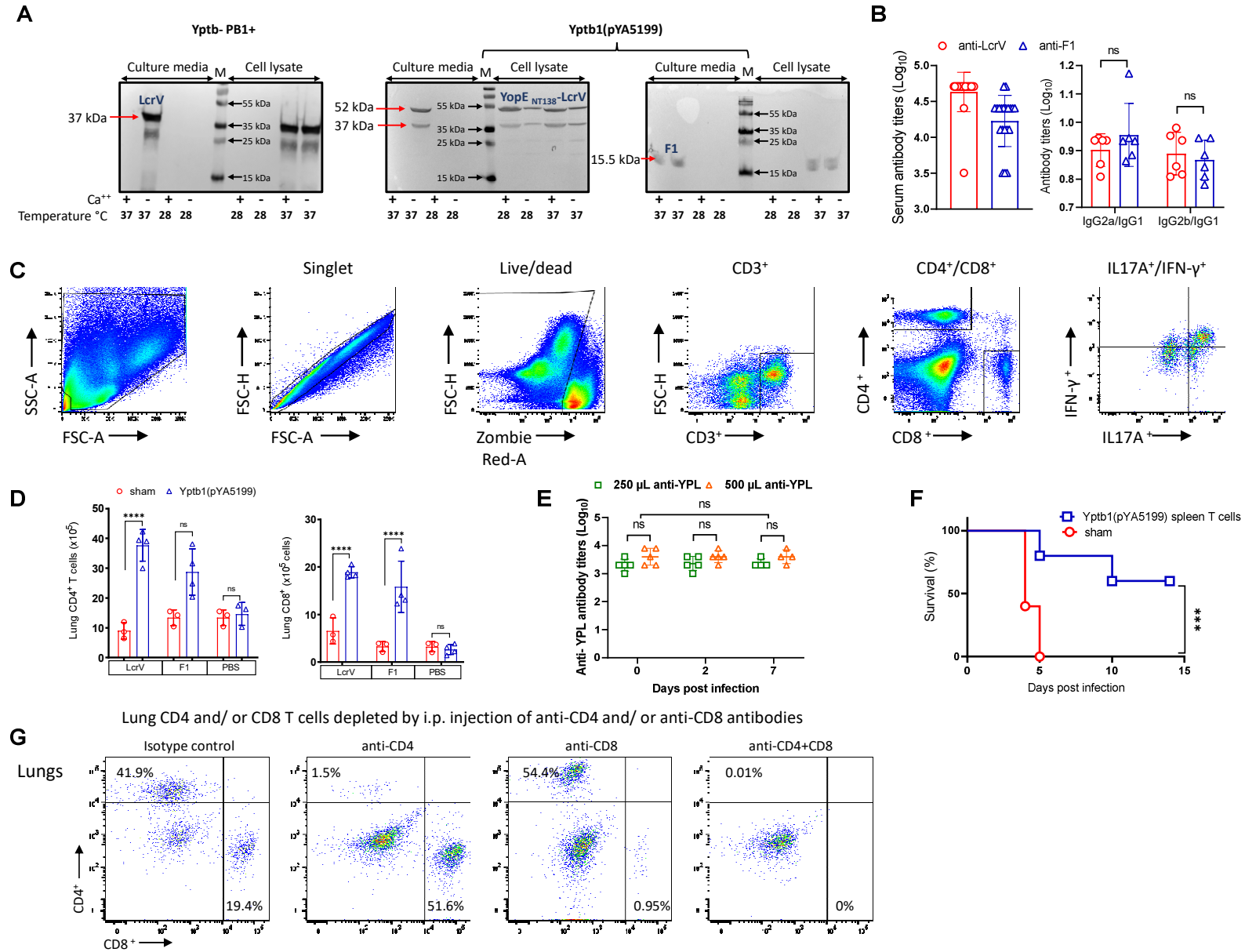
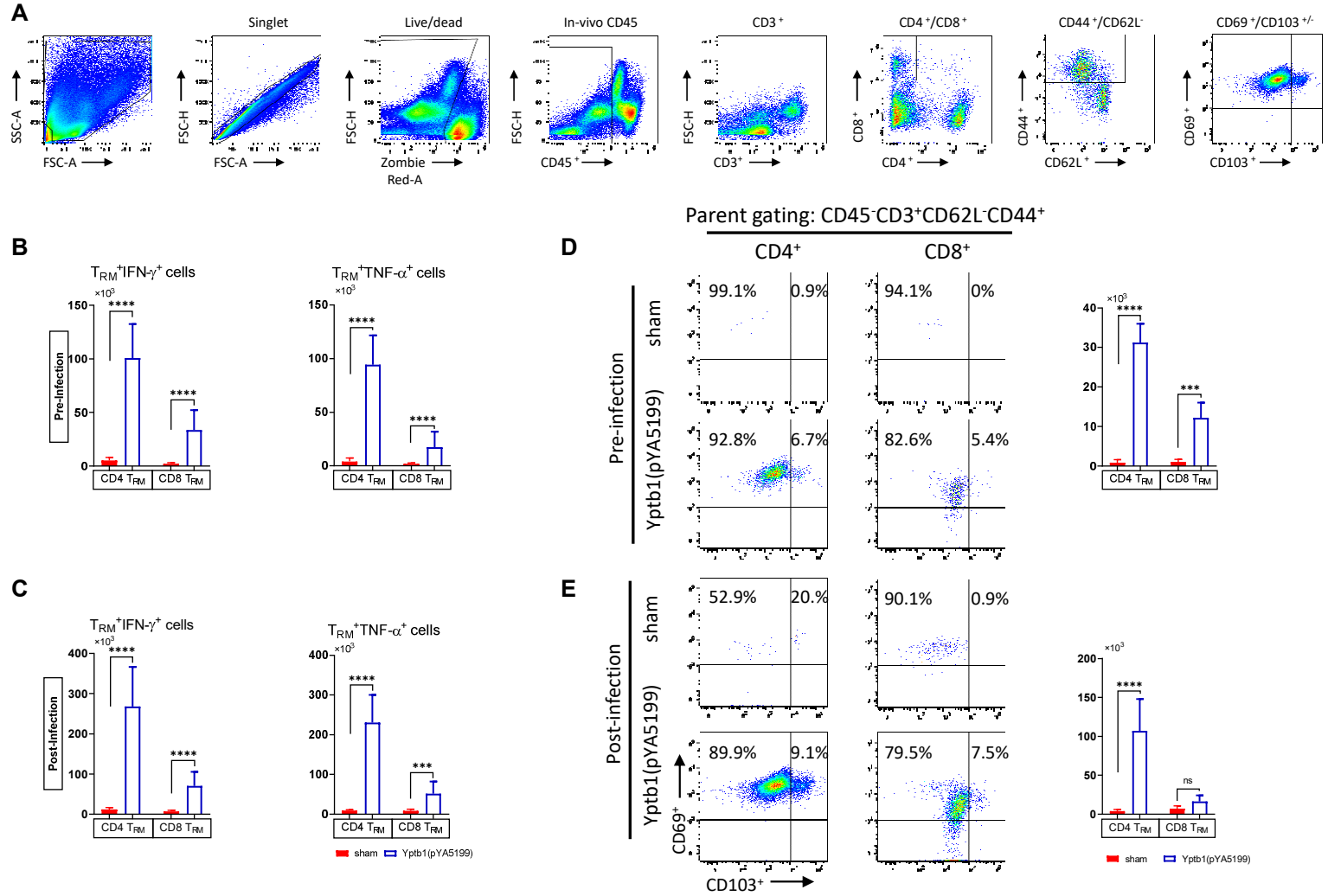


# Figure S1



**Figure S1. Characterization and immunogenicity of Yptb1(pYA5199) in Swiss webster mice.** (A) Western blot analysis of YopE<sub>NT138</sub>-LcrV, LcrV, and F1 synthesis in Yptb1(pYA5199) that was cultured in a medium replete with or deprived of Ca<sup>2+</sup>. (B) Antibody responses to *Y. pestis* LcrV and F1 antigens in Yptb1(pYA5199)-immunized mice (n=15, mixed males and females) at 28 dpv. Serum total IgG titers to LcrV and F1 and ratios of IgG2a/IgG1 and IgG2b/IgG1 to a respective antigen. (C) Gating strategy for CD4<sup>+</sup>/CD8<sup>+</sup> T cells with IFN-γ, TNF-α, and/or IL-17A production. (D) Quantitative analysis of LcrV- or F1-specific CD4<sup>+</sup> and CD8<sup>+</sup> T cells in the lungs of Yptb1(pYA5199)-immunized mice (n=6 females) at 42 dpv. (E) Serum anti-YPL antibody titers in naïve mice (n=5 females) that adoptively received 250 μl and 500 μl of serum from Yptb1(pYA5199)-immunized mice were measured at days 0, 2, and 7 post-pulmonary *Y. pestis* challenge (10 LD<sub>50</sub>). (F) The protective role of spleen T cells against pneumonic plague. Naïve irradiated (5 Gy) mice were i.v. injected with purified spleen (CD4<sup>+</sup> + CD8<sup>+</sup>) T cells isolated from Yptb1(pYA5199)-immunized and sham mice at 42 dpv. At 24 h post-transfer, mice (n=5 females) were intranasally infected with 10 LD<sub>50</sub> of *Y. pestis*, and survival was recorded for 14 days. (G) A representative flow plot showing depletion of CD4<sup>+</sup> and CD8<sup>+</sup> T cells in the lungs by i.p. injection of 500 μg of mouse anti-CD4 and anti-CD8 monoclonal antibodies (mAbs). Each symbol in the bar graph represents a data point obtained from an individual mouse. Data obtained from experiments are presented as the mean ± SD. The statistical analysis is described in the Materials and Methods.

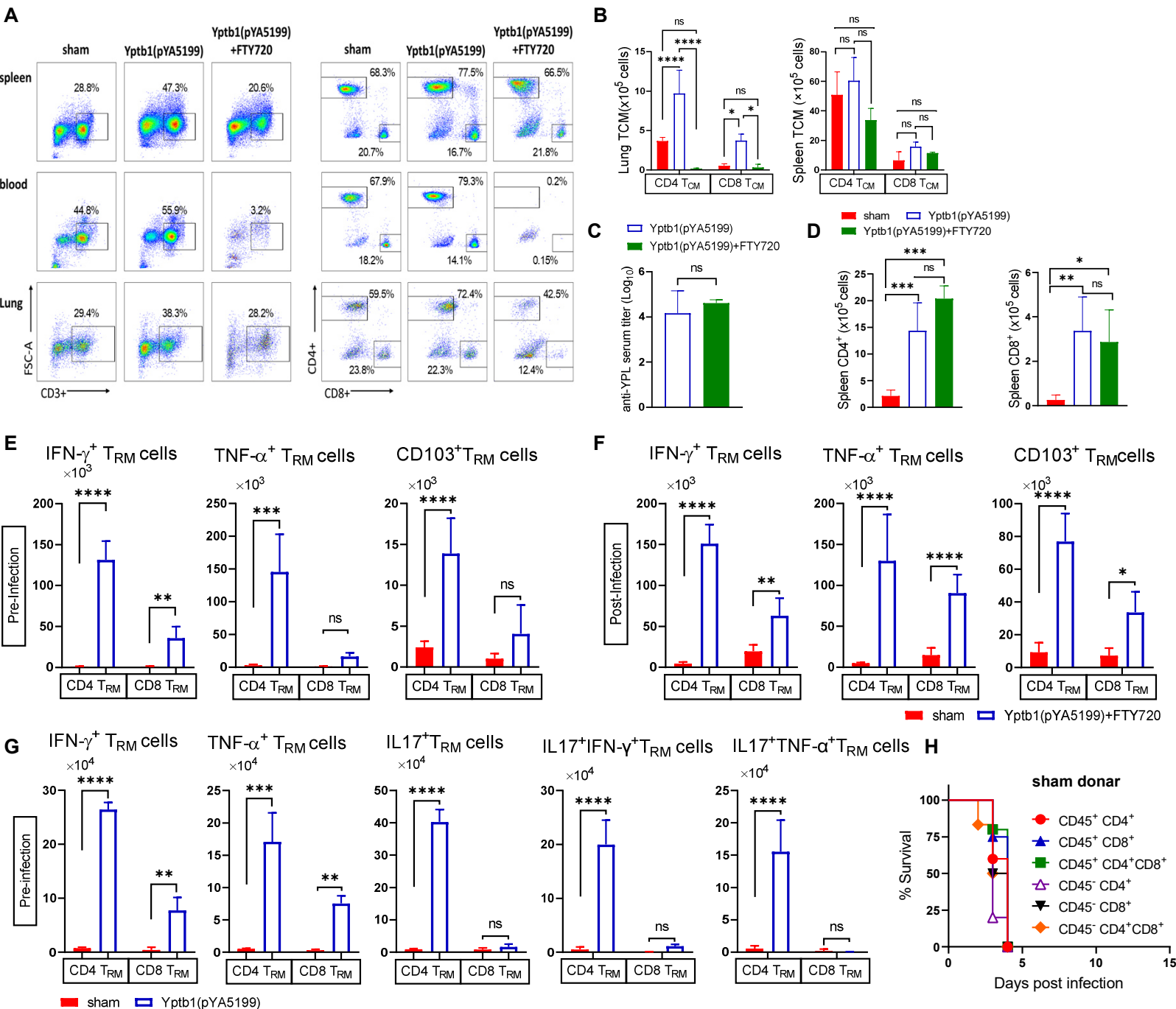
# Figure S2



**Figure S2: Activation of lung CD4<sup>+</sup> and CD8<sup>+</sup>  $T_{RM}$  cells in Yptb1(pYA5199)-immunized mice.** (A) A gating strategy for  $T_{RM}$  cells in the lung. The number of CD4<sup>+</sup> and CD8<sup>+</sup>  $T_{RM}$  cells expressing IFN- $\gamma$ <sup>+</sup> or TNF- $\alpha$ <sup>+</sup> before infection (B) and after infection (C). Lung single cells isolated from mice (n=5 females) at 42 dpv (pre-infection) or at 2 dpi with *Y. pestis* (post-infection) were stained for  $T_{RM}$  surface markers (CD45<sup>+</sup>, CD4<sup>+</sup>/CD8<sup>+</sup>, CD44<sup>+</sup>, and CD69<sup>+</sup>) and intracellular cytokines (IFN- $\gamma$  or TNF- $\alpha$ ) for flow cytometry. (D) Representative flow plots showing lung CD4<sup>+</sup> and CD8<sup>+</sup>  $T_{RM}$  cells with CD103<sup>+</sup> expression in pre-infected mice (n=6 females). (E) Lung CD4<sup>+</sup> and CD8<sup>+</sup>  $T_{RM}$  cells with CD103<sup>+</sup> expression at 2 dpi. Bar graphs showing quantitative analysis of CD4<sup>+</sup> or CD8<sup>+</sup>  $T_{RM}$  cells expressing CD103<sup>+</sup>. Data obtained from experiments were pooled and analyzed and are presented as the mean  $\pm$  SD. The statistical analysis is described in the Materials and Methods.

# Figure S3

sham Yptb1(pYA5199)  
Yptb1(pYA5199)+FTY720

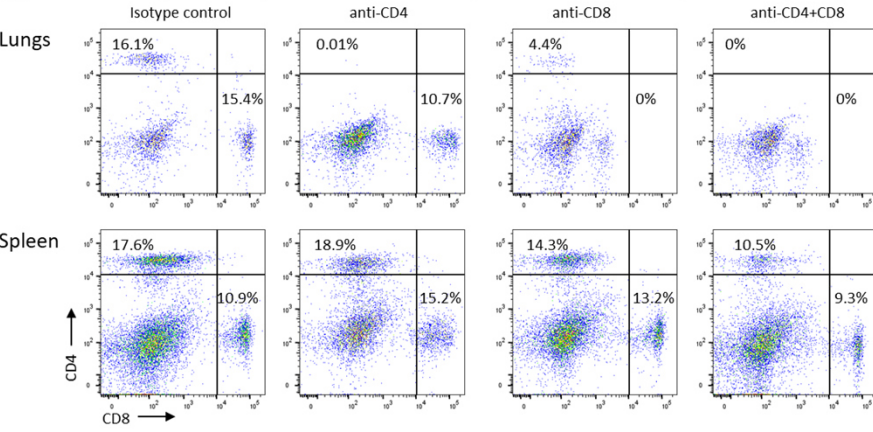


**Figure S3. Resident and central memory T cells in Yptb1(pYA5199)-immunized mice treated with or without FTY720.** (A) Flow cytometric analysis of the ability of FTY720 treatment to block circulating T cells. Flow plot showing CD3<sup>+</sup>, CD4<sup>+</sup>, and CD8<sup>+</sup> T cells in the blood, lung, and spleen obtained from sham, Yptb1(pYA5199)-immunized, and FTY720-treated Yptb1(pYA5199)-immunized mice (n=6 females). (B) Quantitative analysis of T<sub>CM</sub> cells (CD62L<sup>+</sup>) in the lung and spleen of sham mice and immunized mice with and without FTY720 treatment. (C) Total anti-YPL IgG titers in Yptb1(pYA5199)-immunized mice (n=10, mixed males and females) with and without FTY720 treatment. (D) Spleen CD4<sup>+</sup> and CD8<sup>+</sup> T-cell responses in Yptb1(pYA5199)-immunized mice (n=6 females) with and without FTY720 treatment at 42 dpv. (E) Lung CD4<sup>+</sup> and CD8<sup>+</sup> T<sub>RM</sub> cells (CD45<sup>-</sup> CD44<sup>+</sup> CD69<sup>+</sup> CD4<sup>+</sup>/CD8<sup>+</sup>) expressing IFN-γ, TNF-α, or CD103 in sham and Yptb1(pYA5199)+FTY720 mice (n=5 females) at 42 dpv (preinfection). (F) Lung CD4<sup>+</sup> and CD8<sup>+</sup> T<sub>RM</sub> cells expressing IFN-γ, TNF-α, or CD103 at 2 dpi (postinfection). (G) Lung T cells FACS sorted at 42 dpv were induced *in vitro* with 20 μg of LcrV for 48 h. Cells were surface stained with CD4<sup>+</sup> and CD8<sup>+</sup> T cells expressing IL17A, IFN-γ, and TNF-α markers. (H) Adoptive transfer of FACS-sorted lung circulating (CD45<sup>+</sup>) T cells or T<sub>RM</sub> cells (CD45<sup>-</sup>) from sham mice. At 24 h post intratracheal administration, recipient mice (n=5 females) were i.n. challenged with 10 LD<sub>50</sub> of *Y. pestis* and survival was recorded for 14 days. Data obtained from experiments were pooled and analyzed and are presented as the mean ± SD. The statistical analysis is described in the Materials and Methods.

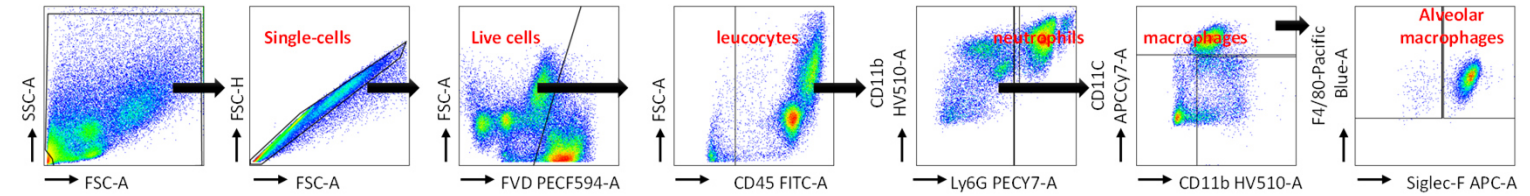
# Figure S4

**A**

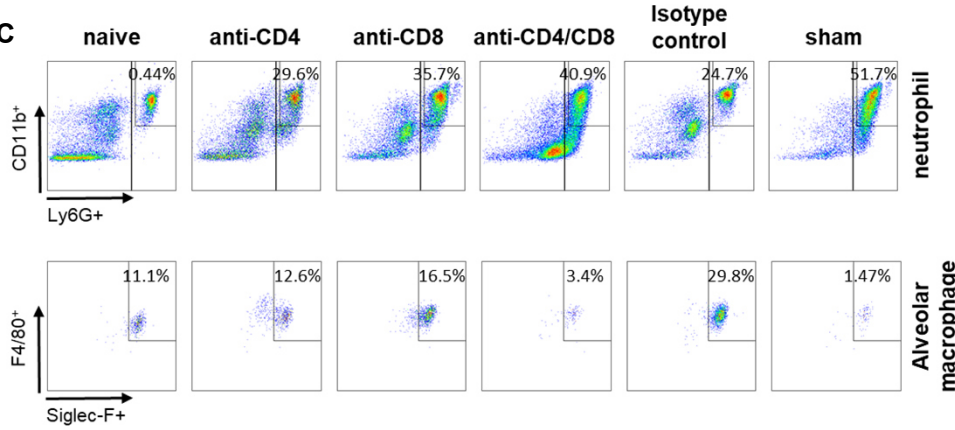
Lung CD4 and/or CD8 T cells depleted by i.n. injection of anti-CD4 and/or anti-CD8 antibodies



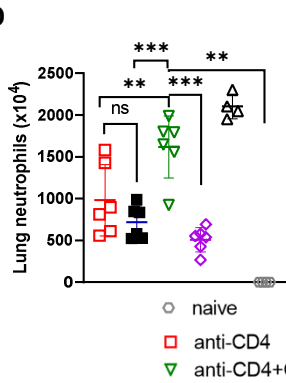
**B**



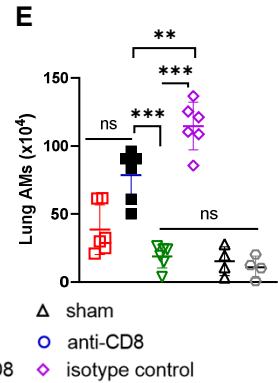
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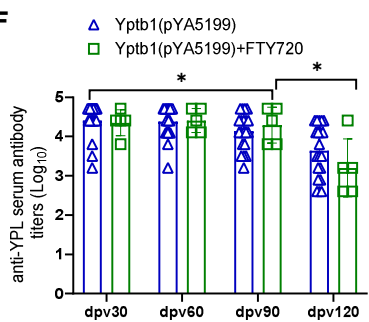
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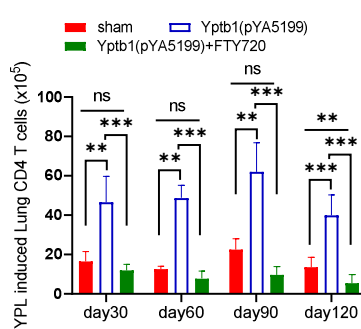
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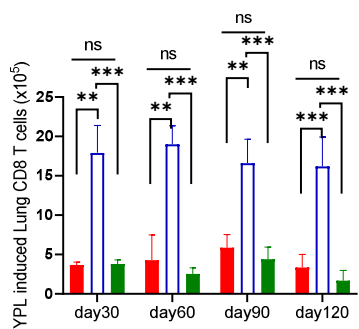
**F**



**G**



**H**



**Figure S4. Alterations in lung neutrophil and alveolar macrophage populations in T cells depleted mice and the assessment of long-term immune responses in Yptb1(pYA5199) and Yptb1(pYA5199)+FTY720 mice. (A)** A representative flow plot showing depletion of CD4<sup>+</sup> and CD8<sup>+</sup> T cells in the lungs by i.n. injection of 200  $\mu$ g of mouse anti-CD4 and anti-CD8 monoclonal antibodies (mAbs). **(B)** Gating strategy to analyze neutrophil and macrophage populations in the lung and BAL fluid. **(C)** Representative flow plots showing the percentages of lung neutrophil and alveolar macrophage populations in naïve, sham, anti-CD4 mAb-treated, anti-CD8 mAb-treated, both anti-CD4 and anti-CD8 mAb-treated, and isotype IgG-treated mice (n=6 females.) at 2 dpi. **(D)** The number of lung neutrophils and **(E)** alveolar macrophages (AMs) in Yptb1(pYA5199)+FTY720 mice after different treatments. Sham and naïve mice were considered controls. **(F)** Kinetics of anti-YPL serum antibodies and **(G)** YPL-specific CD4<sup>+</sup> and **(H)** CD8<sup>+</sup> T cells in the lung of Yptb1(pYA5199) and Yptb1(pYA5199)+FTY720 mice (n=10, equal number of males and females) at 30, 60, 90, and 120 dpv. The statistical analysis is described in the Materials and Methods.