Supplementary Materials

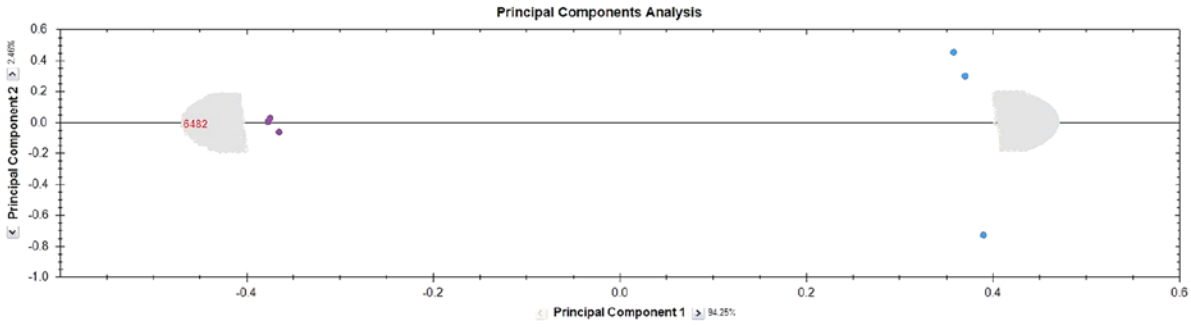
Materials and Methods

4 and 14 days of IGFBP7 treatment on fibroblasts for osteoblastic reprogramming

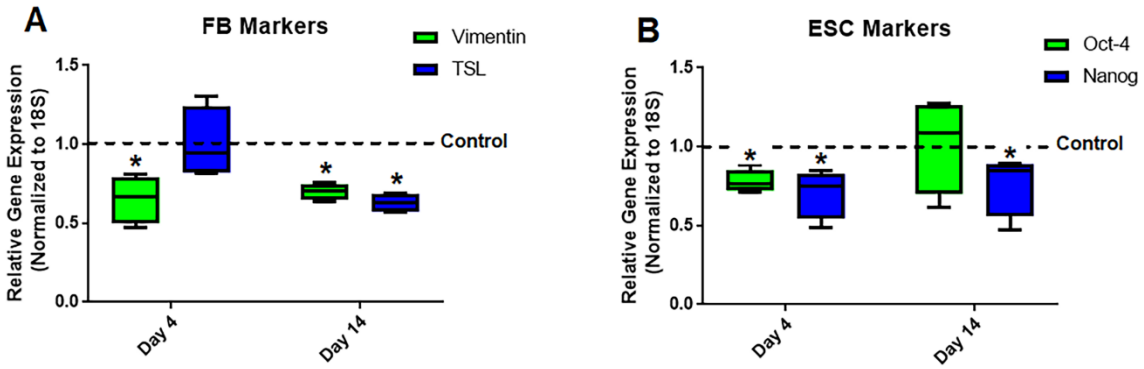
Fibroblasts were seeded in 12-well plates with 100,000 cells/well and cultured in DMEM medium supplemented with 10 % FCS, 30 mg/ml penicillin and 100 mg/ml streptomycin overnight. When reached 80-90% confluence, cells were washed three times with PBS followed by addition of DMEM containing 2% FCS, osteogenic components and IGFBP7 (1000 ng/ml). Cells were treated with IGFBP7 for 4 days, followed by withdrawal of IGFBP7 by replenishing cells in fresh DMEM containing 2% FCS and osteogenic components. Cells were collected at day 14 and 28 days for gene expression and alizarin red staining, respectively.

rhBMP-2 treatment on fibroblasts for osteoblastic reprogramming

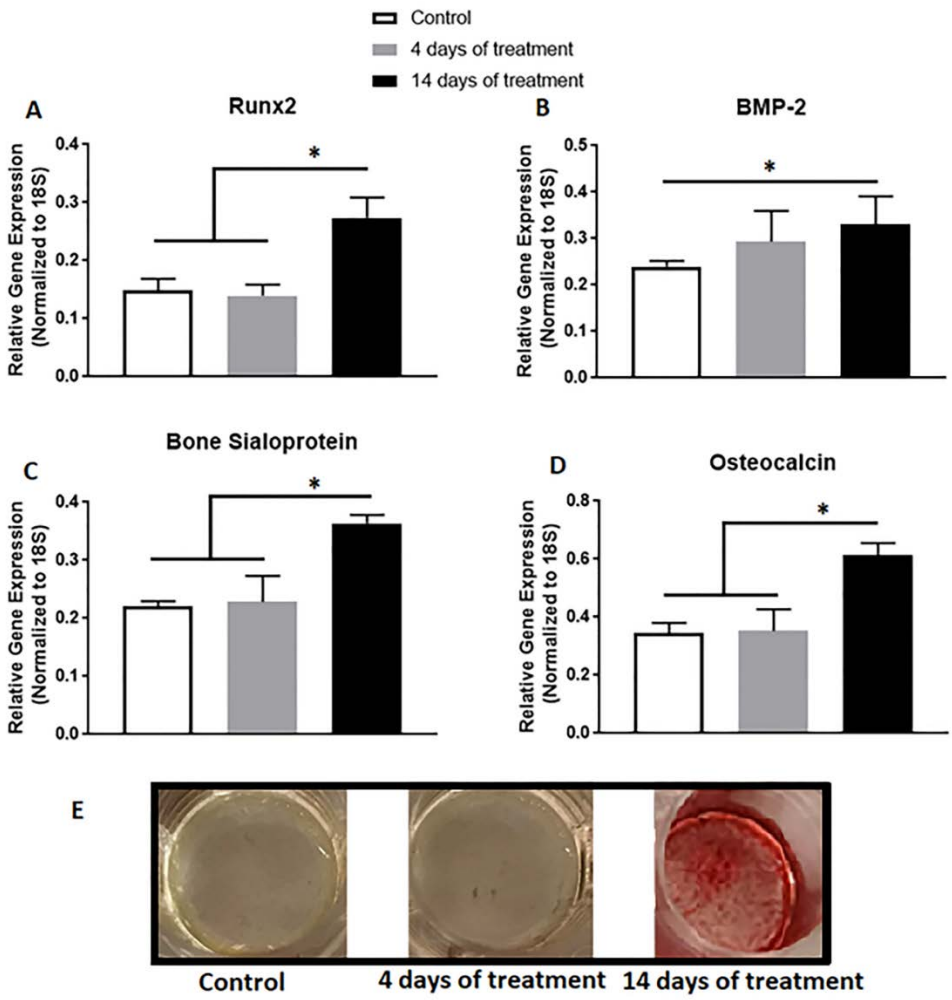
Fibroblasts were seeded in 12-well plates with 100,000 cells/well and cultured in DMEM medium supplemented with 10 % FCS, 30 mg/ml penicillin and 100 mg/ml streptomycin overnight. The cells at 80-90% confluence were washed three times with PBS followed by addition of DMEM containing 2% FCS, osteogenic components and rhBMP-2 (100 ng/ml, Medtronic). After 14 days of treatment, cells were collected for osteogenic gene expression analysis.



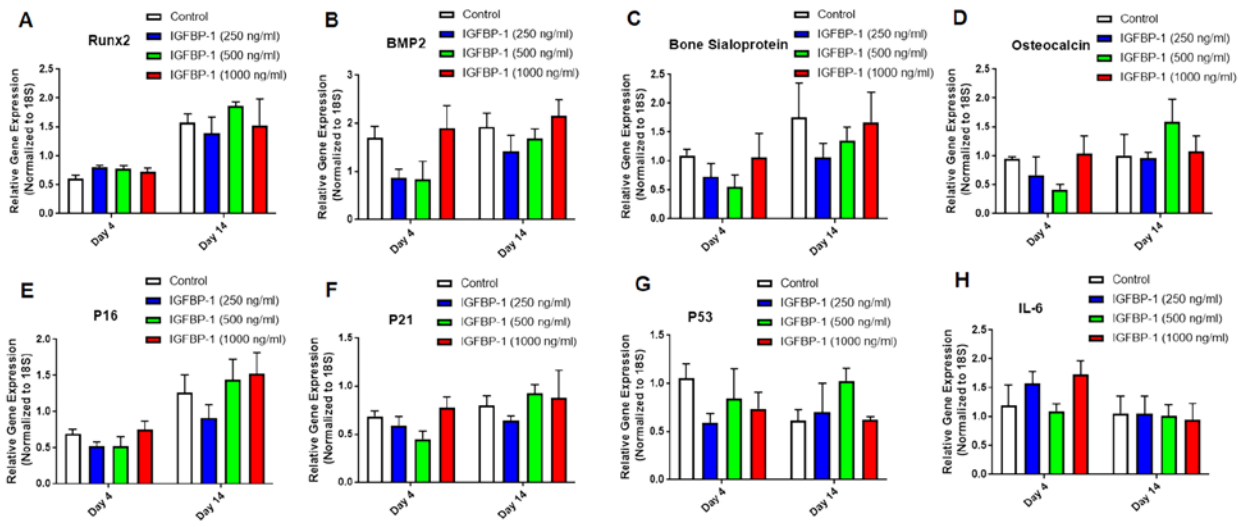
Supplementary Figure 1 Principal component analysis was used to analyse the protein clusters in OB-CM and FB-CM. Principal component analysis identified that conditioned medium was the greatest variation in the experiment. Peptides ions with p-value ≤ 0.05 formed two distinct clusters corresponding to fibroblast or osteoblast medium which were 94.25% different, while variation between replicates was 2.46%. Data were replicated in two independent experiments.



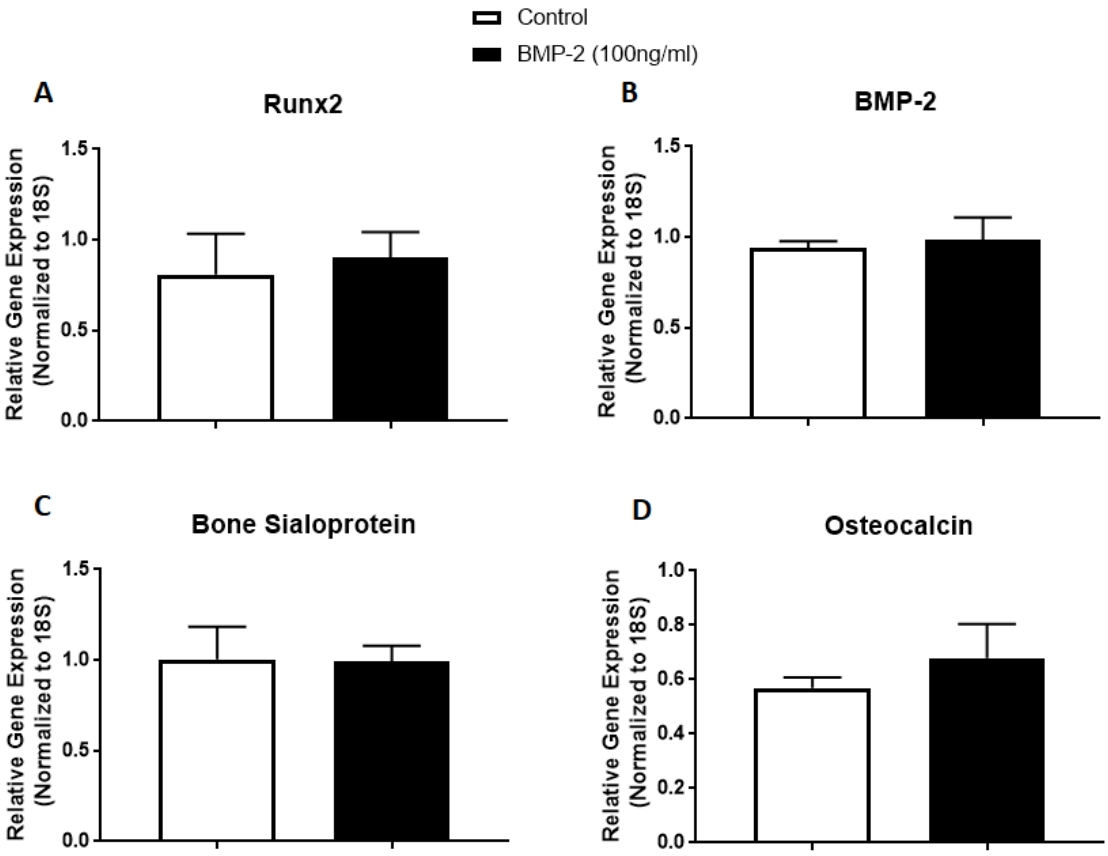
Supplementary Figure 2 IGFBP7 treatment of fibroblasts led to down-regulation of fibroblastic and embryonic stem cell markers. The gene expression levels of vimentin and thymic stromal lymphopoietin (TSL) (A), as well as Oct-4 and Nonag (B) were significantly lower in rOBs induced by IGFBP7 than those in untreated fibroblasts at day 4 and day 14. * indicates $p \leq 0.05$ compared to control. Mean ± SE and 5-95% CI of four independent experiments is shown.



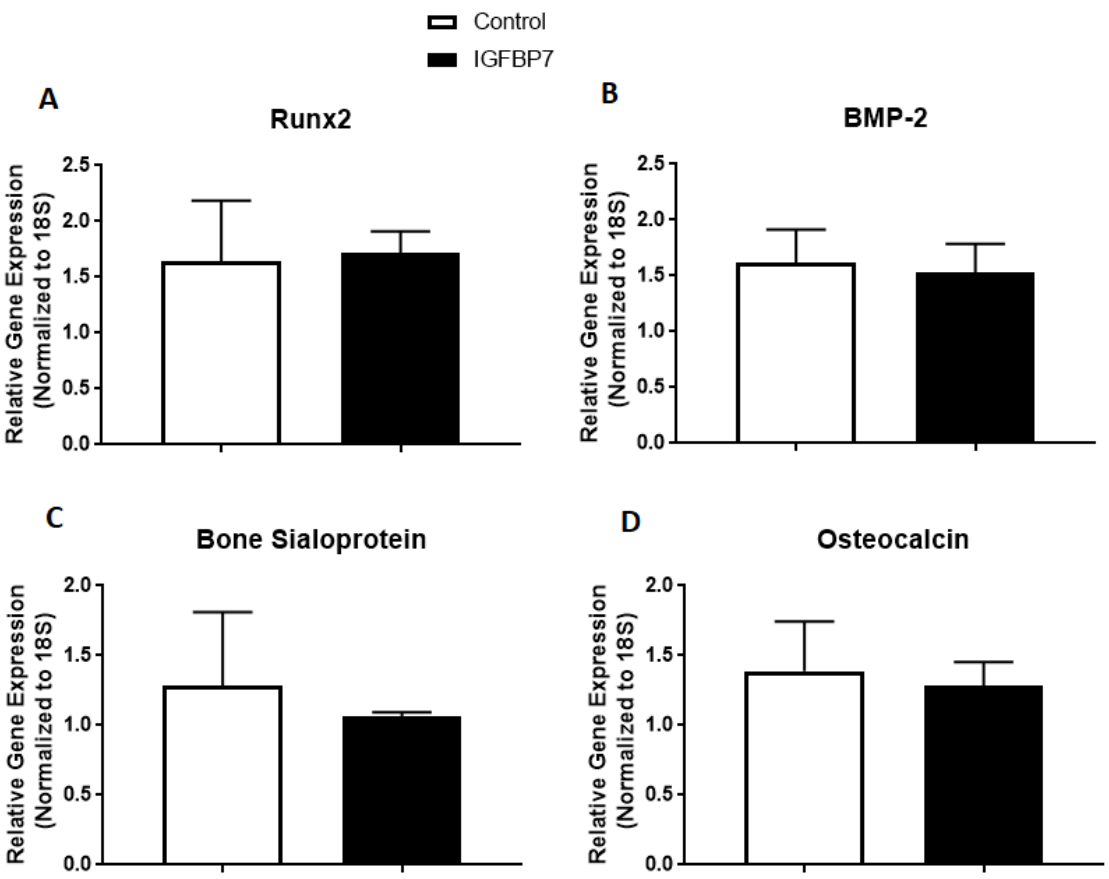
Supplementary Figure 3 Treatment with IGFBP7 for 14 days induced sustained reprograming of fibroblasts into osteoblasts. Fibroblasts were treated with IGFBP7 (1 μg/ml) for 4 or 14 days followed by withdrawal of IGFBP7 from the culturing media till 28 days of culturing to determine whether IGFBP7-induced osteoblastic reprogramming of fibroblast is a transient or long-term effect. Osteogenic gene expression levels was quantified at day 14. Bone nodule formation was measured by Alizarin Red S staining at day 28. Compared with control, no significant change in osteogenic gene expression (A-D) or bone nodule formation was observed in fibroblasts treated for 4 days with IGFBP7 (E), while osteogenic gene expression and bone nodule formation was significantly increased in fibroblasts treated for 14 days with IGFBP7. * indicates $p \leq 0.05$. Mean ± SE of three independent experiments is shown.



Supplementary Figure 4 IGFBP1 did not alter gene expression level for cell senescence and osteoblastic markers in fibroblasts. Fibroblasts were treated with 250, 500 or 1000 ng/ml IGFBP1 and osteogenic gene expression was quantified by qPCR. IGFBP1 did not alter change the gene expression levels of osteoblastic makers (A-D), or cell senescence-associated regulatory (P16, P21 and P53, E-G) and SASP (IL-6, F) genes. Mean ± SE of four independent experiments is shown.



Supplementary Figure 5 BMP-2 did not alter gene expression osteoblastic markers in fibroblasts. Fibroblasts were treated with BMP-2 (100 ng/ml) and the osteogenic gene expression was measured by qPCR. BMP-2 treatment did not lead to elevation of osteogenic gene expression levels in fibroblasts at day 14 post treatment (A-D). Mean ± SE of three independent experiments is shown.



Supplementary Figure 6 IGFBP7 did not change gene expression of osteoblastic markers in late passaged fibroblasts (P20). Fibroblasts at passage 20 were treated with IGFBP7 (100 ng/ml) for 14 days and osteogenic gene expression levels were quantified by qPCR. No elevation of osteogenic gene expression levels was observed in late passaged fibroblasts (A-D)**.** Mean ± SE of three independent experiments is shown.