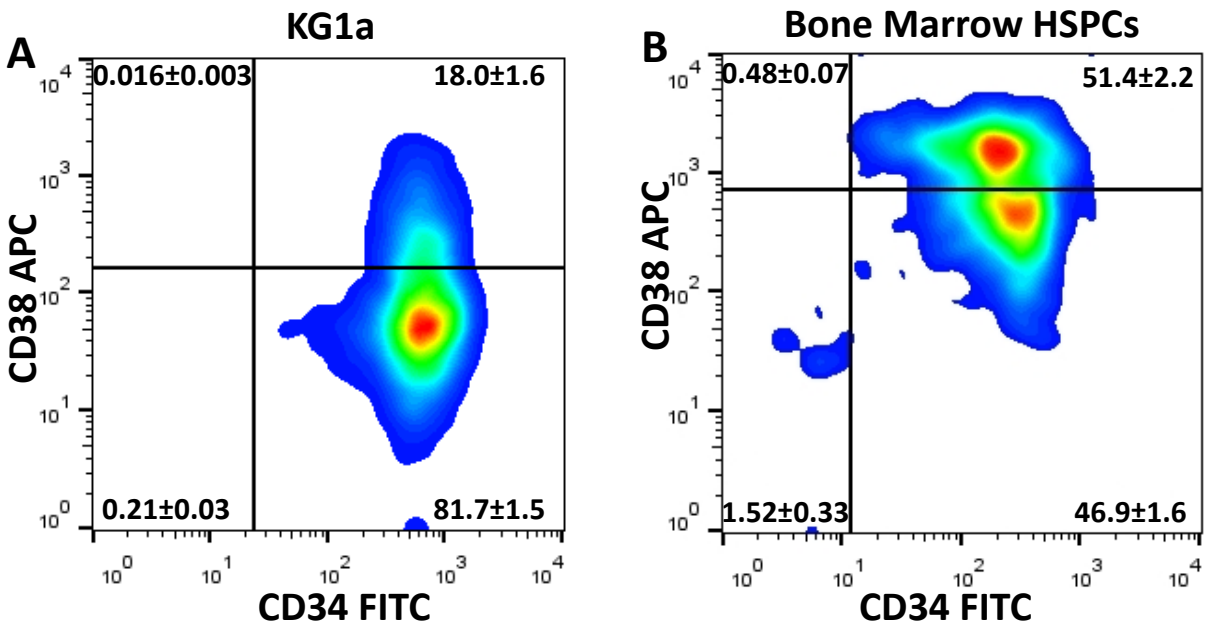
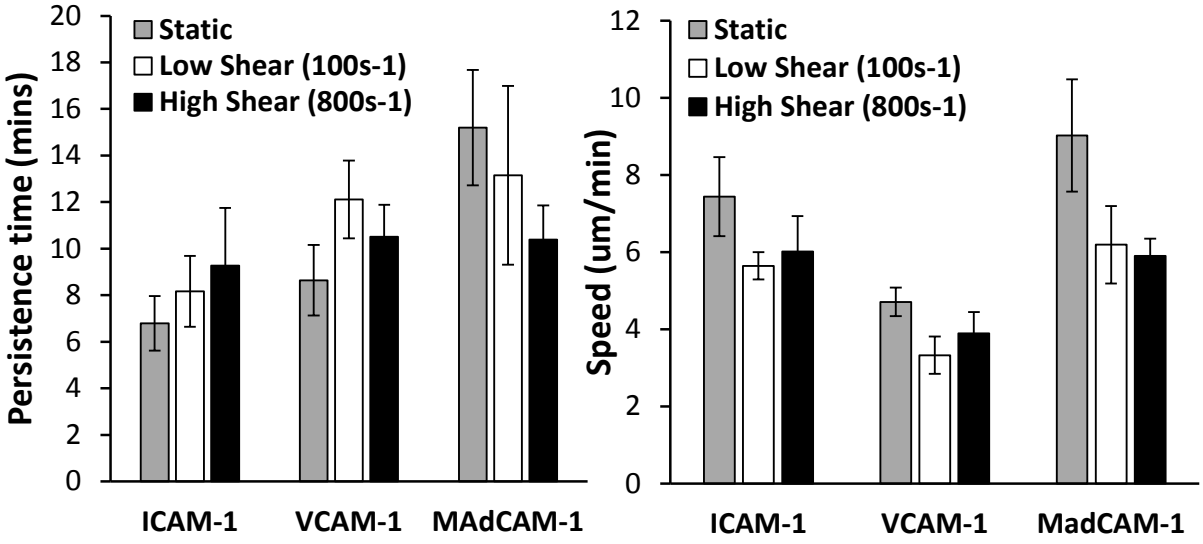
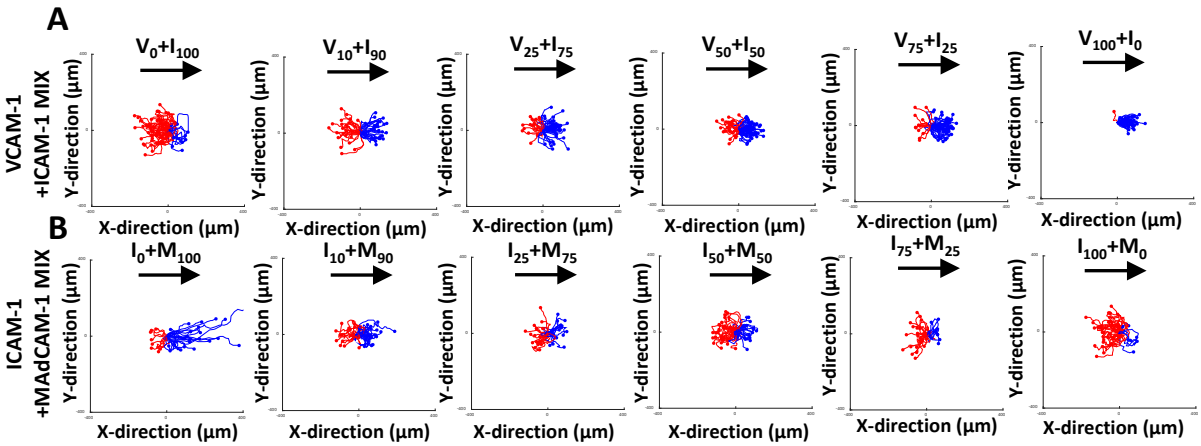


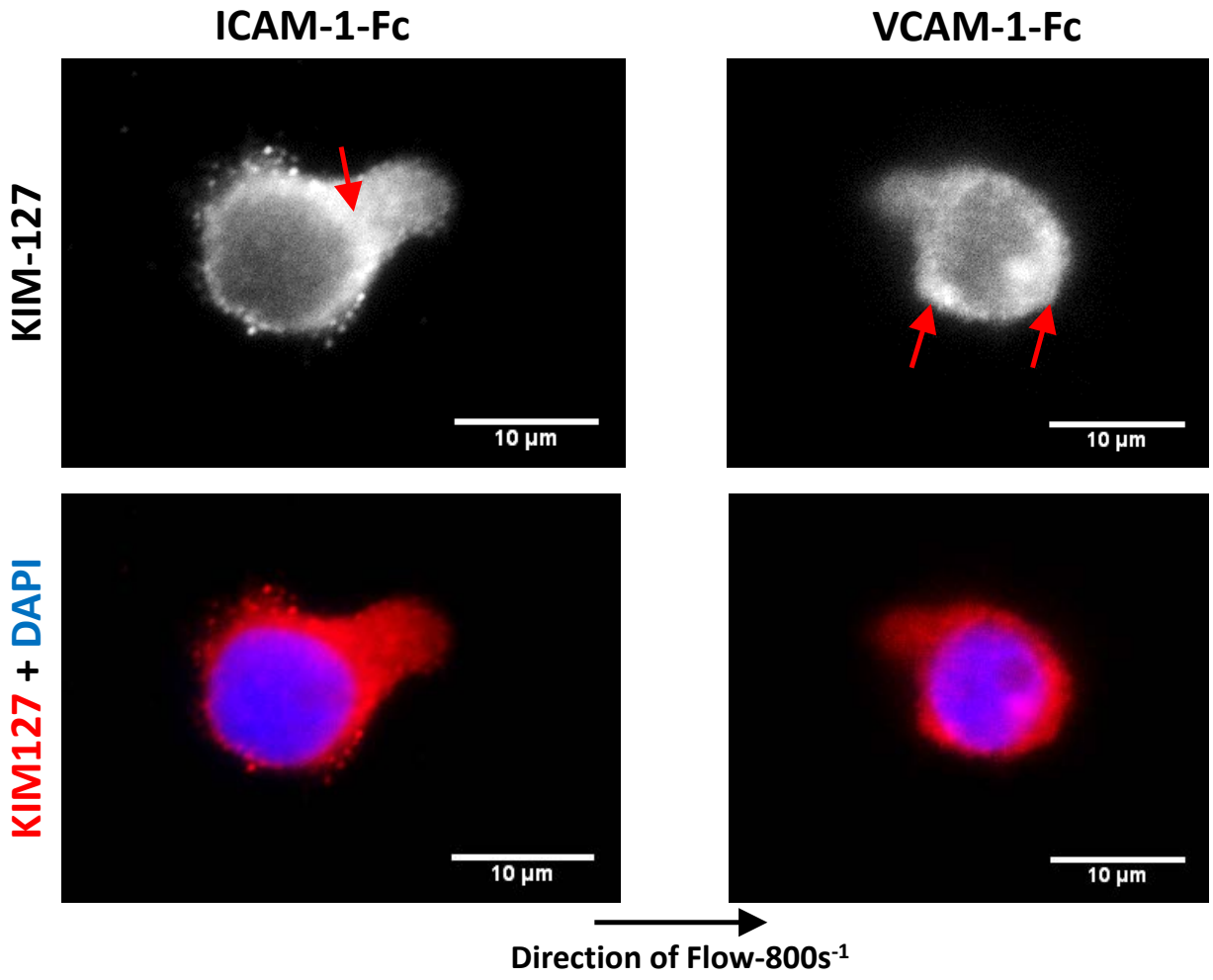
Supplemental Fig. 1. KG1a cells and HSPCs express similar levels of CD34^{+}: Density plot of (A) Kg1a cells and (B) primary bone marrow HSPCs of stem cell markers CD34 and CD38. Quadrant values correspond to percentage of cells in each respective quadrant ± SEM (n=4).



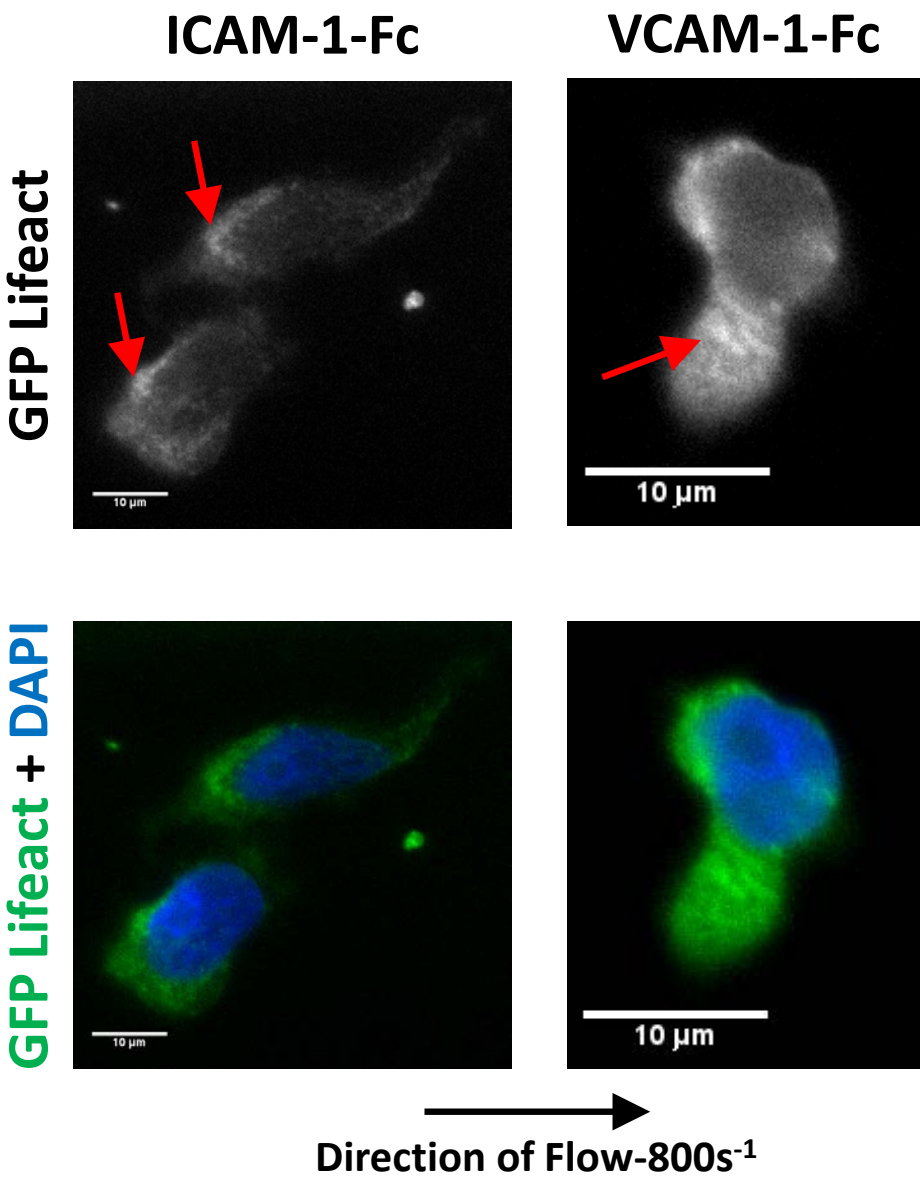
Supplemental Figure 2: Persistence time and speed are unchanged under various shear rates on all three CAMs: Persistence time and speed of KG1a cells under static conditions and both $100s^{-1}$ and $800s^{-1}$ shear on ICAM-1, VCAM-1, and MAdCAM-1 at a concentration of 2.5μg/ml. In all, the speed and persistence time are unchanged between low shear rate and high shear rate on each respective CAM surface. N=4 independent experiments of at least 70 cells analyzed per experiment for each CAM.



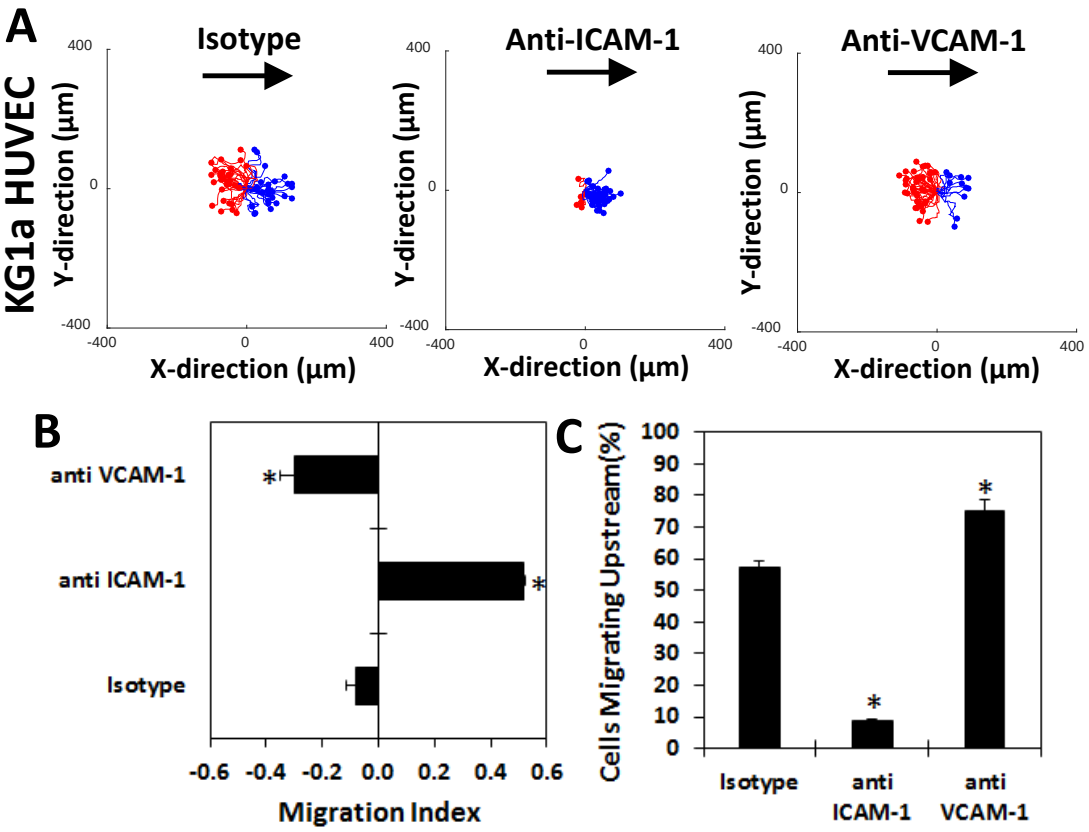
Supplemental Fig. 3. Cell tracks of KG1a cell migration on mixed surfaces at varied mass ratios: Cell traces of KG1a cells on mixed surfaces of varying ratios of (E) VCAM-1 and ICAM-1 and (G) ICAM-1 and MAdCAM-1 at both 800s^{-1} shear rate at a fixed total protein concentration of 5µg/ml. The numbers in subscript refer to the percentage of each respective CAM in the total concentration. The traces depicted are the cumulative tracks of two independent experiments and have units of µm.



Supplemental Fig. 4. Active LFA-1 localizes differently during upstream and downstream migration*:* KG1a cells were fixed after exposure to a 800s^{-1} shear rate for 30mins and stained with the LFA-1 conformation-sensitive antibody (KIM-127) which recognizes both the intermediate and high affinity form of the integrin. The data suggest that active LFA-1 localizes to the uropod and the trailing edge during upstream migration on ICAM-1 surfaces, while it localizes at the leading edge during downstream migration on VCAM-1 surfaces. Red arrows denote areas of high intensity staining. These are representative images of at least 10 analyzed cells per experiment over three independent experiments. Scale bars = 10µm.



Supplemental Fig. 5. F-actin localizes differently during upstream and downstream migration and opposite to active LFA-1*:* KG1a cells stably expressing GFP-Lifeact (which recognizes F-actin) were fixed after exposure to a $800s^{-1}$ shear rate for 30mins. The data suggest that F-actin localizes to the uropod and the trailing edge during downstream migration on VCAM-1 surfaces while localizing at the leading edge during upstream migration on ICAM-1 surfaces. Red arrows denote areas of high intensity staining. These are representative images of at least 20 analyzed cells per experiment over three independent experiments. Scale bars = 10µm.



Supplemental Figure 6*: Blocking ICAM-1 removes upstream migration while blocking VCAM-1 promotes upstream migration of KG1a cells on HUVEC monolayers:* (A) Cell traces of KG1a cells on IL-β stimulated HUVEC surfaces under isotype (first plot), anti-ICAM-1 blocking (second plot), or VCAM-1 blocking (third plot). (B) Migration index and (C) percentage of cells migrating upstream on these surfaces at $100s^{-1}$. KG1a cells migrate downstream upon blocking ICAM-1 and more robustly upstream upon blocking VCAM-1 on the HUVEC surface. N=3 independent experiments of at least 30 cells analyzed per experiment for each CAM. *$p < 0.05$ with respect to isotype control.

Supplemental Movie 1: KG1a cells migrating upstream on an ICAM-1 surface at a shear rate of $800s^{-1}$

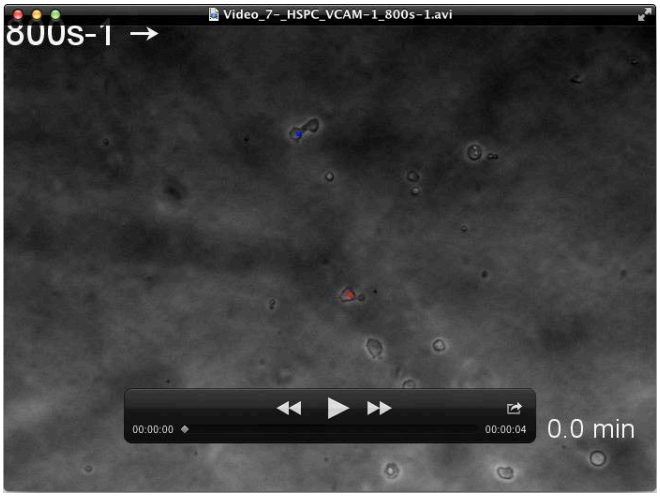
Supplemental Movie 2: KG1a cells migrating downstream on a VCAM-1 surface at a shear rate of $800s^{-1}$

Supplemental Movie 3: KG1a cells migrating in both directions on a MAdCAM-1 surface at a shear rate of $800s^{-1}$

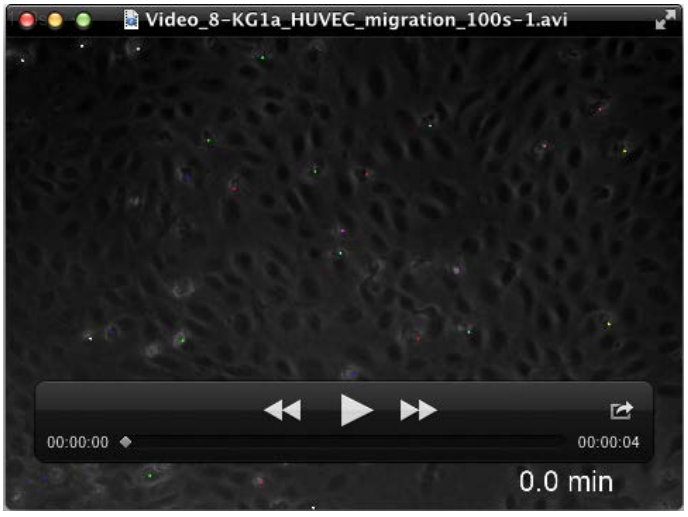
Supplemental Movie 4: KG1a cells migrating upstream on an ICAM-1 surface at a shear rate of $800s^{-1}$ with isotype blocking antibody

Supplemental Movie 5: KG1a cells migrating downstream on an ICAM-1 surface at a shear rate of $800s^{-1}$ with LFA-1 blocking antibody

Supplemental Movie 6: Primary HSPCs migrating upstream on an ICAM-1 surface at a shear rate of $800s^{-1}$



Supplemental Movie 7: Primary HSPCs migrating downstream on a VCAM-1 surface at a shear rate of 800s^{-1}



Supplemental Movie 8: KG1a cells migrating upstream on a stimulated HUVEC monolayer at a shear rate of 100s^{-1}