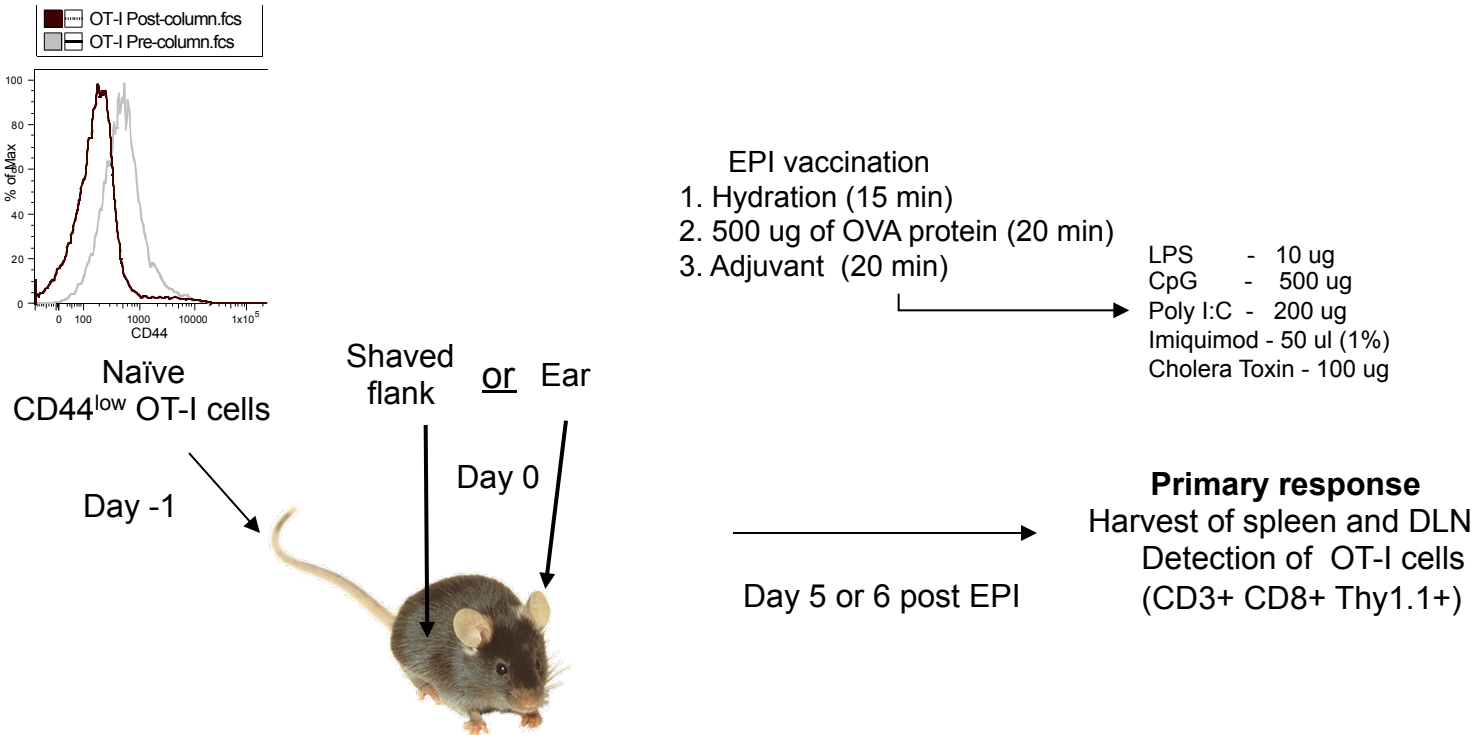
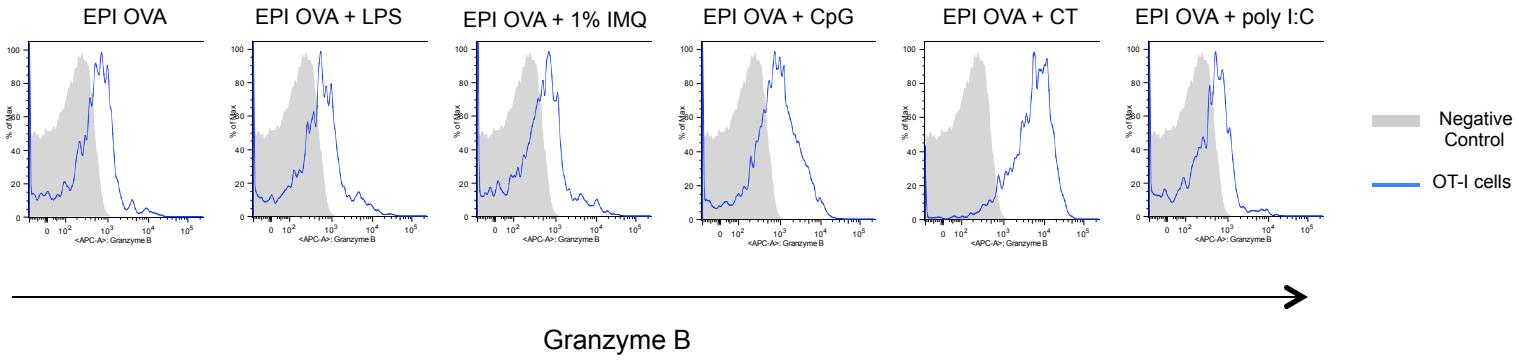
Supplementary Figure 1



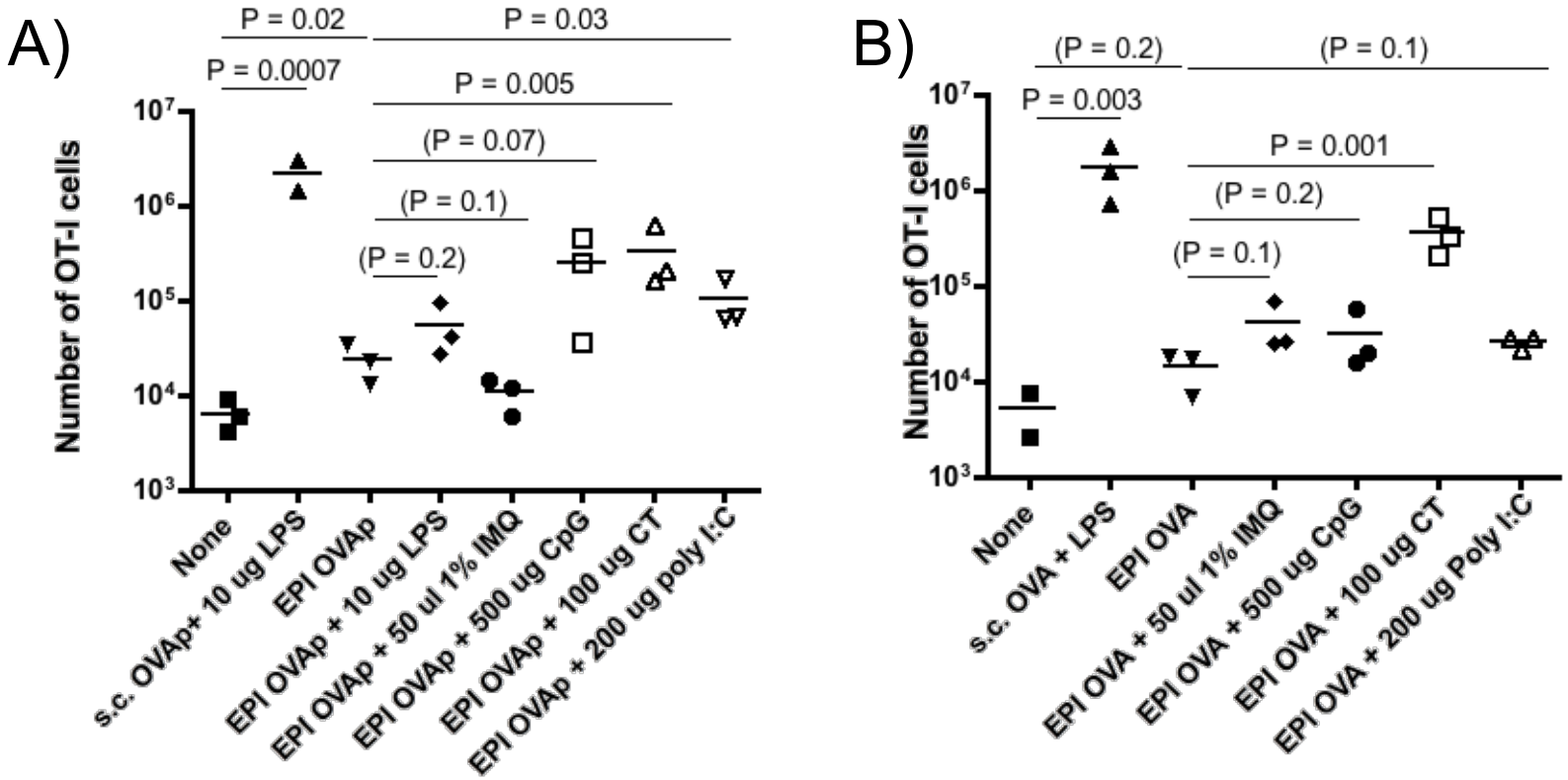
Supplementary Figure 1. **Model of epicutaneous vaccination.** C57BL/6 mice received 2.5 X 10^5 naive OT-I cells one day before immunization. Animals were vaccinated on the unshaved ear or on shaved flank. The flank was shaved one day before EPI immunization. On the day of vaccination, animals were anesthetized, and the skin hydrated with water for 15 minutes. On some animals, 500 ug of OVA protein (in PBS) was then applied to the skin and left for 20 minutes, after which the administration site was washed extensively with water. Next, the indicated adjuvants were applied to the same site and left for an additional 20 minutes, following which the skin was washed with water. Note preliminary data indicated that application of commercial preparations of imiquimod (Aldara: 5% imiquimod) caused mouse skin to become serotic and broken, prompting our use of lower concentration creams. As a negative control, animals were anesthetized, hydrated, but neither OVA nor adjuvant was applied., and for positive control mice were immunized s.c. LPS (10 ug) plus OVA (500 ug).

Supplementary Figure 2



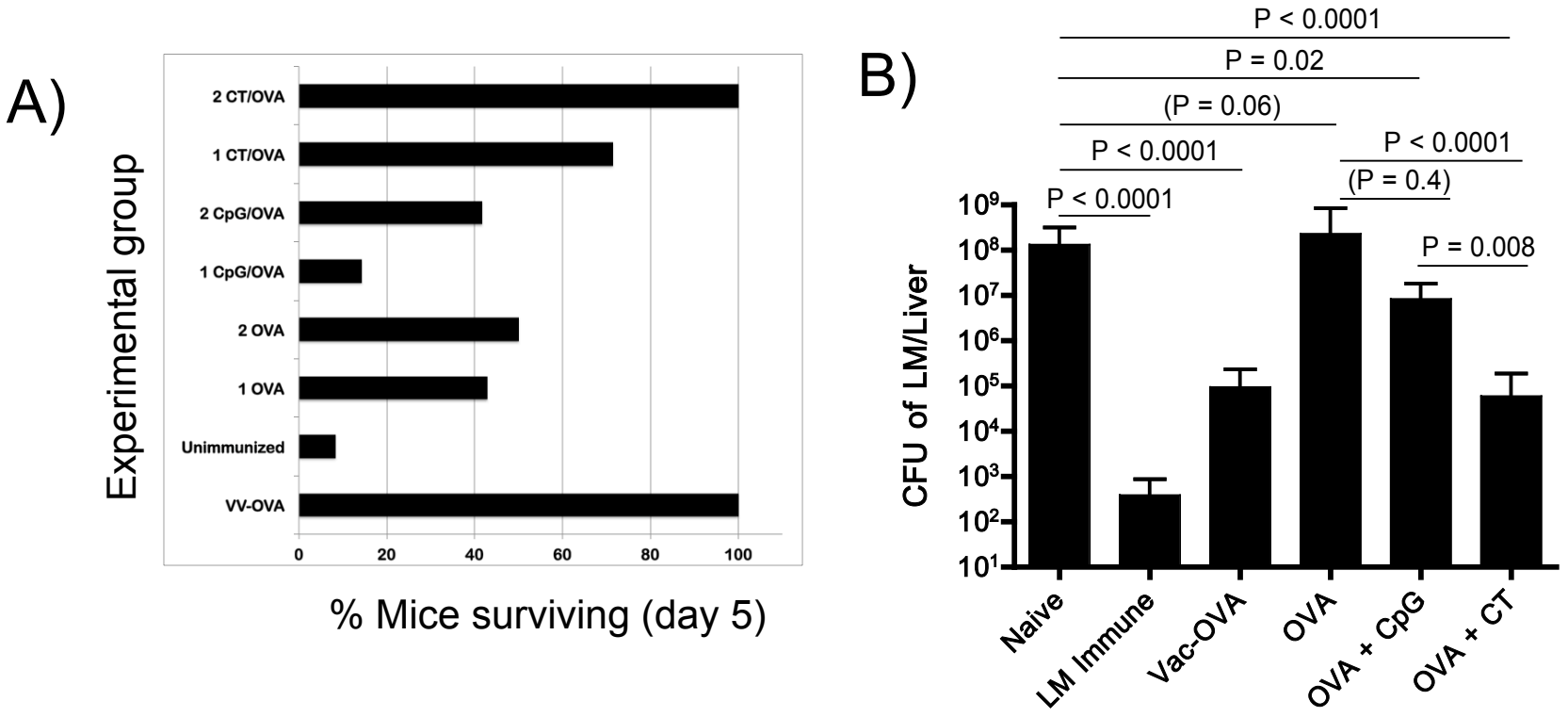
Supplementary Figure 2. **EPI vaccination using Cholera toxin induces high expression of Granzyme B compared with other adjuvants.** C57BL/6 mice received 2.5 X 10^5 naive OT-I cells one day before immunization. Animals were left unimmunized or vaccinated using 500 ug of OVA protein in the presence of different adjuvants: LPS (10 ug), 1% IMQ (50 ul), CpG (500 ug), CT (100 ug), poly I:C (200 ug) as indicated. At day six, granzyme B expression was evaluated in expanded OT-I cells (blue line). The negative control (grey shaded histogram) represents staining of OT-I cells from unimmunized mice. Abbreviations: OVA: ovalbumin; LPS:lipopolysaccharide, IMQ: imiquimod, CT: cholera toxin. These data are representative of at least three independent experiments.

Supplementary Figure 3



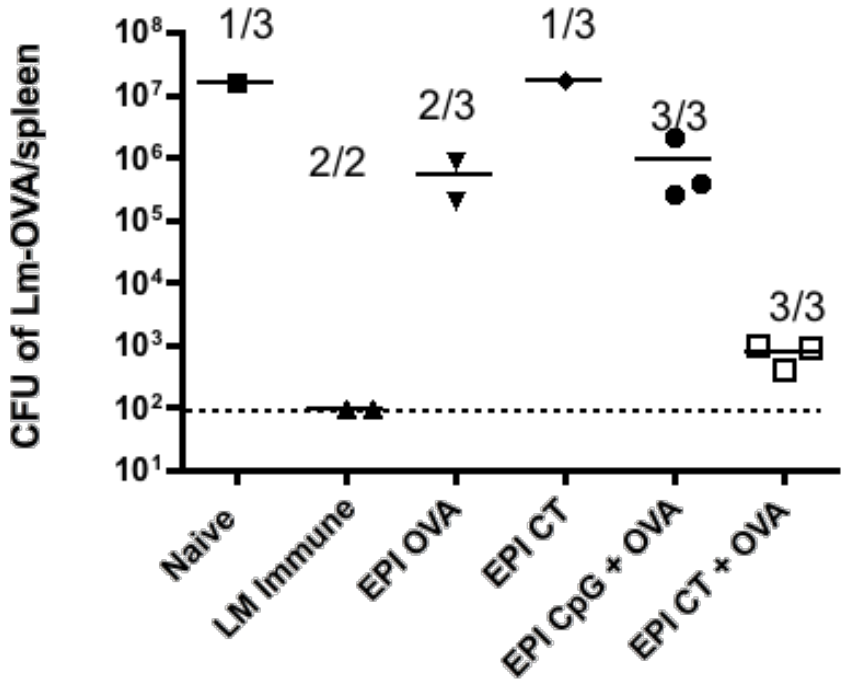
Supplementary Figure 3. **EPI immunization with OVA peptide and OVA protein using various adjuvants.** C57BL/6 mice received 2.5 X 10^5 naive OT-I cells one day before immunization. (A) Animals were vaccinated using 10 ug of OVA peptide (SIINFEKL) in presence or absence of different adjuvants: Cholera toxin (CT), LPS, Imiquimod (IMQ), CpG, or poly I:C as indicated. As positive controls, a group of mice were immunized s.c. with OVAp plus 10 ug LPS. Expansion of OT-I cells in spleen was determined on day 6 after immunization. Each symbol represents an individual mouse. These data are representative of at least three similar experiments. (B) Animals were shaved on the flank one day before EPI immunization and were primed using OVA protein and the indicated adjuvants on flank skin. Expansion of OT-I cells was determined 6 days post-immunization. Abbreviations: s.c. subcutaneous; EPI: epicutaneous; OVA: ovalbumin (500 ug); IMQ: imiquimod, CT: cholera toxin. B6 OT-I: mice that received only OT-I cells., neither OVA nor adjuvant. Each symbol represents an individual mouse. These data are representative of three similar experiments.

Supplementary Figure 4



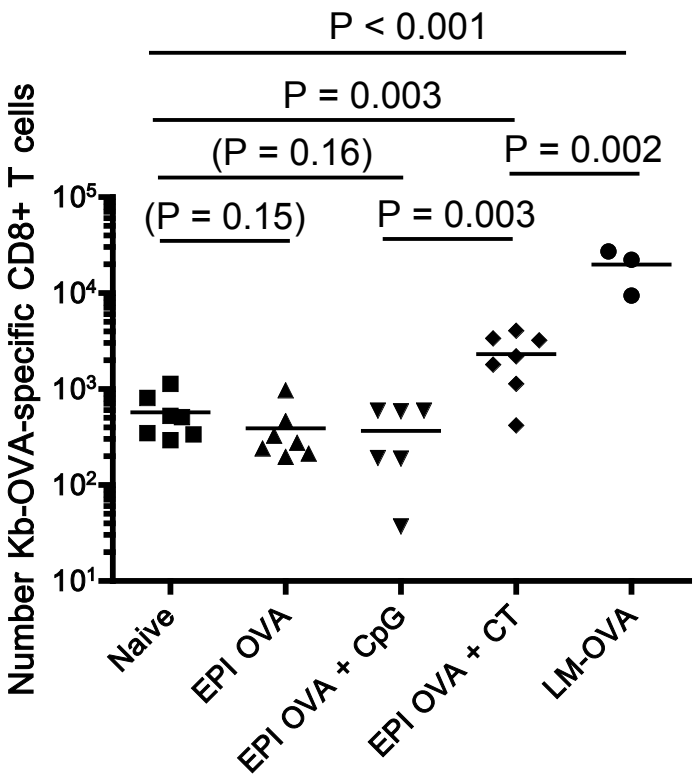
Supplementary Figure 4. Protective CD8 T cell responses generated by epicutaneous prime/boost vaccination. C57BL/6 were epicutaneously vaccinated on the ear with OVA protein, with or without CT or CpG as adjuvant as described in Material and Methods. Mice immunized previously with VV-OVA were used as a positive control for protective immunity. (A) Mice were infected with 1.0 - 2.0 x 10^5 CFU of LM-OVA and the percentage of mice surviving at day 5 post infection is shown. The x axis indicates whether the mice received OVA alone, with CpG or with CT. The number prior to the immunization key indicates whether the mice were immunized once ("1") or were primed and boosted ("2"). These data arose from studies primarily aimed at determining protective immunity in terms of reduced LM-OVA CFU in the spleen and liver. Data are compiled from 2-4 experiments (depending on group) with 2-4 mice per group. For the compiled experiments the number of mice per group ranged from 6 to 12 animals. In (B) mice were primed and boosted by EPI using OVA and the adjuvants listed. The mice were challenged with 1.0 x 10^5 CFU LM-OVA and Listeria CFU in the liver determined on day 5 post-infection. For details see Figure 1B of the main figures.

Supplementary Figure 5



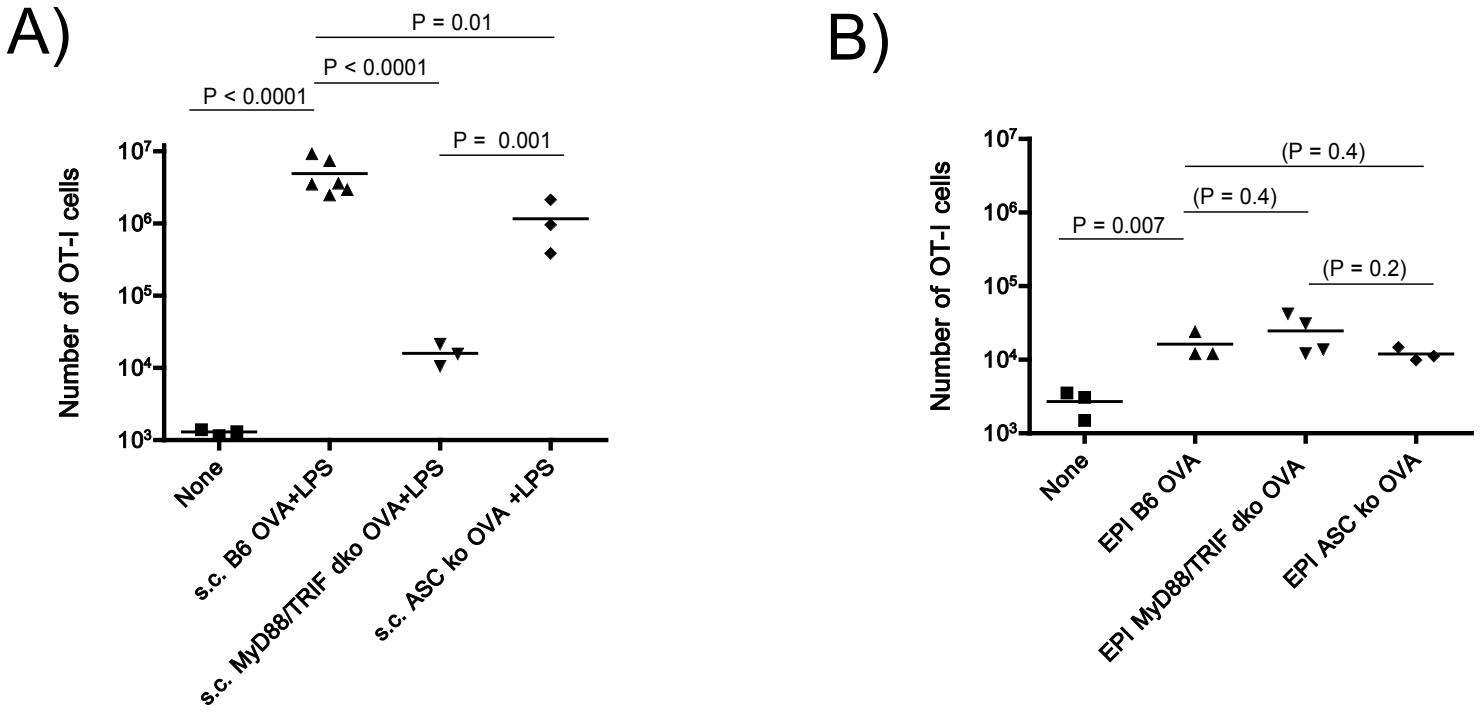
Supplementary Figure 5. **Epicutaneous immunization with CT alone does not lead to protective immunity against Listeria.** C57BL/6 were primed and boosted (30 days later) by EPI with OVA protein alone ("EPI OVA"), CT alone ("EPI CT"), or with OVA protein and CpG or CT, as indicated in the final 2 columns. Mice immunized previously with LM-OVA was used as a positive control of protection, using the scheme of vaccination mentioned in Material and Methods. Thirty days after the last immunization, mice were challenged with LM-OVA, and bacterial counts in the spleen determined five days later. Numbers above each column indicate how many mice in each group survived to the day of sacrifice. Similar results were obtained in an independent experiment.

Supplementary Figure 6



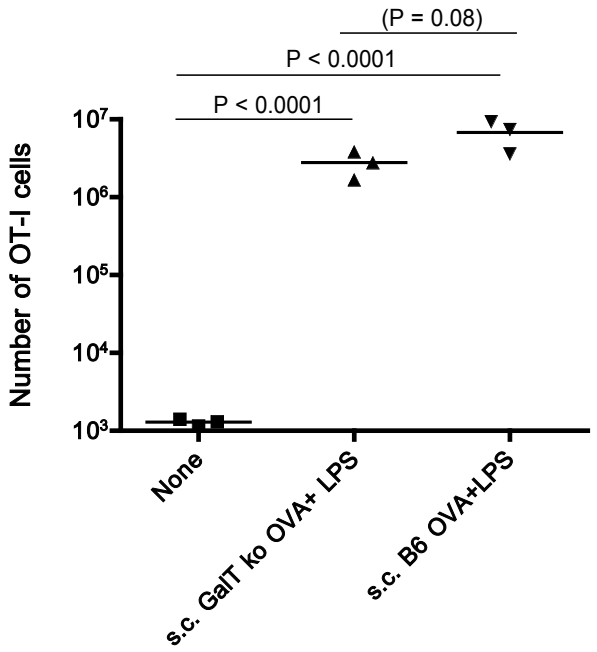
Supplementary Figure 6. EPI using CT induces a long-lived expansion of antigen-specific CD8 T cells.
Normal C57BL/6 were primed and boosted by epicutaneous immunization on the ear skin, using OVA protein, with or without CT or CpG as adjuvants. As controls, naïve mice and mice infected with LM-OVA >30 days earlier was included. More than 30 days after the final immunization, spleens and lymph nodes were recovered and subjected to Ova/K^b tetramer enrichment. The absolute number of Ova/K^b specific CD8 T cells was determined. Data are compiled from 2 separate experiments and are representative of an additional independent experiment.

Supplementary Figure 7



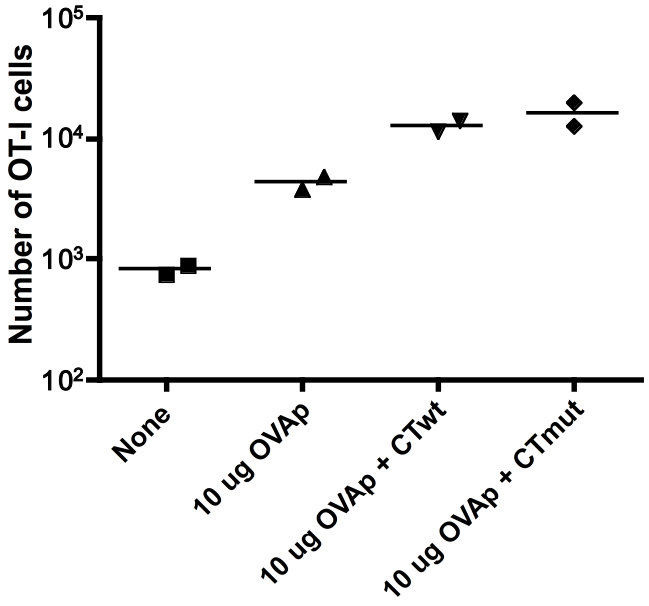
Supplementary Figure 7. **OT-I priming using LPS but not OVA protein alone is inhibited in MyD88/TRIF dko and ASC KO host mice.** B6, MyD88/TRIF dKO and ASC KO mice were adoptively transferred with naïve OT-I cells one day before immunization. In (A) mice were primed with 10 ug of LPS and 500 ug of OVA protein s.c. In (B) mice were primed with OVA protein alone via EPI. Five days later, expansion of OT-I cells were determined. Groups labeled "none" received OT-I cells but were not immunized. These data are representative of three independent experiments.

Supplementary Figure 8



Supplementary Figure 8. **GalT KO mice support OT-I immune responses to LPS+OVA.** GalT KO mice adoptively transferred with naïve OT-I cells were s.c immunized on the following day with LPS (10 ug) and 500 ug of OVA protein. Five days post-immunization, OT-I cell numbers were detected in spleen. These data are representative of three similar experiments.

Supplementary Figure 9



Supplementary Figure 9 . **CT enzymatically inactive and CT wt are able to induce Ag-specific expansion.** B6 mice were adoptively transferred with naive OT-I cells one day before immunization. Then, 10 ug of OVA peptide (SIINFEKL) followed by 10 ug of CTwt or CT mut administration were used for immunization. Five days later,OT-I expansion was evaluated. These data are representative of two independent experiments and similar results were obtained in an independent experiment using 3ug instead of 10ug OVAp.