**Supplementary information**

**Bioinformatics analyses**

The mRNA input into the Ingenuity Pathway core analysis was generated by selecting mRNAs which were targeted by 6 or more of the top ten most significant up- and down-regulated miRNAs, and in which the target prediction was made by 2 or more search engines that comprised TargetScan, MiRanda, RNA22, miRWalk, PICTAR4, PITA, DIANA-mT and RNAhybrid. This was done using a pearl script that extended miRWalk files created with the ‘extendAndFilterMiRWalk.pl’ and created an output file that showed if an mRNA is a predicted target of the input microRNAs and also how many prediction tools generated the prediction.

# Cell culture

Cell lines (RKO, HCT116, SW480, SW620 and A375) were obtained either from the ATCC or the DSMZ (Germany) and cultured routinely in T25 flasks at 370C in the presence of 5% CO2 and 90% humidity. Culture media was cell line specific and supplemented with 10% FCS and 1% penicillin/streptomycin.

# Site- directed mutagenesis

The QuikChange II XL site-directed mutagenesis kit (Stratagene) was used to create the sequence mutations in the binding motifs of miRs-135b, -210 and -218 in the respective 3’UTRs of target genes *SETD2, SIAH1, N-CADHERIN, FOXN3* and *ZEB2*.

## RT-PCR

RT-PCR was carried out using the Quantitect Primer assays (Qiagen, Germany) and SYBR green detection system on a LightCycler 480 (Roche) and the delta delta CT algorithm. Messenger RNAs were normalized to beta-actin, GAPDH or Tata box binding protein (TBP) whereas miRNAs were normalized to U6 or Sno72. The primers used include miR-135b (Cat. No MS00003472); miR-210 (Cat No MS00003801); miR-218 (Cat. No MS00006769); U6 (Cat. No MS00033740); SNO72 (Cat. No MS00014021); ZEB2 (Cat. No QT00008554); FOXN3 (Cat. No QT00049525); CDH1 (Cat. No QT00080143) and N-Cadherin (Cat. No QT00063196).

**RT-PCR primers**

The RT primers used were purchased from Qiagen, Germany, and include miR-135b (Cat. No MS00003472); miR-210 (Cat No MS00003801); miR-218 (Cat. No MS00006769); U6 (Cat. No MS00033740); SNO72 (Cat. No MS00014021); ZEB2 (Cat. No QT00008554); FOXN3 (Cat. No QT00049525); CDH1 (Cat. No QT00080143) and N-Cadherin (Cat. No QT00063196).

**Antibodies**

The antibodies used include; SETD2 (Abcam; ab69836), SIAH-1 (Santa Cruz; sc-5505), FoxN3 (Abcam; #ab12221), ZEB2 (Santa Cruz; sc-5505), N-cadherin (Abcam; #ab12221), E-cadherin (Cell Signaling; #3195) and β-actin (Santa Cruz; sc-1616).

**siRNAs, oligonucleotides, plasmids and viral vectors**

siRNAs targeting SETD2 (ID:s26424) and SIAH1(ID:s12835) were purchased from Ambion. miRNA mimics and anti-miRs for miRs 218 (ID:PM10328), -210 (ID:MC10516) and 135b (ID:MC13044) were also obtained from Ambion. The FOXN3 expression clone in pDEST vector was purchased from Source Bioscience (UK). N-cadherin promoter constructs were a generous gift from Pierre Marie, National Centre for Scientific Research, France. The miR-218 viral expression constructs LentimiR-hsa-218-1 (Cat No mh40291) and pLent-III-mir control vectors (Cat No m003) were obtained from Applied Biological Materials (Richmond, Canada). Details of the all primer sequences used are contained in supplementary primer list.

**Preparation of protein extracts and immunoblotting**

Cells were washed with PBS and lysed in extraction buffer (Biosource). The protein concentration was determined using the BCA (bicinchoninic acid) kit (Pierce). For immunoblotting, samples (40 μg/lane) were heated for 5 min, separated via SDS/PAGE (10% gels), and transferred on to PVDF membranes. After transfer, the membranes were blocked with 5% (w/v) non-fat dried skimmed milk powder in TTBS (Tris-buffered saline with 0.1% Tween 20) for 3 h at room temperature (25°C), and then probed with the indicated primary antibodies against SETD2 (Abcam; ab69836), SIAH-1 (Santa Cruz; sc-5505), FoxN3 (Abcam; #ab12221), ZEB2 (Santa Cruz; sc-5505), N-cadherin (Abcam; #ab12221), E-cadherin (Cell Signaling; #3195) and β-actin (Santa Cruz; sc-1616) for 2 h at room temperature. After three washes with TTBS, the blots were incubated at 25°C with respective horseradish-peroxidase-conjugated secondary antibodies for 2 hours. After final washes with TTBS, the membranes were exposed to film after use of ECL® (enhanced chemiluminescence) (Amersham Biosciences).

# Reporter gene assays

**Promoter assay**

The N-cadherin promoter (a kind gift from Prof Pierre J. Marie, INSERM UMR 606 and University Paris Diderot, Sorbonne Paris Cité Hôpital Lariboisière, France) in pGL3 vector was co-transfected together with a FOXN3 expression plasmid (Source Bioscience, UK) or empty vector. 3 x104 cells were seeded in white 96 well plates (Nunc) 24hrs prior to transfection. Luminescent signals were measured with a microplate reader (TECAN Trading AG, Switzerland). All experimental setups were performed in quadruplicate and repeated in at least 3 independent trials.

**3’UTR assays**

In the 3’UTR luciferase reporter assay, the 3’UTR of the mRNA of interest (SETD2, SIAH1, FOXN3, N-cadherin, and ZEB2) was cloned downstream of a reporter luciferase gene (firefly/renilla) into a multiple cloning site that is located between the stop codon and the (synthetic) poly A tail of the luciferase gene in a plasmid that has a constitutively active promoter. Co-transfection of the plasmid with the mimic or inhibitor of the miRNA of interest results in a suppression of luciferase activity with the mimic, or enhancement of activity with an inhibitor. In our case, based on the suitability of the multiple cloning sites, the pLightswitch 3’UTR plasmid (Switch Gear Genomics) or the pMIR-REPORT plasmid (Applied Biosystems) were used. For the assay, 3 x104 cells were seeded in white 96 well plates (Nunc) 24hrs prior to transfection. 3’-UTR reporter assays were performed by transfecting the respective 3’-UTR constructs together with the respective miRNA mimics, anti-miRs and controls. Luminescent signals were measured with a microplate reader (TECAN Trading AG, Switzerland). All experimental setups were performed in quadruplicate and repeated in at least 3 independent trials.

# Migration and invasion assays

The Boyden chamber, without and with a matrigel coating was used for migration and invasion assays respectively. Migrated/invaded cells were measured with the CellTiter-Glo cell viability assay kit (PromegaCorporataion, USA) according to the manufacturer’s protocol. A cell count of 5 x 104 and 1 x 105 cells were used for migration and invasion assays respectively. The cells were plated in 8 μM transwell plates (Corning Incorporated, USA) in 100 μl of basal medium with a matrigel coating (20 μg/well), in the case of the invasion assay. 500 μl of complete medium containing 10% FCS was used as a chemo- attractant in the lower transwell chamber. The cells were incubated for 24 hrs at 37°C in 5% C02. The migrated/invaded cells were detached into 500 μl of trypsin in a separate 24 well plate, while the non-migrated/invaded cells in the upper chamber were removed by adding 100 μl of trypsin into the upper well. The dislodged cells from both upper and lower transwells were transferred into a white 96 well plate (Nunc), centrifuged, and the trypsin decanted. 50 μl of CellTiterGlo was added to each well, and the luminescent signals were measured with a microplate reader (TECAN Trading, Switzerland). Relative migration/invasion was calculated by dividing the migrated/invaded cell values with the combined total for the particular set up.

**CAM Assay**

The chicken embryo chorionallantoic membrane (CAM) metastasis assay is used to in the evaluation of in vivo tumor growth, intravasation as well as distant metastasis. Treated/transfected and control cells are placed on the upper CAM of fertilized special pathogen free (SPF) eggs after 10 days of incubation. Access to the upper CAM is obtained by cutting a small window in the shell using the egg air sac as a pressure outlet to lower the CAM and prevent damage to the membrane. The artificially created window is then covered with adhesive tape, and the eggs are then incubated for a further 7 days in the incubator. On the 17th day of the experiment, the eggshells are cut open along the latitudinal axis in the midline with a pair of dissecting scissors, and the chicken embryos decapitated. The lungs and liver are harvested and genomic DNA is isolated from these organs. The number of metastasized cells is evaluated quantitatively using a real time PCR method that identifies human specific Alu sequences on a background of chicken DNA (van der Horst et al., 2004).

**Microarray data analysis**

Data analyses were performed using the linear model for microarray analysis (Limma). All statistical analyses were performed within the R statistical software environment (R version 2.15.3 using the R packages limma, version 3.14.4. MicroRNAs with a fold change of ≥ 1.5 and p-value ≤ 0.1 were considered significant.

**Mouse Experiments**

Significance of differences in the mean survival times for both cell lines was calculated by Student’s t-test (significance defined as p<0.05). Kaplan Meier survival analysis together with Mantel-Cox log rank statistics was applied to calculate significant differences in the course of survival of the mice. Mantel-Cox test results were considered significant with p≤ 0.05.

**Supplementary primer list**

All primer sequences are in the 5‘ to 3‘ direction.

1. SETD2\_UTR\_For: GAATCTAGAggatgggtggtcaggtaaga
2. SETD2\_UTR\_Rev: GAATCTAGAtagggaacacacatgccaag
3. SETD2\_RT\_For: ccaatgttcaaaggtgttgc
4. SETD2\_RT\_Rev: tgccaagtgcagtgagaaac
5. SETD2\_Mut\_For: CTTGGCGGTGCTGTCAG*TCTAGA*GACCCATGCGCATCCCCAC
6. SETD2\_Mut\_For: GTGGGGATGCGCATGGGTCTCTAGACTGACAGCACCGCCAA

GG

1. SIAH-1\_UTR\_For: GAATCTAGActctttcggtaggtggaagc
2. SIAH-1\_UTR\_Rev: GAATCTAGAaaacaaaagcctgatttgcag
3. SIAH-1\_RT\_For: gcacaactgcatccaacaat
4. SIAH-1\_RT\_Rev: atagccaagttgcgaatgga
5. SIAH-1\_Mut\_For: GGGTTTTTTTCCTTTAACTGACATCTAGACTTGAGTGGTC

ATGGGCCACTGC

1. SIAH-1\_Mut\_Rev: GCAGTGGCCCATGACCACTCAAGTCTAGATGTCAGTTAA

AGGAAAAAAACCC

1. FOXN3-UTR-For: GAATCTAGAtgatttgttttgaacttacgacca
2. FOXN3-UTR-Rev: GAATCTAGAtttttcacttggatcaaatagtttt
3. FOXN3\_M1\_For: ATTTTTAAAGGAGATTGAAGCAAAAGAACTCATATTGAC

ACTCAGC

1. FOXN3\_M1\_Rev: GCTGAGTGTCAATATGAGTTCTTTTGCTTCAATCTCCT

TTAAAAAT

1. FOXN3\_M2\_For:TTAAGAAAATGTTCCAAAAGCAAAAAGCCTGAAGATTGGCCCTG
2. FOXN3\_M1\_Rev:CAGGGCCAATCTTCAGGCTTTTTGCTTTTGGAACATTTTCTTAA
3. FOXN3\_210\_For: GCCCTGTGCACGCACGCTTTCACACACACACACACACAC
4. FOXN3\_210\_Rev: TGTGTGTGTGTGTGTGTGAAAGCGTGCGTGCACAGGGCC
5. β-Actin\_For: TCCCTGGAGAAGAGCTACG
6. β-Actin\_Rev: GTAGTTTCGTGGATGCCACA