

Article

# Alternative Vaccination Routes against Paratuberculosis Modulate Local Immune Response and Interference with Tuberculosis Diagnosis in Laboratory Animal Models

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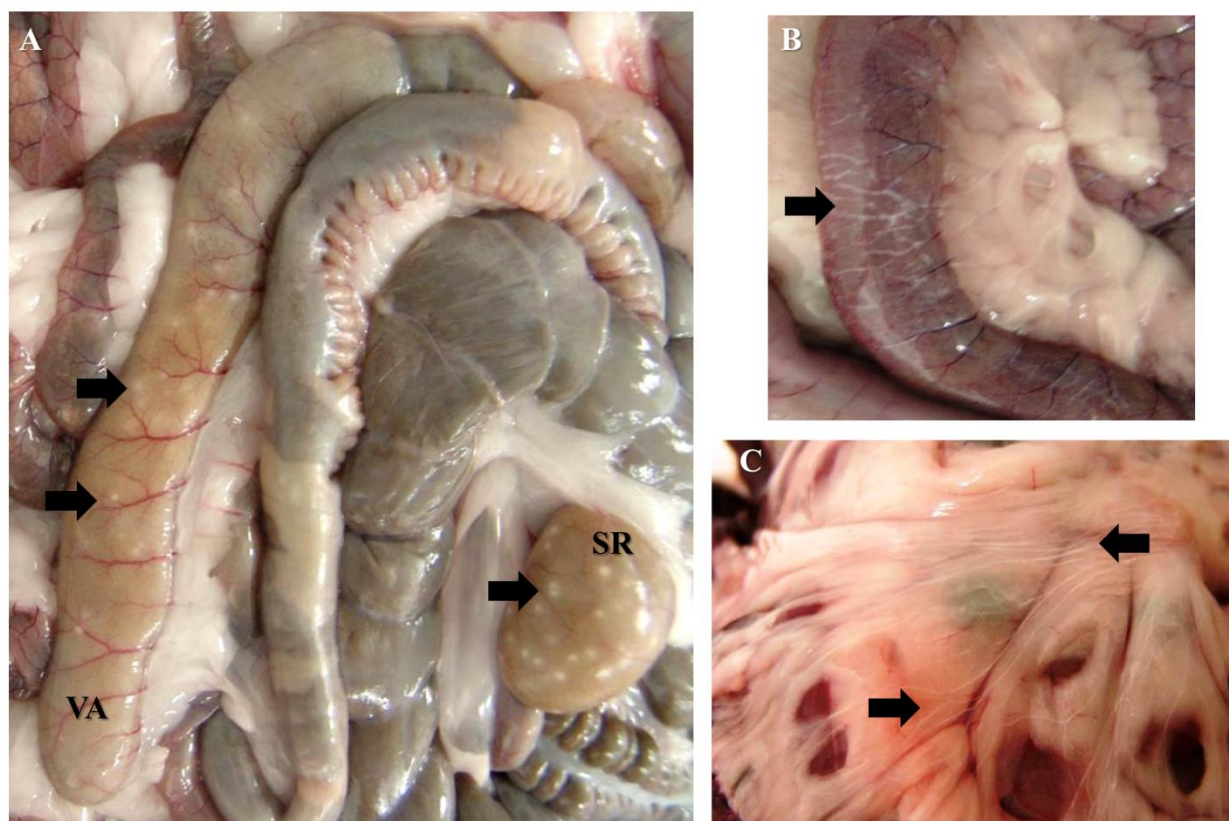
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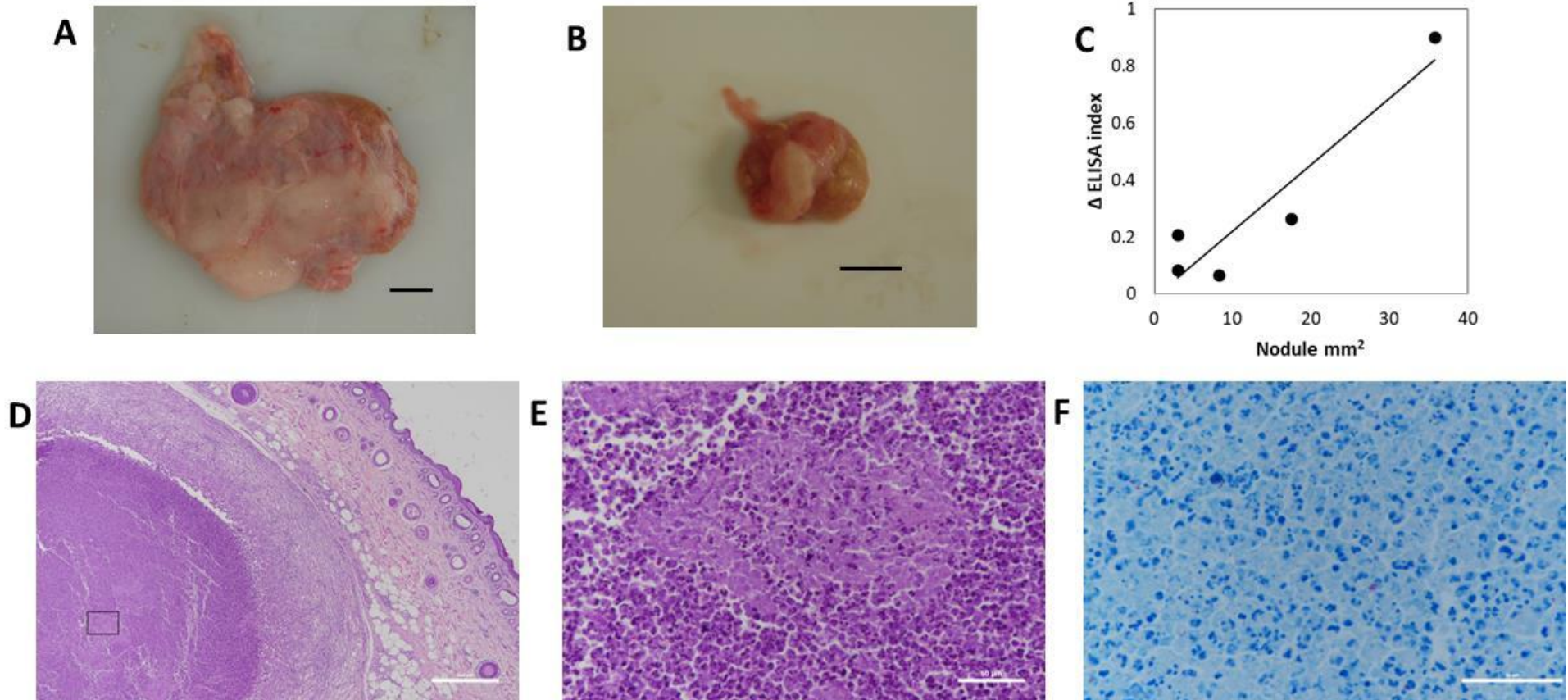
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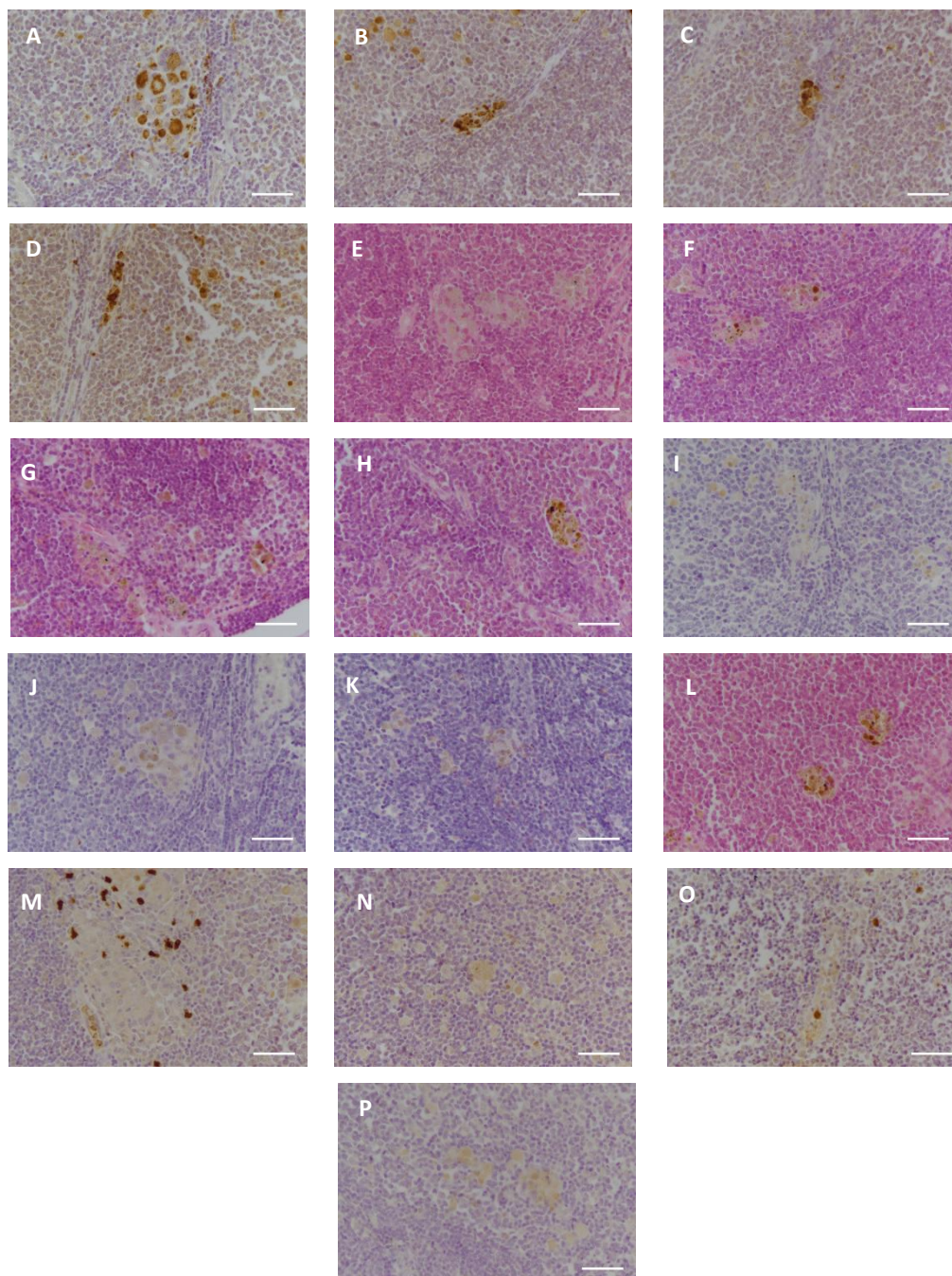
## Supplementary Materials



**Figure S1.** Gross pathology observed in the digestive system. (A) Pale white spots in sacculus rotundus (SR) and vermiform appendix (VA). (B) Dilatation of lymphatic vessels (lymphangiectasia) of intestinal serosa. (C) Lymphangiectasia in the mesentery.



**Figure S2.** Vaccination inoculation sites. (A,B) representative photographs of nodule formed in subcutaneously vaccinated rabbits (bars are equivalent to 1 cm), (C) dot plot showing correlation between vaccination nodule and ELISA index (S6–S0) in the subcutaneously vaccinated group (Pearson: 0.935,  $p$ : 0.02). The trend line represents the least square estimation. (D) Cutaneous pyogranuloma in intradermic vaccination site on guinea pig (haematoxylin-eosin staining; bar = 500  $\mu$ m). (E) Magnification of (D), necrosis and neutrophil inflammatory infiltrate in intradermic injection site (haematoxylin-eosin staining; bar = 50  $\mu$ m). (F) Acid-fast bacilli in necrotic areas of intradermic injection site (Ziehl-Nelsen staining; bar = 50  $\mu$ m).



**Figure S3.** Macrophage polarization status. Representative figures of the immunostaining in sacculus rotundus for each marker and experimental group (bar = 50  $\mu$ m). (A–D) IFN- $\gamma$ , (E–H) TNF- $\alpha$ , (I–L) CD163 and (M–P) calprotectin. A, E, I and M are infected controls (IC); B, F, J and N are experimental intradermal vaccine animals (VID); C, G, K and O are experimental oral vaccine animals (VOR), D, H, L and P are control subcutaneous vaccine animals (VSC). Bars in micrographs represent 50  $\mu$ m.