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Supporting Information

3D encapsulation and inflammatory licensing of mesenchymal stromal cells alter the expression of common reference genes used in real-time RT-qPCR

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Supporting figures

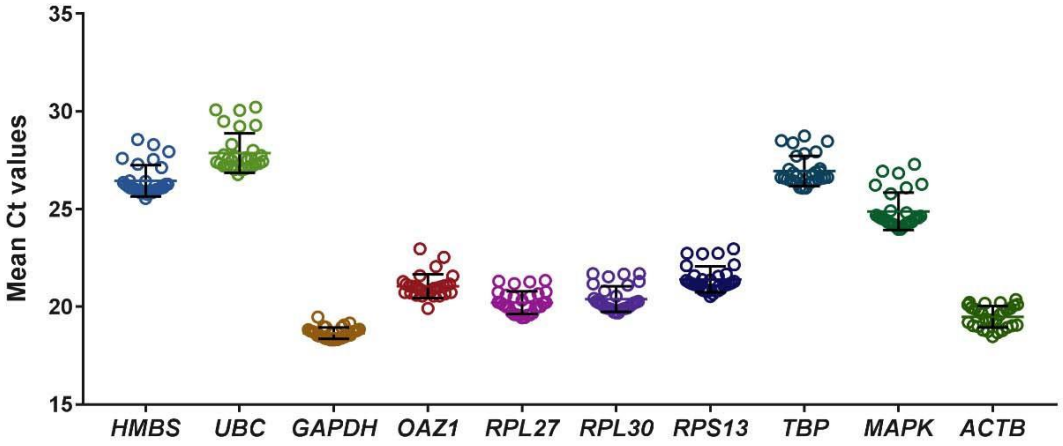

Figure S1. Expression levels of candidate reference genes.

Supporting tables

Table S1. MIQE guidelines

ITEM TO CHECK	IMPORTANCE	INCLUDED?	COMMENTS
EXPERIMENTAL DESIGN			
Definition of experimental and control groups	**E**	**Y**	See Methods
Number within each group	**E**	**Y**	
Assay carried out by core lab or investigator's lab?	D	Y	
Acknowledgement of authors' contributions	D	Y	See Acknowledgedments
SAMPLE			
Description	**E**	Y	See Methods
Volume/mass of sample processed	D	Y	
Microdissection or macrodissection	**E**	N/A	
Processing procedure	**E**	Y	
If frozen - how and how quickly?	**E**	N/A	
If fixed - with what, how quickly?	**E**	N/A	
Sample storage conditions and duration (especially for FFPE	**E**	Y	
NUCLEIC ACID EXTRACTION			
Procedure and/or instrumentation	**E**	Y	See Methods
Name of kit and details of any modifications	**E**	Y	
Source of additional reagents used	D	Y	
Details of DNase or RNAse treatment	**E**	Y	
Contamination assessment (DNA or RNA)	**E**	Y	
Nucleic acid quantification	**E**	Y	
Instrument and method	**E**	Y	
Purity (A260/A280)	D	Y	Table S2
Yield	D	Y	
RNA integrity method/instrument	**E**	Y	See Methods
RIN/RQI or Cq of 3' and 5' transcripts	**E**	Y	Table S2
Electrophoresis traces	D	Y	Table S2
Inhibition testing (Cq dilutions, spike or other)	**E**	Y	See Methods
REVERSE TRANSCRIPTION			
Complete reaction conditions	**E**	Y	See Methods
Amount of RNA and reaction volume	**E**	Y	
Priming oligonucleotide (if using GSP) and concentration	**E**	N/A	
Reverse transcriptase and concentration	**E**	Y	
Temperature and time	**E**	Y	
Manufacturer of reagents and catalogue numbers	D	Y	
Cqs with and without RT	D*	Y	
Storage conditions of cDNA	D	Y	
qPCR TARGET INFORMATION			
If multiplex, efficiency and LOD of each assay.	**E**	N/A	See Methods and Table S3
Sequence accession number	**E**	Y	
Location of amplicon	D	N	
Amplicon length	**E**	Y	
In silico specificity screen (BLAST, etc)	**E**	Y	
Pseudogenes, retropseudogenes or other	D	Y	
	D	N	
Secondary structure analysis of amplicon	D	N	
Location of each primer by exon or intron (if applicable)	**E**	N/A	
What splice variants are targeted?	**E**	Y	
qPCR OLIGONUCLEOTIDES			
Primer sequences	**E**	Y	See Methods and Table S3
RTPrimerDB Identification Number	D	N/A	
Probe sequences	D**	N/A	
Location and identity of any modifications	**E**	N/A	
Manufacturer of oligonucleotides	D	Y	
Purification method	D	Y	
qPCR PROTOCOL			
Complete reaction conditions	**E**	Y	See Methods
Reaction volume and amount of cDNA/DNA	**E**	Y	
Primer, (probe), Mg++ and dNTP concentrations	**E**	N*	
Polymerase identity and concentration	**E**	Y	
Buffer/kit identity and manufacturer	**E**	Y	
Exact chemical constitution of the buffer	D	N	
Additives (SYBR Green I, DMSO, etc.)	**E**	Y	
Manufacturer of plates/tubes and catalog number	D	Y	
Complete thermocycling parameters	**E**	Y	
Reaction setup (manual/robotic)	D	Y	
Manufacturer of qPCR instrument	**E**	Y	
qPCR VALIDATION			
Evidence of optimisation (from gradients)	D	N	
Specificity (gel, sequence, melt, or digest)	**E**	Y	See Methods
For SYBR Green I, Cq of the NTC	**E**	Y	
Standard curves with slope and y-intercept	**E**	Y	See Methods and Tables S3
PCR efficiency calculated from slope	**E**	Y	
Confidence interval for PCR efficiency or standard error	D	N	
r2 of standard curve	**E**	Y	
Linear dynamic range	**E**	Y	
Cq variation at lower limit	**E**	N	
Confidence intervals throughout range	D	N	
Evidence for limit of detection	**E**	Y	See Methods
If multiplex, efficiency and LOD of each assay.	**E**	N/A	
DATA ANALYSIS			
qPCR analysis program (source, version)	**E**	Y	See Methods
Cq method determination	**E**	Y	
Outlier identification and disposition	**E**	N/A	
Results of NTCs	**E**	Y	See Methods
Justification of number and choice of reference genes	**E**	Y	See Introduction
Description of normalisation method	**E**	Y	See Methods
Number and concordance of biological replicates	D	Y	
Number and stage (RT or qPCR) of technical replicates	**E**	Y	
Repeatability (intra-assay variation)	E	N	
Reproducibility (inter-assay variation, %CV)	D	N	
Power analysis	D	N	
Statistical methods for result significance	**E**	Y	See Methods
Software (source, version)	E	Y	
Cq or raw data submission using RDML	**D**	N	

E= essential information, D= desirable information, Y= yes (included in teh manuscript), N= no (not inlcuded), N/A= not applicable. * Propietary information, not provided by the manufacturer

Table S2. RNA yield and integrity determination.

Sample ID	RIN	28S/18S (Area)	$A_{260/280}$	$A_{260/230}$	RNA Conc. (ng μl^{-1})
1. TCP Ctrl_1	8,3	1,2	2,05	1,94	206
2. TCP Ctrl_2	8,7	1,3	2,06	2,02	161
3. TCP Ctrl _3	8,2	1,1	2,07	2,31	282
4. TCP Stim_1	8,1	1,5	2,04	2,34	203
5. TCP Stim_2	8,4	1,6	2,06	1,2	221
6. TCP Stim_3	8,1	1,3	2,03	2,32	156
7. VE soft Ctrl_1	8,9	2,0	2,05	0,39	58,4
8. VE soft Ctrl_2	8,7	1,8	1,97	1,6	62,7
9. VE soft Ctrl_3	9,2	2,3	1,99	2,32	78,9
10. VE stiff Ctrl_1	8,8	2,0	2	1,12	61,5
11. VE stiff Ctrl_2	8,9	2,0	1,93	2,55	75,9
12. VE stiff Ctrl_3	9,1	2,2	2,03	2,19	85,6
13. EL soft Ctrl_1	9,2	2,1	2,05	1,55	126
14. EL soft Ctrl_2	8,9	1,9	2	2,24	159
15. EL soft Ctrl_3	9,0	2,5	1,98	1,58	96,5
16. EL stiff Ctrl_1	9,0	2,2	1,98	1,98	94,6
17. EL stiff Ctrl_2	9,2	1,8	1,94	2,91	60,2
18. EL stiff Ctrl_3	8,9	1,8	1,98	2,4	144
19. VE soft Stim_1	9,2	2,1	2,07	1,3	62,7
20. VE soft Stim_2	9,0	2,2	2,05	2,26	99,6
21. VE soft Stim_3	9,0	2,1	2,07	1,3	93,1
22. VE stiff Stim_1	6,7	1,1	1,92	0,97	17,9
23. VE stiff Stim_2	8,6	1,8	1,99	0,93	34,7
24. VE stiff Stim_3	8,7	1,8	2,05	0,9	42,1
25. EL soft Stim_1	9,3	2,2	2,05	1,64	107
26. EL soft Stim_2	9,2	1,9	2,03	1,71	86,2
27. EL soft Stim_3	9,2	2,3	2,01	1,54	56,5
28. EL stiff Stim_1	9,1	2,0	2,05	1,22	47,1
29. EL stiff Stim_2	9,0	2,0	2,04	1,96	55,0
30. EL stiff Stim_3	9,0	1,8	2,09	0,58	40,0

28S/18S ratios and RIN values were calculated by means of electrophoretic separation after RNA denaturation **(Fig. S1)**. $A_{260/A280}$ and $A_{260/230}$ were calculated by spectroscopy. n = 3 samples per experimental condition. RIN, RNA integrity number. TCP, tissue culture plate (2D). Ctrl, control. Stim, stimulated. VE, viscoelastic. EL, elastic.

Table S3. Primer pairs employed for the reference gene study.

Gene	Accession number (mRNA)	Primer sequences	Amplicon size (bp)	Amplification factor (E)	R^2
RPS13	NM_001017.2	**Fwd** CGCTCTCCTTTCGTTGCCT **Rv** CGCTGCGTCGATAGGGTAAA	96	1.97	0.9975
RPL27	NM_000988.3	**Fwd** ATCGCCAAGAGATCAAAGATAA **Rv** TCTGAAGACATCCTTATTGACG	123	1.97	0.999
RPL30	NM_000989.3	**Fwd** ACAGCATGCGGAAAATACTAC **Rv** AAAGGAAAATTTTGCAGGTTT	158	1.95	0.9977
OAZ1	NM_004152.3 NM_001301020.1	**Fwd** CTCCACTGCTGTAGTAACCCG Rv GATCCCTCTGACTATTCCCTCG	104	1.97	0.9993
ACTB	NM_001101.3	**Fwd** AGCACAGAGCCTCGCCTTT **Rv** GAGCGCGGCGATATCATCA	82	1.97	0.9993
GAPDH	NM_001289746.1 NM_001289745.1 NM_001256799.2 NM_002046.5	**Fwd** CCACATGGCCTCCAAGGAGTAAGAC **Rv** AGGAGGGGAGATTCAGTGTGGTGGG	131	1.96	0.9998
MAPK1	NM_002745.4 NM_138957.3	**Fwd** TCCCAAATGCTGACTCCAAAG **Rv** CATGTCGAACTTGAATGGTGC	164	1.98	0.9994
UBC	NM_021009.6	**Fwd** GCCTTAGAACCCCAGTATCAG **Rv** AAGAAAACCAGTGCCCTAGAG	74	2.05	0.9999
HMBS	NM_000190.3 NM_001024382.1 NM_001258208.1 NM_001258209.1 XM_017017629.1 XM_005271531.1	**Fwd** AGCTTGCTCGCATACAGACG **Rv** AGCTCCTTGGTAAACAGGCTT	157	1.93	0.9973

	XM_005271532.1 XM_005271533.3 XM_011542796.1				
TBP	NM_003194.4 NM_001172085.1	**Fwd** CCACTCACAGACTCTCACAAC **Rv** CTGCGGTACAATCCCAGAACT	127	1.94	0,9987

Primer pairs amplified all transcription variants with equal amplicon length. E, efficiency of qPCR reaction; R^2, coefficient of determination from linear regression of Cq values (cDNA serial dilution).

Table S4. Cq values determined by Bestkeeper algorithm.

Gene	Geo. Mean [Cq]	SD [± Cq]	CV [% Cq]
GAPDH	18.64	0.24	1.30
OAZ1	21.05	0.40	1.88
TBP	26.94	0.61	2.26
RPL27	20.20	0.46	2.30
HMBS	26.44	0.62	2.33
RPS13	21.39	0.51	2.36
ACTB	19.48	0.49	2.49
RPL30	20.38	0.53	2.61
UBC	27.86	0.78	2.79
MAPK1	24.87	0.75	3.03

Geo, geometric. SD, standard deviation. CV, coefficient of variance.

Table S5. Stability values of reference genes determined by NormFinder algorithm.

Gene	Stability value
RPS13 + TBP	0.116
RPL30	0.175
HMBS	0.179
RPS13	0.182
OAZ1	0.200
TBP	0.226
GAPDH	0.248
RPL27	0.269
MAPK1	0.271
ACTB	0.350
UBC	0.365

Table S6. Stability values of reference genes determined by geNorm.

Gene	Stability value (M)
RPS13 + RPL30	0.268
RPS13	0.267
RPL30	0.271
HMBS	0.296
MAPK1	0.3
TBP	0.391
RPL27	0.433
GAPDH	0.471
OAZ1	0.497
UBC	0.541
ACTB	0.6