ADVANCED FUNCTIONAL MATERIALS

Supporting Information

for *Adv. Funct. Mater.,* DOI: 10.1002/adfm.201910442

Controlled Apoptosis of Stromal Cells to Engineer Human Microlivers

*Amanda X. Chen, Arnav Chhabra, Hyun-Ho Greco Song, Heather E. Fleming, Christopher S. Chen, and Sangeeta N. Bhatia**

Supporting Information

Controlled Apoptosis of Stromal Cells to Engineer Human Microlivers

*Amanda X. Chen, Arnav Chhabra, H.-H. Greco Song, Heather E. Fleming, Christopher S. Chen, Sangeeta N. Bhatia**

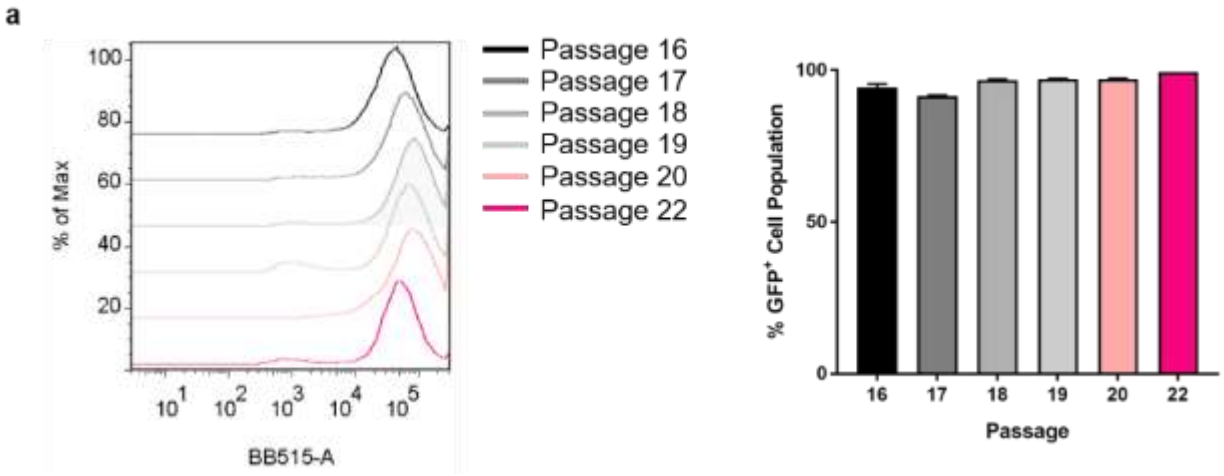

Figure S1. iCasp9-GFP J2s are activated by CID and uniformly eliminated by apoptosis. iCasp9-GFP J2s maintain stable expression of GFP by flow cytometry analysis (a).

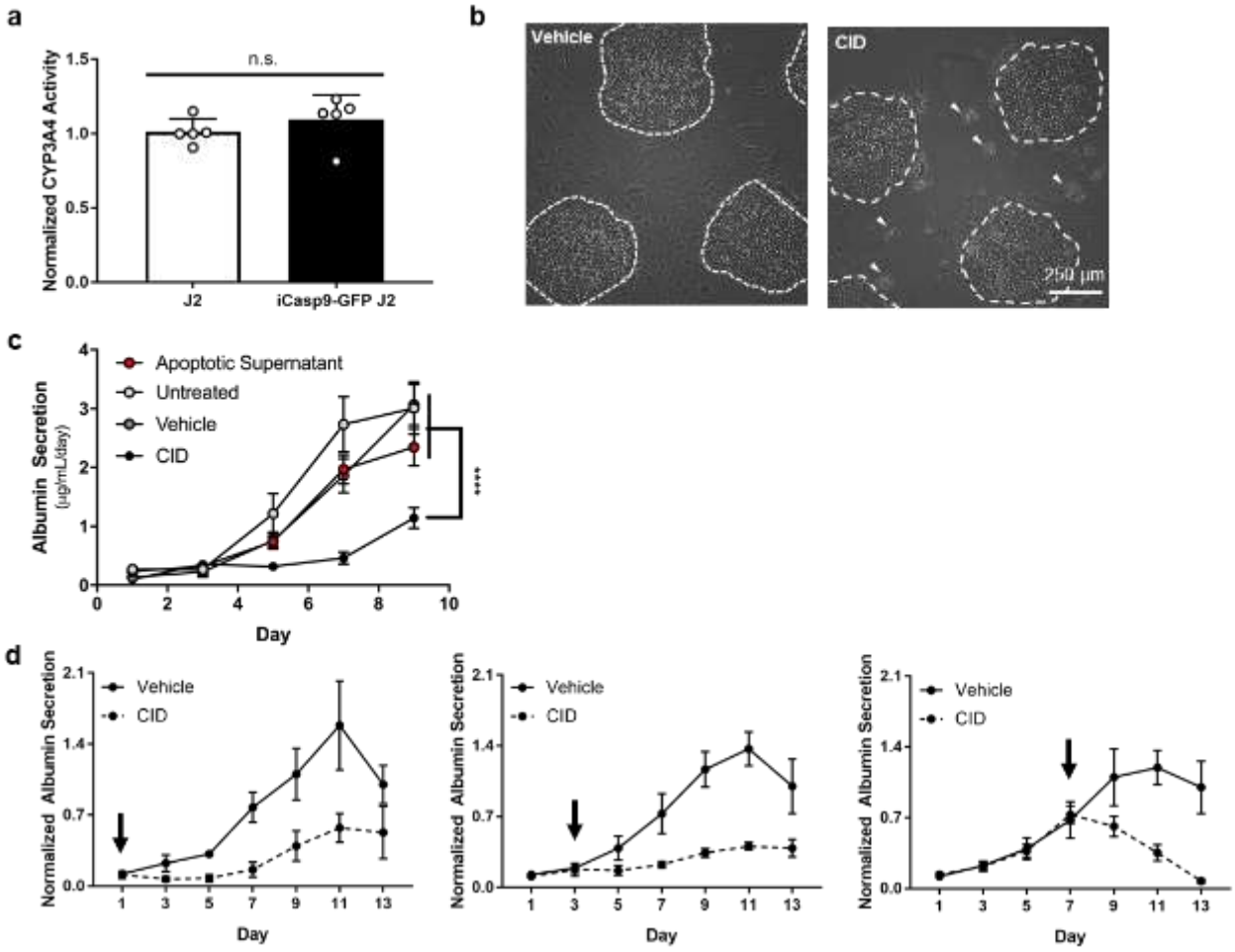

Figure S2. 2D MPCC cultures depend on the sustained presence of stromal cells. MPCCs cultured with J2s or iCasp9-GFP J2s and assayed for basal expression of CYP3A4 (a, n=5). iCasp9-GFP J2s underwent apoptosis in CID-treated MPCCs (b, arrows indicate apoptotic bodies, dotted lines demarcate hepatocyte islands). MPCCs were treated with conditioned apoptotic media, vehicle, CID or untreated and assayed for albumin secretion rate (c, n=4, ****p<0.0001 vs. CID). Experimental repeat showing reproducibility of fibroblast dependence experiment in MPCC from Figure 2f (d, n=5, normalized to day 13, arrows indicate dose day).

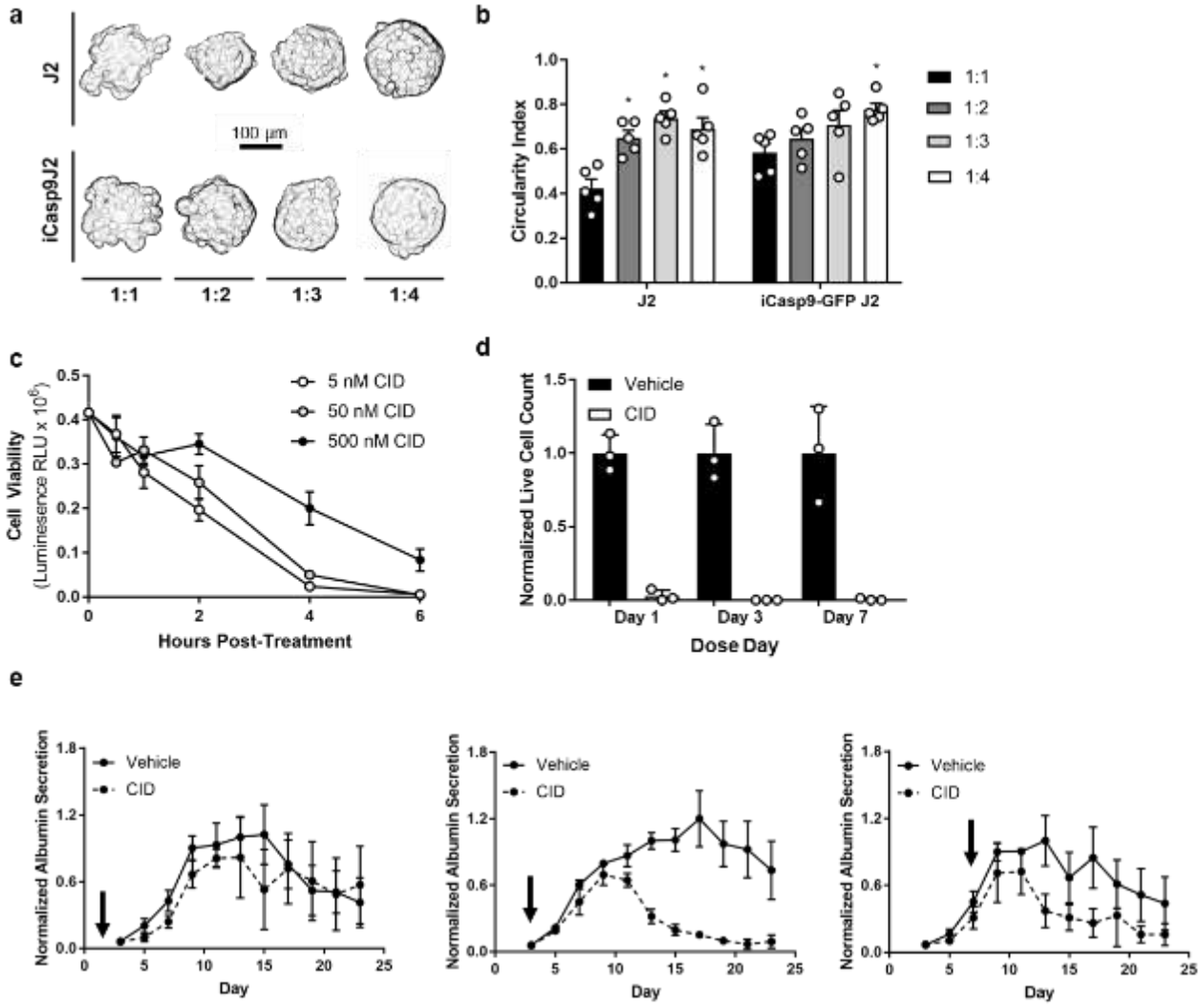

Figure S3. Fibroblasts are not required to maintain hepatocyte function in 3D spheroid-laden cultures. Hepatocytes and fibroblasts were cultured in microwells at a range of hepatocyte:fibroblast ratios and imaged after 24 hours (a) to enable quantification of compaction by circularity (b, n=5, *p<0.05 vs stromal cell-matched 1:1 spheroids). Encapsulated iCasp9-GFP fibroblasts were treated with CID and assayed for viability (c, n=3). Encapsulated iCasp9-GFP fibroblasts were treated with CID at day 1, 3, or 7 post-encapsulation and fixed after 3 weeks of culture, then stained with Hoechst for nuclei-counting (d, n=3). Spheroid-laden hydrogels were treated with CID at day 1, 3, or 7 after initiating co-culture and assayed for albumin secretion rate (e, n=9, normalized to day 15, arrows indicate dose day).

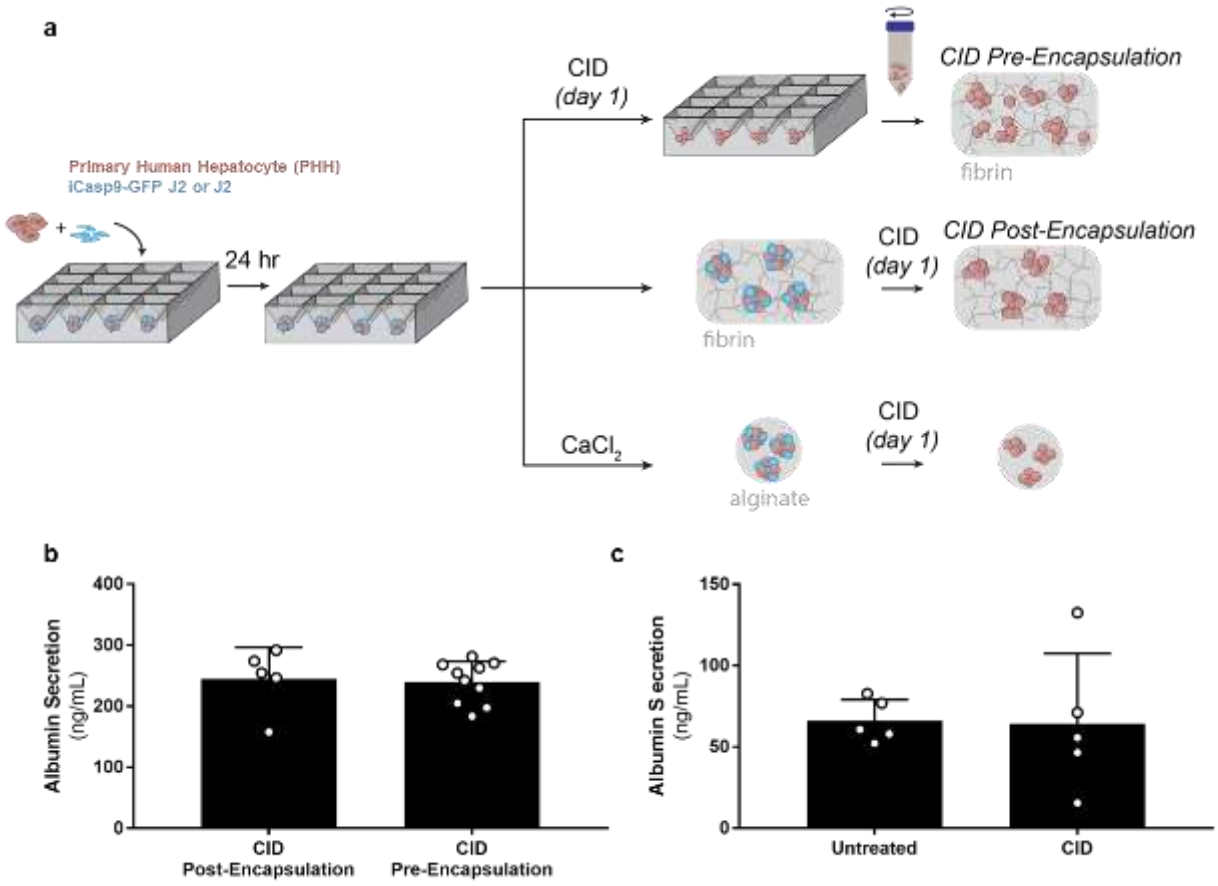

Figure S4. Retention of apoptotic debris and other fibroblast-secreted proteins do not drive maintenance of hepatocyte phenotypic stability in 3D. Hepatocytes were aggregated with fibroblasts in microwell molds. Resulting spheroids were treated with CID prior to harvest from the microwell molds (followed by subsequent removal of apoptotic debris and conditioned supernatant via centrifugation, 60x*g*, 6 minutes, 3 rounds; "Pre-Encapsulation"), encapsulated in fibrin and then treated with CID ("Post-Encapsulation"), or resuspended in 2 w/v % alginate and crosslinked in a warmed 2 w/v % $CaCl_2$ bath and then treated with CID. All cultures were dosed with CID on day 1 (a). Collected supernatant was assayed for albumin secretion rate at day 3 for pre- and post-CID dosed spheroids encapsulated in fibrin hydrogels (b) and day 13 for alginate-encapsulated spheroids (c) (n=5-10, n.s. between groups).

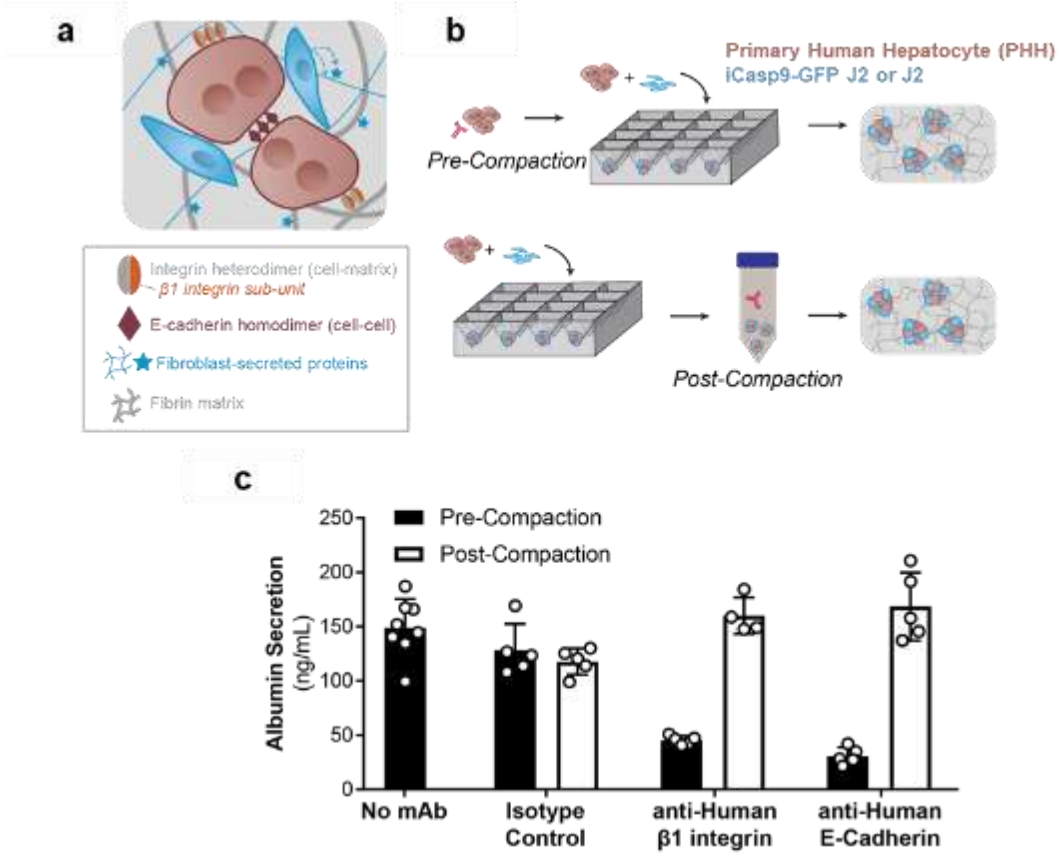

Figure S5. Early provision of β1 integrin-mediated cell-matrix interactions and E-cadherin-mediated cell-cell interactions was required for hepatocyte phenotypic stability in 3D. Hepatocyte (brown) and fibroblast (blue) co-culture and encapsulation in fibrin (grey) hydrogels enabled the formation of integrin-mediated (dimer including orange β1 integrin subunit) cell-matrix interactions and E-cadherin-mediated (brown diamonds) cell-cell interactions. Fibroblasts modified the ECM by depositing matrix (blue). Secreted soluble factors (blue stars) can be bound to the matrix, which can act as a reservoir of paracrine factors (a). Hepatocytes were incubated with function-blocking monoclonal antibodies against β1 integrin, E-cadherin or an isotype control before aggregation ("Pre-Compaction") or after compaction ("Post-Compaction"). After brief incubation (10 μg/ml, 20 minutes at 37 °C) with the antibody, excess reagent was removed by centrifugation washes (b). Resulting spheroids were encapsulated in fibrin hydrogels. Supernatant was

analyzed for secreted human albumin on day 3 (n=2-5) (c).

Movie S1. CID treatment selectively and uniformly depleted iCasp9-bearing fibroblasts from multicellular hepatic microtissues. Time-lapse movie of spheroid-laden hydrogel consisting of primary human hepatocytes (CellTracker, red) co-cultured with iCasp9-GFP J2 fibroblasts (GFP, green). Tissues were treated with CID at the beginning of image capture.