Supplemental Material and Methods:

S1.1 Cell culture and characterization of cell morphology on 2D functionalized glass surfaces

The cells were cultured at 37 °C, 5% CO_2 in Dulbecco's Modified Eagle's medium (DMEM), (Sigma Aldrich, St. Louis, MO, USA) with 10% fetal bovine serum (FBS) (Gibco, Grand Island, NY, USA), 2% GlutaMAX (Gibco), and 1% penicillin streptomycin (Gibco). MDA-MB-231 cell morphology on functionalized glass coverslips was determined using a solution of 800,000-1,000,000 cells ml^{-1} in cell imaging media made from confluent cultures (70-90%). Imaging media consisted of DMEM without phenol red, supplemented with 10% FBS, 2% Gluta-max, 1% penicillin/streptomycin and 12 mM HEPES (Sigma Aldrich). Images were taken at 2, 4 and 16 hrs at 10x magnification (*NA* = 0.3, Nikon, Tokyo, Japan) and captured with a CCD (The Imaging Source, DMK 41AU02, Charlotte, NC, US or Photometrics CoolSNAP HQ2, Tuscan, AZ, US). Aspect ratio, a parameter calculated by dividing the length of a cell by the width of that same cell and cell spreading area were measured using the ImageJ software (**Supplemental Fig. 1**).

S1.2 Fixing and staining of cells

To analyze the cell behavior in a 3D environment, cells were fixed and stained for F-actin. The samples prepared in the above procedure were incubated at 37 °C and 5% CO_2. The cytoskeleton buffer is a 10 mM MES (Sigma Life Sciences, MO, USA), 3 mM $MgCl_2$ (Fisher), 138 mM KCl (Fisher), and 2 mM EGTA solution at pH adjusted to 6.1. While fixing, the samples were placed on heating plates set at 37 ^oC. First, cells were fixed in 4% paraformaldehyde (Fisher) w/v, in cytoskeleton buffer solution. Second, cells were treated with 0.1 M glycine (Fisher Scientific) solution for 10 mins. Third, the samples were treated with a 0.5% triton X solution 1x for 10 mins. Both glycine and triton X were diluted in cytoskeleton buffer. Fifth, cells were washed with Tris-buffered saline (TBS) containing 150 mM NaCl (Fisher Scientific) and 20 mM Tris-Cl pH 7.4 3x for 5 mins each. The blocking buffer was made with TBS, 0.1% v/v tween-20, and 2% w/v bovine serum albumin (BSA, Sigma Aldrich) and contained a 1:200 dilution of alexa 488-phalloidin (Molecular Probes). Cells were blocked and treated with phalloidin for one hr at room temperature and protected from light. After staining, the cells were washed in TBS and the samples were sealed with nail polish followed by valap and stored at 4 °C before imaging. Both fluorescent and transmitted images were collected from each sample at various heights within the collagen matrix using the *z*-stack feature on µManager 1.3 software. Images were taken every 5 µm for the 100 µm chambers and every 10 µm for the 240 µm chambers. The images were taken with a 20 x objective lens (*NA* = 0.5, Nikon) with a charge-coupled device (CoolSNAP HQ2, Photometrics) attached to an inverted microscope (Eclipse Ti, Nikon). Image equipment was controlled by µManager. The

images were analyzed for cell body area, protrusion length, F-actin intensity, and cell shape using ImageJ software.

S1.3 Cell migration and morphology data analysis

Cell migration was analyzed using the MTrackJ plugin [1] in ImageJ. Using the images collected from the time lapse, the courses of the cells were traced and the position coordinates (x, y) were recorded for each j^{th} time interval, τ. Instantaneous cell migration speed, S_i, (i refers to instantaneous) was calculated over 2 minutes for each cell using the following equation:

$$S_i = \frac{1}{N}\sum_{j=1}^{N}\frac{\sqrt{\Delta x_j^{\,2} + \Delta y_j^{\,2}}}{\tau}, \qquad (1)$$

where N is the number of time intervals contained for each track and Δx_j and Δy_j are the x and y displacements between two time points ($\tau = t_{j+1} - t_j$). For each condition, an average S_i was constructed by averaging among cells. The mean squared displacement was fitted to the following equation:

$$d^2(t) = nS_m^{\,2}P_m\left(t - P_m\left(1 - e^{\left(\frac{t}{P_m}\right)}\right)\right), \qquad (2)$$

where n is the dimension of cell motility and P_m, persistence time, characterizes the average time over which there is no change in the direction and speed of the cell movement. The model based speed, S_m (m refers to model) was also calculated. The parameters, P_m and S_m were calculated using a custom-made MATLAB script. Furthermore, the motility coefficient (μ), a random migration parameter was calculated using the following equation:

$$\mu = \frac{S_m^{\,2}P_m}{n}. \qquad (3)$$

In some instances, means-squared displacement was linear. Under this situation, μ can be known, but S_m and P_m can take on numerous compensatory values. Consequently, in order to calculate a persistence time, P we used information about the instantaneous speed, S_i. The persistence time, P_m was plotted as a function of the ratio of S_m to S_i. P_m ranging between 2-160 mins (smallest to longest experimental time

interval) was used for fitting the data. A S_m was calculated based on the P_m and the S_i of a particular cell. This allows for an estimation of S_m for mean squared displacement graphs with only one feature. This new S_m and the μ were used to calculate the persistence time, P.

In the 3D gel with step change in chamber thickness, the cell orientation was measured using θ ($0 \leq \theta \leq \pi/2$), the angle between the orientation of the cell and the direction pointing from the thick side to the thin side of the chamber. In order to quantify the orientation of cells with respect to the change in the gel thickness, an orientation directionality index (DI_o) (o refers to orientation) defined as

$$DI_o = \cos 2\theta \qquad (4)$$

was calculated using the measured angles. A directionality index of 1 implied that the cells are oriented parallel to the direction of change in gel thickness,0 implied that the orientation was random, whereas -1 implied that the cells were positioned perpendicular to direction of change in thickness. DI_o was calculated as a function of distance from the boundary of the step change for both the thick and thin sides after 2 hrs and 16 hrs. Further, DI_o averaged over the cells at different distances from the boundary was also calculated. For the study examining cell migration from the thick side to the thin side of the gel, a directional migration parameter, DI_m (m refers to migration) over a time interval of 12 mins was estimated by calculating the projection of the cell migration in this direction, using a custom-made MATLAB script. A uniform unit vector field in a direction from the thick side to the thin side of the chamber was created to compare the cell movement direction in direction of the vector to calculate directionality using the following equation:

$$DI_m = \frac{1}{N}\sum_{i=1}^{N} \cos\left(\theta_i\right), \qquad (5)$$

where N is the number of time intervals contained in an individual cell track and θ_i is the angle formed during cell movement with respect to the vector (mentioned above) between two time intervals ($t_{i+1} - t_i = \tau$). This value was then averaged among cells to construct an average DI_m for a given condition.

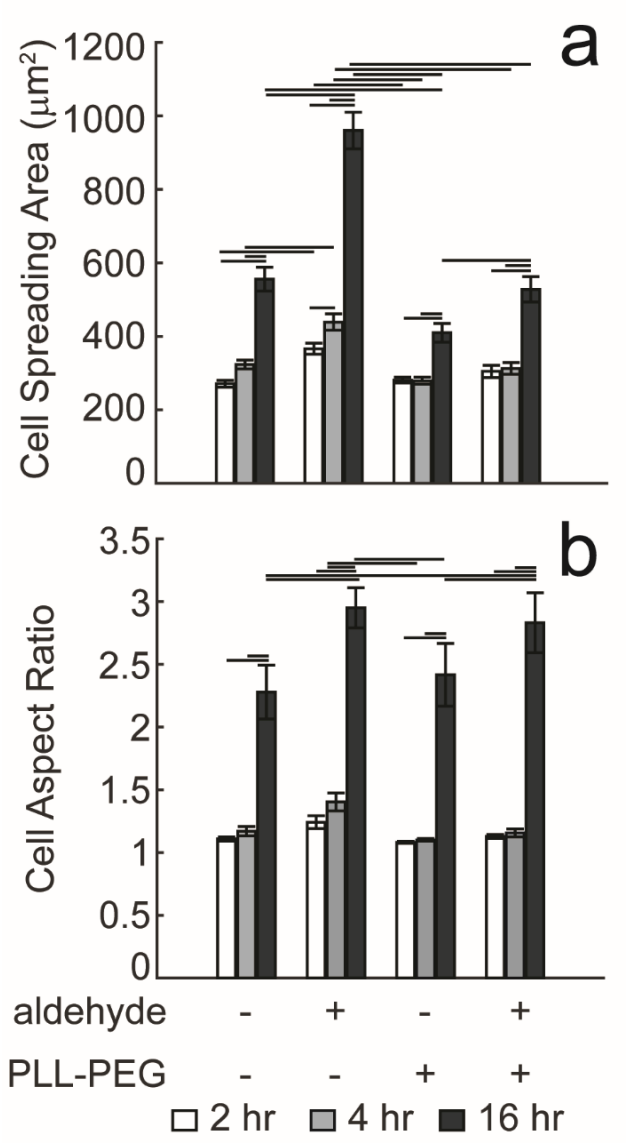
S1.4 Point source modeling of ligands close to reflecting surfaces

A cell was modeled as a point source secreting a ligand at a constant rate of 500 # cell^{-1} min^{-1} [2]. This ligand diffuses at a rate of $2\text{x}10^{-6}$ cm^2 s^{-1}. The following solution was used for a point source

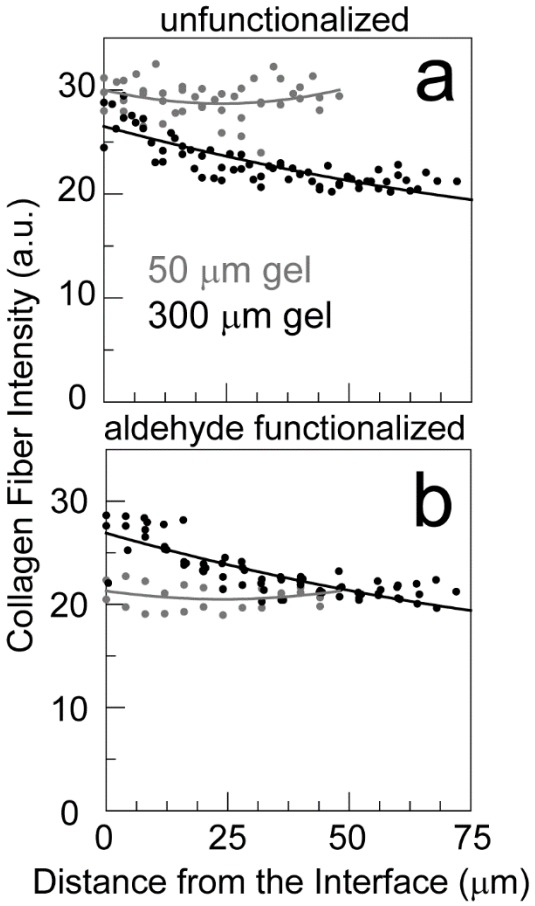
$$\bar{C} = \frac{V}{2\pi D(r-L)} erfc\left(\frac{r-L}{2\sqrt{Dt}}\right) \text{ [3]} \qquad (6),$$

where V is the constant ligand secretion rate, D is the diffusion coefficient, t is time after initiation of secretion, L is the distance from the interface, r is the distance from the point source and L is the distance from the reflecting boundary and $\bar{C}$ is the average concentration. The concentration at the point source was considered (r = 0) and this function was allowed to come to steady-state (t ~ 10 s) and average concentration was plotted as a function of distance from the interface.

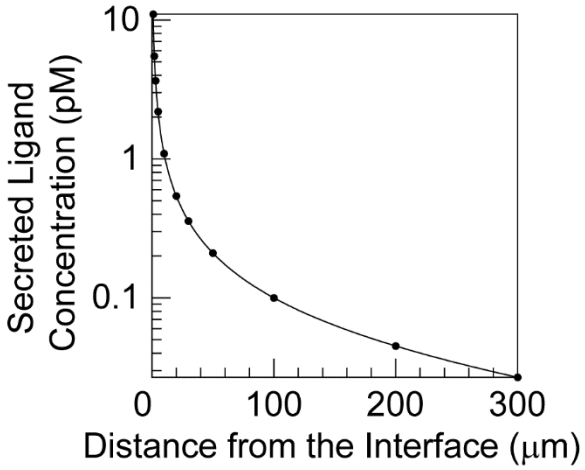
Supplemental Figure Legends:



Supplemental Fig. 1. Characterization of cell spreading on functionalized glass surfaces. a) Cell spreading area and b) cell aspect ratio of MDA-MD231 cells observed after 2, 4 and 16 hrs for different chemical functionalization on glass surfaces ($N_{samples} > 3$, $N_{cells} > 226$). The error bars represent the 95% confidence intervals. Lines over bars indicate that conditions were statistically significantly different as determined using analysis of variance ANOVA test with $p = 0.05$.



Supplemental Fig. 2. Modeling of a point source close to a reflecting boundary. a) and b) The collagen intensity observed at intervals of 4 μm from the glass surface (N_{stacks} = 2-4). For the 50 μm chambers, this was quatified for all the planes between the glass surfaces, whereas for the 300 μm chambers, it was calculated for upto 75 μm from one end of the chamber. The lines are a quadratic fit to the data with symmetry about 50 or 300 μm.



Supplemental Fig. 3. Modeling of a point source close to a reflecting boundary. A point source model of a constant ligand secreted rate was used to calculate the concentration very close to the point source at steady state. This ligand concentration was plotted as a function of distance from the surface.

[1] E. Meijering, O. Dzyubachyk, I. Smal, Methods for cell and particle tracking, Methods Enzymol. 504 (2012) 183–200. doi:10.1016/B978-0-12-391857-4.00009-4.

[2] M. Přibyl, C.B. Muratov, S.Y. Shvartsman, Discrete models of autocrine cell communication in epithelial layers, Biophys. J. 84 (2003) 3624–3635. doi:10.1016/S0006-3495(03)75093-0.

[3] W.M. Deen, Analysis of Transport Phenomena, Oxford University Press, n.d.