which is associated with loss of SAR at increasing
234 temperatures.
235
236 Similar trends in warm temperature-suppression of NHP biosynthetic gene
237 expression and/or NHP metabolite levels were observed in tomato plants (Figures
238 2F-H) and rapeseed plants (Figures 2I-J). Furthermore, as shown in Figures 2K-L,
239 systemic (uninfected) leaves of *Arabidopsis* plants at 2 dpi exhibited lower *ALD1* and
240 *FMO1* gene expression levels at 28°C compared to those at 23°C. Finally, we also
241 observed loss of *Arabidopsis* SAR at intermediate temperatures 24.5°C and 26.5°C
242 (Figure S2), which was associated with loss of pathogen-induced *ALD1* gene
243 expression in *Arabidopsis* plants (Figure S3). Considering that the plant species
244 tested in this study belong to phylogenetically separate families (Brassicaceae and
245 Solanaceae), our findings highlight the remarkable conservation of heat-mediated
246 suppression of NHP biosynthesis in diverse plant lineages.
247
248 Because SA is functionally linked and mutually amplified with Pip and NHP during
249 plant systemic immunity (Hartmann and Zeier, 2019; Huang et al., 2020; Zeier, 2021;
250 Shields et al., 2022), we also measured SA biosynthetic gene expression (Figure 3A)
251 in upper, systemic leaves after primary pathogen challenge. As shown in Figures 3B
252 and 3C, systemic expression of the SA biosynthetic gene *ICS1* (Wildermuth et al.,
253 2001) was induced at 2 days after virulent *Pst* DC3000 or avirulent *Pst*
254 DC3000/AvrRpt2 inoculation at 23°C. However, *ICS1* transcript levels in systemic
255 tissues were comparable between mock and pathogen treatments at 28°C (Figures
256 3A-B), suggesting that elevated temperature downregulates SA accumulation
257 systemically. *ICS1* transcript levels in pathogen-induced plant tissues were also
258 downregulated at elevated temperature (Figure 3D), consistent with previous studies
259 (Huot et al., 2017; Kim et al., 2022).
260
261 Immune-induced *ICS1* gene expression is redundantly regulated by the master
262 transcription factors CBP60g and SARD1 (Wang et al., 2009; Zhang et al., 2010; Wang
263 et al., 2011; Figure 3A). In addition, CBP60g and SARD1 also directly regulate NHP
264 biosynthetic gene expression (*ALD1, SARD4, FMO1*) after pathogen infection (Sun et
265 al., 2015; Sun et al., 2018). Therefore, we also quantified *CBP60g* and *SARD1*
266 expression by RT-qPCR analyses. Consistent with *ICS1* suppression under warm
267 conditions, *CBP60g* and *SARD1* transcript levels were induced at 23°C in local and
268 systemic tissues but this immune induction was lost at 28°C (Figures 3E-H).
269 Altogether, these results demonstrate that the SA biosynthetic pathway is

suppressed at higher temperature similarly as NHP. This suggests that mutual amplification of these two important SAR-associated metabolites (SA and NHP) is negatively affected when temperatures increase.

Exogenous NHP restores *Arabidopsis* immune priming at elevated temperature

Having observed that NHP levels were suppressed at elevated temperature, we hypothesized that exogenous supplementation with NHP may restore disease protection at 28°C if NHP production is the rate-limiting step at elevated temperature. We infiltrated leaves of *Arabidopsis* plants with mock or 1 mM NHP and then infected the same leaves with *Pst* DC3000. As shown in Figure 4A-B, disease symptoms and pathogen levels were reduced after NHP treatment compared to mock treatment at 23°C (~13-fold reduction in *Pst* pathogen levels), expectedly indicating that NHP is sufficient to induce immune priming. Remarkably, NHP-induced disease protection was maintained at 28°C with a ~27-fold reduction in *Pst* pathogen levels) (Figure 4A-B), which confirms that NHP production is a rate-limiting step in SAR at elevated temperature.

Because NHP was sufficient to induce immune priming at both temperatures in *Arabidopsis* plants, we investigated whether NHP-mediated immune resilience at warm temperature is due to restored expression of rate-limiting defence genes, such as those involved in SA biosynthesis. As shown in Figure 4C-D, NHP was capable of significantly inducing *SARD1* at both temperatures but not *CBP60g*. Surprisingly, NHP-induced *ICS1* gene expression remained suppressed at elevated temperature (Figure 4E) in spite of the sustained effectiveness of NHP in protecting against *Pst* challenge at 28°C. Similar to the results with NHP-induced disease protection, we also observed reduced pathogen levels after Pip treatment at both 23°C and 28°C (Figure S4). Pip led to a ~3-fold reduction in *Pst* pathogen levels after leaf infiltration at both temperatures and a 3-4-fold decrease in pathogen load after root treatment (Figure S4). Collectively, our results indicate that the temperature-suppression of the Pip-NHP pathway is governed at the level of Pip and NHP biosynthesis. These findings also indicate that NHP-mediated disease protection was preserved at warmer conditions even without a corresponding *ICS1* gene induction by NHP, suggesting ICS1-independent mechanisms of NHP-mediated immune resilience.

***CBP60g* and *SARD1* control temperature-vulnerability of the NHP-SAR pathway**

In addition to chemical supplementation, we next investigated if we could restore systemic immune responses genetically at warm temperatures. We recently showed

307 that CBP60g and its functionally redundant paralog SARD1 control the temperature-
308 sensitivity of plant basal resistance and pathogen-induced SA biosynthesis (Kim et
309 al., 2022). We therefore investigated whether CBP60g and SARD1 also control
310 temperature-sensitive systemic defence responses. As shown in Figure 3E-H,
311 *CBP60g* and *SARD1* transcript levels are induced locally and systemically after *Pst*
312 DC3000 challenge at 23°C, but this induction is lost at 28°C. These suggest that the
313 loss of systemic immunity and NHP biosynthesis in *Arabidopsis* plants at elevated
314 temperature could be due to significantly decreased *CBP60g* and *SARD1* gene
315 expression.
316
317 To show a causative role for *CBP60g/SARD1* downregulation with NHP immune
318 pathway suppression, we used plants constitutively expressing *CBP60g*
319 (*35S::CBP60g*) or *SARD1* (*35S::SARD1*). As shown in Figures 5A-C, *35S::CBP60g* lines
320 restored NHP biosynthetic gene expression (*ALD1, FMO1*) and levels of the NHP
321 precursor Pip. Consistent with the previously defined functional redundancy of
322 CBP60g and SARD1 (Zhang et al., 2010; Wang et al., 2011; Sun et al., 2015; Kim et al.,
323 2022), constitutive *SARD1* expression in *35S::SARD1* plants also led to restored
324 expression of *ALD1* and *FMO1* at warm temperature (Figure 5D-E).
325
326 In terms of the biological relevance of restored NHP biosynthesis with constitutive
327 *CBP60g* expression, we observed effective SAR in *35S::CBP60g* plants at both normal
328 and elevated temperature (Figure 5F), in contrast to wild-type Col-0 plants that lose
329 SAR protection at 28°C (Figure 1A-B). Intriguingly, *35S::SARD1* plants had
330 consistently enhanced disease resistance even without initial SAR priming at both
331 temperatures, which was not accompanied by additional reduction in systemic
332 pathogen levels after SAR priming (Figure S5).Taken together, a major mechanism by
333 which higher temperatures target plant systemic immunity and NHP biosynthesis is
334 through the expression of the master immune transcription factor genes *CBP60g* and
335 *SARD1*.
336
337 **Discussion**
338 In this study, we showed that pathogen-induced SAR and the SAR-activating NHP
339 pathway in *Arabidopsis* plants are sensitive to elevated temperatures. Local
340 infection with both virulent or ETI-activating avirulent pathogens triggers SAR at
341 normal temperatures but not at elevated temperatures. We showed that SAR
342 regulation by temperature is caused by temperature-sensitive NHP pathway in local
343 and systemic tissues. SAR-induced NHP biosynthetic gene expression (*ALD1,*

344 *FMO1*) and levels of both NHP (and its precursor Pip) are downregulated at elevated
345 temperature. Even though multiple SAR signals have been proposed previously (Fu
346 and Dong, 2013; Návarová, et al., 2012; Hartmann et al., 2018; Wang, et al., 2018;
347 Vlot et al., 2021; Zeier, 2021), we show here that exogenous supplementation with
348 NHP or Pip (locally and systemically) was sufficient to restore SAR at elevated
349 temperature. This demonstrates a causative relationship between temperature-
350 suppressed NHP pathway and SAR. In the future, it would be interesting to
351 disentangle the differential temperature effects on both the initial SAR priming with
352 *Pst* DC3000/AvrRpt2 and the secondary pathogen challenge with *Pst* DC3000.
353
354 What is clear is the rate-limiting nature of NHP biosynthesis in conferring immune
355 preparedness in plants, which is a major concern in a changing climate with more
356 sustained heat waves. It is interesting to note that there are both temperature-
357 sensitive (exemplified by *ALD1* and *FMO1* in the NHP pathway) and temperature-
358 insensitive genes associated with generating central SAR signals. These
359 temperature-insensitive SAR nodes include *RBOHD/RBOHF* (for ROS generation),
360 *FIN4* (for $NADP^+$ biosynthesis), *ARF1/3/4* (for tasi-ARF signaling), *NIA2/NOA1* (for NO
361 generation), and *MGD1/DGD1* (for AzA biosynthesis). Because NHP has been
362 shown to work upstream of most of these SAR signals (Wang et al., 2018a; Li et al.,
363 2023), these temperature-regulated trends underscore that indispensability of the
364 NHP in conferring robust systemic immunity under changing environmental
365 conditions. Future studies can then address the individual contributions of these
366 SAR signals downstream of NHP (e.g. $NADP^+$, ROS, AzA and G3P) in conferring
367 systemic disease resistance under variations in temperatures and other climatic
368 factors.
369
370 Because NHP primes systemic SA biosynthesis and immunity (Yildiz et al., 2021;
371 Zeier, 2021; Li et al., 2023), we also observed that systemic SA biosynthetic gene
372 expression is downregulated by elevated temperature. SA induction is important for
373 SAR establishment since SA induction-deficient *sid2* and SA-insensitive *npr1*
374 mutants also have abolished SAR (Fu and Dong, 2013; Huang et al., 2020; Peng et
375 al., 2021). Temperature-suppressed systemic SA pathway agrees with our previous
376 study showing a negative impact of higher temperatures on local pathogen-induced
377 SA biosynthesis and basal resistance (Huot et al., 2017; Kim et al., 2022).
378 Remarkably, we found that NHP or Pip supplementation alone can protect against
379 *Pst* pathogen challenge at elevated temperature, suggesting that Pip-NHP signaling
380 is sufficient in inducing SAR. It is interesting to note that NHP-mediated disease

381 protection at warmer conditions is associated with partial but not full restoration of
382 the SA biosynthetic pathway. While *CBP60g* and *SARD1* gene expression remain
383 temperature-resilient after NHP treatment, NHP only induces *ICS1* transcript levels
384 at normal temperature but not at elevated temperature. These suggest *ICS1*-
385 independent mechanisms of plant immune resilience to climate warming, which
386 has been shown in certain natural accession of *A. thaliana* plants (Rossi et al.,
387 2024).
388
389 A potential mechanism for NHP-mediated immune resilience to changing
390 temperatures can be gleaned from previously demonstrated roles of NHP in
391 inducing MAPK activation (Wang et al., 2018b) and/or *EDS1* expression (Nair et al.,
392 2021). Because sustained MAPK or EDS1 activation can compensate for lack of SA
393 induction at ambient temperature conditions (Venugopal et al., 2009; Tsuda et al.,
394 2013), this could explain why NHP-induced disease resistance is preserved at warm
395 temperature. Alternatively, basal SA levels that remain at elevated temperature,
396 without a corresponding *ICS1* induction, may be sufficient for NHP-mediated
397 immune priming. This possibility agrees with results in SA induction-deficient
398 mutant *sid2*, which can still induce immune gene expression (like *SARD1* and
399 *FMO1*) after NHP treatment (Nair et al., 2021).
400
401 We previously showed that CBP60g and SARD1 controls the temperature-
402 vulnerability of local pathogen-induced SA biosynthesis (Kim et al., 2022). Partially
403 redundant paralogs CBP60g and SARD1 are master transcription factors that
404 directly target the promoters of numerous immunity-related genes (Wang et al.,
405 2009; Zhang et al., 2010; Wang et al., 2011; Sun et al., 2015, Sun et al., 2018),
406 including those important for SA biosynthesis (like *ICS1*) and NHP biosynthesis (like
407 *ALD1* and *FMO1*). In this study, we further demonstrate that CBP60g and SARD1
408 control the temperature-regulation of NHP biosynthesis and systemic immunity.
409 Expression of *CBP60g* and *SARD1* in both local and systemic tissues are
410 downregulated at elevated temperature, which is associated with suppressed NHP
411 pathway. Restoring organism-wide *CBP60g* or *SARD1* expression using
412 constitutively expressing *35S::CBP60g* or *35S::SARD1* plants rescues NHP
413 biosynthetic gene expression and Pip levels under warming conditions.
414
415 In contrast to *35S::CBP60g* plants which exhibits SAR at both 23°C and 28°C,
416 *35S::SARD1* plants seem to already exhibit primed immunity even without SAR
417 induction by initial pathogen infection. This could be due to differences in *CBP60g*

418 and *SARD1* gene or protein regulation. While the CBP60g protein requires
419 interaction with calcium-activated calmodulin protein, SARD1 does not require
420 calmodulin (Wang et al., 2009; Zhang et al., 2010). *SARD1* is also considered a late
421 pathogen-responsive gene, but *CBP60g* is an early immune-elicited gene in terms of
422 expression profiles (Wang et al., 2011). Finally, overexpression of *SARD1* in a
423 previous study has already been shown to exhibit constitutive SAR phenotypes at
424 ambient temperature (Zhang et al., 2010). Altogether, while CBP60g and SARD1
425 redundantly control the temperature-regulation of NHP biosynthesis in *Arabidopsis*,
426 their differential and potentially distinct functions in plant systemic immunity still
427 need to be further unravelled in future studies.
428
429 Overall, changing climatic factors like elevated temperature have a broad and
430 significant impact on the plant immune system, not only at local sites of infection
431 (PTI, ETI, SA) but also on systemic immune priming in distal sites via the central SAR
432 metabolite NHP. In this study, we determined that elevated temperature regulates
433 SAR by influencing the NHP pathway. We further demonstrated that this
434 temperature-regulation of NHP-mediated SAR is also controlled by the
435 temperature-sensitive master regulators CBP60g and SARD1, reminiscent of their
436 roles in local immune responses (Kim et al., 2022). Not only does temperature
437 affect the plant's ability to directly defend against pathogen attacks but also the
438 plant's immune preparedness for future infections. Our discoveries advance our
439 understanding of how the plant immune landscape is regulated by a changing
440 environment. This foundational mechanistic knowledge of the plant disease triangle
441 is important to inform strategies in mitigating the negative impacts of warming
442 temperatures on plant health and to provide a molecular roadmap towards
443 engineering climate-resilient plants.
444
445 **Materials and Methods**
446
447 **Plant materials and growth conditions**
448 *Arabidopsis thaliana* Columbia-0 (Col-0), *35S::CBP60g* (Wan et al., 2012) and
449 *35S::SARD1* seeds (Kim et al., 2022) were surface-sterilized with 70% ethanol for 10
450 minutes and rinsed with autoclaved water. Seeds were suspended in sterile 0.1%
451 agarose and incubated in cold conditions (4°C) for three days. Seeds were sown
452 into pots containing autoclaved soil mix – one-part Promix-PGX soil (Plant Products,
453 Ancaster, Ontario), one-part Turface (Turface Athletics, Buffalo Grove, IL), and one-
454 part Vermiculite Prolite (Therm-O-Rock, New Eagle, PA) – supplemented with

455 100mL of Miracle-Gro solution (The Scotts Company, Mississauga, ON). Tomato
456 (*Solanum lycopersicum*) cultivar Castlemart and Moneymaker plants were grown as
457 described previously (Shivnauth et al., 2023). Rapeseed (*Brassica napus*) cultivar
458 Westar plants were grown as described previously (Kim et al., 2022). Plants were
459 grown in environmentally controlled chambers at 23°C with 60% relative humidity
460 and 12h light [100 ± 20 μmol m^{-2} s^{-1}]/12 dark cycle based on established procedures
461 (Huot et al., 2017; Kim et al., 2022; Rossi et al., 2024).
462
463 **Plant systemic immunity and SAR assays**
464 Four-week-old *Arabidopsis* plants were covered with plastic domes to increase
465 humidity and open the stomata 24 hours before infiltration, Plants were infiltrated
466 with 0.25 mM $MgCl_2$ (mock), *Pst* DC3000 (OD600=0.02) or *Pst* DC3000/AvrRpt2
467 (OD600=0.02) in their lower leaves (Xin et al., 2013; Xin et al., 2018). Following
468 infiltration, plants were further incubated in environmentally growth chambers at
469 either 23°C or 28°C with identical relative humidity (60%) and lighting conditions
470 (12h light/12 dark). For SAR disease assays, upper systemic leaves were or
471 infiltrated with *Pst* DC3000 (OD600 = 0.001). Bacterial levels were quantified 3 days
472 after systemic infiltration based on a previously published protocol (Huot et al.,
473 2017; Kim et al., 2022; Rossi et al., 2024). Briefly, in planta bacterial extracts were
474 plated on rifampicin-containing LM media and log colony forming units (CFUs) cm^{-2}
475 were calculated.
476
477 **Pip treatment and protection assay**
478 Four-week-old *Arabidopsis* plants were infiltrated with mock solution or 1 mM Pip
479 (Sigma) in their lower leaves. In parallel to direct Pip infiltration into leaves, plants
480 were irrigated with 1 mL of mock solution or 1 mM Pip (Sigma) for root inoculations.
481 Following infiltration or irrigation, plants were further incubated in environmentally
482 growth chambers at either 23°C or 28°C with identical relative humidity (60%) and
483 lighting conditions (12h light/12 dark). After two days, the Pip-treated leaves (for
484 local Pip infiltration) or upper systemic leaves (for root irrigation) were further
485 infiltrated with *Pst* DC3000 (OD600 = 0.001). Bacterial levels were quantified 3 days
486 after systemic infiltration as stated in the previous section.
487
488 **NHP treatment and protection assay**
489 Four-week-old *Arabidopsis* plant leaves were infiltrated with mock solution or 1 mM
490 NHP (MedChemExpress) and then incubated in environmentally growth chambers
491 at either 23°C or 28°C with identical relative humidity (60%) and lighting conditions

492 (12h light/12 dark). After one day, the NHP-treated leaves were collected for gene
493 expression analyses or further infiltrated with *Pst* DC3000 (OD600 = 0.001). In the
494 latter case, bacterial levels were quantified 3 days after systemic infiltration as
495 detailed previously.
496
497 **Gene expression analyses**
498 Pathogen-infected leaves and upper systemic leaves at either 23°C or 28°C were
499 harvested at 1 or 2 days after local infection, respectively. NHP-infiltrated leaves at
500 both temperatures were harvested at 1 day after NHP treatment. Tissues were
501 flash-frozen in liquid nitrogen and stored at -80°C before total RNA extraction. Gene
502 expression levels were quantified based on a previously published protocol (Huot et
503 al., 2017; Kim et al., 2022) with slight modifications. RNA was extracted from flash-
504 frozen plant tissues using the Qiagen Plant RNeasy Mini Kit (Qiagen, Toronto, ON) or
505 TRIzol Reagent (Aidlab Biotech, China) according to the manufacturer's protocol.
506 Resulting cDNA was synthesized using qScript cDNA super mix (Quantabio) or M-
507 MLV reverse transcriptase (RT, Vazyme Biotech, China) based on manufacturers'
508 recommendations. Real-time quantitative polymerase chain reaction (qPCR) was
509 performed using PowerTrack SYBR Green master mix (Life Technologies) or iTaq™
510 Universal SYBR® Green Supermix (Bio-Rad, USA) with approximately 1.5-10 ng of
511 template cDNA. Equivalently diluted mRNA without the qScript cDNA mix were
512 used as negative controls. The resulting qPCR mixes were run using the Applied
513 Biosystems QuantStudio3 platform (Life Technologies) or CFX96 Real-Time PCR
514 Detection System (Bio-Rad, USA). The individual Ct values were determined for
515 target genes and the internal control gene: *PP2AA3* for Arabidopsis; *SlACT2* for
516 tomato and *BnaGDI1* for rapeseed (Huot et al., 2017; Kim et al., 2022; Shivnauth et
517 al., 2023). Gene expression values were reported as $2^{-\Delta Ct}$, where ΔCt is $Ct_{target\ gene}$–
518 $Ct_{internal\ control\ gene}$. qPCR was carried out with three technical replicates for each
519 biological sample. Primers used for qPCR are shown in Supplementary Table 1.
520
521 **Transcriptome meta-analyses**
522 The SAR+ gene list was retrieved from a previously published transcriptome dataset
523 (Hartmann et al., 2018). We then interfaced all SAR+ genes with the temperature-
524 regulated transcriptome dataset from Kim et al. (2022). At each temperature (23°C
525 or 28°C), the fold-change induction after *Pst* infection (*Pst*/mock) was first
526 calculated. The 28°C/23°C *Pst* induction ratios were then determined for all SAR+
527 genes. Genes were classified as either downregulated or upregulated at elevated
528 temperature based on a fold-change cutoff greater than 2. The two gene clusters

529 (warm-downregulated and warm-upregulated) were then subjected to Gene
530 Ontology (GO) analyses using David (Sherman et al., 2022)
531
532 **Pip and NHP metabolite extraction and quantification**
533 Pathogen-infected leaves at 23°C or 28°C were harvested at 1 day after infection.
534 Pip extraction and quantification were performed based on previous reports with
535 slight modifications (Yao et al., 2023; Yao et al., 2024). Approximately 100 mg leaf
536 tissue was frozen and ground in liquid nitrogen. Pip and NHP were simultaneously
537 extracted at 4℃ for 1h in 600 µL ice-cold extraction buffer (80% methanol in water,
538 0.1 g/L butylated hydroxytoluene). The extraction step was repeated twice, and a
539 1.2-mL supernatant was speed-dried in a vacuum centrifugal concentrator (Beijing
540 JM Technology). The pellet was resuspended in 240 µL 30% methanol solution. The
541 solution remains undiluted for the quantification of NHP, yet it is diluted tenfold for
542 the quantification of Pip. Pip and NHP levels were quantified using the AB SCIEX
543 QTARP 5500 LC/MS/MS system. Selected ion monitoring (SIM) was conducted in the
544 positive ES channel for Pip (m/z 130.0>84.0) and NHP (m/z 146.1>100.0), which was
545 done using a collision energy of 20V (for Pip) or 14V (for NHP) and a declustering
546 potential of 50V (for Pip) or 40V (for NHP). The instrument control and data
547 acquisition were performed using Analyst 1.6.3 software (AB SCIEX), and data
548 processing was performed using MultiQuant 3.0.2 software (AB SCIEX). Pip and
549 NHP were separated with an ACQUITY XSelect HSS T3 column (2.5 µm, 3.0 x 100
550 mm, Waters) using the method in Supplementary Table 2. Pip and NHP levels were
551 quantified by calculating the area of each individual peak and comparing it to
552 standard curves. Reported Pip and NHP concentrations were normalized by sample
553 fresh weight (FW) in grams.
554
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Author Contributions

C.D.M.C conceptualized and supervised the study. A.S. performed most of the SAR disease assays and gene expression experiments. L.Y.Y. optimized and performed the Pip and NHP metabolite measurements. C.A.M.R. conducted additional SAR assays and gene expression analyses. P.C.C. did the NHP-mediated disease resistance and RT-qPCR assays. J.H.K. performed gene expression experiments of *35S::SARD1* plants. W.M.A.A.T. and E.M. conducted experimental replicates of the SAR assays. V.S. conducted temperature experiments of tomato plants, extracted RNA and synthesized cDNA. S.L. and T.C. performed gene expression analyses of rapeseed plants. L.Y.Y., P.C.C. and S.L. were supervised by X.F.X., S.Y.H. and T.C., respectively. Everyone analyzed the data. A.S. and C.D.M.C. wrote the paper with input from all authors.

Competing Interest Statement

The authors do not declare any known conflicts of interest.

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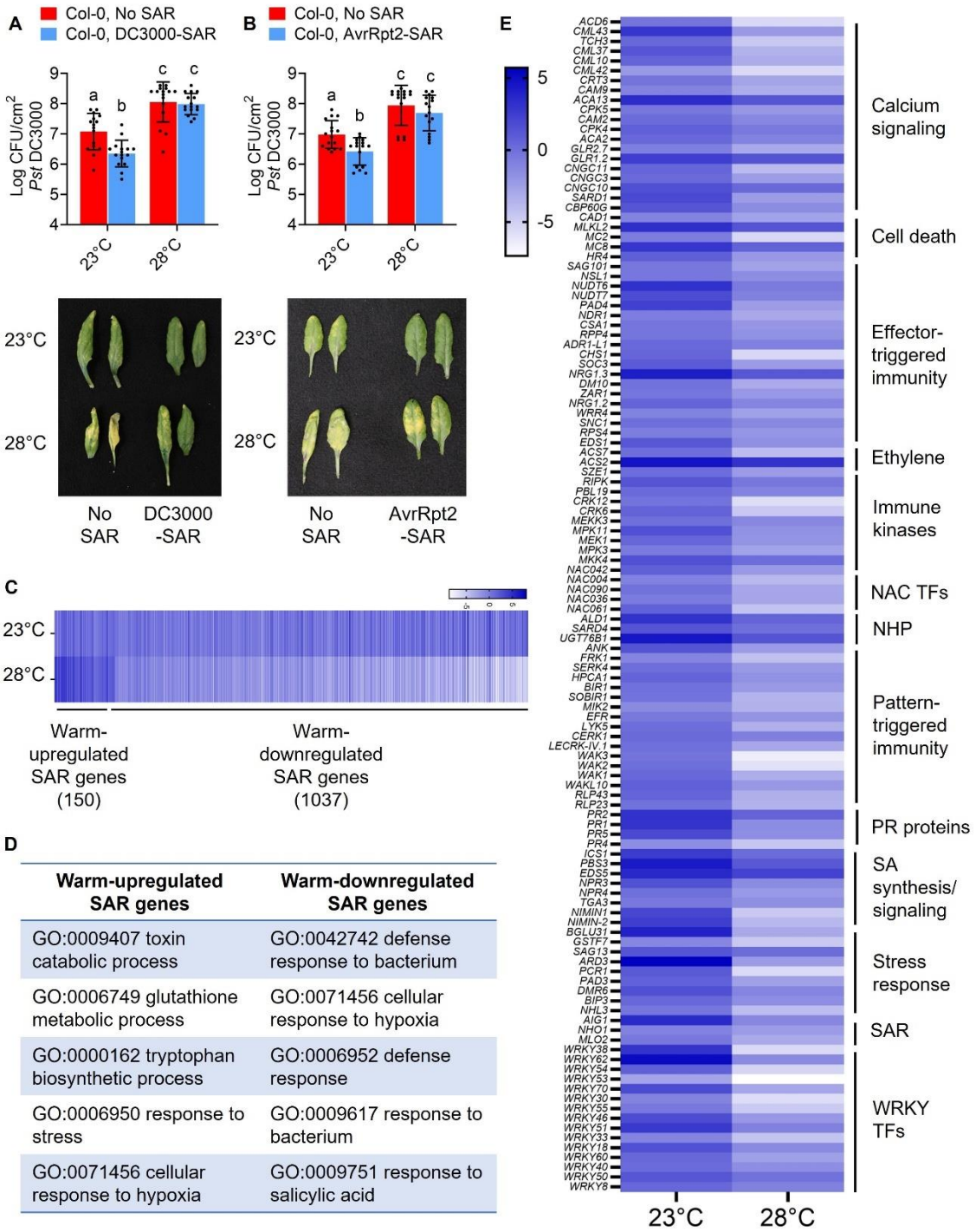
905 **Figures**

906



Warm-upregulated SAR genes	Warm-downregulated SAR genes
GO:0009407 toxin catabolic process	GO:0042742 defense response to bacterium
GO:0006749 glutathione metabolic process	GO:0071456 cellular response to hypoxia
GO:0000162 tryptophan biosynthetic process	GO:0006952 defense response
GO:0006950 response to stress	GO:0009617 response to bacterium
GO:0071456 cellular response to hypoxia	GO:0009751 response to salicylic acid

907

908 **Figure 1. *Arabidopsis* SAR is suppressed at elevated temperature.** (A, B) Lower
909 leaves of four-week-old *Arabidopsis* Col-0 plants were infiltrated with 0.25 mM
910 $MgCl_2$ (mock) and *Pst* DC3000 (OD600 = 0.02) in (A) or *Pst* DC3000/AvrRpt2 (OD600

911 = 0.02) in (B). Plants were then incubated at either 23°C or 28°C. Two days after
912 primary local inoculation, upper systemic leaves were infiltrated with *Pst* DC3000
913 (OD600 = 0.001), and plants were incubated again at their respective temperatures
914 (23°C or 28°C). Bacterial numbers (upper panels) and symptom expression photos
915 (lower panels) were taken at 3 days post-inoculation (dpi) of systemic tissues. Data
916 show the mean log CFU *Pst* DC3000/cm^2 (± S.D.) and individual points (n=16 from 4
917 independent experiments) analyzed with two-way ANOVA and Tukey's Multiple
918 Comparisons test. Statistical differences of means are denoted by different letters.
919 (C) Transcriptome analysis using SAR+ genes from Hartmann et al. (2018)
920 interfaced with temperature-regulated genes from Kim et al. (2022). The number of
921 SAR+ genes that are downregulated and upregulated at elevated temperature are
922 shown (fold change cutoff > 2). The full gene lists are shown in Supplementary Data
923 File 1. (D) Gene Ontology (GO) enrichment analyses of the warm temperature-
924 upregulated SAR+ genes (150) and warm temperature-downregulated SAR+ genes
925 (1037). The full GO results are included in Supplementary Data File 2. (E) Pathogen-
926 induced RPKM values of select plant immunity-associated genes that were
927 downregulated at elevated temperature. For C and E, the heat maps show the log_2-
928 transformed value of the *Pst*/mock RPKM values at each temperature condition.
929

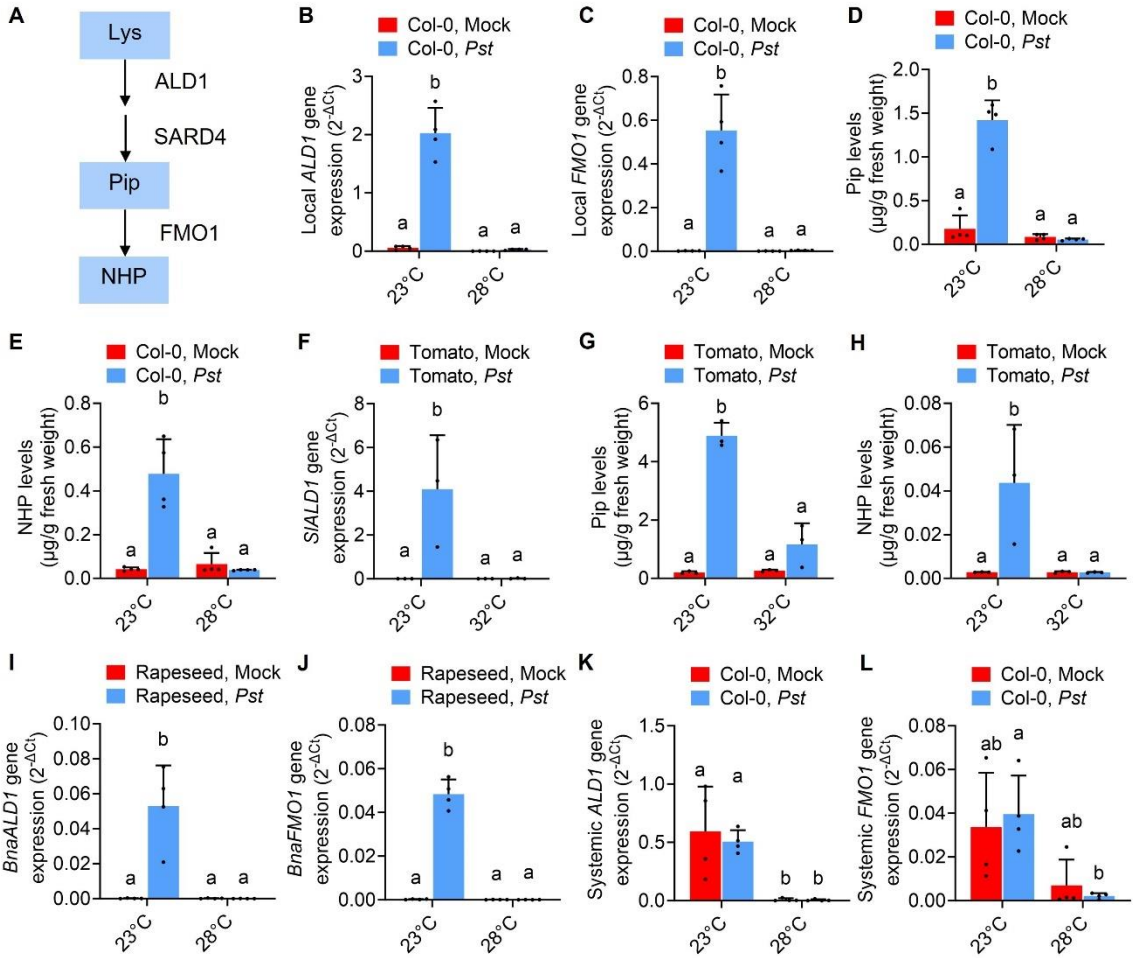


Figure 2. Plant NHP biosynthetic gene expression and NHP production are suppressed at elevated temperature. (A) Simplified diagram of Pip and NHP biosynthesis. (B-E) Leaves of four-week-old *Arabidopsis* Col-0 plants were infiltrated with 0.25 mM $MgCl_2$ (mock) or *Pst* DC3000 (OD600 = 0.001). Plants in were then incubated at either 23°C or 28°C. (B) *ALD1* and (C) *FMO1* transcript levels, as well as (D) Pip and (E) NHP levels of pathogen-inoculated tissues were measured at 1 day post-inoculation (dpi). (F-H) Leaves of four-week-old tomato cultivar Castlemart (F) or Moneymaker plants (G-H) were infiltrated with 0.25 mM $MgCl_2$ (mock) or *Pst* DC3000 (OD600 = 0.001). (F) *SlALD1* transcript levels, (G) Pip and (H) NHP levels of pathogen-inoculated tissues were measured at 1 dpi. (I-J) Leaves of 4- to 5-week-old rapeseed cultivar Westar plants were infiltrated with 0.25 mM $MgCl_2$ (mock) or *Pst* DC3000 (OD600 = 0.0001). (I) *BnaALD1* and (J) *BnaFMO1* transcript levels of pathogen-inoculated tissues were measured at 1 dpi. (K-L) Lower leaves of four-week-old *Arabidopsis* Col-0 plants were infiltrated with 0.25 mM $MgCl_2$ (mock) or *Pst* DC3000 (OD600 = 0.02). Plants in were then incubated at either 23°C or 28°C. (K) *ALD1* and (L) *FMO1* transcript levels in upper systemic

948 tissues were measured at 2 dpi. Data show the means (± S.D.) and individual points
949 (n=3 to 4) analyzed with two-way ANOVA and Tukey's Multiple Comparisons test.
950 Statistical differences of means are denoted by different letters. Experiments were
951 performed at least two times with reproducible results.
952

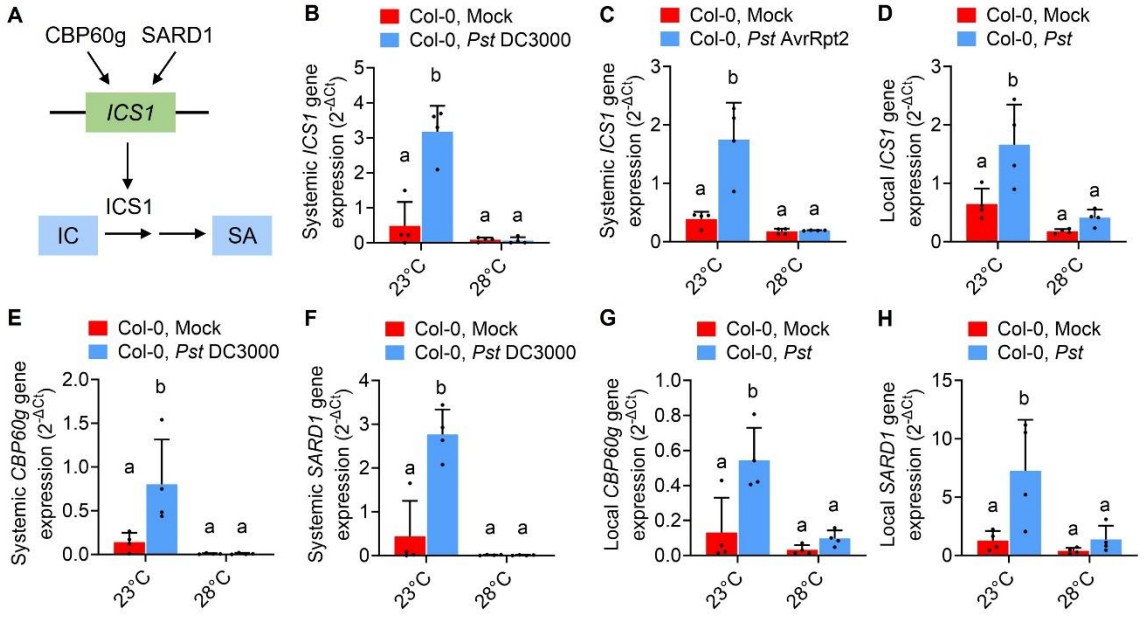


Figure 3. Systemic and local SA biosynthetic gene expression are suppressed at elevated temperature. (A) Simplified diagram of ICS1-mediated SA biosynthesis controlled by master transcription factors CBP60g and SARD1. (B-C) Lower leaves of four-week-old *Arabidopsis* Col-0 plants were infiltrated with 0.25 mM $MgCl_2$ (mock), *Pst* DC3000 (OD600 = 0.02) or *Pst* DC3000 AvrRpt2 (OD600=0.02). Plants were then incubated at either 23°C or 28°C. *ICS1* transcript levels were measured in systemic tissues using RT-qPCR at 2 days post-inoculation (dpi) with *Pst* DC3000 (B) or *Pst* DC3000 AvrRpt2 (C), respectively. (D) Leaves of four-week-old *Arabidopsis* Col-0 plants were infiltrated with 0.25 mM $MgCl_2$ (mock) or *Pst* DC3000 (OD600 = 0.001) and then incubated at either 23°C or 28°C. *ICS1* transcript levels were measured in pathogen-infected tissues using RT-qPCR at 1 dpi. (E-F) *CBP60g* and *SARD1* transcript levels were measured in systemic tissues at 2 dpi with *Pst* DC3000. (G-H) *CBP60g* and *SARD1* transcript levels were measured in pathogen-infected tissues at 1 dpi with *Pst* DC3000. Data show the means (± S.D.) and individual points (n=4) analyzed with two-way ANOVA and Tukey's Multiple Comparisons test. Statistical differences of means are denoted by different letters. Experiments were performed at least two times with reproducible results.

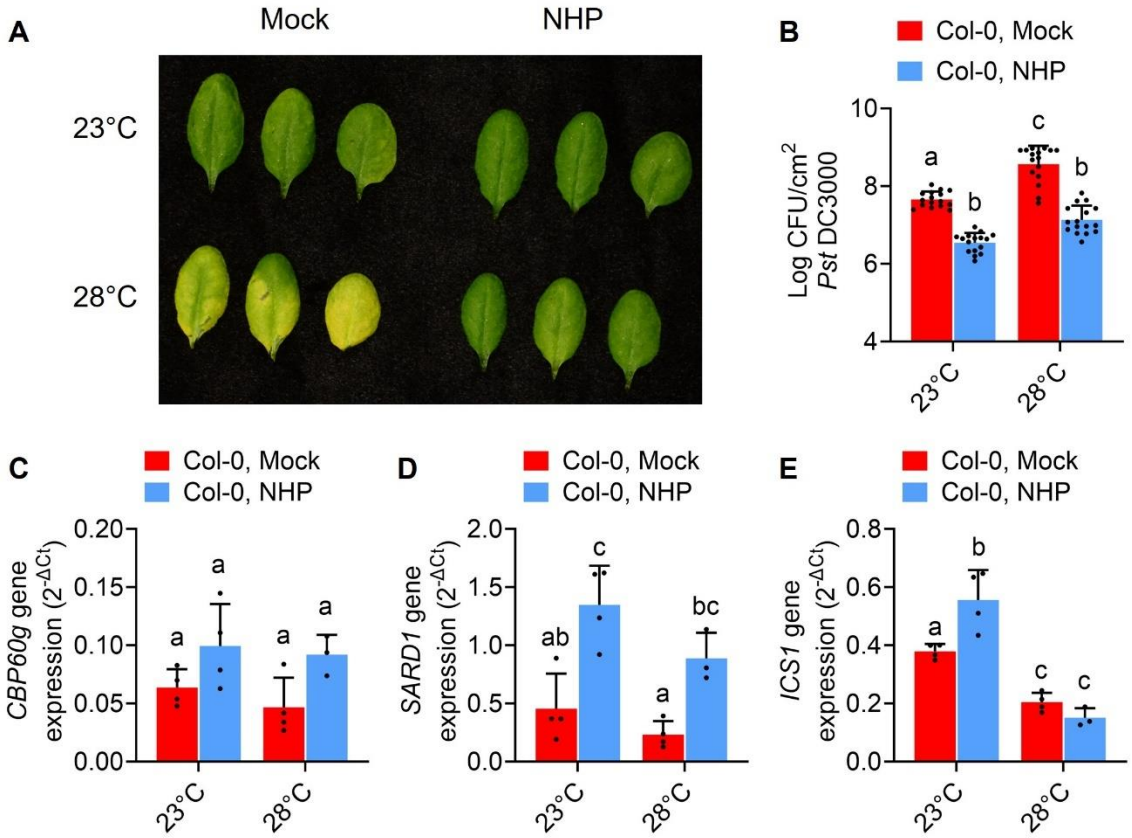


Figure 4. Exogenous NHP treatment restores *Arabidopsis* immune priming at warm temperature. Four-week-old *Arabidopsis* Col-0 plants were treated with mock or 1mM NHP solution. Plants were then incubated at either 23°C or 28°C. (A-B) Two days after NHP treatment, leaves were infiltrated with *Pst* DC3000 (OD600 = 0.001), and plants were incubated again at their respective temperatures (23°C or 28°C). Disease symptoms (A) and bacterial numbers (B) were quantified at 3 days post-inoculation (dpi). Data show the mean log CFU *Pst* DC3000/mL (± S.D.) and individual points (n=16 from 4 independent experiments) analyzed with two-way ANOVA and Tukey's Multiple Comparisons test. (C-E) NHP-treated leaves were collected after 24h. *CBP60g* (C), *SARD1* (D) and *ICS1* (E) transcript levels were measured with RT-qPCR. Data show the means (± S.D.) and individual points (n=4) analyzed with two-way ANOVA and Tukey's Multiple Comparisons test. Statistical differences of means are denoted by different letter. Experiments were performed three times with reproducible results.

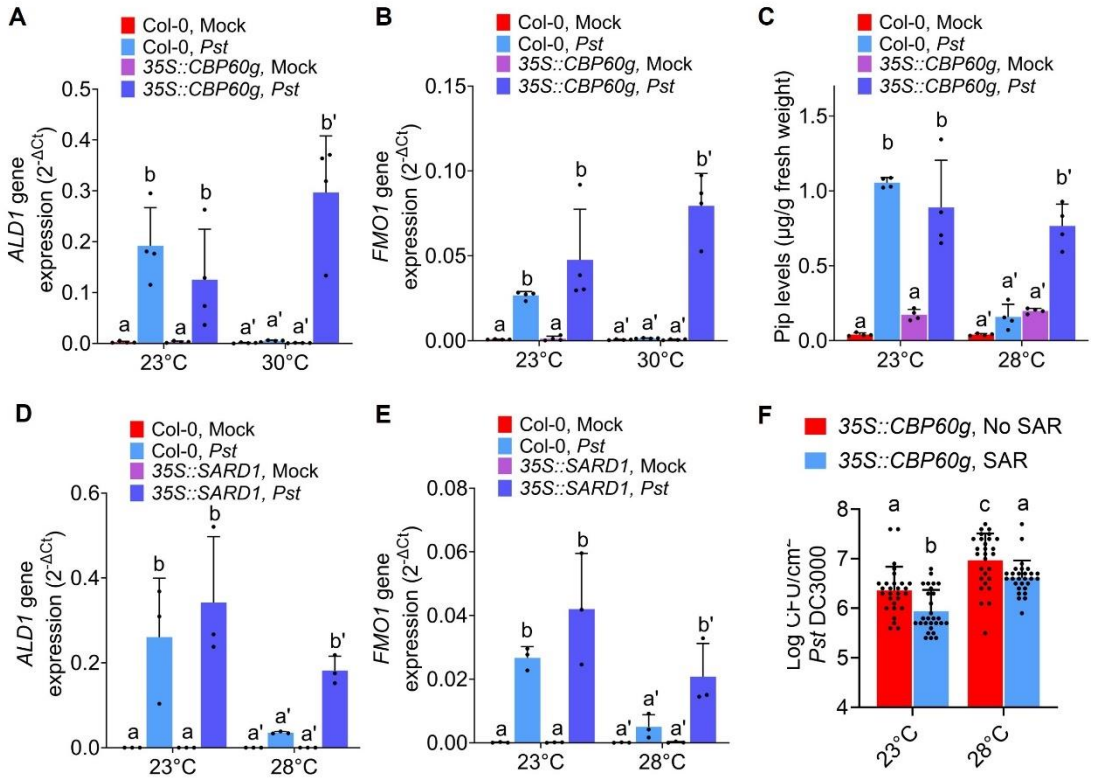
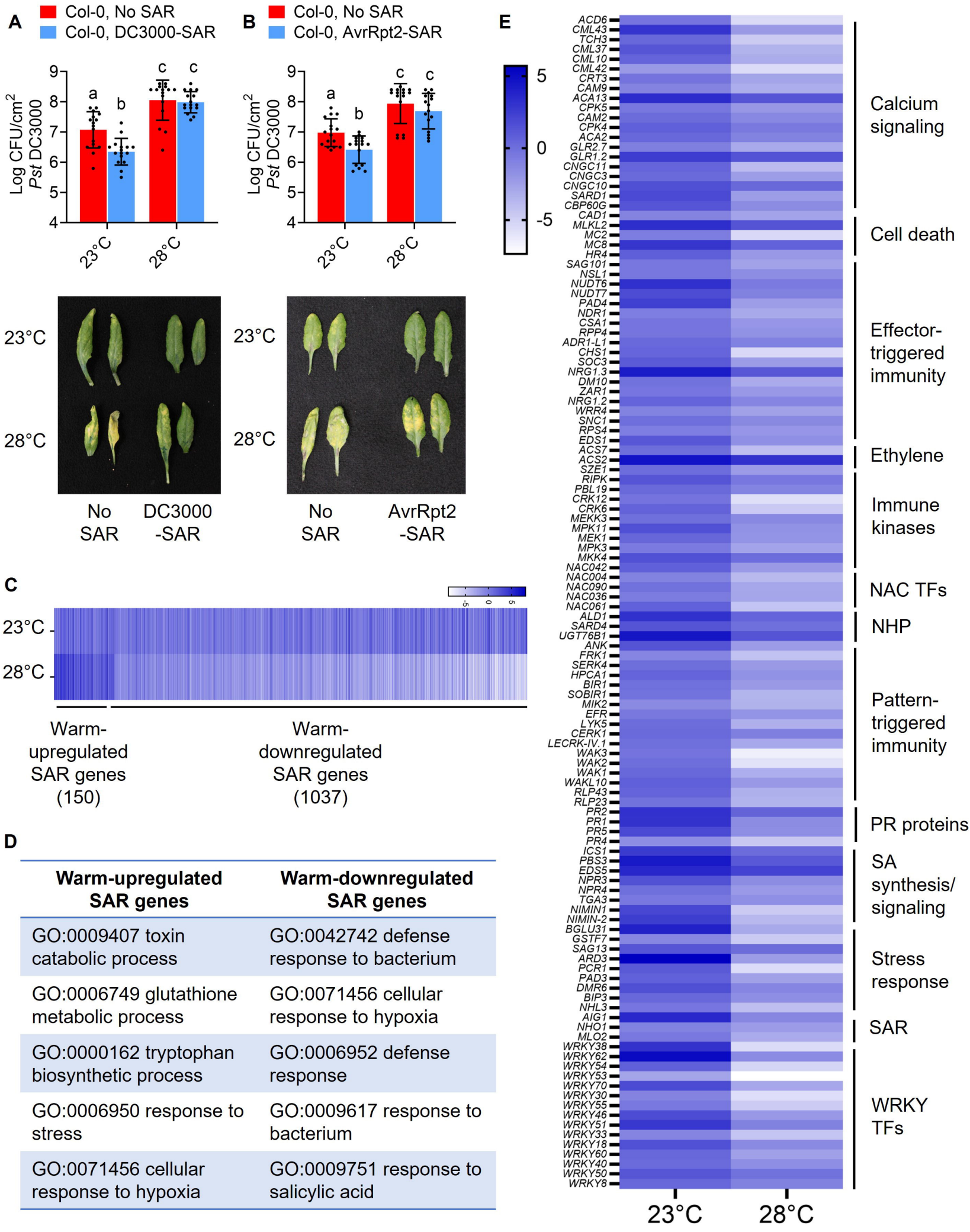


Figure 5. CBP60g and SARD1 control the temperature-vulnerability of NHP-mediated immunity. Leaves of four-week-old *Arabidopsis* Col-0 and *35S::CBP60g* (A-C) or *35S::SARD1* plants (D-E) were infiltrated with 0.25 mM $MgCl_2$ (mock) or *Pst* DC3000 (OD600 = 0.001). Plants were then incubated at either 23°C or 28°C-30°C. (A) *ALD1* and (B) *FMO1* transcript levels were measured using RT-qPCR at 1 day post-inoculation (dpi) of pathogen-inoculated tissues of Col-0 and *35S::CBP60g* plants. (C) Pip levels were measured at 1 dpi in the same tissues. (D) *ALD1* and (E) *FMO1* transcript levels were measured at 1 day post-inoculation (dpi) of pathogen-inoculated tissues of Col-0 and *35S::SARD1* plants. Data show the means (± S.D.) and individual points (n=4 in A-C; n=3 in D-E), with experiments performed three times with reproducible results. (F) Four-week-old *Arabidopsis 35S::CBP60g* plant leaves were infiltrated with 0.25 mM $MgCl_2$ (mock) and *Pst* DC3000/AvrRpt2 (OD600 = 0.02). Plants were then incubated at either 23°C or 28°C. Two days after primary local inoculation, upper systemic leaves were infiltrated with *Pst* DC3000 (OD600 = 0.001), and plants were incubated again at their respective temperatures (23°C or 28°C). Bacterial numbers were taken at 3 days post-inoculation (dpi) of systemic tissues. Data show the mean log CFU *Pst* DC3000/cm^2 (± S.D.) and individual points (n=28 from 7 independent experiments) analyzed with two-way ANOVA and Tukey's Multiple Comparisons test. Statistical differences of means are denoted by different letters.



D

Warm-upregulated SAR genes	Warm-downregulated SAR genes
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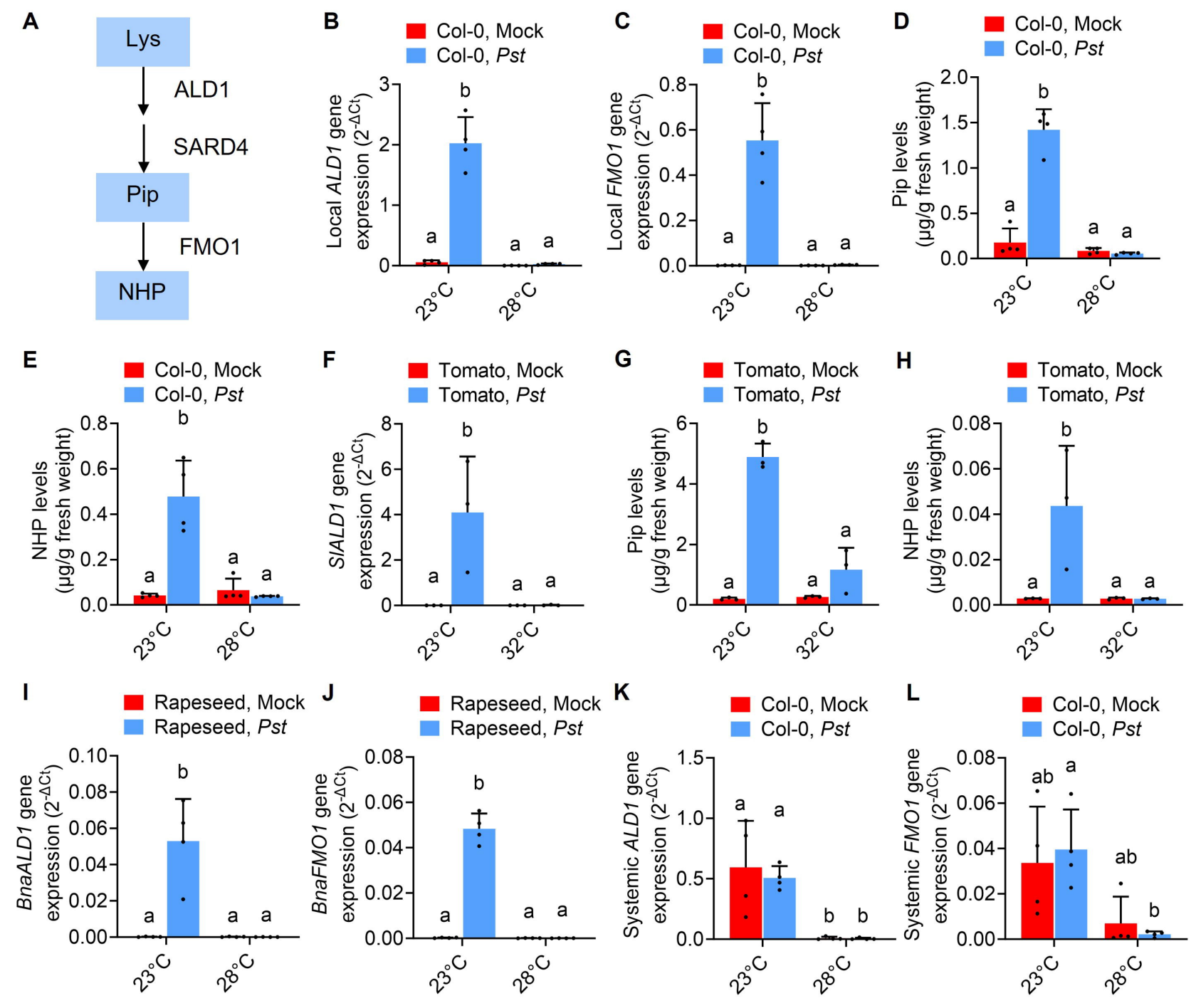
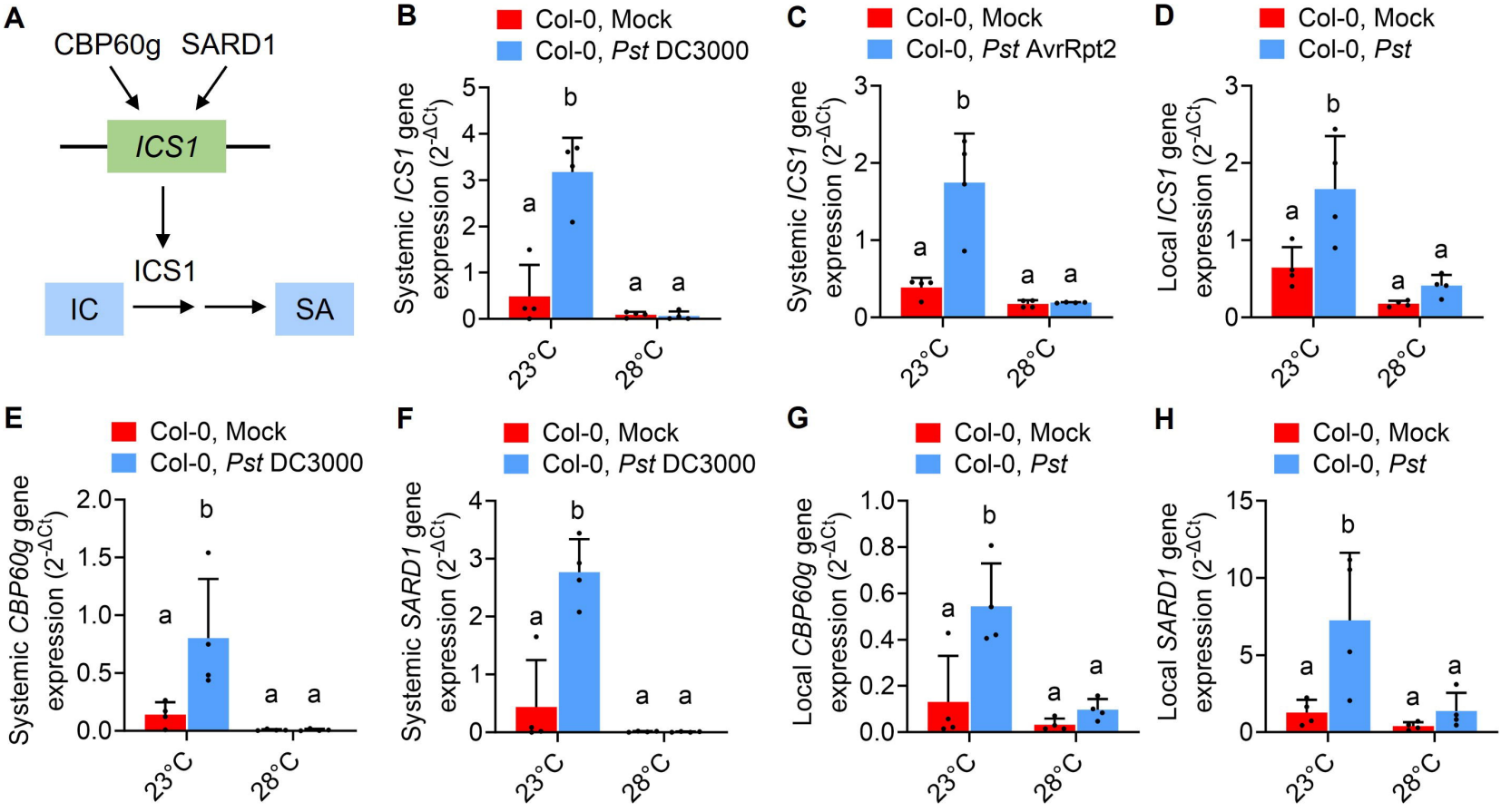
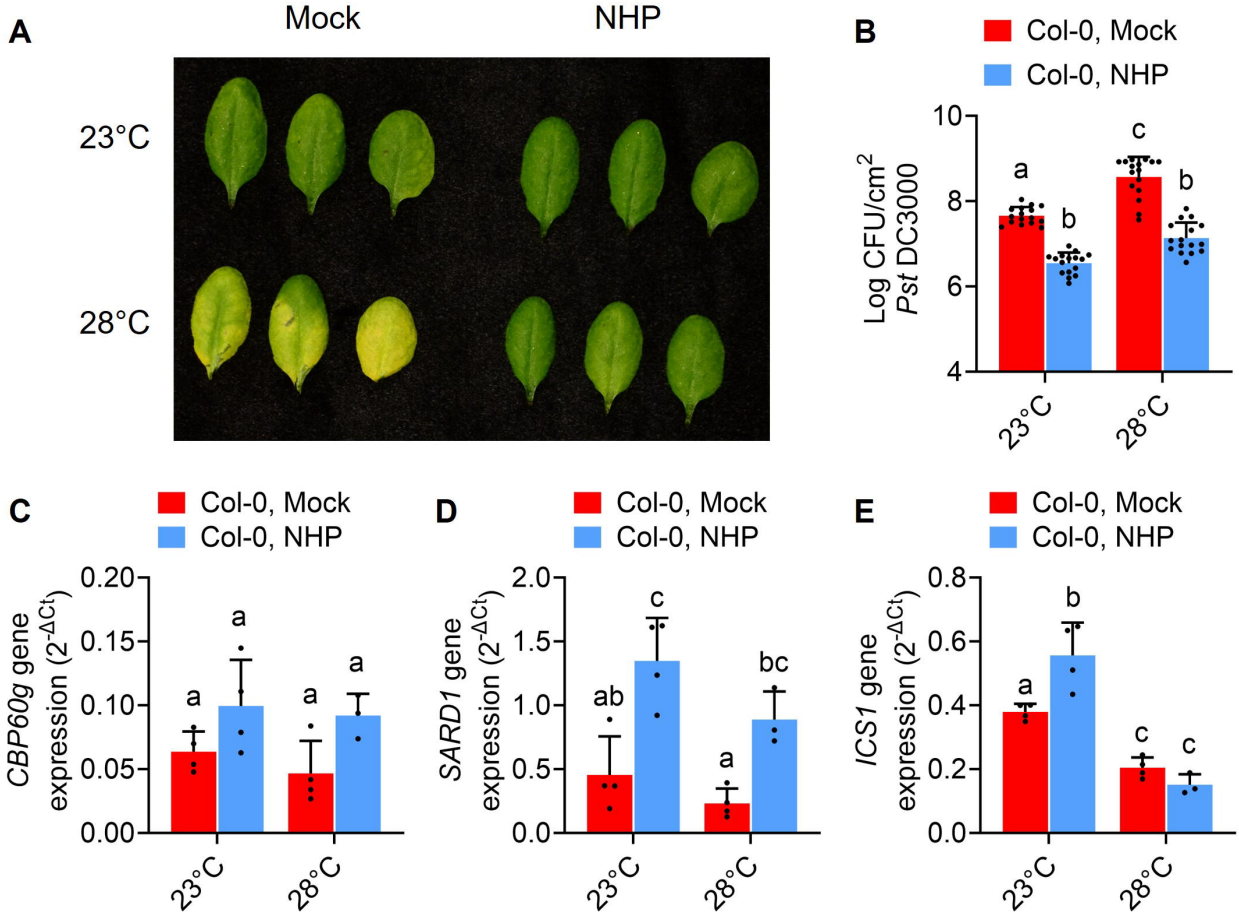
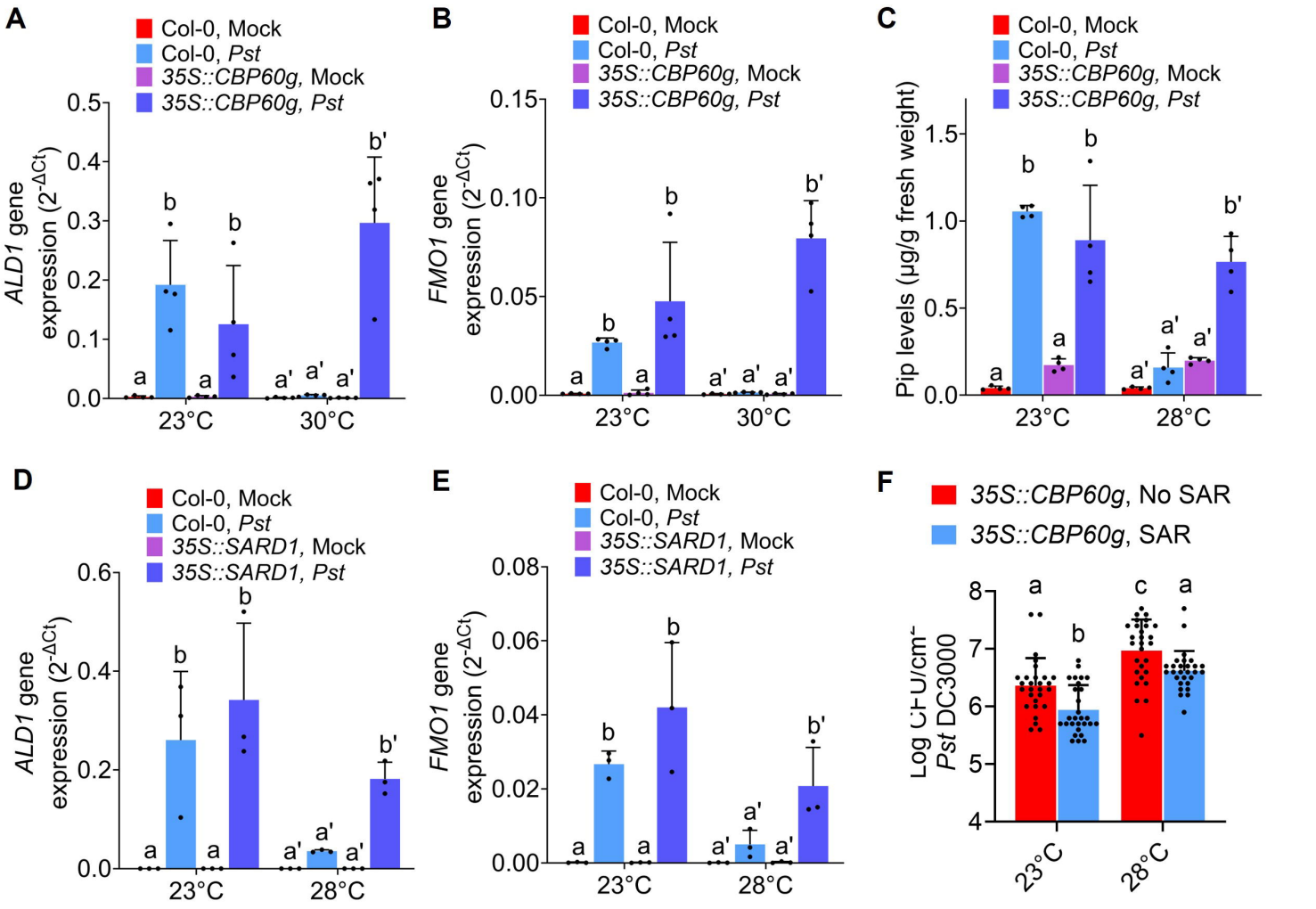

A
Lys
ALD1
SARD4
Pip
FMO1
NHP
B
Col-0, Mock
Col-0, Pst
Local ALD1 gene expression (2^-ΔCt)
23°C
28°C
C
Local FMO1 gene expression (2^-ΔCt)
D
Pip levels (µg/g fresh weight)
E
NHP levels (µg/g fresh weight)
F
Tomato, Mock
Tomato, Pst
SlALD1 gene expression (2^-ΔCt)
32°C
G
H
I
Rapeseed, Mock
Rapeseed, Pst
BnaALD1 gene expression (2^-ΔCt)
J
BnaFMO1 gene expression (2^-ΔCt)
K
Systemic ALD1 gene expression (2^-ΔCt)
L
Systemic FMO1 gene expression (2^-ΔCt)


A
CBP60g
SARD1
ICS1
ICS1
IC
SA
B
Col-0, Mock
Col-0, Pst DC3000
Systemic ICS1 gene expression (2^-ΔCt)
23°C
28°C
C
Col-0, Mock
Col-0, Pst AvrRpt2
Systemic ICS1 gene expression (2^-ΔCt)
D
Col-0, Mock
Col-0, Pst
Local ICS1 gene expression (2^-ΔCt)
E
Col-0, Mock
Col-0, Pst DC3000
Systemic CBP60g gene expression (2^-ΔCt)
F
Col-0, Mock
Col-0, Pst DC3000
Systemic SARD1 gene expression (2^-ΔCt)
G
Col-0, Mock
Col-0, Pst
Local CBP60g gene expression (2^-ΔCt)
H
Col-0, Mock
Col-0, Pst
Local SARD1 gene expression (2^-ΔCt)


A
Mock
NHP
23°C
28°C
B
Col-0, Mock
Col-0, NHP
Log CFU/cm2 Pst DC3000
10
8
6
4
a
b
c
b
23°C
28°C
C
Col-0, Mock
Col-0, NHP
CBP60g gene expression (2-ΔCt)
0.20
0.15
0.10
0.05
0.00
a
a
a
a
23°C
28°C
D
Col-0, Mock
Col-0, NHP
SARD1 gene expression (2-ΔCt)
2.0
1.5
1.0
0.5
0.0
ab
c
a
bc
23°C
28°C
E
Col-0, Mock
Col-0, NHP
ICS1 gene expression (2-ΔCt)
0.8
0.6
0.4
0.2
0.0
a
b
c
c
23°C
28°C


A
Col-0, Mock
Col-0, Pst
35S::CBP60g, Mock
35S::CBP60g, Pst
ALD1 gene expression (2-ΔCt)
0.5
0.4
0.3
0.2
0.1
0.0
a
b
a
b
a'
a'
a'
b'
23°C
30°C
B
Col-0, Mock
Col-0, Pst
35S::CBP60g, Mock
35S::CBP60g, Pst
FMO1 gene expression (2-ΔCt)
0.15
0.10
0.05
0.00
a
b
a
b
a'
a'
a'
b'
23°C
30°C
C
Col-0, Mock
Col-0, Pst
35S::CBP60g, Mock
35S::CBP60g, Pst
Pip levels (µg/g fresh weight)
1.5
1.0
0.5
0.0
a
b
a
b
a'
a'
a'
b'
23°C
28°C
D
Col-0, Mock
Col-0, Pst
35S::SARD1, Mock
35S::SARD1, Pst
ALD1 gene expression (2-ΔCt)
0.6
0.4
0.2
0.0
a
b
a
b
a'
a'
a'
b'
23°C
28°C
E
Col-0, Mock
Col-0, Pst
35S::SARD1, Mock
35S::SARD1, Pst
FMO1 gene expression (2-ΔCt)
0.08
0.06
0.04
0.02
0.00
a
b
a
b
a'
a'
a'
b'
23°C
28°C
F
35S::CBP60g, No SAR
35S::CBP60g, SAR
Log CFU/cm2
Pst DC3000
8
7
6
5
4
a
b
c
a
23°C
28°C