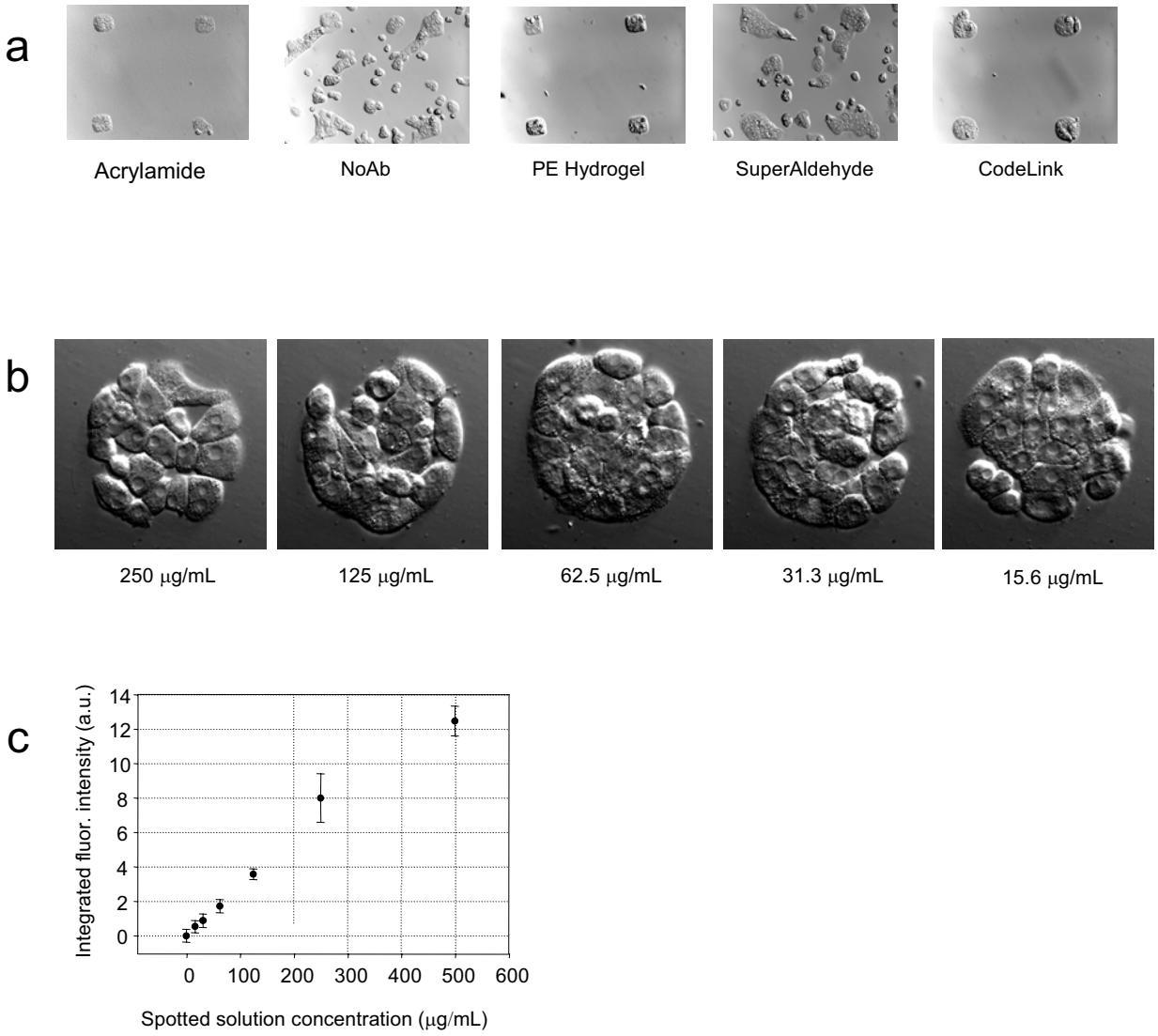

Supplementary Figure 1 Spotted ECM mediates cell attachment. (**a**) Primary hepatocytes attach to collagen I spots, and are spatially confined on the custom acrylamide, CodeLink (Amersham), and Hydrogel (Perkin Elmer) substrates. Superaldehyde (Telechem) and Epoxy Hydrogel (NoAb Diagnostics) allow cell attachment in non-spotted regions. (**b**) Primary hepatocytes attached to collagen I spots made from solutions containing as little as 15.6 μg/mL of protein. Below this concentration no cell attachment was observed. Hepatocytes showed similar attachment to serially diluted collagen III, collagen IV, laminin, and fibronectin (data not shown). (**c**) Immobilized FITC-collagen I signal is linear over a broad range of spotting solution protein concentrations. Error bars represent s.e.m.

SUPPLEMENTARY METHODS

Indirect immunofluoresence. Five identically fabricated ECM microarrays were blocked using a 10% goat serum, 1% BSA solution in PBS. Indirect immunofluorescence was conducted using the following primary antibodies (all raised in rabbits), and an Alexafluor 633 goat anti-rabbit secondary (1:50 dilution, Molecular Probes): anti-rat collagen I (Chemicon), anti-mouse laminin (Chemicon), anti-human collagen III (Biodesign), anti-mouse collagen IV (Biodesign), and anti-human fibronectin (Sigma). Fluorescent images were acquired using a ScanArray 4000 confocal laser scanner (GSI Lumonics).

Cell culture. ECM microarray slides with silicone gaskets were placed in sterile P-100 culture dishes. The gasketed area was filled with 300 L of dH_2O. Protein arrays were sterilized by exposure to UV in a laminar flow hood for 15 minutes, followed by rinsing in sterile culture media.

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Hepatocytes were isolated from 2- to 3-month-old adult female Lewis rats (Charles River Laboratories, Wilmington, MA) weighing 180-200 grams, by a modified procedure of

Seglen[1]. Detailed procedures for isolation and purification of hepatocytes were previously described by Dunn et al.[2]. Routinely, 200-300 million cells were isolated with viability between 85 and 95%, as judged by trypan blue exclusion. Nonparenchymal cells, as judged by their size (<10 m in diameter) and morphology (nonpolygonal or stellate), were less than 1%. Culture medium was Dulbecco's modified Eagle's medium (DMEM, Gibco) supplemented with 10% fetal bovine serum (FBS, Sigma, St. Louis, MO), 0.5 U/mL insulin, 7 ng/mL glucagon, 20 ng/mL epidermal growth factor, 7.5 g/mL hydrocortisone, 100 U/mL penicillin, and 100 g/mL streptomycin. Hepatocytes were suspended at 10^6 cells/mL in culture media. The cell suspension was dispensed onto the ECM microarray in the gasketed region (0.3 mL), and incubated for 2 hours to allow for cell attachment (shaking the plates every 15 minutes to redistribute the cells). The arrays were then gently aspirated to remove unattached cells and fresh culture media was added to the silicone wells. Culture media was changed daily.

The I114 mouse embryonic stem cell line containing a gene-trap[3,4] was propagated in an undifferentiated state on gelatinized flasks in culture media containing 1000 U/mL leukemia inhibitory factor (LIF, Chemicon). ES media consisted of GMEM/BHK21 (Gibco) supplemented with 15% FBS (Hyclone, screened for ES culture), non-essential amino acids, and 10^{-4} M 2-mercaptoethanol (Sigma). Cells were passaged at 80% confluence (approximately every two days). ECM microarrays containing 450,000 cells were cultured for 6-10 hours (without shaking) to allow for cell attachment before rinsing with fresh media. ES cell arrays were cultured for up to six days with either 1000 U/mL LIF or 10^{-6} M all-trans-retinoic acid (Sigma).

Cellular function. Hepatocyte cell viability was assessed on day 1 and day 5 after plating using a live/dead assay (Calcein AM and ethidium homodimer-1, Molecular Probes Inc). Cell arrays were imaged live, and then fixed in 4% paraformaldehyde and mounted in SlowFade Light w/DAPI (Molecular Probes Inc.) for high resolution microscopy.

I114 ES cell alkaline phosphatase activity was assessed by a substrate kit IV (SK-5400, Vector Laboratories). F-Actin was visualized using Phalloidin-TRITC (Sigma). Confocal volume images were aquired at 20x and 40x on a BioRad MRC 1000, and digitally sectioned in the *x-z* plane using Metamorph 6.2r3 software (Universal Imaging Corp.).

Statistics and data analysis. Unless otherwise specified, all data are reported as average value ± standard deviation. We assessed intracellular albumin content of hepatocytes using Metamorph image analysis software. To quantify spot intensity, we calculated the average pixel value within a masked region (each ECM microarray contained 256 spots). After a log transformation, the data appeared to be normally distributed with approximately equal variance. For each matrix mixture, the eight replicate log spot intensities were used to calculate the average signal and standard error for the condition. Two day 1-, and four day 7-arrays were quantified in this manner. The mean signal for each array was adjusted to an arbitrary common value among arrays. All normalized day 7 data were analyzed as a 2^5 full factorial design with 4 blocks (one for each microarray) using Minitab statistical software (Minitab, State College, PA). Main effects, 2-factor, 3-

factor, and 4-factor interactions, along with the statistical significance of each of these properties, were calculated using standard factorial analysis formulae[5]. The residuals were normally distributed with approximately equal variance around a mean value of zero. β-galactosidase analysis of ES cultures was performed similarly using data from 4 day-3 arrays.

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SUPPLEMENTARY DATA

ECM microarray fabrication and characterization

To establish a range of protein concentrations in the spotting solution that were relevant for cell attachment, we deposited each ECM protein at 7 concentrations ranging from 7.81 g/mL to 500 g/mL on the acrylamide gel pad substrate. We found that primary hepatocytes attached to ECM protein spots made from solutions with as little as 15.6 g/mL (**Supplementary Figure 1b** online). Image analysis of serially diluted FITC-collagen I spotted substrates showed a nearly linear trend between spotted protein concentration and immobilized fluorescent collagen I intensity in this range (**Supplementary Figure 1c** online). Spotted substrates and protein mixtures in the source plate could be stored at 4 °C for at least one week without apparent deleterious effects on cell attachment.