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1 **S-nitrosylation of the histone deacetylase HDA19 stimulates its activity to enhance plant**
2 **stress tolerance in Arabidopsis.**

3
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18
19 **Running title:** S-nitrosylation regulates HDA19 function

20 **Short Summary**

21 HDA19 is post-translationally modified by S-nitrosylation at 4 Cysteine (Cys) residues, which
22 is regulated by cellular nitric oxide levels. Cys137 is an important residue for the basal and
23 stress-induced S-nitrosylation that regulates HDA19 activity to deacetylate H3K14 and to
24 repress expression of subsets of genes under stress.

Abstract

Arabidopsis histone deacetylase HDA19 is required for gene expression programs of a large spectrum of plant developmental and stress-responsive pathways. How this enzyme senses cellular environment to control its activity remains unclear. In this work, we show that HDA19 is post-translationally modified by S-nitrosylation at 4 Cysteine (Cys) residues. HDA19 S-nitrosylation depends on the cellular nitric oxide (NO) level which is enhanced under oxidative stress. We find that HDA19 is required for cellular redox homeostasis and plant tolerance to oxidative stress which in turn stimulates its nuclear enrichment, S-nitrosylation and epigenetic functions including binding to genomic targets, histone deacetylation, and gene repression. The Cys137 of the protein is involved in basal and stress-induced S-nitrosylation and is required for HDA19 functions in developmental, stress-responsive, and epigenetic controls. Together, these results indicate that S-nitrosylation regulates HDA19 activity and is a mechanism of redox-sensing for chromatin regulation of plant tolerance to stress.

63 INTRODUCTION

64 Histone acetylation/deacetylation is an important epigenetic modification for chromatin
65 remodeling and gene expression. The dynamic equilibrium of histone acetylation is regulated
66 by histone acetyltransferases (HATs) and histone deacetylases (HDACs) (Mikkelsen et al.,
67 2007; Ueda et al., 2017; Yuan et al., 2013). In Arabidopsis, there are 12 HDACs belonging to
68 the reduced potassium dependency 3 (RPD3/HDA1) family, 4 to the histone deacetylase 2
69 (HD2) group, and 2 to the silent information regulator 2 (SIR2) or sirtuin proteins (Alinsug et
70 al., 2009; Pandey et al., 2002). Many studies have shown that HDA19, a RPD3 family member,
71 plays an essential role in plant growth and development including meristem function;
72 morphology of seedling, leaf, and floral organs; flowering time; fertility; seed maturation and
73 germination (Benhamed et al., 2006; Krogan et al., 2012; Long et al., 2006; Ryu et al., 2014;
74 Tanaka et al., 2008; Tian et al., 2003; Zhou et al., 2013). In addition, HDA19 is also implicated
75 in plant defense and response to environmental stresses (Gao et al., 2015; Gorham et al., 2018;
76 Pi et al., 2015; Ryu et al., 2014; Shen et al., 2019b; Song et al., 2005; Ueda et al., 2018; Zhou et
77 al., 2005). *HDA19* overexpression up-regulates several ethylene/jasmonic acid-induced genes
78 and enhances resistance to a fungal pathogen (Zhou et al., 2005), while its mutation de-
79 represses salicylic acid (SA) biosynthesis and SA-mediated defense gene expression and
80 responses (Jang et al., 2011; Kim et al., 2008). HDA19 is also required for plant response to
81 abiotic stresses such as drought, salt, high temperature and ABA signaling (Mehdi et al., 2016;
82 Perrella et al., 2013; Ryu et al., 2014; Song et al., 2005; Ueda et al., 2017). However, recent
83 data indicate that compared with other HDAC members (i.e. HDA5/14/15/18), HDA19
84 regulates different pathways of plant response to salt stress and high temperature (Shen et al.,
85 2019a; Ueda et al., 2018). HDA19 is reported to form transcriptional repressive complexes with
86 various co-repressors such as TOPLESS (TPL), LEUNIG, SWI-INDEPENDENT3 (SIN3), and
87 SIN3-LIKE (SNL) proteins and to interact with transcription factors such as AP2/EREBP,
88 BES1, SCR-like15, WOX5, and others to repress genes involved in different developmental
89 and stress-responsive pathways through histone deacetylation (Gao et al., 2015; Gonzalez et al.,
90 2007; Krogan et al., 2012; Long et al., 2006; Mehdi et al., 2016; Pi et al., 2015; Ryu et al., 2014;
91 Song et al., 2005; Wang et al., 2013). The versatile function of HDA19 is consistent with its
92 ubiquitous expression pattern (Tian et al., 2003). It was shown that HDA19 is S-sulfenylated at
93 Cys137 in plants treated with SA or flagellin 22 (flg22) (Liu et al., 2015). It remains unclear
94 how HDA19 undergoes redox modifications and whether the modifications control its
95 deacetylase activity and epigenetic function.

96 Under environmental stress, reactive oxygen and nitrogen species (ROS and RNS) are
97 produced from various sources. Thiol (R-SH)-containing cysteine residues (Cys) are
98 particularly prone to oxidation by ROS and RNS, which are often oxidized reversibly to

99 sulfenic acid (R-SOH). Sulfenic groups can further form covalent bonds with nitric oxide (R-S-
100 NO) called S-nitrosylation (Sevilla et al., 2015; Vaahtera et al., 2014). S-nitrosylation affects
101 protein function including stability, biochemical activity, conformation change, subcellular
102 localization, and protein-protein interaction (Astier et al., 2012b; Astier et al., 2011; Hess et al.,
103 2005; Lamotte et al., 2015), and can mediate the physiological activity of NO that plays an
104 important regulatory role in almost all aspects of development as well as response to stress in
105 higher plants (Baxter et al., 2014; Couturier et al., 2013; Stamler et al., 2001; Wrzaczek et al.,
106 2013). In the cell, S-nitrosylated glutathione (GSNO) is a more stable redox form of NO and is
107 considered as a NO reservoir for protein S-nitrosylation (Feng et al., 2019). GSNO levels are
108 controlled by the activity of S-nitrosogluthathione reductase (GSNOR) that catalyzes the NADH-
109 dependent reduction of GSNO to oxidized GSH (GSSG) (Jahnová et al., 2019; Lindermayr,
110 2018). The loss-of-function mutation of the unique gene in Arabidopsis leads to elevated levels
111 of NO, increased protein S-nitrosylation, and pleiotropic effects on plant development and
112 stress response (Feechan et al., 2005; Lee et al., 2008).

113 In this work, we show that HDA19 undergoes constitutive S-nitrosylation *in vivo*. We
114 identified four S-nitrosylated Cys residues in the HDA19 protein purified from Arabidopsis
115 cells by biotin-switch coupled with mass spectrometry. We found that SA and oxidative stress
116 enhance HDA19 S-nitrosylation, binding to target loci, histone deacetylase activity, and gene
117 repression function, in which the S-nitrosylated Cys137 plays an important role. In turn,
118 HDA19 is required for maintaining cellular redox state and plant tolerance to the stress. Finally,
119 our results indicate that HDA19 is not only a target of redox regulation but also controls cellular
120 redox signaling for plant tolerance to stress.

121 122 RESULTS

123 124 HDA19 is S-nitrosylated and Cys137 is an important S-nitrosylated site

125 To study whether HDA19 undergoes S-nitrosylation, we transformed the *hda19* mutant
126 with HDA19-HA under the control of a 2 kb promoter fragment of the gene but failed to get
127 plants with sufficient expression of *HDA19* and to complement the mutant phenotype.
128 Therefore, we transformed the mutant with the *35S::HDA19-HA* construct and obtained several
129 complementation lines, among which lines C1 and C2 that produced a wild type level of
130 *HDA19* transcripts and fully complemented the mutant phenotypes were selected for further
131 analysis (Fig S1A). We isolated HDA19-HA protein by immuno-purification from 10-day-old
132 C1 and C2 seedlings and processed Cys-S-nitrosylation TMT (Tandem Mass Tag) labeling
133 reactions. The TMT biotin-switch analysis revealed a clear S-nitrosylation signal of HDA19-
134 HA purified from the 2 complementation lines (Fig 1A, Fig S1B). To study whether HDA19 S-

1 135 nitrosylation is influenced by NO levels, transgenic plants expressing HDA19-HA were treated
2 136 with the endogenous NO donor GSNO at 0.5 mM and the NO scavenger cPTIO
3 137 (2-4-carboxyphenyl- 4,4,5,5 -tetramethylimidazoline-1-oxyl-3-oxide,) at 0.25 mM for 45 min.
4 138 Exogenously added GSNO enhanced HDA19-HA S-nitrosylation (Fig 1B, Fig S1C), while
5 139 cPTIO treatment abolished the S-nitrosylation signals (Fig 1C, Fig S1D). To confirm the results,
6 140 we transfected *35S::HDA19-HA* into protoplasts prepared from the NO/GSNO accumulation-
7 141 *gsnor* (At5g43940) mutant plants (Feechan et al., 2005), and detected a clear increase of
8 142 HDA19-HA S-nitrosylation (Fig 1D, Fig S1E), indicating that cellular NO accumulation
9 143 stimulates HDA19 S-nitrosylation.

10 144 To detect the detail information of HDA19 S-nitrosylation, mass spectrometry (MS)
11 145 analysis of the protein samples were performed. HDA19 Cys21, Cys137, Cys281 and Cys292
12 146 residues were found to be S-nitrosylated (Fig S2, Supplemental dataset 1). HDA19 Cys281 and
13 147 Cys292 are conserved in Arabidopsis and human HDAC proteins, while Cys137 is conserved in
14 148 Arabidopsis HDA9 and human HDAC3. Cys21 is conserved only in human HDAC2 (Fig S2)
15 149 and it does not located in histone deacetylase domain. Computational analysis of the putative
16 150 HDA19 protein model revealed Cys137 and Cys21 were close to each other (Fig 1E),
17 151 suggesting there might be mutual effect between these two sites. We also made Cys to Ala
18 152 substitution of the 4 Cys residues and expressed the mutant versions
19 153 (C21A/C137A/C281A/C292A-HA) in *hda19* protoplasts to test the mutation effect on HDA19
20 154 S-nitrosylation. TMT biotin-switch assays revealed that the C137A mutation had a stronger
21 155 effect than the others (Fig 1F, Fig S1F). To analyze the role of S-nitrosylation at Cys137, we
22 156 transformed the *hda19* plants with the *35S::HDA19C137A-HA* construct and selected two lines
23 157 (mC1 and mC2) that showed a C137A-HA protein level similar to that of HDA19-HA in C1
24 158 and C2 plants (Fig S1G) and showed S-nitrosylation (Fig S1H) for further analysis. MS
25 159 analysis of the C137A-HA protein purified from mC1 and mC2 plants detected only two S-
26 160 nitrosylated Cys residues (Cys281 and Cys292) (Fig S2, Supplemental dataset 1), suggesting
27 161 that the C137A mutation also affected S-nitrosylation at Cys21.

28 162 Because SA could induce HDA19 S-sulfenylation at Cys137 (Liu et al., 2015), we also
29 163 explored whether SA induces HDA19 S-nitrosylation. We treated the transgenic plants with SA
30 164 (0.5 mM) for 45 min and found that SA enhanced S-nitrosylation of HDA19-HA and NO
31 165 content (Fig 2A and 2B, Fig S3A). However, SA hardly induced S-nitrosylation of C137A-HA,
32 166 similarly the NO content was much lower in C137A-HA than HDA19-HA plants with or
33 167 without SA, indicating that Cys137 is involved in SA-induced S-nitrosylation of the protein and
34 168 also affected the intracellular NO level (Fig 2A and 2B, Fig S3A). To study whether HDA19 S-
35 169 nitrosylation is affected by oxidative stress, we treated leaves of the 19-day-old transgenic
36 170 plants with 3-amino-1, 2, 4-triazole (3-AT), a catalase inhibitor, at 2 mM for 36 hours and

171 found that the treatment increased about 2.5 folds of the HDA19-HA S-nitrosylation levels and,
172 to a lesser extent, that of C137A-HA (Fig 2C, Fig S3B). 3-AT treatment also increased the NO
173 level (Fig 2D). To further detect the effect of intracellular NO levels on HDA19 S-nitrosylation,
174 different concentrations of NO scavenger cPTIO were added in 3-AT treated plants. The results
175 showed that cPTIO significantly abolished the S-nitrosylation signals of HDA19-HA and
176 C137A-HA caused by 3-AT, and at the same concentration the cPTIO inhibitory effect was
177 stronger in C137A-HA than in HDA19-HA (Fig 2E, Fig S3C), confirming the higher NO
178 contents in 3-AT treated HDA19-HA than C137A-HA. To further confirm the oxidative stress
179 effect on HDA19-HA S-nitrosylation, we transformed the *cat2* (At4g35090) mutant (which
180 increased cellular oxidative state in seedlings (Li et al., 2014) with the *35S::HDA19-HA* vector
181 and found that HDA19-HA S-nitrosylation was augmented about 2.4 folds in the *cat2* plants
182 (Fig 2F, Fig S3D). The NO content was also higher in the *cat2* plants than wild type (Fig 2G).

183 Collectively, the results indicate that HDA19 is post-translationally modified by S-
184 nitrosylation and that SA and oxidative stress that enhance intracellular NO levels promote
185 HDA19 S-nitrosylation. The C137A mutation reduced the levels of HDA19 S-nitrosylation
186 under both normal and stressed conditions and showed reduced intracellular NO levels.

187

188 **Stress-induced HDA19 S-nitrosylation occurs mainly in the nucleus**

189 To study HDA19 subcellular localization, we produced transgenic plants of *35S::HDA19-*
190 *EGFP* and *35S::HDA19C137A-EGFP* in the *hda19* background (Fig S4A, B). Confocal
191 microscopy revealed that HDA19-GFP and C137A-GFP proteins were localized in both
192 nucleus and cytoplasm in the transgenic root cells and in transiently transfected protoplasts (Fig
193 3A, Fig S4B). The 3-AT treatment enriched the nuclear localization of the proteins (Fig 3A).
194 DAF-DA analysis detected NO in the nucleus and the cytoplasm (Fig 3A), the levels of which
195 were enhanced by 3-AT treatment (Fig 3A).

196 To further study subcellular localization of HDA19 S-nitrosylation, we isolated the total,
197 cytoplasmic, and nuclear protein fractions from the *HDA19-HA* and *C137A-HA* plants treated
198 with or without 3-AT for biotin-switch analysis. The results revealed that under normal
199 conditions HDA19-HA S-nitrosylation was detected in both nucleus and cytoplasm at a
200 comparable level (Fig 3B, Fig S4C), consistent with the protein distribution detected by
201 microscopy observation (Fig 3A). After the 3-AT treatment, S-nitrosylated HDA19-HA was
202 detected only in the nucleus. The 3-AT-induced nuclear S-nitrosylation levels of the protein
203 were about 4 times higher than the untreated nuclear levels (Fig 3B), in line with the nuclear
204 enrichment of NO and HDA19-EGFP under stress (Fig 3A). The C137A-HA S-nitrosylation
205 was detected only in the nucleus under both normal and stressed condition, probably due to the
206 overall reduced S-nitrosylation levels of the mutant (Fig 2). The 3-AT treatment increased the

207 C137A-HA S-nitrosylation levels only by about 2 folds (Fig 3B). The data indicate that the
208 stress-induced HDA19 S-nitrosylation was enriched in the nucleus and that besides C137 other
209 Cys residues of the protein were also involved in the stress-induced S-nitrosylation.

210
211 **Cys137 is important for HDA19 function in controlling plant development and stress**
212 **response**

213 The *hda19* mutant plants display a number of developmental defects including meristem
214 function, leaf, floral organ morphology, flowering time, plant height, and trichome numbers
215 (Ueda et al., 2018; Zhou et al., 2005) (Fig S5A and B). We found that the mutant phenotypes
216 were complemented by *HDA19-HA*, but only partially by *C137A-HA* (Fig S5A and B),
217 indicating that Cys137 is required for the full function of HDA19 in plant development. To
218 further evaluate the role of Cys137 in HDA19-regulated plant response to oxidative stress, we
219 treated 19-day-old plants of the different genotypes with 3-AT. Based on the characteristic
220 bleaching phenotype induced by 3-AT, we found that the sensitivity of the *hda19* mutant to 3-
221 AT was the highest, while that of the wild type and *HDA19-HA* plants was the lowest (Fig 4A,
222 Fig S5C). The sensitivity of *C137A-HA* plants was at intermediate levels (Fig 4A, Fig S5C),
223 indicating that HDA19 is required for plant tolerance to the oxidative stress at least partly by
224 maintaining cellular redox homeostasis under stress. DAB staining was performed to detect the
225 oxidative stress level in the plants caused by 3-AT treatment. Under normal condition, there
226 were no clearly differences among the plant lines (Fig 4B). However, with 3-AT treatment the
227 highest levels of staining were found in the *hda19* mutant, followed by *C137A-HA*, and the
228 lowest levels were detected in *HDA19-HA* plants (Fig 4B, Fig S5D). Tests of chlorophyll
229 contents of the different samples confirmed the sensibilities of the different plant lines to 3-AT
230 (Fig S5E).

231 To explore the influence of HDA19 on overall cell redox state, we measured the contents
232 of total and oxidized forms of glutathione and determined the percentages of reduction of this
233 key redox molecule in the different plants. Without 3-AT, there was about 92-93% of reduced
234 glutathione in the different genotypes, although higher levels of total glutathione were observed
235 in *hda19* plants (Fig 4C). After 12h treatment, the total glutathione levels were markedly
236 elevated in all genotypes, but the increase was less important in *hda19* and *C137A-HA* plants
237 compared with wild type and *HDA19-HA* plants. In addition, the percentages of reduced
238 glutathione in *hda19* (26.1%) and *C137A-HA* (22.7-22.8%) plants were about the double of
239 wild type and *HDA19-HA* plants (11.0-12.2%). After 48h treatment with 3-AT, the percentages
240 of reduced glutathione in *hda19* (25.8%) were about triple of *C137A-HA* (7.7-7.8%), *HDA19-
241 HA* and wild type plants (6.2-6.9%) (Fig 4C). It is known that during oxidative stress, the
242 glutathione pool is first oxidized by conversion from reduced to oxidized form, which retro-

243 activates the biosynthetic enzyme, γ -glutamyl-cysteine synthetase, leading to an increase in
244 total glutathione. The relatively less important increase in the total glutathione pool and the
245 higher reduced glutathione levels in *hda19* and *C137A-HA* plants treated by 3-AT suggest that
246 HDA19 is required for oxidative stress signaling to maintain cellular redox homeostasis and
247 that Cys137 is important in the processes.

248 To investigate the role of Cys137 in HDA19-regulated oxidative stress-responsive gene
249 expression, we performed RNA-seq analysis of the *hda19*, *HDA19-HA*, *C137A-HA* and the
250 wild type (Ws ecotype) plants (Fig S6A, B). Consistent with previous analysis (Shen et al.,
251 2019a), the *hda19* mutation resulted in a large number of differentially expressed genes (DEG,
252 3,965 up regulated and 1,776 down regulated) relative to wild type (Fig S7A). By contrast, in
253 the *HDA19-HA* complementation plants there were only 20 DEGs (>2 folds, $p < 0.01$) relative to
254 the wild type (Fig S7A, in accordance with the wild type phenotype of the complementation
255 lines (Fig S5). However, in *C137A-HA* complementation plants, we detected more than 200 up
256 and >200 down regulated (>2 folds, $p < 0.01$) genes relative to Ws or *HDA19-HA* plants (Fig
257 S7A). The *C137A-HA* DEGs is enriched for stress responses (Fig S7B). In addition, there were
258 more DEGs in *hda19* relative to Ws or *HDA19-HA* than relative to *C137A-HA* plants (Fig S7A).
259 Collectively, the analysis indicated the C137A mutation partially affected HDA19-dependent
260 gene expression program. Because of the very little difference between Ws and *HDA19-HA*
261 plants, we only treated *C137A-HA*, *hda19* and *HDA19-HA* plants with 3-AT for comparison. 3-
262 AT treatment resulted in 2,685 up and 2,176 down regulated genes in *hda19* plants, much more
263 than those detected in *HDA19-HA* and *C137A-HA* plants (Fig 4D, Fig S7C, Supplemental
264 dataset 2). In *C137A-HA* plants, the number of up regulated DEG was higher than in *HDA19-*
265 *HA* plants (Fig 4D, Fig S7C). About 25% (676/2685) and 28% (77/2685) of the 3-AT-induced
266 genes in *hda19* overlapped with those in *HDA19-HA* and *C137A-HA* plants (similar for 3-AT-
267 repressed genes) respectively, while more than 58% of the HDA19-HA DEGs overlapped with
268 those in C137A-HA (Fig 4D).

269 To further study the specific role of Cys137 for HDA19 function in gene expression, we
270 performed GSEA (Gene Set Enrichment Analysis) of the DEGs. GSEA is a statistical method
271 for determining whether a given gene set is significantly enriched in a list of marker genes
272 ranked by their correlation with a phenotype of interest (Subramanian et al., 2005). The GSEA
273 revealed that in 3-AT treated *HDA19-HA* plants the oxidative stress response-related GO terms
274 (or gene sets) GO:0006979 (response to oxidative stress) and GO: 0000302 (response to
275 reactive oxygen species) ($|\text{NES}| > 1$) were enriched in the 3-AT repressed DEGs ($\text{NES} < 0$) (Fig
276 S8A left). In 3-AT-treated *C137A-HA* lines, only GO: 0000302 (response to reactive oxygen
277 species) was enriched in the down-regulated ($\text{NES} < 0$) DEGs (Fig S8B left). In 3-AT treated
278 *hda19* plants, neither of the GO terms was detected in the DEGs. However, GO:0010167

279 (response to nitrate, NES>0) was found to be enriched in 3-AT-induced genes in *hda19* mutant.
280 The number of genes contributing to GO:0006979 term was the highest in *HDA19-HA*,
281 followed by *C137-HA*, and the *hda19* mutant (Fig S8 A-C, right panels). Conversely, the
282 number of 3-AT-induced genes belonging to GO:0006979 was the highest in the *hda19* mutant,
283 followed by *C137-HA*, and *HDA19-HA* (Fig S8 A-C, right panels). The above analysis indicates
284 that HDA19 has a function to repress subsets of oxidative stress-related genes under the stress
285 and that the C137A mutation partially impairs this function.

287 **S-nitrosylation stimulates HDA19 deacetylase activity**

288 Cys137 is located closely to the HDA19 catalytic residues (Fig S2). To study whether
289 Cys137A mutation affects HDA19 function in histone deacetylation, we compared histone
290 acetylation levels in wild type, *HDA19-HA*, *C137A-HA*, and *hda19* plants by Western blots
291 using antibodies against H3K9ac and H3K14ac marks. The results indicated that the level of
292 H3K14ac, but not H3K9ac, was clearly augmented in the *hda19* mutant compared with the wild
293 type plants (Fig 5A, Fig S9A), indicating that HDA19 is mainly involved in the removal of
294 H3K14ac. H3K14ac levels in *HDA19-HA* plants (C1, C2) were similar to wild type, while
295 those in the *C137A-HA* plants (mC1, mC2) were at the intermediate levels (Fig 5A and Fig
296 S9A), indicating that the C137A mutation partially affected the HDA19-mediated histone
297 deacetylation in plant cells. Treatment of the plants with 3-AT enhanced while with DTT
298 decreased the overall H3K14ac and H3K9ac levels in the different genotypes (Fig 5B, Fig S9B),
299 corroborating previous results that the redox stress inhibited the overall cellular HDAC
300 activities (Mengel et al., 2017). Similarly, GSNO increased while cPTIO decreased the overall
301 H3K14ac and H3K9ac levels in different genotypes. However, the effects of GSNO were the
302 strongest in the *hda19* mutants (Fig 5C, Fig S9C), implying that the HDA19 deacetylase
303 activity in the wild type background was stimulated by 3-AT. The data suggested that the
304 inhibitory effect of GSNO on the overall HDAC activities was overwhelming relative to its
305 stimulating effect on HDA19. To clarify the issue, we isolated the HDA19-HA and C137A-HA
306 proteins by immuno-precipitation from the respective transgenic plants treated with or without
307 3-AT. The assays revealed a lower histone deacetylase activity of C137A-HA than HDA19-HA
308 (Fig 5D). To test whether S-nitrosylation had a direct effect on HDA19 deacetylase activity, we
309 pre-incubated the proteins with the NO donor GSNO or the NO scavenger cPTIO before the
310 activity tests. The presence of GSNO increased the deacetylase activity of HDA19-HA and
311 C137A-HA isolated from untreated plants, and restored or partially restored the lower activities
312 in DTT treated plants. Conversely, cPTIO decreased the deacetylase activity of HDA19-HA
313 isolated from 3-AT-treated and untreated plants, Fig 5D). cPTIO had no clear effect on the
314 deacetylase activity of C137A-HA in the control plants but decreased the activity of the

1 315 proteins isolated from 3-AT treated plants, suggesting that S-nitrosylation stimulates the
2 316 HDA19 deacetylase activity.

3
4 317 Next, we performed H3K14ac ChIP-seq analysis of *hda19* and the complementation plants
5 318 (Table S1, Fig S10-11). Heat-maps of normalized ChIP-seq reads (RPKM) confirmed the
6 319 slightly higher levels of H3K14ac in the *hda19* mutant than the transgenic plants and higher
7 320 levels in *C137A-HA* than *HDA19-HA* plants (Fig S12-13). More genes were found to be marked
8 321 by H3K14ac in *hda19* (16,065) and *C137A-HA* (14,200) than in *HDA19-HA* (10,081) plants
9 322 (Fig S13B, Supplemental dataset 3). Interestingly, 62.6% (5,752/9,188) genes that were marked
10 323 by H3K14ac in *hda19* but not in *HDA19-HA* plants remained to be marked in *C137A-HA* plants
11 324 (Fig S13B), indicating that *C137A* mutation impaired the HDA19-mediated deacetylation from
12 325 a subset of genes. However, in 3-AT treated plants we observed an overall increase of H3K14ac
13 326 in the three genotypes but still with the highest and the lowest levels respectively observed in
14 327 *hda19* and *HDA19-HA* plants (Fig S13A, B). The observations confirmed the Western blot
15 328 results (Fig 5B) and supported the above observation that while inhibiting the overall HDAC
16 329 activities, the oxidative stress imposed by 3-AT stimulated HDA19-mediated H3K14
17 330 deacetylation. These results also confirmed that the *C137A* mutation decreased the HDA19-
18 331 mediated deacetylation. Pearson analysis between expression fold changes of the 317 genes that
19 332 were induced by 3-AT commonly in *hda19* and *C137A-HA* but not in *HDA19-HA* plants, and
20 333 fold changes of gene promoter H3K14ac levels caused by 3-AT in the respective plants
21 334 revealed a moderate correlation ($R=0.55$) in *C137A-HA*, a strong correlation ($R=0.74$) in *hda19*
22 335 mutant, and no correlation ($R=0.11$) in *HDA19-HA* plants (Fig S14), indicating that the *C137A*
23 336 mutation de-repressed these genes by affecting HDA19 deacetylase activity under the oxidative
24 337 stress. GO enrichment analysis of H3K14ac peaks was also performed. The relative gene
25 338 number (represented by gene ratio) was highest in *hda19* mutant, followed by *C137A-HA*, and
26 339 the lowest in *HDA19-HA* with or without 3-AT treatment (Fig S13C), which was in agreement
27 340 with the above observation (Fig S13 A and B). There were two GO terms related to response to
28 341 oxidative stress GO:0016614 (oxidoreductase activity acting on CH-OH group of donors) and
29 342 GO:0008233 (peptidase activity) enriched in *hda19 mutant*, one GO:0008233 (peptidase
30 343 activity) enriched in *C137A-HA* and no related terms enriched in *HDA19-HA*. Moreover, a
31 344 higher relative gene number was induced by 3-AT in these two terms in the *hda19* and *C137A-*
32 345 *HA* mutants than *HDA19-HA* plants (Fig S13C). Moreover, the numbers of GO terms induced
33 346 by 3-AT was the lowest in *HDA19-HA*, followed by *C137A-HA* and the *hda19* mutant. The GO
34 347 analysis of H3K14ac supported the results of western blot (Fig 5B) and HDAC activity analysis
35 348 (Fig 5D). To further study whether S-nitrosylation affects HDA19 binding to genomic loci, we
36 349 performed anti-HA ChIP-seq analysis of the transgenic plants (Fig S10-11). We detected higher
37 350 binding signals in *HDA19-HA* than *C137A-HA* plants (Fig S11, Fig S15A). The anti-HA ChIP-

351 seq peaks corresponded to 2,213 genes in *HDA19-HA* compared with 1,402 genes in *C137A-*
352 *HA* plants (Fig S15B, Supplemental dataset 4). However, 60% (838/1,402) of the C137A-HA
353 binding genes overlapped with those bound by the wild type version, suggesting that the C137A
354 mutation reduced the HDA19 binding to a subset of the target genes, which is in agreement
355 with the higher H3K14ac observed in *C137A-HA* than *HDA19-HA* plants. We found that 3-AT
356 treatment stimulated the binding of both HDA19-HA and C137A-HA (with 2,961 and 2,450
357 genes bound by HDA19-HA and C137A-HA, respectively) and enabled the HDA19-binding to
358 many new targets (Fig S15A). The GO analysis also revealed that 3-AT significantly increased
359 the enriched genes both in *HDA19-HA* and *C137A-HA*. The 3-AT induced more enriched GO
360 terms in *HDA19-HA* than in *C137A-HA* (Fig S15C). Moreover, the relative gene numbers of the
361 GO terms directly related to deacetylation (GO:0019213 deacetylase activity; GO:0004407,
362 histone deacetylase activity; and GO:0033558, protein deacetylase activity) was the highest in
363 3-AT treated-HDA19-HA, followed by 3-AT treated-C137-HA, the control-HDA19-HA, and
364 finally the control-C137A-HA (Fig S15C). Interestingly, other GO terms, GO:0016810
365 (oxidoreductase activity acting on NAD(P)H) and GO:0050136 (NADH dehydrogenase
366 (quinone) activity) were only enriched in 3-AT treated-HDA19-HA (Fig S15C). The results
367 were consistent with the observations that 3-AT enhanced the localization and S-nitrosylation
368 of HDA19 in the nucleus and supported the above data that the oxidative stress stimulated the
369 activity of HDA19 for genome wide deacetylation.

370 Further analysis of the RNA-seq and ChIP-seq data identified 31 genes that showed
371 repressed expression, reduced H3K14ac and increased HDA19-bindings in 3-AT-treated versus
372 untreated *HDA19-HA* plants (Fig 6A, Supplemental data set 5). These genes were related to
373 stress response (Fig 6B), and represented the downstream targets directly repressed by HDA19
374 under the stress. To validate the RNA-seq and the ChIP-seq data, we selected 3 of the target
375 genes (*AtWRKY8*, *AtOZII* and *AtCIPK4*) and 1 HDA19-independent oxidative stress responsive
376 gene (*AtCAT2*) to test the 3-AT effects on their expression, H3K14ac, and HDA19-binding
377 levels by RT-qPCR and ChIP-PCR in wild type, *HDA19-HA*, *C137A-HA*, and *hda19* plants (Fig
378 6). The transcript levels of *AtWRKY8*, *AtOZII* and *AtCIPK4* were higher in *hda19* than in wild
379 type plants under both normal and stressed conditions. In *HDA19-HA* plants, the transcripts
380 decreased back to wild type levels, while in *C137A-HA* plants the transcript levels decreased
381 only partially restored (Fig 6C, upper part). ChIP-qPCR analysis revealed that the H3K14ac
382 levels correlated with the expression levels of the genes in both growth conditions (Fig 6C,
383 middle part). Anti-HA ChIP-qPCR analysis indicated that these genes were bound by both
384 HDA19-HA and C137A-HA and that the C137A-HA binding appeared lower than HDA19-HA
385 (Fig 6C, lower part). Treatment with 3-AT enhanced the binding of HDA19-HA and C137A-
386 HA to these genes (Fig 6C, lower part). By contrast, although 3-AT treatment induced the

1 387 expression of *AtCAT2* no change was detected for H3K14ac or the HDA19 binding in the 3-AT
2 388 treated plants (Fig 6C). Collectively, the analysis confirmed that HDA19 directly represses a
3
4 389 subset of oxidative stress-induced genes under both normal and stressed conditions through
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6 390 H3K14ac deacetylation and that oxidative stress enhances the HDA19-HA-mediated repression
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8 391 by stimulating its HDAC activity and/or binding to the target loci.
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392

10 393 **DISCUSSION**

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12 394 HDA19 is an essential epigenetic regulator involved in a large panel of developmental
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14 395 processes and stress-responsive pathways in Arabidopsis. In this work we show that HDA19 is
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16 396 post-translationally modified by S-nitrosylation and the modification is stimulated by oxidative
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18 397 stress. Since the cellular levels of NO were augmented under the oxidative stress (Fig 3A), we
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20 398 speculate that the increased NO levels stimulated HDA19 S-nitrosylation, which in turn
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22 399 promoted the nuclear enrichment of the S-nitrosylated HDA19 protein. Likely the stress-
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24 400 induced S-nitrosylation mostly occurred in the nucleus, although it is not excluded that S-
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26 401 nitrosylated HDA19 promoted its import in the nucleus under stress. The observations that
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28 402 stress-stimulated HDA19 S-nitrosylation enhances HDA19 binding to genomic loci, histone
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30 403 deacetylase activity, and repression of stress-responsive genes indicate that HDA19 chromatin
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32 404 regulatory function is modulated by S-nitrosylation. The data showing that Cys137 is a major
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34 405 site for both basal and stress-induced HDA19 S-nitrosylation and that the C137A mutation
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36 406 affects plant development and stress response, suggest that Cys137 is important for HDA19
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38 407 function modulated by S-nitrosylation. This is supported by the observation that among the 4
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40 408 Cys residues, the Cys137A mutation had a more important effect on HDA19 S-nitrosylation
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42 409 and that the C137A mutation substantially decreased the 3-AT-induced HDA19 S-nitrosylation
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44 410 in the nucleus (Fig 3B). In addition, the Cys137A mutation also inhibits S-nitrosylation of
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46 411 Cys21 (Fig 1F), which are close in position in the structure model of HDA19 (Fig 1E). Since
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48 412 C137 can be modified by S-sulfenylation (Liu et al., 2015), it is not excluded that the C137A
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50 413 mutation effect on the HDA19 function may be also caused by reduced S-sulfenylation.
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52 414 However, this seems unlikely, since S-sulfenylation is extremely unstable (its identification
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54 415 actually requires prior alkylation to stabilize), which reacts rapidly to produce other forms such
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56 416 S-nitrosylation. Alternatively, the C137A mutation may alter the HDA19 protein structure,
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58 417 HDAC activity, and target binding.

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60 418 Our results are consistent with previous observation that HDA19 plays a distinct role from
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62 419 that of class II RPD3 type HDACs (HDA5/14/15/18) in stress response (Choi et al., 2012; Ueda
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64 420 et al., 2018). This is supported by the requirement of HDA19 for plant cellular redox
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66 421 homeostasis and tolerance to oxidative stress shown in this work. HDA19 together with HDA6
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68 422 and HDA9 belongs to a different RPD3 subgroup (closely related to human class 1 HDAC)

1 423 from HDA5/14/15/18 (related to human class 2 HDAC), which have distinct catalytic residues
2 424 (Yruela Guerrero et al., 2021).

3
4 425 S-nitrosylated proteins are involved in almost all developmental and stress-responsive
5 426 processes (Albertos et al., 2015; Astier et al., 2012a; Astier et al., 2012b; Berger et al., 2016;
6 427 Cui et al., 2018; Iglesias et al., 2018; Lindermayr et al., 2010; Liu et al., 2017; Serrato et al.,
7 428 2018). However, S-nitrosylation of most plant proteins was identified after NO donor treatment
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9 429 (Astier et al., 2012b; Feng et al., 2019), and was based on recombinant proteins produced in
10 430 prokaryotic cells which displays some limitation (Sahdev et al., 2008; Willems et al., 2021).
11
12 431 The present work identified 4 S-nitrosylated Cys residues in the HDA19 protein produced in
13
14 432 plant cells, a highest number of S-nitrosylated Cys residues ever characterized in plant proteins
15 433 (Astier et al., 2012b; Feng et al., 2019). This would suggest that either plant cell-produced
16 434 proteins allow more exhaustive identification of S-nitrosylated sites or the number of S-
17 435 nitrosylated Cys residues in HDA19 is particularly high. It is suggested that S-nitrosylation of
18 436 target proteins can be subtly regulated by biotic and abiotic stresses (Fares et al., 2011; Lee et
19 437 al., 2008; Lindermayr et al., 2010; Liu et al., 2015; Tanou et al., 2009; Wang et al., 2009). The
20 438 present data showing that HDA19 S-nitrosylation is constitutive at a lower level but increased
21 439 by stress indicate that S-nitrosylation is a robust posttranslational modification of HDA19. S-
22 440 nitrosylation regulates protein activity, stability, localization, and protein-protein interactions
23 441 (Feng et al., 2019; Willems et al., 2021). For instance, S-nitrosylation facilitates NPR1
24 442 oligomerization in the cytoplasm to regulate immune response (Tada et al., 2008), represses
25 443 AHP1 phosphorylation to control cytokinin response (Feng et al., 2013), enhances APX1
26 444 activity to increase resistance to oxidative stress (Yang et al., 2015), stimulates ASK1 binding
27 445 to CUL1-TIR1 to take part in response to auxin (Iglesias et al., 2018), induces autophagic
28 446 degradation of GSNOR1 to adjust hypoxia response (Zhan et al., 2018), and increases activity
29 447 of protein arginine methyltransferase5 (PRMT5) in stress tolerance (Hu et al., 2017). The
30 448 observations that S-nitrosylation enhances the HDA19 genomic binding and deacetylase
31 449 activity are reminiscent of the observation that S-nitrosylation at Cys125 of PRMT5 enhances
32 450 its arginine methyltransferase activity (Hu et al., 2017). However, S-nitrosylation appeared to
33 451 have distinct or opposing effects on mammalian HDAC proteins (Ago et al., 2008), suggesting
34 452 that the effect of S-nitrosylation of HDACs may dependent on the structural context of target
35 453 proteins. Among conserved Cys residues, only the counterparts of HDA19 Cys 281 and 292 are
36 454 acetylated in human HDACs (Ago et al., 2008). S-nitrosylation of the N-terminal Cys21 and
37 455 Cys137 that are not all conserved in plant or human HDACs (Fig 1B), may be specific to
38 456 HDA19 and may affect HDA19 activity differently from other HDACs, especially Cys137 that
39 457 is located in the histone deacetylase activity motif (Fig S2).

Collectively, our results indicate that oxidative stress such as exogenously applied 3-AT enhanced the cellular NO levels that stimulated S-nitrosylation of HDA19. HDA19 S-nitrosylation promoted its nuclear enrichment and enhanced its histone deacetylase activity to repress expression of subsets of genes to improve plant tolerance to the stress (Fig 4, Fig 7). We speculate that HDA19-mediated histone deacetylation may be required to repress genes whose expression is unfavorable for stress tolerance, or to attenuate the acute induction of stress-responsive genes whose excessive or continuous expression might be harmful for the cell. Alternatively, HDA19 may repress the expression of transcription factors or metabolic or redox genes that play negative roles in stress tolerance.

MATERIALS AND METHODS

Plant materials, production of transgenic plants, treatments and phenotype analysis

The Arabidopsis ecotype Wassileskija (*Ws*) was used as wild type in this study. The *hda19* (At4g38130, *hda19* or *athd1*, in *Ws* background), *gsnor* (At5g43940, *gsnor-1*, in *Columbia-0*, *Col.0* background) and *cat* (At4g35090, *cat2-1*, in *Col.0* background) T-DNA mutants were previously reported (Feechan et al., 2005; Li et al., 2014; Shen et al., 2019a). To produce complete complementation plants, the full-length cDNA of AtHDA19 was amplified and cloned into p1301-35S-HA and p1301-35S-EGFP vector (F: AAAGTCGACGATACTGGCGGCAATTCGCTG; R: TTTGCGGCCGCGCTTATGTTTTAGGAGGAAACGC). These vectors were transformed by Agrobacterium-mediated infection of *hda19* mutant plants to obtain over-expression (complementation HDA19-HA lines). Cysteine 137 of HDA19 cDNA was mutated to alanine (C137A) using the QuikChange Site-Directed Mutagenesis Kit (Agilent Technologies; cat#210518) and the 35S::C137A-HA construct was transformed into *hda19* plants to obtain point the C137A-HA lines. After surface-sterilization in 30% bleach, Arabidopsis seeds were kept at 4 °C for 48 h before sowing. The seeds were grown in vitro on half-strength Murashige Skoog with 0.5% sucrose media (pH 5.7, 1.2% agar) in a growth chamber (20 °C) under white light (120 μ mol m⁻² s⁻¹ photons) in 16 h light/day photoperiods for 10 days, and transplanted into soil or sampled for further analysis. For trichome density analysis, trichomes were counted and calculated from the largest leaf of four plants randomly selected from each genotype.

For SA treatment, 10-day old seedlings grown in MS medium were treated with 0.5 mM SA for 3 h. For GSNO and cPTIO treatment, 10-day old seedlings grown in 1/2 Murashige Skoog medium were treated with 0.5 mM GSNO or 0.25 mM cPTIO for 45 min. For redox treatments,

1 493 19-day old plants (a stage that plants became tolerant to the treatment) grown in soil were
2 494 treated by spraying the leaves with 2 mM 3-AT or 10 mM DTT. After 36 h, plants were
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4 495 photographed and harvested for mRNA, proteins or chromatin extractions or measurements of
5 496 glutathione contents and chlorophyll content or DAB staining. The total and oxidized
6 497 glutathione levels were measured according to previously reported methods (Zheng et al., 2020).
7 498 The DAB staining was conducted as previous described (Torres et al., 2002). For chlorophyll
8 499 content determination, about 100mg leaves excised into 2-3cm length, and then were immersed
9 500 in the extract solution (45% ethanol+45% acetone+10% water) at room temperature until the
10 501 leaves were bleached. The absorbance of the extracts was read at 647 and 665nm. The total
11 502 chlorophyll content was then calculated (Inskeep and Bloom, 1985).
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19 504 Determination of endogenous NO content

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21 505 The endogenous NO level was analyzed by using an NO-sensitive dye, DAF-FM DA
22 506 (Chandok et al., 2003). For DAF-FM DA imaging, Arabidopsis samples were collected 8 h
23 507 after dawn, incubated in a solution containing 0.1 mM CaCl₂, 10 mM KCl, 10 mM MES-Tris,
24 508 pH 5.6 for 2 h, stained with 20 mM DAF-FM DA (Abcam, ab145388) for 1 h, and rinsed three
25 509 times with distilled water. The treated leaves were analyzed using confocal (Leica SP8). The
26 510 excitation was provided at 560 nm, and the emission images at 595 nm were obtained with a
27 511 constant acquisition time. To avoid fluorescent color cascades as much as possible, GFP
28 512 fluorescence was excited at 480 nm and detected at 507 nm. The plot files of different channels
29 513 were calculated by the Leica SP8 software. The relative content of NO was determined as
30 514 described previously (Besson-Bard et al., 2009). To follow NO production in leaf discs, 7 mm
31 515 diameter leaf discs excised from Arabidopsis plants grown on soil were infiltrated for 30 min
32 516 with 10 mM DAF-FM DA dissolved in the solution described above. Then, leaf discs were
33 517 incubated for 1 h in the dark, rinsed three times with distilled water, and transferred in the dark
34 518 into a 96-well plate (one disc per well) containing 200 ml of distilled water. NO production was
35 519 measured by using a fluorometer (Molecular Device Company, Spectra Max M2) with 485 nm
36 520 excitation and 510 nm emission filters. Twelve leaf discs were used for each treatment.
37 521 Fluorescence was expressed as relative fluorescence units (RFU). All experiments were
38 522 repeated at least three times.
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52 524 In vivo S-nitrosylation detection using TMT Switch assay

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54 525 All the proteins used for S-nitrosylation detection including total proteins, nuclear proteins
55 526 and cytoplasmic proteins were extracted from 15 g seedling tissues (for Western blot) or 10 ×
56 527 108 protoplasts (for LC-MS and Western blot) of 35S::HDA19-HA and
57 528 35S::C21A/C137A/C281A/C292A-HA plants using the extraction kit (BestBio, cat#BB319905,
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1 529 cat#BB319906) according to the manufacturer ' s instructions. To improve the efficiency of
2 530 protoplast transformation, 5 μ l of carrier DNA (10 μ g/ μ l) was added to 50 μ l of transformation
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4 531 system. About 300 μ g isolated native proteins were immunoprecipitated with 6 μ l commercial
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6 532 Anti-HA Magnetic Beads (ThermoFisher scientific; cat#88838) at 4 °C overnight to purify
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8 533 HDA19-HA or C137A-HA protein followed the non-denaturing purification protocol from
9
10 534 manufacturer instructions with purification buffer (BestBio, cat#BB3204). To maintain native
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12 535 structure of HDA19-HA/C137A-HA for S-nitrosylation analysis, all the above commercial
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14 536 buffers were free of reductive agent. After being concentrated, proteins were used for S-
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16 537 nitrosylation detection by an improved biotin switch technique (ThermoFisher scientific;
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18 538 cat#90105). The brief pipeline of S-nitrosylation detection biotin switch assay was as following:
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20 539 (i) blocking of native -SHs by MMTS (Methyl methanethiosulfonate); (ii) reduction of Cys-
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22 540 SNOs by ascorbate with the help of Cu²⁺; and (iii) labeling of the newly generated Cys-SHs
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24 541 from Cys-SNOs with sufficient iodoTMT.

25 542 For LC-MS analysis, the TMT-labeled S-nitrosylated proteins were enriched by
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27 543 vacuum freeze-drying before Western blot detection using anti-TMT (ThermoFisher scientific;
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29 544 cat#90075) or LC-MS analysis. An aliquot of the (5 μ l) proteins was used for western blot to
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31 545 confirm the S-nitrosylation signal, the rest was purified (from coomassie-stained gels) for LC-
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33 546 MS analysis. After digestion at 37 °C for 4 h using trypsin and desalination using C18 spin tips,
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35 547 Cys-TMT proteins were resuspended in TBS and enriched using anti-TMT antibody resin
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37 548 (ThermoFisher scientific; cat#90076) overnight with end-over-end shaking at 4 °C . After
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39 549 collection of the unbound peptides, the resin was washed 4 times with 4M Urea/TBS, 4 times
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41 550 with 0.05% CHAPS, 4 times with TBS, and 4 times with water. Finally, labeling peptides were
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43 551 eluted 4 times with 50% acetonitrile, 0.4% TFA and then dried under vacuum before LC-
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45 552 MS/MS analysis.

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47 554 Western blot

48 555 For HDA19 subcellular localization analysis, nuclear and cytoplasmic proteins of the same
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50 556 amounts of seedlings as for total protein extraction were extracted using Plant Nuclear and
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52 557 Cytoplasmic Protein Extraction Kit (BestBio; cat#BB-3152-1). Protein extracts were separated
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54 558 by SDS-PAGE or with non-reducing native gels (Criterion™ XT Tris-Acetate Precast Gels;
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56 559 Cat# 3450129 and Native Sample Buffer for Protein Gels; Cat# 1610738) and analyzed by
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58 560 Western blots using Anti-HA (Abcam, ab9110, 1:5000) to detect the HDA19-HA. As controls,
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60 561 Anti-actin (cytoplasmic fraction, Abcam, ab197345, 1:5000) and Anti-H3 (nuclear fraction,
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62 562 Abcam, ab1791, 1:5000) were used.

563 For histone acetylation analysis, histone proteins were extracted from 1.5 g plant tissues as
564 using Plant Histone extraction kit (BestBio, cat#BB31171). The following histone antibodies
565 were used: H3K14ac (Millipore; 2686926, 1:2000), H3K9ac (Millipore; 2686925, 1:2000), H3
566 (Abcam, ab1791, 1:5000). Because the different anti-histone H3 acetylation antibodies detect
567 the same protein/band, we had to use individual gel/membrane for each immunoblot. For the
568 sample loading controls, we first performed anti-H3 Western blots and used the signals to
569 calibrate sample loading for the experiments with the antibodies of acetylated H3.

570 All Western blots were developed using the ECL Super signal system with 50-100 μ g
571 protein sample. Western blot bands were measured and quantified by the ImageJ software. The
572 ratio of the integrated band density of the target protein to that of the reference protein or input
573 was used to normalize band intensities. Each test was performed with three independent
574 experiments and the multiple comparisons using LSD (Fisher's least significant difference)
575 method were conducted to compare significant differences among samples.

577 Tests of HDAC activity Assay

578 The HDAC activity was determined by following the previous protocol (51). The HDAC
579 inhibitor TSA (100 nM) was used as control. The absorbance was read on a microplate reader
580 within 2 to 10 min at 450 nm. Total nuclear proteins (about 5 μ g) extracted from 35S::HDA19-
581 HA, 35S::C137A-HA and *hda19* seedlings were used to test total HDAC activity. HDA19
582 proteins (0.2 μ g) Immuno-purified from 35S::HDA19-HA and 35S::C137A-HA plants grown
583 under normal and stressed conditions were used to test in the HDAC activity. To determine the
584 direct impact of S-nitrosylation on HDAC activity of HDA19, purified proteins from normal
585 and 3-AT-treated samples were incubated with 250 μ M cPTIO for 30 min, and proteins from
586 normal and DTT-treated samples were incubated with 250 μ M GSNO for 30 min before the
587 activity tests.

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589 Protein structural modeling

590 Homology modeling of three-dimensional structure HDA19 based on their sequence
591 similarity to five proteins of known structure downloaded from PDB database
592 (<http://www.rcsb.org/>), Homology modeling was performed with MODELLER
593 (<https://salilab.org/modeller/>). This model with the lowest energy and highest score was
594 selected for further docking analysis. ZDOCK (<http://zdock.umassmed.edu/>) was used for
595 primary docking and the top1 model produced by ZDOCK was optimized by PyROSETTA
596 (<http://www.pyrosetta.org/>) according to the software's protein-protein interaction
597 protocol. Structural figures were prepared using PyMOL (<https://pymol.org/2/>).

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599 RNA-Seq and RT-qPCR experiments

1
2 600 Total RNA was extracted from seedlings using Trizol reagents and for RNA-Seq analysis.
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4 601 For RNA-seq library construction, 2 μ g of total RNA were used for mRNA purification and
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6 602 cDNA synthesis. Then the mRNA was enriched by using oligo (dT) magnetic beads. Mixed
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8 603 with the fragmentation buffer, the mRNA was broken into short fragments (about 200 bp). The
9
10 604 first strand of cDNA was synthesized by using random hexamer primer. Buffer, dNTPs, RNase
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12 605 H and DNA polymerase I were added to synthesize the second strand. The double-strand cDNA
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14 606 was purified with magnetic beads. End reparation and 3' -end single nucleotide A (adenine)
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16 607 addition was performed. Finally, sequencing adaptors were ligated to the fragments. The
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18 608 fragments were enriched by PCR amplification. The library products were sequenced with the
19
20 609 Illumina HiSeq 2000 platform, and the library construction and sequencing were completed at
21
22 610 Novogene (Tianjin, China). Two independent biological replicates were produced.

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24 611 For quantitative real-time PCR (qRT-PCR) assays, the cDNA was synthesized from 1 μ g
25
26 612 of total RNA using the First Strand cDNA Synthesis Kit (Transgene Biotech). Transcript levels
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28 613 were normalized against the average of the reference genes: the tubulin and actin genes
29
30 614 (AT5G62690 and AT3G18780). The Ct values and the real-time PCR efficiencies were
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32 615 obtained using LinRegPCR (12.X) software, and then the normalized relative quantities and
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34 616 standard errors for each sample were obtained by pBaseplus software. The relative fold
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36 617 differences for genes expression in different samples were calculated on the base of the
37
38 618 normalized relative quantities obtained above, with the normalized relative quantity of wild
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40 619 type (control) as 1. There were two independent biological replicates, and each biological
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42 620 replicate had three technical repetitions, and reaction mixtures with double distilled water as
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44 621 templates were used as negative controls to evaluate the specificity of each real-time PCR.

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46 623 ChIP-Seq experiments

47 624 Chromatin fragments were isolated from seedlings of wild type and mutant or transgenic
48 625 plants and immunoprecipitated with the following antibodies: H3K14ac (Millipore, 2686926)
49 626 and HA (Abcam, ab9110) according to the previous method (Cui et al., 2021). IgG (Abcam,
50 627 ab171870) was used as negative control.

51 628 ChIP-Seq products were sequenced by Novogene (Tianjin, China). The DNA was
52 629 combined with End Repair Mix and incubated at 20 °C for 30 min, purified the end-repaired
53 630 DNA with QIAquick PCR Purification Kit (Qiagen), added A-Tailing Mix and incubated at
54 631 37°C for 30 min. We combined the purified Adenylate 3'Ends DNA, Adapter and Ligation Mix
55 632 and incubated the ligation reaction at 20°C for 15 min. Several rounds of PCR amplification
56 633 with PCR Primer Cocktail and PCR Master Mix were performed to enrich the Adapter-ligated

634 DNA fragments. Then the PCR products were selected (about 100-500 bp, including adaptor
635 sequence) by running a 2% agarose gel to recover the fragments. We purified the gel with
636 QIAquick Gel Extraction kit (QIAGEN). The final libraries were sequenced with the Illumina
637 HiSeq 2000 platform. The amounts of immunoprecipitated chromatin fragments relative to
638 input chromatin were also determined by qPCR analysis using primer pairs specific to target
639 genes, and calculated according to the $2^{-\Delta\Delta CT}$ method. Each test was performed with three
640 biological replicates.

641

642 Bioinformatic analysis of high throughput sequence reads

643 For bioinformatics analysis of the RNA-seq and ChIP-seq data, clean reads were obtained
644 by removing reads containing adapter, reads containing poly-N and low-quality reads from raw
645 data and were aligned to the Arabidopsis genome (The Arabidopsis Information Resource,
646 <http://www.arabidopsis.org/index.jsp>) using FastQC software. The software subread,
647 featureCounts, and Deseq were subsequently used for RNA-seq data analysis, and genes with
648 an $FDR \leq 0.01$ and Fold Change ≥ 2 were designated as differentially expressed genes (DEGs).
649 The software Bowtie2, MACS, IGV, ChIPseeker were used for Chip-Seq data analysis. The
650 input group was used as a positive control and IgG as a negative control for ChIP. IP signal/
651 Input signal were calculated to perform gene-body enrichment analysis. GO analysis were
652 performed with clusterProfile and R software. For Pearson correlation analysis, the up-
653 regulated peaks of H3K14ac were found by MACS2 and normalized by Deseq with foldchange
654 >1 and $q < 0.05$. The normalized fold change of up-regulation peaks of H3K14ac and log2 fold
655 change value of DEGs were then used for further analysis.

656

657 LC-MS/MS and data processing

658 For protein complex analysis, immunoprecipitation proteins were freezing-dried at $-70^{\circ} C$
659 for enrichment. After fully trypsinized, the proteins were analyzed on Thermo Fisher LTQ
660 Orbitrap ETD mass spectrometry. Briefly, samples were loaded onto an HPLC chromatography
661 system named Thermo Fisher Easy-nLC 1000 equipped with a C18 column (1.8 mm, $0.15 \times$
662 1,00 mm). Solvent A contained 0.1% formic acid and solvent B contained 100% acetonitrile.
663 The elution gradient was from 4% to 18% in 182 min, 18% to 90% in 13 min solvent B at a
664 flow rate of 300 nL/min. Mass spectrometry analysis were carried out at the AIMS Scientific
665 Co. Ltd. (Shanghai, China) in the positive-ion mode with an automated data-dependent MS/MS
666 analysis with full scans (350-1600 m/z) acquired using FTMS at a mass resolution of 30,000
667 and the ten most intense precursor ions were selected for MS/MS. The MS/MS was acquired
668 using higher-energy collision dissociation at 35% collision energy at a mass resolution of

1 669 15,000. Raw MS files were analyzed by Proteome Discoverer 1.4 with TAIR data (uniprot-
2 670 Arabidopsis thaliana.fasta). The related parameters used for raw MS files analyzed were as
3
4 671 follow: EnFLAGme Name was Trypsin (full); Max.Missed Cleavage Sites were 2; Static
5
6 672 Modification was Carbamidomethyl (C); Dynamic Modification were Oxidation (M),
7
8 673 Deamidated (N, Q), Acetyl(K); Precursor Mass Tolerance was 20 ppm; Fragment Mass
9
10 674 Tolerance was 0.05 D; Validation based on q-Value. Each sample had two biological replicates
11
12 675 to ensure the accuracy.

13 676

14 677 Data Availability

15
16 678 The RNA-seq and the ChIP-seq data are deposited to National Genomics Data Center
17
18 679 databases (PRJCA005948/PRJCA005893). The MS files are uploaded to the iProX databases
19
20 680 (IPX0003322000/PXD027558).

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34 688 declare that they have no conflict of interest

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905 AUTHOR CONTRIBUTIONS

906 YZ, ZL and XC performed most of the experiments, AD, EIB, CB, ZY, XL, LP & GCI
907 participated in experimentations; DXZ, EIB, JPR, GN, & HV designed the experiments and
908 analyzed the data, DXZ & EIB supervised the work, DXZ & ZY wrote the paper.

911 FIGURE LEGENDS

912 Figure 1. Identification of S-nitrosylated Cys residues in HDA19.

913 A. Detection of HDA19 S-nitrosylation. HDA19-HA was immuno-purified with anti-HA from
914 the HDA19-HA complementation plants (C1 and C2 lines) and was analyzed by TMT-labeling
915 biotin-switch Western blots. The absence of ascorbate sodium (Asc) and 10 $\mu\text{mol Cu}^{2+}$ was

916 used as technological negative control (-). Input protein levels were detected by anti-HA. **B, C,**
 917 S-nitrosylation levels of HDA19-HA isolated from the complementation plants (C1 and C2
 918 combined) treated with or without 0.5 mM GSNO (B) or 0.25mM cPTIO (C) detected by TMT-
 919 labeling biotin-switch assays as in A. **D.** S-nitrosylation levels of HDA19-HA isolated from the
 920 *hda19* and *gsnor* mutant protoplasts transfected by the 35S::HDA19-HA vector. **E.**
 921 Computational modeling of the HDA19 protein. The four S-nitrosylated Cys residues (Cys21,
 922 Cys137, Cys281, and Cys292) of HDA19 revealed by mass spectrometry are indicated by red
 923 balls. **F.** Tests of Cys to Ala mutation effect at the 4 Cys residues on HDA19 S-nitrosylation by
 924 TMT-labeling biotin-switch assays. The proteins with point mutations were produced in and
 925 purified from transfected protoplasts of *hda19* plants. The other replicates of the immunoblots
 926 are shown in Fig S1. Western blot bands were quantified by ImageJ. Relative (optical density
 927 units relative to that of the inputs) average values from the three replicates are shown. The
 928 values of the wild type protein (purified from line C2) are set at 1. Bars represent standard
 929 errors from three biological replicates (the value of each biological replicate was shown as
 930 black dots). Significant differences (represented by different letters) among multiple
 931 comparisons were tested by LSD (Fisher's least significant difference).

932

933 **Figure 2. HDA19 S-nitrosylation is enhanced by SA and oxidative stress.**

934 **A.** S-nitrosylation levels of HDA19-HA and C137A-HA proteins isolated from the
 935 complementation plants treated with or without 0.5 mM SA. **B.** NO levels in the HDA19-HA
 936 and C137A-HA complementation plants treated with or without SA. The relative NO content
 937 was expressed with the fluorescence of the wild type (purified from line C2) set as 100%. **C.** S-
 938 nitrosylation levels of HDA19-HA and C137A-HA proteins isolated from the complementation
 939 plants treated with or without 2 mM 3-AT. **D.** NO levels in the *HDA19-HA* and *C137A-HA*
 940 plants treated with or without 3-AT as determined in B. **E.** S-nitrosylation levels of HDA19-HA
 941 and C137A-HA proteins isolated from 2 mM 3-AT-treated plants after incubation with cPTIO
 942 at the different concentrations for 30 min. **F.** HDA19-HA S-nitrosylation levels in the *cat2*
 943 mutant background. **G.** NO level in the *hda19* and *cat2* mutant backgrounds.

944 The S-nitrosylation levels were analyzed by TMT-labeling biotin-switch Western blots and the
 945 Western blot bands were quantified by ImageJ. The relative values (to the inputs) are averages
 946 of three separate experiments. (The other two replicates of the immunoblots are shown in Fig
 947 S3). The NO content was detected using DAF-FM DA, and the relative NO content was
 948 expressed relative to the wild type fluorescence (purified from line C2) set as 100%. Bars are
 949 means +/- SD from three independent experiments and significant differences (represented by
 950 different letters) among multiple comparisons were tested by LSD (Fisher's least significant
 951 difference).

952
1
2 953 **Figure 3. Stress-induced HDA19 S-nitrosylation takes place mainly in the nucleus.**
3
4 954 **A.** The subcellular localization of HDA19-EGFP, C137A-EGFP and NO detected by DAF-DA
5 955 in HDA19-EGFP and C137A-EGFP complementation plants treated with or without 2 mM 3-
6 956 AT. The red arrows indicated the co-enrichment of NO and EGFP signals. The fluorescent plot
7 957 files of different channels were calculated by the Leica SP8 software, the yellow areas represent
8 958 S-nitrosylated HDA19 in cytoplasm (co-enrichment of EGFP and NO) and the gray areas
9 959 represented S-nitrosylated HDA19 in the nucleus (co-enrichment of EGFP, DAPI and NO)
10 960 Bars=10 μm . **B.** HDA19 S-nitrosylation levels in the cytoplasm and nucleus. Biotin-switch
11 961 assays of S-nitrosylation levels of HDA19-HA and C137A-HA proteins immunoprecipitated
12 962 from nuclear and cytoplasmic fractions extracted from plants treated with or without 2mM 3-
13 963 AT. The absence of ascorbate sodium (Asc) and 10 μmol Cu^{2+} are used as technological
14 964 negative control (-). Input protein levels were detected by anti-HA. The experiments were
15 965 repeated independently 3 times, the other two replicates are shown in Fig S4C. The Western
16 966 blot bands were quantified by ImageJ. The relative values (optical density units relative to the
17 967 optical density of the inputs) are averages of three separate experiments. The values of nuclear
18 968 HDA19-HA or C137A-HA in the controls are set as 1. Bars represent standard errors from three
19 969 biological replicates and significant differences (represented by different letters) among
20 970 multiple comparisons were tested by LSD (Fisher's least significant difference).

21 971
22
23 972 **Figure 4. Cys137 is important for HDA19 function in oxidative stress tolerance and redox**
24 973 **homeostasis.**

25 974 **A-B.** Leaf phenotypes and DAB (diaminobezidin) staining of leaves isolated from 19-day-old
26 975 wild type (WT), complementation lines, and the *hda19* mutant plants grown under normal
27 976 conditions (control) or treated with 2 mM 3-AT for 36 h. Values represent quantification of
28 977 DAB staining from 3 experiments measured using the luminosity function in Photoshop in
29 978 arbitrary units with means \pm 2 s.e.. **C.** Contents of total and oxidized glutathione in the plants of
30 979 the above genotypes grown under normal (0 h) and oxidative stress conditions during 12 h and
31 980 48 h. Blue and red blocks indicate respectively reduced and oxidized forms of glutathione.
32 981 Percentages of the reduced form versus the total contents are indicated. Bars show means +/-
33 982 SD from three independent experiments (the value of each experiment was shown as black
34 983 rectangles or triangles). **D.** Venn Diagrams of up-regulated and down-regulated genes in
35 984 HDA19-HA (right), C137A-HA (middle) and *hda19* plants (left) treated by 3-AT.

36 985
37
38 986 **Figure 5. S-nitrosylation enhances HDA19 histone deacetylase activity.**

987 **A.** Histone H3K14 and H3K9 acetylation levels in wild type (WT), HDA19-HA (C1, C2) and
 988 C137-HA (mC1, mC2) complementation, and *hda19* mutant plants grown under normal
 989 conditions. Histone acetylation levels were detected by immunoblots with anti-H3K14ac and
 990 anti-H3K9ac. Anti-H3 was used as control. **B.** Histone H3K14 and H3K9 acetylation levels in
 991 the plants treated with 3-AT (36 h) or DTT (36 h). **C.** Histone H3K14 and H3K9 acetylation
 992 levels in the plants treated with GSNO (45 min) or cPTIO (45 min). The Western blot bands
 993 were quantified by Image J. The relative values (optical density units relative to that of H3
 994 histone) are averaged from three separate experiments (the other replicates are shown in Fig
 995 S8). The values of wild type are set at 1. Bars represent standard errors from three biological
 996 replicates. Significant differences (represented by different letters) among multiple comparisons
 997 were tested by LSD. **D.** Tests of deacetylase activity of HDA19-HA and C137A-HA proteins
 998 (0.2 μ g) purified by anti-HA immunoprecipitation from plants treated with or without 3-AT or
 999 DTT. The activities were also tested after incubation with 250 μ M cPTIO for 30 min of the
 1000 proteins from the 3-AT treated (3AT+cPTIO) or with 250 μ M GSNO for 30 min of proteins
 1001 from DTT treated (DTT+GSNO) and untreated control (C) (C+ cPTIO or C+GSNO) plants.
 1002 HDA19-HA and C137A-HA proteins purified from control plants were incubated with the
 1003 HDAC inhibitor TSA at 100 μ M as negative controls in the tests (C1 or C2+ TSA). The
 1004 deacetylase activity was expressed as absorbance at 450 nm read on a microplate reader. Bars
 1005 represent standard errors of five biological replicates (the value of each biological replicate was
 1006 shown as black dots) and significant differences (represented by different letters) among
 1007 multiple comparisons were tested by LSD.

Figure 6. HDA19 directly represses several oxidative-stress responsive genes under both
 normal and stress conditions through histone H3K14 deacetylation.

A. Venn diagram of genes with down-regulated expression, hypo H3K14ac and HDA19-HA
 binding in HDA19-HA plants induced by 3-AT in HDA19-HA. **B.** GO analysis of the 31
 overlapping genes in A. **C.** RT-qPCR tests of transcript levels, ChIP-PCR of H3K14ac levels
 and direct association of HDA19-HA and HDA19C137A-HA proteins of three of the 31 genes
 (*AtWRKY8*, *AtOZII*, and *AtCIPK4*) and a unrelated control gene (*AtCAT2*) in the different
 genotypes under normal or stressed (3-AT) conditions. IgG was used as control for ChIP-PCR
 assays. Bars represent means \pm SD from three biological replicates and multiple comparisons
 were conducted to compare significant differences among samples by LSD.

Figure 7. Proposed model of HDA19 response to oxidative stress.

Oxidative stress (such as 3-AT treatment) leads to NO accumulation in cells which enhances S-
 nitrosylation of HDA19. Stress induced S-nitrosylation stimulates HDA19 nuclear enrichment

1023 and histone deacetylase activity to remove H3K14ac and to repress the expression of a set of
1024 genes of which an excessive or continuous expression is unfavorable for plant tolerance. Red:
1025 functions of S-nitrosylated HDA19.

1026

1027 **Supplementary Information**

1028 **Figure S1. Production of HDA19-HA and HDA19C137A-HA transgenic plants in hda19** 1029 **mutant background.**

1030 **A.** Characterization of HDA19-HA complementation transgenic plants. 35S promoter was used
1031 to drive HDA19 cDNA translationally linked to 6 x HA. The vectors were transformed into the
1032 hda19 plants. Five positive lines were tested by qRT-PCR for HDA19 expression in comparison
1033 with the WT and hda19 mutant plants. C1 and C2 lines that show a HDA19 expression similar
1034 to the WT were selected. **B-E.** The other 2 replicates of Fig 1A-D. **F.** The other replicates of Fig
1035 1F. Vectors for production of HDA19-HA C21A, C137A, C281A and C292A mutant proteins
1036 are shown. **G.** Protein levels in two independent complementation lines of HDA19-HA and
1037 HDA19C137A-HA transgenes (in hda19 mutant background) were detected by western blot
1038 using anti-HA. **H.** Detection of S-nitrosylation of HDA19C137A-HA protein immuno-purified
1039 with anti-HA from the complementation plants (mC1 and mC2 lines) by biotin-switch assays.

1040

1041 **Figure S2. Sequence alignment of Arabidopsis HDA19, HDA6, HDA9 and HDA15** 1042 **together with human HDAC2 and HDAC3.**

1043 The four S-nitrosylated Cys residues (Cys21, Cys137, Cys281, and Cys292) of HDA19
1044 revealed by mass spectrometry are indicated by red arrows. The HDA19 putative catalytic
1045 residues His148, His149 and Try311 are indicated by blue arrows and the putative Zn²⁺-
1046 binding residues are indicated by purple arrows. Conserved Cys residues and their flanking
1047 sequences are highlighted by yellow. The red box represents the histone deacetylase domain
1048 superfamily (IPR003084).

1049

1050 **Figure S3. Time course of SA and 3-AT treatment to determine the optimal timing for** 1051 **sampling and the other 2 replicates of Fig 2.**

1052

1053 **Figure S4. Characterization of HDA19-EGFP and C137A-EGFP plants and other 2** 1054 **replicates of Fig 3.**

1055 **A.** Characterization of HDA19-EGFP and C137A-EGFP complementation transgenic plants.
1056 The 35S promoter was used to drive HDA19 cDNA translationally linked to EGFP. The
1057 vectors were transformed into the hda19 plants. Four positive lines were tested by western blot
1058 for HDA19-EGFP expression in comparison with the WT and hda19 mutant plants. 19E1 and

1059 C137AE2 were selected in this study. **B.** The subcellular localization of HDA19-EGFP and
 1060 C137A-EGFP in Arabidopsis protoplasts. **C.** The other 2 replicates of Fig 3B.

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1062 **Figure S5. Phenotype of HDA19-HA and C137A-HA transgenic plants in the *hda19***
 1063 **mutant background.**

1064 **A.** Rosette phenotypes of wild type (WT), *35S::HDA19-HA* (C1, C2), *35S::C137A-HA* (mC1,
 1065 mC2), and the *hda19* mutant plants. The trichome densities were counted and calculated for one
 1066 leaf of four 19 day-old plants randomly selected from each genotype (n=4). Bars represent
 1067 means +/- SD from the five (plants) replicates (the value of each replicate was shown as black
 1068 dots) and multiple comparisons were conducted to compare significant differences among
 1069 samples. **B.** Flower, silique and mature plant phenotypes of the complementation lines (C1,
 1070 mC1) compared with wild type and *hda19* mutant plants. **C-D.** The other two repeated
 1071 experiments of Fig 4 A and B, respectively. **E.** Chlorophyll contents of different samples. Bars
 1072 represent means +/- SD from the five (plants) replicates (the value of each replicate was shown
 1073 as black dots) and multiple comparisons were conducted to compare significant differences
 1074 among samples.

1075

1076 **Figure S6. RNA-seq analysis of wild type, *hda19* mutant and the complementation plants.**

1077 **A.** RNA-seq reads of HDA19-HA, C137A-HA transgenic and *hda19* mutant seedling grown at
 1078 20°C for 19 days, then treated 3-AT or not treated for 36 hours before RNA extraction. **B.** Pair-
 1079 wise scatter plots of RNA-seq reads between three biological repeats. Each point represents one
 1080 gene locus. RPKM, Reads Per Kilobase per Million reads. Correlation coefficient R² is
 1081 indicated.

1082

1083 **Figure S7. Differential gene expression in *HDA19-HA*, *C137A-HA* and *hda19* plants.**

1084 **A.** Volcano map of differentially expressed genes in between wild type, *hda19*, *HDA19-HA* and
 1085 *C137A-HA* plants as indicated. **B.** GESA of DEGs of C137A-HA vs Ws. The top ten increased
 1086 and decreased significant gene sets were listed according to their NES (normalized enrichment
 1087 score). The color of each bar represents the p value of the gene set. The significance of each
 1088 gene set was classified by a threshold of p<0.05. **C.** Volcano map of differentially expressed
 1089 genes (DEGs, red dots) between control and 3-AT treatment of *HDA19-HA* (right), *C137A-*
 1090 *HA* (middle) and *hda19* plants (left). **D.** Venn Diagrams of up-regulated and down-regulated
 1091 DEGs in C.

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1093 **Figure S8. Differential gene expression in *HDA19-HA*, *C137A-HA* and *hda19* plants.**

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1094 Left panels: GESA analysis of DEGs of 3-AT treatment versus control in *HDA19-HA* (A),
 1095 *C137A-HA* (B) and *hda19* (C) plants. The top ten increased and decreased significant gene sets
 1096 were listed according to their NES (normalized enrichment score). The color of each bar
 1097 represents the p value of the gene set. The significance of each gene set was classified by a
 1098 threshold of $p < 0.05$. Right panels: The GESA analysis of the 3-AT caused DEGs belonging to
 1099 the GO term of oxidative stress response (GO:0006979) in HDA19-HA (A), C137A-HA (B)
 1100 and *hda19* (C) plants. The positive/negative values of Y-axis (Ranked list metric) represent
 1101 up/down regulated DEGs induced by 3-AT, and the X-axis (Position in ranked list of genes)
 1102 represented the list of DEGs belonging to GO:0006979. The red dot vertical lines indicate the
 1103 enrichment score (ES) values of DEGs in GO:0006979.

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 1105 **Figure S9. The other 2 replicates of data shown in Fig 6 A-C.**

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 1107 **Figure S10. Correlation analysis of anti-H3K14ac and anti-HA ChIP-seq data.**

1108 **A-C.** Analysis of anti-H3K14ac and anti-HA ChIP-seq reads of HDA19-HA, C137A-HA plants
 1109 and *hda19* mutant plants. Seedling were grown at 20°C for 19 days, then treated with or without
 1110 3-AT for 36 hours. IgG was used use control for the ChIP experiments. Pairwise scatter plots of
 1111 ChIP-seq reads between two biological repeats are shown. Each point represents one gene
 1112 locus. RPKM, Reads Per Kilobase per Million reads. Correlation coefficient R2 indicated.

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 1114 **Figure S11. IDR analysis of anti-H3K14ac and anti-HA ChIP-seq reads.**

1115 Pairwise scatter plots of ChIP-seq IDR log10 score between two biological repeats are shown.
 1116 Red meant ≥ 0.05 IDR. The ratio of peaks passing IDR cutoff of 0.05 indicated. A, HDA19-
 1117 HA; B, C137A-HA; C, *hda19*.

1118 **Figure S12. ChIP-seq analysis of H3K14ac and HDA19-HA and C137A-HA binding**
 1119 **profiles in *hda19* and/or HDA19-HA and C137A-HA plants.** Left: metaplots of anti-
 1120 H3K14ac and anti-HA Chip-seq reads near the transcriptional start sites (TSS). The Y-axis is
 1121 \log_2 (IP /Input signal). Right: IGV of the anti-H3K14ac peaks at AT3G46487 and AT5G07700
 1122 loci (upper part) and the anti- HA peaks at AT5G08130 and AT5G08139 loci (scales are
 1123 indicated).

1124 **Figure S13. S-nitrosylation induced by oxidative stress enhances total H3K14ac level.**

1125 A. Heatmaps of the H3K14ac ChIP-seq reads (normalized RPKM) in the *hda19* mutant and the
 1126 complementation (by HDA19-HA or C137-HA) plants treated with or without 3-AT. TSS:
 1127 transcription start site, TES: transcription end site. B. Venn diagram of gene promoter H3K14ac

1 1128 peaks in plants of in the *hda19* mutant and the complementation plants treated with or without
 2 1129 3-AT. **C.** GO enrichment analysis of H3K14ac ChIP peaks among different samples under
 3
 4 1130 control (C) or 3-AT treatment (T) using peaks annotation analysis according to ChIP-Seq data.
 5 1131 The top 12 enrichment gene sets were listed by p values. The area of dots represents the gene
 6 1132 ratio, and the color of dots represents the p value of the gene set. The significance of each gene
 7 1133 set was classified by a threshold of $p < 0.05$ for H3K14ac. The red letters represented the crucial
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 9 1134 GO terms of response to oxidative stress.

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 14 1136 **Figure S14.** Pearson correlation analysis of expression and H3K14ac levels of the 317 up-
 15 1137 regulated genes only observed in C137A-HA and *hda19* mutant. The (Y axis) stands for log₂
 16 1138 fold change of gene expression levels and the X axis stands for normalized up-regulated fold
 17 1139 change of H3K14ac peaks in their promoter region caused by 3-AT. The dots whose value < 1
 18
 19 1140 was not drawn in the plot.

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 24 1142 **Figure S15. S-nitrosylation induced by oxidative stress enhances HDA19 binding activity.**

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 26 1143 **A.** Heatmaps of the genome-wide enrichment of anti-HA ChIP-seq reads (normalized RPKM)
 27 1144 in the *HDA19-HA* and *C137A-HA* plants treated with or without 3-AT. **B.** Overlapping between
 28 1145 HDA19-HA- and C137A-HA binding genes under the control and 3-AT treatment are shown
 29 1146 by the Venn diagrams on the left. Overlapping of HDA19-HA- or C137A-HA-binding genes
 30 1147 between untreated and 3-AT-treated conditions are shown by the Venn diagrams on the right. **C.**
 31 1148 GO enrichment analysis of HA ChIP peaks among different samples under control (C) or 3-AT
 32 1149 treatment (T) using peaks annotation analysis according to ChIP-Seq data. The top 12
 33 1150 enrichment gene sets were listed by p values. The area of dots represents the gene ratio, and the
 34 1151 color of dots represents the p value of the gene set. The significance of each gene set was
 35 1152 classified by a threshold of $p < 0.05$ for HA. The red letters represented the crucial GO terms
 36 1153 related to deacetylation.

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40 1155 **Table S1.** ChIP-seq reads and correlation coefficients between the replicates.

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44 1157 Supplemental Dataset 1. MS graphs of SNO peptides of HDA19-HA and C137A-HA

45 1158 Supplemental Dataset 2. Lists of differentially expression genes in HDA19-HA compared with
 46 1159 wild type under normal conditions and HDA19-HA, C137A-HA and *hda19* plants treated with
 47 1160 and without 3-AT.

48 1161 Supplemental Dataset 3. Anti-H3K14ac ChIP-seq annotation gene list

49 1162 Supplemental Dataset 4. Anti-HA ChIP-seq annotation gene list

50 1163 Supplemental Dataset 5. The information of 31 overlapping genes in Fig 6A

1 1164 Supplemental Dataset 6. The full western blot images in this study.

2 1165 Supplemental Dataset 7. The primers used in this study.

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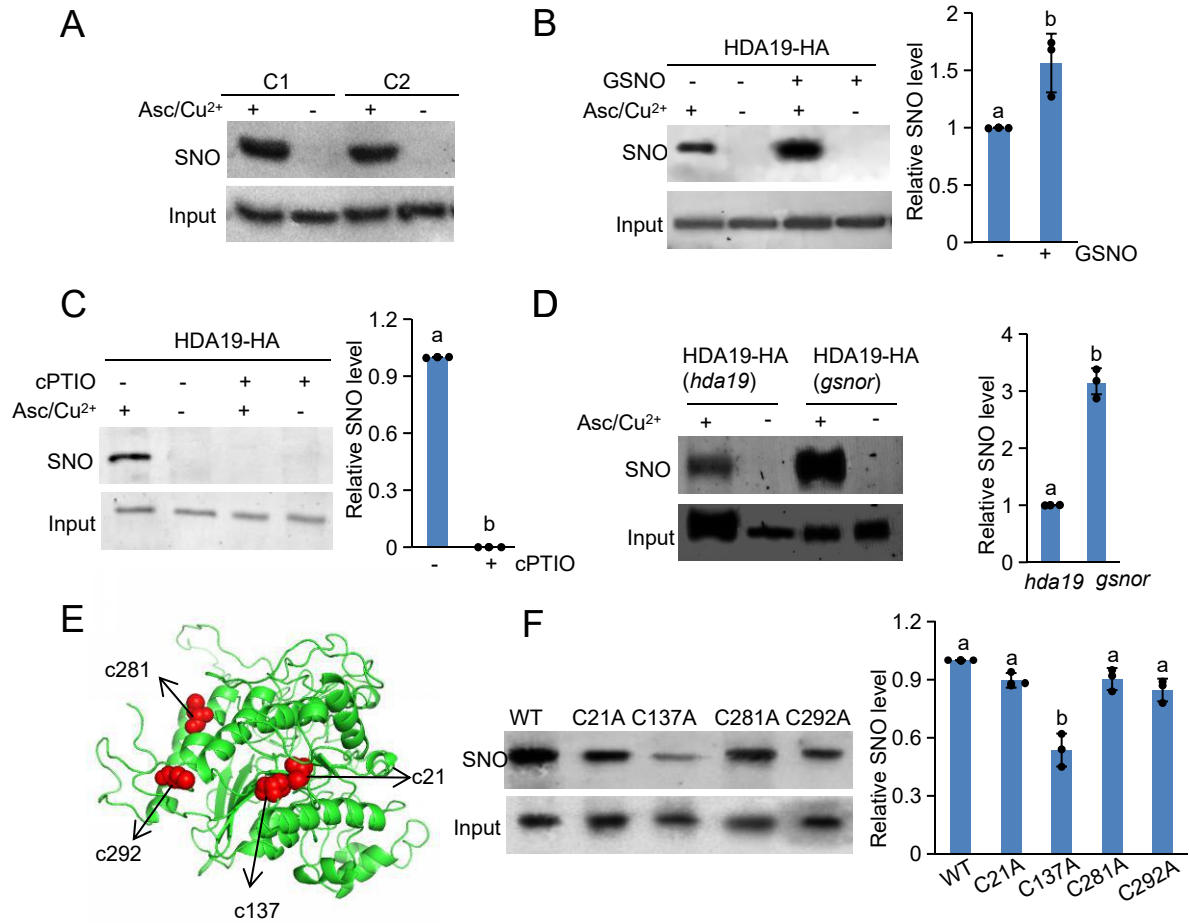


Figure 1. Identification of S-nitrosylated Cys residues in HDA19.

A. Detection of HDA19 S-nitrosylation. HDA19-HA was immuno-purified with anti-HA from the HDA19-HA complementation plants (C1 and C2 lines) and was analyzed by TMT-labeling biotin-switch Western blots. The absence of ascorbate sodium (Asc) and 10 μ M Cu²⁺ was used as technological negative control (-). Input protein levels were detected by anti-HA. **B, C.** S-nitrosylation levels of HDA19-HA isolated from the complementation plants (C1 and C2 combined) treated with or without 0.5 mM GSNO (B) or 0.25mM cPTIO (C) detected by TMT-labeling biotin-switch assays as in A. **D.** S-nitrosylation levels of HDA19-HA isolated from the *hda19* and *gsnor* mutant protoplasts transfected by the 35S::HDA19-HA vector. **E.** Computational modeling of the HDA19 protein. The four S-nitrosylated Cys residues (Cys21, Cys137, Cys281, and Cys292) of HDA19 revealed by mass spectrometry are indicated by red balls. **F.** Tests of Cys to Ala mutation effect at the 4 Cys residues on HDA19 S-nitrosylation by TMT-labeling biotin-switch assays. The proteins with point mutations were produced in and purified from transfected protoplasts of *hda19* plants. The other replicates of the immunoblots are shown in Fig S1. Western blot bands were quantified by ImageJ. Relative (optical density units relative to that of the inputs) average values from the three replicates are shown. The values of the wild type protein (purified from line C2) are set at 1. Bars represent standard errors from three biological replicates (the value of each biological replicate was shown as black dots). Significant differences (represented by different letters) among multiple comparisons were tested by LSD (Fisher's least significant difference).

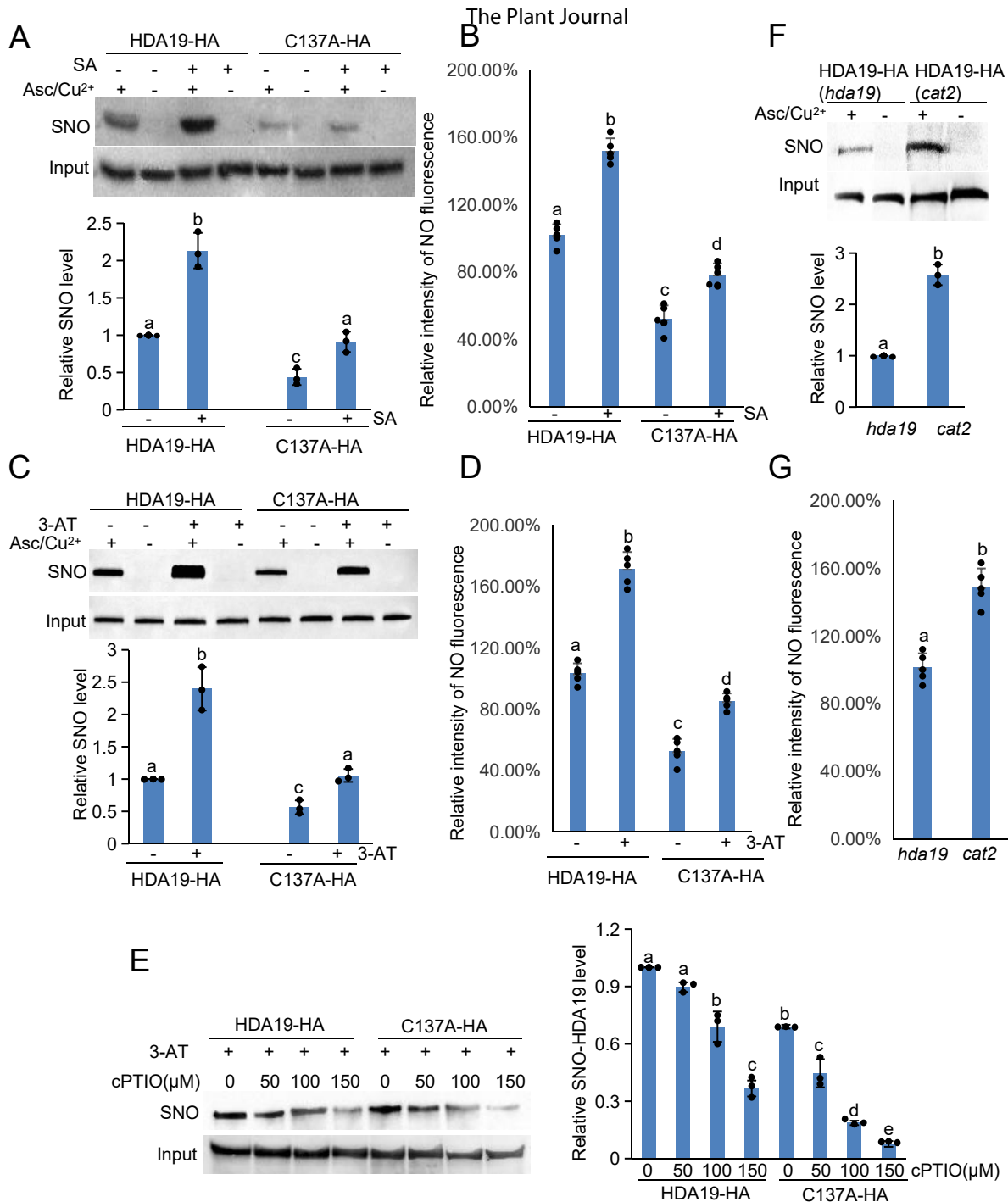
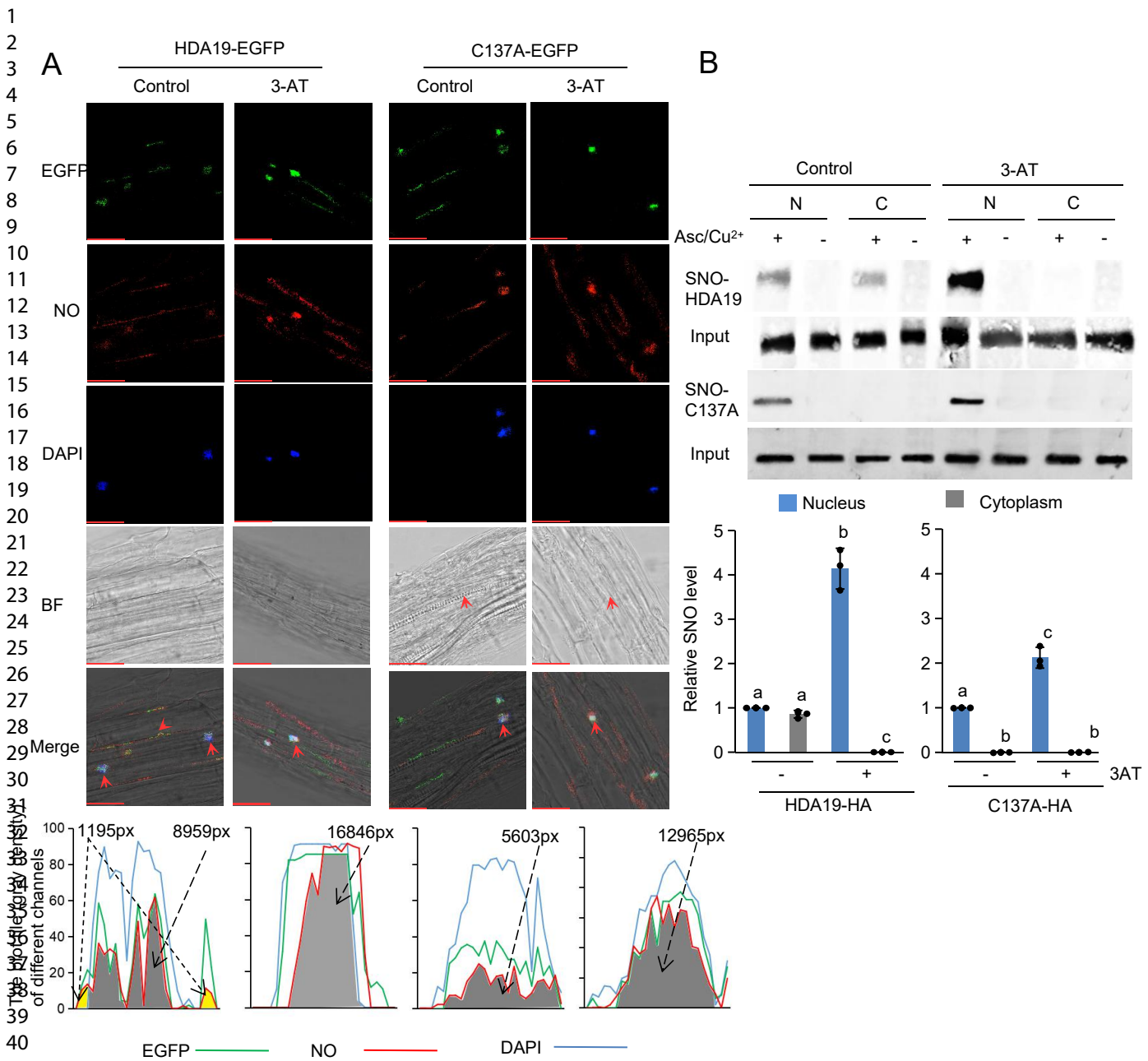


Figure 2. HDA19 S-nitrosylation is enhanced by SA and oxidative stress.

A. S-nitrosylation levels of HDA19-HA and C137A-HA proteins isolated from the complementation plants treated with or without 0.5 mM SA. **B.** NO levels in the *HDA19-HA* and *C137A-HA* complementation plants treated with or without SA. The relative NO content was expressed with the fluorescence of the wild type (purified from line C2) set as 100%. **C.** S-nitrosylation levels of HDA19-HA and C137A-HA proteins isolated from the complementation plants treated with or without 2 mM 3-AT. **D.** NO levels in the *HDA19-HA* and *C137A-HA* plants treated with or without 3-AT as determined in B. **E.** S-nitrosylation levels of HDA19-HA and HDA19C137A-HA proteins isolated from 2 mM 3-AT-treated plants after incubation with cPTIO at the different concentrations for 30 min. **F.** HDA19-HA S-nitrosylation levels in the *cat2* mutant background. **G.** NO level in the *hda19* and *cat2* mutant backgrounds. The S-nitrosylation levels were analyzed by TMT-labeling biotin-switch Western blots and the Western blot bands were quantified by ImageJ. The relative values (to the inputs) are averages of three separate experiments. (The other two replicates of the immunoblots are shown in Fig S3). The NO content was detected using DAF-FM DA, and the relative NO content was expressed relative to the wild type fluorescence (purified from line C2) set as 100%. Bars are means \pm SD from three independent experiments and significant differences (represented by different letters) among multiple comparisons were tested by LSD (Fisher's least significant difference).

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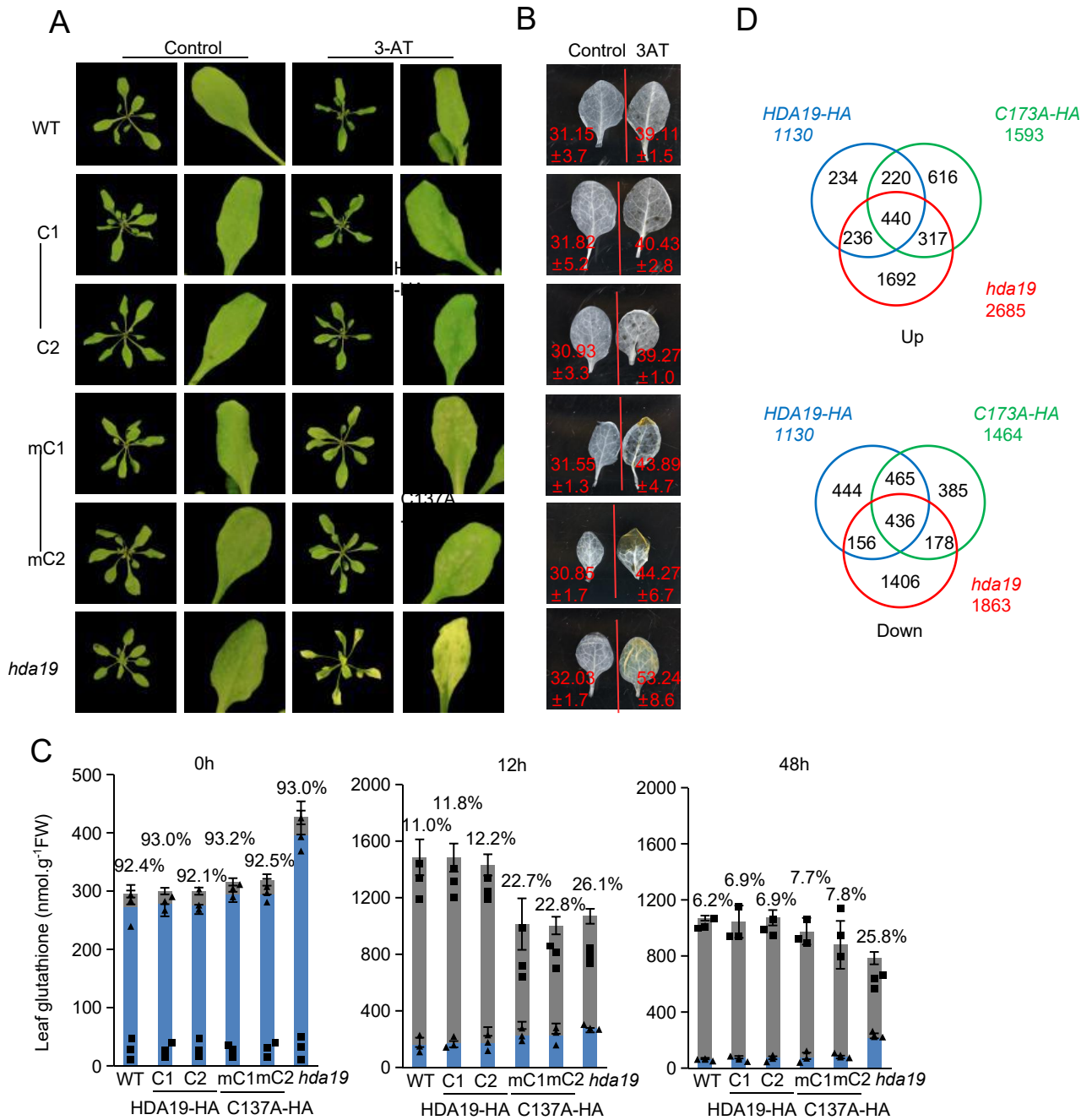


Figure 4. Cys137 is important for HDA19 function in oxidative stress tolerance and redox homeostasis.

A-B. Leaf phenotypes and DAB (diaminobezidin) staining of leaves isolated from 19-day-old wild type (WT), complementation lines, and the *hda19* mutant plants grown under normal conditions (control) or treated with 2 mM 3-AT for 36 h. Values represent quantification of DAB staining from 3 experiments measured using the luminosity function in Photoshop in arbitrary units with means \pm 2 s.e. **C.** Contents of total and oxidized glutathione in the plants of the above genotypes grown under normal (0 h) and oxidative stress conditions during 12 h and 48 h. Blue and red blocks indicate respectively reduced and oxidized forms of glutathione. Percentages of the reduced form versus the total contents are indicated. Bars show means \pm SD from three independent experiments (the value of each experiment was shown as black rectangles or triangles). **D.** Venn Diagrams of up-regulated and down-regulated genes in HDA19-HA (right), C137A-HA (middle) and *hda19* plants (left) treated by 3-AT.

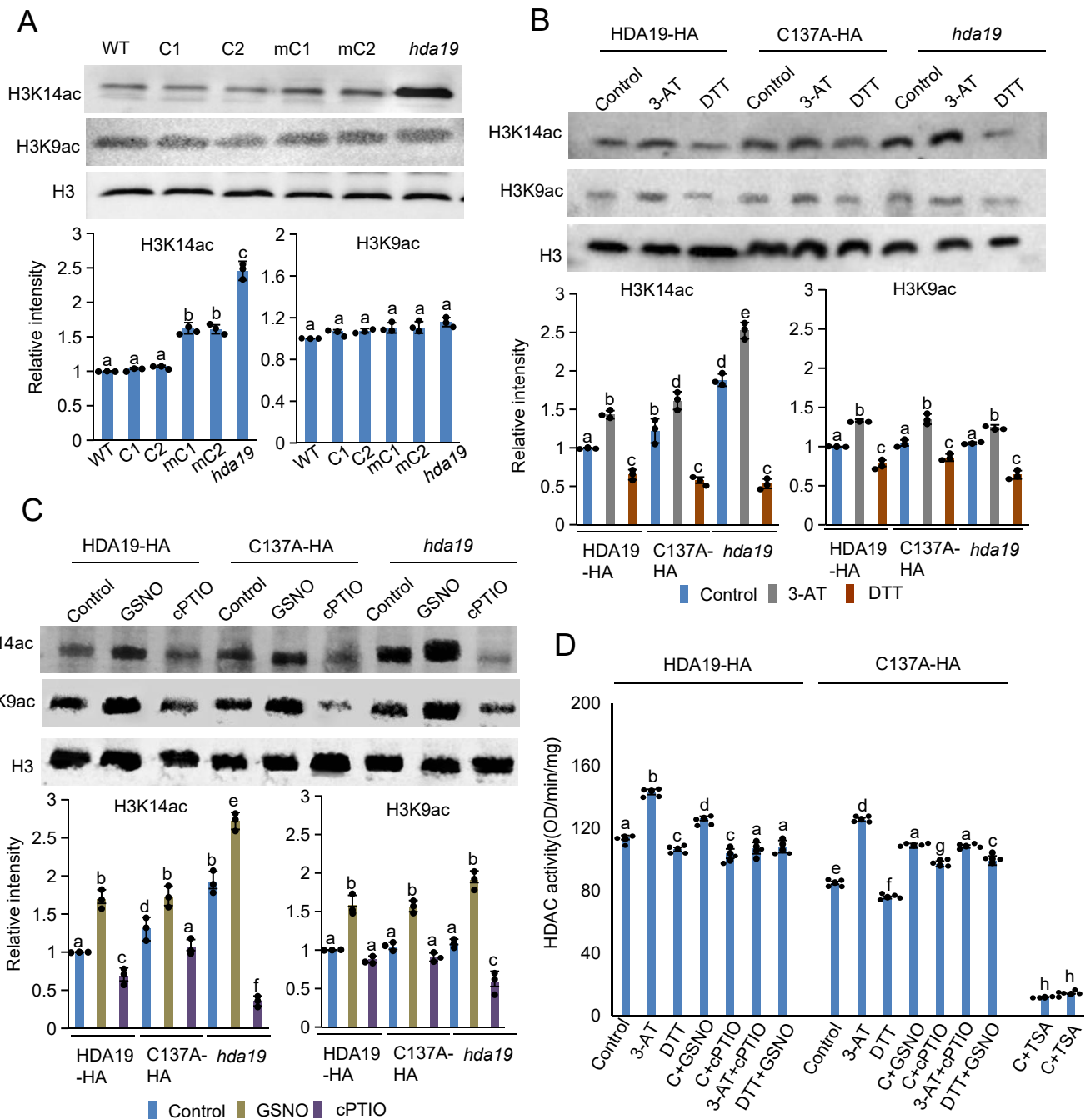


Figure 5. S-nitrosylation enhances HDA19 histone deacetylase activity.

A. Histone H3K14 and H3K9 acetylation levels in wild type (WT), HDA19-HA (C1, C2) and C137A-HA (mC1, mC2) complementation, and *hda19* mutant plants grown under normal conditions. Histone acetylation levels were detected by immunoblots with anti-H3K14ac and anti-H3K9ac. Anti-H3 was used as control. **B.** Histone H3K14 and H3K9 acetylation levels in the plants treated with 3-AT (36 h) or DTT (36 h). **C.** Histone H3K14 and H3K9 acetylation levels in the plants treated with GSNO (45 min) or cPTIO (45 min). The Western blot bands were quantified by Image J. The relative values (optical density units relative to that of H3 histone) are averaged from three separate experiments (the other replicates are shown in Fig S8). The values of wild type are set at 1. Bars represent standard errors from three biological replicates. Significant differences (represented by different letters) among multiple comparisons were tested by LSD. **D.** Tests of deacetylase activity of HDA19-HA and C137A-HA proteins (0.2 μ g) purified by anti-HA immunoprecipitation from plants treated with or without 3-AT or DTT. The activities were also tested after incubation with 250 μ M cPTIO for 30 min of the proteins from the 3-AT treated (3AT+cPTIO) or with 250 μ M GSNO for 30 min of proteins from DTT treated (DTT+GSNO) and untreated control (C) (C+ cPTIO or C+GSNO) plants. HDA19-HA and C137A-HA proteins purified from control plants were incubated with the HDAC inhibitor TSA at 100 μ M as negative controls in the tests (C1 or C2+ TSA). The deacetylase activity was expressed as absorbance at 450 nm read on a microplate reader. Bars represent standard errors of five biological replicates (the value of each biological replicate was shown as black dots) and significant differences (represented by different letters) among multiple comparisons were tested by LSD.

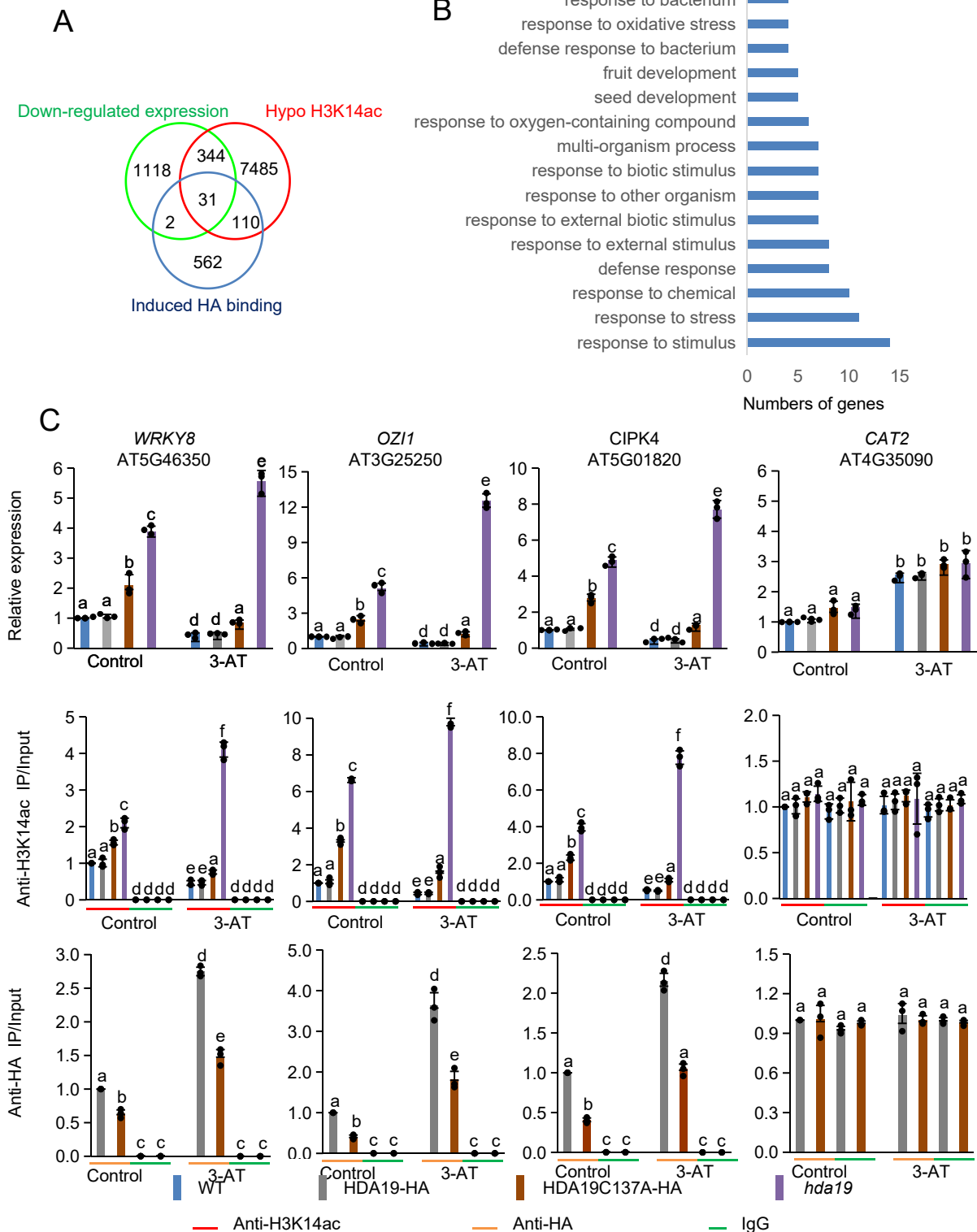


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A. Venn diagram of genes with down-regulated expression, hypo H3K14ac and HDA19-HA binding in HDA19-HA plants induced by 3-AT in HDA19-HA. **B.** GO analysis of the 31 overlapping genes in A. **C.** RT-qPCR tests of transcript levels, ChIP-PCR of H3K14ac levels and direct association of HDA19-HA and HDA19C137A-HA proteins of three of the 31 genes (*AtWRKY8*, *AtOZI1*, and *AtCIPK4*) and a unrelated control gene (*AtCAT2*) in the different genotypes under normal or stressed (3-AT) conditions. IgG was used as control for ChIP-PCR assays. Bars represent means \pm SD from three biological replicates and multiple comparisons were conducted to compare significant differences among samples by LSD.

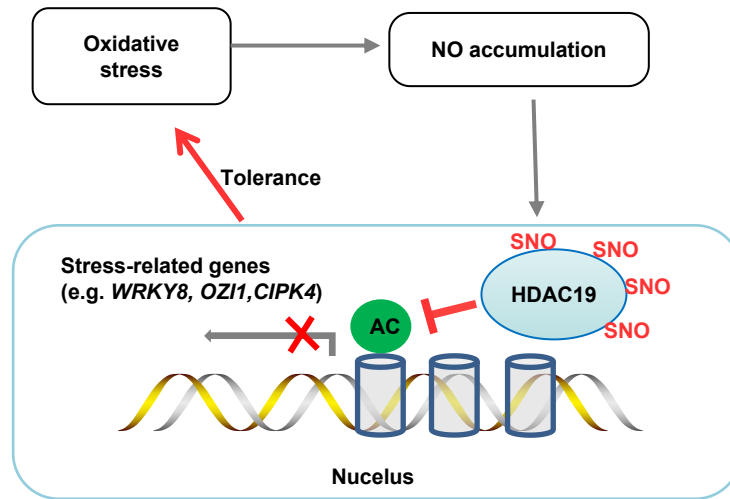


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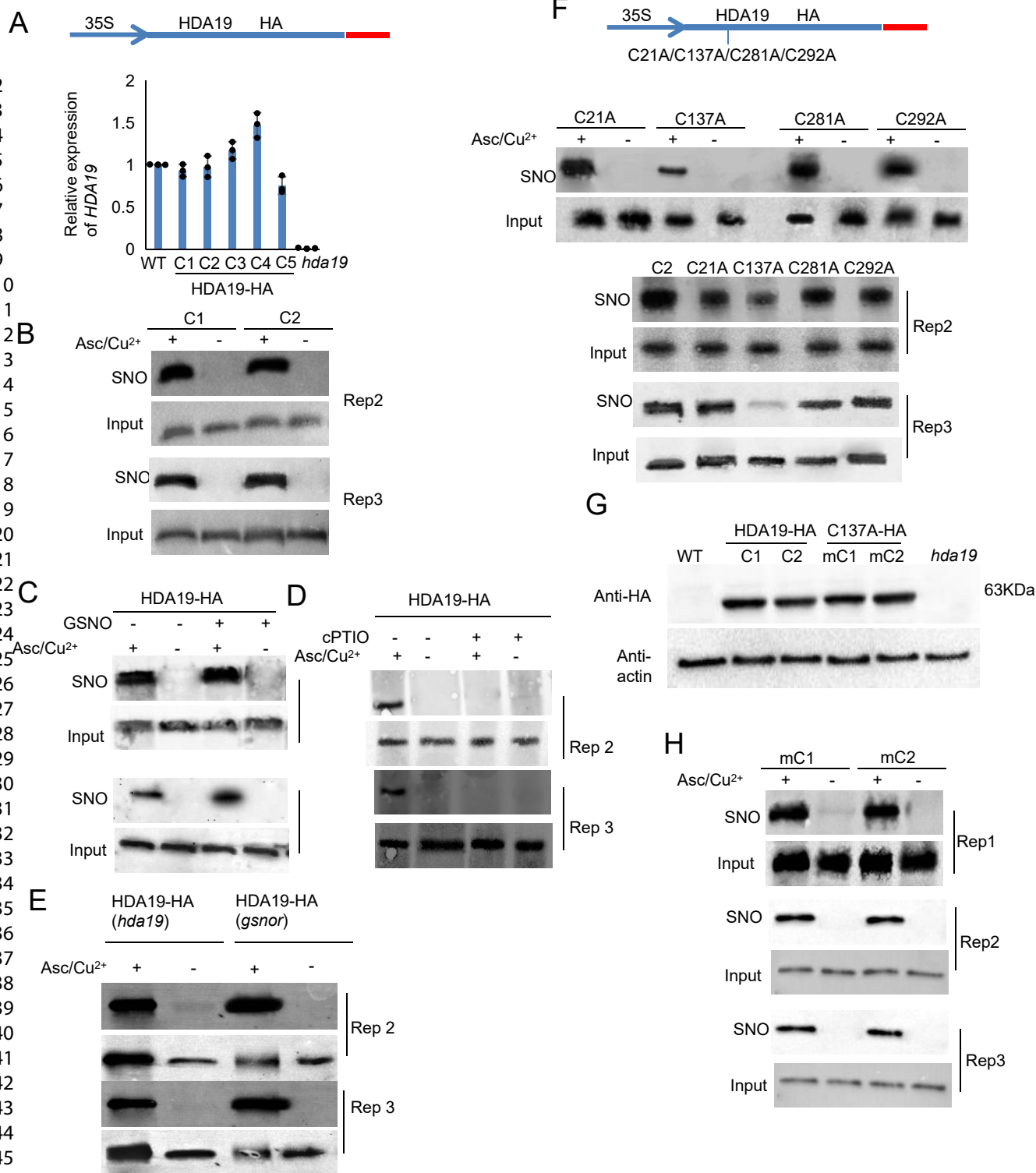


Figure S1. Production of HDA19-HA and HDA19C137A-HA transgenic plants in the *hda19* mutant background.

A. Characterization of HDA19-HA complementation transgenic plants. 35S promoter was used to drive HDA19 cDNA translationally linked to 6 x HA. The vectors were transformed into the *hda19* plants. Five positive lines were tested by qRT-PCR for HDA19 expression in comparison with the WT and *hda19* mutant plants. C1 and C2 lines that show a HDA19 expression similar to the WT were selected. **B-E.** The other 2 replicates of Fig 1A-D. **F.** The other replicates of Fig 1F. Vectors for production of HDA19-HA C21A, C137A, C281A and C292A mutant proteins are shown. **G.** Protein levels in two independent complementation lines of *HDA19-HA* and *HDA19C137A-HA* transgenes (in the *hda19* mutant background) were detected by western blot using anti-HA. **H.** Detection of S-nitrosylation of HDA19C137A-HA protein immuno-purified with anti-HA from the complementation plants (mC1 and mC2 lines) by biotin-switch assays.

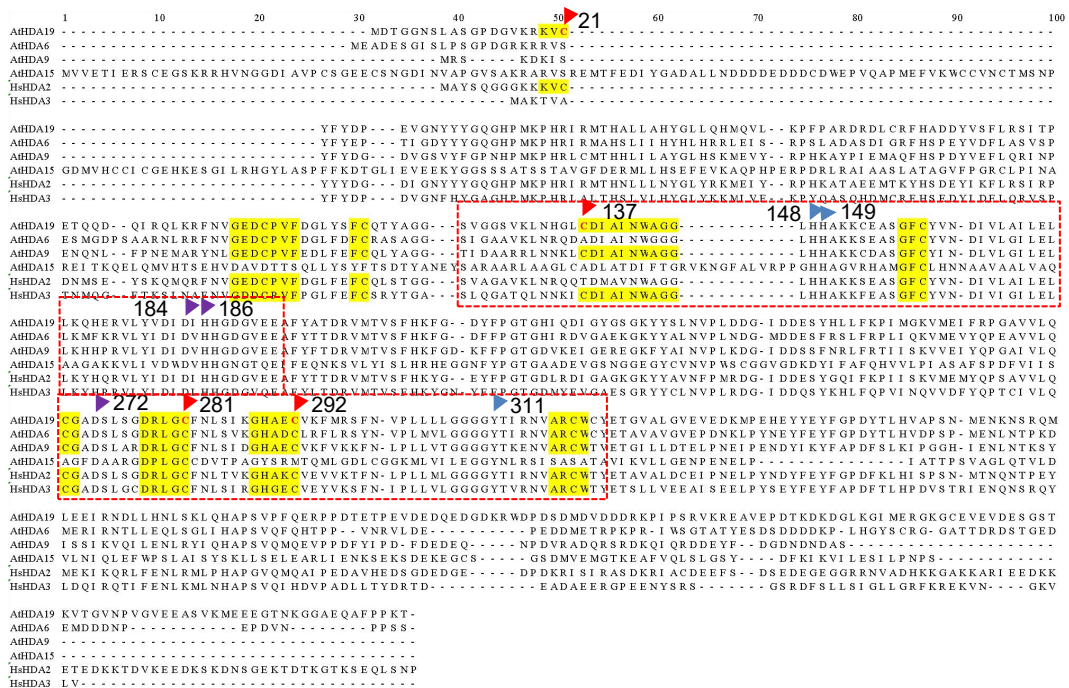


Figure S2. Sequence alignment of Arabidopsis HDA19, HDA6, HDA9 and HDA15 together with human HDAC2 and HDAC3.

The four S-nitrosylated Cys residues (Cys21, Cys137, Cys281, and Cys292) of HDA19 revealed by mass spectrometry are indicated by red arrows. The HDA19 putative catalytic residues His148, His149 and Try311 are indicated by blue arrows and the putative Zn²⁺-binding residues are indicated by purple arrows. Conserved Cys residues and their flanking sequences are highlighted by yellow. The red box represents the histone deacetylase domain superfamily (IPR003084)

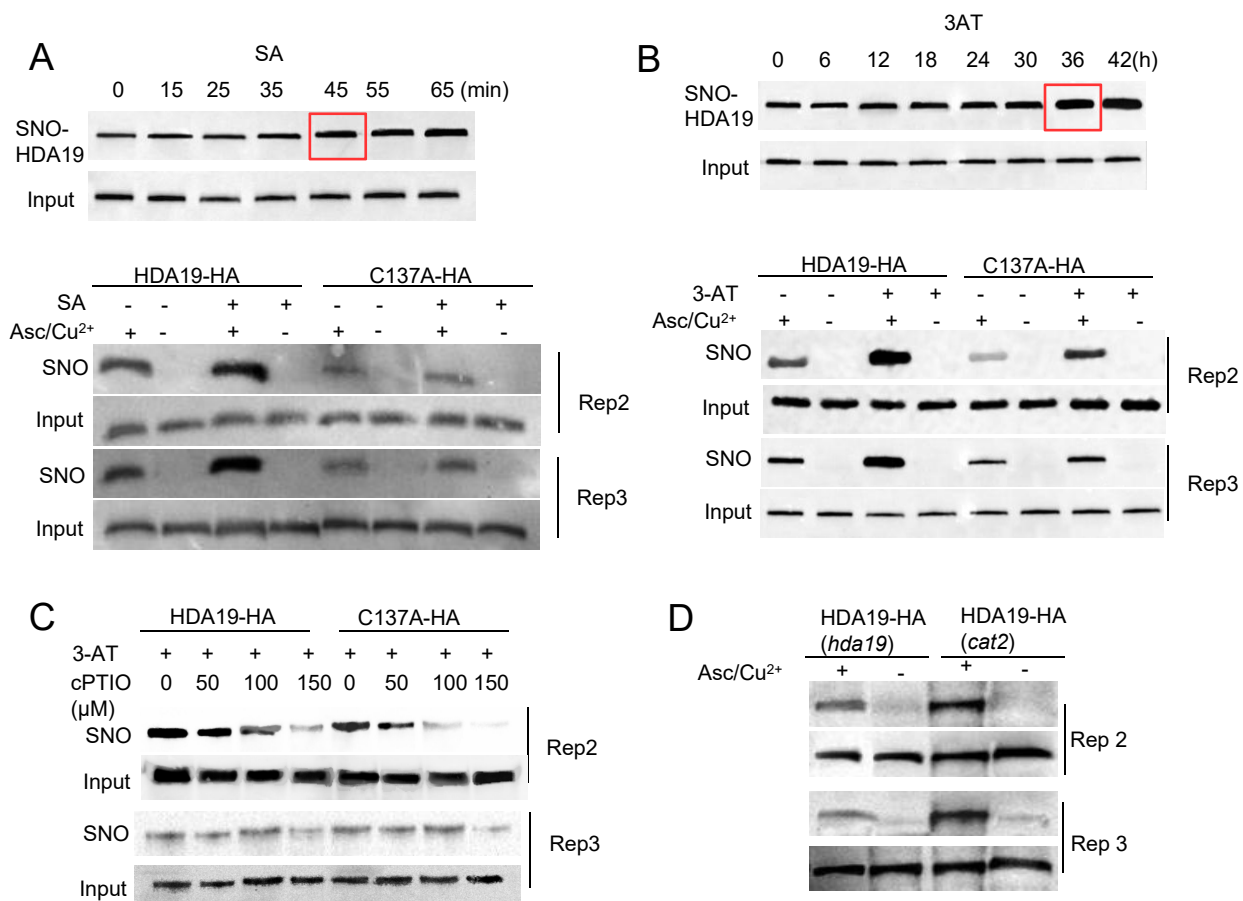


Figure S3. Time course of SA and 3-AT treatment to determine the optimal timing for sampling and the other 2 replicates of Fig 2.

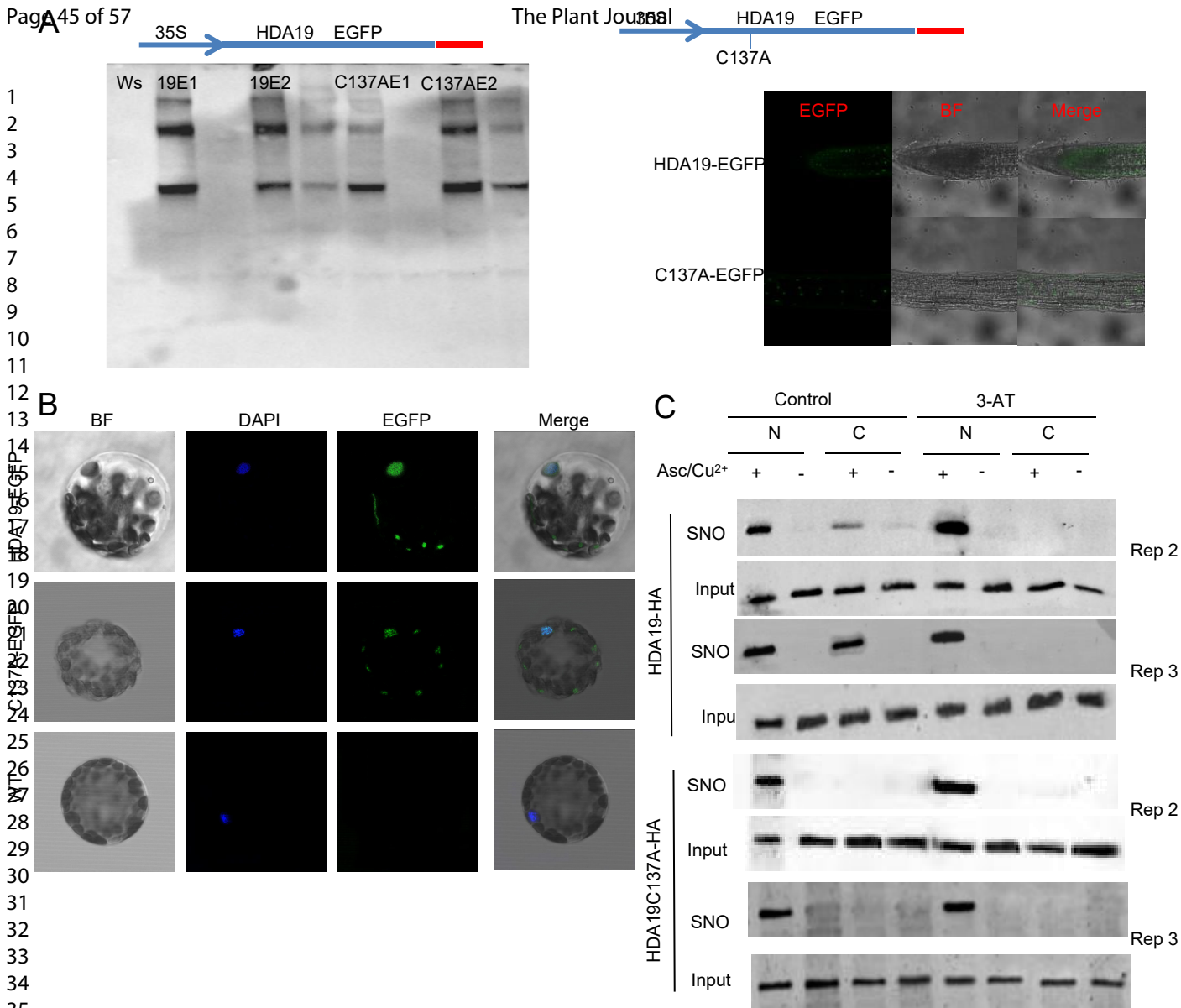
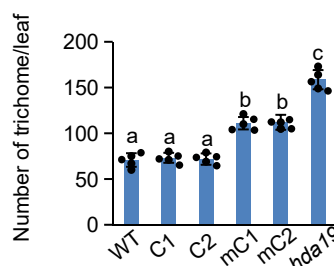
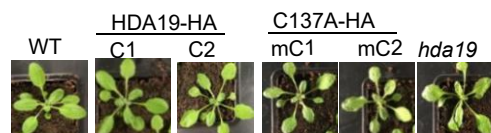
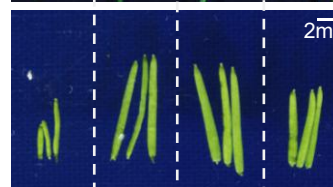


Figure S4. Characterization and sublocalization of HDA19-EGFP and C137A-EGFP plants and other 2 replicates of Fig 3.

A. Characterization of HDA19-EGFP and C137A-EGFP complementation transgenic plants. The 35S promoter was used to drive HDA19 cDNA translationally linked to EGFP. The vectors were transformed into the *hda19* plants. Four positive lines were tested by western blot for HDA19-EGFP expression in comparison with the WT and *hda19* mutant plants. 19E1 and C137AE2 were selected in this study. **B.** The sublocalization of HDA19-EGFP and C137A-EGFP using protoplasts. **C.** The other 2 replicates of Fig 3B.



hda19 WT C1 mC1

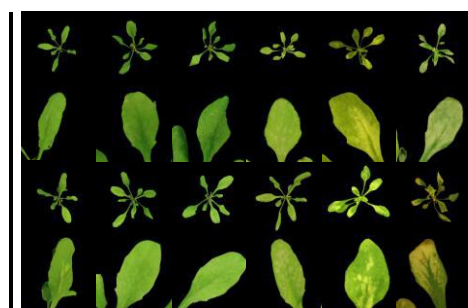


HDA19-HA C137A-HA
WT C1 C2 mC1 mC2 *hda19*



Experiment 2

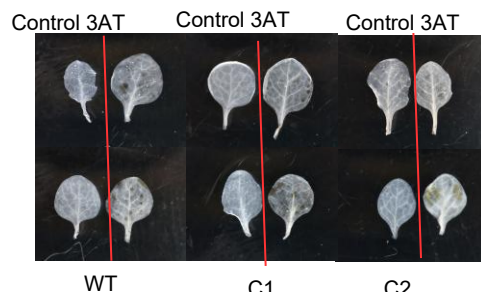
Experiment 3



Experiment 2

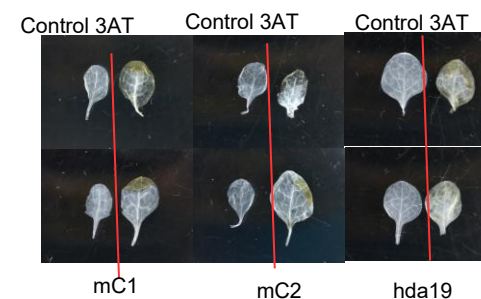
Experiment 3

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Experiment 2

Experiment 3



Experiment 2

Experiment 3

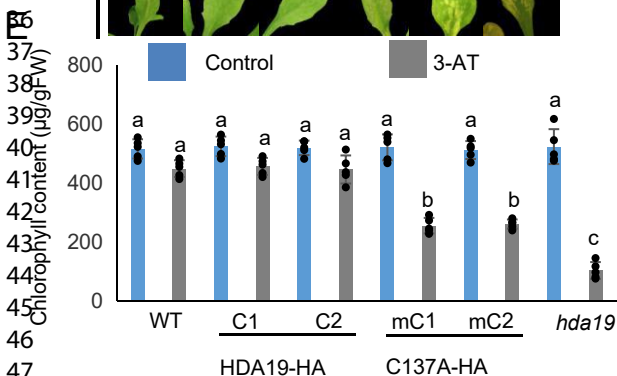


Figure S5. Phenotype of HDA19-HA and C137A-HA transgenic plants in the *hda19* mutant background.

A. Rosette phenotypes of wild type (WT), *35S::HDA19-HA* (C1, C2), *35S::C137A-HA* (mC1, mC2), and the *hda19* mutant plants. The trichome densities were counted and calculated for one leaf of four 19 day-old plants randomly selected from each genotype (n=4). Bars represent means +/- SD from the five (plants) replicates (the value of each replicate was shown as black dots) and multiple comparisons were conducted to compare significant differences among samples. **B.** Flower, silique and mature plant phenotypes of the complementation lines (C1, mC1) compared with wild type and *hda19* mutant plants. **C-D.** The other two repeated experiments of Fig 4 A and B, respectively. **E.** Chlorophyll contents of different samples. Bars represent means +/- SD from the five (plants) replicates (the value of each replicate was shown as black dots) and multiple comparisons were conducted to compare significant differences among samples.

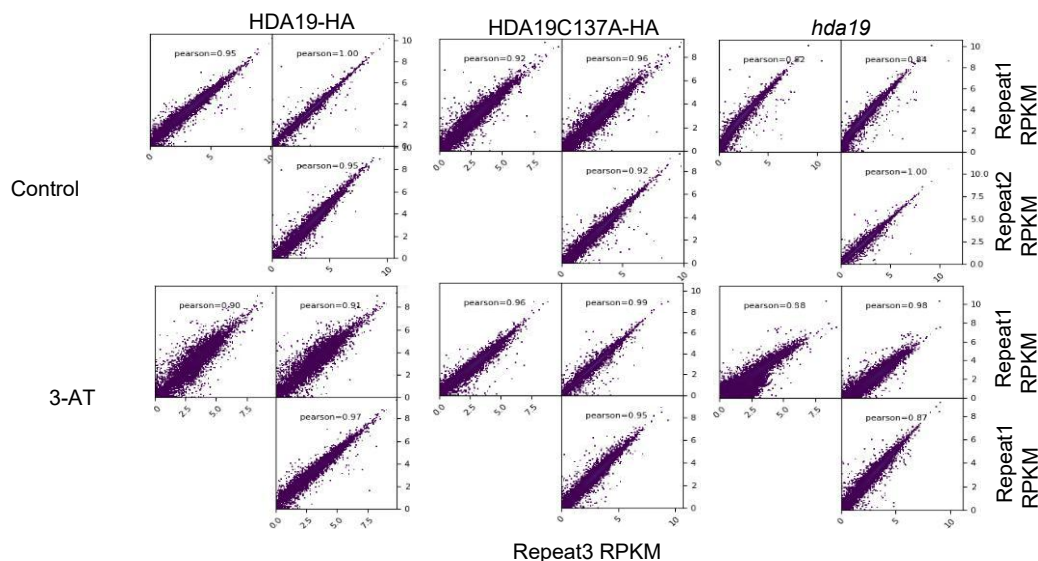
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A

	repeat	raw read	clean reads	mapping alignment reads
HDA19-HA-C	1	22595608	11481277(50.81%)	9341167 (81.36%)
	2	21473845	11119533(51.78%)	9283698 (83.49%)
	3	23459720	12201401(52.01%)	10177189(83.41%)
HDA19-HA-T	1	21571493	11170639(51.78%)	8373511 (74.96%)
	2	18766944	11521097(61.39%)	8163849 (70.86%)
	3	24732239	19031718(76.95%)	13771351(72.36%)
HDA19C137A-HA-C	1	12882114	8662727(67.24%)	5632505 (65.02%)
	2	20088731	10382636 (51.68%)	7862770(75.73%)
	3	16137382	10055203(62.31%)	7502187(74.61%)
HDA19C137A-HA-T	1	15174863	7351439(48.44%)	4894588 (66.58%)
	2	14346547	7930048 (55.27%)	4625597 (58.33%)
	3	14572100	7597893(52.14%)	4921915(64.78%)
HDA19-C	1	18271701	10443377 (57.16%)	7868040 (75.34%)
	2	22259381	11276996 (50.66%)	7999901(70.94%)
	3	22659884	11876045(52.41%)	8597069(72.39%)
HDA19-T	1	18476791	7759308 (41.99%)	6604723 (85.12%)
	2	19686152	7580491(38.50%)	6096231 (80.42%)
	3	17334697	7342978(42.36%)	6051348(82.41%)

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Figure S6. RNA-seq analysis of wild type, *hda19* mutant and the complementation plants.

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A. RNA-seq reads of HDA19-HA, C137A-HA transgenic and *hda19* mutant seedling grown at 20°C for 19 days, then treated 3-AT or not treated for 36 hours before RNA extraction. **B.** Pair-wise scatter plots of RNA-seq reads between three biological repeats. Each point represents one gene locus. RPKM, Reads Per Kilobase per Million reads. Correlation coefficient R2 is indicated.

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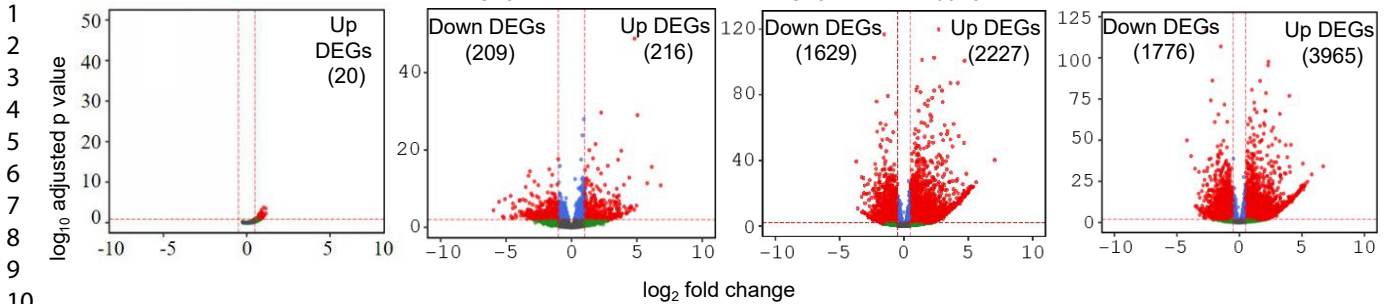
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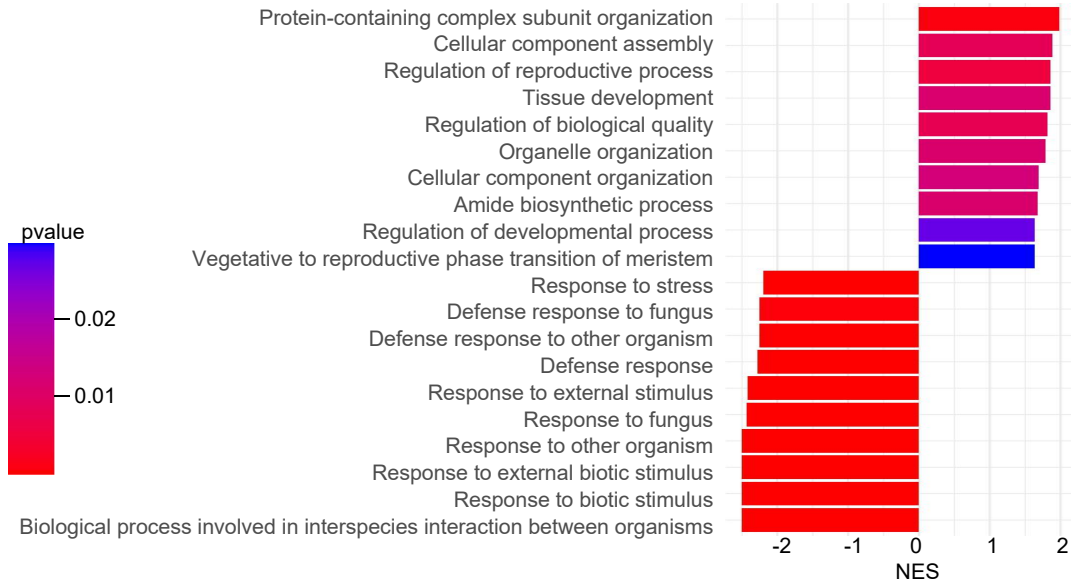
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● NS ($p > 0.01$ & $\log_2FC < 1$)● NS ($p > 0.01$ & $\log_2FC > 1$)● DEGs ($p < 0.01$ & $\log_2FC > 1$)*HDA19-HA* vs Ws*C137A-HA* vs WT*C137A-HA* vs *hda19**HDA19-HA* vs *hda19*

B



C

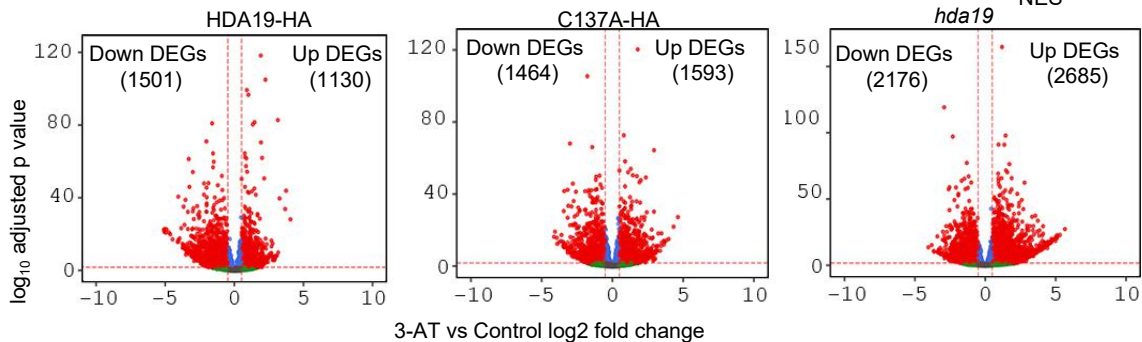


Figure S7. Differential gene expression in *HDA19-HA*, *C137A-HA* and *hda19* plants.

A. Volcano map of differentially expressed genes in between wild type, *hda19*, *HDA19-HA* and *C137A-HA* plants as indicated.

B. GESA of DEGs of *C137A-HA* vs Ws. The top ten increased and decreased significant gene sets were listed according to their NES (normalized enrichment score). The color of each bar represents the p value of the gene set. The significance of each gene set was classified by a threshold of $p < 0.05$. **C.** Volcano map of differentially expressed genes (DEGs, red dots) between control and 3-AT treatment of *HDA19-HA* (right), *C137A-HA* (middle) and *hda19* plants (left).

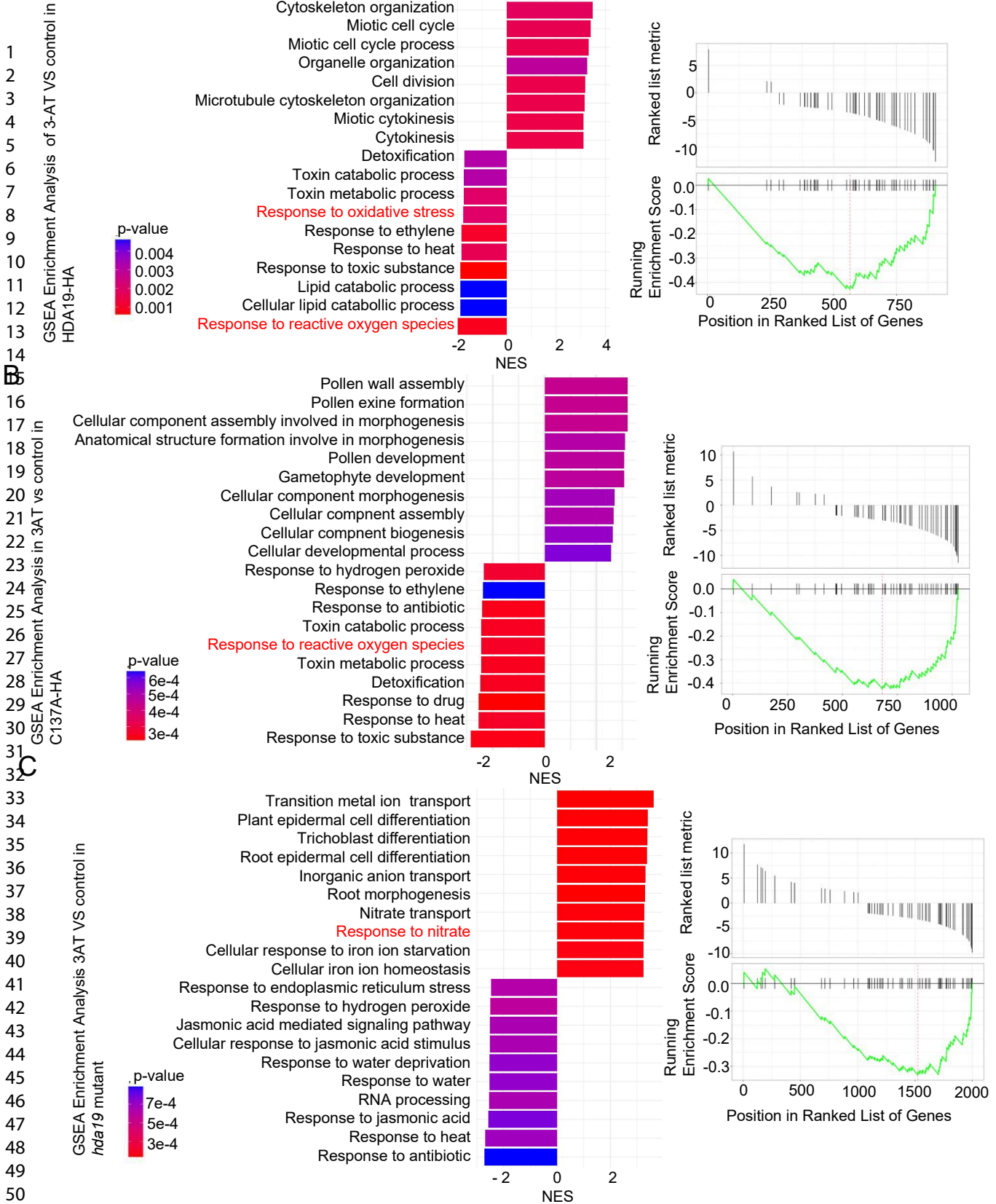


Figure S8. Differential gene expression in HDA19-HA, C137A-HA and hda19 plants with/without oxidative stress. Left panels: GSEA analysis of DEGs of 3-AT treatment versus control in HDA19-HA (A), C137A-HA (B) and *hda19* (C) plants. The top ten increased and decreased significant gene sets were listed according to their NES (normalized enrichment score). The color of each bar represents the p value of the gene set. The significance of each gene set was classified by a threshold of $p < 0.05$. Right panels: The GSEA analysis of the 3-AT caused DEGs belonging to the GO term of oxidative stress response (GO:0006979) in HDA19-HA (A), C137A-HA (B) and *hda19* (C) plants. The positive/negative values of Y-axis (Ranked list metric) represent up/down regulated DEGs induced by 3-AT, and the X-axis (Position in ranked list of genes) represented the list of DEGs belong to GO:0006979. The red dot vertical lines indicate the enrichment score (ES) values of DEGs in GO:0006979.

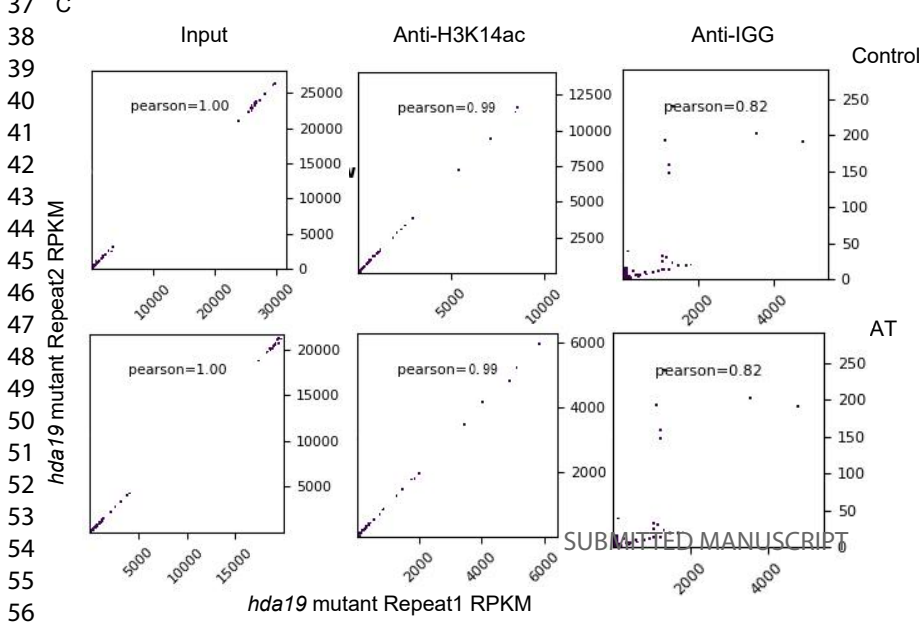
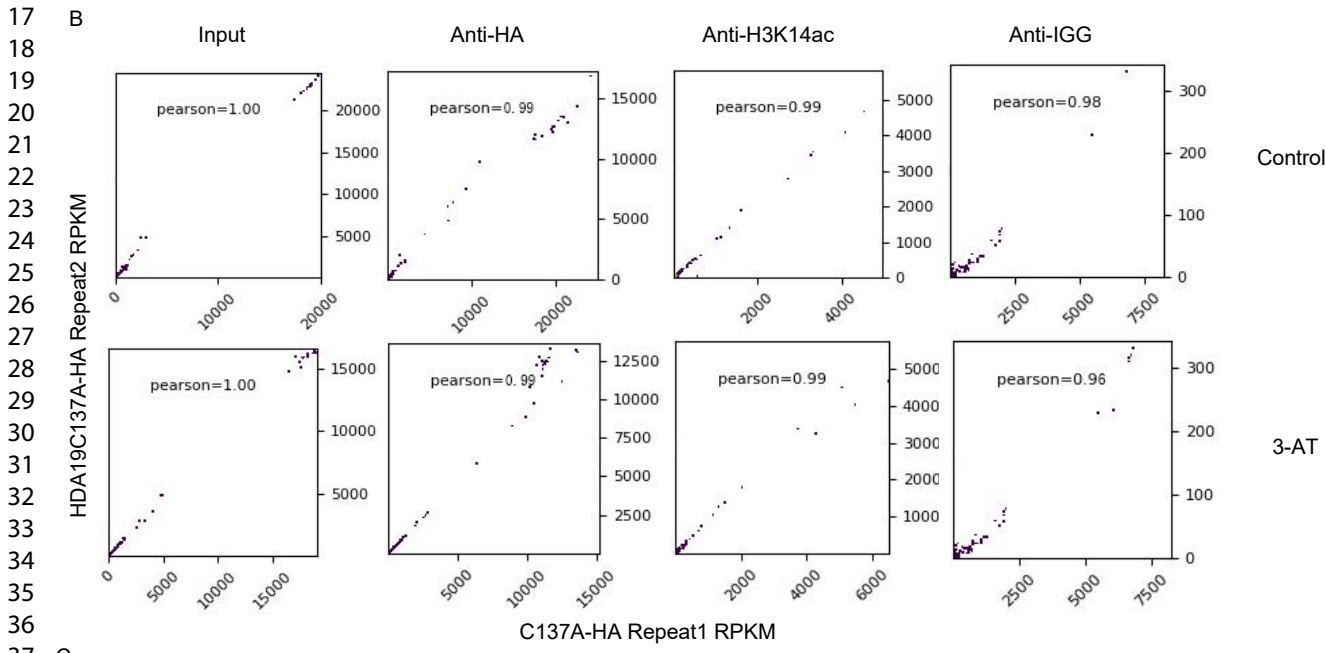
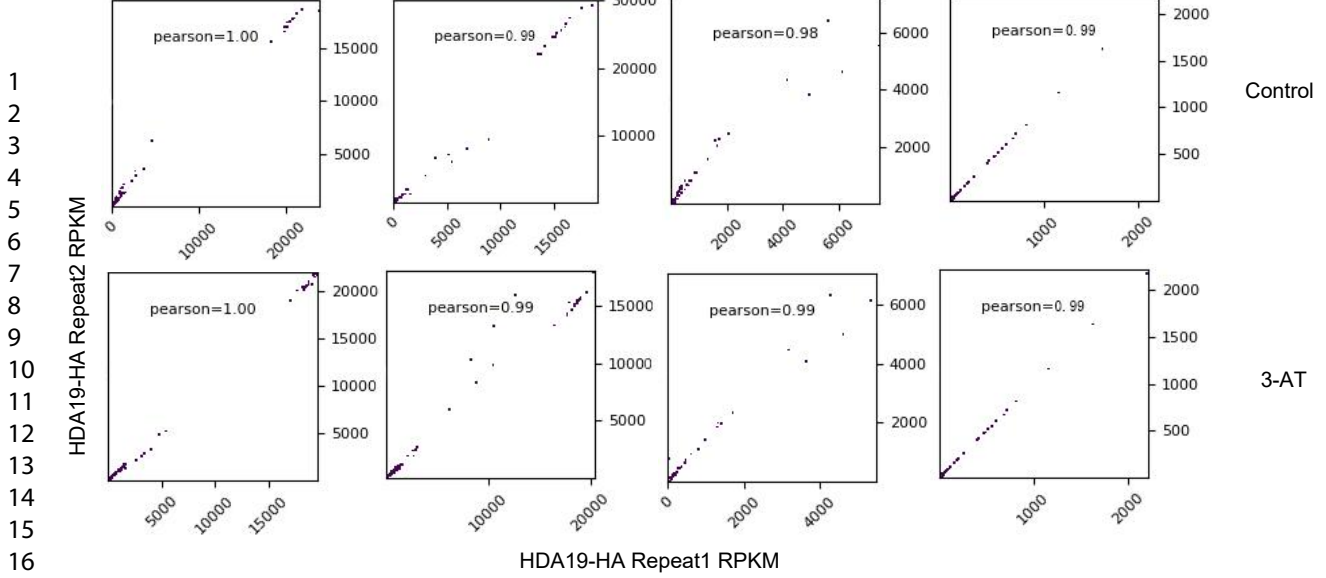
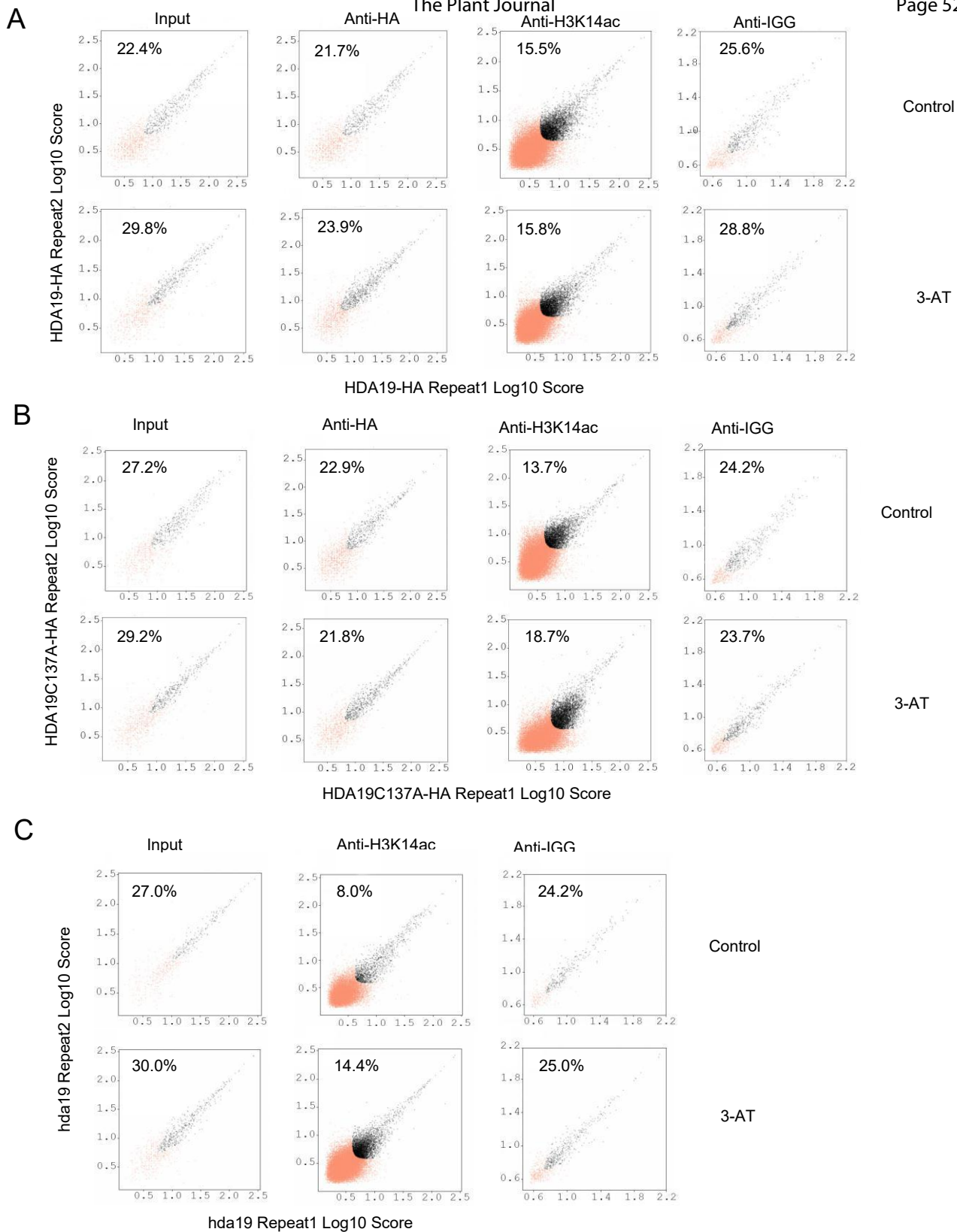


Figure S10. Correlation analysis of anti-H3K14ac and anti-HA ChIP-seq data.
A-C. Analysis of anti-H3K14ac and anti-HA ChIP-seq reads of HDA19-HA, C137A-HA plants and *hda19* mutant plants. Seedling were grown at 20°C for 19 days, then treated with or without 3-AT for 36 hours. IgG was used use control for the ChIP experiments. Pairwise scatter plots of ChIP-seq reads between two biological repeats are shown. Each point represents one gene locus. RPKM, Reads Per Kilobase per Million reads. Correlation coefficient R2 indicated.



51 **Figure S11. IDR analysis of anti-H3K14ac and anti-HA ChIP-seq reads.**

52 Pairwise scatter plots of ChIP-seq IDR log10 score between two biological repeats are shown. Red meant ≥ 0.05 IDR. The ratio of peaks
53 passing IDR cutoff of 0.05 indicated. A, HDA19-HA; B, C137A-HA ; C, *hda19*.

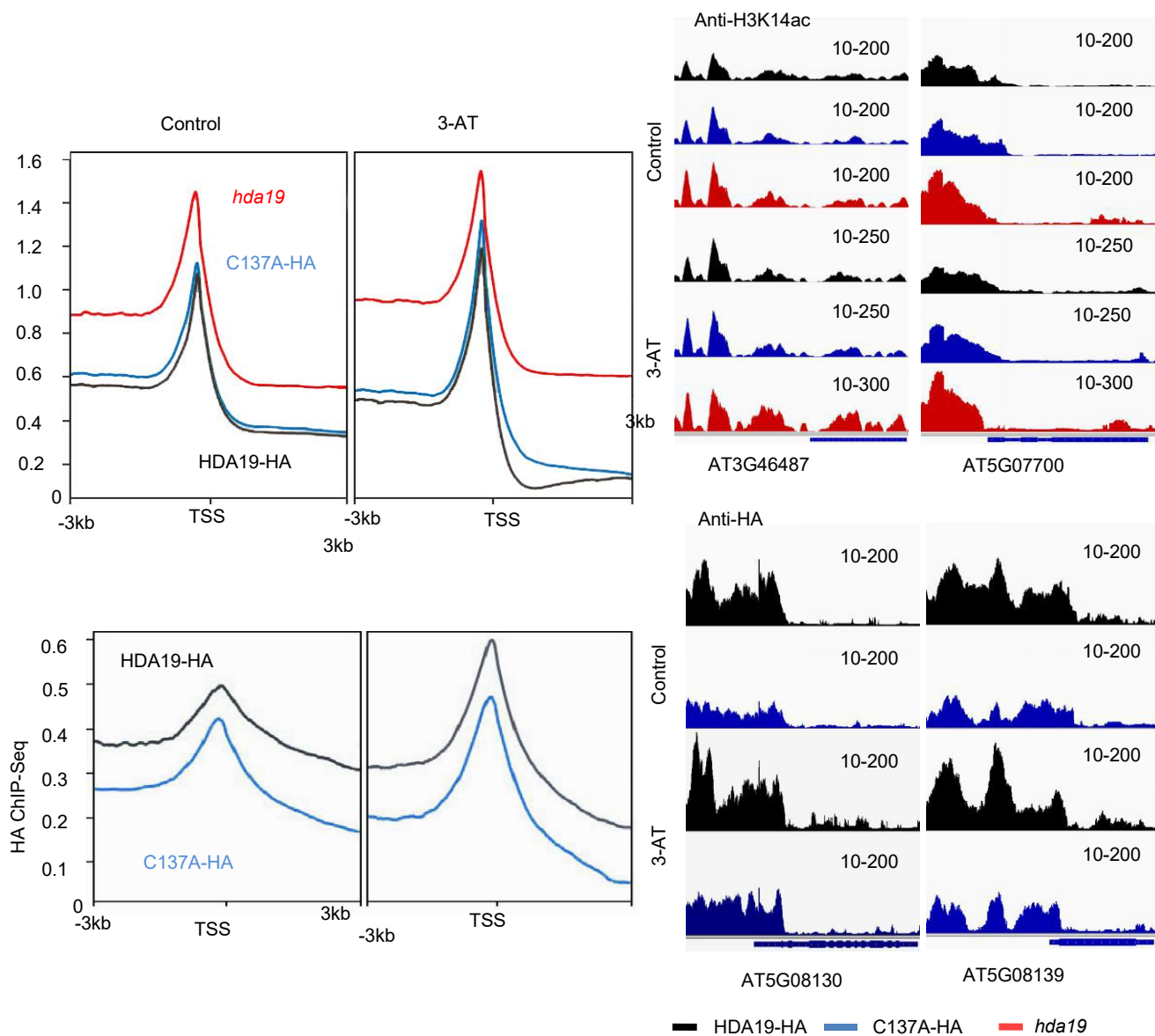


Figure S12. ChIP-seq analysis of H3K14ac and HDA19-HA and C137A-HA binding profiles in *hda19* and/or HDA19-HA and C137A-HA plants. Left: metaplots of anti-H3K14ac and anti-HA ChIP-seq reads near the transcriptional start sites (TSS). The Y-axis is log₂ (IP/Input signal). Right: IGV of the anti-H3K14ac peaks at AT3G46487 and AT5G07700 loci (upper part) and the anti-HA peaks at AT5G08130 and AT5G08139 loci (scales are indicated).

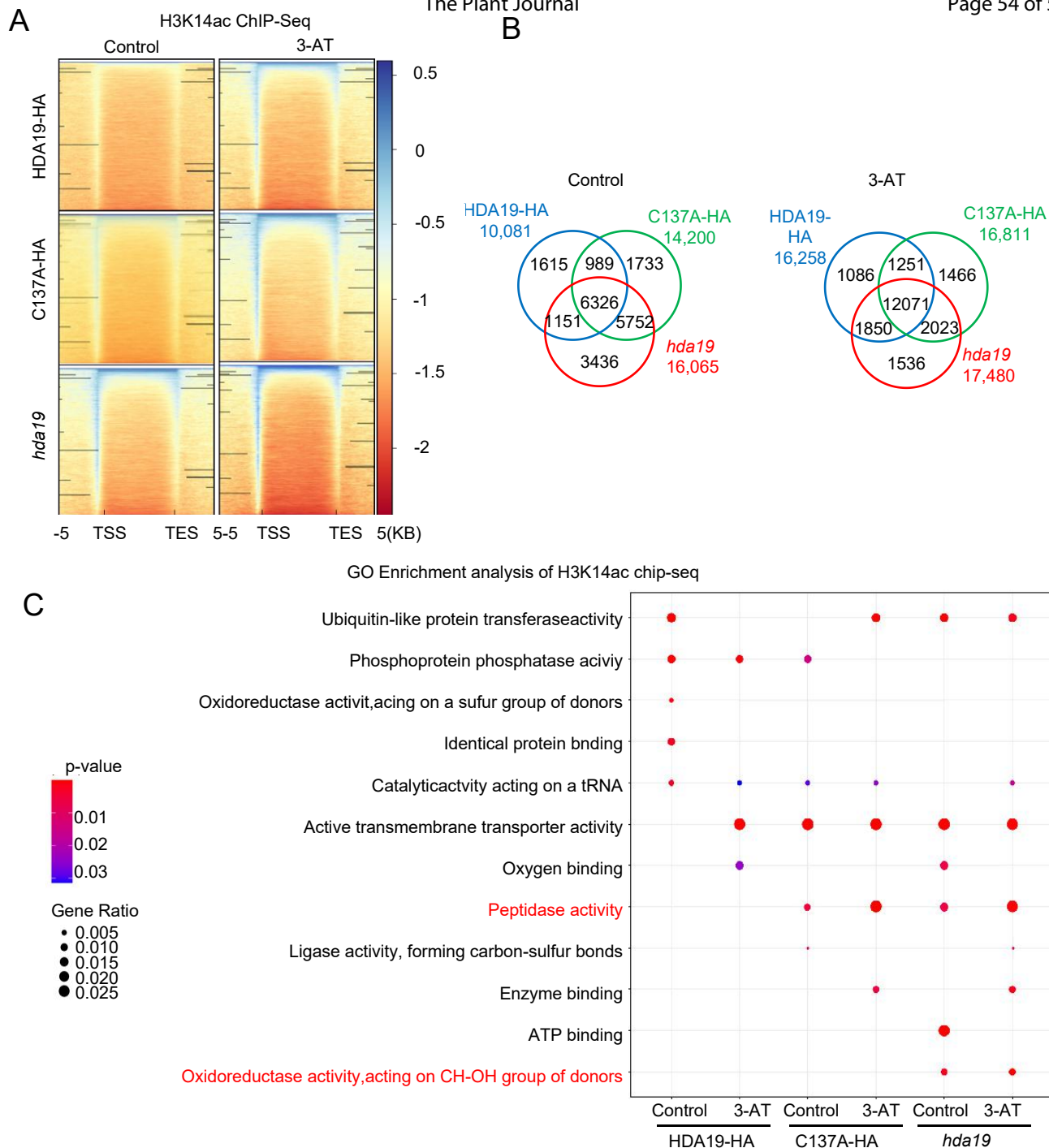


Figure S13. S-nitrosylation induced by oxidative stress enhances total H3K14ac level.

A. Heatmaps of the H3K14ac ChIP-seq reads (normalized RPKM) in the *hda19* mutant and the complementation (by HDA19-HA or C137-HA) plants treated with or without 3-AT. TSS: transcription start site, TES: transcription end site. **B.** Venn diagram of gene promoter H3K14ac peaks in plants of in the *hda19* mutant and the complementation plants treated with or without 3-AT. **C.** GO enrichment analysis of H3K14ac ChIP peaks among different samples under control (C) or 3-AT treatment (T) using peaks annotation analysis according to ChIP-Seq data. The top 12 enrichment gene sets were listed by p values. The area of dots represents the gene ratio, and the color of dots represent the p value of the gene set. The significance of each gene set was classified by a threshold of $p < 0.05$ for H3K14ac. The red letters represented the crucial GO terms of response to oxidative stress.

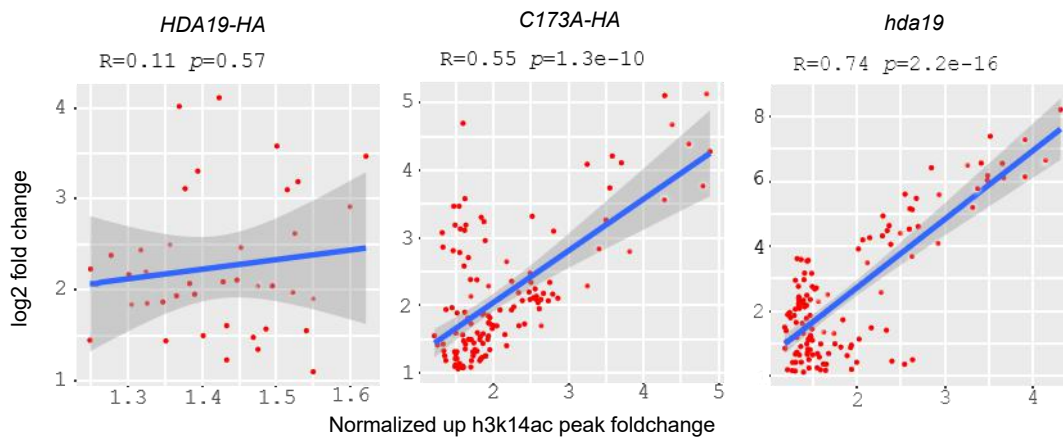


Figure S14. Pearson correlation analysis of expression and H3K14ac levels of the 317 up-regulated genes only observed in C137A-HA and *hda19* mutant. The (Y axis) stands for log₂ fold change of gene expression levels and the X axis stands for normalized up-regulated fold change of H3K14ac peaks in their promoter region caused by 3-AT. The dots whose value <1 were not drawn in the plot.

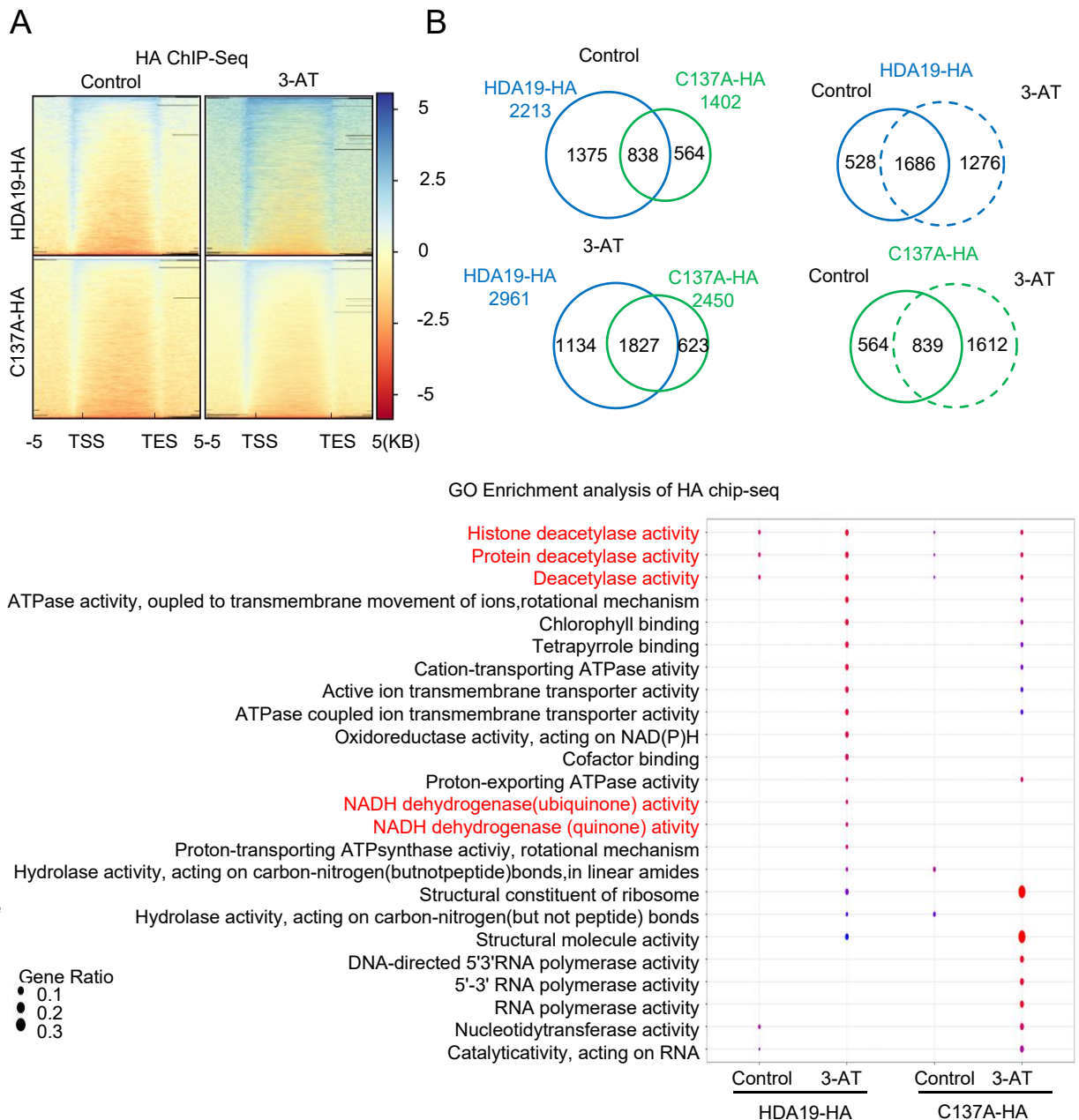


Figure S15. S-nitrosylation induced by oxidative stress enhances HDA19 binding activity.

A. Heatmaps of the genome-wide enrichment of anti-HA ChIP-seq reads (normalized RPKM) in the *HDA19-HA* and *C137A-HA* plants treated with or without 3-AT. **B.** Overlapping between *HDA19-HA*- and *C137A-HA* binding genes under the control and 3-AT treatment are shown by the Venn diagrams on the left. Overlapping of *HDA19-HA*- or *C137A-HA*-binding genes between untreated and 3-AT-treated conditions are shown by the Venn diagrams on the right. **C.** GO enrichment analysis of HA ChIP-seq peaks among different samples under control (C) or 3-AT treatment (T) using peaks annotation analysis according to ChIP-Seq data. The top 12 enrichment gene sets were listed by p values. The area of dots represents the gene ratio, and the color of dots represents the p value of the gene set. The significance of each gene set was classified by a threshold of $p < 0.05$ for HA. The red letters represented the crucial GO terms related to deacetylation.

Table S1. ChIP-seq reads and correlation coefficients between the replicates

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2			Repeat	Raw read	Clean reads	Mapping alignment reads	Pearson Corr
3	HDA19-HA-C	Input	1	23654153	16444367 (69.52%)	15990502 (97.24%)	0.9979
4			2	22921389	16925154(73.84%)	16549416 (97.78%)	
5		Anti-	1	24887157	19471712(78.24%)	18182685(93.38%)	0.9755
6		H3K14	2	24501563	19270479(78.65%)	18416797(95.57%)	
7		Anti-HA	1	29564434	23417988(79.21%)	17301210(73.88%)	0.9976
8			2	26955786	21200726(78.65%)	15092797(71.19%)	
9		Anti-IGG	1	24581167	19566233(79.60%)	17850274(91.23%)	0.9945
10			2	24490256	19481382(79.55%)	17761365(91.17%)	
11	HDA19-HA-T	Input	1	25148485	17661781(70.23%)	17073644(96.67%)	0.9995
12			2	23447375	17930208(76.47%)	17322374(96.61%)	
13		Anti-	1	28004089	22176438(79.19%)	21375869(96.39%)	0.9919
14		H3K14	2	21398331	16652181(77.82%)	16021063(96.21%)	
15		Anti-HA	1	26538606	21037153(79.27%)	17050613(81.05%)	0.9928
16			2	29125062	22129222(75.98%)	18225627(82.36%)	
17		Anti-IGG	1	27432923	21106891(76.94%)	19274813(91.32%)	0.9923
18			2	26985765	20811422(77.12%)	19114400(90.56%)	
19	HDA19C137A-	Input	1	25288604	17719725(70.07%)	17418490 (98.30%)	0.9996
20	HA-C		2	20694867	14567117(70.39%)	13937818(95.68%)	
21		Anti-	1	23659307	18340695(77.52%)	17636412(96.16%)	0.9945
22		H3K14	2	27075701	20006236(73.89%)	19324023(96.59%)	
23		Anti-HA	1	24134978	18709435(77.52%)	15605540(83.41%)	0.9970
24			2	29050680	23234734(79.98%)	16436251(70.74%)	
25		Anti-IGG	1	22768216	18481574(81.17%)	16675924(90.66%)	0.9782
26			2	23179127	18770463(80.98%)	17200765(91.12%)	
27	HDA19C137A-	Input	1	23616827	16057081(67.99%)	15535226(96.75%)	0.9996
28	HA-T		2	22062908	16886950 (76.54%)	16476597(97.57%)	
29		Anti-	1	25543794	19349424(75.75%)	18737982(96.84%)	0.9917
30		H3K14	2	20274632	16485303(81.31%)	15974259(96.90%)	
31		Anti-HA	1	25346635	19945267(78.69%)	17553829(88.01%)	0.9960
32			2	26126070	20537704(78.61%)	16329528(79.51%)	
33		Anti-IGG	1	21131301	17164955(81.23%)	15656155(91.20%)	0.9614
34			2	20740948	16907872(81.52%)	15121670(89.44%)	
35	HDA19-C	Input	1	22202304	17688576 (79.67%)	16552969 (93.58%)	0.9999
36			2	24484577	19808023(80.90%)	18593791(93.87%)	
37		Anti-	1	28777134	23205881(80.64%)	22558437(97.21%)	0.9999
38		H3K14	2	22288220	17603236(78.98%)	17106825(97.18%)	
39		Anti-IGG	1	25929607	22062619(85.09%)	20023314(90.76%)	0.8243
40			2	26726969	21710316(81.23%)	19801979(91.21%)	
41	HDA19-T	Input	1	23433518	17132245(73.11%)	16570307 (96.72%)	0.9669
42			2	22536443	16314131 (72.39%)	15757819(96.59%)	
43		Anti-	1	22165831	17610753(79.45%)	17124696(97.24%)	0.9996
44		H3K14	2	21769084	17108323(78.59%)	16634422(97.23%)	
45		Anti-IGG	1	22712956	18049734(79.47%)	16063162(88.99%)	0.8231
46			2	23047722	17321664(75.16%)	15299089(88.32%)	

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