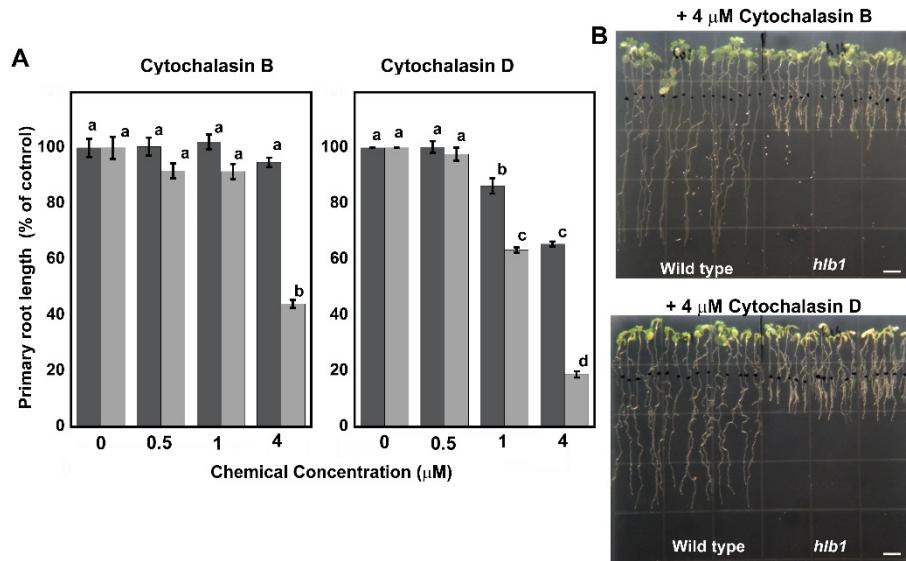


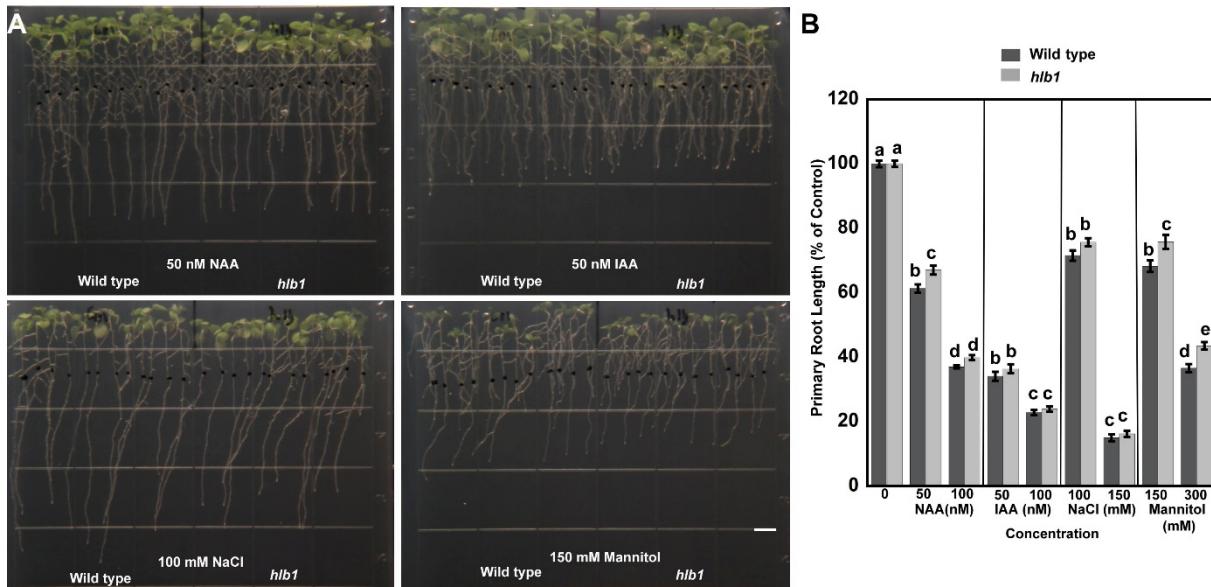
Supplemental Figure 1. Hypocotyl and Root Hair Growth of *hlb1* are Hypersensitive to LatB. (A) to (C) Representative images of 7 d-old dark-grown wild-type and *hlb1* seedlings in 0, 50 and 100 nM LatB. Bar in C = 5 mm.
(D) and (E) Root hairs of light-grown wild-type seedlings in 20 and 30 nM LatB.
(F) and (G) Root hairs of light-grown *hlb1* seedlings in 20 and 30 nM LatB. Note that root hairs of *hlb1* are more severely inhibited by LatB compared to wild type. Bar in G= 100 μ m.
(H) Quantification of hypocotyl and root hair length in wild type and *hlb1* expressed as percent of control. Statistical significance was determined by one way ANOVA. Values are means (n=19 to 36 hypocotyls; 47 to 68 root hairs) \pm S.E. Different letters indicate significant differences among means ($P<0.05$, Tukey's test).



Supplemental Figure 2. *hlb1* is Hypersensitive to Cytochalasin B and D.

(A) Dose response curve of *hlb1* primary root length in cytochalasin B and D. Note that compared to LatB, higher ($>1 \mu\text{M}$) concentrations of cytochalasins are needed to elicit a clear inhibition of root growth. Statistical significance was determined by one way ANOVA. Values are means ($n=30$ to 35 roots) \pm S.E. Different letters indicate significant differences among means ($P<0.05$, Tukey's test).

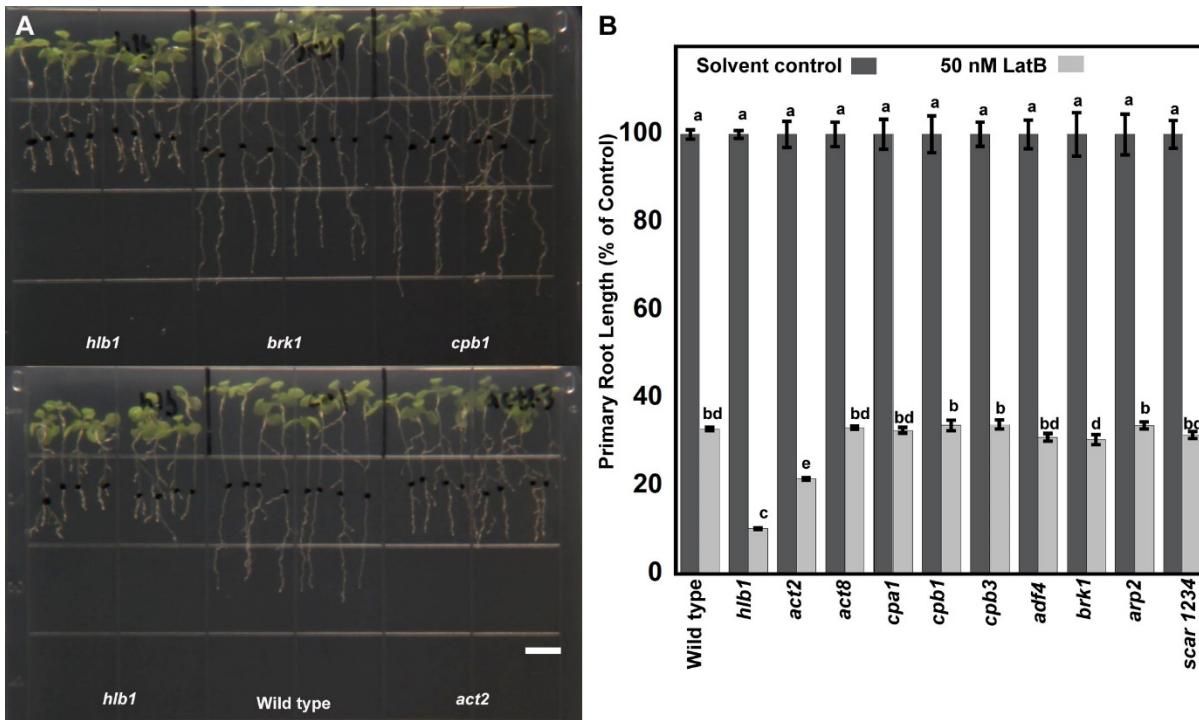
(B) Wild-type and *hlb1* seedlings transplanted on cytochalasin B or D. 3-d-old seedlings were transplanted on media with 4 μM cytochalasin and images were taken 5 days later. Bars= 5 mm.



Supplemental Figure 3. *hlb1* is not Hypersensitive to Other Treatments that Inhibit Primary Root Growth.

(A) Wild-type and *hlb1* seedlings transplanted on media supplemented with NAA, IAA, NaCl or mannitol. 4-d-old seedlings were transplanted on media with the chemicals and images were taken 7 days later. Bars= 5 mm.

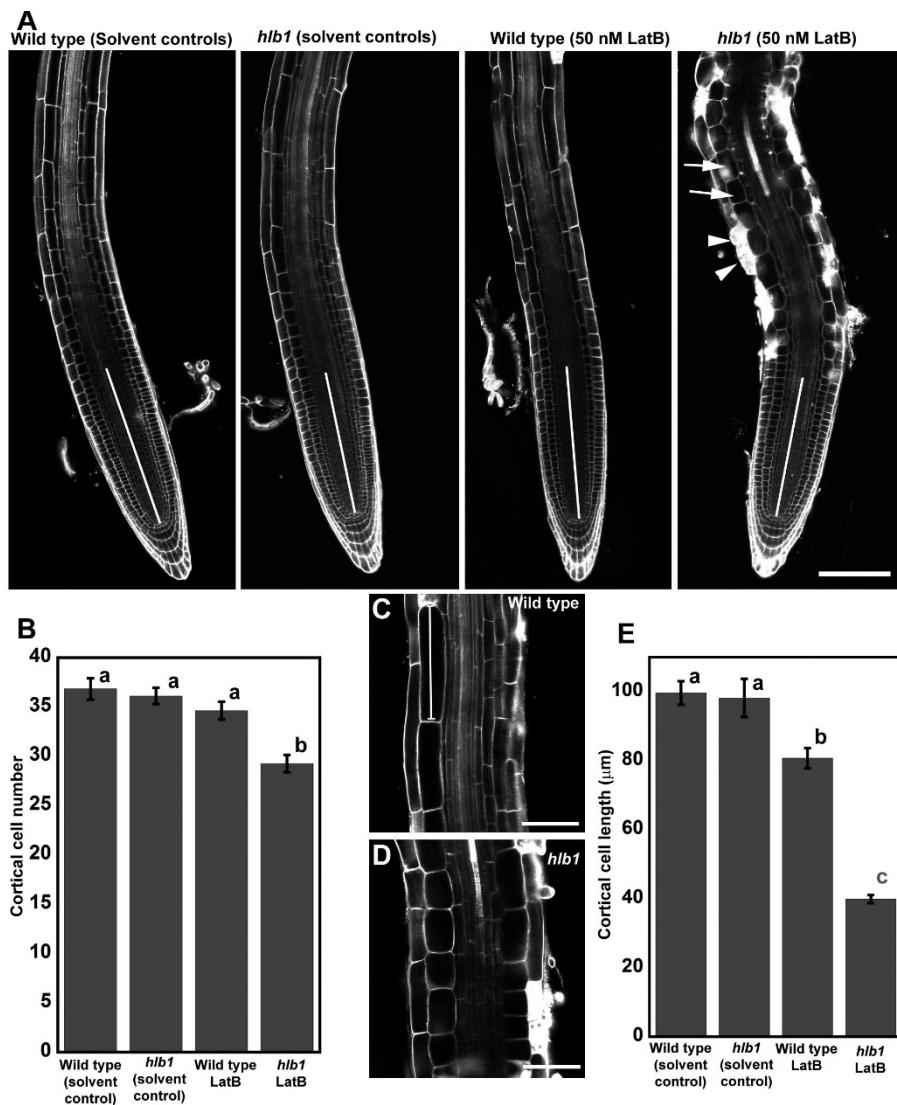
(B) Quantification of *hlb1* primary root length in response to various concentrations of NAA, IAA, NaCl or mannitol. Statistical significance was determined by one way ANOVA. Values are means ($n=29$ to 48 roots) \pm S.E. Different letters indicate significant differences among means ($P<0.05$, Tukey's test). For clarity vertical bars in the graph were placed to indicate a specific treatment. Letters of significance should be compared only to mean values lying within these bars and to 0 concentration.



Supplemental Figure 4. *hlb1* is More Sensitive to LatB Compared to Other Mutants that Directly Affect Actin Function.

(A) Wild type, *hlb1* and representative actin and actin regulatory protein mutants transplanted onto media supplemented with 50 nM LatB. *act2* (*actin2*), *brk1* (*brick1*), *cpb1* (*capping protein b1*). 4-d-old seedlings were transplanted on LatB and images were taken 7 days later. Bar= 5 mm.

(B) Quantification of primary root length among wild type, *hlb1* and various actin and actin regulatory protein mutants. *act8* (*actin8*), *cpa1* (*capping protein a1*), *cpb3* (*capping protein b3*), *adf4* (*actin depolymerizing factor4*), *arp2* (*actin related protein 2*), *scar1234* (*scar 1,2,3,4 quadruple mutant*). Statistical significance was determined by one way ANOVA. Values are means (n= 31 to 119 roots) \pm S.E. Different letters indicate significant differences among means ($P<0.05$; Tukey's test).



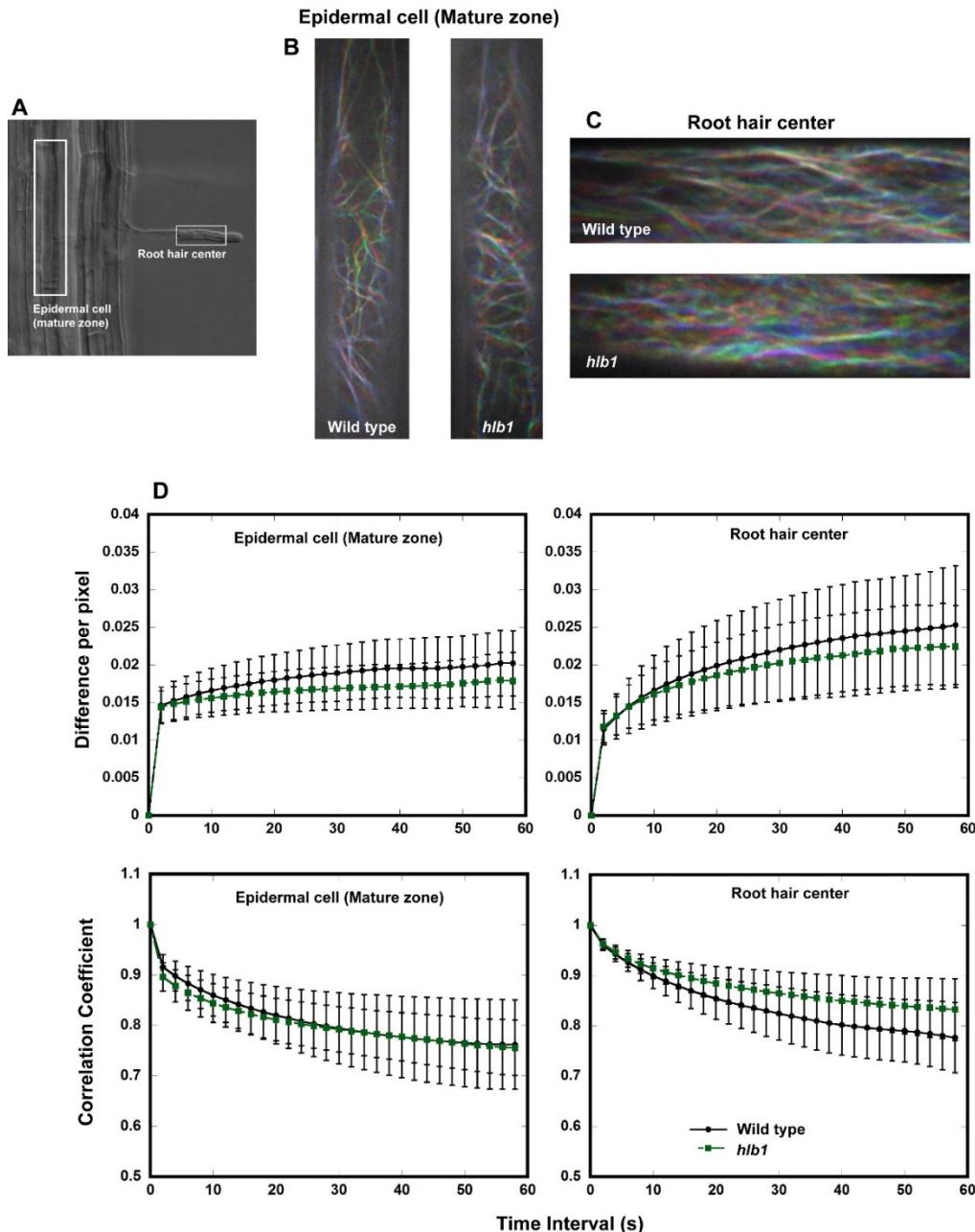
Supplemental Figure 5. Cell Division and Expansion of *hlb1* Roots is Strongly Inhibited by LatB.

(A) Primary roots of wild type and *hlb1* stained with propidium iodide and imaged with a confocal microscope 2 days after transplanting 3-d-old seedlings to growth media with and without (solvent controls) 50 nM LatB. The white vertical line at the center of each root indicates the length of the meristem and marks the region used for counting cortical cell number. Bar= 100 μ m

(B) Average cortical cell number from the area of the root indicated by the vertical lines shown in panel **A**. Statistical significance was determined by one way ANOVA. Means ($n = 16$ to 23 roots) \pm S.E. with different letters are significantly different ($P < 0.05$ by Tukey's test).

(C) and **(D)** High magnification confocal images of cortical cells in the root elongation zone showing differences in length between wild type and *hlb1*. White vertical line in **C** indicates how cell length numbers shown in panel **E** were obtained. Bar = 50 μ m.

(E) Average cortical cell length of wild type and *hlb1*. Statistical significance was determined by one way ANOVA. Means ($n = 16$ to 32) \pm S.E. with different letters are significantly different ($P < 0.05$ by Tukey's test).

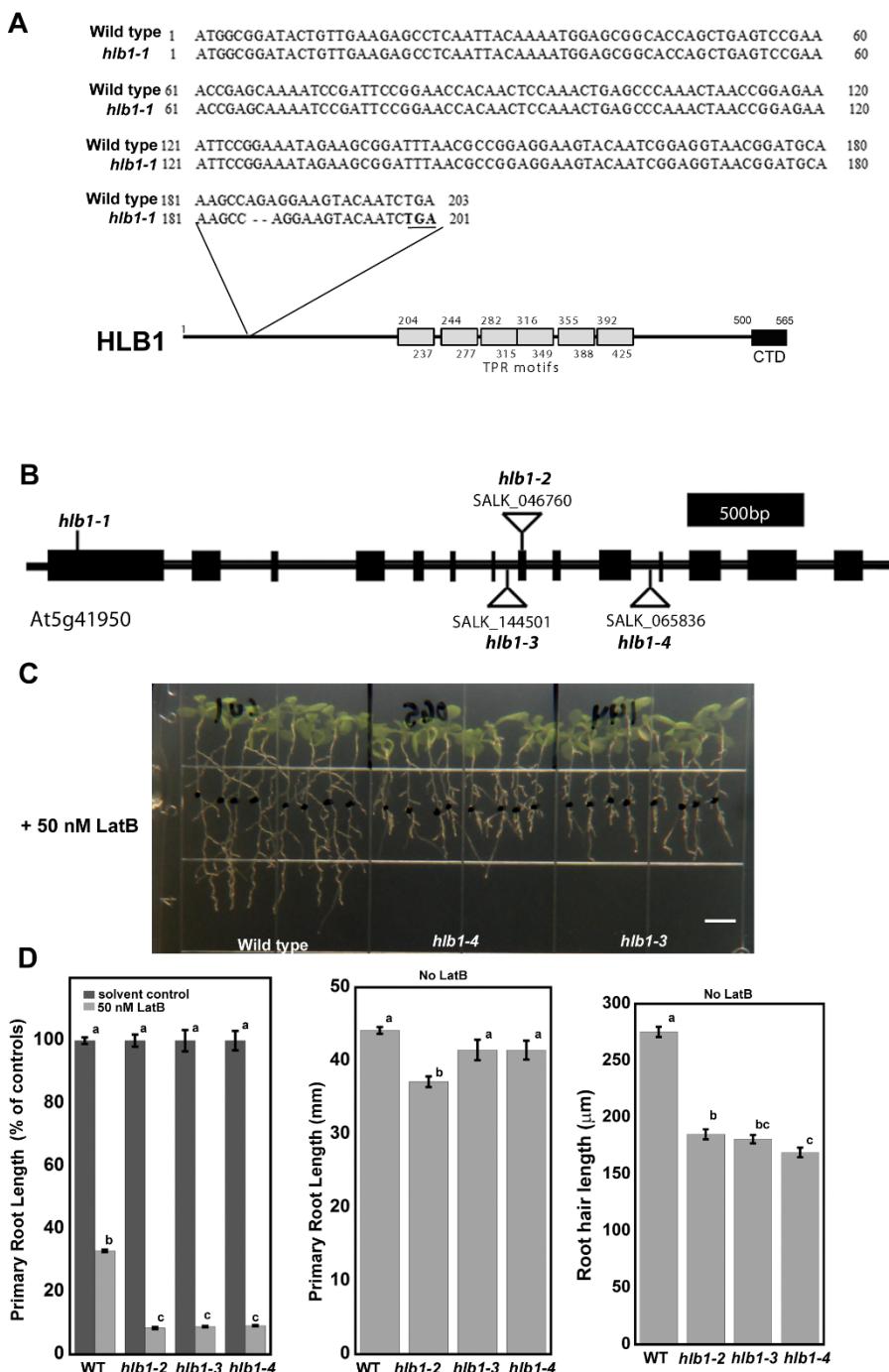


Supplemental Figure 6. Quantitative Analysis of Global F-actin Dynamics in Root Epidermal Cells and Root Hairs.

(A) Brightfield image of the root maturation zone. White rectangles indicate cells where time-lapse movies used for quantification of global F-actin dynamics were obtained.

(B) and **(C)** Representative images of three time points (20, 40 and 60 s) presented as separate color channels in red, green and blue (RGB) image. See Supplemental Movie 3 for a corresponding time-lapse sequence used to generate the RGB image for quantifying global F-actin dynamics.

(D) Quantification of global F-actin dynamics using change in pixel total difference per pixel (top graphs) and decay in correlation coefficient (bottom graphs) following the metrics of Vidali et al. (2010). Values are means ($n = 15$ time-lapse movie sequences) \pm S.D.



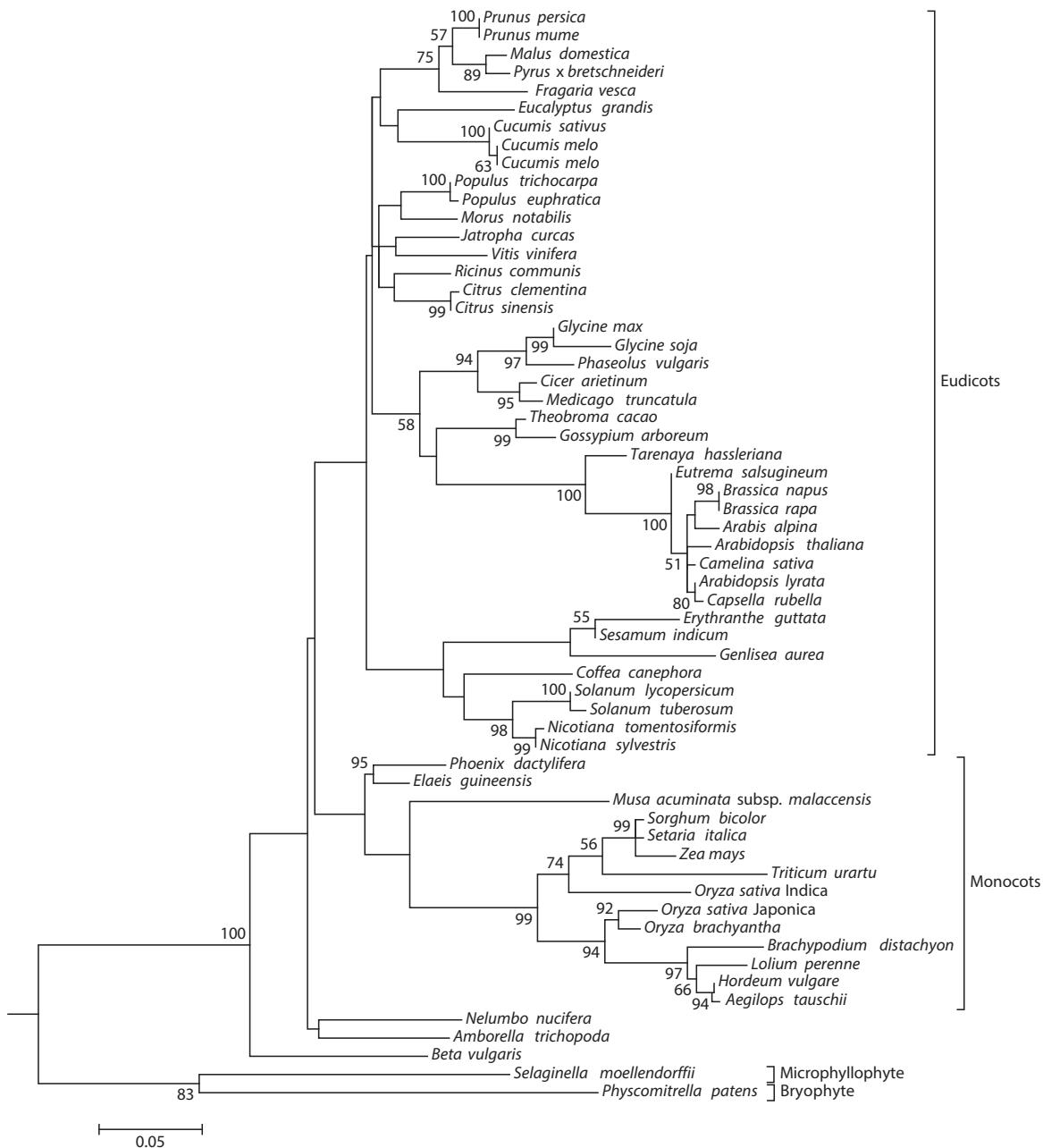
Supplemental Figure 7. Nature of *HLB1* Mutation and Characterization of Other *hbl1* Alleles.

(A) *hbl1-1* has a two base deletion in the first exon of the *HLB1* gene corresponding to amino acid 63. This results in a premature stop codon (underlined) before the six TPR motifs, potentially leading to a severely truncated protein.

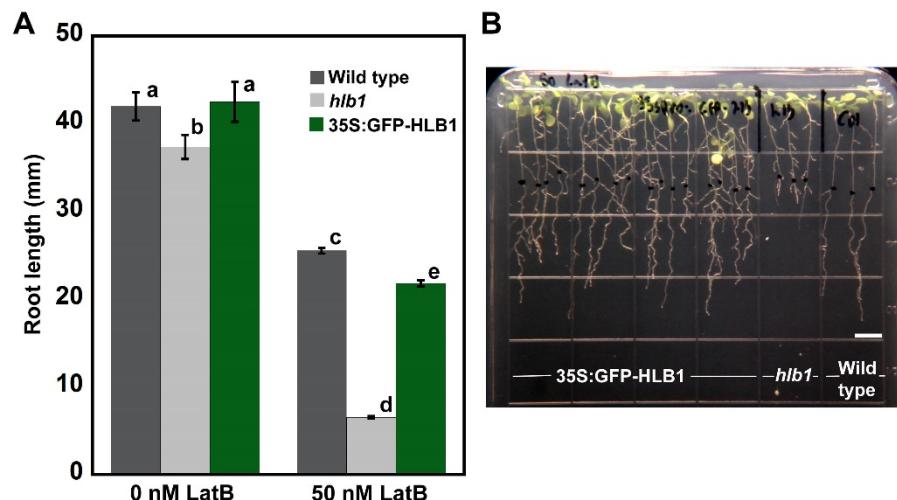
(B) Schematic diagram of *HLB1* gene structure. Boxes indicate exons. Position of the deletion site for the original *hbl1-1* mutant (the vertical line in the first exon) and T-DNA insertion sites for the other *hbl1* mutant alleles (triangles) are indicated.

(C) Representative images of seedlings of two *hlb1* mutant alleles (*hlb1-3* and *hlb1-4*) in comparison to wild type. 4-d-old seedlings were transplanted onto 50 nM LatB and images were taken 7 days after. Bar= 5 mm.

(D) Quantification of LatB hypersensitivity, primary root length and root hair length in 3 other *hlb1* mutant alleles. Note that all 3 mutant alleles show hypersensitivity to LatB and reduced root hair length in the absence of LatB. In regard to primary root length, only the *hlb1-2* (insertion in 2nd exon) shows shorter primary root length in the absence of LatB that is significantly different from wild type. The primary root lengths of the intronic insertional mutants (*hlb1-3* and *hlb1-4*) are not significantly different from that of wild type (WT). Statistical significance was determined by one way ANOVA. Values are means (n= 31 to 216 roots; 82 to 120 root hairs) ± S.E. Different letters indicate significant differences among means (P<0.05; Tukey's test).



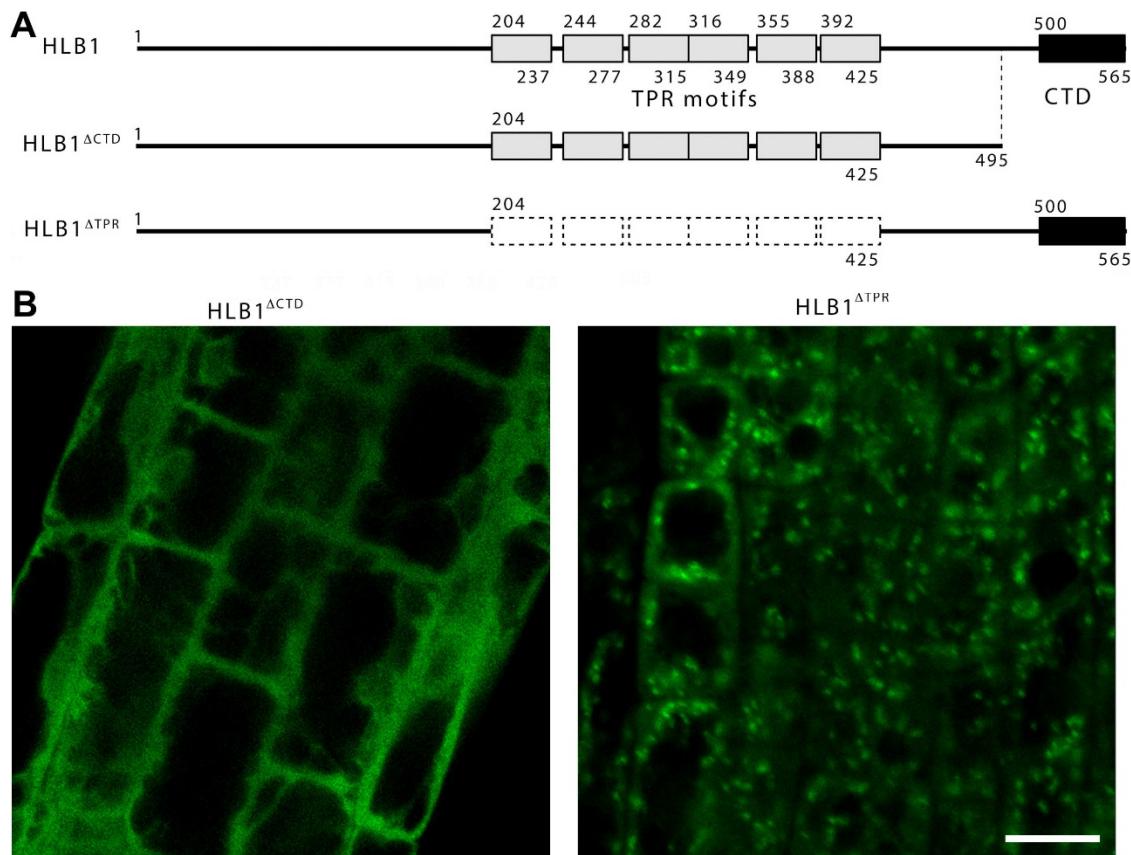
Supplemental Figure 8. Phylogenetic Analysis of HLB1 Sequences in Land Plants. The tree is rooted to four proteins from green algae and drawn to scale with branch lengths measured in the number of substitutions per site. Bootstrap values larger than 50 (1,000 trials) are indicated.



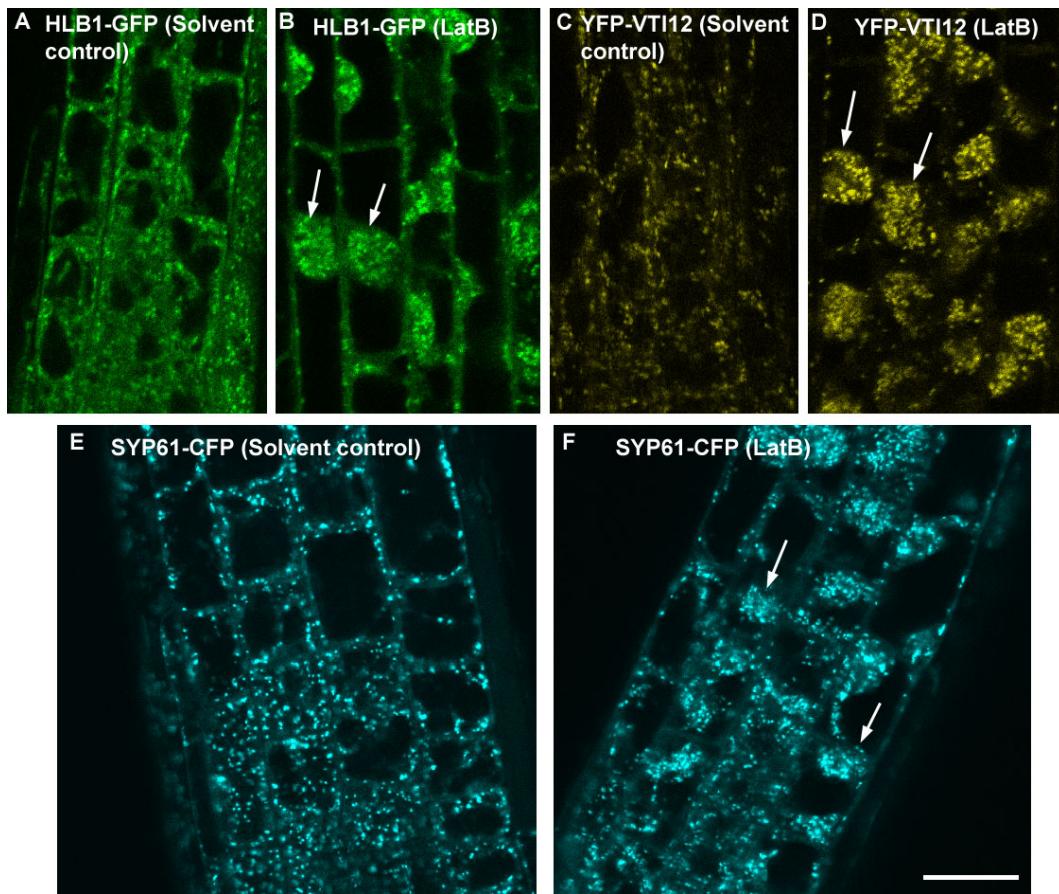
Supplemental Figure 9. *35Spro:GFP-HLB1* Complements the Hypersensitivity to LatB and Short Primary Root Phenotypes of *hlb1-1*.

(A) Quantification of primary root length among wild type, *hlb1* and *35Spro:HLB1-GFP* complemented lines with and without 50 nM LatB. Statistical significance was determined by one way ANOVA. Values are means ($n = 30$ roots) \pm S.E. Different letters indicate significant differences among means ($P < 0.05$; Tukey's test).

(B) Wild type, *hlb1* and *35Spro:GFP-HLB1* complemented lines transplanted onto medium supplemented with 50 nM LatB. 4-d-old seedlings were transplanted on LatB and images were taken 7 days later. Bar= 5 mm.



Supplemental Figure 10. The C-terminal domain targets HLB1 to the TGN/EE.
(A) Schematic diagram of HLB1^{ΔCTD} and HLB1^{ΔTPR} compared to full length HLB1.
(B) HLB1^{ΔTPR}-GFP fusion protein localizes to both the cytoplasm and TGN/EE foci, while HLB1^{ΔCTD} localizes exclusively to the cytoplasm. Bar= 20 μ m.



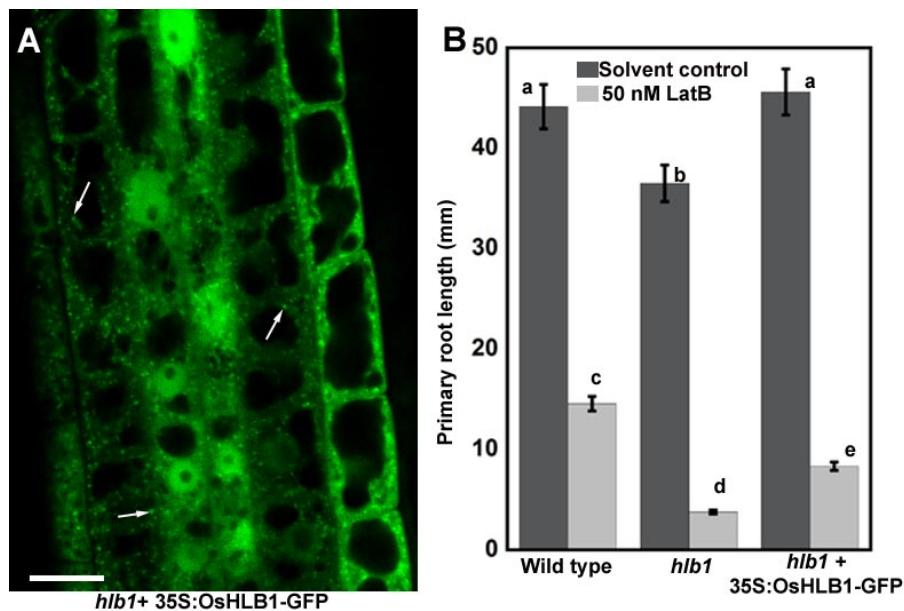
Supplemental Figure 11. HLB1-GFP and the TGN/EE markers (YFP-VTI12 and SYP61-CFP) in the root elongation zone of wild-type seedlings form intracellular agglomerates (arrows) within 10 minutes of exposure to LatB.

(A) and (B) HLB1-GFP in solvent control and 50 nM LatB.

(C) and (D) YFP-VTI12 in solvent control and 50 nM LatB.

(E) and (F) SYP61-CFP in solvent control and 50 nM LatB.

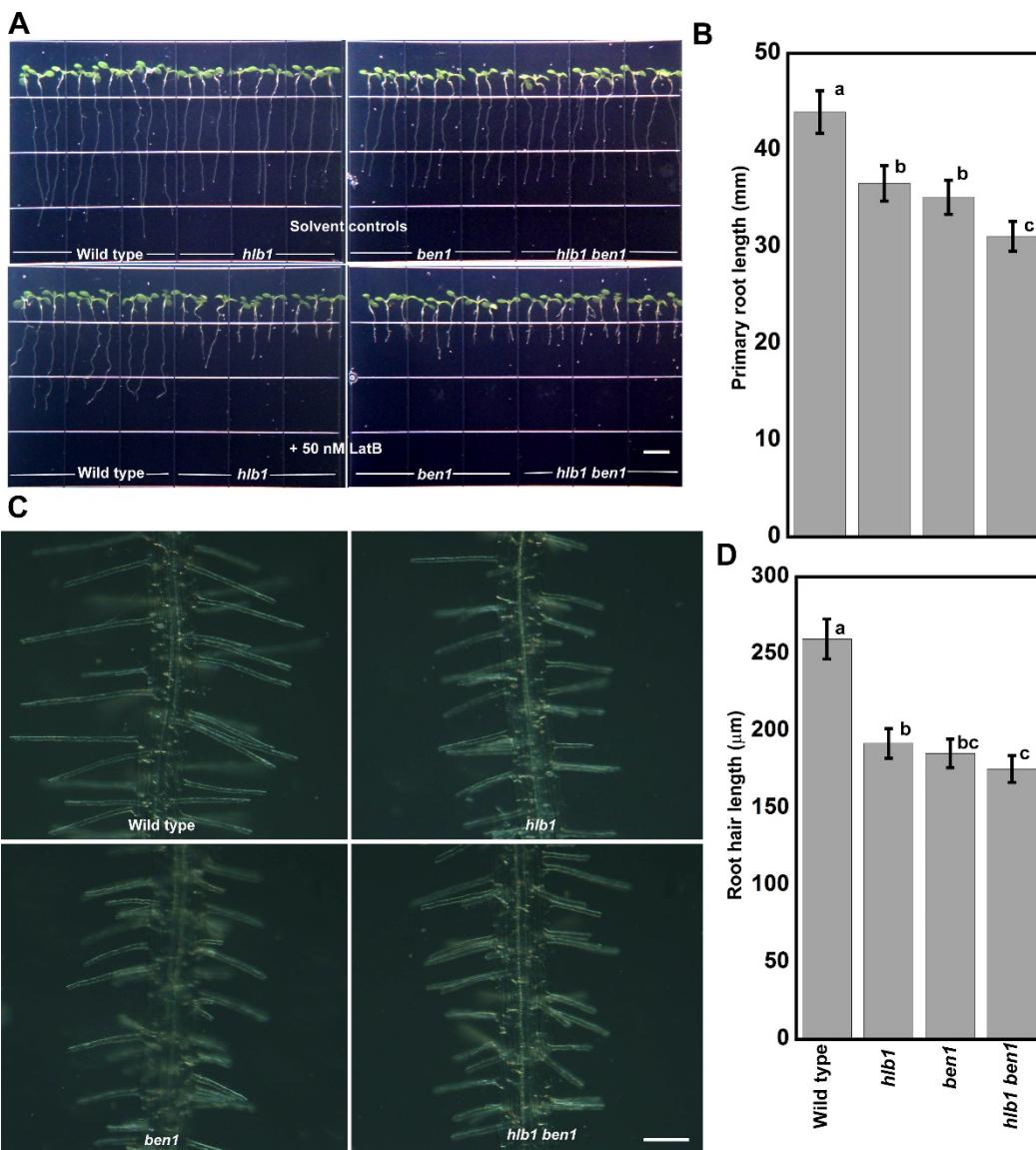
Bars = 20 μ m.



Supplemental Figure 12. *Oryza sativa HLB1* Partially Complements the Arabidopsis *hlb1* Mutant.

(A) OsHLB1-GFP localizes to the cytoplasm and decorates similar punctate bodies (arrows) as Arabidopsis HLB1-GFP. Bar= 20 μ m.

(B) 35S_{pro}:OsHLB1-GFP fully complements the primary root growth defects and partially complements the LatB hypersensitivity phenotype of *hlb1-1*. Statistical significance was determined by one way ANOVA. Values are means ($n= 60$ to 226 roots) \pm S.E. Different letters indicate significant differences among means ($P<0.05$; Tukey's test).

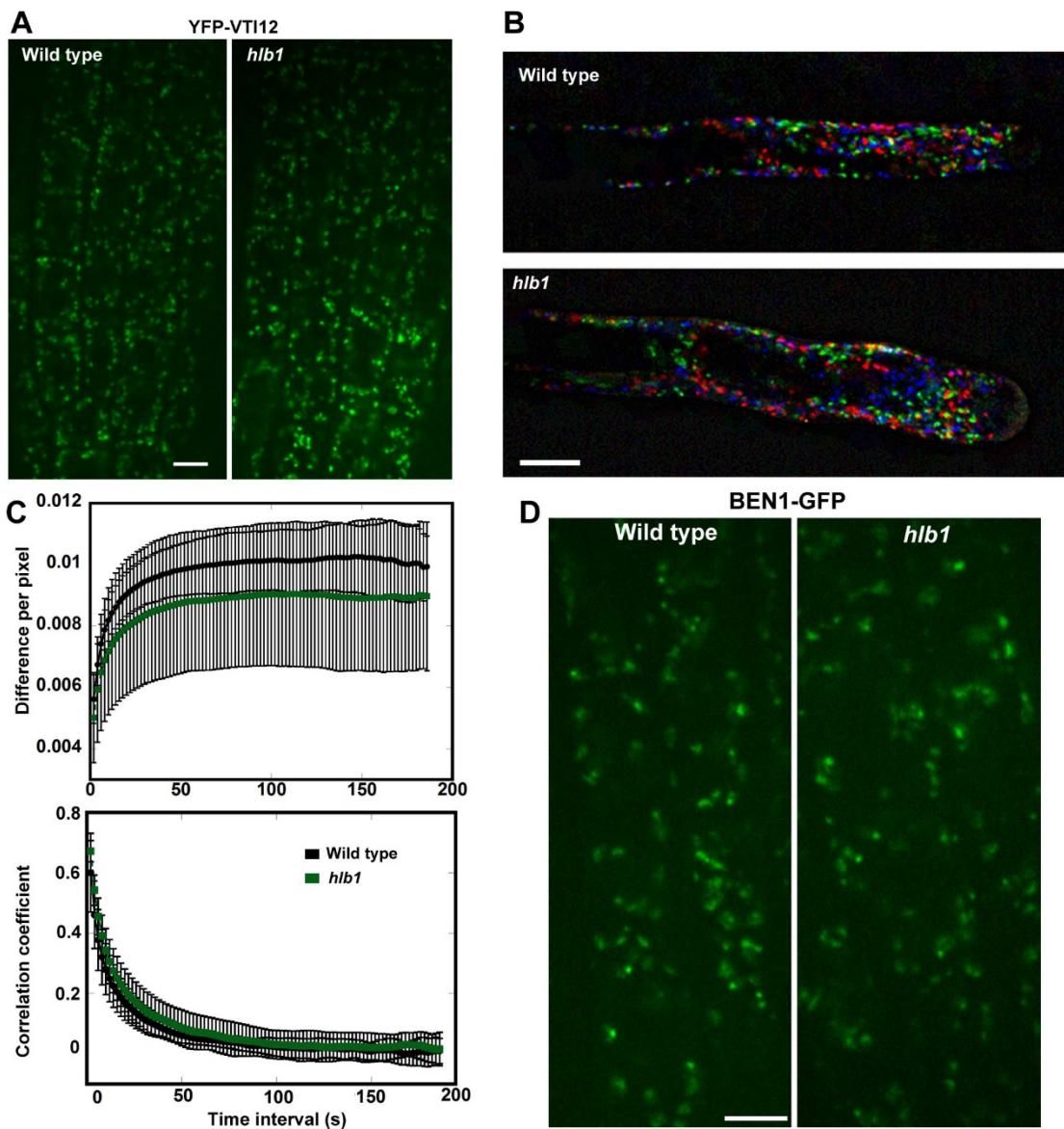


Supplemental Figure 13. HLB1 Functions in Similar Genetic Pathways as BEN1.

(A) Seedlings of wild type and *hlb1* transplanted onto plates with or without 50 nM LatB (solvent controls). Images were taken 4 d after transplant. Note that *hlb1*, *ben1* and *hlb1 ben1* exhibit similar hypersensitive responses of their primary roots to LatB. Bar = 5 mm.

(B) Average primary root length of 7-d-old wild-type, *hlb1*, *ben1* and *hlb1 ben1* seedlings in the absence of LatB. Statistical significance was determined by one way ANOVA. Values are means ($n > 25$ roots) \pm S.E. Different letters indicate significant differences among means ($P < 0.05$; Tukey's test).

(C) and (D) Root hairs of *hlb1*, *ben1* and *hlb1 ben1* are shorter than those of wild type in LatB-free medium. Statistical significance was determined by one way ANOVA. Values are means ($n = 103$ to 137 root hairs) \pm S.E. Different letters indicate significant differences among means ($P < 0.05$; Tukey's test). Bar = 20 μ m.



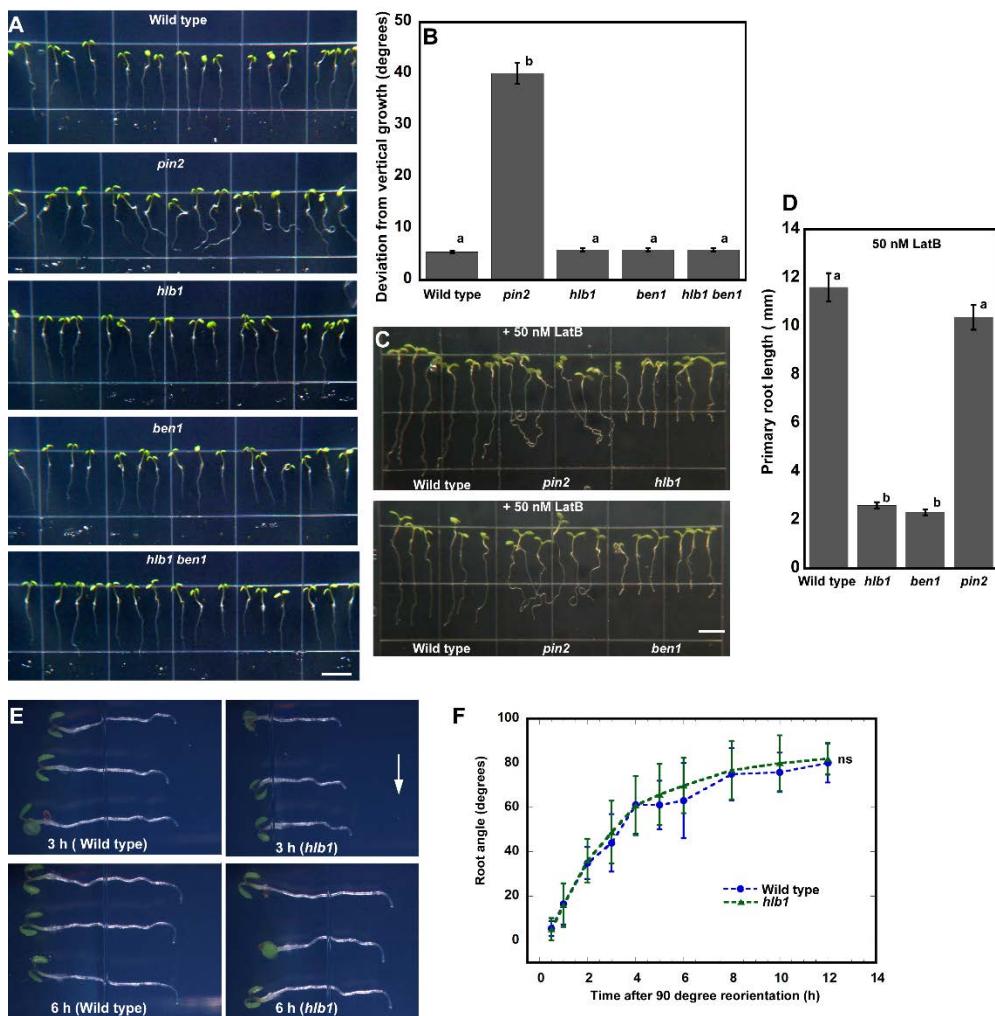
Supplemental Figure 14. Global Organization and Dynamics of YFP-VTI12 and MIN7/BEN1-GFP in *hlb1*.

(A) YFP-VTI12 localization in peripheral cap cells. Bar= 5 μ m.

(B) Representative images of three time points (20, 40 and 60 s) of growing root hairs presented as separate color channels in red, green and blue (RGB) image. See Supplemental Movie 12 for the corresponding time-lapse sequences used to generate the RGB image for quantifying global YFP-VTI12 dynamics. Bar= 10 μ m.

(C) Quantification of global YFP-VTI12 dynamics in root hairs using change in pixel total difference per pixel (top graph) and decay in correlation coefficient (bottom graph) following the metrics of Vidali et al. (2010). Values are means ($n = 10$ time-lapse movie sequences) \pm S.D.

(D) MIN7/BEN1-GFP localization in peripheral cap cells. Bar= 5 μ m.



Supplemental Figure 15. Quantification of Root Growth Orientation and Gravitropism in *hlb1* Seedlings.

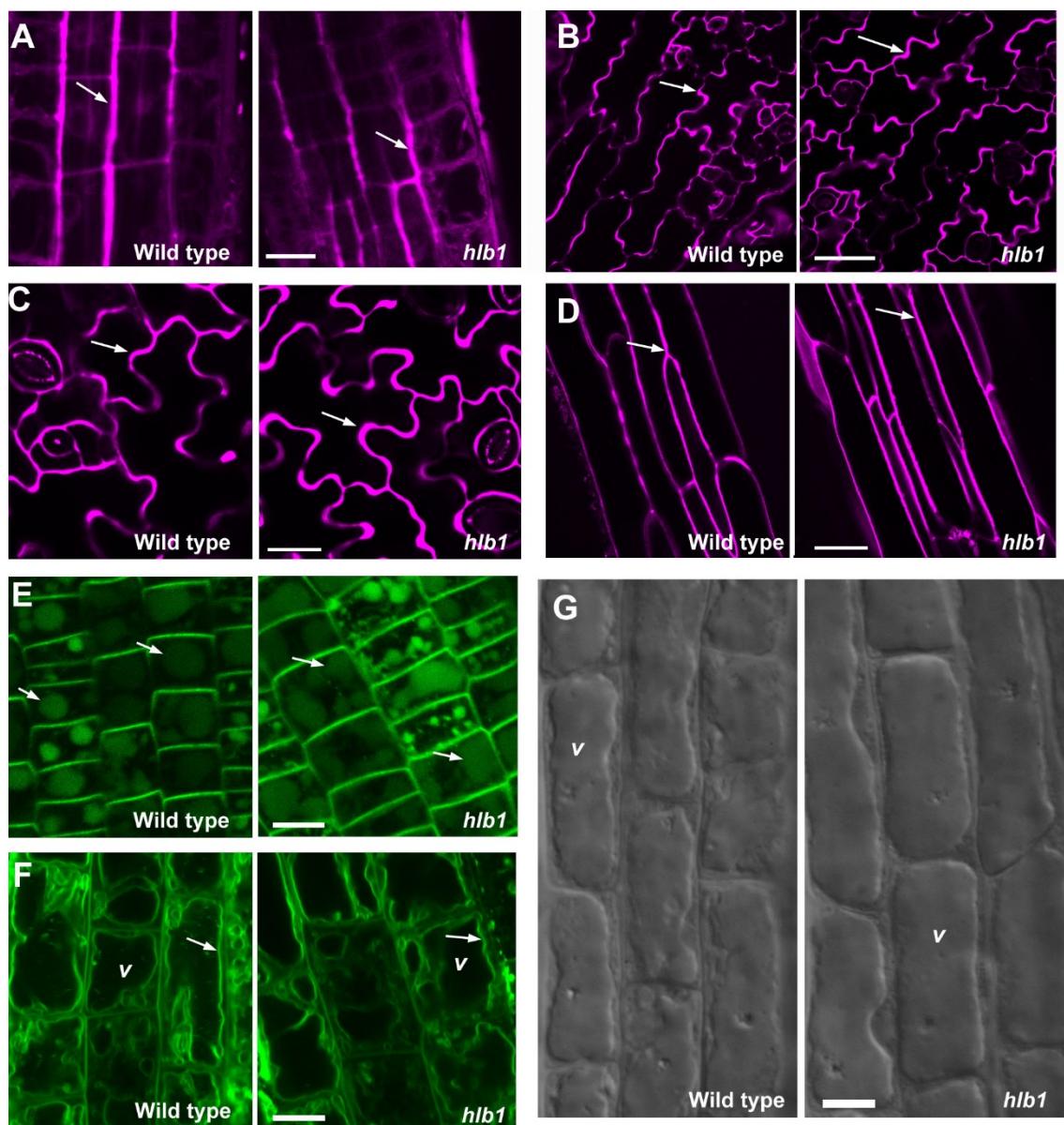
(A) Roots of 5-d-old seedlings of wild type, *hlb1*, *ben1* and *hlb1 ben1* maintain straight growth when kept vertical on agar plates while roots of *pin2* mutants show distinct deviation from the vertical. Bar = 5 mm.

(B) Quantification of deviation from vertical root growth. Statistical significance was determined by one way ANOVA. Values are means ($n = 16$ roots) \pm S.E. Different letters indicate significant differences among means ($P < 0.05$; Tukey's test).

(C) and (D) Primary root growth of *pin2* shows wild-type sensitivity to 50 nM LatB. Statistical significance was determined by one way ANOVA. Values are means ($n=18\text{-}30$ roots) \pm S.E. Different letters indicate significant differences among means ($P < 0.05$; Tukey's test). Bar in C = 5 mm.

(E) Representative images of 4-d-old wild-type and *hlb1* seedlings at two time points after a 90 degree reorientation. White arrow indicates the direction of gravity. Note that at 3 and 6 h after reorientation, roots of both genotypes redirect their growth downward.

(F) Time course of root gravitropism in 4-d-old wild-type and *hlb1* seedlings. Values are means of 24 - 60 roots \pm S.D. from two independent experiments (*t*-test, n.s., not significant).



Supplemental Figure 16. *hlb1* Has Mild Defects in Bulk Protein Secretion and Trafficking to the Vacuole.

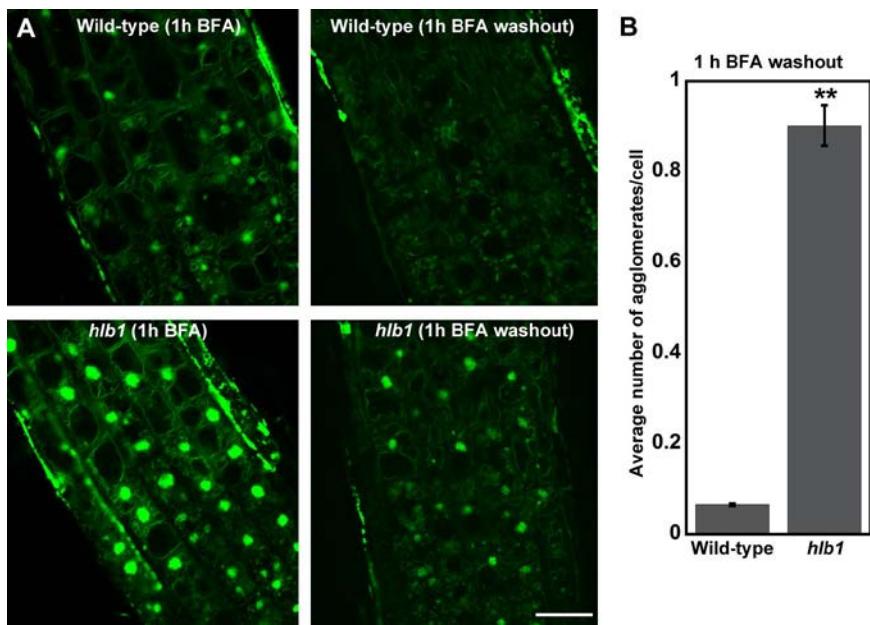
(A) to **(B)** SEC-RFP (arrows) localizes to the apoplast in roots **(A)** and cotyledon pavement cells **(B)** of 5-d-old wild-type and *hlb1* seedlings. Bar in **(A)** = 20 μ m; **(B)** = 50 μ m.

(C) to **(D)** SEC-RFP (arrows) localizes to the apoplast in epidermal pavement cells of the first true leaves **(C)** and mid-vein **(B)** of 14-d-old wild-type and *hlb1* seedlings. Bar = 20 μ m.

(E) A 12 h dark treatment causes PIN2-GFP to accumulate into lytic vacuoles (arrows) of wild type and *hlb1* roots. Bar = 10 μ m.

(F) FM1-43 labels the tonoplast (arrows) in wild-type and *hlb1* root cells. Bar = 20 μ m.

(G) Differential interference contrast microscopy of peripheral root cap cells reveals no dramatic differences in vacuole (v) morphology between wild type and *hlb1*. Bar = 10 μ m.



Supplemental Figure 17. FM1-43 Agglomerates Persist in *hlb1* after BFA Washout. **(A)** Representative images of FM1-43 agglomerates in wild-type and *hlb1* root cells after treatment with 50 μ M BFA for 1 h and 1 h after BFA washout. Note that agglomerates are still visible in many cells of *hlb1* after BFA washout. Bar= 20 μ m. **(B)** Quantification of FM1-43 agglomerates in wild type and *hlb1* after BFA washout. Statistical significance was determined by one way ANOVA. Values are means (n = 145 to 183 cells) \pm S.E. from at least 20 independent seedlings (t -test, $P<0.001^{**}$).