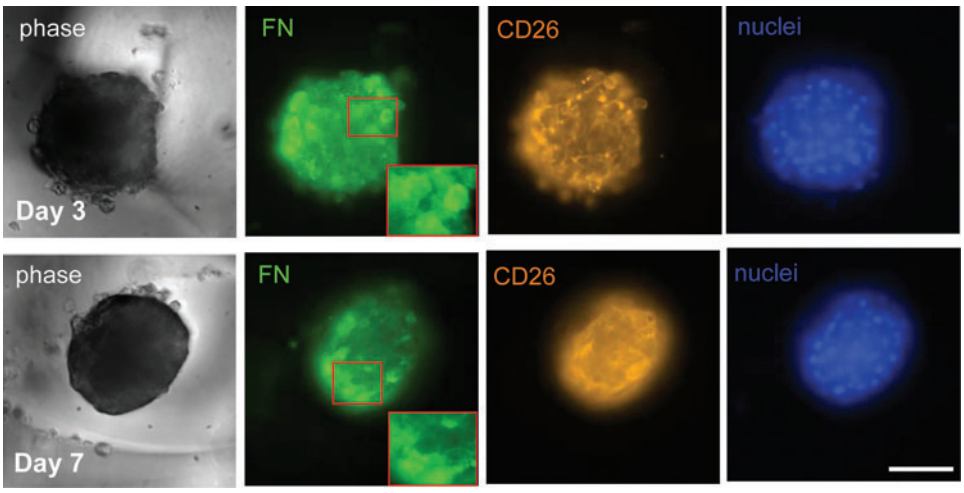
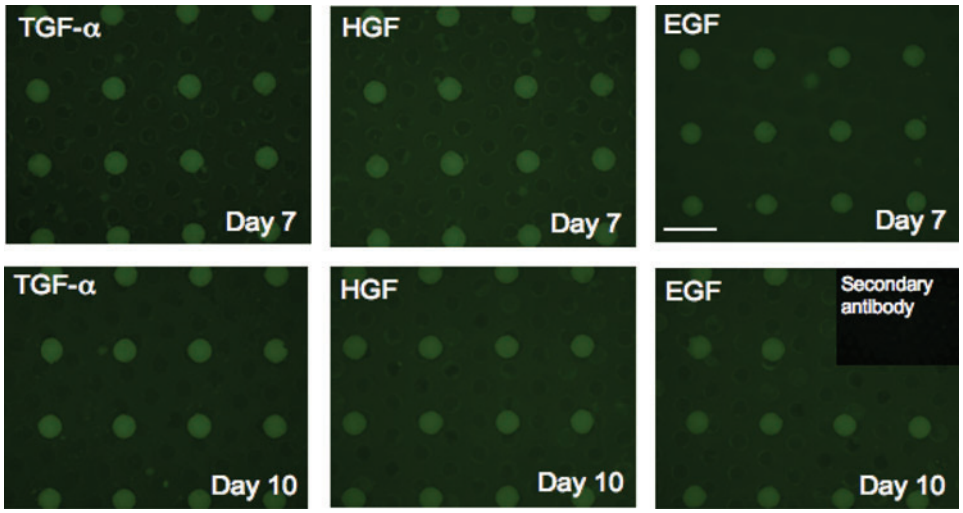
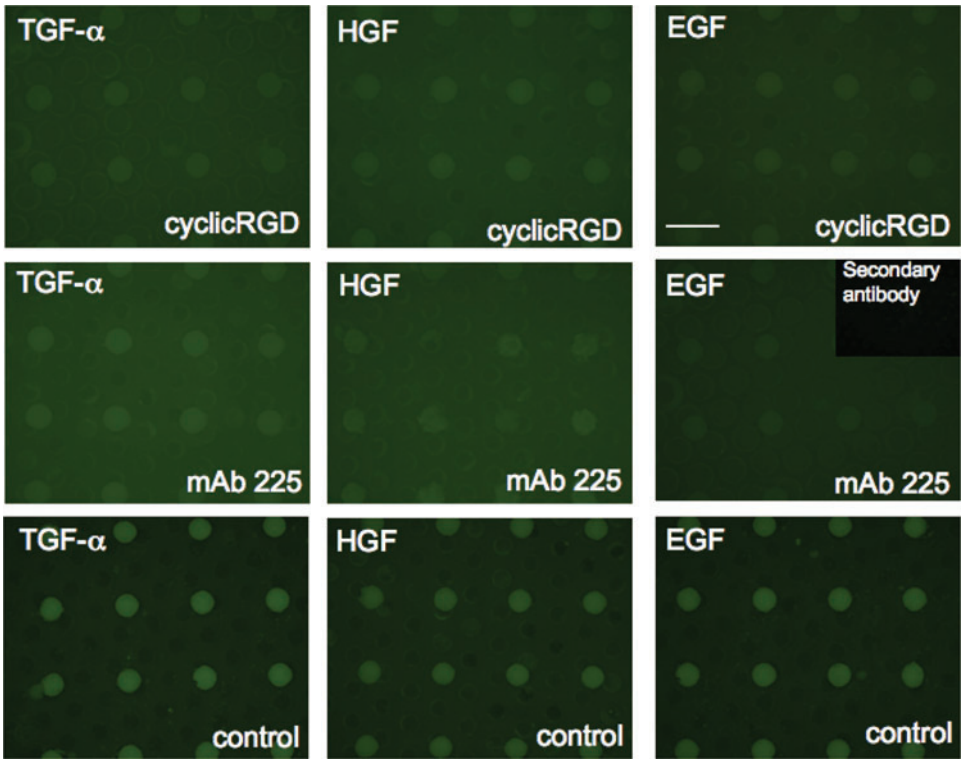
Supplementary Data



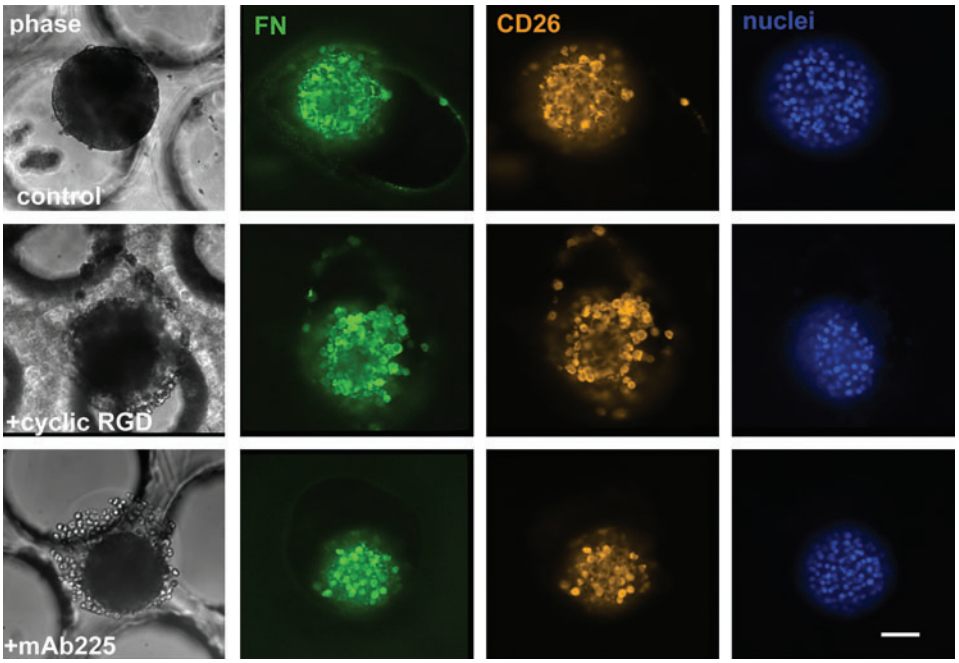
SUPPLEMENTARY FIG. S1. PEGDA gels fail to retain cell-secreted FN. Freshly isolated primary hepatocytes were plated onto molded PEGDA gels at a density of 100,000 cells/cm^2 in hepatocyte growth medium lacking growth factors and cultured for 3 and 7 days, and then cultures were fixed and stained for FN (green), CD26 (orange), and nuclei (blue). Images depict a single confocal cross-section taken within the centermost 25% of the tissue structure (digital zoom of red highlighted regions is illustrated in bottom right insets). Note the absence of soluble FN retention in the microwell wall and the irregular structure of the aggregates (scalloped edges due to failure of cells to form compact aggregate, aggregate perimeter that is noncircular, loose cells at outer edges). For comparison of PEGDA phase image with PEG–fibrinogen microwells, see Figures 1 and 6 (day 3) and Supplementary Figure S4 (day 7). Scale bar = 100 μm. FN, fibronectin; PEG, polyethylene glycol; PEGDA, PEG-diacrylate.



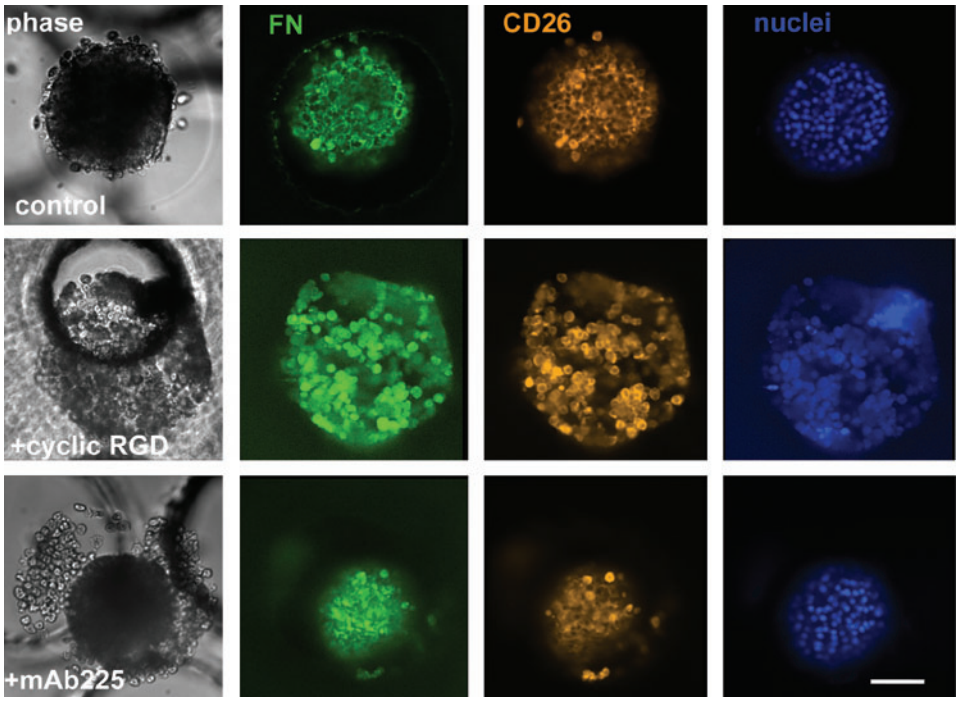
SUPPLEMENTARY FIG. S2. Primary hepatocytes show sustained retention of autocrine growth factors in long-term culture within micropatterned wells. Cells were cultured, as previously described, in the absence of exogenous growth factors. After 7 and 10 days, samples were fixed and stained with antibodies against EGF, HGF, and TGF-α. As a negative control, a sample was incubated with secondary antibody alone to confirm that the signal was from primary antibody. Fluorescence indicates retention of TGF-α, EGF, and HGF. Scale bar = 1 mm (magnification: 35×). For day 3 images, see Figure 3. EGF, epidermal growth factor; HGF, hepatocyte growth factor; TGF, transforming growth factor.



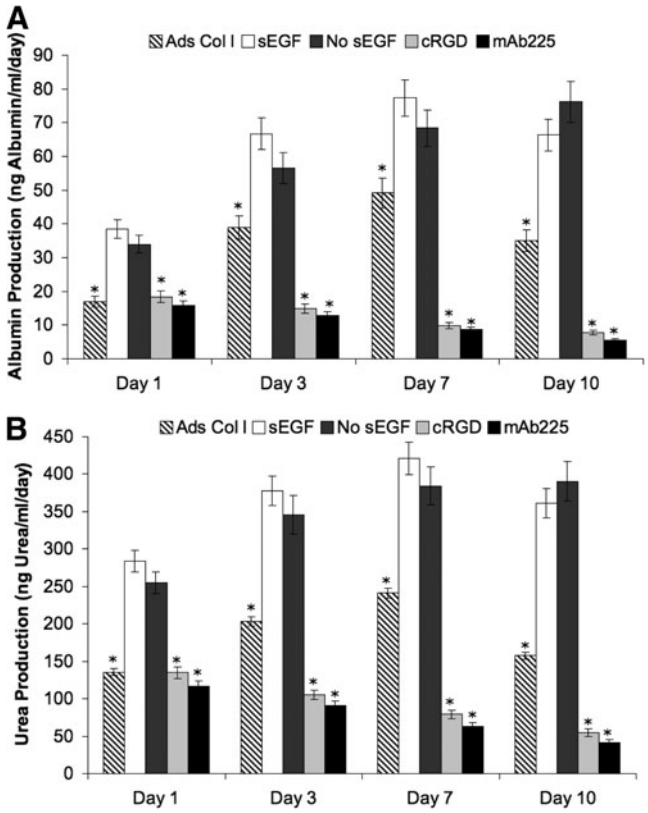
SUPPLEMENTARY FIG. S3. Blocking α5β1 integrin and EGFR binding in primary hepatocytes disrupts autocrine growth factor retention in micropatterned wells. Cells were cultured for 3 days as previously described in the presence of 10 μM cRGD (blocks α5β1) or mAb225 (blocks EGFR) and in the absence of serum or exogenous growth factors. After 3 days, samples were fixed and stained with antibodies against EGF, HGF, and TGF-α. As a negative control, a sample was incubated with secondary antibody alone to confirm that signal was from primary antibody. Matching pictures from untreated cells are shown in the bottom row as positive control. Fluorescence indicates retention of TGFα, EGF, and HGF. Scale bar = 1 mm (magnification: 35×). Note that mAb225 and cRGD show decreased staining for the growth factors. cRGD, cyclic arginine–glycine–aspartate; EGFR, EGF receptor.



SUPPLEMENTARY FIG. S4. Blocking α5β1 integrin signaling from FN disrupts tissue-like structures (day 7). Freshly isolated primary hepatocytes were plated onto molded gels at a density of 100,000 cells/cm^2 in hepatocyte growth medium lacking growth factors and in the absence or presence of 10 μM cRGD peptide or mAb225. After 7 days, cultures were fixed and stained for FN (green), CD26 (orange), and nuclei (blue), depicted here as single confocal cross sections taken within the centermost 25% of the tissue structure. Note the absence of FN fibrils and the loose, dissociated cells present in the microwells of cultures with cRGD and the progressive loss of structure compared with day 3 (Fig. 6). Also note the smooth edges of the aggregates, loose cells in the bottom of the well, diminished staining for FN fibrils relative to control cultures, and CD26 staining similar to control cultures in the microwells of cultures with mAb225. For reference, support scaffold channels have $d = 340$ μm. Scale bar = 100 μm.



SUPPLEMENTARY FIG. S5. Blocking α5β1 integrin signaling from FN disrupts tissue-like structures (day 10). Freshly isolated primary hepatocytes were plated onto molded gels at a density of 100,000 cells/cm^2 in hepatocyte growth medium lacking growth factors and in the absence or presence of 10 μM cRGD peptide or mAb225. After 10 days, cultures were fixed and stained for FN (green), CD26 (orange), and nuclei (blue), depicted here as single confocal cross sections taken within the centermost 25% of the tissue structure. Note the absence of FN fibrils and the loose, dissociated cells present in the microwells of cultures with cRGD and the progressive loss of structure compared with day 3 (Fig. 6) and day 7 (Supplementary Fig. S4). Also note the smooth edges of the aggregates, loose cells in the bottom of the well, diminished staining for FN fibrils relative to control cultures, and CD26 staining similar to control cultures in the microwells of cultures with mAb225. For reference, support scaffold channels have $d = 340$ μm. Scale bar = 100 μm.



SUPPLEMENTARY FIG. S6. PEG–fibrinogen hydrogels promote maintenance of hepatocyte metabolic functions *in vitro*. Primary hepatocytes were cultured on micromolded PEG–fibrinogen gels or adsorbed collagen I as described in the Materials and Methods section. Conditioned medium was collected at the indicated time points and metabolites of interest were quantified as described in the Materials and Methods section. Samples, standards, and controls were tested in duplicate. **(A)** Albumin synthesis; **(B)** urea synthesis. *Statistically significant difference from sEGF supplemented PEG–fibrinogen samples at a specific day; $p < 0.05$, $n > 3$. Data has not been normalized to number of viable cell at each day. For data normalized to cell number at day of sample collection, see Figure 7.