

Supplemental Materials

Molecular Biology of the Cell

Oudin et al.

Supplementary figures:

Figure S1

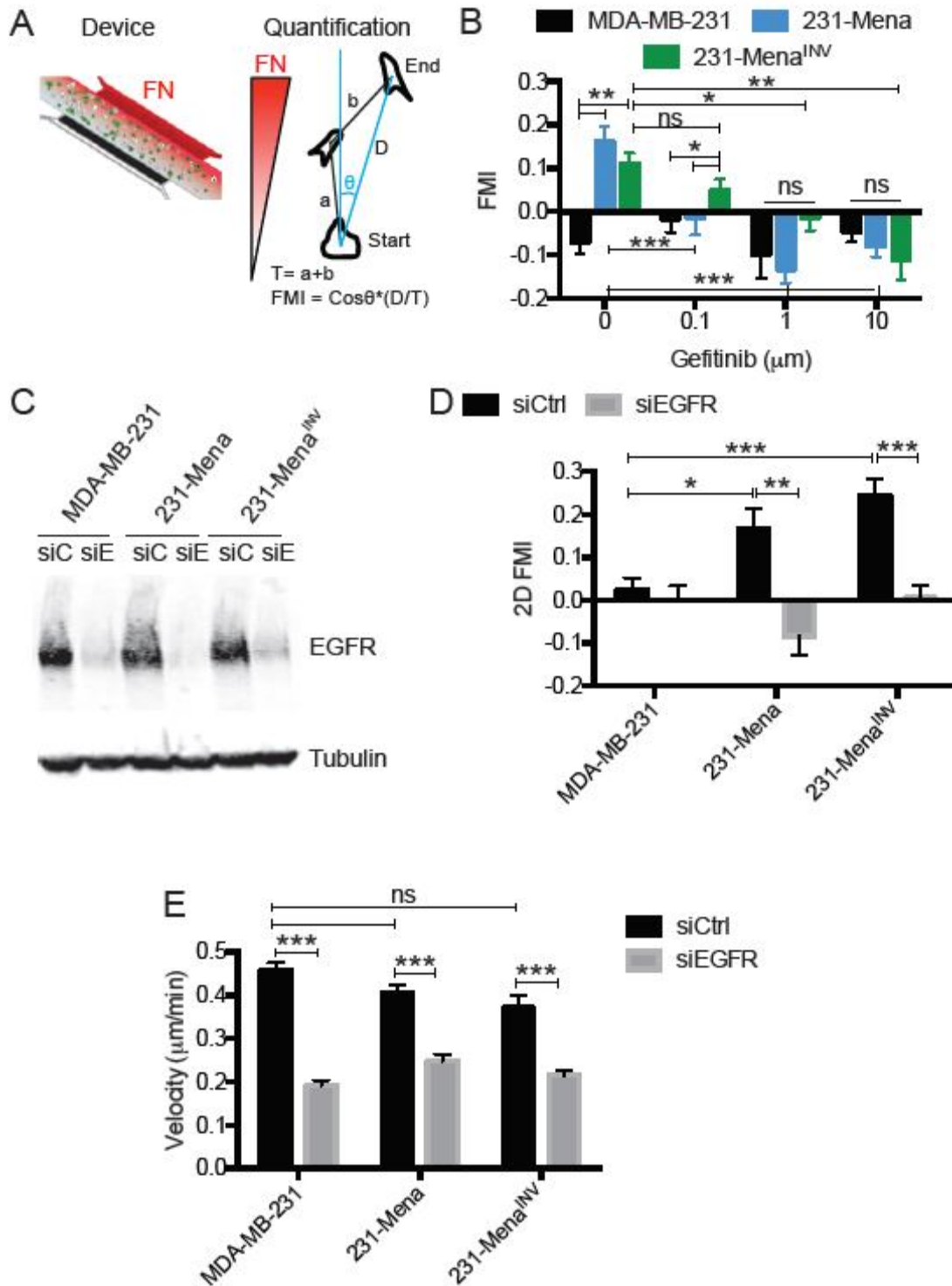


Fig S1: Confirming the role of EGFR in Mena^{INV}-driven haptotaxis

(A) Schematic of microfluidic device used for haptotaxis assay to generate FN gradients, as well as quantification of Forward Migration Index used to measure

directional migration relative to the FN gradient. B) Inhibition of EGFR with different concentrations of Gefitinib decreased Mena- and Mena^{INV}-driven haptotaxis on a 2D 125µg/ml low FN gradient. (C) Representative WB showing EGFR levels in MDA-MB-231, 231-Mena and 231-Mena^{INV} cells transiently transfected with siCtrl or siEGFR for 48hrs. (D) FMI of MDA-MB-231, 231-Mena and 231-Mena^{INV} when transfected with siCtrl or siEGFR. (E) Velocity of MDA-MB-231, 231-Mena and 231-Mena^{INV} when transfected with siCtrl or siEGFR. Data pooled from at least 3 experiments, with over 80 cells tracked per condition. Results show mean ± SEM, significance determined by one way ANOVA, *** p<0.005.

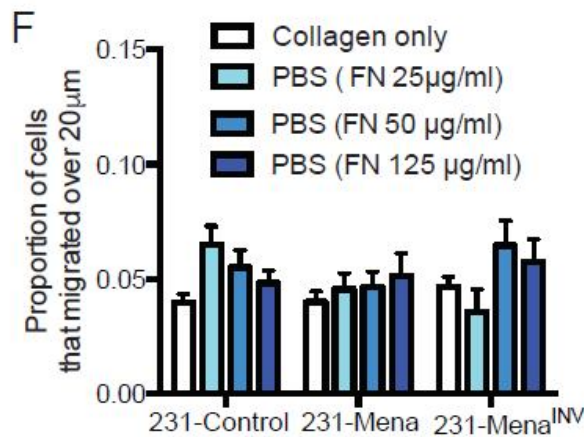
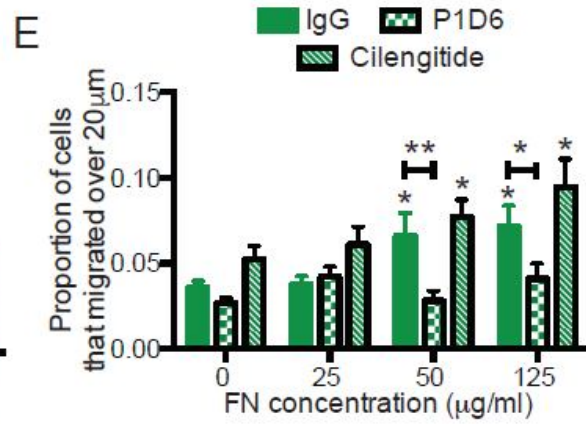
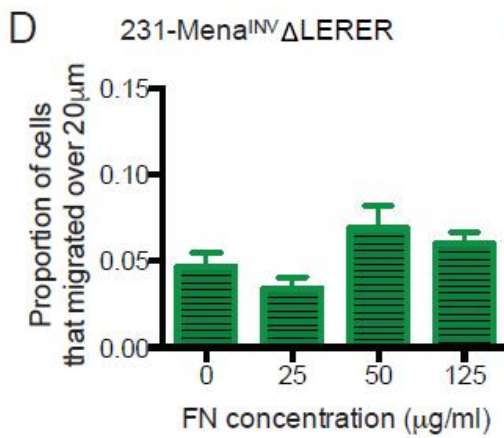
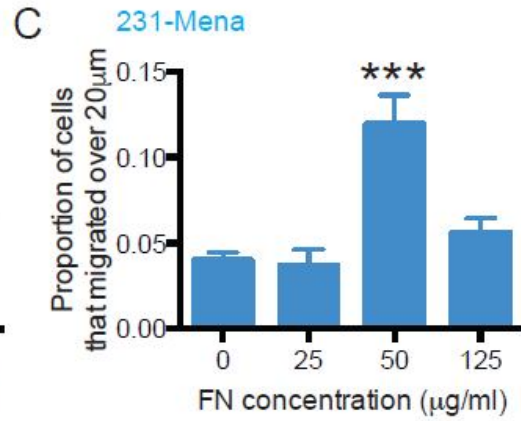
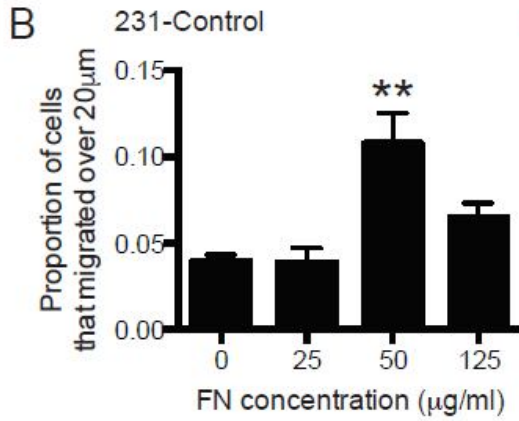
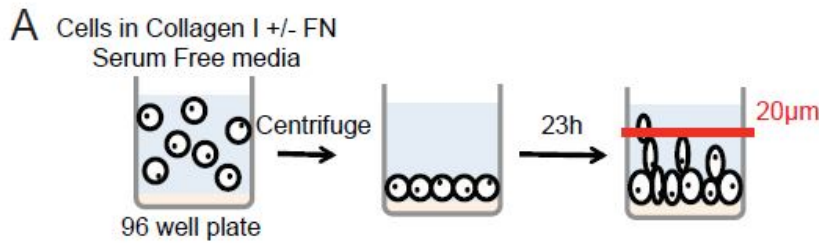


Fig S2: Mena^{INV}-driven FN haptokinesis requires the interaction between Mena^{INV} and $\alpha 5$

(A) Schematic of 3D invasion assay: Cells are plated in a 96-well plate in collagen gels with or without FN, centrifuged briefly so the cells form a uniform layer on the bottom of the well, then treated with media and fixed 24h later *In vitro* invasion assay in a 3D collagen gel with increasing concentrations of FN (0, 25, 50, 125, 175, 250 $\mu\text{g/ml}$) 231-Control (B) and 231-Mena (C) treated with serum-free media for 24h. The proportion of cells migrating 20 μm above baseline was quantified. (D) 231-Mena^{INV} Δ LERER cells did not respond to the increasing FN concentration in the gels. (E) Inhibition of $\alpha 5$ with P1D6, but not αv with Cilengitide, inhibited the Mena^{INV}-driven pro invasive effect in response to increasing FN concentrations. (F) Invasion assay where cells were plated in collagen and the volume of PBS corresponding to 25,50 and 125 $\mu\text{g/ml}$ of FN. PBS did not induce a migratory response. Results show mean \pm SEM, data pooled from at least 3 experiments.

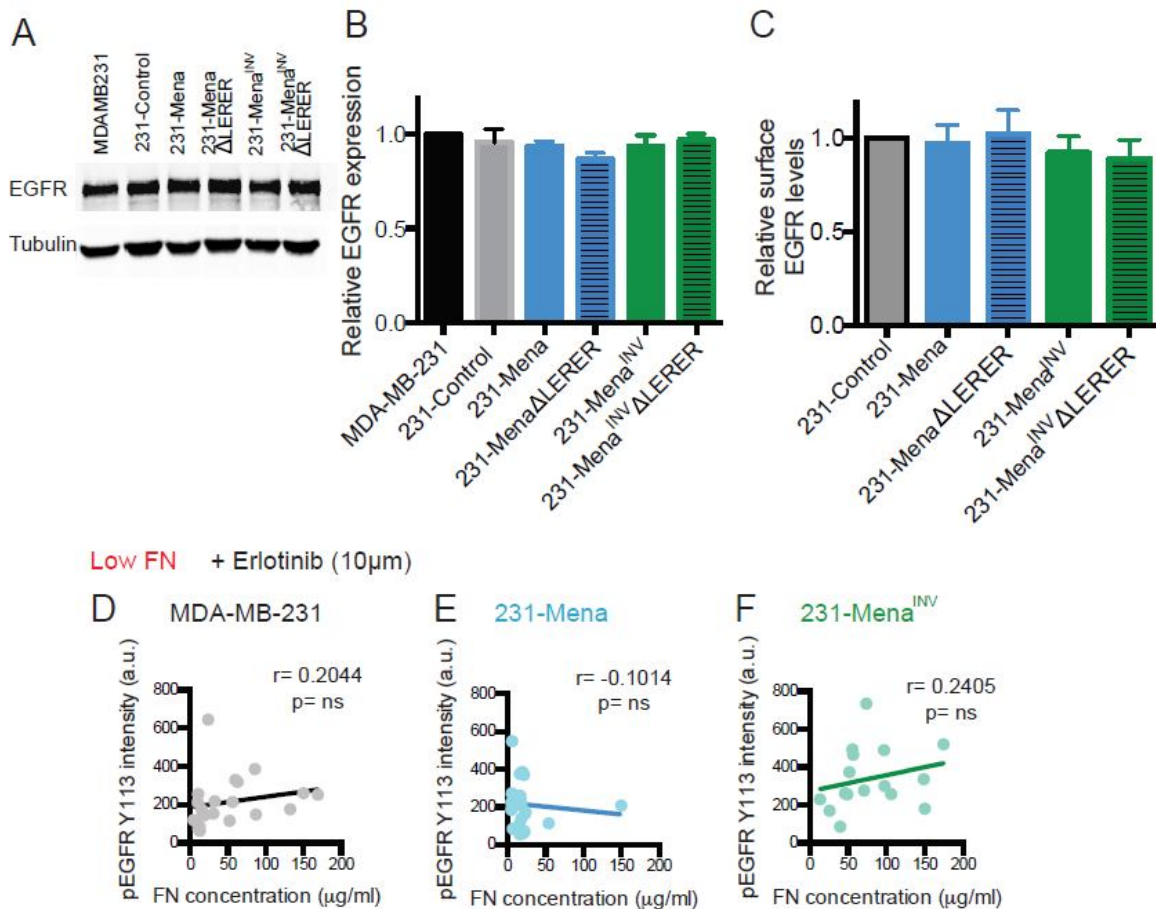


Fig S3: EGFR signaling in haptotaxis

A) Representative Western Blot of EGFR protein expression in lysates from MDA-MB-231 cells expressing different GFP-tagged Mena isoform. B) Quantification of EGFR total protein in lysates from MDA-MB-231 cells expressing different GFP-tagged Mena isoform. C) EGFR surface levels measured by FACS analysis in MDA-MB-231 cells expressing different Mena isoforms. Correlation between intensity in pEGFR at Y1173

and FN in 231-Control (D), Mena (E) or Mena^{INV} (F) cells plated on a low FN 2D gradient with 10uM Erlotinib. Each dot represents a single cell. J) Knockdown of p53 and RCP inhibits Mena^{INV}-driven 3D haptotaxis, while also affecting velocity (K).

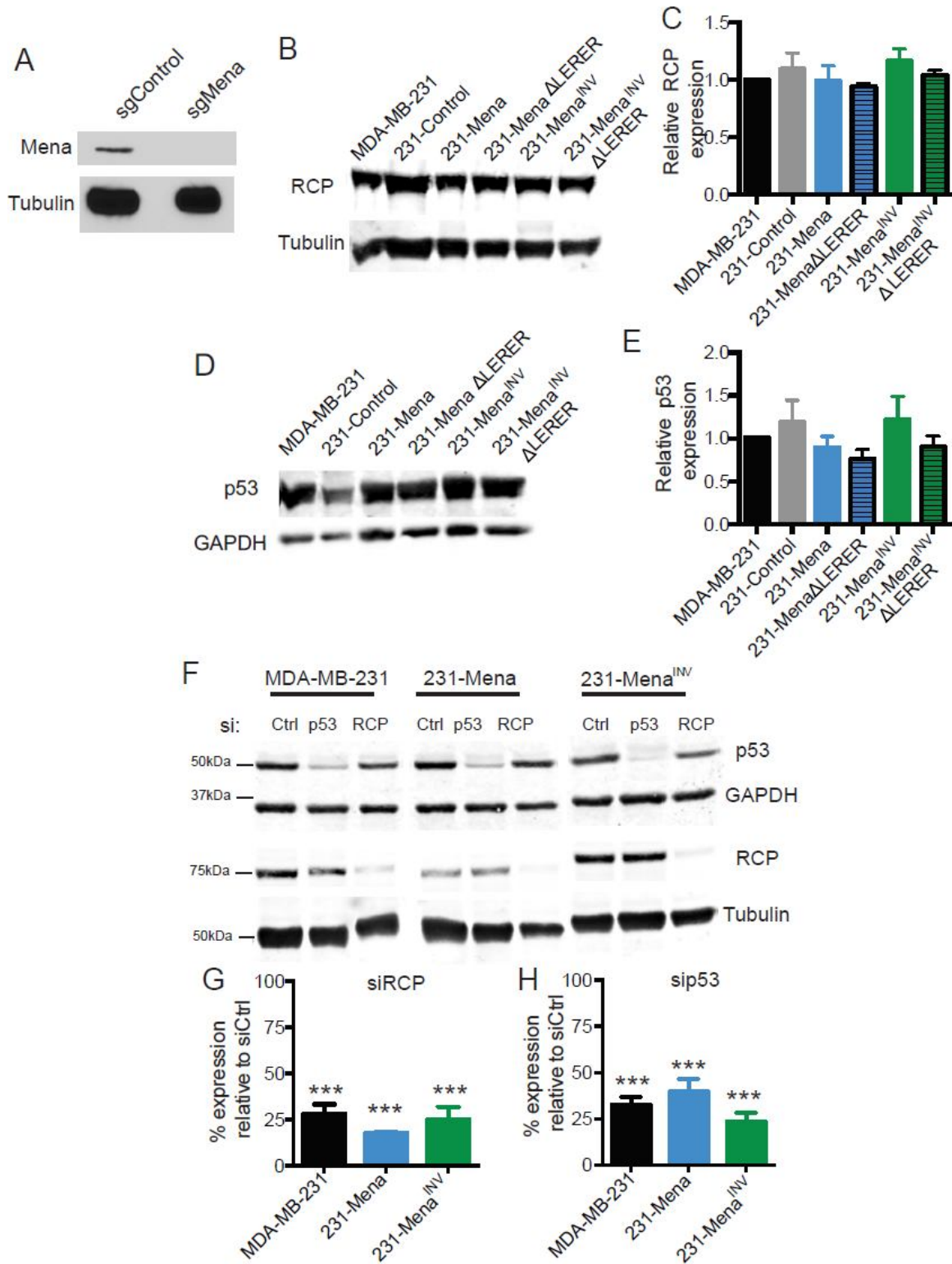


Fig S4: Knockdown controls for Mena, RCP and p53

(B) Representative knockdown for expression of Mena in MDA-MB-231 cells with stable sgControl or sgMena. (B) Representative WB for expression of RCP in MDA-MB-231 cells expressing different Mena isoforms. (C) Quantification of RCP levels relative to loading control Tubulin, data pooled from 3 experiments. (D) Representative WB for expression of p53 in MDA-MB-231 cells expressing different Mena isoforms. (E) Quantification of RCP levels relative to loading control GAPDH, data pooled from 3 experiments. (F) Representative WB for expression of RCP and p53 with siCtrl and sip53 or siRCP 72hrs after transfection in MDAMB231, 231-Mena and 231-Mena^{INV} cells. (G) Quantification of p53 expression levels in MDAMB231, 231-Mena and 231-Mena^{INV} cells 72hrs after transfection with with siCtrl or sip53. (H) Quantification of RCP expression levels in MDAMB231, 231-Mena and 231-Mena^{INV} cells 72hrs after transfection with siCtrl or siRCP. Results show mean \pm SEM, significance determined by one way ANOVA, *** $p < 0.005$.

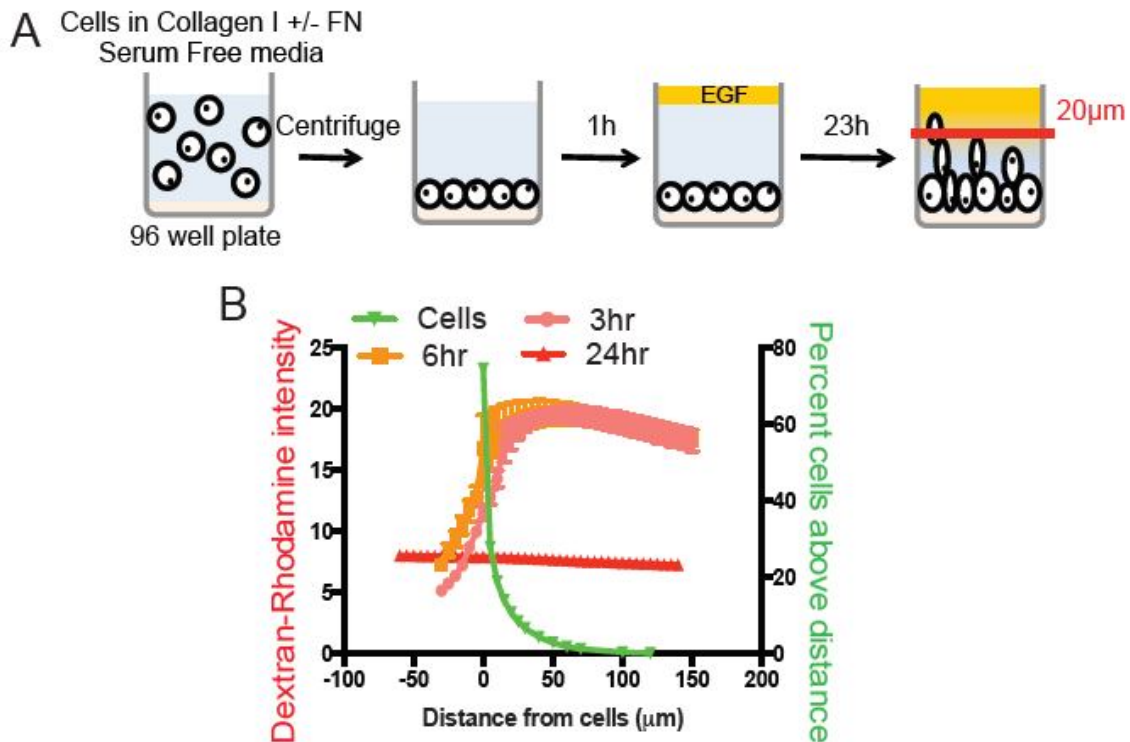


Fig S5: Characterization of the chemotactic gradient in the *in vitro* 3D invasion assay

A) Schematic of 3D invasion assay: Cells are plated in a 96-well plate in collagen gels with or without FN, centrifuged briefly so the cells form a uniform layer on the bottom of the well, then treated with media or EGF an hour later, and fixed 24h later. B) Graph showing the percentage of cells invading in the collagen gel, relative to the slope of the gradient, imaged at 3hr, 6h and 24h post-plating.

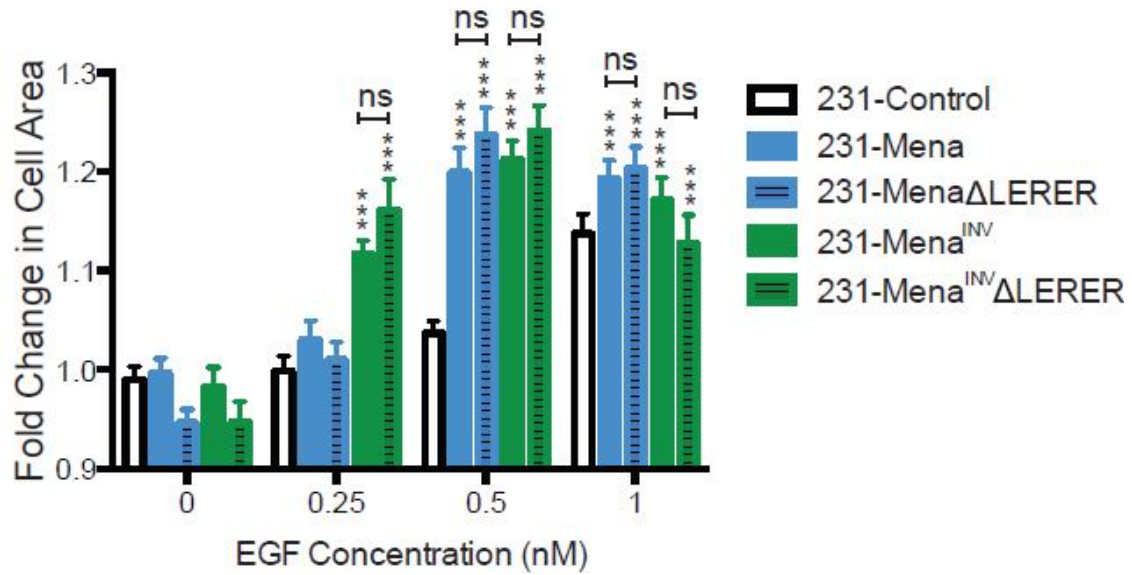


Fig S6: The interaction between Mena/Mena^{INV} and α 5 is not required for protrusion in response to EGF

Lamellopodial protrusion assay, where MDA-MB-231 cells expressing different Mena isoforms plated on 0.1mg/ml collagen and 0.2% Matrigel, starved for 4hrs and treated with increasing EGF concentration for 8 min. Cell area was measured before and after EGFR treatment. Data pooled from 3 experiments with at least 20 cells per experiment. Results show mean \pm SEM, significance determined by one way ANOVA, *** $p < 0.005$.