Transient exposure to miR-203 expands the differentiation capacity of pluripotent stem cells

By Salazar-Roa et al.

List of Appendix Material

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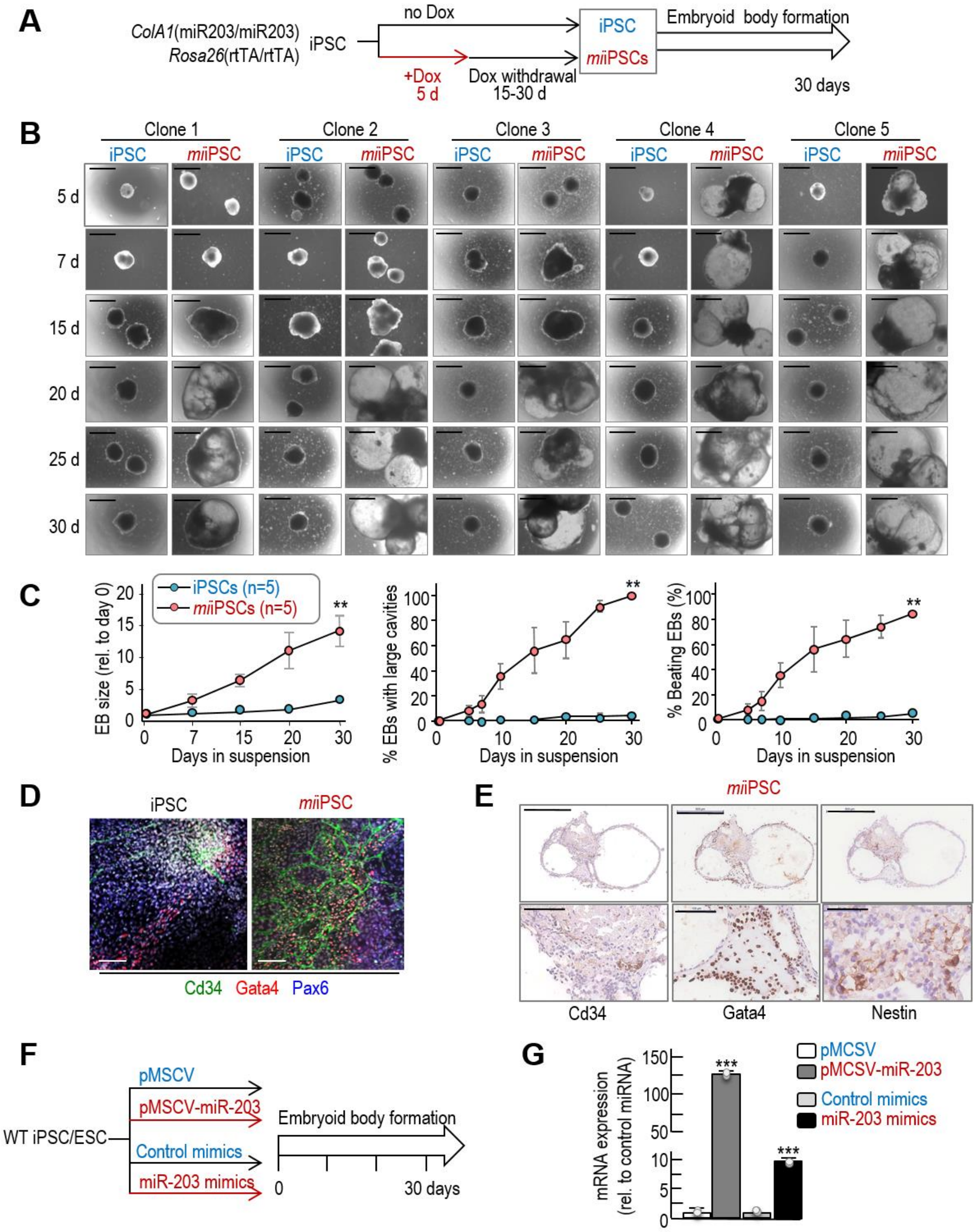
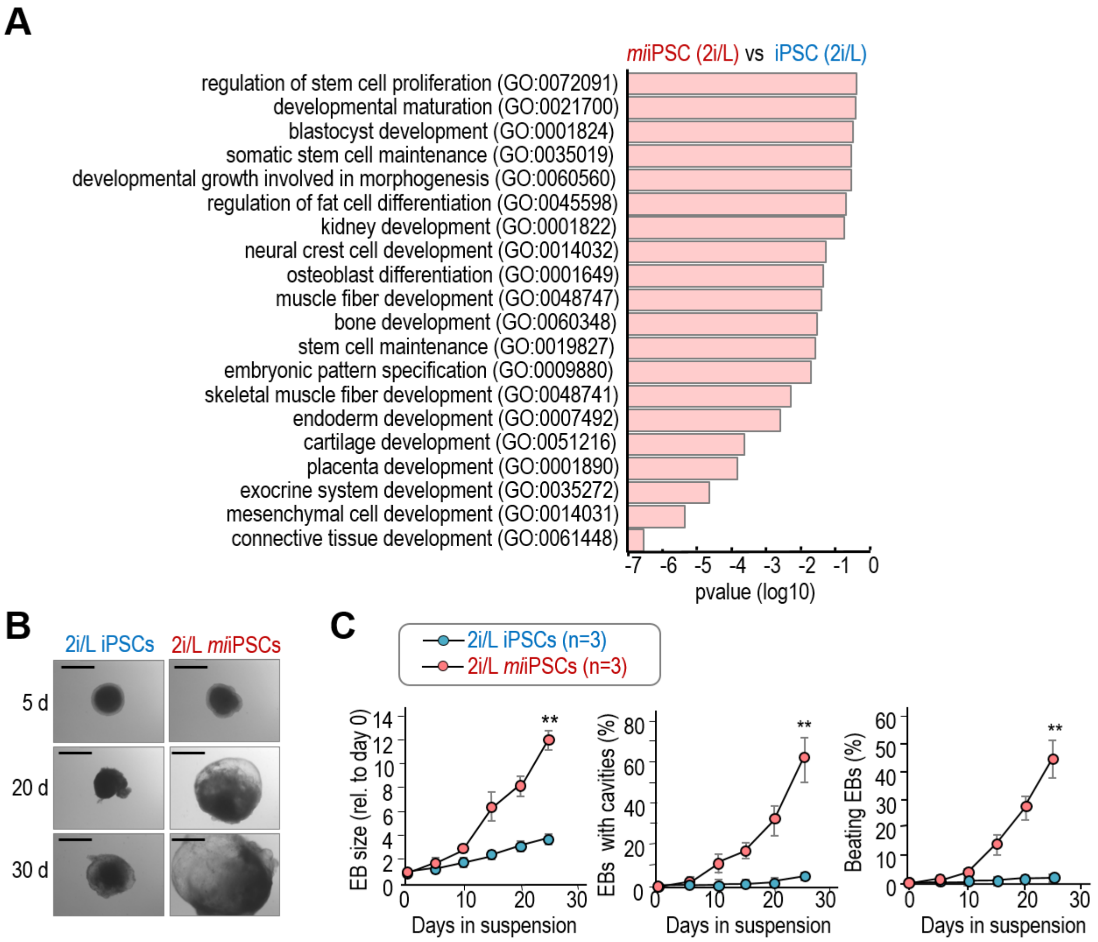
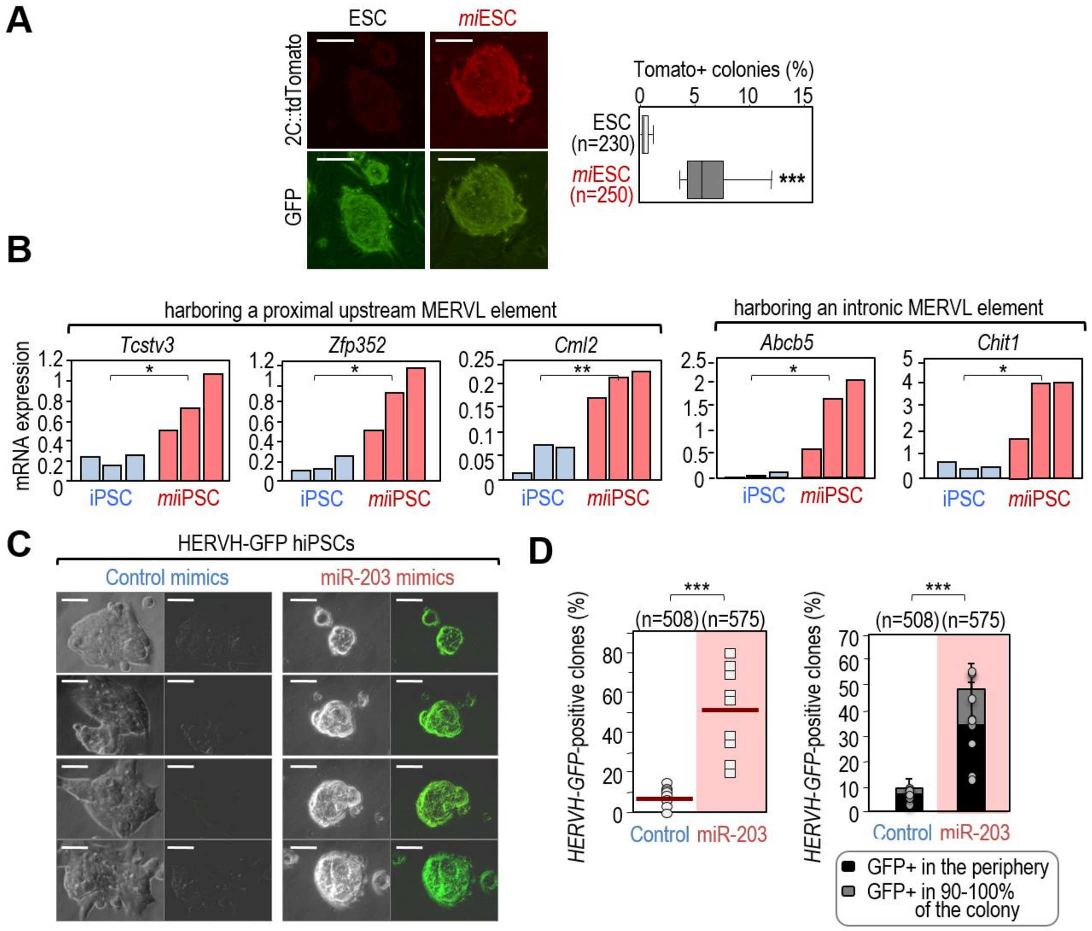


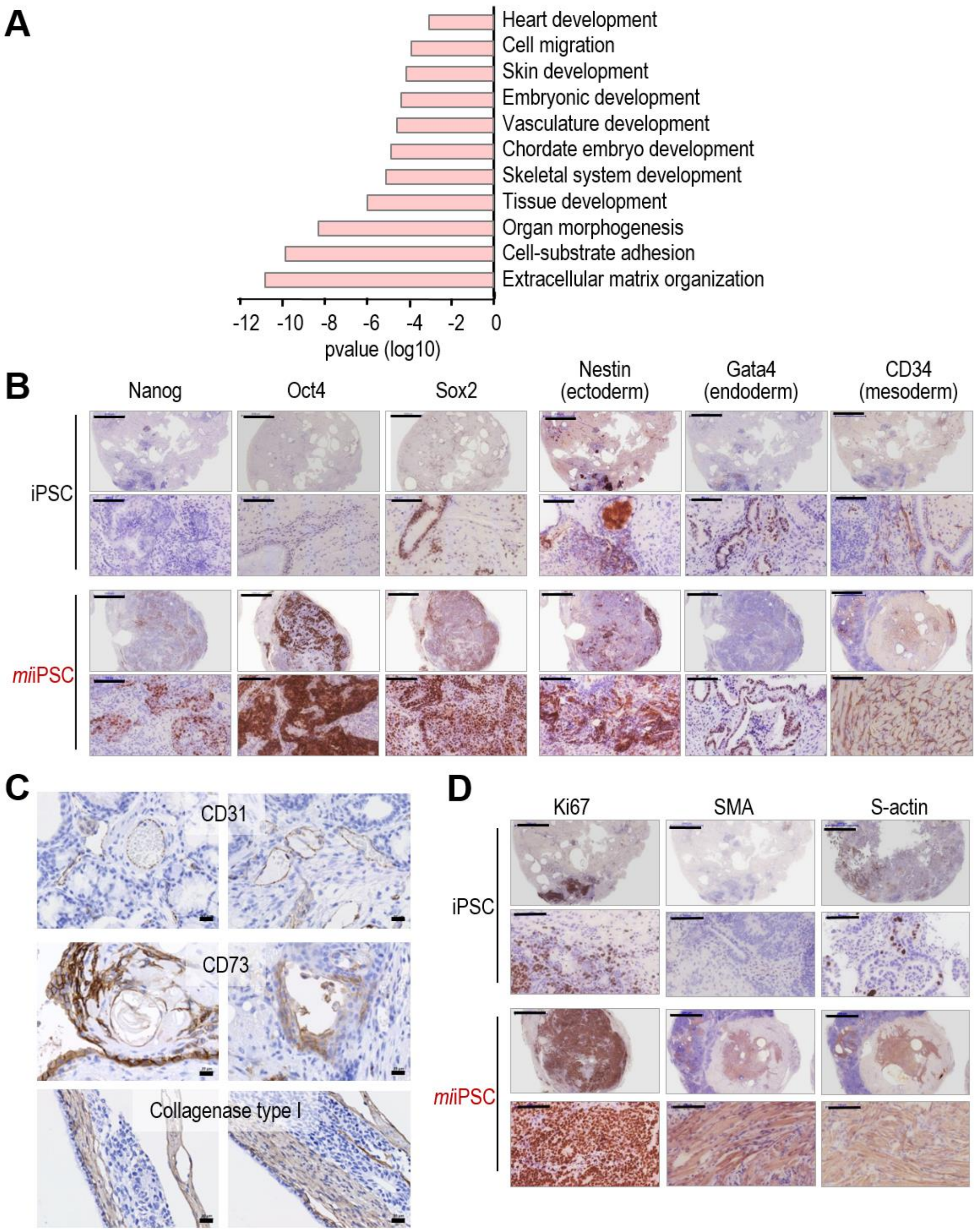
Appendix Figure S1. Effect of transient exposure to miR-203 in embryoid body formation. A, Experimental design to generate embryoid bodies (EBs) from wild-type iPSCs or *mi*iPSCs. Cells were treated or not with Dox during 5 days and Dox was then removed during the following 15-30 days before starting the embryoid body formation assays. **B,** Representative images of embryoid bodies derived from 5 independent clones of un-induced iPSCs or *mi*iPSCs at different time points of the differentiation process. Please note that Clone 4 has been chosen as a representative clone in Fig. 1F. This clone in reproduced here for better comparison among clones. Scale bar, 500 μm. **C,** Quantification of EB size from panel **B**, and percentage of EBs with internal large cavities or beating EBs during the differentiation process. Data are mean ± s.e.m. (n=5 independent experiments). ***P*<0.01 (Student´s *t*-test). **D**, Representative immunofluorescence detection of Cd34 (mesoderm; green), Gata4 (endoderm; red) and Pax6 (ectoderm; blue) in EBs derived from wild-type iPSCs or *mi*iPSCs. Scale bar, 20 μm. **E**, Immunohistochemical detection of Cd34 (mesoderm), Gata4 (endoderm) and Nestin (ectoderm) in *mi*iPSC-derived EBs. Scale bars are 500 μm for the upper images and 100 μm (Cd34, Gata4) or 50 μm (Nestin) for the lower images. **F,** Schematic representation of the experimental design used to generate embryoid bodies (EBs) from wild-type iPSCs or ESCs, either transduced with retroviruses or transfected with miRNA mimics for transient expression of miR-203. **G,** miR-203 RNA expression in wild-type iPSCs transduced with pMCSV or pMCSV-miR-203, or transfected with control mimics or miR-203 mimics. RNA expression is normalized by a control miRNA (miR-142). Data are mean ± s.e.m. (n=3 independent experiments) ****P*<0.001 (Student´s t-test).



Appendix Figure S2. Effect of a transient exogenous expression of miR-203 in PSCs cultured in 2i/L conditions. A, Pathways from the Gene Ontology Database significantly deregulated in *mi*iPSCs versus un-induced iPSC, both cultured on 2i/L conditions. **B,** Representative images of EBs derived from un-induced iPSCs or *mi*iPSCs, cultured in 2i/L conditions during 10 passages, at different time points of the differentiation process. Micrographs are representative of three different experiments. Scale bars, 500 μm. **C,** Quantification of EBs size and the percentage of EBs presenting internal large cavities or beating from panel **B.** Data are mean ± s.e.m. (n=3 independent experiments). ***P*<0.01 (Student´s t-test).



Appendix Figure S3. Transient exposure to miR-203 induces 2-cell-like markers. A, Representative immunofluorescence analysis of ESC colonies stably expressing the *2C::tdTomato* reporter, and transiently transduced with GFP (ESC) or miR-203-GFP (*mi*ESC) viruses. Scale bar, 10 µm. Right plot shows the percentage of tdTomato-positive colonies out of the total GFP-positive colonies, 24 h after transduction. Data are represented as mean ± s.e.m. (n=3 independent experiments). ****P*<0.001 (Student´s *t*-test). **B**, Early RNA expression as determined by RNA sequencing (RPKM) of the indicated transcripts, known to be harboring a proximal upstream MERVL element (*Tcstv3*, *Zfp352* and *Cml2*) or an intronic MERVL element (*Abcb5* and *Chit1*). Data show three independent wild-type iPSC (blue) or *mi*iPSCs (red) clones. **P*<0.05; ***P*<0.01 (Student´s t-test). **C**, Representative images of human pluripotent stem cells (hiPSCs) expressing a long terminal repeat (LTR7) of HERVH endogenous retrovirus tagged by GFP. Cells were transfected with either control (left) or miR-203 mimics (right). White field and GFP expression for the same colonies are shown. Scale bars, 10 µm. **D,** Left plot shows the percentage of HERVH-GFP-positive colonies in the assay described in panel **C**, five days after the miRNA transfection. Right plot shows the percentage of HERVH-GFP-positive colonies in which GFP is expressed only in the periphery of the clone (black) or in the majority of the cells constituting the colony (grey). Data are mean ± s.e.m. (n=3 independent experiments; as indicated, 508 colonies for control mimics and 575 colonies for miR-203 mimics were counted). ****P*<0.001 (Student´s t-test).



Appendix Figure S4. Differentiation properties in teratomas generated from *mi*iPSCs. A, Gene Ontology Analysis of genes significantly altered in *mi*iPSC-derived teratomas compared to teratomas derived from un-induced iPSCs. See Supplementary Table 2 for details. **B,** Immunohistochemical detection of pluripotency markers (Nanog, Oct4, Sox2), and markers of differentiation to the three germ layers (Nestin, Gata4, CD34) in teratomas derived from un-induced iPSCs or *mi*iPSCs. Scale bars, 500 μm and 100 μm for higher magnifications. **C,** Immunodetection of CD31 (bone marrow), CD73 (pancreas) and collagenase type I (cartilage) in teratomas generated from *mi*iPSCs. Scale bars, 20 μm. **D,** Immunohistochemical analysis of teratomas derived from wild-type iPSCs or *mi*iPSCs. Antibodies against the proliferation marker Ki67 or terminal differentiation to smooth muscle actin (SMA) or skeletal muscle actin (S-actin) were used. Scale bars, 500 μm and 100 μm for higher magnifications.

Formation of chimeras

Cells	**iPSC (n=2)**	***mi*iPSC (n=2)**	**ESC (n=2)**	***mi*ESC (n=2)**
Transferred embryos	135	137	68	69
Chimeras/born pups	2/4	8/10	8/12	19/19
Chimerism efficiency	50%	80%	67%	100%
Adult chimeras with G.T. (mice with 100% chimerism)	1 (0)	5 (2)	1 (1)	10 (7)

Appendix Figure S5. Transient exposure to miR-203 in vitro improves the in vivo developmental potential of iPSCs and ESCs in chimera assays. Table showing the frequency of chimeric contribution exhibited by un-induced iPSCs and ESCs or *mi*iPCs and *mi*ESCs previously exposed to miR-203 (2 independent clones were analyzed per condition).

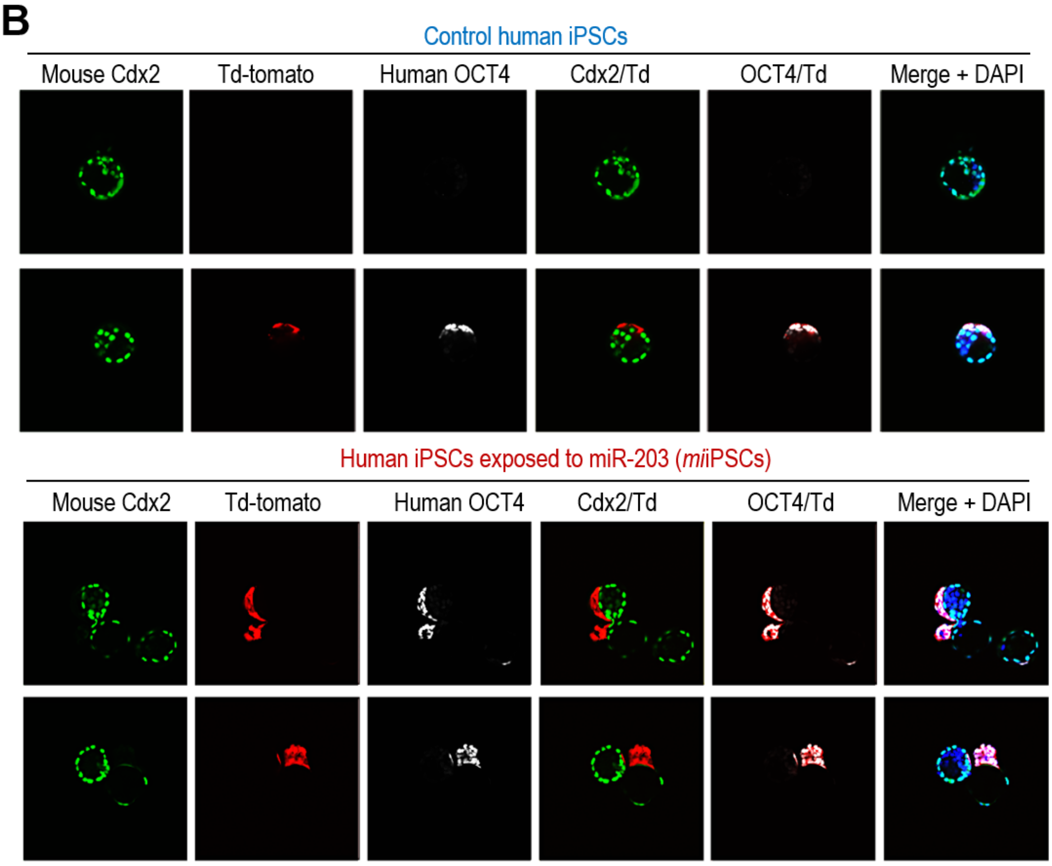
A

	Total embryos	Blastocysts with Td+	Blastocysts with Td+/OCT4+	Percentage of efficiency
Control hiPSCs P31	10	6	6	60%
Human *mi*iPSCs P27+2	11	11	11	100%
Human *mi*iPSCs P30+0	11	11	11	100%

P=0.03679

P=0.03679

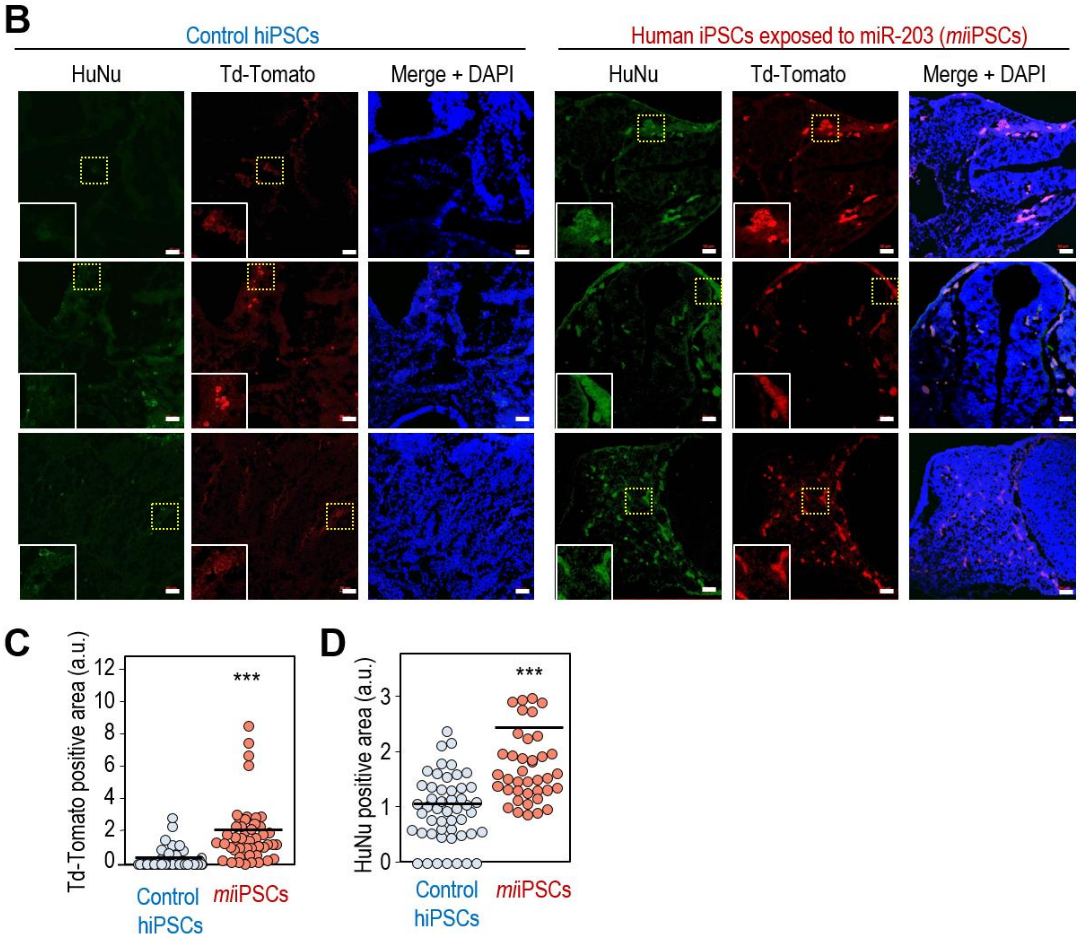
B



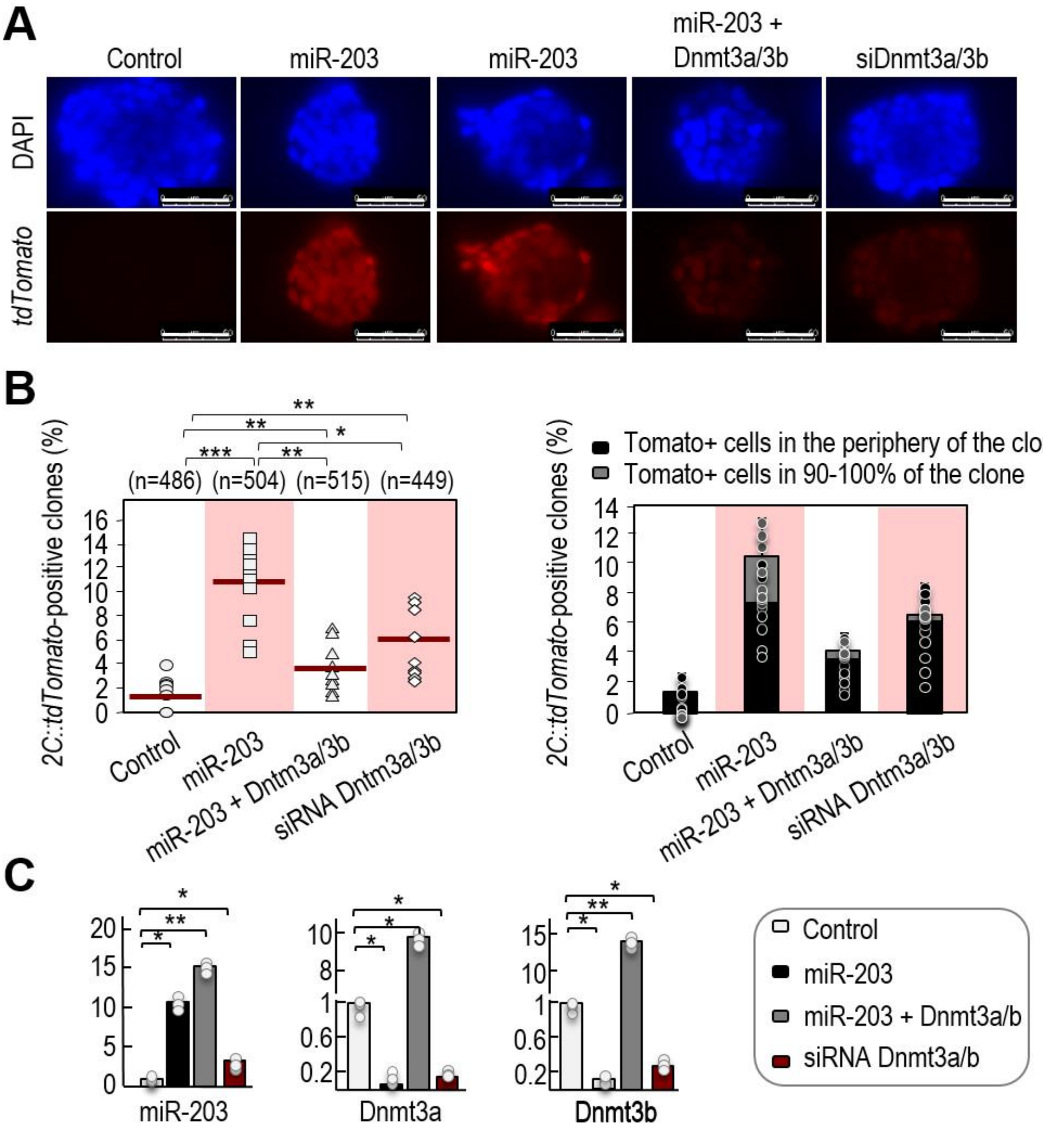
Appendix Figure S6. Human *mi*iPSCs efficiently contribute to chimerism in 8C-stage mouse embryos (15 human cell injected). A, Summary of chimera assays in which 15 Td-Tomato fluorescent-labeled human iPSCs, either control or transiently transfected with miR-203-expressing vectors (*mi*iPSCs) were injected into 8C-stage mouse embryos and their contribution analyzed 48-60 h later. **B,** Representative images showing the contribution of human control iPSCs or *mi*iPSCs cells to mouse blastocysts. These structures were co-immunostained with (human) anti-OCT4 and (human/mouse) anti-Cdx2 antibodies. Td-Tomato, red; Scale bars, 20 μm.

A

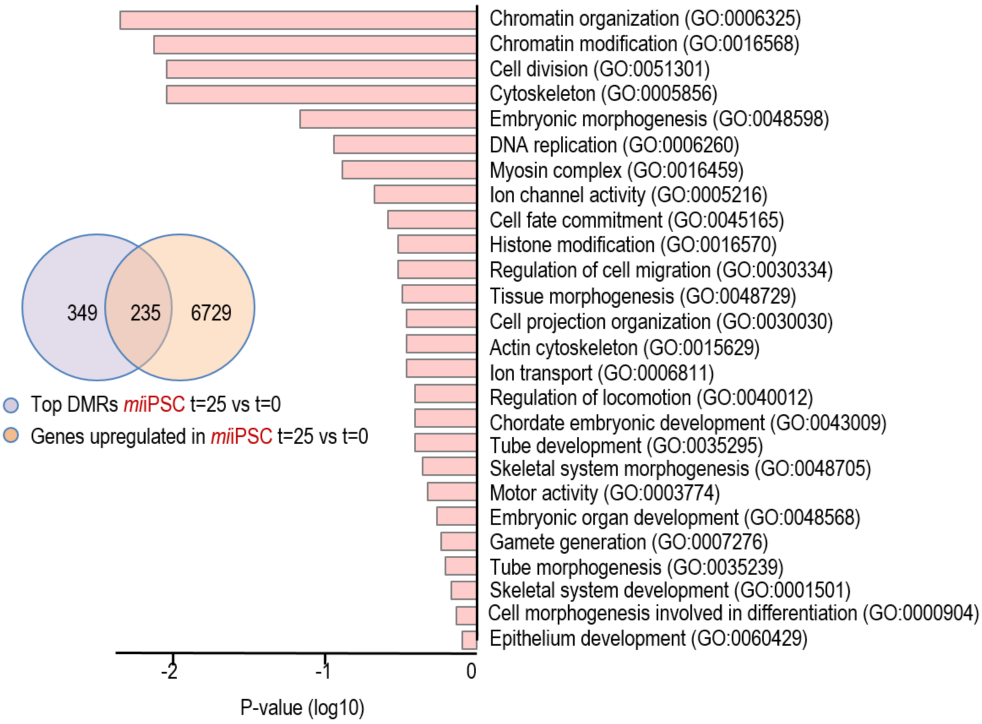
	Injected embryos	Tranferred embryos	Recovered embryos	Normal size embryos	Small size embryos	Implantation sites
Control hiPSCs P34	22	22	7	5 (71.5%)	2 (28.5%)	6 (27.3%)
Human *mi*iPSCs P27+6	26	25	12	8 (66.7%)	4 (33.3%)	9 (36%)
Human *mi*iPSCs P29+4	27	25	4	3 (75%)	1 (25%)	2 (8%)
Human *mi*iPSCs P30+4	30	28	8	6 (75%)	2 (25%)	16 (57.1%)



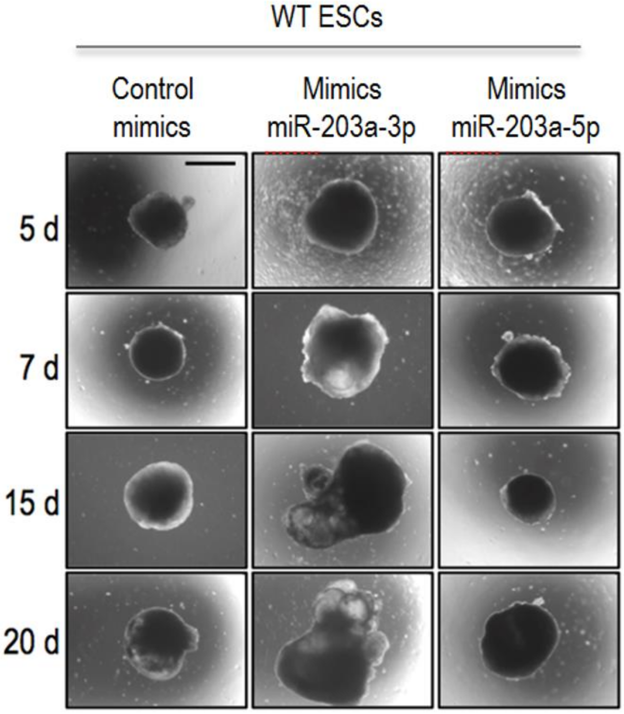
Appendix Figure S7. Human *mi*iPSCs efficiently contribute to chimerism in post-implantation mouse conceptuses (15 human cell injected). A, Summary of interspecies chimera assays in which 15 Td-Tomato fluorescent-labeled human iPSCs, either control or transiently transfected with miR-203-expressing vectors (*mi*iPSCs), were injected into 8C-stage mouse embryos. These embryos were cultured in vitro for 48 h to reach the blastocyst stage and then transfected to 2.5 day post coitum pseudo-pregnant females. The post-implantation mouse conceptuses were dissected at the E10.5 developmental stage and analyzed by immunofluorescence. **B**, Interspecies chimera assays in which 8C-stage mouse embryos were injected with 15 human iPSCs, cultured in vitro for 48 h to reach the blastocyst stage and then transfected to 2.5 day post coitum pseudo-pregnant females. The post-implantation mouse conceptuses were dissected at the E10.5 developmental stage and analyzed by immunofluorescence. Representative images showing the integration of control human iPSCs (left) or *mi*iPSCs (right) into mouse E10.5 embryos. Anti-human HuNu antibody was co-stained with Td-Tomato direct fluorescence to detect human cells in E10.5 mouse embryos. The insets are enlargements of the yellow boxes. Scale bars, 50 μm. **C,D,** Quantification of the Td-Tomato- (**C**) or HuNu- (**D**) positive area in 40-50 different cryosections of these embryos randomly and blindly selected for IF analysis. ***$P < 0.001$ (Student´s t-test).



Appendix Figure S8. DNA methyltransferases 3a and 3b are miR-203 targets involved in its induction of the 2C-like stage. A, Detection of tdTomato and DAPI in ESCs stably expressing the *2C::tdTomato* reporter, and transiently transfected with either control mimics, miR-203 mimics, miR-203 mimics + Dnmt3a and Dnmt3b cDNAs or siRNA against both *Dnmt3a* and *Dnmt3b* transcripts. Scale bar, 50 μm. **B,** The left histogram shows the percentage of Tomato-positive cells in the assays in panel **A** five days after transfection. The right histogram shows the percentage of Tomato-positive colonies in which Tomato is expressed only in the periphery of the clone (black) or in the majority of the cells constituting the colony (grey). Data are mean ± s.e.m. (n=3 independent experiments; 486 colonies for control mimics, 504 colonies for miR-203 mimics, 515 colonies for miR-203 mimics + Dnmt3a/3b cDNA and 449 colonies for *Dnmt3a/3b* siRNAs). **C,** miR-203, *Dnmt3a* and *Dnmt3b* transcript expression in *2C::tdTomato* ESCs transiently transfected as indicated in **A**. RNA expression was measured 24 hours after the transfection protocols and was normalized by a control miRNA (miR-142) or GAPDH mRNA, respectively. Data are mean ± s.e.m. (n=3 independent experiments) **P*<0.05; ***P*<0.01; ****P*<0.001 (Student´s t-test).



Appendix Figure S9. Common genes up-regulated and hypomethylated in *mi*iPSC 20 days after Dox withdrawal. Venn Diagrams representing the common genes up-regulated (data from RNA seq studies) and hypomethylated (data from genome-wide methylation studies) in *mi*iPSC 20 days after Dox withdrawal. A total of 235 genes (**Appendix Table S5**) were both DNA-hypomethylated and up-regulated in such conditions. Gene Ontology Analysis of this list is presented in the right panel and extended in **Appendix Table S6**.



Appendix Figure S10. Improved embryoid body formation after transient exogenous expression of miR-203a-3p *versus* miR-203a-5p. Representative images of EBs derived from wild-type ESCs transfected with control mimics, miR-203a-3p or miR-203a-5p mimics, at different time points during the differentiation process. Scale bars, 500 µm.

Appendix Tables

Appendix Table S1 | Gene Ontology Analysis of genes significantly deregulated in *mi*iPSC-derived teratomas vs. un-induced iPSC-derived teratomas.

Gene Ontology	log10 (p-value)	Transcripts
Extracellular matrix organization (GO:0030198)	-10.9	*Col1a1, Nid1, Col5a1, Postn, Lox, Col3a1, Lamc1, Nepn, Col1a2, Fn1, Serpinh1, Hspg2, Cyr61, Adamts2, Col11a1, Col5a2, Foxf1a, Itga8, Klf4, Pdgfra, Vmo1*
Cell-substrate adhesion (GO:0031589)	-10.0	*Col1a1, Nid1, Thbs1, Col3a1, Lamc1, Ctgf, Fn1, Sox17, Tek, Cyr61, Fbln2, Itgbl1, Klf4, Nid2, Rell2*
Organ morphogenesis (GO:0009887)	-8.4	*Col1a1, Igf2, Lama1, Thbs1, Col5a1, Hand1, Afp, Gata4, Myl3, Ctgf, Fst, Col4a2, Col1a2, Phlda2, Figf, Snai1, Tgfbr3, Hand2, Shank3, Sox17, Tek, Mybpc3, Hspg2, Cyr61, Enpep, Cited1, Edn1, Actc1, Ahsg, Alx3, Ang, Bmp4, Ccl2, Cfc1, Col11a1, Col5a2, Cryaa, Crygs, Fgf3, Flt1, Foxf1a, Ghr, Hoxa13, Hoxc11, Hoxd9, Itga4, Itga8, Klf4, Mmp2, Msx2, Myh6, Myl2, Pdgfra, Rbp4, Tbx20, Tbxa2r, Wnt6*
Cell adhesion (GO:0007155)	-7.4	*Col1a1, Lama1, Nid1, Thbs1, Col5a1, Postn, Col3a1, Lamc1, Ctgf, Svep1, Wisp1, Fn1, Pcdh11x, Sox17, Spon2, Tek, Mybpc3, Hspg2, Cyr61, Col12a1, Lamc3, Podxl, Adam12, B4galnt2, Cdh3, Cntnap1, Cntnap5b, Col11a1, Col6a1, Col9a1, Dpp4, Fbln2, Hapln1, Igsf9b, Itga4, Itga8, Itgbl1, Kcp, Klf4, Ncan, Nid2, Pcdh10, Pcdh19, Rell2, Vnn1*
Tissue development (GO:0009888)	-6.1	*Col1a1, Lama1, Col5a1, Gata6, Postn, Hand1, Col3a1, Gata4, Myl3, Ctgf, Fst, Col1a2, Snai1, Pth1r, Tgfbr3, Hand2, Igfbp1, Shank3, Sox17, Mybpc3, Cyp26a1, Hspg2, Adamts2, Edn1, Actc1, Ahsg, Bmp4, Cdkn2a, Cfc1, Col11a1, Col5a2, Col9a1, Crygs, Foxf1a, Ghr, Ghsr, Hoxd9, Klf4, Myh6, Myl2, Ntf3, Pitx1, Pln, Rbp4, Sprr2k, Sprr4, Srpk3, Tcf23, Trpv1, Unc45b, Upk3a*
Embryonic skeletal system development (GO:0048706)	-5.4	*Col1a1, Hspg2, Alx3, Col11a1, Hoxb9, Hoxd9, Klf4, Pcsk5, Rbp4*
Skeletal system development (GO:0001501)	-5.1	*Col1a1, Igf2, Col3a1, Ctgf, Col1a2, Pth1r, Hspg2, Edn1, Ahsg, Alx3, Bmp4, Casr, Col11a1, Col5a2, Col9a1, Ghr, Hoxb9, Hoxc11, Hoxd9, Klf4, Mmp2, Nppc, Pcsk5, Pdgfra, Pitx1, Rbp4, Twist2*
Chordate embryonic development (GO:0043009)	-4.9	*Col1a1, Gata6, Hand1, Gata4, Tgfbr3, Hand2, Sox17, Hspg2, Cited1, Edn1, Serpina1b, Cdx2, Alx3, Apba1, Bmp4, Col11a1, Dab2, Foxf1a, Hoxb9, Hoxd9, Insl3, Klf1, Klf4, Myh6, Pcsk5, Pdgfra, Rbp4*
Embryonic development ending in birth or egg hatching (GO:0009792)	-4.8	*Col1a1, Gata6, Hand1, Gata4, Tgfbr3, Hand2, Sox17, Hspg2, Cited1, Edn1, Serpina1b, Cdx2, Alx3, Apba1, Bmp4, Col11a1, Dab2, Evx1, Foxf1a, Hoxb9, Hoxd9, Insl3, Klf1, Klf4, Myh6, Pcsk5, Pdgfra, Rbp4*
Blood vessel development (GO:0001568)	-4.7	*Col1a1, Thbs1, Col5a1, Hand1, Lox, Col3a1, Ctgf, Col4a2, Col1a2, Figf, Tgfbr3, Hand2, Sox17, Tek, Cyr61, Enpep, Cited1, Edn1, Cdx2, Ang, Bmp4, Flt1, Foxf1a, Gja4, Itga4, Klf4, Mmp2, Tbx20, Tbxa2r*
Vasculature development (GO:0001944)	-4.7	*Col1a1, Thbs1, Col5a1, Hand1, Lox, Col3a1, Ctgf, Col4a2, Col1a2, Figf, Tgfbr3, Hand2, Sox17, Tek, Cyr61, Enpep, Cited1, Edn1, Cdx2, Ang, Bmp4, Flt1, Foxf1a, Gja4, Itga4, Klf4, Mmp2, Tbx20, Tbxa2r*
Skeletal system morphogenesis (GO:0048705)	-4.6	*Col1a1, Ctgf, Hspg2, Alx3, Col11a1, Ghr, Hoxd9, Klf4, Mmp2, Pdgfra*
Regulation of cell adhesion (GO:0030155)	-4.5	*Col1a1, Lama1, Nid1, Thbs1, Cyr61, Podxl, B4galnt2, Dpp4, Fbln2, Klf4, Rell2*
Collagen fibril	-4.4	*Col1a1, Col5a1, Lox, Col3a1, Col1a2, Serpinh1, Adamts2, Col11a1, Col5a2, Klf4*

organization (GO:0030199)		
Embryonic development (GO:0009790)	-4.1	*Col1a1, Lama1, Gata6, Hand1, Gata4, Tgfbr3, Hand2, Shank3, Sox17, Hspg2, Cited1, Edn1, Serpina1b, Cdx2, Alx3, Apba1, Bmp4, Cfc1, Col11a1, Dab2, Evx1, Foxf1a, Hoxa13, Hoxb9, Hoxc11, Hoxd9, Insl3, Itga8, Klf1, Klf4, Msx2, Myh6, Pcsk5, Pdgfra, Rbp4, Tbx20, Thbd*
Skin development (GO:0043588)	-4.0	*Col1a1, Col5a1, Col3a1, Col1a2, Adamts2, Col5a2, Klf4*
Positive regulation of cell-substrate adhesion (GO:0010811)	-3.9	*Nid1, Thbs1, Cyr61, Fbln2, Klf4, Rell2*
Cell-matrix adhesion (GO:0007160)	-3.8	*Nid1, Col3a1, Ctgf, Fn1, Sox17, Tek, Itgbl1, Klf4, Nid2*
Cell development (GO:0048468)	-3.6	*Lamc1, Prdm6, Pth1r, Tgfbr3, Hand2, Sox17, Gdf9, Mybpc3, Hba-x, Cyp26a1, Edn1, Cdx2, Actc1, Bmp4, Col11a1, Cryaa, D3Bwg0562e, Dab2, Evx1, Grin2a, Hoxd9, Insl3, Klf1, Klf4, Lgi4, Lmx1a, Myh6, Myl2, Ntf3, Pde3a, Pdgfra, Pitx1, Rbp4, Rtn4rl1*
Collagen biosynthetic process (GO:0032964)	-3.4	*Col1a1, Col5a1, Serpinh1, F2*
Heart development (GO:0007507)	-3.2	*Col5a1, Gata6, Hand1, Col3a1, Gata4, Myl3, Tgfbr3, Hand2, Sox17, Mybpc3, Hspg2, Edn1, Actc1, Bmp4, Cfc1, Col11a1, Itga4, Klf4, Msx2, Myh6, Myl2, Pcsk5, Pln, Rbp4, Tbx20*
Behavior (GO:0007610)	-3.2	*Lama1, Thbs1, Lamc1, Alb, Slc6a3, Sox17, Cyr61, Gabra5, a, Adcy1, Apba1, Apln, Aplnr, Casr, Ccl2, Ccl7, Chrna4, Cyp11a1, Fpr1, Ghsr, Gpr34, Grin2a, Hoxd9, Itga8, Klf4, Trpv1*
Anatomical structure formation involved in morphogenesis (GO:0048646)	-3.1	*Col1a1, Thbs1, Hand1, Ctgf, Col4a2, Figf, Tgfbr3, Hand2, Shank3, Sox17, Tek, Mybpc3, Cyr61, Enpep, Edn1, Cdx2, Actc1, Ang, Bmp4, Fgf3, Flt1, Hoxc11, Klf4, Mmp2, Myh6, Myl2, Rbp4, Tbx20, Tbxa2r*

Appendix Table S2 | List of miR-203 predicted targets among the transcripts downregulated in *mi*iPSCs and involved in the epigenetic regulation of gene expression (GO0040029).

Transcript	Predicted miR-203 targets		
	***mi*iPSC vs iPSC log2(fold_change)**	**Agreement[a]**	**Computational Predictions (method|target-site|start|stop|score)**
Dnmt3a	-0.53	1.43	Miranda|7mer-m8|5437|5458|1.42576278100035|
Dnmt3b	-2.36	1.33	Miranda|Offset 3-8 6mer|612|634|1.32739957876685|
Uhrf2	-0.52	1.31	Miranda|N/A|790|809|1.31334769273349|
Trim27	-0.74	1.29	Miranda|Offset 3-8 6mer|87|107|1.28524392066678|
Tet1	-1.05	1.29	Miranda|8mer|128|149|1.28524392066678|
Apobec1	-0.67	1.26	Miranda|N/A|679|700|1.25714014860006|
Ctcf	-0.65	1.24	Miranda|6mer|925|947|1.24308826256671|
Hells	-1.40	1.24	Miranda|7mer-m8|2779|2799|1.24308826256671|
Klf2	-1.11	1.23	Miranda|N/A|414|437|1.22903637653335|
Mier1	-0.86	1.21	Miranda|6mer|2450|2471|1.21498449049999|
Smarca5	-0.63	1.20	Miranda|N/A|1405|1426|1.20093260446663|
Dnd1	-1.34	1.20	Miranda|Offset 3-8 6mer|247|267|1.20093260446663|
Brca1	-1.51	1.20	Miranda|6mer|692|713|1.20093260446663|
Dpy30	-0.90	0.27	Miranda|N/A|98|118|1.25714014860006| Rnahybrid|Offset 1-7 8mer|109|117|-0.759910346569166|
Rlim	-0.51	-0.75	Pita|8mer|520|526|-0.752279554170068|
Rbm3	-1.04	-0.76	Pita|8mer|442|448|-0.756298393575608|
H2afy	-0.54	-0.76	Targetscan|7mer-m8|474|480|-0.764286890785572|

[a] The agreement score of the target, which is a weighted mean (using each method's experimental AUC) of the computational methods scores in the prediction group.

Appendix Table S3 | Antibodies used in this work.

Antibodies				
Antigen	**Catalog Number**	**Clone number**	**Source Ig**	**Source**
AFP	AF5369	-	goat	R&D Systems
Cd31	Ab28364	-	rabbit	Abcam
Cd34	Ab8158	MEC14.7	rat	Abcam
Cd73	13160	D7F9A	rabbit	Cell Signaling Technology
CK8	AM-TROMA I	-	mouse	CNIO Monoclonal Antibodies Core Unit
Collagenase type I	600-401-103S	-	rabbit	Rockland
Dnmt3a	NB120-13888	64B1446	mouse	Novus Biological
Dnmt3b	NB100-56514	52A1018	mouse	Novus Biological
Gata4 (C-20)	Sc-1237	-	goat	Santa Cruz Biotechnology
GFP	11 814 460 001	7.1+13.1	mouse	Roche
Histone 3 (phospho-Ser10)	06-570	-	rabbit	Millipore
HuNu	NBP2-34342	-	mouse	Novus
Ki67	0003110QD	SP6	rabbit	Master Diagnostica
Nestin	MAB353	RAT401	mouse	Millipore
Nanog	8822	D2A3	rabbit	Cell Signaling Technology
Oct4	Ab19857	-	rabbit	Abcam
Oct4	C30A3	-	rabbit	Cell Signalling
Pax6	Ab5790	-	rabbit	Abcam
Skeletal muscle actin	M0635	HHF35	mouse	Dako
Smooth muscle actin	RB-9010-PO	-	rabbit	Thermo Scientific
Sox17	AF1924	-	human	R&D Systems
Sox2	AF2018	-	h/mouse/rat	R&D Systems
Sox2	3728	C70B1	rabbit	Cell Signaling Technology
Ter119 (LY-76)	550565	Ter119	rat	BD Bioscience
Troponin T	Ab8295	1C11	mouse	Abcam
Vinculin	V9131	h-VIN1	mouse	Sigma Aldrich

Appendix Table S4 | Oligonucleotides used in this work.

Gene	Forward Oligonucleotide (5'-3)	Reverse Oligonucleotide (5'-3)
qRT-PCR (mouse genes)		
Dazl	GGTTTTACCACCCGAACTCTG	TGTGGTTGCTGATGAAGACTG
Dnmt1	CAGAGACTCCCGAGGACAGA	TTTACGTGTCGTTTTTCGTCTC
Dnmt3a	AAACGGAAACGGGATGAGT	ACTGCAATTACCTTGGCTTTCT
Dnmt3a2	GGGCAAACTGAAGTAGTGATGA	TTACACGGCACCTGCTGA
Dnmt3b	CCCTCCCCCATCCATAGT	TCTGCTGTCTCCCTTCATTGT
Dnmt3l	AAGTGAACCGACGGAGCAT	CCGAGTGTACACCTGGAGAGTT
Ecat1	TGTGGGGCCCTGAAAGGCGAGCTGAGAT	ATGGGCCGCCATACGACGACGCTCAACT
Eras	ACTGCCCCTCATCAGACTGCTACT	CACTGCCTTGTACTCGGGTAGCTG
Esg1	GAAGTCTGGTTCCTTGGCAGGATG	ACTCGATACACTGGCCTAGC
Fgf4	CGTGGTGAGCATCTTCGGAGTGG	CCTTCTTGGTCCGCCCGTTCTTA
Gapdh	AGGTCGGTGTGAACGGATTTG	TGTAGACCATGTAGTTGAGGTCA
Gata6	ACCTTATGGCGTAGAAATGCTGAGGGTG	CTGAATACTTGAGGTCACTGTTCTCGGG
Gdf3	GTTCCAACCTGTGCCTCGCGTCTT	AGCGAGGCATGGAGAGAGCGGAGCAG
Hcn1	TGAAGCTGACAGATGGCTCTT	CTGGCAGTACGACGTCCTTT
Isl1	TTGTACGGGATCAAATGCGCCAAG	AGGCCACACAGCGGAAACA
Kcna4	TCATTGCTCTGACCTGATGC	TCACTCAGCTCCCTCAGGAT
Kcnh2	ACGCTTACTGCCAGGGTGAC	GCCGACTGGCAACCAGAG
Myh	CTCAAGCTCATGGCCACTCT	GCCTCCTTTGCTTTTACCACT
Nanog	CAGGTGTTTGAGGGTAGCTC	CGGTTCATCATGGTACAGTC
Nppa	GAACCAGAGGGGAGAGACAGAG	CCCTCAGCTTGCTTTTTAGGAG
Tbx5	AAATGAAACCCAGCATAGGAGCTGGC	ACACTCAGCCTCACATCTTACCCT
Tnnt2	GGCAGCGGAAGAGGATGCTGAA	GAGGCACCAAGTTGGGCATGAACGA
qRT-PCR (rat genes)		
Ccnb1	GGAGATGAAGATTCTGAGAGTTCTG	GTATGCTGCTCCACATCGAC
Gapdh	GGCAAGTTCAATGGCACAGT	TGGTGAAGACGCCAGTAGACTC
Myh6	GGGCTGGAGCACTGAGAG	GAGAGAGGAACAGGCAGGAA
Myh7	ATGGCGGATCGAGAGATG	GGTCAAAGGGCCTGGTCT
DNA methylation analysis (mouse genes)		
Elf5 (a)	TAAAGGTTGTAATGAATAGATATTAGGTT	AACTACTTACTTAAAAACAAATAATAACTAAA
Elf5 (b)	TAAAGGTTGTAATGAATAGATATTAGGTT	AAATAATAACTAAATCCAAACAAAAAA
Sirt6	TTTGGTTTTTTTTAGGTTATGTTAGGATTT	CACTTACCTCTACCTCCCAATAAAAAA

Appendix Table S5 | List of genes transcriptionally induced with differentially methylated regions (DMRs) in their promoter regions.

Gene	log2 (fold_change)	Chromosome location	no.cpgs[a]	minfdr[b]	meanbetafc[c]
Card9	3.0310	chr2:26361346-26362230	6	0.0030	-0.4396
1500009C09Rik	2.3588	chr15:82251014-82252426	17	0.0010	-0.2494
Tex13	2.3359	chrX:140812121-140813081	27	2.42E-06	-0.3500
Lgals4	1.4802	chr7:28831931-28832324	9	4.8360	-0.3927
Sap25	1.2865	chr5:137638374-137640152	34	4.20E+06	-0.3430
Mapk15	0.9872	chr15:75994558-75995081	8	0.8020	-0.3733
Nos1	0.9719	chr5:117866360-117867152	24	0.0001	-0.4193
Soat2	0.9490	chr15:102151110-102151341	6	0.0007	-0.4320
Lpar5	0.9400	chr6:125071498-125071516	6	0.0390	-0.5524
Prtn3	0.8944	chr10:79882608-79882829	7	0.8630	-0.3877
Dnajc28	0.8890	chr16:91620043-91621346	8	0.0010	-0.4510
Ceacam19	0.8198	chr7:19888419-19888594	8	8.3210	-0.5751
Apoc1	0.8002	chr7:19691828-19691867	8	0.3060	-0.5509
Tmem44	0.7737	chr16:30540825-30540981	7	0.0010	-0.4173
Dnd1	0.7659	chr18:36765802-36768800	50	0.0020	-0.1943
Fry	0.7580	chr5:150429942-150430220	8	0.0001	-0.3863
Sema6c	0.7013	chr3:95170148-95170396	7	0.0010	-0.3566
Tfeb	0.6646	chr17:47764154-47764330	7	0.0140	-0.4370
Ptch2	0.6619	chr4:117109577-117110528	6	0.0020	-0.4515
B4galnt4	0.6574	chr7:141061951-141062856	10	1.93E-07	-0.4494
Kdm2b	0.6456	chr5:122888623-122888738	13	3.77E-04	-0.5278
Morc1	0.6422	chr16:48431394-48432028	11	0.0020	-0.4749
Sumf2	0.6154	chr5:129850790-129850841	6	5.05E-06	-0.6486
Zbtb8b	0.5967	chr4:129442499-129443802	16	1.7880	-0.2598
Tas1r1	0.5588	chr4:152037421-152037714	6	0.0010	-0.4722
2410021H03Rik	0.5357	chr17:69277011-69279087	17	0.0760	-0.3687
Hk2	0.5190	chr6:82726890-82728420	31	0.1470	-0.4785
Slc25a19	0.5129	chr11:115625780-115626268	10	0.1270	-0.4684
Fblim1	0.5024	chr4:141595395-141595880	7	0.0020	-0.3900
Isyna1	0.4849	chr8:70595767-70596309	7	6.5780	-0.3871
Dtx1	0.4821	chr5:120680962-120680998	6	0.8520	-0.5594
Bcar3	0.4743	chr3:122505007-122505061	7	9.93E-08	-0.6311
Dhx16	0.4722	chr17:35889547-35892069	21	0.0630	-0.2437
Brsk1	0.4713	chr7:4692920-4693021	8	1.47E+09	-0.5558
Chaf1b	0.4604	chr16:93902698-93902909	12	0.0800	-0.5051
Mical3	0.4550	chr6:121001902-121002594	16	6.5660	-0.4533
Nfam1	0.4456	chr15:83023787-83024139	7	0.3060	-0.5867
Prune	0.4431	chr3:95279872-95280422	10	0.0001	-0.4312
Mybl2	0.4419	chr2:163074868-163075964	9	0.6760	-0.4909
Bcam	0.4301	chr7:19758423-19760076	17	3.53E+05	-0.5122
Zfp704	0.4122	chr3:9566601-9568084	9	0.0001	-0.4371
Cdc123	0.4090	chr2:5811880-5812644	16	0.0210	-0.3887
Impa2	0.4084	chr18:67287190-67287362	6	0.0010	-0.5031
Dpf1	0.4082	chr7:29315648-29316092	17	4.54E+06	-0.4304
Cenpa	0.4058	chr5:30668129-30668188	8	0.2960	-0.5096
Foxp4	0.4022	chr17:47878936-47878973	7	1.35E-06	-0.7005
Epha2	0.3852	chr4:141307288-141308695	31	0.0840	-0.3077
Cit	0.3787	chr5:115952556-115953328	17	5.44E-05	-0.4788
Hnf1a	0.3768	chr5:114970850-114970910	6	1.21E-06	-0.6123

Pole	0.3493	chr5:110331107-110331873	9	0.0200	-0.3229
Pou2f1	0.3475	chr1:165880236-165880462	12	0.0004	-0.4728
Rps12	0.3450	chr2:122219923-122219969	9	0.0690	-0.5073
Sh3tc1	0.3445	chr5:35720947-35722044	10	8.3050	-0.3891
Pbx4	0.3368	chr8:69846889-69848885	11	0.0010	-0.2221
Dnajc21	0.3360	chr15:10463618-10467308	28	8.8070	-0.3132
Ncapd2	0.3344	chr6:125174149-125175677	20	0.0010	-0.3534
Mrps24	0.3335	chr11:5716872-5717200	10	0.0003	-0.5126
Tonsl	0.3330	chr15:76633104-76633169	8	9.3910	-0.4776
Naa11	0.3283	chr5:97391967-97392011	7	0.0005	-0.6730
Nrxn2	0.3282	chr19:6454954-6455257	7	0.0010	-0.4474
Crocc	0.3211	chr4:141029712-141029796	7	0.7690	-0.6701
Lrwd1	0.3209	chr5:136134007-136134770	18	2.3530	-0.3000
Rfc2	0.3117	chr5:134595288-134595389	7	0.0001	-0.5346
Zfp532	0.3103	chr18:65627134-65627383	7	2.7160	-0.4654
Zfp593	0.3070	chr4:134244052-134244683	12	8.7590	-0.4690
Hectd3	0.3019	chr4:116999149-116999555	10	0.5230	-0.5729
Cd3eap	0.2939	chr7:19357718-19357912	9	0.0006	-0.3733
Polr1c	0.2900	chr17:46245704-46246139	7	2.26E+08	-0.4857
Clstn3	0.2882	chr6:124446690-124448402	18	2.7050	-0.2512
Sirt6	0.2839	chr10:81624612-81625750	24	0.0380	-0.4846
Dvl2	0.2837	chr11:70006361-70006406	7	0.0470	-0.6015
Vps16	0.2817	chr2:130442204-130442461	6	0.0002	-0.3796
Acin1	0.2767	chr14:54643976-54644009	7	0.2460	-0.3959
Itgb7	0.2757	chr15:102230501-102230851	11	0.0007	-0.4679
Tcf3	0.2745	chr10:80410271-80415504	103	0.115	-0.2554
Sox13	0.2739	chr1:133383042-133383626	9	7.12E-11	-0.4723
Amn	0.2735	chr12:111269764-111270757	6	1.5150	-0.5448
Rps11	0.2725	chr11:116382105-116382347	7	0.0007	-0.3943
Odf2	0.2722	chr2:29914743-29914913	8	0.0002	-0.3648
Nap1l1	0.2716	chr10:111478164-111478910	7	8.9270	-0.4955
Zmiz2	0.2697	chr11:6397777-6399854	11	0.0140	-0.4916
Lrfn1	0.2682	chr7:28458783-28458949	14	0.0001	-0.2905
Mks1	0.2627	chr11:87856432-87856653	10	0.0050	-0.4992
Gse1	0.2621	chr8:120572314-120572775	8	0.0120	-0.6164
Uck2	0.2608	chr1:167236596-167236652	6	0.5220	-0.6910
Cacnb1	0.2588	chr11:98011206-98014598	69	0.4200	-0.2062
Myo10	0.2578	chr15:25768670-25768729	7	0.0450	-0.4493
Slc25a12	0.2571	chr2:71366259-71366749	7	0.0100	-0.4947
Tns3	0.2567	chr11:8466716-8466927	8	0.1420	-0.5812
Zc3hc1	0.2555	chr6:30374853-30375008	7	0.0010	-0.4496
Cks1b	0.2549	chr3:89414585-89416536	33	0.0170	-0.4376
Rps8	0.2529	chr7:13333106-13333184	9	0.0003	-0.5923
Cntrob	0.2503	chr11:69319459-69319995	12	0.0010	-0.2533
Thrap3	0.2468	chr4:126165523-126165568	6	1.0830	-0.5680
Rpl21	0.2449	chr6:90846491-90847412	8	0.0040	-0.0763
Pak4	0.2434	chr7:28594517-28596147	21	4.82E-13	-0.4828
Uchl1	0.2409	chr5:66678486-66678753	8	0.0110	-0.5205
Trf	0.2374	chr9:103227874-103227970	6	0.0010	-0.4441
Nif3l1	0.2353	chr1:58456266-58456635	8	2.6680	-0.3932
Park7	0.2345	chr4:150900352-150901445	21	4.50E+06	-0.4359
Trim2	0.2345	chr3:84303967-84304240	8	0.0008	-0.3292
Jmjd6	0.2331	chr11:116839751-116840233	12	0.0050	-0.4929
Nbeal2	0.2305	chr9:110638420-110638536	10	0.0010	-0.5547

Plagl1	0.2303	chr10:13112825-13122663	90	0.0040	-0.2826
Smchd1	0.2298	chr17:71393041-71396092	26	0.0040	-0.1220
Trmu	0.2285	chr15:85877278-85877567	6	0.0002	-0.6153
Zc3h15	0.2280	chr2:83655204-83655519	10	0.0001	-0.3716
Fastkd1	0.2266	chr2:69712291-69712474	12	6.89E+06	-0.5398
Gfm2	0.2256	chr13:97161099-97161825	18	0.0720	-0.1601
Phf10	0.2256	chr17:14944229-14945883	8	0.0006	-0.2638
Dhx30	0.2247	chr9:110084571-110084637	6	2.7820	-0.6059
Ptov1	0.2243	chr7:44870982-44871042	6	0.0050	-0.6223
Racgap1	0.2221	chr15:99639637-99639790	10	0.1260	-0.5171
Esrrb	0.2220	chr12:86505820-86505961	9	0.0010	-0.4875
Zbtb48	0.2212	chr4:152020406-152020687	8	0.0003	-0.4390
Jakmip1	0.2185	chr5:37085385-37085514	8	3.16E+08	-0.5586
Gtpbp4	0.2163	chr13:8994607-8994658	6	0.0003	-0.6035
Abt1	0.2135	chr13:23422043-23422602	26	4.31E-08	-0.4918
Vars	0.2117	chr17:35011265-35012491	12	1.48E+06	-0.5346
Got1	0.2097	chr19:43516007-43516515	14	0.0007	-0.3548
Myl7	0.2065	chr11:5899323-5899371	6	0.0020	-0.4525
Exosc10	0.2063	chr4:148563038-148564654	36	4.13E-04	-0.2574
Prex1	0.2016	chr2:166598038-166601919	22	1.00E+09	-0.4438
Top3a	0.2014	chr11:60752044-60753293	24	0.0005	-0.3589
Ncaph2	0.2011	chr15:89371716-89372226	14	2.54E+06	-0.4929
Alpl	0.1979	chr4:137794259-137795052	13	8.84E+07	-0.4604
Agbl5	0.1933	chr5:30891131-30892018	17	0.0015	-0.4101
Akap9	0.1906	chr5:4059568-4060361	11	3.5750	-0.4169
Atad5	0.1885	chr11:80103891-80103913	10	2.8790	-0.6230
Det1	0.1879	chr7:78843191-78843858	12	0.0670	-0.6086
Dnajc11	0.1834	chr4:151967333-151968570	8	0.0002	-0.5787
Iffo1	0.1818	chr6:125150967-125151153	7	0.1310	-0.4687
Mc5r	0.1813	chr18:68339054-68339604	20	2.66E-04	-0.5260
Dus1l	0.1808	chr11:120787527-120788151	6	0.0090	-0.4824
Gtpbp6	0.1772	chr5:110103744-110104347	21	0.4050	-0.3806
Shkbp1	0.1762	chr7:27351821-27353273	30	0.0004	-0.2702
Zfp60	0.1752	chr7:27737408-27738831	7	0.0006	-0.3710
Secisbp2	0.1741	chr13:51682031-51682142	7	0.0040	-0.6759
Ptbp1	0.1740	chr10:79861773-79862188	15	1.3530	-0.4227
Prkar2a	0.1729	chr9:108746985-108747991	17	3.17E+03	-0.4623
Filip1	0.1724	chr9:79830256-79830338	7	0.657	-0.6139
Iars2	0.1676	chr1:185287299-185287371	10	4.22E-06	-0.5598
Radil	0.1655	chr5:142475300-142485935	139	0.0620	-0.1954
Aars	0.1638	chr8:111042668-111042815	14	2.25E+05	-0.4703
Ccnt1	0.1632	chr15:98544549-98545004	13	0.0070	-0.4779
Tsr1	0.1602	chr11:74900020-74901322	13	4.5920	-0.4345
Tnrc18	0.1546	chr5:142762696-142764773	39	0.0005	-0.2937
Vangl2	0.1511	chr1:172007981-172009236	33	0.0011	-0.3544
Atp5g3	0.1492	chr2:73907602-73909937	17	0.0004	-0.3202
Ubap2	0.1449	chr4:41201473-41203374	18	1.0110	-0.2629
Iars	0.1447	chr13:49703042-49704932	18	1.46E+08	-0.3757
Slc38a3	0.1437	chr9:107657872-107658004	7	7.8380	-0.4992
BC021614	0.1388	chr19:4059680-4060040	8	0.0020	-0.4783
Traf3ip1	0.1363	chr1:91518727-91519400	13	0.0010	-0.2607
Smpd2	0.1326	chr10:41487060-41488055	6	0.1260	-0.4790
Aldh7a1	0.1316	chr18:56528350-56529182	10	0.2550	-0.5182
Top1mt	0.1300	chr15:75676582-75676912	9	1.4720	-0.3281

Clcn3	0.1288	chr8:60956331-60957191	17	0.0010	-0.2728
Psrc1	0.1280	chr3:108387230-108387356	7	5.5720	-0.5486
Tnpo1	0.1271	chr13:98889338-98889354	9	0.0030	-0.6069
Chmp7	0.1241	chr14:69730221-69731301	17	0.0001	-0.3227
2700060E02Rik	0.1238	chr14:19822664-19826559	126	0.0002	-0.1172
Ppef2	0.1234	chr5:92229344-92235736	75	0.4080	-0.1644
Dapk3	0.1227	chr10:81184084-81185082	13	0.0035	-0.3535
Cdk2ap1	0.1211	chr5:124349404-124349443	6	0.0011	-0.4364
Krt42	0.1209	chr11:100263299-100265351	38	0.0310	-0.4086
Myo1b	0.1207	chr1:51896163-51896224	6	0.0001	-0.5081
Ddx6	0.1199	chr9:44606787-44607581	7	0.1670	-0.4295
Prkab1	0.1153	chr5:116021558-116021689	8	0.1530	-0.5096
Pomt1	0.1075	chr2:32229084-32235234	79	1.4770	-0.3072
Zc3h18	0.1047	chr8:122408007-122408692	7	0.0005	-0.4486
Rtf1	0.1046	chr2:119709485-119710363	7	0.0034	-0.2687
Top3b	0.1040	chr16:16872546-16874776	16	0.0002	-0.2461
Arl6ip1	0.1019	chr7:118126877-118128245	10	6.2640	-0.4010
Eid2	0.1013	chr7:28269825-28274860	36	5.0430	-0.2739
Parl	0.1008	chr16:20297449-20299212	21	0.0012	-0.4763
Timm17a	0.0991	chr1:135309837-135310477	7	3.82E-06	-0.4633
Tdg	0.0974	chr10:82644346-82644394	7	0.0010	-0.6260
Men1	0.0960	chr19:6338886-6339016	8	2.9010	-0.3396
Tbc1d24	0.0959	chr17:24198261-24198855	6	8.3620	-0.5230
Prrc2b	0.0948	chr2:32213479-32214968	15	0.0240	-0.5174
Klf3	0.0932	chr5:64815428-64817322	33	0.0800	-0.3528
Cox4i1	0.0928	chr8:120672644-120672694	6	0.6360	-0.5053
Cpsf6	0.0906	chr10:117356042-117356122	9	2.4170	-0.5502
R3hdm2	0.0904	chr10:127418049-127424591	50	0.0150	-0.1616
Vdac1	0.0901	chr11:52376482-52386901	105	0.0021	-0.2257
Zwilch	0.0893	chr9:64148962-64149781	8	0.0006	-0.4712
Nav1	0.0879	chr1:135465846-135465950	8	0.1820	-0.5859
E2f7	0.0877	chr10:110754202-110754291	6	0.0001	-0.5316
Drg2	0.0857	chr11:60460762-60460839	8	3.1860	-0.4773
Pycr2	0.0843	chr1:180906215-180907783	48	0.0050	-0.2529
Anapc13	0.0842	chr9:102624917-102625037	6	9.4800	-0.5179
Lsm4	0.0817	chr8:70677853-70678480	7	0.0002	-0.3966
Tmem63b	0.0811	chr17:45665139-45665728	15	0.0770	-0.5041
Hspa9	0.0770	chr18:34950400-34950768	9	5.3070	-0.4960
Kdm4b	0.0768	chr17:56370706-56371155	11	8.53E+04	-0.5164
Dcaf15	0.0755	chr8:84102177-84102999	17	4.4500	-0.3282
Scai	0.0752	chr2:39105994-39107017	11	0.0003	-0.1717
Fbxo31	0.0709	chr8:121558124-121558989	7	0.0003	-0.4397
Smarca5	0.0670	chr8:80724816-80726986	10	0.0645	-0.4076
Hsp90aa1	0.0645	chr12:110704558-110704774	8	6.48E-07	-0.4935
2210016L21Rik	0.0619	chr5:114944212-114945943	7	0.0003	-0.5815
Ddx5	0.0616	chr11:106782217-106782484	6	0.0003	-0.3658
Csnk2b	0.0593	chr17:35118996-35120084	11	0.0020	-0.1855
Pias3	0.0574	chr3:96702794-96703600	10	0.0020	-0.4792
Sumo3	0.0571	chr10:77616211-77616338	7	1.26E+09	-0.4807
Alkbh4	0.0557	chr5:136140744-136142305	14	0.1270	-0.2995
Tardbp	0.0557	chr4:148623274-148624465	17	0.0140	-0.3295
Cln3	0.0556	chr7:126572254-126572634	7	0.0020	-0.3839
Shf	0.0553	chr2:122355520-122356667	12	0.1120	-0.3649
Msi1	0.0512	chr5:115431814-115432737	18	0.0002	-0.3109

Coro1c	0.0487	chr5:113880763-113881947	34	0.0008	-0.3454
Son	0.0479	chr16:91659908-91660031	11	5.0060	-0.4297
Cd9	0.0455	chr6:125460674-125464025	64	0.0002	-0.2510
Zfp335	0.0412	chr2:164907220-164907667	18	1.22E+06	-0.4374
Slmo2	0.0394	chr2:174473945-174479354	42	0.0006	-0.2251
Rbpms	0.0369	chr8:33841731-33841752	7	0.0001	-0.5871
Ube2v1	0.0337	chr2:167610327-167612130	21	1.47E+09	-0.4094
Vdac2	0.0325	chr14:21840491-21841860	13	8.6210	-0.3576
Ppp2r5b	0.0321	chr19:6229036-6238486	164	0.0006	-0.1795
P2rx7	0.0295	chr5:122648592-122649715	20	0.0060	-0.4035
Fam161b	0.0289	chr12:84354884-84354967	7	0.0060	-0.5422
Arhgdia	0.0268	chr11:120579214-120579687	14	3.3140	-0.3679
Impact	0.0261	chr18:12974077-12974132	7	0.0001	-0.7501
Nisch	0.0250	chr14:31185002-31185220	9	0.0001	-0.4134
Ptprf	0.0228	chr4:118214221-118215801	14	1.8050	-0.3547
Eya1	0.0224	chr1:14304450-14305238	22	9.41E-11	-0.5070
Nucks1	0.0211	chr1:131928102-131928202	5	0.0004	-0.4003
Npr1	0.0190	chr3:90459070-90459836	11	0.0002	-0.3366
Ccz1	0.0180	chr5:143992596-143993168	16	0.0010	-0.5465
Fryl	0.0165	chr5:73089057-73089316	11	0.0001	-0.5508
Ywhaq	0.0115	chr5:114829129-114829240	10	0.0390	-0.5325
Osbp	0.0063	chr19:11967584-11968641	12	0.0002	-0.2007
Hip1r	0.0047	chr5:123999922-123999964	6	0.0510	-0.3374
Rars2	0.0017	chr4:34647898-34648282	13	0.0001	-0.4473

[a] no.cpgs: Number of CpG sites constituting the DMR.

[b] minfdr: Minimum adjusted p-value from the CpGs constituting the significant region.

[c] meanbetafc: Mean beta fold change within the region.

Appendix Table S6 | Gene Ontology Analysis of genes up-regulated and hypomethylated in *mi*PSCs (+ transient treatment with miR-203) versus control PSCs.

Gene Ontology	Log10 (p-value)	Molecules
Chromosome organization (GO:0051276)	-4.0	*Acin1, Smchd1, Ncapd2, Ncaph2, CenpA, Nap1l1, Men1, Hnf1a, Tcf3, Kdm4b, Smarce1, Kdm2b, Jmjd6Dapk3*
DNA topoisomerase type I activity (GO:0003917)	-3.1	*Top3A, Top3B, Top1MT, POLE, Chaf1B, Tcf3, Isyna1*
Cell cycle (GO:0007049)	-2.7	*Psrc1, Men, Crocc, Cdk2ap1, Chaf1b, E2f7, Fbxo31, Tcf3, Brsk1, Ccnt1, Cks1b Cdc28, Cntrob, Racgap1, Racgap1, Anapc13, Ncapd2, citron, Zc3hc1*
DNA topoisomerase activity (GO:0003916)	-2.7	*Top3A, Top3B, Top1MT, POLE, Chaf1B, Tcf3, Isyna1*
DNA topological change (GO:0006265)	-2.5	*Top3A, Top3B, Top1MT, POLE, Chaf1B, Tcf3, Isyna1*
Chromatin organization (GO:0006325)	-2.3	*Acin1, Smchd1, Ncapd2, Ncaph2, CenpA, Nap1l1, Men1, Hnf1a, Tcf3, Kdm4b, Smarce1, Kdm2b, Jmjd6Dapk3*
DNA unwinding during replication (GO:0006268)	-2.1	*Top3A, Top3B, Top1MT, POLE, Chaf1B, Tcf3, Isyna1*
Chromatin modification (GO:0016568)	-2.1	*Acin1, Smchd1, Ncapd2, Ncaph2, CenpA, Nap1l1, Men1, Hnf1a, Tcf3, Kdm4b, Smarce1, Kdm2b, Jmjd6, Dapk3*
DNA duplex unwinding (GO:0032508)	-2.1	*Top3A, Top3B, Top1MT, POLE, Chaf1B, Tcf3, Isyna1*
Cell division (GO:0051301)	-2.0	*Psrc1, Men, Crocc, Cdk2ap1, Chaf1b, E2f7, Fbxo31, Tcf3, Brsk1, Ccnt1, Cks1b Cdc28, Cntrob, Racgap1, Racgap1, Anapc13, Ncapd2, citron, Zc3hc1*
Cytoskeleton (GO:0005856)	-2.0	*Trim2, Itgb7, Nos1, Dvl2, citron, Hip1r, Coro1a, Mical3, Fblim1, String, Krt42, Myl7, Myo10, Myo1b, Akap9, Traf3ip2, Psrc1, Odf2, Nav, Cntrob, Jakmip1, Racgap1*
DNA geometric change (GO:0032392)	-2.0	*Top3A, Top3B, Top1MT, POLE, Chaf1B, Tcf3, Isyna1*
Voltage-gated anion channel activity (GO:0008308)	-1.7	*Vdac1, Vdac2, Cic3, Cacnb1, P2rx7, Sh3kbp1, Trsf, Atp5g2, Slc38a3, Slc25a12, Racgap1, Timm17b, Slc25a19, Osbp, Chmp7, Jakmip1, Tnpo1, Apoc1, Nos1*
Cell cycle process (GO:0022402)	-1.3	*Psrc1, Men, Crocc, Cdk2ap1, Chaf1b, E2f7, Fbxo31, Tcf3, Brsk1, Ccnt1, Cks1b Cdc28, Cntrob, Racgap1, Racgap1, Anapc13, Ncapd2, citron, Zc3hc1*
DNA-dependent DNA replication (GO:0006261)	-1.2	*Top3A, Top3B, Top1MT, POLE, Chaf1B, Tcf3, Isyna1*
Embryonic morphogenesis (GO:0048598)	-1.2	*P2rx7, Pou2f1, Foxp4, Esrrb, Hnf1a, Dvl2, Vangl2, Epha2*
Mitotic cell cycle (GO:0000278)	-1.1	*Psrc1, Men, Crocc, Cdk2ap1, Chaf1b, E2f7, Fbxo31, Tcf3, Brsk1, Ccnt1, Cks1b Cdc28, Cntrob, Racgap1, Racgap1, Anapc13, Ncapd2, citron, Zc3hc1*
Microtubule cytoskeleton (GO:0015630)	-1.1	*Trim2, Itgb7, Nos1, Dvl2, citron, Hip1r, Coro1a, Mical3, Fblim1, String, Krt42, Myl7, Myo10, Myo1b, Akap9, Traf3ip2, Psrc1, Odf2, Nav, Cntrob, Jakmip1, Racgap1*
DNA replication (GO:0006260)	-1.0	*Top3A, Top3B, Top1MT, POLE, Chaf1B, Tcf3, Isyna1*
Myosin complex (GO:0016459)	-0.9	*Myo10, citron, Myo1b, Myl9*

Cytoskeletal protein binding (GO:0008092)	-0.9	*Trim2, Itgb7, Nos1, Dvl2, citron, Hip1r, Coro1a, Mical3, Fblim1, String, Krt42, Myl7, Myo10, Myo1b, Akap9, Traf3ip2, Psrc1, Odf2, Nav, Cntrob, Jakmip1, Racgap1*
Cytoskeletal part (GO:0044430)	-0.8	*Trim2, Itgb7, Nos1, Dvl2, citron, Hip1r, Coro1a, Mical3, Fblim1, String, Krt42, Myl7, Myo10, Myo1b, Akap9, Traf3ip2, Psrc1, Odf2, Nav, Cntrob, Jakmip1, Racgap1*
Voltage-gated ion channel activity (GO:0005244)	-0.7	*Vdac1, Vdac2, Cic3, Cacnb1, P2rx7, Sh3kbp1, Trsf, Atp5g2, Slc38a3, Slc25a12, Racgap1, Timm17b, Slc25a19, Osbp, Chmp7, Jakmip1, Tnpo1, Apoc1, Nos1*
Nuclear division (GO:0000280)	-0.7	*Psrc1, Men, Crocc, Cdk2ap1, Chaf1b, E2f7, Fbxo31, Tcf3, Brsk1, Ccnt1, Cks1b Cdc28, Cntrob, Racgap1, Racgap1, Anapc13, Ncapd2, citron, Zc3hc1*
Mitosis (GO:0007067)	-0.7	*Psrc1, Men, Crocc, Cdk2ap1, Chaf1b, E2f7, Fbxo31, Tcf3, Brsk1, Ccnt1, Cks1b Cdc28, Cntrob, Racgap1, Racgap1, Anapc13, Ncapd2, citron, Zc3hc1*
M phase of mitotic cell cycle (GO:0000087)	-0.7	*Psrc1, Men, Crocc, Cdk2ap1, Chaf1b, E2f7, Fbxo31, Tcf3, Brsk1, Ccnt1, Cks1b Cdc28, Cntrob, Racgap1, Racgap1, Anapc13, Ncapd2, citron, Zc3hc1*
Anion transport (GO:0006820)	-0.7	*Vdac1, Vdac2, Cic3, Cacnb1, P2rx7, Sh3kbp1, Trsf, Atp5g2, Slc38a3, Slc25a12, Racgap1, Timm17b, Slc25a19, Osbp, Chmp7, J akmip1, Tnpo1, Apoc1, Nos1*
Cell cycle phase (GO:0022403)	-0.7	*Psrc1, Men, Crocc, Cdk2ap1, Chaf1b, E2f7, Fbxo31, Tcf3, Brsk1, Ccnt1, Cks1b Cdc28, Cntrob, Racgap1, Racgap1, Anapc13, Ncapd2, citron, Zc3hc1*
Ion channel activity (GO:0005216)	-0.7	*Vdac1, Vdac2, Cic3, Cacnb1, P2rx7, Sh3kbp1, Trsf, Atp5g2, Slc38a3, Slc25a12, Racgap1, Timm17b, Slc25a19, Osbp, Chmp7, J akmip1, Tnpo1, Apoc1, Nos1*
Organelle fission (GO:0048285)	-0.7	*Psrc1, Men, Crocc, Cdk2ap1, Chaf1b, E2f7, Fbxo31, Tcf3, Brsk1, Ccnt1, Cks1b Cdc28, Cntrob, Racgap1, Racgap1, Anapc13, Ncapd2, citron, Zc3hc1*
DNA metabolic process (GO:0006259)	-0.6	*Top3A, Top3B, Top1MT, POLE, Chaf1B, Tcf3, Isyna1*
Cellular component morphogenesis (GO:0032989)	-0.6	*Citron, Crocc, Cd9, Brsk1, Esrrb, P2rx7, Vangl2, Sema6c, Uchl1*
Neural tube development (GO:0021915)	-0.6	*P2rx7, Pou2f1, Foxp4, Esrrb, Hnf1a, Dvl2, Vangl2, Epha2*
Gated channel activity (GO:0022836)	-0.6	*Vdac1, Vdac2, Cic3, Cacnb1, P2rx7, Sh3kbp1, Trsf, Atp5g2, Slc38a3, Slc25a12, Racgap1, Timm17b, Slc25a19, Osbp, Chmp7, Jakmip1, Tnpo1, Apoc1, Nos1*
Cell fate commitment (GO:0045165)	-0.6	*Citron, Crocc, Cd9, Brsk1, Esrrb, P2rx7, Vangl2, Sema6c, Uchl1*
Histone modification (GO:0016570)	-0.5	*Pou2f1, Eya1, Tcf3*
Regulation of cell migration (GO:0030334)	-0.5	*Gtpbp4, Nisch*
Cell morphogenesis (GO:0000902)	-0.5	*Citron, Crocc, Cd9, Brsk1, Esrrb, P2rx7, Vangl2, Sema6c, Uchl1*
Covalent chromatin modification (GO:0016569)	-0.5	*Acin1, Smchd1, Ncapd2, Ncaph2, CenpA, Nap1l1, Men1, Hnf1a, Tcf3, Kdm4b, Smarce1, Kdm2b, Jmjd6Dapk3*
Tissue morphogenesis (GO:0048729)	-0.5	*P2rx7, Pou2f1, Foxp4, Esrrb, Hnf1a, Dvl2, Vangl2, Epha2*
Cell projection organization (GO:0030030)	-0.5	*Citron, Crocc, Cd9, Brsk1, Esrrb, P2rx7, Vangl2, Sema6c, Uchl1*
Ion transport (GO:0006811)	-0.5	*Vdac1, Vdac2, Cic3, Cacnb1, P2rx7, Sh3kbp1, Trsf, Atp5g2, Slc38a3, Slc25a12, Racgap1, Timm17b, Slc25a19, Osbp, Chmp7, Jakmip1, Tnpo1, Apoc1, Nos1*

Actin cytoskeleton (GO:0015629)	-0.5	*Trim2, Itgb7, Nos1, Dvl2, citron, Hip1r, Coro1a, Mical3, Fblim1, String, Krt42, Myl7, Myo10, Myo1b, Akap9, Traf3ip2, Psrc1, Odf2, Nav, Cntrob, Jakmip1, Racgap1*
Multicellular organism reproduction (GO:0032504)	-0.4	*Cd9, Dvl2, Eya1, Vangl2, Radil, Amn, Hk2, Eid2, Nav1, Sema6c, Jmjd6, Dnd1, citron, Morc1, Racgap1, Odf2*
Reproductive process in a multicellular organism (GO:0048609)	-0.4	*Cd9, Dvl2, Eya1, Vangl2, Radil, Amn, Hk2, Eid2, Nav1, Sema6c, Jmjd6, Dnd1, citron, Morc1, Racgap1, Odf2*
Regulation of cell motion (GO:0051270)	-0.4	*Gtpbp4, Nisch*
Regulation of locomotion (GO:0040012)	-0.4	*Gtpbp4, Nisch*
Tube development (GO:0035295)	-0.4	*P2rx7, Pou2f1, Foxp4, Esrrb, Hnf1a, Dvl2, Vangl2, Epha2*
Chordate embryonic development (GO:0043009)	-0.4	*P2rx7, Pou2f1, Foxp4, Esrrb, Hnf1a, Dvl2, Vangl2, Epha2*
Epithelial tube morphogenesis (GO:0060562)	-0.4	*P2rx7, Pou2f1, Foxp4, Esrrb, Hnf1a, Dvl2, Vangl2, Epha2*
Chordate embryonic development (GO:0043009)	-0.4	*P2rx7, Pou2f1, Foxp4, Esrrb, Hnf1a, Dvl2, Vangl2, Epha2*
Embryonic development ending in birth or egg hatching (GO:0009792)	-0.4	*P2rx7, Pou2f1, Foxp4, Esrrb, Hnf1a, Dvl2, Vangl2, Epha2*
Calcium signaling pathway (mmu04020)	-0.4	*Vdac1, Vdac2, Cic3, Cacnb1, P2rx7, Sh3kbp1, Trsf, Atp5g2, Slc38a3, Slc25a12, Racgap1, Timm17b, Slc25a19, Osbp, Chmp7, Jakmip1, Tnpo1, Apoc1, Nos1*
M phase (GO:0000279)	-0.4	*Psrc1, Men, Crocc, Cdk2ap1, Chaf1b, E2f7, Fbxo31, Tcf3, Brsk1, Ccnt1, Cks1b Cdc28, Cntrob, Racgap1, Racgap1, Anapc13, Ncapd2, citron, Zc3hc1*
Substrate specific channel activity (GO:0022838)	-0.3	*Vdac1, Vdac2, Cic3, Cacnb1, P2rx7, Sh3kbp1, Trsf, Atp5g2, Slc38a3, Slc25a12, Racgap1, Timm17b, Slc25a19, Osbp, Chmp7, Jakmip1, Tnpo1, Apoc1, Nos1*
Skeletal system morphogenesis (GO:0048705)	-0.3	*P2rx7, Pou2f1, Foxp4, Esrrb, Hnf1a, Dvl2, Vangl2, Epha2*
Channel activity (GO:0015267)	-0.3	*Vdac1, Vdac2, Cic3, Cacnb1, P2rx7, Sh3kbp1, Trsf, Atp5g2, Slc38a3, Slc25a12, Racgap1, Timm17b, Slc25a19, Osbp, Chmp7, Jakmip1, Tnpo1, Apoc1, Nos1*
Sexual reproduction (GO:0019953)	-0.3	*Cd9, Dvl2, Eya1, Vangl2, Radil, Amn, Hk2, Eid2, Nav1, Sema6c, Jmjd6, Dnd1, citron, Morc1, Racgap1, Odf2*
Motor activity (GO:0003774)	-0.3	*Myo10, citron, Myo1b, Myl9*
Gamete generation (GO:0007276)	-0.3	*Cd9, Dvl2, Eya1, Vangl2, Radil, Amn, Hk2, Eid2, Nav1, Sema6c, Jmjd6, Dnd1, citron, Morc1, Racgap1, Odf2*
Embryonic organ development (GO:0048568)	-0.3	*P2rx7, Pou2f1, Foxp4, Esrrb, Hnf1a, Dvl2, Vangl2, Epha2*
Embryonic organ	-0.2	*P2rx7, Pou2f1, Foxp4, Esrrb, Hnf1a, Dvl2, Vangl2, Epha2*

morphogenesis (GO:0048562)		
Spermatogenesis (GO:0007283)	-0.2	*Cd9, Dvl2, Eya1, Vangl2, Radil, Amn, Hk2, Eid2, Nav1, Sema6c, Jmjd6, Dnd1, citron, Morc1, Racgap1, Odf2*
Male gamete generation (GO:0048232)	-0.2	*Cd9, Dvl2, Eya1, Vangl2, Radil, Amn, Hk2, Eid2, Nav1, Sema6c, Jmjd6, Dnd1, citron, Morc1, Racgap1, Odf2*
Tube morphogenesis (GO:003523)	-0.2	*P2rx7, Pou2f1, Foxp4, Esrrb, Hnf1a, Dvl2, Vangl2, Epha2*
Morphogenesis of an epithelium (GO:0002009)	-0.2	*Jmjd6, Tns3, Esrrb, Epha2, Hnf1, Eya1, Vangl2, Dvl2*
Skeletal system development (GO:0001501)	-0.2	*P2rx7, Pou2f1, Foxp4, Esrrb, Hnf1a, Dvl2, Vangl2, Epha2*
Cell projection morphogenesis (GO:0048858)	-0.2	*Citron, Crocc, Cd9, Brsk1, Esrrb, P2rx7, Vangl2, Sema6c, Uchl1*
Cell morphogenesis involved in differentiation (GO:0000904)	-0.1	*Citron, Crocc, Cd9, Brsk1, Esrrb, P2rx7, Vangl2, Sema6c, Uchl1*
Cell part morphogenesis (GO:0032990)	-0.1	*Citron, Crocc, Cd9, Brsk1, Esrrb, P2rx7, Vangl2, Sema6c, Uchl1*
Cation transport (GO:0006812)	-0.1	*Vdac1, Vdac2, Cic3, Cacnb1, P2rx7, Sh3kbp1, Trsf, Atp5g2, Slc38a3, Slc25a12, Racgap1, Timm17b, Slc25a19, Osbp, Chmp7, Jakmip1, Tnpo1, Apoc1, Nos1*
Metal ion transport (GO:0030001)	-0.1	*Vdac1, Vdac2, Cic3, Cacnb1, P2rx7, Sh3kbp1, Trsf, Atp5g2, Slc38a3, Slc25a12, Racgap1, Timm17b, Slc25a19, Osbp, Chmp7, Jakmip1, Tnpo1, Apoc1, Nos1*
Epithelium development (GO:0060429)	-0.1	*Jmjd6, Tns3, Esrrb, Epha2, Hnf1, Eya1, Vangl2, Dvl2*