Supplementary Information

Stromal Cell Adhesion Predicts Severity of Metastatic Disease

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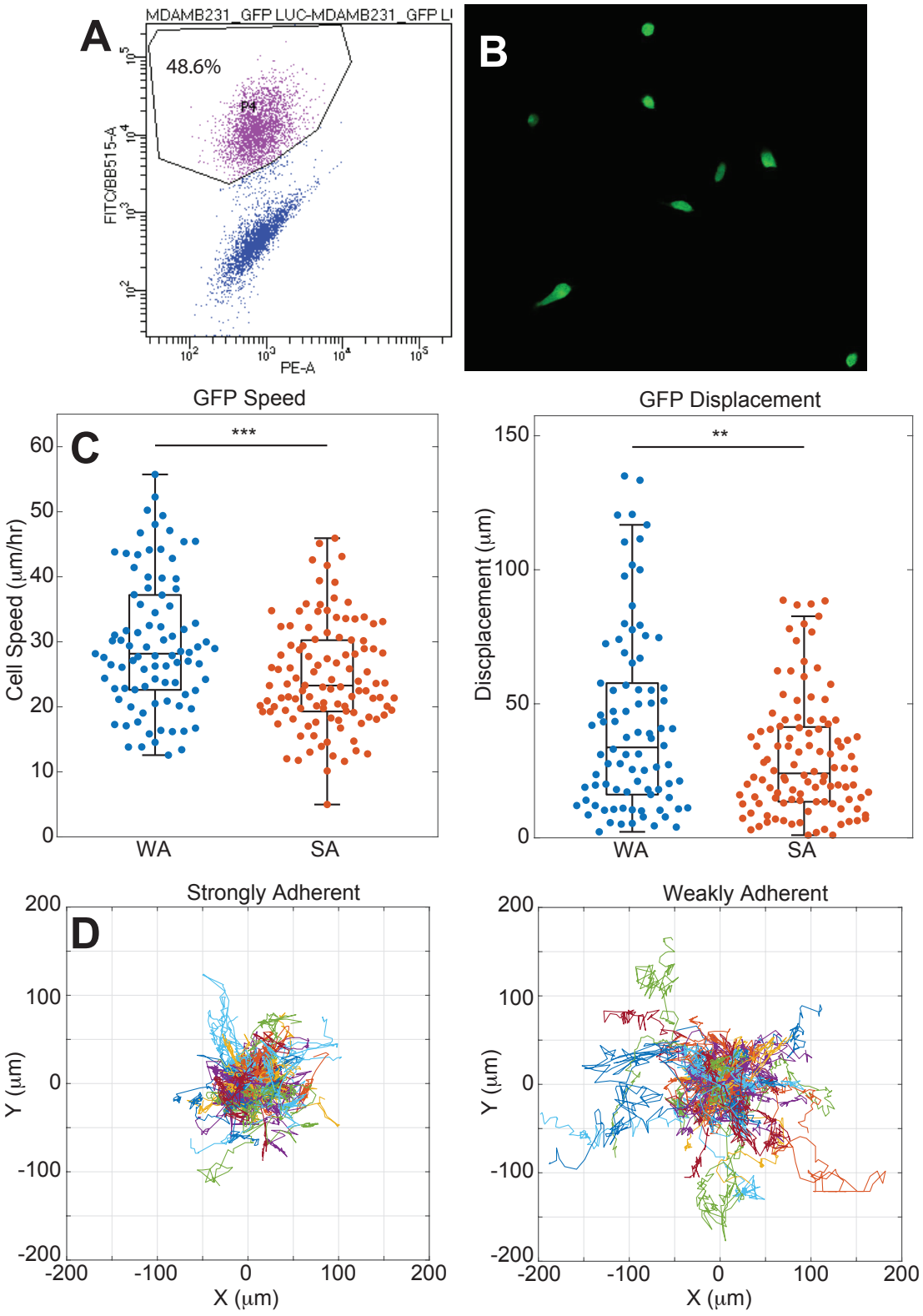
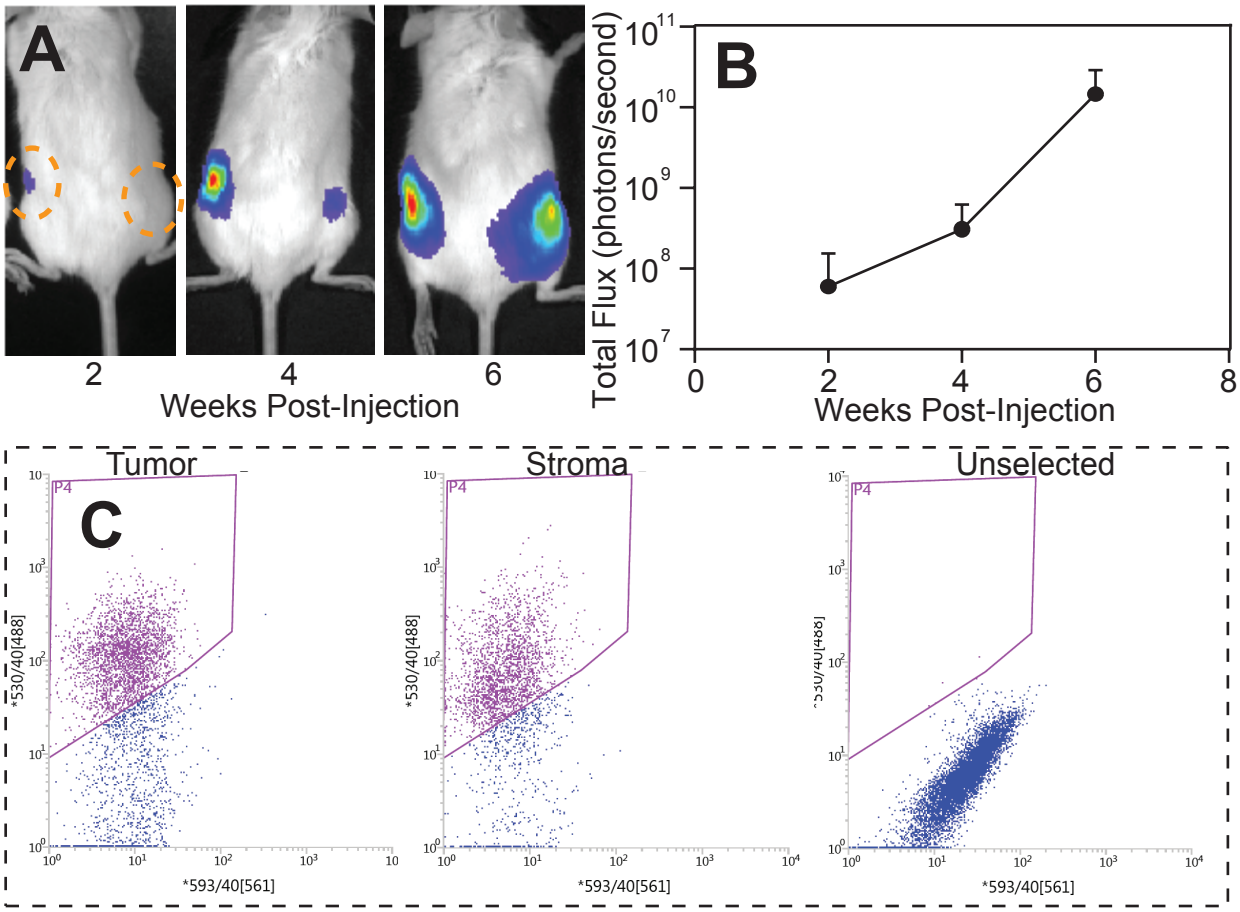
Running Title

Metastatic Disease with Adhesion

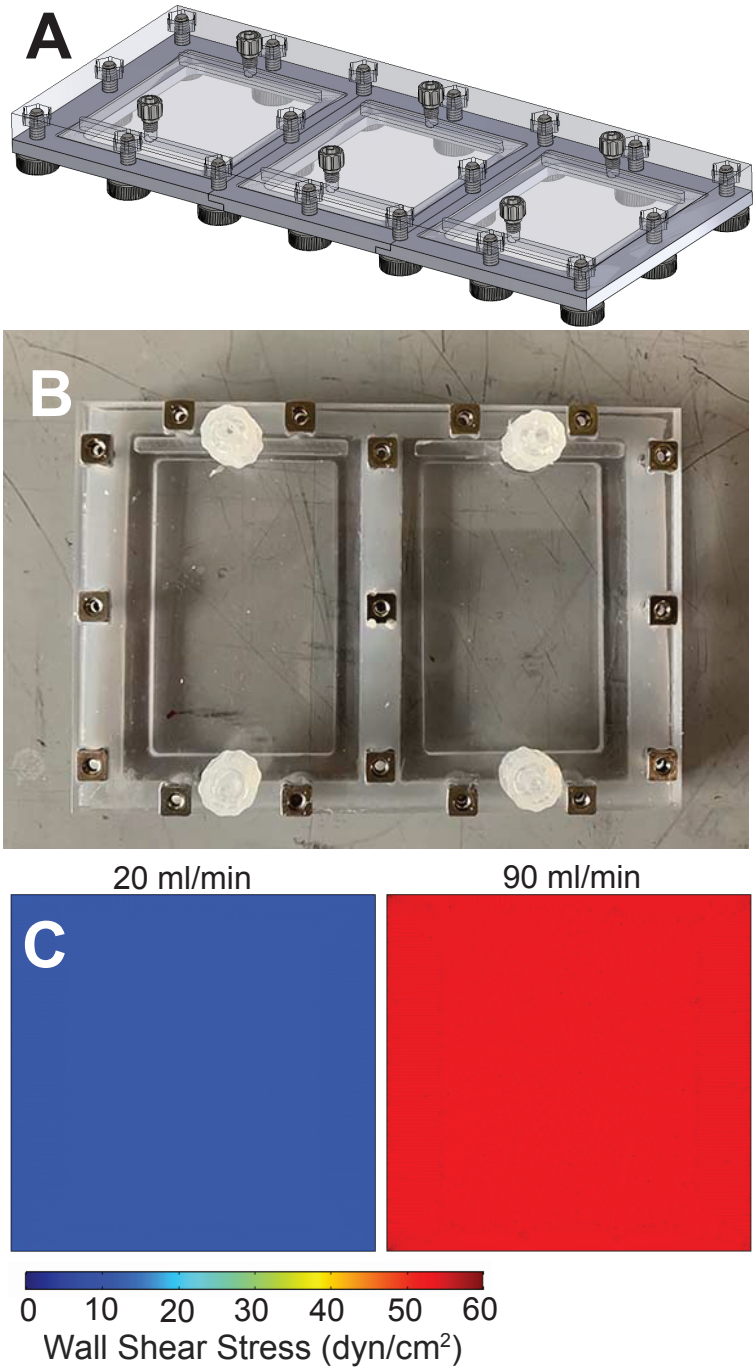
Supplemental Figures



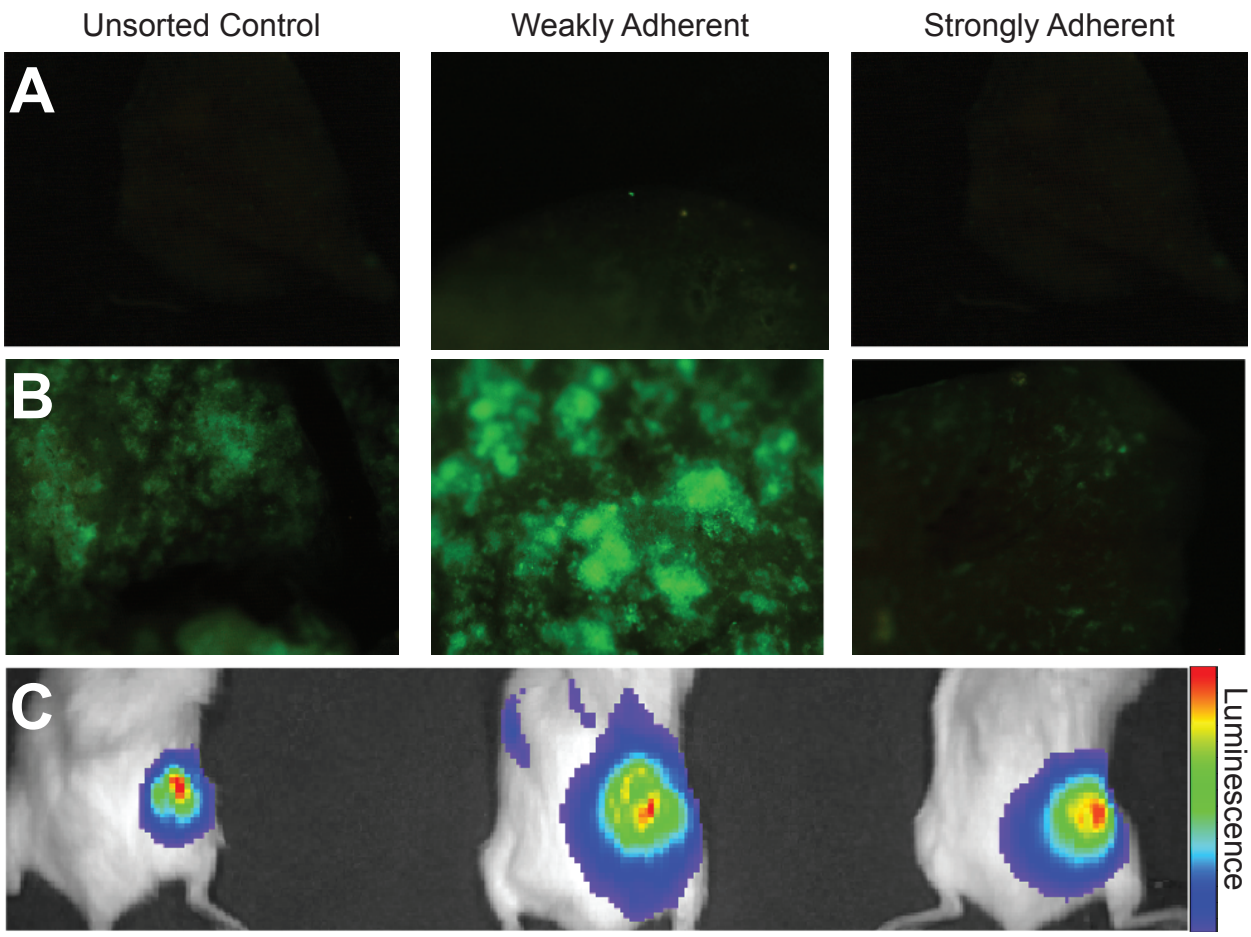
Supplemental Figure 1: GFP-Luciferase lentiviral transduction does not alter inherent heterogeneity of MDA-MB231 cells. (A) After treatment with puromycin to select for cells that expressed Luciferase, cells were sorted using FACS for GFP+ signal (y-axis). Gating strategy is based on excluding cells from an unlabeled control sample. (B) GFP expression was verified using fluorescence microscopy. (C,D) Weakly adherent cells were more migratory than their strongly adherent counterparts, consistent with previous findings. (n=89 and 111 for weakly and strongly adherent, respectively). (C) Statistical analysis via unpaired t-test. ** represents $p<0.01$. *** represents $p<0.001$.



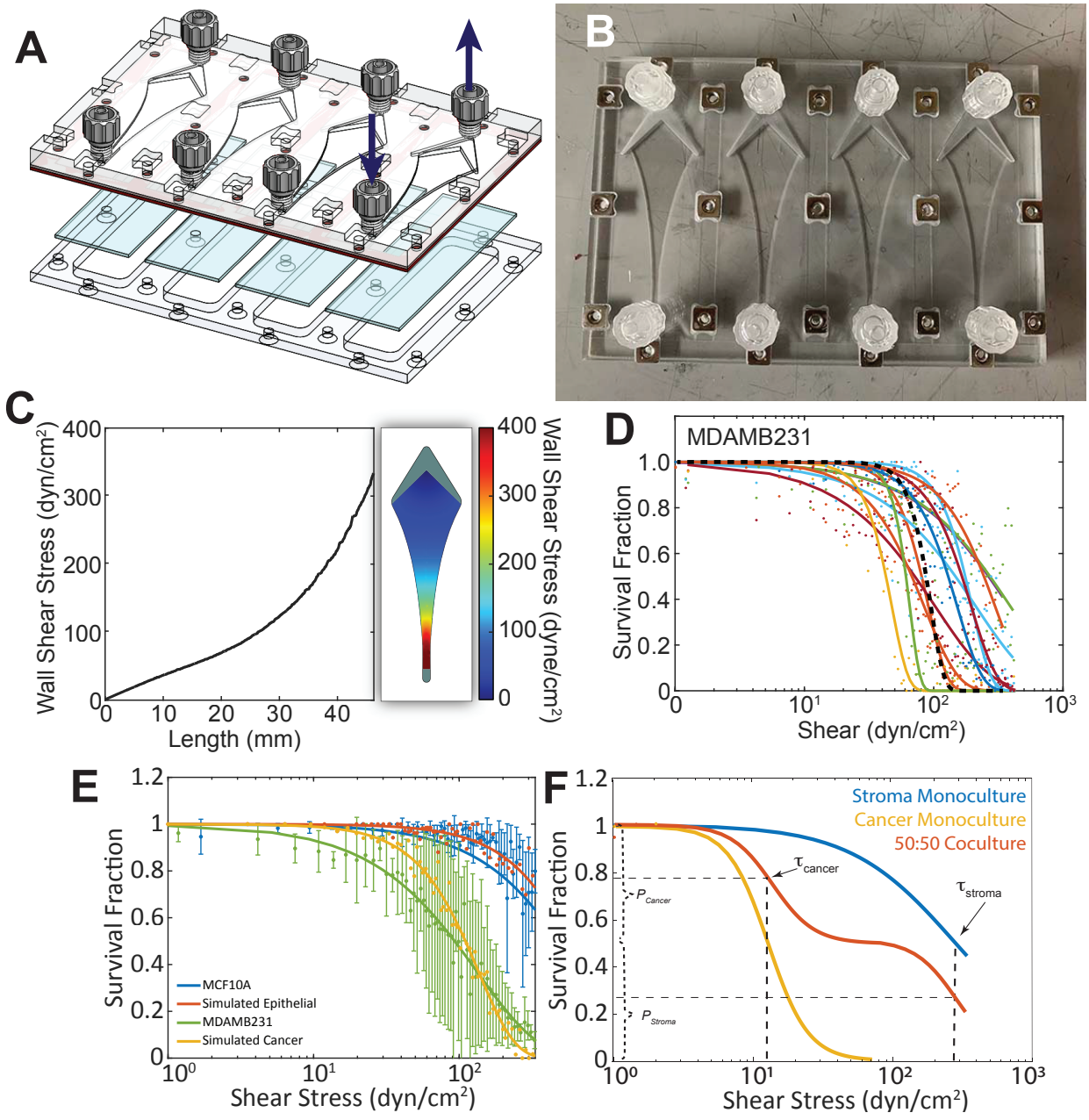
Supplemental Figure 2: Tumor growth was monitored using IVIS and GFP+ cells can be sorted from tumor and stroma. (A) Tumor growth was monitored using IVIS at 2-week intervals using (B) total flux as a measurement (n=6 mice). (C) After manual separation of the stiff tumor from the surrounding stroma and dissociation into single cells, GFP+ cells (y-axis) could be isolated from both tissue fractions using FACS.



Supplemental Figure 3: Parallel plate flow chamber applies a uniform shear stress to cells seeded in the device. (A) SolidWorks design and (B) top-down view of a straight walled parallel plate flow chamber (PPFC). (C) COMSOL simulation of the shear stress profile through the straight-walled parallel plate flow chamber (PPFC).

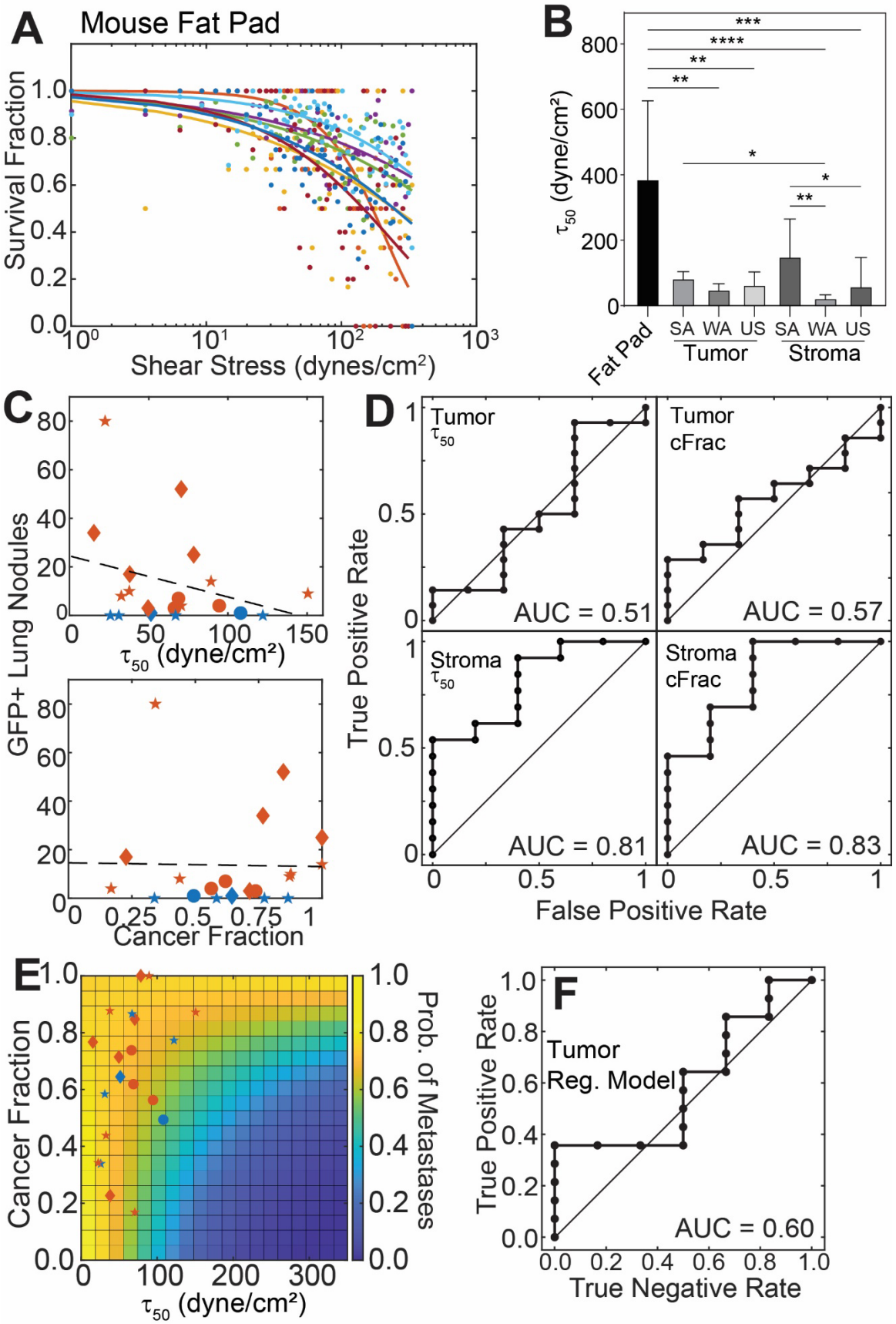


Supplemental Figure 4: Few lung metastases were seen 4 weeks post-injection, but lungs were saturated with metastases 8 weeks post-injection. Representative images of GFP+ lung metastases in mice sacrificed (A) 4 weeks or (B) 8 weeks post-injection. (B) Representative IVIS image of mice 8 weeks post-injection.



Supplemental Figure 5: Divergent parallel plate flow chamber applies an increasing shear stress to cells seeded in the device. (A) SolidWorks design, (B) top-down view, and (C) COMSOL simulation of the shear stress profile through the imaging, divergent parallel plate flow chamber (dPPFC). (D) Percent of attached cells versus shear stress value in the imaging PPFC for various replicates of perfusion of MDAMB231 cells. Black dashed line represents the average shear stress plot of the replicates (n=12 replicates; >500 cells/replicate). (E) Comparison of statistically generated adhesion profiles and

67 experimental adhesion profiles (n=3 replicates/cell line). (F) Schematic of adhesion profile
68 to highlight how cancer fraction, P_c, and adhesion strength are plotted on an adhesion
69 metric with the dPPFC; dashed lines indicate P_{cancer} and P_{Stroma} and darker dashed lines
70 represent media cell adhesion strength for a given population.


A
Mouse Fat Pad
Survival Fraction
Shear Stress (dynes/cm²)
B
τ50 (dyne/cm²)
Fat Pad
SA WA US SA WA US
Tumor
Stroma
C
GFP+ Lung Nodules
τ50 (dyne/cm²)
Cancer Fraction
D
Tumor τ50
Tumor cFrac
Stroma τ50
Stroma cFrac
AUC = 0.51
AUC = 0.57
AUC = 0.81
AUC = 0.83
True Positive Rate
False Positive Rate
E
Cancer Fraction
τ50 (dyne/cm²)
Prob. of Metastases
F
Tumor Reg. Model
AUC = 0.60
True Positive Rate
True Negative Rate

Supplemental Figure 6: Raw data from mammary epithelial cancer adhesion assays. (A) Shear plots of replicates of cells dissociated from the contralateral mammary fat pad (n=7 mice). (B) Average shear stress of cells dissociated from the contralateral mammary fat pads, tumors, and surrounding stroma (n=7 mice/condition). (C) Average shear stress and cancer fraction vs. GFP+ lung nodules from resected tumor samples (n=20 lungs). (D) ROC curves of metastatic risk predictions for average shear stress or cancer fraction for resected tumor and stroma samples. (E) Logistic regression model showing probability estimate of a mouse having ≥2 tumor based on the average shear stress and cancer fraction for tumor sample. (F) ROC curve of metastatic risk predictions based on model's probability estimates. Red points are classified as high metastatic risk and blue are low.

Supplemental Table Legends

Supplemental Table 1: DEGs for Weakly and Strongly Adherent Cells in Tumors In Vivo. Table showing changes in the transcriptome of MDA-MB-231 cells sorted by adhesion and injected as primary tumors. Data listed is annotated for common gene name followed by (left to right): gene name, gene description, fold change for the comparison of weak/strong adhesion, log_2 Fold Change, p-Value, p-Adj, Average Log2 Expression, gene ID, and any gene aliases, if available.

Supplemental Table 2: Intersection of GO terms between primary tumors and pre-sorted cells. DEGs from primary tumors sequenced here and pre-sorted cells sequenced previously (GEO number GSE135515) in vitro were analyzed for GO terms assigned by Panther. Listed in this table are (left to right): In Vitro - Biological Process, In Vitro - Cell Component, In Vivo - Biological Process, and In Vivo - Cell Component.

Supplemental Table 3: Intersection of migration and locomotion GO terms between primary tumors and pre-sorted cells. Top GO terms (as assigned by Panther) between in vitro and in vivo primary tumors and their corresponding p-Values as plotted in Figure 2F. Data is ordered by in vivo p-value.

Supplemental Table 4: Cell line culture conditions. Media formulation for each cell line. Note the following abbreviations: Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum (FBS), penicillin/streptomycin (P/S), hEGF, and horse serum (HS).