Fig. S1 pre-miR-LRRK2-5 and pre-miR-LRRK2-5NB direct silencing of a complementary dual-luciferase target sequence A) Dual-luciferase reporter assay showing knockdown of LRRK2 target sequence following co-transfection with indicated pre-miRNA variants. Values represent mean ratios of *Renilla*:Firefly luciferase +/- S.D. from n=6. Pre-miRNA variants are normalised to cells transfected with a non-specific U6 pre-miRNA hairpin. *= P<0.05 relative to normalising control.

Fig. S2 mirt-LRRK2-5NB directs silencing in a sequence-specific manner A) Codon-modified scrambled target sequence to mirt-LRRK2-5NB. B) Dual-luciferase reporter assays showing knockdown of a codon-modified scrambled LRRK2 target following co-transfection with indicated mirtron (left panel) and pre-miRNA control (right panel) variants. Values represent mean ratios of *Renilla*:Firefly luciferase +/- S.D. from n=6. Mirtron variants are normalized to cells transfected with the NAD variant. Pre-miRNA variants are normalised to cells transfected with a non-specific U6 pre-miRNA hairpin. *= P<0.05 relative to respective normalising control.

Fig. S3 Direct comparison of synthetic mirtrons to U6-transcribed pre-miRNAs (shRNAs) A) Dual-luciferase reporter assays showing knockdown of a LRRK2 target following co-transfection with indicated mirtron (left panel) and pre-miRNA control (right panel) variants at equivalent hairpin concentrations of 2.5 *f*m. Values represent mean ratios of *Renilla*:Firefly luciferase +/- S.D. from n=3. Mirtron variants are normalized to cells transfected with the NAD variant. Pre-miRNA variants are normalised to cells transfected with a non-specific U6 pre-miRNA hairpin. *= P<0.05 relative to respective normalising control.

Fig. S4 Algorithm design of a synthetic mirtron targeting α-synuclein. A) Predicted hairpin alignments of α-synuclein targeting synthetic mirtron, α-syn-mirt-1, designed using an in-house algorithm. B) Representative fluorescent microscopy images of different intron variants 48hrs after transfection in HEK-293 cells. C) Quantification of eGFP fluorescence following expression indicated intron variants in HEK-293 cells. Values represent mean fluorescence +/- S.D. from n=3. *= P<0.05 relative to non-transfected cells. D) Dual-luciferase reporter assays showing

knockdown of an α-synuclein target following co-transfection with indicated mirtron (left panel) and pre-miRNA control (right panel) variants. Values represent mean ratios of *Renilla*:Firefly luciferase +/- S.D. from n=3. Mirtron variants are normalized to cells transfected with the NAD variant. Pre-miRNA variants are normalised to cells transfected with a non-specific U6 pre-miRNA hairpin. *= P<0.05 relative to respective normalising control. E) Quantification of mCherry tagged α-synuclein fluorescence following co-expression with the NAD intron or mirt-α-Syn-1 in HEK-293 cells. Values represent mean fluorescence +/- S.D. from n=3. Mirt-α-Syn-1 fluorescence is normalized to cells transfected with the NAD intron. *= P<0.05 relative to respective normalising control.

Fig. S5 A) Quantification of eGFP fluorescence following expression of indicated intron variants in SH-SY5Y cells and Hela cells. Values represent mean fluorescence +/- S.D. from n=3. *= P<0.05 relative to non-transfected cells.

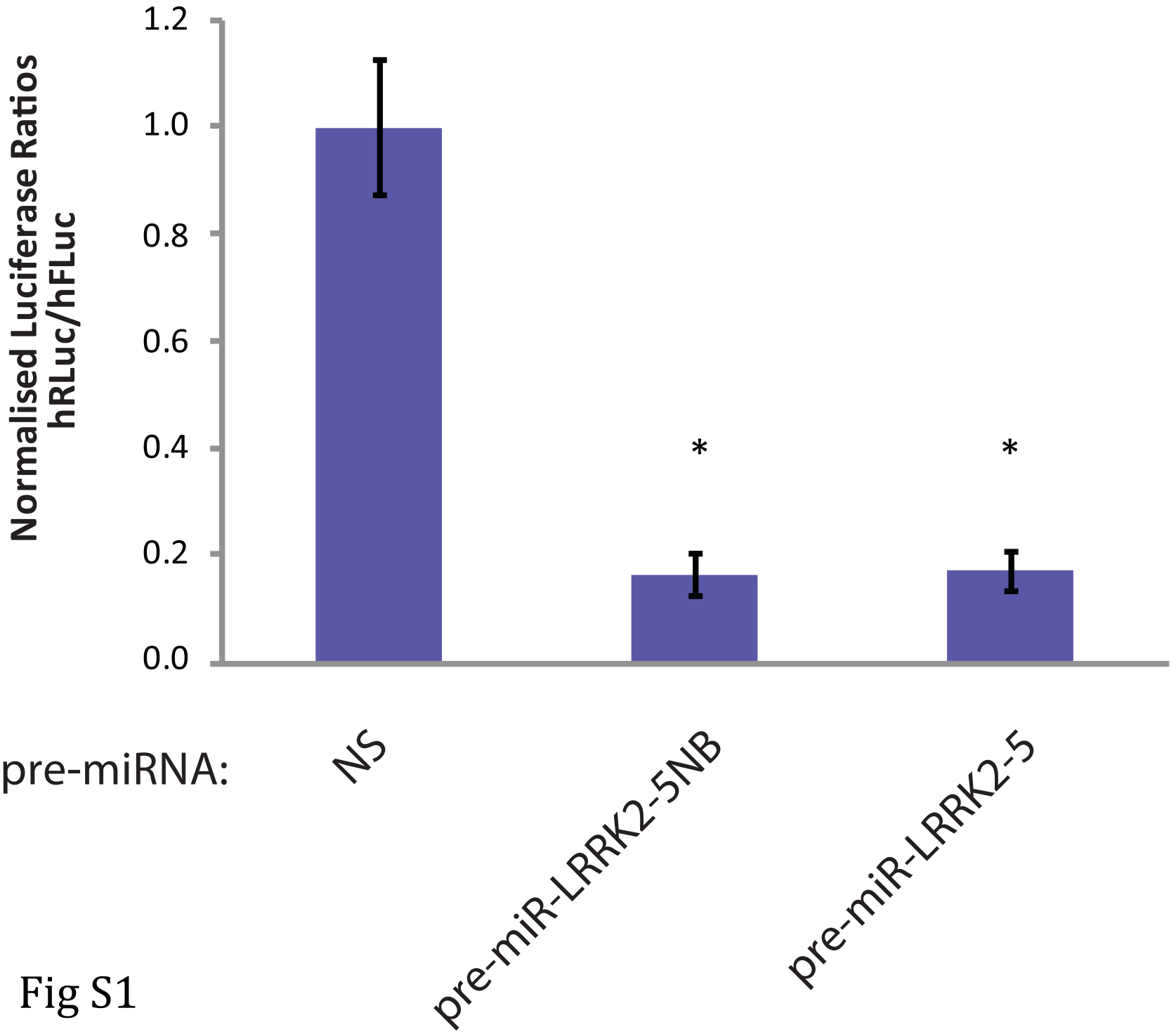
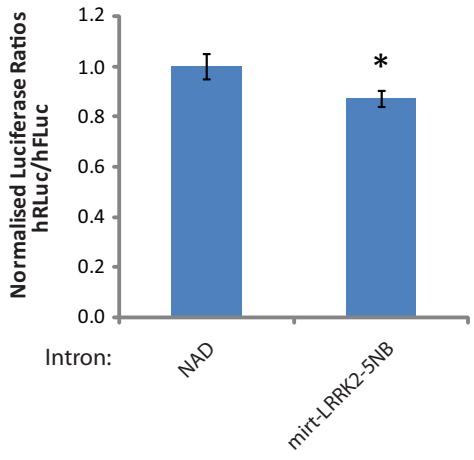

Normalised Luciferase Ratios
hRLuc/hFLuc
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
*
pre-miRNA:
NS
pre-miR-LRRK2-5NB
pre-miR-LRRK2-5

Fig S1

A.

```
LRRK2 sequence: acc ttt att cct gac tct tct atg
Amino-acid seq: Thr Phe Ile Pro Asp Ser Ser Met
Codon replaced: acG ttC atC ccG gaT tcG tcG atg
```

B.



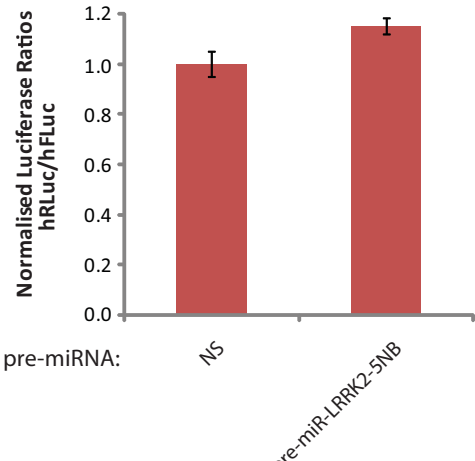


Fig S2

A.

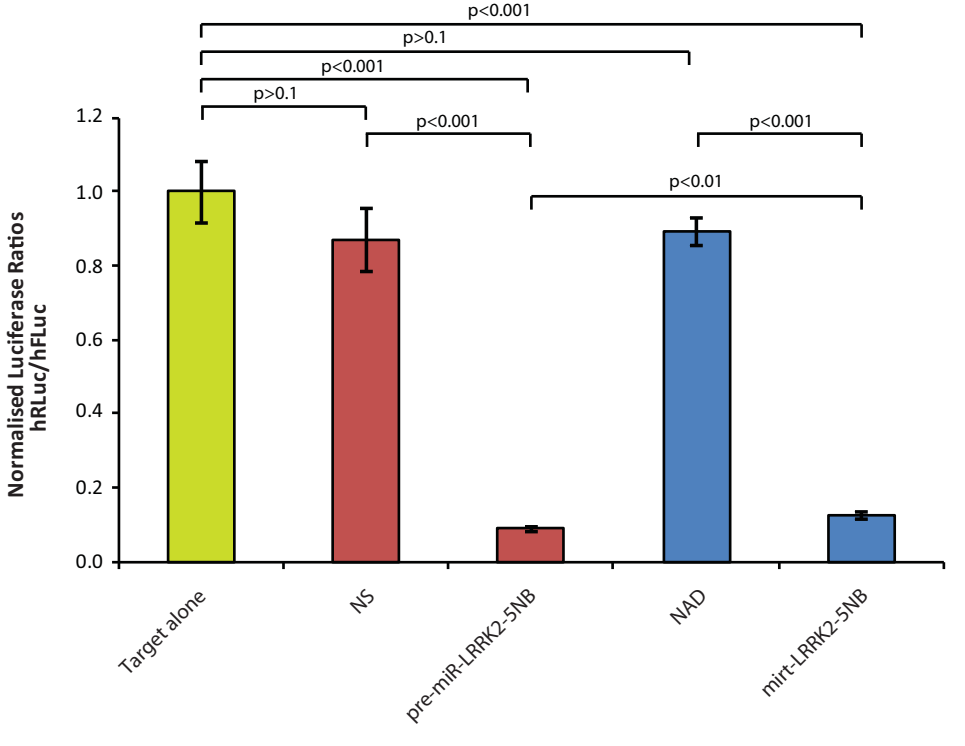

p<0.001
p>0.1
p<0.001
p>0.1
p<0.001
p<0.001
p<0.01
Normalised Luciferase Ratios
hRLuc/hFLuc
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Target alone
NS
pre-miR-LRRK2-5NB
NAD
mirt-LRRK2-5NB
pre-miRNA
Intron

Fig S3

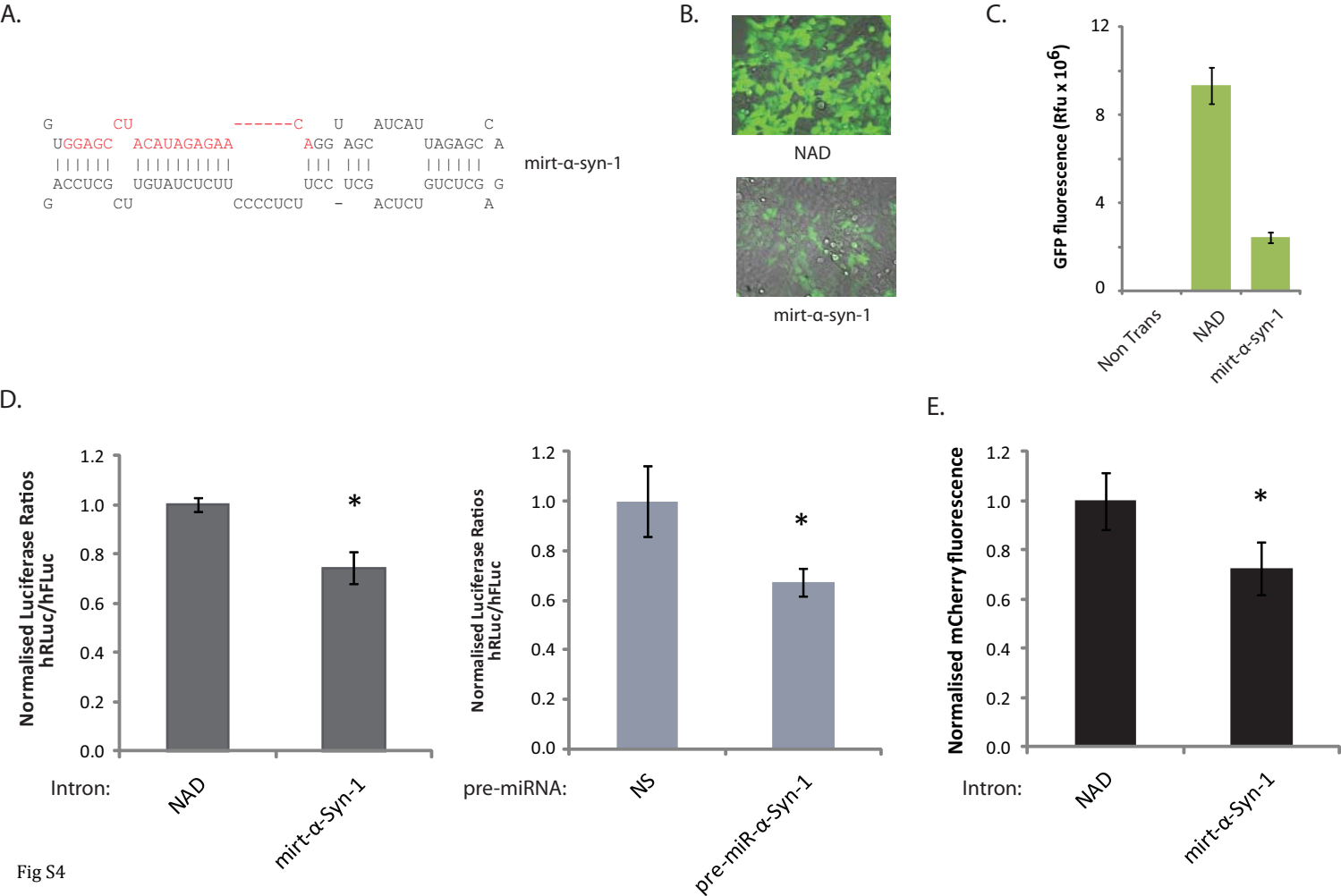

A.
G CU ------C U AUCAU C
UGGAGC ACAUAGAGAA AGG AGC UAGAGC A
ACCUCG UGUAUCUCUU UCC UCG GUCUCG G
G CU CCCCUCU - ACUCU A
mirt-α-syn-1
B.
NAD
mirt-α-syn-1
C.
GFP fluorescence (Rfu x 10^6)
12
8
4
0
Non Trans
NAD
mirt-α-syn-1
D.
Normalised Luciferase Ratios hRLuc/hFLuc
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
Intron:
NAD
mirt-α-Syn-1
pre-miRNA:
NS
pre-miR-α-Syn-1
E.
Normalised mCherry fluorescence
Intron:
NAD
mirt-α-Syn-1

Fig S4

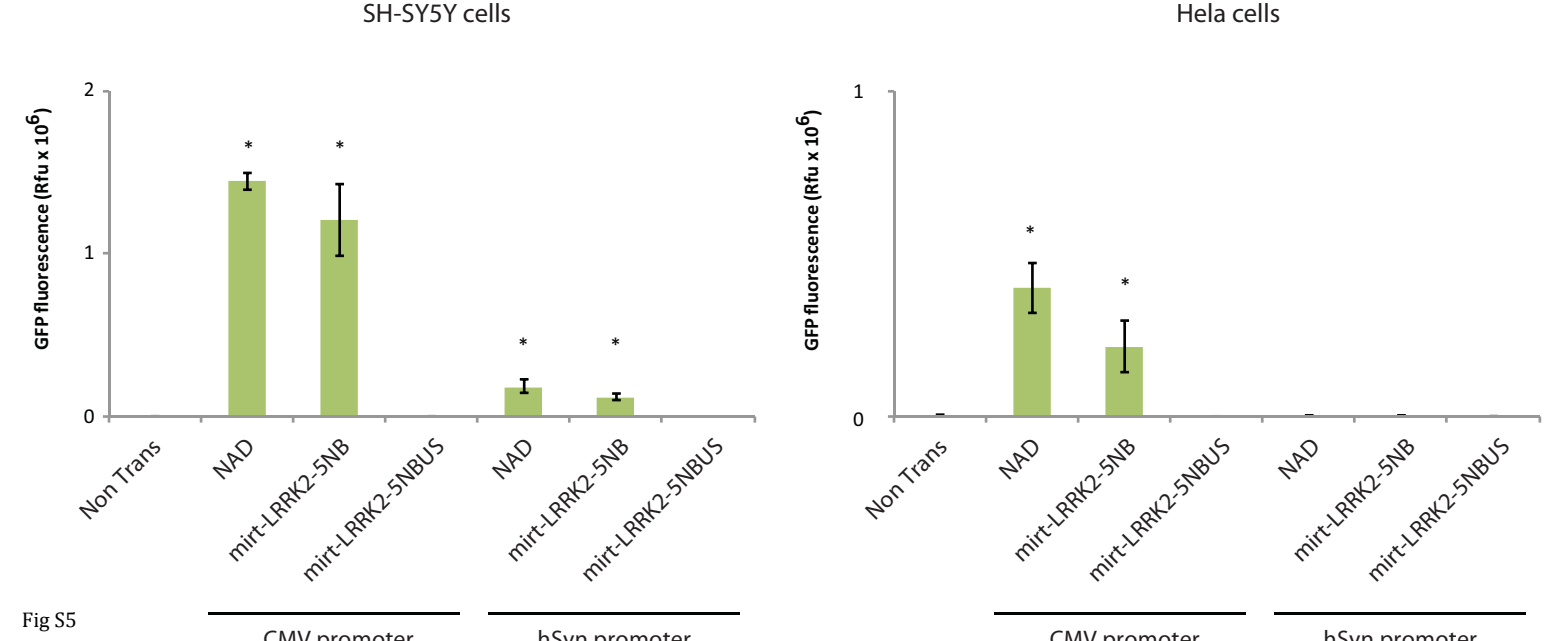

SH-SY5Y cells
Hela cells
GFP fluorescence (Rfu x 10^6)
2
1
0
*
Non Trans
NAD
mirt-LRRK2-5NB
mirt-LRRK2-5NBUS
CMV promoter
hSyn promoter

Fig S5