Supplemental Figure Legend

Figure S1. STING is essential for CDG, but not alum, adjuvanted IgG response. A-D. WT or STING$^{-/-}$ mice (four mice per group) were immunized (*i.p.*) with OVA (20μg) or OVA(20μg)/CDG(15μg) or OVA(20μg)/alum (200μg) twice (14 days apart). Sera were collected 14 days after the last immunization. Sera from the same group were pooled. OVA-specific IgGs were measured in serum as in Figure 1. n=3. Graph present means ± standard error from three independent experiments. Significance is represented by an asterisk, where $p<0.05$. **E-F**. WT or STING$^{-/-}$ mice were injected (*i.p.*) with indicated amount of CDG (E) or alum (F). Peritoneal lavages were performed 15hr later. Collected cells were stained with indicated Abs. Live cells were gated and underwent FACS analysis. n=3.

Figure S2 STING is essential for CDG-induced DC maturation. A-B. BMDC from WT and STING$^{-/-}$ (KO) were activated by CDG (10μg/ml) or LPS (200ng/ml) or medium alone (mock) for 18hr. The surface markers CD80, CD86 were analyzed by flow cytometry. **C&E** Cytokine or nitric oxide was measured in the supernatants using commercial ELISA kits or Griess Reagent Kits (Invitrogen, G7921), according to the manufacturer's protocol. Graph present means ± standard error from three independent experiments. Significance is represented by an asterisk, where $p<0.05$. **D.** iNOS production was measured in whole cell lysate (WCL). n=3. **F&G.** BMDC were activated with CDG(10μg/ml)/OVA(100μg/ml) or LPS(200ng/ml)/OVA (100μg/ml) or medium alone for 18hrs. OVA-specific CD4+ T cells were isolated from OT II mice using the CD4+ T cell isolation kit (Miltenyi, cat#130-095-248). Isolated CD4^{+} T cells were labeled with CFSE (1μM, Molecular probes, cat#C34554). Activated BMDC were co-cultured with CFSE labeled OVA-specific OT II T cells for 3 days. IL-2 productions were

measured in the supernatant. Cell proliferation was determined by CFSE dilution using flow cytometry. n=3. Graph present means ± standard error from three independent experiments. Significance is represented by an asterisk, where $p<0.05$.

Figure S3 TNF-α signaling is critical for the mucosal adjuvant activity of CDA. A-D. WT, $STING^{-/-}$ and $TNFR1^{-/-}$ mice (four mice per group) were vaccinated (*i.n.*) with two doses (14 days apart) of OVA (20μg) together with CDA (5μg, Invivogen, cat# vac-cda) or OVA (20μg) alone. Blood and nasal wash samples were collected 14 days after the last immunization (day 28). Antibodies in the blood and nasal wash samples from individual mouse were determined by ELISA. n=1. Graph present means ± standard deviation from the same group of mice. Significance is represented by an asterisk, where $p<0.05$.

Figure S4. CDG activates TNF-α production in TBK1 knockdown cells. A-B. TBK1 expression in BMDM or BMDC was knocked-down by Dharmacon® siGENOME SMARTpool (Thermo scientific, cat# M-063162) according to the manufactory's instructions. Briefly, 25nM TBK1 siRNA or siGENOME Non-targeting siRNA pool (Thermo Scientific, cat# D-001206-13) were transfected into BMDM or BMDC using Dharmafect 4 (Thermon scientific, cat# T-2004-01). The next day, medium were changed and cells were left in culture for 2 more days. The knockdown efficiency was examined 3 days after the siRNA transfection by Western blot. Cells were then activated by CDG (10μg/ml) or medium alone for 6hr. TNF-α, IFN-α were measured in the supernatant. n=3. Graph present means ± standard error from three independent experiments. Significance is represented by an asterisk, where $p<0.05$.

Fig S1. STING is essential for CDG-, but not Alum-, induced IgG response

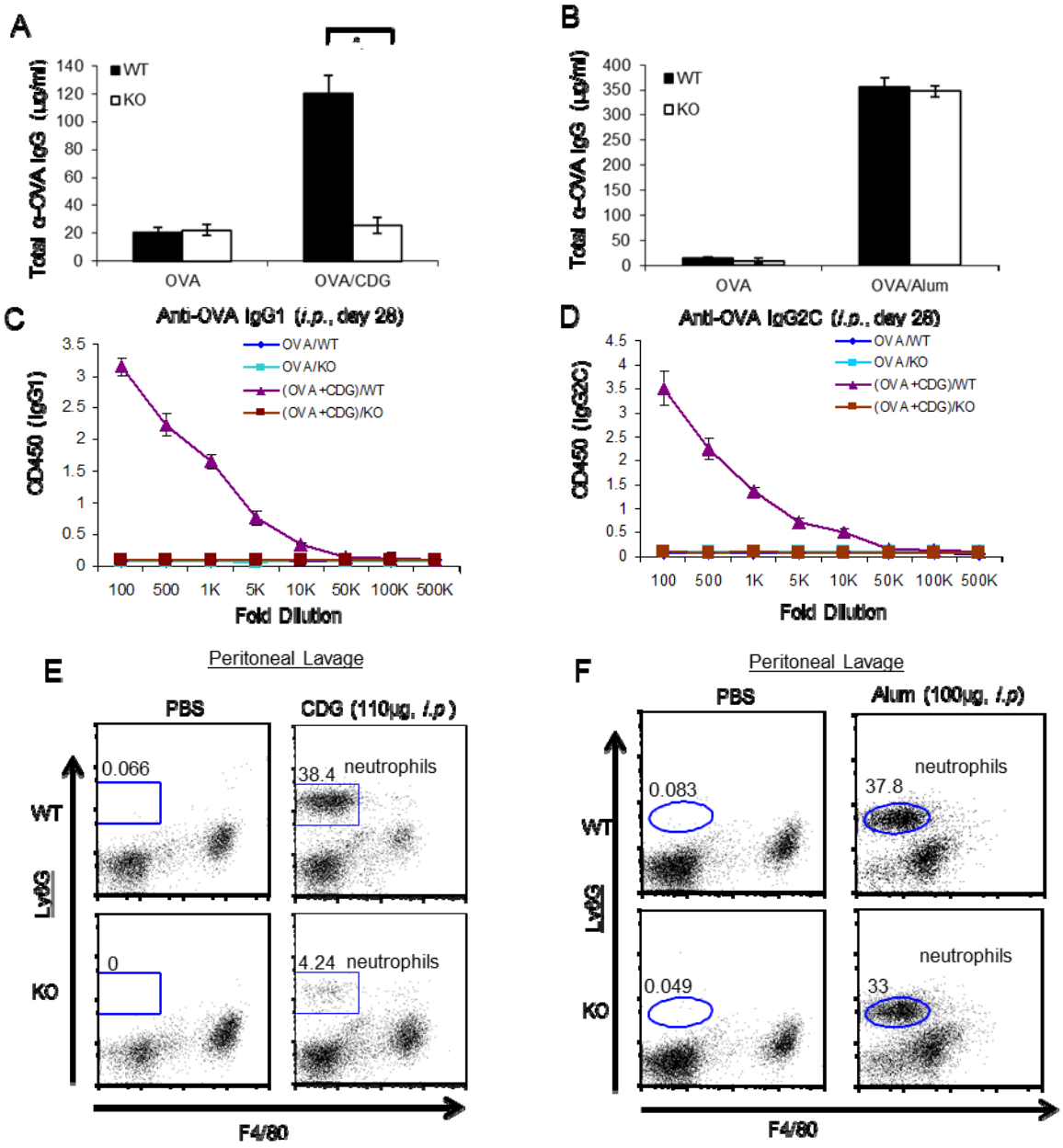

Fig S2 STING is essential for CDG-induced DC maturation

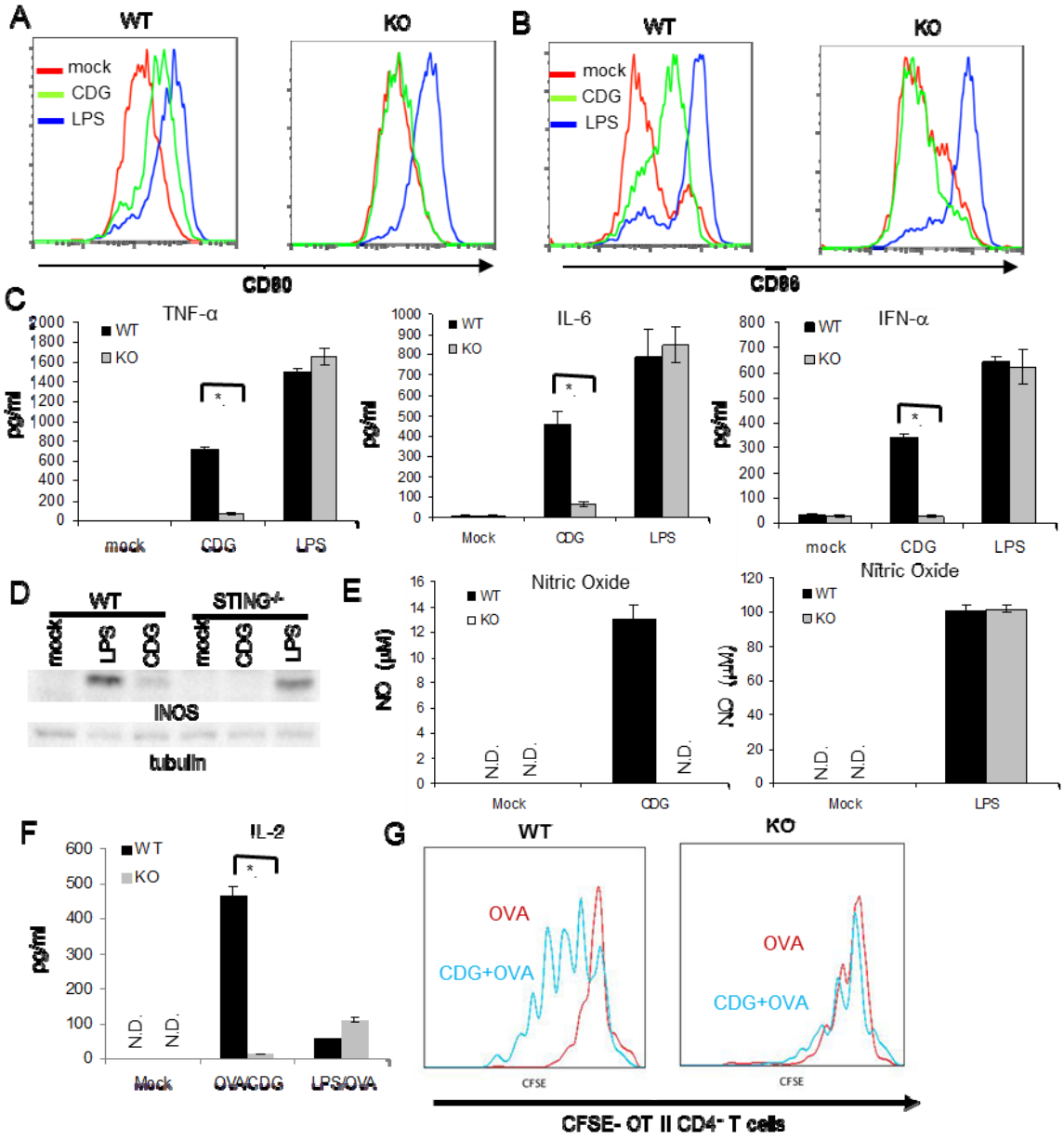

Fig S3. STING and TNF-α signaling are critical for the mucosal adjuvant activity of CDA

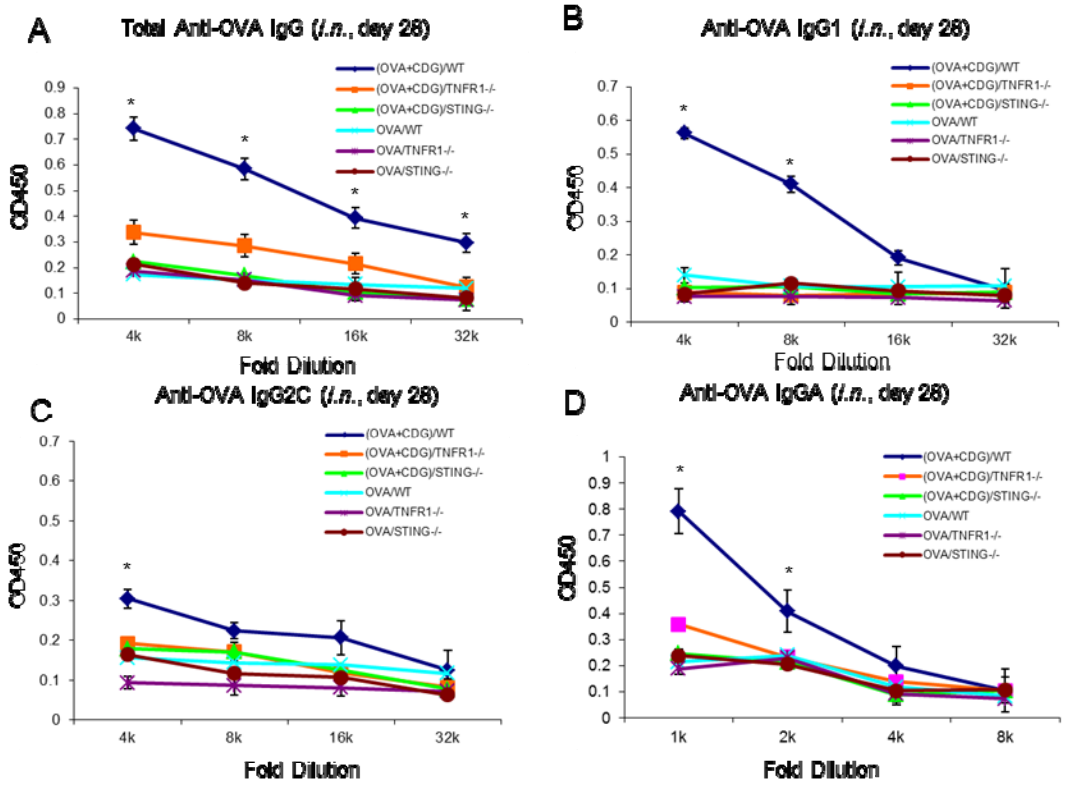

Fig S4. CDG activates TNF-α production in TBK1 knockdown cells