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Supplemental Information

Efficient Knockdown and Lack of Passenger Strand Activity by Dicer-Independent shRNAs Expressed from Pol II-Driven MicroRNA Scaffolds

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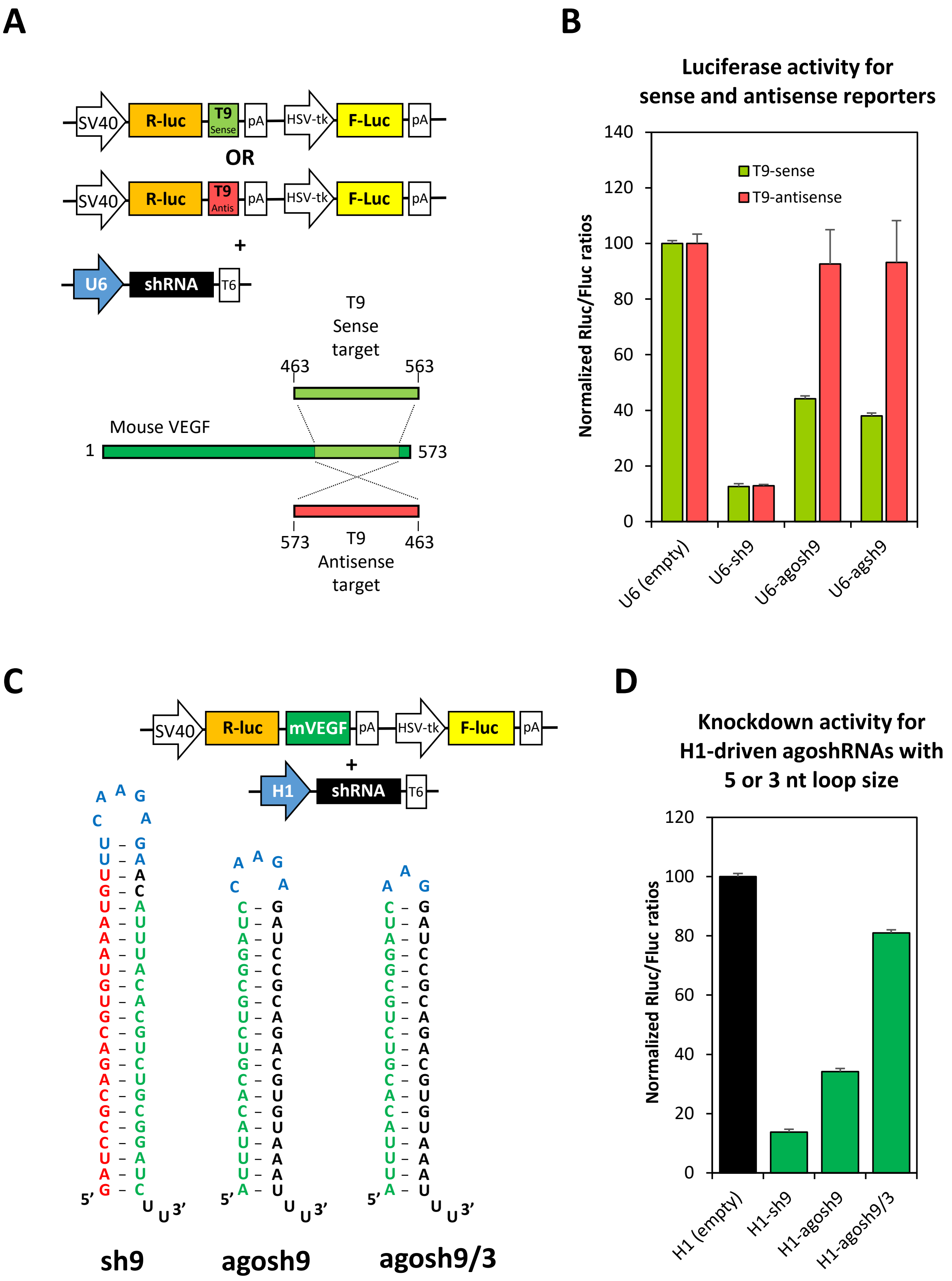
Supplemental Figure 1



Supplemental Figure 2

A

miR451 stem-loop structure

```
     A        GA                 A
UUGGG AUGGCAAG  AACCGUUACCAUUACUG G
GACCC UAUCGUUC  UUGGUAAUGGUAAUGAU U
     A        UC                 U
```

miR451-12.3 stem-loop structure

```
     A        GA                 C
UUGGG AUGGCAAG  GUAGGAAGCUCAUCUCU C
GACCC UAUCGUUC  CAUCCUUCGAGUAGAGA U
     A        UC                 A
```

B

miR324 stem-loop structure

```
      GC     C   U C   A     U   U  AAAG
GACUAU  CUCCC GCA C CCU GGGCA UGG GU    C
CUGAUG  GGGGG CGU G GGA CCCGU ACC CA    U
      UU     U   C U   C     C   -  GAGG
```

miR324-12.3 stem-loop structure

```
      GC     A                 C
GACUAU  CUCCC GUAGGAAGCUCAUCUCU C
CUGAUG  GGGGG CAUCCUUCGAGUAGAGA U
      UU     C                 A
```

C

miR215 stem-loop structure

```
   A      ------      A A  A    -U   U          UAUAG
GAA UGGUAU      ACAGGA A UG CCUA  GAA UGACAGACAA     C
CUU AUCGUG      UGUCUU U AC GGAU  CUU ACUGUCUGUU    U
   C      UCAGUA      A A  C    UU   U          UGAG
```

miR215-12.3 stem-loop structure

```
   A      ------      AAA                 C
GAA UGGUAU      ACAGGA   GUAGGAAGCUCAUCUCU C
CUU AUCGUG      UGUCUU   CAUCCUUCGAGUAGAGA U
   C      UCAGUA      AUC                 A
```

Supplemental Figure 2

D

miR409 stem-loop structure

```
     U  GG   A    AC          -    - AUC
GGUAC CG  GAG GGUU  CCGAGCAAC UUUG C   U
CUAUG GC  UUC CCAA  GGCUCGUUG AAGC G   G
     -  UU   C    GU          U    A CAG
```

miR409-12.3 stem-loop structure

```
     U  GG   A                 C
GGUAC CG  GAG GUAGGAAGCUCAUCUCU C
CUAUG GC  UUC CAUCCUUCGAGUAGAGA U
     -  UU   C                 A
```

E

miR30a stem-loop structure

```
   A            UC           -----   A
GCG CUGUAAACAUCC  GACUGGAAGCU     GUG A
CGU GACGUUUGUAGG  CUGACUUUCGG     CAC G
   C            --           GUAGA   C
```

miR30a-v1-12.3 stem-loop structure

```
   A                   C
GCG CAGUAGGAAGCUCAUCUCU C
CGU GUCAUCCUUCGAGUAGAGA U
   C                   A
```

miR30a-v2-12.3 stem-loop structure

```
   ACA                 C
GCG   GUAGGAAGCUCAUCUCU C
CGU   CAUCCUUCGAGUAGAGA U
   CGC                 A
```

Supplemental Figure 2

F

miR106b stem-loop structure

```
     C     -UA       G       A A    --  UC
CCUGC GGGGC   AAGUGCU ACAGUGC G UAGU  GG  C
GGACG CCUCG   UUCAUGG UGUCACG C AUCG  CC  U
     A     UCG       G       C -    UG  UC
```

miR106b-v1-12.3 stem-loop structure

```
     C     AG                 C
CCUGC GGGGC   UAGGAAGCUCAUCUCU C
GGACG CCUCG   AUCCUUCGAGUAGAGA U
     A     AGA                A
```

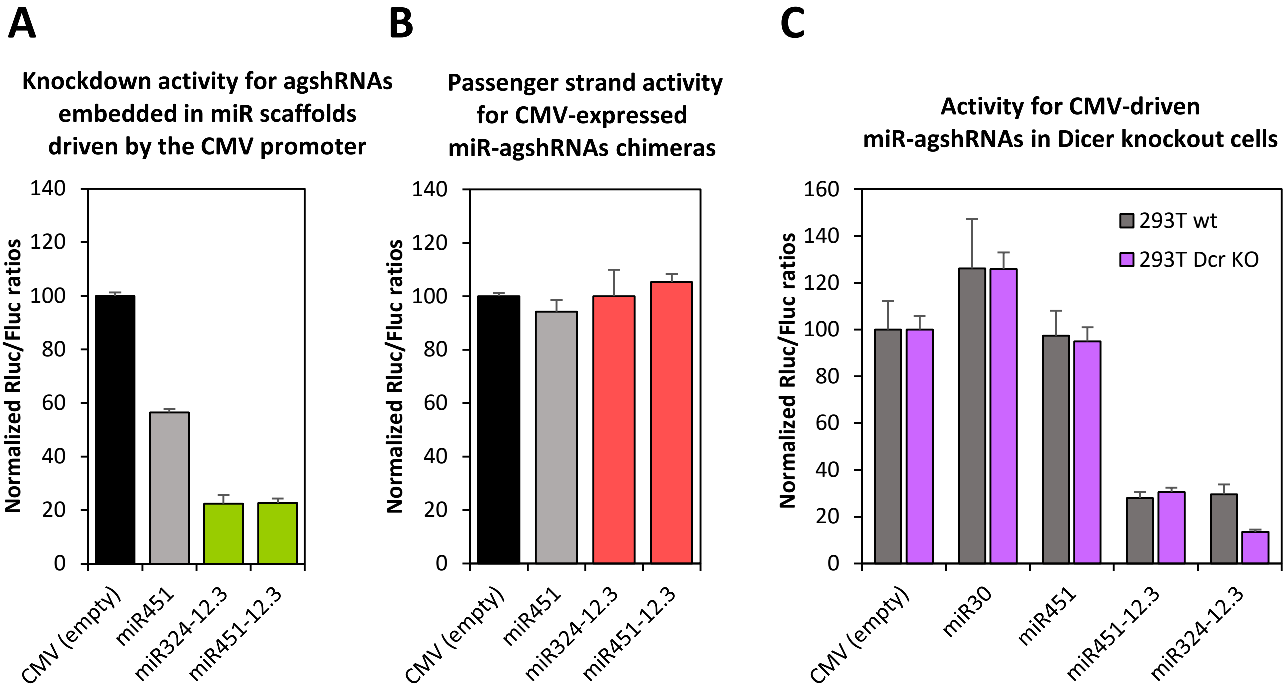
miR106b-v2-12.3 stem-loop structure

```
     C     A                  C
CCUGC GGGGC  GUAGGAAGCUCAUCUCU C
GGACG CCUCG  CAUCCUUCGAGUAGAGA U
     A     AC                 A
```

miR106b-v3-12.3 stem-loop structure

```
     C    CA                 C
CCUGC GGGG  GUAGGAAGCUCAUCUCU C
GGACG CCUU  CAUCCUUCGAGUAGAGA U
     A    UC                 A
```

Supplemental Figure 3

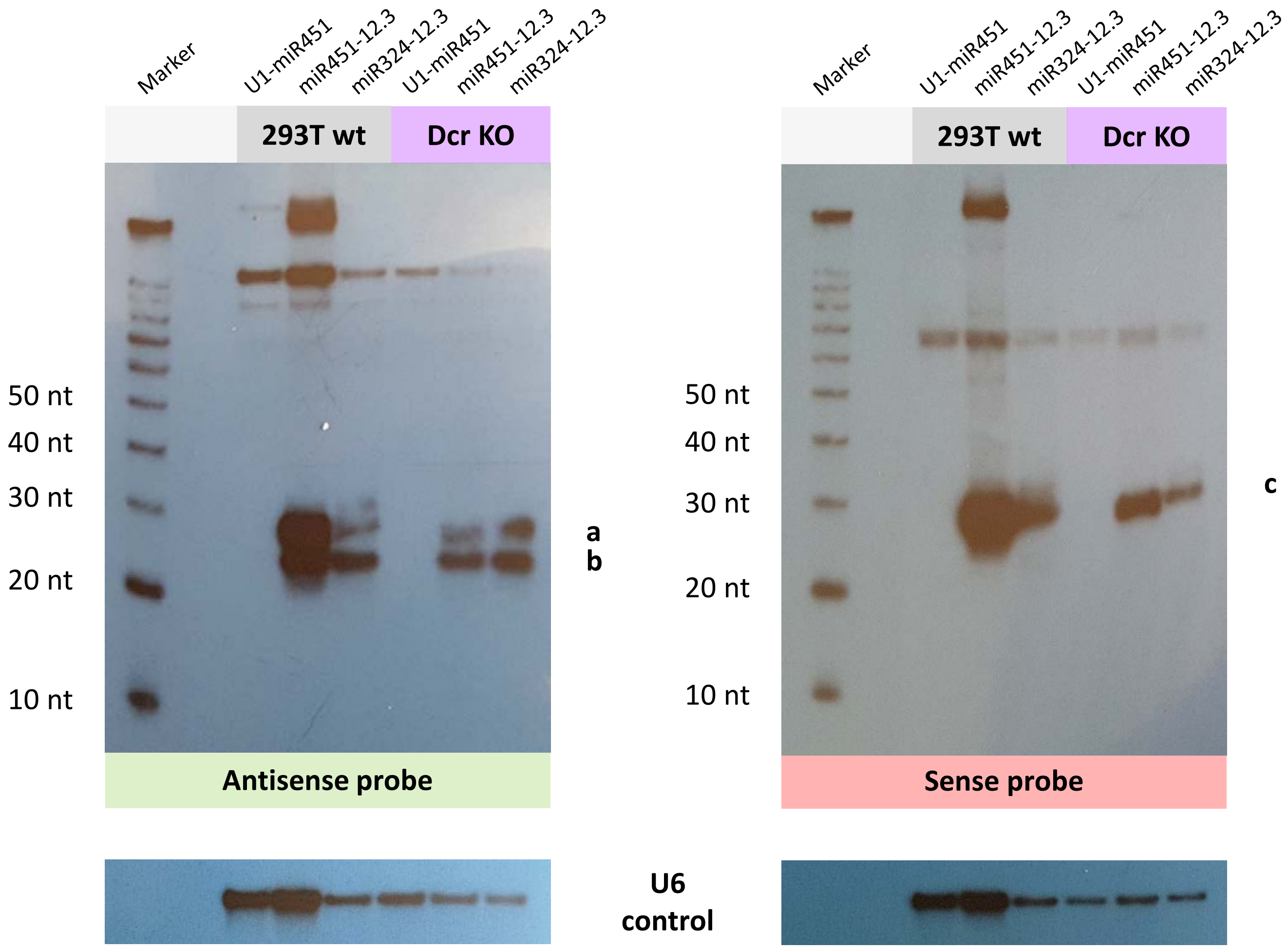


A Knockdown activity for agshRNAs embedded in miR scaffolds driven by the CMV promoter

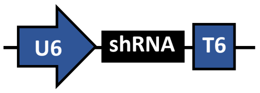
B Passenger strand activity for CMV-expressed miR-agshRNAs chimeras

C Activity for CMV-driven miR-agshRNAs in Dicer knockout cells

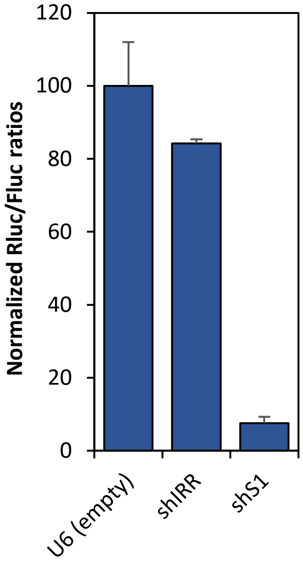
Supplemental Figure 4



Supplemental Figure 5



Knockdown activity
for the U6-driven shRNA
targeting HIV-1 ‘site 1’ (S1)



Supplemental Figure 1: Comparison of conventional and Dicer-independent shRNA designs and two agoshRNA designs with varying loop size. A) Schematic diagram of the dedicated T9-sense and T9-antisense psiCHECK2-based luciferase reporters used for comparing guide and passenger strand activity, respectively, for the various shRNA designs shown in Figure 1A. The illustration at the bottom shows the 100 nt long cDNA region of mVEGF (dubbed T9) encompassing 'site 9' which was cloned into the psiCHECK2-based reporter shown above in sense or antisense orientation (numbers indicate cDNA position). **B)** Knockdown activity of the shRNA designs depicted in Figure 1A when expressed by the human U6 promoter (pFTR-U6),[29] estimated by co-transfection a psiCHECK2-based Renilla luciferase reporter fused to a 100 nt stretch of the VEGF gene (T9-sense) or a corresponding antisense reporter (T9-antisense) as illustrated in panel A. Renilla luciferase (Rluc) and Firefly luciferase (Fluc) activity was measured in relative units of light (RLU). The Rluc/Fluc ratio was normalized to the empty control and plotted as the mean of three replicates plus standard deviations. **C)** Predicted secondary structure of the conventionally designed shRNAs targeting 'site 9' in the VEGF gene[26] (sh9, left side), or two loop variants of the Ago2-dependent agoshRNA structure, either a '19/5' (agosh9, middle part) or a '19/3' (agosh9/3, right side) design as reported by the Berkhout lab.[64] The guide and passenger strands are depicted in green and red, respectively. The ‘Brummelkamp’ loop is shown in blue. The diagrams above show the constructs used in co-transfection assays. Upper part the psiCHECK2-based reporter fused to the full-length mVEGF cDNA, lower part the pSUPERretro-based H1-driven shRNA expression plasmid.[26] **D)** Knockdown activity of the shRNA designs shown in panel C when expressed by the human H1 promoter. The Rluc/Fluc ratio was normalized to the empty control and plotted as the mean of three replicates plus standard deviations. Abbreviations: Fluc, Firefly luciferase; HSV-tk, Herpes simplex virus thymidine kinase promoter; mVEGF, murine Vascular endothelial growth factor; pA, polyadenylation signal; R-luc, Renilla luciferase; shRNA, short hairpin RNA; SV40, simian virus 40 promoter; T6, T-rich pol-III termination signal; U6, human U6 snRNA promoter.

Supplemental Figure 2: Predicted secondary structure of the stem loop region of native pri-miRs and VEGF 12.3 targeting derivatives. A) Upper diagram depicts the stem loop structure of miR-451. The mature miR-451 is highlighted in pink. Lower diagram shows the structure of the miR-451 scaffold re-targeted towards VEGF (site 12.3). The VEGF targeting guide strand (21 nt) is shown in green. **B)** Upper diagram shows central part of pri-miR-324 and the location of the mature species (miR-324-5p and -3p, both highlighted in pink). Bottom diagram shows how the agsh12.3 stem loops structure was embedded in the pri-miR-324 scaffold at the predicted Drosha cleave site (VEGF targeting guide strand shown in green). **C)** Structure of the stem loop part of pri-miR-215 (top) and the corresponding miR215-agsh12.3 chimera (bottom). Mature miR strands and the 12.3 guide strand is shown in pink and green, respectively. **D)** Structure of the stem loop part of pri-miR-409 (top) and the corresponding miR409-agsh12.3 chimera (bottom). Colors as described above. **E)** Upper diagram shows the stem loop structure of pri-miR-30a. Diagrams below shows how the agsh12.3 stem loops structure was embedded in the pri-miR-30a scaffold at the predicted Drosha cleave site. The first version (v1) mimics the miR-30a structure at the Drosha cleavage site and does not include the basal A-C ‘fork’ from the agsh12.3 ‘pre-miR structure’, while the second version (v2) includes the A-C ‘fork’. **F)** Upper diagram shows the stem loop structure of pri-miR-106b. The diagrams below show three different strategies to embed agsh12.3. The first version (v1) mimics the miR-106b large bulge at the Drosha cleavage site and does not include the basal A-C ‘fork’ from the agsh12.3 hairpin. The second (v2) includes the A-C ‘fork’, while the third version (v3) includes an A-CUU ‘fork’ reminiscent of pol-III termination (T_6 box). All stem loop structures and prominent Drosha/Dicer cleavage sites shown in this figure, are based on data from miRBase[67] and the RNA secondary structure predictions algorithms MFOLD.[68]

Supplemental Figure 3: Knockdown of VEGF in luciferase-based reporters using Dicer-independent shRNAs expressed as CMV-driven pri-miR-324 or -451 transcripts. A) Knockdown activity of VEGF-targeting CMV-expressed agshRNA embedded in various miR scaffolds, estimated by co-transfection with psiCHECK-mVEGF-T12-sense. The Rluc/Fluc ratio was normalized to the empty control (black bar) and plotted as the mean of three replicates plus standard deviations. Endogenous miR-451 was included as a non-targeting control (grey bar). **B)** Passenger strand activity for CMV-driven miR324- and miR451-agshRNAs as measured using the psiCHECK-mVEGF-T12-antisense reporter and plotted as described in panel A. **C)** Knockdown levels of CMV-expressed miR-agsh12.3 constructs in Dicer knockout cells[35] (purple bars) and the parental 293T control cells (grey bars) as based on psiCHECK-mVEGF-T12-sense reporter and plotted as described in panel A, including miR-30 as a non-targeting control.

Supplemental Figure 4: Processing of miR-324- and miR-451-agshRNAs transcripts is Dicer-independent: Northern blot analysis of small RNA from transfected 293T cells and DcrKO 293T cells (NoDice 2-20) using probes detecting guide strand RNA (antisense probe, autoradiogram on left side) or passenger strand RNA (sense probe, autoradiogram on right side). The autoradiograms are short time exposures of the cropped images seen in Figure 2D (293T cells) and Figure 3C (Dcr KO cells). Size of 10, 20, 30, 40, and 50 nt band of the RNA decade marker is indicated, and agsh12.3-specific bands are marked by arrows and labeled a-c. A cropped image with detection of the native U6 snRNA band (loading control) is shown below.

Supplemental Figure 5: Knockdown activity of the U6-driven shS1: Knockdown activity of the conventionally designed (pBluescriptIISK-based) shS1 construct targeting the HIV-1 tat/rev transcript (site 1)[34], using experimental conditions similar to the data presented in Figure 4 for the pol-II driven miR-agshS1 constructs. The Rluc/Fluc ratio as measured by the psiCHECK-HIV-S1 reporter was normalized to the empty control (pBluescriptIISK-based) and plotted as the mean of three replicates plus standard deviations. An irrelevant HIV-1 ‘site 2’ targeting shRNA (shIRR) was included as a non-targeting control.

Supplemental table 1

Oligo ID	**Name**	**Sequence (5’-3’)**
Sequencing primers		
350	CMV-F	GCAAATGGGCGGTAGGCGTGTAC
351	BGH-R	AGGGGCAAACAACAGATGGC
352	BGH-R2	GAAGGCACAGTCGAGGCTGATCA
353	5AMP-R	AAGGGAATAAGGGCGACACG

354	3hLuc-F	GAGGACGCTCCAGATGAAATG
355	5TK-R	CGTCAGACAAACCCTAACCAC
350	CMV-F	GCAAATGGGCGGTAGGCGTGTAC
489	pcDNA3-5prom-F	TAAGCTACAACAAGGCAAGGCT
Cloning of psiCHECK2.2 based reporters		
487	mVEGF-R3-NotI-F	GAGA**GCGGCCGC**CATCACCATGCAGATCATGC
488	mVEGF-R3-NotI-R	GAGA**GCGGCCGC**TCTGTCTTTCTTTGGTCTGC
361	mVEGF-R4-NotI-F	GAGAG**CGGCCGCG**AGAAAGCATTTGTTTGTCCAAG
362	mVEGF-R4-NotI-R	GAGA**GCGGCCG**CTCACCGCCTTGGCTTGTCAC
481	RRM2-887-A	**TCGA**AGGCTACCTATGGTGAACGTGTTGTAGC
482	RRM2-887-B	**GGCC**GCTACAACACGTTCACCATAGGTAGCCT
483	RRM2-1354-A	**TCGA**GTGATGTCAAGTCCAACAGAGAATTCTT
484	RRM2-1354-B	**GGCC**AAGAATTCTCTGTTGGACTTGACATCAC
	HIV-S1-A	**TCGA**AAGCGGAGACAGCGACGAAGAGCT
	HIV-S1-B	**GGCC**AGCTCTTCGTCGCTGTCTCCGCTT
Cloning of pcDNA3-based pri-miR contructs		
465	miR106b-F	ATATA**CTCGAG**TGGTAAGTGCCCAAATTGCTG
466	miR106b-R	TGTGT**ACCGGT**GGATCTAGGACACATGGAGTG
467	miR451-F	ATATA**CTCGAG**CCAGCTCTGGAGCCTGACAAG
468	miR451-R	TGTGT**ACCGGT**ACCCCTGCCTTGTTTGAGCTG
469	miR30a-F	ATATA**CTCGAG**CAGAATCGTTGCCTGCACATC
470	miR30a-R	TGTGT**ACCGGT**ATGTATCAAAGAGATAGCAAGGT
Cloning of pcDNA3-based promoter and MCS variants		
207	MCS-A	**CTAG**CCTCGAGGGATCCGAATTCTCCGGATTTTTTACCGGTGGGCC
208	MCS-B	**CACC**GGTAAAAAATCCGGAGAATTCGGATCCCTCGAGG
209	U1prom-MluI-F	CAACAA**ACGCGT**CTAAGGACCAGCTTCTTTGGGAGA
210	U1term-ApaI-R	CAACAA**GGGCCC**GTCTACTTTTGAAACTCCAGAAAGTGGATCACCGGTCTGCAGGAATTCGATATCAAGC
Cloning of pSUPERretro-based H1 constructs		
451	agosh9-A	**GATC**CCCATTTACACGTCTGCGGATCCAAGAGATCCGCAGACGTGTAAATTTTTTC
452	agosh9-B	**TCGA**GAAAAAATTTACACGTCTGCGGATCTCTTGGATCCGCAGACGTGTAAATGGG
447	agosh9/3-A	**GATC**CCCATTTACACGTCTGCGGATCAGAGATCCGCAGACCTGTAAATTTTTTC
448	agosh9/3-B	**TCGA**GAAAAAATTTACACGTCTGCGGATCTCTGATCCGCAGACGTGTAAATGGG
Cloning of pFRT-U6-based constructs		
429	sh9-A	**GATC**GATCCGCAGACGTGTAAATGTTTCAAGAGAACATTTACACGTCTGCGGATCTTTTT
430	sh9-B	**TCGA**AAAAACATCCGCAGACGTGTAAATGTTCTCTTGAAACATTTACACGTCTGCGGATC
425	agosh9-A	**GATC**ATTTACACGTCTGCGGATCCAAGAGATCCGCAGACGTGTAAATTTTTT

426	agosh9-B	**TCGA**AAAAAATTTACACGTCTGCGGATCTCTTGGATCCGCAGACGTGTAAAT
475	agsh9-A	**GATC**AATTTACACGTCTGCGGATCTTTCCGCAGACGTGTAAATCTTTTT
476	agsh9-B	**TCGA**AAAAAGATTTACACGTCTGCGGAAAGATCCGCAGACGTGTAAATT
427	agsh11-A	**GATC**AACATTTACACGTCTGCGGATCCGCAGACGTGTAAATGTCTTTTT
428	agsh11-B	**TCGA**AAAAAGACATTTACACGTCTGCGGATCCGCAGACGTGTAAATGTT
433	agsh12.1-A	**GATC**ACTGTAGGAAGCTCATCTCTCCAGATGAGCTTCCTACAGCTTTTT
434	agsh12.1-B	**TCGA**AAAAAGCTGTAGGAAGCTCATCTGGAGAGATGAGCTTCCTACAGT
435	agsh12.2-A	**GATC**ATGTAGGAAGCTCATCTCTCCTGAGATGAGCTTCCTACACTTTTT
436	agsh12.2-B	**TCGA**AAAAAGTGTAGGAAGCTCATCTCAGGAGAGATGAGCTTCCTACAT
437	agsh12.3-A	**GATC**AGTAGGAAGCTCATCTCTCCTAAGAGATGAGCTTCCTACCTTTTT
438	agsh12.3-B	**TCGA**AAAAAGGTAGGAAGCTCATCTCTTAGGAGAGATGAGCTTCCTACT
477	sh12.3-A	**GATC**GGAGAGATGAGCTTCCTACTTCAAGAGAGTAGGAAGCTCATCTCTCCTTTTT
478	sh12.3-B	**TCGA**AAAAAGGAGAGATGAGCTTCCTACTCTCTTGAAGTAGGAAGCTCATCTCTCC
471	agsh13-A	**GATC**AAAGTACGTTCGTTTAACTCAAGTTAAACGAACGTACTTCTTTTT
472	agsh13-B	**TCGA**AAAAAGAAGTACGTTCGTTTAACTTGAGTTAAACGAACGTACTTT
Synthetic pri-miR-dishRNA inserts (pUC57-based)		
	miR30a-v1-12.3	**CTCGAG**ACAGAATCGTTGCCTGCACATCTTGGAAACACTTGCTGGGATTACTTCTTCAGGTTAACCCAACAGAAGGCTAAAGAAGGTATATTGCTGTTGACAGTGAGCGACAGTAGGAAGCTCATCTCTCCTAAGAGATGAGCTTCCTACTGCTGCCTACTGCCTCGGACTTCAAGGGGCTACTTTAGGAGCAATTATCTTGTTTACTAAAACTGAATACCTTGCTATCTCTTTGATACATTTTTACAAAGCTGAA**ACCGGT**
	miR30a-v2-12.3	**CTCGAG**ACAGAATCGTTGCCTGCACATCTTGGAAACACTTGCTGGGATTACTTCTTCAGGTTAACCCAACAGAAGGCTAAAGAAGGTATATTGCTGTTGACAGTGACCGACAGTAGGAAGCTCATCTCTCCTAAGAGATGAGCTTCCTACCGCTGGCTACTGCCTCGGACTTCAAGGGGCTACTTTAGGAGCAATTATCTTGTTTACTAAAACTGAATACCTTGCTATCTCTTTGATACATTTTTACAAAGCTGAA**ACCGGT**
	miR106b-v1-12.3	**CTCGAG**TGGTAAGTGCCCAAATTGCTGGAGGGCCATCTGTTTTGACCCTTAAAGGGGTAGCTCCTTACCGTGCTCTC

		ATTGCCGCCTCCCCACCTCCCGCTCCAGCCCTGCCGGGGCAGTAGGAAGCTCATCTCTCCTAAGAGATGAGCTTCCTAAGAGCTCCAGCAGGGCACGCACAGCGTCCGTGGAGGGAAAGGCCTTTTCCCCACTTCTTAACCTTCACTGAGAGGGTGGTTGGGGTCTGTTTCACTCCATGTGTCCTAGATCCT**ACCGGT**
	miR106b-v2-12.3	**CTCGAG**TGGTAAGTGCCCAAATTGCTGGAGGGCCATCTGTTTTGACCCTTAAAGGGGTAGCTCCTTACCGTGCTCTCATTGCCGCCTCCCCACCTCCCGCTCCAGCCCTGCCGGGGCAGTAGGAAGCTCATCTCTCCTAAGAGATGAGCTTCCTACCAGCTCCAGCAGGGCACGCACAGCGTCCGTGGAGGGAAAGGCCTTTTCCCCACTTCTTAACCTTCACTGAGAGGGTGGTTGGGGTCTGTTTCACTCCATGTGTCCTAGATCCT**ACCGGT**
	miR106b-v3-12.3	**CTCGAG**TGGTAAGTGCCCAAATTGCTGGAGGGCCATCTGTTTTGACCCTTAAAGGGGTAGCTCCTTACCGTGCTCTCATTGCCGCCTCCCCACCTCCCGCTCCAGCCCTGCCGGGGCAGTAGGAAGCTCATCTCTCCTAAGAGATGAGCTTCCTACCTTTCCAGCAGGGCACGCACAGCGTCCGTGGAGGGAAAGGCCTTTTCCCCACTTCTTAACCTTCACTGAGAGGGTGGTTGGGGTCTGTTTCACTCCATGTGTCCTAGATCCT**ACCGGT**
	miR215-12.3	**CTCGAG**GTTTTATAAAATTAACAAATGATTAAGAATTAATATCAATTTTCTTAAATTCAAGTTTTGTAACACCAAAAAGATCCAATAATGGAAGAGGATTAAAGTCATCATTCAGAAATGGTATACAGGAAAAGTAGGAAGCTCATCTCTCCTAAGAGATGAGCTTCCTACCTATTCTGTATGACTGTGCTACTTCAATATCAGAAATCGACTAACACCACGCAACCAACGCAATGGCAGGTACACAGAAGATAATCTGTAACACTACCATGTAAATGTATTGAGTAAATAAAACATA**ACCGGT**
	miR324-12.3	**CTCGAG**TTCTTAAAAGGGGTGGATGTAAGGGATGAGGTAGAATTAACTTCTGGTACTGCTGGCAGGCACCTGAGCAGAACATCATTGCTGTCTCTCTTCGCAGAAGCTGAGCTGACTATGCCTCCCAGTAGGAAGCTCATCTCTCCTAAGAGATGAGCTTCCTACCGGGGGTTGTAGTCTGACCCGACTGGGAAGAAAGCCCCAGGGCTCCAGGGAGAGGGGCTTGGGAGGCCCTCACCTCAGTTACATACTGCAGCATAACCATCCGTGCCAGCTTCTCCTGGATCAGCCCAAAGTTGTGAA**ACCGGT**
	miR409-12.3	**CTCGAG**TCTGGGCTCTGAATGCCCAGACCTTGTGCTGCCCTTGGGGGAGGGTCTTCTGCAAGCACAGCCGCCTGCAAGCATTCACCTTAGTCCGAGCATCTGAGCCTGGTACTCGGGGAGAGTAGGAAGCTCATCTCTCCTAAGAGATGAGCTTCCTACCCTTTTCGGTATCAGCTGGGGCACCTCGGGGAAGGACGCCGGCATCAGCACCATTCTGGGGTACGGGGATGGATGGTCGACCAGTTGGAAAGTAATTGTTTCTAATGTACTTCA**ACCGGT**

	miR451-12.3	**CTCGAG**CCAGCTCTGGAGCCTGACAAGGAGGACAGGAGAGATGCTGCAAGCCCAAGAAGCTCTCTGCTCAGCCTGTCACAACCTACTGACTGCCAGGGCACTTGGGAATGGCAAGGAGTAGGAAGCTCATCTCTCCTAAGAGATGAGCTTCCTACCTCTTGCTATACCCAGAAAACGTGCCAGGAAGAGAACTCAGGACCCTGAAGCAGACTACTGGAAGGGAGACTCCAGCTCAAACAAGGCAGGGGT**ACCGGT**
	miR324-HIV-S1	**CTCGAG**TTCTTAAAAGGGGTGGATGTAAGGGATGAGGTAGAATTAACTTCTGGTACTGCTGGCAGGCACCTGAGCAGAACATCATTGCTGTCTCTCTTCGCAGAAGCTGAGCTGACTATGCCTCCCATCTTCGTCGCTGTCTCCGCTTGGAGACAGCGACGAAGACGGGGGTTGTAGTCTGACCCGACTGGGAAGAAAGCCCCAGGGCTCCAGGGAGAGGGGCTTGGGAGGCCCTCACCTCAGTTACATACTGCAGCATAACCATCCGTGCCAGCTTCTCCTGGATCAGCCCAAAGTTGTGAA**ACCGGT**
	miR451-HIV-S1	**CTCGAG**CCAGCTCTGGAGCCTGACAAGGAGGACAGGAGAGATGCTGCAAGCCCAAGAAGCTCTCTGCTCAGCCTGTCACAACCTACTGACTGCCAGGGCACTTGGGAATGGCAAGGATCTTCGTCGCTGTCTCCGCTTGGAGACAGCGACGAAGACTCTTGCTATACCCAGAAAACGTGCCAGGAAGAGAACTCAGGACCCTGAAGCAGACTACTGGAAGGGAGACTCCAGCTCAAACAAGGCAGGGGT**ACCGGT**
	miR215-RRM2-887	**CTCGAG**GTTTTATAAAATTAACAAATGATTAAGAATTAATATCAATTTTCTTAAATTCAAGTTTTGTAACACCAAAAAGATCCAATAATGGAAGAGGATTAAAGTCATCATTCAGAAATGGTATACAGGAAAACAACACGTTCACCATAGGTAGTTATGGTGAACGTGTTGCTATTCTGTATGACTGTGCTACTTCAATATCAGAAATCGACTAACACCACGCAACCAACGCAATGGCAGGTACACAGAAGATAATCTGTAACACTACCATGTAAATGTATTGAGTAAATAAAACATA**ACCGGT**
	miR324-RRM2-887	**CTCGAG**TTCTTAAAAGGGGTGGATGTAAGGGATGAGGTAGAATTAACTTCTGGTACTGCTGGCAGGCACCTGAGCAGAACATCATTGCTGTCTCTCTTCGCAGAAGCTGAGCTGACTATGCCTCCCACAACACGTTCACCATAGGTAGTTATGGTGAACGTGTTGCGGGGGTTGTAGTCTGACCCGACTGGGAAGAAAGCCCCAGGGCTCCAGGGAGAGGGGCTTGGGAGGCCCTCACCTCAGTTACATACTGCAGCATAACCATCCGTGCCAGCTTCTCCTGGATCAGCCCAAAGTTGTGAA**ACCGGT**
	miR409-RRM2-887	**CTCGAG**TCTGGGCTCTGAATGCCCAGACCTTGTGCTGCCCTTGGGGGAGGGTCTTCTGCAAGCACAGCCGCCTGCAAGCATTCACCTTAGTCCGAGCATCTGAGCCTGGTACTCGGGGAGACAACACGTTCACCATAGGTAGTTATGGTGAACGTGTTGCCTTTTCGGTATCAGCTGGGGCACCTCGGGGAAGGACGCCGGCATCAGCACCATTCTGGGGTACGGGGATGGATGGTCGACCAGTTGGAAAGTAATTGTTTCTAATGTACTTCA**ACCGGT**

	miR451-RRM2-887	**CTCGAG**CCAGCTCTGGAGCCTGACAAGGAGGACAGGAGAGATGCTGCAAGCCCAAGAAGCTCTCTGCTCAGCCTGTCACAACCTACTGACTGCCAGGGCACTTGGGAATGGCAAGGACAACACGTTCACCATAGGTAGTTATGGTGAACGTGTTGCTCTTGCTATACCCAGAAAACGTGCCAGGAAGAGAACTCAGGACCCTGAAGCAGACTACTGGAAGGGAGACTCCAGCTCAAACAAGGCAGGGGT**ACCGGT**
	miR215-RRM2-1354	**CTCGAG**GTTTTATAAAATTAACAAATGATTAAGAATTAATATCAATTTTCTTAAATTCAAGTTTTGTAACACCAAAAAGATCCAATAATGGAAGAGGATTAAAGTCATCATTCAGAAATGGTATACAGGAAAAATTCTCTGTTGGACTTGACATTAAGTCCAACAGAGAATCTATTCTGTATGACTGTGCTACTTCAATATCAGAAATCGACTAACACCACGCAACCAACGCAATGGCAGGTACACAGAAGATAATCTGTAACACTACCATGTAAATGTATTGAGTAAATAAAACATA**ACCGGT**
	miR324-RRM2-1354	**CTCGAG**TTCTTAAAAGGGGTGGATGTAAGGGATGAGGTAGAATTAACTTCTGGTACTGCTGGCAGGCACCTGAGCAGAACATCATTGCTGTCTCTCTTCGCAGAAGCTGAGCTGACTATGCCTCCCAATTCTCTGTTGGACTTGACATTAAGTCCAACAGAGAATCGGGGGTTGTAGTCTGACCCGACTGGGAAGAAAGCCCCAGGGCTCCAGGGAGAGGGGCTTGGGAGGCCCTCACCTCAGTTACATACTGCAGCATAACCATCCGTGCCAGCTTCTCCTGGATCAGCCCAAAGTTGTGAA**ACCGGT**
	miR409-RRM2-1354	**CTCGAG**TCTGGGCTCTGAATGCCCAGACCTTGTGCTGCCCTTGGGGGAGGGTCTTCTGCAAGCACAGCCGCCTGCAAGCATTCACCTTAGTCCGAGCATCTGAGCCTGGTACTCGGGGAGAATTCTCTGTTGGACTTGACATTAAGTCCAACAGAGAATCCTTTTCGGTATCAGCTGGGGCACCTCGGGGAAGGACGCCGGCATCAGCACCATTCTGGGGTACGGGGATGGATGGTCGACCAGTTGGAAAGTAATTGTTTCTAATGTACTTCA**ACCGGT**
	miR451-RRM2-1354	**CTCGAG**CCAGCTCTGGAGCCTGACAAGGAGGACAGGAGAGATGCTGCAAGCCCAAGAAGCTCTCTGCTCAGCCTGTCACAACCTACTGACTGCCAGGGCACTTGGGAATGGCAAGGAATTCTCTGTTGGACTTGACATTAAGTCCAACAGAGAATCTCTTGCTATACCCAGAAAACGTGCCAGGAAGAGAACTCAGGACCCTGAAGCAGACTACTGGAAGGGAGACTCCAGCTCAAACAAGGCAGGGGT**ACCGGT**
Northern blot probes		
382	U6	TATGGAACGCTTCTCGAATT
C146	12.3-antisense	AGAGATGAGCTTCCTAC
C145	12.3-sense	GTAGGAAGCTCATCTCT
C147	12.3-sense-short	GTAGGAAGCTCAT

Restriction site tags or compatible overhangs marked in bold. LNAs are underlined.

3’