Electronic Supplementary Material (ESI) for Integrative Biology.

Stiff substrates enhance monocytic cell capture through E-selectin but not P-selectin

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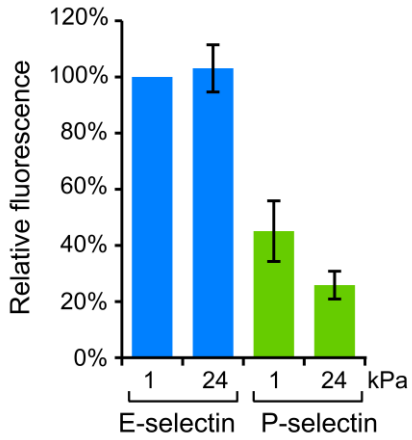

Figure S1. Similar levels of E-selectin on soft and stiff gels, but low levels of P-selectin. E-selectin and P-selectin coated gels were stained with monoclonal antibody that recognizes both E-selectin and P-selectin (clone BBIG-E6), followed by Alexa Fluor 488-labelled secondary antibody. Confocal images were taken at the surface of each gel, and the average fluorescence intensity was calculated relative to 1 kPa gels coated with E-selectin (n = 4 experiments, mean ± s.e.). The levels of E-selectin on 1 kPa and 24 kPa gels were not significantly different from each other (t-test: p = 0.98), and the levels of P-selectin on 1 kPa and 24 kPa gels were not significantly different from each other (t-test: p = 0.16).

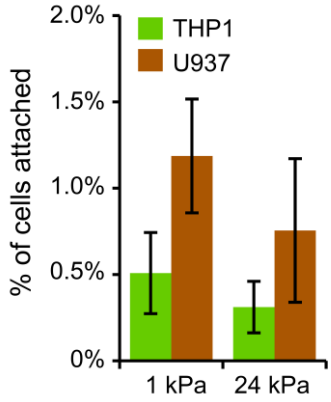

Figure S2. Gels coated with P-selectin do not support cell attachment. THP-1 or U937 cells were perfused at 100 s^{-1} across 1 kPa or 24 kPa gels coated with 4 µM P-selectin, but fewer than 2 cells attached to each gel (n = 5 experiments, mean ± s.e.).

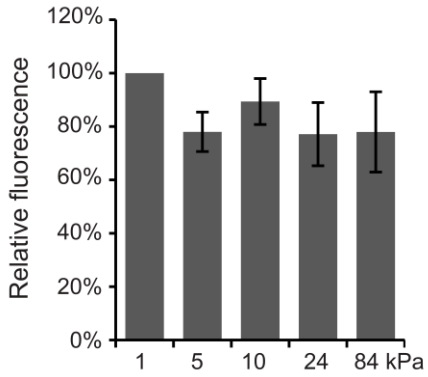

Figure S3. Similar levels of human IgG_1 bind to gels coated with protein A/G, regardless of stiffness. Gels were coated with protein A/G and then human IgG_1 labelled with Alexa Fluor 555. Confocal images were taken at the surface of each gel, and the average fluorescence intensity was calculated relative to 1 kPa gels (n = 4 experiments, mean ± s.e., t-test for 1 vs 24 kPa: p = 0.17).

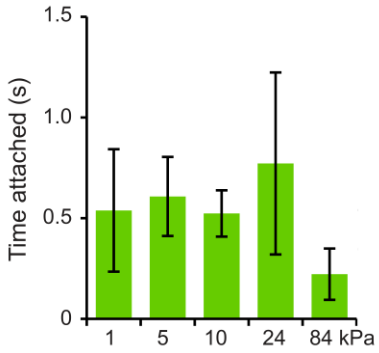

Figure S4. THP-1 cells attach to gels coated with P-selectin/Fc at 16 molecules/μm^2 for similar time periods, regardless of stiffness. THP-1 cells were perfused at 100 s^{-1}, and the average time that cells spent attached to the gels was recorded (n = 3 experiments, mean ± s.e., t-test for 1 vs 24 kPa: p = 0.69).

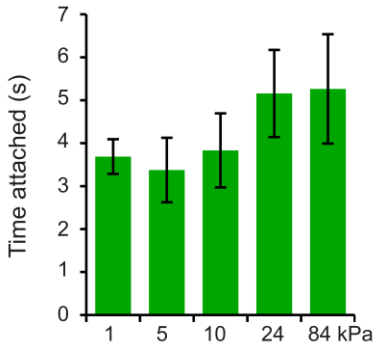

Figure S5. THP-1 cells attach to gels coated with P-selectin/Fc at 32 molecules/μm^2 for similar time periods, regardless of stiffness. THP-1 cells were perfused at 100 s^{-1}, and the average time that cells spent attached to the gels was recorded (n = 3 experiments, mean ± s.e., t-test for 1 vs 24 kPa: p = 0.24).

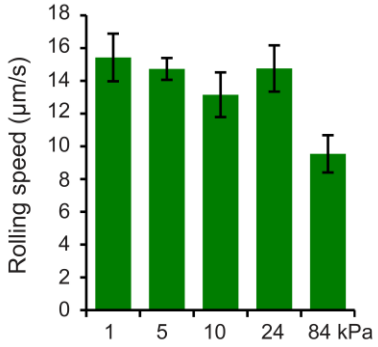

Figure S6. THP-1 cells roll at similar speeds on gels coated with P-selectin/Fc at 178 molecules/μm^2, regardless of stiffness. THP-1 cells were perfused at 100 s^{-1}, and the average rolling speed of attached cells was calculated (n = 3 experiments, mean ± s.e., t-test for 1 vs 24 kPa: p = 0.76).

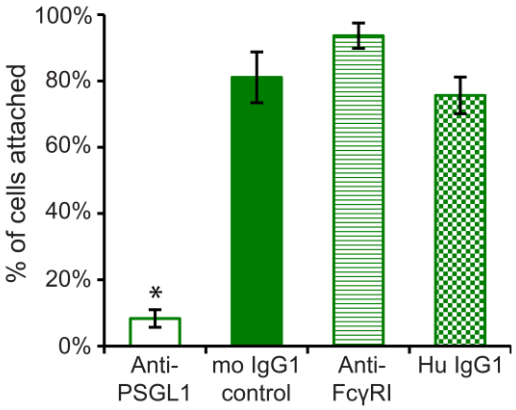

Figure S7. THP-1 cells bind P-selectin/Fc coated gels through PSGL-1. Before flow experiments, THP-1 cells were pretreated for 30 minutes with anti-PSGL-1 blocking antibody, mouse IgG_1 isotype control antibody, anti-FcγRI blocking antibody, or human IgG_1. The cells were then perfused at 100 s^{-1} across 24 kPa gels coated with P-selectin/Fc at 178 molecules/μm^2. Only treatment with anti-PSGL-1 blocking antibody reduced the percentage of attached cells (n = 3 experiments, mean ± s.e., t-test: p = 0.012).

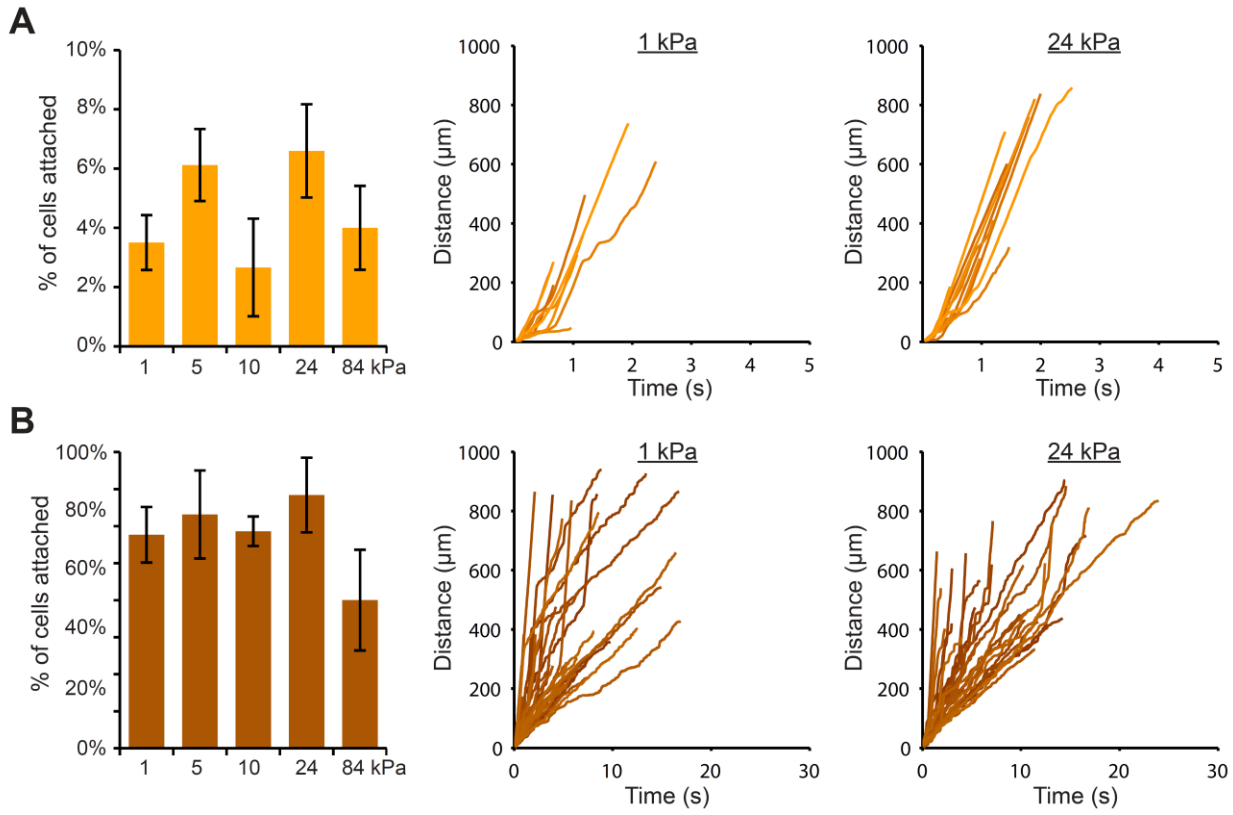

Figure S8. U937 cell attachment through P-selectin/Fc is not sensitive to substrate stiffness. U937 cells were perfused at 100 s^{-1} across gels coated with different densities of P-selectin/Fc: A) 16 molecules/µm^2 and B) 32 molecules/µm^2. For both P-selectin/Fc densities, the percentage of attached cells did not vary with substrate stiffness (n = 3 experiments, mean ± s.e., t-test for 1 vs 24 kPa: p = 0.19 (A), p = 0.49 (B)). Representative cell tracks are shown plotted as distance travelled versus time.

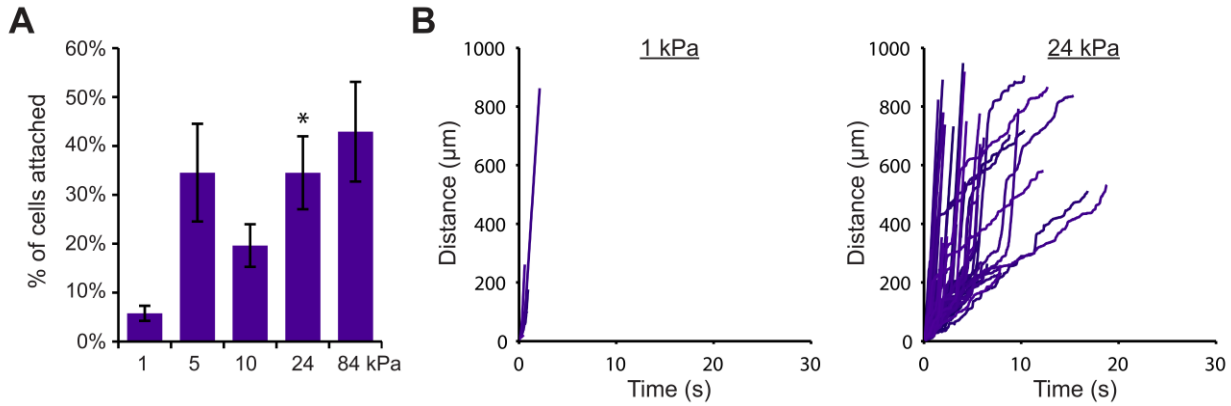

Figure S9. U937 cell attachment through E-selectin/Fc is enhanced on stiff substrates. U937 cells were perfused at 100 s^{-1} across gels coated with E-selectin/Fc at 20 molecules/µm^2. A) The percentage of attached cells increased with increasing substrate stiffness (n = 4 experiments, mean ± s.e., t-test for 1 vs 24 kPa: p = 0.031). B) Representative cell tracks plotted as distance travelled versus time.

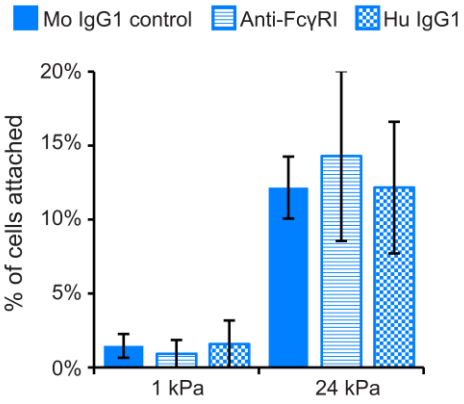

Figure S10. Blocking Fc receptors does not alter THP-1 cell attachment to gels coated with E-selectin/Fc at 20 molecules/µm^2. Before flow experiments, THP-1 cells were pretreated for 30 minutes with mouse IgG_1 isotype control antibody, anti-FcγRI blocking antibody, or human IgG_1. The cells were then perfused at 100 s^{-1} across 1 kPa and 24 kPa gels coated with E-selectin/Fc at 20 molecules/µm^2. The percentage of attached cells was higher on stiff gels, but unaffected by blocking Fc receptors with anti-FcγRI or human IgG_1 (n = 3 experiments, mean ± s.e.).

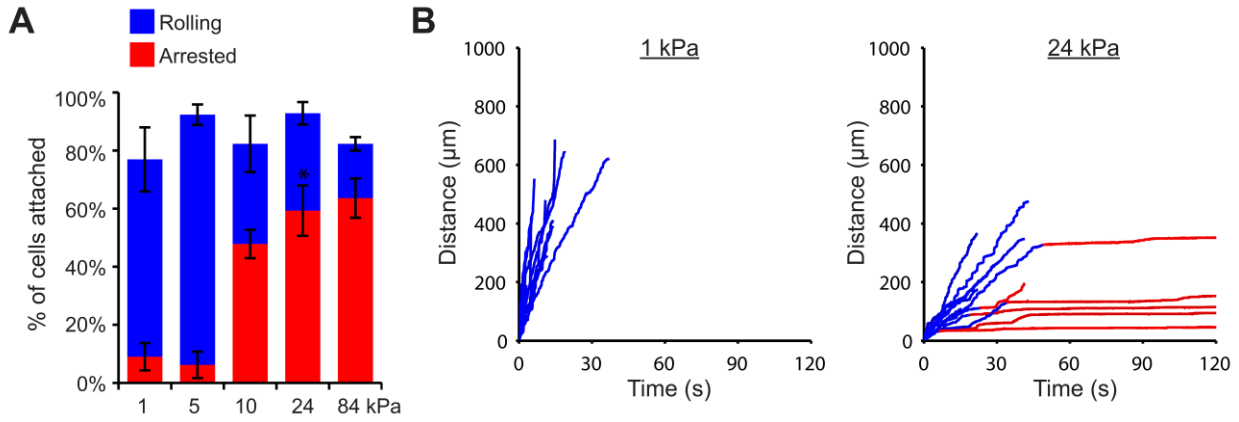

Figure S11. THP-1 cells arrest on stiff gels coated with a high E-selectin/Fc density. THP-1 cells were perfused at 100 s^{-1} across gels coated with E-selectin/Fc at 152 molecules/μm^2. A) The total percentage of attached cells was not sensitive to substrate stiffness (blue bar + red bar; n = 4 experiments, mean ± s.e., t-test for 1 vs 24 kPa: p = 0.31). However, a higher percentage of cells arrested on stiffer gels (red bars, t-test for 1 vs 24 kPa: p = 0.015). B) Representative cell tracks plotted as distance travelled versus time. The tracks are colored red for the time period that cells were arrested.

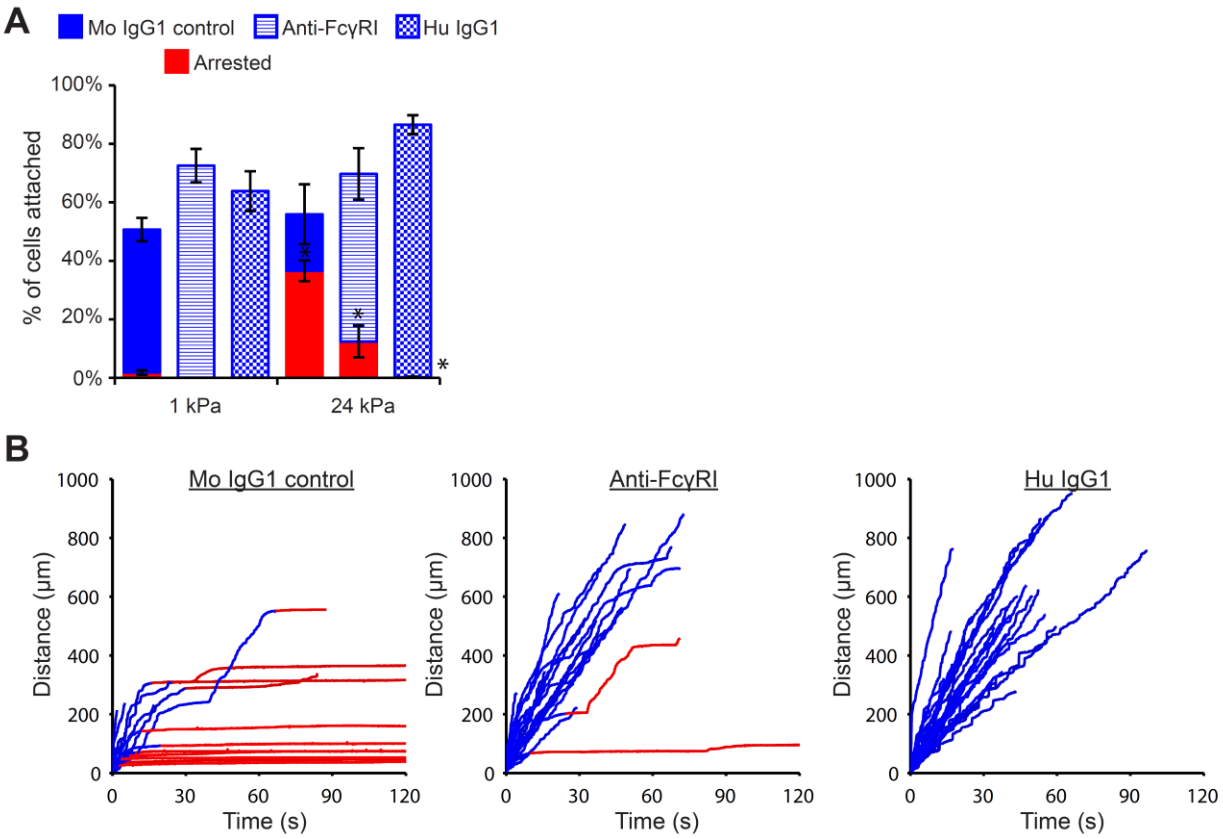

Figure S12. Cell arrest on stiff E-selectin/Fc gels is prevented by blocking Fc receptors. Before flow experiments, THP-1 cells were pretreated for 30 minutes with mouse IgG_1 isotype control antibody, anti-FcγRI blocking antibody, or human IgG_1. The cells were then perfused at 100 s^{-1} across 1 kPa and 24 kPa gels coated with E-selectin/Fc at 152 molecules/μm^2. A) The total percentage of attached cells was not sensitive to substrate stiffness or antibody treatment (blue bar + red bar; n = 4 experiments, mean ± s.e.). However, a higher percentage of control cells arrested on stiff gels (red bar under solid blue bar, t-test: p = 0.0017), which was reduced by treatment with anti-FcγRI blocking antibody (red bar under striped blue bar, t-test: p = 0.008) and completely inhibited by treatment with human IgG_1 (red bar under checkered blue bar, t-test: p = 0.0013). B) Representative cell tracks on 24 kPa gels for the three antibody conditions, plotted as distance travelled versus time. The tracks are colored red for the time period that cells were arrested.