SUPPLEMENTAL DATA

Figure S1

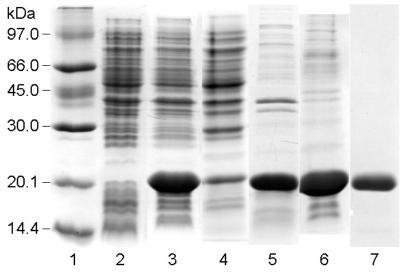
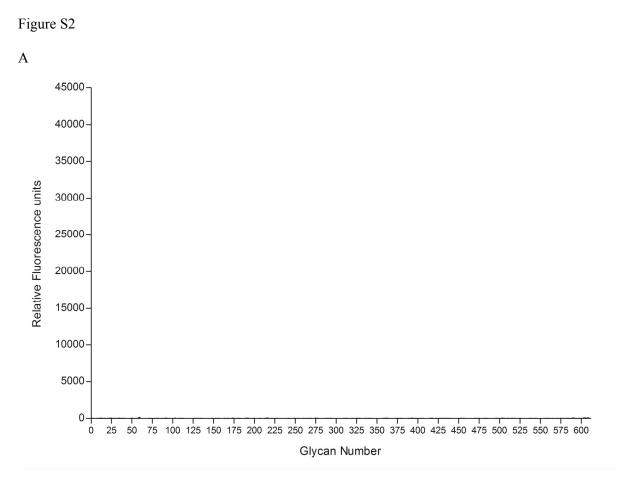
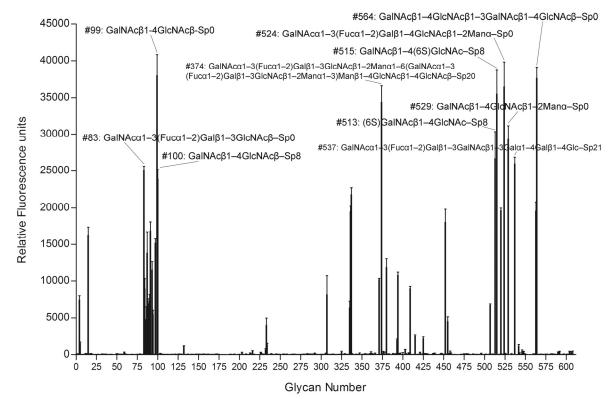


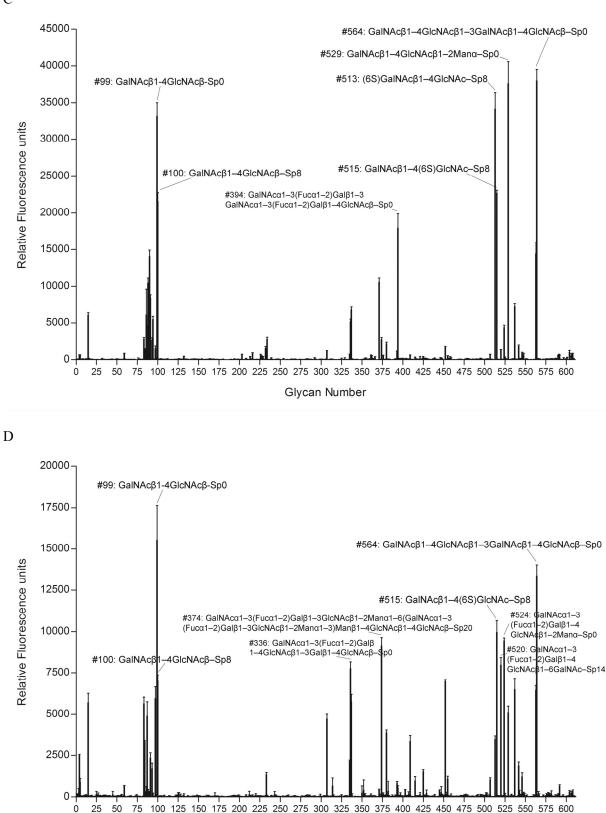
FIGURE S1. **SDS-PAGE analysis of expression at 37°C and purification of recombinant CNL.** *Lane 1*, low molecular weight standard proteins; crude cell extract of *E. coli* BL21(DE3) containing pET11a::rCNL before (*lane 2*) and after (*lane 3*) induction; *lane 4*, soluble fraction of cell lysate; *lane 5*, soluble fraction after 1 M urea solubilization; *lane 6*, soluble fraction after 3 M urea solubilization; and *lane 7*, purified rCNL by gel filtration and lactose-affinity chromatography.



В



С



Glycan Number

FIGURE S2. **Glycan-binding specificity of CNL mutants.** Glycan microarray analyses of a nonsugar-binding mutant 0-CNL with a concentration of 200 μ g/ml (*A*), and non-dimerizing monovalent mutants, Mono1-CNL (100 μ g/ml; *B*), Mono2R-CNL (200 μ g/ml; *C*), and Mono2W-CNL (200 μ g/ml; *D*), were performed using Mammalian Printed Array version 5.0 of the Consortium for Functional

Glycomics. *Bars* represent relative fluorescence units indicating the specificity of a mutant for individual glycans and *error bars* represent standard deviations of the mean fluorescence. The high-affinity glycans with designated numbers on the glycan microarray are presented.

Pohleven et al., Recombinant LacdiNAc-specific Clitocybe nebularis Lectin, Supplemental Data, p. 5

MW rCNL 0-CNL Mono2R-CNL kDa 100-70-55-35-25-15-2 2 2 1 1 1

FIGURE S3. **SDS-PAGE analysis of rCNL and 0-CNL and Mono2R-CNL mutants expressed in** *Escherichia coli* **BL21(DE3) strain overnight at 23°C.** *Lanes* indicated by *1* represent whole cell extracts, and *2*, soluble fractions of the crude *E. coli* extract. *MW* indicates a prestained standard protein ladder (Fermentas).

Figure S3

Figure S4

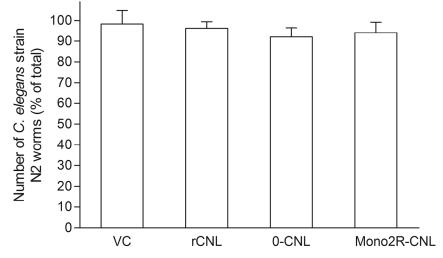


FIGURE S4. Biotoxicity assay of rCNL and mutants on wild-type N2 *C. elegans* nematodes. *Bars* represent means of percentage of total numbers of larval stage L4 or adult animals after 48 h at 20°C in five replicates and *error bars* represent standard deviations. *VC* stands for vector control – *E. coli* containing empty vector. Groups of treated larvae or adults were compared pairwise with vector controls using the two-sample *t*-test. No statistically significant differences were found.