Helicase	**Motif** Ia	**Motif** Ib	**Motif** II	**Motif** III	**Motif** IV	**Motif** V	**Motif** VI
	`          * **     *`	`     * *`	`    ** *`	`     ***`	`  *`	`    *     *        *`	`     *    ** **`
GBV-Amys	YREAPLYLPTGSGKSTR-IP	HRVLVLNPSIATVRAM	VVICDECH	LLFATATPP	LLFCHSKVE	CVCATDALSTGFTG-NFDTVT	TVPAPAELRSQRRGR
GBV-Atri	FTEAPLYLPTGSGKSTR-IP	HKVLVLNPSIATVRAM	IVICDECH	LLFATATPP	LLFCHSKVE	CVCATDALSTGYTG-GFSTVT	TTPAPAELRAQRRGR
GBV-Alab	YREAPLYLPTGSGKSTR-IP	HKVLVLNPSIATVRAM	VVICDECH	LLFATATPP	LLFCHSKVE	CVCATDALSTGYSG-NFDSVT	TVPAPAELRMQRRGR
GBV-A	YREAPLFLPTGAGKSTR-VP	HKVLVLNPSIATVRAM	VVICDELH	LLFATATPP	LLFCHSKVE	CVCATDALSTGYTG-NFDTVT	TVPAPAELRAQRRGR
GBV-B	YSVQILIAPTGSGKSTK-LP	YEVLVLNPSVATTASM	VIICDECH	VVLATATPP	LIFEATKKH	VVVATDALCTGYTG-DFDSVY	VCGVSAIVKGQRRGR
GBV-C gt1	FKEAPLFMPTGAGKSTR-VP	HKVLILNPSVATVRAM	VVICDECH	VLYATATPP	LVFCHSKAE	VVCATDALSTGYTG-NFDSVT	TVPASAELSMQRRGR
GBV-C gt2	FKEAPLFMPTGAGKSTR-VP	HKVLILNPSVATVRAM	VVICDECH	VLYATATPP	LVFCHSKAE	VVCATDALSTGYTG-NFDSVT	TVPASAELSMQRRGR
GBV-C gt3	K-EAPLFMPTGAGKSTR-VP	HKVLILNPSVATVRAM	VVICDECH	VLYATATPP	LVFCHSKAE	VVCATDALSTGYTG-NFDSVT	TVPASAELSMQRRGR
GBV-C gt4	FKEALLFIPTGPGKSIR-VP	HKVLILNPSVATVRAM	VVICDECH	VLYATATPP	LVFCHSKAE	VVCATDALSTGYTG-NFDSVT	TVPASAELSMQRRGR
GBV-C gt5	FKEAPLFMPTGAGKSTR-VP	HKVLILNPSVATVRAM	VVICDECH	VLYATATPP	LVFCHSKAE	VVCATDALSTGYTG-NFDSVT	TVPASAELSMQRRGR
GBV-Ccpz	FKEAPLFLPTGSGKSTR-IP	HKVLILNPSVATVRKM	VVICDECH	VLYATATPP	LVFCHSKAE	VVCATDALSTGYSG-NFDSVT	TVPASAELSMQRRGR
GBV-D	FVEAPLFVPTGSGKSTK-IP	HNVLVINPSVATTMAM	VVVCDEAH	VLYATATPA	LVFCHSKEL	VVVATDALMTGYTG-DFDTVT	SGPATADVRMQRRGR
HCV gt1	VAHL--HAPTGSGKSTK-VP	YKVLVLNPSVAATLGF	IIICDECH	VVLATATPP	LIFCHSKKK	VVVATDALMTGYTG-DFDSVI	TLPQDAVSRTQRRGR
HCV gt2	VGYL--HAPTGSGKSTK-VP	YKVLVLNPSVAATLGF	IIICDECH	TVLATATPP	LIFCHSKKK	VVVATDALMTGYTG-DFDSVI	TVPQDAVSRSQRRGR
HCV gt3	VGYL--HAPTGSGKSTK-VP	YNVLVLNPSVAATLGF	VIICDECH	TVLATATPP	LIFCHSKKK	VVCATDALMTGFTG-DFDSVI	TAPQDAVSRSQRRGR
HCV gt4	VAHL--HAPTGSGKSTK-VP	YKVLVLNPSVAATLGF	IIICDECY	TVLATATPP	LIFCHSKKK	VVCATDALMTGFTG-DFDSVI	TVPQDAVSRSQRRGR
HCV gt5	VGHL--HAPTGSGKSTK-VP	YKVLVLNPSVAATFGF	VIICDECH	VVLATAIPP	LIFCHSKKK	VVCSTDALMTGFTG-DFDSVI	TVPQDAVSRSQRRGR
HCV gt6	VGYL--HAPTGSGKSTR-VP	YKVLVLNPSVAATLSF	IIICDECH	TVLPTATPP	LIFCHSKKK	VVCATDALMTGYTG-DFDSVI	TVPQDAVSRSQRRGR
DV2	LTIM--DLHPGAGKTKRYLP	LRTLILAPTRVVAAEM	LIIMDEAH	GIFMTATPP	VWFVPSIKA	FVDTTDISEMGANF-KAERVI	THSSAA-QRRGRIGR
JEV	MTVL--DLHPGSGKTRKILP	LRTAVLAPTRVVAAEM	LFVMDEAH	AIFMTATPP	VWFVASVKM	FVITTDISEMGANF-GASRVI	SPITSA-SAAQRRGR
WNV	ITVL--DLHPGAGKTRRILP	LRTAVLAPTRVVAAEM	LFVMDEAH	AIFMTATPP	VWFVPSVKM	FVITTDISEMGANF-KASRVI	SAVTAA-SAAQRRGR
YFV	TTIL--DFHPGAGKTRRFLP	LRTLVLAPTRVVLSEM	VIIMDEAH	TILMTATPP	AWFLPSIRA	FILATDIAEMGANL-CVERVL	SASSAA-QRRGRIGR
TBEV	ITVL--DMHPGSGKTHRVLP	LRTLVLAPTRVVLKEM	VAIMDEAH	LVLMTATPP	AWFVPSIAK	FVVTTDISEMGANL-DVSRVI	TTASAA-QRRGRVGR
HogCV	FRQI--TLATGAGKTTE-LP	KRVLVLIPLRAAAESV	FIFLDEYH	VVAMTATPA	LVFVPTRNM	-VVATNAIESGVTLPDLDVVV	TIGEQA-QRRGRVGR
Border DV	FKQI--TLATGAGKTTE-LP	KRVLVLIPLRAAAESV	YIFLDEYH	VVAMTATPA	LVFVPTRNM	VVVATNAIESGVTLPDLDVVV	TIGEQA-QRRGRVGR
BVDV (1)	FKQI--TLATGAGKTTE-LP	KRVLVLIPLRAAAESV	YIFLDEYH	VVAMTATPA	LVFVPTRNM	VIVATNAIESGVTLPDLDTVI	TVGEQA-QRRGRVGR
BVDV (2)	FKQI--TLATGAGKTTE-LP	KRVLVLIPLRAAAESV	YIFLDEYH	VVAMTATPA	LVFVPTRNM	VVVTTNAIESGVTLPDLDTVV	TIGEQA-QRRGRVGR
Bat Flavi	FVL-----KCGAGKTRGVIK	VKVLVLVPTRVVANEA	LIIVDESH	VMYLTATGY	VYFVASGPE	LILTTNISEMGANY-NADIVM	TVCTRA-SKIQRRGR

RdRp	Motif I	Motif II	Motif III	Motif IV	Motif V	Motif VI	Motif VII	Motif VIII
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GBV-Amys	VTKREV	RKPPRFIVYP-PLDFRI--AEKM-ILGDPGIV	LFQYTPNQRVKLL	VDATCFDSSID	RKCRSSGVLTTSSANSITCYIKVKAA-C	FFIAGDDCLI	DTAECCSAY	WWLSTDMRKPLAR
GBV-Atri	VTKREV	RKPPRFICFP-PLDFRI--AEKM-ILGDPGLV	LFQYTPNQRVKLM	VDATCFDSSID	RKCRSSGVLTTSSANSITCYIKVKAA-A	FLIAGDDCVI	DTAESCSSY	WWLSTDMRKPLAR
GBV-Alab	VTKREV	RKPPRFIVFP-PLDFRI--AEKM-ILGDPGIV	LFQYTPNQRVKAL	VDATCFDSSID	RACRSSGVLTTSSANSXTCYIKXXAA-X	FXIAGDDCLI	DTAECCSAY	WWLSTDMRKPLAR
GBV-A	VTKREV	RKPPRFIVFP-PLDFRI--AEKM-ILGDPGIV	LFQYTPNQRVKAL	VDATCFDSSID	RQCRSSGVLTTSSANSITCYIKVSAA-C	FFIAGDDCLI	DTAECCSAY	WWLSTDMRKPLAR
GBV-B	VPKEEV	KKPPRLISYP-HLEMRC--VEKMYYGQVAPDV	GFVDPRTRVKRLL	CDTVCFDSTIT	RRCRSSGVYTTSSSNSLTCWLKVNAA-A	FLICGDDCTV	EELTSCSSN	YFLTRDPRIPLGR
GBV-C gt1	TVKKEV	EKAPRLIVFP-PLDFRI--AEKL-ILGDPGRV	AFQYTPNQRVKKM	VDATCFDSSIT	RYCRSSGVLTTSASNCLTCYIKVKAA-C	LLIAGDDCLI	DTAPFCSTW	FFLTTDFRRPLAR
GBV-C gt2	TVKKEV	EKAPRLIVFP-PLDFRI--AEKL-ILGDPGRV	AFQYTPNQRVREM	VDATCFDSSIT	RYCRSSGVLTTSASNCLTCYIKVKAA-C	LLIAGDDGLI	DTAPFCSTW	FFLTTDFRRPLAR
GBV-C gt3	TVKKEV	EKAPRLIVFP-PLDFRI--AEKL-ILGDPGRV	AFQYTPNQRVKEM	VDATCFDSSIT	RYCRSSGVLTTSASNCLTCYIKVKAA-C	LLIAGDDCLI	DTAPFCSTW	FFLTTDFRRPLAR
GBV-C gt4	TVKKEV	EKAPRLIVFP-PLDFRI--AEKL-ILGDPGRV	AFQYTPNQRVKEM	VDTTCFDSSIT	RYCRSSGVLTTSASNCLTCYIKVKAA-C	LLIAGDDCLI	DTAPFCSTW	FFLTTDFRRPLAR
GBV-C gt5	TVKKEV	EKAPRLIVFP-PLDFRI--AEKL-ILGDPGRV	AFQYTPNQRVREM	VDATCFDSSIT	RYCRSSGVLTTSASNCLTCYIKVKAA-C	LLIAGDDCLI	DTAPFCSTW	FFLTTDFRRPLAR
GBV-Ctrog	TVKKEV	EKAPRLIVFP-PLDFRV--AEKM-IMGDPGRV	AFQYTPNQRVKKM	VDAKCFDSSIN	RFCRSSGVLTTSSSNCITCYIKVSAA-C	LLIAGDDCLI	DTAPFCSTW	FFLTTDFRRPLAR
GBV-D	VTKDRK	LKPPRLICYP-SLEFRV--AEKM-ILGDPSVV	GFQYTPVQRVGFL	VDAICFDSCIT	RACRASGVLTTSSSNCISSFLKVSAA-C	FLIHGDDVMI	DTAQSCSAW	YFLSTDFRRVLAR
HCV gt1	MAKNEV	RKPARLIVYP-DLGVRV--CEKM-ALYDVTQK	GFQYSPNQRVEYL	YDTRCFDSTVT	RRCRASGVLPTSMGNTLTCYLKAQAA-C	MLVCGDDLVV	ELITSCSSN	YYLTRDCTTPLAR
HCV gt2	MAKNEV	KKPARLIVYP-DLGVRV--CEKM-ALYDITQK	GFQYSPAQRVEYL	YDTRCFDSTVT	RRCRASGVLTTSMGNTITCYVKALAA-C	MLVCGDDLVV	ELITSCSSN	YYLTRDPTTPLAR
HCV gt3	MAKNEV	RKPARLIVYP-DLGVRV--CEKR-ALYDVIQK	GFQYSPQQRVERL	YDTRCFDSTVT	RRCRASGVLPTSFGNTITCYIKATAA-A	FLVCGDDLVV	ELITSCSSN	YYLTRDATTPLAR
HCV gt4	MAKNEV	KKPARLIVYP-DLGVRV--CEKR-ALYDVAQK	GFQYSPAQRVDFL	YDTRCFDSTIT	RRCRASGVFTTSMGNTMTCYIKALAS-C	LLVCGDDLVA	ELVTSCSSN	YYLTRDPQVPLAK
HCV gt5	MAKNEV	RKPARLIVFP-DLGVRV--CEKM-ALYDVVTK	GFQYSPGQRVEFL	YDTRCFDSTVT	RRCRASGVLTTSCGNTLTCYIKARAA-C	MLVCGDDLVV	ELITSCSSN	YYLTRDPTTPLAR
HCV gt6	MAKNEV	RKPARLIVYP-DLGSRV--CEKR-ALHDVIKK	GFQYSPAQRVEFL	YDTRCFDSTVT	RRCRATGVYTTSFGNTLTCYLKATAA-I	MLVCGDDLVV	ELITSCSSN	YYLTRDPETPLAR
DV2	MGKREK	AKGSRAIWYMW-LGARFL-EFEA-LGFLNEDH	VEGEGLHKLGYIL	DDTAGWDTRIT	RDQRGSGQVGTYGLNTFTNMGAQLIRQM	MAISGDDCVV	TQVPFCSHH	VVPCRNQDELIGR
JEV	MGKREK	AKGSRAIWFMW-LGARYL-EFEA-LGFLNEDH	VEGSGVQKLGYIL	DDTAGWDTRIT	EDQRGSGQVVTYALNTFTNIAVQLVRLM	MAISGDDCVV	QQVPFCSNH	VVPCRGQDELIGR
WNV	MGKREK	AKGSRAIWYMW-LGARYL-EFEA-LGFLNEDH	VEGIGLQYLGYVI	DDTAGWDTRIT	RDQRGSGQVVTYALNTITNLKVQLIRMA	MAVSGDDCVV	ENVPFCSHH	VVPCREQDELIGR
YFV	MGKREK	AKGSRAIWYMW-LGSRFL-EFEA-LGFLNEDH	VEGISLNYLGWHL	DDTAGWDTKVT	RDQRGSGQVVTYALNTLTNIKVQLIRMM	MLVSGDDCVV	EEVPFCSHH	VVPCRDQDELVGR
TBEV	MGKREK	AKGSRAIWFMWLGARFLEFEALGFLNEDHWLG	LGLQKLGYILREV	DDTAGWDTRIT	EDQRGSGQVVTYALNTFTNLAVQLVRMM	MAVSGDDCVV	QQVPFCSNH	VVPCRGQDELVGR
HogCV	IPKNEK	EKKPRVIQYP-EAKTRL--AITK-VMYKWVKQ	EGKTPLFQIFDKV	FDTKAWDTQVT	KGQRGSGQPDTSAGNSMLNVLTMIYAFC	IHVCGDDGFL	DDIEFCSHT	YMPGRNTTTILAK
Border DV	IPKNEK	EKKPRVIQYP-EAKVRL--AITK-VMYKWVKQ	EGKTPLFEIFDKV	FDTKAWDTQVT	RGQRGSGQPDTSAGNSMLNVLTMIYAFC	IHVCGDDGFL	EDIEFCSHT	YMPGRNTATILAK
BVDV (1)	IPKNEK	EKRPRVIQYP-EAKTRL--AITK-VMYNWVKQ	EGKTPLFNIFDKV	FDTKAWDTQVT	NGQRGSGQPDTSAGNSMLNVLTMMYGFC	IHVCGDDGFL	EDIEFCSHT	HMAGRDTAVILSK
BVDV (2)	IPKNEK	EKKPRVIQYP-DAKVRL--AIAK-VMYKWVKQ	EGKTPLFDIFNKV	FDTKAWDTQVT	NGQRGSGQPDTSAGNSMLNVLTMIYAFC	IHVCGDDGFL	EDIEFCSHT	YMAGRSTATILAK
Bat Flavi	IGKKEK	PKGSRLIMYLD-LVERFL-EHKY-LGFLNSDH	SAVSPYEYPRMIV	DDTAGWDTRLH	KGQRCSGTVVTYSMNTITNTVVQMMRMQ	MMVSGDDCLL	KDWKNISFC	WTLGKNEAEIIGK

Supplementary Fig. S1. Alignment of the amino acid sequence of conserved regions of *Flaviviridae* helicase. Amino acid alignment of the six conserved motifs within the helicase coding region for representative virus species are shown with their GenBank accession numbers in parentheses: GB virus A (GBV-A$_{\mathrm{myx}}$) (AF023424), GBV-A$_{\mathrm{tri}}$ (AF023425), GBV-A$_{\mathrm{lab}}$ (U92241), GBV-A (U22303), GBV-B (NC_001655), GBV-C genotype (gt) 1 (GBV-C gt1) (AB003291), GBV-C gt2 (AF081782), GBV-C gt3 (D90601), GBV-C gt4 (Ab021287), GBV-C gt5 (AY949771), GBV-C$_{\mathrm{tro}}$ (AF70476), GBV-D (GU566735), hepatitis C virus (HCV) gt 1 (M62321), HCV gt 2 (AB047639), HCV gt 3 (D17763), HCV gt 4 (Y11604), HCV gt 5 (Y13184), HCV gt 6 (Y12083), dengue virus serotype 2 (DV2) (AF359579), Japanese encephalitis virus (JEV) (I78128), West Nile virus (WNV) (DQ211652), yellow fever virus (YFV) (U21055), tick-borne encephalitis virus (TBEV) (I40361), classical swine fever virus (HogCV) (D49532), border disease virus (border DV) (U70263), bovine viral diarrhea virus (group 1) (BVDV [1]) (NC_001461), BVDV (2) (U18059), and Entebbe bat virus (Bat; NC_003996). Sequences were manually aligned using published helicase alignments of Koonin, as described in the text. *conserved amino acids.

Supplementary Fig. S2. Alignment of the amino acid sequence of conserved regions of *Flaviviridae* RdRp. Amino acid alignment of conserved motifs within the RdRp coding region. *Flaviviridae* species and GenBank numbers are the same as listed in Fig. 2. Sequences were manually aligned using published RdRp alignments of Koonin, as described in the text. * conserved amino acids.

Stapleton, J. T., Foung, S., Muerhoff, A. S., Bukh, J. and Simmonds, P. (2011). The GB viruses: a review and proposed classification of GBV-A, GBV-C (HGV), and GBV-D in genus *Pegivirus* within the family *Flaviviridae*. *J Gen Virol* **92**, 233–246.