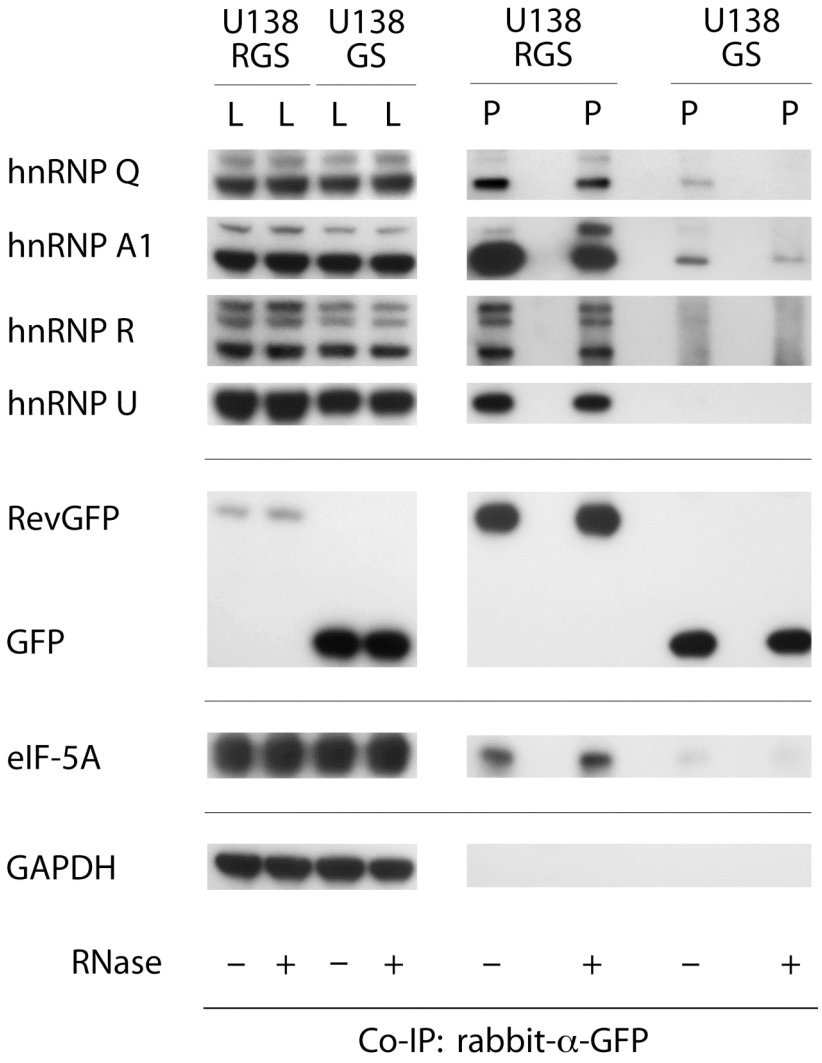
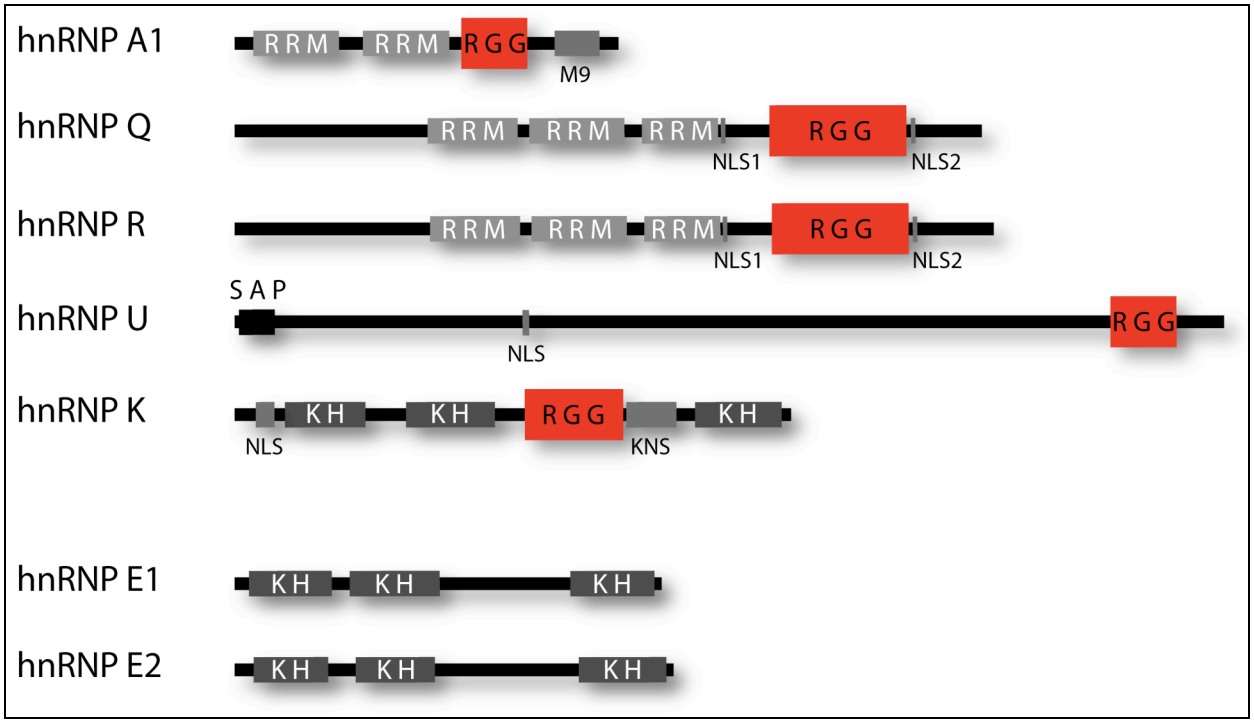
Supplemental Data Hadian et al.

Supplemental Figure S1



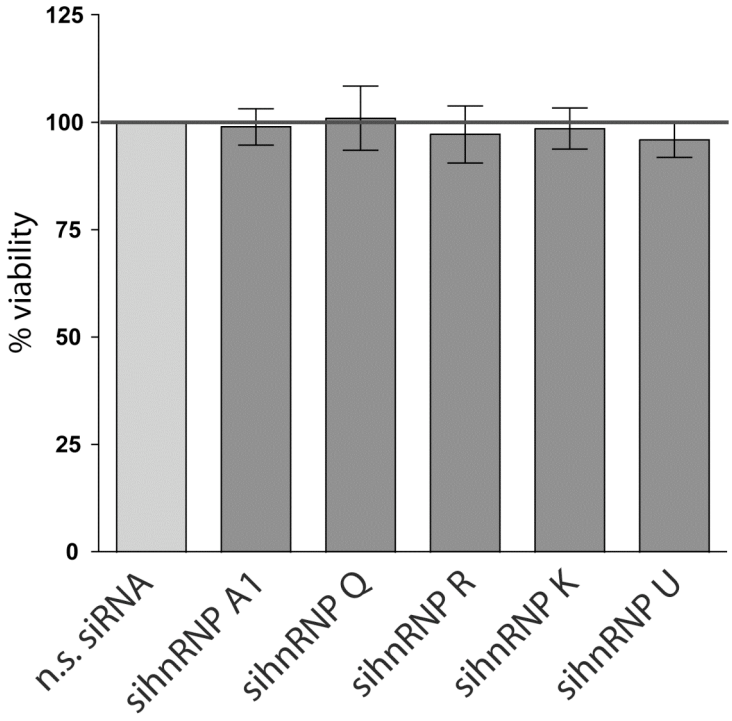
Supplemental Figure S1. Co-immunoprecipitation analysis with RNase A treated cell lysates demonstrates RNA-independent interaction of Rev with hnRNP Q, A1, R and U. Lysates (L) were generated from U138MG cells stably expressing RevGFP-*Strep*TagII (RGS) or GFP-*Strep*TagII (GS). Co-immunoprecipitation assays were performed with cell lysates preincubated with 200 µg/ml RNase A (as described in (1)) and a rabbit-anti-GFP antibody. All investigated proteins were detected in all cell lysates before (-) and after (+) RNase treatment. Analysis of the precipitates (P) from RNase A treated as well as untreated lysates revealed co-precipitation of hnRNP Q, A1, R and U with RevGFP-*Strep*TagII (RGS), but hardly with GFP-*Strep*TagII (GS). RGS and GS were efficiently precipitated. EIF-5A served as a control for a known Rev-interactor and GAPDH as a control for a non-interacting protein.

Supplemental Figure S2



Supplemental Figure S2. Protein domains of hnRNP A1, Q, R, U, K, E1 and E2.
KH = K-homology domain; KNS = K nuclear shuttling domain; NLS = nuclear localization domain; M9 = shuttle sequence; RGG = Arginine-Glycin-rich motif; RRM = RNA recognition motif; SAP = SAFA/B-Acinus-PIAS DNA binding domain

Supplemental Figure S3

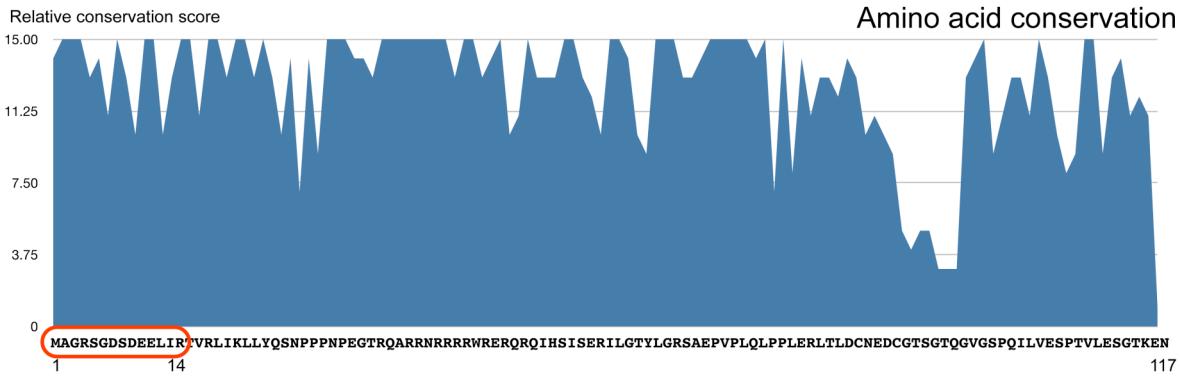


Supplemental Figure S3. Measurement of cytotoxic effects of the investigated siRNAs. All siRNAs were transfected in TH4-7-5 cells and cytotoxicity of the siRNAs were investigated using the MTT test. The non-silencing control was set to 100% viability and all other siRNAs were referred to this control. Results from the MTT test demonstrate that the investigated siRNAs had no effects on cell viability.

Supplemental Figure S4 (next page). Conservation of Rev AA 1-14 amongst HIV-1 clades of the M-group. *A*. The diagram shows the conservation profile of Rev protein sequences (amino acid similarities) amongst HIV-1 clades of the M-group. Conservation was determined from the sequences of the M-group taken from the HIV database (http://www.hiv.lanl.gov/content/index). 38 clade representative protein sequences were used. The alignment was carried out using DiAlign (Genomatix software, Munich), and the maximum similarity measure was set to 15. The sequence of the Rev protein used in this study is indicated below the conservation profile as reference. The N-terminal region of Rev is highlighted. *B*. Conservation of individual amino acid positions in the Rev AA 1-14 region.

Supplemental Figure S4

A



B

Clade	Amio acid (AA)	1	2	3	4	5	6	7	8	9	10	11	12	13	14
	Charge conservation (%)	97	100	100	100	92	95	84	92	100	87	68	100	100	63
	Most conserved AA	M	A	G	R	S	G	D	S	D	E	D/E	L	L/I	R
	%	97	100	100	100	92	95	84	55	97	82	68	100	97	34
A1	Ref.A1.AU.03.PS1055	M	A	G	R	S	G	D	S	D	E	E	L	L	K
	Ref.A1.KE.94.Q23_17	M	A	G	R	S	G	D	S	D	E	E	L	L	R
	Ref.A1.RW.92.92RW008	M	A	G	R	S	G	D	S	D	E	D	L	L	R
	Ref.A1.UG.92.92UG037	M	A	G	R	S	G	N	P	D	E	E	L	L	R
A2	Ref.A2.CD.97.97CDKTB	M	A	G	R	S	G	D	P	D	E	D	L	I	R
	Ref.A2.CY.94.94CY017	T	A	G	R	S	D	D	P	D	E	S	L	L	Q
B	Ref.B.FR.83.HXB2_LAI	M	A	G	R	S	G	D	S	D	E	D	L	I	R
	Ref.B.NL.00.671_00T3	M	A	G	R	S	G	D	S	D	E	E	L	L	R
	Ref.B.B.TH.90.BK123.AY	M	A	G	R	S	G	D	S	D	E	E	L	L	R
	Ref.B.US.98.1058_11	M	A	G	R	S	G	D	R	D	E	E	L	L	Q
C	Ref.C.BR.92.BR025_d	M	A	G	R	S	G	D	S	D	E	A	L	L	Q
	Ref.C.ET.86.ETH2220.	M	A	G	R	S	G	D	S	D	E	E	L	L	K
	Ref.C.IN.95.95IN2106	M	A	G	R	S	G	D	S	D	E	A	L	L	K
	Ref.C.ZA.04.SK164B1.	M	A	G	R	S	G	D	S	D	E	A	L	L	Q
D	Ref.D.CD.83.ELI.K034	M	A	G	R	S	G	D	S	D	E	D	L	L	K
	Ref.D.CM.01.01CM_441	M	A	G	R	R	E	D	S	D	E	D	L	L	K
	Ref.D.TZ.01.A280.AY2	M	A	G	R	S	G	D	S	D	E	E	L	L	R
	Ref.D.UG.94.94UG114	M	A	G	R	S	G	D	R	D	E	E	L	L	Q
F1	Ref.F1.BE.93.VI850.A	M	A	G	R	S	G	D	S	D	T	E	L	L	K
	Ref.F1.BR.93.93BR020	M	A	G	R	S	G	D	S	D	Q	E	L	L	K
	Ref.F1.F1.93.FIN9363	M	A	G	R	S	G	D	S	D	T	E	L	L	K
	Ref.F1.FR.96.MP411.A	M	A	G	R	S	G	D	N	D	E	E	L	L	R
F2	Ref.F2.CM.02.02CM_00	M	A	G	R	S	G	D	S	D	E	A	L	L	T
	Ref.F2.CM.95.MP255.A	M	A	G	R	S	G	D	S	D	E	D	L	L	K
	Ref.F2.CM.95.MP257A.A	M	A	G	R	S	G	D	R	D	E	E	L	L	K
	Ref.F2.CM.97.CM53657	M	A	G	R	S	G	D	S	D	E	E	L	L	K
G	Ref.G.BE.96.DRCBL.AF	M	A	G	R	S	G	S	T	D	E	E	L	L	T
	Ref.G.KE.93.HH8793_1	M	A	G	R	S	G	S	T	D	E	D	L	L	R
	Ref.G.NG.92.92NG083	M	A	G	R	S	G	D	P	D	E	D	L	L	R
	Ref.G.PT.x.PT2695.AY	M	A	G	R	S	G	S	T	D	E	D	L	L	R
H	Ref.H.BE.93.VI991.AF	M	A	G	R	S	G	D	N	D	E	G	L	L	R
	Ref.H.BE.93.VI997.AF	M	A	G	R	S	G	A	G	D	E	Q	L	P	Q
	Ref.H.CF.90.056.AF00	M	A	G	R	S	G	A	S	D	T	E	L	L	Q
J	Ref.J.CD.97.J_97DC_K	M	A	G	R	S	G	D	S	D	E	Q	L	L	L
	Ref.J.SE.93.SE7887.A	M	A	G	R	S	G	D	S	D	D	Q	L	L	L
	Ref.J.SE.94.SE7022.A	M	A	G	R	S	G	D	N	D	D	Q	L	L	A
K	Ref.K.CD.97.WQTB11C	M	A	G	R	R	G	D	S	E	Q	Q	L	L	T
	Ref.K.CM.96.MP535.AJ	M	A	G	R	R	G	D	P	D	E	Q	L	L	T

Supplemental Table

Full designations of GO-categories indicated in Figure 5

Short designation in Figure 5	GO-category designation
RNA processing/splicing	mRNA processing / metabolic process
	mRNA metabolic process / RNA processing / cellular component organization and biogenesis / interspecies interaction between organisms
Nucleobase metabolism	nucleobase, nucleoside, nucleotide and nucleic acid metabolic process
	nucleobase, nucleoside, nucleotide and nucleic acid metabolic process, biopolymer metabolic process
Translation	translational initiation
DNA repair	DNA repair
DNA metabolism	regulation of DNA metabolic process
	positive regulation of DNA metabolic process
TF activity regulation	positive regulation of transcription factor activity
Cytokine production/signaling	cytokine production/secretion/ regulation of secretion
	cytokine and chemokine mediated signaling pathway
Defense response	defense response
Negative regulation of apoptosis	negative regulation of cell death
Cell cycle arrest	cell cycle arrest
Protein kinase cascades	protein kinase cascade / post-translational protein modification / protein amino acid phosphorylation
Oxygen metabolic process	oxygen and reactive oxygen species metabolic process
Gas transport	gas transport
Neg. regulation of development	negative regulation of developmental process
Interspecies interactions	interspecies interaction between organisms

Supplemental Reference

1. Huang, Y., Gattoni, R., Stevenin, J., and Steitz, J. A. (2003) *Mol Cell* **11**(3), 837-843