SUPPLEMENTARY DATA

Supplementary Text. Sequence and structure of RNAs used in this study. The sequence of

mature miRNAs is indicated in red.

Synthetic siRNA duplexes and ss siRNAs

- GFP siRNA
  ```
  5'-pGGCUACGUCCAGGAGCGCACC
     UUCCGAUGCAGGUCCUCGCGUp-5'
  ```

- CLP ss siRNA
  ```
  5'-pAAAUUCUGUACGACCUCCUdtdt
  ```

- Dicer ss siRNA
  ```
  5'-pUCCAGAGCUGCUUCAAGCAdtdt
  ```

- Rluc siRNA (siRluc)
  ```
  5'-pGGCCUUUCACUACUCCUACdtdt
     dtdtCCGGAAAGUGAUGAGGAUGp-5'
  ```

- Ctl siRNA
  ```
  5'-pGUAUAAUACACCGCGCUACdtdt
     dtdtCAUAUUAUGUGGCGCGAUGp-5'
  ```

- Rluc antisense ss siRNA (asRluc)
  ```
  5'-pGUAGGAGUAGUGAAAGGCCdtdt
  ```

- Ctl antisense ss siRNA
  ```
  5'-pGUAGCGCGGUGUAUUAUACdtdt
  ```

Synthetic pre-miRNAs, miRNA:miRNA* duplexes and miRNAs

- pre-hsa-miR-31
  ```
               G   C            -U   GAA
      5'-GGCAA AUG UGGCAUAGC  GUU    C
         CCGUU UAC ACCGUAUCG   CAA   U
      3'-UA    A   A           UC  GGG
  ```
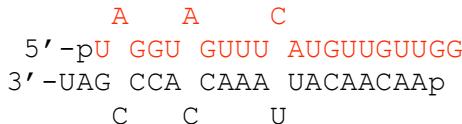
- hsa-miR-31
  ```
  5'-pGGCAAGAUGCUGGCAUAGCUG
  ```

- cel-let-7
  ```
  5'-pUGAGGUAGUAGGUUGUAUAGUU
  ```

- hsa-miR-196-1 miRNA:miRNA*

```
            A   A    C
  5'-pU GGU GUUU AUGUUGUUGG
  3'-UAG CCA CAAA UACAACAAp
        C   C      U
```
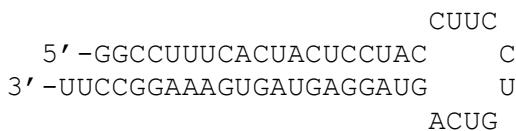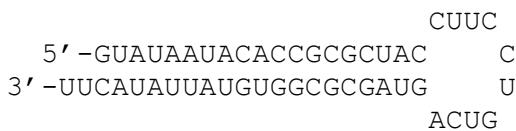
- hsa-miR-196-1
  ```
  5'-pUAGGUAGUUUCAUGUUGUUGG
  ```


psiSTRIKE-derived shRNAs and pre-miRNA

- Rluc shRNA
  ```
                          CUUC
    5'-GGCCUUUCACUACUCCUAC    C
    3'-UUCCGGAAAGUGAUGAGGAUG  U
                          ACUG
  ```

- Control shRNA
  ```
                          CUUC
    5'-GUAUAAUACACCGCGCUAC    C
    3'-UUCAUAUUAUGUGGCGCGAUG  U
                          ACUG
  ```


Target RNAs (synthesized by *in vitro* transcription)

- CLP RNA target (104 nt, CLP nt 414-514)
  ```
  5'-GGGCUGCAGCGCGCCAAAACCGGGACGGACAAGACCCUGGUGAAGGAGGUCGUAC
  AGAAUUUCGCUAAGGAGUUUGUGAUCAGUGAUCGGAAGGAGCUGGAGGA
  ```

- Dicer RNA target (104 nt, Dicer nt 294-394)
  ```
  5'-GGGCAUGAUAACAUUUAUACGCCAAGAAAAUAUCAGGUUGAACUGCUUGAAGCAG
  CUCUGGAUCAUAAUACCAUCGUCUGUUUAAACACUGGCUCAGGGAAGAC
  ```

- *lin-41* RNA target
  ```
  5'-GGGGCACCAACTCAAGTATACCTTTTATACAACCGTTCTACACTCAACGCGATGT
  AAATATCGCAA
  ```
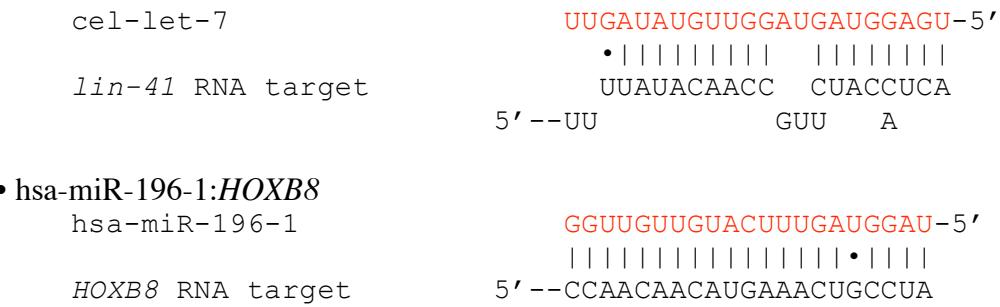
- *HOXB8* RNA target
  ```
  5'-GGGCCGATTTCTGCTAAGTTCTCCCAACAACATGAAACTGCCTATTCACGCCG
  TAATTCTTTCT
  ```

The first 3 G's are incorporated by the T7 polymerase during transcription. The siRNA/miRNA binding sites are underlined.


miRNA:target base pairing

- cel-let-7:*lin-41*

```
cel-let-7                               UUGAUAUGUUGGAUGAUGGAGU-5'
                                        •||||||||| |||||||||
        lin-41 RNA target               UUAUACAACC  CUACCUCA
                                5'--UU           GUU    A
```

• hsa-miR-196-1:*HOXB8*

```
        hsa-miR-196-1                   GGUUGUUGUACUUUGAUGGAU-5'
                                        ||||||||||||||||•||||
        HOXB8 RNA target        5'--CCAACAACAUGAAACUGCCUA
```

<u>siRNA:target base pairing</u>

All the siRNA:target combinations are perfectly complementarity.