Supplementary Information

Table S1. List of reference genes used in 25 published articles involving gene expression studies under ES.

Title, [ref]	First Author	Reference Gene	Cell type
Electrical stimulation enhanced MSC gene expression for orthopaedic tissue repair, [17].	Richard Balint	18S	human MSC
Novel effect of biphasic electric current on in vitro osteogenesis and cytokine production in human MSCs, [39].	In Sook Kim	ACTB	human MSC
Electrical stimulation to promote osteogenesis using conductive polypyrrole films, [40].	Wei Wen Hu	ACTB	rat MSC
Electrical stimulation drives chondrogenesis of MSCs in the absence of exogenous growth factors, [41].	Hyuck Joon Kwon	ACTB	murine MSC
Electrically driven intracellular and extracellular nanomanipulators evoke neurogenic/cardiomyogenic differentiation, [32].	Greeshma Thrivikraman	GAPDH	human MSC
Combined treatment with electrical stimulation and insulin-like growth factor-1 promotes bone regeneration in vitro, [42].	Zhiping Qi	GAPDH	MC3T3-E1
Up-regulation of expression of selected genes in human bone cells with specific capacitively coupled electric fields, [33].	Charles C. Clark	GAPDH	human osteoblasts
Physical manipulation of calcium oscillations facilitates osteodifferentiation of human MSCs, [43].	Shan Sun	GAPDH	human MSC
Study of electrical stimulation with different electric-field intensities in the regulation of the differentiation of PC12 cells, [44].	Wei Jing	GAPDH	PC12 cells
Primary cilia are sensors of electrical field stimulation to induce osteogenesis of human adipose-derived stem cells, [45].	Shaobo Cai	GAPDH	human MSC (adipose-derived)
Intermittent electrical stimuli for guidance of human MSC lineage commitment towards neural-like cells on electroconductive substrates, [46].	Greeshma Thrivikraman	GAPDH	human MSC
Synergy of substrate conductivity and intermittent electrical stimulation towards osteogenic differentiation of human MSCs, [36].	K. Ravikumar	GAPDH	human MSC
Directly induced neural differentiation of human adipose-derived stem cells using three-dimensional culture system of conductive microwell with electrical stimulation, [47].	Dong Nyoung Heo	GAPDH	human MSC (adipose-derived)
Electrical stimulation of adipose-derived MSCs and endothelial cells co-cultured in a conductive scaffold for potential orthopaedic applications, [48].	Jieyu Zhang	GAPDH	human MSC (adipose-derived)
A novel condition of mild electrical stimulation exerts immunosuppression via hydrogen peroxide production that controls multiple signaling pathway, [59].	Mariam Piruzyan	GAPDH	Jurkat T cells

Electrical stimulation by enzymatic biofuel cell to promote proliferation, migration and differentiation of muscle precursor cells, [9].	Jae Ho Lee	GAPDH	C2C12 cells
Electrical stimulation induces direct reprogramming of human dermal fibroblasts into hyalinte chondrogenic cells, [50].	Gyu Seok Lee	GAPDH	human dermal fibroblasts
Biomimetic electrical stimulation induces rate bone marrow MSCs to differentiate into cardiomyocyte-like cells via TGF-beta 1 in vitro, [51].	Xueling He	GAPDH	rat MSC
Self-powered electrical stimulation for enhancing neural differentiation of MSCs on graphene-poly(3,4-ethylenedioxythiophene) hybrid microfibers, [52].	Weibo Guo	GAPDH	rat MSC
Pulsed electrical stimulation benefits wound healing by activating skin fibroblasts through the TGFβ1/ERK/NF-κB axis, [53].	Yongliang Wang	GAPDH	human skin fibroblasts
Direct electrical stimulation enhances osteogenesis by inducing Bmp2 and Spp1 expressions from macrophages and preosteoblasts, [16].	Kasama Srirussamee	GAPDH	J774A.1, MC3T3-E1
Electrical stimulation induces the level of TGF-β1 mRNA in osteoblastic cells by a mechanism involving calcium/calmodulin pathway, [54].	Hongming Zhuang	GAPDH	MC3T3-E1
Effect of electric stimulation on human chondrocytes and MSCs under normoxia and hypoxia, [55].	Bettina Hiemer	HPRT	human chondrocytes, human MSCs
In vitro effect of direct current electrical stimulation on rat MSCs, [14].	Sahba Mobini	RPLP0	rat MSC
Pretreating MSCs with ES causes sustained long-lasting pro-osteogenic effects, [15].	Maria Eischen-Loges	RPLP1, YWHAZ	rat MSC

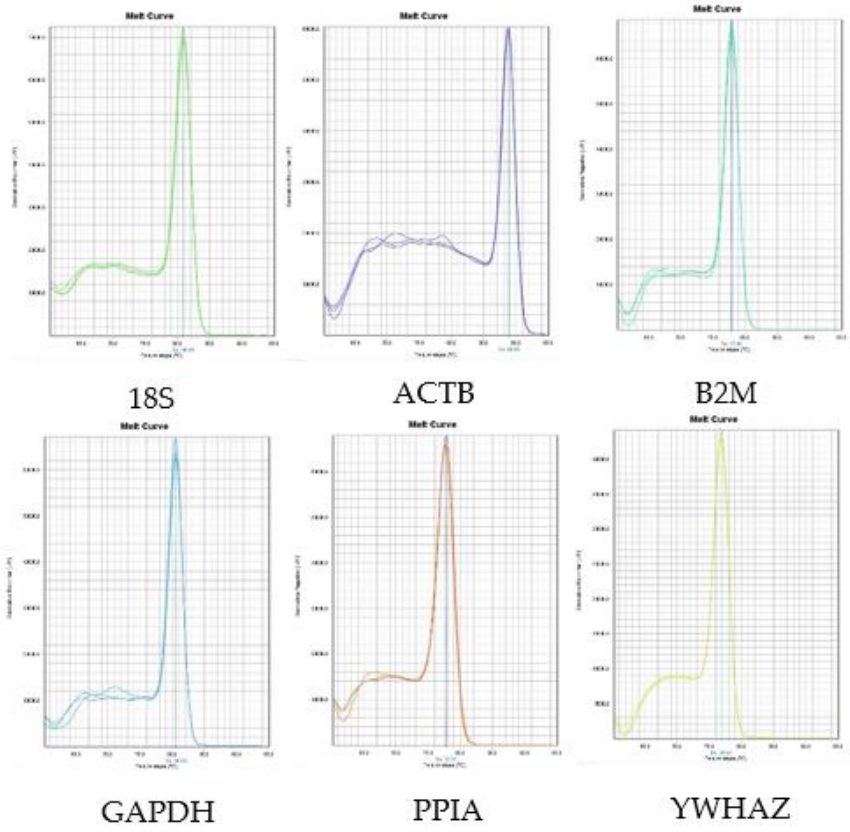


Figure S1. Melt curves for each of the reference genes used in this study.

Table S2. BestKeeper descriptive statistics for each reference gene in the 5000 cells/cm^2 group.

	PPIA	YWHAZ	18S	B2M	GAPDH	ACTB
N	5	5	5	5	5	5
GM, [C_T]	23.98	23.01	12.52	23.43	23.23	24.59
AR, [C_T]	23.98	23.01	12.52	23.44	23.23	24.59
Min, [C_T]	23.52	22.57	12.18	22.79	22.92	24.24
Max, [C_T]	24.54	23.51	12.90	24.46	23.59	25.17
Std Dev [±C_T]	0.43	0.37	0.26	0.68	0.27	0.27
CV [% C_T]	1.80	1.60	2.05	2.90	1.16	1.08
Min, [x-fold]	−1.37	−1.35	−1.26	−1.56	−1.23	−1.27
Max, [x-fold]	1.48	1.42	1.30	2.05	1.29	1.50
Std Dev [± x-fold]	1.35	1.29	1.19	1.60	1.20	1.20

GM = geometric mean; AR = arithmetic mean; Min = minimum; Max = maximum; Std Dev = standard deviation; CV = coefficient of variance; x-fold refers to the over- or under-expression of samples vs. the geometric mean [25].

Table S3. BestKeeper descriptive statistics for each reference gene in the 10,000 cells/cm^2 group.

	PPIA	YWHAZ	18S	B2M	GAPDH	ACTB
N	5	5	5	5	5	5
GM, [C_T]	23.43	22.43	11.74	23.65	22.44	25.41
AR, [C_T]	23.43	22.44	11.76	23.66	22.45	25.42
Min, [C_T]	22.58	21.44	10.85	22.57	21.40	24.48
Max, [C_T]	23.96	22.96	12.47	24.65	23.12	26.43

Std Dev [±C_T]	0.44	0.51	0.59	0.50	0.68	0.67
CV [% C_T]	1.86	2.26	5.04	2.10	3.02	2.63
Min, [x-fold]	−1.80	−1.99	−1.86	−2.11	−2.05	−1.90
Max, [x-fold]	1.44	1.44	1.65	2.00	1.61	2.02
Std Dev [± x-fold]	1.35	1.42	1.51	1.41	1.60	1.59

GM = geometric mean; C_T = threshold cycle value from qRT-PCR data; AR = arithmetic mean; Min = minimum; Max = maximum; Std Dev = standard deviation; CV = coefficient of variance; x-fold refers to the over- or under-expression of samples vs. the geometric mean [25].