Verification of questionable information about the COVID-19 vaccines

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Abstract

Since the rollout of large-scale COVID-19 vaccination programs, misinformation has often been disseminated through social media, particularly regarding mRNA vaccines. Even scientists have sometimes exacerbated the problem and undermined public trust in health policies by proposing questionable theories. In this study, we present experimental and statistical analyses that address these claims. Animal experiments were conducted to evaluate the hypothesis that self-amplifying RNA vaccines can replicate and spread between individuals, a theory that our findings strongly refute. We also examined the claim that the presence of DNA contamination in mRNA vaccine formulations leads to the integration of DNA fragments into the genome, thereby altering gene function or expression to induce various adverse events, a theory that is also refuted by our findings. Additionally, we conducted statistical comparisons between mRNA and conventional influenza vaccines, challenging the narrative promoted by mainstream scientists and physicians that mRNA vaccines are categorically safer. Our findings underscore the need for scientists to rigorously investigate and transparently communicate about such claims, thereby helping to rebuild public trust in vaccination efforts.

Introduction

Four years have passed since large-scale vaccination against COVID-19 began worldwide. In the meantime, a great deal of misinformation about COVID-19 vaccines—especially mRNA-based vaccines, a newly developed technology—has spread, primarily through social media. Much of this misinformation is absurd (such as the claim that mRNA vaccines contain nanotechnology devices to control vaccinated individuals) and has not been taken seriously by the general public. However, a significant number of people fully believe these claims.

In addition, some scientists have contributed to the spread of misinformation by using technical terms that sound scientific, leading to public confusion and societal division. Misinformation about healthcare—particularly when it originates from scientists—can seriously undermine public confidence in health policies.

For this reason, scientists have a responsibility to actively investigate unverified claims that appear scientific and to communicate accurate information to the general public. Here, we report the results of our experimental and statistical evaluation of several scientifically questionable claims regarding the alleged harms of the COVID-19 vaccine.

Do Replicon Vaccines Cause Fatal Infections?

In October 2024, a newly developed vaccine—called a self-amplifying mRNA (saRNA) vaccine, or replicon vaccine—was launched in Japan. This type of mRNA vaccine encodes the SARS-CoV-2 Spike protein and a self-replicating RNA polymerase, which allows the production of large amounts of antigen in the recipient's body, eliciting an immune response even at low doses (1).

However, several months before the saRNA vaccine was introduced, some scientists and physicians—primarily in Japan—began to argue that the saRNA molecules in the vaccine could replicate indefinitely within recipients' bodies, producing virus-like, infectious particles coated with Spike proteins. They claimed these particles could potentially be transmitted to other people or animals, posing a serious threat to public health (2–4).

In December 2024, Meiji Seika Pharma, the pharmaceutical company that manufactures and sells the saRNA vaccine, filed a lawsuit against former minister and Diet member Kazuhiro Haraguchi. Haraguchi had publicly criticized the company, claiming the saRNA vaccine was infectious and dangerous, likening it to a biological weapon (5).

saRNA contains an RNA polymerase-dependent self-replication mechanism, but it does not encode structural proteins or packaging signals. Therefore, it is considered highly unlikely to produce virus-like infectious particles. Conventional research laboratories and clinical trials do not assume the production of infectious particles from saRNA vaccines and, as a result, have not tested for this possibility.

The theory of a "replicon vaccine pandemic," as described above, should be experimentally verifiable. If feasible, scientists should investigate it. We conducted such a verification through animal experiments.

The saRNA vaccine Kostaive, developed by Arcturus Therapeutics and marketed by Meiji Seika Pharma, encodes the Spike protein of the SARS-CoV-2 JN.1 variant, inducing the production of anti-Spike antibodies in both humans and animals. BALB/c mice are susceptible to infection with SARS-CoV-2 strains bearing the JN.1-type Spike protein, making them a suitable animal model for testing whether virus-like particles

capable of transmission to other animals are produced following administration of Kostaive. Mice produce anti-Spike antibodies when administered Spike-encoding saRNA. Therefore, by examining anti-Spike antibody levels in mice co-housed with those vaccinated with Kostaive, it is possible to monitor whether the Spike-encoding saRNA has been transmitted.

We investigated whether BALB/c mice co-housed in the same cage with BALB/c mice vaccinated with 1 μg of Kostaive (equivalent to approximately 600 times the human dose per body weight) would acquire saRNA through animal-to-animal transmission and subsequently produce anti-Spike antibodies (**Figure 1A**).

Our results showed that the anti-Spike antibody titers in the serum of mice co-housed for 28 days with mice vaccinated with 1 μg of Kostaive were not significantly higher ($P = 0.4669$) than those in saline-inoculated control mice (**Figure 1B**). Furthermore, the antibody titers in the co-housed mice were significantly lower ($P = 0.0007$) than in mice directly vaccinated with 0.1 ng of Kostaive (1/10,000 of the dose received by the co-housed mice' partners). These findings indicate that, even after four weeks of sharing air, food, water, and bedding with vaccinated mice, there was no evidence of saRNA transmission.

Additionally, we examined whether the injected Kostaive RNA was released as virus-like particles and remained detectable in the bloodstream of vaccinated mice over time. On day 1 post-vaccination with 1 μg of Kostaive, Spike-encoding RNA was detected in both the draining lymph nodes and serum (**Figure 1C**). This suggests that, as in humans who receive mRNA vaccines, lipid nanoparticles from Kostaive circulate beyond the injection site (6). However, by day 18, Spike RNA was no longer detectable in the serum. Notably, the vaccinated mice had developed a strong immune response, producing high

levels of anti-Spike antibodies by day 28 (**Figure 1B**), indicating that the saRNA is cleared from circulation within a few weeks after administration.

Therefore, it is highly unlikely that Kostaive replicates indefinitely in host cells or is released from them as virus-like particles. Furthermore, no epidemiological evidence of person-to-person transmission has been reported, despite Kostaive being used in clinical trials in Singapore, Japan, Vietnam, and other countries for over two years, with an estimated administration to more than 16,000 individuals (7-10).

Recently, saRNA vaccines have been approved in several countries, including Japan, and may soon be used more widely. A situation similar to the one that occurred in Japan—triggered by public statements from scientists—could also arise elsewhere, potentially leading to public distrust in science and medicine, as well as societal division.

Our present data indicate that the probability of animal-to-animal transmission of the saRNA vaccine is extremely low, if it exists at all. However, this study may still be criticized for limitations in its design or scale. If pharmaceutical companies or research institutions have conducted similar experiments on a larger scale, the resulting data should be made publicly available.

Although the theory that saRNA vaccines are infectious and cause serious health problems can be dismissed based on current evidence, saRNA vaccines—like conventional mRNA vaccines—are believed to have inherent limitations, such as their inability to provide long-term suppression of viral infection or robust immunity against emerging variants. Recently, adverse events and chronic illnesses following COVID-19 vaccination have drawn attention from both immunological and social perspectives (11, 12). We should exercise caution when using mRNA-based vaccines to combat COVID-19, which has now become similar to a common cold in many cases.

Does DNA contamination in mRNA vaccines cause adverse events?

Since the report by Kevin McKernan in the spring of 2023, concerns have been raised about DNA contamination in mRNA vaccines (13, 14). mRNA vaccines are produced via in vitro transcription using DNA as a template (15). After transcription, the template DNA is fragmented using deoxyribonuclease I (DNase I), and the resulting DNA fragments are removed through ultrafiltration. However, due to limitations in DNase I digestion efficiency and the filtration process, DNA fragments of a certain size—averaging about 100 base pairs (bp)—can remain in the final mRNA product. These residual DNA fragments may be encapsulated within lipid nanoparticles (LNPs) along with the mRNA.

According to regulations set by the European Medicines Agency (EMA), residual DNA in mRNA vaccine products should be less than 10 ng per dose. McKernan et al. initially published data suggesting that some mRNA vaccines contained up to 1,000 times more DNA than the EMA's regulatory limit (13). Following that report, several other researchers have also found significant levels of residual DNA in mRNA vaccines—reportedly several hundred times greater than the regulatory threshold (16, 17). They argue that such large amounts of residual DNA are unacceptable and could potentially lead to various health problems following COVID-19 vaccination.

To quantify the DNA content in mRNA vaccines, they employed a method using fluorochromes that bind to DNA molecules. However, the fluorochromes used in these assays can also bind weakly to RNA and emit fluorescence. Given that mRNA vaccines contain a much larger quantity of RNA relative to DNA, fluorescence resulting from RNA binding may have led to an overestimation of the actual DNA content (18).

Our experiments also showed that the amount of DNA was overestimated by dozens of times when a mixture containing a large amount of RNA and a small amount of DNA was measured using a DNA-specific fluorochrome (**Figure 2A,B**). Not only that, signals indicating the presence of DNA were falsely detected, even when the RNA in the RNA:DNA mixture was degraded by ribonuclease A (RNase A). This overestimation occurs because the fluorochrome binds to oligonucleotides and mononucleotides generated by RNase A digestion. Therefore, when measuring DNA using fluorochromes, it is necessary to degrade RNA in the samples with RNase A, remove the resulting oligo(mono)nucleotides, and purify the remaining DNA before measurement. To date, no measurements using such a method have been reported. Furthermore, the use of fluorochromes to measure residual DNA has not been approved by regulatory agencies in any country.

The currently approved method for measuring residual DNA in mRNA vaccines is quantitative polymerase chain reaction (qPCR). This method uses DNA primers complementary to DNA sequences potentially present in mRNA vaccines to amplify and detect the target DNA, theoretically enabling quantification of a single DNA molecule of approximately 60 base pairs or longer. When measured using qPCR by McKernan et al., no residual DNA above the regulatory limit was detected in the Pfizer and Moderna mRNA vaccines (19). The amount of residual DNA in Pfizer's mRNA vaccine tended to be higher than in Moderna's, though still only about one-third of the regulatory limit set by the EMA.

Traditionally, DNA contamination in pharmacological products, including vaccines, has been considered a risk based on the assumption that harmful genes—such as oncogenes derived from cell lines used in the production of live vaccines—could be

present in the final product and affect the cells of vaccinated individuals (20). The current regulatory limit for residual DNA (10 ng/dose) is more lenient than the previous limit (10 pg/dose). This relaxation is based on the assumption that fragmented DNA, if sufficiently small, cannot function as a protein-coding gene.

However, some scientists have raised concerns that this safety threshold may be inadequate, as even small DNA fragments could potentially enter the nucleus and integrate into the genomic DNA of host cells, possibly altering gene function or expression (19). Current safety standards do not sufficiently consider how such small DNA fragments—like those potentially found in mRNA vaccines—might affect human cells.

In this study, we sought to determine the extent to which small DNA fragments introduced into cells could be incorporated into the genome. We used the HEK293 cell line, known for its high efficiency in gene transduction. HEK293 cells (1×10^6 cells) were transfected with a 160 base pair (bp) DNA fragment (1 μg) using lipofection. The cells were then serially passaged, and intracellular DNA levels were measured by quantitative PCR (qPCR) (**Figure 3A**). The copy number of DNA fragments per cell was estimated by comparing it to that of a housekeeping gene in the genome.

Eight hours after transfection, approximately 100,000 copies of the DNA fragment per cell were detected (**Figure 3B**). While most of these fragments are expected to be degraded or diluted during cell division, fragments that integrate into the genome would be maintained at a constant copy number in the cell population. As cell culture continued and division progressed, the number of detectable DNA fragments gradually declined. By day 28, 0.02 copies per cell were detected—a number that remained constant through day 35. This suggests that, on average, one DNA fragment was inserted into the genome of

approximately 1 in 50 cells. Thus, in this highly permissive cell line, it was estimated that if 100,000 DNA fragments were introduced per cell, about 2% of the cells would ultimately acquire at least one integrated fragment.

In normal primary cells, the efficiency of genomic integration would likely be several orders of magnitude lower. Furthermore, under physiological conditions, the introduction of 100,000 foreign DNA molecules into a single cell is highly unlikely. A single lipid nanoparticle (LNP) in mRNA vaccines typically encapsulates hundreds to thousands of mRNA molecules, and even if DNA contamination is present up to the regulatory limit (10 ng/dose), a single LNP would likely contain only 1–10 DNA molecules.

Therefore, the probability that residual DNA fragments in mRNA vaccines would integrate into the genome and alter gene function or expression is extraordinarily low—so low that it is unlikely to be a plausible cause of the various adverse events reported in some vaccinated individuals.

Whether the current regulatory limit for residual DNA is adequate, and whether DNA below that threshold poses any meaningful risk, should be evaluated based on empirical scientific data and rational interpretation. We hope that the results of this experiment will serve as a foundation for informed discussion on this topic.

Are mRNA Vaccines Safe?

Many experts, physicians, and politicians have spread incorrect information about mRNA vaccines against COVID-19, often overestimating their efficacy while underestimating their potential toxicity. For example, Taro Kono, the Minister in Charge of Vaccine Promotion at the time, stated, “there have been no deaths from the COVID-19 vaccination (21).” Additionally, some scientists and doctors recommending COVID-

19 vaccination have claimed that “the side effects of the COVID-19 vaccines are very minor, and symptoms such as fever will definitely subside within a few days (22).” As a result, over 80% of the population in Japan received two or more doses, and the total number of vaccinations administered exceeded 400 million.

However, according to the Ministry of Health, Labour, and Welfare of Japan, more than 9,200 people have been officially certified as having suffered health deterioration due to COVID-19 vaccination, and 1,031 of these individuals have died (23). The number of certified cases of health deterioration is approximately 2.6 times higher, and the number of deaths is 6.8 times higher, than those reported for all regular vaccinations in Japan over the past 45 years (24).

To more accurately assess the health risks caused by COVID-19 vaccines, it is necessary to compare these numbers while adjusting for factors such as the total number and age distribution of vaccinated individuals. Therefore, we compared the number of deaths certified for people aged 65 and over under the same reporting system for COVID-19 vaccines and influenza vaccines. From 2011 to 2021, influenza vaccines were administered 194 million times, with four deaths certified (25, 26, 27, 28). In contrast, COVID-19 vaccines—mainly mRNA vaccines—were administered 196 million times from 2021 to April 2024, with 770 deaths certified (data through the August 26, 2025 release) (27, 28, 29). The Ministry of Health, Labour, and Welfare has stated that its criteria for recognizing vaccine-related health damage have not changed for COVID-19 vaccines.

In simple terms, the risk of death from COVID-19 vaccines appears to be more than 190 times higher than that associated with the Flu vaccine. Therefore, the answer to the headline question must be “no.”

What makes COVID-19 vaccines harmful to some vaccinated individuals? The self-amplifying characteristic is clearly not the cause of damage, as most doses administered so far have been conventional mRNA vaccines, not saRNA vaccines. DNA contamination is also unlikely to be responsible for side effects, as demonstrated by the experiments reported in this paper. Numerous studies have investigated the causes of COVID-19 vaccine side effects, with most attributing them to the effects of Spike proteins—often produced in uncontrolled amounts and in unintended regions of the body (30). Further research is needed to precisely identify the mechanisms by which COVID-19 vaccines may cause harm to recipients (31).

Conclusion

Misinformation about COVID-19 has spread both globally and within local communities, ranging from widely dismissed conspiracy theories to seemingly scientific claims that have prompted large-scale public response. More concerningly, some scientists have exploited such misinformation for personal or financial gain. Those who uphold a genuine scientific mindset should not ignore misleading claims that masquerade science, but should instead verify it using scientific methods and clearly communicate their findings to the public. It is also important to recognize that the overstatement of vaccine efficacy and the understatement of potential risks by some experts have contributed to public mistrust, pushing some individuals toward extreme views and conspiracy theories. This environment benefits pseudo-scientific figures and deepens societal divisions. Restoring public trust in science requires transparency, accountability, and ongoing engagement with the public.

Author contributions

TN and HK conceived the ideas and wrote the initial draft of the manuscript. TN, TK, and YM performed the experiments. YK obtained and analyzed public data on COVID-19 vaccinations in Japan. TT and TM provided advice on experimental design and data interpretation. All authors made substantial contributions to writing and editing the manuscript, and approved the final version for publication.

Declaration of interests

The authors declare no competing interest.

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Figures

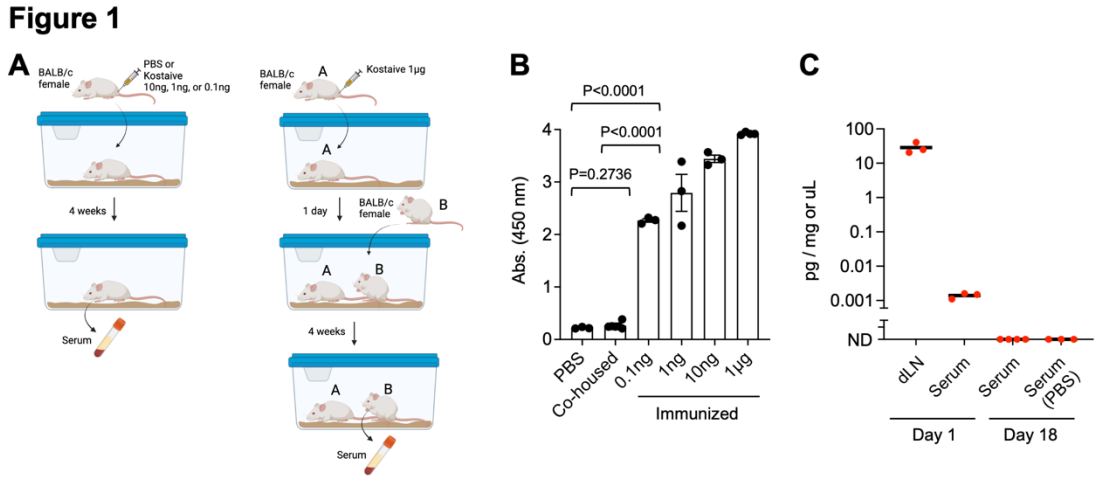

Figure 1. Undetectable transmission and lack of persistence of saRNA vaccine in mice.

A, Scheme of the experiments. Nine-week-old female BALB/c mice (purchased from SLC Inc.) were used. All mice were maintained under specific pathogen-free conditions, and all experiments were approved by the Animal Care and Use Committee of the Tokyo University of Science.

B, Anti-Spike IgG antibody titers in the serum from the mice injected with PBS, co-housed mice (mouse B in panel **A**), and mice immunized with different doses of Kostaive (0.1 ng, 1 ng, 10 ng, or 1 µg). Sera diluted 200- to 6,400-fold were incubated with JN.1 Spike protein (ACROBiosystems) immobilized on a 96-well plate and anti-Spike IgG antibody was measured by ELISA using peroxidase-conjugated anti-mouse IgG antibodies. Serum collection from mice and ELISA were performed by independent researchers at different laboratories. ELISA was performed at sample-blinded manner

by investigators from two independent institutes. The graph shows representative results with 800-fold diluted serum samples.

C, Distribution of Spike mRNA in draining lymph node (dLN) and serum at day 1 or 18 after Kostaive immunization measured by RT-PCR. Total RNA was extraqcted from weighted and homogenized lymph nodes or from 10-30 μl of serum using RNeasy Micro Kit (QIAGEN) and reverse-transcribed with Superscript III (Invitrogen). RNA purified from Kostaive was diluted serially and served as the standards. Quantitative PCR (qPCR) was performed with TB Green Premix ExTaq II (Takara) and the QuantStudio PCR System (Life Technologies, Thermo Fisher Scientific). The following PCR primers were used to amplify the 100 bp Spike sequence: forward, 5'-GGCTGAACGAGGTGGCCAAG-3'; reverse, 5'- AGCCAGATGTACCAGGGCC-3'. Agarose gel electrophoresis of the PCR products confirmed that 100 bp DNA was specifically amplified from the day 1 samples, but not from the day 18 samples. ND:not detected (less than 0.25 ag / μl).

Figure 2

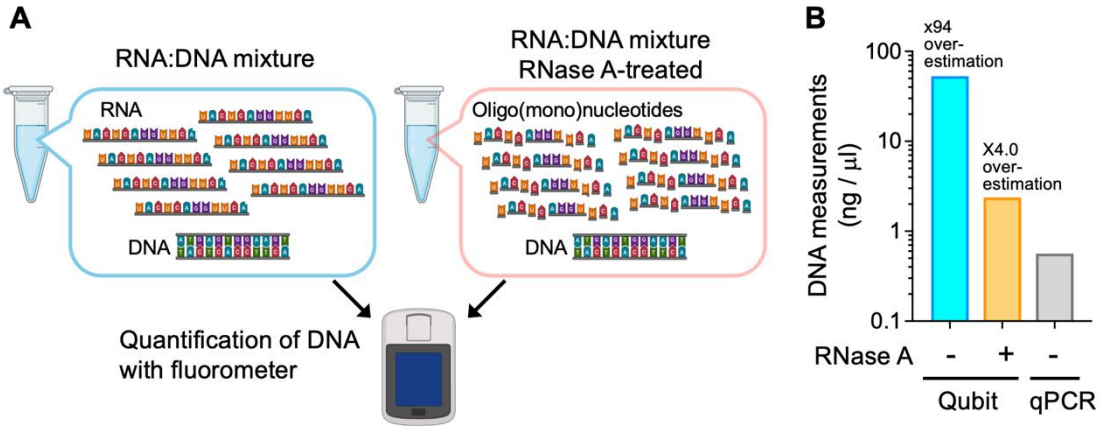

Figure 2. Fluorometer overestimates DNA content in the presence of large amounts of RNA.

A, mRNA was generated by in vitro transcription from template DNA using IVTpro mRNA Synthesis System (TaKaRa), and the resultant nucleotides (RNA:DNA mixture) were purified by acid-phenol extraction without DNase I treatment. A portion of the mixture was treated with RNase A (250 µg/mL, at 37 ˚C for 30 min). RNase A is an endoribonuclease that cleaves RNA at the 3′ end of pyrimidine residues, generating mononucleotides and oligoribonucleotides that contain purine residues or poly(A), that may bind fluorochromes and lead to an overestimation of DNA content.

B, DNA concentration was quantified using a Qubit 4 fluorometer with AccuGreen High Sensitivity dsDNA Quantification kit (Biotium). The exact concentration of template DNA contained in the RNA:DNA mixture was measured by qPCR with PCR primers that amplify the template DNA, and the degrees of overestimation of the Qubit

4 measurement were shown. The graph shows representative results from two independent experiments.

Figure 3

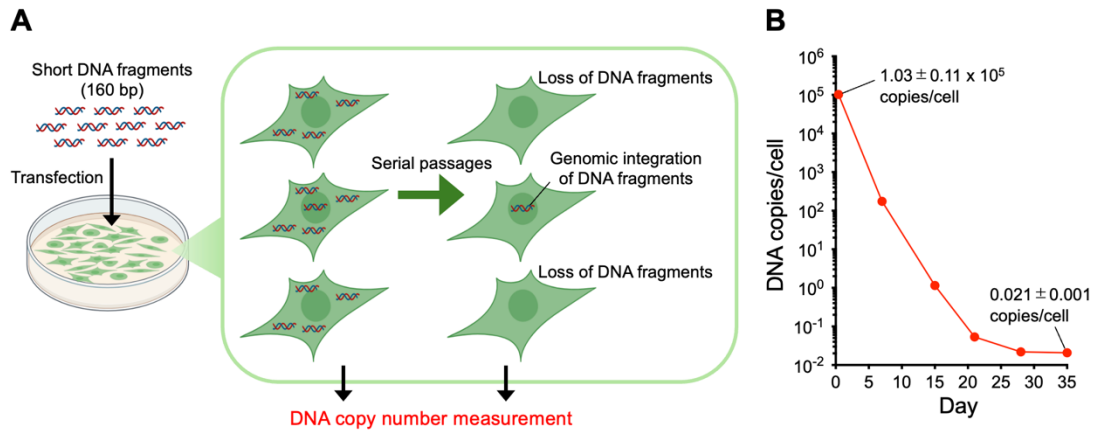

Figure 3. Genomic integration of small DNA fragments in a human cell line.

A, Scheme of the experiments. HEK293 cells were transfected with 1 μ g of 160 bp DNA fragment using Lipofectamine 3000 (Invitrogen). Ten hours after transfection, cells were washed, supplemented with new medium, and thereafter, passaged every 3-4 days. Most of the DNA fragments introduced into the cells are lost due to degradation by cellular DNase and dilution along cell division. If DNA fragments were integrated into the genomic DNA, a certain number of copies of DNA fragments would be maintained in the cell population even after cell division.

B, At different time points after transfection, cells were collected and extracted DNA was used for qPCR. The relative amounts of DNA was quantified using the ΔΔCt method. Since HEK293 cells are triploid, the copy number of transfected DNA per cell was calculated using the PSMA6 gene copy number as 3/cell. The DNA fragment used was a portion of the coding sequence of SARS-CoV-2 Spike, amplified using PCR primers (Fw, 5'-CCCGACGTGGACCTGGGCG-3'; Rv, 5'-

AGCCAGATGTACCAGGGCC-3'). For qPCR detection of the DNA fragments, PCR primers (Fw, 5'-CTGGGCGATATCAGCGGAATC-3'; Rv, 5'-CTGCTCGTACTTCCCCAGTTC-3') were used to amplify 129 bp of DNA excluding the both ends. The 130 bp human PSMA6 DNA was amplified using the following primers: Fw, 5'-GGTATAGATGAAGAGCAAGGCCC-3'; Rv, 5'-TCAAGGAAGCTGGTTGACTCAG-3'.