Figure EV1. Mammosphere formation and clonal capacities of CD44⁺ cells.
A. Representative images of spheres formed by MCF-7 cells under non-adherent (mammosphere) conditions from unsorted and CD44-sorted cell fractions (scale bars: 100 μm).
B. Representative images of spheres formed by MDA-MB-231 cells under non-adherent (mammosphere) conditions from unsorted and EpCAM-sorted cell fractions (scale bars: 100 μm).
C. Extreme limiting dilution assays (ELDAs). Graphs show the curve fit of the log fraction of non-responding cells (continuous line) and confidence intervals (dotted line) versus the number of cells tested. Left panel: MCF-7 cell line; CD44⁺ and CD44⁻ cells are depicted in black and red, respectively. Right panel: MDA-MB-231 cell line. EpCam⁺ and EpCam⁻ cells are depicted in black and red, respectively. The P-value for the difference in stem cell frequency between the two types of cells is shown using a Chi square test.
Figure EV2. Regulation of miR-10b control migration and CD44 expression.
A Wound-healing assays performed on MDA-MB-231 cells transiently transfected with anti-miR molecules. Images were obtained 36 h after wounding.
B Upper panel, left: MCF-7/CD44+ cells were transfected with anti-miR-10b or a scrambled control, and qRT-PCR was performed. Upper panel, right: An aliquot of these cells was subjected to colony-forming assays. The graph shows the mean ± SEM of the number of colonies formed in three independent experiments. Lower panel, left: Expression of miR-10b relative to RNU6B in MCF-7 cells stably transfected with a plasmid containing the miR-10b gene versus an empty vector. Results are the mean ± SEM values from three independent experiments. Lower panel, right: miR-10b-overexpressing MCF-7 cells analyzed by colony-forming assays. The mean ± SEM of the number of colonies in three independent experiments was plotted on each bar.
C Representative flow cytometry histograms for CD44 in single-cell suspensions of MCF-7 breast cancer cells. Wild-type (blue), stably transfected miR-Scr-OE (green) and stably transfected miR-10b-OE (red).
D Representative colonies of MCF-7-miR-10b-OE and control MCF-7-miR-Scr-OE seeded on soft agar.
E Representative ALDH+ flow cytometry analyses. SKBR3-control and SKBR3-miR-10b-OE mammospheres where disaggregated and ALDH enzymatic activity was measured using the ALDEFLUOR assay. The ALDH inhibitor DEAB was used as a control. Upper panels show a dot plot of SKBR3 and SKBR3-miR-10b-OE ALDH-positive cells (P3 population). Lower panels show a dot plot of these cells incubated with the ALDH inhibitor. The mean percentage of three independent experiments was 12.07% ± 0.78 in SKBR3 and 16.67% ± 0.98 in SKBR3-miR-10b-OE.
F Extreme limiting dilution assays (ELDAs). Graphs show the curve fit of the log fraction of non-responding cells (continuous line) and confidence intervals (dotted line) versus the number of cells tested. Left panel: MCF-7 cell line overexpressing miR-10b and control cells are depicted in black and red, respectively. Right panel: SKBR-3 cell line. Overexpressing and control cells are depicted in black and red, respectively. The P-value for the difference in stem cell frequency between the two types of cells is shown.
G Representative immunohistochemistry of E-cadherin expression in SKBR-3 cells (upper panel) and SKBR-3-miR-10b-OE expression cells (lower panel).
Figure EV3. In vivo morphology of tumors overexpressing miR-10b and miR-10b levels in breast cancer cells.

A, B Hematoxylin and eosin analysis of MCF-7-miR-10b-Scr (A) and MCF-7-miR-Scr-OE (B) tumor xenografts.

C Expression of miR-10b in four breast cancer cell lines by qRT-PCR. Bars represent mean ± SEM.
Figure EV4. PTEN and miR-10b expression correlates with worse prognosis in breast cancer patients.

A Kaplan–Meier relapse-free survival curves generated from Affymetrix expression data of an unselected compound cohort of 3,554 breast cancer patients, using the 222176_at probe set as a marker [31]. Patients with high expression are marked in red; patients with low expression are depicted in black. Log-rank $P = 0.00075$.

B Kaplan–Meier distant relapse-free survival curves generated from Affymetrix expression data of the Buffa et al. cohort (GSE22216) [30] using the SurvMicro tool [32]. High-risk patients are marked in red, low-risk patients are depicted in black. Log-rank $P = 0.000669$. The risk correlated with miR-10b expression, as shown in (C) ($P = 3.61^{-28}$).