Meyer et al., http://www.jcb.org/cgi/content/full/jcb.201201003/DC1

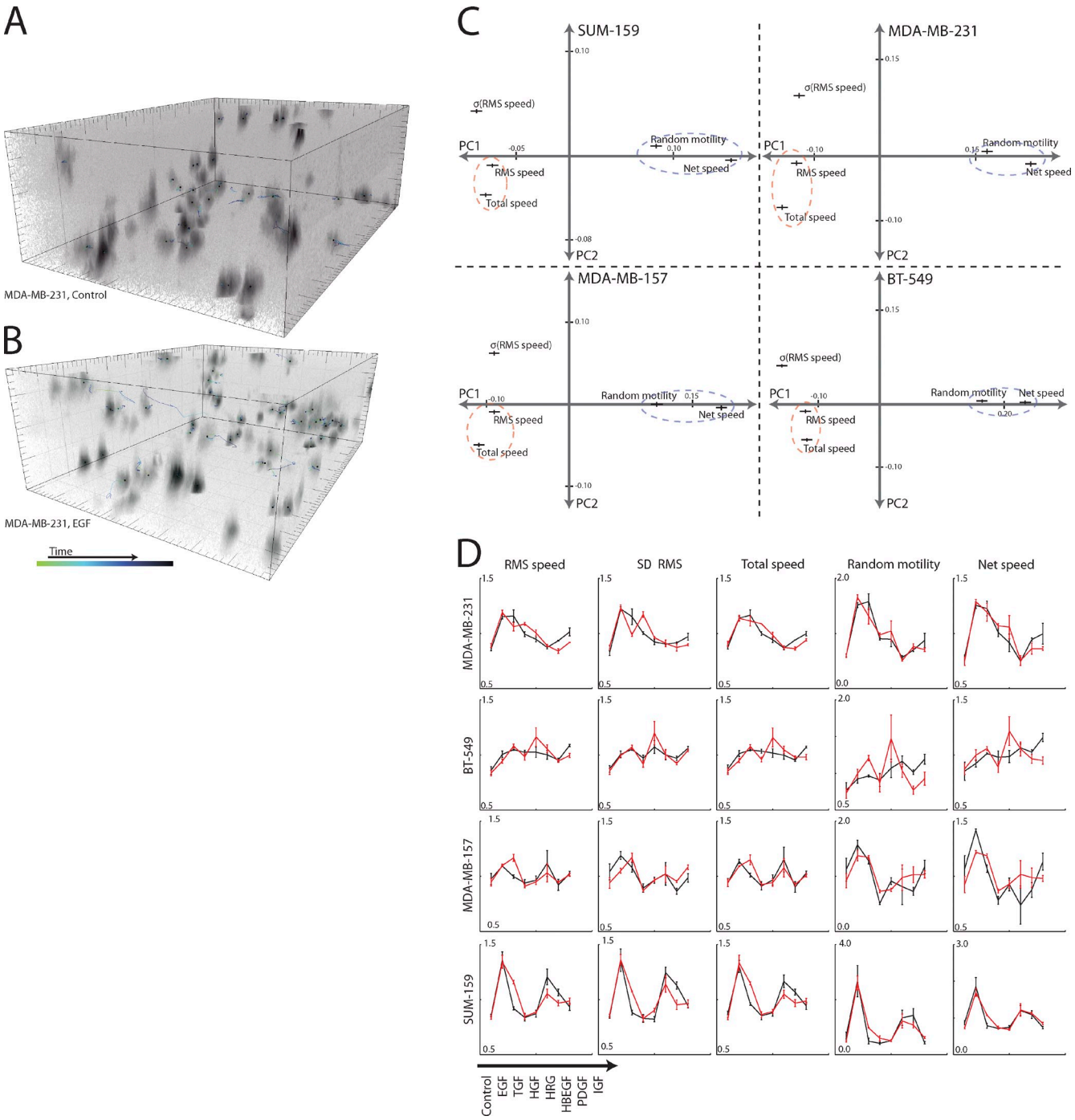

Figure S1. **Fluorescence contrast images of cells migrating within collagen I gels.** (A and B) Tracks were produced using Imaris and show the migration of MDA-MB-231 cells over 16 h with (bottom) or without (top) stimulation with 100 ng/ml EGF 4 h before imaging. Stacks consist of 70 slices measured 665 × 665 µm taken 3 µm apart. Shown is the maximal 45° perspective projection, with darker colors indicating higher fluorescence intensity. Fig. 1 C is a wind rose plot of these conditions. (C) Pairwise distances between each metric were calculated using the inverse Spearman rank correlation (1 − ρ)across individual cells, and condensed by multidimensional scaling. This method seeks to preserve the distances between each motility metric while representing all distances in two dimensions. Two dimensions (PC1 and PC2) captured >99% of the distance quantities. Speed- and persistence-related metrics are circled in red and blue, respectively. Error bars are SEM. (D) Plots for each motility metric across growth factor conditions are shown. Black and red lines indicate the median and 90th percentile responses, respectively. Each independent experiment was mean centered across growth factor conditions. The mean and SEM of all experiments ($n \geq 3$) is shown. IGF, insulin-like growth factor 1; HRG, Heregulin β1; HBEGF, heparin-binding EGF-like growth factor; HGF, hepatocyte growth factor.

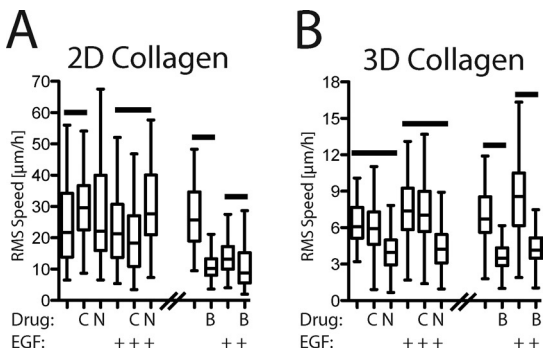

Figure S2. **MDA-MB-231 cells show differing sensitivity to cytoskeletal perturbation in 2D and 3D.** (A) RMS speed of cells treated with three cytoskeleton-related inhibitors with or without EGF stimulation on stiff collagen matrix. (B) RMS speed of cells treated similarly within 3D collagen gels. Bars on top indicate significant differences with respect to the no inhibitor control ($P < 0.05$). The lines indicate the median. The box is bound by the 25th and 75th quantiles. The whiskers extend to either the maximum and minimum values or two thirds the interquartile range, depending on which is closer to the median. C, cytochalasin D; N, nocodazole; B, blebbistatin.

Table S1. **Table of cell lines tested for motility in collagen I gels**

Cell line	Subtype	Markers	Migratory in 3D
MDA-MB-231	Basal B	V	+
SUM-159	Basal B	N, V	+
BT-549	Basal B	N, V	+
MDA-MB-157	Basal B	V	+
SUM-1315	Basal B	V	+
BT-483	Luminal	E, ER, PR, HER2	–
T47D	Luminal	E, N, ER, PR	–
MCF7	Luminal	E, ER, PR	–
MDA-MB-453	Luminal	None	–
SKBR3	Luminal	HER2	–

Cell lines were examined in 3D. The Markers column indicates which of the following clinical markers are present: E-cadherin (E), N-cadherin (N), vimentin (V), estrogen receptor (ER), progesterone receptor (PR), and HER2 (Nieman et al., 1999; Pishvaian et al., 1999; Lacroix and Leclercq, 2004; Neve et al., 2006; Rusnak et al., 2007; Hollestelle et al., 2010).

References

Hollestelle, A., J.H.A. Nagel, M. Smid, S. Lam, F. Elstrodt, M. Wasielewski, S.S. Ng, P.J. French, J.K. Peeters, M.J. Rozendaal, et al. 2010. Distinct gene mutation profiles among luminal-type and basal-type breast cancer cell lines. *Breast Cancer Res. Treat.* 121:53–64. http://dx.doi.org/10.1007/s10549-009-0460-8

Lacroix, M., and G. Leclercq. 2004. Relevance of breast cancer cell lines as models for breast tumours: an update. *Breast Cancer Res. Treat.* 83:249–289. http://dx.doi.org/10.1023/B:BREA.0000014042.54925.cc

Neve, R.M., K. Chin, J. Fridlyand, J. Yeh, F.L. Baehner, T. Fevr, L. Clark, N. Bayani, J.-P. Coppe, F. Tong, et al. 2006. A collection of breast cancer cell lines for the study of functionally distinct cancer subtypes. *Cancer Cell.* 10:515–527. http://dx.doi.org/10.1016/j.ccr.2006.10.008

Nieman, M.T., R.S. Prudoff, K.R. Johnson, and M.J. Wheelock. 1999. N-cadherin promotes motility in human breast cancer cells regardless of their E-cadherin expression. *J. Cell Biol.* 147:631–644. http://dx.doi.org/10.1083/jcb.147.3.631

Pishvaian, M.J., C.M. Feltes, P. Thompson, M.J. Bussemakers, J.A. Schalken, and S.W. Byers. 1999. Cadherin-11 is expressed in invasive breast cancer cell lines. *Cancer Res.* 59:947–952.

Rusnak, D.W., K.J. Alligood, R.J. Mullin, G.M. Spehar, C. Arenas-Elliott, A.-M. Martin, Y. Degenhardt, S.K. Rudolph, T.F. Haws Jr., B.L. Hudson-Curtis, and T.M. Gilmer. 2007. Assessment of epidermal growth factor receptor (EGFR, ErbB1) and HER2 (ErbB2) protein expression levels and response to lapatinib (Tykerb, GW572016) in an expanded panel of human normal and tumour cell lines. *Cell Prolif.* 40:580–594. http://dx.doi.org/10.1111/j.1365-2184.2007.00455.x