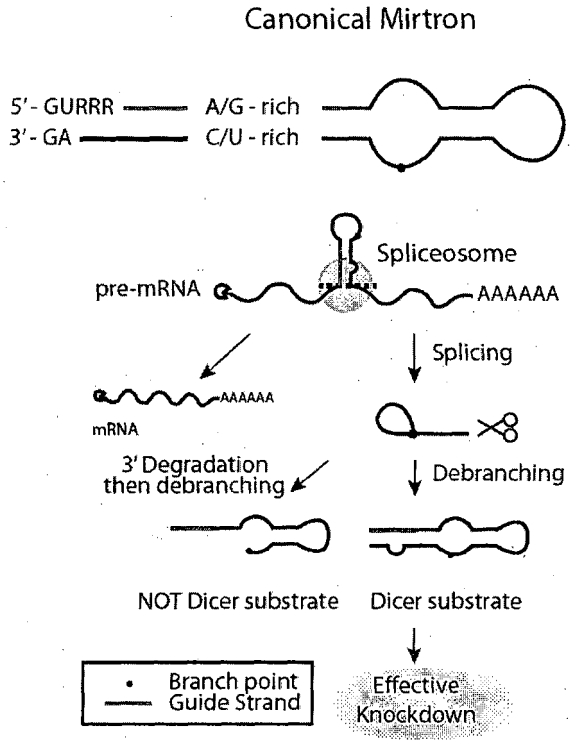

Canonical Mirtron
5'- GURRR
A/G - rich
3'- GA
C/U - rich
Spliceosome
pre-mRNA
AAAAAA
Splicing
AAAAAA
mRNA
3' Degradation
then debranching
Debranching
NOT Dicer substrate
Dicer substrate
Branch point
Guide Strand
Effective
Knockdown

Fig. 1a

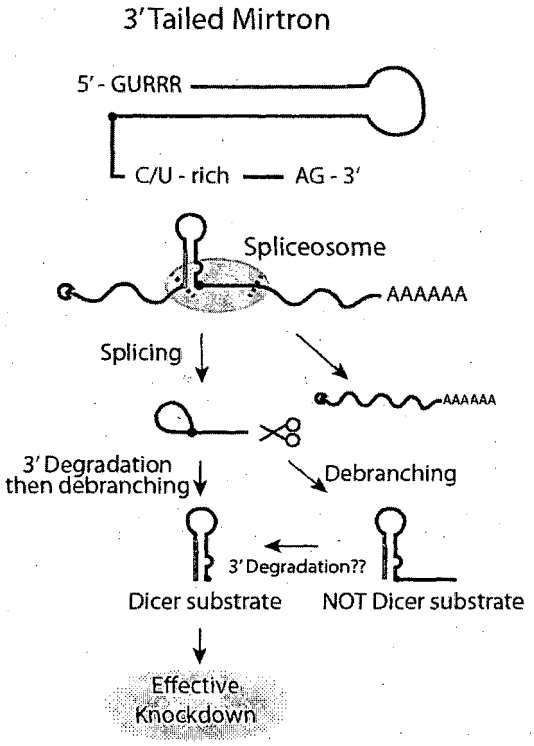
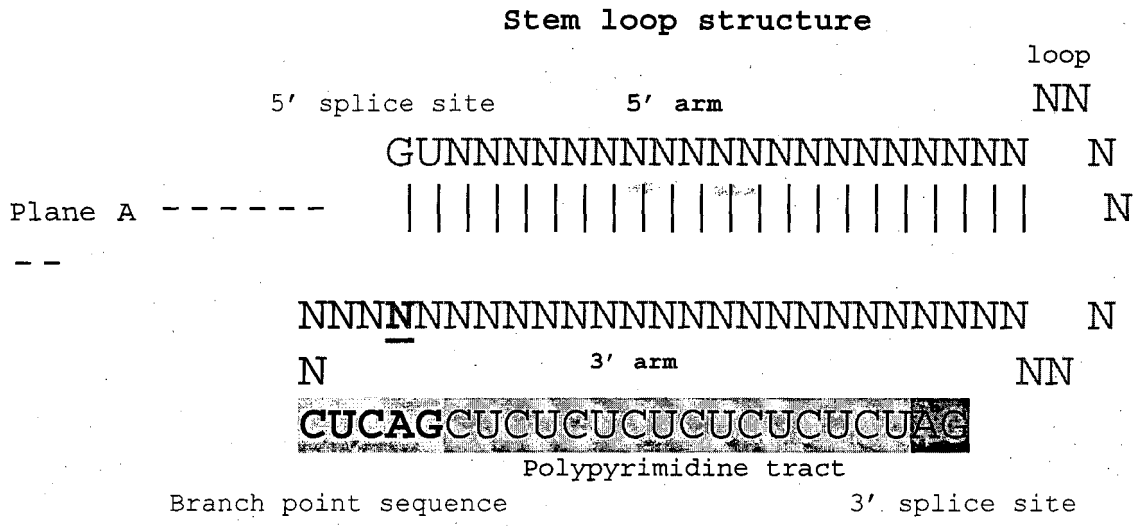

3'Tailed Mirtron
5'- GURRR
C/U - rich
AG - 3'
Spliceosome
AAAAAA
Splicing
AAAAAA
3' Degradation
then debranching
Debranching
3' Degradation??
Dicer substrate
NOT Dicer substrate
Effective
Knockdown

Fig. 1b



SEQ ID NO: 34

Fig. 2

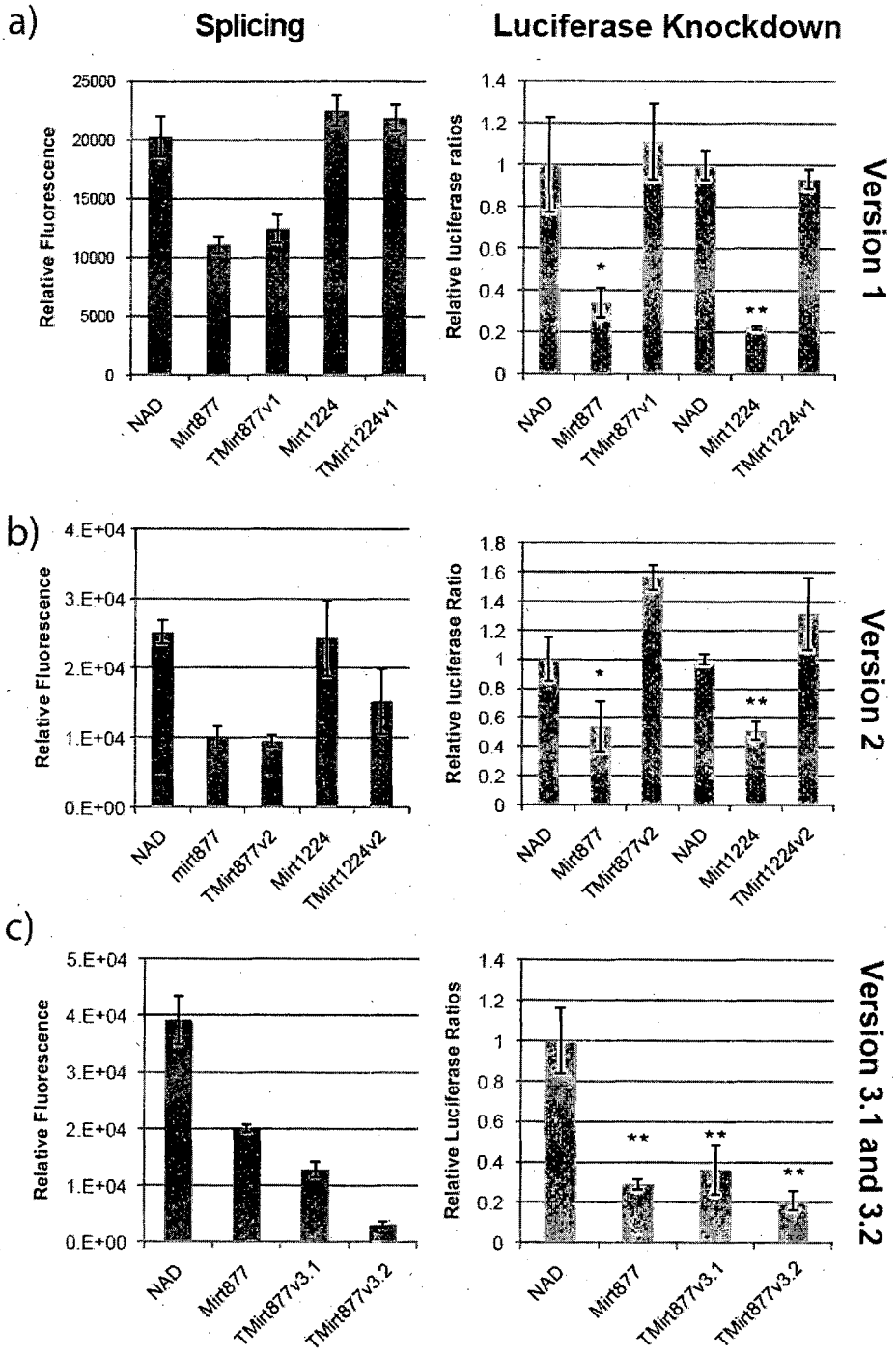
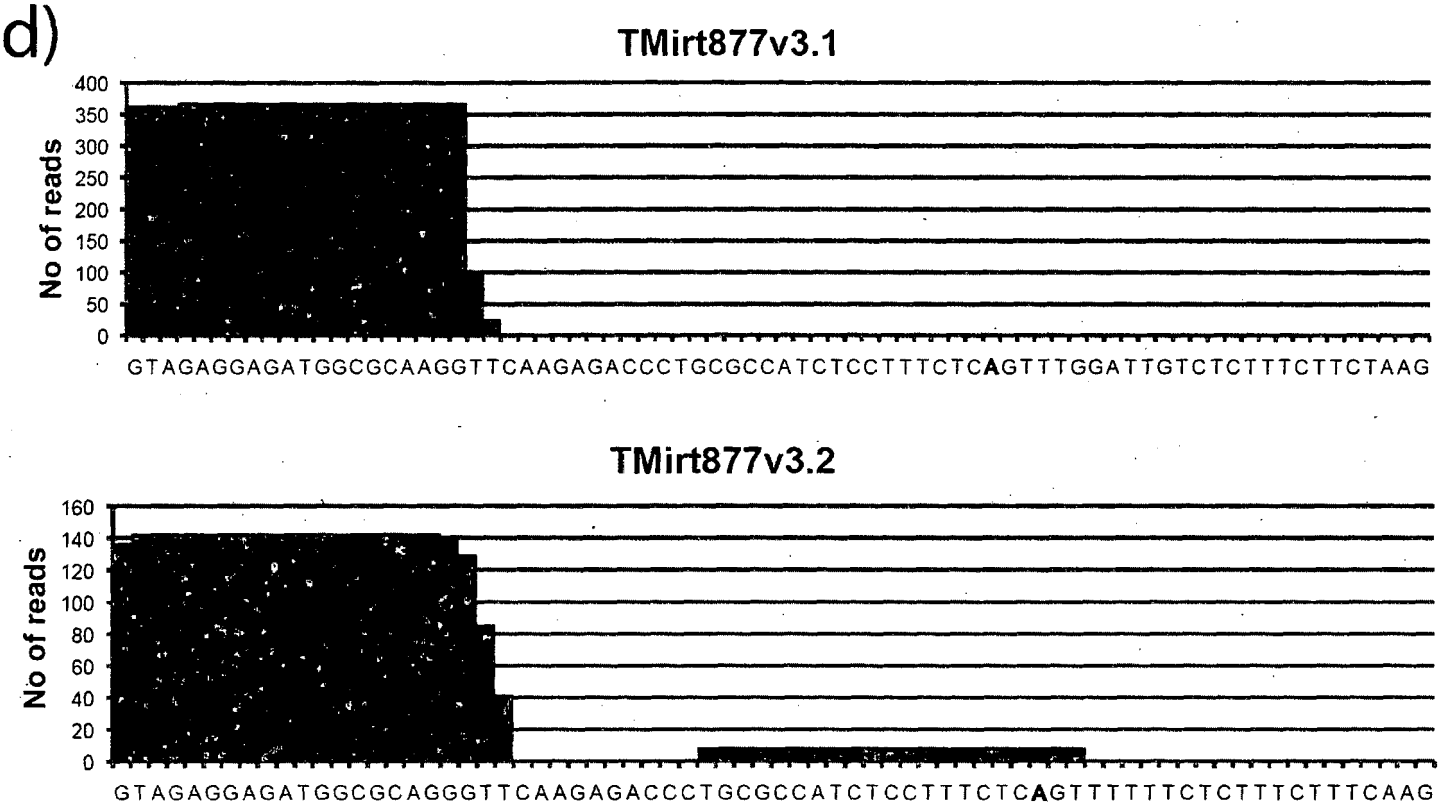


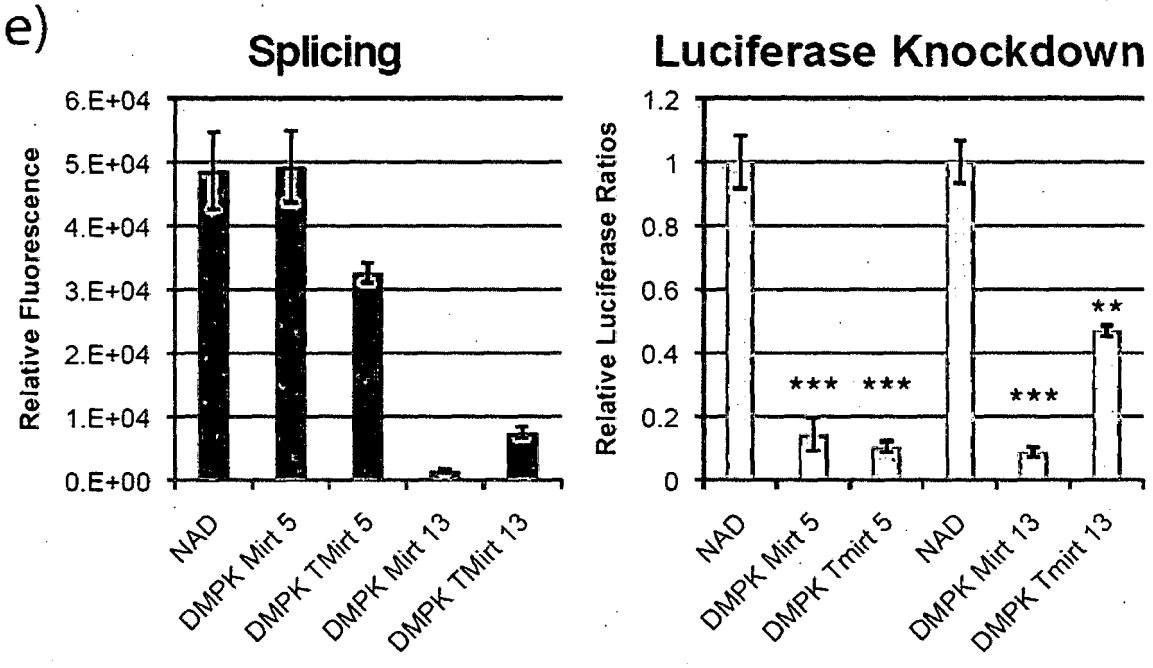
Fig. 3 a-c (continued)

5

d)



e)



5

Fig. 3 d-e (continued)

f)

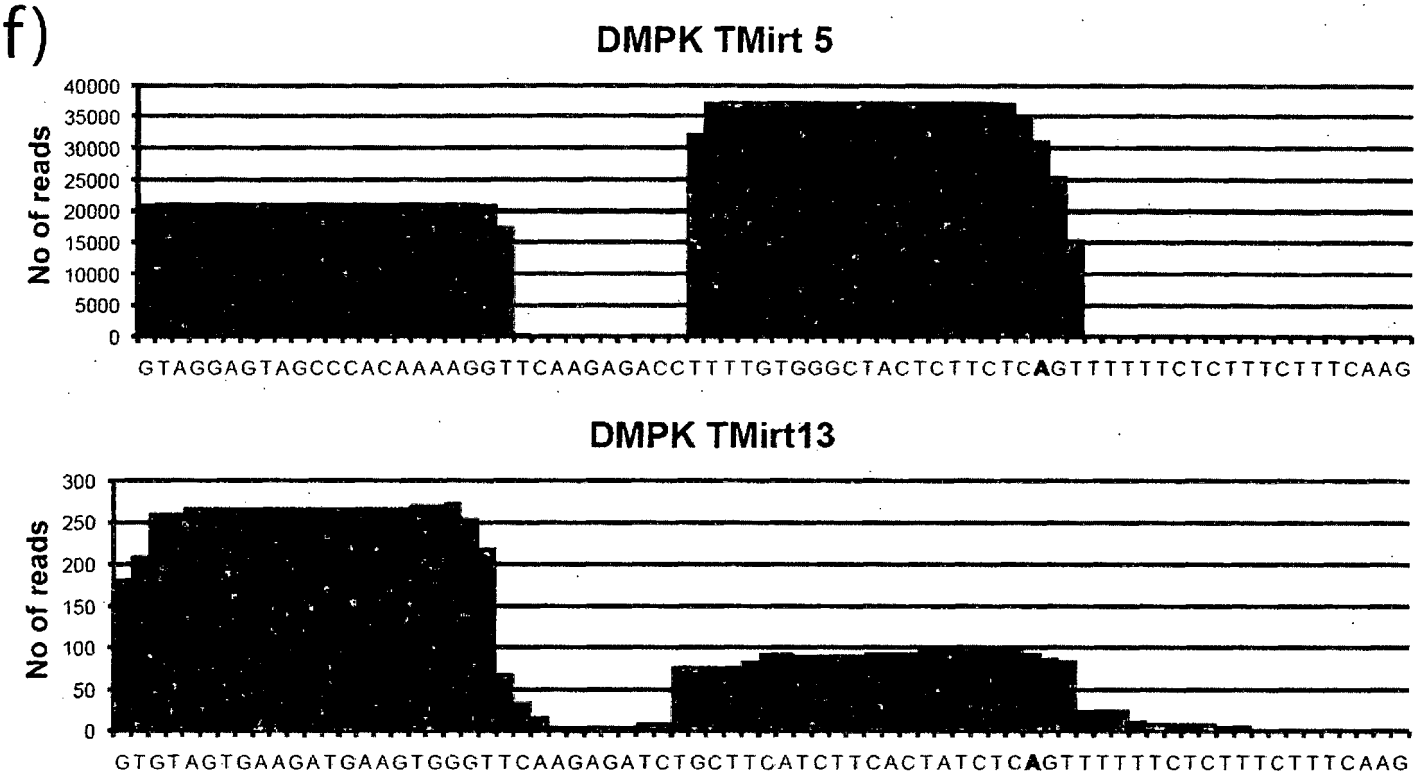


Fig. 3 f (continued)

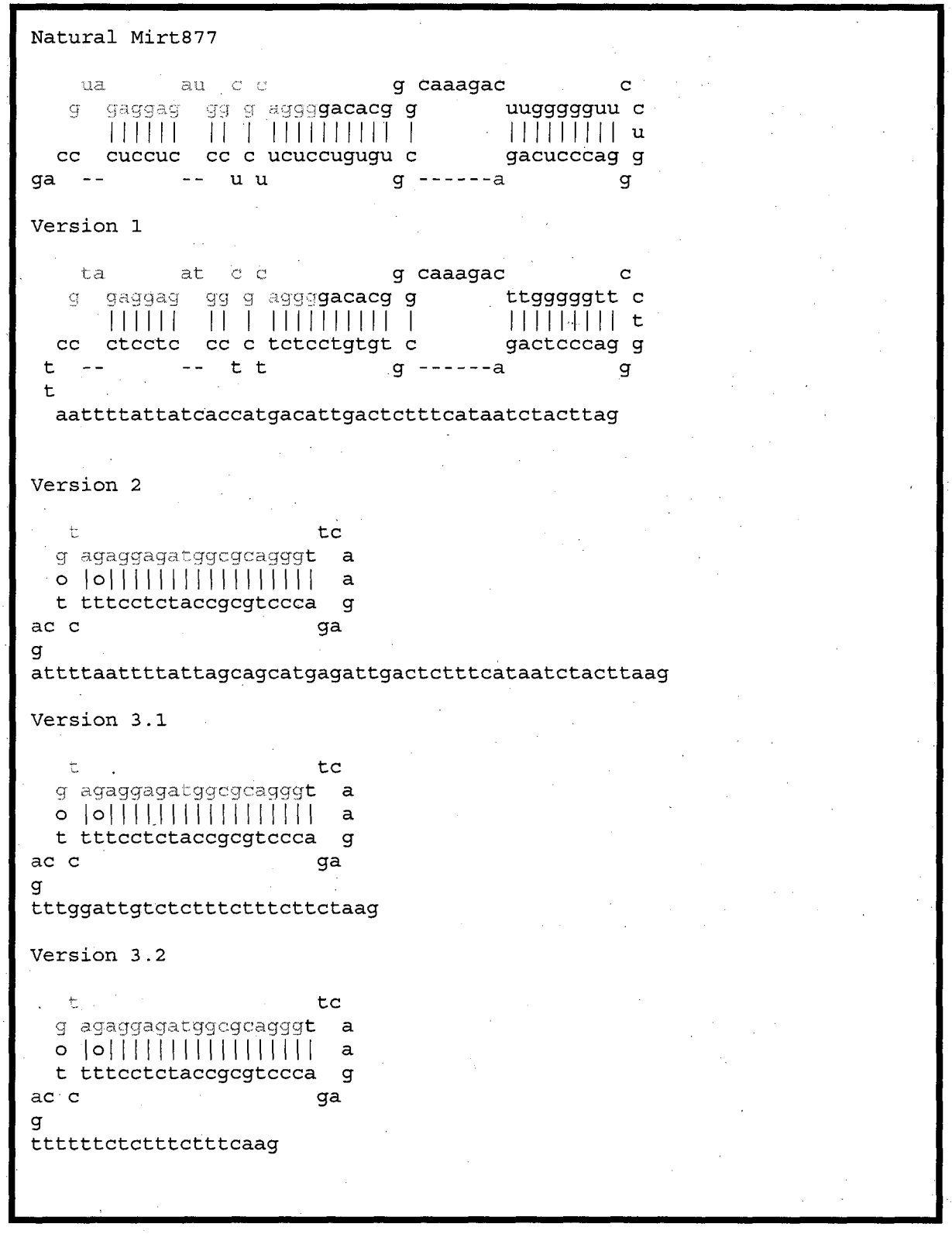

Natural Mirt877
ua au c c g caaagac c
g gaggag gg g aggggacacg g uuggggguu c
cc cuccuc cc c ucuccugugu c gacucccag g
ga -- -- u u g ------a g
Version 1
ta at c c g caaagac c
g gaggag gg g aggggacacg g ttgggggtt c
cc ctcctc cc c tctcctgtgt c gactcccag g
t -- -- t t g ------a g
t
aattttattatcaccatgacattgactctttcataatctacttag
Version 2
t tc
g agaggagatggcgcagggt a
o |o| a
t tttcctctaccgcgtccca g
ac c ga
g
attttaattttattagcagcatgagattgactctttcataatctacttaag
Version 3.1
t tc
g agaggagatggcgcagggt a
o |o| a
t tttcctctaccgcgtccca g
ac c ga
g
tttggattgtctctttctttcttctaag
Version 3.2
t tc
g agaggagatggcgcagggt a
o |o| a
t tttcctctaccgcgtccca g
ac c ga
g
ttttttctctttctttcaag

Fig.3g

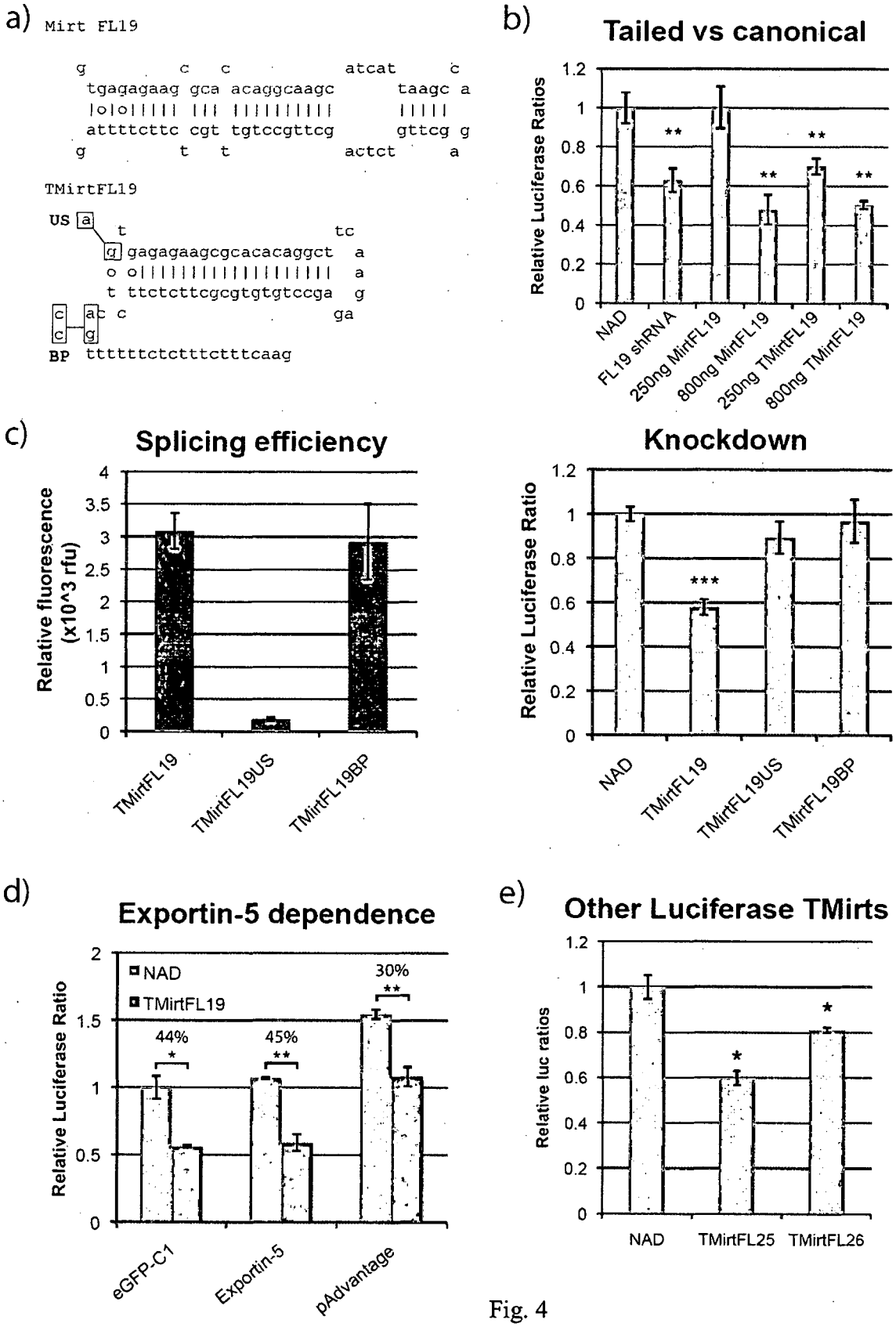

a)
Mirt FL19
TMirtFL19
US
BP
b)
Tailed vs canonical
Relative Luciferase Ratios
NAD
FL19 shRNA
250ng MirtFL19
800ng MirtFL19
250ng TMirtFL19
800ng TMirtFL19
c)
Splicing efficiency
Relative fluorescence (x10^3 rfu)
TMirtFL19
TMirtFL19US
TMirtFL19BP
Knockdown
Relative Luciferase Ratio
NAD
TMirtFL19
TMirtFL19US
TMirtFL19BP
d)
Exportin-5 dependence
Relative Luciferase Ratio
NAD
TMirtFL19
44%
45%
30%
eGFP-C1
Exportin-5
pAdvantage
e)
Other Luciferase TMirts
Relative luc ratios
NAD
TMirtFL25
TMirtFL26

Fig. 4

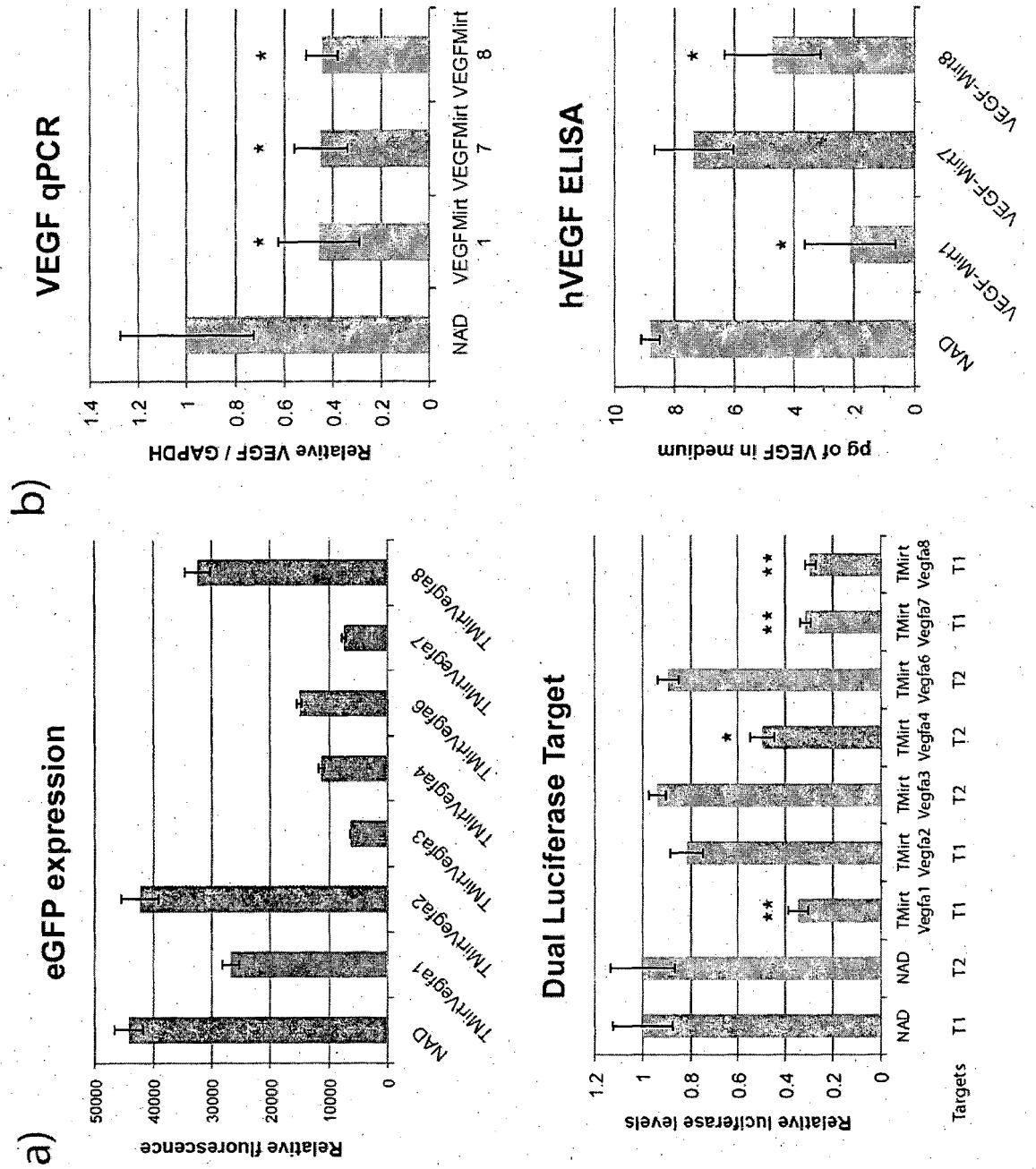
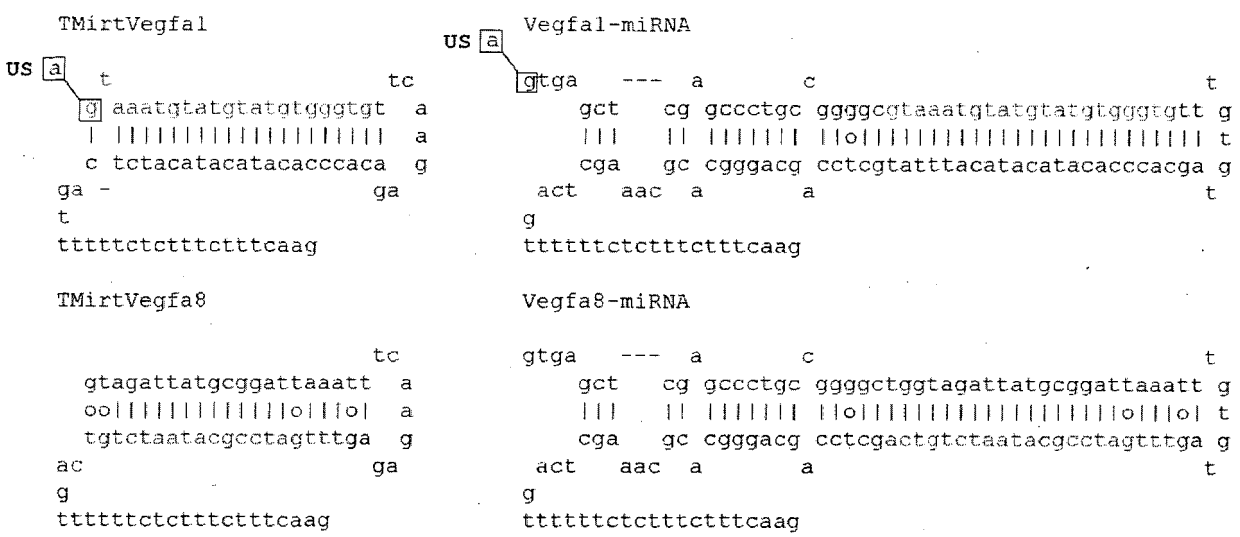
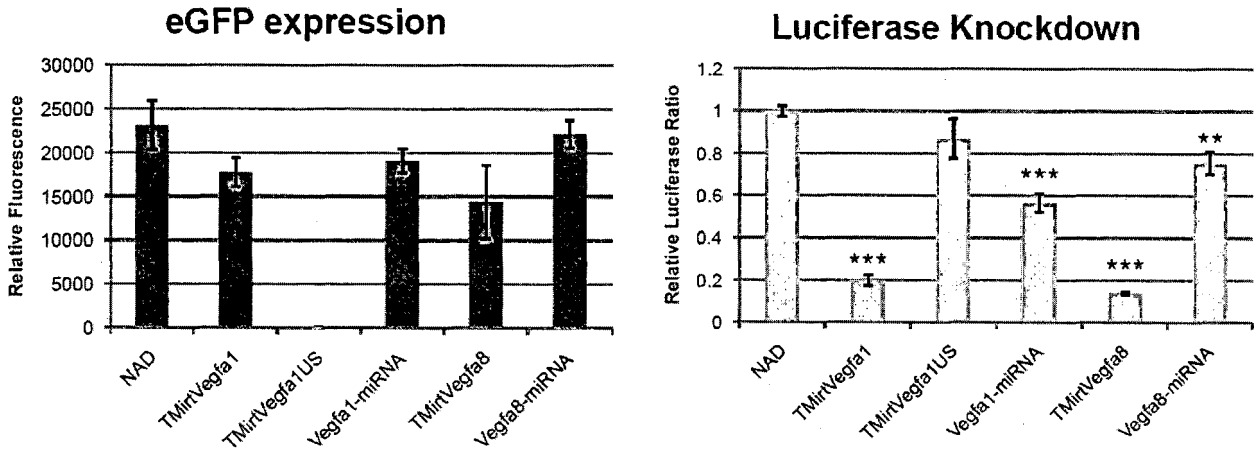


Fig. 5a-b (continued)

c)


TMirtVegfa1
US a
t tc
g aaatgtatgtatgtgggtgt a
a
c tctacatacatacacccaca g
ga - ga
t
ttttttctctttctttcaag
TMirtVegfa8
tc
gtagattatgcggattaaatt a
a
tgtctaatacgcctagtttga g
ac ga
g
ttttttctctttctttcaag
Vegfa1-miRNA
US a
gtga --- a c t
gct cg gccctgc ggggcgtaaatgtatgtatgtgggtgtt g
cga gc cgggacg cctcgtatttacatacatacacccacga g
act aac a a t
g
ttttttctctttctttcaag
Vegfa8-miRNA
gtga --- a c t
gct cg gccctgc ggggctggtagattatgcggattaaatt g
cga gc cgggacg cctcgactgtctaatacgcctagtttga g
act aac a a t
g
ttttttctctttctttcaag

d)


eGFP expression
Relative Fluorescence
30000
25000
20000
15000
10000
5000
0
NAD
TMirtVegfa1
TMirtVegfa1US
Vegfa1-miRNA
TMirtVegfa8
Vegfa8-miRNA
Luciferase Knockdown
Relative Luciferase Ratio
1.2
1
0.8
0.6
0.4
0.2
0
***
**

e)

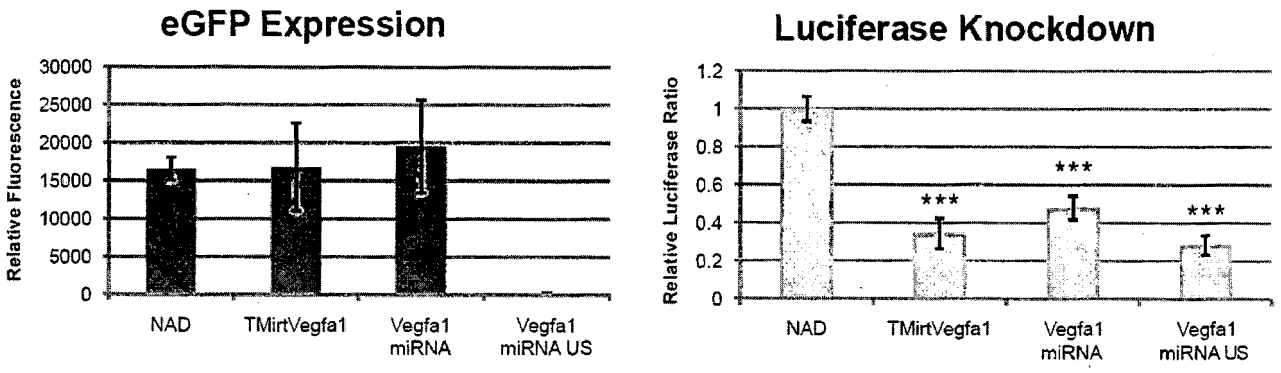

eGFP Expression
Relative Fluorescence
30000
25000
20000
15000
10000
5000
0
NAD
TMirtVegfa1
Vegfa1 miRNA
Vegfa1 miRNA US
Luciferase Knockdown
Relative Luciferase Ratio
1.2
1
0.8
0.6
0.4
0.2
0
***

Fig. 5c-e

Examples of

TMirts with 5′ guide strands SEQ ID NO: 41

```
   t                    tc
  g RRRNNNNNNNNNNNNNNNNt  a
  | ||||||||||||||||||||  a
  c cNNNNNNNNNNNNNNNNNNa  g
ga t                     ga
t
ttttctctttctttcaag
```

TMirts with 3′ guide strands SEQ ID NO: 42

```
   t                    tc
  g RRRNNNNNNNNNNNNNNNNt  a
  o ||||||||||||||o|||||  a
  c cNNNNNNNNNNNNNNNNNNa  g
ga t                     ga
t
ttttctctttctttcaag
```

Fig. 6

gtgagtgccggtgcctgccctgggggccgtccctg**cgcagggggccgggtgctcaccg**catctgccccgcag
gtgggttgggcgggctctgggggatttggtctca**cagcccggatcccagcccacttacctttggtt**actctcctccttcctttctag
gtgagc**aggcgaggctgggctgaa**acccgtgggtgaggagt**gcagcccagctgaggcctctgc**tgtctcttatctgtctcctacag
gtgagtgggctcccgggacggcgcccgccctggccctggcccggcgacgtctcacggt**c**tccctctcccctcag
gtgagtgggagccggtgggctgggagtaagggcacgcccgggggct**gcccccacctgctgaccacc**ctccccccacag
gtgagtgtggatttggcgggttccgggggttccgacggc**gacctcggcgacccctcactc**accgcttcctctttccgcag
gtaagcagggctctgggtgatgtgaggagcaacaggcacc**ctcctccacagccctgctcatt**cctgctgcccccccacccctcccactgcctcacctctag
gtgcggaacgctggccgggcgggaggggaagggacg**cccggccgcggaacgccgcact**cacgccgcctctgcccacag
gtgaggagggctggcagggacccctccaagttgggggacgg**cagccagccctgctcacccctc**gccttccttgtctcctctgcccaccttgtcctcacactag
gtgagtagtggcgcgcggcggctcggagtacctctgc**cgccgcgcgcatcggctcagca**tgcccgtctctgtctcctcag
gtgagtcagggtgggggctggccccctgcttcgtgccc**atccgcgctctgactctctgc**ccacacctgcag
gtgcggacgcggcccagtcctgcccaaggaccgggagcgggaggcccactgggtcc**ctgggctgagcccgtcccca**ccccctccccag
gtactagggcctggaggtttgggctccaagagaagcttgacag**agcccacgcccgacccctact**tctgtttctttcctaag
gtcagtgtg**tgggtgtcgggggtgggggca**tggcggacc**tgcgacctgaccctgacacggg**gcctcctttccactgcag
gtgagcagggctgggggtacctcccccaacctagg**acaccccacttgcctgctgacc**cccagctgtcccggtgtag
gtgagtgggcacggaggaggctgctgctcccctcggccaccgtgagcagggagc**cgccctcacacccccctcctc**ccacag
gtcagtgggaccagacaaggggaggtggttgtct**ctctgcctgggaaactga**ccatctttgttttttgtaattcttcag

Mature miRNA species in **bold**. Possible branch point(s) in red.

Fig. 7

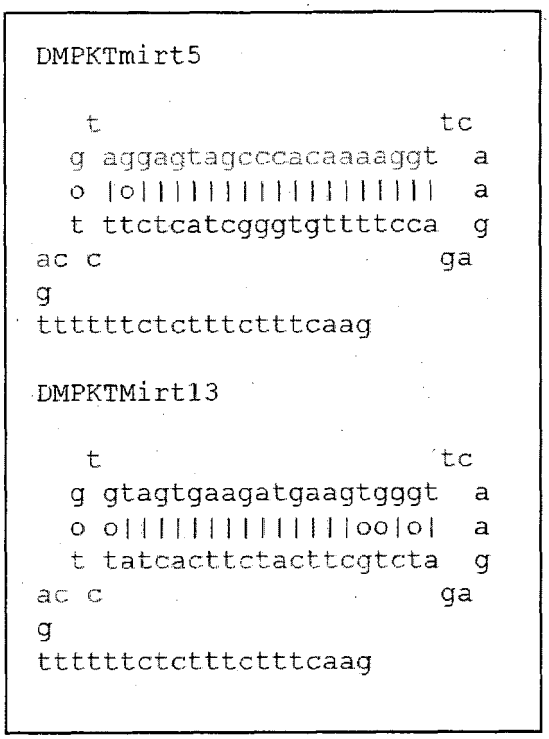

DMPKTmirt5
t tc
g aggagtagcccacaaaaggt a
o |o|||||||||||||||||| a
t ttctcatcgggtgttttcca g
ac c ga
g
ttttttctctttctttcaag
DMPKTMirt13
t tc
g gtagtgaagatgaagtgggt a
o o|||||||||||||oo|o| a
t tatcacttctacttcgtcta g
ac c ga
g
ttttttctctttctttcaag

Fig. 8

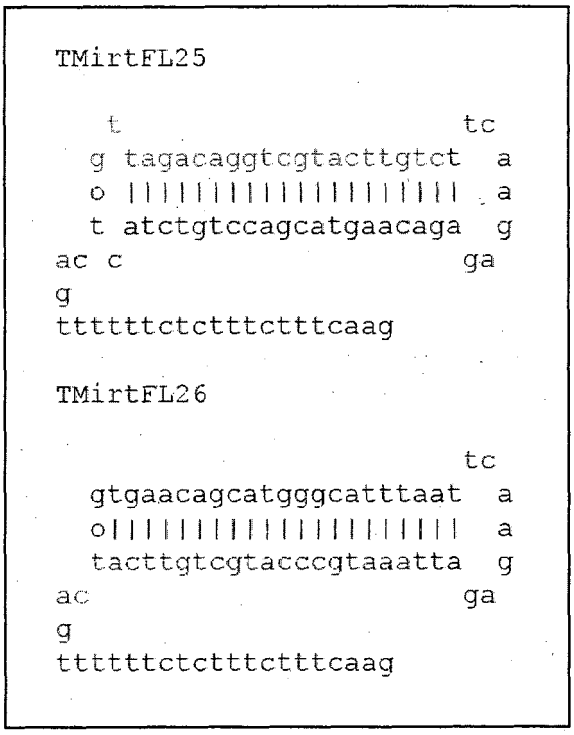

TMirtFL25
t tc
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o |||||||||||||||||| a
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ac c ga
g
ttttttctctttctttcaag
TMirtFL26
tc
gtgaacagcatgggcatttaat a
o|||||||||||||||||||| a
tacttgtcgtacccgtaaatta g
ac ga
g
ttttttctctttctttcaag

Fig. 9

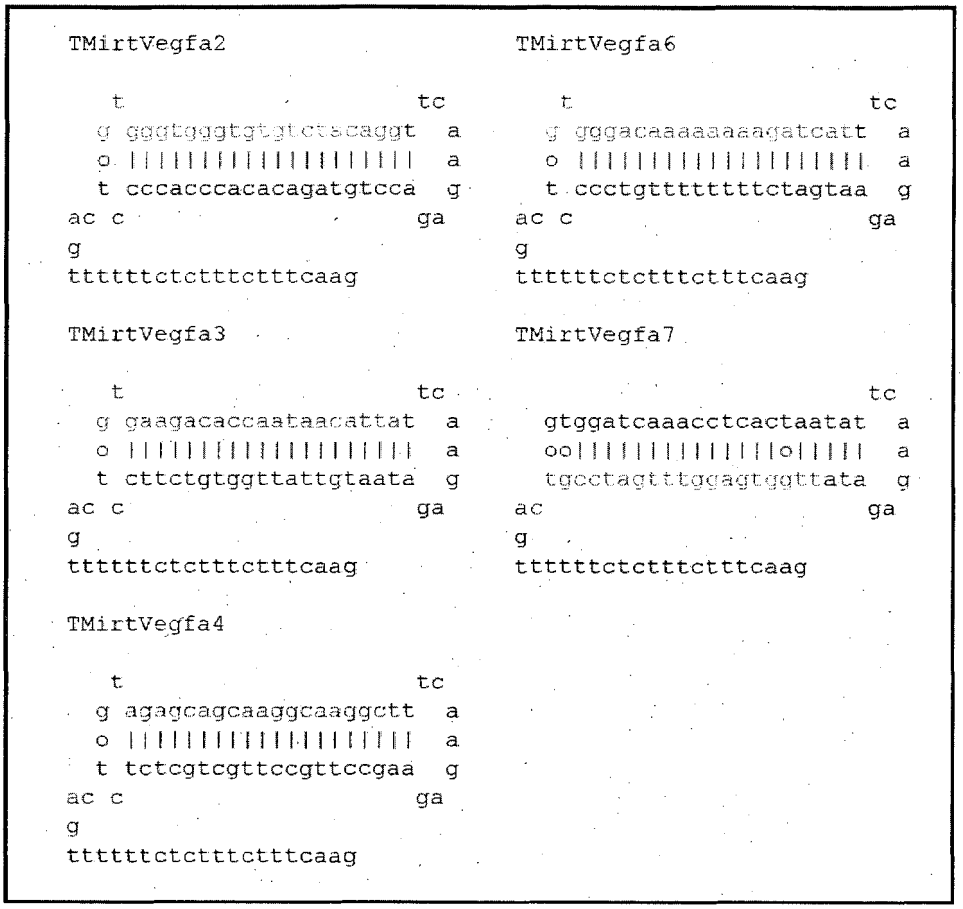

TMirtVegfa2
t tc
g gggtgggtgtgtctcacaggt a
o ||||||||||||||||||||| a
t cccacccacacagatgtcca g
ac c ga
g
ttttttctctttctttcaag
TMirtVegfa3
t tc
g gaagacaccaataacattat a
o ||||||||||||||||||||| a
t cttctgtggttattgtaata g
ac c ga
g
ttttttctctttctttcaag
TMirtVegfa4
t tc
g agagcagcaaggcaaggctt a
o ||||||||||||||||||||| a
t tctcgtcgttccgttccgaa g
ac c ga
g
ttttttctctttctttcaag
TMirtVegfa6
t tc
g gggacaaaaaaaagatcatt a
o ||||||||||||||||||||| a
t ccctgtttttttttctagtaa g
ac c ga
g
ttttttctctttctttcaag
TMirtVegfa7
tc
gtggatcaaacctcactaatat a
oo|||||||||||||||o||||| a
tgcctagtttggagtggttata g
ac ga
g
ttttttctctttctttcaag

Fig. 10

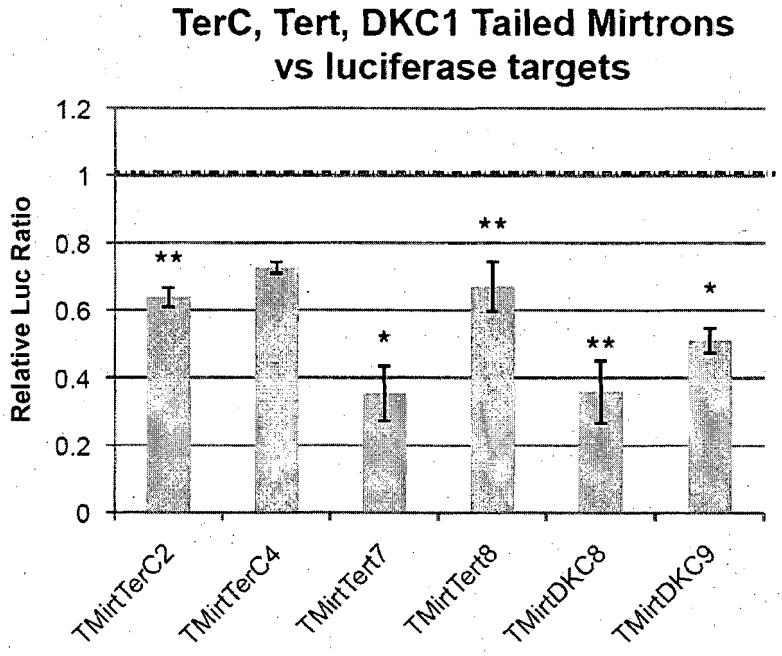

TerC, Tert, DKC1 Tailed Mirtrons
vs luciferase targets
Relative Luc Ratio
1.2
1
0.8
0.6
0.4
0.2
0
**
*
**
**
*
TMirtTerC2
TMirtTerC4
TMirtTert7
TMirtTert8
TMirtDKC8
TMirtDKC9

Fig. 11

Sequences

SEQ ID NO:1: TMirt877v3.1
GUAGAGGAGAUGGCGCAGGGUUCAAGAGACCCUGCGCCAUCUCCUUUCUCAGUUUGGAUUGU
CUCUUUCUUUCUUCUAAG

SEQ ID NO:2: TMirt877v3.2
GUAGAGGAGAUGGCGCAGGGUUCAAGAGACCCUGCGCCAUCUCCUUUCUCAGUUUUUUCUCU
UUCUUUCAAG

SEQ ID NO:3: DMPKTMirt5
GUAGGAGUAGCCCACAAAAGGUUCAAGAGACCUUUUGUGGGCUACUCUUCUCAGUUUUUUCU
CUUUCUUUCAAG

SEQ ID NO:4: DMPKTMirt13
GUGUAGUGAAGAUGAAGUGGGUUCAAGAGAUCUGCUUCAUCUUCACUAUCUCAGUUUUUUCU
CUUUCUUUCAAG

SEQ ID NO:5: TMirtFL19
GUGAGAGAAGCGCACACAGGCUUCAAGAGAGCCUGUGUGCGCUUCUCUUCUCAGUUUUUUCU
CUUUCUUUCAAG

SEQ ID NO:6: TMirtVegfa1
GUAAAUGUAUGUAUGUGGGUGUUCAAGAGACACCCACACACAUACAUCUCAGUUUUUUCUCU
UUCUUUCAG

SEQ ID NO:7: TMirtVegfa4
GUAGAGCAGCAAGGCAAGGCUUUCAAGAGAAGCCUUGCCUUGCUGCUCUCUCAGUUUUUUCU
CUUUCUUUCAG

SEQ ID NO:8: TMirtVegfa7
GUGGAUCAAACCUCACUAAUAUUCAAGAGAUAUUGGUGAGGUUUGAUCCGUCAGUUUUUUCU
CUUUCUUUCAG

SEQ ID NO:9: TMirtVegfa8
GUAGAUUAUGCGGAUUAAAUUUCAAGAGAGUUUGAUCCGCAUAAUCUGUCAGUUUUUUCUCU
UUCUUUCAG

SEQ ID NO:10: TMIrtTerC2
GUGAGAACCGAGUUCCGGGUGUUCAAGAGACACCCGGAACUCGGUUCUUCUCAGUUUUUUCU
CUUUCUUUCAG

SEQ ID NO:11: TMirtTerC4
GUAAGACACCGAACACGGGGAUUCAAGAGAUCCCCGUGUUCGGUGUCUUCUCAGUUUUUUCU
CUUUCUUUCAG

Fig 12 (continued)

SEQ ID NO:12: TMirtTert7
GUAGAUGACAGAAUGAGCAGCUUCAAGAGAGCUGCUCAUUCUGUCAUUCUCAGUUUUUUCUC
UUUCUUUCAG

SEQ ID NO:13: TMirtTert8
GUAAGAACCUAAGGUUAGUGAUUCAAGAGAUCACUAACCUUAGGUUCUUCUCAGUUUUUUCU
CUUUCUUUCAG

SEQ ID NO:14: TMirtDKC8
GUAAAUAGCUGCAGAUAGUUAUUCAAGAGAUAACUAUCUGCAGCUAUUUCUCAGUUUUUUCU
CUUUCUUUCAG

SEQ ID NO:15: TMirtDKC9
GUAGGAGAGGACUAUCUAUGGUUCAAGAGACCAUAGAUAGUCCUCUCUUCUCAGUUUUUUCU
CUUUCUUUCAG

SEQ ID NO: 16
CTCAG

SEQ ID NO: 17
CUCAG

SEQ ID NO: 18
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNCUCAGNN
NNNNNN

SEQ ID NO: 19
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNCUCAGNNNNNNNNNNNNNNN
N

SEQ ID NO: 20
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NYUNAYNNNNNNNN

SEQ ID NO: 21
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNYUNAYNNNNNNNNNNNNNNNNNNNNN
N

SEQ ID NO: 22
NNNNNNNNNNNNNNNNNNTT

SEQ ID NO: 23
NNNNNNNNNNNNNNNNNNTT

Fig. 12 (continued)

SEQ ID NO: 24 VEGF T1 Primer
TCGATGCAGATTATGCGGATCAAACCTGTAGACACACCCACCCACCCACATACATACATTTA
TGCGGATCAAACCTCACCAA

SEQ ID NO: 25
GGCCTTGGTGAGGTTTGATCCGCATAAATGTATGTATGTGGGTGGGTGGGTGTGTCTACAGG
TTTGATCCGCATAATCTGCA

SEQ ID NO: 26 VEGF T2 PRIMER
TCGAGCCTTGCCTTGCTGCTCTACCTCCACATGATCTTTTTTTTTGTCCCACTAATGTTATTG
GTGTCTTCAC

SEQ ID NO: 27
GGCCGTGAAGACACCAATAACATTAGTGGGACAAAAAAAAAGATCATGTGGAGGTAGAGCAGC
AAGGCAAGGC

SEQ ID NO: 28 GGTGTTTCGTCCTTTCCACAA

SEQ ID NO: 29 U6F
GGCTGCAGGTCGACGGAT

SEQ ID NO: 30 hGAPDH F
AAGGTGAAGGTCGGAGTCAA

SEQ ID NO: 31 hGAPDH R
GAAGATGGTGATGGGATTTC

SEQ ID NO: 32 VEGFA F
CGGATCAAACCTCACCAAGGC

SEQ ID NO: 33 VEGFA R
AGGGAGGCTCCTTCCTCCT

SEQ ID NO: 34
GUNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNCUCAGCU
CUCUCUCUCUCUCUAG

SEQ ID NO: 35 (natural TMirt 877)
GUAGAGGAGAUGGCGCAGGGGACACGGGCAAAGACUUGGGGUUCCUGGGACCCUCAGACGUG
UGUCCUCUUCUCCCUCCUCCCGA

Fig. 12 (Continued)

SEQ ID NO: 36 TMirt877v1
GTAGAGGAGATGGCGCAGGGGACACGGGCAAAGACTTGGGGGTTCCTGGGACCCTCAGACGT
GTGTCCTCTTCTCCCTCCTCCCTTAATTTTATTATCACCATGACATTGACTCTTTCATAATC
TACTTAG

SEQ ID NO: 37 TMirt877v2
GTAGAGGAGATGGCGCAGGGTTCAAGAGACCCTGCGCCATCTCCTTTCTCAGATTTTATTTT
ATTAGCAGCATGAGATTGACTCTTTCATAATCTACTTAAG

SEQ ID NO: 38 MirtFL19
GTGAGAGAAGCGCACACAGGCAAGCATCATTAAGCCAGAGCTTGTCTCAGCTTGCCTGTTTG
CTCTTCTTTTAG

SEQ ID NO:39 Vegfa1-miRNA
GTGAGCTCGAGCCCTGCCGGGCGTAAATGTATGTATGTGGGTGTTTGTGTAGCACCCACATA
CATACATTTATGCTCCAGCAGGGCACGCAAAGCTCAGTTTTTTCTCTTTCTTTCAAG

SEQ ID NO: 40 Vegfa8-miRNA
GTGAGCTCGAGCCCTGCCGGGGCTGGTAGATTATGCGGATTAAATTTGTGTAGTTTGATCCG
CATAATCTGTCAGCTCCAGCAGGGCACGCAAAGCTCAGTTTTTTCTCTTTCTTTCAAG

SEQ ID NO: 41
GTRRRNNNNNNNNNNNNNNNTCAAGAGNNNNNNNNNNNNNNNNNNCTCAGTTTTTTCTCTT
TCTTTCAAG

SEQ ID NO: 42
GTRRRNNNNNNNNNNNNNNNTTCAAGAGANNNNNNNNNNNNNNNNNNCTCAGTTTTTTCTCTT
TCTTTCAAG

SEQ ID NO: 43 Mirtron1
GTGAGTGCCGGTGCCTGCCCTGGGGCCGTCCCTGCGCAGGGGCCGGGTGCTCACCGCATCTG
CCCCGCAG

SEQ ID NO: 44 Mirtron2
GTGGGTTGGGGCGGGCTCTGGGGATTTGGTCTCACAGCCCGGATCCCAGCCCACTTACCTTG
GTTACTCTCCTTCCTTCTAG

SEQ ID NO:45 Mirtron 3
GTGAGCAGGCGAGGCTGGGCTGAACCCGTGGGTGAGGAGTGCAGCCCAGCTGAGGCCTCTGC
TGTCTTATCTGTCTCCTACAG

SEQ ID NO:46 Mirtron 4
GTGAGTGGGGCTCCCGGGACGGCGCCCGCCCTGGCCCTGGCCCGGCGACGTCTCACGGTCCC
TCTCCCCTCAG

Fig.12 (continued)

SEQ ID NO:47 Mirtron 5
GTGAGTGGGAGCCGGTGGGGCTGGAGTAAGGGCACGCCCGGGGCTGCCCCACCTGCTGACCA
CCCTCCCCCCACAG

SEQ ID NO:48 Mirtron 6
GTGAGTGTGGATTTGGCGGGGTTCGGGGGTTCCGACGGCGACCTCGGCGACCCCTCACTCAC
CGCTTCCTCTTTCCGCAG

SEQ ID NO:49 Mirtron 7
GTAAGCAGGGGCTCTGGGTGATGTGAGGAGCAACAGGCACCCTCCTCCACAGCCCCTGCTCA
TTCCTGCTGCCCCCCACCCCCTCCCACTGCCTCACCCTCTAG

SEQ ID NO:50 Mirtron 8
GTGCGGAACGCTGGCCGGGGCGGGAGGGGAAGGGACGCCCGGCCGGAACGCCGCACTCACGC
CGCCTCTGCCCACAG

SEQ ID NO:51 Mirtron 9
GTGAGGAGGGGCTGGCAGGGACCCCTCCAAGTTGGGGACGGCAGCCAGCCCCTGCTCACCCC
TCGCCTTCCTTGTCTCCTCTGCCCACCTTGTCCTCACACTAG

SEQ ID NO:52 Mirtron 10
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CCGTCTCTGTCTCCTCAG

SEQ ID NO:53 Mirtron 11
GTGAGTCAGGGTGGGGCTGGCCCCCTGCTTCGTGCCCATCCGCGCTCTGACTCTCTGCCCAC
CTGCAG

SEQ ID NO: 54 Mirtron 12
GTGCGGACGCGGCCCAGTCCTGCCCAAGGACCGGGGAGCGGGAGGCCCACTGGGGTCCCTGG
GCTGAGCCCGTCCCCACCCCTCCCCAG

SEQ ID NO:55 Mirtron 13
GTACTAGGGGCCTGGAGGTTTGGGCTCCAAGAGAAGCTTGACAGAGCCCACGCCCGACCCCT
ACTTCTGTTTCTTCCTAAG

SEQ ID NO:56 Mirtron 14
GTCAGTGTGTGGGTGTCGGGGGTGGGGGCATGGCGGACCTGCGACCTGACCCCTGACACGGG
CCTCCTTTCCACTGCAG

SEQ ID NO:57 Mirtron 15
GTGAGCAGGGCTGGGGTACCTCCCCCAACCTAGGACACCCCACTTGCCCTGCTGACCCCAGC
TGTCCCGGTGTAG

SEQ ID NO:58 Mirtron 16
GTGAGTGGGGCACGGAGGCTGCTGCTCCCCTCGGCCACCGTGAGCAGGGAGCCGCCCTCACA
CCCCCTCCTCTCCACAG

Fig.12 (continued)

SEQ ID NO:59 Mirtron 17
GTCAGTGGGACCAGACAAGGGGAGTGGTGGTTGTCTCTGCCTGGGAAACTGACCATCTTTGT
TTTTGTAATTCTTCAG

SEQ ID NO:60 TMirtFL25
GTTAGACAGGTCGTACTTGTCTTCAAGAGAGACAAGTACGACCTGTCTACTCAGTTTTTTCT
CTTTCTTTCAAG

SEQ ID NO:61 TMirtFL26
GTGAACAGCATGGGCATTTAATTCAAGAGATTAAATGCCCATGCTGTTCATCAGTTTTTTCT
CTTTCTTTCAAG

SEQ ID NO:62 TMirtVegfa2
GTGGGTGGGTGTGTCTACAGGTTCAAGAGACCTGTAGACACACCCACCCCTCAGTTTTTTCT
CTTTCTTTCAAG

SEQ ID NO:63 TMirtVegfa3
GTGAAGACACCAATAACATTATTCAAGAGATAATGTTATTGGTGTCTTCCTCAGTTTTTTCT
CTTTCTTTCAAG

SEQ ID NO:64 TMirtVegfa6
GTGGGCAAAAAAAAAGATCATTTCAAGAGAATGATCTTTTTTTTTGTCCCCTCAGTTTTTTCTC
TTTCTTTCAAG

Fig.12