

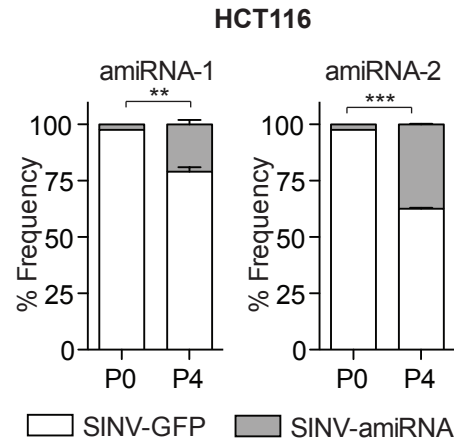
**Table S1**

**Table S1: Frequency of reads of the top ten most enriched amiRNAs in each replicate after four rounds of serial passage in CT26WT cells.**

| amiRNA ID | Sequence               | Frequency of Reads (%) |         |         |         |         |         |
|-----------|------------------------|------------------------|---------|---------|---------|---------|---------|
|           |                        | P0                     | P4-R1   | P4-R2   | P4-R3   | P4-R4   | P4-R5   |
| 1         | GTGGGAGAGGAGACATGTTGGC | 0.0343                 | 26.1698 | 34.9893 | 28.8833 | 58.5099 | 36.5393 |
| 2         | ATGTTGTTGGAGAATAAGACAA | N.D.                   | 39.8990 | 40.1095 | 4.2531  | 7.2182  | 4.4809  |
| 3         | ATGCAGTATGACACTCTCTGCA | N.D.                   | 2.4948  | 4.5950  | 8.2516  | 12.0567 | 9.3752  |
| 4         | TTAACGTGACAGACATGGCGGC | N.D.                   | 0.0058  | 0.2513  | 26.6349 | 0.0433  | N.D.    |
| 5         | AGGCACAGACAGGTGTCTTCCA | N.D.                   | N.D.    | 9.1190  | 1.7738  | 1.6772  | 4.5425  |
| 6         | ATGAGCAAGAGAGATGGATGTC | N.D.                   | 0.7116  | 1.2709  | N.D.    | 1.4508  | 7.8865  |
| 7         | ATTCCTTGGCTTCATTTGTGCT | N.D.                   | 6.4415  | 0.3972  | 0.2905  | 3.9819  | N.D.    |
| 8         | GTTTCCACCTATGGCCACTGGT | N.D.                   | 0.9696  | 0.9448  | 4.3227  | 1.2053  | 0.4803  |
| 9         | TATTGAATGGAGGTCCTAAGGG | N.D.                   | N.D.    | N.D.    | N.D.    | 1.9710  | 5.7629  |
| 10        | TTCGATGAACATGGATTCTGCA | N.D.                   | 0.0238  | N.D.    | 3.6999  | 0.0024  | 3.7873  |
| 11        | TAAATGGGTCTGACACTTAGAA | N.D.                   | N.D.    | N.D.    | N.D.    | 0.0075  | 6.1047  |
| 12        | TTGGTGTGGGTTCTGGTCAGCA | N.D.                   | 0.2364  | 2.4662  | N.D.    | 1.0219  | 2.2367  |
| 13        | TTTCGGCTTCAGTTCACTCCGG | N.D.                   | 0.9158  | 0.1483  | 0.1926  | 0.4143  | 3.2441  |
| 14        | TGGCATTCTGAATGGACCTGCC | N.D.                   | 4.5921  | N.D.    | N.D.    | N.D.    | N.D.    |
| 15        | TGTGAACAGCATGGTTTGGCAC | N.D.                   | 4.3619  | N.D.    | N.D.    | N.D.    | N.D.    |
| 16        | TTTGACTCTGGCTATTAGTCCG | N.D.                   | N.D.    | N.D.    | N.D.    | 4.3512  | N.D.    |
| 17        | TTTCCTGAGGATATTATTGGGG | N.D.                   | N.D.    | N.D.    | N.D.    | N.D.    | 4.2352  |
| 18        | TACTGTAAAGATTCAAGACCC  | 0.0250                 | 1.5417  | N.D.    | 1.8560  | N.D.    | N.D.    |
| 19        | TTCTGCAGGAATGAGATCACCA | N.D.                   | N.D.    | N.D.    | 3.2761  | N.D.    | N.D.    |
| 20        | GTGGTGACGGAGAGGTAGGGCC | N.D.                   | N.D.    | N.D.    | 2.6890  | N.D.    | N.D.    |
| 21        | TTTGGCACTGAAGTCATGTCCA | N.D.                   | 2.4590  | N.D.    | N.D.    | N.D.    | N.D.    |
| 22        | GATTCATGTGGAACACCAACAC | N.D.                   | N.D.    | N.D.    | 2.2136  | N.D.    | N.D.    |
| 23        | TGTTGGATGCCTCTTCAGGCCC | N.D.                   | N.D.    | N.D.    | 2.0613  | N.D.    | N.D.    |
| 24        | ATGCAGTTGGATTGACTTGCCT | N.D.                   | N.D.    | 1.9530  | N.D.    | N.D.    | N.D.    |
| 25        | TTGCTCAATCGAGTTCAGGGCC | N.D.                   | 1.7812  | N.D.    | N.D.    | N.D.    | N.D.    |
| 26        | TTGGAAGTTGGATACCATGGAG | N.D.                   | N.D.    | N.D.    | N.D.    | 1.4934  | N.D.    |
| 27        | CAGCTCATACTGGAATCTGGC  | N.D.                   | N.D.    | 0.4907  | 0.0092  | N.D.    | N.D.    |
| 28        | TTGCAGCAGCATGGGAGTGCCC | N.D.                   | N.D.    | 0.4452  | N.D.    | N.D.    | N.D.    |

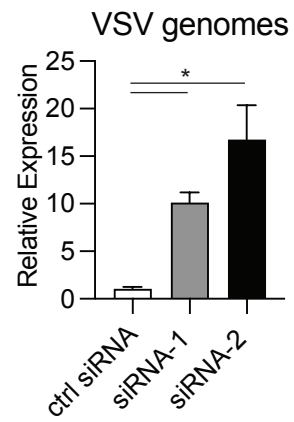
P: passage ; R: replicate ; N.D.: not detected

**Figure S1**



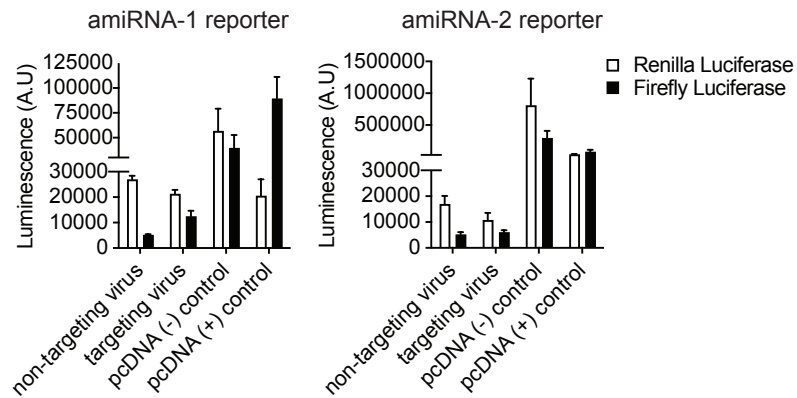
**Supplemental Figure S1: SINV-expressing amiRNA viruses outcompete parental virus in HCT116 cells.** Enrichment of amiRNA-expressing of SINV from competition experiments against parental virus SINV-GFP. The percentage of each virus is shown before competition (P0) and after 4 rounds of competition (P4) (n=2). Data are presented as means  $\pm$  SEM. Student's unpaired, one-tailed t-test was performed; \*\*:  $p < 0.01$ , \*\*\*:  $p < 0.001$ .

**Figure S2**



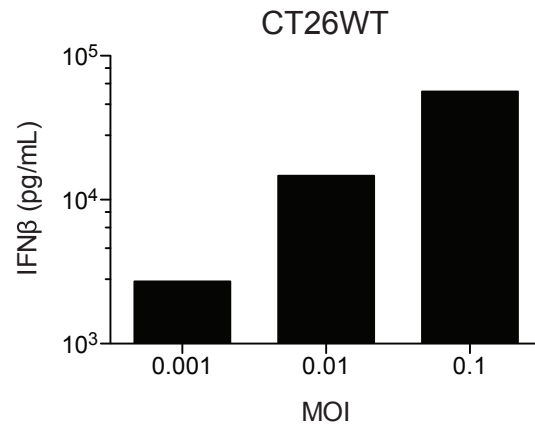
**Supplemental Figure S2: siRNA-1 and -2 increase viral genome copies in infected cells.** RT-qPCR analysis of VSV genomes following VSV infection of siRNA-treated CT26WT cells (n=3). Data are presented as means  $\pm$  SEM. Student's unpaired, two-tailed t-test with Welch's correction was performed; \*:  $p < 0.05$ .

**Figure S3**



**Supplemental Figure S3: Dual-luciferase reporter assay.** BHK cells were transfected with the indicated reporter plasmids possessing target sites for the enriched amiRNAs in the 3' UTR of the Renilla luciferase reporter and subsequently infected with the indicated VSV viruses. Renilla and Firefly luciferase activity was measured 24h post-infection. Raw, non-normalized luminescence values are shown (n=3).

**Figure S4**



**Supplemental Figure S4: IFN- $\beta$  production correlates with VSV $\Delta$ 51 replication.** CT26WT cells were infected with VSV $\Delta$ 51 at the indicated MOIs and IFN- $\beta$  concentrations in the culture supernatants 24h post-infection were measured by ELISA (n=1).

**Table S2****Table S2: List of primers used for qPCR analysis.**

| <b>Gene Name</b>              | <b>Forward Primer (5'-3')</b> | <b>Reverse Primer (5'-3')</b> |
|-------------------------------|-------------------------------|-------------------------------|
| <b>APLN</b>                   | GTGGATCCTGACATGGTTCTATAC      | GTCCTTTGGGCTCTGACTTT          |
| <b>ASPN</b>                   | GATACAAAAGGACACGTTCAAGG       | GAGGTTAGTTTTGCTTCAGCG         |
| <b>DKK2</b>                   | GAAGAATGAGGGATGTGGTAAGA       | CATAACGGAAGCACTGGTAGTA        |
| <b>GAPDH</b>                  | ACCACAGTCCATGCCATCAC          | TCCACCACCCTGTTGCTGTA          |
| <b>IFI44</b>                  | TCCATCCCTTTAGAGTGCTA          | GCAAGCAGAACTAAGCTCAT          |
| <b>IFIT3</b>                  | TCTCAGAAGCTCAGGCTTAC          | ACATTTTTGCTCGTTCATTT          |
| <b>IFN-<math>\beta</math></b> | GATGACGGAGAAGATGCAGAAG        | ACCCAGTGCTGGAGAAATTG          |
| <b>MX2</b>                    | ATCAATGCTTTTAACCAGGA          | TTTTCGATCTCCTTACTCCA          |
| <b>OASL2</b>                  | TAAGCTGCTTTTCCAGTTTC          | AGGACAATGATGTTGTAGGC          |
| <b>OGN</b>                    | AGCCCAAGAGCACCATTT            | CACCTTCCTCTGAAGCTTAGTT        |
| <b>OMD</b>                    | ATCCAGTACACCATCACCATTT        | GTGTATACGAGGGAAGCAGAAG        |
| <b>VSV</b>                    | GATAGTACCGGAGGATTGACGACTA     | TCAAACCATCCGAGCCATTC          |