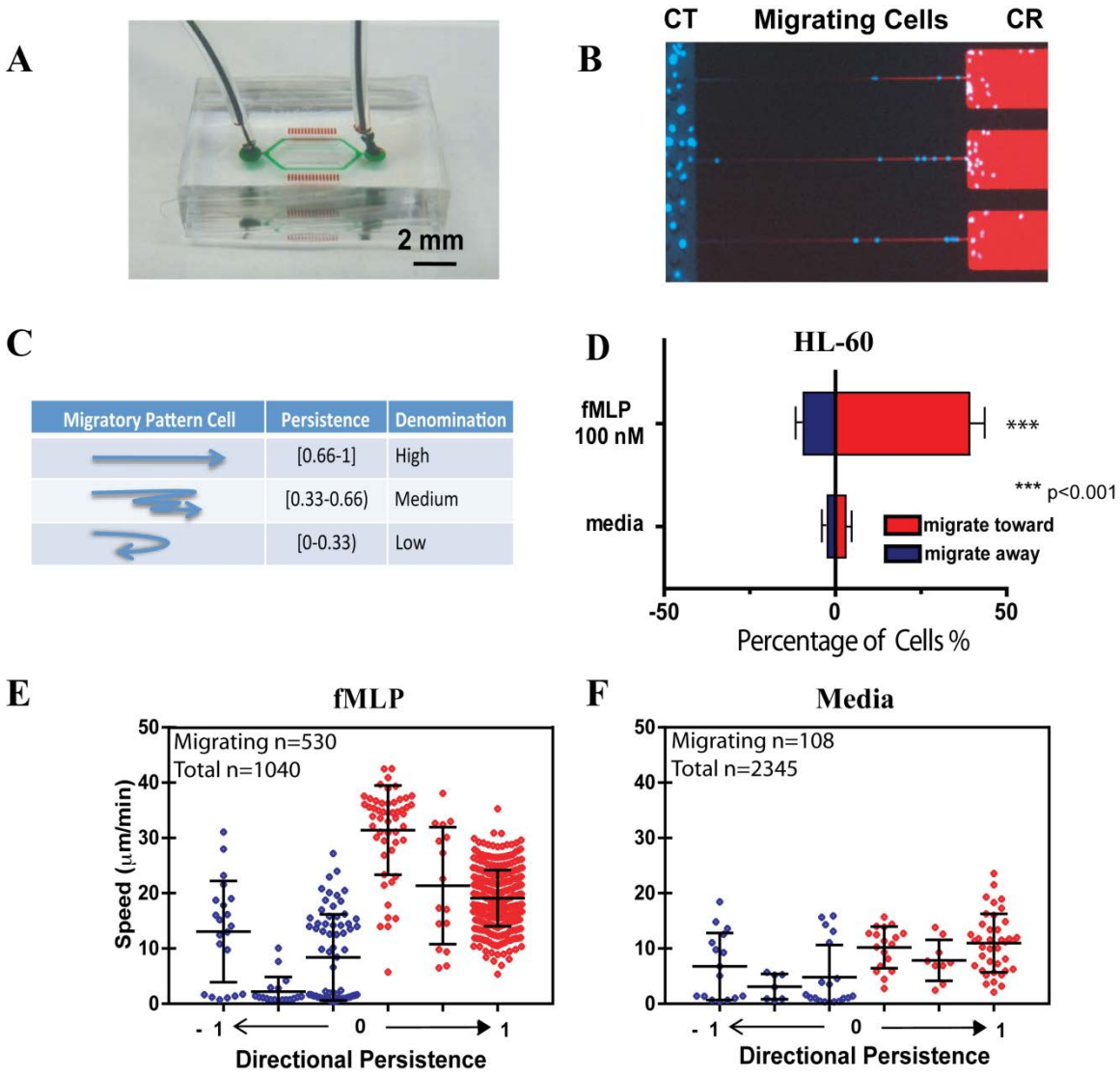
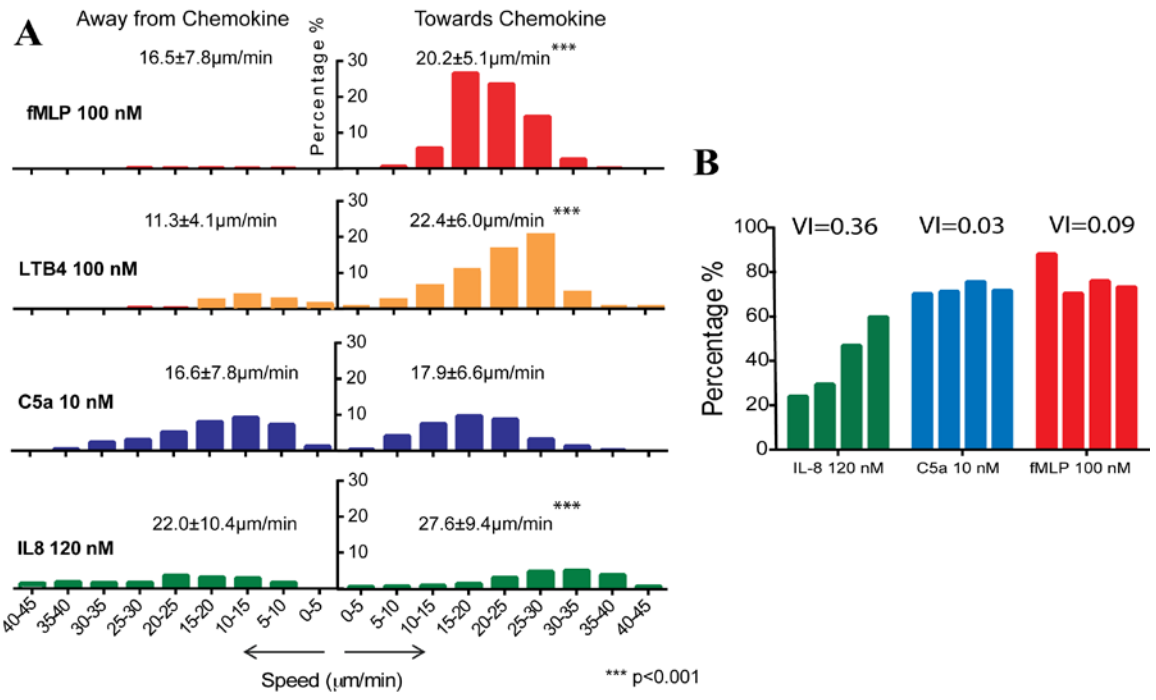
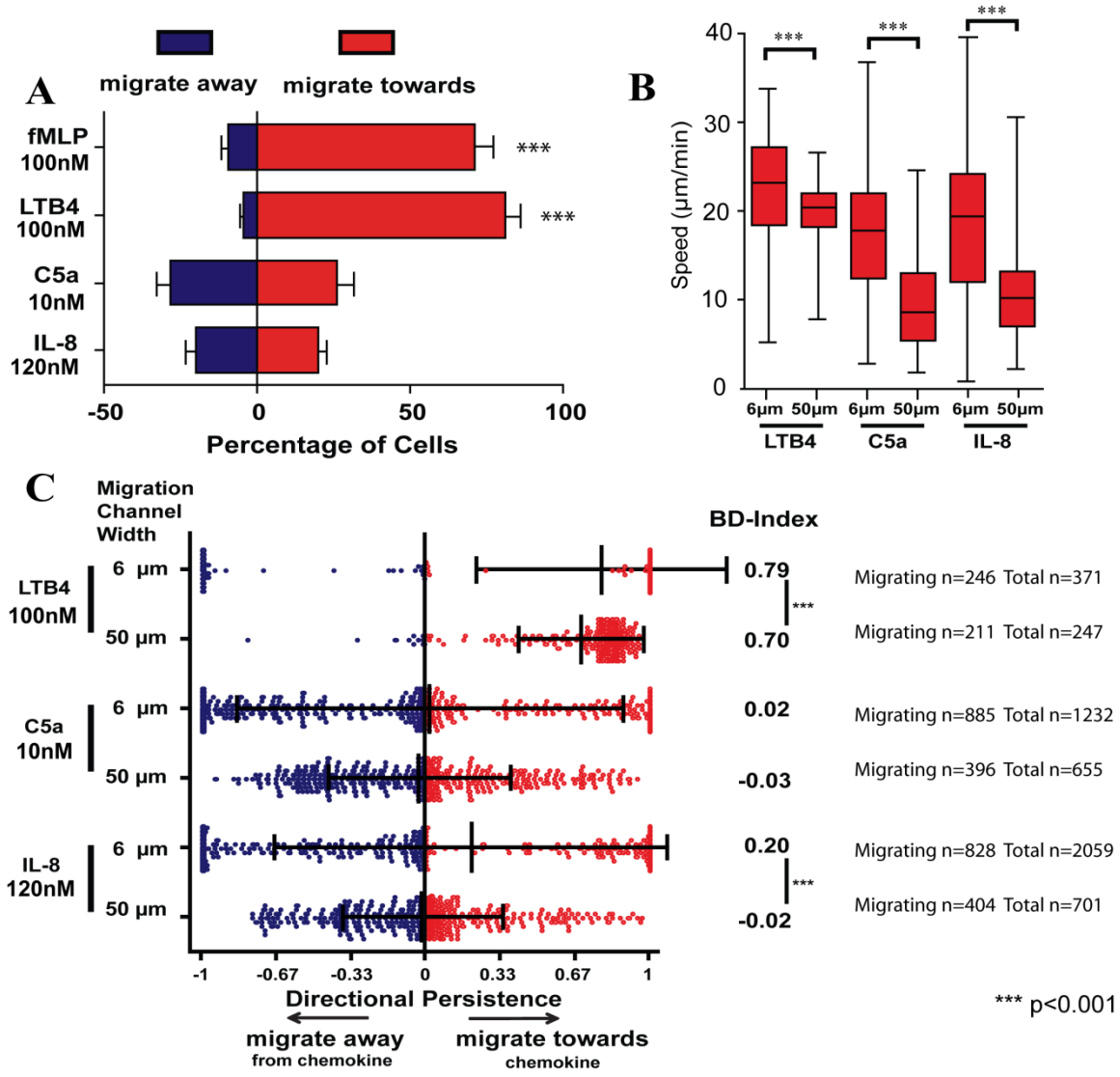


Supplementary Figure 1. Classification of migration patterns in microfluidc channels
A) Photograph of the microfluidic device. Two assays are run in parallel in one device. Each device is consisted of four major elements: 1) a central cell-loading channel with cell traps (CT - green), 2) two arrays of migration channels, perpendicular on both sides of the cell loading chamber, which were 6 × 6 × 300 and 50 × 6 × 300 μm for neutrophils and HL-60 cells, and 10 × 6 × 3000 μm for T-lymphocytes, 3) an array of dead-end chemokine reservoirs (CR - red) connected to individual migration channels on one side of the cell-loading chamber, and 4) a buffer channel (BC - green) connecting the migration channels to the opposite side of the cell loading chamber. B) Fluorescent image showing DAPI stained HL-60 cells migrating towards fMLP. For visualization, fMLP was mixed Texas Red fluorophore. C) Directional Persistence: relative displacement divided by total travel distance; divided in three groups: low [0-0.33), medium [0.33-0.66) and high [0.66-1]. D) HL-60 cells responding to standard chemoattractant fMLP. HL-60 migrate predominantly towards fMLP ($p<0.001$, Student's t test). E) Distribution of Directional Persistence of migrating HL-60 cells (n=530, 3 independent experiments) in response to fMLP ($-1 \leqslant BI \leqslant 1$) out of (n=1040) total cells and F) media without chemokine (control, 3 independent experiments). Bars represent mean +/- SD.

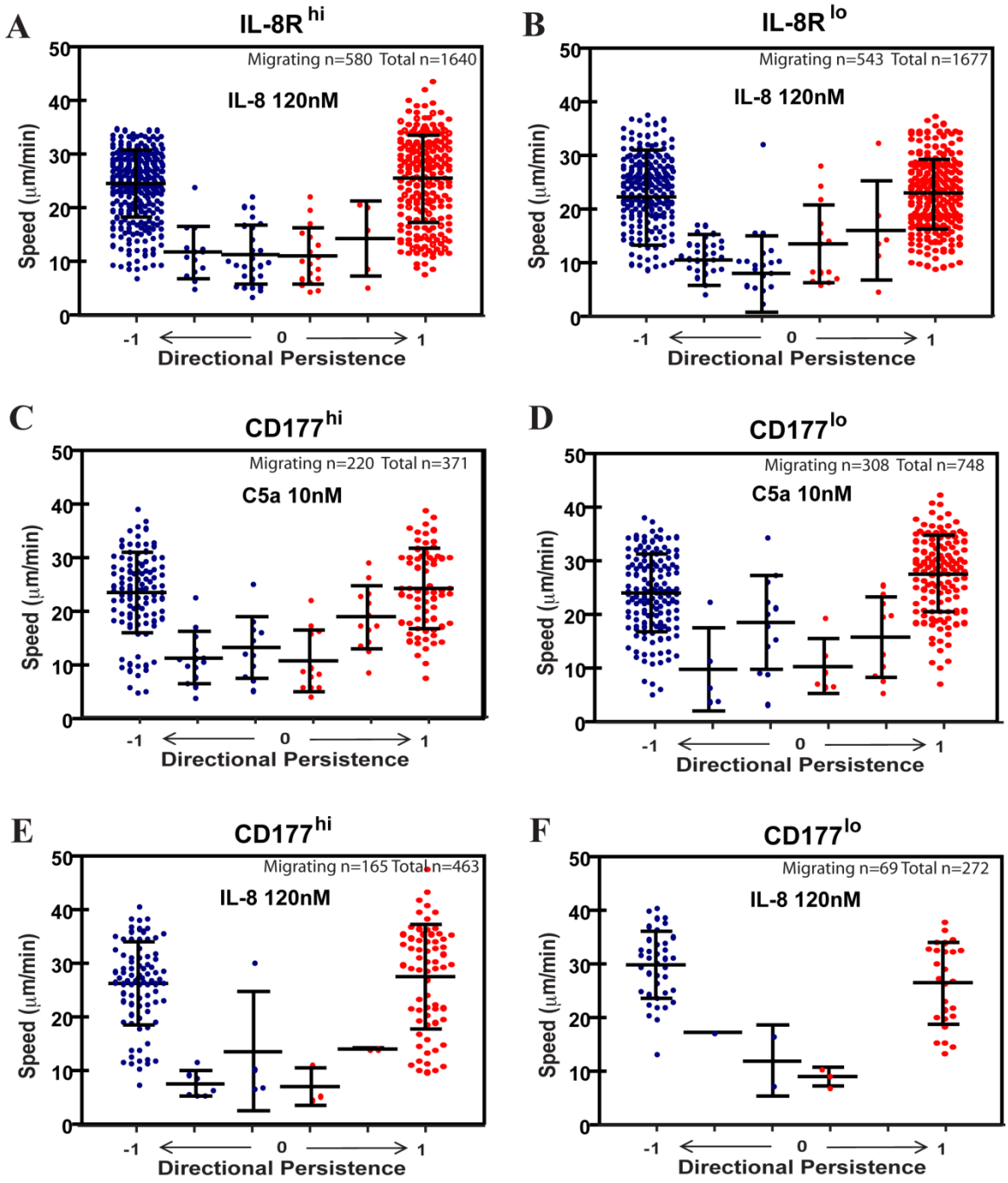


Supplementary Figure 2. Migratory speed of neutrophils and variability in migration
A) Migratory speed of human neutrophils responding to fMLP, LTB4, C5a and IL-8 in both directions. Neutrophils migrate significantly faster towards fMLP (N=4), LTB4 (N=3) and IL-8 (N=4) than away from it ($p<0.001$, Student's t test). Neutrophils responding to C5a migrate at similar speeds in both directions (N=4). B) Percentage of responding neutrophils from 4 different donors to IL-8, C5a, and fMLP. VI=Variety Index, defined as the ratio between sample standard deviation and sample mean.

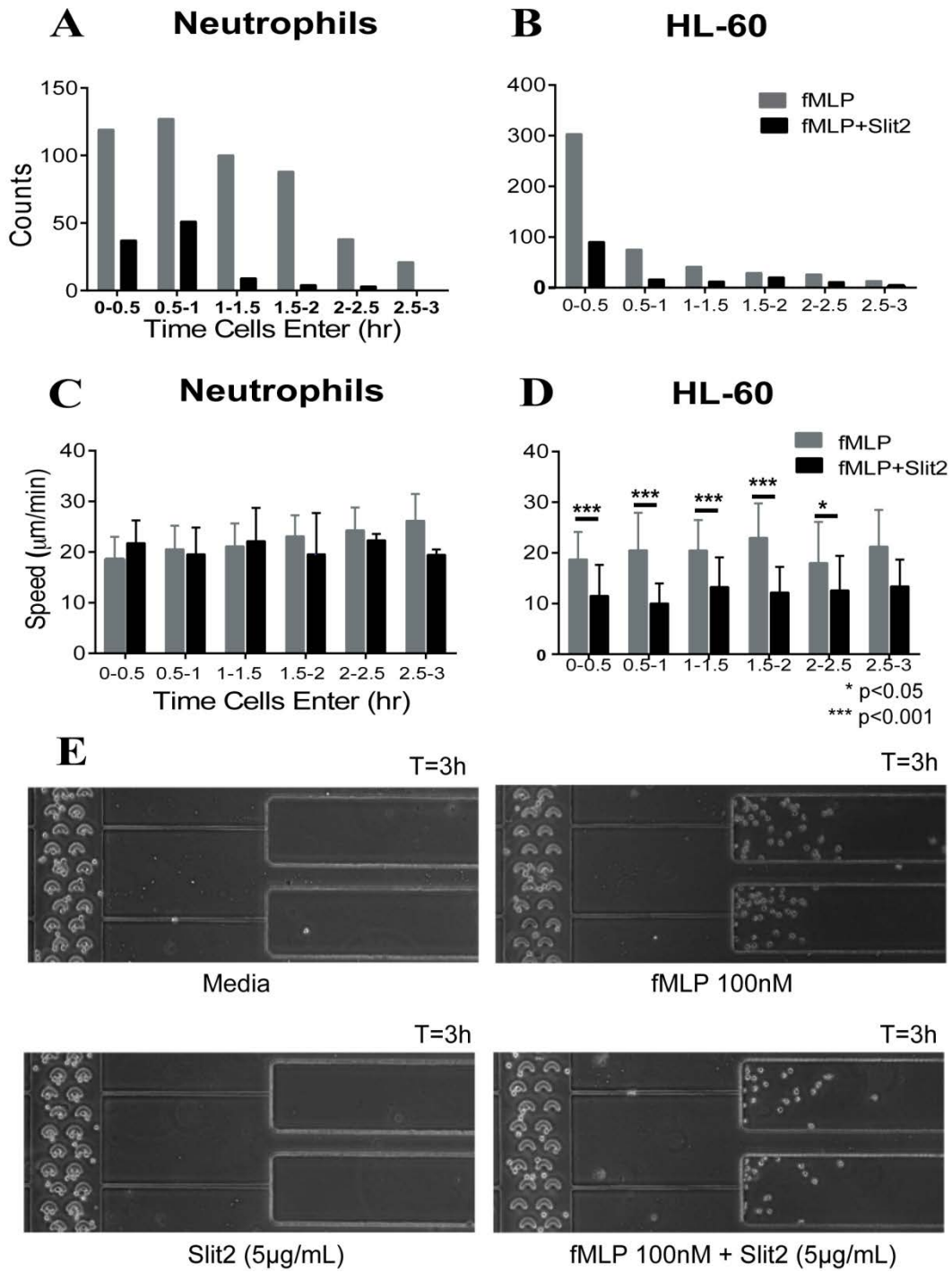


Supplementary Figure 3. Comparison between small and wide migration channels

A) Percentage of human neutrophils loaded in the cell loading chamber that migrate towards or away from fMLP, LTB4, C5a and IL-8 in 50µm wide migration channels. Significant more cells migrate towards fMLP and LTB4 than away from it ($p<0.001$, Student's t test), while cells migrate in equal numbers towards and away from C5a and IL-8. Combined data from N=3 experiments. B) Box-and-Whisker plots showing average migratory speed for neutrophils responding towards LTB4, C5a and IL-8 in small (6µm) and wide (50µm) channels. Cells migrating in wide channels are significantly slower in all conditions ($p<0.001$, Student's t test). C) Scatterplot of migratory persistence of human neutrophils in both directions for 6 and 50µm migration channels, cells migrating in wide channels have lower directional persistence. Neutrophils responding to C5a or IL-8 have similar migratory patterns towards or away from these chemokines. Bars represent mean +/- SD. For LTB4, total 246 migrating cells in 6µm channels analyzed/211 in 50µm channels; 885/396 cells for C5a; 828/404 cells for IL-8; N=3-4.



Supplementary Figure 4. Migration patterns of IL-8R (CXCR1/CD181) and CD177 sorted human neutrophils in response to IL-8 and C5a. Directionality and speed of A) IL-8R^{hi} and B) IL-8R^{lo} expressing neutrophils migrating in response IL-8 (N=4). Directionality and speed of CD177^{hi} expressing neutrophils responding to C) C5a (N=3) and E) IL-8 (N=2). Directionality and speed of CD177^{lo} expressing neutrophils responding to D) C5a (N=3) and F) IL-8 (N=2).



Supplementary Figure 5. Effect of Slit2 on fMLP induced cell migration. Cell counts of A) human neutrophils and B) HL-60 that enter the migration channel (towards fMLP or fMLP+Slit2) as a function of time. Most HL-60 migrate within 30 minutes and Slit2 drastically reduces the number of early responders. Histograms of mean speed of C) human neutrophils cells and D) HL-60 cells that entered the migration channels (towards fMLP or fMLP+Slit2) at time: 0-30, 30-60, 60-90, and 90-120 min. Slit2 inhibits HL-60 migratory speed at every timepoint ($p<0.05$, Student's t test), but does not decrease migratory speed in neutrophils. Combined data of N=4 independent experiments. E) Images of inhibitory effect of Slit2 on fMLP induced migration in neutrophils, seen at t=3 hours.