Supporting Information

Nanofiber Scaffolds with Gradients in Mineral Content for Spatial Control of Osteogenesis

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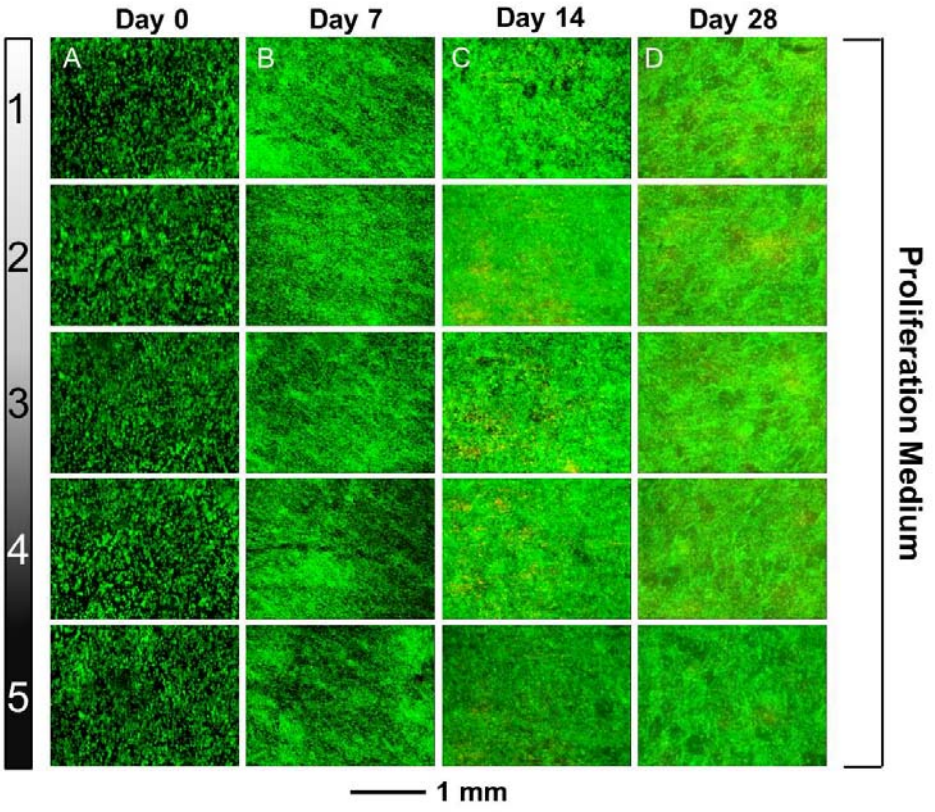

Figure S1. Live/Dead staining of ASCs seeded on aligned nanofibers with a spatial gradient in mineral (1-5) after incubation in proliferation medium for (A) 2 h, (B) 7 days, (C) 14 days, and (D) 28 days. Cells remained viable through the culture period, with few apparent dead (red) cells.

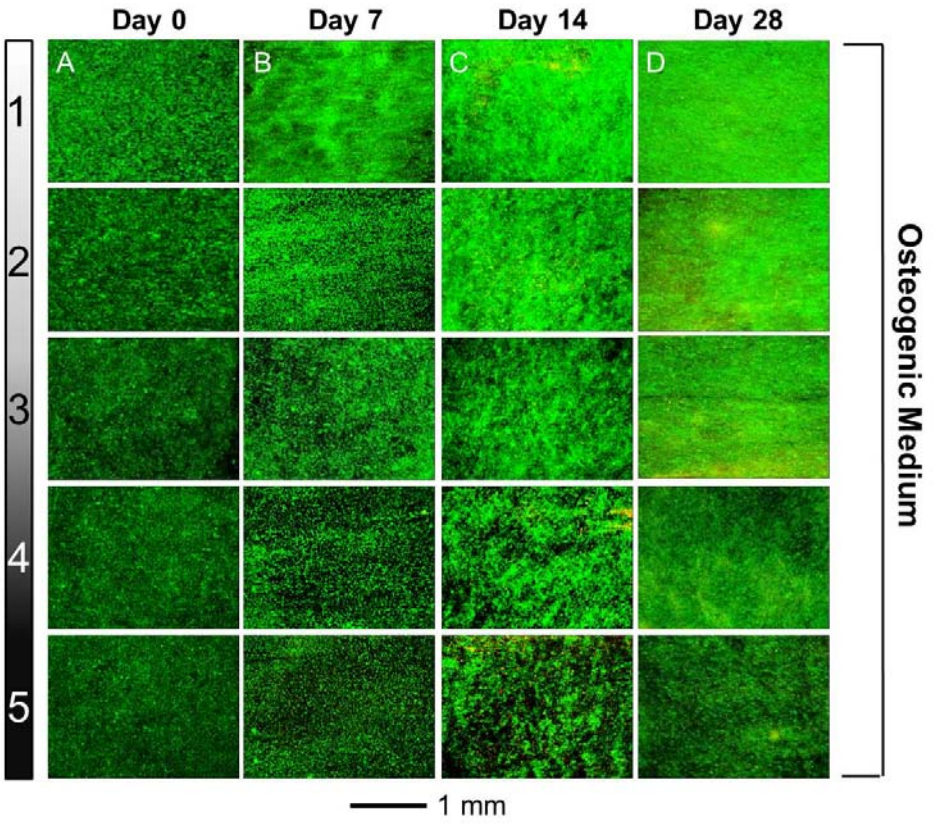

Figure S2. Live/Dead staining of ASCs seeded on aligned nanofibers with a spatial gradient in mineral (1–5) after incubation in osteogenic medium for (A) 2 h, (B) 7 days, (C) 14 days, and (D) 28 days. Cells remained viable through the culture period, with few apparent dead (red) cells.

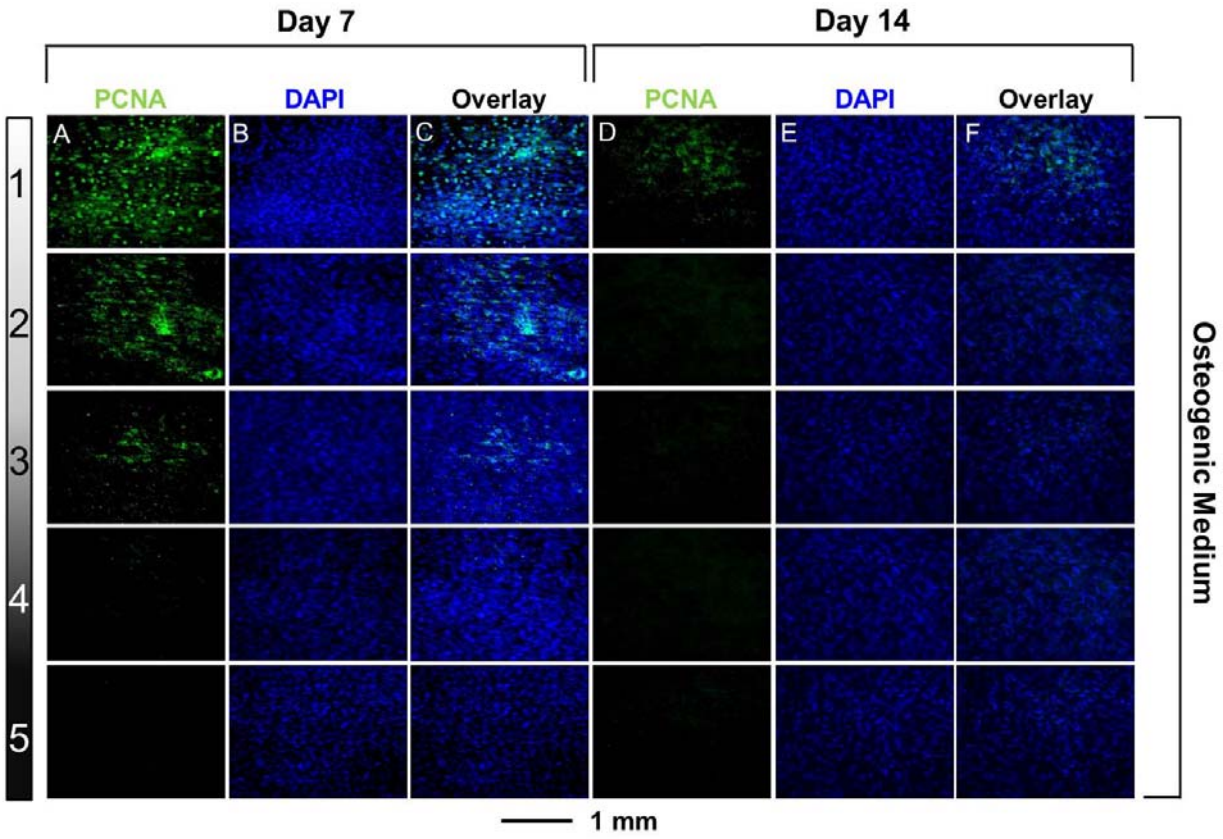

Figure S3. PCNA staining of ASCs seeded on aligned nanofibers with a spatial gradient of mineral after incubation in osteogenic medium for (columns A–C) 7 days and (columns D–F) 14 days. (A, D) PCNA staining of ASCs on day 7 and 14, respectively. (B, E) DAPI staining of ASCs on day 7 and 14, respectively. (C) Superimposed images of (A) and (B). (F) Superimposed images of (D) and (E). Cellular density (as visualized with DAPI) was similar along the length of the scaffold and over time. PCNA staining was negatively associated with mineral content.

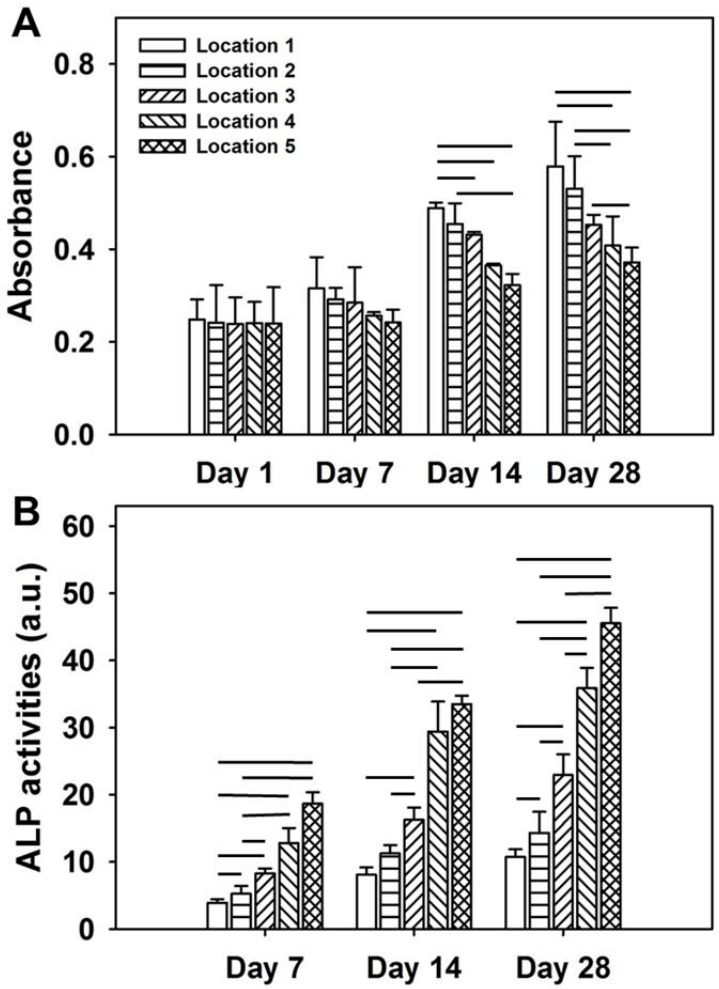

Figure S4. (A) Quantification of cell proliferation on aligned nanofibers with a graded coating of mineral for 1, 7, 14, and 28 days in osteogenic medium. The data were obtained using the MTT assay. Proliferation was negatively associated with mineral content under both culture conditions. (B) Quantification of ALP activity of ASCs seeded on aligned nanofibers with a graded coating of mineral for 7, 14 and 28 days in osteogenic medium. There was an increase in ALP expression by ASCs over time and this expression was positively associated with mineral content. Significance indicated by lines over the bars.

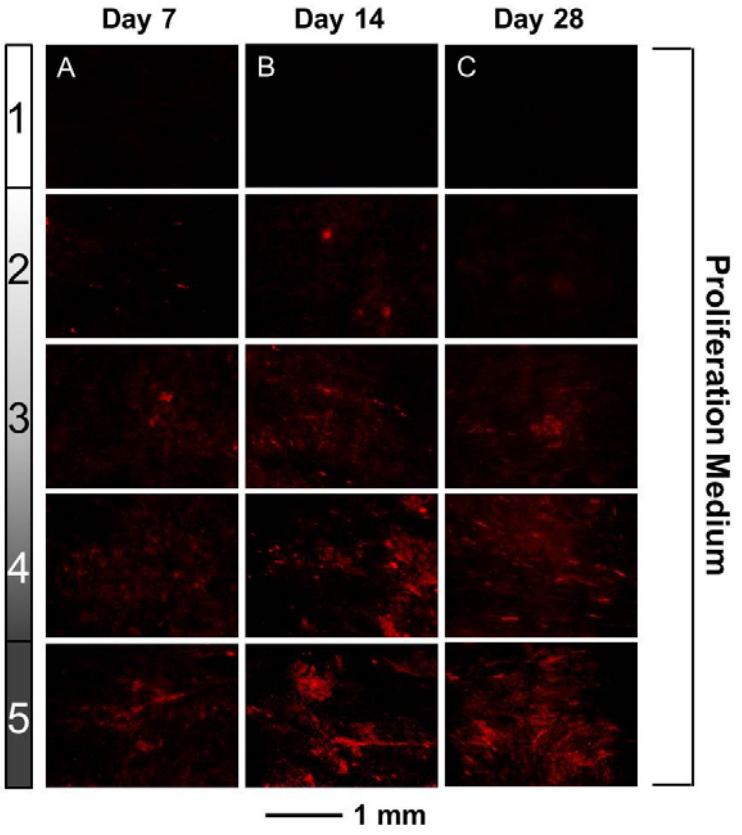

Figure S5. ALP staining of ASCs seeded on aligned nanofibers with a spatial gradient in mineral after incubation in proliferation medium for (A) 7, (B) 14, and (C) 28 days. There was an increase in ALP expression by ASCs over time and this expression was positively associated with mineral content.

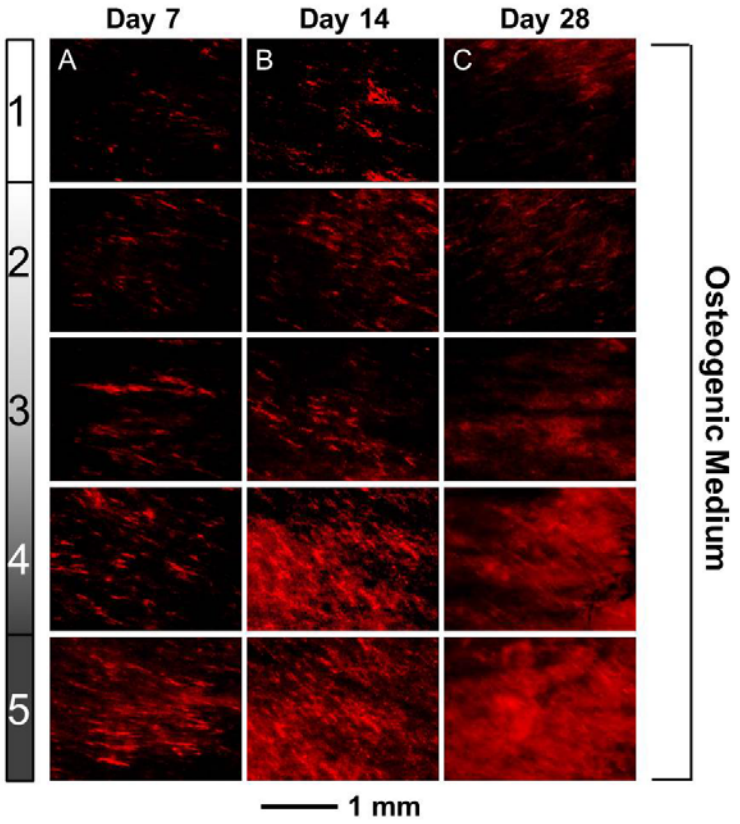

Figure S6. ALP staining of ASCs seeded on aligned nanofibers with a spatial gradient in mineral after incubation in osteogenic medium for (A) 7, (B) 14, and (C) 28 days. There was an increase in ALP expression by ASCs over time and this expression was positively associated with mineral content.

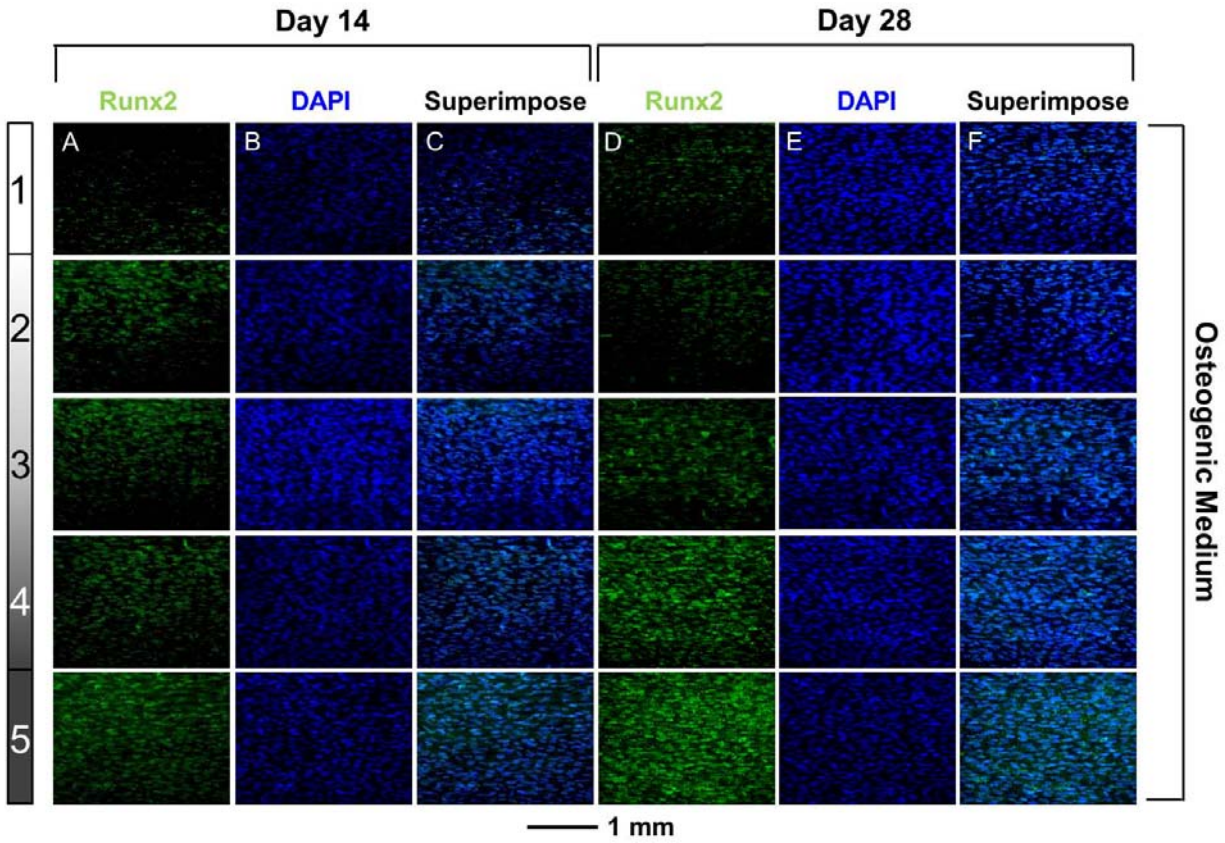

Figure S7. Runx2 staining of ASCs seeded on aligned nanofibers with a spatial gradient in mineral after incubation in osteogenic medium for (A–C) 14 and (D–F) 28 days. (A, D) Runx2 staining of ASCs on day 14 and 28, respectively. (B, E) DAPI staining of ASCs on day 14 and 28, respectively. (C) Superimposed images of (A) and (B). (F) Superimposed images of (D) and (E). Runx2 staining was positively associated with increasing mineral content and increased with culture time.

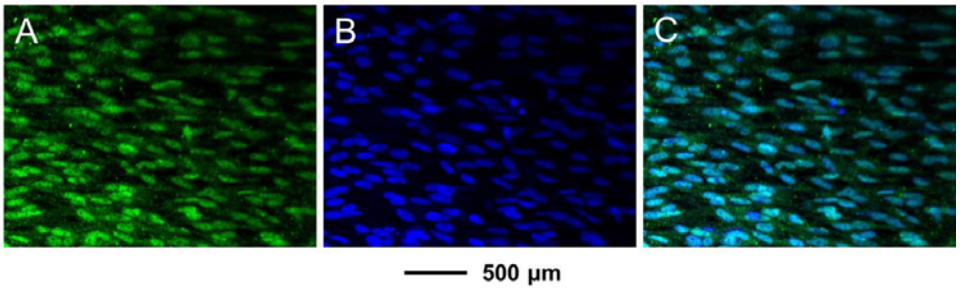

Figure S8. Representative high-magnification images of Runx2 and DAPI staining of ASCs cultured on a nanofiber scaffold with a spatial gradient in mineral content. Runx2 expression was localized to cell nuclei.

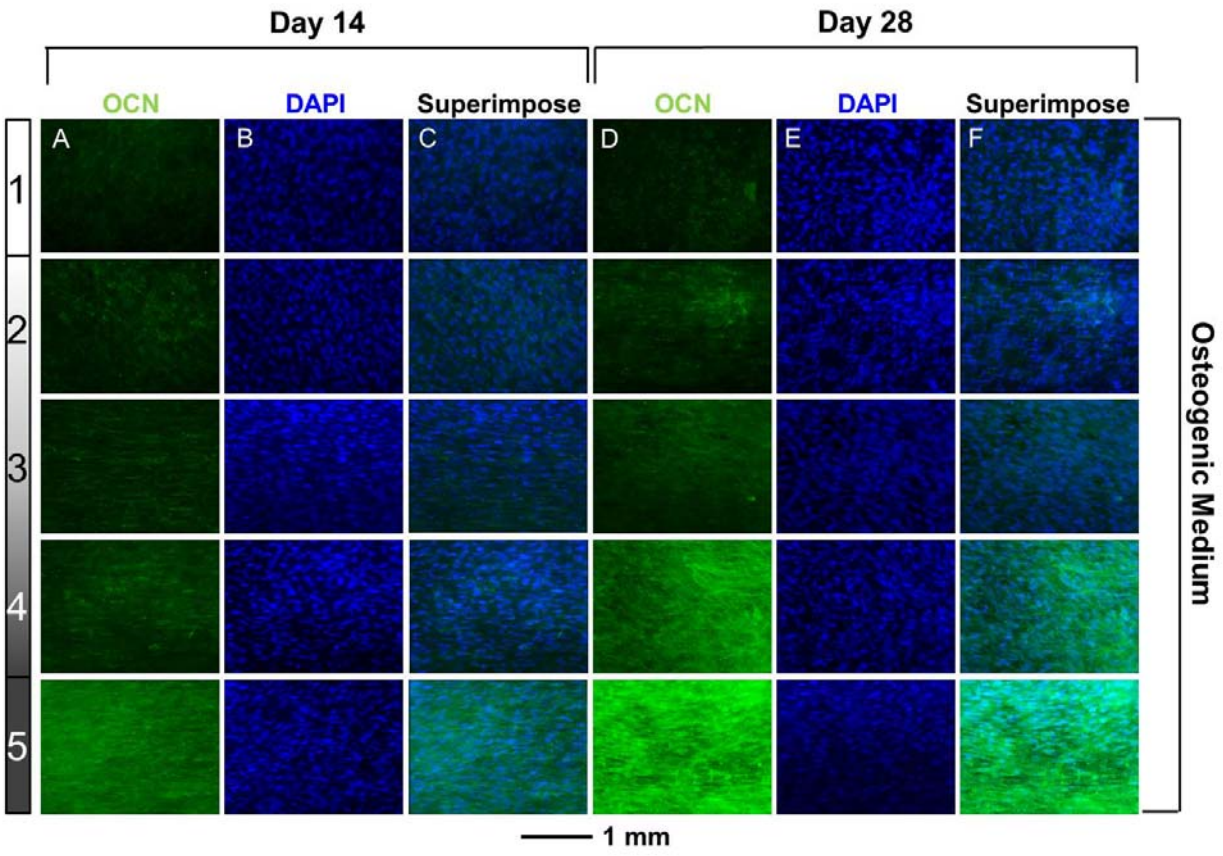

Figure S9. OCN staining of ASCs seeded on aligned nanofibers with a spatial gradient in mineral after incubation in osteogenic medium for (A–C) 14 and (D–F) 28 days. (A, D) OCN staining of ASCs on day 14 and 28, respectively. (B, E) DAPI staining of ASCs on day 14 and 28, respectively. (C) Superimposed images of (A) and (B). (F) Superimposed images of (D) and (E). Runx2 staining was positively associated with increasing mineral content and increased with culture time.

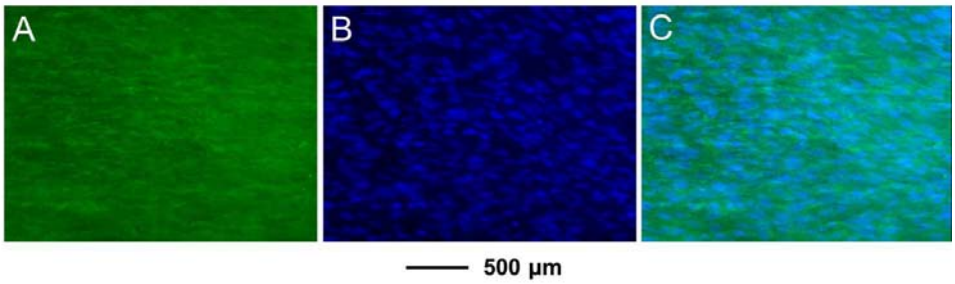

Figure S10. Representative high-magnification images of OCN and DAPI staining of ASCs cultured on a nanofiber scaffold with a spatial gradient in mineral content. OCN was localized to the ECM.