

Figure S1. Total and neutralizing antibody titers against FMDV A/Arg/01 at 30 dpv according to the antigenic payload, strain composition or number of doses. Total FMDV-specific antibodies were measured by LPBE and neutralizing antibodies (NAb) were measured by VNT. Animals were grouped according to: (a and b) the antigenic payload of the formulation (10 µg/dose including the *A24 10µg* group vs. 40 µg/dose including the *A24/C31/O1C* and *A24 40µg* groups), (c and d) the strain composition in the vaccine (trivalent including the *A24/C31/O1C* group vs. monovalent including the *A24 10µg* and *A24 40µg* groups), and number of doses with the lowest antigenic dose (*A24 10µg* group vs. *A24 10µg x2* group). Results are expressed as the log₁₀ of the mean titers against heterologous strain, A/Arg/01. Dotted lines depict the titers corresponding to the EPP₇₅ for FMDV A/Arg/01 strain for each serological assay. Bars represent mean value ± SD.

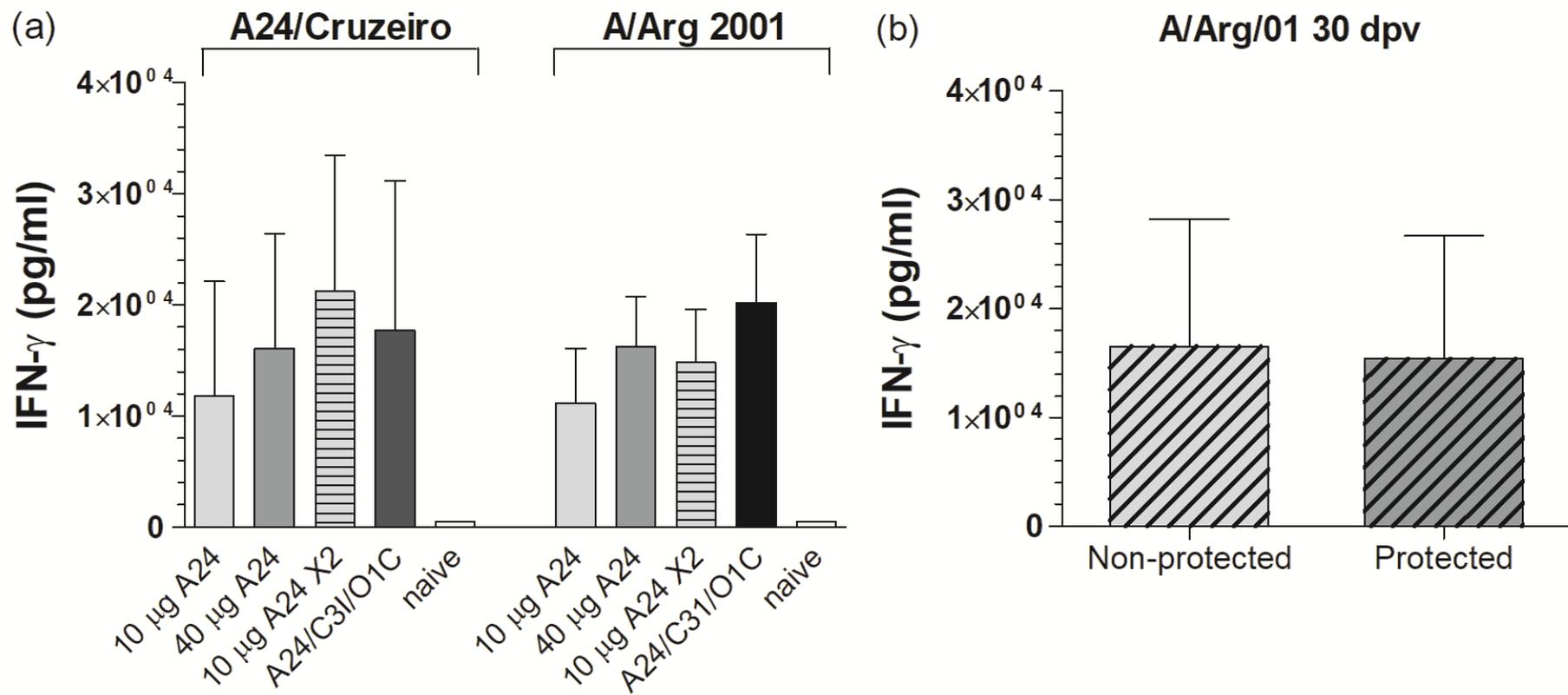


Figure S2. FMDV-specific interferon (IFN)- γ production per experimental group and clinical status after FMDV A/Arg/01 challenge. (a) *Ex-vivo* production of IFN- γ in experimental and control groups was measured by ELISA on stimulated plasma from whole blood samples incubated with 10 μ g/mL of inactivated FMDV A24/Cruzeiro or A/Arg/0 strains, as indicated. (b) IFN- γ production in samples stimulated with the challenge strain, grouped according to the protective status after heterologous infection. Cell viability was assessed for each sample by incubating with 10 μ g/mL of PWM (not shown). Bars represent the mean production of INF- γ \pm SD for each group.

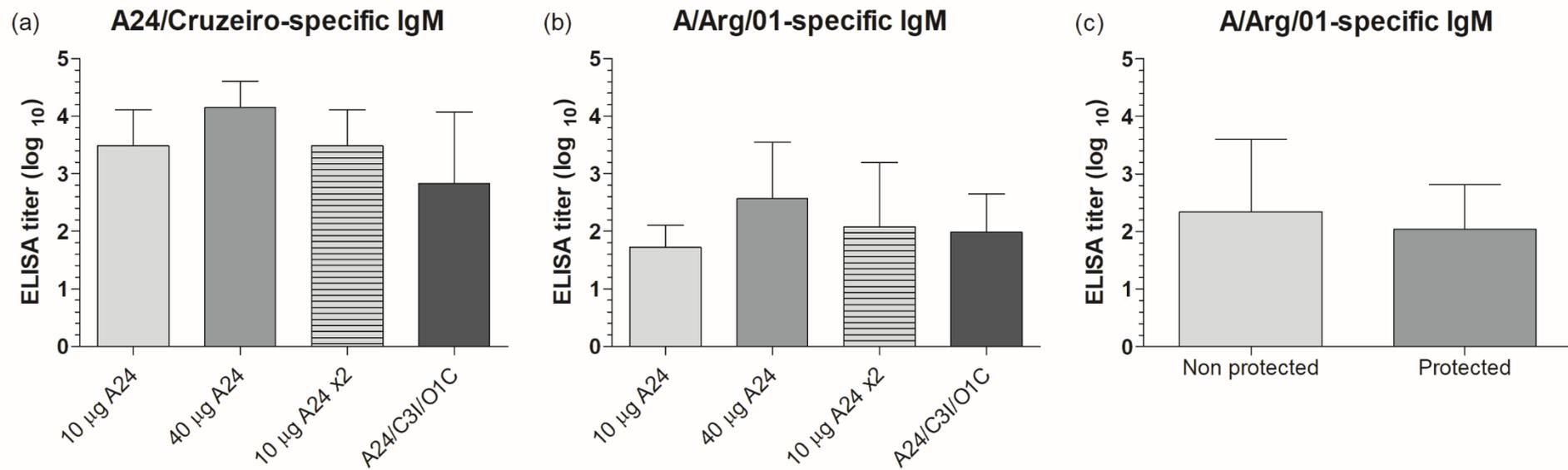


Figure S3. IgM titers against FMDV A24/Cruzeiro and A/Arg/01 strains per experimental group and clinical status after FMDV A/Arg/01 challenge. FMDV-specific IgM responses were measured by ELISA against the A24/Cruzeiro (a) and A/Arg/01 (b) strains. (c) Mean IgM antibody titers against the A/Arg/01 strain were also compared in animals grouped according to their protection status. Results are expressed as the log₁₀ of the mean titer for each experimental group at 30 dpv, and bars represent the mean value of each experimental group ± SD.

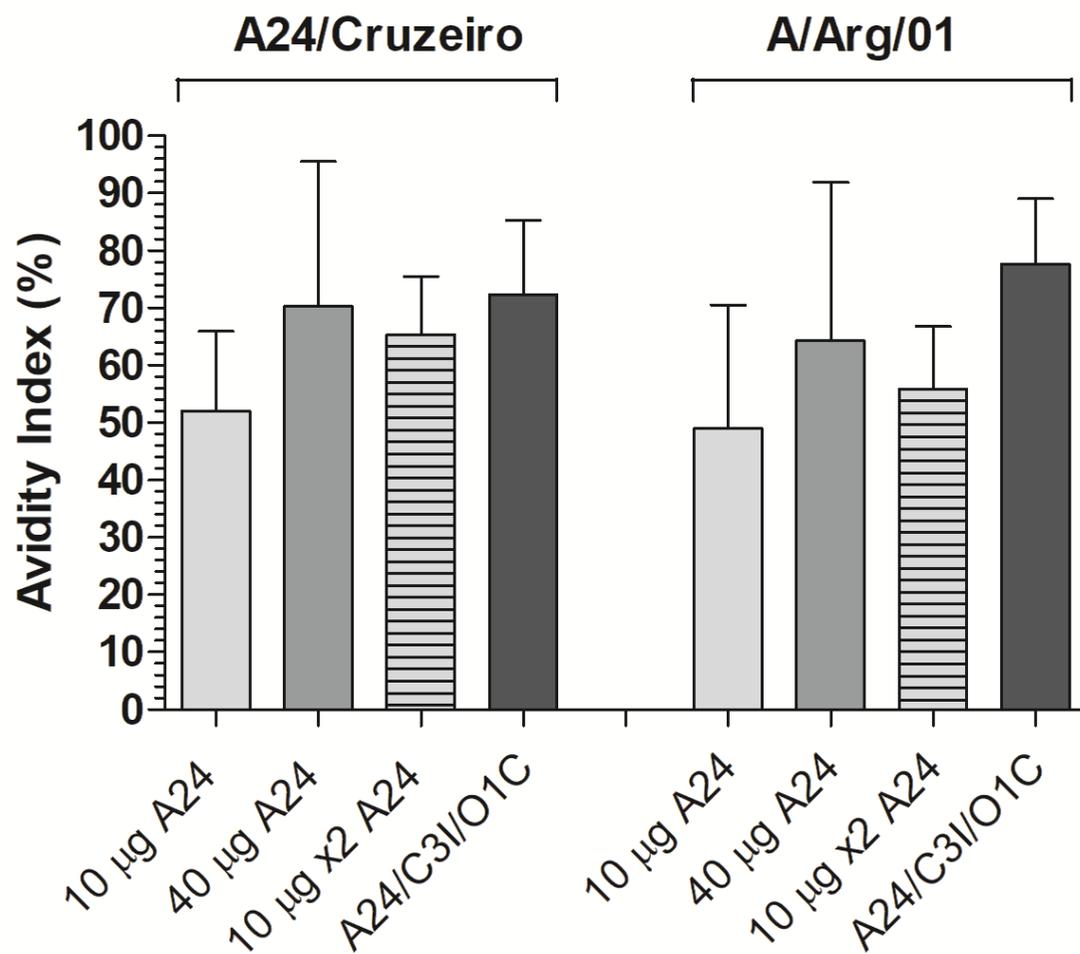


Figure S4. Avidity of the IgG antibodies against the FMDV A24/Cruzeiro and A/Arg/01 strains at 30 dpv per experimental group. The avidity of the FMDV-specific IgG antibody responses against the A24/Cruzeiro and A/Arg/01 strains were measured by Avidity ELISA. Results are expressed as the mean AI per experimental group and bars represent the mean value \pm SD. Statistical differences between experimental groups were studied by ANOVA.

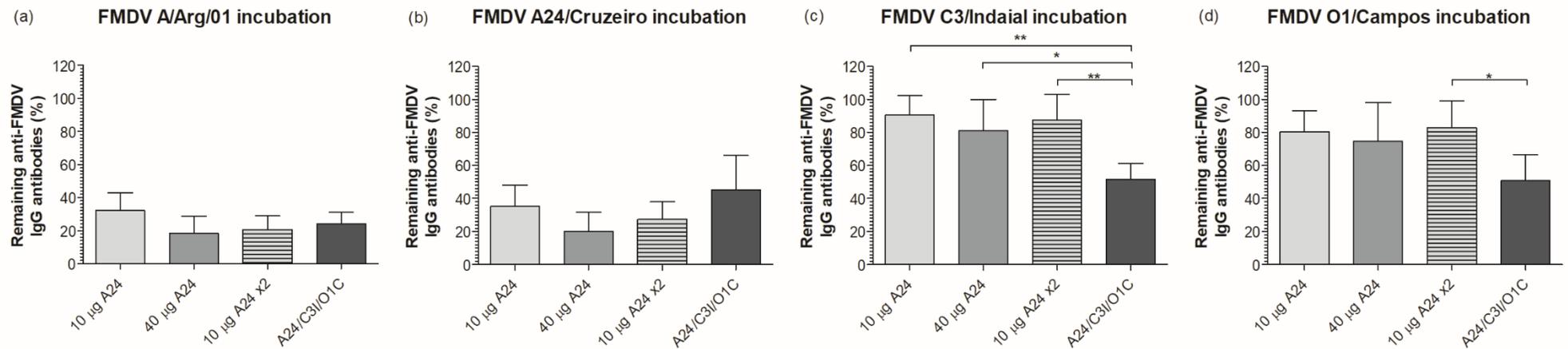


Figure S5. Percentage of total IgG antibodies against FMDV A/Arg/01 remaining in sera from vaccinated animals after incubation with different FMDV strains. Serum samples from vaccinated steers were incubated with PBS, or with inactivated purified FMDV antigens from the A/Arg/01 (a), A24/Cruzeiro (b), C3/Indaial (c) or O1/Campos (d) strains. Virus-incubated serum samples and their PBS-incubated replicates, were then evaluated for the presence of IgG antibodies against the A/Arg/01 strain by an indirect ELISA, using plates coated with purified inactivated FMDV A/Arg/01 (see Appendix A for methodology details). Results are expressed as the percentage of residual activity of the serum sample after virus incubation, relative to that of PBS-incubated sample (OD sample incubated with virus/OD sample incubated with PBS \times 100). Bars represent mean values \pm SD per experimental group. Asterisks denote statistical differences between experimental groups (ANOVA) * $p \leq 0.05$, ** $p \leq 0.01$.