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Supplementary Materials for

Chemoattractant concentration–dependent tuning of ERK signaling dynamics in migrating neutrophils

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The PDF file includes:

Other Supplementary Material for this manuscript includes the following:
(available at www.sciencemagsignaling.org/cgi/content/full/9/458/ra122/DC1)

Movie S6 (.avi format). SCH772984-treated HL-60 cells migrating in a gradient of 0 to 500 nM *f*MLP.
Movie S7 (.avi format). SB203580-treated HL-60 cells migrating in a gradient of 0 to 500 nM *f*MLP.
Movie S8 (.avi format). BIRB-796–treated HL-60 cells migrating in a gradient of 0 to 500 nM *f*MLP.

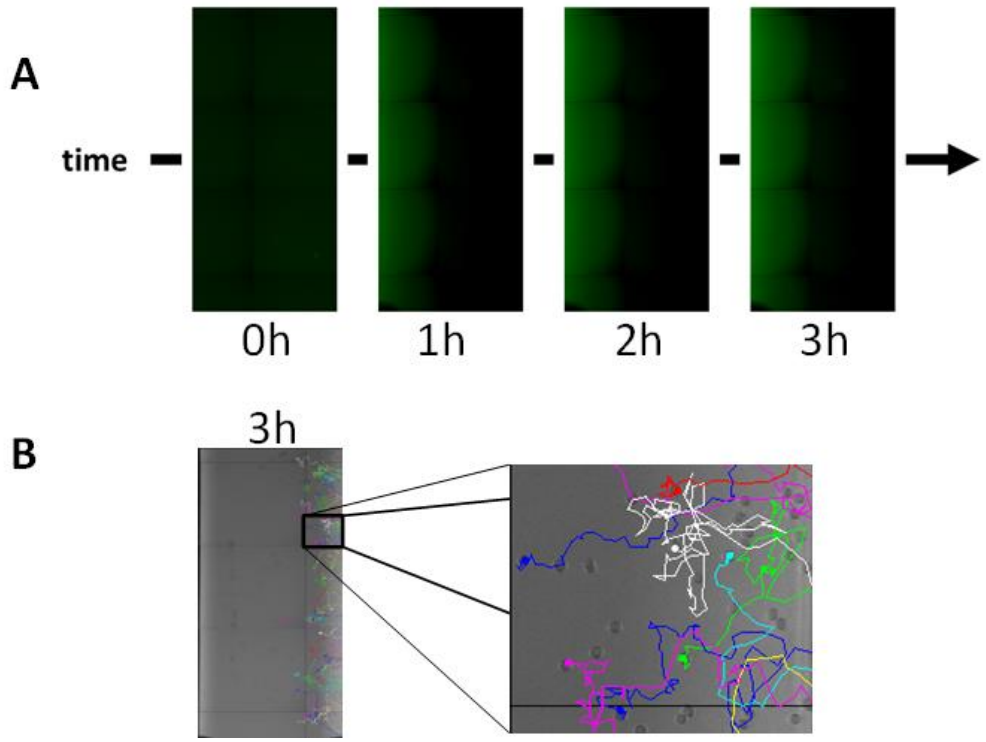

Fig. S1. Migration of cells across a linear gradient of *f*MLP. (**A**) Analysis of the stability and linearity of chemoattractant gradients throughout the 3 hours of live-cell microscopy, as read out by fluorescein-formyl-Nle-Leu-Phe-Nle-Tyr-Lys. (**B**) At approximately 100 nM *f*MLP within an *f*MLP gradient, cell migration remains active, but ceases to be productive. Individual cell traces are represented by colored lines. Data are representative of three experiments.

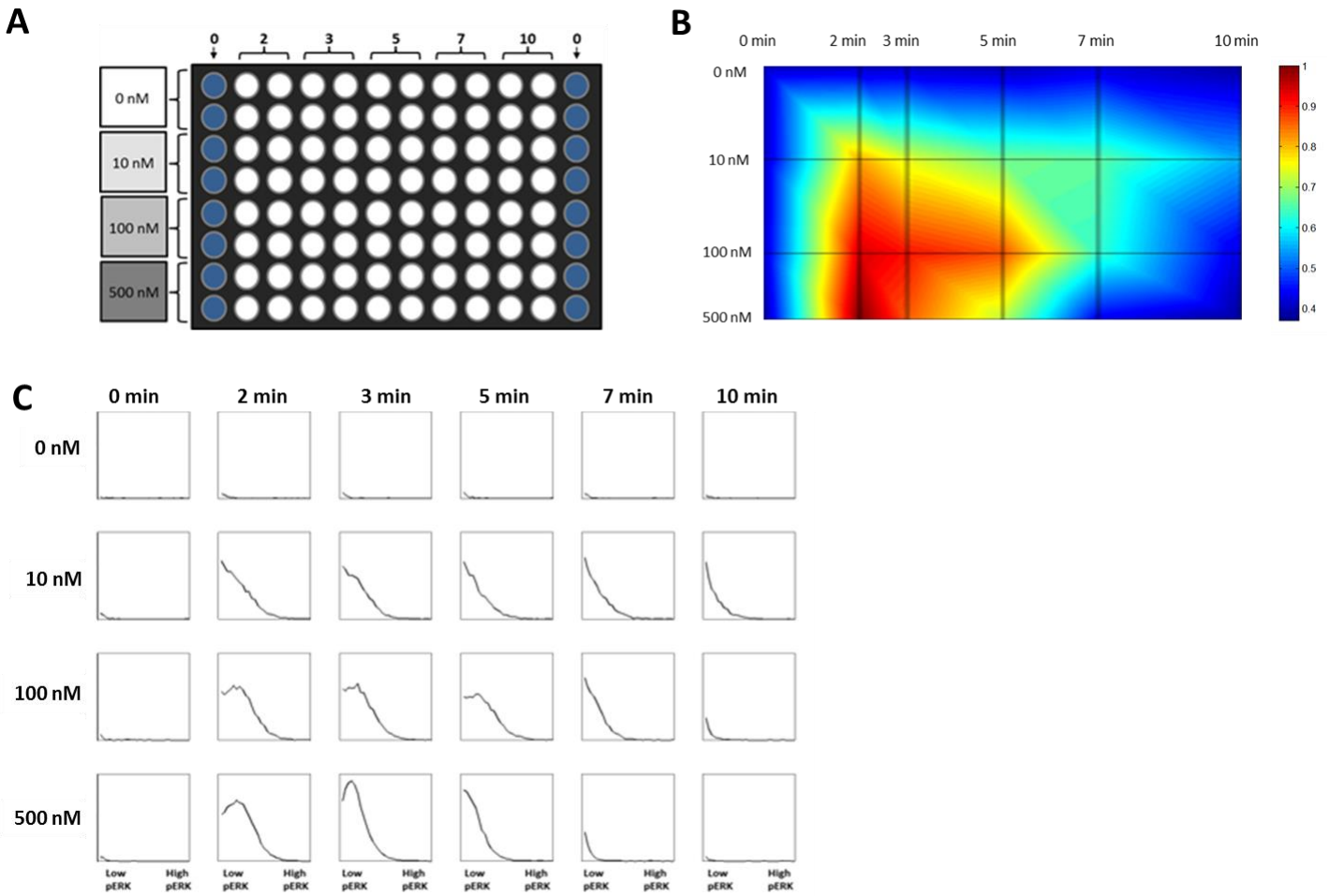

Fig. S2. ERK activation remains increased in cells responding to 10 nM *f*MLP. (**A**) HL-60 cells (168,837 cells) were stimulated with 0, 10, 100, or 500 nM *f*MLP for the indicated times (with at least 1,790 cells per condition). (**B**) Surface plots of pERK fluorescence intensity in HL-60 cells (n = 168,837) across the indicated *f*MLP concentrations and times. (**C**) Histograms show pERK fluorescence intensity across the indicated *f*MLP concentrations and times. Data are representative of four replicate experiments.

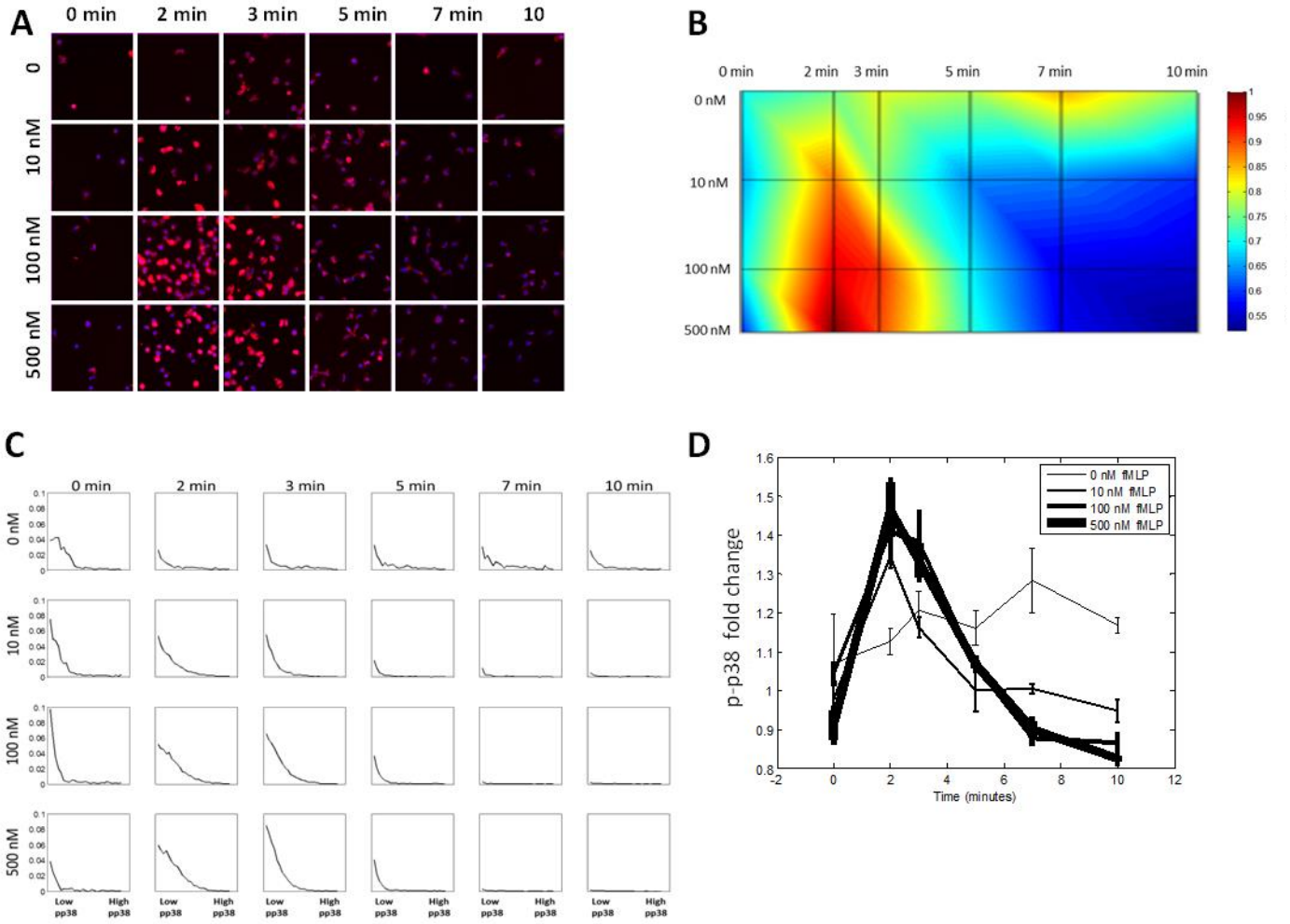

Fig. S3. The abundance of pp38 is reduced below basal amounts within 10 min of *f*MLP stimulation. (**A**) HL-60 cells (168,837 cells) were treated for the indicated times with the indicated concentrations of *f*MLP (with at least 1,790 cells per condition) before being fixed, incubated with fluorescent antibodies against pp38, and then imaged. Each frame is representative of 36 frames per well, and four replicate wells per condition were analyzed. (**B**) Surface plot of the cell-averaged pp38 fluorescence intensity in HL-60 cells (n = 168,837) across the indicated *f*MLP concentrations and times. (**C**) Histograms of pp38 fluorescence intensity in HL-60 cells across the indicated *f*MLP concentrations and times. (**D**) Analysis of pp38 fluorescence intensity in 168,837 cells exposed to the indicated concentrations of *f*MLP over time. Data are means ± SEM of four replicate experiments (with at least 1,790 cells per condition).

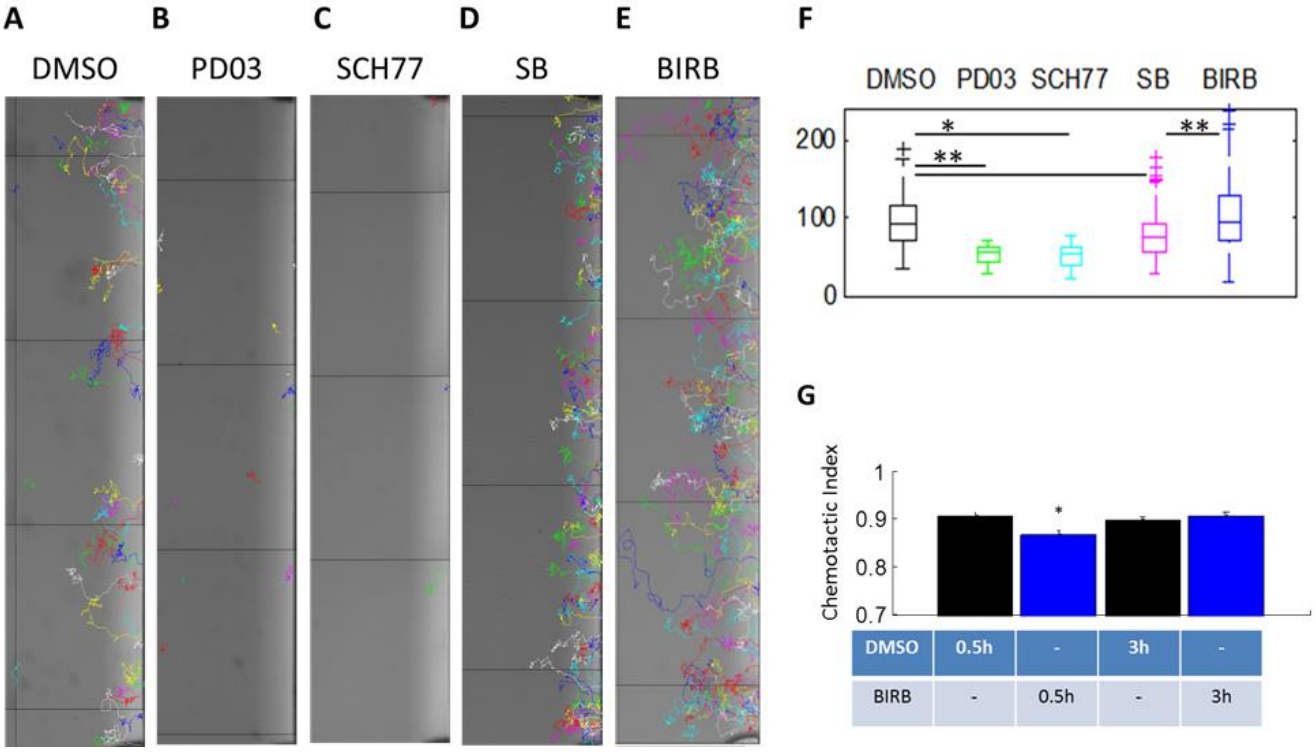

Fig. S4. ERK promotes, whereas p38 inhibits, the migration of neutrophils toward *f*MLP. (**A** to **E**) Representative migration tracks (multicolored) of differentiated HL-60 cells treated with (A) DMSO, (B) 100 nM PD0325901, (C) 100 nM SCH772984, (D) 10 μM SB203580, or (E) 10 nM BIRB-796 and then allowed to migrate across a gradient of 0 to 500 nM *f*MLP. (**F**) Quantification of migration tracks, with three replicate experiments combined within each boxplot. **P* < 0.05 and ***P* < 0.01 as compared to controls by two-sample Kolmogorov-Smirnov test. (**G**) Human neutrophils were treated with DMSO or the p38 inhibitor BIRB-796 and then allowed to migrate for 3 hours in a gradient of 0 to 500 nM *f*MLP. Chemotactic index was determined at 30 min and at 3 hours. Data are means ± SEM of three replicate experiments. **P* < 0.05 compared to DMSO by two-sample Kolmogorov-Smirnov test.

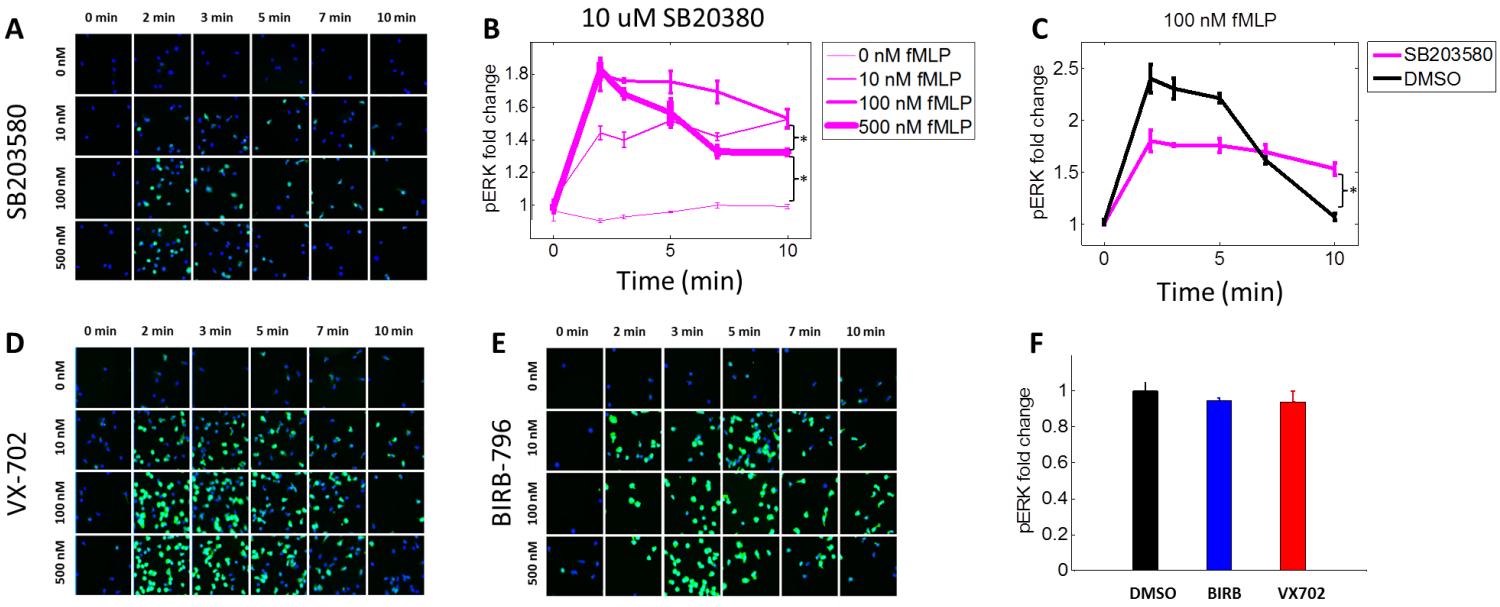

Fig. S5. Inhibition of p38 results in increased pERK abundance in neutrophils at late times after exposure to 10 or 100 nM *f*MLP. (**A**) HL-60 cells (143,275 cells) treated with 10 μM SB203580 were then exposed to the indicated concentrations of *f*MLP for the indicated times before being fixed, incubated with fluorescent antibodies against pERK, and then imaged. Each frame is representative of 36 frames per well, and four replicate wells per condition were analyzed (with at least 3,337 cells per condition). (**B**) Quantification of the fluorescence intensity of pERK in SB203580-treated 143,275 cells exposed to the indicated concentrations of *f*MLP over time. Data are means ± SEM of four replicate experiments (with at least 3,337 cells per condition). **P* < 0.05 as compared to DMSO-treated cells by two-sample Kolmogorov-Smirnov test. (**C**) HL-60 cells (94,960 cells) treated with DMSO or SB203580 were then incubated with 100 nM *f*MLP and the fluorescence intensity of pERK was measured over time. Data are means ± SEM of four replicate experiments (with at least 2,569 cells per condition). **P* < 0.05 as compared to DMSO-treated cells by two-sample Kolmogorov-Smirnov test. (**D** and **E**) HL-60 cells (263,712 cells) treated with 10 nM BIRB-796 (D) or 100 nM VX-702 (E) were then exposed to the indicated concentrations of *f*MLP for the indicated times before being fixed, incubated with fluorescent antibodies against pERK, and then imaged. Each frame is representative of 36 frames per well, and four replicate wells per condition were analyzed (with at least 502 cells per condition). (**F**) Analysis of basal pERK abundance in HL-60 cells (5,494 cells) treated with DMSO, BIRB-796, or VX-702. Data are means ± SEM of four replicate experiments (with at least 1,335 cells per condition).

Movie S1. DMSO-treated human neutrophils migrating in a gradient of 0 to 500 nM *f*MLP. Movie is representative of three replicate experiments.

Movie S2. PD0325901-treated human neutrophils migrating in a gradient of 0 to 500 nM *f*MLP. Movie is representative of three replicate experiments.

Movie S3. BIRB-796–treated human neutrophils migrating in a gradient of 0 to 500 nM *f*MLP. Movie is representative of three replicate experiments.

Movie S4. DMSO-treated control HL-60 cells migrating in a gradient of 0 to 500 nM *f*MLP. Movie is representative of three replicate experiments.

Movie S5. PD0325901-treated HL-60 cells migrating in a gradient of 0 to 500 nM *f*MLP. Movie is representative of three replicate experiments.

Movie S6. SCH772984-treated HL-60 cells migrating in a gradient of 0 to 500 nM *f*MLP. Movie is representative of three replicate experiments.

Movie S7. SB203580-treated HL-60 cells migrating in a gradient of 0 to 500 nM *f*MLP. Movie is representative of three replicate experiments.

Movie S8. BIRB-796–treated HL-60 cells migrating in a gradient of 0 to 500 nM *f*MLP. Movie is representative of three replicate experiments.