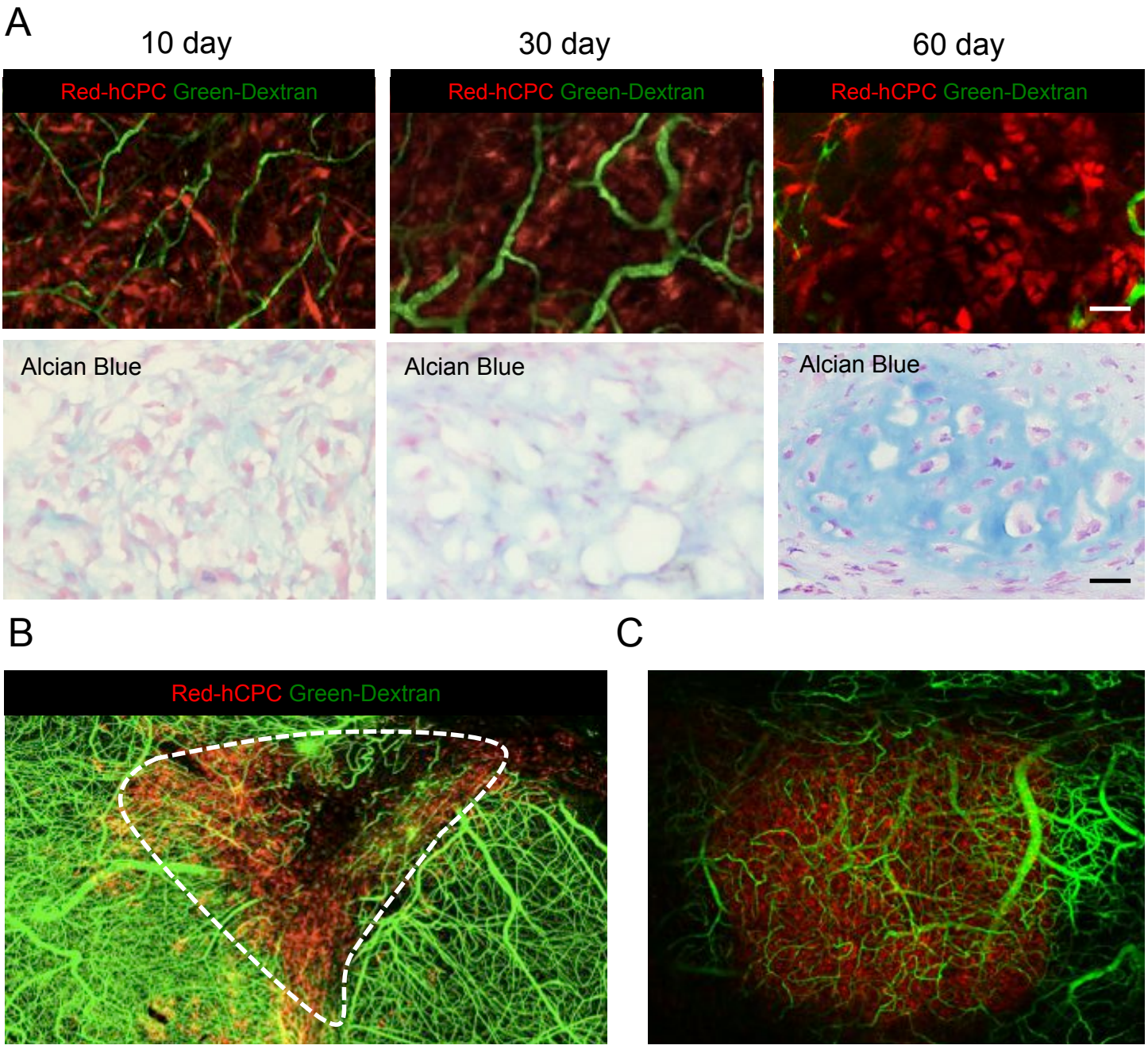
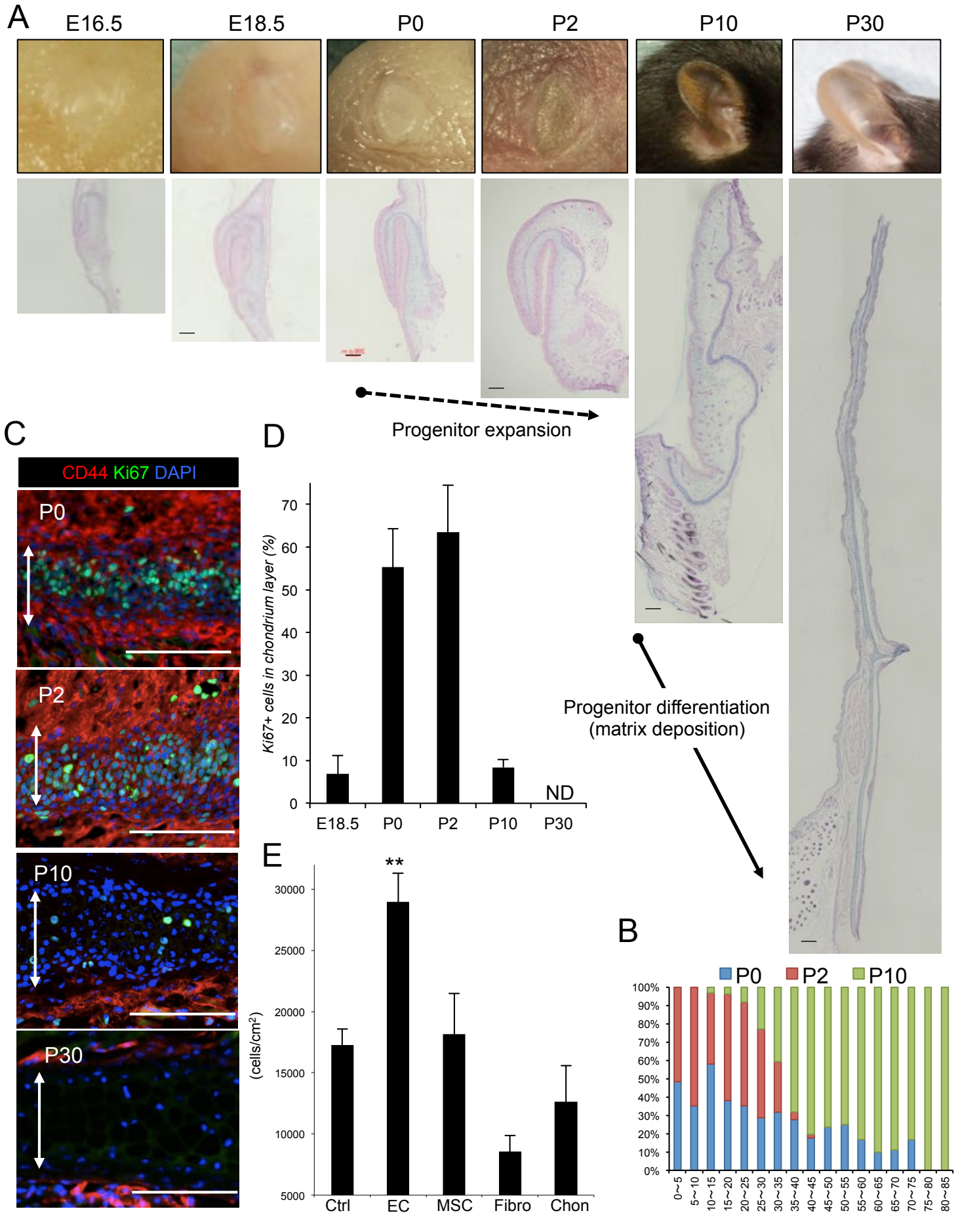
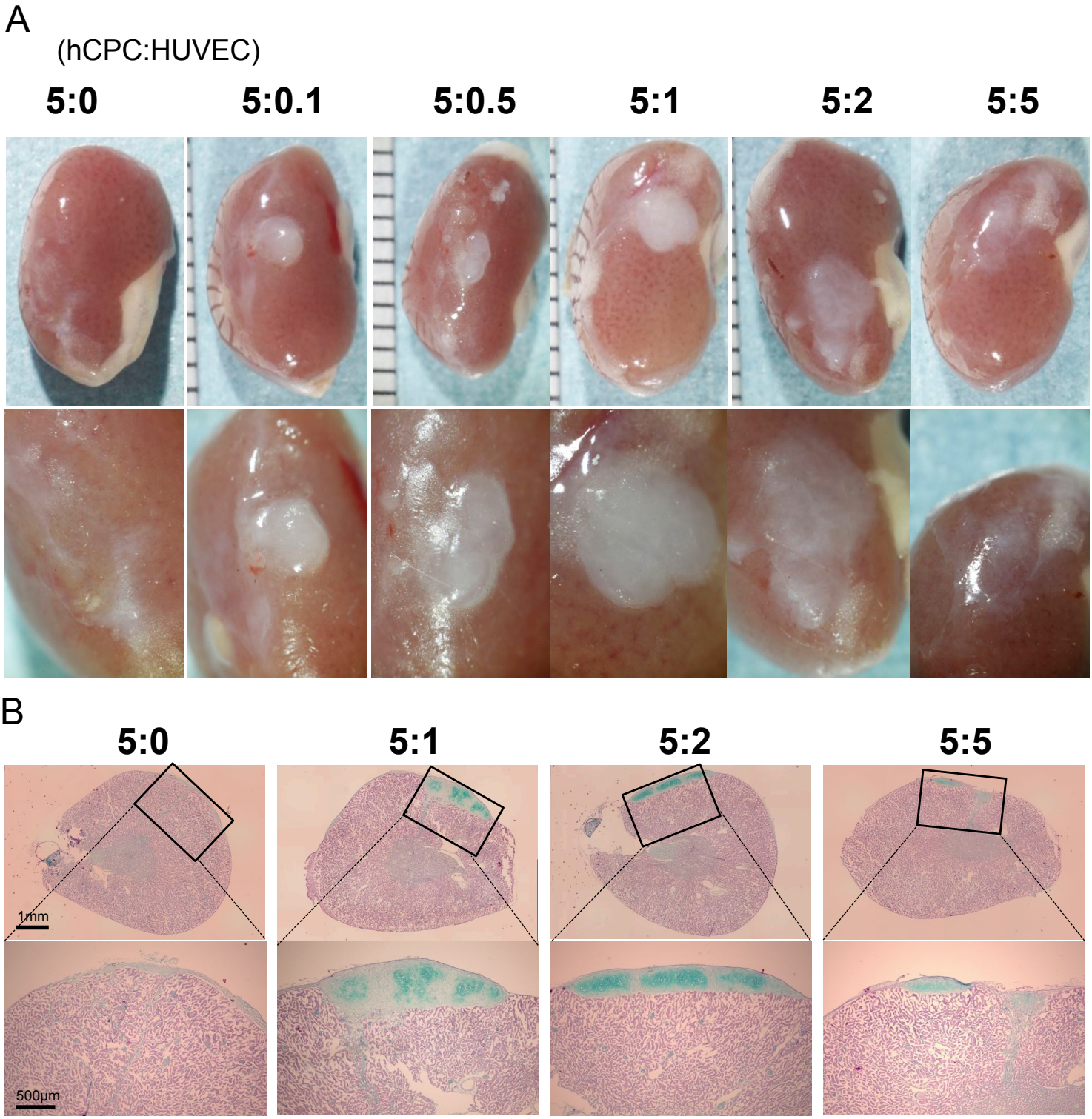
Supplementary Figure1 Cartilage regeneration is also preceded by transient vascularization *in vivo*.



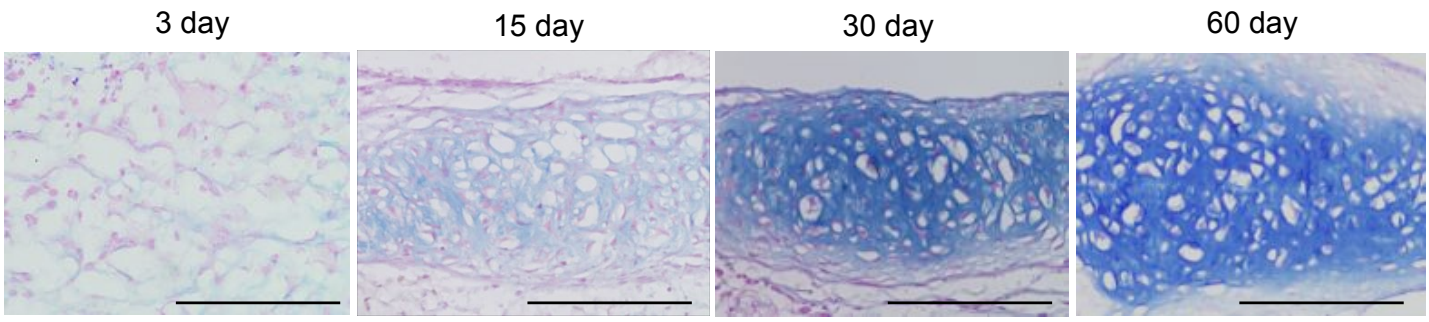
Supplementary Figure2 Expansion of mesenchymal progenitors in an early condensed stage prior to cartilage maturation.



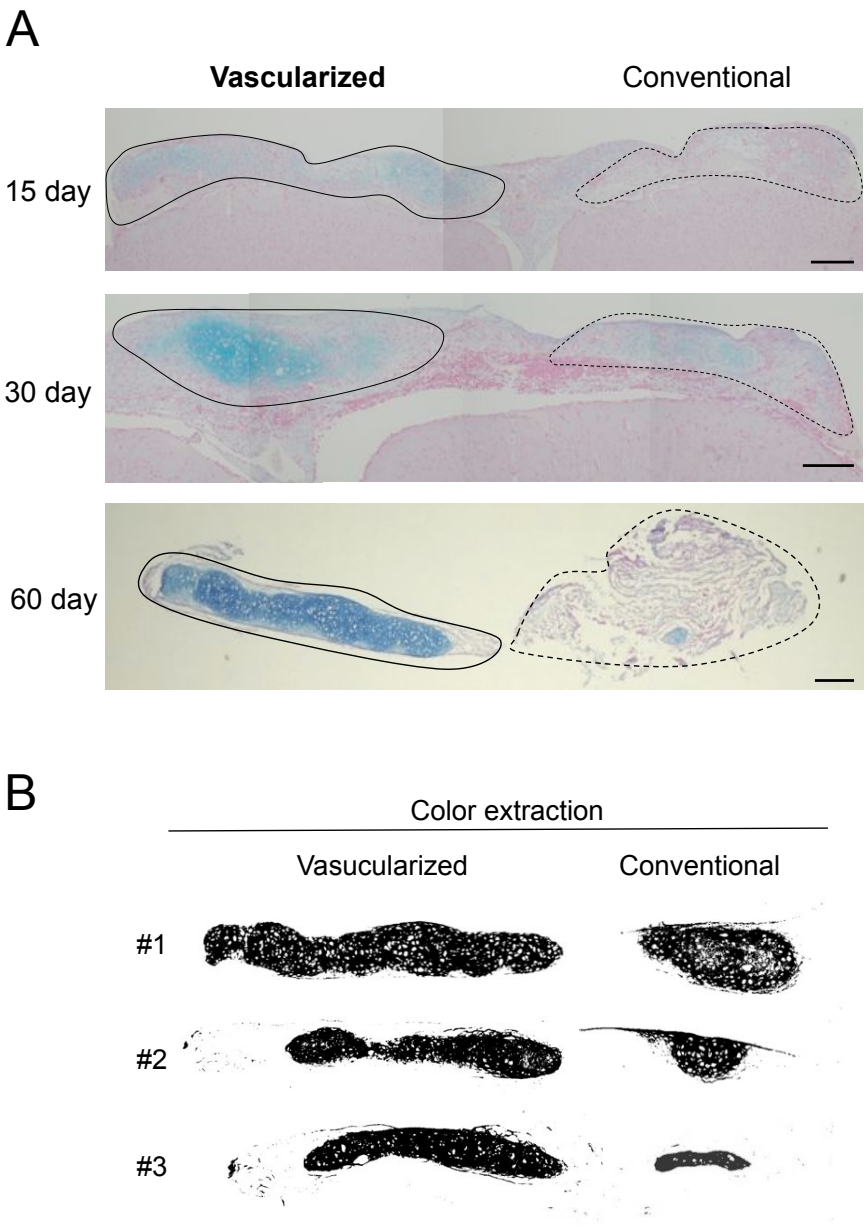
Supplementary Figure3 Comparison of cartilage regeneration efficiency for multiple mixture ratios in culture.



Supplementary Figure4 Alcian blue staining of vascularized cartilage progenitor transplants at multiple time points.

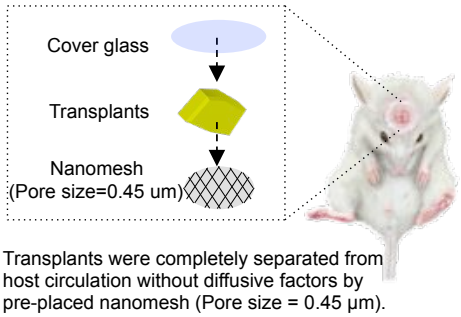


Supplementary Figure5 Comparison of cartilage regeneration efficiency between vascularized- and pellet- cultured cartilage progenitors.

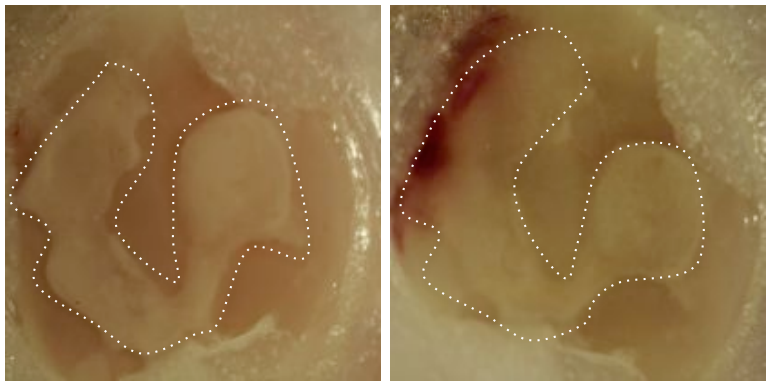


Supplementary Figure6 Suppression of cartilage formation from human progenitor cells *in vivo* by the inhibition of functional vessel formation.

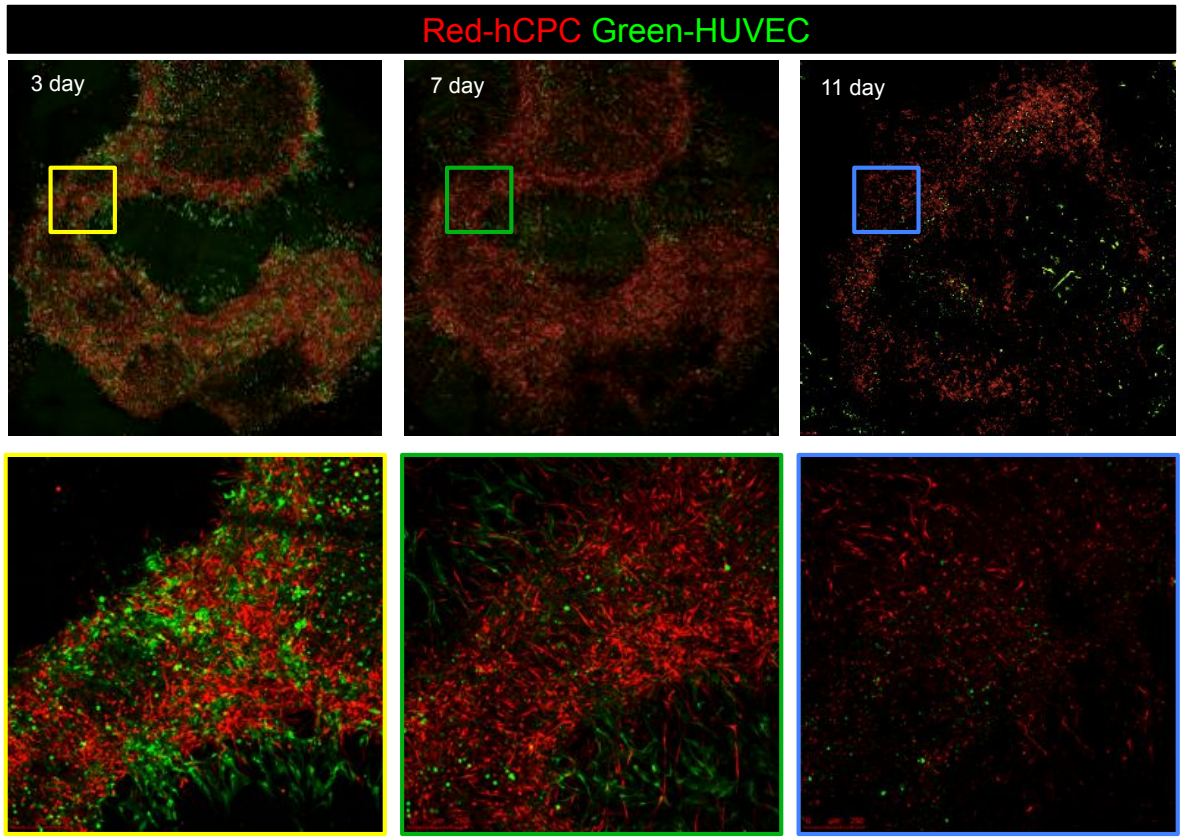
A



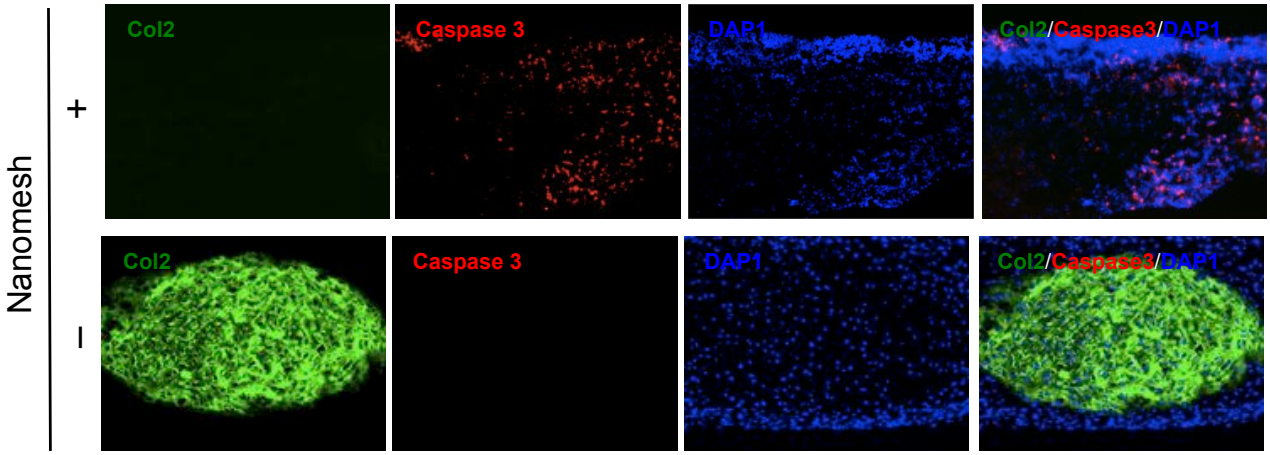
B



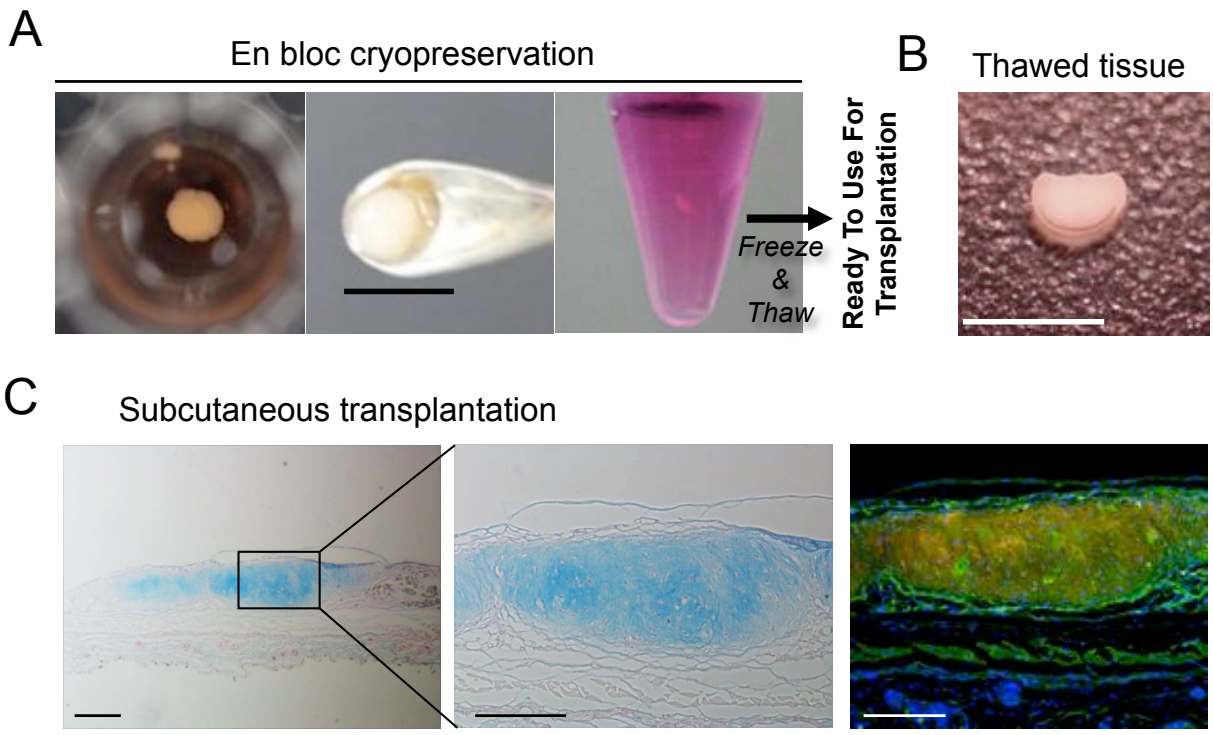
C



D



Supplementary Figure7 En-bloc cryopreservation of the self-aggregated three-dimensional tissues.



SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure 1. Cartilage regeneration is also preceded by transient vascularization *in vivo*.

(**A**) Cartilage progenitor cells (CPCs) subjected to **our previously developed layered** chondrogenic differentiation protocol were transplanted under a cranial window. Intravital imaging showed functional blood perfusion at days 10 and 30. Established vascular networks regressed from the site at which cartilage was regenerated at day 60. Mature cartilage formation was confirmed by Alcian blue staining. (**B**) Low magnification images around the region of cartilage formation are shown on the bottom. Green, dextran; Red, human cartilage from CPCs. (**C**) Transplanted human CPC pellets were intravitally imaged. At day 15, the presence of clear patent vessels inside the **conventional** pellet transplants was similarly demonstrated.

Supplemental Figure 2. Expansion of mesenchymal progenitors in an early condensed stage prior to cartilage maturation.

(**A**) Alcian blue staining of the developing ear cartilage at multiple stages. The lower

panels show the gross morphology of the ear at the corresponding stages. Scale bars, 200 μm. (**B**) Analysis of the distribution of distances from the cells in the chondrium layer to the endothelial cells. The distance from the cells in the chondrium layer to the closest vessels was measured at **postnatal days 0, 2, and 10. Immunostained sections were used for manual measurements of the cells (N=5, results were obtained from over 200 cells per animal).** (**C**) Proliferation of CD44+ cartilage progenitor cells (CPCs) in the auricular cartilage at P0, P2, P10 and P30. Cells in the P0 and P2 chondrium layers proliferated extensively, but those at P10 did not. No proliferating cells were detected in the P30 chondrium. Scale bars, 100 μm. (**D**) Quantification of the number of cycling cells in the chondrium layer. The data are shown as the mean ± s.d. (N = 5). (**E**) Endothelial cell-specific proliferation of CPCs *in vitro*. The Y-axis shows the number of cells/cm^2 counted 11 days after initiating the co-culture. The data are shown as the mean ± s.d. (N = 5, **: P < 0.01). Ctrl: Control; EC: endothelial cells; MSC: mesenchymal stem cells; Fibro: dermal fibroblasts; Chon: chondrocytes.

Supplemental Figure 3. Comparison of cartilage regeneration efficiency for multiple mixture ratios in culture.

(**A**) In vitro-grown condensed tissues comprising mixtures of 2 × 106 cells at multiple ratios (hCPC:HUVEC = 5:5, 5:2, 5:1, 5:0.5, 5:0.1 and 5:0) were transplanted into a subrenal capsular site. Grafts harvested 30 days after transplantation are shown in this panel. (**B**) The formation of cartilage was confirmed by Alcian blue staining. Scale bars, 1mm & 500μm.

Supplementary Figure 4. Alcian blue staining of vascularized cartilage progenitor transplants at multiple time points.

Supplementary Figure 5. Comparison of cartilage regeneration efficiency between vascularized- and pellet-cultured cartilage progenitors.

(**A**) Alcian blue staining at day 15 showed that the vascularized progenitor transplants began to deposit proteoglycans earlier than the conventional pellet culture transplants. At days 30 and 60, the tissue regenerated from the vascularized progenitor transplants,

but not from the pellet culture transplants, was homogenously composed of mature chondrocytes in lacunae that produced high levels of proteoglycan. Scale bars, 200 μm. (**B**) Color extraction from the Alcian blue-stained sections of the vascularized progenitor and pellet transplants at day 60. The extracted data were quantified by Image J software.

Supplemental Figure 6. Suppression of cartilage formation from human progenitor cells *in vivo* by the inhibition of functional vessel formation.

(**A**) A schematic diagram of the transplant model for inhibiting functional vascularization. Vascularized cartilage progenitor cells (CPCs) were transplanted into the cranium and kept separate from the host blood vessels using a 0.45-μm nanomesh. (**B**) Gross observation of the transplants at 0 and 15 days. Host blood perfusion into the transplants was completely inhibited. (**C**) Intravital confocal images of the same field of view in day 3, 7 and 11 transplants showing the failure of human CPC engraftment. The lower panel shows higher magnification images of the corresponding field of view in the upper image. (**D**) Caspase 3 and Col2 immunostaining of the transplants with (upper) or

without (lower) the nanomesh at 15 and 30 days, respectively.

Supplemental Figure 7. En-bloc cryopreservation of the self-aggregated three-dimensional tissues.

(**A**) Process for the en-bloc cryopreservation of the self-aggregated 3-D tissues. Self-aggregated tissues (left) were retrieved with a spatula (middle) and moved into tubes (right) filled with TC-protector. Scale bar, 3 mm. (**B**) Macroscopic view of thawed tissues after 1 month of preservation at -80ºC. Scale bar, 3 mm. (**C**) Histological analysis of transplanted preserved three-dimensional vascularized condensed tissue at 30 days post-transplantation, showing the formation of elastic cartilage *in vivo*. The left two panels show Alcian blue staining. The right panel shows aggrecan (green) and collagen II (red) co-immunostaining. Scale bars, 200 μm.

SUPPLEMENTAL VIDEO LEGEND

Supplemental Video 1. Time-lapse movie showing the self-formation of vascularized cartilaginous tissue *in vitro*.

This video shows the formation of human cartilage progenitor cell-derived vascularized and 3-D tissues by recapitulating endothelial cell interactions.