## Supplemental material

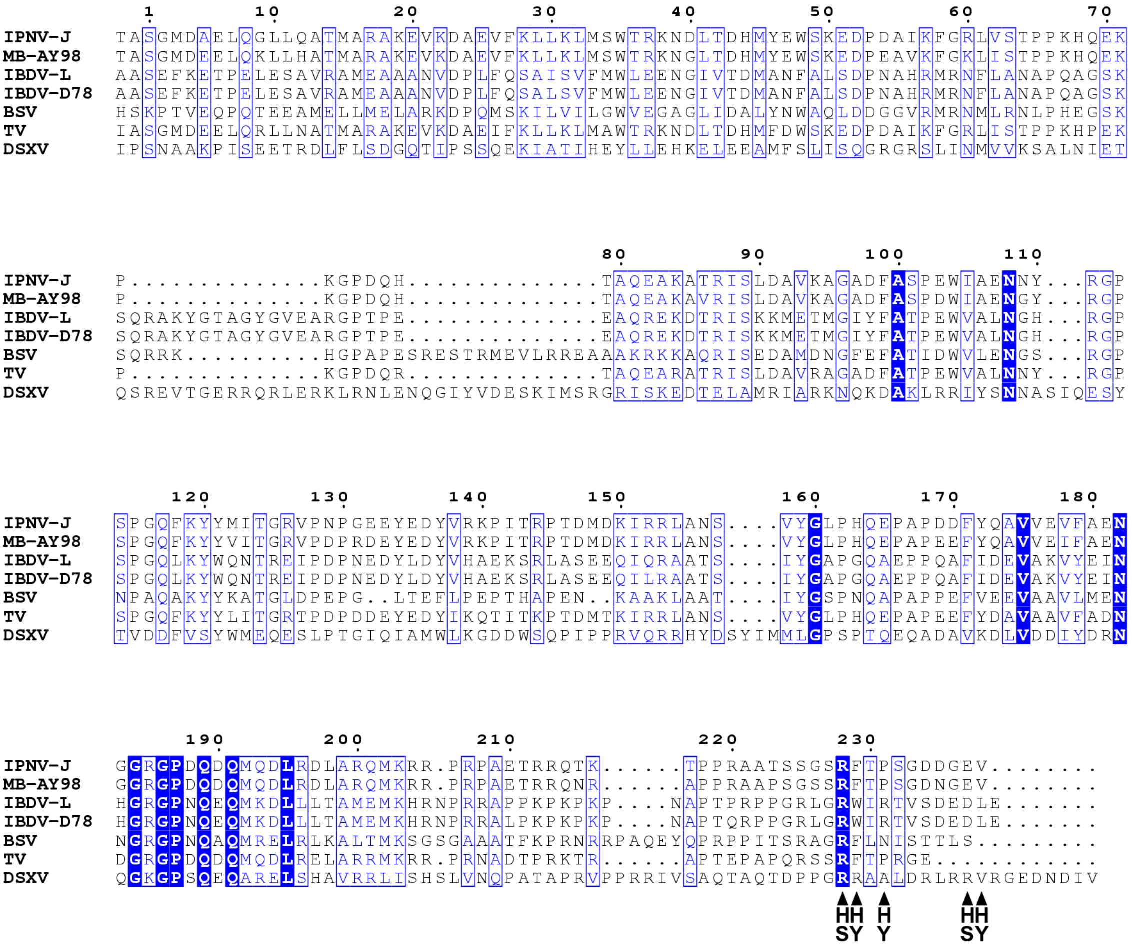
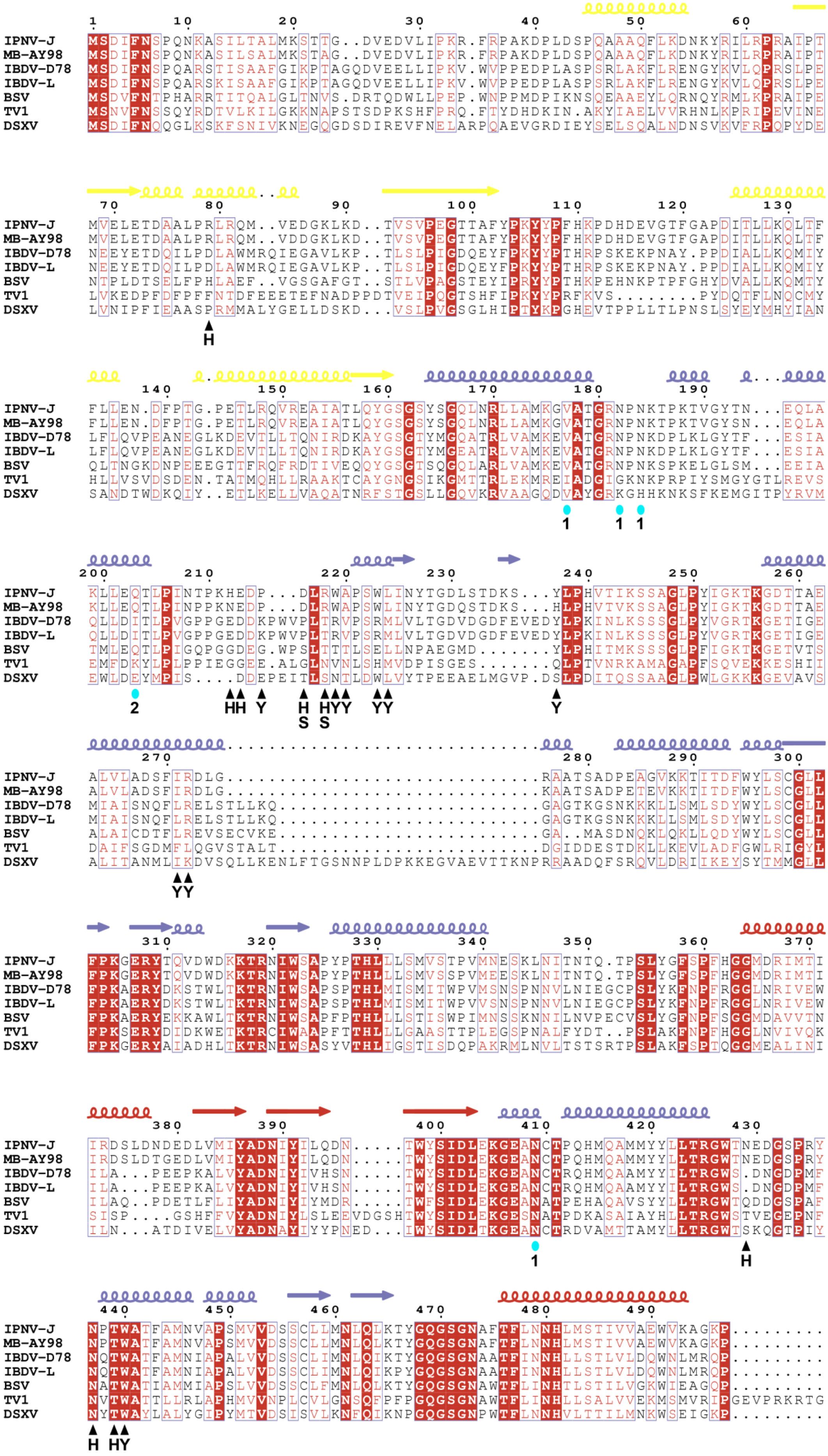


**Fig. S1. Birnavirus VP3 sequence alignment.** Selected birnavirus VP3 sequences are shown as follows (UniProt IDs in parentheses): IPNV-J, Infectious Pancreatic Necrosis virus strain Jasper (P05844); MB-AY98, Marine Birnavirus isolated from *Plecoglossus altivelis* (Q7TLB5); IBDV-L, Infectious Bursal Disease virus strain Lurket (Q49HH5); IBDV-D78, IBDV strain D78 (Q77DJ3); BSV, Blotched Snakehead virus (Q8AZM0); TV, Tellina virus (Q990P7); DSXV, Drosophila X virus (Q96724). Numbering along the top of the alignment is for IPNV-J. Strictly conserved residues are shaded blue with white face, and moderately conserved residues are boxed with blue face. VP3 residues that bind VP1 are marked with a black triangle and labelled according to their interaction mode (H, Hydrogen bond; S, Salt-bridge; Y, Hydrophobic interaction). Note the conservation of R228 that interacts with VP1.


1 10 20 30 40 50 60
IPNV-J MSDIFNSPQNKASILTALMKSTTG..DVEDVLIPKR.FRPAKDPLDSPQAAAQFLKDNKYRILRPRAIPT
MB-AY98 MSDIFNSPQNKASILSALMKSTAG..DVEDVLIPKR.FRPAKDPLDSPQAAAQFLKDNKYRILRPRAIPT
IBDV-D78 MSDIFNSPQARSTISAAFGIKPTAGQDVEELLIPKV.WVPPEDPLASPSRLAKFLRENGYKVLQPRSLPE
IBDV-L MSDIFNSPQARSKISAAFGIKPTAGQDVEELLIPKV.WVPPEDPLASPSRLAKFLRENGYKVLQPRSLPE
BSV MSDVFNTPHARRTITQALGLTNVS.DRTQDWLLPEP.WNPPMDPIKNSQEAAEYLQRNQYRMLKPRAIPE
TV1 MSNVFNSSQYRDTVLKILGKKNAPSTSDPKSHFPRQ.FTYDHDKIN.AKYIAELVVRHNLKPRIPEVINE
DSXV MSDIFNQQGLKSKFSNIVKNEGQGDSDIREVFNELARPQAEVGRDIEYSELSQALNDNSVKVFRPQPYDE
70 80 90 100 110 120 130
IPNV-J MVELETDAALPRLRQM..VEDGKLKD..TVSVPEGTTAFYPKYYPFHKPDHDEVGTFGAPDITLLKQLTF
MB-AY98 MVELETDAALPRLRQM..VDDGKLKD..TVSVPEGTTAFYPKYYPFHKPDHDEVGTFGAPDITLLKQLTF
IBDV-D78 NEEYETDQILPDLAWMRQIEGAVLKP..TLSLPIGDQEYFPKYYPTHRPSKEKPNAY.PPDIALLKQMIY
IBDV-L NEEYETDQILPDLAWMRQIEGAVLKP..TLSLPIGDQEYFPKYYPTHRPSKEKPNAY.PPDIALLKQMIY
BSV NTPLDTSELFPHLAEF..VGSGAFGT..STLVPAGSTEYIPRYYPTHKPEHNKPTPFGHYDVALLKQMTY
TV1 LVKEDPFDFPFFNTDFEEETEFNADPPDTVEIPQGTSHFIPKYYPRFKVS........PYDQTFLNQCMY
DSXV LVNIPFIEAASPRMMALYGELLDSKD...VSLPVGSGLHIPTYKPGHEVTPPLLTLPNSLSYEYMHYIAN
H
140 150 160 170 180 190
IPNV-J FLLEN.DFPTG.PETLRQVREAIATLQYGSGSYSGQLNRLLAMKGVATGRNPNKTPKTVGYTN...EQLA
MB-AY98 FLLEN.DFPTG.PETLRQVREAIATLQYGSGSYSGQLNRLLAMKGVATGRNPNKTPKTVGYSN...EQLA
IBDV-D78 LFLQVPEANEGLKDEVTLLTQNIRDKAYGSGTYMGQATRLVAMKEVATGRNPNKDPLKLGYTF...ESIA
IBDV-L LFLQVPEANEGLKDEVTLLTQNIRDKAYGSGTYMGEATRLVAMKEVATGRNPNKDPLKLGYTF...ESIA
BSV QLTNGKDNPEEEGTTFRQFRDTIVEQQYGSGTSQGQLARLVAMKEVATGRNPNKSPKELGLSM...EEIA
TV1 HLLVSVDSDEN.TATMQHLLRAAKTCAYGNGSIKGMTTRLEKMREIADGIGKNKPRPIYSMGYGTLREVS
DSXV SANDTWDKQIY..ETLKELLVAQATNRFSTGSLLGQVKRVAAGQDVAYGRKGHHKNKSFKEMGITPYRVM
1 1 1
200 210 220 230 240 250 260
IPNV-J KLLEQTLPINTPKHEDP...DLRWAPSWLINYTGDLSTDKS...YLPHVTIKSSAGLPYIGKTKGDTTAE
MB-AY98 KLLEQTLPINPPKNEDP...DLRWAPSWLINYTGDQSTDKS...HLPHVTVKSSAGLPYIGKTKGDTTAE
IBDV-D78 QLLDITLPVGPPGEDDKPWVPLTRVPSRMLVLTGDVDGDFEVEDYLPKINLKSSSGLPYVGRTKGETIGE
IBDV-L QLLDITLPVGPPGEDDKPWVPLTRVPSRMLVLTGDVDGDFEVEDYLPKINLKSSSGLPYVGRTKGETIGE
BSV TMLEQTLPIGQPGGDEG.WPSLTTTLSELLNPAEGMD.......YLPHITMKSSSGLPFIGKTKGETVTS
TV1 EMFDKYLPLPPIEGGEE.ALGLNVNLSHMVDPISGES.......QLPTVNRKAMAGAPFSQVEKKESHII
DSXV EWLDEYMPIS....DDEPEITLSNTLDWLVYTPEEAELMGVP.DSLPDITQSSAAGLPWLGKKKGEVAVS
2
HH Y
H HYY
S S
YY
Y
270 280 290 300
IPNV-J ALVLADSFIRDLG.............................RAATSADPEAGVKKTITDFWYLSCGLL
MB-AY98 ALVLADSFIRDLG.............................KAATSADPETEVKKTITDFWYLSCGLL
IBDV-D78 MIAISNQFLRELSTLLKQ........................GAGTKGSNKKKLLSMLSDYWYLSCGLL
IBDV-L MIAISNQFLRELSTLLKQ........................GAGTKGSNKKKLLSMLSDYWYLSCGLL
BSV ALAICDTFLREVSECVKE........................GA..MASDNQKLQKLLQDYWYLSCGLL
TV1 DAIFSGDMFLQGVSTALT........................DGIDDESTDKLLKEVLADFGWLRIGYL
DSXV ALITANMLIKDVSQLLKENLFTGSNNPLDPKKEGVAEVTTKNPRRAADQFSRQVLDRIIKEYSYTMMGLL
YY
310 320 330 340 350 360 370
IPNV-J FPKGERYTQVDWDKKTRNIWSAPYPTHLLLSMVSTPVMNESKLNITNTQ.TPSLYGFSPFHGGMDRIMTI
MB-AY98 FPKGERYTQVDWDKKTRNIWSAPYPTHLLLSMVSSPVMEESKLNITNTQ.TPSLYGFSPFHGGMDRIMTI
IBDV-D78 FPKAERYDKSTWLTKTRNIWSAPSPTHLMISMITWPVMSNSPNNVLNIEGCPSLYKFNPFRGGLNRIVEW
IBDV-L FPKAERYDKSTWLTKTRNIWSAPSPTHLMISMITWPVVSNSPNNVLNIEGCPSLYKFNPFRGGLNRIVEW
BSV FPKAERYEKKAWLTKTRNIWSAPFPTHLLLSTISWPIMNSSKNNILNVPECVSLYGFNPFSGGMDAVVTN
TV1 FPKSERYDIDKWETKTRCIWAAPFTTHLLLGAASTTPLEGSPNALFYDT..PSLAKFNPFHGGLNVIVQK
DSXV FPKGERYAIADHLTKTRNIWSASYVTHLIGSTISDQPAKRMLNVLTSTSRTPSLAKFSPTQGGMEALINI
380 390 400 410 420 430
IPNV-J IRDSLDNDEDLVMIYADNIYILQDN.....TWYSIDLEKGEANCTPQHMQAMMYYLLTRGWTNEDGSPRY
MB-AY98 IRDSLDTGEDLVMIYADNIYILQDN.....TWYSIDLEKGEANCTPQHMQAMMYYLLTRGWTNEDGSPRY
IBDV-D78 ILA...PEEPKALVYADNIYIVHSN.....TWYSIDLEKGEANCTRQHMQAAMYYILTRGWS.DNGDPMF
IBDV-L ILA...PEEPKALVYADNIYIVHSN.....TWYSIDLEKGEANCTRQHMQAAMYYILTRGWS.DNGDPMF
BSV ILAQ..PDETLFLIYADNIYIYMDR.....TWFSIDLEKGEANATPEHAQAVSYYLLTRGWTQDDGSPAF
TV1 SISP....GSHFFVYADNIYLSLEEVDGSHTWYSIDLEKGESNATPDKASAIAYHLLTRGWSTVEGEPNF
DSXV ILN...ATDIVELVYADNAYIYYPNED...IWYSIDLTKGEANCTRDVAMTTAMYLLTRGWTSKQGTPIY
1
H
440 450 460 470 480 490
IPNV-J NPTWATFAMNVAPSMVVDSSCLLMNLQLKTYGQGSGNAFTFLNNHLMSTIVVAEWVKAGKP.........
MB-AY98 NPTWATFAMNVAPSMVVDSSCLLMNLQLKTYGQGSGNAFTFLNNHLMSTIVVAEWVKAGKP.........
IBDV-D78 NQTWATFAMNIAPALVVDSSCLIMNLQIKTYGQGSGNAATFINNHLLSTLVLDQWNLMRQP.........
IBDV-L NQTWATFAMNIAPALVVDSSCLIMNLQIKPYGQGSGNAATFINNHLLSTLVLDQWNLMRQP.........
BSV NATWATIAMMIAPSLVVDSSCLFMNLQLKTYGQGSGNAWTFLINHTLSTILVGKWIEAGQP.........
TV1 NVTWATTLLRLAPHMVVNPLCVLGNSQFPFPGQGSGNAWTFLINHLLSALVVEKMSMVRIPGEVPRKRTG
DSXV NYTWAYLALYGIPYMTVDSISVLKNFQIKNPGQGSGNPWTFLNNHVLTTILMNKWSEIGKP.........
H HY

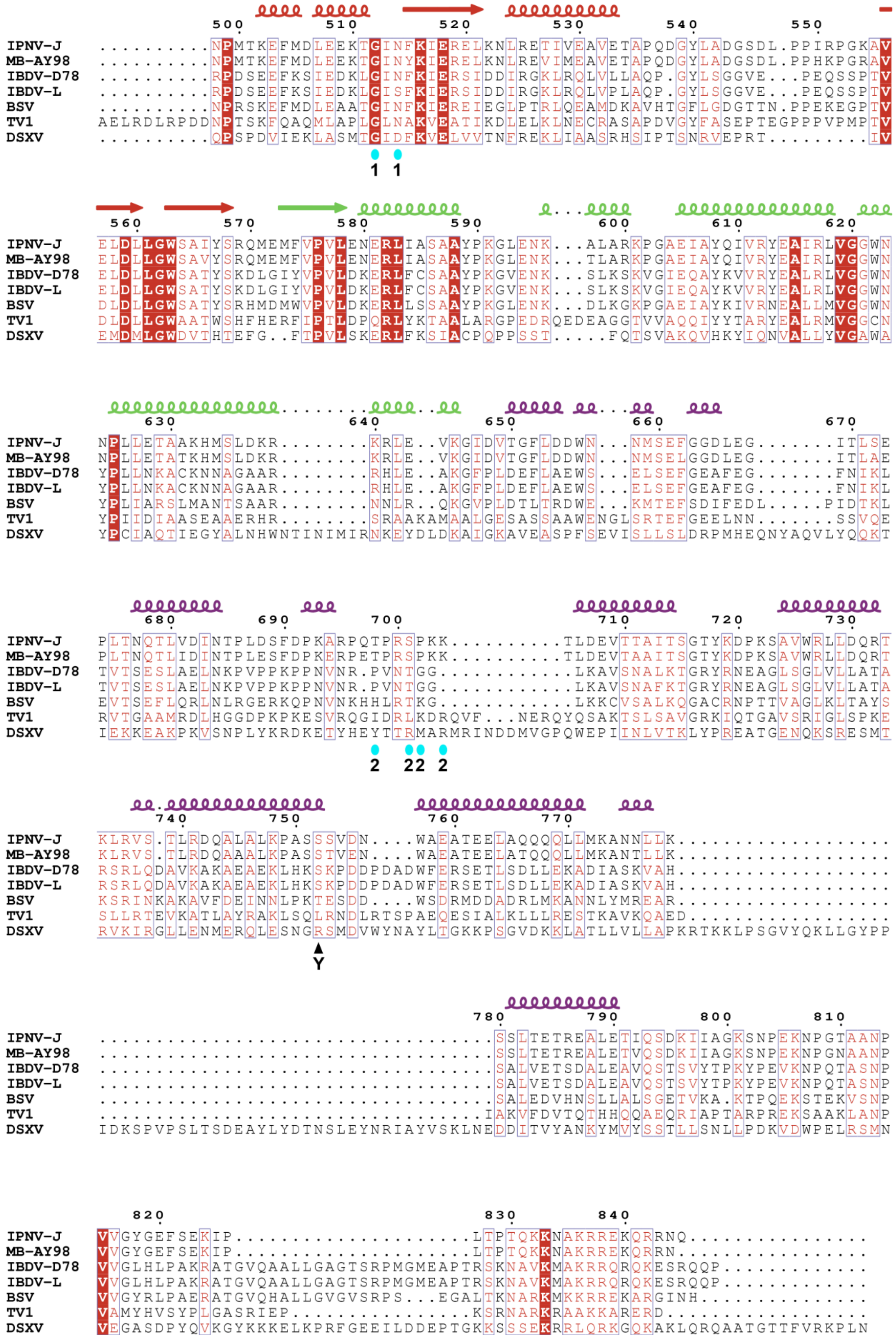


**Fig. S2. Birnavirus VP1 sequence alignment.** Selected birnavirus VP1 sequences are shown as follows (UniProt IDs in parentheses): IPNV-J, Infectious Pancreatic Necrosis virus strain

Jasper (P22173); MB-AY98, Marine Birnavirus isolated from *Plecoglossus altivelis* (Q7T8V1); IBDV-D78, Infectious Bursal Disease virus strain D78 (Q9Q6Q5); IBDV-L, IBDV strain Lurket (Q49HH7); BSV, Blotched Snakehead virus (Q8AZL8); TV1, Tellina virus 1 (Q2PBR4); DSXV, Drosophila X virus (Q91CD5). Numbering along the top of the alignment is for the IPNV strain Jasper VP1 sequence. Strictly conserved residues are shaded red with white face, and moderately conserved residues are boxed with red face. Secondary structure elements for the VP1 structure are shown along the top of the alignment as spirals (α helices) and arrows (β strands), and are coloured by domain as in Fig. 1C. VP1 residues that bind to the VP3 peptide are marked with a black triangle and labelled according to their interaction mode (H, Hydrogen bond; S, Salt-bridge; Y, Hydrophobic interaction). Residues that form the $K^+$ ion binding sites are marked with a cyan circle and are grouped “1” or “2” depending on their position in $K^+$-binding site 1 or site 2, respectively.

**Table S1. Image densitometry analysis of IPNV VP1:VP3 catalyzed RNA synthesis (normalized against IPNV VP1 reaction).**

| Molar ratio of VP3 | Total lane | Unbound dsRNA product | RNA:protein complex |
|---|---:|---:|---:|
| 0 | 100.0 % | 100.0 % | 100.0 % |
| 0.1 | 98.0 % | 96.5 % | 80.3 % |
| 0.2 | 97.4 % | 91.6 % | 78.8 % |
| 0.5 | 96.4 % | 92.5 % | 50.2 % |
| 0.7 | 94.1 % | 81.7 % | 120.3 % |
| 1.0 | 95.4 % | 69.6 % | 236.5 % |
| 1.5 | 117.8 % | 49.2 % | 736.3 % |
| 2.0 | 116.4 % | 39.5 % | 739.2 % |
| 3.0 | 117.6 % | 35.6 % | 980.5 % |
| 5.0 | 108.2 % | 33.6 % | 965.7 % |
| 10 | 114.2 % | 38.1 % | 706.3 % |
| 50 | 68.6 % | 18.6 % | 3423.8 % |

The quantification data is from one representative experiment out of three independent repeats.

**IPNV VP3 synthetic gene nucleotide sequence (synthesised by Geneart).**

GGTACCACCGCAAGCGGTATGGATGCAGAACTGCAGGGTCTGCTGCAGGCAACCATGGCA

CGTGCAAAAGAAGTTAAAGACGCCGAAGTGTTTAAACTGCTGAAACTGATGAGCTGGACC

CGTAAAAATGATCTGACCGATCATATGTATGAATGGTCTAAAGAAGATCCGGACGCCATT

AAATTTGGTCGTCTGGTTAGCACCCCTCCGAAACATCAGGAAAAACCGAAAGGTCCGGAT

CAGCATACCGCACAGGAAGCAAAAGCAACCCGTATTAGCCTGGATGCAGTTAAAGCCGGT

GCAGATTTTGCATCTCCGGAATGGATTGCCGAAAATAATTATCGTGGTCCGTCCCCTGGT

CAGTTTAAATATTATATGATTACCGGTCGTGTTCCGAATCCGGGTGAAGAATATGAAGAT

TATGTGCGTAAACCGATTACCCGTCCGACCGATATGGACAAAATTCGTCGTCTGGCCAAT

AGCGTTTATGGTCTGCCGCATCAGGAACCGGCACCGGACGATTTTTATCAGGCCGTGGTT

GAAGTTTTTGCAGAAAATGGTGGTCGTGGTCCGGATCAGGATCAGATGCAGGATCTGCGT

GATCTGGCACGTCAGATGAAACGTCGTCCGCGTCCGGCAGAAACCCGTCGTCAGACCAAA

ACACCTCCGCGTGCAGCAACCAGCAGCGGTAGCCGTTTTACCCCGAGCGGTGATGATGGT

GAAGTTGAGCTC