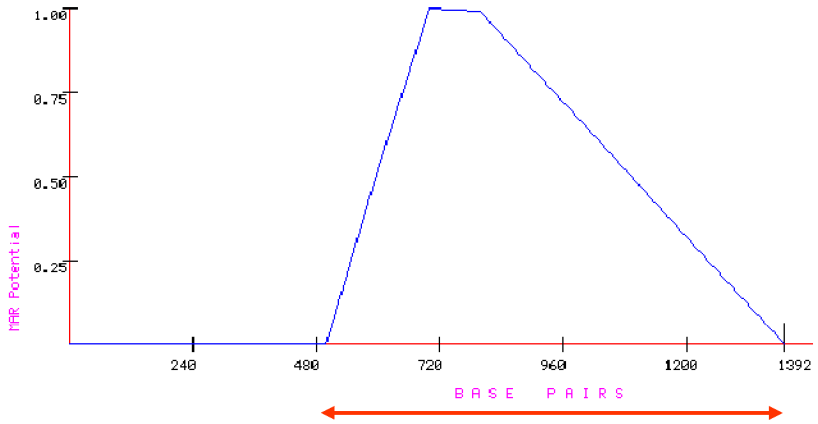
Supplementary Figure 1. Potential MeCP2 binding sites were identified in the *EGR2* intron by: a. Identifying Matrix Attachment Regions (MARS) using MAR-Wiz (Futuresoft at http://www.futuresoft.org/MAR-Wiz) b. Identifying CpGs located near A/T runs within a high-scoring MAR. ChIP showed MeCP2 binding within the amplicon in brackets.



EGR2 Intron

taagccgcgtgtcttcgcaggcccggacctgcgcggcccggaggtggtggatggggtgca
gtgggtgtgcgggaatcgcaggagaggattggggctccaggtggacggtgctgactgcac
tggctttgaagactggaagtgggtgcaggaggaacctgcgacagctgagaccagggcgcg
cgggcagatgcgccaggtcgccagcaaaccggcgtgcgtgcgccccggcgcgcccagccc
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Gggagttgggaaaagctcattctgctttttttggaggtagttcttgagttccatactgtca
Atgggttgccacagttgggctggcctcttcttacagtctgtgggcctcaatttccccacc
Ttttggactttgccacagctctacagtgctgagactctcggccttggaaatggacctatg
Tgagcgggggtgagaagggagtcctggctgtattttcattgttccacctccattctgcct
ttcccctccccag[1391]

Matrix attachment region (predicted by MAR-Wiz)

ChIP primer sites

Relevant CpGs

A/T runs

Supplementary Figure 2. EGR2 binding sites were predicted in two sites of interest: near the *MECP2* core promoter (A) and in an enhancer region 3' of the *MECP2* gene (B). EGR2 bound the site in the core promoter as shown by ChIP (see Figure 1) but did not bind the predicted site in the enhancer region (data not shown).

A *MECP2* core promoter (Liu and Francke, 2006)

ttttccggacggct**tttaccacagccctctctcc**gagaggagggagcgcgcgcgcc
gccgacgccgggaccccgcacggccgacgtcgcgccccgccctcttggccggcccgc
gcgctgctgcacctg**cgcgcccgc**gccccgccctcggcgccgctgcgagcccgcccg
tcattggctgtgatggccgggctgggacggggaggacgggggagggggaggtctca
gcggcgatgccgtca**attggcgagatttcctgtcc**atttcggccagggaaaaggggg
cggacccctccagctgttgattggctgctttcggccgtcgtttggggaaaagaggcg
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cccaccctggagcctgacgttgccccagctgaggggccttggtttcagaggcctact
caatgcattatttcatccgagcctgcccagtgctcacactctcatagaggcggggaa
taaactgacaacaaatcgcaaccctggctttcgcagaactctggcgggggaagagag
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gtgaataaggggcaatgttagctctgcctggaagatatgatttgggagctgagatcc
agcaatttttcactttcctagaagagttgatttggagctgagatccagcaattgtgt
tttgggaaaataagagtatgagaaaactgttagctaaccaggtgttcaggacaggag
tgtttgttaaggggaccttaccctgatgccagtcattatccaatccattcaatgatc
cttctgttagttctatgcttagcaagaaaacctgattctgatttggctgcttgcc
(antisense strand)

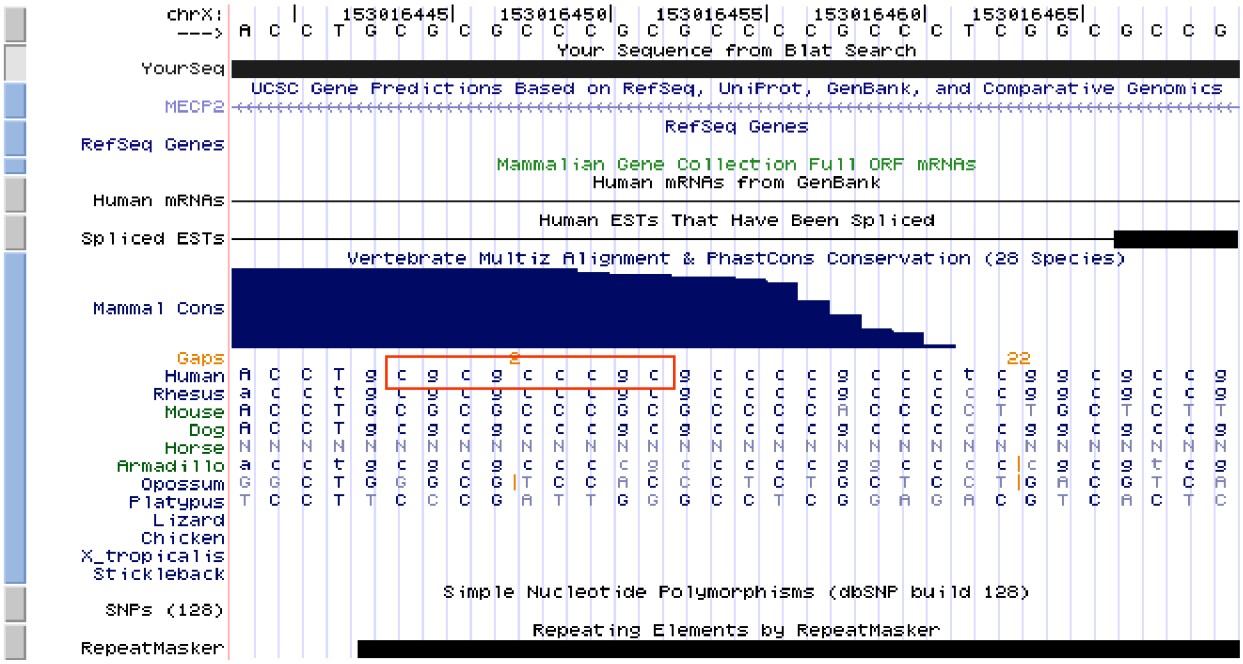
B *MECP2* enhancer S21 (Liu and Francke, 2006)

Ctcggagattatcgccacctaccacgttgactttgaaaggctggtgggttgtctgtg
accgtttttcgttccaggtctctcagcacctgttgcctccagggagcagggcctttc
tcagcagcccccgg**gcgggggcg**cttggtg**gttggtggtgccccgcgcc**

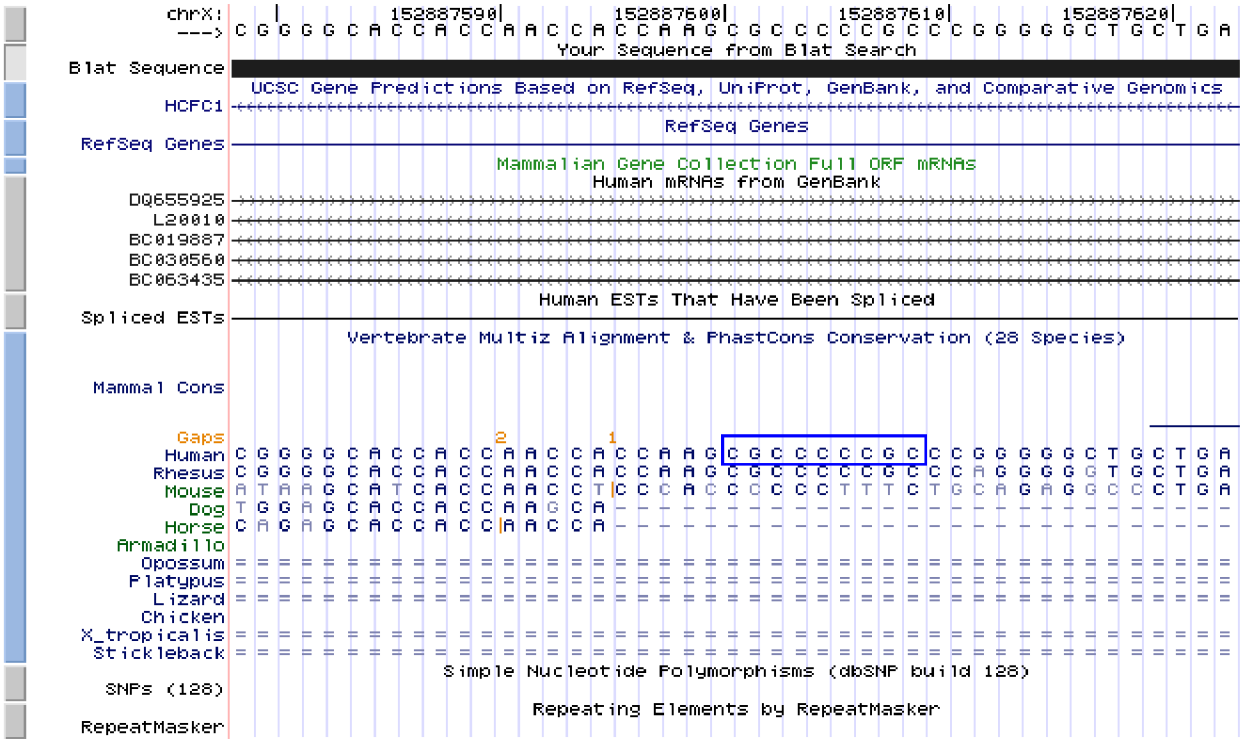
Blue—primer sites
Red—predicted Egr2 binding sites

Supplementary Figure 3. TESS predicted EGR2 binding sites in the *MECP2* promoter region (A) and an enhancer sequence in the *MECP2* 3' UTR identified by Liu and Francke, 2006 (B). The predicted EGR2 binding site located in the MECP2 promoter region is highly conserved whereas the predicted site in the *MECP2* 3' UTR is not.

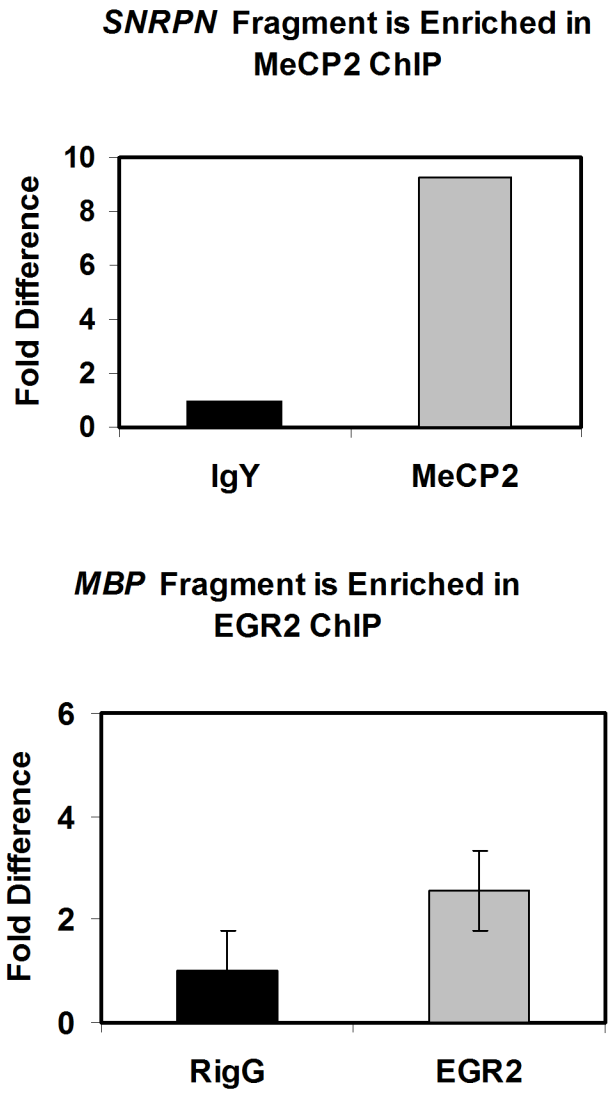
A



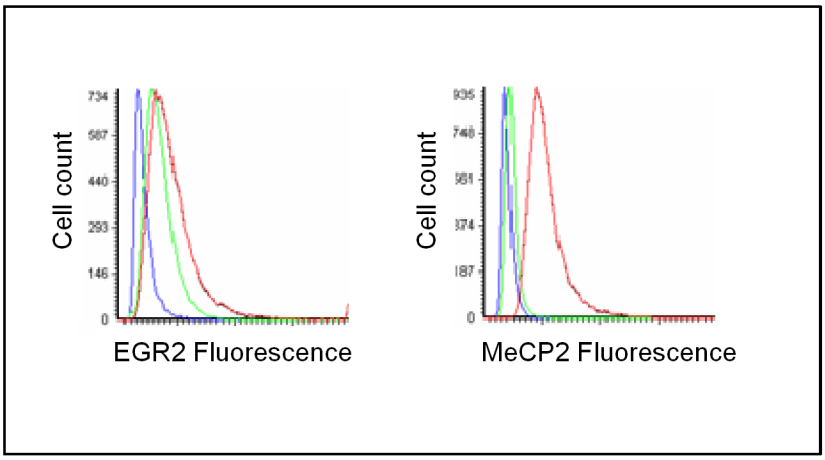
B



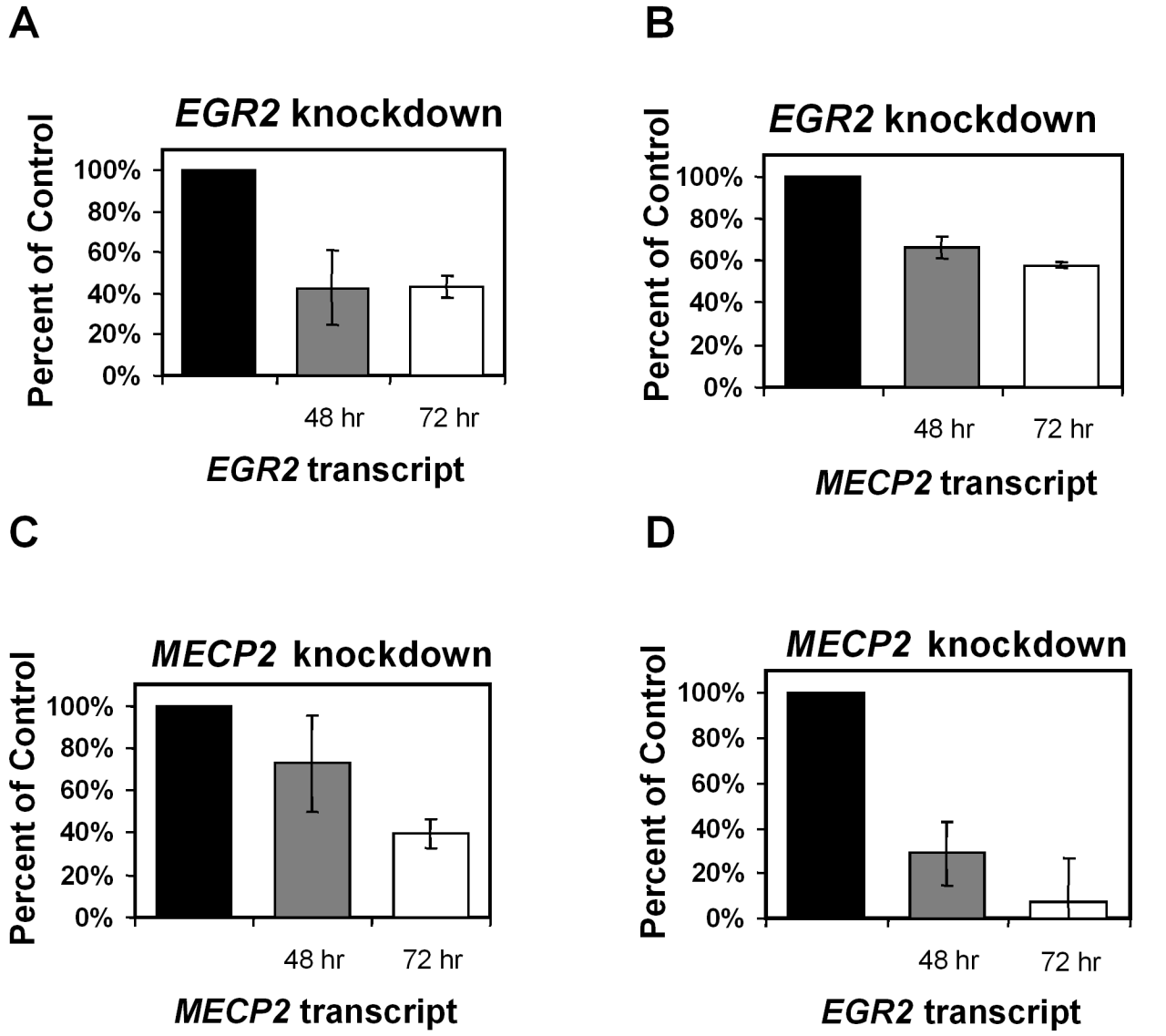
Supplementary Figure 4. Positive controls for known EGR2 and MeCP2 binding sites. A. *SNRPN* showed expected enrichment compared to the appropriate non-specific antibody control. B. Myelin Basic Protein (*MBP*) also showed enrichment compared to its non-specific antibody control.



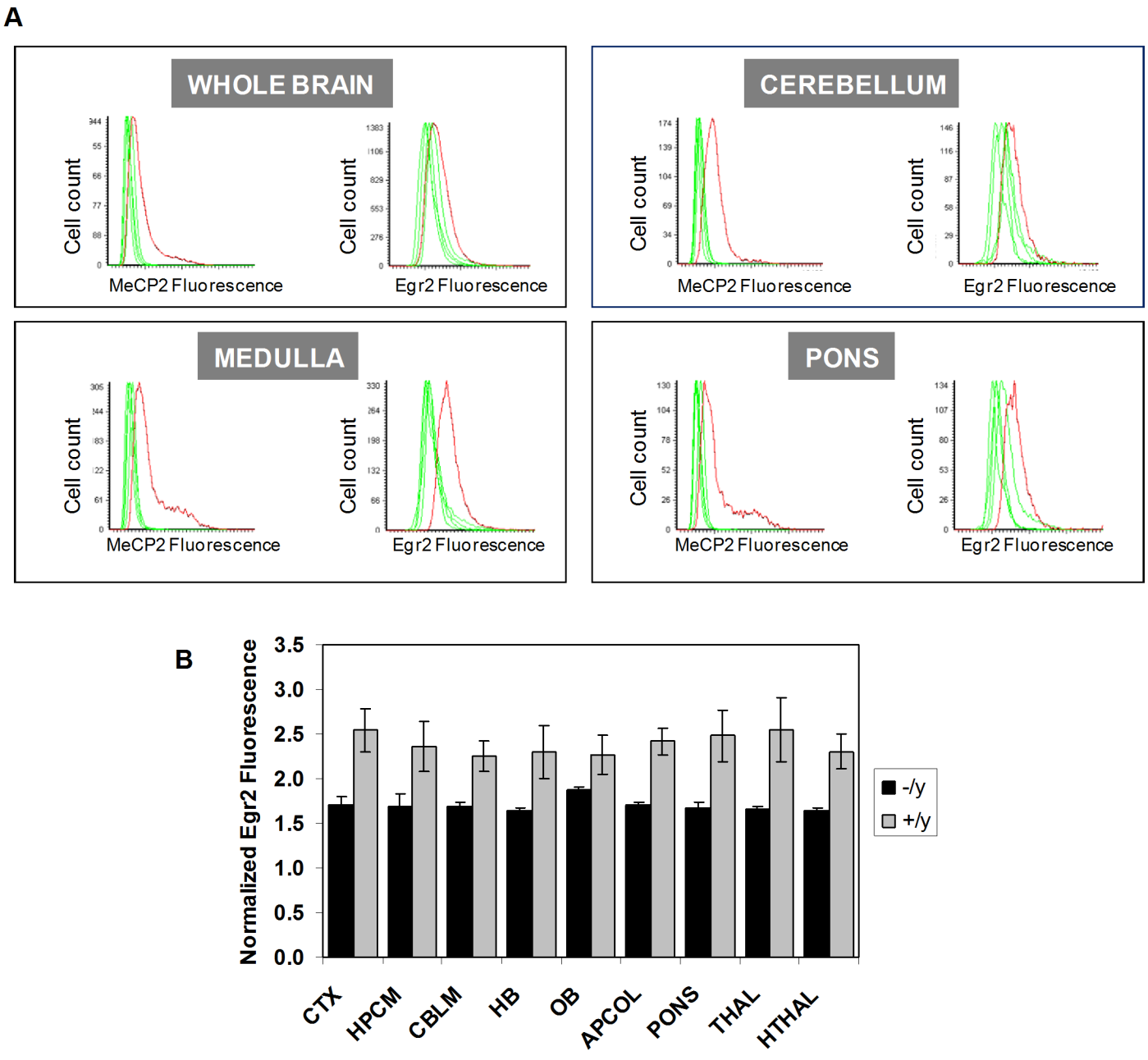
Supplementary Figure 5. EGR2 and MeCP2 protein expression in undifferentiated and differentiated SH-SY5Y cells. Treatment with PMA signals SH-SY5Y cells to differentiate and up-regulates MeCP2 and EGR2 protein. Blue: protein expression in undifferentiated control shows baseline levels of MeCP2 and EGR2. Green: protein expression 20 hours after PMA treatment show a slight but significant increase in MeCP2 and a larger >2-fold increase in EGR2. Red: 68 hours after PMA treatment, high levels of both MeCP2 and EGR2 are observed.



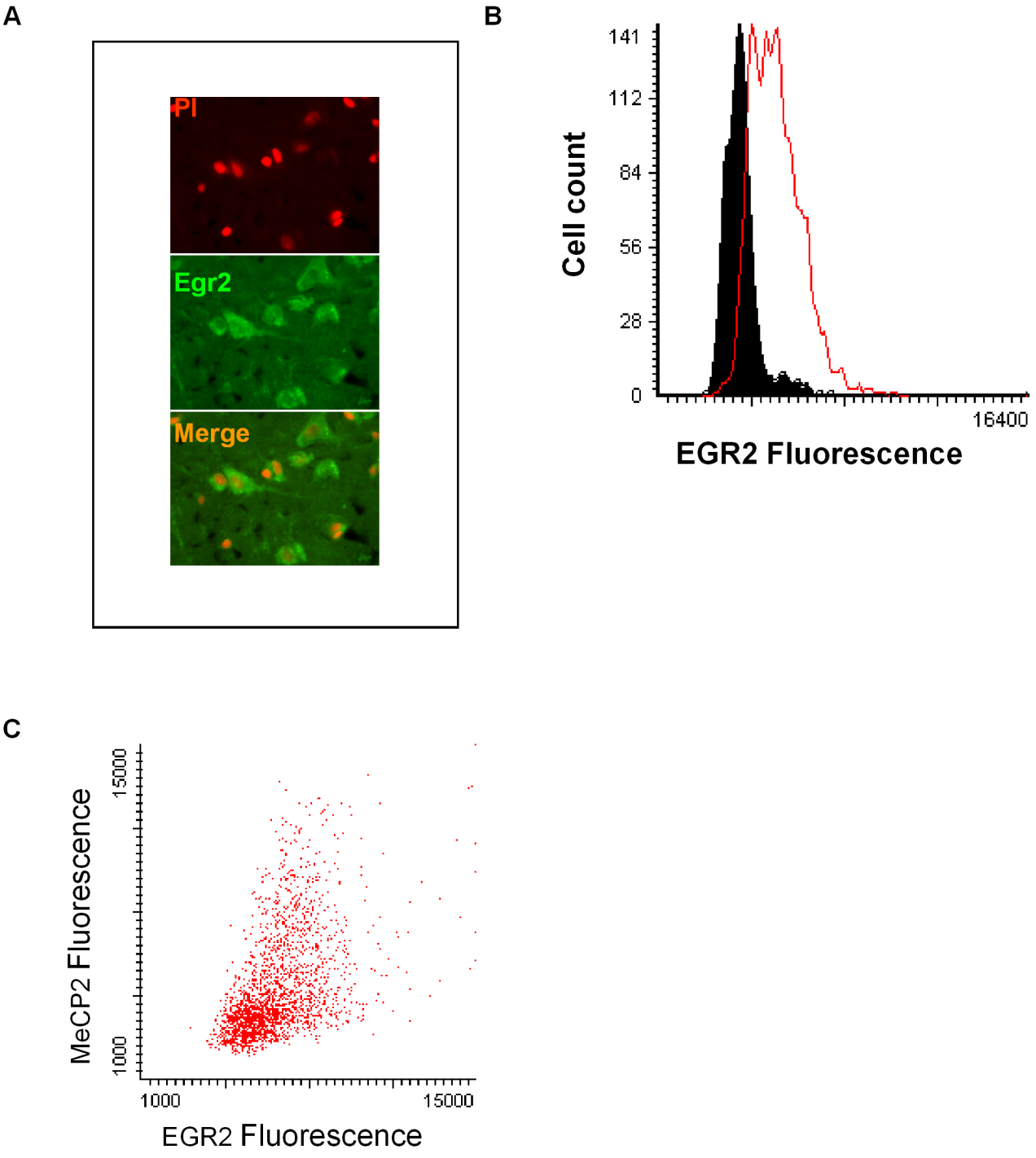
Supplementary Figure 6. Time course of *MECP2* and *EGR2* siRNA and assessment of transcript levels by qRT-PCR. A. SH-SY5Y human neuroblastoma cells were transfected with *EGR2* siRNA (grey bars or white bars) or control (black bars) then stimulated 4 hours later with PMA. Results are shown as the mean±SEM of a minimum of 3 replicates with the mean control normalized to 100%. **A**. 48 and 72 hr post-transfection EGR2 protein levels were quantified by qRT-PCR demonstrating a specific reduction of EGR2 at both time points. **B**. 48 and 72 hr post-transfection *MECP2* transcript levels were also quantified, demonstrating a reduction in *MECP2* for both time points. **C**. While some reduction in *MECP2* transcript was observed at 48 h, 72 h post-transfection *MECP2* levels were reduced to ~40% of control. **D**. Strong reductions in *EGR2* transcript levels were observed following *MECP2* siRNA knockdown at both 48 and 72 h time points.



Supplementary Figure 7. Egr2 expression is reduced in the Mecp2$^{tm1Bird/y}$ mouse brain compared to expression in the wildtype brain. A. Sagittal brain sections of four-week (P28) wild-type (Mecp2 $^{+/+}$ or $^{+/y}$) and MeCP2-null (*Mecp2* $^{tm1Bird/y}$) littermates were stained using immunohistochemical techniques and scanned with the LSC. Histograms on the left side of each panel show MeCP2 staining in a wildtype female littermate (red outline) compared to background staining in four Mecp2$^{tm1Bird/y}$ littermates (green outline). The histograms on the right side of each panel show Egr2 expression in the same Mecp2$^{tm1Bird/y}$ littermates (green outline) compared to the wildtype female littermate (red outline). Reduced Egr2 expression was observed in the whole brain and specific regions including the medulla, the cerebellum and the pons. B. Sagittal brain sections from *Mecp2*-null (Mecp2$^{tm1Bird/y}$) mice were also compared to non-littermate wildtype male mice. Bar graph showing statistically significant reduction of Egr2 protein in the brain of a representative *Mecp2*-null male compared to a representative non-littermate wildtype male control ($p<0.001$). At least three replicates for each individual were used in the analyses. Cortex (CTX), hippocampus (HPCM), hindbrain (HB), olfactory bulb (OB), Anterior and posterior coliculi (APCOL), pons (PONS), thalamus (THAL), hypothalamus (HTHAL).



Supplementary Figure 8. A. Green EGR2 immunofluorescence in human cortical neurons counterstained with PI (red). B. LSC histogram showing fluorescence data from IgG staining control (black histogram) and EGR2 staining (red-outlined histogram). C. LSC scattergram of MeCP2 and EGR2 immunofluorescence showing a high correlation between MeCP2 and EGR2 levels in individual cells within the human cortex.



Supplemental Table 1. Analyses of MeCP2 and EGR2 in RTT and autism cortex (infant to adult) values normalized to control histone H1

Phenotype	Sample ID	Age	Sex	Normalized Egr2 *ab*	Normalized Mecp2 *ab*
Early postnatal to 2 years					
typically developed	1157	20 days	F	1.46 ± 0.03	1.56 ± 0.06
typically developed	1321	62 days	F	1.57 ± 0.06	1.39 ± 0.05
typically developed	125	76 days	M	1.50 ± 0.05	1.99 ± 0.12
typically developed	1055	96 days	M	1.16 ± 0.14	.92 ± 0.09
typically developed	135	120 days	M	1.67 ± 0.05	1.57 ± 0.08
typically developed	1257	2 y	F	1.52 ± 0.06	1.56 ± 0.09
mean (n=6)				**1.48 ± 0.07**	**1.50 ± 0.14**
Childhood to adult					
typically developed	229	4 y	M	2.05 ± 0.02	1.34 ± 0.06
typically developed	1377	6 y	F	1.75 ± 0.03	1.51 ± 0.10
typically developed	3835	9 y	F	1.68 ± 0.05	1.53 ± 0.08
typically developed	662	12 y	F	2.03 ± 0.06	1.73 ± 0.06
typically developed	1065	15 y	M	1.82 ± 0.04	1.47 ± 0.09
typically developed	812	18 y	F	1.86 ± 0.04	1.66 ± 0.11
typically developed	1027	22 y	M	1.89 ± 0.03	1.54 ± 0.09
typically developed	602	27 y	M	1.77 ± 0.04	1.35 ± 0.05
typically developed	1029	29 y	M	1.82 ± 0.03	1.62 ± 0.05
typically developed	1136	33 y	F	1.94 ± 0.06	1.76 ± 0.13
typically developed	1104	35 y	M	2.07 ± 0.07	1.63 ± 0.05
typically developed	1406	38 y	F	1.93 ± 0.05	1.57 ± 0.09
typically developed	1135	42 y	M	2.05 ± 0.04	1.61 ± 0.05
mean (n=13)				**1.90 ± 0.03**	**1.52 ± 0.04**

Phenotype	Sample ID	Age	Sex	Normalized Egr2 *ab*	Normalized Mecp2 *ab*
Early postnatal to 2 years					
RTT	1238	1 y	M	1.58 ± 0.04	1.13 ± 0.04
Childhood to adult					
RTT	B4687	8 y	F	1.75 ± 0.02*	1.15 ± 0.02***
RTT	B5214	10 y	F	1.60 ± 0.06***	1.16 ± 0.06***
RTT	1815	18 y	F	1.78 ± 0.06	1.31 ± 0.03***
RTT	B5075	20y	F	1.54 ± 0.06***	1.11 ± 0.06***
RTT	1420	21 y	F	1.78 ± 0.06	1.35 ± 0.06*
RTT	1748	22 y	F	1.85 ± 0.04	1.08 ± 0.02***
RTT	3381	22 y	F	1.88 ± 0.02	1.35 ± 0.02***
RTT	4321	23 y	F	1.79 ± 0.05	1.53 ± 0.05
RTT	4312	24 y	F	1.53 ± 0.06**	1.13 ± 0.06***
RTT	B5020	24 y	F	1.46 ± 0.05***	1.00 ± 0.05***
mean (n=10)				**1.68 ± 0.04****	**1.21 ± 0.05*****
autism	3871	5 y	M	1.68 ± 0.06*	1.47 ± 0.06
autism	1174	7 y	F	1.55 ± 0.14**	1.31 ± 0.14
autism	B4925	9 y	M	1.57 ± 0.05**	1.17± 0.005***
autism	797	9y	M	1.58 ± 0.04**	1.38 ± 0.04***
autism	1182	9y	F	1.59 ± 0.02	1.51 ± 0.02
autism	B5342	11 y	F	1.57 ± 0.05**	1.23 ± 0.05***
autism (suspected)	732	15 y	M	1.68 ± 0.03*	1.35 ± 0.03***
autism	3924	16 y	F	1.86 ± 0.03	1.48 ± 0.03*
autism	1638	20y	F	1.87 ± 0.03	1.31 ± 0.03***
autism	B5144	20y	M	1.80 ± 0.04	1.09 ± 0.04***
autism	B5000	27y	M	1.70 ± 0.02	1.42 ± 0.02**
autism	B5173	30y	M	1.72 ± 0.07	1.54 ± 0.07
autism (suspected)	967	32y	M	1.55 ± 0.05*	1.54 ± 0.05*
mean (n=13)				**1.67 ± 0.03*****	**1.37 ± 0.04*****

Group comparisons by t-test (Childhood to adult samples)	Egr2	Mecp2
TD/RTT	p <0.005	P<0.0001
TD/Aut	p< 0.00005	p<0.05

Comparison to three closest age-matched controls by t-test
* p ≤ 0.05
** p ≤ 0.01
*** p ≤ 0.001

Supplementary Table 2-Sequences of Dharmacon® siRNA Oligos

	MECP2 siRNA
oligo 1	GGAAAGGACUGAAGACCUG
oligo 2	ACACAUCCCUGGACCCUAA
oligo 3	GCUCUAAAGUGGAGUUGAU
oligo 4	GCAGAAACCACCUAAGAAG
	EGR2 siRNA
oligo 1	GAAGGCAUAAUCAAUAUUG
oligo 2	CUACUGUGGCCGAAAGUUU
oligo 3	GAAACCAGACCUUCACUUA
oligo 4	GAGAAGAGGUCGUUGGAUC
	Non-targeting Control
oligo 1	UGGUUUACAUGUCGACUUA
oligo 2	UGGUUUACAUGUUGUGUG
oligo 3	UGGUUUACAUGUUUUCUGA
oligo 4	UGGUUUACAUGUUUUCCUA

Supplementary Table 3—Primers for ChIP and Q-PCR

EGR2 intron	
forward (5'-3')	atggtgccaaaccgagag
reverse (5'-3')	cggactccttcctctacatcc
MECP2 promoter region	
forward (5'-3')	tttaccacagccctctctcc
reverse (5'-3')	ggacaggaaatctcgccaat
MECP2 Enhancer	
forward (5'-3')	ctcggagattatcgccacctacca
reverse (5'-3')	ggcgcggggcaccaccaac
MBP (Myelin Basic Protein)	
forward (5'-3')	tggtcgccacttagcctatgt
reverse (5'-3')	cagaaggcccttgcatttcag
GAPDH	
forward (5'-3')	catgggtggaatcatattgga
reverse (5'-3')	gagtcaacggatttggtcgt
SNRPN	
forward (5'-3')	tcggtatttaggggtgttg
reverse (5'-3')	tcggtcactgcgacgaat