Supplementary Material

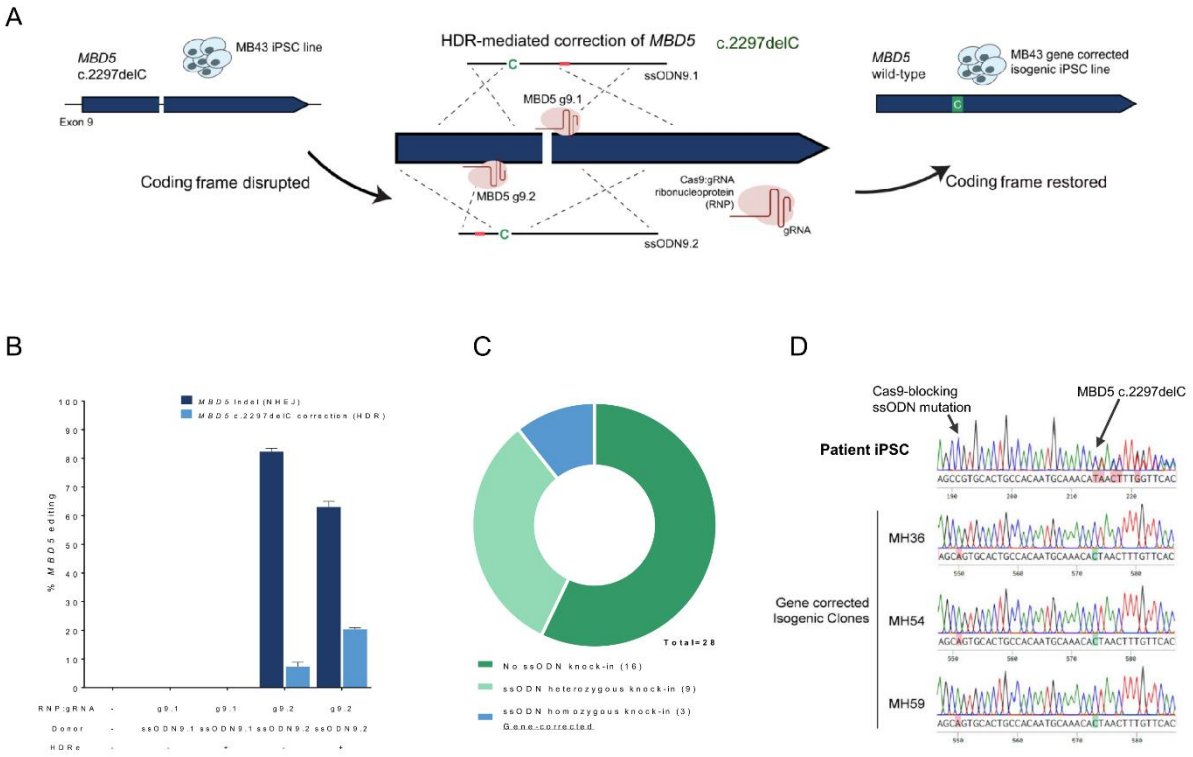

Figure S1 – Correction of *MBD5* c.2297delC in patient-derived induced pluripotent stem cells by CRISPR/Cas9-mediated homology-directed repair (HDR). A) Schematic illustration of the HDR-mediated correction of *MBD5* c.2297delC. B) Estimation of *MBD5* editing by Sanger sequence analysis of genomic DNA from patient-derived iPSCs treated with *MBD5*-targeting RNP and respective ssODN template, in the presence or absence of small-molecule HDR enhancer (HDR). Data represented as mean ± SD (N=2). C) Genotype of *MBD5* editing in iPS cell clones. Single cell-derived clones from the MBD9.2/ssODN9.2 treated population were isolated and genotyped by Sanger sequencing. D) Sequence alignment of *MBD5* gene-corrected isogenic iPS cell clones (MH36, MH54 and MH59) against parental patient-derived iPS cell line. Sequence position of c.2297delC and ssODN Cas9-blocking mutations are illustrated. Red boxes highlight variations against wild-type *MBD5*, while green boxes highlight the correction of c.2297delC in isogenic iPS cell lines.

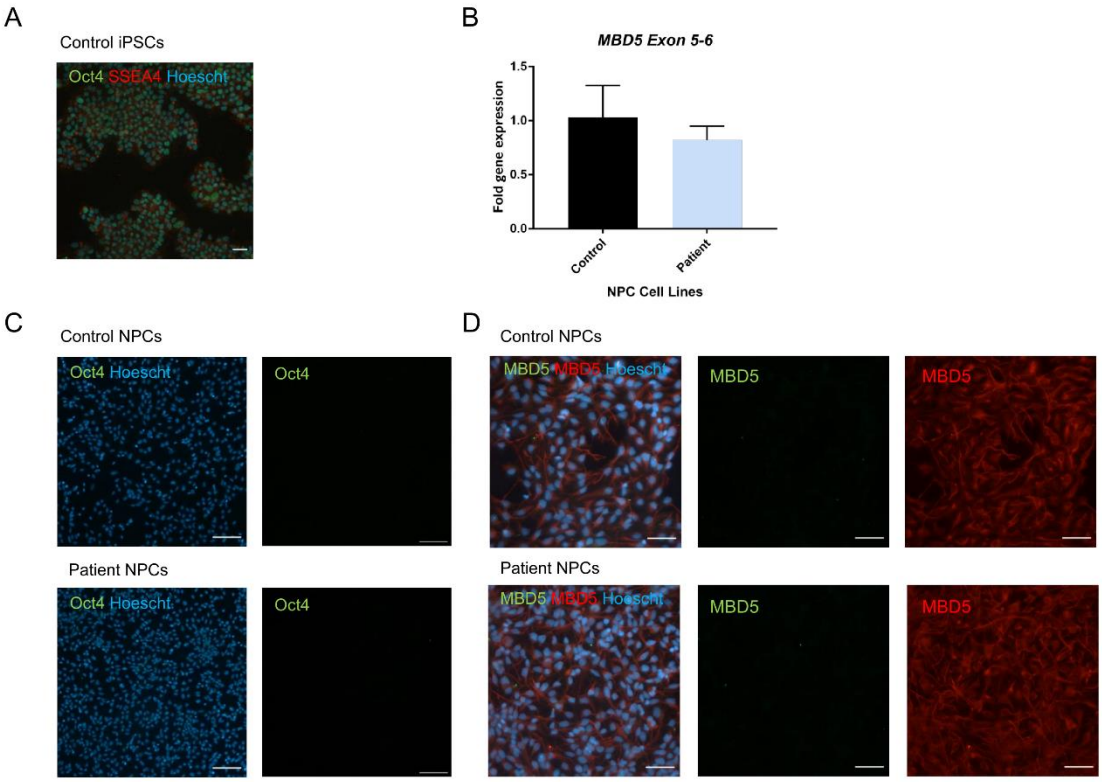

Figure S2 - A) CRISPR-Cas9-edited iPS cells maintain expression of pluripotency markers OCT4 and SSEA4 (objective: 20x, scale bar: 50 µm); B) *MBD5* expression profile of patient-derived and control NPCs, upstream of the deletion site; C) NPCs reveal absence of pluripotency marker OCT4 (objective: 20x, scale bar: 100 µm); D) NPCs do not reveal specific MBD5 staining (green: anti-MBD5 Proteintech, 15961-1-AP; red: anti-MBD5 Sigma-Aldrich WH0055777M1; scale bar: 100 µm).

Table S1 - *MBD5* variant-associated phenotypes reported in the literature.

MBD5 Variant	Sex	Reported Phenotype							Reference
		Developmental Delay	Intellectual Disability	Seizures	Autistic-like behaviors	Dysmorphic features	Behavior Problems	Hypotonia	
Interstitial deletion at 2q21.1-2q23.3	M			X		X			[57]
Interstitial deletion at 2q23.1	F	X	X		X	X	X		[58]
	F	X	X		X	X	X		
Interstitial duplication at 2q23.1	M	X	X		X	X			
Interstitial deletion at 2q23.1	F	X	X		X	X	X		
Nucleotide substitution (c.440C>G)	M	X	X	X	X	X		X	
Interstitial Deletion at 2q22.3-2q23.3	M	X		X		X		X	[59]
Nucleotide substitution (c.397+1G>C)	F	X		X				X	[60]
Interstitial deletion at 2q23.1–23.3	F	X		X		X	X		[61]
Duplication at 2q23.1–2q23.2	F	X	X		X	X	X	X	[62]
Duplication at 2q22.3q23.2	M	X	X		X	X		X	

MBD5 Variant	Sex	Reported Phenotype							Reference
		Developmental Delay	Intellectual Disability	Seizures	Autistic-like behaviors	Dysmorphic features	Behavior Problems	Hypotonia	
Nucleotide substitution (c.1327G>A)	M				X				[63]
Nucleotide substitution (c.3740T>C)	F	X			X				
Nucleotide substitution (c.3806A>G)	F	X	X	X	X		X		
	M	X	X		X		X		
	M	X			X		X		
Nucleotide substitution (c.3896G>A)	M				X				
Deletion at 2q23.1q23.2	F	X		X		X	X		[64]
Deletion at 2q23.1	F	X		X	X	X	X	X	[65]
Deletion at 2q23.1	F		X	X		X			[66]
Nucleotide substitution (c.3595G>A)	F	X		X	X	X			[67]
Deletion in *MBD5* (c.340_347del.)	M	X	X	X				X	[68]
Frameshift variant (c.254_255delGA)	F	X	X	X	X	X	X		[69]
	M			X					

MBD5 Variant	Sex	Reported Phenotype							Reference
		Developmental Delay	Intellectual Disability	Seizures	Autistic-like behaviors	Dysmorphic features	Behavior Problems	Hypotonia	
Nucleotide substitution (c.-443G>A)	M				X		X		[70]
Nucleotide substitution (c.1862A>T)	F				X				
Nucleotide substitution (c.2550A>G)	M			X					
Nucleotide substitution (c.4045G>A)	M	X					X		
Interstitial deletion at 2q23.1	M	X		X		X	X	X	[71]
Interstitial deletion at 2q22.3-2q23.1	M	X		X	X	X	X		
***MBD5* deletion**	M	X	X	X	X		X		[72]
	F	X	X	X	X				
	M	X	X	X	X				
	M	X	X	X	X				
	F	X	X	X	X				
Nucleotide substitution (c.217-1G>C)	M	X	X	X	X				[73]
Deletion at 2q23.1–2q23.2	M	X	X	X		X			[74]

MBD5 Variant	Sex	Reported Phenotype							Reference
		Developmental Delay	Intellectual Disability	Seizures	Autistic-like behaviors	Dysmorphic features	Behavior Problems	Hypotonia	
Duplication at 2q23.1	F	X		X	X	X	X	X	[75]
Duplication at 2q23.1	M	X		X	X	X	X	X	
Duplication including 2q22.3- 2q23.3	F	X		X	X	X	X	X	
Duplication including 2q22.3- 2q23.2	M	X			X	X	X	X	
Duplication including 2q22.3- 2q23.1	M	X			X	X		X	
Deletion at 2q23.1	M	X		X	X	X	X	X	[76]
Complete deletion of *MBD5*	M	X	X	X			X		[77]
Proximal deletion of *MBD5*	M	X	X	X					
Distal deletion of *MBD5*	M	X	X	X			X		
Proximal deletion of *MBD5*	F	X	X	X			X		
p.Thr157Glnfs*4 truncation	F	X	X	X			X		
Proximal deletion of *MBD5*	F	X	X	X			X		

MBD5 Variant	Sex	Reported Phenotype							Reference
		Developmental Delay	Intellectual Disability	Seizures	Autistic-like behaviors	Dysmorphic features	Behavior Problems	Hypotonia	
Proximal deletion of *MBD5*	M	X	X	X			X		
Proximal deletion of *MBD5*	M	X	X	X			X		
Complete deletion of *MBD5*	M	X	X	X					
Proximal deletion of *MBD5*	F	X	X	X					
Complete deletion of *MBD5*	F	X	X	X			X		
Proximal deletion of *MBD5*	M	X	X	X					
Proximal deletion of *MBD5*	F	X	X	X			X		
Proximal deletion of *MBD5*	M	X	X	X			X		
Proximal deletion of *MBD5*	M	X	X	X			X		
Proximal deletion of *MBD5*	M	X	X	X			X		
Distal duplication	F	X		X					
Distal deletion of *MBD5*	M	X	X	X			X		

MBD5 Variant	Sex	Reported Phenotype							Reference
		Developmental Delay	Intellectual Disability	Seizures	Autistic-like behaviors	Dysmorphic features	Behavior Problems	Hypotonia	
Nucleotide substitution (c.598C>T)	M	X	X	X			X		
Proximal deletion of *MBD5*	M	X	X	X			X		
Complete deletion of *MBD5*	M	X	X	X					
Intragenic duplication	F	X	X	X					
Distal deletion of *MBD5*	M	X	X	X			X		
Deletion at 2q23.1	F	X		X		X		X	[78]
Deletion of *MBD5*	M	X	X	X	X	X			[79]
Deletion leading to a frameshift in *MBD5* (c.2579del.)	M	X	X		X	X			[80]
	M	X	X	X		X			
	F		X						
Reciprocal translocation between chromosomes 2 and 5, deletion at *MBD5*	F	X		X	X	X	X	X	[81]
Deletion at 2q23.1	F	X		X					[82]
Deletion at 2q23.1	M	X		X		X			

MBD5 Variant	Sex	Reported Phenotype							Reference
		Developmental Delay	Intellectual Disability	Seizures	Autistic-like behaviors	Dysmorphic features	Behavior Problems	Hypotonia	
	F	X				X			
	F	X				X			
Deletion at 2q23.1	M		X	X	X				
Deletion at q22.3-q23.1	M	X							
***MBD5*-specific disruptions and 2q23.1 deletions**	Cohort of 65 M and F	X		X	X	X	X	X	[6]
Intronic deletion of MBD5					X				[83]
Deletions at 2q23.1	F	X		X	X	X			[84]
	F	X		X	X	X			
	F	X			X	X			
	M	X		X	X	X	X	X	
	F	X		X	X	X	X		
	M	X		X	X	X			
	M	X		X	X	X			
	M	X			X	X	X	X	

MBD5 Variant	Sex	Reported Phenotype							Reference
		Developmental Delay	Intellectual Disability	Seizures	Autistic-like behaviors	Dysmorphic features	Behavior Problems	Hypotonia	
	F	X		X	X	X		X	
	F	X		X	X	X	X		
	M	X		X					
Frameshift variant (c.2173del.)	F	X	X	X	X		X		[85]
***MBD5* deletion**	M	X		X					[86]
Nucleotide substitution (c.365C>T)	*F*		*X*	*X*		*X*			[87]
Nucleotide substitution (c.1885A>G)	M	X		X					
Deletion at 2q23.1	F	X	X	X		X	X	X	[88]
Deletion of chromosome 2q22.3–q23.3	F	X	X	X		X	X	X	
***MBD5* deletion**	M		X		X				[89]

Table S2 - Primers used for Sanger Sequencing

Name	**Sequence 5' - 3'**	**Tm (°C)**
***MBD5* Ex9-F**	TGCTAAGGAAGCAGGGTCAGG	57.99
***MBD5* Ex9-F2**	TCGAGCAGCACTAAGAGATAAGC	59.17
***MBD5* Ex9-R**	ATCTTCCCAATAGACAAATCCCTAGC	56.15
***MBD5* Ex9-R2**	ACAAACCAGGAAACCCTGCC	57.09

Table S3 - *MBD5*-targeting guide RNA (gRNA) and single-stranded oligodeoxynucleotide (ssODN) sequences used for MBD5 c.2297delC correction.

gRNAs and ssODNs	**Sequences 5' - 3'**
***MBD5* g9.1**	ACAAAGTTATGTTTGCATTG
***MBD5* ssODN9.1**	CTGCTAACCAGCTGCATTTTACAGATCCCAGTATGAACTCTAGT GTTCTTCAGAACATACCTTTAAGAGGGGAAGCCGTGCACTGTC ACAATGCAAACACTAACTTTGTTCACAGTAACAGTCCAGT
***MBD5* g9.2**	GTTTGCATTGTGGCAGTGCA
***MBD5* ssODN9.2**	TTGCCTTGCTCTGCTAACCAGCTGCATTTTACAGATCCCAGTAT GAACTCTAGTGTTCTTCAGAACATACCTTTAAGAGGGGAAGCA GTGCACTGCCACAATGCAAACACTAACTTTGTTCACAGTA

Table S4 - Primers used for genotyping *MBD5* c.2297Cdel correction.

Primers	**Sequences 5' - 3'**	**Tm (°C)**
***MBD5* Ex9-F3**	TTGAACCAGCACAATGCTGC	59.97
***MBD5* Ex9-R**	ATCTTCCCAATAGACAAATCCCTAGC	60.47

Table S5 - Primers used for *MBD5* gene expression assessment.

Name	**FW Sequence 5' - 3'**	**RV Sequence 5' - 3'**	**Product Size (bps)**
MBD5 - Exon 12	TTCAGAACTTTCAGGTG AGAATGC	TTGCAACTCACAAGGTG TATCTTG	140
GAPDH	CATGAGAAGTATGACAA CAGCCT	AGTCCTTCCACGATACC AAAGT	113

Table S6 – NGS panel of sequenced genes.

Genes assessed via array panel
AARS, ACY1, AKT3, ALDH7A1, ALG13, AMT, AP3B2, ARFGEF2, ARMGEF15, ARHGEF9, ARV1, ARX, ATPIA2, ATPGAP2, AUH, BCKDK, BRAT1, BSCL2, CACNA1A, CACNA1, CACNA2D2, CACNB4, CAD, CDK5, CDKL5, CHD2, CHRNA2, CHRNA4, CHRNA7, CHRNB2, CLCN2, CLCN4, CLN3, CLN5, CLN6, CLN8, CNTNAP2, COL4Al, COL4A2, COX15, CPA6, CPT2, CRADD, CSF1R, CSTB, CTSD, CTSF, DARS2, DCX, DENND5A, DEPDC5, DNAJC5, DNM1, DNM1L, DOCK7, DYNC1H1, DYRK1A, EEF1A2, EFHC1, EIF2Bl, EIF2B2, EIF2B3, EIF2B4, EIF2B5, EMX2, EPM2A, ERBB4, ETHE1, FADD, FARS2, FBXL4, FGF12, FGFR1, FLNA, FOLRl, FOXGJ, FRRS1L, GABRAl, GABRBl, GABRB2, GABRB3, GABRD, GABRG2, GAMT, GATM, GCSH, GLDC, GLI2, GNAOl, GOSR2, GRIN1, GRIN2A, GRIN2B, GRIN2D, GUFl, HCNl, HCN2, HEPACAM, HNRNPU, HTR2A, HTRA1, IQSEC2, IRF3, ITPA, KANSL1, KARS, KATNBl, KCNA2, KCNB1, KCNH2, KCNH5, KCNJlO, KCNQ2, KCNQ3, KCNTl, KCNT2, KCTD7, KPNA7, LAMBl, LGI3, LIAS, LIPT2, MAGI2, MBD5. MDH2, MECP2. MFF2C, MFF, MFSD8, MLC1, MTOR, NAXE, NDEl, NDP, NECAPl, NEDD4L, NHLRC1, NOTCH3, NR2F1, NRXNl, NTRK2, PAFAH1B1, PCDH19, PIGA, PIGP, PIGQ, PIGV, PIK3CA, PIK3R2, PLCBl, PNKP, PNPO, POLG, PPP3CA, PPT1, PRICKLE1, PRRT2, PTCHl, PTEN, QARS, RANBP2, RBFOXl, RBFOX3, RELN, RNASET2, ROGDI, RRM2B, SCARB2, SCNlA, SCNlB, SCN2A, SCN3A, SCN5A, SCN8A, SCN9A, SCO2, SERACl, SERPINI1, SHH, SIKl, SIX3, SLC12A5, SLC13A5, SLC19A3, SLC1A2, SLC25A12, SLC25A22, SLC2A1, SLC35A2, SLC6Al, SLC6A8, SlC6A9, SLC9A6, SPTAN1, SRPX2, ST3GAL3, ST3GAL5, STRADA, STXlB, STXBPl, SUCLA2, SYNl, SYNGAPl, SYNE1, SZT2, TANGO2, TBC1D24, TBC1D7, TBCD, TBCE, TCF4, TMTC3, TNK2, TPK1, TPP1, TRAF3, TRAPPC12, TSC1, TSC2, TUBAlA, UBA5, UBE3A, WDR45, WWOX, YWHAG, ZEB2