

## **Supplementary Materials**

### **Gamma-irradiated SARS-CoV-2 vaccine candidate, OZG-38.61.3, confers protection from SARS-CoV-2 challenge in human ACEII-transgenic mice**

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Supplementary figures/tables

Study groups	Earring No	Day 0	Day 5	Day 9	Day 14	Day 21	Day 26/1	Day 26/2	Day 27/1	Day 27/2	Day 28/1	Day 28/2	Day 29	
Negative control	40	29,00	29,30	25,60	32,10	33,00	NA	NA	NA	NA	NA	NA	33,90	
	41	24,20	24,70	31,20	26,20	27,00	NA	NA	NA	NA	NA	NA	27,30	
	42	19,00	20,20	21,10	21,30	22,40	NA	NA	NA	NA	NA	NA	21,90	
	43	22,00	23,00	23,10	23,10	24,10	NA	NA	NA	NA	NA	NA	24,60	
	44	20,10	21,10	22,00	22,20	23,30	NA	NA	NA	NA	NA	NA	24,00	
	Average	22,86	23,66	24,60	24,98	25,96	NA	NA	NA	NA	NA	NA	NA	26,34
	Standard deviation	3,96	3,60	4,06	4,39	4,30	NA	NA	NA	NA	NA	NA	NA	4,64
Positive Control	45	21,30	22,00	23,10	23,00	24,20	24,60	24,10	24,30	24,00	23,10	22,70	23,70	
	46	17,80	17,60	18,90	19,30	19,90	19,50	19,50	19,40	20,40	19,90	19,60	19,00	
	47	23,80	24,50	26,10	26,70	27,00	27,40	26,90	27,10	26,80	27,40	26,70	26,70	
	48	25,20	26,20	27,50	27,30	28,40	29,10	28,10	28,90	28,40	28,40	28,00	28,50	
	49	26,60	28,00	29,00	30,30	30,80	31,00	30,50	31,00	30,80	30,50	30,60	31,20	
	50	22,20	23,00	24,00	25,00	25,00	25,20	25,10	25,60	26,10	25,40	25,20	25,50	
	Average	22,82	23,55	24,77	25,27	25,88	26,13	25,70	26,05	26,08	25,78	25,47	25,77	
	Standard deviation	3,12	3,63	3,60	3,80	3,77	4,03	3,78	4,03	3,60	3,84	3,91	4,20	
Do se 1 x	51	28,80	29,00	30,60	30,50	31,30	31,80	31,20	31,50	31,00	31,20	25,30	32,00	

	<b>52</b>	22,10	22,70	23,90	24,90	24,70	25,00	25,10	25,10	25,70	25,40	30,80	25,70
	<b>53</b>	27,80	28,50	30,10	30,30	30,80	32,10	31,20	31,50	31,20	30,80	31,00	31,70
	<b>54</b>	26,40	27,80	30,60	30,20	30,60	31,30	31,40	31,40	31,20	31,50	30,90	31,30
	<b>55</b>	25,20	26,10	27,30	27,30	27,60	28,60	28,20	28,70	29,10	28,20	27,60	28,40
	<b>56</b>	25,10	25,70	27,70	27,60	27,80	28,90	29,00	29,20	29,00	29,30	28,70	29,70
	<b>57</b>	30,10	31,20	33,20	33,30	34,00	35,00	31,00	35,00	33,80	34,80	34,10	34,30
	<b>Average</b>	26,50	27,29	29,06	29,16	29,54	30,39	29,59	30,34	30,14	30,17	29,77	30,44
	<b>Standard deviation</b>	2,68	2,74	3,01	2,75	3,05	3,20	2,34	3,08	2,53	2,95	2,84	2,79
<b>Dose 1 x 10<sup>14</sup></b>	<b>58</b>	23,20	24,10	24,50	25,40	26,00	26,10	25,80	26,70	26,10	26,20	26,20	26,00
	<b>59</b>	27,90	28,80	30,10	31,20	31,90	32,50	31,60	32,50	32,20	31,80	31,70	32,40
	<b>60</b>	20,50	21,20	21,70	22,70	23,50	23,70	23,20	23,50	22,80	23,40	22,90	23,30
	<b>61</b>	28,50	28,90	30,20	31,30	31,80	32,00	32,70	32,10	32,30	31,90	32,00	32,60
	<b>62</b>	24,60	24,80	26,20	26,40	27,10	27,70	27,60	28,00	27,30	27,50	27,90	27,30
	<b>63</b>	26,10	26,70	28,40	28,60	29,50	29,50	29,50	29,70	29,80	29,50	29,30	30,20
	<b>64</b>	25,30	26,10	27,90	28,30	29,20	30,00	29,90	30,12	29,70	29,60	29,40	21,70
	<b>Average</b>	25,16	25,80	27,00	27,70	28,43	28,79	28,61	28,95	28,60	28,56	28,49	27,64
	<b>Standard deviation</b>	2,75	2,73	3,10	3,12	3,09	3,17	3,32	3,17	3,44	3,08	3,19	4,30

**Supplementary Table 1: Viral Challenge Individual Weight (gr) Changes Evaluation Chart.**

<b>Nucleotide Position</b>	<b>Gene/Region</b>	<b>Gene Product</b>	<b>Nucleotide Exchange (Ref/Alt)</b>	<b>Amino acid exchange</b>	<b>Mutation Type</b>
241	5' UTR	NA	C/T	NA	Non-coding
913	ORF1ab	Nsp2	C/T	S36S	Synonymous
3037	ORF1ab	Nsp3	C/T	F106F	Synonymous
12809	ORF1ab	Nsp9	C/T	L42F	Missense
13170	ORF1ab	Nsp10	C/T	T49I	Missense
14408	ORF1ab	RNA-dependent RNA Polymerase	C/T	P323L	Missense
14676	ORF1ab	RNA-dependent RNA Polymerase	C/T	P412P	Synonymous
21648	S	Spike glycoprotein	C/T	T29I	Missense
21789	S	Spike glycoprotein	C/T	T76I	Missense
21846	S	Spike glycoprotein	C/T	T95I	Missense
22036	S	Spike glycoprotein	A/C	R158S	Missense
22117	S	Spike glycoprotein	T/G	N185K	Missense
23014	S	Spike glycoprotein	A/T	E484D	Missense
23403	S	Spike glycoprotein	A/G	D614G	Missense
23520	S	Spike glycoprotein	C/T	E653D	Missense
23997	S	Spike glycoprotein	C/T	P812L	Missense
24538	S	Spike glycoprotein	A/C	Q992H	Missense
26324	E	Envelope protein	T/C	L27S	Missense
26542	M	Membrane glycoprotein	C/T	T7I	Missense
26559	M	Membrane glycoprotein	C/T	L13F	Missense
28881	N	Nucleocapsid phosphoprotein	G/A	R204K	Missense
28882	N	Nucleocapsid phosphoprotein	G/A	R204K	Missense
28883	N	Nucleocapsid phosphoprotein	G/C	G204R	Missense

**Supplementary Table 2:** Viral Genome Sequencing analysis of fractionated SARS-CoV-2 strains forming OZG-38.61.3 vaccine candidate.

<b>Parameter</b>	<b>Method</b>	<b>Limit</b>	<b>Result</b>
<b>Appearance</b>	Visual inspection	Dry Grayish Powder	Passed
<b>Identity</b>	Single Radial Immunodiffusion (SRID)	Positive	Positive
	TEM	Positive	Positive
<b>Size And Particle Analysis</b>	Nanosight Zetasizer	Size <200 Nm Particle >1x10 <sup>8</sup> MI	Passed
<b>Virus Copy Number/Dose Analysis</b>	qRT-PCR	*≥ 1x10 <sup>13</sup> *≥ 1x10 <sup>14</sup>	Passed
<b>Biochemical Analysis</b>	Ph, Na, Cl, K, Ca, Glucose, Total Protein, Albumin	Reference Interval ≥ 70 µg total protein/dose	Passed
<b>Relative Humidity</b>	Moisture Analyzer	<%3	Passed
<b>Purity</b>	Vero Protein ELISA	<4 ng	Passed
	Vero Dna Nanodrop	<10 ng	Passed
<b>Replicative Virus Test</b>	Real-Time Cell Analysis (RTCA) or MTT	-	Passed
<b>Proteome</b>	Mass Spectrometer	-	Passed
<b>Microbiological Quality Control</b>	Culture	Negative	Negative
	Gram Stain	Negative	Negative
<b>Fungi</b>	Culture	Negative	Negative
<b>Mycoplasma</b>	PCR	Negative	Negative
<b>Endotoxin</b>	Lal Test	<0.5 IU/ml	Passed
<b>Stability/Efficiency</b>	Single Radial Immunodiffusion (SRID)	Positive	Efficiency analysis of

			0,1,6,12,24 and 26 months in 4-24°C storage conditions.
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**Supplementary Table 3:** Quality Control tests of the lyophilized product of OZG-38.61.3 vaccine candidate. \* Infective SARS-CoV-2 viral copy number in a dose.

Accession	Description	Coverage [%]	# PSM	# Peptide	# AAs	MW [kDa]	calc. pI	Score Sequest HT
<b>A0A6G7SLW8</b>	Orf1ab polyprotein OS=Severe acute respiratory syndrome coronavirus 2	1,02860	19	4	7097	793,7	6,76	0
<b>A0A6C0T6Z7</b>	Nucleoprotein OS=Severe acute respiratory syndrome coronavirus 2 O	42,0047	30	12	419	45,6	10,07	56,69
<b>A0A679G9E9</b>	S protein OS=Severe acute respiratory syndrome coronavirus 2	0,54988	1	1	1273	141,1	6,65	0
<b>A0A6C0N6C6</b>	Nonstructural protein NS8 OS=Severe acute respiratory syndrome coronavirus2	23,9669	2	2	121	13,8	5,73	0
<b>A0A6H2LA46</b>	ORF1ab polyprotein OS=Severe acute respiratory syndrome coronavirus 2	1,02874	19	4	7096	792,5	6,7	0
<b>A0A6H2LAX8</b>	ORF1ab polyprotein OS=Severe acute respiratory syndrome coronavirus 2	1,02874	19	4	7096	793,5	6,76	0
<b>A0A6H1PMM5</b>	Surface glycoprotein OS=Severe acute respiratory syndrome coronavirus 2	0,54988	1	1	1273	141,1	6,61	0
<b>A0A6H2L905</b>	ORF1ab polyprotein OS=Severe acute respiratory syndrome coronavirus 2	1,02874	19	4	7096	793,5	6,76	0
<b>A0A6H2LC46</b>	Surface glycoprotein OS=Severe acute respiratory syndrome coronavirus 2	0,54988	1	1	1273	140,3	6,76	0
<b>A0A6H2LCV0</b>	ORF1ab polyprotein OS=Severe acute respiratory syndrome coronavirus 2	1,02874	19	4	7096	793,5	6,76	0
<b>A0A6H2L673</b>	Surface glycoprotein OS=Severe acute respiratory syndrome coronavirus 2	0,54988	1	1	1273	141,1	6,73	0
<b>A0A6H2L7S6</b>	ORF1ab polyprotein OS=Severe acute respiratory syndrome coronavirus 2	1,02874	19	4	7096	793,7	6,76	0

<b>A0A6H2L8K8</b>	ORF1ab polyprotein OS=Severe acute respiratory syndrome coronavirus 2	1,02874	19	4	7096	793,5	6,62	0
<b>A0A6H2L9B8</b>	ORF1ab polyprotein OS=Severe acute respiratory syndrome coronavirus 2	1,02874	19	4	7096	793,6	6,76	0
<b>A0A6H2LBL6</b>	ORF1ab polyprotein OS=Severe acute respiratory syndrome coronavirus 2	1,02874	19	4	7096	793,6	6,76	0
<b>A0A6H2LDA7</b>	ORF1ab polyprotein OS=Severe acute respiratory syndrome coronavirus 2	1,02874	19	4	7096	792,9	6,64	0
<b>A0A6H2LBC7</b>	ORF1ab polyprotein OS=Severe acute respiratory syndrome coronavirus 2	1,02874	19	4	7096	793,2	6,68	0
<b>A0A6H2LA93</b>	Nucleocapsid phosphoprotein OS=Severe acute respiratory syndrome coronavirus 2	42,0047	30	12	419	45,6	10,07	56,69
<b>A0A6H2L973</b>	ORF1ab polyprotein OS=Severe acute respiratory syndrome coronavirus 2	1,02874	19	4	7096	793	6,81	0
<b>A0A6H2L8H9</b>	ORF1ab polyprotein OS=Severe acute respiratory syndrome coronavirus 2	1,02874	19	4	7096	793,6	6,76	0
<b>A0A6H2LAC5</b>	ORF1ab polyprotein OS=Severe acute respiratory syndrome coronavirus 2	1,02874	19	4	7096	793,3	6,71	0
<b>A0A6H2LBD2</b>	Surface glycoprotein OS=Severe acute respiratory syndrome coronavirus 2	0,54988	1	1	1273	141	6,73	0
<b>A0A6H2L6R1</b>	ORF1ab polyprotein (Fragment) OS=Severe acute respiratory syndrome coronavirus 2	1,04539	19	4	6983	780,6	6,77	0
<b>A0A6H2L7I9</b>	ORF1ab polyprotein OS=Severe acute respiratory syndrome coronavirus 2	1,02874	19	4	7096	793,2	6,7	0
<b>A0A6H2LC40</b>	Surface glycoprotein OS=Severe acute respiratory syndrome coronavirus 2	0,54988	1	1	1273	140,9	6,73	0
<b>A0A6H2LBU1</b>	Surface glycoprotein (Fragment) OS=Severe acute respiratory syndrome coronavirus 2	0,61946	1	1	1130	124,9	6,44	0
<b>A0A6H2LC49</b>	ORF1ab polyprotein OS=Severe acute respiratory syndrome coronavirus 2	1,02874	19	4	7096	793,6	6,76	0
<b>A0A6H2L683</b>	Surface glycoprotein OS=Severe acute respiratory syndrome coronavirus 2	0,54988	1	1	1273	141,1	6,81	0
<b>A0A6H2LDI3</b>	ORF1ab polyprotein OS=Severe acute respiratory syndrome coronavirus 2	1,02874	19	4	7096	793,5	6,73	0
<b>A0A6H2L877</b>	ORF1ab polyprotein OS=Severe acute respiratory syndrome coronavirus 2	1,02874	19	4	7096	793,6	6,76	0
<b>A0A6H2L915</b>	Nucleocapsid phosphoprotein OS=Severe acute respiratory syndrome coronavirus 2	42,0047	30	12	419	45,7	10,05	56,69

<b>A0A6H2LBN3</b>	ORF8 protein OS=Severe acute respiratory syndrome coronavirus 2	23,9669	2	2	121	13,8	5,73	0
<b>A0A6H2L880</b>	ORF1ab polyprotein OS=Severe acute respiratory syndrome coronavirus 2	1,02874	19	4	7096	793,1	6,62	0
<b>A0A6H2LAL1</b>	ORF1ab polyprotein OS=Severe acute respiratory syndrome coronavirus 2	1,02874	19	4	7096	793,6	6,76	0
<b>A0A6H2L964</b>	Surface glycoprotein OS=Severe acute respiratory syndrome coronavirus 2	0,54988	1	1	1273	141,1	6,73	0
<b>A0A6H2L9W1</b>	ORF1ab polyprotein OS=Severe acute respiratory syndrome coronavirus 2	1,02874	19	4	7096	793,6	6,76	0
<b>A0A6H2LCH0</b>	ORF1ab polyprotein OS=Severe acute respiratory syndrome coronavirus 2	1,02874	19	4	7096	793,6	6,76	0
<b>A0A6H2L702</b>	Surface glycoprotein OS=Severe acute respiratory syndrome coronavirus 2	0,54988	1	1	1273	141,1	6,65	0
<b>A0A6H1PRW5</b>	ORF1ab polyprotein OS=Severe acute respiratory syndrome coronavirus 2	1,02874	19	4	7096	793,6	6,76	0
<b>A0A6H2L7Z5</b>	Nucleocapsid phosphoprotein OS=Severe acute respiratory syndrome coronavirus 2	42,0047	30	12	419	45,6	10,07	56,69
<b>A0A6H2L6T0</b>	Nucleocapsid phosphoprotein OS=Severe acute respiratory syndrome coronavirus 2	42,0047	30	12	419	45,6	10,07	56,69
<b>A0A6H2LA09</b>	ORF1ab polyprotein OS=Severe acute respiratory syndrome coronavirus 2	1,02874	19	4	7096	793	6,71	0
<b>A0A6H2LCI8</b>	ORF1ab polyprotein OS=Severe acute respiratory syndrome coronavirus 2	1,02874	19	4	7096	793,6	6,76	0
<b>A0A6H2L7Z4</b>	ORF1ab polyprotein OS=Severe acute respiratory syndrome coronavirus 2	1,02874	19	4	7096	793,7	6,76	0
<b>A0A6H2LA55</b>	ORF1ab polyprotein OS=Severe acute respiratory syndrome coronavirus 2	1,02874	19	4	7096	793,6	6,8	0
<b>A0A6H2L7V1</b>	Nucleocapsid phosphoprotein OS=Severe acute respiratory syndrome coronavirus 2	42,0047	30	12	419	45,6	10,07	56,69
<b>A0A6H2LD79</b>	ORF1ab polyprotein OS=Severe acute respiratory syndrome coronavirus 2	1,02874	19	4	7096	793,6	6,76	0
<b>A0A6H2L9L2</b>	ORF1ab polyprotein OS=Severe acute respiratory syndrome coronavirus 2	1,02874	19	4	7096	793,2	6,71	0
<b>A0A6H2L6I9</b>	ORF1ab polyprotein OS=Severe acute respiratory syndrome coronavirus 2	1,02874	19	4	7096	792,9	6,7	0
<b>A0A6H2LAG5</b>	Surface glycoprotein OS=Severe acute respiratory syndrome coronavirus 2	0,54988	1	1	1273	140,6	6,04	0
<b>A0A6H2L8N1</b>	ORF1ab polyprotein OS=Severe acute respiratory syndrome coronavirus 2	1,02874	19	4	7096	793,6	6,76	0



<b>A0A6G7SLV1</b>	Orf1ab polyprotein OS=Severe acute respiratory syndrome coronavirus 2	1,02874	19	4	7096	793,6	6,76	0
<b>A0A6H2EH11</b>	ORF1ab polyprotein OS=Severe acute respiratory syndrome coronavirus 2	1,02874	19	4	7096	793,5	6,76	0
<b>A0A6H0QT63</b>	ORF1ab polyprotein OS=Severe acute respiratory syndrome coronavirus 2	1,02874	19	4	7096	793,5	6,76	0
<b>A0A6H2L7L3</b>	Surface glycoprotein OS=Severe acute respiratory syndrome coronavirus 2	0,54988	1	1	1273	141	6,81	0
<b>A0A6H2L5M8</b>	Nucleocapsid phosphoprotein OS=Severe acute respiratory syndrome coronavirus 2	42,0047	30	12	419	45,7	10,08	56,69
<b>A0A6H2LAI7</b>	ORF1ab polyprotein OS=Severe acute respiratory syndrome coronavirus 2	1,02874	19	4	7096	793,6	6,76	0
<b>A0A6H2LEE1</b>	ORF1ab polyprotein OS=Severe acute respiratory syndrome coronavirus 2	1,02874	19	4	7096	793,6	6,76	0
<b>A0A6H2LBP4</b>	ORF1ab polyprotein OS=Severe acute respiratory syndrome coronavirus 2	1,02874	19	4	7096	793,6	6,74	0
<b>A0A6H2L794</b>	ORF1ab polyprotein OS=Severe acute respiratory syndrome coronavirus 2	1,02874	19	4	7096	793,6	6,76	0
<b>A0A6H2LCV8</b>	ORF1ab polyprotein OS=Severe acute respiratory syndrome coronavirus 2	1,02874	19	4	7096	793,6	6,76	0
<b>A0A6C0RS15</b>	2'-O-methyltransferase OS=Severe acute respiratory syndrome coronavirus2	1,028749	19	4	7096	793,6	6,76	0
<b>A0A6G7SLW0</b>	Orf1ab polyprotein OS=Severe acute respiratory syndrome coronavirus 2	1,02874	19	4	7096	793,5	6,74	0
<b>A0A6H2LB79</b>	ORF1ab polyprotein OS=Severe acute respiratory syndrome coronavirus 2	1,028749	19	4	7096	793,6	6,76	0
<b>A0A6H2LB01</b>	ORF1ab polyprotein OS=Severe acute respiratory syndrome coronavirus 2	1,02874	19	4	7096	792,8	6,71	0

**Supplementary Table 4:** LC-MS/MS results of candidate vaccine sample developed against the SARS-CoV-2 virus. OX=2697049.

**Table 5a:**

	<b>Control</b>	<b>1x10<sup>13</sup></b>	<b>1x10<sup>14</sup></b>
<b>Total Body Weight</b>	35,16±0,9	35,16±0,8	35,16±0,7
<b>Lung</b>	1,34±0,01	1,34±0,03	1,343±0,02
<b>Liver</b>	2,59±0,14	2,59±0,19	2,59±0,18
<b>Spleen</b>	1,22±0,01	1,22±0,01	1,22±0,01
<b>Kidney</b>	2,64±0,03	2,64±0,04	2,64±0,05

**Table 5b:**

	<b>Control</b>	<b>1x10<sup>13</sup></b>	<b>1x10<sup>14</sup></b>
<b>Inflammation</b>	2/5*	1/5*	1/5*
<b>Hemorrhage</b>	0/5	0/5	0/5
<b>Eosinophil</b>	0/5	1/5	0/5

**Table 5c:**

	<b>Control</b>	<b>1x10<sup>13</sup></b>	<b>1x10<sup>14</sup></b>
<b>Inflammation</b>	0/5	2/5*	0/5
<b>Hemorrhage</b>	0/5	0/5	0/5
<b>Eosinophil</b>	0/5	0/5	0/5

**Table 5d:**

	<b>Control</b>	<b>1x10<sup>13</sup></b>	<b>1x10<sup>14</sup></b>
<b>Inflammation</b>	1/5*	1/5*	0/5
<b>Hemorrhage</b>	0/5	0/5	0/5
<b>Eosinophil</b>	0/5	0/5	0/5

**Supplementary Table 5: Organ weights and histopathological analysis vaccinated Balb/c mice. A.** Organ Weights Chart. **B.** Histological Analysis of the lung. **C.** the kidney **D.** the liver tissues. Control mice, n=5, Dose 10<sup>13</sup> vaccinated mice, n=5, and Dose 10<sup>14</sup> vaccinated mice, n=5.  
\*mild inflammation

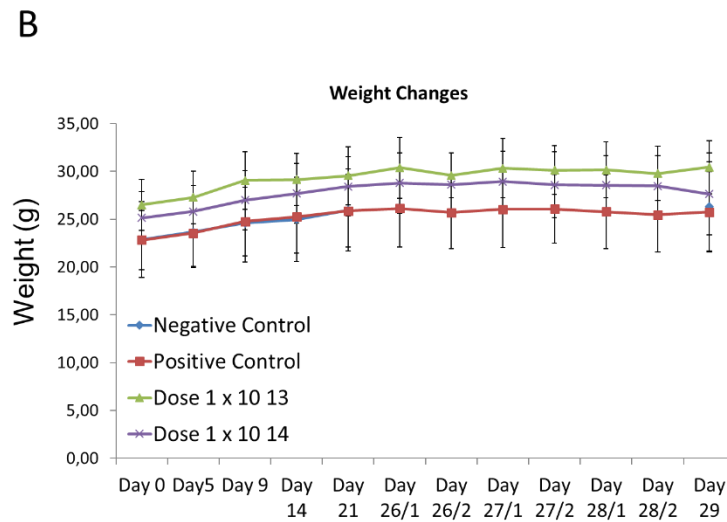
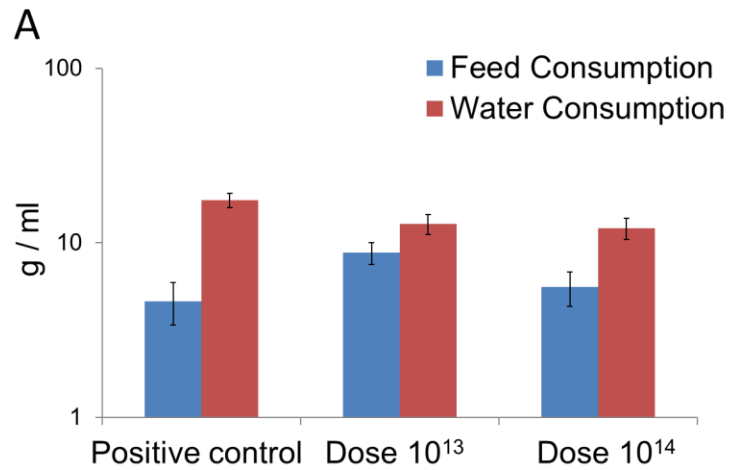
<b>Groups</b>	<b>Earring no</b>	<b>Lung H-Score</b>
<b>Negative Control</b> <b>(Human lung and placental tissue)</b>	<b>40</b>	0,00
	<b>41</b>	0,00
	<b>42</b>	1,00
	<b>43</b>	2,00
	<b>44</b>	0,00
	<b>Average</b>	0,60
	<b>Standard deviation</b>	0,89
<b>Positive Control</b> <b>(SARS-CoV-2 infected mice)</b>	<b>45</b>	46,00
	<b>46</b>	76,00
	<b>47</b>	59,00
	<b>48</b>	34,00
	<b>49</b>	71,00
	<b>50</b>	95,00
	<b>Average</b>	63,50
<b>Dose 1 x 10<sup>13</sup></b> <b>administrated mice</b>	<b>Standard deviation</b>	21,92
	<b>51</b>	26,00
	<b>52</b>	42,00
	<b>53</b>	38,00
	<b>54</b>	29,00
	<b>55</b>	28,00

	<b>56</b>	33,00
	<b>57</b>	37,00
	<b>Ortalama</b>	33,29
	<b>Standard deviation</b>	5,94
<b>Dose 1 x 10<sup>14</sup></b> <b>administrated mice</b>	<b>58</b>	0,00
	<b>59</b>	1,00
	<b>60</b>	3,00
	<b>61</b>	2,00
	<b>62</b>	0,00
	<b>63</b>	0,00
	<b>64</b>	0,00
	<b>Average</b>	0,86
	<b>Standard deviation</b>	1,21
<b>Overall</b>	<b>Average</b>	24,92
	<b>Standard deviation</b>	28,18

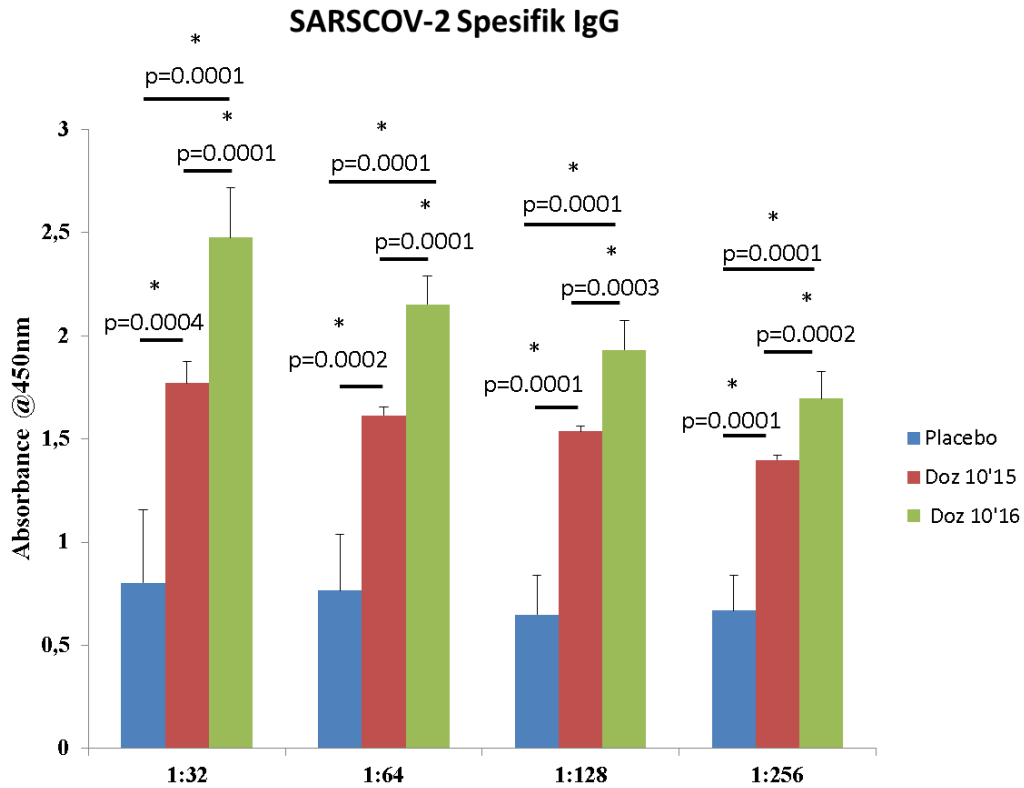
**Supplementary Table 6. Lung Tissue Immunohistochemistry Image (H-score Analysis) Individual Evaluation Chart.** In the statistical analysis of these analyzes, the control groups and dose groups were compared using the Student's t-test method. Significance between groups was added to the graph. ( $p < 0.05$ ).

	Cq	Formül	Anti-Log	x 5x10 <sup>3</sup> copy/ml dilution factor	x 5x10 <sup>4</sup> copy/ml dilution factor	x 5x10 <sup>5</sup> copy/ml dilution factor	x 5x10 <sup>6</sup> copy/ml dilution factor
1st test							
SC2 10 <sup>0</sup>	18,79	6,69	4920796,42				
SC2 10 <sup>1</sup>	21,14	5,65	448964,90	2,24x10 <sup>9</sup>			
SC2 10 <sup>2</sup>	24,33	4,24	17406,36		8,7x10 <sup>8</sup>		
SC2 10 <sup>3</sup>	27,48	2,85	702,91			35x10 <sup>7</sup>	
2nd test							
SC2 10 <sup>2</sup>	24,85	4,01	10247,54		5,12x10 <sup>8</sup>		
SC2 10 <sup>3</sup>	28,38	2,45	280,98			14x10 <sup>7</sup>	
SC2 10 <sup>4</sup>	31,33	1,14	13,91				6,95x10 <sup>7</sup>

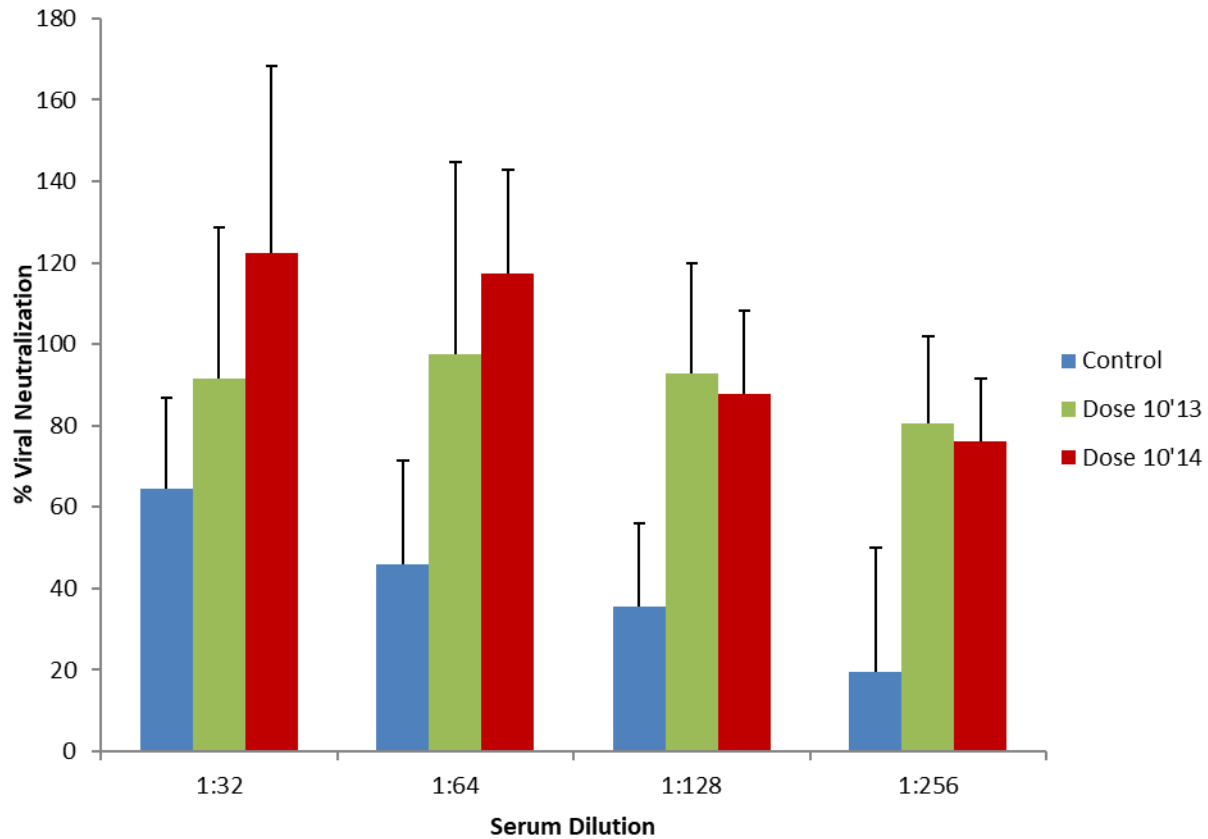
**Supplementary Table 7. Calculation of copy number based on Cq value based on World Health Organization (WHO) SARS-CoV-2 RNA copy number standard analysis (International Unit per ml; IU/ml).**



**Supplementary Figure 1. Health state of challenge test mice. A.** Feed and Water consumption per day post-infection of the positive control group, dose  $1 \times 10^{13}$ , and  $1 \times 10^{14}$  groups. **B.** Monitoring. Bodyweight changes during challenge experiments.

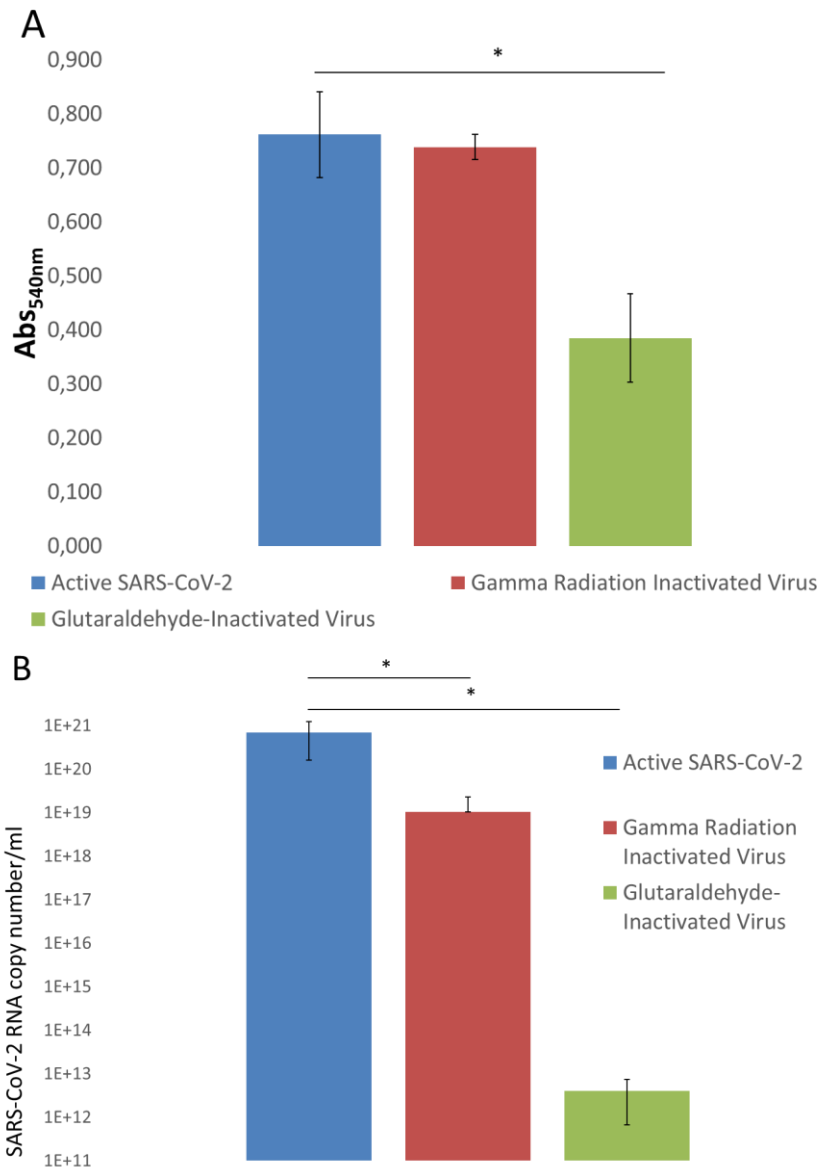


**Supplementary Figure 2. Repeat Dose Study in Rodents Immune Responses (SARS-CoV-2 Specific IgG Analysis).** The 21-day administrated rodent serums were studied with the Creative Diagnostic SARS-CoV-2 Specific IgG Elisa kit by diluting in 1:32, 1:64, 1:128, 1:256 dilution factors of test groups including placebo, dose  $1 \times 10^{15}$ , dose  $1 \times 10^{16}$  vaccine. Measured absorbance at 450nm, absorbance averages, and absorbance standard deviation values are indicated.



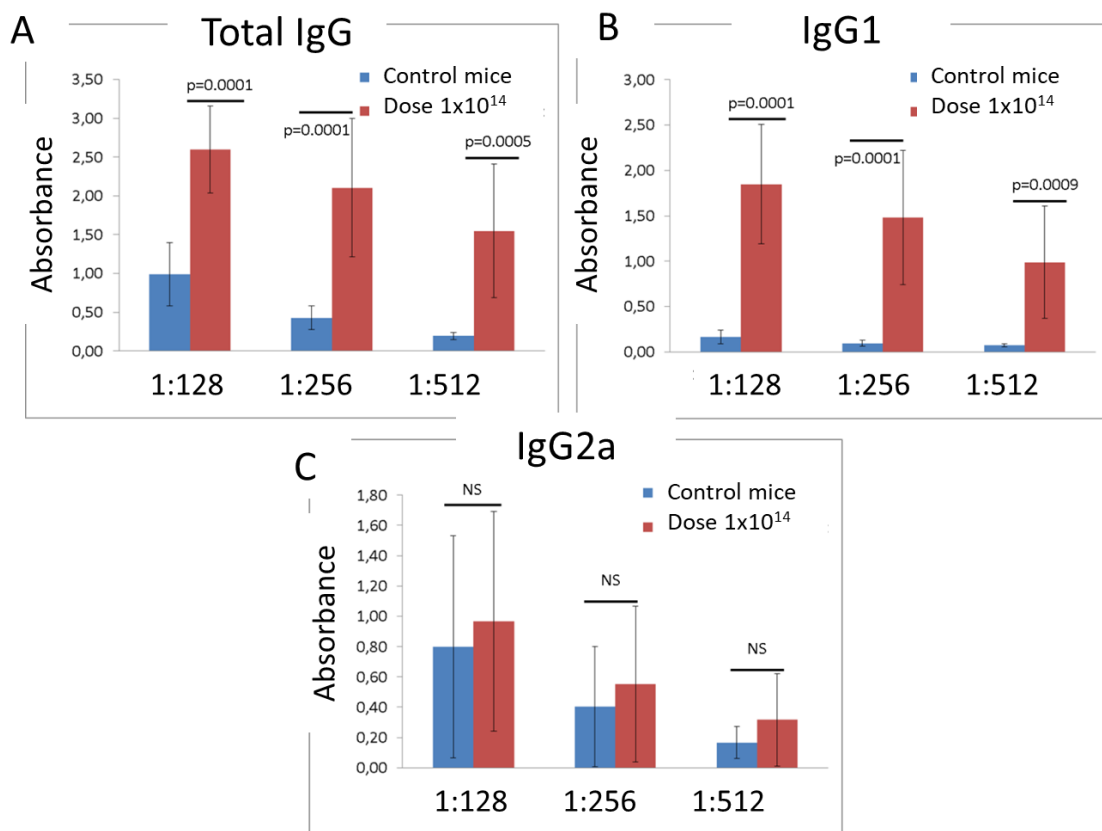
**Supplementary Figure 3. Neutralization analysis in repeated dose administrated rodents.** In a repeated-dose study in rodents, rats were treated with placebo, dose 10<sup>13</sup>, and dose 10<sup>14</sup> vaccine were diluted in 1:32, 1:64, 1:128, 1:256 dilution factors and cultured on Vero cells after treatment with 1000 TCID<sub>50</sub> Brazilian strain of SARS-CoV-2 virus. 96 hours later, neutralization efficacy was determined by MTT analysis.





**Supplementary Figure 4. The difference in loss of Viral load upon Gamma-irradiation and the Glutaraldehyde-based chemical inactivation process.** An equal dose of SARS-CoV-2 virus was inactivated either with 2.5% glutaraldehyde in PBS (0.1 M, pH 7.2) for 2.5 h and isolated through 100 kDa Amicon filter or 25 kGy gamma-irradiation. Next, Single-radial-immunodiffusion (SRID) assay was performed to determine functional Spike protein in the

specimens by anti-SARS-CoV-2 Spike monoclonal antibody (Elabscience, E-AB-V1002). **A.** Spike protein-mono-clonal antibody interaction was assessed by microplate reader at 540nm absorbance. **B.** SARS-CoV-2 RNA copy number of the specimens which were untreated control, gamma-irradiated, or chemically inactivated determined by RT-PCR. Experiments were repeated three times. \* $p < 0.05$



**Supplementary Figure 5. Subgroups of SARS-CoV-2 antibody produced upon immunization of BALB/c mice. A. Total IgG. B. IgG1 and C. IgG2a**