TOR mediates cytokinin-driven development and defense cues

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Abstract

Target of Rapamycin (TOR) kinase is a conserved energy sensor that regulates plant growth and development in response to nutritional and environmental inputs. TOR is also involved in the regulation of plant immunity. TOR downregulation, for example, results in enhanced plant defense responses and plant resistance to several pathogens in *Arabidopsis*, rice, and tomato. Similarly, the plant hormone cytokinin (CK) has also been demonstrated to mediate both development and defense processes. Although TOR is positioned at the interface between development and defense, little is known about the mechanisms in which TOR may potentially regulate the switch between these two modalities. Here, we investigated the interplay between TOR-mediated development and TOR-mediated defense. We show that *TOR*-silencing or inhibition led to enhanced defense responses and disease resistance in WT or CK-deficient backgrounds. However, in a high-CK background, which is already immuno-activated and disease resistant, TOR-inhibition reduced defense responses and disease resistance. *TOR*-silencing resulted in the normalization of developmental phenotypes associated with high or low CK levels, as well as of other classical developmental mutants, demonstrating that TOR is required for the execution of developmental cues. We found that CK represses TOR activity, suggesting the existence of a cross talk mechanism between the two pathways. Our results demonstrate that TOR likely acts downstream to CK signaling and hormonal signaling in general, executing signaling cues resulting from both high and low CK, in both defense and development. Thus, differential regulation of TOR or TOR-mediated processes could underlie a development-defense switch.

Introduction

To survive, plants have developed sophisticated strategies and molecular mechanisms to protect them against attacks by pathogens (Jiang et al., 2020). When plants recognize that they are being attacked by a pathogen, they activate an array of cellular and molecular processes which include the production of defense proteins and metabolites. The activation of plant defense responses is energetically costly, and as a result may be associated with growth arrest, due to resource restrictions. Thus, plants must constantly regulate growth and defense responses, and balance between them. To do so, plants coordinate inputs from the environment with their metabolism, growth, and development, mostly by using hormonal pathways (Huot et al., 2014).

The plant hormone Cytokinin (CK) regulates many aspects of the plant growth and development including cell division, leaf senescence, apical dominance, vascular differentiation, chloroplast biogenesis, root development and stress responses (Zürcher and Müller, 2016). CKs have been previously shown to 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., 2020b). CKs were reported to promote resistance through the SA signaling pathway (Choi et al., 2010; Naseem et al., 2012). 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, and against

bacterial pathogens, in a SA-defendant manner in tomato (Gupta et al., 2020b). Moreover, we have also 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., 2020a).

The Target of Rapamycin (TOR) kinase is an energy sensor that was shown, in contrast to CK, to act as a negative regulator of plant immunity. TOR is a conserved Ser/The protein kinase that coordinates between environmental cues and nutritional information to metabolism, protein synthesis, ribosome biogenesis, and development (Dobrenel et al., 2016). Generally, when nutrients are abundant, TOR is active and promotes anabolism and developmental processes, and when nutrients are limited, TOR is inactive and promotes catabolic processes (Saxton and Sabatini, 2017). Previous studies have demonstrated that TOR plays a role in plant immunity. In rice, for example, TOR was shown to act as a negative regulator of immunity by antagonizing the defense hormones JA and SA (de Vleesschauwer et al., 2018). In agreement with this, mutants impaired in TOR complex and TOR-inhibited *wt Arabidopsis* plants were more resistant to *Fusarium* (Aznar et al., 2018). In citrus spp., TOR inhibition was found to attenuate the growth of *Xanthomonas citri* (Soprano et al., 2018). In another study, *TOR* expression was downregulated upon NB-LRR activation. Moreover, suppression of *TOR* expression enhanced disease resistance, whereas *TOR* overexpression decreased it, suggesting that translational regulation executed by TOR plays an important role in the switch from growth to defense.

(Meteignier et al., 2017). TOR inhibition was also found to block growth and to activate the SA signaling pathway in *Arabidopsis* (Moreau et al., 2012; Dong et al., 2015).

In agreement with this, we have recently shown that TOR inhibition or *TOR*-silencing promotes resistance against *Xcv*, *TMV, On* and *Bc* in tomato and *N. benthamiana*, through the activation of plant defense responses in a SA-dependent manner (Marash et al., 2022), although the exact mechanism by which the inhibition of TOR primes resistance, is still unclear. Some explanations for the effect of TOR on immunity include selective translational control during plant immunity (Meteignier et al., 2018), or negative regulation of autophagy in plants, as was reported in yeast and mammals (Liu and Bassham, 2010).

In *Arabidopsis*, TOR inhibition was shown to alter the expression of hundreds of genes, including genes that are linked to plant hormones signaling networks (Dong et al., 2015). The TOR signaling pathway was later shown to interact with plant hormones. For example, the TOR signaling pathway interacts with the brassinosteroid signaling pathway during hypocotyl elongation through the BZR1 transcription factor (Zhang et al., 2016) and activates ABA receptors by phosphorylation (Wang et al., 2018). Moreover, The Auxin efflux facilitator PIN2 is phosphorylated and stabilized by TOR, which influences the gradient distribution of PIN2 in *Arabidopsis* primary root (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). Therefore, 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).

Considering that TOR and CK regulate growth and development, and since they were both implicated in SA-dependent plant responses to pathogens, it appears possible that CK and TOR may interact or share similar defense response pathways. To date, there is limited evidence of the crosstalk between TOR and CK. For example, (Dong et al., 2015) showed that *TOR* downregulated the expression of genes involved in the CK pathway. These findings demonstrate the existence of a relationship between TOR and CK signaling, yet they did not address the role of TOR in CK-mediated immunity.

Here, we assessed the involvement of TOR in CK-mediated immunity by testing whether *TOR* downregulation affects CK signaling or CK-mediated disease resistance in tomato. We found that defense responses and disease resistance are induced in the CK-deficient line *pFIL>>CKX* upon *TOR* downregulation, whereas they are reduced in the high CK *pBLS>>IPT* line. We found a similar relationship between TOR inhibition and CK-driven developmental cues, whereby leaf patterning was dependent on TOR levels in both high and low CK transgenic lines, as well as in a series of classical tomato developmental mutants.

Transcript levels of classical CK-signaling genes and developmental genes were altered in response to TOR inhibition, and TOR activity was reduced in response to exogenous CK-treatment, demonstrating a partially antagonistic relationship between the TOR and CK signaling pathways. Collectively, our results suggest that CK-mediated immunity and developmental processes likely function via TOR, and strengthen the notion that TOR functions as a master switch, controlling plant growth and defense programs.

Results

TOR inhibition mediates CK-driven immunity

To date, roles for TOR in CK-mediated immunity have not been reported. To determine whether TOR is involved in CK-mediated immunity, we first tested the effect of *TOR*-silencing and inhibition on pathogen resistance in tomato lines with altered CK levels. We used *pBLS>>IPT* and *pFIL>>CKX* as lines with high and low CK levels, respectively, and performed *Bc* pathogenicity assays as previously described in (Gupta et al., 2020b). As can be seen in **Figure 1**, compared with the background line M82, we observed significantly enhanced *Bc* resistance in plants with elevated CK levels, and reduced resistance in plant with low CK levels, which is in

agreement with our previous results (Gupta et al., 2020b). Silencing of the tomato *SlTOR* gene by virus-induced gene silencing (VIGS) resulted in a reduction of *Bc* disease symptoms in the M82 background, as we previously reported. This silencing methodology inhibits *TOR* transcription by about 50% (Marash et al., 2022). Similar reduction was observed in the CK-deficient plants CKX, as *TOR*-silencing reduced disease symptom by more than **50%**. However, when *TOR* was silenced in IPT plants, we observed a small but significant increase in disease levels **(Figure 1)**. Notably, disease levels following *TOR*-silencing were similar in all three

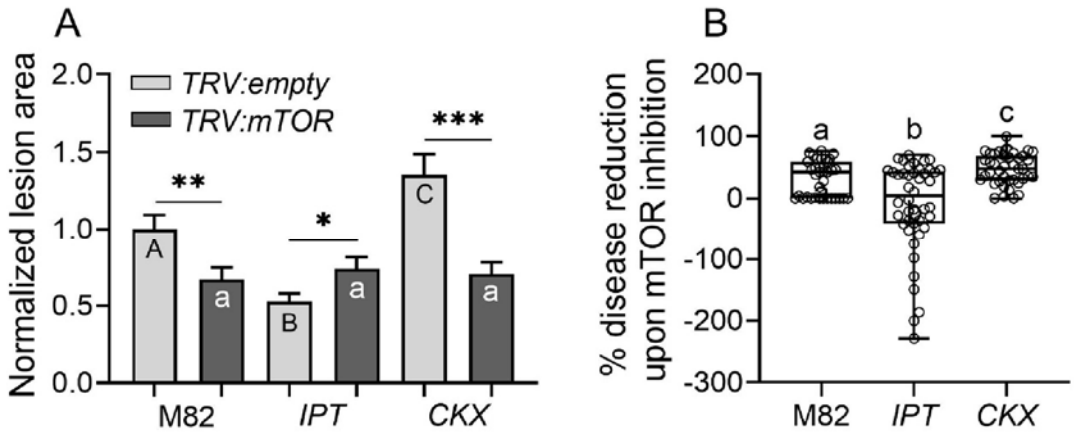

Figure 1: *TOR* inhibition mediates disease resistance downstream to CK

S. lycopersicum plants of the increased CK line *pBLS>>IPT7* ("*IPT*"), the decreased CK line *pFIL>>CKX3* ("*CKX*"), and their WT background M82, were *TOR*- silenced using the VIGS system. Plants were challenged with *B. cinerea (Bc)* mycelia from a 72h old-culture 4 weeks after VIGS, on leaflets derived from leaves 5-6. **A**: *Bc* necrotic lesion size. **B**: percentage of disease reduction following *TOR* silencing in the different genotypes. Bars represent mean ±SEM; boxplots represent inner quartile ranges (box), outer quartile ranges (whiskers), median (line in box), all points shown. Experiments were repeated 4 independent times.

A: Asterisks indicate statistically significant disease reduction upon *TOR* silencing when compared with empty vector ("EV") silencing. Different letters indicate statistically significant differences among samples, upper case letters for Mock altered CK genotypes and lower case letters for samples with silenced TOR, in a one-way ANOVA with a Tukey post hoc test, N=12, $p<0.035$ (*$p<0.05$, **$p<0.01$, ***$p<0.001$). **B:** Different letters indicate statistically significant differences between samples in one-way ANOVA with a Tukey post-hoc test, N=36, $p<0.025$.

genotypes, irrespective of CK levels. These results were further confirmed by TOR inhibition using specific TOR inhibitors (**Figure S1**), where disease symptoms decreased in *pFIL>>CKX* and M82 in the presence of the TOR specific inhibitors Torin2 and WYE132. No significant increase was observed in the IPT line when using TOR inhibitors **(Figure S1)**. This difference could be related to different levels of TOR inhibition in the different methodologies used. Us and others have previously demonstrated the specificity of Torin2 and WYE132 (Li et al., 2017; Montané and Menand, 2013; Marash et al., 2022).

To further examine the relationship between TOR inhibition and CK in plant defense, we assessed *Bc* sensitivity of M82 plants upon Torin2 and 6-BAP treatment. Plants were either treated with Torin2 or with 6-BAP, or both. As expected, both 6-BAP and Torin2 treatments promoted disease resistance, as lesion size was reduced by about 40% with either Torin2 or CK respectively **(Figure S2A)**. 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 S2B,C).** ROS accumulation and ion leakage were increased by either 6-BAP or Torin2 treatment. However, no additive effect on induction of defense responses upon combined treatment with both Torin2 and 6-BAP was observed. Collectively, these results suggest that TOR inhibition likely promotes plant defense and disease resistance downstream to CK.

Both high endogenous CK levels and *TOR* downregulation were shown by us and others to induce plant defense responses (Gupta et al., 2020b; Marash et al., 2022). Given that *TOR* downregulation increased disease susceptibility in resistant high-CK plants, we hypothesized that CK and TOR effects on plant defense responses might be dependent. To test this hypothesis, we examined the effect of TOR inhibition in lines with different endogenous CK levels on ROS accumulation. In the absence of Torin2, consistent with our previous report characterizing CK-mediated immunity (Gupta et al., 2020b), flg-22 elicited ROS levels were higher in the IPT line, and lower in CKX plants. TOR inhibition using Torin2 increased ROS accumulation in both CKX and WT plants **(Figure 2)**. By contrast, ROS accumulation significantly decreased in IPT plants upon Torin2 application. Similar results were obtained when quantifying

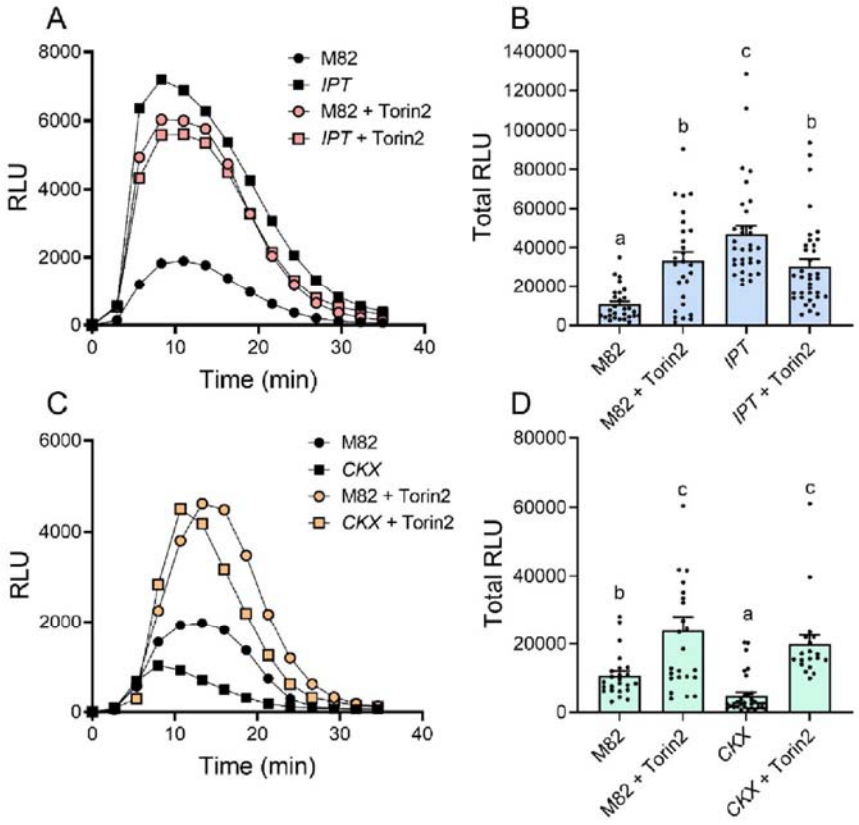

Figure 2: TOR inhibition mediates increased immunity downstream to CK

S. lycopersicum plants of the increased CK line *pBLS>>IPT7* ("*IPT*") (**A, B**), the decreased CK line *pFIL>>CKX3* ("*CKX*") (**C, D**), and their WT background M82, were treated with Mock (1:5000 DMSO in DDW), or 2 µM Torin2. 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). (**B, D**) Average total RLU per treatment. Bars represent mean ±SEM, all points shown. Experiments were repeated 3 independent times. Different letters indicate statistically significant differences between samples in Welch's ANOVA with a Dunnett post-hoc test, **B:** N=28, p<0.0001. **D:** N=20, p<0.031.

ion leakage, an -increase of 36% and 76% in M82 and CKX respectively, and a decrease by 46% in IPT, were observed **(Figure S3)**. These results further support the notion that TOR acts downstream to CK in promoting plant defense.

TOR mediates CK-driven developmental cues

Transgenic tomato lines with altered leaf CK content have altered developmental programs, resulting in quantifiable phenotypic changes to leaf development. IPT has significantly more complex leaves, while CKX has significantly simpler leaves, when compared with their M82 background (Shani et al., 2010). To investigate whether TOR plays a role in CK-mediated leaf development, we compared the leaf complexity of lines with different CK levels upon *TOR*-silencing. Interestingly, whereas the leaves in the WT M82 plants did not show any significant phenotypical changes in response to *TOR*-silencing, we observed a reduction in leaf complexity in the highly complex IPT plants, and an increase in leaf complexity in the simple-leaved CKX plants **(Figure 3)**, suggesting that TOR is required to execute the developmental cues generated by CK. We wondered whether TOR activity could account for other phenotypic changes resulting from alterations to leaf developmental programs observed in "classical" developmental mutants which have not been directly linked to CK levels. Interestingly, *TOR* inhibition “normalized” the phenotypes observed in several mutants (**Figure 4**), suggesting that the execution of developmental cues is mediated by TOR as a general mechanism.

TOR inhibition alters CK response

A recently deposited BiorXiv paper has shown that TOR inhibition reduces CK levels by the induction of the CK deactivating CKX enzyme genes in *Arabidopsis* (Janocha et al., 2021). In agreement with this observation, we found that both CKX2 and CKX5 mRNA levels were upregulated in response to Torin2 treatment, while the level of the CK-mediated response regulator gene TRR3/4 was reduced, suggesting that TOR inhibition results in a corresponding effect on CK signaling **(Figure 5)**. TOR Inhibition also induced expression of the KNOX2

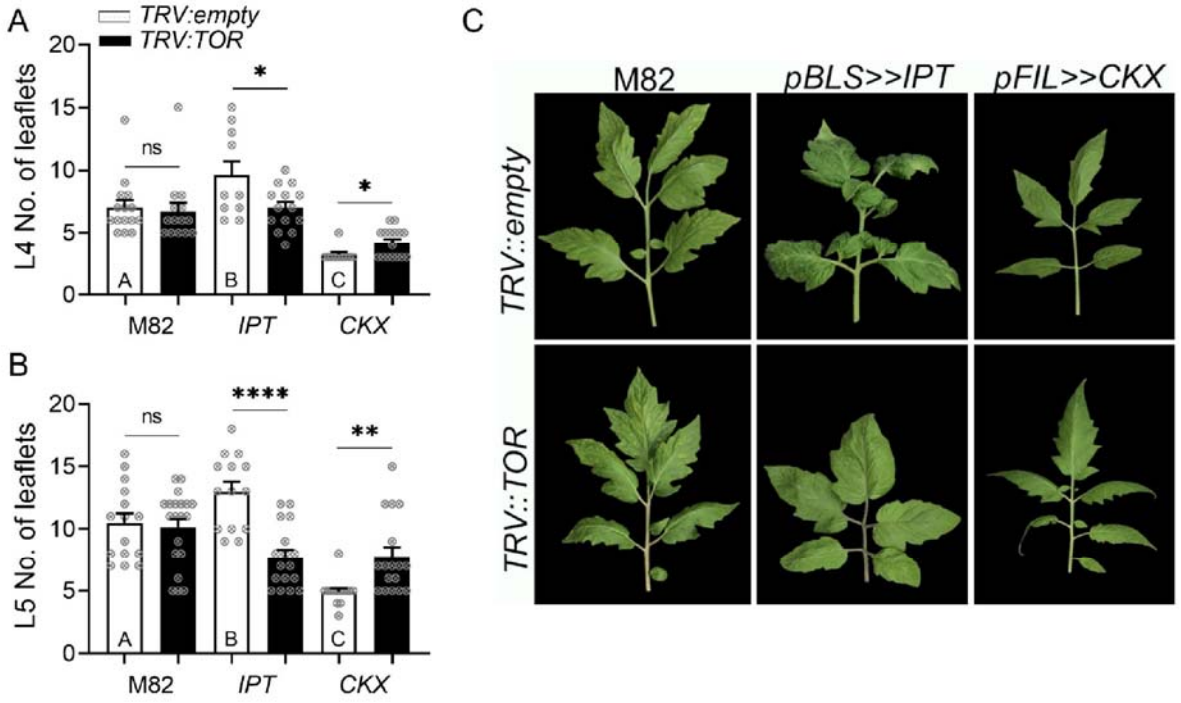

Figure 3: *TOR* silencing affects leaf development downstream to CK

S. lycopersicum plants of the increased CK line *pBLS>>IPT7* ("*IPT*"), the decreased CK line *pFIL>>CKX3* ("*CKX*"), 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, C**) and 5 (**B**). Experiment was conducted 3 times. Bars represent Mean ±SEM, all points shown. Asterisks indicate statistically significant changes in leaf complexity upon *TOR* silencing, and different letters indicate statistically significant differences among the control samples, in a one-way ANOVA with a Holm-Sidak post hoc test. **A:** N>10 individual plants, *p<0.05, ns- non significant. **B:** N>14 individual plants, **p<0.01, ****p<0.0001, ns- non significant.

179 homeobox transcription factor TKN2, that is responsible for meristem maintenance (Shani et
180 al., 2009), and reduced expression of the MYB transcription factor *clausa*, that promotes
181 differentiation (Bar et al., 2016), both of which could correspond with a more juvenile
182 developmental state (**Figure 5**).
183 Pathogen infection has been previously demonstrated to alter the expression of CK pathway
184 genes (Argueso et al., 2012; Gupta et al., 2020b, 2020a).

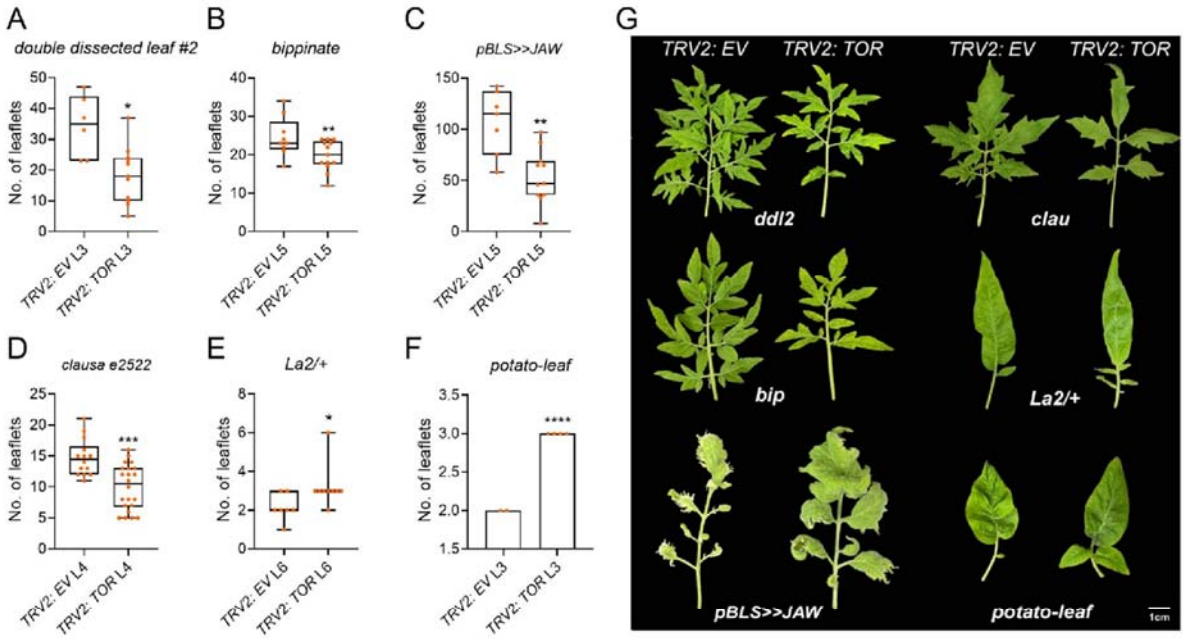

Figure 4: TOR is required for the execution of developmental cues in the leaf

S. lycopersicum plants mutated in the BELL transcription factors *Double Dissected Leaf*: *ddl2* (**A, G**), *Bippinate*: *bip* (**B, G**), the MYB transcription factors, *CLAU*: *clausa* (**D, G**), and *C: potato leaf* (**F, G**), the TCP transcription factor *Lanceolate*: *La2/+* (**E, G**), or overexpressing miR390 under the leaf specific promoter BLS: *pBLS>>JAW* (**C, G**), were *TOR*-silenced using VIGS. 4 weeks after silencing, leaf complexity was quantified by counting the leaflets on leaves 3, 4, 5 or 6- as indicated.

Experiment was conducted 3 times. Boxplots represent inner quartile ranges (box), outer quartile ranges (whiskers), median (line in box), (A-E), or, bars represent Mean ±SEM (F), all points shown. Asterisks indicate statistically significant changes in leaf complexity upon TOR silencing as compared with the same leaf in empty-vector VIGSed plants, in a two-tailed t-test or a Mann-Whitney U test. **A:** N=6-11 individual plants, *p<0.05. **B:** N=9-14 individual plants, **p<0.01. **C**: N=7-11 individual plants, **p<0.01. **D**: N=14-22 individual plants, ***p<0.001. **E**: N=7-9 individual plants, *p<0.05. **F**: N=3-4 individual plants, ****p<0.0001.

To investigate possible effects of TOR status on pathogen-driven alterations in CK pathway genes, we examined gene expression following infection with *Botrytis cinerea* (Bc), with and without Torin2 treatment (**Figure S4**). CK-mediated signaling (examined by assaying expression of the response regulators TRR3/4 and TRR5/6/7) showed the expected increase following Bc treatment (**Figure S4A,B**). TRR3/4 is reduced by Torin2 treatment (**Figure 5, S4B**), and following

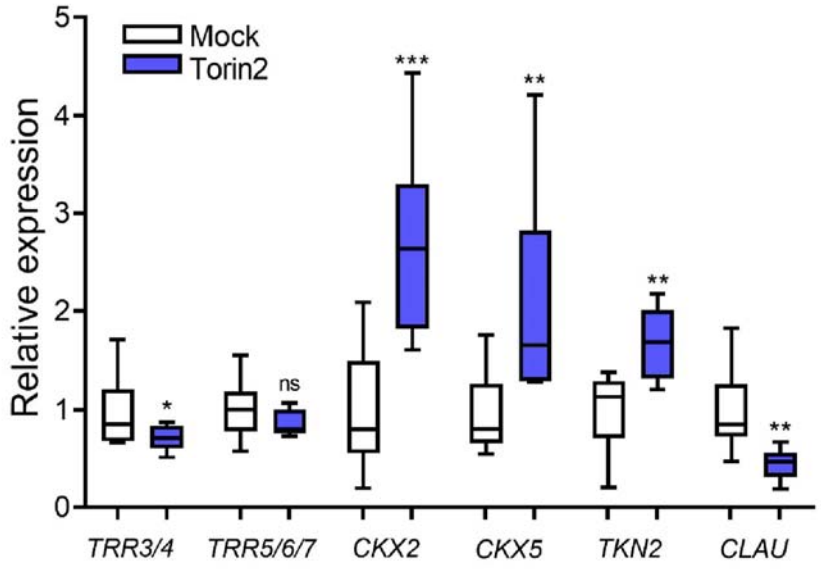

Figure 5: TOR inhibition alters CK pathway and developmental gene expression

Gene expression analysis of the indicated CK pathway and developmental 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 mean between the gene copy number obtained for three reference genes: RPL8 (Solyc10g006580), EXP (Solyc07g025390), and CYP (Solyc01g111170), and normalized to Mock treatment. 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.

Bc and Torin2 co-treatment, returns to background levels (**Figure S4A**). TRR5/6/7 is not affected by Torin2 (**Figure 5, S4B**), however, upon combined treatment, it increases beyond the levels elicited by Bc alone, suggesting that TOR mediated effects on the CK pathway may be differential through interactions with different CK pathway genes. CKX gene expression is reduced in response to Bc infection (**Figure S4C,D**), as was observed following Torin2 treatment (**Figure 5**), and the combination of both Bc infection and Torin2 treatment does not further augment this reduction in CKX expression. The developmental meristem-maintenance KNOX gene TKN2 is induced by Torin2 and repressed by Bc infection, and in this case, the infection process has a dominant effect (**Figure S4E**), indicating that defense processes may take

precedence over meristem maintenance in this case. The effect of TOR inhibition on meristem maintenance is likely also dependent on the developmental stage of the plant.

To assess the effect of TOR inhibition on CK signaling in young developing shoots, we next examined the effect of TOR inhibition on CK signaling by using the CK-response transgenic reporter line TCSv2::3XVENUS, that expresses VENUS under the control of the CK responsive synthetic promoter TCS (Steiner et al., 2016; Zürcher et al., 2013; Steiner et al., 2020).

We found that in the presence of Torin2, there is a reduction in CK signaling in the meristem zone, as treated TCSv2::3XVENUS meristems showed a significant reduction in signal relative to untreated meristems **(Figure 6)**. This is consistent with the increase in CKX gene expression upon Torin2 treatment (**Figure 5**). Similar results were achieved in TCS expressing plants in which *TOR* was silenced by VIGS **(Figure S5)**. To further investigate whether TOR is required for CK signaling, we used *pFIL>>CKX* plants expressing the CK responsive promotor TCSv2.

Similarly to disease reduction (**Figure 1**) and defense responses (**Figure 2,S2,S3**), we observed an increase in TCS driven Venus signal in the meristem of Torin2 treated *pFIL>>CKX* plants, in comparison with untreated plants **(Figure S6)**. These results suggest that TOR activity is required for an intact CK signaling pathway.

CK antagonizes TOR

To further probe the relationship between CK and TOR, we next examined the effect of CK application on the phosphorylation of a downstream target of TOR. We hypothesized that if TOR is indeed downstream to CK signal transduction, CK might affect TOR activity. We used an antibody against the phosphorylated form of S6K1, one of the classical TOR targets that has

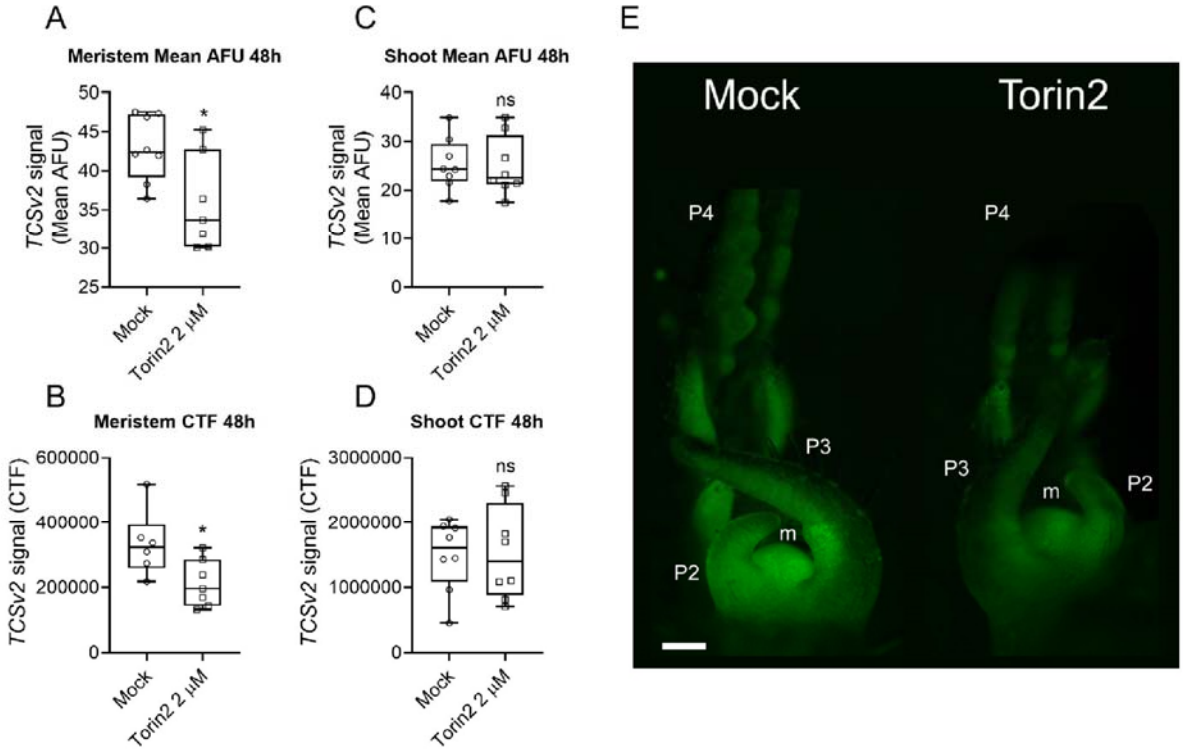

Figure 6: TOR inhibition reduces CK response in the meristem of young shoots

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.

TCSv2 driven total Venus fluorescence in the meristem (**A,B**) or total shoot (**C, D**) was measured as mean arbitrary fluorescent units (AFU, the strength of the signal, **A, C**) or corrected total fluorescence (CTF, a quantification taking into account the fluorescent area, **B, D**), in images captured under identical conditions in shoots comprising the 4 youngest primordia. 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, ns- non significant.

(**E**) 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.

been previously used as an indication of TOR activity in plants (Li et al., 2017). We found that CK application reduces the phosphorylation of S6K1 by TOR, suggesting that CK negatively regulates TOR activity **(Figure 7A,B)**. This was further verified in the *pBLS>>IPT* and *pFIL>>CKX*

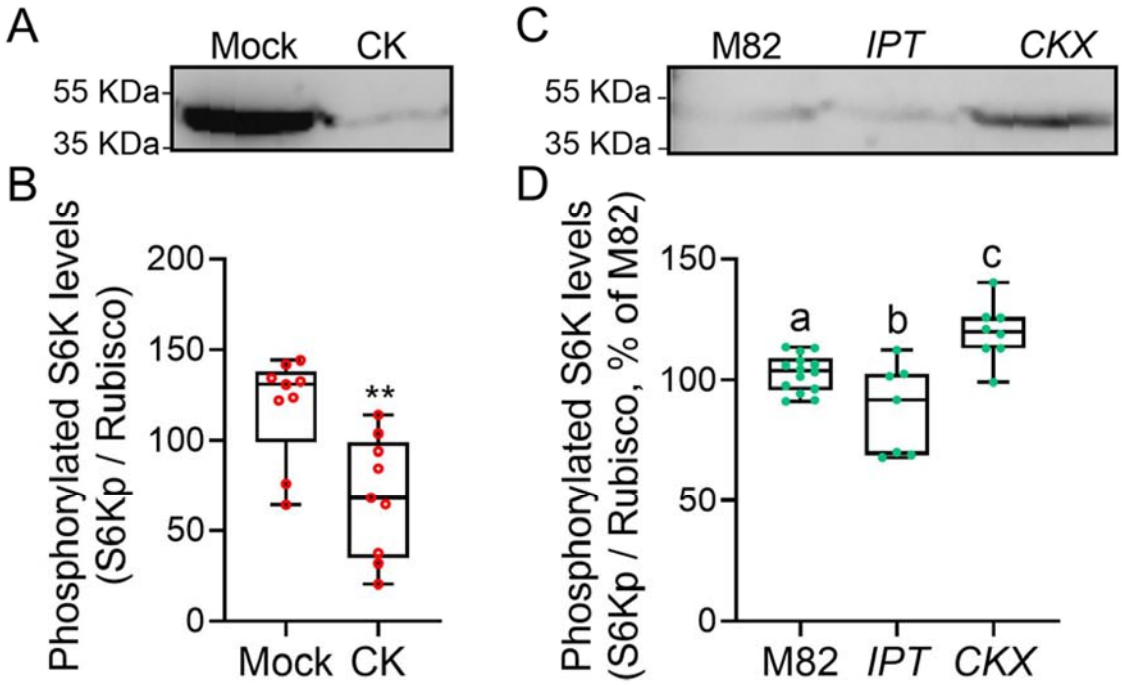

Figure 7: CK reduces TOR activation

A-B *S. lycopersicum* cv. M82 10 day-old seedlings were treated with Mock (10 µM NaOH) or 10 µM of the CK 6-benzylaminopurine (6-BAP). 24 h after treatment, total cellular proteins were preapared from 3 biological replicas of 10 plants each, and TOR activation was assessed by detection of phosphorylated S6K using specific antibodies.

C-D Total cellular proteins were prepared from *S. lycopersicum* cv. M82, and *pBLS>>IPT* or *pFIL>>CKX* transgenic lines in the M82 background (3 biological replicas of 10 plants each). TOR activation was assessed by detection of phosphorylated S6K using specific antibodies.

Experiment was repeated 3 times. Boxplots represent inner quartile ranges (box), outer quartile ranges (whiskers), median (line in box), all points shown. **B** Asterisks indicates statistically significant reduction in phosphorylated S6K in a Mann Whitney U test, N=9, $p<0.01$. **D** Different letters indicate staistically significant differences between samples in a one-way ANOVA with a Tukey post hoc test, $*p<0.05$, N=7-14.

224 transgenic lines, as the phosphorylation of S6K1 was reduced in *pBLS>>IPT* and enhanced in

225 *pFIL>>CKX* in comparison to the background M82 **(Figure C,D)**.

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Discussion

The role of TOR as a development-defense switch

Plants constantly perceive and process environmental signals and balance between the energetic demands of growth and defense. Generally, it is believed that the growth arrest upon pathogen attack is the result of a redirection of the plants' metabolic resources towards the activation of plant defense responses at the expense of growth (Campos et al., 2016). Indeed, growth was found to be prioritized over defense in plants grown under light deprivation, and they were reportedly more susceptible to attacks by *Pseudomonas syringae* and *Botrytis cinerea* (Huot et al., 2014). It is still unclear, however, whether the shift in the metabolic resources toward immunity is the sole factor of growth inhibition, or whether it is an adaptive response regulated by growth and defense signaling networks (Guo et al. 2018). The ability of TOR to sense many different inputs such as hormone levels, nutrients, energy, light, and abiotic or biotic stress signals, enables concerted regulation of downstream anabolic and catabolic processes, resulting in the optimization of plant growth (Dobrenel et al., 2016). TOR and has been previously suggested to act at the intersection of growth and defense as a molecular 'switch' that activates cell proliferation and plant growth at the expense of defense (de Vleesschauwer et al., 2018). Likewise, we have recently reported that TOR negatively regulates plant immunity and defense responses in tomato (Marash et al., 2022). The main objective of this study was to provide insights on the relationships between TOR and CK in the mediation of immunity. We discovered that TOR executes signaling cues resulting from both high and low CK, in both defense and development. We hypothesize that the crosstalk between TOR and CK

signaling is likely involved in the ability of plants to "turn off" growth and developmental programs upon pathogen attack, and thus enable a faster activation of plant defense responses. It was shown previously that TOR interacts with plant hormones (Wu et al., 2019; Dong et al., 2015). For example, a link between the CK and TOR pathways was recently demonstrated by Janocha et al., 2021, who provided evidence that TOR suppresses CK signaling in the shhot apical meristem of Arabidopsis, by promoting the expression of CKXs genes. In addition, because most of the evolutionarily conserved regulators of TOR are absent in land plants, it was suggested that in plants, hormones play a more substantial role in the upstream regulation of TOR (Schepetilnikov and Ryabova, 2018), and that plants, unlike other eukaryotic species, have thus evolved a distinct regulatory mechanism (van Leene et al., 2019).

Involvement of TOR in CK-mediated defense and development

We used plants with different CK levels to test the idea that TOR is required for CK-mediated immunity. We found that *TOR*-silencing promotes *Bc* resistance in CK-deficient CKX overexpressing plants. By contrast, plants with high CK levels showed an opposite trend as they became more susceptible to *Bc* **(Figure 1)**, implying that CK-mediated immunity requires TOR activity for execution. Another line of evidence supporting the role of TOR in CK-mediated immunity comes from the observation that defense responses were induced upon TOR inhibition in *pFIL>>CKX3* plants, whereas they were reduced in the high CK line *pBLS>>IPT* **(Figures 2 and S2-S3)**. This could also explain why TOR inhibition did not further enhance defense responses or *Bc* resistance when combined with CK application **(Figure S2)**.

Both TOR (Eltschinger and Loewith, 2016) and CK (Werner and Schmülling, 2009; Keshishian and Rashotte, 2015) are growth regulators that were shown to play a role in plant immunity; TOR inhibition (Marash et al., 2022) or high endogenous CK levels (Gupta et al., 2020b) promote pathogen resistance in a SA-dependent manner in tomato. Thus, in addition to TOR sensing being required for the achievement of developmental and defense phenotypes determined by signals stemming from hormone levels, it is likely that TOR and CK signaling pathways coordinately regulate plant defense responses through the modulation of SA.

TOR is required for the patterning of leaf organs programmed by CK. The increased leaf complexity in the high-CK overexpressing IPT, and the diminished leaf complexity in the CKX over-expressor which has low CK levels, were both tempered to WT M82 levels by the inhibition of TOR **(Figure 3)**. This phenotypic change might suggest that CK 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. These changes in phenotypes likely stem from the fact that TOR is a translation regulator that acts downstream of CK mediated signals in both defense and development contexts. Interestingly, we found that TOR inhibition decreases the severity of developmental phenotypes of several additional mutants which have not necessarily classically been associated with CK levels **(Figure 4)**. This brings forth the notion that TOR mediates signals from additional hormones, or rather, 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 (Israeli et al., 2021; Greenboim-Wainberg et al., 2005).

Possible mechanisms by which TOR mediates developmental and defense cues

As previously reviewed in (Du et al., 2018) light and sugar, i.e. plant food, generate signals of a positive energy status that are required to enable organ formation in the SAM. These signals converge on TOR (Pfeiffer et al., 2016; Li et al., 2017). Auxin biosynthesis is activated by light in the shoot apex, while auxin mediates light-dependent activation of TOR (Li et al., 2017). The light signal is then relayed through cytokinin signaling pathways (Pfeiffer et al., 2016; Yoshida et al., 2011). Thus, light regulates leaf initiation by activating cytokinin signaling and affecting auxin gradients (Yoshida et al., 2011). As TOR functions in concert with light and positive metabolic status to promote cell growth and division, it has been suggested that active growth and cell-cycle progression are required for the formation of the hormonal axis, involving auxin and cytokinin, that is required for organ formation and patterning during plant development (Du et al., 2018).

Differences in leaf outgrowth are typically attributed to two processes: cell division and cell expansion (reviewed in Kalve et al., 2014). Dividing cells grow by increasing their cytoplasmic volume, which is an energy demanding process that requires the synthesis of macromolecules. The TOR pathway plays a central role in ensuring an adequate supply of elementary building blocks. TOR integrates growth related genes to glucose signaling and is thereby an important regulatory hub in the development of organs. Several previous studies indicated that TOR plays a role during leaf development in *Arabidopsis*. For example, *TOR* downregulation has been shown to result in the production of smaller leaves with fewer cells (Caldana et al., 2013) whereas *TOR* overexpression results in the production of bigger leaves with larger cells

(Deprost et al., 2007). Likewise, mutation in *LST8*, a member of the TOR complex, results in a reduction in the number of leaves and in leaf size (Moreau et al., 2012), and mutation in *AtRAPTOR1B*, another component of the TORC1 complex, stalls leaf initiation (Anderson et al., 2005). By contrast, we did not observe any significant phenotypic lterations in the tomato WT M82 cultivar upon *TOR*-silencing. This could be ascribed to the different inhibition methods used, or the different plant species. We found that *TOR*-silencing caused tempering of the phenotypes of several developmental mutants, with both simple-leaved and complex-leaved mutants becoming closer to WT leaf phenotypes following *TOR*-silencing. We thus conclude that TOR is required for the execution of a variety of cues that are integrated to form a cohesive leaf developmental program.

TOR plays a key role as a regulator of translation and ribosome biogenesis in mammals and plants, and therefore its activity is tightly regulated (Pereyra et al., 2020). Interestingly, proteome analysis of cytokinin activity in *Arabidopsis* demonstrated extensive differential regulation of ribosomal proteins in response to CK (Brenner and Schmülling, 2012). In another proteomic study, the functional classification 'Ribosome biogenesis' was found to be strongly differential in response to CK depletion or overproduction (Černý et al., 2013). CKs are known to alter leaf development and morphology (Werner et al., 2003; Hay and Tsiantis, 2010; Shani et al., 2010), and the differentially regulated ribosomal proteins are probably involved in the underlying molecular mechanism. Horiguchi et al., 2011 demonstrated that ribosomal proteins play a key role in *Arabidopsis* leaf development, supporting this notion. Thus, it is likely that TOR is involved in the execution of CK-mediated signals, in both defense and development, by

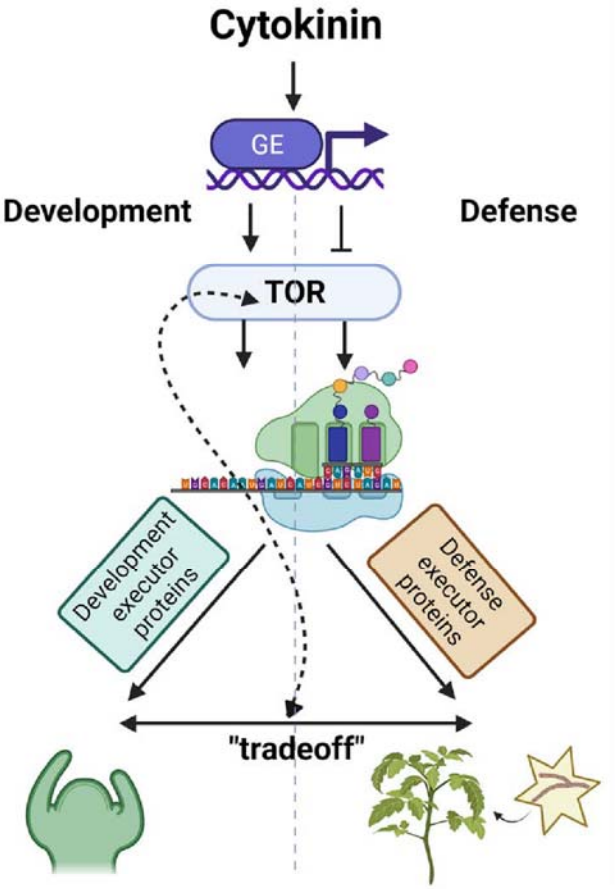

Figure 8: Model describing the role of TOR as a mediator of both development and defense cues, and the tradeoff between them.

Cytokinin promotes both development and defense. Balanced CK levels are required to achieve "normal" developmental patterning and disease resistance.
Under low CK, development and defense are inhibited, resulting in reduced patterning (Fig. 3), and increased disease susceptibitlity (Fig. 1). Under high CK, development and defense are promoted, resulting in increased patterning (Fig. 3) and disease resistance (Fig. 1).
Inhibition of TOR results in rescue of both low and high CK pehnotypes, resulting in return to baseline developmental patterning and disease resistance, and suggesting that TOR mediates these develpmental and defense cues originating from CK.
In the tradeoff between development and defense, high CK causes downregulation of TOR (Fig. 6), resulting in a shift towards defense. The switch between development and defense may be modulated by cross-talk between environmental sensing and TOR status.

its direct or indirect regulation of proteins required for the execution of these processes (**Figure 8**).

339 ***TOR activity is needed for normal CK signaling***

340 When plants are exposed to pathogens, the CK signaling pathway is activated (Argueso et al.,
341 2012; Gupta et al., 2020b) leading to down-regulation of TOR activity **(Figure 6)**. Our results
342 support the notion that down-regulation of TOR could be the mechanism executing CK-
343 mediated induced immunity and disease resistance, at least in part. Our results demonstrating
344 that TOR inhibition results in a decrease in CK signal intensity in the meristem of
345 *pTCSv2::VENUS* plants **(Figure 5)**, as was recently similarly found in *Arabidopsis* (Janocha et al.,
346 2021), indicate that TOR activity is required for a normal CK signaling pathway. The increase of
347 TCSv2 signal in *pFIL>>CKX* plants that were *TOR*-silenced **(Figure S6)**, also demonstrates that
348 TOR is responsible for CK signaling output, and that in its absence, a more "normal" CK signal is
349 generated in the otherwise CK-deficient CKX overexpressing plants, as was evident also by their
350 increase in leaf complexity (**Figure 3**). The observation that CK application results in a decrease
351 in TOR activation in the meristem might suggest the existence of a mechanism that balances
352 between TOR activity and CK signal. Interestingly, an opposite result was reported in
353 *Arabidopsis* cell suspensions, where kinetin and auxin both induced the phosphorylation of
354 AtS6k (Turck et al., 2004). Our data show that TOR is likely downstream to CK-signaling in
355 plants, and that the ability of CK to induce immunity is related to TOR status. Therefore,
356 increased CK leads to a reduction in TOR activity. The difference between our report and the
357 previous one likely relates to a cell suspension being used in the previous report, or to the
358 different plant hosts being examined.
359

We propose a model by which TOR modulates CK signaling and pathogen resistance. Under standard growth conditions, CK and TOR work together to carry out developmental programs, with TOR mediating the execution of CK-derived signals. Upon pathogen attack, metabolic resources are redirected toward the activation of defense mechanisms at the cost of growth (Campos et al., 2016). The decrease in available resources is sensed by TOR, and that leads to a decrease in TOR activity in the meristem and to the subsequent upregulation in the expression of CKX genes and decrease in CK levels. Thus, TOR, through CK, actively halts growth during pathogen attack. The upregulation in CK degrading genes CKX2 and CKX5, as well as the downregulation of the CK-responsive response regulator gene TRR3/4 upon TOR inhibition, could also be associated with a transition from a "growth" to "defense" state. CK-mediated differential phenotypic output is diminished upon TOR inhibition, indicating that TOR is required for CK-mediated phenotypes in both defense and development. **Figure 8** provides a model for the role of TOR as a mediator of both developmental and defense cues downstream of CK. Inhibition of TOR results in rescue of both low and high CK phenotypes, resulting in return to baseline developmental patterning and disease resistance, and suggesting that TOR mediates these developmental and defense cues originating from CK. Its involvement in both development and defense poises TOR as a prime regulator of tradeoffs between these two important aspects of plant life. It will be interesting to investigate how different metabolic states and biotic and abiotic stresses alter the cross talk between CK and TOR signaling in the future.

Acknowledgements

We thank members of the Spiegelman group for technical assistance, Dr. Barak Rotblat for helpful discussions, and members of the Bar group for continuous support.

Author Contributions

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

Competing Interests Statement

The authors declare no competing interests.

Materials and methods

Plant material and growth conditions

S. lycopersicum tomato cultivar M82 plants were used in this study. Genotypes used, all in the cv. M82 background, were as follows: M82 background line; *pBLS>>IPT7*, which contains elevated endogenous levels of CK- referred to hereinafter as "*pBLS>>IPT* "or "*IPT*"; and the CK depleted *pFIL>>CKX3*, referred to hereinafter as "*pFIL>>CKX*" or "*CKX*". The lines used in this work overexpress Arabidopsis AtIPT7 or AtCKX3 from the leaf specific promoters pFIL and pBLS, in tomato cv M82, and were previously characterized (Shani et al., 2010). The transgenic plants have normal early development and are viable and fertile (Shani et al., 2010). TCSv2:3XVENUS overexpressing plants were also previously described (Steiner et al., 2016; Steiner et al., 2020;

Zürcher et al., 2013). Plants were grown in soil (Green 443; Even-Ari Green, Ashdod, Israel) in a growth chamber set to long-day conditions (16/8 light/dark) at 24°C, or in a greenhouse under natural day length conditions.

Torin2 and WYE132 treatments

Torin2 (SML1224 Sigma-Aldrich) or WYE132 (PZ0321 Sigma-Aldrich), were applied to detached tomato leaves through the petiole for 24 hours prior to pathogen inoculation (both inhibitors), defense response quantification (only Torin2), or RNA preparation (only Torin2). For both inhibitors, a 10 mM stock solution was prepared in concentrated DMSO (P0037 SIGMA-Aldrich) and diluted to 2 µM in water. Mock leaves were treated with water containing 1:5000 of DMSO.

6-BAP treatment

Two weeks old M82 plants were sprayed with 100mM 6-benzyl purine (6-BAP) solution (Sigma-Aldrich) or mock solution 24h prior to analysis. Stock solution was prepared in NaOH and diluted with water.

Virus Induced Gene Silencing (VIGS)

VIGS was performed as previously described (Liu et al., 2002). The *SlTOR* silencing construct was generated as previously described (Marash et al., 2022). The *TRV2:TOR* construct, as well as an empty TRV RNA2 for control, were introduced into *A. tumefaciens* strain GV3101::pMP90. TRV RNA1 was mixed in at a ratio of 1:1 with RNA2 (either empty or *TRV2:TOR*) in infiltration buffer, and infiltrated into tomato cotyledons. Fifth leaves of six-week-old tomato plants were used for

pathogenicity assays, measurement of defense responses, complexity quantification and TCSv2 driven Venus quantification.

Pathogenesis assays

Botrytis cinerea pathogenicity assays were performed as previously described (Gupta et al., 2020b). Briefly, *B. cinerea* isolate Bcl16 was maintained on potato dextrose agar (PDA; Difco) plates in 22C. 0.4 cm diameter agar discs were pierced from colony margins and used to inoculate detached leaves. Inoculated leaves were kept in a humid chamber at 22^{o}C under long-day conditions. Necrotic lesion size was measured 5 days post inoculation using ImageJ. For Bc inoculation prior to RT-qPCR analysis, Bc spores were collected in 1 mg/ml glucose and 1 mg/ml K_2HPO_4, filtered through cheesecloth, and tomato leaves were then spray inoculated with the spore suspension. Mock plants were sprayed with similar concentrations of glucose and K_2HPO_4. RNA was prepared 24 h after Bc inoculation.

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 250 ul distilled water in a 96-well plate (SPL Life Science) at room temperature with agitation. After 4 hours, the water was removed and 50 µL of distilled water were added. Right before measurement, 100 µL of distilled water with or without 1 µM flg22 (PhytoTechLabs #P6622) were added. Light emission was measured using a luminometer (GloMax® Discover, Promega, United States).

448 Ion leakage (conductivity) measurement

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

455

456 RNA extraction and RT-qPCR

457 Isolation of total RNA was performed according to the TRI reagent (Sigma-Aldrich) procedure,
458 with application of DNAse (EN0521 ThermoFisher) to remove genomic DNA. 1 µg of RNA was
459 used for cDNA synthesis using Maxima reverse transcriptase (ThermoFisher). RT-qPCR was
460 conducted with Power SYBR Green Mix (Life Technologies), using specific primers
461 (Supplemental Table 1) in a Rotor-Gene Q machine (Qiagen). Standard curves were achieved by
462 dilutions of one cDNA sample. Relative expression was quantified by dividing the expression of
463 the relevant gene by the geometric mean of the expression of three normalizers: ribosomal
464 protein RPL8 (Solyc10g006580), Cyclophillin CYP (Solyc01g111170) and EXPRESSED EXP
465 (Solyc07g025390). All primer pairs had efficiencies in the range of 0.97-1.03 (see supplemental
466 Table 1).

467

468 Protein purification and Western Blotting

25 mg of tissue was collected from shoots of 4-week-old tomato plants and ground in liquid nitrogen with 3 volumes of extraction buffer (100mM MOPS pH 7.6, 100mM NaCl, 40mM ß-MeOH, 5% SDS, 10% Glycerol, 4mM EDTA, 2mM PMSF). The samples were boiled for 5 minutes and centrifuged at 10,000 rpm for 10 minutes, to remove cell debris. Protein concentration in each sample was determined using the Bradford assay with BSA calibration curves. Samples with Equal amounts of protein were then separated on 15% SDS acrylamide gels, transferred to nitrocellulose membranes (Protran, #10401380), and blocked with 3% skimmed milk in Tris buffer saline (TBS)- Tween.

Membranes were probed overnight with Anti-S6K1 p-Thr449 polyclonal antibody (AB-ab207399, Abcam) (dilution 1:1000) at 4^{o}C. A goat-anti-rabbit IgG HRP antibody (AB-ab205718, Abcam) was used as secondary antibody (dilution 1:10,000). Chemiluminescence was observed using Elistar Supernova as substrate (Cyanagen, #XLSE2) and images of protein bands were acquired and quantified using the Alliance UVITEC software.

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 were assessed using a post-hoc test. Tukey or Holm-Sidak 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 Prism8.

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.

Supplemental data files

Supplemental Figures S1-S7

Supplemental Figure S1: TOR inhibition with drugs mediates disease resistance downstream to CK.

Supplemental Figure S2: TOR inhibition mediates disease resistance downstream to CK: co-application of CK and Torin2.

Supplemental Figure S3: TOR inhibition mediates increased immunity downstream to CK- ion leakage.

513 Supplemental Figure S4: TOR inhibition and Bc infection have differential effects on CK pathway

514 genes.

515 Supplemental Figure S5: TOR inhibition by VIGS reduces CK response in the meristem of young

516 shoots.

517 Supplemental Figure S6: TOR inhibition increases CK response in the meristem of cytokinin

518 deficient *pFIL>>CKX* shoots.

519 Supplemental Figure S7: Original uncropped blots used in Figure 7.

520 Supplemental Table 1: RT-qPCR primers used in this work.

Figure Legends

Figure 1: *TOR* inhibition mediates disease resistance downstream to CK

S. lycopersicum plants of the increased CK line *pBLS>>IPT7* ("*IPT*"), the decreased CK line *pFIL>>CKX3* ("*CKX*"), and their WT background M82, were *TOR*- silenced using the VIGS system. Plants were challenged with *B. cinerea (Bc)* mycelia from a 72h old-culture 4 weeks after VIGS, on leaflets derived from leaves 5-6. **A**: *Bc* necrotic lesion size. **B**: percentage of disease reduction following *TOR* silencing in the different genotypes. Bars represent mean ±SEM; boxplots represent inner quartile ranges (box), outer quartile ranges (whiskers), median (line in box), all points shown. Experiments were repeated 4 independent times.

A: Asterisks indicate statistically significant disease reduction upon *TOR* silencing when compared with empty vector ("EV") silencing. Different letters indicate statistically significant differences among samples, upper case letters for Mock altered CK genotypes and lower case letters for samples with silenced TOR, in a one-way ANOVA with a Tukey post hoc test, N=12, $p<0.035$ (*$p<0.05$, **$p<0.01$, ***$p<0.001$). **B:** Different letters indicate statistically significant differences between samples in one-way ANOVA with a Tukey post-hoc test, N=36, $p<0.025$.

Figure 2: TOR inhibition mediates increased immunity downstream to CK

S. lycopersicum plants of the increased CK line *pBLS>>IPT7* ("*IPT*") (**A, B**), the decreased CK line *pFIL>>CKX3* ("*CKX*") (**C, D**), and their WT background M82, were treated with Mock (1:5000 DMSO in DDW), or 2 µM Torin2. 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). (**B, D**) Average total RLU per treatment. Bars represent mean ±SEM, all points shown. Experiments were repeated 3 independent times. Different letters indicate statistically

significant differences between samples in Welch's ANOVA with a Dunnett post-hoc test, **B:** N=28, p<0.0001. **D:** N=20, p<0.031.

Figure 3: *TOR* silencing affects leaf development downstream to CK

S. lycopersicum plants of the increased CK line *pBLS>>IPT7* ("*IPT*"), the decreased CK line *pFIL>>CKX3* ("*CKX*"), 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, C**) and 5 (**B**). Experiment was conducted 3 times. Bars represent Mean ±SEM, all points shown. Asterisks indicate statistically significant changes in leaf complexity upon *TOR* silencing, and different letters indicate statistically significant differences among the control samples, in a one-way ANOVA with a Holm-Sidak post hoc test. **A:** N>10 individual plants, *p<0.05, ns- non significant. **B:** N>14 individual plants, **p<0.01, ****p<0.0001, ns- non significant.

Figure 4: TOR is required for the execution of developmental cues in the leaf

S. lycopersicum plants mutated in the BELL transcription factors *Double Dissected Leaf*: *ddl2* (**A, G**), *Bippinate*: *bip* (**B, G**), the MYB transcription factors, *CLAU*: *clausa* (**D, G**), and *C: potato leaf* (**F, G**), the TCP transcription factor *Lanceolate*: *La2/+* (**E, G**), or overexpressing miR390 under the leaf specific promoter BLS: *pBLS>>JAW* (**C, G**), were *TOR*-silenced using VIGS. 4 weeks after silencing, leaf complexity was quantified by counting the leaflets on leaves 3, 4, 5 or 6- as indicated.

Experiment was conducted 3 times. Boxplots represent inner quartile ranges (box), outer quartile ranges (whiskers), median (line in box), (A-E), or, bars represent Mean ±SEM (F), all points shown. Asterisks indicate statistically significant changes in leaf complexity upon TOR silencing as compared with the same leaf in empty-vector VIGSed plants, in a two-tailed t-test or a Mann-Whitney U test. **A:** N=6-11 individual plants, *p<0.05. **B:** N=9-14 individual plants, **p<0.01. **C**: N=7-11 individual plants, **p<0.01. **D**: N=14-22 individual plants, ***p<0.001. **E**: N=7-9 individual plants, *p<0.05. **F**: N=3-4 individual plants, ****p<0.0001.

Figure 5: TOR inhibition alters CK pathway and developmental gene expression

Gene expression analysis of the indicated CK pathway and developmental 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 mean between the gene copy number obtained for three reference genes: RPL8 (Solyc10g006580), EXP (Solyc07g025390), and CYP (Solyc01g111170), and normalized to Mock treatment. 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.

Figure 6: TOR inhibition reduces CK response in the meristem of young shoots

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.

TCSv2 driven total Venus fluorescence in the meristem (**A,B**) or total shoot (**C, D**) was measured as mean arbitrary fluorescent units (AFU, the strength of the signal, **A, C**) or corrected total fluorescence (CTF, a quantification taking into account the fluorescent area, **B, D**), in images captured under identical conditions in shoots comprising the 4 youngest primordia. 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, ns- non significant.

(**E**) 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.

Figure 7: CK reduces TOR activation

A-B *S. lycopersicum* cv. M82 10 day-old seedlings were treated with Mock (10 µM NaOH) or 10 µM of the CK 6-benzylaminopurine (6-BAP). 24 h after treatment, total cellular proteins were preapared from 3 biological replicas of 10 plants each, and TOR activation was assessed by detection of phosphorylated S6K using specific antibodies.

C-D Total cellular proteins were prepared from *S. lycopersicum* cv. M82, and *pBLS>>IPT* or *pFIL>>CKX* transgenic lines in the M82 background (3 biological replicas of 10 plants each). TOR activation was assessed by detection of phosphorylated S6K using specific antibodies.

Experiment was repeated 3 times. Boxplots represent inner quartile ranges (box), outer quartile ranges (whiskers), median (line in box), all points shown. **B** Asterisks indicates statistically significant reduction in phosphorylated S6K in a Mann Whitney U test, N=9, p<0.01. **D** Different letters indicate staistically significant differences between samples in a one-way ANOVA with a Tukey post hoc test, *p<0.05, N=7-14.

Figure 8: Model describing the role of TOR as a mediator of both development and defense cues, and the tradeoff between them.

Cytokinin promotes both development and defense. Balanced CK levels are required to achieve "normal" developmental patterning and disease resistance.

Under low CK, development and defense are inhibited, resulting in reduced patterning (Fig. 3), and increased disease susceptibitlity (Fig. 1). Under high CK, development and defense are promoted, resulting in increased patterning (Fig. 3) and disease resistance (Fig. 1).

Inhibition of TOR results in rescue of both low and high CK pehnotypes, resulting in return to baseline developmental patterning and disease resistance, and suggesting that TOR mediates these develpmental and defense cues originating from CK.

In the tradeoff between development and defense, high CK causes downregulation of TOR (Fig. 6), resulting in a shift towards defense. The switch between development and defense may be modulated by cross-talk between environmental sensing and TOR status.

Supplemental materials

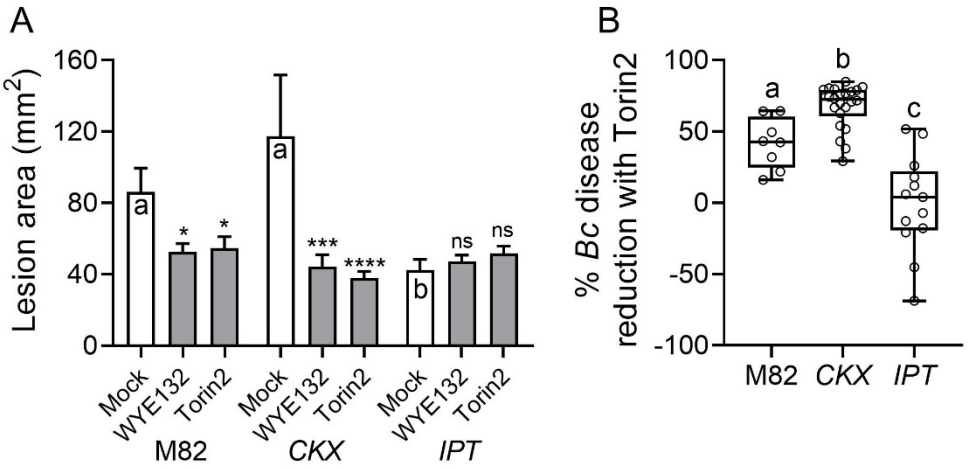

Figure S1: TOR inhibition with drugs mediates disease resistance downstream to CK

S. lycopersicum plants of the increased CK line *pBLS>>IPT7* ("*IPT*"), the decreased CK line *pFIL>>CKX3* ("*CKX*"), and their WT background M82, were treated with Mock (1:5000 DMSO in DDW) or 2 µM of the TOR inhibitors Torin2 (**A, B**) or WYE132 (**A**). Plants were challenged with *B. cinerea (Bc)* mycelia from a 72h old-culture, 24 h after drug treatment. **A**: *Bc* necrotic lesion size. **B**: percentage of disease reduction following TOR inhibition in the different genotypes. Bars represent mean ±SEM; boxplots represent inner quartile ranges (box), outer quartile ranges (whiskers), median (line in box), all points shown. Experiments were repeated 3 independent times.

A: Asterisks indicate statistically significant disease reduction with Torin2 or WYE132 treatment, and different letters indicate statistically significant differences among samples, in a one-way ANOVA with a Tukey post hoc test, N=9, $p<0.05$ (****$p<.0001$, ***$p<0.001$, *$p<0.05$, ns- not significant). **B:** Different letters indicate statistically significant differences between samples in ANOVA with a Tukey post-hoc test, N=9, $p<0.047$.

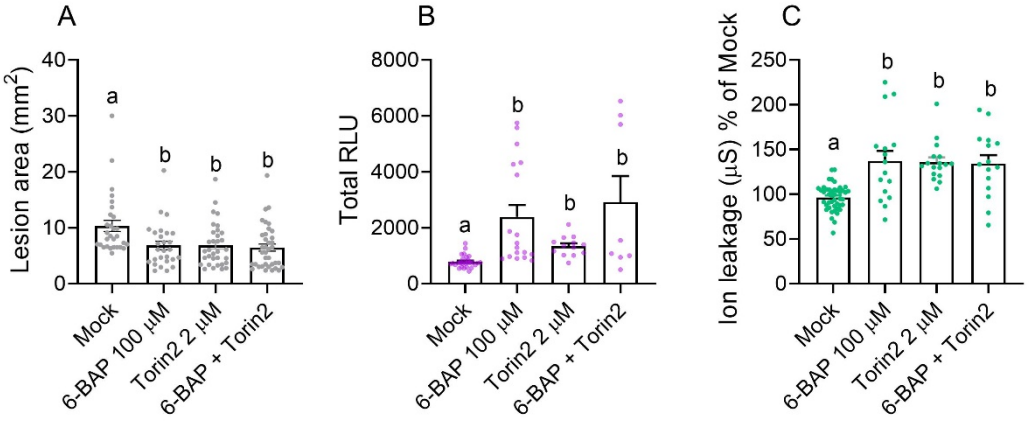

Figure S2: TOR inhibition mediates disease resistance downstream to CK: co-application of CK and Torin2.

S. lycopersicum cv M82 plants were treated with 100 µM of the CK 6-benzylaminopurine (6-BAP), 2 µM Torin2, or a combination of both. Plants were challenged with *B. cinerea (Bc)* mycelia from a 72h old-culture (**A**), or with the immunity elicitors flg-22 (1 µM) (**B**) or Xylanase (1 µg mL^{-1})(**C**), 24 h after CK and Torin2 treatments. **A** Lesion area was quantified 72 h after *Bc* inocculation. Boxplots represent inner quartile ranges (box), outer quartile ranges (whiskers), median (line in box), all points shown. Different letters indicate statistically significant differences in a one-way ANOVA with a Tukey post hoc test, N>25, p<0.017. **B** ROS production was measured immediately after flg-22 application every three minutes, using the HRP-luminol method, and expressed as the total Relative Luminescent Units (RLU) generated. Floating bars represent minimum to maximum values, line indicates median. Different letters indicate statistically significant differences in a one-way ANOVA with a Holm-Sidak post hoc test, N>8, p<0.01. **C** Conductivity of samples immersed in water for 40 h was measured. Mock average conductivity was defined as 100%. Bars represent mean ±SEM, all points shown. Different letters indicate statistically significant differences among samples in Welch's ANOVA with a Dunnett post hoc test, N>15, p<0.02.

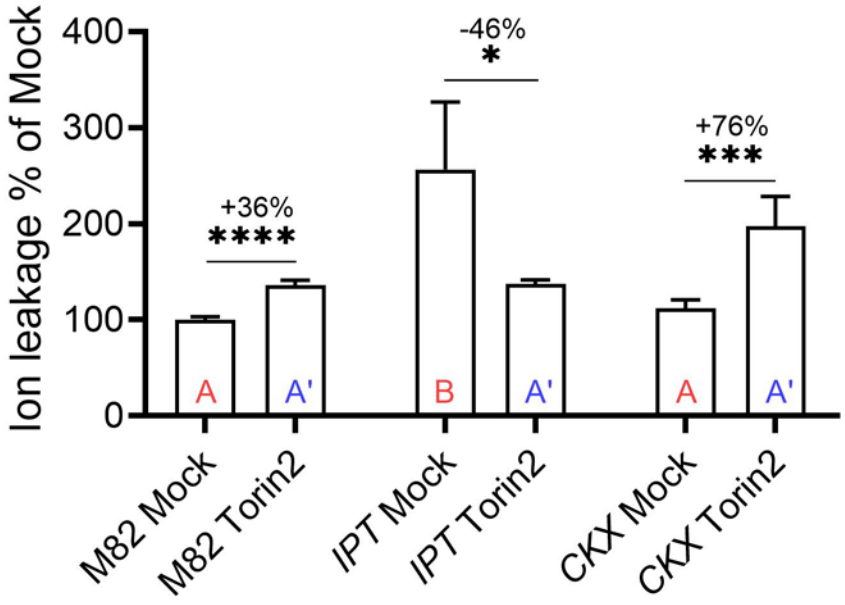

Figure S3: TOR inhibition mediates increased immunity downstream to CK- ion leakage

S. lycopersicum plants of the increased CK line *pBLS>>IPT7* ("*IPT*"), the decreased CK line *pFIL>>CKX3* ("*CKX*"), and their WT background M82, were treated with Mock (1:5000 DMSO in DDW) or 2 µM Torin2. Leaf discs were prepared 24 h after Torin2 treatment, and ion leakage sue to wounding, measured as conductivity of samples immersed in water for 40 h, was quantified. Mock average conductivity was defined as 100%. Bars represent mean ±SEM. Experiments were repeated 3 independent times, on at least 5 plants per experiment per treatment. Asterisks indicate significant differences in conductivity within each genotype following Torin2 treatment, and different letters indicate statistically significant differences between samples in Welch's ANOVA with a Dunnett post-hoc test, red letters for baseline differences in the wounding-response of the different Mock-treated genotypes, N=10, $p<0.0058$, and blue letters for the wounding response following Torin2 treatment, N=10, no significant differences. The percent change in the conductivity of each genotype following Torin2 treatment is indicated above the relevant bar.

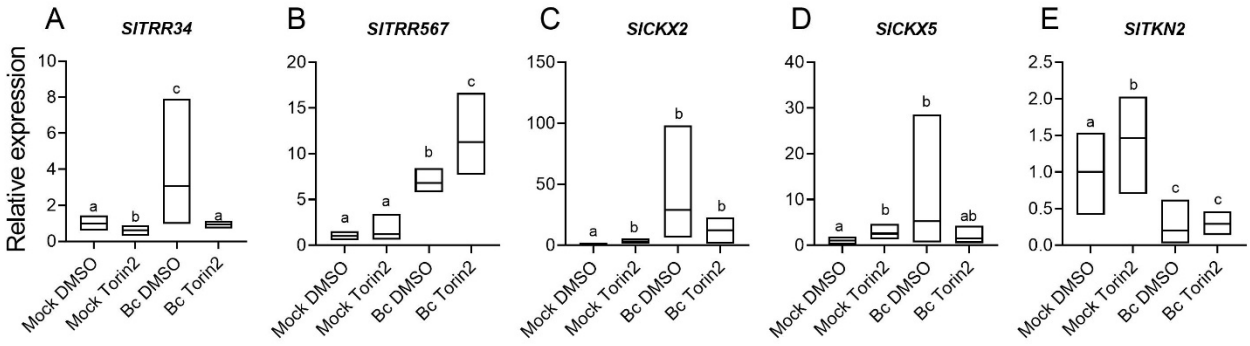

Figure S4: TOR inhibition and Bc infection have differential effects on CK pathway genes

Gene expression analysis of the indicated CK pathway and developmental genes, with and without Torin2 (2 µM) treatment, Bc infection, or combined Bc infection and Torin 2 treatment, was measured by RT-qPCR. Relative expression was calculated using the mean between the gene copy number obtained for three reference genes: RPL8 (Solyc10g006580), EXP (Solyc07g025390), and CYP (Solyc01g111170), and normalized to Mock treatment. Analysis was conducted on 6 biological samples comprised of 10 plants each, and repeated twice, N=12. Floating bars represent minimum to maximum values, line in bar represents the median. Different letters indicate statistically significant differences among samples in Welch's ANOVA (A, B), one-way ANOVA (E), or Kruskal Wallis ANOVA (C, D) tests comparing each gene, A: $p<0.013$; B: $p<0.047$; C: $p<0.001$; D: $p<0.008$; E: $p<0.05$.

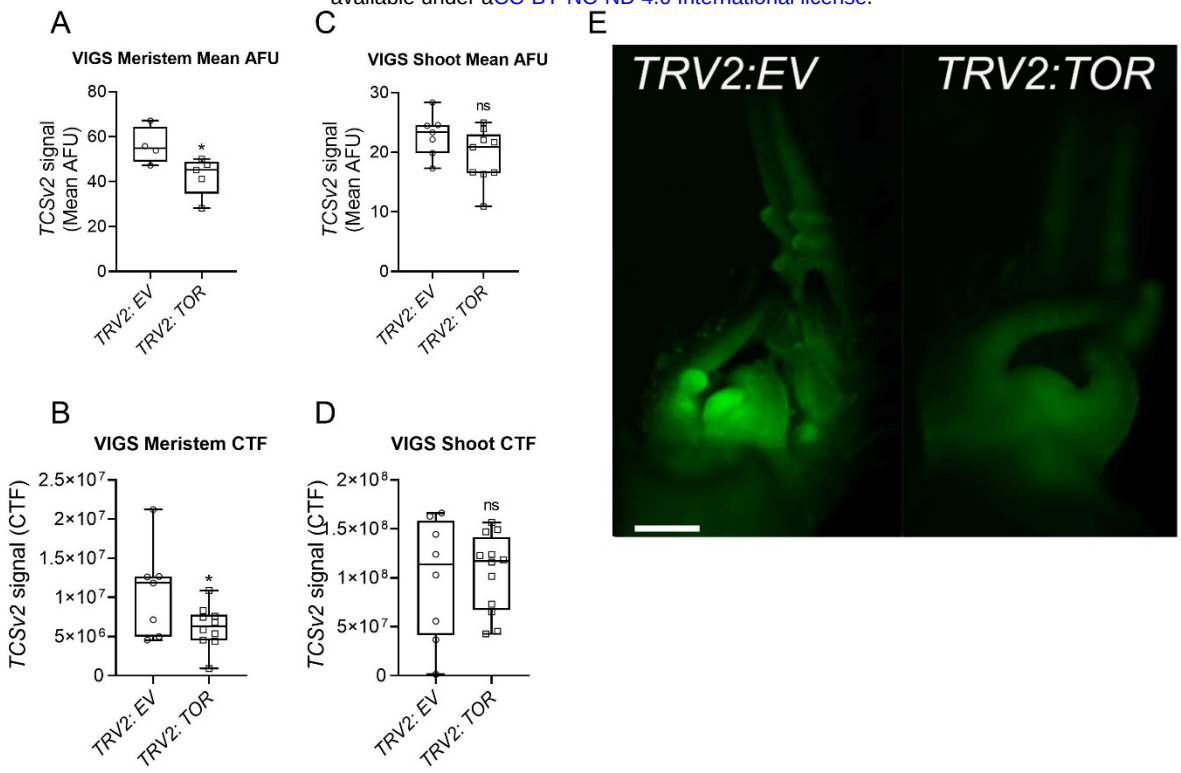

Figure S5: *TOR* silencing by VIGS reduces CK response in the meristem of young shoots

S. lycopersicum cv. M82 seedlings expressing VENUS driven by the cytokinin responsive promoter TCSv2 were *TOR*-silenced using VIGS. 2 weeks after silencing, TCSv2 driven total Venus fluorescence in the meristem (**A,B**) or total shoot (**C, D**) was measured as mean arbitrary fluorescent units (AFU, the strength of the signal, **A, C**) or corrected total fluorescence (CTF, quantification taking into account the fluorescent area, **B, D**), in images captured under identical conditions in shoots comprising the 4 youngest primordia. Boxplots represent inner quartile ranges (box), outer quartile ranges (whiskers), median (line in box), all points shown. Asterisks indicate significant TCSv2 signal reduction upon TOR silencing in a t-test, N>4, *p<0.05, ns- non significant. Typical Mock silenced (**E**) and TOR silenced (**F**) shoots are depicted. Bar- 1000 µM.

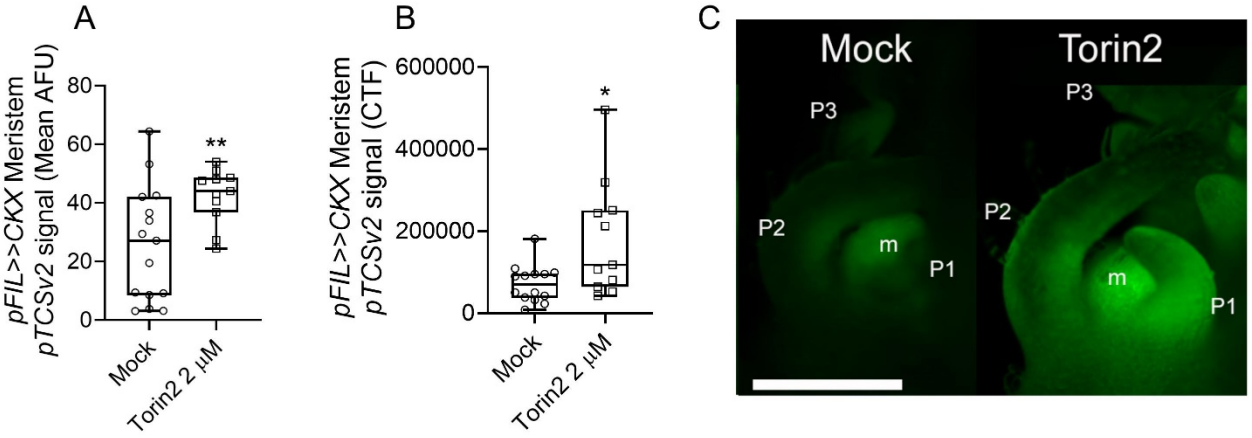

Figure S6: TOR inhibition increases CK response in the meristem of cytokinin deficient *pFIL>>CKX* shoots

S. lycopersicum pFIL>>CKX 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.

TCSv2 driven total Venus fluorescence in the meristem was measured as mean arbitrary fluorescent units (AFU, the strength of the signal, **A**) or corrected total fluorescence (CTF, quantification taking into account the fluorescent area, **B**), in images captured under identical conditions in shoots comprising the 3 youngest primordia. Boxplots represent inner quartile ranges (box), outer quartile ranges (whiskers), median (line in box), all points shown. Asterisks indicate significant TCSv2 signal increase upon Torin2 treatment in a t-test with Welch's correction, N>11, **p<0.01, *p<0.05.

Typical Mock treated and Torin2 treated shoots are depicted in **C**. Images captured under identical conditions. The meristem (m), first (P1), second (P2) and third (P3) youngest leaf primordia are indicated. Bar= 100 µM.

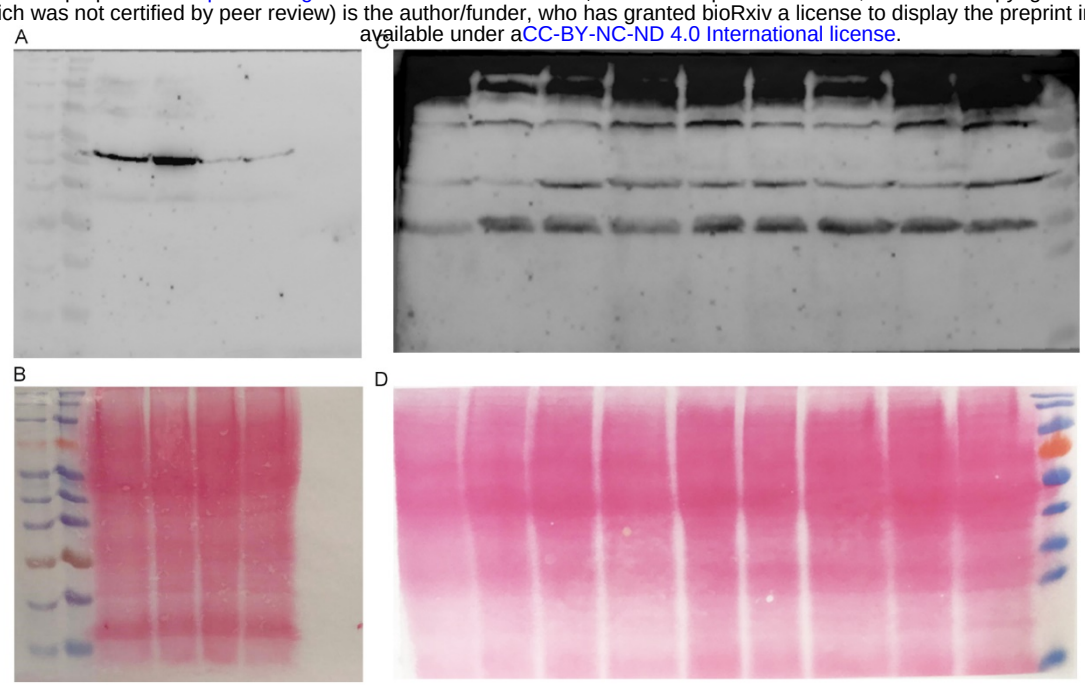

Figure S7: Original uncropped blots used in Figure 7.

A, C Anti-S6K1 p-Thr449 polyclonal antibody (AB-ab207399, Abcam).

B, D PonceauS staining.

Table S1: Primer pairs used for RT-qPCR

Locus	Name	Forward	Reverse
Solyc05g006420	TRR3/4	CGTCCCCTAAAGCATTCTCA	CGTCTTGTTGGTGATGTTGG
Solyc03g113720	TRR5/6/7	GGGATTGATGGTTTGAAGGT	ATCTTGCTCAACACCGATGA
Solyc01g088160	CKX2	CCCCGAAAATGGTGAAATG	CAAAGTGGCTTGCTTGAACA
Solyc04g016430	CKX5	TGTCACTGGTAAAGGAGAGGTG	GAGCAATCCTAGCCCTTGTG
Solyc10g006580	RPL8	TGGAGGGCGTACTGAGAAAC	TCATAGCAACACCACGAACC
Solyc01g111170	*CYP*	TGAGTGGCTCAACGGAAAGC	CCAACAGCCTCTGCCTTCTTA
Solyc07g025390	*EXP*	TGGGTGTGCCTTTCTGAATG	GCTAAGAACGCTGGACCTAATG
Solyc01g106770	*TOR* (VIGS)	GGTCTAGAATGGCTGCCACCGTTCAGGCGATCCG	GGGGATCCTTCGCTGATGGTGACATCTAT
Solyc02g081120	*TKN2*	CCATATCCATCGGAATCTCAG	TGGTTTCCAATGCCTCTTTC
Solyc04g008480	*CLAUSA*	CCTCTCACAACAAGCAATGAACTT	AGGACGATGCAATGAGAGAGAC

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