## 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 (Q900P7); 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, Saltbridge; Y, Hydrophobic interaction). Note the conservation of R228 that interacts with VP1.

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IPNV-J MB-AY98 IBDV-D78 IBDV-L BSV	MSDIFN MSDIFN MSDIFN MSDIFN MSDVFN	S P Q N S P Q N S P Q A S P Q A T P H A	KASI KASI RSTI RSKI RRTI	LTA LSA SAA SAA TQA	LMKS LMKS FGIK FGIK LGLT	TTG TAG PTA PTA NVS	I GQI GQI .DF	VE VE VE VE VE	DVI DVI ELI ELI DWI	, L P I ' I P I ' I P I ' I P I ' I P I	KR. KR. KV. KV. EP.	FRI FRI WVI WVI WNI	2 A K 2 A K 2 P E 2 P E 2 P E 2 P M	DPL DPL DPL DPL DPI	DS DS AS AS KN	PQI PQI PSI PSI SQI	AAA AAA RLA RLA EAA	QF QF KF KF EY	LKI LKI LRI LRI LQI	DNF DNF ENG ENG RNG	YR YR YK YK YK YR	ILR ILR VLQ VLQ MLK	PR PR PR PR PR	AIPT AIPT SLPE SLPE AIPE
TV1 DSXV	MSNVFN MSDIFN	SSQY QQGL	RDTV KSKF	SNI	LGKK VKNE	N A P G <mark>Q</mark> G	STS DSE	DPI	K S H E V F	IFPI NEI	RQ. LAR	FTY PQZ	Y D H A E V	DKI GRD	N. IE	AK Y <mark>S</mark> I	YIA ELS	E L QA	V V I L N I	RHN DNS	VK VK	PRI VFR	ΡΕ ΡQ	VINE PYDE
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BSV TV1 DSXV	N TPLDT LVKEDP LVNIPF	SELF FDFP IEAA	PHLA FFNT SPRM	EF. DFE MAL	.VGS EETE YGEL	GAF	GT. DPE KD.	.s dt	TLV VEI VSI	PA PQ PV	GSŤ GTS GSG	EYI HFI LHI	IPR IPK IPT	Y Y P Y Y P Y K P	TH RF GH	K P I K V S E V S	E H N 5 7 P P	KP LL	T P H	FGH F PNS	YD YD SLS	V A L Q T F Y E Y	LK LN MH	OMTY OCMY YIAN
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IBDV-L BSV	LFLQVP QLTNGK	EANE	GLKD GLKD EEGT	EVT		NIR TIV		AYG QYG	SGI SGI SGI	YM SQ	GEA GQL	TRI ARI	LVA LVA	MKE	VA VA VA	TG TG	RNP	NK	DPI DPI SPI	LKI KEI	GY	TF. SM.		ESIA
DSXV	H L L V S V S A <mark>N</mark> D T W	DSDE DKQI	N.TA YE	TLK	HLLR ELLV	A A K A Q A	T C A T N F	AYGI RFS'	NGS TGS	LL	GM T GQ V	KR	JAA	MRE G <mark>Q</mark> D	LA VA	DG. Y <mark>G</mark> I	RKG	. N K H H	P R I K N I	Y I Y K S F	'KEI	GYG MGI	TP	KEVS YRVM
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MB-AY98 IBDV-D78 IBDV-L	KLLEQT QLLDIT QLLDIT	LPIN LPVG LPVG	P P K N P P G E P P G E	IEDP IDDK IDDK	D PWVP PWVP	LRW LTR	APS VPS VPS	SWL SRM SRM	INY LVI LVI	TGI TGI TGI	DQS DVD DVD	TDH GDH GDH	KS. FEV FEV	H EDY EDY	LP LP LP	HV: KII KII	N L K N L K	SS SS SS	AGI SGI SGI	LPY LPY LPY	IGI VGI VGI	K T <mark>K</mark> R T <mark>K</mark> R T <mark>K</mark>	GD GE GE	FTAE FIGE FIGE
BSV TV1 DSXV	TMLEQT EMFDKY EWLDEY	LPIG LPLP MPIS	QPGG PIEG	GEG GEE DDE	.WPS .ALG PEIT	LTT LNV LSN	TLS NLS TLI	SEL: SHM SWL	LNP VDP VYI	PAE	GMD GES EAE	 LM(	  GVP	Y Q .DS	LP LP LP	HI: TVI DI:	FMK NRK FQS	SS AM SA	SGI AGZ AGI	L P F A P F L P W	IGI SQ LGI	K T <mark>K</mark> V E <b>K</b> K K <b>K</b>	GE KE GE	TVTS SHII VAVS
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DSXV	ALITAN	MLIK	DVSQ	LLK	ENLF	TGS	NNE	LDI	PKK	ŒGV	VAE	VTI	ΓKΝ	PRR	ΑA	DQI	F S R	QV	LDI	RII	KE	YSY	TM	GLL
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IBDV-D78 IBDV-L	FPKAER FPKAER	YDKS	TWLT	KTR KTR	NIWS	APS APS	PTH	ILM ILM	ISMISM		WPV WPV	MSI	NSP NSP		LNLN	IE( IE(	GCP GCP	SL	Y K Y K Y K	E N E E N E	FR	GGL	NR NR	IVEW
BSV TV1 DSXV	F P K A E R F P K S E R F P K <mark>G</mark> E R	Y E K K Y D I D Y A I A	AWLI KWET DHLT	KTR KTR KTR	NIWS CIWA NIWS	APF APF ASY	TTH VTH		LSI LGA G <mark>S</mark> I	ASI	NPI ITP DQP	MN S LEC AK	SSK SSP RML	N N I N A L N V L	L N F Y T S	DT TSI	P RTP	SL SL SL	Y G A K A K	E N E E N E E S E	F H F H T Q	GGM GGL GGM	NV EA	UV TN IVQK LINI
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IBDV-L BSV TV1	ILA ILAQ SISP	PEEP PDET GS	KALV LFLI HFFV	YAD YAD YAD	N I Y I N I Y I N I Y I	VHS YMD SLE	N R E V I		T W T W H T W	IYS: IFS: IYS:	IDL IDL IDL	EK( EK( EK(	GEA GEA GES	N C T N A T N A T	RQ PE PD	H M ( H A ( K A (	QAA QAV SAI	MY SY AY	YI] YL] HL]	6 T F 6 T F 6 T F	RGW RGW RGW	S.D TQD STV	NG DG EG	DPMF SPAF EPNF
DSXV	ILN	ATDI	VELV	YAD	NAYI	ΥΥΡ	NEI	)	.IW	Y <mark>s</mark> :	IDL	TKO	GEA		RD	VA	TTN	ΑM	Υ <mark>Ι</mark>	LTF	GW	TSK ▲	Q <mark>G</mark>	T <mark>PIY</mark>
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IBDV-D78 IBDV-L BSV	NQTWAT NQTWAT NATWAT	FAMN	IAPA	LVV	DSSC	LIM	NLQ	IK	ΤΥG ΡΥG		SGN SGN	AA AA	CFI CFI	N N H N N H		STI STI	LVL	DQ	WN WN WN	LMF	QP	· · ·	· · · ·	· · · · ·
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IPNV-J	680 PLINOTLVD	690 TNTPLDSFDP	700 KARPOTPRSPK	к	710 . TLDEVTTA	720 ITSGTYKDPKS	730 AVWRLLDORT
MB-AY98 IBDV-D78 IBDV-L BSV TV1 DSXV	PLTNQTLID TVTSESLAE TVTSESLAE EVTSEFLQR RVTGAAMRD IEKKEAKPK	INTPLESFDP LNKPVPPKPP LNKPVPPKPP LNLRGERKQP LHGGDPKPKE VSNPLYKRDK	KERPETPRSPK NVNR.PVNTGG NVNR.PVNTGG NVNKHHLRTKG SVRQGIDRLKD ETYHEYTTRMA	K RQVFNEF RMRINDDMVG	. TLDEVTAA . LKAVSNA . LKAVSNA . LKKCVSA QYQSAKTSL GPQWEPIINL	I T SGTYKDPKS LKTGRYRNEAG FKTGRYRNEAG LKQGACRNPTT SAVGRKIQTGA VTKLYPREATG	AVWR LLD QRT LSGLVLLATA LSGLVLLATA VAGLKLTAYS VSRIGLSPKE ENQKSRESMT
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IPNV-J MB-AY98 IBDV-D78 IBDV-L BSV TV1 DSXV	IDKSPVPSL	TSDEAYLYDT	NSLEYNRIAYV	SSLTE SSLTE SSLTE SALVE SALVE SKLNEDDITV	TREALETIQ TREALETVQ TSDALEAVQ TSDALEAVQ VHNSLLALS VHNSLLALS VYANKYMVYS	SDKILAGKSNP SDKILAGKSNP STSVYTPKYPE STSVYTPKYPE GETVKA.KTPQ EQRIAPTARPR STLLSNLLPDK	EKNPGNAANP EKNPGNAANP VKNPQTASNP VKNPQTASNP EKSTEKVSNP EKSAAKLANP VDWPEL <u>RSM</u> N
IPNV-J ME-AY98 IBDV-D78 IBDV-L BSV TV1 DSXV	820 VVGYGEFSE VVGLHLPAK VVGLHLPAK VVGLHLPAK VAMYHVSYP VEGASDPYO	KIP RATGVQAALL RATGVQAALL RATGVQAALL LGASRIEP VKGYKKKELK	GAGTSRPMGME GAGTSRPMGME GVGVSRPS PRFGEEILDDE	830 LTPTQKK LTPTQKK APTRSKNAVK APTRSKNAVK EGALTKNARK KSRNARK PTGKKSSSEK	840 NAKRREKQR MAKRRQRQK MAKRRQRQK MKRREKAR RAAKKARER RAAKKARER	RNQ RN ESRQQP GINH D AKLQRQAATGT	TFVRKPLN

**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 ( $\alpha$  helices) and arrows ( $\beta$  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<sup>+</sup> ion binding sites are marked with a cyan circle and are grouped "1" or "2" depending on their position in K<sup>+</sup>-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).