

## Supplementary Materials and Methods

**The CellTiterGlo Assay.** Cells ( $10^3$ /well) were seeded in triplicate onto 384-well plates. The next day, cells were either left untreated or treated with compounds using a 3-fold dilution “dose matrix”, such that each plate contained a dose response curve of both drugs in a two-dimensional matrix. The plates were incubated at 37°C for 5 days followed by addition of CellTiterGlo reagent. Percent growth inhibition was calculated by normalizing raw luminescence values of treated wells to that of untreated wells. To evaluate combination effects of LJM716 with BYL719 studies were conducted using a “dose matrix”. The percent growth inhibition and excess inhibition were analyzed using the Chalice software (Zalocus Inc., Cambridge MA). Synergy was calculated by comparing a combination’s response to those of its single agents, against the drug-with-itself dose-additive reference model (1).

**Xenograft studies.** To establish BT474 xenografts, a 21-day  $17\beta$ -estradiol pellet (Innovative Research of America) was inserted subcutaneously (s.c.) in the dorsum of 4- to 6-week-old female athymic mice (Harlan Sprague Dawley Inc.) one day before tumor cell injection. Approximately  $5 \times 10^6$  BT474, MDA453, NCI-N87 or HCC1954 cells were injected s.c. into the mouse right flank. Once tumors reached a volume  $\geq 200$ -400 mm<sup>3</sup>, mice were randomized to different treatments including the following: 20 mg/kg trastuzumab, 20 mg/kg LJM716, 100 mg/kg lapatinib or 12.5-30 mg/kg BYL719. Trastuzumab and LJM716 were diluted in PBS and given two (trastuzumab) or three (LJM716) times per week i.p. Lapatinib and BYL719 were suspended in 0.5% methyl cellulose and administered by orogastric gavage daily. Tumor diameters were measured serially with calipers and volume in mm<sup>3</sup> calculated as described (2). For all these studies, there was no apparent drug-related toxicity in any of the treatment arms. At the completion of treatment, animals were anesthetized with 1.5% isoflurane-air mixture and sacrificed by cervical dislocation. Tumors were harvested and divided in multiple fragments that were either flash frozen in liquid N<sub>2</sub> or formalin fixed and paraffin embedded for further analysis.

**Supplementary Figure 1.** LJM716 and BYL719 were screened in combination using a 6x6 dose-response matrix to capture a wide range of concentrations and ratios. Single agent responses at varying concentrations of BYL719 and LJM716 are shown along the bottom and left edges of the dose matrix marked “Inhibition”. Furthermore, the combination of LJM716 and BYL719 at different concentrations is shown in the “Inhibition” matrices using colors that run from black (no effect) through the rainbow to pink/white (total effect). Using the data collected for the two single agents, combination data was modeled for a null interaction between the components of the combination using the Loewe Additivity model. Loewe dose additivity is the expected response if both agents inhibit the same molecular target by the same mechanism (3). This null interaction matrix is subtracted from the experimentally-derived data (matrix marked as “Inhibition”) to identify activity values in excess (matrix marked as “ADD Excess Inhibition”) of what would be expected if there is no interaction between the components.

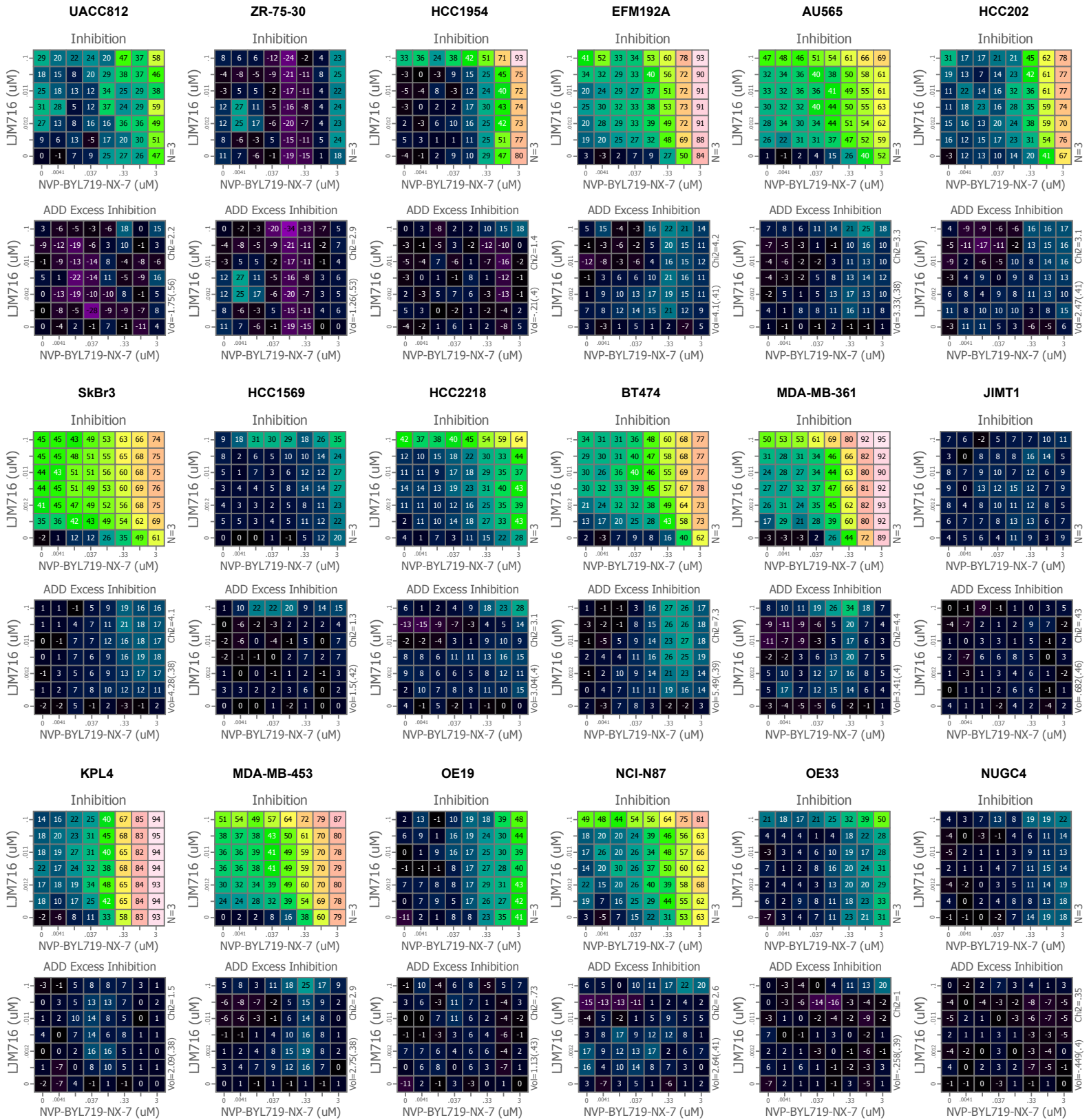
**Supplementary Figure 2.** Cells were plated in 6-well plates and treated in triplicate with DMSO, 10 µg/ml LJM716 and/or 1 µM BYL719. Media and drugs were replenished every 3-4 days. Cells were stained with crystal violet when the DMSO-treated (control) monolayers became confluent, ranging from 14-21 days. Representative images and quantification of integrated intensity (% control) are shown (\*, p<0.05, t test).

**Supplementary Figure 3.** Cells were seeded in Matrigel and allowed to grow in the absence or presence of 10 µg/ml LJM716 and/or 1 µM BYL719 as indicated. Cell media and drugs were replenished every 3 days. Images shown were recorded 15-19 days after cell seeding. Colony number and volume was quantified using the GelCount system. Each bar graph represents the mean ± SEM of triplicate samples (\*, p<0.05, t test.)

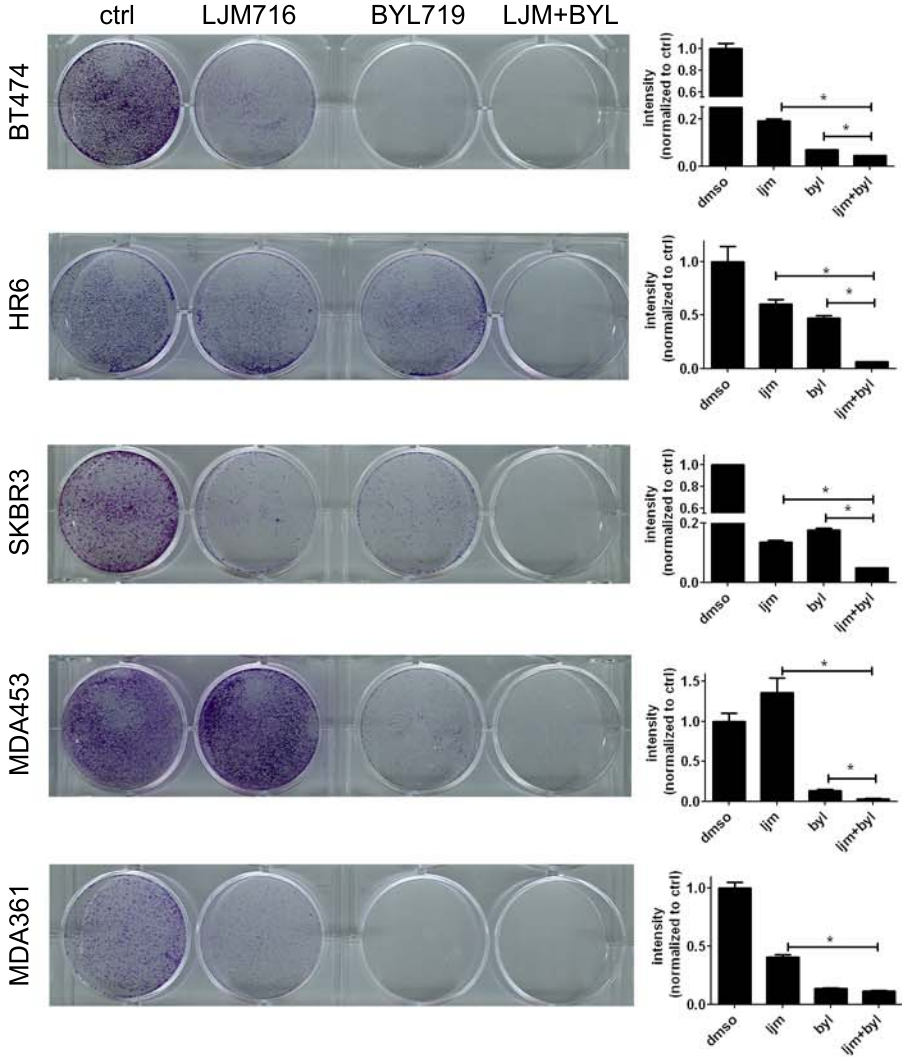
**Supplementary Figure 4.** Mice bearing NCI-N87 xenografts were treated with IgG (20 mg/kg q 2 days), LJM716 (20 mg/kg q 2 days), BYL719 (12.5 mg/kg daily) or LJM716/BYL719. Treatment of mice in the control and LJM716-treated groups was terminated at 34 days. The remaining groups were kept on treatment for an additional 14 days before the study was terminated. Mice received treatment 2 hours before sacrifice and extraction of tumor. Tumor AKT phosphorylation levels were measured using a Meso Scale Discovery AKT S473 phosphorylation kit according to the manufacturer’s instructions.

1. Lehar J, Krueger AS, Avery W, Heilbut AM, Johansen LM, Price ER, et al. Synergistic drug combinations tend to improve therapeutically relevant selectivity. *Nat Biotechnol.* 2009;27:659-66.
2. Garrett JT, Olivares MG, Rinehart C, Granja-Ingram ND, Sanchez V, Chakrabarty A, et al. Transcriptional and posttranslational up-regulation of HER3 (ErbB3) compensates for inhibition of the HER2 tyrosine kinase. *Proc Natl Acad Sci U S A.* 2011;108:5021-6.
3. Rickles RJ, Tam WF, Giordano TP, 3rd, Pierce LT, Farwell M, McMillin DW, et al. Adenosine A2A and beta-2 adrenergic receptor agonists: novel selective and synergistic multiple myeloma targets discovered through systematic combination screening. *Mol Cancer Ther.* 2012;11:1432-42.

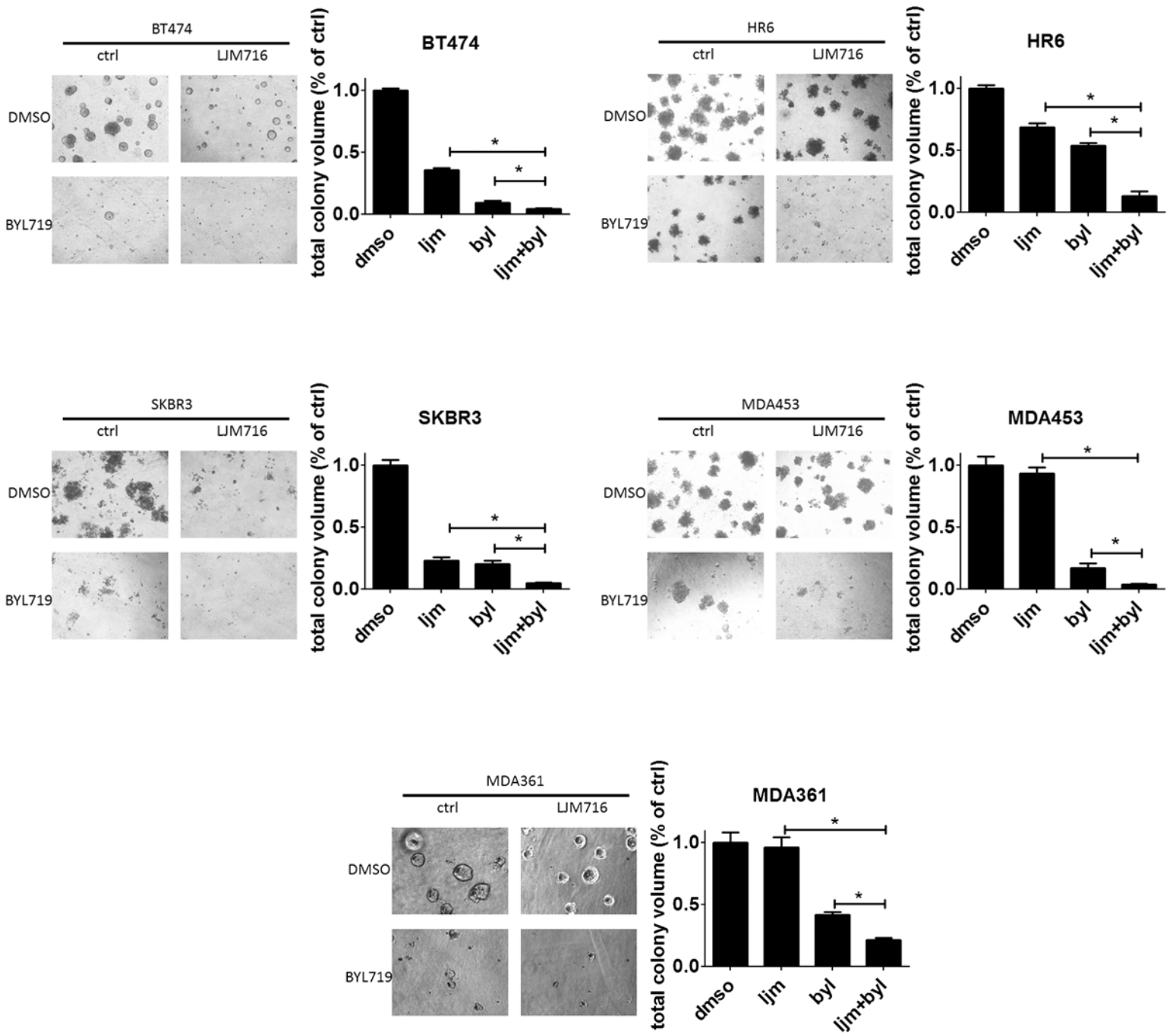
Supplementary Figure 1.



Supplementary Figure 2.



Supplementary Figure 3.



Supplementary Figure 4.

