

Supplementary information

Systematic analysis of COVID-19 mRNA vaccines using four orthogonal approaches demonstrates no excessive DNA impurities

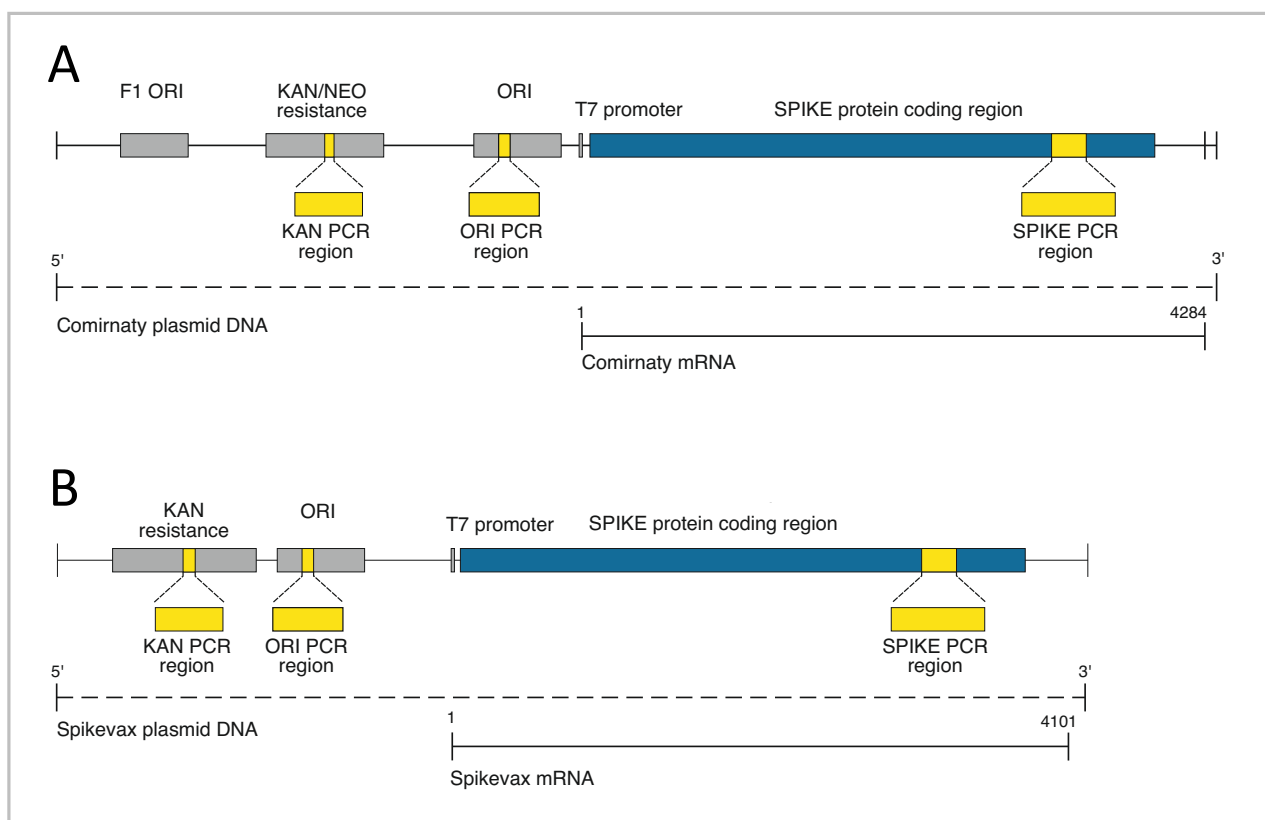


Figure S1. | Schematic illustrations of DNA templates used for production of mRNA for Comirnaty and Spikevax vaccines with indicated position of regions amplified in qPCR. Template structures were derived from the sequences obtained in this study and were used for the design of primers and probes.

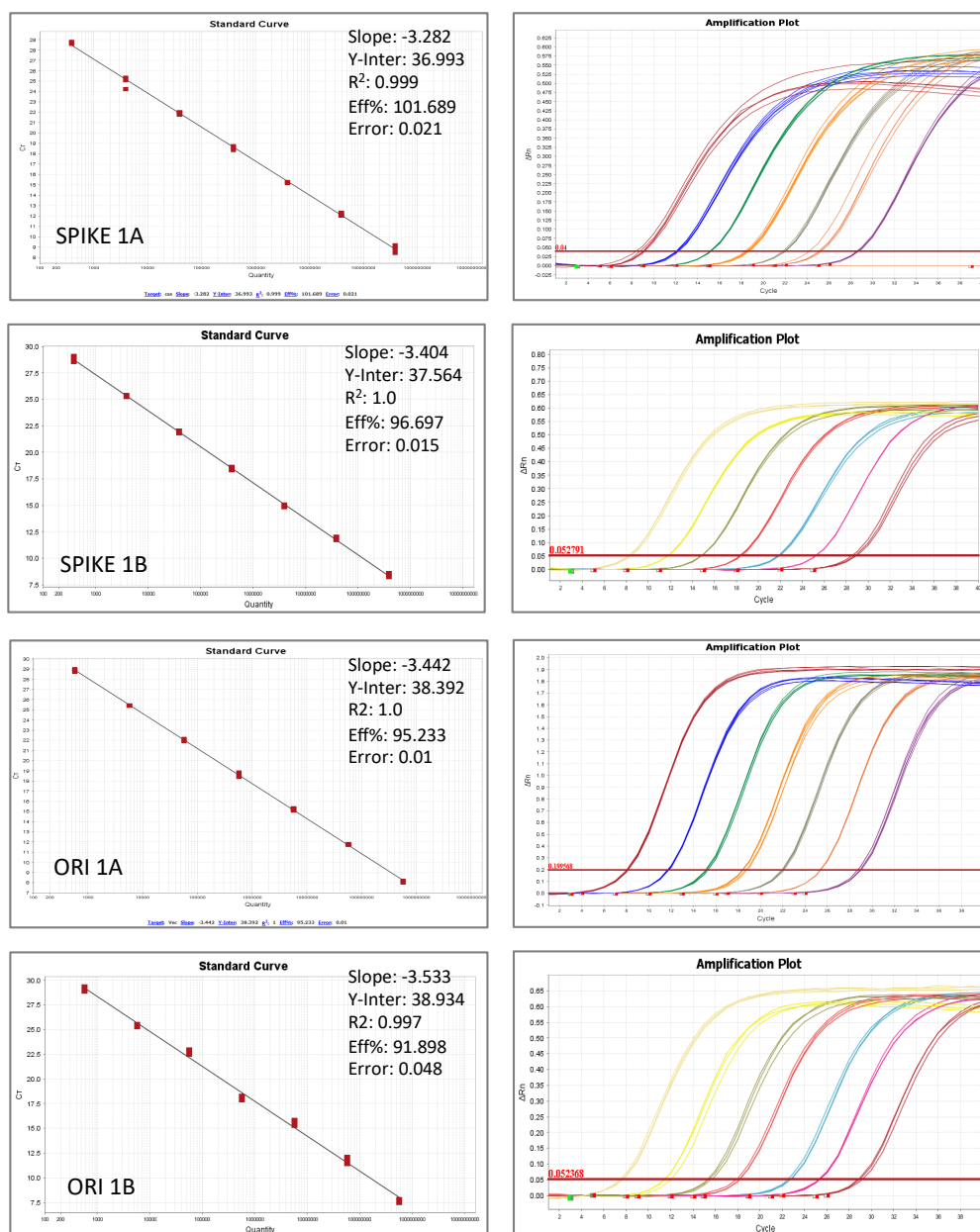


Figure S2. Part 1 | Calibration graphs and amplification curves for assessing qPCR efficiency of the primers used in the vaccine analysis and ensuring reliable quantification of target regions. Calibration graphs were generated from serial tenfold dilutions of known concentrations of DNA standards (from 100 pg to 0.1 fg) corresponding in size to individual PCR amplicons. Inlets in calibration graphs indicate parameters of each designed qPCR assay. Amplifications curves visualize the progress of qPCR reactions.

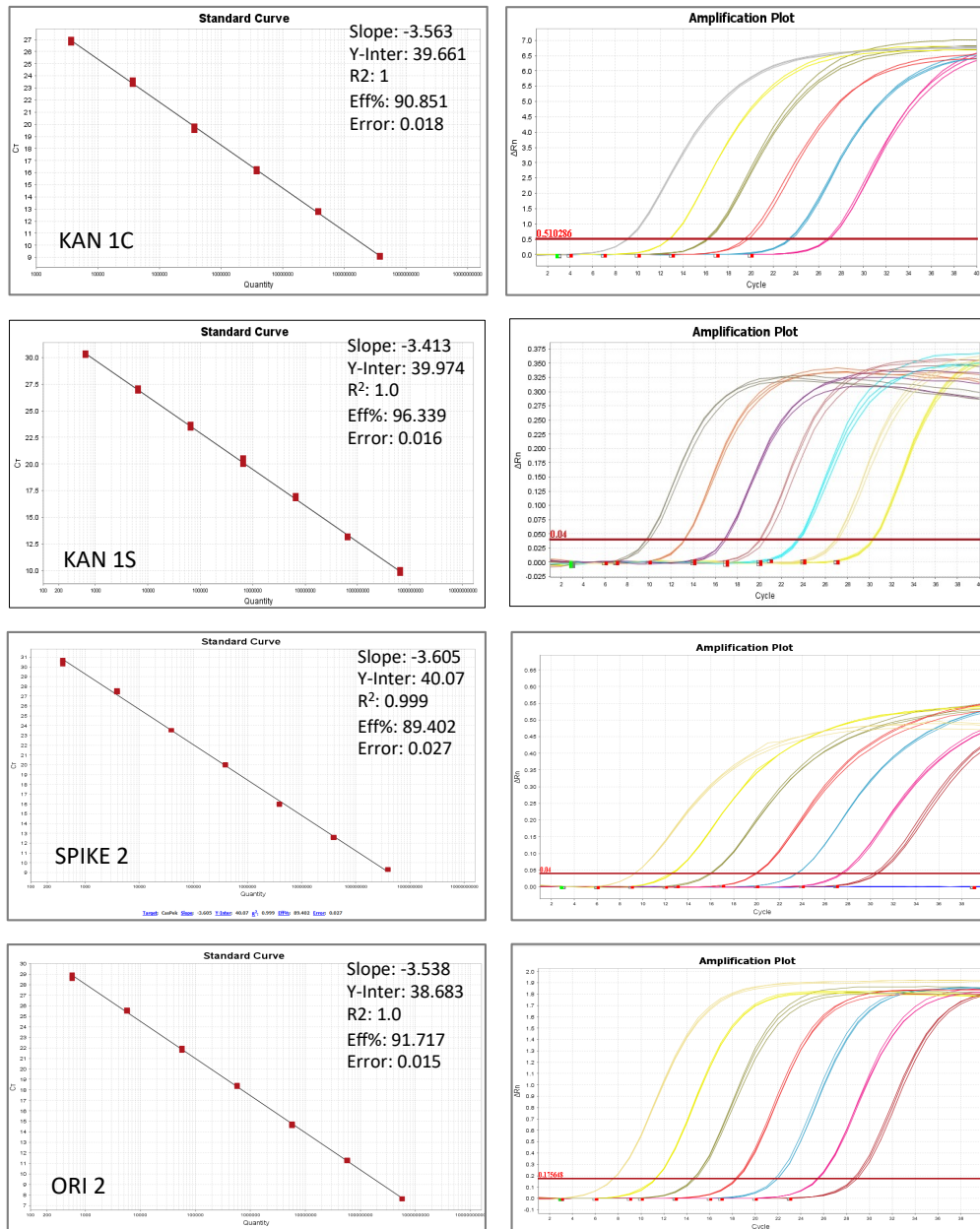


Figure S2. Part 2 | Calibration graphs and amplification curves for assessing qPCR efficiency of the primers used in the vaccine analysis and ensuring reliable quantification of target regions. Calibration graphs were generated from serial tenfold dilutions of known concentrations of DNA standards (from 100 pg to 0.1 fg) corresponding in size to individual PCR amplicons. Inlets in calibration graphs indicate parameters of each designed qPCR assay. Amplifications curves visualize the progress of qPCR reactions.

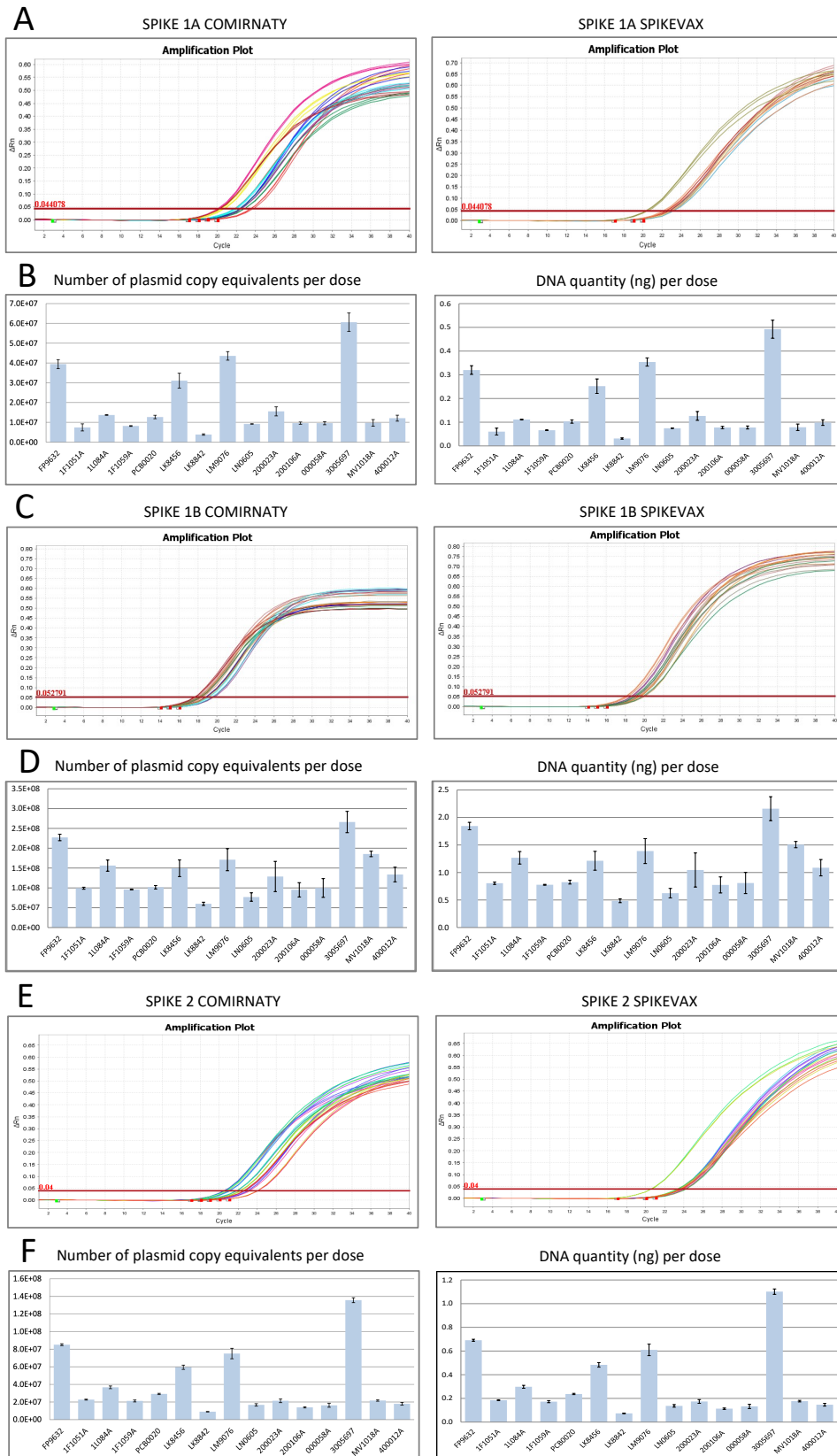


Figure S3. | Analysis of all vaccine batches by qPCR SPIKE assays. (A, C) Amplification curves of the the 3' end of S protein coding sequence using SPIKE 1A and SPIKE 2 primers. (B, D) Graphs illustrating calculated numbers of plasmid copy equivalents and their conversion to quantity of residual DNA in nanograms per vaccine dose.

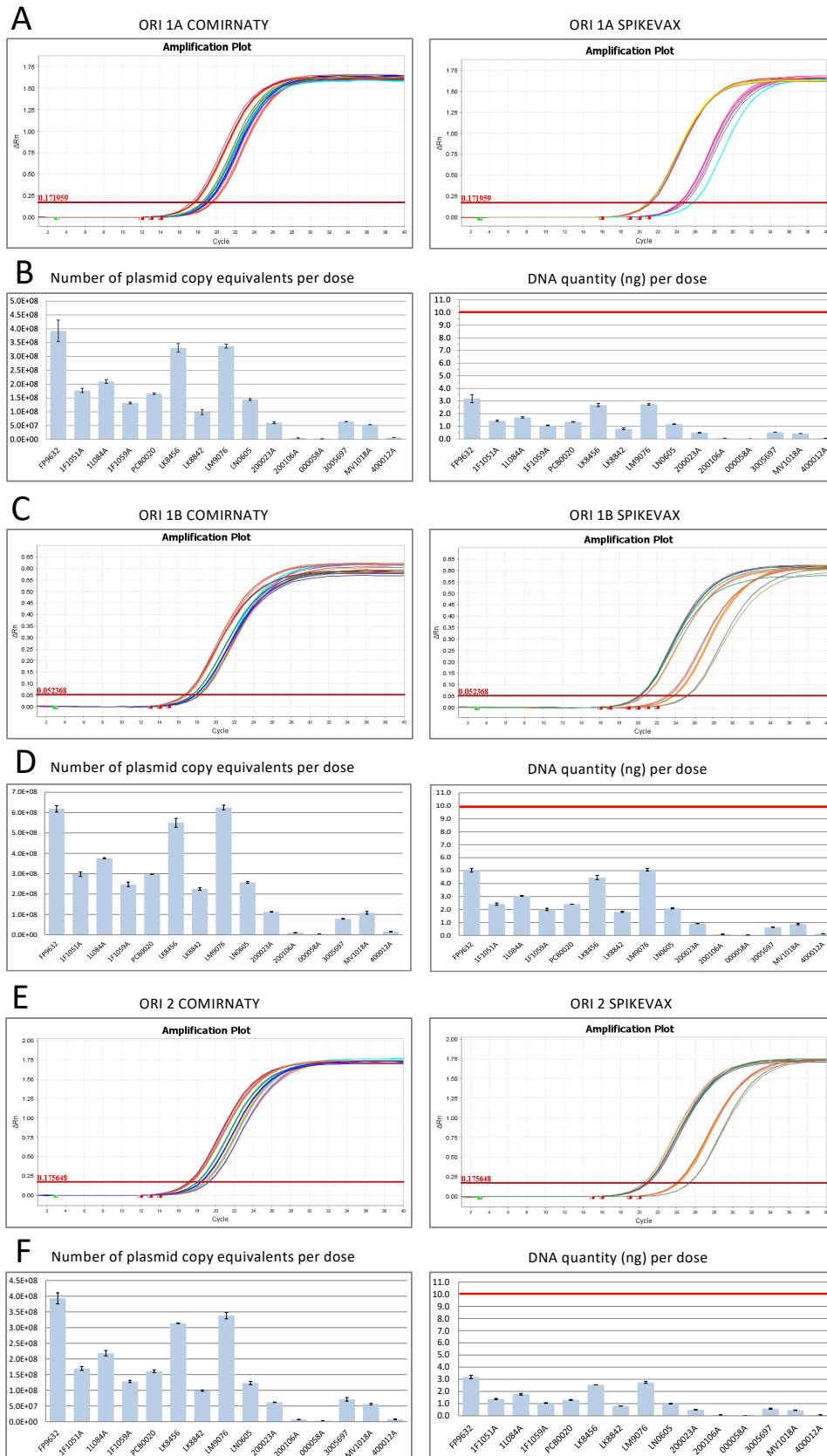


Figure S4. | Analysis of all vaccine batches by qPCR ORI assays. (A, C, E) Amplification curves of the region containing origin of plasmid replication using ORI 1A, ORI 1B and ORI 2 primers. (B, D, F) Graphs illustrating calculated numbers of plasmid copy equivalents and their conversion to quantity of residual DNA in nanograms per vaccine dose. Red line indicates a limit of 10 ng/dose.

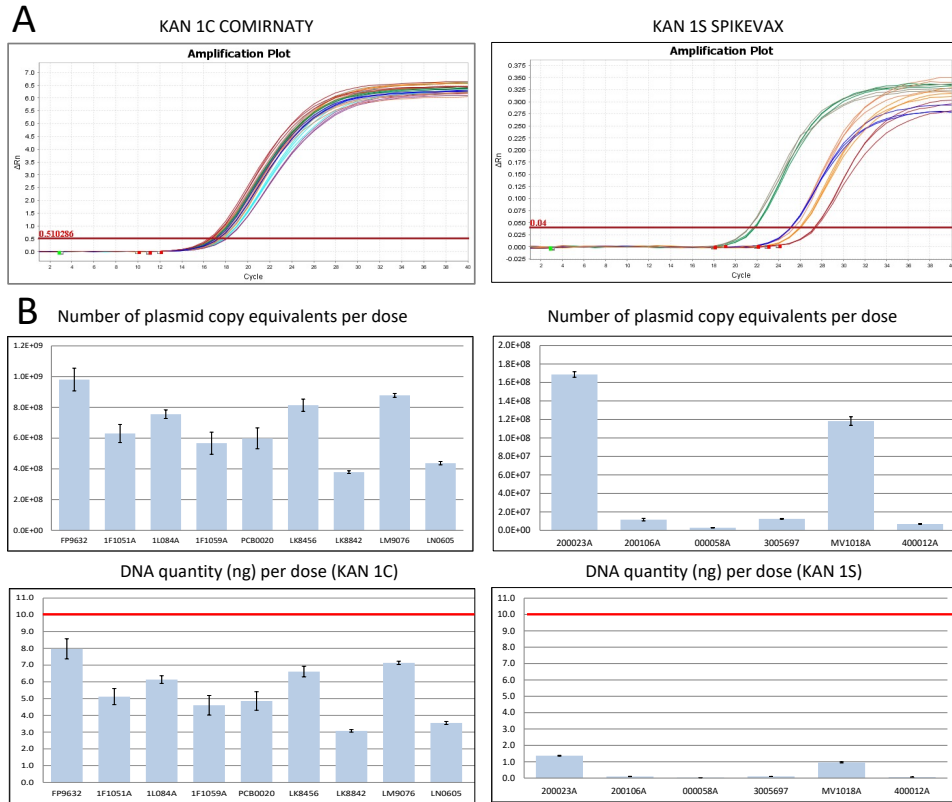


Figure S5. | Analysis of Comirnaty vaccine batches by qPCR KAN assay. (A) Amplification curves of the region that confers resistance to kanamycin using KAN 1C primers. (B) Graphs illustrating calculated numbers of plasmid copy equivalents and their conversion to quantity of residual DNA in nanograms per vaccine dose. Red line indicates a limit of 10 ng/dose.

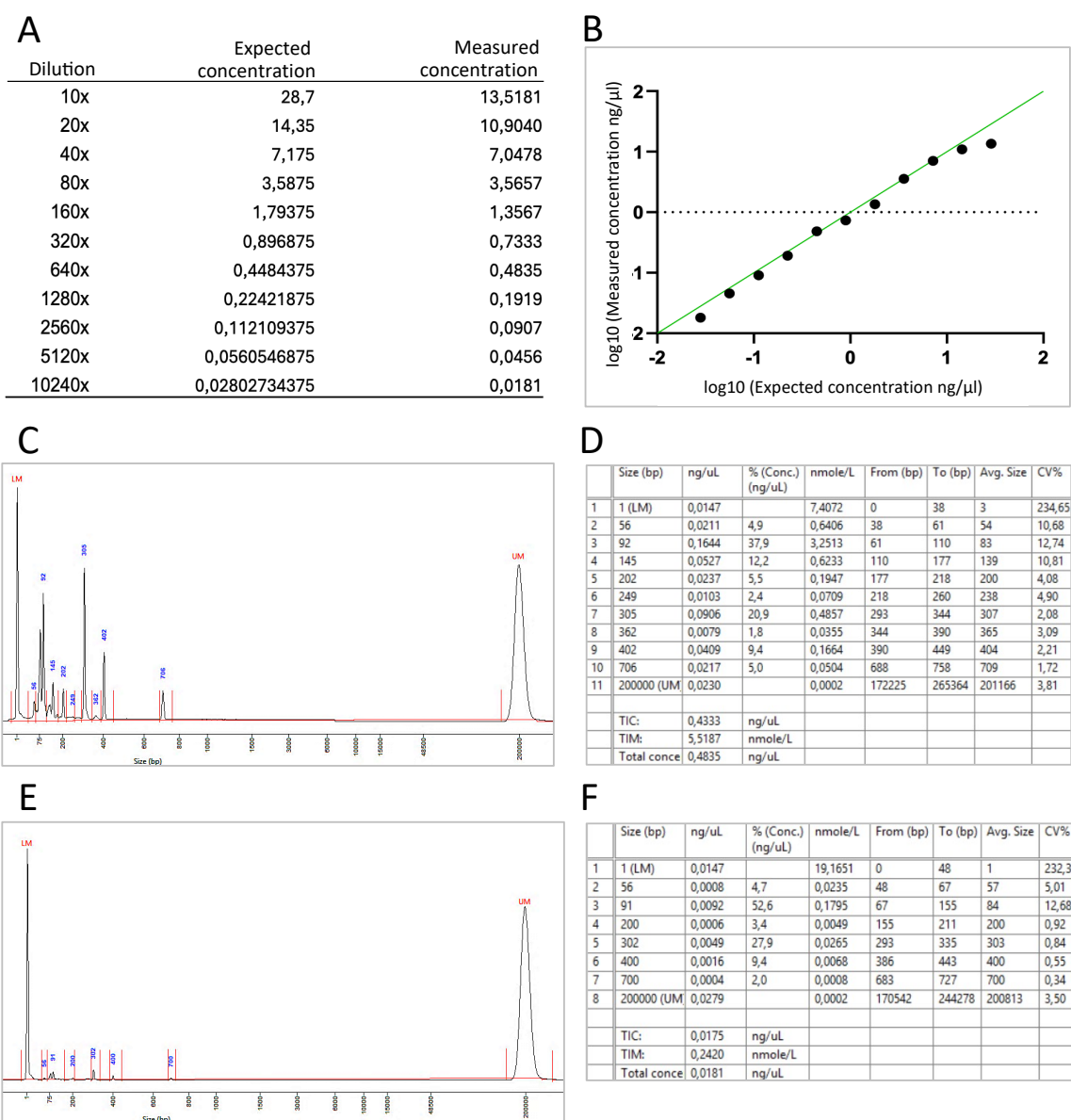


Figure S6. | Quantification of control ladder DNA by capillary electrophoresis on Fragment Analyser System 5200 using DNF-464 HS DNA Fragment Kit. (A) List of control DNA dilutions, expected concentrations and actually measured concentrations. (B) Graphical comparison of expected and measured values. (C) Electropherogram resulting from the fragment analysis of ladder DNA diluted 640-fold (LM – lower marker of 1 bp, UM – upper marker of 20,000 bp) and (D) table with corresponding quantitative values for each size marker of the ladder DNA. (E) Electropherogram of control DNA diluted 10,240-fold and (F) corresponding table with quantitative values for measurable size markers.

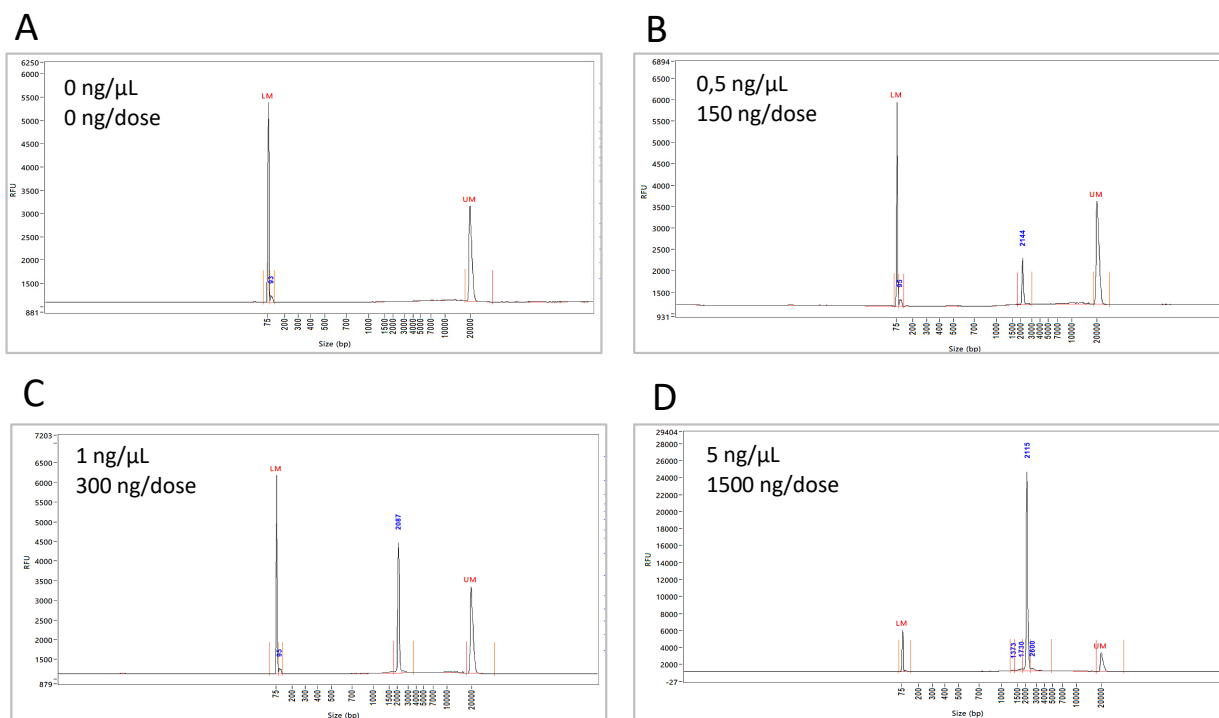


Figure S7. | Visualisation of spiked external plasmid DNA added to negative control. Capillary electrophoresis was performed on Fragment Analyser System 5200 using DNF-930 Large Fragment DNA Kit. (A) Blank, negative control with no added DNA (LM depict 75 bp, UM depicts 20,000 bp). (B – D) Samples with addition of increasing quantities of external plasmid DNA. DNA signal was detected as a single peak of 2,100 bp with the height corresponding to the amount of added DNA.

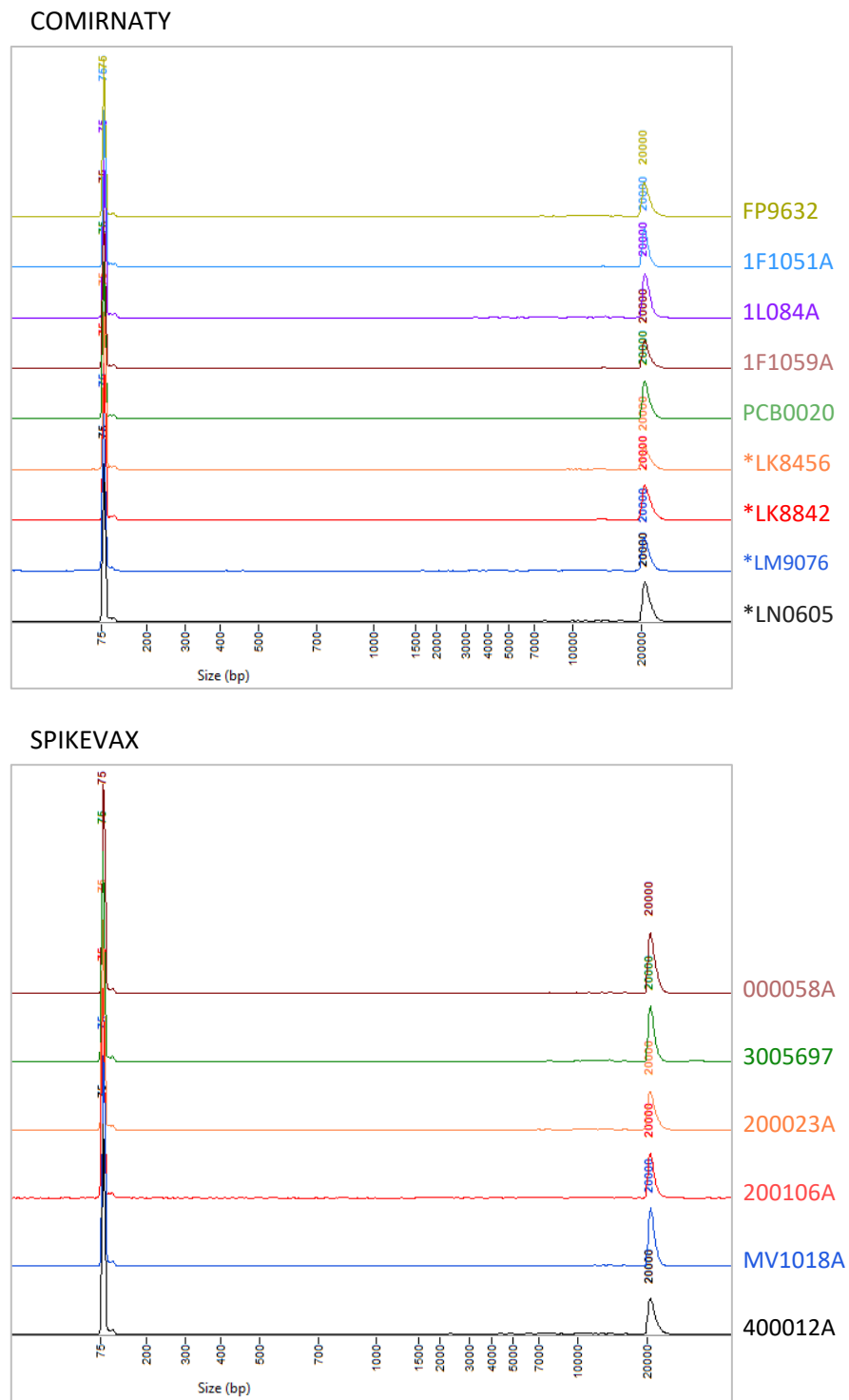
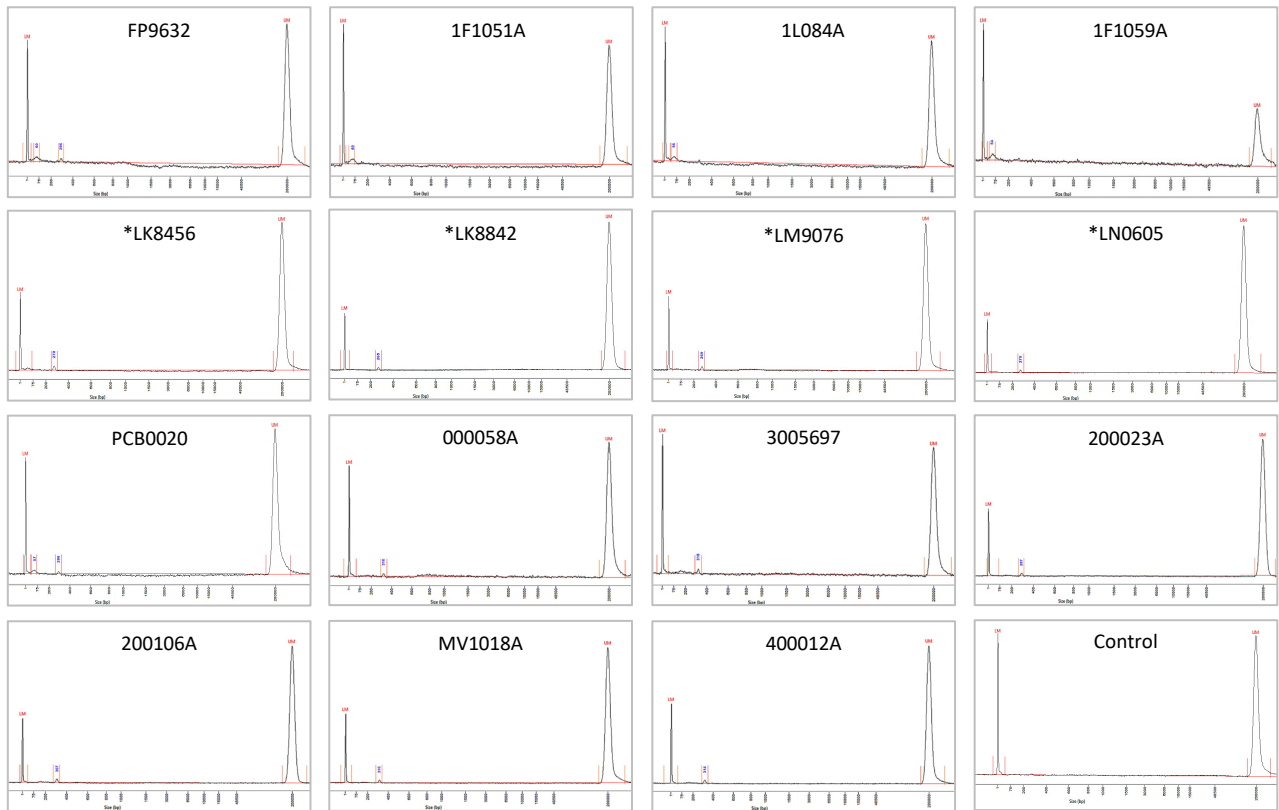


Figure S8. | Fragment analysis of Comirnaty and Spikevax vaccines using the DNF-930 dsDNA 930 Reagent Kit. Representative electropherograms of all batches show no specific peaks indicating absence of DNA quantities within the detection range of the kit.

A



B

Figure S9. | Densitograms and corresponding quantitative data from the fragment analysis of the batches of Comirnaty and Spikevax vaccines on Fragment Analyzer 5200 using DNF-464 HS DNA Fragment Kit. Measurements were accomplished with 3 biological and 2-3 technical replicates. (A) Representative electropherograms from each analysed vaccine batch. (B) Corresponding quantitative assessment of the total DNA concentrations per 1 μL of vaccine sample).

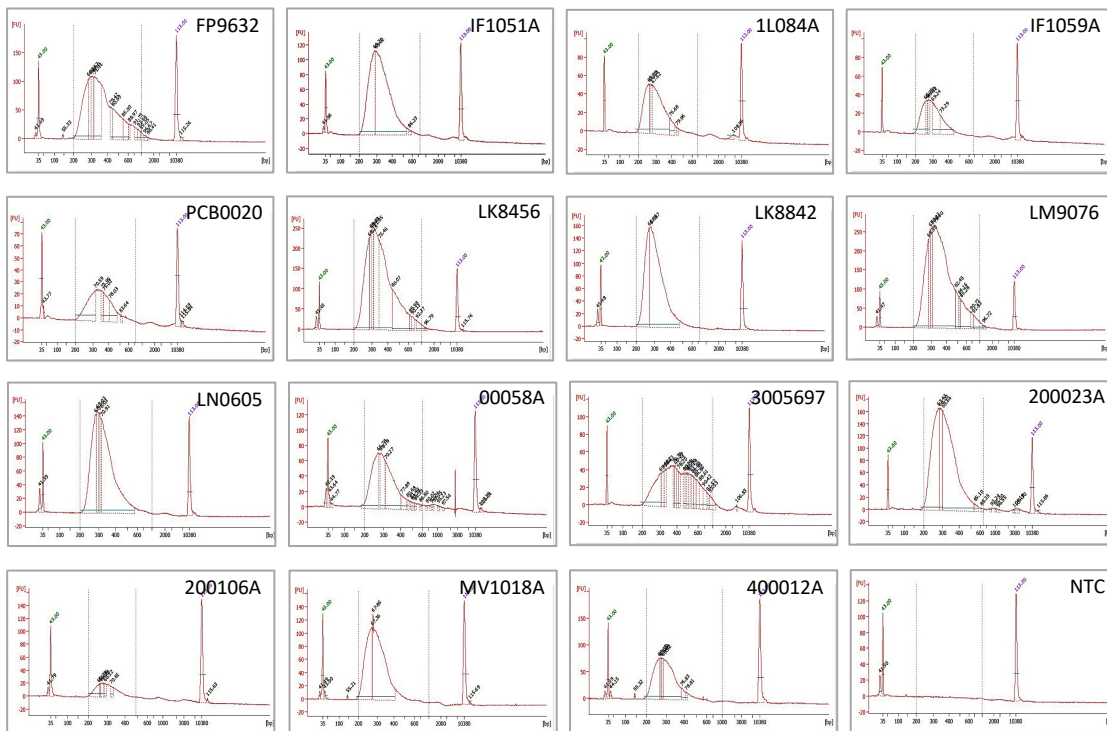


Figure S10. | Fragment lengths profiles of sequencing libraries prepared using TruSeq DNA Nano kit according to modified protocol from the analysed vaccine batches. Fragment analysis was performed on Agilent Bioanalyzer 2100 using High Sensitivity DNA kit with detection range of 100 pg/μl for the fragmented DNA.

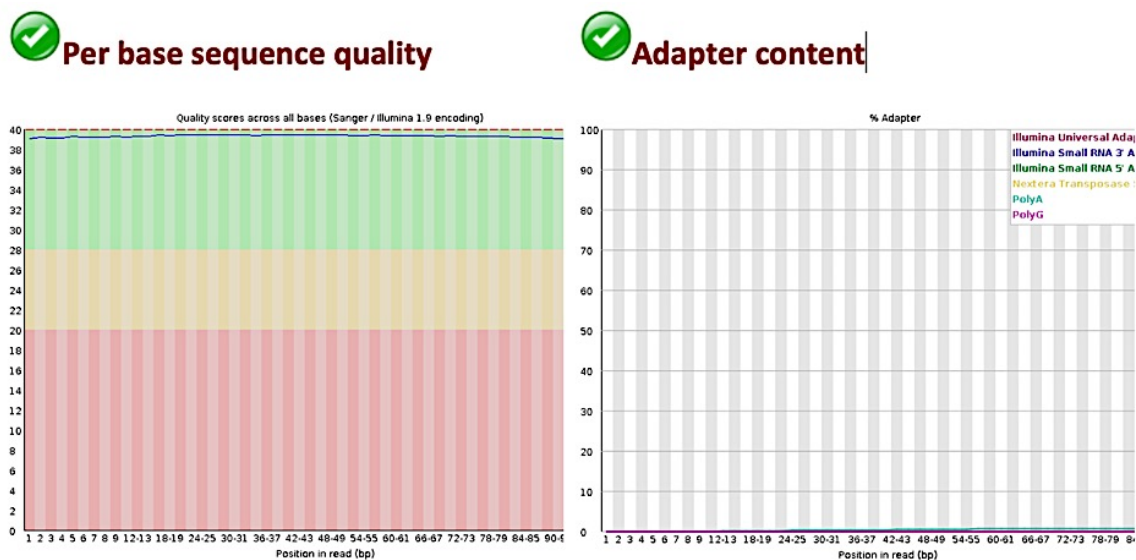


Figure S11. | FastQC quality control output for short-read sequencing data.