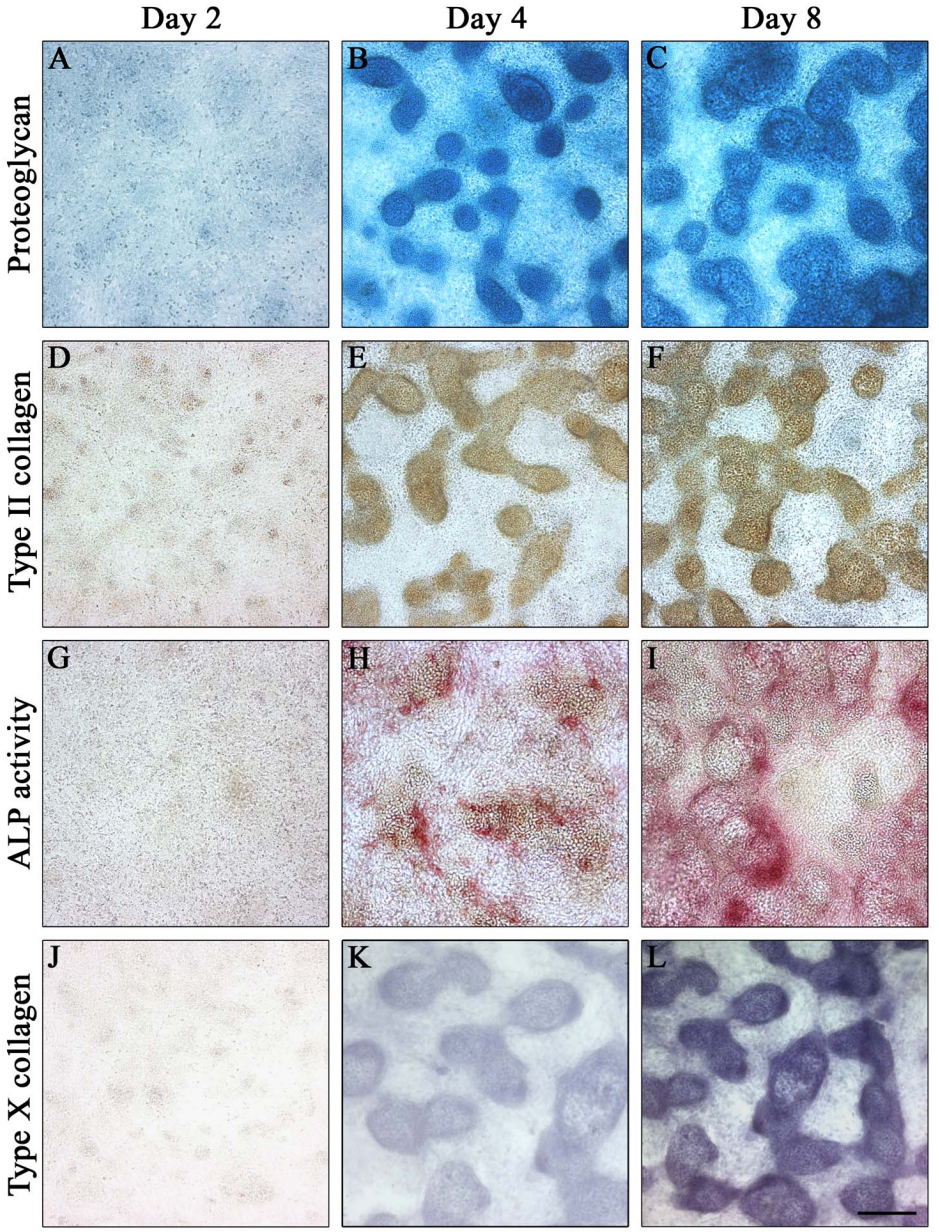
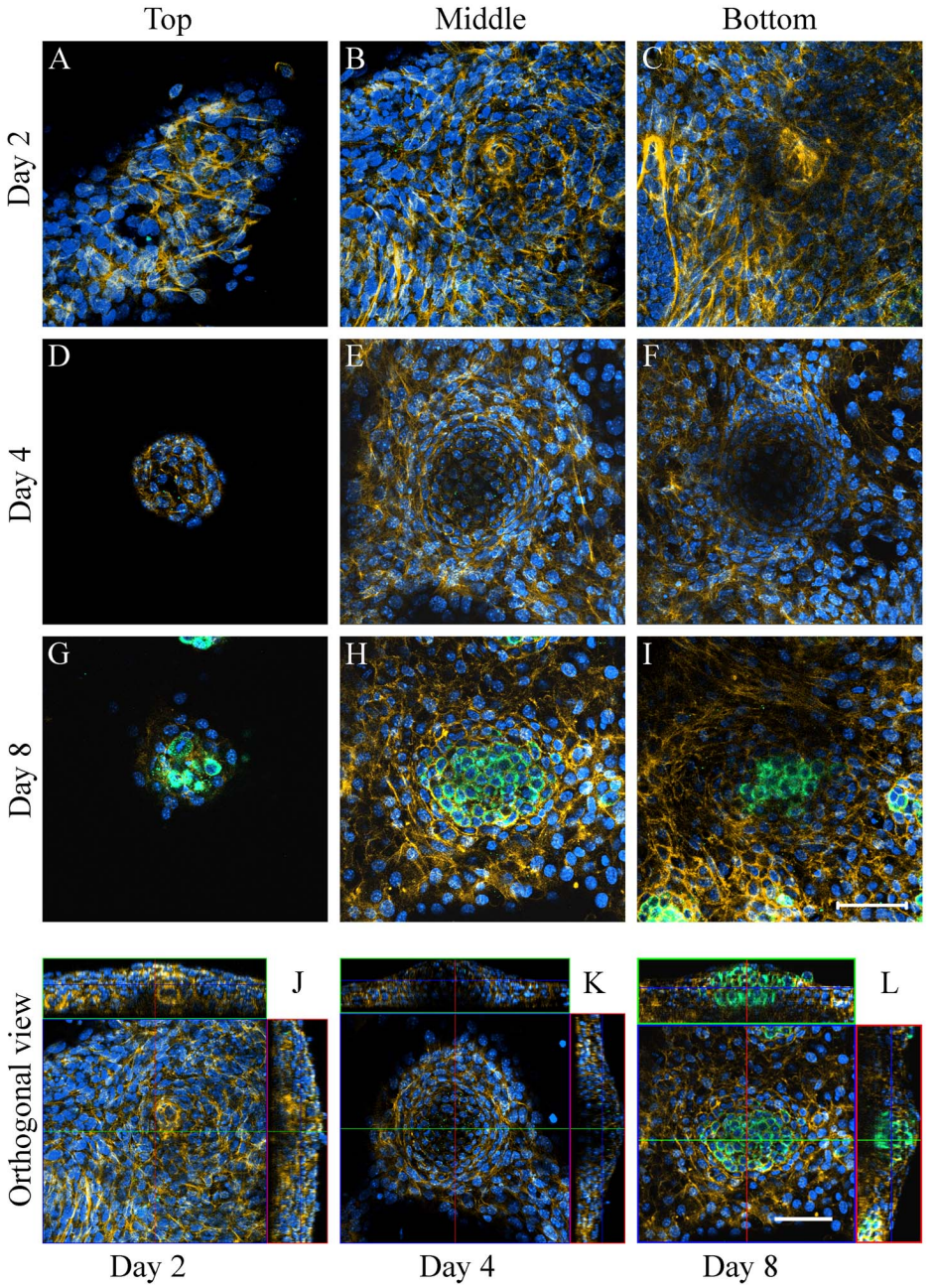
Supplemental Data

Fig S1 Molecular markers of chondrocyte differentiation



Supplemental Data

Fig S2 Confocal laser scanning micrographs of cartilage nodules



Supplemental Data Fig 1 Expression of molecular markers of chondrocyte differentiation in limb bud cell cultures
Cells released from the limb buds of E11.5 mice were plated at a density of 10^7 cells/mL and maintained for 2, 4 or 8 days in medium containing 2% FBS. At the indicated times cultures were fixed with 4% paraformaldehyde and stained with alcian blue **(A-C)** or with a monoclonal antibody raised against type II collagen **(D-F),** to identify cells undergoing chondrogenic differentiation. Fully differentiated, hypertrophic cells were identified by histochemical staining for alkaline phosphatase activity (ALP) **(G-I)** or by in situ hybridization with a DIG-conjugated type X collagen anti-sense probe **(J-L)**. Scale bar represents 100 μm.

Supplemental Data Fig 2 Confocal laser scanning microscopy of cartilage nodules
Cultures were plated and maintained as described in Supplemental Data Fig S1 for 2, 4 or 8 days before fixing with 4% paraformaldehyde and treating with proteases and triton X to permeabilize. Triple staining was performed with Hoescht 33258 (blue, nuclei), TRITC-phalloidin (orange, actin fibres) and a type II collagen antibody that was visualized with an Alexa 488-conjugated secondary antibody (green). Images from the top **(A, D, G),** middle **(B, E, H)** and bottom **(C, F, I)** of each stack show condensations of mesenchymal cells at Day 2 and formation of 3 dimensional nodules by Day 4, which increase in breadth and depth by Day 8. The architecture of the nodules can be seen in the orthogonal view images of Day 2, Day 4 and Day 8 cultures described above **(J-L).** Scale bar represents 50μm.