METHODS

Cell Culture. The HMLE immortalized human mammary epithelial cells were described previously[28]. Cell lines MCF7, MDA-MB-231 and 4T1 were from ATCC and cultured under conditions provided by the manufacturer. Cell lines SUM149 and SUM159 were from Dr. S. Ethier and grown as described (http://www.asterand.com/Asterand/human_tissues/149PT.htm). MCF7-RAS cells were described previously[37]. The *MYCN*-normal copy cell line SH-EP carrying an expression vector encoding for MYCN fused to an estrogen responsive domain (referred to as SHEP-MYCN-ER)[42] was cultured in complete RPMI medium (Invitrogen) supplemented with 10% fetal calf serum, 1% penicillin/streptomycin and 1% kanamycin. SHEP-MYCN-ER cells were induced with 4-hydroxy-tamoxifen (4-OHT, 200 nM) for 48 hours.

Plasmids and SiRNA. The MDH1-PGK-GFP 2.0 vector for ectopic expression of miRNAs was described previously[6,51]. The binding site for miR-9, or a partial *CDH1* 3'UTR sequence (819 bp), was cloned into the pMIR-REPORT luciferase construct[6,52]. The mutant construct of the *CDH1* 3'UTR was generated using a QuikChange Site-Directed Mutagenesis Kit (Stratagene). Vectors expressing E-cadherin or ΔN90β-catenin were used as described[27]. E-cadherin and β-catenin shRNA constructs were described previously[27]. The MYC-expressing pBabe-puro vector is from lab stock. The Topflash and Fopflash constructs were described previously[33].

miRNA sponge. The miR-9 sponge was constructed using a method modified from previous reports[11,36]: annealed oligonucleotides for tandem miR-9 binding sites were ligated into the pcDNA5-CMV-d2eGFP vector (Invitrogen) digested with XhoI and ApaI. The *gfp* mRNA along

with the miR-9 sponge sequence in the 3'UTR was then subcloned into the pBabe-puro vector digested with BamHI and SalI. The control sponge was described previously[11,36].

RNA isolation and miRNA quantification. Total RNA from cultured cells, with efficient recovery of small RNAs, was isolated using the mirVana miRNA Isolation Kit (Ambion). Quantification of the mature form of miRNAs was performed using either the mirVana qRT-PCR miRNA Detection Kit or the TaqMan MicroRNA Assay Kit (data obtained using these two kits are comparable), according to the manufacturer's instructions (Applied Biosystems). The U6 small nuclear RNA was used as an internal control.

Real-time RT-PCR of mRNAs. Total RNA was reverse transcribed with an iScript cDNA synthesis kit (Bio-Rad). The resulting cDNA was used for PCR using the SYBR-Green Master PCR Mix (Applied Biosystems) in triplicates. PCR and data collection were performed on iCycler (Bio-Rad). The expression levels of samples were determined using the standard curve method. Data were normalized to an endogenous control β-actin. Primer sequences are listed in supplementary materials. For *VEGFA*, we used real-time RT-PCR primers that are specific to common exons of the various *VEGFA* splice isoforms and therefore are capable of detecting the total levels of the *VEGFA* mRNA.

miRNA gene cloning and ectopic expression. The human *mir-9-3* and *mir-10b* genes were PCR amplified from normal genomic DNA and cloned into the MDH1-PGK-GFP 2.0 retroviral vector. The production of amphotropic viruses and infection of target cells were described previously[53].

***In Vitro* migration and invasion Assays.** Transwell migration assays and Matrigel invasion assays were performed as described previously[6].

Luciferase reporter assay. Luciferase reporter assays were performed as described previously[6].

Immunoblotting. Western blot analysis was performed as described previously[6], using the following antibodies: anti-E-cadherin (#610182, 1:1000, BD Transduction), anti-vimentin V9 (MS-129-P, 1:1000, Neomarkers), anti-phospho-β-catenin (#9561S, Ser33/37/Thr41, 1:1000, Cell Signaling Technology), anti-β-catenin (#610154, 1:1000, BD Transduction), and anti-β-actin (ab8226-100, 1:5000, Abcam). The ImageGauge Program was used for densitometric analysis of Western blots.

Immunofluorescence. Immunofluorescence was performed as described previously[27], using an anti-β-catenin (#610154, 1:1000, BD Transduction) primary antibody and an Alexa Fluor-594-conjugated anti-mouse secondary antibody (A11005, 1:500, Invitrogen). DAPI was used for nucleus staining.

Surgery, necropsy, histopathology, and immunohistochemistry. All animal experiments were performed in accordance with a protocol approved by the MIT committee on Animal Care. Surgery (mammary fat pad implantation), necropsy, and histological analysis were performed essentially as described[6]. Six- to eight-week-old female mice (from Jackson Laboratory) were used for surgery: NOD-SCID mice were used for human tumor cells; immunocompetent Balb/c

mice were used for 4T1 cells. The IHC detection using anti-Ki-67 (1:50, Pharmingen) and anti-MECA-32 (1:50, from University of Iowa) antibodies was performed on paraffin sections, using a BioGenex i6000 automated stainer in the Histology Core Lab at MIT. The IHC detection using anti-vimentin (human specific, pre-diluted per the manufacturer, Ventana platform) and anti-cytokeratin antibodies AE1/AE3 (1:800, BioGenex) was performed on paraffin sections in the Histology Core Lab at MSKCC.

ELISA. Blood was harvested from tumor-bearing mice using EDTA-treated tubes (BD Biosciences). Plasma was obtained by removing blood cells through centrifugation. The plasma levels of VEGF were measured using an ELISA (enzyme-linked immunosorbent assay) kit (R&D Systems) that is specific for human VEGF165, according to the manufacturer's instructions. (Use of this kit revealed that the VEGF level in the plasma of non-tumor-bearing mice is negligible.)

Genome-wide ChIP-on-chip. Chromatin immunoprecipitation was performed as described previously[54,55]. ChIP-DNA templates from Kelly (*MYCN*-amplified) or SJ-NB-12 (*MYC*-amplified) cells were immunoprecipitated using 10 μg of the MYCN (sc-53993, Santa Cruz) or MYC (sc-764, Santa Cruz) antibody, and were amplified for DNA microarray analysis (Agilent Human Promoter ChIP-chip Set 244K) using WGA (Sigma) method as previously described[54]. DNA labeling, array hybridization and measurement were performed according to Agilent mammalian ChIP-chip protocol. In brief, amplified ChIP-DNA and input DNA were labeled with Cy-5 and Cy-3, respectively, and hybridized together on the promoter array. After scanning and feature extraction, the data were normalized using the variance stabilization algorithm, and

then the log2 ratios were calculated for ChIP-DNA versus input DNA. Positive binding was defined using the following criteria, based on a validated set of 153 MYCN/MYC target genes: a center probe is ≥2 fold enriched (log2 ratio ≥1) and the two neighboring probes are ≥1.5 fold enriched (log2 ratio ≥0.58). For the visualization of ChIP-chip results, the cureos package v0.2 for R was used (available upon request). Histone marks for active transcription (H3K4me3; ab8580, Abcam) and repressed transcription (H3K27me3; 07-449, Upstate) were assessed together with MYC binding. Negative control regions are shown in Supplementary Information, Fig. S9 and in a related study[54,55].

Patient samples and miRNA expression profiling. RNA samples from primary breast tumors were described previously[6]. A total of 45 primary neuroblastoma tumor samples were collected at the Ghent University Hospital (Ghent, Belgium) and the Medical School of Valencia (Valencia, Spain) prior to therapeutic treatment. Patients were staged according to the International Neuroblastoma Staging System[56]. Informed consent was obtained in all cases. Total RNA was extracted using the miRNeasy kit (Qiagen) according to the manufacturer's instructions. RNA integrity was assessed on the Experion (Bio-Rad) and evaluated by means of the RNA quality index (RQI). For all samples the RQI was higher than 5. MiRNA expression was profiled as described previously[57,58]. In brief, 20 ng of total RNA was reverse transcribed using the Megaplex RT stem-loop primer pool (Applied Biosystems). Subsequently, Megaplex RT product was pre-amplified and quantified using miRNA specific hydrolysis probes and primers (Applied Biosystems). Reactions were performed on the 7900HT RT-qPCR system (Applied Biosystems).

Statistical analysis. Unless otherwise noted, data are presented as mean ± s.e.m, and Student's *t* Test (two-tailed) was used to compare two groups ($p < 0.05$ was considered significant) for independent samples, assuming equal variances on all experimental data sets.

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