Supplementary Information

Functional Assessment of Migration and Adhesion to Quantify Cancer Cell Aggression

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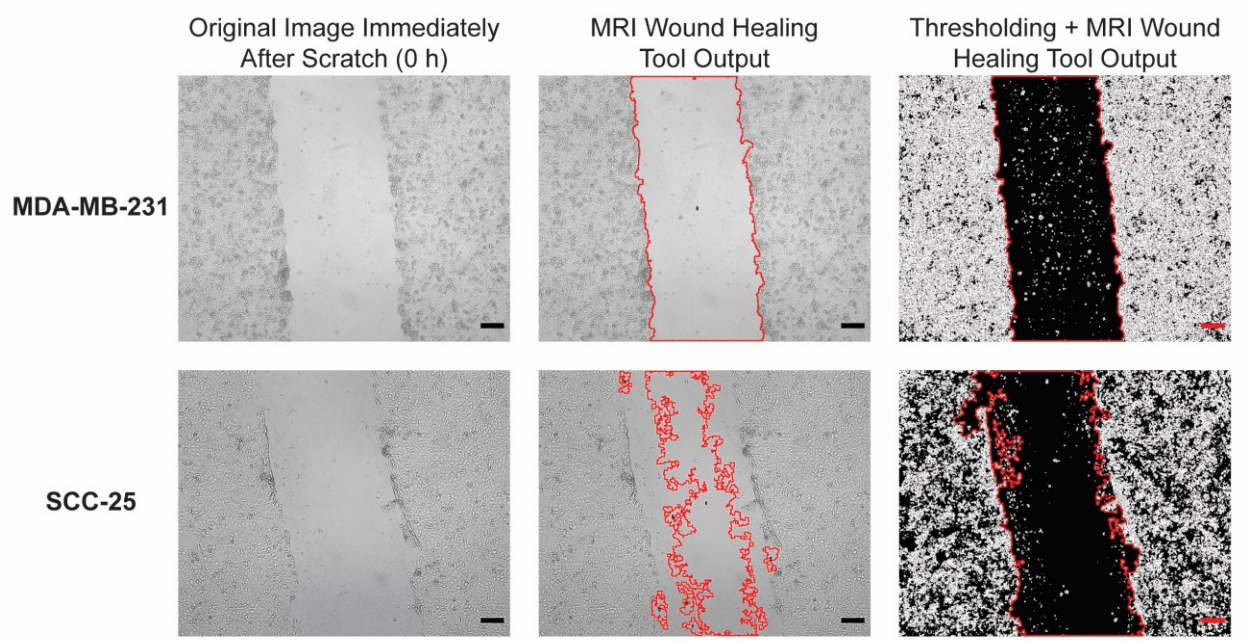
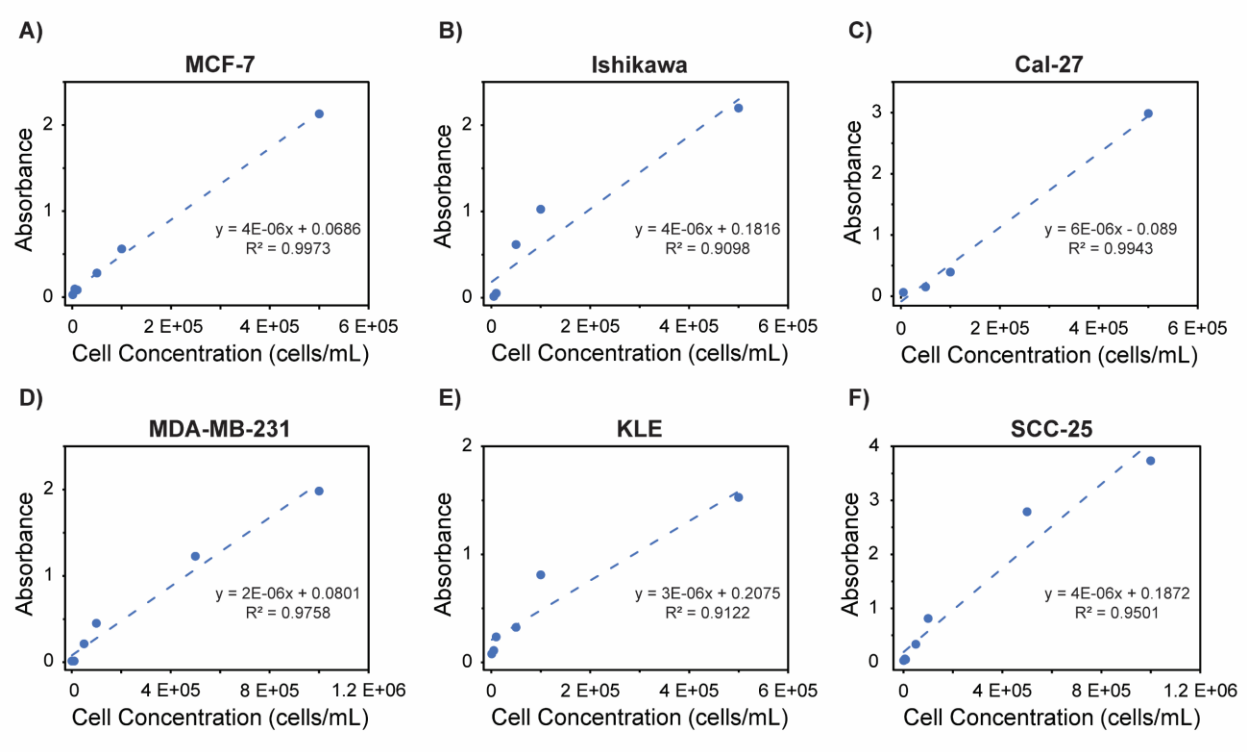
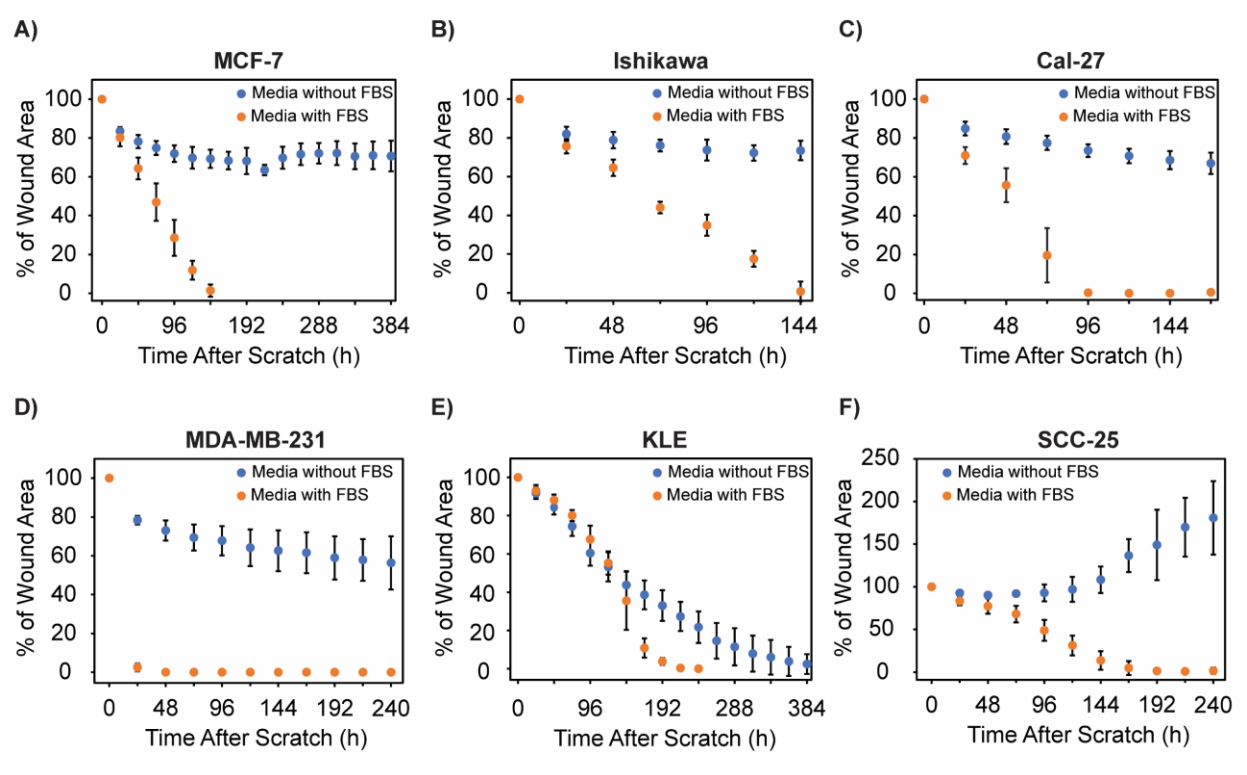


S1 Fig. Wound Healing Assay Analysis Methods. Example of the ImageJ output using the MRI Wound Healing Tool of the original image and the output of the MRI Wound Healing Tool when the image was thresholded. A comparison of these outputs was created for a cell line with good contrast (MDA-MB-231) versus poor contrast (SCC-25) between the cells and background. Scale bars represent 200 μm.



S2 Fig. MTT Calibration Curves. (A-F) Calibration curve relating cell concentration (cells/mL) to absorbance from MTT assay for each cell line.



S3 Fig. Extended Analysis of Wound Closure Study. Extended timepoints of the percentage of wound area over time based on the wound being cultured in serum-containing or serum-free media.

S1 Table. Wound Closure with Serum-free/Low Serum Comparison to Literature. Summary of current literature of wound closure assays with serum-free or low-serum culture media for the six cell lines used in this work in comparison to observed data.

Tissue of Origin	Wound Closure from Data (This Work)	Wound Closure from Literature
Breast	• MDA-MB-231 had significantly faster initial wound closure in first 12 h compared to MCF-7 • Neither cell lines fully closed the wound • MCF-7 closed ~17% of the wound in 24 h and ~29% of the wound in 144 h • MDA-MB-231 closed ~27% of the wound in 24 h and ~38% of the wound in 144 h	• MDA-MB-231 had faster wound closure compared to MCF-7[1] • MDA-MB-231 closed ~40% of the wound in 24 h[1] • MCF-7 closed ~42% of the wound in 48 h[1]
Endometrium	• Ishikawa had significantly faster initial wound closure in first 12 h compared to KLE • Ishikawa did not fully close the wound • Ishikawa closed ~18% of the wound in 24 h and ~26% of the wound in 144 h • KLE closed ~10% of the wound in first 24 h and fully closed the wound within 384 h	• In low-serum media, Ishikawa closed ~15% of the wound in 72 h[2] • Did not find appropriate KLE literature in low/serum-free conditions for comparison
Tongue	• Cal-27 had faster initial wound closure in first 12 h compared to SCC-25 (not significant) • Neither cell lines fully closed the wound • Cal-27 closed ~15% of the wound in 24 h and ~44% of the wound in 144 h • SCC-25 closed ~7% of the wound in 24 h • After 72 h, SCC-25 cells detached from surface, causing an increase in the wound area	• Did not find appropriate literature in low/serum-free conditions for comparison

S2 Table. Wound Closure with Serum Comparison to Literature. Summary of current literature of wound closure assays with serum containing culture media for the six cell lines used in this work in comparison to observed data.

Tissue of Origin	Wound Closure from Data (This Work)	Wound Closure from Literature
Breast	• MDA-MB-231 had significantly faster initial wound closure in first 12 h compared to MCF-7 • MDA-MB-231 completely closed the wound and MCF-7 closed over 99% of wound in 144 h • MCF-7 closed ~20% of the wound in 24 h and >99% of the wound in 144 h • MDA-MB-231 closed >98% of the wound in 24 h and completely closed the wound within 48 h	• MDA-MB-231 had faster wound closure compared to MCF-7 • MCF-7 closed ~23% of the wound in 24 h[3] • MDA-MB-231 closed ~68% of the wound in 24 h[3] • Additional studies show that MDA-MB-231 close the wound within 24 h[4, 5]
Endometrium	• Ishikawa had significantly faster initial wound closure in first 12 h compared to KLE • Ishikawa closed ~25% of the wound in 24 h and >99% of the wound in 144 h • KLE closed ~7% of the wound in 24 h and ~72% of the wound in 144 h	• Ishikawa had ~40% of wound closure in 48 h[6] • KLE had ~30% wound closure in 30 h[7]
Tongue	• Cal-27 had faster initial wound closure in first 12 h compared to SCC-25 (not significant) • Cal-27 closed ~30% of the wound in 24 h and completely closed the wound within 144 h • SCC-25 closed ~17% of the wound in 24 h and ~87% of the wound in 144 h	• Cal-27 had faster wound closure compared to SCC-25 • Cal-27 closed ~25% of the wound in 24 h[8] • SCC-25 closed ~15% of the wound in 24 h[8]

S3 Table. Cell Detachment Comparison to Literature. Summary of current literature of E-cadherin and N-cadherin biomarker expression for the six cell lines used in this work in comparison to observed cell detachment data of individual cell populations.

Tissue of Origin	Individual Population Cell Detachment from Data (This Work)	Relative E-Cadherin Expression from Literature	Relative N-Cadherin Expression from Literature
Breast	• MDA-MB-231 had significantly greater cell detachment at each timepoint	• E-cadherin expression is high in MCF-7 and low in MDA-MB-231[9-12]	• N-cadherin expression is low to moderate in MCF-7 and high in in MDA-MB-231[9-12]
Endometrium	• KLE had greater detachment at each timepoint (not significant)	• E-cadherin expression is high in Ishikawa and low in KLE[6, 13-15]	• N-cadherin expression is low in Ishikawa and high in KLE[6, 13-15]
Tongue	• SCC-25 had greater detachment at each timepoint (not significant) • More similar cell detachment between SCC-25 and Cal-22 compared to cell lines from other tissue origins	• E-cadherin expression is moderate to high in Cal-27 and low to moderate in SCC-25[16-19]	• N-cadherin expression is low to moderate in Cal-27 and moderate to high in SCC-25[16-19]

S4 Table. Significance Testing of Wound Closure Study. Summary of significance testing using one-way ANOVA to determine significance between the 12 h wound closure average migration velocity of cell lines when cultured in media with the presence or absence of serum using JMP. Significance was determined as * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Comparison	P-Value	Significant?
Cell Lines without Serum – Migration Velocity	<0.001	Yes
Cell Line with Serum – Migration Velocity	<0.001	Yes
Interaction Between Presence of Serum – Cell Line	<0.001	Yes

S5 Table. Significance Testing of Wound Closure Study using Post-Hoc Testing. Summary of adjusted p-values using Tukey-Kramer HSD for multiple comparisons in JMP. Significance was determined as * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Cell Type 1	Cell Type 2	Variable	P-Value
MCF-7	MDA-MB-231	Average Migration Velocity Without Serum	6E-04
Ishikawa	KLE	Average Migration Velocity Without Serum	5E-04
Cal-27	SCC-25	Average Migration Velocity Without Serum	0.74
MCF-7	MDA-MB-231	Average Migration Velocity With Serum	<1E-4
Ishikawa	KLE	Average Migration Velocity With Serum	1.2E-03
Cal-27	SCC-25	Average Migration Velocity With Serum	1.0
MCF-7	MCF-7	Average Migration Velocity With Serum vs Without Serum	0.79
MDA-MB-231	MDA-MB-231	Average Migration Velocity With Serum vs Without Serum	<1E-4
Ishikawa	Ishikawa	Average Migration Velocity With Serum vs Without Serum	0.63
KLE	KLE	Average Migration Velocity With Serum vs Without Serum	1.0
Cal-27	Cal-27	Average Migration Velocity With Serum vs Without Serum	0.26
SCC-25	SCC-25	Average Migration Velocity With Serum vs Without Serum	0.088

S6 Table. Summary of Wound Closure Migration Velocity. Summary of the average wound closure migration velocity in the first 12 h after the wound was created and standard deviation of each cell line in serum-containing or serum-free media.

Cell Type	Serum Present	Average Migration Velocity (μm/h)	St. Dev.
MCF-7	Yes	2.34	1.82
Ishikawa	Yes	6.45	1.36
Cal-27	Yes	4.75	1.21
MDA-MB-231	Yes	21.8	1.93
KLE	Yes	1.53	1.11
SCC-25	Yes	4.54	2.24
MCF-7	No	3.87	0.27
Ishikawa	No	4.50	1.10
Cal-27	No	2.70	0.83
MDA-MB-231	No	6.86	1.47
KLE	No	1.47	0.73
SCC-25	No	1.87	0.91

S7 Table. Summary of Cell Population Doubling Time. Summary of the average population doubling time and standard deviation of each cell line determined from the MTT assay.

Cell Type	Average Population Doubling Time (h)	St. Dev.
MCF-7	59.6	14.6
Ishikawa	31.8	2.22
Cal-27	33.5	4.69
MDA-MB-231	28.0	3.07
KLE	33.4	1.06
SCC-25	40.4	7.11

S8 Table. Significance Testing of Cell Adhesion of Individual Cell Populations. Summary of significance testing using an independent two-group Student's t-test between cell lines for the percentage of cells retained after exposure to 50 mL/min shear flow for 15 s, 30 s, 45 s, and 60 s. Significance was determined as * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Cell Type 1	Cell Type 2	Variable	P-Value
MCF-7	MDA-MB-231	% Cell Retention at 15 s	5.3E-03
MCF-7	MDA-MB-231	% Cell Retention at 30 s	0.015
MCF-7	MDA-MB-231	% Cell Retention at 45 s	0.021
MCF-7	MDA-MB-231	% Cell Retention at 60 s	0.023
Ishikawa	KLE	% Cell Retention at 15 s	0.16
Ishikawa	KLE	% Cell Retention at 30 s	0.12
Ishikawa	KLE	% Cell Retention at 45 s	0.13
Ishikawa	KLE	% Cell Retention at 60 s	0.11
Cal-27	SCC-25	% Cell Retention at 15 s	0.53
Cal-27	SCC-25	% Cell Retention at 30 s	0.73
Cal-27	SCC-25	% Cell Retention at 45 s	0.32
Cal-27	SCC-25	% Cell Retention at 60 s	0.35

S9 Table. Significance Testing of Cell Adhesion of Co-Cultures. Summary of significance testing using an independent two-group Student's t-test between cell lines in co-culture for the percentage of cells retained after exposure to 50 mL/min shear flow for 60 s. Significance was determined as * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Cell Type 1	Cell Type 2	Variable	P-Value
MCF-7	MDA-MB-231	% Cell Retention at 60 s	8.3E-03
Ishikawa	KLE	% Cell Retention at 60 s	0.96
Cal-27	SCC-25	% Cell Retention at 60 s	0.72

S10 Table. Significance Testing of Cell Adhesion of each Cell Line Cultured as Homogenous Populations versus in Co-Culture using Post-Hoc Testing. Significance testing was performed using one-way ANOVA to determine differences between the percentage of cells retained after exposure to 50 mL/min shear flow for 60 s between culture conditions. Post-hoc testing using Tukey-Kramer HSD was used to adjust the p-values for multiple comparisons in JMP. Significance was determined as * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Homogenous Population	Co-Culture	Variable	P-Value
MCF-7	MCF-7	% Cell Retention at 60 s	<1E-4
MDA-MB-231	MDA-MB-231	% Cell Retention at 60 s	1E-4
Ishikawa	Ishikawa	% Cell Retention at 60 s	0.5857
KLE	KLE	% Cell Retention at 60 s	0.9921
Cal-27	Cal-27	% Cell Retention at 60 s	0.8057
SCC-25	SCC-25	% Cell Retention at 60 s	0.9616

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