

Figure S1. Study design. ALDH, aldehyde dehydrogenase; Cl, cleaved; ALT, alanine aminotransferase; AST, aspartate amino-transferase; BUN, blood urea nitrogen; TCGA, The Cancer Genome Atlas; CSC, cancer stem cell; PDB, protein data bank; KD, kinase domain; CETSA, cellular thermal shift assay.

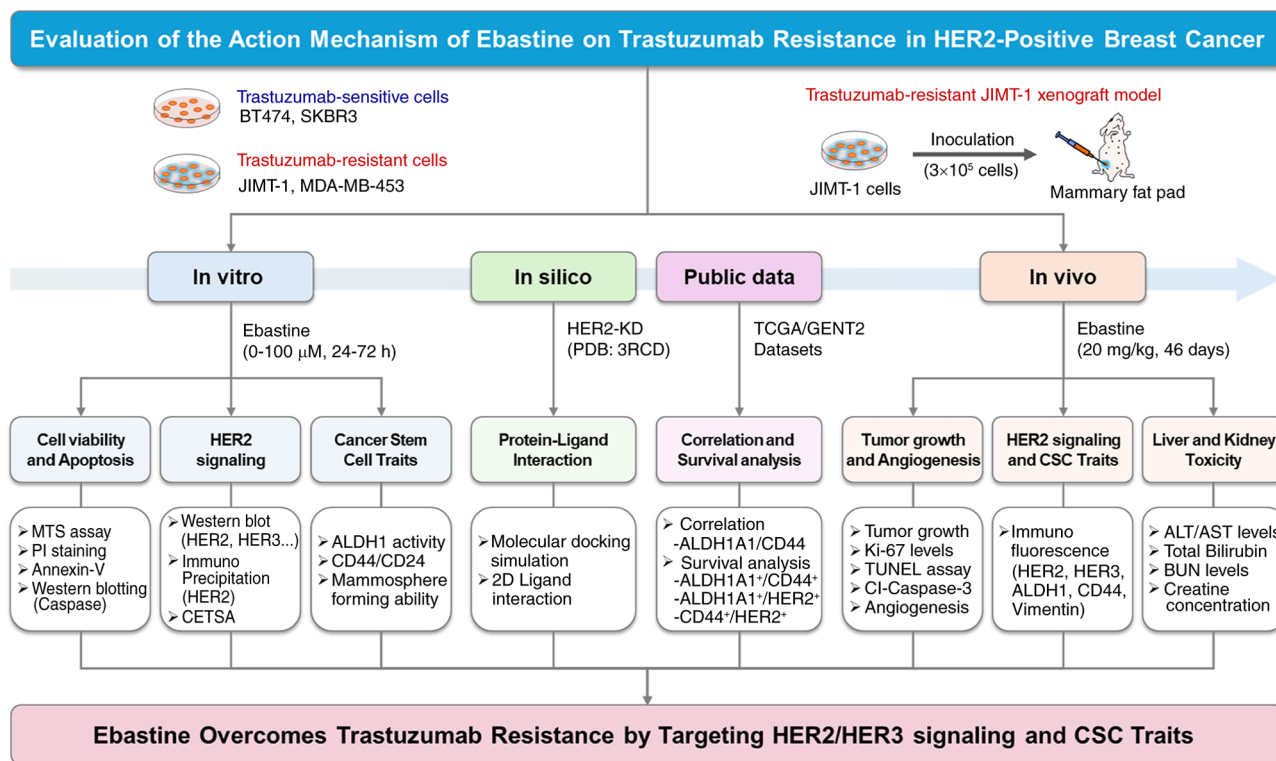


Figure S2. HER2 and HER3 expression levels in trastuzumab-sensitive and -resistant HER2-positive breast cancer cell lines. Immunoblot analysis of HER2, p-HER2, HER3, p-HER3 in trastuzumab-sensitive (SKBR3 and BT474) and -resistant (MDA-MB-453 and JIMT-1) cells.  $\beta$ -tubulin was used as a loading control. p-, phosphorylated.

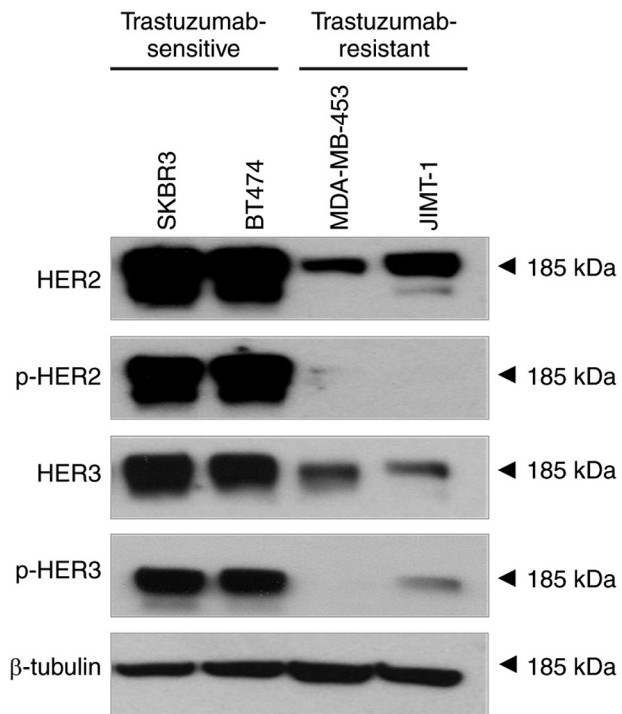


Figure S3. EBA induces apoptosis and modulates apoptosis-associated proteins in BT474 and SKBR3 cells. (A) BT474 and (B) SKBR3 cells were treated with EBA or vehicle control (DMSO). Cell viability and IC<sub>50</sub> values were determined by MTS assay. (C) Representative phase-contrast microscopy of BT474 cells following treatment with EBA. BT474 and SKBR3 cells were treated with EBA (5 and 10  $\mu$ M) for 48 h. (D) Proportion of cells in the sub-G1 phase was quantified by flow cytometry. (E) Early and late apoptotic cells were determined by Annexin V/PI staining. Effects of EBA (48 h) on the expression of PARP, cleaved PARP, caspase-3, -7 and -8 and cleaved caspase-3, -7, -8 in (F) BT474 and (G) SKBR3 cells. Expression of Bcl-2 family proteins (Bcl-2 and Bax) following EBA treatment (48 h) in (H) BT474 and (I) SKBR3 cells. GAPDH was used as a loading control. \*P<0.05 and, \*\*P<0.01, \*\*\*P<0.001 and \*\*\*\*P<0.0001 vs. control (vehicle-treated). EBA, ebastine.

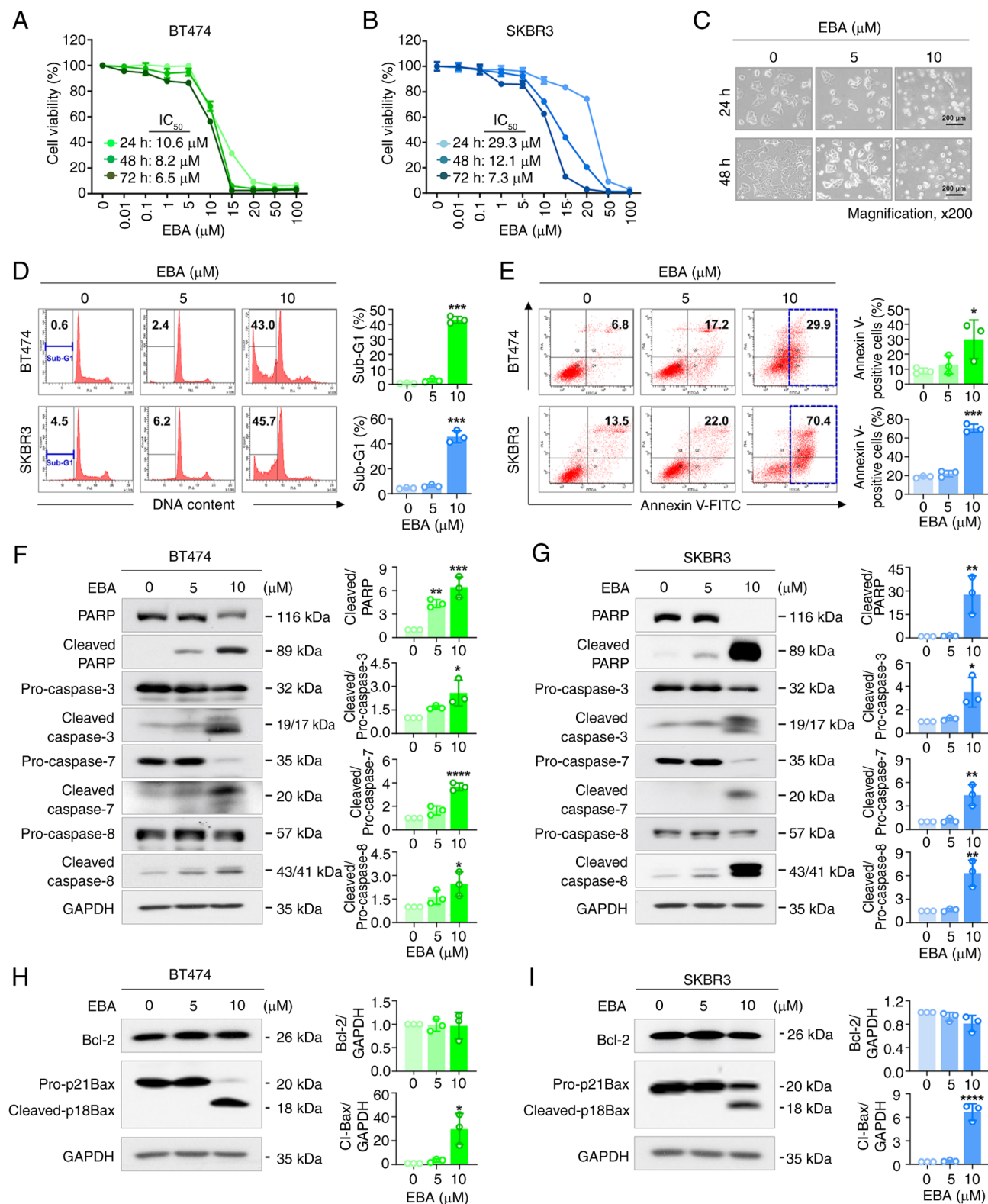


Figure S4. EBA suppresses HER2/HER3 and AKT signaling in BT474 and SKBR3 cells. Immunoblot analysis of HER2, p95HER2, p-HER2 (Y1221/1222), HER3, p-HER3 (Y1289), AKT, and p-AKT following treatment with EBA (48 h) in (A) BT474 and (B) SKBR3 cells. \*P<0.05, \*\*P<0.01, \*\*\*P<0.001 and \*\*\*\*P<0.0001 vs. vehicle-treated control (0  $\mu$ M EBA). p-, phosphorylated; EBA, ebastine.

