

Supplemental Information for:

Noninvasive measurement of respiratory activity of mouse embryoid bodies and its correlation with mRNA levels of undifferentiation/differentiation markers

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Table S1. Primer list for gene expression analyses of mEBs for 1 to 6 days of culture and those that underwent further cultivation on gelatin-coated dishes for 10 days. ES cells at the different passages and those cultured in different media before mEB formation were also evaluated.

Table S2. Primer list for gene expression analyses of mEBs with the same radius for 3 different culture conditions.

Figure S1. The average expression levels of 48 genes for mEBs with an initial cell number per drop of 500 cells/drop for days 1, 2, 3, 4, and 6.

Figure S2. The average expression levels of 6 genes (mesoderm) as a function of length of cultivation.

Figure S3. The average expression levels of 48 genes for embryonic stem (ES) cells (passages 19 and 40) cultured with +LIF, -FBS medium. 2-dimensionally differentiated cells with -LIF, +FBS medium, and embryoid bodies (EBs) on day 1 cultured with -LIF, +FBS medium.

Figure S4. The gene expression levels vs. F_{O_2} for the other 23 marker genes except for those shown in **Fig. 2-C**.

Figure S5. (A) The rate of the beating sample number for each culture condition. 24 samples of mEBs were evaluated for each culture condition. **(B)** The percentage of the beating area of the mEB-derived beating sample normal to the whole area of the outgrowth on gelatin-coated dish. The areas of the beating and whole outgrowth were estimated on day 10 or 11 by using Image J software.

Figure S6. The respiratory activities (F_{O_2}) and radii (r_s) of mEBs cultured for 1 to 6 days of culture as a function of each PC score axis.

Figure S7. The respiratory activities (F_{O_2}) and radii (r_s) of mEBs with the same radii for the 3 different culture conditions were plotted as a function of each PC score axis.

Table S1. Primer list for gene expression analyses of mEBs for 1 to 6 days of culture and those that underwent further cultivation on gelatin-coated dishes for 10 days. ES cells at the different passages and those cultured in different media before mEB formation were also evaluated.

Gene Type	Gene Symbol	Left Primer 5'→3'	Right Primer 5'→3'	Notes (Optional)	ID
Pluripotency	<i>Lifr</i>	GCTAATTCCAAGAAAGAGTGAGG	AGCAACATGGTAAGTTGAATGCT	leukemia inhibitory factor receptor	ENSMUST00000067190.4
	<i>Nanog</i>	TTCTTGCTTACAAGGGTCTGC	AGAGGAAGGGGCGAGGAGA	Nanog homeobox	ENSMUST00000112581.1
	<i>Pou5f1</i>	GTTGGAGAAGGTGGAACCAA	CTCCTTCTGCAAGGGCTTTTC	POU domain, class 5, transcription factor 1	ENSMUST00000025271.7
	<i>Sox2</i>	TCCAAAACCTAATCACAACAATCG	GAAGTGCAATTGGGATGAAAA	SRY-box containing gene 2	ENSMUST00000099151.2
	<i>Stat3</i>	GGAAATAACGGTGAAGGTGCT	CATGTCAAACGTGAGCGAAT	signal transducer and activator of transcription 3	ENSMUST00000092671.4
Endoderm	<i>Rex1</i>	GAGTCCACCCAGCGTGAAGA	GTCTTCTCCTCAGCCTTCTCC	RNA exonuclease 1 homolog	NM_025852.3
	<i>Gata6</i>	GGTCTCTACAGCAAGATGAATGG	TGGCACAGGACAGTCCAAAG	GATA binding protein 6	ENSMUST00000047762.7
	<i>Afp</i>	AAGGAACCTCACAGCAGCACTG	GCACAGACAATTGTAACTGTAGCA	Alpha-fetoprotein	ENSMUST00000042755.2
	<i>Sox17</i>	CACAACGACAGAGCTAAGCAA	CGCTTCTCTGCCAAGGTC	Transcription factor SOX-17	ENSMUST00000027035.3
	<i>Lamb1</i>	TTGCGTGTGTTTGTGATCCT	ATCCAGAGGACAGTCAATCA	laminin B1	ENSMUST00000002979.7
Mesoderm	<i>Foxa2</i>	GAGCAGCAACATCACCCACAG	CGTAGGCCTTGAGGTCCAT	Hepatocyte nuclear factor 3-beta	ENSMUST00000047315.1
	<i>Sox7</i>	CACCGTGCCTGAGAAAAAC	GGGAGTACTCACCCTGTGTC	Transcription factor SOX-7	ENSMUST00000079652.5
	<i>Pdgfra</i>	CACCAGCAGCGTCAAGTG	TTCTGACATCTCCACTTTTGG	Platelet-derived growth factor subunit A	ENSMUST00000076085.6
	<i>Gsc</i>	GGAGACGAAGTACCCAGACG	CGGCGGTTCTTAAACGAG	Homeobox protein goosecoid	ENSMUST00000021513.2
	<i>T</i>	ACTCCTGGAGGTGGAGAGG	TCTTCCCTGCCTCTCTG	Brachyury protein	ENSMUST00000074667.2
Cardiomyocyte	<i>Kdr</i>	CCCCAAATTCATTATGACAA	CGGCTCTTTGCTTACTGTT	Vascular endothelial growth factor receptor 2	ENSMUST00000113516.1
	<i>Foxf1a</i>	AGCATCTCCACGCCACTCC	TGTGAGTGATACCGAGGGATG	Forkhead box protein F1	ENSMUST00000050684.1
	<i>Gata1</i>	CCCTGAACCTCGTACACCACT	GGGAACACTGGGGTTGAA	Erythroid transcription factor	ENSMUST00000033502.8
	<i>Gata4</i>	GGAGACACCCCAATCTCG	CATGGCCCCACAATTGAC	Gata4 Transcription factor	ENSMUST00000118022.1
	<i>Actc1</i>	GGTCATCACCAATTGGCAAC	ATGCCAGCAGATTCCATACC	Actin, alpha cardiac muscle 1	ENSMUST00000090289.5
Vascular endothelium	<i>Nkx2-5</i>	GACGTAGCCTGGTGTCTCG	GTGTGGAATCCGTCGAAAGT	Homeobox protein Nkx-2.5	ENSMUST00000015723.2
	<i>Myh6</i>	CGCATCAAGGAGCTCAC	CCTGCAGCCGATTAAGT	Myosin heavy chain 6	ENSMUST000000081857.5
	<i>Myh7b</i>	GCCTCTGCGGACATTGATAG	GGGCAGCTGGAAGATCACT	Myosin heavy chain 7B	ENSMUST00000092995.4
	<i>Tnnt2</i>	ATGTCTGACGCCGAGGAG	CACCTGCCTCTCTTGTCTCT	Troponin T	ENSMUST00000027671.5
	<i>Angpt1</i>	GGAGATGGAAGCCTGGAT	ACCAGAGGGATTCCCAAAAC	Angiopoietin-1	ENSMUST00000110296.1
Ectoderm	<i>Angpt2</i>	CACACTGACCTTCCCCAAT	CCCAAGTCCATGTCACAGTA	Angiopoietin-2	ENSMUST00000033846.5
	<i>Mash1</i>	TCTCCTGGGAATGGAATTTG	CGTTGGCGAGAAACACTAAAG	Ascl1-001 Achaete-scute homolog 1	ENSMUST00000020243.6
	<i>Pax6</i>	ACCCGGCAGAAGATCGTAG	TTTGCATCTGCATGGGTCT	Paired box protein Pax-6	ENSMUST00000111087.1
	<i>Sox1</i>	GCGAGATGATCAGCATGTACC	TAGTGCTGTGGCAGCGAGT	Transcription factor SOX-1	ENSMUST00000116111.1
	<i>Otx2</i>	GACTGCAGGGCAGAGACG	GGTAGATTGGAGTGACGGAAC	Homeobox protein OTX2	ENSMUST00000118578.1
Neurons	<i>Trp63</i>	CACCTCTCCATGCCCTCCA	GCCCCAACCTTGCTAAGAACT	Tumor protein 63	ENSMUST00000040231.5
	<i>Hes1</i>	ACACCGGACAAACCAAGAC	CGCCTCTCTTCCATGATAGG	Transcription factor HES-1	ENSMUST00000023171.7
	<i>Nes</i>	CTGCAGGCCACTGAAAAGTT	TCTGACTCTGTAGACCCCTGCTTC	Nestin	ENSMUST00000090973.4
	<i>Map2</i>	GGTTC AAGGATAACATCAACA	CATT TGGATGTACATGGCTTA	Microtubule-associated protein 2	ENSMUST00000114017.1
	<i>Neurod1</i>	CGCAGAAGGCAAGGTGTC	TTTGGTCATGTTTCCACTTCC	Neurogenic differentiation factor 1	ENSMUST00000040109.3
Hypoxic response	<i>Tubb3</i>	GCGCATCAGCGTATACTACAA	CATGGTTCCAGGTTCCAAGT	Tubulin beta-3 chain	ENSMUST00000071134.2
	<i>Fgf5</i>	AAAACCTGGTGACCCCTAGA	CATCACATTCGCCAATTAAGC	Fibroblast growth factor 5	NM_010203.4
	<i>Wnt3a</i>	CTTAGTGCTCTGCAGCCTGA	GAGTGCTCAGAGAGGAGTACTGG	wingless-related MMTV integration site 3A	NM_009522.2
	<i>Hnf4a</i>	CAGCAATGGACAGATGTGTGA	TGGTGATGGCTGTGGAGTC	Hepatocyte nuclear factor 4-alpha	ENSMUST00000109411.1
	<i>Eno1</i>	GAGGACACTTTCATCGCAGAC	CCAGCTCTTCTCAATTCTGA	Alpha-enolase	ENSMUST00000080926.5
Apoptosis	<i>Aldoa</i>	TGCCAGTATGTTACTGAGAAGTC	CATGTTGGGCTTCAGCAA	Fructose-bisphosphate aldolase A	ENSMUST00000032934.4
	<i>Hif1a</i>	GCCTAGACAAAGTTCCACCTGAGA	CGCTATCCACATCAAAAGCAA	Hypoxia-inducible factor 1 alpha	ENSMUST00000021530.6
	<i>Casp8</i>	TTGAACAATGAGATCCCCAAA	CCATTTCTACAAAAATTTCAAGCAG	Caspase-8	ENSMUST00000027189.7
	<i>Casp3</i>	ACTCGTGAAGACATTTTGAATTA	TCACCATGGCTT AGAATCACA	Caspase-3	ENSMUST00000009351.7.4
House Keeping	<i>Tbp</i>	CGGTGCGCTGATTTTCTC	GGGTTATCTTTCACACACCATGA	TATA box binding protein	ENSMUST00000119879.1
	<i>Tfrc</i>	TCCTTTCTTGCATATCTGG	CCAAATAAGGATAGTCTGCATCC	Transferrin receptor protein 1	ENSMUST000000023486.7
	<i>Actb</i>	CTAAGGCCAACCGTGAAAG	ACCAGAGGCATACAGGGACA	Actin, cytoplasmic 1	ENSMUST00000100497.3
	<i>Gapdh</i>	GGGTTCTTATAAATACGGAATGC	CCATT TTTGCTACGGGACGA	glyceraldehyde-3-phosphate dehydrogenase	ENSMUST00000118875.1

Table S2. Primer list for gene expression analyses of mEBs with the same radius for 3 different culture conditions.

Gene Type	Gene Symbol	Left Primer 5'→3'	Right Primer 5'→3'	Notes (Optional)	ID
Pluripotency	<i>Nanog</i>	TTCTTGCTTACAAGGGTCTGC	AGAGGAAGGGCGAGGAGA	Nanog homeobox	ENSMUST00000112581.1
	<i>Pou5f1</i>	GTTGGAGAAGGTGGAACCAA	CTCCTTCTGCAGGCTTTTC	POU domain, class 5, transcription factor 1	ENSMUST00000025271.7
	<i>Sox2</i>	TCCAAAACTAATCACAACAATCG	GAAGTGCAATTGGGATGAAAA	SRY-box containing gene 2	ENSMUST00000099151.2
	<i>Stat3</i>	GGAAATAACGGTGAAGGTGCT	CATGTCAAACGTGAGCGACT	signal transducer and activator of transcription 3	ENSMUST000000092671.4
	<i>Rexo1</i>	GAGTCCACGACGTGAAGA	GTCTTCTCCTCAGCCTTCTCC	RNA exonuclease 1 homolog	NM_025852.3
	<i>Zfp57</i>	CCTCAAGTAGGGCTGTCGAG	ATTTGTTGGTGCCCACTTCT	Zinc finger protein 57	ENSMUST000000089250.5
Endoderm	<i>Gata6</i>	GGTCTCTACAGCAAGATGAATGG	TGGCACAGGACAGTCCAAAG	GATA binding protein 6	ENSMUST00000047762.7
	<i>Afp</i>	GTTCTGGCATGCTGCAAAAG	GGATGCTCTCTTTGTCTGGAA	Alpha-fetoprotein	ENSMUST000000042755.2
	<i>Sox17</i>	CACAACGCAAGGCTAAGCAA	CGCTTCTCTGCACAAGGTC	Transcription factor SOX-17	ENSMUST00000027035.3
	<i>Foxa2</i>	GAGCAGCAACATCACCAAG	CGTAGGCCTTGAGGTCCAT	Hepatocyte nuclear factor 3-beta	ENSMUST00000047315.1
	<i>Lefty1</i>	TGACCGGAGAGCAGATCCT	GACTGGCGGTTGATCGAG	Left-right determination factor 1	ENSMUST000000037361.7
	<i>Cer1</i>	TCAGCTGCTTGTCTCTTTC	ACTCTGGCAGCCATCCAC	Cerberus-related protein	ENSMUST000000048430.2
Hepatocyte	<i>Hhex</i>	GACATCGAGGGGATAAAGG	TCTCTAAACATGCCCGATGAC	Hematopoietically-expressed homeobox protein	ENSMUST00000025944.1
	<i>Dlk1</i>	GGGACTCCAGCCCTAAGTGT	TGCACGCACTCAGAATACAA	Protein delta homolog 1	ENSMUST000000056110.7
Mesoderm	<i>Ttr</i>	ATTTCCTCGTTCCATGAAT	GATGGTGTAGTGGCGATGG	Transferrin	ENSMUST00000075312.2
	<i>Gsc</i>	GGAGACGAAGTACCCAGACG	CGGCGGTTCTTAAACCAAG	Homeobox protein goosecoid	ENSMUST000000021513.2
	<i>T</i>	ACTCCTGGAAGGTGGAGAGG	TCTTCCTCTGCCTCTCTG	Brachyury protein	ENSMUST000000074667.2
	<i>Kdr</i>	CCCCAAATTCATTATGACAA	CGGCTCTTTGCTTACTGTT	Vascular endothelial growth factor receptor 2	ENSMUST00000113516.1
	<i>Gata1</i>	CCCTGAACCTCGTCATACCACT	GGGAACACTGGGGTTGAA	Erythroid transcription factor	ENSMUST000000033502.6
	<i>Bmp4</i>	GAGGAGTTTCCATCACGAAGA	GCTCTGCCGAGGAGATCA	Bone morphogenetic protein 4	ENSMUST000000074077.4
Cardiomyocyte	<i>Dll1</i>	AGCAAACGTGACACCAAGTG	TAAGTGTTGGGGCGATCTTC	Delta-like protein 1	ENSMUST000000014917.6
	<i>Gata4</i>	GGAGACACCCCAATCTCG	CATGCCCCCACAATTGAC	Gata4 Transcription factor	ENSMUST00000118022.1
	<i>Actc1</i>	GGTCATCACCAATTGGCAAC	ATGCGAGCAGATTCCATACC	Actin, alpha cardiac muscle 1	ENSMUST000000090269.5
	<i>Nkx2-5</i>	GACGTAGCCTGGTGTCTCG	GTGTGGAATCCGTCCGAAAGT	Homeobox protein Nkx-2.5	ENSMUST000000015723.2
	<i>Myl2</i>	GCCTCAGGTGACCCACAG	GCCAGAGCCCAAGACTTCCT	Myosin regulatory light chain 2	ENSMUST000000014080.5
	<i>Sox1</i>	GCGAGATGATCAGCATGTACC	TAGTGTCTGTGGCAGCGAGT	Transcription factor SOX-1	ENSMUST00000116111.1
Ectoderm	<i>Otx2</i>	GACTGCAGGGCAGAGACG	GGTAGATTTGGAGTGAOAGGAA	Homeobox protein OTX2	ENSMUST00000118578.1
	<i>Zic2</i>	GATCCACAAAAGAACTCATACAGG	CTTCTTCTCTGCTGCTGT	Zinc finger protein ZIC 2	ENSMUST000000075888.4
	<i>Zic3</i>	ACGTGGACAACAACCAAGTTC	GGTCTGCACGGCCAAATA	Zinc finger protein ZIC 3	ENSMUST000000088627.3
	<i>Fgf8</i>	GCTGAGCTGCCTGCTGTT	AGCTCGGAGCAGGGAAGT	Fibroblast growth factor 8	ENSMUST000000026240.6
	<i>Nes</i>	CTGCAGGCCACTGAAAAGTT	TCTGACTCTGTAGACCCTGCTTC	Nestin	ENSMUST000000090973.4
	<i>Neurod1</i>	CGCAGAAGGCCAAGGTGTC	TTTGGTCATGTTTCCACTTCC	Neurogenic differentiation factor 1	ENSMUST000000041099.3
Neurons	<i>Tubb3</i>	GCGCATCAGCGTATACTACAA	CATGGTTCCAGGTTCGAAGT	Tubulin beta-3 chain	ENSMUST000000071134.2
	<i>Fgf5</i>	AAAACCTGGTGACCCCTAGA	CATCACATTCCGGAATTAAGC	Fibroblast growth factor 5	NM_010203.4
Hypoxic response	<i>Foxd3</i>	CCCCAACACTGACCAACAG	GTTTGCTCCGCCAGCTTA	Forkhead box protein D3	ENSMUST000000087285.3
	<i>Hif1a</i>	GCACTAGACAAAGTTCACTGAGA	CGCTATCCACATCAAAGCAA	Hypoxia-inducible factor 1 alpha	ENSMUST000000021530.6
Apoptosis	<i>Casp8</i>	TTGAACAATGAGATCCCCAAA	CCATTTCTACAAAATTTCAAGCAG	Caspase-8	ENSMUST000000027189.7
House Keeping	<i>Gapdh</i>	GGGTTCCTATAAATACGGACTGC	CCATTTTGTCTACGGGACGA	glyceraldehyde-3-phosphate dehydrogenase	ENSMUST00000118875.1

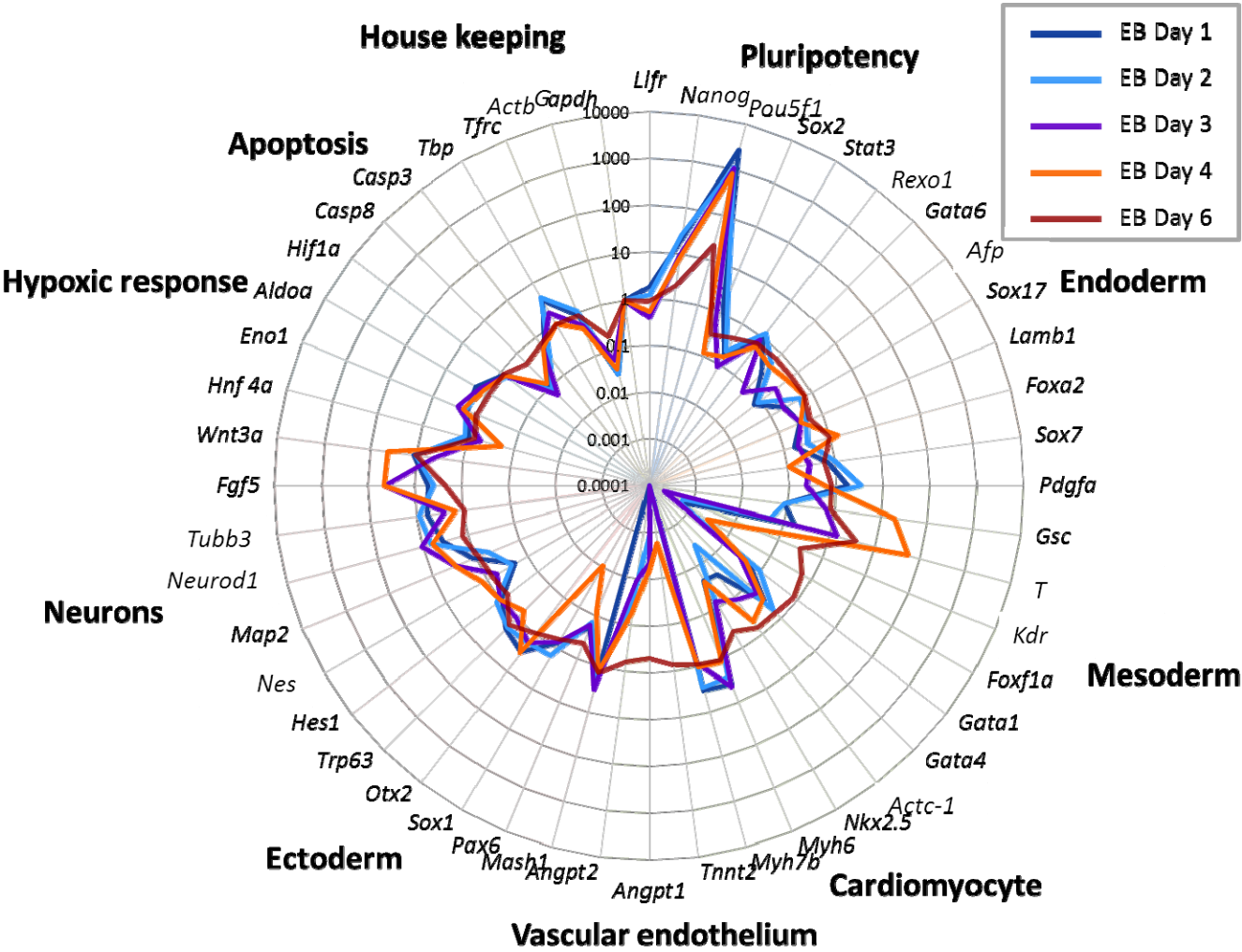


Figure S1. The average expression levels of 48 genes for mEBs with an initial cell number per drop of 500 cells/drop for days 1, 2, 3, 4, and 6.

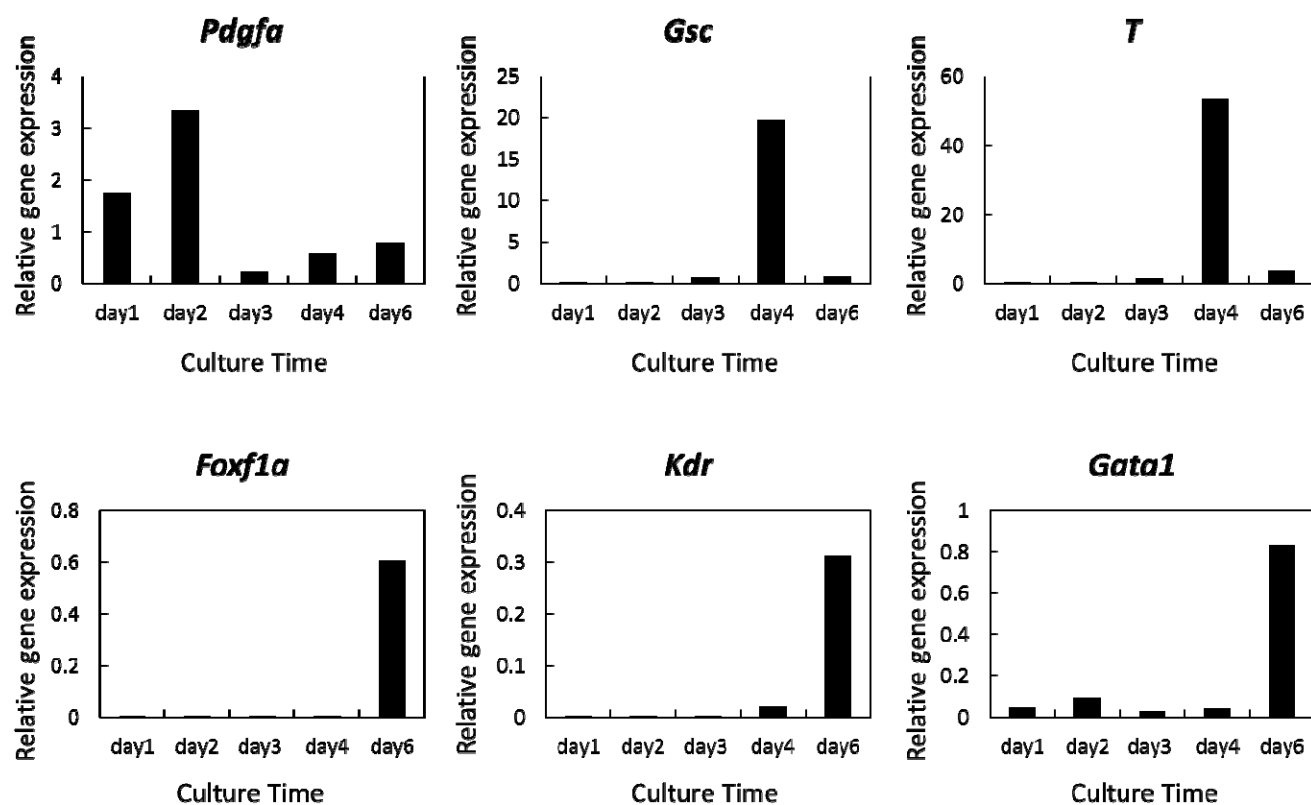


Figure S2. The average expression levels of 6 genes (mesoderm) as a function of length of cultivation.

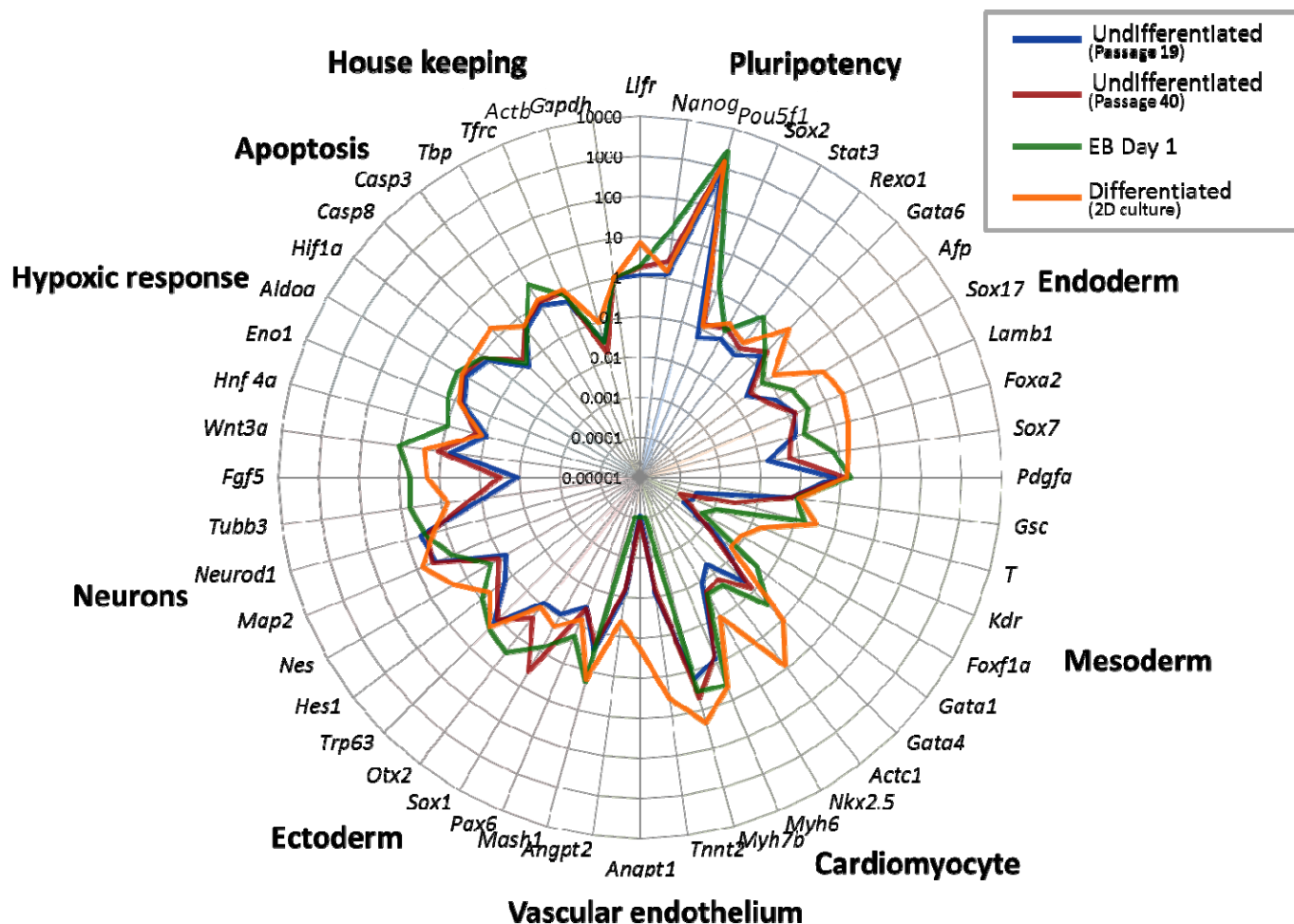


Figure S3. The average expression levels of 48 genes for embryonic stem (ES) cells (passages 19 and 40) cultured with +LIF, -FBS medium. 2-dimensionally differentiated cells with -LIF, +FBS medium, and embryoid bodies (EBs) on day 1 cultured with -LIF, +FBS medium.

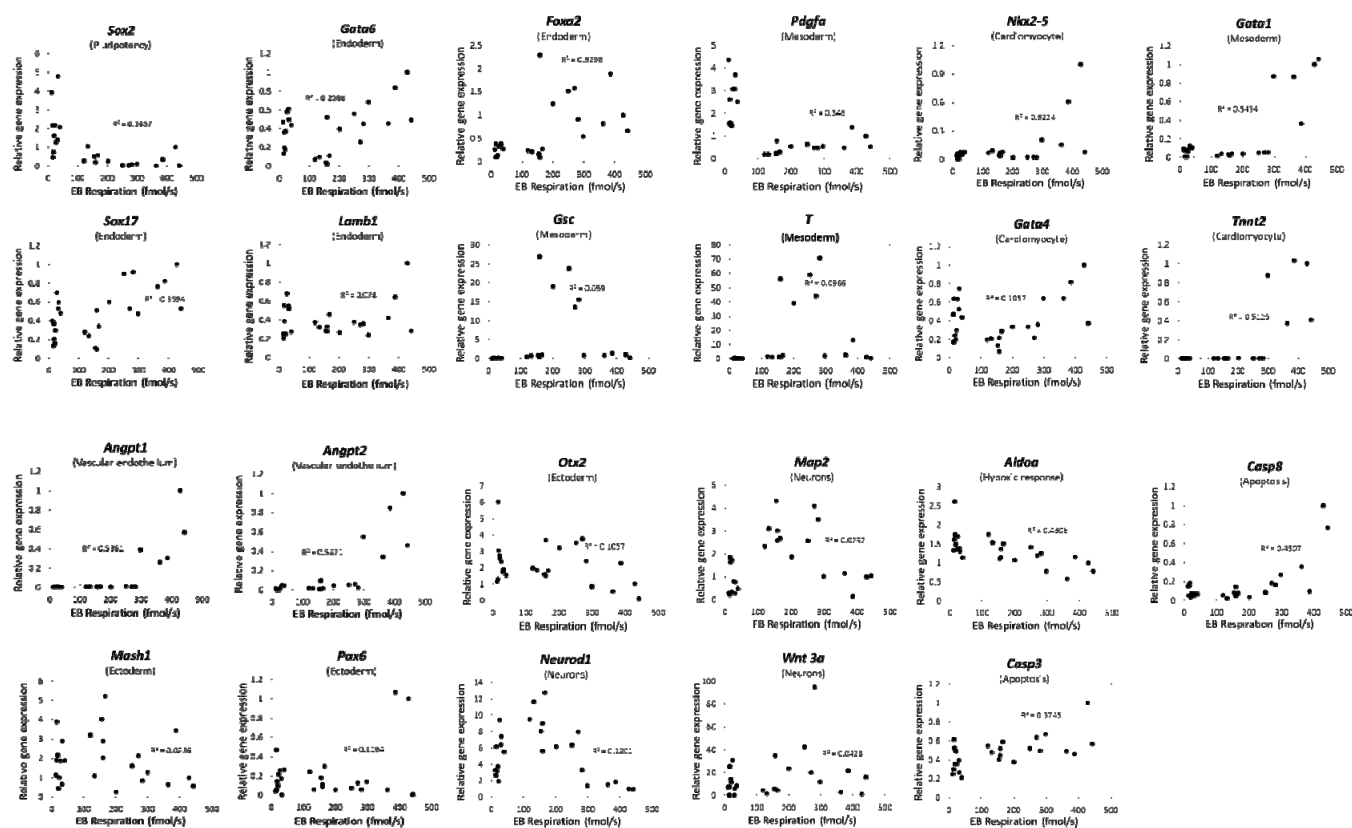


Figure S4. The gene expression levels vs. F_{O_2} for the other 23 marker genes except for those shown in Fig. 2-C.

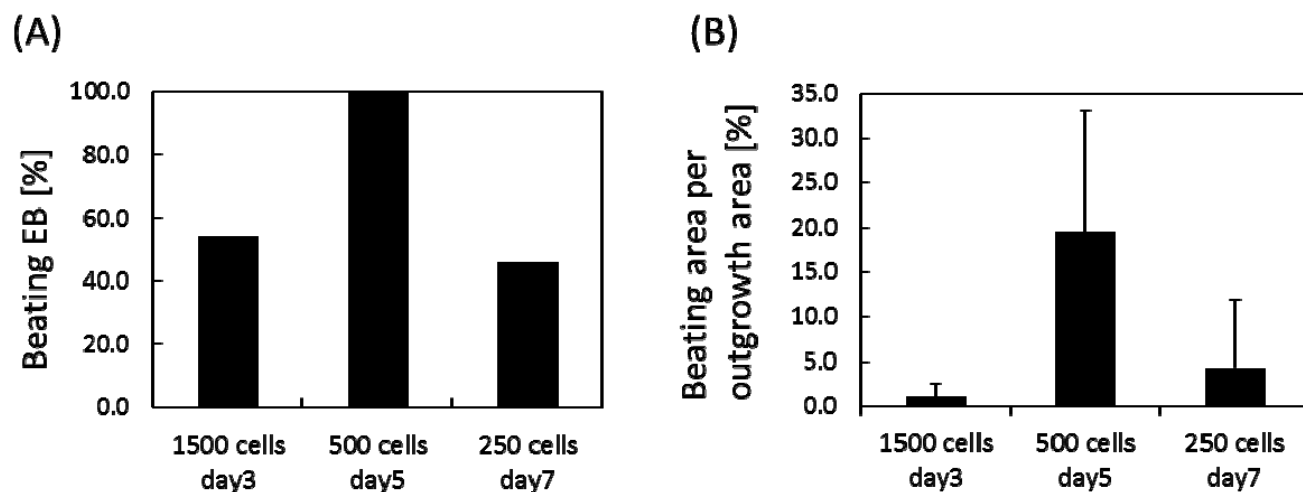


Figure S5. (A) The rate of the beating sample number for each culture condition. 24 samples of mEBs were evaluated for each culture condition. (B) The percentage of the beating area of the mEB-derived beating sample normal to the whole area of the outgrowth on gelatin-coated dish. The areas of the beating and whole outgrowth were estimated on day 10 or 11 by using Image J software.

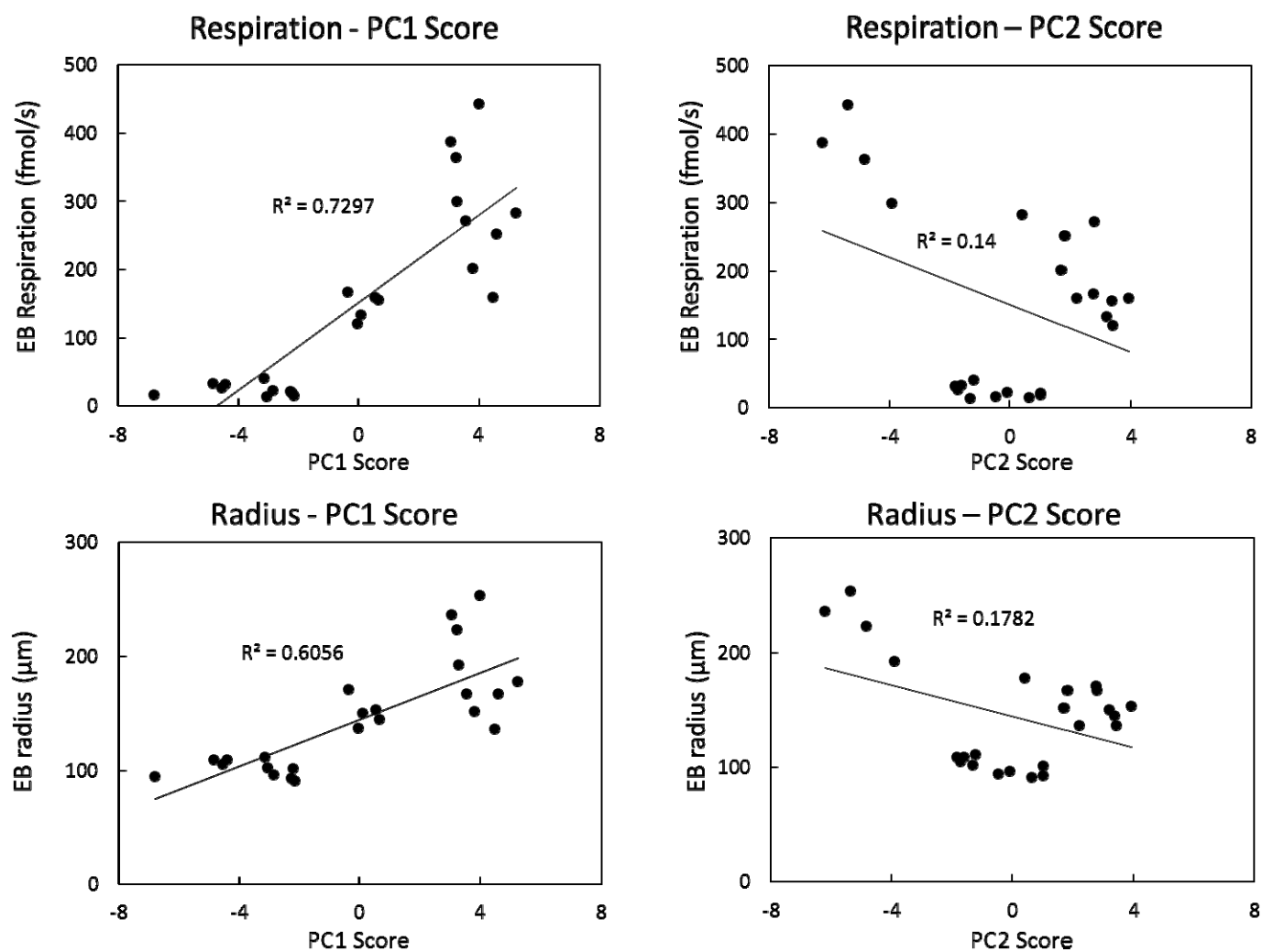


Figure S6. The respiratory activities (F_{O_2}) and radii (r_s) of mEBs cultured for 1 to 6 days of culture as a function of each PC score axis.

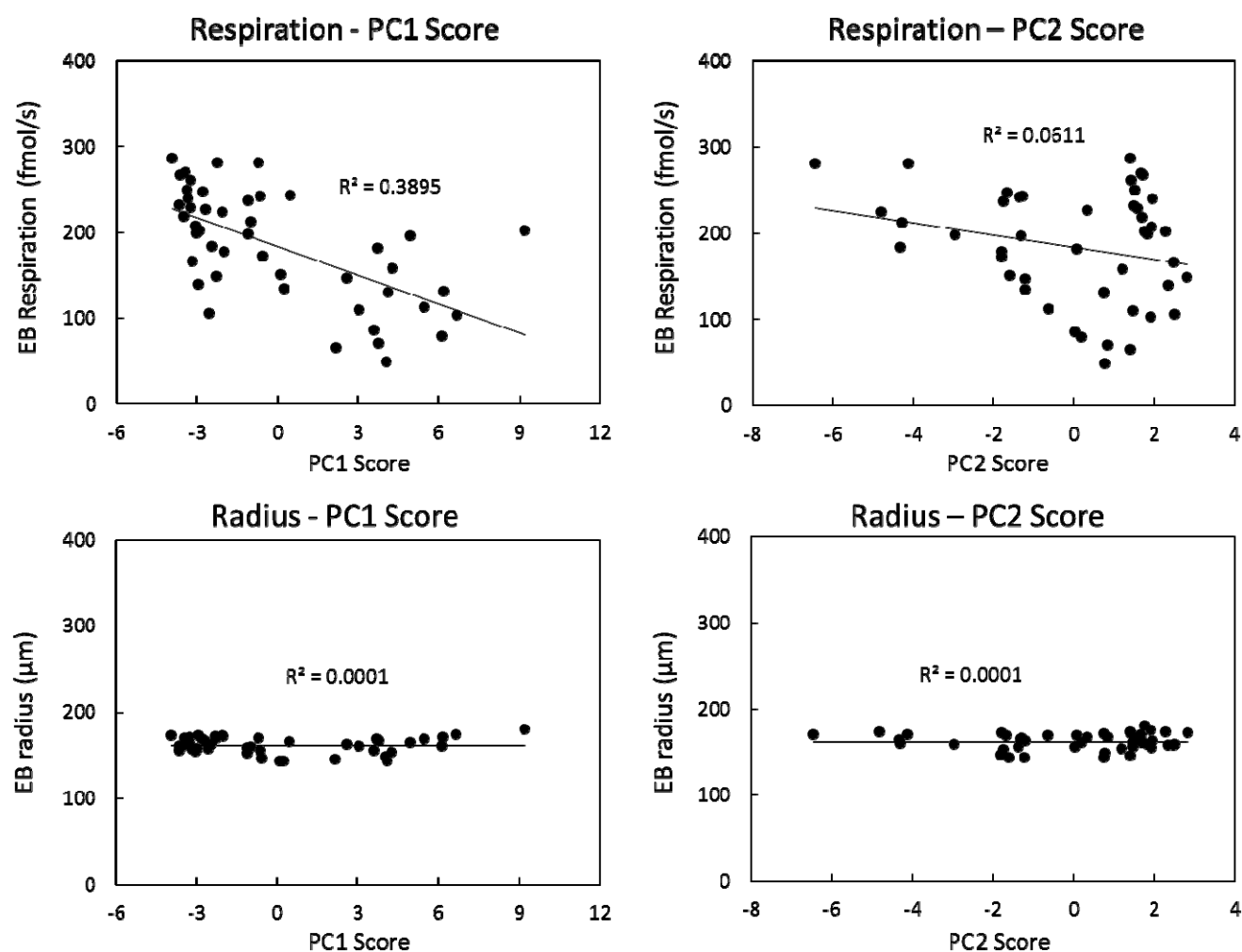


Figure S7. The respiratory activities (F_{O_2}) and radii (r_s) of mEBs with the same radii for the 3 different culture conditions were plotted as a function of each PC score axis.