Supplementary Material

Intranasal vaccination with recombinant antigen-FLIPr fusion protein alone induces long-lasting systemic antibody responses and broad T cell responses

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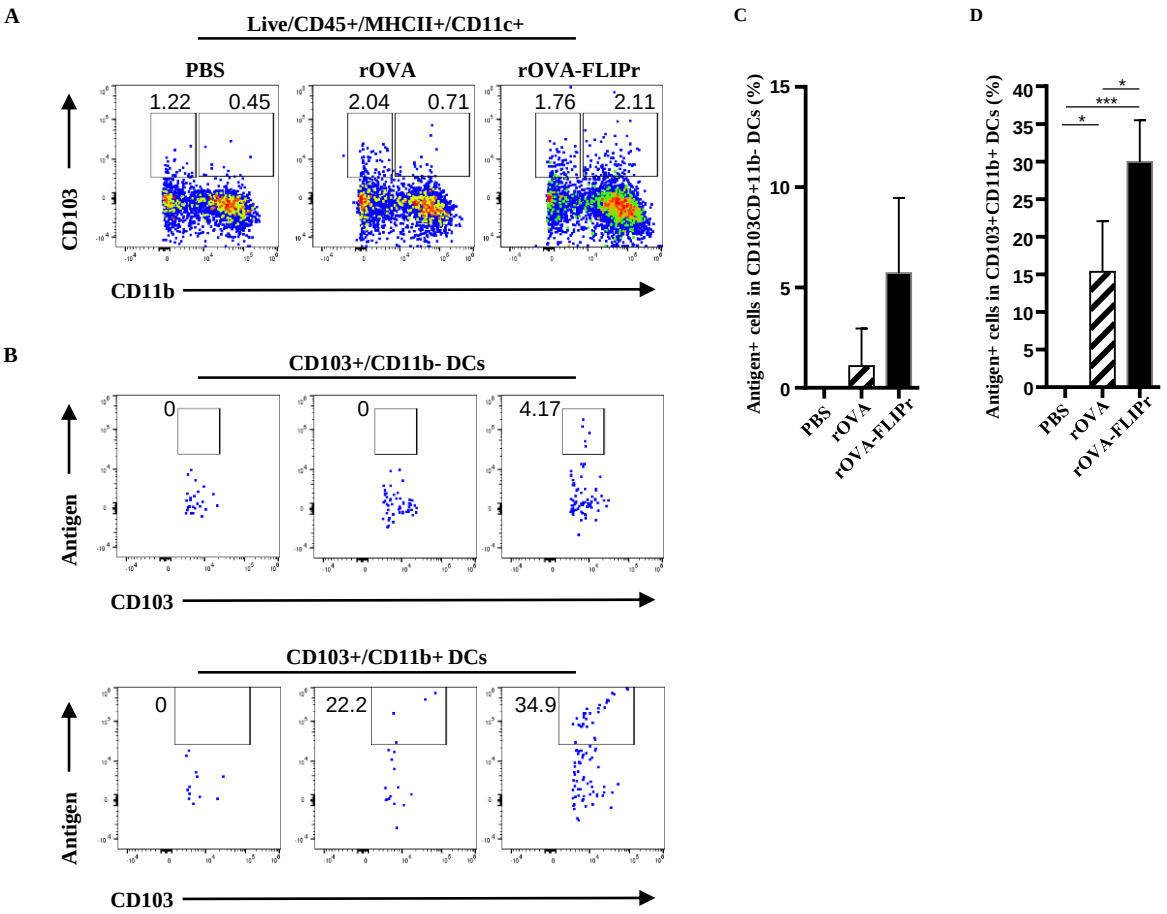
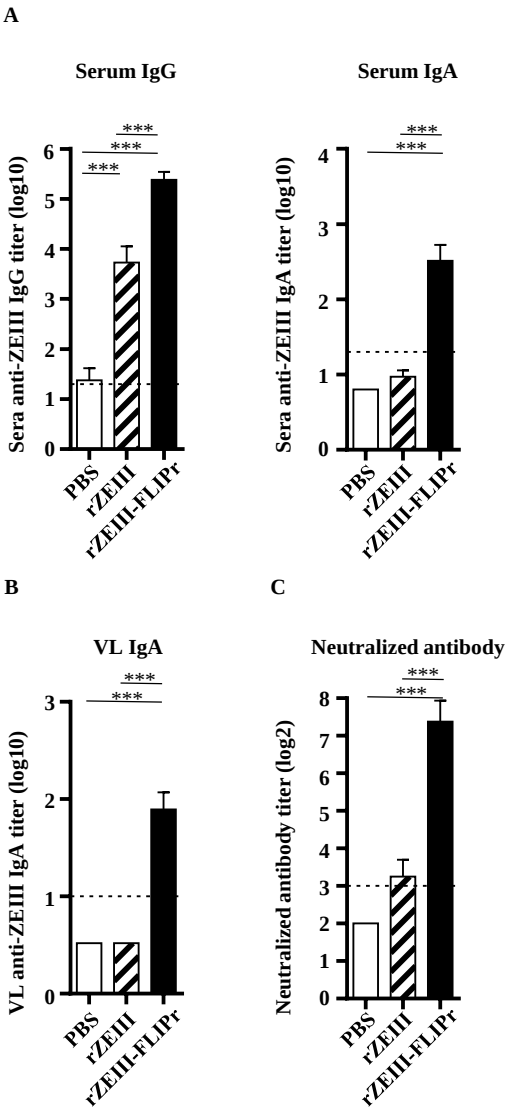


Supplementary Figure S1. Ovalbumin fusion with FLIPr efficiently delivers to NALT CD103$^+$ dendritic cells via intranasal administration. (A) NALT DCs gated on live/CD45+/CD11c+MHCII+ cells are separated by the expression of CD103 and CD11b. **(B)** The representative figure of antigen-labeled CD103$^+$CD11b$^-$ or CD103$^+$CD11b$^+$ DCs. The box representative the antigen positive cells. **(C)**The frequencies of antigen-labeled CD103$^+$CD11b$^-$ DCs. **(D)** The frequencies of antigen-labeled CD11c$^+$MHCII$^+$CD103$^+$CD11b$^-$ DCs. Cumulative data from three individual experiments are quantified here. The data are presented as the mean ± SEM (n=3).



Supplementary Figure S2. Antibody responses induced by intranasal administration of rZEIII-FLIPr in C57BL/6 mice. Immunocompetent C57BL/6 mice (n=8/group) were vaccinated three times with PBS, rZEIII, or rZEIII-FLIPr (30 μg per dose) via the intranasal route at two-week intervals. Serum and VL samples were collected from vaccinated mice 6 weeks after the first vaccination. **(A)** The titers of anti-rZEIII IgG and IgA antibodies in the serum were determined by ELISA. **(B)** The titers of anti-rZEIII IgA antibodies in VL were determined by ELISA. **(C)** The Zika virus-neutralizing capacity of the serum samples was determined by FRNT. The neutralizing antibody titer was defined as the reciprocal of the highest dilution that resulted in a 50% reduction in FFUs compared to the FFUs of control samples containing the virus alone. Data represent the mean ± SE of a total of 9 mice per group, which were pooled from 2 independent experiments. The detection limit is indicated by the dotted line on the y-axis, which indicates the initial dilution factor of the sample. Statistical significance was determined using the Kruskal-Wallis test with Dunn's multiple comparison test. ***$p < 0.001$.