Rolny et al., http://www.jem.org/cgi/content/full/jem.20072509/DC1

MTT assays. A549 cells were seeded in multiple 96-well plates (1,000 cells/well). Each day of the culture, cell number and viability were evaluated in one multiwell plate by measuring the mitochondrial-dependent conversion of the tetrazolium salt MTT (Sigma-Aldrich) into a colored formazan product. In brief, the cells were rinsed with PBS, and 100 μl of fresh RPMI 1640 medium without FBS was added to each well. 10 μl of MTT solution in 5 mg/ml PBS was then added to each well, and the dishes were incubated for 2 h at 37 °C in a 5% CO_2 humidified incubator. The medium was discarded, the cells were rinsed with PBS, and 100 μl of DMSO was added to each well to extract the dye (followed shaking for 20 min). Dye absorbance was eventually measured at 570 nm.

HUVEC co-culture experiments. HUVECs were from Clonetics and were grown in endothelial cell basal media from Cambrex. For co-culture experiments, 10^5 HUVECs were plated on gelatin-coated cover slips for 24 h. 10^3 MDA-EV and MDA-3B cells were labeled with Vybrant CellTracker green (Invitrogen) for 15 min and then plated on a confluent monolayer of HUVECs for 24 h. Cells were fixed with methanol and stained for CD31.

Endothelial cell migration. HUVEC motility was assayed using Transwell chamber inserts (8-μM pore size; Costar). The lower surface of the porous membrane was coated by incubation with 10 μg/ml fibronectin overnight. HUVECs were detached with 1 mM EDTA in PBS and resuspended in serum-free medium. 10^5 cells were added to the upper side of the porous membrane and allowed to migrate toward the lower chamber for 4 h. The migrated cells to the lower side were fixed with 11% glutaraldehyde and stained with crystal violet. Cells were photographed and the dye was solubilized in 10% acetic acid to measure absorbance at 562 nm with a microplate reader.

REFERENCES

1. van 't Veer, L.J., H. Dai, M.J. van de Vijver, Y.D. He, A.A. Hart, M. Mao, H.L. Peterse, K. van der Kooy, M.J. Marton, A.T. Witteveen, et al. 2002. Gene expression profiling predicts clinical outcome of breast cancer. *Nature*. 415:530–536.
2. Bhattacharjee, A., W.G. Richards, J. Staunton, C. Li, S. Monti, P. Vasa, C. Ladd, J. Beheshti, R. Bueno, M. Gillette, et al. 2001. Classification of human lung carcinomas by mRNA expression profiling reveals distinct adenocarcinoma subclasses. *Proc. Natl. Acad. Sci. USA*. 98:13790–13795.
3. Talantov, D., A. Mazumder, J.X. Yu, T. Briggs, Y. Jiang, J. Backus, D. Atkins, and Y. Wang. 2005. Novel genes associated with malignant melanoma but not benign melanocytic lesions. *Clin. Cancer Res*. 11:7234–7242.
4. Li, A., S. Dubey, M.L. Varney, B.J. Dave, and R.K. Singh. 2003. IL-8 directly enhanced endothelial cell survival, proliferation, and matrix metalloproteinases production and regulated angiogenesis. *J. Immunol*. 170:3369–3376.