SUPPLEMENTARY DATA

The chaperone-like activity of the hepatitis C virus IRES and CRE elements regulates genome dimerization

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Supplementary Table 1. Oligonucleotides used to construct DNA plasmids described in this study.

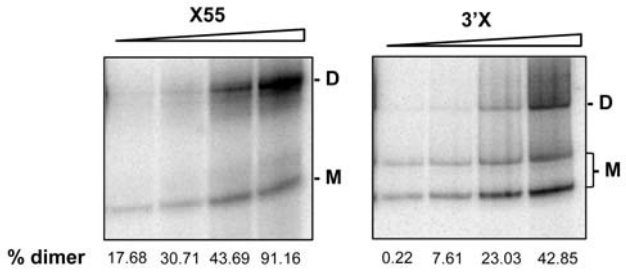
Construct	Oligonucleotides	Sequence (5'-3')
pGLI+CU_A288U	A288U	GGCCTTGTGGTTCTGCCTGATAGGGT
	asHCV-276	TTTCGCGACCCAACACTACTCGGCTA
pGLI+CU_U297A	U297A	GGCCTTGTGGTACTGCCTGAAAGGGT
	asHCV-276	TTTCGCGACCCAACACTACTCGGCTA
pGLI+CU_PK2R	A288U/U297A	GGCCTTGTGGTTCTGCCTGAAAGGGT
	asHCV-276	TTTCGCGACCCAACACTACTCGGCTA
pGLI+CU_G150C	G150C	TAGTGGTCTCCGGAACCGGTGAGTA
	asHCV-140	TGGCTCTCCCGGGAGGGGGGGTCCTGGA
pGLI+CU_Mut3	C242A	GGGCGUGCCCCCGAAGACTGCTA
	asHCV-228	AAATCTCCAGGCATTGAGCGGGTT
pGLI+CU_3R	C242G	GGGCGTGCCCCCGGAAGACTGCTA
	asHCV-228	AAATCTCCAGGCATTGAGCGGGTT
pGLI+CU_3c	3c_BiloopAG	GGGCAGGCCCCCGCAAGACTGCTA
	asHCV-228	AAATCTCCAGGCATTGAGCGGGTT
pGLI+CU_d3d	asHCV-252	TAGCAGTCTTGCGGGGGCACGCCCAA
	HCV-284	GTGGTACTGCCTGATAGGGTGCTTGCGAGT
pGLI+CU_dIL3d	HCVInser_275CT	GCCGAGTAGTGTTGGGTCGCGACTAGGCCTT
	asHCV-252	TAGCAGTCTTGCGGGGGCACGCCCAA
pGLI+CU_Pu	HCV-311mut319	GTGCCCCGCCTCCTCTCGTAGACCGT
	asHCV-310	TCGCAAGCACCCTATCAGGCAGTA
pGLI+CU_Py	HCV-120mut125	CCCCCGGAGGCGGGAAAGCCATAGT
	asHCV-119	TCCTGGAGGCTGCACGACACTCAT
pGLI+CU_Py/Pu	HCV-311mut319	GTGCCCCGCCTCCTCTCGTAGACCGT
	asHCV-310	TCGCAAGCACCCTATCAGGCAGTA
pGLI+CU_d3.1	HCV-9260	TTACAGCGGGGGAGACATATATCACAG
	asHCV-9215	GATTGGAGTGAGTTTGAGCTTGGTCCT
pGLI+CU_d3.2	HCV-9311	GTTCATGTGGTGCCTACTCCTACTTTCTGTA
	asHCV-9262	TAACCAGCAACGAACCAGCTGGATAAATCCAA
pGLI+CU_d3.3	HCV-9352	TCTATCTACTCCCCAACCGATGAACGG
	asHCV-9320	CCACATGAACCAGCGGGGTCGGGCA
pGLI+CU_d3.4	HCV-9384	TAAACACTCCAGGCCAATAGGCCA
	asHCV-9358	GCTCCCCGTTCATCGGTT
pGLI+CU_dHV	HCV-9507	TATGGTGGCTCCATCTTAGCCCTA
	asHCV-9384muta	AGCTCCCCGTTCATCGGTTGGGGAGT
pGLI+CU_dimUU	HCV-9553	CGTGAGCCGCATGACTGCAGAGA
	Dim_Mut_UU	GACCTTTCACAGCAAGCCGTGATTA
pGLI+CU_d3'SL1	5'T7pHCV	TAATACGACTCACTATAGGGATGGAAGCCAAAAACA
	asHCV-9560	GGCTCACGGACCTTTCACAGCTA

Supplementary Table 2. Oligonucleotides used in this study.

Oligonucleotide	Sequence (5'-3')
5'T7pHCV	TAATACGACTCACTATAGCCAGCCCCCTGATGG
5'T7pHCV-9181	TAATACGACTCACTATAGGGCAGTAAGGACCAAGCTCAA
3'HCV	ACTTGATCTGCAGAGAGGCCA
3'HCV_cassette	GAACCGGACCGAAGCCCGATTTGGATCCGGCGAACCGGATCGAACAT GATCTGCAGAGAGGCCAGTA
asHCV-9585	GTATCAGCACTCTCTGCAGT
Primer Std	GAACCGGACCGAAGCCCG

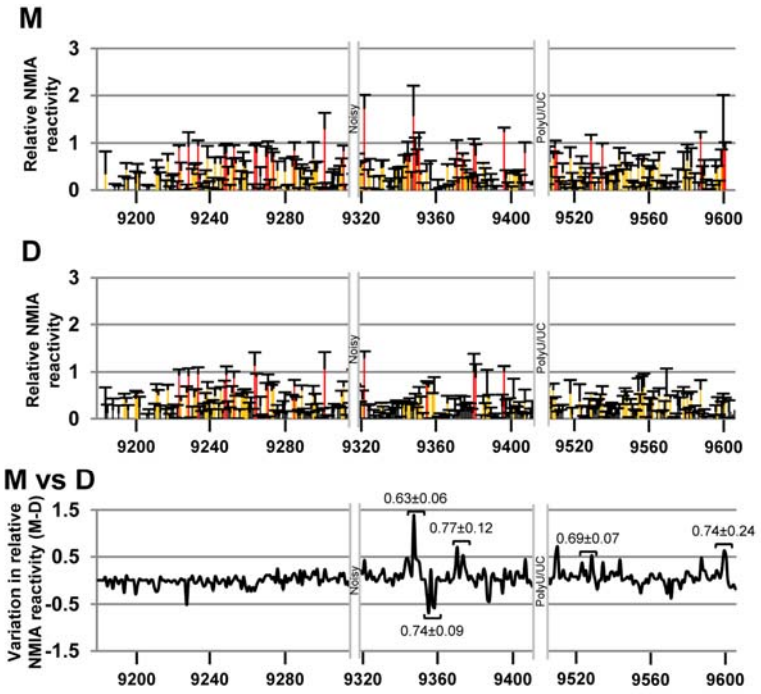
Promoter sequence for the T7 RNA polymerase is underlined.

Supplementary Figure S1. The 3'SLI interferes with the efficient dimer formation. Dimerization efficiency was assayed at increasing concentrations of 3'X and X55, which contains only the 3'SLIII and 3'SLII domains required for HCV genomic dimerization (40). Dimeric products were resolved by native TBM polyacrylamide gel electrophoresis. A representative image of the electrophoretic mobility shift assays is shown. Relative dimer formation for each transcript was quantified. M, monomer; D, dimer.

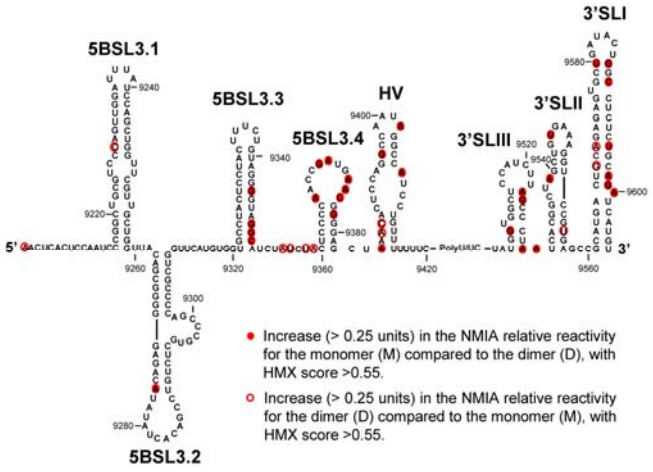


Supplementary Figure S2. Identification of nucleotides influencing dimer formation in the CRE region of the HCV genome. Nucleotides required for dimer formation were identified by 2'-hydroxyl molecular interference (HMX). A) Molecule CU was modified with NMIA under denaturing conditions. The monomeric and dimeric populations were then partitioned by native polyacrylamide gel electrophoresis. Modified positions were detected as stops in a reverse transcription reaction. Histograms show the NMIA reactivity profile for each of the isolated pools, M (monomer) and D (dimer). Data are the mean of three independent experiments ± standard deviation. Different accessibility values are colour coded as indicated. HMX profiles shown in the bottom panel correspond to difference in NMIA reactivity between the monomeric and the dimeric conformations (M-D) for the CU transcript. HMX scores indicated at precise positions were calculated from the reactivity profiles of the monomeric and dimeric isoform, as previously described {Homan, 2014 #2794}. B) Sequence and secondary structure of CU, summarizing the HMX results. Filled circles: increase (>0.25 units) in NMIA relative reactivity for the monomer (M) compared to the dimer (D), with HMX score >0.55; open circles: increase (>0.25 units) in NMIA relative reactivity for the dimer (D) compared to the monomer (M), with HMX score >0.55.

A)



B)



Supplementary Figure S3. Line graph showing the differential SHAPE reactivities for the transcripts CU and I+CU calculated by scaling 1M6 to NMIA relative values and then subtracting corrected 1M6 from NMIA reactivity data, under monomeric (A) or dimeric (B) conditions.