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## Supplemental Information

# CD209a Synergizes with Dectin-2 and Mincle to Drive Severe Th17 Cell-Mediated Schistosome Egg-Induced Immunopathology

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## *Figure S1. Related to Figure 4*

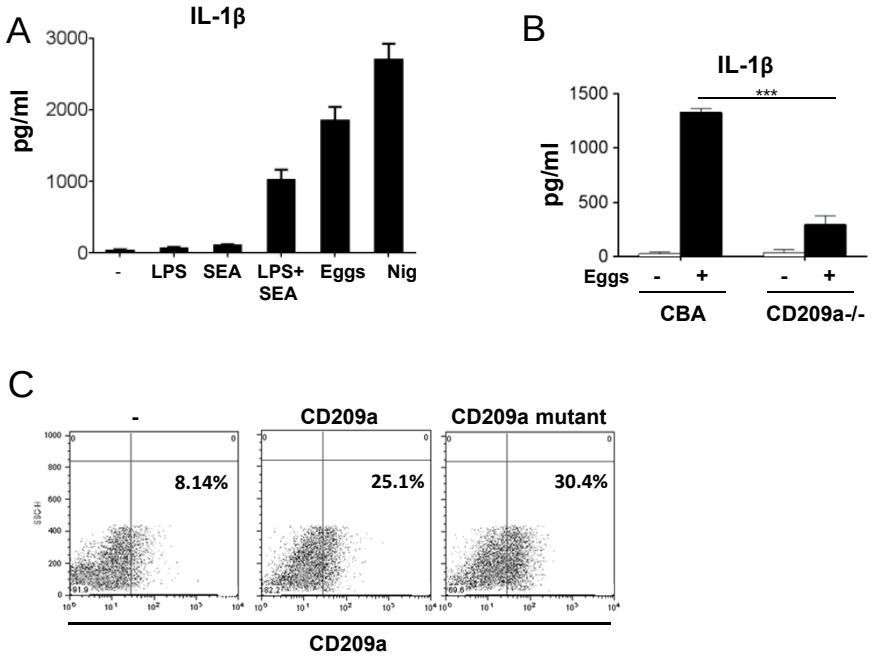


**Figure S1 (Related to Figure 3): (A) Live schistosome eggs, but not SEA, induce IL-1β production in the absence of LPS priming.** CBA BMDCs were cultured with LPS (100ng/ml), SEA (15μg/ml), both, 80 live eggs, or LPS plus nigericin (Nig) (10μM). IL-1β was measured in 24h cell supernatants by ELISA. **(B) IL-1β production is reduced in CD209a$^{-/-}$ BMDC-T cell co-cultures.** CBA and CD209a$^{-/-}$ BMDCs were co-cultured with 80 live schistosome eggs, naïve CD4+ T cells and anti-CD3/CD28-coated beads. IL-1β was measured in 72h cell supernatants by ELISA. Bars are as described in Fig.3. **(C) CD209a mutant is expressed on cell surface.** BL/6 BMDCs were transduced with CD209a or CD209a mutant. Cell surface expression of CD209a and CD209a mutant was assessed by flow cytometric analysis.

# *Figure S2. Related to Figures 4,5,6,7*

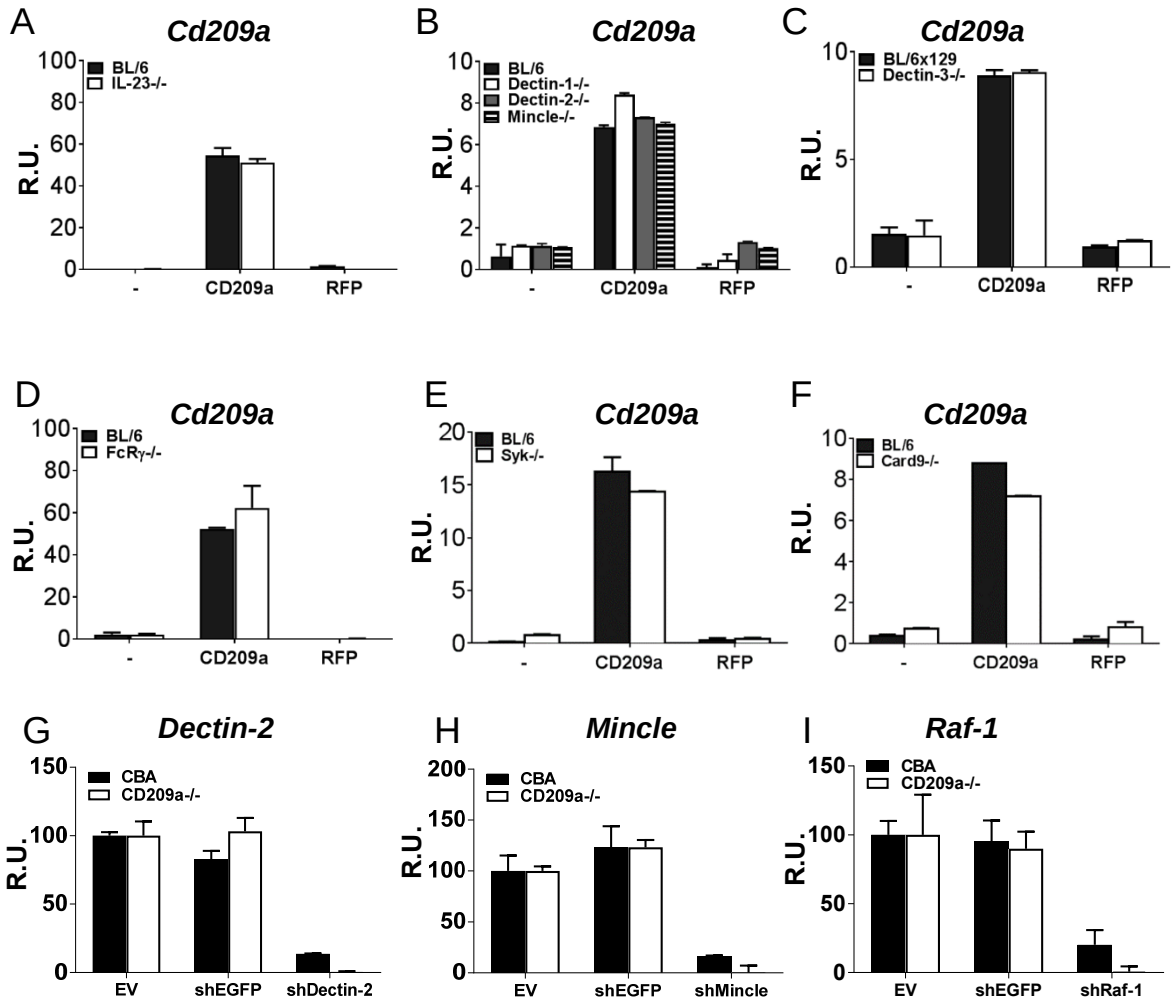


**Figure S2 (Related to Figures 4, 5, 6, 7 and S3): Verification of CD209a overexpression and Dectin-2, Mincle and Raf-1 knockdown in BMDCs**. BL/6 and (A) IL-23p19$^{-/-}$, (B) Dectin-1$^{-/-}$, Dectin-2$^{-/-}$ and Mincle$^{-/-}$, (C) BL/6x129 and Dectin 3$^{-/-}$, (D) FcRγ$^{-/-}$, (E) Syk$^{-/-}$, and (F) Card9$^{-/-}$ BMDCs were not transduced or were transduced with CD209a and control RFP. CD209a overexpression was assessed by qRT-PCR. CBA or CD209a$^{-/-}$ BMDCs were transduced with empty vector (EV), EGFP shRNA (shEGFP), Dectin-2 shRNA (shDectin-2) (G), Mincle shRNA (shMincle) (H) and Raf-1 shRNA (shRaf-1) (I). The mRNA levels of Dectin-2 (G), Mincle (H) and Raf-1 (I) were set at 100% in cells transduced with EV. Dectin-2, Mincle and Raf-1 mRNA levels were assessed by qRT-PCR. Bars represent the mean ± S.D. CD209a, Dectin-2, Mincle and Raf-1 relative units (R.U.) of three biological replicates from one representative experiment of three with similar results.

# *Figure S3. Related to Figure 5*

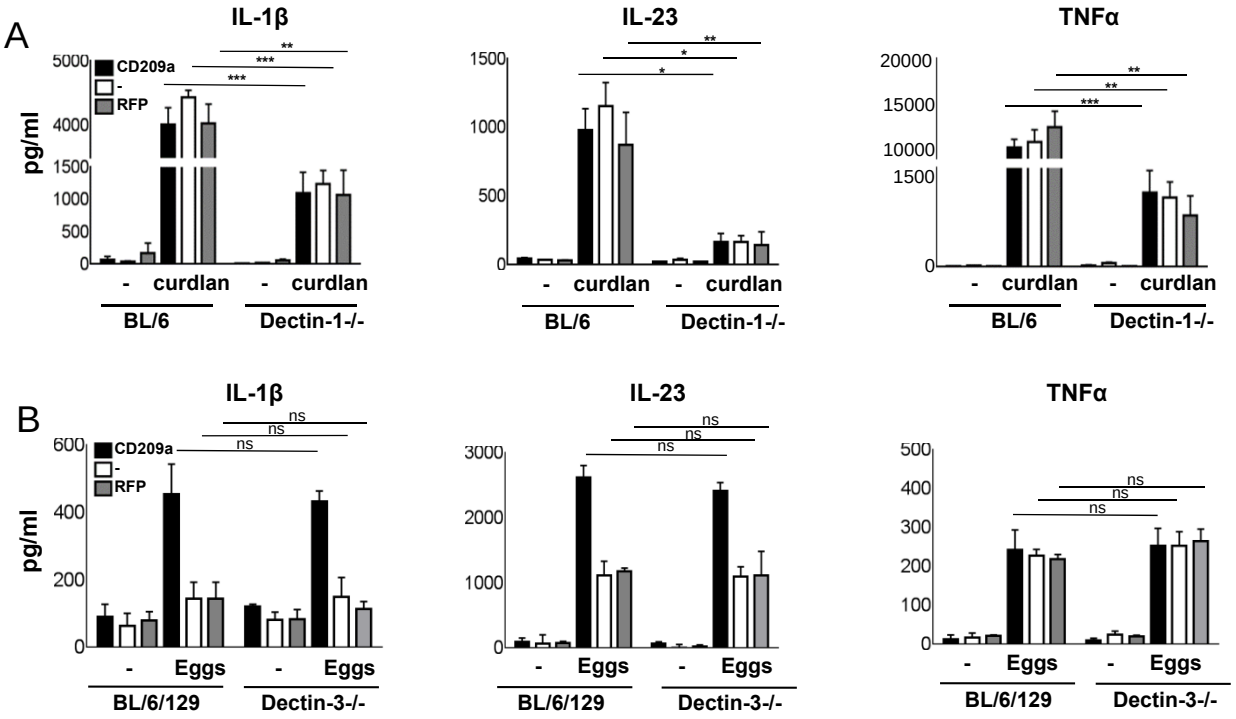


**Figure S3 (Related to Figure 5): (A) Cytokine production to curdlan is not affected by CD209a but is inhibited in the absence of Dectin-1.** BL/6 and Dectin-1$^{-/-}$ BMDCs were transduced with CD209a or control RFP and cultured with curdlan (100ug/ml). **(B) The absence of Dectin-3 does not affect cytokine production.** BL/6x129 and BL/6x129 Dectin 3$^{-/-}$ BMDCs were transduced with CD209a or control RFP and cultured with 80 live eggs. In (A), (B) IL-1β, IL-23 and TNFα were measured in 24h supernatants by ELISA. Bars are as described in Fig. 3.

# *Figure S4. Related to Figure 6*

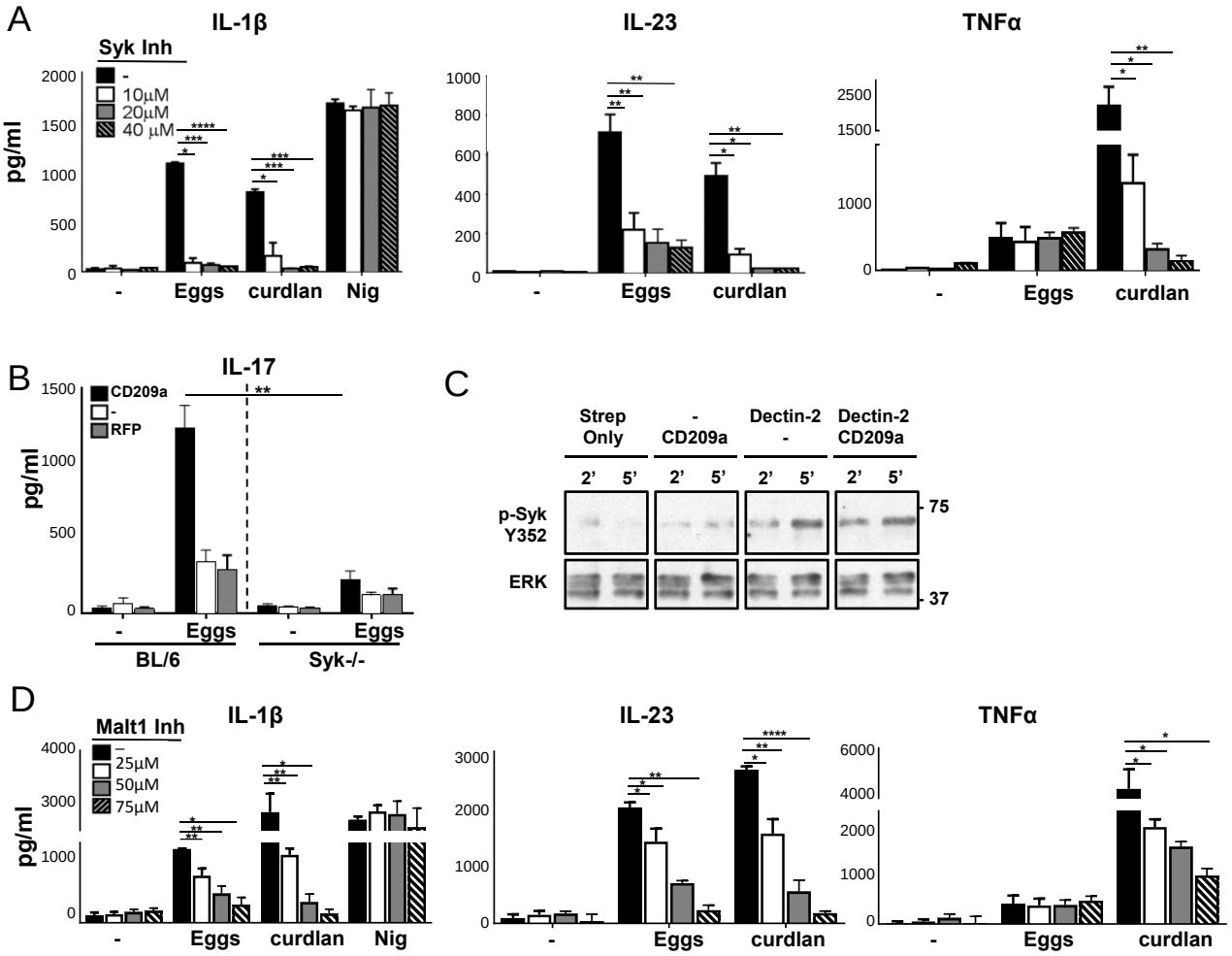


**Figure S4 (Related to Figure 6): Pharmacologic inhibition of Syk and MALT1 abrogates egg-induced production of IL-1β and IL-23 but not TNFα, and curdlan-induced production of all cytokines.** CBA BMDCs were treated with various concentrations of **(A)** Syk inhibitor piceatannol or **(D)** Malt1 inhibitor Z-VRPR-FMK for 1h and then cultured with live 80 eggs, curdlan (100ug/ml) or LPS (100ng/ml) plus Nigericin (Nig) (10uM). Cytokines in 24 h supernatants were measured by ELISA. **(B) CD209a-induced increase in IL-17 is dependent on Syk.** BL/6 and Syk$^{-/-}$ BMDCs were cultured with T cells and 80 live eggs. IL-17 in 72h supernatants was measured by ELISA. Bars are as described in Fig. 3. **(C) Triggering Dectin-2, but not CD209a, induces Syk phophorylation.** 1x10$^6$ CBA BMDCs were plated in 12-well plates and incubated for 2 and 5 min with biotinylated antibodies against Dectin-2, CD209a, or both, followed by streptavidin (Strep). Cell lysates were used for western blot analysis using antibodies against phosphorylated Syk (Tyr352) and total ERK. Results are representative of 2 independent experiments.

## SUPPLEMENTAL EXPERIMENTAL PROCEDURES

**Flow cytometry:** Cells were blocked with rat IgG and stained with biotin-conjugated anti-CD209a (BD Biosciences) followed by Alexa Fluor 647-conjugated streptavidin (Invitrogen). Data were acquired with a FACSCallibur flow cytometer and CellQuest software version 3.2.1 (Becton Dickinson) and analyzed with FlowJo software. Cells were gated for viability by propidium iodide exclusion.