Supplementary Materials and methods

For northern blots, 15 μg total RNA were run through 1.2% agarose gels, transferred to a Hybond-N membrane (Amersham, Arlington Heights, IL) and hybridized with a random-primed ^{32}P-ATP (Amersham)-labeled VEGF, c-Myc, and 18s cDNAs probe at 42^{o}C for 24h as described previously (Amundadottir et al., 1995).

RNA from 5 Myc and 5 Myc/VEGF tumors were used to compare gene expression profiles between Myc and Myc/VEGF primary tumors. RNA for Myc tumors was pooled and used as a reference to compare expression profiles of each of the 5 Myc/VEGF tumors. The preparation of the cDNA labeled probes was performed using the MICROMAX^{TM} system (NEN Life Science Products, Boston, MA), according to the manufacturer's protocol, as previously described (Calvo et al., 2002). Hybridizations were carried out on the Incyte Genomics mouse GEM1 set of cDNA clones, spotted at the National Cancer Institute Advanced Technology Center. The gene list is available at http://nciarray.nci.nih.gov. Normalization, statistical analyses and hierarchical clustering were performed as described earlier (Calvo et al., 2002).

Biological interpretation of the filtered genes was carried out by Gene Ontology (GO) enrichment analysis using the GARBAN™ software (Genomic Analysis for Rapid Biological Annotation, http://garban.tecnun.es/garban2/index.php). Signaling pathways were analyzed with Ingenuity™ software (Ingenuity Systems, Redwood City, CA). Gene expression profiles of human breast tumors were retrieved from Gene Expression Omnibus (GEO, accession number GSE2603) (Minn et al., 2005) and cross-compared with the mouse dataset.

For the migration experiment using MV630 cells, the following reagents were used: VEGF-R1 specific inhibitor Flt-1/Fc chimera (100 ng/mL) (R&D Systems); anti-

TNC blocking antibody (10 μg/mL) (Chemicon); and the VEGF-R2 inhibitor GW654652 (1 μM) (GlaxoSmithKline).

Cell lines from a Myc/VEGF tumor (designated MV630, and MVT1) were established following previously published protocols (Pei et al., 2004a). The cell line Myc83 was previously established from a Myc transgenic mammary tumor (Pei et al., 2004b). For transplantation experiments, Mammary Myc or Myc/VEGF tumor pieces ($1mm^3$ in size) were transplanted subcutaneously into the right and left flanks of either nude mice, or wild type FVB/N mice. Animals were followed up for tumor progression and lung metastases.

Orthotopic injection of either 10^6 cells MDA-MB-435 parental cells, or cell clones were injected with Matrigel™ in the mammary fat pads of athymic female nude mice (5 mice per group). When tumors reached 1.5 cm in diameter, surgical procedures were performed to remove primary tumors and animals were then observed for tumor relapse for an additional 8 weeks. Tumor volume was measured with the formula: The largest diameter x (the smallest diameter)^2 x 0.4. For lung metastasis assays, 2 x 10^4 cells were injected in the tail vein of female athymic nude mice (5 mice per group). Two weeks after injection, animals were sacrificed and lungs were removed and processed for histological analysis. The total number of metastatic foci per reference area (35 mm^2) was quantified.

For quantification of the vascular density, 15 random images per slide were captured electronically from CD-31 stained tumors and image analysis was performed with Analysis™ software. The area occupied by the vessels was divided by the total area of the field, and final results are given as the percentage of vascular area compared to the reference area (Mean Vascular Density).