Supplemental 3

Supplemental Figures legends:

Supplemental Figures 1. Increased dimerization of HER2/HER3 in SKBR3/100-8 cells. The protein was extracted from SKBR3 and SKBR3/100-8 cells. A total 250μg protein lysis was immunoprecipitated with HER2 antibody and followed by Western blot analysis with the indicated antibodies.

Supplemental Figures 2. Pathway signaling differentially expressed in SKBR3/100-8 compared to SKBR3 revealed by IPA. The gene profile from microarray analysis was analyzed by Ingenuity IPA Software. The bars indicated the differentially regulated breast cancer related pathways between SKBR3/100-8 and SKBR3 cells revealed by IPA analysis. The red color indicated percentage of upregulated genes and green indicated percentage of downregulated genes. The numbers of top of each bar indicated the total genes related to the indicated pathway analyzed by IPA.

Supplemental Figures 3. Confirmation of Wnt pathway gene expression by RT-Q-PCR. A, Relative expression of the indicated Wnt ligands and FZDs in SKBR3 (open bar) and SKBR3/100-8 (dark bar) was determined by RT-Q-PCR. Each bar indicates mean±SD from three determinations. *$p<0.05$ compared to SKBR3. B, Relative expression of Wnt3 and Wnt6 in BT474 (open bar) and BT474/100-2 (dark bar) was determined by RT-Q-PCR. Each bar indicates mean±SD from three determinations. *$p<0.05$ compared to BT474.

Supplemental Figures 4. Confirmation of Wnt/β-catenin signaling pathway genes and protein expression. A, Relative expression of the indicated genes in SKBR3 (open bar) and SKBR3/100-8 (dark bar) was determined by RT-Q-PCR. Each bar indicates mean±SD from three

determinations. *p<0.05 compared to SKBR3. B, Protein levels of β-catenin, MMP7 and VEGF in SKBR3 and SKBR3/100-8 were determined by Western blot analysis and β-actin was used as loading control.

Supplemental Figures 5. E-cadherin and β-catenin expression in SKBR3 and SKBR3/100-8 cells. A, E-cadherin expressions in SKBR3 (a) and SKBR3/100-8 (b) were determined by immunocytochemistry analysis as described in supplemental methods. The arrows indicate positive membrane staining of E-cadherin. B, The N-cadherin expressions were determined by IF with anti-N-cadherin and followed by FITC conjugated second antibody (green). The nuclei were labeled by DAPI (blue) and merged with N-cadherin-FITC labeling demonstrated in a (SKBR3) and b (SKBR3/100-8). The arrows indicate positive staining of N-cadherin. C, The localizations of β-catenin in SKBR3 (a) and SKBR3/100-8 (c) were determined by Immunofluorescence analysis (IF) with anti-β-catenin and followed by FITC conjugated second anti-body (green). The nuclei were labeled by DAPI (blue). The white arrows indicate cytoplasmic and membrane expression of β-catenin and red arrows indicate nuclear expression of β-catenin. The merged β-catenin-FITC labeling and DAPI staining in SKBR3 and SKBR3/100-8 were showed in b and d respectively.

Supplemental Figures 6. siRNA knocked down EGFR in SKBR3/100-8 cells. SKBR3/100-8 cells were treated with either siRNA EGFR or negative sequence for 72 hrs and RT-Q-PCR was performed. The bars indicate relative expression of EGFR and Wnt3 in the cells treated with siRNA-EGFR (dark bar) and negative sequence (Mock) (open bar). Each bar indicates mean±SD from three determinations. *p<0.05 compared Mock cells.

Supplemental Figures 7. siRNA knocked down HER3 sensitizes SKBR3/100-8 cell response to trastuzumab-induced cell growth inhibition. The cells were treated with either siRNA HER3 or negative sequence (Mock) for 48 hrs. A, the cell invasiveness was determined by Boyden Chamber Invasion assay as described in Method. The invasive cells (mean+SD from 5 different areas) were counted. *$p<0.05$ compared to untreated cells. B, The cells were treated with or without transtuzumab (10μg/ml) for 3 days after treated with siRNA-HER3 for 48hrs and MTT assay was performed. The bars indicate percentage of cells growth (mean±SD) from 6 determinations. *$p<0.05$ compared to control and Mock cells.