Supporting Information

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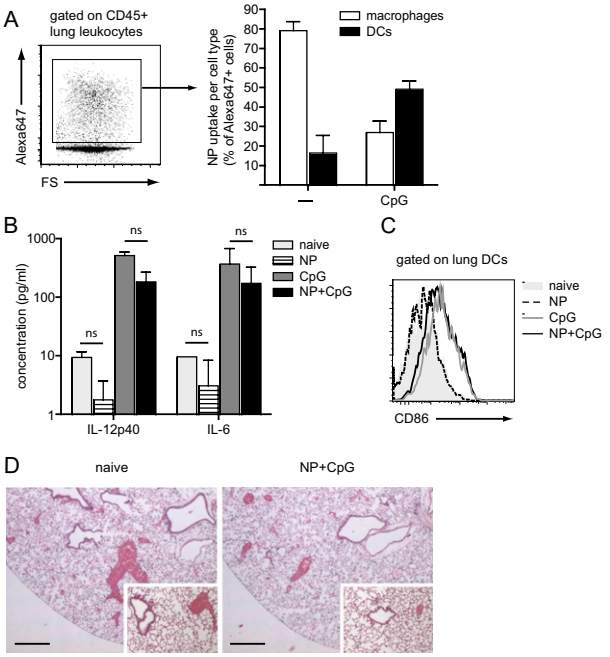


Fig. S1. Following pulmonary delivery, NPs are taken up by lung macrophages and DCs but do not induce inflammation. Alexa 647-labeled NPs (200 μg) were administered, with or without the addition of 2.5 μg CpG, in the lungs of C57BL/6 mice. (*A*) Uptake by lung macrophages and DCs was evaluated 24 h after delivery by flow cytometry. (*B*) Secretion of IL-12p40 and IL-6 in the BAL was determined by ELISA 24 h after the co-delivery of NPs and CpG. (*C*) Surface expression of CD86 by lung DCs at 24 h following pulmonary NP and CpG administration. (*D*) H&E staining on lung sections 30 d after co-delivery of NPs and 2.5 μg CpG. (Scale bar, 500 μm.) Results are presented as mean ± SD. Experiments were repeated two or three times with three mice per group.

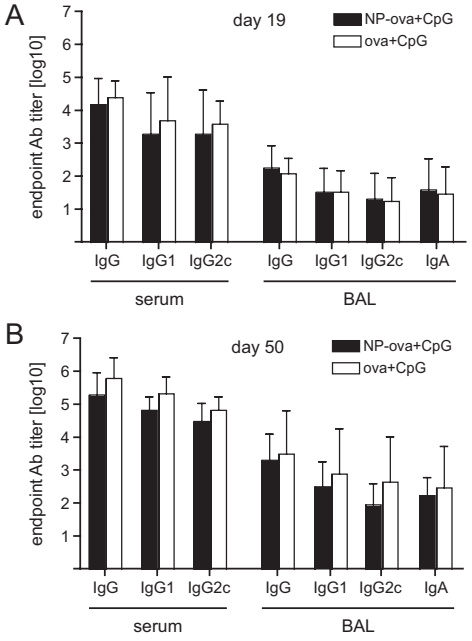


Fig. S2. Ova conjugation to NPs does not influence antigen-specific systemic and mucosal antibody responses. Mice were immunized as described in Fig. 1. Ova-specific antibody titers were determined on day 19 (*A*) and day 50 (*B*) in the serum and the BAL by ELISA. Results are presented as mean ± SD. Experiments were repeated with 10 mice per group.

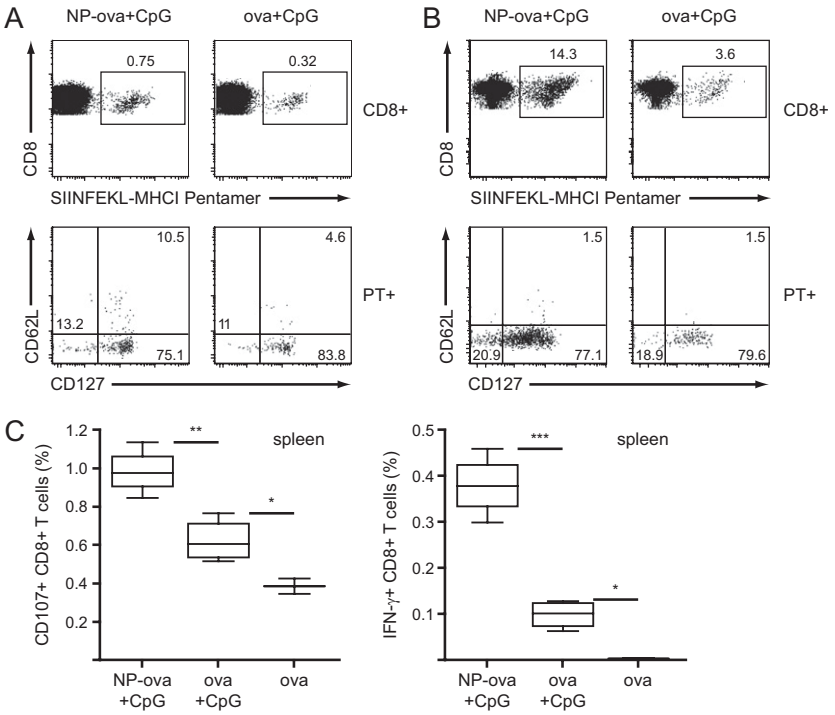
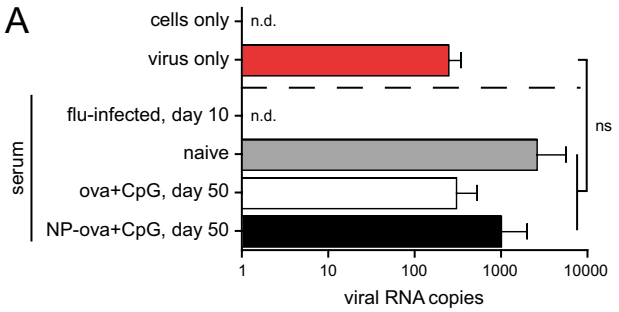


Fig. S3. Antigen-specific CD8$^+$ T cells are increased in frequencies in NP-immunized mice on day 50 and mainly display effector memory phenotype. Mice were immunized as described in Fig. 1; recall CD8$^+$ T-cell responses were analyzed on day 50. The memory phenotype of PT positive CD8$^+$ T cells was determined in the spleen (*A*) and lung (*B*) by CD62L and CD127 surface staining and flow cytometry. Values in dot plots represent the percentage of cells detected in each gate. (*C*) CD107a and IFN-γ expression by splenic CD8$^+$ T cells was analyzed after 6 h restimulation in the presence of SIINFEKL. Experiments were repeated two times with a total of four to 12 mice per group.



B

serum	NP-ova +CpG	ova +CpG	flu-infected	naive
dilution factor	—	—	320	—

Fig. S4. Antibodies generated after vaccination with NP-ova and CpG do not neutralize influenza-ova virus. To exclude neutralization of influenza-ova by antibodies generated against NP-ova, a neutralization assay was performed. As a positive control for neutralizing antibodies, serum collected on day 10 from mice infected with 50 $TCID_{50}$ PR8 influenza was used. Serum from mice immunized as described in Fig. 1 with NP-ova and CpG or ova and CpG was isolated on day 50 before infection with influenza-ova, and preincubated with the virus. MDCK cells were then added to the virus/serum mixture and cultured for 72 h. (*A*) Viral RNA copy numbers in the wells were measured by real-time PCR after total RNA isolation. Results for 1:50 serum dilution are presented as mean ± SD. (*B*) Alternatively, chicken red blood cells were added to the virus and MDCK cultures and the presence of agglutination was determined. Results are presented as the highest serum dilution factor at which no agglutination was observed. A dash represents no neutralization; i.e., agglutination was observed for all serum dilutions tested. Experiments were repeated two times with serum from four different mice per group.

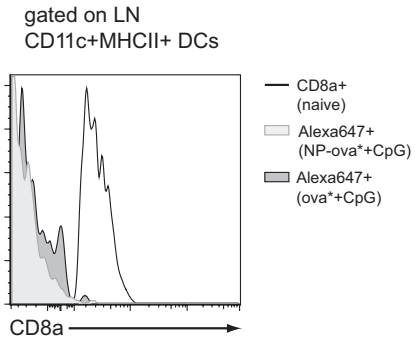


Fig. S5. Ova reaches the LN within DCs migrating from the lung. Twenty-four hours after delivery of NP-ova* plus CpG or ova* plus CpG, CD8a expression of LN Alexa 647^{+} CD11c^{+} MHC II^{+} was analyzed. As a positive control, CD11c^{+} MHCII^{+} CD8a^{+} DCs of a naive mouse LN are shown in the histogram overlay. Experiment was repeated two times with three mice per group.