

Primer	Sequence
pEGFP-Mirt, psiCheck2.2, shRNA and DMPK Cloning	
Mirtron cloning	5'-caag – mirtron sequence forward-3' and 5'-cgtc – mirtron sequence reverse-3'
VEGFA T1 for (Mirt 1, 2, 7, 8)	5'-tcgatgcagattatgcggatcaaacctgtagacacaccacccaccacaca tacatacatttatgcggatcaaacctcacc-3'
	5'-ggcctgggtagggttgatccgcataaatgtatgtatgtgggtgggtggg tgtgtctacaggttgatccgcataaatctgca-3'
VEGFA T2 for (Mirt 3, 4, 5, 6)	5'-tcgagcctgacctgctgctctacctccacatgatctttttgt cccactaatgtattggtgtcttcac-3'
	5'-ggcctggaagacaccaataacattagtgaggacaaaaaaagatcat gtggaggtagagcagcaaggcaaggc-3'
shRNA U6 Reverse	5'-gggtttcgtccttccacaa – <i>mirtron sequence reverse</i> -3'
U6 Forward	5'-ggctgcaggtcgacggat-3'
Quantitative PCR	
hGAPDH F	5'-aaggtgaaggtcggagtcaa-3'
hGAPDH R	5'-gaagatggtgatggatttc-3'
VEGFA F	5'-cggatcaaacctcacc-3'
VEGFA R	5'-aggaggctcctcctcct-3'

Supplementary Table S1. Oligonucleotides used in this study.

Natural Mirt877

```
      ta      at  c c          g caaagac          c
g  gaggag  gg g aggggacacg g          ttggggggtt c
      |||||  || | ||||| ||||| |          ||||| ||||| t
cc  ctctc  cc c tctctgtgt c          gactcccag g
ga  --      --  t t          g -----A          g
```

Natural Mirt1224

```
g      cu          -----  u  aucau          c
ugagga  ggggaggugg          aggg agc          uagagc a
|||||  ||||| |||||          |||  |||          |||||
acuccu  cuucuccacc          uccc ucg          gucucg g
g      cu          cccuc  -  acucu          a
```

TMirt1224 Version 1

```
g      ct          -----  u  aucau          c
tgagga  ggggaggtgg          aggg agc          uagagc a
|||||  ||||| |||||          |||  |||          |||||
acuccu  cuucuccacc          uccc ucg          gucucg g
t      cu          cccuc  -  acucu          a
t
aattttattatcaccatgacattgactctttcataatctacttag
```

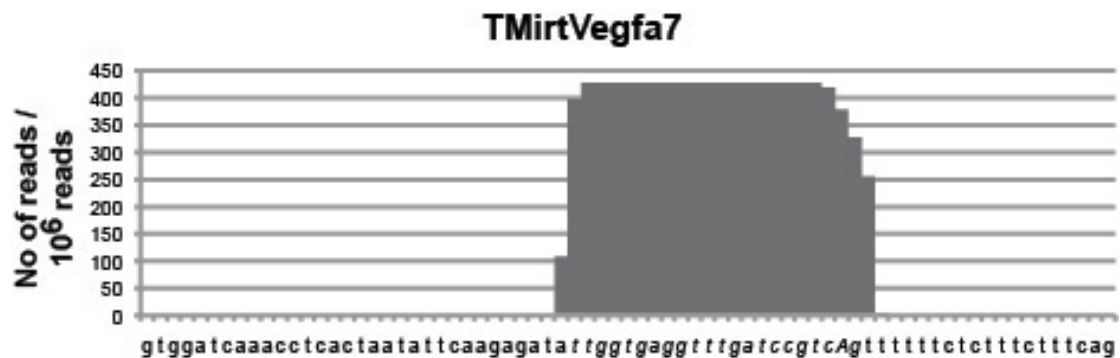
TMirt1224 Version 2

```
      t          tc
g  gaggactggggaggtgt a
| o||o||||| ||||| ||||| a
c  tccttgaccctccacca g
Act t          ga
g
atTTTaattttatttagcagcatgagattgactctttcataatctacttaag
```

Supplementary Figure S1. Mirt-877 and Mirt-1224 Natural and Tailed Mirtron designs. Cyan nucleotides indicate guide strand. Red nucleotides denotes branch point.



Supplementary Figure S4. Human VegfA Tailed Mirtron designs. Cyan nucleotides indicate guide strand. Red nucleotides denotes branch point.



Supplementary Figure S5. High throughput sequencing of small RNA from cells transfected with 500ng peGFP-TMirtVegfa7. Nucleotides in italics indicate guide strand; capital letter denotes branch point.