

## Supplementary Material For: Modeling halotropism: A key role for root tip architecture and reflux loop remodeling in redistributing auxin

Thea van den Berg, Ruud Korver, Christa Testerink, Kirsten ten Tusscher

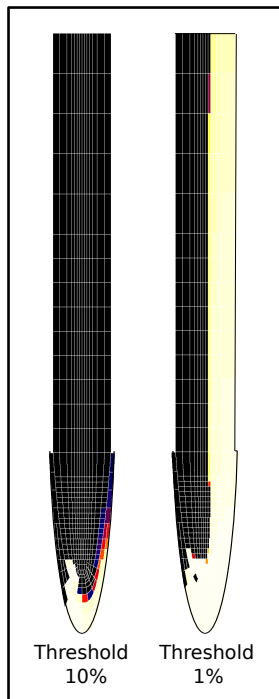


Figure S1: **Influence of measurement threshold on displayed auxin rerouting.** Auxin rerouting maps for auxin elevations of 10% or more (same as in Figure 2C, right) (left) and for auxin elevations of 1% or more (right).

distance from lateral root cap ( $\mu m$ )	significance value
2.5	0.000193054
7.5	0.157423
12.5	0.0118692
17.5	$1.18449 * 10^{-8}$
22.5	$3.61994 * 10^{-7}$
27.5	$1.38665 * 10^{-8}$
32.5	0.000122297
37.5	$7.38984 * 10^{-9}$
42.5	$3.1453 * 10^{-10}$
47.5	$3.44796 * 10^{-11}$
52.5	$6.1504 * 10^{-8}$
57.5	0.0000609791
62.5	$4.14202 * 10^{-13}$
67.5	$9.73117 * 10^{-12}$
72.5	$2.40387 * 10^{-20}$
77.5	$9.78448 * 10^{-15}$
82.5	$5.25836 * 10^{-15}$
87.5	$2.01565 * 10^{-17}$
92.5	$2.02167 * 10^{-14}$
97.5	$1.80546 * 10^{-10}$
102.5	$4.7292 * 10^{-10}$
107.5	$1.61581 * 10^{-12}$
112.5	$2.65674 * 10^{-10}$
117.5	$2.95543 * 10^{-7}$
122.5	$1.39511 * 10^{-8}$
127.5	$6.17551 * 10^{-6}$
132.5	0.0000357128

Table S1: Significance values for AUX1 asymmetry in salt-gradient exposed versus non exposed roots. Significance values were computed for a double sided T-test performed on AUX1 fluorescence level ratios between salt-exposed and non-salt exposed plants. Ratios were binned per 5  $\mu m$  intervals, indicated distances represent the midpoint of the bin.

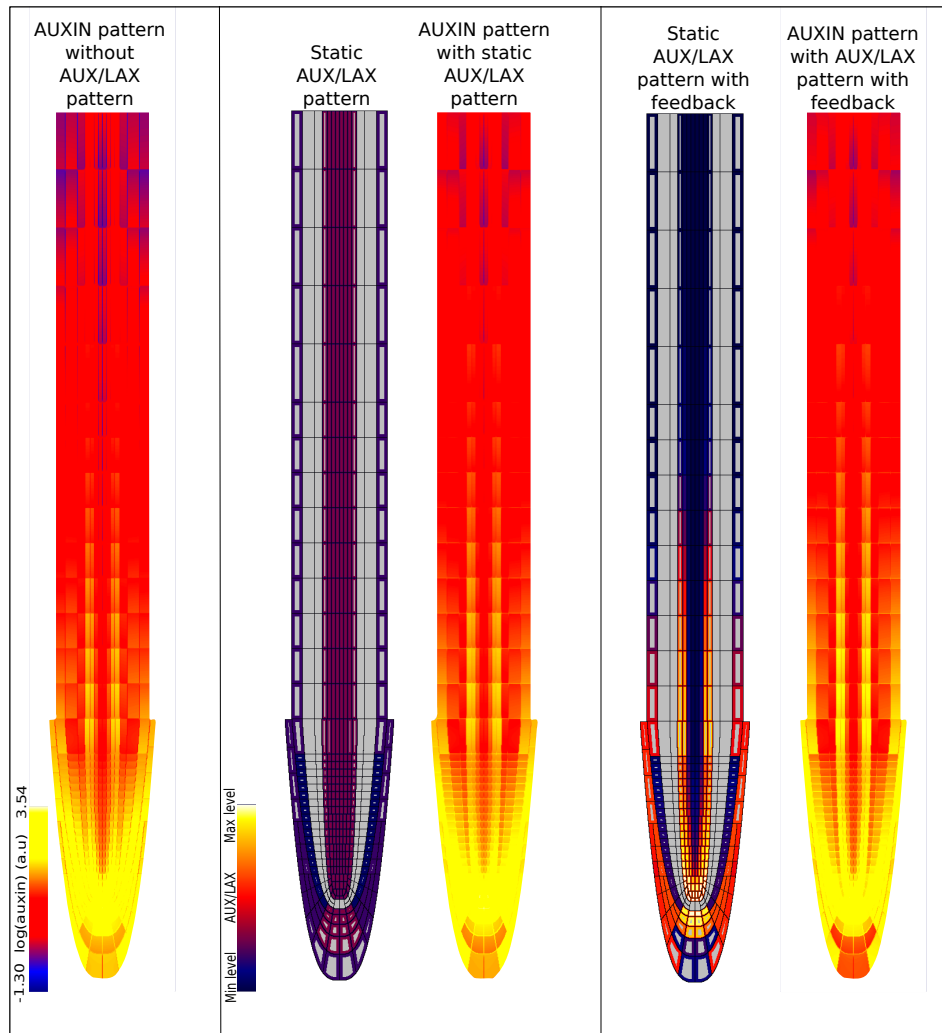
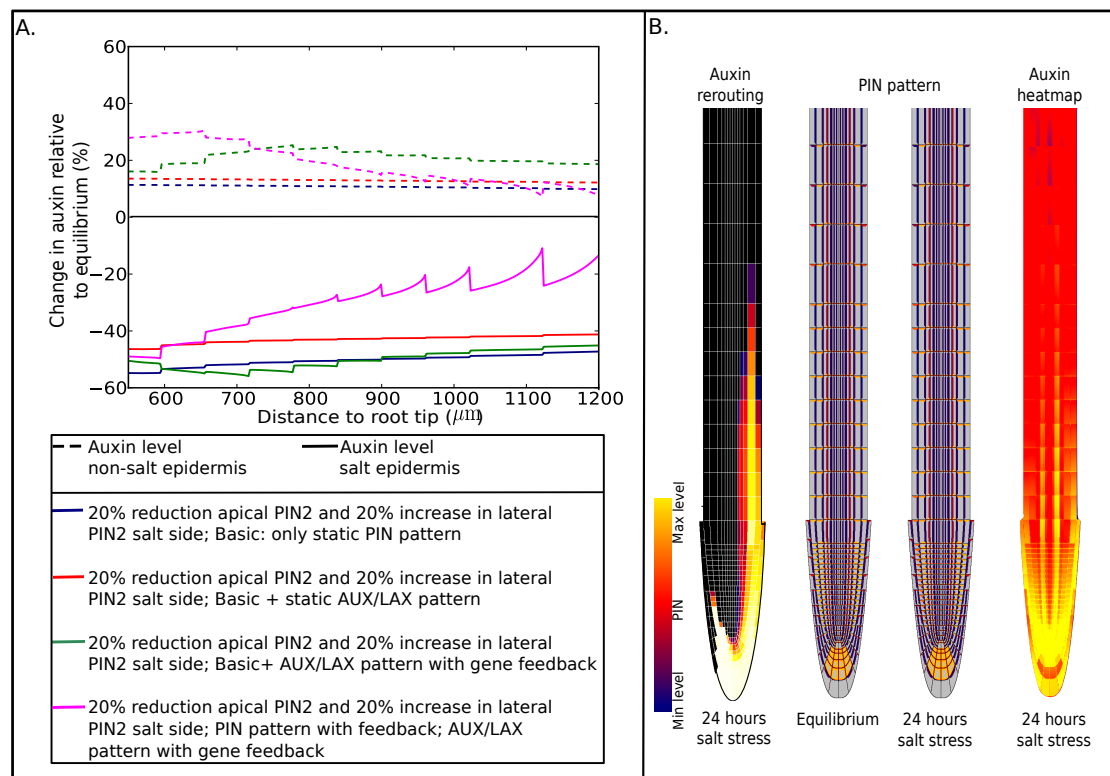
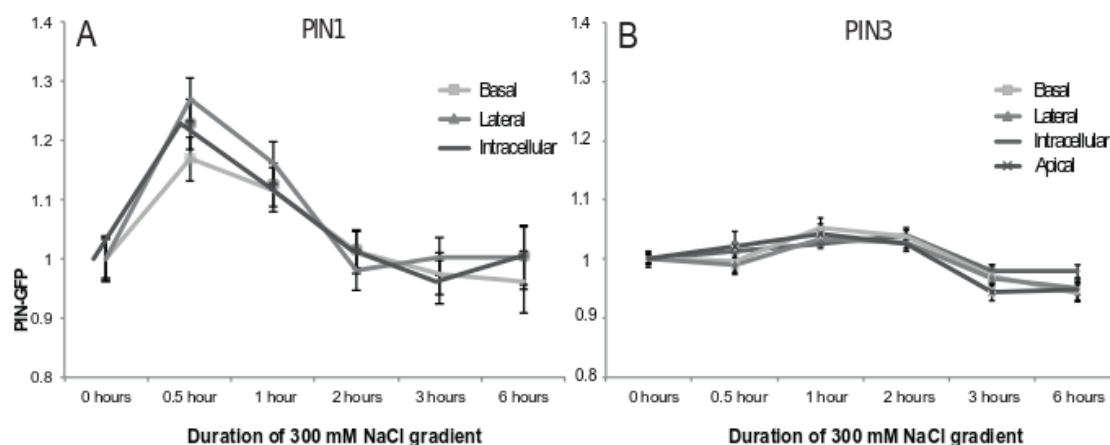


Figure S2: **Influence of AUX/LAX on root tip auxin pattern.** Left: auxin pattern in absence of AUX/LAX prepattern; Middle: AUX/LAX pattern with static AUX/LAX levels and resulting auxin pattern; Right: AUX/LAX pattern with auxin dependent AUX/LAX expression and resulting auxin pattern.



**Figure S3: Influence of auxin feedback on PIN2.** **A** Impact of positive feedback of auxin on PIN2 membrane occupancy on epidermal auxin asymmetry after 24 hours of applying salt stress by reducing apical and increasing lateral PIN2 levels. For comparison purposes auxin asymmetry under several other conditions are also shown. We can see that while auxin induced upregulation of AUX/LAX expression substantially elevated the auxin asymmetry, the added effect of auxin feedback on PIN2 appears more subtle, increasing auxin asymmetry in the lower part and reducing asymmetry in the higher parts of the elongation zone. Important to consider here is that we take the PIN2 situation as observed after 6 hours of salt stress as a starting point for our simulations. As a consequence, we start our simulations from a situation in which most if not all PIN2 dynamics, directly salt induced as well as secondary auxin-feedback dependent- has most likely already taken place. In retrospect, adding auxin dependent feedback of PIN2 on top of this should not be expected to have too much effect. Indeed, auxin dependence of PIN2 might be more important for the initial establishment of the PIN2 asymmetry. **B** Auxin rerouting, change in PIN membrane occupancy pattern and resulting auxin asymmetry in presence of auxin feedback on both AUX/LAX expression and PIN2 membrane occupancy.



**Figure S4: PIN1 and no PIN3 re-distribution during a 300 mM NaCl gradient.** **A** PIN1-GFP and **B** PIN3-GFP intensities compared to control on the basal and lateral sides of the membrane and inside of the cell. GFP-intensities on the individual membranes and cell interior follow the same pattern as the total GFP-intensity (Fig 4), implying that no redistribution of PIN1 or PIN3 occurs during 6 hours of exposure to a 300mM NaCL gradient. Note that in our earlier work we found substantial upregulation of both PIN1 and PIN3. The differences in salt-induced upregulation of PIN1 and PIN3 found in our current and earlier experiments can be explained by differences in experimental set-up. In the Galvan-Ampudia *et al.* study roots were dipped in liquid 100 mM NaCL medium for an hour, generating a uniform and severe salt stress for the root. In contrast, in the current study roots were grown in solid medium containing a salt-gradient with a maximum of 300 mM NaCL. These conditions are more representative for naturally occurring growth conditions. Extrapolating from measurements of similar gradients in our earlier study (Galvan-Ampudia *et al.* Curr Biol 2013), we derive that salt concentrations at the tip of the root will not exceed 75 mM after 24 hours. Thus, in the current experiments roots are exposed to non-uniform and considerably lower salt-stress, explaining the reduced upregulation of PIN1 and PIN3.

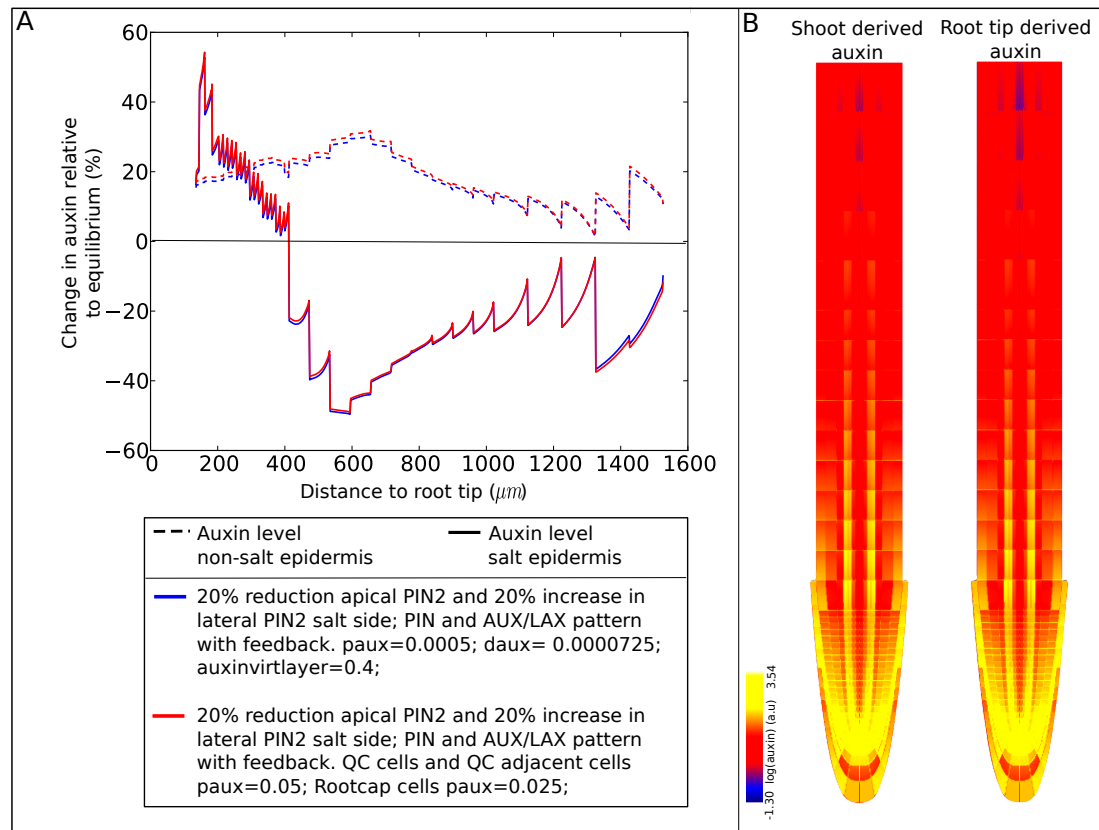


Figure S5: **Robustness to variation in location of major auxin source.** In the default model, all cells have a similar capacity to produce and degrade auxin and there is a substantial flux of auxin from the shoot. Here we reduced shoot derived auxin influx by a factor 2, while increasing auxin production in the QC by a factor 100 and in the root cap increasing auxin production a factor 50 and decreasing degradation by a factor 2. We compare the epidermal auxin asymmetry and overall auxin pattern generated during halotropism with that of the default model.

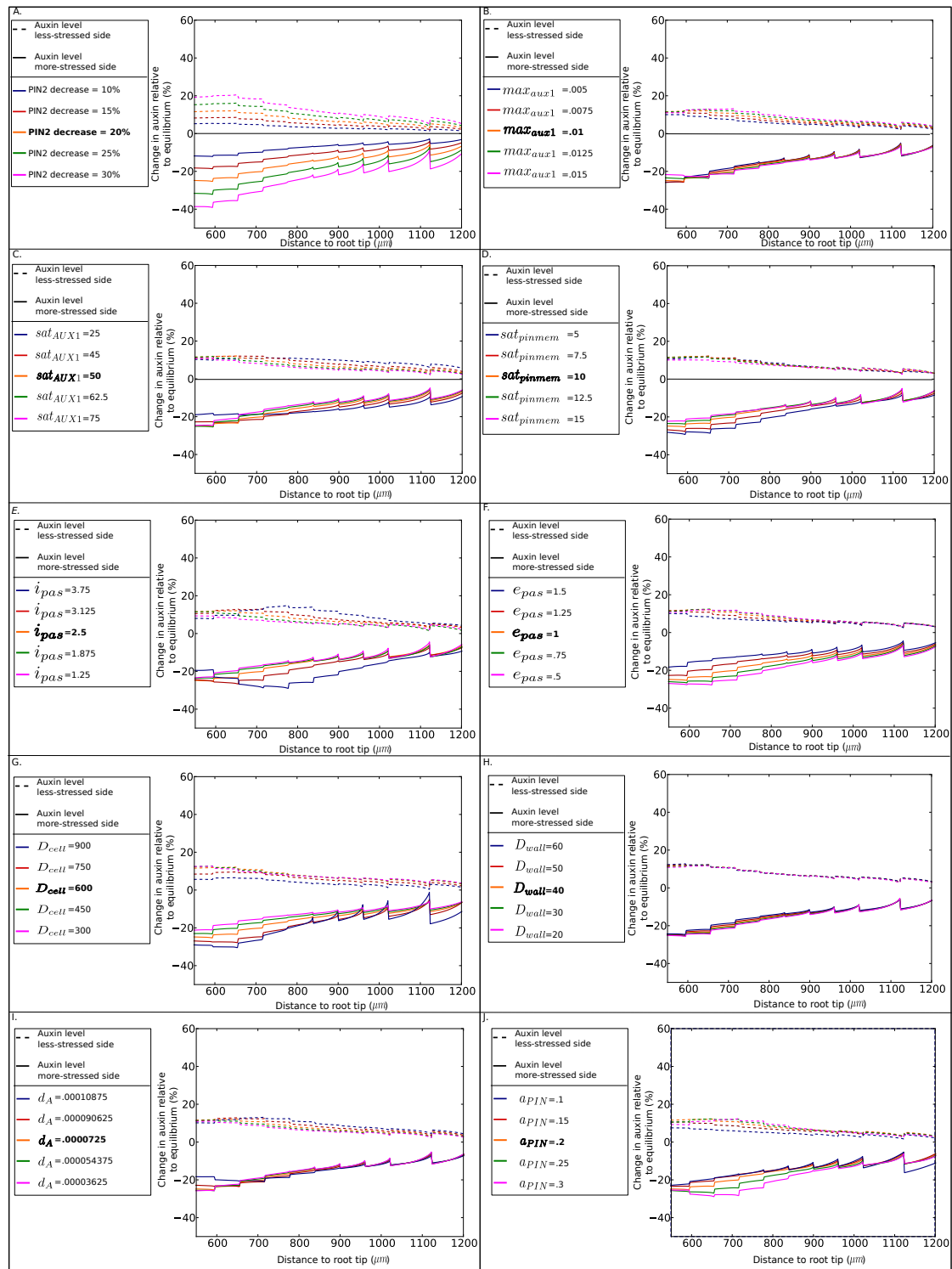


Figure S6: **Robustness to variation in parameter values.** Robustness of simulation outcomes for changes in the reduction of apical PIN2 at the salt-stressed side (A), changes in  $max_{AUX1}$  (B), changes in  $sat_{AUX1}$  (C), changes in  $sat_{pinmem}$  (D), changes in  $i_{pas}$  (E), changes in  $e_{pas}$  (F), changes in  $D_{cell}$  (G), changes in  $D_{wall}$  (H), changes in  $d_a$  (I) and changes in  $a_{PIN}$  (J). Parameter values were varied within a range of a factor 0.5 to 1.5 of the original values. All simulations were performed with the an apical PIN2 reduction of 20%, and without AUX/LAX prepatter and feedback on auxin transporters. Simulations were run for 24 hours, auxin levels during stress were compared to equilibrium levels, and plotted for the elongation zone.