Supplementary methods:

Reagents with catalogue numbers and manufacturer information, cell culture conditions:
MCF-10A and MDA-MB-231 cells were maintained in DMEM/F12 (with 5% FCS, 10 
µg/ml Insulin, 20 ng/ml EGF, 0.5 µg/ml hydrocortisone) and MEM with 10% FCS,
respectively. Cells were transfected with C5A cDNA using the Lipofectamine 2000 
reagent and selected using Zeocin to generate stable cell lines (Invitrogen, Carlsbad, CA,
USA). Retroviral vector containing validated shRNA for ANTXR1 (Cat # FI326646) as 
well as the control shRNA vector with non-effective 29-mer scrambled sequence were 
purchased from Origene Technologies, Inc. (Rockville, MD, USA). Lentiviral vectors 
containing three independent siRNAs (piLenti-siRNA-GFP) were purchased from 
Applied Biological Materials, Inc. (Cat #i001078). Lentivirus infected cells were selected 
using puromycin. siRNAs for transient expression were purchased from Santa Cruz 
Biotechnology (SC-44144, Santa Cruz, CA, USA).

Primary human breast epithelial cell isolation
Following surgical resection, breast tissue was collected in DMEM/F12, minced with 
scalpels and transferred into 100 ml of media containing 5% FCS, 5 g/ml insulin, 0.5 
g/ml hydrocortisone, 300 U/ml collagenase and 100 U/ml hyaluronidase. After six 
hours incubation at 37 C, cells were resuspended in the same media, washed, and 
cultured overnight for flow cytometry sorting in majority of experiments.

Flow cytometry analyses sorting
MCF-10A Cells were incubated with FITC-conjugated CD44 (Cat. # 555487) and PE-conjugated CD24 antibody (Cat # 555428) (BD Biosciences, San Jose, CA, USA). Primary cells were incubated with FITC conjugated CD49f (Cat # 555735), APC conjugated EpCAM (Cat # 130-091-254, Miltenyi Biotech GmbH), and PE conjugated lineage markers CD31 (Cat # 130-092-653, Miltenyi), CD45 (130-080-201, Miltenyi), and CD140b antibodies (Cat # 558233, BD Biosciences).

Primer sequences used for qRT-PCR are listed below.

**ANTXR1:**
F 5’-TGCAACACAGAAATGCTCTGCTGCCTG-3’  
R 5’-TTTATCCCTGGGTGATGAAGCCCA-3’

**AXIN2:**
F 5’-ACAACAGCATTGTCTCACAAGCAGC-3’  
R 5’-GCGCCTGGTCAACATGATGGAAT-3’

**BMP4:**
F 5’-TTCCGGACTACATGCAGATCTCTTT-3’  
R 5’-ATGTTCTTCTGGAATGCCTCCT-3’

**β-ACTIN:**
F 5’-AATGGRGGCCGAGGACTTTTGATTG-3’  
R 5’-AGGATGGCAAGGGACTTCTCTGTAA-3’

**HSPA1A:**
F 5’-TGCTGGACAAGTGCTCAAGAGG-3’  
R 5’-TCTCGTGGCTGGACGCCAACC-3’

**Keratin 14:**
F 5’-GTGGGTGGAATGTCATT-3’  
R 5’-CATCCTTGATGGAATGCCT-3’

**LRP6:**
F 5’-CCGAGTCAGACCTGGAAATAC-3’  
R 5’-CTCCAAACTGATCCCATCTAATC-3’

**MSX2:**
F 5’-CGGTCAAAGTGGAAATTC-3’  
R 5’-GAGGAGCTGGGTAGTGTA-3’

*Antibodies and other reagents*
Antibodies against p-GSK3β (Cat # 9331), LRP6 (Cat #3395), pβ-Catenin (Cat # 610154) (Cell Signaling Technologies, Danvers, MA, USA), β-actin (Cat # A1978, Sigma, St. Louis, MO, USA), Cyclin D1 (Cat # Sc-718), cMYC (Cat # Sc-764), ZEB1 (Cat # Sc-25388) (Santa Cruz Biotechnologies, Santa Cruz, CA, USA), ANTXR1 (Cat # ab21270, Abcam, Cambridge, MA, USA or Cat # 6677, Abnova, Walnut, CA, USA), β-catenin (Cat # 610154, BD Biosciences), Survivin (Cat # AF886, R & D systems, Minneapolis, MN, USA) were used for western blot analyses as per instructions from manufacturers. After indicated treatments, cells were washed in PBS and lysed in RIPA buffer with protease/phosphatase inhibitors. Thirty micrograms of protein was separated by SDS-PAGE and then transferred to PVDF membranes. The membranes were probed with the indicated antibodies and developed with Piece Western Blotting Substrate (Thermo Scientific, Rockford, IL, USA). PA antigen (Cat # 171A), DKK (Cat # pro-673-a) and Wnt3a (Cat # 1324-WIN) were purchased from List Biological Laboratories (Campbell, CA, USA), Prospec-Tany Technologies (East Brunswick, NJ, USA), and R&D Systems, respectively.

**Legend for supplementary information:**

Supplementary Figure 1: ANTXR1 DNA copy number and expression in subtypes of breast cancer. A) ANTXR1 copy number units in TCGA dataset. Oncomine database was used for this analysis. Medullary breast carcinomas have higher copy numbers. B) ANTXR1 expression in normal, ERα-negative, and ERα-positive breast cancer in the TCGA dataset. C) ANTXR1 expression in normal, ERBB2-negative and ERBB2-positive
breast cancer. D) ANTXR1 expression in normal, triple negative and rest of the breast cancers.

Supplementary Figures 2: Ingenuity pathway analysis of genes differentially expressed in control and ANTXR1 shRNA expressing TMD-231 cells. Genes labeled in red are expressed at higher levels, whereas genes labeled in green are expressed at lower levels in ANTXR1 shRNA cells.

Supplementary Figure 3: Prognostic value of ANTXR1 and HSPA1A combination in breast cancer. Kaplan-Meier survival curves demonstrate elevated expression of these two genes correlating with unfavorable recurrence-free, distant metastasis-free, and overall survival in all patients (A) and in patients who did not receive systemic therapy (B).

Supplementary Figure 4: The role of ANTXR1 in mammosphere formation. A) Mammosphere formation by a single cell. TMD-231 cells with control or ANTXR1 shRNA were sorted into 96-well plate, single cell per well and mammosphere formation was analyzed after 10 days. B) Secondary mammosphere formation by control and ANTXR1 shRNA expressing cells. 5000 cells from primary mammospheres were replated. C) Tertiary mammosphere formation. 5000 cells from secondary mammospheres were replated. After 10 days, mammospheres were filtered and stained using Wright-Giemsa. D) ANTXR1-positive cells from TMD-231 cells were sorted (top).
and subjected to mammospheres. Individual mammospheres (bottom left) and total mammospheres (bottom right) are shown.

Supplementary Figure 5: Characteristics of control and ANTXR1 shRNA expressing primary tumors. A) H&E staining of tumors (two each) derived from control shRNA (top) and ANTXR1 shRNA (bottom) infected TMD-231 cells. B) Proliferation rate of control shRNA and ANTXR1 shRNA infected cells in vitro. BrDU-incorporation ELISA was used to measure proliferation rate.

Supplementary Figure 6: Tumor growth rate and metastasis of TMD-231 with vector control or ANTXR1 shRNA from an independent experiment. A) Tumor growth rate. Note that cells used in this experiment were cultured for a longer time than cells used in experiments described in Figure 7. B) Lung metastasis index of animals implanted with indicated cells.

Supplementary Table 1: Genes differentially expressed in control shRNA and ANTXR1 shRNA expressing TMD-231 cells. Genes with positive fold-change values are overexpressed in ANTXR1 shRNA cells, whereas genes with negative fold-change values are overexpressed in control cells.