

Table S1 Detailed information about the proportion of glycosylation modification of different GMS globally.

	HA1														
Glycosylation forms	NSTE	NCTI	NGTC	NVSY/ NVTY	NGTS	NNTY/ NNSY/ NGSY	NTTQ/ NDTQ	NDTT/ NDTS	NRTF	NGSL	NGSC	NCTV/ NSSG	NTTL/ NSTL	NVSK/ NISK/ VSR	NCSK
Glycosylated sites	11-13	54-56	87-89	123-125	127-129	148-150	178-180	188-190	200-202	238-240	265-267	267-269	280-282	287-289	295-297
Number of glycosylated modification	7691	5	1097	7865	269	48	19	109	1968	2	2	23	7588	7819	5821
The proportion of glycosylation modification(total number:7888)	97.50%	0.06%	13.91%	99.71%	3.41%	0.61%	0.24%	1.38%	24.95%	0.03%	0.03%	0.29%	96.20%	99.13%	73.80%

Table S2 Detailed information about the proportion of glycosylation modification of different GMS in domestic.

	HA1														
Glycosylation forms	NSTE	NCTI	NGTC	NVSY/ NVTY	NGTS	NNTV	NTTD	NDTT	NRTF	NGSL	NGSC	NCTV	NTTL	NVSK/ NISK	NCSK
Glycosylated sites	11-13	54-56	87-89	123-125	127-129	148-150	178-180	188-190	200-202	238-240	265-267	267-269	280-282	287-289	295-297
Number of glycosylated modification	6182	6	10	6486	257	20	14	70	1837	2	3	24	6140	6317	5590
The proportion of glycosylation modification(total number:6519)	94.83%	0.09%	0.15%	99.49%	3.94%	0.31%	0.21%	1.07%	28.18%	0.03%	0.05%	0.37%	94.19%	96.90%	85.75%

Downloaded the HA sequences from 1994 to 2021 of H9N2 avian influenza virus globally or in domestic from GISAID database (<https://www.gisaid.org>). There were 7888 sequences globally and 6519 sequences in domestic, and domestic sequences were included in the global sequences. Summarized the glycosylation modification proportion of different GMS, as illustrated in Table S1 and Table S2.

Table S3 Detailed primers sequences information about specific GMS

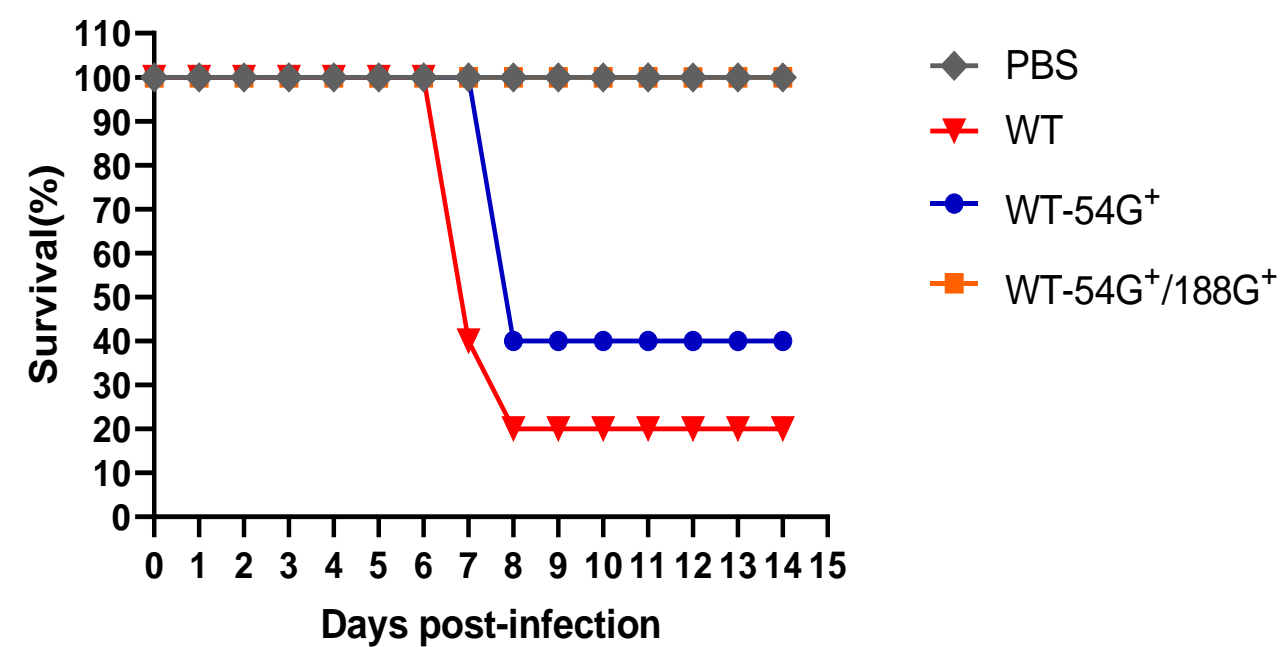
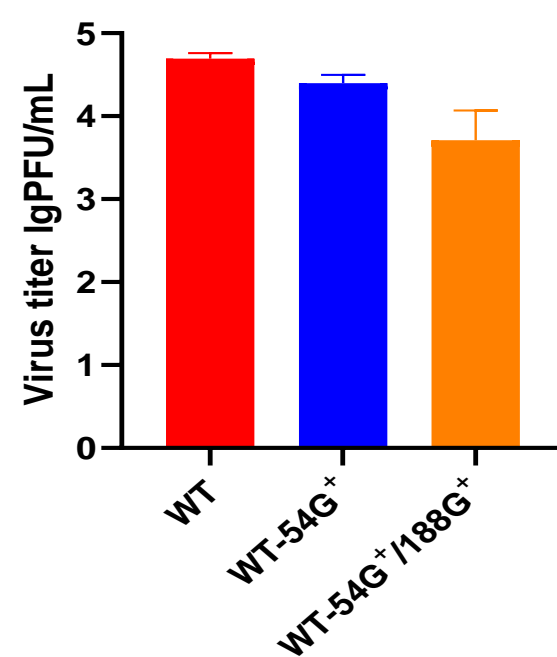
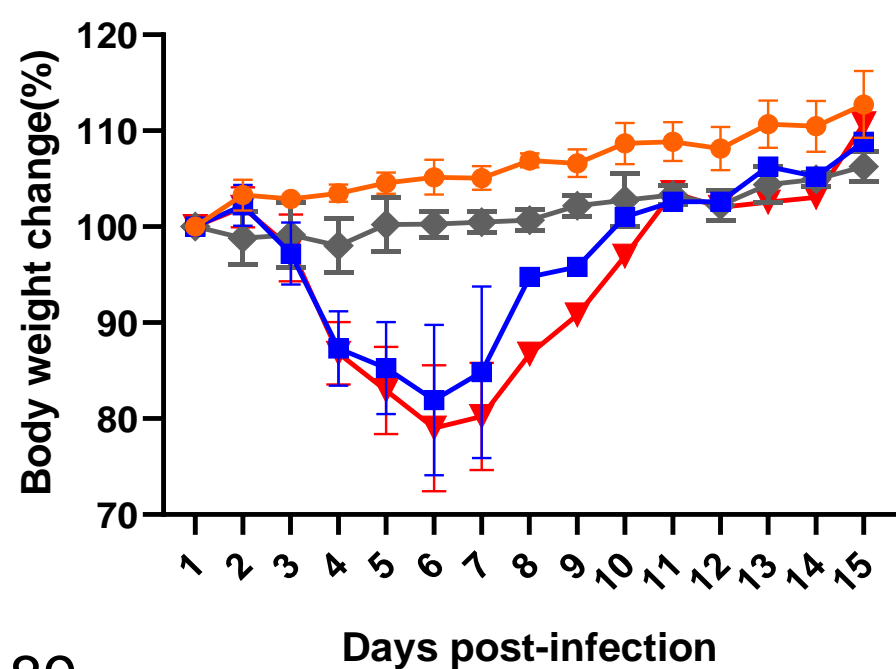
	Amino acid position	Primers used	Primers sequences(5'-3')	
Removal of the GMS	11-13	F	AATCAACAAACTCCGCGGAAACTGTGGACACACT	
		R	AGTGTGTCCACAGTTTCCGCGGAGTTTGTTGATT	
	123-125	F	ACACAATCTGGAGTGTGACTTACAGTGGGACAAGC	
		R	GCTTGTCCCCTGTAAGTCACACTCCAGATTGTGT	
	280-282	F	GAAAGGTGGCTTAAACACAATATTGCCCTTCCAAAATG	
		R	CATTTTGGAAGGGCAATATTGTGTTTAAGCCACCTTC	
	287-289	F	AACATTGCCCTTCCAAAAGGTAAGTAAGTATGCATTTG	
		R	CAAATGCATACTTACTTACCTTTTGGAAGGGCAATGTT	
	295-297	F	GCATTTGGAAACTGCCCAAAGTACATTGGTGTA	
		R	TACACCAATGTACTTTGGGCAGTTTCCAAATGC	
	Addition of the GMS	54-56	F	CTCTTATTCTAGACAACCTGCACCATTGAAGGGC
			R	GCCCTTCAATGGTGCAGTTGTCTAGAATAAGAG
87-89		F	TCAGCTGTTAACGGAACGTGTTATCCCGGGAATGT	
		R	ACATTCCCGGGATAACAGTATCCGTTAACAGCTGA	
127-129		F	TGGAATGTGTCTTACAATGGGACAAGCAAAGC	
		R	GCTTTGCTTGTCCCATTGTAAGACACATTCCA	
148-150		F	TCAAAAGAACAACACCTACCCAACCCAAGATGCT	
		R	AGCATCTTGGGTTGGGTAGGTGTTGTTCTTTTGA	
178-180		F	AAATCACCCACCCACCAATACTACGCAGACAA	
		R	TTGTCTGCGTAGTATTGGTGGGTGGGTGATTT	
188-190		F	AAATCTGTACACCAGAAACGACACAACAACGA	
		R	TCGTTGTTGTGTCGTTTCTGGTGTACAGATTT	
200-202		F	GGAAATGAATAGGACCTTTAAACCATTGATAG	
		R	CTATCAATGGTTTAAAGGTCCTATTCATTTCC	
238-240		F	ACTGCGGATAAAATCTAATGGGAGTCTAATAGCTCCAT	
		R	ATGGAGCTATTAGACTCCCACCAGATTTTATCCGCAGT	

Table S4 Primers of quantitative real-time RT-PCR assay

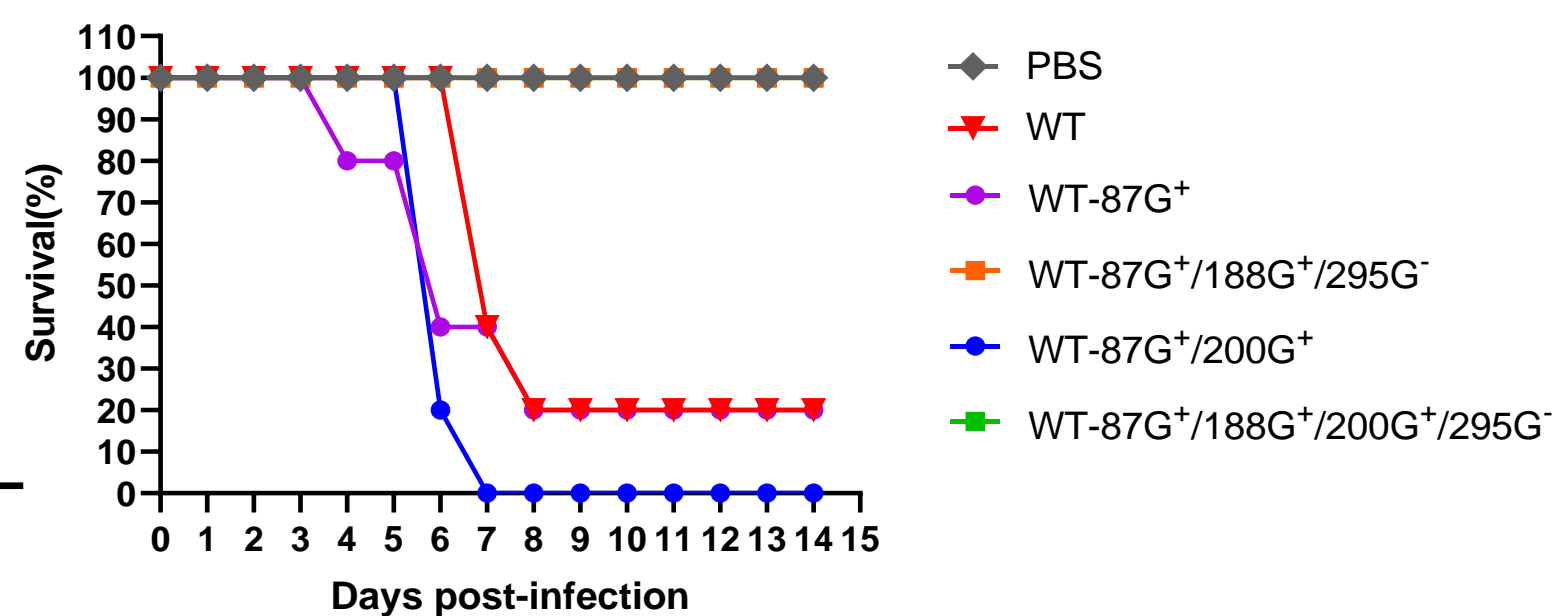
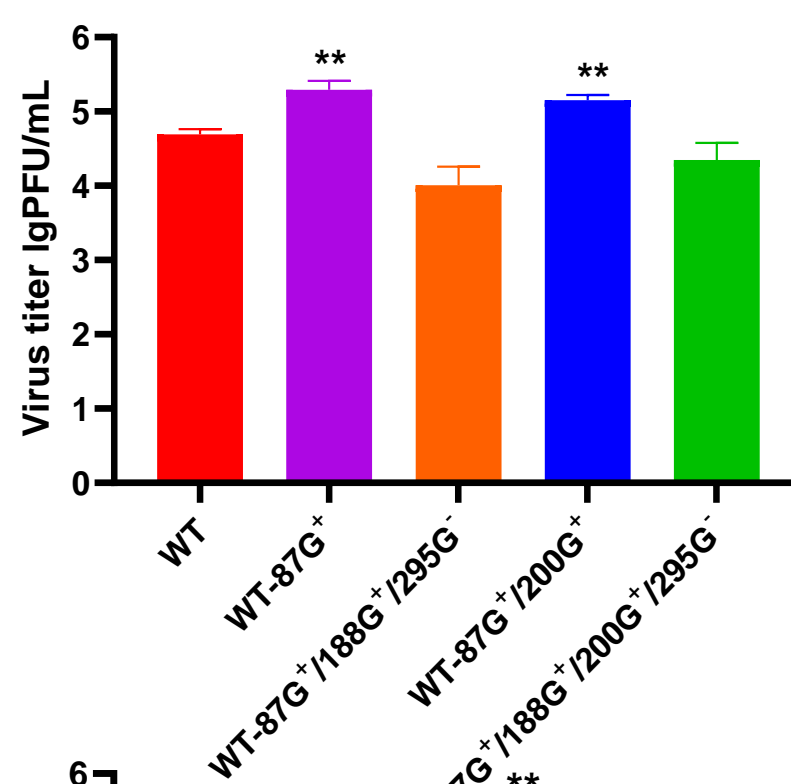
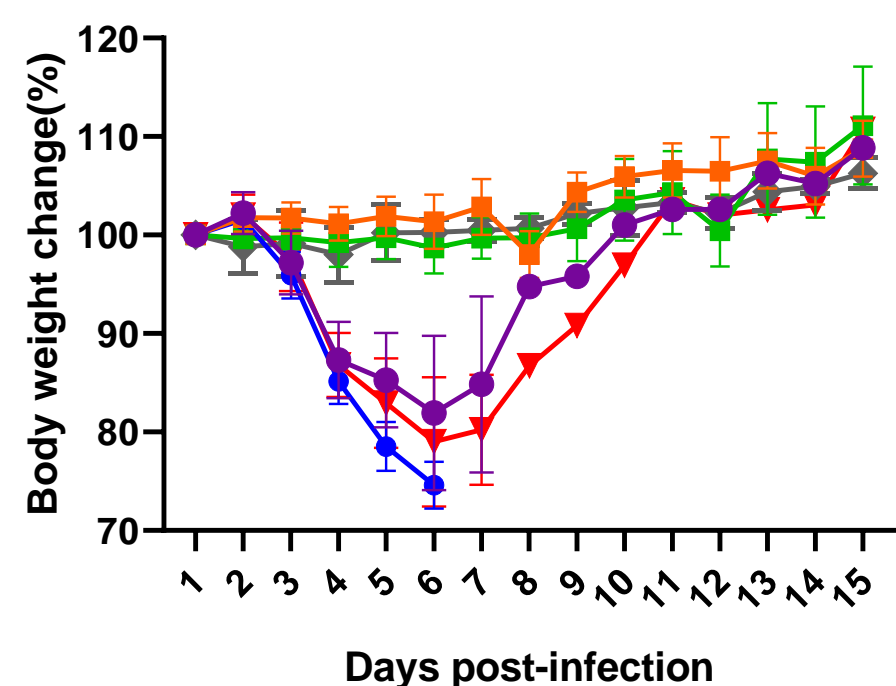
Cytokines	Primers used	Primers sequences(5'-3')
IL-1 $\beta$	F	GAAATGCCACCTTTTGACAGTG
	R	TGGATGCTCTCATCAGGACAG
IL-6	F	CTGCAAGAGACTTCCATCCAG
	R	AGTGGTATAGACAGGTCTGTTGG
TNF- $\alpha$	F	CCTGTAGCCCACGTCGTAG
	R	GGGAGTAGACAAGGTACAACCC
IFN- $\beta$	F	TGGGTGGAATGAGACTATTGTTG
	R	CTCCACGTCAATCTTTCCTC
GAPDH	F	TGACCTCAACTACATGGTCTACA
	R	CTTCCCATTCTCGGCCTTG

# Figure S1

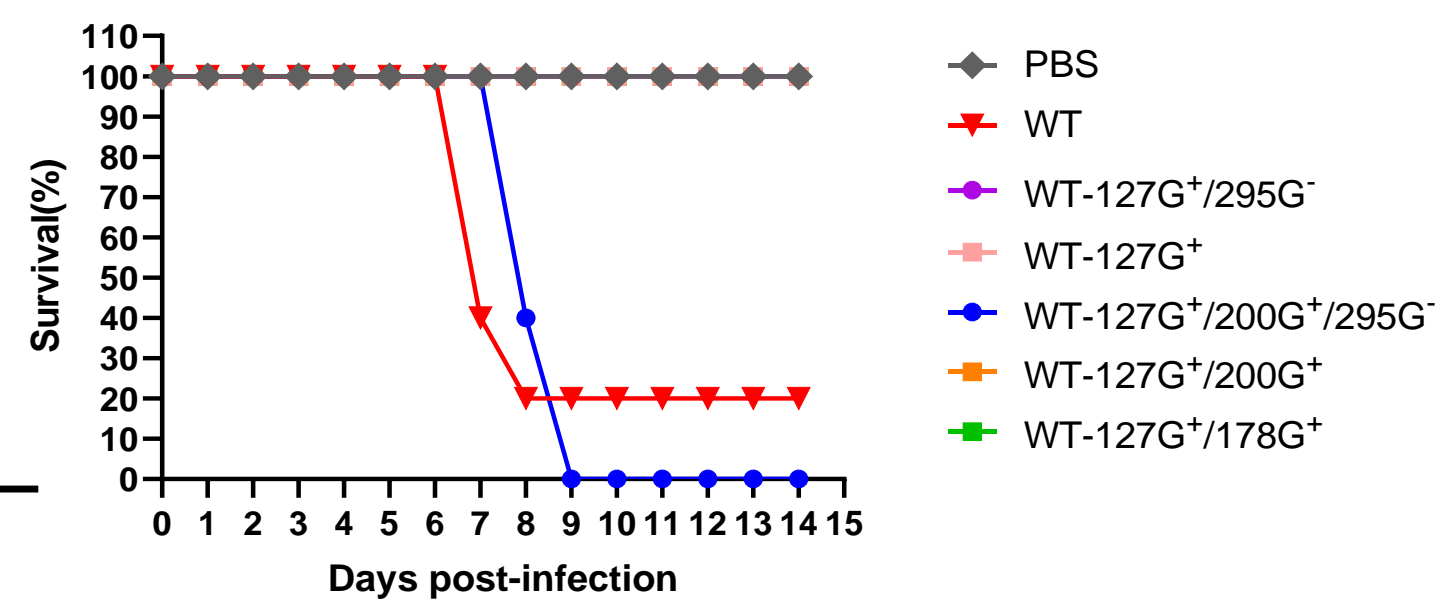
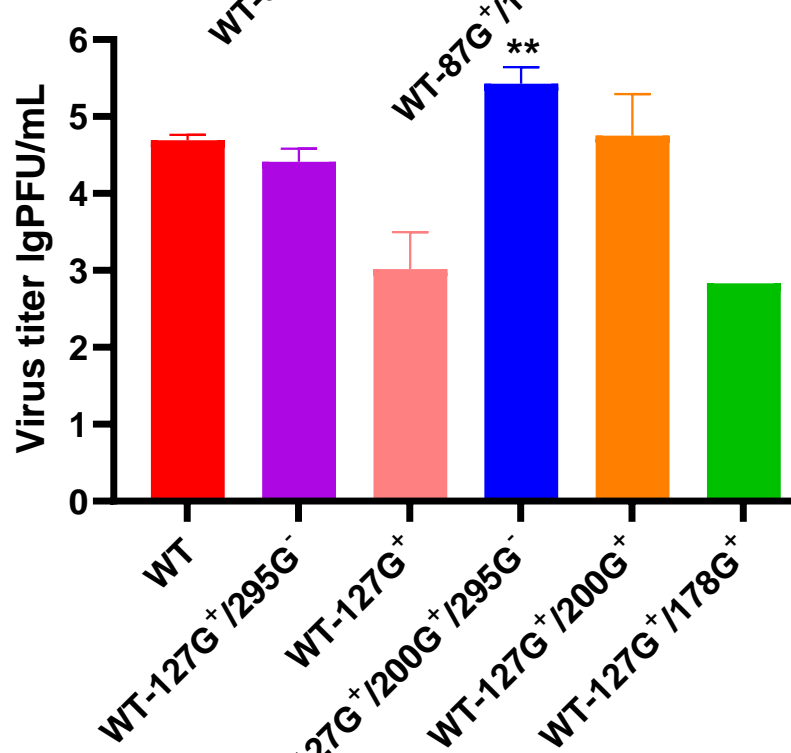
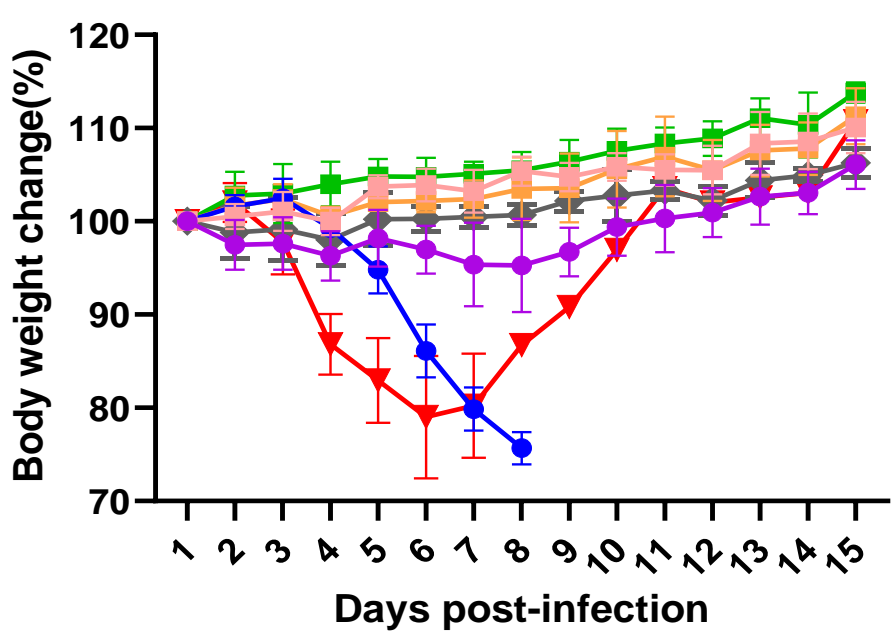
a 54-56



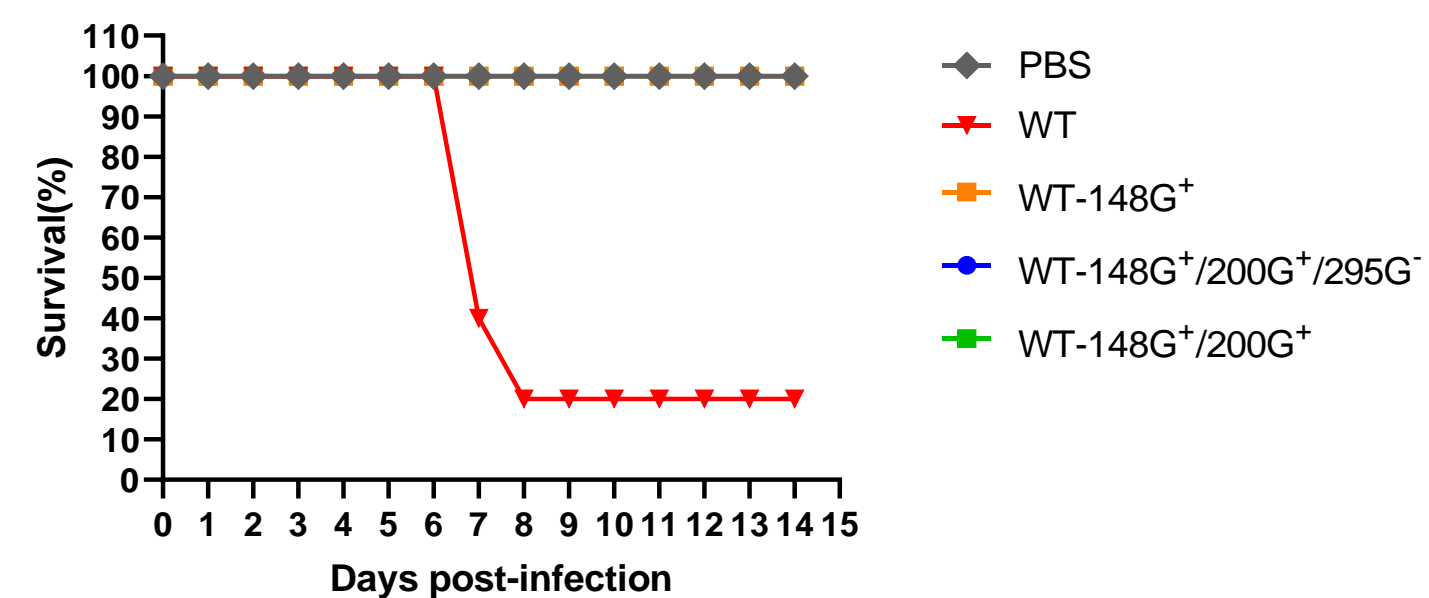
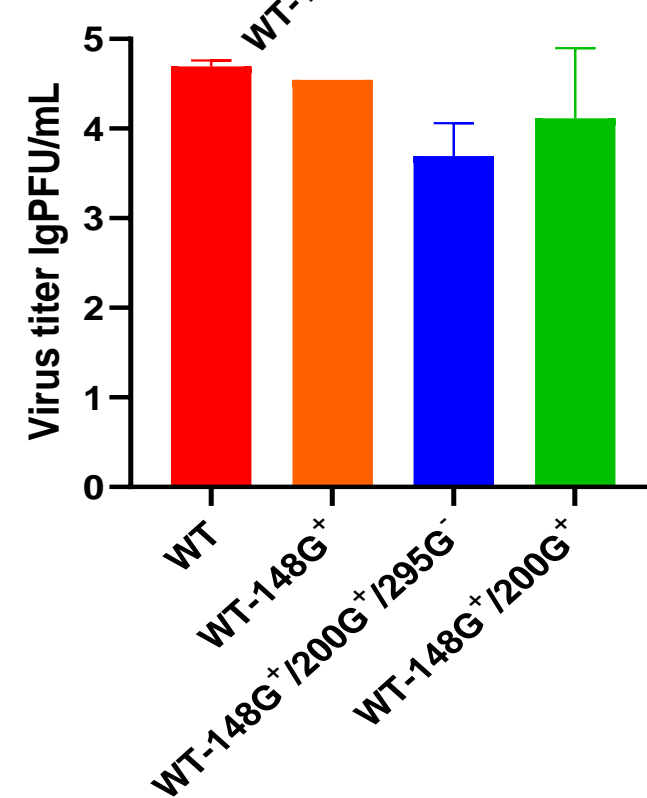
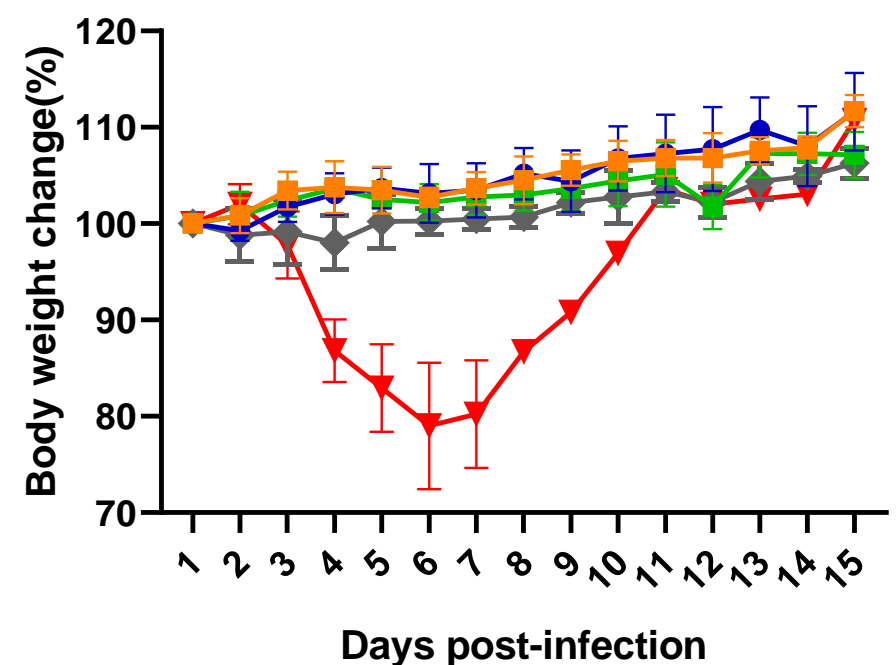
b 87-89



c 127-129

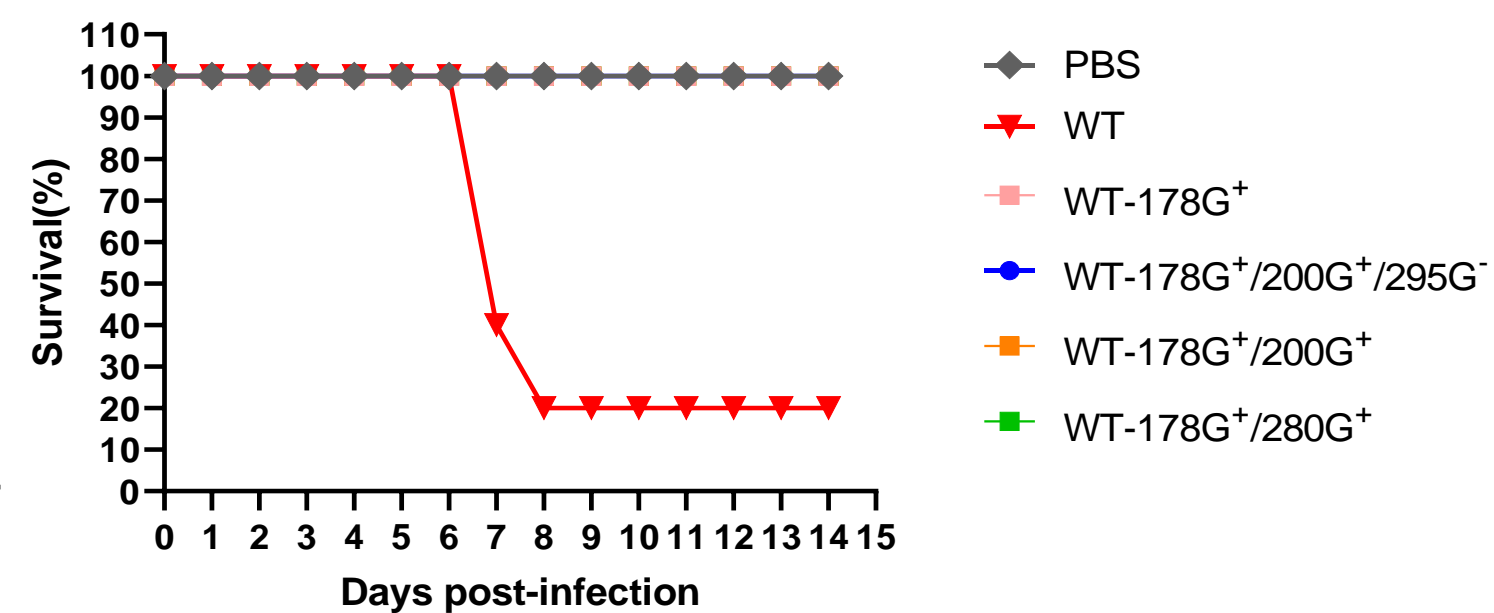
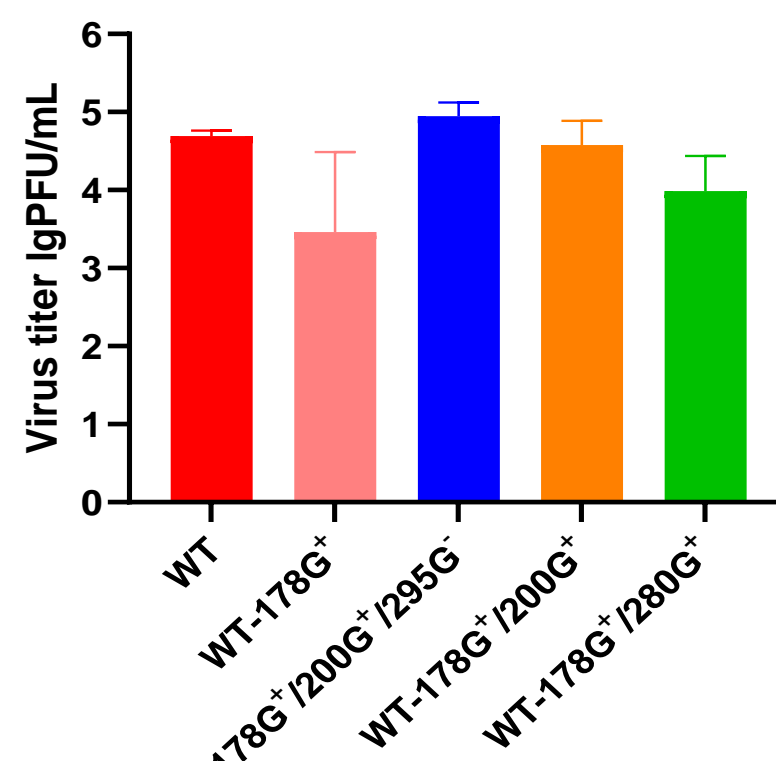
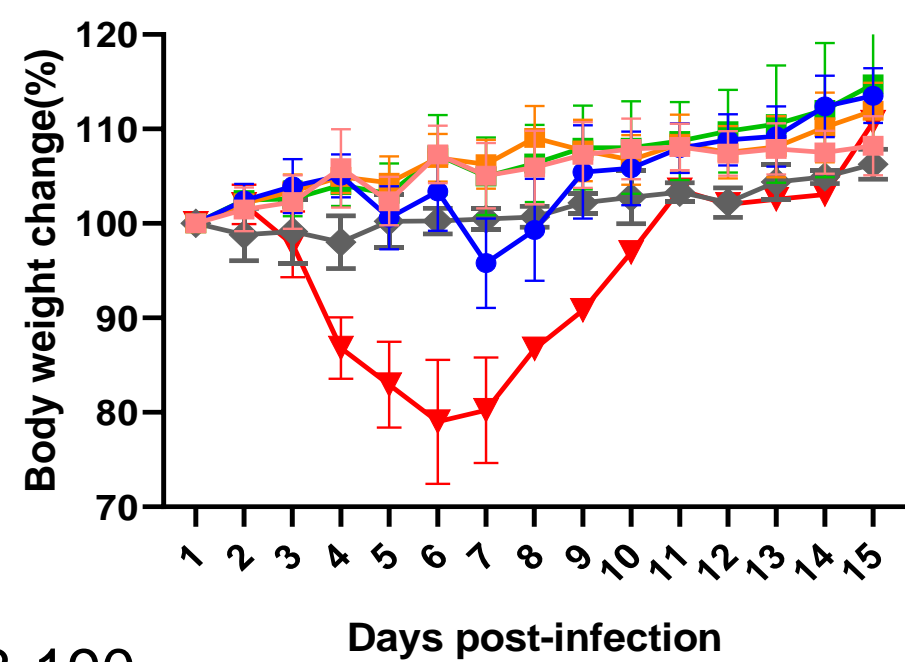


d 148-150

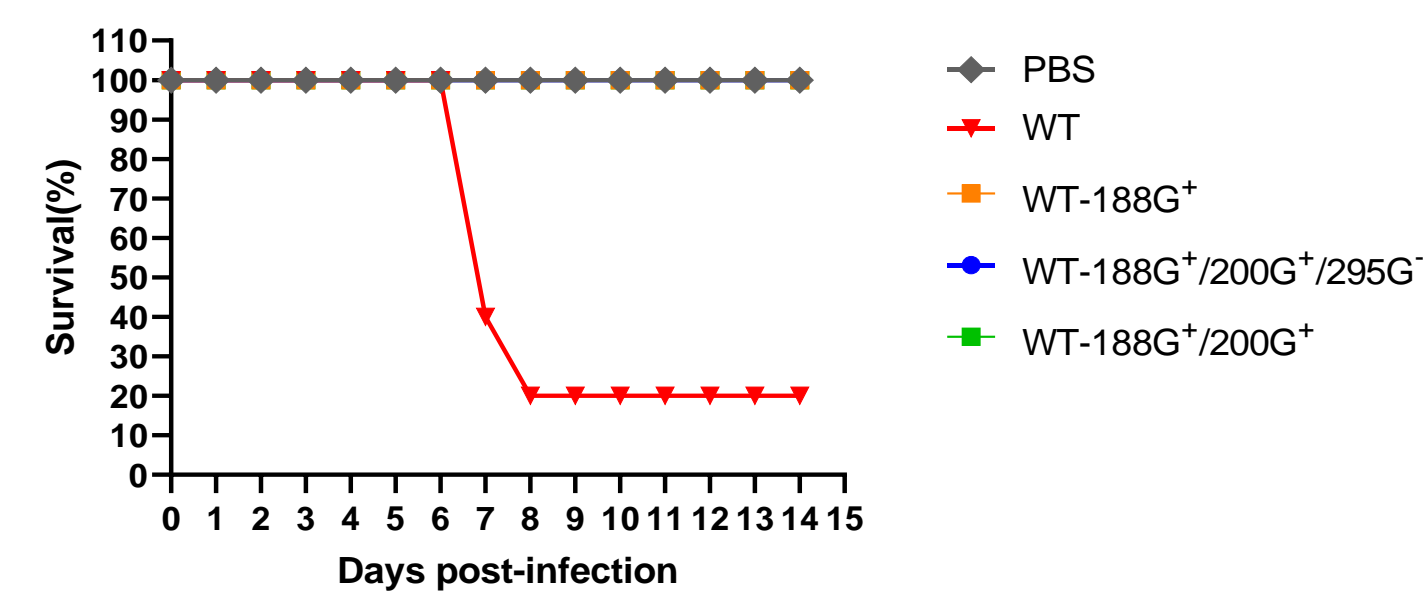
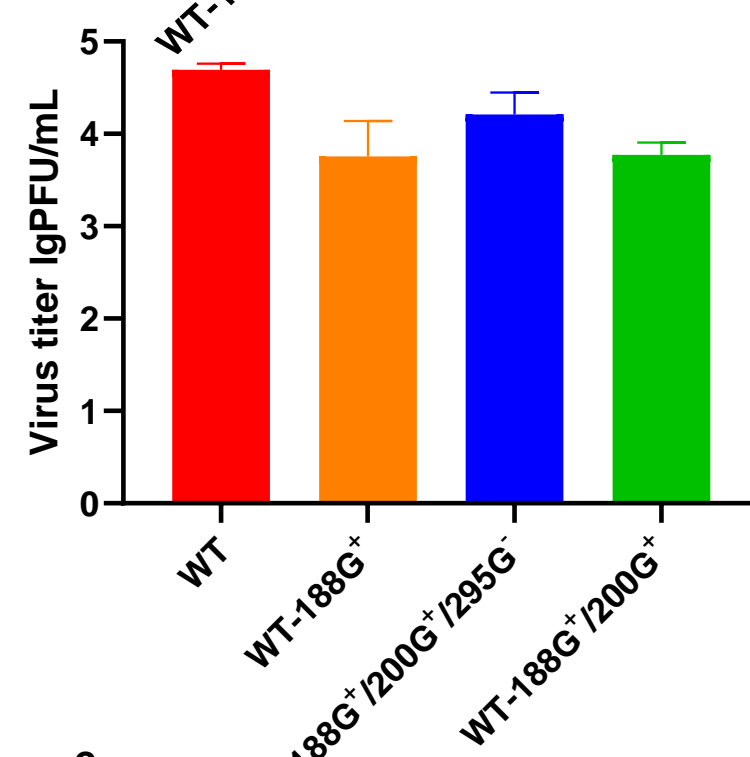
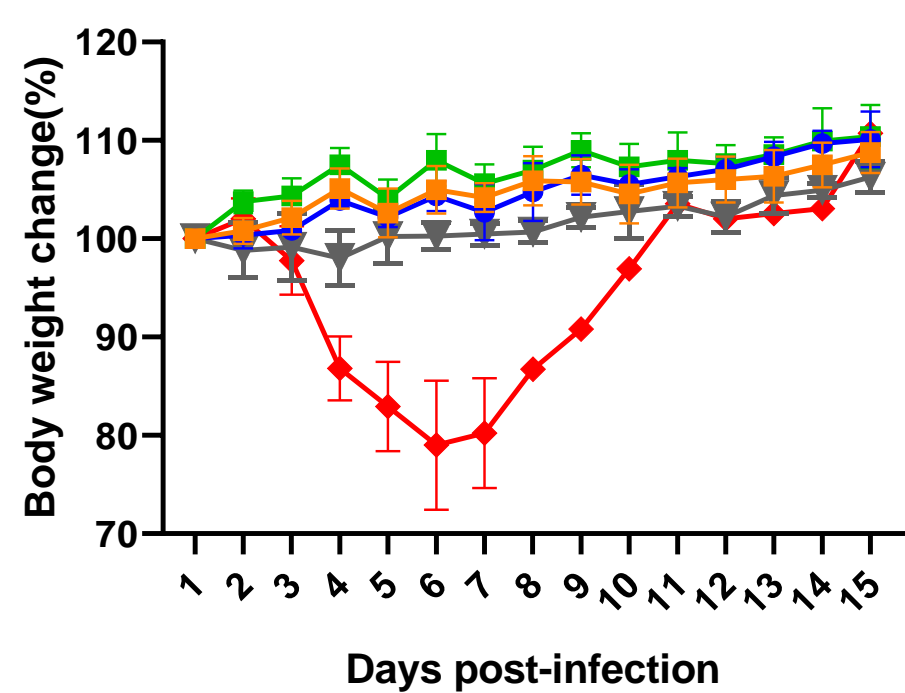


# Figure S1

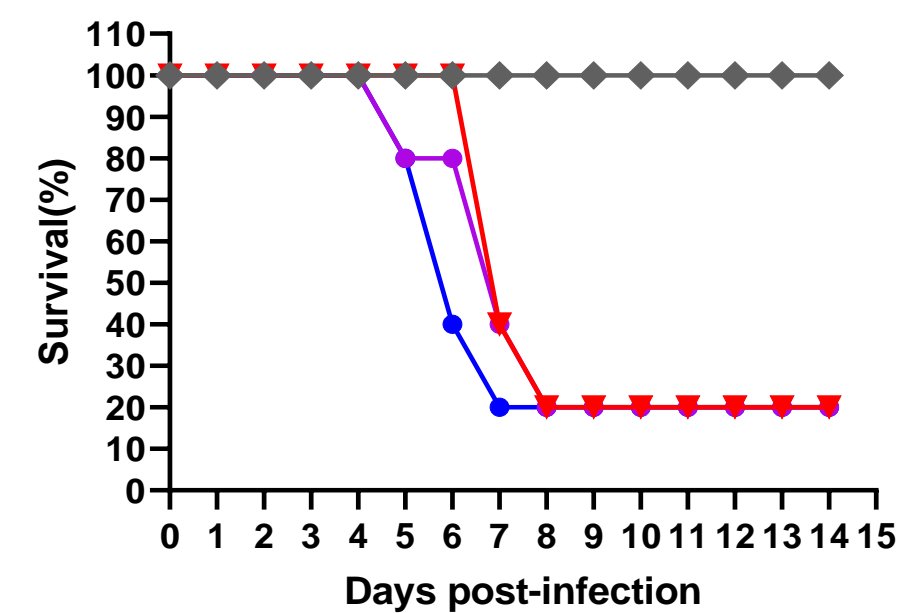
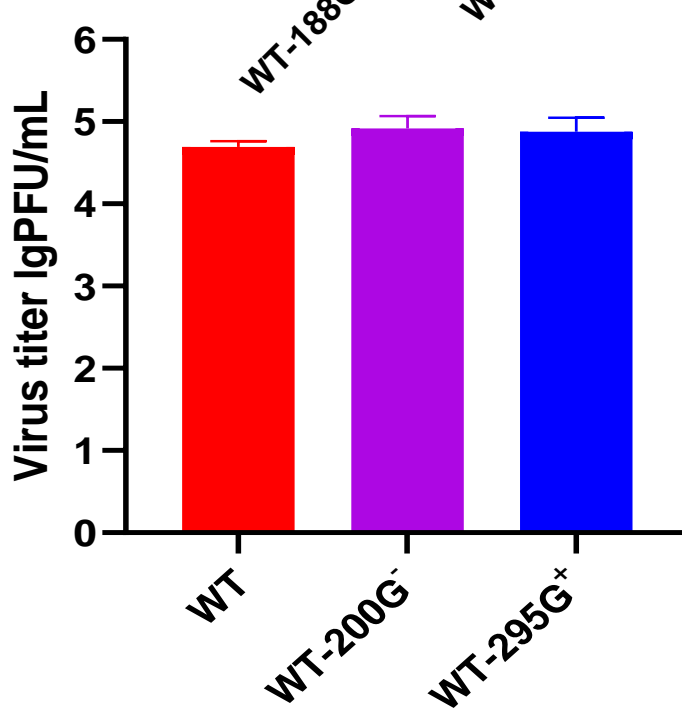
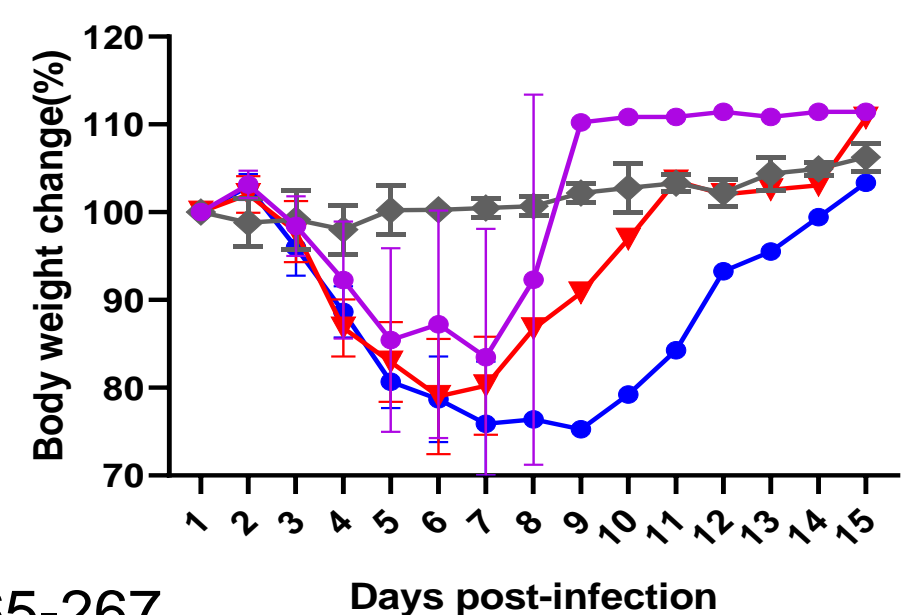
e 178-180



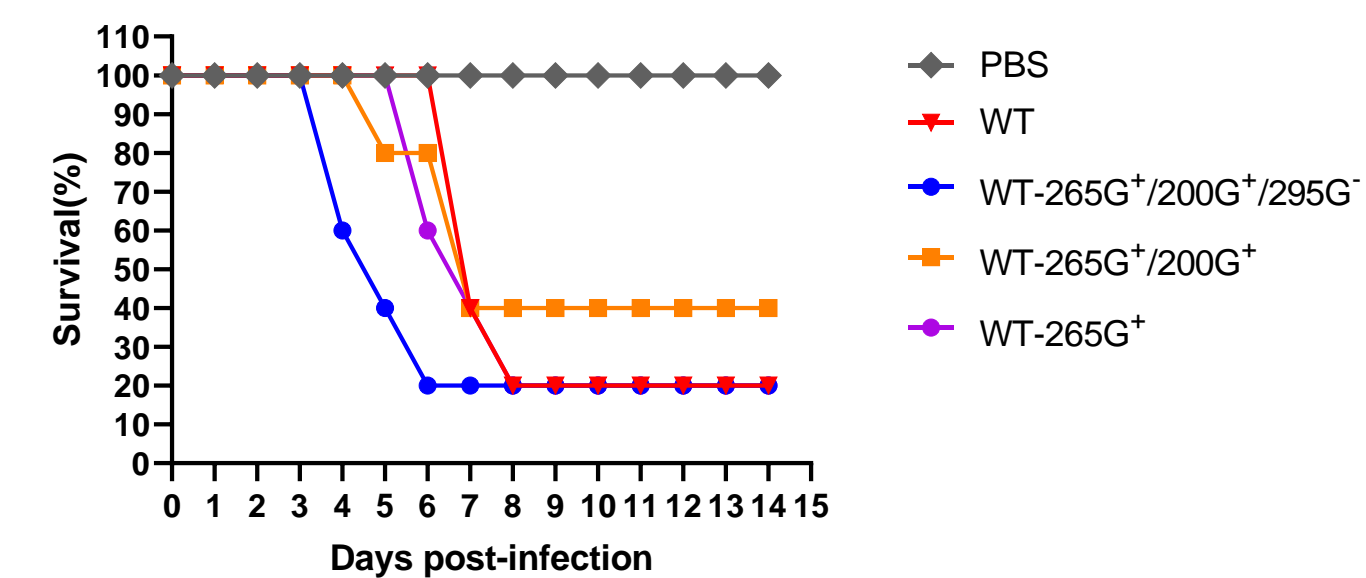
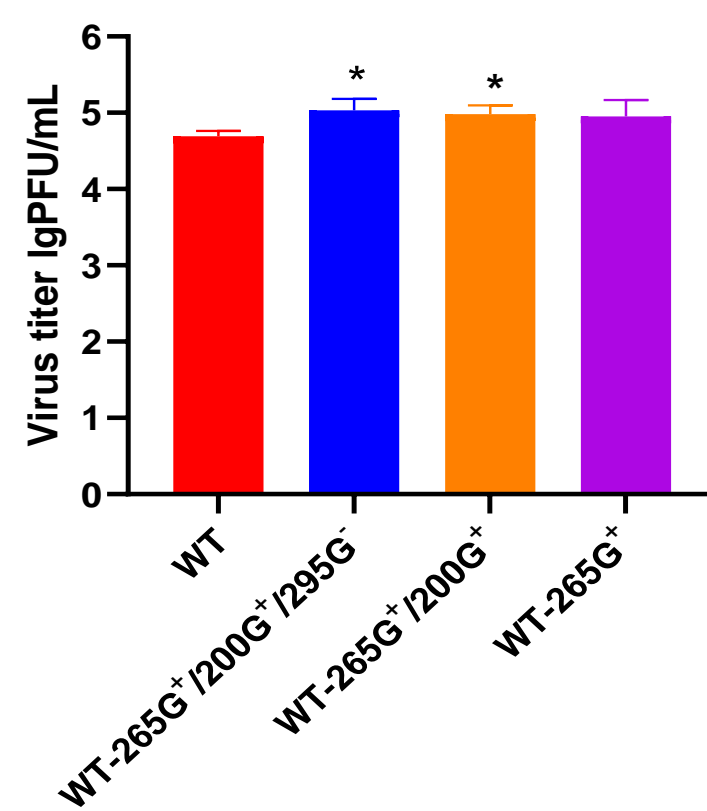
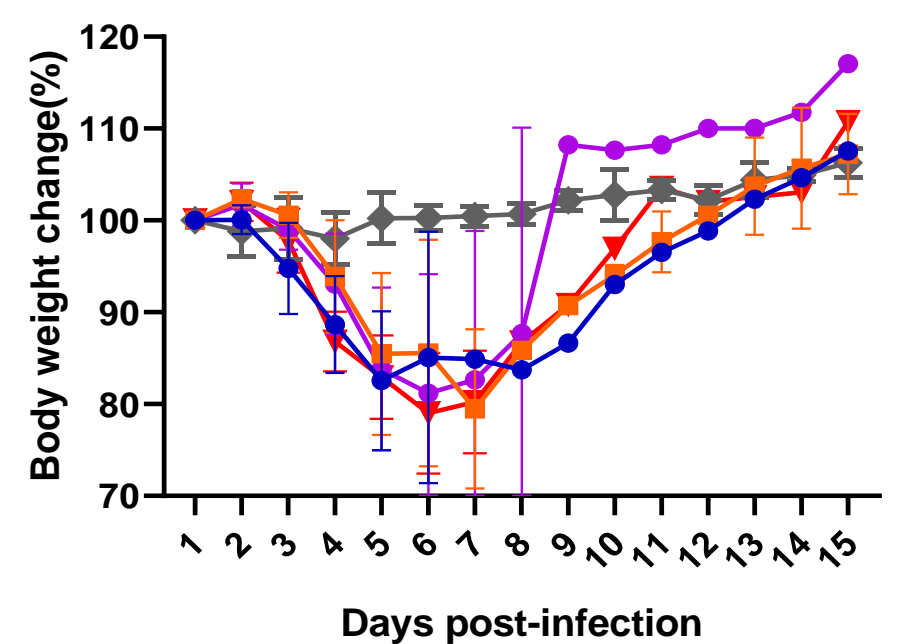
f 188-190



g 200-202 295-297

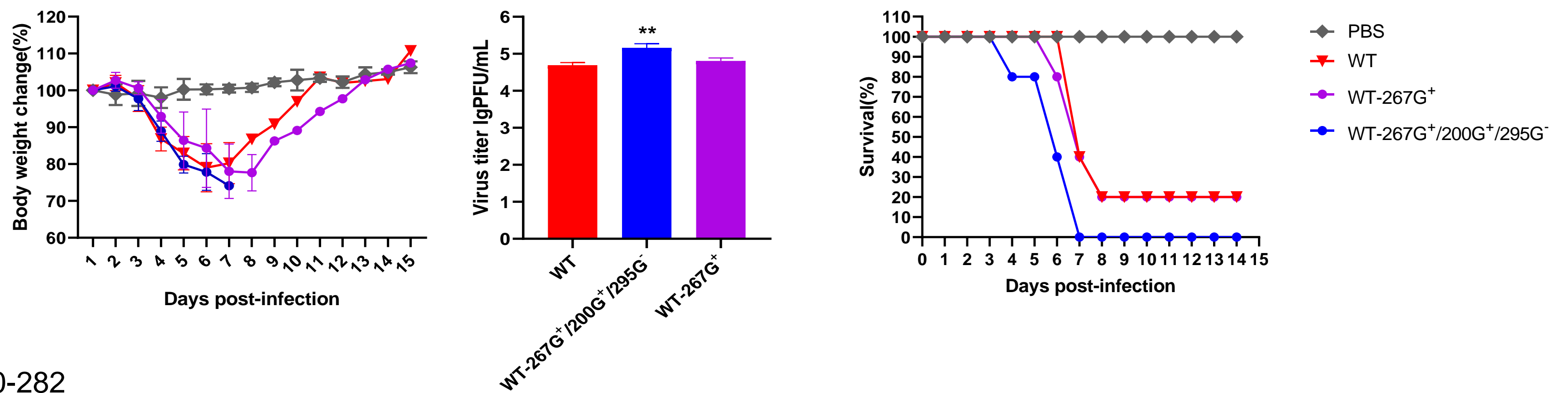


h 265-267



# Figure S1

i 267-269



j 280-282

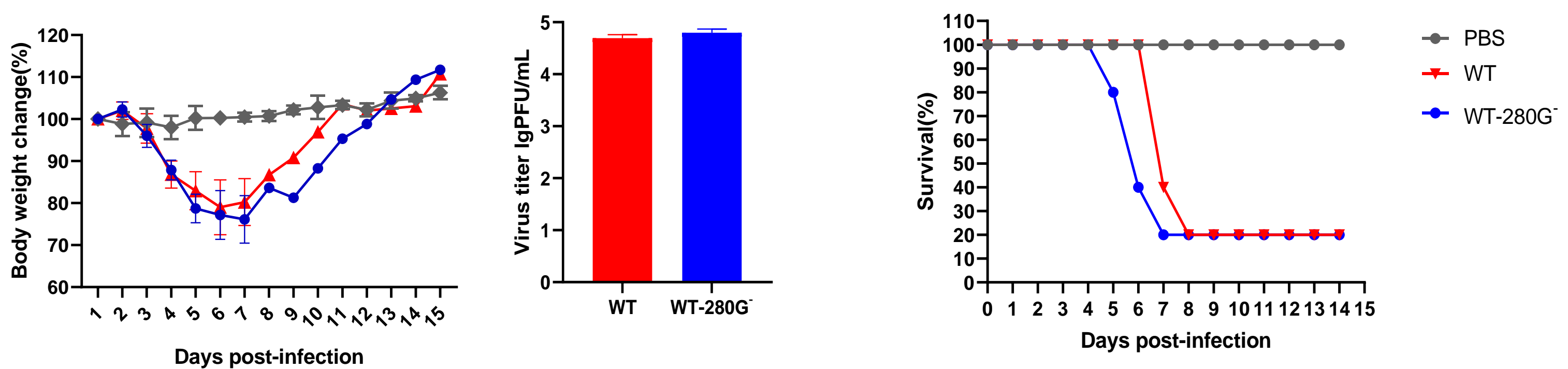


Figure S1: The body weight change, viral titers of lungs and fatality rate of every group of GMS viruses. Six-week-old SPF Balb/c mice were inoculated intranasally with  $4 \times 10^5$  PFU all GMS viruses, continuously monitored changes in mouse body weight until 14 days after challenge and lungs were collected on day 3 post-infection for virus titration in MDCK cells. Different letters from a to j represented different GMS groups, every group contained single site GMS virus and its related combination viruses. Summarized the weight changes, lung viral titers and fatality rate of each group GMS virus for comprehensive analysis. a: 54-56; b: 87-89; c: 127-129; d: 148-150; e: 178-180; f: 188-190; g: 200-202 and 295-297; h: 265-267; i: 267-269; j: 280-282; WT and PBS in every group were colored with red and grey. In every group, different GMS viruses were represented with various colors according to the pathogenicity to mice. Among them, dark blue represented the strongest pathogenicity in this group, followed by purple, next orange and green, and finally light pink, indicating the weakest pathogenicity to mice in this group.