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Supporting Information for

The balance between Gasdermin D and STING signaling shapes the severity of schistosome immunopathology.

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Figures S1 to S5

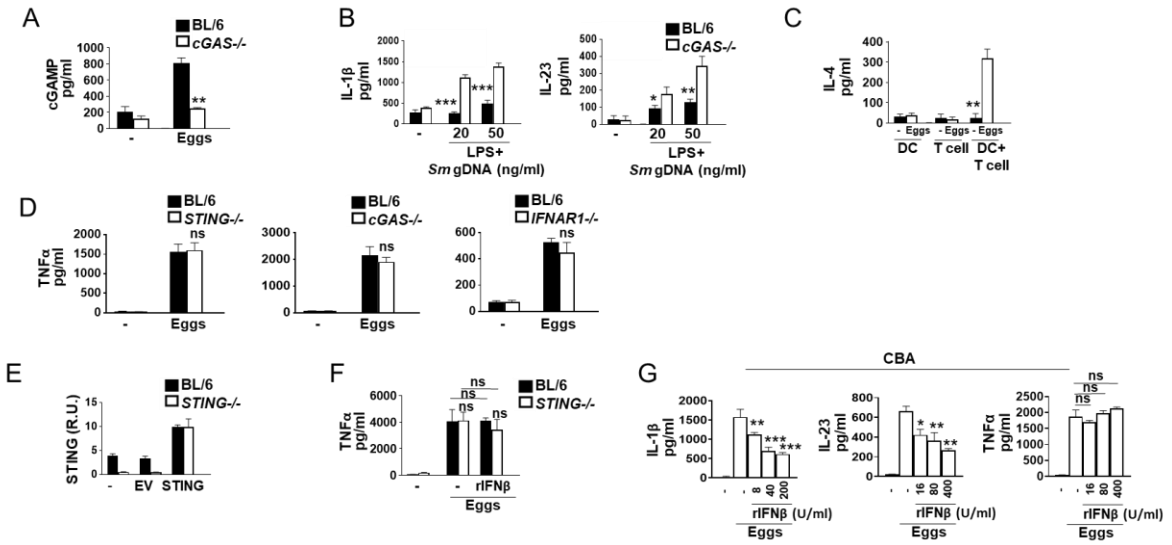

Fig. S1. (Related to Fig. 1). (A) cGAMP intracellular levels were diminished in egg-stimulated *cGAS*$^{-/-}$ BMDCs. 2x 10^6 BL/6 and *cGAS*$^{-/-}$ BMDCs were plated in six-well plates and cultured with or without 1000 eggs per well for 24h. cGAMP was then measured in cell lysates using ELISA **(B) *Sm* gDNA-mediated proinflammatory cytokine production was enhanced in the absence of cGAS**. BL/6 and *cGAS*$^{-/-}$ BMDCs were treated with LPS (100ng/ml) and transfected with indicated amounts of *Sm* gDNA using Lipofectamine 2000. IL-1β (left) and IL-23 (right) in the supernatants were measured by ELISA. **(C) CD4+ T cells are the source of IL-4 in BMDC-T cell-egg cocultures.** BL/6 BMDCs or CD4+ T cells, or both were cocultured with or without eggs for 72h and IL-4 in the supernatants was measured by ELISA. **(D) The absence of STING, cGAS and IFNAR1 does not affect TNFα production in egg- stimulated BMDCs.** BL/6 and *STING*$^{-/-}$ (left), *cGAS*$^{-/-}$ (center) and *IFNAR1*$^{-/-}$ (right) BMDCs were cultured with 80 live eggs and TNFα in the supernatants were measured by ELISA. **(E) Verification of STING overexpression**. BL/6 and *STING*$^{-/-}$ BMDCs were transduced with empty vector (EV) or STING. STING overexpression was assessed by qRT-PCR. The mRNA level of STING was set at 10 in BL/6 BMDCs transduced with STING. Bars represent the mean ± S.D. STING relative units (R.U.) of three biological replicates from one representative experiment of three with similar results. **(F) Pretreatment of BL/6 and *STING*$^{-/-}$ BMDCs with rIFNβ does not affect TNFα production in response to eggs.** BL/6 and STING$^{-/-}$ BMDCs were pretreated with rIFNβ for 1h followed by egg stimulation and TNFα in the supernatants was measured by ELISA. **(G) Pretreatment of egg-stimulated CBA BMDCs with rIFNβ leads to significant decrease in proinflammatory cytokine production.** CBA BMDCs were pretreated with rIFNβ for 4h followed by egg stimulation for 24h. Bars in A, B, C, D, F and G represent the mean ±SD cytokine levels of three biological replicates from one representative experiment of three with similar results. For this and all figures, unless specified: * p ≤ 0.05, ** p ≤0.005, *** p ≤0.0005, ns: not significant.

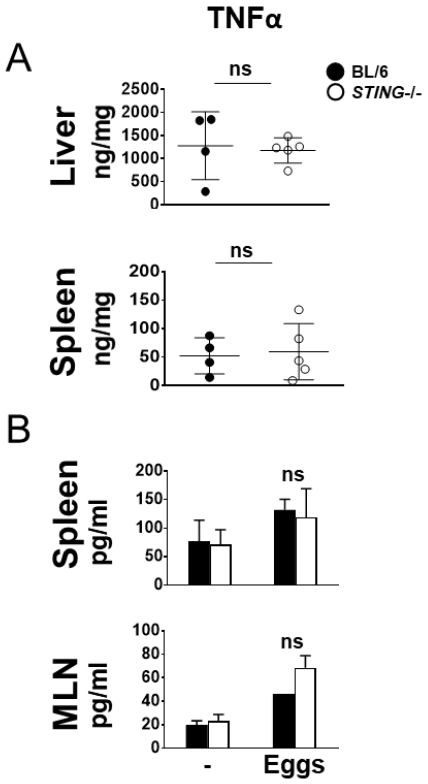

Fig. S2 (Related to Fig. 3). The absence of STING does not affect TNFα production *in vivo* and *ex vivo*. (A) Livers and spleens from infected mice were homogenized and TNFα levels were assessed by ELISA. (B) Bulk spleen and MLN and from 4-5 mice per group were pooled and cultured with live eggs. TNFα in 72h supernatants was measured by ELISA. Data in A is representative of one of three independent experiments with similar results. Data in A and B are representative of one of three independent experiments with similar results. Each dot (A) and bar (B) represents mean ± S.D. cytokine levels of triplicate determinations per mouse. ns: not significant.

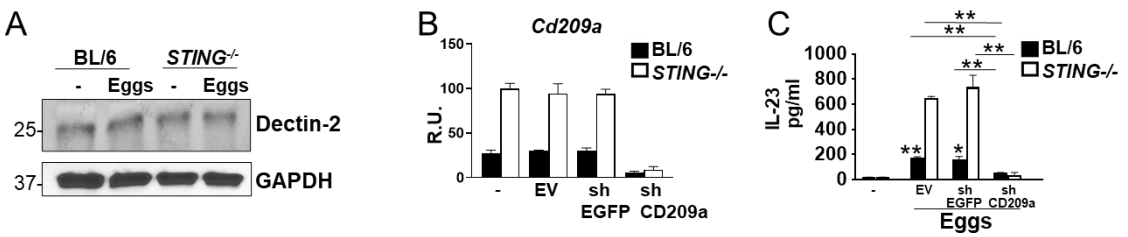

Fig. S3 (Related to Fig. 4). (A) The loss of STING does not impact Dectin-2 expression. 2x 10^6 BL/6 and *STING*$^{-/-}$ BMDCs were plated in six-well plates and cultured with or without 1000 eggs per well for 24h. Cell lysates were used for western blot analysis using Abs against Dectin-2 and GAPDH. **(B) Verification of CD209a knockdown in BMDCs.** BL/6 and *STING*$^{-/-}$ BMDCs were transduced with empty vector (EV), EGFP shRNA (shEGFP) and CD209a shRNA (shCD209a). The mRNA levels of CD209a were assessed by qRT-PCR. The mRNA level of CD209a was set at 100 in *STING*$^{-/-}$ BMDCs transduced with EV. Bars represent the mean ± S.D. CD209a relative units (R.U.) of three biological replicates from one representative experiment of three with similar results. **(C) CD209a silencing resulted in a marked reduction of IL-23 in BMDCs.** BMDCs from BL/6 and *STING*$^{-/-}$ mice were transduced with EV, shRNA against Enhanced Green Fluorescent Protein (EGFP) (shEGFP) or shRNA against CD209a (shCD209a) and then stimulated with 100 eggs for 24h. IL-23 in culture supernatants were measured by ELISA. Bars represent the mean ±SD cytokine levels of three biological replicates from one representative experiment of three with similar results. * $p \leq 0.05$, ** $p \leq 0.005$.

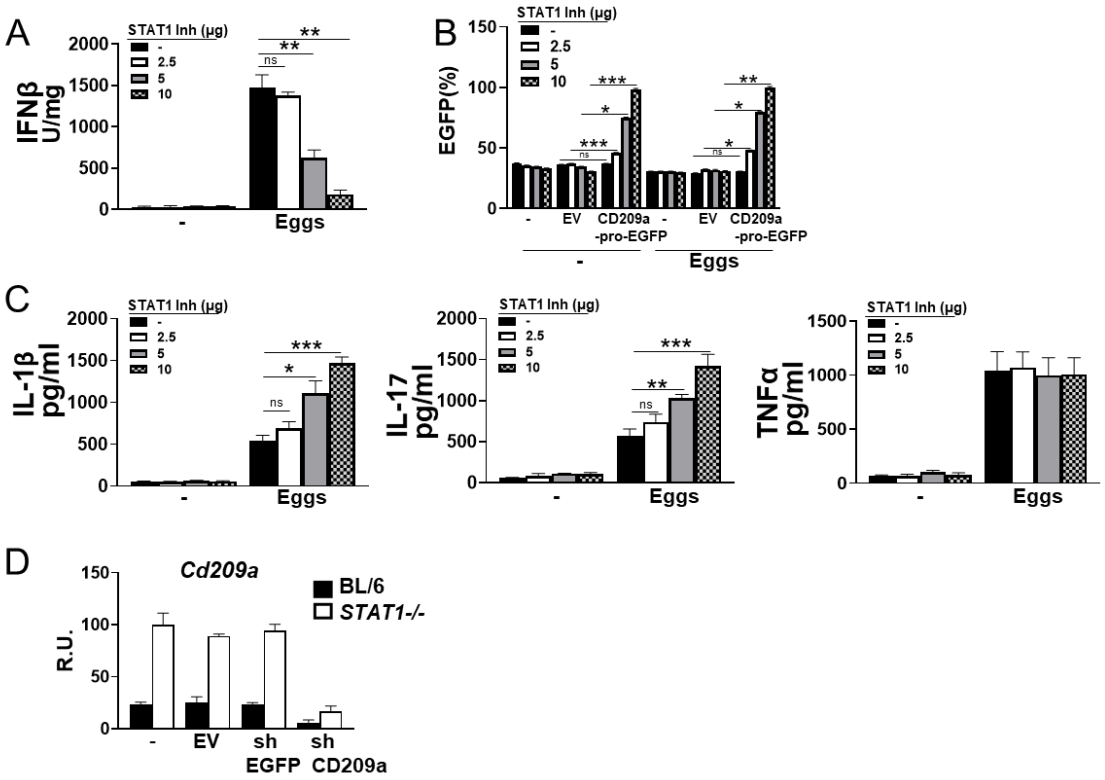

Fig. S4 (Related to Fig. 5). (A) STAT1 is required for IFN-I production in DCs. BMDCs from BL/6 mice were pretreated with various concentrations of STAT1 inhibitor Fludarabine phosphate before stimulation with 100 eggs for 24h. IFNβ levels were then assessed in the supernatants by ELISA. **(B) STAT1 suppresses CD209a promoter activity.** BMDCs from BL/6 mice were transduced with EV or CD209a-promoter (pro)-EGFP and then pretreated with various concentrations of STAT1 inhibitor Fludarabine phosphate before stimulation with 100 eggs for 24h. EGFP levels were measured using a plate reader. Data presented as the percentage of EGFP, with EGFP levels in STAT1 Inhibitor (Inh) (10µg) treated BMDCs being set at 100%. **(C) Inhibition of STAT1 leads to an enhancement of IL-1β and IL-17 but does not affect TNFα production.** BMDCs from BL/6 mice were pretreated with various concentrations of STAT1 inhibitor Fludarabine phosphate before stimulation with 100 eggs for 24h. IL-1β (left), IL-17 (center) and TNFα (right) levels were then assessed in the supernatants by ELISA. Bars in A, B and C represent the mean ±SD of three biological replicates from one representative experiment of three with similar results. **(D) Verification of CD209a knockdown in BMDCs.** BL/6 and *STAT1*$^{-/-}$ BMDCs were transduced with empty vector (EV), EGFP shRNA (shEGFP) and CD209a shRNA (shCD209a). The mRNA levels of CD209a were assessed by qRT-PCR. The mRNA level of CD209a was set at 100 in *STAT1*$^{-/-}$ BMDCs transduced with EV. Bars represent the mean ± S.D. CD209a relative units (R.U.) of three biological replicates from one representative experiment of three with similar results. * $p \leq 0.05$, ** $p \leq 0.005$, *** $p \leq 0.0005$, ns: not significant.

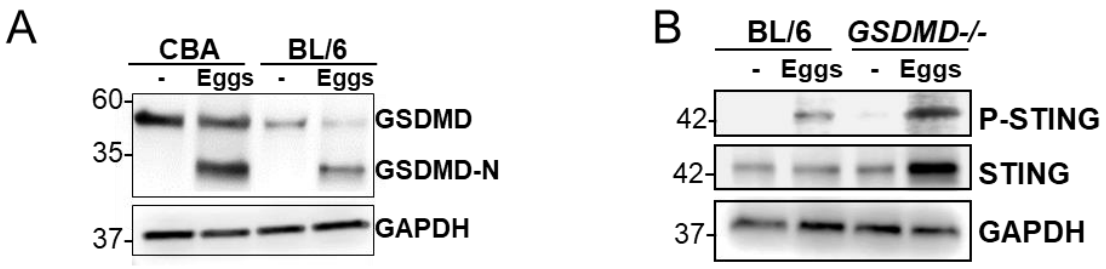

Fig. S5 (Related to Fig. 6). (A) Gsdmd is highly expressed in CBA BMDCs. Immunoblot analysis of $2x10^6$ CBA and BL/6 BMDCs plated in six-well plates and stimulated with 1000 live eggs per well for 24h. Cell lysates were used for western blot analysis using Abs against GSDMD and GAPDH. Results are a representative of three independent experiments. (**B) STING is highly phosphorylated in egg-stimulated *GSDMD*$^{-/-}$ BMDCs.** Immunoblot analysis of $2x10^6$ CBA and *GSDMD*$^{-/-}$ BMDCs plated in six-well plates and stimulated with 1000 live eggs per well for 24h. Cell lysates were used for western blot analysis using Abs against Phospho-STING, STING and GAPDH. Results are a representative of three independent experiments.