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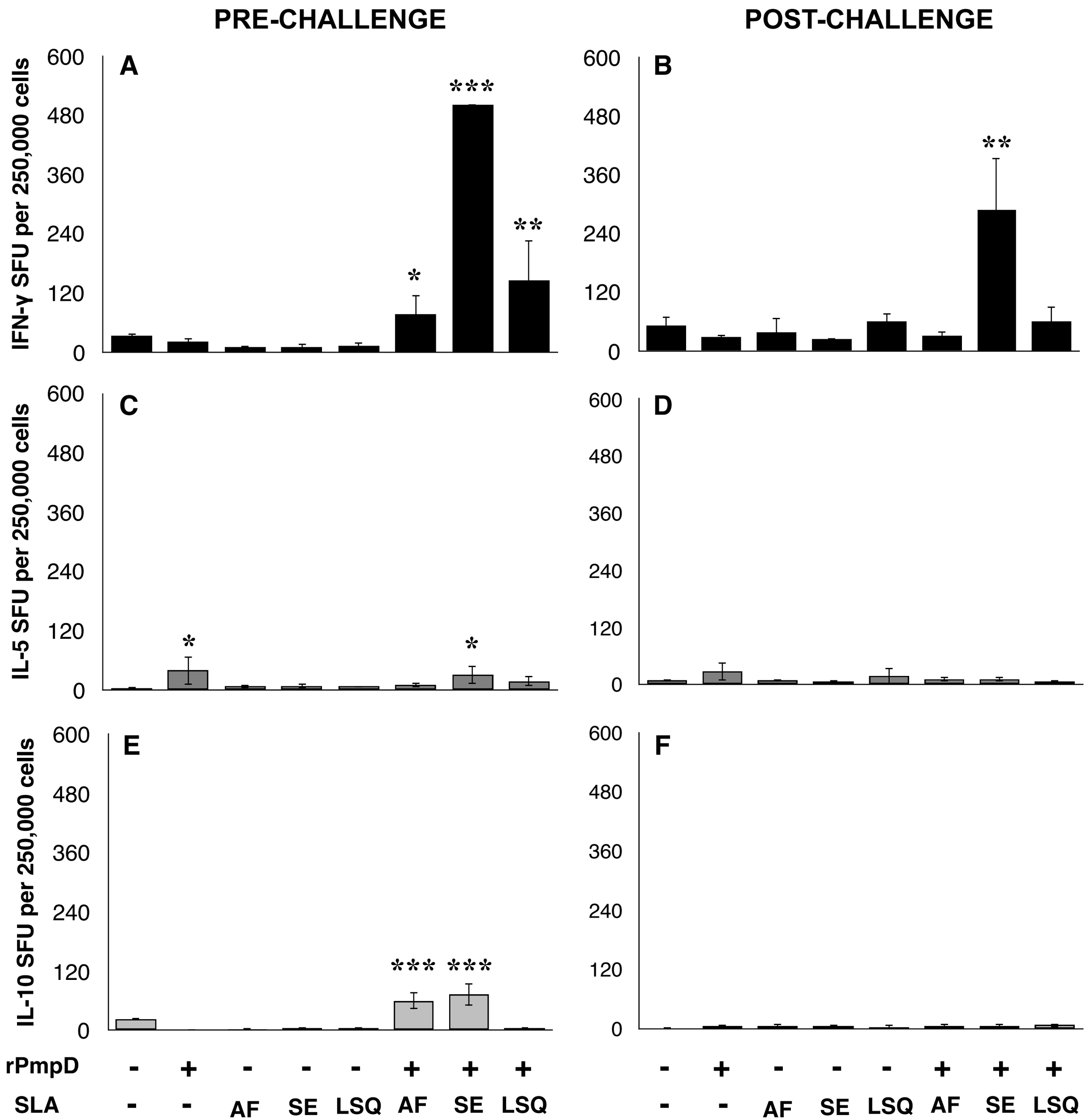

Figure 1. rPmpD-specific cytokine responses following immunization with different vaccine formulations in C57BL/6 mice. Splenocytes were harvested and stimulated with rPmpD two weeks after the final immunization (**A, C, E**) or six weeks post-challenge (**B, D, F**), and assessed for IFNγ, IL-5 or IL-10 secretion. SLA formulation-specific differences in cytokine profiles are observed. The results are expressed as the mean ± the standard deviation for groups of 5 mice measured in triplicate from two experiments (*P≤0.05, **P≤0.01, ***P≤0.001 relative to PBS-immunized mice).

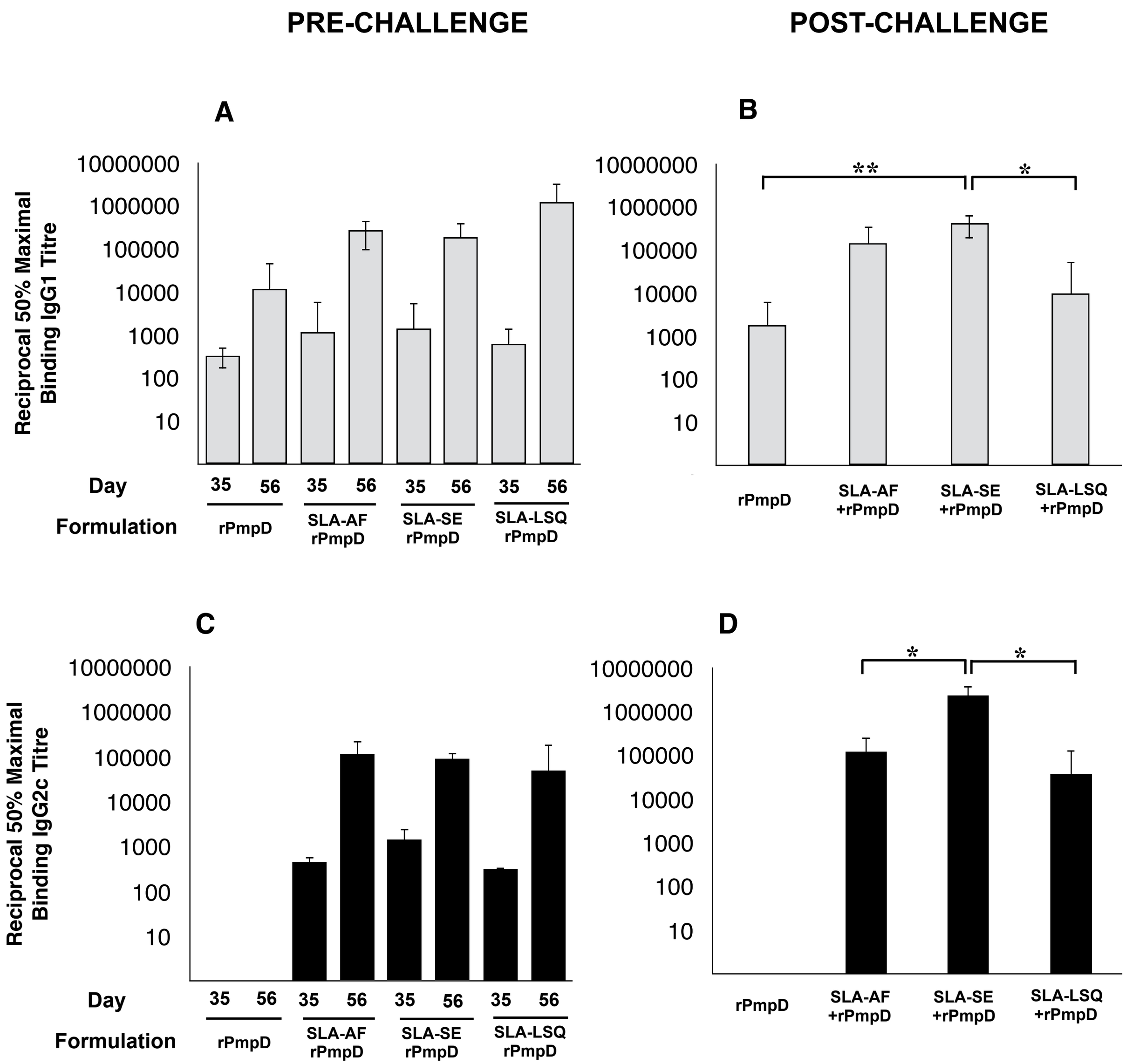

Figure 2. rPmpD-specific IgG1 and IgG2c responses following immunization with different rPmpD vaccine formulations in C57BL/6 mice. Anti-rPmpD serum IgG1 and IgG2c titres were measured two weeks following penultimate and final immunisations (**A, C**) or six weeks post-challenge (**B, D**). Results are expressed as the geometric mean ± the standard deviation for groups of 5 mice measured in triplicate, and are representative of two separate experiments. (* $P \leq 0.05$, ** $P \leq 0.01$).

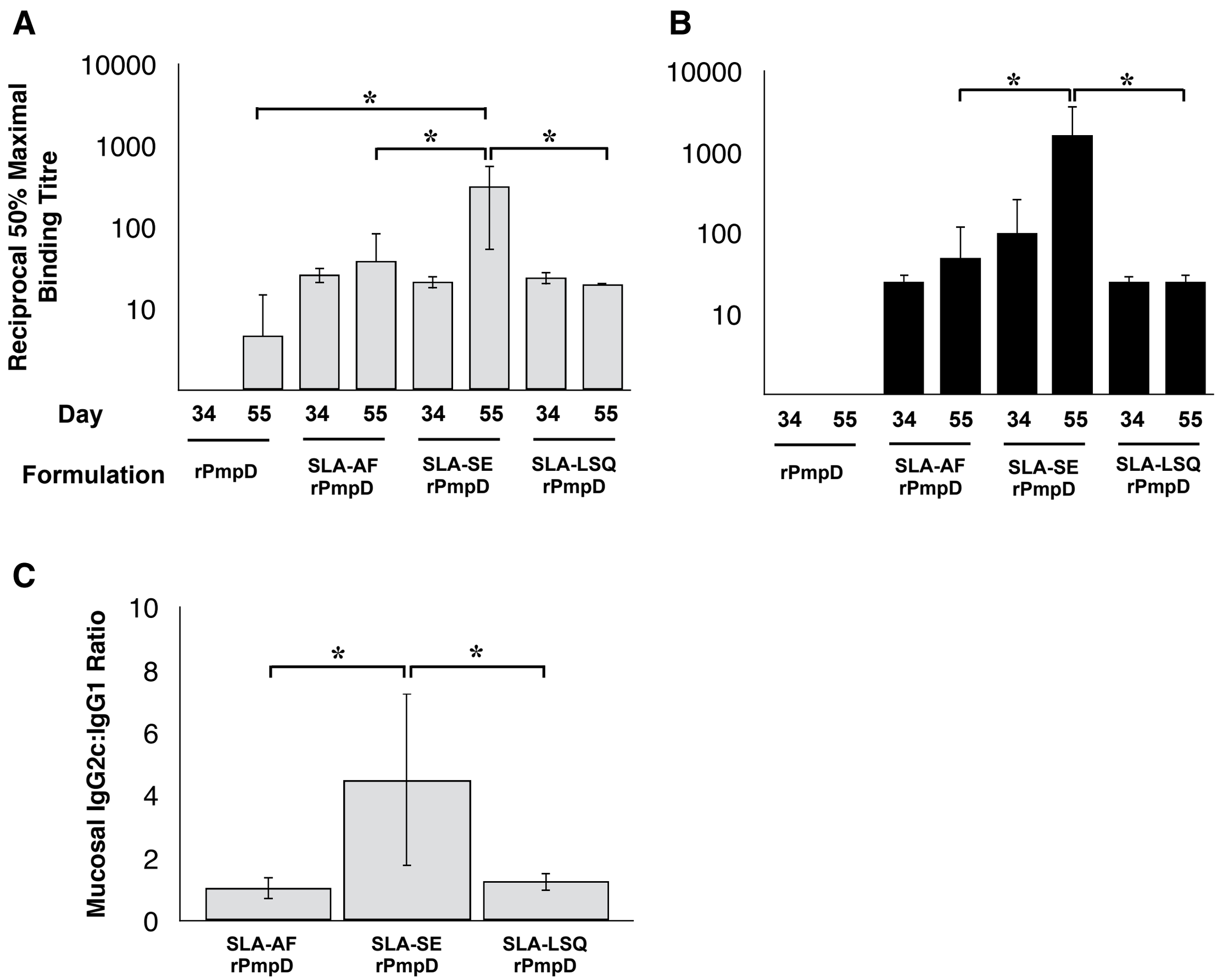

Figure 3. Immunization with rPmpD elicits robust cervico-vaginal antigen-specific IgG titres. C57BL/6 mice were immunized with all rPmpD formulations and mucosal anti-rPmpD IgG1 (**A**) and IgG2c (**B**) titres were measured at the indicated time points prior to *Ct* challenge. Results are expressed as the geometric mean ± the standard deviation for groups of 5 mice measured in triplicate, and are representative of two separate experiments. (**C**) Data are also shown as IgG2c:IgG1 titre ratios two weeks following final immunization, and are representative of two separate experiments (*P≤0.05). Data shown are for the same animals as in **Fig.2**.

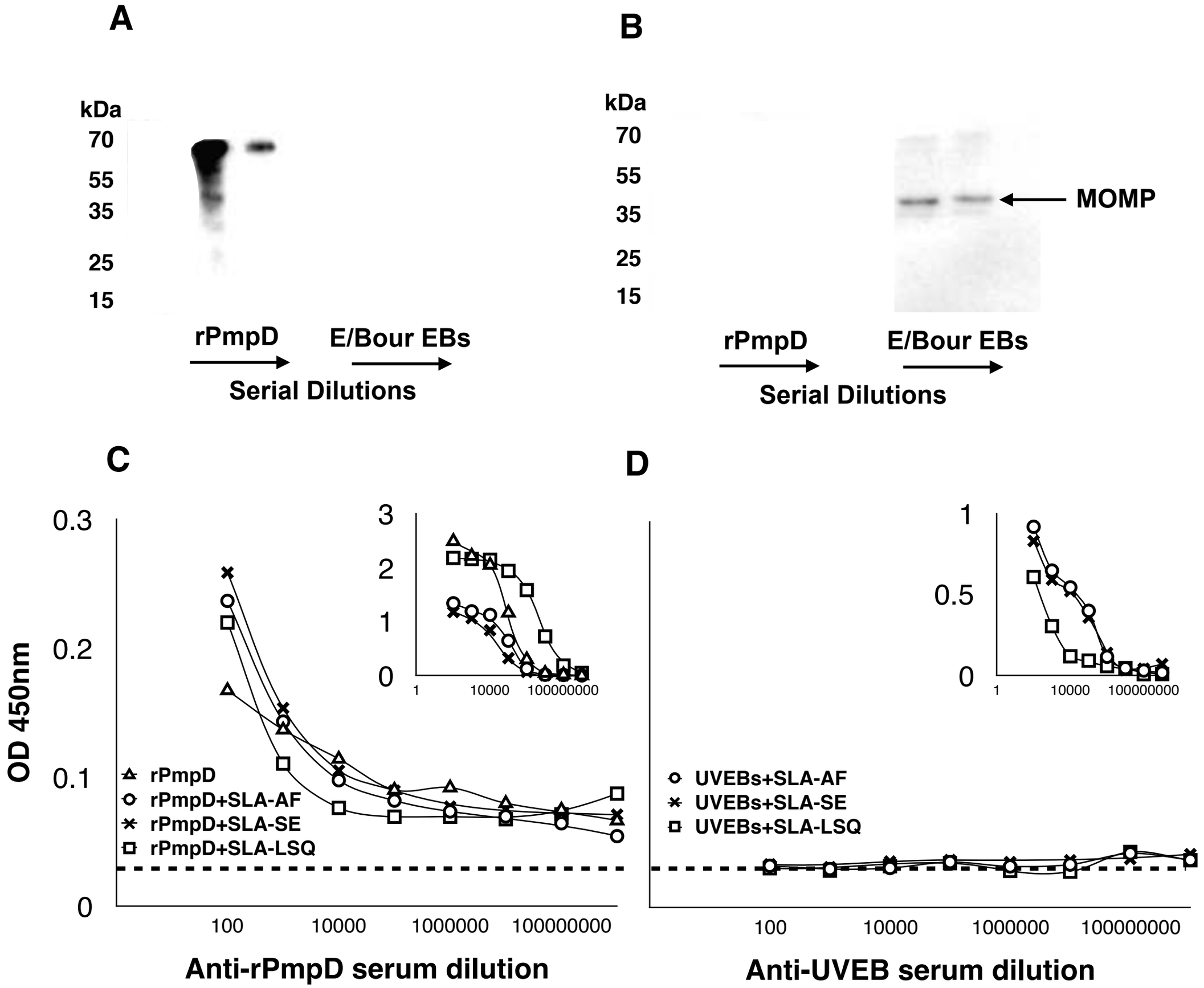

Figure 4. Reactivity of anti-rPmpD and anti-UVEB serum. Serum from mice immunized with rPmpD (**A**) or UVEBs (**B**) was tested for reactivity against each antigen in western blots. (**C**) Anti-rPmpD serum reacts with UVEBs in an indirect ELISA (inset shows reactivity against rPmpD). (**D**) Anti-UVEB serum failed to react with rPmpD (inset shows reactivity against UVEBs). Dotted lines represent the OD_{450} cut-off value equivalent to the mean plus two standard deviations of control pre-immune serum.

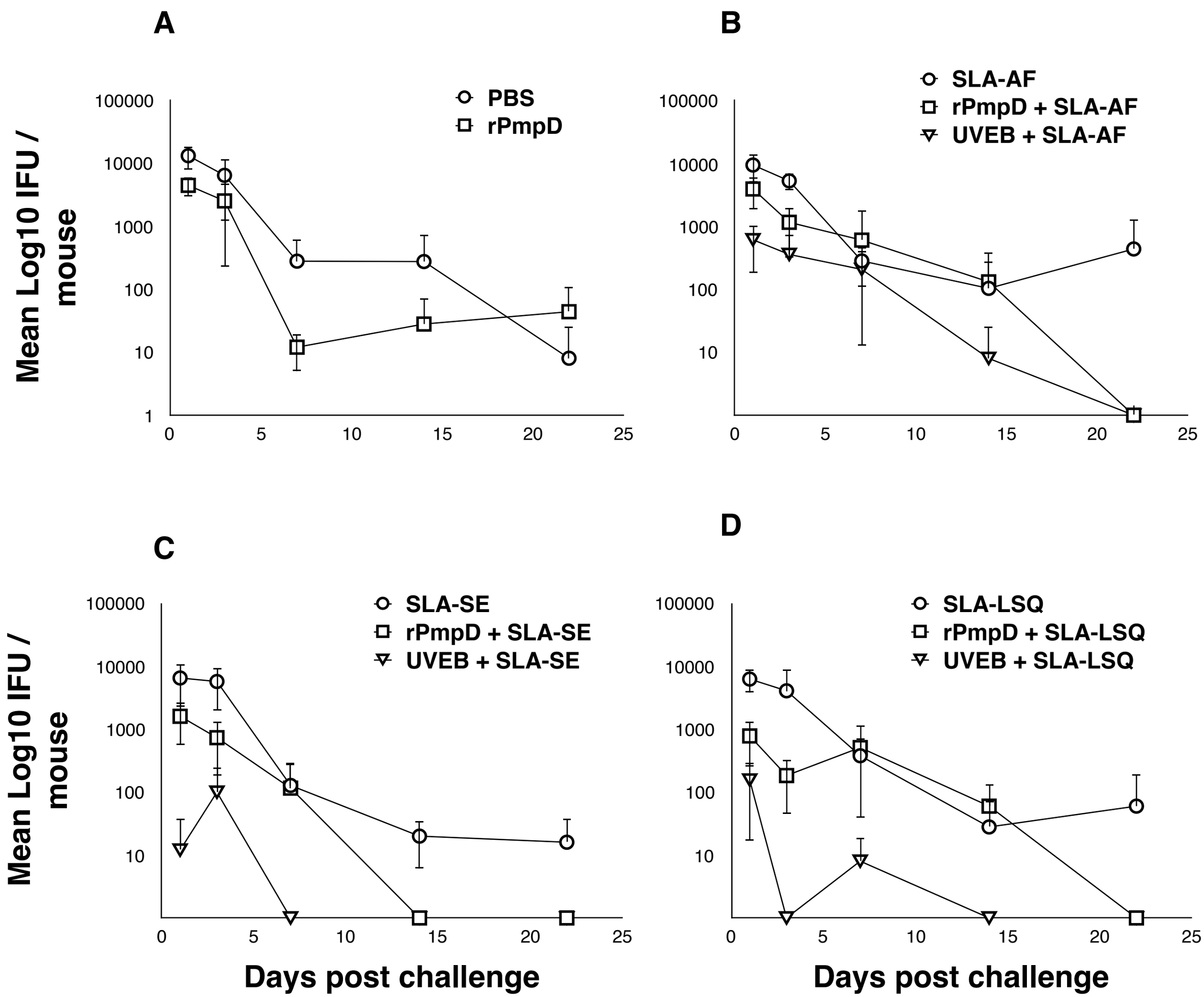

Figure 5. Infection curves showing bacterial shedding following challenge of vaccinated mice. C57BL/6 mice were infected intra-vaginally with *Ct* serovar D/UW3/Cx three weeks post final immunization with either rPmpD or *Ct* serovar E/Bour UVEBs (positive control). Individuals were swabbed on days 1, 3, 7, 14 and 22 post infection, and recoverable cervico-vaginal IFU assessed on Hak cell monolayers. Culture-negative mice were assigned a cut-off value of <10 IFU. Formulation- and antigen-specific differences in time to complete resolution of infection are observed. Results are expressed as the mean ± the standard deviation for groups of 5 mice.

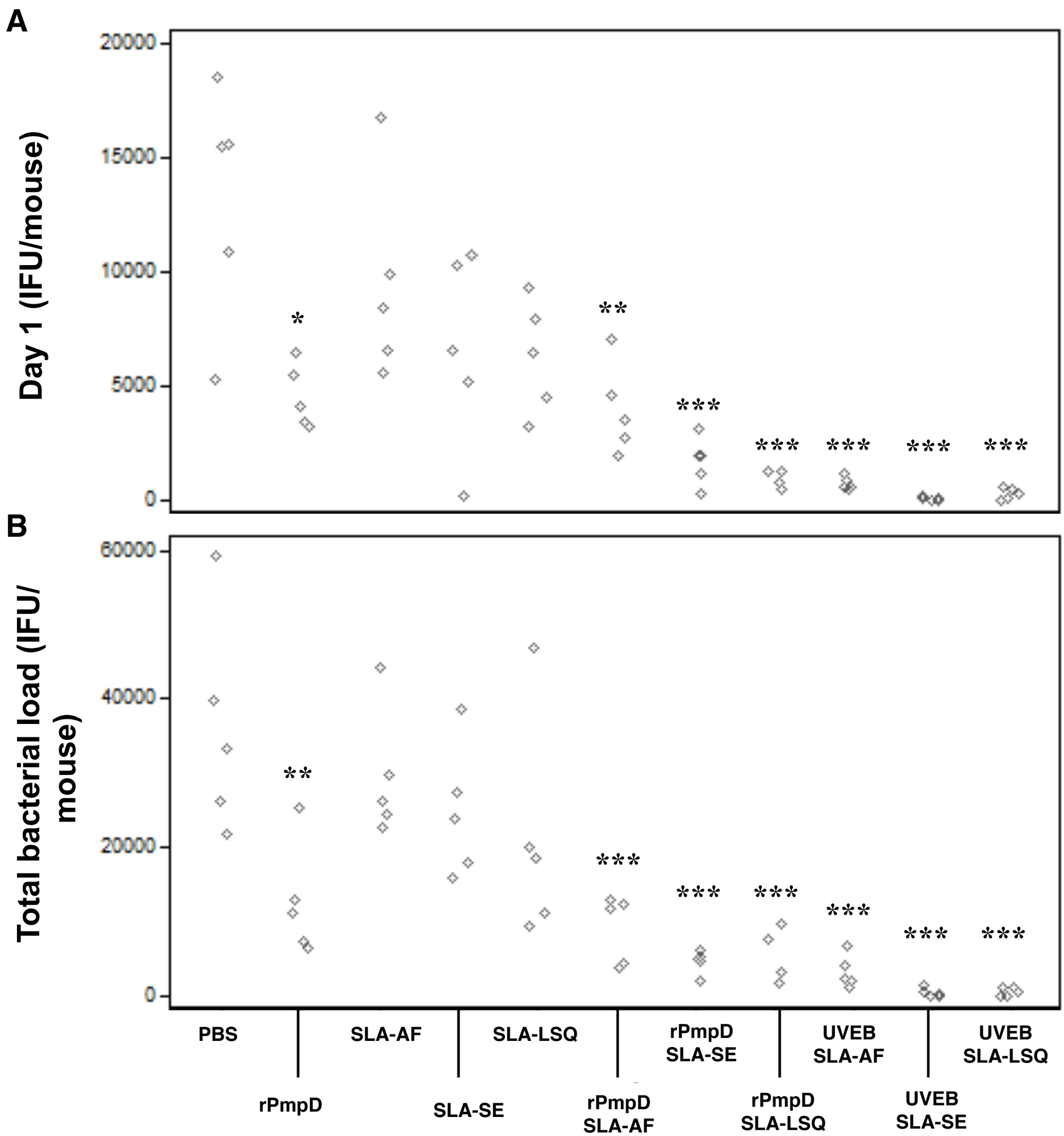

Figure 6. Quantification of vaccine elicited protection against *Ct* challenge. (**A**) Resistance to infection was assessed by swabbing individuals on day 1 and determining recoverable IFU on Hak cell monolayers. (**B**) Total bacterial load over the 22 day time period was quantified by integrating the area under the shedding curves (depicted in Fig.5) for individual mice. Data were analysed using a one-way analysis of variance (ANOVA) for groups of five mice. Significance values displayed are relative to PBS-immunized mice (*P≤0.05, **P≤0.01, ***P≤0.001).