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Supplemental Information

Human Neutrophils Will Crawl Upstream on ICAM-1 If Mac-1 Is Blocked

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Human neutrophils will crawl upstream on ICAM-1 if Mac-1 is blocked

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Contents:

SUPPLEMENTAL METHODS

Flow cytometric profiling of inflammatory cells: Immunofluorescent staining and flow cytometric analysis of cells were performed as described previously (1-3). Cells (0.5×10^6–1×10^6) were washed in PBS twice before labeling. Samples were incubated singly with fluorescently labeled antibodies HI111 (anti-CD11a/LFA-1), ICRF44 (anti–CD11b/Mac-1), 9F10 (anti-CD49d), TS1/18 (anti-CD18/β_2-integrin), TS2/16 (anti-CD29/β_1-integrin) for 15 minutes before analysis. All antibodies were from Biolegend. Flow cytometric analysis was performed using a Calibur flow cytometer (Becton Dickinson Immunocytometry Systems) and the FACS Diva software package and histograms were generated using the FlowJo software.

SUPPLEMENTAL REFERENCES

1. Buffone, A., N. Mondal, R. Gupta, K. P. McHugh, J. T. Y. Lau, and S. Neelamegham. 2013. Silencing α1,3-Fucosyltransferases in Human Leukocytes Reveals a Role for FUT9 Enzyme during E-selectin-mediated Cell Adhesion. Journal of Biological Chemistry 288(3):1620-1633.
2. Buffone, A., N. R. Anderson, and D. A. Hammer. 2018. Migration against the direction of flow is LFA-1-dependent in human hematopoietic stem and progenitor cells. Journal of Cell Science 131(1). 10.1242/jcs.205575.
3. Dominguez, G. A., N. R. Anderson, and D. A. Hammer. 2015. The direction of migration of T-lymphocytes under flow depends upon which adhesion receptors are engaged(). Integrative biology : quantitative biosciences from nano to macro 7(3):345-355.

Supplemental Figure 1

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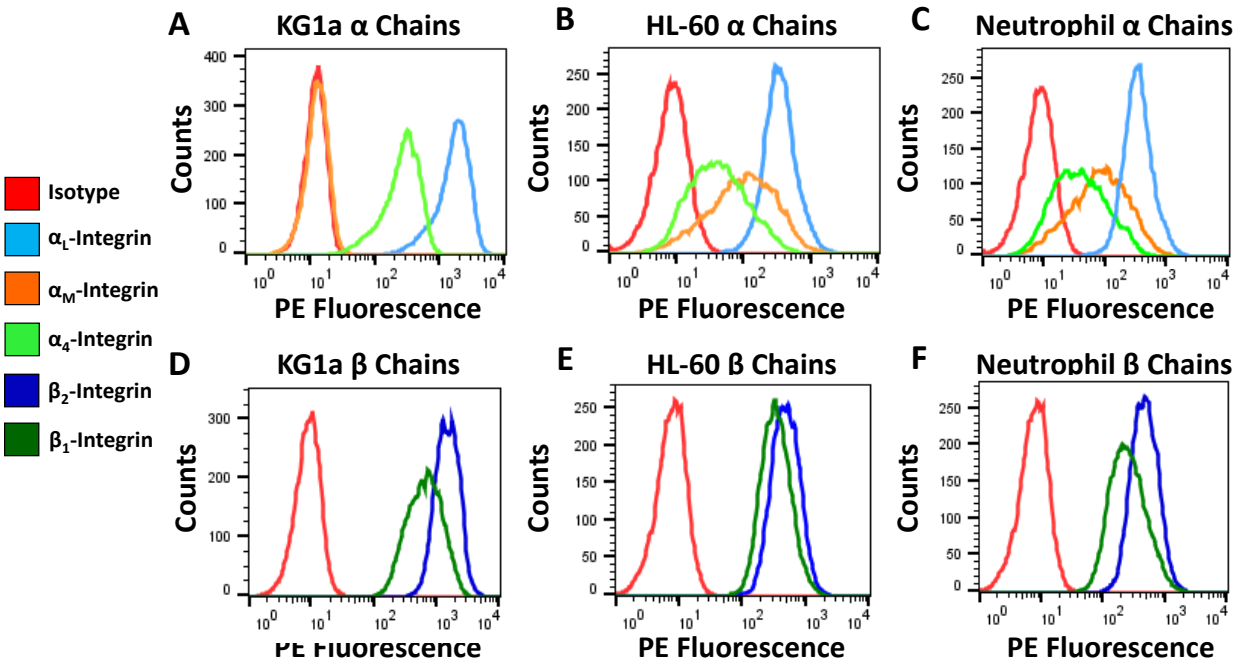

Supplemental Figure 1: ***Expression of integrin subunits on HL-60 cells and KG1a cells***: 10^5 stem cell-like KG1a cells, neutrophil-like HL-60 cells or primary neutrophils from whole blood were assayed for expression of integrin α chains (A-C) and integrin β chains (D-F). Expression of integrins α_L (light blue), α_M (orange), and α_4 (light green) on (A) KG1a cells, (B) HL-60 cells, and (C) primary neutrophils. Expression of integrins β_1 (dark green) and β_2 (dark blue) on (D) KG1a cells, (E) HL-60 cells, and (F) primary neutrophils. Isotype controls are depicted in red. In sum, these data show KG1a cells, HL-60 cells, and primary neutrophils express the cell-borne integrins LFA-1 and VLA-4 but only HL-60 and primary neutrophils cells express Mac-1.

Supplemental Figure 2

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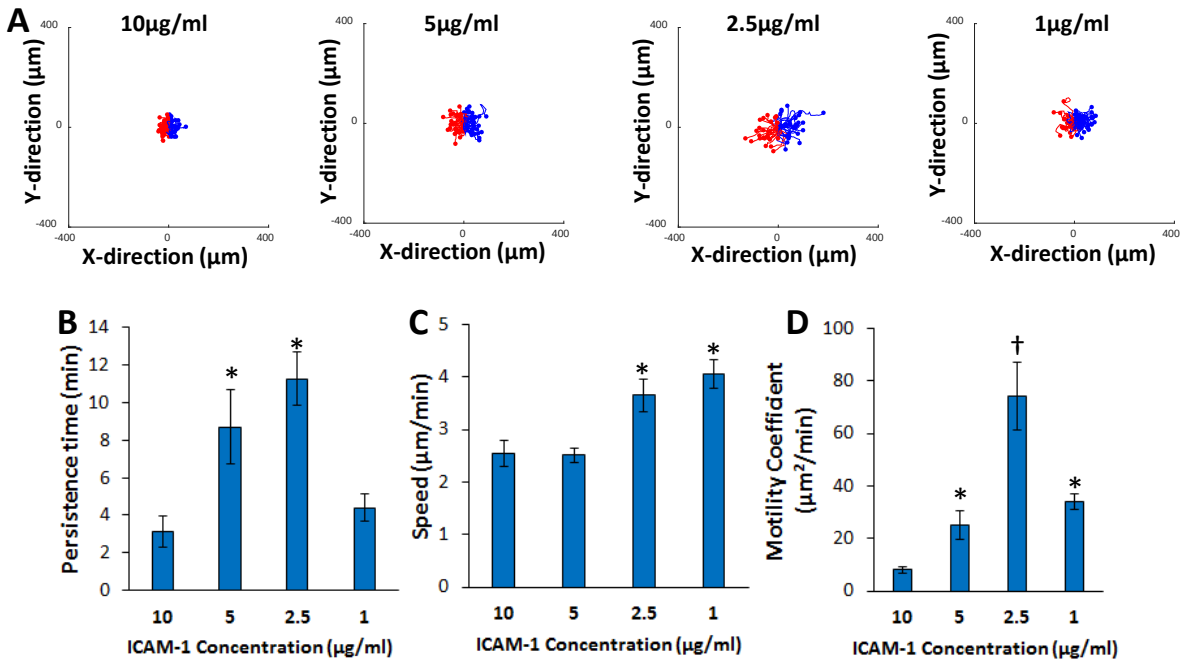

Supplemental Figure 2: ***Motility on ICAM Surfaces under Static Conditions:*** (A) Cell traces of HL-60 cells under static conditions on ICAM-1 at concentrations of 10, 5, 2.5, or 1ug/ml. The traces depicted are the cumulative tracks of two independent experiments and have units of µm. Blue traces indicate cells that traveled to the left while red traces indicate cells that traveled to the right. HL-60 cells migrating at any of the four concentrations had a migration index (MI) of -0.05<MI<0.05 indicating random motility. The persistence time (min) (D), speed (µm/min) (E), and random motility coefficient (μm^2/min) (F) were calculated for each concentration. In all, the area explored by HL-60 in the traces is greatest at 2.5ug/mL. N=4 independent experiments of at least 75 cells analyzed per experiment for each CAM. *p < 0.05 with respect to 10ug/mL. †p < 0.05 with respect to all other concentrations.

Supplemental Figure 3

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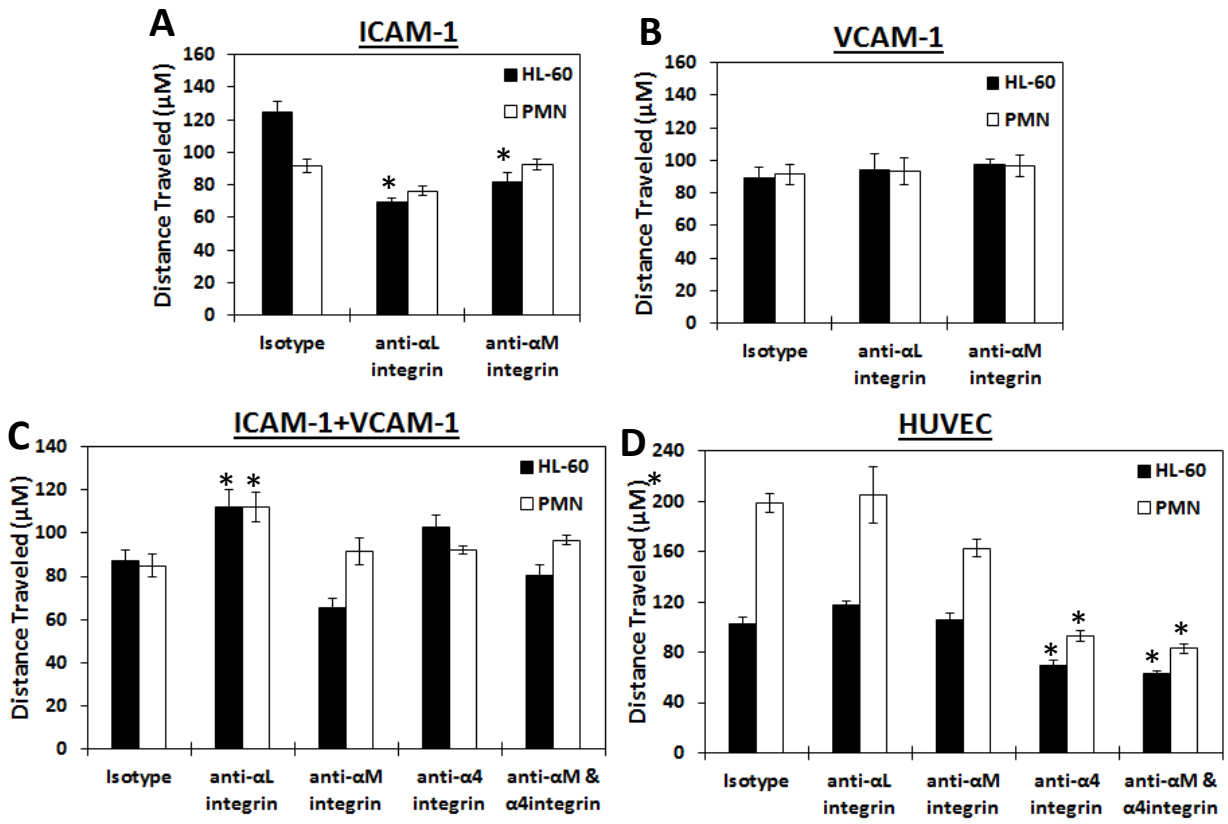

Supplemental Figure 3: ***Average Distance traveled for HL-60 and neutrophils on ICAM, VCAM-1, Mixed Surfaces, and HUVECs:*** Average distance traveled for HL-60 and primary neutrophils under all blocking conditions on (A) ICAM-1, (B) VCAM-1, (C) ICAM-1+VCAM-1 at a concentration of 2.5ug/ml, or (D) a monolayer of IL-1β stimulated HUVECs. N=4 independent experiments of at least 60 cells analyzed per experiment for each CAM. *$p < 0.05$ with respect to isotype control.

Supplemental Figure 4

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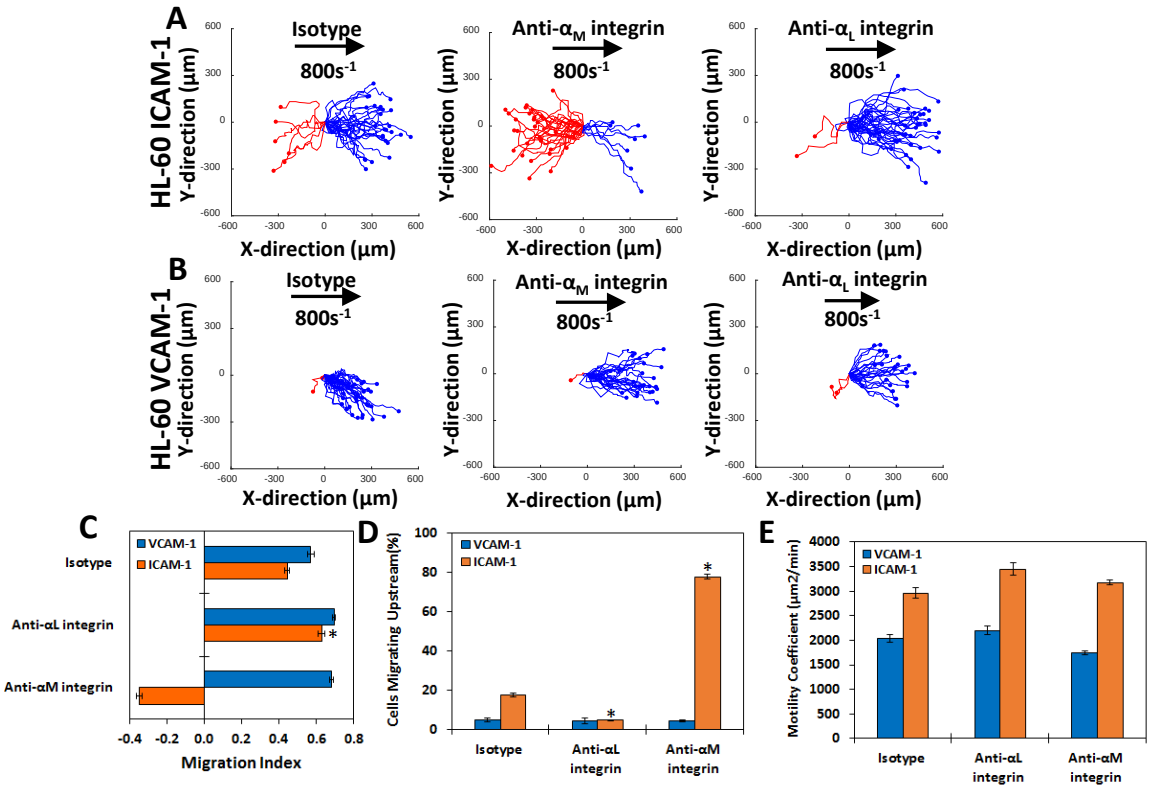

Supplemental Figure 4*: Stimulated HL-60 cells exhibit similar directional migration profiles but are more motile than unstimulated HL-60 on ICAM-1 and VCAM-1*: Cell traces of HL-60 cells stimulated with 10ng/ml fmlp on (A) ICAM-1 and (B) VCAM-1 under isotype (First column), with anti-α_M integrin blocking (second column), or with anti-α_L integrin blocking (third column) at $800s^{-1}$ shear rate at a concentration of 2.5 μg/mL. The traces depicted are the cumulative tracks of two independent experiments and have units of um. Blue traces indicate downstream migration (with flow) while red traces indicate upstream migration (against flow). The direction of flow is from left to right in these traces and the traces have units of μm. (C) The direction of HL-60 cell migration under shear flow as expressed by the migration index (MI) under isotype, anti-α_M integrin, or anti-α_L integrin blocking at 800 s^{-1} shear rate on ICAM-1 or VCAM-1. A negative MI indicates migration against the flow (upstream) while a positive MI indicates migration with the flow (downstream). (D) Percentage of migrating cells traveling upstream under isotype, anti-α_L integrin blocking, or anti-α_M integrin blocking at 800 s^{-1} shear rate on ICAM-1 or VCAM-1. (E) Motility coefficient of stimulated HL-60 on ICAM-1 or VCAM-1 surfaces under isotype, anti-α_L integrin blocking, or anti-α_M integrin blocking at 800 s^{-1} shear rate. HL-60 cells stimulated with fmlp exhibit similar migrational direction preferences as unstimulated HL-60. The stimulated HL-60 are significantly more motile than the unstimulated HL-60. N=3 independent experiments of at least 30 cells analyzed per experiment for each CAM. *$p < 0.05$ with respect to isotype condition

Supplemental Figure 5

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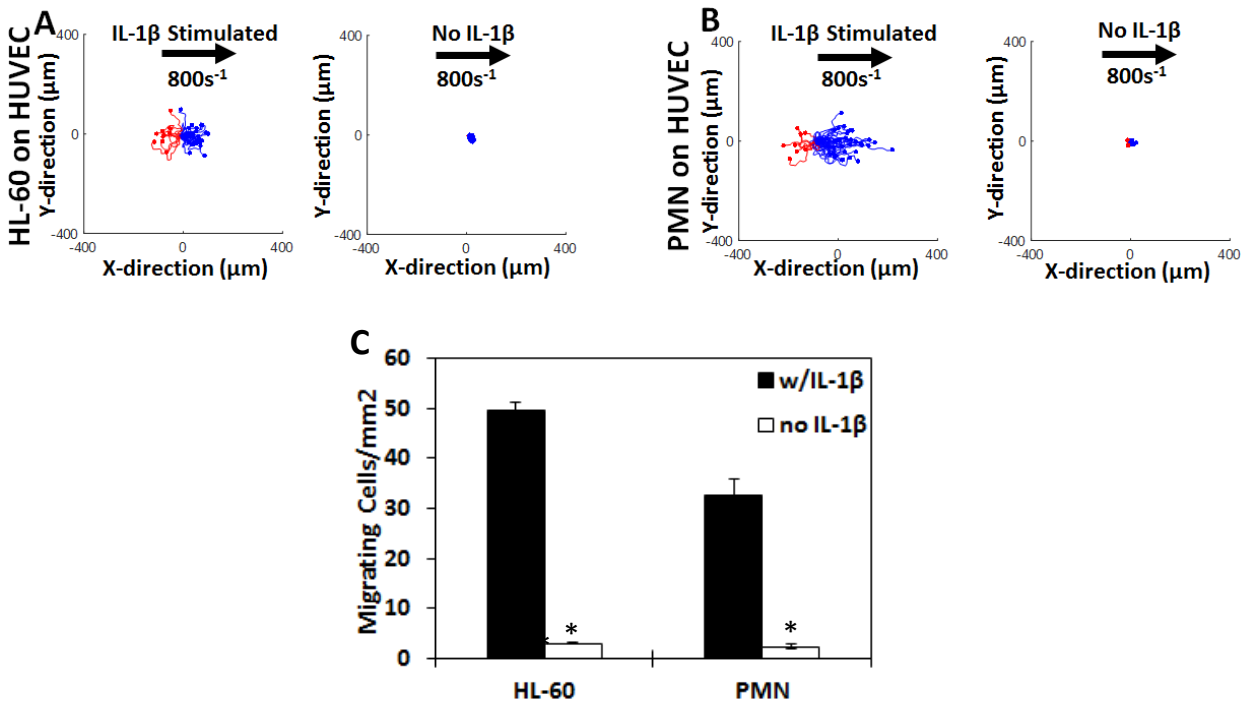

Supplemental Figure 5: ***Migration of HL-60 and PMN on HUVECs with and without IL-1β stimulation:*** Cell traces of (A) HL-60 cells and (B) primary neutrophils under 100s-1 shear on HUVEC monolayers with or without IL-1β stimulation The traces depicted are the cumulative tracks of three independent experiments and have units of μm. Blue traces indicate cells that traveled with the direction of flow while red traces indicate cells that traveled against the direction of flow. (C) The number of migrating cells per mm^2 for HL-60 and PMN with or without IL-1β. In all, HL-60 and PMN attach 90% less to unstimulated HUVECs and those that do attach, do not migrate. N=3 independent experiments of at least 50 cells analyzed per experiment. *$p < 0.05$ with respect to HUVECs with IL-1β Stimulation.

Supplemental Movie 1: HL-60 cells migrating downstream on an ICAM-1 surface at a shear rate of $800s^{-1}$ with an isotype blocking antibody.

Supplemental Movie 2: HL-60 cells migrating upstream on an ICAM-1 surface at a shear rate of $800s^{-1}$ with a Mac-1 blocking antibody.

Supplemental Movie 3: Primary neutrophils migrating downstream on an ICAM-1 surface at a shear rate of $800s^{-1}$ with an isotype blocking antibody.

Supplemental Movie 4: Primary neutrophils migrating upstream on an ICAM-1 surface at a shear rate of $800s^{-1}$ with a Mac-1 blocking antibody.

Supplemental Movie 5: HL-60 cells migrating upstream on a stimulated HUVEC monolayer at a shear rate of $100s^{-1}$ with a Mac-1 blocking antibody.