Supplemental File

Supplemental Methods:

For quantification of free IAA levels, 5 day old low light seedlings were transferred to high light conditions with or without excision, and hypocotyl tissues were harvested at indicated time points and frozen in liquid nitrogen. The intact samples included the whole hypocotyl, while in the root-excised samples the bottom half of the hypocotyls were removed, during the process of excision, About 50-80 mg of frozen tissues were homogenized using 150µl of homogenization buffer (65% isopropanol, 35% 0.2 M imidazole, pH 7), incubated with internal standard ($^{13}C_6$)-IAA, followed by centrifugation at 10,000 g for 8 min. Free IAA was extracted by running through two automated successive columns followed by methylation, drying and redissolving in ethyl acetate (Barkawi et al., 2008). Quantification was done using GC-SIM-MS through isotope dilution analysis and values are reported relative to fresh weight (ng/g).

Supplemental Tables and Figures

Supplemental Table I. Auxin from the shoot is important for adventitious root formation.

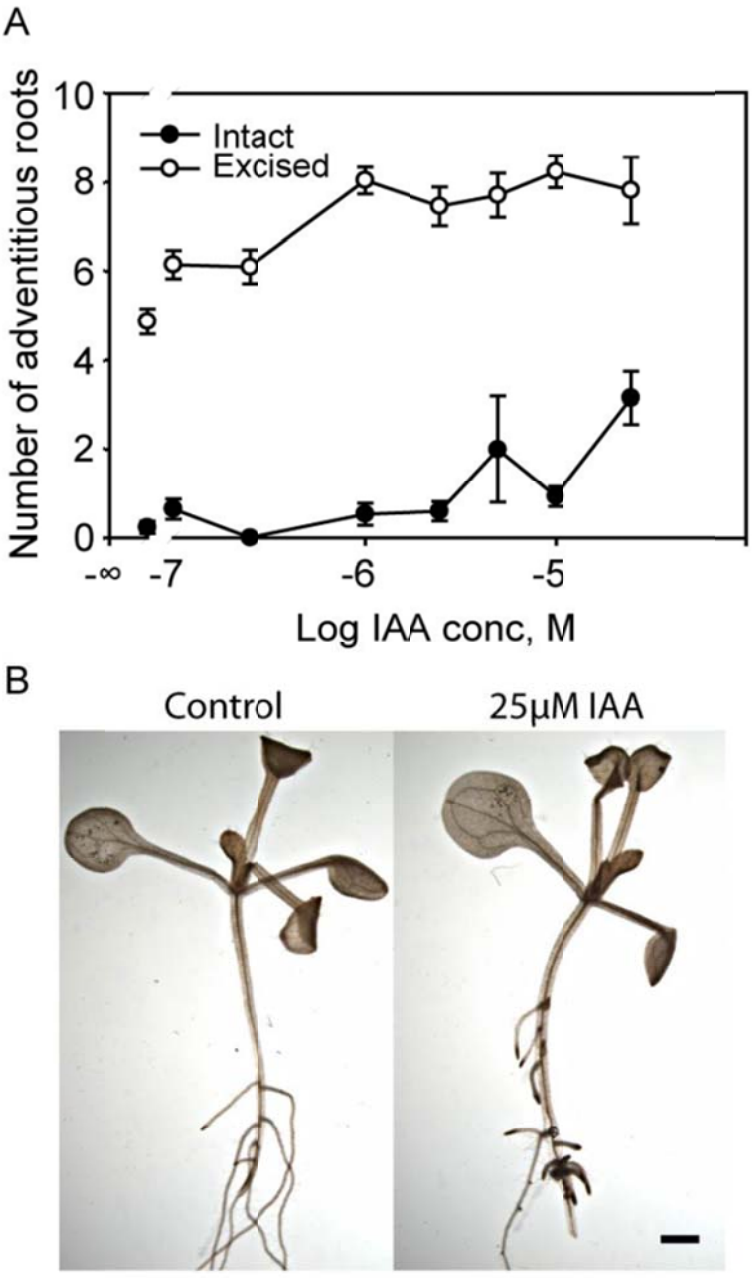
Shoot apex	Treatment	Number of adventitious roots[a]
Present	None	5.8 ± 0.4
Present	10μM NPA[b]	0 ± 0[c]
Absent	None	0 ± 0[c]
Absent	100μM IAA[c]	4.8 ± 0.4
Absent	100μM IAA+100μM NPA[c]	1.1 ± 0.3[d]

[a]The average and standard error of each sample with n=18-20.

[b]Seedlings treated globally.

[c]Seedlings treated locally with agar containing these compounds applied at the shoot apex.

[d] Values that are significantly different from intact seedlings were determined by student's t-test with $p<0.05$

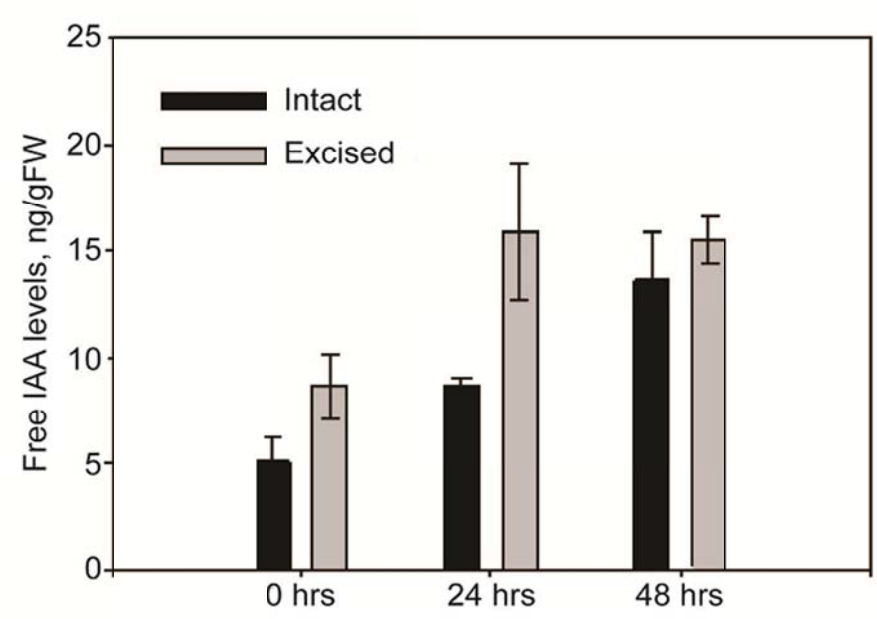


Supplemental Figure 1. The effect of IAA on adventitious root formation and IAA transport in hypocotyls.

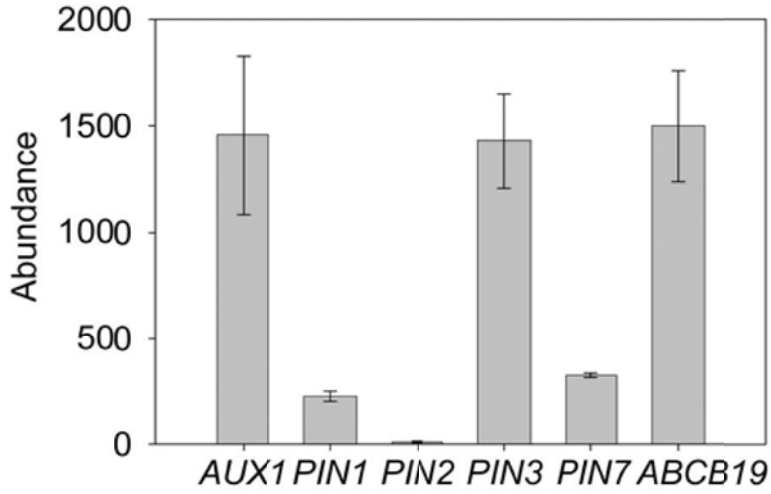
A. The number of adventitious roots formed in control or seedlings treated with a range of concentrations of IAA. n= 10-31.

B. Images of cleared wild-type hypocotyls; control and treated with 25 µM IAA, 7 d after excision. Scale bar=1 mm.

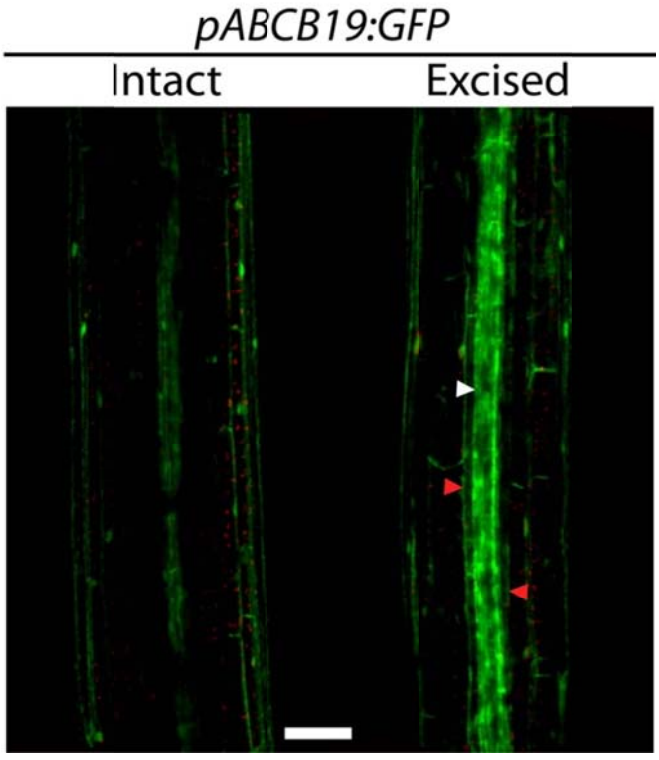
Supplemental Figure 2. Hypocotyls of At*GH3-2:GUS* transgenic seedlings were stained prior to excision, at the time of excision, and at 24 h after root excision with or without 10 µM NPA added. Scale bar is 1 mm.



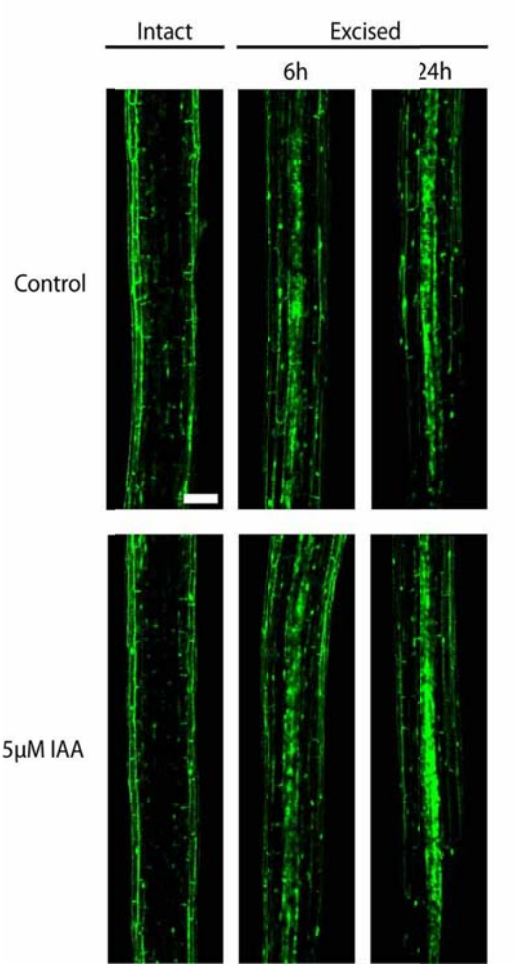
Supplemental Figure 3. Free IAA levels were quantified using IAA extraction followed by detection using GC-MS. The average and SE of 3 to 5 samples are shown. None of the values are statistically different between intact and excised samples. The increase in the intact shows developmental changes in free IAA levels over this time frame.



Supplemental Figure 4. Transcript abundance of genes encoding IAA signaling and IAA transport proteins from a previously published microarray data set using hypocotyl tissues (Van Hoewyk et al., 2008).



Supplemental Figure 5: *pABCB19; GFP* fluorescence is found in vascular and pericycle cells. The p*ABCB19:GFP* fluorescence was examined in intact and excised hypocotyls. White arrowhead points to the vascular bundle and red arrowheads point to pericycle cells. Scale bar = 100 µm.



Supplemental Figure 6. The effect of IAA on GFP fluorescence in the *pABCB19:GFP* line. Central cylinders of hypocotyls are shown above the point of excision in both intact hypocotyls and 6 or 24 h after excision with or without local treatment of 5 μM IAA. Scale bar = 200 μm.