Supplemental Information

Distribution and structural diversity of Type IV internal ribosome entry sites

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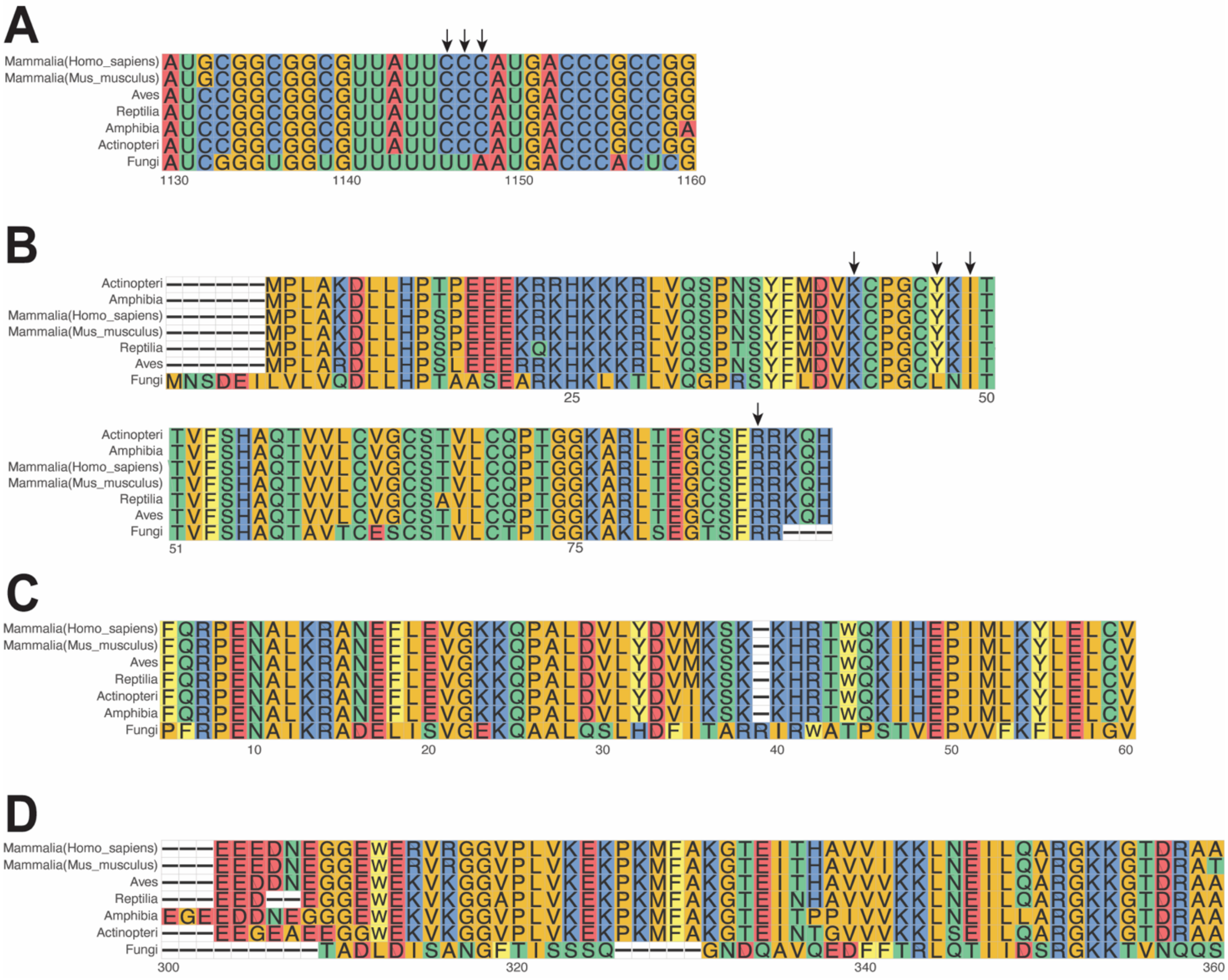


Figure S1. Conservation of key rRNA and translation initiation factor sequences in vertebrates. **(A)** Alignment of 18S rRNA sequences focused on expansion segment 7 across representative vertebrate examples. Arrows show conserved 'CCC' motif. Nucleotides are colored by identity. **(B)** Alignment of ribosomal protein eS27 across representative vertebrate examples. Arrows show residues that are implicated in HCV IRES interaction (*Quade. et al. 2015*). **(C)** Alignment of eIF3A N-terminal region, containing helix-loop-helix motif that is suggested to directly bind the HCV IRES (Sun et al. 2013). **(D)** Alignment of eIF3C helix-loop-helix motif that is suggested to directly bind the HCV IRES (Sun et al. 2013).

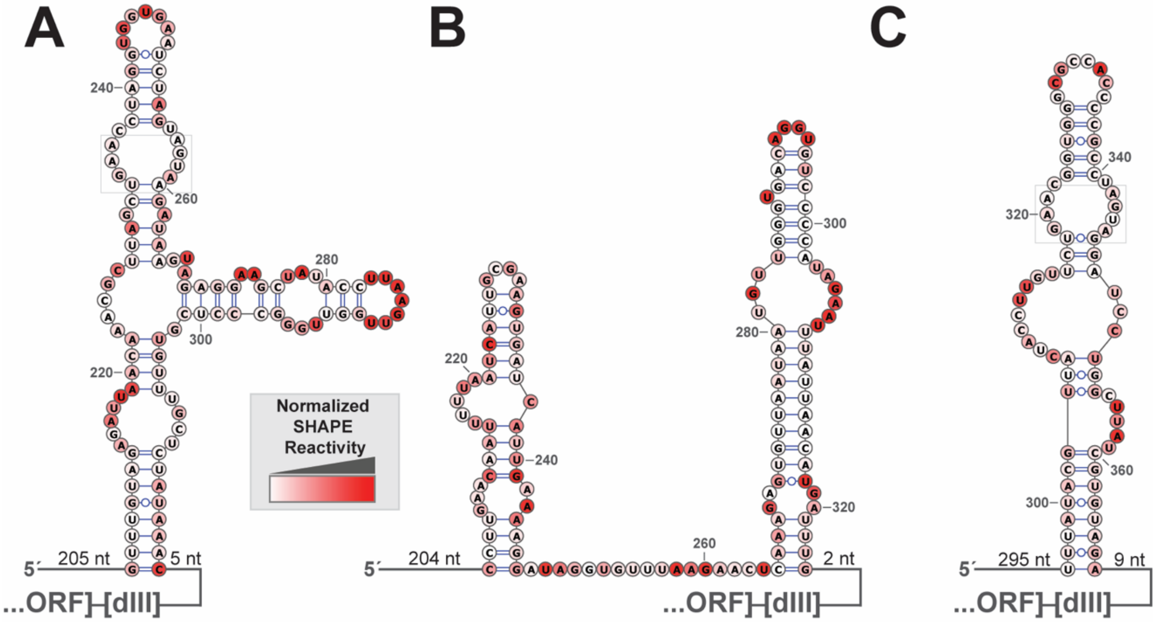


Figure S2. Chemical probing of domain II. Chemical probing results mapped onto secondary structure of domain II from different IRESs: **(A)** hepatovirus C (NC_038313:206-318), **(B)** limnipivirus C (NC_039212:205-324), and **(C)** megrivirus E (NC_039004:295-366). The color of each nucleotide indicates how reactive it was to the probing agent, from low reactivity (white) to high (red). Domain II secondary structure for hepatovirus C and limnipivirus C was predicted with chemical probing data and sequence using the RNAprobing Webserver (Hofacker 2003; Deigan et al. 2009; Zarringhalam et al. 2012). Domain II secondary structure for megrivirus E was pulled directly from the alignment. The number of nucleotides upstream of domain II and between domain II and domain III are indicated. Loop E motifs are boxed in gray.

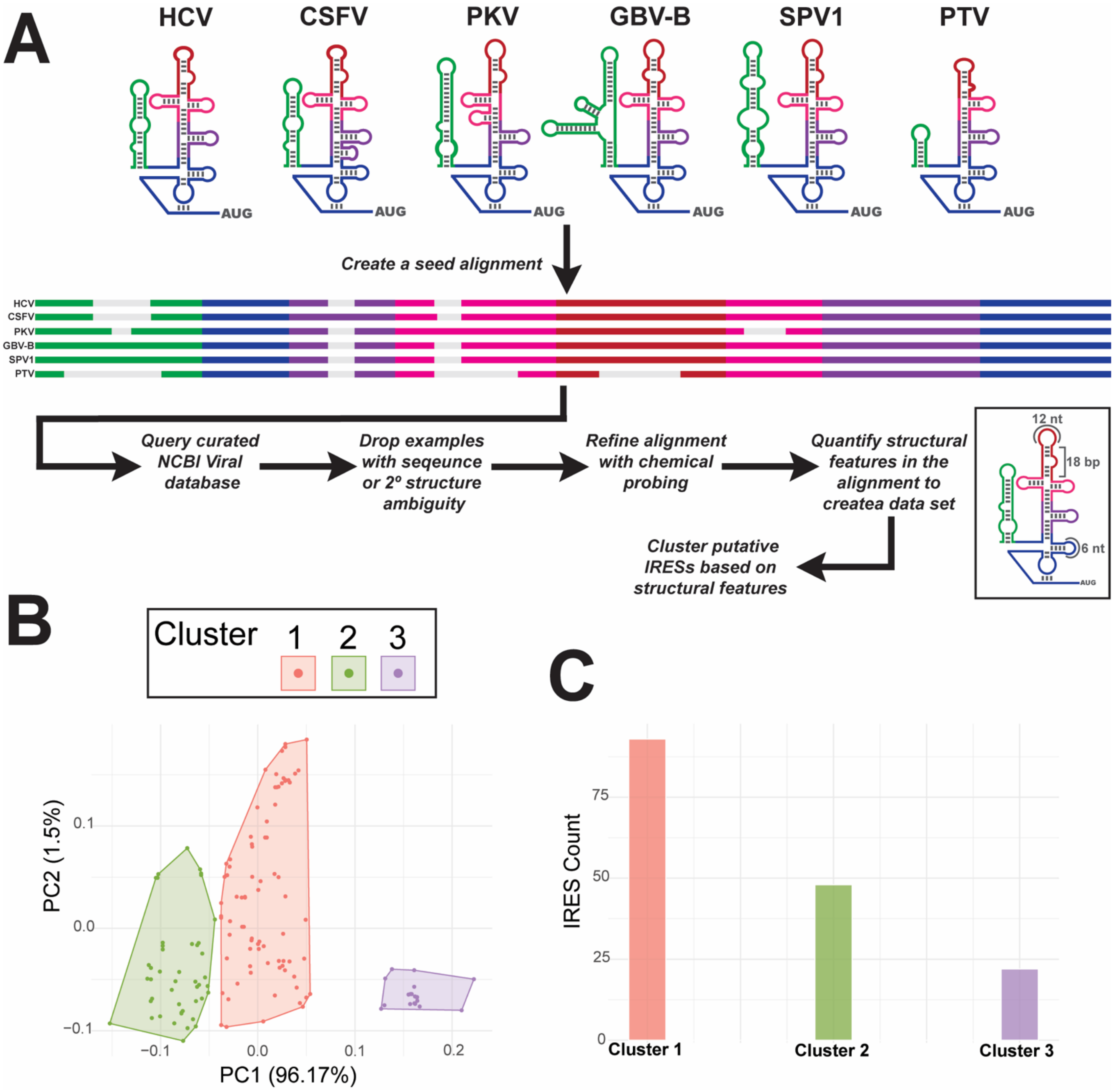


Figure S3. Sub-grouping of structurally similar Type IV IRESs using cluster analysis. (A) Workflow showing generation of the data set for analysis. Six Type IV IRESs were aligned based on secondary structure and important sequence features to create a seed alignment. The seed was used to search a curated NCBI database, limited to roughly one example per viral species. Of the 176 hits, 13 were dropped due to structure or sequence ambiguity. Chemical probing data on a diverse selection of putative IRESs was used to further refine the alignment. Subdomain features (i.e. stems and loops) were quantified using custom python scripts, which counted the number of base pairs or nucleotides present in a feature for each IRES. This dataset detailing structural features for each IRES was then used for clustering analysis. **(B)** Principal component analysis (PCA) plot of clustering results. Data was clustered into 3 groups using the CLARA algorithm (detailed in Methods). The first two principal components (PC1 and PC2), computed via PCA, are shown on the x- and y-axes. Each point represents an individual data observation, and colors indicate the assigned cluster membership. Convex hulls enclose points belonging to the same cluster for visualization purposes. **(C)** Graphical representation of the number of IRESs in each subgroup.

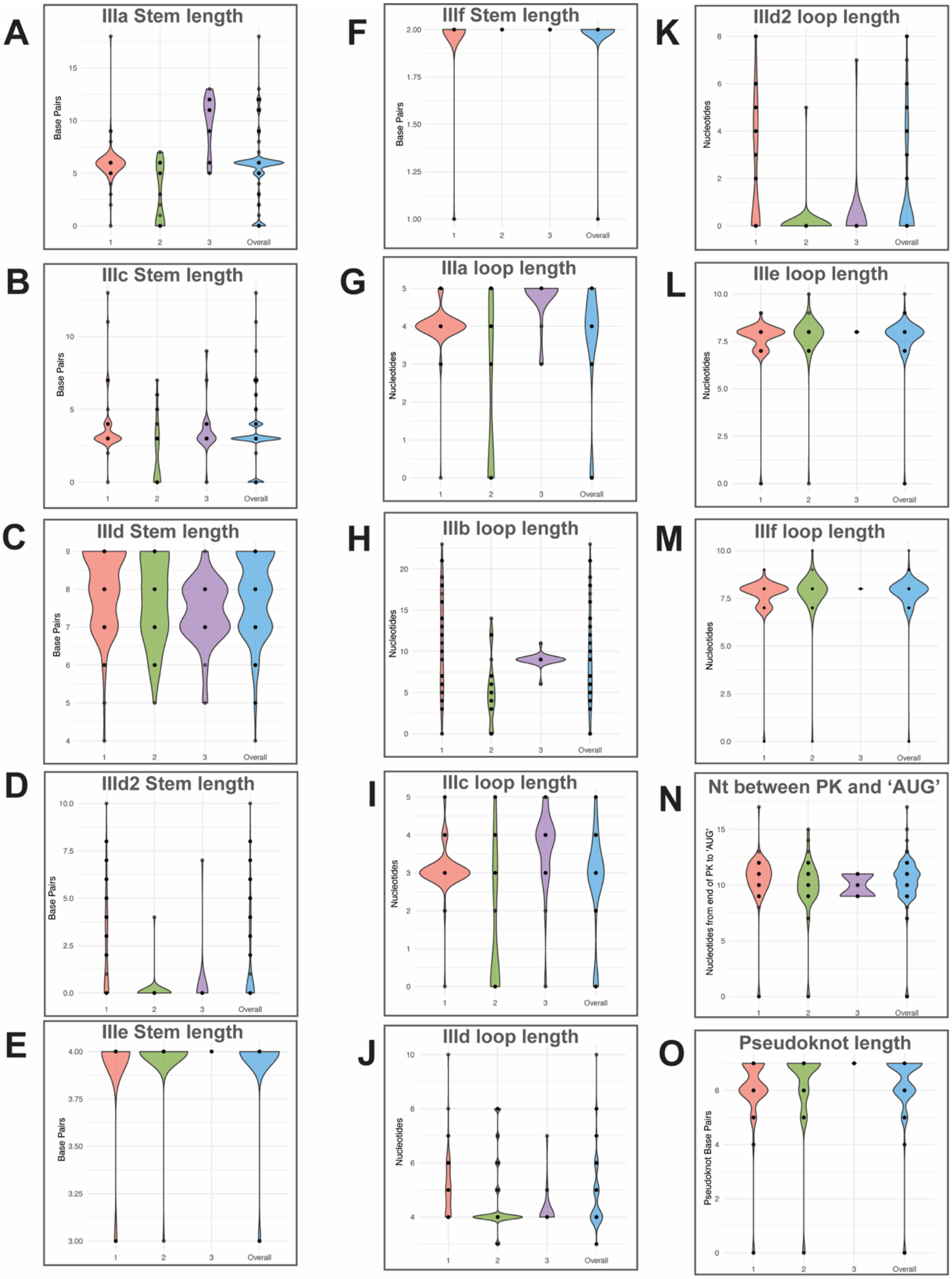


Figure S4. Additional data on sub-groups secondary structural features. Violin plots of the distribution of sub-domain stem lengths as measured in base pairs: **(A)** IIIa, **(B)** IIIc, **(C)** IIId, **(D)** IIId2, **(E)** IIIe, **(F)** IIIf. Violin plots of distribution of sub-domain loop lengths as measured in nucleotides: **(G)** IIIa, **(H)** IIIb, **(I)** IIIc, **(J)** IIId, **(K)** IIId2, **(L)** IIIe, **(M)** IIIf. **(N)** Violin plot of distribution of nucleotides between the end of the pseudoknot and the start codon (AUG). **(O)** Violin plot of distribution of pseudoknot length as measured in base pairs.

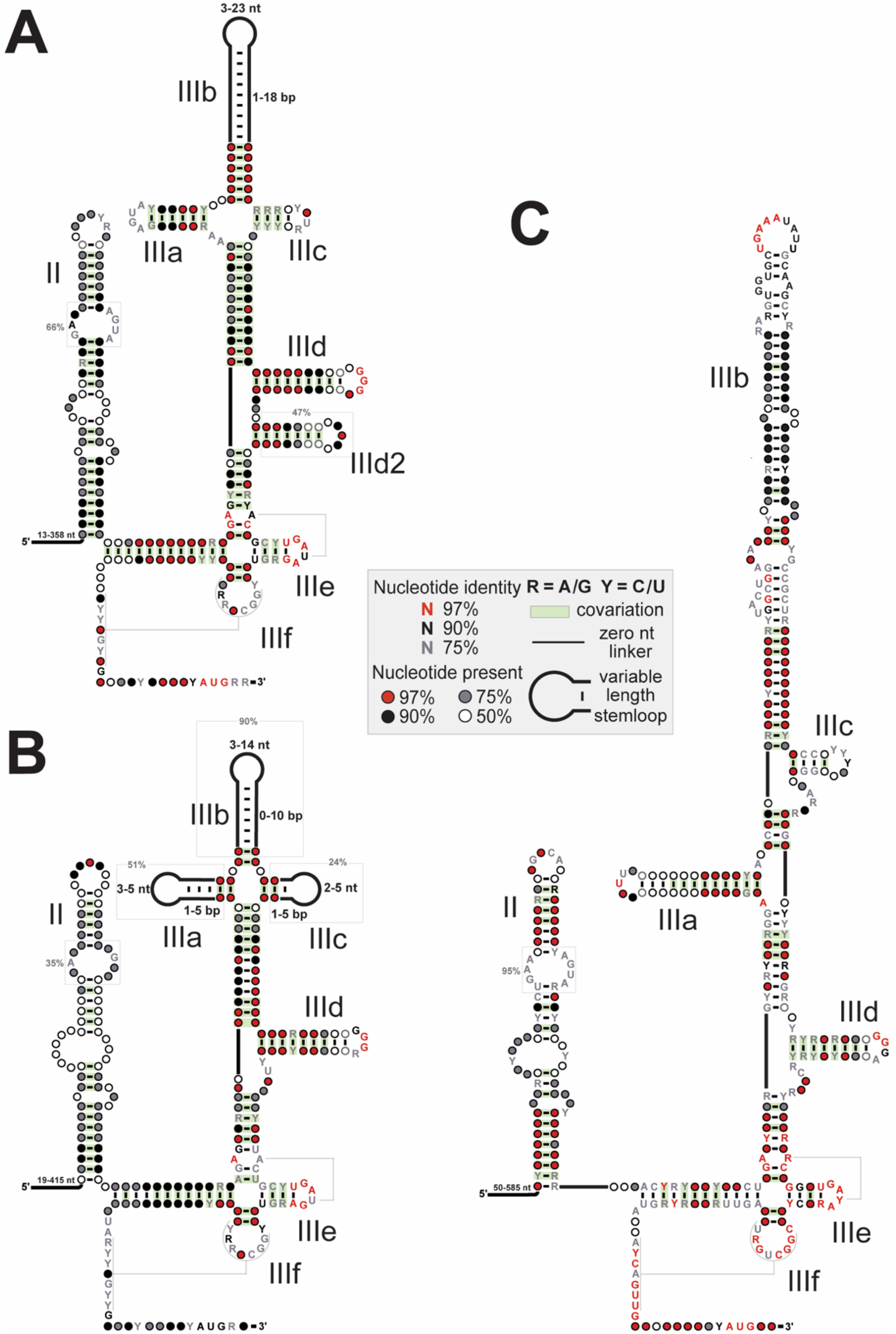
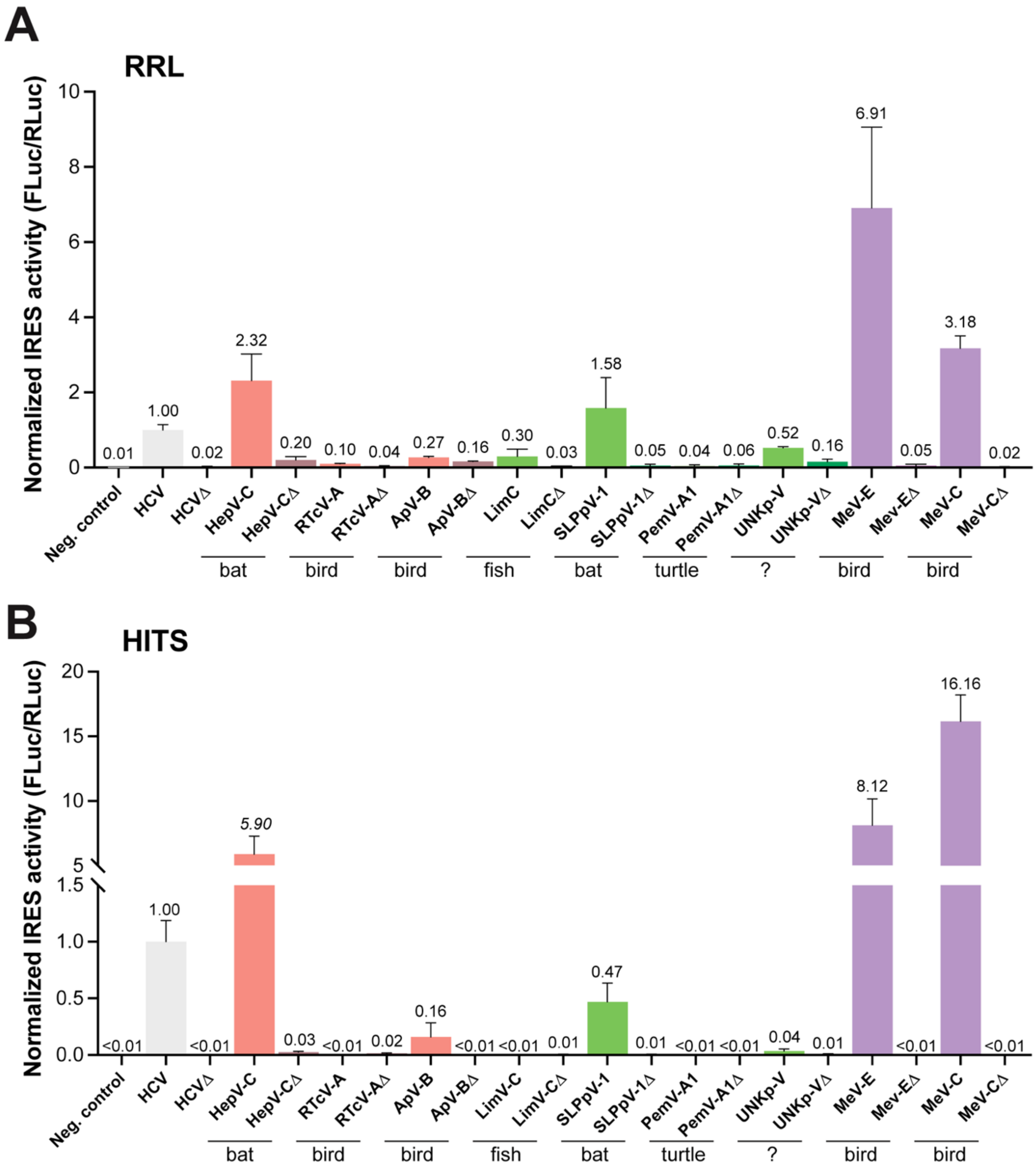
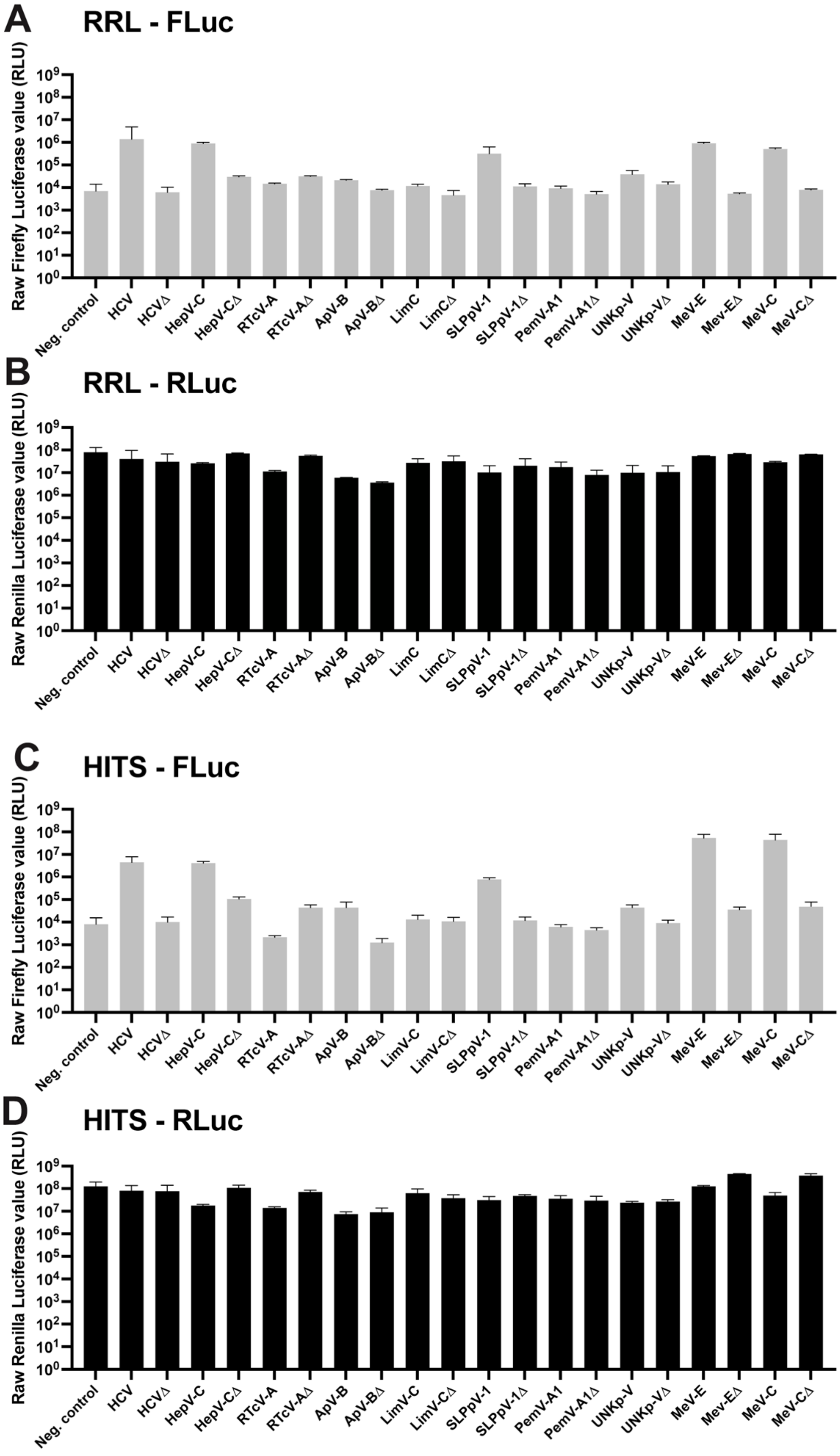


Figure S5. Cluster consensus/covariation models. Sequence and secondary structure consensus models for **(A)** Cluster 1 IRESs, **(B)** Cluster 2 IRESs, and **(C)** Cluster 3 IRESs, detailing conserved structure, as determined by statistically significant covariation, and sequence features. Models generated using R-scape (Rivas 2020; Weinberg and Breaker 2011).



Supplemental Figure S6. Functional data on putative IRESs in *in vitro* mammalian systems. *In vitro* translation assays of IRES activity using dual luciferase constructs in rabbit reticulocyte lysate (RRL, **A**) and human *in vitro* translation system (HITS, **B**). Dual luciferase constructs are as described in Figure 2. The graph contains the results of translation assays of both wild-type and mutant (Δ) IRES sequences. Ratios were generated by FLuc signal /RLuc signal. Ratios were then normalized to wild type HCV IRES. The IRESs shown are as follows: hepatovirus C (HepV-C), Ruddy turnstone calicivirus A (RTcV-A, MH453861:200-581), avocet picornavirus B MW14 (ApV-B, MH453809:95-458), limnipivirus C (LimV-C), sapelo-like bat picornavirus 1 (SLPpV-1, HQ595341:16-350), Pemapivirus A1 (PemV-A1, MG600106:394-713), picornaviridae sp. isolate wftcra74pic2 (UNKp-V, MT138373:249-558), megrivirus E (MeV-E), megrivirus C (MeV-C, HM751199:50-506). The color of the bars indicates the Cluster each IRES belongs to; pink for Cluster 1, green for cluster 2, purple for Cluster 3.



Supplemental Figure S7. Raw luciferase values for in vitro translation assays. (A&C) Raw Firefly and **(B&D)** *Renilla* luciferase values for each construct tested in the RRL and HITS systems. Virus names and genomic information of IRESs are as described above and in Figure 2.

Accession number	**Genomic Coordinates**	**Reason dropped**
NC_021153	87-429	ambiguious SS
MF352427	236-560	ambiguious SS
NC_038314	237-576	ambiguious SS
KY312542	37-275	ambiguious SS
MH824542	39-356	ambiguious SS
MT210612	145-541	ambiguious seq
MT210615	6-342	ambiguious seq
MT210611	95-491	ambiguious seq
MT210606	11-335	ambiguious seq
MT210613	71-397	ambiguious seq
MT210607	168-491	ambiguious seq
NC_012812	58-410	ambiguious seq

Table S1. Sequences discarded from the covariation and clustering analysis. Of the 176 putative IRESs identified, 13 were dropped from further analysis due to ambiguous secondary structure (ambiguous SS) or degenerate sequencing (ambiguous seq).

	Flaviviridae	*Picornaviridae*	*Caliciviridae*	*Picobirnaviridae*	*Unassigned*	***Total***
Cluster 1	56	35	1	1	-	93
Cluster 2	3	33	-	-	12	48
Cluster 3	-	21	-	-	1	22

	Mammalia	*Aves*	*Reptilia*	*Aamphibia*	*Actinopteri*	*Insect*	*Unknown*	***Total***
Cluster 1	81	9	-	-	-	1	2	93
Cluster 2	23	14	5	1	5	-	-	48
Cluster 3	-	21	1	-	-	-	-	22

Table S2. Taxonomic information on viral family and host class between Clusters.

Table S3. DNA oligonucleotide sequences used in this study.

DNA sequence	Description
GGGCCCTACGGGCCCTACTCGAGCTAAAGCTTGGCAATCCGGTACTGTTGGTAAAGCCACCGCCAGCCCCCTGATGGGGGCGACACTCCACCATGAATCACTCCCCTGTGAGGAACTACTGTCTTCACGCAGAAAGCGTCTAGCCATGGCGTTAGTATGAGTGTCGTGCAGCCTCCAGGACCCCCCCTCCCGGGAGAGCCATAGTGGTCTGCGGAACCGGTGAGTACACCGGAATTGCCAGGACGACCGGGTCCTTTCTTGGATAAACCCGCTCAATGCCTGGAGATTTGGGCGTGCCCCCGCAAGACTGCTAGCCGAGTAGTGTTGGGTCGCGAAAGGCCTTGTGGTACTGCCTGATAGGGTGCTTGCGAGTGCCCCGGGAGGTCTCGTAGACCGTGCACCATGAGCACGAATCCTAAACCTCAAAGAAAAACCAAACGTAACACCAGATCTGAGGCACGGCATAAGC	Gene block (dsDNA) fragment including the WT HCV IRES sequence with flanking restriction sites for cloning into pSGDLuc v3.0 to create dual luciferase reporter mRNAs
GGGCCCTACTCGAGCTAAAGCTTGGCAATCCGGTACTGTTGGTAAAGCCACCGGACTAATTCCTATGTTTATACGTTACTACCTTGTTCTGAACGGTGGGCGCCACCCCGCCTAGTAGGATCCTGGCTTATCGTGTAGACCTCTAGGGACCACATTAGCTAGAGTGTAGGCTGCTATGGATGGAGTAGTGACCCCTTTTTGGGTATCACTCTCTAAGACTCCGGAATGTGTCATAGTACGCTGGAAATCCTTACTTGTTTTTCCATGAGGGGGAGGTGGTGCTGAAATATTGCAAGCCACCCCTCGGTTAAAACAGTTTGGTGCCGCTTATGCCATATTACCGCCCCTTGTAGTTGGGCTGTTTTTGCAGCTCCGGGTTAGTAGAGTACCATAGTGGACGCGGTGTTGGGAATCACCGCCTTGGCTGCACACTGCTTGATAGAGCTGCGGCTGGTCAAGCTAATTGTGGTATAACCAGTTGATTTGGCATATGGATTCTAGACTTACCTTCTTACAAGATTTTCTTAAGGAACATAGATCTGAGGCACGGCATAAGC	Gene block (dsDNA) fragment including the WT MeV-E IRES sequence with flanking restriction sites for cloning into pSGDLuc v3.0 to create dual luciferase reporter mRNAs
GGGCCCTACGGGCCCTACTCGAGCTAAAGCTTGGCAATCCGGTACTGTTGGTAAAGCCACCTCGTATGCTATCTCCTTGAACAATTTTTAATCATTGCGAAGTGATCATTGAAAAGGATAGGTGTTTAAGAACTCAAAGAGTGTTAATAATGTTGGGTGACAGGTGTCCCCATAGAATTTATTAACATGATTTGGACTGGTTATCTAGTAAGAAGAACCATCGAACGCACGAGCGAGCATTGCTTGCGGGGCAGTTACCCTGCGTCGATGTAAGTGTGTACCGGGGGGTGCACATGTTGATTCTTTATGGCCTGATAGGGTGCGTCATTCGCGCCTAGATAATTAGTATAATGCGAATGGAATAAATTTACATGGCTTCTATAATTGAAAATTTGACCACAACTTTTGCATCATCAATGCTGGGAACAGCTGAGGATGCTGTCAGAAGATCTGAGGCACGGCATAAGC	Gene block (dsDNA) fragment including the WT LimV-C IRES sequence with flanking restriction sites for cloning into pSGDLuc v3.0 to create dual luciferase reporter mRNAs
GGGCCCTACGGGCCCTACTCGAGCTAAAGCTTGGCAATCCGGTACTGTTGGTAAAGCCACCTCCTACAAATGCACATGAAGAACAGTTTGTAGAGATTAACAAACGC	Gene block (dsDNA) fragment including the HepV-C IRES sequence with flanking restriction sites for cloning into pSGDLuc

TTAGCTGAACCTAGGTGGTGAATCTAGTAGTAAGATAAGTAGAGGAAGCTATACCTTAAGTTGGTTGGGCCCTCGTGTTTGCTCTATAAACAAAACCAAGTGAGTAGAGTGGATGAACAGTACTAAATCCCTGAGTACAGGGAACCTCACAGGTGTGATACACTTATGTCTATGTGACCTGGTTGGAGGTTGGGCGTGCCCTATGATACTGGAGTGGGAGATCTTTTGGGGAACCCACGTTTTCACACTGCCTGATAGGGTCTTGCCGAGAGACTCACTTGTTTCGGCTGTACTTGTAACATGGAGAATAAAAATAAAGGAATTTTTCAAACTGTTGGAGAGAGTTTGGATGGAATTTTGACTTTGGCTGATAGAAGATCTGAGGCACGGCATAAGC	v3.0 to create dual luciferase reporter mRNAs
GGGCCCTACGGGCCCTACTCGAGCTAAAGCTTGGCAATCCGGTACTGTTGGTAAAGCCACCTCGTATGCTATCTCCTTGAACAATTTTTAATCATTGCGAAGTGATCATTGAAAAGGATAGGTGTTTAAGAACTCAAAGAGTGTTAATAATGTTGGGTGACAGGTGTCCCCATAGAATTTATTAACATGATTTGGACTGGTTATCTAGTAAGAAGAACCATCGAACGCACGAGCGAGCATTGCTTGCGGGGCAGTTACCCTGCGTCGATGTAAGTGTGTACCGCCCCGTGCACATGTTGATTCTTTATGGCCTGATAGGGTGCGTCATTCGCGCCTAGATAATTAGTATAATGCGAATGGAATAAATTTACATGGCTTCTATAATTGAAAATTTGACCACAACTTTTGCATCATCAATGCTGGGAACAGCTGAGGATGCTGTCAGAAGATCTGAGGCACGGCATAAGC	Gene block (dsDNA) fragment including the LimV-C ΔGGG IRES sequence with flanking restriction sites for cloning into pSGDLuc v3.0 to create dual luciferase reporter mRNAs
GGGCCCTACGGGCCCTACTCGAGCTAAAGCTTGGCAATCCGGTACTGTTGGTAAAGCCACCTCCTACAAATGCACATGAAGAACAGTTTGTAGAGATTAACAAACGCTTAGCTGAACCTAGGTGGTGAATCTAGTAGTAAGATAAGTAGAGGAAGCTATACCTTAAGTTGGTTGGGCCCTCGTGTTTGCTCTATAAACAAAACCAAGTGAGTAGAGTGGATGAACAGTACTAAATCCCTGAGTACAGGGAACCTCACAGGTGTGATACACTTATGTCTATGTGACCTGGTTGGAGGTTGGGCGTGCCCTATGATACTGGAGTGGGAGATCTTTTCCCGAACCCACGTTTTCACACTGCCTGATAGGGTCTTGCCGAGAGACTCACTTGTTTCGGCTGTACTTGTAACATGGAGAATAAAAATAAAGGAATTTTTCAAACTGTTGGAGAGAGTTTGGATGGAATTTTGACTTTGGCTGATAGAAGATCTGAGGCACGGCATAAGC	Gene block (dsDNA) fragment including the HepV-C ΔGGG IRES sequence with flanking restriction sites for cloning into pSGDLuc v3.0 to create dual luciferase reporter mRNAs
GGGCCCTACGGGCCCTACTCGAGCTAAAGCTTGGCAATCCGGTACTGTTGGTAAAGCCACCGGGGAAATCTGGATCATGGGATTCCGCCTGCAGTACGGAAAAGTACCGAGTACTTAGTATGTTCTGTAGTGCCCCATGTGAAGGGACCGCCTGAGCTAGAGCTACGGGCTTGGAAGGTAAGGTAGGACGCCTTTTTGGCTATCCTACGATCGTCCGGCGCCGATTGCGTGGCCTCGTGCCCCTCTCCGGTTCCGCCCCGATGATGCAGGATTTCCGCTAGGGGTTTGTGACGCCTCCAGGTACGTGAGGATGGGAAGCCTCACCAATGCGTAGTGCTGGGTGACAGCCTGC	Gene block (dsDNA) fragment including the RTcV-A IRES sequence with flanking restriction sites for cloning into pSGDLuc v3.0 to create dual luciferase reporter mRNAs

CGGCTGGCGCAGTTCAGTGTGGTATAGCCAGTTGATTACCGCCATGGCTTGCTTTCGTTCAACTCTCTCAACCCCAAAAAACAACTCTGCAAGAAGATCTGAGGCACGGCATAAGC	
GGGCCCTACGGGCCCTACTCGAGCTAAAGCTTGGCAATCCGGTACTGTTGGTAAAGCCACCGGGGAAATCTGGATCATGGGATTCCGCCTGCAGTACGGAAAAGTACCGAGTACTTAGTATGTTCTGTAGTGCCCCATGTGAAGGGACCGCCTGAGCTAGAGCTACGGGCTTGGAAGGTAAGGTAGGACGCCTTTTTGGCTATCCTACGATCGTCCGGCGCCGATTGCGTGGCCTCGTGCCCCTCTCCGGTTCCGCCCCGATGATGCAGGATTTCCGCTAGGGGTTTGTGACGCCTCCAGGTACGTGAGGATCCCAAGCCTCACCAATGCGTAGTGCTGGGTGACAGCCTGCCGGCTGGCGCAGTTCAGTGTGGTATAGCCAGTTGATTACCGCCATGGCTTGCTTTCGTTCAACTCTCTCAACCCCAAAAAACAACTCTGCAAGAAGATCTGAGGCACGGCATAAGC	Gene block (dsDNA) fragment including the RTcV-A ΔGGG IRES sequence with flanking restriction sites for cloning into pSGDLuc v3.0 to create dual luciferase reporter mRNAs
GGGCCCTACGGGCCCTACTCGAGCTAAAGCTTGGCAATCCGGTACTGTTGGTAAAGCCACCGAATTCAGGAATTCTGGAACTGGGGGACTTCGCCCAAGCTGTGAACCGGATTACCGATGTAGCTCCTTGGGTGCCTCAGGGAAGGGACCAGTAGTTCTAGAGCACATGTGAAGCACTGACAAGGGGACTGCGTTGCATATCCCCGACCCCGGACTCTGTGTGATGATGCTGGATTGCCGCTAGCACAGAGAAACCACCCTTTTAGGGCTCGCAGGGCAGTGTGTAGCACCTAGTTTGGGAAAGCTAGGCTGGTGTGTACTGGATGATAGTCTCGCGGCGGATCGAGTTCTACTGGTATAATCCGTTGTATCTTATCATGGATTCTCGCTACCTGACCTCTGATGTAACTGAACTCACAAGCTACAGAAGATCTGAGGCACGGCATAAGC	Gene block (dsDNA) fragment including the ApV-B ΔGGG IRES sequence with flanking restriction sites for cloning into pSGDLuc v3.0 to create dual luciferase reporter mRNAs
GGGCCCTACGGGCCCTACTCGAGCTAAAGCTTGGCAATCCGGTACTGTTGGTAAAGCCACCGAATTCAGGAATTCTGGAACTGGGGGACTTCGCCCAAGCTGTGAACCGGATTACCGATGTAGCTCCTTGGGTGCCTCAGGGAAGGGACCAGTAGTTCTAGAGCACATGTGAAGCACTGACAAGGGGACTGCGTTGCATATCCCCGACCCCGGACTCTGTGTGATGATGCTGGATTGCCGCTAGCACAGAGAAACCACCCTTTTAGGGCTCGCAGGGCAGTGTGTAGCACCTAGTTTCCCAAAGCTAGGCTGGTGTGTACTGGATGATAGTCTCGCGGCGGATCGAGTTCTACTGGTATAATCCGTTGTATCTTATCATGGATTCTCGCTACCTGACCTCTGATGTAACTGAACTCACAAGCTACAGAAGATCTGAGGCACGGCATAAGC	Gene block (dsDNA) fragment including the ApV-B ΔGGG IRES sequence with flanking restriction sites for cloning into pSGDLuc v3.0 to create dual luciferase reporter mRNAs
GGGCCCTACGGGCCCTACTCGAGCTAAAGCTTGGCAATCCGGTACTGTTGGTAAAGCCACCGGAATAACGGCAATTGTTTGTATGTGGGAGGTCAAGCCTGCCTAGCCGATAACCTTTGACCGGGTGTGTAGGATAGAACAGGAACCCACTACAGGCGACAGGTTATGGTAGAGTGGATACCTAGCCAGGGGCAATGGGACTGCGTTGCAT	Gene block (dsDNA) fragment including the SLpV-1 IRES sequence with flanking restriction sites for cloning into pSGDLuc v3.0 to create dual luciferase reporter mRNAs

ATCCCTAATGAGCCATCGAGATTTCTCTGGCCATTACCCGGTGATGGTTGTGTGGGGGGGGCCCCACACACTAGATCCATACTGCCTGATAGGGTCGCGGCTGGCCGACCATAACCTGTATAGTCAGTTGACTTTGAATCATGGTTTTCTTCTTCAACTCCAACAAGACTACTGAGACTGCCCAAAGAAGATCTGAGGCACGGCATAAGC	
GGGCCCTACGGGCCCTACTCGAGCTAAAGCTTGGCAATCCGGTACTGTTGGTAAAGCCACCGGAATAACGGCAATTGTTTGTATGTGGGAGGTCAAGCCTGCCTAGCCGATAACCTTTGACCGGGTGTGTAGGATAGAACAGGAACCCACTACAGGCGACAGGTTATGGTAGAGTGGATACCTAGCCAGGGGCAATGGGACTGCGTTGCATATCCCTAATGAGCCATCGAGATTTCTCTGGCCATTACCCGGTGATGGTTGTGTGGGGCCCGCCCCACACACTAGATCCATACTGCCTGATAGGGTCGCGGCTGGCCGACCATAACCTGTATAGTCAGTTGACTTTGAATCATGGTTTTCTTCTTCAACTCCAACAAGACTACTGAGACTGCCCAA AGAAGATCTGAGGCACGGCATAAGC	Gene block (dsDNA) fragment including the SLpV-1 ∆GGG IRES sequence with flanking restriction sites for cloning into pSGDLuc v3.0 to create dual luciferase reporter mRNAs
GGGCCCTACGGGCCCTACTCGAGCTAAAGCTTGGCAATCCGGTACTGTTGGTAAAGCCACCAGTAGAAATCGCAATCTTATGTCATAGGGAACCCGTGGCCGATAACCGGTAACCAATCCGGTGTGTAGGACACGGAGTAAACTAACACTCTATGGTAAACATAAGGACTGATGCGAGAAGAGCAGAAGCTGTAGACTCGTTTGGGACCCAATTTGGTATCCCTTGCCTAGTAGCGCGAGCTATGGACTCCGGGCTCCACTCCCGGATTTGATTCTGTACTTCCTGATAGGGACGCGGATGGCCGTCTCGCATTAGTATAGTCATTTGCAACTTACATGGACTCTAATACTATTTCAAATGTAACCAACAAAAACATACTGAGAAGATCTGAGGCACGGCATAAGC	Gene block (dsDNA) fragment including the PemV-A1 ∆GGG IRES sequence with flanking restriction sites for cloning into pSGDLuc v3.0 to create dual luciferase reporter mRNAs
GGGCCCTACGGGCCCTACTCGAGCTAAAGCTTGGCAATCCGGTACTGTTGGTAAAGCCACCAAAGGTAAAAGGGTAGATTGCTACCACTTATGGCATGTGAACTGGATTACCAATGTAGCATGGACCCTCTAGGTGGGGAATGGCAATCGACTATCCTAGCTAGATTGGACCACTCATGGGCTTTGGGCGCGAATTACGCAGAACCTACCGACAACATGTCTGGAAACCCCATGAGTGGAGGGGAATTGGGAAAACCCCTTTGATCCAAACTGCCTGATAGGGTCGCGGCTGGCCGAGTTAGGATTAGTAGTCAGTAGAATTTAACATGGAAACTCTTGGTAACGTTGTGATGAATGAAGTAACAGAAGCTAGAAGATCTGAGGCACGGCATAAGC	Gene block (dsDNA) fragment including the UNKp-V IRES sequence with flanking restriction sites for cloning into pSGDLuc v3.0 to create dual luciferase reporter mRNAs
GGGCCCTACGGGCCCTACTCGAGCTAAAGCTTGGCAATCCGGTACTGTTGGTAAAGCCACCACACTCTCTCCACAAGTAGGCGCCGTGGAGTTCATGCTCGTTCTGAACCGGGAATGGCACAACCCGTAGTAGGATCTTGCCTCTGCCACGTGCACCTGCGCCTGTTGCTTCTAGACTATGGGCTGCTAAGGATGATCACAGACTCCTTTTTGGACAATCTGTGTCAAGTCTACTGTTACATCTGATGTACGCTGGAAATCCTCGTACTGGAGGCAATACCTG	Gene block (dsDNA) fragment including the MeV-C IRES sequence with flanking restriction sites for cloning into pSGDLuc v3.0 to create dual luciferase reporter mRNAs

GAGGTGGTGCTGAAATATTGCAAGCCACAGGTGTGTTTCTAGTCTTGGTGCCGCTATCAGATGTCGCACCCTCTACGGGCAAATGTAGAACCTTAGTGGACGCGTGAGATGGGAATCCACGCCTGGCCATAGACTGGCTGATAAGCTCGCGGCTGATCGAGTTGCAACAGTAATCAGTTGATTTGCCACTATGTCGTACAAACCTGAAGATAATGCTCCACTCCAGTCTCAACAAAGAAGATCTGAGGCACGGCATAAGC	
GGGCCCTACGGGCCCTACTCGAGCTAAAGCTTGGCAATCCGGTACTGTTGGTAAAGCCACCACACTCTCTCCACAAGTAGGCGCCGTGGAGTTCATGCTCGTTCTGAACCGGGAATGGCACAACCCGTAGTAGGATCTTGCCTCTGCCACGTGCACCTGCGCCTGTTGCTTCTAGACTATGGGCTGCTAAGGATGATCACAGACTCCTTTTTGGACAATCTGTGTCAAGTCTACTGTTACATCTGATGTACGCTGGAAATCCTCGTACTGGAGGCAATACCTGGAGGTGGTGCTGAAATATTGCAAGCCACAGGTGTGTTTCTAGTCTTGGTGCCGCTATCAGATGTCGCACCCTCTACGGGCAAATGTAGAACCTTAGTGGACGCGTGAGATCCCAATCCACGCCTGGCCATAGACTGGCTGATAAGCTCGCGGCTGATCGAGTTGCAACAGTAATCAGTTGATTTGCCACTATGTCGTACAAACCTGAAGATAATGCTCCACTCCAGTCTCAACAAAGAAGATCTGAGGCACGGCATAAGC	Gene block (dsDNA) fragment including the MeV-C ΔGGG IRES sequence with flanking restriction sites for cloning into pSGDLuc v3.0 to create dual luciferase reporter mRNAs
GCTAGCCGAGTAGTGTTCCCTCGCGAAAGGCCTTGTG	Forward ssDNA primer to introduce the ΔGGG into the HCV IRES sequence within pSGDLuc v3.0 via Q5 site-directed mutagenesis
AGTCTTGCGGGGGCACGC	Forward ssDNA primer to introduce the ΔGGG into the HCV IRES sequence within pSGDLuc v3.0 via Q5 site-directed mutagenesis
TAATACGACTCACTATAGGACTAATTCCTATGTTTATACGTTACTACCTTGTTCTG	Forward ssDNA primer to introduce the ΔGGG into the MeV-E IRES sequence within pSGDLuc v3.0 via Q5 site-directed mutagenesis
GTAAGAAGGTAAGTCTAGAATCCATATGCCAAATC	Reverse ssDNA primer to introduce the ΔGGG into the MeV-E IRES sequence within pSGDLuc v3.0 via Q5 site-directed mutagenesis
TAATACGACTCACTATAGGACTAATTCCTATGTTTATACGTTACTACCTTGTTCTG	Forward ssDNA primer to amplify MegE-V IRES templates for transcription of RNAs for SHAPE-MaP
GTAAGAAGGTAAGTCTAGAATCCATATGCCAAATC	Reverse ssDNA primer to amplify MegE-V IRES templates for transcription of RNAs for SHAPE-MaP

TAATACGACTCACTATAGGCTCCTACAAATGCACATGAAGAACAGTTTGTAG	Forward ssDNA primer to amplify HepV-C IRES templates for transcription of RNAs for SHAPE-MaP
AAGTCAAAATTCCATCCAAACTCTCTCCAAC	Reverse ssDNA primer to amplify HepV-C IRES templates for transcription of RNAs for SHAPE-MaP
TAATACGACTCACTATAGGGTTTCTGAGCACTGGTAAGAGCTTAGAC	Forward ssDNA primer to amplify LimV-C IRES templates for transcription of RNAs for SHAPE-MaP
TGATGATGCAAAAGTTGTGGTCAAATTTTCAATTATAG	Reverse ssDNA primer to amplify LimV-C IRES templates for transcription of RNAs for SHAPE-MaP
GCTATGGACTGGGGGCTCCACTC	Forward ssDNA primer to introduce the wild type GGG into the PemV-A1 ΔGGG IRES sequence within pSGDLuc v3.0 via Q5 site-directed mutagenesis
TCGCGCTACTAGGCA	Reverse ssDNA primer to introduce the wild type GGG into the PemV-A1 ΔGGG IRES sequence within pSGDLuc v3.0 via Q5 site-directed mutagenesis
GAGGGGAATTCCCAAAACCCCTTTGATC	Forward ssDNA primer to introduce the ΔGGG into the UNKp-V IRES sequence within pSGDLuc v3.0 via Q5 site-directed mutagenesis
CACTCATGGGGTTTCCAG	Forward ssDNA primer to introduce the ΔGGG into the UNKp-V IRES sequence within pSGDLuc v3.0 via Q5 site-directed mutagenesis