Imprinting fidelity in mouse iPSCs depends on sex of donor cell and medium formulation

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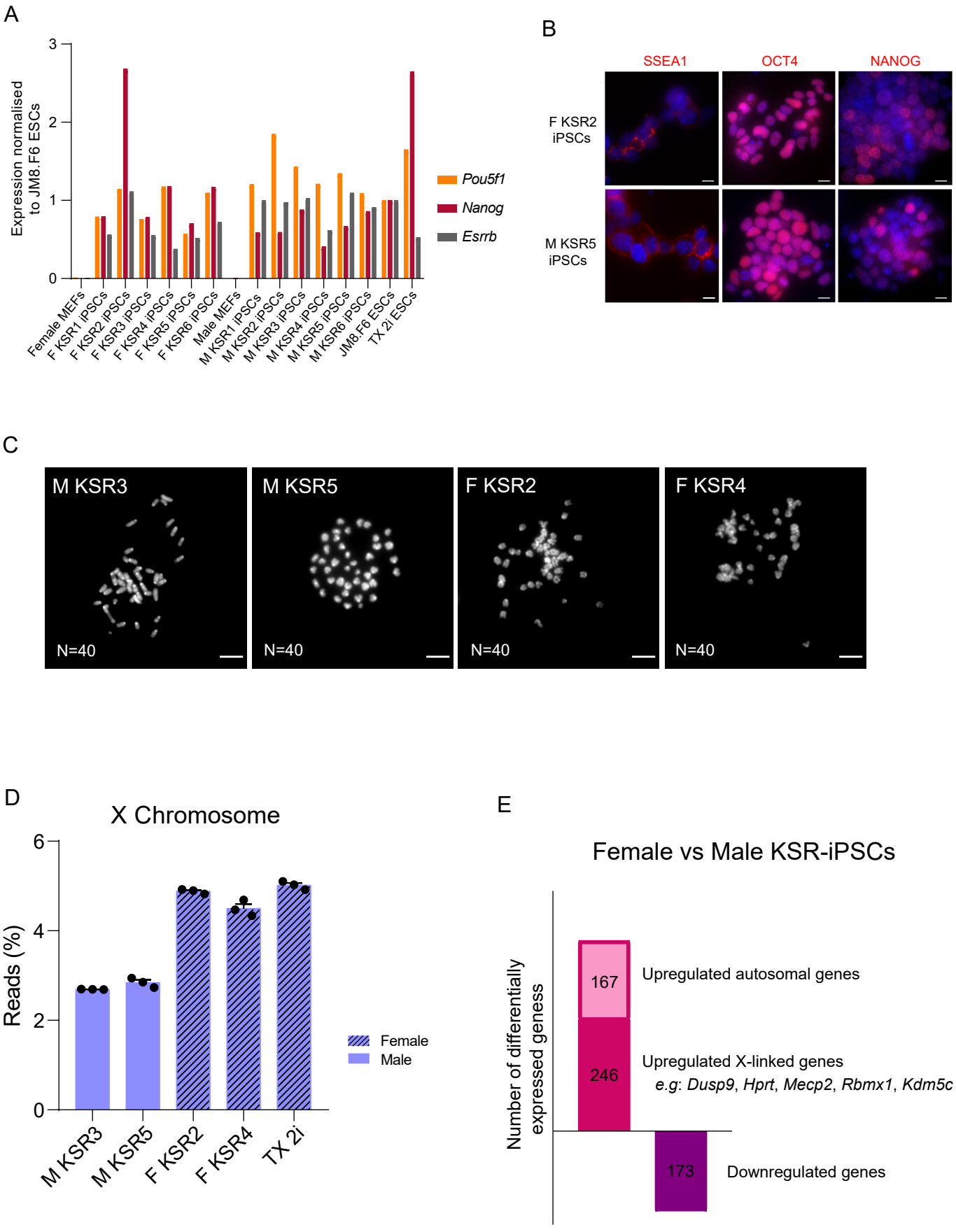
SUMMARY OF SUPPLEMENTARY INFORMATION

Supplementary Fig. 1



Supplementary Figure 1 – Expression analysis of pluripotent markers in F1 hybrid KSR-iPSCs

A. RT-qPCR expression analyses of pluripotent markers (*Pou5f1*, *Nanog* and *Esrrb*) normalised with the *Gapdh* housekeeping gene in female MEFs, female (F KSR1-F KSR6), male (M KSR1-M KSR6) iPSCs and JM8.F6 and TX 2i ESCs; Values for each gene were normalised to the JM8.F6 ESCs; each bar represents data from only one biological replicate. Source data are provided as a Source Data file.

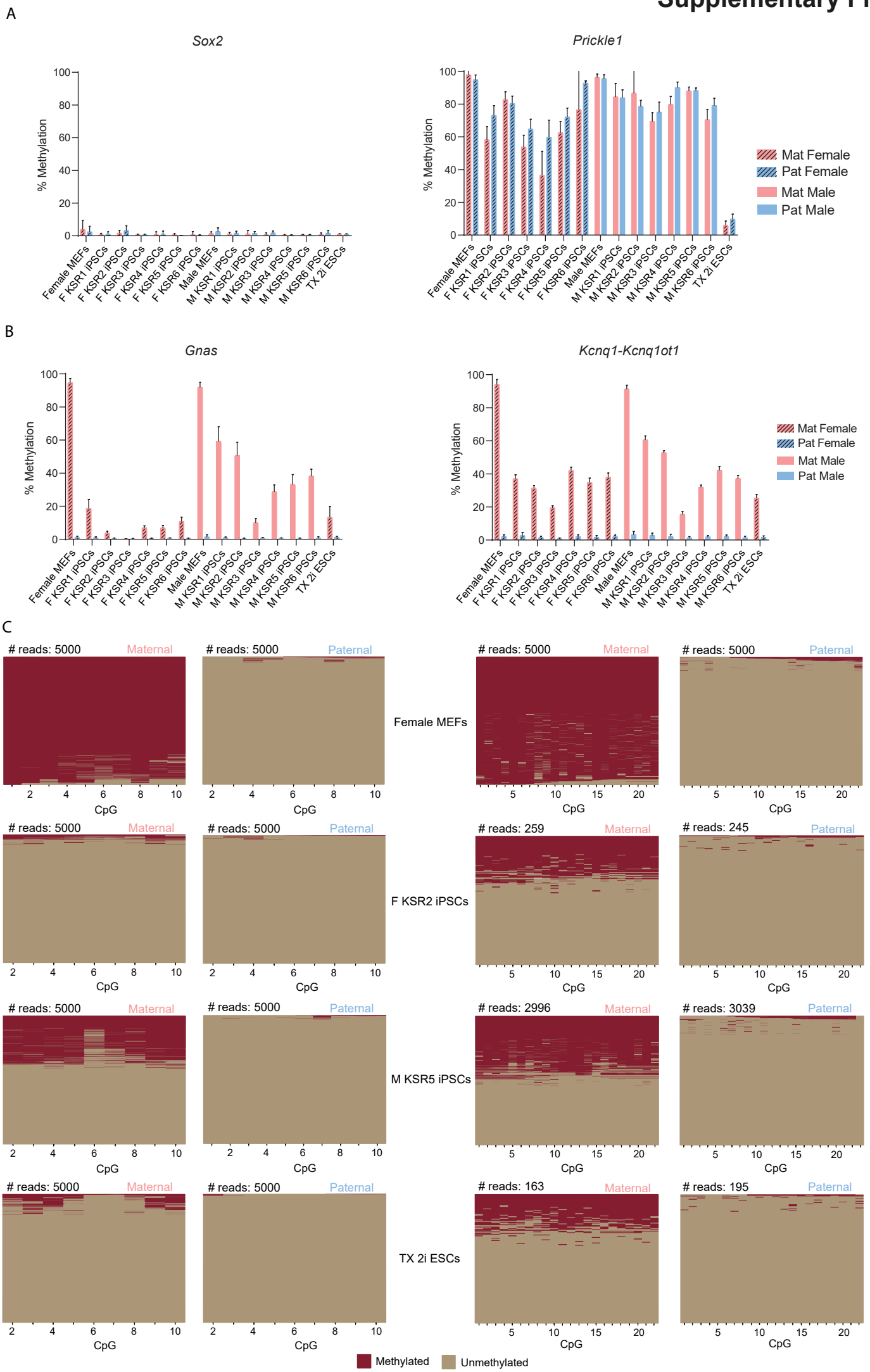
B. Representative immunofluorescence (IF) images for three pluripotent markers (SSEA1, OCT4 and NANOG) in red and nuclei in blue (DAPI staining) in F KSR2 and M KSR5 iPSCs; This experiment was performed once per cell line for each antibody. Scale bars correspond to 10 µm.

C. Representative karyotypes of M KSR3, M KSR5, F KSR2 and F KSR4 iPSC lines with 40 chromosomes each (N=40); Scale bars correspond to 10 µm; A normal karyotype was considered when most of the metaphases counted presented 40 chromosomes (see Methods).

D. Percentage of total RNAseq reads mapping on the X chromosome in biological triplicates of male (M KSR3 and M KSR5), female (F KSR2 and F KSR4) iPSCs and TX 2i ESCs. Graph represents the mean percentage ± SEM of reads mapping on the X chromosome per cell line. Source data are provided as a Source Data file.

E. Differential expression analysis between female and male KSR-iPSCs using both EdgeR and intensity difference filter (*p*-value < 0.05 with multiple testing correction using Benjamini and Hochberg correction for both). Graph represents the number of upregulated and downregulated genes in female versus male KSR-iPSCs. The number of differentially expressed genes from the X chromosome is highlighted. There are no downregulated X-linked genes. Source data are provided as a Supplementary Data 1.

Supplementary Fig. 2



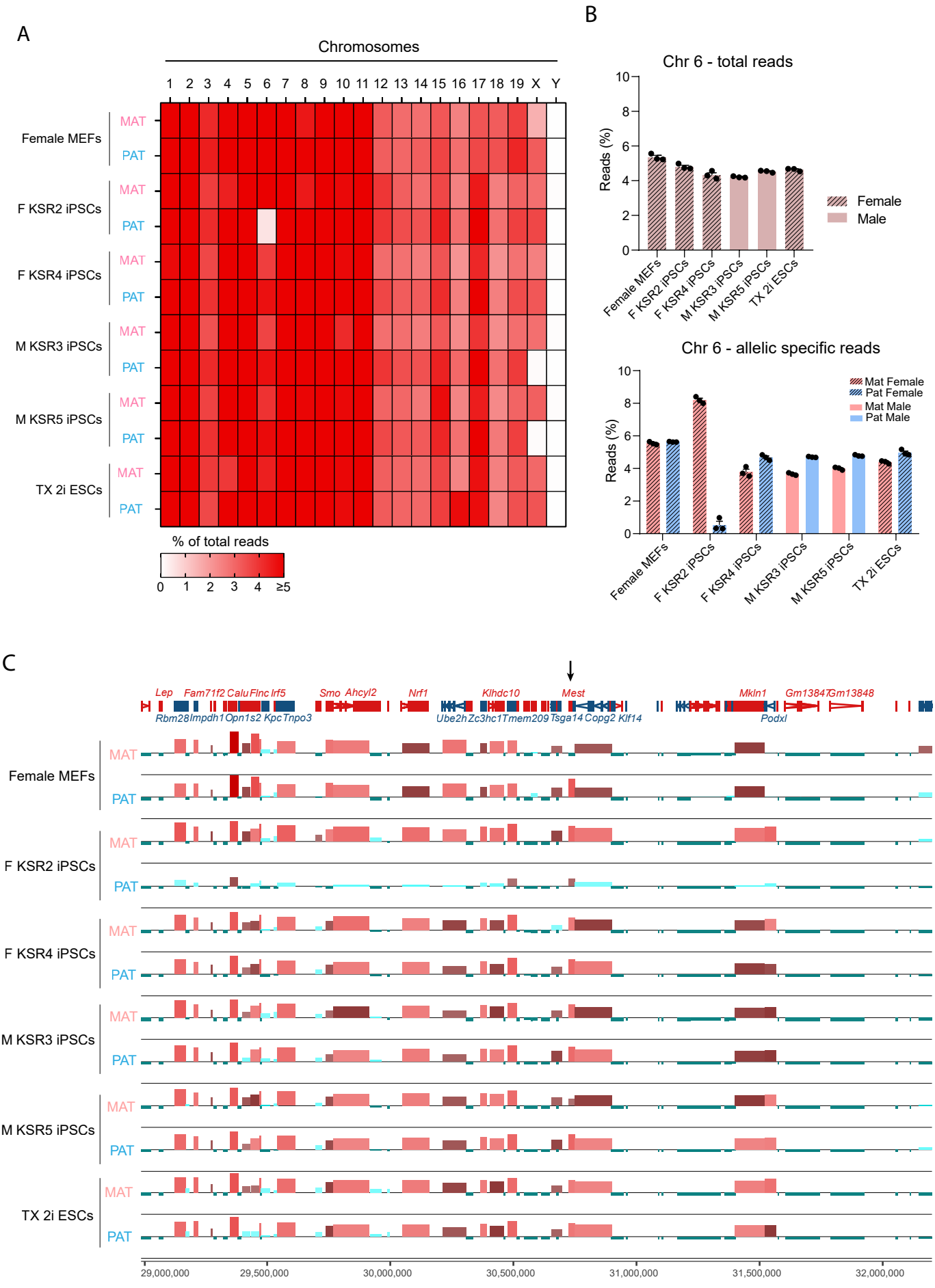
Supplementary Figure 2 – Widespread imprinting methylation defects in KSR-iPSCs

A. Methylation analysis of *Sox2* (unmethylated control) and *Prickle1* (methylated control) in male and female MEFs, female (F KSR1-F KSR6) and male (M KSR1-M KSR6) iPSCs and TX 2i ESCs; Each graph represents the mean ± SD methylation levels measured at each CpG within different genomic regions per parental allele for each sample (number of CpG per locus - *Sox2*: n=14; *Prickle1*: n=10); Source data are provided as Supplementary Data 2.

B. Methylation analysis of *Gnas* and *Kcnq1-Kcnq1ot1* imprinted loci in female and male MEFs, female (F KSR1-F KSR6) and male (M KSR1-M KSR6) iPSCs and TX 2i ESCs; Each graph represents the mean ± SD methylation levels measured at each CpG within different genomic regions per parental allele for each sample (number of CpG per locus - *Gnas*: n=10; *Kcnq1-Kcnq1ot1:* n=23); Source data are provided as Supplementary Data 2.

C. Plots display methylated and unmethylated CpGs for each CpG position (in columns) in all the individual reads (in rows) for both maternal and paternal alleles of *Gnas* and *Kcnq1-Kcnq1ot1* imprinted loci in female MEFs, F KSR2 and M KSR5 iPSCs and TX 2i ESCs.

Supplementary Fig. 3



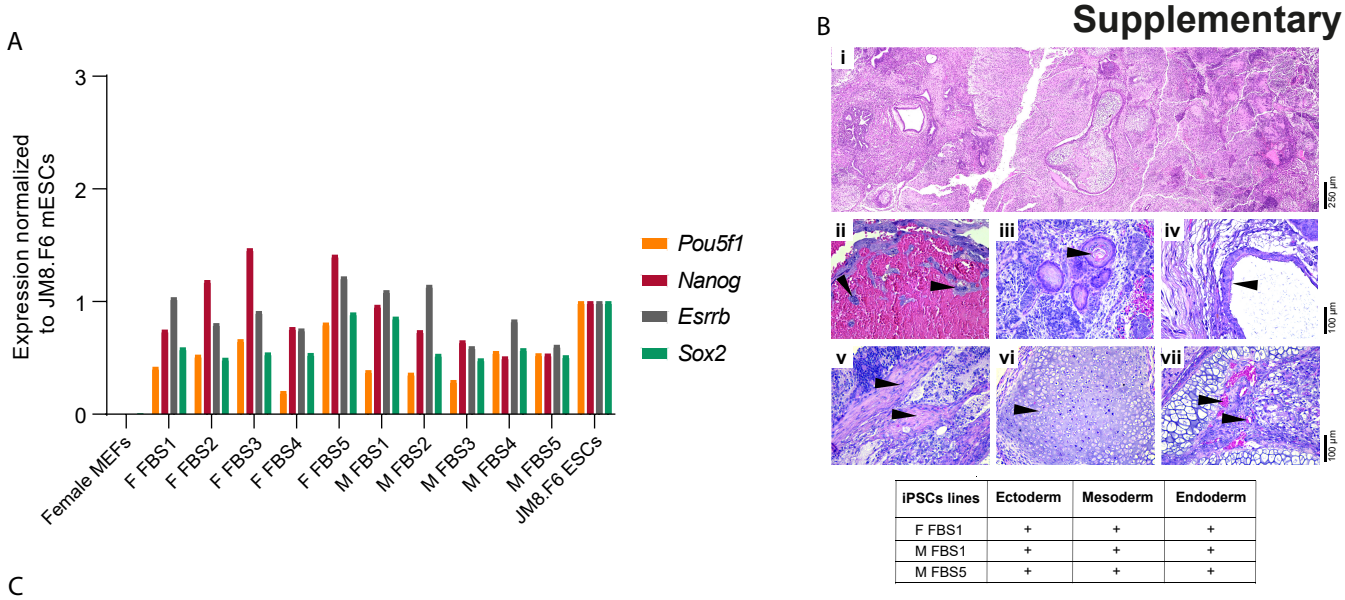
Supplementary Figure 3 - RNAseq data of KSR-iPSCs

A. Heatmap representing the percentage of total reads of each parental chromosome (MAT - maternal; PAT - paternal) in biological triplicates of female MEFs, female (F KSR2 and F KSR4) male (M KSR3 and M KSR5) iPSCs and TX 2i ESCs. Note: absence of Y chromosome in M KSR3 and M KSR5 is due to low number of confident SNPs for this chromosome (1132) compared to others (e.g, X chromosome – 636442 SNPs). Source data are provided as a Source Data file.

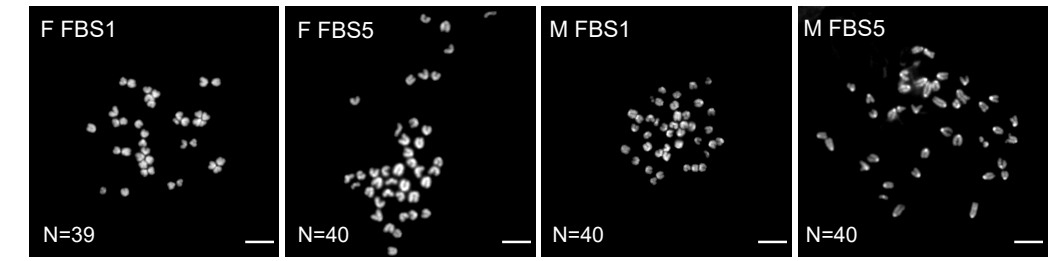
B. Percentage of total RNAseq reads of chromosome 6 (top) and of allele-specific reads distinguishing the two parental chromosomes 6 (bottom) in biological triplicates of female MEFs, female (F KSR2 and F KSR4), male (M KSR3 and M KSR5) iPSCs and TX 2i ESCs. Graphs represent the mean percentage ± SEM of total (top) and allele-specific (bottom) reads mapping on the chromosome 6 per cell line. Source data are provided as a Source Data file.

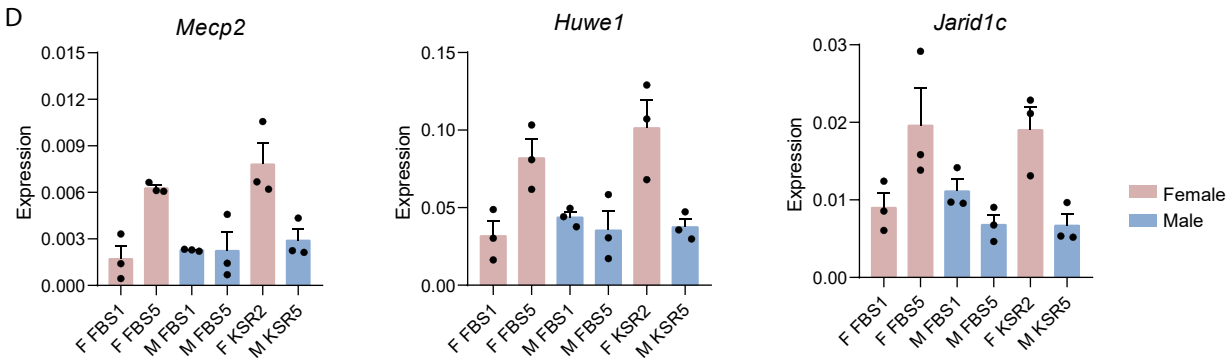
C. Genome browser view of chromosome 6 region containing *Mest* imprinted gene (denoted by black arrow); Height of bars correspond to log2 RPKM values for each gene on either maternal (MAT) or paternal (PAT) inherited allele for biological triplicates of female MEFs, female (F KSR2 and F KSR4) male (M KSR3 and M KSR5) iPSCs and TX 2i ESCs. Selected genes names on sense (red) and antisense (dark blue) are shown.

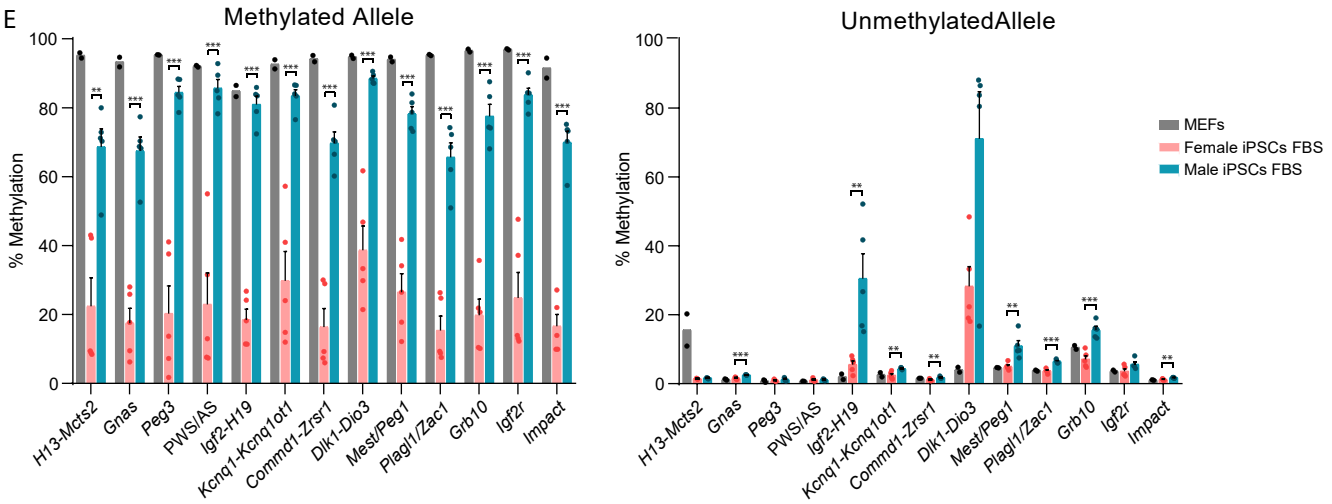
Supplementary Fig. 4

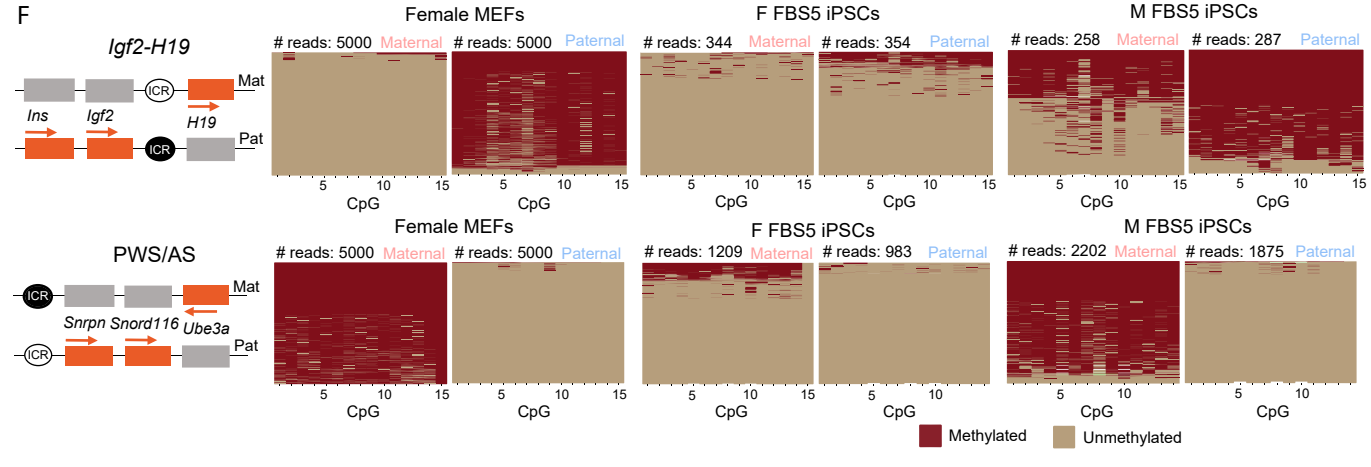


iPSCs lines	Ectoderm	Mesoderm	Endoderm
F FBS1	+	+	+
M FBS1	+	+	+
M FBS5	+	+	+









Supplementary Figure 4 – Generation of F1 hybrid FBS-iPSCs

A. RT-qPCR of pluripotent markers (*Pou5f1*, *Nanog, Esrrb* and *Sox2*) normalised to the *Gapdh* housekeeping gene in female MEFs, female and male FBS-iPSCs and JM8.F6 ESCs; Values are normalised to JM8.F6 ESCs; Each bar represents data from only one biological replicate. Source data are provided as a Source Data file.

B. Table and representative H&E staining of teratomas derived from FBS-iPSCs; **i**, Low magnification of a mature teratoma. **ii**, Trophectoderm-derived trophoblast giant cells, associated with large vascular spaces (black arrowhead). **iii**, Ectodermal components corresponding to squamous epithelium (black arrowhead). **iv**, Endodermal components corresponding to ciliated respiratory epithelium (black arrowhead). **v, vi, vii**, Mesodermal components (black arrowhead) corresponding to fibrous tissue, cartilage, and blood vessels; Table summarises the successful generation of trilineage teratomas from F FBS1, M FBS1 and M FBS5 iPSCs. Two teratomas per cell line were analysed by H&E staining.

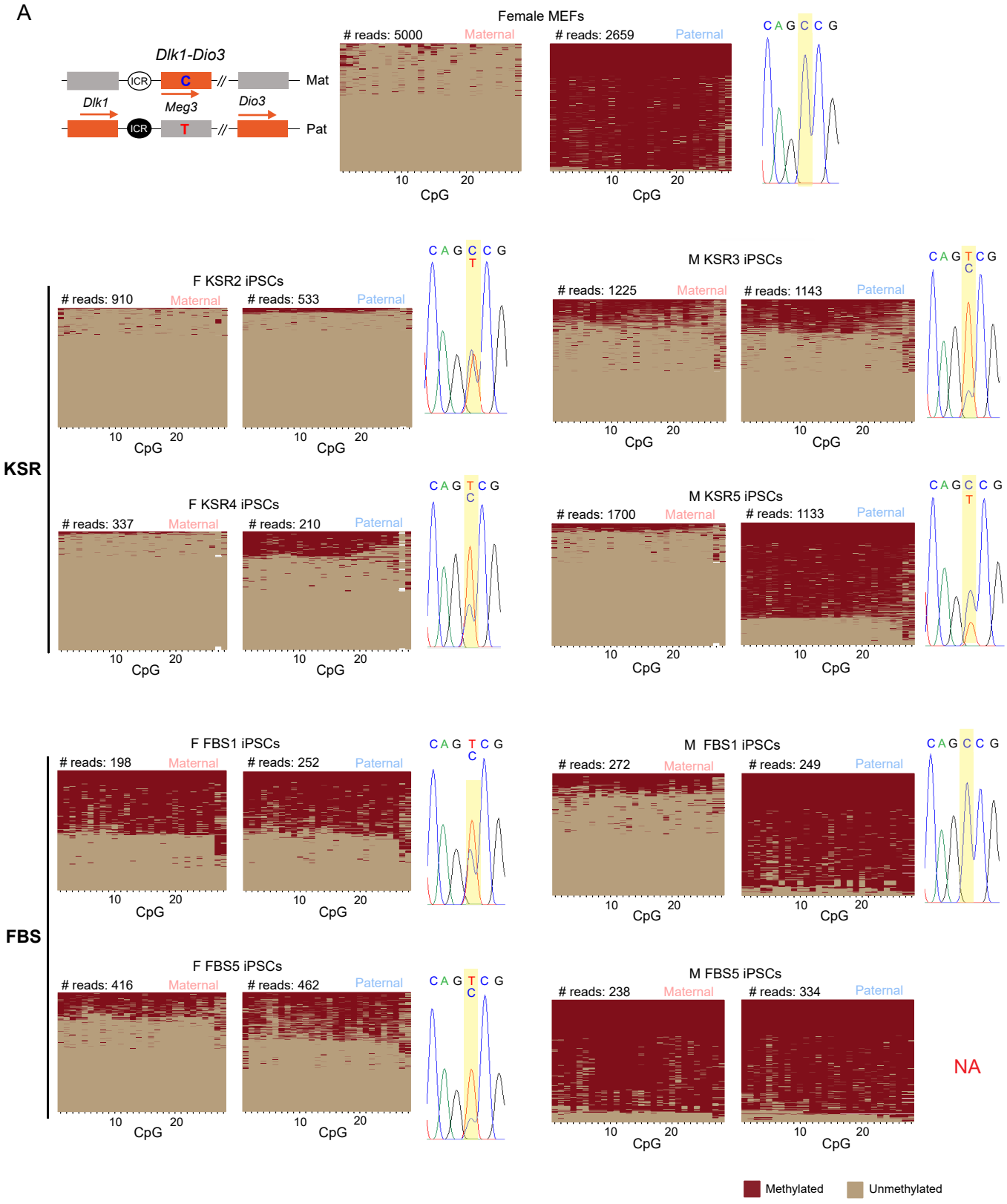
C. Representative karyotypes of F FBS1, F FBS5, M FBS1 and M FBS5 iPSC lines with 39 chromosomes for the F FBS1 and 40 for the others. Scale bars correspond to 10 μm; Number of chromosomes considered per each line corresponds to the most frequent pattern present in the metaphases counted (see Methods);

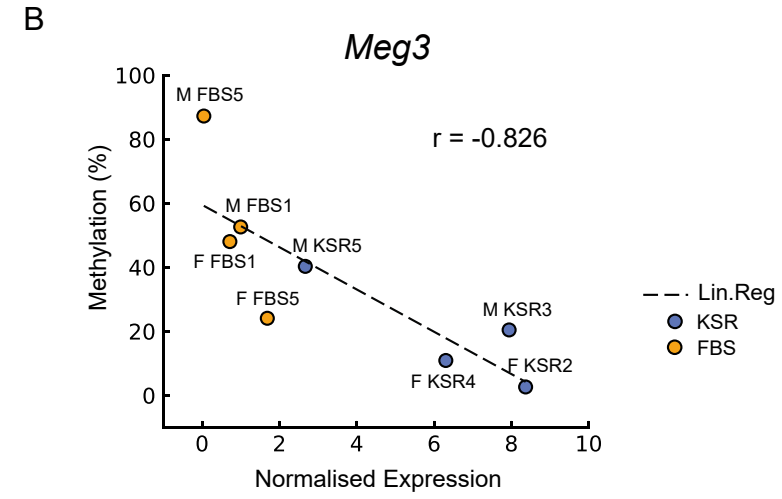
D. Graph represents mean expression ± SEM measured by RT-qPCR of X-linked genes (*Mecp2*, *Huwe1* and *Jarid1c*) normalised to the *Gapdh* gene in F FBS1, F FBS5, M FBS1, M FBS5, F KSR2 and M KSR5 iPSCs (n=3 biological replicates). Source data are provided as a Source Data file.

E. Graph represents the mean percentage of methylation ± SEM at methylated and unmethylated alleles of ICRs in both parental MEFs (note: same data as in Fig. 2B, n=2 independent cell lines), female and male FBS-iPSCs (n=5 independent cell lines each); Statistically significant differences between female and male

FBS-iPSCs are indicated as ** $p < 0.01$; *** $p < 0.001$ (unpaired two-tailed Student's *t*-test). Source data are provided as Supplementary Data 2.

F. Plots displaying methylated and unmethylated CpGs for each CpG position (columns) in all the individual reads (rows) for both maternal and paternal alleles of *Igf2-H19* and PWS/AS loci in female MEFs, F FBS5 and M FBS5 iPSCs; Schemes on the left represent the normal methylation status in *Igf2-H19* and PWS/AS loci (as in Fig. 2A).


A
Dlk1-Dio3
ICR
C
T
Mat
Pat
Dlk1
Meg3
Dio3
Female MEFs
reads: 5000
Maternal
reads: 2659
Paternal
CpG
KSR
F KSR2 iPSCs
reads: 910
reads: 533
M KSR3 iPSCs
reads: 1225
reads: 1143
F KSR4 iPSCs
reads: 337
reads: 210
M KSR5 iPSCs
reads: 1700
reads: 1133
FBS
F FBS1 iPSCs
reads: 198
reads: 252
M FBS1 iPSCs
reads: 272
reads: 249
F FBS5 iPSCs
reads: 416
reads: 462
M FBS5 iPSCs
reads: 238
reads: 334
NA
Methylated
Unmethylated

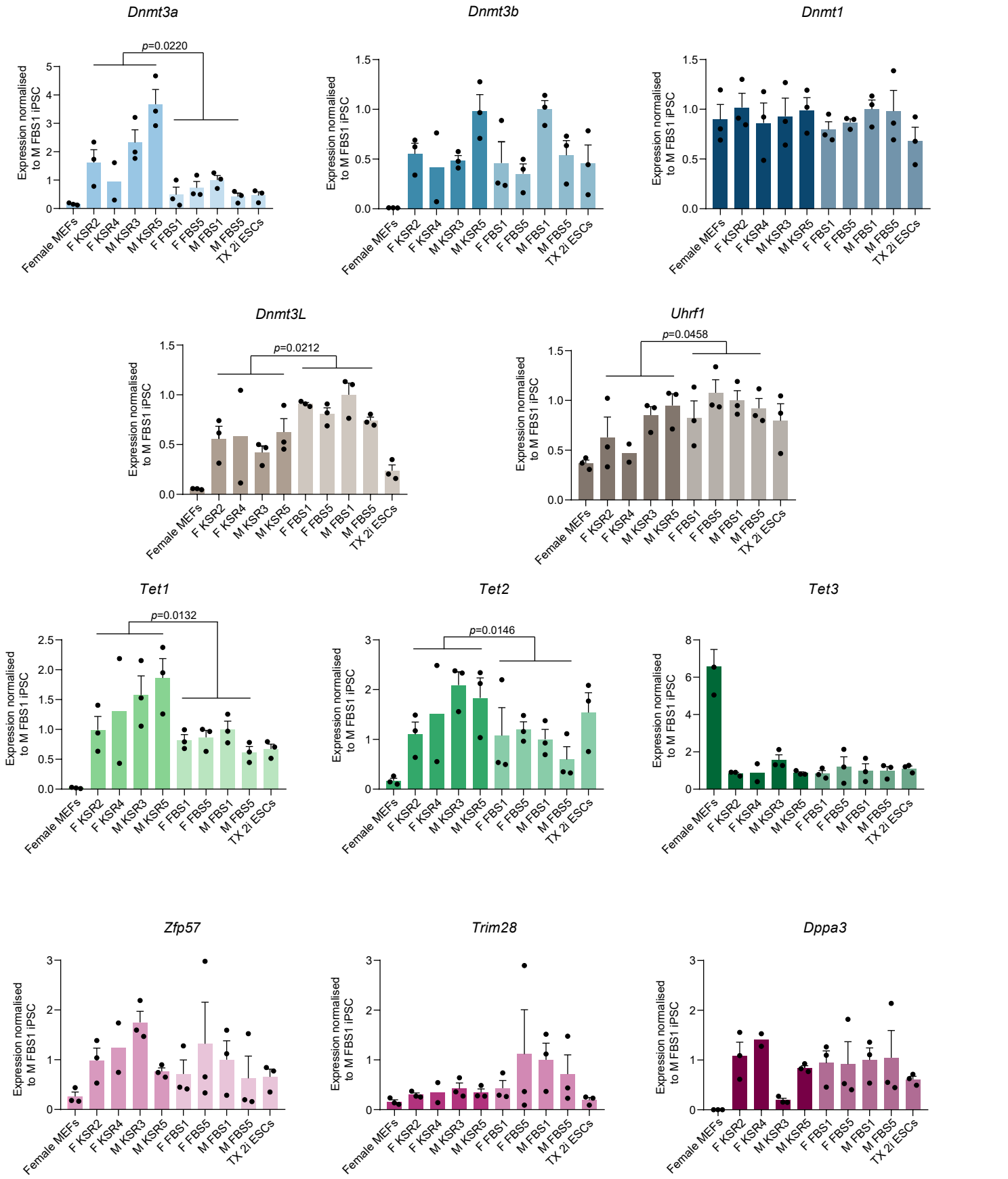

B
Meg3
r = -0.826
Methylation (%)
Normalised Expression
M FBS5
M FBS1
F FBS1
M KSR5
F FBS5
M KSR3
F KSR4
F KSR2
Lin.Reg
KSR
FBS

Supplementary Figure 5 – Imprinting defects in the *Dlk1-Dio3* locus in female and male KSR- and FBS-iPSCs

A. Plots displaying methylated and unmethylated CpGs for each CpG position (in columns) in all the individual reads (in rows) for both maternal and paternal alleles of *Dlk1-Dio3* locus in female MEFs, female and male KSR-iPSCs (F KSR2, F KSR4, M KSR3, M KSR5) and female and male FBS-iPSCs (F FBS1, F FBS5, M FBS1, M FBS5) with the correspondent chromatograms displaying allelic-specific *Meg3* expression assayed by Sanger sequencing on their right. M FBS5 does not express *Meg3*, hence allelic expression was not performed (NA - not applicable); scheme on the left of female MEFs plot represent the normal imprinting status of *Dlk1-Dio3* locus with normal *Meg3* expression and with the associated SNP (in blue) of each allele; white circle – unmethylated ICR; black circle – methylated ICR; Mat – maternal allele; Pat – paternal allele; orange rectangle – expressed gene; grey rectangle –silenced gene; region is not drawn to scale.

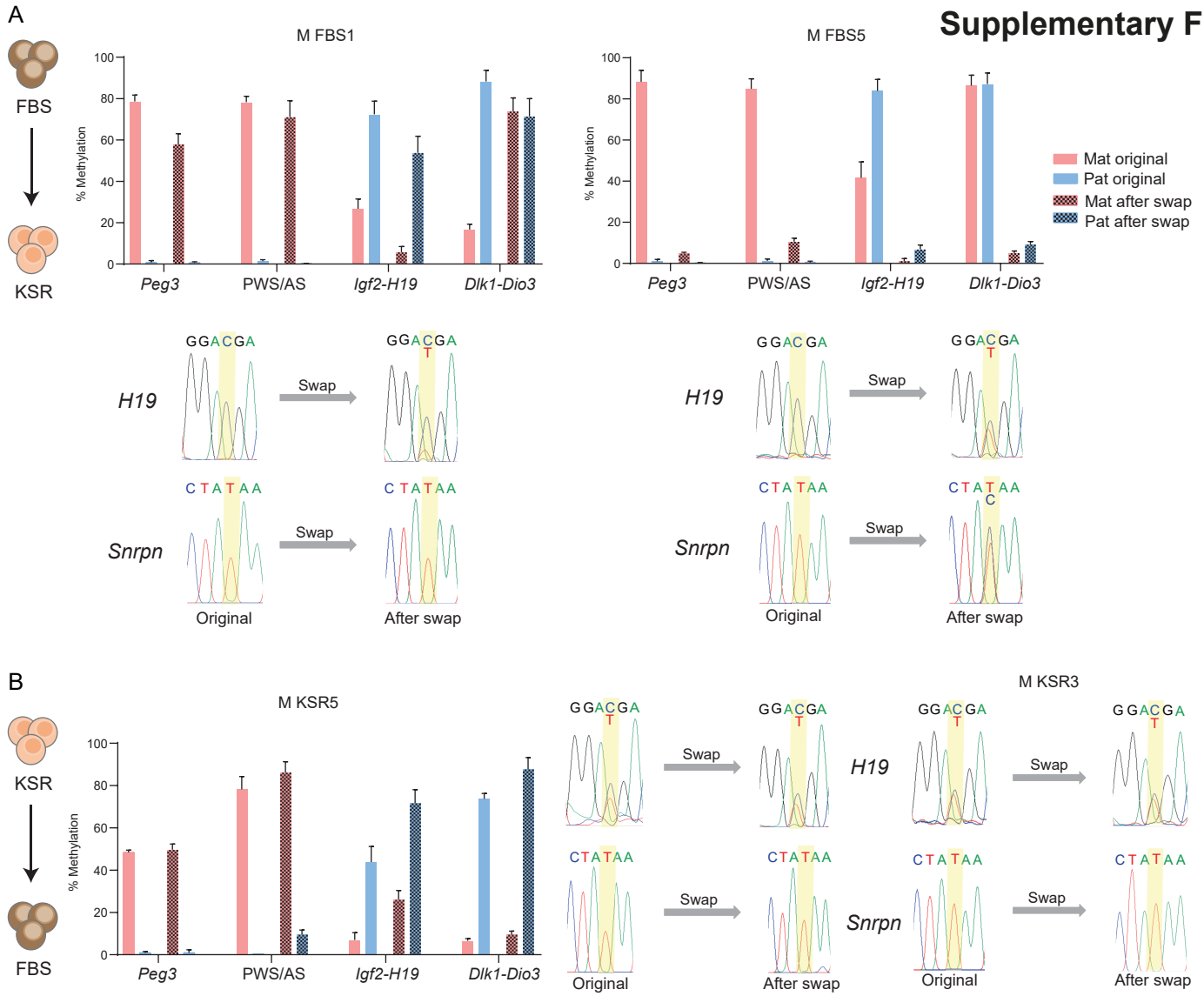
B. Scatter plot representing the correlation between *Dlk1-Dio3* ICR methylation and *Meg3* expression for the samples: F KSR2, F KSR4, M KSR3, M KSR5, F FBS1, F FBS5, M FBS1 and M FBS5. The X-axis represents the average methylation levels of the *Dlk1-Dio3* ICR considering both parental alleles. The Y-axis represents the *Meg3/Gapdh* values measured by RT-qPCR expression analysis and normalised to the M FBS1 iPSC line (n=3 biological independent replicates). *r* represents Pearson's correlation between methylation levels of *Dlk1-Dio3* locus measured by IMPLICON and *Meg3* expression data measured by RT-qPCR. Source data are provided as a Source Data file.

Supplementary Fig. 6



Supplementary Figure 6 – Expression analysis of genes involved in the DNA methylation machinery and imprinting protection

RT-qPCR expression analyses for *Dnmt3a*, *Dnmt3b*, *Dnmt1*, *Dnmt3L, Uhrf1, Tet1*, *Tet2*, *Tet3*, *Zfp57*, *Trim28* and *Dppa3* normalised with the *Gapdh* housekeeping gene in female MEFs, F KSR2, F KSR4, M KSR3, M KSR5, F FBS1, F FBS5, M FBS1, M FBS5 iPSCs (n=3 biological independent replicates; except for F KSR4 iPSC, where n=3 only for *Dnmt1* and n=2 biological replicates for all the other genes). Graph represents the mean expression ± SEM for each cell line. Values for each gene were normalised to the M FBS1 iPSC. *P*-values < 0.5 comparing KSR- and FBS-iPSCs are indicated on top of the bars (two-way ANOVA). Source data are provided as a Source Data file.


A
FBS
KSR
M FBS1
M FBS5
% Methylation
Peg3
PWS/AS
Igf2-H19
Dlk1-Dio3
Mat original
Pat original
Mat after swap
Pat after swap
H19
Snrpn
Swap
Original
After swap
B
KSR
FBS
M KSR5
M KSR3
C
F FBS day12 (2)
S+/T-
11.8%
Mix
20.1%
S-/T+
14.8%
SSEA1
Thy1
D
Pluripotency genes
Expression
Nanog
Esrrb
F FBS d12 T+/S- (2)
F FBS d12 T-/S+ (2)
F FBS d24 T+/S- cl C
F FBS d24 cl A
F FBS d24 cl B
F FBS2
F FBS4
E
5mC/C
5hmC/C
%
F FBS day12 T+/S- (1)
F FBS day12 T-/S+
F FBS day24 T-/S+
F FBS-iPSCs day50
M FBS day12 T+/S-
M FBS day12 T-/S+
M FBS day24 T-/S+
M FBS-iPSCs day50
F
Prickle1
% Methylation
G
H19
Snrpn
M FBS day24 cl A
M FBS2 day50

Supplementary Figure 7 – Imprinting and global 5mC/5hmC dynamics during reprogramming and maintenance of iPSCs

A-B. FBS-to-KSR (A) and KSR-to-FBS swap (B) experiments – briefly, M FBS1 and M FBS5 previously generated in Foetal Bovine Serum-based medium (FBS) were cultured for 10 passages (~20 days) in Knockout Serum Replacement-based medium (KSR), while M KSR3 and M KSR5 previously generated in KSR medium were cultured in FBS medium. Imprinting methylation was measured after swap by IMPLICON and imprinting expression was measured by Sanger Sequencing. Graphs on top in A and B show mean ± SD methylation levels measured at each CpG within different imprinted regions per parental allele for each sample (number of CpG per locus - *Peg3*: n=24; *Dlk1-Dio3:* n=27; *Igf2-H19:* n=16*;* PWS/AS: n=15) before and after swap (note: same data as in Fig. 4B for M FBS1 and M FBS5 iPSCs and as in Fig. 2A for M KSR5 iPSC before swap); On the bottom in A and B, chromatograms of *H19* and *Snrpn* genes are shown for M FBS1 and M FBS5 as well as M KSR3 and M KSR5, respectively, before and after medium swap (note: same chromatograms as in Fig. 4C for M FBS5). Source data are provided as Supplementary Data 2.

C. Representative flow cytometry plot for F FBS day12 (2) showing where gates were placed for collecting reprogramming [SSEA1+/THY1- (S+/T-) colored in green] and non-reprogramming [SSEA1-/THY1+ (S-/T+) colored in blue] intermediates. Mixed population is colored in orange.

D. RT-qPCR expression analyses of pluripotent markers (*Nanog* and *Esrrb*) normalised to the *Gapdh* gene in female cells collected during reprogramming (day12 and day24: T+/S- non-reprogramming intermediates; T-/S+ reprogramming intermediates) as well as in fully reprogrammed day50 F FBS2 and F FBS4 iPSCs; each bar represents data from only one biological replicate; Source data are provided as a Source Data file.

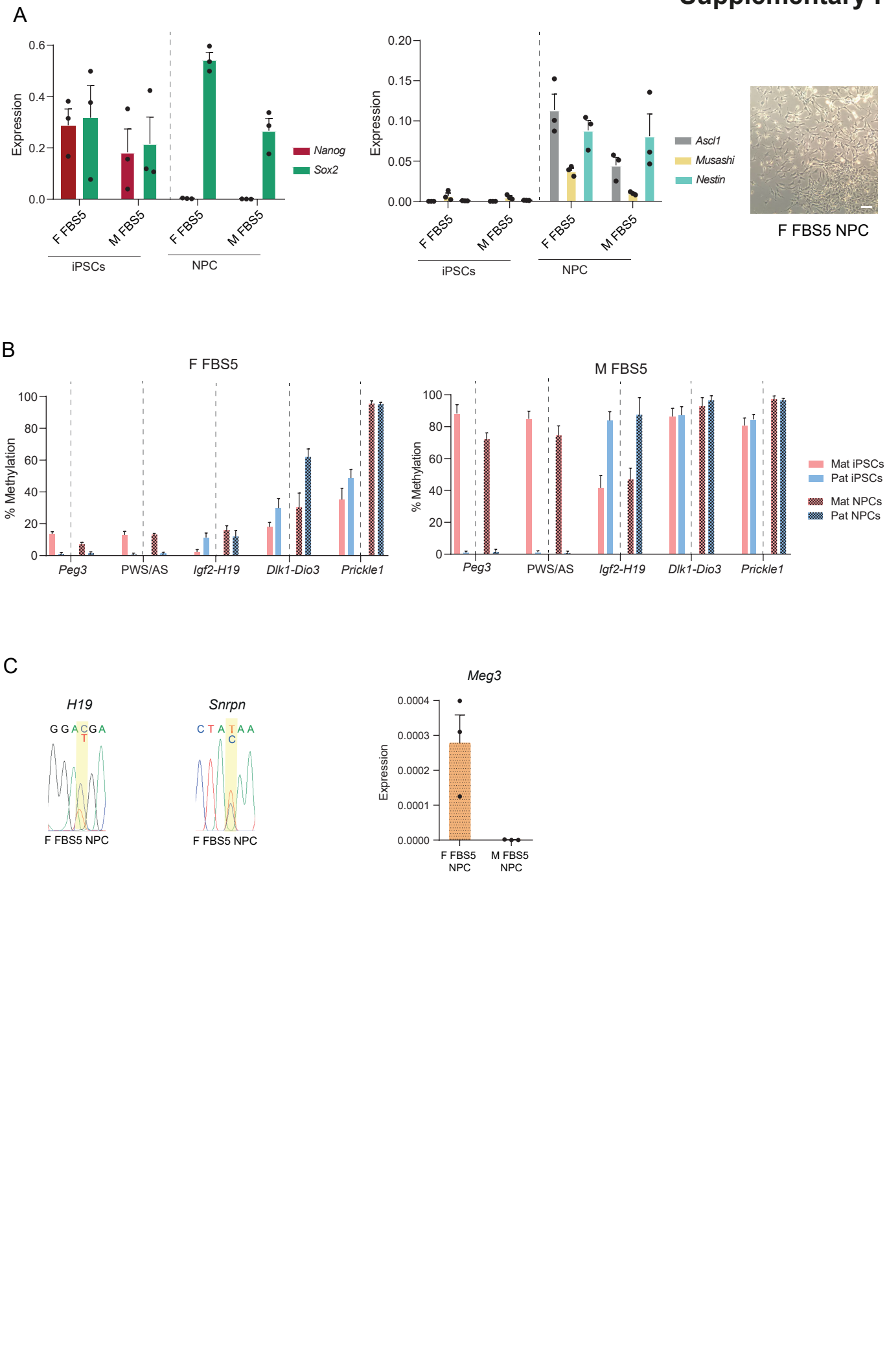
E. Graphs represent the average percentage ± SEM of 5mC and 5hmC per total cytosines in female and male cells sorted at day12 (T+/S-, n=1 for both biological sexes; female T-/S+, n=2; male T-/S+, n=1) and at day24 (female T-/S+, n=3;

male T-/S+, n=2), as well as in female and male day50 FBS-iPSCs (note: same data as in Fig. 5B, D for FBS-iPSCs, n=5 for both biological sexes); Source data are provided as a Source Data file.

F. Graph represents the mean percentage of methylation ± SEM considering both parental alleles of *Prickle1* gene in female and male cells sorted at day12 (T+/S-, n=1 for both biological sexes; female T-/S+, n=2; male T-/S+, n=1) and day24 (T-/S+, n=2 for both biological sexes) as well as in female and male day50 iPSCs (n=5 for both biological sexes); Source data are provided as Supplementary Data 2.

G. Allele-specific *H19* and *Snrpn* expression analysis assayed by Sanger sequencing. Chromatograms are shown for M FBS day24 clA and M FBS2.

Supplementary Fig. 8



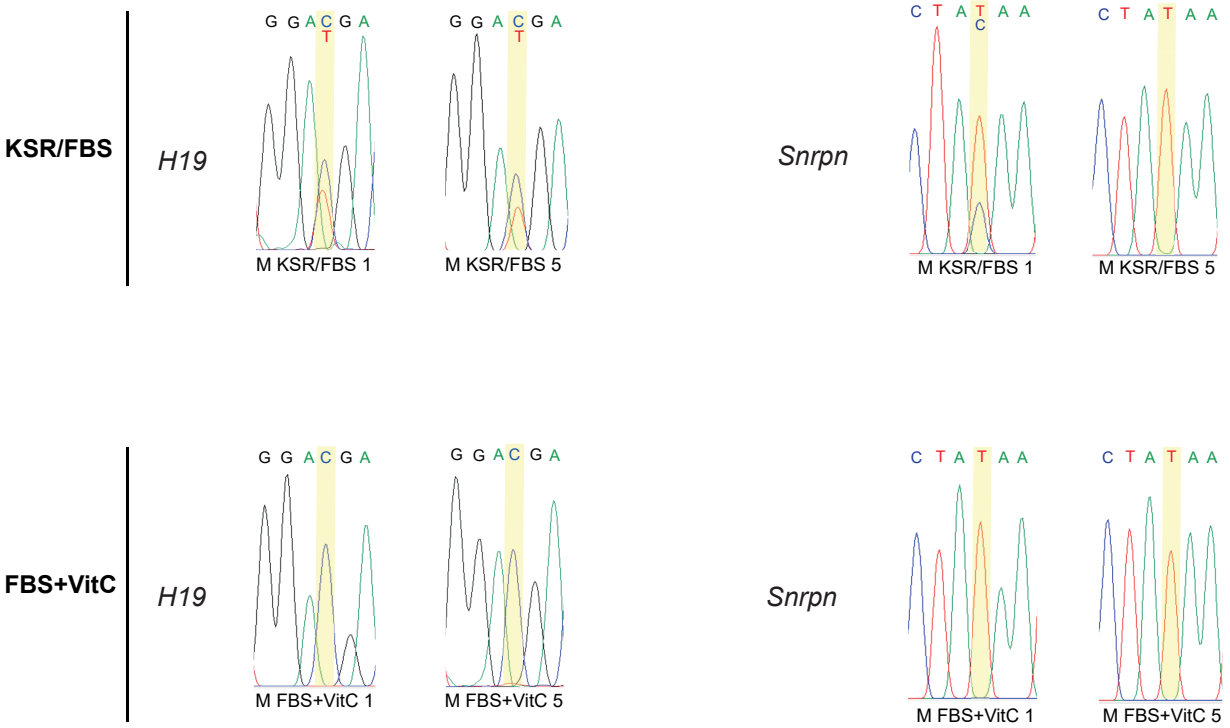
Supplementary Figure 8 – Persistence of imprinting errors in iPSC-derived NPCs

A. On the left, RT-qPCR expression analyses of pluripotent marker (*Nanog*), pluripotent and Neuronal Precursor Cell (NPC) marker (*Sox2*) and NPC-specific markers (*Ascl1*, *Musashi*, *Nestin*) normalised to the *Gapdh* housekeeping gene in F FBS5, M FBS5 and their correspondent NPCs (n=3 biological independent replicates); Graph represents the mean expression ± SEM in iPSCs and corresponding NPCs. On the right, a representative bright-field image of the F FBS5 NPCs. Scale bar corresponds to 100 µm. Source data are provided as a Source Data file.

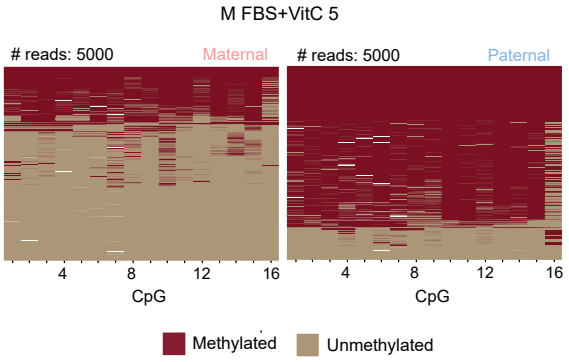
B. Methylation analysis of *Peg3*, PWS/AS, *Igf2-H19*, *Dlk1-Dio3* imprinted regions and *Prickle1* (methylated control) in F FBS5, M FBS5 iPSCs and correspondent NPCs (note: same data as in Fig. 4B for F FBS5 and M FBS5 iPSC lines); Each graph represents the mean ± SD methylation levels measured at each CpG within different genomic regions per parental allele for each sample (number of CpG per locus - *Peg3*: n=24; PWS/AS: n=15; *Igf2-H19:* n=16*; Dlk1-Dio3:* n=27; *Prickle1*: n=10); Source data are provided as Supplementary Data 2.

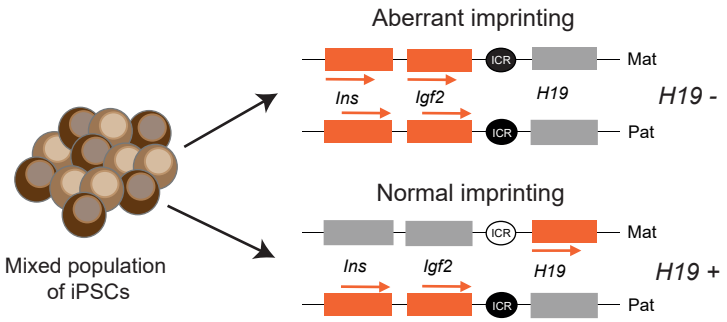
C. Allele-specific *H19* and *Snrpn* expression analysis assayed by Sanger sequencing and RT-qPCR expression analysis of *Meg3* normalised to the *Gapdh* housekeeping gene in F FBS5 and M FBS5 NPCs (n=3 biological independent replicates). Graph represents the mean expression ± SEM in F FBS 5 and M FBS 5 NPCs. Source data are provided as a Source Data file.

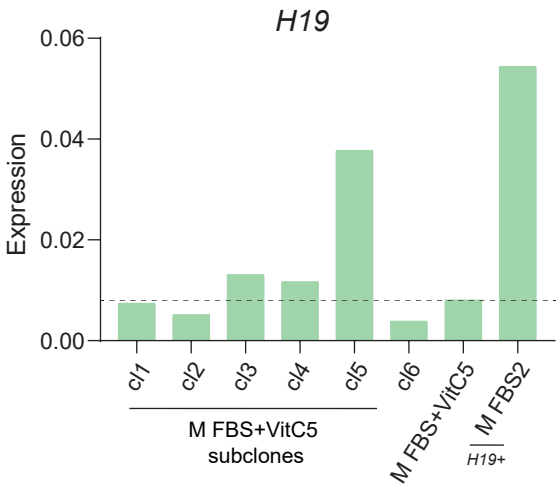
A


KSR/FBS
H19
M KSR/FBS 1
M KSR/FBS 5
Snrpn
M KSR/FBS 1
M KSR/FBS 5
FBS+VitC
H19
M FBS+VitC 1
M FBS+VitC 5
Snrpn
M FBS+VitC 1
M FBS+VitC 5

B


M FBS+VitC 5
reads: 5000
Maternal
reads: 5000
Paternal
4
8
12
16
CpG
Methylated
Unmethylated


Aberrant imprinting
Mixed population
of iPSCs
Ins
Igf2
ICR
H19
Mat
Pat
H19 -
Normal imprinting
H19 +

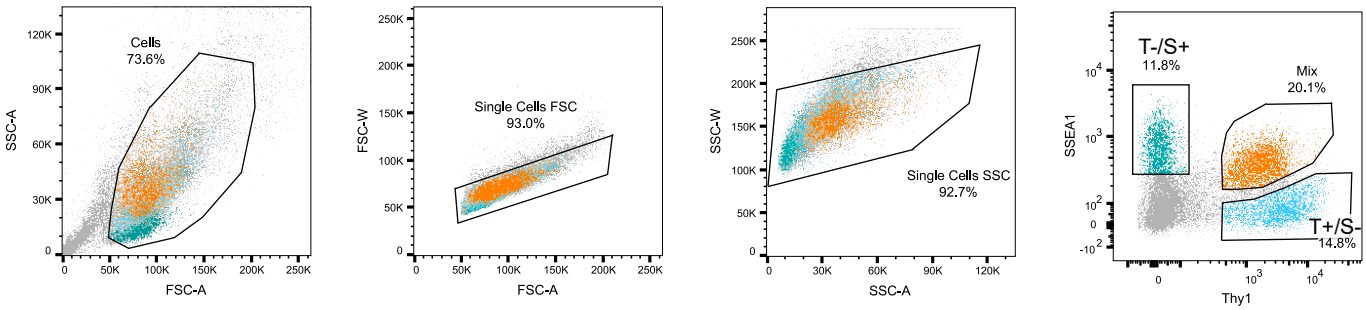

H19
Expression
0.06
0.04
0.02
0.00
cl1
cl2
cl3
cl4
cl5
cl6
M FBS+VitC5
M FBS2
H19+
M FBS+VitC5
subclones

Supplementary Figure 9 – Expression of imprinted genes in KSR/FBS- and FBS+VitC-iPSCs

A. Allele-specific *H19* and *Snrpn* expression analysis assayed by Sanger sequencing in KSR/FBS- and FBS+VitC-iPSCs. Chromatograms for M KSR/FBS1 and M KSR/FBS5 (top) and for M FBS+VitC1 and M FBS+VitC5 (bottom) are shown.

B. On the left, plots displaying methylated and unmethylated CpGs for each CpG position (in columns) in all the individual reads (in rows) for both maternal and paternal alleles of *Igf2-H19* locus in M FBS+VitC5. On top right, the scheme represents M FBS+VitC5 iPSCs with a mixed population of cells with normal and abnormal imprinting as estimated by the plots presented on the left. The status of *Igf2-H19* ICR with normal and abnormal (hypermethylation) imprinting are also displayed (white circle – unmethylated ICR; black circle – methylated ICR; Mat – maternal allele; Pat – paternal allele; orange rectangles – expressed genes; grey rectangles – silenced genes; regions are not drawn to scale). On the bottom right, graph represents the RT-qPCR expression analysis of *H19* expression normalised with the *Gapdh* housekeeping gene in the subclones of M FBS+VitC5, original M FBS+VitC5 and M FBS2 (normal imprinting); dashed line represents the *Meg3* expression level of the parental M FBS+VitC5 iPSC line; each bar represents data from only one biological replicate. Source data are provided as a Source Data file.

FACS gating strategy



Supplementary Figure 10 – FACS gating strategy

Gating strategy used for FACS-sorting of THY1+/SSEA1- (T+/S-) non-reprogramming intermediates and THY1-/SSEA1+ (T-/S+) reprogramming intermediates. Cells were gated in an FSC and SSC intensity dot plot to eliminate debris. Doublets were excluded based on FCS-W, FSC-A and SSC-W, SSC-A. Then non-reprogramming and reprogramming intermediates populations were discriminated based on CD90.2/Thy1.2 and CD15/SSEA-1 expression (note: last plot on the right is the same data as in Supplementary Fig. 7C). FSC: Forward scatter, SSC: Side scatter.

	Article	Stadtfeld et al., (2010)	Sun, et al., (2012)	Takikawa et al., (2013)	Yagi,et al., (2019)
Reprogramming	System	DOX-inducible, OKSM in *Col1a1* locus + *rtTA* in *Rosa26* locus	Retrovirus vsv-pseudotyped expressing individual human K,O,S,M	DOX-inducible, OSKM in *Col1a1* locus + *rtTA* in *Rosa26* locus	*PiggyBac* vector containing a DOX-inducible OSKM + *rtTA*
	Culture Conditions	15% FBS	20% KSR	15% FBS	15% FBS
iPSCs lines	Strain	————	Reciprocal crosses of C57BL/6J x Cast/Ei	(F)DBA/2 x (M)129	(F)129X1/SvJ x (M) MSM/Ms
	Number	6	8	12	5 (and 1 pre-iPSC)
	Gender	Not defined	Female and Male	Not defined	Male
Imprinting analyses	Imprinted clusters analysed	*Dlk1-Dio3*	PWS/AS, *Peg3*, *Kcqn1-Kcnq1ot1* , *Dlk1-Dio3*, *Igf2-H19*, *Mcts2-H13*	*Dlk1-Dio3*, *Igf2-H19, Rasgrf1*, *Mest/Peg1*, *Peg3*, *Sgce-Peg10*, PWS/AS, *Plagl1/Zac1*	Virtually all
	Methylation method	Bisulfite Pyrosequencing	Bisulfite Pyrosequencing	COBRA / Bisulfite sequencing	Bisulfite sequencing MethylC-seq
	Expression	Affymetrix mRNA expression microarray & RT-qPCR	Pyrosequencing	Allele-specific RT-PCR	RNA-seq
	Parental allele distinction	No	No	Yes, only for *Zim1* and *Snrpn* genes	Yes, large number of genes with SNPs
	Output	Hypermethylation of *Dlk1-Dio3*	Hypomethylation of PWS/AS, *Peg3* & *Kcnq1-Kcnq1ot1* (only female); *Dlk1-Dio3*, *Mcts2-H13* and *Igf2-H19* (male + female)	Hypomethylation of *Dlk1-Dio3*, *Igf2-H19, Rasgrf1*, *Peg3*, *Sgce-Peg10*, PWS/AS, *Plagl1/Zac1* Hypermethylation of *Mest/Peg1*	Hypermethylation of *Dlk1-Dio3* and *Igf2-H19*

Supplementary Table 1 – Previous studies addressing imprinting defects in mouse iPSCs

Abbreviations: DOX - Doxycycline; OSKM – *Oct4*, *Sox2*, *Klf4*, *c-Myc*; FBS – Fetal Bovine Serum; KSR – Knockout Serum Replacement; F - Female; M – Male.

	Region	Primer name	Primer sequences	PCR conditions
Pyrosequencing	*Line1-A*	L1-A_F	AGATTGAGGTATATAGGGAAGTAGGTT	Tm: 55C
		L1-A_R-[Btn]	[biot]ATCCACTCACCAAAAATCTTAAAAT	
		L1-A seq	GGTATATAGGGAAGTAGGTTA	
	Line1-T	L1-T_F	GGTTGGGGAGGAGGTTTAAGTTATA	Tm: 55C
		L1-T_R-[Btn]	[biot]CTACCTATTCCAAAAACTATCAAATTCTCT	
		L1-T seq	GGGAGGAGGTTTAAGTTATAGTA	
	IAP	IAP F	GAGGGTGGTTTTTTATTTTATGTGT	Tm: 58C
		IAP R [Btn]	[biot]ATCACTCCCTAATTAACTACAACC	
		IAP seq	TTTTTATTTTATGTGTTTTGTTTTT	
RT-qPCR	*Pou5f1*	mPou5f1 Fw	CCGGAAGAGAAAGCGAACTA	Tm: 60C
		mPou5f1 Rv	CGCCGGTTACAGAACCATAC	
	Nanog	mNanog Fw	CCAGTCCCAAACAAAAGCTC	Tm: 60C
		mNanog Rv	ATCTGCTGGAGGCTGAGGTA	
	Esrrb	mEsrbb3 Fw	TCTCATCTTGGGCATCGTGT	Tm: 60C
		mEsrbb31 Rv	AGTTTCTTGTACCTGCGCAC	
	Gapdh	mGapdh Fw	AACTTTGGCATTGTGGAAGG	Tm: 60C
		mGapdh Rv	ACACATTGGGGGTAGGAACA	
	Meg3	mMeg3 Fw	TTGCACATTTCCTGTGGGAC	Tm: 60C
		mMeg3 Rv	AAGCACCATGAGCCACTAGG	
	Dnmt1	mDnmt1 Fw	ATCAGGTGTCAGAGCCCAAAG	Tm: 60C
		mDnmt1 Rv	TGGTGGAATCCTTCCGATAAC	
	Dnmt3a	mDnmt3a Fw	AGACGTCTCCAACATGAGCC	Tm: 60C
		mDnmt3a Rv	TTTCTCTTCTGGGTGCTGAAC	
	Dnmt3b	Dnmt3b Fw	CCCTTGAAGGACTACTTTGCC	Tm: 60C
		Dnmt3b Rv	AGAGAACATGAGCACCATGC	
	Dnmt3L	Dnmt3L Fw	ATGGACAATCTGCTGCTGACTG	Tm: 60C
		Dnmt3L Rv	CGCATAGCATTCTGGTAGTCTCTG	
	Tet1	mTet1 Fw	TTTGGTTCGTGAGCGTGTAG	Tm: 60C
		mTet1 Rv	TGCAGGTACGCTTTTTGTTG	
	Tet2	mTet2 Fw	AACCTGGCTACTGTCATTGCTCCA	Tm: 60C
		mTet2 Rv	AGATGTTCTGCTGGTCTCTGTGGGAA	
	Tet3	mTet3 Fw	TGCGATTGTGTCGAACAAATAGT	Tm: 60C
		mTet3 Rv	TCCATACCGATCCTCCATGAG	
	Zfp57	mZfp57 Fw	CACAAATCCACAAAGCCGCAA	Tm: 60C
		mZfp57 Rv	TGAACGGGGCCTATAACCTAAA	
	Trim28	mTrim28 Fw	CGCATGTATCAGGCATGAAG	Tm: 60C
		mTrim28 Rv	CTTCCAGGAAAGACCTTGAAGA	
	Dppa3	mDppa3 Fw	GACCCAATGAAGGACCCTGAA	Tm: 60C
		mDppa3 Rv	GCTTGACACCGGGGTTTAG	
	Uhrf1	mUhrf1 Fw	CCCAGGTGGTCCAGGTACAG	Tm: 60C
		mUhrf1 Rv	CACGAGCACGGACATTCTTG	
	Sox2	mSox2 Fw	ATGCACCGCTACGACGTCA	Tm: 60C
		mSox2 Rv	CTTTTTGCACCCCTCCCAATT	
	Xist	mXist Fw	GCTGGTTCGTCTATCTTGTGGG	Tm: 60C
		mXist Rv	CAGAGTAGCGAGGACTTGAAGAG	
	Mecp2	mMecp2 Fw	TGACTTCACGGTAACTGGGAG	Tm: 60C
		mMecp2 Rv	TTTCACCTGAACACCTTCTGATG	
	Huwe1	mHuwe1 Fw	GAGGGCGTAAACATACAGAGAAG	Tm: 60C
		mHuwe1 Rv	CGCTGCTGTGTAAAGTGGC	
	Jarid1c	mJarid1c Fw	GAGGCCCAGACAAGAGTGAAA	Tm: 60C
		mJarid1c Rv	TTGGGAATCTTTAAGGATGAGCC	
	Ascl1	mMash1 Fw	CCAACTACTCCAACGACTTGAACTC	Tm: 60C
		mMash1 Rv	TCCTGCCATCCTGCTTCCAAAG	
	Musashi	mMsi1 Fw	CCGGAGTTACACAGGCCTTG	Tm: 60C
		mMsi1 Rv	GGGATAGCTGTGAGCTCGGG	
	Nestin	mNestin Fw	CTCCTGTGACAGCCTTTCTGAAG	Tm: 60C

		mNestin Rv	AGGATAGGGAGCCTCAGACATAGG	
Genotyping	Yamanaka cassette	mPpary Fw	CAGCATCAAATGGCTCGGTA	Tm: 59C
		mLenti Rv	GCACCATCCAAAGGTCAGTG	
	rtTA cassette (*Rosa26 locus*)	mRosa26 wt Fw	AAAGTCGCTCTGAGTTGTTAT	Tm: 59C
		mRosa26 wt Rv	GGAGCGGGAGAAATGGAATG	
		mRosa26 mut Rv	GCGAAGAGTTTGTCCTCAACC	
Allelic expression	*H19*	mH19 Fw	GCAATGCTGCCCCAGTAC	Tm: 61C
		mH19 Rv	GACTAGGCGAGGGGAAGGC	
	Snrpn	mSnrpn Fw	CATTATGGCTCCTCCACCTG	Tm: 61C
		mSnrpn Rv	GGGTCAAAAAGCTTGCAGGTAC	
	Meg3	mMeg3 Fw	CTTGCTGGCCCTGGAGAT	Tm: 61C
		mMeg3 Rv	AACGTGTTGTGCGTGAAGTC	

Supplementary Table 2 – Primers used for pyrosequencing, RT-qPCR, genotyping and allelic expression experiments

	Locus	Primer name	Primer sequences	PCR conditions	Coordinates of CpGs analysed	Type of ICR
IMPLICON	***Sox2***	mSox2 Fw	CTACACGACGCTCTTCCGATCTTAGTTAGTTATATGGGTAGAGGATT	Tm: 55C	chr3:34649370-34649554	N/A
		mSox2 Rv	TGCTGAACCGCTCTTCCGATCTNNNNNNNNAATATTTCAAAAAACTAACAAAACC			
	Klf4	mKlf4 Fw	CTACACGACGCTCTTCCGATCTGGTTTTTTTTGTTAATATTGATGAT	Tm: 55C	chr4:55530381-55530595	N/A
		mKlf4 Rv	TGCTGAACCGCTCTTCCGATCTNNNNNNNNACAAATAAAAAACTCCTCTACAACC			
	Prickle1	mPrickle1 Fw	CTACACGACGCTCTTCCGATCTGTTGGTTAAGGAAATAGTTTTTTTT	Tm: 55C	chr15:93500632-93500729	N/A
		m Prickle1 Rv	TGCTGAACCGCTCTTCCGATCTNNNNNNNNCAACCTCTATAACCAATATACCCATAC			
	H13-Mcts2	mH13DMR Fw	CTACACGACGCTCTTCCGATCTAGTATTAGAATATTGGGGGATTTT	Tm: 57C	chr2:152686786-152686933	Maternal
		mH13DMR Rv	TGCTGAACCGCTCTTCCGATCTNNNNNNNNAATTAAAAAATAAACAACCACTTC			
	Gnas	mGnasDMR Fw	CTACACGACGCTCTTCCGATCTATTTTGGATTAAAGTTTAAAGTGTT	Tm: 54C	chr2:174295708-174295902	Maternal
		mGnasDMR Rv	TGCTGAACCGCTCTTCCGATCTNNNNNNNNACAATTATACACCATACAAATAAATATATT			
	Peg3	mPeg3DMR Fw	CTACACGACGCTCTTCCGATCTTTTTGTAGAGGATTTTGATAAGGAG	Tm: 60C	Chr7:6730141-6730468	Maternal
		mPeg3DMR Rv	TGCTGAACCGCTCTTCCGATCTNNNNNNNNCAACCTTATCAATTACCCTTAAAAA			
	PWS/AS	mSnrpnDMR Fw	CTACACGACGCTCTTCCGATCTAATTTGTGTGATGTTTGTAATTATTTGG	Tm: 60C	Chr7:60005043-60005284	Maternal
		mSnrpnDMR Rv	TGCTGAACCGCTCTTCCGATCTNNNNNNNNATAAAATACACTTTCACTACTAAAATCC			
	Igf2-H19	mH19DMR Fw	CTACACGACGCTCTTCCGATCTGTAAGGAGATTATGTTTTATTTTTGG	Tm: 56C	Chr7:142581761-142582087	Paternal
		mH19DMR Rv	TGCTGAACCGCTCTTCCGATCTNNNNNNNNCCTCATAAAACCCATAACTAT			
	Kcnq1-Kcnq1ot1	mKvDMR Fw	CTACACGACGCTCTTCCGATCTTAAGGTGAGTGGTTTAGGAT	Tm: 60C	Chr7:143295094-143295470	Paternal
		mKvDMR Rv	TGCTGAACCGCTCTTCCGATCTNNNNNNNNAATCCCCCACACCTAAATTC			
	Commd1-Zrsr1	mCommd1DMR Fw	CTACACGACGCTCTTCCGATCTTAGGAGTATTTTTATTGTAGGGGG	Tm: 60C	chr11:22971952-22972131	Maternal
		mCommd1DMR Rv	TGCTGAACCGCTCTTCCGATCTNNNNNNNNCTAAAACAATAATTATCTACCTTACC			
	Dlk1-Dio3	mIG-DMR Fw	CTACACGACGCTCTTCCGATCTGTGGTTTGTTATGGGTAAGTTT	Tm: 60C	Chr12:109528253-109528471	Paternal
		mIG-DMR Rv	TGCTGAACCGCTCTTCCGATCTNNNNNNNNCCCTTCCCTCACTCCAAAAATTAA			
	Mest/Peg1	mPeg1 DMR Fw	CTACACGACGCTCTTCCGATCTTTTTTGTTATTATGGGTTAAGGG	Tm: 54C	Chr6:30737609-30737809	Maternal
		mPeg1 DMR Rv	TGCTGAACCGCTCTTCCGATCTNNNNNNNNCCTCTTTCCTTATAAATCTCTAA			
	Plagl1/Zac1	mPlagl1 Fw	CTACACGACGCTCTTCCGATCTTTGTAGTTAGAGATGTAGTAAAAG	Tm: 54C	Chr10:13091188-13091317	Maternal
		mPlagl1 Rv	TGCTGAACCGCTCTTCCGATCTNNNNNNNNTTCCCCAAAAAAACACAAAT			
	Grb10	mGrb10 Fw	CTACACGACGCTCTTCCGATCTTTTTAAGGAGAAAAAAGGTT	Tm: 50C	Chr11:12025411-12025700	Maternal
		mGrb10 Rv	TGCTGAACCGCTCTTCCGATCTNNNNNNNNCCAAATAATAAACAACTCCT			
	Igf2r	mIgf2r Fw	CTACACGACGCTCTTCCGATCTAGGGTGAAAAGTTGTATAAG	Tm: 56C	Chr17:12742173-12742488	Maternal
		mIgf2r Rv	TGCTGAACCGCTCTTCCGATCTNNNNNNNNAATTTACACCCTCAAAATAC			
	Impact	mImpact Fw	CTACACGACGCTCTTCCGATCTGTGGATGAGGTGTATAATTT	Tm: 56C	Chr18:12972868-12973155	Maternal
		mImpact Rv	TGCTGAACCGCTCTTCCGATCTNNNNNNNNACAAAACAAAACTAAACCTAC			

Supplementary Table 3 – Primers used for IMPLICON experiments