

Supplemental Data

**DYRK1A-Dosage Imbalance Perturbs NRSF/REST Levels,
Deregulating Pluripotency and Embryonic Stem Cell Fate
in Down Syndrome**

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Figure S1. Significantly Increased or Decreased Probe Sets

(1) Numbers of probe sets from the total of 12488 on MGU74Av2 mouse expression microarrays (Affymetrix) found to be significantly increased (Inc.) or decreased (Dec.) in 47-1 compared to D3 RNA, using two different search strategies (MAS= search based on Affymetrix MicroArray Suite change calls; ANOVA = search based on Analysis Of Variance in Genespring; see Methods in paper for details). Green areas indicate numbers of genes found by both search strategies, and numbers in parentheses indicate how many genes are expected in the intersection by chance alone.

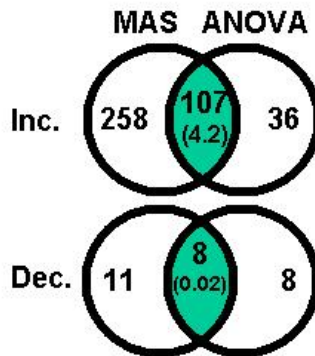


Figure S2. qRT-PCR Analysis of Rest and Selected Rest Downstream Target Genes

qRT-PCR analysis of Rest and selected Rest downstream target genes in tissues of adult Ts1Cje mice and their WT littermates (n = 6). Means and standard errors are shown.

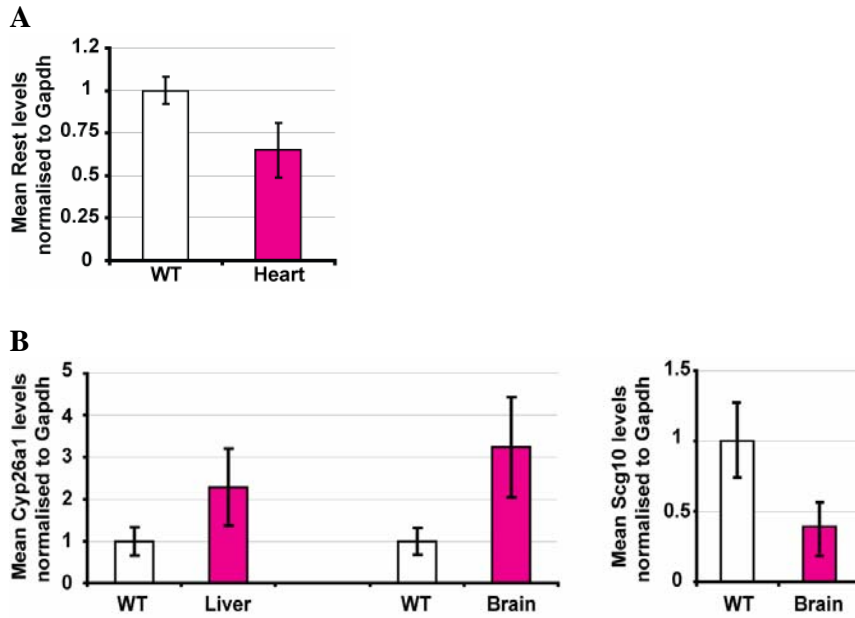


Figure S3. Efficiency of Gene Silencing

Verification of the efficiency of gene(s) silencing with specific RNAi in mouse E14ES cells (Figure 2C in the main text). Means and standard errors are shown.

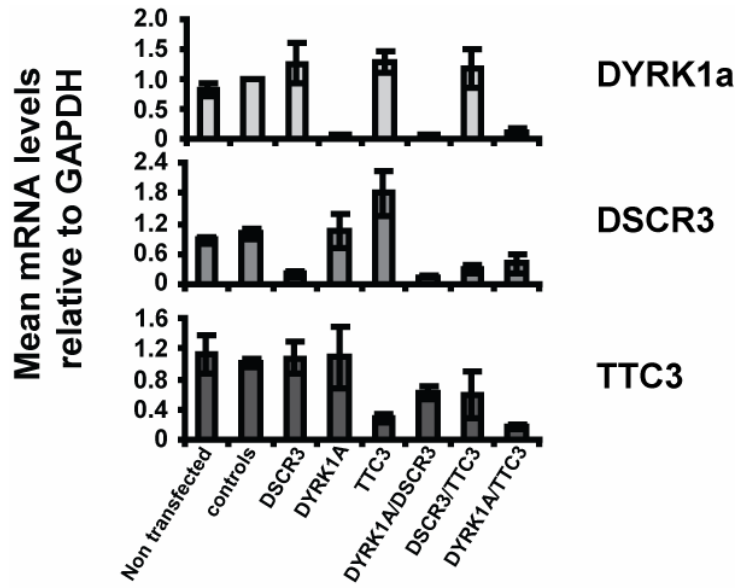


Figure S4. qRT-PCR Analysis of Rest and Cbr1 and Rest and Setd4

qRT-PCR analysis of Rest and Cbr1 (panel a) and of Rest and Setd4 (panel b) mRNA levels in undifferentiated mouse D3 ES cells transfected with RNAi targeting, respectively, Cbr1 and Setd4. Means and standard errors are shown.

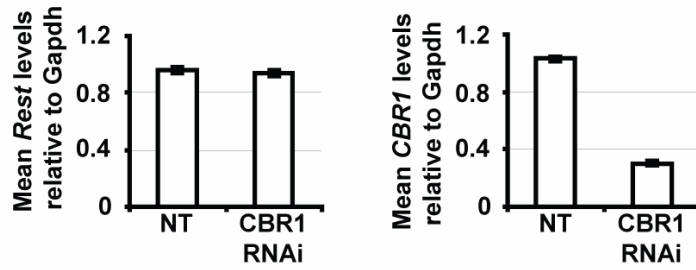
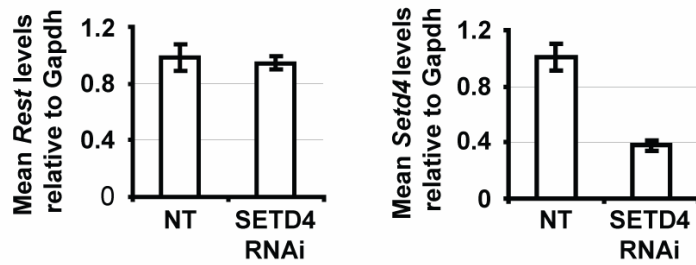
a**b**

Figure S5. Secondary versus Primary Neurites

Mean numbers of secondary neurites per number of primary neurites are shown in D3/47-1 after three days of neuronal differentiation in N2 medium. Means and standard errors are shown (n = 3 independent experiments).

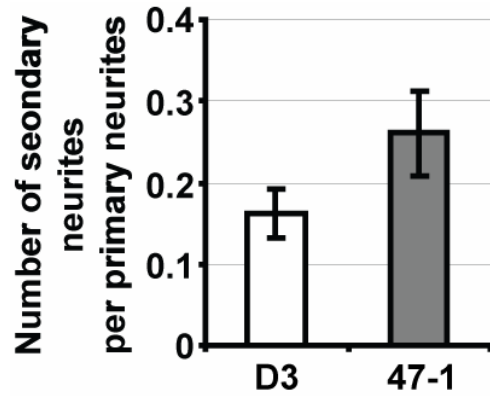


Table S3. Primers and Probes used for qRT-PCR

Gene	Oligonucleotide Sequences	ABI Cat. Number
Mouse Rest 1 (SYBR)	ACTCATT CAGGTGAGAAGCC, TGAACCTGTCTTGCATGTCC	
Mouse Rest 4 (SYBR)	GTGTGATCTAGCTGGGTATG TGAACCTGTCTTGCATGTCC	
Gapdh (SYBR)	TCACCACCATGGAGAAGCC, GCTAAGCAGTTGGTGGTGCA	
Mouse Rest	CAGGAAAGTCTACACCTGCA, TTGAGTAAGGACAAAGTTCACA	
Taqman Gapdh	-	Mm99999915_g1
Taqman Map2	-	Mm00485230_m1
Taqman Nestin		Mm00450205_m1
Tubb3 (SYBR)	GCTGTCCGCCTGCCTTTT, GACCTCCCAGAACTTGGCC	
Human REST	GACATATGCGTACTCATT CAGGTGA, TGGCGGGT TACTTCATGTTG PROBE: TTAGAGGCCACATAACTGCACTGATCACATTTAA	
mDscr3 (SYBR)	GAGATGCCACAGAGATTCAG GTGGTCAGCATGAAGGAGG	
mTtc3 (SYBR)	TCATTCCGGCTGCCTCTGTG TTTCATGGCATAATCTCACAGG	
hmDyrk1a (SYBR)	CCTCTGTT CAGTGGTGCC CCGTTTTCCATCTTTGGTC	
mOct4 (SYBR)	CACGAGTGGAAAGCAACTCA TTCATGTCCTGGGACTCCTC	
mNanog (SYBR)	CTTACGTACAGTTGCAGCAAAACT GTGGAGTCACAGAGTAGTTCAGGA	
mGata4 (SYBR)	TCGTATATATTTT GAGCGAGTTGG ACTGTCACCAAAAATCAAAACAAA	
mGata6 (SYBR)	TCTGAGGTC ACTTGG AATTTGTAA GCAAATGATTTAACACTGATTGCT	
mFoxa2 (SYBR)	C GACTGGAGCAGCTACTAC GCGGACATGCTCATGTAGTG	
mPitx2 (SYBR)	CGGCAGAGGACTCATTTCAC CTTCCGTAAGGTTGGTCCAC	
mSnai1 (SYBR)	ACTGGTGAGAAGCCATTCTCCT CTGGTATCTTTCACATCCGAGT	
mSox2 (SYBR)	GAGAGCAAGTACTGGCAAGACCG ATATCAACCTGCATGGACATTTTT	
mSetd4 (SYBR)	AACAGGAAGAGGGCTGATGA GAGACACAGGAGGCTTCCAC	
mCbr1 (SYBR)	ATCACTCGTGACCTGTGTCCG CCTCCGTATTCCTTGAGCAG	

Table S4. List of RNAi Oligos

RNAi target	Oligonucleotide sequences
Nonspecific control	UAAUGUAUUGGAACGCAUATT
<i>Dyrk1Aa</i>	CUUUGAACCUAACACGAAA
<i>Dyrk1Ab</i>	GCCACUUUAUGUUUCGAAA
<i>Dyrk1Ac</i>	GGAACCCGUAACUUCUAU
<i>Dscr3a</i>	GGUCCUGUAUGAGACUUAUACTT
<i>Dscr3b</i>	CCAGCAAGCUCUCUGAUUGTT
<i>Ttc3a</i>	GCAGUGGAGUAUGUUAUCGTT
<i>Ttc3b</i>	AGAAUGGAAGAGGACUUAATT
Human-specific <i>DYRK1A</i> (3' UTR)	AAGUUGACUCCUUAUUGGTT
<i>Setd4</i>	siGENOME SMARTpool L-052980-01-0005
<i>Cbr1</i>	siGENOME SMARTpool M-044203-00-0005

Table S5. Mapping Data for ES Cell Lines

	40-2	46-1	47-1	D3
SETD4	-	-	+	-
MORC3	-	-	+	-
HLCS	-	-	+	-
DSCR6	-	-	+	-
DSCR5	-	-	+	-
TTC3	+	-	+	-
DYRK1A	+	-	+	-
KCNJ6	+	+	+	-
D21S55	+	+	+	-
KCNJ15	-	-	+	-

Mapping data for the transchromosomal ES cell lines using human-specific marker sequences for the genes/markers in column 1. The mapping is shown only for the critical region that is the most significant eQTL for the control of Rest in human LBL (see Figure 2a and 2b in the main paper). The mapping for the remainder of the HSA21 is based on (Hernandez et al. 1999; O'Doherty et al. 2005, Mensah et al. 2007) and unpublished data using additional markers.