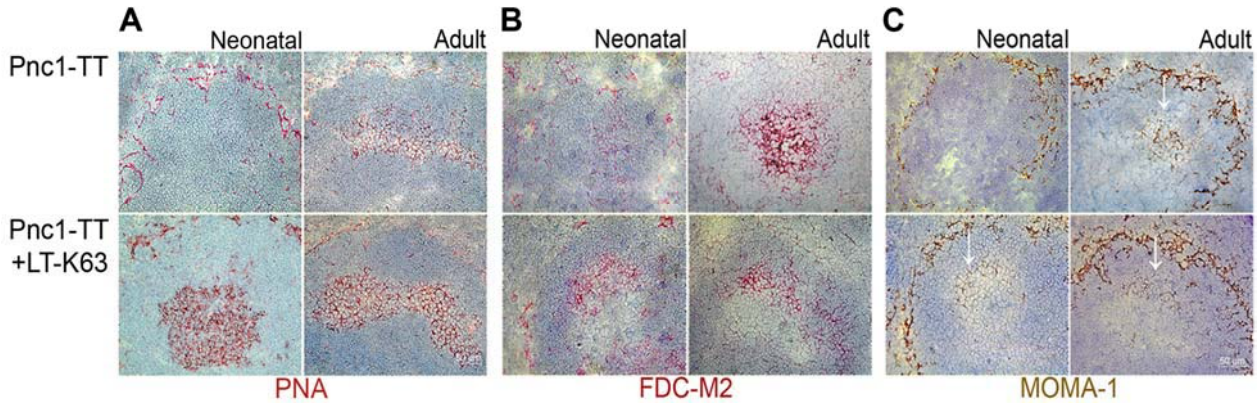
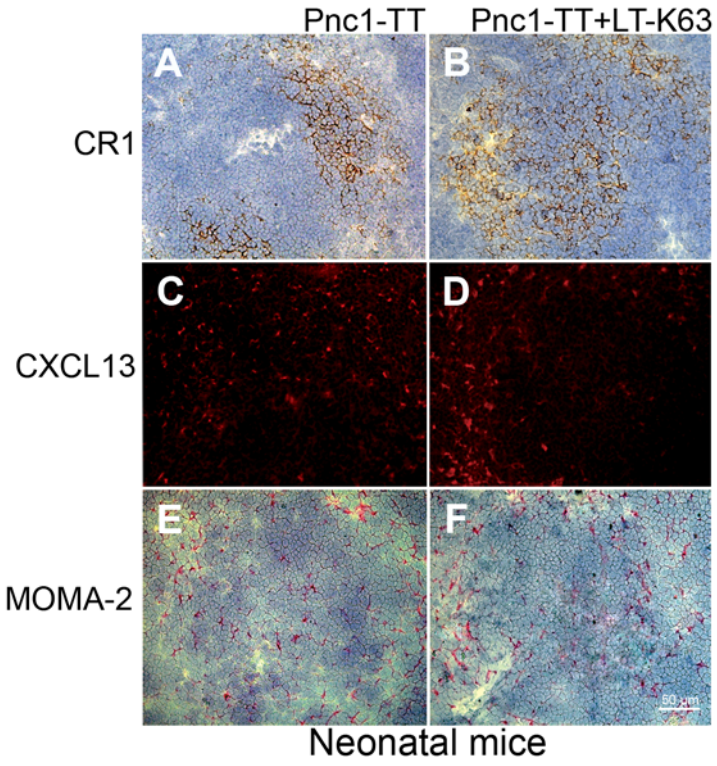
Data Supplement

Supplemental Figures



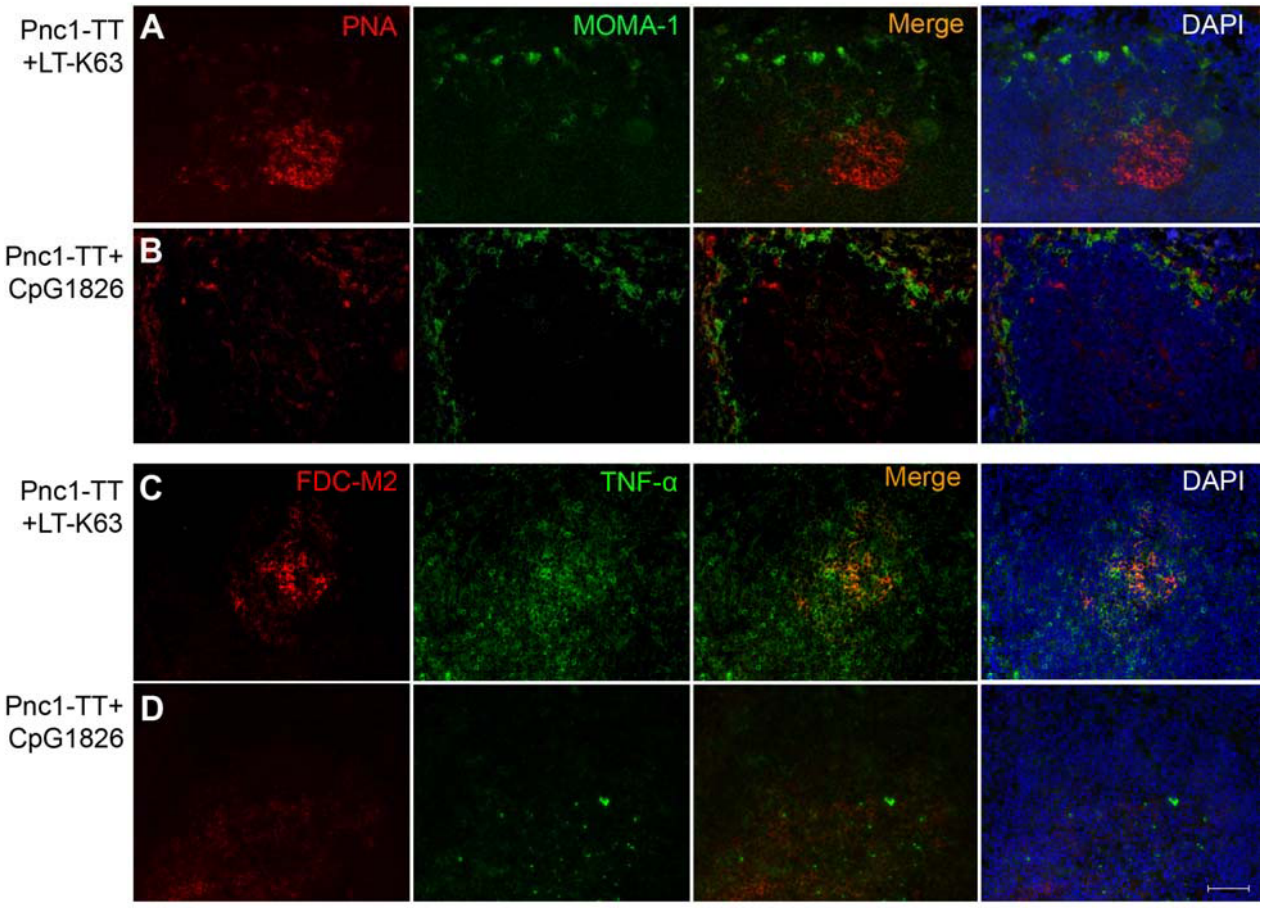
Supplemental Figure 1. LT-K63 overcomes limited germinal center (GCs) induction in early life by accelerating the maturation of FDCs and migration of MOMA-1$^+$ MMM into activated GCs.

To detect activated germinal centers, mature FDCs and marginal metallophilic macrophages spleen sections were stained with PNA (A), FDC-M2 (B), MOMA-1 (C), respectively, 14 or 10 days after priming of neonatal and adult mice with 0.5 μg Pnc1-TT (upper panels) or 0.5 μg Pnc1-TT with 5.0 μg LT-K63 (lower panels). 7 μm spleen sections were prepared from four different levels in the spleen, the first started 700 μm into the tissue and the levels were separated by 210 μm. One representative section from each group is shown. Magnification x40 and scale bar is 50 μm. The results shown in A–D are from three experiments for neonatal and two experiments for adult mice (n=16 per group) with eight mice per group in each experiment.



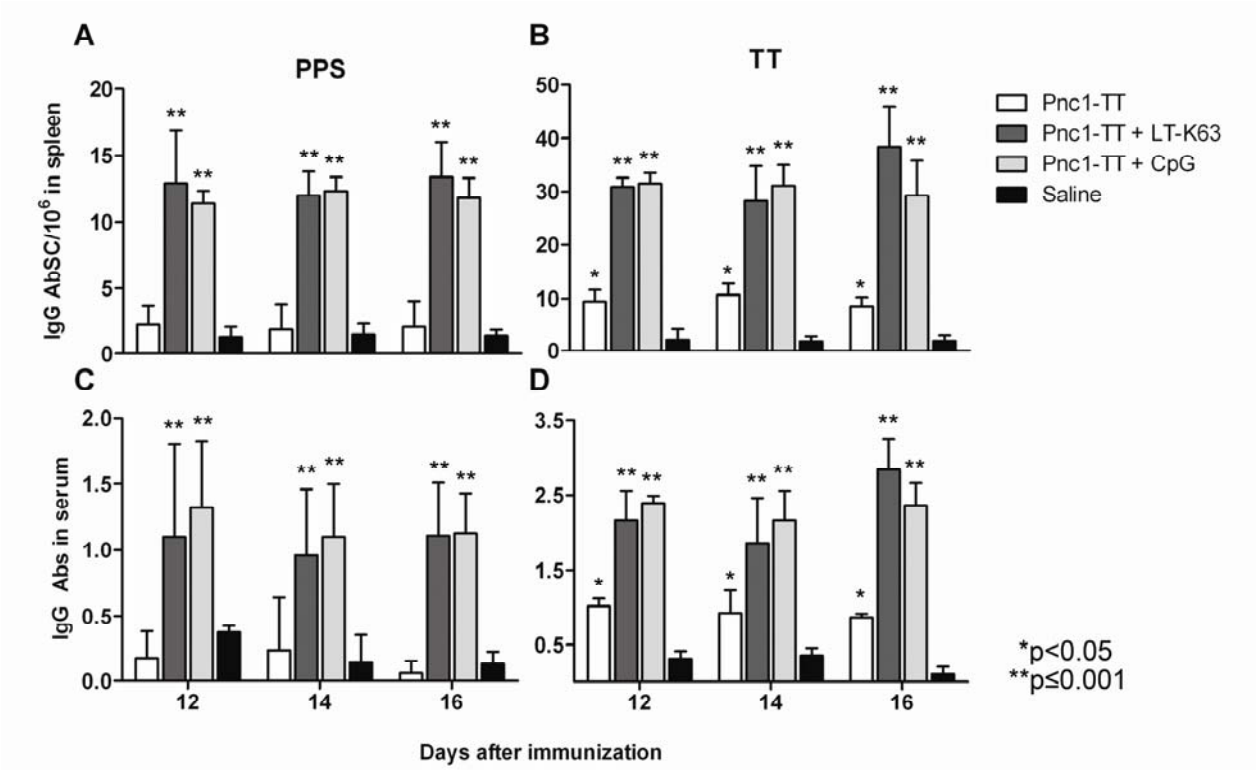
Supplemental Figure 2. CR1$^+$, CXCL13$^+$ FDC network and MOMA-2$^+$ macrophages were detected in mice immunized with Pnc1-TT as neonates.

To detect mature FDCs spleen sections were stained with CR1 (top panel, A and B), anti-CXCL-13 (middle panel, C and D) and MOMA-2 (bottom panel, E and F) to detect C3 producing macrophages, 14 days after priming of neonatal with 0.5 µg Pnc1-TT (left panel) or 0.5 µg Pnc1-TT+5.0 µg LT-K63 (right panel). 7 µm spleen sections were prepared from four different levels in the spleen, the first started 700 µm into the tissue and the levels were separated by 210 µm. One representative section from each group is shown. Magnification x40 and scale bar is 50 µm. The results shown in A–D are from three experiments for neonatal (n=24 per group) with eight mice per group in each experiment.



Supplemental Figure 3. A weak TNF-α staining was detected in follicles 16 days after mice were immunized with Pnc1-TT+CpG1826 as neonates.

Spleen sections were stained with (A and B) PNA (red), (A and B) MOMA-1 (green), (C and D) FDC-M2 (red), (C and D) Ab to TNF-α (green) or DAPI to visualize the nuclei (blue) 16 days after priming of neonatal mice with 0.5 µg Pnc1-TT+5.0 µg LT-K63 (A and C) or 0.5 µg Pnc1-TT+20.0 µg CpG1826 (B and D). 7 µm spleen sections were prepared from four different levels in the spleen, the first started 700 µm into the tissue and the levels were separated by 210 µm. One representative section from each group is shown. Magnification x40 and scale bar is 50 µm. Results shown in A–D are from two experiments in neonatal mice with 9 mice per group.



Supplemental Figure 4. LT-K63 and CpG1826 enhanced vaccine specific IgG$^+$ AbSCs in spleen and Abs in serum when co-administered with Pnc1-TT in neonatal mice.

PPS-1- and TT-specific IgG$^+$ AbSC measured by ELISPOT shown as number of spots (mean±SD) per 10^6 cells (A and B) and PPS-1- and TT-specific IgG levels (mean EU/mL±SD) in serum measured by ELISA (C and D). Results are shown for neonatal mice immunized with 0.5 μg Pnc1-TT alone (white column), with 5.0 μg of LT-K63 (dark grey column) or with 20 μg of CpG1826 (light grey column) or saline (black column). Statistical differences between test groups and control mice (that received saline) are shown; * $p<0.05$; ** $p\leq 0.001$. Results in A–D are from one experiment with 9 mice per group.