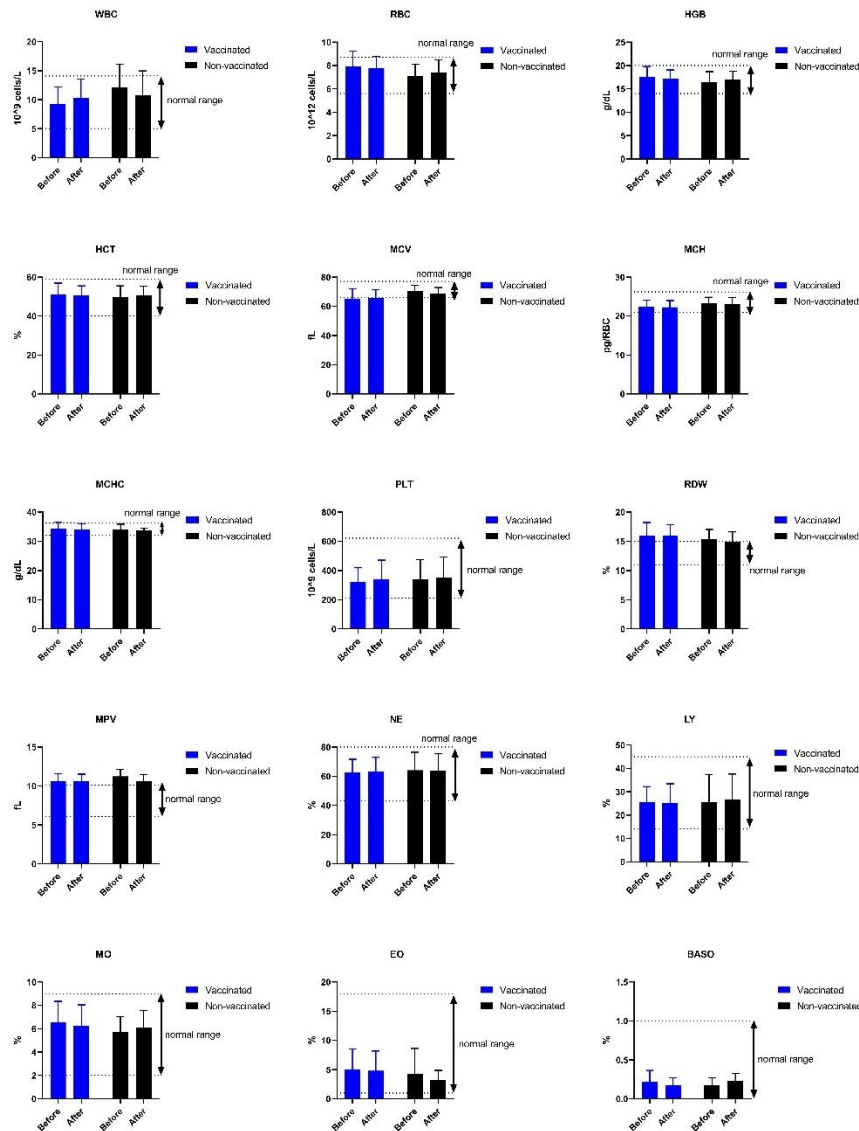
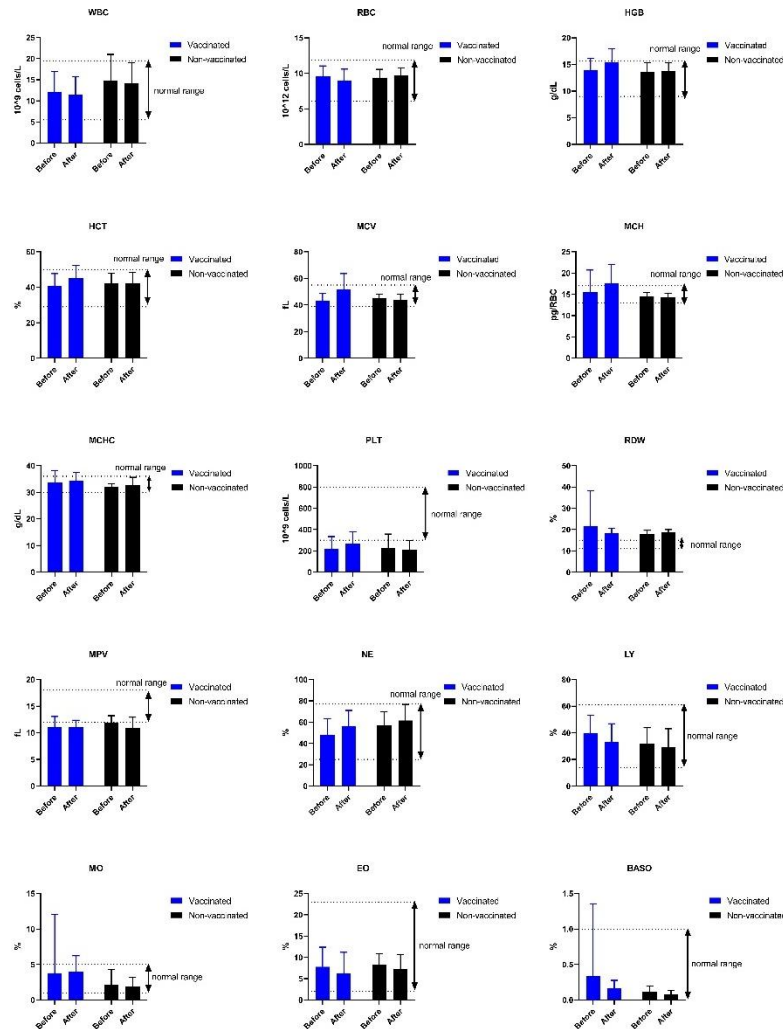


- 1 **Additional information**
- 2 **Supplementary information**
- 3



Additional file 1. Complete blood count (CBC) tests of dogs in clinical trial with companion animals

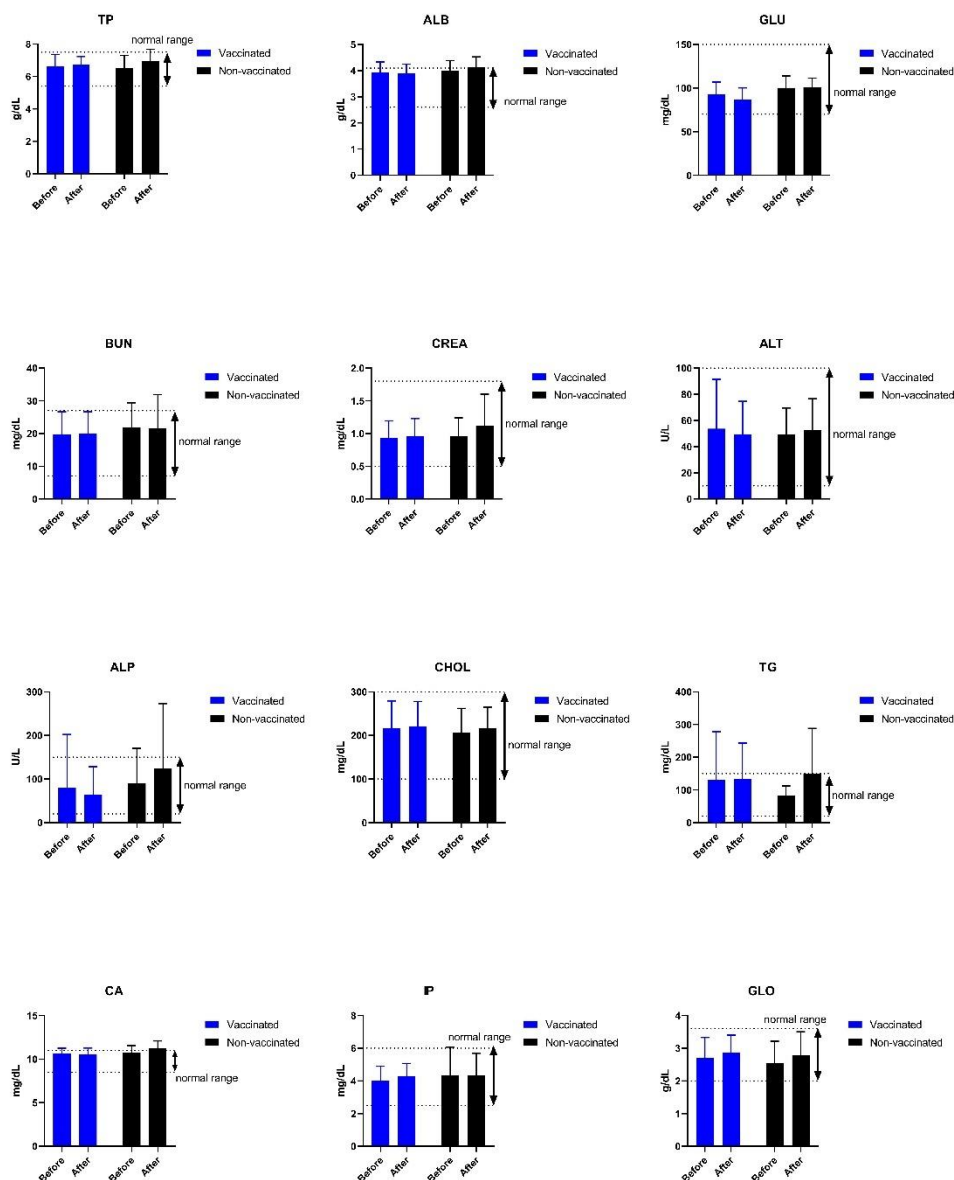
CBC tests were performed on dogs participating in the clinical trial to monitor their overall health status associated with vaccination. The parameters analyzed included WBC, RBC, HGB, HCT, MCV, MCH, MCHC, PLT, RDW, MPV, NE%, LY%, MO%, EO%, and BASO%. Across all parameters, most values remained within the normal range for dogs. Although a small number of individual animals exhibited values outside the normal range, these deviations were not associated with vaccination. Overall, the results indicate that the vaccine did not induce any adverse hematological effects in the tested dogs.



14

15 **Additional file 2. Complete blood count (CBC) tests of cats in clinical trial with companion**
 16 **animals**

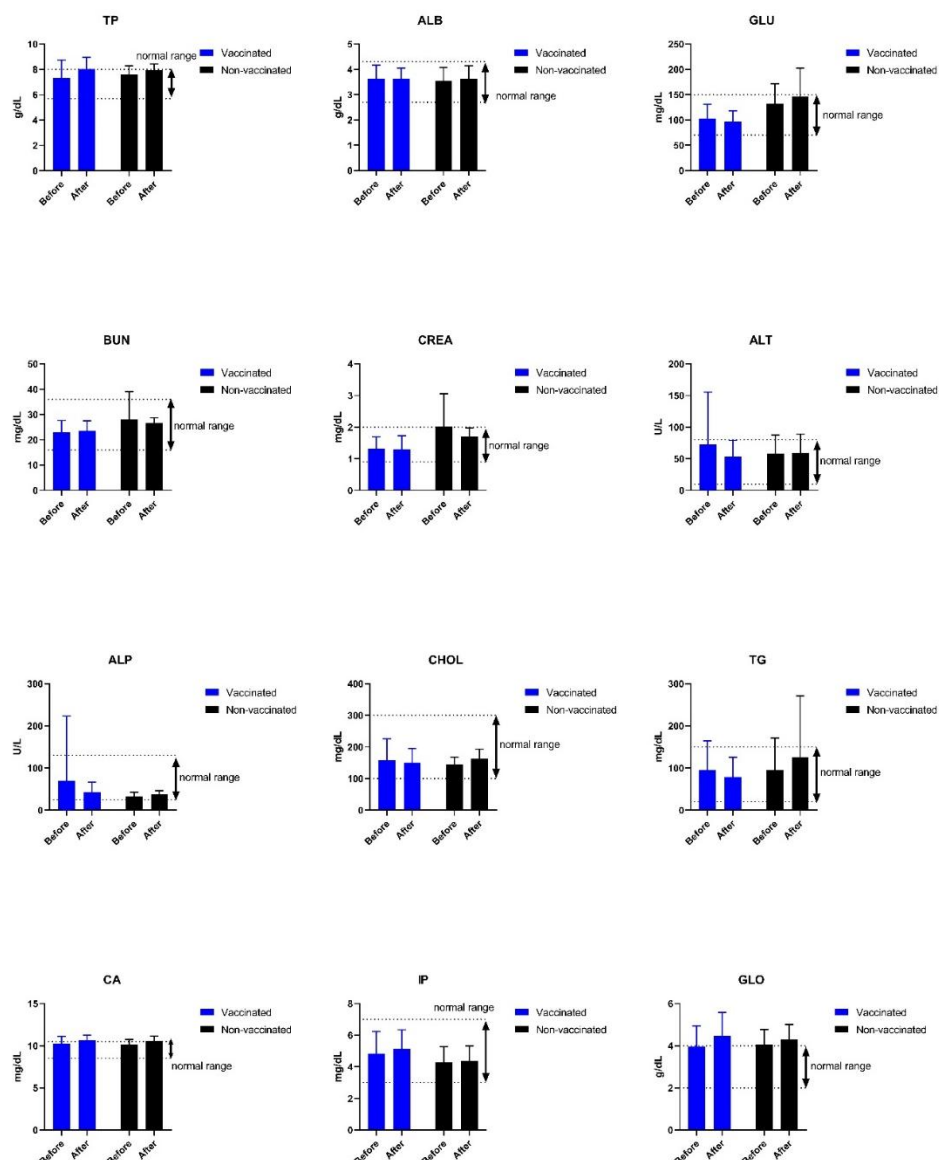
17 CBC tests were performed on cats participating in the clinical trial to monitor their overall health status
 18 associated with vaccination. The parameters analyzed included WBC, RBC, HGB, HCT, MCV, MCH,
 19 MCHC, PLT, RDW, MPV, NE%, LY%, MO%, EO%, and BASO%. Across all parameters, most values
 20 remained within the normal range for cats. Although a small number of individual animals exhibited
 21 values outside the normal range, these deviations were not associated with vaccination. Overall, the
 22 results indicate that the vaccine did not induce any adverse hematological effects in the tested cats.



23

24 Additional file 3. Blood chemistry analysis of dogs in clinical trial with companion animals

25 Blood chemistry analysis was conducted on dogs to evaluate their overall health status associated with
 26 vaccination. The parameters analyzed included TP, ALB, GLU, BUN, CREA, ALT, ALP, CHOL, TG,
 27 CA, IP, and GLO. Across all parameters, most values remained within the normal range for dogs.
 28 Although a small number of individual animals exhibited values outside the normal range, these
 29 deviations were not associated with vaccination status. Overall, the blood chemistry analysis confirmed
 30 that vaccination did not induce any adverse effects on metabolic or organ functions in dogs.

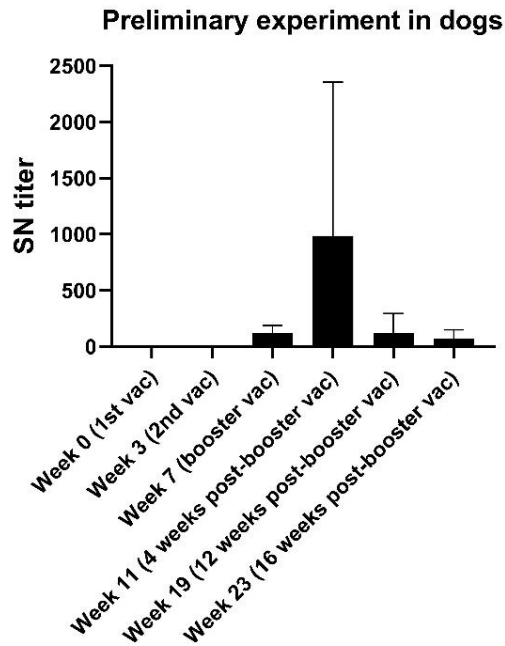


31

32 Additional file 4. Blood chemistry analysis of cats in clinical trial with companion animals

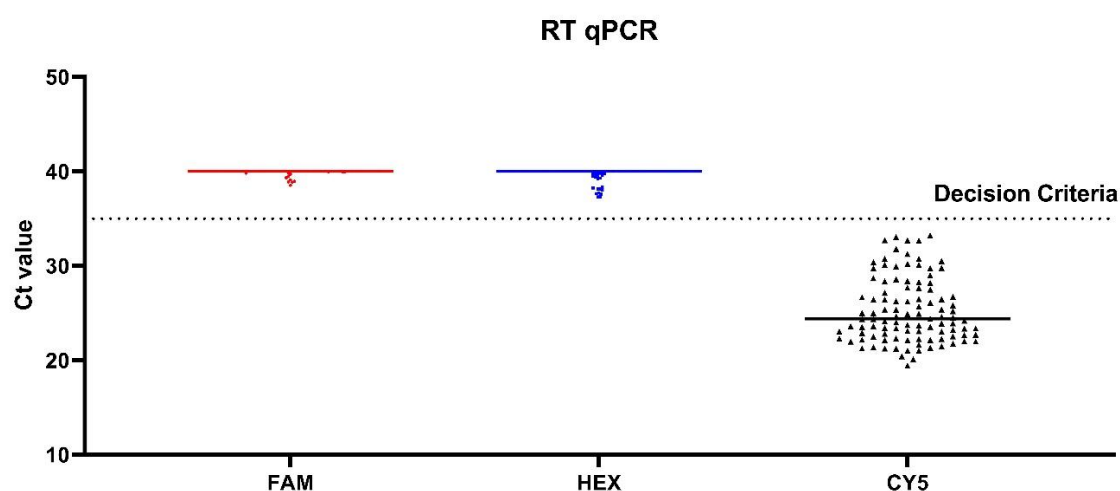
33 Blood chemistry analysis was conducted on cats to evaluate their overall health status associated with
 34 vaccination. The parameters analyzed included TP, ALB, GLU, BUN, CREA, ALT, ALP, CHOL, TG,
 35 CA, IP, and GLO. Across all parameters, most values remained within the normal range for cats.
 36 Although a small number of individual animals exhibited values outside the normal range, these
 37 deviations were not associated with vaccination status. Overall, the blood chemistry analysis confirmed
 38 that vaccination did not induce any adverse effects on metabolic or organ functions in cats.

39



Additional file 5. Preliminary experiment for determining vaccination and follow-up monitoring schedules

Preliminary evaluation of serum neutralizing (SN) antibody titers in (A) dogs (n=3) following administration of the vaccine candidate. Data are presented as mean SN titers at each indicated week post-vaccination. The results showed that a marked increase in SN titers occurred after administration of the booster vaccination, emphasizing the necessity of completing the booster dose to achieve a robust antibody response. In addition, neutralizing antibody titers declined markedly by week 19 (12 weeks post-booster vaccination) and became nearly undetectable by week 23 (16 weeks post-booster vaccination). Statistical analyses were not performed, as the primary objective was to characterize overall trends.



Additional file 6. SARS-CoV-2 antigen test by RT-qPCR prior to vaccination in clinical trial with companion animals

RT-qPCR was performed to detect the presence of SARS-CoV-2 RNA in recruited companion animals prior to vaccination. The assay utilized three fluorescence channels: FAM, HEX, and CY5, corresponding to target detection and internal controls (FAM for the RdRp gene, HEX for the E gene, and Cy5 for the internal control). Ct values for all samples were analyzed and compared against the decision criteria (36 Ct value) for positivity. As shown in the graph, all samples tested negative for SARS-CoV-2, confirming that none of the recruited companion animals were infected at the time of sample collection.

63 **Data availability**

64 All data generated or analyzed during this study are included in this published article and its
65 supplementary information files.