Supplementary figure 1. Laminarin reduces Nb larval recognition by M(IL-4) and *ex vivo* challenge-infected lung macrophages in a dose dependent manner. (a-b) Bone marrow derived macrophages were generated from C57BL/6 (B6). Macrophages were stimulated with IL-4 (10 ng mL⁻¹) and (b) pre-incubated at 37°C for 1 hour with Invivogen brand laminarin at various concentrations shown. (c) Interstitial macrophages were isolated from the lungs of challenge-infected mice at day 2 of secondary infection after removal of alveolar macrophages by lavage. Adherent cells were cultured for 2 days with no additional stimulation. (a-c) Nb L3 were then added to the culture for 24 hours. The percentage of larvae attacked by macrophages was then quantified by microscopy and manual counting of live and motile larvae. Data are pooled from a-b two and c one independent experiment(s) with technical and biological triplicates. NS, not significant; untr, untreated; *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001.
Supplementary figure 2. Laminarin does not affect macrophage viability or IL-4 polarization. (a) Bone marrow derived macrophages were generated from C57BL/6 (B6). Macrophages were stimulated with IL-4 (10 ng mL⁻¹) and pre-incubated at 37°C for 1 hour with laminarin (invivogen) ligands at different concentrations as indicated in the figure. Nb L3 were then added to the culture for 24 hours. The percentage of larvae attacked by macrophages was then quantified by microscopy and manual counting of live and motile larvae. Data are pooled from 5 independent experiments with technical triplicates. (a-b) MO (unstimulated macrophages), M(IL-4) (IL-4-stimulated macrophages, 10 ng mL⁻¹) and M(IL-4) + laminarin (invivogen, 5 mg mL⁻¹) were stained as indicated in the figures and analyzed by flow cytometry. (c) Bone marrow derived macrophages were stimulated for 24 hours with IL-4 and laminarin (5 mg mL⁻¹). Extensive washing with complete medium were then performed to remove laminarin from the solution (pretreated M). (d) Nb L3 were pretreated with laminarin (5 mg mL⁻¹) for 24 hours at 37°C then extensively washed to remove unbound laminarin. (c-d) Cells were incubated with Nb L3 for 24 hours. The percentage of larvae attacked by macrophages was quantified 24 hours later by microscopy and manual counting of live and motile larvae. Data are pooled from 2 independent experiments in biological duplicate with technical triplicates. NS, not significant; untr, untreated; *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001.
Supplementary figure 3. The β-glucan binding sites of CD11b, and to a lesser extent EphA2, contribute to M(IL-4) macrophage recognition of Nb L3. Bone marrow derived macrophages were generated from (a-b) C57BL/6 or (c) EphrinA2/-/ mice and stimulated with IL-4 (10 ng mL⁻¹). Cells were pre-incubated at 37°C for 1 hour with αCD11b (20 μg mL⁻¹) and/or laminarin (5 mg mL⁻¹). Nb L3 were then added to the culture for 24 hours. (a) The percentage of larvae attacked by macrophages was quantified 24 hours later by microscopy and manual counting of live and motile larvae. (b) In the same experiments, the proportion of larvae high attack (>30 macrophages/larvae) or low attack (<30 macrophages/larvae) was counted by microscopy. Data are pooled from 9 independent experiments in a and b with technical triplicates, and b 1 experiment with biological duplicate and technical quadruplicate. NS, not significant; untr, untreated; *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001.