

Figure S1. Description of the experiment setting. The 3D- μ -Slide migration chamber consists of three channels with their associated reservoirs. The channels were filled with a gelling cell-collagen-matrix and incubated for 35 min at 37 °C and 5 % CO₂. After consolidation of the gel, the left reservoir was filled with 60 μ l either of CA in medium or medium (1640 RPMI with 10 % FCS) alone. The right reservoir was filled with medium alone. After filling, cell movement was observed under a microscope for 180 min with 1 picture taken every minute. The displayed graphic is downloaded from the IBIDI web site

(http://ibidi.com/fileadmin/products/labware/channel_slides/S_8032X_Slide_Chemo3D/S_8032X_Slide_Chemo3D_image1.jpg, 22.02.16; 10:35 a.m.).

Table S1. Parameters for Chemotaxis.

Parameter	Direction	Formula
Centre of Mass (CoM)*, μ m	\parallel	$xCoM = \frac{1}{n} \sum_{i=1}^n (x_{i,end})$
	\perp	$yCoM = \frac{1}{n} \sum_{i=1}^n (y_{i,end})$

<i>Forward Migration Index (FMI)†</i>		$xFMI = \frac{1}{n} \sum_{i=1}^n \frac{x_{i,end}}{d_{i,accum}}$
	⊥	$yFMI = \frac{1}{n} \sum_{i=1}^n \frac{y_{i,end}}{d_{i,accum}}$
<i>Directionality‡</i>		$D = \frac{1}{n} \sum_{i=1}^n \frac{d_{i,euclid}}{d_{i,accum}}$
<i>Mean Velocity</i>		$V = \frac{1}{n} \sum_{i=1}^n \frac{d_{i,accum}}{\Delta t}$
<p>Parameters measure effects on the cells in direction either parallel () or perpendicular (⊥) to the CA. $x_{i,end}$ and $y_{i,end}$ are the endpoints of each single cell in x- or y-direction. $d_{i,accum}$ describes the accumulated way of each cell; $d_{i, Euclid}$ is the cell's Euclidean way; $n \leq 20$ cells.</p> <p>*The CoM indicates the average displacement of the cells in relation to either the x-direction or the y-direction.</p> <p>†The FMI indicates the direction and efficiency of any movement in either x- or y-direction. Values range from 0 (not effective movement) to 1/-1 (very effective movement).</p> <p>‡Directionality measures the efficiency of any movement without indicating its direction. Values range from 0 (not effective movement) to 1 (very effective movement).</p>		

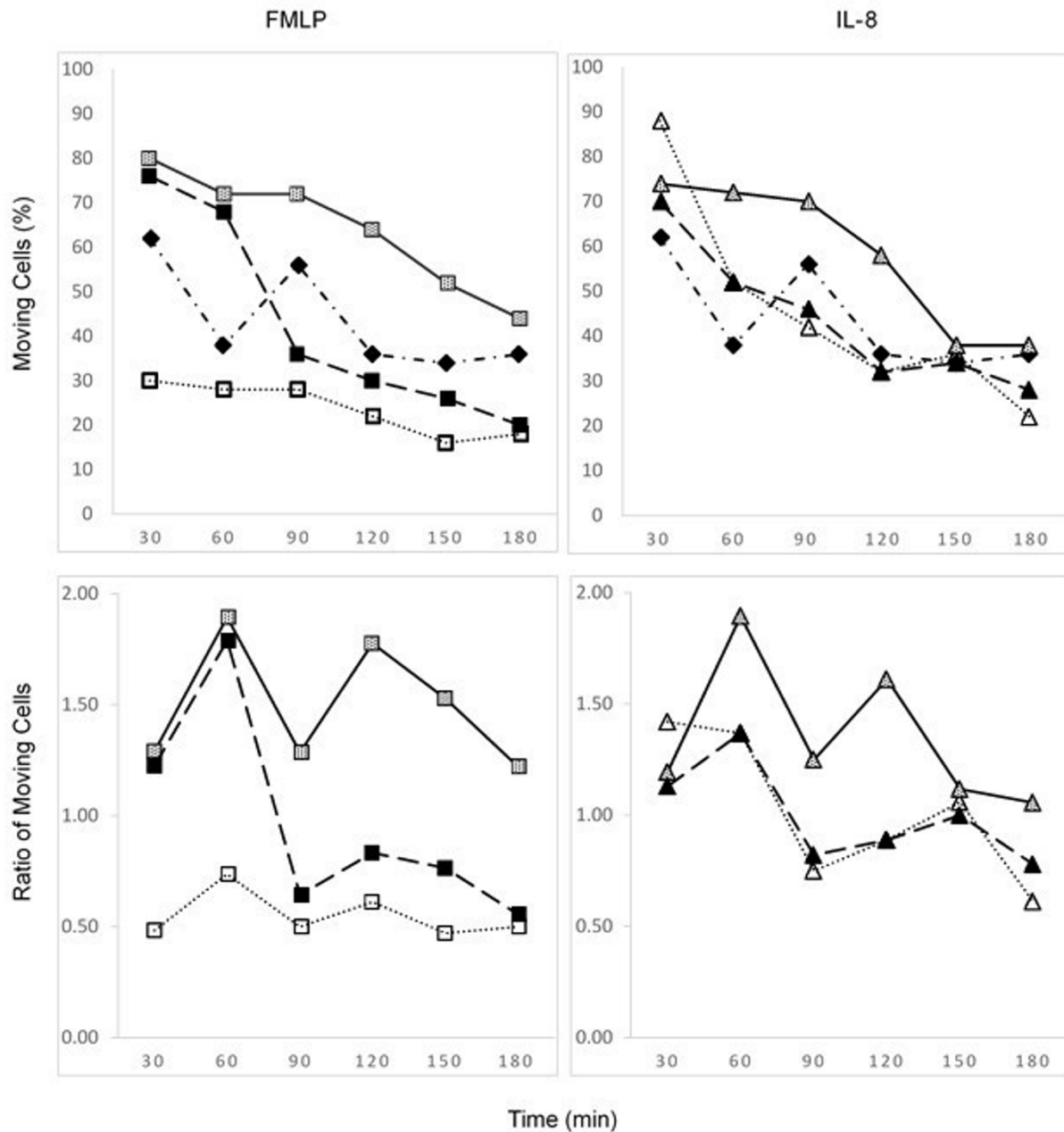


Figure S2. Moving cells and ratio of moving cells. The percentage of moving cells (top) and the ratio of moving cells (bottom) are displayed either for FMLP (left) or for IL-8 (right). CA-free cases (◆) as reference for the ratio of moving cells have a constant ratio of 1 and are not displayed in the lower row graphs. Different concentrations of CAs were used: FMLP in 1 (□), 10 (◻) and 100 (■) nM and IL-8 in 1 (Δ), 10 (◻), 100 (▲) nM concentrations. For each concentration of CA and the CA-free cases 5 experiments were performed. The median absolute deviation was in a range from 2.4 – 18.8 %.

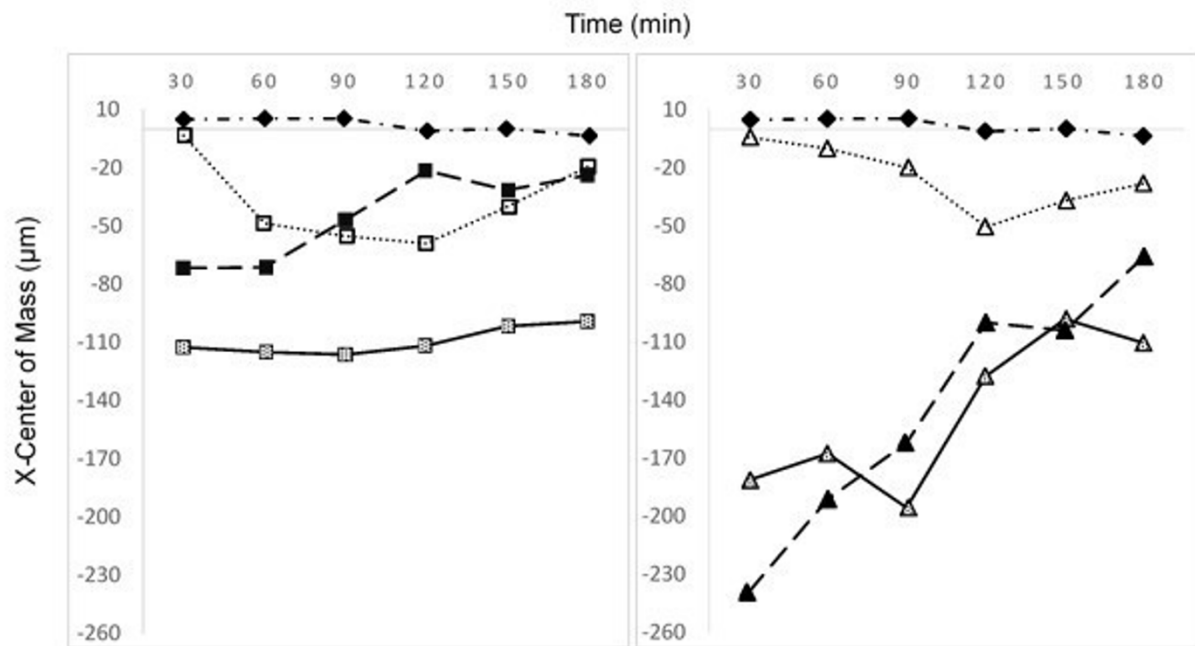


Figure S3. X-Centre of Mass. The time course of the xCoM (μm) during observation is displayed for different concentrations of CAs: 1 (□), 10 (▣) and 100 (■) nM FMLP (left); 1 (Δ), 10 (▴), 100 (▲) nM of IL-8 (right) and CA-free cases (◆). For each concentration of CA and the CA-free cases 5 experiments were performed. The median absolute deviation was in a range from 1.74 – 60.88 μm.

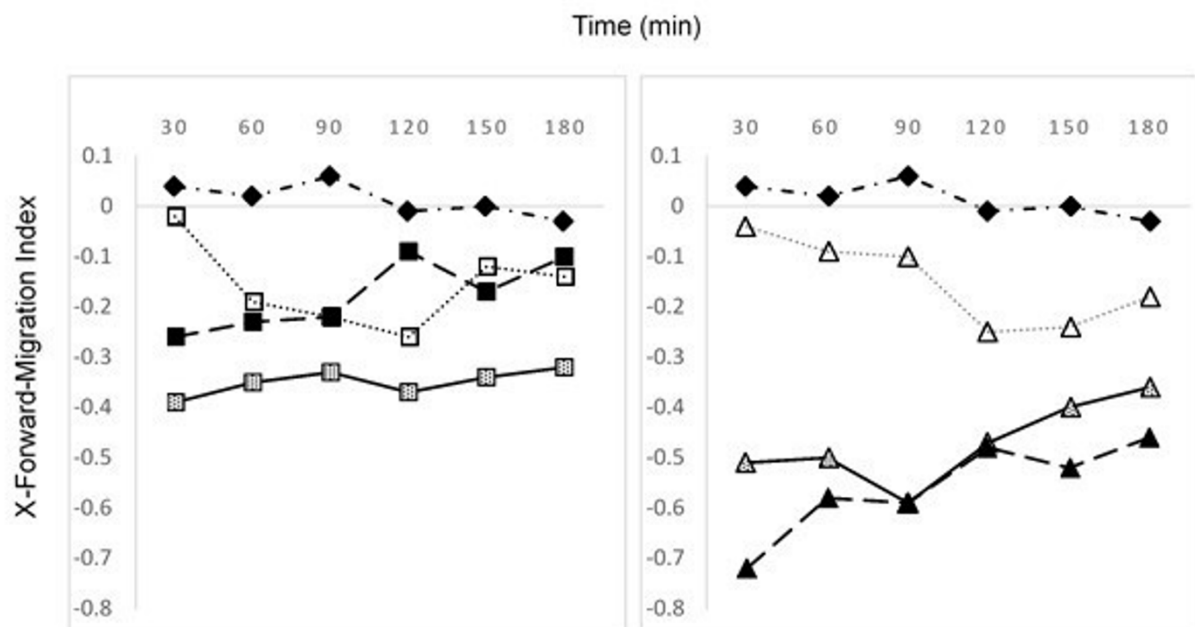


Figure S4. X-Forward-Migration Index. The time course of the xFMI during observation is displayed for different concentrations of CAs: 1 (□), 10 (▣) and 100 (■) nM FMLP (left); 1 (Δ), 10 (▴), 100 (▲) nM of IL-8 (right) and CA-free cases (◆). For each concentration of CA and the CA-free cases 5 experiments were performed. The median absolute deviation was in a range from 0.02 – 0.21.

Table S2. Time course of the Directionality parameter.

Time, min	CA, nM						
	Without	FMLP			IL-8		
		1	10	100	1	10	100
0–30	0.20	0.40	0.45	0.37	0.43	0.57*	0.75*
30–60	0.25	0.38	0.45	0.37	0.48*	0.55*	0.66*
60–90	0.21	0.44*	0.45*	0.35*	0.37*	0.63*	0.61*
90–120	0.17	0.40	0.46	0.28	0.38*	0.50*	0.53*
120–150	0.22	0.36	0.42	0.34	0.41*	0.55*	0.57*
150–180	0.24	0.40*	0.45*	0.34	0.34	0.45*	0.53*
Average	0.22	0.40	0.45	0.34	0.39	0.55	0.58

The median standard deviation was in a range from 0.01 – 0.10.

*Value is significant ($P < .05$) different from corresponding value in cases without CA.