Linking Invasive Motility to Protein Expression in Single Tumor Cells

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### SUPPORTING MATERIAL

#### **Supplementary Methods**

#### Silicon Master Fabrication

After the silicon wafer was pre-cleaned and baked, it was allowed to cool to room temperature. A 15 µm layer of SU-8 was created by pouring 7 milliliters of SU-8 2010 on the wafer and spun at 1600 rpm for 30 s (Brewer Science). The wafer was then soft baked on a hot plate (Corning) for 1 min at 65°C and then for 3 min at 95°C. The wafer was cooled to room temperature and the SU-8 was exposed to 365-nm UV light at a dose of 140 mJ/cm<sup>2</sup> (OAI Series 200 aligner, San Jose, California) under the "migratory microchannel photomask". The wafer was then baked for 1 min at 65°C and then for 3 min at 95°C and cooled to room temperature. Then, a 50 µm layer of SU-8 was spun on top of the previous SU-8 layer by pouring 7 milliliters of SU-8 2025 on the existing SU-8 layer and spun at 1500 rpm for 30 s. The wafer was then soft baked again for 1 min at 65°C and then for 6 min at 95°C. The "gradient-generating channel photomask" was carefully aligned to the previously photopatterned migratory microchannels and exposed to UV light at a dose of 160 mJ/cm<sup>2</sup>. The wafer was baked again for 1 min at 65°C and then for 6 min at 95°C. The patterns were then developed using SU-8 Developer (Microchem, Boston, MA) and the wafers were hard baked for 30 min at 150°C. After slowly cooling to room temperature, the final wafer was coated with dimethyldichloromethylsilane (Sigma-Aldrich) using the vapor deposition method.

### **Supplementary Figures**



**Supplementary Figure 1. SCAMPR Device Design and Fabrication.** a) Cross section of the SCAMPR device. The dashed red line indicates the where the schematic of cross-section is take from on the device. b) Device fabrication. A 1.5 mm thick layer of polydimethylsiloxane (PDMS), with holes punched for cell seeding, is placed on top of a micropatterned polyacrylamide (PA) gel. The PDMS/PA device is then subjected to a brief vacuum treatment to "seal" the two layers together.



Supplementary Figure 2. Fluorescent Intensity Plots of the 10-hour FITC-Dextran Gradient in 3 separate SCAMPR Devices.(a)-(c) Fluorescence intensity curves for each hour of the 10-hour FITC-Dextran gradient for one microchannel in a SCAMPR device for three different devices (Coefficient of variation for (a), (b), and (c) are 9.40%, 20.0% and 2.98%, respectively).



**Supplementary Figure 3. DN Rac1 cells are significantly slower than Empty Vector cells on a 2D glass substrate.** A dot plot of cell speed for Empty Vector and the DN Rac1 cell lines shows that the DN Rac1 cells are significantly slower than Empty Vector cells (\*Welch's t-test, p<0.0001, t=10, df=41.76). Cells were plated on a fibronectin coated 2D glass substrate and motility was measured using time-lapse images acquired every 15 minutes over a 6-hour period. The centroid of each cell was tracked from one frame to another to yield instantaneous migration speeds, which were then averaged over the entire time course of the experiment to yield the migration speed of a cell. D'Agostino-Pearson omnibus normality test was used to determine normality of the two populations. Using the F test, variances in the two populations were determined to be significantly different (F-statistic=8.714, p<0.0001, degrees of freedom: (32,22)). n=33 and 23 cells for Empty Vector and DN Rac1, respectively.



Supplementary Figure 4. The rank of persistence, but not the rank of aspect ratio, correlates with the rank of TIC speed in the SCAMPR device. Scatter plots reveal that that the rank of TIC persistence (b), but not the rank of aspect ratio (b) positively correlates with the rank of TIC speed (Spearman's rank  $r_{Persistence}$ =0.664, p<0.0001, n=64 cells;  $r_{Aspect Ratio}$ =-0.134, p=0.430, n=37 cells).



Supplementary Figure 5. TCGA analysis reveals elevated RNA expression levels of Nestin, STAT3, EphA2 and  $\beta$ -tubulin in classical GBM tissue compared to Normal Brain Tissue. (a)-(d) Analysis of mRNA expression in Classical GBM and normal brain tissue samples reveal statistically significantly higher expression levels of Nestin, STAT3, EphA2, and  $\beta$ -tubulin in GBM samples compared to normal brain tissue (Nestin, STAT3, EphA2,  $\beta$ -tubulin: Mann-Whitney Test, p<0.001; Nestin: Mann-Whitney U=0, n=11, 54 for normal brain and classical GBM, respectively; STAT3: Mann-Whitney U=2, n=11, 54 for normal brain and classical GBM, respectively; EphA2: Mann-Whitney U=107, n=11, 54 for normal brain and classical GBM, respectively; Bann-Whitney U=67, n=10, 53 for normal brain and classical GBM, respectively). Each point represents one patient tumor.



Supplementary Figure 6. TCGA analysis reveals positive correlations of RNA expression between EphA2 and Nestin as well as between STAT3 and  $\beta$ -tubulin. Analysis of TCGA data reveals that (a) EphA2, but not (b) STAT3 nor (c)  $\beta$ -tubulin expression correlates with Nestin expression (Spearman's rank  $r_{EphA2}$ =0.395, p=0.004, n=52 tumors;  $r_{STAT3}$ =-0.062, p=0.661, n=52 tumors;  $r_{\beta$ -tubulin}=0.220, p=0.113, n=53 tumors). A similar analysis reveals that STAT3 does not correlate with (d) EphA2, but does correlate with (e)  $\beta$ -tubulin (Spearman's rank  $r_{EphA2}$ =-0.019, p=0.894, n=53 tumors;  $r_{\beta$ -tubulin}=0.303, p=0.027, n=53 tumors). f) Scatter plot of EphA2 and  $\beta$ -tubulin expression levels show no correlation (Spearman's rank r=-0.006, p=0.961, n=53 tumors). Each point represents one patient tumor.



Supplementary Figure 7. Ranking TCGA derived RNA expression values shows a correlation between the ranks of EphA2 and Nestin as well as between the ranks of STAT3 and  $\beta$ -tubulin. Scatter plots reveal that the rank of EphA2 (a), but not the rank of STAT3 nor the rank of  $\beta$ -tubulin TCGA derived RNA expression values correlates with the rank of Nestin expression (Spearman's rank r<sub>EphA2</sub>=0.398, p=0.004, n=52 tumors; r<sub>STAT3</sub>=-0.142, p=0.317, n=52 tumors; r<sub> $\beta$ -tubulin</sub>=0.219, p=0.116, n=53 tumors). A similar analysis reveals that the rank of STAT3 expression does not correlate with (d) the rank of EphA2 expression, but does correlate with (e) the rank of  $\beta$ -tubulin expression (Spearman's rank r<sub>EphA2</sub>=-0.019, p=0.894, n=53 tumors; r<sub> $\beta$ -tubulin</sub>=0.303, p=0.028, n=53 tumors). f) Scatter plot of the rank of EphA2 and  $\beta$ -tubulin expression levels show no correlation (Spearman's rank r=-0.005, p=0.974, n=53 tumors). Each point represents one patient tumor.



Supplementary Figure 8. Ranking SCAMPR protein expression values reveals a correlation between the ranks of Nestin and  $\beta$ -tubulin. Scatter plots do not reveal a correlation between (a) the ranks of Nestin and EphA2 expression nor between (b) the ranks of Nestin and STAT3 expression, but does reveal a positive correlation between (c) the ranks of Nestin and  $\beta$ -tubulin (Spearman's rank  $r_{EphA2-Nestin}=-0.351$ , p=0.118, n=21 cells;  $r_{STAT3-Nestin}=0.295$ , p=0.055, n=43 cells;  $r_{\beta-tubulin-Nestin}=0.503$ , p=0.002, n=34 cells). A similar analysis does not reveal a correlation between (d) the ranks of STAT3 and EphA2 expression, (e) the ranks of STAT3 and  $\beta$ -tubulin expression, and (f) the ranks of EphA2 and  $\beta$ -tubulin expression (Spearman's rank  $r_{STAT3-EphA2:}=0.263$ , p=0.276, n=19 cells;  $r_{STAT3-\beta-tubulin}=0.362$ , p=0.054, n=29 cells;  $r_{EphA2-\beta-tubulin}=0.453$ , p=0.069, n=17 cells)



Supplementary Figure 9. SCAMPR assay reveals that the ranks of Nestin and EphA2 correlate with the rank of cell speed. Scatter plots of individual TICs show that the ranks of (a) Nestin and (b) EphA2 are positively correlated with the rank of TIC speed (Spearman's rank  $r_{Nestin}$ =0.383, p=0.001, n=68 cells;  $r_{EphA2}$ =0.451, p=0.040, n=21 cells). However, the ranks of (c) STAT3 and (d)  $\beta$ -tubulin are not correlated with the rank of TIC speed (Spearman's rank  $r_{STAT3}$ =0.031, p=0.0842, n=43 cells;  $r_{\beta$ -tubulin=-0.001, p=0.997, n=34 cells).



Supplementary Figure 10. ICC quantification of Nestin expression reveals no significant correlation between Nestin and TIC speed. Scatter plot of ICC-quantified Nestin expression and TIC motility in the SCAMPR device shows no statistically significant correlation between these two parameters (Spearman's rank r=0.053, p=0.696, n=56 cells).



**Supplementary Figure 11. Low EphA2 expressing TICs are significantly slower than High EphA2 expressing TICs in the SCAMPR device.** A dot plot of cell speed for Low EphA2 (bottom 5% expression level) and High EphA2 (top 5% expression level) TIC subpopulations shows that the Low EphA2 cells are significantly slower than the High EphA2 cells in the SCAMPR device (\*Mann-Whitney Test, p<0.0001, n=18,24 for the low and high EphA2 subpopulations, respectively). The cells in this figure represent subpopulations of the cell line quantified in Fig. 4a.

# Phenotypes

Speed	0.0023
Persistence	0.2069
Aspect Ratio	-0.0074

### **Proteins**

Nestin	0.102
STAT3	0.006
EphA2	0.004
β-Tubulin	-0.006

## b

# Phenotypes

Speed	0.925
Persistence	0.738
Aspect Ratio	-0.261

## Proteins

Nestin	0.881
STAT3	0.309
EphA2	0.420
β-Tubulin	-0.253

**Supplementary Figure 12. Canonical Correlation Analysis of phenotypic and proteotypic sets of variables.** (a) Canonical Correlation Analysis coefficients for the 3 phenotypic and 4 proteotypic variables. (b) Pearson correlation coefficient for each variable and their respective canonical component (phenotype or proteotype).

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Supplemental Photomask

Supplementary Photomask 1: Migratory microchannel photomask

Supplementary Photomask 2: Gradient-generating channel photomask

### Supplementary Videos

Supplementary Video 1. Representative time-lapse of GBM TICs migrating in the SCAMPR device. Scale bar is 100  $\mu m.$