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| **Supplementary Table 1: Correlation in Expression of Different Marker Genes in Differentiating MSCs** | | | |
| **Timepoint/Gene Pair** | **r2** | **Pearson’s correlation coefficient** | **p-value** |
| **Day 1 aggrecan - osteopontin** | 0.24 | 0.49 | 1.0e-07 |
| **Day 21 aggrecan - osteopontin** | .11 | .34 | .0019 |
| **Day 1 aggrecan-LPL** | 7.5e-5 | .0087 | .92 |
| **Day 21 aggrecan-LPL** | .0022 | .047 | .67 |
| **Day 1 osteopontin-LPL** | 1.9e-5 | .0044 | .96 |
| **Day 21 osteopontin-LPL** | .04 | .21 | .059 |

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| **Supplementary Table 2: Coefficient of Variation of RNA Count in Small MSC Colonies** | | | | |
| **Colony** | **A** | **B** | **C** | **D** |
| **Aggrecan CV** | 1.71 | 1.34 | 1.07 | 0.91 |
| **GAPDH CV** | 0.32 | 0.35 | 0.31 | 0.20 |

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| | **Supplementary Table 3**  **P-values for Figure 1E:**  **Aggrecan RNA Count,**  **MSCs with Differentiation** | | | --- | --- | | **Comparison** | **Adjusted P-value** | | **-TGFB vs +TGFB, Day 01** | 0.0067 | | **-TGFB vs +TGFB, Day 04** | 0.0043 | | **-TGFB vs +TGFB, Day 07** | 0.0020 | | **-TGFB vs +TGFB, Day 14** | 0.0017 | | **-TGFB vs +TGFB, Day 21** | 0.0031 | | **+TGFB, Day 1 vs Day 4** | 0.0006 | | **+TGFB, Day 1 vs Day 7** | 0.0002 | | **+TGFB, Day 1 vs Day 14** | 0.0047 | | **+TGFB, Day 1 vs Day 21** | 0.0002 | | **+TGFB, Day 4 vs Day 7** | 0.0004 | | **+TGFB, Day 4 vs Day 14** | 0.0021 | | **+TGFB, Day 4 vs Day 21** | 0.0002 | | **+TGFB, Day 7 vs Day 14** | 0.0002 | | **+TGFB, Day 7 vs Day 21** | <.0001 | | **+TGFB, Day 14 vs Day 21** | 0.0002 | |  | | **Supplementary Table 4**  **P-values for Figure S1B:**  **GAPDH RNA Count ,**  **MSCs with Differentiation** | | | --- | --- | | **Comparison** | **Adjusted P-value** | | **-TGFB vs +TGFB, Day 01** | 0.3572 | | **-TGFB vs +TGFB, Day 04** | 0.0827 | | **-TGFB vs +TGFB, Day 07** | 0.0361 | | **-TGFB vs +TGFB, Day 14** | 0.9479 | | **-TGFB vs +TGFB, Day 21** | 0.9980 | | **+TGFB, Day 1 vs Day 4** | 0.0022 | | **+TGFB, Day 1 vs Day 7** | 0.4637 | | **+TGFB, Day 1 vs Day 14** | <.0001 | | **+TGFB, Day 1 vs Day 21** | 0.0002 | | **+TGFB, Day 4 vs Day 7** | 0.0019 | | **+TGFB, Day 4 vs Day 14** | 0.0002 | | **+TGFB, Day 4 vs Day 21** | 0.0009 | | **+TGFB, Day 7 vs Day 14** | <.0001 | | **+TGFB, Day 7 vs Day 21** | 0.0002 | | **+TGFB, Day 14 vs Day 21** | 0.0016 | |

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| | **Supplementary Table 5 P-values for Figure S3:**  **Aggrecan Difference Between Sister Cells** | | | | --- | --- | --- | | **Comparison** | | **Adjusted P-value** | | **< 12 hrs** | **[12-23 hrs]** | <.0001 | | **< 12 hrs** | **[24-35 hrs]** | <.0001 | | **< 12 hrs** | **[36-47 hrs]** | <.0001 | | **< 12 hrs** | **> 47 hrs** | <.0001 | | **[12-23 hrs]** | **[24-35 hrs]** | 0.8759 | | **[12-23 hrs]** | **[36-47 hrs]** | 0.7564 | | **[12-23 hrs]** | **> 47 hrs** | 0.9997 | | **[24-35 hrs]** | **[36-47 hrs]** | <.0001 | | **[24-35 hrs]** | **> 47 hrs** | <.0001 | | **[36-47 hrs]** | **> 47 hrs** | <.0001 | |  | | **Supplementary Table 6 P-values for Figure S3:**  **GAPDH Difference Between Sister Cells** | | | | --- | --- | --- | | **Comparison** | | **Adjusted P-value** | | **< 12 hrs** | **[12-23 hrs]** | 0.1649 | | **< 12 hrs** | **[24-35 hrs]** | 0.6435 | | **< 12 hrs** | **[36-47 hrs]** | <.0001 | | **< 12 hrs** | **> 47 hrs** | <.0001 | | **[12-23 hrs]** | **[24-35 hrs]** | 0.0173 | | **[12-23 hrs]** | **[36-47 hrs]** | <.0001 | | **[12-23 hrs]** | **> 47 hrs** | <.0001 | | **[24-35 hrs]** | **[36-47 hrs]** | <.0001 | | **[24-35 hrs]** | **> 47 hrs** | <.0001 | | **[36-47 hrs]** | **> 47 hrs** | <.0001 | |

| **Supplementary Table7**  **P-values for Figure 4B-D**  **Chondrocyte Gene Expression During Dedifferentiation** | | | | |
| --- | --- | --- | --- | --- |
| **Passage Comparison** | | **Aggrecan/GAPDH (Pooled Population)**  **Adjusted P-value** | **Aggrecan per Cell**  **Adjusted P-value** | **GAPDH per Cell**  **Adjusted P-value** |
| 0 | 1 | 0.0079 | 0.2108 | 0.0006 |
| 0 | 3 | 0.0004 | 0.9342 | 0.0006 |
| 0 | 5 | 0.0002 | 0.9999 | 0.0008 |
| 0 | 7 | 0.0002 | 1.0000 | 0.0008 |
| 0 | 9 | 0.0011 | 0.9984 | 0.0025 |
| 1 | 3 | 0.7036 | 0.6621 | 1.0000 |
| 1 | 5 | 0.5040 | 0.2891 | 0.9945 |
| 1 | 7 | 0.4593 | 0.1658 | 0.9929 |
| 1 | 9 | 0.9377 | 0.3623 | 0.7026 |
| 3 | 5 | 0.9993 | 0.9783 | 0.9981 |
| 3 | 7 | 0.9982 | 0.8850 | 0.9972 |
| 3 | 9 | 0.9944 | 0.9934 | 0.7784 |
| 5 | 7 | 1.0000 | 0.9988 | 1.0000 |
| 5 | 9 | 0.9510 | 0.9999 | 0.9343 |
| 7 | 9 | 0.9309 | 0.9941 | 0.9431 |

| **Supplementary Table 8**  **P-values for Figure 4H-J**  **Chondrocyte RNA Count During Redifferentiation** | | | |
| --- | --- | --- | --- |
| **Comparison** | **Aggrecan per Cell**  **Adjusted P-value** | **GAPDH per Cell**  **Adjusted P-value** | **Aggrecan/GAPDH  per Cell**  **Adjusted P-value** |
| **Day 1, P0 vs P5** | 0.0016 | 0.0406 | 0.9983 |
| **P0, Day 1 vs 14** | 0.0897 | 0.0006 | 0.0176 |
| **Day 14, P0 vs P5** | 0.0002 | 0.6714 | 0.0588 |
| **P5, Day 1 vs 14** | <.0001 | <.0001 | 0.1982 |

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| **Supplementary Table 9: RNA-FISH probe sequences.** | | | |
| **Aggrecan** | **GAPDH** | **Osteopontin** | **Lipoprotein Lipase** |
| gtccttgtctccatagcaac  acacgtcataggtttcgttg  cttggaaggtgaacttctcc  tttctgggatgtccacaaag  cccttacttcagggacaaac  actgatgtcctctactccag  gcagagatttctggaccttc  actgatgtcctctactccag  aaggtcctctactccagaag  agaaggaagtccactaaggt  agtctgctgagatcctctac  tctactccagaagcagagac  actgatgtcctctactccag  caccagaaggaagaacactg  actgatgtcctctactccag  actgatgtcctctactccag  cctccagaaggaagtctact  agaaggaagtccactaaggt  gaaggtattctgccaaggtc  gtccactgaaatcaggaagg  caaagagtccagaggtttcc  ccactaatgtcaggaaaccc  gtcacgccagatatttctcc  gatttctggttgtccagagg  caattgctccagaagacagt  aagttccagaatgcctgaag  ccaaagatcccaaatggtct  accactggattcaaaaagct  tgggactgatgacacttcta  cagatcagcttcatggaagg  ggattcgatttctagacgcg  attgatctcgtatcggtcct | agaattaaaagcagccctgg  tcattgatggcgacgatgtc  agaccatgtagtgaaggtca  ttgactgtgccgttgaactt  ctttccattgatgacgagct  gatctcgctcctggaagatg  tctccatggtagtgaagacc  agagatgatgaccctcttgg  ttgttatacttctcgtggtt  gaggcattgctgacaatctt  ccaaagtggtcatggatgac  aagcagggatgatattctgg  ttgagctcagggatgacctt  gtcagatccacaacagacac  catacttggcaggtttctcc  gtcctcagtgtagcctagaa  tgagtgtcgctgttgaagtc  aatgagcttgacaaagtggt  ttgctgtagccgaattcatt  tactccttggaggccatgtg | cccaagaggcagaagcaaat  gtttaactggaagggcggag  tacagcatctgggtatttgt  aagtctgcttctgagatggg  gacacagaattctgtggtgc  gggtattttgtttgttgtca  ggctttcattggacttactt  gtcatctagatcgtctgttt  cgtcggagtcattagagttg  aatggtcagggtcatcagtg  tcagaatggtgagactcgtc  gcgattgttggaatatcagt  ccgtagggataaacggagtg  tcttgacttcagtccgtaag  ttagatcggcggaacttctt  tgtggcatctggactctgaa  ctgactcgtcttcttaggtg  tggcgtgagttctttggaaa  gaatgcttgttcttatcctt  tttcctgactctcaatcaga  aattcttggctgagtttgga  atgatctaggtctagcttgt  tttcaggtgtttgtcttctt  attgacctcagaagaggcac | ggagtcctgagagcaaattt  caggaatgaggtggcaagtg  tgctgtggttgaagtgacag  caactctcatacattcctgt  gacactggataatgctgctg  ataaacttggccacatcctg  agttaaattcatccgccatc  atcccaagagatgcacattg  ttcttattggtcagacttcc  catactcgaagttaggtcca  caggagaaaggcgacttgga  ccctggtgaatgtgtgtaaa  ttctggattccgatacttcg  cgttagggtaaatgtccaca  caatgttacatcctggttgg  ctctgcaatcacacggagag  taggccttacttggattttc  ggcagagacctttctcaaag  tgaccttgttgatctcgtag  tacatcttgctgcttctttt  gcatctgagaacgagtcttc  cctggttggtgtatgtatta  cagtgccatacagagagatc  aaaagggatgttctcgctct  ttgtggaaacttcaggcagg  aatatccacctccgtgtaaa  ggaccagctgaagtaggaat  cacctttttttgagtctctc  gataagacattttctcccgg  tatcacaggtgactttcctt  acttgtcatggcatttcaca  cagccagactttctattcag |