Supplementary Table and Figure Legends

Supplementary Table 1. Synergistic/Antagonistic growth inhibition by **A)** KX-01 + PAX **B)** KX-01 + DOX, demonstrated by calculation of the combinational index (CI) in ER/PR/HER2-negative breast cancer cells (48 h). Synergism was calculated using the Calcusyn software that calculates the CI to confirm synergy between two compounds for growth inhibition. CI < 1 indicates synergism; CI = 1 indicates additivity; CI > 1 denotes antagonism.

Supplementary Figure 1. Comparison of apoptosis in ER/PR/HER2- negative MDA-MB-468 breast cancer cells following treatment with KX-01 (**A)** and clinical Src kinase inhibitor dasatinib **(B)**. MDA-MB-468 cells (5x 10^3 cells) were incubated with vehicle, KX-01, dasatinib, at concentrations (10 nmol/L to 250 nmol/L) for 24 h. Apoptosis was measured by detection of nucleosomes in cytoplasmic fractions of MDA-MB-468 cells using a cell death detection ELISA kit (Roche, Indianapolis, IN). The data are presented as the mean ± SD.*, P < 0.05, compared with vehicle incubated samples.

Supplementary Figure 2. Tumor xenograft studies in NUDE mice. **A)** NUDE mice bearing MDA-MB-157 xenografts (~100 mm^3) were separated into three treatment groups randomly with 5 mice/group (two tumors/mouse) for a total of *n* = 10 tumors/group. The first group (vehicle) received ultra pure distilled water for each treatment application. The second and third groups were treated with two different doses of KX-01 (1 and 5 mg/kg) by oral gavage twice daily for 28 days. Tumors were measured twice a week by caliper and tumor volumes were calculated as described in the 'Materials and Methods'. Data is represented as mean tumor volume in mm^3 ± SD. *, p < 0.05, significantly different from vehicle control (VC). **B)** For combination treatment, mice in fourth group were treated with doxorubicin (DOX) alone (3

mg/kg) once a week (day 1, 8, 15, 22) by i.p. injection as indicated by the arrows. The fifth group was treated with KX-01 + DOX. **C)** NUDE mice bearing MDA-MB-231 xenografts (~80-100 mm^3) received ultra pure distilled water for each treatment administration, the second group was treated with KX-01 alone (5 mg/kg) by oral gavage twice daily [same used in figure 3], third group was treated with doxorubicin (DOX) alone (3 mg/kg) once a week (day 1, 8, 15, 22, 29, 36) by i.p. injection as indicated by the arrows and fourth group was treated with KX-01 + DOX. Tumors were measured twice a week by caliper and tumor volumes were calculated as described in the 'Materials and Methods'. Data are presented as mean tumor volume in mm^3 ± SD. *, $P < 0.05$ compared to either drug alone.

Supplementary Figure 3. Effect of KX-01 and paclitaxel on mice body weight and tumor histology (Hematoxylin and Eosin staining). **A)** Mice bearing MDA-MB-231 tumor xenografts were treated with Vehicle (ultra pure distilled water), KX-01 alone (5 mg/kg) by oral gavage twice daily, paclitaxel (PAX) (3 mg/kg) once a week (day 1, 8, 15, 22, 29, 36) by intraperitoneal (i.p.) injection, and KX-01 + PAX with above mentioned doses were weighed every ten days and the weight of mice in grams were graphed. **B)** Tumors obtained from above mentioned treatment groups were fixed, paraffin embedded and sectioned. The slides were then stained for H&E. Bright field microscopic images were taken and representative images are represented in the panel (original magnification, x 100). Arrows indicate acelluar, necrotic areas in the tumor sections from the combination treatment groups. **C)** Mice bearing established tumor were treated with vehicle (ultra pure distilled water), KX-01 (15 mg/kg) by oral gavage once daily, paclitaxel (PAX, 20 mg/kg) once a week by i.p. injection (days 1, 8, 15, 22), and KX-01 + PAX combination were weighed once week and graphed. **D)** Tumors obtained from above mentioned treatment group (Suppl. 3C) were fixed, paraffin embedded and sectioned. The slides were

stained for H&E. Bright field microscopic images were taken and representative photomicrographs are presented in the panel (original magnification, x 100). Arrows indicate acelluar, necrotic areas in the tumor sections from the combination treatment group.

Supplementary Figure 4. KX-01 suppressed microtubule formation and inhibited migration and invasion in MDA-MB-231 cells *in vitro*. MDA-MB-231 cells were plated in 8 well chamber slides with DMEM medium supplemented with 10% FBS. Cells were incubated with 200 nmol/L KX-01 for 24 hours. Cells were fixed in 3.7% paraformaldehyde in PBS, and then permeabilized in 0.3% Triton X-100 in PBS. After 1% BSA block, cells were immunostained for 2 hours at room temperature with alpha-tubulin antibody conjugated with Alexa fluor 488 (Invitrogen #32-2588) and nuclei were stained with DAPI. Fluorescence image was captured with a confocal microscope (BD Pathway 855) at 60x objective lens using Atto Vision software. **B)** MDA-MB-231 cells were seeded on the upper surface of the transwell insert coated with Matrigel. Chemoattractant media (DMEM containing 10% FBS) was added to lower chamber of the transwell plate. Cells were incubated with vehicle (VC) or KX-01 (10, 25, 50 nmol/L) for 24 h and the number of cells invaded was quantified and normalized to cell number. Representative images from invasion assay were shown. **C)** MDA-MB-231 cell migration was measured using an *in vitro* wound healing/scratch assay. Monolayer cell cultures of MDA-MB-231 cells were gently scratched with a pipette tip to produce a wound. Photographs of the cultures are taken immediately after the scratch at 0 h and after 24 h of exposure to vehicle or KX-01 (5, 10, 25 nmol/L). The extent of wound closure was determined after 24 h as described in the Materials and Methods. Representative images from migration assay were shown.

Supplementary Figure 5. A) Standard curves (SC) for number of human cells per mouse organ were created by addition of human MCF-7 breast cancer cells to mouse whole lung, bone marrow (from one femur), or mouse whole liver. Using real-time RT-PCR to measure the human chromosome -17, standard curves (SC) for number of human cells within a mouse organ were generated by serial additions of human MCF-7 cells (10^6, 10^5, 10^4, 10^3, 10^2, and 0 cells [shown in log scale in the graphs 6, 5, 4, 3, 2] to mouse whole lung, bone marrow (from one femur), and whole liver just prior to homogenization of the tissues and isolation of DNA. Mouse organs were derived from untreated female NUDE mice at the same age as mice from the treatment groups. Real-time PCR for human chromosome -17 was performed as described in the Materials and Methods. **B)** Quantification of micrometastasis of MDA-MB-231 tumors in mouse lung, bone marrow and liver. After necropsy, lung, bone marrow (from femur) and liver were collected from treatment groups described in Supplementary figure 2C [vehicle control (VC), KX-01, DOX, KX-01+DOX (n=5 mice/group)] and genomic DNA isolated. Micrometastases originating from human primary MDA-MB-231 xenografts were detected by quantitative real-time RT-PCR for an α-satellite DNA sequence of the centromere region of human chromosome-17. Test samples (the number of human cells in each mouse tissue sample) were interpolated from a standard curve as described in Materials and Methods. Graphs represent the number of human cells in intact mouse lung , bone marrow from one femur and intact mouse liver . *, $P<0.05$, significantly different compared to VC.