Supporting Information

Bulmer et al. 10.1073/pnas.0904063106

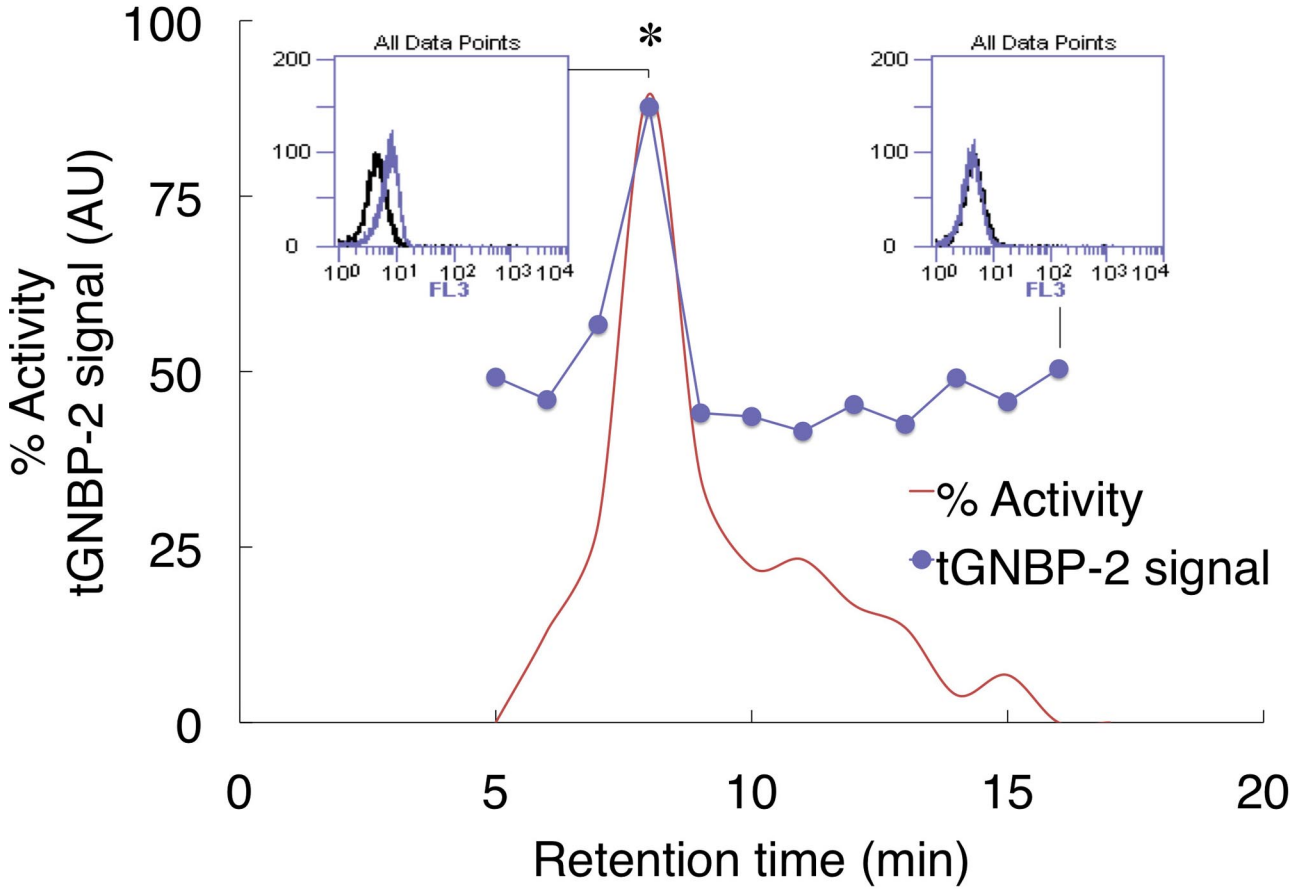

Fig. S1. tGNBP-2 coincides with β(1,3)-glucanase activity in termites, as measured by flow cytometry (AU = 10 $\times$ [mean fluorescent intensity]; *, $P < 0.05$ vs. adjacent fractions). For this assay, HPLC fractions were adsorbed on 3.0-μm-diameter polystyrene microspheres by a 3-h incubation in boric acid (0.1 M, pH 8.5), followed by 3 washes in cold PBS; the microspheres were stained using anti-tGNBP-2 as described and analyzed by flow cytometry. (*Insets*) Representative histograms used to calculate signal intensities. Activity curve is the same curve used in Fig. 1*D*.

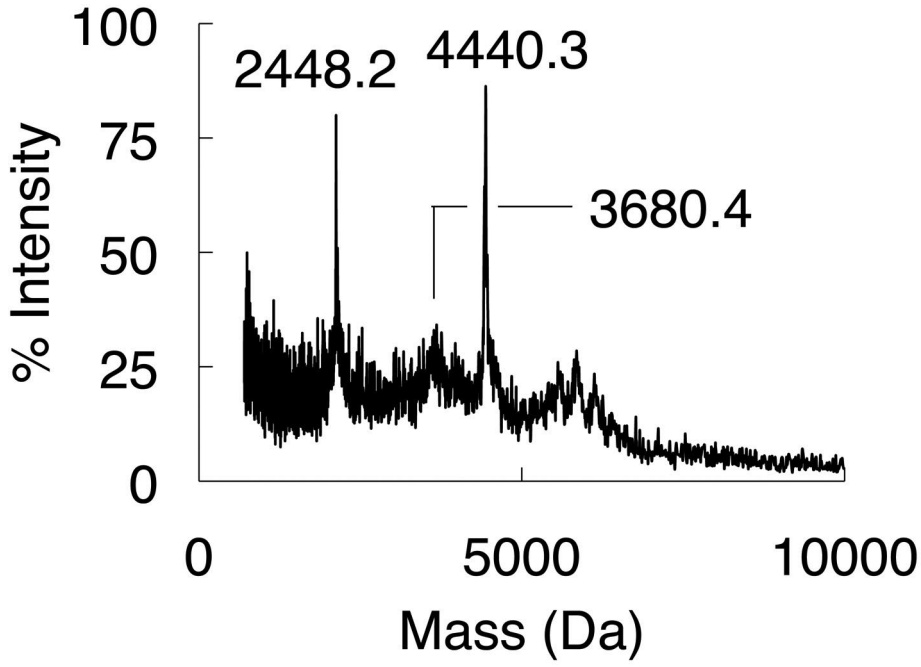

Fig. S2. MALDI-MS analysis of the termite fraction marked by an asterisk in Fig. 2*I*, showing prominent peaks highly correlating with masses for previously reported antimicrobial peptides (masses in daltons). For this analysis, termite extract was diluted with ultrapure water and analyzed on a Voyager DE MALDI mass spectrometer (Applied Biosystems) in positive linear mode with α-cyano-4-hydroxycinnamic acid (CHCA) as the matrix (30% 10 mg/mL CHCA, 70% acetonitrile), accelerating voltage 20 kV, grid voltage 95%, guide wire 0.05%, extraction delay 75 ns.

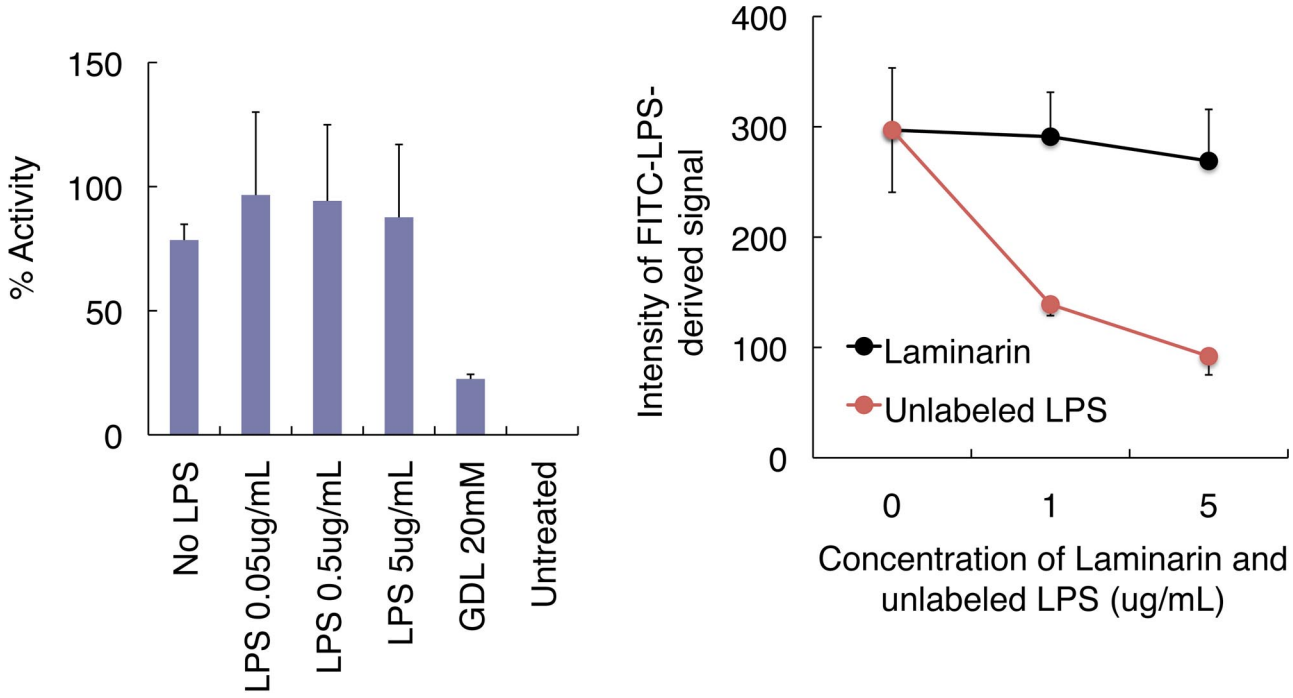

Fig. S3. Binding of LPS and catalysis of β(1,3)-D-glucans do not cross-interfere with each other. The activity of tGNBP-2 in the presence of 0.05–5 μg/mL LPS was measured by flow cytometry (Fig. S3). Binding of FITC-labeled LPS (FITC-LPS; Sigma) to matrix-immobilized tGNBP-2 was measured in the presence of either laminarin or unlabeled LPS and measured by flow cytometry. For this assay, tGNBP-2-adsorbed beads were incubated with either LPS or laminarin at various concentrations, then directly stained with FITC-labeled LPS (Sigma) and analyzed by flow cytometry.

Fig. S4. Representative dead termites following 4-day postmortem incubation.

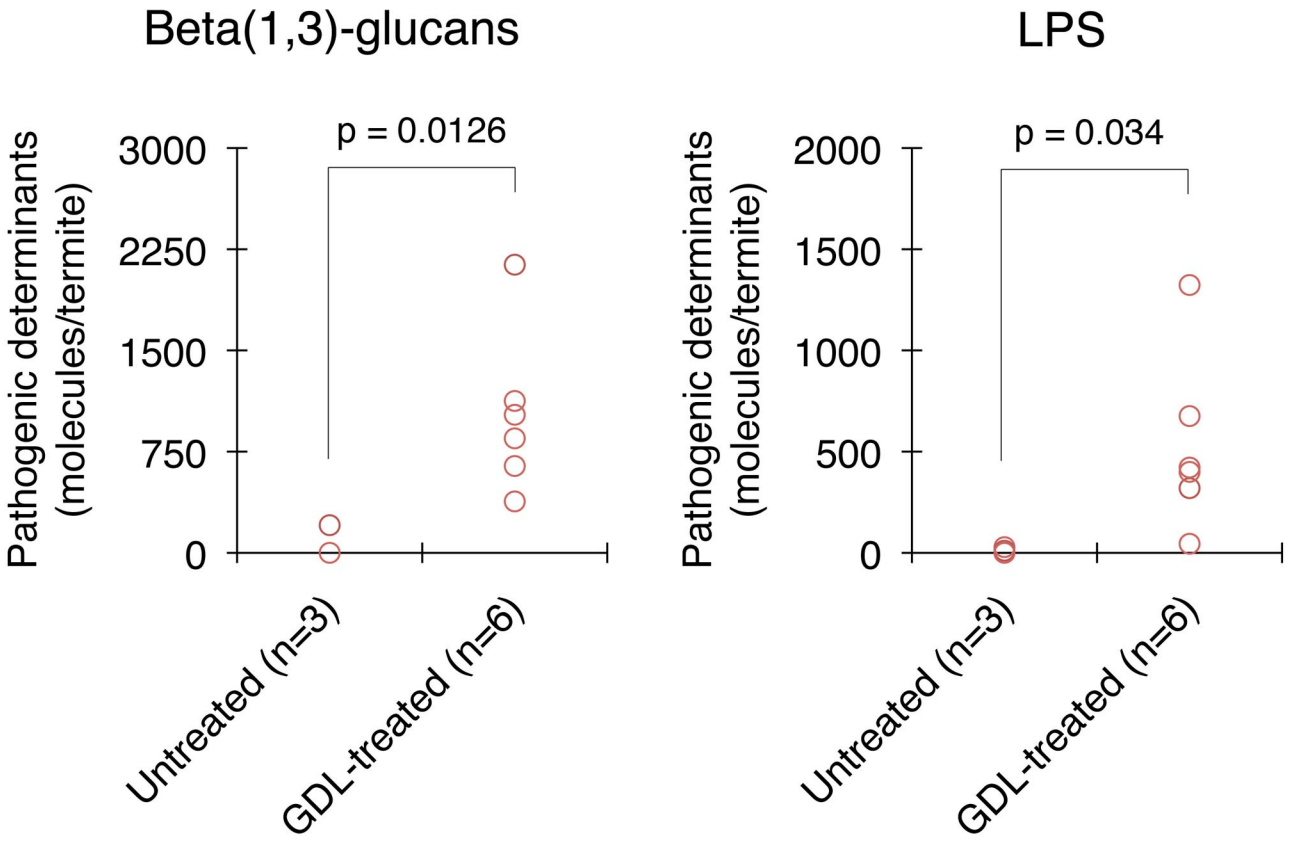

Fig. S5. Postmortem quantification of pathogenic determinants in dead, GDL-treated termites. Dead termites were extracted in 50 mM borate buffer (pH 8.6) and mounted on 3.0-μm-diameter flow cytometry matrix. Recombinant human CD14 (R&D Systems) and human Dectin-1 (as a murine Fc fusion protein; Alexis) were labeled with FITC (3.77 mol FITC/mol rhCD14, 1.44 mol FITC/mol mFc-hDectin-1; Pierce) and used to detect Gram-negative bacteria-derived LPS and fungal β(1,3)-glucans, respectively. MESF calibration standards (PolySciences) were used to quantify signal intensities. Untreated termites, $n = 3$. GDL-treated termites, $n = 6$. *P* values are stated in the graph.

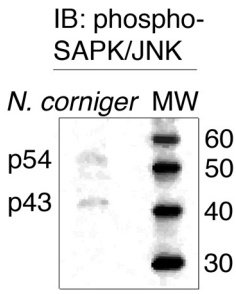

Fig. S6. Cross-reactivity of anti-phospho SAPK/JNK antibodies with termites as shown by Western blot. Termite extract was run by SDS/PAGE and blotted vs. anti-phospho SAPK/JNK (at 1:1,000), demonstrating p43/p54 proteins.

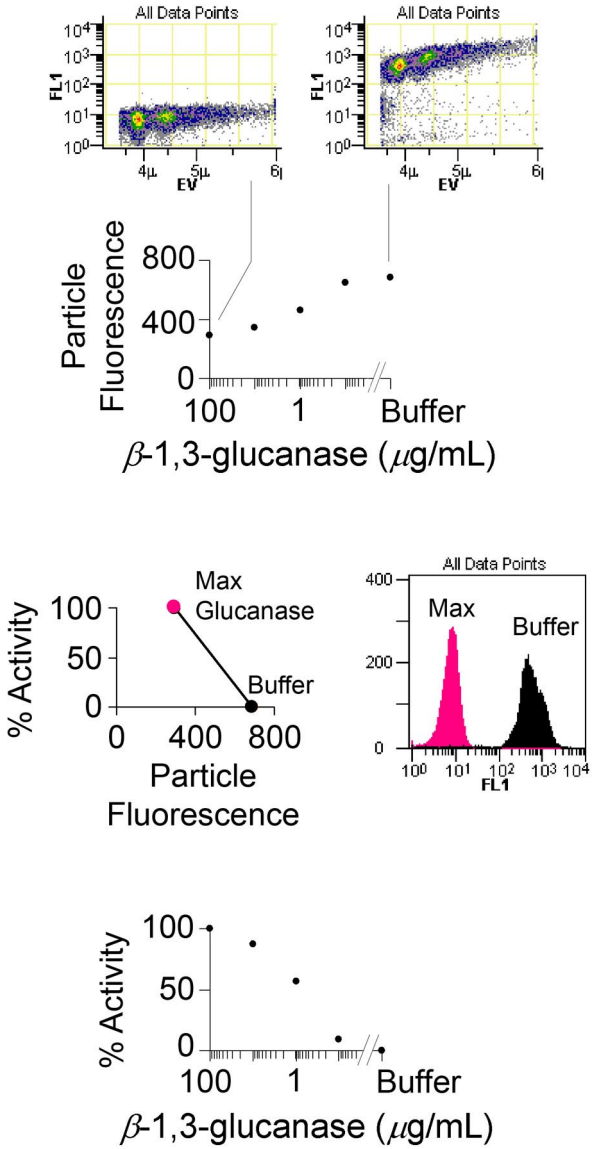

Fig. S7. Microsphere-based flow cytometric assay for β(1,3)-glucanase activity. Laminarin was labeled at the reducing end with rhodamine green X-succinyl ester as described, stably adsorbed on 3.0-μm-diameter polystyrene microspheres in carbonate/bicarbonate buffer at pH 9.6, and washed in 100 mM sodium acetate pH 5.5. Samples were then incubated with the fluorescent microspheres for varying time periods starting from 15 min and analyzed by flow cytometry. Purified β(1,3)-glucanase at 100 μg/mL (0.1 milliunits/mL) and sodium acetate buffer alone were used as positive and negative controls, respectively. Finally, fluorescence data are transformed to percentage activity and plotted vs. samples or concentrations.

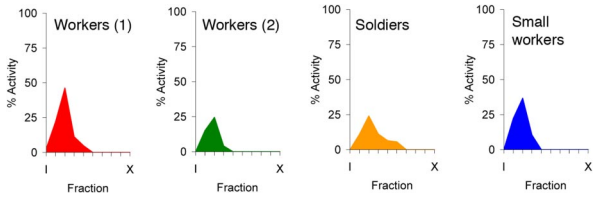

Fig. S8. β(1,3)-glucanase activity in termites does not vary among castes. Termites of different castes (workers, soldiers, small workers) were extracted and separated by HPLC as described in *Size Exclusion HPLC* (*Methods*). β(1,3)-glucanase activity was measured by flow cytometry as described in *β(1,3)-Glucanase Assays* (*Methods*).

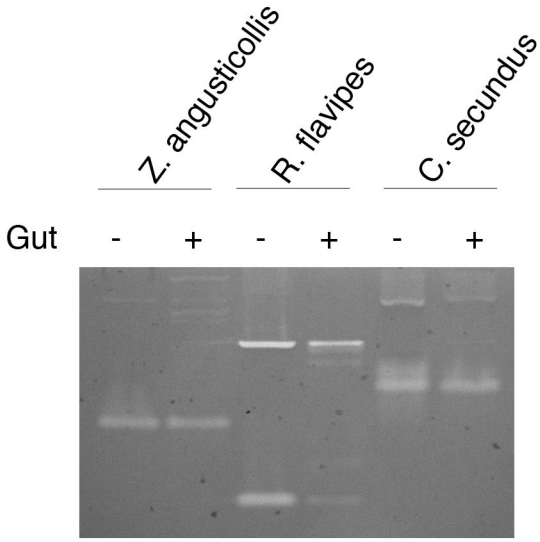

Fig. S9. β(1,3)-glucanase activity is shared by different termite species. Termites from different species (*Zootermopsis angusticollis*, *Reticulotermes flavipes*, and *Cryptotermes secundus*) were extracted and β(1,3)-glucanase activity was measured on a CM-curdlan-RBB gel as described in *β(1,3)-Glucanase Assays*.

Other Supporting Information Files

Table S1 (PDF)