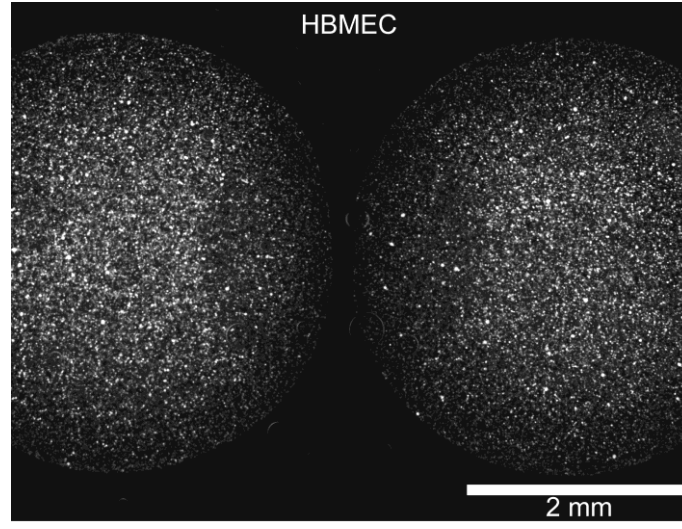
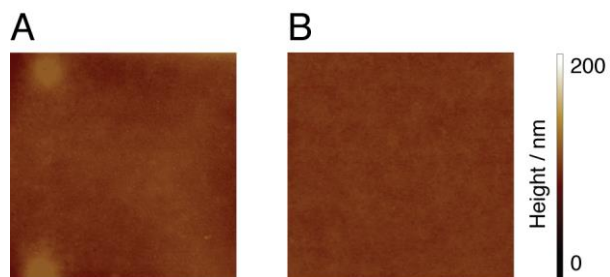


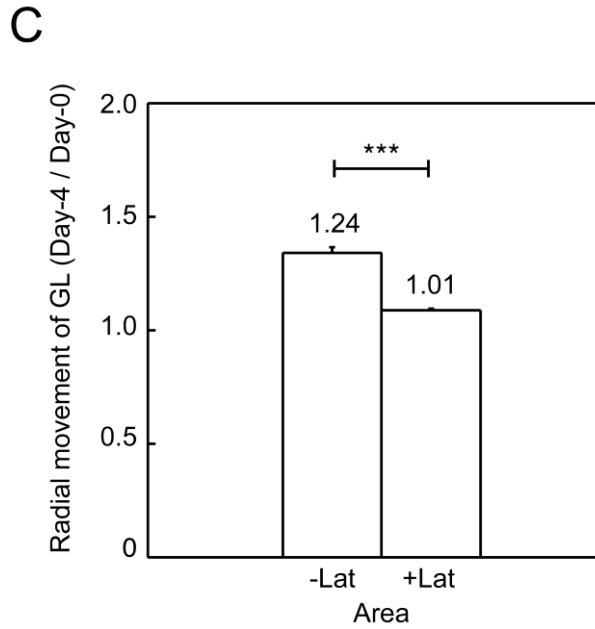
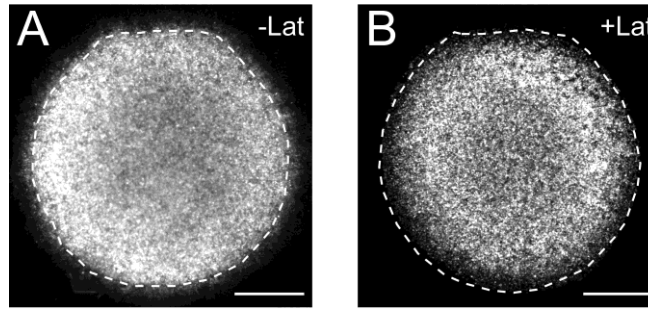
## Supplementary Figures



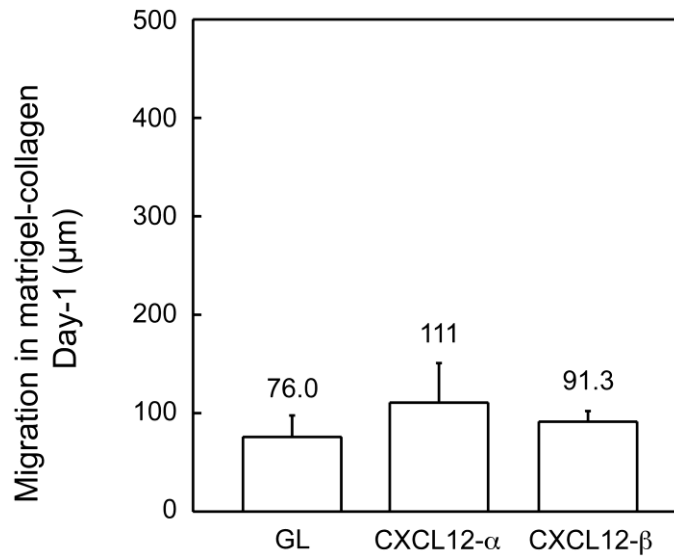
**Figure S1.** HBME cells in the patterned hydrogel droplets overlaid by matrigel with 200  $\mu\text{m}$  spacing.



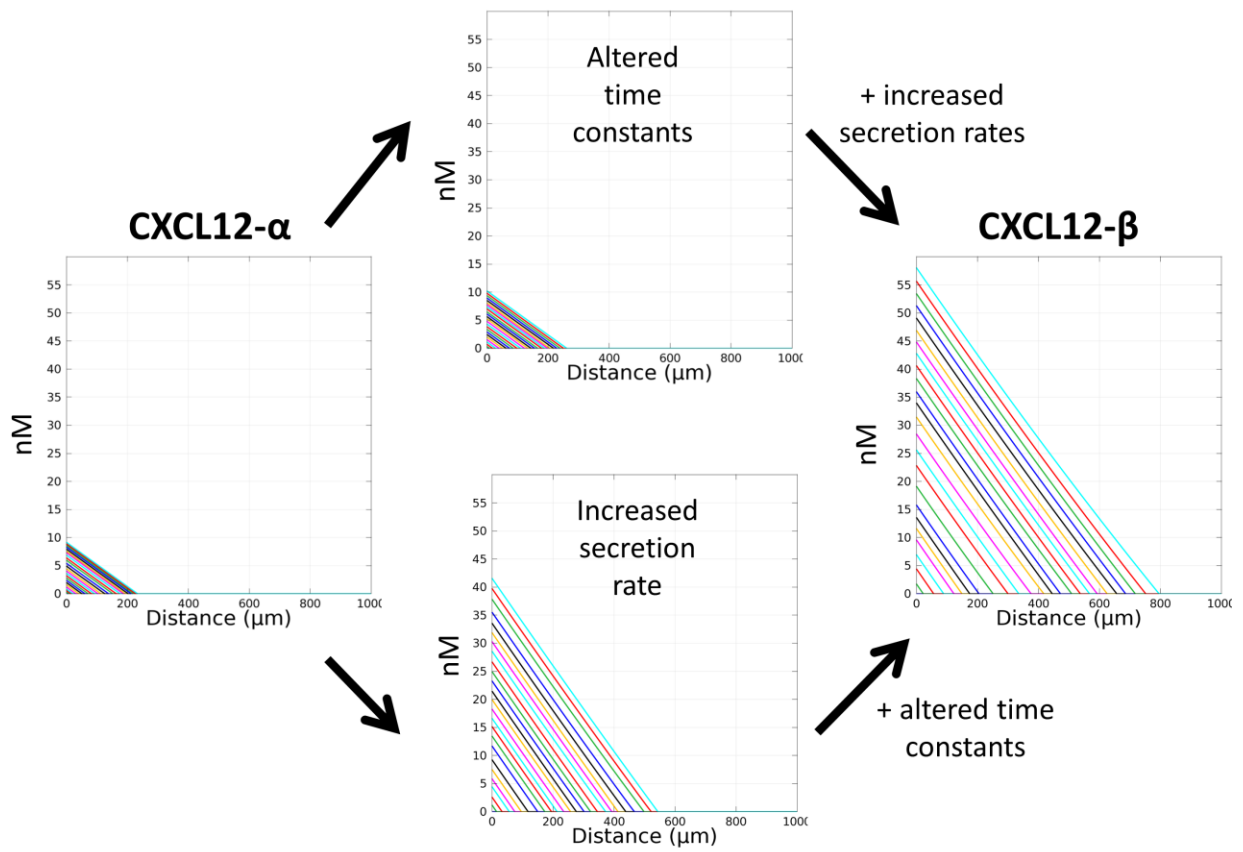
**Figure S2.** Surface topography of intact and oxidized PAA-coated PDMS. Surface height profile of (A) intact and (B) oxidized PAA-coated PDMS measured by AFM (Area  $10\ \mu\text{m} \times 10\ \mu\text{m}$ ). Surfaces do not show significantly different topographies between the intact and oxidized regions.



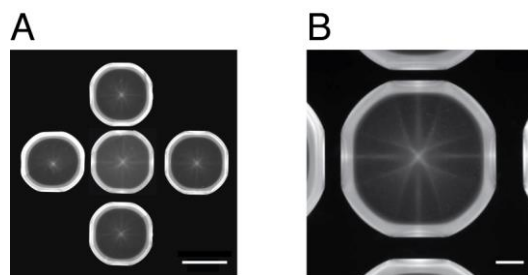
**Figure S3.** Soluble factors can be used to inhibit migration of MDA-MB-231 cells in the patterned hydrogel system. Fluorescent images of GL-expressing cells in the (A) absence and (B) presence of Latrunculin (50  $\mu$ M) at day4. White dotted line represents initial cell area at day0. (C) Comparison of spread area at day 4 reveals that migration was significantly inhibited by the presence of latrunculin. Area at day4 was normalized by area at day0 (\*\* $p < 0.001$ ). Scale bar 1 mm.



**Figure S4.** Migration assay of CXCR4-CXCL12 (GL, CXCL12- $\alpha$ , and CXCL12- $\beta$ ) at day1 within a matrigel-supplemented collagen matrix with 500  $\mu\text{m}$  spacing. Migration values are expressed as a mean  $\pm$  standard deviation ( $n = 3$  for GL,  $n = 3$  for CXCL12- $\alpha$ ,  $n = 3$  for CXCL12- $\beta$ ). In contrast to similar experiments conducted with spacings of 250  $\mu\text{m}$ , no significant differences in migration were observed between the three cell types ( $p > 0.35$ ).



**Figure S5.** Varied simulation parameters to identify the different diffusion profiles for  $\alpha$  and  $\beta$  isoforms of CXCL12. The combination of increased secretion rates and altered  $K_{on}$  and  $K_{off}$  values results in significantly accelerated diffusion profiles over 24 hours between CXCL12- $\alpha$  and CXCL12- $\beta$ .



**Figure S6.** Multiplex gel patterning in air. (A) Stitched fluorescent image of multiple hydrogels in air encapsulating fluorescent beads having different pattern-to-pattern distances (200  $\mu\text{m}$ , 300  $\mu\text{m}$ , 400  $\mu\text{m}$ , and 500  $\mu\text{m}$ ). Scale bar 500  $\mu\text{m}$ .