Supporting Information

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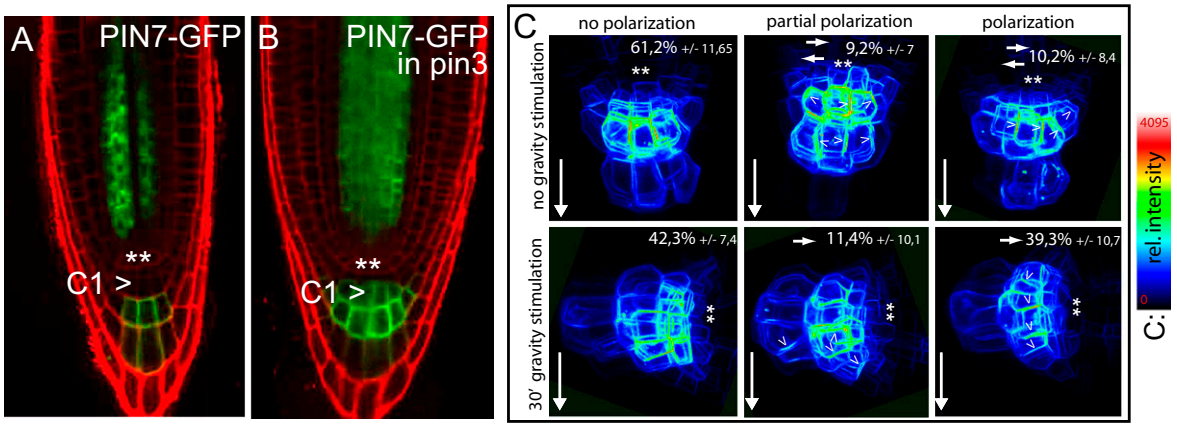

Fig. S1. PIN7 and PIN3 localization in gravity-sensing columella cells. (*A* and *B*) PIN7-GFP expression (in green; red propidium iodide for counterstaining) in wild-type (*A*) and *pin3* mutant (*B*). Arrowheads indicate C1 columella cells and asterisks mark the quiescent center. (*C*) PIN3-GFP polarity was assessed with semiquantitative confocal imaging. Seedlings were rated as not polarized, partially polarized (at least two polarized adjacent cells), or polarized (at least three polarized neighboring cells). Twenty nonstimulated and 20 gravity-stimulated seedlings were analyzed. The overall percentage of roots that were assigned to each category is indicated and the SD as well. Short and long arrows mark the directions of the preferential polarization and gravity vector, respectively.

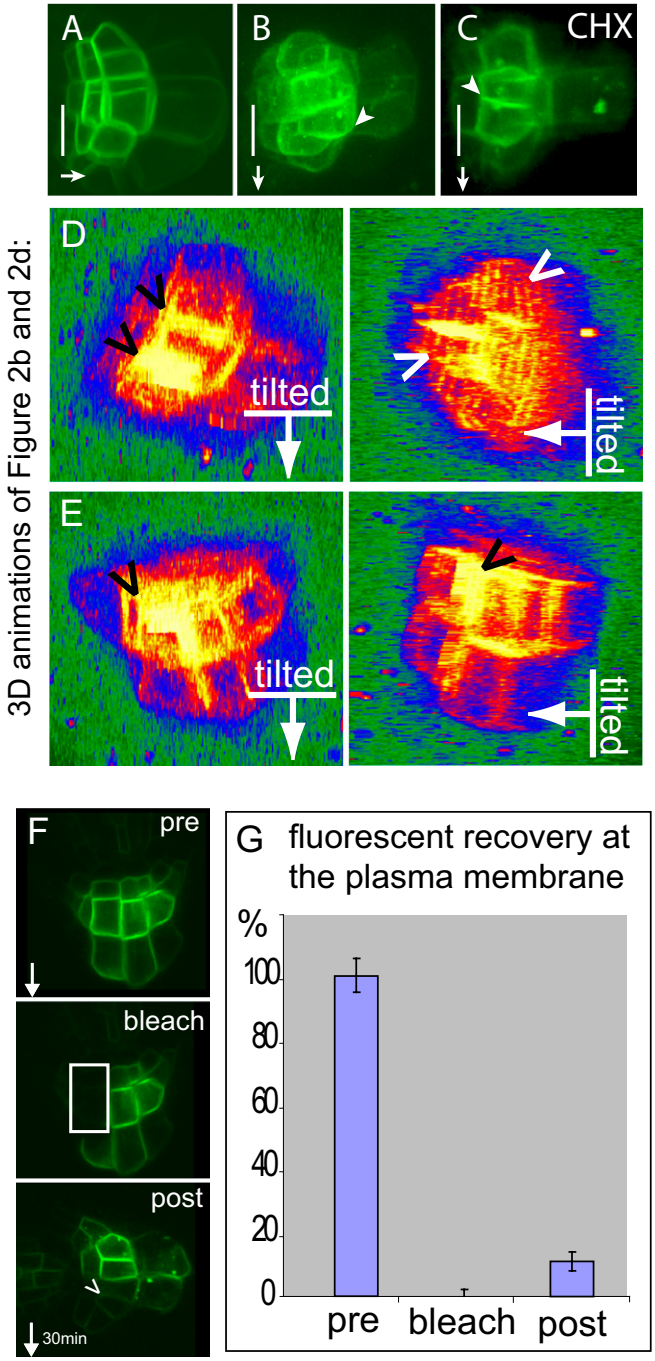

Fig. S2. PIN3 de-novo protein biosynthesis is not required for gravity-induced polarization. (*A*–*C*) Nonpolarly distributed PIN3-GFP (*A*) is translocated to the new lower cell side in response to (30 min) gravity (*B*). Cycloheximide (CHX)-dependent inhibition of the protein biosynthesis does not interfere with PIN3 polarization in response to gravity (*C*). Arrowheads indicate PIN polarization. (*D* and *E*) Three-deimensional projection (horizontally and vertically tilted) of photoconverted PIN3EosFP with (*D*) and without (*E*) gravity stimulation reveals pronounced gravity-induced and de novo secretion-independent PIN3 polarization. Black arrowheads highlight PIN3-GFP-containing cell faces, and white arrowheads indicate the absence of PIN3. (*F* and *G*) A *PIN3-GFP*-expressing columella cell was photobleached and subsequently gravity stimulated for 30 min (*F*). Only mild fluorescent recovery was observed (*G*), indicating that PIN3 secretion does not significantly contribute to early PIN3 polarization events. Arrows mark gravity vector. (Scale bars, 10 μm.)

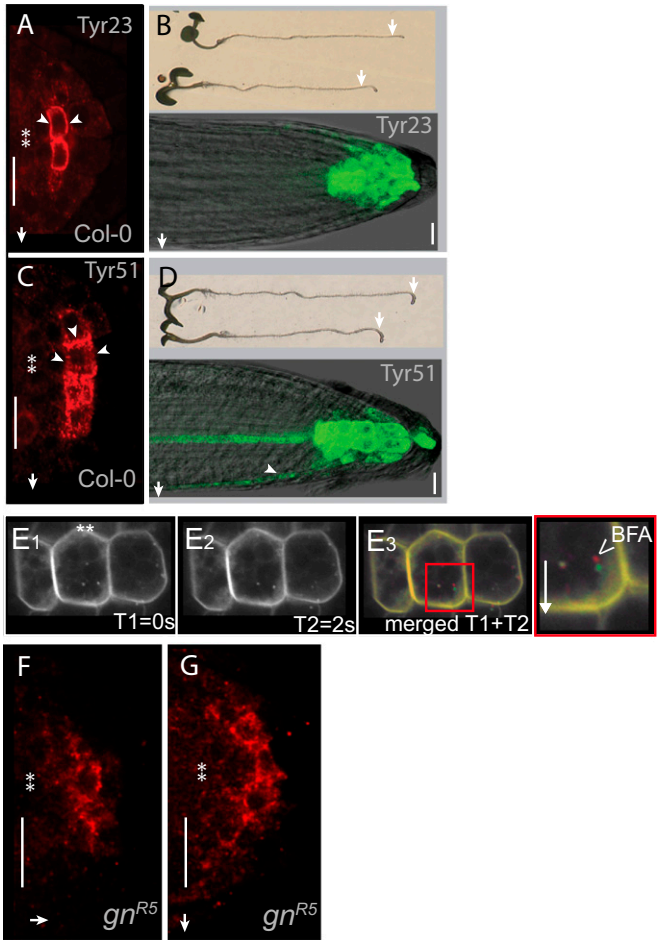

Fig. S3. Pharmacological and genetic interference with PIN3 targeting during the gravitropic response. (*A–D*) Tyrphostin A23 (Tyr23)-dependent interference with clathrin function inhibited gravity-induced PIN3 polarization (*A*) and reduced the asymmetric auxin distribution (*DR5rev::GFP*) and the gravity response (*B*). In contrast, the inactive analog tyrphostin A51 did not interfere with PIN3 polarization (*C*), asymmetric auxin distribution (DR5rev::GFP), or the gravitropic response (*D*). (*E*) Nonstimulated seedlings were treated with brefeldin A. Two consecutive frames E1 (t = 0 s) and E2 (t = 2 s) and their merged overlay in E3 (*Inset*, *Right*) display distinct red and green endosomes, indicating endosomal trafficking. (*F* and *G*) PIN3 antibody labeling of root columella cells in unstimulated (*F*) and gravity stimulated (*G*) weak *gnom*^{R5} mutants. Arrows mark the gravity vector, arrowheads the asymmetric DR5 signal, PIN3 accumulation, and endosomal movement, and asterisks the position of the quiescent center. (Scale bars, 10 μm.)