Supplementary material

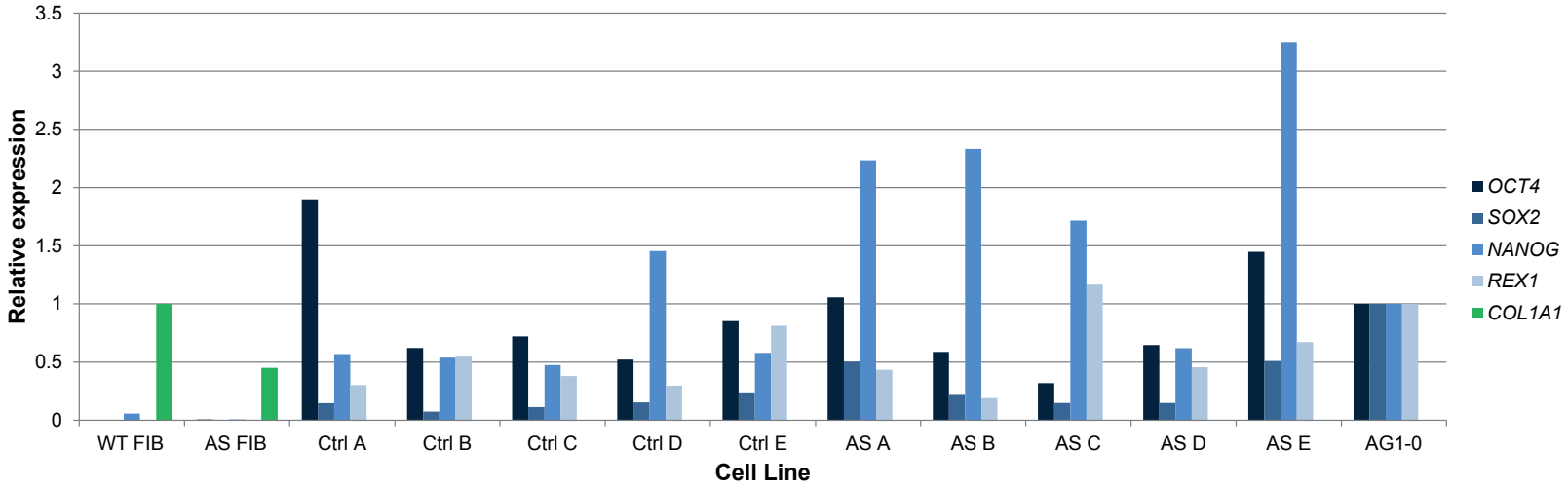
The supplementary material contains:

- Supplementary figures 1-3;
- Supplementary tables 1-4;
- Legends to the supplementary figures 1-3.

A

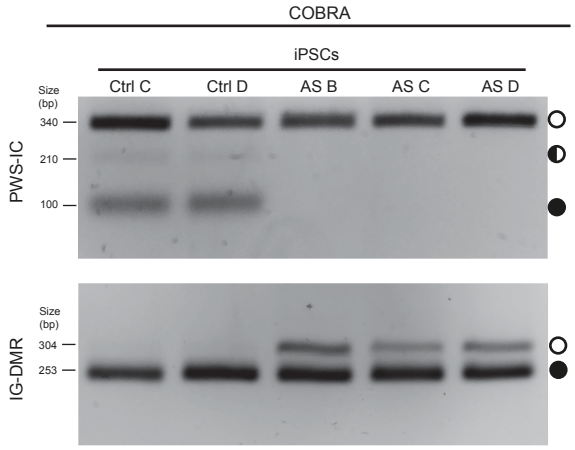
Line	Karyotype
Ctrl A	46 XX
Ctrl B	46 XX
Ctrl C	46 XX, inv(6)/p23q13)
Ctrl D	46 XX
Ctrl E	46 XX
AS A	46 XX
AS B	46 XX (telomeric association between chr 13, 14, 15, 21 and 22 in 55% of the cells)
AS C	n/d
AS D	46 XX
AS E	n/d

B



Suppl. Fig. 1

A

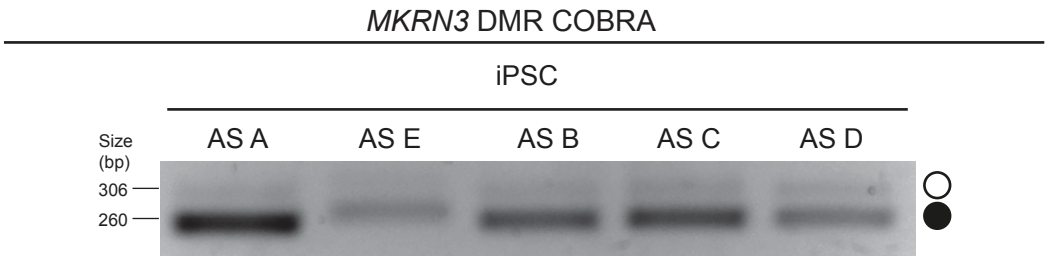


B

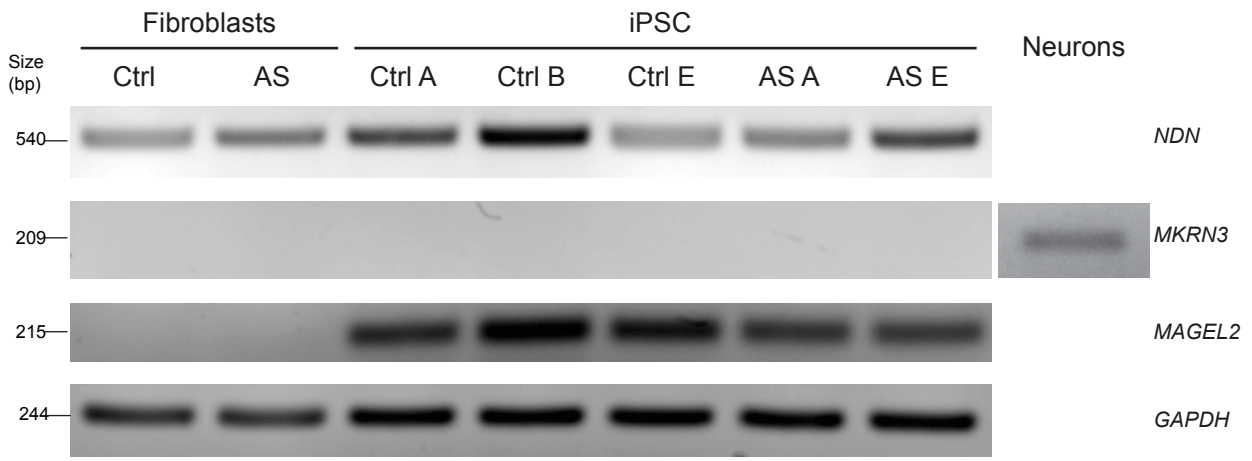
Cell Line	DNA methylation	*SNRPN* allelic status	*SNORD116* allelic status	*UBE3A* allelic status
iPSC Ctrl A	Unmeth/Meth	Monoallelic	n/d	n/d
iPSC Ctrl B	Unmeth/Unmeth	Biallelic	Biallelic	Biallelic
iPSC Ctrl C	Unmeth/Meth	n/d	n/d	n/d
iPSC Ctrl D	Unmeth/Meth	Monoallelic	n/d	n/d
iPSC Ctrl E	Unmeth/Meth	n/d	Monoallelic	Biallelic
iPSC AS A	Unmeth/-	n/d	n/d	n/d
iPSC AS B	Unmeth/-	n/d	n/d	n/d
iPSC AS C	Unmeth/-	n/d	n/d	n/d
iPSC AS D	Unmeth/-	Monoallelic	n/d	n/d
iPSC AS E	Unmeth/-	Monoallelic	Monoallelic	Monoallelic

Suppl. Fig. 2

A



B



Suppl. Fig. 3

SUPPL. TABLE 1. COmbined Bisulfite Restriction Analysis (COBRA) conditions for PWS-IC, IG-DMR, *NDN* and *MKRN3* DMR

DMR	Name	Sequence	PCR conditions	Origin	COBRA expected sizes (in bp)
PWS-IC	SNRPN DMR F1	GGTTTTTTTTTATTGTAATAGTGTTGTGGGG	Nested PCR: 1st PCR: F1/R1: Tm 51ºC 35 cycles; 2nd PCR: 5% of 1st PCR product, F2/R2 Tm 53ºC, 25 cycles; BioTaq enzyme	Rugg-Gunn et al., 2007	Uncut band: 340 Cut bands: 22, 93, 110, 115
	SNRPN DMR R1	GGTTTTAGGGGTTTAGTAGTTTTTTTTTTTTAG			
	SNRPN DMR F2	CTCCAAAACAAAAAACTTTAAAACCCAAA			
	SNRPN DMR R2	CAATACTCCAAATCCTAAAAACTTAAAATATCTA			
IG-DMR	IG DMR F	TTTTATTATTGAATTGGGTTTGTTAGT	Nested PCR: 1st PCR: F/R1: Tm 54ºC 35 cycles; 2nd PCR: 5% of 1st PCR product, F/R2 Tm 54ºC, 25 cycles; BioTaq enzyme	Mo et al., 2015	Uncut band: 304 Cut bands: 33, 51, 253, 271
	IG DMR R1	ACAATTCCTACTACAAAATTTCAACA			
	IGDMR R2	TCCTACTACAAAATTTCAACAAATAC		Customized	
MKRN3 DMR	MKRN3 DMR F	GGAAGGAAAAAGAGATGTATATT	Tm 55ºC, 35 cycles, Kapa Uracil enzyme		Uncut band: 306 Cut bands: 46, 260
	MKRN3 DMR R	ACAAAAAAAAAAACCCAACC			
NDN DMR	NDN DMR F	AAGGTGGAGTGTTTTTTTTA	Tm 55ºC, 35 cycles, Kapa Uracil enzyme		Uncut band: 282 Cut bands: 75, 207
	NDN DMR R	ATAATACAAAAACATCCTCC			

Legend: DMR – Differentially Methylated Region; F – forward primer; R – reverse primer; PCR – Polymerase Chain Reaction; Tm – melting Temperature; bp – base pairs.

SUPPL. TABLE 2. Primers used for expression analysis

Name	Sequence	Origin	Experiments	PCR Conditions (non-quantitative)
UBE3A F	AGCCGGAATCTAGATTTCCA	Ahmad et al., 2012	RT-qPCR	
UBE3A R	TGTCTGTGCCCGTTGTAAACT			
SNRPN F	CTTCTGCCCAGCTTGCAT	Hogart et al., 2007	RT-qPCR and allelic-specific expression analysis (primer R used for sequencing)	Tm 55ºC, 35 cycles, BioTaq enzyme
SNRPN R	TGAAGATTCGGCCATCTTGC			
NDN F	GCCCGAATACGAGTTCTTTT	MacDonald & Wevrick, 1997	RT-PCR and allelic-specific expression analysis (primer F used for sequencing)	Tm 55ºC, 35 cycles, NZYlong polymerase
NDN R	CACACATCATCAGTCCCATA			
MKRN3 F	GAAGCCGAGAGAGACAATGC	Customized	RT-PCR	Tm 60ºC, 35 cycles, NZYlong polymerase
MKRN3 R	CCCCTGGAAGCATAATAGCA			
MAGEL F	AAAGCCAGCACAAAGCTGAT		RT-PCR	Tm 60ºC, 35 cycles, NZYlong polymerase
MAGEL R	TTCCTGGTGTTTGTGTTCCA			
OCT4 F	CTGAGGGCGAAGCAGGAGTC	Jezierski et al., 2010	RT-qPCR	
OCT4 R	CTTGGCAAATTGCTCGAGTT			
NANOG F	GCAGAAGGCCTCAGCACCTA			
NANOG R	AGGTTCCCAGTCGGGTTCA			
SOX2 F	ATGCACCGCTACGACGTGA	Ginis et al., 2004		
SOX2 R	CTTTTGCACCCCTCCCATTT			
REX1 F	CAGATCCTAAACAGCTCGCAGAAT	Takahashi et al., 2007		
REX1 R	GCGTACGCAAATTAAAGTCCAGA			
COL1A1 F	GAGGGCCAAGACGAAGACATC	Hu et al., 2015		
COL1A1 R	CAGATCACGTCATCGCACAAC			
GAPDH F	GTCGTGGAGTCCACTGGCGTC	Huira et al., 2013	RT-PCR and RT-qPCR	Tm 60ºC, 30 cycles, NZYlong polymerase
GAPDH R	TCATGAGTCCTTCCACGATAC			

Legend: DMR – Differentially Methylated Region; F – forward primer; R – reverse primer; PCR – Polymerase Chain Reaction; RT-qPCR – Reverse Transcription, quantitative PCR; RT-PCR – Reverse Transcription PCR; Tm – melting Temperature. BioTaq enzyme from Bioline and NZYlong polymerase from NZYTech.

SUPPL. TABLE 3. Sequence of Stellaris™ RNA FISH oligo probes

Probe #	Probe Sequence	Probe #	Probe Sequence
1	ATGGAGTAGAGGTCTAACCT	25	GGCATGCAACATTGATTTCT
2	TGGACTACACAGGACATGGA	26	AAGTGTTTCTGGTACTTCGG
3	CGATCATCTCTAGCTAGTGA	27	CAGGGCTACTCTGATTTTTA
4	TACCAAATCCTTCTTTTGCT	28	CACACTCGTTGTAACTACCA
5	CATGATGTGTGATTCTGGGT	29	TCACACTGTGCTCTATGAGA
6	CCAACTGTTCCAATATACCA	30	ACCTAGCAACCAGAAATGGT
7	CACAGGTCAGGAAAACCGTG	31	CTCTTTCTCTAAAAGGGGCT
8	TACCTATATTAAGCCCCAAG	32	AAGAAACACCCTGCTTCTTG
9	CGTAAGCAAGCTCAGTTACT	33	AATCATGAGGTTCCTGCTTA
10	TACCTTCAGTGGAAACCTTT	34	TTTGCTTGTTATGTTCTGGG
11	TATGCACACTCCTCACAATT	35	GAATATTTGGTAAGCTGCCT
12	CGTCGTCTTATGTATCTCTG	36	AGGAAGTCCCAAGTTTTCTA
13	TGATACTTCTACTGCCATCA	37	ACAATTCTCTGTTACAGCCA
14	TCTCATGTTCCAGGGAGAAA	38	CATGACTTTTTGCAGACACC
15	CTGAAGTTCCATTTTAGGCA	39	CAAAATCACACACCCCTTTG
16	TTCCTTGGAACCAAGAGCAA	40	GTGAGTTTGCTTATTTGGGA
17	GTTCAAGCTGACAATGGTGT	41	CGCCAAATGCAGGAGATTAC
18	GCCAAGTTACAACATCCATG	42	GGAACCTCTACATCTTTCTG
19	TTTCCTTGATCACTAGGAGT	43	AGTAGTAAGGGCATTTGCTT
20	GGAGGCCTTTTTTATATCAC	44	ACTTGAACCAGTCTTAGCAG
21	ATGGTCTTCACTATGCAAGC	45	AACTTCCAGGAAGGTACGTG
22	ATATCTGTACCACACGTACT	46	CTTAGAGCAATAGCCTTTGG
23	ATATTCATGCTTTATCCACC	47	CCACATACCACGACGAACAA
24	TGGTGGCATCGGGTAGAAAA	48	AGCACAGAGGTTGCTAAGAG

SUPPL. TABLE 4. Antibodies for IF

Name	Dilution	Supplier
α-SOX2	1:50	R&Dsystens (MAB2018)
α-NANOG	1:100	Affymetrix eBioscience (14-5768)

Suppl. Fig. 1 – Karyotype and stem cell marker characterization of all the isogenic Ctrl and AS iPSCs

A. Karyotype status of Ctrl and AS iPSCs.
B. RT-qPCR analyses for the fibroblast marker (*COL1A1*) and the stem cell markers (*OCT4*, *SOX2*, *NANOG* and *REX1*) in only one representative sample for Ctrl and AS FIBs and all the generated isogenic Ctrl and AS iPSCs and the published AG1-0 iPSC line. Relative expression has been normalized to the *GAPDH* housekeeping gene; *COL1A1* RT-qPCR values were normalized to 1 for the Ctrl FIB sample, while *OCT4*, *SOX2*, *NANOG* and *REX1* RT-qPCR values were normalized to 1 for the AG1-0 iPSC sample;

Suppl. Fig. 2 – Characterization of the imprinting status of the chr15q11-q13 region in isogenic Ctrl and AS iPSCs

A. PWS-IC and IG-DMR COBRA for Ctrl C, Ctrl D, AS B, AS C and AS D iPSCs. White circle – unmethylated band; half black circle – partially methylated band; black circle – fully methylated band.
B. Summary table of the imprinting analysis performed on the different isogenic Ctrl and AS iPSC lines.

Suppl. Fig. 3 - Characterization of the imprinting status and expression analysis of genes at the proximal region of the chr15q11-q13 region in iPSCs

A. *MKRN3* DMR COBRA for all the isogenic AS iPSCs. White circle – unmethylated band; black circle – methylated band.

B. RT-PCR expression analysis of the *NDN*, *MKRN3* and *MAGEL2* in Ctrl and AS FIBs, several isogenic iPSCs lines and brain cortex (neurons).