

1 **The transcriptional landscape of *Arabidopsis thaliana* pattern-triggered**
2 **immunity**

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12
13 **Summary**

14 **Plants tailor their metabolism to environmental conditions, in part through recognition of a**
15 **wide array of self and non-self molecules. In particular, the perception of microbial or plant-**
16 **derived molecular patterns by cell surface-localized pattern recognition receptors (PRRs)**
17 **induces pattern-triggered immunity, which includes massive transcriptional**
18 **reprogramming¹. While an increasing number of plant PRRs and corresponding ligands are**
19 **known, whether plants tune their immune outputs to patterns of different biological origins**
20 **or of different biochemical nature remains mostly unclear. Here, we performed a detailed**
21 **transcriptomic analysis in an early time-series focused to study rapid signaling**

22 transcriptional outputs induced by well-characterized patterns in the model plant
23 *Arabidopsis thaliana*. This revealed that the transcriptional response to diverse patterns –
24 independent of their origin, biochemical nature, or type of PRR – is remarkably congruent.
25 Moreover, many of the genes most rapidly and commonly up-regulated by patterns are also
26 induced by abiotic stresses, suggesting that the early transcriptional response to patterns is
27 part of the plant general stress response (GSR). As such, plant cells' response is in the first
28 instance mostly to danger. Notably, genetic impairment of the GSR reduces pattern-induced
29 anti-bacterial immunity, confirming the biological relevance of this initial danger response.
30 Importantly, the definition of a small subset of 'core immunity response' genes common and
31 specific to pattern response revealed the function of previously uncharacterized
32 GLUTAMATE RECEPTOR-LIKE (GLR) calcium-permeable channels in immunity. This
33 study thus illustrates general and unique properties of early immune transcriptional
34 reprogramming that uncovered important components of plant immunity.

36 Main Text:

37 Plants are challenged by a wide variety of potentially pathogenic organisms; their health relies on
38 their ability to recognize and respond to this plethora of challenges. This recognition is partly
39 accomplished through cell surface-localized pattern recognition receptors (PRRs), which
40 recognize pathogen-associated molecular patterns (PAMPs) or host-derived damage-associated
41 molecular patterns (DAMPs), leading to pattern-triggered immunity (PTI)². While a wide variety
42 of PRRs with an equivalent variety of cognate ligands have been identified in various plant
43 species³, it is still unclear to what extent plants discriminate among patterns from different source
44 organism, of different chemical nature, or that are recognized by different PRR classes. Notably,

45 while a few studies have compared transcriptional responses (as a proxy of a dynamic large
46 immune cellular output) triggered by two or three patterns together⁴⁻⁶, or used meta analyses to
47 compare responses^{7,8}, these studies were limited in scale or utilized different experimental
48 conditions, which hinders meaningful comparisons.

49

50 To ascertain the timing and degree of discrimination among pattern-triggered transcriptional
51 responses, we selected a panel of seven patterns with known PRRs, representing a variety of source
52 organism, chemical composition, and recognition mechanisms. This included bacterial flg22 (a
53 22-amino acid epitope derived from bacterial flagellin) recognized by the leucine-rich repeat
54 receptor kinase (LRR-RK) FLAGELLIN SENSING 2 (FLS2)⁹, elf18 (an 18-amino acid epitope
55 derived from bacterial elongation factor Tu) recognized by the LRR-RK EF-TU RECEPTOR
56 (EFR)⁸, Pep1 (a 23-amino acid peptide potentially released as DAMP upon cellular damage)
57 recognized by the LRR-RKs PEP1-RECEPTOR (PEPR1) and PEPR2¹⁰⁻¹², nlp20 (a 20-amino acid
58 peptide derived from bacterial, oomycete, and fungal NECROSIS AND ETHYLENE-INDUCING
59 PEPTIDE 1 -LIKE PROTEINS) recognized by the LRR-receptor protein RECEPTOR-LIKE
60 PROTEIN 23 (RLP23)¹³, chitooctase (CO8, an octamer fragment of fungal cell walls) recognized
61 by the LysM-RKs LYSM-CONTAINING RECEPTOR KINASE 4 (LYK4) and LYK5¹⁴, 3-OH-
62 FA (a bacterial hydroxylated fatty acid) recognized by the S-lectin-RK
63 LIPOOLIGOSACCHARIDE-SPECIFIC REDUCED ELICITATION (LORE)^{15,16}, and
64 oligogalacturonides (OGs, derived from the plant cell wall) proposed to be recognized by the
65 epidermal growth factor receptor-like-RK WALL-ASSOCIATED KINASE 1 (WAK1)¹⁷. Both
66 Pep1 and OGs are considered DAMPs, while the other patterns are PAMPs. Each pattern was
67 applied in four replicate experiments to two-week-old *Arabidopsis thaliana* (hereafter

68 Arabidopsis) seedlings grown in liquid culture, at concentrations either previously used in
69 transcriptomics studies or shown to be saturating for upstream signaling responses^{8,15,18–20}. Each
70 pattern was applied to Col-0 wild-type (WT) or cognate receptor mutant, and seedlings were flash
71 frozen for RNA extraction at 0, 5, 10, 30, 90, and 180 min post-treatment (Fig. 1a). Note that *wak1*
72 mutants are not viable¹⁷, and thus OG treatment was paired with a mock water treatment.

73

74 Transcript abundance was assessed by RNA-seq and differentially expressed genes (DEGs) were
75 identified by comparison with time 0 [$\log_2(\text{fold change, FC}) > 1$, $p_{\text{adj}} < 0.05$], resulting in a total of
76 10,730 DEGs throughout the experiment (5,718 up-regulated; 5,012 down-regulated), with the
77 strongest treatment being flg22 (8,451 DEGs; 4,816 up and 3,635 down) and the weakest being 3-
78 OH-FA (1,633 DEGs; 1,246 up and 387 down; Supplementary Tables 1 & 2; Fig. 1b). One
79 selection criterion for treatments chosen here was saturation of upstream signaling outputs (*e.g.*
80 ROS, Ca^{2+} influx), but it cannot be ruled out that higher concentrations of ‘weaker’ patterns would
81 match responses observed here for ‘stronger’ patterns. Treatments in this study were also selected
82 to match previously published transcriptomics experiments – indeed, $\log_2(\text{FC})$ expression values
83 were similar to those published with single patterns^{4,7,8}, supporting the experimental and analysis
84 setups used here (Extended Data Fig. 1a, b). Principal component analysis (PCA) of DEGs
85 revealed strong responses at 30, 90, and 180 min in WT plants that are absent in receptor mutant
86 or mock controls (Extended Data Fig. 1c). Any genes behaving similarly in WT and controls were
87 removed from further analysis. Similar to the PCA, correlation analysis implicated time post
88 treatment as the main factor determining transcriptome response; WT samples became highly
89 correlated at later time points (Extended Data Fig. 1d; Pearson correlation at 5 min, 0.08; at 10
90 min, 0.49; at 30 min, 0.89; at 90 min, 0.80; at 180 min, 0.71).

91
92 We then collected the set of DEGs up- or down-regulated by each pattern at each time point, and
93 subdivided these sets by the number of patterns similarly affecting each gene (Fig. 1b). This
94 revealed a large set of DEGs induced by all tested patterns (n=970; Supplementary Table 3; Fig.
95 1b, darkest bar segment). Furthermore, with the exception of flg22, no pattern induced or repressed
96 a large number of genes uniquely (Extended Data Fig. 2; Supplementary Table 4). To ascertain
97 whether there exist sets induced specifically by pattern subclasses (*e.g.* by PRR type, pattern
98 origin, etc.), we identified DEGs induced or repressed by all possible combinations of patterns
99 (Fig. S3), and determined the extent to which the relative sizes of these sets departed from that of
100 a random assortment of genes among patterns (deviation)²¹. To avoid potential effects of
101 accelerated or delayed induction, we collected all DEGs induced by a pattern in this experiment
102 into one representative set. As expected, this confirmed that the largest two sets were DEGs
103 induced uniquely by flg22 (n=1,041) or commonly by all tested patterns (Fig. 1c; Extended Data
104 Fig. 3). Both of these sets were larger than would be expected by chance (deviation 0.16 for each).
105 The next two largest sets comprised DEGs induced by at least five of the tested patterns – indeed
106 the treatment of CO8 and 3-OH-FA in this experiment were relatively weaker than other patterns
107 (Fig. 1b), suggesting that DEGs in these sets may also be induced by all patterns under specific
108 conditions. Remarkably, none of the pattern subsets we identified *a priori* induced unique sets of
109 DEGs much larger or smaller than would be expected by chance (Extended Data Fig. 3). Taken
110 together, these results suggest that gene induction within the first three hours mostly constitutes a
111 general pattern-triggered response (against ‘non-self’ or ‘damaged-self’), rather than being pattern-
112 or pattern-subclass-specific.

113

114 To explore the set of ~1,000 DEGs up-regulated commonly by all treatments, we first
115 hierarchically clustered these genes according to their $\log_2(\text{FC})$ values for each pattern/time
116 combination (Fig. 1d). This revealed four clusters with characteristic expression patterns,
117 described here as ‘Very rapid’, ‘Rapid transient’, ‘Rapid stable’, and ‘Late’ (Fig. 1e). Interestingly,
118 though all tested patterns induced all DEGs and the overall expression patterns were similar, some
119 differences in timing of gene induction could be observed. Among the ‘Very rapid’ and ‘Rapid’
120 sets OGs, flg22, elf18 and Pep1 induced gene expression already at 5 min, only detectable in
121 response to nlp20, 3-OH-FA and CO8 after 10 min. This partially correlated with the total number
122 of DEGs up-regulated (Fig. 1b), suggesting a potential relationship between amplitude and rapidity
123 of transcriptional response, similar to that observed in some earlier steps of PTI signaling^{22,23}. Of
124 note, differences in diffusion cannot be excluded as contributing to this observation.

125
126 A similar analysis of down-regulated DEGs revealed no similar congruence in pattern response –
127 indeed, most sets had similar sizes to those expected by chance (deviation -0.03 – 0.11). There are
128 approximately 100 DEGs down-regulated by all tested patterns (Supplementary Table 5).
129 Although this set was not significantly larger or smaller than expected by chance, we nevertheless
130 clustered these genes to identify characteristic expression patterns, finding differences in kinetics
131 similar to up-regulated genes (Extended Data Fig. 4). Taken together, these results show that
132 expression patterns in response to pattern perception are dominated by a small number of pattern-
133 specific responses, and a large set of commonly-induced genes.

134
135 In order to investigate transcriptional regulators controlling this response, we expanded this
136 analysis from the genes up-regulated by all patterns to the entire dataset. As timing was the

137 dominant effect in pattern-induced transcriptome reprogramming (Fig. 1; Extended Data Fig. 1),
138 we grouped the up-regulated DEGs by the time at which they first became induced, regardless of
139 the inducing pattern, as previously done in response to other stimuli²⁴. GO term enrichment of
140 these five gene sets supports progressive waves of transcriptional response (Fig. 2a). A *cis*-element
141 enrichment analysis revealed enrichment of binding sites for a large number of WRKY
142 transcription factors (TFs) in the promoters of DEGs first induced at 10-30 min post-elicitation
143 (Fig. 2b). This is in line with the established roles of many WRKY TFs in PTI²⁵. In contrast, among
144 genes first induced at 5 or 10 min post-elicitation, there is enrichment in the binding sites for
145 CALMODULIN-BINDING TRANSCRIPTIONAL ACTIVATORS (CAMTAs; Fig. 2b). TFs of
146 the CAMTA family bind the core element vCGCGb, and are the major transcriptional regulators
147 of the plant general stress response (GSR) – a rapid and transient induction of a core set of genes
148 in response to a wide variety of stimuli^{26–28}. Given the congruence of pattern-induced gene sets,
149 and the presence of CAMTA binding sites in promoters of rapidly up-regulated DEGs, we sought
150 to ascertain the degree to which pattern-induced genes are also affected by varied abiotic stresses.
151 To do this, we utilized the published AtGenExpress dataset of Arabidopsis seedling response to
152 cold, drought, genotoxic stress, heat, osmotic stress, salt, UVB irradiation, or wounding²⁹. We then
153 classified each of the DEGs up-regulated in this study according to (i) the time at which it is first
154 induced, (ii) the number of patterns that induce it throughout the experiment, and (iii) the number
155 of abiotic stresses tested in the AtGenExpress experiment that induce it within 3 h. Plotting each
156 DEG according to these criteria, with the color of the point determined by the maximum $\log_2(\text{FC})$
157 observed in this study, revealed that rapidly induced genes tend to be strongly induced by all tested
158 patterns, and induced by most tested abiotic stresses (Fig. 2c). This analysis extended the
159 observation of a common set of genes induced by all tested patterns to the conclusion that the rapid

160 transcriptional response to pattern perception is dominated by the GSR. As such, our analysis of
161 transcriptional responses indicated that plant cells mostly respond to ‘stress’.

162
163 A similar analysis of down-regulated DEGs revealed mostly later responses than for up-regulated
164 DEGs, with notably no down-regulated DEGs identified at 5 min ($p < 0.05$). Comparison with gene
165 repression under abiotic stress treatment did not reveal a trend like the GSR; though, interestingly,
166 the most strongly affected genes do tend to be down-regulated commonly by most or all tested
167 patterns (Extended Data Fig. 5). Finally, while relatively few GO terms or TF binding sites were
168 enriched in down-regulated genes found, many enriched GO terms were associated with growth
169 hormones and response to light, consistent with previous reports that pattern treatment impedes
170 photosynthesis^{30,31}.

171
172 We next sought to test whether the GSR is required for PTI. CAMTA3 is the primary member of
173 the CAMTA family in inducing the GSR²⁷. The genetic analysis of a role of CAMTA3 in PTI is
174 however confounded by the autoimmune phenotype of *camta3* loss-of-function mutants, due at
175 least in part to activation of the two nucleotide-binding leucine-rich repeat receptor proteins
176 (NLRs) DOMINANT SUPPRESSOR OF CAMTA3 1 (DSC1) and DSC2³². We thus utilized the
177 *camta3/dsc1/dsc2* triple mutant; while WT plants were able to mount an effective flg22-induced
178 resistance to the bacterium *Pseudomonas syringae* pv. tomato DC3000 (Pto), this effect was almost
179 completely lost in the GSR-deficient *camta3/dsc1/dsc2* ($p = 0.0007$, Fig. 2d), consistent with
180 similar results obtained with the dominant-negative *camta3D* allele³³. Interestingly, basal
181 susceptibility to Pto was also significantly reduced in *camta3/dsc1/dsc2* compared to WT

182 (p=0.0008, Supplementary Note 1), in contrast to *camta3D* but in line with studies showing a
183 negative role for CAMTA3 in salicylic acid-mediated immunity regardless of DSC1/2³⁴⁻³⁶.

184

185 Beyond highlighting the importance of the GSR in PTI, our comparison with AtGenExpress
186 (extended to selected abiotic stress RNA-seq studies)³⁷⁻³⁹ further identified DEGs up-regulated
187 commonly by all tested patterns, but not by abiotic stresses. Notably, among these 39 ‘core
188 immunity response’ (*CIR*) genes (Supplementary Table 6), the most strongly up-regulated gene
189 encodes GLUTAMATE RECEPTOR 2.9 (*GLR2.9*), and *GLR2.7* is also among the *CIR* set.
190 *GLR2.7* and *2.9* are closely related and are present in a tandem repeat on the genome with
191 *GLR2.8*⁴⁰, which is similarly induced by all tested patterns (Supplementary Table 3). GLRs are
192 Ca²⁺-permeable channels of which Arabidopsis GLR3 clade members, for example, are key for
193 wound-responsive signaling⁴¹⁻⁴³. In contrast, GLR2 clade members – to which *GLR2.7*, *2.8* and
194 *2.9* belong – are poorly characterized. Notably, previous pharmacological studies showed that
195 GLRs contribute to pattern-induced Ca²⁺ influx in Arabidopsis⁴⁴, but the identity of relevant GLRs
196 is still unknown. Given the high sequence similarity between *GLR2.7*, *2.8* and *2.9*, as well as their
197 chromosomal clustering, we generated a *glr2.7/2.8/2.9* triple mutant using CRISPR-Cas9 in both
198 Col-0 WT and a genetically encoded YELLOW CHAMELEON 3.6 (YC3.6) indicator line. In both
199 backgrounds, this resulted in a large deletion in the *GLR2.7-2.9* genomic region (Extended Data
200 Fig. 6). Interestingly, the increase of [Ca²⁺]_{cyt} triggered by *flg22*, *elf18* and *Pep1* was
201 approximately 25 % reduced in *glr2.7/2.8/2.9* relative to the YC3.6 parental line in 12 day-old
202 seedlings and leaf discs taken from 5-6 week old plants (Fig. 3a; Extended Data Fig.7a, b). In line
203 with this reduced immune output, *glr2.7/2.8/2.9* plants (in both WT Col-0 and YC3.6
204 backgrounds) were more susceptible to *Pto* infection by infiltration, to a similar degree as the

205 immune-deficient *bak1-5* mutant (Fig. 3c)⁴⁵. Notably, consistent with the specific regulation of
206 *GLR2.7* and *2.9* by pattern perception, but not by abiotic stresses, *glr2.7/2.8/2.9* plants were not
207 impaired in salt or ice water-induced $[Ca^{2+}]_{cyt}$ increase (Fig. 3b; Extended Data Fig. 7c).
208 Altogether, these results implicate the *GLR2.7/2.8/2.9* clade of GLRs in PTI.

209

210 We recently reported that Ca^{2+} -permeable channels from another family, OSCA1.3 and 1.7,
211 contribute to pattern-induced stomatal immunity⁴⁶. In contrast, *glr2.7/2.8/2.9* was not
212 compromised in pattern-induced stomatal closure (Extended Data Fig. 7d), nor was this mutant
213 more susceptible to Pto WT or a coronatine-deficient mutant upon surface-inoculation by spraying
214 (Extended Data Fig. 7e,f). *GLR2.7/2.8/2.9* are not strongly expressed prior to elicitation, and unlike
215 OSCA1.3 and CNGC2/4 – calcium-permeable channels previously shown to play roles in PTI –
216 they do not show strong preference for/against stomatal expression (Extended Data Fig. 8). Also,
217 the previously reported role of CNGC2/4 is only apparent under high external $[Ca^{2+}]$
218 conditions^{47,48}, indicating that additional calcium-permeable channels must be involved in PTI
219 during normal conditions. These findings substantiate the emerging concept that multiple channels
220 belonging to distinct Arabidopsis families (*e.g.* CNGCs, OSCAs, GLRs) contribute to the overall
221 pattern-induced calcium response observed at the whole plant level.

222

223 The *CIR* gene set includes several other genes associated with immunity (Supplementary Table
224 6)⁴⁹⁻⁵². We have here shown the utility of this transcriptomic dataset in identifying signaling and
225 regulatory components of general stress and immune responses in Arabidopsis. The future
226 characterization of other *CIR* genes with yet uncharacterized functions or unknown roles in

227 immunity may thus reveal additional PTI players, and for understanding of how the plant
228 transitions from the rapid general stress response to later immunity-specific responses.

229

230

231 **Materials and Methods**

232 Arabidopsis growth conditions

233 Arabidopsis growth conditions followed standard protocols⁴⁶. For *in vitro* culture Arabidopsis
234 seeds were surface-sterilized, stratified 3-5 days at 4 °C, then plated on full-strength MS medium,
235 1 % sucrose 0.8 % agar. Plates were placed at 22 °C, 16 h/8 h light/dark. After four days,
236 germinated seedlings were transferred to liquid culture. For RNA-seq, seedlings were placed, two-
237 per-well, in 24-well plates with 1 mL of MS media lacking agar, and plates were sealed with porous
238 tape. For seedling Ca²⁺ measurements, seedlings were transferred, 30-50 per plate, to sterile 9 cm
239 petri dishes containing ca. 25 mL MS media lacking agar, and plates were sealed with porous tape.
240 For soil growth Arabidopsis seeds were lightly surface-sterilized, stratified 3-5 days, and planted
241 on soil. Plants were grown for four-to-six weeks at 20 °C, 60 % humidity, 10 h/14 h light/dark
242 before assays were performed.

243 Lines used in this project include Col-0 used as WT control, *fls2c* (SAIL_691_C04)⁵³, *efr-1*
244 (SALK_044334)⁸, *pepr1-1/2-1* (SALK_059281 / SALK_036564)¹¹, *rlp23-1* (SALK_034225)¹³,
245 *lyk4/5* (WiscDsLox297300_01C / SALK_131911c, seeds obtained from Gary Stacey)¹⁴, *sd1-29*
246 (*lore*, SAIL_857_E06, seeds obtained from Stefanie Ranf)¹⁶, *bak1-5* (BAK1^{C408Y})⁴⁵,
247 *camta3/dsc1/dsc2* (SALK_001152/SAIL_49_C05/FLAG014A11, seeds obtained from Morten
248 Petersen) (*NB*: while the FLAG collection was generated in the Ws-2 background, containing a
249 mutated *FLS2*, the *camta3/dsc1/dsc2* line contains a Col-0-version, functional *FLS2* gene)³², and
250 YC3.6 (obtained from Myriam Charpentier). The *glr2.7/2.8/2.9* lines generated in this study are
251 described in Extended Data Fig. 6.

252

253 RNA-seq treatment

254 Each plate contained an equal number of wells of Col-0 wild type and PRR mutant control, with
255 the exception of a single plate for combined OG/mock treatment. After nine days growth in liquid
256 MS medium, sealing tape was removed from plates, media removed from wells, and replaced with
257 0.6 mL liquid MS per well. The following day, when seedlings were 14 days post-stratification,
258 400 μ L of 2.5x pattern solution was added to each well. Two wells, for a total of four seedlings,
259 were harvested for each genotype/treatment/time combination. Final pattern concentrations were
260 1 μ M flg22^{18,53} (Scilight-Peptide), 1 μ M elf18⁸ (Scilight-Peptide), 1 μ M Pep1⁵⁴ (Scilight-Peptide),
261 1 μ M nlp20¹³ (provided by Thorsten Nürnberger), 100 μ g/mL OGs DP10/15^{4,55} (elicityl GAT114),
262 1 μ M CO8¹⁹ (IsoSep 57/12-001), and 1 μ M 3-OH-FA¹⁵ (provided by Stefanie Ranf).

263

264 Tissue harvest, library preparation, and sequencing

265 Samples were collected and libraries prepared using a combination of published high-throughput
266 protocols⁵⁶⁻⁵⁹. Briefly, two wells per genotype/treatment/time combination were pooled at each of
267 0, 5, 10, 30, 90, or 180 min following treatment. Seedlings were blotted dry and flash-frozen in
268 liquid nitrogen. Tissue was pulverized while frozen via two one-minute pulses in a BioRad
269 TissueLyser, and divided in half for library preparation. Divided powder was further disrupted for
270 one minute, prior to addition of extraction buffer, and disrupted in buffer for a further two one-
271 minute pulses. Samples were spun down and lysate collected and incubated with biotin-oligo-dT
272 and streptavidin magnetic beads. The full set of RNA washes and elution was performed twice,
273 with DNase I treatment in-between, to minimize rRNA and gDNA contamination. cDNA
274 synthesis was performed as described, with the exception that only 2 μ L of DNA Pol I was used.
275 Serapure-cleaned dscDNA was quantified via SYBR-green based plate assay and normalized to 2
276 ng/ μ L for tagmentation^{60,61}. Tagmentation was performed in 5 μ L reactions containing 0.2 μ L Tn-

277 5 transposase, and the entire reaction used as template for PCR⁵⁸. PCR was performed using in-
278 house primers to add 5' and 3' tags and the NEBnext 2x polymerase mix, amplifying for 10 cycles.
279 Libraries were again Serapure cleaned, SYBR quantified, and normalized to 0.5 μ M for pooling
280 and sequencing. Pooled libraries were run on 2-3 flowcells of a NextSeq500, and pooling adjusted
281 after each run to maximize overall read density per sample.

282

283 Read mapping and differential expression analysis

284 Read data was analyzed using FastQC, trimmed using trimmomatic⁶², and mapped to the
285 Arabidopsis TAIR10 genome via TopHat2^{63,64}. Mapped reads were assigned to genes, and
286 differential expression analysis performed using DESeq2⁶⁵. Prior to differential expression
287 analysis, a total of 17/336 libraries were removed from later analysis, primarily for poor
288 sequencing leading to few mapped reads. For each sample, differential expression was determined
289 relative to the same genotype-treatment combination at time 0. To account for time and mechanical
290 stress, for WT samples, genes were removed if also differentially expressed in PRR mutant
291 controls, with the exception of OG-treated samples, which were filtered based on differential gene
292 expression in mock-treated WT. For data exploration (*e.g.* PCA, correlation, GO term and *cis*-
293 element enrichment) a relatively loose cutoff of $|\log_2(\text{FC})| > 1$, $p_{\text{adj}} < 0.1$ was used to obtain a broad
294 landscape of DEGs. For analyses in which specific genes of interest would be analyzed (*e.g.* CIR
295 gene set), a more stringent cutoff of $|\log_2(\text{FC})| > 1$, $p_{\text{adj}} < 0.05$ was used. Data manipulation was done
296 in R^{66,67}, using functions from the tidyverse⁶⁸.

297

298 Exploratory data analysis

299 Principal component analysis was performed using the `prcomp` function in R and sample
300 correlation was determined via the Pearson method, using the `cor` function in R. Visualization of
301 genes induced by various combinations of patterns was done via user-modified adaptations of the
302 `UpSetR` and `SuperExactTest` R packages^{69,70}, and deviation was calculated as described²¹.
303 Expression of the core set of genes up- or down-regulated by pattern treatment was clustered using
304 the `hclust` function, with extra functionality from the `dendextend` package in R⁷¹.
305 Gene induction specific to individual pattern treatments was determined using a modification of
306 tissue-specific gene expression assignment^{72,73}. Briefly, normalized pseudocount data were first
307 filtered to genes significantly upregulated ($p < 0.1$, $\log_2(\text{FC}) > 1$) in at least one condition. Filtered
308 pseudocounts were next averaged across all replicates, then summed across all time points for each
309 pattern. For each gene and each pattern the fraction of total counts for that gene attributed to that
310 pattern was calculated (specificity measure, SPM). Data were finally filtered to those genes with
311 $\text{SPM} > 0.33$ for at least one pattern (approximately 1/3 total reads in experiment attributable to one
312 pattern, determined empirically to find reasonably specific expression).

313

314 GO term and *cis*-element enrichment

315 GO term enrichment was performed using the library `TopGO` in R, using GO terms obtained from
316 TAIR, searching for enrichment in each gene set relative to the complete set of genes detected in
317 this experiment, and determining enriched GO terms using the `weight01` method with the Fisher
318 statistic⁷⁴.

319 *cis*-element enrichment analysis was performed using AME, part of the MEME suite⁷⁵, using a
320 published library of TF binding sites found via DAPseq⁷⁶.

321

322 Comparison with AtGenExpress abiotic stress microarray data analysis

323 As the AtGenExpress experiment was performed using the ATH1 microarray, we first restricted
324 induced genes to those present on the array. Normalized abiotic stress microarray data (intensity)
325 was obtained from <http://jsp.weigelworld.org/AtGenExpress/resources/> in 2017 and analyzed
326 using limma (*NB*: data are no longer hosted here, but CEL files can be downloaded through
327 <https://www.arabidopsis.org/portals/expression/microarray/ATGenExpress.jsp>)^{29,77}. We did not
328 consider the oxidative stress treatment for filtering pattern-responsive genes, as most patterns
329 induce production of reactive oxygen species¹. To facilitate comparisons with this study's RNA-
330 seq data, only time points from the first three hours were considered, and comparisons for
331 differential expression were first made between each treatment and time 0, then between each
332 treatment and mock at the same time, considering only genes that were differentially expressed
333 [$\log_2(\text{FC}) > 1$, $p_{\text{adj}} < 0.05$] under both criteria.

334
335 *CIR* genes were selected according to the following criteria: (i) significantly induced in at least
336 one time by all seven patterns tested here (ii) not significantly induced at any time point by any of
337 the selected stresses in the AtGenExpress Dataset (iii) Uniquely targeted by at least one probe in
338 the ATH1 microarray (iv) not significantly induced in selected abiotic stress experiments (3 hr
339 proteotoxic stress, 4 h darkness, 4 h flooding, 3 h 50, 150, or 200 mM NaCl) assayed using RNA-
340 seq³⁷⁻³⁹. This resulted in a set of 40 DEGs. Among these, one highly upregulated gene,
341 *AT3G32090*, is a suspected pseudogene with strong homology to *WRKY40* in one region. All of
342 the reads assigned to *AT3G32090* mapped to only this region (Extended Data Figure 9). As
343 *WRKY40* is both highly expressed and strongly upregulated by pattern treatment, we suspected
344 these reads were mistakenly assigned to *AT3G32090*, and removed it from the *CIR* set.

345

346 Measurement of intracellular Ca²⁺ concentration in seedlings

347 After six days growth in liquid MS medium, sealing tape was removed from plates and seedlings
348 rinsed in sterile water and transferred one-per-well to black 96-well plates containing 150 μ L
349 sterile water. Seedlings were gently pressed to ensure the majority of the seedling was submerged,
350 and plates were incubated in the dark under bench conditions overnight. The following day, when
351 seedlings were 11 days post-stratification, plates were imaged in a Tecan SPARK microplate
352 reader at two conditions: excitation 440 nM, emission 480 nM (Cyan Fluorescent Protein, CFP)
353 and excitation 440 nM emission 530 nM (Yellow Fluorescent Protein, YFP). In the ratiometric
354 YC3.6 reporter, Ca²⁺ binding increases fluorescence resonance energy transfer from CFP to YFP;
355 thus YFP/CFP ratio (R) is proportional to [Ca²⁺]. Initial ratios can vary from well-to-well:
356 accordingly YC3.6 ratios are normalized to initial ratio (R₀) and reported as (R-R₀)/R₀. Pattern
357 treatment was performed through addition of 38 μ L of 5x solution injected after 5 min visualization
358 by the microplate reader. The focal plane for fluorescence measurements was set to a single point
359 in the center of each well, and moved up 0.5 mm post-injection to accommodate increased volume
360 in wells. Despite this adjustment, overall fluorescence intensity and thus ratio was frequently
361 altered post-injection, as seedlings did not uniformly fill well. Due to this change, and the generally
362 slow pattern response, we normalized all subsequent fluorescence ratios to the first ratio measured
363 post-injection (R₀), as (R-R₀)/R₀. Wells were manually rejected if pre-injection fluorescence was
364 not stable or vastly different than R₀.

365 Salt (NaCl) treatment was performed similar to pattern treatment, with the following changes: to
366 accommodate the faster response, injection and imaging was performed on a well-by-well basis
367 rather than across a subsection of the plate. Due to the faster response, the first measurement post-

368 injection already reflects the beginning of plant response - R_0 was thus defined as pretreatment
369 fluorescence ratio, though this resulted in more noise in the final data. For cold treatment, the plate
370 was first pre-imaged for baseline fluorescence, then removed from the plate reader, the overnight
371 water removed, and 150 μ L fresh water at 22 or 4 $^{\circ}$ C (ice water bath) added. Plates were
372 immediately placed back in plate reader and imaged 5 minutes. As with salt response, the speed of
373 the cold response necessitated defining R_0 as pretreatment fluorescence levels, though this
374 combined with removal and addition of fresh water resulted in noise in final peak levels.

375 As some silencing was observed both in parent YC3.6 lines and YC3.6 *glr2.7/2.8/2.9* lines, only
376 seedlings with visible fluorescence at four days were transferred to liquid culture, and following
377 treatment, only seedlings (wells) with pre-treatment fluorescence in both wavelengths greater than
378 3x that of a non-fluorescent Col-0 control were considered. Total seedlings imaged were as
379 follows: YC3.6 mock: 56, YC3.6 *flg22*: 54, YC3.6 *elf18*: 52, YC3.6 *Pep1*: 55, YC3.6
380 *glr2.7/2.8/2.9* mock: 48, YC3.6 *glr2.7/2.8/2.9 flg22*: 43, YC3.6 *glr2.7/2.8/2.9 elf18*: 36, YC3.6
381 *glr2.7/2.8/2.9 Pep1*: 43, YC3.6 mock (NaCl): 56, YC3.6 NaCl: 51, YC3.6 *glr2.7/2.8/2.9* mock
382 (NaCl): 38, YC3.6 *glr2.7/2.8/2.9* NaCl: 29, YC3.6 22 $^{\circ}$ C water: 56, YC3.6 4 $^{\circ}$ C water: 54, YC3.6
383 *glr2.7/2.8/2.9* 22 $^{\circ}$ C water: 44, YC3.6 *glr2.7/2.8/2.9* 22 $^{\circ}$ C water: 42.

384

385 Measurement of intracellular Ca^{2+} concentration in leaf discs

386 Four leaf discs per plant were collected from five-to-six-week-old soil-grown Arabidopsis plants
387 and incubated overnight on 100 μ L sterile ultrapure water in black 96-well plates. As for seedlings,
388 plates were imaged in a Tecan SPARK microplate reader at two conditions: excitation 440 nM,
389 emission 480 nM (CFP) and excitation 440 nM emission 530 nM (YFP). *flg22* treatment was
390 performed through addition of 25 μ L of 5x solution injected after 5 min visualization by the

391 microplate reader. The focal plane for fluorescence measurements was set to a single point in the
392 center of each well, and moved up 0.5 mm post-injection to accommodate increased volume in
393 wells. Wells were manually rejected if pre-injection fluorescence was not stable or vastly different
394 than R₀.

395

396 Reporter expression in the YC3.6 *glr2.7/2.8/2.9* line used for seedling imaging is completely
397 silenced by 5-6 weeks old. Accordingly, a different line with slightly different CRISPR deletion
398 (Extended Data Fig. 6) was used for soil-grown assays. Reporter expression in this line is generally
399 only ~1/5 the level of parent YC3.6. Only leaf discs with pre-treatment fluorescence in both
400 wavelengths greater than 3x that of a non-fluorescent Col-0 control were considered. Leaf discs
401 passing filter were averaged to get one value for each plant. Total plants imaged were as follows:
402 YC3.6 flg22: 73, YC3.6 *glr2.7/2.8/2.9* flg22: 38.

403

404 Bacterial infection assays

405 For all infection assays, Arabidopsis plants were treated when four- to five-weeks old, and bacteria
406 grown overnight in Kings B medium liquid culture, refreshed via a 1-2 h subculture in the morning,
407 spun down and resuspended in 10 mM MgCl₂. For induced resistance⁵³, three leaves from each
408 plant were infiltrated with either 1 μM flg22 or water in the morning. The following morning,
409 selected leaves were re-infiltrated with *Pseudomonas syringae* pv. tomato DC3000 (Pto)
410 expressing luciferase⁷⁸ at OD₆₀₀=0.0002 or ~1x10⁵ colony-forming units (CFU)/mL. Plants were
411 covered and infection allowed to proceed for two days. For infiltration infection assays, infection
412 was performed similarly with the following differences: WT Pto was used rather than the
413 luciferase-expressing strain; trays were incubated uncovered; and there was no mock or pattern

414 pretreatment. For spray infection, Pto was diluted to $OD_{600}=0.2$ or $\sim 1 \times 10^8$ CFU/mL in $MgCl_2$,
415 Silwet L-77 added to 0.04 %, and plants sprayed to surface saturation (~ 4 mL per plant).
416 For all infection assays, after approximately 48 h leaf discs were collected (infiltration: two from
417 each infiltrated leaf; spray: 6 from 6 separate leaves), ground in 10 mM $MgCl_2$, and serial dilutions
418 from 1×10^{-1} to 1×10^{-5} plated to count CFU.
419 Following infection, $\log_{10}(CFU)$ follow an approximately normal distribution. ANOVA was
420 performed using the `glm` and `anova` functions in R, and post-hoc tests via `emmeans` package⁷⁹.
421 Sample numbers are as follows: for induced resistance $n=12$ plants for all genotype/treatment
422 combinations. For infiltration infection total plants counted were Col-0: 17, Col-0 *glr2.7/2.8/2.9*:
423 19, *bak1-5*: 18, YC3.6: 12, YC3.6 *glr2.7/2.8/2.9*: 12. For spray infection $n=18$ for all
424 genotype/treatment combinations.

425

426 Stomatal aperture measurements

427 For each experiment, three leaf discs were taken from each of 6 plants per line. The three leaf discs
428 were floated one-per-well in 100 μ M stomatal opening buffer (10 mM MES-KOH pH 6.15, 50
429 mM KCl, 10 μ M $CaCl_2$, 0.01 % Tween-20) in white 96-well plates for 2 h in the growth chamber.
430 Subsequently, one leaf disc from each plant was treated with 5 μ M flg22, 10 μ M ABA, or mock
431 through addition of stock solution to stomatal opening buffer. Leaf discs were incubated 2-3 h
432 further, then imaged on a Leica DMR microscope and photographed with the equipped Leica
433 DFC320 camera. Stomata length and width were annotated in ImageJ. The experiment was
434 repeated twice. Total number of stomata counted per genotype/treatment combination are as
435 follows: Col-0 mock: 581; Col-0 flg22: 529; Col-0 ABA: 519; *glr2.7/2.8/2.9* mock: 461;

436 *glr2.7/2.8/2.9 flg22*: 503; *glr2.7/2.8/2.9 ABA*: 426; *bak1-5 mock*: 567; *bak1-5 flg22*: 607; *bak1-5*
437 *ABA*: 719.

438 Stomatal aperture (width/length) followed an approximately square normal distribution. ANOVA
439 was performed on square-root transformed ratios using the *glm* and *anova* functions in R, and post-
440 hoc tests via *emmeans* package⁷⁹.

441

442 Tissue expression patterns of genes encoding calcium channels implicated in PTI

443 Tissue-specific expression datasets containing aerial (rosette) tissue were selected in
444 Genevestigator, comprising datasets from⁸⁰⁻⁸³.

445

446 Data availability

447 The RNA-seq datasets generated and analyzed in the current study have been deposited in the
448 ArrayExpress database at EMBL-EBI (www.ebi.ac.uk/arrayexpress) under accession number E-
449 MTAB-9694. Markdowns documenting the steps in filtering, visualizing, and analyzing the data
450 in all figures and tables are available in Supplementary Note 1. Source data is available for Figures
451 2, 3 and Extended Data Figures 5 and 7.

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- 650

651

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667 **Additional information**

668 Supplementary Information is available for this paper.

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672

673 **Fig. 1 | Rapid pattern-triggered transcriptional responses are largely common, with**
674 **characteristic kinetics. a**, Arabidopsis seedlings were treated with a panel of patterns, and tissue
675 harvested for RNA extraction at indicated times. **b**, Genes up- or down-regulated ($|\log_2(\text{FC})| > 1$
676 and $p_{\text{adj}} < 0.05$) are shown for each time point within each pattern treatment (total height of bars).
677 Bars are subdivided by the number of patterns affecting each gene set at that time, with darker
678 colors representing more patterns co-regulating. **c**, UpSet diagram showing the size of gene sets
679 induced by each pattern (left, single gene list from all times combined) and the top 15 intersections
680 (bottom right) by size (top right). Bars for set sizes are colored by deviation from size predicted
681 by random mixing. **d**, Heat map of expression of the genes commonly induced by all tested
682 patterns. Genes are hierarchically clustered according to their behavior across all pattern/time
683 combinations, and cut into four clusters. **e**, Visualization of average $\log_2(\text{FC})$ patterns of the four
684 clusters identified in **d**, showing different approximate patterns of expression (time points spaced
685 evenly to visualize early times). Error bars represent standard error of the mean.

686

687 **Fig. 2 | Pattern-triggered transcriptional responses act in time-resolved waves, with the first**
688 **wave constituting a general stress response important for immune activation. a**, GO term and
689 **b** *cis*-element enrichment analysis of induced genes, categorized according the time point at which
690 they first passed induction threshold, regardless of which pattern caused induction. The top three
691 GO terms for each time point are indicated. **c**, Distribution of up-regulated genes. Each gene
692 induced in this study was plotted according to the time it is first induced (panels from top to
693 bottom), the number of tested patterns which induce it (x axis) and the number of abiotic stresses
694 in the AtGenExpress dataset which also induce it within the first three hours (y axis). The color of
695 each dot indicates the maximum $\log_2(\text{FC})$ observed in this study. **d**, Box-and-beeswarm plots of

696 flg22-induced resistance to Pto infection. Box plots center on the median, with box extending to
697 the first and third quartile, and whiskers extending to the lesser value of the furthest point or 1.5x
698 the inter-quartile range. Data were obtained from three independent experiments (point shapes),
699 n=4 per genotype/treatment combination in each experiment. Data were analyzed in R: Two-way
700 ANOVA with experiment as a blocking factor, and p value reports the interaction between
701 treatment and genotype.

702

703 **Fig. 3 | A *glr2.7/2.8/2.9* triple mutant is compromised in pattern-induced Ca²⁺ influx and**
704 **bacterial disease resistance. a, b,** Parent (darker shades) or *glr2.7/2.8/2.9* (lighter shades) YC3.6
705 reporter lines were assayed for response to a variety of patterns, salt (NaCl) or cold (4 °C)
706 treatment; peak Ca²⁺ signal reported by YC3.6 within 25 min (patterns), 1 min (salt), or 5 min
707 (cold) is shown. Each point represents peak ratio of YFP to CFP (proportional to Ca²⁺
708 concentration) for a single seedling, normalized to initial ratio. Different shapes represent 3-4
709 independent experiments, n=10-20 for each experiment/line/treatment combination. **c,** Parent and
710 *glr2.7/2.8/2.9* mutants in Col-0 and YC3.6 background were assayed for bacterial susceptibility,
711 alongside the hypersusceptible *bak1-5* mutant. Colony forming units (CFU) were counted two
712 days post infiltration. Each point represents one infected plant and different shapes represent 3
713 independent experiments, n=5-7 for each experiment/line/treatment combination. Box plots center
714 on the median, with box extending to the first and third quartile, and whiskers extending to the
715 lesser value of the furthest point or 1.5x the inter-quartile range. Statistical tests were performed
716 in R: ANOVA with experiment as a blocking factor, on square root of peak normalized Ca²⁺
717 response or log₁₀(CFU). Post-hoc tests were performed using the emmeans package in R: In **a** and

718 **b** *glr2.7/2.8/2.9* was compared to parent under each treatment, and in **c** (left), each genotype was
719 compared to Col-0 (dunnett method) and (right) YC3.6 *glr2.7/2.8/2.9* was compared to YC3.6.
720

