

Supplementary Information

Supplementary Figures 1 – 5

Supplementary Tables 1

Human DMPK Mirtron

5' arm Mirtron Target Finder
Target Sequences (9) [score]

ttcgccatgaaatgttctat	[7] DMPK 7
gccacggatccaccttcccat	[7] DMPK 8
ggtcccaggccccacacccat	[6] DMPK 9
ggacagcgtgccccctttac	[5] DMPK 1
ccttttgtgggtactcctac	[5] DMPK 5
gaccttctcctggcggtccac	[4] DMPK 10
gcccttctacgcggattccac	[4] DMPK 6
tggagccctcggtgtccccac	[4] DMPK 4
gccaccgcctgccagttcac	[4] DMPK 2

3' arm Mirtron Target Finder
Target Sequences (8) [score]

gcgaggtagcggtagtgaaga	[6] DMPK 11
aggtagcggtagtgaagatga	[6] DMPK 12
ggtagcggtagtgaagatgaa	[5]
cggtagtgaagatgaagcaga	[5] DMPK 13
gaagatcatgaacaagtggga	[5] DMPK 14
aggagagggacgtgttggtga	[5]
ggagagggacgtgttggtgaa	[5]
gagcggggcggggagacact	[5]

Mmu-miR-1224

```

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

```

DMPK Mirt 1

```

g      c      u-----      c      u      aucau      c
ugaaggggggca gc      guc gg agc      uagagc a
|||oo|||  |||  |||  |||  |||
acuuuuccccgu ug      cag cc ucg      gucucg g
g      c      uccuuu      u      -      acucu      a

```

DMPK Mirt 4

```

g      c      cuc      aucau      c
uggggaca cgaggg      caggaaagc      uagagc a
|o|o|  |||  |||||  |||||
aucucgu gcuucc      guccuuucg      gucucg g
g      u      uu      acucu      a

```

DMPK Mirt 5

```

gu      cc      aucau      c
aggaguag caaaaaggaaagc      uagagc a
|oo|||  |||||  |||||
uuuucauc      guuuuuccuuucg      gucucg g
gau      uu      acucu      a

```

DMPK Mirt 6

```

g      uc      c      aucau      c
uggaa cg      guagaaggcgaaagc      uagagc a
|o||  ||  |||||  |||||
aucuu gc      caucuuuccgcuuuucg      gucucg g
g      uu      u      acucu      a

```

DMPK Mirt 7

```

g      c      uc      aucau      c
uagaa auu      auaggcgaaagc      uagagc a
||||  |||  |||||  |||||
aucuu uaa      uauccgcuuuucg      gucucg g
g      c      uu      acucu      a

```

DMPK Mirt 8

```

g      u      c      aucau      c
ugggaagg ggau      cgaggcaagc      uagagc a
|o|o|||  |||  |||||  |||||
aucuuucc      ccua      guccguucg      gucucg g
g      u      u      acucu      a

```

DMPK Mirt 9

```

g      u      cc      aucau      c
uggg      gugggg      ugggaccaagc      uagagc a
|o||  |o|||  |||||  |||||
aucc      caucc      accugguucg      gucucg g
g      u      uu      acucu      a

```

DMPK Mirt 10

```

g      cc      aucau      c
uggacg      caggagaagguaagc      uagagc a
|o|||  |||||  |||||
aucugc      guccucuuccaguucg      gucucg g
g      uu      acucu      a

```

DMPK Mirt 11

```

g      uc      aucau      c
ugagguagc      uagugaggaagc      uagagc a
|||||  |||||  |||||
acuccaucg      aucacuucucg      gucucg g
g      cc      acucu      a

```

DMPK Mirt 12

```

g      g      c      aucau      c
ug      agcgguagugaa      guggaagc      uagagc a
||  |||||  |||||  |||||
ac      ucgccaucacu      uacuuucg      gucucg g
g      a      c      acucu      a

```

DMPK Mirt 13

```

g      uu      aucau      c
uguagugaagau      agcgggaagc      uagagc a
|||||  |||||  |||||
acaucauucua      ucgucuuucg      gucucg g
g      cu      acucu      a

```

DMPK Mirt 14

```

g      u      aucau      c
uagaucaugaacaggu      ggaagc      uagagc a
|||||  |||||  |||||
aucuaguacuuguuca      ccuucg      gucucg g
g      c      acucu      a

```

Figure S1. Output from the selection algorithm against human DMPK and corresponding mirtrons

Threshold scores were set at 4 for hDMPK (obtained from Genbank accession number NM_004409.3). The number in brackets indicates the total number of sequences that scored above that threshold. Mirtrons were then designed against those targets with a miR-1224 backbone. The guide strand is highlighted in red.

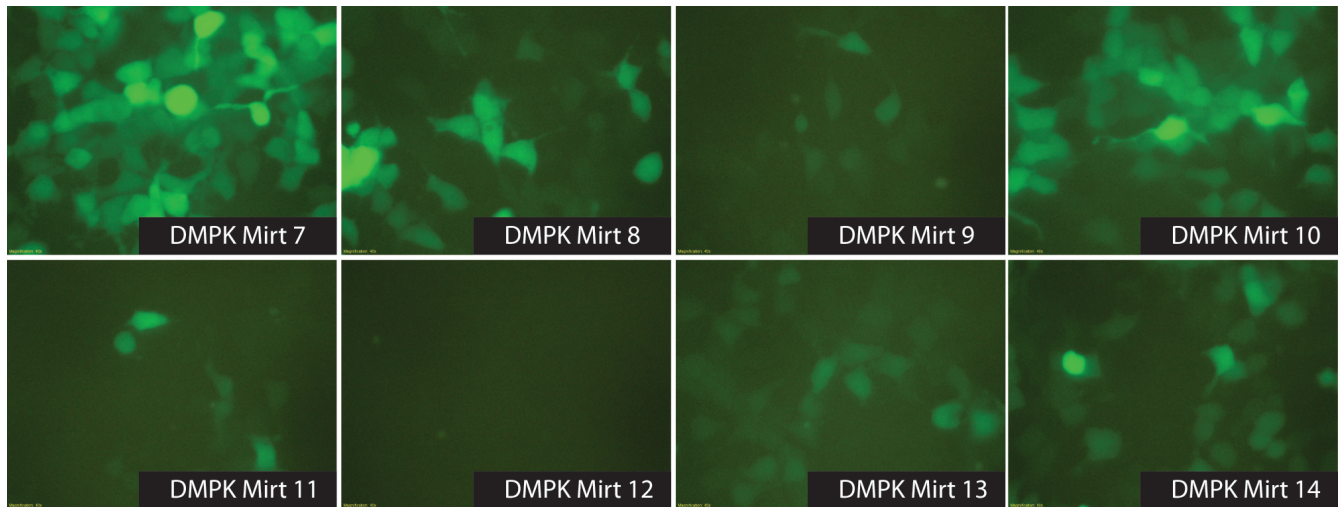


Figure S2. Fluorescent images of HEK cells transfected with pEGFP-Mirt containing DMPK-Mirt 7-14.

HEK cells were transfected with equimolar amounts of each mirtron (500ng of plasmid in 24 well plates) and imaged 48 hours post transfection using the same setting. EGFP fluorescence corresponds to the amount of coding transcript produced when the mirtron is spliced out of the EGFP open reading frame.



Figure S3. Deep sequencing results for DMPK-Mirt 6, 8, 9, 10.

Deep sequencing was performed with 18-25nt RNA from HEK cells transfected with DMPK-Mirt 5, 6, 8, 9, 10 and 13. Sequences that were aligned to mirtron sequences were collected, 3' tail removed and aligned to chart the frequency of individual nucleotides appearing in the small RNA species harvested from the cells. Red nucleotides denote active guide strand; green nucleotides denote flanking exonic sequences.

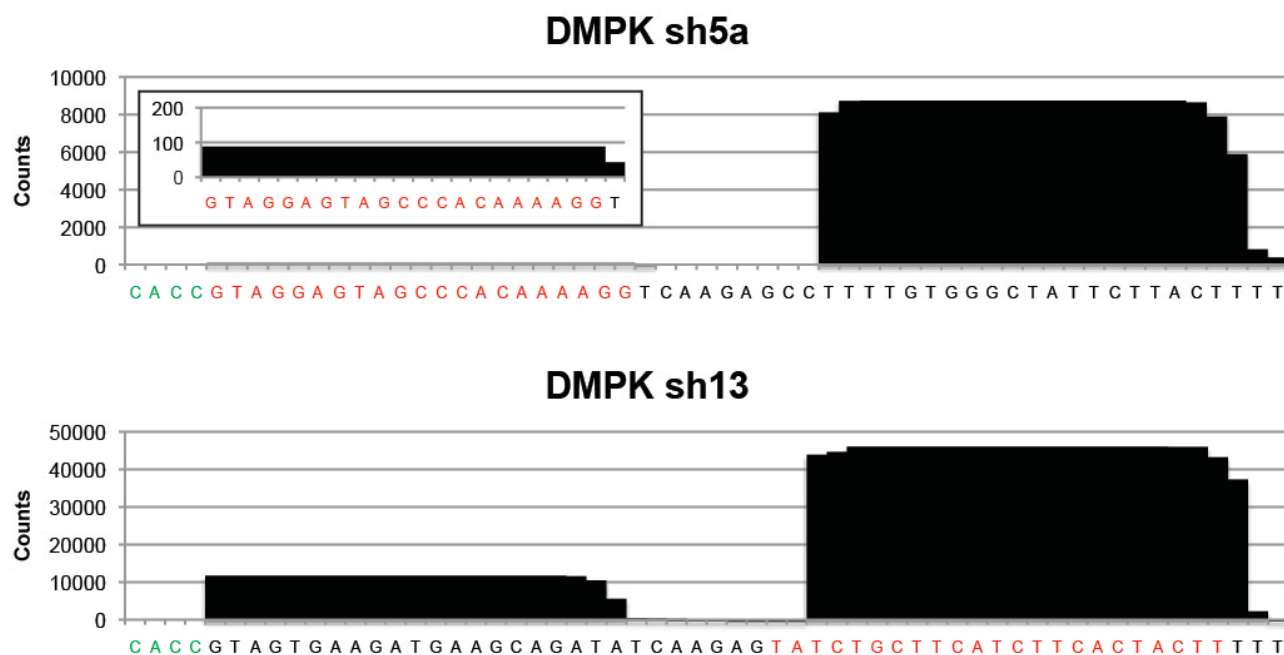


Figure S4. Deep sequencing results for DMPK shRNAs.

Deep sequencing was performed with 18-25nt RNA from HEK cells transfected with DMPK sh5a and DMPK sh13. Sequences that were aligned to the shRNA sequences were collected, 3' tail removed and aligned to chart the frequency of individual nucleotides appearing in the small RNA species harvested from the cells. Red nucleotides denote active guide strand; green nucleotides denote U6 promoter sequence. Insert is a blow-up of the graph it is nested in to illustrate the counts of the nucleotides that make up the guide strand.

a

```
5' tcttcttcaagTAGTCTTCTCGAGAAGACTTgacgacggc 3'
3' agaagaagttcATCAGAAGAGCTCTTCTGAActgctgccg 5'
      exon  <-BbsI      BbsI-> exon
              XhoI
```

b

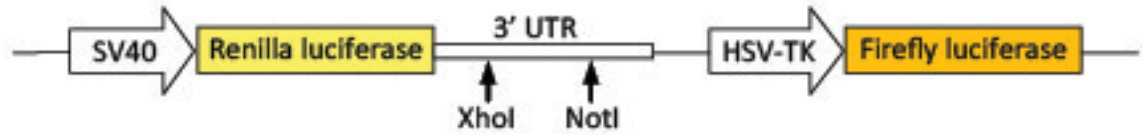


Figure S5. Plasmid Construction (a) Unique overhangs in the eGFP coding region (green nucleotides) that allows for the insertion of intronic sequences. (b) psiCheck2.2 sequence and restriction sites

Table S1. List of primers used

Primer	Sequence
pEGFP-Mirt, psiCheck2.2, shRNA and DMPK Cloning	
Mirt5F	5'-agatccgctagcgctaccggtc-3'
Mirt5R	5'-gtctactcgagaagactacttgaagaagatggtgcgc-3'
Mirt3F	5'-gtcttctcgagaagacttgacgacggcaactacaaga-3'
Mirt3R	5'-atcgaagcttactgtacagctcgtccat-3'
NADintF	5'-ctgtgaagactgcaaggatgtagtagaattctgtcaat-3'
NADintR	5'-ctgagaagacagcgctccttaagtagattatgaaagagtc-3'
Mirtron cloning	5'-caag – mirtron sequence forward-3' and 5'-cgtc – mirtron sequence reverse-3'
Target cloning	5'-tcga – target sequence(s) forward-3' and 5'-ggcc – target sequence(s) reverse-3'
DMPK-Mirt 1,4,5,6 target cloning	5'-tcgagcccttctacgaggattcactggagccctcggtgtccacgacagc gtgccccctttacctttgtggctactcctac-3'
	5'-ggccgtaggtagtcccacaaaaggtaaagggggcacgctgtccgtgggacacc gagggctccagtggaatccgctagaagggc-3'
DMPK-Mirt 7-10 target cloning	5'-tcgagccacggatccaccttccattcgctatgaaatgttctatggtcccaggccccaca cccatgaccttctctggcggtccac-3'
	5'-ggccgtggacgcccagagaagggtcatgggtgtgggcctgggaccatagaacatttcata ggcgaatgggaagggtggatccgtggc-3'
DMPK-Mirt 11-14 target cloning	5'-tcgagcgaggtagcggtagtagaagatcatgaacaagtgggaggtagcggtagtgaagatg aagcaga-3'
	5'-ggcctctgctcatcttctactaccgctacctcccactgttcatgatcttctactaccgctacctcgc-3'
shRNA cloning	5'-ggtgttctgctcttccacaa – <i>mirtron sequence reverse-3'</i>
U6F	5'-ggctgcaggtcgacggat-3'
BspEI XPO5 F	5'-acttccggaatggcgatggatcaagtaaac-3'
XhoI XPO5 R	5'-agtctcgagtcaggggtcaaagatggtgg-3'
NheI Dmpk F	5'-atccgctagcgtcgccaccatgtcagccgaggtgcgg-3'
HindIII Dmpk-R	5'-attcaagcttgggagcgcgggcggt-3'
Quantitative PCR	
mGAPDH F	HK-SY-mo-600 against murine GAPDH (PrimerDesign)
mGAPDH R	
hDMPK F	5'-ccggatttcaagggtgccac-3'
hDMPK R	5'-cacctcccgaatgtccgac-3'
18S F	5'-gtaaccggtgaacccatt-3'
18S R	5'-ccatccaatcggtagtagcg-3'
Intron-spanning PCR	
eGFP	5'-tcttcaagtccgcatgcc-3'
	5'-tgtcgccctcgaacttcac-3'
Serca-1 (Lin, 2006)	5'-gctcatggtctcaagatctcac-3'
	5'-gggtcagtcctcagctttg-3'