Supplementary Figure 1. Malat1 RNA expression level varies in a tissue-specific manner. Relative levels of MALAT1 RNA in various mouse tissues are depicted as a histogram showing the highest levels in Heart, Kidney and Brain and lowest in spleen and skeletal muscle. In culture, Malat1 RNA is detected ≈2.5 PCR cycles after β-actin mRNA in Neuro2A cells and after ≈4 cycles in MEFs (not shown).

Supplementary Figure 2. Malat1 expression is high in Purkinje cells of the cerebellum, in neurons of the substantia nigra and in motoneurons. A. Malat1 ncRNA *in situ* hybridization signal is detected at a high level in Purkinje cells of the cerebellum (p) and at a low level in the granular neurons (gl). No signal is found in the molecular layer (ml). **B**. Malat1 signal is also detected in neurons of the substantia nigra (sn). The highly expressing neurons are dopaminergic (data not shown). **C-D**. Malat1 is expressed at a high level in the neurons of the spinal cord ventral horn (vh). Malat1 *in situ* hybridization shows that large neurons of the ventral horn (arrows) display a stronger signal than smaller dorsal neurons (arrowhead). The large neurons were identified as motoneurons by their expression of choline acetyltransferease (data not shown). Scale bar: 100 μm.

Supplementary Figure 3. SF2/ASF relocalizes to nuclear speckles during transcription reactivation. A-D. Co-immunolabeling using antibodies against SF2/ASF (A) and SON (B) in control HeLa cells shows complete co-localization of both proteins at nuclear speckles (C-D). **E-H**. A significant fraction of SF2/ASF (E) localizes

around nucleoli in DRB-treated cells (RNA pol II mediated transcription inhibitor). Note, a major population of SON protein (F) localizes to nuclear speckles. **I-L.** Following the removal of DRB from the medium (5 mins rec), SF2/ASF (I) continues to distribute around nucleoli and also shows homogenous nuclear distribution. **M-T.** SF2/ASF (M and Q) relocalizes to nuclear speckles within 15 mins (M-P) and 30 mins (Q-T) post-washout of DRB from the medium and co-localizes with SON (N & R). DNA is counterstained with DAPI in blue (D,H,L,P, T). Scale bar: 5 μm.

Supplementary Figure 4. Malat1 ncRNA is not involved in the recruitment of transcription factors to the active transcription site. The transcriptional activator rtTa-YFP (green; A & D), CDK9 (green; G & J) and RNA pol II large subunit (green; M & P) are recruited to the transcriptionally active locus in cells treated with scrambled oligonucleotide (A-C, G-I, M-O) or Malat1 AS oligonucleotide (D-F, J-L, P-R), upon doxycycline addition (3 hr DOX). The DNA is counterstained with DAPI (C, F, O, R). The scale bar equals 5 μm.

Supplementary Figure 5. Malat1 ncRNA modulates the association of SC35 SR splicing factor to an active transcription site. Cells treated with a scrambled oligonucleotide (A-C; G-I) or Malat1 specific oligonucleotide (D-F; J-L) in the absence of doxycycline (0 hr DOX) do not show SC35 (A and G) at the transcriptionally inactive reporter gene locus (B, C and H, I). Upon addition of doxycycline (3 hr DOX), the transcriptionally active locus (E) showed enrichment of SC35 (D and F). In the absence of Malat1 ncRNA, upon addition of doxycycline (3 hr DOX), a significantly reduced level

of SC35 (J and L) was associated with the transcriptionally active locus (K). The inset in Figures B, E, H and K represents the magnified reporter locus. Scale bar: 5 μm.

Supplementary Figure 6. Malat1 depletion does not affect constitutive splicing of the reporter gene pre-mRNA. RT-PCR using exon specific primers against rabbit β-globin (reporter transcript) in RNA from Malat1 depleted cells showed constitutive splicing of the reporter pre-mRNA. A weak band observed in the upper portion of the top panel represents low levels of unspliced pre-mRNA. RT-PCR using Malat1 specific primers indicated efficient knock down of Malat1 transcripts in Malat1 AS oligonucleotide treated cells. Gapdh was used as loading control.

Supplementary Figure 7. Inhibition and overexpression of Malat1 ncRNA in mouse hippocampal neurons. A-B. Malat1 expression is decreased in cultured hippocampal neurons upon transfection with antisense oligodeoxynucleotides (AS). When neurons are transfected with scrambled oligodeoxynucleotides, Malat1 ncRNA is still detectable in the speckles by *in situ* hybridization (A, arrows). Upon transfection with a mix of two AS, a significantly reduced Malat1 ncRNA signal is detectable (B). Scale bar: 25 μm**. C.** Quantification of Malat1 ncRNA level in cultured neurons by quantitative RT-PCR. Transfection of scrambled oligodeoxynucleotide (Scr) does not significantly alter the level of Malat1 ncRNA in neuronal culture as compared to control untransfected cultures (Ctl). In contrast, transfection with two AS strongly decreases the level of Malat1 ncRNA. **D-E** Malat1 expression is increased in cultured hippocampal neurons upon transfection with an overexpression vector. Neurons were cotransfected

with a EGFP expressing vector along with an empty pCAGG vector (D) or the Malat1 overexpressing vector (E). When neurons were not transfected (arrows), or transfected with the empty vector (D, arrowhead), Malat1 ncRNA localizes to the nuclear speckles. Upon over-expression (E, double arrow), Malat1 ncRNA signal is stronger and extends throughout the nucleus.

Supplementary Table 1. Gene Ontology (GO) of genes downregulated in Malat1 knock-down Neuro2A cells. For each GO category, the first row lists its sub-root (biological process, molecular function, or cellular component). The second row lists the category name with indications of number of reference genes in the category (C), number of genes in the category that are down regulated by Malat1 knowk-down (O), expected number in the ca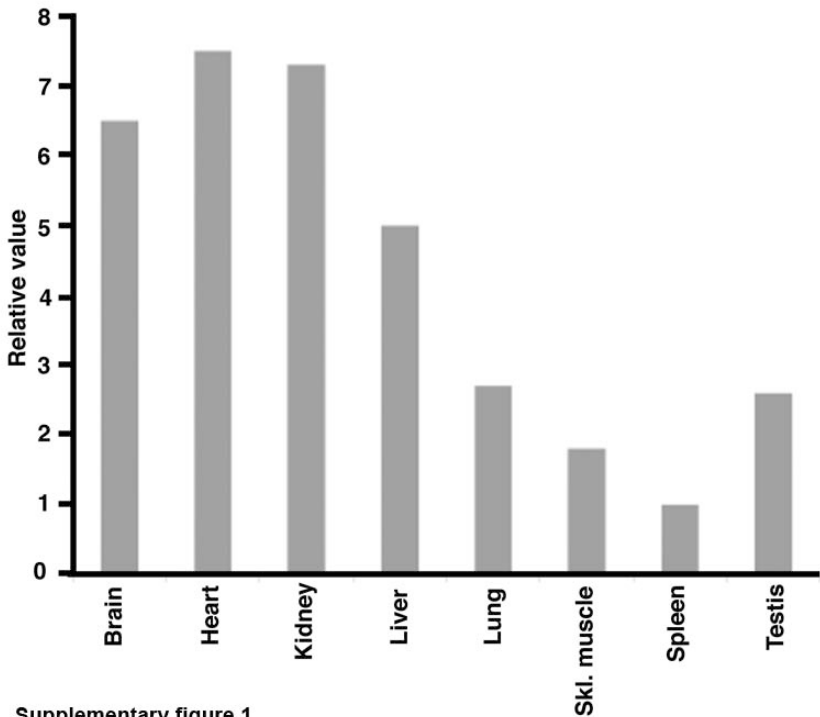tegory (E), Ratio of enrichment (R), p value from hypergeometric test (rawP), and p value adjusted by the multiple test adjustment (adjP). The genes in the category are listed. For each gene, the table lists the ENSEMBL Gene ID with the GO ID; the Gene symbol, the description, the Entrez ID and finally, the Agilent ID.

Supplementary Table 2. Gene Ontology (GO) of genes upregulated in Malat1 knock-down Neuro2A cells. For each GO category, the first row lists its sub-root (biological process, molecular function, or cellular component). The second row lists the category name with indications of number of reference genes in the category (C), number of genes in the category that are down regulated by Malat1 knowk-down (O), expected number in the category (E), Ratio of enrichment (R), p value from

hypergeometric test (rawP), and p value adjusted by the multiple test adjustment (adjP). The genes in the category are listed. For each gene, the table lists the ENSEMBL Gene ID with the GO ID; the Gene symbol, the description, the Entrez ID and finally, the Agilent ID.

Supplementary Table 3 : Validation of DNA microarray experiments with qPCR. For the 15 genes listed in this table (first column), the values are : second column, the log of the ratio calculated in the microarray experiments (Antisense / Scramble); third column: the ratio measured by qPCR using the RNA that were used for the micro-array experiments; the fourth and fifth columns indicate the RNA levels relative to GAPDH (Mean ± SEM) in Neuro2A cells transfected with the scrambled or antisense oligonucleotide respectively.

Supplementary Table 4 : Primers used for qPCR
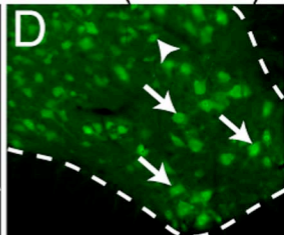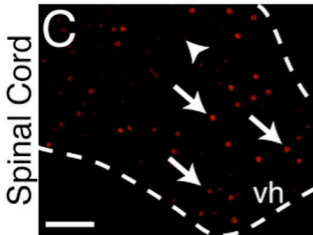
Supplementary figure 1
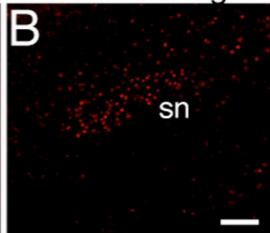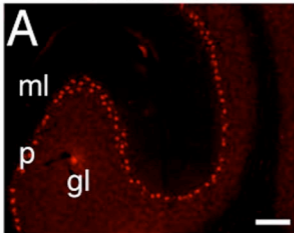
Supplementary figure 2

Supplementary figure 3

rtTA-YFP | LacI-Cherry | Merge+DAPI

A **B** **C** — 3 hr DOX — Scr. oligo

D **E** **F** — 3 hr DOX — Malat1 AS oligo

CDK9 | LacI-Cherry | Merge

G **H** **I** — 3 hr DOX — Scr. oligo

J **K** **L** — 3 hr DOX — Malat1 AS oligo

RNA pol II | LacI-Cherry | Merge+DAPI

M **N** **O** — 3 hr DOX — Scr. oligo

P **Q** **R** — 3 hr DOX — Malat1 AS oligo
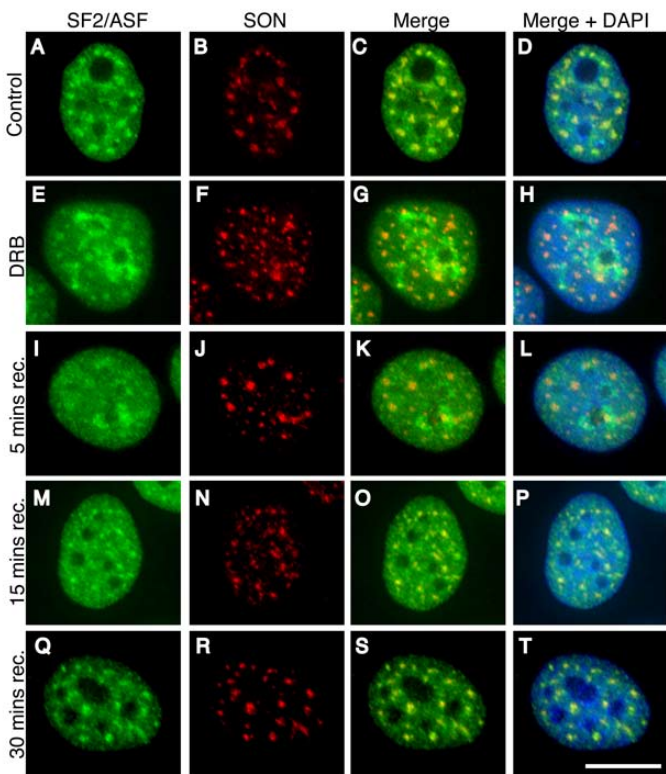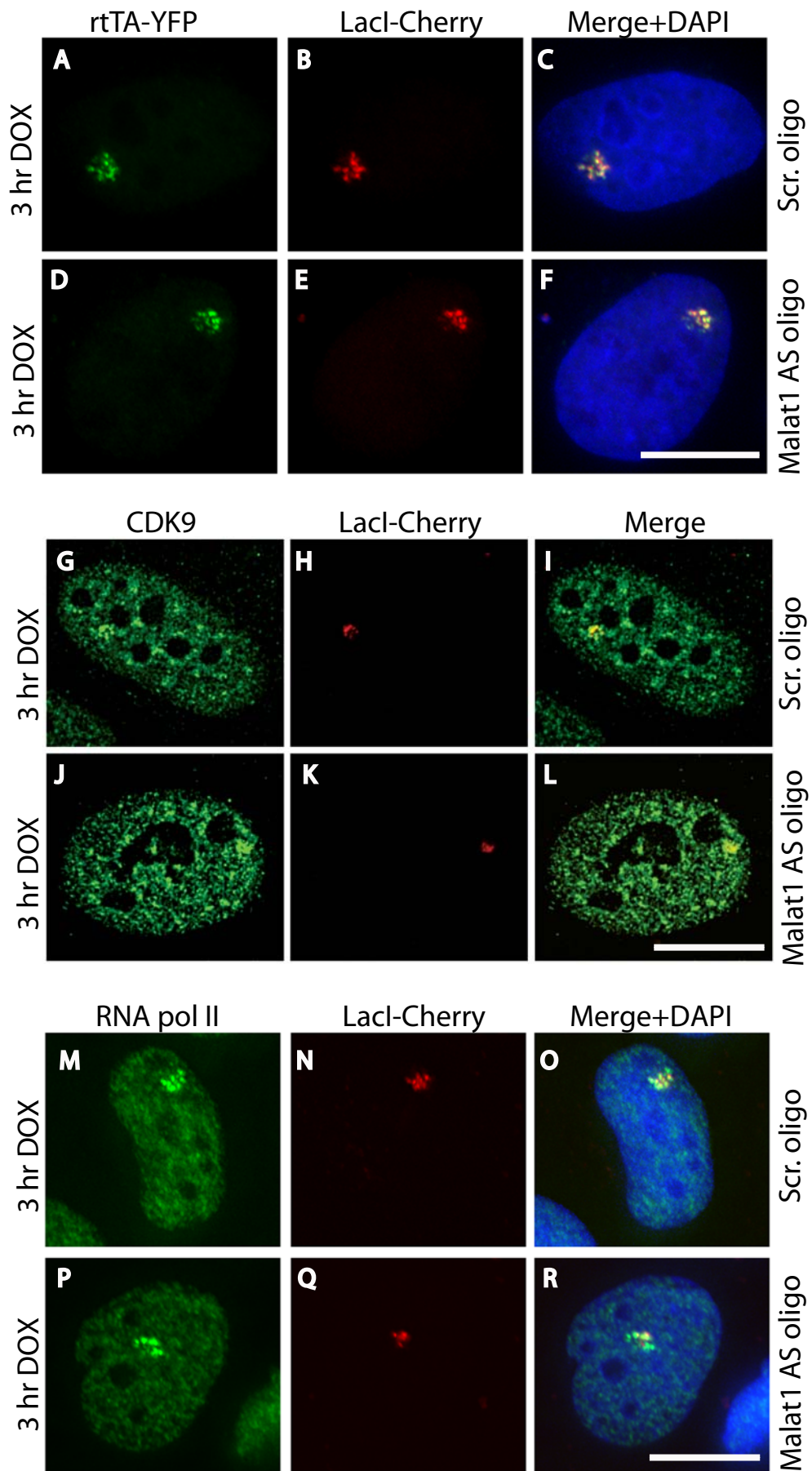
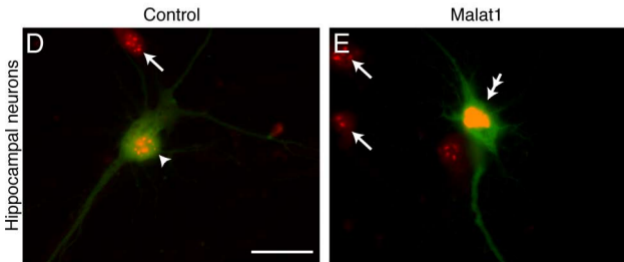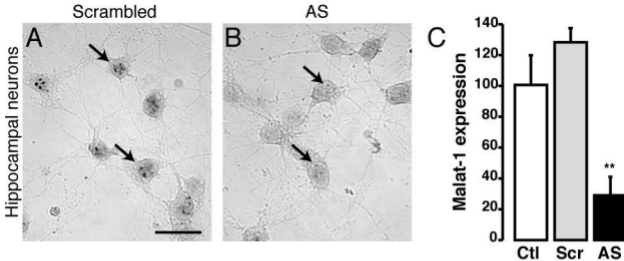Supplementary figure 4 (Figure S4)
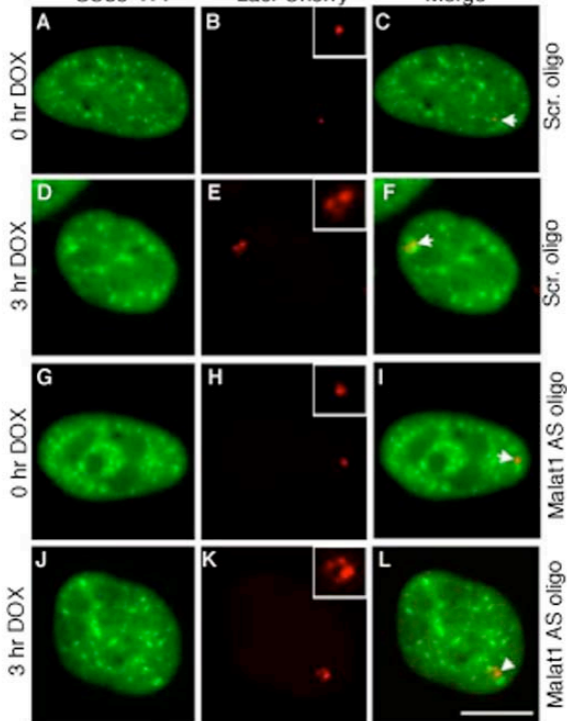
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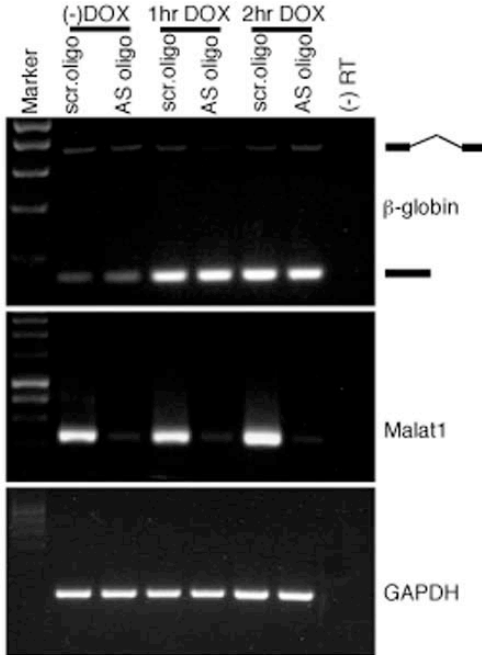
Supplementary figure 6

Supplementary figure 7