Supporting information

Supplemental Table 1

siRNAs used in this study

name	sequence
control siRNA	GGCUACGUCCAGGAGCGCACCdTdT
siPRMT1-1	CGUCAAAGCCAACAAGUUAdTdT
siPRMT1-2	CGUGUAUGGCUUCGACAUGdTdT

Supplemental Table 2

DNA oligonucleotides used in this study

name	sequence
SUMO expression plasmids	
PRMT1RBSFor	GAAGGAGATATACATATGGCGGCAGCCGAGGCCGCGAAC
PRMT1RBSRev	TCAGCGCATCCGGTAGTCGGTGGAG
pETSUMORBSFor	GAAGGAGATATACATATGGGCAGC
pETSUMORBSRev	TTAAAGTTAAACAAAATTATTTCTAGAGGGGAATTG
PRMT1v1v2BsaIFor	ATGGTCTCATGGTGCGGCAGCCGAGGCCGCGAAC
PRMT1v3BsaIFor	ATGGTCTCATGGTGTAGGCGTGGCTGAGGTGTCC
PRMT1v5BsaIFor	ATGGTCTCATGGTGAGGTGTCCTGTGGCCAGG
PRMT1XhoI	ATCTCGAGTCAGCGCATCCGGTAGTCGGTGGAG
qRT-PCR	
Reverse transcription	
qRT-3UTRRev	CGCAGACTGCACTCTCCG
OligodT	TTTTTTTTTTTTTTTTTTT
PCR	
qRTNS5For	AGAGTTCGGAAAGGCCAAG
qRTNS5Rev	CAGAGCCTCGAACTCCAGAA
GAPDHfw	AGCCTCAAGATCATCAGCAATGC
GAPDHrw	ATGGCATGGACTGTGGTCATG

Supplemental Table 2 continued	
WNV-INEID-f1-wob	AGTAGTTCGCCTGTGTGARC
WNV-INEID-r1-mod	GCCCTCCTGGTTTCTTAGAC
WNV-INEID-probe	FAM-AATCCTCACAAACACTACTAAGTTTGTCA-TAMRA
unrelated RNA for filter binding assay	
T7Top	TAATACGACTCACTATAG
SL Bottom	CGCACGCACGCGCACGCACGCACGCACGCACGCGCACGATATCGTGCGC GTGCGTGCGTGCGTGCGTGCGCGTGCGTGCGCTATAGTGAGTCGTATTA

Supplemental Table 3

RNA oligonucleotides used in this study

name	**sequence**
3'SLtrunc	Cy5-AGAUCUUCUGCUCUGCACAAUGGUGCGAGAACACAGGAUCU-BHQ
5'UAR	UCUUAGCACGAAGAUCU
5UAR WNV Cy5	Cy5-UUAGCACGAAGAUCUC
3UAR WNV Cy3	Cy3-AGGAGAUCUUCUGCU
5CS WNV Cy5	Cy5-UGUCAAUAUGCUAA
3CS WNV Cy3	Cy3-AGCAUAUUGACACC

Sequence of the unrelated RNA used for filter binding assay.

CGCACGCACGCGCACGCACGCACGCACGCACGCGCACGAUAUCGUGCGCG
UGCGUGCGUGCGUGCGUGCGCGUGCGUGCG

Supplemental Figures

Fig. S1. PRMT1 co-precipitates with FLAG-AUF1 p45. A plasmid encoding a FLAG-tagged version of AUF1 isoform p45 or an empty vector (control) was transfected in Huh7 cells. 48 h later, the cells were harvested and the cytoplasmic extracts were subjected to immune-precipitation (IP) *via* anti-FLAG antibody followed by elution with a FLAG peptide. The eluates of the FLAG-AUF1 p45 IP and the control IP were analyzed by SDS-PAGE and silver staining. The protein band corresponding to FLAG-AUF1 p45 is marked by an arrow. A dominant protein band with a molecular weight of approximately 40 kDa consistently co-purified with FLAG-AUF1 p45. Subsequent analysis by Mass Spectrometry (MS) identified this protein band as arginine-methyltransferase PRMT1 (data not shown; PRMT1 marked by an arrow).

Supplemental Figure S1

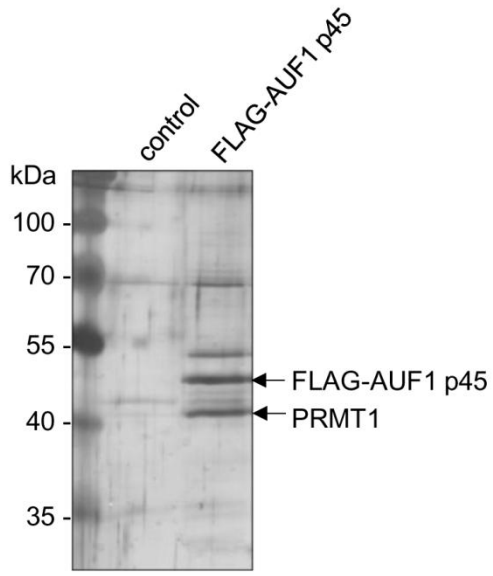


Fig. S2. *In vitro* methylation of AUF1 p45 by different PRMT1 isoforms. (A) N-terminal amino acid sequences representing the main differences between the different PRMT1 isoforms. **(B)** The genes of the PRMT1 isoforms v1, v2, v3 and v5 were cloned and expressed in *E. coli* and the proteins purified as described in supplemental Materials and Methods. About 1 µg of each PRMT1 isoform was analyzed on a Coomassie-stained SDS-PAGE in comparison to protein markers. **(C)** *In vitro* methylation of recombinant AUF1 p45, which was expressed in and purified from *E. coli*, by the different PRMT1 isoforms. The methylation assay was performed with 13 pmol of AUF1 p45 and 5 pmol of PRMT1 v1, v2, v3 or v5, respectively, in the presence of 40 µM [*S*-^{14}C] adenosylmethionine as described in Materials and Methods. Samples were taken at the indicated time points and analyzed by SDS-PAGE and phosphorimaging.

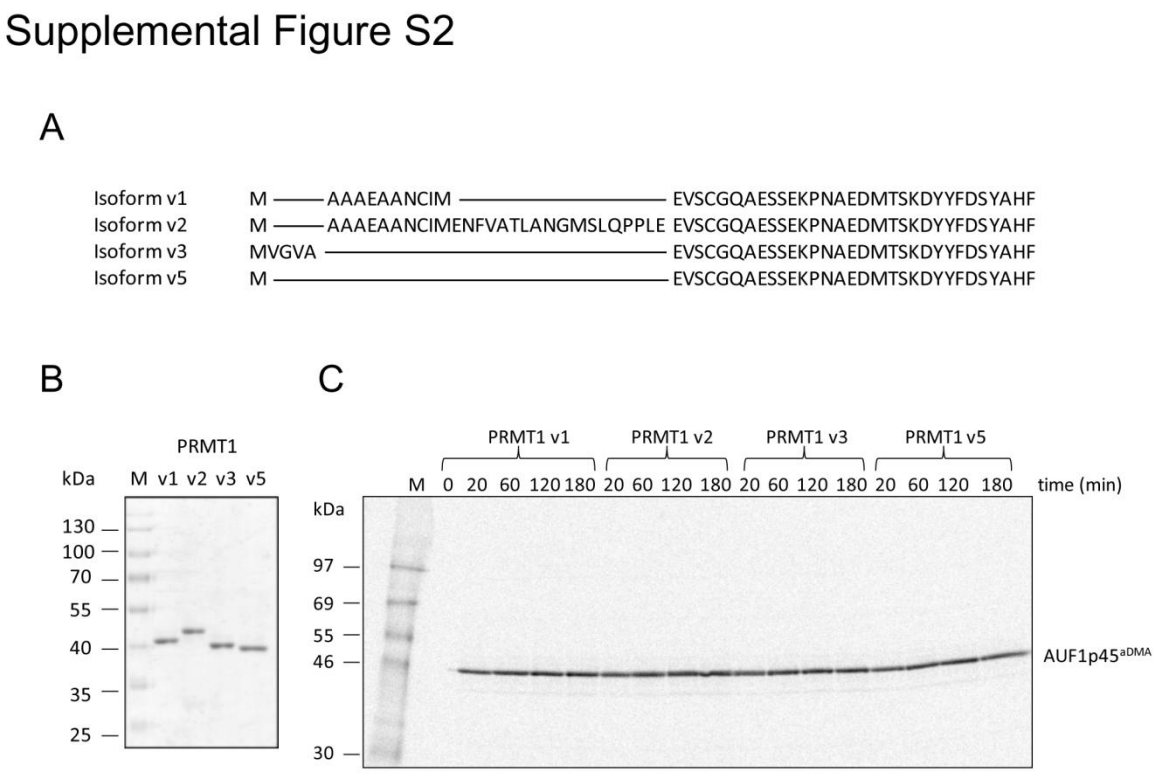


Fig. S3. Cell viability upon RNAi-mediated depletion of PRMT1. Huh7 cells were transfected with siRNAs targeting PRMT1 (siPRMT1-1 or siPRMT1-2) or a control siRNA (siC). 72 h post transfection, the cells were re-transfected with the same siRNAs. Another 48 h later, cells were analyzed by Trypan blue exclusion assay. Trypsinized cells were mixed with equal volumes of Trypan blue solution (Sigma) and incubated for 10 min at room temperature. Viable cells were counted in a Neubauer chamber.

Supplemental Figure S3

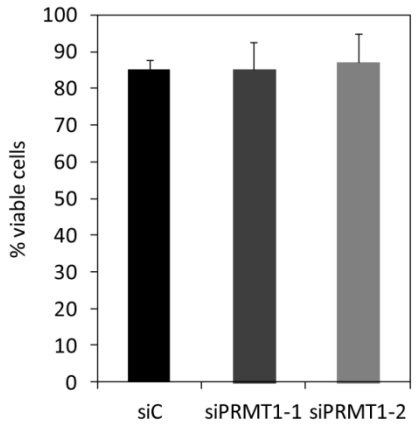


Fig. S4. Peptide fragmentation analysis of dimethylated AUF1 p45 proteolytic peptides. MS-analysis followed by peptide fragmentation was performed with chymotrypsin-digested recombinant methylated AUF1 p45 that was co-expressed with PRMT1 v1 in and purified from *E. coli*. Peptide fragmentation analysis of dimethylated peptide AG**R**A**R**G**R**GGGPSQNW indicated the original peptide mass being 1610.8 Da. Following fragmentation, masses of 1565.8 Da and 1520.8 Da were detected, which indicated the loss of dimethylammonium ions (45 Da) from the methylated arginine residues. Dimethylammonium ions are formed exclusively from asymmetrically dimethylated peptides.

Supplemental Figure S4

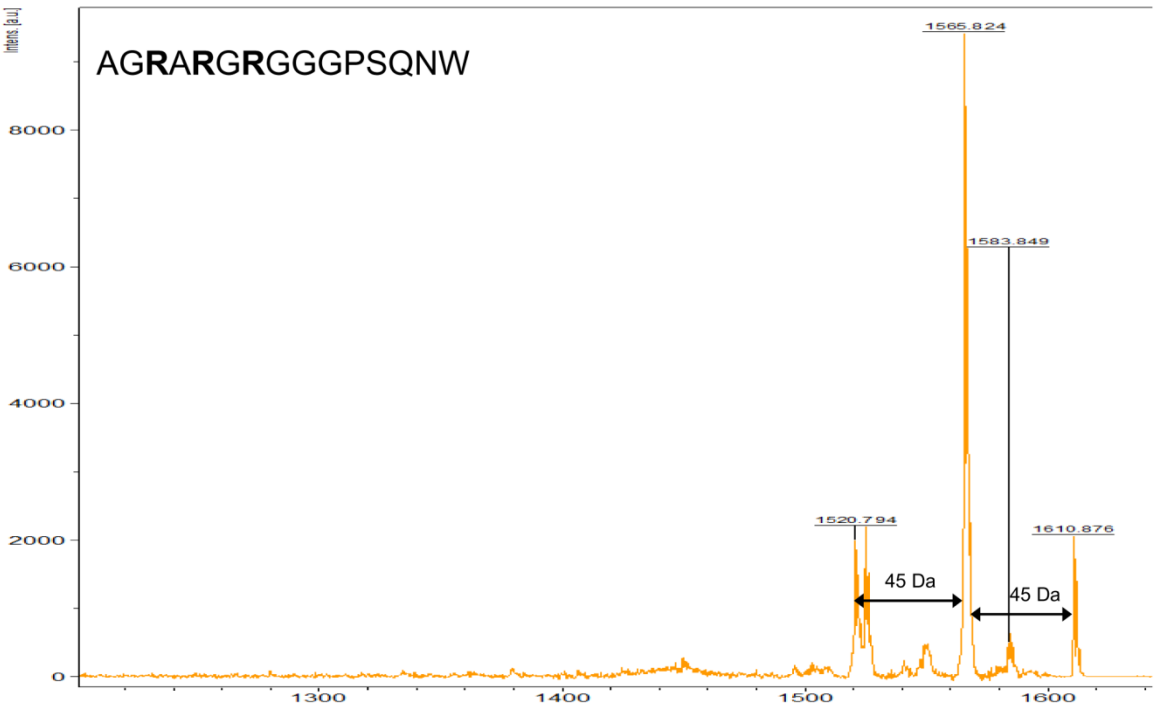


Fig. S5. Interaction of AUF1 p45^{aDMA} and the WNV sgRNA monitored by a filter binding assay. ^{32}P-labeled WNV sgRNA (0.1 nM) was exposed to increasing concentrations of AUF1 p45^{aDMA}. Formed RNA-protein complexes were applied to a nitrocellulose membrane and visualized by autoradiography (left panel, bound). The unbound RNA was recovered by DE81 ion exchange paper (left panel, free). The radioactive signals retained on the nitrocellulose membrane were quantified and plotted as a function of the concentration of AUF1 p45^{aDMA} and fitted using Equation 1 (Materials and Methods, solid line) (right panel). For comparison, the optimal fit to a single-site binding model (Friedrich et al. 2014) is also shown (dotted line).

Supplemental Figure S5

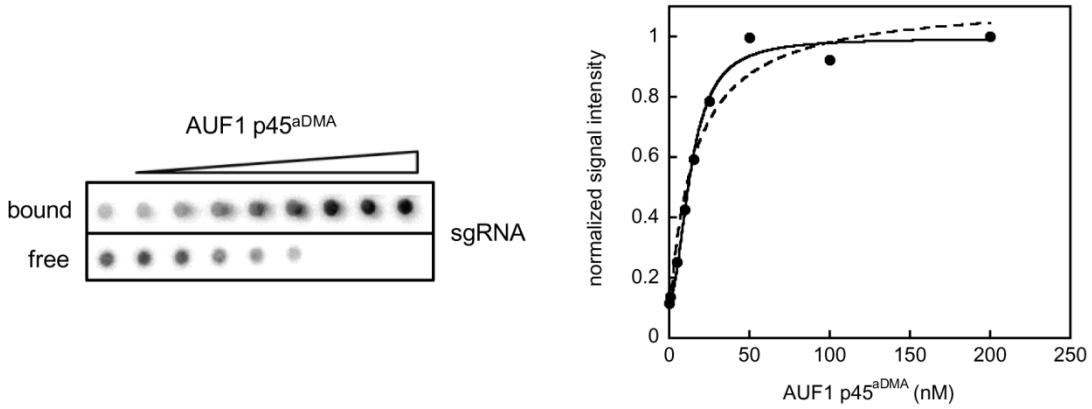
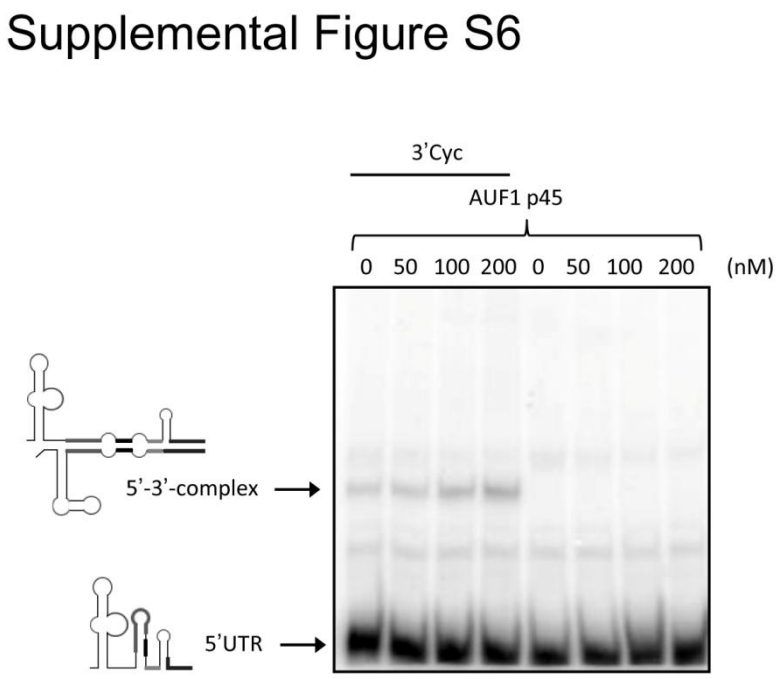


Fig. S6. Interactions of the 3'Cyc and 5'UTR RNAs, tested in the absence and presence of *E. coli*-purified AUF1 p45. A total of 2 nM 3'Cyc RNA (lane 1-4) was exposed to the indicated amounts of recombinant protein followed by the addition of 2 nM ^{32}P-labeled 5'UTR RNA. A control reaction was performed without the 3'Cyc RNA (lane 5-8). The migration positions of the unbound 5'UTR RNA and of the 5'-3' RNA complex are indicated.



Supplemental Table 4

Observed rate constants k_{obs} (s^{-1}) with the 3'SL^{trunc}-5'UAR interaction assay

	k_{obs} (s^{-1})		
Protein (nM)	**AUF1p45**[a]	**AUF1p45^{aDMA}**	**hnRNPH1**[a]
0	0.0062	0.0062	0.0076
10	n.d.	0.01	0.0061
20	0.0071	0.012	0.0082
35	n.d.	0.025	n.d.
50	0.012	0.058	0.009
75	n.d.	n.a.	n.d.
100	0.024	n.a.	0.0086
240	0.064	n.d.	0.0067

The observed rate constants k_{obs} (s^{-1}) for the 3'SL^{trunc} and 5'UAR interaction reaction in the absence and presence of different concentrations of AUF1 p45, AUF1 p45^{aDMA} and hnRNPH1 were determined with equation 2 and 3 (materials and methods). Note that due to the lack of data points at the beginning of the reaction, no rate constants were determined for reactions where AUF1 p45^{aDMA} was present at 75 and 100 nM, respectively (n.a. = not analyzed, n.d. = not done). [a]The observed rate constants k_{obs} (s^{-1}) for AUF1 p45 and hnRNPH1 were taken from Friedrich et al. (2014).

Supplemental Table 5

Observed rate constants k_{obs} (s^{-1}) with the 5'UAR-3'UAR RNA annealing assay

	k_{obs} (s^{-1})		
Protein (nM)	**AUF1p45**[a]	**AUF1p45^{aDMA}**	**hnRNPH1**
0	0.0029	0.0029	0.0029
40	0.0075	0.0061	0.0029
100	0.01	0.0075	0.0027
150	0.014	0.011	0.0028
230	0.019	0.013	0.0029

The observed rate constants k_{obs} (s^{-1}) for the 5'UAR-3'UAR RNA annealing in the absence and presence of different concentrations of AUF1 p45, AUF1 p45^{aDMA} and hnRNPH1 were determined with equation 3 (materials and methods).

Supplemental Table 6

Observed rate constants k_{obs} (s^{-1}) with the 5'CS-3'CS RNA annealing assay

	k_{obs} (s^{-1})		
Protein (nM)	**AUF1p45**[a]	**AUF1p45^{aDMA}**	**hnRNPH1**
0	0.017	0.017	0.017
5	0.027	0.021	0.017
10	0.043	0.044	0.016
15	0.071	0.05	n.d.
20	0.11	0.085	0.017

The observed rate constants k_{obs} (s^{-1}) for the 5'CS-3'CS RNA annealing in the absence and presence of different concentrations of AUF1 p45, AUF1 p45^{aDMA} and hnRNPH1 were determined with equation 3 (materials and methods). n.d. not determined.

References

Friedrich, S, Schmidt, T, Geissler, R, Lilie, H, Chabierski, S, Ulbert, S, Liebert, UG, Golbik, RP, Behrens, SE. 2014. AUF1 p45 promotes West Nile virus replication by an RNA chaperone activity that supports cyclization of the viral genome. *J Virol* **88**: 11586-11599.