

Supplementary Table S1. Primer sequences used for RT-qPCR.

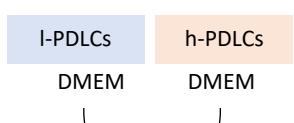
Gene		Primer sequence 5'-3'	Reactions Conditions
<i>ACTB</i>	Forward	CCAACCGCGAGAAGATGA	95 °C – 10"
	Reverse	CCAGAGGCGTACAGGGATAG	61 °C - 10" 72 °C – 10"
<i>OCT4</i>	Forward	CGCAAGCCCTCATTCAC	95 °C – 10"
	Reverse	CATCACCTCCACCACCTG	60 °C – 06" 72 °C – 06"
<i>PPARG</i>	Forward	GCTTATCTATGACAGATGTGATCT	95 °C – 10"
	Reverse	AGCTTCTCCTTCTCGGC	55 °C – 10" 72 °C – 10"
<i>SIX3</i>	Forward	CCTCCTCCTCTTCCTCCTCT	95 °C – 10"
	Reverse	AGTGGTAGATGGTGGTTGGG	60 °C – 20" 72 °C – 10"
<i>SP7</i>	Forward	ATGAGTGGAAAAGGGAGGG	95 °C – 10"
	Reverse	TCAACAACTCTGGGCAAAGC	60 °C – 10" 72 °C – 20"

Supplementary Table S2. Top 60 most differentially expressed genes at basal level between I- and h-PDLCs.

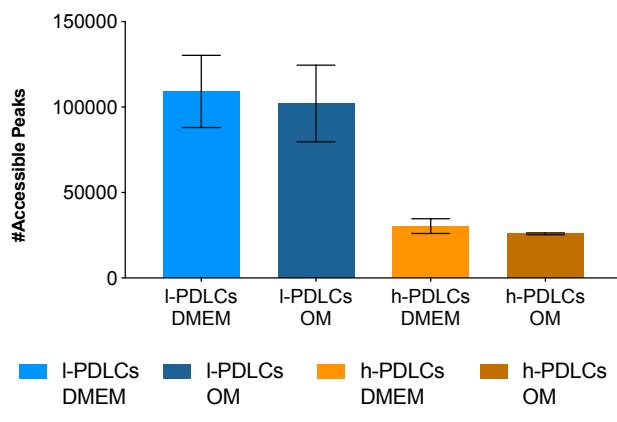
Symbol	Log2FC_High	Log2FC_Low	
C1QL3	7.625	-6.019	Symbol: gene identification
CX3CR1	5.019	-5.220	Log2FC_High: log2 fold change in h-PDLCs
DLX4	9.064	-7.405	Log2FC_Low: log2 fold change in I-PDLCs
EGFLAM	8.784	-5.718	Negative Log2FC: gene downregulated
FABP7	5.019	-5.746	Positive Log2FC: gene upregulated
FOXO6	6.893	-5.496	
GRM3	5.996	-5.535	
KRT18	7.231	-5.661	
KRT81	7.441	-5.304	
LRRC18	7.154	-5.384	
PCDH1	5.535	-7.860	
PLD4	6.688	-5.081	
PLEKHG1	7.566	-5.788	
PLVAP	6.450	-5.336	
PSMB9	5.996	-7.824	
SP7	8.370	-6.736	
THNSL2	8.386	-9.274	
TNNC1	5.306	-5.222	
TSPAN18	6.010	-6.372	
ABLIM2	-5.641	5.194	
ACTN3	-7.207	5.628	
ADH1C	-5.055	6.301	
ADORA1	-8.120	6.640	
BCHE	-5.931	8.511	
BEX1	-6.627	5.087	
BMP5	-6.847	5.351	
CASQ1	-7.207	5.663	
CDH18	-8.811	6.780	
CHGB	-7.207	5.556	
DDX25	-5.070	5.158	
DNAJB7	-5.070	5.193	
EPHA6	-6.847	5.058	
ERICH5	-7.207	6.679	
FIBCD1	-8.616	6.901	
GALNT13	-12.680	11.179	
GRID2	-6.847	5.191	
IFNE	-6.627	5.085	
IGFALS	-7.358	5.769	
IGSF3	-7.558	5.722	
IL1RN	-6.847	5.519	
IL4	-6.847	6.034	
IRX6	-7.940	6.395	
JPH1	-7.125	5.697	
L1TD1	-6.627	5.372	
LRRTM1	-5.070	5.511	
MAB21L2	-6.937	7.049	
NKD1	-5.070	7.181	
OCM	-7.207	5.886	
OR5K2	-6.627	5.165	
PCDH10	-5.899	5.883	
PSG1	-7.734	5.329	
PSMA8	-6.627	5.189	
RSPH1	-5.641	5.084	
SCN7A	-7.678	6.244	
SIX3	-9.369	5.630	
SLTRK5	-5.140	5.146	
SMOC1	-6.733	6.101	
TMEM196	-6.627	5.144	
TMEM74	-6.847	5.340	
TP53TG3D	-7.358	5.367	

Supplementary Table S3. Top 150 most differentially expressed genes at induced levels in I- and h-PDLCs

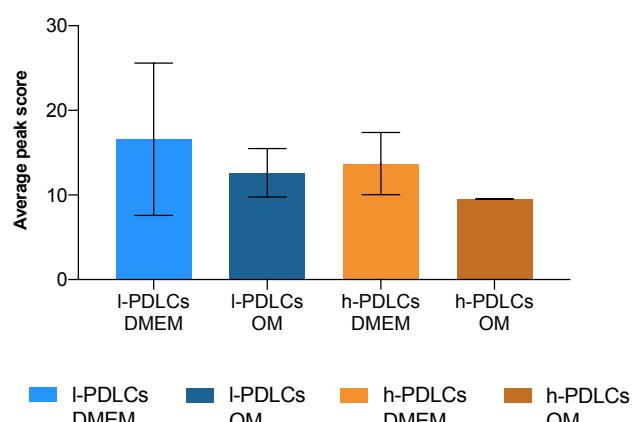
Symbol	Log2FC Low	Log2FC High	Symbol	Log2FC Low	Log2FC High	Symbol	Log2FC Low	Log2FC High
BST2	7.196	5.918	AC069368.1	6.340	-2.353	EPHB6	0.174	11.538
CHKB-CPT1B	6.436	5.774	CD101	8.129	-1.489	GOLGA8A	-2.813	12.699
FGL1	4.698	2.577	ASB2	4.445	-2.998	HNRNPAB	5.902	8.637
ART3	8.085	4.343	ATP6AP1	4.401	-3.091	FAM117B	6.236	8.179
COMTD1	7.158	2.758	DLEC1	4.908	-4.146	CEMP1	4.420	9.641
CRABP1	4.860	4.305	CTAGE15	4.561	-4.268	CXCR5	4.826	12.415
FCMR	4.653	4.074	AC004223.3	4.328	-4.160	EXPH5	-2.612	5.034
GPM6B	4.503	3.449	HSD17B11	4.375	-4.287	FUT6	-2.601	4.897
CEMIP	4.321	3.487	CHRFAM7A	5.545	-3.811	AL049844.3	-2.823	5.179
FKBP5	5.762	3.715	AMDHD1	6.250	-4.564	HLA-DOA	-2.826	4.986
ANOS3	4.541	2.968	GMNC	8.830	-4.738	CATSPERG	-2.219	5.532
FCHO1	5.449	5.612	ARHGAP20	8.498	-4.993	DRGX	-2.687	5.468
DGKB	4.289	6.676	BORCS8-	7.399	-4.885	FOXE3	-3.786	5.844
FBXO43	3.639	6.930	MEF2B			CRLF2	-3.797	5.754
ADGRF2	4.348	6.116	ANKRD26	9.161	-7.281	DND1	-3.791	5.254
DPEP1	3.946	5.837	GNL1	8.281	-7.396	C1QTNF3-AMACR	-1.725	6.259
CXCL8	3.315	6.063	AC012184.2	7.842	-7.396	GPAT2	-2.709	6.267
INMT-MINDY4	2.813	6.040	CORO7-PAM16	6.791	-6.630	CELF2	-4.461	5.599
	2.550	6.567	AC090527.2	6.118	-5.955	AC104662.2	-4.501	5.009
	3.819	5.213	AL022318.4	5.650	-6.168	C19ORF67	-5.132	4.898
	3.542	5.073	ITIH5	5.371	-7.351	CKAP2L	-5.383	6.646
	2.980	5.238	FAM183A	-0.368	5.432	GCH1	-6.244	6.206
	4.621	0.199	GAL3ST3	-0.368	5.391	KIF24	-8.416	8.937
	4.582	0.199	AC068946.1	-0.368	5.474	FAM213B	-9.038	6.174
	4.691	0.199	GNLY	-0.368	5.256			
	4.873	0.169	BOP1	-0.368	5.226			
	4.822	-0.156	CYTH4	-0.368	5.068	Symbol: gene identification		
HLA-A	4.570	-0.092	FAM166B	-0.368	5.029	Log2FC_High: log2 fold change in h-PDLCs		
C3	4.406	0.199	CLDN34	-0.368	4.952	Log2FC_Low: log2 fold change in I-PDLCs		
CHI3L1	4.397	0.199	CTXN3	-0.860	5.095	Negative Log2FC: gene downregulated		
FPR1	4.445	0.200	FIBCD1	-0.982	5.131	Positive Log2FC: gene upregulated		
GNGT1	4.378	0.199	FOXD4L1	-0.697	5.036			
ATP13A4	4.353	0.250	FAM189A2	-0.677	5.387			
IFITM5	4.502	0.199	KIF18B	-0.771	5.406			
ADAMDEC1	4.455	0.199	HYDIN	-1.106	5.829			
ALOX15B	5.365	0.199	CENPA	-1.052	5.669			
AL121758.1	5.245	0.199	BLOC1S5-TXND5	-0.711	5.980			
	5.119	-0.275	HLA-B	-1.021	AF241726.2	-1.364	5.210	
	5.194	-1.021	CHRM3	-0.427	5.887			
	4.974	-0.990	JSRP1	-0.368	5.811			
	5.131	-0.804	AGAP2	-0.247	5.683			
	5.134	-1.310	ALDOB	-0.417	5.583			
	4.718	-1.161	CHGB	-0.042	6.048			
	4.688	-0.785	AC008758.4	-0.567	6.896			
	4.400	-0.656	ELMO3	-0.368	6.649			
	5.942	0.199	CRYM	1.656	5.119			
BIN2	5.888	0.199	GPR82	1.597	5.131			
ADH4	6.249	0.199	ADGRV1	1.565	5.501			
DMKN	6.238	-0.170	IGKV2-30	2.087	5.809			
CASC1	6.114	-0.425	AC104109.3	2.057	5.267			
KCNT1	5.617	-0.751	C1ORF87	2.374	4.869			
CDH5	4.438	1.773	APOBEC3B	0.697	5.478			
HKDC1	4.367	1.499	DUXA	0.551	5.471			
HPD	4.822	0.974	ASF1B	0.508	5.667			
FGR	4.419	1.145	BLID	0.566	6.014			
ABCA2	5.364	0.584	CDC45	1.091	5.678			
ANKDD1A	5.164	0.753	BMP7	0.059	5.225			
CCDC13	5.945	0.965	GYS2	0.311	4.899			
GRIA2	6.679	0.707	HNRNPA1L2	-0.700	8.914			
C17ORF100	6.654	0.346	KIAA1107	-0.956	9.016			
	7.906	0.290	CNNM1	-0.368	8.265			
	9.535	1.259	CYP19A1	2.071	7.976			
	6.593	-1.408	CDC25C	1.035	6.983			
ERVMER34-1	6.227	-1.335	COL26A1					



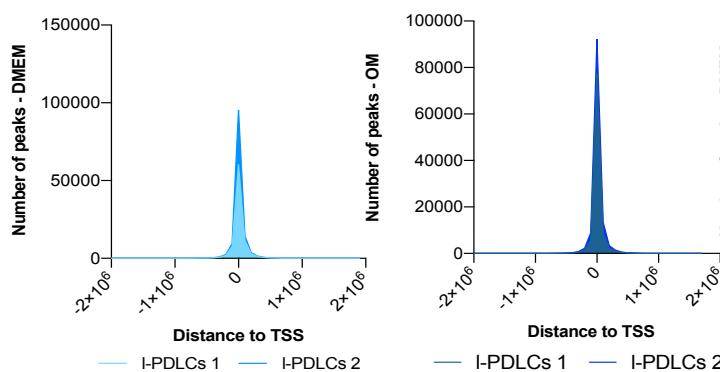
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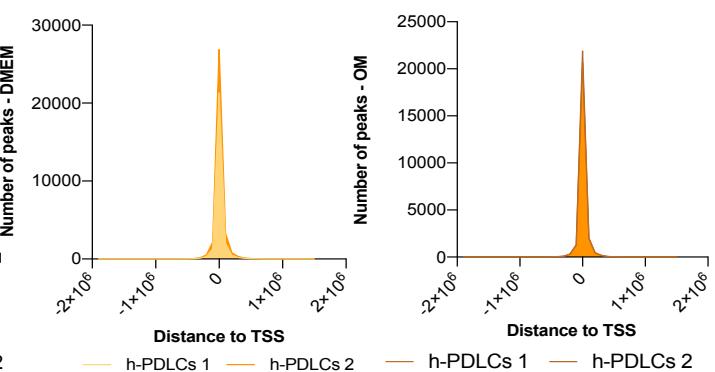
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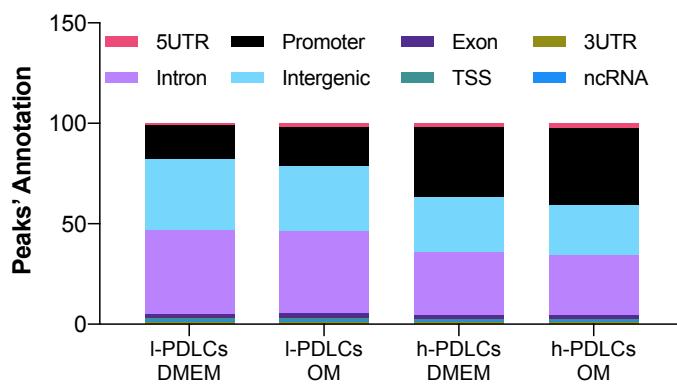
C



D



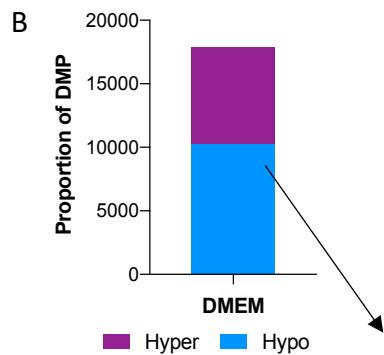
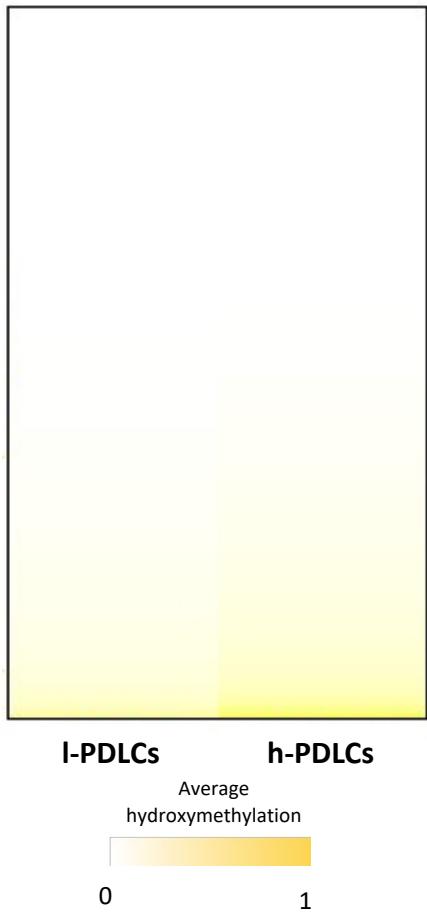
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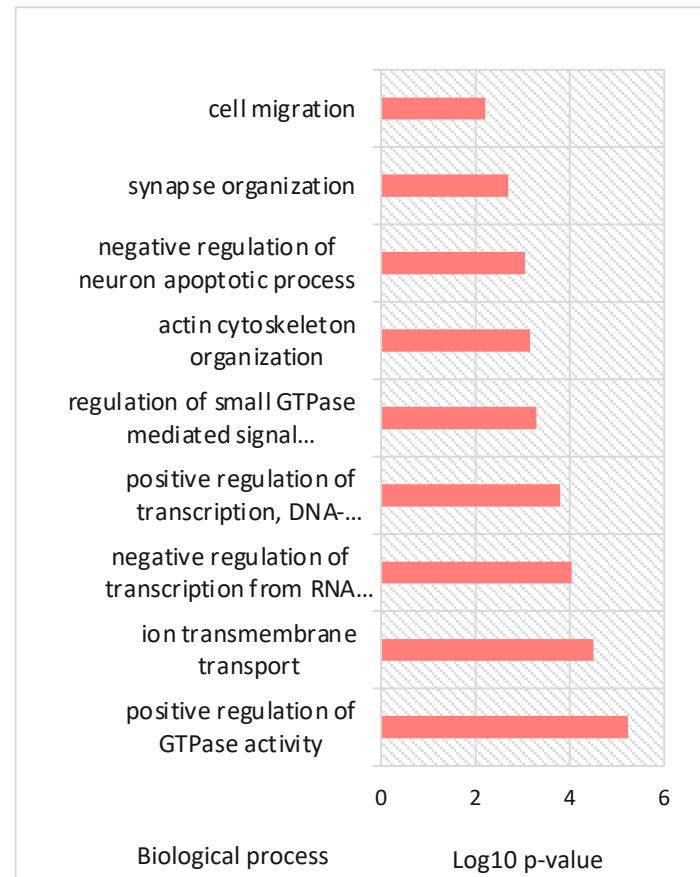
**Supplementary figure S1. Chromatin accessibility in PDLCs.** (A) Number of accessible chromatin peaks in I-PDLCs and h-PDLCs. (B) Average peak score at basal levels for I-PDLCs and h-PDLCs. (C, D) Distribution of peaks in relation to the nearest transcription start site (TSS) for each ATAC-seq replicate and experimental condition in I-PDLCs (C) and h-PDLCs (D). (E) Peaks' annotations shown as percentage of all peaks and compared between the experimental conditions. I-PDLCs, periodontal ligament cells showing low osteogenic potential *in vitro*; h-PDLCs, periodontal ligament cells showing high osteogenic potential *in vitro*.

I-PDLCs h-PDLCs  
DMEM DMEM

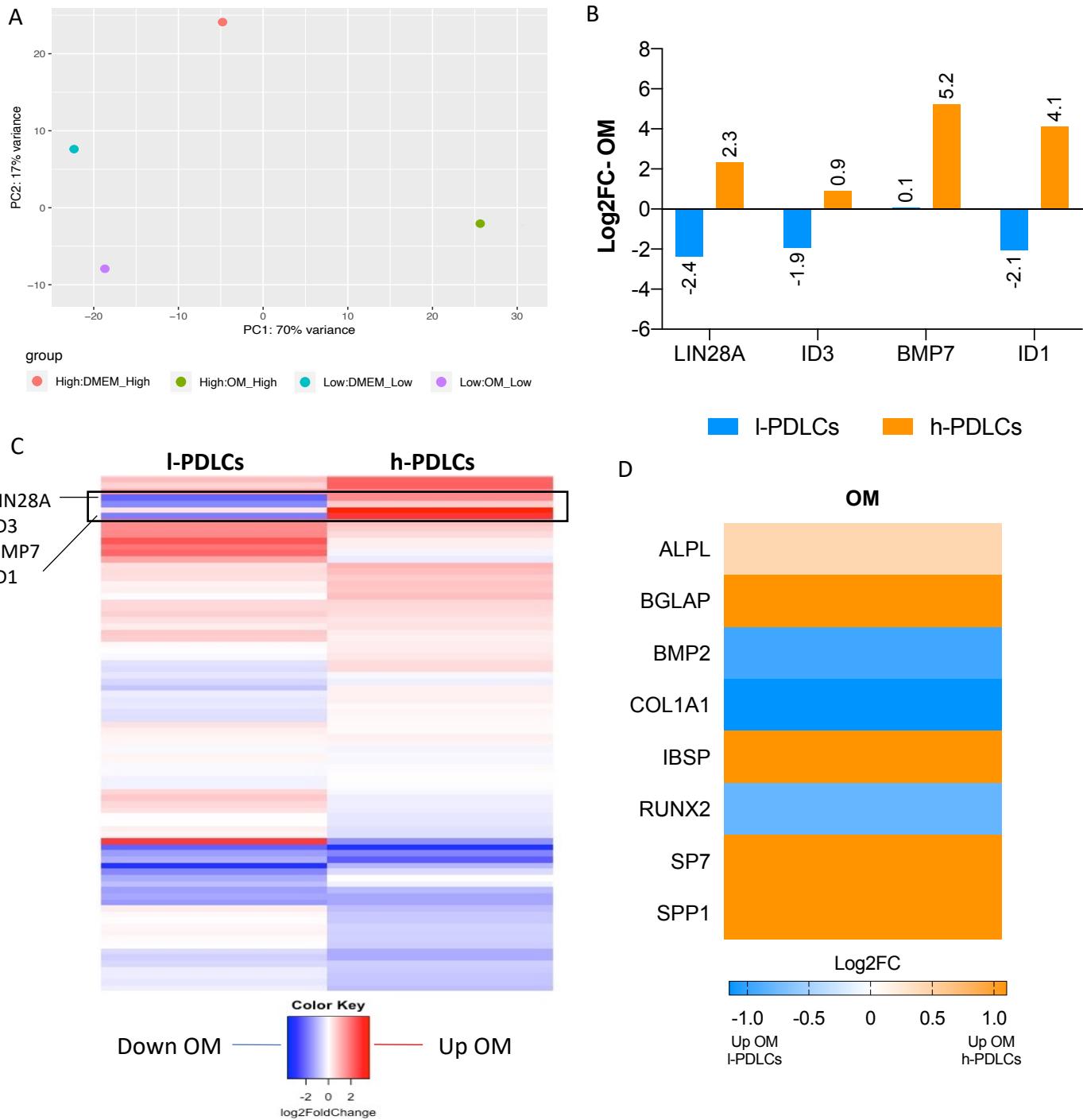
### A Hydroxymethylation levels



### Hypomethylated probes in I-PDLCs



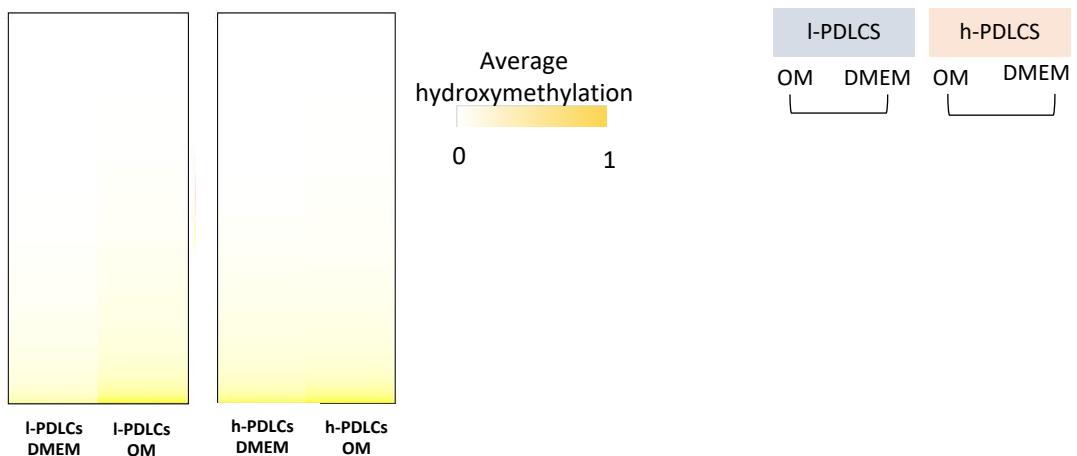
**Supplementary figure S2. DNA hydroxymethylation of PDLCs at basal levels.** (A) Heatmap showing DNA hydroxymethylation levels of all DNA methylation sites. (B) The proportion of 14,194 differentially methylated probes at day 10 of osteogenic stimulations *in vitro* in I-PDLCs (upper graph) and biological processes related to this subset. I-PDLCs, periodontal ligament cells showing low osteogenic potential *in vitro*; h-PDLCs, periodontal ligament cells showing high osteogenic potential *in vitro*.



**Supplementary figure S3. Transcriptional analysis of osteogenic markers.** (A) PCA plot of RNA-seq data showing the four experimental conditions: h-PDLCs in DMEM, h-PDLCs in OM, I-PDLCs in DMEM and I-PDLCs in OM. For each condition, three independent replicates were first performed and then pooled for sequencing. The variability represented by the first component clearly separates the h-PDLCs after OM stimulation from the other samples. (B) OM-induced gene expression changes in RNA-seq data in I-PDLCs and h-PDLCs. The panel represents genes responsible for controlling osteoblast lineage progression as described in *"Genetic and transcriptional control of bone formation"* by Javed et al. (2010). (C) Gene expression changes for four genes most differentially up-regulated by OM in h-PDLCs (as highlighted in (B)). (D) Comparison of gene expression levels of classical osteogenic markers between I- and h-PDLCs at day 10 of osteogenic stimulation. I-PDLCs, periodontal ligament cells showing low osteogenic potential *in vitro*; h-PDLCs, periodontal ligament cells showing high osteogenic potential *in vitro*.

## Hydroxymethylation levels

A



B

OM

Probe	I-PDLCs DeltaBeta	Gene	Role
cg09391665	0.594	SEC23IP	The encoded protein is localized to endoplasmic reticulum exit sites and plays a critical role in ER-Golgi transport as part of the multimeric coat protein II complex
cg11382054	0.457	PPFIBP1	May regulate the disassembly of focal adhesions.
cg03771702	0.425	HM13	Catalyzes intramembrane proteolysis of some signal peptides after they have been cleaved from a preprotein, resulting in the release of the fragment from the ER membrane into the cytoplasm.
cg01832737	0.416	PDE10A	Plays a role in signal transduction by regulating the intracellular concentration of cyclic nucleotides
cg05960822	0.391	EBF1	For instance, positively regulates many B-cell specific genes including BCR or CD40 while repressing genes that direct cells into alternative lineages, including GATA3 and TCF7 for the T-cell lineage. In addition to its role during lymphopoiesis, controls the thermogenic gene program in adipocytes during development and in response to environmental cold

C

OM

Probe	h-PDLCs Delta Beta	Gene	Role
cg14683065	0.46622582	LRRK1	The encoded protein is thought to play a role in the regulation of bone mass. Mice lacking a similar gene showed severe osteopetrosis, increased bone mineralization and decreased bone resorption.
cg04049542	0.35763966	C11orf80	Together with SPO11, mediates DNA cleavage that forms the double-strand breaks (DSB) that initiate meiotic recombination. The complex promotes relaxation of negative and positive supercoiled DNA and DNA decatenation through cleavage and ligation cycles.
cg01997461	0.35682086	FARP1	Functions as guanine nucleotide exchange factor for RAC1. Plays a role in the assembly and disassembly of dendritic filopodia, the formation of dendritic spines, regulation of dendrite length and ultimately the formation of synapses.
cg14313797	0.34804238	CAMK1D	This gene is a member of the calcium/calmodulin-dependent protein kinase 1 family, a subfamily of the serine/threonine kinases. In vitro, phosphorylates transcription factor CREM isoform Beta.
cg10582795	0.34776656	RBMS1	These proteins have been implicated in such diverse functions as DNA replication, gene transcription, cell cycle progression and apoptosis. Probably has a role in DNA replication.

**Supplementary figure S4. DNA hydroxymethylation in PDLCs following osteogenic induction.** (A) Heatmap showing DNA hydroxymethylation levels of 850,000 DNA methylation sites at basal state and after 10 days of osteogenic induction. (B) Top 5 most DNA hydroxymethylated probes in I-PDLCs after osteogenic induction. (C) Top 5 most hydroxymethylated probes in h-PDLCs at induced levels. I-PDLCs, periodontal ligament cells showing low osteogenic potential *in vitro*; h-PDLCs, periodontal ligament cells showing high osteogenic potential *in vitro*. DMEM, standard culture medium; OM, osteogenic medium.