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Adhesive Dynamics simulations quantitatively predict effects of kindlin-3 deficiency on T-cell homing

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Abstract

Leukocyte adhesion is important for the proper functioning of the immune system. While leukocyte homing is mediated by adhesion receptors, the activation of these receptors is modulated by intracellular signaling molecules. In Leukocyte Adhesion Deficiency Type 3, the loss of the kindlin-3 prevents the activation of Leukocyte Function-associated Antigen-1 (LFA-1), which leads to a defect in adhesion, causing recurrent infections and bleeding disorders. Here, we use Integrated Signaling Adhesive Dynamics, a computer model of leukocyte rolling and adhesion combined with a simulated intracellular signaling cascade, to predict the response of T cells to depletion of kindlin-3. Our model predicts that cell adhesion is hypersensitive to the amount of kindlin-3 in the cell, while the rolling velocity is independent of kindlin-3 concentration. In addition, our simulation predicted that the time to stop, an important metric of adhesion, would increase with decreasing kindlin-3 expression. These predictions were confirmed experimentally in experiments using Jurkat cells with reduced expression of kindlin-3. These results suggest that Adhesive Dynamics is a versatile tool for quantifying adhesion in the immune response and predicting the effects of engineering cellular components.

Introduction

To perform necessary homeostasis and effector functions, immune and vascular cells must be able to control when and where immune cells adhere to the vasculature. The multi-step process of cell adhesion in the vasculature is normally referred to as the leukocyte adhesion cascade¹, which is modulated by the inside-out activation of adhesion receptors, most notably integrins². In the leukocyte adhesion cascade, immune cells progress from tethering, to rolling, to firm adhesion on the endothelium¹. The steps of initial capture and rolling are controlled by binding between selectins and selectin receptors expressed on the surface of activated endothelium and the leukocyte, respectively³. While the cell is rolling, it is exposed to chemokines which bind to chemokine receptors on the leukocyte and trigger

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intracellular signaling cascades which activate integrins on the leukocyte surface⁴. These activated integrins then bind to adhesion ligands, such as Intercellular Adhesion Molecule-1 and Vascular Cell Adhesion Molecule-1 (ICAM-1 and VCAM-1), which lead to the firm binding of the leukocyte under shear flow⁵. The cascade is then completed when the leukocyte transmigrates across the endothelium to perform appropriate immune functions in adjacent tissues¹.

The details of the intracellular signaling cascade that controls integrin activation is being elucidated. Although the specific molecules involved differ, the basic structure of the cascade is shared similar among all leukocytes⁴. Initially, chemokines bind to chemokine receptors, which are typically G-protein coupled receptors (GPCRs), causing exchange of the G α -bound GDP for GTP and leading to the activation of the G proteins. This activation then leads to the creation of second messengers, such as, but not limited to, IP₃ and diacylglycerol (DAG), causing the activation of GTPases, such as Rap, which activate integrins on the cell surface^{6,7}.

The hematopoietic cell-specific integrin activator kindlin-3 is critical to the activation of the integrin LFA-1 ($\alpha_L\beta_2$)⁸. Kindlin-3 works with talin to convert integrins from a resting, inactive conformation to an open, extended, highly active conformation in all leukocytes. Talin binds to LFA-1 first, partially activating LFA-1 so that it binds ICAM-1 with an intermediate affinity. Kindlin-3 then binds to the partially activated integrin, converting it to the high affinity state^{9–11}. The role of kindlin-3 in the activation of other integrins besides LFA-1 is disputed, with some models suggesting a requirement of kindlin-3 for activation of integrins containing β_1 , β_2 , or β_3 subunits¹² and other reports suggesting no role for kindlin-3 in activation of physiologically important integrins such as VLA-4 ($\alpha_4\beta_1$)¹³. A loss of function mutation of kindlin-3 causes an immune disorder known as Leukocyte Adhesion Deficiency III (LAD III). LAD III is a rare genetic disorder, characterized by neutrophilia, recurrent infections, and an associated bleeding disorder¹⁴. The two other commonly accepted LADs, LAD I and II, also impact the ability of leukocytes to adhere to the endothelium, albeit through different mechanisms. LAD I is caused by the loss of the integrin β_2 , preventing the formation of the integrin-based adhesion required for cell stopping^{15,16}. In contrast, LAD II is a defect in the glycosyltransferases of the leukocyte, which prevents the formation of the glycan structures needed to mediate rolling interactions¹⁶. We chose to focus on LAD III because it impacts an intracellular “control switch” which allows for the integration of physical adhesion and intracellular signaling¹⁷. Previous studies have shown that the stabilization of the high affinity form of LFA-1 by kindlin-3 is required for cells to resist shear forces applied to them both *in vitro* and *in vivo*^{8,12,13,18}. In addition, studies using mouse models have suggested adhesion is hypersensitive to the amount of kindlin-3 in cells, with as little as 5% of WT levels allowing for normal cell function¹⁹.

Our lab has previously developed Adhesive Dynamics (AD) to study the biophysics of cell rolling and adhesion^{20,21}. Previous studies have highlighted its predictive power regarding the adhesion of leukocytes under flow^{22–24}. In an important modification, we have developed Integrated Signaling Adhesive Dynamics (ISAD), which integrates a simulated intracellular signaling network within a mechanically accurate simulation of adhesion²⁵.

ISAD modeling was able to recapitulate the rolling and stopping of a lymphocyte cell line on surfaces of recombinant protein²⁵, as well as predict that the deletion of diacylglycerol kinase would lead to a gain of adhesive function²⁶.

Here, we extend ISAD to simulate the effect of reductions in the level of kindlin-3 on lymphocyte adhesion under flow. Our model predicts adhesion is hypersensitive to the amount of kindlin-3, which has been reported in the literature¹⁹ and which we confirm experimentally. ISAD predicted decreases in kindlin-3 will increase the time for a lymphocyte to stop, without decreasing the level of firm binding. In addition, we predict kindlin-3 has no effect on the rolling velocity. Both of these predictions are confirmed experimentally. Our results suggest that ISAD is an accurate simulator of lymphocyte homing which could ultimately be used to improve the targeting of leukocytes to sites of infection and inflammation.

Materials and Methods:

Jurkat Cells.

Wild type Jurkat E6.1 cells were maintained in RPMI media supplemented with 10% fetal bovine serum and 100 IU/mL of penicillin/streptomycin. Jurkat E6.1 shkindlin-3 cells were graciously donated by Dr. Janis K. Burkhardt (Children's Hospital of Philadelphia, Philadelphia, PA) and were maintained in the same media. Cells were stimulated for 48 hours with 1 μ g/mL phytohemagglutinin (PHA) and 100 nM phorbol 12-myristate 13-acetate (PMA) to stimulate expression of sialyl-Lewis X (sLex) to allow for true rolling interactions.

Flow cytometry.

Cells were washed three times in PBS before labeling and resuspended at a final concentration of 10^6 cells mL^{-1} . Samples were then incubated individually with each of the following antibodies: IgG1 κ (clone MOPC-21, Biolegend, San Diego, CA), IgG2 κ (clone MOPC-173, Biolegend), anti-CD11a (α L, clone HI111, Biolengend), anti-CD184 (CXCR4, clone 12G5, Biolegend), anti-CD162 (PSGL-1, clone KPL-1, Biolegend), IgM κ (clone G155-228, BD Biosciences, San Jose, CA), and anti-sialyl-Lewis X (clone CSLex1, BD Biosciences). After incubation according to manufacturer's directions, cells were washed three times in PBS to remove unbound antibodies. Flow cytometric analysis was performed on a BD Accuri C6 flow cytometer (BD Biosciences) and the CFlow software package. Histograms were generated using FlowJo (FlowJo, Ashland, OR).

Adsorption of Protein A/G and SDF-1 α .

Non-tissue culture treated polystyrene petri dishes (Corning, Corning, NY) were enclosed using a single well FlexiPerm gasket (Sigma-Aldrich, St. Louis, MO). A solution of 2 μ g mL^{-1} of Protein A/G (Thermo-Fisher Scientific, Waltham, MA) and 1 μ g mL^{-1} of SDF-1 α (R&D Systems, Minneapolis, MN) was applied and incubated overnight at 4°C. The surfaces were then washed three times with PBS before a 30-minute incubation of a 0.2% w/v solution of Pluronic F-127 (Sigma-Aldrich). The surfaces were washed again three times with PBS. Next, a solution of 8 μ g/mL ICAM-1/Fc and 2 μ g/mL P-selectin/Fc

chimeras (R&D Systems) was applied to the surface and incubated for three hours at room temperature. The completed surfaces were then washed again three times with PBS before use.

Flow Chamber Assays.

Flow chamber experiments were performed in a circular parallel plate flow chamber (GlycoTech, Gaithersburg, MD) with a gasket width of 0.25 cm, thickness of 127 μm , and length of 2 cm. Before flow chamber experiments, a functionalized dish was washed with prewarmed PBS and media to remove air bubbles in the flow path. The assembled flow chamber was mounted on an inverted Axiovert 200 (Carl Zeiss, Gottigen, Germany) enclosed by a XL-3 microscope incubator (PeCon, Ulm, Germany). All experiments were performed at 37°C. Cells were suspended at a concentration of $5 \times 10^5 \text{ mL}^{-1}$ and were perfused into the flow chamber via syringe pump (Harvard Apparatus, Holliston, MA) at a flow rate corresponding to a calculated wall shear rate of 100 s^{-1} . Rolling and adhesion of T cells on the immobilized ICAM-1 and P-selectin was observed via phase contrast microscopy under a 10X objective (NA = 0.2, Type A-Plan, Carl Zeiss).

Data Acquisition and Cell Tracking.

A CCD camera (QImaging, Surrey, BC, Canada) was used to monitor cell adhesion events with adhesive P-selectin/ICAM-1/SDF-1 α substrates. Adhesion of Jurkat cells was recorded on DVD+RW discs for cell tracking analyses. Cell adhesion videos were redigitized to 640X480 pixels at 29.97 frames s^{-1} and deinterlaced with HandBrake software (<http://handbrake.fr/>) then converted to image stacks with MATLAB (MathWorks, Natick, MA). The stacked images were thresholded and converted to binary images. The coordinates of the centroid of interacting cells with a surface every video frame were then acquired using the MTrack2 plugin (<http://valelab.ucsf.edu/~nico/IJplugins/MTrack2.html>) in the ImageJ program (<http://imagej.nih.gov/ij/>; National Institutes of Health).

Analysis of Cellular Adhesion.

MATLAB was used to analyze the tracked centroids obtained from the MTrack2 plugin. Rolling was defined as the movement of an interacting cell over the substrate at an instantaneous velocity in any frame less than 20% of the calculated free stream velocity of a noninteracting cell²⁷ while remaining in the field of view. Cell stopping was defined as reduction of its instantaneous velocity below 5% of the free stream velocity. Adherent T lymphocytes were further classified as tethering, rolling or firmly arrested cells based on their instantaneous velocities and the duration of their stopping. A tethering cell is defined as a cell that “rolls” less than 30 video frames (1 s) in a rolling period within the field of view. A firmly arrested cell is defined as a cell that “stops” stably more than 90 frames (3 s). Time and distance to stop were both calculated based on the initial time and location of the cell rolling. Values for figures were calculated based on cells that interacted with the surface. Cells which were tracked but did not interact with the surface were excluded from the analysis.

Integrated Signaling Adhesive Dynamics.

In this study, we updated a previously developed ISAD model, which was originally designed to simulate the loss of the protein diacylglycerol kinase in T lymphocytes and its effect on T cell homing^{25,26}. The major updates are listed below and the parameters used in the model included in Tables 1 and 2:

- i. An intermediate affinity form of LFA-1 was added to the model to allow for determination of the effect of integrin activation on homing. It is assumed in this model that the binding of talin to the inactive form of LFA-1 converts it directly to the intermediate affinity form^{2,9,10}.
- ii. Kindlin-3 was added to convert intermediate affinity LFA-1 to high affinity, fully active LFA-1, in line with previous studies^{8,10}. Talin and kindlin-3 are present in equi-molar amounts¹⁹. Kindlin-3 is presumed to have no other role in the cell other than serving as an integrin activator and is assumed to be distributed evenly in the cytosol. Kindlin-3 is also assumed to have the same diffusivity as talin.
- iii. The reaction rates of talin binding to LFA-1 remained unchanged from previous iterations of the ISAD model^{25,26}. Kindlin-3 was assumed to react with intermediate affinity LFA-1 with a forward reaction rate of $10^3 \mu\text{M}^{-1} \text{s}^{-1}$ and a reverse reaction rate of 0.1s^{-1} . As there is no information regarding the reactivity of kindlin-3, we assumed a very fast forward reaction rate and a slow reverse reaction rate. These choices did not affect the dynamics of the signaling network (data not shown).
- iv. The outer radius of the cell was increased to $5.75 \mu\text{m}$ and the radius of the nucleus increased to $4.7 \mu\text{m}$ to match previously published values for Jurkat cells²⁸ and our own measurements (data not shown).

The Next Subvolume Method of ISAD stochastically simulates both the reactions and diffusion of the various molecular components of the signaling cascade. In contrast, Adhesive Dynamics is solely concerned with the physical actions occurring at the cell-substrate interface. The simulations begin with the cell close to, but not interacting with, a surface containing simulated chemokine, P-selectin, and ICAM-1 at a shear rate of 100s^{-1} . The NSM updates the intracellular reactions due to the binding of chemokine to the receptors on the cell surface. As the reaction proceed, the NSM can also change the number of extracellular receptors as integrins change activation states. Ultimately, AD determines the trajectory of the cell over the surface based on the binding and unbinding of the PSGL-1 and low, intermediate, and high affinity LFA-1 on the surface of the cell. The two systems share information via shared data structures which are accessible at any point during the simulation. Intracellular protein amounts were updated in each subvolume according to the Gillespie algorithm, while the timestep for AD was fixed at 10^{-6}s . These simulations were programmed in C++ using Xcode 9 (Apple, Cupertino, CA) and run on a Mac Pro with 3.0 GHz 8-core Intel Xeon processor. Results were then summarized using a custom written R script.

Results:

Figure 1 shows the general structure of the ISAD simulation. The cell is simulated as a rigid sphere coated with receptors on microvilli moving across a flat surface coated with ligand at set densities. PSGL-1 is clustered on the tips of the microvilli, while LFA-1 is distributed equally across the surface of the cell. The LFA-1/ICAM-1 and PSGL-1/P-selectin bonds are assumed to have catch-slip behavior, which is simulated using the Evans two-state model²⁹. Microvilli are allowed to deform viscoelastically, in line with previous reports^{25,26,30}. Bonds are tested for formation and breakage in a stochastically, with on and off rates dependent on the deviation from the resting length of each receptor. The cytosol of the cell is discretized into subvolumes based on a spherical lattice. Each subvolume, where appropriate, allows for all reactions of the signaling cascade to occur inside. The signaling cascade is a generalized model of the signaling occurring to activate leukocyte integrins. It begins with the binding of chemokine to chemokine receptors on the cell surface, which triggers the release of G proteins. These proteins can interact with other molecular species to catalyze the creation of second messengers like IP₃ and DAG. DAG then activates Rap1 GTPase, which triggers the association of Rap1 and talin, leading to the intermediate activation of LFA-1. Finally, kindlin-3 binds to intermediate affinity LFA-1 to convert it to the fully active, high affinity form of LFA-1. We hypothesized that the quantitative depletion of kindlin-3 would reduce the level of adhesion by inhibiting the creation of high affinity LFA-1.

The effects of kindlin-3 depletion on lymphocyte adhesion are shown in Figure 2. Our model predicts that the level of cell adhesion is hypersensitive to the amount of kindlin-3 in the cell. There was no significant effect on adhesion when kindlin-3 was depleted to a level of 20% of normal WT expression levels. Below this level, the level of adhesion significantly decreased such that there was no adhesion in the simulated kindlin-3 KO cells, as highlighted in Figure 2A. Our model also predicted that kindlin-3 levels would not affect the rolling velocity of cells (Figure 2B). Interestingly, the model predicts an increase in the time to stop for the cells as the level of kindlin-3 decreased in the cell, as shown in Figure 2C. The time to stop was inversely logarithmic with the level of kindlin-3. Finally, Figure 2D shows how the distance to stop is affected by kindlin-3, which combines the effects of the rolling velocity and the time to stop. The distance to stop showed a linear increase with a small slope kindlin-3 expression as long as the level of kindlin-3 was above 20% of the expression of WT cells. However, the rate of change in distance to stop with kindlin-3 levels was greater once the expression of kindlin-3 fell below 20% of the WT expression levels. These results suggest that expression of about 20% is the “tipping point” below which the ability of the cells to adhere to the endothelial surface is significantly degraded.

In order to confirm our simulation results, we compared the adhesion of WT and a shkindlin-3 knock down Jurkat T lymphocytes for their ability to adhere to a surface coated with recombinant adhesion proteins and chemokines. A representative western blot of kindlin-3 levels in Jurkat cells is shown in Figure 3A, showing an approximately 84% reduction in kindlin-3 expression in shkindlin-3 knock down Jurkat T lymphocytes. Figure 3B shows the quantification of three independent blots. In addition, shkindlin-3 knock down Jurkat cells had similar levels of expression of both LFA-1 and PSGL-1 to the WT Jurkat cells, as shown in Figure 3C. Our experiments showed that there was a significant decrease

in the level of adhesion of the shkindlin-3 cells compared to the WT control, as shown in Figure 3D. In the absence of chemokine, neither shkindlin-3 nor WT Jurkat cells adhered to the surface (data not shown). There was no significant difference in the rolling velocity between the shkindlin-3 knock down and WT cells, consistent with predictions (Figure 3E). Also, as shown in Figure 3F, there was a significant difference in the time to stop when comparing the shkindlin-3 Jurkat cells to the WT Jurkat cells. Finally, although there was an increase in the distance to stop, as predicted by the model, this difference was not statistically significant (Figure 3G). A direct comparison between the simulation predictions and experimental results can be seen in Table 3.

Discussion:

In this manuscript, we have presented a simulation using ISAD which predicts the effect of quantitative reductions of expression of the hematopoietic-cell specific integrin activator kindlin-3. We compared the simulations to experiments using WT and shkindlin-3 Jurkat cells to confirm the predictions of the model. Our results suggest that ISAD modeling can accurately predict the effect of a decrease in the expression level of kindlin-3 quantitatively.

Much work has focused on the effect of a loss of kindlin-3 on the function of leukocytes and platelets³¹. Kindlin-3 has been implicated in the activation of LFA-1, along with the clustering of integrins, on platelets^{8,12}. While knock-out systems provide a snapshot of what happens when a molecule is eliminated, it is often more instructive to understand what might happen with quantitative reductions which can be achieved through siRNA, shRNA, or engineering of mice. In our simulation, the binding of chemokine to the chemokine receptor triggers an intracellular signaling cascade that results in the activation of LFA-1 on the surface of the cell. This model is somewhat simplified from the known biology, as several intermediate steps known to occur in leukocytes (e.g. Rap1-GTP-interacting adaptor molecule linking talin and Rap1^{GTP} to promote talin membrane localization³²⁻³⁴) are not explicitly included. In addition, our model does not incorporate the possibility of outside-in signaling through PSGL-1, which has been shown previously to activate integrins that lead to reductions in rolling velocities of leukocytes³⁵⁻³⁷. Ca²⁺ signaling might also play an important role in integrin activation, but was not explicitly included here^{38,39}. Despite these limitations, the model was able to accurately simulate the rolling and adhesion of cells on a flat surface.

Previous experiments have shown that as little as 5% of WT kindlin-3 expression levels can rescue the LAD III phenotype in mice, and that cell adhesion is hypersensitive to the level of kindlin-3 in the cell¹⁹. These findings are consistent with our simulation results showing that at 5% WT kindlin-3 expression, approximately half of the cells will be able to stop, while showing no effect on cell stopping for kindlin-3 levels above 20% of WT expression. These results also are consistent with our experimental findings that kindlin-3 KD of ~84% results in only a 17% reduction in adhesion. In addition, Moser and colleagues found no change in the *in vivo* rolling velocities of WT and kindlin-3 KO cells in stimulated mouse cremaster muscles¹². This matches our findings from simulations that the selectin-mediated rolling of leukocytes is not perturbed by the absence of kindlin-3.

Our model also predicted an increase in the time to stop when kindlin-3 expression was decreased. This increase is driven by the local depletion of kindlin-3 closest to the plasma membrane, requiring increased diffusion from interior pools of kindlin-3 in the cytosol. The time delay is due to the time for kindlin-3 to diffuse to the plasma membrane. We also confirmed the increase in the time to stop experimentally, although the magnitude of the change was larger than the model predicted. Our model predicted an increase of about 30%, but the experiments showed an increase of more than twice the prediction. The basis of this discrepancy is unclear but could be related to specific localization of kindlin-3 or a lower diffusivity than was assumed.

In this paper, we directly compared the results of ISAD simulations to results from an *in vitro* experimental system. Our experimental system used a flat surface and defined molecular components to more closely match the simulations. It might be more physiologically relevant to include surface roughness from the shape of endothelial cells, which would require solving for the complex motion of a deformable sphere caused by non-laminar flow. In addition, the wider variety of molecular interactions would require understanding all of the possible interactions, as well as the effect of clustering of adhesion molecules on the apical surface. Also, our model did not include the effect that kindlin-3 has on integrin clustering^{9,40}, but we suspect this becomes only a major effect after the leukocyte has come to a halt. Finally, our model does not allow for force applied to integrin-ligand bonds to change the affinity state of the integrins, as has been shown previously⁴¹. These complications would require additional effort and resources and could be the basis of future work.

In conclusion, we have used an ISAD simulation to predict *a priori* the effect of a kindlin-3 depletion on the ability of lymphocytes to adhere to a simulated endothelium. Our results suggest that the hypersensitivity of cell adhesion to kindlin-3 results from its ability to stimulate a sufficient number of integrin interactions to secure adhesion. Finally, this manuscript highlights the ability of ISAD to accurately and quantitatively simulation leukocyte adhesion and highlights its ability to predict the effect of intracellular modifications on cell adhesion.

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Insight Box

Lymphocyte homing is important for immune homeostasis. Integrative Signaling Adhesive Dynamics, or ISAD, is a method to simulate cell adhesion that integrates the effects of signaling pathways with the mechanics of adhesion to understand how intracellular pathways can affect cell adhesion and response. We use ISAD to simulate the effects of reduction of kindlin-3, an intracellular modulator of integrin affinity whose absence is responsible for Leukocyte Adhesion Deficiency Type III, and verify those predictions in a cell line in which kindlin-3 is knocked down. The correspondence between simulation and experiment suggests very little kindlin-3 needs to be replaced to restore proper homing.

Figure 1 –.

Diagram of integrated signaling adhesive dynamics simulations. (A) Model geometry for simulation of a T lymphocyte interacting with a functionalized surface in shear flow. The contact zone (highlighted by dashed box) allows for the stochastic formation and breakage of bonds between adhesive receptors on the cell and ligands on the flat surface. Adhesive receptors are located on viscoelastic microvilli. Shear flow is in the X direction, causing a translational velocity U and rotational velocity Ω . (B) Discretization of the lymphocyte membrane and cytosol into subvolumes using a spherical lattice. (C) Signaling cascade for integrin activation. Binding of chemokine to chemokine receptors releases G proteins to activate PLC. PLC then converts PIP₂ to IP₃ and DAG, which binds to a GEF. This molecule can then trigger the exchange of GDP for GTP in Rap1, allowing it to recruit talin to the membrane to initially activate LFA-1. Kindlin-3 then binds to convert LFA-1 into its high affinity form. Species in parentheses are not explicitly enumerated in the model.

Figure 2 –.

Results of ISAD simulations. (A) Graph showing the effect of kindlin-3 expression (normalized to WT) on the fraction of cells stopping. (B) Plot showing the effect of kindlin-3 expression on the rolling velocity of cells. (C) Graph showing the effect of kindlin-3 expression on the time to stop of simulated cells. (D) Plot showing the effect of kindlin-3 expression on the distance to stop of simulated cells. All plots are the mean $\pm$ SEM of 500 independent simulated cells.

Figure 3 –.

Results of kindlin-3 KD in Jurkat cells. (A) Representative western blot showing knockdown of kindlin-3 in Jurkat cells. (B) Quantification of kindlin-3 knock down. Overall KD was 83.7 ± 1.6 % (mean $\pm$ SEM, $n = 3$). (C) Results of flow cytometry staining of shkindlin-3 (grey) and WT (black) cells. Dashed lines are isotype controls. Plots comparing the fraction of cells stopping (D), rolling velocity (E), time to stop (F), and distance to stop (G). Stars indicate $p < 0.05$ according to a two-tailed Student's t test. All plots have shkindlin-3 cells in grey and WT cells in black, showing mean $\pm$ SEM ($n = 4$).

Table 1:

ISAD parameters used in simulations

Parameter	Definition	Value ²⁶
R	Cell radius (μm)	5.75
R_N	Nucleus radius (μm)	4.7
$\dot{\gamma}$	Shear rate (s^{-1})	100
CXCR4	Chemokine receptor at the plasma membrane (per cell)	4.8×10^4
Talin	Talin in the cytosol (per cell)	1×10^4
Kindlin-3	Kindlin-3 in the cytosol (per cell)	1×10^4
LFA-1 _{Low}	Low affinity LFA-1 at the cell surface (per cell)	4.8×10^4
PSGL-1	PSGL-1 at the cell surface (per cell)	7.5×10^4

Table 2:

Receptor-ligand kinetics constants used in simulations

Parameter	Definition	PSGL-1 + P-selectin ²⁶	LFA-1 _{Low} + ICAM-1 ²⁶	LFA-1 _{Int} + ICAM-1 ⁴²	LFA-1 _{High} + ICAM-1 ²⁶
λ	Equilibrium bond length (nm)	70	24	44	44
σ	Bond spring constant (pN nm ⁻¹)	5	50	50	50
a	Radius of reactivity (μm)	0.002	5×10^{-4}	0.0015	0.003
k_{on}	Intrinsic on rate (*10 ⁵ sec ⁻¹)	4.1	0.1	0.1	0.1
L	Ligand density (μm ⁻²)	10.2	50	100	500
k_{rup}	Fast pathway dissociation rate (s ⁻¹)	10	19	13	17
k_2	Unstressed slow pathway dissociation rate (s ⁻¹)	0.37	0.55	0.41	0.02
f_{10}	Thermal energy divided by the position of the first transition state (pN)	N/A	$k_B T / 0.049$	$k_B T / 0.056$	$k_B T / 0.056$
f_{β}	Thermal energy divided by the position of the second transition state (pN)	18	$k_B T / 0.26$	$k_B T / 0.29$	$k_B T / 0.35$
f_{12}	Difference between the two pathways	5	N/A	N/A	N/A
Φ_0	Equilibrium constant between the two states at zero force	100	N/A	N/A	N/A

Table 3:

Comparison of simulated and actual differences between WT and shkindlin-3 cells

Metric	Simulation prediction (n = 500)	Experimental result (n = 4)
Fraction stopping	-13.9%	-17.2 ± 6.6%
Rolling velocity	-1.0 ± 2.0%	+9.6 ± 5.5% *
Time to stop	+29.7 ± 2.2%	+62.8 ± 16.1%
Distance to stop	+27.2 ± 4.4%	+45.4 ± 11.4% *

All values mean ± SEM.

* Difference was not statistically significant