Electronic Supplementary Information for Barney et al., 2014

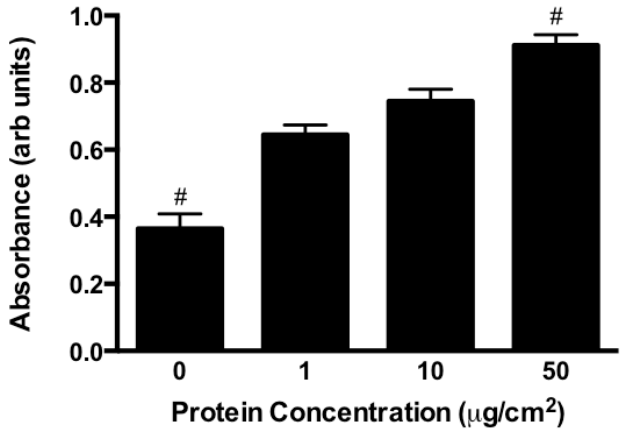

Figure S1. ECMs have controlled protein binding. Binding of Collagen III to our surfaces was measured using a modified ELISA. # identifies conditions that are significantly different from all others. Data are mean ± SEM. N = 3 independent replicates.

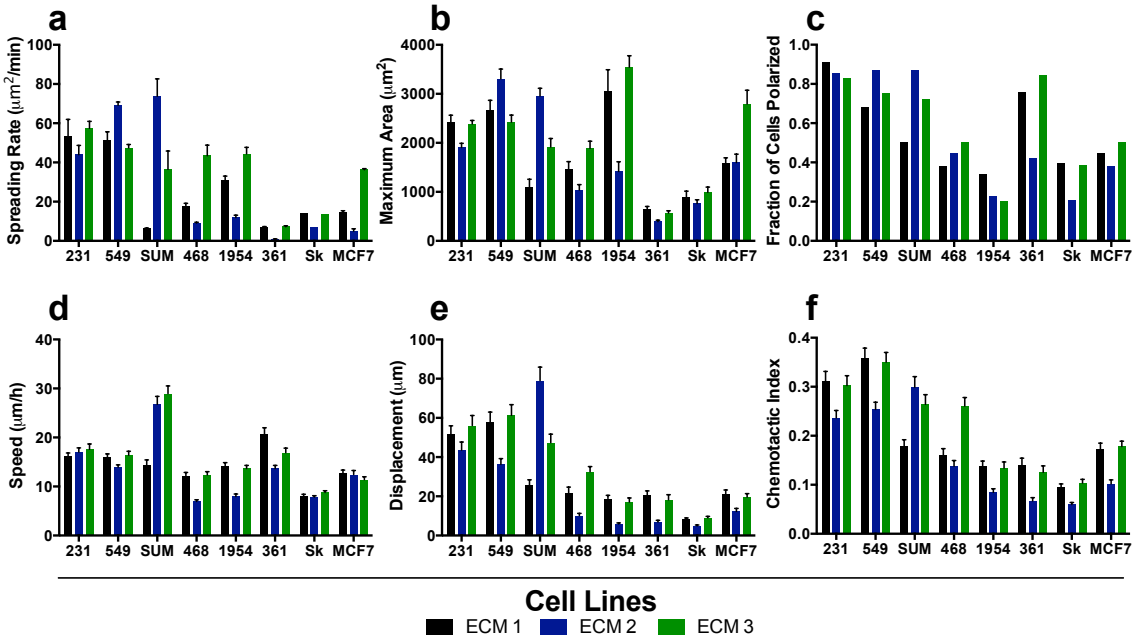

Figure S2. Detailed view of cell adhesion and motility across all cell lines. (a) Spreading rate, (b) maximum area, and (c) fraction of cells polarized. (d) Migration speed, (e) displacement, and (f) chemotactic index. Black bars: ECM 1; blue: ECM 2; green: ECM 3. Abbreviations: 231: MDA-MB-231; 549: BT549; SUM: SUM1315 MO2; 468: MDA-MB-468; 1954: HCC 1954; 361: MDA-MB-361; Sk: SkBr3. N ≥ 2 biological replicates. (a-c) N ≥ 40 cells/condition, (d-f) N ≥ 90 cells/condition.

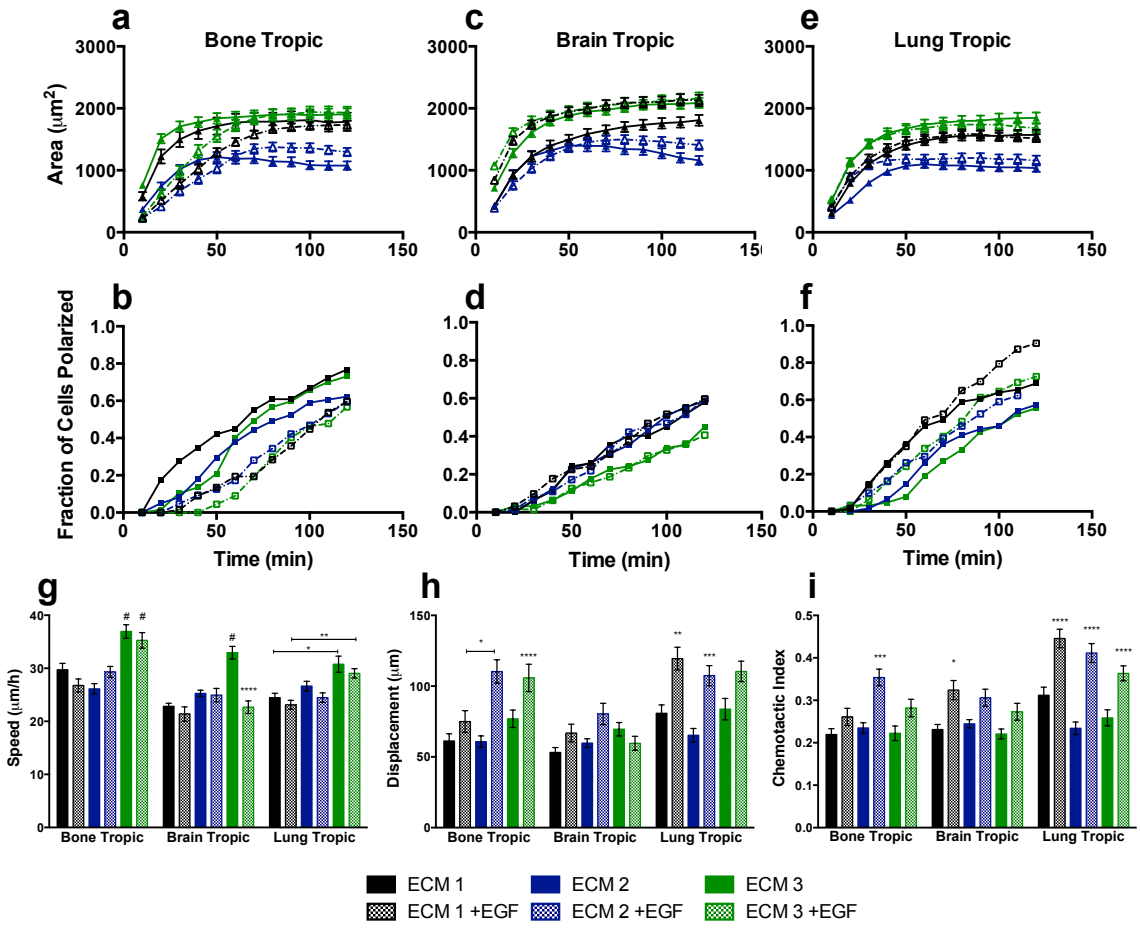

Figure S3. Detailed view of all measurements for the tropic cell line subpopulations. (a, c, e) Cell area and (b, d, f) polarization measurements at 10 minute intervals after seeding for the bone (a, b), brain (c, d) and lung (e, f) tropic subpopulations. Long-term migration (g) speed, (h) displacement, and (i) chemotactic index for the tropic cell lines. Asterisks directly over EGF stimulated conditions indicate significance between the EGF-stimulated and normal measurements on the same ECM. # identifies an ECM condition that is significantly different than the other two corresponding normal or EGF ECM conditions. N ≥ 2 biological replicates. (a-f) N ≥ 40 cells/condition, (g-i) N ≥ 90 cells/condition.

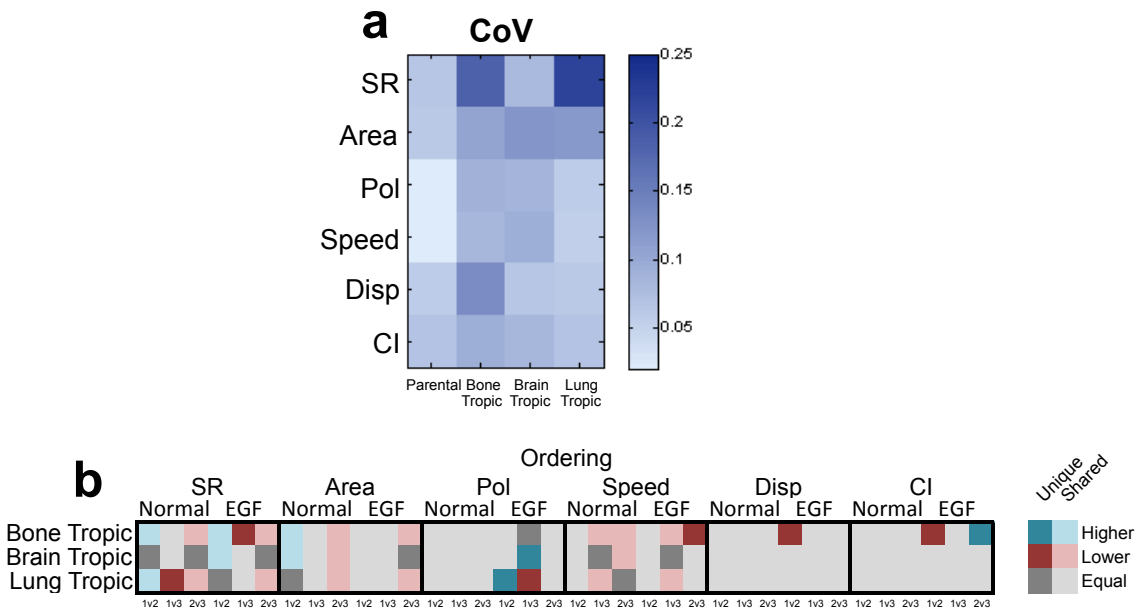

Figure S4. Tropic cell line subpopulations are more sensitive to engineered ECMs than parental cell line. (a) Heat map displaying coefficient of variation calculations for adhesion and motility metrics of parental and tropic cell lines. Darker colors indicate higher CoV values. (b) Pairwise ordering features of the phenotypic fingerprints, expanded from Figure 2, capturing statistical ordering of measurements. Dark colors indicate unique features. 1v2: ECM 1 versus ECM 2; 1v3: ECM 1 versus ECM 3; 2v3: ECM 2 versus ECM 3. Blue: first measurement is higher; red: first measurement is lower; grey: both are equal. Abbreviations: 1: ECM 1; 2: ECM 2; 3: ECM 3; SR: spreading rate; Pol: polarization; Disp: displacement; CI: chemotactic index.

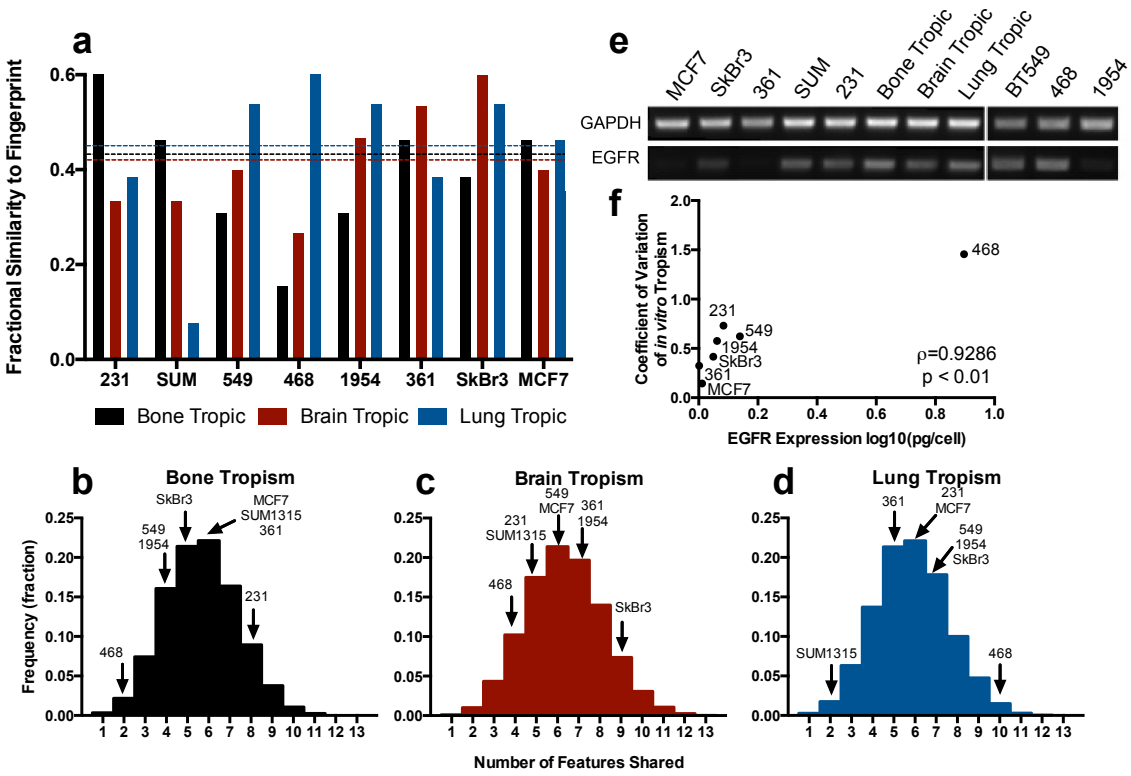

Figure S5. Fingerprinting of heterogeneous cell lines. (a) Raw fractional similarity of heterogeneous cell lines to the bone, brain, and lung tropic fingerprints. Dashed lines indicate the average of the null distribution, comprised of 10,000 randomly generated cell lines. Black: bone tropic, red: brain tropic, blue: lung tropic. (b-d) Histograms of the null distributions (b: bone tropism, c: brain tropism, d: lung tropism), with heterogeneous cell lines overlaid at their associated experimentally observed value. (e) RT-PCR for EGFR expression in cell lines. (f) Correlation between coefficient of variation calculated from tropism predictions (percentiles from Fig. 2g) and basal EGFR expression retrieved from Niepel et al. (ref. 41 in text). Abbreviations: 361: MDA-MB-361; SUM: SUM1315 MO2; 231: MDA-MB-231; 468: MDA-MB-468; 1954: HCC 1954; 549: BT549.

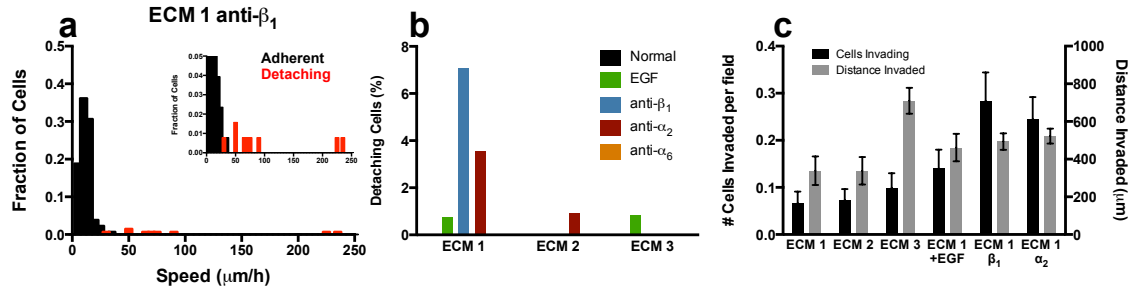

Figure S6. Integrin antibody treatment causes SkBr3 detachment and invasion. (a) Histogram of SkBr3 cell speeds on ECM 1 with β_1 integrin antibody treatment. The detaching cells are highlighted in red. Inset: magnified y-axis. (b) Fraction of total cells observed detaching during migration in all conditions. (c) Mean number of SkBr3 cells invading into an overlaid collagen gel, and average distance invaded after 48 hours.

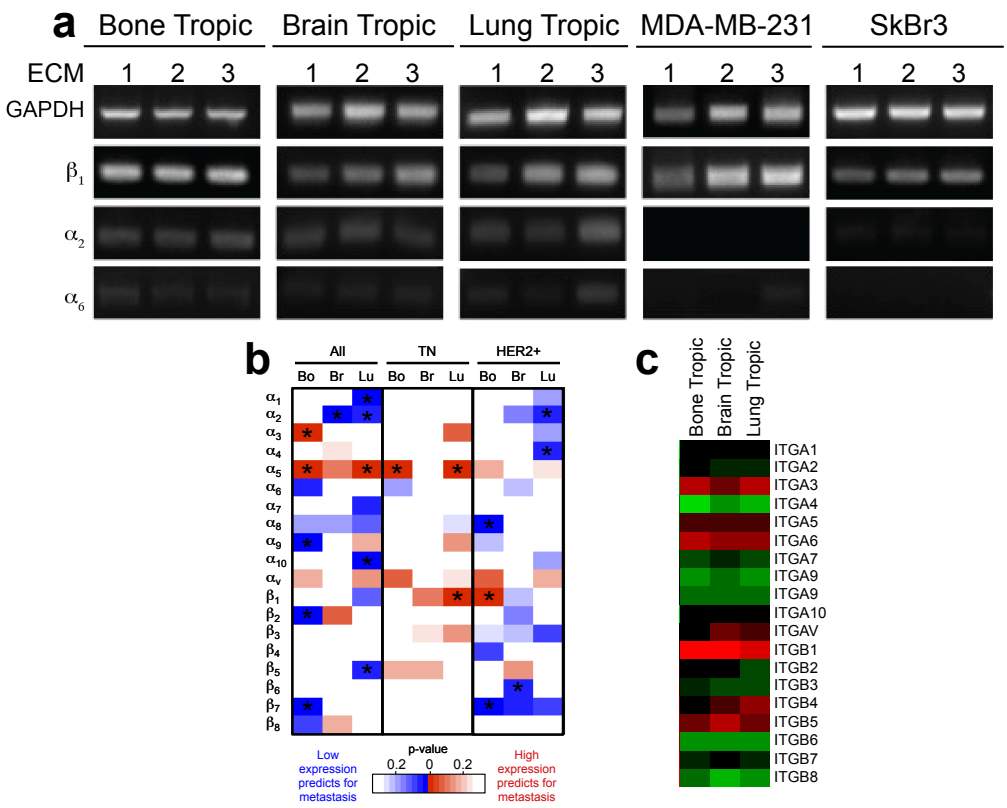

Figure S7. Integrin gene expression *in vitro* and clinical metastasis predictions. (a) RT-PCR for β_1, α_2, and α_6 integrin genes for the three tropic subpopulations, the parental MDA-MB-231, and the SkBr3 cell lines on the ECMs. (b) Heat map displaying integrin genes that significantly predict for tissue-specific metastasis in patients. Compared to Fig. 4g, this heat map is modified to include larger p-values to further display trends in data despite lack of statistical significance. Blue: low expression of the gene predicts for increased risk of metastasis; red: high expression of the gene predicts for increased risk of metastasis; white: no effect on metastasis. Asterisks indicate statistically significant relationships. Abbreviations: Bo: bone metastasis; Br: brain metastasis; Lu: lung metastasis. (c) Integrin gene expression heat map of the tropic cell lines used in this work, compiled from GSE 2603 and GSE 12237.

Movie S1. SkBr3 cells detach when treated with β_1 integrin antibodies. Length of time is 12 hours; scale bar is 25 μm.