**Supplementary Figure Legends**

**Supplementary Figure 1. Tissue Distribution of HS131 and HS152 in 4T1 Tumor-bearing Mice.**

**A) Tissue distribution of HS131 and control HS152 in 4T1-tumor bearing mice 24h after intravenous injection.** 4T1 cells were subcutaneously implanted to the flank of BALB/c mice. When tumor size reached about 10 mm in diameter, HS131 or HS152 (10 nmol in 20µL) was intravenously injected via tail vein (3 mice for each Hsp90 probe). Twenty four hours later, mice were euthanized and tissues/organs were harvested. Mice without injection of Hsp90 compounds were used as negative controls. Eyes, brain, heart, lung, liver, spleen, kidney, gut, urinary bladder, skin, and 4T1 tumor were collected from mice. nIR signal was detected ex vivo by IVIS machine (640 nm, Cy5.5, 1.0 sec exposure).

**B) Average florescence intensity of organs harvested from mice 24 h after HS131 or HS152 injection.** Average of fluorescence intensity for each organ (3 mice) was calculated for each Hsp90 probe. Error Bar: Standard deviation.

**Supplementary Figure 2. nIR signal in 4T1 or MDA-MB-231 tumor-bearing mice administered with HS131, HS198 or HS152.**

4T1 cells (A) or MDA-MB-231 cells (B) were implanted subcutaneously to the flank of female BALB/c mice or SCID-beige mice, respectively. When the tumor sizes reached around 10 mm in diameter, mice received administration of HS131, HS198 or HS152 (10 nmol/mouse) via tail vein. nIR signals from these mice were analyzed over time (immediate, 3h, 6h, 12h, and 24h after compound injection) with LI-COR Pearl Imager using 700 nm channel. Three mice were analyzed for each Hsp90 probe. In the right panels, temporal dynamics of nIR signal of tumor area for each Hsp90 probe is shown.

**Supplementary Figure 3. nIR Imaging of Intraductally and subcutaneously Implanted 4T1 cells.**

**A) Imaging of intraductally and subcutaneously implanted 4T1 tumor cells using HS131**. Unlabeled 4T1 cells (100 M cells/ml, 10 µl) were intraductally injected to the mammary duct of left 2nd mammary glands (yellow arrows) in BALB/c mice. Right mammary glands were injected with saline only (10 µl) without 4T1 cells (negative control). On day 1, HS131 or HS152 (25 nmol/20 µl DMSO) were injected via tail vein, and 6 hours later, nIR images were taken by IVIS machine (ex 640 nm, em Cy5.5 filter, 0.2 sec exp). The same number of unlabeled 4T1 cells (100 M cells/ml, 10 µl) were subcutaneously injected to the flank (yellow arrows) of BALB/c mice. On day 1, nIR images were taken by IVIS machine after HS131 or HS152 injection to mice. Five mice were intraductally implanted with 4T1 cells, among them 2 were given intravenous injection of HS131, another 2 mice with HS152, and one mouse without compound injection.

**B) nIR signal analysis of excised 4T1 cell-implanted mammary glands, control mammary glands and subcutaneous cell aggregates.** On day 1, when the imaging of mice was completed after the administration of HS131 or HS152, mice were euthanized. Bilateral 2nd mammary glands as well as tumor cell aggregates formed at the sites of subcutaneous injection were excised and nIR signals were analyzed ex vivo.

**C) In vivo nIR signal analysis of intraductally and subcutaneously implanted 4T1 tumor cells.** nIR signal levels in 4T1 cells implanted mammary glands (left side, yellow arrow) and control mammary glands (right side) were analyzed in vivo by IVIS imager and shown in the left panel. nIR signal levels of the subcutaneous 4T1 cells aggregates were analyzed and shown in right panel.

**D) Ex vivo nIR signal analysis of intraductally and subcutaneously implanted 4T1 tumor cells.** As shown in Figure 4B, 4T1 cell implanted mammary glands (left side, cyan arrow) and control mammary glands (right side) as well as tumor cell aggregates at the sites of subcutaneous injections were excised from mice, and nIR signal levels were analyzed ex vivo by IVIS imager.

**Supplementary Figure 4. Cell Surface Expression of Hsp90 by Breast Cancer Cell Lines.**

Breast cancer cell lines were stained with PE-conjugated anti-Hsp90 mAb, or PE-conjugated control IgG for 30 min at 4C. After wash with PBS, cells were acquired by LSRII flow cytometry machine. Open histogram: anti-Hsp90, filled histogram: IgG control. Median fluorescence intensity for Hsp90 staining is shown in each histogram.