

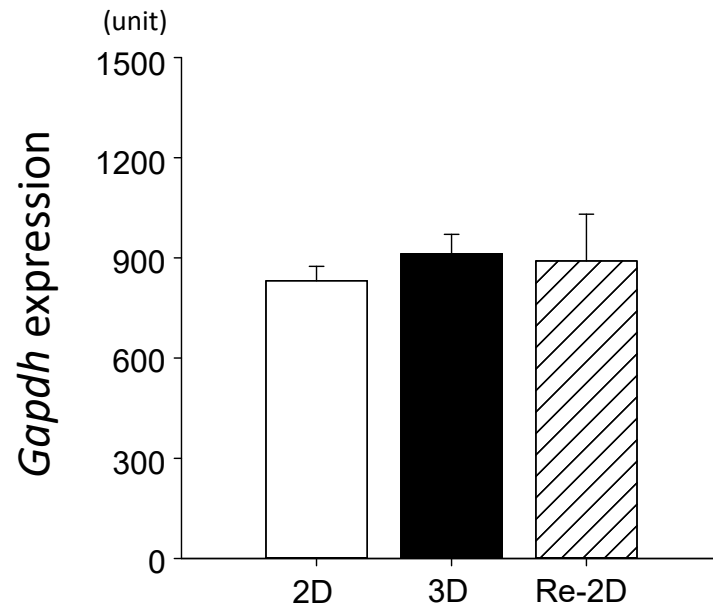
## Supplemental information

### Alternating Differentiation and Dedifferentiation between Mature Osteoblasts and Osteocytes

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Suppl. Table S1. qPCR Primers

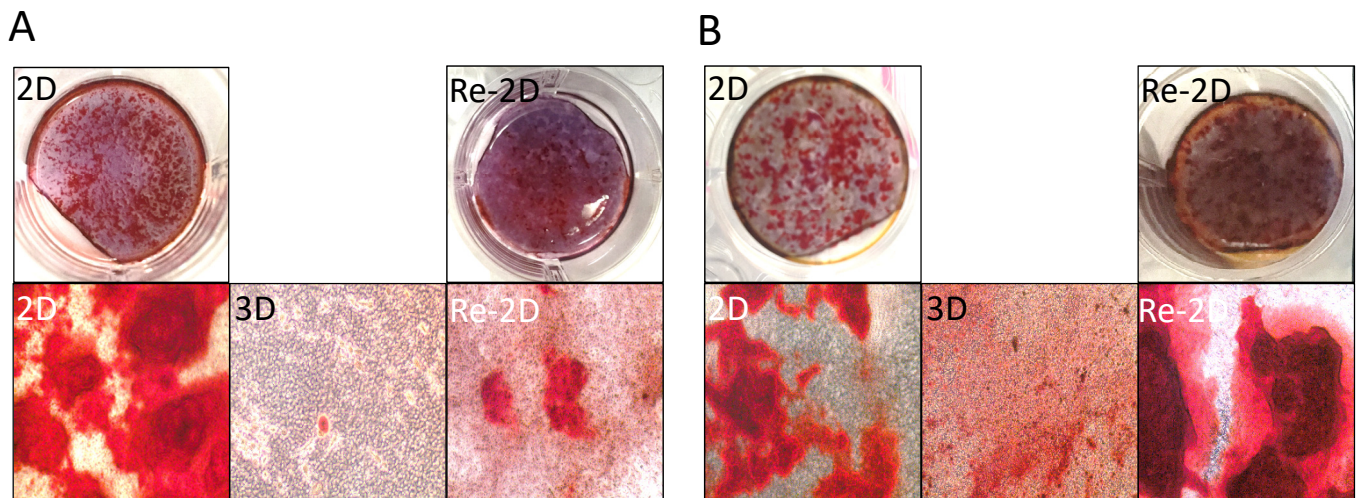
| Gene             | Forward primer sequence (5'-3') | Reverse primer sequence (5'-3') |
|------------------|---------------------------------|---------------------------------|
| <i>Alpl</i>      | AGGCAGGATTGACCACGG              | TGTAGTTCTGCTCATGGA              |
| <i>Sp7</i>       | TGCTTGAGGAGGAAGTTCAC            | AGGTCACTGCCCACAGAGTA            |
| <i>Sost</i>      | CTTCAGGAATGATGCCACAGAGGT        | ATCTTTGGCGTCATAGGGATGGTG        |
| <i>Fgf23</i>     | ACTTGTCGCAGAAGCATC              | GTGGGCGAACAGTGTAGAA             |
| <i>Dmp1</i>      | GGCTGTCCTGTGCTCTCCAG            | GGTCACTATTTGCCTGTGCCTC          |
| <i>Tnfsf11</i>   | CAAGCTCCGAGCTGGTGAAG            | CCTGAACTTTGAAAGCCCCA            |
| <i>Tnfrsf11b</i> | AAGAGCAAACCTTCCAGCTGC           | CACGCTGCTTTCACAGAGGTC           |
| <i>Gapdh</i>     | ACCCAGAAGACTGTGGATGG            | CAGATTGGGGGTAGGAACAC            |
| <i>Ccna2</i>     | CCTGCCTTCACTCATTGCTG            | GTGGCGCTTTGAGGTAGGT             |
| <i>Ccne1</i>     | GTGTCAAATGGATGGTTC              | GGAGAAATCCTATTCTGTTC            |



**Suppl. Fig. S1.** The amount of the *Gapdh* expression per 2  $\mu$ g of total RNA. No significant differences were noted between 2D, 3D, and Re-2D conditions.

### Alizarin red staining

Cells were cultured in osteogenic medium ( $\alpha$ MEM, 50  $\mu$ g/ml ascorbic acid, 10 mM  $\beta$ -glycerophosphate) to stimulate calcium deposition (Figure 1C). Alizarin red staining was performed to visualize calcium deposits. At the end of cell culture in osteogenic medium, cells were fixed in 70% ethanol followed by incubation with 40 mM Alizarin red, pH 4.2, for 10 minutes. Cell cultures were washed to remove excess alizarin red staining.



**Suppl. Fig. S2.** Representative photomicrographs of Alizarin red stained calcium deposits. Primary osteoblast cultures (A) and MC3T3-E1 cell cultures (B) are shown.