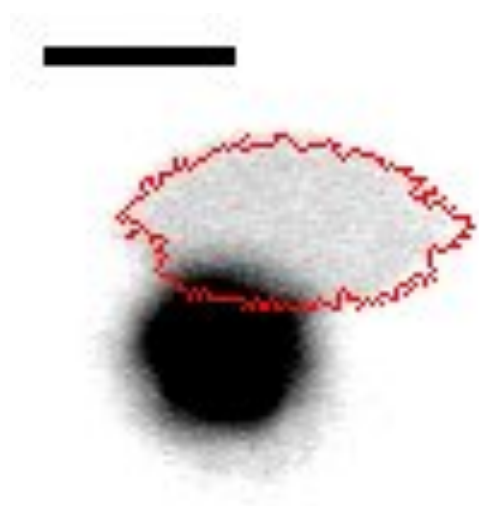


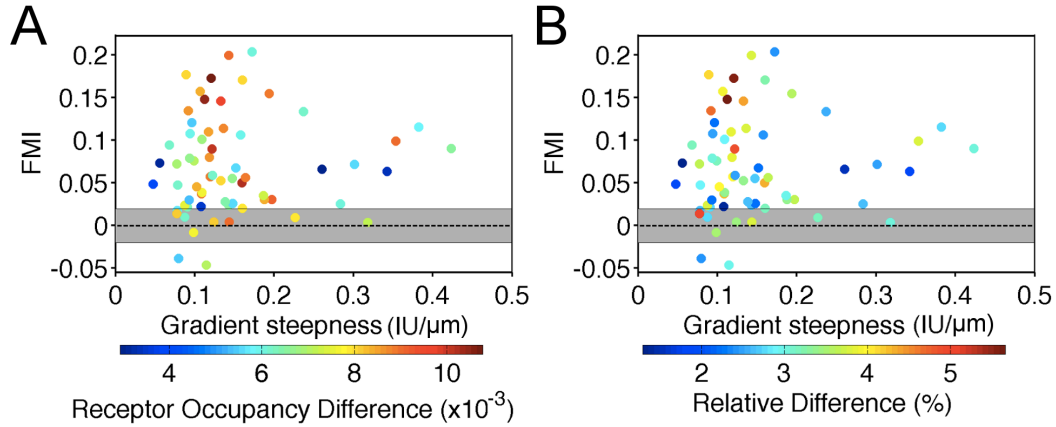
## Quantitative analysis of B-lymphocyte migration directed by CXCL13

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### SUPPLEMENTARY MATERIALS



**Supplementary Figure S1.** Overlaid epifluorescence (inverted grayscale) and TIRF (red outline) images of a representative B cell migrating on a surface presenting uniform CXCL13 and ICAM-1. Scale bar: 10  $\mu\text{m}$ .



**Supplementary Figure S2.** The data in Fig. 4A are replotted in the contexts of the absolute sensing with saturation (A) and relative sensing (B) models of haptotactic sensitivity. In A, the data are color-coded according to the receptor occupancy difference, expressed as a fraction of the total cell-surface receptor density, across a typical cell length of 10  $\mu\text{m}$ . The local receptor occupancy,  $b$ , as a fraction of total receptor density, is given by

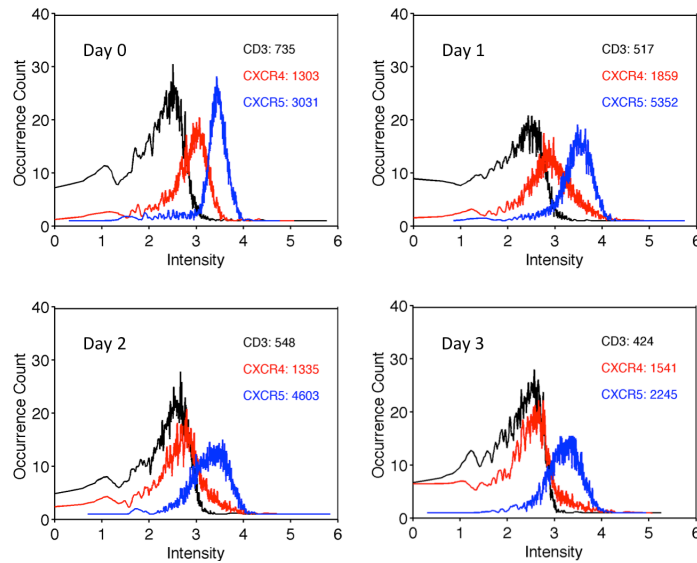
$$b = \frac{C}{K_d + C}.$$

And so the fractional occupancy difference is calculated as

$$(10 \mu\text{m}) \frac{db}{dx} = (10 \mu\text{m}) \frac{K_d}{(K_d + C)^2} \frac{dC}{dx},$$

with  $K_d = 60.9$  IU based on the fit shown in Fig. 4D. In B, the data are color-coded according to the relative % difference in chemokine density, given by

$$(10 \mu\text{m}) \left( \frac{1}{C} \frac{dC}{dx} \right) (100\%).$$



**Supplementary Figure S3.** Flow cytometry results showing CXCR4 and CXCR5 expression as a function of time in culture. Anti-CD3 staining serves as a negative control. Fluorescence is expressed as log<sub>10</sub>, and the mean intensities are shown.

## MOVIE CAPTIONS

### Supplementary Movie S1

Movie of a representative B cell randomly migrating on a surface presenting uniform CXCL13 and ICAM-1. The field is under TIRF illumination, with intensity represented as inverted grayscale. 1 second in the movie corresponds to 1 minute real-time. Scale bar: 10  $\mu\text{m}$ .

### Supplementary Movie S2

Overlaid epifluorescence (inverted grayscale) and TIRF (red outline) movie of a representative B cell migrating on a surface presenting uniform CXCL13 and ICAM-1. 1 second in the movie corresponds to 1 minute real-time. Scale bar: 10  $\mu\text{m}$ .

### Supplementary Movie S3

Movie of a representative B cell migrating on a surface-bound CXCL13 gradient with uniform ICAM-1. The source concentration of CXCL13 was 5  $\mu\text{g/ml}$ , and the CXCL13 density increases from top-to-bottom. The field is under TIRF illumination, with intensity represented as inverted grayscale. 1 second in the movie corresponds to 1 minute real-time. Scale bar: 10  $\mu\text{m}$ .