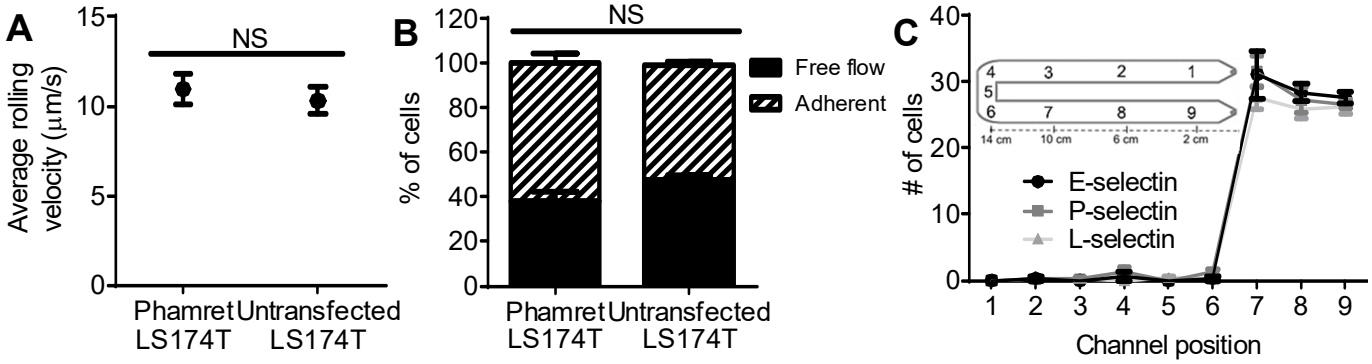
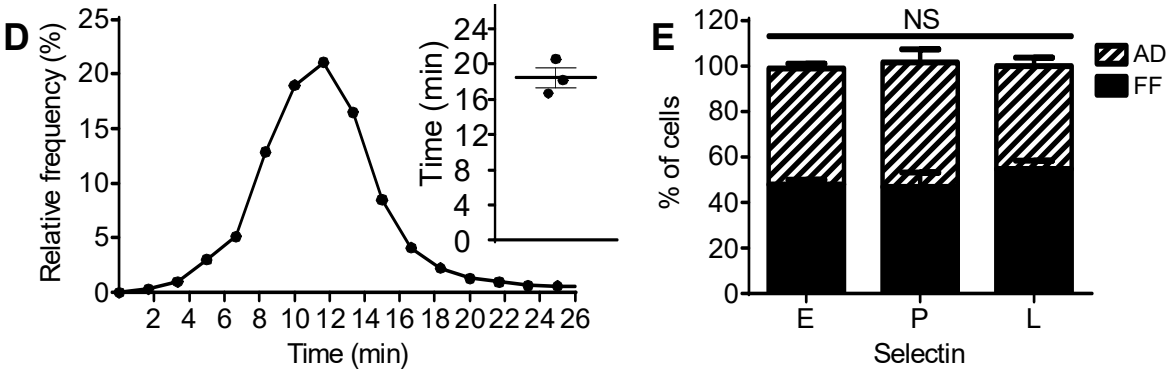
Electronic Supplementary Material (ESI) for Lab on a Chip.

Figure S1. Photoconvertible LS174T cell line and adhesive chromatography microfluidic characterization. (A,B) Comparison of (A) average rolling velocity and (B) percent of cells collected in each fraction from a cell pulse of either 250,000 control LS174T cells or Phamret-expressing LS174T cells over 2.5 μg mL^{-1} E-selectin at 1 dyn cm^{-2}. (C) Number of interacting cells along the length of the channel during the sorting phase in an E-, P-, or L-selectin functionalized channel. (D) Representative relative frequency distribution of elution time of a cell pulse infused at 0.5 dyn cm^{-2}. Inset depicts the mean time at which 95% of the cell pulse has eluted ± s.e.m. (E) Percent of cells collected in each fraction after perfusion in an E-, P-, or L-selectin functionalized channel. (F) Viability of cells in each collected Free flow or Adherent fraction. (C-F) Perfusion and separation of a Phamret-expressing LS174T cell pulse of 250,000 cells over 2.5 μg mL^{-1} E-selectin at 1 dyn cm^{-2} or 25 μg mL^{-1} L- or P-selectin at 0.5 dyn cm^{-2}. (A-E) Each dot or bar represents the mean ± s.e.m. of n≥3 individually run experiments. Statistical analysis (A,B,F) by paired t-test or (E) one-way ANOVA with post hoc t-test with Bonferroni corrections for multiple comparisons.





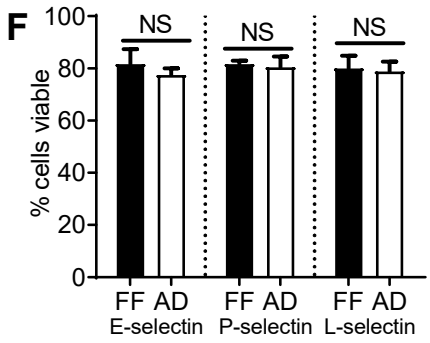


Figure S2. Elution time determination for collection of multiple AD fractions. (A) Relative cumulative frequency of elution time of the interacting cells in a perfused cell pulse. Orange, blue, and green lines represent elution of 33%, 50%, and 66% of the interacting cells, respectively. (B,C) Percent of cells in each fraction when either (B) 2 or (C) 3 Adherent fractions are collected. (A-C) Perfusion of 250,000 Phamret-expressing LS174T cells over 2.5 μg mL^{-1} E-selectin at 1 dyn cm^{-2}. (B,C) Each bar represents the mean ± s.e.m. of n≥3 individually run experiments.

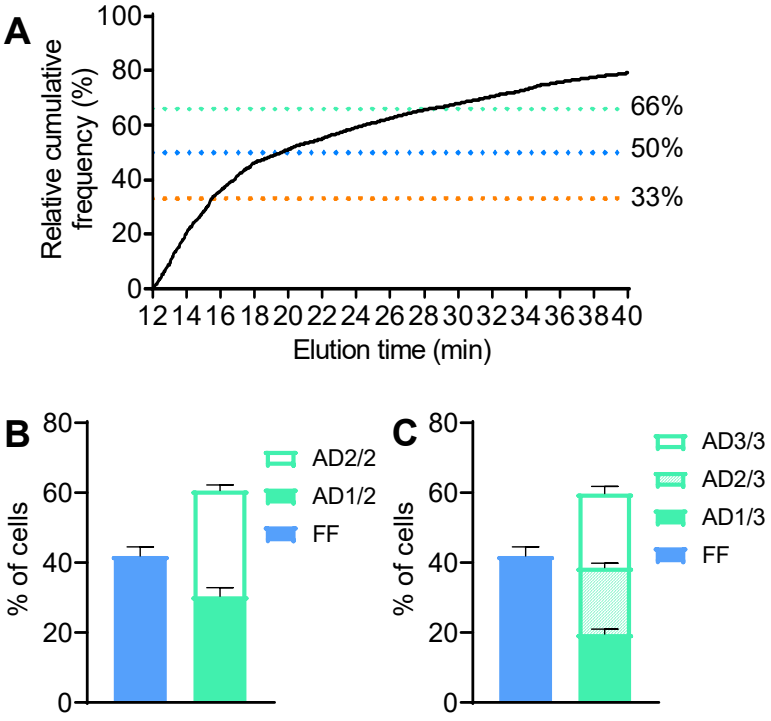


Figure S3. No correlations between ligand expression and GFP/CFP ratios among PC- cells within any collected cell fraction. (A-E) The extent of photoconversion (GFP/CFP ratio) of FF PC- cells and (A-C) 2 or (D-E) 3 AD fractions related to the expression of (A,D) sLe^x reactive HECA452, (B,E) CD44, and (C,F) CEA. (A-F) Binned, flow cytometry data of PC- cells was pooled from independent experiments and plotted with corresponding linear fits; C_F and C_{AD} represent the regression slope of the PC- cells in the Adherent and Free flow fractions, respectively. Outlier tests with criteria Q = 1 were conducted on all data sets, and outliers were removed prior to regression and statistical analysis. Perfusion, photoconversion, and separation of a Phamret-expressing LS174T cell pulse of 250,000 cell over 2.5 μg mL^{-1} E-selectin at 1 dyn cm^{-2}.

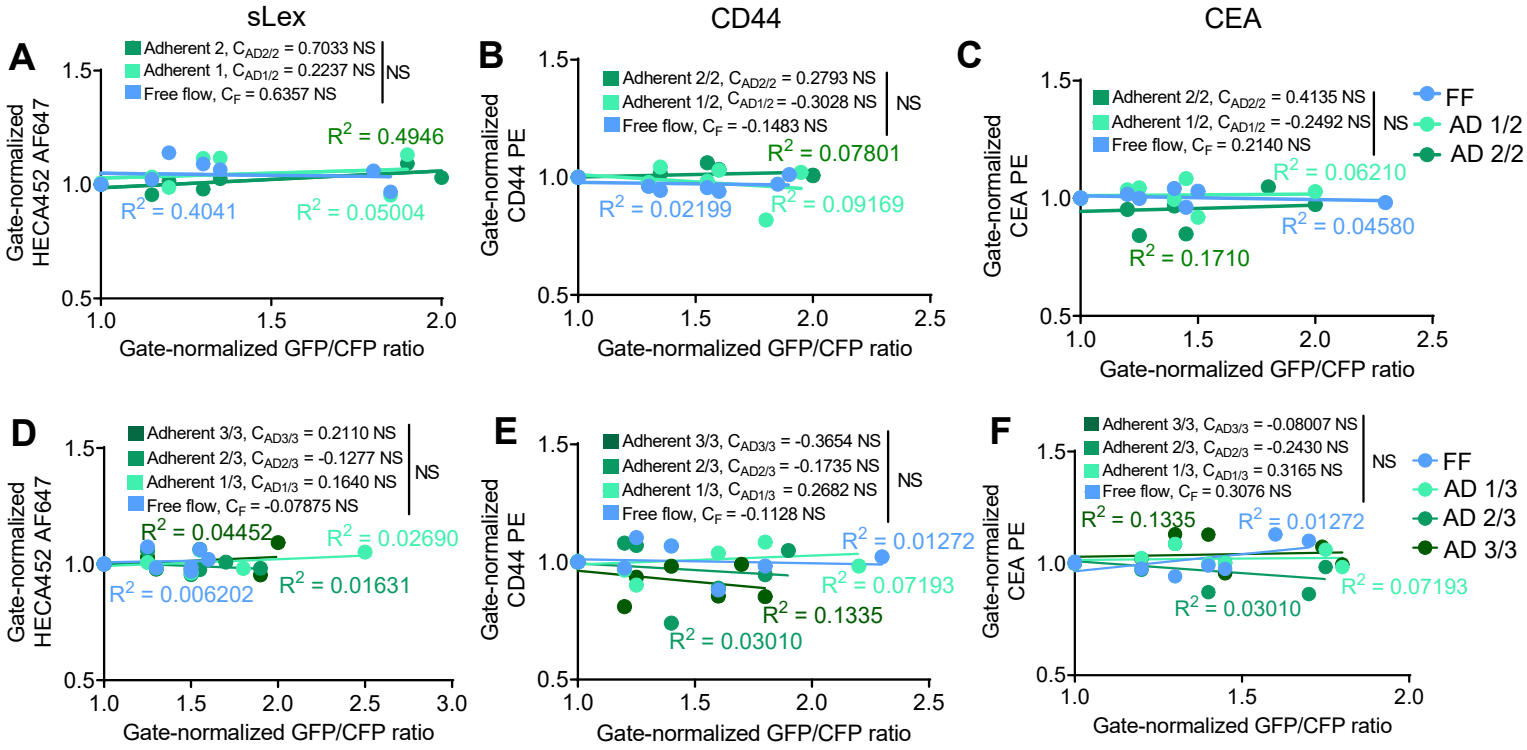


Figure S4. Effects of pre-photoconversion on cells pre and post perfusion. (A) Percent of viable Phamret-expressing LS174T cells after exposure to the 405 nm laser for different lengths of static pre-photoconversion time. Gray line represents 75% cell viability, black line represents 50% cell viability. (B,C) Percent of cells viable (B) 4 or (C) 8 min of pre-photoconversion. FF denotes Free flow, AD denotes Adherent. PC denotes photoconversion. (D,E) Percent of cells collected in each fraction after either (D) 4 or (E) 8 minutes of static photoconversion, followed by perfusion without photoconversion and fractionation. (A-E) Pre-photoconversion and perfusion of a Phamret-expressing LS174T cell pulse of 250,000 cell over 2.5 μg mL^{-1} E-selectin at 1 dyn cm^{-2}. (A-E) Each bar represents the mean ± s.e.m. of n≥3 individually run experiments. (B,C) ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ by one-way ANOVA with post hoc t-test with Bonferroni corrections for multiple comparisons. (D,E) Two sample t-test comparing percent of cells in each fraction compared to percent of cells in each fraction when cells are not pre-photoconverted (Figure S2B). NS denotes no significance.

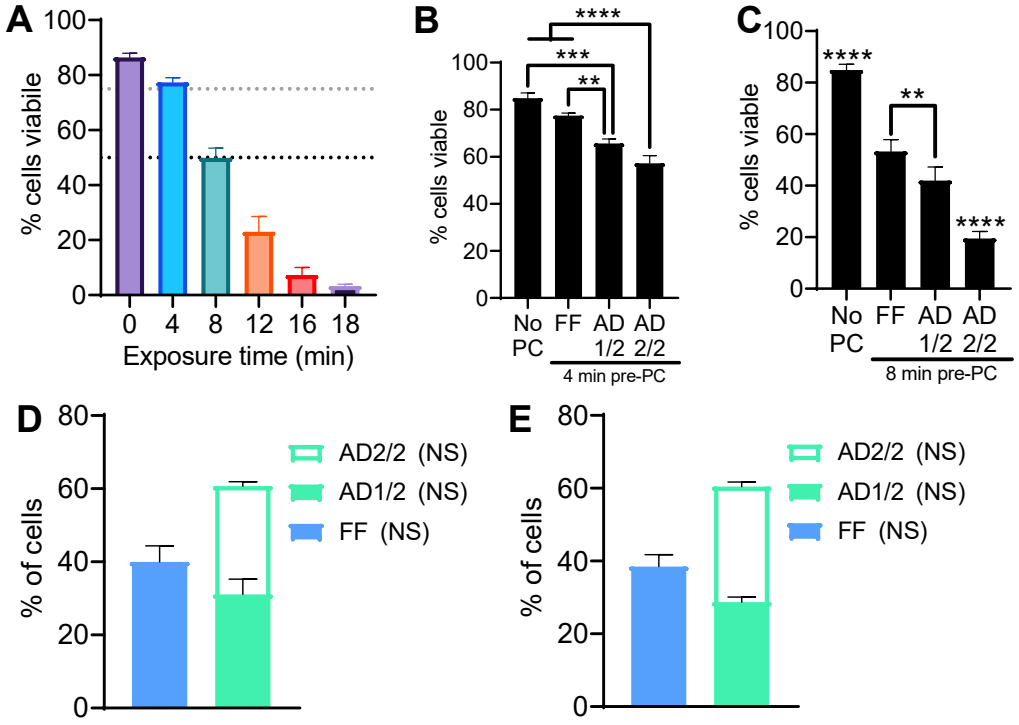


Figure S5. No correlation between selectin ligand expression and GFP/CFP ratios among cells that remain unphotoconverted. The extent of photoconversion (GFP/CFP ratio) of PC- cells within FF and AD fractions related to the expression of (A) sLe^x (HECA452), (B) CD44, and (C) CEA sorted over E-, P-, or L-selectin. (A-C) Binned, flow cytometry data of PC- cells was pooled from independent experiments and plotted with corresponding linear fits; C_{AD} and C_F represent the regression slope of the PC- cells in the AD and FF fractions, respectively. Outlier tests with criteria Q = 1 were conducted on all data sets, and outliers were removed prior to regression and statistical analysis. Perfusion, photoconversion, and separation of a Phamret-expressing LS174T cell pulse of 250,000 cell over 2.5 μg mL^{-1} E-selectin at 1 dyn cm^{-2} or 25 μg mL^{-1} P- or L-selectin at 0.5 dyn cm^{-2}.

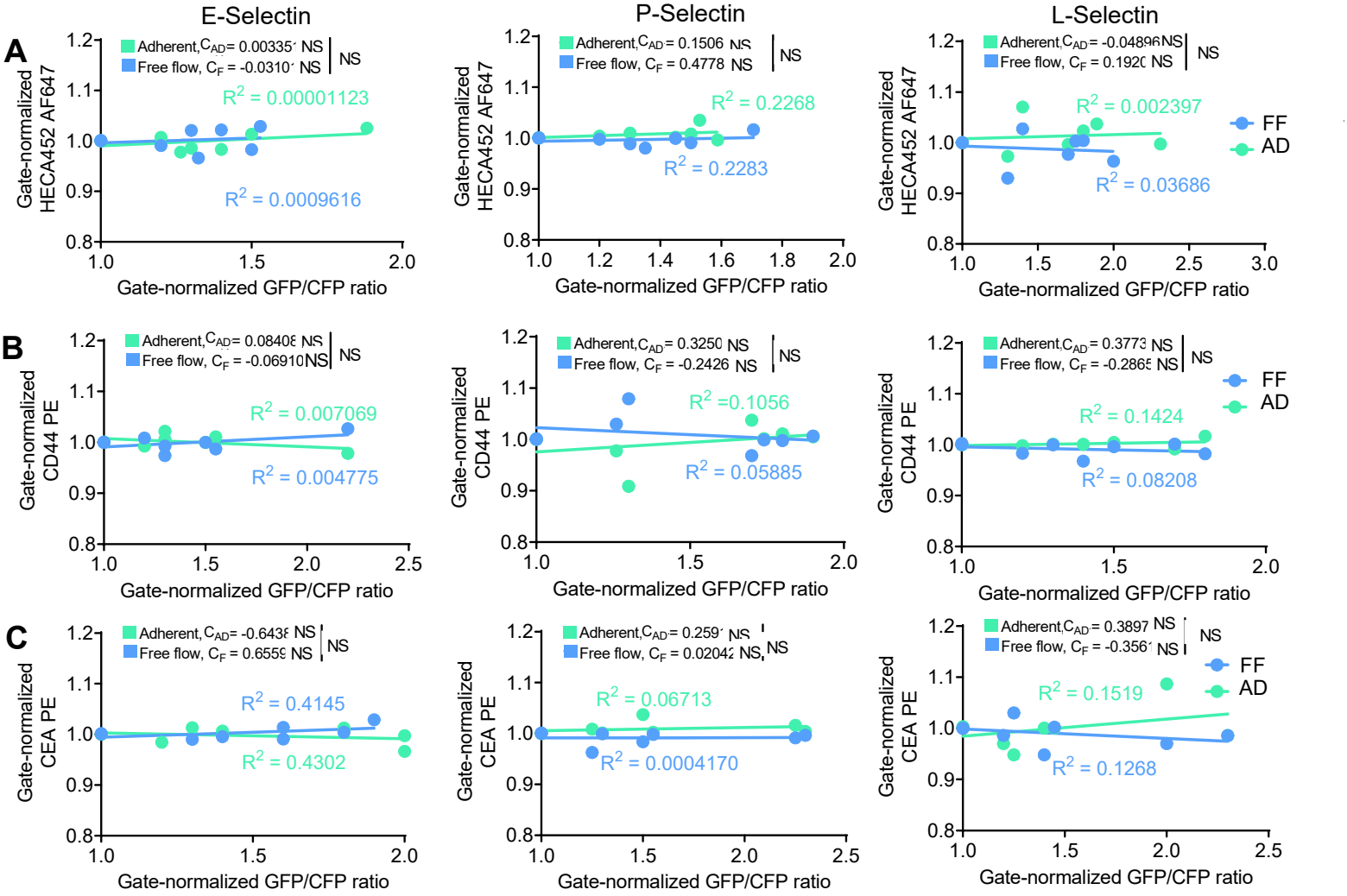


Figure S6. Neuraminidase treatment effects on cell adhesion in flow to P- and L-selectin. (A,C) Percent of cells collected in the FF and AD fractions. (B,D) Percent of cells photoconverted in the AD fraction normalized to the FF fraction after perfusion of a cell pulse of 250,000 Phamret-expressing LS174T cells over 25 μg mL^{-1} (A,B) P- or (C,D) L-selectin at 0.5 dyn cm^{-2}. FF denotes Each dot or bar represents the mean ± s.e.m. of n≥3 individually run experiments. **** $p < 0.0001$ by (A,C) one-way ANOVA with post hoc t-test with Bonferroni corrections for multiple comparisons or (B,D) one sample t-test with $h_0 = 1$; dashed line at y = 1.

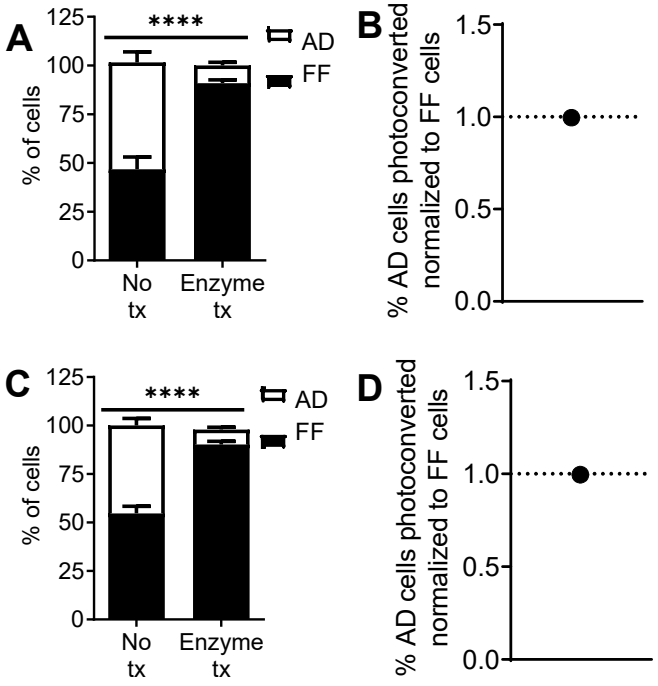


Figure S7. Comparison of interpolated standard curves. (A) Overlay of photoconversion standard curves created either by statically photoconverting 250,000 Phamret-expressing LS174T cells for various amounts of time (gray line) or perfusing a cell pulse of 250,000 Phamret-expressing LS174T cells in an unfunctionalized channel at various flow rates (black line). (B) Static standard curve (orange) and perfusion standard curve (black) from (A) overlaid with a perfusion standard curve from a population of Phamret-expressing LS174T cells (teal) thawed at a different time than the cells used to create the standard curves from (A).

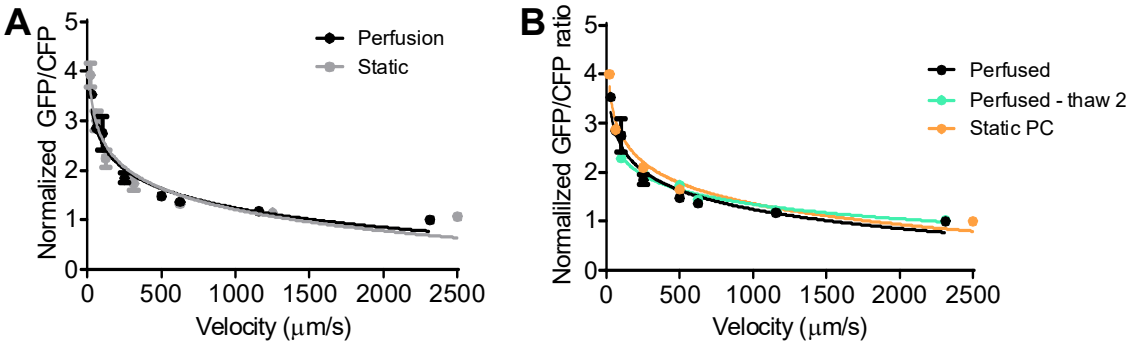


Figure S8. Verification of anti-IgG functionalization after perfusion. (A) Standard curve of mean fluorescence intensity (MFI) of FITC labeled anti-IgG at different concentrations with MFI of perfusion media collected after either functionalization and perfusion with 2.5 μg/mL anti-IgG at 1 dyn cm^{-2} for 28 minutes (purple circle) or 25 μg/mL anti-IgG at 0.5 dyn cm^{-2} for 20 minutes (orange square).