Legends to Supplementary Figures

Figure S1. FACS sorting of HER2-enriched fraction of SKBR3 and analysis of same by FACS in parental derivatives

A, SKBR3 parental cells analyzed with secondary antibody only (control). B, Sorting of SKBR3 cells according to HER2 levels; the HER2 enriched boxed R6 fraction was FACS sorted and subsequently analyzed by FACS to confirm the elevated HER2 level (see Panel D). C, FACS analysis of R6 fraction in SKR6 clonal derivative with secondary antibody only (control for D-F). Panels D-F, FACS analysis for HER2 levels in SKR6, SKR6CA and SKR6LR cells, respectively upon staining with anti-HER2 primary antibody + secondary antibody. The number of events (counts) are shown on the Y axis and the HER2-staining intensity is shown on the X axis. Note SKR6 is the sorted fraction with elevated level of HER2 staining at 10^3 to 10^4 intensity, that is elevated in comparison to the total population of the parental SKBR3 cells. The same elevated HER2 intensity is stably retained in the SKR6CA and SKR6LR cells similar to SKR6.

Figure S2. Inhibition of HER2 signaling by Lapatinib in SKR6 and SKR6LR cells.

Western blots were done with antibodies to phospho-HER2 (Tyr 877), anti-HER2 and anti-actin used as a loading control. Cells were grown in rich medium and Lapatinib was added for 24 hours prior to analysis; vehicle is DMSO. A, SKR6. B, SKR6LR. Lapatinib inhibits the auto-phosphorylation of HER2, the first step in HER2 signaling, in both SKR6 and its Lapatinib resistant derivative at nanomolar doses, indicating the drug is reaching its target kinase and capable of inhibiting its activity in both cells.

Figure S3. Xenograft histopathology.

Paraffin sections of xenograft tumors were stained with hematoxylin and eosin and shown at 20X magnification. A, SKR6. B, SKR6CA. C, SKR6LR. Both SKR6CA and SKR6LR xenografts were highly proliferative and showed confluent areas of necrosis in larger tumors.

Figure S4. NF-κB DNA binding activity by electrophoretic mobility shift assay (EMSA**).**

NF−κB activation in SK-BR3 and derivative cell lines and xenografts was determined by its DNA-binding activity by electrophoretic mobility shift assays (EMSA).

A shows NF-κB DNA-binding activity by electrophoretic mobility shift assay in 5 µg of nuclear extracts from parental SKBR3 cells and derivatives.

B shows NF−κB DNA biding activity by EMSA in 5 µg of whole tumor tissue extracts from xenogragfts of three cell lines. Each tumor was confirmed to be human SKBR3-derived by genotyping.

Figure S5. Geno Ontology (GO) terms for genes found in clusters II, III and IV from Figure 5B

Figure S6. Hypothesis: Constitutive activation of NF-kB accelerates tumor growth by evading drug sensitivity resulting in poor clinical outcome.

The HER2 dependent SKR6 cells are sensitive to anti-HER2 drugs. Most interestingly, expression of a designed constitutively activated NF-κB renders SKR6 cells resistant to anti-HER2 drugs, trastruzumab and Lapatinib. Independently isolated Lapatinib resistant cells up regulate NF-kB activity. Both cell types are profoundly anti-apoptotic and have elevated levels of anti-apoptosis related genes. Cells expressing the constitutively active derivative of *RelA* (SKR6CA) phenocopies Lapatinib resistance, high expression of anti-apoptotic genes and accelerated tumor growth in nude mice observed in SKR6LR cells. Taken together, these results converge on anti-apoptosis driven by hyperactivation of NF-kB as a central mechanism of oncogenesis, drug resistance and relapse. Most relevant in a clinical setting is the sensitivity of the drug resistant SKR6LR cells to a combination of Lapatinib and NBD (an inhibitor of NF-kB signaling), suggesting a potential dual targeting of HER2-NF-kB that would result in improved clinical outcome.