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*Non-specific activities of the major herbicide-resistance gene BAR*

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1 **Nonspecific activities of the major herbicide-resistance gene BAR**

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14           **Bialaphos resistance (BAR) and phosphinothricin acetyltransferase (PAT)**  
15 **genes, which convey resistance to the broad-spectrum herbicide phosphinothricin**  
16 **(also known as glufosinate) via N-acetylation, have been globally used in basic plant**  
17 **research and genetically engineered crops<sup>1-4</sup>. Although early in vitro enzyme assays**  
18 **showed that recombinant BAR and PAT exhibit substrate preference toward**  
19 **phosphinothricin over the 20 proteinogenic amino acids<sup>1</sup>, indirect effects of BAR-**  
20 **containing transgenes *in planta*, including modified amino acid levels, have been**  
21 **seen but without the identification of their direct causes<sup>5,6</sup>. Combining**  
22 **metabolomics, plant genetics, and biochemical approaches, we show that transgenic**  
23 **BAR indeed converts two plant endogenous amino acids, aminoadipate and**  
24 **tryptophan, to their respective N-acetylated products in several plant species**  
25 **examined. We report the crystal structures of BAR, and further delineate structural**  
26 **basis for its substrate selectivity and catalytic mechanism. Through structure-**  
27 **guided protein engineering, we generated several BAR variants that display**  
28 **significantly reduced nonspecific activities compared to its wild-type counterpart *in***  
29 ***vivo*. Our results demonstrate that transgenic expression of enzymes can result in**  
30 **unintended off-target metabolism arising from enzyme promiscuity. Understanding**  
31 **of such phenomena at the mechanistic level can facilitate the design of maximally**  
32 **insulated systems featuring heterologously expressed enzymes.**

33           Phosphinothricin is a naturally occurring herbicide derived from the tripeptide  
34 antibiotic bialaphos produced by species of *Streptomyces* soil bacteria. Phosphinothricin  
35 is a structural analog of glutamate, and thereby inhibits glutamine synthetase, an essential  
36 enzyme for glutamine synthesis and ammonia detoxification in plants, giving rise to its

37 herbicidal activity<sup>3</sup>. In the 1980s, the bialaphos resistance (*BAR*) gene and its closely  
38 related homolog phosphinothricin acetyltransferase (*PAT*) gene were isolated from  
39 *Streptomyces hygroscopicus* and *Streptomyces viridochromogenes*, respectively, and  
40 were later broadly used as transgenes to confer herbicide resistance in a variety of major  
41 genetically-engineered (GE) crops, including corn, soybean, canola, and cotton<sup>7</sup>. In  
42 addition, *BAR* and *PAT* have also gained much utility in basic research as selection  
43 markers for generating transgenic plants<sup>1</sup>. Despite the prevalent use of *BAR* and *PAT* in  
44 the context of generating herbicide-resistant transgenic plants, whether these bacteria-  
45 derived enzymes may possibly interfere with plant endogenous metabolism has not been  
46 rigorously investigated.

47 In research not initially intended to address this issue regarding phosphinothricin-  
48 resistance trait, we carried out untargeted metabolomics analysis on senescent leaf  
49 extracts prepared from the *Arabidopsis thaliana clh2-1* mutant (FLAG\_76H05, referred  
50 to as FLAG-1 hereafter), which contains a transfer DNA (T-DNA) insertion that  
51 abolishes the *CHLOROPHYLLASE 2* gene<sup>8</sup>. This analysis revealed two metabolites that  
52 were ectopically accumulated at high levels in *clh2-1* compared to wild type (Fig. 1a).  
53 Using liquid chromatography-tandem mass spectrometry (LC-MS<sup>2</sup>), we identified these  
54 two metabolites as N-acetyl-aminoadipate and N-acetyl-tryptophan (referred to as acetyl-  
55 aminoadipate and acetyl-tryptophan, respectively, hereafter; Fig. 1a and Supplementary  
56 Fig. 1). Because the deficiency of *CHLOROPHYLLASE 2*, a serine esterase<sup>8</sup>, in *clh2-1*  
57 does not explain the accumulation of these acetylated metabolites, we hypothesized that  
58 the *BAR* gene present on the T-DNA as a selection marker in *clh2-1* might be responsible  
59 for their formation. To test this, we extended our metabolomics analysis to additional

60 Arabidopsis T-DNA insertional mutants unrelated to chlorophyll metabolism that carry  
61 either *BAR* (e.g. mutants from the FLAG<sup>9</sup> and SAIL<sup>10</sup> collections) or alternative antibiotic  
62 selection markers (e.g. mutants from the SALK (*NTPH*, kanamycin resistance)<sup>11</sup> and  
63 GABI (*SULI*, sulfadiazine resistance)<sup>12</sup> collections (Supplementary Table 1). Senescent  
64 leaves of all six T-DNA mutants carrying *BAR* manifested accumulation of acetyl-  
65 aminoadipate and acetyl-tryptophan, while these metabolites were significantly lower or  
66 not detected in wild-type plants and T-DNA mutants containing alternative selection  
67 markers (Fig. 1b). These results indicate that the ectopic accumulation of these  
68 metabolites is likely resulted from the nonspecific N-acetyltransferase activities of  
69 transgenic *BAR* acting upon plant endogenous amino acids.

70 We quantified the absolute concentrations of acetyl-aminoadipate and acetyl-  
71 tryptophan in senescent leaves of *BAR*-containing transgenic Arabidopsis to range from  
72 306 to 845 nmole/g and from 14 to 76 nmole/g fresh weight, respectively (Supplementary  
73 Fig. 2). While trace level of acetyl-tryptophan can be detected in wild-type Arabidopsis,  
74 acetyl-aminoadipate was undetectable in wild-type samples (Supplementary Fig. 2). The  
75 ectopic accumulation of acetyl-aminoadipate and acetyl-tryptophan in *BAR*-containing  
76 transgenic Arabidopsis is substantial given that the concentrations of free aminoadipate  
77 and tryptophan in senescent leaves of these Arabidopsis lines are in the ranges of 61 to  
78 122 nmole/g and from 1566 to 2663 nmole/g fresh weight, respectively (Supplementary  
79 Fig. 2). On the other hand, the concentrations of free amino acids in senescent leaves do  
80 not seem to be significantly affected by the expression of *BAR*, as revealed by the  
81 quantification of 21 other amino acids (Supplementary Fig. 2).

82 To assess whether the nonspecific activities of transgenic BAR also manifest in  
83 other plant hosts, we performed metabolic profiling of various tissue samples from  
84 phosphinothricin-resistant soybean (*Glycine max*), canola (*Brassica napus*), mustard  
85 (*Brassica juncea*) and wheat (*Triticum aestivum*). Substantially increased accumulation  
86 of acetyl-aminoadipate and acetyl-tryptophan was also detected in some tissues of these  
87 transgenic lines (Supplementary Fig. 3), indicating that our findings regarding the *in vivo*  
88 nonspecific activities of BAR may apply broadly to a wide range of *BAR*-containing  
89 transgenic plants.

90 The concentration of free tryptophan is low in photosynthetically active leaves,  
91 but increases significantly in senescent leaves<sup>13</sup>. This is due to enhanced proteolysis  
92 during senescence, facilitating remobilization of protein-bound nitrogen and other  
93 nutrients to sink organs, such as seeds<sup>14</sup>. Aminoadipate, an intermediate of lysine  
94 degradation, also exhibits a similar accumulation pattern during leaf senescence<sup>15</sup>. To test  
95 whether the *BAR*-catalyzed production of acetyl-aminoadipate depends on lysine  
96 degradation, we analyzed an *Arabidopsis* mutant from the FLAG collection,  
97 FLAG\_ikrsdh, in which the *BAR*-containing T-DNA disrupts *At4g33150* encoding the  
98 *Arabidopsis* bifunctional lysine-ketoglutarate reductase/saccharopine dehydrogenase  
99 (LKR/SDH, Supplementary Fig. 4)<sup>16</sup>. LKR/SDH catalyzes the first committed step of  
100 lysine degradation, and, together with the subsequent aminoadipate semialdehyde  
101 dehydrogenase (AADH), converts lysine to aminoadipate (Fig. 2a). In a segregating  
102 population for the FLAG\_ikrsdh locus, heterozygous, homozygous and wild-type plants  
103 were identified by genotyping, and subjected to LC-MS metabolic profiling after  
104 senescence induction (Fig. 2b, Supplementary Fig. 4). Acetyl-aminoadipate occurred at

105 the highest level in the heterozygous mutant, but was greatly reduced in the homozygous  
106 mutant, suggesting that the ectopic accumulation of acetyl-aminoadipate in *BAR*-  
107 containing plants is dependent on the activity of LKR/SDH in the lysine degradation  
108 pathway in senescent leaves (Fig. 2a). By contrast, the relative abundance of acetyl-  
109 tryptophan in the segregating population of *FLAG\_lkrstdh* generally reflected the copy  
110 number of the *BAR*-containing T-DNA transgene, with approximately 2-fold acetyl-  
111 tryptophan level observed in the homozygotes compared to the heterozygotes (Fig. 2b).  
112 Furthermore, acetyl-aminoadipate and acetyl-tryptophan levels were approximately 10-20  
113 fold higher in senescent leaves than those in green leaves (Fig. 2b), which is likely due to  
114 the increased availability of the corresponding free amino acids during senescence.  
115 Consistent with these observations in leaves, ectopic accumulation of acetyl-  
116 aminoadipate and acetyl-tryptophan was also observed in seeds of multiple *BAR*-  
117 containing T-DNA mutant lines compared to the wild-type controls (Supplementary Fig.  
118 5).

119 To shed light on the kinetic properties of *BAR*, we carried out pseudo-first-order  
120 enzyme kinetic assays using recombinant *BAR* against several native and non-native  
121 amino acid substrates (Fig. 3 and Supplementary Fig. 6). Similar to published data<sup>1,3,17</sup>,  
122 N-acetylation of phosphinothricin exhibits Michaelis-Menten kinetics with an apparent  
123  $K_m$  of approximately 132  $\mu$ M (Fig. 3). Although *BAR* clearly showed N-acetyltransferase  
124 activities toward aminoadipate and tryptophan,  $K_m$  values for these non-native substrates  
125 could not be established, as both substrates reached solubility limit before reaching  
126 saturation concentration for *BAR*.  $V_{max}/K_m$  values of *BAR* against aminoadipate and  
127 tryptophan, which were inferred from Lineweaver-Burk plots, reveals that these two side

128 reactions are less favorable than the acetylation of phosphinothricin. BAR also exhibited  
129 relatively higher catalytic activity toward amino adipate than tryptophan *in vitro* (Fig. 3).

130 To reveal the structural basis for substrate selectivity and catalytic mechanism of  
131 BAR that would enable structure-guided protein engineering, we determined the crystal  
132 structures of the BAR/acetyl-CoA holocomplex and the BAR/CoA/phosphinothricin  
133 ternary complex (see Supplementary Table 2 for data collection and refinement  
134 statistics). Our refined structures revealed that BAR is an  $\alpha\beta$  protein harboring a globular  
135 tertiary structure resembling the previously reported Gcn5-related N-acetyltransferase  
136 (GNAT) structures (Supplementary Fig. 7)<sup>18-21</sup>. BAR crystallizes as a homodimer with  
137 two active sites symmetrically distributed around the dimer interface inside a large open  
138 cavity (Fig. 4a and Supplementary Fig. 8). The cofactor acetyl-CoA binds to a cleft  
139 between  $\alpha 4$  and  $\alpha 5$  on the opposite side of the dimer interface with the acetyl group  
140 pointing toward the catalytic center (Fig. 4a). Close examination of the BAR/acetyl-CoA  
141 and BAR/CoA/phosphinothricin structures illuminates the catalytic mechanism of BAR  
142 (Fig. 4b, 4c and Supplementary Fig. 9). Similar to other GNATs, BAR utilizes a  
143 conserved catalytic Glu88 as a general base to deprotonate the amino group of  
144 phosphinothricin through a water molecule as the proton shuttle (Fig. 4b, 4c, and  
145 Supplementary Fig. 9)<sup>21</sup>. The deprotonated amino group then undergoes nucleophilic  
146 attack on the carbonyl carbon of acetyl-CoA to produce a tetrahedral intermediate, which  
147 is further stabilized by an oxyanion hole formed by a positively charged H137 and its  
148 proton donor Y107 (Fig. 4c and Supplementary Fig. 9). Interestingly, the structural  
149 feature underlying this oxyanion hole in BAR must have arisen independently from the  
150 functionally analogous oxyanion hole previously described in the histone



151 acetyltransferase GCN5, featuring a backbone amide nitrogen instead<sup>21</sup>. In the final step  
152 of the catalytic cycle, coenzyme A is released from the tetrahedral intermediate as a  
153 leaving group to produce acetyl-phosphinothricin (Fig. 4c).

154 The BAR/CoA/phosphinothricin ternary structure also reveals active-site residues  
155 involved in phosphinothricin binding. Within each active site, the methylphosphoryl  
156 group of the substrate engages hydrophobic interactions with the surrounding F36, G127,  
157 and V161 from the same monomer, whereas the two phosphoryl oxygen atoms are  
158 coordinated by K78, R80, and Y83 from the  $\beta$ 3-loop- $\alpha$ 3 region of the neighboring  
159 monomer via a set of hydrogen bonds and electrostatic interactions (Fig. 4b).  
160 Furthermore, the amino acid group of phosphinothricin is properly positioned at the  
161 catalytic center by a hydrogen-bond network involving the backbone carbonyl group of  
162 V125 and the side chains of T90 and Y92 (Fig. 4b). Despite various attempts using co-  
163 crystallization and soaking techniques, structures of BAR containing aminoadipate or  
164 tryptophan could not be obtained, reflecting the low affinity of these nonspecific  
165 substrates to BAR. Simulated docking of these substrates within the active site of the  
166 BAR/CoA/phosphinothricin structure reveals fewer favorable interactions as well as  
167 potential steric clashes with the surrounding residues compared to phosphinothricin (Fig.  
168 4d).

169 Site-directed mutagenesis followed by biochemical assays confirmed the roles of  
170 many active-site residues predicted by structural analysis (Fig. 4e and Supplementary  
171 Fig. 10). Mutating the catalytic E88 to alanine or glutamine greatly reduces the activity of  
172 BAR toward phosphinothricin and aminoadipate. Nevertheless, these mutants exhibit  
173 higher activity toward tryptophan than that of the wild-type enzyme at the substrate

174 concentration tested (Fig. 4e), suggesting that tryptophan may be deprotonated through  
175 an alternative mechanism independent of E88 and/or the first deprotonation step is not  
176 rate-limiting for BAR-catalyzed acetyl-tryptophan formation. H137A and Y107F mutants  
177 failed to yield sufficient soluble recombinant protein (Supplementary Fig. 10), preventing  
178 the role of the oxyanion hole in catalysis to be directly assessed. We thus probed this  
179 indirectly by mutating S133, a residue that forms a hydrogen bond with the imidazole  
180 ring  $\pi$ -nitrogen of H137 (Fig. 4b). The resulting S133A mutant exhibits completely  
181 abolished N-acetyltransferase activity toward the three tested substrates, suggesting an  
182 essential role of S133 in catalysis, likely through proper positioning of the imidazole  
183 ring of the histidine within the oxyanion hole (Fig. 4e and Supplementary Fig. 9).  
184 Mutants affecting phosphinothricin-binding residues, including F36A, K78A, R80A,  
185 Y83F, Y92F, generally show significantly reduced activity toward phosphinothricin and  
186 amino adipate, while K78A and Y83F display increased activity toward the more  
187 hydrophobic substrate tryptophan compared to the wild-type enzyme (Fig. 4e).

188         With the structural information of BAR in hand, we sought to engineer BAR  
189 through structure-guided mutagenesis to repress its undesired nonspecific activities  
190 toward amino adipate and tryptophan while maintaining its native activity against  
191 phosphinothricin. We selected residue positions N35, Y73, T90, Y92, and V125 for  
192 targeted mutagenesis based on structural analysis as well as multiple sequence alignment  
193 containing BAR, PAT, and other closely related homologs from bacteria (Fig. 4b, 4d and  
194 Supplementary Fig. 11). A set of eleven mutants was first characterized *in vitro* (Fig. 4e),  
195 and eight of them were further tested in transgenic Arabidopsis (Fig. 4f and 4g). All eight  
196 BAR mutants confer phosphinothricin resistance in Arabidopsis T1 and T2 generations

197 (Fig. 4f, Supplementary Fig. 12-14). Metabolic profiling of these transgenic lines  
198 confirmed that mutations in select active-site residues of BAR can modulate the *in vivo*  
199 nonspecific activities of BAR toward amino adipate and tryptophan (Fig. 4g). Notably,  
200 transgenic Arabidopsis plants containing Y73F, Y92F, N35T, N35D, T90A, V125L, or  
201 V125I BAR mutants display significantly reduced levels of acetyl-amino adipate  
202 compared to plants containing wild-type BAR (Fig. 4g). Moreover, plants expressing  
203 Y73F, Y92F or T90A BAR mutants exhibit significantly reduced levels of both acetyl-  
204 amino adipate and acetyl-tryptophan compared to plants containing wild-type BAR. These  
205 observed differences in acetyl-amino adipate and acetyl-tryptophan levels are not due to  
206 BAR protein levels in transgenic plants (Supplementary Fig. 15), but are consistent with  
207 the altered catalytic activities of various BAR mutants measured *in vitro* (Fig. 4e and  
208 Supplementary Fig. 16). Subsequent analysis of N35T and Y92F revealed that both  
209 mutants exhibit compromised affinity toward native substrate phosphinothricin *in vitro*  
210 compared to wild-type BAR. However, N35T and Y92F retain largely unaltered catalytic  
211 speed *in vitro* and confer level of resistance to phosphinothricin *in planta* similar to that  
212 of wild-type BAR (Supplementary Fig. 16a and Supplementary Fig. 14). Furthermore,  
213 both mutants show more pronounced reduced catalytic activity toward one or both non-  
214 native substrates as compared to phosphinothricin (Supplementary Fig. 16).

215 Transgenic expression of enzymes catalyzing a variety of desirable biochemical  
216 reactions in heterologous hosts is a common strategy in both basic biological research  
217 and translational biotechnology. Prominent examples include reporter enzymes, such as  
218 firefly luciferase and  $\beta$ -glucuronidase, antibiotic/herbicide markers, such as  
219 aminoglycoside kinase that confers kanamycin resistance and BAR, and many enzymes

220 used for metabolic engineering purposes in microbes and higher eukaryotes<sup>22</sup>. Although  
221 enzymes are generally considered as perfected catalysts with superior substrate  
222 specificity and predictable catalytic mechanism, increasing evidences have raised  
223 awareness of the unpredictable behaviors of enzymes and their profound implication in  
224 natural and directed evolution of new enzymatic functions<sup>23</sup>. However, whether and how  
225 heterologous expression of a foreign enzyme would interfere with the native metabolic  
226 system remains an open question to be addressed on a case-by-case basis.

227         In this study, we discovered that transgenic expression of the herbicide-resistance  
228 enzyme BAR of bacterial origin indeed acetylate two endogenous amino acids, resulting  
229 in the ectopic accumulation of acetyl-aminoadipate and acetyl-tryptophan. While acetyl-  
230 tryptophan is a naturally occurring metabolite found in numerous plant species, including  
231 *Arabidopsis*, *Salsola collina*, *Glycine max*, *Solanum lycopersicum*, *Cocos nucifera*, and  
232 *Ginkgo biloba*<sup>24,25</sup>, to the best of our knowledge, acetyl-aminoadipate has never been  
233 reported as an endogenous plant metabolite. Interestingly, in line with our findings, a  
234 recent study reported the ectopic accumulation of acetyl-aminoadipate in the flower  
235 tissue of a BAR-containing T-DNA mutant of *Arabidopsis*, which could not be  
236 rationalized by the mutated gene<sup>26</sup>. Despite the widespread use of BAR in GE crops<sup>2,27</sup>  
237 and the extensive testing and deregulation processes associated with this trait over the  
238 past few decades<sup>1,3,17,28,29</sup>, such phenomenon was not reported elsewhere, probably due to  
239 technological limitation in metabolic profiling in the past. Studies have demonstrated  
240 indirect effects of BAR-containing transgenes in transgenic lines with high BAR  
241 expression, such as reduced fitness and modified amino acid levels, but without  
242 identifying their direct causes<sup>5,6</sup>. However, the implications of our findings about the

243 nonspecific activities of BAR on crop fitness and human/animal health are yet to be  
244 evaluated in future studies.

245 Our findings suggest that untargeted metabolomics analysis could be a useful  
246 methodology for future assessment of GE plants<sup>7</sup>. This study also provides solutions to  
247 reduce the nonspecific activities of BAR through structure-guided enzyme engineering so  
248 that its intended herbicide-degrading activity can be maximally insulated from the  
249 metabolome of the host.

250

#### 251 CORRESPONDING AUTHORS

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253

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270

271 AUTHOR CONTRIBUTIONS

272 B.C., S.A., S.H. and J.K.W. designed experiments; B.C., R.H., L.G., R.F. and S.A.  
273 performed experiments; B.C., R.H., L.G. and J.K.W. analyzed data; B.C., S.H., S.A. and  
274 J.K.W. wrote the manuscript.

275

276 COMPETING FINANCIAL INTERESTS

277 B.C. and J.K.W. have filed a patent application on BAR and PAT mutants described in  
278 this paper that show altered acetyltransferase activity.

279

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## 281 REFERENCES

- 282 1 Wehrmann, A., Van Vliet, A., Opsomer, C., Botterman, J. & Schulz, A. The similarities of *BAR*  
283 and *PAT* gene products make them equally applicable for plant engineers. *Nat. Biotechnol.* **14**,  
284 1274-1278 (1996).
- 285 2 Duke, S. O. Taking stock of herbicide-resistant crops ten years after introduction. *Pest. Manag.*  
286 *Sci.* **61**, 211-218 (2005).
- 287 3 Thompson, C. J. *et al.* Characterization of the herbicide-resistance gene *BAR* from *Streptomyces*  
288 *hygroscopicus*. *EMBO J.* **6**, 2519-2523 (1987).
- 289 4 Wohlleben, W. *et al.* Nucleotide sequence of the phosphinothricin N-acetyltransferase gene from  
290 *Streptomyces viridochromogenes* Tü494 and its expression in *Nicotiana tabacum*. *Gene* **70**, 25-37  
291 (1988).
- 292 5 Ren, Y. F. *et al.* A comparative proteomics approach to detect unintended effects in transgenic  
293 *Arabidopsis*. *J. Genet. Genomics* **36**, 629-639 (2009).
- 294 6 Brown, R. H., Raboy, V. & Bregitzer, P. Unintended consequences: high phosphinothricin  
295 acetyltransferase activity related to reduced fitness in barley. *In Vitro Cell. Dev. Biol. Plant* **49**,  
296 240-247 (2013).
- 297 7 The National Academies. *Genetically Engineered Crops: Experiences and Prospects*. (National  
298 Academies Press, 2016).
- 299 8 Schenk, N. *et al.* The chlorophyllases *AtCLH1* and *AtCLH2* are not essential for senescence-  
300 related chlorophyll breakdown in *Arabidopsis thaliana*. *FEBS Lett.* **581**, 5517-5525 (2007).
- 301 9 Samson, F. *et al.* FLAGdb/FST: a database of mapped flanking insertion sites (FSTs) of  
302 *Arabidopsis thaliana* T-DNA transformants. *Nucleic Acids Res.* **30**, 94-97 (2002).
- 303 10 Sessions, A. *et al.* A high-throughput *Arabidopsis* reverse genetics system. *Plant Cell* **14**, 2985-  
304 2994 (2002).
- 305 11 Alonso, J. M. *et al.* Genome-wide insertional mutagenesis of *Arabidopsis thaliana*. *Science* **301**,  
306 653-657 (2003).
- 307 12 Rosso, M. G. *et al.* An *Arabidopsis thaliana* T-DNA mutagenized population (GABI-Kat) for  
308 flanking sequence tag-based reverse genetics. *Plant Mol. Biol.* **53**, 247-259 (2003).
- 309 13 Soudry, E., Ulitzur, S. & Gepstein, S. Accumulation and remobilization of amino acids during  
310 senescence of detached and attached leaves: *in planta* analysis of tryptophan levels by  
311 recombinant luminescent bacteria. *J. Exp. Bot.* **56**, 695-702 (2005).
- 312 14 Hörtensteiner, S. & Feller, U. Nitrogen metabolism and remobilization during senescence. *J. Exp.*  
313 *Bot.* **53**, 927-937 (2002).
- 314 15 Arruda, P., Kemper, E. L., Papes, F. & Leite, A. Regulation of lysine catabolism in higher plants.  
315 *Trends Plant Sci.* **5**, 324-330 (2000).
- 316 16 Zhu, X., Tang, G., Granier, F., Bouchez, D. & Galili, G. A T-DNA insertion knockout of the  
317 bifunctional *LYSINE-KETOGLUTARATE REDUCTASE/SACCHAROPINE DEHYDROGENASE*  
318 gene elevates lysine levels in *Arabidopsis* seeds. *Plant Physiol.* **126**, 1539-1545 (2001).
- 319 17 Vinnemeier, J., DrogeLaser, W., Pistorius, E. K. & Broer, I. Purification and partial  
320 characterization of the *Streptomyces viridochromogenes* Tü494 phosphinothricin-N-  
321 acetyltransferase mediating resistance to the herbicide phosphinothricin in transgenic plants. *Z.*  
322 *Naturforsch. C.* **50**, 796-805 (1995).
- 323 18 Dyda, F., Klein, D. C. & Hickman, A. B. GCN5-related N-acetyltransferases: a structural  
324 overview. *Annu. Rev. Bioph. Biom.* **29**, 81-103 (2000).
- 325 19 Vetting, M. W. *et al.* Structure and functions of the GNAT superfamily of acetyltransferases.  
326 *Arch. Biochem. Biophys.* **433**, 212-226 (2005).
- 327 20 Srivastava, P. *et al.* Structural characterization of a GCN5-related N-acetyltransferase from  
328 *Staphylococcus aureus*. *PLoS ONE* **9** (2014).
- 329 21 Rojas, J. R. *et al.* Structure of Tetrahymena GCN5 bound to coenzyme A and a histone H3  
330 peptide. *Nature* **401**, 93-98 (1999).
- 331 22 Woolston, B. M., Edgar, S. & Stephanopoulos, G. Metabolic engineering: past and future. *Annu.*  
332 *Rev. Chem. Biomol. Eng.* **4**, 259-288 (2013).
- 333 23 Weng, J. K. & Noel, J. P. The remarkable pliability and promiscuity of specialized metabolism.  
334 *Cold Spring Harb. Symp. Quant. Biol.* **77**, 309-320 (2012).



335 24 Jin, Y.-S. *et al.* Chemical and biologically active constituents of *Salsola collina*. *Chem. Nat.*  
336 *Compd.* **47**, 257-260 (2011).

337 25 Yu, P., Hegeman, A. D. & Cohen, J. D. A facile means for the identification of indolic compounds  
338 from plant tissues. *Plant J.* **79**, 1065-1075 (2014).

339 26 Bruckhoff, V. *et al.* Functional characterization of CYP94-genes and identification of a novel  
340 jasmonate catabolite in flowers. *PLoS One* **11**, e0159875 (2016).

341 27 Green, J. M. & Owen, M. D. Herbicide-resistant crops: utilities and limitations for herbicide-  
342 resistant weed management. *J. Agric. Food Chem.* **59**, 5819-5829 (2011).

343 28 Herouet, C. *et al.* Safety evaluation of the phosphinothricin acetyltransferase proteins encoded by  
344 the *PAT* and *BAR* sequences that confer tolerance to glufosinate-ammonium herbicide in  
345 transgenic plants. *Regul. Toxicol. Pharm.* **41**, 134-149 (2005).

346 29 Dan, Y. *Plant transformation technology revolution in last three decades: historical technology*  
347 *developments*. (Bentham Science Publishers, 2012).

348 30 Song, W. Y., Choi, K. S., Alexis de, A., Martinoia, E. & Lee, Y. Brassica juncea plant cadmium  
349 resistance 1 protein (BjPCR1) facilitates the radial transport of calcium in the root. *Proc. Natl.*  
350 *Acad. Sci. U.S.A.* **108**, 19808-19813 (2011).

351 31 Foetzki, A. *et al.* Surveying of pollen-mediated crop-to-crop gene flow from a wheat field trial as  
352 a biosafety measure. *GM Crops Food* **3**, 115-122 (2012).

353 32 Czechowski, T., Stitt, M., Altmann, T., Udvardi, M. K. & Scheible, W. R. Genome-wide  
354 identification and testing of superior reference genes for transcript normalization in Arabidopsis.  
355 *Plant Physiol.* **139**, 5-17 (2005).

356 33 Chambers, M. C. *et al.* A cross-platform toolkit for mass spectrometry and proteomics. *Nat.*  
357 *Biotechnol.* **30**, 918-920 (2012).

358 34 Gowda, H. *et al.* Interactive XCMS Online: simplifying advanced metabolomic data processing  
359 and subsequent statistical analyses. *Anal. Chem.* **86**, 6931-6939 (2014).

360 35 Smith, C. A. *et al.* METLIN: a metabolite mass spectral database. *Ther. Drug Monit.* **27**, 747-751  
361 (2005).

362 36 Tropea, J. E., Cherry, S. & Waugh, D. S. Expression and purification of soluble His(6)-tagged  
363 TEV protease. *Methods Mol. Biol.* **498**, 297-307 (2009).

364 37 Batty, T. G., Kontogiannis, L., Johnson, O., Powell, H. R. & Leslie, A. G. iMOSFLM: a new  
365 graphical interface for diffraction-image processing with MOSFLM. *Acta Crystallogr. D* **67**, 271-  
366 281 (2011).

367 38 Evans, P. Scaling and assessment of data quality. *Acta Crystallogr. D* **62**, 72-82 (2006).

368 39 Adams, P. D. *et al.* PHENIX: a comprehensive Python-based system for macromolecular structure  
369 solution. *Acta Crystallogr. D* **66**, 213-221 (2010).

370 40 Emsley, P. & Cowtan, K. Coot: model-building tools for molecular graphics. *Acta Crystallogr. D*  
371 **60**, 2126-2132 (2004).

372 41 Goldstein, A. L. & McCusker, J. H. Three new dominant drug resistance cassettes for gene  
373 disruption in *Saccharomyces cerevisiae*. *Yeast* **15**, 1541-1553 (1999).

374 42 Engler, C. *et al.* A Golden Gate modular cloning toolbox for plants. *ACS Synth. Biol.* (2014).

375 43 Clough, S. J. & Bent, A. F. Floral dip: a simplified method for *Agrobacterium*-mediated  
376 transformation of *Arabidopsis thaliana*. *Plant J.* **16**, 735-743 (1998).

377 44 Schelbert, S. *et al.* Pheophytin pheophorbide hydrolase (pheophytinase) is involved in chlorophyll  
378 breakdown during leaf senescence in Arabidopsis. *Plant Cell* **21**, 767-785 (2009).

379 45 Christ, B. *et al.* MES16, a member of the methyltransferase protein family, specifically demethylates  
380 fluorescent chlorophyll catabolites during chlorophyll breakdown in Arabidopsis. *Plant Physiol.*  
381 **158**, 628-641 (2012).

382 46 Guyer, L. *Characterization of dephytylation and dechelation, two early steps of chlorophyll*  
383 *breakdown in leaves and fruits* PhD thesis, Zurich, (2015).

384 47 Christ, B. *et al.* Cytochrome P450 CYP89A9 is involved in the formation of major chlorophyll  
385 catabolites during leaf senescence in Arabidopsis. *Plant Cell* **25**, 1868-1880 (2013).

386 48 Perez-Perez, J. M. *et al.* Functional redundancy and divergence within the Arabidopsis  
387 RETICULATA-RELATED gene family. *Plant Physiol.* **162**, 589-603 (2013).

388 49 Christ, B. *Chlorophyll breakdown: modifications of colorless chlorophyll catabolites* PhD thesis,  
389 Zurich, (2013).

390 50 Zufferey, M. *et al.* The novel chloroplast outer membrane kinase KOC1 is a required component  
391 of the plastid protein import machinery. *J. Biol. Chem.* **292**, 6952-6964 (2017).  
392 51 Pulido, P., Llamas, E. & Rodriguez-Concepcion, M. Both Hsp70 chaperone and Clp protease  
393 plastidial systems are required for protection against oxidative stress. *Plant Signal. Behav.* **12**  
394 (2017).  
395 52 Waterhouse, A. M., Procter, J. B., Martin, D. M., Clamp, M. & Barton, G. J. Jalview Version 2 - a  
396 multiple sequence alignment editor and analysis workbench. *Bioinformatics* **25**, 1189-1191  
397 (2009).  
398

399 **FIGURE LEGENDS**

400 **Figure 1 | Accumulation of acetyl-aminoadipate and acetyl-tryptophan in senescent**  
401 **leaves of Arabidopsis carrying the BAR transgene. a**, Metabolite profiles of senescent  
402 leaves from Wassilewskija (Ws) and *clh2-1* (FLAG-1), displayed as base peak  
403 chromatograms (BPC), reveal the ectopic accumulation of acetyl-aminoadipate (**1**) and  
404 acetyl-tryptophan (**2**). BPC traces of four biological replicates are displayed. **b**,  
405 Comparative levels of acetyl-aminoadipate and acetyl-tryptophan in Arabidopsis mutants  
406 from different insertional mutant collections that contain either BAR (SAIL and FLAG)  
407 or alternative selection marker genes (SALK (*NTPII*, kanamycin resistance) and GABI  
408 (*SULI*, sulfadiazine resistance)). Error bars, mean  $\pm$  s.d. (n = 3 biological replicates). This  
409 experiment was repeated at least three times with similar results. See Supplementary Fig.  
410 2 for absolute quantification. a.u., arbitrary unit; FW, fresh weight; n.d., not detected

411

412 **Figure 2 | BAR-dependent accumulation of acetyl-aminoadipate and acetyl-**  
413 **tryptophan is linked to nitrogen remobilization during senescence. a**, Aminoadipate  
414 is derived from the lysine degradation pathway in plants, which can be metabolized by  
415 BAR as a nonspecific substrate. **b**, Comparative levels of acetyl-aminoadipate and acetyl-  
416 tryptophan in green and senescent leaves from the heterozygous (He) and homozygous  
417 (Ho) FLAG\_ *lkrsdh* mutant, harboring a BAR-containing T-DNA that abolishes the

418 Arabidopsis *LKR/SDH* gene. Error bars, mean  $\pm$  s.d. (n = 4 biological replicates). a.u.,  
419 arbitrary unit; n.d., not detected; Ws, Wassilewskija wild-type plants.

420

421 **Figure 3 | In vitro enzyme kinetic assays of BAR against native and non-native**  
422 **substrates.** An apparent  $K_M$  value of  $132 \pm 19.2 \mu\text{M}$  was obtained for phosphinothricin,  
423 similar to previously published data<sup>1,3,17</sup>.  $V_{max}$ ,  $k_{cat}$ ,  $k_{cat}/K_m$  and  $V_{max}/K_m$  values for  
424 phosphinothricin are also indicated, as well  $V_{max}/K_m$  values for aminoadipate and  
425 tryptophan (estimated from Lineweaver-Burk plots). Aminoadipate and tryptophan are in  
426 vitro substrates of BAR but both substrates reached solubility limit before reaching  
427 saturation concentration for BAR. Negative controls (open circles) were performed in  
428 absence of BAR at the highest substrate concentration tested (20 mM).

429

430 **Figure 4 | Structural basis for amino acid N-acetylation catalyzed by BAR and**  
431 **structure-guided engineering of BAR with reduced nonspecific activities. a,** Cartoon  
432 representation of BAR homodimer in complex with phosphinothricin and CoA. Two  
433 monomers of the dimer are colored in blue and yellow respectively. **b,** Close-up view of  
434 the BAR active site. The  $|2\text{Fo}-\text{Fc}|$  omit electron density map (contoured at  $3.0 \sigma$ ) is  
435 shown for phosphinothricin. **c,** Proposed catalytic mechanism of BAR. **d,** Docking of  
436 tryptophan and aminoadipate within the BAR active site reveals reduced favorable  
437 contacts compared to phosphinothricin. **e,** Enzyme activity assays using purified BAR  
438 mutant proteins against phosphinothricin (0.2mM), aminoadipate (1 mM) and tryptophan  
439 (1 mM). Wild-type BAR (WT BAR) and PAT from *Streptomyces viridochromogenes*  
440 were also examined as controls. Assays were terminated during the initial linear rate of

441 product formation. The relative amount of product formed by each BAR mutant was  
442 normalized to WT BAR for each substrate (value of 1). Error bars, mean  $\pm$  s.d. (n = 3  
443 technical replicates). **f**, Photographs of Arabidopsis T1 lines transformed with select BAR  
444 mutants 10 days after phosphinothricin treatment (see also Supplementary Fig. 12-14).  
445 Scale bar = 0.3 cm. **g**, Comparative levels of acetyl-aminoadipate and acetyl-tryptophan  
446 in phosphinothricin-resistant T2 Arabidopsis plants transformed with selected BAR  
447 mutants. Error bars, mean  $\pm$  s.d. (n = 5-6 biological replicates: Y73F (6), Y92F (6), N35T  
448 (5), N35S (5), T90A (6), V125T (5), V125L (6), V125I (6), WT BAR (5), PAT (5)).  
449 Significance levels were indicated based on one-way ANOVA with Dunnett's test for  
450 multiple comparisons to WT BAR. a, p-value < 0.1; b, p-value < 0.05; c, p-value < 0.01;  
451 a.u., arbitrary unit.

452

## 453 **Materials and Methods**

### 454 **Plant materials**

455 Arabidopsis (*Arabidopsis thaliana*) Columbia-0 (Col-0) and Wassilewskija (Ws) were  
456 used as wild types. T-DNA insertion lines were from the following collections: SALK  
457 lines<sup>11</sup>: SALK\_130606 (SALK\_1), SALK\_051823C (SALK\_2), SALK\_110649  
458 (SALK\_3); SAIL lines<sup>10</sup>: SAIL\_1165\_B02 (SAIL\_1), SAIL\_503\_C03 (SAIL\_2),  
459 SAIL\_1235\_D10 (SAIL\_3); GABI lines<sup>12</sup>: GABI\_453E01 (GABI\_1), GABI\_833F02  
460 (GABI\_2), GABI\_453A08 (GABI\_3); FLAG lines<sup>9</sup>: FLAG\_076H05 (*chl2-I*<sup>8</sup>; FLAG\_1),  
461 FLAG\_271B02 (FLAG\_2), FLAG\_495A09 (FLAG\_3), FLAG\_271B12 (FLAG\_1*kr**sdh*).  
462 SALK, SAIL and GABI lines were obtained from the European Arabidopsis Stock  
463 Center (<http://arabidopsis.info/>). The FLAG lines were obtained from the INRA

464 Versailles Arabidopsis Stock Center (<http://publiclines.versailles.inra.fr/>). Homozygous  
465 (and heterozygous for FLAG\_ *lkr*sdh) plants were identified by PCR using T-DNA- and  
466 gene-specific primers.

467 Arabidopsis T-DNA lines used for untargeted metabolomics and relative  
468 quantification of acetyl-aminoadipate and acetyl-tryptophan were grown on soil under a  
469 12-h-light/12-h-dark photoperiod with fluorescent light of 80 to 120  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$   
470 at 22°C and 60% relative humidity. For senescence induction, leaves from 5-week-old  
471 plants were excised and incubated in permanent darkness on wet filter paper for 8 d at  
472 ambient temperature. Transgenic Arabidopsis lines transformed with BAR mutants and  
473 Arabidopsis T-DNA lines used for absolute quantification of acetyl-aminoadipate and  
474 acetyl-tryptophan were grown on soil under a 16-h-light/8-h-dark photoperiod with  
475 fluorescent light of 80 to 120  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$  at 22°C and 60% relative humidity.  
476 For senescence induction, leaves from phosphinothricin-resistant, 4-week-old plants were  
477 excised and incubated in permanent darkness on wet filter paper for 6 d at ambient  
478 temperature. For measuring the expression of LKR/SDH in FLAG\_271B12, seedlings  
479 were grown for 7 days on ½ Murashige and Skoog (MS) plates containing 1% sucrose.

480 Phosphinothricin-resistant *Glycine max* (Liberty Link trait A2704-12, 283 Morrill  
481 MC-116, CredeNZ CZ 3841 LL, Bayer CropScience), wild-type (non-isogenic) *Glycine*  
482 *max* (Chiba Green; High Mowing Organic Seed), lines were grown on soil under a 16-h-  
483 light/8-h-dark photoperiod with fluorescent light of 80 to 120  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$  at  
484 22°C and 60% relative humidity. Green and senescent leaf samples were collected from  
485 40-days old plants. This experiment was repeated once with similar results.

486 Phosphinothricin-resistant *Brassica napus* (Liberty Link trait L252, Bayer  
487 CropScience) and wild-type (non-isogenic) *Brassica napus* (NDC-E12131, NDC-E13285  
488 and NDC-E12027) lines were grown on soil under a 16-h-light/8-h-dark photoperiod with  
489 fluorescent light of 80 to 120  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$  at 22°C and 60% relative humidity.  
490 For senescence induction, fully developed cotyledons were excised and incubated in  
491 permanent darkness on wet filter paper for 5-7 days at ambient temperature. This  
492 experiment was done once.

493 Wild-type (isogenic) and phosphinothricin-resistant *Brassica juncea*<sup>30</sup> were  
494 grown on soil under a 16-h-light/8-h-dark photoperiod with fluorescent light of 80 to 120  
495  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$  at 22°C and 60% relative humidity. For senescence induction, fully  
496 developed cotyledons were excised and incubated in permanent darkness on wet filter  
497 paper for 5-7 d at ambient temperature.

498 Wild-type (isogenic) and phosphinothricin-resistant *Triticum aestivum*<sup>31</sup> were  
499 grown on soil under a 16-h-light/8-h-dark photoperiod with fluorescent light of 80 to 120  
500  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$  at 22°C and 60% relative humidity. For senescence induction,  
501 leaves were excised and incubated in permanent darkness on wet filter paper for 5-7 d at  
502 ambient temperature.

503

#### 504 **RNA Isolation and qRT-PCR**

505 Total RNA was extracted using a Qiagen RNeasy Plant Mini Kit according to  
506 manufacturer's instructions (DNase treatment was performed on-column). The  
507 concentration and purity of RNA were determined by absorbance at 260/280 nm. First-  
508 strand cDNA was synthesized from 1  $\mu\text{g}$  of RNA using SuperScript III Reverse

509 Transcriptase with Oligo dT primers (Thermo Scientific). Reactions were run on a  
510 QuantStudio 6 system machine (Thermo Scientific) using Sybr Green Master Mix  
511 (Thermo Scientific) using primer listed in Supplementary Fig. 4 and Supplementary  
512 Table 3. Gene expression values were calculated using Ct values and normalized using  
513 the reference gene At1g13320<sup>32</sup>.

514

### 515 **Metabolite extraction**

516 Arabidopsis and *Brassica napus* samples were collected in 2 mL Eppendorf tubes  
517 containing 500 µL of 1.5 mm glass beads, weighted and snap-frozen in liquid nitrogen.  
518 The frozen samples were ground using a MM300 Mixer Mill (Retsch) at 30 Hz for 5 min  
519 and stored at -80°C until further processing. *Glycine max* samples were snap-frozen in  
520 liquid nitrogen and ground with a mortar and pestle. Metabolites were extracted using 5-  
521 10 (for leaf samples) or 10 -50 volumes (for seed samples; w/v) of ice-cold extraction  
522 buffer (80% methanol, 20% water, 0.1% formic acid (v/v/v)). Extracts were homogenized  
523 at 30 Hz for 5 min and centrifuged (14,000-16,000 g, 4°C). After re-centrifugation,  
524 supernatants were transferred to LC vials and analyzed by LC-MS.

525

### 526 **LC-MS analysis of Arabidopsis T-DNA mutants, *Brassica juncea* and *Triticum*** 527 ***aestivum* (untargeted metabolomics and relative quantification of acetyl-** 528 **aminoadipate and acetyl-tryptophan)**

529 The LC-MS instrument was composed of an Ultimate 3000 Rapid Separation LC  
530 system (Thermo Scientific) coupled to a Bruker Compact ESI-Q-TOF (Bruker  
531 Daltonics). The reverse-phase chromatography system consisted of an 150 mm C18

532 column (ACQUITY UPLC™ BEH, 1.7  $\mu\text{m}$ , 2.1 x 150 mm, Waters), which was  
533 developed using LC-MS solvents (Chemie Brunschwig) with a gradient (flow rate of 0.3  
534  $\text{mL min}^{-1}$ ) of solvent B (acetonitrile with 0.1% (v/v) formic acid) in solvent A (water with  
535 0.1% (v/v) formic acid) as follows (all (v/v)): 5% for 0.5 min, 5% to 100% in 11.5 min,  
536 100% for 4 min, 100% to 5% in 1 min and 5% for 1 min. Electrospray ionization (ESI)  
537 source conditions were set as follows: gas temperature, 220°C; drying gas, 9  $\text{L min}^{-1}$ ;  
538 nebulizer, 2.2 BAR; capillary voltage, 4500 V; end plate offset, 500 V. Tuning conditions  
539 were set as follows: funnel 1 RF, 250 Vpp; funnel 2 RF, 150 Vpp; isCID energy, 0 eV;  
540 hexapole RF, 50 Vpp; quadrupole ion energy, 3.0 eV; quadrupole low mass, 90 m/z;  
541 collision cell, 6 eV; pre-pulse storage time, 3  $\mu\text{s}$ . The instrument was set to acquire over  
542 the m/z range 50-1300, with an acquisition rate of 4 spectra  $\text{s}^{-1}$ . Conditions for MS<sup>2</sup> of  
543 automatically selected precursors (data-dependent MS<sup>2</sup>) were set as follows: threshold,  
544 1000 counts; active smart exclusion (5x); active exclusion (exclude after 3 spectra,  
545 release after 0.2 min, reconsider precursor if current intensity/previous intensity is  $\geq 5$ );  
546 number of precursors, 3; active stepping (basic mode, timing 50%-50%, collision RF  
547 from 350 to 450 Vpp, transfer time from 65 to 80  $\mu\text{s}$ , collision energy from 80 to 120%).  
548 All data were recalibrated internally using pre-run injection of sodium formate (10 mM  
549 sodium hydroxide in 0.2% formic acid, 49.8% water, 50% isopropanol (v/v/v)). After  
550 data recalibration using DataAnalysis (version 4.2, Bruker Daltonics) and data conversion  
551 to mzXML format using ProteoWizard MSConvert<sup>33</sup>, metabolite features detected in Ws  
552 and FLAG\_076H05 (senescent leaves, four replicates) were aligned according to  
553 retention time and relatively quantified using XCMS online<sup>34</sup> (pairwise comparison using  
554 XCMS online pre-set parameters “UPLC/Bruker Q-TOF”). Up-regulated features in



555 FLAG\_076H05 were identified at retention times of 2.8 min (labeled as “1” in Fig. 1a,  
556 m/z 204.086 (fold change  $\geq 10$ , p-value  $\leq 0.005$ , intensity threshold 800,000)) and 6.5 min  
557 (labeled as “2” in Fig. 1a, m/z 247.108 (fold change  $\geq 10$ , p-value  $\leq 0.005$ , intensity  
558 threshold 100,000)) and further characterized as ions derived from N-acetyl-D/L-  
559 amino adipate and N-acetyl-D/L-tryptophan, respectively, by database searches in  
560 METLIN<sup>35</sup> using MS and MS<sup>2</sup> spectra. Relative quantification of acetyl-amino adipate  
561 and acetyl-tryptophan in Arabidopsis mutants from different insertion mutant collections  
562 was carried out by QuantAnalysis (version 2.2, Bruker Daltonics) using extracted ion  
563 chromatogram (EIC) traces ( $[M+H]^+$ ). Metabolomics data generated in this study have  
564 been uploaded to the EBI MetaboLights database (<http://www.ebi.ac.uk/metabolights/>)  
565 with the following accession number (MTBLS553).

566

### 567 **Absolute quantification of free amino acids in senescent leaves of Arabidopsis T-** 568 **DNA mutants**

569 The LC-MS instrument was composed of an Ultimate 3000 Rapid Separation LC system  
570 (Thermo Scientific) coupled to a Q-Exactive mass spectrometer (Thermo Scientific). The  
571 HILIC chromatography system consisted of SeQuant ZIC-pHILIC Polymeric column  
572 ( $2.1 \times 150$  mm,  $5 \mu\text{M}$ , EMD Millipore), which was developed using Optima<sup>TM</sup> LC/MS  
573 solvents (Fisher Chemical) with a gradient (flow rate of  $0.15 \text{ mL min}^{-1}$ ) of solvent B  
574 (acetonitrile) in solvent A (20 mM ammonium carbonate, 0.1% ammonium hydroxide) as  
575 follows (all (v/v)): 80% to 20% in 20 min, 80% to 20% in 0.5 min and 80% for 7.5 min.

576 The mass spectrometer was operated in full-scan (resolution,  $70,000$ ; AGC  
577 target,  $1e6$ ; Maximum IT, 20ms) polarity switch mode with the spray voltage set to +/-

578 3.0 kV, the heated capillary held at 275C, and the HESI probe held at 350C. Seventeen  
579 labeled amino acids (MSK-A2-1.2, Cambridge Isotope Laboratories) were added to the  
580 extraction solvent (80% methanol, 20% water) and used as internal standards. Standard  
581 curves were performed for each 25 amino acids. Acetyl-aminoadipate was synthesized  
582 using recombinant BAR as described below and all 24 other amino acids were purchased  
583 from Sigma-Aldrich. Data analysis was performed with Xcalibur (Thermo Scientific).  
584 Note that values for a few amino acids are shown as relative levels in Supplementary Fig.  
585 2 because their concentrations in some samples were more than 10-fold higher than the  
586 highest concentration of the standard.

587

588 **Absolute quantification of acetyl-aminoadipate and acetyl-tryptophan in seeds of**  
589 ***Arabidopsis* T-DNA mutants and various tissues of *Glycine max* and *Brassica napus***

590 Metabolites were extracted as described above and then analyzed on an Ultimate  
591 3000 Rapid Separation LC system (Thermo Scientific) coupled to a TSQ Quantum  
592 Access MAX triple-quadrupole mass spectrometer (Thermo Scientific). The reverse-  
593 phase chromatography system consisted of an 150 mm C18 column (Kinetex 2.6  $\mu\text{m}$   
594 silica core shell C18 100Å pore, Phenomenex) which was developed using Optima™  
595 LC/MS solvents (Fisher Chemical) with a gradient (flow rate of 0.6 mL min<sup>-1</sup>) of solvent  
596 B (acetonitrile with 0.1% (v/v) formic acid) in solvent A (water with 0.1% (v/v) formic  
597 acid) as follows (all (v/v)): 2% for 3 min, 2% to 99% in 9 min, 99% for 4 min, 99% to  
598 2% in 1 min and 2% for 1 min. The mass spectrometer was configured to perform two  
599 selected-reaction-monitoring scans, each for 0.5 seconds, for acetyl-aminoadipate and  
600 acetyl-tryptophan. The m/z resolution of Q1 was set to 0.4 FWHM, the nitrogen collision

601 gas pressure of Q2 was set to 1.5 mTorr, and the Q3 scan width was set to 0.500 m/z in  
602 both cases. Selected reaction monitoring for acetyl-aminoadipate was as follows:  
603 precursor ion selection at 204.086 m/z on positive ion mode, fragmentation at 10 V, and  
604 product ion selection at 144.065 m/z. Selected reaction monitoring for acetyl-tryptophan  
605 was as follows: precursor ion selection at 247.107 m/z on positive ion mode,  
606 fragmentation at 20 V, and product ion selection at 188.070 m/z. Acetyl-aminoadipate  
607 was synthesized using recombinant BAR as described below and used as standard. Pure  
608 acetyl-tryptophan was purchased from Sigma-Aldrich.

609

#### 610 **Heterologous expression of wild-type BAR and activity determination**

611 The BAR coding sequence was amplified by PCR (KaPa HiFi HotStart  
612 polymerase; KaPa Biosystems) from genomic DNA extracted from homozygous plants of  
613 the SAIL line SAIL\_1165\_B02 using primers SAIL\_BAR\_F\_pPROEX and  
614 SAIL\_BAR\_R\_pPROEX (see Table S3) and then cloned into pProEX Hta (Invitrogen)  
615 via *EcoRI* and *HindIII* resulting in a 6xHis-BAR fusion construct.

616 6xHis-tagged BAR protein was expressed in *E. coli* BL21(DE3) grown in Terrific  
617 Broth medium. At an optical density at 600 nm of 0.6, protein expression was induced  
618 with 1.0 mM IPTG and cells were grown at 37°C for 2.5 h. Cells from 1 L culture were  
619 harvested by centrifugation and resuspended in 25 mL binding buffer (50 mM Tris-HCl  
620 pH 8, 500 mM NaCl, 30 mM imidazole). All the following steps were carried out at 4°C.  
621 Cell lysis was performed using a microfluidizer (HC-8000, Microfluidics). The lysate  
622 was centrifuged (16,000 g) for 20 min, and the 6xHis-tagged BAR protein was purified  
623 by metal affinity (5-ml HisTrap HP column, GE Healthcare) and size-exclusion

624 chromatography (HiLoad 16/600 Superdex 200 pg, GE Healthcare) using an ÄKTA Pure  
625 FPLC system (GE Healthcare). The 6xHis-TEV tag was removed from BAR prior to  
626 size-exclusion chromatography by overnight incubation with 1  $\mu$ g of 6xHis-TEV  
627 protease<sup>36</sup> per 10  $\mu$ g protein in 50 mM Tris-HCl pH 8, 500 mM NaCl, 1 mM  
628 dithiothreitol, followed passage through HisTrap HP column. Purified recombinant BAR  
629 was dialyzed in storage buffer (12.5 mM Tris-HCl pH 8, 50 mM NaCl, 2 mM  
630 dithiothreitol) and concentrated to 13 mg/mL using an ultra-centrifugal filter (10,000 Da  
631 MWCO, Amicon EMD Millipore). The purity of recombinant BAR was assessed by  
632 SDS-PAGE (Supplementary Fig. 6a). Purified BAR was aliquoted, snap-frozen in liquid  
633 nitrogen and stored at -80°C until further use.

634         Enzyme assays were carried out in 2 mM Tris-HCl pH 8 and 10 mM acetyl-CoA  
635 (Sigma-Aldrich; final volume 25  $\mu$ l). Before determining the kinetics of BAR with  
636 different substrates, time-dependent activity of the purified protein was tested at substrate  
637 concentrations of 500  $\mu$ M L-phosphinothricin (glufosinate ammonium, considered as a  
638 1:1 mixture of L- and D- enantiomers; Sigma-Aldrich) or 1 mM (L-aminoadipate and L-  
639 tryptophan; Sigma-Aldrich). Reactions were initiated by the addition of purified BAR at  
640 0.26  $\mu$ M (assays with L-phosphinothricin) or 150  $\mu$ M (assays with aminoadipate or  
641 tryptophan) and incubated at 25°C for the indicated times (Supplementary Fig.3 b-d).  
642 Reactions were stopped by the addition of four volumes of 10% water, 90% acetonitrile  
643 (v/v), 5 mM ammonium formate pH 3. Likewise, substrate concentration-dependence  
644 was determined by incubating assays for 25 min (assays with L-phosphinothricin), 3 h  
645 (assays with aminoadipate) or 7 h (assays with tryptophan; Fig. 3). Stock solutions of  
646 aminoadipate and tryptophan at 60 mM were made in 2 mM Tris-HCl pH 8 supplemented

647 with 1 mM N-nonyl  $\beta$ -D-glucopyranoside and substrate concentration-dependence assays  
648 employing these two substrates contained 0.33 mM N-nonyl  $\beta$ -D-glucopyranoside.  
649 Control assays (Fig. 3) were performed with aminoadipate and tryptophan at 20 mM, but  
650 in the absence of BAR.

651 The assays were analyzed on an Ultimate 3000 Rapid Separation LC system  
652 (Thermo Scientific) coupled to a TSQ Quantum Access MAX triple-quadrupole mass  
653 spectrometer (Thermo Scientific). Assays on phosphinothricin were analyzed as follows.  
654 The normal-phase chromatography system consisted of an 150 mm HILIC column  
655 (Kinetex 2.6  $\mu$ m silica core shell HILIC 100Å pore, Phenomenex), which was developed  
656 using Optima™ LC/MS solvents (Fisher Chemical) with a gradient (flow rate of 0.8 mL  
657  $\text{min}^{-1}$ ) of solvent B (50% water, 50% acetonitrile (v/v), 5 mM ammonium formate pH 3)  
658 in solvent A (10% water, 90% acetonitrile (v/v), 5 mM ammonium formate pH 3) as  
659 follows (all (v/v)): 0% for 2 min, 0% to 70% in 10 min, 70% to 100% in 30 sec, 100% for  
660 90 sec, 100% to 0% in 30 sec and 0% for 3.5 min. The mass spectrometer was configured  
661 to perform selected-ion-monitoring scans of 0.5 seconds using Q3 (center mass m/z:  
662 224.068, scan width 1.0 m/z, scan time 0.5 sec). Assays on aminoadipate and tryptophan  
663 were analyzed as described above for the absolute quantification of acetyl-aminoadipate  
664 and acetyl-tryptophan in planta. Product formation was quantified using standards  
665 synthesized using recombinant BAR (acetyl-phosphinothricin and acetyl-aminoadipate)  
666 or commercially available (acetyl-tryptophan, Sigma-Aldrich).  $K_m$  and  $V_{max}$  value for  
667 phosphinothricin were inferred using the Michaelis-Menten kinetics nonlinear regression  
668 function under Prism 6 (GraphPad).

669

670 **X-ray crystallography**

671 Purified BAR protein was incubated with 1 mM acetyl-CoA for >2 hour prior to  
672 setting crystal trays. Crystals of BAR were obtained after 3 days at 20 °C in hanging  
673 drops containing 1 µL of protein solution (7.5 mg/mL) and 1 µL of reservoir solution  
674 (0.18 M calcium acetate, 0.1 M Tris-HCl pH 7, 18% (w/v) PEG 3000, 0.2% (v/v) N-  
675 nonyl β-D-glucopyranoside, 1 mM acetyl-CoA). Several crystals were soaked in reservoir  
676 solution supplemented with 30 mM L-phosphinothricin for 30-60 min before freezing.  
677 Crystals were frozen in reservoir solution supplemented with 15% (v/v) ethylene glycol.  
678 Acetylation of phosphinothricin occurred during soaking as no density for the acetyl  
679 group of acetyl-CoA was observed in the BAR/CoA/phosphinothricin ternary complex.

680 X-ray diffraction data were collected on the 24-ID-C beam line of the Structural  
681 Biology Center at the Advanced Photon Source (Argonne National Laboratory) equipped  
682 with a Pixel Array Detector (Pilatus-6MF). Diffraction intensities were indexed,  
683 integrated, and scaled with the iMosflm<sup>37</sup> and SCALA<sup>38</sup> programs. Initial phases were  
684 determined by molecular replacement using Phaser under Phenix<sup>39</sup>. The search model  
685 was an ensemble model generated with Ensembler using 8 protein structures homologous  
686 to BAR (PBD codes and % identity to BAR: 2JLM (28%), 3DR8 (35%), 4J3G (31%),  
687 4JXQ (33%), 4MBU (30%), 1VHS (30%), 1YR0 (29%) and 1YVO (35%)). Subsequent  
688 structural building and refinements utilized Phenix programs (TSL was used in early  
689 rounds of refinement)<sup>39</sup>. Coot was used for graphical map inspection and manual  
690 rebuilding of atomic models<sup>40</sup>. Crystallographic calculations were performed using  
691 Phenix. Molecular graphics were produced with the program PyMol.

692

693 **Heterologous expression of BAR mutants and activity determination**

694 Single amino acid mutants of BAR were generated using the QuikChange II site-  
695 directed mutagenesis kit (Agilent Technologies) and 6xHis-BAR in pProEX Hta as  
696 template (see Supplementary Table 3 for primer sequences). PAT from *Streptomyces*  
697 *viridochromogenes* was amplified using primers BAC0327 and BAC0328 from pAG31  
698 vector <sup>41</sup> (Addgene 35124) and cloned into *Bam*HI/*Hind*III-linearized pProEX Hta by  
699 Gibson assembly (New England Biolabs). Wild-type 6xHis-BAR, 6xHis-BAR mutants  
700 and 6xHis-PAT were expressed in *E. coli* BL21(DE3) grown in Terrific Broth medium.  
701 At an optical density at 600 nm of 0.6, protein expression was induced with 1.0 mM  
702 IPTG and cells were grown at 37°C for 2.5 h. Cells from a 150 mL cultures were  
703 harvested by centrifugation, lysed using B-PER™ Bacterial Protein Extraction Reagent  
704 (Thermo Scientific) and purified by metal affinity using Ni-NTA Agarose (Qiagen).  
705 Purified recombinant proteins were concentrated and buffer-exchanged using storage  
706 buffer (10 mM Tris-HCl pH 8.0, 0.2 M NaCl, 10% (v/v) glycerol, 1 mM dithiothreitol)  
707 and ultra-centrifugal filters (10,000 Da MWCO, Amicon EMD Millipore). The purity of  
708 the recombinant proteins was assessed by SDS-PAGE. Final protein concentrations were  
709 determined and normalized using a NanoDrop 2000 UV-VIS spectrometer (extinction  
710 coefficient: 43430 M<sup>-1</sup> cm<sup>-1</sup>, Thermo Scientific).

711 Enzyme assays for comparing the relative activity of the purified BAR mutants  
712 were carried out in 2 mM Tris-HCl pH 8 and 5 mM acetyl-CoA (Sigma-Aldrich) (final  
713 reaction volume 12 µL). Reactions were initiated by the addition of purified recombinant  
714 protein at 0.2 µM (assays with L-phosphinothricin at 0.2 mM) or 150 µM (assays with  
715 amino adipate or tryptophan at 1 mM) and incubated at 25°C for 15 min

716 (phosphinothricin), 165 min (aminoadipate), or 330 min (L-tryptophan). Substrate  
717 concentration-dependences toward phosphinothricin, aminoadipate and tryptophan were  
718 determined for the BAR mutants Y92F and N35T in 2 mM Tris-HCl pH 8 and 10 mM  
719 acetyl-CoA (Sigma-Aldrich). Note that assays on aminoadipate and tryptophan were  
720 supplemented with 0.33 mM of N-nonyl  $\beta$ -D-glucopyranoside (see also above).  
721 Reactions were stopped by the addition of four volumes of 10% water, 90% acetonitrile  
722 (v/v), 5 mM ammonium formate pH 3, centrifuged for 2 min (14,000-16,000 g), and  
723 transferred to LC vials.

724 The assays were analyzed on an Ultimate 3000 Rapid Separation LC system  
725 (Thermo Scientific) coupled to a TSQ Quantum Access MAX triple-quadrupole mass  
726 spectrometer (Thermo Scientific). Assays on phosphinothricin were analyzed as  
727 described above. Assays on aminoadipate were analyzed as follows. The reverse-phase  
728 chromatography system consisted of an 150 mm C18 column (Kinetex 2.6  $\mu$ m silica core  
729 shell C18 100Å pore, Phenomenex), which was developed using Optima™ LC/MS  
730 solvents (Fisher Chemical) with a gradient (flow rate of 0.6 mL min<sup>-1</sup>) of solvent B  
731 (acetonitrile with 0.1% (v/v) formic acid) in solvent A (water with 0.1% (v/v) formic  
732 acid) as follows (all v/v): 1% for 2 min, 1% to 30% in 9 min, 30% to 99% in 30 sec, 99%  
733 for 30 sec, 99% to 1% in 1 min and 1% for 2 min. The mass spectrometer was configured  
734 to perform selected-ion-monitoring scans of 0.5 seconds using Q3 (center mass m/z:  
735 204.086, scan width 0.5 m/z, scan time 0.5 sec). Assays on tryptophan were analyzed as  
736 follow: the reverse-phase chromatography system consisted of an 150 mm C18 column  
737 (Kinetex 2.6  $\mu$ m silica core shell C18 100Å pore, Phenomenex) which was developed  
738 using Optima™ LC/MS solvents (Fisher Chemical) with a gradient (flow rate of 0.7 mL



739 min<sup>-1</sup>) of solvent B (acetonitrile with 0.1% (v/v) formic acid) in solvent A (water with  
740 0.1% (v/v) formic acid) as follows (all v/v): 5% for 1 min, 5% to 99% in 9 min, 99% for  
741 2 min, 99% to 5% in 2 min and 5% for 1 min. The mass spectrometer was configured to  
742 perform selected-ion-monitoring scans of 0.5 seconds using Q3 (center mass m/z:  
743 247.108, scan width 0.5 m/z, scan time 0.5 sec).

744

#### 745 **Analysis of BAR mutants in planta**

746 Wild-type BAR from *Streptomyces hygroscopicus*, selected BAR mutants and wild-type  
747 PAT from *Streptomyces viridochromogenes* were amplified by PCR (Phusion  
748 polymerase; New England Biolabs) from pProEX Hta clones (see above) using primers  
749 listed in Table S3 and cloned into *BpiI*-linearized pICH41308<sup>42</sup> (Golden Gate entry  
750 vector) by Gibson assembly (New England Biolabs). BAR and PAT coding sequences  
751 were fused with *Agrobacterium tumefaciens* mannopine synthase promoter (from  
752 pICH85281) and terminator (from pICH77901) into the empty binary vector pICH47732  
753 by Golden Gate assembly<sup>42</sup>. pICH47732 constructs were transformed into *Agrobacterium*  
754 *tumefaciens* GV3130 strain by electroporation and transformed into Arabidopsis Col-0 by  
755 the floral dip method<sup>43</sup>. 90 mg of T1 seeds were sown on soil and transformants were  
756 selected with Finale® (contains 11.33% glufosinate ammonium; Bayer CropScience)  
757 diluted 1:500 in water. Photographs were taken 10 days after herbicide treatment (**Fig. 4**  
758 **and Supplementary Fig. 12**). This experiment was repeated once with similar results. T2  
759 seeds from 5 to 6 T1 plants were collected for each BAR mutants, sown on soil and  
760 transgenic individuals were selected with Finale® (contains 11.33% glufosinate  
761 ammonium; Bayer CropScience) diluted 1:500 in water (**Supplementary Fig. 13**). This

762 experiment was done once. Metabolites were extracted from dark-incubated leaves  
763 collected from T2 phosphinothricin-resistant individuals (senescent leaves from 8-9  
764 individuals were pooled for each T2 population) and then analyzed as described above  
765 for the absolute quantification of acetyl-aminoadipate and acetyl-tryptophan in *Glycine*  
766 *max* and *Brassica napus*.

767 To further compare the phosphinothricin tolerance in T2 lines transformed with  
768 Y92F, N35T and wild-type BAR, seeds from 5-6 independent lines were germinated on  
769  $\frac{1}{2}$  MS medium containing 1% sucrose and 8  $\mu\text{g}/\text{mL}$  glufosinate ammonium (45520-  
770 Sigma-Aldrich). Seven-days old seedlings were then transformed on soil and further  
771 grown for 10 days. Photographs were taken before treatment with four different  
772 concentrations of Finale® (0, 0.2X, 1X and 5X; see **Supplementary Fig. 14** for further  
773 details on the herbicide concentrations). Plants were further grown for 8 days,  
774 photographs were taken and the average aerial mass of each T2 populations was  
775 measured (average from 8-9 individuals). This experiment was done once.

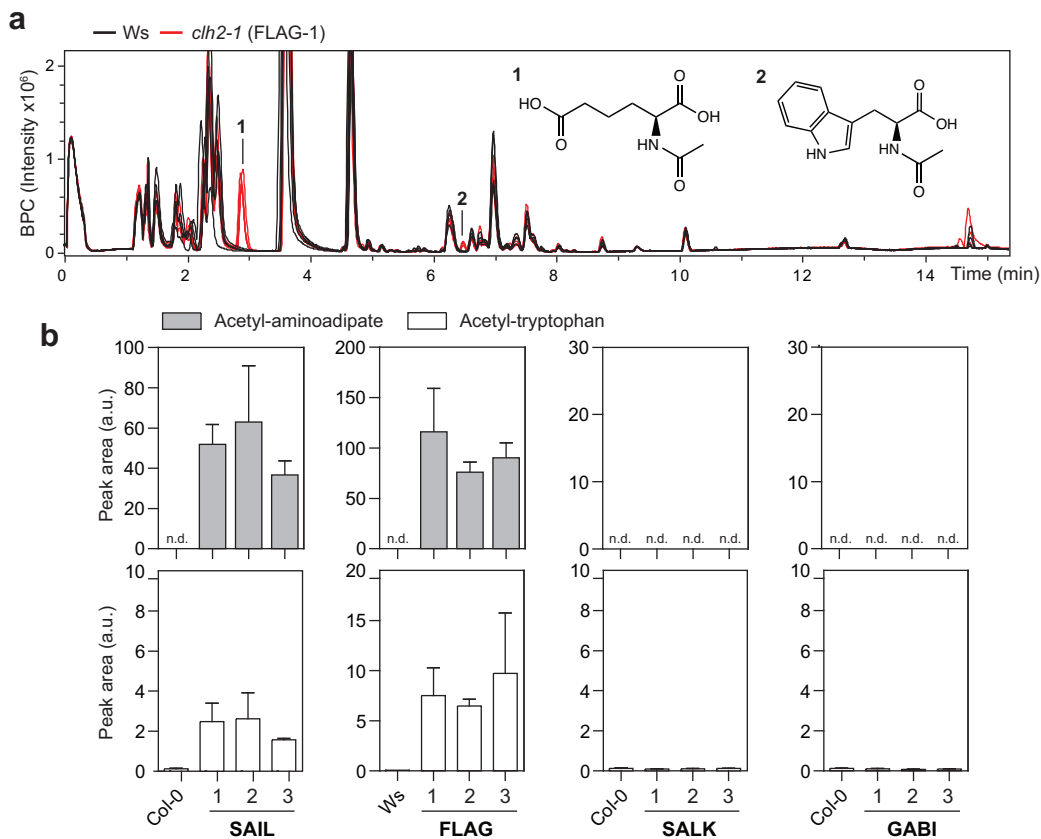
776 Protein levels of the BAR mutants in T2 lines were measured as follow. For each  
777 protein extraction, equal amounts of aerial tissues from 5-6 transgenic T2 populations  
778 were pooled. Total proteins were isolated from frozen samples by homogenization in 5  
779 volumes of ice-cold extraction buffer [50 mM Tris-HCl pH 8, 100 mM NaCl, 0.5% (v/v)  
780 TritonX-100, 2mM  $\beta$ -mercaptoethanol] complemented with a protease inhibitor cocktail  
781 (Complete; Roche Diagnostics). Samples were centrifuged at 12,000 g for 5 min and  
782 protein concentration of the supernatant was determined using the Bradford Assay (Bio-  
783 Rad). Proteins were subsequently precipitated with chloroform-methanol and 10  $\mu\text{g}$  were  
784 analyzed by SDS-PAGE and immunoblotting as described <sup>44</sup>. The following antibodies

785 were used for immunoblot analysis: a primary polyclonal antibody against BAR from  
786 *Streptomyces hygroscopicus* produced in rabbit (1:1000; P0374-Sigma-Aldrich) and a  
787 polyclonal horseradish peroxidase conjugated goat anti-rabbit IgG as the secondary  
788 antibody (1:50000; A0545-Sigma-Aldrich). Substrate detection was performed by  
789 chemiluminescence (ECL Western Blotting Substrate™ (Pierce)) and film exposure. This  
790 experiment was done once.

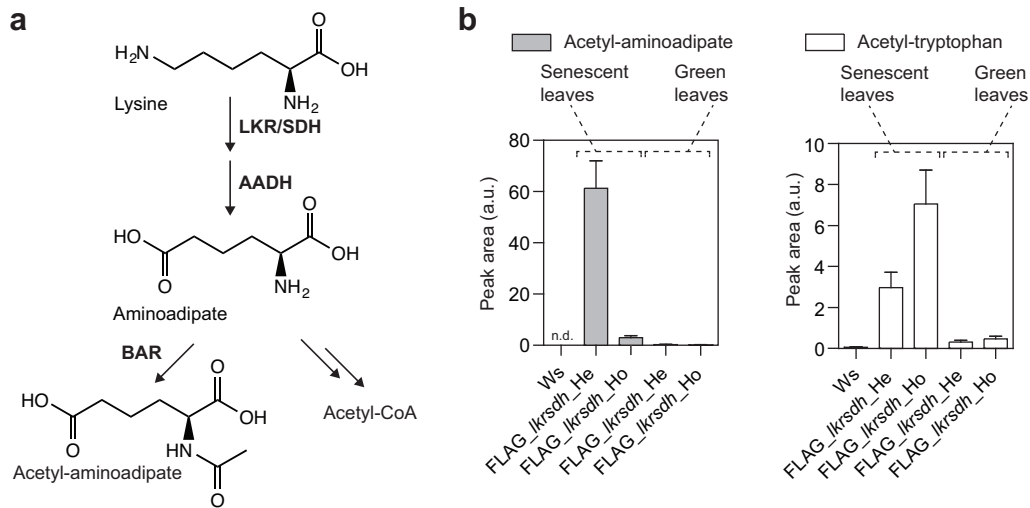
791

#### 792 **Data availability**

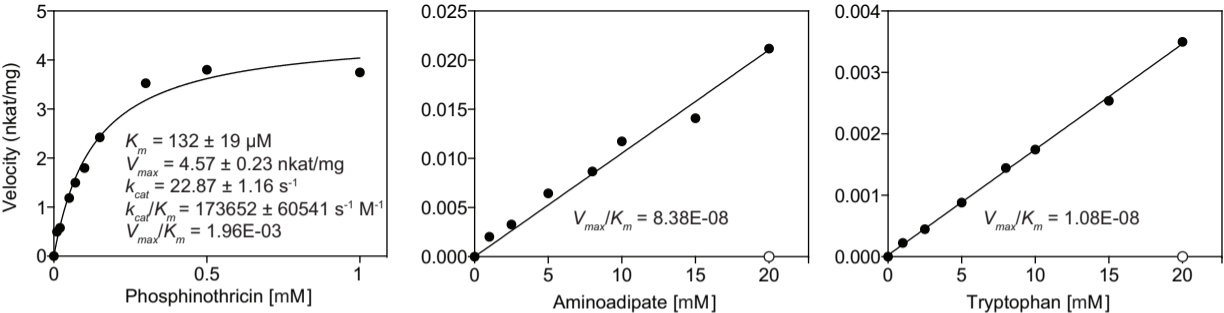
793 The data that support the findings of this study are available from the corresponding  
794 authors upon reasonable request.



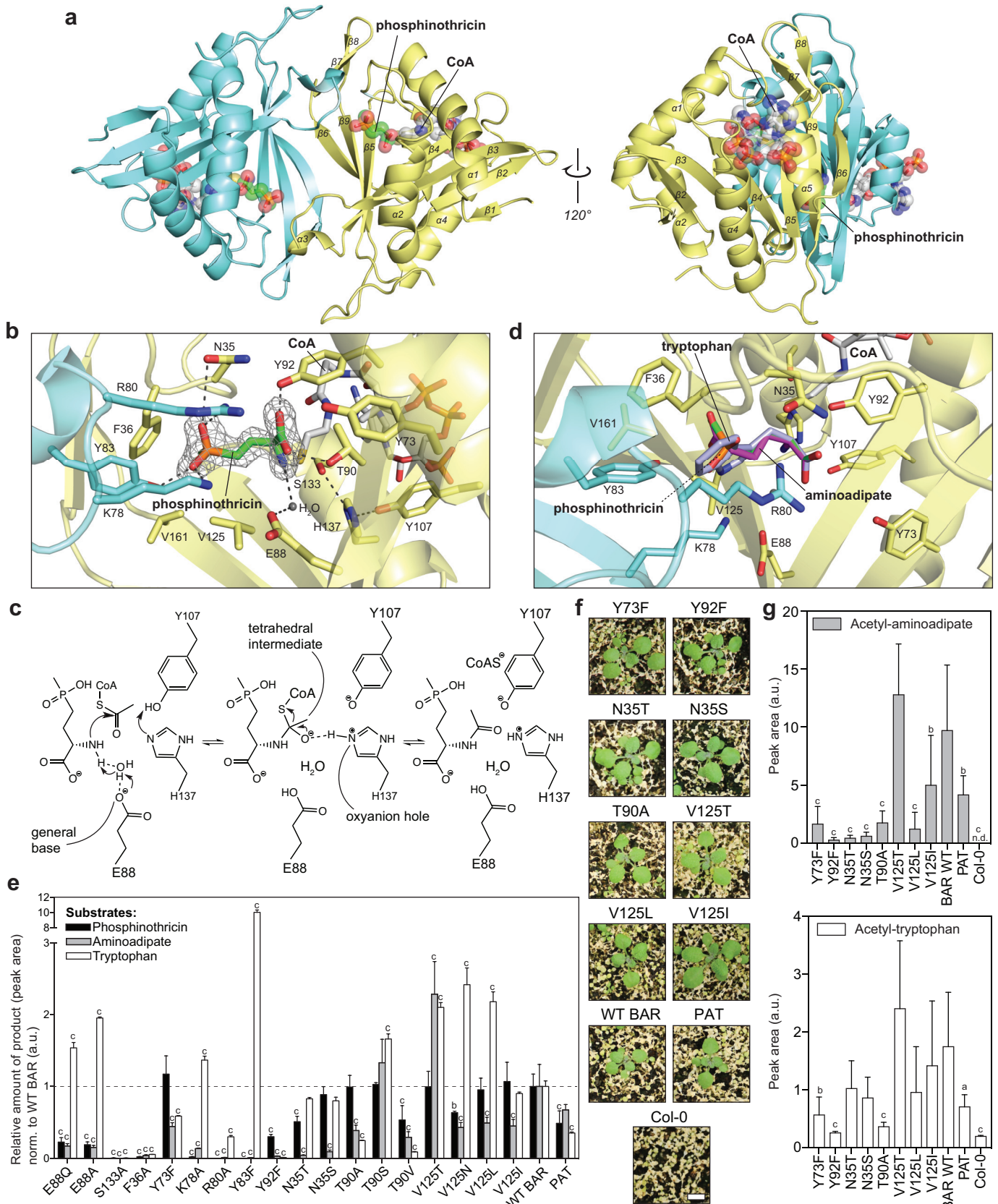
**Figure 1 | Accumulation of acetyl-aminoadipate and acetyl-tryptophan in senescent leaves of Arabidopsis carrying the BAR transgene.** **a**, Metabolite profiles of senescent leaves from Wassilewskija (*Ws*) and *chl2-1* (FLAG-1), displayed as base peak chromatograms (BPC), reveal the ectopic accumulation of acetyl-aminoadipate ('1') and acetyl-tryptophan ('2'). BPC traces of four biological replicates are displayed. **b**, Comparative levels of acetyl-aminoadipate and acetyl-tryptophan in Arabidopsis mutants from different insertional mutant collections that contain either BAR (SAIL and FLAG) or alternative selection marker genes (SALK (*NTP11*, kanamycin resistance) and GABI (*SUL1*, sulfadiazine resistance)). Error bars, mean  $\pm$  s.d. ( $n = 3$  biological replicates). This experiment was repeated at least three times with similar results. See Supplementary Figure 2 for absolute quantification. a.u., arbitrary unit; FW, fresh weight; n.d., not detected



**Figure 2 | BAR-dependent accumulation of acetyl-aminoadipate and acetyl-tryptophan is linked to nitrogen remobilization during senescence.** **a**, Aminoadipate is derived from the lysine degradation pathway in plants, which can be metabolized by BAR as a nonspecific substrate. **b**, Comparative levels of acetyl-aminoadipate and acetyl-tryptophan in green and senescent leaves from the heterozygous (He) and homozygous (Ho) FLAG\_ikrsdh mutant, harboring a BAR-containing T-DNA that abolishes the Arabidopsis LKR/SDH gene. Error bars, mean  $\pm$  s.d. (n = 4 biological replicates). a.u., arbitrary unit; n.d., not detected; Ws, Wassilewskija wild-type plants.



**Figure 3 | *In vitro* enzyme kinetic assays of BAR against native and non-native substrates.** An apparent  $K_M$  value of  $132 \pm 19.2 \mu\text{M}$  was obtained for phosphinothricin, similar to previously published data<sup>1, 3, 17</sup>.  $V_{max}$ ,  $k_{cat}$ ,  $k_{cat}/K_m$  and  $V_{max}/K_m$  values for phosphinothricin are also indicated, as well  $V_{max}/K_m$  values for amino adipate and tryptophan (estimated from Lineweaver-Burk plots). Amino adipate and tryptophan are *in vitro* substrates of BAR but both substrates reached solubility limit before reaching saturation concentration for BAR. Negative controls (open circles) were performed in absence of BAR at the highest substrate concentration tested (20 mM).



**Figure 4 | Structural basis for amino acid N-acetylation catalyzed by BAR and structure-guided engineering of BAR with reduced nonspecific activities.** **a**, Cartoon representation of BAR homodimer in complex with phosphinothricin and CoA. Two monomers of the dimer are colored in blue and yellow respectively. **b**, Close-up view of the BAR active site. The  $|2Fo-Fc|$  omit electron density map (contoured at  $3.0 \sigma$ ) is shown for phosphinothricin. **c**, Proposed catalytic mechanism of BAR. **d**, Docking of tryptophan and aminoacidate within the BAR active site reveals reduced favorable contacts compared to phosphinothricin. **e**, Enzyme activity assays using purified BAR mutant proteins against phosphinothricin (0.2 mM), aminoacidate (1 mM) and tryptophan (1 mM). Wild-type BAR (WT BAR) and PAT from *Streptomyces viridochromogenes* were also examined as controls. Assays were terminated during the initial linear rate of product formation. The relative amount of product formed by each BAR mutant was normalized to WT BAR for each substrate (value of 1). Error bars, mean  $\pm$  s.d. ( $n = 3$  technical replicates). **f**, Photographs of Arabidopsis T1 lines transformed with select BAR mutants 10 days after phosphinothricin treatment (see also Supplementary Fig. 12, 13 and 14). Scale bar = 0.3 cm. **g**, Comparative levels of acetyl-aminoacidate and acetyl-tryptophan in phosphinothricin-resistant T2 Arabidopsis plants transformed with selected BAR mutants. Error bars, mean  $\pm$  s.d. ( $n = 5-6$  biological replicates (Y73F (6), Y92F (6), N35T (5), N35S (5), T90A (6), V125T (5), V125L (6), V125I (6), WT BAR (5), PAT (5)). Significance levels were indicated based on one-way ANOVA with Dunnett's test for multiple comparisons to WT BAR. a,  $p$ -value $<0.1$ ; b,  $p$ -value $<0.05$ ; c,  $p$ -value $<0.01$ ; a.u., arbitrary unit.