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Structural Maintenance of Chromosome 3 interacts with the Topoisomerase VI complex and contributes to the oxidative stress response in *Arabidopsis thaliana*.

1

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11

12 Summary

13 In plants adverse environmental conditions can induce the accumulation of reactive oxygen species,
14 such as singlet oxygen or hydrogen peroxide, at the level of the photosynthetic apparatus. The
15 coordinated action of nucleus-encoded genes is required for containing the deleterious effects of
16 reactive oxygen species. The regulation of such genes follows a molecular signalling process between
17 the chloroplast and the nucleus called retrograde signalling. Previously, we proposed that the
18 Topoisomerase VI (Topo VI) complex participates in the singlet oxygen stress response by regulating
19 the expression of specific subsets of nuclear genes. However, the underlying molecular mechanisms
20 remain unresolved. In this study, we demonstrate that the Topo VI subunit BIN4 interacts with the
21 cohesin subunit AtSMC3. We also show that, similarly to Topo VI mutants, a line suppressing AtSMC3
22 shows constitutive activation of singlet oxygen response genes and enhanced tolerance to
23 photooxidative stress. Together, these results suggest that Topo VI and AtSMC3 control the expression
24 of singlet oxygen response genes and are possibly involved in the acclimation of plants to
25 photooxidative stress conditions.

26 **Key words:** Cohesin / Oxidative Stress / Structural Maintenance of Chromosome / Topoisomerase VI /
27 co-suppression /

28

29 INTRODUCTION

30

31 Abrupt changes in environmental conditions are a source of stress that can disrupt cellular homeostasis
32 and affect the integrity of organisms. One of the consequences common to most environmental
33 stresses is the production of reactive oxygen species (ROS), such as hydrogen peroxide (H₂O₂) or singlet
34 oxygen (¹O₂) (Baxter, Mittler and Suzuki, 2014). Although the oxidizing power of ROS can be deleterious
35 for the function of a wide range of macromolecules, they can also act as signals and promote the
36 induction of several stress responsive genes, at lower concentrations (Laloi and Havaux, 2015;
37 Exposito-Rodriguez *et al.*, 2017). In plants, ROS accumulation arise mainly from an imbalance between
38 energy harvesting and dissipation at the level of the photosynthetic chain in the chloroplasts (Pinnola
39 and Bassi, 2018). Therefore, ROS signalling is among the principal actor of inter-organelle
40 communication from chloroplasts to the nucleus, namely retrograde signal.

41 The less reactive H₂O₂ has been shown to diffuse out of isolated chloroplasts, probably through
42 aquaporins (Mubarakshina *et al.*, 2010; Bienert and Chaumont, 2014). Furthermore, direct transfers of
43 H₂O₂ from chloroplasts to nucleus have been highlighted in *Nicotiana benthamiana* during high light
44 stress, followed by an activation of *NbAPXa*, a H₂O₂ responsive gene, suggesting that H₂O₂ could act as
45 a signal itself (Exposito-Rodriguez *et al.*, 2017). Because of its short lifetime and high reactivity, ¹O₂ is
46 unlikely to follow the same signalling scheme as H₂O₂. In the presence of ¹O₂, induction of cell death
47 can be mediated by two independent pathways involving EXECUTERS or OXI1 proteins (Laloi and
48 Havaux, 2015). Moreover, the activation of photooxidative stress tolerance nuclear genes rather relies
49 on the production of β-cyclocitral (β-CC), a product of the oxidation of β-carotene (Ramel *et al.*, 2012).
50 Following this, METHYLENE-BLUE SENSITIVITY 1 (MBS1) and SCARCROW LIKE14 (SCL14), two
51 transcriptional regulators induced by β-CC, regulate the acclimation process through two independent
52 pathways (Shumbe *et al.*, 2017; D'alessandro, Ksas and Havaux, 2018; Dmitrieva, Tyutereva and
53 Voitsekhovskaja, 2020). The action of such transcriptional regulators on the nuclear genome is
54 intrinsically regulated by chromatin topology. Indeed, studies describing the involvement of chromatin
55 remodelling complexes and topoisomerases in stress response are increasing (Vriet, Hennig and Laloi,
56 2015; Song, Liu and Han, 2021), thus revealing the importance of chromatin architecture regulation in
57 the completion of retrograde signalling.

58 To identify ¹O₂ dependant retrograde signalling compounds, we previously performed a
59 genetic screen that allowed the isolation of constitutive activators (*caa*) as well as non-activators (*naa*)
60 of the AAA-ATPase (AAA) / AT3G28580, a ¹O₂-responsive gene (Baruah *et al.*, 2009). We reported that
61 a weak mutant allele of the A subunit of the topoisomerase VI (Topo VI) complex, *caa39*, constitutively

62 activates a transcriptional response to $^1\text{O}_2$ that cannot be further enhanced under stress conditions
63 (Šimková *et al.*, 2012). Moreover, chromatin immunoprecipitation experiments showed that two
64 subunits of the Topoisomerase VI (TopoVIA and RHL1) bind the proximal regions of $^1\text{O}_2$ -responsive
65 gene promoters during high light stress (Šimková *et al.*, 2012). Interestingly, H_2O_2 response genes are
66 stimulated in the *caa39* mutant under photooxidative stress (Šimková *et al.*, 2012). Considering the
67 antagonistic effects of the *caa39* mutation on H_2O_2 and $^1\text{O}_2$ pathways under photoinhibitory
68 conditions, we proposed Topo VI to be a molecular switch that might relay both H_2O_2 and $^1\text{O}_2$
69 responses. To date, the mechanistic insights of the Topo VI-dependent retrograde signalling and its
70 connexion with the above-mentioned retrograde signalling components have not been completely
71 elucidated.

72 In this study, we show that the Topo VI subunit BIN4 interacts with the cohesin subunit SMC3,
73 likely through the hinge domain of SMC3. Since knockout of *SMC3* is embryonically lethal (Schubert *et al.*,
74 2009), we isolated *SMC3* co-suppression lines to understand the genetic interaction between Topo
75 VI and SMC3. The transcriptomic analysis of *SMC3* co-suppression lines and *caa39* mutants revealed
76 that both lines constitutively activate $^1\text{O}_2$ -responsive genes. Finally, we show that *caa39* and *SMC3* co-
77 suppression lines display enhanced resistance to high light stress conditions, which is correlated with
78 the activation of the non-photochemical quenching mechanisms.

79

80 MATERIALS AND METHODS

81

82 Plant material and growth conditions

83

84 *Nicotiana benthamiana* was used for transient expression of recombinant proteins. *caa39* mutant and
85 SMC3 suppressors are in *Arabidopsis thaliana* Col-0 ecotype. Both *Arabidopsis* and *Nicotiana* plants
86 were grown in a controlled environment at $120 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ illumination with an 8 h (short
87 days) or 16 h (long days) photoperiod at 22°C day / 20°C night, and 55% day / 75% night relative
88 humidity. The photoperiod used for each experiment is indicated in the figure legends.

89

90 Cloning

91

92 Multigenic BiFC vectors containing a repressor of silencing (P19), a transformation marker (OEP7-
93 mTRQ), and the two coding sequences of interest fused with the two different parts of the 174/175
94 YFP split were made using the cloning toolbox and the protocol described in (Engler *et al.*, 2014; Velay
95 *et al.*, 2022). The chimeric CDS of *AtSMC3* was cloned following an optimized protocol due to the high
96 instability of the construct in bacterial strains. Notably, the addition of genomic introns n°1, 24, 25, 26,
97 27 was found to help improve stability. Reducing the number of bacteria spread on selective media
98 (maximum 10 colonies per Petri dish) also helped to dramatically reduce the frequency of
99 recombination events within the *AtSMC3* CDS. Moreover, due to the strong propensity of positive
100 colonies grown on solid media to quickly evict the *AtSMC3*-containing plasmid, after each cloning step,
101 the positive clones were constantly kept in a liquid culture containing the appropriate antibiotic and
102 refreshed every 4 days. After transformation of the final plasmid into *Agrobacterium tumefaciens*, no
103 notable instability was detected.

104

105 Yeast two-hybrid screen

106

107 The yeast two-hybrid screen was performed by Hybrigenics using the Arabidopsis RP1 library. The full-
108 length *BIN4* cDNA (*AT5G24630.3/4*) was used as bait.

109

110 Stable and transient expression by agroinfiltration

111

112 Transient expression in *Nicotiana benthamiana* leaves was performed following the protocol described
113 in Velay *et al.* 2022. For Arabidopsis stable expression, *Agrobacterium tumefaciens* GV3101
114 transformed with the plasmid containing GFP-SMC3 were grown at 28°C in LB medium supplemented
115 with rifampicin and kanamycin. Arabidopsis flowers were then dipped in 50 mL of *Agrobacterium*
116 suspension OD₆₀₀ = 0.8 containing 5 % sucrose and 0.05 % SILWET L-77 (Clough and Bent, 1998). The
117 transformed plants were covered and kept in the dark for 24 h after what they were manually watered
118 every 4 days until the seeds were harvested. Bialaphos resistant primary transformants were selected
119 by spraying a solution containing 150 mg/L ammonium-glufosinate on 6-day-old seedlings.

120

121 BiFC assay

122

123 BiFC assays were carried out in *Nicotiana benthamiana* lower epidermal cells. Sample preparation,
124 imaging and analysis were performed following the protocol described in (Velay *et al.*, 2022). Exposure
125 time and post-acquisition analyses were similar between all samples.

126

127 Protein extraction, antibody production and Immunoblotting

128

129 For total protein extraction, 6-day-old plant aerial parts; 2-week-old plant aerial parts; 4-week-old
130 plant mature rosette leaves were harvested, and ground in liquid nitrogen. Tissue powder was
131 resuspended in SDS loading buffer, heated at 85°C 10 min and centrifuged at 16000 g, 10 min at room
132 temperature. The proteins contained in the supernatant were then separated by SDS-PAGE,
133 transferred onto a nitrocellulose membrane and probed with specific antibodies. To generate the anti-
134 AtSMC3 antibody, we synthesized the peptide C-QALDFIEKDQSHDT, corresponding to the C-terminal
135 region of AtSMC3, and used it to raise a polyclonal antibody in rabbit (Genscript). Anti-AtSMC3 and
136 anti-GFP (Roche 11814460001) antibodies were diluted 1:500 and 1:1000 in PBST milk 5 %,
137 respectively. Total protein staining was performed using SYPRO ruby protein stain (Thermofisher).

138

139 RNA extraction and RT-qPCR

140

141 Total RNA was extracted using TRI Reagent (MRC). 500 ng of RNA was treated with DNaseI (Takara)
142 and then used for RT reactions using the PrimeScript RT Reagent Kit (Takara), with an equimolar mix
143 of random hexamers and polyA primers. The efficiency of RT reaction and absence of residual genomic
144 DNA were confirmed by semi-quantitative RT-PCR. cDNAs were then diluted 4 times with ultra-pure
145 water. Quantitative RT-PCR were carried out using TB Green Premix Ex Taq II (Takara). 1 µL of diluted
146 cDNA was used for each reaction in a final volume of 15 µL. *PP2A* (*AT1G13320*) and *PRF1* (*AT2G19760*)
147 were used as control genes in all RT-qPCR experiments.

148

149 RNA-seq library preparation and sequencing

150

151 Two independent biological replicates were produced. For each biological repetition, RNA samples
152 were obtained by pooling RNAs from 4 plants. Aerials parts were collected on plants at developmental
153 growth stage 1.04 (Boyes *et al.*, 2001) cultivated as described above. Total RNA was extracted using
154 TRI Reagent (MRC) and purified using RNA clean and concentrator-25 (Zymo Research) according to

155 the supplier's instructions. Libraries were prepared and sequenced at BGI (China) on a DNBSEQ™
156 sequencing platform in paired-end (PE) with a read length of 100 bases. Approximately 30 million of
157 PE reads by sample were generated.

158

159 RNA-seq bioinformatic treatment and analyses

160

161 Each RNA-Seq sample was analysed using the same workflow. Read preprocessing criteria were
162 assessed using FastQC (v0.11.9). Bowtie 2 (v 2.4.4) (Langmead and Salzberg, 2012) was used to align
163 reads against the *Arabidopsis thaliana* transcriptome
164 (Araport11_cdna_20160703_representative_gene_model.fa). The reads count was calculated by a
165 local script adapted from Van Verk *et al.*, 2013 (Van Verk *et al.*, 2013). Gene expression analysis was
166 performed using SarTools (v1.7.4) (Varet *et al.*, 2016) with EdgeR in R (v4.1.2) (Robinson, McCarthy
167 and Smyth, 2009). Cluster analysis was performed using a script derived from [https://2-](https://2-bitbio.com/2017/10/clustering-rnaseq-data-using-k-means.html)
168 [bitbio.com/2017/10/clustering-rnaseq-data-using-k-means.html](https://2-bitbio.com/2017/10/clustering-rnaseq-data-using-k-means.html) (Method S1). The optimal number of
169 clusters was calculated using four different methods: sum of squared error, average silhouette width,
170 Calinsky criterion and gap statistic (Calinski and Harabasz, 1974; Rousseeuw, 1987; Tibshirani, Walther
171 and Hastie, 2001). GO enrichment analysis of the RNA-seq data was performed using a custom script
172 (prepare_gene_ontology.pl, <https://github.com/cecile-lecampion/gene-ontology-analysis-and-graph>)
173 which automatically uses PANTHER and REVIGO for the identification and simplification of enriched
174 GO terms according to the procedure proposed by Bonnot *et al.*, 2019 (Bonnot, Gillard and Nagel,
175 2019). Results were plotted using ggplot2.

176

177 High light stress and photosynthetic parameter measurements

178

179 2-week or 4-week-old plants grown in short-day conditions were submitted to two high light (HL) stress
180 periods consisting of 1500 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ illumination at 20°C with a 8 h light / 16 h dark
181 photoperiod (Ramel *et al.*, 2012). Plants were mounted on a grill to allow air circulation at the base of
182 plants. Photosynthetic parameters were acquired on dark adapted plants using a FluorCam (Photon
183 System Instruments) before HL, after HL and after 16 h of recovery period in the dark. Plants were
184 rewatered at the beginning of the recovery period.

185

186 Data analysis

187

188 Graphs and statistical tests were generated in Python (Python Software
189 Foundation, <https://www.python.org/>) using the Panda (Reback *et al.*, 2022), Matplotlib (Hunter,
190 2007), Seaborn (Waskom, 2021) and Pingouin (Vallat, 2018) libraries. A Games-Howell Post-hoc test
191 was adopted for non-parametric data comparisons and a pairwise T-test for data with normal
192 distributions. For multiple comparisons, Games-Howell Post-hoc test was adopted for non-parametric
193 data and a pairwise T-test using the Benjamini/Hochberg FDR correction for data with normal
194 distributions.

195

196 RESULTS

197

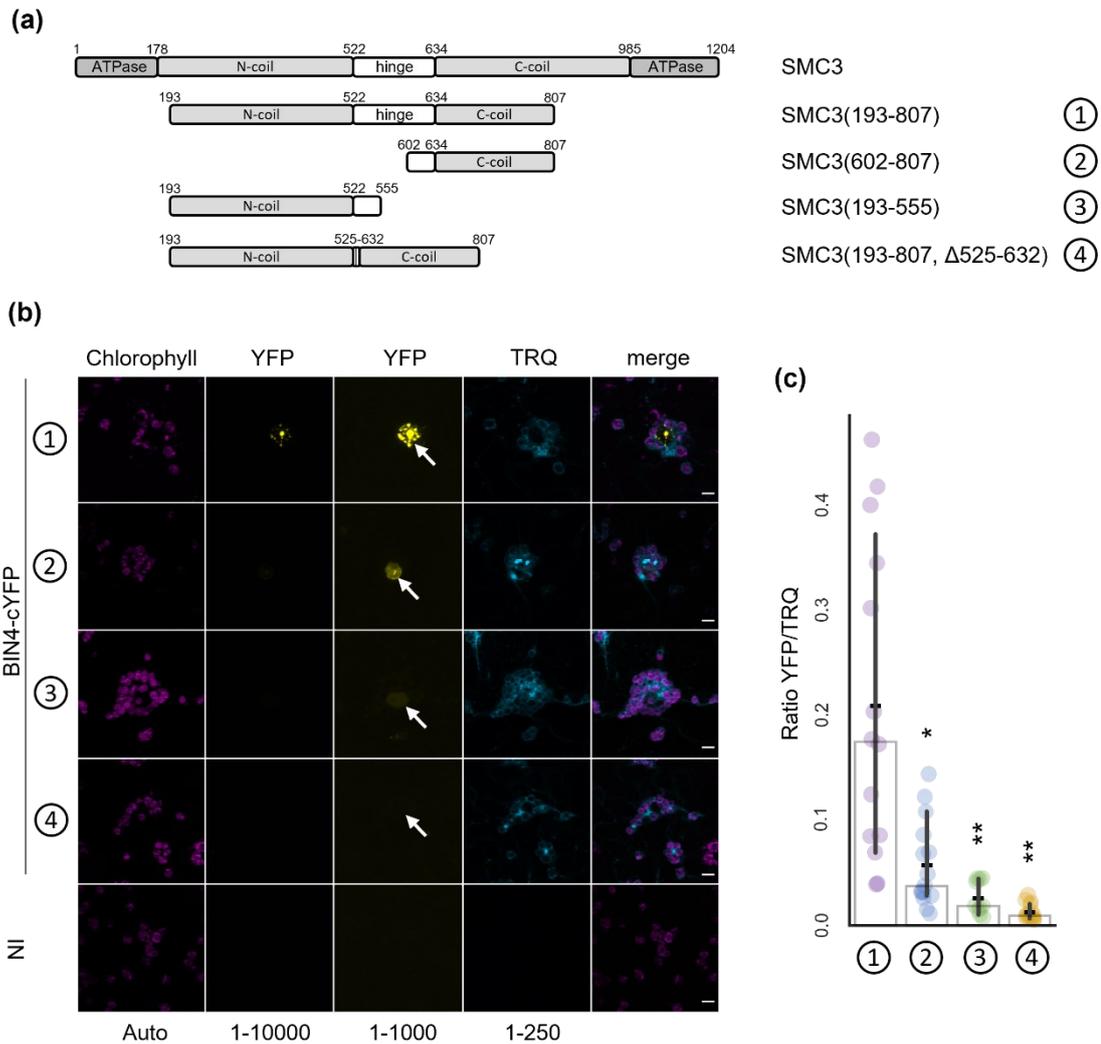
198 The Topoisomerase VI subunit BIN4 interacts with SMC3.

199

200 To gain insight into the molecular mechanism by which Topo VI controls the expression of oxidative
201 stress responsive genes, we performed a yeast two hybrid (Y2H) screen with the BIN4 subunit as bait.
202 The screen was performed under two different stringency conditions, 2.0 and 0.5 mM 3AT
203 (Hybrigenics, Table S1). We detected a strong interaction with the Topo VI subunit RHL1, which
204 supports the reliability of the screening procedure (Table S1). Among the eleven additional interacting
205 partners, TTN7 (TITAN7) / SMC3 (STRUCTURAL MAINTENANCE OF CHROMOSOMES 3) / AT2G27170
206 was identified under the two stringency conditions (Table S1). *AtSMC3* encodes a subunit of cohesin,
207 a ring shape chromatin architectural protein complex involved in sister chromatid cohesion (Tao *et al.*,
208 2007; Schubert *et al.*, 2009; Bolaños-Villegas *et al.*, 2013; Morales and Losada, 2018), gene expression
209 (Dorsett and Merckenschlager, 2013), DNA repair (Sjögren and Ström, 2010; Bolaños-Villegas *et al.*,
210 2013; da Costa-Nunes *et al.*, 2014; Pradillo *et al.*, 2015), loop extrusion (Rowley and Corces, 2018) and
211 many other processes related to chromatin dynamics (Cheng, Zhang and Pati, 2020; Bolaños-Villegas,
212 2021). The *AtSMC3* protein is composed of two globular domains located at the C- and N-terminal
213 extremities, forming an ATPase head necessary for the proper conformation of the cohesin ring during
214 DNA entrapment or release (Arumugam *et al.*, 2003; Murayama and Uhlmann, 2014; Muir *et al.*, 2020).
215 The overall rod shape structure of *AtSMC3* is due to the presence of two coil domains, separated by a
216 hinge, having the capacity to associate to each other, forming a coiled-coil region (Haering *et al.*, 2002;
217 Jumper *et al.*, 2021). Among the two clones isolated in Y2H, the clone pB27A-36 encodes a truncated

218 N-terminal coil, the entire hinge domain, and a truncated C-terminal coil (SMC3 193-807) meanwhile
219 the second clone would only encode a truncated hinge domain and a truncated C-terminal coil.

220 We then sought to confirm the biochemical interaction between AtSMC3 and BIN4 *in planta*
221 using Bimolecular Fluorescence Complementation (BiFC). We designed two recombinant CDSs, BIN4
222 fused with the C-terminus of YFP as well as the CDS of SMC3(193-807) fused with the N-terminus of
223 YFP and supplemented with a Simian-Virus 40 Nuclear Localization Signal (NLS) to ensure the nuclear
224 location of the truncated SMC3 peptide. We then added both CDS of interest to the same multigenic
225 expression vector that also contains the silencing repressor P19 and the chloroplast outer membrane
226 OEP7-mTRQ as a transformation marker (Yong Jik Lee *et al.*, 2001; Scholthof, 2006; Velay *et al.*, 2022).
227 Expression of BIN4-cYFP and SMC3(193-807)-nYFP resulted in reconstitution of YFP fluorescence in the
228 nuclei of epidermal cells of *Nicotiana benthamiana* (Fig. 1). To determine which part of SMC3 is
229 necessary for the interaction, we designed 3 truncated versions of SMC3(193-807): SMC3(602-807),
230 lacking the N-terminal coil and the N-terminal half of the hinge domain; SMC3(193-555), lacking the C-
231 terminal coil and the C-terminal half of the hinge domain; SMC3(193-807, Δ 525-632) lacking the entire
232 hinge domain (Fig. 1a). Expression of SMC3(602-807)-nYFP with BIN4-cYFP led to a significant drop in
233 YFP fluorescence compared to experiments using SMC3(193-807)-nYFP (Fig. 1b,c). The use of
234 SMC3(193-555)-nYFP resulted in an even greater decrease in YFP fluorescence. Finally, the SMC3(193-
235 807, Δ 525-632)-nYFP/BIN4-cYFP pair produced only background fluorescence in the transformed
236 nuclei (Fig. 1b,c). Collectively, these results show that BIN4 and SMC3 can interact *in planta* and that
237 the hinge domain of SMC3 is necessary for this interaction.



238

239 **Fig. 1** SMC3 interacts with BIN4 *in planta*. (a) Schematic representation of the different domains of
 240 AtSMC3; (1) SMC3(193-807); (2) SMC3(602-807); (3) SMC3(193-555); (4) SMC3(193-807, Δ525-632).
 241 (b) BiFC assay in *Nicotiana benthamiana* epidermal cells showing (1) the nuclear interaction of BIN4-
 242 cYFP and NLS-SMC3(193-807)-nYFP; (2) the weak interaction of BIN4-cYFP and NLS-SMC3(602-807)-
 243 nYFP; (3) the weaker interaction of BIN4-cYFP and NLS-SMC3(193-555)-nYFP; (4) the absence of
 244 interaction between BIN4-cYFP and NLS-SMC3(193-807, Δ525-632)-nYFP. The two YFP columns
 245 correspond to two distinct histogram levels. Histogram levels are indicated below each column. The
 246 positions of the nuclei are indicated by the white arrows. NI, not inoculated; scale bar, 10 μm. (c)
 247 Normalized BiFC signal consisting of the ratio between the YFP and the TRQ fluorescences. (1), (2), (3)
 248 and (4) as in (b) and (c). Bars indicate mean and crosses indicate median +/- 95% confidence interval
 249 (n=10-15 nuclei). Statistical tests (Games-Howell Post-hoc test) shown against (1), * $P < 0,05$, ** $P < 0,01$.
 250 The whole experiment was repeated twice with similar results.

251

252 Generation and characterization of *35S::GFP-SMC3* transgenic lines

253

254 We initially aimed to generate transgenic Arabidopsis plants overexpressing tagged SMC3 to allow
 255 biochemical studies of SMC3. For this purpose, we cloned the full CDS of *AtSMC3* following an

256 optimized protocol due to the high instability of the construct in bacterial strains. Notably, the addition
257 of genomic introns n°1, 24, 25, 26, 27 was found to help improve stability. We transformed *Arabidopsis*
258 *thaliana* plants with the synthetic transcription unit *35S::GFP-SMC3* associated with a Bialaphos
259 Resistance (BaR) cassette. Seven out of sixteen independent T1 plants showed a similar morphological
260 phenotype with varying degrees of severity among individuals: reduced growth, short petioles,
261 anthocyanin accumulation and reduced fertility. The most severely affected plants were sterile (Fig.
262 S1a). Based on the BaR segregation in the T2 generations from the fertile T1 plants, we selected four
263 independent transformant lines likely containing a single T-DNA insertion. Similarly, we then isolated
264 homozygous individuals based on BaR segregation in the T3s. Among the four lines isolated this way,
265 three displayed an almost identical morphological phenotype characterized by reduced growth, short
266 petioles, anthocyanin accumulation, reduced fertility and, after 5 weeks, leaf necrotic spots,
267 reminiscent of hypersensitive response (Fig. S1b). The fourth line displayed a less severe morphological
268 phenotype only visible after 4 weeks of growth. Suspecting that the phenotype was due to the co-
269 suppression of *GFP-SMC3* and *AtSMC3*, we named the lines: late suppressor of SMC3 (*lss*), the line
270 whose particular phenotype was visible after 4 weeks and which showed reduced fertility (Figs 2a,
271 S1b); and early suppressor of SMC3 (*ess*), the three lines whose phenotype emerged after 2 weeks and
272 which were sterile in the homozygous state (Figs 2a, S1b). Interestingly, the morphological phenotype
273 of heterozygous *ess* plants was indistinguishable from that of homozygous *lss* plants, supporting the
274 idea that *lss* and *ess* phenotype was attributable to the transgene (Fig. S1b).

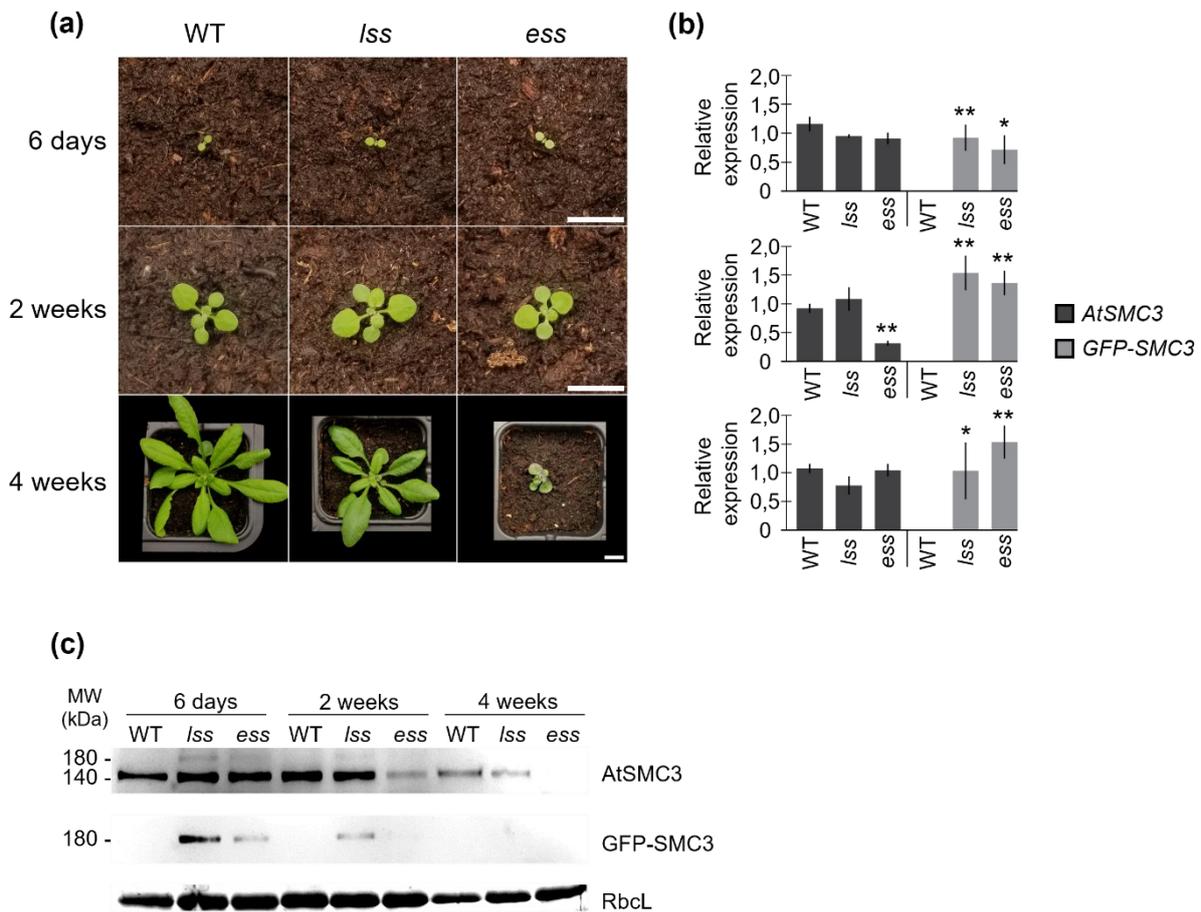
275

276 [The developmental phenotype of *35S::GFP-SMC3* lines is associated with co-](#)
277 [suppression of *GFP-SMC3* and *AtSMC3*](#)

278

279 To test whether *lss* and *ess* lines were indeed co-suppression lines, we first quantified *GFP-SMC3* and
280 *AtSMC3* transcript levels by RT-qPCR at different developmental stages in *lss* and one of the three *ess*
281 lines (Fig. 2b). Similar levels of *GFP-SMC3* transcripts were detected in 6-day-old seedlings, 2-week-old
282 plants, and rosette leaves of 4-week-old plants. *AtSMC3* transcripts were also detected in all three
283 tested conditions. The only notable decrease of *AtSMC3* expression was transiently detected in the *ess*
284 line at 2 weeks but recovered to WT level in 4-week-old rosette leaves. Although this transient drop in
285 *AtSMC3* transcript level is significant, it cannot explain the persistent phenotype observed in *ess* at 4
286 weeks. Moreover, the relatively constant level of *AtSMC3* transcripts in *lss* was not consistent with the
287 hypothesis of a transcriptional or post-transcriptional co-silencing. Therefore, to test whether the
288 putative co-silencing event could take place at a translational or post-translational level, we raised an
289 antibody against the C-terminus region of *AtSMC3*. In accordance with the theoretical size of *AtSMC3*

290 (The Arabidopsis Genome Initiative, 2000; Lam, Yang and Makaroff, 2005), immunoblotting using the
 291 anti-AtSMC3 antibody detected a 140 kDa protein with similar abundance in each lines at 6 days (Fig.
 292 2c). At 2 weeks, the signal was similar in WT and *lss* lines but strongly reduced in the *ess* line. The
 293 decrease in AtSMC3 levels is therefore correlated with the early appearance of a morphological
 294 phenotype in *ess* (Fig. 2a,c). At 4 weeks, the AtSMC3 signal also decreased in the *lss* line, which again
 295 is correlated with the later appearance of the morphological phenotype in this line. Immunoblots using
 296 anti-GFP antibody revealed that the GFP-SMC3 protein was already undetectable at 2 weeks in *ess* and
 297 became undetectable at 4 weeks in the *lss* line (Fig. 2c). These results confirm that expression of *GFP-*
 298 *SMC3* in the *lss* and *ess* lines triggers stable translational or post translational co-silencing of AtSMC3
 299 and GFP-SMC3. Furthermore, we confirmed that silencing of AtSMC3 is triggered earlier in *ess*, leading
 300 to a severe morphological phenotype that is essentially characterized by growth arrest and sterility
 301 (Fig. 2a).



302
 303 **Fig. 2** Late Suppressor of SMC3 (*lss*) and Early Suppressor of SMC3 (*ess*) show co-silencing of GFP-SMC3
 304 and AtSMC3. (a) Photographs of long day grown WT, homozygous *lss* and *ess* at 6 days, 2 weeks and 4
 305 weeks. Scale bar, 1 cm. (b) RT-qPCR analysis of *AtSMC3* and *GFP-SMC3* transcript abundance at 6 days,
 306 2 weeks and 4 weeks in WT, *lss* and *ess* genotypes. A segregating population of *ess* was analysed at 6
 307 days. Bars indicate mean and lines indicate the standard error ($n = 3$ independent biological replicates).
 308 Statistical tests (pairwise t-test) shown against respective WT controls, * $P < 0.05$, ** $P < 0.01$. (c)
 309 Representative immunoblot with specific antibodies against AtSMC3 and GFP-SMC3 performed on

310 total protein extracts of the indicated genotypes at 6 days, 2 weeks and 4 weeks. A segregating
311 population of *ess* was analysed at 6 days. Rubisco (RbCL) revealed by total protein staining was used as
312 loading control.

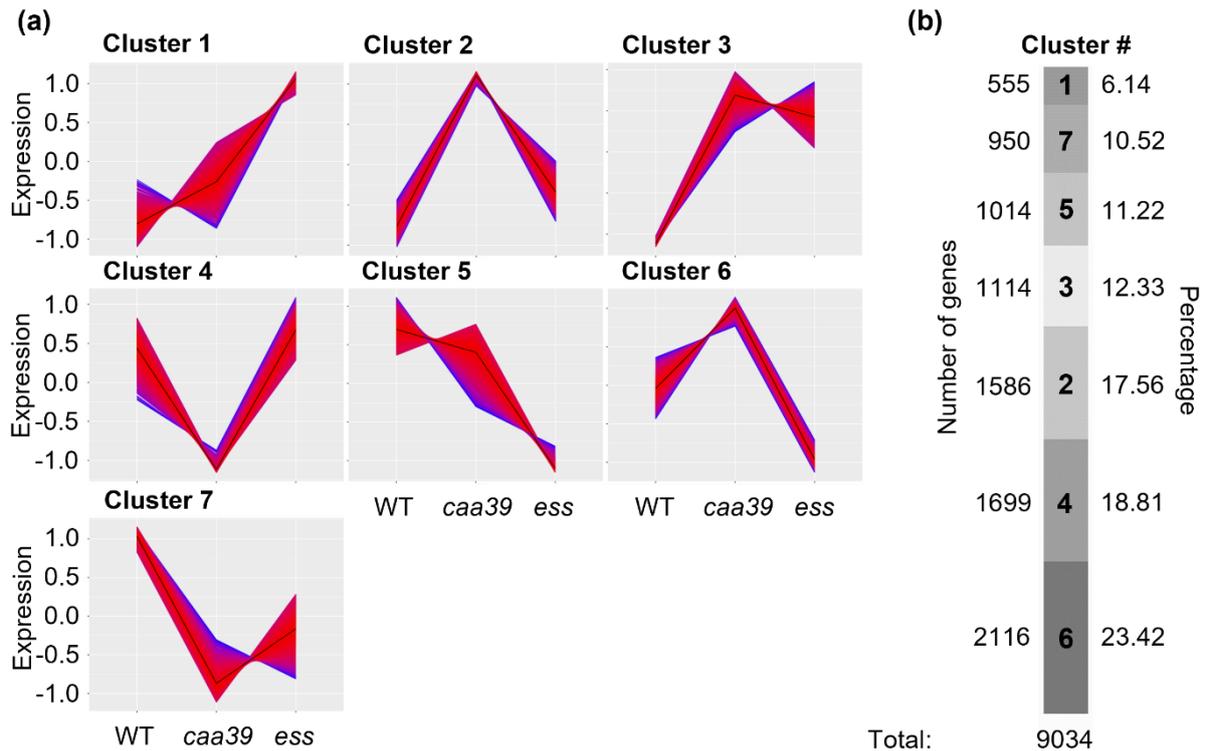
313

314 Transcriptomic analysis of *ess* and *caa39* reveals a common upregulation of oxidative
315 stress response genes.

316

317 To date, the only reported *AtSMC3* mutant lines contained T-DNA insertions in exons that lead to
318 embryo lethality in the homozygous state (Liu *et al.*, 2002), and almost no decrease in *AtSMC3*
319 expression and a WT-like morphological phenotype in the heterozygous state (Schubert *et al.*, 2009).
320 These insertion mutants are therefore not convenient for genetic analysis of *AtSMC3* in plants. The *lss*
321 and *ess* lines where *AtSMC3* decreases during plant development appear to be more suitable tools to
322 study the consequences of reduced accumulation of *AtSMC3* in plants. We therefore decided to
323 compare the impact of *AtSMC3* silencing and a hypomorphic Topo VI mutation on gene expression by
324 RNA-seq analysis of *ess* and *caa39* lines at 2 weeks. At this developmental stage, *ess* displays reduced
325 *AtSMC3* protein levels, undetectable GFP-SMC3 levels, and only slight morphological defects. We
326 decided not to include 4-week-old *ess* plants in this analysis because of the severity of its morphological
327 phenotype.

328 To identify commonly and differently regulated genes and pathways in *caa39* and *ess*, we
329 performed gene clustering based on gene expression profiles in *caa39*, *ess* and the WT control. By
330 combining four different methods, seven main gene clusters could be identified, each cluster
331 corresponding to a typical expression pattern (Fig. 3a, S2, Table S2). Clusters 1, 2 and 3 correspond to
332 genes up regulated in both *caa39* and *ess* (Fig. 3a) and contain 36% of the 9034 genes that are
333 differentially regulated in at least one line (Fig. 3b). Conversely, clusters 5 and 7 contain genes down
334 regulated in both lines and represent about 22% of the genes (Fig. 3a, b). Thus, 58% of the genes belong
335 to clusters containing commonly deregulated genes between *caa39* and *ess*, suggesting a consistent
336 overlap between Topo VI and *AtSMC3* functions. The remaining 42% of genes are distributed in two
337 clusters, 4 and 6, which correspond to genes specifically repressed in *caa39* or *ess*, respectively.



338

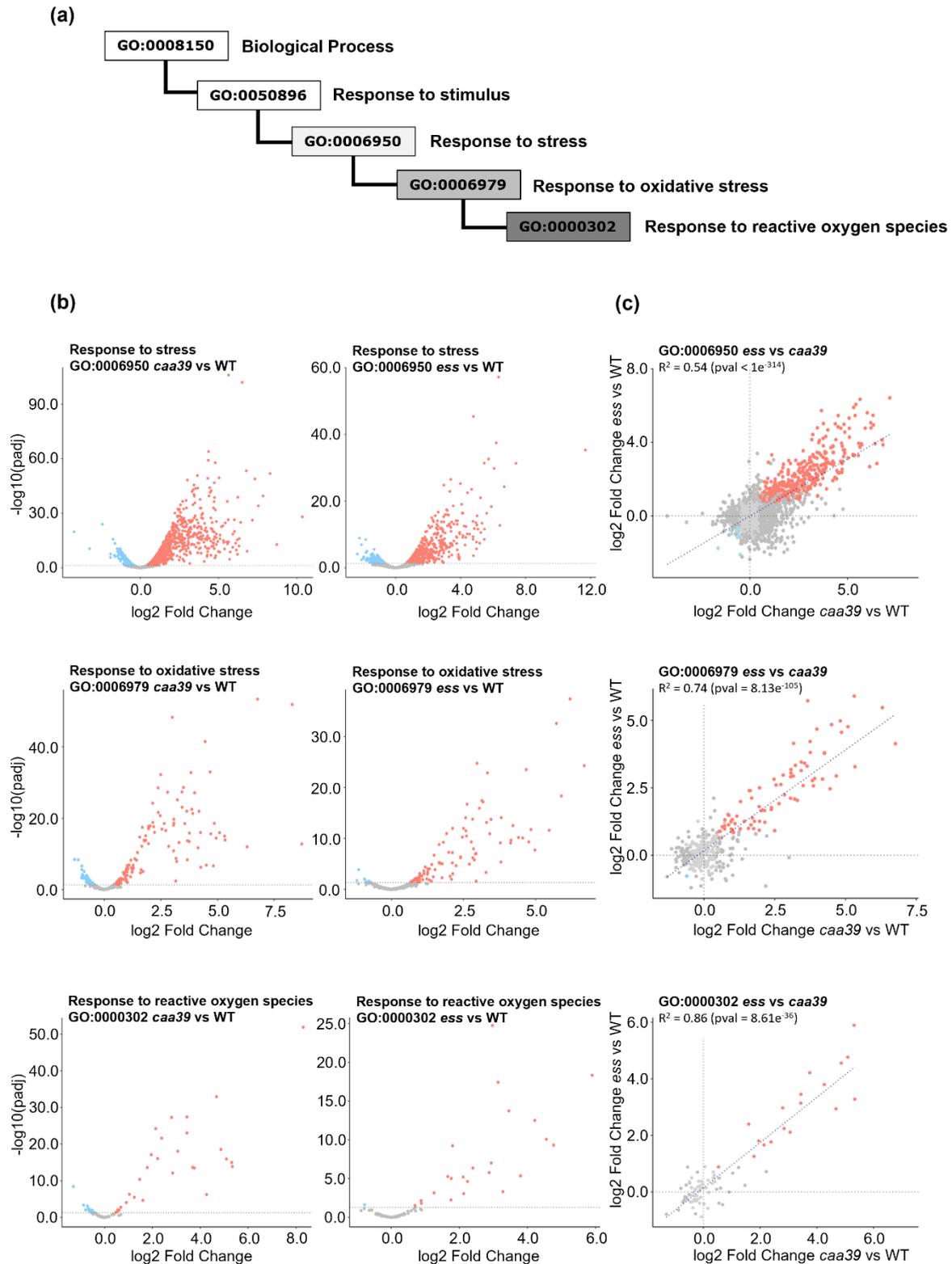
339 **Fig.3** Gene clustering of RNA-seq transcriptomic data for 2-week-old WT, *caa39* and *ess* lines. (a)
 340 Cluster 1 corresponds to genes induced in *caa39* and *ess* with different intensities. Cluster 2
 341 corresponds to genes induced in *caa39* and slightly induced in *ess*. Cluster 3 corresponds to genes
 342 almost equivalently induced in *caa39* and *ess*. Cluster 4 corresponds to genes only repressed in *caa39*.
 343 Cluster 5 corresponds to genes repressed in *ess*. Cluster 6 corresponds to genes upregulated in *caa39*
 344 and repressed in *ess*. Finally, cluster 7 corresponds to genes repressed in *caa39* and *ess*. Genes whose
 345 expression profile is close to the cluster trend are represented by a red line. Those whose expression
 346 profile is more divergent are represented by a blue trace. The exact blue/red scale of each cluster is
 347 provided in figure S2. (b) Number and percentage of genes associated with the different clusters. Two
 348 biological replicates were performed for each genotype.
 349

350

We then performed a Gene Ontology (GO) term enrichment analysis to identify biological
 351 processes enriched in each cluster. Clusters 5 and 6, which contain genes strongly repressed in *ess* (Fig.
 352 3), are enriched for genes involved in microtubule dynamics, especially in the context of cellular
 353 division (Fig. S2). Such down regulation of microtubule related genes could arise from arrest of the cell
 354 cycle at an early phase, as has been observed in breast cancer cells where the gene encoding cohesin
 355 loader NIPPED-B-LIKE (NIPBL) is silenced (Zhou *et al.*, 2017). This hypothesis is further supported by
 356 the growth arrest of *ess* plants after 2 weeks (Fig. 2a). Clusters 4 and 7 correspond to genes repressed
 357 in *caa39* or in both *caa39* and *ess*, and are enriched for genes involved in several chloroplast functions
 358 including light harvesting (Fig. S2, Table S2). Considering that a decrease in photosynthetic efficiency
 359 and chloroplast translation is a common response to exposure to diverse stresses (Grennan and Ort,
 360 2007; Upadhyaya and Rao, 2019; Romand *et al.*, 2022), the downregulation of genes for chloroplast
 361 function could result from a global misregulation of stress signalling in *caa39* and *ess*. Supporting this

362 idea, clusters 1, 2 and 3, which contain genes upregulated in both *caa39* and *ess*, are strongly enriched
363 for genes involved in various stress responses, such as hypoxia, iron starvation or pathogenesis (Fig.
364 S2). This suggests that Topo VI and SMC3 could participate in the regulation of several metabolic or
365 signalling pathways related to stress responses. Importantly, cluster 1 is also specifically enriched for
366 a GO term related to oxidative stress (GO:0006979) (Fig. S2), a feature characteristic of most
367 environmental stresses (Apel and Hirt, 2004). The GO term "Response to oxidative stress" belongs to
368 a more general class called "Response to stress" (GO:0006950) and is subdivided into more specific
369 subclasses among which "response to reactive oxygen species" contains the largest number of genes
370 (GO:0000302) (Fig. 4a).

371 We then performed the reciprocal analysis to determine if an oxidative stress response is
372 activated in *caa39* and *ess*, by analysing the expression of all genes assigned to each of these three GO
373 terms in *caa39* and *ess*. Genes assigned to GO "Response to stress" and more specifically "Response
374 to oxidative stress" and "Response to reactive oxygen species" were in the vast majority activated in
375 both mutant lines compared to WT (Fig. 4b, left and right panels). To further determine whether these
376 genes are commonly regulated in *caa39* and *ess*, we directly compared their fold changes in these two
377 lines. The expression of genes belonging to general GO term "stress response" appeared to be
378 moderately correlated between *caa39* and *ess* ($R^2=0.54$) (Fig. 4c). However, the correlation between
379 the two lines increased when considering only the subclass "response to oxidative stress" ($R^2=0.74$)
380 and the most specific subclass "response to reactive oxygen species" ($R^2=0.86$) (Fig. 4c), suggesting that
381 most oxidative stress related genes are commonly regulated by Topo VI and SMC3.



382

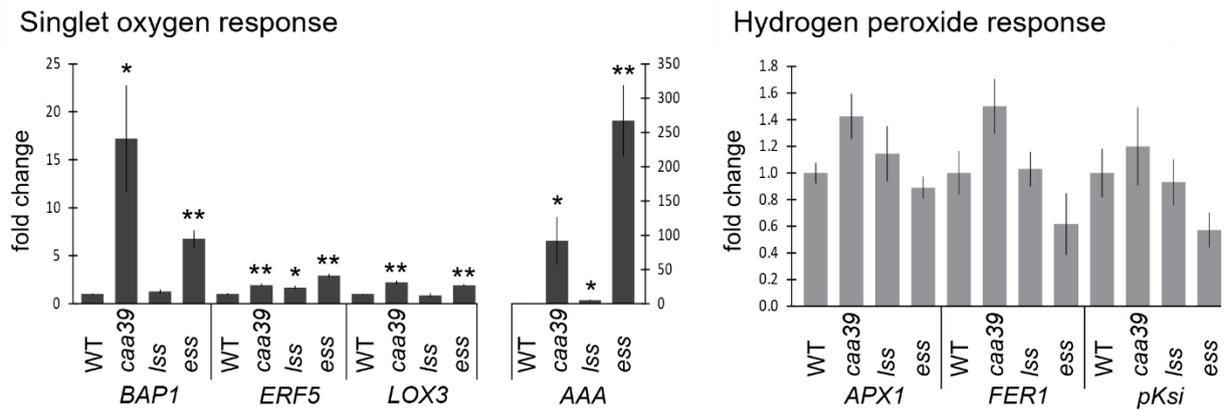
383 **Fig. 4** ROS responsive genes are similarly activated in *caa39* and *ess* under normal growth conditions.
 384 (a) Diagram representing the hierarchy of the 5 GO: « Response to reactive oxygen species »
 385 (GO:0000302) is part of « Response to oxidative stress » (GO:0006979), which is part of « Response to
 386 stress » (GO:0006950), which is part of « Response to stimulus » (GO:0050896), which is part of
 387 « Biological process » (GO:0008150). (b) Expression profiles of the genes belonging to GO:0006950,
 388 GO:0006979 and GO:0000302 in *caa39* (left panel) and *ess* (right panel) compared to the WT at 2

389 weeks. The p-value is calculated using an exact test for negative binomial distribution and corrected
390 based on Benjamini-Hochberg test. Red dots indicate genes significantly up-regulated, blue dots
391 indicate genes significantly down-regulated, grey dots indicate genes whose expression is not
392 significantly altered. (c) Scatterplots of fold changes versus WT of genes belonging to GO:0006950,
393 GO:0006979 and GO:0000302 for *caa39* and *ess*. Line of best fit and R^2 with corresponding p-value are
394 indicated. Red dots indicate genes similarly up-regulated, blue dots indicate genes similarly down
395 regulated, grey dots indicate genes differently regulated or genes whose expression is not significantly
396 altered. Two biological replicates were performed for each genotype.
397

398 *caa39* and SMC3 co-suppression lines show constitutive activation of $^1\text{O}_2$ -responsive
399 genes under normal light conditions.

400

401 To test whether induction of oxidative stress response genes could be attributed to SMC3 co-silencing,
402 we measured the expression of several $^1\text{O}_2$ -responsive marker genes (*BON ASSOCIATION PROTEIN 1* /
403 *AT3G61190*, *BAP1*; *ETHYLENE RESPONSIVE ELEMENT BINDING FACTOR 5* / *AT5G47230*, *ERF5*;
404 *LIPOXYGENASE 3* / *AT1G17420*, *LOX3*; *AAA-ATPase* / *AT3G28580*, *AAA*) and H_2O_2 -responsive genes
405 (*ASCORBATE PEROXIDASE 1* / *AT1G07890*, *APX1*; *FERRETIN 1* / *AT5G01600*, *FER1*; *AT3G49160*, *pKsi*)
406 (Op Den Camp *et al.*, 2003; Šimková *et al.*, 2012) by RT-qPCR at 6 days and 2 weeks in *lss*, *ess* and
407 *caa39*. In previous studies, 6-day-old *caa39* plants were reported to show selective activation of a set
408 of $^1\text{O}_2$ -responsive genes (Šimková *et al.*, 2012). As expected, 6-day-old *caa39* seedlings strongly
409 accumulated transcripts for the $^1\text{O}_2$ -responsive genes *BAP1* and *AAA*, whereas they displayed very
410 slight variations in the expression of H_2O_2 -responsive genes (Fig. S3). However, *lss* and *ess* lines showed
411 transcript levels similar to the WT control for both H_2O_2 and $^1\text{O}_2$ markers, suggesting that over-
412 expression of *GFP-SMC3* at this stage does not trigger oxidative stress response under normal light
413 conditions (Fig. S3). In agreement with the RNA-seq data, 2-week-old *caa39* plants displayed significant
414 increased transcript levels of $^1\text{O}_2$ marker genes. Similarly, 2-week-old *ess* line accumulated high
415 amount of all tested $^1\text{O}_2$ marker transcripts, up to over 250-fold activation for *AAA* expression.
416 Interestingly, *lss* line only partially activated the $^1\text{O}_2$ markers with a slight induction of *AAA* gene (7.3
417 fold change) and *ERF5* gene (1.73 fold change) (Fig. 5). No significant increase in the expression of
418 H_2O_2 -responsive genes was observed, suggesting a specific and constitutive activation of the $^1\text{O}_2$
419 pathway in AtSMC3 suppressors and *caa39*.



420

421 **Fig. 5** The suppressors of SMC3 and *caa39* show activation of the $^1\text{O}_2$ response. RT-qPCR analysis
422 measuring the transcript abundance of the $^1\text{O}_2$ -responsive genes *BAP1*, *ERF5*, *LOX3*, *AAA* and the H_2O_2 -
423 responsive genes *APX1*, *FER1*, *pKsi* at 2 weeks of growth. Bars indicate mean and lines indicate the
424 standard error (n = 3 independent biological replicates). Statistical tests (pairwise t-test for data
425 following a parametric distribution; Games-Howell Post-hoc test for data not following a parametric
426 distribution) shown against respective WT controls, * $P < 0.05$, ** $P < 0.01$.

427

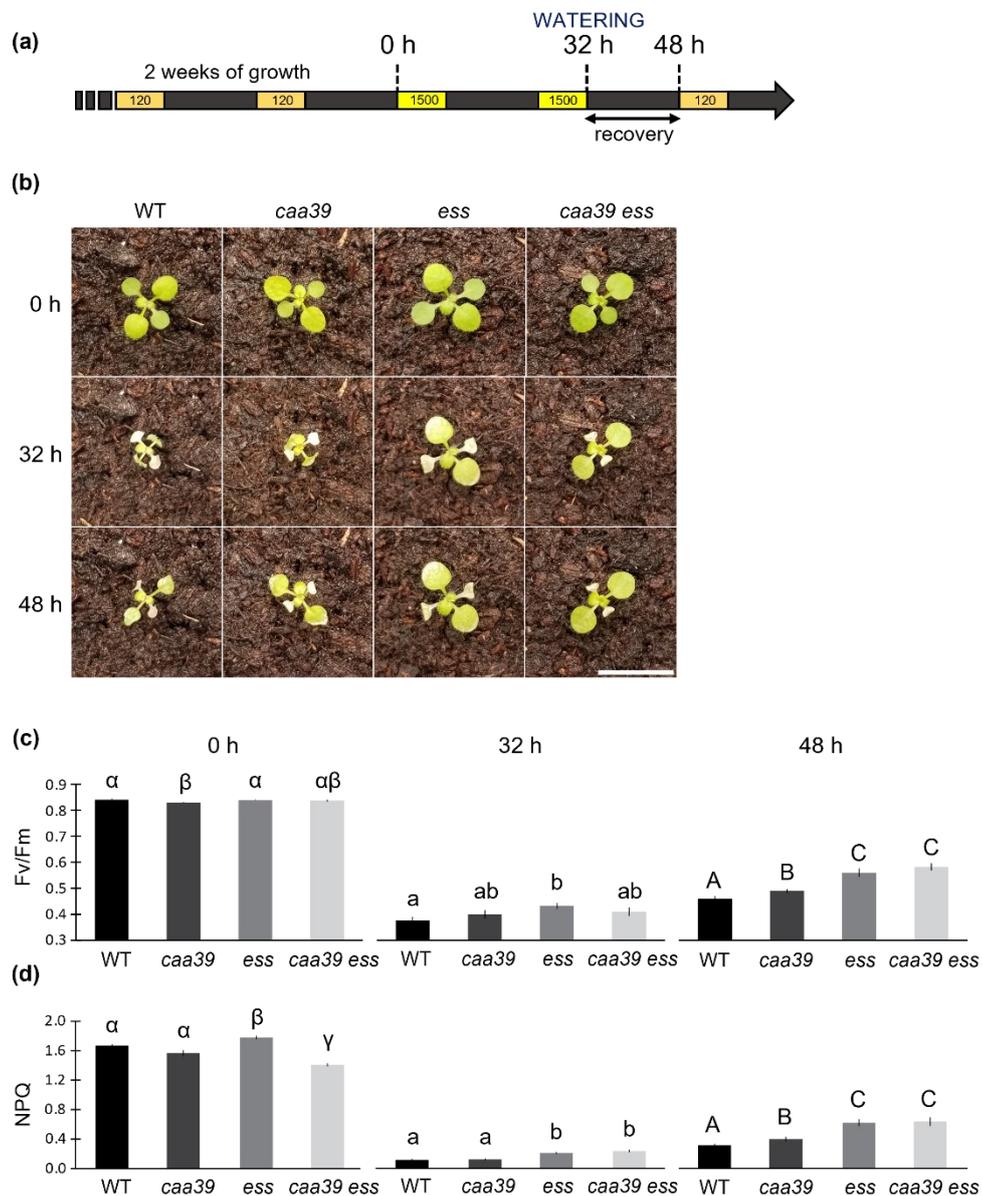
428 [SMC3 co-suppression and *caa39* lines display enhanced resistance to high light stress.](#)

429

430 To further investigate the interplay between Topo VI and AtSMC3 in the constitutive activation of the
431 $^1\text{O}_2$ response, we crossed *lss* and *ess* plants with the *caa39* mutant to obtain the *caa39 lss* and *caa39*
432 *ess* lines. The *caa39* mutation coupled to SMC3 suppression led to an additive phenotype combining
433 the morphological characteristics of each parental lines, leading to severe growth defects (Fig. S4). To
434 investigate the physiological response to the production of $^1\text{O}_2$ we exposed the SMC3 suppressors, the
435 *caa39* mutant, and the double *caa39 lss* and *caa39 ess* lines to high light photooxidative stress
436 conditions (1500 $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$, 4°C, 48 h, 8 h photoperiod, Fig. 6a) that are specifically
437 designed to generate a burst of $^1\text{O}_2$ production (Ramel *et al.*, 2012). We performed the high light stress
438 on 2-week-old *ess* and 4-week-old *lss* in order to limit indirect effects due to morphological differences
439 or residual expression of GFP-SMC3.

440 After 32 h high light stress, 2-week-old plantlets of all tested genotypes displayed photo-
441 bleaching on the entire surface of the cotyledons and partly on the first and second leaves (Fig. 6b).
442 Although the WT and *caa39* plantlets appeared to be more severely affected and desiccated, as shown
443 by down-curved leaves after the stress period, the Fv/Fm measurements indicated PSII photoinhibition
444 was similar in all lines (Fig. 6c, middle panel "32 h"). Interestingly, after the recovery period of 16 h in
445 the dark, *caa39* displayed a slightly higher Fv/Fm ratio compared to the WT line (Fig. 6c, right panel
446 "48 h"). Following a similar trend, the SMC3 suppressor lines, *ess* and *caa39 ess* displayed an even

447 greater Fv/Fm ratio indicating that there is a more efficient recovery of PSII capacity in the absence of
 448 AtSMC3 (Fig. 6c). To better understand the high light tolerance of SMC3 suppressor, we measured non-
 449 photochemical quenching (NPQ) before and after exposure to oxidative stress. Surprisingly, under
 450 normal light conditions, NPQ was lower in *caa39 ess* compared to the WT and *caa39*, whereas the *ess*
 451 line had the highest level of NPQ (Fig. 6d, left panel “0 h”). However, after exposure to photoinhibitory
 452 conditions, the NPQ values of both SMC3 suppressing lines clustered and became substantially higher
 453 than WT and *caa39* (Fig. 6d, middle panel “32 h”). Finally, after the 16 h recovery in the dark, NPQ
 454 remained higher in SMC3 suppressors than in the other lines, thus correlating the tolerance to
 455 photooxidative stress with the activation of NPQ (Fig. 6d, right panel “48 h”). These results show that
 456 the silencing of SMC3 and, to a lesser extent, the *caa39* mutation led to a resistance to high light, likely
 457 through an overactivation of the NPQ pathways.



458

459 **Fig. 6** SMC3 suppressors show enhanced tolerance to photooxidative stress. (a) Timescale of the high
460 light stress experiment performed on two-week-old plantlets. Orange, 8 h normal light periods (120
461 $\mu\text{mol m}^{-2} \text{s}^{-1}$); yellow, 8 h high light periods ($1500 \mu\text{mol m}^{-2} \text{s}^{-1}$); black, 16 h dark periods. 0 h, onset of
462 the high light stress; 32 h, end of the second high light illumination period; 48 h, end of the dark period
463 following the high light stress. (b) Pictures of representative 2-week-old WT, *caa39*, *ess* and *caa39 ess*
464 plantlets at 0 h, steady state; 32 h, after high-light exposure; 48 h, following a 16 h recovery period in
465 the dark. Scale bar, 1 cm. (c) PSII efficiency (Fv/Fm) of WT, *caa39*, *ess* and *caa39 ess* at 0 h, 32 h and
466 48 h. Black lines indicate the standard error (n = 12 plants from 3 independent biological replicates).
467 Compact letter display represents statistic groups and was generated using a pairwise t-test corrected
468 with the Benjamini/Hochberg FDR method. (d) Non-Photochemical Quenching (NPQ) measurements.
469 Black lines indicate the standard error (n = 12 plants from 3 independent biological replicates).
470 Compact letter display represents statistic groups and was generated using a pairwise t-test corrected
471 with the Benjamini/Hochberg FDR method.
472

473 To confirm that these physiological observations are a consequence of SMC3 silencing, we
474 repeated this experiment using 4-week-old WT, *caa39*, *lss* and *caa39 lss* plants. After applying the
475 same stress conditions on 4-week-old plants, we observed that the oldest WT leaves were partly
476 bleached. However, control plants remained mostly green, showing the higher tolerance of 4-week-
477 old plants than 2-week-old plantlets to these photoinhibitory conditions (Fig. S5a). Immediately after
478 exposure to high light, Fv/Fm was slightly but significantly higher in *lss* and *caa39 lss* than in WT and
479 *caa39* (Fig. S5b). In agreement with observations in 2-week-old *caa39 ess*, NPQ under normal light
480 conditions was lower in *caa39 lss* compared to the WT. (Fig. 6d, left panel “0 h”). However, after
481 exposure to photoinhibitory conditions, the NPQ appeared to be not significantly higher in *lss* lines
482 than in WT and *caa39* (Fig. 6d, middle panel “32 h”). These results show that SMC3 suppressors and to
483 a lesser extent *caa39*, are resistant to photoinhibitory conditions.
484

485 DISCUSSION

486
487 Photooxidative stress conditions trigger retrograde signalling to activate expression of nuclear stress
488 responsive genes essential to prevent damages to macromolecules and to detoxify the cell (Gill and
489 Tuteja, 2010; Kerchev and Van Breusegem, 2022). The coordinated expression of these genes requires
490 the activity of transcription factors (Dubos *et al.*, 2010; Jiang *et al.*, 2017; Feng *et al.*, 2020), chromatin
491 remodelling factors (Song, Liu and Han, 2021) and topoisomerases (Šimková *et al.*, 2012). However,
492 interactions between these different factors remain unclear. In this study, we presented two SMC3
493 silencing lines, *lss* and *ess*, where the $^1\text{O}_2$ -responsive gene AAA is constitutively activated under normal
494 light conditions. This result echoes a previous study showing that *caa39*, a hypomorphic mutant of
495 Topo VI, constitutively activates several $^1\text{O}_2$ -responsive genes including AAA (Baruah *et al.*, 2009;

496 Šimková *et al.*, 2012). Additionally, RNA-seq analysis revealed that genes overexpressed in both *caa39*
497 and *ess* are enriched for oxidative stress responsive genes. The confirmation of RNA-seq data by RT-
498 qPCR revealed that many ¹O₂-responsive genes, like *BAP1*, *ERF5* or *LOX3* (Op Den Camp *et al.*, 2003;
499 Danon *et al.*, 2005) are upregulated in *caa39* and *ess*. The similarities of the transcriptomic features
500 between *caa39* and SMC3 suppressors raises the idea that Topo VI and the cohesin complex could be
501 involved in a common pathway regulating the response to ¹O₂.

502 Supporting this idea, we demonstrated that the BIN4 subunit of Topo VI directly interacts with
503 SMC3(193-807). SMC3(193-807) lacks the N and C-terminal extremities which assemble in the ATP
504 binding cassette of SMC3. This domain contains the interaction site with the α-kleisin subunit SCC1
505 which participates in the formation of the cohesin ring (Gligoris *et al.*, 2014). Moreover, the enclosure
506 and opening of the SMC3/SCC1 interface require the binding and hydrolysis of ATP (Marcos-Alcalde *et*
507 *al.*, 2017; Muir *et al.*, 2020), a process which cannot be achieved in SMC3(193-807). This suggests that
508 the interaction between SMC3(193-807) and BIN4 can take place without the integration of SMC3 into
509 a functional cohesin complex. In regard of the complete loss of BiFC signal obtained with the pair BIN4-
510 cYFP associated with SMC3(193-807, Δ525-632)-nYFP lacking the hinge domain of SMC3, we also
511 propose that BIN4 interacts with the hinge domain of SMC3. Indeed, several studies have shown that
512 this domain is prone to interact with partner proteins, as is the case for SMC1 (Chiu, Revenkova and
513 Jessberger, 2004) and for hinderin (Patel and Ghiselli, 2005).

514 At a physiological level, 2-week-old *ess* and *caa39 ess* plants displayed an enhanced recovery
515 of PSII efficiency, accompanied by activation of NPQ, after recovery from severe photoinhibitory
516 conditions. Moreover, 4-week-old *lss* and *caa39 lss* displayed reduced PSII photoinhibition after high-
517 light treatment compared to WT plants. The enhanced resistance of SMC3 suppressors could be
518 attributed to lower light-harvesting efficiency or could arise from a constitutive stress state that might
519 prime the plants for further stress exposure. However, under normal light conditions, the similar PSII
520 efficiency of WT, *lss* and *ess* shows that SMC3 suppressors are not pre-stressed and have no defect in
521 light harvesting. We therefore propose that SMC3 is required for the regulation of the photooxidative
522 stress response in *Arabidopsis thaliana*. Unlike 6-day-old *caa39* seedlings (Baruah *et al.*, 2009; Šimková
523 *et al.*, 2012), 2-week and 4-week-old *caa39* plants did not show pronounced tolerance to
524 photooxidative stress under our conditions. This suggests that *caa39* is sensitive to variations in
525 experimental setup or developmental stages.

526 Limited signs of photodamage after high light treatment in *ess*, combined with the
527 spontaneous appearance of necrotic spots very similar to hypersensitive response on 5-week-old-
528 leaves, suggest that SMC3 could participate in the regulation of a wider range of stress responses than

529 Topo VI. Several studies support this idea. For instance, in Cornelia de Lange Syndrome, cell lines
530 carrying mutations in *SMC1A* or *SMC3* exhibit higher level of protein carbonylation, reflecting
531 increased global oxidative stress (Gimigliano *et al.*, 2012). In budding yeast, temperature sensitive
532 mutants of the cohesin complex show induction of the cell wall stress responsive genes CHITIN
533 SYNTHASE 3 (*CHS3*) and the β -1,3-GLUCAN SYNTHASE (*FKS2*), display increased amount of chitin in
534 their cell wall, and are sensitive to cell wall stress-inducing agents (Kothiwal, Gopinath and Laloraya,
535 2021). Finally, in embryonic stem cells, the cohesin complex was reported to participate in the
536 establishment of the heat stress response by modifying the local chromatin architecture around heat
537 stress-activated enhancers (Lyu, Rowley and Corces, 2018).

538 From a technical perspective, this study generated new and efficient tools that mimic
539 conditional knockout or knockdown lines and are useful for studying the function of AtSMC3 in plants.
540 Indeed, the SMC3 silencing lines *lss* and *ess* drive a robust translational or post-translational silencing
541 of AtSMC3 and can be crossed with other mutant lines while conserving their ability to silence AtSMC3.
542 Furthermore, the overexpression of GFP-SMC3 in 6-day-old *lss* and *ess* and the new anti-AtSMC3
543 antibody open the field for biochemical studies of AtSMC3.

544

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549

550 CONFLICT OF INTEREST

551 The authors declare that they have no conflict of interest.

552

553 AUTHOR CONTRIBUTIONS

554 FV, DA, C Laloi performed the experiments. C Lecampion performed the bioinformatic analyses. FV,
555 SD, BF and C Laloi analysed the data. NK and BF contributed to material and methods. FV and C Laloi
556 designed the research. FV and C Laloi wrote the manuscript. All authors read and approved the final
557 manuscript.

558

559 DATA AVAILABILITY

560 RNA-seq data are available at <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE211131>
561 (reviewer token: wfydqwyqrbgdfir).

562

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739

740 SUPPORTING INFORMATION

741

742 **Fig. S1** SMC3 suppressors display growth defects, anthocyanin accumulation and localized cell death
743 throughout the silencing of AtSMC3.

744

745 **Fig. S2** Expression profil and GO term enrichment of the 7 gene clusters.

746

747 **Fig. S3** 6-day-old *lss* and *ess* are not impaired in the oxidative stress response.

748

749 **Fig. S4** Suppressing AtSMC3 and AtTopoVI A leads to additive morphological phenotypes.

750

751 **Fig. S5** 4-week-old *lss* displays a slight tolerance to photooxidative stress.

752

753 **Table S1** BIN4 interacts with SMC3 in Y2H.

754

755 **Table S2** Complete list of genes associated with clusters 1-7.

756

757 **Method S1** Script used for the generation of gene clusters and analyses of GO term enrichments.