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Supplementary Materials for

Decellularized extracellular matrix scaffolds identify full-length collagen VI as a driver of breast cancer cell invasion in obesity and metastasis

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The PDF file includes:

Other Supplementary Material for this manuscript includes the following:

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Supplementary Figures

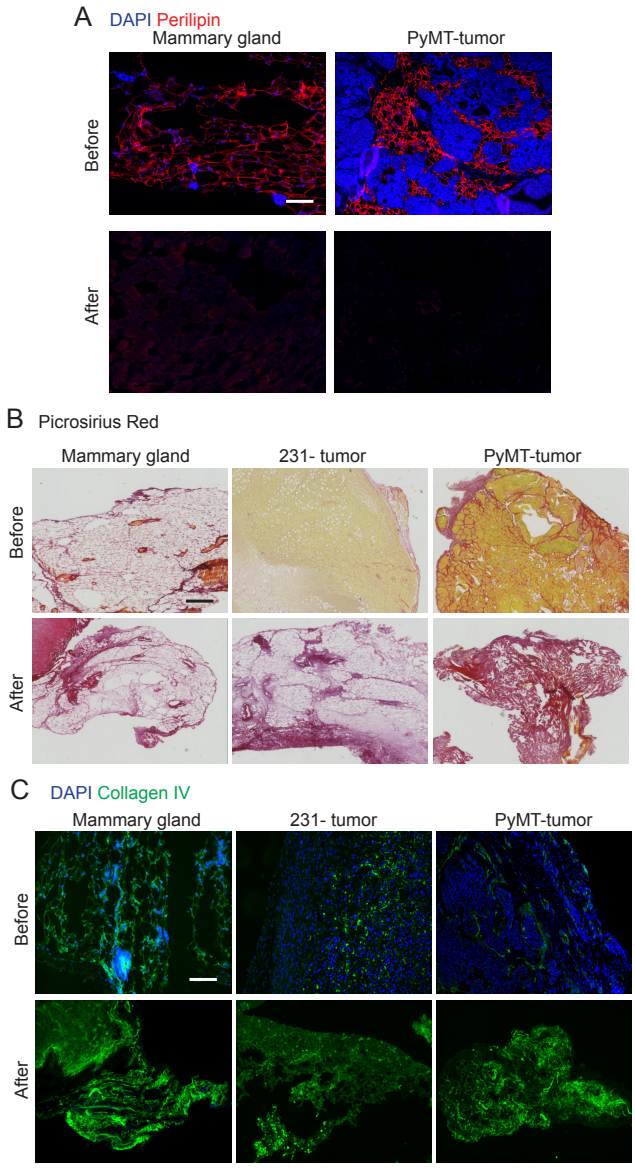

Figure S1: Validation of decellularization method. Tissue sections from healthy mammary gland, xenograft 231-tumor and PyMT-tumors from before and after decellularization stained with A) Perilipin and DAPI, B) Picrosirius Red and C) Collagen IV and DAPI. Scale bars is 100μm for A,C and 200μm for B.

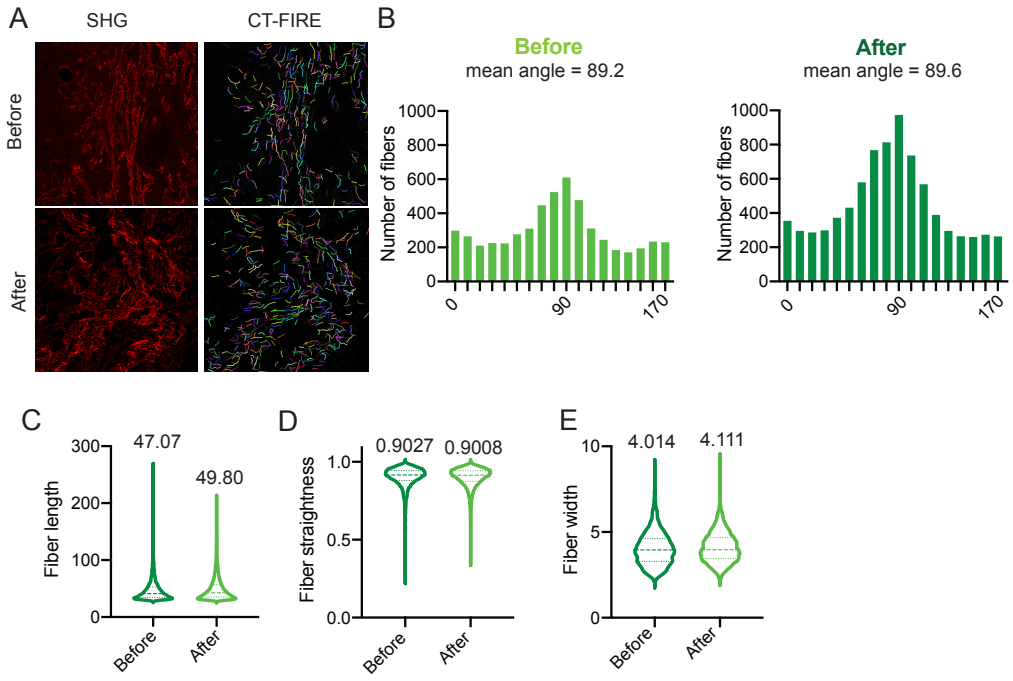

Figure S2: Decellularization does not alter Collagen fiber organization. Tissue sections from PyMT-tumors from before and after decellularization were imaged via second harmonic to visualize Collagen fibers and images were analyzed with CT-FIRE (A). Analysis shows that decellularization does not alter B) the mean angle and distribution, C) length, D) straightness and E) width of Collagen I fibers. Over 5000 Collagen I fibers analyzed per condition.

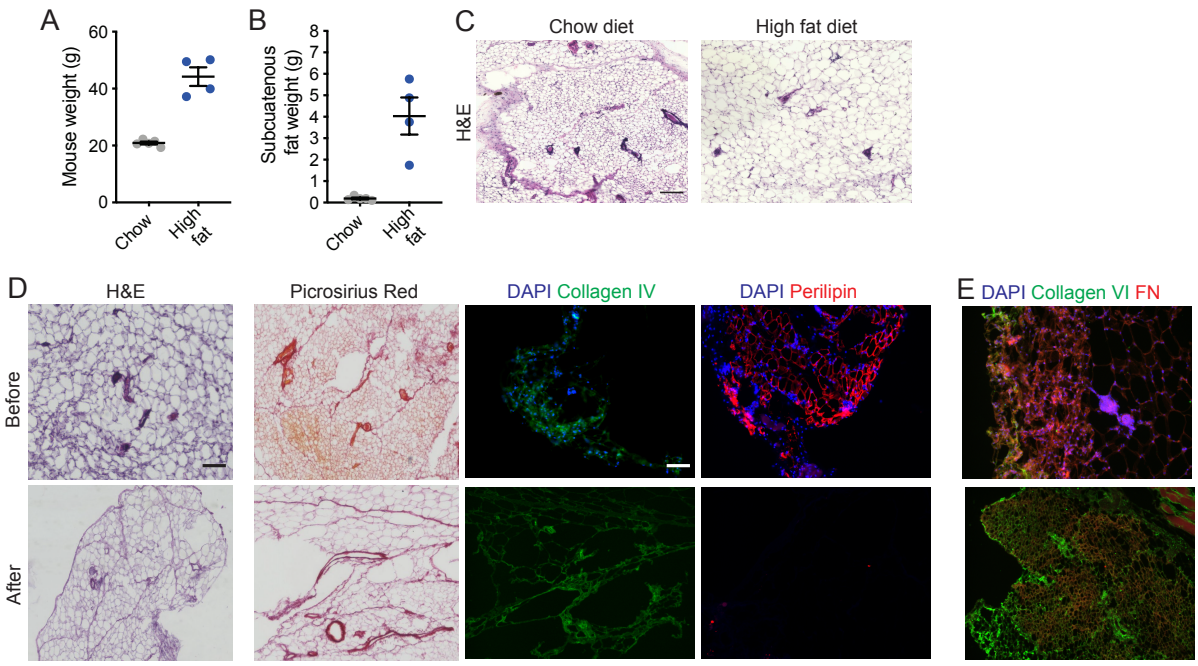

Figure S3: Diet-induced obesity model and staining validation for obese mice. C57BL/6 female mice were fed on chow (n=4) or high fat diet (n=4) for 16 weeks after which both total body weight (A) and subcutaneous fat weight (B) were measured. C) H&E staining sections of mammary gland from chow and high fat diet fed mice were H&E processed, revealing significant hypertrophy of adipocytes (typical of fatty tissue) in the glands of mice fed with high fat diet (Scale bar, 200μm). Tissue sections from mammary gland from obese mice from before and after decellularization stained with D) H&E, Picrosirius Red, Collagen IV and Perilipin and E) Collagen VI and FN and DAPI. Scale bars is 200μm for chromogenic stains and 100μm for immunofluorescence.

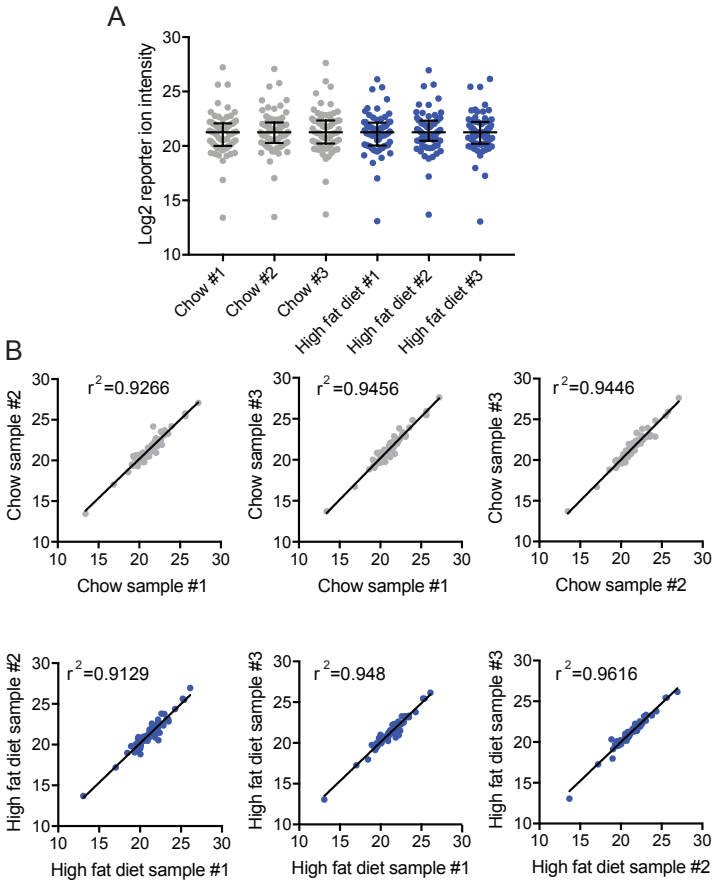

Figure S4: ECM proteomics data normalization and biological group correlations.
A) ECM reporter ion intensities were median-centered and log2-transformed, the bars represent median with interquartile range. This normalizes the data, correcting for any potential inter-sample protein loading variances on the mass spectrometer, to enable accurate comparisons between abundance of ECM proteins. B) Scatterplots with r^2 correlation values between biological replicates reveal a strong correlation between ECM proteins within the chow diet fed mice group and also between ECM proteins within the high fat diet fed mice group (values on x and y axes are log2 of reporter ion intensity).

A

Core Matrisome

ECM Glycoproteins

Entrez Gene Symbol	Obese
TGFBI	25.44
LAMC1	25.44
DPT	23.31
LAMA2	23.18
NID1	23.14
FBN1	22.67
FN1	22.45
EMILIN2	22.15
LAMB3	21.76
FGG	22.11
MFGE8	21.38
LAMA3	21.04
PAPLN	21.25
MFAP4	21.09
NID2	21.30
EMILIN1	20.83
FGA	21.23
VTN	20.88
LAMB1	20.72
LAMA4	20.78
FGB	20.57
ELN	20.09
VWA1	19.98
LAMB2	19.84
TINAGL1	19.55
FBLN5	19.51
LAMA5	19.53

Collagens

Entrez Gene Symbol	Obese
COL1A1	26.47
COL3A1	21.99
COL7A1	21.89
COL6A6	22.10
COL6A5	22.13
COL5A2	21.79
COL2A1	21.74
COL11A1	21.71
COL15A1	21.57
COL6A1	21.07
COL12A1	21.43
COL6A2	21.02
COL5A1	20.27
COL4A1	20.29
COL4A2	19.86
COL14A1	20.03
COL16A1	19.17
COL1A2	13.31

Proteoglycans

Entrez Gene Symbol	Obese
BGN	24.17
PRG2	22.47
PRELP	21.83
OGN	21.86
ASPN	21.51
LUM	21.37
DCN	18.53
HSPG2	17.16

B

Matrisome-associated

ECM-affiliated Proteins

Entrez Gene Symbol	Obese
ANXA2	23.38
LGALS1	23.30
ANXA1	22.60
ANXA5	21.73
LMAN1	22.54
ANXA4	20.89
ANXA7	20.51
ANXA6	20.57
ANXA11	19.86
ANXA3	19.90

Secreted factors

Entrez Gene Symbol	Obese
S100A11	20.51
POSTN	19.53

ECM regulators

Entrez Gene Symbol	Obese
ITIH5	21.89
TGM2	21.46
SERPINH1	19.80

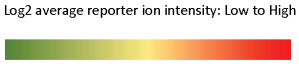

Figure S5: Matrisome profile of obese mammary fat pad.
A) Core matrisome list, including ECM glycoproteins, collagens and proteoglycans. B) Matrisome-associated list including ECM-affiliated proteins, secreted factors and ECM regulators. Proteins were annotated according to the Matrisome Project. Data are the result of 3 biological replicates with the average log2 reporter ion intensity reported.

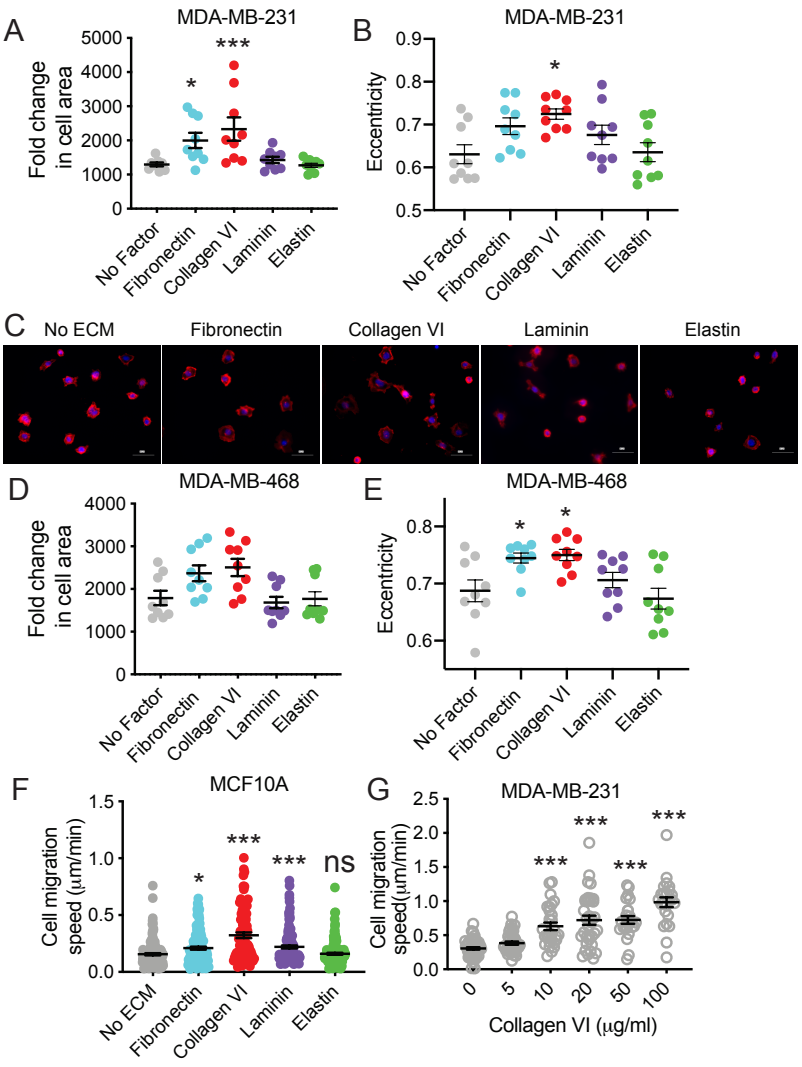

Figure S6: Collagen VI drives adhesion and migration of several breast cancer cell lines
Quantification of cell area (A) and cell eccentricity (B) for MDA-MB-231 cells seeded onto wells coated with 20μg/ml Fibronectin, Collagen VI, Laminin or Elastin and allowed to adhere for 2hrs. C) Representative images of MDA-MB-468 breast cancer cells seeded on the different substrates (Scale bar = 50μm). Quantification of cell area (D) and cell eccentricity (E) for MDA-MB-468 cells on 20μg/ml Fibronectin, Collagen VI, Laminin or Elastin and allowed to adhere for 2hrs. Graphs show data from 3 independent experiments and average from 3 different fields of view per experiment. Significance was determined by a nonparametric Kruskal-Wallis test with Dunn's multiple testing correction, with *p<0.05, ***p<0.005. F) Cell migration speed of pre-neoplastic mammary epithelial cell line MCF10A cells on 20μg/ml Fibronectin, Collagen VI, Laminin or Elastin and allowed to adhere for 2hrs. Each point represents the average speed of a cell over the time course (16hrs). G) Cell migration speed of MDA-MB-231 cells on different concentrations of Collagen VI. Graphs show individual cell data and mean ± SEM. Significance was determined by a nonparametric Kruskal-Wallis test with Dunn's multiple testing correction, with *p<0.05 and ***p<0.005. For each experiment, data pooled from at least 3 independent experiments.

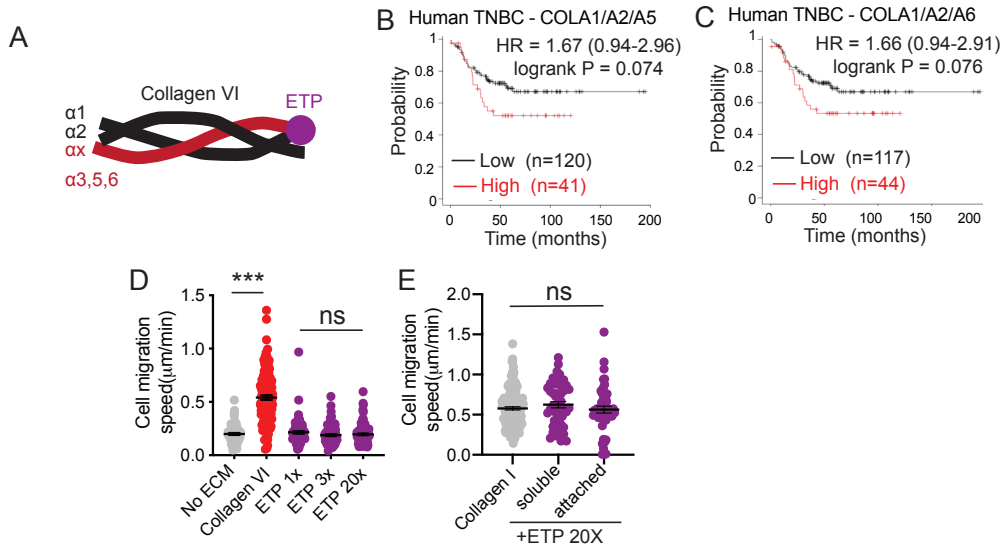

Figure S7: Collagen VI chains and ETP in TNBC

A) Endotrophin (ETP) is a small terminal peptide on the α3 chain. Kaplan-Meier curve of human TNBC breast cancer patients with low vs high mRNA of COL6A1/COL6A2/COL6A4 (B) and COL6A1/COL6A2/COL6A5 (C), data from (37). D) Cell migration speed of MDA-MB-231 cells on full length Collagen VI or ETP (at 1x, 3x and 20x the relative amount to the ETP present on a full length Collagen VI strand). E) Cell migration speed of MDA-MB-231 cells seeded on Collagen I in combination with ETP or with ETP in the media.

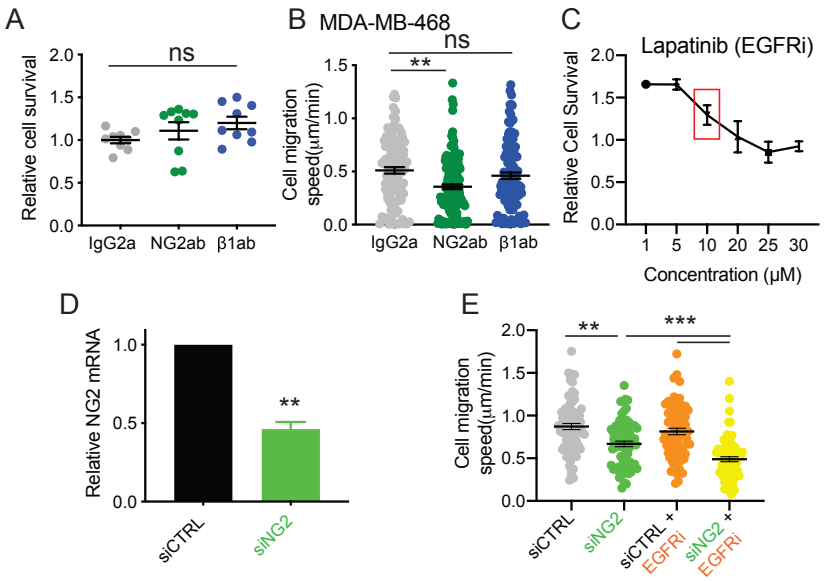

Figure S8: Investigation into receptors mediating Collagen VI-driven cancer cell migration
A) Cell survival of MDA-MB-231 cells treated with IgG2a, NG2 targeting antibody (NG2ab) or β1 integrin inhibitory antibody (β1ab) for 16hrs. B) Cell migration speed of MDA-MB-468 cells seeded on 20μg/ml Collagen VI and treated with IgG2a, NG2 targeting antibody (NG2ab) or β1 integrin inhibitory antibody (β1ab). B) Graph shows individual cell data and mean ± SEM. Significance was determined by a nonparametric Kruskal-Wallis test with Dunn's multiple testing correction, with **p<0.01. For each experiment, data pooled from at least 3 independent experiments. C) Dose response curves plotting the effect of Lapatinib on MDA-MB-231 cell survival after 16hrs. Box represents dose chosen for experiments in Figure 5 & 6. D) Relative mRNA levels of NG2 measured by qPCR in MDA-MB-231 cells 2h after being transfected with siCTRL and siNG2. E) Cell migration speed of MDA-MB-231 cells seeded on 20μg/ml Collagen VI overnight 72h after transfection with siCTRL or siNG2, alone or in combination with 10μM Lapatinib. Significance was determined by a nonparametric Kruskal-Wallis test with Dunn's multiple testing correction, with **p<0.01. For each experiment, data pooled from 3 independent experiments.

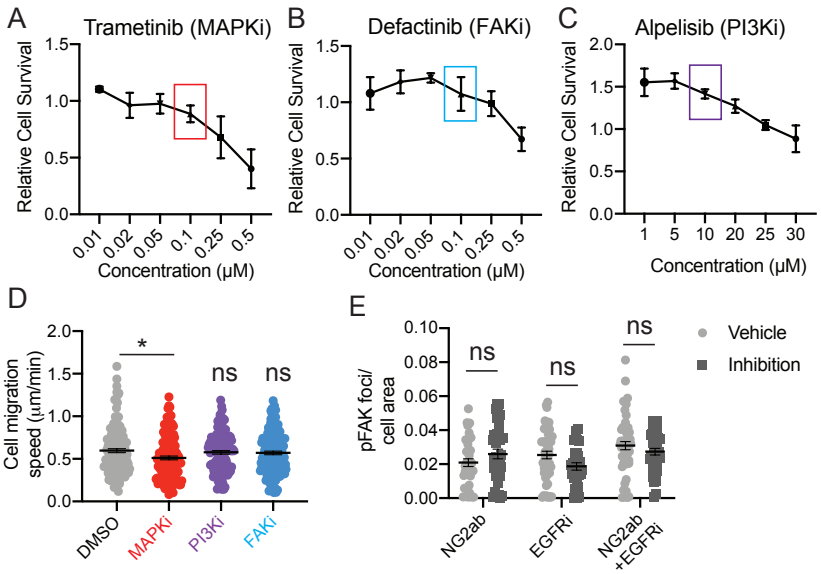

Figure S9: Investigation into downstream signaling pathways mediating Collagen VI-driven cancer cell migration

Dose response curves plotting the effect of A) Trametinib (MAPK inhibitor), B) Defactinib (FAK inhibitor), C) Alpesilib (PI3K inhibitor) on MDA-MB-231 cell survival after 16hrs. Box represents dose chosen for experiments in Figure 5 & 6. D) Cell migration speed of MDA-MB-231 cells plated on Collagen VI and treated with Alpesilib (PI3K inhibitor, 10μM), Defactinib (FAK inhibitor, 0.1μM) and Trametinib (MEK inhibitor, 0.1μM). Graph shows individual cell data and mean ± SEM. Significance was determined by a nonparametric Kruskal-Wallis test with Dunn's multiple testing correction, with *p<0.05. For each experiment, data pooled from at least 3 independent experiments. E) Number pFAK397 in breast cancer cells plated on Collagen VI for 2hrs and treated with NG2ab, EGFR inhibitor Lapatinib (10μM) or a combination of both. Data pooled for 3 experiments.

Supplementary Videos:

Movie S1: 231-GFP cells seeded onto dECM obtained from healthy mammary gland. Cells imaged every 10 mins for 16h.

Movie S2: 231-GFP cells seeded onto dECM obtained from 231-tumor xenografts. Cells imaged every 10 mins for 16h.

Movie S3: PyMT-GFP cells seeded onto dECM obtained from the mammary gland of healthy FVB mice. Cells imaged every 10 mins for 16h.

Movie S4: PyMT-GFP cells seeded onto dECM obtained from PyMT-MMTV mammary tumors. Cells imaged every 10 mins for 16h.

Movie S5: 231-GFP cells seeded onto dECM obtained from the mammary glands of obese C57Bl6 mice. Cells imaged every 10 mins for 16h.

Movie S6: 231-GFP cells plated on glass and imaged every 10 mins for 16h.

Movie S7: 231-GFP cells plated on Collagen VI (20ug/ml) and imaged every 10 mins for 16h.

Movie S8: 231-GFP cells seeded onto dECM obtained from the mammary glands of obese C57Bl6 mice and treated with IgG2a. Cells imaged every 10 mins for 16h.

Movie S9: 231-GFP cells seeded onto dECM obtained from the mammary glands of obese C57Bl6 mice and treated with NG2ab. Cells imaged every 10 mins for 16h.