Supplemental Figures

Figure S1: Effects on haptotaxis are independent of effects on cell velocity A) Velocity of MV^{D7} fibroblasts expressing different GFP-tagged Ena/VASP family proteins in the haptotaxis device left overnight to migrate on a 2D FN gradient (125µg/ml)**.** B) Western Blot of MDA-MB-231 cells expressing different GFP-tagged Mena isoforms showing GFP, Mena (endogenous and GFP-tagged) and tubulin. The epitope for the Mena antibody is absent in the ΔLERER cells, which explains the lack of bands at the higher molecular weight. C) Western Blot of SUM-159 cells expressing different GFP-tagged Mena isoforms showing GFP, Mena (endogenous and GFP-tagged) and tubulin. D) 231-Mena cells plated in a 2D VN gradient and subjected to increasing concentrations of VN at the top of the gradient. E) Velocity of MDAMB231 and 231-Mena cells in the haptotaxis device left overnight to migrate on increasing concentrations of a 2D VN gradient. F) 231-Mena cells plated in a 2D LN gradient and subjected to increasing concentrations of LN at the top of the gradient. G) Velocity of MDAMB231 and 231-Mena cells in the haptotaxis device left overnight to migrate on increasing concentrations of a 2D LN gradient. H) Velocity of MDAMB231 and Mena in the haptotaxis device left overnight to migrate on a 2D FN gradient. I) Velocity of MDAMB231 and Mena in the haptotaxis device left overnight to migrate in a 3D collagen gel and increasing concentrations of a 3D FN gradient**.** (J) Cell counts for MDA-MB-231 cells expressing different GFP-tagged Mena isoforms after 72hrs, show no difference in 2D proliferation. K) Cell area for MDA-MB-231 cells expressing different GFP-tagged Mena isoforms of cells at steady state plated on 0.1 mg/ml collagen I and 0.25% Matrigel for 24h. L) Deletion of LERER region in Mena in MVD7 fibroblasts inhibits 2D haptotaxis on a FN gradient. M) Velocity of MV^{D7} fibroblasts expressing different GFP-tagged Mena

and MenaΔLERER. All data pooled from at least 3 different experiments. Results show mean ± SEM, significance determined by one way ANOVA, * p<0.5, ** p<0.01, *** 0.005.

Figure S2: Mena^{INV} drives haptotaxis at high FN concentrations

(A) Velocity of MDAMB231 and Mena^{INV} in the haptotaxis device left overnight to migrate on a 2D 125µg/ml FN gradient (B) Velocity of MDAMB231 and 231-Mena^{INV} in the haptotaxis device left overnight to migrate in a 3D collagen gel and increasing concentrations of a 3D FN gradient**.** C) DMSO (0.1%), IgG and no drug controls for MDAMB231, 231-Mena and 231-Mena^{INV}-driven haptotaxis in a 3D125µg/ml FN gradient. D) 231-Mena^{INV} cells plated in a 2D LN gradient and subjected to increasing concentrations of LN at the top of the gradient. E) 231-Mena^{INV} cells plated in a 2D VN gradient and subjected to increasing concentrations of VN at the top of the gradient. (F) FMI of SUM159, 159-Mena^{INV} and 159-Mena^{INV}ΔLERER cells plated on a 2D 125µg/ml FN gradient (G) Velocity of SUM159, 159-Mena^{INV} and 159-Mena^{INV}ΔLERER cells plated on a 2D 125µg/ml FN gradient. (H) FMI of SUM159, 159-Mena^{INV} and 159-Mena^{INV}ΔLERER cells plated in a 3D 125µg/ml FN gradient. (I) Velocity of SUM159, 159-Mena^{INV} and 159-Mena^{INV}ΔLERER cells plated in a 3D 125µg/ml FN. (J) Representative images of a control gradient with fluorescently labeled dextran (150kD) (K) Representative images of a time course from an intravital movie of 231- Mena^{INV} and 231-Mena^{INV}ΔLERER tumors in response to a FN gradient. White arrows point towards cells moving towards FN gradient over time. Cell tracks showing cell movement are shown in image on the right for each cell type. Scale bar is 100µm, 25µm in insets. (L) Quantification of motile cells from an hour-long movie of MDAMB231 cells expressing different isoforms in naïve tumors or in the presence of a device with dextran or FN. Stars show significance relative to control tumors for each condition. (M) Quantification of motile cells from an hour-long movie of SUM-159 cells expressing different isoforms in

naïve tumors or in the presence of a device with dextran or FN. For L and M, stars show significance relative to Control tumors for each condition. (data pooled ≥8 movies from ≥2 mice per condition). Results show mean ± SEM, significance determined by one way ANOVA, * p<0.5, ** p<0.01, *** 0.005.

Figure S3: TCGA analysis supplementary analysis and characterization of Mena^{INV}-specific monoclonal antibody.

Kaplan-Meier curves for survival of entire 1060 breast cancer patients in the TCGA cohort binned by quartile of Mena (A) or Mena^{INV} (B) mRNA levels. Tables (C&D) show median survival, hazard ratio (HR), 95% confidence interval and p-value for each quartiles (Q2, Q3, Q4) relative to the top1/4 (Q1). Data from 1060 breast cancer cases from TCGA dataset. Significance calculated by log-rank Mantel-Cox test, hazard ratio calculated by logrank test. C) COX regression carried out to assess the relationship between Mena or Mena^{INV} and time to death in breast cancer patients (total cohort or patients with 10-year follow-up). D) Logistic regression carried out to assess the relationship between Mena or Mena^{INV} and survival in breast cancer patients (total cohort or patients with 10-year follow-up). (E) Kaplan Meier curves from the patients in the TCGA breast cancer patient cohort with node-negative breast cancer with high (top 1/4) or low (bottom 3/4) Mena^{INV} expression. (F) Table showing correlations (R by Spearman and p-value) between Mena or Mena^{INV} and FN and α5. Table showing correlations (R by Spearman and p-value) between Mena or Mena^{INV} and FN and α5 in entire TCGA breast cancer cohort in patients with 10 year follow-up that survived (G) or were deceased (H). I) Kaplan-Meier curve for survival of patients in each quartile of anti-Mena^{INV} signal in the TMA. Cohort was separated into quartiles (75 patients each; Q1, Q2, Q3 and Q4) according to levels of Mena^{INV} expression. J) Immunofluorescence images of representative samples within each quartile of Mena^{INV} signal in the TMA are

shown. K) Relationship between relative Mena^{INV} expression and percent of patients with recurrent disease in each quartile.

Figure S4: Deletion of LERER in Mena^{INV} affects tumor growth in a xenograft model (A) Volume of tumors grown in NOD-SCID mice generated from MDA-MB-231 cells expressing different GFP-tagged Mena isoforms. (B) Volume of tumors grown in NOD-SCID mice generated from SUM-159 cells expressing different GFP-tagged Mena isoforms. (C) Final tumor weight for 231 tumors after 8 weeks. (D) Quantification of Ki67-positive cells in FFPE sections from tumors generated from MDA-MB-231 cells expressing different GFP-tagged Mena isoforms. (E) Quantification of cleaved Caspase3-positive cells in FFPE sections from tumors generated from MDA-MB-231 cells expressing different GFP-tagged Mena isoforms. F) Lung metastatic index of SUM159 tumors expressing Control, Mena^{INV} or Mena^{INV}ΔLERER 8 weeks after cell injection (n=3 mice per group). (G) Representative Western Blot for Mena and Tubulin expression in cultured MDAMB231 cells with shCtrl and shMena. (H) Quantification of Mena knockdown in cultured MDAMB231 cells with shCtrl and shMena. (I) Volume of tumors grown in NOD-SCID mice generated from MDA-MB-231 cells expressing shCtrl and shMena (3 mice per group). (J) Representative Western Blot for Mena and Tubulin expression in tumors MDAMB231 cells with shCtrl and shMena 8 weeks after cell injection. (K) Quantification of Mena knockdown in MDAMB231 tumors with shCtrl and shMena. (L) Number of cells moving in 231-shCtrl and shMena as visualized by intravital imaging (M) Percentage of mice with spontaneous lung metastases from 231-shCtrl and shMena tumors. Results show mean ± SEM, significance determined by one way ANOVA, * $p<0.5$, ** $p<0.01$, *** 0.005.

Figure S5: Mena isoform expression does not affect Integrin αv, β1 or FAK levels

(A) Western Blot of α5 protein expression in lysates from MDA-MB-231 cells expressing different GFP-tagged Mena isoform. (B) Quantification of blot shown in (A) showing increased expression of α5 in 231-Mena^{INV} cells. (C) Surface expression of α5 as measured by FACS is increased in 231-Mena^{INV} cells. Surface αv (D) and β1 (E) protein levels measured by FACS analysis from MDA-MB-231 cells expressing different Mena isoforms. Total αv (F) and β1 (G) protein levels measured by Western Blot from whole cell lysates of MDA-MB-231 cells expressing different Mena isoforms. (H) Total FAK protein levels measured by Western Blot from whole cell lysates of MDAMB231 cells expressing different Mena isoforms. All data from at least 3 experiments. I) Number of GFP-positive adhesions relative to cell area in cells plated on a low 2D 125 μg/ml FN gradient**.** (J) FMI for MDAMB231, 231-Mena and 231-Mena^{INV} cells migrating on 2D low 125μg/ml FN and high 500μg/ml FN gradient.

Figure S6: Mena^{INV}-driven ECM reorganization *in vitro* and *in vivo*. A) Number of protrusions of 231-Control, Mena and Mena^{INV} cells in 3D low (125μg/ml) and high (500μg/ml) FN gradients. B) Representative images of cells at the leading edge of tumors generated with 231-Control, Mena and Mena^{INV}, with a FN gradient. C) Quantification of the cell length/width ratio for cells at the leading edge, with and without a FN gradient. D) Representative images of a 231-Mena^{INV} cell plated on 0.1mg/ml collagen I and 50μg/ml FN for 1hr in the presence of a control peptide or the 70kD fragment that inhibits fibrillogenesis, showing Mena^{INV} as labeled by GFP-tag, α5 and phalloidin. Scale bar = 5μM. Quantification of cell area (μm^2) (E), number of α5-positive adhesion relative to cell area (F), and level of α5 in Mena-positive adhesions (G) in 231-Mena^{INV} cells the presence of the control peptide of the 70kD fragment. At high FN gradient concentrations, inhibition of ROCK with Y-27632 (10μM) but not MMP inhibition

with Marimistat reduced collagen (H) and FN (I) accumulation. (J) Quantification of collagen capsule width surrounding 231-tumors expressing different Mena isoforms, as measured by intravital imaging. (K) Quantification of collagen capsule width surrounding 231-tumors expressing shControl of shMena. (L) Representative images of collagen organization (shown in magenta) of MDA-MB-231 xenograft tumors expressing GFP-tagged MenaΔLERER and Mena^{INV}ΔLERER (shown in green) taken by intravital imaging. Scale bar is 100μm. (M) Distribution of collagen fiber orientation relative to tumors edge comparing 231-Mena and 231-MenaΔLERER expressing tumors, as well as from tumors generated from 231-Mena^{INV}ΔLERER cells. (N) Representative images of collagen organization (shown in magenta) of SUM-159 xenograft tumors expressing Control-GFP and Mena^{INV} (shown in green) taken by intravital imaging. Scale bar is 100μm. (O) Distribution of collagen fiber orientation relative to tumors edge comparing 159-Control, Mena^{INV} and Mena^{INV}ΔLERER tumors.

Supplemental videos:

Supplementary Video 1: Mena^{INV} drives haptotaxis *in vivo*: Representative movie of cells (shown in green) from 231-Mena^{INV} tumor at the edge of the tumor in the presence of a FN gradient (not shown here). Movie from on Z-position, captured by intravital imaging for 1hr, frames every 2 min, 7fps.

Supplementary Video 2: Mena^{INV} drives haptotaxis via its interaction with α5β1 *in vivo*: Representative movie of cells (shown in green) from 231-Mena^{INV}ΔLERER tumor in the presence of a FN gradient (not shown here). Movie from on Z-position, captured by intravital imaging for 1hr, frames every 2 min, 7fps.