Figure S1

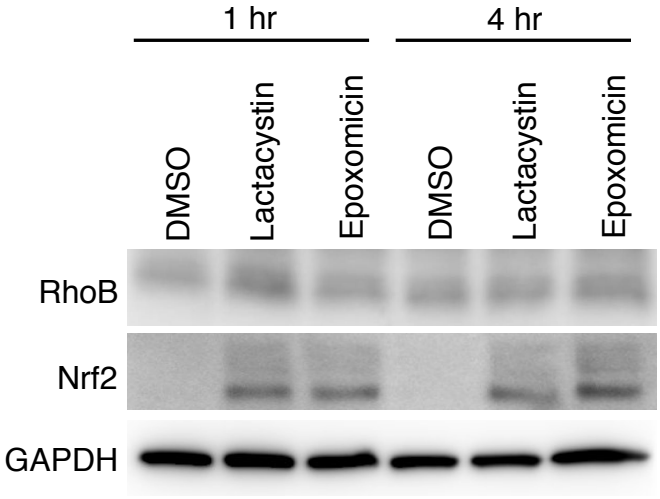

Fig. S1. Effects of other proteasome inhibitors on protein expression of RhoB. Western blots of cell lysates of SKBR-3 cells treated with 10 μM lactacystin or 10 μM epoxomicin for indicated time. Nrf2 was a positive control to examine the effect of proteasome inhibition.

Figure S2

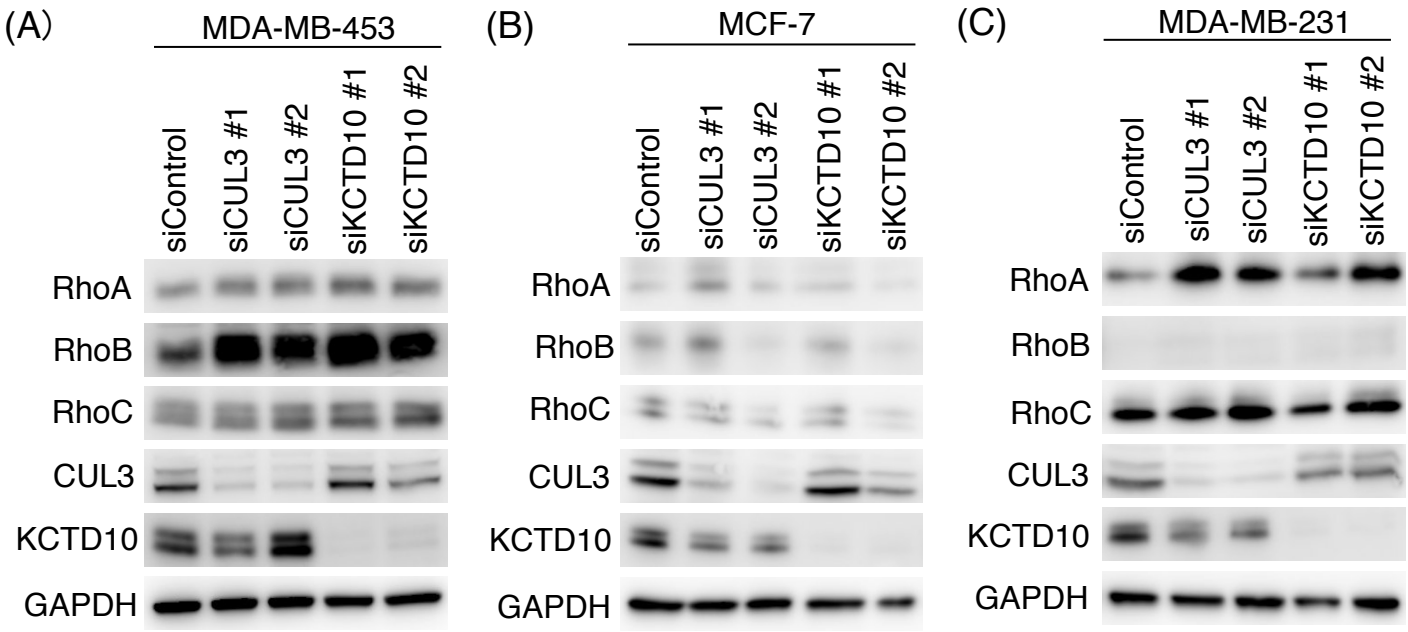

Fig. S2. Effects of CUL3 or KCTD10 knockdown on expression of Rho proteins in other BC cell lines. Western blots of MDA-MB-453 (HER2-positive type; A), MCF-7 (luminal type; B) and MDA-MB-231 (basal type; C) cell lysates 72 h post-transfection with siRNAs.

Figure S3

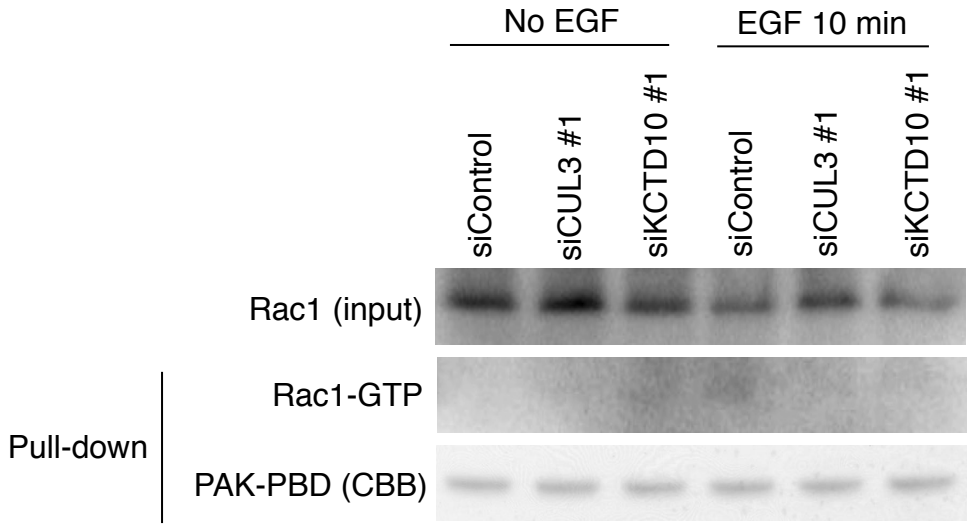

Fig. S3. Rac1-GTP pull-down assay. SKBR-3 cells were incubated with or without EGF for 10 min. Cell lysates were incubated with PAK-PBD affinity beads. Proteins bound to PAK-PBD were pulled down and detected by western blotting for Rac1. CBB staining was used to visualize PAK-PBD.

Figure S4

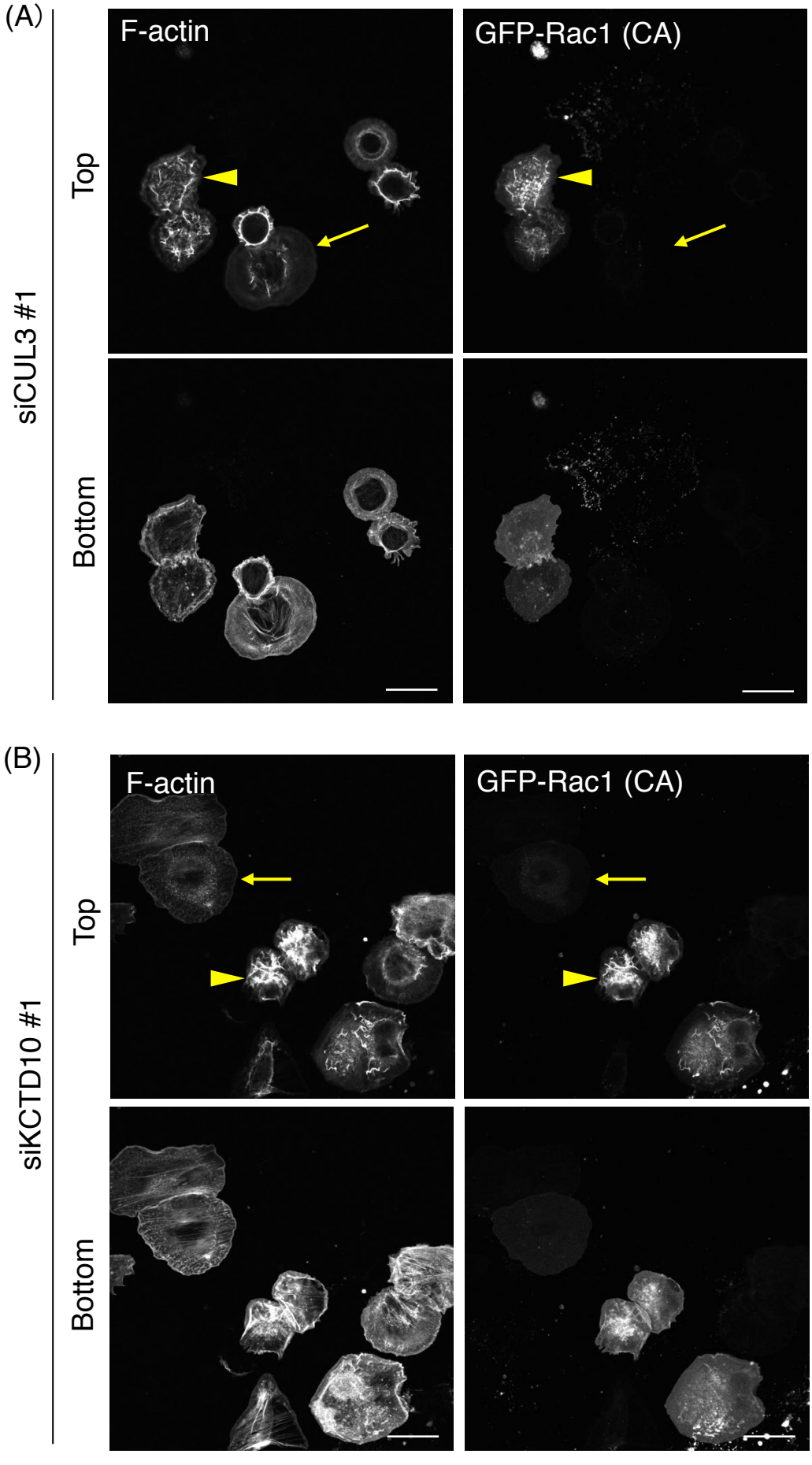

Fig. S4. Enforced expression of constitutive-active Rac1 (Rac1-CA) restored formation of dorsal membrane ruffles in CUL3- or KCTD10-depleted SKBR-3 cells. (A, B) F-actin staining by phalloidin in GFP-Rac1 (CA)-expressing in CUL3- (A) or KCTD10 (B)-knockdown SKBR-3 cells 15 min after EGF stimulation. Confocal images at the top and bottom sections of cells are shown. Representative cells expressing GFP-Rac1 (CA) exogenously were indicated by yellow arrowheads, and non-transfected cells were indicated by yellow arrows. Bars; 20 μm.

Figure S5

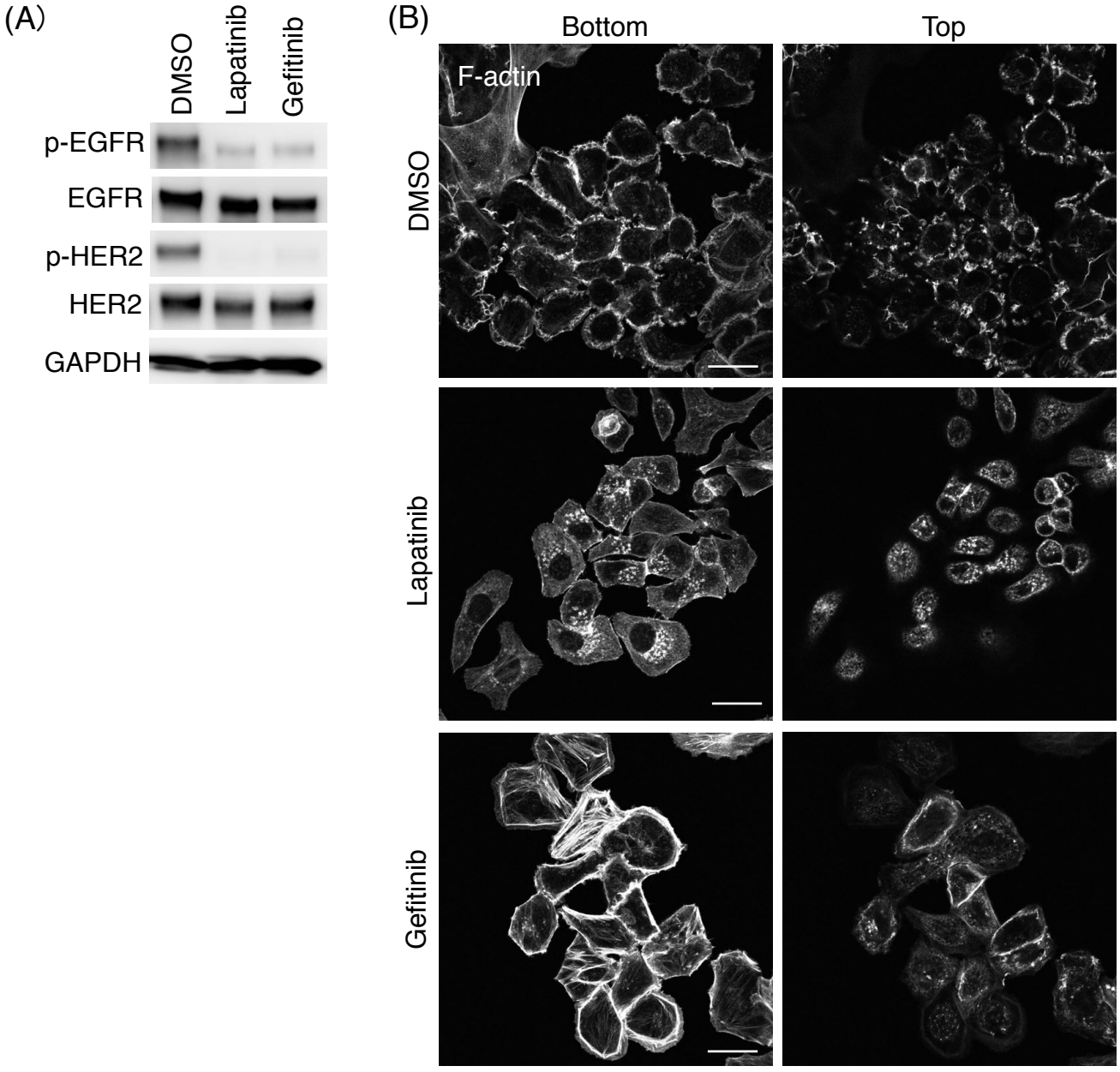

Fig. S5. HER2 signaling is essential for the dorsal membrane ruffle formation. (A) Western blots of cell lysates of SKBR-3 cells stimulated by EGF for 15 min. Cells were pre-treated with 10 μM lapatinib for 60 min or 20 μM gefitinib for 60 min before EGF stimulation. (B) F-actin staining by phalloidin in SKBR-3 cells treated with 10 μM lapatinib for 60 min or 20 μM gefitinib for 60 min. Confocal images 15 min after EGF stimulation at the top and bottom sections of cells are shown. Bars; 20 μm.

Figure S6

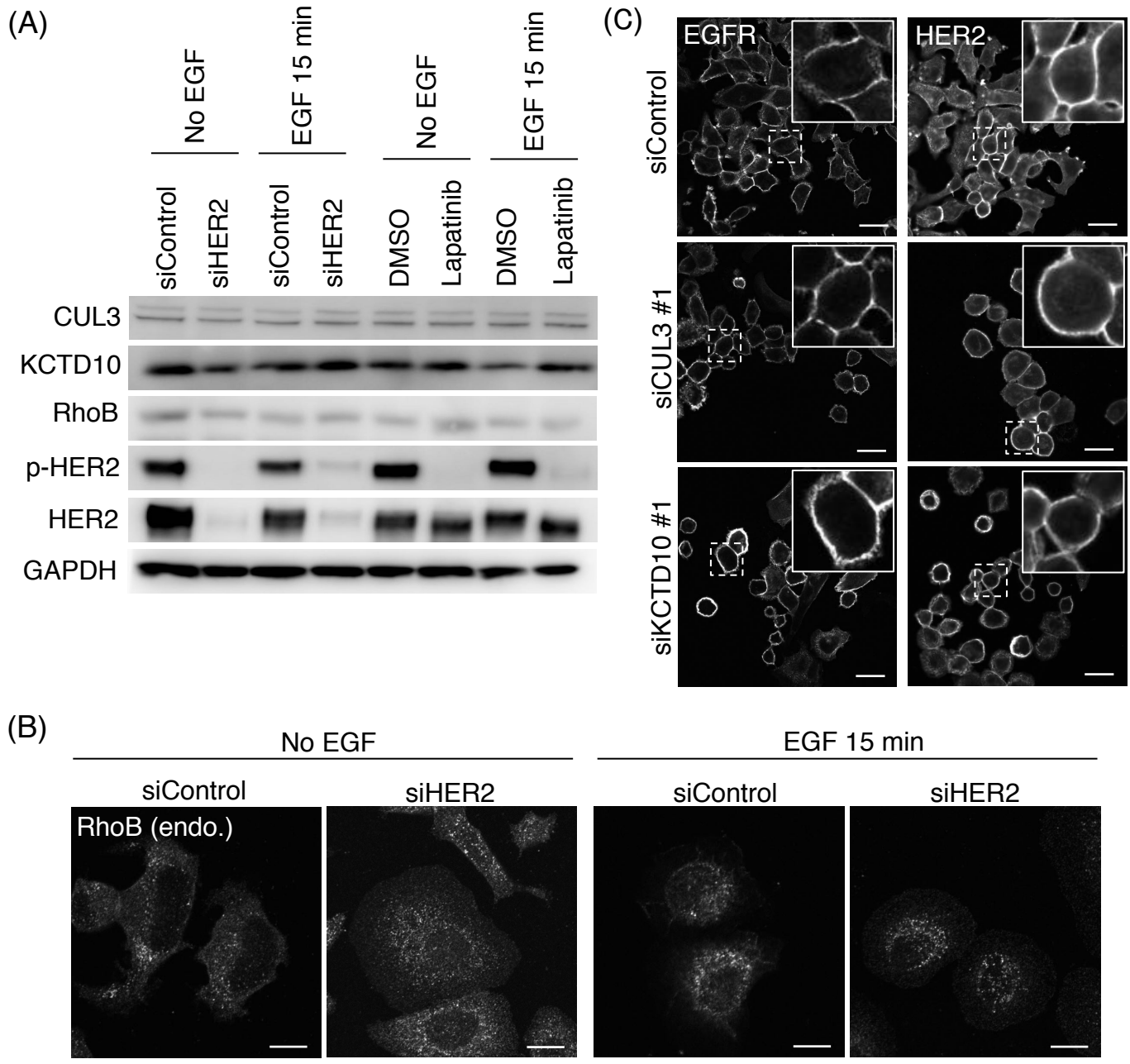

Fig. S6. Relationship between HER2 signaling and CUL3/KCTD10-mediated degradation pathway. (A) Western blots of cell lysates of HER2 knockdown SKBR-3 cells or cell lysates of SKBR-3 cells treated with 10 μM lapatinib for 60 min. (B) Confocal images of SKBR-3 cells treated with control siRNA or HER2 siRNA for 72 h. Cells were incubated with or without EGF, fixed, permeabilized, and stained for RhoB. Bars; 10 μm. (C) Confocal images of EGFR and HER2. SKBR-3 cells were fixed after 72 h transfection with siRNAs. Cells were serum-starved for 24 h before fixation. Inset; magnifications of the area indicated by a dashed square. Bars; 20 μm.

Figure S7

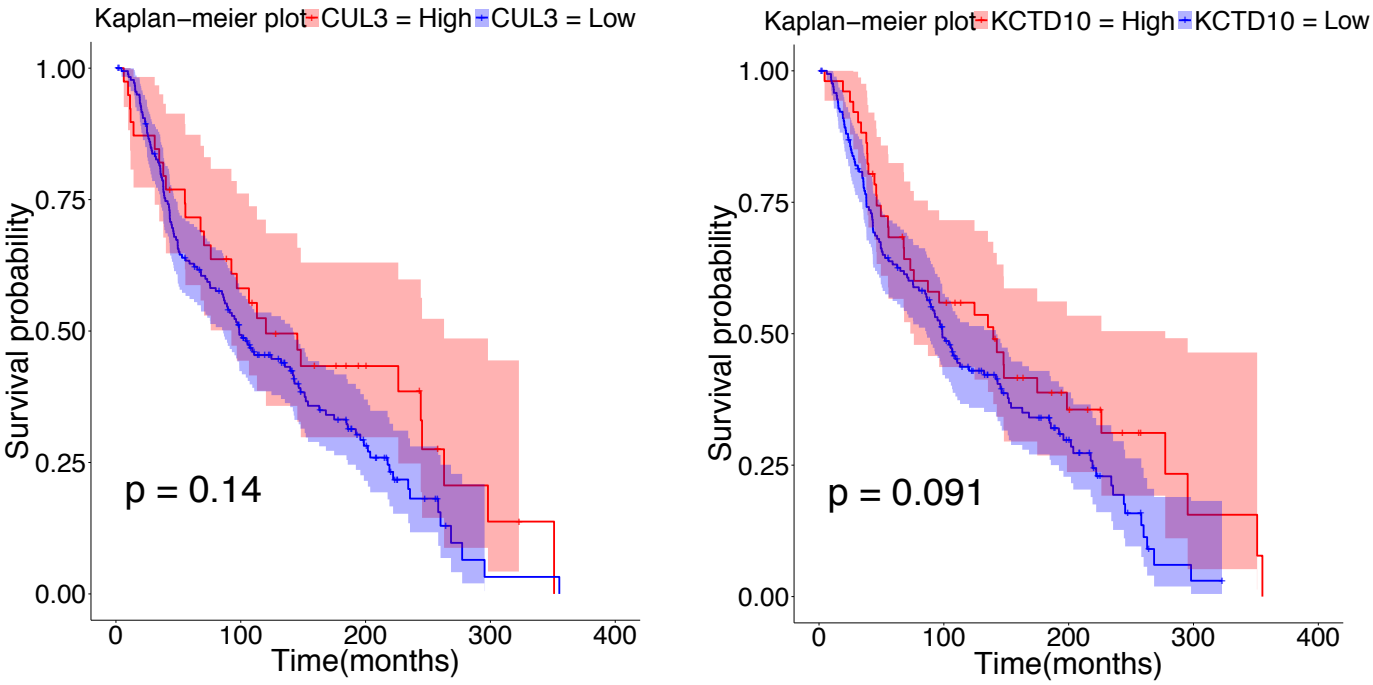

Fig. S7. Survival analysis with CUL3 or KCTD10 expression profiles in human HER2-positive BC. Kaplan-Meier plot with CUL3 or KCTD10 expression profiles in METABRIC cohort using overall survival status were shown. Shading along the curve showed 95 % confidential interval. In HER2-positive subtype, neither CUL3 (High: n=39, Low: n=181) nor KCTD10 (High: n=51, Low: n=169) expression was correlated with poor prognosis.