Supplementary Files

Supplementary Figure Legends

Supplementary Fig S1. Ago-HITS-CLIP successfully purifies endogenous and transfected AGO-bound miRNAs. (a) Immunoprecipitation of endogenous Argonaute (left) and resulting depletion of Argonaute (right) in cross-linked cell extracts. (b) Preparation of DNA libraries for illumina sequencing. (c) Endogenous miRNAs, and transfected miR-200a and miR-200b, (x axis) were co-immunoprecipitated with Argonaute and their abundance represented as a proportion of the total miRNA sequencing reads (y axis).

Supplementary Fig S2. Examples of non-canonical targeting identified by Ago-HITS-CLIP. Histograms of examples of various non-canonical modes of targeting are shown. The y axis shows the number of unique sequencing reads comprising the peak and the x axis indicates the position of the peak within the 3’UTR. The locations of potential seed sites (black arrows, 8-mers; purple arrows, 7-mers; yellow arrows, 6-mers; asterisk, central-paired) are indicated. Sequence alignments of miR-200a and miR-200b with the target sites identified by Ago-HITS-CLIP are shown.

Supplementary Fig S3. Cytoskeletal remodeling genes are strongly enriched in the miR-200a and miR-200b targets identified using Ago-HITS-CLIP. Pathway analysis was performed using GeneGo and the top 10 pathway maps (by P value) displayed for the genes targeted by miR-200a and miR-200b within 3’UTRs and coding regions (CDS).
Supplementary Fig S4. miR-200 promotes cortical actin and E-cadherin localization and inhibits migration and invasion. (a) Staining to show localization of E-cadherin (immunofluorescence, green), actin (phalloidin, red) and nuclei (DAPI, Blue) in MDA-MB-231 cells transfected with control or miR-200 mimics. Scale bars represent 0.01 mm. (b-d) MDA-MB-231 cells were transfected with control or miR-200a and -200b mimics. Cell migration was assessed by (b) transwell migration and (c) real time imaging of scratch wound assays. (d) Cell invasion was assessed by matrigel transwell invasion assay. Error bars represent SD.

Supplementary Fig S5. miR-200 does not effect early steps of invadopodia formation. MDA-MB-231 cells were transfected with 20 nM control or 10 nM miR-200a plus 10 nM miR-200b mimics and the effects on the early stages of invadopodia formation assessed by (a) western blotting for phospho-Src (Y416), (b) blotting for Tks5 or cortactin after anti-phosphotyrosine IP and (c) anti-phospho-tyrosine western blotting after cortactin immunoprecipitation. (d) representative images showing colocalisation (yellow puncta, arrowheads) of actin (red puncta) and TKS5 (green puncta), markers of invadopodia. Scale bars represent 0.01 mm.

Supplementary Fig S6. miR-200 target gene expression correlates negatively with miR-200 across cell lines and breast cancers. Spearman correlation coefficients were calculated for the expression of miR-200 target genes identified by HITS-CLIP (classified into 8mer, 7mer and 6mer sites within the 3’UTR or CDS) with miR-200a, -200b and -200c across (a) 59 cell lines from the NCI-60 cancer cell line panel and (b) 934 breast cancers from the Cancer Genome Atlas (TCGA) dataset. Plots represent q values of the correlations (in log scale) on the y-axis plotted against the correlation coefficients on the x-axis. The vertical red line indicates the median
Supplementary Fig S7. miR-200 target gene expression is not negatively correlated with miR-24 across cell lines and breast cancers. Spearman correlation coefficients were calculated for the expression of miR-200 target genes identified by HITS-CLIP with miR-24 across (a) 59 cell lines from the NCI-60 cancer cell line panel and (b) 934 breast cancers from the Cancer Genome Atlas (TCGA) dataset. Plots represent q values of the correlations (in log scale) on the y-axis plotted against the correlation coefficients on the x-axis. The vertical red line indicates the median correlation coefficient. Shaded regions represent the 25th and 75th median percentiles, with blue showing regions of positive correlation and negative correlations shown as red.
Supplementary Table S1. Complete gene lists of all putative miR-200a and miR-200b targets identified by Ago-HITS-CLIP. Complete gene lists of all targets identified by Ago-HITS-CLIP for miR-200a and miR-200b are listed, subdivided into perfect 6-mer, 7-mer and 8-mer seed matches within 3’UTRs, CDS and 5’UTRs. Transcripts with multiple peaks are listed separately for each peak.

Supplementary Movie S1. MiR-200 stabilises focal adhesions. Live-cell imaging of MDA-MB-231 cells stably overexpressing GFP-tagged vinculin (pLL GFP-moVincFL) to indicate focal adhesions and transiently transfected either with (a) control miR showing dynamic rearrangements of focal adhesions or (b) miR-200b (cells within circle overexpress miR-200b and have undergone an MET indicated by change in morphology and presence of cell-cell adhesion) showing decreased rate of change of focal adhesion rearrangements. Cells were imaged using an Olympus CV1000 spinning-disk confocal microscope at 37°C, 5% CO₂. Images were captured at 30 sec intervals for 2h and movies played at 14 frames/sec.