Supplementary Table 1

DNA oligonucleotides used in this study

name	sequence
cloning of cDNAs encoding different WNV replicon RNA	
3SLMut1For	GGTGCGAGAACAGAGGATCTGGGTCG
3SLMut1Rev	CGACCCAGATCCTCTGTTCTCGCACC
3SLMut2For	GGTGGTGCGAGAGCACAGGATCTGG
3SLMut2Rev	CCAGATCCTGTGCTCTCGCACCACC
3SLMut3For	GGTGGTGCGAGAGCAGAGGATCTGGGTCG
3SLMut3Rev	CGACCCAGATCCTCTGCTCTCGCACCACC
PCR templates for in vitro transcription	
5UTRWestFor	ATAAGCTTTAATACGACTCACTATAGGAGTAGTTCGCCTGTGTGAGCTGA
3UTRnativRev	AGATCCTGTGTTCTCGCACCACCAGCC
3'UTRRevMut1	AGATCCTCTGTTCTCGCACCACC
3'UTRRevMut2	AGATCCTGTGCTCTCGCACCACC
3'UTRRevMut3	AGATCCTCTGCTCTCGCACCACC
Exon-intron PCR	
C636 Exon4 Fw	AAGGAGGTGGACGTCAAGAAGG
C636 Intron4 Rv	GCTTCAGTTTTCAAGCACTGAAGG
SUMO expression plasmids	
AUF1 Aedes BsaIFor	ATGGTCTCATGGTGCCGATCAGGATCAAGAG
AUF1 Aedes BamHIRev	ACGGATCCTTAGTACGGCGTATGCCTTGG
FLAG-p30 and p32 fusion transcribed from pSinRep5	
C636AUF1XbaFw	ATTCTAGAGCCACCATGGATTACAAGGATGACGACGATAAGGCCCGG GCGGATGCCGATCAGGATCAAGAGATG
C636AUF1ApaIRv	ATGGGCCCTTAGTACGGCGTATGCCTTGGCTG
cloning of cDNAs encoding WNV replicon NS5mut RNA	
NS5PolQuikFor	GCTGTCAGTGGAGATGCCTGTGTGGTAAAGCCC
NS5PolQuikRev	GGGCTTTACCACACAGGCATCTCCACTGACAGC

Supplementary Table 2

RNA oligonucleotides used in this study

name	sequence
3'SL^{trunc}	Cy5-AGAUCUUCUGCUCUGCACAAUGGUGCGAGAACACAGGAUCU-BHQ
5'UAR	UCUUAGCACGAAGAUCU
AU/GU-rich RNA	FAM-EX-5'-UAUUUAGUGGUGUUAG-3'
random RNA	FAM-EX-5'-CUAAGAUGCUCGCUGC-3'
5CS WNV Cy5	Cy5-UGUCAAUAUGCUAA
3CS WNV Cy3	Cy3-AGCAUAUUGACACC

Supplementary methods

Plasmid encoding WNV NS5mut replicon

To obtain replicon-encoding cDNAs with mutations in the NS5 gene, the SpeI-SacII fragment of pWNV (Shi et al., 2002) was subloned into pGEM-T Easy. Site-directed mutagenesis was carried out using primers NS5PolQuikFor and NS5PolQuikRev. The SpeI-SacII fragment with the corresponding mutation was cloned into pWNVRluc.

RNA gel-shift assay

RNA-RNA interactions were analysed by electrophoretic mobility shift assays as performed earlier (Friedrich et al., 2014). The binding reaction contained 5 mM Hepes/NaOH, pH 7.9, 100 mM KCl, 5 mM $MgCl_2$, 1 mM dithiothreitol, uniformly ^{32}P-labelled 5'-RNA (5' 162 Nt) (2 nM) and non-labelled 3'-RNA (3' 111 Nt) (2 nM) in a final volume of 20 µl. The binding reaction was performed at the indicated temperatures for 2 h. The RNA-RNA complexes were resolved by native 5% polyacrylamide gel electrophoresis in 1 x TBE at 4°C, analysed by phosphor imaging and quantified by ImageQuant Software (GE). Signal intensities were blotted as a function of 3'-RNA concentration and fitted by KaleidaGraph (Synergy) to a single-site binding model to determine the dissociation constant K_D:

$$S = S_{max} \bullet x / (K_D + x)$$

S - radioactive signal; S_{max} - maximal signal amplitude; x - concentration of the 3'-RNA; K_D - dissociation constant.

Supplementary Figures

Figure S1

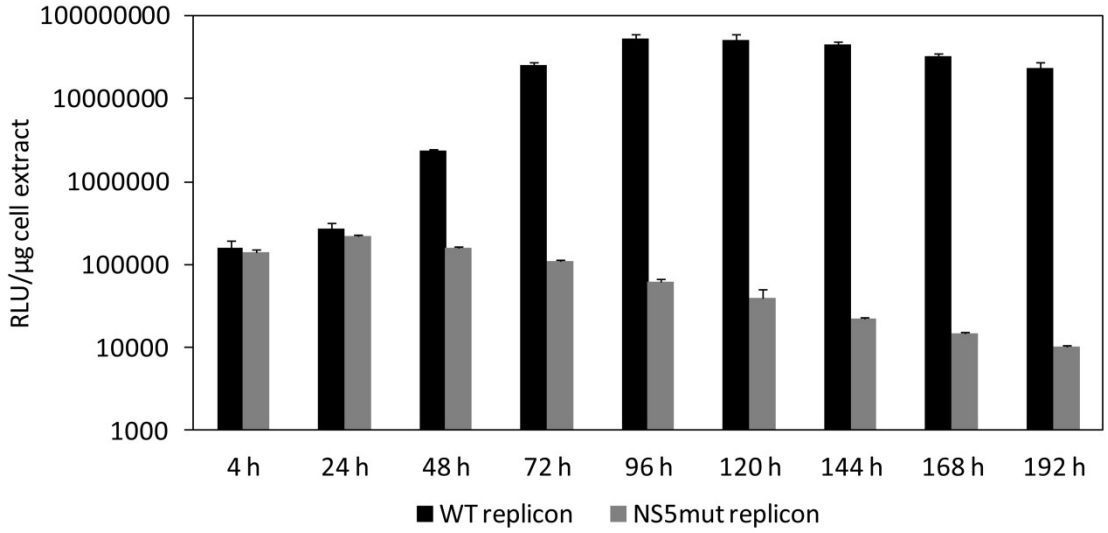

Fig. S1. Time course of Rluc activity in cells transfected with WNV replicons. Huh7 cells were transfected with wild-type and NS5mut WNVRluc replicon RNAs, subsequently cultivated at 28°C and analysed for luciferase reporter activity at the indicated time-points post transfection. Results from two independent transfections with two technical replicates are shown; error bars reflect standard deviations. Please note that the values for the Rluc activity are higher compared to results from the main manuscript due to a different luminometer used.

Figure S2

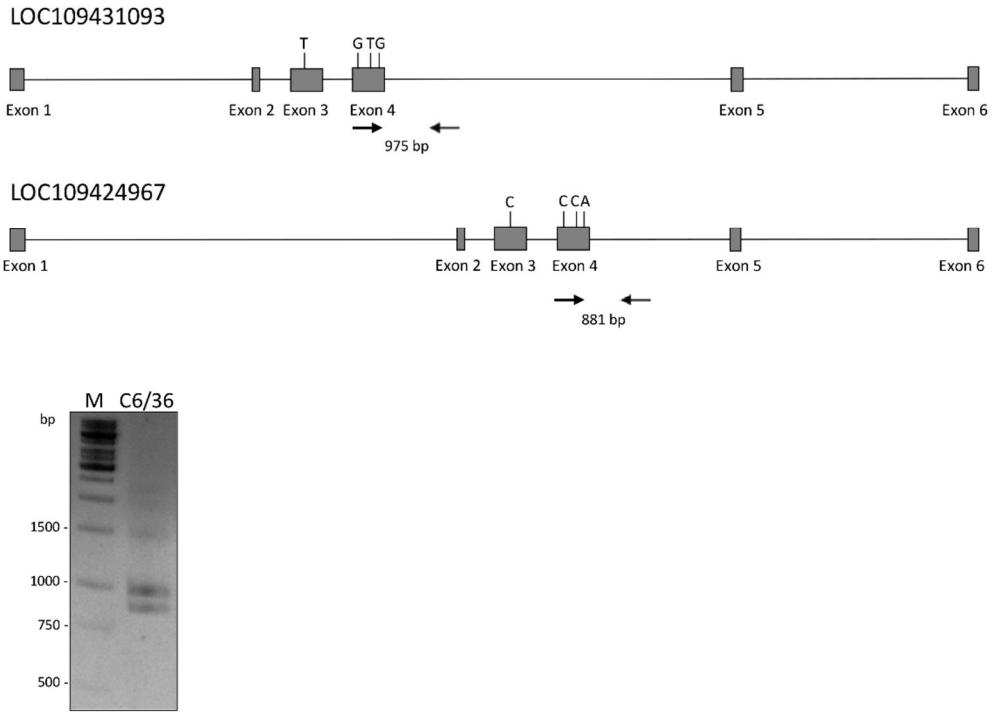

Fig. S2. Exon-intron organization of two genomic loci in C6/36 cells encoding AUF1-homologous proteins. (Top) The two loci mainly differ in their intron sequence. Nucleotide differences in exons 3 and 4 do not change the amino acid sequence. Arrows indicate the location of PCR primer binding sites. PCR product sizes are indicated. (Bottom) The PCR products from the exon-intron PCR were analysed on an agarose gel in parallel with a molecular weight marker (M).

Figure S3

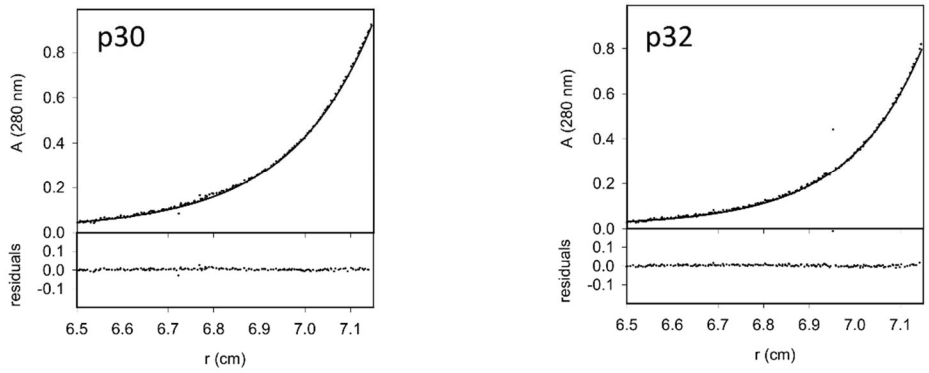

Fig. S3. Mosquito squid p30 and p32 are monomeric proteins. In sedimentation equilibrium experiments (analytical ultracentrifugation) 12 µM (p30) and 7 µM (p32) of protein was analyzed at 14000 rpm at 20°C. No aggregation of the proteins was observed in the course of the experiment.

Figure S4

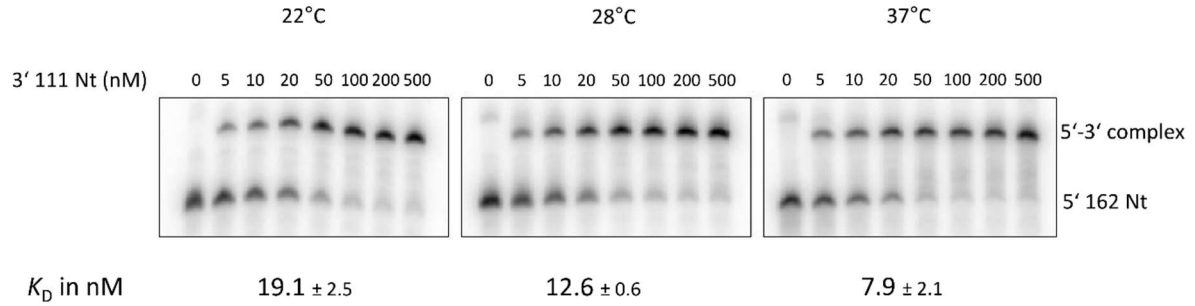

Fig. S4. 5’-3’ RNA-RNA interaction is increased at higher temperatures. ^{32}P-labelled 5’-RNA (5’ 162 Nt) (2 nM) and non-labelled 3’-RNA (3’ 111 Nt) (2 nM) were incubated at the indicated temperatures for 2 h. The RNA-RNA complexes were resolved by native 5% polyacrylamide gel electrophoresis, analysed by phosphor imaging and quantified by ImageQuant Software (GE). The signal intensities were fitted as a function of 3’-RNA concentration to obtain the binding affinities (shown below). Average results and standard deviations (n = 3) are shown.

References

1. Friedrich, S.; Schmidt, T.; Geissler, R.; Lilie, H.; Chabierski, S.; Ulbert, S.; Liebert, U.G.; Golbik, R.P.; Behrens, S.E. AUF1 p45 promotes West Nile virus replication by an RNA chaperone activity that supports cyclization of the viral genome. J Virol 2014, 88, 11586-11599, doi:JVI.01283-14
2. Shi, P.Y.; Tilgner, M.; Lo, M.K. Construction and characterization of subgenomic replicons of New York strain of West Nile virus. Virology 2002, 296, 219-233