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Supplemental Information

Identification of Imprinted Differentially Methylated Regions by Global Analysis of Human-Parthenogenetic-Induced Pluripotent Stem Cells

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Figure S1

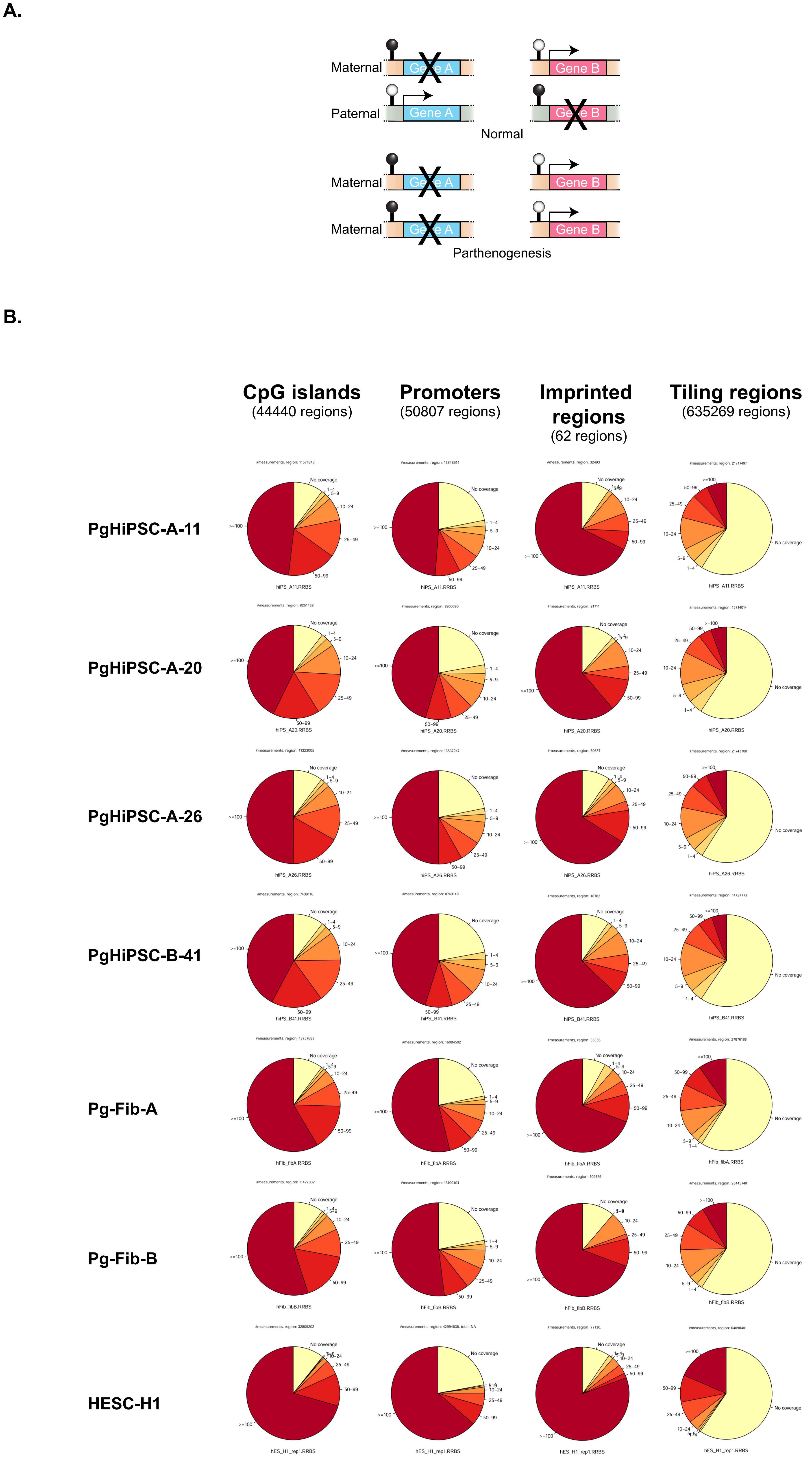

A.
Maternal
Gene A
Gene B
Paternal
Gene A
Gene B
Normal
Maternal
Gene A
Gene B
Maternal
Gene A
Gene B
Parthenogenesis
B.
CpG islands
(44440 regions)
Promoters
(50807 regions)
Imprinted regions
(62 regions)
Tiling regions
(635269 regions)
PgHiPSC-A-11
hiPS_A11.RRBS
PgHiPSC-A-20
hiPS_A20.RRBS
PgHiPSC-A-26
hiPS_A26.RRBS
PgHiPSC-B-41
hiPS_B41.RRBS
Pg-Fib-A
hFib_fibA.RRBS
Pg-Fib-B
hFib_fibB.RRBS
HESC-H1
hES_H1_rep1.RRBS
No coverage
1–4
5–9
10–24
25–49
50–99
>=100

Figure S2

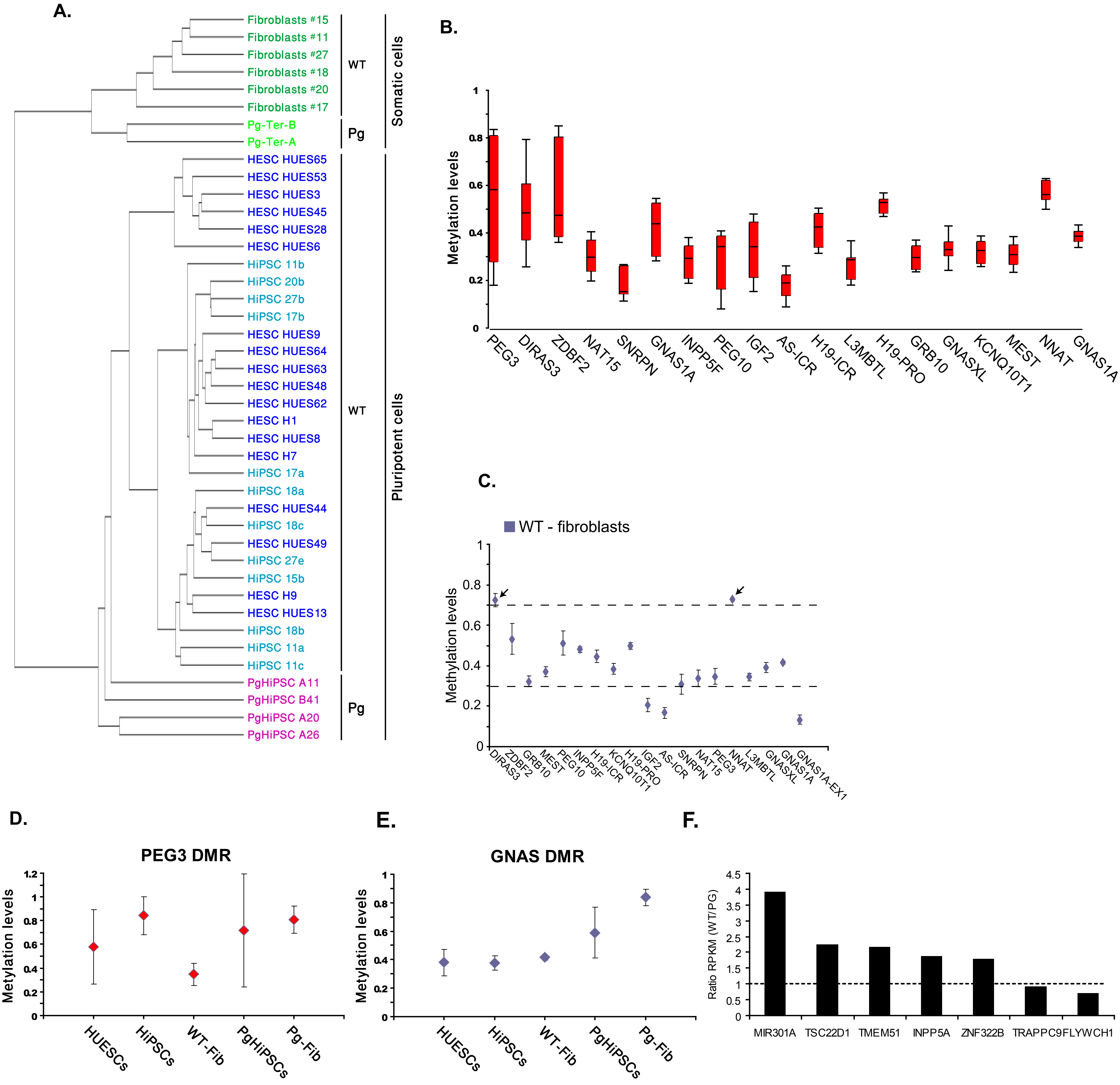

A.
Fibroblasts #15
Fibroblasts #11
Fibroblasts #27
Fibroblasts #18
Fibroblasts #20
Fibroblasts #17
Pg-Ter-B
Pg-Ter-A
HESC HUES65
HESC HUES53
HESC HUES3
HESC HUES45
HESC HUES28
HESC HUES6
HiPSC 11b
HiPSC 20b
HiPSC 27b
HiPSC 17b
HESC HUES9
HESC HUES64
HESC HUES63
HESC HUES48
HESC HUES62
HESC H1
HESC HUES8
HESC H7
HiPSC 17a
HiPSC 18a
HESC HUES44
HiPSC 18c
HESC HUES49
HiPSC 27e
HiPSC 15b
HESC H9
HESC HUES13
HiPSC 18b
HiPSC 11a
HiPSC 11c
PgHiPSC A11
PgHiPSC B41
PgHiPSC A20
PgHiPSC A26
WT
Pg
Somatic cells
WT
Pg
Pluripotent cells
B.
Metylation levels
1
0.8
0.6
0.4
0.2
0
PEG3
DIRAS3
ZDBF2
NAT15
SNRPN
GNAS1A
INPP5F
PEG10
IGF2
AS-ICR
H19-ICR
L3MBTL
H19-PRO
GRB10
GNASXL
KCNQ10T1
MEST
NNAT
GNAS1A
C.
WT - fibroblasts
Methylation levels
1
0.8
0.6
0.4
0.2
0
DIRAS3
ZDBF2
GRB10
MEST
PEG10
INPP5F
H19-ICR
KCNQ10T1
H19-PRO
IGF2
AS-ICR
SNRPN
NAT15
PEG3
NNAT
L3MBTL
GNASXL
GNAS1A
GNAS1A-EX1
D.
PEG3 DMR
Metylation levels
1.2
1
0.8
0.6
0.4
0.2
0
HUESCs
HiPSCs
WT-Fib
PgHiPSCs
Pg-Fib
E.
GNAS DMR
Metylation levels
1
0.8
0.6
0.4
0.2
0
HUESCs
HiPSCs
WT-Fib
PgHiPSCs
Pg-Fib
F.
Ratio RPKM (WT/PG)
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
MIR301A
TSC22D1
TMEM51
INPP5A
ZNF322B
TRAPPC9
FLYWCH1

Figure S3

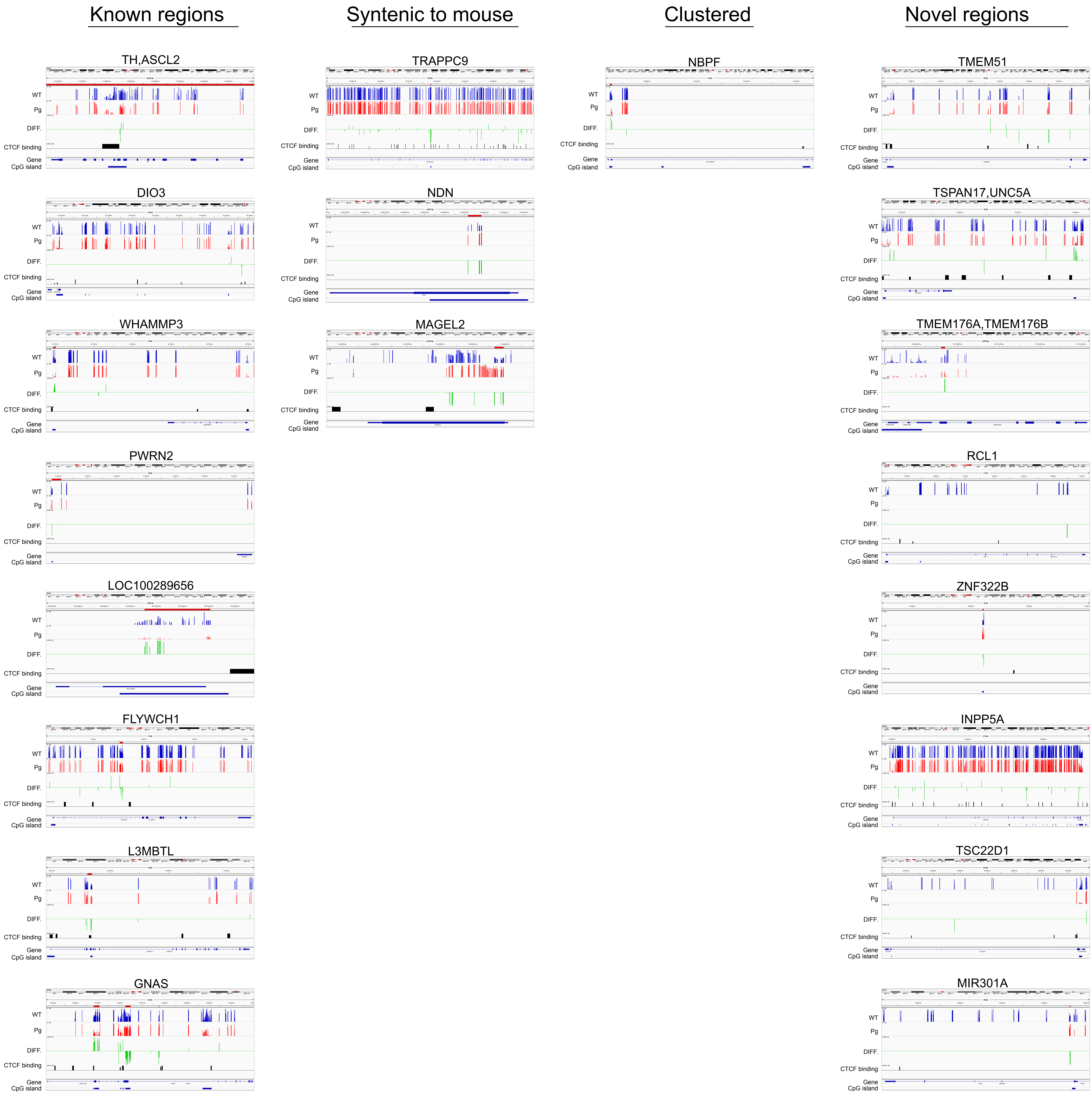
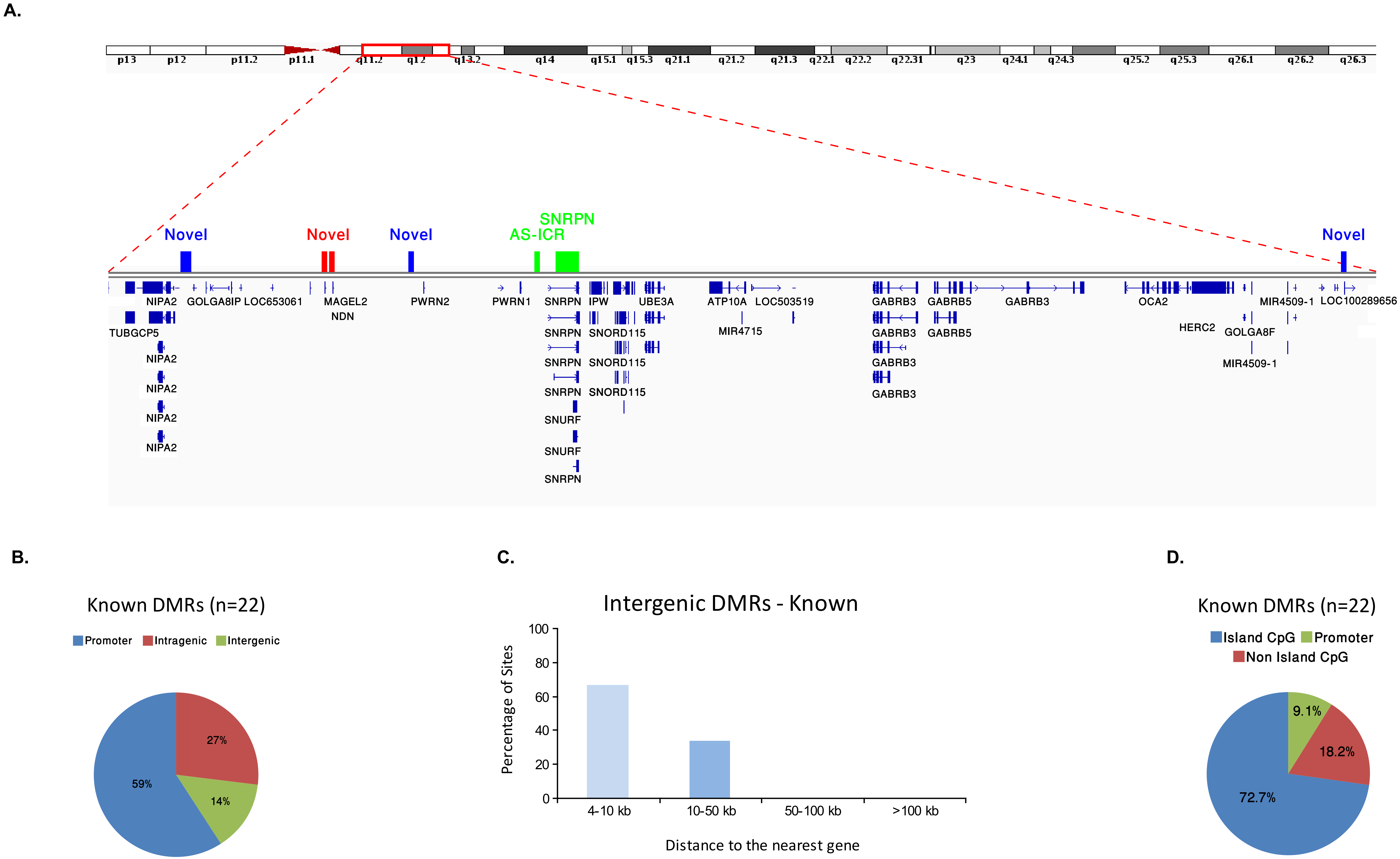

Known regions
Syntenic to mouse
Clustered
Novel regions
TH,ASCL2
DIO3
WHAMMP3
PWRN2
LOC100289656
FLYWCH1
L3MBTL
GNAS
TRAPPC9
NDN
MAGEL2
NBPF
TMEM51
TSPAN17,UNC5A
TMEM176A,TMEM176B
RCL1
ZNF322B
INPP5A
TSC22D1
MIR301A
WT
Pg
DIFF.
CTCF binding
Gene
CpG island

Figure S4


A.
p13
p12
p11.2
p11.1
q11.2
q12
q13.2
q14
q15.1
q15.3
q21.1
q21.2
q21.3
q22.1
q22.2
q22.31
q23
q24.1
q24.3
q25.2
q25.3
q26.1
q26.2
q26.3
Novel
Novel
Novel
SNRPN
AS-ICR
Novel
NIPA2
GOLGA8IP
LOC653061
MAGEL2
NDN
PWRN2
PWRN1
SNRPN
IPW
UBE3A
ATP10A
LOC503519
GABRB3
GABRB5
GABRB3
OCA2
MIR4509-1
LOC100289656
TUBGCP5
SNRPN
SNORD115
MIR4715
GABRB3
GABRB5
HERC2
GOLGA8F
NIPA2
SNRPN
SNORD115
GABRB3
MIR4509-1
NIPA2
SNRPN
SNORD115
GABRB3
NIPA2
SNURF
NIPA2
SNURF
SNRPN
B.
Known DMRs (n=22)
Promoter
Intragenic
Intergenic
27%
59%
14%
C.
Intergenic DMRs - Known
Percentage of Sites
100
80
60
40
20
0
4-10 kb
10-50 kb
50-100 kb
>100 kb
Distance to the nearest gene
D.
Known DMRs (n=22)
Island CpG
Promoter
Non Island CpG
9.1%
18.2%
72.7%

Legends for Supplementary Figures

Figure S1 - related to Figure 1. Conceptual frame-work and analysis of reduced representation libraries. **(A)** Schematic representation of the conceptual frame-work used in this study. **(B)** Statistical analysis of reduced representation library read coverage; Pie chart indicating the number of individual CpG measured in each type of genomic region. Regions were calculated as previously described in Gu et. al. (Gu et al., 2011); shown are all parthenogenetic cell lines presented in this study.

Figure S2 – related to Figure 1. Characterization of global methylation profiles and known imprinted DMRs in different cell types. (**A**) Hierarchical clustering based on global DNA methylation profiles of HESCs, HiPSCs, PgHiPSCs, normal and parthenogenetic fibroblasts.(**B**) Distribution of DNA methylation in 12 low-passage (< passage 25) HESCs lines in known imprinted DMRs. (**C**) Average methylation calls ± S.E of normal fibroblast cells in known imprinted DMRs. DMRs are ordered by chromosome numbers (X axis) (**D+E**) Average methylation calls ± SD in different cell types representing (**D**) hyper-methylation in HiPSCs and several PgHiPSC lines in *PEG3* DMR and (**E**) hypo-methylation of several PgHiPSC lines in *GNAS* DMR following reprogramming. **(F)** Expression profile of genes associated with novel intra-genic and promoter DMRs; Shown is the ratio in RPKM between normal PSCs and PgHiPSCs.

Figure S3 – related to Figure 2 and Table 1. Regional view of all novel DMRs identified in this study**.** Average methylation values for wild type PSC (Blue) and PgHiPSCs (red) of all CpG calls. Green track indicates the difference between hemi-methylated wild type PSCs and PgHiPSCs CpGs. *CTCF* binding sites in H1 ES cells from the ENCODE project is depicted in Black and CpG islands (UCSC) in dark green.

Figure S4 – related to Figure 3. Characterization of previously identified imprinted DMRs. (**A)** Schematic representation of the genomic organization of all imprinted DMRs identified in the Prader-Willi/Angelman region in chromosome 15. pDMRs are depicted in blue rectangles; Red rectangles – mDMRs. The previously identified DMRs, *SNRPN* and Angelman syndrome imprinted control region (AS-ICR), are highlighted in green. **(B+C)** Characterization of previously identified imprinted

DMRs. (**B**) Pi-chart representing the different genomic properties of previously (**C**) Pi-chart representing the different genomic properties of the previously identified imprinted DMRs. identified imprinted DMRs. (**D**) Distribution of distances of the imprinted inter-genic DMRs to their nearest gene.

Table S1

Genes associated with intragenic DMRs

Genes	Ratio RPKM	PgHiPSCs (RPKM)	WT-HiPSCs (RPKM)
SNORD116-5	30.3	45.669	1385.773
SNORD116-3	30.3	46.248	1401.206
SNORD116-1	29.4	93.010	2738.360
SNORD116-2	28.1	72.213	2032.450
SNORD116-8	26.9	39.622	1067.274
SNORD116-6	25.6	23.808	609.657
SNORD116-24	25.4	37.492	951.107
SNORD116-23	22.3	59.182	1316.893
SNORD116-22	22.0	16.732	367.633
SNORD116-16	21.8	23.106	503.020
SNORD116-20	21.7	26.116	567.527
PEG10	21.2	0.193	4.098
SNORD116-15	21.1	22.929	484.401
SNURF	19.9	5.635	112.060
SNORD116-18	19.3	29.879	575.443
SNORD116-11	18.6	23.505	436.349
SNORD116-21	18.6	21.911	406.580
IGF2	18.0	0.214	3.857
SNORD116-17	17.3	14.541	251.079
SNORD116-19	17.3	14.541	251.079
SNORD116-29	16.9	39.941	675.126
INS-IGF2	16.3	0.063	1.029
SNORD116-25	16.3	18.326	298.937
SNORD116-14	15.8	40.237	633.736
SNORD116-4	15.6	14.132	220.532
PAR5	15.0	2.907	43.676
SNORD116-26	14.6	6.111	89.387
SNORD116-27	13.4	25.098	337.319
SNORD116-13	13.3	59.979	796.673
SNRPN	13.2	0.554	7.310
IPW	13.2	1.006	13.236
SNORD107	12.2	16.038	195.453
SNORD109A	12.0	8.295	99.409
SNORD109A	12.0	8.295	99.409
SNORD108	11.4	3.571	40.796
MAGEL2	11.2	0.062	0.696
NDN	10.2	0.828	8.407
SNORD116-28	10.0	0.000	1004784500
IGF2AS	5.2	0.036	0.188
DIRAS3	4.5	0.399	1.804
NAP1L5	4.4	0.238	1.049
MIR301A	3.9	0.862	3.373
KIAA1026	3.8	0.083	0.316
ZDBF2	2.7	1.608	4.300
DLK1	2.5	2.396	6.034
TSC22D1	2.2	389.494	873.141
TMEM51	2.2	0.264	0.574
INPP5A	1.9	3.946	7.386
ZNF322B	1.8	175.000	315.500
GRB10	1.6	2.208	3.495
SMG8	1.5	5.380	8.088

GNAS	1.5	16.565	24.717
COPG2	1.3	8.988	11.797
TRIM37	1.3	2.589	3.365
MEST	1.2	12.001	14.877
EFHD2	1.1	9.300	10.475
KCNQ1OT1	1.1	0.892	0.965
INPP5F	1.1	9.901	10.580
SKA2	1.0	4.204	4.125
PPM1E	1.0	1.254	1.210
CTSL2	0.7	69.819	51.091
FLYWCH1	0.7	1.822	1.298
TRAPPC9	0.5	2.650	1.445
PRR11	0.5	17.604	9.521

Table S2

Genes associated with intergenic DMRs

Genes	Ratio RPKM	PgHiPSCs (RPKM)	WT-HiPSCs (RPKM)
ANKRD9	0.48	3.246	1.553
APBA2	1.33	1.203	1.605
CLTB	1.40	63.665	74.228
CYFIP1	0.89	13.941	12.433
DIO3	0.83	4.747	3.957
FAF2	1.45	7.896	11.484
GPRIN1	0.69	1.282	0.886
HERC2P2	1.22	2.980	3.631
HSP90AA1	0.92	217.467	199.550
NDNL2	1.33	11.126	14.794
NIPA1	1.05	2.580	2.721
NIPA2	1.97	1.804	3.555
OCA2	1.20	0.173	0.208
PPP2R5C	0.90	11.607	10.458
RNF44	1.27	11.547	14.664
TJP1	1.16	8.323	9.636
TSPAN17	1.33	2.688	3.563
TUBGCP5	1.05	3.346	3.529
UIMC1	0.89	5.705	5.051
UNC5A	0.89	0.532	0.476
WDR20	0.65	2.392	1.551
ZNF346	1.07	3.511	3.745
ZNF839	1.09	2.344	2.554

Table S3

Mouse Vs. Human

	DMRs in mouse adopted from Xie et al. 2012				
Chromosome	Locus	Status in Mouse	Synteny in Human	Same position of synteny relative to gene	DMR in Human
1	*Gpr1/Zdbf2*	Known	Yes	Yes	Yes
2	*Mcts2/H13*	Known	Yes	Yes	No
2	*H13* DMR2 (3' end)	Novel	Yes	No	No
2	*Nesp*	Known	Yes	Yes	Yes
2	*Nespas/Gnasxl*	Known	Yes	Yes	Yes
2	*Gnas1a*	Known	Yes	Yes	Yes
6	*Peg10/Sgce*	Known	Yes	Yes	Yes
6	*Vwde* promoter	Novel	Yes	Yes	No
6	*Mest (Peg1)*	Known	Yes	Yes	Yes
6	*Herc3/Nap1l5*	Known	Yes	Yes	N/A
6	*Casc1* intragenic	Novel	No	-	No
7	*Peg3/Usp29*	Known	Yes	Yes	Yes
7	*6330408a02Rik* 3' end	Novel	Yes	No	No
7	*Snurf/Snrpn*	Known	Yes	Yes	Yes
7	*Snrpn* U exon	Novel	No	-	No
7	*U80893* 5' upstream	Novel	No	-	No
7	*mir344b*	Novel	No	-	No
7	*mir344c*	Novel	No	-	No
7	*mir344*	Novel	No	-	No
7	*mir344-2*	Novel	No	-	No
7	*mir344g*	Novel	No	-	No
7	*AK086712* promoter	Novel	Yes	Yes	No
7	*Ndn*	Known	Yes	Yes	Yes
7	*Magel2*	Known	Yes	Yes	Yes
7	*Magel2-Mrkn3* intergenic	Novel	No	-	No
7	*Mkrn3*	Known	Yes	Yes	No
7	*Peg12*	Known	Yes	Yes	No
7	*Inpp5f*	Known	No	-	Yes
7	*H19* promoter	Known	Yes	Yes	Yes
7	*H19* ICR	Known	Yes	Yes	Yes
7	*igf2 DMR*	Known	Yes	Yes	Yes
7	*Kcnq1ot1*	Known	Yes	Yes	Yes
7	*Cdkn1c*	Known	Yes	Yes	No
7	*Cdkn1c* upstream	Known	Yes	No	No
9	*Rasgrf1*	Known	Yes	Yes	N/A
10	*Plagl1*	Known	Yes	Yes	Yes
10	*Neurog3* upstream	Novel	Yes	Yes	N/A
11	*Grb10*	Known	Yes	Yes	Yes
11	*Grb10* DMR2 (intragenic)	Novel	Yes	Yes	N/A
11	*Zrsr1/Commd1*	Known	Yes	No	No
11	*Commd1* DMR2 (intragenic)	Novel	No	-	No
11	*FR149454* promoter	Novel	No	-	No
12	*FR085584* promoter	Novel	Yes	Yes	No
12	*Dlk1*	Known	Yes	Yes	No
12	*Dlk1-Gtl2* IG	Known	Yes	Yes	Yes
12	*Gtl2*	Known	Yes	Yes	Yes
12	*Gtl2-Mirg* diffuse DMR	Novel	Yes	Yes	No
13	*Nhlrc1* downstream	Novel	Yes	No	No
15	*Myo10* intragenic	Novel	Yes	Yes	No
15	*Pvt1* promoter	Novel	Yes	Yes	N/A
15	*Peg13/Trappc9*	Known	Yes	Yes	Yes
15	*Eif2c2* diffuse DMR	Novel	Yes	Yes	No
15	*Slc38a4*	Known	Yes	No	No
17	*Airn/Igf2r*	Known	No	No	No
17	*Igf2r*	Known	Yes	Yes	No
18	*Impact*	Known	Yes	Yes	No

Human Specific Vs. Mouse

	Human Specific DMRs			
Chromosome	Locus	Status in Human	Synteny in Mouse	Same position of synteny relative to gene
1	TMEM51	Novel	Yes	Yes
1	NBPF1,NBPF10,NBPF20,NBPF14	Novel	No	-
1	NBPF1,NBPF10,NBPF20,NBPF15	Novel	No	-
5	TSPAN17,UNC5A	Novel	Yes	Yes
7	TMEM176A,TMEM176B	Novel	Yes	Yes
9	RCL1	Novel	No	-
9	ZNF322B	Novel	Yes	No
10	INPP5A	Novel	No	-
11	TH,ASCL2	Novel	No	-
13	TSC22D1	Novel	Yes	Yes
14	DIO3	Novel	No	-
15	WHAMMP3,NIPA1,NIPA2	Novel	Yes	No
15	PWRN2	Novel	No	-
15	LOC100289656	Novel	Yes	No
16	NAT15,ZNF597	Known	Yes	Yes
16	FLYWCH1	Novel	No	-
17	FAM33A,SKA2,MIR301A,PRP11	Novel	Yes	Yes
20	BLCAP,NNAT	Known	Yes	Yes
20	TOX2,GTSF1L,MYBL2	Novel	Yes	Yes
20	GNAS	Novel	Yes	Yes