Supplementary Figure 1: Pharmacological delivery of antagonir-10b inhibits metastasis.

(a) Schematic representation of the antagonir administration schedule for mice with mammary fat pad implantation of 4T1 cells.

(b) Relative miR-10b expression in primary breast tumors (left panel) and livers (right panel) of 4T1 tumor-bearing mice treated with PBS or antagonir-10b. The TaqMan qPCR assays of breast tumor samples and liver samples were performed separately, using a breast tumor sample and a liver sample as standards (both express detectable miR-10b), respectively. Data are presented as mean ± s.e.m. (n = 6 mice in each group; each data point represents the mean expression value of triplicates of the sample from one mouse).

(c) Immunoblotting of Hoxd10 in primary breast tumors of 4T1 tumor-bearing mice treated with PBS or antagonir-10b. SE: short exposure; LE: long exposure. Full-length blots and molecular weight markers are presented in Supplementary Figure 6.

(d) Primary tumor weight of 4T1 tumor-bearing mice treated with PBS or antagonir-10b, at 4 weeks after orthotopic implantation.

(e) Bright field imaging and H&E staining of the lungs from 4T1 tumor-bearing mice treated with PBS or antagonir-10b, at 4 weeks after orthotopic implantation. Arrows indicate lung metastases. Scale bars, 800 μm for bright field imaging; 200 μm for H&E staining.

(f,g) Number of visible lung metastases (f) and metastasis index (= metastasis number divided by primary tumor weight, g) in 4T1 tumor-bearing mice treated with PBS or antagonir-10b, at 4 weeks after orthotopic implantation. Data in d, f, and g are presented as mean ± s.e.m. (n = 10 mice in the PBS group and n = 8 in the antagonir-10b group).

(h) miR-10b expression levels (X-axis) in primary breast tumors positively correlate with lung metastasis numbers (Y-axis) in both PBS-treated and antagonir-10b-treated mice (n = 6 mice per group). The value of the X-axis represents the mean miR-10b expression value of triplicates of the sample from one mouse.
Supplementary Figure 2: Antagomir-10b treatment does not affect expression levels of miR-10a, miR-9 and miR-21.

Real-time RT-PCR of miR-10a (a), miR-9 (b) and miR-21 (c) in primary breast tumors of 4T1 tumor-bearing mice treated with PBS or antagomir-10b. Data are presented as mean ± s.e.m. (n = 6 mice in each group; each data point represents the mean expression value of triplicates of the sample from one mouse).
Supplementary Figure 3: miR-10b is highly expressed in metastatic tumors compared to normal tissues.
Relative miR-10b expression in primary breast tumors and paired livers of 4T1 tumor-bearing mice treated with PBS or antagonir-10b. The TaqMan qPCR assays of breast tumor samples and paired liver samples were performed together, using a breast tumor sample with detectable miR-10b as the common standard. Data are presented as mean ± s.e.m. Each bar represents triplicates of the sample from one mouse. The expression values are rescaled relative to the one with the highest miR-10b expression among all different tissue samples examined.
Supplementary Figure 4: Weight of vital organs following intravenous delivery of antagonir-10b in normal mice.

Weight of lung (a), heart (b), liver (c) and spleen (d) was measured in normal Balb/c mice after treatment with PBS or six doses of 50 mg/kg antagonir-10b or antagonir-10b_mm. Data are presented as mean ± s.e.m. (n = 5 mice in each group).
Supplementary Figure 5: Blood chemistry assessment following intravenous delivery of antagonim-10b in normal mice.
Levels of albumin (a), ALT (b), AST (c), cholesterol (d), BUN (e), and total bilirubin (f) were measured in normal Balb/c mice after treatment with PBS or six doses of 50 mg/kg antagonim-10b or antagonim-10b_mm. Data are presented as mean ± s.e.m. (n = 5 mice in each group). Normal ranges are from http://www.ahc.umn.edu/rar/refvalues.html.
Supplementary Figure 6: Full-length gel blots for cropped images.
Supplementary Figures 5a-c are full-length blots for Fig. 1b, Supplementary Fig. 1c, and Fig. 2b, respectively. In each case, the blot was cut into two halves after transfer: the top half was incubated with the anti-Hsp90 antibody (as a loading control), and the bottom half was incubated with the anti-Hoxd10 antibody. The anti-Hoxd10 antibody was validated using a human HOXD10 overexpression construct as described previously.¹²