frontiers

Supplementary Material

Sex Differences in Immune Protection in Mice Conferred by Heterologous Vaccines for Pneumonic Plague

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Supplementary Data

1.1 Supplementary Tables

Supplementary Tables 1-10 are in separate tabs of the accompanying Excel file.

1.2 Supplementary Figures

Supplementary Figure 1. Median lethal dose (LD_{50}) estimations for *Y. pestis* C12 aerosol challenge in male (black) and female (red) BALB/c mice. LD_{50} values were estimated by probit regressions and graphing was performed in SAS version 9.4. The doses delivered to the male mice to calculate the LD_{50} were approximately $2.13x10^3$, $1.57x10^4$, $3.18x10^5$, and $2.50x10^6$ inhaled CFUs.

Supplementary Figure 2. Examples of regression lines comparing tissue cytokine level to bacterial burden in male and female lungs. The data in **Table 2** was natural log (ln)-transformed and plotted for linear regression. **(A)** In the regression using CFU/g to predict tissue G-CSF level, regression coefficients for females (F) were intercept: -0.10 and slope: 0.28. Regression coefficients for males (M) were intercept: -0.41 and slope: 0.29, statistically similar to females. **(B)** In the regression using CFU/g to predict tissue IL-13 level, regression coefficients for females were intercept: 3.42 and slope: -0.03. Regression coefficients for males were intercept: 1.60 and slope: 0.05, both significantly different from females based on the method of least squares.

Supplementary Figure 3. Cytokine levels 3 dpi in lungs of mice immunized with Δ*caf1* or Δ*yopD*/Δ*caf1* regimens and challenged with aerosolized *Y. pestis* C12. The seven cytokines whose expression in lungs was not significantly correlated with bacterial burden in **Table 2** were plotted to look for differences between sexes and vaccine groups. **(A)** Cytokine levels were compared across groups of mice given Δ*caf1* vaccine regimens and challenged with aerosolized C12 (4.7×10^5 CFU in females, 5.7×10^5 CFU in males). **(B)** Cytokine levels were compared across groups of mice given Δ*yopD*/Δ*caf1* vaccine regimens and challenged with aerosolized C12 (2.0×10^5 CFU in females, 2.4×10^5 CFU in males). Graphs show each data point and lines representing geometric means. * $p <$ 0.05 in Mann-Whitney test comparing males and females in same immunization group.

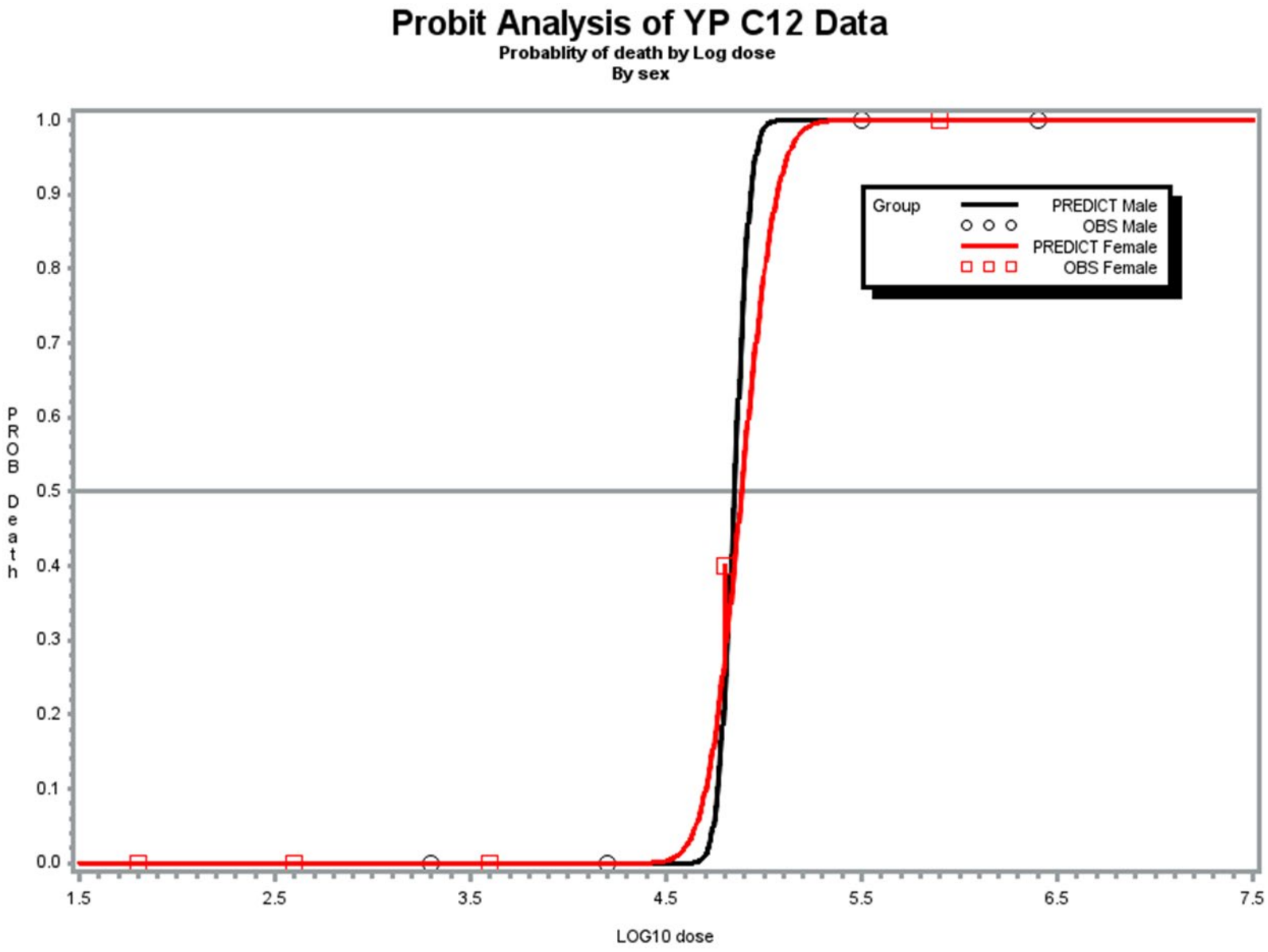
Supplementary Figure 4. Serum levels of complement proteins in mice vaccinated with Δ*caf1* and Δ*yopD*/Δ*caf1* regimens and challenged with aerosolized *Y. pestis* C12. Three dpi, male (M)

and female (F) mice were euthanized; lung and spleen homogenates were plated to quantify bacteria (CFU/g), and ELISA was used to measure serum samples for the anaphylatoxin C3a **(A-C)** and C5b-9 soluble membrane attack complex **(D-F)**. Serum complement protein level for each mouse was plotted against bacterial burden in lungs **(A,B)** and spleens **(D,E)**. Females and males sham-vaccinated with PBS, and females and males that received any of the heterologous vaccine regimens, were compared for serum levels of C3a **(C)** and C5b-9 **(F)**. ** $p < 0.01$ in Mann-Whitney test.

Supplementary Figure 5. ELISpot assays for IFN-γ-secreting splenocytes from mice given Δ*caf1* and Δ*yopD*/Δ*caf1* vaccine regimens, after *ex vivo* re-stimulation with *Y. pestis* antigens. Four weeks post-boost, female (red) and male (blue) mice were euthanized and splenocytes re-stimulated for 24 h with rF1-V **(A,E)**, rV **(B,F)**, or temperature-shifted whole-cell CO92 **(C,G)** and C12 **(D,H)** antigens, followed by quantification of spots representing cells secreting IFN-γ. Splenocytes from mice given Δ*caf1* vaccine regimens are shown in **A-D**; splenocytes from mice given Δ*yopD*/Δ*caf1* vaccine regimens are shown in **E-H**. Graphs show each data point and lines representing geometric means. * $p < 0.05$, * $p < 0.01$, * $p < 0.001$ in Mann-Whitney test.

Supplementary Figure 6. Sample gating strategy for upregulation of CD44 surface expression. Cryopreserved splenocytes were thawed, incubated with 1:200 Mouse FcBlock, stained with surface antibody cocktail, and fixed in 2% formaldehyde. Samples were run on a FACSCanto II and data analyzed in FlowJo v10.8.

Supplementary Figure 1. **Median lethal dose (LD_{50}) estimations for *Y. pestis* C12 aerosol challenge in male (black) and female (red) BALB/c mice.** LD_{50} values were estimated by probit regressions and graphing was performed in SAS version 9.4. The doses delivered to the male mice to calculate the LD_{50} were approximately $2.13x10^3$, $1.57x10^4$, $3.18x10^5$, and $2.50x10^6$ inhaled CFUs.



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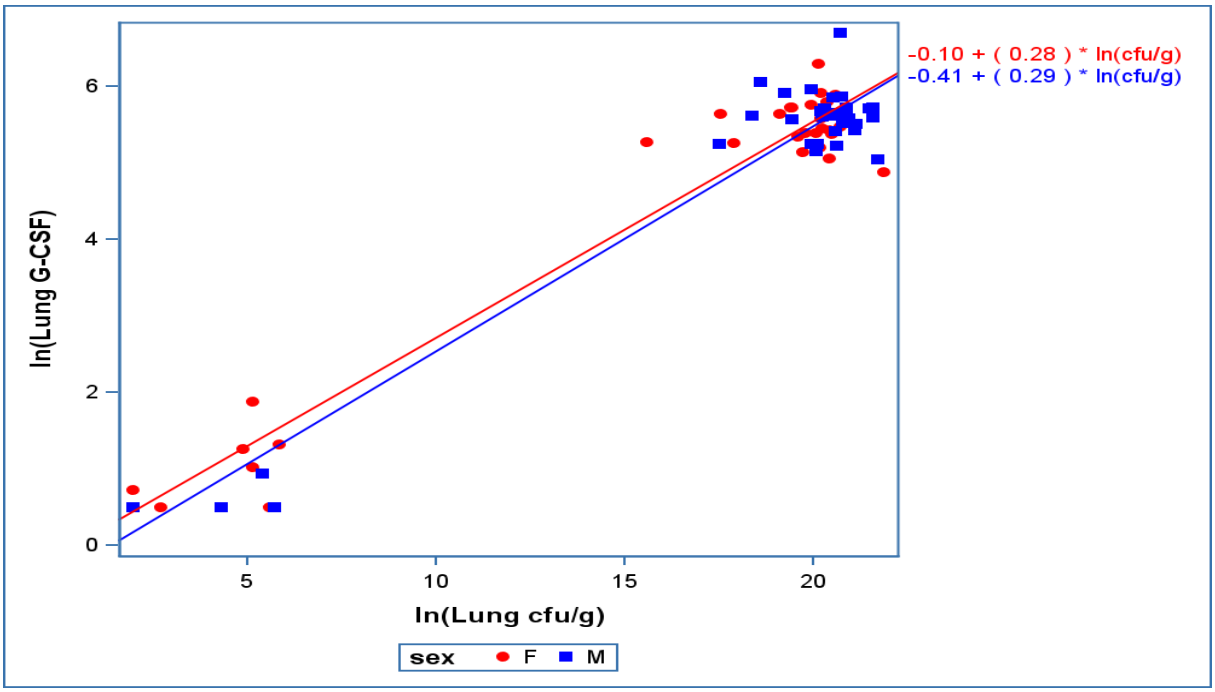
A



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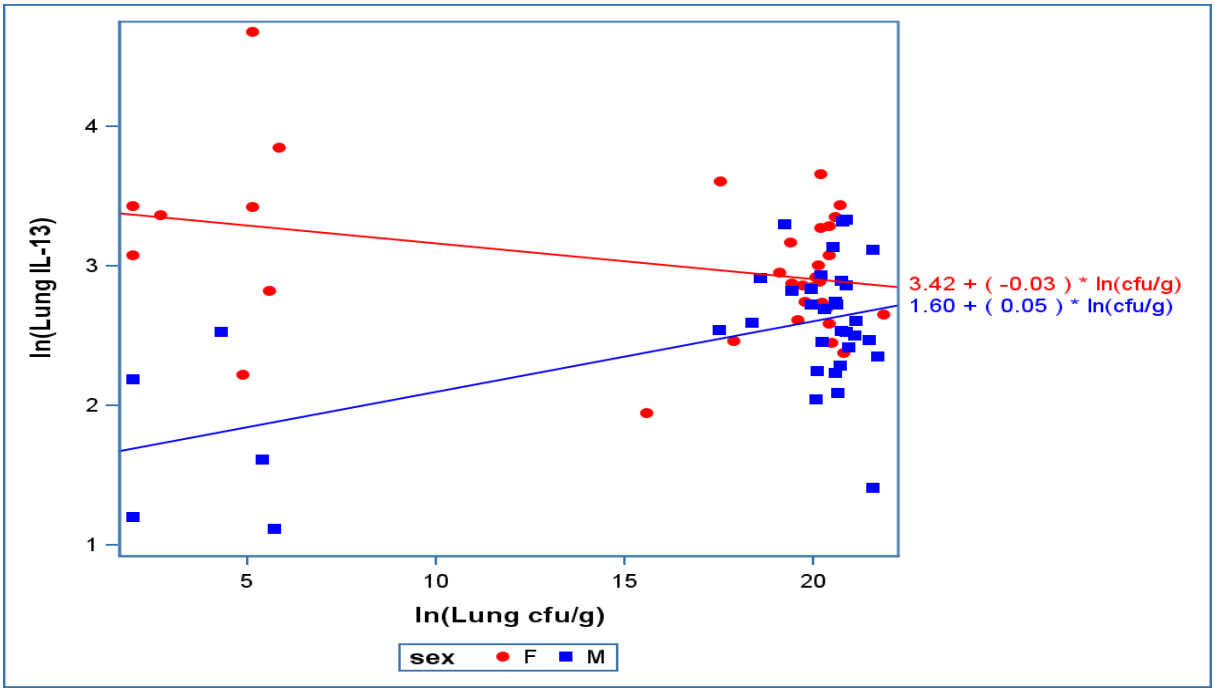

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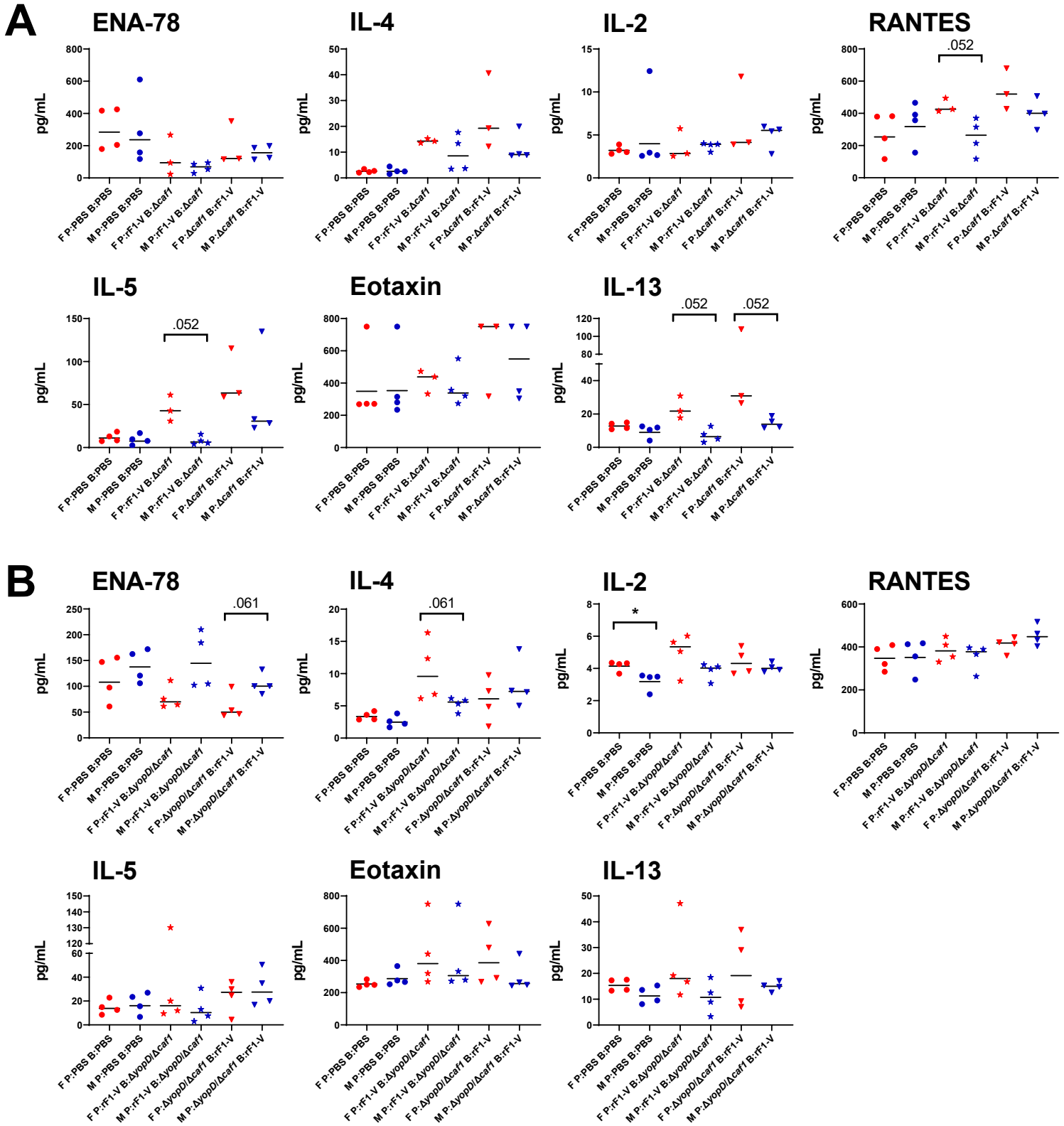

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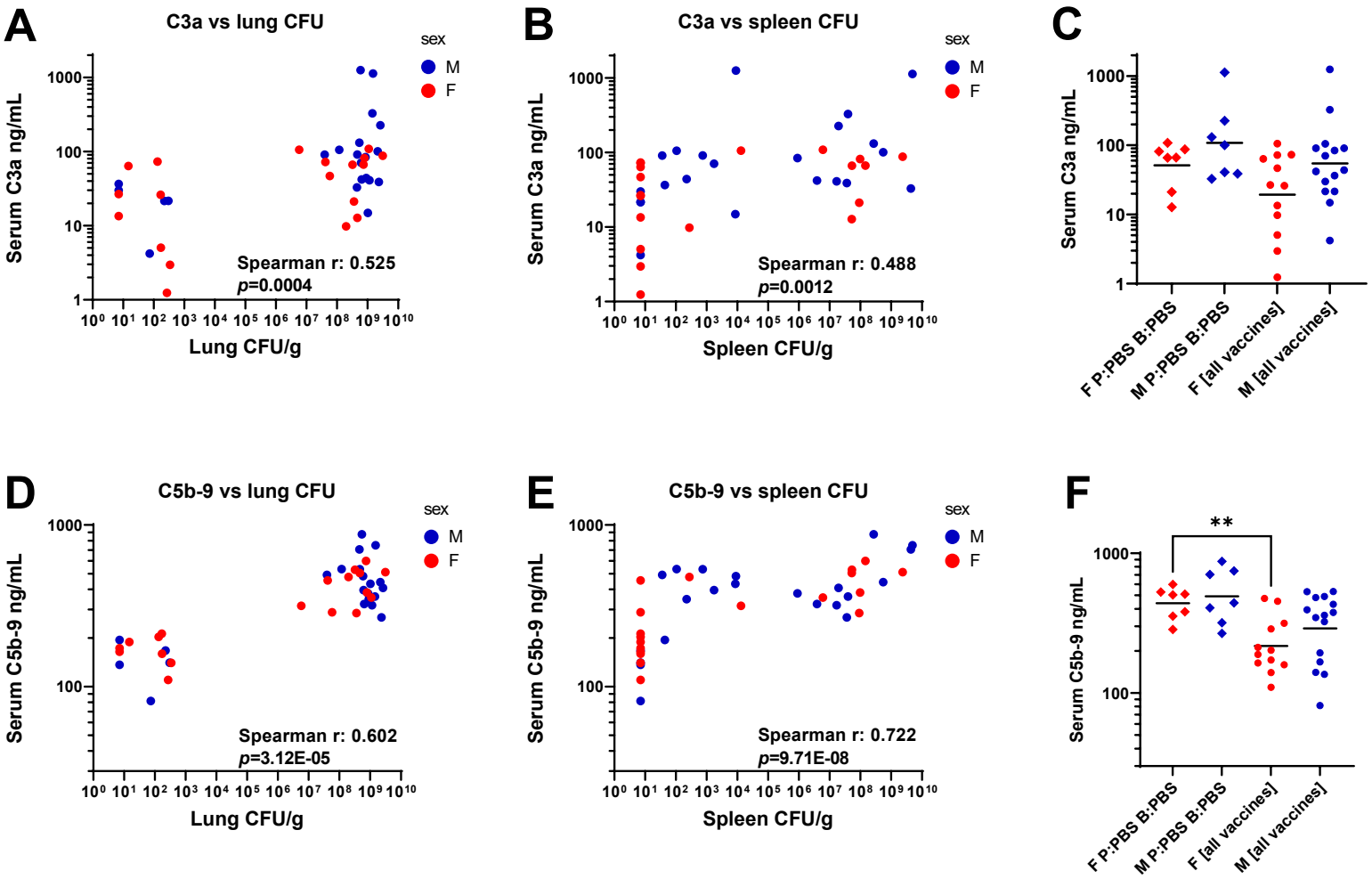

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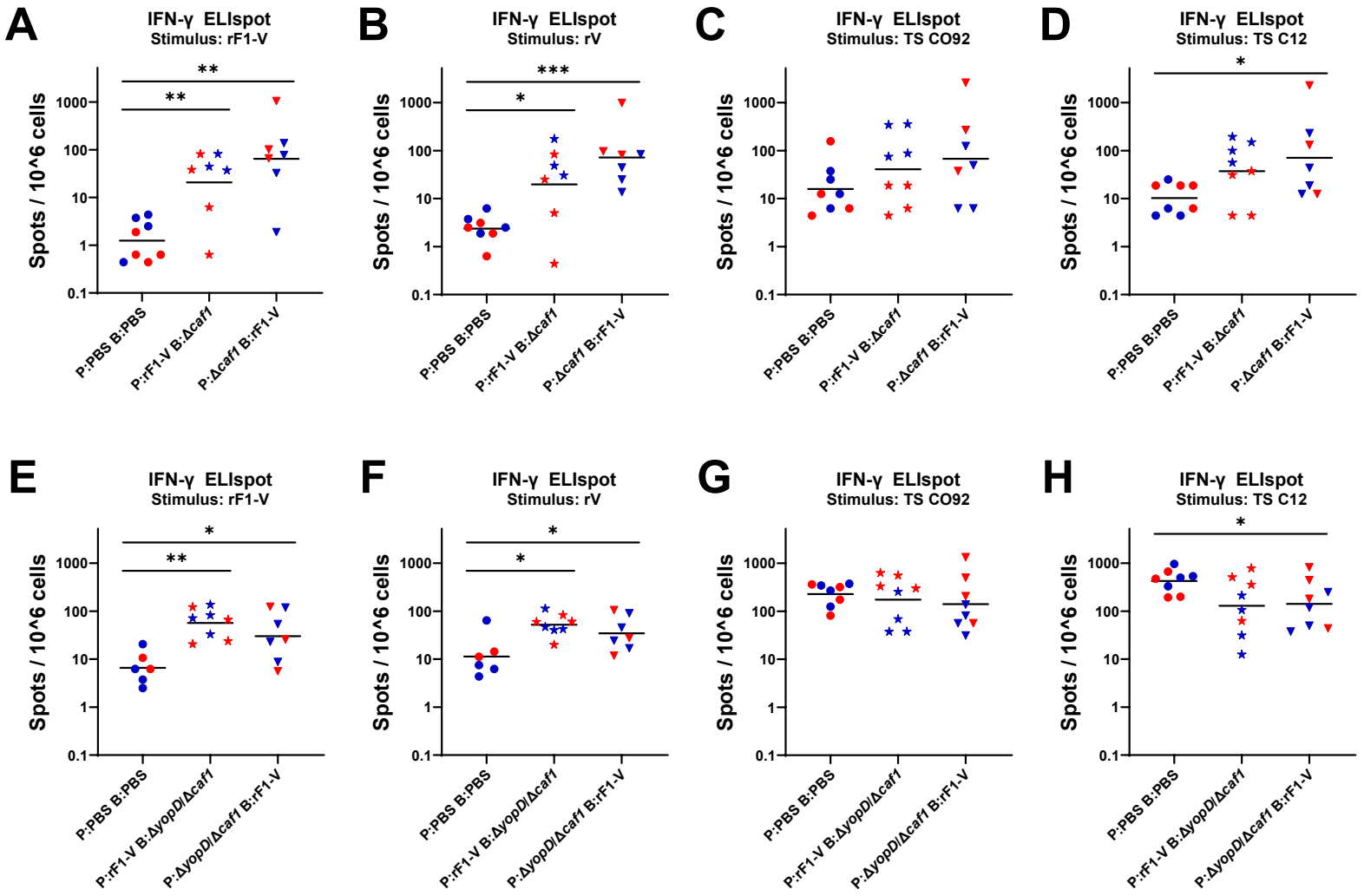

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