Pluripotent stem cells as a source of osteoblasts for tissue regeneration

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Supplementary information

Supplementary figure legends

Fig. S1. qRT-PCR validation of osteoblast and bone related gene upregulation and Gene Ontology Analysis of significantly downregulated genes in ESC-OB versus ESC. (A) qRT-PCR analysis of osteoblast and bone related genes on ESC-OB and ESC normalized to ß-actin. *, $p<.05$; **, $p<.005$; #, $p<5\text{x}10^{-4}$ (relative to ESC). (B) Gene Ontology Analysis of biological process for significantly downregulated genes in mouse ESC-OB versus ESC population. Gene fold enrichment and $-\log_{10}$ (P value) of top 20 annotation terms were shown.

Fig. S2. qRT-PCR validation of osteoblast and bone related gene upregulation and Gene Ontology Analysis of significantly downregulated genes in iPSC-OB versus iPSC. (A) qRT-PCR analysis of osteoblast and bone related genes on iPSC-OB and iPSC normalized to ß-actin. **, $p<.005$; #, $p<5\text{x}10^{-4}$; +, $p<5\text{x}10^{-5}$ (relative to iPSC). (B) Gene Ontology Analysis of biological process for significantly downregulated genes in mouse iPSC-OB versus iPSC population. Gene fold enrichment and $-\log_{10}$ (P value) of top 20 annotation terms were shown.

Fig. S3. Gene Ontology Analysis of significantly downregulated genes in ESC-OB GFP$^+$ versus ESC. Gene Ontology Analysis of biological process for significantly downregulated genes in mouse ESC-OB GFP+ versus ESC population. Gene fold enrichment and $-\log_{10}$ (P value) of top 20 annotation terms were shown.

Supplementary Table information

Table S1. ESC-OB vs ESC significantly upregulated genes Signaling Pathway Analysis (Top 50 Terms)

Signaling Pathway	Gene Number	P value
Focal adhesion	135	-1.44E-25
ECM-receptor interaction	72	-2.55E-23
Pathways in cancer	178	-1.81E-18
Dilated cardiomyopathy	65	-1.54E-15
Axon guidance	84	-2.50E-14
Hypertrophic cardiomyopathy (HCM)	60	-4.19E-14
Lysosome	79	-6.79E-14
MAPK signaling pathway	138	-7.77E-12
Endocytosis	117	-3.04E-11
Amoebiasis	71	-6.49E-11
Regulation of actin cytoskeleton	113	-2.54E-10
Osteoclast differentiation	70	-6.47E-10
Arrhythmogenic right ventricular cardiomyopathy (ARVC)	49	-1.31E-09
Vascular smooth muscle contraction	71	-2.60E-09
Chagas disease (American trypanosomiasis)	60	-5.95E-09
Small cell lung cancer	52	-4.29E-08
Cytokine-cytokine receptor interaction	118	-5.71E-08
TGF-beta signaling pathway	51	-8.28E-08
Toxoplasmosis	69	-1.23E-07
Apoptosis	51	-1.41E-07
Protein processing in endoplasmic reticulum	86	-1.87E-07
Leukocyte transendothelial migration	65	-3.31E-07
Gastric acid secretion	44	-5.36E-07

Calcium signaling pathway	88	-6.99E-07
Tight junction	70	-1.06E-06
Pancreatic cancer	42	-1.15E-06
Salivary secretion	45	-1.36E-06
Melanogenesis	55	-1.40E-06
SNARE interactions in vesicular transport	25	-1.86E-06
Hepatitis C	70	-2.14E-06
Chronic myeloid leukemia	42	-5.32E-06
Wnt signaling pathway	75	-6.88E-06
Hedgehog signaling pathway	33	-9.33E-06
Adipocytokine signaling pathway	39	-1.27E-05
Toll-like receptor signaling pathway	53	-1.37E-05
Aldosterone-regulated sodium reabsorption	28	-1.50E-05
PPAR signaling pathway	44	-1.57E-05
Circadian rhythm	17	-1.63E-05
Complement and coagulation cascades	42	-2.09E-05
Neurotrophin signaling pathway	64	-3.66E-05
Chemokine signaling pathway	85	-3.99E-05
Renal cell carcinoma	39	-4.92E-05
Jak-STAT signaling pathway	71	-0.000115
NOD-like receptor signaling pathway	32	-0.000133
Adherens junction	39	-0.000163
Drug metabolism - cytochrome P450	44	-0.000221
ErbB signaling pathway	44	-0.000221
Protein digestion and absorption	40	-0.00029
Phosphatidylinositol signaling system	40	-0.00029
Bacterial invasion of epithelial cells	37	-0.000321

Table S2. iPSC-OB vs iPSC significantly upregulated genes Signaling Pathway Analysis (Top 50 Terms)

Signaling Pathway	Gene Number	P value
Lysosome	70	-7.25E-18
Focal adhesion	97	-2.03E-17

ECM-receptor interaction	51	-3.78E-14
Complement and coagulation cascades	47	-4.12E-14
Protein processing in endoplasmic reticulum	73	-2.27E-10
Pathways in cancer	118	-4.80E-10
Endocytosis	86	-2.54E-09
Hypertrophic cardiomyopathy (HCM)	41	-2.21E-08
Dilated cardiomyopathy	43	-2.32E-08
Pancreatic cancer	35	-1.62E-07
Amoebiasis	49	-4.92E-07
Axon guidance	53	-8.75E-07
Small cell lung cancer	38	-2.54E-06
Protein digestion and absorption	35	-4.11E-06
Regulation of actin cytoskeleton	74	-1.23E-05
Sphingolipid metabolism	21	-3.10E-05
N-Glycan biosynthesis	24	-3.44E-05
Mucin type O-Glycan biosynthesis	16	-4.86E-05
Hepatitis C	50	-5.09E-05
Apoptosis	35	-5.39E-05
Chronic myeloid leukemia	31	-5.43E-05
Chagas disease (American trypanosomiasis)	39	-6.41E-05
Staphylococcus aureus infection	23	-0.000115
PPAR signaling pathway	32	-0.000164
Melanogenesis	37	-0.000343
Osteoclast differentiation	42	-0.000371
Calcium signaling pathway	58	-0.000477
Arrhythmogenic right ventricular cardiomyopathy (ARVC)	29	-0.000492
Vascular smooth muscle contraction	43	-0.000493
Toxoplasmosis	44	-0.000532
ErbB signaling pathway	32	-0.000947
Other glycan degradation	10	-0.000994
TGF-beta signaling pathway	31	-0.00133
Prostate cancer	32	-0.00147
Glycosphingolipid biosynthesis - ganglio series	9	-0.00149
ABC transporters	19	-0.00161
Glycosaminoglycan degradation	11	-0.002
Tight junction	44	-0.00219
Salivary secretion	28	-0.00234
Insulin signaling pathway	44	-0.003
Renin-angiotensin system	10	-0.00307
Aldosterone-regulated sodium reabsorption	18	-0.00322
Adipocytokine signaling pathway	25	-0.00331
Glioma	24	-0.00372
Fc gamma R-mediated phagocytosis	31	-0.00372

Glycosaminoglycan biosynthesis - heparan sulfate / heparin	12	-0.00486
Proximal tubule bicarbonate reclamation	10	-0.00495
Type II diabetes mellitus	19	-0.00508
Carbohydrate digestion and absorption	16	-0.00518
MAPK signaling pathway	76	-0.00527

Table S3. ESC-OB GFP+ vs ESC significantly upregulated genes Signaling Pathway Analysis (Top 50 Terms)

Signaling Pathway	Gene Number	P value
Focal adhesion	124	-4.02E-18
ECM-receptor interaction	67	-4.52E-18
Lysosome	79	-1.93E-13
Pathways in cancer	165	-1.69E-12
Dilated cardiomyopathy	57	-8.72E-10
Hypertrophic cardiomyopathy (HCM)	54	-1.03E-09
Endocytosis	113	-3.33E-09
Axon guidance	74	-1.05E-08
Osteoclast differentiation	66	-1.10E-07
Toxoplasmosis	69	-2.60E-07
MAPK signaling pathway	126	-2.66E-07
Rheumatoid arthritis	48	-6.03E-07
Cytokine-cytokine receptor interaction	116	-6.48E-07
Vascular smooth muscle contraction	66	-8.63E-07
Amoebiasis	62	-2.20E-06
Complement and coagulation cascades	44	-4.24E-06
Protein processing in endoplasmic reticulum	83	-4.40E-06
Leukocyte transendothelial migration	62	-9.48E-06
Protein digestion and absorption	44	-1.07E-05
Regulation of actin cytoskeleton	100	-1.29E-05
SNARE interactions in vesicular transport	24	-1.30E-05
Chagas disease (American trypanosomiasis)	53	-1.64E-05
Apoptosis	47	-1.71E-05
Malaria	29	-2.00E-05
Hepatitis C	68	-2.05E-05
TGF-beta signaling pathway	46	-2.93E-05
Arrhythmogenic right ventricular cardiomyopathy (ARVC)	41	-3.80E-05

Small cell lung cancer	46	-4.33E-05
Pancreatic cancer	39	-4.95E-05
Salivary secretion	42	-5.05E-05
Calcium signaling pathway	83	-5.09E-05
Drug metabolism - cytochrome P450	46	-6.33E-05
Metabolic pathways	437	-0.000123
NOD-like receptor signaling pathway	32	-0.000192
Tight junction	64	-0.000201
Toll-like receptor signaling pathway	50	-0.00027
PPAR signaling pathway	41	-0.000375
Adipocytokine signaling pathway	36	-0.000376
Gastric acid secretion	38	-0.000407
Melanogenesis	49	-0.000421
Chemokine signaling pathway	82	-0.000453
Renal cell carcinoma	37	-0.00047
Leishmaniasis	34	-0.000498
N-Glycan biosynthesis	28	-0.000504
Circadian rhythm	15	-0.000646
Jak-STAT signaling pathway	69	-7.00E-04
Bacterial invasion of epithelial cells	36	-0.00108
Sphingolipid metabolism	23	-0.00153
Cell adhesion molecules (CAMs)	66	-0.0016
Glycerophospholipid metabolism	39	-0.00178

Table S4. qRT-PCR primer sequences

Gene	Forward primer (5’-3’)	Reverse primer (5’-3’)
Gata4	TTCCTGCTCGGACTTGGGAC	TTCCCAGGCAGGTGGAGAATAAG
Gata6	CTCAGGGGTAGGGGCATCA	GAGGACAGACTGACACCTATGTA
Flk1	CCTGGTCAAACAGCTCATCA	AAGCGTCTGCCTCAATCACT
α-SMA	GTCCCAGACATCAGGGAGTAA	TCGGATACTTCAGCGTCAGGA
Cxcl12	CTTCCTCCCAGAAGTCAGTCATCC	ACACAACACTGAACCCATCGCTG
Mash1	ACTTGAACTCTATGGCGGGTT	CCAGTTGGTAAAGTCCAGCAG

Cdx2	AGGCTGAGCCATGAGGAGTA	CGAGGTCCATAATTCCACTCA
Col1a1	CACCCTCAAGAGCCTGAGTC	GTTCGGGCTGATGTACCAGT
Col1a2	TCGGGCCTGCTGGTGTTCGTG	TGGGCGCGGCTGTATGAGTTCTTC
Twist1	AGATGTCATTGTTTCCAGAGAAGGA	TTAGTTATCCAGCTCCAGAGTCTCTAGA
Msx2	AACACAAGACCAACCGGAAG	GCAGCCATTTTCAGCTTTTC
Runx2	AACCCACGGCCCTCCCTGAACTCT	ACTGGCGGGGTGTAGGTAAAGGTG
Bglap	TCTCTCTGCTCACTCTGCTGGCC	TTTGTCAGACTCAGGGCCGC
Ibsp	TACCGGCCACGCTACTTTCTTTAT	GACCGCCAGCTCGTTTTCATCC