

Claims

1. A construct comprising:
- a promoter;
 - 5 - at least one non-mammalian or synthetic miRNA is expressed within an intron, wherein the synthetic miRNA is a sequence which is not naturally occurring;
 - a transgene;
 - 10 - at least one non-mammalian or synthetic miRNA binding site(s) which provides for control of the expression of the transgene, wherein the synthetic miRNA binding site(s) is a sequence which is not naturally occurring; and
 - a polyadenylation signal.
2. A construct of claim 1 wherein the miRNA binding site(s) which provide for control of the expression of the transgene is provided within the 3' UTR, or the 5' UTR.
3. A construct of claim 1 wherein the non-mammalian or synthetic miRNA binding site(s) is provided within the transgene.
4. A construct as claimed in any preceding claim wherein the construct provides a single gene circuit to provide a relatively fixed level of expression of the transgene across cells receiving different levels of vector-derived transgene (i.e. dosage-insensitivity).
5. A construct as claimed in any preceding claim, wherein the at least one synthetic or non-mammalian miRNA exhibits no off-target binding effects.
6. A construct as claimed in any preceding claim wherein the non-mammalian or synthetic miRNA is expressed in an intron provided by
- human_EF1a_intron_A (SEQ ID NO: 5)
- ```
gtaagtccggtgtgtgggtcccgcgggcctggcctctttacgggttatggcccttgcgtgccttgaattactccacgcccc
tggctgcagtacgtgattctgatcccgagcttcgggttgaagtgggtgggagagttcgaggccttgcgcttaaggag
ccccttcgcctcgctgcttgagttgagcctggctgggcgctggggccgccgctgcgaatctggtggcaccttcgcgc
ctgtctcgctgcttgcataagtctctagccatttaaaattttgatgacctgctgcgacgctttttctggcaagatagctctgt
```

aaatgcgggccaagatctgcacactggtatttcggttttggggccgcgggacggggcccgtgctcccagc  
gcacatgttcggcgaggcggggctgcgagcgcgccaccgagaatcgagcggggtagtctcaagctggccgg  
cctgctctggtgctggcctcgcgccgctgtatcgccccgctggcggaaggctggcccggtcggcaccagt  
tgcgtgagcggaaagatggccgctcccggccctgctgcaggagctcaaaatggaggacgcggcgctcgggag  
5 agcgggcggtgagtcacccacacaaaggaaaaggccttccgtcctcagccgtcgctcatgtgactccacgga  
gtaccggcgccgtccaggcacctcgattagttctcgagccttttgagtagctcgtcttaggtgggggaggggttta  
tgcgatggagttccccacactgagtggtggagactgaagtaggccagctggcactgatgtaattctccttgaatt  
tgccttttgagttggatcttggtcattctcaagcctcagacagtgggtcaaagtttttctccattcag  
>MINIX\_artificial\_intron (SEQ ID NO: 6)  
10 Gtaagagcctagcatgtagaactggttacctgcagcccaagcttgcgcacgtctagggctaccgggttccttgatg  
aggtaccgacatactatcctgtccctttttccacag

7. A construct as claimed in any preceding claim wherein the miRNA is non-mammalian miRNA derived from an insect miRNA, optionally wherein the miRNA is  
15 capable of specifically binding to firefly luciferase (ffluc1) miRNA binding site.

8. A construct as claimed in any preceding claim wherein there are a plurality of miRNA binding sites provided in the construct, optionally three miRNA binding sites, at least four miRNA binding sites, at least five miRNA binding sites, at least six  
20 miRNA binding sites.

9. A construct as claimed in any preceding claims wherein there are a plurality of non-mammalian or synthetic miRNAs expressed in a construct.

25 10. A construct as claimed in any preceding claim wherein the non-mammalian firefly luciferase miRNA is a sequence selected from

>ffluc1 (SEQ ID NO:9)

tggttgatgaggcttcagtactttacagaatcggtgcctgcacatcttggaacacttgctgggattactcgactcttaa  
cccaacagaaggctcgagaaggatattgctgttgacagtgcgaaacgatatgggctgaatacaatagtgagc  
30 cacagatgtattgtattcagccatcgtgtgcctactgcctcggactcaaggggctagaattcgagcaattatctgtt  
tactaaaactgaatacctgtctatctcttgatacatctttacaaagctgaattaaaatgtataaataaatcattt

>ffluc9 (SEQ ID NO: 10)

tggttgatgaggcttcagtactttacagaatcggtgcctgcacatcttggaacacttgctgggattactcgactcttaa  
cccaacagaaggctcgagaaggatattgctgttgacagtgcgaaacgtgaattgctcaacagtatagtgagcc

acagatgtatactgttgagcaattcacgttgctgactgctcggactcaaggggctagaattcgagcaattatctgtt  
tactaaaactgaataccttgctatctcttgatacattttacaaagctgaattaaaatggataaaataatcacttt

>ffluc18 (SEQ ID NO: 11)

5 tgtttgaatgaggcttcagtactttacagaatcgttgctgcacatcttggaacacttgctgggattactcgacttcttaa  
cccaacagaaggctcgagaaggtatattgctgttgacagtgagcgacccgacgatgacgccggtgaatagtgaa  
ccacagatgtattcaccggcgctcatcgctggggtgctactgctcggactcaaggggctagaattcgagcaattatc  
ttgttactaaaactgaataccttgctatctcttgatacattttacaaagctgaattaaaatggataaaataatcacttt  
>ffluc22 (SEQ ID NO: 12)

10 tgtttgaatgaggcttcagtactttacagaatcgttgctgcacatcttggaacacttgctgggattactcgacttcttaa  
cccaacagaaggctcgagaaggtatattgctgttgacagtgagcgattcgatagggacaagacaatttagtgaa  
acagatgtaaattgtctgtccctatcgaagtcctactgctcggactcaaggggctagaattcgagcaattatctgtt  
tactaaaactgaataccttgctatctcttgatacattttacaaagctgaattaaaatggataaaataatcacttt.

11. A construct as claimed in any of claims 1 to 6 or 8 to 9 wherein the synthetic  
15 miRNA is a sequence selected from

>novel\_seq\_1 (SEQ ID NO: 13)

tgtttgaatgaggcttcagtactttacagaatcgttgctgcacatcttggaacacttgctgggattactcgacttcttaa  
cccaacagaaggctcgagaaggtatattgctgttgacagtgagcgagcatgttacgggacttcttattagtgaa  
cagatgtaataagaagtcccgttaacatgcgtgctactgctcggactcaaggggctagaattcgagcaattatcttg  
20 ttactaaaactgaataccttgctatctcttgatacattttacaaagctgaattaaaatggataaaataatcacttt

>novel\_seq\_2 (SEQ ID NO: 14)

tgtttgaatgaggcttcagtactttacagaatcgttgctgcacatcttggaacacttgctgggattactcgacttcttaa  
cccaacagaaggctcgagaaggtatattgctgttgacagtgagcgagtgaggagcagcgatcttaatagtgaa  
cacagatgtattaagatccgctgctcctcacgtgctactgctcggactcaaggggctagaattcgagcaattatctt  
25 gttactaaaactgaataccttgctatctcttgatacattttacaaagctgaattaaaatggataaaataatcacttt

>novel\_seq\_3 (SEQ ID NO: 15)

tgtttgaatgaggcttcagtactttacagaatcgttgctgcacatcttggaacacttgctgggattactcgacttcttaa  
cccaacagaaggctcgagaaggtatattgctgttgacagtgagcgagtcagtcacggaacttatagtgaa  
acagatgtataagttccgtgacgacatgacgtgctactgctcggactcaaggggctagaattcgagcaattatctt  
30 gttactaaaactgaataccttgctatctcttgatacattttacaaagctgaattaaaatggataaaataatcacttt

>novel\_seq\_4 (SEQ ID NO: 16)

tgtttgaatgaggcttcagtactttacagaatcgttgctgcacatcttggaacacttgctgggattactcgacttcttaa  
cccaacagaaggctcgagaaggtatattgctgttgacagtgagcgagagaagtgcggatttcgtatttagtgaa  
acagatgtataacgaaatccgcacttctcgtgctactgctcggactcaaggggctagaattcgagcaattatcttg  
35 ttactaaaactgaataccttgctatctcttgatacattttacaaagctgaattaaaatggataaaataatcacttt

>novel\_seq\_5 (ran1g) (SEQ ID NO: 17)  
tggttgaaatgaggcttcagttactttacagaatcggtgcctgcacatcttgaaacacttgctgggattactcgacttcttaa  
cccaacagaaggctcgagaaggatattgctgttgacagtgcgaaagcgccaaaggagctgtgataggaagc  
cacagatgtatcacagactccttggcgcttgctcactgcctcgactcaaggggctagaattcgagcaattatcttg  
5 tttactaaaactgaataccttgctatctcttgatacatctttacaaagctgaattaaaatggataaaattaaatcacttt  
>novel\_seq\_6 (ran2g) (SEQ ID NO: 18)  
tggttgaaatgaggcttcagttactttacagaatcggtgcctgcacatcttgaaacacttgctgggattactcgacttcttaa  
cccaacagaaggctcgagaaggatattgctgttgacagtgcgaaagtcggatttcgtattgctaggaagcca  
cagatgtagcaaatccgaatccgcacttgctcactgcctcgactcaaggggctagaattcgagcaattatctgt  
10 tttactaaaactgaataccttgctatctcttgatacatctttacaaagctgaattaaaatggataaaattaaatcacttt  
>novel\_seq\_7 (SEQ ID NO: 19)  
tggttgaaatgaggcttcagttactttacagaatcggtgcctgcacatcttgaaacacttgctgggattactcgacttcttaa  
cccaacagaaggctcgagaaggatattgctgttgacagtgcgaaagtcggatgcgatgcgattgctaggaagcc  
acagatgtagcaatccgcacttgctcactgcctcgactcaaggggctagaattcgagcaattatcttg  
15 tttactaaaactgaataccttgctatctcttgatacatctttacaaagctgaattaaaatggataaaattaaatcacttt  
>novel\_seq\_8 (SEQ ID NO: 20)  
Tggttgaaatgaggcttcagttactttacagaatcggtgcctgcacatcttgaaacacttgctgggattactcgacttcttaa  
cccaacagaaggctcgagaaggatattgctgttgacagtgcgaaacggatccgcaactgcgataggaagc  
cacagatgtatcgaaagtcggatccgttgctcactgcctcgactcaaggggctagaattcgagcaattatctt  
20 gttactaaaactgaataccttgctatctcttgatacatctttacaaagctgaattaaaatggataaaattaaatcacttt.

12. A construct as claimed in any one of the preceding claims wherein the synthetic  
miRNA is targeted against the coding sequence of a target gene and is selected  
from

25 >MECP2\_coding\_1 (SEQ ID NO: 21)  
tggttgaaatgaggcttcagttactttacagaatcggtgcctgcacatcttgaaacacttgctgggattactcgacttcttaa  
cccaacagaaggctcgagaaggatattgctgttgacagtgcgaaagtcgatgttacttgatctaggaagcca  
cagatgtagatcaagtaaacatcgacttgctcactgcctcgactcaaggggctagaattcgagcaattatctgtt  
tactaaaactgaataccttgctatctcttgatacatctttacaaagctgaattaaaatggataaaattaaatcacttt  
30 >MECP2\_coding\_2 (SEQ ID NO: 22)  
tggttgaaatgaggcttcagttactttacagaatcggtgcctgcacatcttgaaacacttgctgggattactcgacttcttaa  
cccaacagaaggctcgagaaggatattgctgttgacagtgcgaaagccgctcttggtctctacataggaagcca  
cagatgtagtagagaccaagagcggttgctcactgcctcgactcaaggggctagaattcgagcaattatctt  
gttactaaaactgaataccttgctatctcttgatacatctttacaaagctgaattaaaatggataaaattaaatcacttt  
35 >MECP2\_coding\_3 (SEQ ID NO: 23)

- tgtttgatgaggcttcagtactttacagaatcgttgctgcacatcttggaacacttgctgggattactcgacttctaa  
cccaacagaaggctcgagaaggtatattgctgttgacagtgagcgaaagtcgaagatcaagacctgtagtgaagc  
cacagatgtacaggcttgatcttcggacttgctcactgcctcggactcaaggggctagaattcgagcaattatctgt  
ttactaaaactgaataccttgctatctcttgatacatctttacaaagctgaattaaaatggtataaattaaatcacttt
- 5 >MECP2\_coding\_4 (SEQ ID NO: 24)
- tgtttgatgaggcttcagtactttacagaatcgttgctgcacatcttggaacacttgctgggattactcgacttctaa  
cccaacagaaggctcgagaaggtatattgctgttgacagtgagcgagagtcaggtatatacgagtgtagtgaagcc  
acagatgtatacactgcgtatactggactcgtgcctactgcctcggactcaaggggctagaattcgagcaattatctgt  
ttactaaaactgaataccttgctatctcttgatacatctttacaaagctgaattaaaatggtataaattaaatcacttt
- 10 > MECP2\_coding\_5 (SEQ ID NO: 25)
- tgtttgatgaggcttcagtactttacagaatcgttgctgcacatcttggaacacttgctgggattactcgacttctaa  
cccaacagaaggctcgagaaggtatattgctgttgacagtgagcgagtcaggtatatacgagtgtagtgaagcc  
acagatgtatgtacactgcgtatactggactcgtgcctactgcctcggactcaaggggctagaattcgagcaattatctgt  
ttactaaaactgaataccttgctatctcttgatacatctttacaaagctgaattaaaatggtataaattaaatcacttt
- 15 > MECP2\_coding\_6 (SEQ ID NO: 26)
- Tgtttgatgaggcttcagtactttacagaatcgttgctgcacatcttggaacacttgctgggattactcgacttctaa  
cccaacagaaggctcgagaaggtatattgctgttgacagtgagcgagactcgggaaaccgtagtattagtgagcc  
acagatgtaactaactacggttcccgagtcgtgcctactgcctcggactcaaggggctagaattcgagcaattatctgt  
ttactaaaactgaataccttgctatctcttgatacatctttacaaagctgaattaaaatggtataaattaaatcacttt
- 20 >SYNGAP1\_coding\_1 (SEQ ID NO: 27 )
- tgtttgatgaggcttcagtactttacagaatcgttgctgcacatcttggaacacttgctgggattactcgacttctaa  
cccaacagaaggctcgagaaggtatattgctgttgacagtgagcgagcgcttaagtcagcataaattagtgagc  
cacagatgtattatgctggacttaaggcgctgcctactgcctcggactcaaggggctagaattcgagcaattatctt  
gttactaaaactgaataccttgctatctcttgatacatctttacaaagctgaattaaaatggtataaattaaatcacttt
- 25 > SYNGAP1\_coding\_2 (SEQ ID NO: 28)
- tgtttgatgaggcttcagtactttacagaatcgttgctgcacatcttggaacacttgctgggattactcgacttctaa  
cccaacagaaggctcgagaaggtatattgctgttgacagtgagcgagagttctgttcgaagttagtgagccac  
agatgtaagtaactcgaaacagaactcgtgcctactgcctcggactcaaggggctagaattcgagcaattatctgtt  
tactaaaactgaataccttgctatctcttgatacatctttacaaagctgaattaaaatggtataaattaaatcacttt
- 30 > SYNGAP1\_coding\_3 (SEQ ID NO: 29)
- tgtttgatgaggcttcagtactttacagaatcgttgctgcacatcttggaacacttgctgggattactcgacttctaa  
cccaacagaaggctcgagaaggtatattgctgttgacagtgagcgaggcgagcactcgagttcaattagtgagcc  
acagatgtaattgaactcgaagtgtcgccgtgcctactgcctcggactcaaggggctagaattcgagcaattatctgt  
ttactaaaactgaataccttgctatctcttgatacatctttacaaagctgaattaaaatggtataaattaaatcacttt
- 35 > SYNGAP1\_coding\_4 (SEQ ID NO: 30)

tgtttgatgaggcttcagtactttacagaatcgttgctgcacatcttggaacacttgctgggattactcgacttctaa  
 cccaacagaaggctcgagaaggatattgctgttgacagtgagcgaaagatccgaatctagcagaaatagtggaagc  
 cacagatgtatttctgtagattcggatcttgcctactgcctcggactcaaggggctagaattcgagcaattatctgtt  
 tactaaaactgaataccttgctatctcttggatacattttacaaagctgaattaaaatggataaaataatcacttt

5 > SYNGAP1\_coding\_5 (SEQ ID NO: 31)

tgtttgatgaggcttcagtactttacagaatcgttgctgcacatcttggaacacttgctgggattactcgacttctaa  
 cccaacagaaggctcgagaaggatattgctgttgacagtgagcgaaaggtcgtaaacagccactgttagtggaagc  
 cacagatgtaacagtggctgtttacgacctgtgcctactgcctcggactcaaggggctagaattcgagcaattatcttg  
 ttactaaaactgaataccttgctatctcttggatacattttacaaagctgaattaaaatggataaaataatcacttt

10 > SYNGAP1\_coding\_6 (SEQ ID NO: 32)

tgtttgatgaggcttcagtactttacagaatcgttgctgcacatcttggaacacttgctgggattactcgacttctaa  
 cccaacagaaggctcgagaaggatattgctgttgacagtgagcgaaagaggaaatacactctctgatagtggaagc  
 cacagatgtatcagagagtgtatttctctgtgcctactgcctcggactcaaggggctagaattcgagcaattatctgt  
 ttactaaaactgaataccttgctatctcttggatacattttacaaagctgaattaaaatggataaaataatcacttt

15

13. A construct as claimed in any one of the preceding claims wherein the non-mammalian or synthetic miRNA binding site is selected from

>ffluc1\_x1\_binding\_site (SEQ ID NO: 33)

gctatgaaacgatatgggctgaatacaaatcacag

20 >ffluc1\_x 3\_binding\_sites (SEQ ID NO: 34)

gctatgaaacgatatgggctgaatacaaatcacaggctatgaaacgatatgggctgaatacaaatcacaggctatg  
 aaacgatatgggctgaatacaaatcacag

>ffluc1\_x6\_binding\_sites (SEQ ID NO: 35)

gctatgaaacgatatgggctgaatacaaatcacaggctatgaaacgatatgggctgaatacaaatcacaggctatg  
 25 aaacgatatgggctgaatacaaatcacaggctatgaaacgatatgggctgaatacaaatcacaggctatgaaacg  
 atatgggctgaatacaaatcacaggctatgaaacgatatgggctgaatacaaatcacag

>ffluc1\_x3\_binding\_sites\_1bp\_central\_mismatch (SEQ ID NO: 36)

gctatgaaacgatatgggcggaatacaaatcacaggctatgaaacgatatgggcggaatacaaatcacaggctat  
 gaaacgatatgggcggaatacaaatcacag

30 > ffluc1\_x3\_binding\_sites\_3bp\_central\_mismatch (SEQ ID NO: 37)

gctatgaaacgatatgttatgaatacaaatcacaggctatgaaacgatatgttatgaatacaaatcacaggctatgaa  
 acgatatgttatgaatacaaatcacag

>ffluc1\_x3\_binding\_sites\_3'\_mismatch (SEQ ID NO: 38)

gctatgacccaaactgtgaagaatacaaatcacaggctatgagtgctatcacccgaatacaaatcacaggctatga  
 35 ctaggcccgtttcgaatacaaatcacag

- >ffluc1\_x3\_binding\_sites\_mutant\_1 (SEQ ID NO: 39)  
gctatgaaacgatatgcgctgaatacaaatcacaggctatgaaacgatatgcgctgaatacaaatcacaggctatga  
aacgatatgcgctgaatacaaatcacag
- >ffluc1\_binding\_sites\_mutant\_2 (SEQ ID NO: 40)  
5 gctatgaaacgatatgggctgaatacaaatcacaggctatgaaacgatatgggctgaatacaaatcacaggctatg  
aaacgatatgggctgaatacaaatcacag
- >ffluc1\_x3\_binding\_sites\_mutant\_3 (SEQ ID NO: 41)  
gctatgaaacgatatgggctcaatacaaatcacaggctatgaaacgatatgggctcaatacaaatcacaggctatga  
aacgatatgggctcaatacaaatcacag
- 10 >ffluc1\_x3\_binding\_sites\_mutant\_4 (SEQ ID NO: 42)  
gctatgaaacgatatgggctgattacaaatcacaggctatgaaacgatatgggctgattacaaatcacaggctatga  
aacgatatgggctgattacaaatcacag
- >ffluc1\_x3\_binding\_sites\_mutant\_5 (SEQ ID NO: 43)  
gctatgaaacgatatgggctgaattcaaatcacaggctatgaaacgatatgggctgaattcaaatcacaggctatga  
15 aacgatatgggctgaattcaaatcacag
- >ffluc1\_x3\_binding\_sites\_mutant\_6 (SEQ ID NO: 44)  
Gctatgaaacgatatgggctgaataactaatcacaggctatgaaacgatatgggctgaataactaatcacaggctatga  
aacgatatgggctgaataactaatcacag
- >ffluc9\_x3\_binding\_sites (SEQ ID NO: 45)  
20 gctatgaaacgtgaattgctcaacagtaatcacaggctatgaaacgtgaattgctcaacagtaatcacaggctatgaa  
acgtgaattgctcaacagtaatcacag
- >ffluc18\_x3\_binding\_sites (SEQ ID NO: 46)  
gctatgacccgacgatgacgccggtgaaatcacaggctatgacccgacgatgacgccggtgaaatcacaggctat  
gacccgacgatgacgccggtgaaatcacag
- 25 >ffluc22\_x3\_binding\_sites (SEQ ID NO: 47)  
gctatgattcgatagggacaagacaattatcacaggctatgattcgatagggacaagacaattatcacaggctatgat  
tcgatagggacaagacaattatcacag
- >Novel\_seq\_1\_3x\_binding\_sites (SEQ ID NO: 48)  
gctatgagcatgttacgggacttcttatatcacaggctatgagcatgttacgggacttcttatatcacaggctatgagcat  
30 gttacgggacttcttatatcacag
- >Novel\_seq\_2\_3x\_binding\_sites (SEQ ID NO: 49)  
gctatgagtgaggagcagcggtcttaaatcacaggctatgagtgaggagcagcggtcttaaatcacaggctatg  
agtgaggagcagcggtcttaaatcacag
- >Novel\_seq\_3\_3x\_binding\_sites (SEQ ID NO: 50)

- gctatgagtcacggaacttaacacaggctatgagtcacggaacttaacacaggctatgagtcacggaacttaacacag  
catgtcgtcacggaacttaacacag
- >Novel\_seq\_4\_3x\_binding\_sites (SEQ ID NO: 51)
- gctatgagagaagtcggttcgtattatcacaggctatgagagaagtcggttcgtattatcacaggctatgaga  
5 gaagtcggttcgtattatcacag
- >Novel\_seq\_5\_3x\_binding\_sites (ran1g binding sites) (SEQ ID NO: 52)
- gctatgaaagcgccaaaggagtcgtgaatcacaggctatgaaagcgccaaaggagtcgtgaatcacaggctatg  
aaagcgccaaaggagtcgtgaatcacag
- >Novel\_seq\_6\_3x\_binding\_sites (ran2g binding sites) (SEQ ID NO: 53)
- 10 gctatgaaagtcggttcgtattatcacaggctatgaaagtcggttcgtattatcacaggctatgaaagt  
gcggttcgtattatcacag
- >Novel\_seq\_7\_3x\_binding\_sites (SEQ ID NO: 54)
- gctatgaaagtcggttcgtattatcacaggctatgaaagtcggttcgtattatcacaggctatga  
aagtcggttcgtattatcacag
- 15 >Novel\_seq\_8\_3x\_binding\_sites (SEQ ID NO: 55)
- gctatgaaagtcggttcgtattatcacaggctatgaaagtcggttcgtattatcacaggctatga  
aacggttcggttcgtattatcacag
13. A construct as claimed in claim 12 wherein the synthetic miRNA is targeted  
20 against the coding sequence of a target gene and the synthetic miRNA binding site  
is selected from
- >MECP2\_coding\_1\_binding\_site (SEQ ID NO: 56)
- aagtcggttcgtattatcacag
- > MECP2\_coding\_2\_binding\_site (SEQ ID NO: 57)
- 25 aagtcggttcgtattatcacag
- > MECP2\_coding\_3\_binding\_site (SEQ ID NO: 58)
- aagtcggttcgtattatcacag
- > MECP2\_coding\_4\_binding\_site (SEQ ID NO: 59)
- gagtcggttcgtattatcacag
- 30 > MECP2\_coding\_5\_binding\_site (SEQ ID NO: 60)
- gttcggttcgtattatcacag
- > MECP2\_coding\_6\_binding\_site (SEQ ID NO: 61)
- gagtcggttcgtattatcacag
- >SYNGAP1\_coding\_1\_binding\_site (SEQ ID NO: 62)
- 35 ggccttaagtcggttcgtattatcacag

- > SYNGAP1\_coding\_2\_binding\_site (SEQ ID NO: 63)  
gagttctgttcgaagtact
- > SYNGAP1\_coding\_3\_binding\_site (SEQ ID NO: 64)  
ggcgagcacttcgagttcaat
- 5 > SYNGAP1\_coding\_4\_binding\_site (SEQ ID NO: 65)  
Aagatccgaatctagcagaaa
- > SYNGAP1\_coding\_5\_binding\_site (SEQ ID NO: 66)  
aaggtcgtaaacagccactgt
- > SYNGAP1\_coding\_6\_binding\_site (SEQ ID NO: 67)  
10 aagaggaaatacactctctga
14. A construct as claimed in any preceding claim wherein the promoter is selected from a constitutive or conditional promoter, optionally wherein the promoter is tissue specific.
- 15
15. A construct as claimed in any preceding claim wherein the promoter is selected from >EF1a\_promoter (SEQ ID NO: 68)  
Agtaattcatacaaaaggactcgcccctgccttgggggaatcccaggacgctcgttaaactcccactaacgtagaac  
ccagagatcgctgcgttccgccccctcaccgcccgcctctcgtcatcactgagggtggagaagagcatgcgtgagggc  
20 tccggtgccgctcagtgggcagagcgacatcgccacagtcgccgagaagttggggggaggggtcggaattga  
accggtgcctagagaagggtggcggggttaaactgggaaagtatgctgtactggctccgccttttccgaggggt  
gggggagaaccgtatataagtgcagtagtcgccgtgaacgttcttttcgcaacgggttgccgccagaacacag  
>Jet\_promoter (SEQ ID NO: 69)  
gggcggagtagggcggagccaatcagcgtgcgccgttccgaaagttgccttttatggctgggcggagaatgggcg  
25 gtgaacgccgatgattatataaggacgcgccgggtgtggcacagctagttccgtcgcagccgggattgggtcgcg  
ttctgtttgt
16. A construct as claimed in any preceding claim wherein the polyA sequence is selected from
- 30 >sv40 polyA signal (SEQ ID NO: 70 )  
Aactgtttattgcagcttataatggttacaataaagcaatagcatcacaatttcacaataaagcattttttcactgc  
attctagtgtgtgtgtccaaactcatcaatgtatctta  
>BGH polyA signal(SEQ ID NO: 71)

Ctgtgccttctagtgtccagccatctgtgtttgcccctccccgccttccttgaccctggaaggtgccactcccactgtc  
 ctttcctaataaaatgaggaaattgcatcgcatgtctgagtaggtgcattctattctgggggtgggtggggcagga  
 cagcaagggggaggattgggaagacaatagcaggcatgctgggatgcggtgggctctatgg, and  
 >SpA (SEQ ID NO: 72)

5 Aataaagagctcagatgcatcgatcagagtgtgtgtttttgtgtg

17. The construct of claims 1-16, further comprising a stability element, wherein the stability element is located in the 3'UTR.

10 18. The construct of claim 17, wherein the stability element is selected from  
 > Woodchuck Hepatitis Virus (WHV) Posttranscriptional Regulatory Element (WPRE)  
 (SEQ ID NO: 74)

AATCAACCTCTGGATTACAAAATTTGTGAAAGATTGACTGGTATTCTTAACTATG  
 TTGCTCCTTTTACGCTATGTGGATACGCTGCTTTAATGCCTTTGTATCATGCTAT  
 15 TGCTTCCCGTATGGCTTTCATTTTCTCCTCCTTGTATAAATCCTGGTTGCTGTCT  
 CTTTATGAGGAGTTGTGGCCCGTTGTCAGGCAACGTGGCGTGGTGTGCACTGT  
 GTTTGCTGACGCAACCCCCACTGGTTGGGGCATTGCCACCACCTGTCAGCTCC  
 TTTCCGGGACTTTTCGCTTTCCCCCTCCCTATTGCCACGGCGGAACATCGCCG  
 CCTGCCTTGCCCGCTGCTGGACAGGGGCTCGGCTGTTGGGCACTGACAATTCC  
 20 GTGGTGTTCGCGGGAAATCATCGTCCTTTCTTGGCTGCTCGCCTGTGTTGCC  
 ACCTGGATTCTGCGCGGGACGTCCTTCTGCTACGTCCCTTCGGCCCTCAATCC  
 AGCGGACCTTCTTCCCGCGGCCTGCTGCCGGCTCTGCGGCCTCTTCCGCGT  
 CTTGCGCTTCGCCCTCAGACGAGTCGGATCTCCCTTTGGGCCGCTCCCCGC  
 > a truncated version of WPRE (WPRE3) (SEQ ID NO: 75)

25 ATAATCAACCTCTGGATTACAAAATTTGTGAAAGATTGACTGGTATTCTTAACTAT  
 GTTGCTCCTTTTACGCTATGTGGATACGCTGCTTTAATGCCTTTGTATCATGCTA  
 TTGCTTCCCGTATGGCTTTCATTTTCTCCTCCTTGTATAAATCCTGGTTAGTTCTT  
 GCCACGGCGGAACATCATCGCCGCTGCTTGGCCGCTGCTGGACAGGGGCTC  
 GGCTGTTGGGCACTGACAATTCCGTGGT.

30

19. The construct of any preceding claim, wherein the construct further comprises ,  
 the Kozak sequence GCCACCATGG (SEQ ID NO: 73).

20. The construct of any preceding claim, wherein the miRNA binding site has been  
 35 designed to partially ameliorate miRNA binding.

21. A vector comprising a construct of any one of claims 1 to 20.
22. The vector of claim 21, wherein the vector is an AAV or lentiviral vector,  
optionally wherein the vector is an AAV vector, optionally wherein the construct is  
5 operably linked to expression control elements, and the expression control elements  
and the construct are together flanked by 5' and 3' AAV inverted terminal repeats  
(ITR).
23. The vector of any one of claims 21-22, packaged into a virion, optionally wherein  
the vector when packaged into the viron does not affect the quality of the construct.
- 10 24. The vector of any one of claims 21-22, formulated in a nanoparticle.
25. A method of using a construct of any one of claims 1 to 20 or a vector of claim 21  
to 24 to express a transgene, optionally to express a transgene in a specific  
mammalian cell type or types.
- 15 26. A method of treating a disorder in a subject, the method comprising the step of  
providing a construct of any one of claims 1 to 20, or a vector of claim 21 to 24 to the  
subject.
- 20 27. A composition comprising a construct of any one of claims 1 to 20, or a vector of  
claim 21 to 24 for use in the treatment of a disorder caused by insufficient expression  
of a gene in a subject.
- 25 28. A method of claim 26 or a composition of claim 27 wherein the disorder is any  
monogenic disorder in which controlled expression of the corrective gene is desired,  
optionally wherein the monogenic disorder is selected from the group consisting of  
Rett Syndrome, Fragile X syndrome, Angelman syndrome, Syngap-related  
intellectual disability, CDK15 deficiency, Friedrich's ataxia, Spinal muscular dystrophy,  
Haemophilia and Diabetes.
- 30 29. A method of claim 26 or a composition of claim 27 wherein the disorder is treated  
by expression of a gene selected from the list comprising: PRKCZ, TTC34, PRDM16,

ARHGEF16, PARK7, PRDM2, IGSF21, PTCH2, NFIA, ST6GALNAC3, DPYD, COL11A1, PDZK1, GPR89A, NBPFF11, GPR89B, KCNT2, CFHR2, ASPM, PTPRC, GPATCH2, DUSP10, GPR137B, RYR2, CHRM3, RGS7, AKT3, KIF26B, SMYD3, LPIN1, EPCAM, MSH2, NRXN1, XPO1, LRP1B, ZEB2, ACVR2A, MBD5, KIF5C, 5 SCN1A, COL3A1, PMS1, PLCL1, SATB2, PARD3B, EPHA4, SPHKAP, CHL1, GRM7, TRANK1, DOCK3, FAM19A1, FOXP1, ROBO1, CADM2, FOXL2, SOX2, LPP, RASGEF1B, GRID2, FAT4, NR3C2, LRBA, FGA, GALNTL6, WWC2, TLR3, IRX2, IRX1, CDH12, CDH9, NIPBL, HEXB, MEF2C, GRAMD3, FBN2, PRELID2, TCOF1, GABRG2, MSX2, NSD1, FOXC1, CDYL, TBC1D7, RUNX2, MUT, RIMS1, 10 NKAIN2, LAMA2, ARID1B, PARK2, PACRG, QKI, TNRC18, FBXL18, SUGCT, GLI3, AUTS2, MLXIPL, COL1A2, PPP1R9A, CFTR, TSPAN12, GRM8, CNTNAP2, MNX1, CSMD1, MCPH1, LPL, ANK1, IMPAD1, CHD7, VCPIP1, TRPS1, PARP10, DOCK8, KANK1, GLIS3, PTPRD, MLLT3, ROR2, PTCH1, AL162389.1, ARRDC1, EHMT1, PCDH15, CTNNA3, ADK, BMPR1A, PAX2, BTRC, INPP5A, MRPL23, ELP4, PAX6, 15 CPT1A, DYNC2H1, KIRREL3, WNK1, CACNA1C, PPFIBP1, TBX5, MED13L, NALCN, CHD8, MYH7, TTC6, DAAM1, NRXN3, MTA1, SNRPN, UBE3A, OCA2, HERC2, CHRFAM7A, ARHGAP11B, OTUD7A, FBN1, HEXA, SNUPN, NRG4, AC112693.2, IGF1R, LRRC28, HBA2, HBQ1, CREBBP, RBFOX1, CDR2, CDH13, CYBA, NXN, YWHAE, SMG6, METTL16, PAFAH1B1, ADORA2B, NT5M, RAI1, 20 NF1, C17orf67, PITPNC1, ACOX1, TCF4, DOCK6, CACNA1A, LPHN1, ZSCAN5A, BMP2, MYT1, PEX26, USP18, DGCR6L, USP41, UBE2L3, NF2, LARGE, BRD1, SHANK3 CDKL5, FXN, SMN1, F8 and INS.