SUPPLEMENTARY FIGURE LEGENDS

Supplementary Table 1. Mutational status of twenty-five NSCLC cell lines.

KRAS mutational status of the cell lines in the panel used in this study together with additional genetic mutation information derived from the Cancer Genome Project (http://www.sanger.ac.uk/genetics/CGP/CellLines/). MUT: mutant, WT: wild-type and nd: not determined.

Figure S1. Single agent drug effects in twenty-five NSCLC cell lines.

(A) Selected drugs that show a statistically significant reduction of cell viability in *KRAS* mutant cells versus wild-type cells. Panels show curves representing average values for each *KRAS* genotype (mean ± SEM).

(B) Selected drugs that show no differential effect when comparing reduction of cell viability in *KRAS* mutant cells versus wild-type cells. Panels show curves representing average values for each *KRAS* genotype (mean ± SEM).

(C) Heat-map displaying hierarchical cluster analysis of relative drug sensitivities across the cell panel. Clustering of RAF, MEK and IGF1R inhibitors is highlighted.

Figure S2. Effects of MEK inhibitors on signal transduction pathways in NSCLC cell lines.

KRAS mutant and *KRAS* wild-type cells were treated for 4 h or 24 h with 100 nM PD-0325901 and cell lysates were probed with the indicated antibodies.

Figure S3. Effects of IGF1R inhibitors on signal transduction pathways in NSCLC cell lines.

(A) *KRAS* mutant and *KRAS* wild-type cells were treated for 4 h or 24 h with 1 μM NVP-AEW541 and cell lysates were probed with the indicated antibodies.

(B) Levels of phospho-/total AKT in six *KRAS* mutant and six *KRAS* wild-type NSCLC cell lines treated with an AKT inhibitor (5 μM Akti1/2) or a PI3K

inhibitor (1 μM GDC0941) for 4 h. Data was normalized to vehicle-treated cells.

Figure S4. Effects of combining MEK and IGF1R inhibitors on signal transduction pathways in NSCLC cell lines.

KRAS mutant and *KRAS* wild-type cells were treated for 4 h with either 1 μ M NVP-AEW541, 10nM PD-0325901, or both together, and cell lysates were probed with the indicated antibodies.

Figure S5. Effects of combining MEK and IGF1R inhibitors on cell viability and apoptosis in NSCLC cell lines.

(A-B) Representative cell lines of each *KRAS* genotype treated for 72 h with serial dilutions of IGF1R inhibitor NVP-AEW541 together with a low dose of MEK inhibitor (5 nM or 20nM PD-0325901). Relative cell viability (A) and apoptosis (B) were measured. The Chou-Talalay Combination Index (CI) measure, with values less than 1 indicating drug synergy (1), was calculated for all three *KRAS* mutant cell lines using a fixed dose ratio of the two drugs. Insufficient response of the *KRAS* wild-type cells produces no CI value (n.d.).

Figure S6. Drug combinations across the NSCLC cell line panel.

(A) IC_{60} values of NCLSC cell lines treated for 72 h with serial dilutions of IGF1R inhibitor NVP-AEW541 together with a low dose of MEK inhibitor (5 nM PD-0325901).

(B) *KRAS* mutant and *KRAS* wild-type NSCLC cells were treated for 72 h with serial dilutions of IGF1R inhibitor NVP-AEW541 together with a low dose of MEK inhibitor (1 nM Trametinib). Curves represent average values for each *KRAS* genotype (mean ± SEM).

(C-D) *KRAS* mutant and *KRAS* wild-type NSCLC cells were treated for 72 h with serial dilutions of IGF1R inhibitor OSI-906 together with low doses of MEK (C) or RAF (D) inhibitor (5 nM PD-0325901 or 100 nM AZ628). Curves represent average values for each *KRAS* genotype (mean ± SEM). Right-hand panel of S6C shows single data-points representing viability of individual

cell lines at two representative doses of IGF1R inhibitor in the presence or absence of MEK inhibitor PD-0325901.

(E) IC_{60} values of NCLSC cell lines treated for 72 h with serial dilutions of IGF1R inhibitor OSI-906 together with low a dose of MEK inhibitor (5 nM PD-0325901).

(F) *KRAS* mutant and *KRAS* wild-type NSCLC cells were treated for 72 h with serial dilutions of IGF1R inhibitor OSI-906 together with a low dose of MEK inhibitor (1 nM Trametinib). Curves represent average values for each *KRAS* genotype (mean ± SEM).

(G) Six *KRAS* mutant (MUT) and six *KRAS* wild-type (WT) NSCLC cell lines were treated with IGF1R and/or MEK inhibitors for 12 days. Viability was measured by crystal violet staining.

(H) IC_{60} values of NCLSC cell lines treated for 72 h with serial dilutions of PI3K inhibitor GDC0941 together with a low dose of MEK inhibitor (5 nM PD-0325901).

(I) *KRAS* mutant and *KRAS* wild-type NSCLC cells were treated for 72 h with serial dilutions of PI3K inhibitor GDC0941 together with a low dose of MEK inhibitor (90 nM Selumetinib). Curves represent average values for each *KRAS* genotype (mean ± SEM).

Figure S7. Indicators of IGF1R or INSR depletion in NSCLC cell lines and of combination drug treatment in *Kras*$^{LA2\text{-}G12D}$ mice.

(A) Six *KRAS* mutant and four *KRAS* wild-type NSCLC cell lines were transfected with IGF1R, INSR or control siRNAs for 48 h and cell lysates were probed with the indicated antibodies.

(B) Individual lung tumors from *Kras*$^{LA2\text{-}G12D/+}$mice, treated with either vehicle or the MEK inhibitor/IGF1R inhibitor combination for 40 d, were isolated at the conclusion of the experiment and cell lysates were probed with the indicated antibodies.

Figure S8. Indicators of IGF1R pathway activity in NSCLC cell lines.

(A) Six *KRAS* mutant and six *KRAS* wild-type NSCLC cell lines growing at steady-state were deprived of serum for 24 h and then stimulated with either 20 ng/ml IGF1 or 20 ng/ml EGF for 30 min. 10% indicates steady-state cells (10% FBS) for comparison. Cell lysates were probed with the indicated antibodies.

(B) Six *KRAS* mutant and six *KRAS* wild-type NSCLC cell lines growing at steady-state were treated for 4 h with either 1 μM NVP-AEW541 or 1 μM erlotinib and cell lysates were probed with the indicated antibodies.

(C) Cell lysates from six *KRAS* mutant and six *KRAS* wild-type cells growing at steady-state were probed with the indicated antibodies in order to compare protein expression levels between the two genotypes.

(D) Oncomine (https://www.oncomine.com/resource/) was used to extract gene expression data from publically available datasets (2, 3). Data derived from lung cancer cell lines was analysed for expression of IRS1 by comparing *KRAS* mutant versus *KRAS* wild-type cells following exclusion of cell lines carrying *EGFR* mutations. Panels show single data-points representing relative IRS1 expression in individual cell lines as measured on probe set 204686_at.

(E) Oncomine (https://www.oncomine.com/resource/) was used to extract gene expression data from publically available datasets (2-4). Data derived from lung cancer cell lines and lung adenocarcinoma tumor samples was analysed for expression of IGF1R, IRS1 and IRS2 by comparing *KRAS* mutant versus *KRAS* wild-type cells following exclusion of cell lines carrying *EGFR* mutations. p values derived from these comparisons across different probe sets are displayed.

Figure S9. KRAS is required for both MEK/ERK and PI3K/AKT signaling in *KRAS* mutant NSCLC cells.

(A) *KRAS* mutant and *KRAS* wild-type cell lines were transfected with KRAS, KRAS-OTP or control siRNAs for 48 h and cell lysates were probed with the indicated antibodies.

(B) *KRAS* mutant cell lines were transfected with KRAS or control siRNAs for 48 h. 24h after transfection cells were treated with either DMSO or 100 nM rapamycin. Cell lysates were probed with the indicated antibodies.

Figure S10. Acute oncogenic RAS signaling in MCF10A epithelial cells.
(A) MCF10A/ER:HRAS V12 cells were deprived of growth factors for 24 h and treated with vehicle or 100 nM 4-OHT for 0, 15, 30, 60, 120 or 240 min. Cell lysates were probed with the indicated antibodies.
(B) MCF10A/ER:KRAS V12 cells were deprived of growth factors for 24 h and treated with vehicle or 250 nM 4-OHT for 4 h following a 20 min pre-treatment with either DMSO (Ctrl), 1 μM NVP-AEW541, 1 μM OSI-906 or 5 nM PD-0325901. Cell lysates were probed with the indicated antibodies.

SUPPLEMENTARY REFERENCES

1. Chou TC, Talalay P. Quantitative analysis of dose-effect relationships: the combined effects of multiple drugs or enzyme inhibitors. Advances in enzyme regulation 1984; 22: 27-55.
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