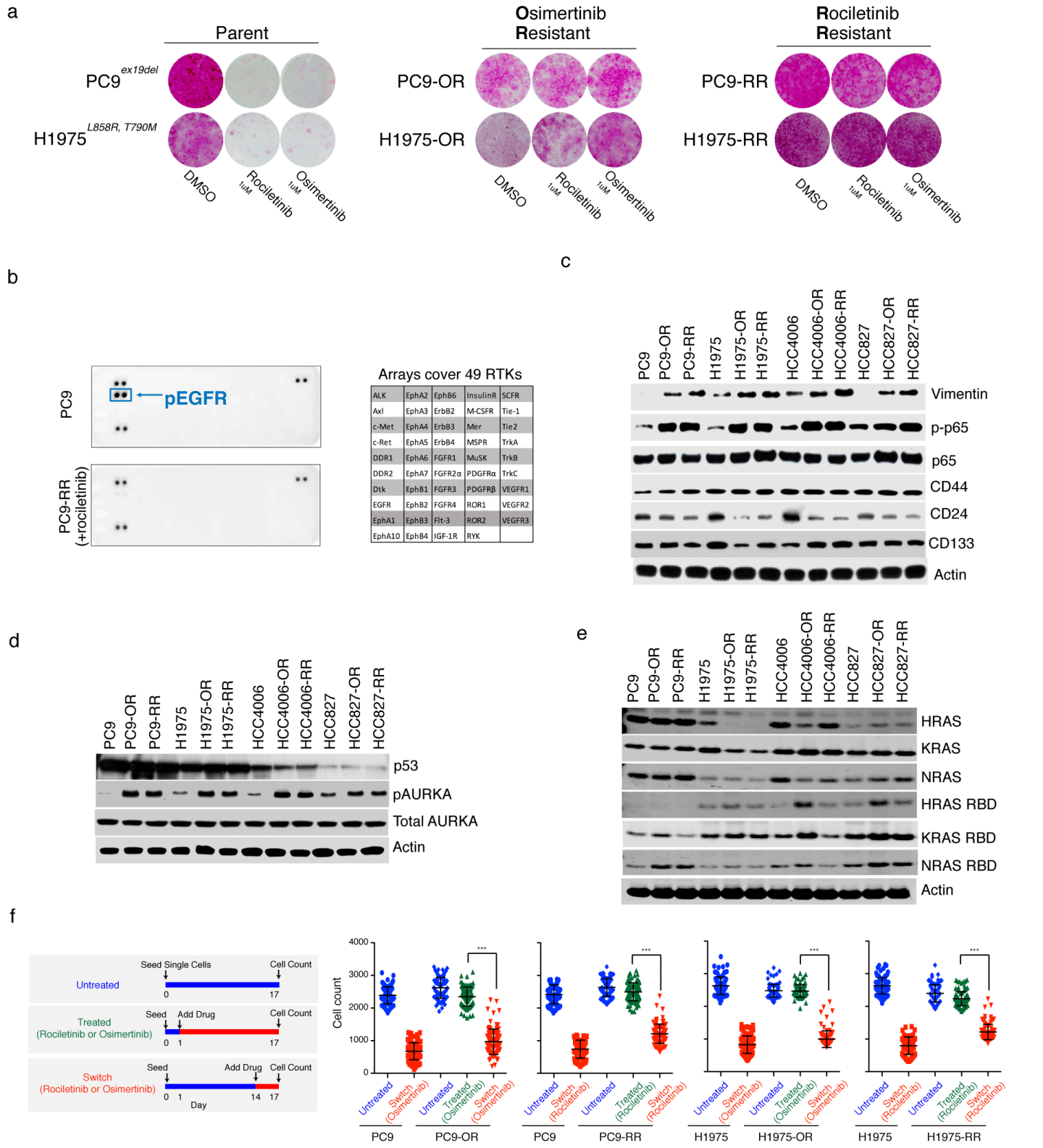
SUPPLEMENTARY TABLES

Supplementary Table 1: Results of drug synergy screen. Synergy scores from drug screen in H1975-RR cells treated with 2uM rociletinib. Data based on n=4 biologically independent samples, see methods.

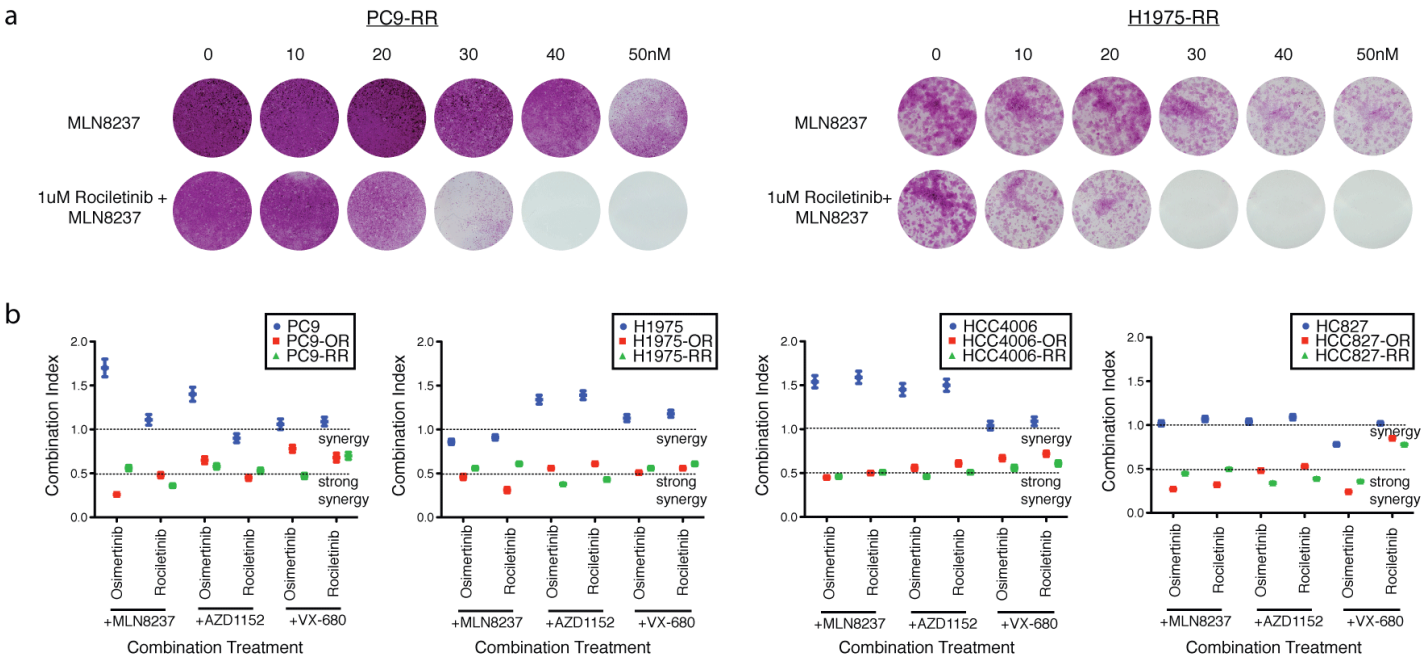
Supplementary Table 2: Mitotic defects after EGFR-TKI treatment. Percentages of specific types of mitotic defects observed in mitotic cells after EGFR-TKI treatment. Data based on 60 cells from two independent experiments.

SUPPLEMENTARY FIGURES

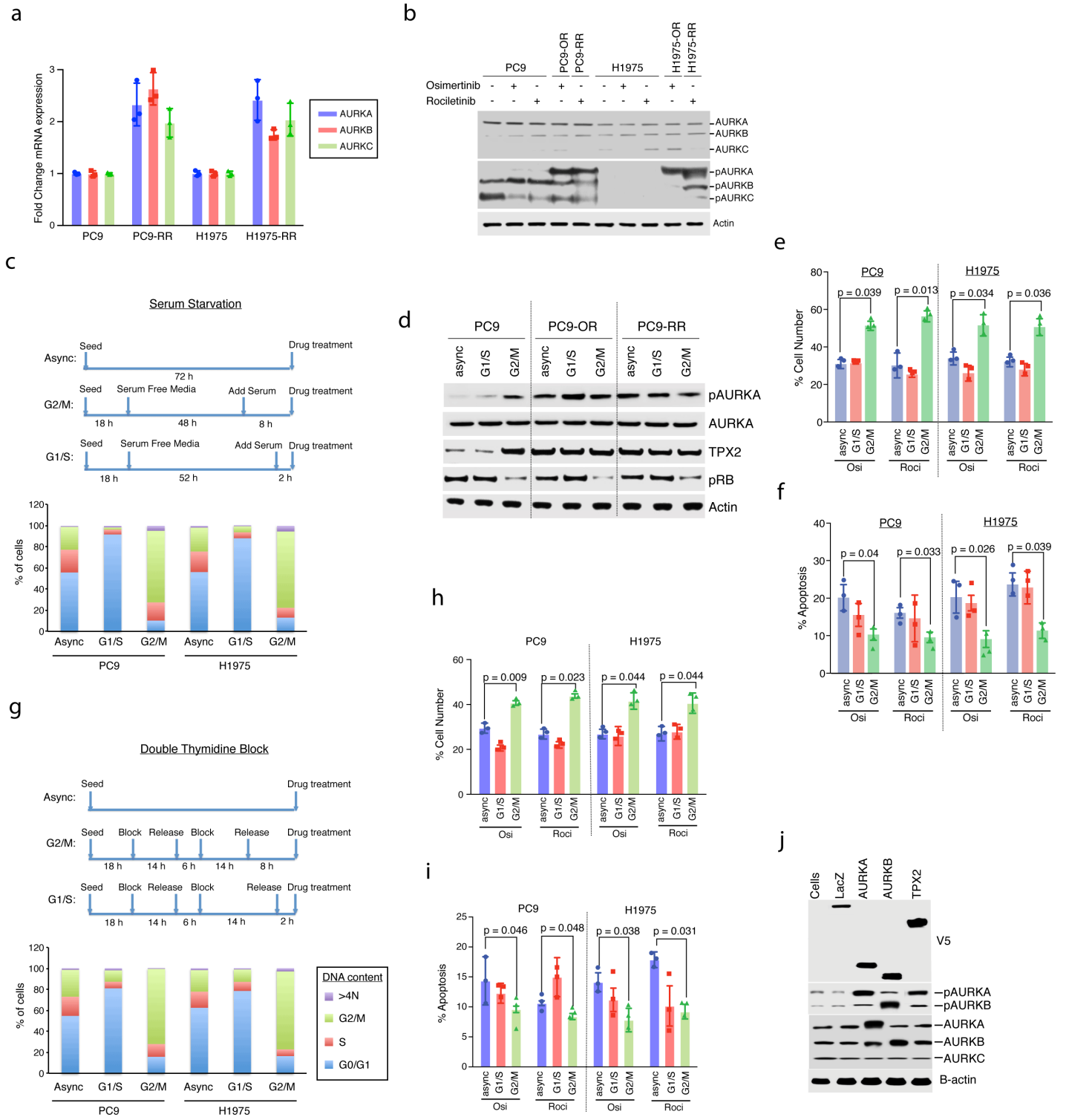


Supplementary Figure 1: Characterization of acquired resistant cell lines. a Colony formation assays of PC9 and H1975 parental and acquired resistant cell lines grown for 9 d in the presence of 1uM of the indicated EGFR TKI. Representative images are shown from two independent experiments. **b** Phospho-receptor tyrosine kinase (RTK) array analysis of lysates from PC9 cells and PC9-RR cells. Reduction in phospho-EGFR highlighted. A list of RTKs measured within the array are shown. **c** Immunoblot of lysates from parental and acquired resistant cell lines. Levels of Vimentin the NFkB pathway effector, phospho-p65 (p-p65)

and p65, and stem cell markers CD44 and CD133 (usually high) and CD24 (usually low). **d** Immunoblot of lysates from parental and acquired resistant cell lines for P53. AURKA and pAURKA are the same as in Figure 2f. **e** Immunoblot of lysates from parental and acquired resistant cell lines for H-, K- and N-RAS and GTP levels in the same molecules determined by RAF-RBD binding. **f** Single cell clones from each of the indicated cell lines were plated and allowed to proliferate without TKI (blue), in the continued presence of EGFR TKI (green, 1uM rociletinib for –RR cell lines or 1uM osimertinib for –OR lines) or plated with no TKI for 14 days and switched to TKI for 3 days (red) before counting. In the case of genetic resistance, switching on and off treatment should not impact drug sensitivity. Mean cell numbers were calculated from n=96 independent single cell clones upon drug treatment. Each dot represents one single cell clone with 96 clones used in each condition. Error bars s.d. *** $p<0.0001$ based on a two-tailed Student's t-test. Blots are representative of a single experiment.

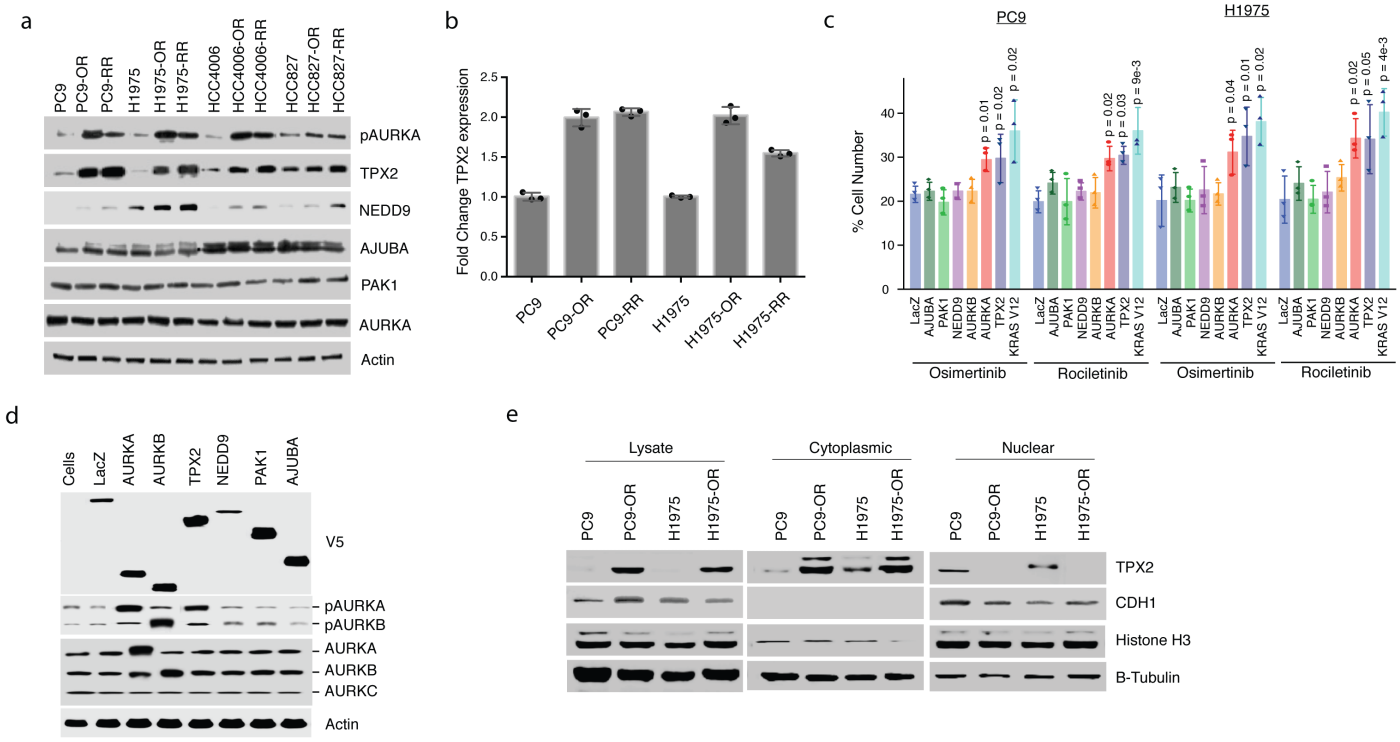


Supplementary Figure 2: Synergy between EGFR TKIs and Aurora kinase inhibitors in acquired resistant lines. a Growth of acquired resistant cell lines for 9 d in the presence of single agent MLN8237 at the indicated doses or the combination of MLN8237 with 1uM of rociletinib. Images are representative of two independent experiments. **b** Combination index analysis or the indicated cell lines grown in the presence of 30nM of Aurora kinase inhibitor (MLN8237, AZD1152 or VX680) in combination with 1uM of either EGFR TKI, osimertinib or rociletinib, for 3 d. Shown are CI values calculated from mean of cell proliferation from n=3 independent biological experiments. Error bars mean±s.e.m.

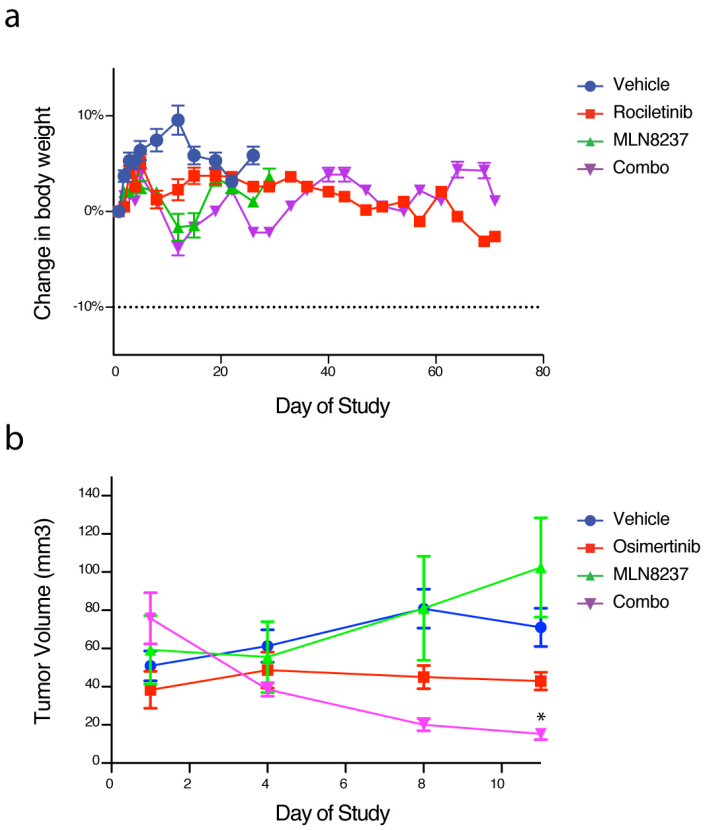


Supplementary Figure 3: Contribution of Aurora kinases on resistance to EGFR TKI. a RT-PCR of the indicated genes in parental and rociletinib acquired resistant cell lines. Mean fold changes indicated from n =4 biologically independent samples. **b** Immunoblot of lysates from cells treated with 1uM of the indicated agents for 24hrs. **c** Schematic of strategy for cell synchronization by serum starvation and validation of strategy using FACS. **d** Immunoblot analysis of lysates from cell lines grown asynchronously or synchronized into the indicated cell cycle phase by serum starvation. Phosphorylated RB (pRB) used as a marker of G1/S phase cells. **e** Proliferation relative to DMSO for asynchronous cells or cells synchronized by serum starvation into G1/S or G2/M phase and then treated for 72 h with 1uM of the indicated EGFR TKI. Data shown represents mean calculated from n=4 biologically independent samples. **f** Measurement of early apoptosis by YO-PRO-1 positivity in asynchronous cells or cells synchronized by serum starvation into G1/S or G2/M phase and then treated for 48 h with 1uM of the indicated EGFR TKI. Data shown represents mean calculated from n=4

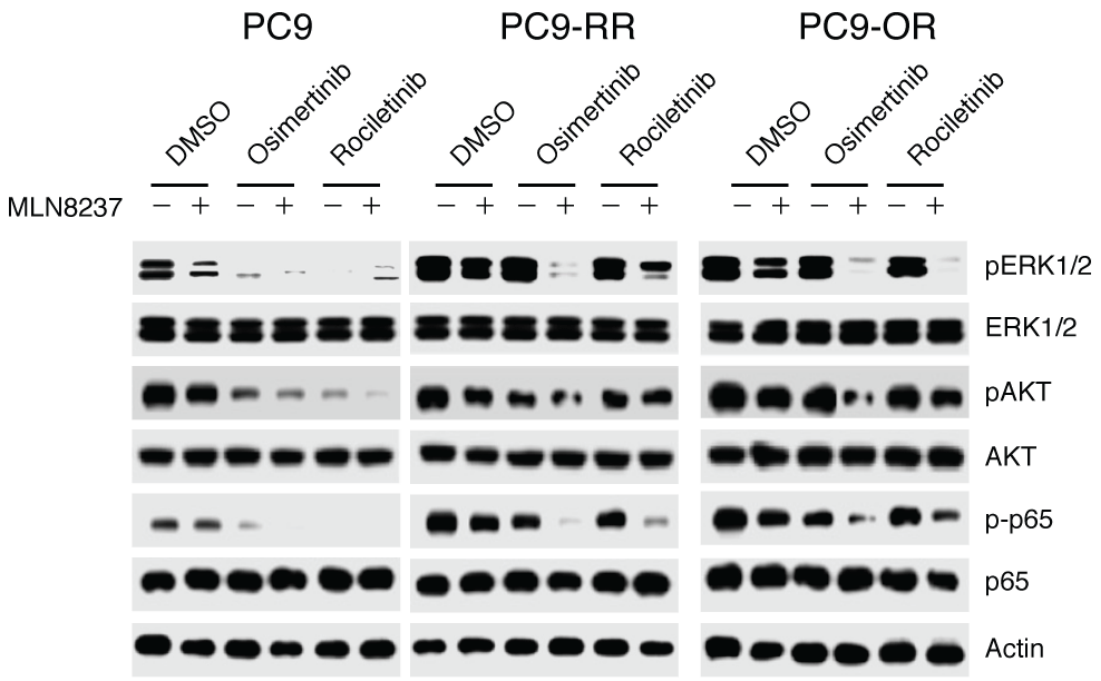
biologically independent samples. **g** Schematic of strategy for cell synchronization by double thymidine block and validation of strategy using FACS. **h** Proliferation relative to DMSO for asynchronous cells or cells synchronized by double thymidine block into the indicated cell cycle phase and then treated for 72 hours with 1uM EGFR TKI. Data shown represents mean calculated from n=4 independent experiments. **i** Measurement of early apoptosis by YO-PRO-1 positivity in asynchronous cells or cells synchronized by double thymidine block into the indicated cell cycle phase and then treated for 48 h with 1uM EGFR TKI. Data shown represents mean calculated from n=4 biologically independent samples. **j** Immunoblot of lysates from PC9 cells or cells transfected with the indicated constructs. Error bars s.d. P-values based on a two tailed Student's t-test. Blots representative of two independent experiments.



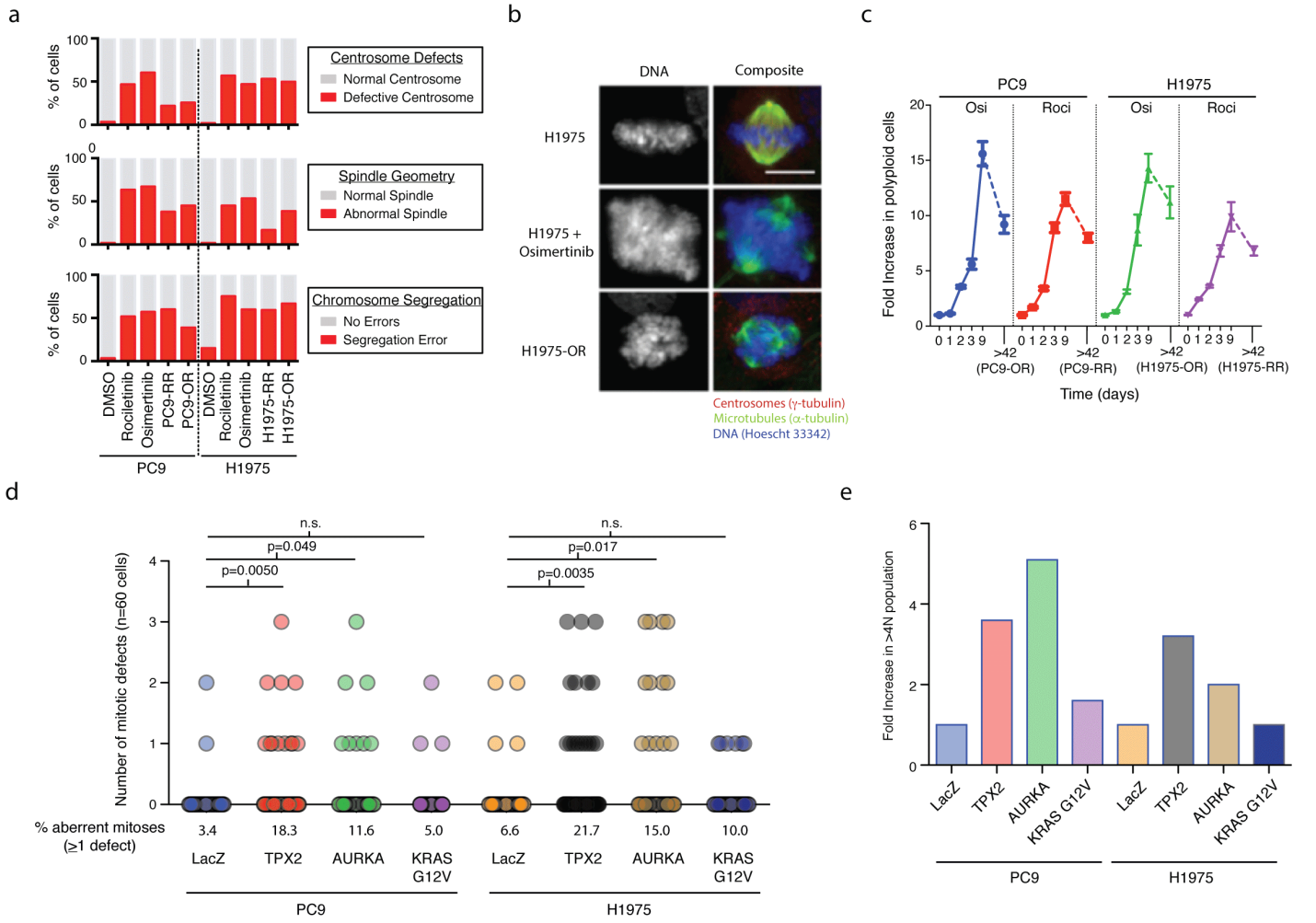
Supplementary Figure 4: Analysis of AURKA activators in acquired resistant cells. a Immunoblot of lysates from indicated cell lines for AURKA, phospho-AURKA and reported activators of AURKA. Blot was performed once. **b** RT-PCR of TPX2 levels in the indicated cell lines normalized to parental cells. Mean fold changes from n=3 biologically independent samples. **c** Relative proliferation of PC9 or H1975 cells transfected with plasmids expressing the indicated genes and treated 1uM EGFR TKI for 72 h compared to DMSO treated cells. Significance based on comparison to LacZ control. Data are mean over n=3 biologically independent samples. A portion of this panel is the same as Figure 2e. **d** Immunoblot of PC9 cells or PC9 cells transfected with the indicated vectors. This blot is an expanded version of Supplementary Figure 3g. Blot was performed once. **e** Immunoblot of results from subcellular fractionation of PC9 or H1975 parental and acquired resistant cells. Histone H3 is used to indicate nuclear fraction. Blot is a single film separated for clarity and was performed with two independent experiments with similar results. Error bars s.d. P-values based two-tailed Student's t-test.



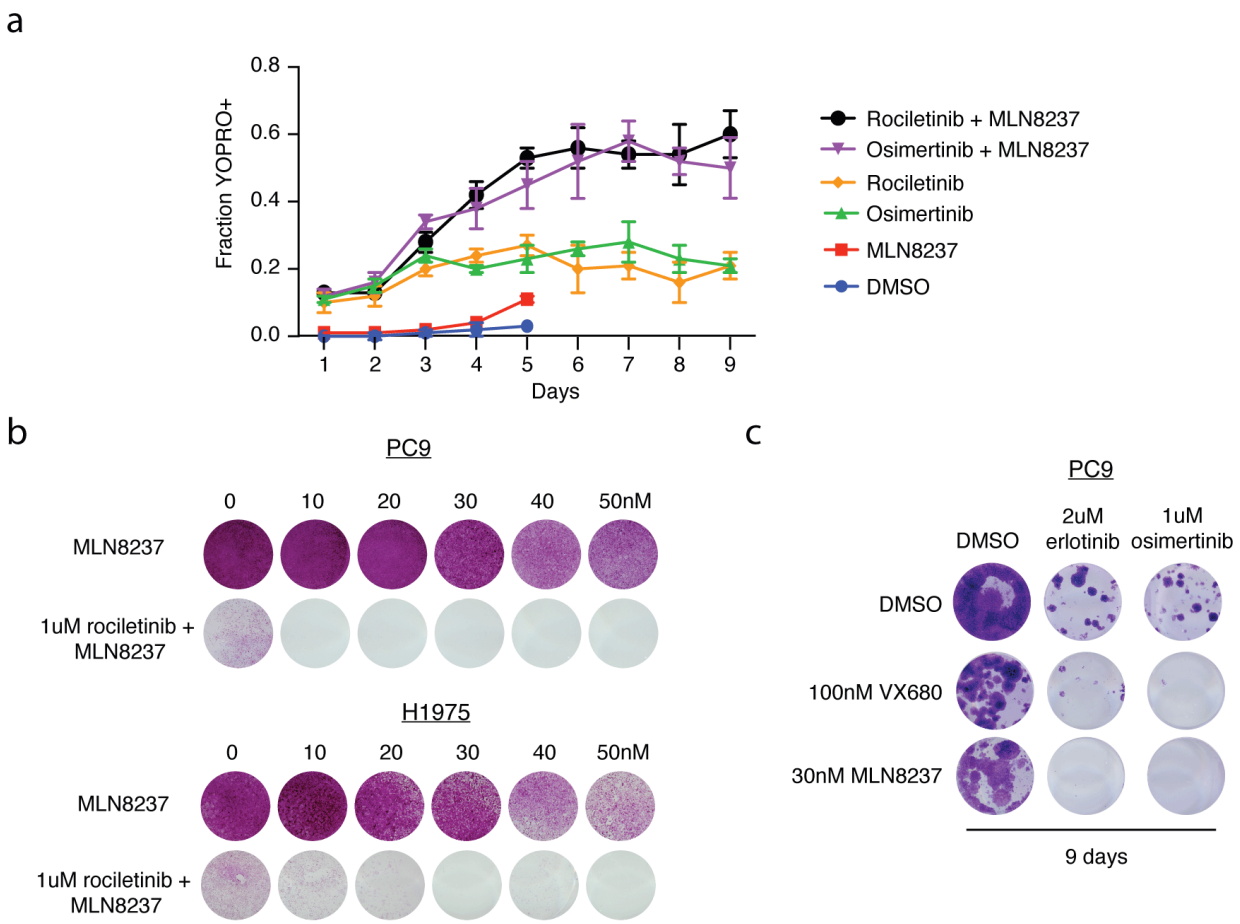
Supplementary Figure 5: Cell line xenograft studies. a Mean changes in body weight for n=10 mice per group vehicle, rociletinib (100mg/kg), and n=7 mice for MLN8237 (10mg/kg) and the combination. **b** Mean tumor volume measurements for the xenograft study from 10 tumors per treatment arm in Figure 2g. * p = 0.02 for combination compared to osimertinib by two-tailed t-test. Error bars s.e.m



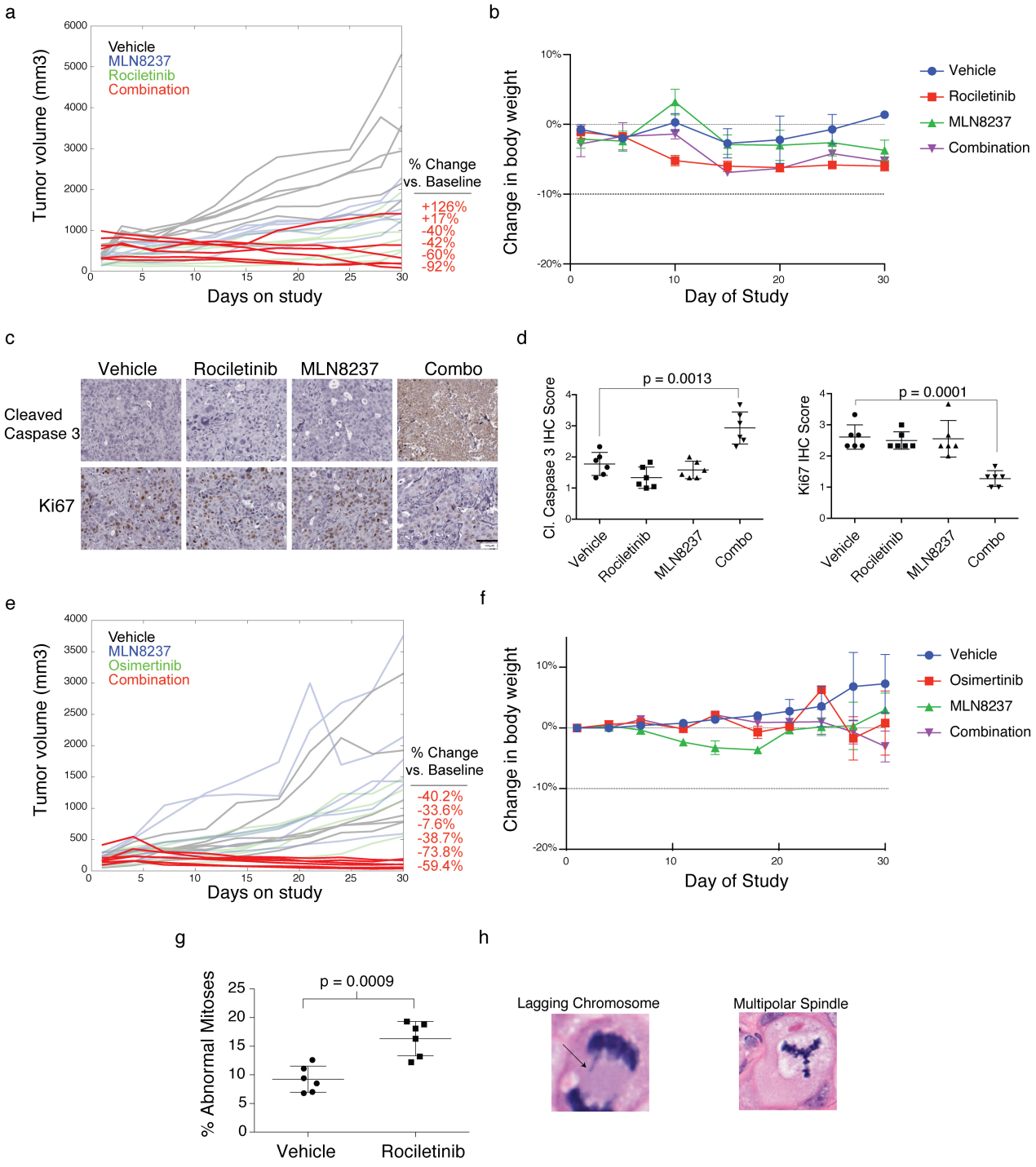
Supplementary Figure 6: Aurora kinase regulates ERK and NF-kB signaling in acquired resistant lines. Immunoblot of protein lysates harvested from the indicated cell lines treated with 1uM of EGFR kinase inhibitors osimertinib or rociletinib with or without 30nM MLN8237 for 24 hours. All samples are on the same gel and separated for clarity. For comparison purposes, pERK1/2, ERK1/2 and B-actin panels are duplicated in Figure 3c. Blot is representative of two independent experiments.



Supplementary Figure 7: EGFR inhibition and TPX2 or AURKA over-expression causes mitotic defects and polyploidy. a Measurement of mitotic defects based on γ-tubulin and α-tubulin staining in the indicated PC9 and H1975 cells in response to DMSO or 1uM EGFR TKI for 72 h. Shown are the percentage of a minimum of n=60 counted cells with defects in centrosome formation, microtubule spindle geometry and chromosome segregation. **b** Representative immunofluorescence images from two independent experiments of normal or aberrant mitoses present in untreated H1975, H1975 cells treated for 72 h with 1 uM osimertinib, or H1975-OR cells. DNA is stained by Hoescht 33342. Scale bar, 10µM. **c** Increase in polypoid cells, defined as having >4N DNA content measured by flow-cytometry, after treatment with 1uM of the indicated drug over time or in acquired resistant lines. Data are mean fold changes from three replicate experiments and are normalized relative to the untreated parental cell line (PC9 or H1975). **d** The number of defects per mitotic event in cells transiently transfected with the indicated constructs for 48 hours at treated with 1uM osimertinib for 24h. Each mitotic cell was evaluated for defects in three categories: centrosome defects, spindle geometry errors and chromosomal segregation errors. Each point represents a single mitosis and is scored based on the number of categories that defects were observed in (maximum of three). n=60 mitoses were evaluated in each treatment and the percentage with a defect in at least one of these categories is indicated. P-values based on a two-tailed rank-sum test. **e** Fold increase in >4N cells for cells expressing the indicated constructs compared to LacZ control. Error bars s.e.m.

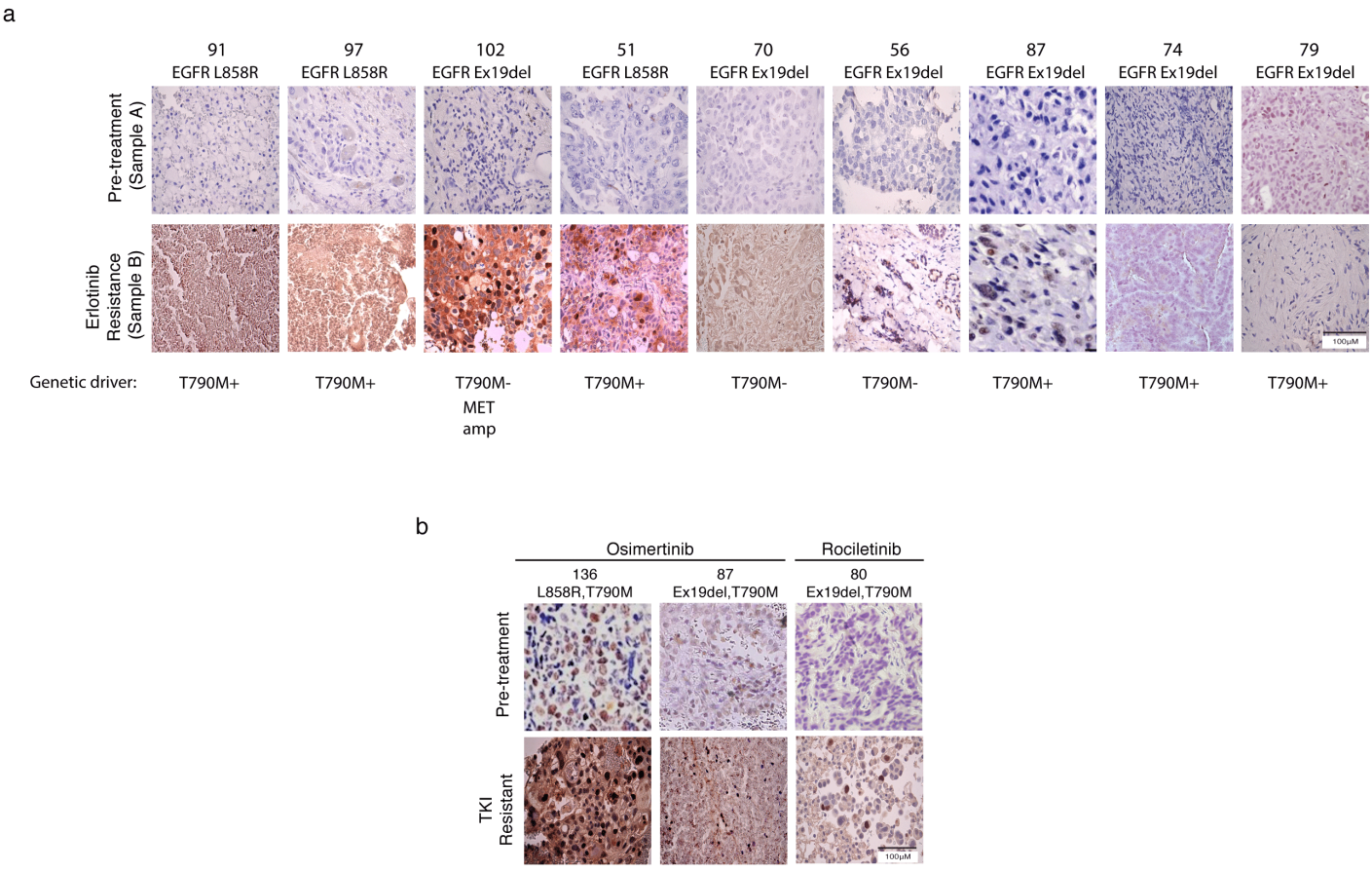


Supplementary Figure 8: Reduced formation of drug tolerant cells in the presence of MLN8237. a Mean proportion apoptotic cells determined by YO-PRO-1 positivity after treatment with EGFR TKI, MLN8237 or the combination for the indicated period of time. DMSO and MLN8237 treated groups reached confluence after 5 days and were not analyzed further. Data represents mean of apoptotic cells from n=6 biologically independent experiments per time point. **b** The indicated cell lines were grown for a period of 9 days in the presence of the indicated dose of MLN8237 alone or in combination with 1uM rociletinib and stained using crystal violet. **c** Crystal violet stain of PC9 cells treated with Aurora kinase inhibitors VX680 or MLN8237 in combination with erlotinib or osimertinib for 9 days to measure the formation of drug tolerant persister cells. Colony formation images representative of at least two independent experiments. Error bars s.e.m.



Supplementary Figure 9: Analysis of treatment in the EGFR mutant erlotinib residual disease PDX model. a Individual tumor volume measurements for PDX mice treated with the indicated agents. Percent change for combination treated tumors are indicated. **b** Mean changes in the mouse weights with 3 mice per group shown for vehicle, rociletinib (100mg/kg), MLN8237 (10mg/kg) or the combination. **c** Immunohistochemical (IHC) analysis of cleaved caspase-3 and Ki67 from tumor tissue from PDX tumors treated with the indicated agents for 30 days. Images taken at 20x magnification, bar represents 100μM. **d** Quantification of mean IHC score and each point represents a single tumor (n=6 independent tumors in total) with scores averaged over 3 fields. **e** Individual tumor volumes for PDX mice treated with the indicated agents. Percent change for combination treated tumors are shown. **f** Mean changes in the mouse weights with 3 mice per group shown for vehicle, osimertinib (1mg/kg), MLN8237 (10mg/kg) or the combination **g** Measurement of the percent of abnormal mitoses per PDX tumor expressed as a percentage of all mitoses identified. Data

represent mean from n=6 biologically independent tumors. **h** Representative image of abnormal mitoses identified in haemotoxylin and eosin (H&E) stained PDX tumors treated with rociletinib. Images taken at 60x magnification. Arrow points to lagging chromosome. P-values based on two-tailed Student's t-test. d,g error bars are mean±s.d, b,f error bars are mean±s.e.m.



Supplementary Figure 10: Pre- and post-TKI matched samples stained with TPX2. Images of EGFR-mutant non-small cell lung cancer samples collected from patients before and after progression on **a** erlotinib. or **b** third-generation inhibitors and stained with TPX2 antibody. The initial EGFR mutation is indicated. Genetic characterization of genetic drivers of resistance are indicated. Images are representative of 3 independent images from each sample. Images taken at 10X magnification. Scale bar, 100μM.

Figure 1c

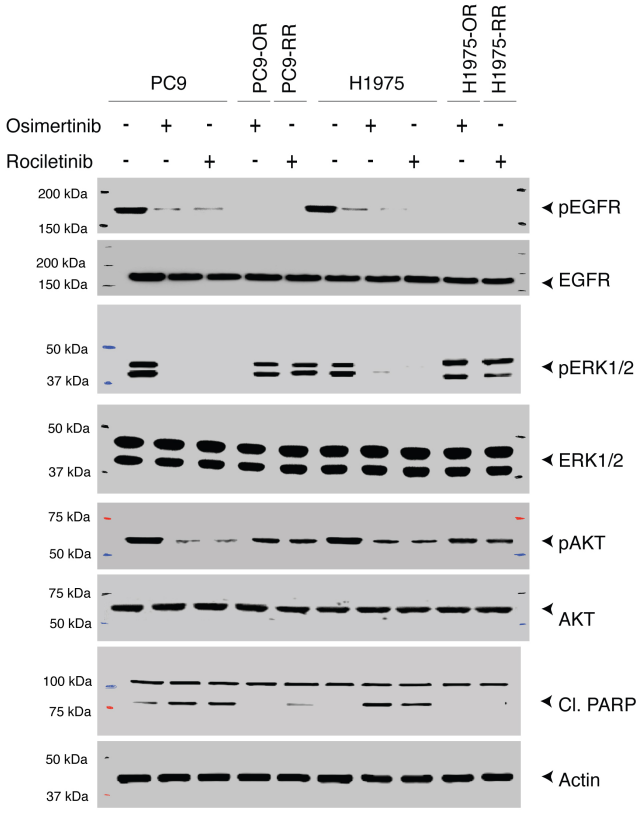


Figure 2a

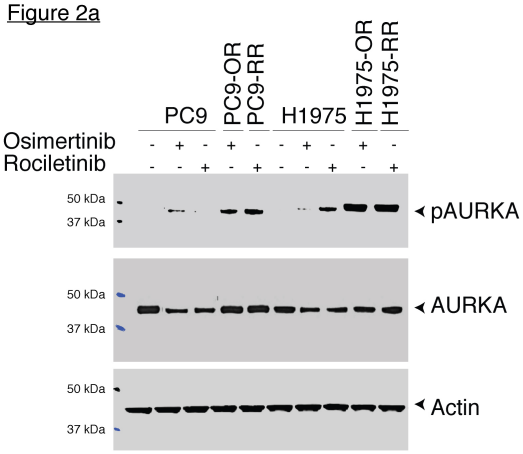


Figure 2c

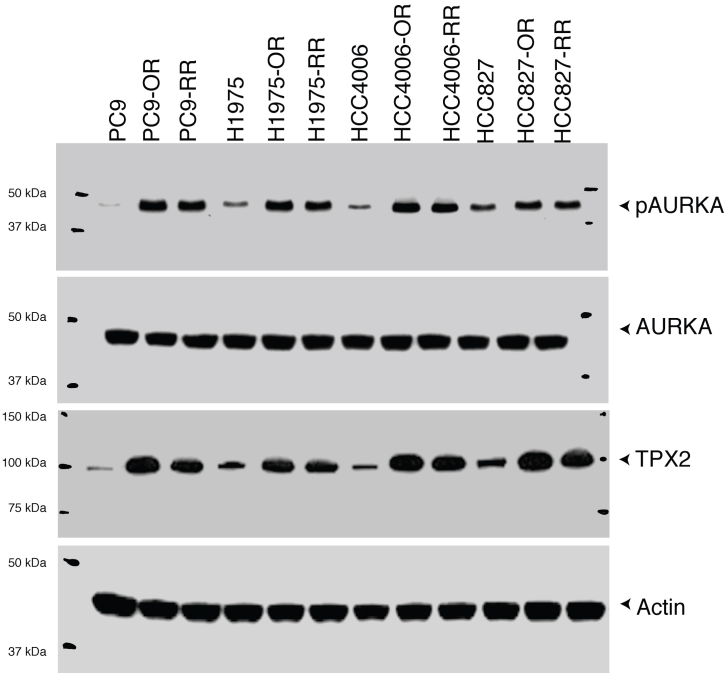


Figure 2h

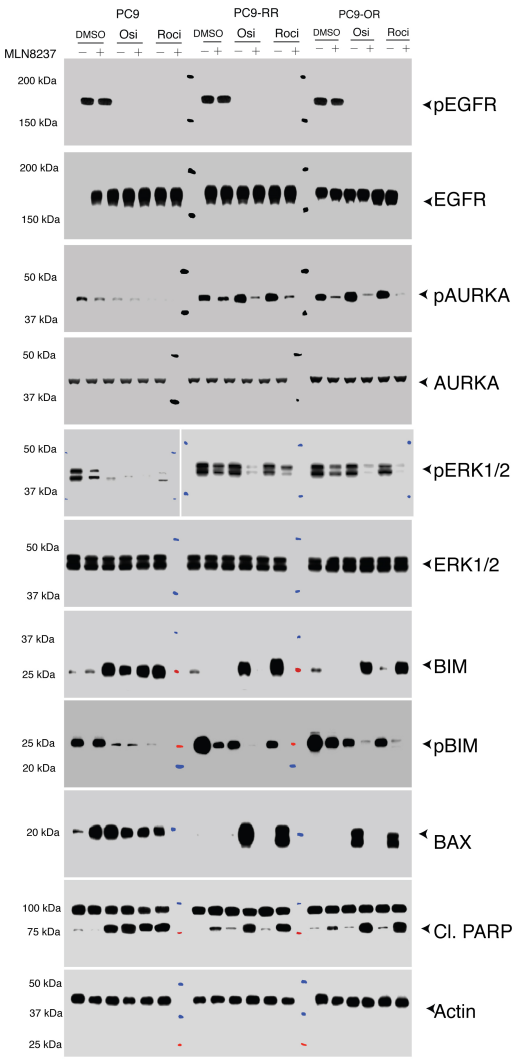


Figure 3a

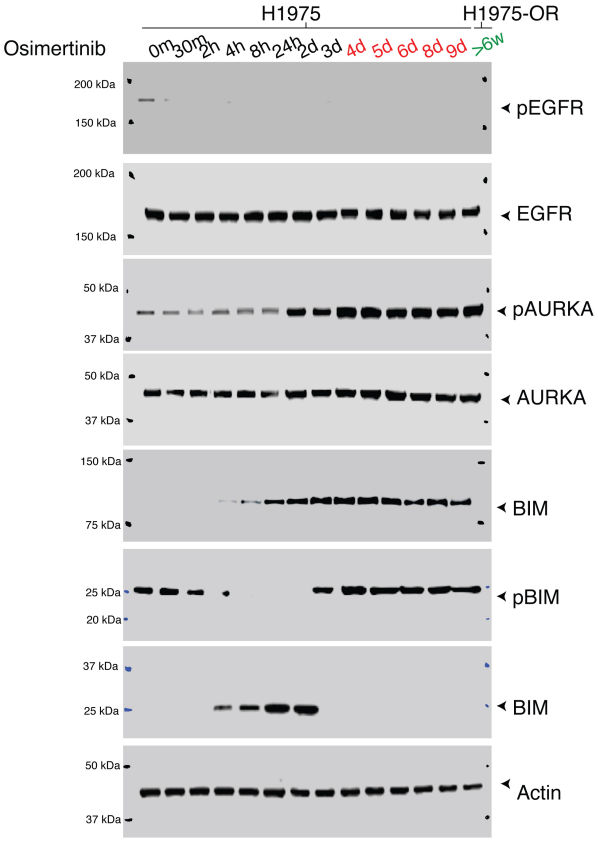


Figure 4c

Figure 3c

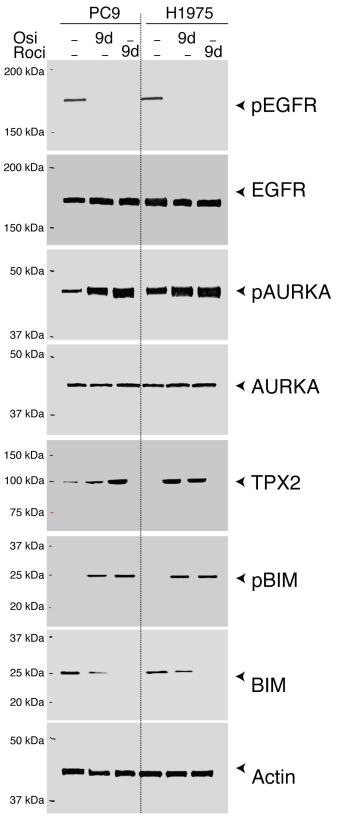
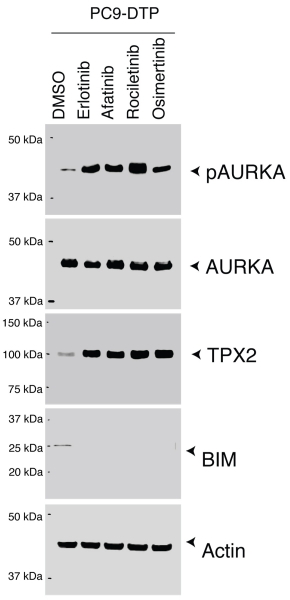
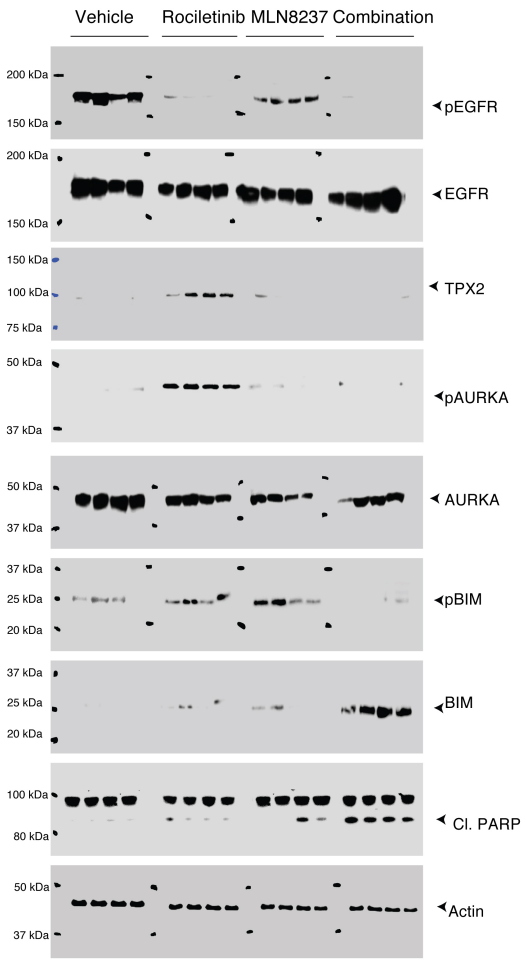


Figure 3d





Supplementary Figure 11: Uncropped gel images. Gel source data for immunoblots presented in the main figures including molecular weight (kDa).