

# ER-anchored protein sorting controls the fate of two proteasome activators for intracellular organelle communication during proteotoxic stress

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1 **ER-anchored protein sorting controls the fate of two proteasome activators**  
2 **for intracellular organelle communication during proteotoxic stress**

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16

17 **Summary**

18 Proteotoxic stress, characterized by the accumulation of damaged proteins, poses a significant  
19 challenge to cellular homeostasis. To mitigate proteotoxicity eukaryotes employ the proteasome  
20 that is regulated by proteasome activators, e.g. transcription factors that promote gene  
21 expression of proteasome subunits. As proteotoxicity originates in different compartments, cells  
22 need to perceive signals from various locations. Understanding which components integrate  
23 signals to address proteotoxicity is essential to develop strategies to cope with proteotoxicity  
24 but remain elusive. Here, we identify that the proteasome autoregulatory feedback loop acts as  
25 a gatekeeper to facilitate the communication between nucleus and chloroplast. We reveal that  
26 the ER-anchored protein sorting system (ERAPS) controls the proteasomal degradation or  
27 nuclear translocation of proteasome activators NAC53 and NAC78. While both transcription  
28 factors activate the proteasome gene expression, they repress photosynthesis-associated nuclear  
29 genes during proteotoxicity through association with a conserved cis-element. Our data  
30 implicate a general trade-off between proteasome function and energy metabolism unravelling  
31 an unprecedented mechanism of how eukaryotic cells cope with proteotoxicity. Collectively,  
32 our discoveries provide a novel conceptual framework in which the proteasome autoregulatory  
33 feedback loop coordinates subcellular proteostasis and the trade-off between growth and  
34 defence.

35

## 36 **Main**

37 Protein homeostasis, hereafter proteostasis, is defined as the synthesis of proteins and their  
38 regulated degradation. This intimate balance between protein synthesis and degradation is  
39 tightly regulated in all organisms to respond to several environmental stimuli<sup>1,2</sup>. Various  
40 perturbations can alter this equilibrium, leading to proteotoxic stress, the accumulation of  
41 aberrant proteins that cause ultimately irreversible cellular damage. These perturbations can  
42 range from ageing to pathological diseases such as Parkinson and Alzheimer as well as infection  
43 by pathogens<sup>1,3</sup>. The latter is a consequence of the onset and maintenance of defense responses  
44 during microbial infections and microbes' ability to manipulate proteostasis to their own  
45 benefit<sup>3,4</sup>.

46 To mitigate deleterious effects due to excessive proteotoxic stress, cells employ various protein  
47 quality control machineries. One of the major protein quality control machineries across the  
48 tree of life is the ubiquitin-proteasome system (UPS). The UPS controls proteostasis through  
49 selective elimination of defective proteins and short-lived regulatory proteins<sup>5</sup>. To rapidly react  
50 to proteotoxic stress, all the constituents of the 26S proteasome are under a tight transcriptional  
51 control. All eukaryotic kingdoms possess transcription factors (TFs) required for proteasome  
52 gene activation<sup>6</sup>. Yeast utilizes Rpn4, mammals Nrf1/2 and plants the pair of NAC TFs (NAM,  
53 ATAF and CUC) NAC53/NAC78 as a conserved mechanism to cope with proteotoxicity. In  
54 yeast and animals, proteasome activators Rpn4 and Nrf1/2 have been shown to be UPS targets  
55 themselves, being degraded by the proteasome in the cytosol or ER<sup>6</sup>. Consequently, proteasome  
56 malfunction impairs their degradation leading to their stabilization and subsequent nuclear  
57 translocation to activate proteasome gene expression<sup>6</sup>. In plants, NAC78 mediates the  
58 transcriptional activation of proteasome promoters through the association with the proteasome  
59 regulatory cis element PRCE [TGGGC]<sup>7</sup>. In addition, loss of the NAC53/78 TF pair rendered  
60 plants hypersensitive to proteasome inhibition<sup>8</sup>. Therefore, a similar proteasome regulatory  
61 feedback loop has been proposed in plants<sup>6</sup>; however, to date, no evidence supports proteasomal  
62 removal of NAC53/78.

63 The involvement of the proteasome in various cellular pathways<sup>9-11</sup>, suggests that the  
64 autoregulatory feedback loop is a central mechanism to precisely integrate and coordinate stress  
65 responses through proteotoxic stress sensing. Indeed, a growing body of evidence suggests that  
66 localized proteotoxic stress in organelles causes the activation of respective proteasome-  
67 mediated degradation pathways<sup>12-16</sup>. This implies a multi-layered signaling role of the  
68 proteasome in governing subcellular proteostasis. This aspect is particularly relevant for semi-  
69 autonomous organellar nuclear-encoded proteins, as their regulation also involves cytosolic

70 proteasomal degradation prior to organellar import<sup>17,18</sup>. As such, proteasome activators could  
71 act as gatekeepers of the communication between the nucleus and energy-producing organelles  
72 to maintain subcellular proteostasis. This could link the proteasome directly to energy  
73 metabolism as well as to the trade-off between growth and defense. However, to date it is  
74 unknown whether proteasome activators exert these functions.

75 Here we report that proteasome activators, TFs NAC53 and 78, orchestrate the communication  
76 between the nucleus and chloroplast during various stress conditions. We reveal that ER-  
77 anchored protein sorting (ERAPS) controls the proteasomal degradation and nuclear  
78 translocation of NAC53 and NAC78. While both TFs activate the proteasome gene expression,  
79 they repress photosynthesis-associated nuclear genes during proteotoxicity. The trade-off  
80 between proteasome activation and photosynthesis downregulation seems to be a general  
81 feature as it occurs in response to various environmental and developmental cues.

82

### 83 **NAC53/78 are central integrators of various proteotoxic stress conditions**

84 Proteotoxicity is triggered by a wide range of physicochemical stresses at different subcellular  
85 locations often leading to accumulation of proteins that are not imported into these organelles  
86 <sup>17,19</sup>. To understand whether proteasome activators might play a role in integrating signals from  
87 different locations, we used the proteasome activator mutant *nac53-1 78-1*<sup>8</sup> to disrupt cellular  
88 proteostasis through a variety of approaches (Fig.1a): (i) inhibiting the proteasome via  
89 bortezomib (BTZ) and bacterial infection with *Pseudomonas syringae* *pv. tomato* DC3000  
90 (Pst)<sup>20,21</sup>, (ii) inhibiting the segregase CDC48 (CB-5083)<sup>16,22</sup>, (iii) targeting HSP90 (GDA)<sup>23</sup>,  
91 which plays a critical role in the import and degradation of chloroplastic/mitochondrial  
92 precursors, (iv) inducing ER stress with DTT and TM, and (v) targeting semi-autonomous  
93 organelles such as chloroplasts and mitochondria (Lin, Cml, MV)<sup>23</sup>.

94 Perturbation of the proteasome by chemicals, bacteria, CDC48 inhibition and induced ER stress  
95 resulted in an increased susceptibility of the *nac53-1 78-1* mutant (Fig.1b), suggesting that both  
96 proteasome activators are involved in a general regulation of subcellular proteostasis.  
97 Strikingly, all other treatments perturbing organelle function (Lin, Cml, MV, GDA) resulted in  
98 increased tolerance of *nac53-1 78-1* (Fig.1b). Overall, our phenotypic screen implies that  
99 NAC53 and NAC78 integrate signals from different compartments to adjust their impact on  
100 plant response to stress.

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103

## 104 **NAC53/78 are part of an autoregulatory proteasome feedback loop**

105 To elucidate how both TFs might integrate signals from different compartments and organelles,  
106 we aimed to decipher their regulation through interactome analysis. Transgenic Arabidopsis  
107 GFP-NAC53 and 78 lines (under the control of UBQ10 promoter) were only detectable for the  
108 GFP fusion proteins upon proteasome inhibition by BTZ (Fig. 2,b), implying a high  
109 proteasomal turnover of both NACs. Thus, we used *Agrobacterium*-mediated transient  
110 expression in *Nicotiana benthamiana* to boost the expression of NACs for our interactome  
111 studies. Transient expression of GFP-NAC53 and 78 (sTag construct) decorated the ER, being  
112 consistent with the predicted transmembrane domain (TM) at the C-terminus of both proteins  
113 (Fig. 2c and Extended Data Fig.1a). We additionally employed a chimeric construct (dTag-  
114 NACs) tagged with fluorophores at the N and C-terminus following the TM domain (Extended  
115 Data Fig.1a) to monitor subcellular interaction partners, as the RFP signal remained strictly in  
116 the ER (Fig. 2d). BTZ treatment, stabilized all NAC constructs, supporting the notion of  
117 NAC53/78 as proteasome targets and validating the functionality of the system in *N.*  
118 *benthamiana* (Extended Data Fig.1b,c).

119 Next, we performed immuno-precipitation (IP) followed by tandem mass spectrometry  
120 (MS/MS) with both constructs. We processed a total of 4 IP-MS/MS conditions for both NACs  
121 among two experiments. We performed IP of sTag-NACs in mock conditions and at 8 hours  
122 after bacterial infection, to induce proteotoxic stress (Extended data Fig.1D). In parallel, we  
123 performed IP of dTag-NACs enriching for the N- or C-terminus end of the proteins, which  
124 allowed us to characterize the interactome at a subcellular resolution (Extended data Fig.1E).  
125 For our downstream analysis, we focused on the common interactors for both NACs. Out of  
126 the 4 conditions we identified 245 unique *A. thaliana* orthologous proteins as interactor  
127 candidates (Fig. 2e, Supplementary Table 1). The Gene Ontology (GO) annotations for cellular  
128 components and the displayed enrichment for terms mainly associated with the proteasome  
129 complex and plastid components (Extended Data Fig.1f). Investigating the SUBA5 annotation  
130 of NAC53/78 interacting candidates, we found enrichment for following compartments: plastid,  
131 peroxisome, Golgi, and ER (Extended Data Fig.1g). Further GO analysis for biological  
132 processes revealed that possible candidates were enriched in proteins related to three main  
133 biological process: proteolysis, trafficking and translation (Fig. 2f). We extracted the  
134 corresponding proteins and performed a protein network analysis (Fig. 2g), highlighting the  
135 presence of UPS components (Fig. 2g; Extended Data Fig.1h-k). However, proteasome  
136 components were strongly reduced by bacterial infection and mainly enriched when targeting  
137 the ER-anchored end of the TFs (C-ter IP) (Extended Fig.1h), indicating that both proteasome

138 regulators are degraded at the ER. The latter was further supported by the presence of several  
139 ER-associated degradation (ERAD) components in the interactome (Fig.2g and Extended Data  
140 Fig.1i). In summary, our interactome analysis validates the role of NAC53/78 as integrators of  
141 subcellular proteostasis disruptions, affirming their regulation by the proteasome  
142 autoregulatory feedback loop in plants.

143

#### 144 **ER-anchored protein sorting (ERAPS) regulates the subcellular fate of NAC53 & NAC78**

145 Our interactome approach revealed that NAC53/78 associated with candidates that belong to  
146 the ERAD machinery, including membrane bound E3 ligase HRD1 and CDC48 (Fig. 2g and  
147 Extended data Fig.1i). To test whether ERAD is required to turnover the NACs, we took  
148 advantage of pharmacological drugs targeting individual steps in ERAD: we treated the GFP-  
149 NAC53 and GFP-NAC78 transgenic lines with CB-5083 and LS-102, inhibitors of CDC48 and  
150 HRD1 proteins, respectively<sup>22,24</sup>. We confirmed that previously uncharacterized LS-102 can  
151 efficiently inhibit both *A. thaliana* orthologs AtHRD1a and AtHRD1b *in vitro* (Extended data  
152 Fig. 2a,b). Similar to BTZ and CDC48, HRD1 inhibition led to a stabilization of both proteins;  
153 confirmed by confocal microscopy and immunoblotting (Fig. 3a,b). Collectively, these results  
154 indicate that NAC53/78 are targets of ERAD. Interaction studies of NACs with HRD1 isoforms  
155 revealed that GFP-NACs strongly associated with RFP-HRD1a/b in localization and co-IP  
156 experiments (Fig. 3c,d). The association was largely reduced upon deletion of the TM domain  
157 of both NAC TFs ( $\Delta$ TM, Fig. 3c). Interestingly, loss of ER association led to a strong  
158 stabilization of the proteins, supporting ERAD-dependent turnover for both TFs (Fig. 3e).  
159 Subsequent *in vitro* ubiquitination assays using purified GST-HRD1a/b isoforms and MBP-  
160 NAC53/78 proteins confirmed that both NACs are ubiquitinated by HRDs, indicated by the  
161 appearance of higher molecular weight bands of MBP-NAC proteins (Fig. 3f). We could  
162 confirm the presence of ubiquitination marks at different sites via MS/MS analysis of the *in*  
163 *vitro* ubiquitination reactions (Extended Data Fig. 2c,e). In addition, ubiquitin marks were  
164 consistently inhibited by the addition of the inhibitor LS-102 to the *in vitro* ubiquitination assay  
165 (Extended data Fig.2f). The *in vitro* data was corroborated by the identification of several  
166 ubiquitination sites on NAC53 and 78 using *in vivo* IP-MS/MS analysis (Extended data Fig.  
167 2d,e). This could be confirmed by performing ubiquitin IP on *N. benthamiana* leaves transiently  
168 expressing the NAC constructs (Extended data Fig. 2g). Mutation of identified lysine residues  
169 substantially reduced the association with anti-ubiquitin beads *in planta* (Extended data Fig.  
170 **2h,i**). Altogether, our *in vivo* and *in vitro* analysis demonstrate that NAC53 and NAC78 are  
171 directed to proteasomal degradation via ERAD through HRD1 ubiquitination.

172 During our analysis we observed that NAC53 and 78 responded different to the tested  
173 inhibitors: while BTZ led to a strong signal in the nucleus, CDC48 inhibition led to the  
174 appearance of cytoplasmic dot-like structures (Fig. 2a) that was not due to differences in drug  
175 potency depending on the concentration of the drugs (Extended data Fig. 3a). Co-expression of  
176 CDC48 isoform AtCDC48c with both NACs revealed that both proteins associated in dot-like  
177 structures (Extended data Fig. 3b), suggesting that NAC53/78 accumulate at sites of CDC48  
178 action upon CDC48 inhibition. Given the role of CDC48 in retro-translocation of ER proteins  
179 to the cytosol we tested whether inhibition of its action can also perturb the subcellular sorting  
180 of NACs comparing the GFP signal intensity in the nucleus and cytoplasm upon BTZ or CB-  
181 5083 treatment. The proportion of the GFP signal in the nucleus was significantly lower when  
182 plants were treated with CB-5083, supported by the lowered ratio of nucleus/cytoplasm GFP  
183 intensity of plants subjected to the same treatment (Extended data Fig. 3c-f).  
184 Together, our findings demonstrate the recycling of both NACs by the ERAD machinery,  
185 regulated by HRD1 and CDC48, with the latter facilitating their nuclear translocation (Fig. 3g).  
186 Thus, we propose to refer to this mechanism as ERAPS (ER-anchored protein sorting).

187

### 188 **The transcriptional landscape of proteotoxic stress**

189 To understand why the *nac53-1 78-1* mutant is more tolerant to organelle-directed proteotoxic  
190 stress while it is hypersensitive to general proteotoxic stress we undertook a transcriptomic  
191 analysis. To this end we exploited the *Pst*-mediated proteotoxic stress caused by the suppression  
192 of proteasome activity and broad modification of chloroplast proteome<sup>20,21,25</sup>. Our analysis  
193 revealed that *Pst* induced proteotoxic stress hallmarks were dependent on NAC53/78 (Fig.  
194 4a,b,c, Extended data Fig.4a) and stabilized both NAC TFs in *N. benthamiana* and *A. thaliana*  
195 (Fig. 4e,f). As such, this system is ideal to unveil the function of the proteasome autoregulatory  
196 feedback loop in response to environmental perturbation. Next, we analysed the transcriptomic  
197 profile of *nac53-1 78-1* infected with *Pst*, which revealed 945 differentially expressed genes  
198 (DEGs). Strikingly, apart from the proteasome cluster most of the DEGs displayed a positive  
199 Log<sub>2</sub>FC (Fig. 4g,h and Extended data Fig. 4a). This suggests that NAC53/78 are major  
200 transcriptional repressors upon bacteria-induced proteotoxic stress. Further investigation of  
201 which biological processes are enriched within the DEGs revealed photosynthesis,  
202 glucosinolates, cell wall and auxin signalling as major terms (Fig. 4i). To obtain a global view  
203 of the transcriptional landscape during proteotoxicity, we included two other datasets: (i)  
204 transcriptome analysis of proteasome mutant *rpt2a-2* in response to *Pseudomonas syringae* pv.  
205 *maculicola* (*Pma*) infection (Extended data Fig.4b-e) and (ii) previously characterized

206 transcriptome analysis on Col-0 plants upon MG132 treatments<sup>8</sup>. The comparison of all three  
207 datasets allowed us to extract a core set of 538 DEGs present in the *nac53-1 78-1* dataset and  
208 at least one of the other datasets (Fig. 4j and Supplementary Table 2). Extracting the genes  
209 associated with these processes from the *nac53-1 78-1* 945 DEGs, we could define the core  
210 transcriptional network associated with proteotoxic stress (Fig. 4l). This network is comprised  
211 of 4 modules: the proteasome genes, a major cluster of photosynthesis associated nuclear genes  
212 (PhANGs), glucosinolate and auxin signalling pathways (Fig. 4k,l). Only the proteasome and  
213 photosynthesis cluster were present in all three datasets (Extended data Fig.4f). This suggests  
214 that PhANGs transcriptional repression is a recurrent response to proteotoxic stress. Altogether  
215 our analysis revealed a so far unidentified link between NAC53/78 and the transcriptional  
216 regulation of PhANGs in response to proteotoxic stress.

217

### 218 **NAC53 and NAC78 coordinate the regulation of proteasome and photosynthesis gene** 219 **expression through the same cis-element**

220 The global transcriptional profile during proteotoxic stress indicates a strong link between  
221 proteasome activation and repression of photosynthesis. Thus, we hypothesized NAC53/78 can  
222 act as novel repressors of PhANGs. Detailed analysis of distinct photosynthetic processes  
223 revealed that while many processes were downregulated during proteotoxic stress, loss of  
224 NAC53/78 had a general effect on PhANGs transcriptional regulation (Extended data Fig. 5a).  
225 Enrichment analysis for specific promoter cis-elements in the 4 gene clusters of the core  
226 transcriptional network (Fig. 4k,l) revealed that the proteasome genes and PhANGs share a  
227 common cis-element characterized by the PRCE-like [TGGGC] core motif (Fig. 5a). In line  
228 with this, PRCE-like elements appeared homogeneously distributed among PhANGs promoters  
229 and enriched close to the transcription starting site (Extended data Fig. 5b,c). We then extracted  
230 the promoter regions of selected candidates, LHCA3 and PSAD1, (Fig. 5b), members of the  
231 light harvesting complex I and photosystem I<sup>26</sup>. Performing electromobility shift assay  
232 (EMSA), we could confirm that NAC53 and NAC78 directly bind to these elements (Fig. 5c),  
233 dependent on the presence of their respective DNA recognition motif (Fig. 5c). Furthermore,  
234 we confirmed the TGGGC motif as being the driver of this association, as mutated oligos were  
235 unable to outcompete the association of NAC53/78 with the probes (Fig. 5d). Chromatin IP  
236 followed by qPCR corroborated our findings, as both TFs were only able to associate to the  
237 selected promoter regions when plants were challenged with bacteria-induced proteotoxic stress  
238 (Fig. 5e). Performing luciferase reporter assays in protoplasts<sup>27</sup>, we revealed that the PSAD1  
239 promoter activity was consistently repressed by NAC53 and 78, which did not occur in *nac53-*



240 *1 78-1* mutant protoplasts (Extended data Fig. 5d). Deletion of the PRCE motif in PSAD1  
241 abolished NAC78-mediated repression, while NAC53 led to an increase in promoter activity  
242 (Extended data Fig. 5e). In contrast, LHCA3 promoter activity appeared to be rather induced by  
243 NAC53 and unchanged by NAC78 (Extended data Fig. 5d), with NAC53-mediated activation  
244 being dependent on the PRCE motif (Extended data Fig. 5e). This difference might be due to  
245 the fact that both TFs are expressed without a TM domain, bypassing ERAPS, in a rather  
246 artificial system. To circumvent this, we analysed the gene expression of *LHCA3* and *PSAD1* in  
247 the transgenic NAC53 and 78 lines upon BTZ treatment. Proteasome inhibition in the  
248 transgenic NAC53 and 78 lines partially enhanced the repression of PhANGs, supporting the  
249 notion that NAC53/78 act as repressors of photosynthesis during proteotoxic stress (Extended  
250 data Fig. 5f). In addition, both lines showed an increase in *PBA1* and *RPT2a* mRNA (Extended  
251 data Fig. 5g), confirming that NAC53/78 can act concomitantly as transcriptional repressor and  
252 activator. These results confirmed the importance of the PRCE-like element in PhANGs  
253 promoters, suggesting that NAC53 and NAC78 possess the ability to modulate each other's  
254 activity and highlights the complexity of the NAC53/78-PRCE regulatory module mediating  
255 transcriptional repression of PhANGs and activation proteasome genes. To confirm that  
256 NAC53/78 act indeed as novel repressors of photosynthesis we monitored the abundance of  
257 photosynthesis proteins, PSII activity and photosynthetic pigment content upon bacteria-  
258 induced proteotoxicity. To summarize, we detected that bacterial infection had a significantly  
259 lesser impact on all measured photosynthesis readouts in the *nac53-1 78-1* double mutant  
260 compared to Col-0 (Fig. 5f-j). Consistently with these findings, subjecting the NAC53/78  
261 transgenics to constant proteasome inhibition resulted in strong developmental defect  
262 (Extended data Fig.5h,i), suggesting that the role of NAC53/78 on photosynthesis repression is  
263 not restricted to bacterial infection. Altogether, we demonstrate the unique ability of NAC53  
264 and NAC78 to mediate activation and repression of target genes through the same PRCE  
265 element. This makes them novel repressors of photosynthesis during proteotoxic stress and  
266 explains our previous findings that the NAC mutant was more tolerant to organelle-directed  
267 proteotoxic stress.

268

### 269 **The proteasome autoregulatory feedback loop monitors photosynthesis homeostasis** 270 **during stress responses**

271 Our results suggest that photosynthesis repression by NAC53/78 is a general feature of the  
272 proteasome autoregulatory feedback loop to cope with proteotoxicity. In line with this, recent  
273 evidence indicates that loss of proteasome function in a chloroplast import deficient mutant

274 improved photosynthesis due to a lack of chloroplast precursor protein degradation<sup>28</sup>. To  
275 analyze to which extent NAC53/78 are involved in the response to chloroplast perturbation, we  
276 investigated photosynthesis readouts upon BTZ and Lincomycin treatments in *nac53-1 78-1*  
277 plants. All readouts (pigment content, PhANGs expression, PSII activity) were significantly  
278 less impaired in *nac53-1 78-1* (Fig. 6a, b and Extended data Fig. 6a), suggesting that NAC53/78  
279 are indeed monitoring PhANGs expression during chloroplast stress. We then repeated the  
280 phenotypic screen introduced in Fig. 1 using the *hrd1a 1b* double mutant<sup>29</sup> to investigate the  
281 direct involvement of the proteasome autoregulatory feedback loop in coordinating cellular  
282 proteostasis and photosynthesis. Proteasome inhibition rendered the *hrd1a 1b* more tolerant in  
283 comparison to Col-0 (Fig. 6c), which is consistent with its role in negatively regulating  
284 NAC53/78 stability (Fig. 3g). In contrast, all other tested drugs or bacterial infection rendered  
285 the *hrd1a 1b* mutant more susceptible (Fig. 6c,d). The reduced growth of *hrd1a 1b* upon  
286 chloroplastic perturbations (Cml and Lin) are consistent with our previous results (Fig. 1b), as  
287 stabilisation of NACs would lead to increased repression of PhANGs and growth reduction  
288 (Extended data Fig. 5a, h,I ). The impaired performance of *hrd1a 1b* upon CDC48 inhibition,  
289 pathogen infection or ER stress induction might be due to the general function of HRDs in  
290 ERAD<sup>30</sup>. Analysis of the photosystem II activity revealed a stronger reduction in the *hrd1a 1b*  
291 double mutant (Extended data Fig. 6b) upon infection corroborating our findings that ERAPS  
292 coordinates proteasome and photosynthesis through NAC53/78 stability. In addition, these data  
293 suggest that organellar perturbation would lead the NAC53/78 activation. Indeed, subjecting  
294 GFP-NACs transgenics to several organellar perturbators led to stabilization and nuclear  
295 localization of both TFs (Extended data Fig. 6c). Taken together our analysis illustrates the  
296 importance of the proteasome autoregulatory feedback loop in maintaining the fine equilibrium  
297 of proteostasis to ensure normal response to proteotoxic stress and suggests that it is involved  
298 in other biological contexts. To answer this, we performed a large-scale meta-transcriptomic  
299 analysis using the recently developed Arabidopsis RNAseq data base<sup>31</sup>. Using our pipeline (see  
300 methods and Supplementary Table 3) we investigated the correlation of transcript abundance  
301 between the 54 genes encoding 26S proteasome subunits and the 68 PhANGs associated with  
302 proteotoxic stress. We could observe that genes within the same cluster displayed a strong  
303 positive correlation ( $\rho \approx 0.6$  and  $\rho \approx 0.5$  for 26S Proteasome and PhANGs, respectively).  
304 Strikingly, analysing the relationship between the two clusters Proteasome vs. PhANGs showed  
305 a significant negative correlation ( $\rho \approx -0.4$ ) (Fig. 6e), supporting the notion that this is a general  
306 phenomenon. To investigate this in more detail, we extracted 43 projects in which 26S  
307 Proteasome and PhANGs appear transcriptionally co-regulated (Supplementary Table 3). From

308 these 43 projects, 35 displayed an opposite regulation for 26S Proteasome and PhANGs mRNA  
309 level (Extended data Fig. 6d), confirming that both clusters undergo recurrent contrasting co-  
310 regulation. Amongst them pathogen attack, abiotic stress (cold, dark, drought), circadian  
311 rhythm, nutrient stress, hormone treatment (SA, auxin) as well as treatment with phytotoxins  
312 (trans-chalcone) resulted in a negative correlation. It suggests that coordination of  
313 PhANGs/proteasome is a general feature of plant stress responses and developmental processes.  
314 Thus, the coordination of proteasome activation and PhANGs repression is a key feature of the  
315 *A. thaliana* transcriptome in response to multiple biotic, abiotic, and developmental cues.

316

## 317 **Discussion**

318 Understanding how the proteasome can degrade proteins from different compartments and how  
319 these different subcellular signals are integrated upon external cues and to maintain overall  
320 proteostasis was a long-standing question. Our study discovered that the NAC53/78 module  
321 acts as a gatekeeper to facilitate the communication between the chloroplast-proteasome-  
322 nucleus during proteotoxic stress (Extended Data Fig. 7). We have identified ERAPS as the key  
323 regulatory node of the proteasome autoregulatory feedback loop that shapes and maintains  
324 subcellular proteostasis in plants. Considering this loop is mechanistically conserved across  
325 kingdoms, similar control mechanisms of subcellular proteostasis are possible in yeast and in  
326 animals<sup>6</sup>. Interestingly, connections between ER-anchored TFs and proteasome activation have  
327 previously been observed in animals, whilst separate studies identified an indirect link between  
328 proteasomal, and mitochondrial transcriptional regulation in yeast<sup>19</sup>. However, a unified  
329 mechanism that explains the direct crosstalk between these seemingly disparate processes has  
330 been lacking. This implies a divergence between yeast and plants on subcellular proteostasis  
331 coordination.

332 Our findings highlight that subcellular proteostasis is governed by the ERAPS-dependent  
333 autoregulatory pathway permitting a rapid integration of proteotoxic signals from various  
334 compartments. As such, it allows the communication between different organelles such as  
335 chloroplast or mitochondria with the nucleus, a process referred as retrograde signaling<sup>32</sup>. This  
336 communication is essential to respond to sudden changes in the environment. Retrograde  
337 signaling from mitochondria or chloroplast to the nucleus involves massive transcriptional  
338 changes that in turn influence organelle function<sup>33</sup>. Some nuclear communicators have been  
339 identified to mediate retrograde signaling<sup>33</sup>. However, to date, transcription factors GLK1 and  
340 2, are the only known PhANGs activators<sup>34</sup>, while putative repressors ABI4 and PTM could not  
341 be verified as retrograde signaling components<sup>35-38</sup>. Here we have identified that plants have

342 evolved a dual-regulatory system through the proteasome autoregulatory feedback loop: the  
343 NAC53/78-PRCE module acts as a novel retrograde signaling component, repressing PhANGs  
344 expression and activating proteasome genes via the same cis-element during proteotoxic stress.  
345 In this scenario, (i) repression of PhANGs by NAC53/78 may be a strategy to cope with the  
346 excess of precursor accumulation due to proteasomal stress (Extended Data Fig. 7) which is  
347 corroborated by our findings that the *nac53-1 78-1* mutant is more tolerant to photosynthesis  
348 stress and HSP90 inhibition which activate NAC53/78 nuclear localization but also (ii) to  
349 counteract the overactivation of PhANGs by GLK1 that is degraded by the proteasome<sup>39</sup>. To  
350 our knowledge, a similar system has not been found in other multicellular organisms such as  
351 animals. However, findings in animals that connect proteasome activators and mitochondrial  
352 biogenesis<sup>40</sup>, strongly suggest a tight link between proteasome and energy metabolism in  
353 animals.

354 Various environmental perturbations impede the balance between protein translation, sorting  
355 and degradation resulting in proteotoxicity. Mounting evidence highlights a role for the  
356 proteasome in clearing organelle-associated proteins and immune components, mediating the  
357 growth-defense trade-off. Thus, it is essential to decipher how signals from different  
358 compartments and organelles are integrated to maintain proteostasis during various stress  
359 conditions. Our findings provide a new conceptual framework for understanding the integral  
360 role of the proteasome complex as a *bona fide* signalling hub, to manage cellular proteostasis  
361 under environmental stress. This framework could be potentially universally employed across  
362 various organisms to adapt to internal and external disruptions.

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374 **FIGURE LEGENDS**

375 **Fig. 1. NAC53/78 are central integrators of various proteotoxic stress conditions.**

376 (A) The role of the 26S Proteasome in degradation of proteins from distinct subcellular  
377 compartments. Prime site of action of the several treatments used in this study: Blunt head  
378 arrows indicate enzymatic activity suppression; pointed head arrows indicate trigger of  
379 substrate accumulation.

380 (B) Fresh weight of seedlings grown under the indicated treatments at 10-14 day after  
381 germination (dag). Fresh weight is expressed as a percentage of Col-0 mock conditions.  
382 Boxplots colors refer to the genotype. Statistical significance is assessed by a Wilcoxon-Mann-  
383 Whitney test (p values: n.s. > 0.05; \* < 0.05; \*\* < 0.01). Every treatment has been repeated at  
384 least twice with similar results.

385 (C) Bacterial density in Log10 Colony-Forming-Unit (CFU) per leaf cm<sup>2</sup>. Statistical  
386 significance is assessed by a Wilcoxon-Mann-Whitney test (p values: n.s. > 0.05; \*\* < 0.01).  
387 Boxplots colors refer to the genotype as in panel B. The treatment has been repeated three times  
388 with consistent results.

389 (B-C) Representative phenotypic pictures of Col-0 and *nac53-1 78-1* plants under several  
390 treatments are included.

391

392 **Fig. 2. NAC53/78 are part of an autoregulatory proteasome feedback loop.**

393 (A) Confocal microscopy pictures of roots of GFP-NAC53/78 *Arabidopsis thaliana* transgenics  
394 at 7dag exposed to mock or BTZ 10μM treatment for 3h. Treatments were repeated at least  
395 three times with similar observations.

396 (B) Immunoblot analysis against GFP on crude extract of seedlings related to panel A. Ponceau  
397 staining is used as loading control.

398 (C) Confocal microscopy pictures of transiently co-expressed sTag-NAC53/78 with  
399 prom35S::RFP-HDEL or together in *N. benthamiana* epidermis cells. Pearson index (ρ)  
400 represents co-localization index. Scale bars = 10μm

401 (D) Confocal microscopy pictures of transiently co-expressed sTag-NAC53/78 together or  
402 dTag-NAC53/78 alone in *N. benthamiana* epidermis cells. Scale bars = 10μm

403 (E) Bar plots representing the number of significantly enriched proteins in the 4 IP-MS/MS  
404 conditions for NAC53 only, NAC78 only and NAC53+NAC78 (Common).

405 (F) Cladogram of top 30 gene ontology (GO) terms for biological process (BP) enriched in the  
406 list of *A. thaliana* ortholog proteins found from NAC53/78 common interactome.

407 (G) Protein network of the interacting candidates related to the GO BP terms annotated as  
408 “Transport” and “Proteolysis” in panel F.

409

410 **Extended data Fig. 1. Deciphering the details of NAC53/78 interactome.**

411 (A) Chimeric NAC53/78 constructs generated in this study. Upstream promoter, fluorescent tag  
412 and protein domain of interests are represented.

413 (B) Immunoblot against GFP on crude extracts of *N. benthamiana* leaves transiently expressing  
414 sTag-NACs in addition to mock or 6h BTZ (10μM) treatment. Ponceau staining is used as  
415 loading control.

416 (C) Immunoblot against GFP or RFP on crude extracts of *N. benthamiana* leaves transiently  
417 expressing dTag-NACs in addition to mock or BTZ 10μM for 6h. Ponceau staining is used as  
418 loading control.

419 (D) Immunoblot against GFP after immunoprecipitation (IP) with anti-GFP agarose beads on  
420 *N. benthamiana* leaf extracts transiently expressing sTag-NACs after mock or *Pst*Δ*HopQ*  
421 infection for 8h. The same samples were used for subsequent MS/MS analysis.

422 (E) Immunoblot against GFP or RFP after IP with anti-GFP or anti-RFP agarose beads on *N.*  
423 *benthamiana* leaf extracts transiently expressing dTag-NACs. The same samples were used for  
424 subsequent MS/MS analysis.

425 (F) Cladogram of top 30 GO terms for cellular components enriched in the list of *A. thaliana*  
426 ortholog proteins found in NAC53/78 common interactome.  
427 (G) Subcellular compartments enrichment in the list of *A. thaliana* proteins from NAC53/78  
428 common interactome based on their SUBA5db annotation. Statistical significance is provided  
429 through a chi-square testing.  
430 (H-K) Heatmap representing the peptide enrichment (Log<sub>2</sub>FC) of the protein clusters from the  
431 network Fig. 2G.

432

433 **Fig. 3. ER-Anchored Protein Sorting (ERAPS) coordinates the subcellular fate of NAC53**  
434 **& NAC78.**

435 (A) Confocal microscopy pictures of GFP-NAC53/78 expressing *A. thaliana* roots. 7 dag  
436 seedlings were exposed to mock treatment, BTZ 10 $\mu$ M, CB-5083 10 $\mu$ M or LS-102 100 $\mu$ M for  
437 3h. The treatments were repeated at least three times with similar observations. Scale bars =  
438 10 $\mu$ m

439 (B) Immunoblot against GFP on crude extracts of GFP-NAC53/78 *A. thaliana* adult plants  
440 infiltrated with mock treatment, BTZ 10 $\mu$ M, CB-5083 10 $\mu$ M or LS-102 100 $\mu$ M for 6h. Ponceau  
441 staining is used as loading control.

442 (C) Confocal microscopy pictures of *N. benthamiana* transiently co-expressing GFP-  
443 NAC53/78 full-length (FL) or deleted for the transmembrane domain ( $\Delta$ TM) with RFP-HRD1a  
444 or RFP-HRD1b. Pearson index ( $\rho$ ) indicates correlation index at the orange lines.

445 (D) Co-IP of RFP-HRD1a/b with GFP-NAC53/78. IP was performed with anti-GFP beads.  
446 GFP-HDEL and free-GFP were used as negative control. The experiment was repeated twice  
447 with similar results.

448 (E) Immunoblot against GFP on crude extract of *N. benthamiana* leaves transiently expressing  
449 GFP-NAC53/78FL or  $\Delta$ TM. Ponceau was used as loading control. Boxplots represent the  
450 relative  $\Delta$ TM abundance compared FL.

451 (F) *In vitro* trans-ubiquitination assays of GST-HRD1a/b against MBP-NAC53/78. Removal of  
452 E1 and ATP from the reaction was used as negative control. The experiment was repeated twice  
453 with similar results.

454 (G) Representation of the ER-Anchored Protein Sorting (ERAPS) mechanism. NAC53/78 are  
455 ER-anchored proteins recognized and ubiquitinated by the HRD1 complex. The CDC48  
456 complex subsequently extracts NAC53/78 from the ER to mediate ER-Associated Degradation  
457 or allowing nuclear translocation in the context of proteasomal stress to mitigate proteotoxicity.

458

459 **Extended data Fig. 2. NAC53/78 are subjected to high level of poly-ubiquitination.**

460 (A) *In vitro* analysis of LS-102 effect on HRD1a/b ubiquitination activity. Removal of E1 and  
461 ATP from the reaction was used as negative control.

462 (B) Quantification of the inhibitory effect of LS-102 (100 $\mu$ M) on HRD1 activity. Bar plots  
463 represent the mean of three independent replicates with error bars representing the standard  
464 deviation.

465 (C) Heatmaps representing the mean Log<sub>10</sub> intensity of peptides bearing a di-glycine mark on  
466 the indicated lysin residue identified by MS/MS analysis of *in vitro* trans-ubiquitination  
467 reactions (see Figure 3F).

468 (D) Heatmaps representing the mean Log<sub>10</sub> intensity of peptides bearing a di-glycine mark on  
469 the indicated lysin residue identified by MS/MS analysis of IP samples generated for Figure 2.

470 (E) Alignments of NAC53 and NAC78 peptide sequence regions around the identified lysin  
471 residues in panel C and D. Colors represent the residue conservation (green: conserved, shade  
472 of red: not conserved). All the identified lysins in one protein are conserved in the other.  
473 Underlined residues correspond to the mutated residues generated for the panel H and I.

474 (F) *In vitro* trans-ubiquitination assays of GST-HRD1a/b against MBP-NAC53/78 in the  
475 presence of LS-102 inhibitor. Removal of E1 and ATP from the reaction was used as negative  
476 control.

477 (G) Immunoblot analysis of *N. benthamiana* transiently expressing free-GFP, RFP-HDEL or  
478 dTag-NAC53/78 subjected to IP with anti-ubiquitin beads. The experiment was repeated twice  
479 with similar results.

480 (H) Immunoblot analysis of *N. benthamiana* transiently expressing GFP-NAC53/78 mutated  
481 for 5 lysins (Mut., see panel E) or not (WT) subjected to IP with anti-ubiquitin beads.

482 (I) Quantification of the relative ubiquitination levels assessed by the ratio between GFP signal  
483 and ubiquitin signal after IP as in panel H. Barplots represent the mean ratio of two replicates,  
484 normalized to the ratio of WT constructs with error bars representing the standard deviation.

485

### 486 **Extended data Fig. 3. NAC53/78 association with CDC48 is required for their nuclear** 487 **translocation.**

488 (A) Confocal microscopy pictures of transgenic GFP-NAC53/78 root seedlings exposed to BTZ  
489 10 $\mu$ M, BTZ 20 $\mu$ M, CB-5083 10 $\mu$ M or CB-5083 20 $\mu$ M for 3h. Scale bars = 10 $\mu$ m

490 (B) Confocal microscopy pictures of *N. benthamiana* leaves transiently co-expressing  
491 AtCDC48c-GFP with RFP-NAC53/78. Co-localization is visible in aggregate-like structures.  
492 Scale bars = 1 $\mu$ m

493 (C) Confocal microscopy representative pictures (used for panel D-E) of transgenic GFP-  
494 NAC53/78 roots treated with BTZ 10 $\mu$ M and CB-5083 10 $\mu$ M followed by Propidium Iodide  
495 staining. Scale bars = 10 $\mu$ m

496 (D-F) Boxplot representing the GFP signal quantification after confocal microscopy imaging  
497 of transgenic GFP-NAC53/78 roots exposed to BTZ 10 $\mu$ M or CB-5083 10 $\mu$ M. Black dots  
498 represent one cell. Statistical difference is assessed by a Wilcoxon-Mann-Whitney test.

499

### 500 **Fig. 4. The transcriptional landscape of proteotoxic stress**

501 (A) Log<sub>2</sub>FC mRNA levels of *NAC53* and *NAC78* transcripts after *Pst* infection of adult Col-0  
502 plant leaves. The heatmap represent the mean of 4 biological replicates.

503 (B) Log<sub>2</sub>FC mRNA levels of 26S proteasome transcripts after *Pst* infection of Col-0 or *nac53-1*  
504 *78-1* adult plant leaves. The heatmap represent the mean of 4 biological replicates. The  
505 experiment has been repeated 3 times with consistent results.

506 (C) Immunoblot against multiple 26S proteasome subunits on crude extracts of Col-0 or *nac53-1*  
507 *78-1* adult plant leaves inoculated with *Pst* or mock solutions for 24h. Ponceau staining is  
508 used as loading control.

509 (D) Relative Log<sub>2</sub>FC abundance of representative 26S proteasome subunits in Col-0 or *nac53-1*  
510 *78-1* adult plant leaves. The abundance is calculated relative to the mock conditions.  
511 Statistical differences are assessed via a Welch t.test (p values: n.s > 0.05, \* < 0.05, \*\* < 0.01).

512 (E) Immunoblot against GFP on crude extract of *N. benthamiana* leaves transiently expressing  
513 GFP-NAC53/78 after mock treatment or *Pst* $\Delta$ *HopQ* infection for 8h. Ponceau staining is used  
514 as loading control.

515 (F) Immunoblot against GFP on extract of adult *A. thaliana* transgenics GFP-NAC53/78  
516 subjected to mock treatment or *Pst* infection 8h by vacuum infiltration followed by IP with anti-  
517 GFP beads.

518 (G) Number of differentially expressed genes (DEGs) in the different conditions of the *nac53-1*  
519 *78-1 Pst* RNAseq analysis. Total number of DEGs per condition is indicated. Colors indicate  
520 the proportion of DEGs up-regulated and down-regulated. DEGs are considered when |Log<sub>2</sub>FC|  
521 > 1.5. Genot. mock: *nac53-1 78-1* mock vs. Col-0 mock; WT *Pst*: Col-0 *Pst* vs. Col-0 mock;

522 Mut. *Pst*: *nac53-1 78-1 Pst* vs. *nac53-1 78-1* mock; Genot. *Pst*: *nac53-1 78-1 Pst* vs. Col-0 *Pst*.

523 (H) Level of differential expression (Log<sub>2</sub>FC) of the 945 DEGs found in Genot. *Pst* (see panel  
524 G) for the several conditions analyzed.

525 (I) Cladogram of top 40 GO terms for BP enriched in the list of 945 DEGs. Colors represent  
526 broad GO clusters.  
527 (J) Venn diagram representing the overlap between the 945 DEGs and DEGs from *rpt2a-2 Pma*  
528 ( $|\text{Log2FC}| > 0.5$ ) or Col-0 MG132 24h ( $|\text{Log2FC}| > 0.5$ ).  
529 (K) Cladogram of top 30 GO terms for BP enriched in the list of 538 DEGs extracted from the  
530 combined analysis (panel J). For conciseness terms are hidden and grouped in 4 processes.  
531 (L) Protein network of the genes from the 945 DEGs list related to the GO BP terms from panel  
532 K. Node colors refers to the 4 GO clusters from panel K.  
533

534 **Extended data Fig. 4. Transcriptional analysis of proteotoxic stress reveals a trade-off**  
535 **between 26S proteasome and other biological process.**

536 (A) mRNA level Log2FC of all 26S proteasome genes and associated interactors/chaperones in  
537 several conditions of the *nac53-1 78-1 Pst* RNAseq.  
538 (B) Number of differentially expressed genes (DEGs) in the different conditions of the *nac53-*  
539 *1 78-1 Pst* RNAseq analysis. Total number of DEGs per condition is indicated. Colors indicate  
540 the proportion of DEGs up-regulated and down-regulated. DEGs are considered when  $|\text{Log2FC}|$   
541  $> 1.5$ . Genot. mock: *rpt2a-2* mock vs. Col-0 mock; WT *Pma*: Col-0 *Pma* vs. Col-0 mock; Mut.  
542 *Pma*: *rpt2a-2 Pma* vs. *nac53-1 78-1* mock; Genot. *Pma*: *rpt2a-2 Pma* vs. Col-0 *Pma*.  
543 (C) mRNA levels Log2FC of the 981 DEGs found in Genot. *Pma* (see panel D) for the several  
544 conditions analyzed.  
545 (D) mRNA level Log2FC of all 26S proteasome genes and associated interactors/chaperones in  
546 the several conditions of the *rpt2a-2 Pma* RNAseq.  
547 (E) Cladogram of top 40 GO terms for BP enriched in the list of 945 DEGs. Colors represent  
548 the broad GO clusters.  
549 (F) mRNA level Log2FC of the genes present in the 4 network modules from Figure 4L in the  
550 3 Transcriptome used.  
551

552 **Fig. 5. NAC53 and NAC78 coordinate the regulation of proteasome and photosynthesis**  
553 **gene expression through the same cis-element.**

554 (A) Top 3 motifs found by STREME software in the promoter regions from the 4 network  
555 clusters (Figure 4L). PRCE-like elements from proteasome and PhANGs clusters are  
556 highlighted.  
557 (B) 25bp region comprising the PRCE-like elements from LHCa3 and PSAD1 promoters used  
558 in this figure. The region corresponds to the probe sequence used in panel C and D. The PRCE  
559 is highlighted in red.  
560 (C) Electro-Mobility Shift Assay (EMSA) of MBP-NAC53/78 with LHCa3/PSAD1 probes (see  
561 panel B). As a negative control, probes were incubated without MBP-NAC53/78 (-) or with  
562 MBP-NAC53/78 deleted for their DNA recognition motif ( $\Delta\text{RM}$ ). Experiment was repeated  
563 twice with similar results.  
564 (D) EMSA competition assay of MBP-NAC53/78 association with LHCa3/PSAD1 probes.  
565 Competitor is applied at a concentration gradient (25X, 50X, 75X and 100X) indicated by the  
566 shade of grey. WT and mutated competitors are labelled TGGGC and AAAAA, respectively.  
567 Experiment was repeated twice with similar results.  
568 (E) Boxplot representing association of GFP-NAC53/78 with LHCa3/PSAD1 PRCE in adult  
569 *A. thaliana* transgenics lines infected with *Pst* or mock for 8h after Chromatin  
570 Immunoprecipitation. The association is quantified as % of input and amplification of a region  
571 in the CDS is used as negative control.  
572 (F) Immunoblot against mature PhANGs proteins in Col-0 or *nac53-1 78-1* adult plant leaves  
573 inoculated with *Pst* or mock solutions for 24h. Ponceau staining is used as loading control.



574 (G) Relative Log<sub>2</sub>FC abundance of PhANGs mature proteins immunoblotted in panel F. The  
575 abundance is calculated relative to the mock conditions. Statistical differences are assessed via  
576 a Welch t.test (p values: n.s > 0.05, \* < 0.05, \*\* < 0.01).  
577 (H) Photosystem II (PSII) activity measurement on Col-0 or *nac53-1 78-1* adult plants  
578 inoculated with *Pst* or mock solutions for 24h. Letters indicate the statistical group assessed by  
579 pairwise Welch t.test (p value < 0.05). The experiment was repeated 3 times with consistent  
580 results.  
581 (I) Representative pictures of measurements from panel I. Fv/Fm values are represented by  
582 false color as indicated by the color gradient legend.  
583 (J) Total chlorophylls and carotenoids from Col-0 or *nac53-1 78-1* adult plant leaves inoculated  
584 with *Pst* or mock solutions for 24h. Letters indicate the statistical group assessed by pairwise  
585 Wilcoxon-Mann-Whitney test (p value < 0.05). The experiment was repeated 3 times with  
586 consistent results.  
587

588 **Extended data Fig. 5. Characterization of the NAC53/78-PRCE module in PhANGs**  
589 **promoters.**

590 (A) mRNA level Log<sub>2</sub>FC of the PhANGs clusters from Figure 4L in the 3 transcriptomes used.  
591 Genes are separated based on their associated process in the photosynthetic pathway.  
592 (B) Mapping of the PRCE-like motifs in PhANGs promoter regions from panel A. Promoter  
593 regions are organized according to the order of the genes from panel A. Purple marks represent  
594 the position of the motifs based on their distance from the transcription starting site (TSS). The  
595 two red arrows highlight LHCa3 and PSAD1 promoter regions.  
596 (C) Histograms representing the number of occurrences of the PRCE-like motifs across the 4  
597 genes cluster from the network Figure 4L. Each bar represents a 20bp region, frequency *f* of  
598 promoters bearing at least one motif is indicated as a percentage.  
599 (D) LHCa3/PSAD1 promoter activity measured by luminescence in Col-0 or *nac53-1 78-1*  
600 protoplasts extract transiently co-expressing LHCa3/PSAD1 reporter constructs and  
601 NAC53/78ΔTM. Log<sub>2</sub>FC is calculated from the luminescence level of protoplasts expressing  
602 the reporter construct alone. Replicates are a pool of at least two independent experiments.  
603 Letters indicate the statistical group assessed by pairwise Wilcoxon-Mann-Whitney test (p  
604 value < 0.05).  
605 (E) Promoter activity as calculated in panel D for LHCa3/PSAD1 promoters deleted for their  
606 respective DNA regions from Figure 5B. Replicates are a pool of at least two independent  
607 experiments. Letters indicate the statistical group assessed by pairwise Wilcoxon-Mann-  
608 Whitney test (p value < 0.05).  
609 (F-G) Boxplot representing the Log<sub>2</sub>FC mRNA level the indicated transcripts in Col-0, GFP-  
610 NAC53 or GFP-NAC78 seedlings treated with BTZ 10μM or mock solutions for 6h. Log<sub>2</sub>FC  
611 is calculated from Col-0 mock. Letters indicate the statistical group assessed by pairwise  
612 Wilcoxon-Mann-Whitney test (p value < 0.05).  
613 (H) Fresh weight of seedlings from indicated genotypes grown under the indicated treatments  
614 at 10 days after germination. Letters indicate the statistical group assessed by pairwise  
615 Wilcoxon-Mann-Whitney test (p value < 0.05). Experiment was repeated 3 times with  
616 consistent results.  
617 (I) Representative phenotypic pictures related to panel D.  
618

619 **Fig. 6. The proteasome autoregulatory feedback loop monitors photosynthesis**  
620 **homeostasis during stress responses**

621 (A) Photosynthetic pigment content from Col-0 or *nac53-1 78-1* adult plant leaves infiltrated  
622 with Lin 200μM, BTZ 2μM or mock solutions for 24h. Content is expressed in percentage  
623 relative to Col-0 mock. Letters indicate the statistical group assessed by pairwise Wilcoxon-

624 Mann-Whitney test (p value < 0.05). The experiment was repeated 3 times with consistent  
625 results.

626 (B) Log2FC mRNA level of the indicated transcripts in Col-0, *nac53-1 78-1* adult plants leaves  
627 Lin 200 $\mu$ M, BTZ 2 $\mu$ M or mock solutions for 24h. Log2FC is calculated from Col-0 mock and  
628 *PDHI* is used as negative control. Statistical differences are assessed with a Welch t-test (p  
629 values: n.s. > 0.05, \* < 0.05, \*\* < 0.01). The experiment was repeated twice with consistent  
630 results.

631 (C) Fresh weight of seedlings grown under the indicated treatments at 10-14 day after  
632 germination. The fresh weight is represented as a percentage of Col-0 mock conditions. Letters  
633 indicate the statistical groups assessed by pairwise Wilcoxon-Mann-Whitney test (p value <  
634 0.05). Every treatment has been repeated at least twice with consistent results. Representative  
635 phenotypic pictures are included.

636 (D) Bacterial density shown as Log10 Colony-Forming-Unit (CFU) per leaf cm<sup>2</sup> in Col-0 and  
637 *hrd1a hrd1b* plants. Statistical significance is assessed by a Welch t-test (p values: \* < 0.05).  
638 The experiment has been repeated three times with consistent results.

639 (E) Correlation heatmap of the 54 26S proteasome subunit genes and the expression of 68  
640 PhANGs from publicly available 1223 RNAseq libraries identified in this study.

641

642 **Extended data Fig. 6. The proteasome autoregulatory feedback loop transcriptional**  
643 **signature is a systemic response to environmental cues.**

644 (A) PSII activity measurement on Col-0 and *nac53-1 78-1* treated as indicated in Figure 6A.  
645 Replicates are a pool of 3 independent experiments. Letters indicate the statistical group  
646 assessed by pairwise Wilcoxon-Mann-Whitney test (p value < 0.05).

647 (B) PSII activity measurement on Col-0 and *hrd1a hrd1b* adult plant leaves inoculated with *Pst*  
648 or mock solutions for 24h. Letters indicate the statistical group assessed by pairwise Welch  
649 t.test (p value < 0.05). The experiment was repeated 2 times with consistent results.

650 (C) Confocal microscopy pictures of transgenic GFP-NAC53/78 *A. thaliana* roots exposed to  
651 mock treatment, MV 100 $\mu$ M, GDA 100 $\mu$ M and Lin 100 $\mu$ M for 2h. The treatments were  
652 repeated at least three times with similar observations. Scale bars = 10 $\mu$ m

653 (D) Density functions of the 54 26S proteasome genes and 68 PhANGs transcripts (Log2FC)  
654 in the different transcriptome projects. Log2FC limits are -10 to +10. Treatment types and  
655 relevant condition information are provided in the figure. Plots are separated by treatment  
656 groups and subdivided by experiments.

657

658 **Extended data Fig. 7. ER-anchored protein sorting (ERAPS) controls the fate of two**  
659 **proteasome activators for intracellular organelle communication during proteotoxic**  
660 **stress.**

661 In steady state conditions, the ER-anchored protein sorting system (ERAPS) promotes the  
662 constitutive degradation of NAC53/78 via the 26S proteasome. Meanwhile, PhANGs  
663 expression is activated and subsequent chloroplastic import permits the maintenance of active  
664 photosynthesis. Upon proteotoxic stress, the chloroplast import machinery is targeted for  
665 proteasomal degradation. This leads to an accumulation of PhANGs precursors which are  
666 therefore subjected to proteasomal degradation, inducing proteotoxicity. Thus, to avoid  
667 proteotoxicity, the ERAPS system facilitates the nuclear translocation of NAC53/78, to activate  
668 the production of a new proteasome complex and to repress PhANGs expression to mitigate  
669 substrate accumulation.

670

671

672

## 673 **Methods**

### 674 **Plant materials and growth conditions**

675 *A. thaliana* Columbia-0 (Col-0) ecotype was considered as WT. T-DNA insertion mutants for  
676 *NAC53* (*nac53-1*, SALK\_009578C) and *NAC78* (*nac78-1*, SALK\_025098) were previously  
677 described in Gladman et al., 2016. T-DNA insertion mutants for *RPT2a* was previously  
678 described<sup>20</sup>. T-DNA insertion mutants for *HRD1a* (*hrd1a*, SALK\_032914) and *HRD1b* (*hrd1b*,  
679 SALK\_061776) were obtained from Yasin Dagdas. *promUBQ10::GFP-NAC53* and  
680 *promUBQ10::GFP-NAC78* transgenics lines were obtained by *Agrobacterium tumefaciens*  
681 floral dipping of Col-0 plants. For experiments on seedlings, seeds were surface sterilized  
682 10min with 1.3% sodium hypochlorite. Seeds were sown on ½ Murashige and Skoog (MS)  
683 medium plus 1% sucrose and stratified for 2 days. Plants were grown under long day conditions  
684 (light/dark cycles: 16h 22°C/8h 20°C, 130µmol.mm<sup>2</sup>.s<sup>-1</sup> light intensity, 70% relative humidity).  
685 For experiments on adult plants, plants were grown under short day conditions (light/dark  
686 cycles: 12h 22°C/ 12h 20°C, 90µmol.mm<sup>2</sup>.s<sup>-1</sup> light intensity, 70% relative humidity). Plants  
687 were grown 4-5 weeks until use.

688

### 689 ***Nicotiana benthamiana* growth**

690 *N. benthamiana* plants were grown under long day conditions (light/dark cycles: 16h/8h, at  
691 21°C and 70% humidity) and typically used 4 weeks after germination for confocal microscopy  
692 experiments and 5 weeks after germination for co-immunoprecipitation experiments.

693

### 694 **Bacterial strains**

695 For *Pseudomonas syringae* pv. *tomato* DC3000 wild-type and *Pseudomonas syringae* pv.  
696 *maculicola* strains ES4326 were grown on King's B medium containing rifampicin 100µg.mL<sup>-1</sup>  
697 at 28°C. For floral dipping and transient expression in *Nicotiana benthamiana* leaves,  
698 *Agrobacterium tumefaciens* C58C1 strain was grown in LB broth high salt (Duchefa L1704)  
699 containing the required antibiotics at 28°C. For molecular cloning, *Escherichia coli* Top10  
700 strain was grown in LB broth high salt (Duchefa L1704) containing the required antibiotics at  
701 37°C. For protein purification, *Escherichia coli* BL21 (DE3) was grown in LB broth high salt  
702 (Duchefa L1704) containing the required antibiotics at 37°C or 16°C.

703

### 704 **Gene Accession**

705 Arabidopsis genome initiative (AGI) locus identifier of the principal genes investigated in this  
706 study are the following: *NAC53* (AT3G10500), *NAC78* (AT5G04410), *HRD1a* (AT3G16090),

707 *HRD1b* (AT1G65040), *CDC48c* (AT5G03340), *RPT2a* (AT4G29040), *LHCa3* (AT1G61520),  
708 *PSAD1* (AT4G02770).

709

### 710 **Molecular cloning**

711 For chimeric protein constructs generated in this study, protein coding sequence (CDS) of the  
712 desired genes were amplified from *A. thaliana* cDNA. For Golden Gate based cloning<sup>41</sup>, CDS  
713 were amplified with addition of flanking regions including BpiI/BsaI sites and final constructs  
714 were generated using the LI CDS module of interest and desired other modules and assembled  
715 in the LIIa F 1-2 vector. For GATEWAY™ based cloning, CDS were amplified with addition  
716 of attb1/attb2 flanking regions and final constructs were generated through BP clonase™ II  
717 enzymatic reaction and LR Clonase™ II enzymatic reaction into the desired destination vectors.  
718 All LI and GATEWAY™ entry clones were verified by sanger sequencing.

719

### 720 **Seedlings phenotyping**

721 For phenotyping assay, seeds were sown on ½ MS 1% sucrose round petri dish supplemented  
722 with the indicated concentration of drug or a mock treatment. After 10 to 14 days of growth  
723 fresh weight was measured. For one replicate, 5 representative seedlings were scaled together  
724 on an analytical scale (resolution 0.0001g). Typically, 5 replicates were measured for  
725 subsequent statistical analyses.

726

### 727 **Transient expression in *N. benthamiana* leaves**

728 Bacterial suspension was centrifuged for 4min at 4000g after overnight incubation of *A.*  
729 *tumefaciens* strain carrying the desired expression construct. Supernatant was removed and  
730 pellet was resuspended in 500µl of agroinfiltration buffer (10mM MgCl<sub>2</sub>, 10mM MES pH 5.7).  
731 OD600 was measured and infiltration solutions were diluted to reach final 0.5 OD600 in  
732 agroinfiltration buffer supplemented with 200µM acetoseryingone. Solution were incubated in  
733 dark at least 1h prior to infiltration. Infiltrated tissues were used 30h post infiltration for  
734 subsequent experimental procedures.

735

### 736 **Protein purification**

737 Recombinant proteins were expressed in *E. coli* BL21(DE3) by IPTG induction. Bacterial  
738 solution was centrifuged 20min at max speed and pellet was subjected to sonication after  
739 resuspension in 1mL MBP-buffer (20 mM TRIS-HCl pH 7.5, 200mM NaC, 1mM EDTA) or  
740 1mL IPP50 (10 mM Tris-HCl, pH 8.0. 150mM NaCl, 0.1% NP40). Sonicated samples were

741 used for purification by affinity chromatography using amylose resin (New England Biolabs)  
742 for MBP-NAC53/78 or glutathione sepharose 4B (cytiva) for GST-HRD1a/b. For recombinant  
743 His-UBA1 and His-UBC8 protein were purified using Ni-Ted resin (Macherey-Nagel).

744

#### 745 ***In vitro* ubiquitination assay**

746 In vitro ubiquitination assay was performed as described previously<sup>42</sup>. Samples were separated  
747 by SDS-PAGE electrophoresis using 4–15% Mini-PROTEAN® TGX™ Precast Protein Gels  
748 (BioRad) followed by detection of the ubiquitinated substrate by immunoblotting using anti-  
749 MBP (New England Biolabs), anti-GST and anti-ubiquitin (Santa Cruz Biotechnology)  
750 antibodies.

751

#### 752 **Co-immunoprecipitation**

753 Plant tissue was homogenized in a mortar with liquid nitrogen to keep the sample frozen during  
754 the process. And extracted in 1ml/g of extraction buffer (10% glycerol, 25 mM Tris pH 7.5, 1  
755 mM EDTA, 150 mM NaCl, 1 mM DTT, 0.5% Triton X, 1X protease cocktail inhibitor (Sigma)).  
756 Solution was vortexed for 3min and incubated in cold room on rotator for 10min following a  
757 30min centrifugation at 4000g, 4°C. Supernatant was filtered. 50µl of filtrate was sampled as  
758 input, supplemented with 12.5µL laemmli buffer 4X (Biorad) and boiled for 10min at 95°C.  
759 Next, 10µl/g of ChromoTek GFP-Trap® or RFP-Trap Agarose beads were added and tubes  
760 were incubated 2-3h in cold room. Tubes were then centrifuge at 800g for 1min; supernatant  
761 was carefully removed and pelleted beads were transferred to 1.5mL microcentrifuge tube in  
762 1mL of washing buffer (10% glycerol, 25 mM Tris pH 7.5, 1 mM EDTA, 150 mM NaCl, 0.5%  
763 Triton X-100, 1X protease cocktail inhibitor (Sigma)). Washing was performed by  
764 centrifugation at 800g for 1min using 1mL washing buffer. After last washing, Laemmli buffer  
765 2X (Biorad) was added to equal amounts of beads and sample was boiled 10min at 95°C.

766

#### 767 **RNA isolation & RT-qPCR**

768 Total RNA isolation was performed using RNeasy Plant Mini Kit (Qiagen 74904) according to  
769 manufacturer instructions. To exclude potential contaminant DNA, RNA samples were  
770 subjected to DNase I treatment (Thermo scientific™) following provider instructions. For RNA  
771 sequencing, integrity and RNA concentration was determined (2100 Bioanalyzer, Agilent). For  
772 RT-qPCR sample analysis was performed as described previously<sup>43</sup>; cDNA synthesis was  
773 performed using LunaScript® RT SuperMix Kit (NEB) following provider recommendation.  
774 qPCR was performed using MESA BLUE qPCR 2X MasterMix Plus for SYBR®

775 Assay (Eurogentec) using a 2-step reaction protocol for 40cycles with systematic evaluation of  
776 primer melting curve. mRNA level was quantified based on the  $\Delta\Delta C_t$  method followed by Log2  
777 transformation.

778

### 779 **NanoLC-MS/MS analysis and data processing**

780 For purification, proteins were subjected to a NuPAGE 12% gel (Invitrogen) and in-gel trypsin  
781 digestion was done on Coomassie-stained gel pieces with a modification: *chloroacetamide* was  
782 used instead of iodoacetamide for carbamidomethylation of cysteine residues to avoid  
783 formation of lysine modifications isobaric to two glycine residues left on ubiquitinated lysine  
784 after tryptic digestion. Next, peptides mixture were desalted using C18 Stage tips and run on an  
785 Easy-nLC 1200 system coupled to a Q Exactive HF mass spectrometer (both Thermo Fisher  
786 Scientific) as described elsewhere<sup>44</sup> with some modifications: separation of the peptide  
787 mixtures was done using a 87-min or 127-min segmented gradient from 10-33-50-90% of  
788 HPLC solvent B (80% acetonitrile in 0.1% formic acid) in HPLC solvent A (0.1% formic acid)  
789 at a flow rate of 200 nl/min. The seven most intense precursor ions were sequentially  
790 fragmented in each scan cycle using higher energy collisional dissociation (HCD)  
791 fragmentation. In all measurements, sequenced precursor masses were excluded from further  
792 selection for 30 s. The target values were  $10^5$  charges for MS/MS fragmentation and  $3 \times 10^6$   
793 charges for the MS scan.

794 Acquired MS spectra were processed with MaxQuant software package version 1.5.2.8 with an  
795 integrated Andromeda search engine. For *in vivo* IP samples, database search was performed  
796 against a *N. benthamiana* database containing 74,802 protein entries<sup>45</sup>, the sequences of eGFP-  
797 NAC53/78, mCerulean3-NAC53/78-mCherry and 285 commonly observed contaminants.

798 For *in vitro* ubiquitination samples, database search was performed against a Uniprot *E. coli*  
799 database (4,403 entries, downloaded on 7th of October 2020), the sequences of MBP-  
800 NAC53/78, GST-HRD1a/b and 285 commonly observed contaminants.

801 Endoprotease trypsin was defined as a protease with a maximum of two missed cleavages.  
802 Oxidation of methionine, phosphorylation of serine, threonine, and tyrosine, GlyGly dipeptide  
803 on lysine residues, and N-terminal acetylation were specified as variable modifications.  
804 Carbamidomethylation on cysteine was set as a fixed modification. Initial maximum allowed  
805 mass tolerance was set to 4.5 parts per million (ppm) for precursor ions and 20 ppm for fragment  
806 ions. Peptide, protein, and modification site identifications were reported at a false discovery  
807 rate (FDR) of 0.01, estimated by the target-decoy approach (Elias and Gygi). The iBAQ

808 (Intensity Based Absolute Quantification) and LFQ (Label-Free Quantification) algorithms  
809 were enabled, as was the “match between runs” option<sup>46</sup>.

810

### 811 **Photosynthesis monitoring**

812 For photosynthetic pigment concentration, 2 leaf discs (6mm) were sampled and incubated  
813 over-night in 1mL acetone 100% on a rotating wheel in cold room. Tubes were next centrifuged  
814 for 3min at 400g. 200µl of was then pipetted in a transparent 96-well plate and absorbance was  
815 measured at 470nm, 646nm and 663nm using a plate reader (Tecan Infinite 200 PRO®).  
816 Acetone 100% was used as blank. Concentration was calculated as following the obtained from  
817 Karlsruher Institut für Technologie (KIT, <https://www.jkip.kit.edu/molbio/998.php>,  
818 “Chlorophyll and Carotenoid determination in leaves”). Values were then then expressed as  
819 ng.mm<sup>2</sup>. For photosynthetic efficiency, photosystem II (PSII) activity was measured by  
820 quantifying the maximum photosystem II yield, Fv/Fm via the saturation pulse methods on dark  
821 acclimated plants (Schreiber 2004), a Imaging-PAM chlorophyll fluorometers: Maxi version  
822 v2-46i, for measurements or Dual-PAM100 (Walz GmbH, Effeltrich, Germany).

823

### 824 **Bacterial Infection**

825 *Pst* overnight liquid culture was centrifuged for 15min at 4000g. Bacterial pellet was  
826 resuspended in 5mL of MgCl<sub>2</sub> 10mM prior to OD600 measurement.

827 For qPCR, RNAseq and western blot analysis; a *Pst* solution at OD600=0.2 in 10mM MgCl<sub>2</sub>  
828 was used. Adult plant leaves were syringe infiltrated with the *Pst* solution or a 10mM MgCl<sub>2</sub>  
829 solution for 24h until sampling. For IP and Chromatin-IP on *A. thaliana*, *Pst* infiltration was  
830 prepared as explained above and detached whole rosettes of adult plants were vacuum  
831 infiltrated 2 times 2 min at < 15mbar. For mock, control solution (10mM MgCl<sub>2</sub>) was used  
832 similarly. Rosettes were put back in the growth chamber for 8h on multiple layers of wet tissue.  
833 For assessment of bacterial density, *Pst* infiltration solution was prepared in 10mM MgCl<sub>2</sub> at  
834 an OD600 of 0.0001. Adult plant leaves were syringe infiltrated with the *Pst* infiltration solution  
835 for 72h in high humidity conditions. Next, 2 leaf disks per replicates were sampled and  
836 homogenized in 200µl 10mM MgCl<sub>2</sub> and a serial dilution from 10<sup>-1</sup> to 10<sup>-6</sup> in 200µL was done.  
837 For bacterial counting, 20µL of 10<sup>-5</sup> and 10<sup>-6</sup> dilutions were plated. Growing colony-forming  
838 units were counted and Log<sub>10</sub>CFU/cm<sup>2</sup> was calculated.

839

### 840 **Chromatin Immuno-Precipitation**

841 For Chromatin-IP, after Arabidopsis rosettes were vacuum infiltrated for 30min with a fixation  
842 buffer solution (1% formaldehyde, 10mM KH<sub>2</sub>PO<sub>4</sub> pH 7, 50mM NaCl, 0.1M sucrose, 0.01%  
843 Triton X-100). Plant material (1g) was dried and frozen in liquid nitrogen. Tissue was ground  
844 in 5mL nuclei isolation buffer (20mM Hepes pH8, 250mM sucrose, 1 mM MgCl<sub>2</sub>, 5mM KCl,  
845 40% Glycerol, 0.25% Triton X-100, 0.1mM PMSF, 0.1% 2-mercaptoethanol, 1μM BTZ). The  
846 solution was filtered and subjected to 10min centrifugation at 4°C, 3000g. Pellet was  
847 resuspended in 1mL nuclei isolation buffer and centrifuged 5min, at 4°C, 3000g, two. Pellet  
848 was resuspended in 200μl M3 buffer (10mM KH<sub>2</sub>PO<sub>4</sub> pH7, 0.1mM NaCl, 10mM 2-  
849 mercaptoethanol) and centrifuged 5min, 4°C, 3000g. Nuclei pellet was resuspended in 1mL  
850 sonication buffer (10mM KH<sub>2</sub>PO<sub>4</sub> pH7, 0.1mM NaCl, 0.5% Sarkosyl, 10mM EDTA).  
851 Sonication was performed with an Active Motif Sonicator (Amp 25%; Pulse: 30sec ON – 30sec  
852 OFF; Timer: 300sec). Sonicated sample was centrifuged for 5min, 4°C at max speed.  
853 Supernatant was transferred to a new 1 tube and 10% (v/v) Triton X-100 was added to neutralize  
854 the sarkosyl. 5% (v/v) of this solution was saved as input sample. For IP, 15μL ChromoTek  
855 GFP-Trap® were activated in 15μL IP buffer (50mM Hepes pH 7.5, 150mM NaCl, 5mM  
856 MgCl<sub>2</sub>, 10uM ZnSO<sub>4</sub>, 1% Triton X -100, 0.05% SDS), added to the tube and incubated 3h on  
857 a rotating wheel at 4°C. Beads were washed 4 times by centrifuging for 30sec, at 1000g and  
858 subsequent incubation on rotating wheel in 500μL buffer for 3 min; twice in IP buffer, once in  
859 LNDET buffer (0.25 LiCl, 1% NP-40, 1% deoxycholate, 1mM EDTA pH 8, 10mM Tris pH 8)  
860 and once in TE buffer (10mM Tris pH 8, 1mM EDTA pH 8). After the last washing step, beads  
861 were centrifuged for 30sec at 1000g, resuspended in 200μL EB buffer (50mM Tris pH 8, 10mM  
862 EDTA pH 8, 1% SDS) supplemented with 8μL 5M NaCl to reverse cross-linking and incubated  
863 at 65°C for at least 6h. At this step input sample was added and treated similarly. For protein  
864 digestion, 1μL of Proteinase K (Thermo Scientific) was added and incubated for 1h at 45°C.  
865 Finally, DNA was purified.

866 For DNA quantification, qPCR was performed using MESA BLUE qPCR 2X MasterMix Plus  
867 for SYBR® Assay (Eurogentec) in technical triplicates. For quantification, % of input was  
868 calculated.

869

### 870 **Immunoblot analysis**

871 For immunoblot analysis, sample processing was performed as described previously<sup>43</sup>.

872

### 873 **Electromobility Shift Assay**



874 For EMSA assays, complementary DNA oligos were synthesized by EuroFins Genomics with  
875 ATTO565 dye linked to the 5' end of minus strand for probes. To generate double stranded  
876 probes, complementary oligos were incubated at 2 $\mu$ M in annealing buffer (25mM HEPES-  
877 KOH pH 7.8, 40mM KCl) at 70°C for 5min and cool down to room temperature.

878 EMSA reactions were performed in 20 $\mu$ L reaction buffer (25 mM HEPES-KOH pH 7.8, 40  
879 mM KCl, 1 mM DTT, 10% Glycerol) using 1 $\mu$ g purified MBP-NAC53/78 and 50ng DNA probe  
880 for 30min at 25°C. Reactions were subjected to native polyacrylamide migration with TGX  
881 FastCast 7.5% Acrylamide gels (Biorad) in TAE (Tris-base 40 mM pH 8.3, 20mM acetic acid,  
882 1mM EDTA). After migration, probes were imaged using a Typhoon FLA 9000 Gel Scanner.

883

#### 884 **Luciferase reporter assay in Protoplasts**

885 For promoter activity analysis, *Arabidopsis* mesophyll protoplasts were generated as described  
886 perviously<sup>27</sup>. Luciferase quantification was performed according to Dual-Luciferase® Reporter  
887 Assay System from Promega recommendation (see [link](#)).

888

#### 889 **Data analysis**

890 For all statistical analysis, R programming language was used in the Rstudio environment. For  
891 non-omics data, the statistical test and associated p value threshold for each analysis are  
892 indicated in the figure legends. Data dispersion, medians and quantiles are represented with  
893 boxplots and replicates displayed in every figures. For bar plots in Extended Data Fig. 2,  
894 number of replicates are indicated in the figure legend, error bars represent the standard  
895 deviation from the mean.

896

#### 897 **RNA sequencing**

898 For *rpt2a-2 Pma* transcriptome, sequencing was performed by ATLAS Biolabs, Berlin. For  
899 *nac53-1 78-1 Pst* transcriptome, sequencing was performed by the NGS Competence Center,  
900 Tübingen. After quality control, reads were mapped to the *A. thaliana* TAIR10 reference  
901 genome. The mapped reads were counted with *htseq-count* for subsequent analysis. Log2FC  
902 and false discovery rate (FDR) were determined using R package DEseq2<sup>47</sup> with default  
903 settings. A gene is considered significantly differentially expressed when its FDR < 0.05.  
904 Log2FC threshold is indicated in the figure legends according to the analysis.

905

#### 906 **IP-MS/MS analysis**

907 For IP-MS/MS analysis, Log2FC and false discovery rate (FDR) for each protein group were  
908 determined as described previously<sup>42</sup>. For downstream analysis, to each protein group a unique  
909 Agi-code was assigned by blasting every *N. benthamiana* protein present in the protein group  
910 against *A. thaliana* proteome and taking the most frequent Agi-code with the best E-value. An  
911 E-value threshold of e-10 was used, in case of absence of *A. thaliana* the protein group was not  
912 considered for downstream analysis. For heatmap representation, in case multiple protein  
913 groups correspond to the same *A. thaliana* orthologs the mean Log2FC is represented.

914

### 915 **Meta-Transcriptomic analysis**

916 For meta-transcriptomic analysis in Fig. 6 and Extended data Fig. 6, fragments per kilobase per  
917 million counts were retrieved from the data base (<http://ipf.sustech.edu.cn/pub/athrna/>).  
918 Log2FC for individual genes in every project was calculated based on the control RNAseq  
919 libraries as annotated in the original publication<sup>31</sup>. Filtering of relevant projects for subsequent  
920 correlation analysis was done based on the median |Log2FC|. Median|Log2FC| > 0.5 was used  
921 for identification of projects with at least one gene cluster transcriptionally impacted and  
922 median |Log2FC| > 0.3219 was used for identification of projects in which 26S proteasome and  
923 PhANGs clusters were co-transcriptionally impacted. Pearson correlations were calculated.  
924 Only correlations with p-value < 0.05 were considered significant and used for calculation of  
925 mean/median.

926

### 927 **Omics downstream analysis: Gene ontology, Protein Network, subcellular enrichment &** 928 **cis-element analysis**

929 For Gene Ontology enrichment, the list of Agi-code was uploaded into ShinyGO software  
930 (<http://bioinformatics.sdstate.edu/go/>). Cladogram of the indicated number of top terms for the  
931 indicated type of terms was used for graphical representation. Table of annotations was  
932 extracted for further analysis.

933 For network creation, list of Agi-code was provided to the string database ([https://string-](https://string-db.org/)  
934 [db.org/](https://string-db.org/)). Table of interactions was extracted and uploaded to cytoscape software  
935 (<https://cytoscape.org/>) for network generation, design, and annotation. For purposes of clarity,  
936 network nodes were manually arranged. Edge transparency relate to the strength of interaction  
937 with opaque edges corresponding to strong interactions and transparent edges to weak  
938 interactions. Node color corresponds to manual annotation inferred based on GO annotations  
939 and further curated from the literature.

940 For subcellular compartment enrichment, the list of Agi-code was uploaded into SUBA5db  
941 (<https://suba.live/>). Compartment annotation was extracted, and occurrence of every  
942 compartment was counted to estimate the list distribution. For theoretical distribution 20 Agi-  
943 code lists of the same length were randomly generated and processed similarly. The mean  
944 distribution of the 20 lists was used as theoretical distribution. Enrichment for every compartment  
945 was calculated as a Log2FC of the ratio between the observed proportion and the theoretical one.

946

#### 947 **Cis-Regulatory Element analysis**

948 For cis-regulatory element analysis, promoter sequences of 1000bp upstream of the  
949 transcription starting site were retrieved from plant ensembl database using biomart browser  
950 (<https://plants.ensembl.org/biomart/>). For identification of novel cis-regulatory elements in  
951 Figure 5A, promoter sequences of the several gene clusters were analyzed using the STREME  
952 software (<https://meme-suite.org/meme/tools/streme>) with default settings. For mapping and  
953 counting, promoter sequences were analyzed in R.

954

#### 955 **Immunoblot semi-quantitative analysis**

956 Raw image of the immunoblot signals against the protein of interest and the corresponding loading  
957 control were processed using Fiji software (<https://imagej.net/software/fiji/downloads>). Image  
958 was converted to 8-bit format, background was subtracted. Individual lanes were quantified by  
959 measurement of the peak area after circumscription with the rectangular ROI selection and  
960 “Gels” function. The obtained values for individual bands were normalized by the  
961 corresponding loading control values. Log2FC was calculated using the corresponding mock  
962 sample of the same genotype.

963

#### 964 **Confocal microscopy imaging**

965 Confocal microscopy was done using an inverted Zeiss LSM 880 microscope, an upright Leica  
966 SP8 microscope or an inverted Leica Stellaris 8 microscope. For mCerulean3 imaging,  
967 excitation = 458nm and emission window = 465nm-500nm; for eGFP or sGFP imaging,  
968 excitation = 488nm and emission window = 510nm-540nm; for mRFP or mCherry imaging,  
969 excitation = 561nm and emission window = 590nm- 630nm; in addition, chlorophyll auto  
970 fluorescence was visualized in the far-red wavelength. Images were acquired with a 40X water  
971 immersion objective, pinhole set to 1 airy unit, resolution of acquisition >1024x1024 with a  
972 line average of 4.

973 After acquisition images were processed in Fiji Software. For images obtained from Leica  
974 microsystem devices, a gaussian blur (radius 0.75) was applied. For clarity purposes, contrast  
975 on the individual channels was manually adjusted.

976 For subcellular quantification of GFP signal, all images used were acquired on the Zeiss LSM  
977 880 with a 16bits depth. Prior to imaging seedlings were incubated for 10min in a 10 $\mu$ M  
978 propidium iodide (PI)  $\frac{1}{2}$  MS solution followed by a brief washing in clear  $\frac{1}{2}$  MS. For PI  
979 fluorescence, was excitation = 488nm and emission window = 590nm-660nm. Each cell and  
980 corresponding nucleus were segmented using the polygon ROI selection tool in Fiji software.  
981 Signal proportion in the nucleus was quantified by dividing integrated density of nucleus ROI  
982 by integrated density of whole cell ROI and ratio of signal between nucleus and cytosol was  
983 calculated dividing nucleus ROI mean intensity by cytosolic ROI mean intensity.

984 For calculation of co-localization index the image J plugin “co-localization finder” was used to  
985 calculate pearson index or plot profile of GFP and RFP signals was extracted at the indicated  
986 line using Fiji software and the pearson index was calculated between the two profiles.

987

## 988 **RESOURCE AVAILABILITY**

989 Source code of R and Python3 scripts used for the several computational analysis done in this  
990 study will be put available on <https://github.com/Gogz31> or can be directly requested.

991

## 992 **DATA AVAILABILITY**

993 The mass spectrometry data from this publication will be made available on the PRIDE archive  
994 (<https://www.ebi.ac.uk/pride/archive/>) and with the identifier (PXDXXXXX; and  
995 (PXDXXXXX). All relevant proteomics data are made available in the supplemental information.

996

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1012

### 1013 **Author Contribution**

1014 S.Ü. conceived the project. G.L. performed most experiments, analyzed the data, and performed  
1015 all computational analysis. M.R. performed the *in vitro* ubiquitination assays. D.B performed  
1016 the EMSA, assisted with the Luciferase reporter assay and the molecular cloning. D.S.  
1017 generated the *rpt2a-2 Pma* transcriptome under the supervision of F.B..M.F.W and B.M.  
1018 performed LC-MS/MS analysis. S.Ü. wrote the manuscript together with G.L..  
1019

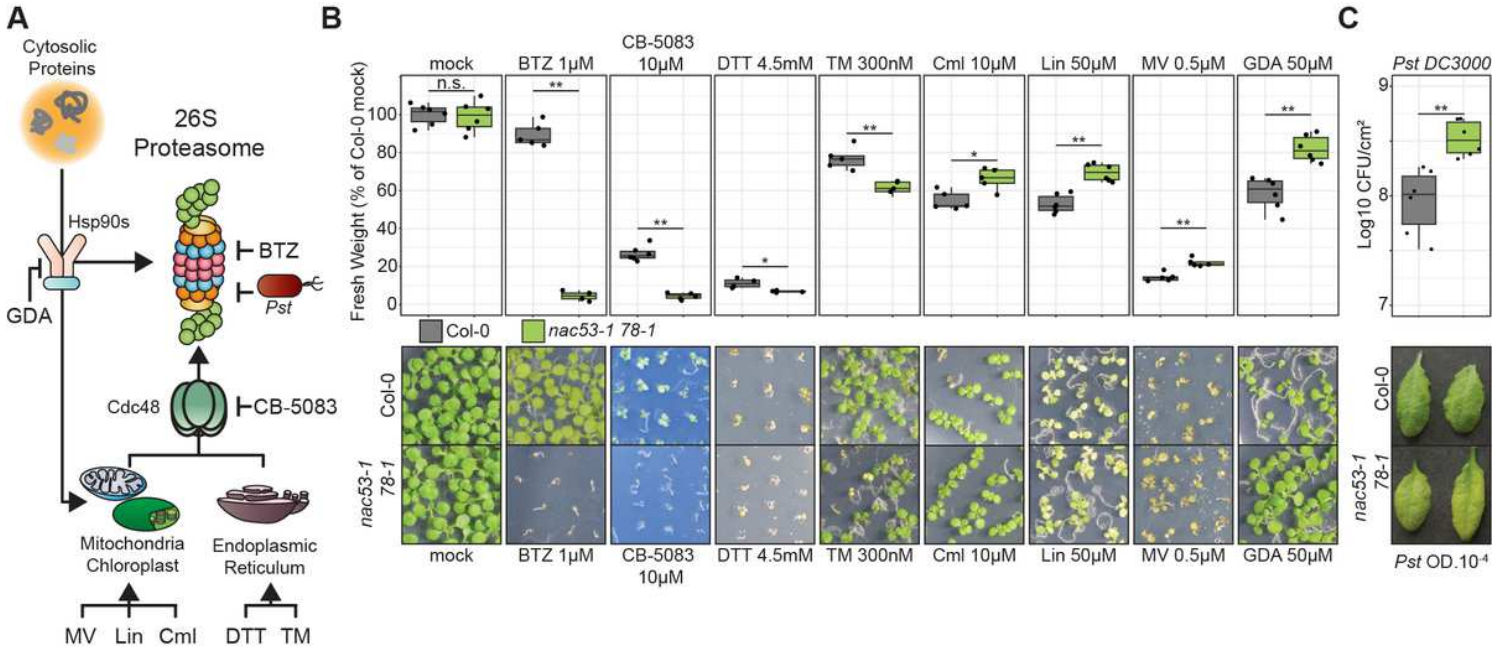
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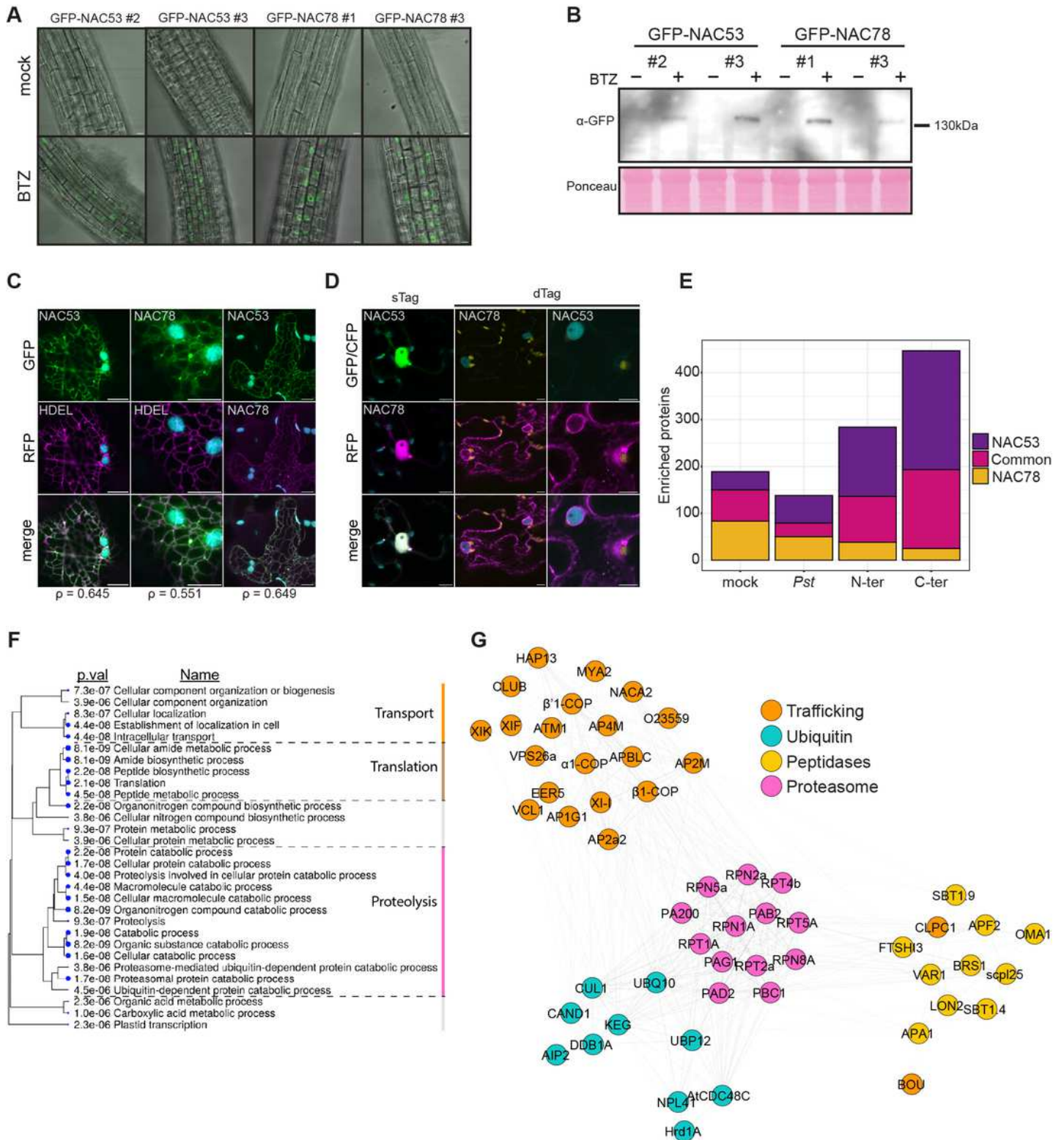
# Figures



**Figure 1**

NAC53/78 are central integrators of various proteotoxic stress conditions. (A) The role of the 26S Proteasome in degradation of proteins from distinct subcellular compartments. Prime site of action of the several treatments used in this study: Blunt head arrows indicate enzymatic activity suppression; pointed head arrows indicate trigger of substrate accumulation. (B) Fresh weight of seedlings grown under the indicated treatments at 10-14 day after germination (dag). Fresh weight is expressed as a percentage of Col-0 mock conditions. Boxplots colors refer to the genotype. Statistical significance is assessed by a Wilcoxon-Mann-Whitney test (p values: n.s. > 0.05; \* < 0.05; \*\* < 0.01). Every treatment has been repeated at least twice with similar results. (C) Bacterial density in Log<sub>10</sub> Colony-Forming-Unit (CFU) per leaf cm<sup>2</sup>. Statistical significance is assessed by a Wilcoxon-Mann-Whitney test (p values: n.s. > 0.05; \*\* < 0.01). Boxplots colors refer to the genotype as in panel B. The treatment has been repeated three times with consistent results. (B-C) Representative phenotypic pictures of Col-0 and *nac53-1 78-1* plants under several treatments are included.

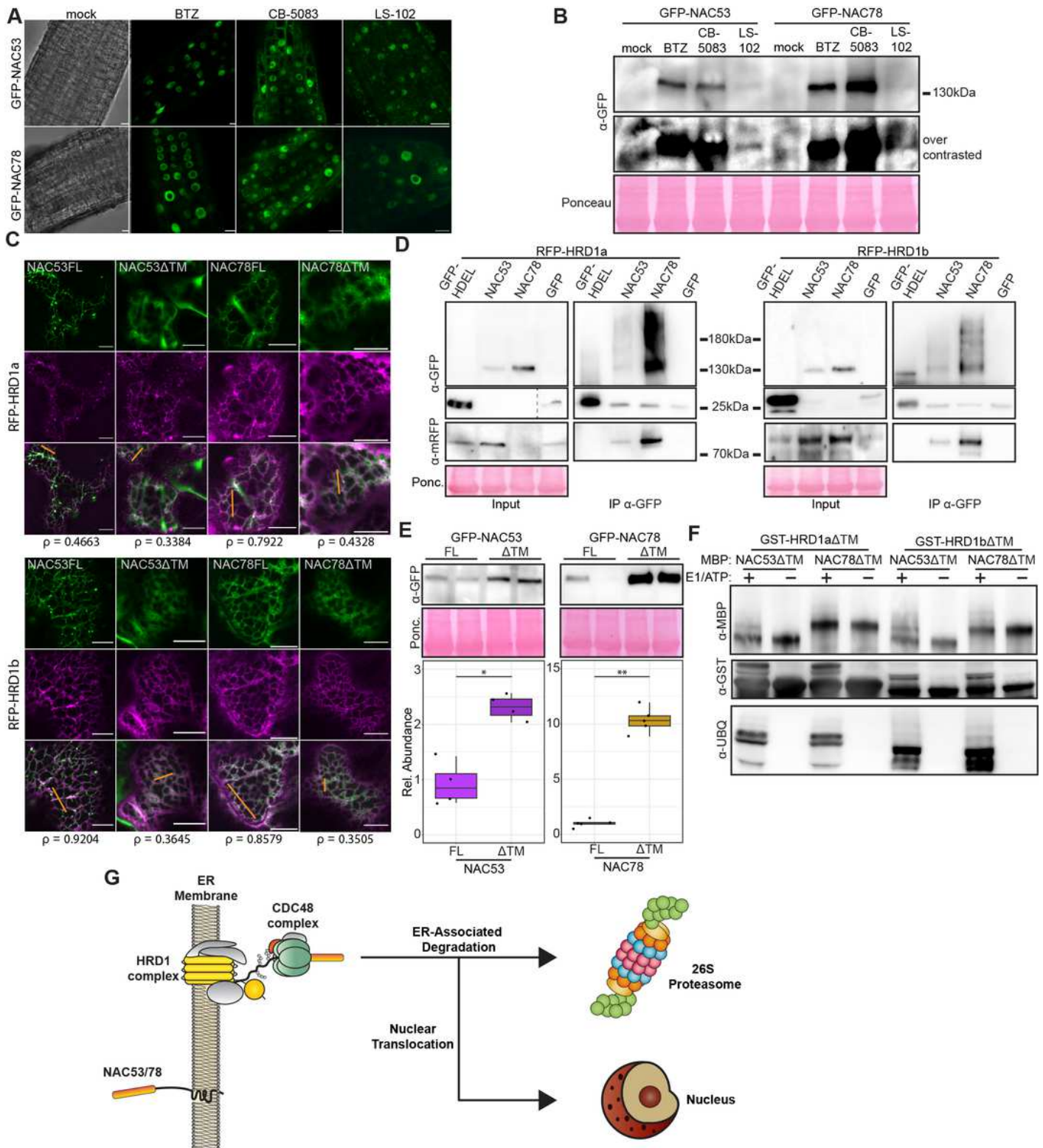




**Figure 2**

NAC53/78 are part of an autoregulatory proteasome feedback loop. (A) Confocal microscopy pictures of roots of GFP-NAC53/78 *Arabidopsis thaliana* transgenics at 7dag exposed to mock or BTZ 10 $\mu$ M treatment for 3h. Treatments were repeated at least three times with similar observations. (B) Immunoblot analysis against GFP on crude extract of seedlings related to panel A. Ponceau staining is used as loading control. (C) Confocal microscopy pictures of transiently co-expressed sTag-NAC53/78 with

prom35S::RFP-HDEL or together in *N. benthamiana* epidermis cells. Pearson index ( $\rho$ ) represents co-localization index. Scale bars = 10 $\mu$ m (D) Confocal microscopy pictures of transiently co-expressed sTag-NAC53/78 together or dTag-NAC53/78 alone in *N. benthamiana* epidermis cells. Scale bars = 10 $\mu$ m (E) Bar plots representing the number of significantly enriched proteins in the 4 IP-MS/MS conditions for NAC53 only, NAC78 only and NAC53+NAC78 (Common). (F) Cladogram of top 30 gene ontology (GO) terms for biological process (BP) enriched in the list of *A. thaliana* ortholog proteins found from NAC53/78 common interactome. (G) Protein network of the interacting candidates related to the GO BP terms annotated as "Transport" and "Proteolysis" in panel F.



**Figure 3**

ER-Anchored Protein Sorting (ERAPS) coordinates the subcellular fate of NAC53 & NAC78. (A) Confocal microscopy pictures of GFP-NAC53/78 expressing *A. thaliana* roots. 7 dag seedlings were exposed to mock treatment, BTZ 10 $\mu$ M, CB-5083 10 $\mu$ M or LS-102 100 $\mu$ M for 3h. The treatments were repeated at least three times with similar observations. Scale bars = 10 $\mu$ m (B) Immunoblot against GFP on crude extracts of GFP-NAC53/78 *A. thaliana* adult plants infiltrated with mock treatment, BTZ 10 $\mu$ M, CB-5083

10 $\mu$ M or LS-102 100 $\mu$ M for 6h. Ponceau staining is used as loading control. (C) Confocal microscopy pictures of *N. benthamiana* transiently co-expressing GFP443 NAC53/78 full-length (FL) or deleted for the transmembrane domain ( $\Delta$ TM) with RFP-HRD1a or RFP-HRD1b. Pearson index ( $\rho$ ) indicates correlation index at the orange lines. (D) Co-IP of RFP-HRD1a/b with GFP-NAC53/78. IP was performed with anti-GFP beads. GFP-HDEL and free-GFP were used as negative control. The experiment was repeated twice with similar results. (E) Immunoblot against GFP on crude extract of *N. benthamiana* leaves transiently expressing GFP-NAC53/78FL or  $\Delta$ TM. Ponceau was used as loading control. Boxplots represent the relative  $\Delta$ TM abundance compared FL. (F) In vitro trans-ubiquitination assays of GST-HRD1a/b against MBP-NAC53/78. Removal of E1 and ATP from the reaction was used as negative control. The experiment was repeated twice with similar results. (G) Representation of the ER-Anchored Protein Sorting (ERAPS) mechanism. NAC53/78 are ER-anchored proteins recognized and ubiquitinated by the HRD1 complex. The CDC48 complex subsequently extracts NAC53/78 from the ER to mediate ER-Associated Degradation or allowing nuclear translocation in the context of proteasomal stress to mitigate proteotoxicity.

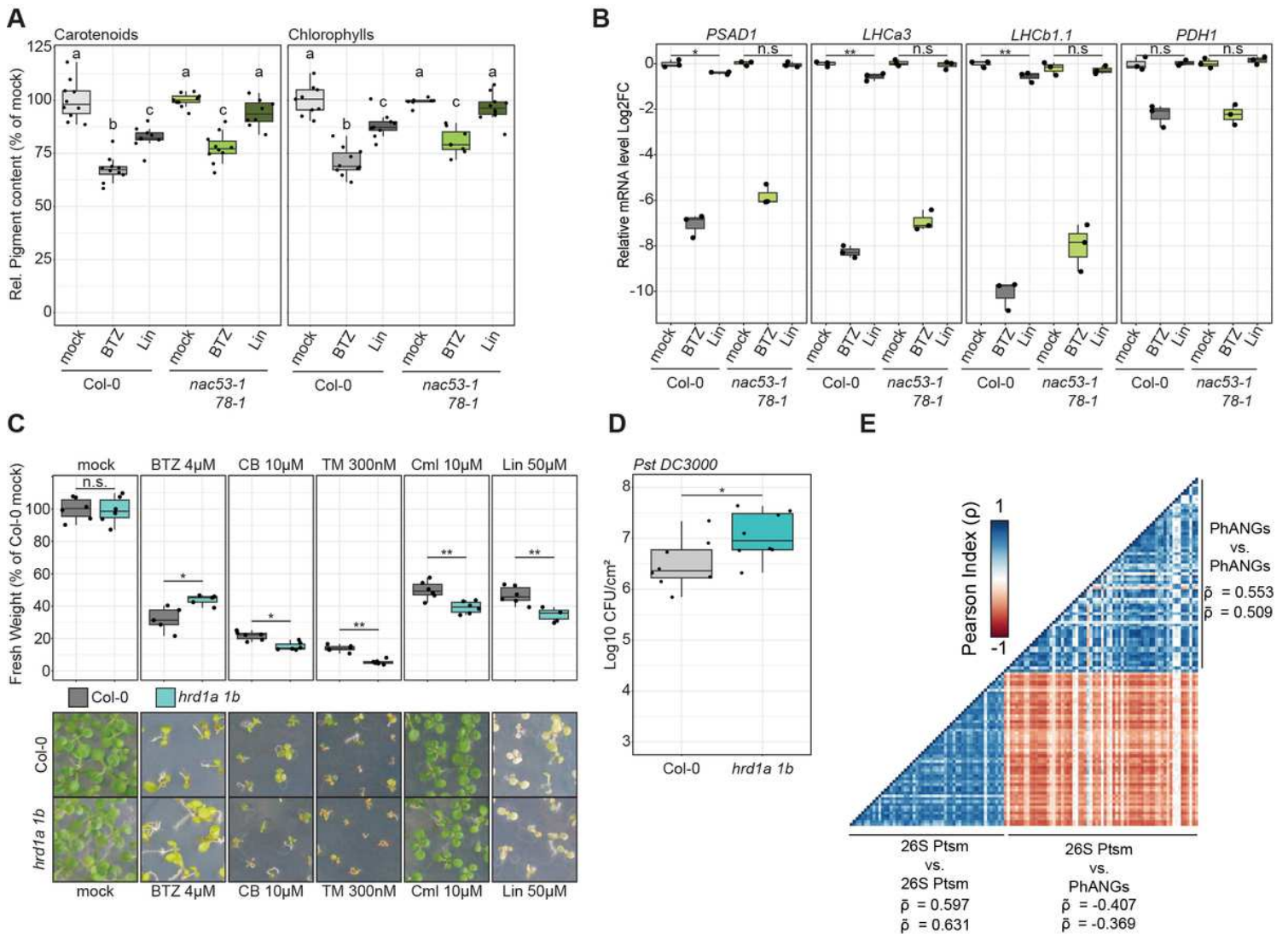


on crude extracts of Col-0 or *nac53-1 78-1* adult plant leaves inoculated with Pst or mock solutions for 24h. Ponceau staining is used as loading control. (D) Relative Log<sub>2</sub>FC abundance of representative 26S proteasome subunits in Col-0 or *nac53-1 78-1* adult plant leaves. The abundance is calculated relative to the mock conditions. Statistical differences are assessed via a Welch t.test (p values: n.s > 0.05, \* < 0.05, \*\* < 0.01). (E) Immunoblot against GFP on crude extract of *N. benthamiana* leaves transiently expressing GFP-NAC53/78 after mock treatment or Pst $\Delta$ HopQ infection for 8h. Ponceau staining is used as loading control. (F) Immunoblot against GFP on extract of adult *A. thaliana* transgenics GFP-NAC53/78 subjected to mock treatment or Pst infection 8h by vacuum infiltration followed by IP with anti-GFP beads. (G) Number of differentially expressed genes (DEGs) in the different conditions of the *nac53-1 78-1* Pst RNAseq analysis. Total number of DEGs per condition is indicated. Colors indicate the proportion of DEGs up-regulated and down-regulated. DEGs are considered when  $|\text{Log}_2\text{FC}| > 1.5$ . Genot. mock: *nac53-1 78-1* mock vs. Col-0 mock; WT Pst: Col-0 Pst vs. Col-0 mock; Mut. Pst: *nac53-1 78-1* Pst vs. *nac53-1 78-1* mock; Genot. Pst: *nac53-1 78-1* Pst vs. Col-0 Pst. (H) Level of differential expression (Log<sub>2</sub>FC) of the 945 DEGs found in Genot. Pst (see panel G) for the several conditions analyzed. (I) Cladogram of top 40 GO terms for BP enriched in the list 525 of 945 DEGs. Colors represent broad GO clusters. (J) Venn diagram representing the overlap between the 945 DEGs and DEGs from *rpt2a-2 Pma* ( $|\text{Log}_2\text{FC}| > 0.5$ ) or Col-0 MG132 24h ( $|\text{Log}_2\text{FC}| > 0.5$ ). (K) Cladogram of top 30 GO terms for BP enriched in the list of 538 DEGs extracted from the combined analysis (panel J). For conciseness terms are hidden and grouped in 4 processes. (L) Protein network of the genes from the 945 DEGs list related to the GO BP terms from panel K. Node colors refers to the 4 GO clusters from panel K.



Electro-Mobility Shift Assay (EMSA) of MBP-NAC53/78 with LHCa3/PSAD1 probes (see panel B). As a negative control, probes were incubated without MBP-NAC53/78 (-) or with MBP-NAC53/78 deleted for their DNA recognition motif ( $\Delta$ RM). Experiment was repeated twice with similar results. (D) EMSA competition assay of MBP-NAC53/78 association with LHCa3/PSAD1 probes. Competitor is applied at a concentration gradient (25X, 50X, 75X and 100X) indicated by the shade of grey. WT and mutated competitors are labelled TGGGC and AAAAA, respectively. Experiment was repeated twice with similar results. (E) Boxplot representing association of GFP-NAC53/78 with LHCa3/PSAD1 PRCE in adult *A. thaliana* transgenics lines infected with Pst or mock for 8h after Chromatin Immunoprecipitation. The association is quantified as % of input and amplification of a region in the CDS is used as negative control. (F) Immunoblot against mature PhANGs proteins in Col-0 or *nac53-1 78-1* adult plant leaves inoculated with Pst or mock solutions for 24h. Ponceau staining is used as loading control. (G) Relative Log<sub>2</sub>FC abundance of PhANGs mature proteins 574 immunoblotted in panel F. The abundance is calculated relative to the mock conditions. Statistical differences are assessed via a Welch t.test (p values: n.s > 0.05, \* < 0.05, \*\* < 0.01). (H) Photosystem II (PSII) activity measurement on Col-0 or *nac53-1 78-1* adult plants inoculated with Pst or mock solutions for 24h. Letters indicate the statistical group assessed by pairwise Welch t.test (p value < 0.05). The experiment was repeated 3 times with consistent results. (I) Representative pictures of measurements from panel I. Fv/Fm values are represented by false color as indicated by the color gradient legend. (J) Total chlorophylls and carotenoids from Col-0 or *nac53-1 78-1* adult plant leaves inoculated with Pst or mock solutions for 24h. Letters indicate the statistical group assessed by pairwise Wilcoxon-Mann-Whitney test (p value < 0.05). The experiment was repeated 3 times with consistent results.





< 0.05). The experiment has been repeated three times with consistent results. (E) Correlation heatmap of the 54 26S proteasome subunit genes and the expression of 68 PhANGs from publicly available 1223 RNAseq libraries identified in this study.

## Supplementary Files

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