Supplementary Online Content

Ruzicka WB, Subburaju S, Benes FM. Circuit- and diagnosis-specific DNA methylation changes at γ-aminobutyric acid–related genes in postmortem human hippocampus in schizophrenia and bipolar disorder. *JAMA Psychiatry*. Published online March 4, 2015. doi:10.1001/jamapsychiatry.2015.49.

This supplementary material has been provided by the authors to give readers additional information about their work.

eTable 1. Demographic variables

Diagnosis	Gender	Age	PMI	pH	COD	Medication Exposures
CON	M	57	22.3	6.19	Cancer	Lorazepam
CON	F	51	30.7	6.50	PE	
CON	F	71	22.0	6.55	MI	
CON	F	71	22.3	6.05	Cancer	
CON	M	88	11.1	6.18	Cancer	
CON	M	49	27.1	6.24	MI	
CON	M	75	20.3	6.69	MI	
CON	F	51	23.1	6.56	CVA	
	4M|4F	64.13	22.35	6.37		
SZ	F	73	28.8	6.08	Cancer	Clozapine, Lithium
SZ	M	77	25.3	6.14	PNA	Clozapine, Quetiapine, Valproate
SZ	F	64	18.5	6.58	Resp Fail	Olanzapine, Lorazepam
SZ	F	73	24.0	6.45	Cancer	Risperidone
SZ	M	83	43.5	6.08	Cancer	Fluphenazine
SZ	M	56	20.0	6.52	MI	Clozapine
SZ	F	85	12.8	6.02	MI	Fluphenazine, Haloperidol
SZ	M	32	38.4	6.27	Suicide	Risperidone, Valproate
	4M|4F	67.88	26.42	6.27		
BD	F	76	22.8	6.60	MI	Lithium, Valproate
BD	M	69	29.5	6.60	PNA	Lithium
BD	M	77	24.2	6.13	Cancer	Lithium, Diazepam
BD	F	51	30.1	6.28	MI	Valproate, Risperidone, Diazepam
BD	M	80	13.4	6.37	PNA	Haloperidol, Lithium, Risperidone
BD	F	76	19.9	6.29	PNA	Lithium, Olanzapine, Fluphenazine
BD	F	51	35.1	6.41	PNA	Clozapine, Lithium, Valproate
BD	F	66	25.0	6.09	Suicide	Olanzapine, Valproate
	3M|5F	68.25	24.98	6.35		
p	0.86	0.82	0.58	0.46		

Listed are the demographic variables for our assembled cohort. Sample groups were not significantly different for age, gender, or PMI. PMI - postmortem interval, COD - cause of death, CVA – cerebrovascular accident, PE - pulmonary embolism, MI - myocardial infarction, PNA – pneumonia. No cases were included with a documented history of illicit drug use.

eTable 2. Pyrosequencing primers

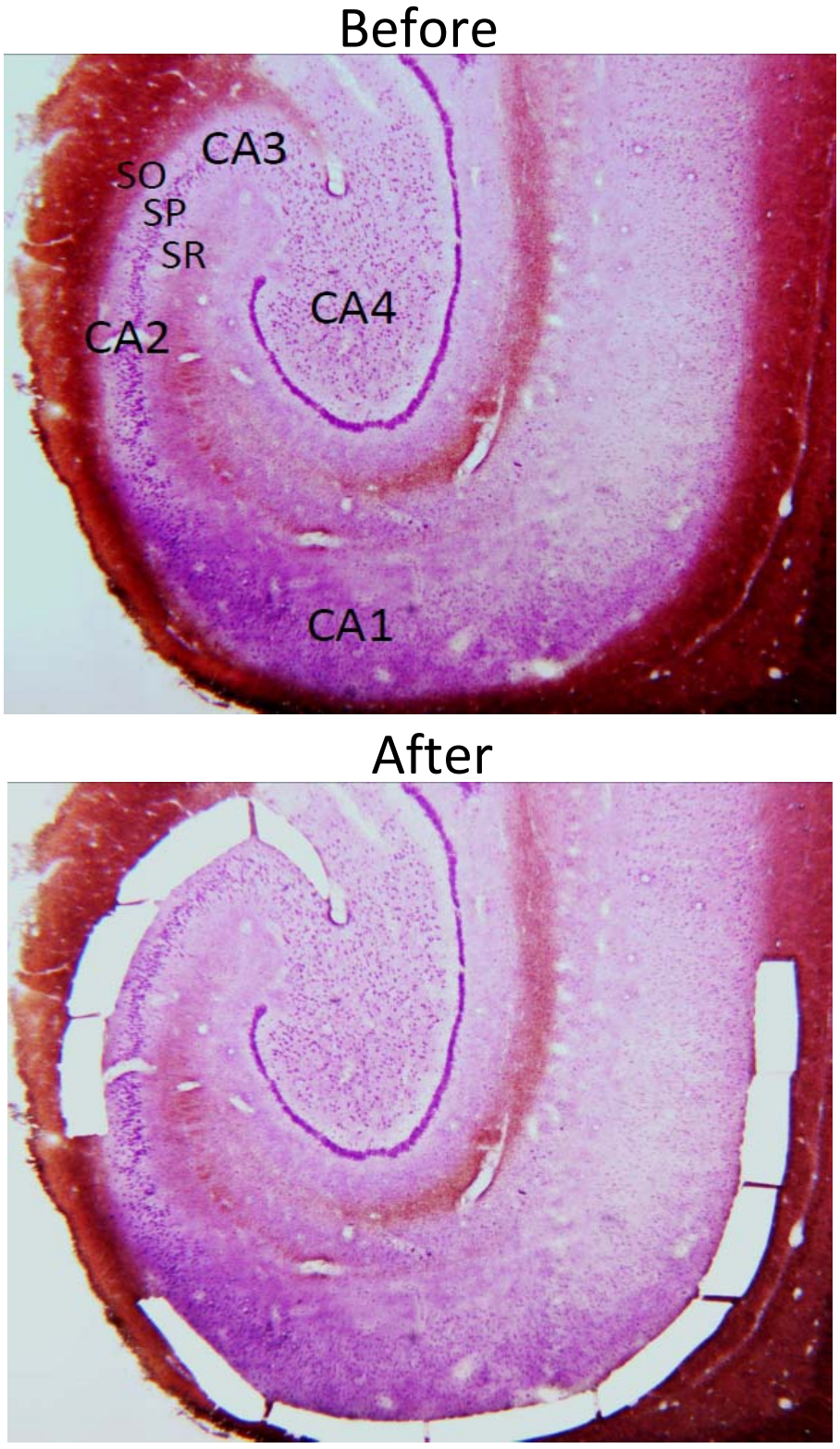
MSX1 Forward Primer	5'- TGT GGA TAG ATG GGA GGT TAT TAA T -3'
MSX1 Reverse Primer	5'- /5BiosG/AT CTC CAA AAA ACA CAA AAA ACT AC -3'
MSX1 Sequencing Primer	5'- GTT TTT AAT TTA TAG TT -3'

FOXG1 Forward Primer	5'- TTT TGT GTT AAG TTT TTG AAG TTA A -3'
FOXG1 Reverse Primer	5'- /5BiosG/AA ACA ACA ATT CAA ATC AAC ACT ATC -3'

RUNX2 Forward Primer	5'- TGG AAT TGA TGT AAG GAT ATA TGG TT -3'
RUNX2 Reverse Primer	5'- /5BiosG/ TT CAA AAC TAA AAA ACC AAA AAA AA -3'

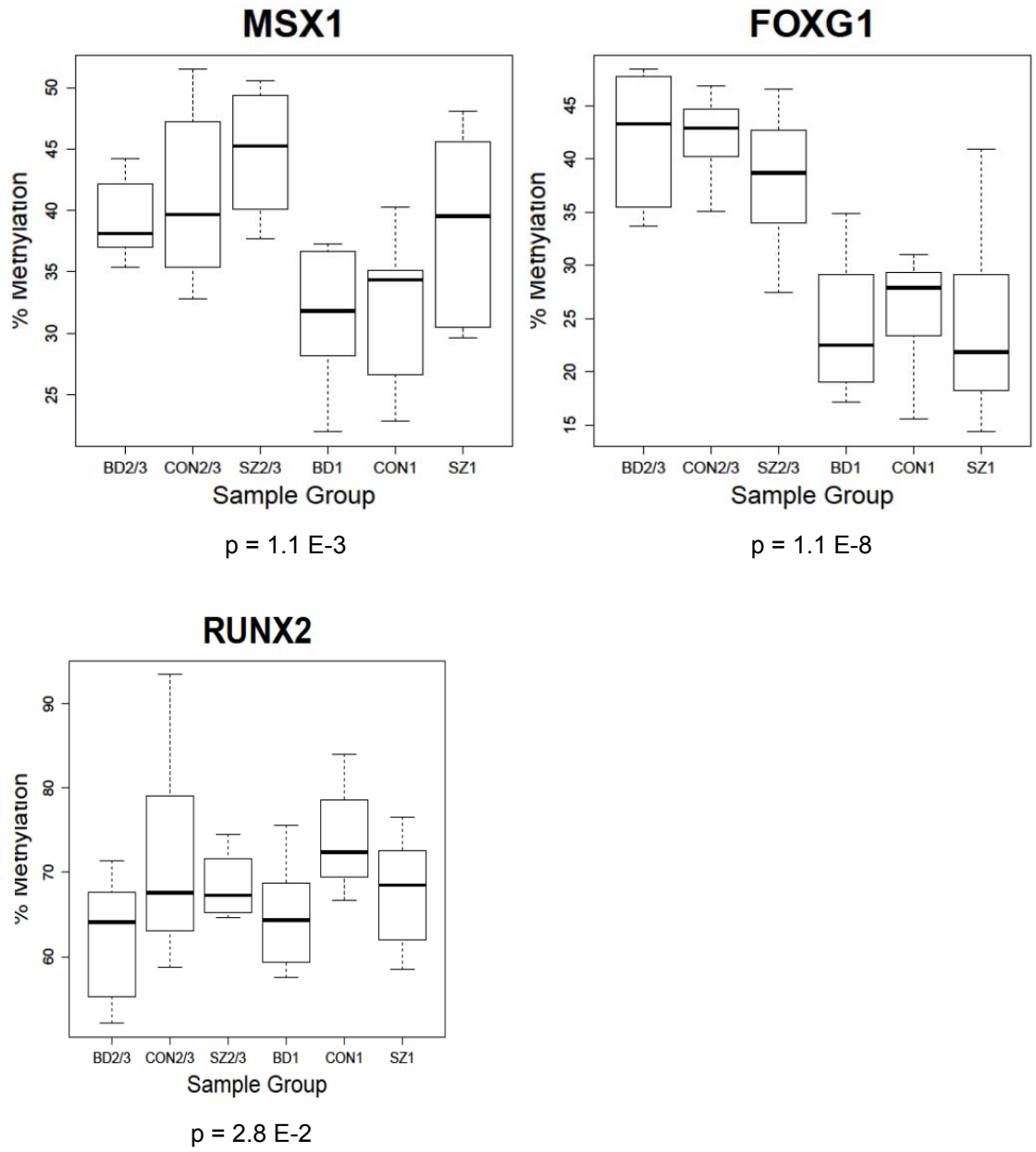
Listed are primer sequences used for replication analysis by bisulfite pyrosequencing. For the FOXG1 and RUNX2 assays the forward primer was used as the sequencing primer. For all three assays PCR conditions were:
95˚C for 15 minutes, (94˚C for 30 seconds, 50˚C for 30 seconds, 72˚C for 30 seconds) for 45 cycles, 72˚C for 10 minutes, 4˚C hold

eFigure 1. Laser microdissection of stratum oriens from CA2/3 and CA1



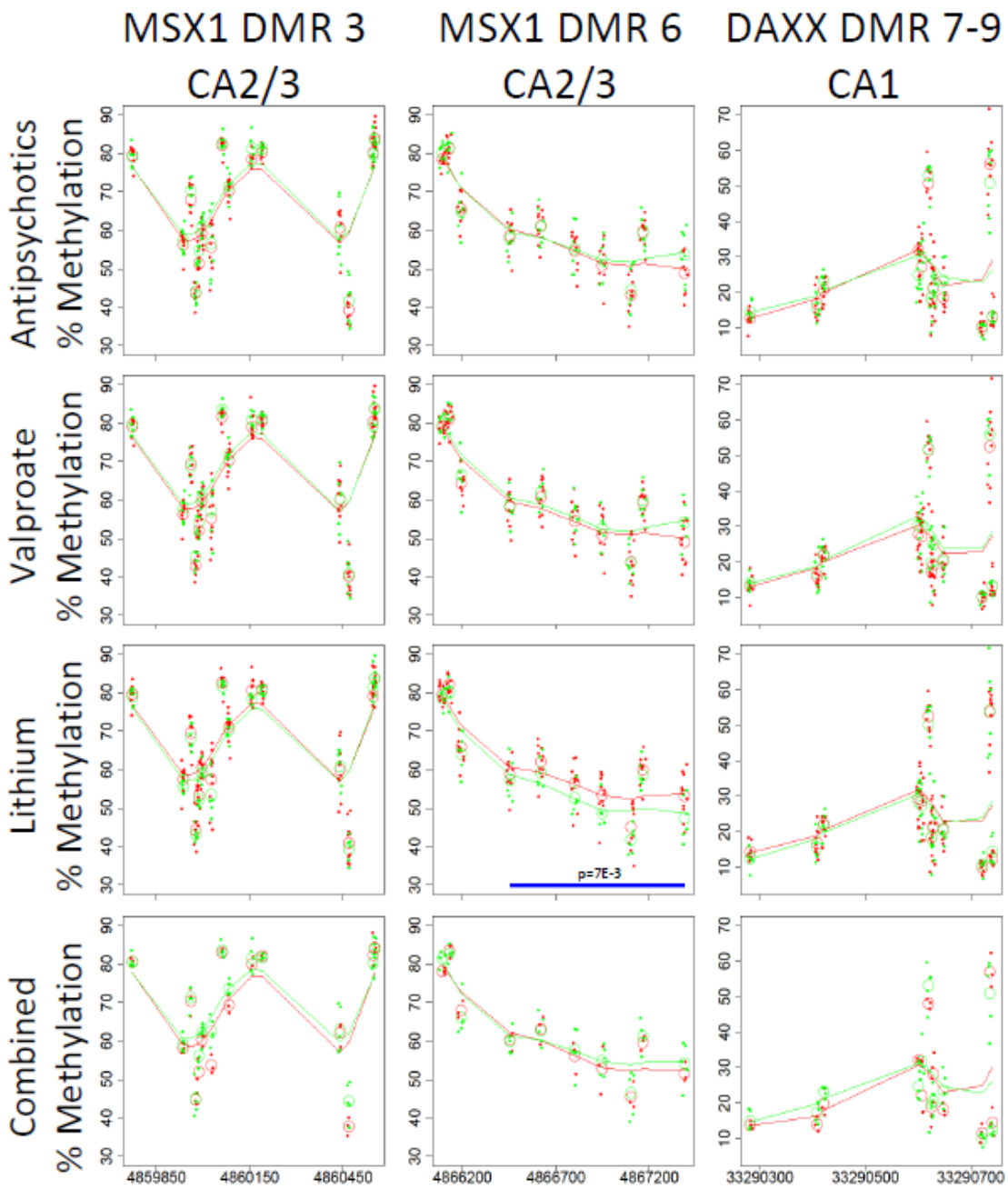
Photomicrographs taken at 4x magnification of a single 30 µm section of postmortem human hippocampus before and after laser microdissection of tissue from stratum oriens of subfield CA1 and subfields CA2/3.

eFigure 2. Bisulfite pyrosequencing replication assay results



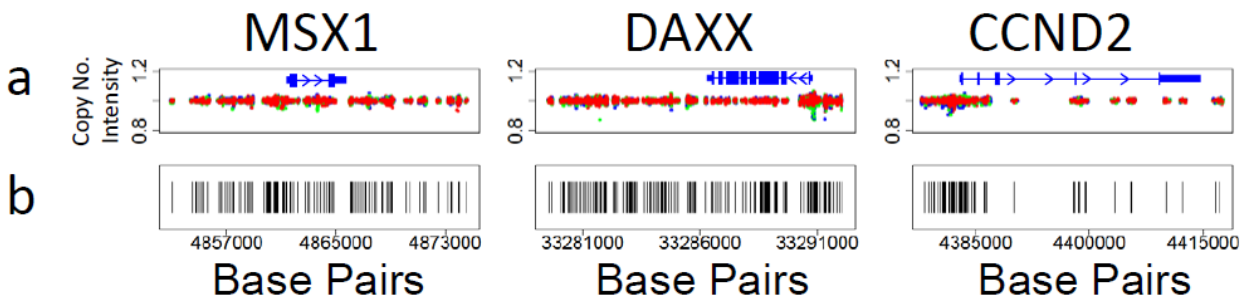
Box plots represent methylation levels measured using pyrosequencing assays with the probes listed in eTable 2.

eFigure 3. Assessment of medication effect at the three most significant differentially methylated regions



Shown are the methylation levels at the three most significant DMRs in the patient cases (SZ and BD together) from single subfields, separated with those cases exposed to the indicated medication in green and cases without that exposure in red. Analogous to figure 2, each column represents a single genomic region, and each row represents a single medication exposure comparison. Open circles represent group averages at each measured site, and the smoothed lines represent running group averages. “Antipsychotics” compares cases exposed to dibenzodiazepine-type antipsychotics to those exposed to no or non-dibenzodiazepine-type antipsychotics. In the final row “Combined” refers to combined medication analysis. As only three SZ cases and no BD cases were not exposed to any of the three assessed medication categories, shown are the SZ cases unexposed to any of the three medication categories (red) and the remainder of the SZ cases demonstrating that this subset does not separate from the rest of the group at these genomic locations.

eFigure 4. Assessment of copy number variation at *MSX1*, *DAXX*, and *CCND2*



A, Copy number intensity for each probe along the length of the gene, calculated as the total intensity of the methylated and unmethylated channels at each probe for each sample divided by that sum averaged across all samples.[27] The proximity of all values to unity indicates that copy number variation is not a driving factor in our results. B, Vertical bars indicate the genomic position of each probe associated with the gene.