Effects of AKT Inhibition on HGF-mediated Erlotinib Resistance in Non-Small Cell Lung Cancer Cell Lines

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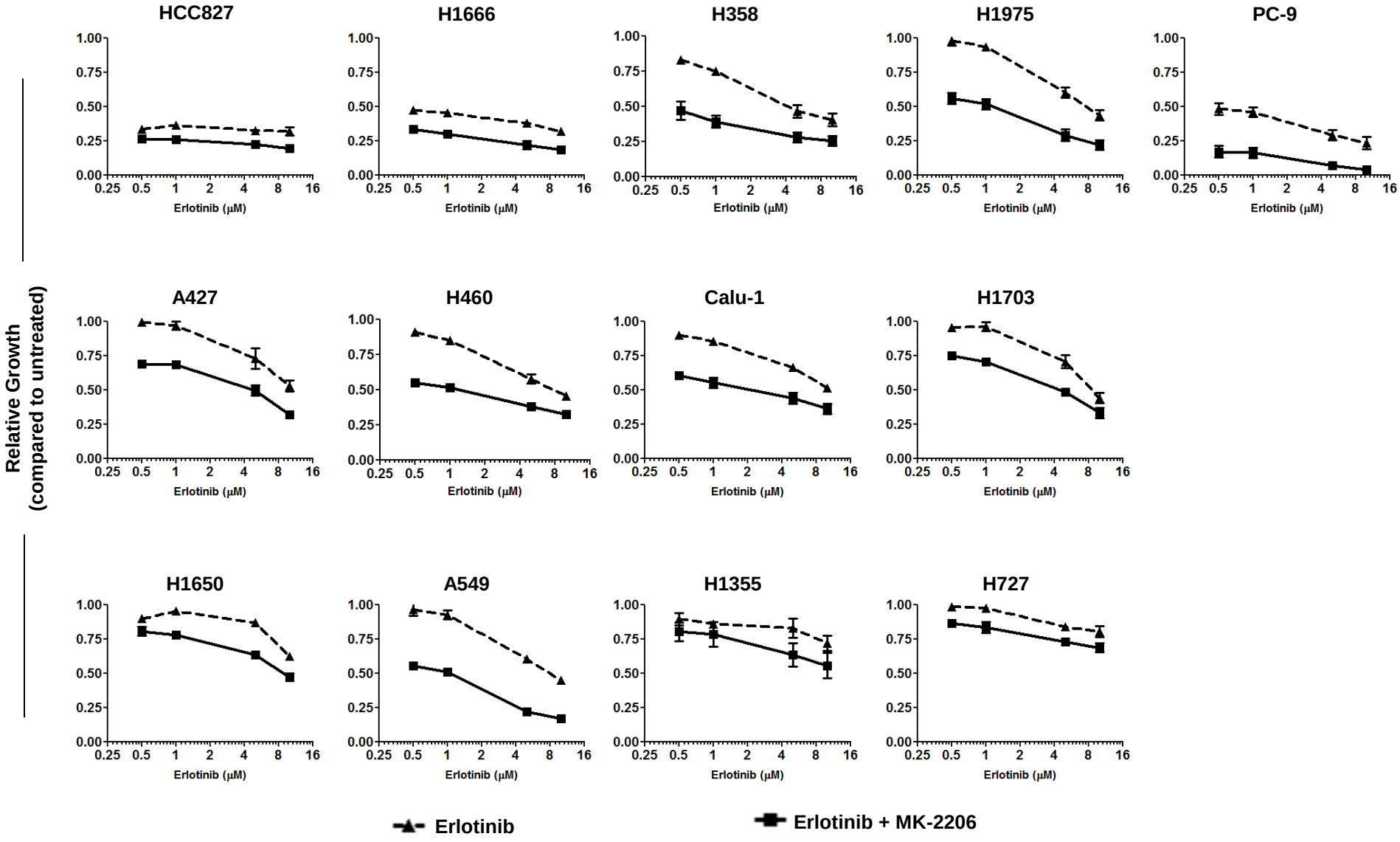
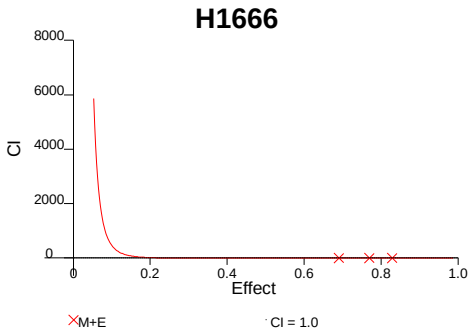
Online Resource 1 Growth curves of combination MK-2206 (fixed dose of 0.5 μM) and erlotinib (increasing doses) in NSCLC cell lines. Cells were treated for 72hrs prior to MTT.

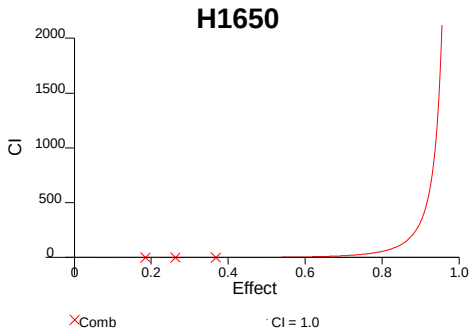


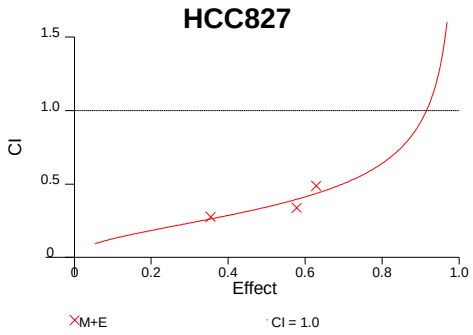
Online Resource 2 Fa-CI plot of combination index (CI) of erlotinib plus MK-2206 for NSCLC cell lines by mutation type. Three combination effects were modeled using 1:1 fixed doses of both agents at 0.5, 2.5, and 5.0 μM concentrations. Graphs show CI values plotted on the y-axis versus the combination effect (e.g. ED_{50}) on the x-axis. The dotted line indicates a CI value of 1. CI values plotted >1 show drug antagonism, =1 show drug additivity and <1 show drug synergy. The three plotted asterisks indicate the experimentally derived effect observed following combination treatment. *Dose effects for H1650 and H1355 do no reach an ED_{50} for either single agent treatments or the combination.

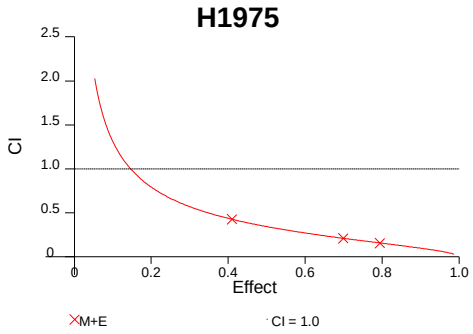
EGFR / KRAS wt



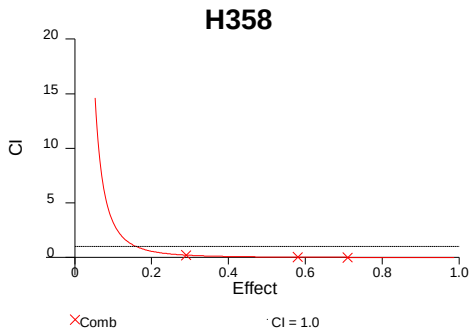
EGFR Mutant

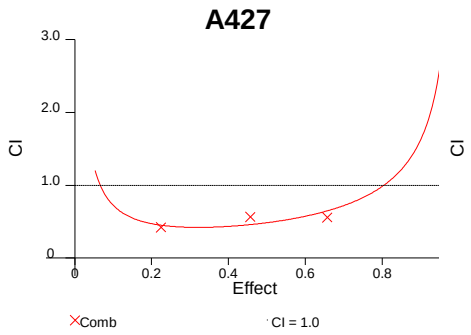


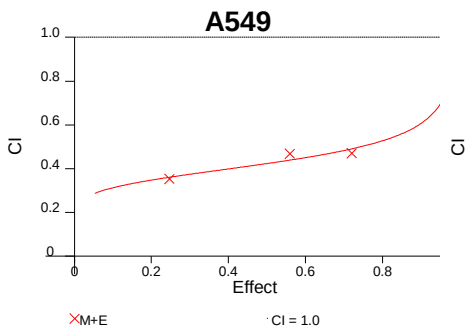


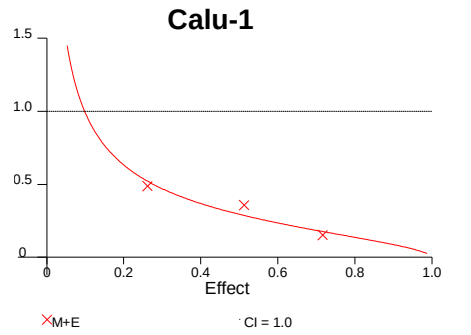


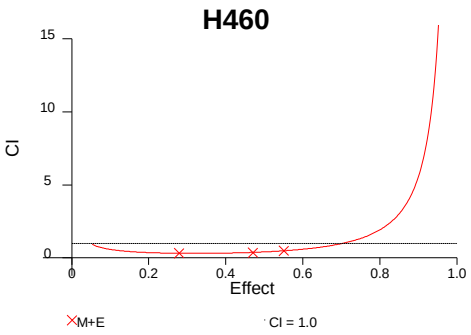
KRAS Mutant

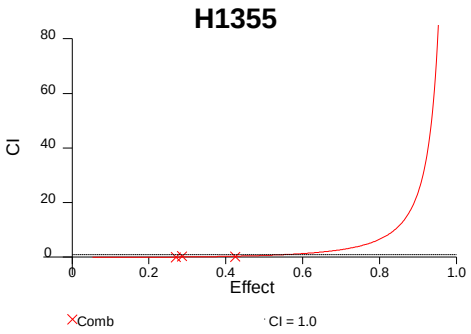




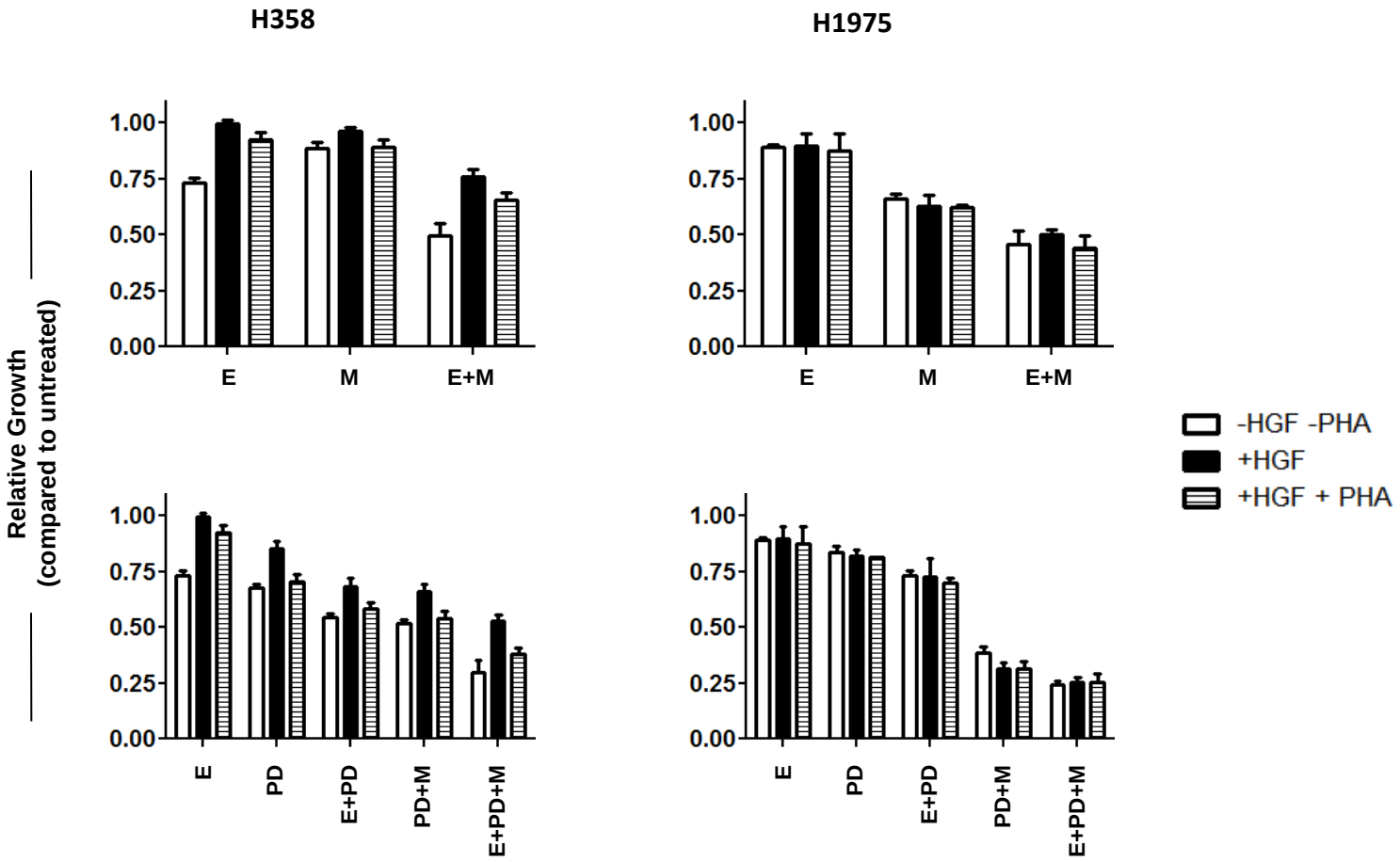






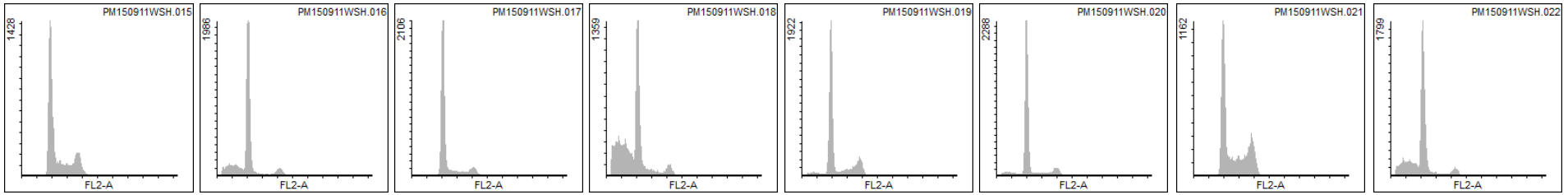


Online Resource 3 Growth response of H1975 and H358 cell line models following 72 hrs of treatment with a higher dose of erlotinib (E) at 2.5 μM MK-2206 (M) at 0.5 μM, HGF at 50 ng/μl, and/or PHA-665752 (PHA) at 0.5 μM using the CellTiter-Fluor Cell Viability Assay. Data is graphed as % growth relative to untreated cells. A (+) indicates addition of HGF or PHA while a (-) indicates it is not included

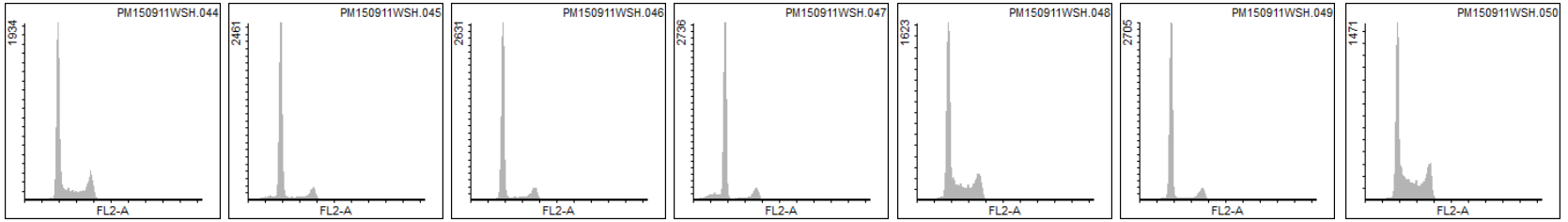


Online Resource 4 Cell cycling effects of MK-2206 on erlotinib treated cells with and without HGF. All cells were treated for 24 hrs with a vehicle control (<0.1% DMSO), erlotinib (0.05 μM for HCC827, 0.5 μM for H1666, and 2.5 μM for H1975 and H358), MK-2206 (0.5 μM), HGF (0.05 μg/ml) and/or PHA665752 (0.5 μM) as indicated. Following treatment, cells were stained with propidium iodide and analyzed by flow cytometry. All treatments were repeated in triplicate.

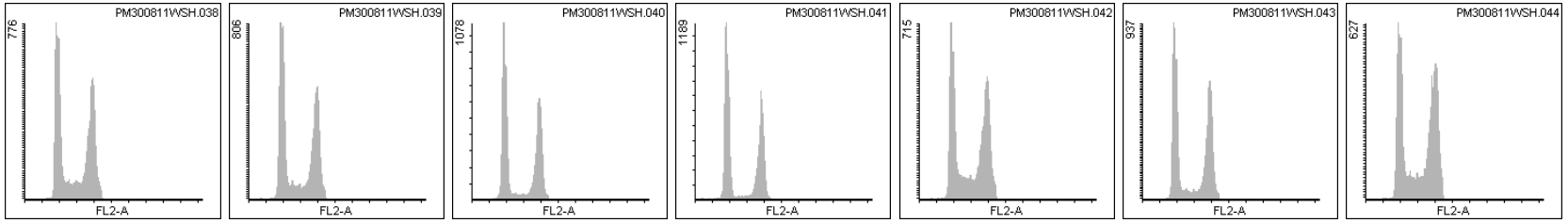
HCC827



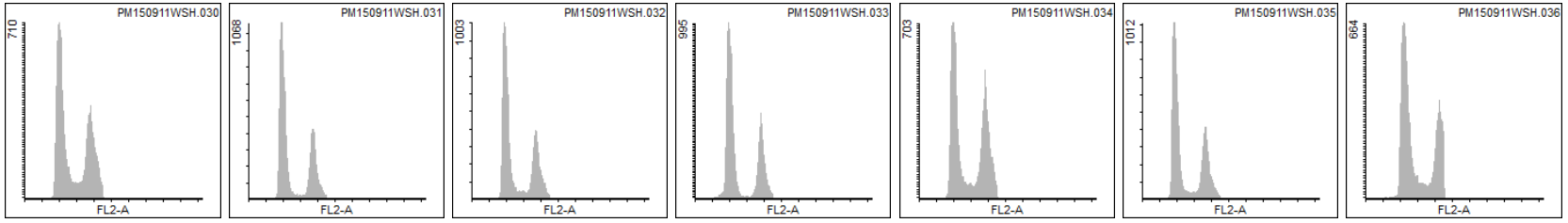
H358



H1975

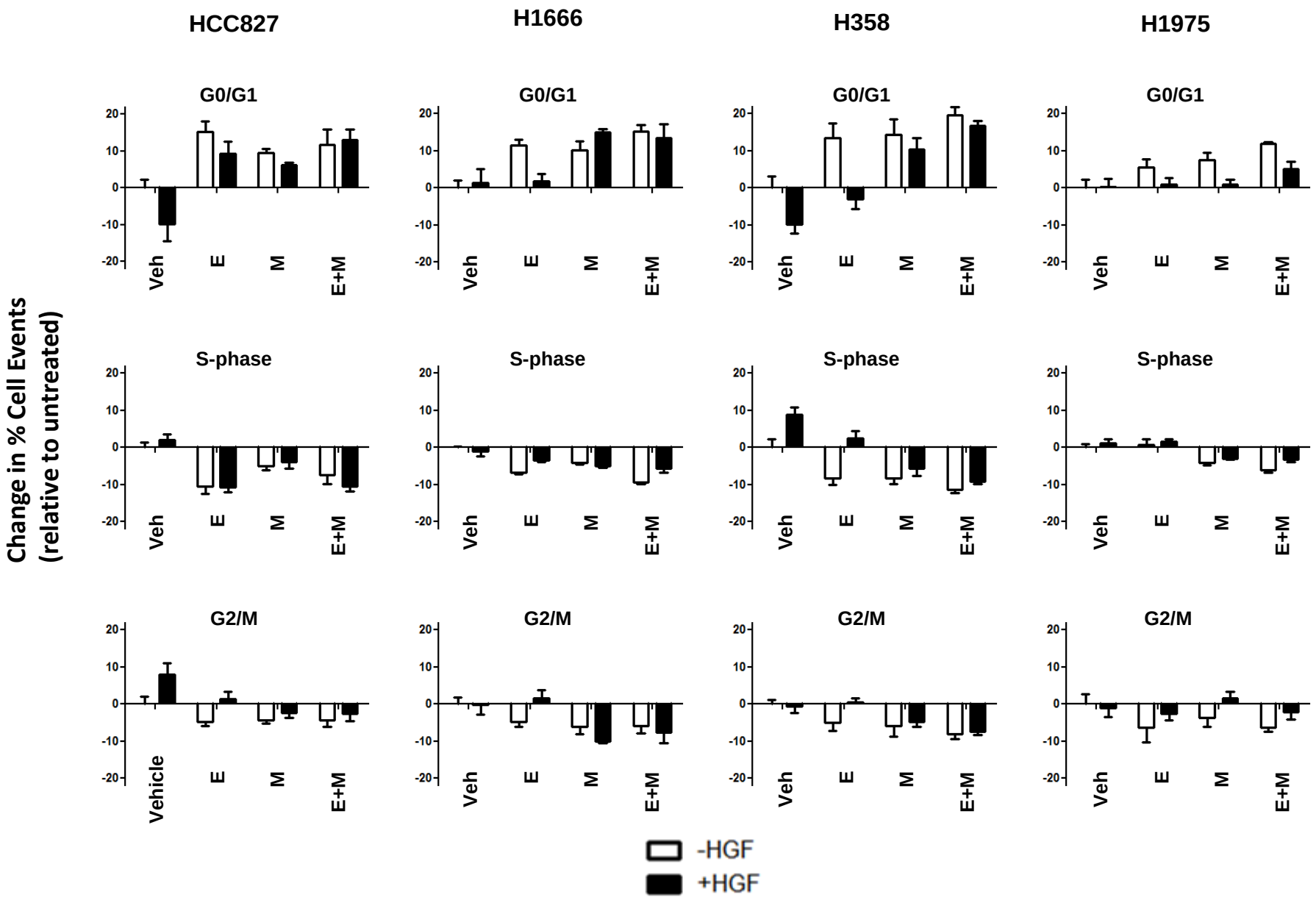


H1666



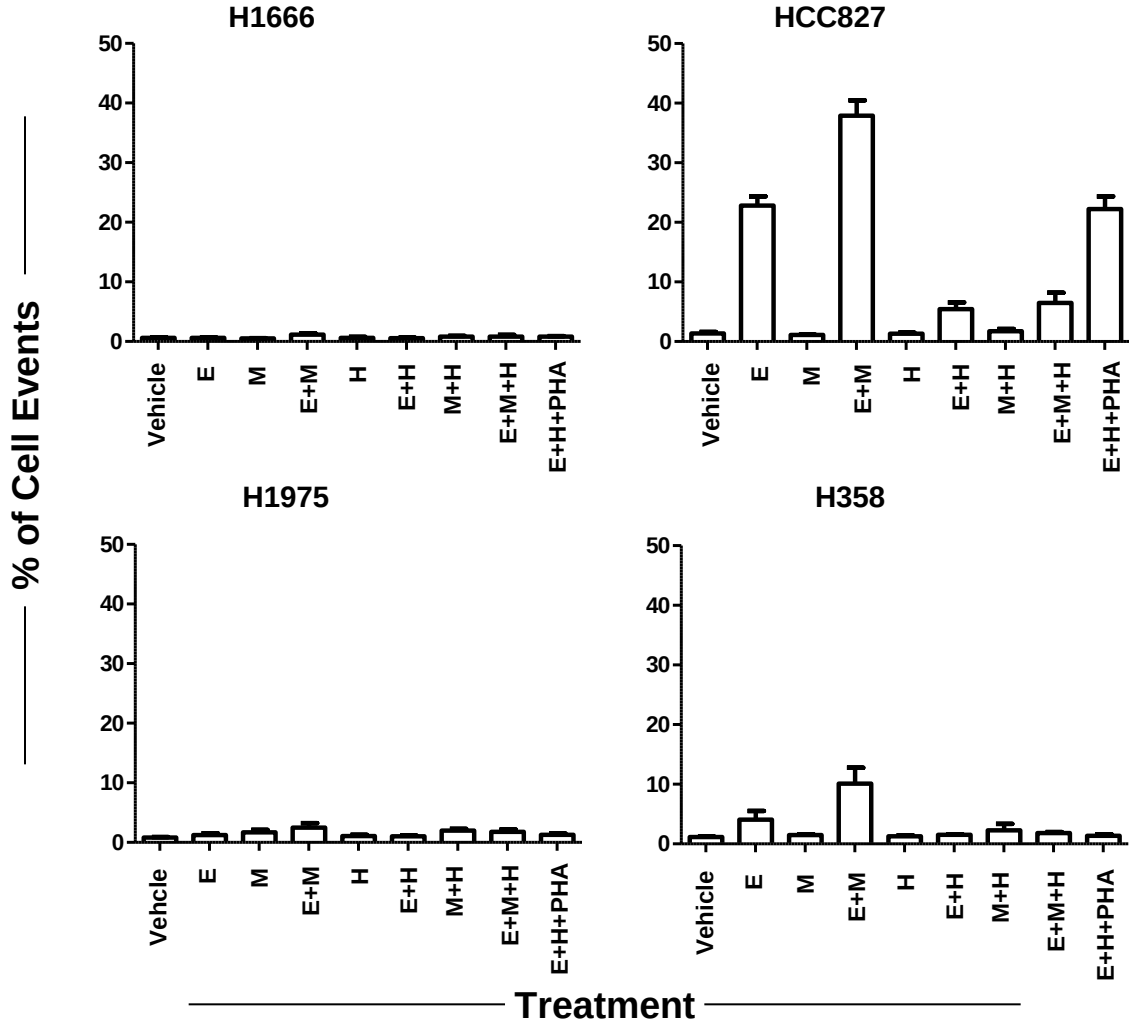
Vehicle | **Erlotinib** | **MK-2206** | **MK-2206 + Erlotinib** | **Erlotinib + HGF** | **MK-2206 + Erlotinib + HGF** | **HGF** | **Erlotinib + HGF + PHA665752**

Online Resource 5 Change in cell cycle distribution of MK-2206 on erlotinib-treated cells with and without HGF. All cells were treated for 24 hrs with vehicle control (Veh) (<0.1% DMSO), erlotinib (E) (0.05 μM for HCC827, 0.5 μM for H1666, and 2.5 μM for H1975 and H358), MK-2206 (M) (0.5 μM), and HGF (0.05 μg/ml) as indicated. Following treatment, cells were stained with propidium iodide and analyzed by flow cytometry. Each treatment represents data collected from three independent experiments.



Online Resource 6 Sub-G1 fractionation of erlotinib (E) plus MK-2206 (M) in NSCLC cell lines with or without HGF (H) or PHA665752 (PHA). All cells were treated for 24 hrs with a vehicle control (<0.1% DMSO), E (0.05 μM for HCC827, 0.5 μM for H1666, and 2.5 μM for H1975 and H358), M (0.5 μM), HGF (0.05 μg/ml) and/or PHA (0.5 μM) as indicated.

A



Online Resource 7 Immunoblotting analysis of phospho- and total MET in HCC827 following 24 hrs of treatment with erlotinib (0.05 μM), MK-2206 (0.5 μM), PD0325901 (0.5 μM) as single agents and in combination in NSCLC cell lines with or without HGF (0.05 μg/ml) and PHA665752 (0.5 μM).