## Supplemental Experimental Procedures

### Construction of lineage lines

The inducible tissue specific lineage system was constructed using two vectors, the first containing CRE-GR under a specific promoter (pTISSUE:CRE) and the second containing the reporter 35S:loxP:CFP, both derived from pJCGLOX (Joubes et. al., 2004). To generate pTISSUE:CRE vectors, the CRE:intron-GR cassette was amplified from pJCGLOX, adding gateway sites to produce AttB1-CRE:GR-AttB2. Promoter sequences were amplified with flanking AttB4-AttB1R sites. Fragments were recombined with the destination vector pH7m34GW,0 in a three-way gateway reaction together with a connector sequence containing AttB2R-AttB3 sites. To generate the 35S:loxP:CFP vector, the HS:CRE:GR was removed from pJCGLOX by digestion with HinIII and Egfp, followed by partial digestion with Ale1. ER-CFP was then recombined in a Gateway reaction. Transformations were performed by double floral dipping with equal concentrations of pTISSUE:CRE and 35S:loxP:CFP vectors.

### Live imaging

To follow clone expansion in individual live roots, plants carrying the lineage markers *pSCR:lineage-CFP* or *pAHP6:lineage-CFP* were induced at 4 days post germination using 15 μM dexamethasone for 24 hours, and then cut at ~130 μm from the tip of the meristem. Cut roots were then placed on a coverslip under a block of high density (2%) agar and mounted on the confocal microscope stage. Imaging was carried out approximately every 6 or 12 hours (for CFP-lineage), using the 405nm laser for excitation of CFP. To track live nuclei at high temporal resolution, plants carrying the nuclear marker *35S:H2B-mCherry* were cut and mounted as above and imaged at 13 minutes intervals with excitation by the 561nm laser. Z-stacks comprised 40 slices, with roughly 1 μm spacing between each slice. Time point registration was performed with Correct 3D Drift (Parslow et al., 2014), and endodermal nuclei were manually false-colored using Adobe After Effects.

### Generation of single cell RNA-Seq libraries

Clones of *AHP6* or *WOL* lineage lines were induced as described in the Methods section. We isolated cells from uncut meristems, and at 3h, 16h, and 46h after the cut. Following recovery, isolated root meristem stumps were treated (~1h-2h) in a cell wall digestion solution (Ip and Birnbaum, 2015). The solution was filtered 3 times through a 40μm falcon screen (BD Biosciences) to remove clumps, and single cells were isolated based on their CFP fluorescence either by hand-picking for preliminary results, or, for the bulk of data, by using fluorescence activated sorting (FACS, BD FACSAria or Sony SY3200) into 96-well plates containing collection buffer for mRNA amplification. Library preparation was carried out according to (Tang et al., 2009) for preliminary data or (Satija et al., 2015) for the bulk of the data, with the following modifications to the Satija et al. protocol: Maxima reverse transcriptase (10U/rxn) was used for reverse transcriptase and Kapa Hotstart PCR Enzyme (6.25U/rxn) used for PCR

amplification. For FACS isolation of single cells, we established stringent forward scatter gates (area vs. width) that eliminated small cell clumps (Figure S7A-B). Seven cells from regenerating roots that had been previously described were added to the analysis (Efroni et al., 2015). Libraries were sequenced using Illumina HiSeq 2500 and resulting reads were aligned to the *Arabidopsis* TAIR10 genome using bowtie2 (*bowtie2 –end-to-end –L 25 –D 20 –all*). Counts were derived using NGSUTILS 0.5.7 (*bamutils count –library unstranded –multiple partial)*, normalized to mean library size and $\log_2$-transformed. Cells with less than 10,000 aligned reads were discarded. Data was submitted to GEO (GSE74488).

**Calling of cell identity**

To calculate spec scores, tissue specific microarray profiles were obtained from GEO (list of datasets are provided in Table S3). Expression values were derived using MAS5 and median expression was normalized to 50. Spec values were calculated as in (Birnbaum and Kussell, 2011; Efroni et al., 2015) using the *getAllSpec* function (Efroni et al., 2015) with the parameters (medianfilter=250, cuts=10, distshape=2). As auxin may have short-term effects on tissue identity, we used samples of auxin-treated tissues as a separate tissue for the Spec analysis. As Spec analysis does not require markers to be exclusive to one tissue, this does not affect the marker selection for the non-treated tissues. We used the *getIdentity* function (Efroni et al., 2015) to calculate ICI scores, using an information threshold of 30 to call markers, using 579 markers over all. To score cell identities, we used a p-value cutoff of 0.1. Of the 238 cells that passed the read filter, 140 scored as single identity, 37 as mixed identity and 61 as unknown (cells in which no identity scored below p-value cutoff). We checked for potential sequencing artifacts that could lead to mixed identities and false positive calls that represented new cell states. First, we confirmed that the identification of mixed identity was not correlated to read count indicating our calling of mixed identity was not due to failed amplification (Figure S7C-D). To verify that the identity transitions occurring during regeneration are not caused by stochastic variations in mRNA copy number leading to false positive identity calls, we profiled 32 single cells from the stele (induced *WOL* clones*)* of uncut roots. The stele clones do not contain QC, columella and Epidermis\LRC identities. Cells from uncut roots had very low background ICI scores for QC, Columella and Epidermis\LRC identities (Figure S7E), confirming that ICI thresholds accurately filtered noise and led to reliable identity calls. We used these background levels as an empirically determined threshold. ICI scores lower than the threshold were discarded. To calculate combined ICI scores for Figure 3, we added the raw marker scores, as in Table S3, and normalized the values, such that the sum of all ICI for each cell equaled 1.

**Supplemental References**

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