

WUSCHEL acts as a rheostat on the auxin pathway to maintain apical stem cells in *Arabidopsis*

Yanfei Ma^{1§}, Andrej Miotk^{1§}, Zoran Šutiković¹, Anna Medzihradzsky¹, Christian Wenzl¹, Olga Ermakova¹, Christophe Gaillochet¹, Joachim Forner¹, Gözde Utan¹, Klaus Brackmann³, Carlos S. Galvan-Ampudia⁴, Teva Vernoux⁴, Thomas Greb² & Jan U. Lohmann^{1*}

¹Department of Stem Cell Biology, Centre for Organismal Studies, Heidelberg University; D-69120 Heidelberg, Germany

²Department of Developmental Physiology, Centre for Organismal Studies, Heidelberg University; D-69120 Heidelberg, Germany

³Gregor Mendel Institute (GMI), Austrian Academy of Sciences, Vienna Biocenter (VBC), Dr. Bohr-Gasse 3, 1030 Vienna, Austria

⁴Laboratoire Reproduction et Développement des Plantes, Univ Lyon, ENS de Lyon, UCB Lyon 1, CNRS, INRA, F-69342 Lyon, France

§ these authors contributed equally and are listed alphabetically

*corresponding author

Mailing address of corresponding author:

Jan U. Lohmann
Department of Stem Cell Biology
University of Heidelberg
Im Neuenheimer Feld 230
D-69120 Heidelberg
Germany

PH: +49 6221 546269
FX: +49 6221 546424
EM: jlohmann@meristemaniamania.org

Abstract

During development and growth, dynamic signals need to be translated into spatially precise and temporally stable gene expression states, which define cell fate. In the context of the apical plant stem cell system, local accumulation of the small, highly mobile phytohormone auxin triggers organ initiation. Here, we show that the WUSCHEL transcription factor locally protects stem cells from differentiation by controlling the auxin signaling and response pathway via regulation of histone acetylation. Conversely, low levels of signaling are required for stem cell maintenance, demonstrating that WUSCHEL acts as a rheostat on the auxin pathway. Our results reveal an important mechanism that allows cells to differentially translate a potent and highly mobile developmental signal into appropriate cell behavior with high spatial precision and temporal robustness.

Main Text

The shoot apical meristem (SAM) is a highly dynamic and continuously active stem cell system responsible for the generation of all above ground tissues of plants. The stem cells are located in the central zone and are maintained by a feedback loop consisting of the stem cell promoting WUSCHEL (WUS) homeodomain transcription factor and the restrictive CLAVATA (CLV) pathway^{1,2}. WUS protein is produced by a group of niche cells, called organizing center, and moves to stem cells via plasmodesmata^{3,4}. Stem cells are surrounded by transient amplifying cells, which are competent to undergo differentiation in response to auxin, a small, mobile signaling molecule with diverse and context specific roles in plant development and physiology (reviewed in ref. 5). Auxin sensing is dependent on nuclear receptors, whose activation triggers the proteolytic degradation of AUX/IAA proteins, such as BODENLOS (BDL), which inhibit the function of activating AUXIN RESPONSE FACTOR (ARF) transcription factors⁶⁻⁸. Intracellular accumulation of auxin is regulated by active polar transport and in the context of the SAM, the export carrier PINFORMED1 (PIN1) determines the sites of lateral organ initiation and thus differentiation^{9,10}. Here we ask how long-term stem cell fate is robustly maintained within such a highly dynamic signaling system geared towards differentiation.

Results

Role of auxin signaling for apical stem cell fate

As a first step, we mapped auxin signaling behavior using the genetically encoded markers R2D2 and DR5v2 (ref. 11). R2D2 is based on fusing the auxin-dependent degradation domain II of an Aux/IAA protein to Venus fluorescent protein, and uses a mutated, non-degradable domain II linked to tdTomato as an internal control¹¹. Hence, R2D2 signal is dictated by the levels of auxin as well as the endogenous receptors and represents a proxy for the auxin signaling input for every cell. Computational analysis of the green to red ratio in plants carrying R2D2 demonstrated that auxin is present and sensed fairly uniformly across the SAM including the central stem cell domain, with local minima only detected at organ boundaries (Fig. 1a, b and refs. 12,13). In contrast, DR5v2, a reporter for auxin signaling output based on a synthetic promoter containing repeats of ARF DNA binding sites¹¹, was strongly activated non-uniformly in wedge shaped zones of differentiation competent cells, but only weakly expressed the center of the SAM (Fig. 1d; and ref. 12). Leveraging the sensitivity of DR5v2 and the expression of the *CLV3* stem cell

marker in the same transgenic line, we observed that the auxin response minimum invariably coincided with a small group of cells in the stem cell domain (Fig. 1c-f).

To test if the auxin output minimum is functionally connected to stem cell identity, we interfered with their maintenance. To this end, we induced symplastic isolation through callose deposition at plasmodesmata of stem cells, which we had shown earlier to induce their differentiation^{4,14}. Following auxin signaling output over time, we observed activation of DR5v2 in the central zone domain after 36 hours of callose synthase (iCalSm) expression and cell expansion, a hallmark of plant cell differentiation, after 72 hours (Fig. 2a-e; Extended Fig. 1-3; Extended Table 1). Thus, stem cell fate and the auxin response minimum appeared to be functionally connected, leading us to hypothesize that manipulation of auxin signaling in the central zone should affect stem cell behavior. To test this directly we designed a transgene, which allowed us to suppress auxin signaling output specifically in stem cells by fusing the dominant auxin signaling output inhibitor *BDL-D* with the glucocorticoid receptor tag. The activity of the resulting fusion protein could be induced by dexamethasone (DEX) treatment, which facilitated the translocation of BDL-D-GR into the nucleus¹⁵. In line with our expectations, we found that inducing *pCLV3:BDL-D-GR* led to an expansion of the DR5v2 minimum in the center of the SAM (Fig. 2 f, g). Surprisingly, long term induction of BDL-D-GR or stem cell specific expression of *BDL-D* caused meristem termination in half of the seedlings (n=90; Fig. 2 k, l), demonstrating that stem cells require active auxin signaling for their maintenance. In contrast, expression of a potent positive signaling component, the auxin response factor *ARF5/MONOPTEROS (MP)*, or its constitutively active form *MPΔ*, which engages the auxin pathway independently of signal perception¹⁶, did not cause relevant reduction in meristem size (Fig. 2 h-j, o and ref. 17). When expressed throughout the entire SAM, *MPΔ* stimulated ectopic organ initiation specifically in the peripheral zone (Fig. 2n; Extended Fig. 4), demonstrating that resistance to auxin was not a general feature of the meristem, but limited to stem cells. Importantly, the DR5v2 reporter, which senses auxin output by providing binding sites for ARF transcription factors, was activated in stem cells of plants expressing *MP* and *MPΔ* (6/8 independent T1 lines; Fig. 2h-j and Extended Fig. 5), suggesting that the resistance to auxin occurs, at least in part, downstream of ARF activity. Taken together, these experiments suggested that auxin signaling is locally gated to permit a low instructive output level, while at the same time protecting stem cells from the differentiation inducing effects of the phytohormone at high signaling levels.

WUSCHEL controls auxin signaling output in stem cells

Since suppressing auxin signaling output in stem cell caused SAM arrest and a phenotype highly similar to *wus* mutants (Fig. 2 k, l), we tested the contribution of *WUS* to controlling auxin responses in diverse genetic backgrounds. Since the *WUS* expression domain is massively enlarged in *clv* mutants^{1,2}, which causes stem cell over-proliferation phenotypes, SAMs from these plants provide an ideal background to elucidate the functional connection of *WUS* and auxin. Therefore, we analyzed auxin output in *clv3* meristems and found the DR5v2 minimum expanded in line with the overaccumulation of *WUS*, however some weak signal remained throughout the SAM (Fig. 3a, b). To test whether auxin signaling is required for stem cell over-proliferation in *clv3* mutants, we locally blocked auxin output by our *pCLV3:BDL-D* transgene and observed stem cell termination phenotypes in almost all seedlings (n=30; Fig. 3c). This result suggested that also in fasciated SAMs of *clv3* mutants, ectopic *WUS* is sufficient to reduce auxin signaling, while at the same time permitting basal output levels. To test the short term effect of enhancing *WUS* levels without the indirect effects of the *clv3* phenotype, we created plants that carry a *pUBI10:mCherry-GR-linker-WUS* (*WUS-GR*) transgene which allowed for experimental induction of ubiquitous *WUS* activity (Extended Fig. 6). After 24 h of DEX treatment the central auxin signaling minimum as well as the *CLV3* domain expanded (Extended Fig. 7), suggesting that *WUS* is indeed sufficient to reduce signaling output in the center of the SAM, but is unable to override active auxin responses at the periphery. To test whether *WUS* is also required to protect stem cells from high signaling levels, which lead to differentiation, we developed a genetic system that allowed us to inducibly remove *WUS* protein from stem cells. To this end, we adapted deGradFP technology¹⁸ and combined switchable stem cell specific expression of an anti-GFP nanobody with a *pWUS:WUS-linker-GFP wus* rescue line⁴. After 24h of induction of nanobody expression, *WUS*-linker-GFP signal was substantially reduced in stem cells of the epidermis and subepidermis (Extended Fig. 8), while at the same time DR5v2 expression had spread into the center of the SAM (8/9 vs. 0/12 in control plants; Fig. 3 d, e). We made similar observations in plants carrying the weak *wus-7* allele, which are able to maintain a functional SAM for some time and only terminate stochastically. In these lines, DR5v2 activity fluctuated substantially and was frequently observed in the central zone (Extended Fig. 9). Taken together, these results demonstrated that *WUS* is required for stably maintaining stem cells in a state of low auxin signaling.

Mechanisms of auxin pathway gating

To address how WUS is able to gate the output of the auxin pathway, we went on to define direct target genes combining new ChIP-seq and RNA-seq experiments using seedlings of our *WUS-GR* line. Interestingly, WUS binding was almost exclusively found in regions of open chromatin¹⁹ and among the WUS targets (Supplementary File 1 and refs. 20,21) we found the gene ontology term “response to auxin” to be most highly enriched within the developmental category (Extended Table 2). Importantly, WUS appeared to control auxin signaling output at all relevant levels, since it was able to bind to the promoters or regulate the expression of a large number of genes involved in auxin biosynthesis, transport, auxin perception, auxin signal transduction, as well as auxin response, which occurs downstream of ARF transcription factors (Fig. 4a and Extended Tables 3 and 4). Since WUS can act as transcriptional activator or repressor dependent on the regulatory environment^{22,23} and our profiling results were based on ectopic expression of WUS in non-stem cells, we were unable to predict how the expression of individual targets would be affected *in vivo*. However, it has been reported that in the SAM, WUS mainly acts as a transcriptional repressor^{20,22,24} and consistently, many auxin signaling components are expressed at high levels only in the periphery of the SAM and exhibit low RNA accumulation in the cells that are positive for WUS protein¹². To test if WUS is required for this pattern, we analyzed the response of *MP* and *TIR1* mRNA accumulation to variations in *WUS* expression. To circumvent morphological defects of stable *wus* mutants, we again made use of our deGradFP line to analyze expression of *MP* after loss of WUS protein activity, but prior to changes in SAM morphology. After 24 h of induction, *MP* mRNA expression had extended from the periphery into the central zone (Fig. 4b, c; Extended Fig. 10, 11), demonstrating that WUS is indeed required for *MP* repression in stem cells. Conversely, ectopic activation of WUS revealed that it is also sufficient to reduce, but not shut down *MP* and *TIR1* transcription even in the periphery of the SAM (Fig. 4 d-e, Extended Fig. 7).

To elucidate the molecular mechanisms responsible for the observed regulatory gating, we asked whether chromatin structure may be changed in response to WUS. WUS physically interacts with TOPLESS (TPL)^{25,26}, a member of the GROUCHO/Top1 family of transcriptional co-repressors. These adaptor proteins mediate interaction with HISTONE DEACETYLASES (HDACs, reviewed in ref. 27), which in turn act to reduce transcriptional activity of chromatin regions via promoting the removal of acetyl modifications from histone tails²⁸. To test whether regulation of chromatin modification

is involved in translating WUS activity into the observed reduction of transcriptional activity of target genes we quantified histone acetylation on H3K9 and methylation on H3K27. After 2 h of induction of our *WUS-GR* line, we observed a significant change in the genome wide histone acetylation patterns, which were spatially correlated with WUS chromatin binding events (2939 out of 6740 WUS bound chromatin regions showed acetylation changes), while histone methylation patterns were largely unaffected (525 out of 6740 WUS bound chromatin regions showed methylation changes). WUS binding events clustered in the proximal promoter regions, while chromatin regions whose acetylation levels were changed after WUS activation were mainly found around the transcriptional start sites and 5'UTRs of genes (Fig. 4f). Zooming in on the 1656 directly repressed WUS targets, we found that 587 of them also showed histone de-acetylation. For the vast majority of these loci the observed reduction was fairly subtle, suggesting that mild de-acetylation may be the mechanism that allows WUS to reduce, but not shut off transcription of target genes. To test whether the observed changes in chromatin state of direct WUS targets also translate to variation in gene expression, we induced WUS activity in the presence of Trichostatin A (TSA), a potent inhibitor of class I and II HDACs²⁹, and recorded the transcriptional response. Strikingly, from the 1656 directly repressed genes, 922 were no longer responsive to WUS-GR induction when TSA was present, underlining the relevance of histone de-acetylation for the genome-wide functional output of WUS. To investigate whether this mechanism is relevant for controlling auxin responses in the SAM, we analyzed DR5v2 reporter activity after TSA and/or auxin treatment. Auxin was insufficient to trigger a transcriptional response in stem cells, likely due to the presence of functional WUS (Fig. 4g). In contrast, inactivation of HDACs and consequently WUS-mediated transcriptional repression by TSA treatment, led to low but consistent DR5v2 signal in the center of the meristem (Fig. 4h). Combining stimulation of the pathway with reduction in WUS function caused substantial DR5v2 response in stem cells (Fig. 4i). Taken together, these results showed that WUS binds to and reduces transcription of the majority of genes involved in auxin signaling and response via de-acetylation of histones and thus is able to maintain pathway activity in stem cells at a basal level.

Pathway wide gating provides robustness to apical stem cell fate

We next wondered what the functional relevance of the observed pathway wide regulatory interaction might be. Therefore, we tested the capacity of *WUS* targets with auxin signaling or response functions to interfere with stem cell activity. Based on their highly localized expression at the periphery of the SAM¹², we selected the signaling components *ARF3*, *ARF4*, *ARF5* (*MP*), *IAA8*, *IAA9*, and *IAA12* (*BDL*) as well as the TIR1 receptor along with transcription factors of the auxin response category including *TARGET OF MONOPTEROS* (*TMO*) and *LATERAL ORGAN BOUNDARIES* (*LOB*) genes that have established roles in other developmental contexts³⁰. Neither of the 17 factors tested caused meristem phenotypes when expressed in stem cells (Fig. 2 and Extended Table 5), highlighting the robustness of stem cell fate in the presence of *WUS* on the one hand and the activity of auxin signaling in these cells on the other hand. This conclusion is based on two observations: 1. The auxin sensitive native version of *BDL* was unable to terminate the SAM in contrast to the auxin insensitive *BDL-D* version (Fig. 2i, j). 2. *pCLV3:MP* plants showed enhanced *DR5v2* activity in stem cells (Fig. 2g, h) demonstrating that *ARF* activity is indeed limiting for transcriptional output in wild-type. However, this transcriptional output registered by the *DR5v2* reporter is not translated into an auxin response, since *WUS* limits the expression of a large fraction of the required downstream genes (Fig. 4a; extended tables 2, 3). Thus, *WUS* seems to act both up- and downstream of the key *ARF* transcription factors.

Since we had found that stem cell specific expression of individual auxin signaling components was not sufficient to interfere with stem cell fate, we wanted to test whether reducing *WUS* function would sensitize stem cells to activation of the entire pathway. To this end, we grew plants segregating for *wus-7* on plates supplemented with auxin. Eleven days after germination, we observed twice as many terminated *wus-7* mutant seedlings than on control plates, whereas wild-type seedlings were unaffected (Fig. 3f, Extended Fig. 12). Thus, reducing *WUS* function allowed activation of auxin responses under conditions that were tolerated in wild type. Taken together, the activation of individual pathway components was insufficient to override the protective effect of *WUS*, however removing the master regulator itself rendered stem cells vulnerable to even mild perturbations in auxin signaling.

Discussion

In conclusion, our results show that WUS restricts auxin signaling in apical stem cells by pathway-wide transcriptional control, while at the same time allowing instructive low levels of signaling output. This rheostatic activity may be based on selective transcriptional repression/activation of a subset of signaling and response components that render the pathway unresponsive to high input levels. Alternatively, WUS may be able to reduce expression of targets rather than to shut off their activity completely, leaving sufficient capacity for low level signaling only. In support of the latter hypothesis, we demonstrate that WUS acts via de-acetylation of histones and that interfering with HDAC activity triggers auxin responses in stem cells. However, there is evidence supporting both scenarios²⁰⁻²³ and likely both mechanisms work hand in hand dependent on the regulatory environment of the individual cell. Thus, a definitive answer will require inducible WUS loss of function approaches in stem cells coupled with time-resolved whole genome transcript profiling at the single cell level. Importantly, in addition to its effects on auxin signaling, WUS enhances cytokinin responses via the repression of negative feedback regulators²⁴. Whereas this interaction can be overridden by expression of dominant cytokinin signaling components²⁴, stem cells remain unresponsive to elements of the auxin pathway. This argues that the regulation of the auxin pathway might be of higher significance than the interaction with cytokinin, which may primarily serve to sustain *WUS* expression^{31,32}. Auxin and cytokinin signaling are directly coupled¹⁷ and balancing their outputs is key to maintaining functional plant stem cell niches^{17,33}. Given the dynamic and self-organizing nature of the auxin system³⁴, the independent spatial input provided by WUS appears to be required to bar differentiation competence from the center of the SAM, while at the same time still allowing to sense this important signal. In light of the recent findings that PIN1 mediated auxin flux in the SAM is directed towards the center³⁵, it is tempting to speculate that auxin may serve as a positional signal not only for organ initiation, but also for stem cells.

Author Contributions:

A. Me. performed in situ hybridizations, C.W. carried out imaging and analyses, J.F. established the WUS-GR line, G.U. and A. M. performed RNA-seq, O.E. performed bioinformatic analyses, K.B. and T.G. established the *pDR5v2:ER-EYFP-HDEL:tAt4g24550* line, C.G. made the *pCLV3:mCherry-NLS:tCLV3* construct, Z.Š., A.M and Y.M. performed all other experiments. C.G.-A. and T.V. designed the TSA treatment of the SAM, Y.M., Z.Š., A.M. and J.U.L. designed all other experiments and wrote the paper with input from all other authors.

Acknowledgments:

We thank Dolf Weijers for sharing R2D2 and DR5v2 resources before publication. This work was supported by the DFG through grants SFB1101 and SFB873 to JUL and TG and by HFSP Grant RPG0054-2013 and ANR-12-BSV6-0005 grant to T.V. Computational analyses have been carried out on heiCLOUD provided by Heidelberg University Computing Centre.

References

1. Schoof, H. *et al.* The stem cell population of Arabidopsis shoot meristems is maintained by a regulatory loop between the CLAVATA and WUSCHEL genes. *Cell* **100**, 635–644 (2000).
2. Brand, U., Fletcher, J. C., Hobe, M., Meyerowitz, E. M. & Simon, R. Dependence of stem cell fate in Arabidopsis on a feedback loop regulated by CLV3 activity. *Science* **289**, 617–619 (2000).
3. Yadav, R. K. *et al.* WUSCHEL protein movement mediates stem cell homeostasis in the Arabidopsis shoot apex. *Genes Dev* **25**, 2025–2030 (2011).
4. Daum, G., Medzihradzky, A., Suzaki, T. & Lohmann, J. U. A mechanistic framework for noncell autonomous stem cell induction in Arabidopsis. *Proc Natl Acad Sci USA* **111**, 14619–14624 (2014).
5. Benjamins, R. & Scheres, B. Auxin: the looping star in plant development. *Annu Rev Plant Biol* **59**, 443–465 (2008).
6. Kepinski, S. & Leyser, O. Auxin-induced SCFTIR1-Aux/IAA interaction involves stable modification of the SCFTIR1 complex. *Proc. Natl. Acad. Sci. U.S.A.* **101**, 12381–12386 (2004).
7. Dharmasiri, N., Dharmasiri, S. & Estelle, M. The F-box protein TIR1 is an auxin receptor. *Nature* **435**, 441–445 (2005).
8. Kepinski, S. & Leyser, O. The Arabidopsis F-box protein TIR1 is an auxin receptor. *Nature* **435**, 446–451 (2005).
9. Vernoux, T., Kronenberger, J., Grandjean, O., Laufs, P. & Traas, J. PIN-FORMED 1 regulates cell fate at the periphery of the shoot apical meristem. *Development* **127**, 5157–5165 (2000).
10. Reinhardt, D. *et al.* Regulation of phyllotaxis by polar auxin transport. *Nature* **426**, 255–260 (2003).
11. Liao, C.-Y. *et al.* Reporters for sensitive and quantitative measurement of auxin response. *Nature Methods* **12**, 207–210 (2015).
12. Vernoux, T. *et al.* The auxin signalling network translates dynamic input into robust patterning at the shoot apex. *Mol Syst Biol* **7**, 508 (2011).
13. Brunoud, G. *et al.* A novel sensor to map auxin response and distribution at high spatio-temporal resolution. *Nature* **482**, 103–106 (2012).
14. Vatén, A. *et al.* Callose biosynthesis regulates symplastic trafficking during root development. *Dev Cell* **21**, 1144–1155 (2011).
15. Lloyd, A. M., Schena, M., Walbot, V. & Davis, R. W. Epidermal cell fate determination in Arabidopsis: patterns defined by a steroid-inducible regulator. *Science* **266**, 436–439 (1994).
16. Hardtke, C. S. & Berleth, T. The Arabidopsis gene MONOPTEROS encodes a transcription factor mediating embryo axis formation and vascular development. *EMBO J* **17**, 1405–1411 (1998).
17. Zhao, Z. *et al.* Hormonal control of the shoot stem-cell niche. *Nature* **465**, 1089–1092 (2010).
18. Caussinus, E., Kanca, O. & Affolter, M. Fluorescent fusion protein knockout mediated by anti-GFP nanobody. *Nature Publishing Group* **19**, 117–121 (2011).
19. Zhang, W., Zhang, T., Wu, Y. & Jiang, J. Genome-wide identification of regulatory DNA elements and protein-binding footprints using signatures of open chromatin in Arabidopsis. *Plant Cell* **24**, 2719–2731 (2012).
20. Busch, W. *et al.* Transcriptional control of a plant stem cell niche. *Dev Cell* **18**, 849–861 (2010).

21. Yadav, R. K. *et al.* Plant stem cell maintenance involves direct transcriptional repression of differentiation program. *Mol Syst Biol* **9**, 654–654 (2013).
22. Ikeda, M., Mitsuda, N. & Ohme-Takagi, M. Arabidopsis WUSCHEL is a bifunctional transcription factor that acts as a repressor in stem cell regulation and as an activator in floral patterning. *Plant Cell* **21**, 3493–3505 (2009).
23. Zhou, Y. *et al.* HAIRY MERISTEM with WUSCHEL confines CLAVATA3 expression to the outer apical meristem layers. *Science* **361**, 502–506 (2018).
24. Leibfried, A. *et al.* WUSCHEL controls meristem function by direct regulation of cytokinin-inducible response regulators. *Nature* **438**, 1172–1175 (2005).
25. Long, J. A., Ohno, C., Smith, Z. R. & Meyerowitz, E. M. TOPLESS regulates apical embryonic fate in Arabidopsis. *Science* **312**, 1520–1523 (2006).
26. Kieffer, M. *et al.* Analysis of the transcription factor WUSCHEL and its functional homologue in Antirrhinum reveals a potential mechanism for their roles in meristem maintenance. *Plant Cell* **18**, 560–573 (2006).
27. Liu, Z. & Karmarkar, V. Groucho/Tup1 family co-repressors in plant development. *Trends Plant Sci* **13**, 137–144 (2008).
28. Taunton, J., Hassig, C. A. & Schreiber, S. L. A mammalian histone deacetylase related to the yeast transcriptional regulator Rpd3p. *Science* **272**, 408–411 (1996).
29. Yoshida, M., Kijima, M., Akita, M. & Beppu, T. Potent and specific inhibition of mammalian histone deacetylase both in vivo and in vitro by trichostatin A. *J Biol Chem* **265**, 17174–17179 (1990).
30. Schlereth, A. *et al.* MONOPTEROS controls embryonic root initiation by regulating a mobile transcription factor. *Nature* **464**, 913–916 (2010).
31. Gordon, S. P., Chickarmane, V. S., Ohno, C. & Meyerowitz, E. M. Multiple feedback loops through cytokinin signaling control stem cell number within the Arabidopsis shoot meristem. *Proceedings of the National Academy of Sciences* **106**, 16529–16534 (2009).
32. Buechel, S. *et al.* Role of A-type ARABIDOPSIS RESPONSE REGULATORS in meristem maintenance and regeneration. *European Journal of Cell Biology* **89**, 279–284 (2010).
33. Müller, B. & Sheen, J. Cytokinin and auxin interaction in root stem-cell specification during early embryogenesis. *Nature* **453**, 1094–1097 (2008).
34. Bhatia, N. *et al.* Auxin Acts through MONOPTEROS to Regulate Plant Cell Polarity and Pattern Phyllotaxis. *Curr Biol* **26**, 3202–3208 (2016).
35. Galván-Ampudia, C. S. *et al.* From spatio-temporal morphogenetic gradients to rhythmic patterning at the shoot apex. *BioRxiv* (2018).
36. Yadav, R. K., Tavakkoli, M., Xie, M., Girke, T. & Reddy, G. V. A high-resolution gene expression map of the Arabidopsis shoot meristem stem cell niche. *Development* **141**, 2735–2744 (2014).
37. Yadav, R. K., Girke, T., Pasala, S., Xie, M. & Reddy, G. V. Gene expression map of the Arabidopsis shoot apical meristem stem cell niche. *Proc Natl Acad Sci USA* **106**, 4941–4946 (2009).
38. Prunet, N. Live Confocal Imaging of Developing Arabidopsis Flowers. *J Vis Exp* e55156–e55156 (2017). doi:10.3791/55156
39. Roslan, H. A. *et al.* Characterization of the ethanol-inducible alc gene-expression system in Arabidopsis thaliana. **28**, 225–235 (2001).
40. Lampropoulos, A. *et al.* GreenGate---a novel, versatile, and efficient cloning system for plant transgenesis. *PLoS ONE* **8**, e83043 (2013).
41. Schindelin, J. *et al.* Fiji: an open-source platform for biological-image analysis. *Nat Meth* **9**, 676–682 (2012).

42. de Reuille, P. B., Robinson, S. & Smith, R. S. Quantifying cell shape and gene expression in the shoot apical meristem using MorphoGraphX. *Methods Mol Biol* **1080**, 121–134 (2014).
43. Sommer, C., Straehle, C., Kothe, U. & Hamprecht, F. A. Ilastik: Interactive learning and segmentation toolkit. in 230–233 (IEEE, 2011). doi:10.1109/ISBI.2011.5872394
44. Berthold, M. R. *et al.* KNIME - the Konstanz information miner: version 2.0 and beyond. *ACM SIGKDD Explorations Newsletter* **11**, 26–31 (2009).
45. Medzihradzky, A., Schneitz, K. & Lohmann, J. U. Detection of mRNA expression patterns by nonradioactive in situ hybridization on histological sections of floral tissue. *Methods Mol Biol* **1110**, 275–293 (2014).
46. Pfeiffer, A. *et al.* Integration of light and metabolic signals for stem cell activation at the shoot apical meristem. *Elife* **5**, 827 (2016).
47. Li, H. & Durbin, R. Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics* **25**, 1754–1760 (2009).
48. Afgan, E. *et al.* The Galaxy platform for accessible, reproducible and collaborative biomedical analyses: 2018 update. *Nucleic Acids Res* **46**, W537–W544 (2018).
49. Starmer, J. & Magnuson, T. Detecting broad domains and narrow peaks in ChIP-seq data with hiddenDomains. *BMC Bioinformatics* **17**, 144 (2016).
50. Huang, W., Loganantharaj, R., Schroeder, B., Fargo, D. & Li, L. PAVIS: a tool for Peak Annotation and Visualization. *Bioinformatics* **29**, 3097–3099 (2013).
51. Kim, D., Langmead, B. & Salzberg, S. L. HISAT: a fast spliced aligner with low memory requirements. *Nat Meth* **12**, 357–360 (2015).
52. Liao, Y., Smyth, G. K. & Shi, W. featureCounts: an efficient general purpose program for assigning sequence reads to genomic features. *Bioinformatics* **30**, 923–930 (2014).
53. Love, M. I., Huber, W. & Anders, S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol* **15**, 550 (2014).

Figures and Legends:

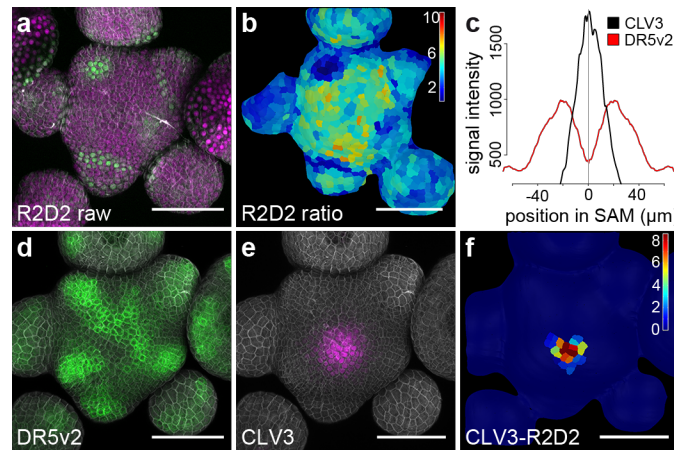


Figure 1: Auxin output minimum correlates with apical stem cells.

a) Confocal readout from R2D2 auxin input sensor. b) Ratiometric representation of R2D2 activity in the epidermal cell layer (L1). c) Quantification of averaged *pDR5v2:ER-eYFP-HDEL* and *pCLV3:mCherry-NLS* distribution (n=5). d) Confocal readout from *pDR5v2:ER-eYFP-HDEL* auxin output reporter. e) *pCLV3:mCherry-NLS* stem cell marker in the same SAM. f) Computational subtraction of L1 signals shown in (d) and (e). Relative signal intensity is shown in arbitrary units. All scale bars, 50 μm.

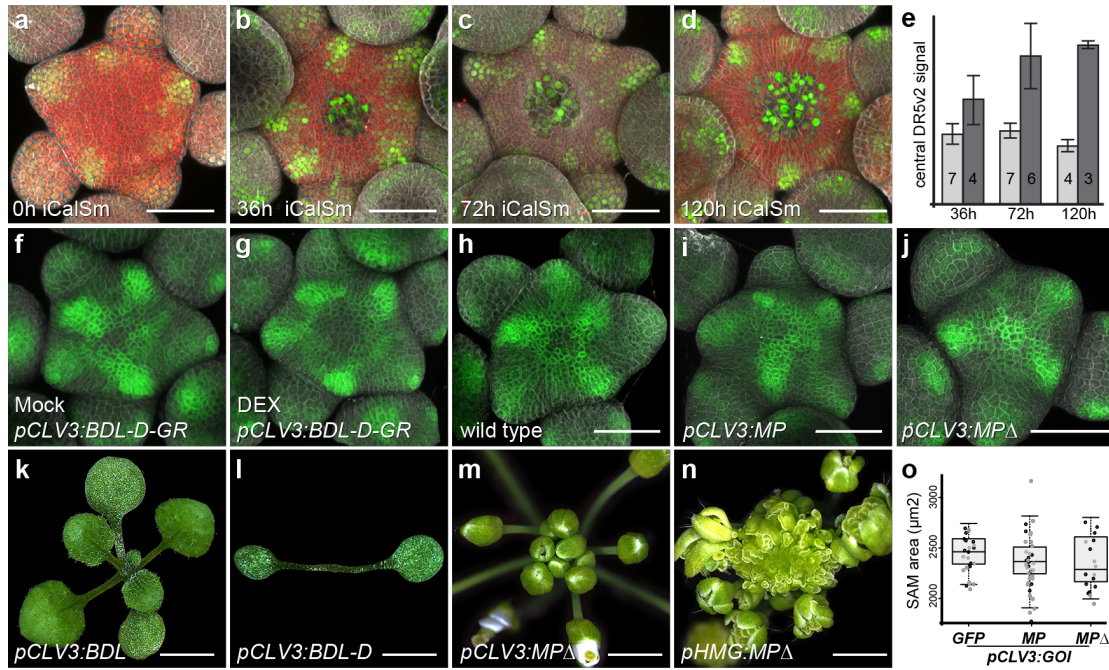


Fig. 2: Apical stem cells are dependent on auxin signaling, but resistant to differentiation.

a-d) *pDR5v2:3xVENUS-NLS* activity after induction of iCalSm. Stem cell differentiation is marked by loss of *pRPS5a:NLS-tdTomato*. **e)** Quantification of DR5v2 signal intensity in the central zone across the experimental cohort. Light grey bars represent uninduced controls, dark grey bars represent plants induced with 1% ethanol. Numbers of analyzed SAMs are indicated. See also Extended Figure 1-3 and Extended Table 1. **f-j)** *pDR5v2:ER-eYFP-HDEL* activity in plants harboring *pCLV3:BDL-D-GR* after 24h of mock treatment (f), *pCLV3:BDL-D-GR* after 24h of DEX treatment (g), wild type (h), *pCLV3:MP* (i) or *pCLV3:MPΔ* (j). **k-n)** Representative phenotypes of lines expressing *pCLV3:BDL* (k), *pCLV3:BDL-D* (l), *pCLV3:MPΔ* (m), or *pHMG:MPΔ* (n). **o)** SAM size quantifications for plants carrying *pCLV3:GFP*, *pCLV3:MP*, or *pCLV3:MPΔ* in two independent T1 populations. All scale bars 50 µm, except k) and i) 3,5 mm; m) and n) 2mm.

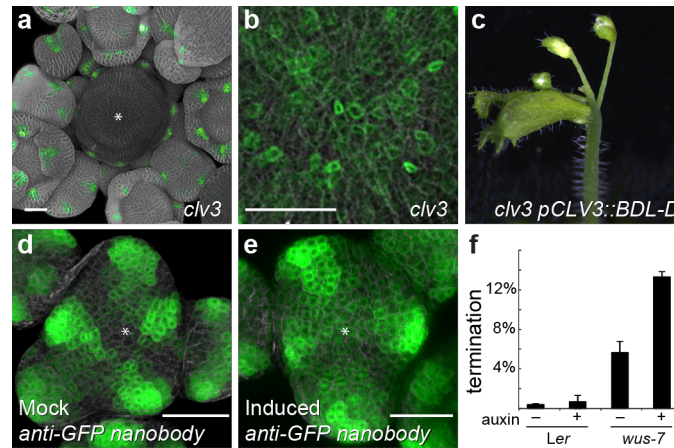


Fig. 3: WUSCHEL maintains low auxin signaling output in stem cells.

a) *pDR5v2:ER-mCherry-HDEL* activity in SAM of *clv3* mutant. Asterisk marks center of SAM. b) Zoom into central SAM area of *clv3* mutants reveals basal *pDR5v2* activity. c) SAM arrest caused by *pCLV3:BDL-D* expression in *clv3*. d, e) Representative *pDR5v2:ER-mCherry-HDEL* signals after 24h of mock treatment (d) or inducible depletion of WUS protein from stem cells by ethanol induction (e). f) Quantification of terminated seedlings grown on auxin plates (10 μ M IAA; $n > 200$ for each genotype and treatment). Genotyping revealed that all arrested plants were homozygous for *wus-7*. Scale bars, 50 μ m.

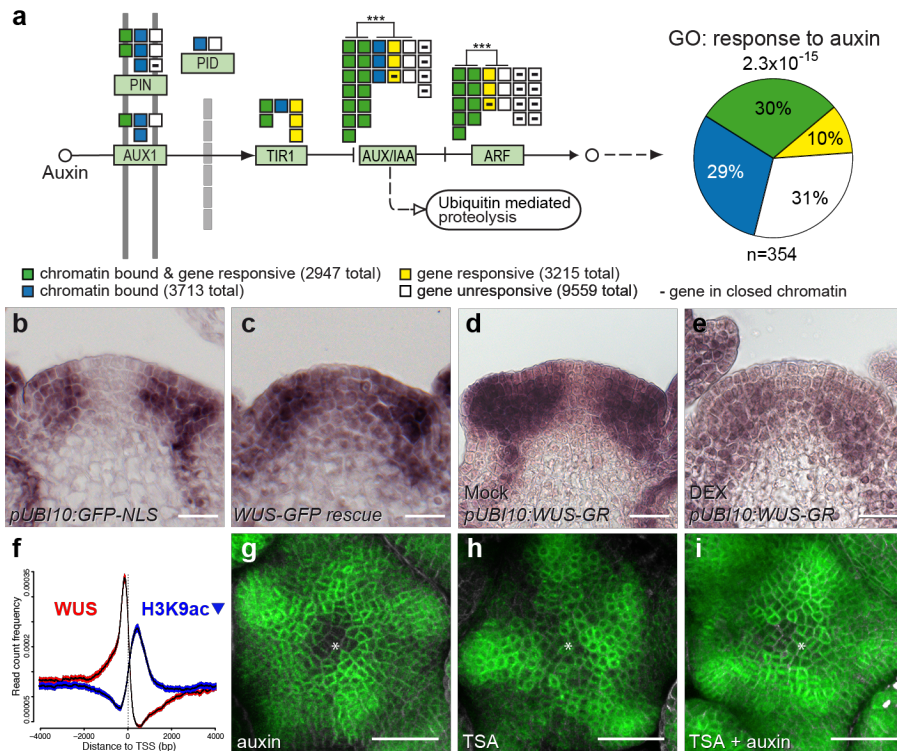
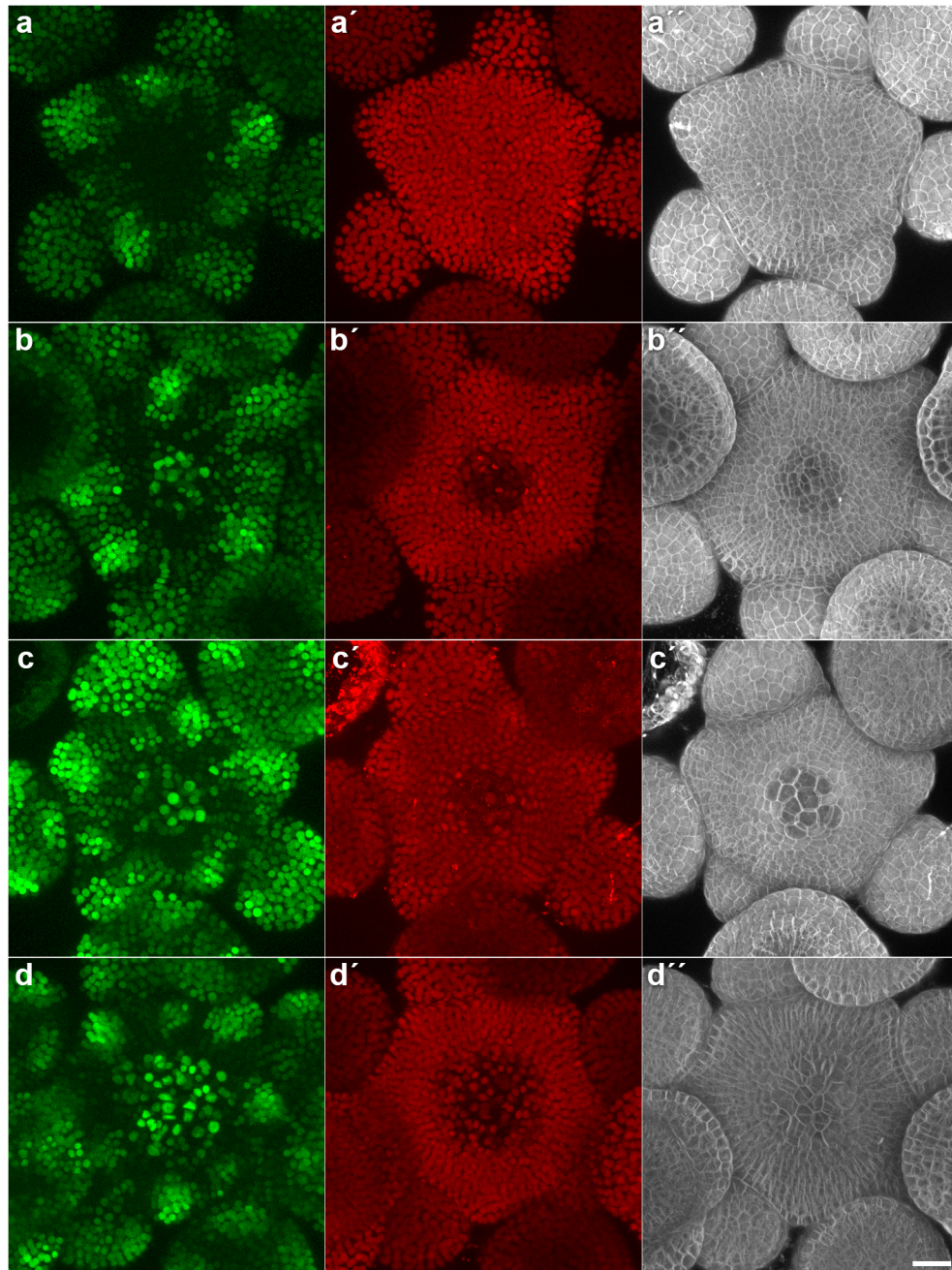


Fig. 4: Pathway level control underlies WUSCHEL mediated gating of auxin signaling.

a) WUS globally affects the auxin pathway, including transport, perception, signal transduction, as well as transcriptional response. Across the entire pathway bound and responsive genes are overrepresented (p -value 9.9×10^{-10}). Within gene family tests are shown. *** p -value by Fisher exact test $< 10^{-4}$. **b, c)** MP RNA accumulation 24 hours post anti-GFP nanobody induction in **b)** a *pUBI10:GFP-NLS* control line and **c)** the *pWUS:WUS-linker-GFP wus* rescue background. **d, e)** Response of MP mRNA to induction of WUS-GR. MP RNA after 24h of mock (**d**) or DEX treatment (**e**). **f)** Spatial correlation between WUS chromatin binding events (red) and regions with reduced histone acetylation (blue) 0.95 confidence intervals are shown. **g-i)** Representative images of *pDR5v2:ER-mCherry-HDEL* activity in response to HDAC inhibition. **g)** auxin treated SAM; **h)** TSA treated SAM; **i)** TSA and auxin treated SAM. Scale bars **b-e**: 20 μ m; **g-i**: 30 μ m.

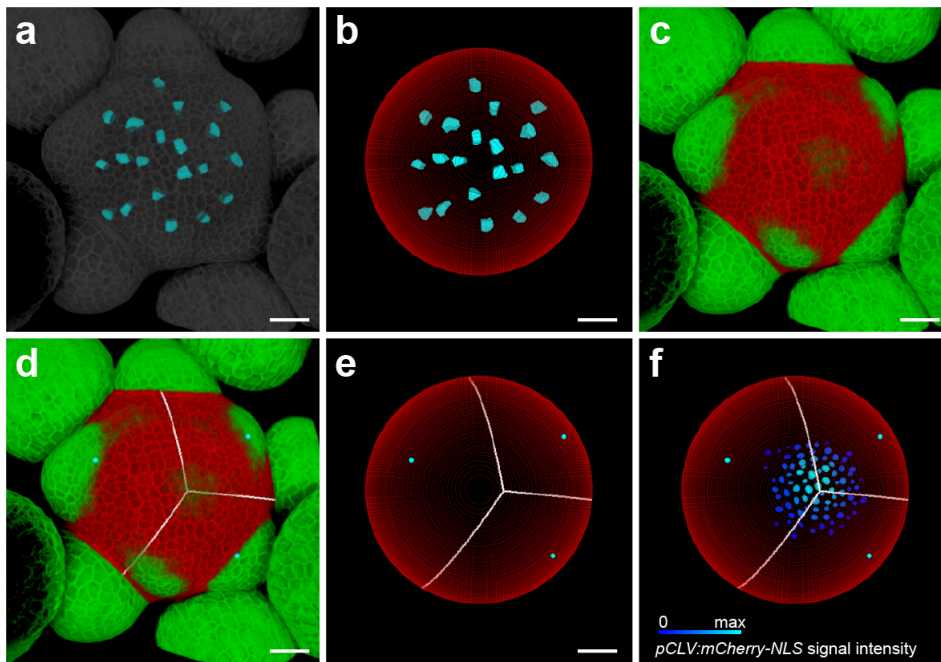
Extended Data

Extended Figures 1-12



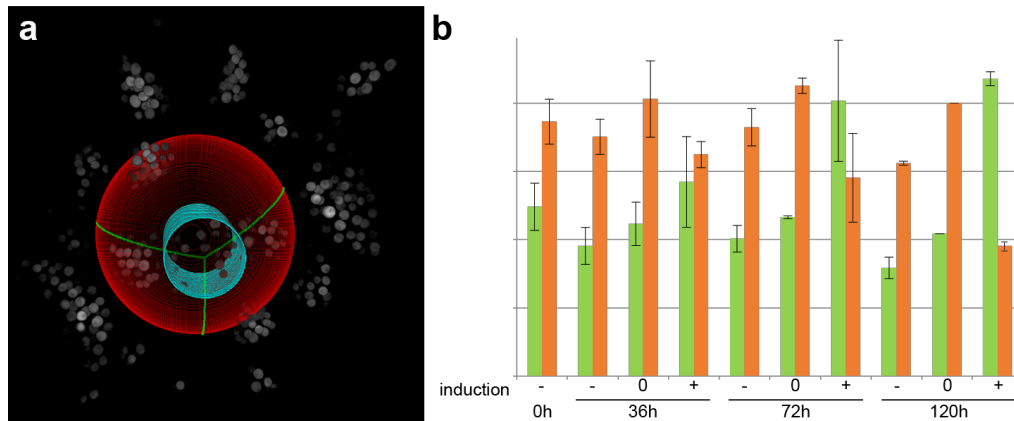
Extended Figure 1: Activation of DR5v2 and differentiation after induced stem cell loss.

Representative SAMs of the imaged cohorts quantified in Extended Table 1. **a)** 0h after induction **b)** 36h after induction **c)** 72h after induction **d)** 120h after induction. Left panels show *pDR5v2:3xVENUS-NLS* signal, middle panels show *pRPS5a:NLS-tdTomato* and right panels show DAPI stained cell walls. Scale bars, 20 μ m.



Extended Figure 2: Computational strategy to identify the stem cell domain.

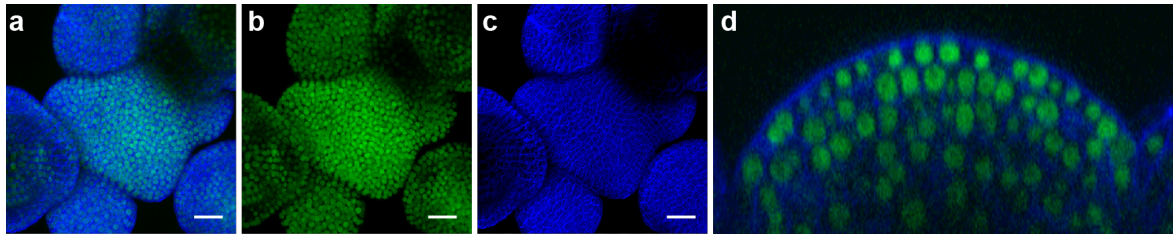
a) In a first step, cells across the L1 of the SAM are segmented. **b)** Based on the position of segmented cells, a perfect sphere is fitted to the SAM. **c)** The sphere is applied to the SAM and organ primordia are identified by emergence through the sphere. **d, e)** Equidistant points between the primordia are calculated and used to triangulate the center of the SAM. **f)** The triangulated center was benchmarked against SAMs harboring *pCLV3* reporter labelled stem cells (n=9). The triangulation invariantly identified one of the most central *pCLV3* positive cells. See also Methods. Scale bars, 20 μ m.



Extended Figure 3: Quantification of signal changes in the stem cell domain following induced stem cell loss.

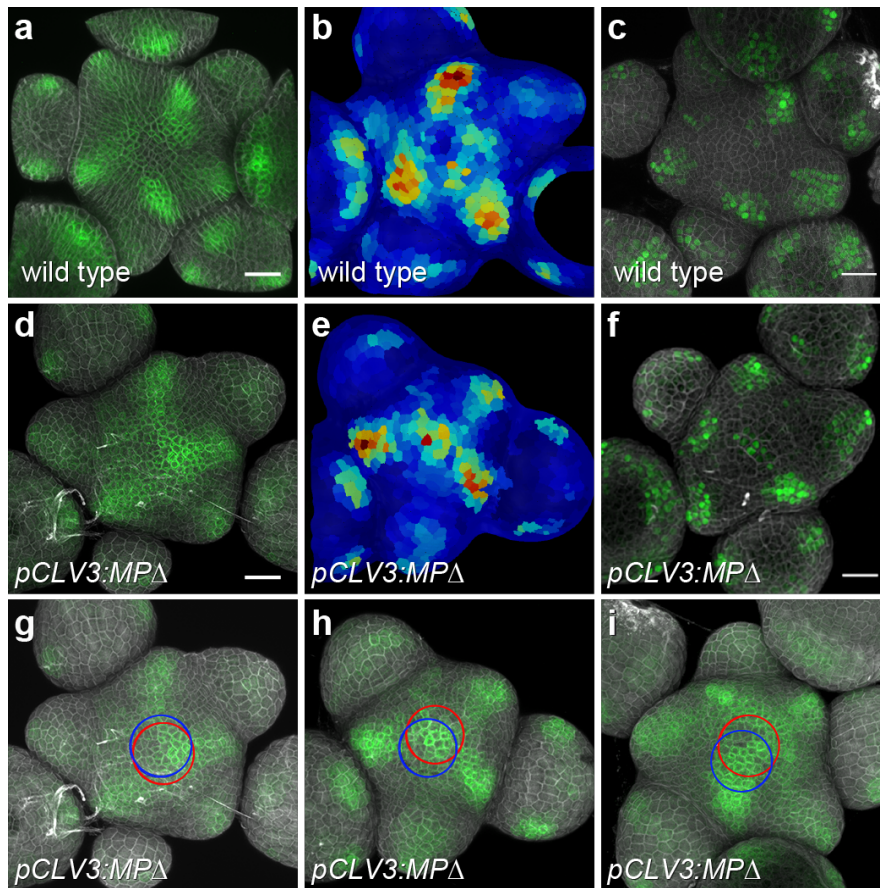
a) For signal quantification in the stem cell domain, a cylinder with radius r_{cyl} ($= 1/3 * r_{sphere}$) mimicking the average size of the *CLV3* domain was placed into the computationally identified center of the SAM and fluorescence intensities were quantified within this narrowly defined subdomain. DR5v2-NLS signals are shown in grey, SAM sphere derived from segmentation in red, triangulation lines in green and quantification cylinder in cyan. **b)** Quantification of fluorescent signals from all SAMs of the stem cell loss experiment described in Extended Table 1. Total fluorescence signal intensities for *pDR5v2:3xVENUS-NLS* and *pRPS5a:NLS-tdTomato* for the inner region (I_{cyl}) and for the peripheral region (I_{sphere}) were extracted from respective image volumes. I_{cyl} was averaged over all plants for each time-point and condition and normalized to the overall signal ($I_{cyl} + I_{sphere}$).

Green bars: DR5v2:3xVENUS-NLS signal, Orange bars: pRPS5a:NLS-tdTomato signal. - : mock treated, 0: ethanol induced, but no observable stem cell loss, + : ethanol induced and stem cell loss.



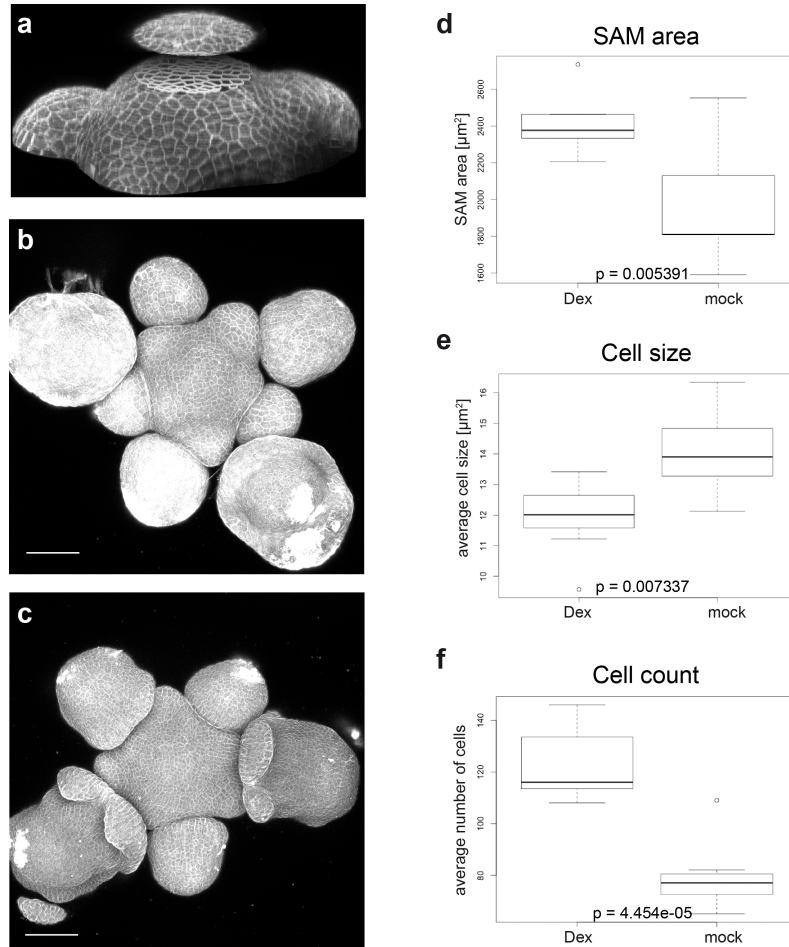
Extended Figure 4: Activity of the *pHMG* promoter.

Transgenic line carrying 1347 bp upstream of the At1g76110 locus fused to the *GFP-NLS* coding sequence. **a)** GFP and DAPI channels. **b)** GFP channel. **c)** DAPI channel. **d)** Side view through a representative SAM. Scale bars, 20 μm .



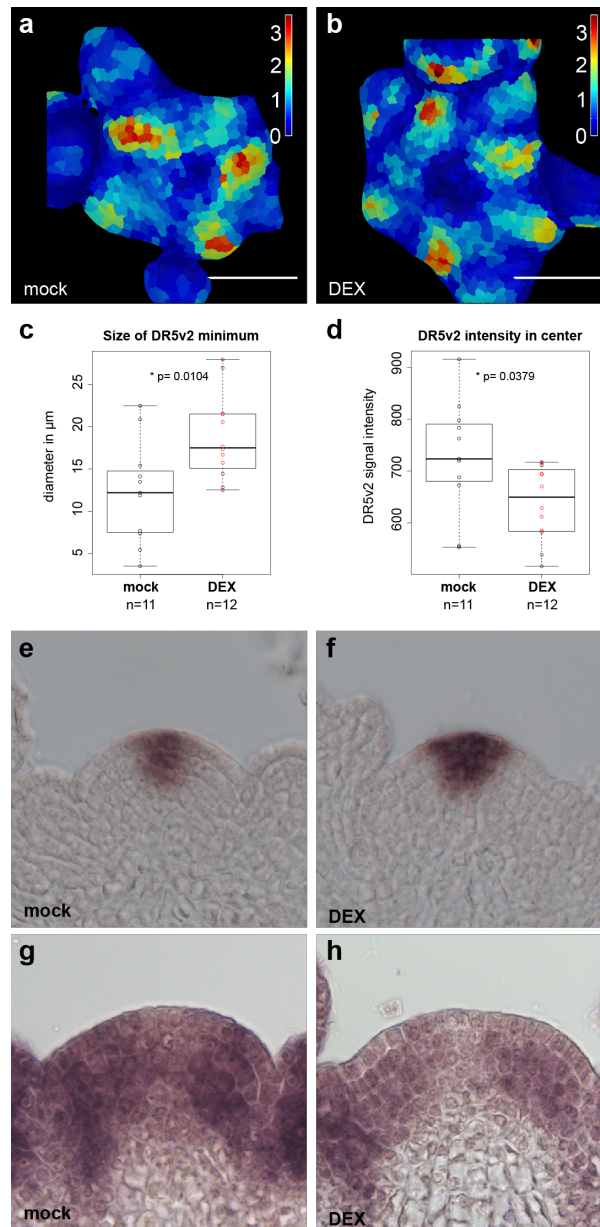
Extended Figure 5: Auxin signaling output in wild type and *pCLV3:MPΔ* lines.

a) *pDR5v2:ER-EYFP-HDEL* in wild type **b)** Quantification of an independent *pDR5v2:ER-EYFP-HDEL* wild-type SAM **c)** *pDR5v2:3xVENUS-NLS* in wild type **d-f)** Auxin signaling output was present in the centre of *pCLV3:MPΔ* lines, indicated by two independent reporters *pDR5v2:ER-EYFP-HDEL* (6 out of 8 independent T1 plants) (d) and *pDR5v2:3xVENUS-NLS* (6 out of 7 independent T1 plants) (f). **e)** Quantification of *pDR5v2:ER-EYFP-HDEL* in an independent *pCLV3:MPΔ* SAM. DR5v2 activity was not observed in the center of wild-type SAMs grown in the same experiments. **g-i)** Computationally derived central zone in L1 (red) and L3 (blue) are superimposed to SAMs of *pDR5v2:ER-EYFP-HDEL* carrying *pCLV3:MPΔ* (g, h) and *pCLV3:MP* (i). DR5v2 signal clearly coincides with central zone. Scale bars, 20 μ m.



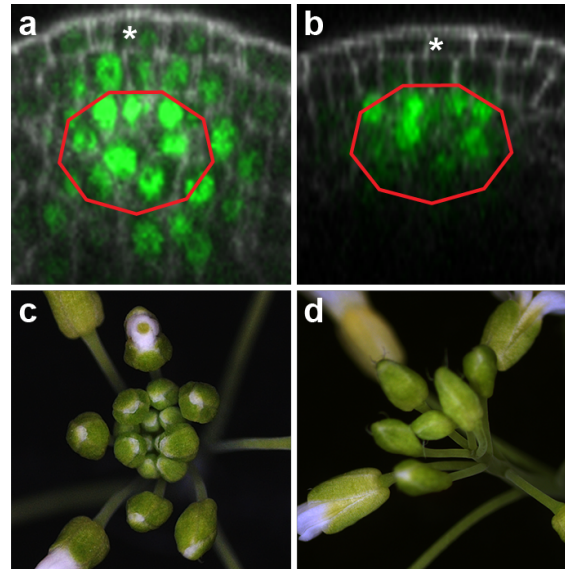
Extended Figure 6: Morphological effects of WUS-GR induction.

a) Visualization of meristem morphology analysis strategy. Meristem size, cell count and average cell size were measured at a constant relative position defined by the image plane in which the L1 to L2 transition became visible. **b)** Mock treated and **c)** DEX induced *pUBI:mCherry-GR-linker-WUS* SAMs four days after local application. Following WUS induction, SAM size increased (**d**), cell size decreased (**e**) and cell number strongly increased (**f**) $n = 7$ and 8 meristems, respectively. Scale bars, $50 \mu\text{m}$.



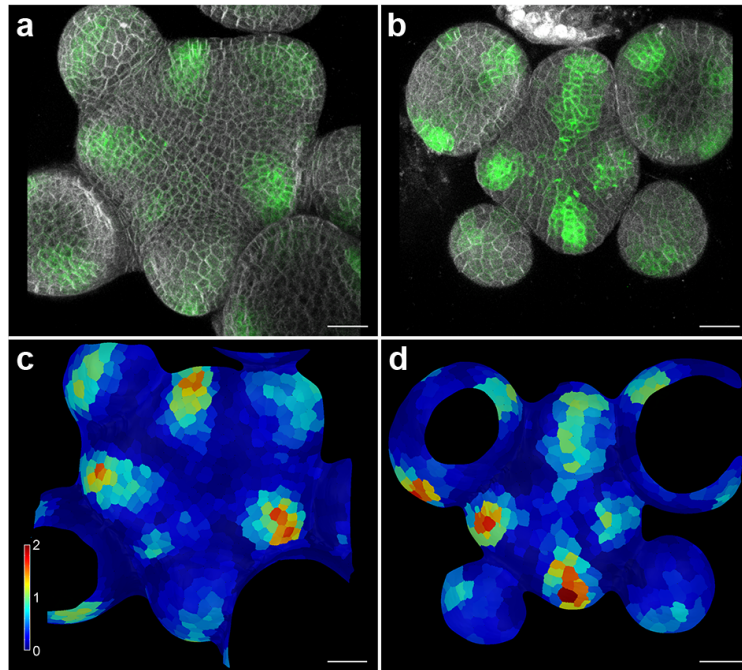
Extended Figure 7: SAM specific molecular responses to ectopic WUS induction.

24 hours after induction of ectopic WUS-GR activity, DR5v2 signal in the central zone was suppressed and *CLV3* mRNA expression was enhanced. Representative in situ quantifications of DR5v2 signal after mock (a) and DEX (b) treatments. c) Quantification of the size of the central DR5v2 minimum. d) Quantification of the average DR5v2 signal intensity in the central zone. e) *CLV3* mRNA expression after 24 hours of mock treatment. f) *CLV3* mRNA expression after 24 hours of DEX treatment. g) *TIR1* mRNA expression after 24 hours of mock treatment. h) *TIR1* mRNA expression after 24 hours of DEX treatment. SAMs of both treatment types were hybridized on the same microscopic slide and imaged under identical settings.



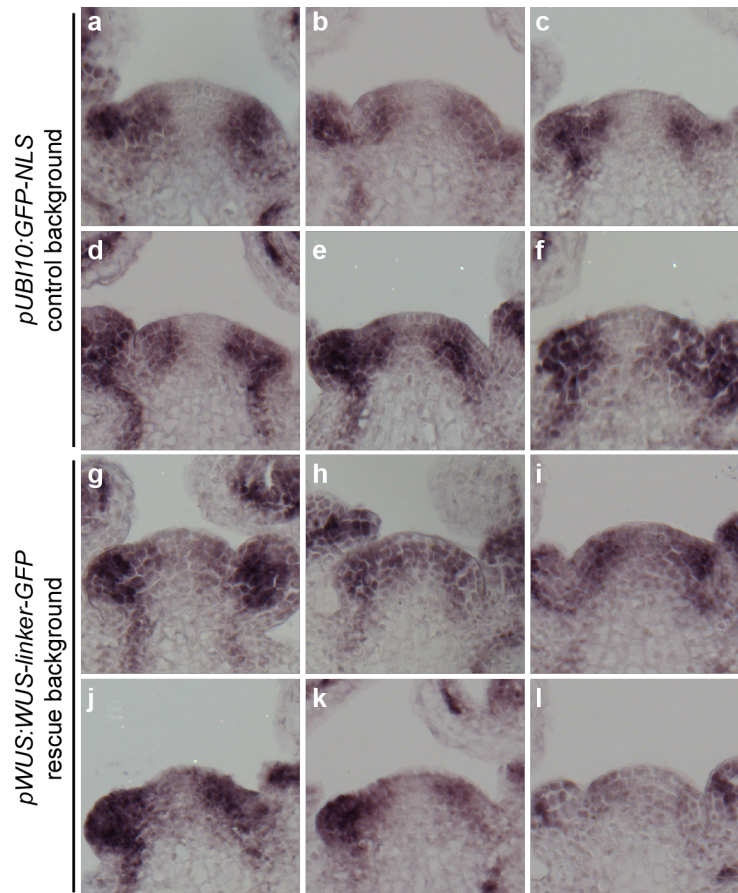
Extended Figure 8: Inducible depletion of WUS protein by stem cell expressing anti-GFP nanobody.

Representative images of a *pWUS:WUS-linker-GFP* rescue line expressing the anti GFP nanobody under the control of *pCLV3:AlcR*. The full genotype of these plants was: *wus/pWUS:WUS-linker-GFP/pCLV3:AlcR/pAlcA:NSlmb-vhhGFP4*. **a)** WUS-linker-GFP signal after 24h of mock treatment. **b)** WUS-linker-GFP signal after 24h of induction with 1% ethanol. Shoot phenotypes after five days of mock (**c**) or ethanol induction (**d**). Red line marks *WUS* mRNA expressing cells of the organizing centre; asterisk denotes epidermal stem cell.



Extended Figure 9: SAMs of *wus-7* plants show auxin signaling output in the stem cell domain.

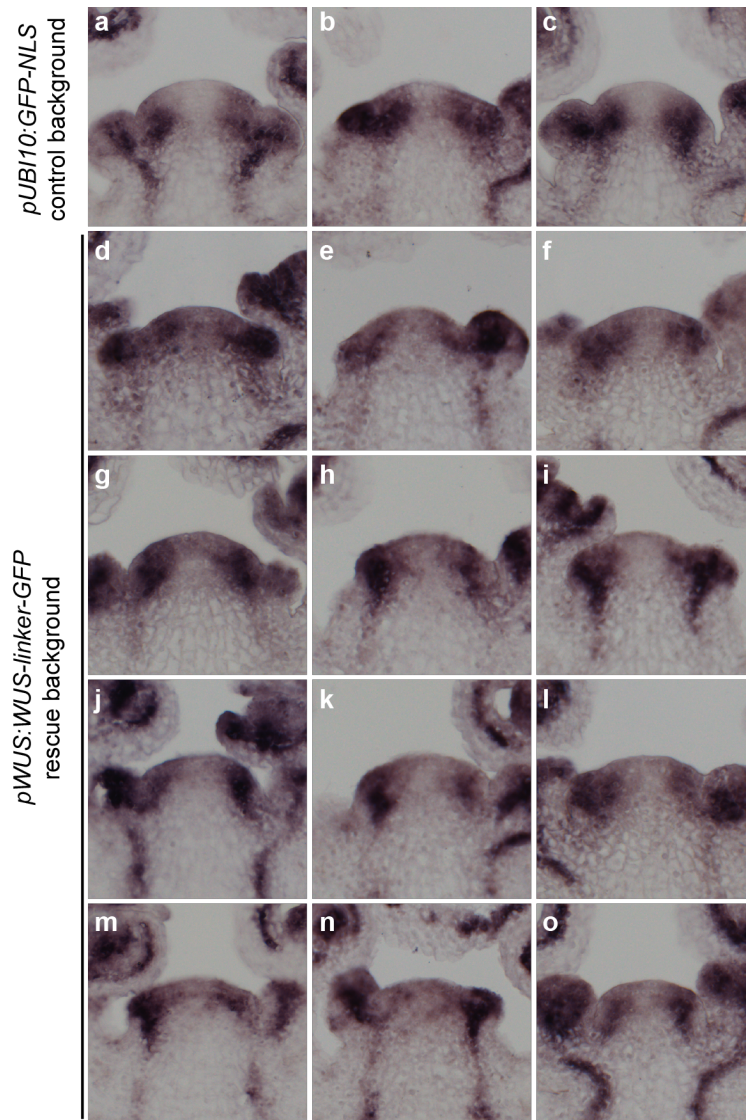
a) Representative image of *pDR5v2:ER-eYFP-HDEL* signal in the SAM of *Ler* wild-type plants. Only 16% of plants showed DR5v2 activity in the center of the SAM (n=38).
b) Representative image of *pDR5v2:ER-eYFP-HDEL* signal in a *wus-7* SAM before termination. 61% of *wus-7* plants showed DR5v2 activity in the center of the SAM (n=13). Per cell quantification of DR5v2 signal in wild type (**c**) and *wus-7* (**d**). Scale bars, 20 μ m



Extended Figure 10: *MP* mRNA expression after induced *WUS* loss of function.

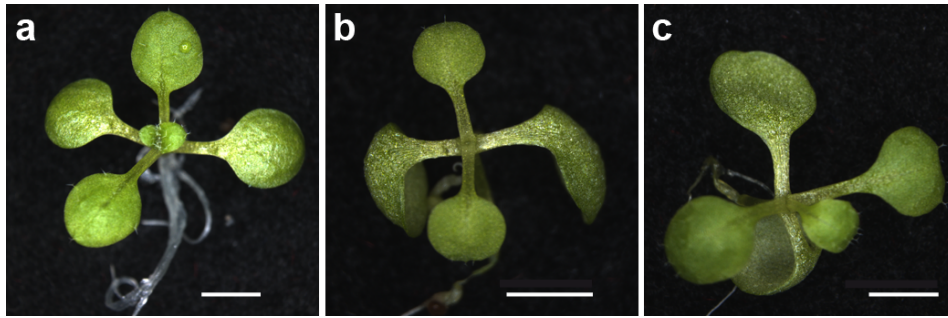
Experiment I.

a-f) In situ detection of *MP* mRNA in *pUBI10:GFP-NLS* control plants carrying *pCLV3:AlcR/AlcA:NSlmb-vhhGFP4* after 24h of ethanol treatment. **g-l)** In situ detection of *MP* mRNA in stable *pWUS:WUS-linker-GFP* *wus* rescue plants carrying *pCLV3:AlcR/AlcA:NSlmb-vhhGFP4* after 24h of ethanol treatment. SAMs of both genotypes were hybridized in a single experiment and imaged under identical settings. Unadjusted images are shown.



Extended Figure 11: *MP* mRNA expression after induced *WUS* loss of function. Experiment II.

a-c) In situ detection of *MP* mRNA in *pUBI10:GFP-NLS* control plants carrying *pCLV3:AlcR/AlcA:NSlmb-vhhGFP4* after 24h of ethanol treatment. **d-o)** In situ detection of *MP* mRNA in stable *pWUS:WUS-linker-GFP wus* rescue plants carrying *pCLV3:AlcR/AlcA:NSlmb-vhhGFP4* after 24h of ethanol treatment. SAMs of both genotypes were hybridized in a single experiment and imaged under identical settings. Unadjusted images are shown.



Extended Figure 12: Seedling phenotypes eleven days after germination on auxin supplemented plates.

Phenotypes of seedlings segregating *wus-7* grown on plates containing 10 μ m IAA ranged from **a)** unaffected, to **b)** arrested at four leaves stage, or **c)** arrested at five leaves stage with a terminal leaf. Scale bars, 1 mm.

Extended Tables 1-5

| | induced | | | untreated | | |
|------|---------------|---------------|----------|---------------|---------------|-------|
| | <i>pRPS5a</i> | <i>pRPS5a</i> | DR5v2 | <i>pRPS5a</i> | <i>pRPS5a</i> | DR5v2 |
| | + | - | + | + | - | + |
| 0h | - | - | - | 7 | 0 | 0 |
| 36h | 3 | 4 | 4 | 7 | 0 | 0 |
| 72h | 2 | 6 | 6 | 7 | 0 | 0 |
| 120h | 2 | 3 | 3 | 4 | 0 | 0 |

Extended Table 1: Quantification of DR5v2 response after induced stem cell loss.

Plants carrying *DR5v2:3xVENUS-NLS*, *pRPS5a:NLS-tdTomato*, as well as *pCLV3:AlcR/AlcA:CalS3m* were either induced with 1% ethanol or maintained as untreated controls and cohorts were scored for loss of *RPS5a* promoter activity from stem cells and DR5v2 expression by confocal imaging. Stem cell loss and associated DR5v2 activation exclusively occurred in induced plants. All plants with reduced *pRPS5a* activity expressed DR5v2. *pRPS5a* + denotes plants with uncompromised *pRPS5a* promoter activity in stem cells. *pRPS5a* - denotes plants with reduced *pRPS5a* promoter activity in stem cells. DR5v2 + denotes plants with DR5v2 activity in stem cells. Table lists number of individual plants showing reporter expression.

| | GO ID | Term | Annotated | Significant | Expected | p-Value |
|----|------------|---|-----------|-------------|----------|-----------|
| 1 | GO:0010200 | response to chitin | 393 | 145 | 55.45 | 2.8E-30 |
| 2 | GO:0009611 | response to wounding | 313 | 109 | 44.16 | 1E-20 |
| 3 | GO:0010363 | regulation of plant-type hypersensitive response | 336 | 111 | 47.41 | 4.6E-19 |
| 4 | GO:0006612 | protein targeting to membrane | 340 | 111 | 47.97 | 1.3E-18 |
| 5 | GO:0009414 | response to water deprivation | 374 | 130 | 52.77 | 5.7E-18 |
| 6 | GO:0009867 | jasmonic acid mediated signaling pathway | 256 | 89 | 36.12 | 1.2E-15 |
| 7 | GO:0009733 | response to auxin | 354 | 107 | 49.95 | 2.3E-15 |
| 8 | GO:0002679 | respiratory burst involved in defense response | 114 | 50 | 16.09 | 1.1E-14 |
| 9 | GO:0009737 | response to abscisic acid | 548 | 174 | 77.32 | 1.1E-14 |
| 10 | GO:0009738 | abscisic acid-activated signaling pathway | 232 | 78 | 32.74 | 1.1E-12 |
| 11 | GO:0009651 | response to salt stress | 704 | 187 | 99.33 | 2.6E-12 |
| 12 | GO:0009695 | jasmonic acid biosynthetic process | 125 | 49 | 17.64 | 3.4E-12 |
| 13 | GO:0006857 | oligopeptide transport | 97 | 41 | 13.69 | 1.1E-11 |
| 14 | GO:0050832 | defense response to fungus | 303 | 84 | 42.75 | 3.3E-10 |
| 15 | GO:0009862 | systemic acquired resistance, salicylic acid mediated signaling pathway | 222 | 66 | 31.32 | 1.2E-9 |
| 16 | GO:0042538 | hyperosmotic salinity response | 152 | 50 | 21.45 | 2.9E-9 |
| 17 | GO:0009612 | response to mechanical stimulus | 59 | 27 | 8.32 | 4.5E-9 |
| 18 | GO:0042742 | defense response to bacterium | 344 | 93 | 48.54 | 4.9E-9 |
| 19 | GO:0009684 | indoleacetic acid biosynthetic process | 94 | 36 | 13.26 | 5.2E-9 |
| 20 | GO:0006569 | tryptophan catabolic process | 67 | 29 | 9.45 | 6E-9 |
| 21 | GO:0009723 | response to ethylene | 325 | 101 | 45.86 | 1.2E-8 |
| 22 | GO:0009753 | response to jasmonic acid | 427 | 141 | 60.25 | 1.2E-8 |
| 23 | GO:0009873 | ethylene-activated signaling pathway | 118 | 41 | 16.65 | 1.3E-8 |
| 24 | GO:0009620 | response to fungus | 440 | 132 | 62.08 | 2.5E-8 |
| 25 | GO:0000165 | MAPK cascade | 197 | 57 | 27.8 | 4.5E-8 |
| 26 | GO:0009963 | positive regulation of flavonoid biosynthetic process | 93 | 34 | 13.12 | 5.3E-8 |
| 27 | GO:0006355 | regulation of transcription, DNA-templated | 1588 | 296 | 224.07 | 7.1E-8 |
| 28 | GO:0043069 | negative regulation of programmed cell death | 158 | 48 | 22.29 | 1E-7 |
| 29 | GO:0009739 | response to gibberellin | 143 | 49 | 20.18 | 1.1E-7 |
| 30 | GO:0031348 | negative regulation of defense response | 246 | 65 | 34.71 | 2.3E-7 |
| 31 | GO:0009409 | response to cold | 539 | 118 | 76.05 | 4.2E-7 |
| 32 | GO:0009750 | response to fructose | 127 | 39 | 17.92 | 0.0000011 |
| 33 | GO:0030968 | endoplasmic reticulum unfolded protein response | 171 | 48 | 24.13 | 0.0000013 |
| 34 | GO:0009693 | ethylene biosynthetic process | 110 | 35 | 15.52 | 0.0000016 |
| 35 | GO:0009805 | coumarin biosynthetic process | 51 | 21 | 7.2 | 0.000002 |
| 36 | GO:0010310 | regulation of hydrogen peroxide metabolic process | 159 | 45 | 22.43 | 0.0000022 |
| 37 | GO:0030003 | cellular cation homeostasis | 146 | 42 | 20.6 | 0.000003 |
| 38 | GO:0007623 | circadian rhythm | 156 | 44 | 22.01 | 0.0000032 |
| 39 | GO:0006833 | water transport | 118 | 36 | 16.65 | 0.0000034 |
| 40 | GO:0009741 | response to brassinosteroid | 102 | 37 | 14.39 | 0.0000036 |
| 41 | GO:0080167 | response to karrikin | 114 | 35 | 16.09 | 0.000004 |

| | | | | | | |
|----|------------|--|-----|-----|--------|-----------|
| 42 | GO:0002237 | response to molecule of bacterial origin | 97 | 31 | 13.69 | 0.0000056 |
| 43 | GO:0006979 | response to oxidative stress | 407 | 90 | 57.43 | 0.0000065 |
| 44 | GO:0006813 | potassium ion transport | 35 | 16 | 4.94 | 0.0000066 |
| 45 | GO:0046777 | protein autophosphorylation | 131 | 37 | 18.48 | 0.000018 |
| 46 | GO:0006598 | polyamine catabolic process | 34 | 15 | 4.8 | 0.000022 |
| 47 | GO:0035556 | intracellular signal transduction | 446 | 133 | 62.93 | 0.000023 |
| 48 | GO:0009269 | response to desiccation | 31 | 14 | 4.37 | 0.00003 |
| 49 | GO:0031347 | regulation of defense response | 485 | 146 | 68.43 | 0.00003 |
| 50 | GO:0009825 | multidimensional cell growth | 96 | 29 | 13.55 | 0.000037 |
| 51 | GO:0009697 | salicylic acid biosynthetic process | 181 | 46 | 25.54 | 0.000037 |
| 52 | GO:0019344 | cysteine biosynthetic process | 181 | 46 | 25.54 | 0.000037 |
| 53 | GO:0006970 | response to osmotic stress | 749 | 207 | 105.68 | 0.000041 |
| 54 | GO:0070838 | divalent metal ion transport | 184 | 53 | 25.96 | 0.000069 |
| 55 | GO:0009627 | systemic acquired resistance | 395 | 109 | 55.73 | 0.000077 |
| 56 | GO:0006949 | syncytium formation | 19 | 10 | 2.68 | 0.000083 |
| 57 | GO:0042398 | cellular modified amino acid biosynthetic process | 50 | 18 | 7.06 | 0.000091 |
| 58 | GO:0009751 | response to salicylic acid | 423 | 122 | 59.69 | 0.000098 |
| 59 | GO:0042631 | cellular response to water deprivation | 59 | 20 | 8.32 | 0.0001 |
| 60 | GO:0009965 | leaf morphogenesis | 186 | 49 | 26.24 | 0.00011 |
| 61 | GO:0010583 | response to cyclopentenone | 132 | 35 | 18.63 | 0.00012 |
| 62 | GO:0001666 | response to hypoxia | 74 | 23 | 10.44 | 0.00014 |
| 63 | GO:0007030 | Golgi organization | 160 | 40 | 22.58 | 0.00017 |
| 64 | GO:0016126 | sterol biosynthetic process | 150 | 38 | 21.17 | 0.00018 |
| 65 | GO:0019748 | secondary metabolic process | 527 | 133 | 74.36 | 0.00022 |
| 66 | GO:0006468 | protein phosphorylation | 620 | 157 | 87.48 | 0.00024 |
| 67 | GO:0006995 | cellular response to nitrogen starvation | 21 | 10 | 2.96 | 0.00024 |
| 68 | GO:0009863 | salicylic acid mediated signaling pathway | 315 | 92 | 44.45 | 0.00028 |
| 69 | GO:0009407 | toxin catabolic process | 180 | 43 | 25.4 | 0.00029 |
| 70 | GO:0009595 | detection of biotic stimulus | 92 | 26 | 12.98 | 0.0003 |
| 71 | GO:0046686 | response to cadmium ion | 415 | 84 | 58.56 | 0.00033 |
| 72 | GO:0006816 | calcium ion transport | 108 | 29 | 15.24 | 0.00036 |
| 73 | GO:0042335 | cuticle development | 42 | 15 | 5.93 | 0.00038 |
| 74 | GO:0009617 | response to bacterium | 499 | 140 | 70.41 | 0.0004 |
| 75 | GO:0010264 | myo-inositol hexakisphosphate biosynthetic process | 51 | 17 | 7.2 | 0.00041 |
| 76 | GO:0010119 | regulation of stomatal movement | 47 | 16 | 6.63 | 0.00046 |
| 77 | GO:0043900 | regulation of multi-organism process | 115 | 30 | 16.23 | 0.00049 |
| 78 | GO:0010017 | red or far-red light signaling pathway | 39 | 14 | 5.5 | 0.00056 |
| 79 | GO:0010260 | animal organ senescence | 27 | 11 | 3.81 | 0.00063 |
| 80 | GO:0009740 | gibberellic acid mediated signaling pathway | 72 | 21 | 10.16 | 0.0007 |
| 81 | GO:0007169 | transmembrane receptor protein tyrosine kinase signaling pathway | 113 | 29 | 15.94 | 0.0008 |
| 82 | GO:0015824 | proline transport | 68 | 20 | 9.59 | 0.00083 |
| 83 | GO:0010227 | floral organ abscission | 32 | 12 | 4.52 | 0.00088 |
| 84 | GO:0052541 | plant-type cell wall cellulose metabolic process | 24 | 10 | 3.39 | 0.0009 |
| 85 | GO:0010158 | abaxial cell fate specification | 7 | 5 | 0.99 | 0.00091 |

| | | | | | | |
|-----|------------|--|-----|----|-------|---------|
| 86 | GO:0009742 | brassinosteroid mediated signaling pathway | 37 | 13 | 5.22 | 0.0011 |
| 87 | GO:0048767 | root hair elongation | 164 | 38 | 23.14 | 0.00117 |
| 88 | GO:0010118 | stomatal movement | 86 | 32 | 12.13 | 0.00168 |
| 89 | GO:0009694 | jasmonic acid metabolic process | 147 | 58 | 20.74 | 0.00171 |
| 90 | GO:0033500 | carbohydrate homeostasis | 12 | 8 | 1.69 | 0.00174 |
| 91 | GO:0007231 | osmosensory signaling pathway | 5 | 4 | 0.71 | 0.00175 |
| 92 | GO:2000022 | regulation of jasmonic acid mediated signaling pathway | 5 | 4 | 0.71 | 0.00175 |
| 93 | GO:0010037 | response to carbon dioxide | 5 | 4 | 0.71 | 0.00175 |
| 94 | GO:0009624 | response to nematode | 72 | 20 | 10.16 | 0.0018 |
| 95 | GO:0006766 | vitamin metabolic process | 77 | 21 | 10.86 | 0.0018 |
| 96 | GO:0006865 | amino acid transport | 228 | 61 | 32.17 | 0.00209 |
| 97 | GO:0000038 | very long-chain fatty acid metabolic process | 44 | 14 | 6.21 | 0.00214 |
| 98 | GO:0046885 | regulation of hormone biosynthetic process | 8 | 5 | 1.13 | 0.00215 |
| 99 | GO:0050801 | ion homeostasis | 205 | 59 | 28.93 | 0.00226 |
| 100 | GO:0052546 | cell wall pectin metabolic process | 40 | 13 | 5.64 | 0.00247 |

Extended Table 2: GO category enrichment analysis of direct WUS targets.

Top 100 enriched categories are shown.

| ID | Term | Annotated | Significant | Expected | p-Value |
|------------|--|-----------|-------------|----------|---------|
| GO:0009733 | response to auxin | 354 | 107 | 49.95 | 2.3E-15 |
| GO:0090354 | regulation of auxin metabolic process | 6 | 4 | 0.85 | 0.00467 |
| GO:0010600 | regulation of auxin biosynthetic process | 5 | 3 | 0.71 | 0.02246 |
| GO:0009926 | auxin polar transport | 90 | 20 | 12.7 | 0.02424 |
| GO:0060918 | auxin transport | 93 | 20 | 13.12 | 0.03351 |

Extended Table 3: Auxin related GO terms enriched among direct WUS targets at $p < 0.05$.

| AGI | Gene Name | WUS peaks | Log2FC | p adj. |
|------------|-----------|-----------|--------------|-------------|
| AT1G59750 | ARF1 | 0 | -0.111597744 | 0.358464723 |
| AT5G62000 | ARF2 | 1 | -0.160244055 | 0.080636193 |
| AT2G33860 | ARF3 | 1 | 0.74121558 | 0.000129974 |
| AT5G60450 | ARF4 | 1 | -1.260779231 | 2.78E-12 |
| AT1G19850 | ARF5 | 1 | 0.824932624 | 0.00041103 |
| AT1G30330 | ARF6 | 5 | 0.273665676 | 0.047298892 |
| AT5G20730 | ARF7 | 0 | 0.150899027 | 0.280076124 |
| AT5G37020 | ARF8 | 1 | -1.563675792 | 1.19E-12 |
| AT4G23980 | ARF9 | 1 | 1.122752639 | 4.50E-17 |
| AT2G28350 | ARF10 | 2 | 0.787777365 | 0.022930942 |
| AT2G46530 | ARF11 | 1 | 0.895198988 | 1.60E-07 |
| AT1G34310* | ARF12 | 0 | 0 | 1 |
| AT1G34170* | ARF13 | 0 | 0 | 1 |
| AT1G35540* | ARF14 | 0 | 0 | 1 |
| AT1G35520* | ARF15 | 0 | 0 | 1 |
| AT4G30080 | ARF16 | 0 | 0.121858649 | 0.716958625 |
| AT1G77850 | ARF17 | 0 | 0.820301179 | 0.002887907 |
| AT3G61830* | ARF18 | 0 | 0.959318681 | 2.10E-08 |
| AT1G19220 | ARF19 | 0 | 1.078934444 | 9.14E-09 |
| AT1G35240* | ARF20 | 0 | 0 | 1 |
| AT1G34410* | ARF21 | 0 | 0 | 1 |
| AT1G34390* | ARF22 | 0 | 0 | 1 |
| AT1G43950* | ARF23 | 0 | 0 | 1 |
| AT4G14560 | IAA1 | 1 | -0.049017547 | 0.917632937 |
| AT3G23030 | IAA2 | 2 | -0.779787625 | 9.22E-17 |
| AT1G04240 | SHY2 | 2 | 3.163647428 | 9.32E-101 |
| AT5G43700 | ATAUX2-11 | 1 | -0.467157239 | 0.000156676 |
| AT1G15580* | IAA5 | 0 | 0 | 1 |
| AT1G52830 | IAA6 | 2 | 0.116034132 | 1 |
| AT3G23050 | IAA7 | 1 | 0.551262546 | 9.12E-10 |
| AT2G22670 | IAA8 | 2 | 1.364381273 | 4.79E-29 |
| AT5G65670 | IAA9 | 2 | 0.105183055 | 0.311631229 |
| AT1G04100* | IAA10 | 0 | -1.683375121 | 2.55E-12 |
| AT4G28640 | IAA11 | 0 | -0.673556893 | 0.092257616 |
| AT1G04550 | IAA12 | 1 | -0.566175948 | 0.043218198 |
| AT2G33310 | IAA13 | 1 | -1.008001464 | 1.12E-12 |
| AT4G14550 | IAA14 | 2 | -0.585607079 | 0.011992423 |
| AT1G80390 | IAA15 | 0 | -0.574441357 | 1 |
| AT3G04730 | IAA16 | 1 | -0.406079449 | 6.14E-08 |
| AT1G04250 | AXR3 | 1 | 0.640284515 | 0.002128125 |
| AT1G51950 | IAA18 | 3 | -0.767731853 | 1.75E-11 |
| AT3G15540 | IAA19 | 2 | 1.265591239 | 0.010725483 |
| AT2G46990 | IAA20 | 1 | 1.864661912 | 1.06E-15 |
| AT3G16500 | PAP1 | 2 | -1.490402367 | 1.73E-25 |
| AT4G29080 | PAP2 | 1 | 0.842174986 | 4.80E-05 |
| AT5G25890 | IAA28 | 0 | -0.291255875 | 0.203663582 |
| AT4G32280 | IAA29 | 0 | 1.771034915 | 8.53E-05 |
| AT3G62100 | IAA30 | 0 | 1.111422364 | 0.000669299 |
| AT3G17600* | IAA31 | 0 | -0.235206969 | 1 |
| AT2G01200* | IAA32 | 0 | 1.100401196 | 0.205623963 |
| AT1G15050* | IAA34 | 0 | -0.423041078 | 0.426177309 |
| AT4G03190 | AFB1 | 0 | -1.525054649 | 4.66E-10 |
| AT3G26810 | AFB2 | 2 | -0.162976188 | 0.363114532 |
| AT1G12820 | AFB3 | 0 | 1.171943219 | 5.46E-33 |
| AT4G24390 | AFB4 | 0 | 0.554308915 | 0.003823998 |
| AT5G49980 | AFB5 | 1 | 0.454882856 | 0.000299204 |
| AT3G62980 | TIR1 | 1 | -0.886064319 | 4.74E-14 |
| AT1G73590 | PIN1 | 1 | 0.097477956 | 0.872138329 |
| AT5G57090 | PIN2 | 0 | 0.609007336 | 0.190482509 |
| AT1G70940 | PIN3 | 2 | -1.183821577 | 5.24E-22 |
| AT2G01420 | PIN4 | 3 | 0.34302747 | 0.001748035 |
| AT5G16530* | PIN5 | 0 | -0.181089018 | 1 |
| AT1G77110 | PIN6 | 1 | 1.037619295 | 0.205625501 |
| AT1G23080 | PIN7 | 2 | -0.188145814 | 0.217710653 |
| AT5G15100 | PIN8 | 0 | 0 | 1 |
| AT2G38120 | AUX1 | 2 | 0.877290156 | 2.64E-09 |
| AT5G01240 | LAX1 | 2 | 0.138928766 | 0.245947254 |
| AT2G21050 | LAX2 | 0 | 0.415613371 | 0.277081161 |
| AT1G77690 | LAX3 | 1 | -0.008043826 | 0.977318196 |
| AT2G34650 | PID | 2 | 0.319524941 | 0.197369125 |
| AT2G26700 | PID2 | 0 | -0.126731923 | 0.818644213 |

Extended Table 4: Response of auxin signalling to WUS.

Adjusted p-value for RNA-seq data was calculated using the Benjamini-Hochberg method in Deseq2 (ref. 46). Asterisks denote genes in regions with closed chromatin¹⁹.

| AGI | Name | Responsive to auxin | Expression PZ>CZ | Promoter bound by WUS | Responsive to WUS |
|-----------|--------------------|---------------------|------------------|-----------------------|-------------------|
| AT3G62980 | <i>TIR1</i> | x | x | x | x |
| AT2G33860 | <i>ARF3</i> | x | x | x | x |
| AT5G60450 | <i>ARF4</i> | x | x | x | x |
| AT1G19850 | <i>ARF5 (MP)</i> | x | x | x | x |
| AT2G22670 | <i>IAA8</i> | x | x | - | x |
| AT5G65670 | <i>IAA9</i> | x | x | x | x |
| AT1G04550 | <i>IAA12 (BDL)</i> | x | x | - | - |
| AT5G60200 | <i>TMO6</i> | x | x | x | x |
| AT1G74500 | <i>TMO7</i> | x | - | - | - |
| AT3G25710 | <i>TMO5</i> | x | - | - | x |
| AT4G23750 | <i>TMO3</i> | x | - | x | x |
| AT1G68510 | <i>LBD42</i> | - | - | x | - |
| AT3G49940 | <i>LBD38</i> | - | - | x | x |
| AT3G58190 | <i>LBD29</i> | x | - | - | - |
| AT3G11280 | | x | x | x | x |
| AT3G28910 | <i>MYB30</i> | x | x | x | x |
| AT5G58900 | | x | x | x | - |

Extended Table 5: WUS targets functionally tested by expression from *pCLV3* promoter.

Expression domains in the SAM are based on refs. ^{12,36,37}.

| Purpose | Gene | Name | Sequence |
|------------|----------------------|--------|--|
| genotyping | <i>wus-7</i> | A05337 | CCGACCAAGAAAGCGGCAACA |
| | | A05338 | AGACGTTCTTGCCCTGAATCTTT |
| Cloning | <i>MP</i> | A04634 | aacaGGTCTCaggctcaacaATGATGGCTTCATTGTCTTGTGTTG |
| | | A04635 | aacaGGTCTCtAGACCCGCATATCGCCTTACGGTA |
| | | A04636 | aacaGGTCTCGGTCTaAGCTCTCAGTTGGTATGAGATTTG |
| | | A04637 | aacaGGTCTCtAGACCGTTCAACTGAGTGTCCCAC |
| | | A04638 | aacaGGTCTCGGTCTaAAGTTTGACCAGTTCAGTCCCCTTG |
| | At4g24550 terminator | 72A4 | ACTAGGATCCTGTTTTTCAGATAATGTTTATCCTTC |
| | | 72A5 | ACTACTCGAGATCGTTGCACCTTTATTTTC |
| | <i>MPΔ</i> | A04640 | aacaGGTCTCtctgaGGTTCGGACGCGGGGTGTCGAATT |
| | | A04634 | aacaGGTCTCaggctcaacaATGATGGCTTCATTGTCTTGTGTTG |
| | <i>TIR1</i> | A04641 | aacaGGTCTCaggctcaacaATGCAGAAGCGAATAGCCTTGTGCT |
| | | A04642 | aacaGGTCTCtAGACCATCGGTGGAGAAGCCTTCG |
| | | A04643 | aacaGGTCTCGGTCTaGCTGTATCGCTGCCACTTGCAGG |
| | | A04644 | aacaGGTCTCcCTCGAGTCCGGTGCACCCCGTTCA |
| | | A04645 | aacaGGTCTCCCGAGgCCAGAGAGTGCCTGTGTGAGAGA |
| | | A04646 | aacaGGTCTCtctgaTAATCCGTTAGTAGTAATGATT |
| | <i>AT5G58900</i> | A05617 | aacaGGTCTCaggctcaacaATGGAGGTTATGAGACCGTTCGACGT |
| | | A05618 | aacaGGTCTCtctgaTAGTTGAAACATGTGTTTTGGGCG |
| | <i>LBD29</i> | A05621 | aacaGGTCTCaggctcaacaATGACTAGTTCCAGCTCTAGCTCTG |
| | | A05622 | aacaGGTCTCtctgaCGAGAAGGAGATGTAGC AAAAATTT |
| | <i>LBD38</i> | A05623 | aacaGGTCTCaggctcaacaATGAGTTGCAATGGTTGTCGAGTTC |
| | | A05624 | aacaGGTCTCtctgaAGCGAAGAGATTGAGCAACTTTGTC |
| | <i>LBD42</i> | A05625 | aacaGGTCTCaggctcaacaATGAGAATCAGTGC AACGGGTGTA |
| | | A05626 | aacaGGTCTCtctgaACCAAGTCTGAGCTCTAAGCCAACC |
| | <i>MYB30</i> | A05627 | aacaGGTCTCaggctcaacaATGGTGAGGCCCTCTTGTGTGACA |
| | | A05628 | aacaGGTCTCtctgaGAAGAAATTAGTGTTCATCCAAT |
| | <i>TMO3</i> | A05629 | aacaGGTCTCaggctcaacaATGGAAGCGGAGAAGAAAATGGTTC |
| | | A05630 | aacaGGTCTCtctgaAACAGCTAAAAGAGGATCCGACCCG |
| | <i>TMO5</i> | A05631 | aacaGGTCTCaggctcaacaATGTACGCAATGAAAGAAGAAGACT |
| | | A05632 | aacaGGTCTCtctgaATTATAACATCGATTACCATCTTA |
| | <i>TMO6</i> | A05633 | aacaGGTCTCaggctcaacaATGGATCATTGTTACAACACCAGG |
| | | A05634 | aacaGGTCTCtctgaCATTAAAGCACCAGAATTAATGTAG |
| | <i>TMO7</i> | A05635 | aacaGGTCTCaggctcaacaATGTCGGGAAGAAGATCACGTTCGA |
| | | A05636 | aacaGGTCTCtctgaTTGGGTAAGTAAGCTTCTGATTAAA |
| | <i>AT3G11280</i> | A05637 | aacaGGTCTCaggctcaacaATGGAGACTCTGCATCCATTCTCTC |
| | | A05638 | aacaGGTCTCtctgaAGCTCCGGCACTGAAGACATTTTCT |
| | <i>ARF3</i> | A06245 | gaacaGGTCTCaggctcaacaATGGGTGGTTTAATCGATCT |
| | | A06246 | gaacaGGTCTCtctgaGAGAGCAATGTCTAGCAACA |
| | <i>ARF4</i> | A06823 | aacaGGTCTCaggctcaacaATGGAATTTGACTTGAATACTGAG |
| | | A06824 | aacaGGTCTCtctgaAACCTAGTGATTTGTAGGAGA |
| | <i>IAA8</i> | A06825 | aacaGGTCTCaggctcaacaATGAGTTCTGGGAACGATAAG |
| | | A06826 | aacaGGTCTCtctgaAACCCGCTCTTTGTTCTTCG |
| | <i>IAA9</i> | A06827 | aacaGGTCTCaggctcaacaATGTCCCGGAAGAGGAGC |
| | | A06828 | aacaGGTCTCtctgaAGCTCTCATCTTCGATTTCTCCATT |
| | <i>IAA12 (BDL)</i> | A04647 | aacaGGTCTCaggctcaacaATGCGTGGTGTGTCAGAATTGGAGG |
| | | A04650 | aacaGGTCTCtctgaAACAGGTTGTTTCTTTGTCTATCC |

Extended Table 6: Oligonucleotides used in this study

METHODS

Plant material and treatments

All plants were grown at 23 °C in long days or continuous light. Ethanol inductions were performed by watering with 1% ethanol and continuous exposure to ethanol vapour, refreshed every 12 hours. WUS-GR was induced by submerging seedlings in 25 μ M dexamethasone, 0.015% Silwet L-70 in 0.5x MS for 2 hours. For local induction at the SAM, 10 μ l induction solution were directly applied to the primary inflorescence meristem. Auxin plates were 0.5x MS, 1% agar, pH 5.7, 10 μ M IAA. For TSA/IAA cotreatments, shoot apical meristems were dissected from about 4 cm high stem and cultured *in vitro* in Apex Growth Medium (AGM) overnight³⁸. AGM was supplemented with vitamins (Duchefa M0409), cytokinin (200 nM 6-Benzylaminopurine), and IAA (3-indole acetic acid, 1 mM) and/or Trichostatin A (TSA, Sigma, T8552, final concentration 5 μ M) or mock before pouring. IAA stock solution (0.1 M in 0.2 M KOH) was diluted with 2 mM M.E.S (pH 5.8) to 1 mM working solution, then added to the plates for 30 min before imaging on the second day.

For WUS-induction with TSA treatments, seedlings were submerged in DEX (10 μ M) or TSA (1 μ M) solution or both, slowly shaken for 2 h, and then harvested for RNA-seq.

All plants were of Col-0 accession apart from *wus-7*, which was in *Ler* background. For experiments involving *wus-7*, *Ler* plants were used as controls.

Transgenes

The *R2D2* and *pDR5v2:3xVENUS-NLS* lines have been described in ref. 11. *pDR5v2:tdTomato-Linker-NLS:trbcS* was transformed into heterozygous *wus-7* plants and *Ler* control plants and activity patterns were scored in T1. A stable single insertion T3 line of *pDR5v2:ER-EYFP-HDEL:tAt4g24550* was used for transformation with *pCLV3:3xmCherry-NLS* and signals were scored in T1. For deGradFP the anti-GFP nanobody coding sequence (*NSlmb-vhhGFP4*)¹⁸ was brought under control of the AlcR/AlcA system³⁹ and transformed into a stable *pWUS:WUS-linker-GFP wus* rescue line (GD44, described in ref. 4) or an *pUBI10:GFP-NLS* line as control. Experiments were performed in stable single insertion T3 lines. Similarly, the *pCLV3:AlcR/AlcA:CaLS3m* line⁴ was crossed to *pDR5v2:3xVENUS-NLS*, *pRPS5a:NLS-tdTomato* and F3 single insertion progeny was used for experiments. For ectopic WUS induction lines *mCherry* was fused N-terminally to the ligand-binding domain of the rat glucocorticoid receptor (GR) and linked by (AAASAIAS[SG]11SAAA)

to the *WUS* coding sequence under control of the *pUBI10* promoter. A single insertion homozygous line was used for crossings, in RNA-seq, and ChIP-seq.

The *pHMG* promoter corresponds to 1347 bp upstream of the AT1g76110 locus. Most constructs were assembled using GreenGate cloning⁴⁰. All oligonucleotides are listed (Extended Table 5).

Microscopy

Confocal microscopy was carried out on a Nikon A1 Confocal with a CFI Apo LWD 25x water immersion objective (Nikon Instruments) as described⁴. 1 mg/ml DAPI was used for cell wall staining.

Image analysis

Quantitative image analysis was done on isotropic image stacks using Fiji (v1.50b)⁴¹, MorphoGraphX⁴², ilastik⁴³, Matlab (Release 2014b, The MathWorks, Inc., United States) and KNIME⁴⁴. Signal quantification methods: all images for an experimental set were captured under identical microscope settings and signal intensities were never adjusted, making intra-experiment signal comparisons possible. MorphographX analysis was performed according to standards defined in the user manual. Averaging and statistical analysis of signals across meristems was performed as follows: histograms of signal intensities along 100 central cross-sections per SAM were (cross-sections rotated by 3.6 degrees successively) were measured by ImageJ standard function. Signals were centered for comparison between individuals. Signals +/- 12.5 μ m around the SAM center were compared between treatment and control and tested for significance by Student's T-test. Distance from center with signal up to 120% of center background signal between treatment and control was determined and tested by Student's T-test.

To determine the center of an inflorescence meristem, 10 to 20 L1 cells located at the meristem summit were segmented using the carving workflow in ilastik. A sphere was fitted through the centroids of these cells using the least squared distances method. The sphere was superimposed on the original DAPI stained image volume to help identifying the newly emerging flower primordia. Three points marking the center of three young flower primordia were manually picked close to the sphere surface, projected onto the sphere and then used as seeds to perform a spheric voronoi tessellation (<https://de.mathworks.com/matlabcentral/fileexchange/40989-voronoi-sphere>). The point P_{center} is equidistant to the three seed points and serves as a good approximation for the meristem center which is marked by the *pCLV3* stem cell reporter. The method was tested using image stacks of nine meristems containing cell

walls stained by DAPI in one channel and the stem cell marker *pCLV3::mCherry-NLS* in the second channel. The computationally estimated meristem center and the one determined by *pCLV3::mCherry-NLS* expression in every case were in the range of one cell diameter. Further details and workflows are available on request.

In situ hybridization

In-situ hybridizations were carried out as described⁴⁵.

ChIP-seq and RNA-seq

All experiments were carried out on 5 day old seedlings grown on 0.5 MS plates after 2 hours of either Dex or mock treatment. ChIP assays were performed from 3g of fresh weight each as described in ref. 46 using RFP-Trap single chain antibodies (Chromotek). Enrichment of specific DNA fragments was validated by qPCR at the *ARR7* promoter region²⁴. Two independent libraries were generated for the *WUS-GR* and control ChIP each using pooled DNA from 6 to 9 individual ChIP preparations. RNA-seq was carried out in biological triplicates. After careful benchmarking of our *WUS-GR* line, we find it to be the most potent and consistent tool for *WUS* induction to date, affording a much higher sensitivity for identifying transcriptional targets. In addition, the use of RFP-trap increased sensitivity of the ChIP assay. Consistently, we were able to identify 5874 genomic regions bound by *WUS* in both ChIP-seq experiments at $p < 0.05$, which corresponded to 4515 genes. This compared to 136 regions we had previously identified by ChIP-chip²⁰, highlighting the increase in power. Previously identified direct targets, such as *ARR7*, *CLV1*, *KAN1*, *KAN2 AS2* and *YAB3*^{20,21,24} were also picked up in our analysis. Because of the medium level ubiquitous expression of *WUS*, both RNA-seq and ChIP-seq capture the global regulatory potential of *WUS*. Since regulatory output of *WUS* is dependent on tissue context, targets identified here might not be relevant for all tissues. In addition, targets might be induced by *WUS* in one tissue and repressed in another, which cannot be resolved by this dataset. All genomic datasets are available under GEO accession: GSE97065

Bioinformatics

ChIP-seq data were mapped to TAIR10 genome by BWA aligner (v0.7.17)⁴⁷ on a local Galaxy instance (v17.09)⁴⁸. Peak calling was performed using Hiddendomains (v3.0)⁴⁹. Peaks were annotated to TAIR10 genes using PAVIS⁵⁰.

Alignment of RNA-seq reads to TAIR10 genome by HISAT2 (v2.1.0)⁵¹ and calculation of count matrices by featureCounts (v1.6.3)⁵² was done on Galaxy instance. Differentially expressed genes were identified with R bioconductor package Deseq2 (1.20.0)⁵³. Gene ontology analysis was carried out using topGO R package (v2.32.0) with all genes annotated to open chromatin¹⁹ as background.