SUPPLEMENTARY FIGURE LEGENDS

Suppl. Fig. 1. Syncrip transcript isoform quantification

Transcript specific reanalysis of a published RNA sequencing dataset in rat cortical neurons (DIV7) (26). Violine plots show that transcript ENSRNOT00000074515 (Ensembl_ID - corresponding to isoform X3 in the NCBI annotation (XM_006243515)) is the highest expressed among Ensembl annotated Syncrip isoforms

Suppl. Fig. 2. Pie chart representing the distribution of iCLIP peaks (Piranha bin 20 – at least 2 replicates) in the defined regions of the complete rat genome (Rnor_6.0).

Different regions of NCBI RefSeq genes are indicated as 5' UTR, coding exons, introns and 3'UTR. Peaks mapping outside NCBI RefSeq genes are divided in two regions – intergenic and 1000 bp downstream of 3'UTR end.

Suppl. Fig. 3. Gene Ontology analysis of SYNCRIP targets

Gene ontology (GO) analysis of molecular function (MF, upper panel) and cellular component (CC, lower panel) terms enriched in SYNCRIP iCLIP target genes. Displayed are the top 15 MF and CC terms based on statistical enrichment (P-values). The number of SYNCRIP iCLIP target genes in each term is highlighted on the right.

Suppl. Fig. 4. A snapshot from NCBI genomic data viewer showing detailed mapping profile of iCLIP unique reads and peaks within selected SYNCRIP iCLIP target genes (DCX, Cd24 and Syt4).

Total unique reads from only one replicate of SYNCRIP iCLIP were used to visualize mapping profile of iCLIP unique reads to rat genome (Rnor_6.0), as an example (unique reads panel). Positions of significant peaks (Piranha peaks, bin 20 – at least 2 replicates) and a number of unique reads identified within each peak are provided as a separate panel.

Suppl. Fig. 5. Schematic representation of luciferase reporter genes containing 2x miR-9 and 2x Let7 binding sites in the 3' UTR.

Sequence of each miRNA binding site (black) together with its complementary mature miRNA (green) are provided. Mutations disrupting the binding of mature miRNA to the respective 3'UTR are indicated as a red box.

Suppl. Fig. 6. Depletion of SYNCRIP leads to upregulation of luciferase reporter lacking poly-A tail.

Luciferase reporter gene assays in rat cortical neurons (DIV 6-9) transfected with the indicated 2xmiR binding site (BS) reporters, containing histone stem loop (HSL) and hammerhead ribozyme (HhR), and shRNA constructs. Relative luciferase activity (firefly/renilla activity) is presented as mean ± SD (n=4). For each reporter gene, results from co-transfection of a vector alone (without shRNA) were set to one. Statistical test is performed using two-way ANOVA and corrected for multiple

comparison with Tukey's post-hoc test (2x miR-9 BS-HSL-HhR: $P<0.05$, 2x miR-9 BS-mut-HSL-HhR: P= n.s.; BS: $F(1, 12) = 3.039$, $P=0.107$; shRNA: $F(1, 12) = 10.81$, $P=0.007$; BS x shRNA: $F(1, 12) = 2.183$, $P=0.165$).

Suppl. Fig. 7. Regulation of miR-9 and Let7 BS luciferase reporter expression by miRNAs occurs mainly at the level of mRNA translation.

Rat cortical neurons (DIV 6) were transfected with the indicated 2xmiR binding site (BS) reporters and lysed at DIV 9 either for RNA extraction or luciferase activity measurements. RNA levels of luciferase reporters were determined using real-time PCR. Relative luciferase activity (firefly/renilla activity) and RNA levels are presented as mean ± SD (n=3). Statistical test is performed using one-sample Student's t-test (p value, * < 0.05, ** < 0.01).

Suppl. Fig. 8. miRNA binding site enrichment analysis

Enrichment plots, highlighting the results of miRNA binding site depletion (left panel) and enrichment (right panel) analyses on SYNCRIP iCLIP target genes against a specific gene expression background. Circle sizes indicate the overlap of predicted miRNA and SYNCRIP target genes. Plotted are all miRNAs with an overlap of at least five and a p-value equal or below 0.05. y-axis – -log10 transformed p-value (hypergeometric test on miRNA binding sites), x-axis – fold enrichment of miRNA binding sites against the background. miRNA expression levels in DIV7 cortical neuronal cultures, as reported in (21), are color-coded.

SUPPLEMENTARY TABLES:

Suppl. Table 1. General analysis pipeline of iCLIP sequencing

Suppl. Table 2. The list of all significant peaks (< 0.05 FDR, present in at least 2 out of 3 replicates) identified using –bin_size=1 parameter of Piranha software.

Suppl. Table 3. The list of all significant peaks (< 0.05 FDR, present in at least 2 out of 3 replicates) identified using –bin_size=20 parameter of Piranha software.

Suppl. Table 4. The number of unique reads and peaks from Suppl. T2 mapping to different genomic positions.

Suppl. Table 5. The number of unique reads and peaks from Suppl. T3 mapping to different genomic positions.

Suppl. Table 6. The list of SYNCRIP iCLIP target genes based on data from Suppl. Table T3 that maps to 3'UTR.

SUPPLEMENTARY MATERIAL:

DNA oligonucleotides

Cloning

Into pEGFP-N1

fw-N1_X3_SalI: CTAGTCGACCGCCACCATGGCTACAGAACATGTTAATGGA

rev-N1_X3_AgeI: GATCACCGGTTGTAACAGGTCAGGACCGG

fw-N1_X7_SalI: CTAGTCGACCGCCACCATGAAGACTTACAGGCAGAGAGAGA

rev-N1_X7_AgeI: same as rev-N1_X3_AgeI

Into pEGFP-C1

fw-C1_X6_XhoI: CTAGCTCGAGCAATGAAGACTTACAGGCAGAGAGAGA

rev-C1_X6_XbaI: CTAGTCTAGACTACTTCCACTGTTGCCCAAAAG

into pIS1:

fw-2xmiR-9 BS:
CATTAACAGCTGATCAACCAAAGACTCCGTTAACAGCTGATCAACCAAAGA

rev-2xmiR-9 BS:
CTAGTCTTTGGTTGATCAGCTGTTAACGGAGTCTTTGGTTGATCAGCTGTTAATG
AGCT

fw-2xmiR-9 BS-mut:
CATTAACAGCTGATCAACCGTTCACTCCGTTAACAGCTGATCAACCGTTCA

rev-2xmiR-9 BS-mut:
CTAGTGAACGGTTGATCAGCTGTTAACGGAGTGAACGGTTGATCAGCTGTTAAT
GAGCT

fw-2xLet7 BS (Hmga2):
CCAATCAAAACACACTACTACCTCTTAAGTCCCAGTATACCTCATTT

rev-2xLet7 BS (Hmga2):
CTAGAAATGAGGTATACTGGGACTTAAGAGGTAGTAGTGTGTTTTGATTGGAGC
T

fw-2xLet7 BS-mut:
CCAATCAAAACACACTACTAACGCTTAAGTCCCAGTATAACGCATTT

rev-2xLet7 BS-mut:
CTAGAAATGCGTTATACTGGGACTTAAGCGTTAGTAGTGTGTTTTGATTGGAGC
T

fw-HSL-HhR: CTAGACTAGTATCTCGAGGCGGCCGC

Rev-HSL-HhR: CTAGATCGATAAGCTTGATATCGAATTCCC

into pSuper:

fw1-SYNCRIP-shRNA-1: GATCCCCTGAGAAAGCTGGACCTA

fw2-SYNCRIP-shRNA-1: TATTCAAGAGATATAGGTCCAGCTTTCTCATTTTTA

rev1- SYNCRIP-shRNA-1: AGCTTAAAAATGAGAAAGCTGGACCTA

rev1- SYNCRIP-shRNA-1: TATCTCTTGAATATAGGTCCAGCTTTCTCAGGG

fw1-SYNCRIP-shRNA-2: GATCCCCGATCAGAAGAGGAAAGA

fw2-SYNCRIP-shRNA-2: AATTCAAGAGATTTCTTTCCTCTTCTGATCTTTTTA

rev1-SYNCRIP-shRNA-2: AGCTTAAAAAGATCAGAAGAGGAAAGA

rev2-SYNCRIP-shRNA-2: AATCTCTTGAATTTCTTTCCTCTTCTGATCGGG

fw1-Cyt-SYNCRIP-shRNA - GATCCCCGATCAGAGCTTATACCT

fw2-Cyt-SYNCRIP-shRNA - AATTCAAGAGATTAGGTATAAGCTCTGATCTTTTTA

rev1-Cyt-SYNCRIP-shRNA - AGCTTAAAAAGATCAGAGCTTATACCT

rev2-Cyt-SYNCRIP-shRNA - AATCTCTTGAATTAGGTATAAGCTCTGATCGGG

Real-time PCR

fw-U6: CTCGCTTCGGCAGCACA

rev-U6: AACGCTTCACGAATTTGCGT

fw_12sRNA: ATACCGCCATCTTCAGCAAAC

rev-12sRNA: TTCTTTCCGCTTCATTGGCTA

fw-GAPDH: GCCTTCTCTTGTGACAAAGTGGA

rev-GAPDH: CCGTGGGTAGAGTCATACTGGAA

fw-HIPK2: AACACAAGCAGCGTGCAGAT

rev-HIPK2: TGTTTTTGGAGGTGGCAGTG

fw-Dclk1: AAATTTCGCTGTTGTCAAGGAA

rev-Dclk1: GACCTCGTTTTGGATCATGTGT

fw-Cd24: TCCTACCCACGCAGATTTATTG

rev-Cd24: GATTTGGGGCAGCAGAGATACT

fw-DCX: GGAGTGCGCTACATTTACACT

rev-DCX: GTCTGAGGAACAGACATAGCTT

fw-Syt4: CGGGGTGAACTTCTGGTCTC

rev-Syt4: TGCCGCGCTTTTAAGACAAC

fw-Firefly: GCTCAGCAAGGAGGTAGGTG

rev-Firefly: TCTTACCGGTGTCCAAGTCC

fw-Renilla: TTATTGAATCGGACCCAGGA

re-Renilla: TTGAGAACTCGCTCAACGAA

RNA-pull down

Templates for in vitro transcription

fw-TAG-pre: AATTTAATACGACTCACTATAG

rev-TAG-pre: AGTGAGTCGTATTAAATT

fw1-TAG-2xmiR-124 BS: GGAGAATAGATAGTCTGTTTTATATCTTAAGAGTGC

fw2-TAG-2xmiR-124 BS: CTTATTATGTTGCTGCCCACATTGTGCCTTTA

rev1-TAG-2xmiR-124 BS:
TAAAGGCACAATGTGGGCAGCAACATAATAAGGCACTCTT

rev2-TAG-2xmiR-124 BS: AAGATATAAAACAGACTATCTATTCTCCCTAT

fw1-TAG-2xmiR-124 BS-mut: same as fw1-TAG-2xmiR-124 BS

fw2-TAG-2xmiR-124 BS-mut: GCCATTATGTTGCTGCCCACATTGTCGGTTTA

rev1-TAG-2xmiR-124 BS-mut:
TAAACCGACAATGTGGGCAGCAACATAATGGCGCACTCTT

rev2-TAG-2xmiR-124 BS-mut: same as rev2-TAG-2xmiR-124 BS

fw1-TAG-2xmiR-9 BS: GGAGAATAGATAGTATTAACAGCTGATCAACC

fw2-TAG-2xmiR-9 BS: AAAGACTCCGTTAACAGCTGATCAACCAAAGA

rev1-TAG-2xmiR-9 BS:
TCTTTGGTTGATCAGCTGTTAACGGAGTCTTTGGTTGATC

rev2-TAG-2xmiR-9 BS: AGCTGTTAATACTATCTATTCTCCCTAT

fw1-TAG-2xmiR-9 BS-mut: same as fw1-TAG-2xmiR-9 BS

fw2-TAG-2xmiR-9 BS-mut: GTTCACTCCGTTAACAGCTGATCAACCGTTCA

rev1-TAG-2xmiR-9 BS-mut:
TGAACGGTTGATCAGCTGTTAACGGAGTGAACGGTTGATC

rev2-TAG-2xmiR-9 BS-mut: same as rev2-TAG-2xmiR-9 BS

DNA adapter

5’ ACTATCTATTCTCCC 3’ biotin

iCLIP (from Sutandy et al., 2016)

L3 linker: /5rApp/AGATCGGAAGAGCGGTTCAG/3ddC/

RT-CLIP1: PHO/NNAACCNNNAGATCGGAAGAGCGTCGTGGATCCTGAACCGC

RT-CLIP2: PHO/NNACAANNNAGATCGGAAGAGCGTCGTGGATCCTGAACCGC

RT-CLIP3: PHO/NNATTGNNNAGATCGGAAGAGCGTCGTGGATCCTGAACCGC

RT-CLIP4: PHO/NNCGCCNNNAGATCGGAAGAGCGTCGTGGATCCTGAACCGC

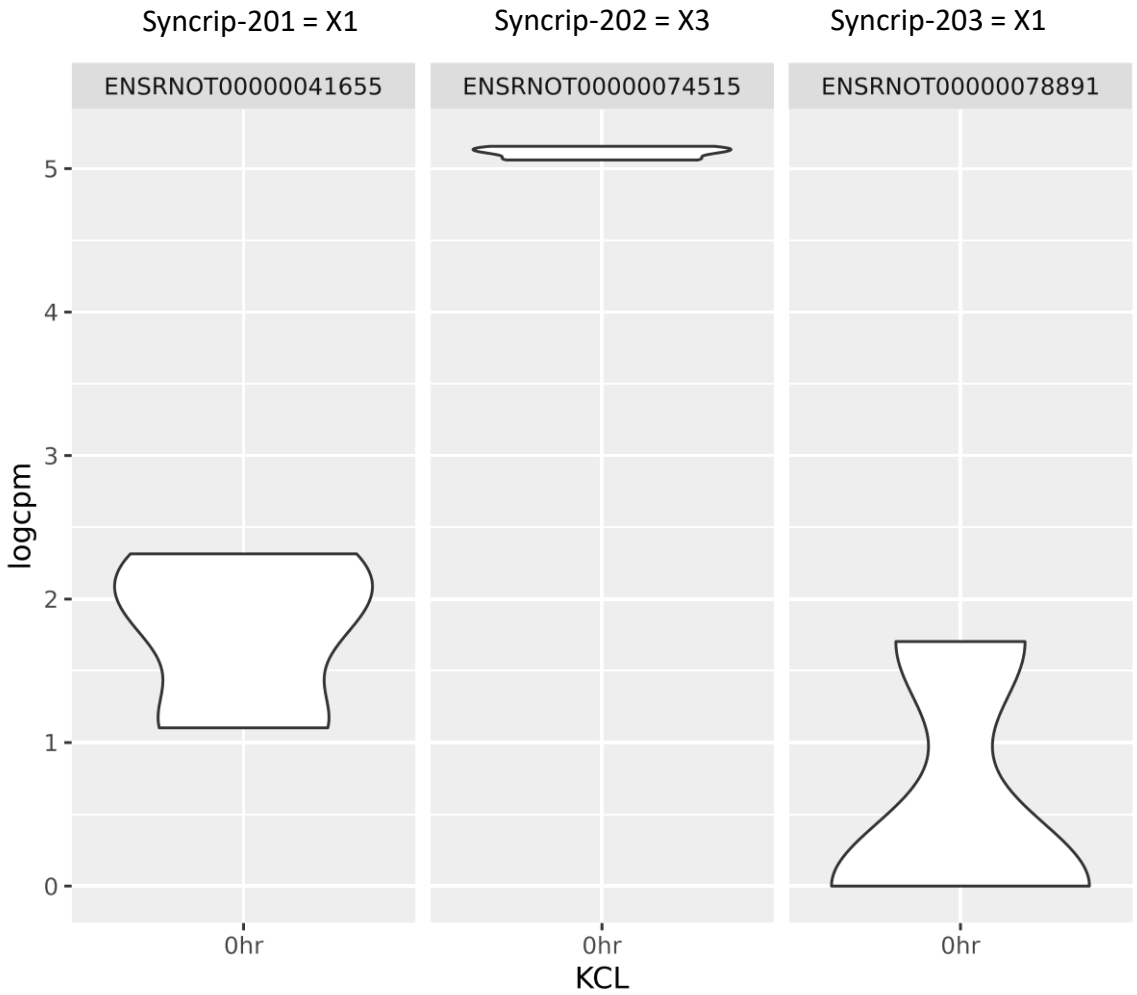
RT-CLIP5: PHO/NNGCCANNNAGATCGGAAGAGCGTCGTGGATCCTGAACCGC

RT-CLIP6: PHO/NNGACTNNNAGATCGGAAGAGCGTCGTGGATCCTGAACCGC

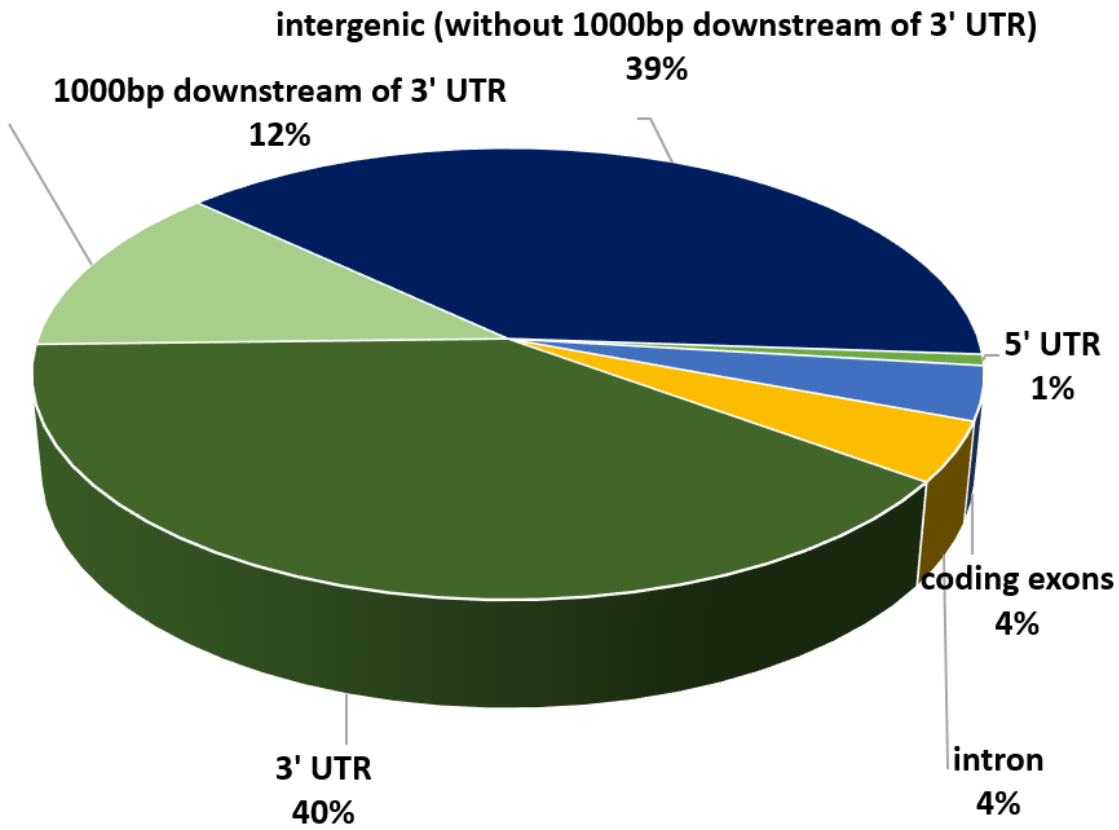
Cut_oligo: GTTCAGGATCCACGACGCTCTTCAAAA

P5solexa:
AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT

P3solexa:
CAAGCAGAAGACGGCATACGAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATCT

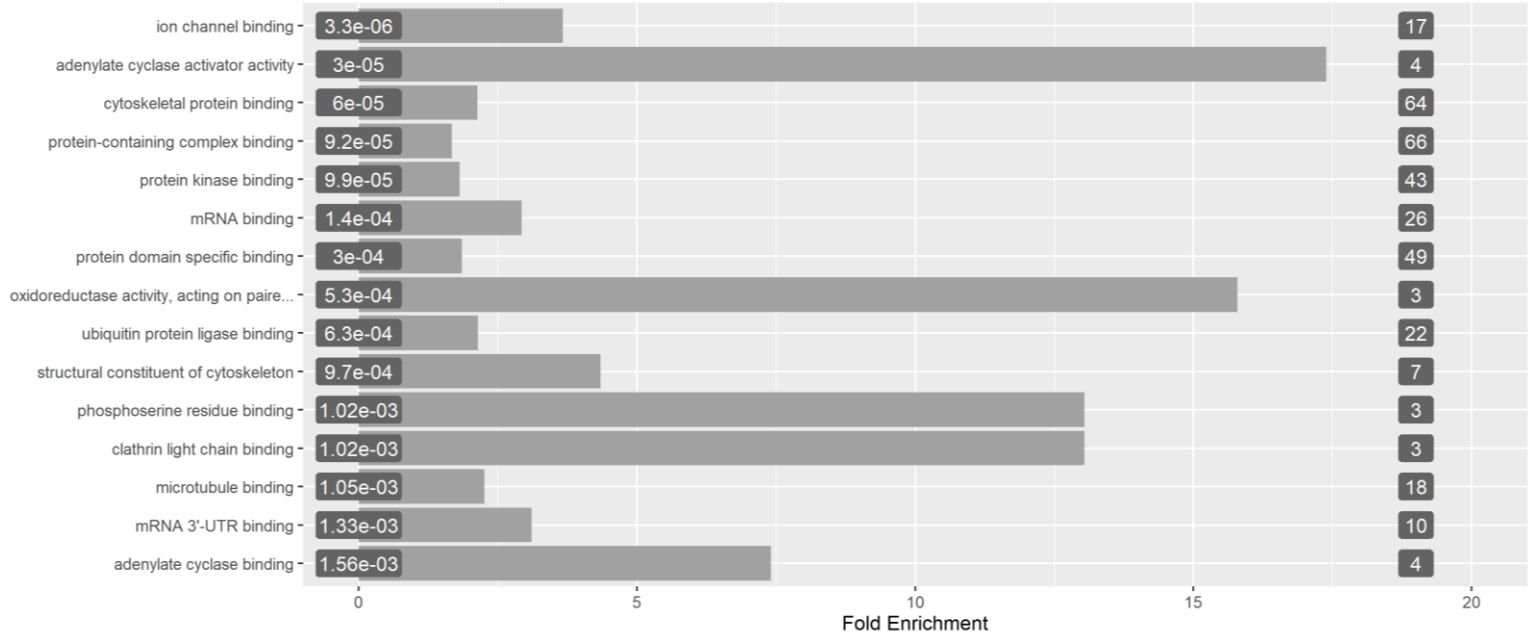

Syncrip-201 = X1
Syncrip-202 = X3
Syncrip-203 = X1
ENSRNOT00000041655
ENSRNOT00000074515
ENSRNOT00000078891
logcpm
5
4
3
2
1
0
0hr
0hr
0hr
KCL

Suppl. Fig. 1

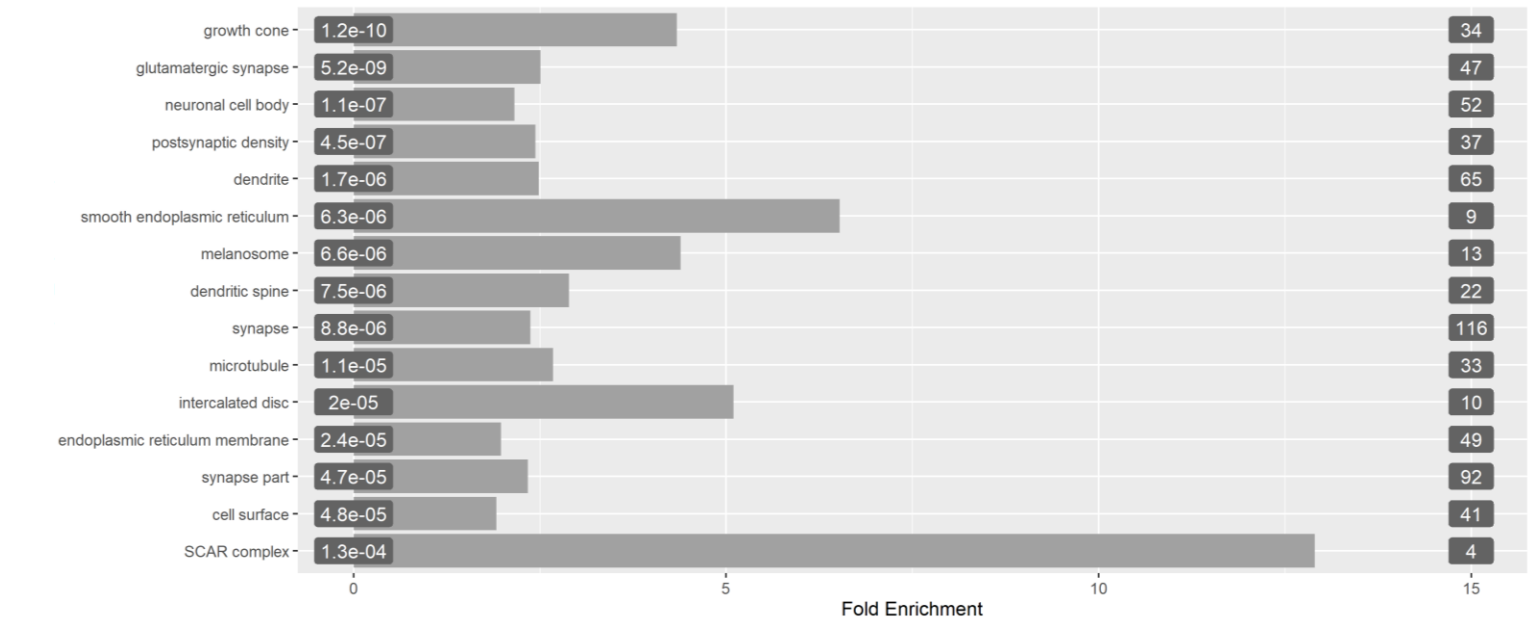

intergenic (without 1000bp downstream of 3' UTR)
39%
1000bp downstream of 3' UTR
12%
5' UTR
1%
coding exons
4%
intron
4%
3' UTR
40%

Suppl. Fig. 2

MF

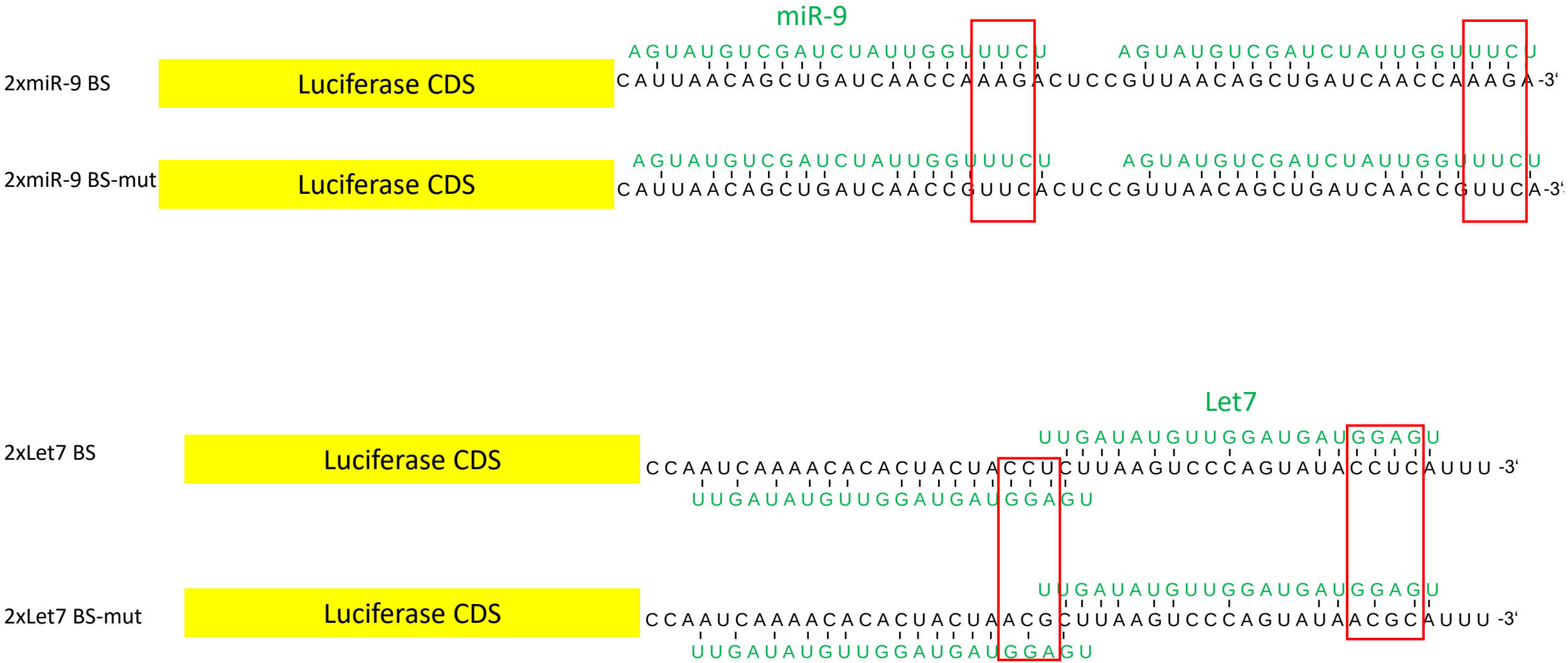


CC



Suppl. Fig. 3

DCX
Unique reads
Piranha bin20 peaks (#unique reads/peak)
Genes, NCBI Rattus norvegicus Annotation Release 106, 2016-07-27
RNA-seq exon coverage, aggregate (filtered), NCBI Rattus norvegicus Annotation Release 106 - log 2 scaled
(U) All_conditions/1hnrnpq
(U) Piranha-bin20
Dcx [+14
Cd24
NM_012752.3
NP_036884.1
Syt4
NP_113881.1
NM_031693.2
Suppl. Fig. 4


miR-9
2xmiR-9 BS
Luciferase CDS
AGUAUGUCGAUCUAUUGGUUUCU
CAUUAACAGCUGAUCAACCAAAGACUCCGUUAACAGCUGAUCAACCAAAGA-3'
AGUAUGUCGAUCUAUUGGUUUCU
2xmiR-9 BS-mut
Luciferase CDS
AGUAUGUCGAUCUAUUGGUUUCU
CAUUAACAGCUGAUCAACCGUUCACUCCGUUAACAGCUGAUCAACCGUUCA-3'
AGUAUGUCGAUCUAUUGGUUUCU
Let7
2xLet7 BS
Luciferase CDS
UUGAUAUGUUGGAUGAUGGAGU
CCAAUCAAAACACACUACUACCUCUUAAGUCCCAGUAUACCUCAUUU-3'
UUGAUAUGUUGGAUGAUGGAGU
2xLet7 BS-mut
Luciferase CDS
UUGAUAUGUUGGAUGAUGGAGU
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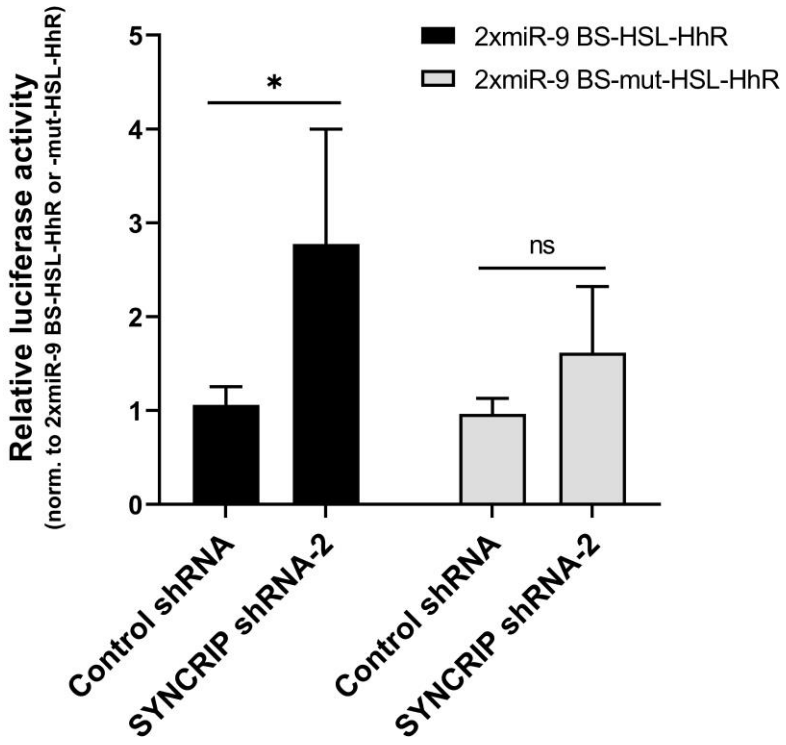
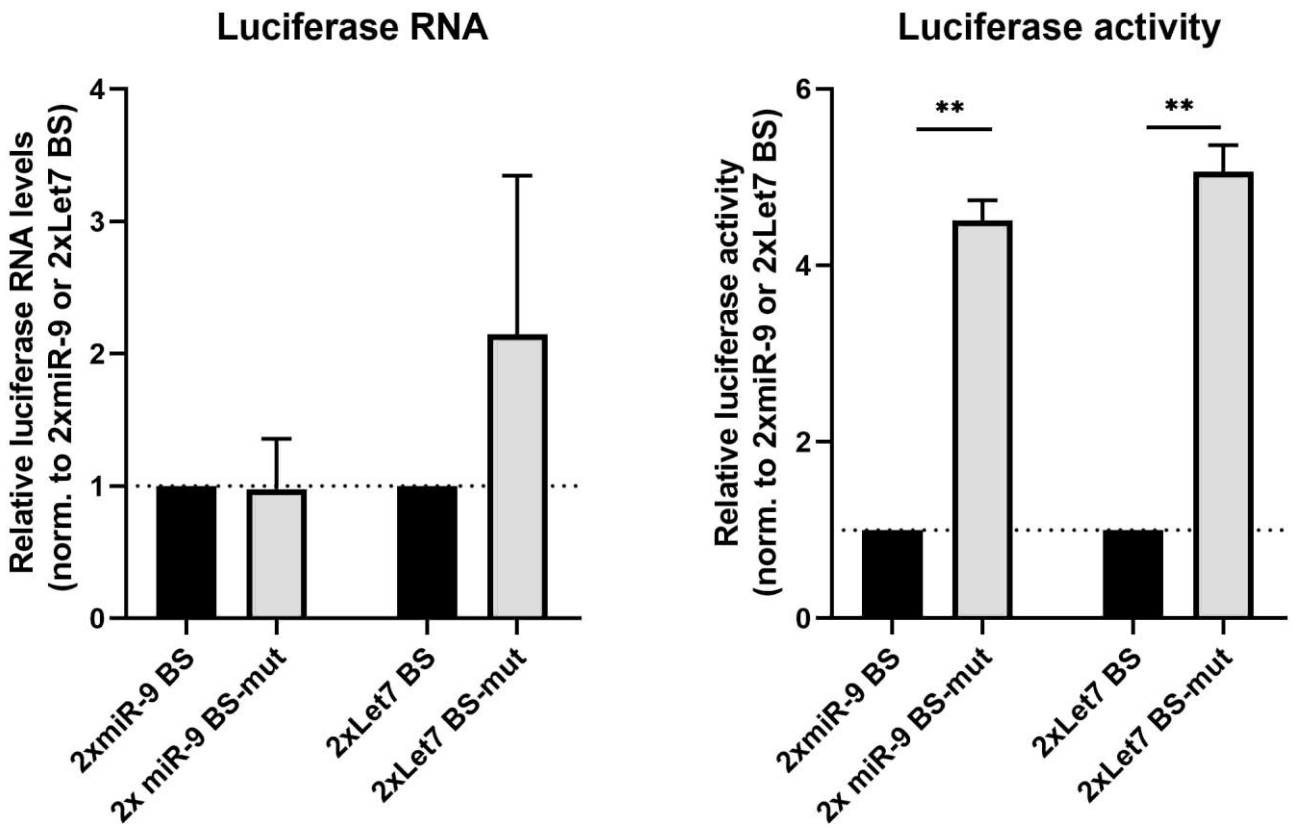
UUGAUAUGUUGGAUGAUGGAGU

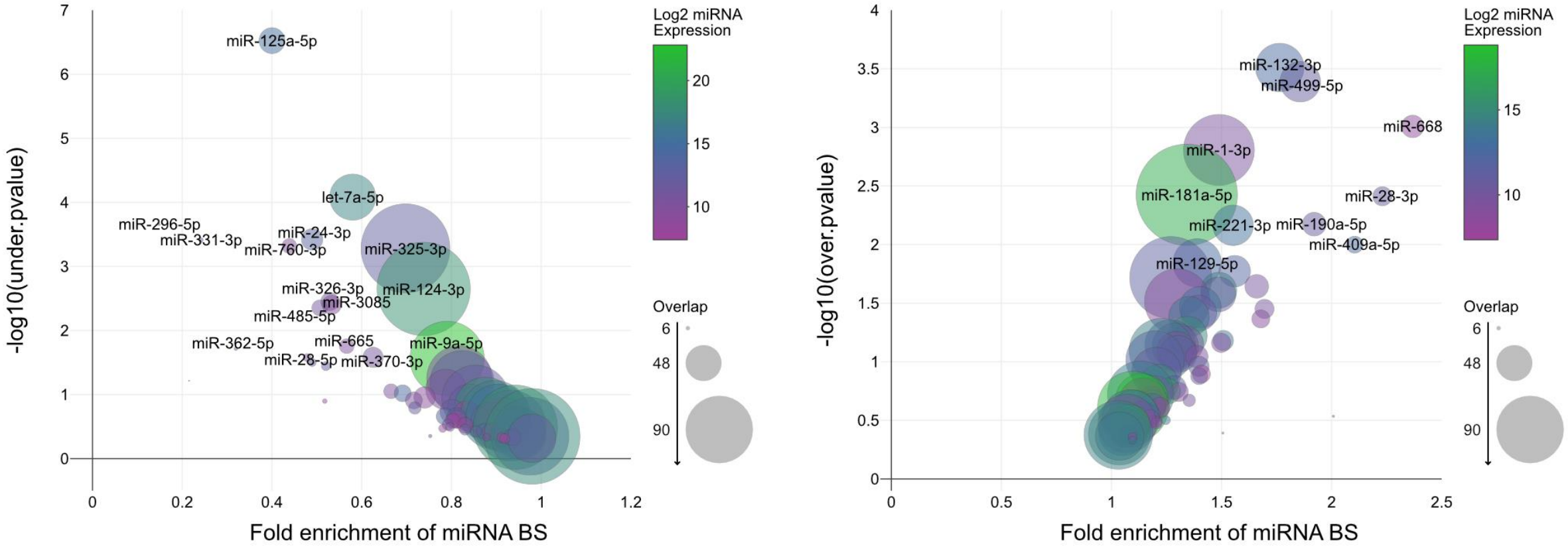
Suppl. Fig. 5


Relative luciferase activity
(norm. to 2xmiR-9 BS-HSL-HhR or -mut-HSL-HhR)
2xmiR-9 BS-HSL-HhR
2xmiR-9 BS-mut-HSL-HhR
*
ns
0
1
2
3
4
5
Control shRNA
SYNCRIP shRNA-2
Control shRNA
SYNCRIP shRNA-2

Suppl. Fig. 6


Luciferase RNA
Relative luciferase RNA levels
(norm. to 2xmiR-9 or 2xLet7 BS)
0
1
2
3
4
2xmiR-9 BS
2x miR-9 BS-mut
2xLet7 BS
2xLet7 BS-mut
Luciferase activity
Relative luciferase activity
(norm. to 2xmiR-9 or 2xLet7 BS)
0
2
4
6
**
**
2xmiR-9 BS
2x miR-9 BS-mut
2xLet7 BS
2xLet7 BS-mut

Suppl. Fig. 7


-log10(under.pvalue)
Fold enrichment of miRNA BS
Log2 miRNA Expression
Overlap
miR-125a-5p
let-7a-5p
miR-296-5p
miR-331-3p
miR-24-3p
miR-760-3p
miR-325-3p
miR-326-3p
miR-124-3p
miR-3085
miR-485-5p
miR-362-5p
miR-665
miR-9a-5p
miR-28-5p
miR-370-3p
-log10(over.pvalue)
Fold enrichment of miRNA BS
Log2 miRNA Expression
Overlap
miR-132-3p
miR-499-5p
miR-668
miR-1-3p
miR-181a-5p
miR-28-3p
miR-221-3p
miR-190a-5p
miR-409a-5p
miR-129-5p

Suppl. Fig. 8