

1 **Short title:** Resource for spatio-temporal expression control

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16 **A Comprehensive Toolkit for Inducible, Cell Type-Specific Gene Expression**

17 **in Arabidopsis**

18

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29 **One sentence summary:** A set of transgenic lines was developed to enable spatio-temporal  
30 control of gene expression in Arabidopsis.

31 **Footnotes**

32 Author contributions

33 A-KS and VL-S generated DNA constructs and transgenic plants; A-KS, VL-S, and ZL  
34 analysed transgenic plants; JF, CW, CG, SA, AVB, MF, MG, JEMV, JL contributed  
35 GreenGate modules; A-KS, VL-S, TG, and SW designed the project; VL-S, TG, and SW  
36 wrote the manuscript with contributions from A-KS and ZL.

37

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49 **Abstract**

50 Understanding the context-specific role of gene function is a key objective of modern  
51 biology. To this end, we generated a resource for inducible cell type-specific trans-activation  
52 in *Arabidopsis thaliana* (*Arabidopsis*) based on the well-established combination of the  
53 chimeric GR-LhG4 transcription factor and the synthetic *pOp* promoter. Harnessing the  
54 flexibility of the GreenGate cloning system, we produced a comprehensive set of transgenic  
55 lines termed GR-LhG4 driver lines targeting most tissues in the *Arabidopsis* shoot and root  
56 with a strong focus on the indeterminate meristems. When we combined these transgenic  
57 lines with effectors under the control of the *pOp* promoter, we observed tight temporal and  
58 spatial control of gene expression. In particular, inducible expression in F<sub>1</sub> plants obtained  
59 from crosses of driver and effector lines allows for rapid assessment of the cell type-specific  
60 impact of an effector with high temporal resolution. Thus, our comprehensive and flexible  
61 method is suitable for overcoming the limitations of ubiquitous genetic approaches, the  
62 outputs of which are often difficult to interpret due to the widespread existence of  
63 compensatory mechanisms and the integration of diverging effects in different cell types.

64

65

## 66 **Introduction**

67 The key to the evolutionary success of multicellularity, which arose independently in plants  
68 and animals, is the division of labour between highly specialized cell types. This requires the  
69 robust specification of cell fate through epigenetic and transcriptional programming, despite  
70 the identical genetic makeup of each cell. In plants, cell fate acquisition is largely based on  
71 positional information, which depends on cell-to-cell communication and medium to long  
72 distance morphogenetic signals that cooperate in organ patterning (Efroni, 2017).  
73 Conversely, individual genes, pathways, and metabolites can have diverse or even opposing  
74 roles depending on the tissue context. A prominent example for context-dependency of a  
75 fundamental patterning process is given by the interplay of the auxin and cytokinin  
76 phytohormones (Furuta et al., 2014; Greb and Lohmann, 2016; Truskina and Vernoux,  
77 2017). In the shoot apical meristem, harbouring the stem cell niche ultimately responsible for  
78 most above-ground plant organs, cytokinin signalling is associated with maintaining a  
79 pluripotent, undifferentiated state, whereas auxin signalling promotes differentiation. In  
80 marked contrast, auxin is required for stem cell maintenance in the root apical meristem  
81 (RAM) (Pacifici et al., 2015; Weijers and Wagner, 2016). Therefore, the global effects of  
82 genetic lesions or of knock-ins can dilute and mask specific functions and are often difficult  
83 to interpret.

84 Routinely, stable genetic gain- and loss-of-function mutants remain the main pillar of the  
85 reductionist approach to biology and the phenotypes of such mutants are assessed to  
86 deduce a function of the mutated locus in the wild type. However, the function of many gene  
87 products is context-specific and thus the phenotypes of mutants or transgenic lines can be  
88 complex. In addition, mutant organisms can undergo life-long adaptation, impeding the  
89 interpretation of their phenotype. Moreover, transgenic and mutational approaches can  
90 interfere with plant vitality, precluding an in-depth analysis.

91 Many of these problems can be overcome by inducible, cell type-specific expression  
92 mediated by two-component transcription activation systems (Moore et al., 2006). An  
93 expression cassette is constructed using a heterologous or synthetic promoter and is hence  
94 silent unless a cognate transcription factor is present. An efficient approach is to generate  
95 transgenic plants called 'driver lines' that express the transcription factor in a spatially and  
96 temporally controlled manner, and a 'responder line' carrying the effector construct. After  
97 crossing of the two lines, expression can be induced and the phenotypic consequences of  
98 the effector can be studied. In the abstract, these expression systems are highly valuable  
99 because they ideally enable cell-type specific or stage-specific complementation or knock-  
100 down, facilitate time-resolved monitoring of the response to a given cue, can overcome the  
101 lethality of constitutive expression, and allow to study cell autonomous and non-cell  
102 autonomous effects with high temporal and spatial resolution. However, the considerable  
103 effort and time requirements for DNA cloning and the generation of stable transgenic plants  
104 are a major bottleneck curtailing their use to date. For the same reason and because distinct  
105 tissue-specific promoters were not always available in the past, attention is usually given to  
106 one tissue or cell type of interest at a time and unbiased approaches targeting a larger  
107 spectrum of individual tissues are rarely followed.

108 Here, we report on the generation of a comprehensive set of *Arabidopsis thaliana* driver  
109 lines suited for tissue-specific trans-activation of an effector cassette in a wide range of cell  
110 types and with the possibility to monitor gene activation in space and time by a fluorescent  
111 promoter reporter. To ensure rapid, stable induction with minimal adverse effects on plant  
112 growth caused by the inducer, our system takes advantage of the widely used LhG4/pOp  
113 system (Moore et al., 1998; Craft et al., 2005; Samalova et al., 2005) combined with the  
114 ligand binding domain of the rat glucocorticoid receptor (GR) (Picard, 1993) (Craft et al.,  
115 2005). LhG4 is a chimeric transcription factor consisting of a mutant version of the  
116 *Escherichia coli lac* repressor with high DNA binding affinity (Lehming et al., 1987) and the  
117 transcription activation domain of yeast Gal4p (Moore et al., 1998). N-terminal fusion with

118 the GR ligand binding-domain renders the transcription factor inactive in the cytosol through  
119 sequestration by HEAT SHOCK PROTEIN90 (HSP90) in the absence of the inducer.  
120 Nuclear import after treatment with the synthetic glucocorticoid dexamethasone (Dex)  
121 (Picard, 1993) results in transcriptional activation of expression cassettes that are under the  
122 control of the synthetic Op 5' regulatory region consisting of a *Cauliflower mosaic virus*  
123 (CaMV) 35S minimal promoter and two upstream *lac* operators (Moore et al., 1998; Craft et  
124 al., 2005). Combining multiple interspersed repeats of the operator elements (*pOp4*; *pOp6*)  
125 and localized expression of LhG4 enable strong overexpression of a target gene in a cell  
126 type-specific manner (Craft et al., 2005).

127 Our work builds on these seminal studies by creating 19 well-characterized and stable driver  
128 lines targeting most cell types in Arabidopsis with a focus on the three main meristems of the  
129 plant, the root apical meristem (RAM), the shoot apical meristem (SAM), and the cambium.  
130 Of note, for several cell types such as the pith in the inflorescence stem or the xylem pole  
131 pericycle cells in the root, inducible expression systems were not available so far. The driver  
132 lines were generated employing the fast and flexible GreenGate cloning system  
133 (Lampropoulos et al., 2013), but are compatible with any vector/transgenic line in which the  
134 expression of an effector is under the control of derivatives of the *pOp* promoter element  
135 (Moore et al., 1998). An important feature of our driver lines is the presence of a fluorescent  
136 reporter amenable to live imaging, which allows monitoring the spatio-temporal dynamics of  
137 gene induction and may serve as a read-out for any effect on the respective tissue identity.  
138 Similarly, it allows us to assess whether the expression of the effector has an impact on the  
139 transcriptional circuitries targeting the promoter it is expressed from. The material described  
140 here allows testing the effect of genetic perturbations in a broad repertoire of individual  
141 tissues on a distinct developmental or physiological process. As trans-activation efficiently  
142 occurs in the presence of the inducer in F1 plants derived from a cross between a driver and  
143 an effector line, the effect of a given expression cassette can be assessed relatively quickly

144 in a wide range of cell types, demonstrating the usefulness of this resource for the broader  
145 research community.

146

## 147 **Results**

### 148 **Design of driver lines with cell type-specific expression of GR-LhG4**

149 To generate a comprehensive set of driver lines expressing the chimeric GR-LhG4  
150 transcription factor under the control of cell type-specific promoters, we made use of the  
151 Golden Gate-type GreenGate cloning system, which enables quick, modular, and scarless  
152 assembly of large constructs (Engler et al., 2008; Lampropoulos et al., 2013). Our design  
153 included, on the same T-DNA, the coding sequence for an mTurquoise2 fluorescent reporter  
154 (Goedhart et al., 2012) targeted to the endoplasmic reticulum (ER) through translational  
155 fusion with an N-terminal signal peptide from sweet potato (*Ipomoea batatas*) Sporamin A  
156 (SP, (Lampropoulos et al., 2013)) and the ER retention motif His-Asp-Glu-Leu (HDEL) under  
157 the control of pOp6 and a minimal CaMV 35S promoter (*pOp6:SP-mTurquoise2-HDEL*) (Fig.  
158 1). In our set up, the GR-LhG4 transcription factor is expressed under the control of a tissue-  
159 or cell type-specific promoter (pTS). Consequently, GR-LhG4 activates the expression of the  
160 mTurquoise2 reporter and any other effector downstream of a *pOp* promoter after Dex  
161 treatment specifically in those tissues (Fig. 1). We anticipate that the most utility can be  
162 obtained from this system if lines harbouring effector cassettes are crossed with driver lines  
163 and analyses are performed with F1 plants. However, other modes such as direct  
164 transformation of multiple driver lines or the introgression into different (mutant) backgrounds  
165 are also conceivable. Notably, even though the mTurquoise2 reporter is expressed from the  
166 same T-DNA as GR-LhG4, there is no mechanistic difference to the activation of an effector  
167 *in trans* (Fig. 1).

168 For establishing a rather comprehensive set of driver lines, we first selected respective  
169 tissue-specific promoters based on literature reports and our own expression data (Table 1).

170 Subsequently, we generated stable transgenic driver lines in the Arabidopsis Col-0  
171 background using 19 specific promoters that cover most cell types in the RAM, the SAM,  
172 and the cambium. Several of the promoters have been previously shown to work robustly in  
173 cell type-specific mis-expression approaches (e.g. Nakajima et al., 2001; Weijers et al.,  
174 2006; Mustroph et al., 2009; Miyashima et al., 2011; Roppolo et al., 2011; Vaten et al., 2011;  
175 Naseer et al., 2012; Cruz-Ramirez et al., 2013; Ohashi-Ito et al., 2014; Wang et al., 2014;  
176 Chaiwanon and Wang, 2015; Serrano-Mislata et al., 2015; Vragovic et al., 2015; Marques-  
177 Bueno et al., 2016; Siligato et al., 2016; Doblas et al., 2017). Next, we generated T3 lines in  
178 which the resistance to the selective agent sulfadiazine appeared homozygous after  
179 segregating as a single locus in the T2 generation based on resistance or standard addition  
180 quantitative real-time PCR (SA-qPCR) analyses (Huang et al., 2013).

#### 181 **Validation of the specificity of driver lines.**

182 To confirm the expected expression patterns in the root, driver lines were germinated on  
183 medium containing 30  $\mu$ M Dex or DMSO and analysed with confocal laser scanning  
184 microscopy (CLSM) five days after germination (DAG). In each case, we recorded  
185 mTurquoise2-derived fluorescence in longitudinal optical sections of the root meristem (Fig.  
186 2 and Supplemental Fig. S1) and, where appropriate, in cross sections through the meristem  
187 or the differentiation zone (Supplemental Fig. S2). To visualize expression in the shoot, lines  
188 were grown on soil in long day conditions and the aerial part of plants with 15 cm tall  
189 inflorescence stems were dipped either in tap water containing 10  $\mu$ M Dex (Fig. 3) or only  
190 the solvent DMSO (Supplemental Fig. S3). After 24 h, freehand sections of the stem were  
191 stained with propidium iodide (PI) to highlight xylem elements and analysed by confocal  
192 microscopy. To analyse expression in the SAM, inflorescence meristems of 15 cm tall plants  
193 were treated with Dex 48 hours before being dissected and imaged with CLSM, again using  
194 PI as a cell wall counterstain (Fig. 4). Reporter gene activities were consistent with the  
195 expected patterns and strictly dependent on the presence of Dex (Supplemental Fig. S1,  
196 Supplemental Fig. S3, and Supplemental Fig. S4). In addition, the complete absence of



197 reporter activity in tissues adjacent to cells in which activity was expected suggested that the  
198 chimeric GR-LhG4 protein does not move between cells. We did not observe any negative  
199 effect of Dex treatment on plant growth (Supplemental Fig. S5).

#### 200 **Characterization of gene activation.**

201 We next tested whether dose-response and induction dynamics previously observed with the  
202 GR-LhG4 system (Craft et al., 2005) were recapitulated in our set up. To this end we  
203 germinated the *pSCARECROW* (*pSCR*) driver line mediating GR-LhG4 expression in the  
204 quiescent centre (QC) and the endodermis (Di Laurenzio et al., 1996; Wysocka-Diller et al.,  
205 2000) on plates containing solvent, 0.1  $\mu\text{M}$ , 1  $\mu\text{M}$ , 10  $\mu\text{M}$ , and 100  $\mu\text{M}$  Dex. Visualizing  
206 reporter fluorescence 5 DAG indeed revealed increasing reporter activity with increasing Dex  
207 concentrations (Fig. 5A), arguing for the possibility to fine tune gene expression by adjusting  
208 the levels of the inducer. We noticed that QC cells showed markedly stronger fluorescence  
209 compared to the endodermis, putatively reflecting higher promoter GR-LhG4/reporter  
210 stability in the QC as this was not observed with previously published lines using the same  
211 promoter fragment (Gallagher et al., 2004; Heidstra et al., 2004; Cruz-Ramirez et al., 2013).  
212 We therefore quantified fluorescence separately in the QC cells and the endodermal initials  
213 (Fig. 5C and Fig. 5D). Whereas the QC did not show a significant difference in fluorescence  
214 intensity between any of the treatments (Fig. 5C), the endodermis fluorescence intensity  
215 correlated with the concentration of the inducer until saturation appeared to be reached  
216 between 10  $\mu\text{M}$  and 100  $\mu\text{M}$  of Dex (Fig. 5D). Consequently, we concluded that, to fine tune  
217 gene expression by applying different Dex concentrations, the appropriate concentration  
218 range has to be determined for each promoter and cell type individually.

219 To further assess induction kinetics, the *pSCR* driver line was germinated on plates with  
220 control medium and transferred onto plates containing 50  $\mu\text{M}$  Dex after five days. As  
221 expected, a time-dependent increase of reporter activity was observed over a period of 24  
222 hours (Fig. 5B). Combined quantification of fluorescence in the QC and the endodermis  
223 initials detected reporter activity six hours after induction (Fig. 5E) and the activity values

224 were close to the values of constitutive Dex treatment after 24 hours (Fig. 5D and Fig. 5E).  
225 These observations suggested that six hours are sufficient to allow nuclear import of GR-  
226 LhG4, the induction of gene transcription, and initial protein translation, and that within 24  
227 hours, protein levels reached a steady-state level. In addition, 5 day old roots that were  
228 either induced at 2 DAG, 3DAG, or 4 DAG showed similar reporter activities, demonstrating  
229 that responsiveness to the inducer is sustainable (Supplemental Fig. S6). To assess the  
230 kinetics of reporter expression after removal of the inducer, we germinated the  
231 *pSCR>GR>mTurquoise2* line on Dex-containing medium and transferred the seedlings to  
232 Dex-free medium 2 DAG. Quantifying reporter fluorescence revealed that one day after  
233 transfer, fluorescence intensity was indistinguishable from control plants transferred to  
234 inducer-containing plates, but declined over the course of the next two days to hardly  
235 detectable levels (Supplemental Fig. S7).

236 To estimate the level of transcription mediated by the GR-LhG4/pOp system we employed a  
237 line expressing *PECTIN METHYLESTERASE INHIBITOR5 (PMEI5)* (Wolf et al., 2012)  
238 under control of the strong and nearly-ubiquitous *35S* promoter (*p35S:PMEI5*). When  
239 comparing roots from the *p35S:PMEI5* line with roots from a Dex-treated GR-LhG4/pOp line  
240 conferring expression of the same *PMEI5* coding sequence in xylem pole pericycle (XPP)  
241 cells (designated as *pXPP>GR>PMEI5* (Craft et al., 2005)), we observed *PMEI5* transcript  
242 levels similar to or slightly exceeding those in the *p35S:PMEI5* line (Supplemental Fig. S8).  
243 This was despite the fact that the *XPP* expression domain contains only approximately six  
244 cell files in the young root (Supplemental Fig. S2). Thus, we concluded that, although  
245 activating transcription in a very local manner, the GR-LhG4/pOp system can lead to strong  
246 expression in the respective cell types.

247 The ER-localized mTurquoise2 reporter present in our driver lines is transcribed from the  
248 same T-DNA that harbours the GR-LhG4 module (Fig. 1). To analyse the response of an  
249 independent T-DNA insertion carrying the *pOp6* element *in trans*, we generated a transgenic  
250 line carrying an ER-targeted mVenus reporter under the control of the *pOp6* promoter

251 (*pOp6:SP-mVenus-HDEL*) and crossed it with the *pSCR* driver line. The resulting F1 plants  
252 did not show any reporter activity when grown on plates without Dex (Fig. 6), again  
253 confirming that the GR-LhG4/pOp system is fully Dex-dependent. After Dex induction, we  
254 visualized both mTurquoise2 and mVenus fluorescence in the root and the stem and  
255 observed a complete congruence of both reporter activities (Fig. 6). Likewise, transgenic  
256 lines expressing a nucleus-targeted triple green fluorescent protein (GFP) fusion protein  
257 under the control of the *pOp6* promoter were generated and crossed with the *pCLAVATA3*  
258 (*CLV3*) driver line mediating expression in stem cells of the SAM (Fletcher et al., 1999). As  
259 expected, upon Dex induction, the 3xGFP-NLS signal was observed in a narrow domain at  
260 the tip of the SAM which also expressed the mTurquoise2 marker (Fig. 6). Together, these  
261 observations confirmed robust and specific trans-activation of transgenes in F1 plants.

#### 262 **Cell type-specific induction of VND7 demonstrates efficacy of trans-activation**

263 To explore the potential of our lines to mediate the expression of a biologically active  
264 effector, we crossed the *pSCR* driver line with a line harbouring the *VASCULAR RELATED*  
265 *NAC-DOMAIN PROTEIN 7 (VND7)* effector fused to the VP16 activation domain able to  
266 induce the formation of xylem vessels in a broad range of cell types (Kubo et al., 2005;  
267 Yamaguchi et al., 2010). F1 plants were grown on control medium for five days and then  
268 transferred to medium containing either 10  $\mu$ M Dex or solvent. Five days later, fully  
269 differentiated vessel-like elements could be observed in the endodermis of the root and  
270 hypocotyl (Fig. 7), whereas in DMSO-treated controls xylem elements were clearly restricted  
271 to the stele. These results demonstrate that this resource for cell type-specific and inducible  
272 trans-activation can be used to study gene function with high spatio-temporal resolution.

273

#### 274 **Discussion**

275 In this study, we combined the proven efficacy of the well-established GR-LhG4/pOp  
276 expression system (Craft et al., 2005; Rutherford et al., 2005; Samalova et al., 2005) with

277 the ease of cloning enabled by the GreenGate system (Lampropoulos et al., 2013) to  
278 provide a comprehensive toolbox for inducible, cell type-specific expression in Arabidopsis.  
279 The driver lines described here cover a large proportion of the known cell types in the three  
280 main meristems of the plant, the RAM, the SAM, and the cambium. Our analysis  
281 demonstrates that this system achieves non-leaky, adjustable, and robust trans-activation of  
282 effectors in the F1 generation after crossing with effector-carrying plants. Therefore,  
283 generating a line harbouring an effector cassette under the control of the *pOp6* promoter  
284 should enable users to rapidly assess a battery of different expression regimes for a wide  
285 range of applications. In most cases, the effector might be the coding region of a gene one  
286 may want to miss-express in a spatially and temporally controlled manner, but other uses  
287 are conceivable, such as adjustable (pulsed) expression of reporters, domain specific knock-  
288 down through artificial microRNAs, cell type-specific complementation studies, the  
289 acquisition of cell type-specific transcriptomes/translatomes/proteomes/epigenomes, or the  
290 local induction of genome editing, for example through expression of Cre recombinase or  
291 clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated  
292 protein 9 (Cas9) (CRISPR/Cas9) modules (e.g. Birnbaum et al., 2003; Brady et al., 2007;  
293 Dinneny et al., 2008; Gifford et al., 2008; Mustroph et al., 2009; Deal and Henikoff, 2011;  
294 Hacham et al., 2011; Iyer-Pascuzzi et al., 2011; Petricka et al., 2012; Fridman et al., 2014;  
295 Adrian et al., 2015; Vragovic et al., 2015; Efroni et al., 2016; Kang et al., 2017). Thus, this  
296 system should be a valuable tool for the generation of inducible genetic perturbations to  
297 overcome the limitations of “endpoint” genetics and study genetic activities in specific tissue  
298 contexts.

### 299 **Design of the trans-activation system**

300 Two-component trans-activation and chemically-induced gene expression systems have  
301 been widely used by plant biologist in the past. For example, a large collection of enhancer  
302 trapping lines based on the yeast Gal4 transcription factor (Haseloff, 1999; Engineer et al.,  
303 2005) are an invaluable tool for constitutive, tissue-specific trans activation in Arabidopsis

304 (Aoyama and Chua, 1997; Sabatini et al., 2003; Weijers et al., 2003; Swarup et al., 2005;  
305 Weijers et al., 2005). In addition, an inducible system based on Gal and cognate upstream  
306 activation sequence (UAS) has been devised (Aoyama and Chua, 1997) but appears to  
307 induce unspecific growth defects (Kang et al., 1999). Trans-activation based on LhG4  
308 (Moore et al., 1998) shows only minimal detrimental effects on plant development, is  
309 thoroughly characterized and optimized (Moore et al., 1998; Baroux et al., 2005; Craft et al.,  
310 2005; Rutherford et al., 2005; Samalova et al., 2005; Moore et al., 2006) and has been used  
311 by the plant community in a number of studies (e.g. Schoof et al., 2000; Baroux et al., 2001;  
312 Eshed et al., 2001; Hay and Tsiantis, 2006; Nodine and Bartel, 2012; Sauret-Gueto et al.,  
313 2013; Hazak et al., 2014; Serrano-Mislata et al., 2015; Jiang and Berger, 2017). Parallel to  
314 the development of these tools for cell type-specific expression, a number of inducible  
315 systems have been conceived to enable temporal control of gene expression (Gatz et al.,  
316 1992; Weinmann et al., 1994; Caddick et al., 1998; Zuo et al., 2000). Subsequently,  
317 combining and optimizing the available technology has succeeded in generating tools to  
318 mediate inducible expression in a cell type-specific manner (Deveaux et al., 2003; Laufs et  
319 al., 2003; Maizel and Weigel, 2004; Craft et al., 2005).

320 For the generation of this resource, we build on ground-breaking previous work establishing  
321 the LhG4 system in combination with the GR ligand binding domain (Craft et al., 2005),  
322 which has since been proven to be a valuable resource (e.g. Reddy and Meyerowitz, 2005;  
323 Ongaro et al., 2008; Ongaro and Leyser, 2008; Heisler et al., 2010; Jiang et al., 2011; Dello  
324 loio et al., 2012; Merelo et al., 2016; Caggiano et al., 2017; Tao et al., 2017). For the  
325 generation of our driver lines, we exploited the power of the GreenGate cloning system  
326 (Lampropoulos et al., 2013). We were able to rapidly assemble a large number of constructs  
327 efficiently, circumventing the bottleneck previously imposed by the challenging generation of  
328 large DNA constructs with varying promoter elements, coding regions, and terminators. The  
329 limiting factor in generating this resource was thus plant transformation, and obtaining single  
330 insertion, homozygous transgenic lines. As a general workflow, we aimed to generate at

331 least 40 T1 transformants, then scored segregation ratios of antibiotic/herbicide resistance  
332 in the T2 generation and maintained lines in which the resistance segregated as a single  
333 locus. These lines usually showed similar characteristics concerning the response to the  
334 inducer and the expression levels achieved through trans-activation (based on fluorescence  
335 intensity). Nevertheless, reporter expression in any set of newly generated driver lines  
336 should be carefully assessed and compared with the literature and within lines, as genome  
337 integration in the vicinity of endogenous promoter and/or enhancer elements might influence  
338 the expression pattern. As expected, we occasionally observed widespread silencing in the  
339 T2 generation of the driver lines, which did not correlate with any particular DNA element  
340 present in multiple constructs

341 An important feature of our driver lines is the incorporation of a reporter amenable to live  
342 imaging, which can be used to monitor the induction and visualize the spatial expression  
343 domain. In addition, it allows us to assess whether the expression of the effector has an  
344 impact on the transcriptional circuitries of the cell type it is expressed from. For some  
345 applications, the internal reporter of the driver lines might also serve as an inducible marker  
346 even in the absence of any further effector expression. We chose mTurquoise2 as a  
347 fluorescent reporter, since its spectral characteristics make it compatible with more widely  
348 used green and red fluorophores, and it displays high photostability, fast maturation, and  
349 high quantum yield (Goedhart et al., 2012). The fluorescent protein was N-terminally fused  
350 with a signal peptide and modified with a C-terminal HDEL motif to mediate retention in the  
351 ER, which in our experience is the preferable subcellular localization for a fluorescent  
352 reporter when cross sections through the highly differentiated cells of the stem are required.

### 353 **Trans-activation characteristics**

354 Our system allows stringent temporal control of gene expression, as indicated by the lack of  
355 reporter expression in the absence of the inducer Dex. Moreover, the trans-activated  
356 reporter faithfully reproduced previously described expression patterns associated with the  
357 respective 5' regulatory regions, suggesting that the chimeric GR-LhG4 transcription factor is

358 not cell-to-cell mobile. However, we noticed that in some cases trans-activation led to slightly  
359 different expression patterns as compared to fusions of the same 5' regulatory region with a  
360 reporter gene *in cis*. For example, expression driven from the *CLV3* promoter seemed  
361 broader than what was described in *pCLV3:XF*P lines, but consistent with a similarly  
362 designed *pCLV3*-driven trans-activation (Serrano-Mislata et al., 2015), possibly because the  
363 multiple binding sites of the *pOp6* promoter increase expression in cells where the *CLV3*  
364 promoter is only weakly active. Alternatively, high protein stability of the chimeric  
365 transcription factor, the reporter, or both, might cause prolonged activity of these proteins in  
366 cells that are already displaced from the stem cell region. This potential issue is less relevant  
367 for organs such as the root, where cells of one cell type also largely have the same clonal  
368 identity (Kidner et al., 2000; Costa, 2016).

369 Our experiments, in agreement with previous results, suggested that GR-LhG4/pOp-  
370 mediated trans-activation can achieve tissue-specific overexpression of the target gene,  
371 dependent on the concentration of the inducer. However, the possibility of “squenching”, the  
372 sequestration of general transcription factors required for other processes by the LhG4  
373 activation domain, must be taken into account at very high expression levels. Consistent with  
374 previous reports (Craft et al., 2005), our analysis of the *pSCR* driver line revealed a linear  
375 dose-response over at least two orders of magnitude, but the induction kinetics might be  
376 affected by the genomic location of the transgene and thus should be empirically determined  
377 for each line. It should be noted that expression of effectors using LhG4/pOp systems can be  
378 quenched by adding Isopropyl  $\beta$ -D-1-thiogalactopyranoside (IPTG) (Craft et al., 2005), which  
379 would allow pulsing experiments. However, we did not test the effect of IPTG in our lines.

### 380 **Distribution of driver lines and DNA constructs**

381 The lines described here, as well as DNA constructs, are available to the community upon  
382 request. While GR-LhG4 and the sulfadiazine resistance gene are constitutively expressed,  
383 care should be taken to amplify seeds only from non-induced plants to minimize the chance

384 of inducing post transcriptional gene silencing through high expression levels of the reporter  
385 (Schubert et al., 2004; Abranches et al., 2005).

386



387 **Material and methods.**

388 **Cloning**

389 All constructs were produced by GreenGate cloning (Lampropoulos et al., 2013) using the  
390 modules described in Supplemental Table S1. The *Eco31I* (*Bsal*) sites of the *SCR*, *PXY* and  
391 *WOX4* promoters were removed by the QuickChange XL Site-Directed Mutagenesis Kit  
392 (Agilent Technologies, USA) using the primers in Supplementary Table S1 following the  
393 manufacturer's instructions. The *Eco31I* site of the *ATHB-8* promoter was removed by  
394 amplifying the 5' part of the promoter up to the endogenous *Eco31I* restriction site, which  
395 was mutated by a single base exchange in the primer. This primer contained an *Eco31I*  
396 restriction site in the 5' overhang. The 3' fragment of the promoter was amplified with a  
397 forward primer directed against the region immediately 3' of the endogenous *Eco31I* site  
398 (containing a *Eco31I* site in the 5' overhang) and the reverse primer binding to the region  
399 immediately upstream of the ATG. The two fragments were amplified separately, digested  
400 with *Eco31I*, and ligated afterwards. As *Eco31I* is a Type IIs restriction enzyme, the  
401 recognition site in the primer overhangs were removed by digestion.

402 The repetitive sequences of the *pOp* promoter increase the likelihood of recombination  
403 events while amplifying the plasmids. To discriminate against clones with shorter *pOp*  
404 sequences, we designed primers that bind in the short flanking sequences at the beginning  
405 and end of *pOp6* (*pOp6\_F* TGCATATGTCGAGCTCAAGAA; *pOp6\_R*  
406 CTTATATAGAGGAAGGGTCTT) for PCR amplification and size assessment through gel  
407 electrophoresis. Final constructs were always confirmed by sequencing in *E. coli* and  
408 *Agrobacterium tumefaciens*. The occasional recombination events were only detected in *E.*  
409 *coli*.

410 **Plant material and growth conditions.**

411 All constructs were transformed by the floral dip method (Clough and Bent, 1998) as  
412 modified by (Zhang et al., 2006) into *Arabidopsis Col-0*. Transformed seeds were selected

413 on ½ MS plates containing 1.875-3.75 µg/ml sulfadiazine or 7.5 µ/ml glufosinate ammonium.  
414 Only single integration lines based on T2 segregation ratios were propagated to T3, in which  
415 plants homozygous for the resistance were selected. All plants were grown in long day  
416 conditions (L16:D8) at 22°C. For root analysis, plants were grown vertically in ½ MS plates  
417 containing 1% sucrose and 0.9% plant agar (Duchefa P1001, Haarlem, The Netherlands).  
418 For the induction treatments in plates, the seeds were sown on plates containing Dex  
419 (Sigma\_D4903, Missouri, United States) in the indicated concentration while the same  
420 volume of DMSO (D139-1, Fisher Scientific, UK) was added for the mock control. For the  
421 trans-activation experiment, seeds were sown on plates without Dex and seedlings were  
422 transferred to Dex-containing plates at 1, 6 and 24 hours before imaging five DAG. For  
423 analysis of the stem, the aerial parts of 15 cm tall plants were dipped for 30 s in either tap  
424 water containing 10 µM Dex with 0.02% Silwet L-77 (Kurt Obermeier GmbH & Co. KG, Bad  
425 Berleburg, Germany) or water with the same volume of DMSO with 0.02% Silwet. After 24  
426 hours, free-hand sections of the stem were performed with a razor blade. Sections were  
427 transferred to a small petri dish (35/10 mm, Greiner Bio-One GmbH, Germany) with 0.25  
428 mg/ml of propidium iodide for 5 min and mounted on microscope slides to be visualized by  
429 CSLM. For SAM imaging, the inflorescence meristems of 25-30 DAG plants were sprayed  
430 with 10 µM Dex, whereas an equal volume of DMSO was added to water sprayed onto the  
431 mock controls. 48 h after the treatment, the inflorescence meristems were dissected by  
432 cutting of the stem, flowers and buds. The SAM was stained in 0.25 mg/ml propidium iodide  
433 (Sigma-Aldrich, P4170) for 5 min and mounted in a 3% agarose small petri dish (35/10mm,  
434 Greiner Bio-One GmbH, Germany) and visualized by CLSM.

### 435 **Microscopy**

436 Root samples were imaged using a Leica TCS SP5 laser scanning confocal microscope with  
437 a HCX PL APO lambda blue 63x water immersion objective. The mTurquoise2 fluorophore  
438 was excited by an argon laser at 458 nm and emission was collected between 460 and 516  
439 nm. The mVenus fluorophore was excited by 514 nm and emission was collected between

440 520 and 580 nm. Cells were counter-stained by PI (Sigma-Aldrich, P4170) and imaged with  
441 488 nm for excitation and emission was collected between 590 and 660 nm.

442 For stem and SAM samples we used a Nikon (Minato, Tokyo, Japan) A1 Confocal with a CFI  
443 Apo LWD 25x water immersion objective. The PI counter-stained cells were imaged with 561  
444 nm for excitation and 570-620 nm for emission. The mTurquoise2 fluorescence was  
445 acquired using excitation at 405 nm and emission was collected between 425-475 nm. For  
446 the trans-activation experiments, the 3xGFP-NLS signal in the SAM was imaged with 488  
447 nm for excitation and 500-550 nm for emission. In the root, mVenus was excited with 514 nm  
448 and the emission was collected between 500-550 nm.

449 For visualization of the xylem, plants were germinated in ½ MS plates and 5 DAG were  
450 transferred to either 10 µM Dex or mock containing ½ MS plates. To visualise the ectopic  
451 xylem formation, plants were collected five days after induction and fixed overnight in a 1:3  
452 acetic acid:ethanol solution. Then, they were cleared in a 8:1:2 chloral hydrate:glycerol:water  
453 solution for at least 3 hours. Samples were mounted on microscope slides containing 50%  
454 glycerol solution and brightfield images were obtained using an Axioimager M1 microscope  
455 equipped with an AxioCamHRc (Carl Zeiss, Jena, Germany).

#### 456 **qPCR and SA-qPCR analysis**

457 Analysis of *PMEI5* expression by qPCR was performed as described (Wolf et al., 2012). For  
458 standard addition quantitative real-time PCR (SA-qPCR), plant DNA extraction was  
459 performed as in (Allen et al., 2006) and SA-qPCR was performed as in (Huang et al., 2013).  
460 Quadruplicate qPCR reactions were performed in a final volume of 12.50 µl, including 6.25  
461 µl of ABSolute qPCR SYBR Green Mix (Thermo Scientific), 0.25 µl of each primer (10 µM), 2  
462 µl of genomic DNA (1.6 ng /µl) with different amounts (0, 1 or 3 µl) of plasmid (0.1 pg/µl) as a  
463 reference. The *Sulfr* resistance gene was amplified with primers Sulfr\_Fwd  
464 GCATGATCTAACCCTCTGTCTC and Sulfr\_Rvs GAAGTCACTCGTTCCCACTAG, plasmid

465 target sequence was amplified with PL\_Fwd GCCGTACTAAACCTCTCATCG and PL\_Rvs  
466 CTGACCGGAAAGTTTGTATTTCG.

467

#### 468 **Accession Numbers**

469 The Arabidopsis Genome Initiative numbers of genes used in this study are: *SCR* (  
470 AT3G54220), *ATHB-8* (AT4G32880), *XPP* (At4g30450), *AHP6* (  
471 AT1G80100), *PXY* (AT5G61480), *TMO5* (AT3G25710), *SMXL5* (  
472 AT5G57130), *CASP1* (AT2G36100), *VND7* (AT1G71930), *APL* (AT1G79430), *NST3*  
473 (AT1G32770), *WOX4* (AT1G46480), *PMEI5* (AT2G31430), *LTP1* (AT2G38540), AT2G3830,  
474 *ML1* (AT4G21750), *CLV3* (AT2G27250), *REV* (AT5G60690), *UFO* (AT1G30950), *CUC2*  
475 (AT5G53950).

476

477

#### 478 **Supplemental Data**

479 **Supplemental Figure S1.** Analysis of DMSO-treated mock controls for driver line seedling  
480 root induction 5 DAG.

481 **Supplemental Figure S2.** Analysis of induced driver lines in 5 DAG seedling root.

482 **Supplemental Figure S3.** Analysis of DMSO-treated driver lines in the stem.

483 **Supplemental Figure S4.** Analysis of DMSO-treated driver lines in the SAM.

484 **Supplemental Figure S5.** Growth on 50  $\mu$ M Dex does not impair root growth of Col-0.

485 **Supplemental Figure S6.** Reporter activation in the *pSCR>GR>mTurquoise2* line is  
486 sustainable.

487 **Supplemental Figure S7.** Kinetics of *pSCR>GR>mTurquoise2* reporter activity after  
488 removal of inducer.

489 **Supplemental Figure S8.** Quantification of GR-LhG4-mediated trans-activation.

490 **Supplemental Table S1.** List of primers used and DNA constructs generated in this study.

491

492

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 499 Programme (WO 1660/2-1).

500

### 501 **Tables**

Promoter		Expression	Reference
<i>pSCR</i>	<i>SCARECROW</i>	Endodermis, quiescent centre in RAM, starch sheath in stem	(Di Laurenzio et al., 1996; Wysocka-Diller et al., 2000)
<i>pATHB-8</i>	<i>HOMEODOMAIN GENE 8</i>	Procambium, xylem precursors and columella in RAM	(Baima et al., 1995)
<i>pXPP</i>	<i>XYLEM POLE PERICYCLE</i>	Xylem pole pericycle cells	(Andersen et al., 2018)
<i>pAHP6</i>	<i>HISTIDINE PHOSPHOTRANSFER PROTEIN 6</i>	Protoxylem precursors, pericycle, organ primordia in the SAM	(Mahonen et al., 2006; Besnard et al., 2014)
<i>pPXY</i>	<i>PHLOEM INTERCALATED WITH XYLEM</i>	(Pro-)cambium	(Fisher and Turner, 2007)
<i>pTMO5</i>	<i>TARGET OF MONOPTEROS 5</i>	Xylem precursors	(Schlereth et al., 2010; De Rybel et al., 2013)
<i>pSMXL5</i>	<i>SMAX1-LIKE 5</i>	Phloem (precursors)	(Wallner et al., 2017)
<i>pCASP1</i>	<i>CASPARIAN STRIP MEMBRANE DOMAIN PROTEIN 1</i>	Endodermis	(Roppolo et al., 2011)
<i>pVND7</i>	<i>VASCULAR RELATED NAC-DOMAIN PROTEIN 7</i>	Protoxylem (differentiating) in root, vessels in stem	(Kubo et al., 2005)
<i>pAPL</i>	<i>ALTERED PHLOEM DEVELOPMENT</i>	Phloem (differentiating)	(Bonke et al., 2003)
<i>pNST3</i>	<i>NAC SECONDARY WALL THICKENING PROMOTING 3</i>	Fibres	(Mitsuda et al., 2007)
<i>pWOX4</i>	<i>WUSCHEL RELATED HOMEODOMAIN 4</i>	(Pro-)cambium	(Hirakawa et al., 2010)
<i>pLTP1</i>	<i>LIPID TRANSFER PROTEIN 1</i>	Epidermis in stem	(Thoma et al., 1994)
<i>pAT2G3830</i>		Pith	(Valerio et al., 2004)
<i>pML1</i>	<i>MERISTEM LAYER 1</i>	L1 layer, epidermis	(Lu et al., 1996)
<i>pCLV3</i>	<i>CLAVATA3</i>	SAM stem cells	(Fletcher et al., 1999)
<i>pREV</i>	<i>REVOLUTA</i>	SAM central zone	(Otsuga et al., 2001)
<i>pUFO</i>	<i>UBUSUAL FLOWER ORGANS</i>	SAM peripheral zone	(Levin and Meyerowitz, 1995)
<i>pCUC2</i>	<i>CUP-SHAPED COTYLEDON 2</i>	Boundaries in SAM and leaf	(Aida et al., 1997)

502 **Table 1:** Overview of promoters utilized in this study.

503 **Figure Legends**

504 **Figure 1.** Overview of the Dex-inducible GR-LhG4/pOp system. In driver lines, expression of  
505 the synthetic transcription factor LhG4 is controlled by a tissue-specific promoter (*pTS*),  
506 whereas translational fusion with the ligand binding domain of rat glucocorticoid receptor  
507 (GR) prevents nuclear translocation in the absence of the inducer (Dex). After crossing with  
508 an effector line harbouring a transcriptional cassette under the control of a *pOp* element and  
509 a TATA box-containing minimal 35S promoter and addition of Dex, GR-LhG4 drives the  
510 expression of the effector as well as the mTurquoise2 reporter encoded by the driver line.

511 **Figure 2.** Analysis of induced driver lines in seedling roots. A, Schematic representation of  
512 root tissue layers. B-I, Induced driver line roots displaying fluorescence from propidium  
513 iodide (PI)-stained cell walls and the mTurquoise2 reporter (see Fig. 1 and Table 1). The  
514 indicated promoters mediate expression in the differentiating endodermis (B, *pCASPARIAN*  
515 *STRIP MEMBRANE DOMAIN PROTEIN 1* (*pCASP1*)), phloem precursor cells and adjacent  
516 pericycle cells (C, *pHISTIDINE PHOSPHOTRANSFER PROTEIN 6* (*pAHP6*)), xylem  
517 precursor cells (D, *pTARGET OF MONOPTEROS 5* (*pTMO5*)), xylem pole pericycle cells (E,  
518 *pXYLEM POLE PERICYCLE* (*pXPP*)), stele initials, cortex/endodermis initial (CEI) and  
519 columella initials (F, *pHOMEobox GENE 8* (*pATHB-8*)), endodermis, CEI and quiescent  
520 centre (G, *pSCARECROW* (*pSCR*)), stele initials, phloem and procambial cells (H, *pSMAX1-*  
521 *LIKE 5*, (*pSMXL5*)), and procambial cells (I, *pPHLOEM INTERCALATED WITH XYLEM*  
522 (*pPXY*)). PI fluorescence is false-coloured in magenta and mTurquoise2 fluorescence in  
523 green. Bars = 50 µm.

524 **Figure 3.** Analysis of induced driver lines in the stem. A, Schematic representation of  
525 inflorescence stem tissue layers. B-I, Induced driver line stems displaying fluorescence from  
526 propidium iodide (PI)-stained cell walls and the mTurquoise2 reporter (see Fig. 1 and Table  
527 1). The promoters mediate expression in differentiated phloem (B, *pALTERED PHLOEM*  
528 *DEVELOPMENT*, (*pAPL*)), xylem fibres and interfascicular fibres (C, *pNAC SECONDARY*  
529 *WALL THICKENING PROMOTING 3* (*pNST3*)), starch sheath (D, *pSCR*), cambium (E,

530 *pWUSCHEL RELATED HOMEBOX 4* (*pWOX4*), xylem vessels (F, *pVASCULAR*  
531 *RELATED NAC DOMAIN PROTEIN 7* (*pVND7*), epidermal cells (G, *pLIPID TRANSFER*  
532 *PROTEIN 1* (*pLTP1*)), the incipient phloem (H, *pSMXL5*), and pith (I, *pAT2G38380*). PI  
533 fluorescence is false-coloured in magenta and mTurquoise2 fluorescence in green. Bars =  
534 50  $\mu\text{m}$ .

535

536 **Figure 4.** Analysis of induced driver lines in the shoot apical meristem (SAM). A, Schematic  
537 representation of cell identity domains in the SAM. B-G, Induced driver line stems displaying  
538 fluorescence from propidium iodide (PI)-stained cell walls and the mTurquoise2 reporter (see  
539 Fig. 1 and Table 1). The left and middle panels are maximum projections of confocal stack,  
540 the right panels consist of a single median confocal xy section and xz and yz view of the  
541 stack. The indicated promoters mediate expression in the L1 layer/epidermis (B,  
542 *pMERISTEM LAYER 1* (*pML1*)), the stem cell domain (C, *pCLV3*), the central zone (D,  
543 *pREVOLUTA* (*pREV*)), the peripheral zone (E, *pUNUSUAL FLOWER ORGANS* (*pUFO*)),  
544 the boundary domain (F, *pCUP-SHAPED COTYLEDON* (*pCUC2*)), and organ primordia (G,  
545 *pAHP6*). PI fluorescence is false-coloured in magenta and mTurquoise2 fluorescence in  
546 green. Bars = 20  $\mu\text{m}$ .

547 **Figure 5.** Dose-response and time course analysis of driver line seedling roots. A, The  
548 *pSCR* driver line was grown on 0, 0.1, 1, 10 and 100  $\mu\text{M}$  Dex and imaged five DAG. B,  
549 Time-course of *pSCR* driver line induction for 1, 6 and 24 hours with 10  $\mu\text{M}$  Dex. C,  
550 Quantification of the mTurquoise2 fluorescence intensity dose-response in quiescent centre  
551 cells and CEI (cells outlined in white in panel A). D, Quantification of mTurquoise2  
552 fluorescence intensity of the first 3 endodermal cells after the CEI (cells outlined in blue in  
553 panel A). E, quantification of the induction time-course (B) in quiescent centre cells, CEI and  
554 the first 3 endodermal cells. Significant differences in (C, D, and E) are based on the results  
555 of a two-tailed t test with  $p < 0.05$ ,  $p < 0.01$ ,  $p < 0.001$ ,  $n=3-6$  roots each. Bars = 50  $\mu\text{m}$ .



556 **Figure 6.** Induction of mTurquoise2 and mVenus/3xGFP fluorescence in the root, stem and  
557 SAM of F1 plants from a driver line-effector line cross. Cells are counter-stained with PI  
558 (which, in the stem, highlights lignified vessel elements and fibres). Fluorescence channels  
559 are false-coloured. Bars = 50 µm for the root and the stem, 40 µm for the SAM.

560 **Figure 7.** Cell-type specific induction demonstrates the efficacy of trans-activation. Plants  
561 expressing VND7-VP16 as an effector in the endodermal cells (*pSCR>GR>VND7-VP16*)  
562 show ectopic vessel formation (white arrows) after 5 days of Dex induction in both root and  
563 hypocotyl endodermis, in contrast to DMSO-treated plants. The spiral secondary cell wall  
564 thickening was observed after fixing and clearing the samples and visualized by DIC  
565 (differential interference contrast microscope). E = endodermis, P = pericycle, X = xylem.  
566 Bars = 20 µm.

567

568

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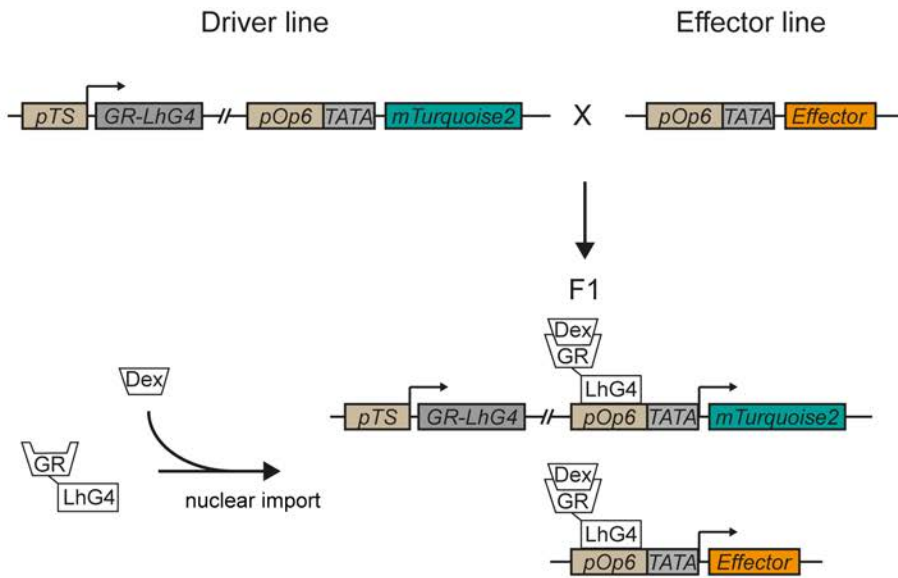
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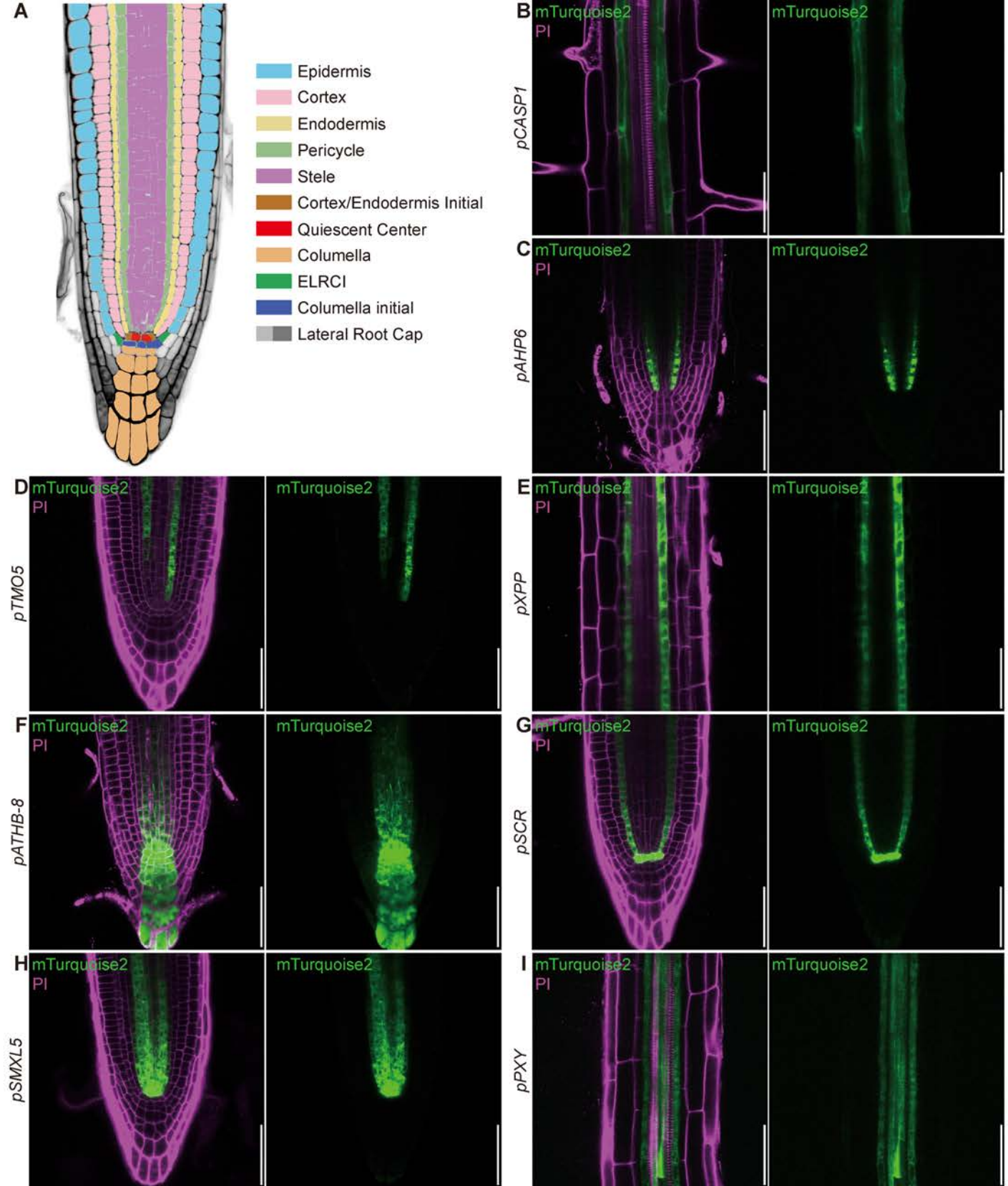
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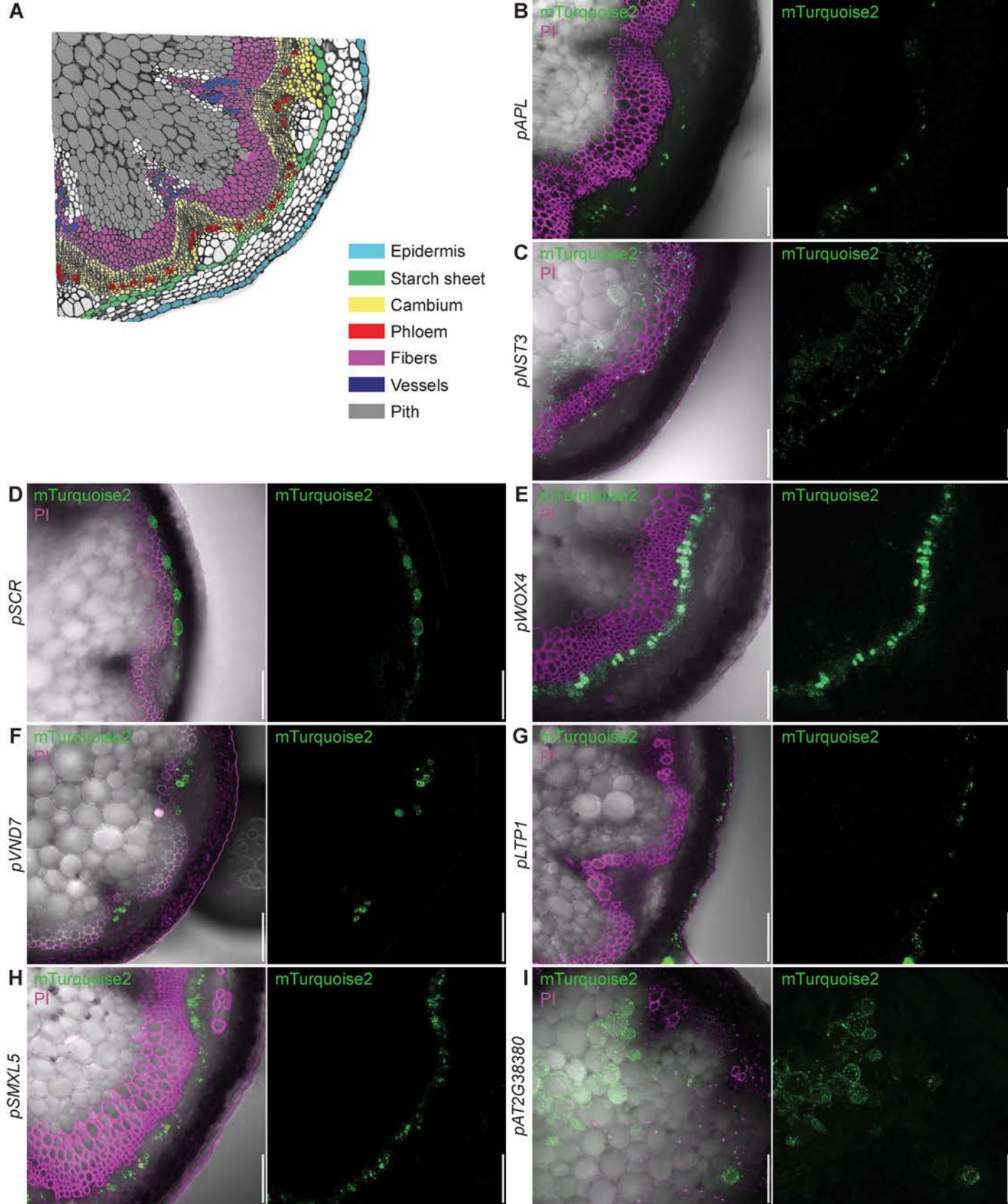
**Figure 1.** Overview of the Dex-inducible GR-LhG4/pOp system. In driver lines, expression of the synthetic transcription factor LhG4 is controlled by a tissue-specific promoter (*pTS*), whereas translational fusion with the ligand binding domain of rat glucocorticoid receptor (GR) prevents nuclear translocation in the absence of the inducer (Dex). After crossing with an effector line harbouring a transcriptional cassette under the control of a *pOp* element and a TATA box-containing minimal 35S promoter and addition of Dex, GR-LhG4 drives the expression of the effector as well as the mTurquoise2 reporter encoded by the driver line.



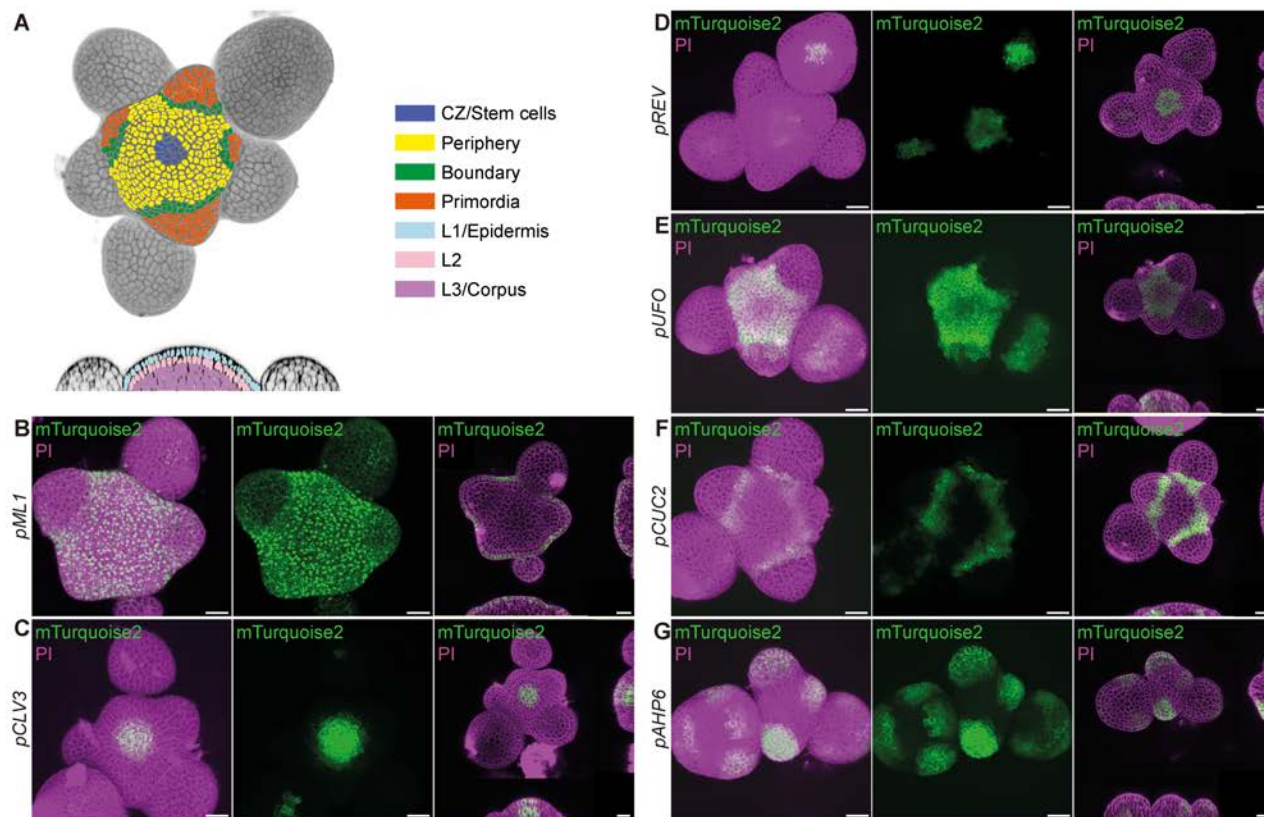


**Figure 2.** Analysis of induced driver lines in seedling roots. A, Schematic representation of root tissue layers. B-I, Induced driver line roots displaying fluorescence from propidium iodide (PI)-stained cell walls and the mTurquoise2 reporter (see Fig. 1 and Table 1). The indicated promoters mediate expression in the differentiating endodermis (B, pCASPARIAN STRIP MEMBRANE DOMAIN PROTEIN 1 (pCASP1)), phloem precursor cells and adjacent pericycle cells (C, pHISTIDINE PHOSPHOTRANSFER PROTEIN 6 (pAHP6)), xylem precursor cells (D, pTARGET OF MONOPTEROS 5 (pTMO5)), xylem pole pericycle cells (E, pXYLEM POLE PERICYCLE (pXPP)), stele initials, cortex/endodermis initial (CEI) and columella initials (F, pHOMEBOX GENE 8 (pATHB-8)), endodermis, CEI and quiescent centre (G, pSCARECROW (pSCR)), stele initials, phloem and procambial cells (H, pSMAX1-LIKE 5 (pSMXL5)) and procambial cells (I, pPHLOEM INTERCALATED WITH XYLEM (pPXY)). PI fluorescence is false-coloured in magenta and mTurquoise2 fluorescence in green. Bars = 50  $\mu$ m.



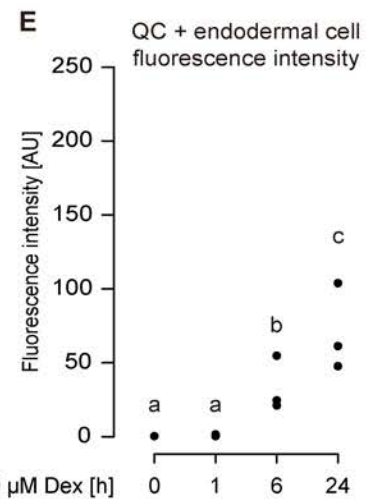
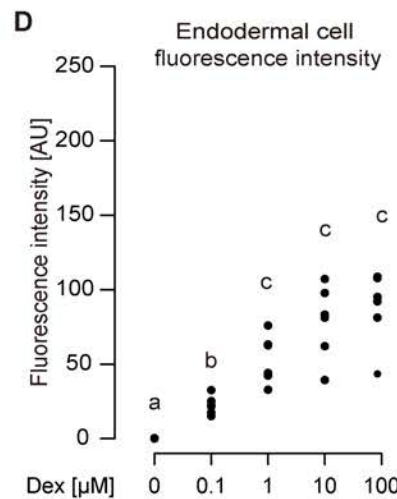
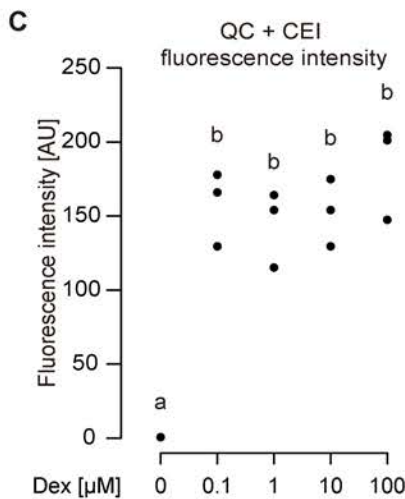
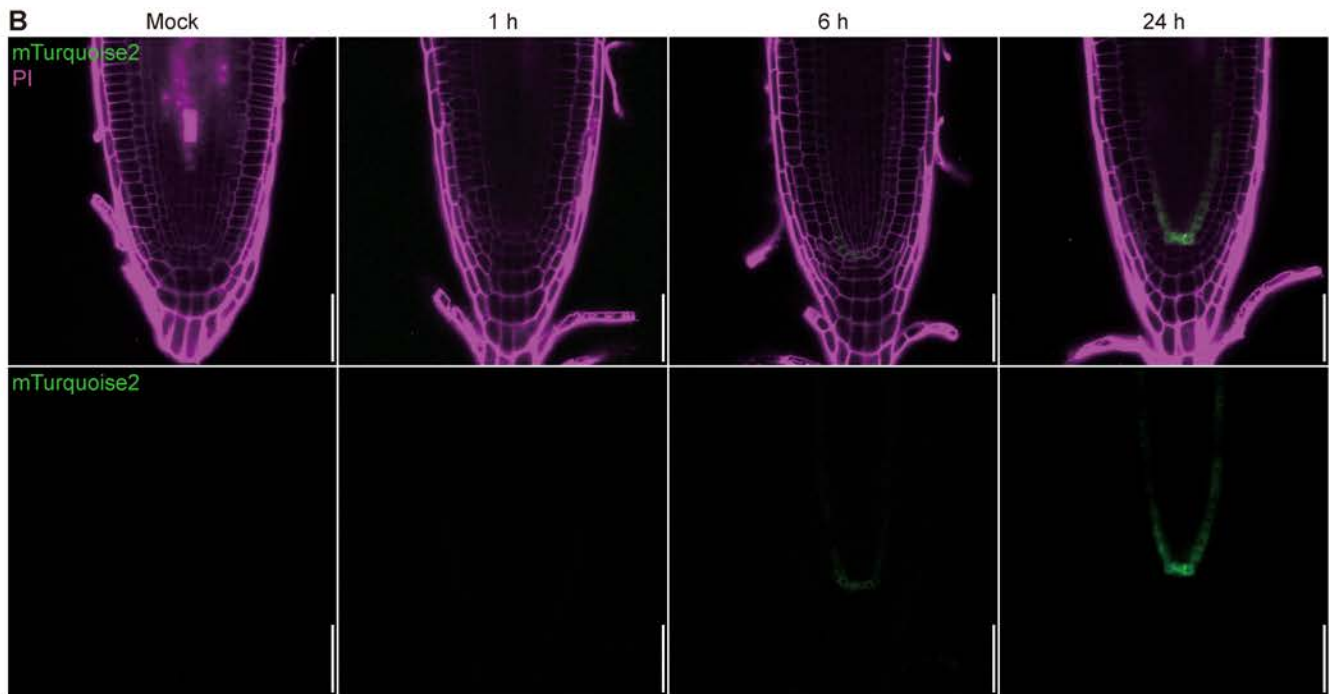
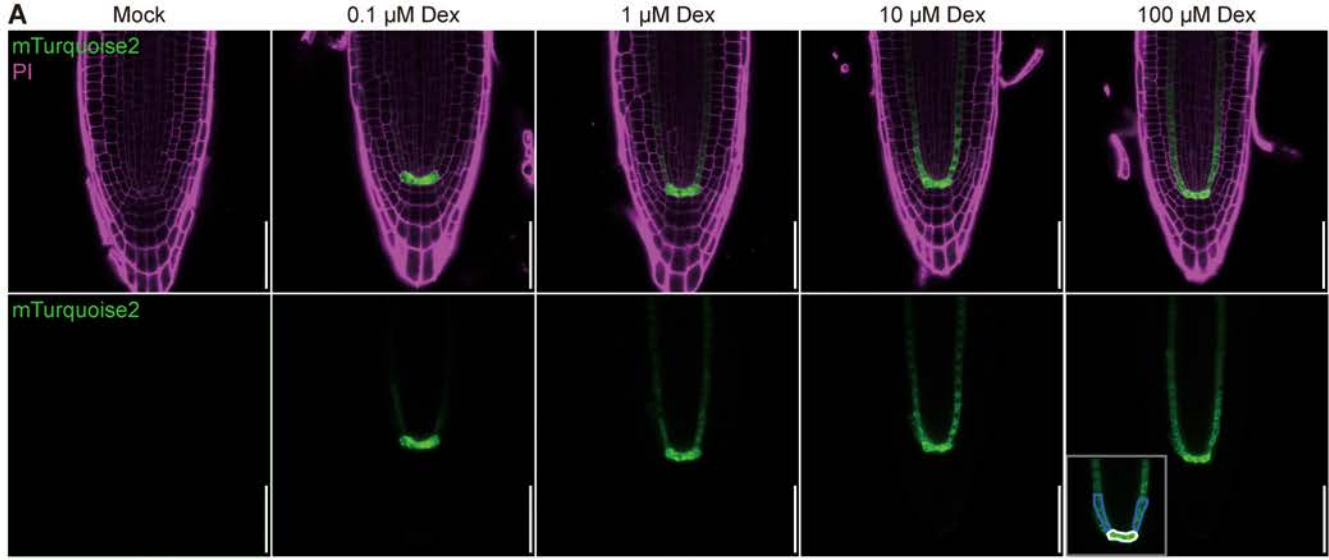


**Figure 3.** Analysis of induced driver lines in the stem. A, Schematic representation of inflorescence stem tissue layers. B-I, Induced driver line stems displaying fluorescence from propidium iodide (PI)-stained cell walls and the mTurquoise2 reporter (see Fig. 1 and Table 1). The promoters mediate expression in differentiated phloem (B, *pALTERED PHLOEM DEVELOPMENT*, (*pAPL*)), xylem fibres and interfascicular fibres (C, *pNAC SECONDARY WALL THICKENING PROMOTING 3* (*pNST3*)), starch sheath (D, *pSCR*), cambium (E, *pWUSCHEL RELATED HOMEBOX 4* (*pWOX4*)), xylem vessels (F, *pVASCULAR-RELATED NAC DOMAIN PROTEIN 7* (*pVND7*)), epidermal cells (G, *pLIPID TRANSFER PROTEIN 1* (*pLTP1*)), the incipient phloem (H, *pSMXL5*), and pith (I, *pAT2G38380*). PI fluorescence is false-coloured in magenta and mTurquoise2 fluorescence in green. Bars = 50  $\mu$ m.

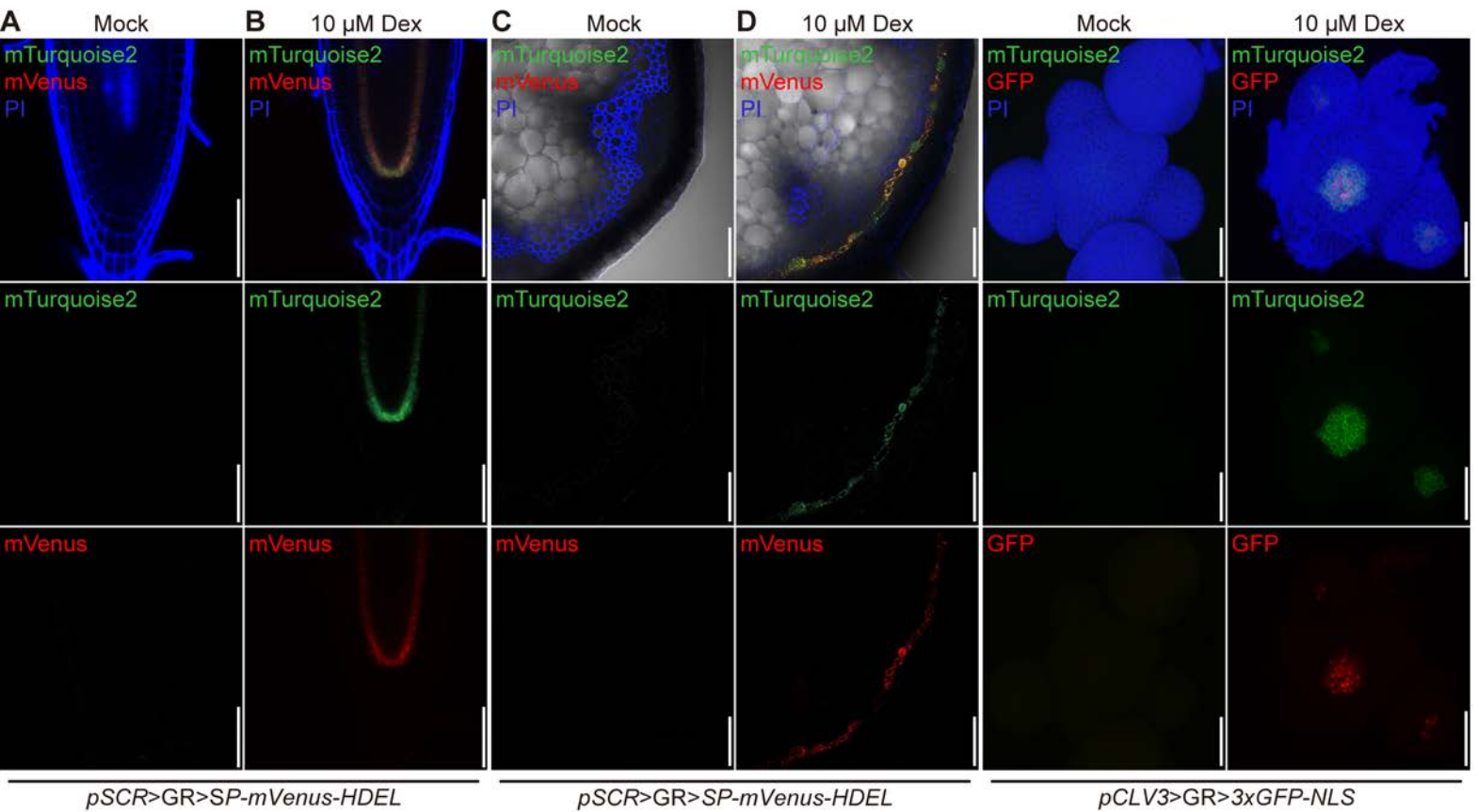


**Figure 4.** Analysis of induced driver lines in the shoot apical meristem (SAM). A, Schematic representation of cell identity domains in the SAM. B-G, Induced driver line stems displaying fluorescence from propidium iodide (PI)-stained cell walls and the mTurquoise2 reporter (see Fig. 1 and Table 1). The left and middle panels are maximum projections of confocal stack, the right panels consist of a single median confocal xy section and xz and yz view of the stack. The indicated promoters mediate expression in the L1 layer/epidermis (B, *pMERISTEM LAYER 1* (*pMML1*)), the stem cell domain (C, *pCLV3*), the central zone (D, *pREVOLUTA* (*pREV*)), the peripheral zone (E, *pUNUSUAL FLOWER ORGANS* (*pUFO*)), the boundary domain (F, *pCUP-SHAPED COTYLEDON* (*pCUC2*)), and organ primordia (G, *pAHP6*). PI fluorescence is false-coloured in magenta and mTurquoise2 fluorescence in green. Bars = 20  $\mu\text{m}$ .

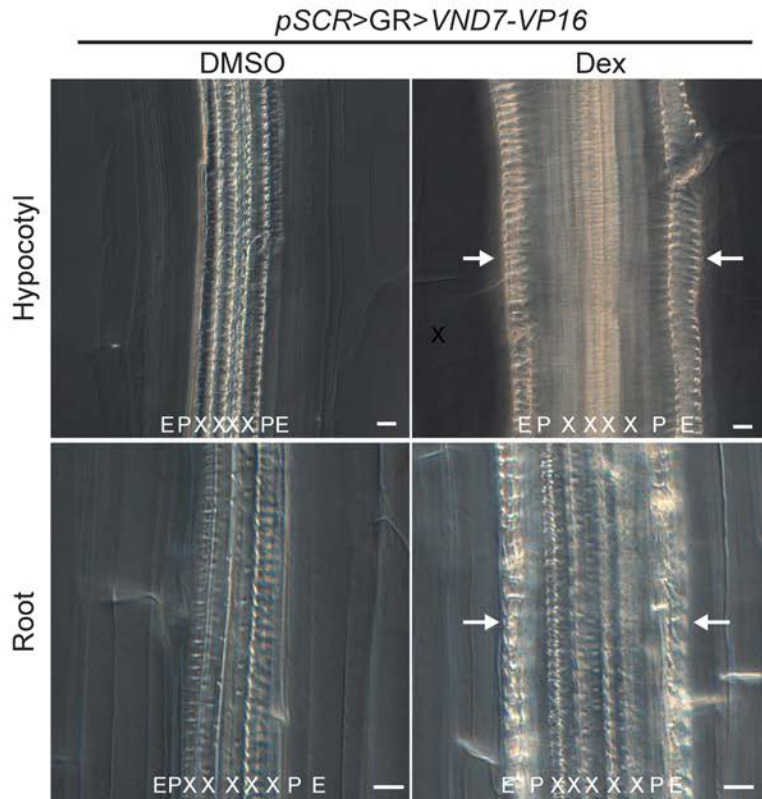




**Figure 5.** Dose-response and time course analysis of driver line seedling roots. **A**, The *pSCR* driver line was grown on 0, 0.1, 1, 10 and 100  $\mu\text{M}$  Dex and imaged five DAG. **B**, Time-course of *pSCR* driver line induction for 1, 6 and 24 hours with 10  $\mu\text{M}$  Dex. **C**, Quantification of the mTurquoise2 fluorescence intensity dose-response in quiescent centre cells and CEI (cells outlined in white in panel A). **D**, Quantification of mTurquoise2 fluorescence intensity of the first 3 endodermal cells after the CEI (cells outlined in blue in panel A). **E**, quantification of the induction time-course (B) in quiescent centre cells, CEI and the first 3 endodermal cells. Significant differences in (C, D, and E) are based on the results of a two-tailed t test with  $p < 0.05$ ,  $p < 0.01$ ,  $p < 0.001$ ,  $n=3-6$  roots each. Bars = 50  $\mu\text{m}$ .



**Figure 6.** Induction of mTurquoise2 and mVenus/3xGFP fluorescence in the root, stem and SAM of F1 plants from a driver line-effector line cross. Cells are counter-stained with PI (which, in the stem, highlights lignified vessel elements and fibres). Fluorescence channels are false-coloured. Bars = 50  $\mu$ m for the root and the stem, 40  $\mu$ m for the SAM.



**Figure 7.** Cell-type specific induction demonstrates the efficacy of trans-activation. Plants expressing VND7-VP16 as an effector in the endodermal cells (*pSCR>GR>VND7-VP16*) show ectopic vessel formation (white arrows) after 5 days of Dex induction in both root and hypocotyl endodermis, in contrast to DMSO-treated plants. The spiral secondary cell wall thickening was observed after fixing and clearing the samples and visualized by DIC (differential interference contrast microscope). E = endodermis, P = pericycle, X = xylem. Bars = 20  $\mu$ m.



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