INVENTORY OF SUPPLEMENTARY DATA

SUPPLEMENTARY METHODS

DNA samples

The samples used in this study consisted of 215 autism, Rett syndrome and intellectual disability patients from the Hospital Clínic, Sant Joan de Deu and Pompeu Fabra University, Barcelona, Spain. DNA was extracted from peripheral blood leukocytes using standard techniques. We measured DNA concentration with the Quant-iT Picogreen (Invitrogen) and then normalized all concentrations to 25-50 ng/µl before proceeding with the Access Array amplification.

Primer design

48 pairs of primers were designed using the Access Array Amplicon Tagging Assay design service from Fluidigm to cover all the 26 exons of the *JMJD1C* gene (NM_032776.1), including exon-intron boundaries. These primers generated 48 fragments varying in size from 360-489 bp. Amplicons were designed of approximately the same length to obtain an optimal sequencing result on the Junior 454. We also used the 96 Access Array Barcode Library (Fluidigm) to identify all the sequences in a pool of samples. This also contained the sequencing adaptors necessary for subsequent sequencing in the 454 GS Junior Sequencer.

Fluidigm access array

The Fluidigm Access Array is a microfluidic chip on which 48 patient samples and 48 primer pairs can be loaded. The outcome is a pool of 48 fragments per patient sample. By incorporating a unique identifier or barcode for each sample and the necessary sequencing adaptors, it is possible to pool the samples on the sequencing platform. Three access arrays were used to amplify the DNA samples in this study. For each experiment, 25-50 ng DNA per sample was used as input for the system. Experiments were performed according to the manufacturer's 4-Primer Amplicon Tagging protocol. Briefly, the target-specific primers were injected into the primer inlets and the sample-specific primers with their unique MID were loaded into the sample inlets along with the DNA samples and the PCR reagents. The primers and DNA mixture were then combined in the reaction chambers in the chip. After PCR, 10 µl of the samples were collected from their original wells, now containing a pool of 48 amplicons.

Verification and quantitation of harvested pcr products

Before running the samples on the GS Junior 454, we verified the amplification of the fragments using an Agilent 2100 BioAnalyzer with DNA 1000 chips, following the manufacturer's instructions. We ran 1 µl from all the amplified samples to ensure that the amplicon size and distribution were within the expected range. We also checked that primer dimer contamination was less than 25%. In addition, we obtained a concentration value used to ensure equimolar pools of amplified samples. 11 pools were obtained, which were sequenced on a 454 Titanium PicoTiterPlate device before purifying the pooled samples with Agencourt AMPure XP system (Beckman Coulter Genomics), following the manufacturer's instructions. This consists of magnetic beads that allow a high level of recovery of amplicons, efficient removal of unincorporated dNTPs, primers, primer dimers and salts.

Multiplex ligation-dependent probe amplification (mlpa)

All samples had been screened for large rearrangements in the *JMJD1C*. We designed 9 MLPA probes specific to the *JMJD1C* gene and 6 control probes according to the instructions provided by MRC-Holland (www.mrc-holland.com/pages/support_desing_synthetic_probespag.html). Probes are

available from the authors on request. Unique sequences were identified using the BLAT program from UCSC (www.genome.ucsc.edu), and care was taken to avoid the presence of known sequence variants in the probe annealing site. Probes were designed to produce PCR products differing by 3 bp to allow correct separation by size. Oligonucleotides were obtained from Sigma–Aldrich (Haverhill, UK). The signal of each probe was adjusted after visual examination of preliminary results by raising or lowering the concentration in the probe mix. PCR products were analyzed on an ABI 3100 capillary sequencer using Gene Mapper software (Applied Biosystems, Foster City, CA, USA). The proportion of each peak relative to the height of all peaks was calculated for each sample and then compared with the proportions of the corresponding peak averaged over a set of at least ten normal DNA samples. Ratios between 0.8-1.2 were considered to have a normal copy number (2n).

Sanger sequencing

The variants were validated by Sanger sequencing using a *BigDye*® Terminator v3.1 Cycle Sequencing Kit in an Applied Biosystems 3730/DNA Analyzer. The raw data were analyzed with Codon Code Aligner Software.

Exome sequencing

The patient and healthy parents were analyzed by whole exome sequencing with TruSeq Sample Preparation Kit (Illumina). Exomes were captured with TruSeq Exome Enrichment Kit (Illumina) and paired-end 100x2 sequenced with the equip HiScan SQ. The raw data were analyzed in Centre Nacional d'Anàlisi Genòmica (CNAG), in Barcelona, Catalonia, Spain. FASTQ files were analyzed as is it follows:

1.Alignment and variant calling. Sequence reads were aligned to the human reference genome build GRCh37 (hg19) by using the Burrows-Wheeler Aligner (BWA) (Li & Durbin, 2009). Properly mapped reads were filtered with SAMtools [Li, 2009], which was also used for sorting and indexing mapping files. GATK [McKenna, 2012] was used to realign the reads around known indels and for base quality score recalibration. Once a satisfactory alignment was achieved, identification of single nucleotide variants and indels was performed using GATK standard multisample variant calling protocol, including variant

recalibration (DePristo et al 2011). For the final exome sequencing analysis report we used the ANNOVAR [Wang et al 2010] annotation tool to provide additional variant information to ease the final selection of candidates. In particular, minor allele frequency (MAF), obtained from dbSNP (Sherry 2001) and 1000 Genomes project was provided to help to select previously undescribed variants in healthy population.

2.SNV. To identified de novo single nucleotide variations, the patient´s variant were filtered first for the parental variants and then for the variants of a pool of controls made up by all healthy parents included in the study. Also SIFT (Kumar 2009) and Polyphen (Adzhubei 2010) damage scores were computed to predict putative impact over protein structures. The successive application of quality control filters and the prioritization by the parameters with potential functional impact was used to construct a list of candidate genes (and variants) ranked by its uniqueness in the cases (or very low frequency in the control population, as derived from the MAFs) and the putative potential impact. The variants were validated by sanger sequencing using BigDye® Terminator v3.1 Cycle Sequencing Kit in a Applied Biosystems 3730/DNA Analyzer

3.CNV. To identify Copy Number Variation, we used the C++ software XHMM (eXome-Hidden Markov Model) (Poultney 2013). The CNV events were filtered by DGV Data Base to remove common CNV and validated by Quantitative PCR using for normalization Type-it CNV KIT primers from QIAGEN and 2 multicopy amplicons with similar results.

Cell culture and vectors

JMJD1C coding sequence in pCMV6-AC-GFP vector was purchased from Origene (RG214878). The mutants were generated with Mutant QuikChange™ Site-Directed Mutagenesis Kit. WT and Pro163Leu, His2336Ala mutant was subcloned in pCMV6-Entry vector to introduce Myc-DDK-tag. shRNAs against the coding sequence of mouse Jmjd1c gene were cloned in pLVX-shRNA2 vector between the BamHI and EcoRI restriction sites (shRNA24 target: CAGAGACTGCTTGAGGAAT). Hek293 cells were cultivated in DMEM 10% FBS. To generate stable WT or mutant clones, Hek293 cells were transfected with Lipofectamine 2000 (Invitrogen), selected with G418 antibiotic, and individual clones were isolated 2 weeks later. For transient expression, 6 mg of

vector were transfected in 35 mm 6-well plates with jetPRIME™ transfection reagent following the manufacturer's instructions. Primary cultures of hippocampal neurons were prepared from neonate mice (P0). Brains were dissected out with forceps on ice and placed in petri dishes containing ice-cold Hibernate® media, meninges were removed under a dissecting microscope and hippocampuses were removed and saved in Hibernate. The tissues were transferred to 15-ml tubes containing trypsin-EDTA (0.025% in PBS). The tubes were incubated in a 37°C chamber for 15 minutes and agitated every 5 minutes. After stopping trypsinization with 5 ml 20% FBS, these tissues were triturated 15 times with a Pasteur pipette. The suspension was filtered through a 75 µm pore-sized filter and centrifuged in a tube for 2 minutes at 1000 *g*. The cells were resuspended in DMEM 10% FSB, Glutamax-Pyruvate and seeded at $2x10^6$ cell/cm^2. Three hours later, the medium was replaced with Neurobasal medium (GIBCO) containing 2% B27, 0.5 mM glutamine, and 50 U/ml penicillin/streptomycin, AraC 5 □M and cultured at 37°C, 5% CO_2. Cultures were infected at 3DIV with lentiviral vectors to express scramble or shRNAs against JMJD1C together with a GFP tracer (pLVX-shRNA2 system). Coverslips were fixed and protein was extracted at 15DIV.

Cellular fractionation

Cell were harvested by scratching, washed in PBS buffer, and incubated 5 minutes in RBS buffer (10mM Tris HCl pH7.6, 10mM NaCl, 1.5mM MgCl2, 0.1% NP40) and centrifuge 1 minute at max speed. Supernatant was considerate cytoplasm fraction. The pellet was washed in once in RBS buffer and then incubated 5 minutes with RIPA (50 mM Tris HCl pH 8.0, 150mM NaCl, 1.0% NP-40, 0.5% Sodium Deoxycholate), the supernatant was considerate nuclear fraction, the pellet containing the chromatin fraction was resuspended en RIPA and sonicated to fragment the DNA.

Immunoprecipitation

750µg of chromatin fraction was diluted 10 fold in IP buffer (5mM Tris-HCl pH 7.6, 15mM HEPES pH 8.0, 1mM EDTA, 1mM EGTA, 0.1% SDS, 1% Triton X-100), incubated with 2µg of antibodies anti-Me-Lysine (Abcam ab23366) overnight at 4°C and 2 hours with PureProteome Protein A/G Magnetic Beads.

Beads were washed twice with IP buffer, twice in RBS NP-40 and eluted in laemli buffer in reduction condition at 70°C by 10 minutes. For the MeCP2 immunoprecipitation procedure, anti-JMJD1C and anti-MeCP2 antibodies were coupled to Dynabeads Protein G (Invitrogen). JMJD1C transfected HEK293F cells were transiently transfected with MeCP2-Flag tagged plasmid and the nuclear fraction was obtained by RIPA buffer (10 mM TRIS-Cl pH 8.0, 1 mM EDTA, 0.5 mM EGTA, 1% TRITON x-100, 0.1 % Sodium deoxycholate, 0.1% SDS and 140 mM NaCl) supplemented with protease inhibitors (complete, ROCHE) and hybridized with the antibodies at 4ºC for 2h. 150 mM NaCl RBS buffer was used for washing. Human IgG was used as negative control. Anti-Flag HRP (M2-SIGMA) antibody was used to visualize the binding.

Western blot

Protein extract of Hek293 cells and primary neuronal culture was obtained in RIPA buffer supplemented with cOmplete Protease Inhibitor Cocktails tablet (Roche) and sonicated. Protein concentrations were determined using a DC Protein Assay kit from Bio-RAD. 50 µg of each protein sample were denatured in Laemli buffer 4% β-mercaptoethanol for 10 minutes at 95ºC and separated on a 7.5% or 15% SDS-polyacrylamide gel, then transferred onto a PVDF membrane (Immobilon-P, Millipore) by liquid electroblotting for 90 minutes at 100 V. The membrane was blocked in 5% nonfat dry milk in TBS-0.05% Tween 20. The antibodies and dilutions used are as follows: rabbit anti-JMJD1C 1:2000 (Millipore 09-817), mouse anti-nucleolin 1:1000 (Santa-Cruz SC-8031), rabbit anti-MeCP2 1:5000 (Sigma M9317), rabbit anti-H3 1:10000 (Abcam AB1791); mouse anti-H3 1:4000 (Abcam AB10799), rabbit anti-H3K9Me2 1:4000 (Abcam AB32521), rabbit anti-MDC1 1:5000 (Abcam AB11171). The blots were developed with Luminata™ Crescendo Western HRP Substrate or with the LiCor Odyssey System.

Immunofluorescence

Cells were fixed in 4% PFA-PBS, quenched in 100 mM glycine-PBS, permeabilized with 0.25% Triton X-100, 1% BSA, PBS. The cells were blocked with 0.2% gelatin, 0.25% triton X-100. Antibody dilutions were prepared in 0.25% Triton X-100, 1% BSA, PBS. The dilutions used were: rabbit anti-

JMJD1C 1:200, chicken anti-Map2 1:5000, anti β-tubulin 1:1500 (Abcam AB21058). Nuclei were stained with 2 mg/ml Hoechst 33342. Coverslips were mounted in ProLong® Gold antifade reagent.

Microscopy

Confocal images were captured with a Leica SP5 confocal microscope. For FRAP analysis the cells were maintained at 37ºC in an atmosphere of 5% CO_2. We captured images every 70 μs at 63x, 128x128 resolution, at 1400Hz with bidirectional acquisition. We captured 25 control images at 3% laser transmission before bleaching, then bleached the ROI inside a nucleus 25 times at nominal level of 100% laser transmission. For this experiment, 150 images were captured after bleaching. The raw data were analyzed with FrapAnalyzer Software (http://actinsim.uni.lu/eng/Downloads/FRAPAnalyser).

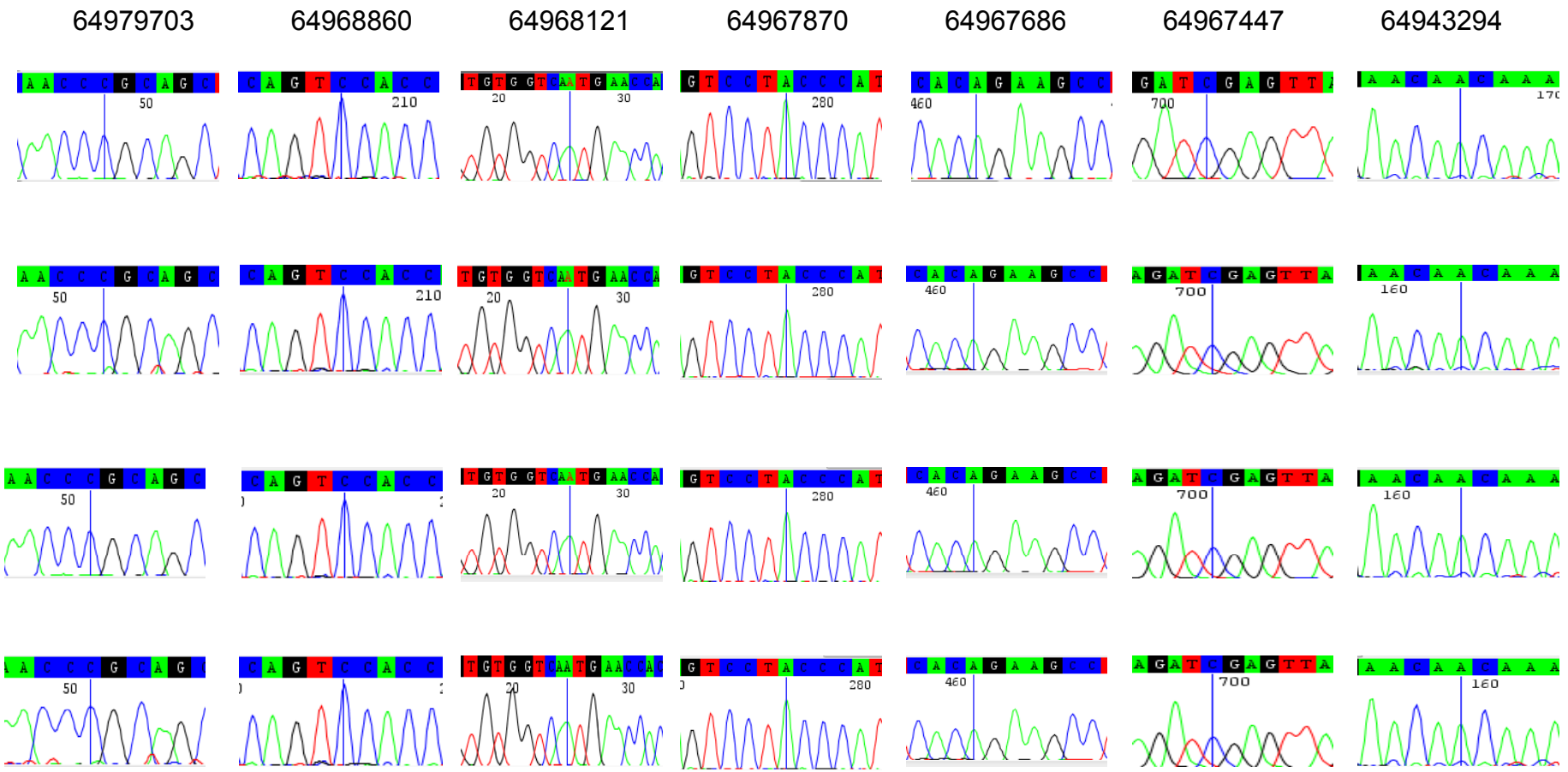
RNA extraction and real-time PCR

Total RNA was extracted from cell lysates using TRIzol Reagent (Invitrogen), purified using the RNeasy Kit (Qiagen) and 2 μg were retrotranscribed using the ThermoScriptTM RT-PCR System (Invitrogen). Real-time PCR reactions were performed in triplicate on an Applied Biosystems 7,900HT Fast Real-Time PCR system using 20 ng cDNA, 5 μl SYBR Green PCR Master Mix (Applied Biosystem) and 150 nM specific primers (sequences are available upon request) in a final volume of 10 μl.

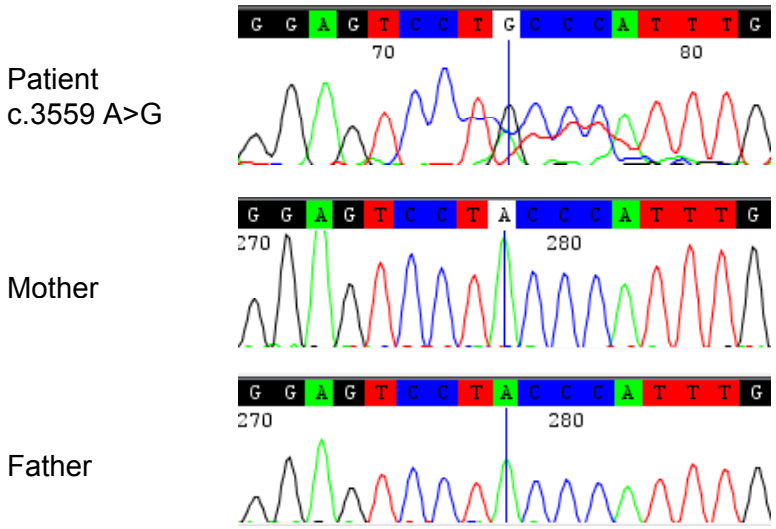
Supplementary Table S1. JMJD1C synonymous variants found in the studied patients					
	Chr 10 position	**Nucleotide Change**	**Exon**	**Amino acid**	**Protein Position**
1	64974871	c. 1056A>G	8	R	352
2	64973860	c. 2067T>A	8	I	689
3	64973890	c.2037T>C	8	H	679
4	64968828	c. 2862T>C	9	H	954
5	64967478	c. 3951T>C	10	S	1317
6	64967445	c. 3984T>A	10	R	1146
7	64966764	c. 4665C>A	10	L	1555
8	64966860	c. 4569C>A	10	I	1523
9	64952834	c.5940G>A	16	P	1980
10	64945364	c. 6789C>T	20	D	2263

Supplementary Table S2. Previously informed JMJD1C variants in studied patients							
	Chr10 position	**Nucleotide Change**	**Exon/ Intron**	**Amino acid change**	**Protein Position**	**Exome Variant Server**	**Patient**
1	64975327	c. 808G>A	Exon 8	V/I	270	1/6019	Intellectual Disability
2	64974224	c. 1703A>T	Exon 8	D/V	568	15/5953	1 Intellectual Disability 1 Autism Spectrum Disorder
3	64973978	c.1949C>T	Exon 8	T/I	650	114/5981	1 Autism Spectrum Disorder 1 Intellectual Disability
4	64967249	c. 4180A>T	Exon 10	T/S	1394	8/6058	1 Intellectual Disability 1 Autism Spectrum Disorder
5	64966621	c. 4808T>C	Exon 10	I/T	1603	2/5954	Intellectual Disability
6	64945336	c. 6817A>G	Exon 20	M/V	2273	1/5918	Autism Spectrum Disorder
7	64948925	c.6570+3G>A	Intron 18	-	-	107/5896	Autism Spectrum Disorder

Supplementary Table S3. JMJD1C mutational status in studied patients									
	Chr 10 position	**Nucleotide Change**	**Exon**	**Amino acid change**	**Protein Position**	**Exome Variant Server**	**Patient**	**Gender**	**Parental Status**
1	64979703	c. 488C>T	4	P/L	163	0/5953	Rett syndrome	Female	Wild Type
2	64968860	c. 2830C>T	9	P/S	944	0/5943	Autism Spectrum Disorder	Male	Not Available
3	64968121	c. 3308A>G	10	N/S	1103	0/5989	Intellectual Disability	Female	Not Available
4	64967870	c. 3559A>G	10	T/A	1187	0/6086	Intellectual Disability	Male	Wild Type
5	64967686	c. 3743A>G	10	Q/R	1248	0/5946	Intellectual Disability	Female	Not Available
6	64967447	c. 3982C>G	10	R/G	1328	0/6000	Autism Spectrum Disorder	Male	Not Available
7	64943294	c. 6997A>G	22	T/A	2333	0/5880	Autism Spectrum Disorder	Male	Not Available



Supplementary Figure S1. Illustrative chromatograms of Sanger sequencing from control population (n=500). Numbers represent chromosome position and nucleotides of interest are marked with anvertical line .



Supplementary Figure S2. Chromatograms of Sanger sequencing showing the *de novo* status of the c.3559 A>G JMJD1C mutation. Parents are wild type for the amino acid.