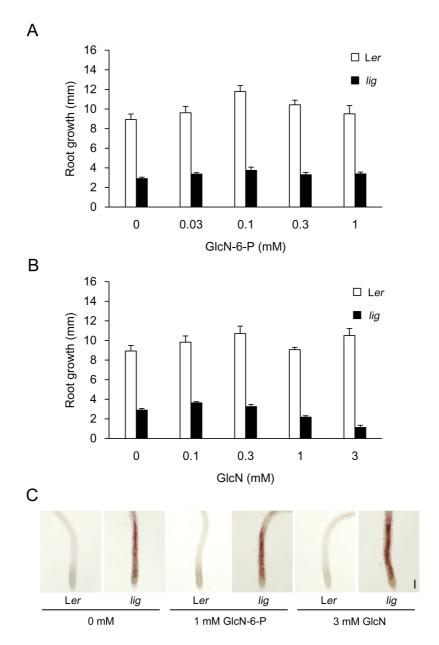


Supplemental Figure 1. Anion Exchange HPLC Elution Profiles for UDP-GlcNAc and UDP-GalNAc Analyses in the *lig* Mutant and Wild-type Seedlings.

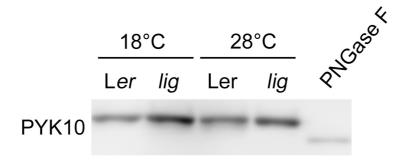
Nucleotide sugars were extracted from the roots of seedlings of the wild type (Ler) and *lig* mutant grown for 5 d at 18°C and then for 3 d at 18°C or 28°C. The eluate was monitored at 254 nm. Arrows indicate cytidine-5'-monophospho-*N*-acetyl-D-neuraminic acid as an internal standard (IS), UDP-GlcNAc, and UDP-GalNAc.



Supplemental Figure 2. Effects of Treatment with Nonacetylated Glucosamine or Glucosamine-6-Phosphate on Root Growth and Ectopic Lignin Deposition in the *lig* Mutant.

Seedlings were cultured on GMA containing various concentrations of glucosamine-6-phosphate (GlcN-6-P) or glucosamine (GlcN) for 3 d at 28°C after 5 d of culture at 18°C.

- (A) (B) Growth of primary roots during 3 d of culture at 28°C was measured. Vertical bars represent SE values for seven to 16 seedlings.
- (C) Primary root tips of the wild-type (Ler) and lig seedlings stained with phloroglucinol-HCl for detection of lignin. Scale bar represents 100 μ m.



Supplemental Figure 3. Immunoblot Analysis with the Anti-PYK10 Antibody.

Proteins were extracted from the roots of seedlings of the wild type (Ler) and lig mutant grown for 6 d at 18°C and then for 3 d at 18°C or 28°C. A portion of the protein sample prepared from the wild type (Ler, 28°C) was incubated in the presence of PNGase F for digestion of N-glycans before SDS-PAGE. Each lane contains proteins equivalent to the extract from 2 mg (fresh weight) of root tissues.

Supplemental Table 1. Segregation of the Adventitious Root Phenotype in the Progeny of the Mutant Line Backcrossed with the Wild-Type Ler.

| Plant line | Number of explants that formed adventitious roots normally at 28°C / Total |
|----------------------------------|--|
| | number of explants examined |
| Ler | 16 / 16 |
| b2337 ^a | 0 / 12 |
| BC_2-1^b | 22 / 32° |
| BC_2-2^b | 23 / 32 ^c |
| BC_2-3^b | 32 / 32 |
| BC_2-4^b | 25 / 27 |
| BC_2-4^b BC_2-5^b BC_2-6^b | 28 / 28 |
| BC_2-6^b | 26 / 32° |

Hypocotyl explants were cultured on RIM at 28°C to examine the temperature sensitivity of adventitious root formation.

^a The original line of *lig*.

^b The b2337 (original *lig*) line was backcrossed two times with the wild-type Ler and the resultant BC₂ plants were allowed to self-pollinate. Seeds were collected separately from each individual BC₂ plant and subjected to examination of the adventitious rooting phenotype.

^c Not significantly different from the ratio of three normal to one temperature-sensitive (P > 0.1, by X^2 test). In these cases, the parental BC₂ plants were assumed to be heterozygous for a single, recessive mutation responsible for the temperature sensitivity of adventitious root formation.

| Supplemental Table 2. Primers Used in RT-PCR Analysis. | | | |
|--|------------------------------|--------|--|
| Primer Name | Sequence | Cycles | |
| PAL1-F | 5'-CACGAGATTGGCGATAGCAG | 26 | |
| PAL1-R | 5'-TCCGTTATCGTAGGCTGCTC | | |
| CCR1-F | 5'-TTGTTGAGATTCTGGCTAAGCTA | 26 | |
| CCR1-R | 5'-TGAAGACTTGACTACAAAATCCATC | | |
| CCR2-F | 5'-TGTTGAGATTCTGGCCAAATTC | 28 | |
| CCR2-R | 5'-ATAAAACCATTGCTTCCATTATCG | | |
| CAD-C-F | 5'-GCACAGGAGCAGATGATG | 26 | |
| CAD-C-R | 5'-CGCCATTAGACCGAAGTG | | |
| CAD-D-F | 5'-GGGGACATAGTTGGAGTTGGT | 26 | |
| CAD-D-R | 5'-GCTCCCCGTTATCACTTTCCT | | |
| UBQ-F | 5'-TAAAAACTTTCTCTCAATTCTCTCT | 24 | |
| UBQ-R | 5'-CAAGAGTTCTGCCATCCTC | | |
| BIP3-F | 5'-AAGGCGAAGAGCAGAAACTG | 28 | |
| BIP3-R | 5'-CCCGTTGGCTCATTGATT | | |
| ACT2-F | 5'-TTAACTCCCGCTATGTATGTC | 24 | |
| ACT2-R | 5'-TTCCATTCCCACAAACGAG | | |