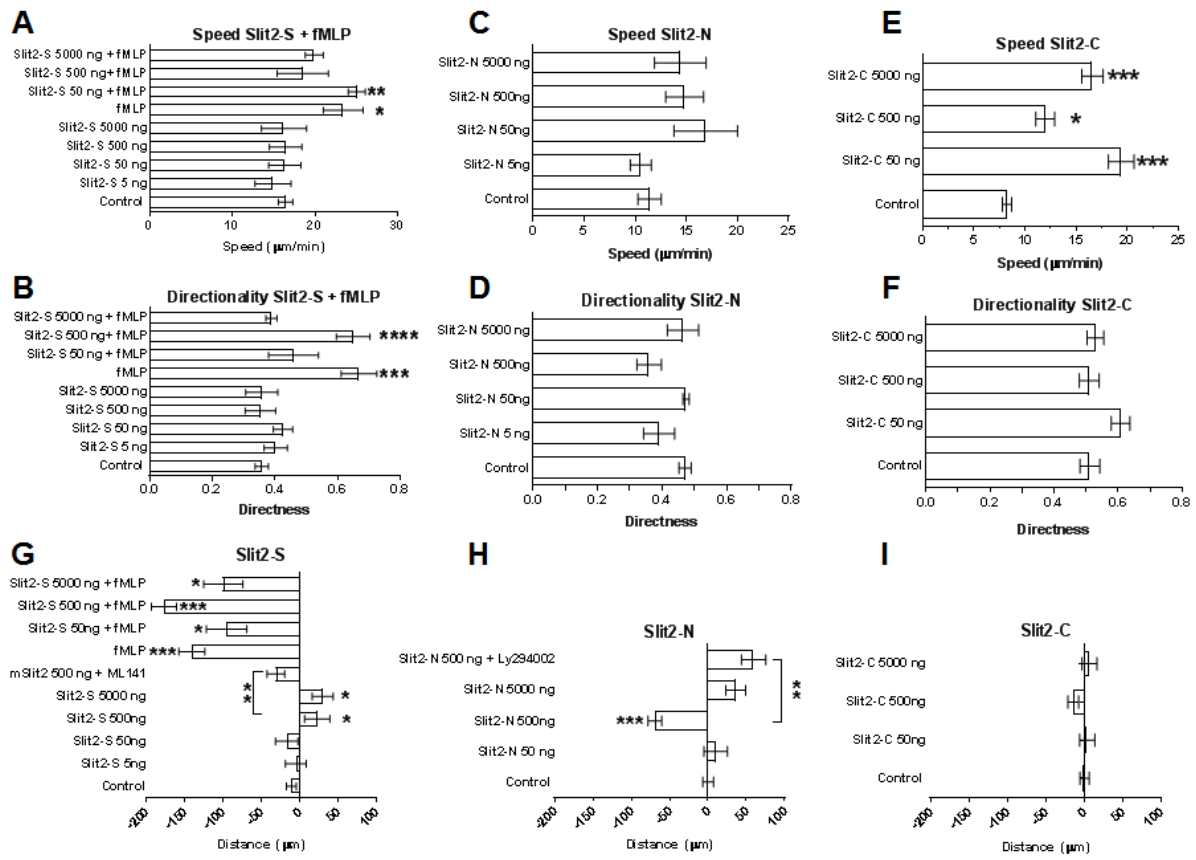
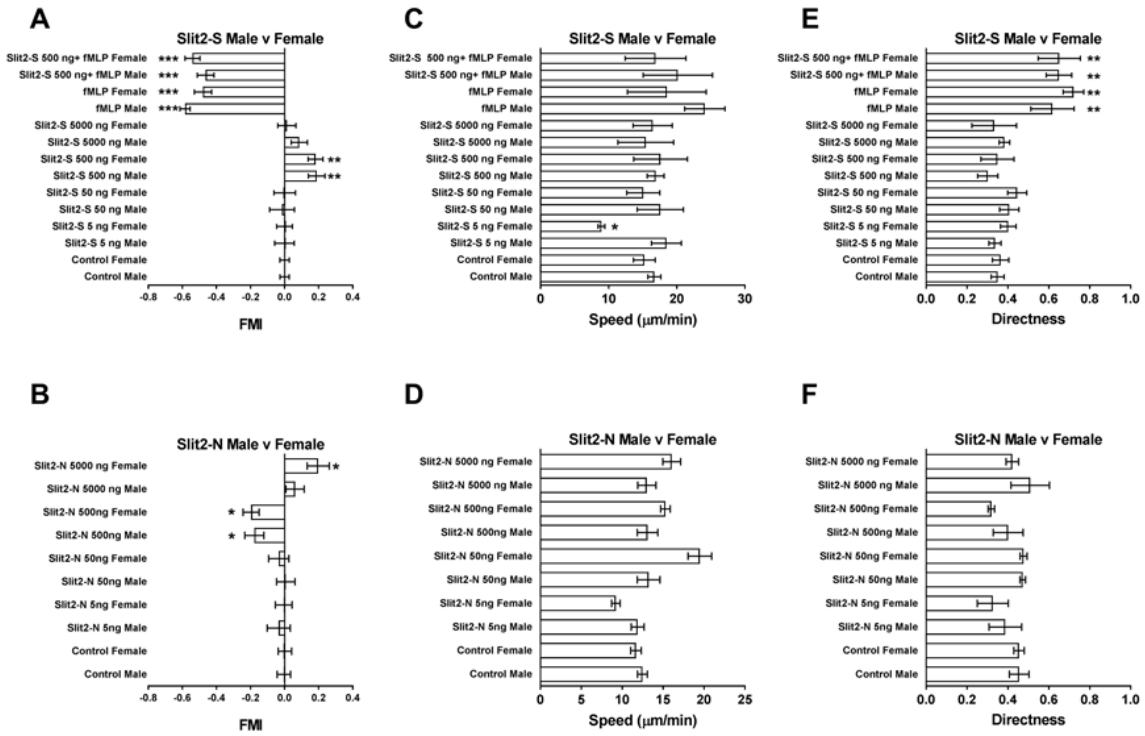
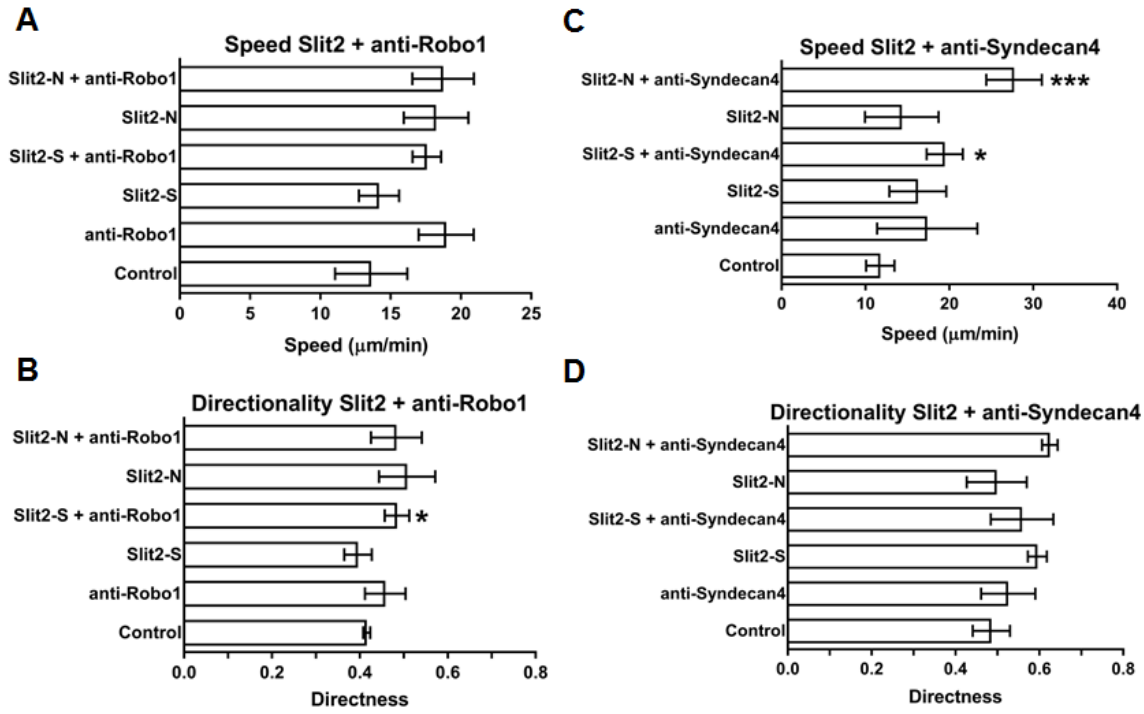


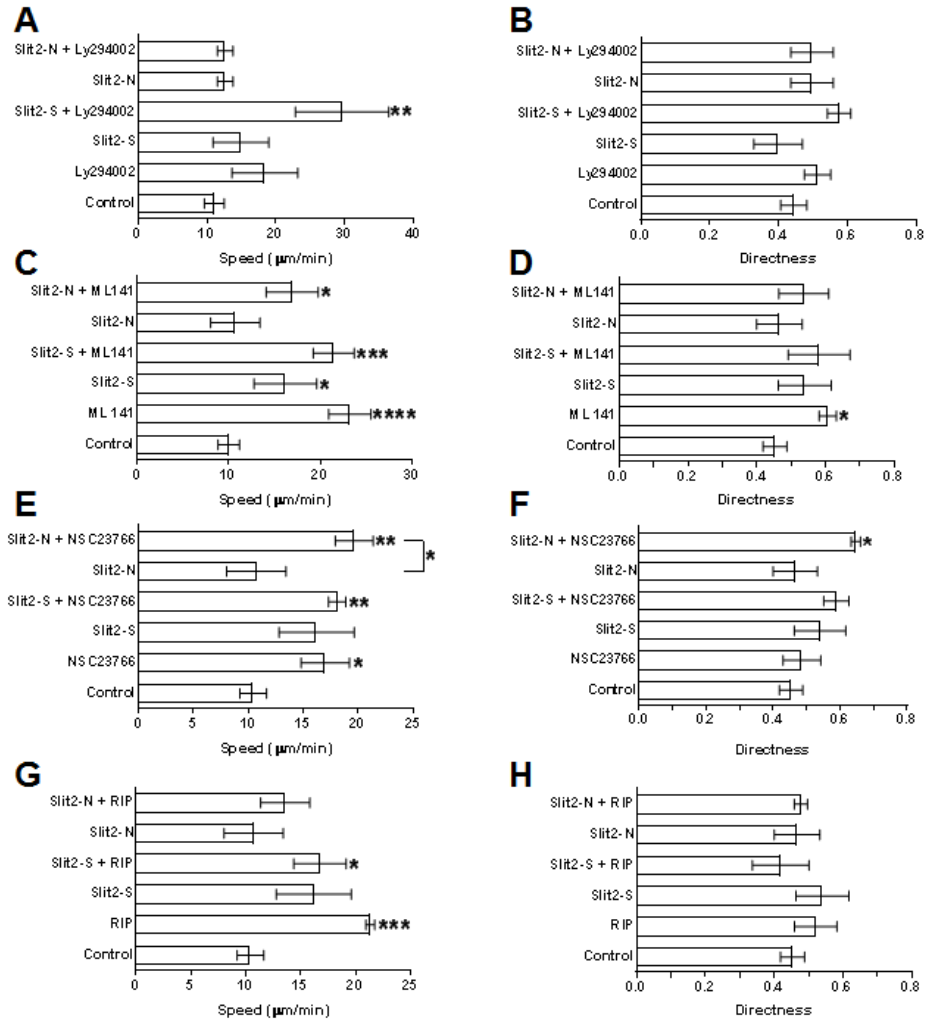
Supplemental Figure 1. Cell speed, directness, and net migration of neutrophils. The data from Figures 1 and 6 was analyzed for the speed of cell movement (µm/minute), directness of cell movement in any direction, and the distance (net migration, µm) cells moved parallel to the gradient over 40 minutes (i.e. if the gradient is along the X axis, the X component of the difference between the starting position and the final position of a cell). Positive values indicate chemorepulsion. At least 10 cells per experiment group for each individual donor were tracked for 40 minutes Values are mean ± SEM from at least 5 different donors. * indicates $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ compared to the no gradient control (1- way ANOVA Dunnett's -test) or for the indicated comparison between two sets (t test).



Supplemental Figure 2. Human neutrophils from both male and female donors show biased movement away from Slit2-S and towards Slit2-N. The data from Figure 1 was also analyzed for differences in neutrophil migration between 3 male and 3 female donors. At least 10 cells per experiment group for each individual donor were tracked for 40 minutes Values are mean ± SEM. * indicates $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$, compared to the no gradient control (1-way ANOVA with Dunnett's -test) .



Supplemental Figure 3. Cell speed and directness of movement for the neutrophils analyzed in Figure 2. The data analyzed for Figure 2 was also analyzed for the speed of cell movement and directness of cell movement in any direction. At least 10 cells per experiment group for each individual donor were tracked for 40 minutes Values are mean ± SEM from at least 5 different donors. * indicates $p < 0.05$ and *** $p < 0.001$ compared to the no gradient control (1-way ANOVA with Dunnett's test).



Supplemental Figure 4. Cell speed and directness of movement for the neutrophils analyzed in Figure 6. The data for Figure 6 was also analyzed for cell speed and directness of cell movement in any direction. Human neutrophils were pre-incubated for 30 minutes with 10 μM inhibitors for **(A-B)** PI3 kinase (LY294002), **(C-D)** Cdc42 (ML141), **(E-F)** Rac (NSC23766), or **(G-H)** Ras (ras inhibitory peptide-RIP) and were then placed in gradients of Slit2, and videomicroscopy was used to record cell movement. At least 10 cells per experiment group for each individual donor were tracked for 40 minutes Values are mean ± SEM from at least 6 different donors. * indicates $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ compared to the no

gradient control (1-way ANOVA with Dunnett's test), or for the indicated comparison between two sets (t test).

Supplemental Videos 1-6

Supplemental video 1. Migration of cells in the absence of a gradient. The horizontal field of view in the video is 0.3 mm.

Supplemental video 2. Migration of cells in a gradient of 0 – 500 ng/ml (0 - 5 nM) Slit2-S. The source of Slit2-S is at the left of the video. The horizontal field of view in the video is 0.3 mm.

Supplemental video 3. Migration of cells in a gradient of 0 - 1 nM fMLP. The source of fMLP is at the left of the video. The horizontal field of view in the video is 0.3 mm.

Supplemental video 4. Migration of cells in a gradient of 0 - 500 ng/ml (0 - 3.6 nM) Slit2-N. The source of Slit2-N is at the left of the video. The horizontal field of view in the video is 0.3 mm.

Supplemental video 5. Migration of cells in a gradient of of 0 - 500 ng/ml Slit2-N in the presence of LY294002. The source of Slit2-N is at the left of the video. The horizontal field of view in the video is 0.3 mm.

Supplemental video 6. Migration of cells in a gradient of 0 - 500 ng/ml Slit2-S in the presence of ML141. The source of Slit2-S is at the left of the video. The horizontal field of view in the video is 0.3 mm.