Supplementary Figure legends

Supplementary Figure 1. Correlation of HER-2/neu with pCREB expression.

(A) shHER-2 transfection strongly down-regulates HER-2/neu expression in MCF-7 cells.

(B) shHER-2/neu-mediated down-regulation of HER-2/neu in HER-2/neu$^+$ MCF-7 cells results in a reduced in CREB phophorylation.

(C) Representative immunohistochemical staining and grading from breast cancer tumor lesions are shown.

Supplementary Figure 2. Effects of shCREB constructs on CREB protein expression.

(A) Efficacy of shCREB knock-down (shCREB-HER-2/neu$^+$ cells) was analyzed using an NC control plasmid and four different shRNA plasmid contructs targeting murine CREB1. The following shRNA sequences were amplified and cloned into a pClip plasmid construct containing a puromycin resistance gene: plasmid #1: TCAGCCGGGTACTACCATTCT; plasmid #2: ACAGGGAGGCAGCAAGAGAAT, plasmid #3: AAGTCCAAACAGTTCAGATTT and plasmid #4: TGCTCCCACTGTAACCTTAGT. Plasmid NC (GGAATCTCATTCGATGCATAC) served as a nonsense control. These plasmids were transfected into HER-2/neu$^+$ cells and CREB protein expression was determined by Western blotting as described in Materials and Methods using an anti-CREB-specific antibody. Arrows point to plasmid constructs that were further used in this study.

(B) mRNA expression of ATF-1 and CREM was analyzed in HER-2/neu$^+$ cells and shCREB-HER-2/neu$^+$ cells using q-PCR. Data are means ±SD (n = 3).

Supplementary Figure 3. Time- and dose-dependent effects of KG-501 on the CREB expression and activity of HER-2/neu$^+$ cells.

(A) mRNA expression of CREB, CBP, bcl2 and bcl-xL was determined over time upon treatment with 10 μM KG-501 using RT-PCR followed by agarose electrophoresis as described in Materials and Methods. Expression of β-actin served as control.

(B) HER-2/neu$^+$ cells were incubated with KG-501 for 48 h at 37°C and analysed as described in Materials and Methods. Western blotting using a phospho(Ser-133) mAb was performed and β-actin mAb served as the control. The right panel demonstrate the quantified densitometry data (right) are means ±SD (n = 3).

(C) CFSE-labeled HER-2/neu$^+$ cells were incubated with KG-501 and fluorescence was determined 48 h later on a FACsCalibur. Red line, untreated cells.

Supplementary Figure 4. Immunohistochemical analysis of HER-2/neu$^+$ cells and shCREB-HER-2/neu$^+$ cells following *in-vivo* tumorigenicity.

(A) Representative images from organs of tumor-bearing mice injected with HER-2/neu$^+$ cells and shCREB-HER-2/neu$^+$ cells are shown. The tumors were fixed, embedded in paraffin, and 5 μm slices were stained as indicated. Magnification, 20×.

(B) Tumor slices were stained with hematoxylin-eosin and analyzed for apoptosis (TUNEL), proliferation (Ki-67) and CREB expression. Following incubation with a horse-radish-peroxidase-linked antibody, the slices were stained with diaminobenzidine and counterstained with methylene blue. Arrows (apoptosis staining), blood vessels; arrowheads, apoptotic cells. Magnification, 20×.

(C) mRNA isolated from tumors injected with HER-2/neu^{+} cells and shCREB-HER-2/neu^{+} cells respectively, was subjected to RT-PCR analysis using HER-2/neu-specific primers. Expression of β-actin served as the control.