

Table S1. Primers for the construction of HiBiT recombinant full-length cDNA of BDV and virus rescue for BVDV-1 and BDV.

Primer	Direction ^a	Sequence (5'→3') ^b
BDV-FNK-RT	R	GGGCTGTTAGGGTTTTTCCTTAATCCAA
BDV-FNK-F	F	GTATACGGGGTAGCTCATGCCCCGTGTACAAAATTGGACTTT
BDV-FNK-R	R	GGGCTGTTAGGGTTTTTCCTTAATCCAACCTCTGGACTTCA
BDV-FNK-15_pBR	F	AGTACCCCGTATACGGCGCCCGCCGTCGACCAATTCTCATGTTTGACAGCTTATC
BDV-FNK-15_pBF	R	AAACCCTAACAGCCCGCGCCGCATCGAATATAACTTCGT
FNK-Erns-HiBiT- F	F	attagcgggagtctggcggctcgagcggGAGAATATAACACAATGGAACC
FNK-Erns-HiBiT- R	R	cttctgaacagccgccagcgcctcacTGGTGCTACAGGCTGCCACATCAG
BDV-T7-F	F	<u>TAATACGACTCACTATA</u> GTATACGGGGTAGCTCATGCCCCGTGTACAAAATTGGACTTT
NCP-T7-F	F	<u>TAATACGACTCACTATA</u> GTATACGAGGTTAGGCAAGTTCTCGTATACATATTGGACAC
NCP-7-R	R	GGGCTGTTAAGGGTTTTCCCTAGTCCAACC

^a “F” indicates forward, and “R” indicates reverse.

^b The underlined sequence indicates the T7 promoter sequence. Lower-case letters indicate the HiBiT and linker sequences.

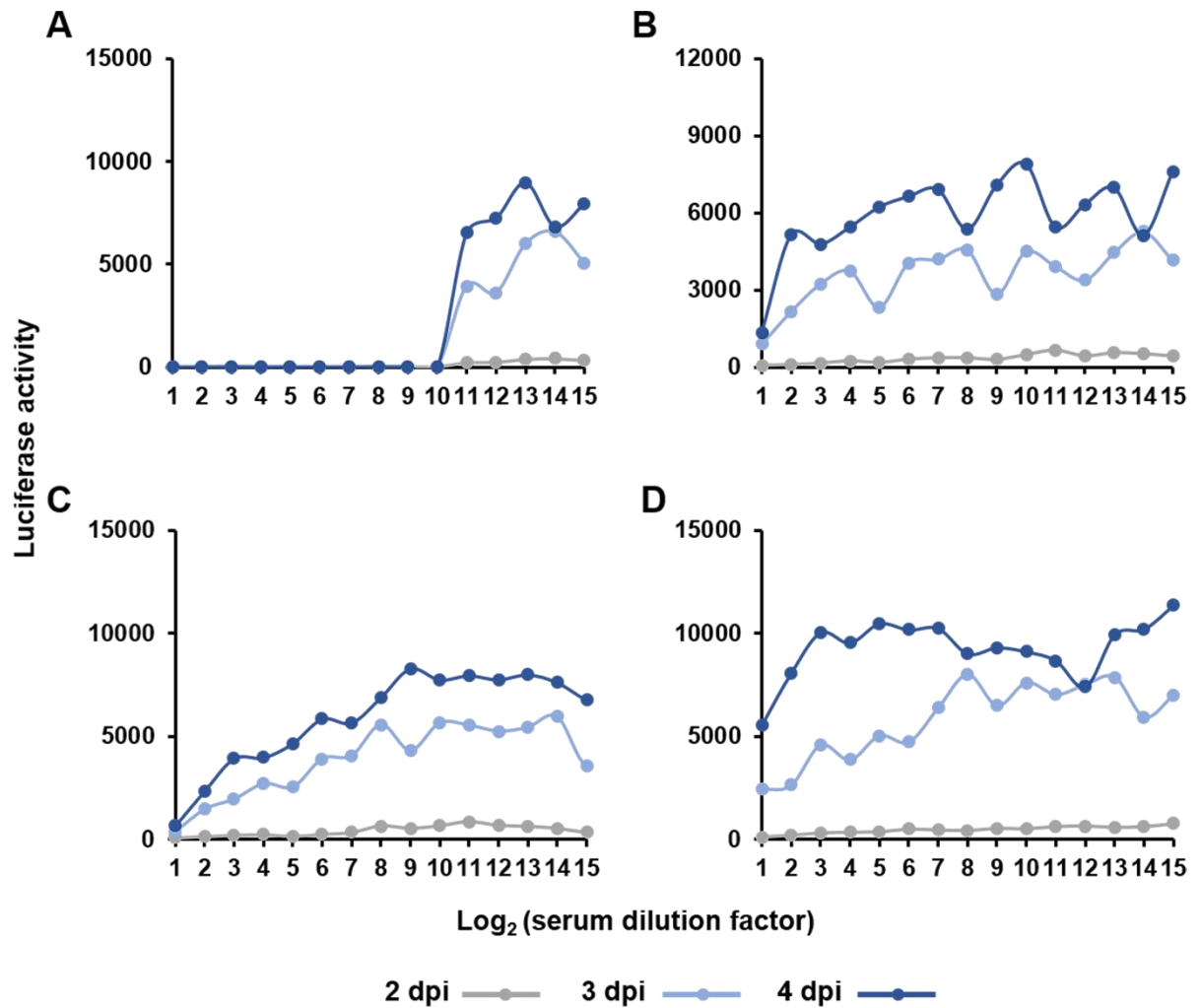


Figure S1. Serum neutralization test using recombinant CSFV carrying HiBiT and anti-pestivirus serum. vCSFV GPE⁻/HiBiT was incubated with two-fold serially diluted anti-CSFV serum (A), anti-BVDV-1 serum (B), anti-BVDV-2 serum (C), or anti-BDV serum (D). Luciferase activity in the culture supernatant of 96-well plates was determined on 2, 3, and 4 days post-infection (dpi). The cut-off value (luciferase activity = 70) to signal complete neutralization was calculated based on that of mock-infected 96-well plates as described in the Materials and Methods. The diagram of obtained luciferase activity was figured out as a sigmoid curve using ImageJ (version 1.52t) for the calculation of 50% effective concentration (EC₅₀).

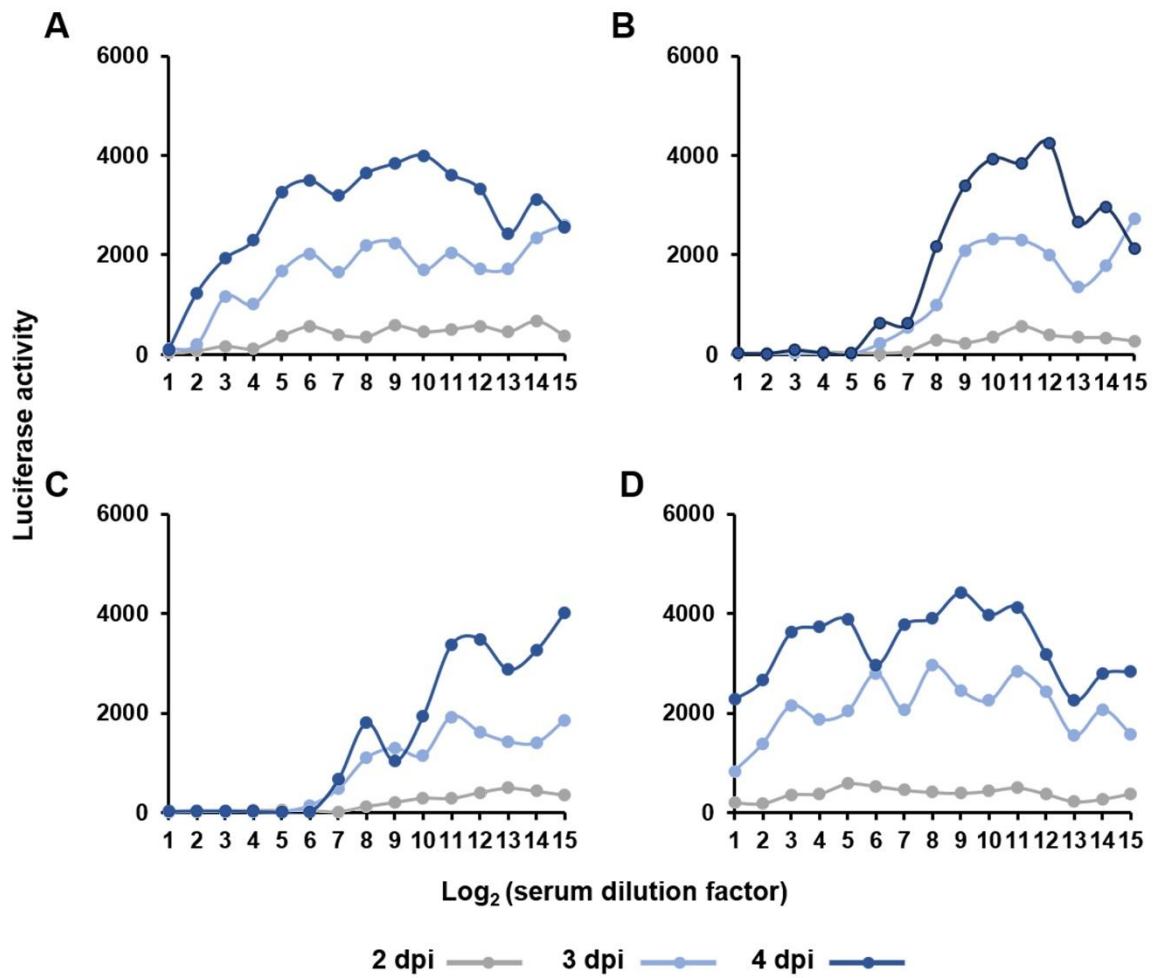


Figure S2. Serum neutralization test using recombinant BVDV-1 carrying HiBiT and anti-pestivirus serum. vBVDV-1 NCP7/HiBiT was incubated with two-fold serially diluted anti-CSFV serum (A), anti-BVDV-1 serum (B), anti-BVDV-2 serum (C), or anti-BDV serum (D). Luciferase activity in the cell lysate of 96-well plates was determined on 2, 3, and 4 days post-infection (dpi). The cut-off value (luciferase activity = 70) to signal complete neutralization was calculated based on that of mock-infected 96-well plates as described in the Materials and Methods. The diagram of obtained luciferase activity was figured out as a sigmoid curve using ImageJ (version 1.52t) for the calculation of 50% effective concentration (EC₅₀).

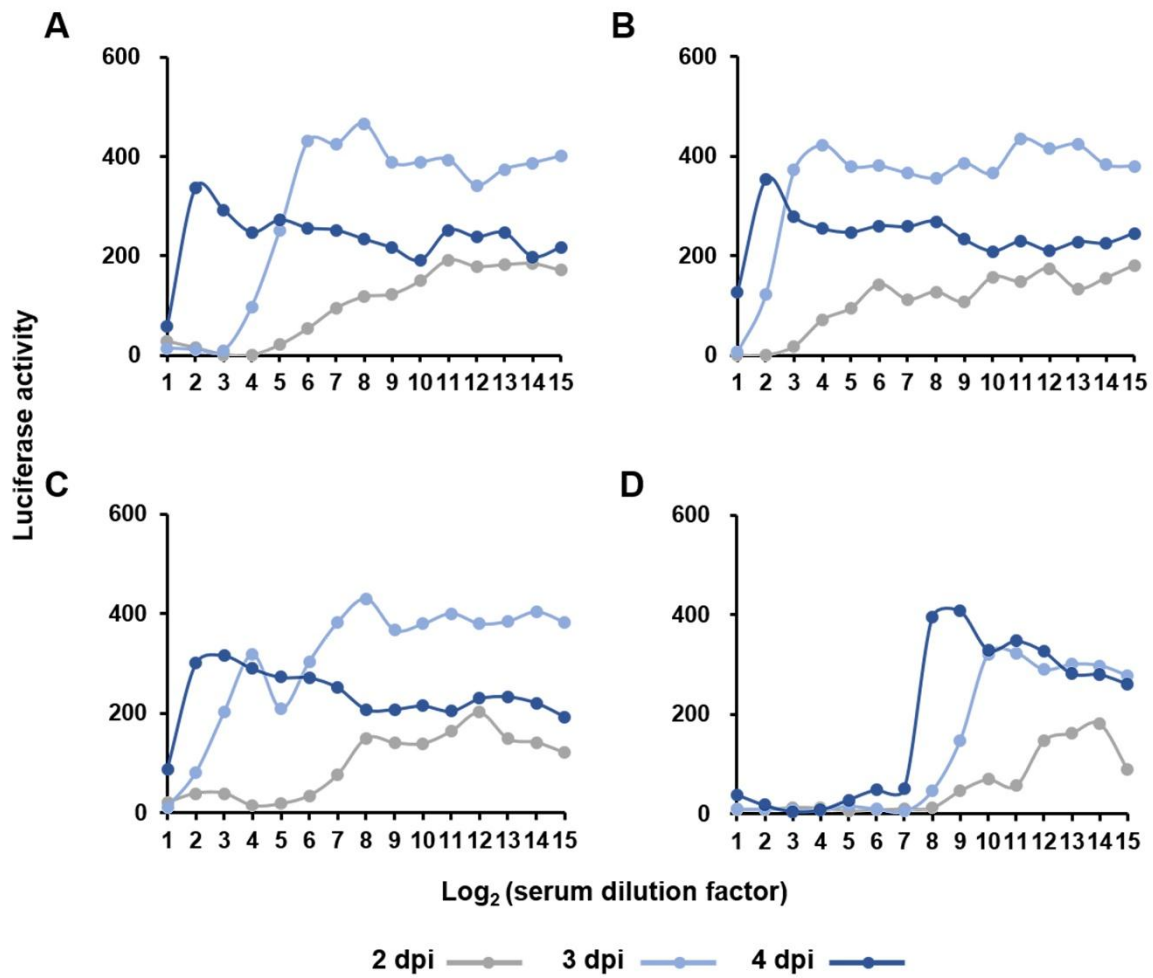


Figure S3. Serum neutralization test using recombinant BDV carrying HiBiT and anti-pestivirus serum. vBDV FNK/HiBiT was incubated with two-fold serially diluted anti-CSFV serum (A), anti-BVDV-1 serum (B), anti-BVDV-2 serum (C), or anti-BDV serum (D). Luciferase activity in the cell lysate of 96-well plates was determined on 2, 3, and 4 days post-infection (dpi). The cut-off value (luciferase activity = 70) to signal complete neutralization was calculated based on that of mock-infected 96-well plates as described in the Materials and Methods. The diagram of obtained luciferase activity was figured out as a sigmoid curve using ImageJ (version 1.52t) for the calculation of 50% effective concentration (EC₅₀).