Indirect immobilized Jagged1 suppresses cell cycle progression and induces odonto/osteogenic differentiation in human dental pulp cells

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Supplementary Table 1. List of the downregulated genes in the cell cycle pathway based on the enriched KEGG pathway analysis.

Gene Symbol	Gene Name	EntrezGene
CCNE2	cyclin E2	9134
MCM6	minichromosome maintenance complex component 6	4175
PTTG1	pituitary tumor-transforming 1	9232
ORC1	origin recognition complex, subunit 1	4998
CCNB1	cyclin B1	891
MCM4	minichromosome maintenance complex component 4	4173
CDKN2C	cyclin-dependent kinase inhibitor 2C (p18, inhibits CDK4)	1031
CCND1	cyclin D1	595
WEE1	WEE1 homolog (S. pombe)	7465
CCNA2	cyclin A2	890
MCM5	minichromosome maintenance complex component 5	4174
TTK	TTK protein kinase	7272
BUB1	budding uninhibited by benzimidazoles 1 homolog (yeast)	699
CDC25A	cell division cycle 25 homolog A (S. pombe)	993
CDC20	cell division cycle 20 homolog (S. cerevisiae)	991
BUB1B	budding uninhibited by benzimidazoles 1 homolog beta (yeast)	701
CDC6	cell division cycle 6 homolog (S. cerevisiae)	990
PKMYT1	protein kinase, membrane associated tyrosine/threonine 1	9088
MCM2	minichromosome maintenance complex component 2	4171
CDK1	cyclin-dependent kinase 1	983
CDC45	cell division cycle 45 homolog (S. cerevisiae)	8318
RBL1	retinoblastoma-like 1 (p107)	5933
MAD2L1	MAD2 mitotic arrest deficient-like 1 (yeast)	4085
TFDP1	transcription factor Dp-1	7027
E2F1	E2F transcription factor 1	1869
SMC1A	structural maintenance of chromosomes 1A	8243
CDK2	cyclin-dependent kinase 2	1017
E2F2	E2F transcription factor 2	1870
YWHAH	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, eta polypeptide	7533
CCNB2	cyclin B2	9133
ESPL1	extra spindle pole bodies homolog 1 (S. cerevisiae)	9700
MCM3	minichromosome maintenance complex component 3	4172
ORC6	origin recognition complex, subunit 6	23594

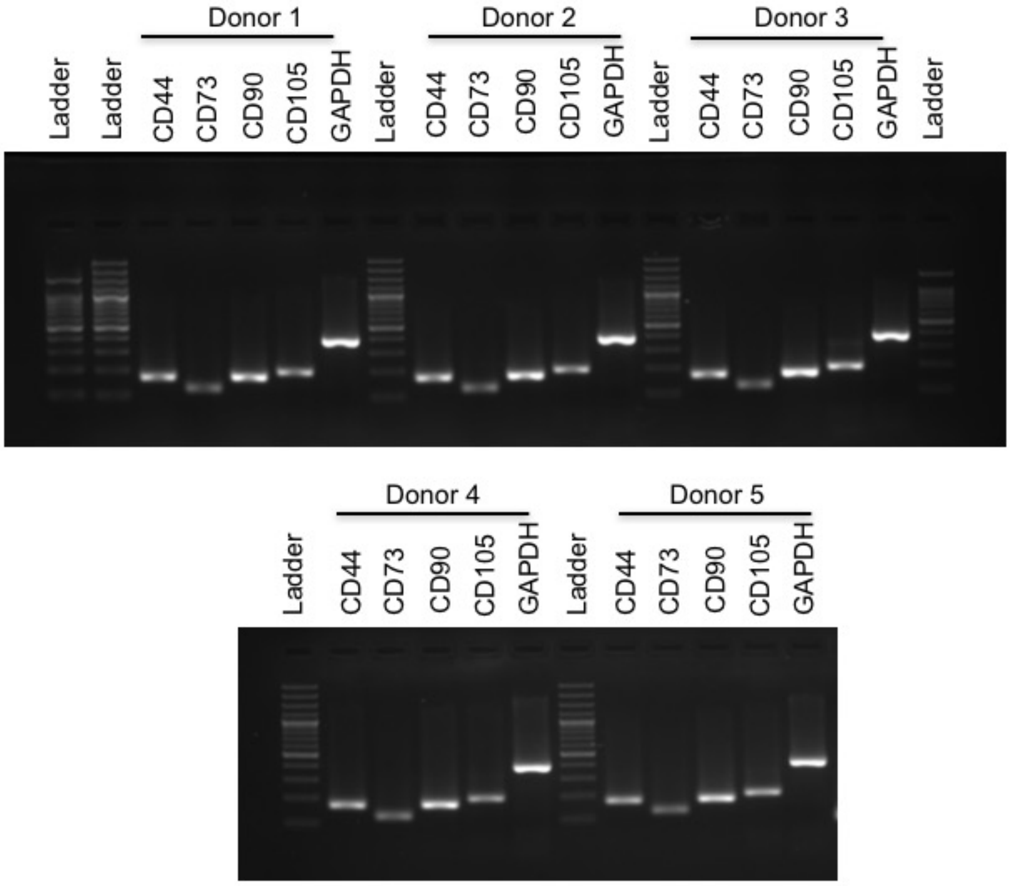
Supplementary Table 2. List of downregulated genes in DNA replication pathway based on enrich KEGG pathway analysis.

Gene Symbol	Gene Name	EntrezGene
MCM4	minichromosome maintenance complex component 4	4173
POLA2	polymerase (DNA directed), alpha 2, accessory subunit	23649
MCM6	minichromosome maintenance complex component 6	4175
RFC3	replication factor C (activator 1) 3, 38kDa	5983
POLE	polymerase (DNA directed), epsilon, catalytic subunit	5426
POLE2	polymerase (DNA directed), epsilon 2, accessory subunit	5427
PRIM2	primase, DNA, polypeptide 2 (58kDa)	5558
POLA1	polymerase (DNA directed), alpha 1, catalytic subunit	5422
MCM5	minichromosome maintenance complex component 5	4174
LIG1	ligase I, DNA, ATP-dependent	3978
RNASEH2A	ribonuclease H2, subunit A	10535
MCM2	minichromosome maintenance complex component 2	4171
FEN1	flap structure-specific endonuclease 1	2237
MCM3	minichromosome maintenance complex component 3	4172
RPA1	replication protein A1, 70kDa	6117

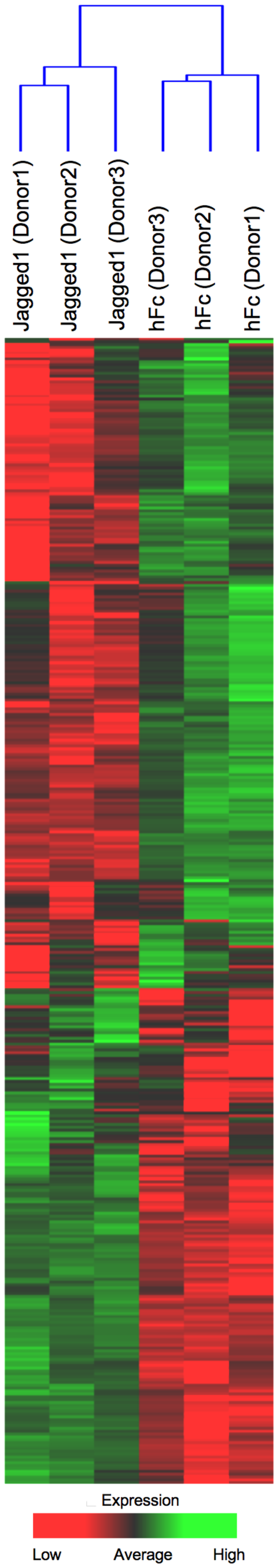
Supplementary Table 3. Primer sequences

Gene	Primer sequences (F=forward, R=Reverse)		Accession number
Real-time PCR			
GAPDH	F	5’-TCATGGGTGTGAACCATGAGAA-3’	NM_002046.3
	R	5’-GGCATGGACTGTGGTCATGAG-3’	
HES1	F	5’-AGGCGGACATTCTGGAAATG-3’	NM_005524.2
	R	5’-CGGTACTTCCCCAGCACACTT-3’	
HEY1	F	5’-CTGCAGATGACCGTGGATCA-3’	NM_012258.3
	R	5’-CCAAACTCCGATAGTCCATAGCAA-3’	
RUNX2	F	5’-ATGATGACACTGCCACCTCTGA-3’	NM_001024630.3
	R	5’-GGCTGGATAGTGCATTCGTG-3’	
OSX	F	5’-GCCAGAAGCTGTGAAACCTC-3’	NM_152860.1
	R	5’-GCTGCAAGCTCTCCATAACC-3’	
MSX2	F	5'-GGTTTCCTCTCCCTCTCCAC-3'	NM_002449.4
	R	5'-GGCTTGGTGCCTCCGCCTAC-3'	
OPN	F	5’-AGGAGGAGGCAGAGCACA-3’	NM_001040060.1
	R	5’-CTGGTATGGCACAGGTGATG-3’	
COL1	F	5’-GTGCTAAAGGTGCCAATGGT-3’	NM_000088.3
	R	5’-ACCAGGTTCACCGCTGTTAC-3’	
OCN	F	5’-CTTTGTGTCCAAGCAGGAGG-3’	NM_199173.4
	R	5’-CTGAAAGCCGATGTGGTCAG-3’	
DMP1	F	5’-CAGGAGCACAGGAAAAGGAG-3’	NM_004407.3
	R	5’-CTGGTGGTATCTTGGGCACT-3’	
DSPP	F	5’-ATATTGAGGGCTGGAATGGGGA-3’	NM_014208.3
	R	5’-TTTGTGGCTCCAGCATTGTCA-3’	
BMP2	F	5’-GCGTGAAAAGAGAGACTGC-3’	NM_001200.2
	R	5’-CCATTGAAAGAGCGTCCAC-3’	
TWIST1	F	5’-TCTTACGAGGAGCTGCAGACGCA-3’	NM_000474.3
	R	5’-ATCTTGGAGTCCAGCTCGTCGCT-3’	
TWIST2	F	5’-GCTGCGCAAGATCATCCC-3’	NM_057179.2
	R	5’-GTAGCTGCAGCTGGTCATC-3’	
ALP	F	5’-CGAGATACAAGCACTCCCACTTC-3’	NM_000478.3
	R	5’-CTGTTCAGCTCGTACTGCATGTC-3’	
NOTCH1	F	5’-AGGACCTCATCAACTCACACGC-3’	NM_017617.3
	R	5’-TCTTTGTTAGCCCCGTTCTTCAG-3’	
NOTCH2	F	5’-CCGTGTTGACTTCTGCTCTCTCAC-3’	NM_024408.3
	R	5’-CCTACTACCCTTGGCATCCTTTG-3’	
NOTCH3	F	5’-TCTCAGACTGGTCCGAATCCAC-3’	NM_000435.2
	R	5’-ACACTTGCCTCTTGGGGGTAAC-3’	
NOTCH4	F	5’-ATGCGAGGAAGATACGGAGTGG-3’	NM_004557.3
	R	5’-TCGGAATGTTGGAGGCAGAAC-3’	
FOXS1	F	5’-CAGGAATGTTCTTCTTTGAGT-3’	NM_004118.3
	R	5’-GTCCTTCGAGAGTTCTTCAG-3’	
PDGFA	F	5’-TCGATGAGATGGAGGGTCG-3’	NM_002607.5
	R	5’-ACCCGGACAGAAATCCAGTCT-3’	
NGF	F	5’-CATGCTGGACCCAAGCTCA-3’	NM_002506.2
	R	5’-GACATTACGCTATGCACCTCAGTG-3’	
DKK2	F	5’-AGTGTGAAGTTGGGAGGTATTGCC-3’	NM_014421.2
	R	5’-TGCCATTATTGCAGCGGGTACTG-3’	
SOST	F	5’-ACTTCAGAGGAGGCAGAAATGG-3’	NM_025237.2
	R	5’-CAAGGGGGAATCTTATCCAACTTTC-3’	

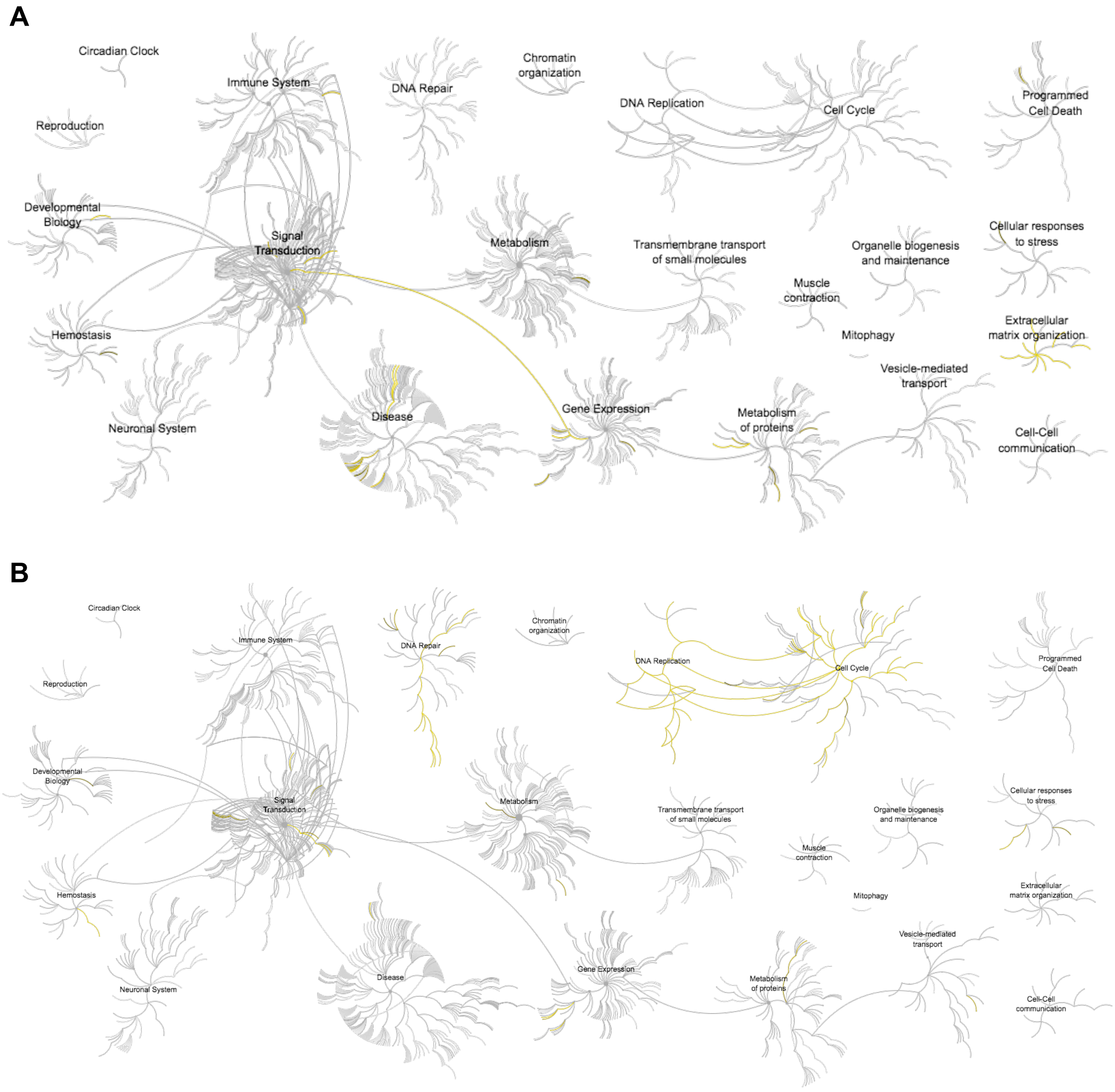
PDGFD	F	5’-GTGGAGGAAATTGTGGCTGT-3’	NM_025208.4
	R	5’-CGTTCATGGTGATCCAACTG-3’	
LRP5	F	5’-ATCAACGTCCACAGGCTGA-3’	NM_002335.3
	R	5’-CCTGCATGTTGGTGAAGTACA-3’	
E2F1	F	5’-GACCCTGACCTGCTGCTCT-3’	NM_005225.2
	R	5’-GGCCAGGTACTGATGGTCA-3’	
E2F2	F	5’-CAAGTTGTGCGATGCCTG-3’	NM_004091.3
	R	5’-TTGGGAACTCAGGGACGA-3’	
MCM2	F	5'-AATTTCGTCCTGGGTCCTTT-3'	NM_004526.3
	R	5'-CACTTTGCCTGGACTCTCCT-3'	
MCM4	F	5'-TTCTTTGACCGTTACCCTGA-3'	NM_005914.3
	R	5'- ACACTTGGCACTGGAAGAAG-3'	
MCM5	F	5'-TATTGCCTACTGCCGAGTGA-3'	NM_006739.3
	R	5'-ACTGTCCCTCTCGTGCTGAC-3'	
MCM8	F	5'-CCAGGCCTAGGAAAAAGTCA-3'	NM_032485.5
	R	5'-GAGGTGGTCGTGGTGTTACC-3'	
MCM10	F	5'-CCGTCTGCAAAAATCCCCTGAGA-3'	NM_018518.4
	R	5'-ATGAGCTTTTGGGATCTGGAGGTG-3'	
CCND1	F	5'-GGCGGAG GAGAACAAACAGA-3'	NM_053056.2
	R	5'-ATGGAGGGCGGATTGGAAA-3'	
CCNE2	F	5'-GCCGAGCGGTAGCTGGTC-3'	NM_057749.2
	R	5'-GGGCTGCTGCTTAGCTTGTAAA-3'	
TGF-β1	F	5’-GGATACCAACTATTGCTTCAGCTCC-3’	NM_000660.4
	R	5’-AGGCTCCAAATGTAGGGGCAGGGCC-3’	
TGFβ2	F	5’-ATTGCCCTCCTACAGACTTGAG-3’	NM_001135599.2
	R	5’-CAGCACAGAAGTTGGCATTGTA-3’	
TGFβ3	F	5’-GGTTTTCCGCTTCAATGTGT-3’	NM_003239.2
	R	5’-GCTCGATCCTCTGCTCATTC-3’	
Conventional PCR			
CD44	F	5’-GGCCCAGATGGAGAAAGCTCTGAGC-3’	NM_00610.3
	R	5’-GAAGCAATATGTGTCATACTGGGAG-3’	
CD73	F	5’-ACACTTGGCCAGTAAAATAGGG-3’	NM_002526.2
	R	5’-ATTGCAAAGTGGTTCAAAGTCA-3’	
CD90	F	5’-GAAGACCCCAGTCCAGATCCA-3’	NM_006288.4
	R	5’-TGCTGGTATTCTCATGGCGG-3’	
CD105	F	5’-CATCACCTTTGGTGCCTTCC-3’	NM_001114753.1
	R	5’-CTATGCCATGCTGCTGGTGGA-3’	
GAPDH	F	5’-TGAAGGTCGGAGTCAACGGAT-3’	NM_002046.3
	R	5’-TCACACCCATGACGAACATGG-3’	



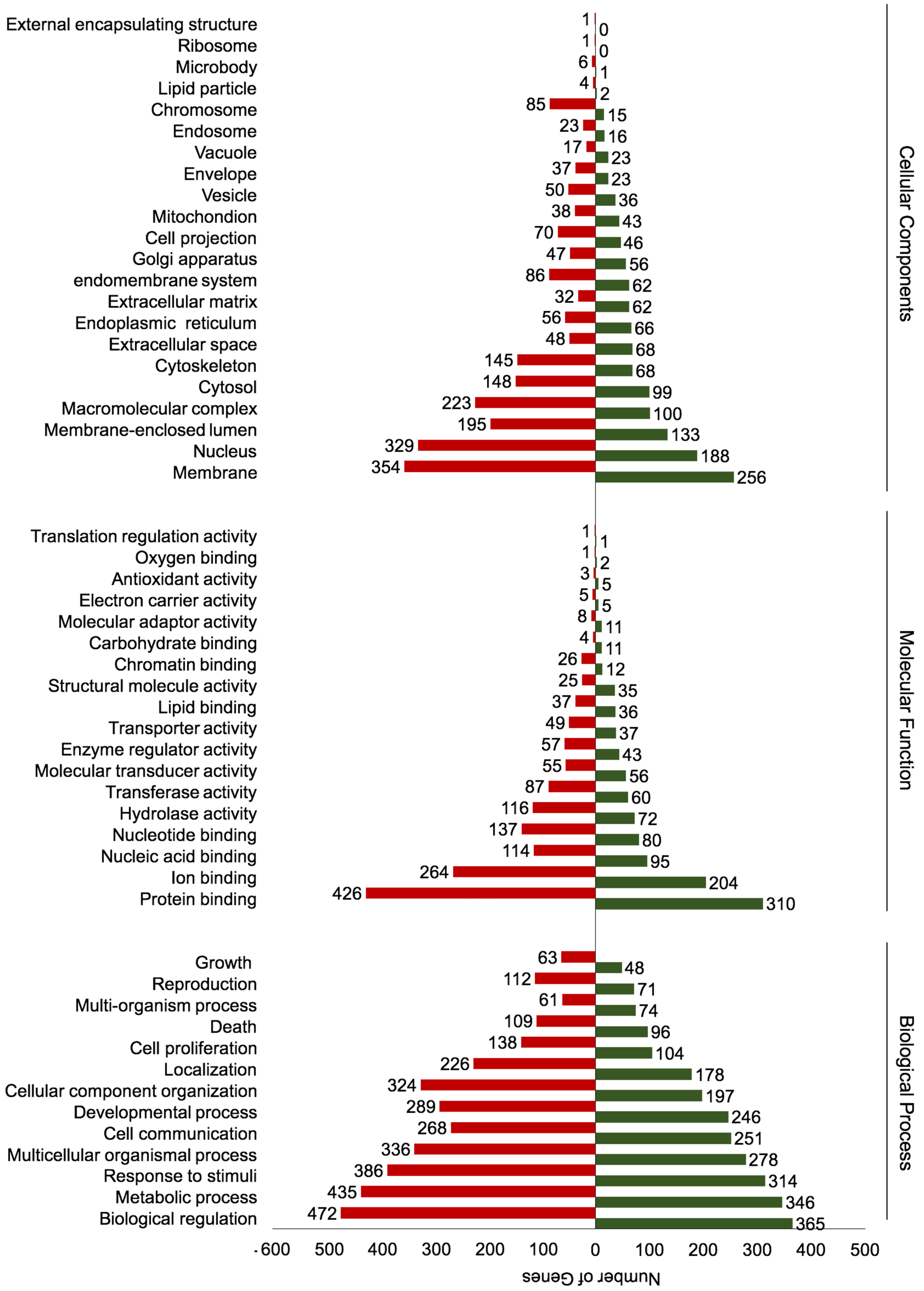
Supplementary Figure 1. Conventional polymerase chain reaction demonstrated the mRNA expression of mesenchymal stem cell marker from 5 donors.



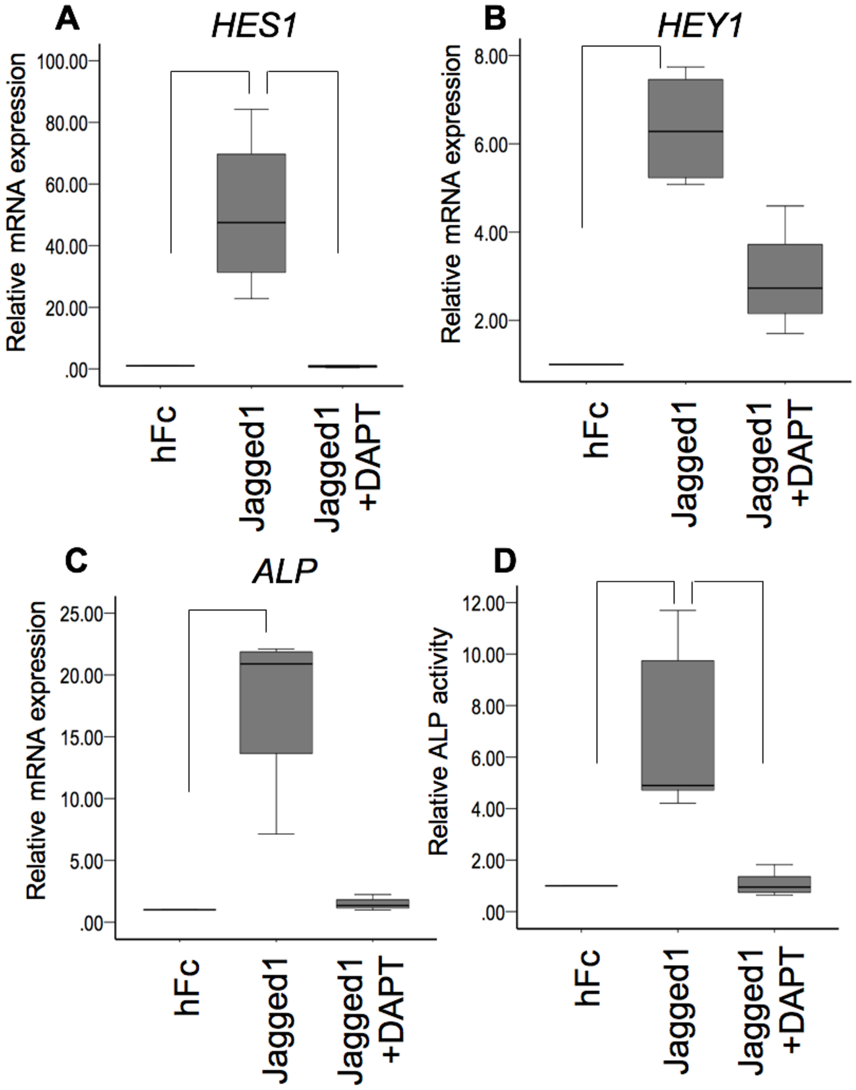
Supplementary Figure 2. Heat map representing the differential gene expression (A). Color codes range from red (low expression) to green (high expression).



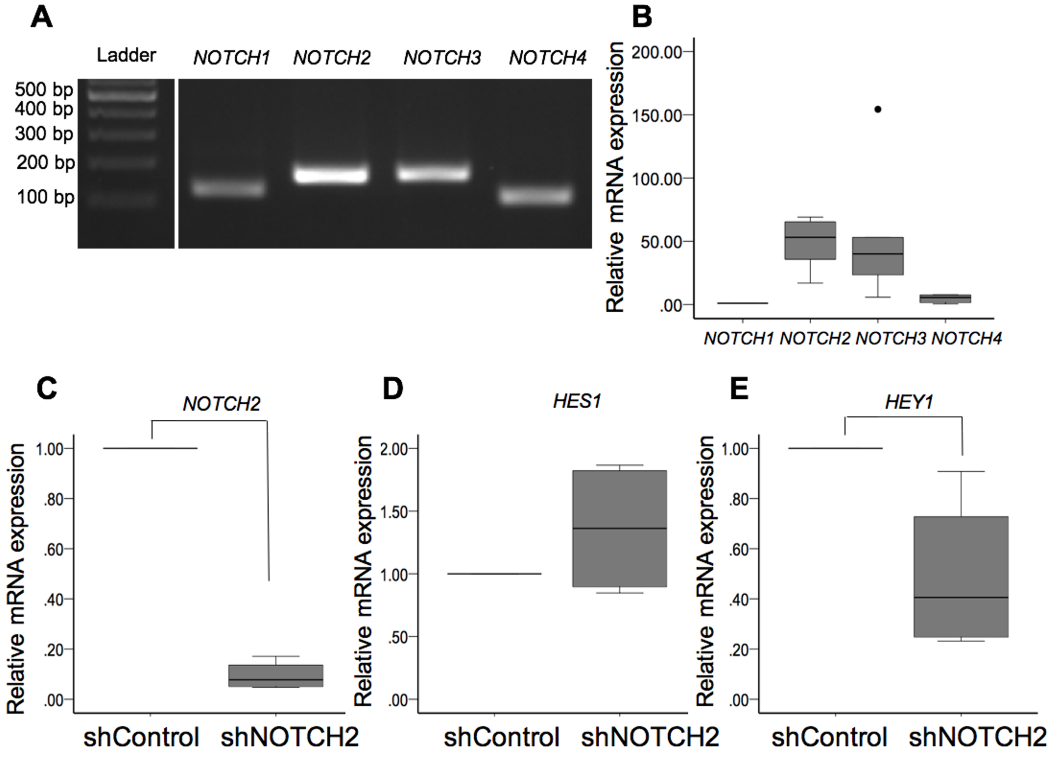
Supplementary Figure 3. Differentially expressed pathways in Jagged1 treated hDPs determined by the Reactome pathway database analysis. The differentially expressed genes were analyzed using an online bioinformatic tool to identify related affected pathways. The diagrams illustrated the upregulated (A) and down regulated (B) pathways.



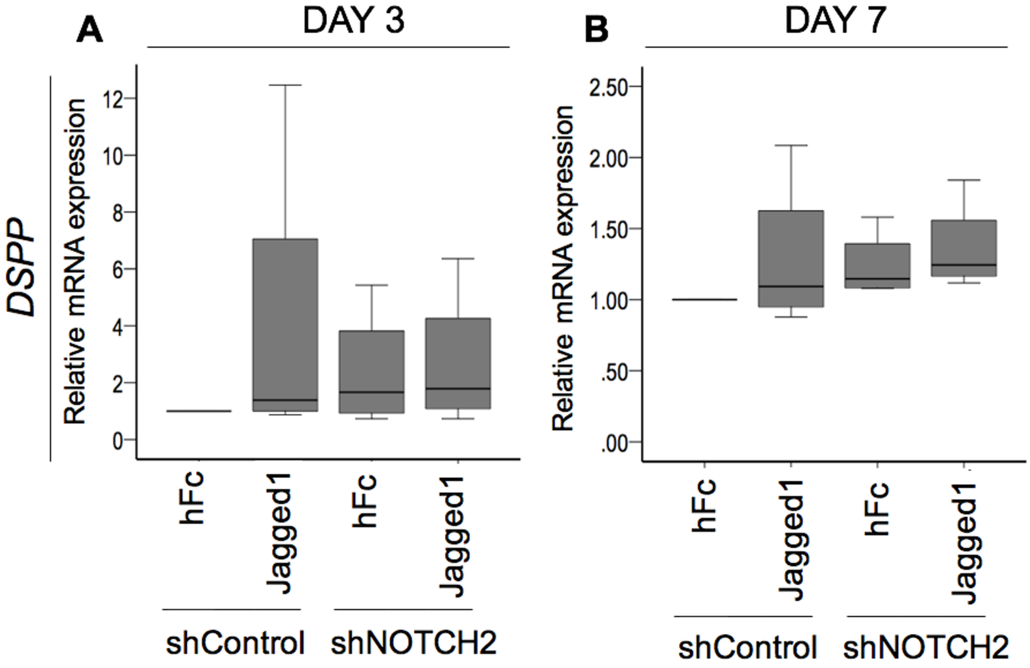
Supplementary Figure 4. Gene ontology evaluation of the differentially expressed genes. Gene ontology classification was analyzed using WebGestalt. Green and red bars represent the upregulated and downregulated genes, respectively.



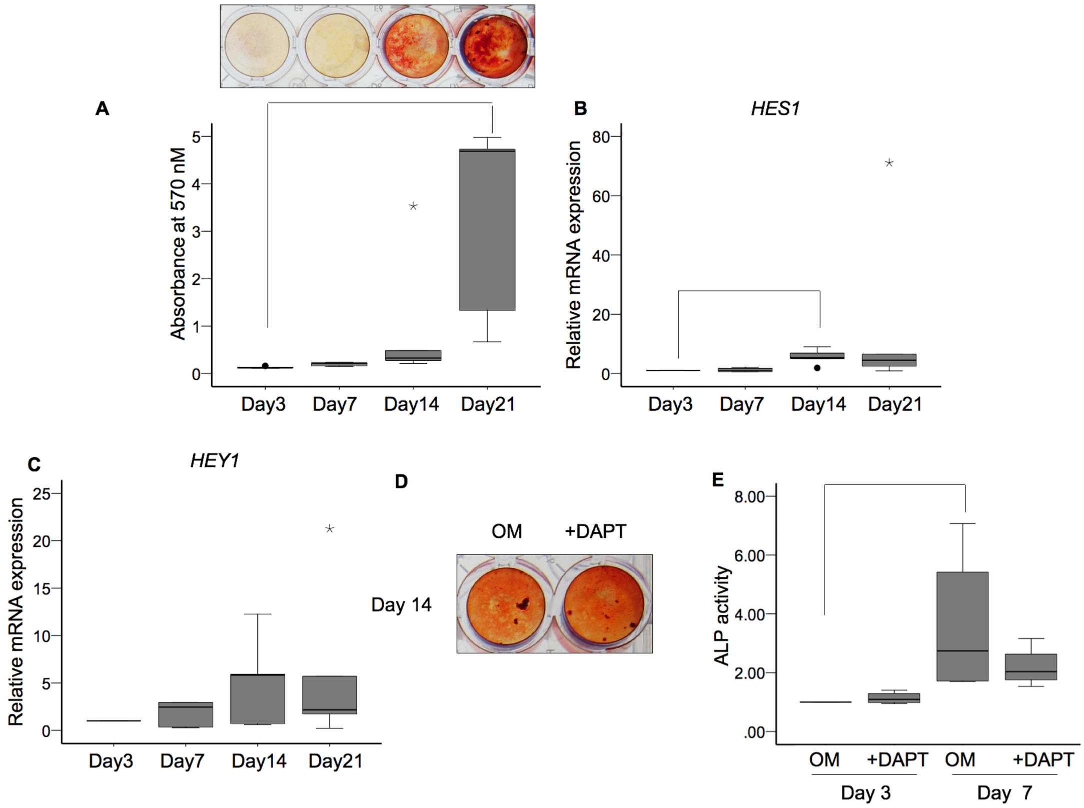
Supplementary Figure 5. γ-secretase inhibitor abolished Jagged1-induced ALP activity and mineral deposition. hDPs were seeded on indirect immobilized Jagged1 surfaces and maintained in osteogenic medium for 7 days. Some cells were pretreated with DAPT, a γ-secretase inhibitor, 30 min prior to Jagged1 exposure. The mRNA levels of *HES1* (A), *HEY1* (B), and *ALP* (C) were measured using real-time polymerase chain reaction. ALP enzymatic activity was evaluated (D). Bars indicate a significant difference between groups (p<0.05).



Supplementary Figure 6. Characterization of the hDPs transduced with shRNA against *NOTCH2*. Baseline mRNA expression of Notch receptors was evaluated using conventional (A) and real-time polymerase chain reaction (B). hDPs were transduced with *NOTCH2* shRNA. The mRNA expression of *HES1* (C), *HEY1* (D), and *NOTCH2* (E) was evaluated using real-time polymerase chain reaction. Bars indicate a significant difference between groups (*p*<0.05). Black dot (•) indicates an outlier data point.



Supplementary Figure 7. NOTCH2 participated in Jagged1 induced odonto/osteogenic differentiation by hDPs. The shNOTCH2 and shControl transduced hDPs were seeded on indirect immobilized Jagged1 or the hFc control surfaces and maintained in osteogenic medium for 3 (A) and 7 days (B). The *DSPP* mRNA expression was observed using real-time polymerase chain reaction.



Supplementary Figure 8. Endogeneous Notch signaling in odonto/osteogenic differentiation by hDPs. hDPs were cultured in osteogenic medium and the mineral deposition was stained by Alizarin Red S staining (A). The mRNA expression of Notch target genes, *HES1* and *HEY1*, was evaluated in hDPs at different time points during odonto/osteogenic induction (B–C). hDPs were cultured in osteogenic medium for 14 days with or without the Notch signaling inhibitor DAPT. Mineral deposition was evaluated using Alizarin Red S staining (D). ALP enzymatic activity was evaluated when the hDPs were cultured in osteogenic medium with or without DAPT for 3 and 7 days (E). Bars indicate a significant difference between groups (p<0.05). Black dots (•) indicate outlier data points. Asterisks (*) indicate extreme outlier data points.