Supplementary Table 1 Probes and reagents used in ISH

Probes and Reagents	Reference	Company
Hsa-miR-17a-5p. miRCURY LNA detection probe	619852-360	Exiqon
Scramble-miR. miRCURY LNA detection control probe	99004-15	Exiqon
U6, hsammurno. miRCURY LNA detection control probe	99002-15	Exiqon
microRNA ISH Bufferset (FFPE)	9000	Exiqon
RiboWash SSPE buffer RUO	5266262001	Roche
Discovery EZ Prep	5279755001	Roche
LCS (Liquid cover slip)	5264839001	Roche
Reaction buffer (10x)	5353955001	Roche
Discovery CC1 RUO	6414575001	Roche
Discovery Antibody block RUO	5268869001	Roche
Anti DIG AP Multimer	7256302001	Roche
ChromoMap Blue kit RUO	526661001	Roche
Red Stain II	5272017001	Roche
Ethanol 96%	20823.362	VWR, Avantor
Etnanol absolute	20821.296	VWR, Avantor
Xylene	28975.291	VWR, Avantor
Histokitt mounting medium	Assistant 1025/250	Sondheim/Rhoen

Supplementary Table 2 ISH protocol

	MiR-17-5p	**U6**	**Scramble miRNA**
Deparaffinization	EZ Prep, 3x12 min, 68ºC	EZ Prep, 3x12 min, 68ºC	EZ Prep, 3x12 min, 68ºC
Heat retrieval	Discovery CC1, 40 min, 95 ºC	Discovery CC1, 40 min, 95 ºC	Discovery CC1, 40 min, 95 ºC
Denaturation	8 min, 90 ºC	8 min, 90 ºC	8 min, 90 ºC
Hybridization Concentration Buffer	60 min, 54 ºC 20 nM microRNA ISH Bufferset	60 min, 55 ºC 1.5nM microRNA ISH Bufferset	60 min, 57 ºC 10nM microRNA ISH Bufferset
Stringency wash	RiboWash, 2x8 min, 54ºC	RiboWash, 2x8 min, 55ºC	RiboWash, 2x8 min, 57ºC
Blocking	AB block, 16 min, 37ºC	AB block, 16 min, 37ºC	AB block, 16 min, 37ºC
Secondary multimer	Anti-DIG-AP, 20 min, 37ºC	Anti-DIG-AP, 20 min, 37ºC	Anti-DIG-AP, 20 min, 37ºC
Detection	Blue kit, 60 min, 37ºC	Blue kit, 60 min, 37ºC	Blue kit, 60 min, 37ºC
Counter stain	Red II, 4 min, 37ºC	Red II, 4 min, 37ºC	Red II, 4 min, 37ºC
Used in multiple steps Coverslip solution Washing buffer	LCS (Liquid cover slip) Reaction buffer (10x)	LCS (Liquid cover slip) Reaction buffer (10x)	LCS (Liquid cover slip) Reaction buffer (10x)

Supplementary Table 3 Cell lines, culture, and transfection

	SK-BR-3	**MDA-MB-231**	**MCF-7**
Molecular subtype represented	HER2-positive	Triple negative	Luminal A
Cell line characteristics	HER2 overexpression, absence of ER and PR	Absence of ER, PR, and HER2 expression	ER and PR positive, low HER2 levels
Concentration in culture	2×10^5 cells/ml		
Culture medium	Opti-MEM I (1×) without phenol red (catalog# 11058-021, GIBCO, RF, UK), supplemented with 5% of fetal bovine serum (FBS) (catalog# S0415, Biochrom, Berlin, Germany) and Penicillin Streptomycin 1% (catalog# 15140-148, Gibco, NY, USA)		
Atmosphere	Humidified, 5% CO2: 95% air		
Temperature	37 °C		
Time	72 h		
Number of passages	<20	<15	<10
Cell confluence at start of experiments	80-95%		
MiR-17-5p mimic	hsa-miR-17-5p Pre-miR™ miRNA Precursor (catalog# PM12412, Thermo Fisher Scientific, USA)		
Negative control	Cy3™ Dye-Labeled Pre-miR Negative Control #1 (catalog# AM17120, Thermo Fisher Scientific, USA)		
Transfection reagent	Lipofectamine™ RNAiMAX (catalog#13778075, Thermo Fisher Scientific, USA).		

Supplementary Table 4 Proliferation, migration and invasion assays

	MTT assay (proliferation)	Wound-healing assay (migration)	Transwell assay (Invasion)
Plate type	96-well plates	24-well plates	24-well plates with ThinCert® chambers (Greiner Bio-one, Kremsmünster, Austria)
Coating (if applicable)	NA	NA	Polyethylene terephthalate membranes (8 µm pore size) pre-coated with 50 mL of phenol red-free Matrigel (Gibco)
Number of cells	5×10^3 cells/well	2×10^5 cells/well	2×10^5 cells/well
Medium	Standard culture medium	Serum-free medium with mitomycin C (10 µg/L)	Serum-free medium in the upper chamber; culture medium with 5% FBS in the lower chamber
Assay-specific steps	Cells were transfected with miR-17-5p mimic or negative control. Cells were treated with 12mM, [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (MTT, 5 mg/ml) (cat.# M6494, Invitrogen, OR, USA) and incubated for 4 h at 37 °C. Formazan crystals were solubilized with 0.01 M HCl/SDS and cells further incubated overnight at 37 °C.	A «wound» was created with a 200 µl sterile pipette tip and washed to remove debris. Cells were incubated for 4 h at 37 °C. Cells were transfected with miR-17-5p mimic or negative control, and incubated for 24 h at 37 °C.	Cells in the upper chamber were transfected and incubated for 48 h at 37 °C. Chambers were washed, fixed in 4% paraformaldehyde (30 min) and stained with 0.2% crystal violet (10 min). Non-invading cells were removed from the upper membrane surface using a cotton swab.
Washing solution	10 mM PBS	10 mM PBS	10 mM PBS
Detection and quantification	Absorbance at 570 nm: CLARIOstar® plate reader (BMG Labtech, Ortenberg, Germany)	Photographs: Nikon Eclipse TS100. Image analysis: Micrometrics SE Premium 4 software	Photographs of invaded cells on the lower membrane surface: Nikon Eclipse TS100. Image analysis: Image J (NIH, Bethesda, MD, USA).
Replicates	Three experiments with four parallel wells per cell line	Three experiments	Images of three random fields captured in duplicate

NA Not applicable; FBS Fetal Bovine Serum; PBS Phosphate-Buffered Saline