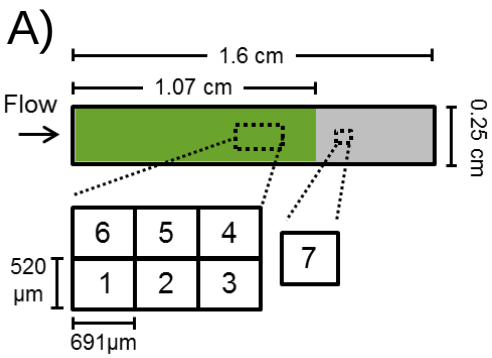
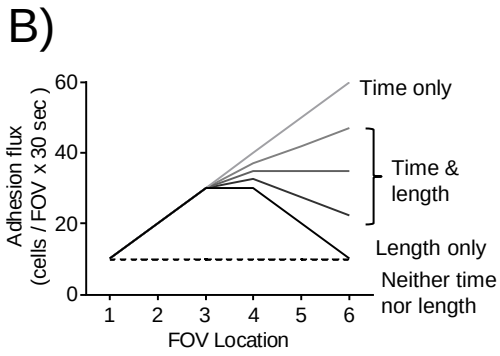
Electronic Supplementary Material (ESI) for Integrative Biology.

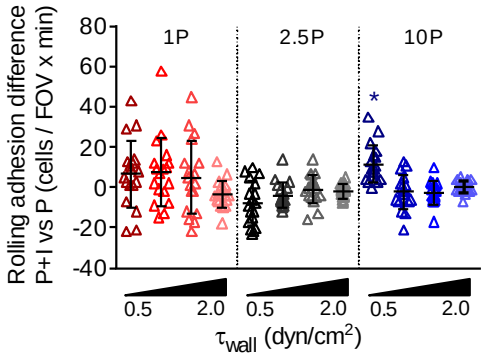
Supplemental Information



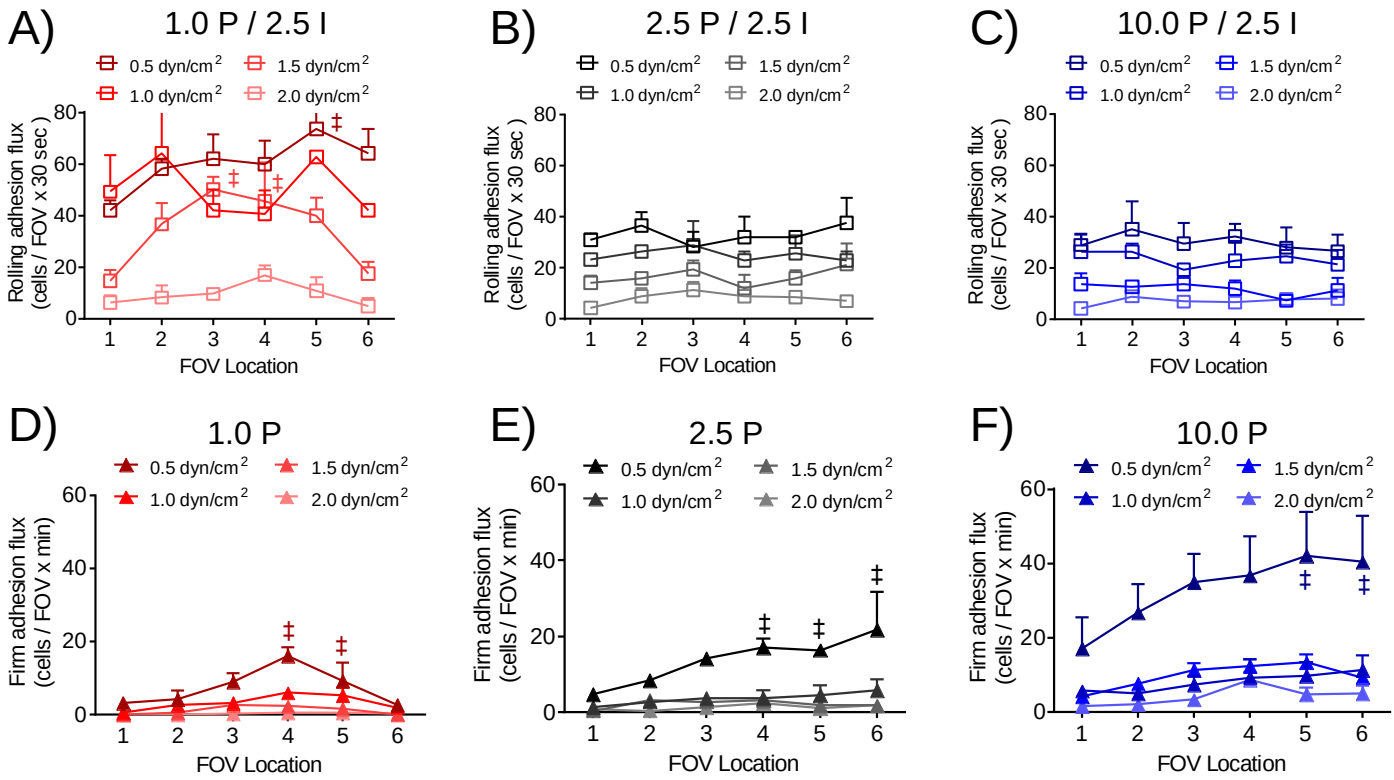
FOV location	Time from initiation of flow (mm:ss)
1	3:01-3:30
2	3:31-4:00
3	4:01-4:30
4	4:31-5:00
5	5:01-5:30
6	5:31-6:00
7 (blocked)	6:01-6:30



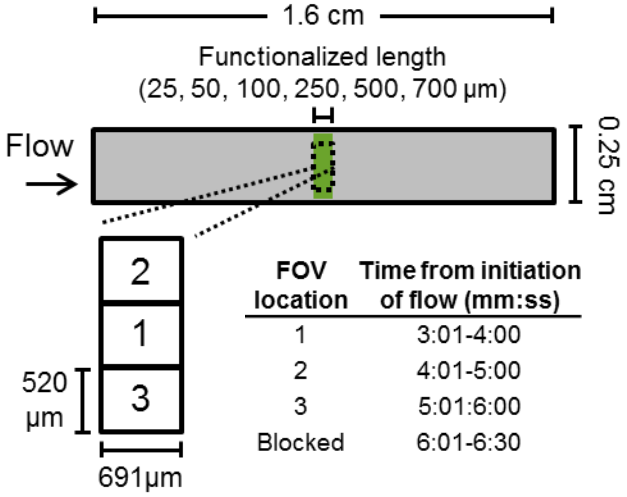
Supplemental Figure S1) Schematic of imaging setup for experiments performed using uniformly functionalized substrates. (A) After 3 minutes of perfusion to allow for flow equilibrium, multiple fields of view (FOV) were imaged in succession for 30 seconds each. Green shading indicates functionalized area; grey indicates area that was only blocked. (B) Given the spatial and temporal variability between each field of view, changes in adhesion levels with FOV may suggest that adhesion is time and/or length dependent.



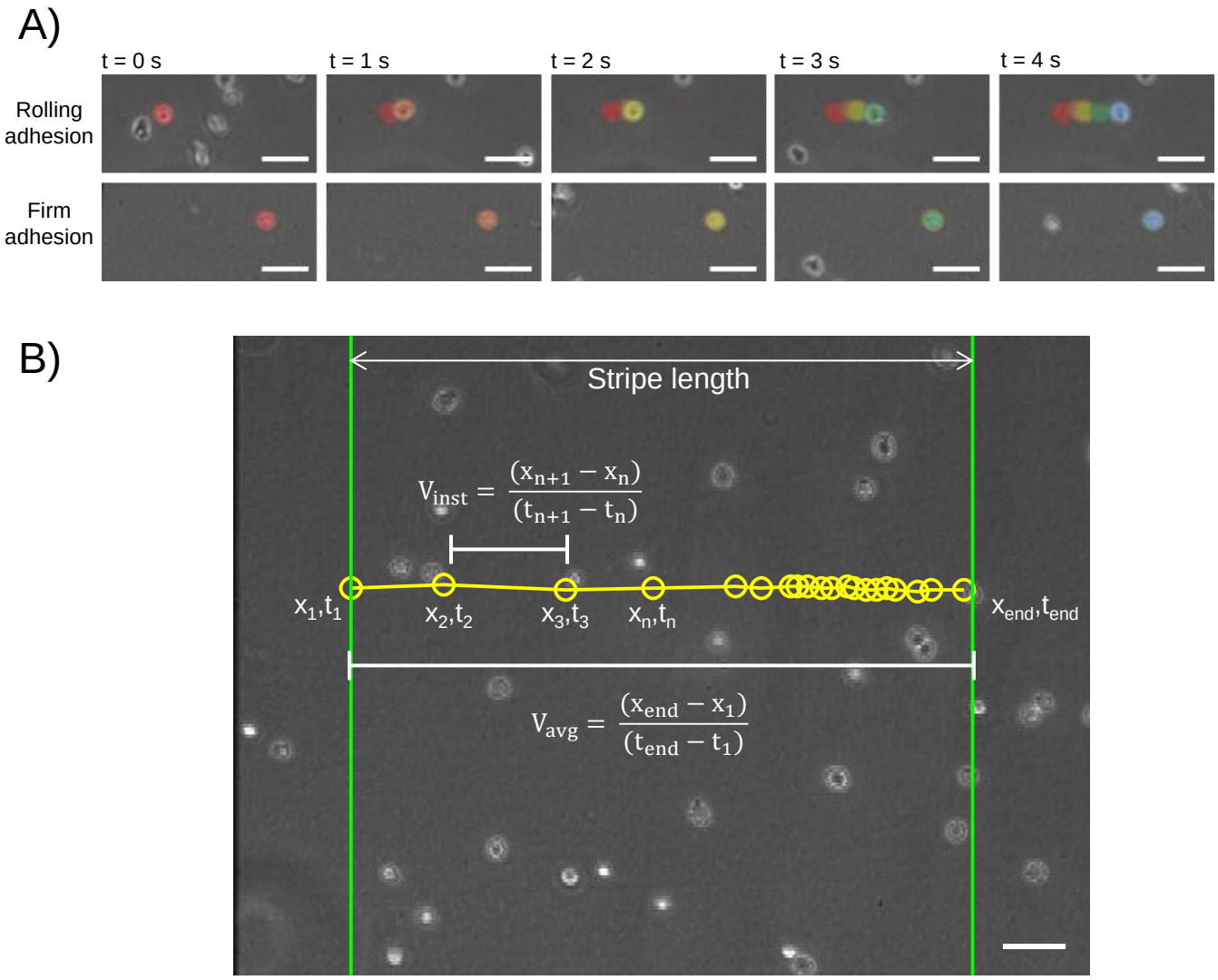
Supplemental Figure S2) Extent of rolling adhesion remains nearly unchanged on P-selectin alone versus P-selectin+ICAM-1. Under all tested conditions, save one, there are no differences in the extent of rolling adhesion on P-selectin+ICAM-1 versus P-selectin alone. Data represent 6 sampled imaging fields of view from 3 independently run experiments, with mean ± SEM indicated for each condition. One-sample t-tests were conducted for all data, where Ho=0 and * indicates significantly non-zero mean that is also greater than zero. 1.0, 2.5, and 10 in legends indicate concentrations of either P-selectin (P) in µg/mL.



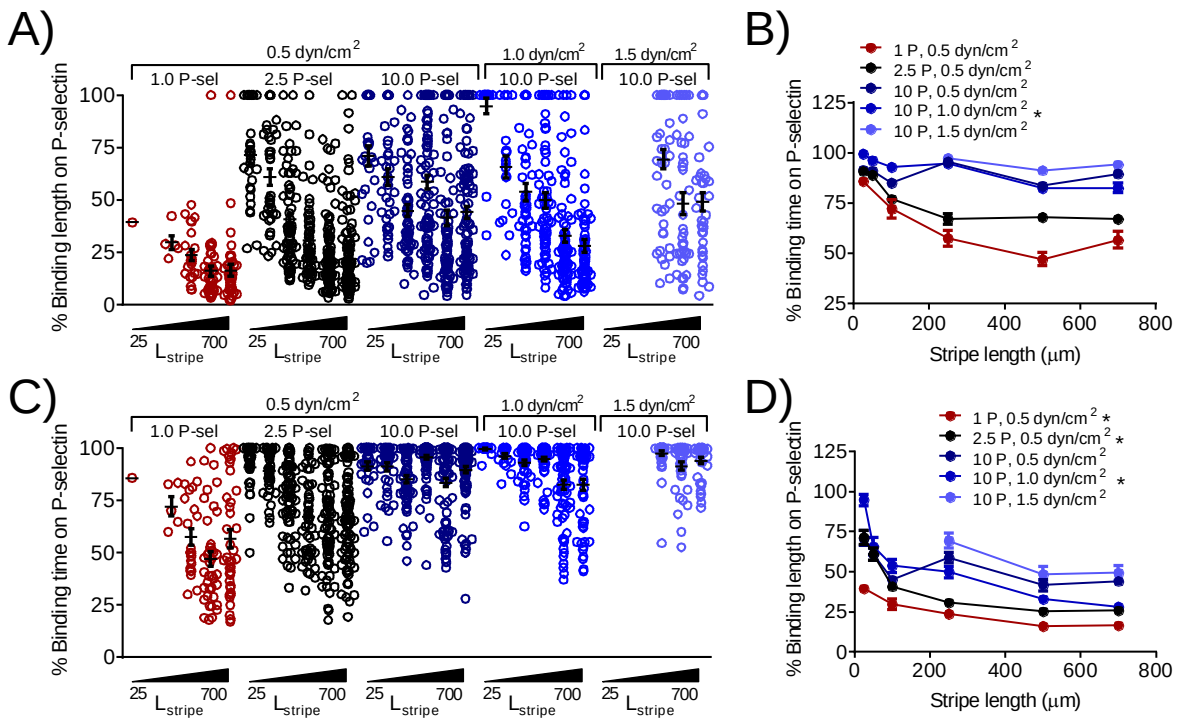
Supplemental Figure S3) THP-1 rolling adhesion on P-selectin + ICAM and firm adhesion on P-selectin alone demonstrate similar trends to rolling and firm adhesion on P-selectin and P-selectin + ICAM-1, respectively. While rolling adhesion levels on P-selectin + ICAM-1 do not vary significantly with FOV location, except at lowest P-selectin concentrations and intermediate wall shear stress (1.0 µg/mL, 1.5 dyn/cm^2) (A-C), extents of firm adhesion on P-selectin differ with FOV location at 0.5 dyn/cm^2 (D-F). Data represent mean ± SEM of 3 independently run samples. Two-way ANOVA with Bonferroni correction for multiple comparisons; ‡ indicates significance relative to first FOV location. (A-F) 1.0, 2.5, and 10 in graph titles indicate concentrations of either P-selectin (P) or ICAM-1 (I) in µg/mL.



Supplemental Figure S4) Schematic of imaging setup for micropatterned stripes. After 3 minutes of perfusion, multiple fields of view (FOV) were imaged in succession for 1 minute each. Green rectangle signifies P-selectin/ICAM-1 functionalized area, centered in the flow path; grey indicates area that was only blocked.



Supplemental Figure S5) Rolling adhesion, firm adhesion, and velocity analyses. (A) Representative frames depicting cell rolling (top row) and firm adhesion (bottom row). Colored labels indicate cell position at successive points in time, where red represents the 0 second time point and blue represents the 4 second time point. (B) Visual description of instantaneous and average velocity calculations. Green outline indicates stripe location, yellow circles indicate recorded positions of a single track. All scale bars 50 µm.



Supplemental Figure S6) Persistence of rolling adhesion is P-selectin concentration and length dependent. Percent binding time (A-B) and percent binding length (C-D) are weakly dependent on stripe length and lowest at longer stripe lengths. (A,C) Each data point represents a single tracked cell. (B,D) Data represent mean ±SEM of singly tracked cells for each condition in each of 3 FOVs from 3 independent experiments; linear regression, * indicates the slope of the relationship is nonzero and m indicates the slope of the linear relationships are significantly different among the indicated group.