

Fig. S1. Related to [Fig. 1](#). SV is a Th2 type antigen. (a–c) On days 0 and 14, C57BL/6J mice ($n = 5$) were immunized i.m. with 50 μg SV alone or together with CpG or alum. One week after the second immunization, splenocytes were prepared from immunized mice and stimulated with MHC class I or II epitope peptides of nucleoprotein for 2 days. IFN- γ , IL-13 and IL-17 in supernatants were measured by ELISA. Results are representative of two separate experiments. Median and SEM are shown for each group. Statistical significances are indicated, $*P < 0.05$, $***P < 0.001$ by Dunnett's Multiple comparison test.

Fig. S2. Related to [Fig. 3](#). Advax fails to induce antibody immune responses to OVA, a Th0 type antigen. On days 0 and 14, (a, b) C57BL/6J mice ($n = 4$ or 5) or (c, d) *Tlr7*^{-/-} mice ($n = 5$) were immunized i.m. with 100 μg OVA alone or together with adjuvant, or 1.5 μg WV alone or together with adjuvant, respectively. Antigen-specific IgG1 and IgG2c titers in sera at days 14 and 28 were measured by ELISA. Results are representative of two or three separate experiments. Median and SEM are shown for each group. Statistical significances are indicated, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ by Dunnett's Multiple comparison test or Student's *t*-test.

Fig. S3. Related to [Fig. 4](#). Advax does not activate cDCs *in vitro*. (a–c) Flt3L induced bone marrow-derived DCs were stimulated with 1 mg/mL alum, 1 mg/mL Advax or 50 ng/mL LPS for 24 h *in vitro*, and then the expression of CD40 on cDCs was evaluated by FACS. Results are representative of three separate experiments.

Fig. S4. Related to [Fig. 4](#). Activation of CD8 α^+ DCs or CD8 α^- DCs is not observed *in vivo* after adjuvant administration. C57BL/6J mice were injected with 0.67 mg alum, 1 mg Advax or 50 ng LPS at the base of the tail. Twenty-four hours after injection, the inguinal lymph nodes were collected, treated with DNase I and collagenase. Then, the cells were stained and the expression of CD40 on (a–c) CD8 α^+ or (d–f) CD8 α^- DCs was analyzed by FACS. Results are representative of three separate experiments.

Fig. S5. Related to [Fig. 6](#). Cytokine production profile induced by injection of Advax or alum alone without antigen Cytokine levels in the peritoneal lavage fluids were measured 1, 3, 6 and 24 h after i.p. administration of (a) 0.67 mg alum or (b) 1 mg Advax, Cytokines that are significantly increased in each time point are shown by red, Statistical difference compared to PBS administration group are determined by Dunnett's Multiple comparison test.

Fig. S6. Related to [Fig. 6](#). IL-1 β is not required in the adjuvant effect of Advax. (a) Peritoneal macrophages and (b) GM-DCs were primed with 50 ng/mL LPS for 15 h and then stimulated with Advax or alum at the indicated concentration for 8 h. IL-1 β secreted into culture supernatants was measured by ELISA. Results are representative of three separate experiments. Median and SEM are shown for each group. Statistical significances compared to non-stimulation group are indicated, *** $P < 0.001$ by Dunnett's Multiple comparison test or Student's t -test.

Fig. S7. Related to [Fig. 6](#). The NLRP3 inflammasome is not required for the adjuvant effect of Advax. On days 0 and 14, (a–c) *Nlrp3*^{+/−} or *Nlrp3*^{−/−} ($n = 3$ or 4) or (d–f) *Caspase1*^{+/−} or *Caspase1*^{−/−} ($n = 5$) were immunized i.d. with 5 μ g HBs alone or together with Advax. (g–i) *Il-1r*^{+/−} or *Il-1r*^{−/−} mice ($n = 3$) were immunized i.m. with 1.5 μ g WV alone or together with Advax. Antigen-specific total IgG, IgG1 and IgG2c titers in sera at days 14 and 28 were measured by ELISA. Results are representative of two or three separate experiments. Median and SEM are shown for each group. Statistical significances were determined by Student's t -test.

Fig. S8 Related to [Fig. 6](#). CLRs-related signal pathways are not required for the adjuvant effect of Advax. On days 0 and 14, (a–c) *Fcrg*^{+/−} or *Fcrg*^{−/−}, (d–f) *Card9*^{+/−} or *Card9*^{−/−}, or (g–i) *Dap12*^{+/−} or *Dap12*^{−/−} mice ($n = 4$ –6) were immunized i.m. with 1.5 μ g WV alone or together with adjuvant. Antigen-specific total IgG, IgG1 and IgG2c titers in sera at days 14 and 28 were measured by ELISA. Results are representative of two separate experiments. Median and SEM are shown for each group. Statistical significances were determined by Student's t -test.

Fig. S9. Related to [Fig. 7](#). TNF- α is essential for the adjuvant effect of Advax. On days 0 and 14, *Tnfa*^{+/−} or *Tnfa*^{−/−} mice ($n = 5$) were immunized i.d. with 5 μ g HBs alone or together with adjuvant. Antigen-specific (a) total IgG, (b) IgG1 and (c) IgG2c titers in sera at days 14 and 28 were measured by ELISA. Results are representative of two separate experiments. Median and SEM are shown for each group. Statistical significances are indicated, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ by Dunnett's Multiple comparison test or Student's t -test.