Supplemental Figure Legends

Figure S1: Pixel intensity values were measured for all of the channels depicted in Figure 2A. The intensities were measured along the length of the representative dotted white line as shown in the center of 12b in Figure 2A. Each curve represents the average of two channels that are the same size, and right next to each other. The exception is channel 12a, because the other 12 micron channel was outside the captured image and could not be measured.

Figure S2: Average cell migration speed is independent of varying mean concentrations and channel widths. **A)** Average cell speed (μm/min) of unpolarized cells migrating toward folic acid in 6 μm channels (gray column) and in 12 μm channels (black column). The standard error of the mean was determined for each column (n=20). **B)** Image of polarized cells migrating toward a micropipette filled with 10μM cAMP in the OMD at t= 0 minutes. **C)** Image of polarized cells migrating toward cAMP within the OMD at t= 9 minutes. **D)** Image of polarized cells migrating toward cAMP (white asterisk) for 18 minutes. Cells migrated through the channels at roughly the same speed in the middle, where the mean concentration was highest (asterisk indicates micropipette tip), and at the top and bottom of the device, where the mean concentration would be much lower. Scale bar is 12 μm.

Figure S3: The stability of the gradient was measured using 10 μM FITC, which was pumped at 50 hPa in front of the wide channel region. FITC has a mass similar to cAMP (332.306 grams/mol versus 329.06 grams/mol, respectively). Time lapse images were captured every 5 minutes for over two hours. The gradient was measured by drawing a line and measuring the pixel intensities in the center of the channel for all the time points using software provided by SlideBook.

Supplemental Movie Legends

Movie S1: Polarized cells migrate to cAMP with similar speeds to one another within different-sized channels containing different mean concentrations of cAMP. The time lapse movie depicts polarized cells in different channels (widths ranging from 6-12 μm) migrating to cAMP with similar speeds over an 18 minute time span. The scale bar represents 12 μm.

Movie S2: Racing mixed cells. Unpolarized cells expressing PH-GFP migrated to folic acid, and polarized cells expressing both LimE-RFP and PH-GFP and chemotaxing towards cAMP, were raced against each other in the narrow channels (widths ranging from 6 - 12 μm). A micropipette filled with a 1:1 ration of 10 μM folic acid and cAMP was used. The pumping pressure was 50 hPa. The time interval between each frame is 15 seconds.

Movie S3: Unpolarized cells expressing PH-GFP were placed in a wide channel that is 100 μm wide. A micropipette containing 10 μM folic acid was used. The pumping pressure was 50 hPa.

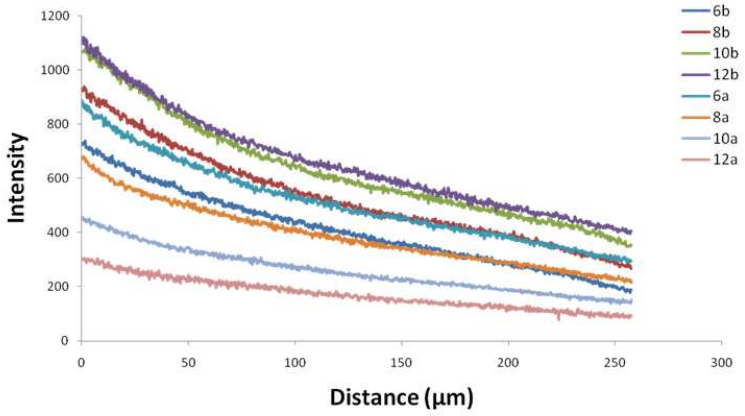
1200
1000
800
600
400
200
0
Intensity
0
50
100
150
200
250
300
Distance (µm)
6b
8b
10b
12b
6a
8a
10a
12a

Figure S1

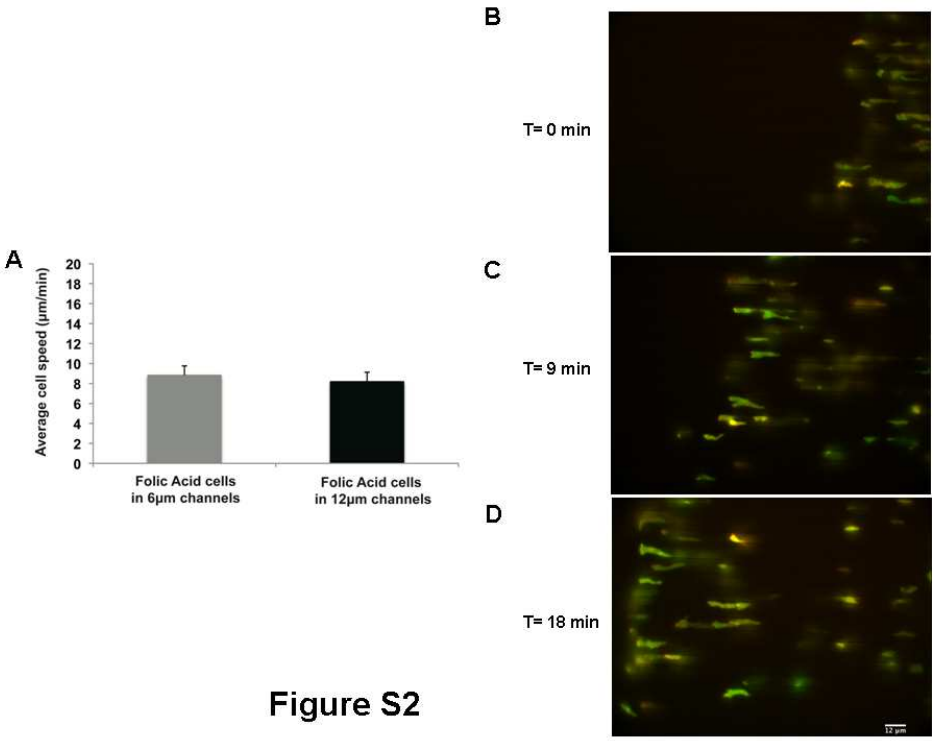
A
Average cell speed (µm/min)
20
18
16
14
12
10
8
6
4
2
0
Folic Acid cells in 6µm channels
Folic Acid cells in 12µm channels
B
T= 0 min
C
T= 9 min
D
T= 18 min

Figure S2

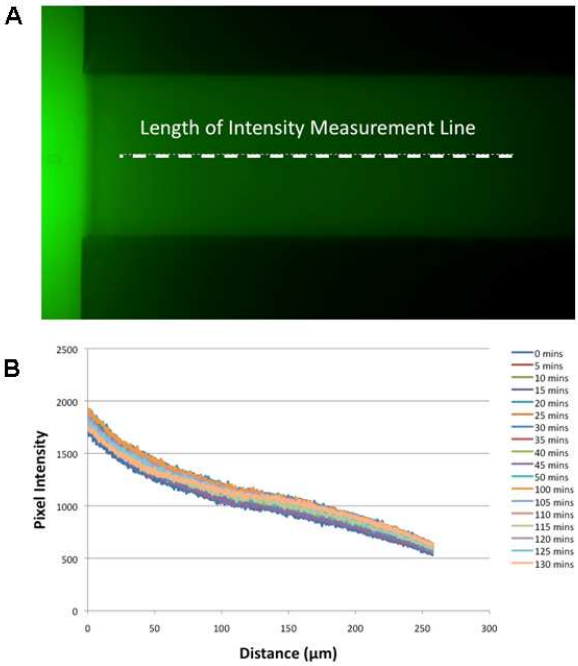
A
Length of Intensity Measurement Line
B
Pixel Intensity
Distance (µm)
0 mins
5 mins
10 mins
15 mins
20 mins
25 mins
30 mins
35 mins
40 mins
45 mins
50 mins
100 mins
105 mins
110 mins
115 mins
120 mins
125 mins
130 mins

Figure S3