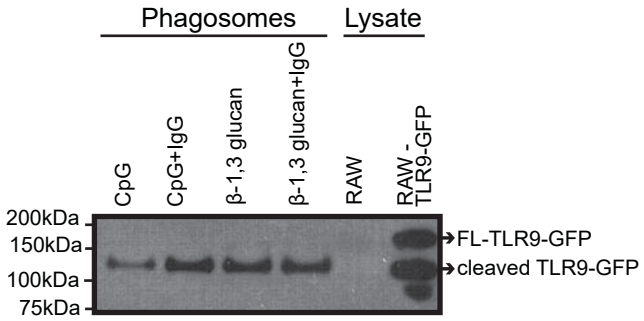
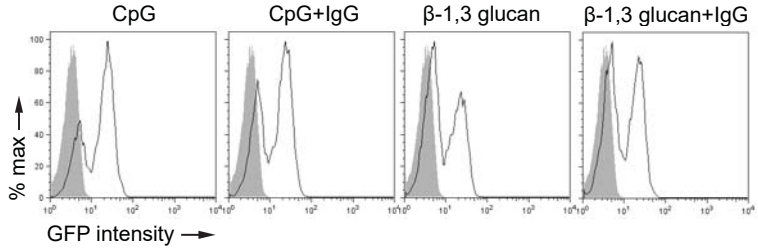
SUPPLEMENTARY FIGURE 1

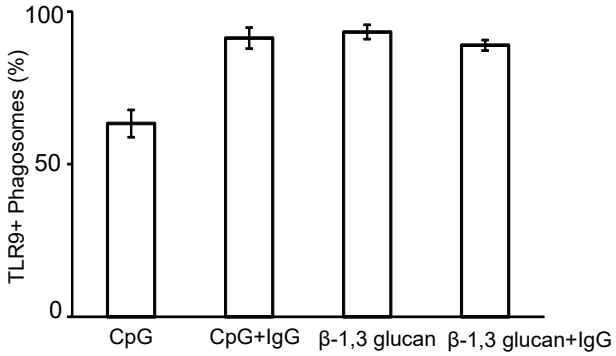
A



B

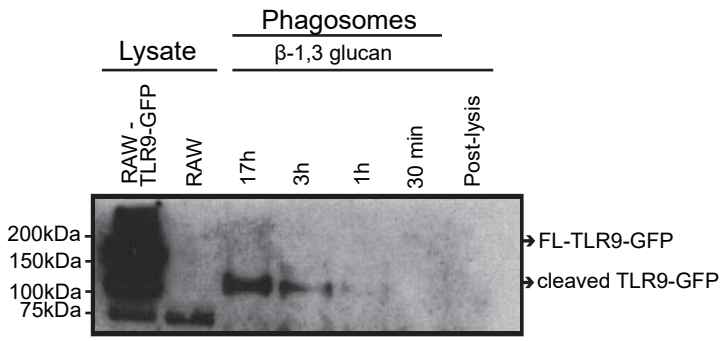


C



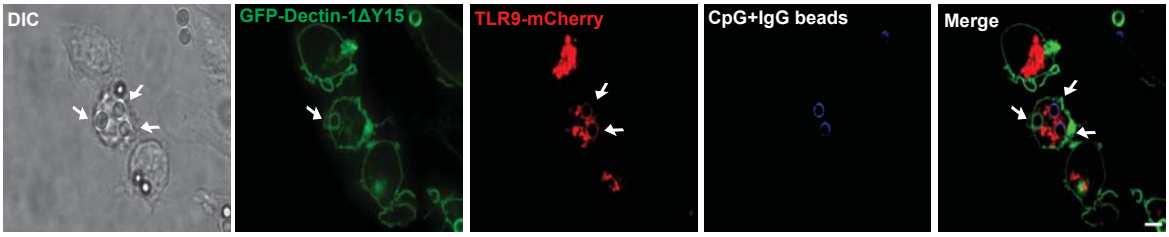
Supplemental Figure 1. TLR9 recruitment is not enhanced by the addition of anti-β-1,3 glucan antibody to β-1,3 glucan beads. (**A-C**) RAW TLR9-GFP incubated with CpG, CpG+IgG, β-1,3 glucan, or β-1,3 glucan+IgG beads for 2 h. (**A**) $1x10^6$ purified phagosomes were resolved by SDS-PAGE and blotted for TLR9-GFP. Lysate controls indicate full-length (FL) TLR9-GFP and cleaved TLR9-GFP. (**B**) Indicated purified phagosomes were analyzed by phagoFACS for TLR9-GFP recruitment (black line) and compared to bead-only control (gray shaded histogram). (**C**) RAW TLR9-GFP cells are treated as described in (A). The percentage of positive TLR9-GFP phagosomes was assessed by confocal microscopy (n≥75). Results are representative of five (A and B) or three (C) independent experiments.

SUPPLEMENTARY FIGURE 2



Supplemental Figure 2. Cleaved portion of TLR9 is recruited to β-1,3 glucan phagosomes. RAW macrophages expressing TLR9-GFP were stimulated with β-1,3 glucan beads for 30 min, 1 h, 3 h and 17 h and addition of β-1,3 glucan beads to cell lysate indicated as post-lysis. TLR9-GFP recruitment to purified phagosomes containing β-1,3 glucan beads were analyzed by immunoblot. Lysate control of indicated cell type were used to indicate FL-TLR9-GFP and cleaved TLR9-GFP. Data are representative of three independent experiments.

SUPPLEMENTARY FIGURE 3



Supplemental Figure 3. Dectin-1 dependent Syk activation requirement for TLR9 recruitment to β-1,3 glucan beads is maintained in the presence of CpG-IgG beads. Confocal microscopy of Dectin-1-knockout macrophages expressing TLR9-mCherry and GFP-Dectin-1ΔY15 co-incubated with β-1,3 glucan and CpG-IgG beads for 3 h. Original magnification X100. Scale bar indicates 5μm. Data are representative of three independent experiments.