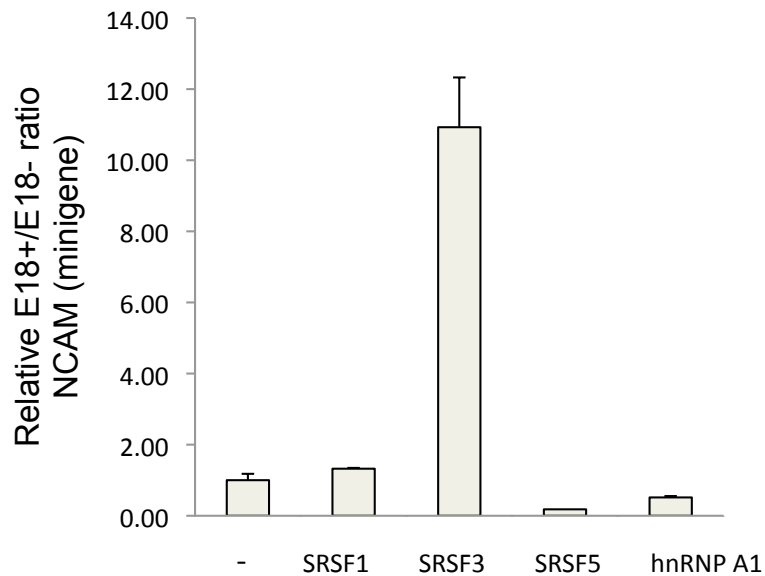


Figure S1. Treatment of differentiated N2a cells with 5aC doesn't affect the expression of differentiation markers. Undifferentiated, differentiated and 5aC-treated differentiated N2a cells were fixed and stained with both anti-Tau (rabbit polyclonal, Santa Cruz) and anti-Neurofilament 200 (mouse monoclonal, Sigma) antibodies, both markers of differentiated neurons. Signal increases with differentiation and remains after treatment with 5aC at the time and concentration used in this work (5 μ M for 3 days)

A

Effect of splicing regulators overexpression on NCAM E18 minigene inclusion

**B**

Levels of splicing regulators mRNAs after treatment of differentiated N2a cells

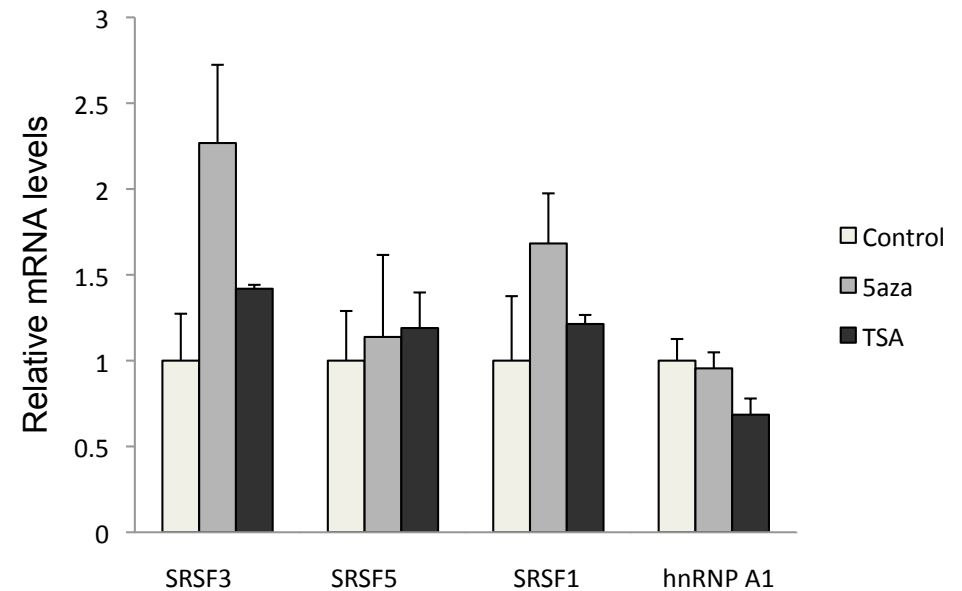


Figure S2. Effect of splicing regulators overexpression on NCAM E18 inclusion and splicing regulators levels after drugs treatments. (A) Effect of splicing regulators overexpression on NCAM E18 minigene inclusion. N2a cells were co-transfected with NCAM minigene and overexpression vectors of splicing regulators. After 48 hours of transfection, alternative splicing pattern of NCAM E18 was analyzed. All values are expressed as mean + SD, relativized to values of cells transfected only with the NCAM minigene. (B) Levels of splicing regulators mRNAs after treatment of differentiated N2a cells. Total splicing regulators mRNA levels were quantificated by real time quantitative PCR after treatment with 5aC, TSA or vehicle (control). Values (means + SD) are expressed in relation to the housekeeping gene HSPCB, and relativized to the mean for untreated cells. Note that the if conspicuous increase in SRSF3 mRNA levels caused by 5aC played a role in E18 inclusion, it would be in the opposite direction to the one observed (Fig. 1C).

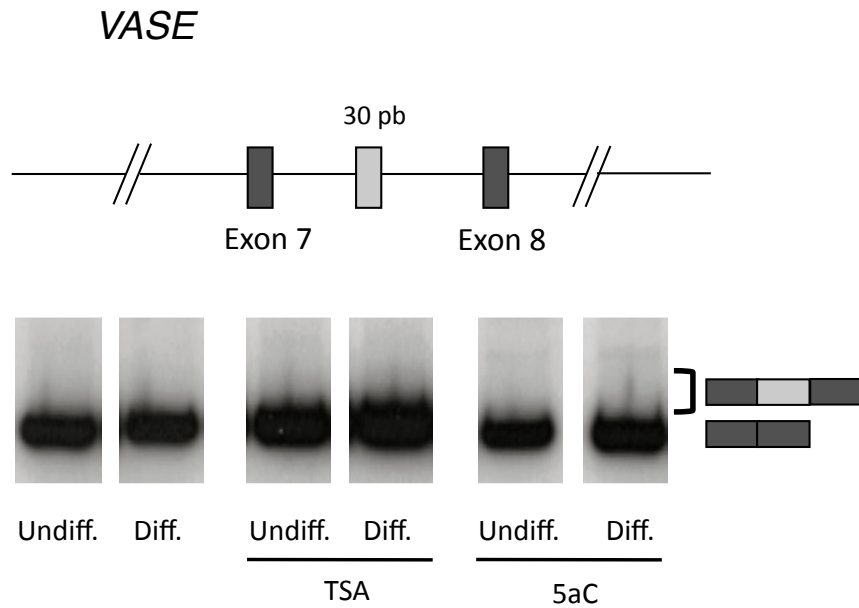
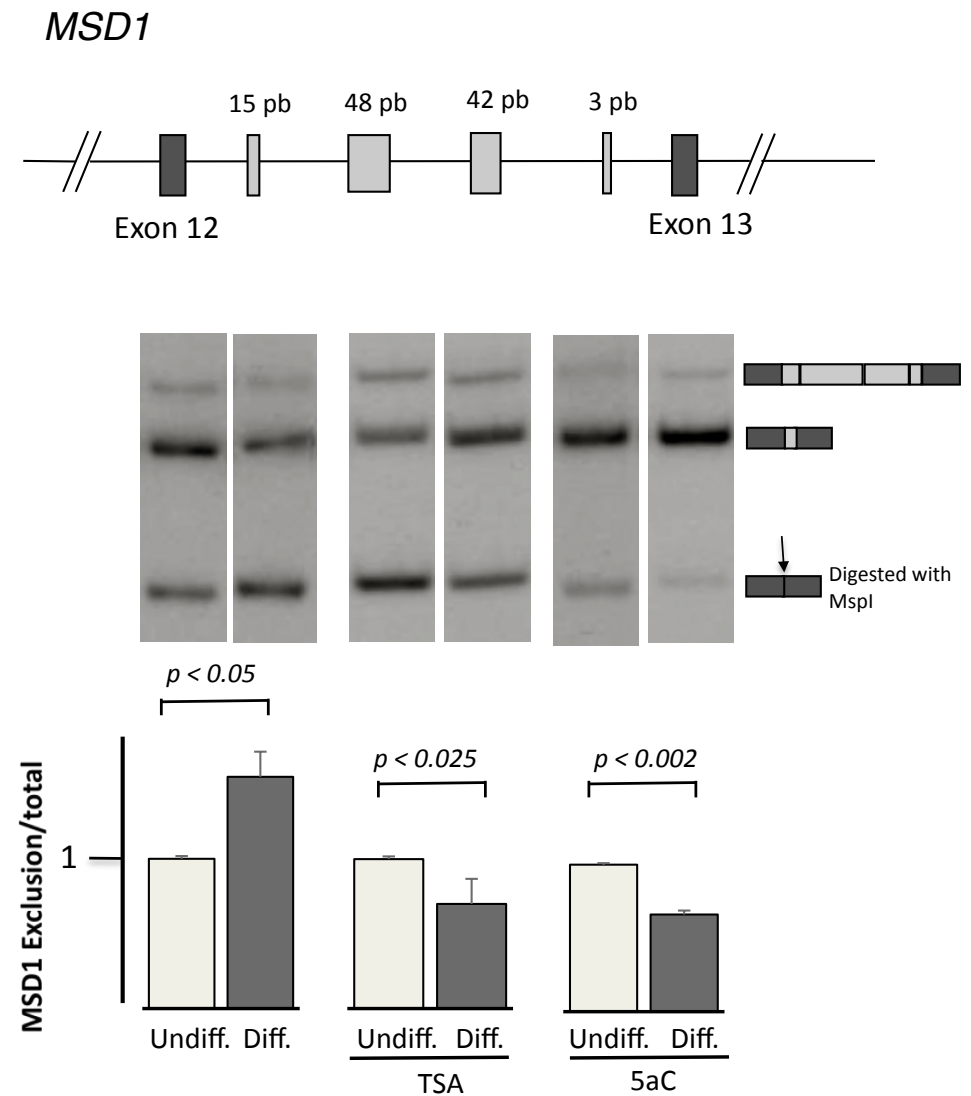
A

Figure S3. Other alternative spliced exons of *NCAM* in undifferentiated and differentiated N2a cells. (A) *NCAM* VASE alternative splicing pattern after differentiation of N2a cells, responsiveness of N2a cells to trichostatin A (TSA) and 5-aza-cytidine (5aC) before and after differentiation. Only exclusion of alternative exon between exons 7 and 8 of *NCAM* are seen. Assessed by radioactive semi-quantitative PCR. (B) Increase in *NCAM* MSD1 total exclusion after differentiation of N2a cells, responsiveness of N2a cells to trichostatin A (TSA) and 5-aza-cytidine (5aC) before and after differentiation. To observe the exclusion of the 4 alternative exons between exon 12 (E12) and exon 13 (E13) of *NCAM*, samples were digested with *MspI* which recognize specifically the E12-E13 boundary. Assessed by radioactive semi-quantitative PCR. Cells were treated with 5 ng/ μ l TSA for 16 hours or with 5 μ M 5aC for 3 days. All values are expressed as mean + SD, relativized to values of control undifferentiated cells.

B

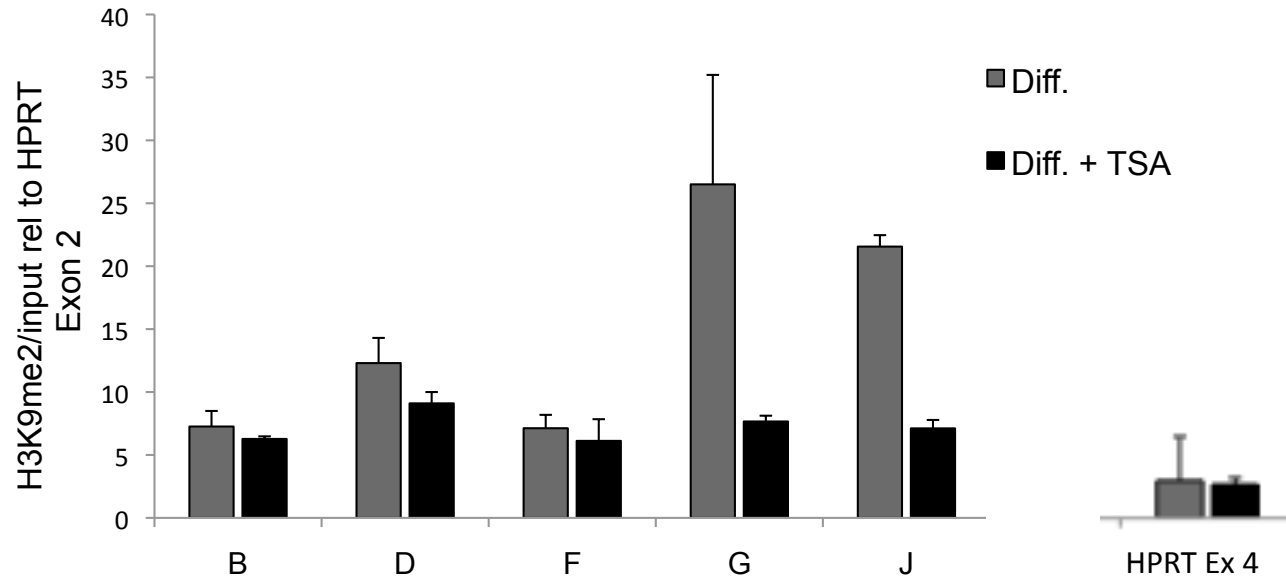
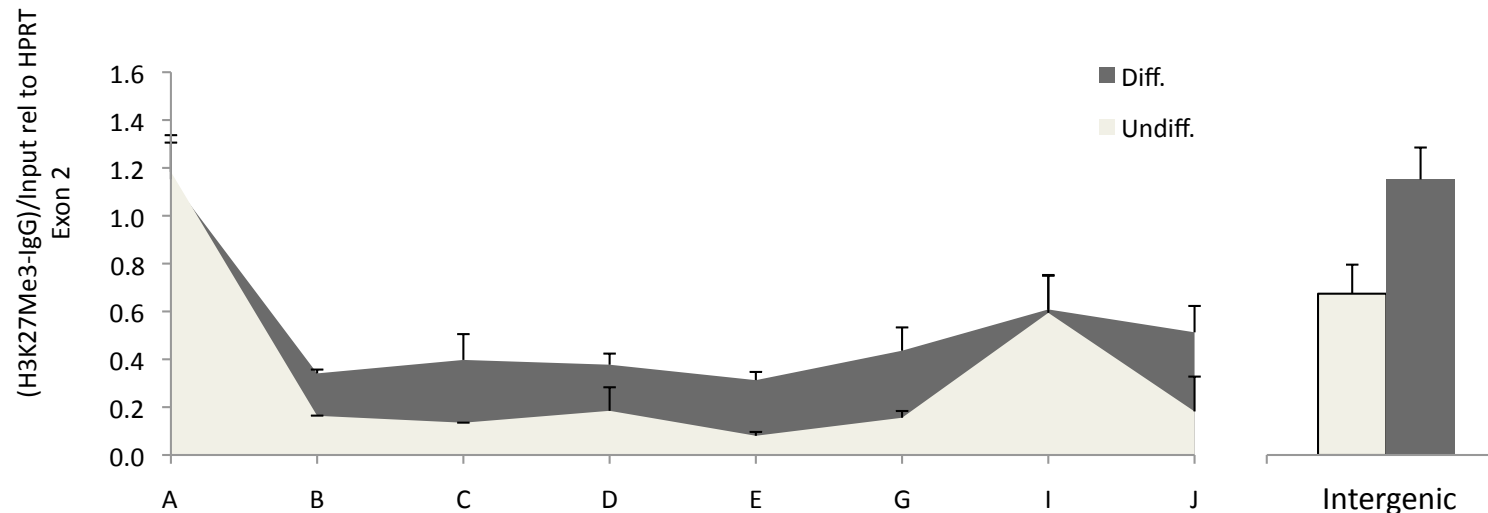


Figure S4. Differentiated N2a cells show increased H3K9me2 mark along the *NCAM* gene compared to TSA-treated differentiated cells. nChIP against H3K9me2 for differentiated and TSA-treated differentiated cells. TSA treatment was as in Figure 1B. Values are means + SD of immunoprecipitation duplicated for Diff. and Diff. + TSA samples. All values are relativized to those of *HPRT* exon 2.

A



B

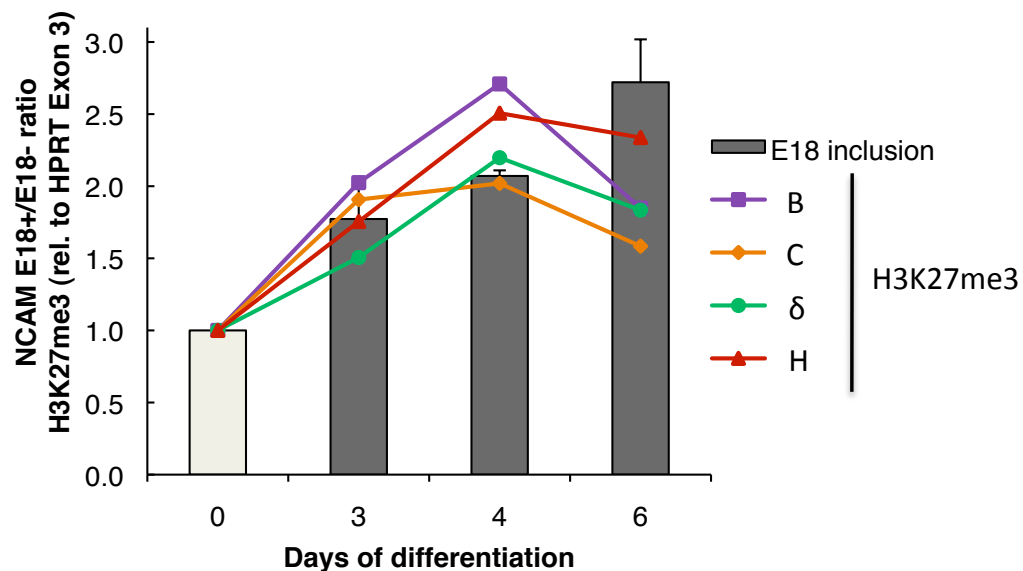


Figure S5. H3K27me3 mark increases in the intragenic region of *NCAM* as H3K9me2 do, peaking before E18 inclusion. (A) Same as Figure 2C, the H3K27me3 is assessed for the amplicons indicated in Fig. 2B. (B) H3K27me3 in different regions of the *NCAM* gene (colored lines) indicated in Fig. 2B and E18 splicing (black bars) were assessed in the same biological samples at different days of differentiation, showing that the change in the chromatin mark increases even before that the E18 inclusion reaches its maximum value, supporting a causative role of the repressive marks on splicing regulation. For the E18 splicing patterns, values are means + SD of biological duplicates, which were pooled for nChIP analysis. All values are relativized to those of undifferentiated cells (day 0).

Supplementary Methods

Oligonucleotide sequences

All DNA oligos listed here are indicated from 5' to 3', and where obtained from RefSeq sequences.

Primers for alternative splicing assessment

PCR conditions for measuring NCAM exon 18 (Schor et al, 2009) and VASE (Qin et al, 2005) inclusion/exclusion ratios were described elsewhere.

NCAM MSD region

Exon 12 (Fw) AGCCACTGAGTTCAAGACAC
Exon 13 (Rv) TTCCCGTCCTCTCCCATC

Primers for steady state mRNA levels assessment

Real time qPCR conditions for measuring total levels of HSPCB (Schor et al, 2009) and hnRNP A1 (Pelisch et al, 2012) were described elsewhere.

Total NCAM levels

Exon 2 (Fw) AGGAGAAATCAGCGTTGGAGAG
Exon 3 (Rv) TTGGCGTTGTAGATGGTGAGG

HP1 α

Exon 4 (Fw) CCAGGGAGAAATCAGAAGGAAAC
Exon 5 (Rv) AACATTAAGTCACCGCAGGAATC

Ago1

Exon 5 (Fw) GCCGCTCCTTCTTCTCACC
Exon 6 (Rv) GTTCGTCTATGTTCCCTGATGTCC

Primers for nChIP

Real time qPCR for measuring NCAM amplicons in the following regions was described in (Schor et al, 2009):

Promoter (A), Exon 2 (B), Exon 5 (C), Exon 14 (F), Intron 17 (G), Exon 18 (H), Intron 18 (I), Exon 19 (J), Intergenic

NCAM exon 8

AGACCCCATCCCCTCCATCAC
GCATCCCACAGCCCTGAGAC

NCAM exon 11
CAGTGAACCGTATTGGACAGGAG
AAGGATGGTGTGGAAGTTGGC

HPRT Exon 2
GCTAAATTCCTCTGTTAACTAAG
CTGGTTCATCATCGCTAATC

HPRT Exon 3
GGGCTATAAGTTCTTTGCTG
AAATCTACAGTCATAGGAATGG

HPRT Exon 4
ACTGGAAAGGTATGTAAGTTGAAG
CCTCTAAGTAAGTGGTTGAAAGC

HPRT Exon 6
AGAGCCAGATAGGTTACAAGTTCC
TTCTAGTATTCCACGACACAATGC

HPRT Exon 8
CAGGGATTTGAATGTAAGTAATGC
ACGAGGTGCTGGAAGGAG

Primers for the elongation rate assay

For the following NCAM regions we used the same primers that for nChIP: β (E2-I2), γ (I4-E5), ε (I17) and η (I18). The same applies for HPRT exon 6-intron 6.

α (I1-E1)
TGCTGCGAACTAAGGATCTCATC
GCAAGCGGACAATTAACAGACC

δ (E17-I17)
GAAGCACACAGAGCCCAACG
GCGACAATCCCTCCTACTCCAC

ζ (E18-I18)
CCCGCACCAAGCAAGACC
GGACATAACACAGCACAGACAC

Small interference RNAs (siRNAs)

Both strands are given. siRNAs were custom-selected and purchased from Invitrogen. For the intronic siRNAs, Stealth technology was used. siRNA for Ago1 was described before (Allo et al, 2009).

HP1 α

GGGAGAAAUCAGAAGGAAATT
UUUCCUUCUGAUUUCUCCCTT

I17as

CAUGCCUGCCUAGGAUUGUAAGUAU
UAUCUUACAAUCCUAGGCAGGCAUG

I18as

CAGGGCUUCUGUGAUACCACUGUCA
UGACAGUGGUAUCACAGAAGCCCUG

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