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Markers (%)	MSC lines					
	H9	SA01	VUB03	3814	FS2	4603
CD29/CD105	99,9	96	100	94,5	96,2	99,8
CD29/CD44	99,9	99,6	97,1	96,3	98,2	97,6
CD105/CD44	99,8	95,8	97,8	94,6	95,8	97,7
CD166/CD73	100	99,3	99,1	90,8	94,9	99,2

Figure S1. Characterization of MPCs derived from human pluripotent stem cells. **(A)** Schematic representation of the protocol used for osteogenic differentiation of hPSCs_MPCs. **(B)** Gating strategy and staining representation of MPC surface antigen marker expression analyzed by flow cytometry. **(C)** Flow cytometry analysis of mesenchymal markers CD73, CD29, CD105, CD44, CD166 in MPCs derived from three human embryonic stem cell lines (H9, SA01 and VUB03) and three induced pluripotent stem cell lines (3814, FS2 and 4603).

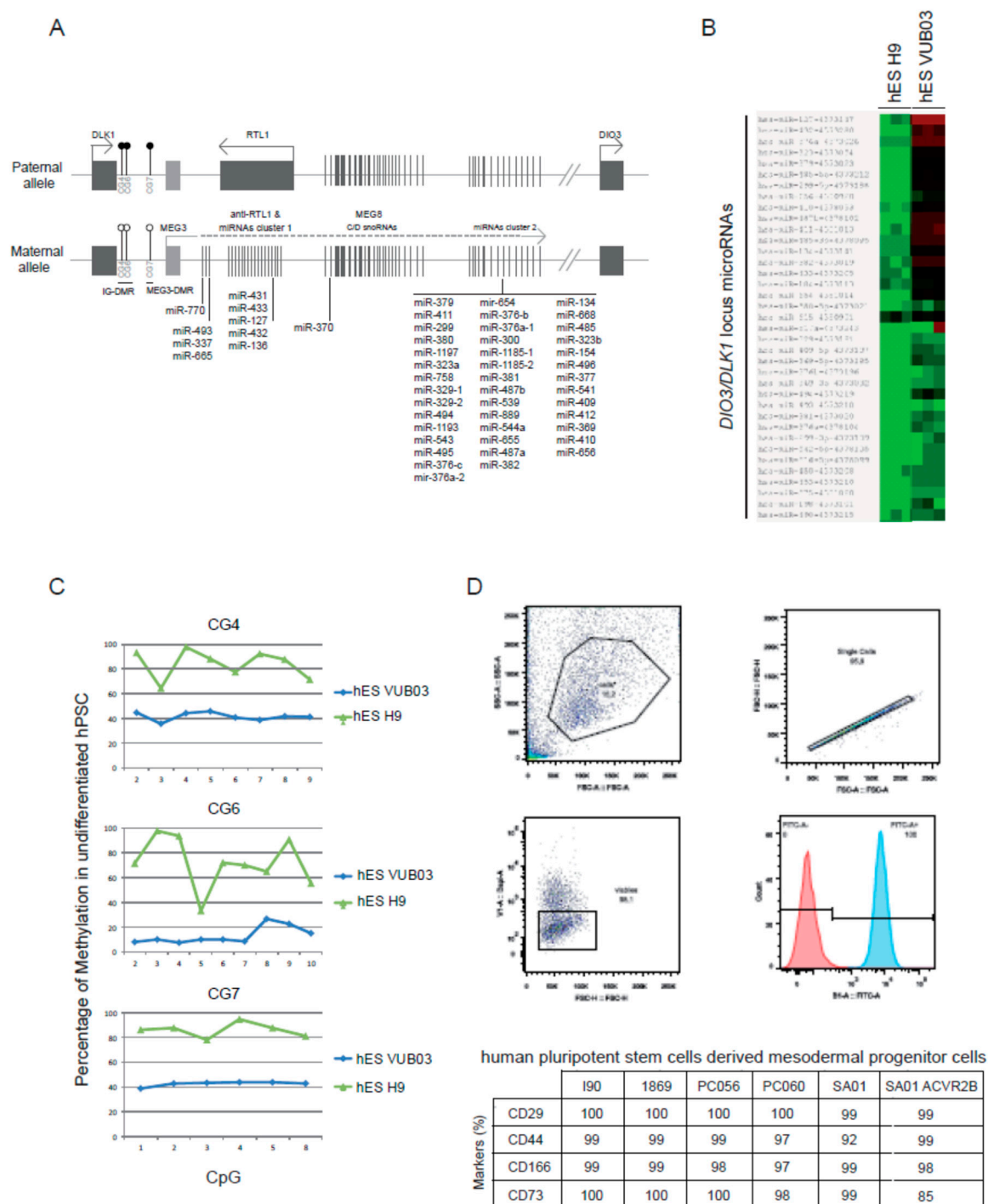


Figure S2. Identification of the differentially expressed miRNAs between hESC lines harboring a variable osteogenic capacity. **(A)** Schematic representation of gene and microRNA organization within the imprinted *DLK1/DIO3* locus. **(B)** Screening of microRNAs differentially expressed in VUB03 and H9 human embryonic stem cell lines by quantitative RT-PCR Taqman Arrays. Data were obtained from 3 independent samples. **(C)** Methylation status analysis of IG-DMR (CG4, CG6) and MEG3-DMR (CG7) CpG islands present in the regulatory sequences of the *DLK1/DIO3* locus from VUB03 and H9 human embryonic stem cell lines. **(D)** Flow cytometry analysis of mesenchymal markers CD29, CD44, CD166 and CD73 in MPCs derived from the four induced pluripotent stem cell lines i90, 1869, PC056 and PC060, the embryonic stem cell line SA01 and the ACVR2B-overexpressing SA01 cell line.

Type 1 TGFb receptors family					Type 2 TGFb receptors family		
ACVR1	ACVR1B	ACVR1C	BMPR1A	BMPR1B	ACVR2A	ACVR2B	BMPR2
DIO3/DLK1 microRNA	miR-770		miR-136		miR-770	miR-770	miR-136
	miR-337					miR-370-3p	
		miR-432				miR-323a-3p	
		miR-494_3p			miR-380	miR-380	miR-329-3p
		miR-543			miR-543	miR-543	miR-494_3p
	miR-496			miR-495-3p		miR-495-3p	
		miR-654-3p					miR-376a-3p
						miR-654-3p	
		miR-487b		miR-300			miR-376b
	miR-1185			miR-381		miR-300	miR-376a
		miR-889-3p		miR-889-3p		miR-1185	miR-300
		miR-889-3p		miR-889-3p	miR-889	miR-381	miR-381
	miR-889	miR-889-3p		miR-889-3p		miR-487b	miR-889-3p
							miR-889-3p
	miR-544					miR-544a-3p	miR-544a-3p
		miR-382					miR-655
		miR-485-3p				miR-323b	
						miR-154	
		miR-541			miR-409-5p	miR-377-3p	
		miR-409-3p				miR-409	
						miR-369-3p	miR-369-3p
		miR-410-3p				miR-410-3p	
	miR-656-3p	miR-656-3p		miR-656-3p			

Figure S3. Table describing the number of miRNA binding sites from the *DLK1/DIO3* locus predicted in the 3'UTR of receptors of the TGFb Pathway.

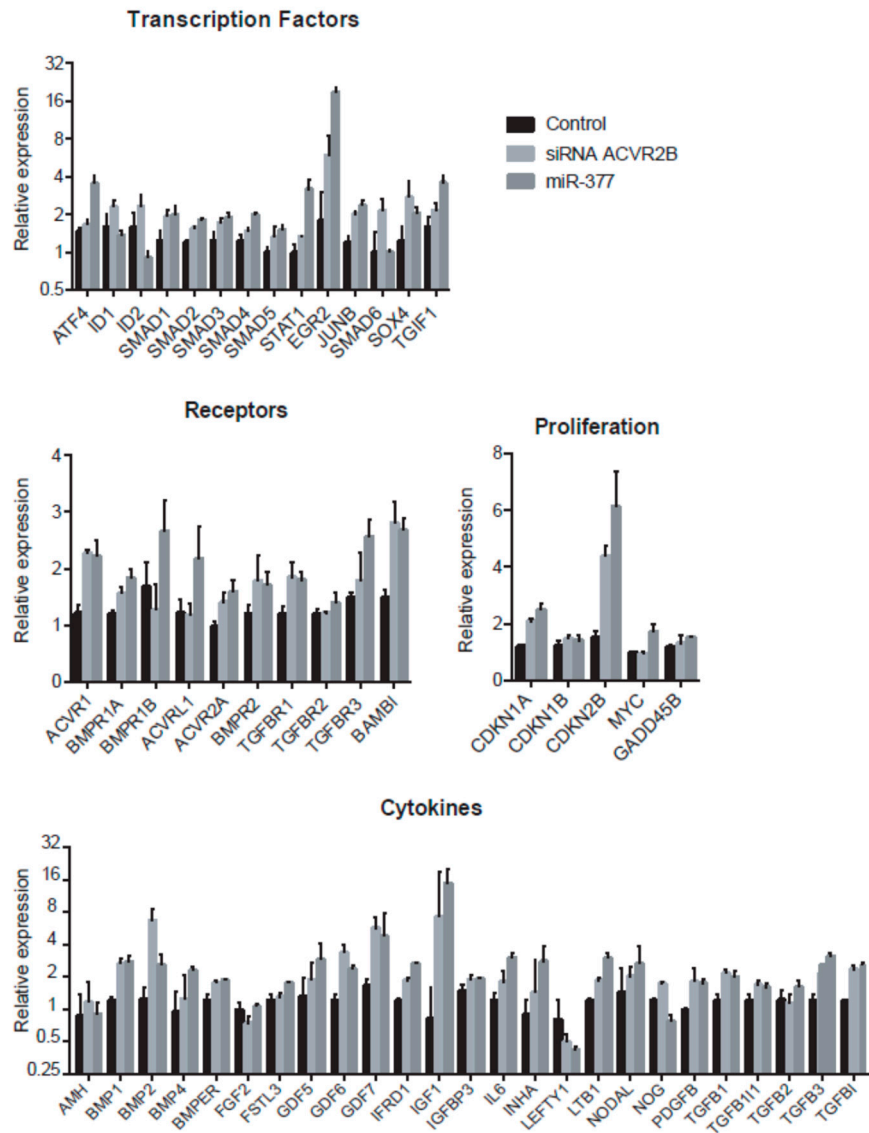


Figure S4. Detail of genes modulation detected using the TGF β /BMP Signaling Pathway Plus PCR Array card. Analysis of gene regulation quantified by RT-QPCR in SA01-MPCS transfected with an siRNA control, siRNA specific for *ACVR2B* or a pre-miR-377-3p. Genes were classified into the following categories: Transcription factors, Receptors, Proliferation and Cytokines. Data in triplicates are represented as the mean \pm SEM.

Table S1. Primer sequences and assays used for genes and microRNAs quantifying.

Gene	Primer	Sequence
<i>RUNX2</i>	RunX2 Fw	5'-ttacttacaccccgcagtc-3'
	RunX2 Rev	5'-tatggagtgtgtgtgtctg-3'
<i>ALPL</i>	ALPL Fw	5'-ccacgtcttcacatttggtg-3'
	ALPL Rev	5'-agactgcgcctgtagttgt-3'
<i>OCN</i>	OCN Fw	5'-gctgagtctgagcagcag-3'
	OCN Rev	5'-ccaatagggcgaggagtgtg-3'
<i>18S</i>	18S Fw	5'-gaggatgaggtggaacgtgt-3'
	18S Rev	5'-tcttcagtcgtccaggtct-3'
miScript miRNA Mimic		Cat. No.
Syn-hsa-miR-300		MSY0004903
Syn-hsa-miR-410-3p		MSY0002171
Syn-hsa-miR-543		MSY0004954
Syn-hsa-miR-377-3p		MSY0000730
Syn-hsa-miR-495-3p		MSY0002817
Syn-hsa-miR-370		MSY0000722
Syn-hsa-miR-544a		MSY0003164
miScript Primer Assay		Cat. No.
Hs_miR-127_1		MS00003437
Hs_miR-377_1		MS00004095
Hs_miR-487b_1		MS00004298
Hs_miR-382_2		MS00031836
Hs_miR-134_2		MS00031437
Hs_RNU6-21		MS00033740