



Supplemental Figure 2: Nanocomposite hydrogel alone does not stimulate Osterix and Osteocalcin expression at 4 and 8 weeks.

(A) Nanocomposite implanted for 4 weeks; (B) Nanocomposite implanted for 8 weeks. A and B show Masson's Trichrome staining and the yellow boxes indicate the area of interest shown in subsequent sections. Collagen deposition stained in blue. C and D show endogenous Osterix expression as red fluorescence. DAPI is shown in blue. E and F show endogenous Osterix expression in red and immunostaining for Osterix in green, which confirms the specificity of the immunostaining by the yellow overlap and is additionally marked with white arrows, indicating the bone surface. G and H images show endogenous Osterix expression in red and areas of Osteocalcin immunostaining in green. The nanocomposite hydrogel appears bright green in immunostained images (due to the secondary antibody binding to the nanocomposite) and gray-brown in C and D, as these sections were not treated with an antibody. The bright green nanocomposite is labeled Gel. Scale bars=500 μ m. Gel = Nanocomposite hydrogel; OB=Osteoblasts; OC=Osteocytes.