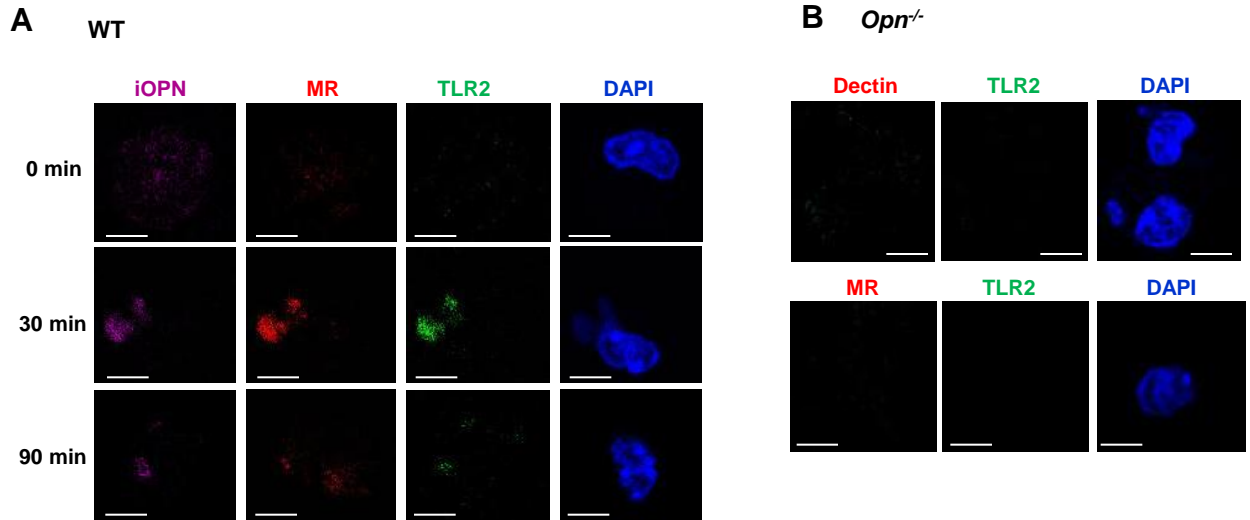


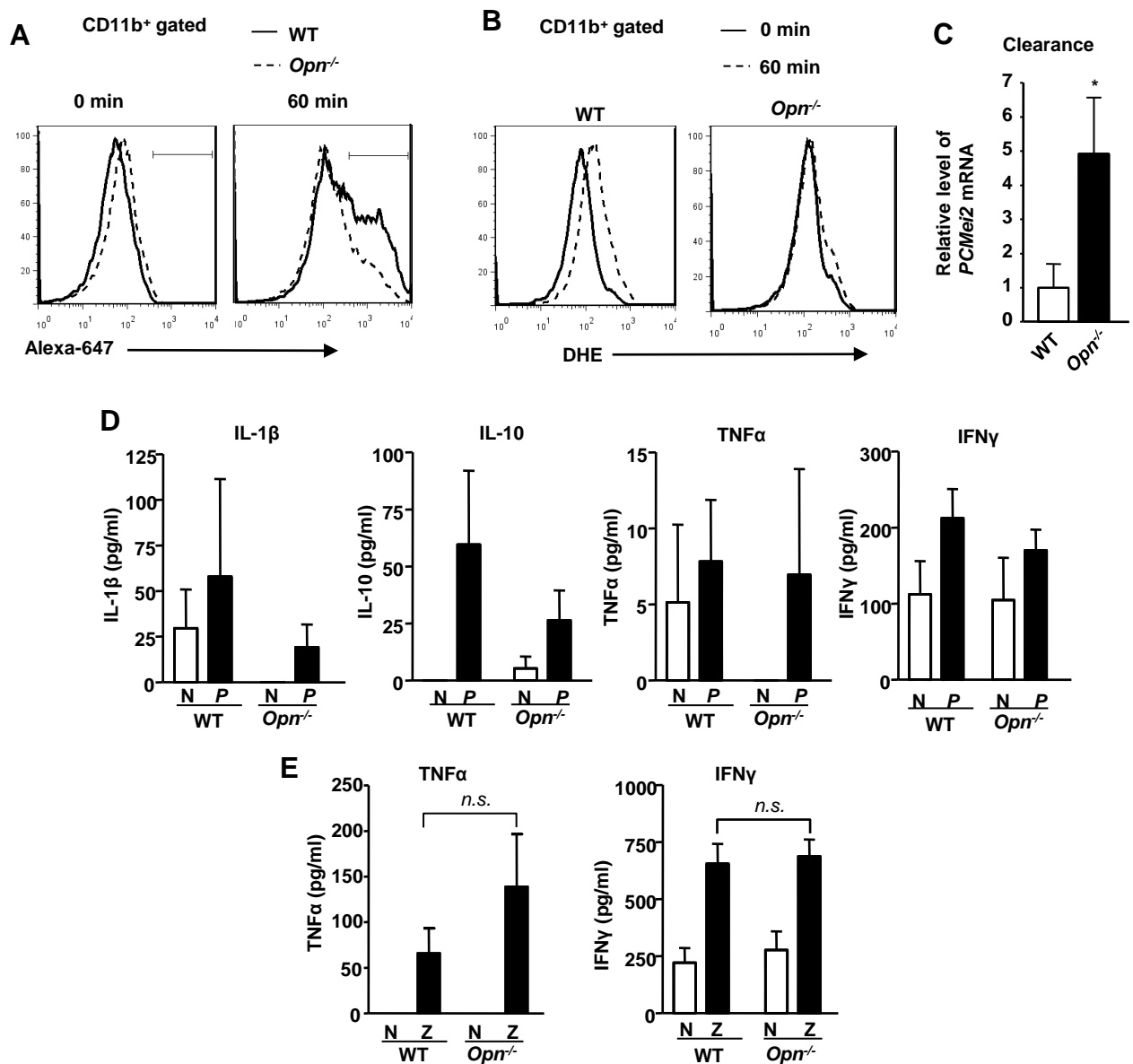
Supplemental Fig. 1 – Involvement of OPN in resistance against *Pneumocystis* infection in innate immunity.

(A) Body weight of four- and 13-week old *Opn*^{+/+}*Rag2*^{-/-} mice (white columns) and *Opn*^{-/-}*Rag2*^{-/-} mice (black columns). *n*=7. (B) *PCMei2* mRNA was detected by real-time qPCR, normalized with β -actin mRNA. First column denotes *PCMei2* levels in the lungs of 4-wk old *Opn*^{-/-}*Rag2*^{-/-} (DKO) mice, otherwise all the samples were obtained from 14-wk old mice of DKO, and single KO of *Rag2*^{-/-} or *Opn*^{-/-}. The last column denotes values from the lung draining LNs from 14-wk old DKO mice.

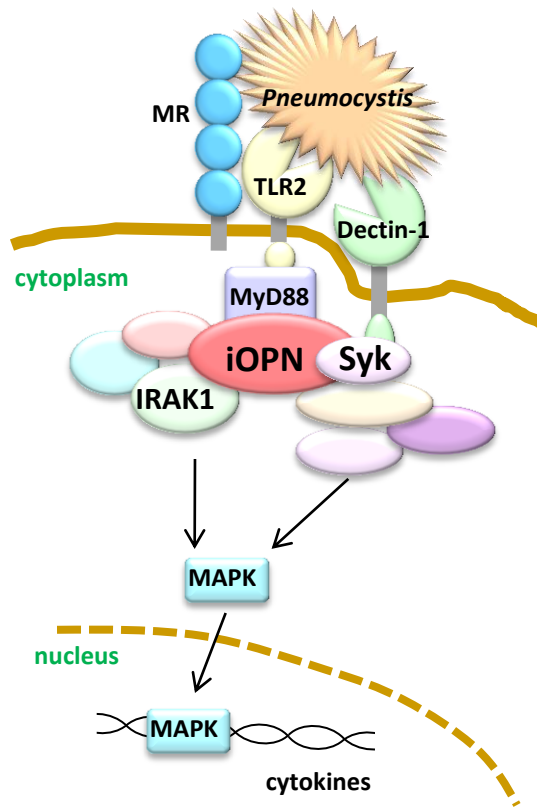


Supplemental Fig. 2 – iOPN is essential for PRR co-localization.

(A) Representative confocal microscopic images of iOPN (magenta), MR (red), TLR2 (green) and DAPI (blue) staining of WT macrophages at indicated time points after co-culture with *Pneumocystis*. Merged images are found in Fig. 2B. (B) Representative confocal microscopic images of dectin-1 or MR (red), TLR2 (green) and DAPI (blue) staining in *Opn*^{-/-} macrophages 30 min after *Pneumocystis* and macrophage co-culture. Merged images are found in Fig. 2C. Scale bars = 5 μ m.



Supplemental Fig. 3 – OPN induces phagocytosis of *Pneumocystis*, ROS generation, and fungal clearance. (A) Phagocytosis of *Pneumocystis* by macrophages. Macrophages (CD11b-labeled) were co-cultured with *Pneumocystis* (Alexa-647-labeled) for indicated duration. CD11b-gated cells were analyzed for phagocytosis of *Pneumocystis* by detecting Alexa-647. Alexa-647/CD11b double-positive cells over total CD11b positive cells were indicated with horizontal bars in flow cytometry panels. (B) ROS production by macrophages was evaluated 0 min (solid lines) and 60 min (broken lines) after co-culture with *Pneumocystis*. Cells gated on CD11b positive were analyzed with DHE staining to detect ROS. Increase of ROS activity from 0 min to 60 min is indicated in Fig. 3C as a percentage increase of MFI values. (C) *Pneumocystis* clearance was detected with PCMei2 mRNA in macrophages by real-time qPCR. (D) Cytokine expression detected in culture supernatants of peritoneal macrophage (1×10^7 cells/ml) stimulated with (shown as “P”) or without (indicated as “N”) *Pneumocystis* (5×10^6 cysts/ml). (E) Expression of TNF α and IFN γ detected in culture supernatants of WT and *Opn*^{-/-} macrophages stimulated with (indicated as “Z”) or without (indicated as “N”) 100 μ g/ml zymosan for 24 hr. Macrophage culture supernatants were analyzed by ELISA. n.s.: not significant.



Supplemental Fig. 4 – Schematic model of iOPN fungal PRR clustering and signal transduction to MAPK activation. *Pneumocystis* is simultaneously detected by dectin-1, MR, and TLR2. iOPN is essential for colocalization of dectin-1, TLR2, and MR. (It is likely that TLR2 is dimerized with TLR6, but we did not include TLR6 here for simplification.) In this figure, three PRRs co-localize and detect *Pneumocystis* for efficient detection and synergistic signal transduction. iOPN associates with IRAK1 (and MyD88 (14)) and Syk when TLR2 and dectin-1 are ligated, respectively. Simultaneous stimulation of TLR2 and dectin-1 requires the presence of iOPN for ERK MAPK activation to produce cytokines.