

1 **Title: Mutual Potentiation of Plant Immunity by Cell-surface and**
2 **Intracellular Receptors**

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10 **The plant immune system involves cell-surface receptors that detect intercellular pathogen-**
11 **derived molecules, and intracellular receptors that activate immunity upon detection of**
12 **pathogen-secreted effectors that act inside the plant cell. Surface receptor-mediated**
13 **immunity has been extensively studied¹, but intracellular receptor-mediated immunity has**
14 **rarely been investigated in the absence of surface receptor-mediated immunity.**
15 **Furthermore, interactions between these two immune pathways are poorly understood. By**
16 **activating intracellular receptors in the absence of surface receptor-mediated immunity, we**
17 **dissected interactions between the two distinct immune systems. Recognition by surface**
18 **receptors activates multiple protein kinases and NADPH oxidases; we find intracellular**
19 **receptors primarily potentiate the activation of these proteins by elevating their abundance**
20 **via multiple mechanisms. Reciprocally, the intracellular receptor-dependent hypersensitive**
21 **response is strongly enhanced by activation of surface receptors. Activation of either immune**
22 **system alone is insufficient to provide effective resistance against the bacterial pathogen**
23 ***Pseudomonas syringae*. Thus, immune pathways activated by cell-surface and intracellular**
24 **receptors mutually potentiate to activate strong defense that thwarts pathogens. These**

25 **findings reshape our understanding of plant immunity and have broad implications for crop**
26 **improvement.**

27 **Main Text**

28 Plant cell-surface pattern-recognition receptors (PRRs) recognize pathogen-associated molecular
29 patterns (PAMPs) and signal *via* plasma-membrane-associated co-receptor kinases, and
30 intracellular protein kinases¹. Ligand-dependent association between PRRs and these protein
31 kinases activates calcium influx, production of reactive oxygen species (ROS) via activation of
32 NADPH oxidases encoded by respiratory burst oxidase homolog (Rboh) genes, activation of
33 mitogen-activated protein kinases (MAPKs) and induction of defense genes¹.

34 Intracellular nucleotide-binding, leucine-rich-repeat-containing (NLR) receptors activate immune
35 responses upon recognition of pathogen effectors. Plant sensor NLRs carry either an N-terminal
36 coiled-coil (CC) domain, or an N-terminal Toll/Interleukin-1 receptor/Resistance protein (TIR)
37 domain^{2,3}. Upon activation, the CC-NLR ZAR1 forms pentameric resistosome complexes,
38 associates with plasma membranes (PMs) and likely perturbs their integrity⁴. The TIR-NLRs Roq1
39 and RPP1 form tetrameric resistosomes with effectors XopQ and ATR1, respectively^{5,6}. Upon
40 activation, plant TIR-NLRs require NADase activity of their TIR domains to activate defense⁷.
41 TIR-NLR signaling involves the lipase-like proteins EDS1, SAG101 and PAD4⁸. PRRs activate
42 pattern-triggered immunity (PTI), and NLRs effector-triggered immunity (ETI)⁹. How PTI and
43 ETI interact to arrest pathogens is poorly understood.

44 **ETI enhances PTI defense responses**

45 To study ETI without PTI, we generated an *Arabidopsis thaliana* (*Arabidopsis*) line with estradiol-
46 inducible expression of bacterial effector AvrRps4 recognized by an intracellular TIR-NLR pair,
47 RRS1 and RPS4 (RRS1/RPS4). Estradiol induces AvrRps4 expression and activates ETI^{AvrRps4}.
48 Pre-activation of ETI^{AvrRps4} elevates plant resistance against *Pseudomonas syringae* pv. *tomato*
49 (*Pst*) DC3000¹⁰. To test if ETI^{AvrRps4} potentiates PTI, we measured ROS production triggered by
50 flagellin-derived peptide flg22 (a bacterial PAMP) after pre-activating ETI^{AvrRps4}. ETI^{AvrRps4} pre-
51 activation elevates ROS production induced by flg22, but induction of ETI^{AvrRps4} alone does not
52 activate ROS production (Extended Fig 1a-b). Estradiol pre-treatment in an *eds1-2* mutant

53 background does not elevate flg22-induced ROS (Extended Fig 1c-d). Thus, ETI^{AvrRps4} enhances,
54 but does not initiate, PTI.

55 During bacterial infection, PTI activation precedes effector delivery. To mimic this, we treated
56 plants with flg22, or estradiol, or “flg22 + estradiol”, to activate PTI, or ETI^{AvrRps4} or “PTI +
57 ETI^{AvrRps4}”. Over 16 hours (h), “PTI + ETI^{AvrRps4}” shows elevated ROS compared to PTI alone,
58 particularly during Phase III of the burst (Fig 1a, b and Extended Fig 1e, f). ETI^{AvrRps4} enhances
59 ROS production triggered by other PAMPs (elf18, C10:0, nlp20 and chitin) and the DAMP pep1
60 (Extended Fig 2). We investigated if ETI mediated by CC-NLRs also potentiates PTI. The CC-
61 NLR RPS2 recognizes bacterial effector AvrRpt2⁹. We found ETI^{AvrRpt2} also elevates flg22-
62 induced ROS (Extended Fig 1h-j). Thus, ETI activated by both TIR- or CC-NLRs can enhance
63 ROS induced by PAMPs.

64 As “PTI + ETI” enhances the ROS burst of PTI alone, we assessed hydrogen peroxide (H₂O₂)
65 levels in leaves after activation of PTI, ETI^{AvrRps4} and “PTI + ETI^{AvrRps4}”. The non-virulent *Pst*
66 DC3000 *hrcC* mutant (*hrcC*⁻) induces PTI. Using diaminobenzidine (DAB) staining, “PTI +
67 ETI^{AvrRps4}”, but not PTI or ETI^{AvrRps4} alone, trigger strong H₂O₂ accumulation after 2 days (Fig 1c).
68 H₂O₂ promotes peroxidase-mediated cross-linking of proteins and phenolics in callose cell wall
69 appositions during PTI¹¹. ETI^{AvrRps4} alone induces some callose deposition (Fig 1d). Callose
70 deposition upon co-activation of PTI and ETI^{AvrRps4} (“PTI + ETI^{AvrRps4}”) is significantly higher
71 than the sum of that induced by PTI and ETI^{AvrRps4} alone (Fig 1d-e). Thus, PTI and ETI together
72 enhance callose deposition. Furthermore, the expression of PTI-responsive genes such as *FRK1*,
73 *NHL10*, *FOX1* is significantly higher 24 h after “PTI + ETI^{AvrRps4}” treatment compared to PTI or
74 ETI^{AvrRps4} alone (Fig 1f and Extended Fig 1g). In summary, PTI-induced physiological changes
75 are potentiated and enhanced by ETI.

76 Upon PAMP recognition, phosphorylation of the receptor-like cytoplasmic kinase subfamily VII
77 (RLCK-VII) member BIK1 activates the NADPH oxidase RbohD *via* phosphorylation at its 39th
78 and 343rd serine residues (S39 and S343). Activated RbohD produces extracellular ROS^{12,13}. PTI
79 also activates MAPKs, such as MPK3 and MPK6, contributing to transcriptional reprogramming
80 (Extended Fig 3a)¹⁴. We compared the activation of BIK1, RbohD and MAPKs during PTI and
81 “PTI + ETI^{AvrRps4}”. Both pre-activation and co-activation of ETI^{AvrRps4} result in prolonged flg22-

82 induced phosphorylation of BIK1, RbohD (at S39 and S343) and MPK3 (Figure 2a, b, Extended
83 Fig 3b-e). However, ETI^{AvrRps4} activation alone does not lead to phosphorylation of RbohD and
84 MAPKs (Figure 2c, d)¹⁰. To investigate how ETI potentiates PTI, we monitored accumulation of
85 BIK1, RbohD and MPK3 proteins during “PTI + ETI^{AvrRps4}” compared to PTI alone (Figure 2a, b
86 and Extended Fig 3d, e). More of these proteins accumulate during “PTI + ETI^{AvrRps4}” than during
87 PTI alone. We assessed protein levels of multiple PTI signaling components during ETI activated
88 in four additional inducible effector-expressing lines: AvrRpp4, AvrRpt2, AvrRpm1 and AvrPphB,
89 which are recognized by TIR-NLR RPP4 and CC-NLRs RPS2, RPM1 and RPS5, respectively
90 (Extended Fig 3f)¹⁵. ETI triggered by these effectors elevates protein accumulation of BAK1,
91 SOBIR1, BIK1, RbohD and MPK3 but not CERK1, FLS2, MPK4 and MPK6 (Extended Fig 3g).

92 Transcription and translation are strongly correlated during ETI¹⁶. We tested if PTI signaling
93 components are elevated by transcriptional induction. ETI triggered by different effectors strongly
94 elevates transcript abundance of *BAK1*, *SOBIR1*, *BIK1*, *RbohD* and *MPK3*, and weakly that of
95 *CERK1*, *FLS2*, *RbohF*, *MPK4* and *MPK6* (Extended Fig 4a, b). Both protein and transcript
96 accumulation of BIK1, RbohD and MPK3 during ETI^{AvrRps4} is EDS1-dependent (Extended Fig 4c,
97 d). Thus, ETI alone boosts transcription of many genes involved in PTI signaling. We performed
98 genome-wide expression profiling 4 h after induction of ETI^{AvrRps4} and found ~10% of the
99 transcriptome shows significant differential gene expression (Extended Fig 5a, b). Most
100 upregulated genes are enriched in immunity-related biological processes, especially PRR signaling
101 pathways (Fig 2c, Extended Fig 5c-e). Additional PTI signaling components such as *EFR*,
102 *PEPR1/2*, *LORE*, *LYK5*, *XLG2*, *CNGC19* and *MKK4/5* are highly upregulated during ETI^{AvrRps4}.
103 Thus, ETI-dependent gene induction elevates the abundance of PTI signaling components.

104 Previous studies suggest substantial overlap between PTI- and “PTI + ETI”-induced
105 transcriptional reprogramming^{17,18}. We tested if increases in PTI signaling components during ETI
106 are solely due to transcriptional activation. Transcript and protein levels of several PTI signaling
107 components were monitored over a 24-h time-course post ETI^{AvrRps4}-induction (Fig 3a, b).
108 Consistent with the protein level, *SOBIR1* and *BAK1* transcripts are highly induced by ETI^{AvrRps4}
109 (Fig 3a, b). However, *BIK1*, *RbohD* and *MPK3* mRNAs are upregulated briefly and then
110 downregulated after 3 h, while increases in their protein levels are sustained over 24 h (Fig 3a, b
111 and Extended Fig 6a-e). *CERK1*, *MPK4* and *MPK6* transcripts are weakly induced without

112 elevating protein abundance. In addition, ETI^{AvrRps4} and “PTI + ETI^{AvrRps4}” both lead to stronger
113 BIK1, RbohD and MPK3 accumulation compared to PTI, but their transcript levels differ only
114 slightly between different conditions (Extended Fig 6f). These results imply that increases in PTI-
115 signaling components during ETI^{AvrRps4} involves both transcriptional and post-transcriptional
116 regulation.

117 We investigated accumulation of PTI-signaling components during ETI^{AvrRps4} using the translation
118 inhibitor cycloheximide (CHX) and/or a proteasome inhibitor MG132. CHX blocks the
119 accumulation of BIK1, RbohD, MPK3 and BAK1 during ETI, but not MPK6 or Actin (Fig 3c and
120 Extended Fig 7a, b). MG132 treatment results in higher accumulation of BIK1 and RbohD but has
121 no effect on MPK3 or BAK1 (Fig 3c and Extended Fig 7a, b). MPK3 accumulation is similar
122 between the combined treatment of CHX and MG132 (“CHX + MG132”) and CHX alone
123 (Extended Fig 7b), suggesting that elevated MPK3 protein accumulation is likely due to increased
124 translation rather than decreased protein degradation. BIK1 and RbohD protein levels increase
125 with “CHX + MG132” treatment compared to those with CHX (Extended Fig 7b), implying that
126 protein turnover of BIK1 and RbohD also plays a role^{19,20}. However, this increase was not observed
127 with FLS2, BAK1 or epitope-tagged RPS4 (Extended Fig 7c). Since translational reprogramming
128 also contributes to immunity²¹, we compared abundance of ribosome-bound transcripts of *ICS1*,
129 *SOBIR1*, *BAK1*, *BIK1*, *RbohD* and *MPK3*, normalized to a housekeeping gene *EF1α* during mock
130 and ETI^{AvrRps4} treatment (Extended Fig 7d-f). ETI-induced increases in mRNA levels for *BIK1*,
131 *RbohD* and *MPK3* are matched by elevation in ribosome-loaded mRNA levels (Extended Fig 7g-
132 h). ETI thus elevates protein levels of PTI signaling components via multiple and distinct
133 mechanisms that will be the subject of future investigations.

134 **ETI functions through PTI**

135 Whether ETI and PTI activate the same or distinct mechanisms is poorly defined, because ETI
136 responses are rarely investigated in the absence of PTI. We tested whether (i) PTI provides the
137 main defense mechanism against pathogens and (ii) ETI enhances PTI by replenishing PTI
138 components, thus restoring effector-attenuated PTI.

139 We challenged plants with non-virulent *Pst* DC3000 *hrcC*⁻ and found protein levels of BIK1 and
140 RbohD are slightly elevated during PTI, and MAPKs are activated and show elevated

141 phosphorylation. After infiltration with a virulent strain *Pst* DC3000, PTI-induced protein
142 accumulation of BIK1 and RbohD, and MAPK activation is reduced compared to *hrcC*⁻, consistent
143 with effector-triggered susceptibility (ETS)⁹. We co-infiltrated plants with DC3000 and estradiol
144 to co-induce ETI^{AvrRps4} which restored protein levels of BIK1, RbohD and MPK3 and prolonged
145 activation of MAPKs (Extended Fig 8a). This indicates that ETI overcomes ETS and restores PTI
146 signaling capacity.

147 During natural infections, ETI is rarely activated without PTI. We hypothesized that ETI provides
148 robust resistance by restoring and elevating the abundance of PTI signaling components,
149 compensating for their turnover upon activation and attenuation by ETS (Extended Fig 8b). This
150 model implies NLR-mediated resistance functions through PTI. We tested if PTI is required for
151 NLR-dependent ETI-enhanced disease resistance by infiltrating the PTI-compromised mutants
152 *bak1-5 bkk1-1* and *fls2 efr* with *Pst* DC3000 delivering AvrRps4 (DC3000:AvrRps4)²².
153 Remarkably, *bak1-5 bkk1-1* is as susceptible as the NLR mutant *rps4-2 rps4b-2* that cannot detect
154 AvrRps4 (Figure 4a and Extended Fig 8c), while *fls2 efr* also showed enhanced susceptibility to
155 DC3000:AvrRps4 compared to wild type (Extended Fig 8d-g). These data show that PTI is
156 required for *RRS1/RPS4*-dependent resistance to bacteria, and that activation of ETI in the absence
157 of PTI is not sufficient for enhanced resistance against *P. syringae* in Arabidopsis. In addition,
158 Yuan *et al* (co-submitted manuscript, 2020-04-06411) provide complementary data, independently
159 showing that PTI is required for induced bacterial resistance mediated by multiple NLRs.

160 **PTI potentiates ETI-induced cell death**

161 ETI in the presence of PTI often culminates in hypersensitive cell death responses (HR).
162 Arabidopsis infiltration with a non-pathogenic *P. fluorescens* Pf0-1 delivering AvrRps4 (Pf0-
163 1:AvrRps4^{WT}) triggers “PTI + ETI^{AvrRps4}” and HR. However, ETI^{AvrRps4} alone does not lead to HR
164 (Extended Fig 9a)¹⁰. We used a Pf0-1 strain delivering a mutant allele of AvrRps4 (Pf0-
165 1:AvrRps4^{mut}) to activate PTI. Co-activation of PTI and ETI^{AvrRps4} results in HR and elevated
166 electrolyte leakage (a widely used indicator of cell death), unlike PTI or ETI^{AvrRps4} alone (Extended
167 Fig 9a, b). To test if other PTI-inducers also potentiate HR, we repeated the experiment with either
168 *hrcC*⁻ strain Pf0-1, a mixture of PAMPs and a DAMP (flg22, elf18 and pep1), or PAMPs or a
169 DAMP alone (flg22, elf18, pep1, C10:0, nlp20 or chitin) to activate PTI¹. In all cases, only PAMP

170 infiltration combined with ETI^{AvrRps4} triggers HR (Figure 4b and Extended Fig 9c). Thus, PTI
171 potentiates ETI-induced HR.

172 Like “PTI + ETI^{AvrRps4}”, co-activation of PTI and ETI^{AvrRpp4} causes HR, but not PTI or ETI^{AvrRpp4}
173 alone (Extended Fig 9e). In contrast, inducible expression of AvrRpt2, AvrRpm1 and AvrPphB
174 that are recognized by CC-NLRs can trigger HR in the absence of PTI (Extended Fig 9d). By
175 reducing levels of estradiol or dexamethasone, we defined sub-lethal levels of AvrRpt2, AvrRpm1
176 and AvrPphB induction. At these levels, CC-NLR mediated HR was also enhanced by PTI co-
177 activation (Extended Fig 9e). Thus, PTI activation enhances HR triggered by multiple NLRs.

178 MAPKs and Rboh proteins promote ETI-dependent HR^{23,24}. To understand PTI-enhanced ETI-
179 associated HR, we investigated the role of MAPKs and Rbohs during ETI alone. We found
180 MAPKs are phosphorylated during ETI^{AvrRpm1}, ETI^{AvrRpt2} and ETI^{AvrPphB}, but not during ETI^{AvrRps4}
181 or ETI^{AvrRpp4} (Fig 2c and Extended Fig 10a). However, none of the inducible ETIs led to RbohD
182 phosphorylation at S39 (Extended Fig 10b). ETI^{AvrRpt2} leads to RbohD phosphorylation at S343
183 and S347²⁵, which might explain why ETI^{AvrRpt2} activates a weak ROS burst (Extended Fig 1h-j).

184 Since ETI potentiates PTI-induced activation of MPK3 and RbohD, and ETI alone leads to weak
185 or no activation of these components, we tested if HR enhancement by PTI involves the ETI-
186 potentiated activity of MAPKs and NADPH oxidases. In an Arabidopsis line *MPK6SR*, an *mpk3*
187 *mpk6* double mutant is complemented by a mutant MPK6 allele (MPK6^{YG})²⁶. Activity of MPK6^{YG}
188 but not the wild-type MPK6 can be inhibited by an ATP analogue 1-NA-PP1²⁶. We tested the
189 response to Pf0-1:AvrRps4^{WT} (“PTI + ETI^{AvrRps4}”) in the *MPK6SR* line in the presence or absence
190 of 1-NA-PP1. Like others²³, we found inhibition of MPK6^{YG} in *MPK6SR* prevents ETI^{AvrRps4}-
191 associated HR even in the presence of PTI (Extended Fig 10c). Furthermore, HR induced by Pf0-
192 1:AvrRps4^{WT} is reduced in the NADPH oxidase mutant *rbohD rbohF* (Extended Fig 10d). Together,
193 these results demonstrate that the activation of MAPK and NADPH oxidases during “PTI +
194 ETI^{AvrRps4}” contributes to HR.

195 Discussion

196 We show here that ETI requires PTI to provide effective resistance. PTI can halt pathogens through
197 nutrient restriction, cell wall fortification, suppression of bacterial type III secretion and induction
198 of antimicrobial compounds^{11,27,28}. ETI enhances PTI-induced defense responses *via* upregulation

199 of PTI signaling components, and transcriptional, translational and/or protein turnover control
200 (Extended Fig 10e). How this is achieved for each PTI component remains to be determined. We
201 also show that the stronger immune response during “PTI + ETI” involves mutual potentiation of
202 these two systems.

203 Our data, and those of Yuan *et al* (~~co-submitted manuscript, 2020-04-06411~~), support a model in
204 which defenses activated by PRR-dependent signaling are the primary source of immunity, and
205 activated NLR receptors act to replenish PRR signaling components and enhance PRR-dependent
206 signaling, counteracting attenuation by turnover upon activation and by pathogen effectors (Fig
207 4c). In turn, PRR-mediated immunity can potentiate ETI outputs such as HR to further restrict
208 pathogen proliferation. These data are highly relevant to elevating crop disease resistance. Many
209 *NLR* genes are semi-dominant, suggesting ETI strength is rate-limiting for resistance²⁹. Thus, when
210 PTI is present, stacks of multiple *NLR* genes should provide physiologically stronger resistance,
211 as well as enhancing genetic durability, and are a potential source of non-host resistance³⁰. Other
212 reports have indicated synergistic functions of cell-surface and intracellular receptors in
213 mammalian immunity^{31,32}, highlighting the relevance of these insights to multiple host-pathogen
214 systems.

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287

288 **Fig. 1 | ETI potentiates PTI responses.** (a) “PTI + ETI^{AvrRps4}” leads to prolonged ROS production
289 from 300-960 mins (Phase III). Solid line represents mean \pm standard error of the mean (S.E.;
290 shaded curve). $n = 40$ leaf disks. (b) Total ROS production in “PTI + ETI^{AvrRps4}”-treated leaves is
291 significantly higher than PTI-treated leaves. $n = 120$ leaf disks from three independent
292 experiments. (c) “PTI + ETI^{AvrRps4}” leads to higher H₂O₂ accumulation than PTI or ETI^{AvrRps4}
293 alone. Scale bars represent 0.5 cm. $n = 12$ leaves. (d) “PTI + ETI^{AvrRps4}” leads to stronger callose
294 deposition than PTI or ETI^{AvrRps4} alone. Numbers represent the mean \pm S.E. (e) Callose deposition
295 in “PTI + ETI^{AvrRps4}”-treated leaves is higher than PTI- or ETI^{AvrRps4}-treated leaves. Mock: $n = 21$
296 leaves; PTI, ETI, “PTI + ETI”: $n = 23$ leaves. (b, e) Centre lines represent medians; bounds of box
297 indicate the 25th and 75th percentiles; whiskers represent 1.5 \times interquartile range from 25th and 75th
298 percentiles. Data points from 3 biological replicates were analyzed with one-sided Kruskal-Wallis
299 test with Holm correction, then followed by post hoc Dunn’s test. Data points with different letters
300 indicate significant differences of $P < 0.05$. P-values were adjusted with Holm correction, and
301 exact P-values can be found in Supplementary Table 5. (f) “PTI + ETI^{AvrRps4}” leads to a stronger
302 *FRK1*, *NHL10*, *FOX1* transcript accumulation compared to PTI or ETI^{AvrRps4} alone. Data points
303 from 3 independent experiments were plotted onto the graphs, with \pm S.E. for error bars. Two-sided
304 Welch’s t-test was used to analyze significant differences between “PTI + ETI^{AvrRps4}” and PTI or
305 ETI^{AvrRps4}. (*, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.005$; ****, $P \leq 0.001$; otherwise, not significant).
306 Exact P-values can be found in Supplementary Table 5. All experiments were repeated at least
307 three times with similar results.

308 **Fig. 2 | ETI potentiates activation of PTI signaling components.** (a) “PTI + ETI^{AvrRps4}” co-
309 activation leads to increased MPK3 accumulation and prolonged phosphorylation compared to
310 PTI. (b) “PTI + ETI^{AvrRps4}” co-activation leads to increased BIK1 and RbohD accumulation and
311 prolonged phosphorylation compared to PTI. (c) ETI^{AvrRps4} activation alone does not trigger
312 RbohD-S39 phosphorylation. (d) ETI^{AvrRps4} alone does not lead to MAPK activation. For (b, c),
313 microsomal fractions from the samples were isolated for immunoblotting. Molecular weight
314 marker (in kDa) is indicated on the left. Ponceau staining (PS) was used as loading control. (e)
315 RNA-seq results of the upregulation of PTI signaling pathway during ETI^{AvrRps4}. Heatmap
316 representing the expression level of PTI signaling pathway genes, salicylic acid (SA) and piperolic
317 acid (PIP) biosynthesis pathway genes and photosynthetic pathway genes at 4 h after ETI^{AvrRps4}

318 induction. Red represents upregulation and blue represents downregulation. All experiments were
319 repeated at least three times with similar results.

320 **Fig. 3 | Accumulation of PTI signaling components during ETI.** (a) Relative mRNA expression
321 changes of *SOBIR1*, *BAK1* (top panel), *BIK1*, *RbohD*, *MPK3* (middle panel), and *CERK1*, *MPK4*,
322 *MPK6* (bottom panel) upon ETI^{AvrRps4} induction. Samples were taken at indicated time points after
323 ETI^{AvrRps4} activation. All samples were normalized against expression of the corresponding genes
324 in untreated samples ($\log_2FC = 0$, dotted line). Solid line represents mean \pm S.E. (shaded band).
325 (b) Protein accumulation of Actin, SOBIR1, BAK1, BAK1, RbohD, MPK3, CERK1, MPK4 and
326 MPK6 at different time points; Actin is the loading control. Molecular weight is indicated on the
327 left. Ponceau staining (PS) was used as additional loading control and shown in Extended Fig 6d.
328 (c) Translation is necessary for the increased protein accumulation of MPK3, RbohD, BIK1, but
329 not MPK6 and Actin. 7-day-old seedlings were pre-activated with ETI^{AvrRps4} for 3 h and
330 subsequently treated with cycloheximide (50 μ M; CHX), MG132 (10 μ M), or both for indicated
331 times (2, 4, 8 h). Actin is loading control. Molecular weight (in kDa) is indicated on the left.
332 Ponceau staining (PS) images of corresponding blots are also shown. All experiments were
333 repeated at least three times with similar results.

334 **Fig. 4 | PTI and ETI function synergistically to provide robust immunity.** (a) Both PTI and
335 ETI^{AvrRps4} are required to provide effective immunity against *P. syringae*. Col-0, *rps4-2 rps4b-2*
336 and *bak1-5 bkk1-1* were infected with *P. syringae* pv. *tomato* (*Pst*) strain DC3000 carrying empty
337 vector (grey) or AvrRps4 (pink). Both *rps4-2 rps4b-2* (no ETI) and *bak1-5 bkk1-1* (PTI-reduced)
338 are insufficient to provide resistance against *Pst* DC3000 carrying AvrRps4 compared to Col-0
339 (“PTI + ETI”). $n = 18$ leaves. Centre lines represent medians; bounds of box indicate the 25th and
340 75th percentiles; whiskers represent 1.5 \times interquartile range from 25th and 75th percentiles. Data
341 points from 3 biological replicates were analyzed with one-way ANOVA, then followed by post
342 hoc Tukey’s HSD test. Data points with different letters indicate significant differences of $P <$
343 0.05. P-values were adjusted with Holm correction, and exact P-values can be found in
344 Supplementary Table 5. (b) ETI^{AvrRps4} leads to macroscopic HR only in the presence of PTI,
345 activated by either non-virulent *Pst* DC3000 *hrcC*, *P. fluorescens* Pf0-1 or mixture of flg22, elf18
346 and pep1 (PAMPs). $n = 18$ leaves. (c) Schematic representation of the plant immune system.
347 PAMPs from pathogens are recognized by plant PRRs and induce PTI (red). Virulent pathogens

348 secrete effectors to suppress PTI (green). Effectors are recognized by NLRs and induce ETI (dark
349 yellow arrow), which potentiates PTI to produce robust immune response (blue arrow). All
350 experiments were repeated at least three times with similar results.

351 **Methods**

352 Plant material and growth conditions

353 *Arabidopsis thaliana* Columbia-0 (Col-0) was used as wild type in this study. Seeds were sown on
354 compost and plants were grown at 21 °C with 10 h under light and 14 h in dark, and at 70%
355 humidity. The light level is approximately 180-200 μmol with fluorescent tubes. Information about
356 all plant materials can be found in the referred literatures^{26,33-37}, and were kindly provided by
357 Jeffery Dangl (Department of Biology, The University of North Carolina at Chapel Hill), Roger
358 Innes (Department of Biology, Indiana University), Shuta Asai (RIKEN, Japan), Shuqun Zhang
359 (Division of Biochemistry, University of Missouri), Xiufang Xin (Shanghai Institutes for Biology
360 Sciences, Chinese Academy of Sciences) and Cyril Zipfel (The Sainsbury Laboratory, UK).

361 ROS burst assay (pre-treatment with ETI)

362 Leaf discs harvested with a 6-mm-diameter cork borer from 5-week-old plants were placed in 96-
363 well plates with 200 μl of deionized water overnight in dark (with abaxial surface of the leaves
364 face down). Leaf discs were then soaked in mock solution (1% DMSO) or 50 μM est (estradiol to
365 trigger ETI^{AvrRps4}) for 6 h. 200 μl of 20 mM luminol (Sigma-Aldrich, A8511), 0.02 mg/ml
366 horseradish peroxidase (Sigma-Aldrich, P6782) and 100 nM flg22 were added in each well. ROS
367 production was measured with a Photek camera (East Sussex, UK). Data from each treatment is
368 represented by 40 leaf discs in one biological replicate. Every plate was measured over 55 mins.

369 ROS burst assay (co-treatment with ETI)

370 Leaf discs harvested with a 6-mm-diameter cork borer from 5-week-old plants were placed in 96-
371 well plates with 200 μl of deionized water overnight in dark (with abaxial surface of the leaves
372 face down). 200 μl of 20 mM luminol (Sigma-Aldrich, A8511), 0.02 mg/ml horseradish peroxidase
373 (Sigma-Aldrich, P6782) and indicated elicitors (concentration indicated in Supplementary Table
374 3) were added in each well. ROS production was measured with a Photek camera (East Sussex,
375 UK). Data from each treatment is represented by 40 leaf discs in one biological replicate. Every
376 plate was measured over the 16 h.

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DAB staining

3,3'-diaminobenzidine (Sigma-Aldrich, D8001) was dissolved in water (1 mg/ml) and the pH is adjusted to 6 with sodium hydroxide. Arabidopsis leaves were infiltrated with indicated solutions (concentration indicated in Supplementary Table 3). Two days after infiltration, leaves were vacuum infiltrated with DAB solution for 30 mins and incubated in room temperature for 2 h. The DAB solution was replaced with 100% ethanol and then boiled for 1 mins. The leaves are then further de-stained with 70% ethanol under room temperature. De-stained leaves were then scanned with EPSON Perfection V600 Photo. Scale bar = 0.5 cm.

Callose quantification

Leaves from 5-week-old Arabidopsis were hand-infiltrated with the indicated solutions (concentration indicated in Supplementary Table 3) and covered for 24 h. Leaves were then hand-infiltrated with 1× PBS buffer containing 0.01% Aniline Blue. Leaf discs were then harvested with a 6-mm-diameter cork borer for imaging. Images were taken by an epifluorescence microscope with UV filter (excitation, 365/10 nm; emission, 460/50 nm). The number of callose dots was calculated by ImageJ software. One leaf disc was harvested per leaf. At least 6 leaves from individual plants were included per treatment in one biological replicate.

Gene expression analysis by reverse transcription-quantitative polymerase chain reaction (RT-qPCR)

Arabidopsis thaliana tissues were treated with indicated solutions (concentration indicated in Supplementary Table 3) for indicated time point. Tissues were then snap-frozen and RNA was isolated by RNeasy Plant Mini Kit (74904; Qiagen) and used for subsequent RT-qPCR analysis. Reverse transcription was carried out with SuperScript IV Reverse Transcriptase (18090050; ThermoFisher Scientific). qPCR was performed with KAPA SYBR® FAST (Roche) using the CFX96 Touch™ Real-Time PCR Detection System. Primers for qPCR analysis are listed in Supplementary Information Table 2. Data were analyzed using the double delta Ct method³⁸.

Immunoblotting (pre-treatment with ETI)

5-week-old Est:AvrRps4 leaves were sprayed with either mock or 50 µM est solution (in 0.01% Silwet L-77) and covered for 6 h. Leaves were then infiltrated with 100 nM flg22. Samples were collected at indicated time points and snap-frozen in liquid nitrogen. Samples were lysed and

406 proteins were extracted using GTEN buffer (10% glycerol, 25 mM Tris pH 7.5, 1 mM EDTA, 150
407 mM NaCl) with 10 mM DTT, 1% NP-40 and protease inhibitor cocktail (cOmplete™, EDTA-free;
408 Merck), phosphatase inhibitor cocktail 2 (Sigma-Aldrich; P5726) and phosphatase inhibitor
409 cocktail 3 (Sigma-Aldrich; P0044). After centrifugation at 13,000× rpm for 10 mins to remove cell
410 debris, protein concentration of each sample was measured using the Bradford assay (Protein
411 Assay Dye Reagent Concentrate; Bio-Rad). After normalization, extracts were incubated with 2×
412 TruPAGE™ LDS Sample Buffer (Sigma-Aldrich) at 70 °C for 10 mins. SDS-PAGE gels of
413 different percentages were used to run protein samples of difference sizes. After transferring
414 proteins from gels to PVDF membranes (Merck-Millipore) using Trans-Blot Turbo System (Bio-
415 Rad), membranes were blocked with 5% nonfat dried milk in TBST for 1h, immunoblotted with
416 antibodies specified in Supplementary Information Table 1. Anti-Rabbit IgG (whole molecule)–
417 Peroxidase antibody produced in goat (A0545; Merck-Sigma-Aldrich) was used as secondary
418 antibody following the use of above antibodies. Ponceau S solution (P7170; Sigma-Aldrich) was
419 used to stain the PVDF membrane for loading control. For RbohD and BIK1, plasma membrane
420 protein was extracted for immunoblotting (see below).

421 Immunoblotting (co-treatment with ETI)

422 5-week-old est:AvrRps4 leaves were infiltrated with indicated solutions (concentration indicated
423 in Supplementary Table 3) for indicated time point. Tissues were then collected and snap-frozen.
424 Proteins were extracted and immunoblotting was performed as stated above. Concentrations of
425 primary antibodies are specified in Supplementary Information Table 1. Anti-Rabbit IgG (whole
426 molecule)–Peroxidase antibody produced in goat (A0545; Merck-Sigma-Aldrich) was used as
427 secondary antibody following the use of above antibodies. Ponceau S solution (P7170; Sigma-
428 Aldrich) was used to stain the PVDF membrane for loading control. For RbohD and BIK1, plasma
429 membrane protein was extracted for immunoblotting (see below).

430 Plasma membrane protein extraction

431 Minute™ Plant Plasma Membrane Protein Isolation Kit (Invent Biotechnologies, SM-005-P) was
432 used to extract total membrane fraction from Arabidopsis samples as instructed. Protein
433 concentration of the cytosolic fraction from each sample was measured using the Bradford assay
434 (Protein Assay Dye Reagent Concentrate; Bio-Rad). After normalization, total membrane fractions
435 were dissolved in 2× TruPAGE™ LDS Sample Buffer (Sigma-Aldrich) at 70 °C for 5 mins (in a

436 minimal volume of 80 μ l). 6% SDS-PAGE gels were used to run the protein samples. After
437 transferring proteins from gels to PVDF membranes (Merck-Millipore) using Trans-Blot Turbo
438 System (Bio-Rad), membranes were blocked with 5% nonfat dried milk in TBST for 1 h,
439 immunoblotted with either BIK1, pS39-RbohD or pS343-RbohD antibodies kindly provided by
440 Jian-Min Zhou (Institute of Genetics and Developmental Biology, Chinese Academy of
441 Sciences)¹³. Concentrations of primary antibodies are specified in Supplementary Information
442 Table 1. Anti-Rabbit IgG (whole molecule)–Peroxidase antibody produced in goat (A0545;
443 Merck-Sigma-Aldrich) was used as secondary antibody. Ponceau S solution (P7170; Sigma-
444 Aldrich) was used to stain the PVDF membrane for loading control.

445 Immunoblotting

446 5-week-old *Arabidopsis thaliana* leaves were treated with indicated solution (concentration
447 indicated in Supplementary Table 3). Tissues were then collected and snap-frozen. Proteins were
448 extracted and immunoblotting was performed as stated above. Concentrations of primary
449 antibodies are specified in Supplementary Information Table 1. Anti-Rabbit IgG (whole
450 molecule)–Peroxidase antibody produced in goat (A0545; Merck-Sigma-Aldrich) was used as
451 secondary antibody following the use of above antibodies. Ponceau S solution (P7170; Sigma-
452 Aldrich) was used to stain the PVDF membrane for loading control.

453 RNA-seq and data analysis

454 Leaves from 5-week-old *Arabidopsis* estradiol-inducible AvrRps4 (est:AvrRps4) or
455 est:AvrRps4^{mut}¹⁰ were hand-infiltrated with 50 μ M estradiol for 0 or 4 h. Samples were collected
456 and total RNA was isolated with TRI Reagent[®] (T9424; Sigma-Aldrich) and RNA Clean &
457 Concentrator-25 Kit (R1018; Zymo Research). RNA samples are processed by BGI and libraries
458 are sequenced with BGISEQ-500 sequencing platform. At least 10 M single-end 50-bp reads are
459 obtained for each RNA-seq library. Adaptor-trimmed clean reads have been uploaded to the
460 European Nucleotide Archive (ENA) (accession ID: PRJEB34955). After FastQC, Kallisto was
461 used to map and quantify RNA-seq reads³⁹, and kallisto_quant output files are submitted to the 3D
462 RNA-seq tool for statistics and data visualization⁴⁰. P-values for differentially expressed (DE)
463 genes were generated with Fisher Z-transformation after Student's *t*-test and were adjusted with
464 Benjamini and Hochberg's (BH) method⁴⁰.

465 Serial dilution to estimate protein abundance

466 Fold changes of BIK1, RbohD and MPK3 protein accumulation upon ETI^{AvrRps4} is estimated by
467 serial dilution. Protein samples of ETI^{AvrRps4} at 8 h were diluted 2× (1/2), 4× (1/4), 8× (1/8), 16×
468 (1/16) and 32× (1/32) in 2× TruPAGE™ LDS Sample Buffer (Sigma-Aldrich). Samples were then
469 loaded together with protein samples of ETI^{AvrRps4} at 0 h and ran on 10% SDS-PAGE gels. After
470 transferring the proteins from gels to PVDF membranes (Merck-Millipore) using Trans-Blot Turbo
471 System (Bio-Rad), membranes were blocked with 5% nonfat dried milk in TBST for 1 h,
472 immunoblotted with antibodies specified in Supplementary Information Table 1. Anti-Rabbit IgG
473 (whole molecule)–Peroxidase antibody produced in goat (A0545; Merck-Sigma-Aldrich) was
474 used as secondary antibody. Ponceau S solution (P7170; Sigma-Aldrich) was used to stain the
475 PVDF membrane for loading control.

476 Cycloheximide and MG132 treatment

477 1-week-old seedlings of est:AvrRps4 Arabidopsis transgenic line were grown in liquid MS
478 supplemented with 1% sucrose were pre-treated with 50 µM estradiol or mock (DMSO) for 3 h.
479 After pre-treatment, cycloheximide (CHX; 50 µM), MG132 (10 µM), or combination of CHX and
480 MG132 were treated to seedlings in addition to estradiol or mock. Seedlings were harvested 2 h,
481 4 h, and 8 h after inhibitor treatments. Upon protein extraction, protein concentration was measured
482 using Bradford assay, and protein samples were analyzed by immunoblotting as described above.

483 Enrichment of ribosome

484 Enrichment of ribosome was performed based on previous publications^{41,42} with modifications. 5-
485 week old Arabidopsis leaves of est:AvrRps4 were infiltrated with mock (1% DMSO) or 50 µM est
486 for 6 h. 0.6 g of leaves were harvested and ground in liquid nitrogen and extracted with 5 ml
487 extraction buffer (0.2 M Tris-HCl, pH 8.4, 50 mM KCl, 25 mM MgCl₂, 0.5% or Nonidet P-40, 50
488 µg/ml cycloheximide, RNase inhibitor (RNasin®, Promega). After centrifugation at 13,000× rpm
489 for 10 mins, supernatant was loaded onto a 1.6 M sucrose cushion. Samples were ultracentrifuged
490 at 170,000× g for 16 h. Pellet samples were resuspended in 1 ml DEPC-treated water, and 800 µl
491 was used for RNA extraction and qPCR analysis and 200 µl for protein extraction as described
492 above.

493 Bacterial growth assay

494 *Pseudomonas syringae* pv. *tomato* strain DC3000 carrying AvrRps4 or empty vector pVSP61 was
495 grown on selective King's B (KB) medium plates for 48 h at 28 °C. Bacteria were resuspended
496 and the concentration was adjusted to 0.001 at OD₆₀₀. Abaxial surfaces of 5-week-old Arabidopsis
497 leaves were infiltrated with bacterial solution by a 1-ml needleless syringe. For quantification, two
498 leaf discs per leaf were harvested with a 6-mm diameter cork borer (with disc area of 0.283 cm²).
499 For "day 0", samples were ground in infiltration buffer (10 mM MgCl₂) and spotted (10 µl/spot)
500 on selective KB medium. For "day 3", samples were ground in infiltration buffer, serially diluted
501 (into 5, 50, 500, 5,000, and 50,000 times), and spotted (6 µl/spot) on selective KB medium. The
502 number of colonies (CFU per drop) was calculated, and bacterial growth was represented as CFU
503 cm⁻² of leaf tissue.

504 HR assay in Arabidopsis

505 *Pseudomonas fluorescens* Pf0-1 engineered with a type III secretion system (Pf0-1 "EtHAn"
506 strains) expressing effectors, AvrRps4, AvrRps4^{KRVY135-138AAAA} (mutant AvrRps4; AvrRps4^{mut})⁴³,
507 or pVSP61 empty vector were grown on selective KB plates for 24 h at 28 °C. Wild-type
508 *Pseudomonas fluorescens* were grown on KB plates with chloramphenicol for 24 h at 28 °C.
509 *Pseudomonas syringae* pv. *tomato* strain DC3000 *hrcC*⁻ or DC3000 were grown on KB plates with
510 kanamycin for 48 h at 28 °C. Bacteria were harvested from the plates, resuspended in infiltration
511 buffer (10 mM MgCl₂) and the concentration was adjusted to indicated OD₆₀₀ (Supplementary
512 Information Table 3). The abaxial surfaces of 5-week-old Arabidopsis leaves were hand infiltrated
513 with indicated solution by a 1-ml needleless syringe. Cell death was monitored at indicated time
514 points after infiltration.

515 Electrolyte leakage assay

516 5-week-old Arabidopsis leaves were infiltrated with indicated solutions (Supplementary
517 Information Table 3) with a 1-ml needleless syringe. Leaf discs were collected with a 2.4-mm-
518 diameter cork borer from infiltrated leaves. Discs were dried and washed in deionized water for
519 1h before being floated on 10 ml deionized water (15 discs per sample, three samples per biological
520 replicate). Electrolyte leakage was measured as water conductivity with a Pocket Water Quality
521 Meters (LAQUAtwin-EC-33; Horiba) at the indicated time points.

522 Statistical data analysis

523 Statistical data were analyzed using the R software (<https://www.r-project.org/>), and the data were
524 plotted using the Origin software. For statistical analysis, all data were tested for homoscedasticity
525 with Levene's test, and normal distribution with Shapiro-Wilk test, and either parametric one-way
526 ANOVA analysis followed by Tukey's post-hoc HSD test, or non-parametric Kruskal-Wallis test
527 followed by Dunn's test were applied for statistical significance. Data points with different letters
528 indicate significant differences of $P < 0.01$ for Tukey's HSD test results, and $P < 0.05$ for Dunn's
529 test. Data points are plotted onto the graph, and number of samples for each data are indicated in
530 corresponding figure legends. Three biological replicates were tested, and individual biological
531 replicates are indicated with different shapes of the data points. qPCR assay results were analyzed
532 using two-sided Welch's t-test for statistical significance (*, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$;
533 ****, $P \leq 0.0001$; otherwise, not significant) between samples. Detailed information of sample
534 number, statistical analysis values for all experiments can be found in the Supplementary Table 5.

535 Generation of schematic figures

536 Schematic figures in Fig 2e, 4c, Extended Data Fig 3b, c, 5a, 7d and 10e were created with
537 BioRender.com.

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583 **Author contributions**

584 B.P.M.N., P.D. and J.D.G.J. conceived and conceptualized the study. B.P.M.N. performed the
585 ROS assay, DAB staining, callose quantification, gene expression analysis, immunoblotting,
586 protein serial dilution, RNA-seq, bacterial growth assay, HR assay and electrolyte leakage assay.
587 B.P.M.N performed plasma membrane protein extraction with assistance from H.K.A.. H.K.A
588 designed and performed the cycloheximide and MG132 experiment. H.K.A. and B.P.M.N.
589 performed enrichment of ribosomes. P.D. performed the RNA-seq analyses. H.K.A. performed the
590 statistical analyses. B.P.M.N. and P.D. wrote the original draft. B.P.M.N., H.K.A., P.D. and
591 J.D.G.J. reviewed and edited the manuscript.

592 **Competing interest declaration**

593 The authors declare no competing interests.

594 **Data availability**

595 All data in this study are available within the article and Supplementary Information. RNA-seq
596 data generated from this study are given in Supplementary Table 4. Statistical analyses of this
597 study are provided in Supplementary Table 5. The original sequence data of RNA-seq that support
598 the findings of this study have been deposited and made publicly available in the European
599 Nucleotide Archive (ENA) with the primary accession code “PRJEB34955”. All original gel blots
600 can be found in the Supplementary Figure 1. Source Data from Fig. 1-4 and Extended Data Fig.
601 1-9 are provided with the paper.

602 **Correspondence and requests for materials should be addressed to P.D. or J.D.G.J.**

603 **Additional Information**

604 Supplementary Information is available for this paper.

605 **Extended Data Fig. 1 | ETI^{AvrRps4} and ETI^{AvrRpt2} potentiates PTI responses. (a)** Estradiol pre-
606 treatment in est:AvrRps4 leads to stronger and prolonged ROS burst compared to mock pre-
607 treatment. n = 40 leaf disks. **(b)** ROS accumulation over 55 mins in ETI^{AvrRps4}-pretreated leaves is
608 significantly higher than mock-pretreated leaves. n = 120 leaves over 3 independent experiments.
609 **(c)** Pre-treatment of estradiol in est:AvrRps4 *eds1-2* does not lead to stronger and prolonged ROS
610 burst compared to mock pre-treatment. n = 40 leaf disks. **(d)** ROS accumulation over 55 mins in

611 ETI^{AvrRps4}-pretreated leaves in the *eds1-2* is comparable to mock-pretreated leaves. $n = 120$ leaves
612 over 3 independent experiments. **(e)** ROS accumulation of PTI, ETI^{AvrRps4} and “PTI + ETI^{AvrRps4}”
613 treated leaves during Phase I (0-60 mins), Phase II (60-300 mins) and Phase III (300-960 mins). n
614 = 120 leaves over 3 independent experiments.. **(f)** Summary table of ROS accumulation in different
615 phases. **(g)** “PTI + ETI^{AvrRps4}” leads to a stronger *PER4*, *WRKY31* transcript accumulation
616 compared to PTI or ETI^{AvrRps4} alone. *ICS1* transcript is induced upon “PTI + ETI^{AvrRps4}” as well as
617 ETI^{AvrRps4} alone. Data points from 3 independent experiments were plotted onto the graphs, with
618 \pm S.E. for error bars. Two-sided Welch’s t-test was used to analyze significance differences
619 between PTI + ETI^{AvrRps4} and PTI or ETI^{AvrRps4} (*, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.005$; ****, P
620 ≤ 0.001 ; otherwise, not significant). Exact P values can be found in Supplementary Table 5. **(h)**
621 PTI + ETI^{AvrRpt2} leads to prolonged ROS production during Phase II. $n = 40$ leaf disks. **(i)** ROS
622 accumulation of PTI, ETI^{AvrRpt2} and PTI+ETI^{AvrRpt2} treated leaves during Phase I, Phase II and
623 Phase III and in total. $n = 120$ leaves over 3 independent experiments. **(j)** Summary table of ROS
624 accumulation in different phases. For **(a)**, **(c)**, **(h)** Solid line represents mean \pm S.E. (shaded curve)
625 from one biological replicate. For **(b)**, **(d)**, **(e)**, **(i)**, data points from 3 independent experiments
626 were analyzed with one-sided Kruskalis-Wallis test followed by post hoc Dunn’s test. Different
627 letters next to the boxplot indicate significant differences of $P < 0.05$. Centre lines represent the
628 medians; bounds of box indicate the 25th and 75th percentiles; whiskers represent 1.5 \times interquartile
629 range from 25th and 75th percentiles. P-values were adjusted using Holm correction, and exact P-
630 values can be found in Supplementary Table 5. All experiments were repeated at least three times
631 with similar results.

632 **Extended Data Fig. 2 | ETI^{AvrRps4} enhances ROS production triggered by different PAMPs**
633 **and DAMP. (a-c)** elf18-triggered ROS production in the presence of ETI^{AvrRps4} is stronger than
634 elf18 treatment alone. **(d-f)** pep1-triggered ROS production in the presence of ETI^{AvrRps4} is stronger
635 than pep1 treatment alone. **(g-i)** C10:0-triggered ROS production in the presence of ETI^{AvrRps4} is
636 stronger than C10:0 treatment alone. **(j-l)** nlp20-triggered ROS production in the presence of
637 ETI^{AvrRps4} is stronger than nlp20 treatment alone. **(m-o)** Chitin-triggered ROS production in the
638 presence of ETI^{AvrRps4} is stronger than chitin treatment alone. Shaded curves in **(a)**, **(d)**, **(g)**, **(j)**,
639 **(m)** represent standard error (S.E.) and solid line represents mean.. $n = 40$ leaf disks. ROS
640 production in Phase I, Phase II, Phase III and total are shown as boxplots in **(b)**, **(e)**, **(h)**, **(k)** and
641 **(n)**. Centre lines represent the medians; bounds of box indicate the 25th and 75th percentiles;

642 whiskers represent 1.5× interquartile range from 25th and 75th percentiles. Data points from 3
643 independent experiments were analyzed with one-sided Kruskalis-Wallis test followed by post hoc
644 Dunn’s test. Different letters next to the boxplot indicate significant differences of $P < 0.05$. $n =$
645 120 leaves over 3 independent experiments. P values were adjusted using Holm correction, and
646 exact P values can be found in Supplementary Table 5. (c), (f), (i), (l), (o) Tabular summary of
647 total ROS production in different phases upon different PAMPs or DAMP treatments with
648 ETI^{AvrRps4} co-activation. All experiments were repeated at least three times with similar results.

649 **Extended Data Fig. 3 | Protein accumulation of PTI signaling components during ETI. (a)**
650 PTI signaling pathway. (b-c) Schematic representation of “natural infection mimicking” and “ETI
651 pre-activation” experimental design. ETI^{AvrRps4} was activated by estradiol treatment. * indicates
652 activated immune system. (red: PTI activation; yellow: ETI activation, blue: PTI and ETI co-
653 activation). (d) Pre-activation of ETI^{AvrRps4} leads to accumulation and prolonged phosphorylation
654 of MPK3 compared to mock pre-treatment. (e) Pre-activation of ETI^{AvrRps4} leads to accumulation
655 and prolonged phosphorylation of BIK1 and RbohD (S39 and S343) compared to mock pre-
656 treatment. Microsomal fractions from each sample were isolated for immunoblotting. Molecular
657 weight marker (in kDa) is indicated on the left. Ponceau staining (PS) was used as loading control.
658 (f) Transcript induction of corresponding effectors and *ICS1* upon induced expression of *AvrRpm1*
659 (dex:AvrRpm1), *AvrRpt2* (est:AvrRpt2), *AvrPphB* (est:AvrPphB), *AvrRps4* (est:AvrRps4) and
660 *AvrRpp4* (est:AvrRpp4). Extracted RNA were analyzed by qPCR and expression level is presented
661 as relative to *EF1α*. Data points from 3 independent experiments were plotted onto the graphs,
662 with ±S.E. for error bars.. Two-sided Welch’s t-test was used to analyze significance in differences
663 of 4 h, 8 h data points from 0h. (*, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.005$; ****, $P \leq 0.001$;
664 otherwise, not significant). Exact P-values can be found in Supplementary Table 5. (g) Protein
665 accumulation of BAK1, SOBIR1, BIK1, RbohD, MPK3, MPK6, FLS2, CERK1 and MPK4 upon
666 ETI activation for 4 h, 8 h in multiple effector inducible lines. 5-week-old leaves of inducible-
667 AvrRpm1, AvrRpt2, AvrPphB, AvrRps4 and AvrRpp4 lines were infiltrated with 50μM dex (for
668 dex:AvrRpm1) or 50μM est. Samples were collected at 0, 4 and 8 h post infiltration (hpi) for
669 protein extraction. Molecular weight marker (in kDa) is indicated on the left. Ponceau staining

670 (PS) were used as loading control. All experiments were repeated at least three times with similar
671 results.

672 **Extended Data Fig. 4 | Transcript accumulation of PTI signaling components during ETI.**

673 (a) Relative gene expression of *BAK1*, *SOBIR1*, *BIK1*, *RbohD*, *MPK3*, *MPK6*, *FLS2*, *CERK1*,
674 *MPK4* and *RbohF* relative to *EF1 α* in multiple effector-inducible lines. 5-week-old leaves of
675 inducible-AvrRpm1, AvrRpt2, AvrPphB, AvrRps4 and AvrRpp4 lines were infiltrated with 50 μ M
676 dex (for dex:AvrRpm1) or 50 μ M est. Samples were collected at 0, 4 and 8 hpi for RNA extraction.
677 (b) Heatmap of fold-changes (\log_2 FC) of *BAK1*, *SOBIR1*, *BIK1*, *RbohD*, *MPK3*, *MPK6*, *FLS2*,
678 *CERK1*, *MPK4* and *RbohF* from (a). Gene expression at 4 h and 8 h was normalized to expression
679 level at 0 h. Red indicates upregulation and blue indicates downregulation. (c) Protein
680 accumulation of BIK1, RbohD, and MPK3 during ETI^{AvrRps4} is abrogated in *eds1-2*. Proteins were
681 extracted from est:AvrRps4 and est:AvrRps4 *eds1-2* upon est treatment for 0 h, 4 h, and 8 h.
682 Molecular weight marker (in kDa) is indicated on the left. Ponceau staining (PS) were used as
683 loading control. (d) Transcript induction of *BIK1*, *RbohD*, and *MPK3* during ETI^{AvrRps4} is
684 abrogated in *eds1-2*. For (a) and (d), extracted RNA were analyzed by qPCR and expression level
685 is presented as relative to *EF1 α* . Data points from 3 independent experiments were plotted onto
686 the graphs, with \pm S.E. for error bars. Two-sided Welch's t-test was used to analyze significance in
687 differences of 4 h, 8 h data points from 0h (*, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.005$; ****, $P \leq$
688 0.001; otherwise, not significant). Exact P-values can be found in Supplementary Table 5. All
689 experiments were repeated at least three times with similar results.

690 **Extended Data Fig. 5 | Genome-wide gene expression profiling of ETI^{AvrRps4}.**

691 (a) Schematic design of RNA-seq analysis. 5-week-old inducible lines of wild-type AvrRps4 (est:AvrRps4) and
692 mutant AvrRps4 (estradiol-inducible AvrRps4^{KRVY135-138AAAA}-expressing line or est:AvrRps4^{mut})
693 were infiltrated with mock or 50 μ M est and samples were collected at 0 h, and 4 h. Samples from
694 three biological replicates were collected for RNA-seq analysis. (b) 2573 differentially expressed
695 (DE) genes were identified as significant in comparison between est:AvrRps4 treated with
696 estradiol for 0 h (est:AvrRps4, Est-0h) and est:AvrRps4 treated with est for 4 h (est:AvrRps4, Est-
697 4h). P values for differentially expressed (DE) genes were generated with Fisher Z-transformation
698 after Student's *t*-test. DE genes with "Benjamini and Hochberg's (BH) method" false discovery
699 rate (FDR) two-sided adjusted P-value (adj.pval) < 0.01 are categorized as significant. Heatmap

700 representing the 2573 DE genes during 5 treatments; est:AvrRps4 (Untreated), est:AvrRps4 treated
701 with est for 0 h (Est-0h), est:AvrRps4 treated with est for 4 h (Est-4h), est:AvrRps4^{mut} treated with
702 est for 0 h (Est-0h) and est:AvrRps4^{mut} treated with est for 4 h (Est-4h). Genes that are specifically
703 upregulated during ETI^{AvrRps4} are in cluster 7 and 8. **(c-e)** GO enrichment analysis of genes from
704 cluster 7 and 8. **(c)** Top three significantly enriched biological process GO-terms in cluster 7 and
705 8. **(d)** Top four significantly enriched molecular function GO-terms in cluster 7 and 8. **(e)** Top four
706 significantly enriched cellular component GO-terms in cluster 7 and 8. For details of GO
707 enrichment analysis refer to Source Data. **(f)** Red (positive log₂FC (fold change)) represents genes
708 that are significantly induced and blue (negative log₂FC) represents genes that are significantly
709 repressed. BH-FDR two-sided adjusted P-value (adj.pval) < 0.05 is considered as significant.
710 Gradient of green color indicates significance of the adjusted P-value. For full list of DE genes
711 refer to Supplementary Table 4.

712 **Extended Data Fig. 6 | Expression dynamics of PTI signaling components during ETI^{AvrRps4}.**
713 **(a)** Transcript induction of *SOBIR1*, *BAK1*, *BIK1*, *RbohD*, *MPK3*, *CERK1*, *MPK4*, *MPK6*, *ICS1*,
714 *PR1* during ETI^{AvrRps4} over 24 h. Transcript levels were normalized to *EF1a*. Data points from 3
715 independent experiments were plotted onto the graphs, with ±S.E. for error bars. Two-sided
716 Welch's t-test was used to analyze significance in differences of data points from ETI^{AvrRps4}-
717 activated samples compared to untreated (UNT) samples (*, P ≤ 0.05; **, P ≤ 0.01; ***, P ≤ 0.005;
718 ****, P ≤ 0.001; otherwise, not significant). Exact P values can be found in Supplementary Table
719 5. **(b)** Relative mRNA expression changes of *ICS1* (green) and *PR1* (black) during ETI^{AvrRps4}.
720 Relative expression changes of the corresponding genes to untreated samples (Log₂FC = 0, dotted
721 line) are shown. Solid line represents mean ± S.E. (shaded band). **(c)** Heatmap representing fold-
722 changes (log₂FC) of transcripts from **(a)**. Gene expression at indicated time points are relative
723 value to untreated samples. Red indicates upregulation and blue indicates downregulation. **(d)**
724 Protein accumulation of PR1 at different time points. Ponceau staining of western blots from Fig.
725 3b are also shown. **(e)** Serial dilution to estimate protein accumulation of BIK1, RbohD and MPK3
726 at 8h after ETI^{AvrRps4} activation compared to 0 h. Red asterisk indicates approximate fold
727 differences between 0 h and 8 h. **(f)** 5-week old Arabidopsis rosette leaves of est:AvrRps4 were
728 treated with *hrcC*, est, or "*hrcC* + est" for indicated timepoints and both RNA and proteins were
729 extracted. Extracted RNA were analyzed by qPCR and expression level is presented as relative to
730 *EF1a*. Data points from 3 independent experiments were plotted onto the graphs, with ±S.E. for

731 error bars (PTI: red; ETI^{AvrRps4}: yellow; “PTI + ETI^{AvrRps4}”: blue). Two-sided Welch’s t-test was
732 used to analyze significance in differences of 4h, 8h data points from 0h (*, $P \leq 0.05$; **, $P \leq 0.01$;
733 ***, $P \leq 0.005$; ****, $P \leq 0.001$; otherwise, not significant). Exact P-values can be found in
734 Supplementary Table 5. For (d), (e), (f), Ponceau staining (PS) was used as loading control.
735 Molecular weight marker (in kDa) is indicated on the left. All experiments were repeated at least
736 three times with similar results.

737 **Extended Data Fig. 7 | Multiple mechanisms are involved in the upregulation of PTI signaling**
738 **components during ETI^{AvrRps4}.** (a) Relative gene expression of *ICS1*, *BIK1*, *RbohD*, *MPK3* and
739 *MPK6* in seedlings pre-activated with ETI^{AvrRps4} for 3 h prior to treatment with cycloheximide
740 (CHX) and MG132. Data points from 3 independent experiments were plotted onto the graphs,
741 with \pm S.E. for error bars.. Two-sided Welch’s t-test was used to analyze significance in differences
742 at 3h compared to 0h (*, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.005$; ****, $P \leq 0.001$; otherwise, not
743 significant). Exact P values can be found in Supplementary Table 5. (b, c) Protein accumulation
744 of MPK3, RbohD, BIK1, MPK6, Actin in seedlings pre-treated with Mock (DMSO) for 3 h (b),
745 and RPS4-HA, FLS2, and BAK1 (c) in seedlings pre-treated with Mock or est, subsequently
746 treated with CHX (50 μ M), MG132 (10 μ M), or both for indicated times (2 h, 4 h, 8 h). Actin was
747 used as loading control. Ponceau staining (PS) of corresponding blots are shown below. For FLS2
748 and Actin, as well as BAK1 and BIK1, immunoblot was performed with membranes cut in half
749 (above 70 kDa for FLS2, BAK1, respectively, below 70 kDa for Actin and BIK1 immunoblot,
750 respectively). Therefore, Ponceau staining (PS) for FLS2 and Actin, BAK1 and BIK1, respectively,
751 are identical. (d) Schematic representation of ribosome enrichment. (e-f) Ribosome was enriched,
752 and (e) total extract (T), supernatant (S), and ribosomal pellet (P) samples were blotted with RPS6
753 and RPL10 antibody. For (b), (c) and (e), Ponceau staining (PS) was used as loading control.
754 Molecular weight marker (in kDa) is indicated on the left. (f) RNA extracted from total extract
755 (Total RNA), and ribosomal pellet (Ribosome RNA) from mock and est-treated est:AvrRps4
756 samples were loaded on an agarose gel. 28S and 18S rRNA are indicated. (g) Relative expression
757 of *ICS1*, *SOBIR1*, *BAK1*, *BIK1*, *RbohD* and *MPK3* to *EF1 α* from total RNA (Total) and ribosomal
758 pellet (Ribosomal). Data points from 3 independent experiments were plotted onto the graphs,
759 with \pm S.E. for error bars. Two-sided Welch’s t-test was used to analyze significance in differences
760 of 6 h compared to 0 h (*, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.005$; ****, $P \leq 0.001$; otherwise, not
761 significant). Exact P-values can be found in Supplementary Table 5. (h) Ratio of ribosomal RNA

762 to total RNA (relative to *EF1α*) of *ICSI*, *SOBIR1*, *BAK1*, *BIK1*, *RbohD* and *MPK3* in mock and
763 ETI samples. Values are calculated from the transcripts retained in the ribosomal samples over
764 total samples. Data points from 3 independent experiments were plotted onto the graphs, with
765 \pm S.E. for error bars. All experiments were repeated at least three times with similar results. Two-
766 sided Welch's t-test was used to analyze significance in differences of the translation efficiency
767 (T.E.) between Mock and ETI-treated samples (*, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.005$; ****, P
768 ≤ 0.001 ; otherwise, not significant). Exact P-values can be found in Supplementary Table 5.

769 **Extended Data Fig. 8 | ETI functions through PTI.** (a) 5-week-old leaves of *est:AvrRps4* were
770 infiltrated with *Pst* strain DC3000 *hrcC*⁻ (*Pst hrcC*⁻; triggers PTI), *Pst* DC3000 (*Pst*; triggers “PTI
771 + ETS”), or “50 μ M *est* + *Pst hrcC*” (triggers “PTI - ETS + ETI^{AvrRps4}”), and samples were
772 collected at the indicated time points for protein extraction and immunoblotting. PTI leads to
773 activation of MAPKs and accumulation of BIK1 and RbohD (red). *Pst* secretes effectors to block
774 PTI (green). Co-activation of PTI and ETI^{AvrRps4} leads to stronger accumulation of MPK3, BIK1
775 and RbohD compared to PTI (blue). MAPKs activation is also prolonged during “PTI +
776 ETI^{AvrRps4}”. (b) Updated version of the “zig-zag-zig” model. (c) Col-0, *rps4-2 rps4b-2* and *bak1-
777 5 bkk1-1* were infected with *Pst* DC3000 carrying AvrRps4 (red) or empty vector (grey). Bacterial
778 growth at 0 dpi as measured. $n = 12$ leaves. (d) Col-0, *rps4-2 rps4b-2* and *fls2 efr* were infected
779 with *Pst* DC3000 carrying AvrRps4 (red) or empty vector (grey). Both *rps4-2 rps4b-2* (No ETI)
780 and *fls2 efr* (PTI-reduced) are insufficient to provide resistance against *Pst* DC3000:AvrRps4
781 compared to Col-0 (“PTI + ETI”). Day 0: $n = 12$ leaves; day 3: $n = 18$ leaves. For (c), (d), data
782 points were analyzed by one-way ANOVA followed by post hoc Tukey's HSD test. Data points
783 with different letters indicate significant differences of $P < 0.01$. (e) flg22-induced ROS burst is
784 not affected in *rps4-2 rps4b-2*. Shaded curve represents standard error (S.E.) and solid line
785 represents average value from 24 leaves in each treatment during $n = 24$ leaves. (f) flg22-induced
786 ROS production over 55 mins in Col-0 and *rps4-2 rps4b-2*. Data points from 3 biological replicates
787 were analyzed with one-sided Kruskal-Wallis test followed by post hoc Dunn's test. Data points
788 with different letters indicate significant differences of $P < 0.05$. $n = 72$ leaves over 3 independent
789 experiments. (g) flg22-induced MPK phosphorylation is not affected in *rps4-2 rps4b-2*. Upon
790 flg22 treatment, samples were taken at indicated time points for immunoblotting. For (a), (g),
791 Ponceau staining (PS) was used as loading control. Molecular weight marker (in kDa) is indicated
792 on the left. All experiments were repeated at least three times with similar results. For (c), (d), (f),

793 centre lines represent the medians; bounds of box indicate the 25th and 75th percentiles; whiskers
794 represent 1.5× interquartile range from 25th and 75th percentiles. Exact P-values can be found in
795 Supplementary Table 5.

796 **Extended Data Fig. 9 | Potentiation of ETI^{AvrRps4}-induced HR by PTI. (a)** Pf0-1:AvrRps4 leads
797 to macroscopic HR in est:AvrRps4 leaves. Both PTI (Pf0-1:AvrRps4^{mut}) or ETI^{AvrRps4} (est) does
798 not lead to macroscopic HR. Coactivation of PTI and ETI^{AvrRps4} (est + Pf0-1:AvrRps4^{mut}) leads to
799 macroscopic HR. The numbers indicate number of leaves displaying HR of the total number of
800 leaves infiltrated. *n* = 18 leaves. **(b)** Est:AvrRps4 leaves were hand-infiltrated with indicated
801 solutions and electrolyte leakage was measured over 48hpi. Combination of “PTI + ETI^{AvrRps4}”
802 (blue dots, “est + Pf0-1:AvrRps4^{mut}”) leads to stronger electrolyte leakage compared to ETI^{AvrRps4}
803 (est) or PTI (Pf0-1:AvrRps4^{mut}) alone. Pf0-1:AvrRps4 (green) acts as a positive control. Data
804 points from 3 biological replicates were analyzed with one-way ANOVA followed by post hoc
805 Tukey’s HSD test. Data point from each biological replicate is indicated with different shapes.
806 Data points with different letters indicate *P* < 0.01. *n* = 9 data points; each represents data from 15
807 leaf discs. Exact P-values can be found in Supplementary Table 5. **(c)** PTI induced by flg22, elf18,
808 pep1, C10:0, nlp20 or chitin does not lead to macroscopic HR. Coactivation of PTI (trigger by
809 these PAMPs or DAMP) with ETI^{AvrRps4} leads to macroscopic HR. The numbers indicate number
810 of leaves displaying HR of the total number of leaves infiltrated. *n* = 18 leaves. **(d)** 5-week-old
811 inducible AvrRpm1 (dex:AvrRpm1), AvrRpt2 (est:AvrRpt2), AvrPphB (est:AvrPphB), AvrRps4
812 (est:AvrRps4) and AvrRpp4 (est:AvrRpp4) Arabidopsis leaves were infiltrated with either dex (for
813 dex:AvrRpm1 only) or est. All pictures were taken at 3 dpi. The numbers indicate the number of
814 leaves displaying HR of the total number of leaves infiltrated. *n* = 18 leaves. **(e)** Combination of
815 “PTI + ETI” leads to stronger macroscopic HR in inducible-AvrRpm1, AvrRpt2, AvrPphB and
816 AvrRpp4 Arabidopsis lines. All pictures were taken 3 dpi. The numbers indicate number of leaves
817 displaying HR of the total number of leaves infiltrated. *n* = 18 leaves. All experiments were
818 repeated at least three times with similar results.

819 **Extended Data Fig. 10 | MAPKs and NADPH oxidases are involved in HR induced by PTI +**
820 **ETI. (a)** MPK phosphorylation during ETI triggered by multiple effectors. Seedlings of
821 dex:AvrRpm1, est:AvrRpt2, est:AvrPphB and est:AvrRpp4 lines were soaked in dex or est,
822 solution respectively for indicated time points (dark yellow). Untreated (UNT) seedlings were used

823 as negative control, seedlings treated with 100 nM flg22 for 15 min (red, flg22) were used as
824 positive control. **(b)** RbohD phosphorylation during ETI triggered by multiple effectors. Seedlings
825 of dex:AvrRpm1, est:AvrRpt2, est:AvrPphB and est:AvrRpp4 were soaked in either mock (black),
826 dex or est solution (dark yellow) for 6 h. Microsomal fraction from seedlings were isolated for
827 immunoblotting. For **(a)**, **(b)**, Ponceau staining (PS) was used as loading control. Molecular weight
828 marker (in kDa) is indicated on the left. **(c)** MPK6SR#58 (*mpk3 mpk6* P_{MPK6}:MPK6^{YG}) is a
829 conditional *mpk3 mpk6* double mutant. MPK6^{YG} has a larger ATP binding pocket than MPK6^{WT}
830 and is sensitive to the inhibitor 1-Naphthyl-PP1 (NA-PP1, ATP analog). Pre-treatment with NA-
831 PP1 inhibits MPK6^{YG} and temporarily generates a *mpk3 mpk6* double mutant. Both Col-0 and
832 MPK6SR#58 leaves were pre-infiltrated with either 1% DMSO (mock) or 10 μ M NA-PP1. After
833 3 h, these leaves were infiltrated with either Pf0-1:empty vector (triggers PTI) or Pf0-1:AvrRps4
834 (triggers “PTI + ETI^{AvrRps4}”). With mock pre-treatment, Pf0-1:AvrRps4 infiltration leads to
835 macroscopic HR in both Col-0 and MPK6SR#58. NA-PP1 pre-treatment attenuates HR caused by
836 Pf0-1:AvrRps4 only in the MPK6SR#58 line. All pictures were taken at 1 dpi. The numbers
837 indicate number of leaves displaying HR of the total number of leaves infiltrated. *n* = 18 leaves.
838 **(d)** Col-0 and *rbohD rbohF* leaves were infiltrated with either Pf0-1:empty vector (triggers PTI) or
839 Pf0-1:AvrRps4 (triggers “PTI + ETI^{AvrRps4}”) at varying OD₆₀₀. With OD₆₀₀ = 0.025, Pf0-
840 1:AvrRps4 infiltration leads to less macroscopic HR in *rbohD rbohF*. All pictures were taken 1 dpi.
841 The numbers indicate number of leaves displaying HR of the total number of leaves infiltrated. *n*
842 = 18 leaves. All experiments were repeated at least three times with similar results. **(e) Model:**
843 Upon ligand detection by PRRs, PTI leads to activation of BIK1, RbohD and MAPKs. Activation
844 of an NLR (ETI without PTI) elevates accumulation of PTI signaling components. Co-activation
845 of both PTI and ETI elevates accumulation and enhances activation of multiple PTI signaling
846 components, enabling a stronger immune response.