TOR coordinates Cytokinin and Gibberellin signals mediating development and defense

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Abstract

Plants constantly perceive and process environmental signals and balance between the energetic demands of growth and defense. Growth arrest upon pathogen attack was previously suggested to result from a redirection of the plants' metabolic resources towards the activation of plant defense. The energy sensor Target of Rapamycin (TOR) kinase is a conserved master coordinator of growth and development in all eukaryotes. Although TOR is positioned at the interface between development and defense, little is known about the mechanisms in which TOR may potentially regulate the relationship between these two modalities. The plant hormones cytokinin (CK) and gibberellin (GA) execute various aspects of plant development and defense. The ratio between CK and GA was reported to determine the outcome of developmental programs. Here, investigating the interplay between TOR-mediated development and TOR-mediated defense in tomato, we found that *TOR* silencing resulted in rescue of several different aberrant developmental phenotypes, demonstrating that TOR is required for the execution of developmental cues. In parallel, *TOR* inhibition enhanced immunity in genotypes with a low CK/GA ratio but not in genotypes with a high CK/GA ratio. TOR-inhibition mediated disease resistance was found to depend on developmental status, and was abolished in strongly morphogenetic leaves, while being strongest in mature, differentiated leaves. CK repressed TOR activity, suggesting that CK-mediated immunity may rely on TOR downregulation. At the same time, TOR activity was promoted by GA , and *TOR* silencing reduced GA sensitivity, indicating that GA signaling requires normal TOR activity. Our results demonstrate that TOR likely acts in concert with CK and GA signaling, executing signaling cues in both defense and development. Thus, differential regulation of TOR or TOR-mediated processes could regulate the required outcome of development-defense prioritization.

Introduction

Plants have developed sophisticated strategies and molecular mechanisms to protect themselves against attacks by pathogens (Jiang et al., 2020). When subjected to biotic stresses, plants activate an array of cellular and molecular processes which include the production of defense proteins and metabolites. However, this activation of defense responses is often energy-demanding, and can suppresses plant growth by diverting energy and resources toward defense at the expense of growth, or by activation of conflicting pathways, or the sharing of components between immune and growth signaling (Eichmann & Schäfer, 2015). This is known as the 'growth-defense tradeoff', a phenomenon in which plants must constantly regulate and balance growth and defense in order to adapt to changes. It is now accepted that the tradeoff between growth and defense is carefully regulated by the plant, rather than a passive process in which energy diverted toward defense is simply not available for other needs (Karasov et al., 2017; Kliebenstein, 2016). Yet, our understanding of the mechanisms that enable plants to balance growth during biotic stress response is still limited.

In recent years, the conserved Target of Rapamycin (TOR) kinase has been established as a central eukaryotic regulatory hub, playing a role in the regulation of various cellular processes including metabolism, mRNA translation and transcription, cell division, rRNAs and ribosomal proteins synthesis, and autophagy (Dobrenel et al., 2016; Zhang et al., 2016). The TOR signaling pathway fine-tunes growth and development by coordinating nutrient availability, energy status and external cues. In plants, TOR signaling is particularly important for embryogenesis, meristem activation, leaf and root growth, senescence, and flowering (McCready et al., 2020). Under nutrient availability and when conditions are favorable for growth, the TOR signaling pathway is activated and developmental and anabolic processes are promoted while catabolic processes are repressed. When nutrients are limited or in the presence of environmental stresses, TOR is inactive and catabolic processes are promoted (Dobrenel et al., 2016; Saxton & Sabatini, 2017). Even though TOR signaling is involved in the regulation of multiple important

46 signaling pathways, there is currently limited evidence of its crosstalk with the plant hormones GA and
47 CK, and its possible involvement in CK-mediated immunity.

48 Recent studies suggest that TOR acts as a negative regulator of plant immunity, as it has been
49 demonstrated to antagonize the defense hormones JA and SA in rice, suggesting that it may act as a
50 switch between these modalities (De Vleesschauwer et al., 2018). Similarly, TOR was reported to
51 negatively regulate JA biosynthesis and response in cotton (Song et al., 2017). Furthermore, mutants
52 impaired in TOR complex and TOR-inhibited WT *Arabidopsis* plants were more resistant to *Fusarium*
53 (Aznar et al., 2018), and in citrus spp., TOR inhibition was found to attenuate the growth of
54 *Xanthomonas citri* (Soprano et al., 2018). In another study, *TOR* expression was downregulated upon
55 NB-LRR activation. Suppression of *TOR* expression enhanced disease resistance, whereas *TOR*
56 overexpression decreased it, suggesting that translational regulation executed by TOR plays an
57 important role in the switch from growth to defense (Meteignier et al., 2018). TOR inhibition was also
58 found to block growth and to activate the SA signaling pathway in *Arabidopsis* (Dong et al., 2015;
59 Moreau et al., 2012). In agreement with this, we previously showed that TOR inhibition or *TOR* silencing
60 promotes resistance against *Xanthomonas*, Tobacco mosaic virus (TMV), *Alternaria alternata* and
61 *Botrytis cinerea* (*Bc*) in tomato and *N. benthamiana*, by SA-dependent activation of plant defense
62 responses (Marash et al., 2022). Although the exact mechanism by which the inhibition of TOR primes
63 resistance is not fully understood, it was suggested to selectively regulate translational control during
64 plant immunity (Meteignier et al., 2018) and/or negatively regulate autophagy in plants, as was reported
65 in yeast and mammals (Liu & Bassham, 2010).

66 Recent studies have shown that the TOR signaling pathway interacts with several plant hormones. TOR
67 signaling interacts with the Brassinosteroid (BR) signaling pathway during hypocotyl elongation through
68 the BZR1 transcription factor (Zhang et al., 2016), and activates Abscisic acid (ABA) receptors by
69 phosphorylation (Wang et al., 2018). Additionally, TOR phosphorylates and stabilizes the Auxin (AUX)

efflux facilitator PIN2, which affects the distribution gradient of PIN2 in *Arabidopsis* primary roots (Yuan et al., 2020). TOR monitors the level of sugar in meristematic regions and halts growth when the sugar level is low, blocking hormone signals that normally promote growth (Xiong et al., 2013). As plant growth rate is dictated by hormones, it seems that energy status and growth are integrated through the activity of TOR (Monson et al., 2022). Furthermore, TOR inhibition was shown to alter the expression of hundreds of genes, including genes that are linked to plant hormone signaling networks. When TOR is inhibited, the expression of genes involved in the signaling of growth hormones (AUX, GA, BR and CK) is repressed, while the expression of stress / growth inhibiting hormones (ABA, JA and SA) is upregulated (Dong et al., 2015). Although these findings demonstrated the existence of a relationship between TOR, GA, and CK signaling, the role of TOR in GA and CK-mediated immunity remains unclear.

Cytokinin (CK) is a plant hormone that regulates many aspects of plant growth and development including cell division, leaf senescence, apical dominance, vascular differentiation, chloroplast biogenesis, root development and stress responses (Zürcher & Müller, 2016). Previous studies have shown that CKs have a role in plant response to biotic stresses in tobacco (Großkinsky et al., 2011), Rice (Jiang et al., 2013) and tomato (Gupta et al., 2020). Several studies have reported that CKs promote resistance through the SA signaling pathway (Choi et al., 2010; Naseem et al., 2012). In tomato, we have previously shown that CK-deficiency results in higher susceptibility to the fungi *Botrytis cinerea* (*Bc*) and *Oidium neolycopersici* (*On*), while high endogenous CK content, as well as external application of CK, confer increased resistance against these fungi, in a SA-defendant manner (Gupta et al., 2020). Moreover, we have shown that CKs directly inhibit the growth, development, and virulence of fungal pathogens (Gupta et al., 2021), and that CKs improve *Xanthomonas campestris pv. Vesicatoria* (*Xcv*) and *Pseudomonas syringae* pv. *tomato Pst* disease outcome in tomato (Gupta et al., 2021).

Gibberellins (GAs) are growth-promoting phytohormones that play critical roles throughout the plant's life cycle, including stem elongation, germination, leaf expansion, flowering, and fruit development

(Davière & Achard, 2013). GAs regulate growth by destabilizing DELLA, a class of nuclear growth-repressing proteins that act as key regulators of the GA signaling and inhibit GA responses by interaction with multiple transcription factors (Locascio et al., 2013). Binding of GA to its receptor GA INSENSITIVE DWARF (GID1) results in degradation of DELLA and activation of responsive genes in the GA signaling pathway (Harberd et al., 2009; Hauvermale et al., 2012). GAs regulate plant growth in response to environmental changes as well as nutrient availability (Colebrook et al., 2014). Previous works demonstrated that GAs act as negative regulators of JA signaling (Campos et al., 2016; Major et al., 2020).

The opposing effects of GA and CK on many aspects of plant growth and development, such as shoot apical meristem formation, shoot and root elongation, and cell differentiation, often lead to their perception as antagonists (Ezura & Harberd, 1995; Jasinski et al., 2005). For instance, treatment with CK reduces GA activity by downregulation of GA biosynthesis genes and upregulation of two DELLA genes, *GAI* and *RGA* (Brenner et al., 2005). In addition, Greenboim-Wainberg et al., 2005 have shown that GA inhibits CK responses in Arabidopsis. The balance between CK and GA is maintained by three main proteins: KNOX, SPY and SEC. KNOX proteins induce CK biosynthesis while inhibiting GA biosynthesis and promoting GA deactivation. On the other hand, SPY and SEC repress GA signal and promote CK signals (Weiss & Ori, 2007). CK and GA have development-reciprocal relations, in which CK inhibits GA biosynthesis and promotes its deactivation by DELLA, and GA inhibits CK response. Normal shoot apical meristem function requires high CK and low GA signal, whereas later developmental stages require the opposite: low CK and high GA signals (Weiss & Ori, 2007). Considering that TOR and CK were both implicated in SA-dependent plant responses to pathogens, it appears possible that TOR and CK may interact or share similar defense pathways.

Here, we assessed the involvement of TOR in the mediation of GA and CK signals in both immunity and development. By inhibiting or down-regulating TOR, we observed partial rescue of abnormal

118 development and defense phenotypes caused by imbalanced CK or GA levels. Our findings suggest that
119 TOR plays a role in the mediation of developmental and defense signals originating from the balance
120 between CK and GA.

121 **Results**

122 ***Leaf age and CK/GA balance affect plant immunity***

123 Age has been previously linked to alterations in disease susceptibility (Goss & Bergelson, 2006). To
124 examine *Botrytis cinerea (Bc)* susceptibility in the context of "leaf developmental age", we first
125 compared *Bc* sensitivity across different leaves on the same plants. We found that younger tomato
126 leaves (L8) are significantly more resistant to *Bc* than older leaves (L5 and L3) **(Figure 1A)**. It is well
127 established that the plant leaf developmental program is primarily influenced by the CK/GA balance
128 (Fleishon et al., 2011; Hay et al., 2004; Shani et al., 2006). Young leaves undergoing morphogenesis are
129 usually high in CK and low in GA, while differentiated organs have an opposite CK/GA balance (Israeli et
130 al., 2021; Shwartz et al., 2016). Plant with high endogenous levels of CK or increased CK signaling age
131 more slowly, have delayed reproduction and senescence, and retain "juvenile" developmental
132 characteristics for longer periods (Gan & Amasino, 1995; Hwang et al., 2006, 2012; Riefler et al., 2006).
133 We therefore hypothesized that the reduction in *Bc* disease severity in young tissues could relate to the
134 CK/GA ratio. To test this hypothesis and to better understand the mechanisms that may confer
135 resistance to plants with high CK levels, we investigated *Bc* susceptibility in leaves of different
136 developmental stages in genotypes with altered CK/GA ratios. We used the following genotypes, all in
137 the M82 background: *pBLS>>IPT7*, which contains elevated endogenous levels of CK (Shani et al., 2010)
138 referred to hereinafter as "*pBLS>>IPT* "or "*IPT*"; *clausa*, which has increased CK sensitivity coupled with
139 decreased CK content (Bar et al., 2016), referred to hereinafter as "*clausa*" or "*clau*"; *pFIL>>CKX3*, which
140 has reduced CK levels (Shani et al., 2010; Shwartz et al., 2016), referred to hereinafter as "*pFIL>>CKX*" or
141 "*CKX*"; *pFIL>>GFP-PROΔ17*, which has low GA signaling, referred to hereinafter as "*pFIL>> PROΔ17*" or

142 "*PROΔ17*" (Nir et al., 2017); *ga20ox3*, which is predicted to have reduced GA levels; and *procera*$^{\Delta GRAS}$,
143 referred to hereinafter as "*procera*" or "*pro*", which has increased GA signaling (Livne et al., 2015). All the
144 genotypes used in this study are detailed and justified in **Table 1** in the materials section.
145 As shown in **Figure 1B**, when compared with the background line M82, genotypes with high CK/GA ratio
146 (*IPT*, *clausa*, *ga20ox*, *PROΔ17*) exhibit significantly higher resistant to *Bc*, whereas genotypes with low
147 CK/GA ratio (*CKX*, *pro*) are significantly more sensitive to *Bc*. This finding is in agreement with our and
148 other previous studies (Bari & Jones, 2009; De Bruyne et al., 2014; Gupta et al., 2020; Wang et al., 2013).
149 To examine the connection between the CK/GA ratio and leaf developmental age-related resistance, we
150 examined the age-related *Bc* sensitivity of genotypes with altered CK/GA ratios. We found that leaf
151 developmental age-related resistance was preserved when the CK/GA ratio was low (*CKX*, *pro*), but
152 abolished when the CK/GA ratio was high (*IPT*, *clausa*, *PROΔ17*) (**Figure 1C**), indicating that CK-mediated
153 resistance supersedes and/or is the same as leaf developmental age-related resistance.
154
155 ***TOR inhibition mediates disease resistance and immunity effected by the CK/GA ratio***
156 In our previous work, we showed found that similar to CK-mediated resistance, downregulation of TOR
157 promotes immunity through the SA pathway (Marash et al., 2022). As it emerged from our results that
158 the CK/GA ratio affects not only development but also disease resistance, we next turned to examine
159 the connection between TOR and the CK/GA ratio in the context of immunity. We used Torin2, a potent
160 ATP-competitive inhibitor of TOR activity in plants (Shi et al., 2018; Xiong et al., 2013; Ye et al., 2022) to
161 inhibit TOR, and found that it reduced *Bc* disease symptoms in the M82 background and in genotypes
162 with low CK/GA ratio (*CKX, pro*). However, we did not observe any additional decrease in *Bc* disease
163 level when TOR was inhibited in genotypes with high CK/GA ratios (*IPT, clau, ga20ox, PROΔ17*) **(Figure**
164 **2A)**. We confirmed these results using another TOR inhibitor, WYE132 (**Figure S1**). We also silenced the
165 expression of the tomato *SITOR* gene by virus-induced gene silencing (VIGS), which reduces *SITOR*

166 transcription level by 50% in *TRV2:SlTOR* leaves (Marash et al., 2022), and observed similar results
167 (**Figure S2**). This suggests that TOR might be required to transmit the output of the GA/CK ratio in
168 defense.
169 As differences in *Bc*-sensitivity could be due to changes in cellular immunity, we examined the effect of
170 TOR inhibition on defense responses in genotypes with an altered CK/GA ratio. In mock treated samples,
171 consistent with our previous report characterizing CK-mediated immunity (Gupta et al., 2020), flg-22
172 elicited ROS levels were higher in *IPT*, and lower in *CKX* plants (**Figures 2C, S3**). Flg-22 elicited ROS
173 production was enhanced in the mock samples of the high CK/GA ratio genotype *PROΔ17*, and
174 interestingly, in the low CK/GA ratio genotype *pro* (**Figures 2C, S3**). This increased ROS production in *pro*
175 could stem from higher levels of SA, similar to the increased SA levels observed in the quadruple-DELLA
176 mutant, which lacks four out of the five DELLA proteins in *Arabidopsis* (Navarro et al., 2008). Torin2
177 treatment led to increased ROS production in M82 plants as previously reported (Marash et al., 2022)
178 and in the low CK/GA ratio genotype *CKX* **(Figures 2C, S3)**. However, Torin2 did not affect any of the the
179 high CK/GA ratio genotypes *IPT*, *ga20ox3*, or *PROΔ17* (**Figures 2C, S3**). We did not observe any
180 significant change in the low CK/GA ratio genotype *pro*, again, possibly due to extremely high initial ROS
181 levels. Similar results were obtained when quantifying ion leakage in response to Torin2, with increased
182 in the background M82 line and in the low CK/GA ratio genotypes *CKX* and *pro*, but not in the high
183 CK/GA genotypes, apart from *ga20ox*3 (**Figure 2D**).
184 We continued to assess the effect of TOR downregulation on disease resistance in lines with an altered
185 CK/GA ratio by examining susceptibility to the hemibiotrophic bacterial pathogen *Xanthomonas*
186 *campestris pv. Vesicatoria* (*Xcv*), the causal agent of bacterial spot disease (Moss et al., 2007), and the
187 obligate biotrophic fungus *Oidium neolycopersici (On)*, the causal agent of powdery mildew in tomato
188 (Jacob et al., 2008), upon silencing of *TOR*. We compared disease symptoms in L5 of *TOR* silenced and
189 non-silenced plants after inoculated with *Xcv* or *On*. Leaves of *TOR* silenced M82 plants showed lower

190 *Xcv* disease symptoms and exhibited a significant decrease in *On* disease severity in comparison to non-
191 silenced plants. With the exception of *CKX*, none of the genotypes displayed a significant reduction in
192 *Xcv* disease symptoms upon silencing (**Figure S4A**). In the case of *On* disease symptoms, *CKX* and *ga20ox*
193 both showed a reduction in disease symptoms (**Figure S4B**).

194 ***TOR mediates CK-driven developmental cues***

195 Transgenic tomato lines with altered leaf CK content have altered developmental programs, resulting in
196 quantifiable phenotypic changes in leaf development. *pBLS>>IPT7* has significantly more complex leaves,
197 while *pFIL>>CKX*3 has significantly simpler leaves, when compared with their M82 background (Shani et
198 al., 2010). Likewise, *pFIL>>PROΔ17* and *clausa* have more complex leaves (Israeli et al., 2021), while
199 $pro^{\Delta GRAS}$ has simpler leaves in comparison to the M82 background (Livne et al., 2015). To investigate
200 whether TOR plays a role in GA and CK-mediated leaf development, we compared the leaf complexity of
201 lines with different GA and CK levels upon *TOR* silencing. Interestingly, while the leaves of the WT M82
202 plants did not show any significant developmental changes in response to *TOR* silencing, we observed a
203 reduction in leaf complexity in the highly complex *IPT, clausa*, *ga20ox3*, and *PROΔ17* plants, and an
204 increase in leaf complexity in the simple-leaved *CKX* and *pro* plants **(Figure 3A-B)**, suggesting that TOR is
205 required to execute the developmental cues generated by CK and GA. In addition, *TOR* silencing
206 significantly promoted shoot length (plant height) in *IPT, clau, ga20ox3* and *PROΔ17*, while it reduced
207 the height of *pro*. No significant difference in plant height was observed in *CKX*.
208 To further examine the importance of TOR in mediating hormonal signals leading to abnormal tomato
209 leaf phenotypes, we downregulated *TOR* in several classical tomato mutants, and examined the effect
210 on leaf phenotypes. A dominant mutation in the TCP transcription factor *LA* (*LANCEOLATE*), known as
211 *La2*, results in highly simple leaves, and overexpression of the *miR* that regulates *LA* expression, *miR*319
212 (as in *pBLS>>JAW*) causes highly complex leaves levels (Ori et al., 2007). *LA* was demonstrated to be

213 involved in the regulation of the balance between CK and GA during leaf development (Israeli et al.,
214 2021), and its activity is mediated in part by positive regulation of GA (Yanai et al., 2011) and negative
215 regulation of CK (Efroni et al., 2013; Israeli et al., 2021). *CLAUSA* (**Figure 3**) and *LA* jointly regulate leaf
216 development through the CK-GA balance (Israeli et al., 2021). The classical mutants *BIPPINATE* (*bip*) and
217 *DOUBLE-DISSECTED LEAF* (*ddl*) were both previously found to be related to the KNOX-BELL machinery
218 (Kimura et al., 2008; Nakayama et al., 2021). BELL proteins negatively regulate KNOX genes, and as such,
219 can affect CK and GA levels (Bolduc & Hake, 2009; Jasinski et al., 2005; Sakamoto et al., 2001; Yanai et
220 al., 2005). *POTATO-LEAF* (*c*) is a MYB transcription factor mutant shown to be involved in branching and
221 boundary formation (Busch et al., 2011). *TOR* inhibition "normalized" the phenotypes observed in all
222 these mutants (**Figure S5**), demonstrating TORs involvement in the mediation of hormonal signals and
223 execution of development, in accordance with previous studies (reviewed in: McCready et al., 2020).

224 ***GA-response is mediated by TOR***

225 Exogenous GA can affect developmental programs and phenotypes (Jasinski et al., 2008; Sun, 2010).
226 Given our results that TOR mediates hormonal signals, we hypothesized that *TOR* silencing would reduce
227 GA sensitivity. Therefore, we next characterized the effect of treatments with different concentrations
228 of GA on growth and immunity phenotypes of *TOR* silenced plants. GA-treated plants showed a
229 concentration-dependent increase in *B. cinerea* disease susceptibility (**Figure 4A**) and plant height
230 (**Figure 4B**), and a reduction in leaf complexity (**Figure 4C-D**) in comparison with untreated plants, as
231 previously described (Fleishon et al., 2011). In response to GA treatment, *TOR* silenced plants showed a
232 milder increase in disease susceptibility and plant height, and a milder reduction in leaf complexity, as
233 compared with non silenced plants (**Figure 4A-D**). *TOR* silenced plants were more resistant to *B. cinerea*
234 infection in the mock treatment, as previously described (Marash et al., 2022). Apart from plant height,
235 which remained significantly differential among control and TOR-silenced plants, GA treatment at the

highest concentration of 100 µM had similar effects on *TOR* silenced and non silenced plants (**Figure 4**). The reduced GA-sensitivity upon *TOR* silencing suggests that GA-response is mediated, at least in part, by TOR.

TOR inhibition mediated immunity and CK mediated immunity do not augment each other

To further examine the relationship between TOR inhibition and CK in plant defense, we assessed *Bc* disease sensitivity of M82 plants upon Torin2 and 6-BAP treatment. Plants were treated either with Torin2 6-BAP, or both. As we previously reported (Gupta et al., 2020; Marash et al., 2022), both 6-BAP and Torin2 treatments promoted disease resistance, as lesion size was reduced by about 40% with either Torin2 or CK **(Figure S6A)**. Treatment with both Torin2 and 6-BAP, however, had no additive effect on disease resistance. To assess the effect of 6-BAP and Torin2 on plant defense responses, we analyzed ROS accumulation and ion leakage with or without Torin2 and 6-BAP treatment **(Figure S6B-C).** ROS accumulation and ion leakage were increased by 6-BAP and Torin2 treatments. However, no additive effect on induction of defense responses was observed upon combined treatment with both Torin2 and 6-BAP, suggesting that TOR inhibition and CK likely promote disease resistance through overlapping pathways.

TOR inhibition alters CK and GA pathway genes and reduces CK response

Since *TOR* inhibition reduced the sensitivity to signaling cues mediated by CK and GA, we analyzed the effect of Torin2 on the expression of CK and GA metabolic and signaling genes. CK is synthesized by IPT enzymes (Kakimoto, 2001; Sakamoto et al., 2006; Takei et al., 2001), activated by LOG enzymes (Kurakawa et al., 2007; Kuroha et al., 2009), and perceived by a response regulator array (Argyros et al., 2008; Ishida et al., 2008). Type-A response regulators (*TRRs* in tomato) are known to be CK-responsive (Fleishon et al., 2011). The deactivation of CKs can happen either through conjugation or irreversible

degradation by *Cytokinin oxidase/dehydrogenase* (*CKX*) (Mok & Mok, 2001; Werner et al., 2006). The expression of the CK-responsive response regulator genes, *TRR3/4* and *TRR16B* was downregulated by Torin2 treatment in developing leaves and unaffected in mature leaves, while the expression of the CK inactivating genes *CKX2* and *CKX5* was upregulated in developing leaves and downregulated in mature leaves (**Fig. 5A-B**). Expression levels of *TRR5/6/7* and *CKX6* was unaffected in both tissues. Expression of the CK-biosynthesis genes *IPT5* and *IPT6* was downregulated in developing leaves upon Torin treatment, and unaffected in mature leaves. *IPT3* was unaffected in developing leaves and downregulated in mature leaves (**Figure 5C-D**). Expression of the CK activating LOG enzymes *LOG5* and *LOG8* was reduced by Torin in developing leaves (**Figure 5C-D**). Torin also induced *LOG8* expression in mature leaves. *LOG4* was unaffected in both tissues. Overall, TOR inhibition causes CK biosynthesis, CK activation, and CK signaling, to be inhibited in developing leaves, and somewhat promoted (apart from *IPT3*) in mature leaves.

The production of GA from Geranylgeranyl diphosphate (GGDP) requires multiple enzymes, including *ent*-kaurene synthase (*KS*), *ent*-kaurene oxidase (*KO*), *ent*-kaurenoic acid oxidase (*KAO*), GA20 oxidase (*GA20ox*), and GA3 oxidase (*GA3ox*). The GA metabolic genes *GA20ox* and *GA3ox* convert precursors of GA into its active form through a series of oxidation reaction, while the *GA2ox* genes are essential for inactivating GA (Hedden, 2020). We found that the expression of key genes involved in the biosynthesis of active forms of GAs, *GA20ox2 and GA3ox2*, was upregulated by Torin2 in developing leaves (**Figure 5E**). *GA3ox2* was downregulated by Torin2 in mature leaves, and *GA20ox1* was unaffected in developing leaves and downregulated in mature leaves, while *GA20ox3* was downregulated in both developing and mature leaves (**Figure 5E-F**). The expression of the GA-inactivating enzymes *GA2ox4* and *GA2ox7* was upregulated in developing leaves and unaffected in mature leaves. (**Figure 5E-F**). The expression levels of genes involved in early GA biosynthesis, *KS*, *KO*, and *KAO,* was elevated by Torin2 in developing leaves, and reduced in mature leaves (except for *KAO* that was unaffected in mature leaves)

282 (**Figure 5G-H**). Likewise, the expression of the GA response and signal transduction genes *PROCERA* and
283 *GAST1* (Shi & Olszewski, 1998) was upregulated in developing leaves, while *GAST1* was downregulated in
284 mature leaves **(Figure 5G-H)**. Overall, TOR inhibition causes GA biosynthesis, GA activation, and GA
285 signaling, to be promoted in developing leaves, and inhibited in mature leaves. These results suggest the
286 existence of a feedback regulatory mechanism between GA, CK and TOR that may help plants to fine-
287 tune the level of these hormones upon pathogen attack.
288 To further examine the effect of TOR inhibition on CK signaling in young developing shoots, we used the
289 CK-response transgenic reporter line *pTCSv2::3xVENUS*, that expresses *VENUS* under the control of the
290 CK-responsive synthetic promoter TCSv2 (Steiner et al., 2016, 2020; Zürcher et al., 2013). We found that
291 in the presence of Torin2, there is a reduction in CK signaling. Torin treated *pTCSv2::3xVENUS* shoots
292 showed a significant reduction in VENUS signal relative to untreated shoots, in the meristem (**Figure 5I,**
293 **K**) and four youngest leaf primordia (**Figure 5 I, L**). Similar results were achieved in TCS expressing plants
294 in which *SlTOR* was silenced by VIGS **(Figure S7)**. This aligns with the reduction in *TRR3/4*, *TRR16B*, *IPT5*,
295 and *IPT6*, and the increase in *CKX*2 and *CKX5* expression in developing leaves upon Torin2 treatment
296 (**Figure 5A, C**).
297 Pathogen infection has been previously demonstrated to alter the expression of CK pathway genes
298 (Argueso et al., 2012; Gupta et al., 2020; Gupta, Leibman-Markus, et al., 2021). To investigate possible
299 effects of TOR status on pathogen-driven alterations in CK pathway genes, we examined gene
300 expression in L5 of 10-week-old WT M82 plants 24h following spray-inoculation with *Bc* spores, with and
301 without Torin2 treatment by petiole feeding (**Figure S8**). CK-mediated signaling (examined by assaying
302 expression of the response regulators *TRR3/4* and *TRR5/6/7*) showed the expected increase following Bc
303 treatment (**Figure S8A-B**). *TRR3/4* was reduced by Torin2 treatment, and following Bc and Torin2 co-
304 treatment, returned to background levels (**Figure S8A**). *TRR5/6/7* was not affected by Torin2, however,
305 upon combined treatment, it increased beyond the levels elicited by *Bc* alone (**Figure S8B**), suggesting

306 that TOR mediated effects on the CK pathway may be differential through interactions with different CK
307 pathway genes. *CKX2* and *CKX5* expression was increased in response to Torin2 or *Bc* infection, and the
308 combination of both *Bc* infection and Torin2 treatment did not further augment this increase in their
309 expression (**Figure S8C-D**).

310 ***CK and GA can affect TOR activity***

311 To further investigate a possible feedback regulatory mechanism between GA, CK and TOR, we
312 examined how CK and GA might modulate TOR activity. To do so, we analyzed the phosphorylation
313 status of S6 kinase 1 (S6K1), a conserved TOR substrate that has been previously used as an indication of
314 TOR activity in plants (Cao et al., 2019; Li et al., 2017; Liu et al., 2021; Song et al., 2022; Xiong & Sheen,
315 2012; Ye et al., 2022). We treated plants with 100 µM GA and CK, separately or simultaneously, and
316 tested the phosphorylation status of S6K1 after 4 hours, in both developing and mature leaves. As
317 shown in **Figure 6**, exogenous CK treatment reduced the phosphorylation of S6K1 by TOR in both
318 developing and mature leaves, suggesting that CK may negatively regulate TOR activity. By contrast, GA
319 treatment, as well as the GA and CK co-treatment, significantly increased the level of TOR-
320 phosphorylated S6K in developing and mature leaves in comparison to mock, suggesting that GA can
321 inhibit CK-mediated reduction of TOR activity.

322 We found that leaf developmental status affected disease resistance (**Figure 1**) and TOR inhibition-
323 mediated gene expression (**Figure 5**). Therefore, we tested whether TOR-inhibition mediated immunity
324 could be dependent on leaf developmental-stage. Interestingly, *Bc* susceptibility decreased with leaf age
325 (**Figure 1A, 7A**). While Torin2 reduced *Bc*-induced disease in L3 and L5, disease level of L8 treated with
326 Torin2 was similar to that observed in untreated leaves (**Figure 7B**). This finding demonstrates that the
327 extent of TOR-mediated immunity increases with leaf developmental age.

Given the results that both CK and GA affect TOR activity (**Figure 6**), and the fact that the ratio between CK and GA changes during leaf development (Weiss & Ori, 2007), we examined how TOR-kinase activity changes throughout leaf maturation. TOR activity was lowest in L8, the youngest, most developmentally morphogenetic leaf, and gradually increased with age, with the highest activity observed in the mature, no longer morphogenetic L3 (**Figure 7C-D**). This is in agreement with earlier report demonstrating that TOR's activity level increases with leaf age in Arabidopsis (Brunkard et al., 2020).

Discussion

The TOR pathway senses many different inputs such as changes in cellular energy status, hormone levels, light, and abiotic or biotic stresses, to regulate growth, metabolism, transcription, and translation (Dobrenel et al., 2016). Various reports have suggested that TOR balances between growth and defense responses in plants (Caldana et al., 2019; De Vleesschauwer et al., 2018; Margalha et al., 2019; Ryabova et al., 2019). Previous work, for example, indicated that in plants, TOR acts as a molecular 'switch' at the intersection of growth and defense, and activates cell proliferation and plant growth at the expense of defense (De Vleesschauwer et al., 2018). Consistent with this, we have recently reported that plant immunity and defense responses are enhanced upon TOR downregulation in tomato (Marash et al., 2022).

TOR mediates plant immunity

Despite the extensive research on the role of the GA signaling in plant growth and development, there has been limited study on its role in plant defense responses (Bari & Jones, 2009; Wang et al., 2013). Previous works in Arabidopsis show that treatment with GA increases the resistance to (hemi)biotrophic bacterial pathogens, but reduces resistance to necrotrophic pathogens (Navarro et al., 2008), whereas in

349 rice, it increases resistance to necrotrophic pathogens, and reduces resistance to (hemi)biotrophic
350 pathogens (de Vleesschauwer et al., 2012, 2016; Qin et al., 2013; Yang et al., 2008). This implies that the
351 effect of GA on plant immunity depends on both the host plant and type of pathogen involved (reviewed
352 in: De Bruyne et al., 2014). As shown here (**Figure 4**), GA likely affects plant immunity in a similar
353 manner in tomato and *Arabidopsis*. Our data suggest that immunity mediated by CK or GA requires
354 inhibition of TOR activity for execution. This could also explain why TOR inhibition did not further
355 enhance defense responses or *Bc* resistance when combined with CK application **(Figure S6)**. In tomato,
356 TOR inhibition (Marash et al., 2022) or high endogenous CK levels (Gupta et al., 2020), promote
357 pathogen resistance in a SA-dependent manner. Thus, a possible mechanism that could explain our
358 results is that TOR and CK signaling pathways coordinately regulate plant defense responses through the
359 modulation of SA. We hypothesize that the crosstalk between TOR and CK signaling could be involved in
360 the ability of plants to modify growth and developmental programs upon pathogen attack, and thus
361 enable a faster activation of plant defense responses. Conversely, the result that TOR downregulation
362 decreased *Bc*-sensitivity in high GA (or low CK) genotypes or upon exogenous GA treatment, whereas it
363 had no significant effect in low GA (or high CK) genotypes, suggests that TOR and the CK and/or GA
364 pathways might share signaling components.

365 ***TOR mediates the leaf developmental program***

366 Leaf development relies on the balance between GA, which promotes differentiation, and CK, which
367 promotes morphogenesis (Hay et al., 2005; Jasinski et al., 2005; Yanai et al., 2005). Thus, CK and GA
368 have a partial antagonistic role in leaf development (Bar et al., 2016; Fleishon et al., 2011). Generally,
369 GAs are considered as differentiation promoting hormones which help to complete developmental
370 programs and regulate the achievement of final organ forms. GA shortens the morphogenetic stage of
371 leaf development by promoting differentiation (Shwartz et al., 2016). The termination of the juvenile

372 phase is associated with an increase in the levels of endogenous GA. This suggests that GAs promote the
373 transition from a juvenile- or developing- state, to an adult- or differentiated- state (Porri et al., 2014) .
374 On the other hand, CKs are known to alter leaf development and morphology (Hay & Tsiantis, 2010;
375 Shani et al., 2010; Werner et al., 2003), and are regarded as factors that promote "juvenility" by
376 promoting morphogenesis and delaying differentiation and senescence (Shwartz et al., 2016). Dividing
377 tissues in the leaf have the highest levels of cytokinin, while bioactive gibberellins peak at transition
378 zone between the division and expansion zone (Nelissen et al., 2012).
379 It has been suggested that active growth and cell-cycle progression are required for the formation of the
380 hormonal axis, involving auxin and cytokinin, that is required for organ formation and patterning during
381 plant development (Du et al., 2018). Accordingly, several studies reported that TOR plays a role during
382 leaf development in *Arabidopsis*. For example, *TOR* downregulation has been shown to result in the
383 production of smaller leaves with fewer cells (Caldana et al., 2013) whereas *TOR* overexpression results
384 in the production of bigger leaves with larger cells (Deprost et al., 2007). Likewise, mutation in *AtLST8*, a
385 member of the TOR complex, results in a reduction in the number of leaves and in leaf size (Moreau et
386 al., 2012), and mutation in *AtRAPTOR1B*, another component of the TORC1 complex, stalls leaf initiation
387 (Anderson et al., 2005). By contrast, we did not observe any significant phenotypic alterations in the
388 tomato WT M82 cultivar upon *TOR* silencing. This could be ascribed to the different inhibition methods
389 used, or the different plant species. While TOR inhibition might affect the translation of the ectopically
390 expressed proteins in transgenic lines, given similar results achieved with several mutants, this would be
391 unlikely to explain our results, however, it should also be noted that our developmental analyses could
392 be limited by use of the VIGS system. Improved systems to comprehensively study plant development
393 upon TOR inhibition will no doubt emerge in the future.

Our work indicates that TOR is required for the developmental response to hormonal signals. Response to exogenous GA treatment, as well as the patterning of leaf organs programmed by CK and GA, were perturbed by TOR inhibition. These findings agree with a previous report demonstrating that mutants in *raptor*, a protein in the TOR complex, were less sensitive to exogenous GA treatment (Zhang et al., 2018). The increased leaf complexity in lines with a high CK/GA ratio, and decreased complexity in lines with a low CK/GA ratio, were both partially rescued to WT M82 levels as a result of *TOR* silencing (**Figure 3**). In general, TOR silencing partially rescued a variety of aberrant leaf developmental phenotypes (**Figures 3, S5**). This brings forth the notion that TOR mediates signals from additional hormones, or that TOR is responsible for the reduction to practice of a variety of cues and signals generated by the balance and crosstalk of several developmental hormones (Greenboim-Wainberg et al., 2005; Israeli et al., 2021). The signaling events that are transmitted from the CK/GA ratio and encode leaf complexity, are no longer properly transmitted or decoded when TOR is attenuated. Thus, TOR supports hormonal signal output, resulting in the typically observed leaf phenotypes. When TOR is inhibited, the signaling output from the aberrant hormonal balance in developmental mutants is no longer supported by TOR, resulting in milder phenotypes. These phenotypic changes could indicate that CK and GA distribution and/or signaling are altered in response to *TOR* silencing, or that factors which execute organ patterning downstream of hormonal cues are dependent on TOR status. It is therefore possible that TOR is required for the execution of a variety of cues that are integrated to form a cohesive leaf developmental program. Notably, TOR inhibition reduced CK signaling and increased GA signaling in developing leaves, and promoted CK signaling and reduced GA signaling in mature leaves (**Figure 5**). Due to the lethality of complete TOR inhibition, and the limitations of the VIGS system, we were not able to assess a full leaf developmental time course upon TOR inhibition. However, we would expect TOR inhibition early in leaf development to result in precocious differentiation and simpler leaves, while TOR inhibition in late development could potentially lengthen the morphogenetic window.

418 The TOR pathway has been implicated in the regulation of translation and ribosome biogenesis in
419 mammals and plants, and therefore its activity is tightly regulated (Pereyra et al., 2020). Interestingly,
420 proteome analysis of CK activity in *Arabidopsis* demonstrated extensive differential regulation of
421 ribosomal proteins in response to CK (Brenner & Schmülling, 2012). In another proteomic study, the
422 functional classification 'Ribosome biogenesis' was found to be strongly differential in response to CK
423 depletion or overproduction (Černý et al., 2013). Thus, the molecular mechanisms underlying the effect
424 of CKs on leaf development and morphology could potentially be mediated by changes in translational
425 processes due to differential regulation of ribosomal proteins. Horiguchi et al., 2011 demonstrated that
426 ribosomal proteins play a key role in *Arabidopsis* leaf development, supporting this notion. Thus, it is
427 likely that TOR is involved in the execution of CK-mediated signals, in both defense and development, by
428 its direct or indirect regulation of proteins required for the execution of these processes.

429 ***Immunity to B. cinerea depends on developmental status***

430 The phenomenon in which developing leaves show stronger resistance than developmentally mature
431 leaves (**Figures 1, 7**) has been described as "leaf-stage associated disease resistance" (Berens et al.,
432 2019; Develey-Rivière & Galiana, 2007; Xu et al., 2018). Zhang & Chen, 2009, for example, showed that
433 as tomato leaves age, they become more susceptible to diseases caused by *B. cinerea* and *Fusarium*
434 *oxysporum*. In *Arabidopsis*, it was proposed that this resistance is due to higher accumulation of SA in
435 young leaves (Zeier, 2005). Our results suggest that high CK or low GA signals prevent the increase in *B.*
436 *cinerea* susceptibility that normally occurs with developmental stage progression. As young organs have
437 a higher CK/GA ratio than mature organs, and since the CK/GA balance, rather than the content or signal
438 of each individual hormone was reported to execute leaf developmental functions (Fleishon et al., 2011;
439 Shani et al., 2006; Shwartz et al., 2016), it is possible that leaf developmental stage-related *B. cinerea*

susceptibility may also depend on the CK/GA balance. This idea corresponds to previous work demonstrating that altered GA levels were not able to prevent the age-related decrease in JA-mediated immunity (Mao et al., 2017). Therefore, disease resistance related to developmental status could potentially be attributed to the CK/GA balance, and further, be mediated by TOR in a manner similar to that we observed for leaf developmental processes. Thus, it emerges from our results that TOR activity supports GA-mediated processes and reduces disease resistance, while a decrease in TOR activity supports CK-mediated processes and increases disease resistance.

Increased B. cinerea resistance in young leaves could be a result of decreased TOR activity

TOR's low activity in young leaves (**Figure 7**) could account for their enhanced *Bc* resistance. The observation that CK application results in a decrease in TOR activation whereas GA application results in an increase in in TOR activation could potentially provide mechanistic insight for how resistance is affected by developmental-status. This indicates that resistance may be depended on TOR activity as well as the relationships between hormonal signals. Additionally, this implies the existence of a feedback mechanism that balances between TOR activity and these hormonal pathways. It is noteworthy that previous work in *Arabidopsis* cell suspensions revealed that kinetin and auxin both induced the phosphorylation of AtS6k (Turck et al., 2004), whereas the activation of *triticum aestivum* TOR (TaTOR) was induced during GA-triggered germination (Smailov et al., 2020). Our data demonstrate that the ability of CK to induce immunity is related to TOR status (**Figures 1, 7**), and that increased CK leads to a reduction in TOR activity (**Figure 6**). Our results align with those obtained in wheat, and differ from those obtained in *Arabidopsis*, possibly due to the use of cell suspensions, or due to differences among plant hosts.

<u>The involvement of TOR in growth-defense tradeoffs</u>

Recent studies demonstrating that growth and defense can be uncoupled suggest that resource reallocation toward immunity is not the sole factor of growth inhibition (Campos et al., 2016; Kliebenstein, 2016; Shchneider 2023). Phytohormones serve as clear mediators of the tradeoff between growth and defense, as they mediate development and defense responses (Berry & Argueso, 2022).

Leaves become more susceptible to necrotrophic diseases with age (**Figure 1**). This increased susceptibility appears to be largely influenced by the CK/GA ratio, which correlates with leaf developmental stage. Based on that observation, we speculate that this might be a mechanism by which old plants, in which the CK/GA ratio is very low, die and allow for the allocation of resources to their offspring. Thus, the change in CK/GA ratio in mature plants is translated into defense hormonal outputs that modulate plant immunity.

Following our findings, we propose a hypothetical model by which TOR modulates CK and GA signaling and acts as a mediator of both developmental and defense processes, potentially regulating development-defense trade-offs (**Figure 8**). Developing leaves have low TOR activity (Brunkard et al., 2020, **Figure 7**), a relatively high CK to GA ratio (Shwartz et al., 2016), and are resistant to *Bc*. Mature differentiated leaves have higher TOR activity, a lower CK to GA ratio, and are more sensitive to *Bc*. Our results suggest that developmental-status related resistance could depend on processes by which TOR transduces signals derived from the CK/GA balance. In mature leaves under standard growth conditions, TOR activity is high (the CK/GA ratio is relatively low), and morphogenesis is largely concluded. The "price" for this is relative disease susceptibility. When pathogens attack, CK increases and GA decreases (Meldau et al., 2012; Gupta et al 2020), and TOR becomes less active (Margalha et al., 2019), promoting disease resistance. The "price" for this disease resistance could be an arrest of growth, until the pathogen is vanquished. The relationship between TOR, GA and CK likely involves complex feedback mechanisms that are based on mutual regulation between these pathways, allowing for a gradual shift between growth and defense that should be addressed in future research.

TORs involvement in both development and defense poises TOR as a prime regulator of tradeoffs between these two important aspects of plant life. Here, we demonstrate that CK and GA can regulate TOR activity, while TOR is required for the interpretation of defense and developmental signals originating from the CK/GA balance. The interaction between TOR, GA and CK could potentially help plants mediate growth and defense tradeoffs to adapt to the environment, with TOR sensing integrating environmental cues and stresses with plant hormonal balances, potentially allowing gradual shifts between growth and defense as a mechanism regulating plant robustness and survival under changing environments and pathogen loads. The relationship between TOR, GA and CK appears to involve complex feedback mechanisms based on mutual regulation between these pathways. It will be interesting to investigate how different metabolic states and biotic and abiotic stresses alter the cross talk between CK, GA and TOR in the future.

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Author Contributions

MB and IM conceived and designed the study. IM, ML-M, RG, GA, NL, DN, AI and MB formulated the methodology and carried out the experiments. IM, ML-M, RG, GA, and MB analyzed the data. All authors contributed to the writing of the manuscript.

Competing Interests Statement

The authors declare no competing interests.

506 **Materials and methods**

507 Plant material and growth conditions

508 Plants were grown in soil (Green 332; Even-Ari Green, Ashdod, Israel) in a growth chamber set to long-
509 day conditions (16/8 light/dark) at 24°C, or in a greenhouse under natural day length conditions.

510 The genotypes used in this study are detailed in the following table. Promoter line selection and
511 hormone content are explained below.

512 **Table 1: Plant genotypes used in this study**

Genotype name	Source	Phenotype	References
S. lycopersicum cultivar M82		WT	
ga20ox3 null mutant Cas9 Knock out of the GA biosynthesis enzyme GA20OX3. M82 background line.	Prof. Naomi Ori	Short stature; increased leaf complexity.	This work
pTCSv2::3×VENUS Overexpression of VENUS driven by the synthetic two-component signaling sensor pTCSv2. M82 background line.	Bar lab	WT	(Bar et al., 2016; Steiner et al., 2020)
pBLS>>IPT7 ("*IPT*"): Transgenic overexpression of IsoPentenyl Transferase7 from the leaf *BLS* (Lifschitz et al., 2006) promoter. Elevated endogenous leaf levels of CK. M82 background line.	Prof. Naomi Ori	Highly complex rugose leaves; short stature; hirsutism; disease [n,b] resistance.	(Bar et al., 2016; Gupta et al., 2020, 2022; Gupta, Leibman-Markus, et al., 2021; Shani et al., 2010)
pFIL>>CKX3 ("CKX")- Transgenic overexpression of Cytokinin Oxidase3 from the leaf *FIL* (Bonaccorso et al., 2012) *promoter.* Decreased endogenous leaf CK content. M82 background line.	Prof. Naomi Ori	Highly simple thin leaves; lack of hairy trichomes; disease [n,b] susceptibility.	(Bar et al., 2016; Gupta et al., 2020, 2022; Shani et al., 2010)
clausa ("clau"): Recessive MYB TF mutant. High CK sensitivity coupled	Prof. Naomi Ori	Highly complex rugose leaves;	(Bar et al., 2016; Gupta et al., 2020,

with low CK content; low GA sensitivity coupled with increased amounts of pre-active GAs. M82 background line.		hirsutism; disease resistance [n,b].	2022; Shani et al., 2010)
pFIL>>GFP-PROΔ17: Transgenic overexpression of a mutated, un-processed version of the DELLA TF *Procera* from the leaf *FIL* promoter. Low GA signal. M82 background line.	Prof. David Weiss	Complex leaves. Disease resistance [n,b] (this work).	(Israeli et al., 2021; Nir et al., 2017)
procera: Recessive mutant in the DELLA TF *Procera*. ΔGRAS allele. High GA signal. M82 background line.	Prof. David Weiss	Simple leaves; tall stature. Disease susceptibility [n]/ resistance [b] (this work; resistance [b] previously reported in Arabidopsis).	(Livne et al., 2015; Navarro et al., 2008)

[n] necrotrophic

[b] hemi/biotrophic

Lines with altered GA signaling: the N'-terminal region of DELLA proteins contains the DELLA domain, which is required for the interaction with the GID1 receptor. Gain-of-function (GOF), dominant mutations in the DELLA domain block the interaction between DELLA and GID1 and prevent DELLA degradation. (Locascio et al., 2013; Murase et al., 2008). The C'-terminal region of DELLA interacts and represses multiple growth-promoting transcription factors (Locascio et al., 2013; Sun et al., 2012). Loss-of-function (LOF), recessive mutations in DELLA's C'-terminus are linked to constitutive GA responses (Achard et al., 2006, 2008; Nir et al., 2017). DELLA proteins promote Jasmonic Acid (JA) signaling and repress salicylic acid (SA) biosynthesis and signaling, promoting susceptibility to biotrophs and resistance to necrotrophs. A DELLA loss-of-function mutant (quadruple *della* mutant, lacking four out of the five *Arabidopsis* DELLA proteins) for example, was shown to be partially insensitive to gene induction by JA, whereas a DELLA gain-of function mutant *gai* (constitutively active dominant DELLA mutant) was shown to be more sensitive (Navarro et al., 2008). There is only one DELLA protein in tomato (Livne et al., 2015). We used the GA deficient mutant *ga20ox3* and the DELLA gain-of-function line *pFIL>>GFP-*

528 *PROΔ17* as lines with low GA signaling, and the DELLA loss-of-function mutant *procera*$^{\Delta GRAS}$ as a line with
529 high GA signaling. The gain-of-function mutant *pFIL>>GFP-PROΔ17* expresses the stable DELLA mutant
530 protein *PROΔ17*, which lacks the DELLA domain. GA responses are constitutively suppressed in this
531 mutant, resulting in a severe GA-deficient phenotype and GA insensitivity (Nir et al., 2017). *pro*$^{\Delta GRAS}$ is a
532 *procera* null mutant that lacks the entire C-terminal region of DELLA and exhibits enhanced GA
533 responses (Livne et al., 2015). The GA deficient mutant *ga20ox3* was generated using CRISPR/CAS9
534 essentially as described in Israeli et al., 2019, using the same constructs and methodology. gRNA primers
535 are detailed in Table S1.
536 Lines with altered CK signaling: We used the high endogenous CK content genotype *pBLS >> IPT7*, which
537 overexpresses the CK biosynthesis gene *ISOPENTYL TRANSFERASE 7 (IPT7)* and the low CK content
538 genotype *pFIL>> CKX3*, which overexpresses the CK degrading enzyme *CKX OXIDASE3 (CKX3)* (Shani et
539 al., 2010). We also used the increased CK sensitivity and decreased GA sensitivity mutant *clausa*.
540 *CLAUSA* (*CLAU*) is a MYB transcription factor that promotes the transition from morphogenesis to
541 differentiation by negatively affecting CK signaling and promoting GA signaling (Bar et al., 2016; Israeli et
542 al., 2021).

543 Rationale for genotype selection and reported CK and GA content in different genotypes
544 We used transgenic lines expressing genes of interest from the leaf specific promoters *FIL* and *BLS*. In
545 tomato, the Arabidopsis *FIL* (filamentous flower) promoter drives expression throughout leaf primordia,
546 starting from initiation (first plastochron), in initiating leaflets, and the abaxial side of the leaves. The *BLS*
547 promoter drives expression later in leaf development, in primordia from about the fourth plastocrhon
548 stage, and in young leaves (Lifschitz et al., 2006). Plants overexpressing *IPT* have been shown to contain
549 increased levels of cytokinin many times, in various plant species (Márquez-López et al., 2019; Redig et
550 al., 1996; Smigocki & Owens, 1988). Strongly increasing CK levels throughout the plant also led in some

551 cases to undesirable phenotypes such as reduced apical dominance, increased lateralization, late
552 flowering, and infertility. Therefore, tissue specific promoters were used to express IPT (Bartrina et al.,
553 2011; Shani et al., 2010; Smigocki et al., 1993). This eliminated the undesired effects, and allowed plants
554 to be viable and fertile, though mild effects of increased CK were occasionally observed in the non-
555 targeted organs as well. Plant overexpressing *CKX* have been shown to contain reduced levels of
556 cytokinin many times, in various plant species. *CKX3* overexpression was specifically shown to cause
557 reduction in CKs in several works (Nishiyama et al., 2011; Reid et al., 2016). Reducing cytokinin levels
558 with *CKX* overexpression led to stunting in Arabidopsis when expressed from the strong 35S promoter
559 (Vercruyssen et al., 2011), but overexpression of *CKX3* in tomato had relatively minimal phenotypes
560 under optimal conditions (Farber et al., 2016). The lines we used, which overexpress Arabidopsis *AtIPT7*
561 or *AtCKX3* from the leaf specific promoters *pFIL* and *pBLS*, in tomato cv M82, have normal early
562 development and are viable (Shani et al., 2010). *pFIL>>IPT7* is mostly infertile, which is why we used
563 *pBLS>>IPT7*, that has a milder phenotype, due to the expression being later in development, and normal
564 fertility (Shani et al., 2010). *pFIL>>CKX3* and *pFIL>>GFP-PROΔ17* are both viable and fertile. We used
565 lines with expression driven from the *FIL* promoter in both these cases because lines driven from the *BLS*
566 promoter had mild to undetectable leaf phenotypes. Thus, *BLS* was used only in the case of IPT, to avoid
567 pleiotropic effects. CK and GA content were previously analyzed in the tomato *clausa* mutant. *clausa* is
568 highly CK sensitive and GA insensitive, and displays meristematic and leaf phenotypes similar to those of
569 overexpression of *IPT*. In terms of hormonal content, *clausa* has a significant reduction in the content of
570 many CK compounds, and a significant increase in several active GA precursors (Israeli et al., 2021).

571 <u>Torin2 and WYE132 treatments</u>

572 It was previously demonstrated that Torin2 and WYE132 are effective and specific inhibitors of TOR (Li
573 et al., 2017; Marash et al., 2022; Montané & Menand, 2013). Torin2 (SML1224 Sigma-Aldrich) or

574 WYE132 (PZ0321 Sigma-Aldrich), were applied to detached tomato leaves through the petiole for 24
575 hours prior to pathogen inoculation (both inhibitors), defense response quantification (only Torin2), or
576 RNA preparation (only Torin2). For both inhibitors, a 10 mM stock solution was prepared in
577 concentrated DMSO (P0037 SIGMA-Aldrich) and diluted to 2 µM in water. Mock leaves were treated
578 with water containing 1:5000 of DMSO. Torin2 was used this study in concentration of 2 µM based on
579 previous studies (Marash et al., 2022; Ye et al., 2022).

580 Hormone treatments

581 For CK treatment (Figure S4), plants were sprayed with 100 µM 6-benzyl purine (6-BAP, Sigma-Aldrich)
582 or Mock solution 24h prior to analysis. The stock solution was prepared in NaOH and diluted with water.
583 Similarly diluted NaOH in water served as Mock.
584 For GA treatment (Figure 4), GA_3 (Sigma-Aldrich) in the indicated concentrations was dissolved in
585 ethanol and applied by spraying three times a week for two weeks. The stock solution was prepared in
586 ethanol and diluted with water.
587 For assessing TOR activity following hormonal treatment (Figure 6), 100 µM 6-BAP or GA_3, or both, were
588 sprayed on developing leaves or mature L5, 4h prior to protein extraction. Mock treatments were dilute
589 NaOH for CK, or ethanol with Tween 20 (100 µl/L) for GA, as described above.

590 Virus Induced Gene Silencing (VIGS)

591 VIGS was performed as previously described (Liu *et al.*, 2002). The *SlTOR* silencing construct was
592 generated as previously described in Marash *et al.*, 2022. The TRV2:TOR construct, as well as an empty
593 TRV RNA2 for control, and the pTRV1 vector were introduced into *A. tumefaciens* strain
594 GV3101::pMP90. The cultures were adjusted to OD_{600}= 0.2 and TRV RNA1 was mixed in at a ratio of 1:1

595 with RNA2 (either empty or *TRV2:TOR*) in infiltration buffer, and infiltrated into cotyledons of 10-day-old
596 seedlings. Analyses were subsequently conducted when plants reached 6 weeks of age.

597 Pathogenesis assays

598 *Botrytis cinerea* (*Bc*) pathogenicity assays were performed as previously described (Gupta et al., 2020b).
599 Briefly, inoculum of *Bc* isolate Bcl16 was maintained on potato dextrose agar (PDA; Difco) plates in an
600 incubator at 22°C. Agar discs with a diameter of 0.4 cm were then pierced from colony margins and used
601 to inoculate detached leaves. Inoculated leaves were kept in a humid chamber at 22°C under long-day
602 conditions. Necrotic lesion size was measured 2-3 days post inoculation using ImageJ. For *Bc* inoculation
603 prior to RT-qPCR analysis, *Bc* spores were collected from 21-day-old culture in 5 ml of 1 mg/ml glucose
604 and 1 mg/ml K_2HPO_4. The spore suspension was vortexed, filtered through cheesecloth to remove
605 mycelial fragments and used to inoculate tomato leaves by spraying. Mock plants were sprayed with
606 similar concentrations of glucose and K_2HPO_4. RNA was prepared 24 h after *Bc* inoculation.
607 *Xanthomonas campestris pv. vesicatoria (Xcv)* was grown at 28°C in Luria Bertani (LB) broth overnight
608 supplemented with Rifampicin (10 µg/ml) (Sigma), diluted to concentration of OD_{600}=0.0002 in 10mM
609 $MgCl_2$, and used to pressure-infiltrate L5 of silenced plants with a 1-ml needleless syringe. Disease was
610 assessed by measuring the water-soaked lesion area as previously described (Teper et al., 2018), 10 days
611 after inoculation.
612 *Oidium neolycopersici (On)* was continuously maintained by randomly placing healthy plants alongside
613 pre-infected plants in a dedicated chamber, and allowing them to be inoculated through air circulation.
614 New plants were introduced every two weeks, upon which wholly covered chlorotic plants were
615 discarded. For inoculation, diseased leaves with 80% On coverage were shaken above L5 of each plant.
616 Two infected leaves were used for each individual. Disease severity was calculated based on the
617 percentage of the leaf surface covered with powdery mildew symptoms 10-14 dpi.

ROS production measurement

ROS measurement was carried out as previously described by (Leibman-Markus et al., 2017). 0.5 cm diameter leaf discs were collected, and each disc was incubated in 250µL distilled water in a 96-well plate (SPL Life Science) at room temperature with gentle shaking. After 4 hours, the water was removed and 50µL of distilled water were added. Right before measurement, 100µL of distilled water with or without 1µM flg22 (PhytoTechLabs #P6622) were added. Light emission was measured using a luminometer (GloMax® Discover, Promega, United States).

Ion leakage (conductivity) measurement

Conductivity was measured according to (Leibman-Markus et al., 2017). 0.9 cm diameter leaf discs were harvested and washed with distilled water for 3 hours in a 50 mL tube. For each sample, five discs were placed in a 10-flask with 1 ml of distilled water, with 2µM Torin2, 6-BAP, or DMSO, for 48 hours at room temperature with gentle shaking. After incubation, 1.5 mL of distilled water were added to each sample, and conductivity was measured using a conductivity meter (AZ® Multiparameter pH/Mv/Cond./Temp Meter 86505, Taiwan).

RNA extraction and RT-qPCR

for RNA extraction, either five 0.9 cm diameter leaf discs were harvested from L5 of 6-week-old plants (Figure 5B, D, F, H) or whole shoots of of developing leaves (m+6) of 3-week-old seedlings with four true leaves (Figure 5A, C, E, G) were used. Isolation of total RNA was performed according to the TRI reagent (Sigma-Aldrich) procedure, with application of DNAse (EN0521 ThermoFisher) to remove genomic DNA. 1 µg of RNA was used for cDNA synthesis using Maxima reverse transcriptase (ThermoFisher). RT-qPCR assays were conducted with Power SYBR Green Mix (Life Technologies), using specific primers

639 (Supplemental Table 1) in a Rotor-Gene Q machine (Qiagen). Standard curves were achieved by dilutions
640 of one cDNA sample. Relative expression was quantified by dividing the expression of the relevant gene
641 by the geometric mean of the expression of the following normalizer genes: for developing leaves: *RPL8*
642 (Solyc10g006580), *EXP* (Solyc07g025390), and *CYP* (Solyc01g111170), and for mature leaves, *RPL8*, *CYP*,
643 and *Actin* (Solyc11g005330). All primer pairs had efficiencies in the range of 0.97-1.03. All the primers
644 used for RT-qPCR are listed in Table S1.

645 Protein purification and Western Blotting

646 For protein purification from mature leaves, whole tomato leaves (Figures 6C-D and 7) were harvested
647 from 6-week-old plants with nine true leaves, and ground in liquid nitrogen. 150 mg ground tissue was
648 used. For developing leaves (Figure 6A,B), 25mg of tissue was collected from shoots of developing leaves
649 (m+6) of 3-week-old seedling with four true leaves. Each sample of developing leaves was composed
650 from a total of 10-12 different plants. The tissues were then ground in liquid nitrogen with 3 volumes of
651 extraction buffer (100mM MOPS pH 7.6, 100mM NaCl, 40mM ß-MeOH, 5% SDS, 10% Glycerol, 4mM
652 EDTA, 2mM PMSF and Pphosphatase Inhibitor (Sigma)), boiled for 5 minutes at 95°C and centrifuged at
653 10,000 rpm for 10 minutes, to remove cell debris. Samples of equal volume were separated on 15% SDS
654 acrylamide gels, transferred to nitrocellulose membranes (Protran, #10401380), stained with Ponceau
655 red as loading and transfer control, and blocked with 3% skimmed milk in Tris buffered saline (TBS) with
656 1% Tween20 for one hour at room temperature with gentle shaking. Membranes were probed with
657 Anti-S6K1 p-Thr449 polyclonal antibody (AB-ab207399, Abcam,1:750), Anti-S6K1/2 (GRS-AS121855,
658 Agrisera, 1:500), or Anti-ACTIN (GRS-AS132640, Agrisera, 1:1000) overnight at 4°C. IgG HRP-conjugated
659 goat-anti-rabbit (AB-ab205718, Abcam, 1:10,000) was used as secondary antibody. Chemiluminescence
660 was observed using Elistar Supernova as substrate (Cyanagen, #XLSE2) and images of protein bands

were acquired and quantified using the Alliance UVITEC software. Phosphorylation status of S6K1 was tested 4 h after GA and CK treatment based on a previous study quantifying the decrease in TOR activity upon inhibition (Upadhyaya et al., 2020).

Statistical analysis

All data are presented as average ±SEM, or as boxplots showing minimum to maximum values, with the box representing inner quartile ranges and the whiskers representing outer quartile ranges. Data sets were analyzed for normality using the Shapiro-Wilk test. For non-Gausian distributed samples, differences between two groups were analyzed for statistical significance using a Mann-Whitney U test, and differences between three groups or more were analyzed using Kruskal-Wallis ANOVA with Dunn's post hoc test. For normally distributed samples, differences between two groups were analyzed for statistical significance using a two tailed t-test, with Welch's correction for samples with unequal variances, where appropriate. Differences among three groups or more were analyzed for statistical significance using one-way ANOVA. Regular ANOVA was used for groups with equal variances, and Welch's ANOVA for groups with unequal variances. When a significant result for a group in an ANOVA was returned, significance in differences between the means of different samples in the group was assessed using a post-hoc test. Tukey's or Bonferroni's tests were employed for samples with equal variances, and Dunnett's test was employed for samples with unequal variances. All statistical analyses were conducted using Prism9™.

Data availability Statement

The authors declare that the data supporting the findings of this study are available within the paper and its Supplementary information files. Raw data is available from the corresponding author upon reasonable request.

683 **Supplementary data**

684 **Figure S1:** TOR inhibition mediated disease resistance depends on the CK/GA balance- additional results.

685 **Figure S2:** *TOR* silencing mediated disease resistance depends on the CK/GA balance- VIGS results.
686
687 **Figure S3:** TOR inhibition mediated disease resistance depends on the CK/GA balance- ROS kinetics.
688
689 **Figure S4:** TOR inhibition mediated disease resistance to *X. campestris p. vesicatoria* and *O. neolycopersici*
690 depends on the CK/GA balance.
691
692 **Figure S5:** TOR is required for the execution of developmental cues in the leaf.
693
694 **Figure S6:** TOR inhibition does not augment CK-mediated disease resistance.
695
696 **Figure S7:** *TOR* silencing by VIGS reduces CK response in the meristem and developing leaf primordia.
697 **Figure S8:** TOR inhibition and *Bc* infection have differential effects on CK pathway genes.
698
699 **Table S1:** Primer pairs used in this work.

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Figure legends

Figure 1: Disease susceptibility depends on leaf age and hormonal balance

A: Different leaves as indicated from *S. lycopersicum* cv. M82 5 week old plants were infected with *B. cinerea*. Asterisks denote staistical significance among indicated samples in Welch's t-test N>35, ****p<0.0001.

B: The fifth leaf of *S. lycopersicum* 5 week old plants of altered CK/GA genotypes: increased CK content *pBLS>>IPT7* ("*IPT*"), decreased CK content *pFIL>>CKX3* ("*CKX*"), increased CK sensitivity and decreased GA sensitivity *clausa* mutant ("*clau*"), decreased GA content mutant ("*ga20ox*"), decreased GA signaling *pFIL>>proΔ17* ("*proΔ17*"), increased GA signaling *procera* ("*pro*") and their WT background M82, were infected with *B. cinerea* mycelia from a 72h old-culture. Different letters indicate staistically significant differences among indicated samples in Welch's ANOVA with Dunnett's post hoc test, N>20, p<0.018.

C: Different leaves as indicated from *S. lycopersicum* 5 week old plants of altered CK/GA genotypes: increased CK content *pBLS>>IPT7* ("*IPT*"), decreased CK content *pFIL>>CKX3* ("*CKX*"), increased CK sensitivity and decreased GA sensitivity *clausa* mutant ("*clau*"), decreased GA signaling *pFIL>>proΔ17* ("*proΔ17*"), increased GA signaling *procera* ("*pro*") and their WT background M82, were infected with *B. cinerea* mycelia from a 72h old-culture. Asterisks denote staistical significance among indicated samples in Welch's ANOVA with Dunnett's post hoc test, N>13, ****p<0.0001, ns- non significant.

Bars represent Mean ±SEM, all points shown.

Figure 2: TOR inhibition mediated disease resistance depends on the CK/GA balance
S. lycopersicum plants of altered CK/GA genotypes: increased CK content *pBLS>>IPT7* ("*IPT*"), decreased CK content *pFIL>>CKX3* ("*CKX*"), increased CK sensitivity and decreased GA sensitivity *clausa* mutant ("*clau*sa"), decreased GA content mutant ("*ga20ox*"), decreased GA signaling *pFIL>>proΔ17* ("*proΔ17*"), increased GA signaling *procera* ("*pro*") and their WT background M82, were treated with Mock (1:5000 DMSO in DDW), or 2 µM Torin2. Plants were challenged with *B. cinerea (Bc)* mycelia from a 72h old-culture 24 h after treatment.

A: *Bc* necrotic lesion size. Asterisks indicate statistically significant disease reduction upon Torin2 treatment when compared with Mock treatment. Different letters indicate statistically significant differences among samples, upper case letters for Mock treated genotypes and lower case letters for samples treated with Torin2, in a one-way ANOVA with a Tukey post hoc test, N>12, p<0.044 (***p<0.001, ****p<0.0001, ns- non significant). Experiments were repeated 6 independent times.

B: Representative *Bc* infected leaf images.

C: Plants were challenged with the immunity elicitor flg-22 (1 µM) 24 h after Torin2 treatment. ROS production was measured immediately after flg-22 application every three minutes, using the HRP-luminol method, and expressed as Relative Luminescent Units (RLU). Average total RLU per treatment, expressed as % of M82 control, is plotted.
D: Conductivity as a results of wounding was measured 24 h after Torin2 treatment.
Bars represent mean ±SEM, all points shown. Experiments were repeated 3 independent times.
C, D: Asterisks indicate statistically significant increases in ROS production or conductivity upon Torin2 treatment when compared with Mock treatment. Different letters indicate statistically significant differences among samples, upper case letters for Mock treated genotypes and lower case letters for samples treated with Torin2 in Kruskal-Wallis ANOVA with Dunn's post-hoc test, N>20, p<0.0001.

Figure 3: The CK/GA balance governs effects of *TOR* silencing on leaf development
S. lycopersicum plants of altered CK/GA genotypes: increased CK content *pBLS>>IPT7* ("*IPT*"), decreased CK content *pFIL>>CKX3* ("*CKX*"), increased CK sensitivity and decreased GA sensitivity *clausa* mutant ("*clau*"), decreased GA content mutant ("*ga20ox*"), decreased GA signaling *pFIL>>proΔ17* ("*PROΔ17*"), increased GA signaling *procera* ("*pro*") and their WT background M82, were *TOR*-silenced using VIGS. 4 weeks after silencing, leaf complexity was quantified by counting the leaflets on leaves 4 (**A, B**), and height was measured (**C**). Experiment was conducted 3 times. Bars represent Mean ±SEM, all points shown. Asterisks indicate statistically significant changes in leaf complexity (B) or plant height (C) upon *TOR* silencing, and different letters indicate statistically significant differences among samples, upper case for control-silenced and lower-case for TOR-silenced, in Welch's ANOVA with Dunnett's post hoc test, or in student's t-test with Welch's correction. **B:** N>5 individual plants, **C:** N>8 individual plants. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, ns- non significant.

Figure 4: *TOR* silencing affects GA response
S. lycopersicum cv M82 plants were *TOR*-silenced using VIGS. One week after silencing, mock and *TOR*-silenced plants were treated with indicated concentration of GA3, three times a week, for 2 weeks, by spraying. 4 weeks after silencing, plants were challenged with *B. cinerea (Bc)* mycelia from a 72h old-culture (**A**), height was measured (**B**), and leaf complexity was quantified by counting the leaflets on

leaves 5 (**C**) and 6 (**D**). Experiment was conducted 3 times. Bars represent Mean ±SEM, all points shown. Asterisks indicate statistically significant changes in B. cinerea disease (**A**), plant height (**B**), or leaf complexity (**C-D**) upon *TOR* silencing, and different letters indicate statistically significant differences among samples, upper case for control-silenced and lower-case for TOR-silenced, in Welch's ANOVA with Dunnett's post hoc test (**A**), one-way ANOVA with Bonferroni's post hoc test (**B**), or in student's t-tests with Welch's correction (**C-D**). **A:** N=30, **B:** N=17, **C:** N=5, **D:** N>3. *p<0.05, **p<0.01, ns- non significant.

Figure 5: *TOR* inhibition alters CK and GA pathway gene expression and reduces CK response in the meristem of young shoots

A-H: Gene expression analysis of the indicated CK (A-D) and GA (E-H) pathway genes, with and without Torin2 (2 µM) treatment, was measured by RT-qPCR. 1:5000 DMSO in DDW served as Mock. Relative expression was calculated using the geometric mean of the gene copy number obtained for three reference genes, and normalized to the expression following Mock treatment. Developing leaves consisted of 3- week-old shoot apexes with 6 youngest primordia, mature leaves the fifth leaf of 6-week-old plants. The following normalizer genes were used: for developing leaves: *RPL8* (Solyc10g006580), *EXP* (Solyc07g025390), and *CYP* (Solyc01g111170), and for mature leaves, *RPL8*, *CYP*, and *Actin* (Solyc11g005330). Analysis was conducted on 6 individual plants. Boxplots represent inner quartile ranges (box), outer quartile ranges (whiskers), median (line in box). Asterisks indicate significant differential regulation upon Torin2 treatment in Welch's t-test comparing each gene, *p<0.05, **p<0.01, ***p<0.001, ns- non significant.

A-B: Tomato response regulators (TRRs) and CK oxidases (CKXs). C-D: Iso-pentenyl transferases (IPTs) and lonely-guy CK activating enzymes (LOGs). E-F: GA20, GA2, and GA3 oxidases. G-H: GA biosyntesis upstream enzymes (KA, KO, KAO) and responsive genes (GAST1, PRO).

I-L: *S. lycopersicum* cv. M82 10 day-old seedlings expressing VENUS driven by the cytokinin responsive promoter TCSv2 were treated with Torin2 (2 µM) or Mock (1:5000 DMSO in DDW) for 48 h.

I Typical Mock treated and Torin2 treated shoots are depicted. Images captured under identical conditions. The meristem (m), second (P2) third (P3) and fourth (P4) youngest leaf primordia are indicated. Bar- 1000 µM.

TCSv2 driven total Venus fluorescence in the meristem (**I-K**) or leaf primordia (P1-P4) (**I-L**) was measured as corrected total fluorescence (CTF), in images captured under identical conditions. Boxplots represent inner quartile ranges (box), outer quartile ranges (whiskers), median (line in box), all points shown. Asterisks indicate significant TCSv2 signal reduction upon Torin2 treatment in an unpaired two-tailed *t*-test, N>7, *p<0.05.

Figure 6: CK and GA affect TOR activation

S. lycopersicum cv. M82 6-week-old plants were treated with Mock (10 µM NaOH), 10 µM of the CK 6-benzylaminopurine (6-BAP), or 10 µM of GA3. 24 h after treatment, total cellular proteins were prepared from developpoing (**A-B**) and mature (**C-D**) leaves. Developing leaves consisted of 3-week-old shoot apexes with 6 youngest primordia (10-12 plants per biological repeat), and the fifth leaf of 6 individual plants 6-week-old plants was used for mature leaves. TOR activation was expressed as the ratio between phosphorylated S6K and total S6K, detected using specific antibodies. Actin was detected as an additional control.

Experiment was repeated two independent times, A-B: N=8, C-D: N=6. Boxplots represent inner quartile ranges (box), outer quartile ranges (whiskers), median (line in box), mean ("+" sign). Different letters

indicate statistically significant differences among samples in a Mann Whitney U test, A: $p<0.045$, C: $p<0.0019$.

Figure 7: *TOR* activity and *TOR* inhibition mediated disease resistance depend on leaf developmetal stage

A: Different leaves as indicated from *S. lycopersicum* cv. M82 5 week old plants were infected with *B. cinerea*. Asterisks denote staistical significance among indicated samples in Welch's ANOVA with Dunnett's post hoc test, N>35, ****$p<0.0001$. Box plots represent inner quartile ranges (box), median (line in box), and outer quartile ranges (whiskers), all points shown.

B: Different leaves as indicated from *S. lycopersicum* cv. M82 5 week old plants, were treated with Mock (1:5000 DMSO in DDW), or 2 µM Torin2. Plants were challenged with *B. cinerea (Bc)* mycelia from a 72h old-culture 24 h after treatment. Bars represent mean ±SEM, all points shown. Experiments were repeated 3 independent times. Asterisks indicate statistically significant decreases in B. cinerea infection upon Torin2 treatment as compared with Mock treatment, and letters indicate statistically significant differences among samples, upper case letters for Mock treated genotypes and lower case letters for samples treated with Torin2, in Welch's ANOVA with Dunnett's post-hoc test, N>40, *$p<0.05$. Percentage of disease reduction is indicated above asterisks.

C-D: Total cellular proteins were preapared from the indicated leaves of *S. lycopersicum* cv. M82 5 week old plants. TOR activation was expressed as the ratio between phosphorylated S6K and total S6K, detected using specific antibodies. Actin was detected as an additional control. Experiment was repeated 4 times, N=8. Boxplots represent inner quartile ranges (box), outer quartile ranges (whiskers), median (line in box), mean ("+" sign), all points shown. Asterisks indicate statistically significant differences among indicated samples in One-way ANOVA with Tukey's post hoc test, **$p<0.01$, ****$p<0.0001$. D: Representative blots.

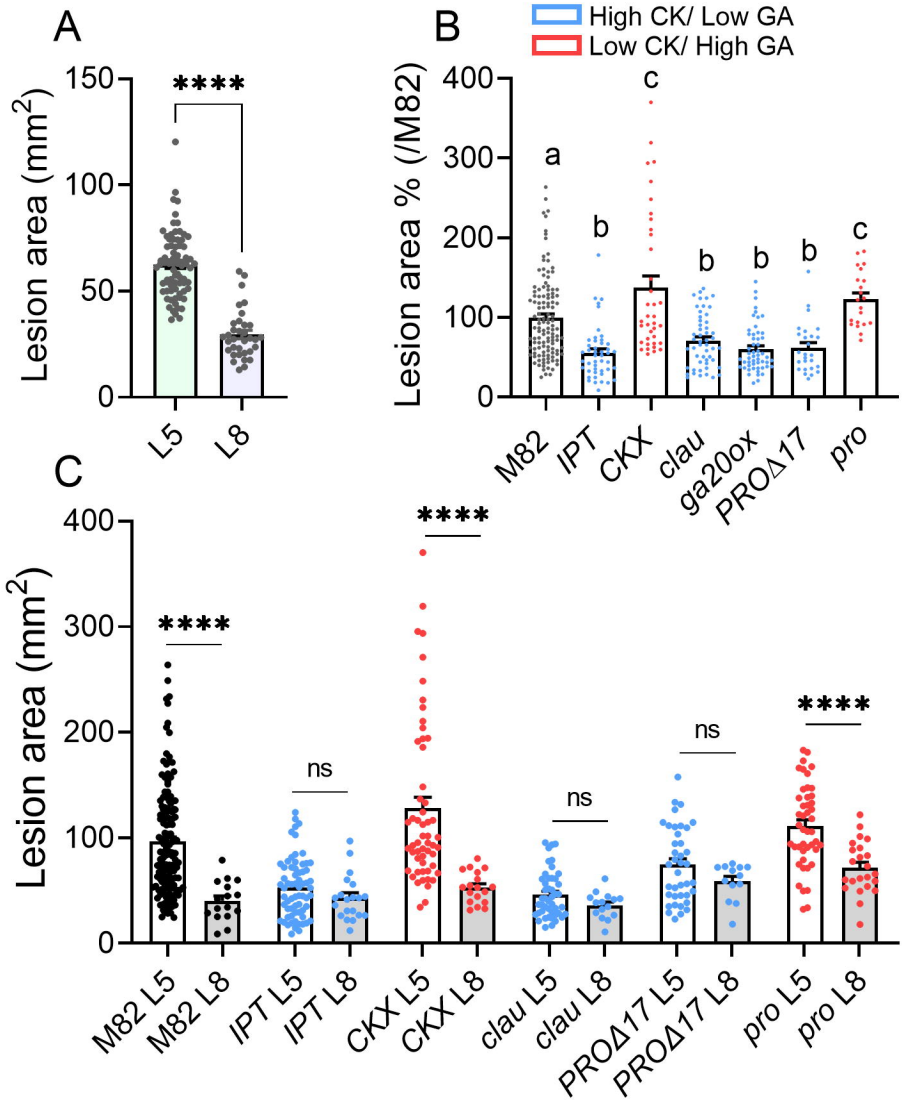
Figure 8: Model describing the interplay between the CK/GA balance and *TOR* in the regulation of development and defense cues.

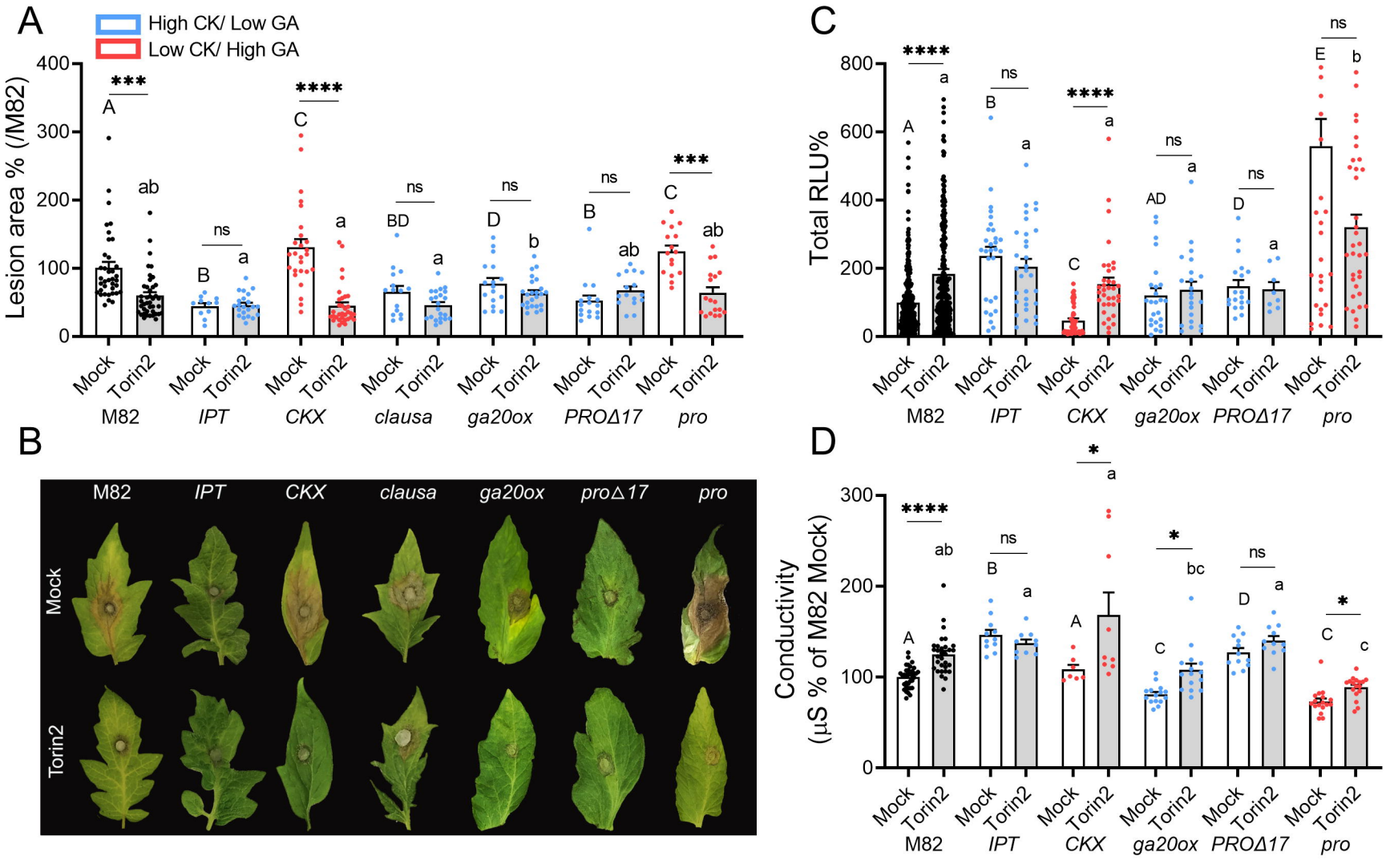
Cytokinin promotes both moprhogenesis and defense. Balanced CK/GA levels are required to achieve "normal" developmental patterning and disease resistance (**Fig.s 1-3**).

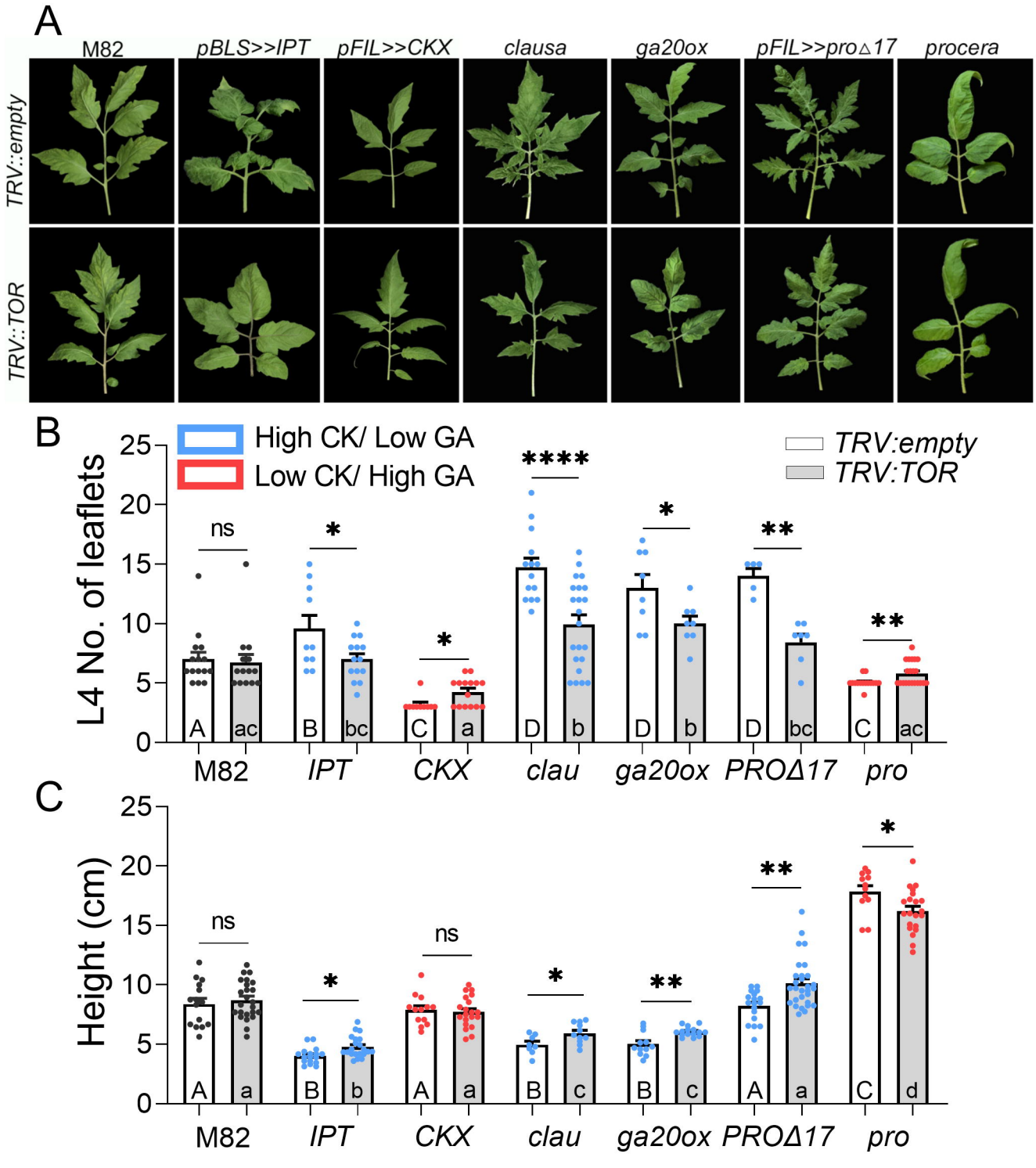
In young leaves undergoing morphogenesis, CK signaling is high and TOR activity is low (**Fig.s 5, 7**), resulting in decreased pathogen susceptibility (**Fig. 1**). As leaves mature developmentally, CK signals are reduced and GA signals increase, the leaf morphogenetic potential declines as it differentiates, and TOR activity and pathogen suceptibility increase (**Fig.s 1, 7**).

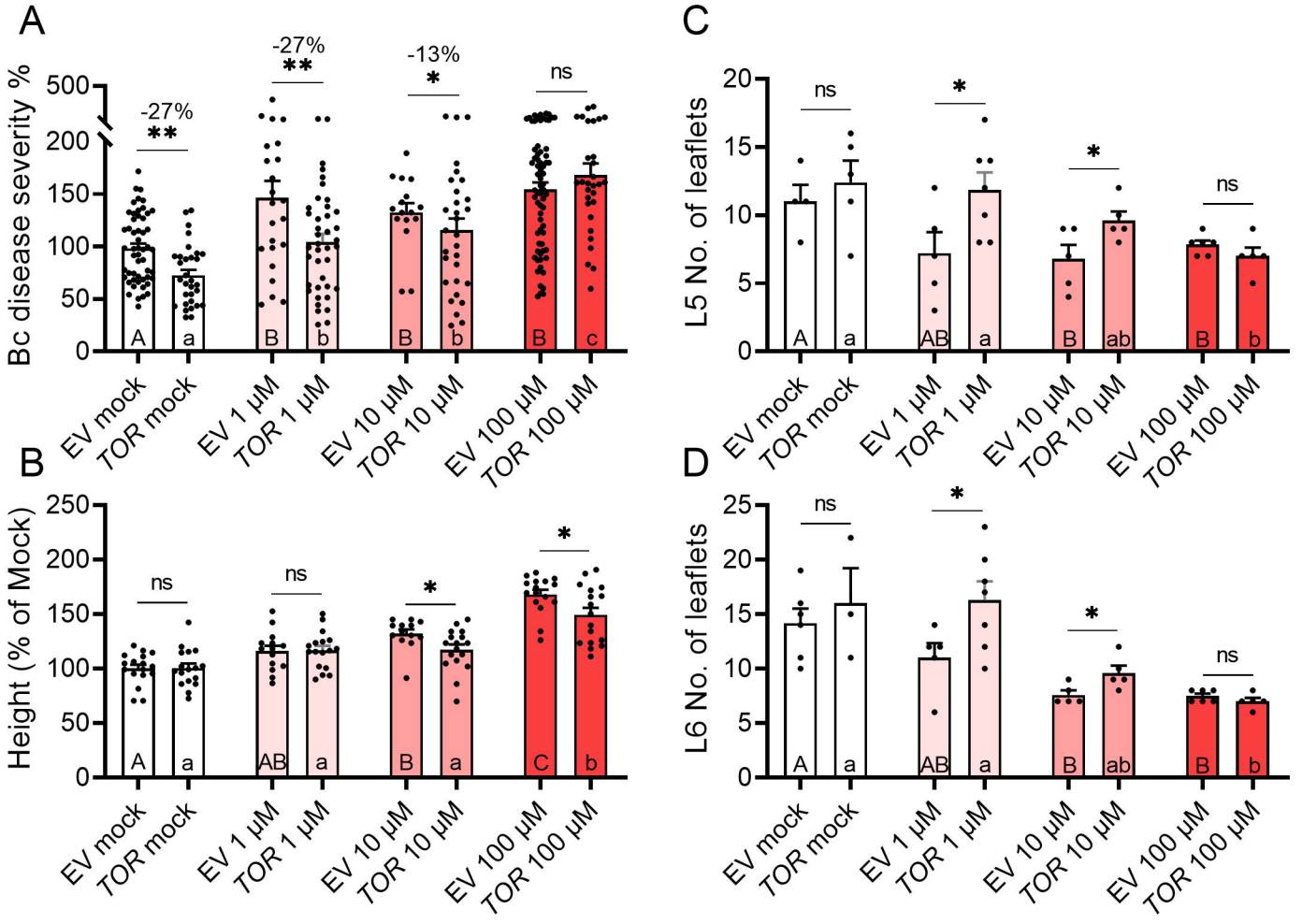
The CK/GA balance underlies both the morphogenetic potential and the disease susceptibility of the leaf. Inhibition of TOR results in rescue of altered CK/GA balances, partially restoring baseline developmental patterning and disease resistance (**Fig.s 3, 7**), and suggesting that TOR mediates these develpmental and defense cues originating from the CK/GA balance.

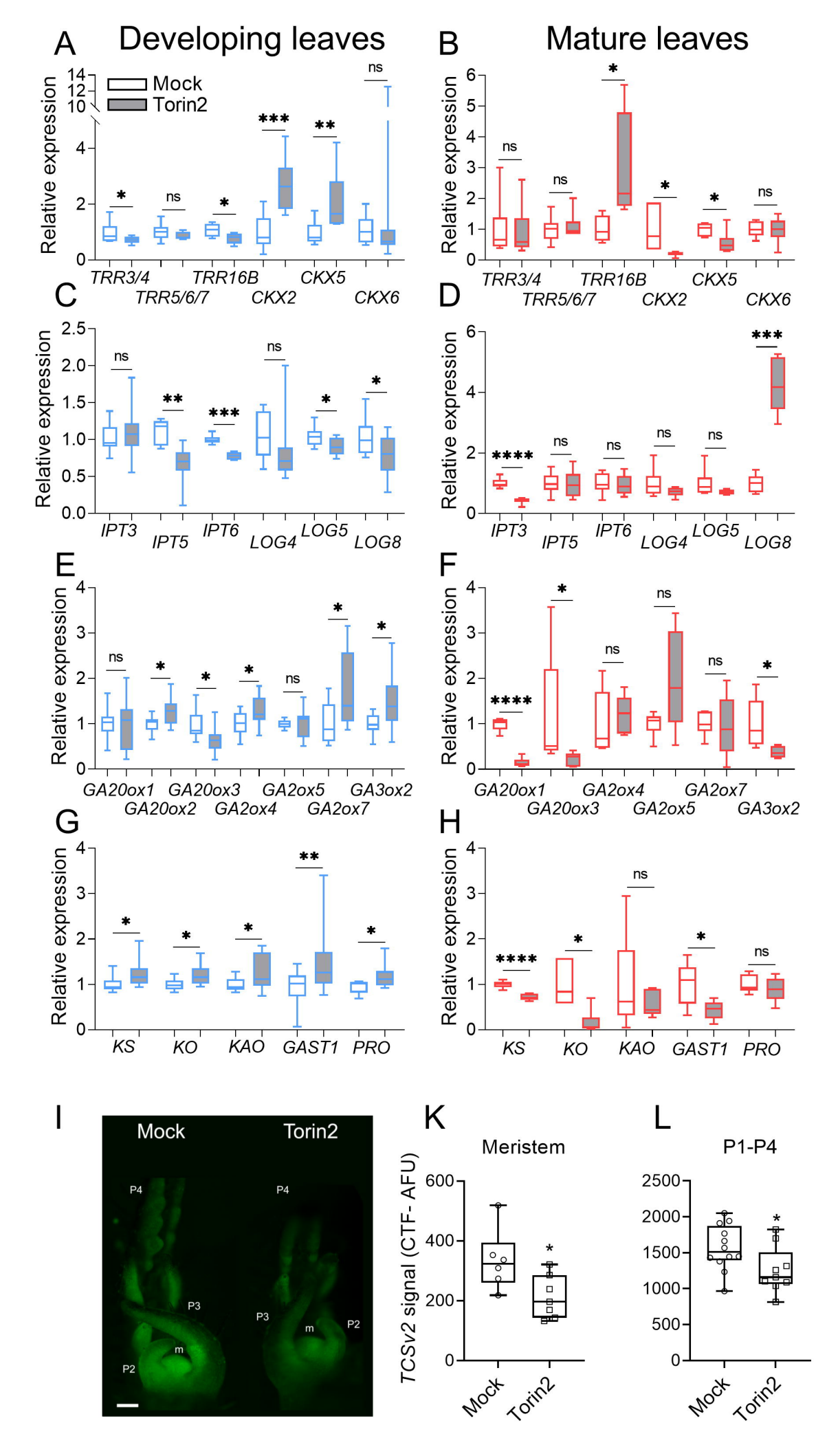
In the tradeoff between development and defense, high CK can cause downregulation of TOR (**Fig. 6**), resulting in a shift towards defense (**Fig.s 1-2**), while high GA results in up-regulation of TOR activity (**Fig. 6**), and increased disese susceptibility (**Fig. 4**). The switch between development and defense may be modulated by cross-talk between environmental sensing and TOR status.

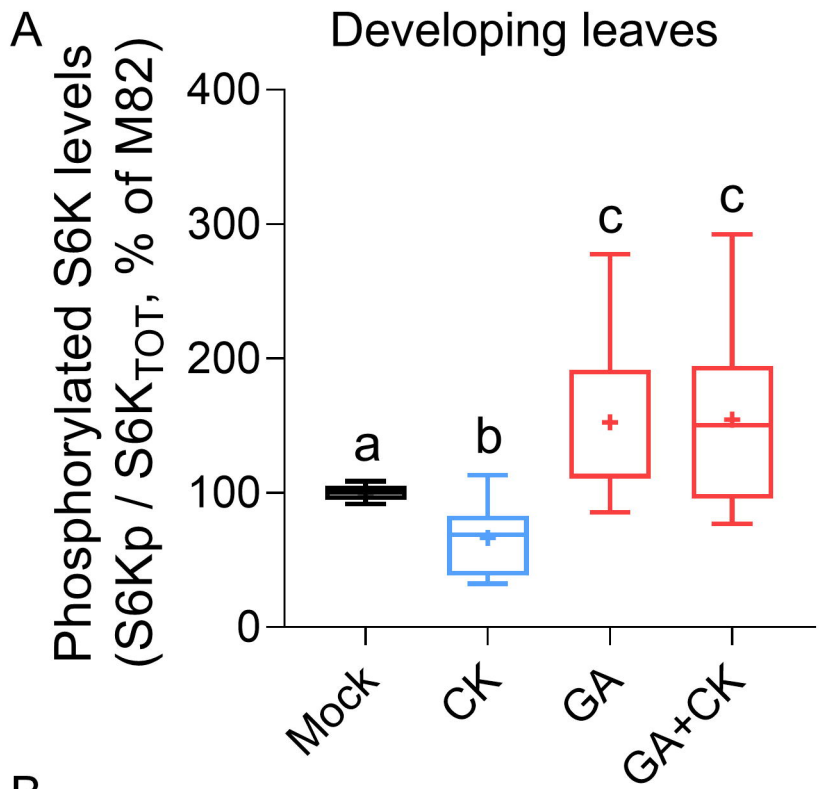

A
Lesion area (mm2)
150
100
50
0
****
L5
L8
B
High CK/ Low GA
Low CK/ High GA
Lesion area % (/M82)
400
300
200
100
0
a
b
c
b
b
b
c
M82
IPT
CKX
clau
ga20ox
PROΔ17
pro
C
Lesion area (mm2)
400
300
200
100
0
****
ns
****
ns
ns
****
M82 L5
M82 L8
IPT L5
IPT L8
CKX L5
CKX L8
clau L5
clau L8
PROΔ17 L5
PROΔ17 L8
pro L5
pro L8

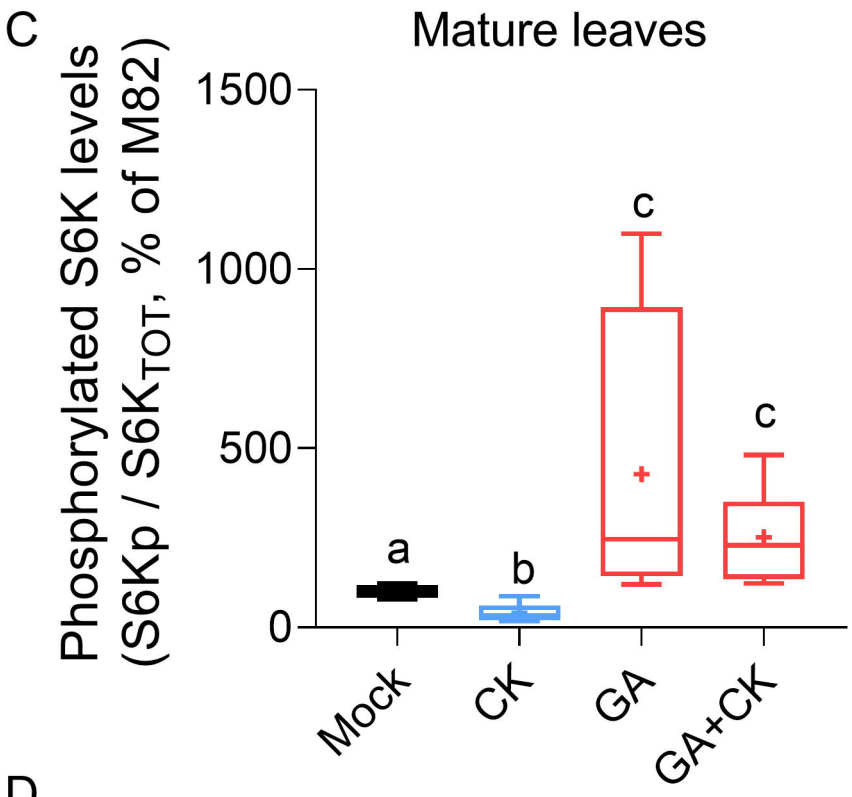

A
High CK/ Low GA
Low CK/ High GA
Lesion area % (/M82)
400
300
200
100
0
***
****
***
ns
A
ab
B
a
C
a
BD
a
D
b
B
ab
C
ab
Mock
Torin2
M82
IPT
CKX
clausa
ga20ox
PROΔ17
pro
B
M82
IPT
CKX
clausa
ga20ox
proΔ17
pro
Mock
Torin2
C
Total RLU%
800
600
400
200
0
****
ns
****
ns
ns
ns
ns
A
a
B
a
C
a
AD
a
D
a
E
b
Mock
Torin2
M82
IPT
CKX
ga20ox
PROΔ17
pro
D
Conductivity
(µS % of M82 Mock)
300
200
100
0
*
****
ns
*
ns
*
ab
B
a
A
a
C
bc
D
a
C
c
Mock
Torin2
M82
IPT
CKX
ga20ox
PROΔ17
pro

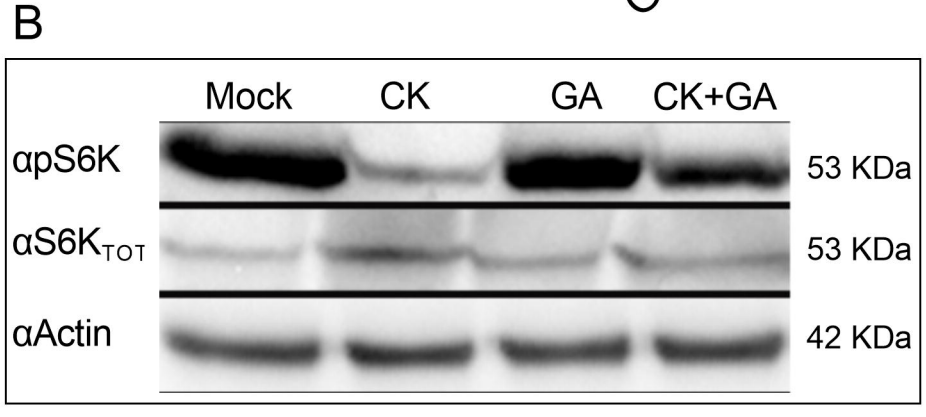

A
M82
pBLS>>IPT
pFIL>>CKX
clausa
ga20ox
pFIL>>proΔ17
procera
TRV::empty
TRV::TOR
B
High CK/ Low GA
Low CK/ High GA
TRV:empty
TRV:TOR
L4 No. of leaflets
ns
*
*
****
*
**
**
A
ac
B
bc
C
a
D
b
D
b
D
bc
C
ac
M82
IPT
CKX
clau
ga20ox
PROΔ17
pro
C
Height (cm)
ns
*
ns
*
**
**
*
A
a
B
b
A
a
B
c
B
c
A
a
C
d
M82
IPT
CKX
clau
ga20ox
PROΔ17
pro

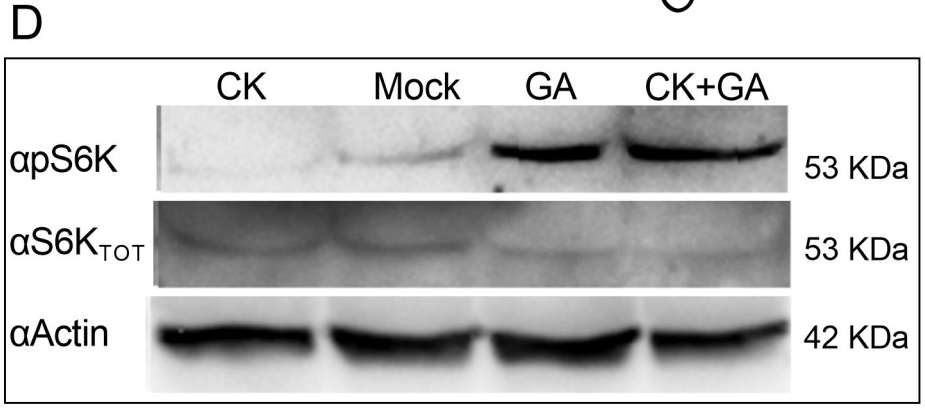

A
Bc disease severity %
500
200
150
100
50
0
-27%
**
-27%
**
-13%
*
ns
A
a
B
b
B
b
B
c
EV mock
TOR mock
EV 1 µM
TOR 1 µM
EV 10 µM
TOR 10 µM
EV 100 µM
TOR 100 µM
B
Height (% of Mock)
250
200
150
100
50
0
ns
ns
*
*
A
a
AB
a
B
a
C
b
C
L5 No. of leaflets
20
15
10
5
0
ns
*
*
ns
A
a
AB
a
B
ab
B
b
D
L6 No. of leaflets
25
20
15
10
5
0
ns
*
*
ns
A
a
AB
a
B
ab
B
b

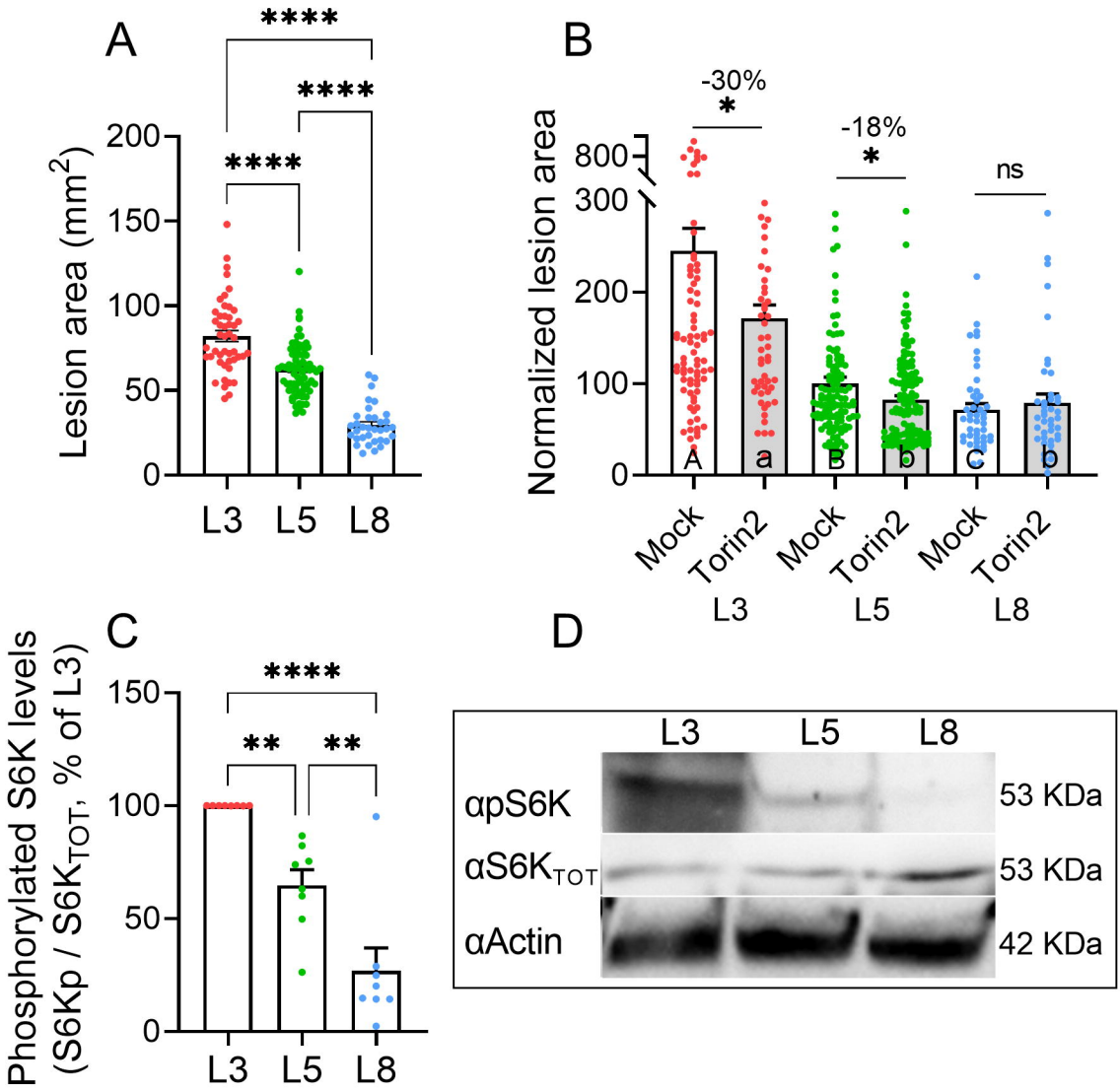

A
Developing leaves
B
Mature leaves
Mock
Torin2
Relative expression
TRR3/4
TRR5/6/7
TRR16B
CKX2
CKX5
CKX6
C
D
IPT3
IPT5
IPT6
LOG4
LOG5
LOG8
E
F
GA20ox1
GA20ox2
GA20ox3
GA2ox4
GA2ox5
GA2ox7
GA3ox2
G
H
KS
KO
KAO
GAST1
PRO
I
Mock
Torin2
P4
P3
P2
m
K
Meristem
TCSv2 signal (CTF- AFU)
L
P1-P4

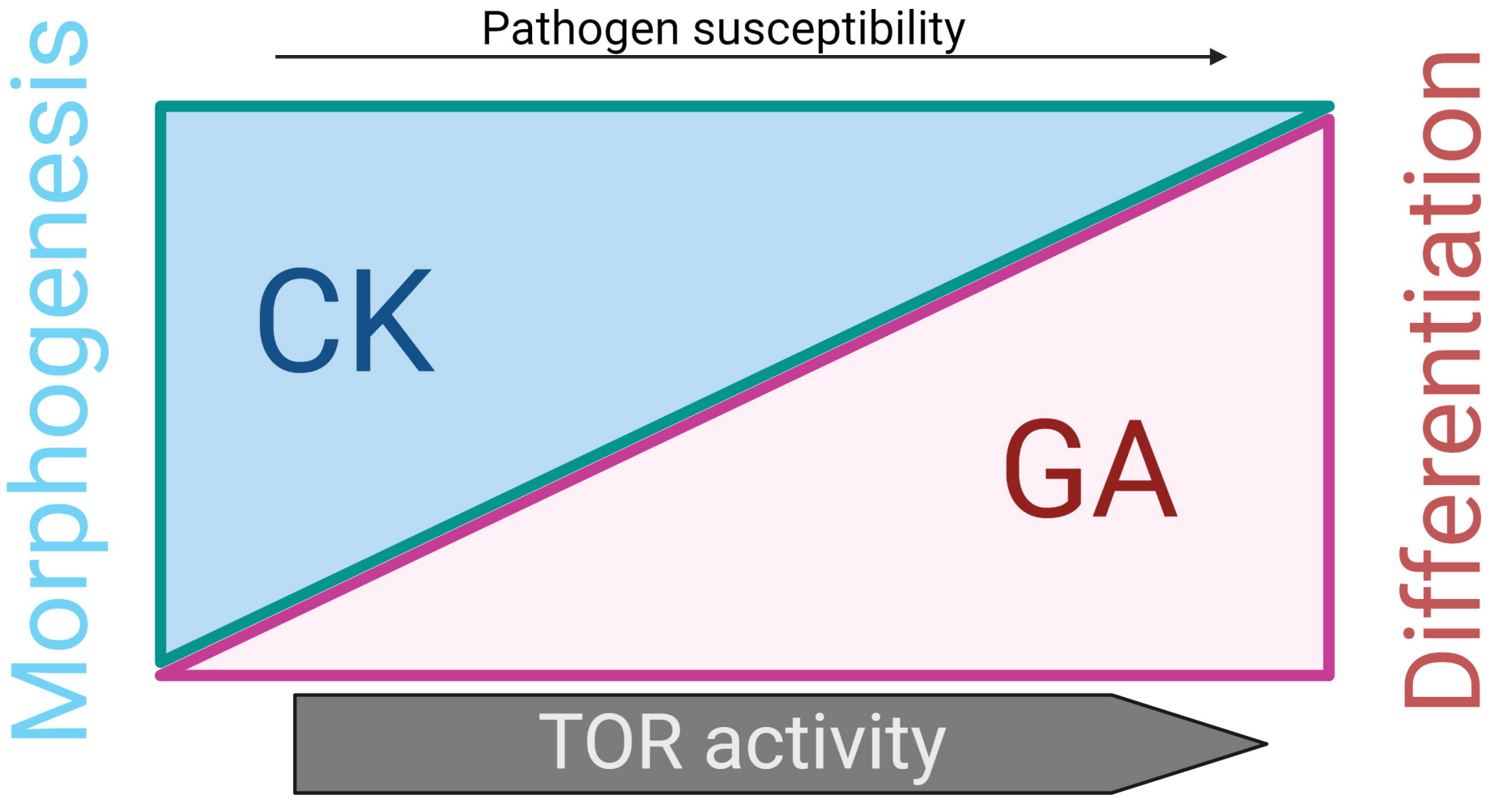

A
Developing leaves
Phosphorylated S6K levels
(S6Kp / S6K$_{TOT}$, % of M82)
400
300
200
100
0
a
b
c
c
Mock
CK
GA
GA+CK


C
Mature leaves
Phosphorylated S6K levels
(S6Kp / S6K$_{TOT}$, % of M82)
1500
1000
500
0
a
b
c
c
Mock
CK
GA
GA+CK


B
Mock
CK
GA
CK+GA
αpS6K
αS6K$_{TOT}$
αActin
53 KDa
53 KDa
42 KDa


D
CK
Mock
GA
CK+GA
αpS6K
αS6K$_{TOT}$
αActin
53 KDa
53 KDa
42 KDa


A
****
****
****
Lesion area (mm2)
200
150
100
50
0
L3
L5
L8
B
-30%
*
-18%
*
ns
Normalized lesion area
800
300
200
100
0
A
a
B
b
C
b
Mock
Torin2
Mock
Torin2
Mock
Torin2
L3
L5
L8
C
****
**
**
Phosphorylated S6K levels
(S6Kp / S6KTOT, % of L3)
150
100
50
0
L3
L5
L8
D
L3
L5
L8
αpS6K
αS6KTOT
αActin
53 KDa
53 KDa
42 KDa


Pathogen susceptibility
Morphogenesis
Differentiation
CK
GA
TOR activity