**Supplemental Information Inventory:**

- **Supplemental Figure Legends**
- **Figure S1, related to Figure 1**
- **Figure S2, related to Figures 2 and 3**
- **Figure S3, related to Figure 4**
- **Figure S4, related to Figure 5**
- **Figure S5, related to Figure 6**
- **Table S1, related to Figure 4**
- **Table S2, related to Figure 5**
- **Supplemental Materials and Methods**
- **List of primers, shRNA and siRNA sequences**

## Supplemental Figure Legends

**Figure S1, related to Figure 1. (A)** Experimental protocol to differentiate SH-SY5Y cells into neuron-like cells. Differentiation was induced by RA for 4 days (from day D0 to D4) and subsequently induced with BDNF in serum-free medium for 5 days (from day D4/B0 to B5). The cell phenotype can be examined with phase-contrast microscopy e.g. a representative picture of differentiated cells at day B5 (scale bar, 100 µm). **(B)** SH-SY5Y cells were induced to differentiation; mRNA levels of LSD1 isoforms were measured by RT-qPCR and normalized to that of GAPDH. Results for total LSD1 mRNA (right) are shown as mean ± SEM from at least five independent experiments. Quantification of LSD1 splicing variants (left) are shown as mean ± SEM from at least three independent experiments. (*) $P \leq 0.035$. **(C)** Stable SH-SY5Y cells infected with control or LSD1+8a shRNA were induced to differentiation; LSD1-8a splicing variants were measured by RT-qPCR and normalized to that of GAPDH. Results for mRNA data are shown as mean ± SEM from at least three independent experiments. (*) $P \leq 0.035$, (NS) non-specific. **(D)** LSD1 immunofluorescent stainings of stable SH-SY5Y cells infected with control or LSD1+8a shRNA (scale bar, 100 µm). **(E)** Stable SH-SY5Y cells infected with control or LSD1+8a shRNA were induced to differentiation; the cell phenotypes were examined with phase-contrast microscopy at day B0 (scale bar, 100 µm). **(F-G)** iNGN cells were induced to differentiation; **(F)** mRNA levels of LSD1+8a and total LSD1 isoforms were measured by RT-qPCR and normalized to that of GAPDH. Results for mRNA data are shown as mean ± SEM from at least three independent experiments. (**) $P \leq 0.001$ (*) $P \leq 0.002$. **(G)** Protein levels were analyzed with a LSD1+8a specific antibody and a LSD1 antibody which recognizes all LSD1 isoforms. Actin was used as a loading control. **(H-K)** Stable iNGN cells infected with control or

LSD1+8a shRNA were induced to differentiation; **(H)** Protein levels at day D4 of differentiation were analyzed with a LSD1+8a specific antibody. Actin was used as a loading control. **(I)** mRNA levels of LSD1+8a and total LSD1 isoforms were measured by RT-qPCR and normalized to that of GAPDH. Results for mRNA data are shown as mean ± SEM from at least three independent experiments. (**) $P \leq 0.001$ (*) $P \leq 0.01$. The cell phenotypes were examined **(J)** with phase-contrast microscopy at day D3 (scale bar, 100 µm), and evaluated **(K)** by quantification of neurite length and number; results are indicated as mean ± SEM. (**) $P \leq 10^{-5}$ (*) $P \leq 0.003$. **(L-M)** SH-SY5Y cells treated or not with 50 µM LSD1 inhibitor (S2101 inhibitor; Millipore) were induced to differentiation. The cell phenotypes were examined **(L)** with phase-contrast microscopy at day B3 (scale bar, 100 µm), and evaluated **(M)** by quantification of neurite length and number; results are indicated as mean ± SEM. (**) $P \leq 10^{-5}$.

**Figure S2, related to Figures 2 and 3. (A)** Gene ontology analysis of down-regulated genes at day B3 of differentiation upon LSD1+8a inhibition. **(B)** Venn diagram showing the overlap between down-regulated genes at day B3 of differentiation upon LSD1+8a inhibition and LSD1+8a target genes in SH-SY5Y cells at day B3 of differentiation. $P \leq 10^{-4}$ assessed using hypergeometric test. **(C)** High frequency motifs in LSD1 binding sites. Motifs were searched with Binding and Expression Target Analysis (BETA) software, in LSD1 binding sites surrounding promoters of up- or down-regulated genes (UP and DOWN respectively). **(D)** Stable iNGN cells infected with control or LSD1+8a shRNA were induced to differentiation. mRNA levels of LSD1+8a target genes were measured by RT-qPCR and normalized to that of GAPDH. Results for mRNA data are shown as mean ± SEM from at least three independent experiments. (**) $P \leq 0.005$ (*) $P \leq 0.01$. **(E)** Genomic distribution of LSD1 binding sites (16,882) in SH-SY5Y cells at day B0 of differentiation. (*) $P \leq 10^{-4}$ assessed using one-sided binomial test. **(F)**

Venn diagram showing the overlap between LSD1 peaks in SH-SY5Y cells at day B0 and B3 of differentiation. $P \leq 10^{-4}$ assessed using hypergeometric test. **(G)** ChIP-seq signal profiles at the promoters of LSD1/LSD1+8a target genes at day B0 and B3 of differentiation. For each target, LSD1, LSD1+8a and input profiles are shown. Peak calling track is displayed (black boxes).

**Figure S3, related to Figure 4. (A)** Histone demethylase activity of LSD1 and LSD1+8a proteins purified from bacteria, on H3K4me2 and H3K9me2 peptides (residues 1 to 21 of histone H3 tail). Activity was examined by MALDI-TOF mass spectrometry. **(B)** LSD1 and LSD1+8a proteins purified from bacteria. Proteins were analyzed with a LSD1+8a specific antibody and a LSD1 antibody which recognizes all LSD1 isoforms. Eluted fraction from non-induced bacteria was used as control. **(C)** Size-exclusion chromatograph of His-tagged LSD1 and LSD1+8a proteins. Proteins were expressed in bacteria, purified by Ni-affinity chromatography and then analyzed on a Superdex 200 gel filtration column. **(D)** ChIP-qPCR analysis of H3 and IgG levels at the promoters of LSD1+8a target genes. Stable SH-SY5Y cells infected with control or LSD1+8a shRNA were induced to differentiation. Experiments were performed before and during the neuronal maturation (day B0 and day B3 respectively). β2 microglobulin (B2M) promoter was used as a control. Results are shown as the relative fold enrichment over the input (mean ± SEM) of at least three independent experiments. **(E)** ChIP-qPCR analysis of H3K4me2 and H3K9me2 levels at the promoters of LSD1+8a target genes. Stable iNGN cells infected with control or LSD1+8a shRNA were induced to differentiation. Experiments were performed at day D3 of differentiation. β2 microglobulin (B2M) promoter was used as a control. Results are shown as the relative fold enrichment over the input (mean ± SEM) of at least three independent experiments. (**) $P \leq 0.01$ (*) $P \leq 0.04$. **(F)** Normalized H3K9me2 tag density distribution across

the gene bodies. Each gene body was normalized to 0%–100%. Normalized tag densities were plotted from 20% upstream the TSS to 20% downstream the TTS. **(G)** Normalized H3K9me2 tag density distribution across the gene bodies. Each gene body was normalized to 0%–100%. Normalized tag density was plotted from 20% upstream the TSS to 20% downstream the TTS. H3K9me2 ChIP-seq datas were downloaded from NCBI SRA database under accession numbers SRR03511 and SRR03512 (Chandra et al., 2012; accession number: GSE38442).

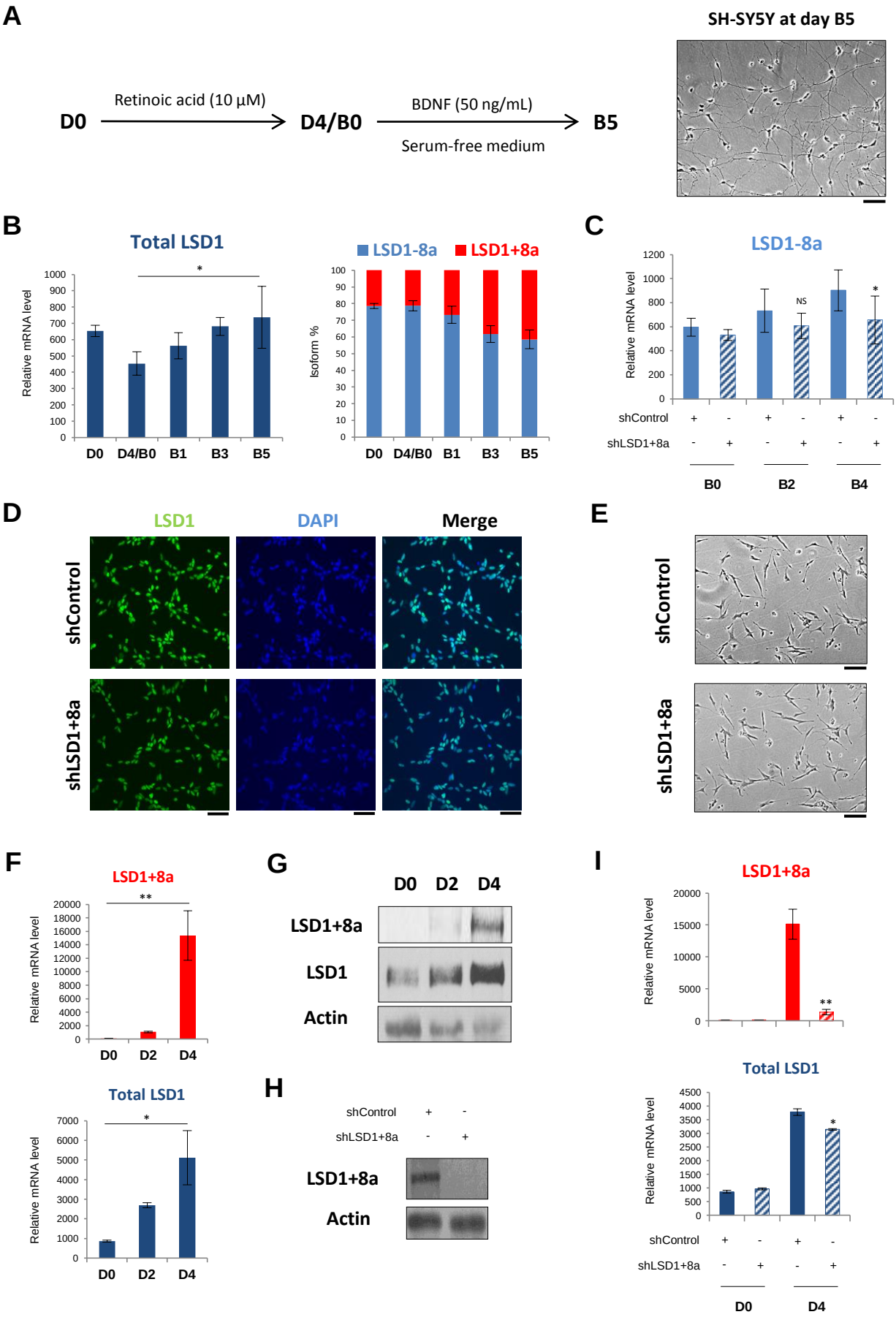
**Figure S4, related to Figure 5. (A)** Schematic representation of bovine supervillin (adapted from Wulfkuhle et al., 1999). Predicted nuclear localization signals (NLSs), steroid receptor and actin binding domains are shown. Deletion mutants of SVIL used in C are indicated. **(B)** Cytoplasmic and nuclear fraction of SH-SY5Y cells were probed with a SVIL and LSD1 antibodies. GATA2 and tubulin were respectively used as control for nuclear and cytoplasmic extraction. **(C)** GFP-tagged SVIL deletion mutants and LSD1+8a vectors were transfected in HEK293T cells, and total lysates were subjected to GFP or IgG IP. Western blotting was then performed with GFP and LSD1 antibodies. **(D)** iNGN cells were induced to differentiation. Protein levels were analyzed with a SVIL antibody. Actin was used as a loading control. **(E-I)** iNGN cells transfected with control or SVIL siRNA were induced to differentiation; **(E)** Protein levels of iNGN cells at day D3 of differentiation were analyzed with a SVIL antibody. Actin was used as a loading control. **(F)** SVIL mRNA level was measured by RT-qPCR and normalized to that of GAPDH. Results for mRNA data are shown as mean ± SEM from at least three independent experiments. (**) $P \leq 10^{-4}$. The cell phenotypes were examined **(G)** with phase-contrast microscopy at day D3 (scale bar, 100 µm), and evaluated **(H)** by quantification of neurite length and number; results are indicated as mean ± SEM. (**) $P \leq 10^{-5}$, (NS) non-specific. **(I)** mRNA level of the neuronal marker MAP2 was measured by RT-qPCR and normalized to that of

GAPDH. Results for mRNA data are shown as mean ± SEM from at least three independent experiments. (**) $P \leq 0.002$.

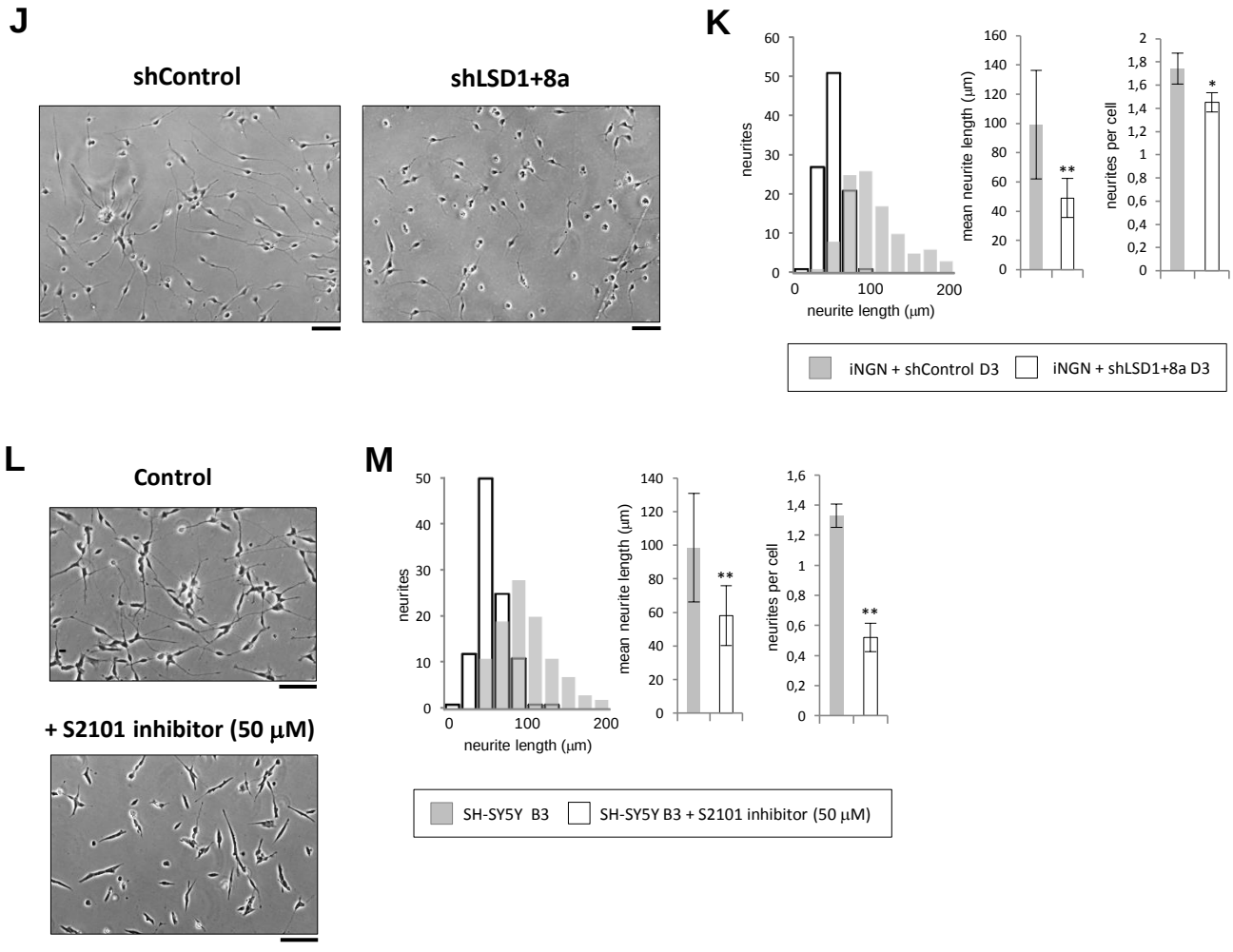
**Figure S5, related to Figure 6. (A)** Genomic distribution of SVIL binding sites (1,452) in SH-SY5Y cells at day B3 of differentiation. (*) $P \leq 10^{-4}$ assessed using one-sided binomial test. **(B)** Normalized SVIL tag density distribution at all genes' promoters, in a relative distance of 5,000 bp from the TSS (left panel). Heatmap analysis of SVIL distribution at promoter regions, in a relative distance of 10 kb from the TSS (right panel). **(C)** Venn diagram showing the overlap between SVIL and LSD1+8a peaks in SH-SY5Y cells at day B3 of differentiation. $P \leq 10^{-4}$ assessed using hypergeometric test. **(D)** Normalized SVIL tag density distribution at promoters of genes up-regulated (up), down-regulated (down) or with stable expression (no change) during neuronal differentiation of SH-SY5Y cells. Normalized tag densities are shown in a relative distance of 5,000 bp from the TSS. **(E)** LSD1+8a, SVIL and input ChIP-seq signal profiles at the promoters of LSD1+8a target genes at day B3 of differentiation. For each target, both SVIL ChIP-seq signal profiles from SH-SY5Y cells infected with control or SVIL shRNA are shown. Peak calling track is displayed (black boxes). **(F)** Histone demethylase assay on calf histones with LSD1+8a proteins purified from sf9 insect cells and Flag-tagged SVIL proteins purified from SH-SY5Y cells. Histone lysates were analyzed with H3K4me2 and H3K9me2 antibodies. H3 was used as a loading control. **(G)** Histone demethylase assay on calf histones with LSD1+8a complexes purified from SH-SY5Y cells stably expressing Flag-HA tagged LSD1+8a. Cells were infected twice with control or SVIL shRNA and after two days, protein complexes were purified on FLAG tag. Histone lysates were analyzed with H3K4me2 and H3K9me2 antibodies. H3 was used as a loading control. **(H-I)** iNGN cells transfected with control or SVIL siRNA were induced to differentiation; **(H)** ChIP-qPCR analysis of H3K4me2 and H3K9me2 levels at the

promoters of LSD1+8a target genes. Experiments were performed at day D3 of differentiation. β2 microglobulin (B2M) promoter was used as a control. Results are shown as the relative fold enrichment over the input (mean ± SEM) of at least three independent experiments. (**) $P \leq 0.0015$, (*) $P \leq 0.025$, (NS) non-specific. **(I)** mRNA levels of LSD1+8a target genes were measured by RT-qPCR and normalized to that of GAPDH. Results for mRNA data are shown as mean ± SEM from at least three independent experiments. (**) $P \leq 10^{-4}$, (*) $P \leq 0.02$. **(J)** Working model of the mechanism by which LSD1+8a and SVIL cooperate to demethylate H3K9 and therefore activate gene expression during neuronal differentiation (adapted from Hu et al., 2008 and Archer et al., 2005). LSD1+8a mediated H3K9 demethylase activity may depend on a cooperative and ligand enhancement effect between SVIL and nuclear receptors. Dynamic binding of SVIL to actin filaments may favor structural maintenance of transcriptional hubs in the nucleus. Combination of protein interactions and nuclear structure rearrangements may coordinate transcription of LSD1+8a target genes during brain development.

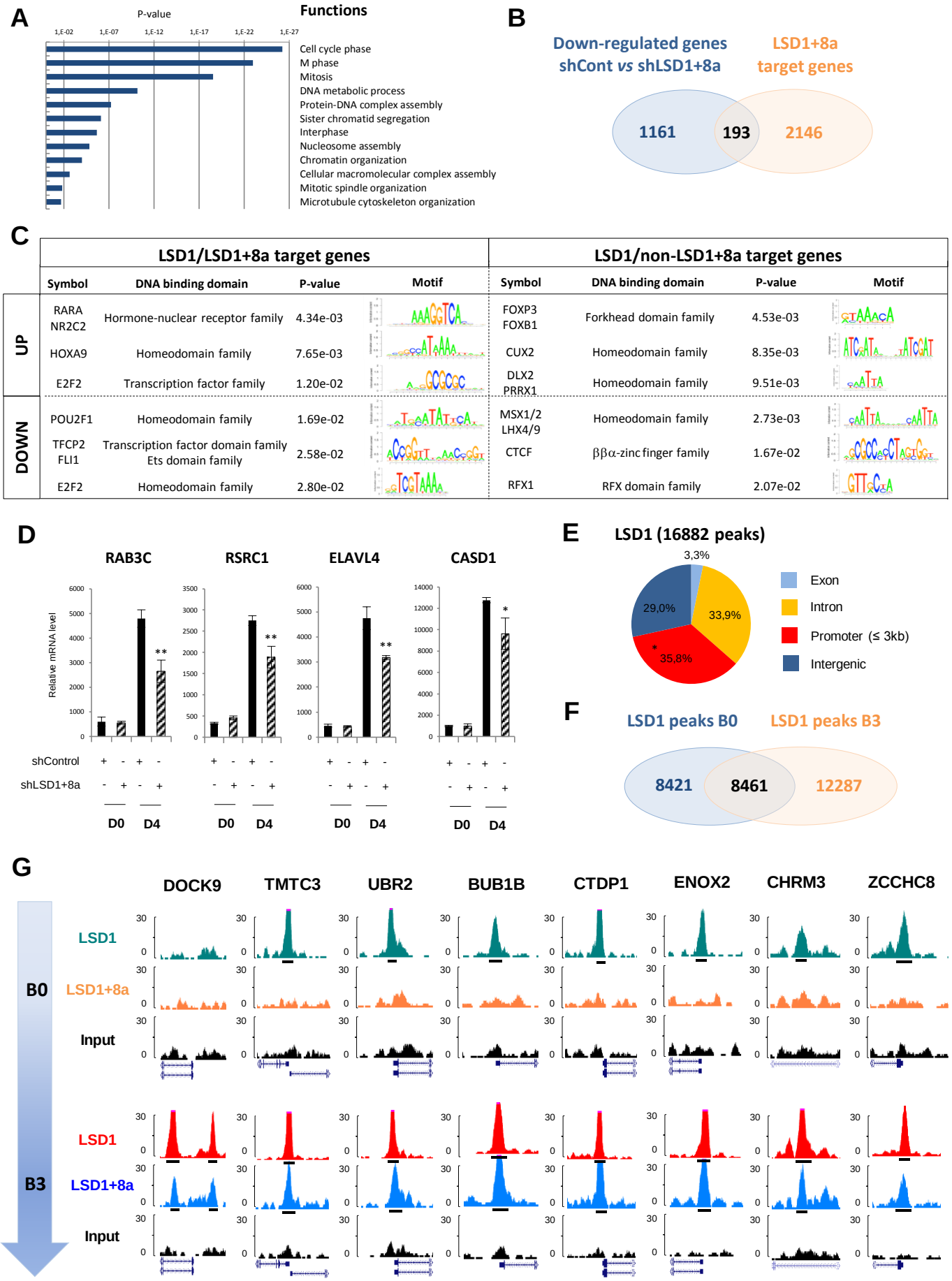
# Figure S1


A
SH-SY5Y at day B5
D0
Retinoic acid (10 µM)
D4/B0
BDNF (50 ng/mL)
Serum-free medium
B5
B
Total LSD1
Relative mRNA level
D0 D4/B0 B1 B3 B5
LSD1-8a
LSD1+8a
Isoform %
C
LSD1-8a
Relative mRNA level
NS
shControl
shLSD1+8a
B0 B2 B4
D
LSD1
DAPI
Merge
shControl
shLSD1+8a
E
shControl
shLSD1+8a
F
LSD1+8a
Relative mRNA level
D0 D2 D4
Total LSD1
G
D0 D2 D4
LSD1+8a
LSD1
Actin
H
shControl
shLSD1+8a
LSD1+8a
Actin
I
LSD1+8a
Relative mRNA level
Total LSD1
shControl
shLSD1+8a
D0 D4

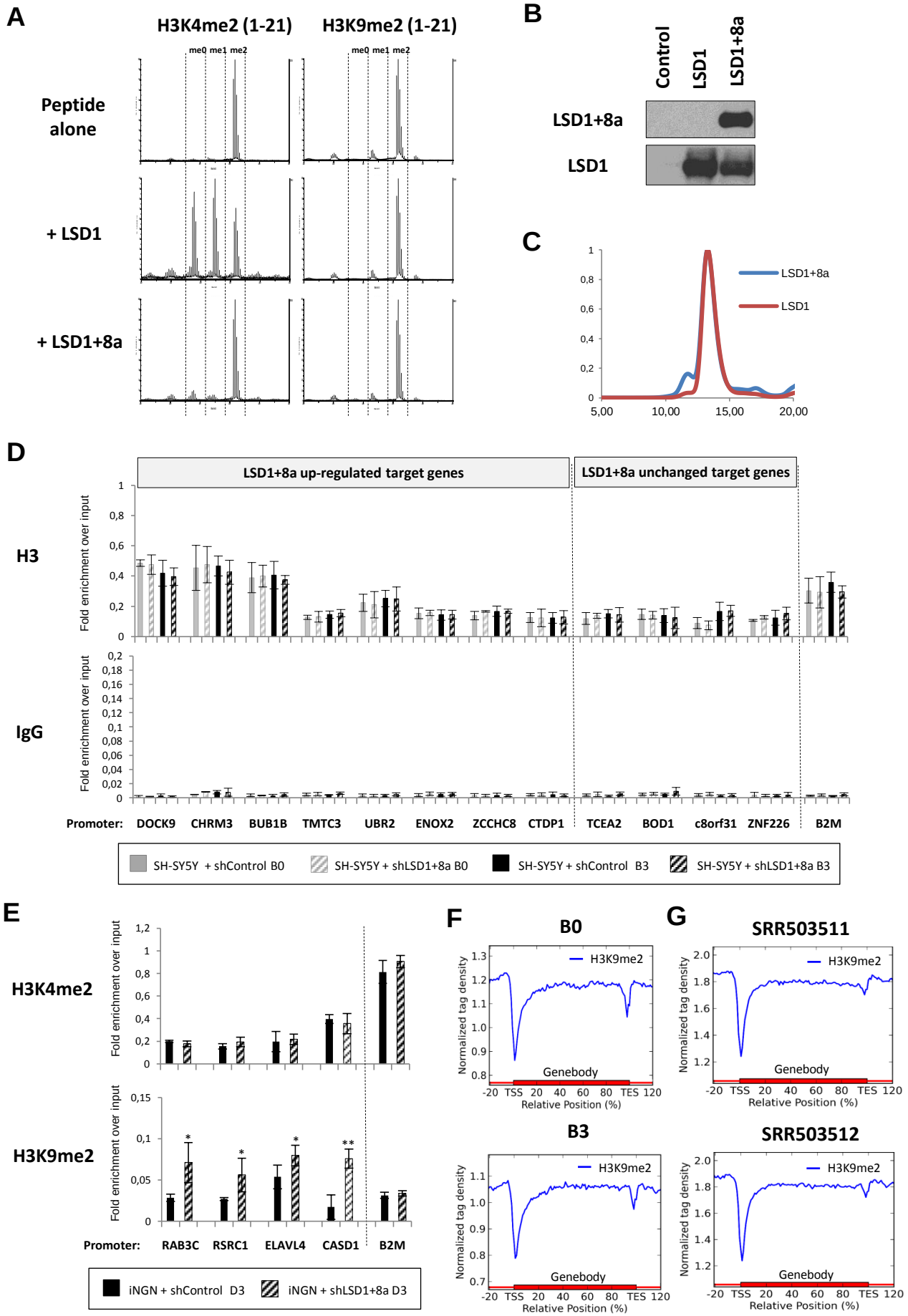
# Figure S1


J
shControl
shLSD1+8a
K
neurites
neurite length (µm)
mean neurite length (µm)
neurites per cell
**
*
iNGN + shControl D3
iNGN + shLSD1+8a D3
L
Control
+ S2101 inhibitor (50 µM)
M
neurites
neurite length (µm)
mean neurite length (µm)
neurites per cell
**
**
SH-SY5Y B3
SH-SY5Y B3 + S2101 inhibitor (50 µM)

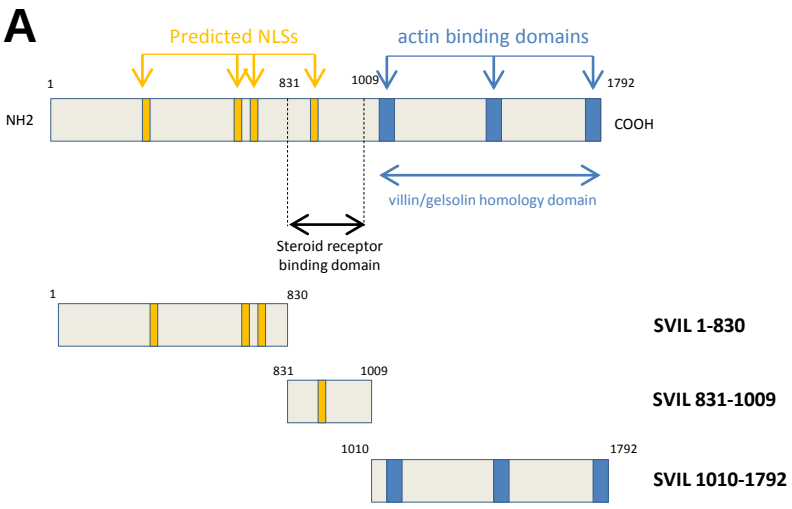
# Figure S2

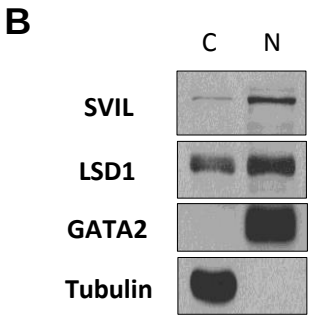

A
P-value
1,E-02
1,E-07
1,E-12
1,E-17
1,E-22
1,E-27
Functions
Cell cycle phase
M phase
Mitosis
DNA metabolic process
Protein-DNA complex assembly
Sister chromatid segregation
Interphase
Nucleosome assembly
Chromatin organization
Cellular macromolecular complex assembly
Mitotic spindle organization
Microtubule cytoskeleton organization
B
Down-regulated genes shCont vs shLSD1+8a
LSD1+8a target genes
1161
193
2146
C
LSD1/LSD1+8a target genes
LSD1/non-LSD1+8a target genes
Symbol
DNA binding domain
P-value
Motif
UP
RARA NR2C2
Hormone-nuclear receptor family
4.34e-03
HOXA9
Homeodomain family
7.65e-03
E2F2
Transcription factor family
1.20e-02
FOXP3 FOXB1
Forkhead domain family
4.53e-03
CUX2
Homeodomain family
8.35e-03
DLX2 PRRX1
Homeodomain family
9.51e-03
DOWN
POU2F1
Homeodomain family
1.69e-02
TFCP2 FLI1
Transcription factor domain family Ets domain family
2.58e-02
E2F2
Homeodomain family
2.80e-02
MSX1/2 LHX4/9
Homeodomain family
2.73e-03
CTCF
ββα-zinc finger family
1.67e-02
RFX1
RFX domain family
2.07e-02
D
RAB3C
RSRC1
ELAVL4
CASD1
Relative mRNA level
shControl
shLSD1+8a
D0
D4
E
LSD1 (16882 peaks)
3,3%
29,0%
33,9%
35,8%
Exon
Intron
Promoter (≤ 3kb)
Intergenic
F
LSD1 peaks B0
LSD1 peaks B3
8421
8461
12287
G
DOCK9
TMTC3
UBR2
BUB1B
CTDP1
ENOX2
CHRM3
ZCCHC8
B0
B3
LSD1
LSD1+8a
Input

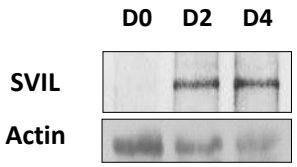
# Figure S3

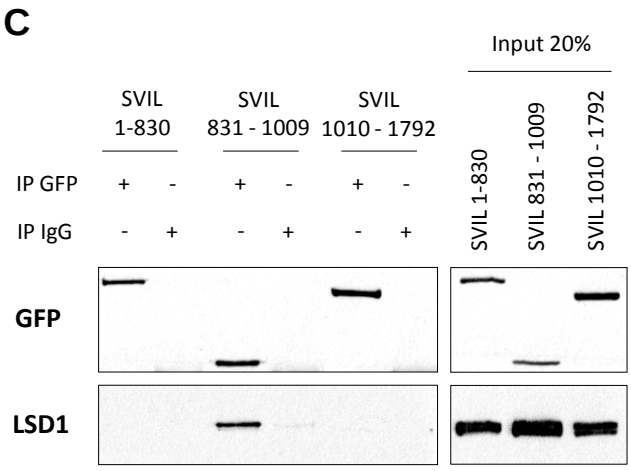

A
H3K4me2 (1-21)
H3K9me2 (1-21)
me0 me1 me2
Peptide alone
+ LSD1
+ LSD1+8a
B
Control
LSD1
LSD1+8a
LSD1+8a
LSD1
C
LSD1+8a
LSD1
D
LSD1+8a up-regulated target genes
LSD1+8a unchanged target genes
H3
IgG
Fold enrichment over input
Promoter: DOCK9 CHRM3 BUB1B TMTC3 UBR2 ENOX2 ZCCHC8 CTDP1 TCEA2 BOD1 c8orf31 ZNF226 B2M
SH-SY5Y + shControl B0
SH-SY5Y + shLSD1+8a B0
SH-SY5Y + shControl B3
SH-SY5Y + shLSD1+8a B3
E
H3K4me2
H3K9me2
Fold enrichment over input
Promoter: RAB3C RSRC1 ELAVL4 CASD1 B2M
iNGN + shControl D3
iNGN + shLSD1+8a D3
F
B0
B3
H3K9me2
Normalized tag density
Genebody
-20 TSS 20 40 60 80 TES 120
Relative Position (%)
G
SRR503511
SRR503512
H3K9me2
Normalized tag density
Genebody
Relative Position (%)

# Figure S4

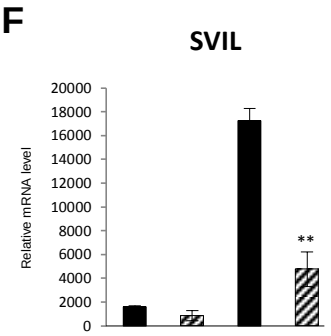

A
Predicted NLSs
actin binding domains
1
831
1009
1792
NH2
COOH
villin/gelsolin homology domain
Steroid receptor binding domain
1
830
SVIL 1-830
831
1009
SVIL 831-1009
1010
1792
SVIL 1010-1792

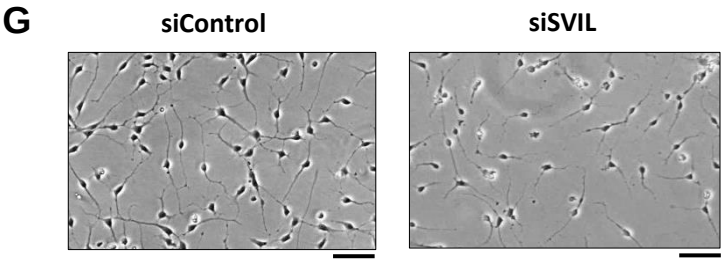

B
C
N
SVIL
LSD1
GATA2
Tubulin

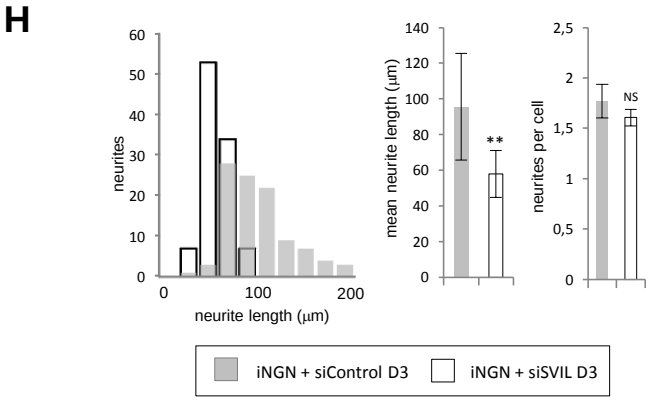

D
D0
D2
D4
SVIL
Actin

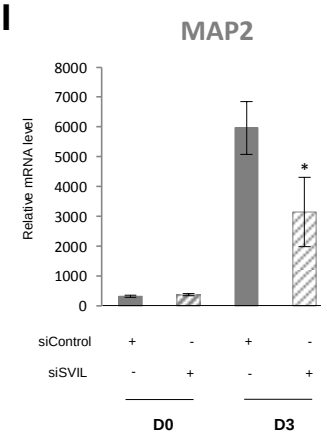

C
Input 20%
SVIL 1-830
SVIL 831 - 1009
SVIL 1010 - 1792
IP GFP
IP IgG
GFP
LSD1


E
siControl
siSVIL
SVIL
Actin

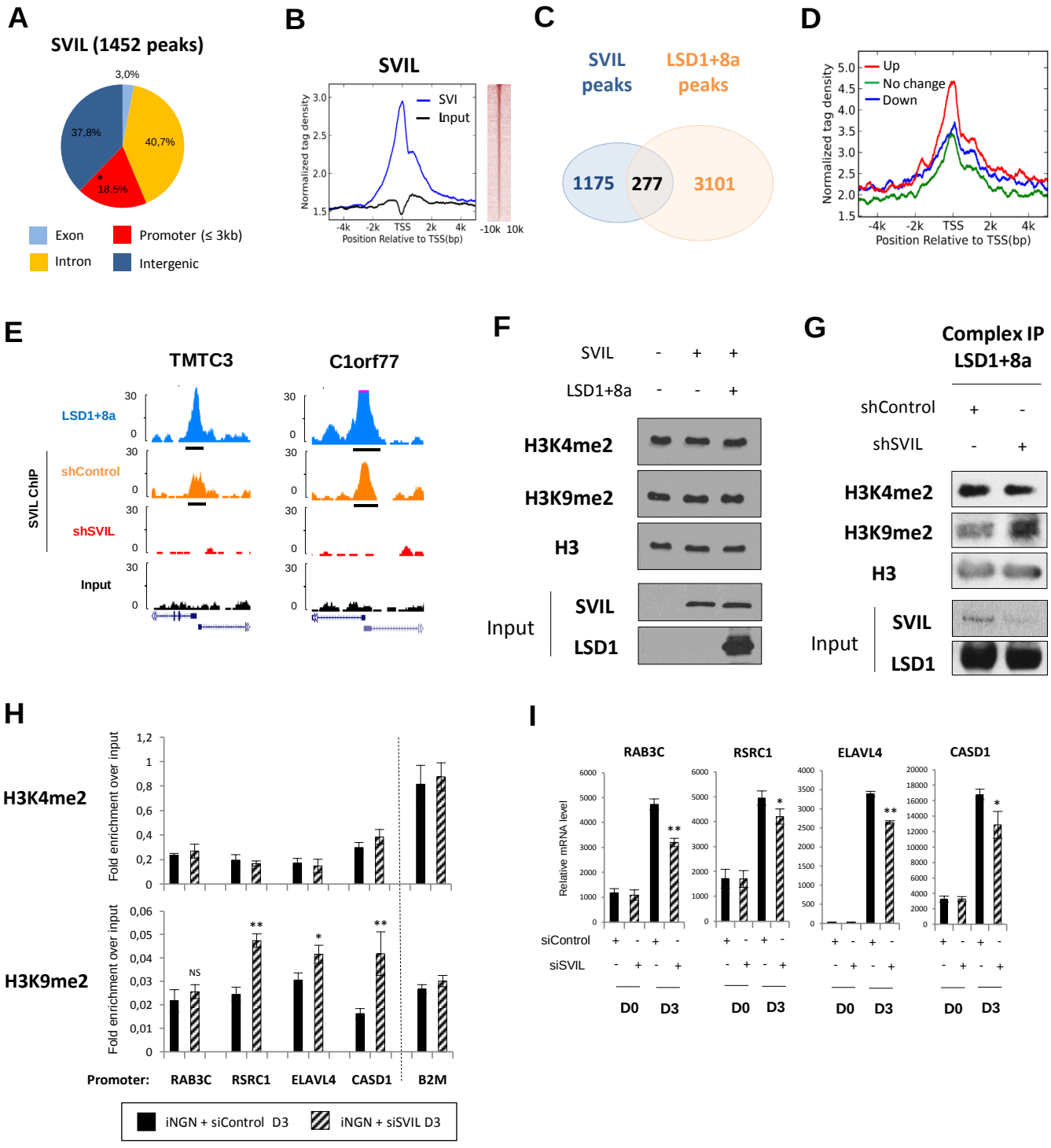

F
SVIL
Relative mRNA level
20000
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
**


G
siControl
siSVIL


H
neurites
neurite length (µm)
mean neurite length (µm)
**
neurites per cell
NS
iNGN + siControl D3
iNGN + siSVIL D3


I
MAP2
Relative mRNA level
8000
7000
6000
5000
4000
3000
2000
1000
0
*
siControl
siSVIL
D0
D3

# Figure S5


A
SVIL (1452 peaks)
3,0%
37,8%
40,7%
18,5%
Exon
Intron
Promoter (≤ 3kb)
Intergenic
B
SVIL
SVI
Input
Normalized tag density
Position Relative to TSS(bp)
-10k 10k
C
SVIL peaks
LSD1+8a peaks
1175
277
3101
D
Up
No change
Down
Normalized tag density
Position Relative to TSS(bp)
E
TMTC3
C1orf77
LSD1+8a
shControl
shSVIL
Input
SVIL ChIP
F
SVIL
LSD1+8a
H3K4me2
H3K9me2
H3
Input
SVIL
LSD1
G
Complex IP
LSD1+8a
shControl
shSVIL
H3K4me2
H3K9me2
H3
Input
SVIL
LSD1
H
H3K4me2
H3K9me2
Fold enrichment over input
NS
Promoter:
RAB3C
RSRC1
ELAVL4
CASD1
B2M
iNGN + siControl D3
iNGN + siSVIL D3
I
RAB3C
RSRC1
ELAVL4
CASD1
Relative mRNA level
siControl
siSVIL
D0
D3

## Supplemental tables

**Table S1, related to Figure 4.** Analysis by MS/MS analysis of histones subjected to a demethylase assay with LSD1 complexes purified from SH-SY5Y cells stably expressing Flag-HA tagged LSD1, LSD1+8a or the catalytic mutant LSD1+8a K665A. Only the relative percentage of methyl events on H3K9 is shown.

| | Control | LSD1 | LSD1+8a | LSD1+8a K665A |
|---|---|---|---|---|
| H3K9me0 | 17% | 13% | 21% | 17% |
| H3K9me1 | 13% | 12% | 28% | 7% |
| H3K9me2 | 42% | 43% | 28% | 42% |
| H3K9me3 | 28% | 33% | 23% | 34% |

**Table S2, related to Figure 5.** Analysis by MS/MS analysis of SVIL complex purified from SH-SY5Y cells transiently expressing or not Flag-tagged SVIL. Only the gene symbols of proteins with more than 2 peptides are shown.

| | Flag IP | |
|---|---|---|
| | + SVIL-Flag | Mock |
| GANAB | 54 | 0 |
| SVIL | 35 | 1 |
| SPTA2 | 30 | 0 |
| NCAM1 | 16 | 0 |
| SEC31A | 13 | 0 |
| RPA1 | 12 | 2 |
| FLNC | 12 | 0 |
| CTNND1 | 11 | 1 |
| HBG1 | 11 | 0 |
| COPG | 10 | 0 |
| USO1 | 9 | 0 |
| AIFM1 | 9 | 0 |
| MYO1B | 9 | 1 |
| SEPT9 | 9 | 0 |
| DOCK7 | 8 | 0 |
| KIAA0090 | 8 | 0 |
| GDE | 8 | 0 |
| LEPRE1 | 8 | 0 |
| ATP1A1 | 7 | 0 |
| LIMA1 | 7 | 0 |
| P4HA1 | 7 | 0 |
| KTN1 | 7 | 0 |
| RENT1 | 7 | 0 |
| DYNC1I2 | 7 | 0 |
| IGF2R | 6 | 1 |
| L1CAM | 6 | 0 |
| JIP4 | 6 | 0 |
| ATAD3A | 6 | 0 |
| IGF2BP3 | 5 | 1 |
| VIGLN | 5 | 1 |
| KHDR3 | 5 | 0 |
| WDR61 | 5 | 0 |
| DPP3 | 5 | 0 |
| CEP170 | 5 | 1 |
| KLC2 | 5 | 0 |
| NCKAP1 | 5 | 1 |
| SMCE1 | 5 | 0 |
| CADH2 | 5 | 0 |
| HBB | 5 | 0 |
| PABPC4 | 5 | 0 |
| AP2A1 | 4 | 0 |
| ELAVL2 | 4 | 0 |
| TPM4 | 4 | 0 |
| SNX9 | 4 | 1 |
| ADD1 | 4 | 1 |
| SMEK1 | 4 | 0 |
| SYNPO2 | 4 | 0 |
| LMAN1 | 4 | 0 |
| RAD23B | 4 | 0 |
| P4HA2 | 4 | 0 |
| JUP | 4 | 1 |
| COX2 | 4 | 1 |
| CAPRIN1 | 4 | 1 |
| SET | 4 | 0 |
| NOMO3 | 4 | 0 |
| ADNP | 4 | 0 |
| RCC1 | 3 | 0 |
| FKBP10 | 3 | 0 |
| CCDC47 | 3 | 0 |
| AP3D1 | 3 | 0 |
| NSUN2 | 3 | 0 |
| PFKP | 3 | 0 |
| SMCA5 | 3 | 0 |
| TSSC1 | 3 | 0 |
| LUC7L2 | 3 | 0 |
| HCFC1 | 3 | 0 |
| TRIM25 | 3 | 0 |
| KIF11 | 3 | 0 |
| HK1 | 3 | 0 |
| SMC4 | 3 | 0 |
| PGAM5 | 3 | 0 |
| PTCD3 | 3 | 0 |
| SLC25A1 | 3 | 0 |
| PFKL | 3 | 0 |
| CLASP2 | 3 | 0 |
| INTS3 | 3 | 0 |
| CKAP5 | 3 | 0 |
| PP2BA | 3 | 0 |
| TCOF1 | 3 | 0 |
| NUDT5 | 3 | 0 |
| PHOX2B | 3 | 0 |
| CLTA | 3 | 0 |
| RNH1 | 3 | 0 |
| RALY | 3 | 0 |
| ATRX | 3 | 0 |
| RCC2 | 3 | 0 |
| KDM1A | 2 | 0 |
| SMARCAD1 | 2 | 0 |
| ANK2 | 2 | 0 |
| PARF | 2 | 0 |
| NRD1 | 2 | 0 |
| PP6R3 | 2 | 0 |
| MAPRE1 | 2 | 0 |
| CD11A | 2 | 0 |
| RO52 | 2 | 0 |
| GOLGA2 | 2 | 0 |
| STAG2 | 2 | 0 |
| GATAD2B | 2 | 0 |
| FAR1 | 2 | 0 |
| GLB1 | 2 | 0 |
| PACSIN2 | 2 | 0 |
| GDAP1 | 2 | 0 |
| WDR1 | 2 | 0 |
| c15orf24 | 2 | 0 |
| PLD3 | 2 | 0 |
| CPSF1 | 2 | 0 |
| COBRA1 | 2 | 0 |
| CRTAP | 2 | 0 |
| TUBG2 | 2 | 0 |
| GAPVD1 | 2 | 0 |
| COPS6 | 2 | 0 |
| HDGFRP2 | 2 | 0 |
| CTSD | 2 | 0 |
| APP | 2 | 0 |
| MEX3A | 2 | 0 |
| CNOT1 | 3 | 0 |
| RANBP3 | 2 | 0 |
| GLCM | 2 | 0 |
| MMS19 | 2 | 0 |
| CDK5 | 2 | 0 |
| IPO4 | 2 | 0 |
| SDF2 | 2 | 0 |
| THOC4 | 2 | 0 |
| GIT1 | 2 | 0 |
| OLA1 | 2 | 0 |
| MGEA5 | 2 | 0 |
| NUDCD3 | 2 | 0 |
| RBBP5 | 2 | 0 |
| HAT1 | 2 | 0 |
| USP14 | 2 | 0 |
| ILVBL | 2 | 0 |
| C6orf174 | 2 | 0 |
| SAE1 | 2 | 0 |
| PPM1B | 2 | 0 |
| LEO1 | 2 | 0 |
| SMU1 | 2 | 0 |
| FSD1 | 2 | 0 |
| MTCH2 | 2 | 0 |
| PYGM | 2 | 0 |
| AKAP12 | 2 | 0 |
| PNPT1 | 2 | 0 |
| PRCC | 2 | 0 |
| HECTD1 | 2 | 0 |
| DHX30 | 2 | 0 |
| KIAA0196 | 2 | 0 |
| STUB1 | 2 | 0 |
| LARP1 | 2 | 0 |
| TNPO1 | 2 | 0 |
| ADRM1 | 2 | 0 |
| CLINT1 | 2 | 0 |

## Supplemental Material and Methods

### Plasmids

LSD1, LSD1+8a and LSD1+2a+8a cDNA were cloned into pENTR-D-TOPO (Invitrogen) and recombined into the pHAGE-CMV-Flag-HA Gateway destination vector for lentiviral expression. Mutations in LSD1 were produced by site-directed mutagenesis and confirmed by sequencing. Constructs encoding various protein fragments of bovine supervillin were provided by Dr Elizabeth J. Luna (University of Massachusetts Medical School, Worcester, MA). Flag-tagged human full length SVIL vector was obtained from the pEGFP-hSVIL construct (from Dr Elizabeth J. Luna) by cloning Flag tag within NheI/SacI restriction sites. Lentiviral shRNA constructs for LSD1+8a and SVIL were obtained by cloning into the pLKO.1 vector. shRNA and siRNA sequences used are described in supplementary material.

### RNA extraction, qPCR and microarray analysis

Total RNAs were extracted from cells by using RNeasy mini kit (Qiagen). Reverse transcription was carried out on 1 μg of total RNA by using Superscript III (Invitrogen) and oligo(dT). cDNAs obtained were diluted accordingly and used for quantitative PCR (qPCR). Each qPCR assay was performed with a standard dilution curve of a calibrator, which was a mixture of different cDNA at different times of neuronal differentiation, to precisely and arbitrarily quantify the transcripts. Gene-specific primers were used together with SYBR green (Roche) for detection on a LightCycler 480 system (Roche). The primer sequences used are described in supplementary material.

Microarray analyses were performed with purified total RNAs. Gene expression profiling was determined by hybridization on Affymetrix microarrays (Human Transcriptome Array 2.0). The microarray dataset was deposited at the Gene Expression Omnibus (GEO) under the subseries entry GSE63097.

**Protein extraction, western-blot and antibodies**

For cell extract preparation, cells were washed once with phosphate-buffered saline (PBS) and incubated for 10 minutes at 4°C in buffer A (10 mM HEPES, pH 7.6, 3 mM $MgCl_2$, 10 mM KCl, 5% glycerol, 0.5% NP-40) containing 1 mM $Na_2VO4$, 20 mM NaF, 1 mM sodium pyrophosphate, 25 mM μ-glycerophosphate and proteinase inhibitors (Roche Diagnostics). After centrifugation, the supernatant (cytoplasmic fraction) was collected and the nuclear pellet was resuspended in buffer A containing 300 mM KCl.

Samples were subjected to SDS-polyacrylamide gel electrophoresis (PAGE) and transferred to nitrocellulose membranes. Filters were blocked overnight in Tris-Buffered Saline (TBS)–0.05% Tween 20 with 5% skimmed milk, and incubated with the appropriate antibody. Membranes were washed four times in TBS–Tween 20 and incubated for 1 hour with the appropriate peroxidase-conjugated secondary antibody. The primary antibodies used were as follows: LSD1 (ab-17721; Abcam), LSD1 (1B2E5) (sc-53875; Santa Cruz), GATA-2 (H-116) (sc-9008; Santa Cruz), α-Tubulin (B-5-1-2) (sc-23948; Santa Cruz), Supervillin (S8695 ; Sigma), Supervillin (B8C1) (sc-53556; Santa Cruz), H3K9me2 (ab1220 ; Abcam), H3K4me2 (07-030; Millipore), H3 (ab1791; Abcam) and GFP (N86/38) (75-132; NeuroMab). The antibody against LSD1+8a isoforms was generated by injecting a peptide containing exon E8a DTVKVPK, into rabbits.

**Immunofluorescence**

Cells were fixed with PBS containing 4% paraformaldehyde for 15 min at room temperature, and then washed extensively with PBS. The fixed cells were next incubated with a blocking buffer containing 5% Bovine Serum Albumin (BSA) and 0.1% triton X-100, for 1h at room temperature, and then stained with the TBB3 antibody (ab7751; Abcam) diluted 1:500 in blocking buffer at 4°C overnight. After staining with secondary antibody (conjugated with Alexa Fluor 488; Millipore), images were captured with a Nikon 80i microscope and a Nikon DS-Qi1Mc camera (Nikon, Tokyo, Japan).

## List of primers, shRNA and siRNA sequences

### Primers for mRNA RT-qPCR

| | |
|---|---|
| GAPDH | F: TGGGATTTCCATTGATGACAA |
| | R: CCACCCATGGCAAATTCC |
| DOCK9 | F: GGCAGCAGACAGTGAAGTGGAAATGG |
| | R: GCTATCTAAACCGGAACCAGAACCTTCC |
| CHRM3 | F: GCTCCTACCTGGAACAGATCCTCATG |
| | R: CACAGGCCAGCTCTGCAACTTATGTTTAGTG |
| BUB1B | F: TCTGCCTGTAACAATACTCTTCAGCAGCAG |
| | R: CTGCTCTGTCCAGCTGATATACCTATCCC |
| TMTC3 | F: GCTGTGTGTATGAAGTGTTTATTGCCCAGG |
| | R: GTGTCTGCAGCATAGAAAATGGAATGCTACCC |
| UBR2 | F: CCTGATGATCATGCTCAGCCGCTTTGAAC |
| | R: CTCTCTCCAACAAGCATTATAATGAGGTATAGC |
| ENOX2 | F: GAGATCATACACTGTAAAAGCTGCACGCTC |
| | R: GCGAATGGCAATGATCTCTCCACACTGCTCG |
| ZCCHC8 | F: GTACCCACCAGGGTGGCTCAAAGAGG |
| | R: CCATTCGTCTGGAATTCCTCTGGGAGTAG |
| TCEA2 | F: CCCGAGTCGGGATGTCTGTCAACGC |
| | R: CATCCCTCGAGGACGTGGGCAGAGG |
| CTDP1 | F: CACAACCGAGCAGCACTGTCAGCAG |
| | R: GGTGAAGACGTGCAGCTCGTACAGC |

| | |
|---|---|
| BOD1 | F: CATCGTGGAGCAGCTCAAGAGCCGG |
| | R: CATTCCTGCTTGTCCAGATGTGTTGACAC |
| C8orf31 | F: TGCCAGGACCACCGCATTGCAGAGG |
| | R: CCCAGAAAGTGAGCGTCTTTGGCAGTGAG |
| ZNF226 | F: GCTGTACCGAGATGTGATGGTGGAGAA |
| | R: CTATAGGTGATACATCTTGTTTGAAGGGTGG |
| MAP2 | F: CAGGTGGCGGACGTGTGAAAATTGAGAGTG |
| | R: CACGCTGGATCTGCCTGGGGACTGTG |
| NCAM | F: ACATCACCTGCTACTTCCTGA |
| | R: CTTGGACTCATCTTTCGAGAAGG |
| LSD1 | F: GAGATGGATGAAAGCTTGG |
| | R: GAAGTCATCCGGTCATGAGG |
| LSD1+8a | F: GCTGTGGTCAGCAAACAAG |
| | R: CTCTTTAGGAACCTTGACAGTGTC |
| LSD1-8a | F: GCCAACGGACAAGCTGTTCC |
| | R: GCCTGGCCAAGGGACACAGGC |
| SVIL | F: TACACGAGTGTGATGAAGGCTCCGAGC |
| | R: CCAGAGGAGCTGCTGAGGATGAACAG |
| RAB3C | F: GATACAGGACTATCACCACAGCCTATTATCG |
| | R: CCAGAATAACTTGGGCATTGTCCCAAGAGTATG |
| RSRC1 | F: GGATAAAGGGAAGGACAAGGAATTACATAACATC |
| | R: ACTTGTTCTACCAGGGTGGCTTGGTC |
| CASD1 | F: CTTGACCTATTCCATCTGGGCTAGCAGTTG |
| | R: GTCCGCTGCCAGCCATATGTGATACTGGC |

| | |
|---|---|
| ELAVL4 | F: CGGCCGTATCATCACCTCACGAATCCT |
| | R: GGCTTCTTCTGCCTCAATCCTCTTATC |

**Primers for ChIP RT-qPCR**

| | |
|---|---|
| B2M | F: CCAGTCTAGTGCATGCCTTCTTAA |
| | R: CAAGCCAGCGACGCAGT |
| DOCK9 | F: CATCAGACAAGTGACCACAGACAGACGAGAC |
| | R: CCCCGAATATCTGCCCTTCCCATCGG |
| CHRM3 | F: CAATGGAGCTAGGCGCCGGCAGG |
| | R: GGTCGTGGCTACTGCTGGCC |
| BUB1B | F: GCCTGCTGCACTTCCACGGCCA |
| | R: GGTCGACGTGGCCACGCCC |
| TMTC3 | F: GGATGATGAGAAGGCGTGGAACGAGGAC |
| | R: CGTCGCAGTCTTACGCGTTGCATGC |
| UBR2 | F: CTACAGTACCCAGAAAGCTCCGCTTCC |
| | R: CGCCCAAGAGTCAAGTCACTGGCTCG |
| ENOX2 | F: GTGCCCACTGCGGCGTCTCCTAT |
| | R: GGAAGAACAGCCTCCAGCGACCTC |
| ZCCHC8 | F: CCAGTCGCTAGAGACTACAACTCCCG |
| | R: CCAGATTGCTTTAGCGCATTCTGACCTAC |
| TCEA2 | F: GGGTATTGTGGGATCACGAGTCACTG |
| | R: CGCTCTGGACCACAACTCCCAGGC |
| CTDP1 | F: CGTAGTCCCGCGACGTTGCGACTC |
| | R: CTACGAGTCCCAGCGTTCAACGCAGG |

| | |
|---|---|
| BOD1 | F: CAGATGGCGTCAACGTTGACCTTTGC |
| | R: GCCTACAGTTCCCATATTACCCCGC |
| C8orf31 | F: CGAGGCTTAATGAGCTCCAGATGTCTC |
| | R: GTAATGACATGTCGCTGATTTAAAGGGAACTG |
| ZNF226 | F: GGTAGTTGGACACTTCCACATCAGAAGG |
| | R: CTTACTGCAGCTGAGCTATGCGCGAC |
| RAB3C | F: GACTGGCGGATCGAGCCTGTTTAATGG |
| | R: CTAACCGGCTCCAACATCAGCACTGG |
| RSRC1 | F: GAAACGACTCAGGGACTGCGGC |
| | R: CACAGACACTGAGCCGCAAACTGC |
| CASD1 | F: CGCACTGTTGTCATGGAGGAACCAAG |
| | R: GAGGTGGCACGCTGCGAGGAGCA |
| ELAVL4 | F: AAGGTCTGCAGCTGTGTTCGGAGGGAC |
| | R: GGCTAAACAGACCTGTTCCATATGACTG |

**List of shRNA and siRNA sequences**

| | |
|---|---|
| LSD1+8a shRNA | AAGCTGACACTGTCAAGGTTC |
| SVIL shRNA | sh1: CCGAGTATTTATCCCGCTATA |
| | sh2: GCTACTTATATCCAAACCATT |
| | sh3: CAAACCATTGAAGAAGGAATT |
| SVIL siRNA – SMARTpool (Dharmacon) | siRNA1: GGACUGAUGUCAAGGCAUA |
| | siRNA2: GAACUUGGUUGUAGAGCUA |
| | siRNA3: CCGAAAGAAUUGCAAGGUA |
| | siRNA4: GCCAUAAGGAAUCUAAAUA |