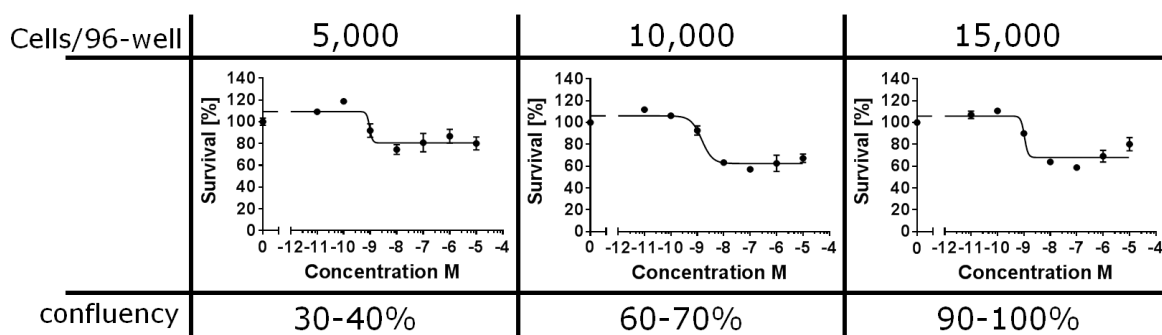


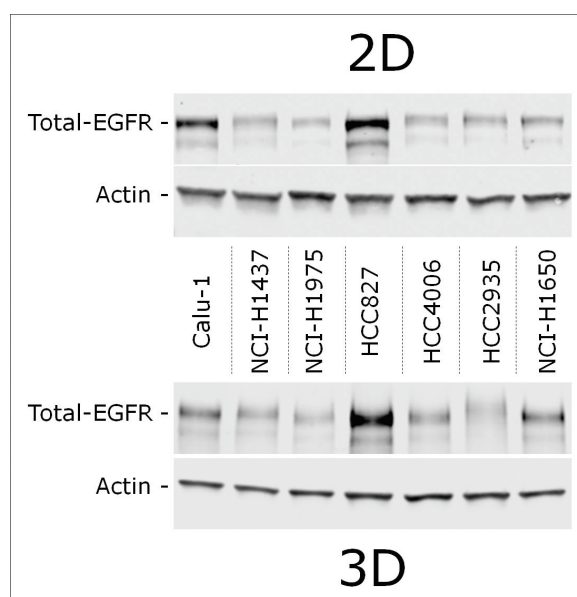
## Organotypic three-dimensional cancer cell cultures mirror drug responses *in vivo*: lessons learned from the inhibition of EGFR signaling

### SUPPLEMENTARY MATERIALS

#### Gefitinib mediated cytotoxicity at different cell densities

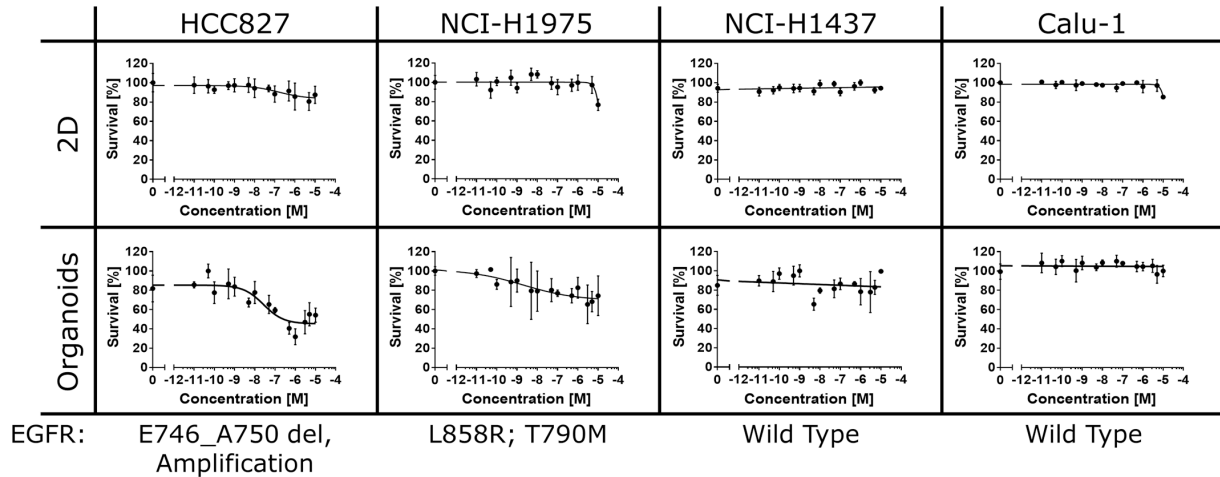


**Supplementary Figure 1: 2D cultures of HCC827 cells are refractory to gefitinib treatment.** HCC827 cells were plated at different cell densities in 96-well plates and treated with varying concentrations of gefitinib for 72 hours. Cell viability, relative to untreated controls, was measured after 72 hours using alamarBlue<sup>®</sup> assay according to manufacturer's instructions for 6 hours at 37°C. Each data point represents the mean  $\pm$  s.d. of at least four independent experiments.



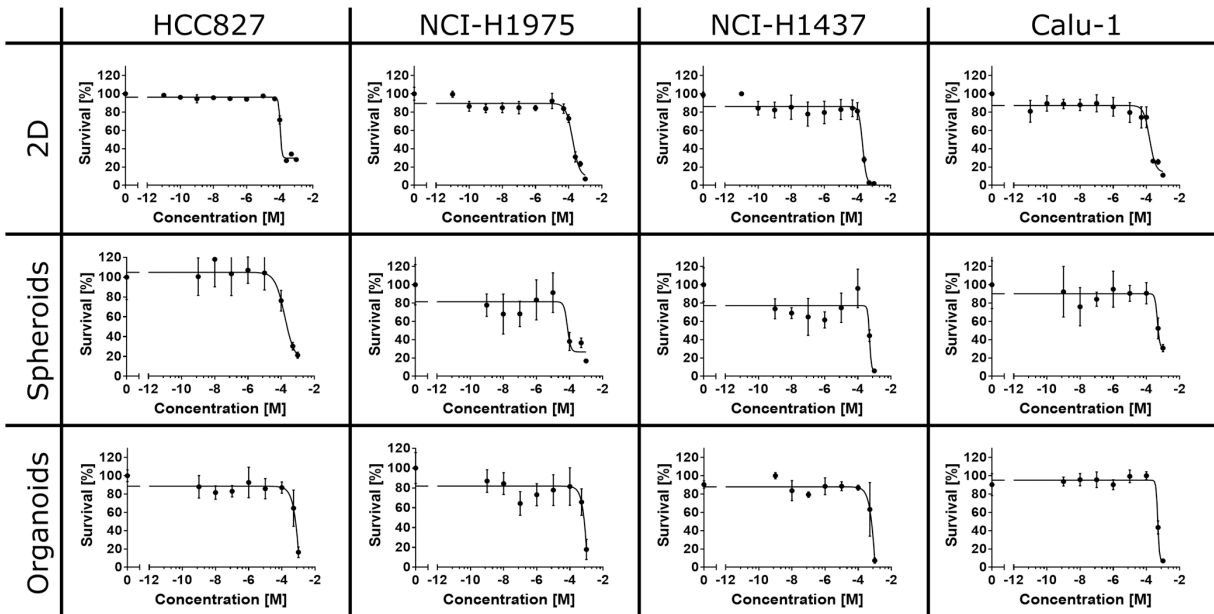
**Supplementary Figure 2: EGFR protein levels in NSCLC cell lines.** Note that EGFR protein levels cannot be compared between the 2D and 3D cultures. Acquisition parameters were optimized for each blot individually.

## Trametinib

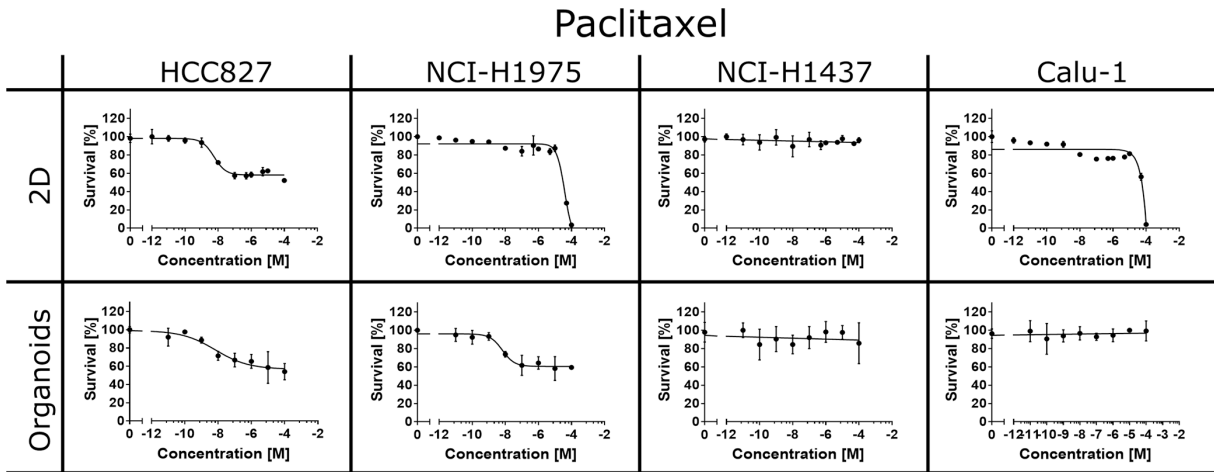


**Supplementary Figure 3: The MEK inhibitor trametinib decreased cell viability in HCC827 cells cultured in 3D.** HCC827, NCI-H1975, NCI-H1437 and Calu-1 cells were treated with different concentrations of the MEK inhibitor trametinib for 72 hours. Cell viability, relative to untreated controls, was measured using the alamarBlue® assay according to manufacturer's instructions for 6 hours at 37°C. Each data point represents the mean ± s.d. of at least four independent experiments.

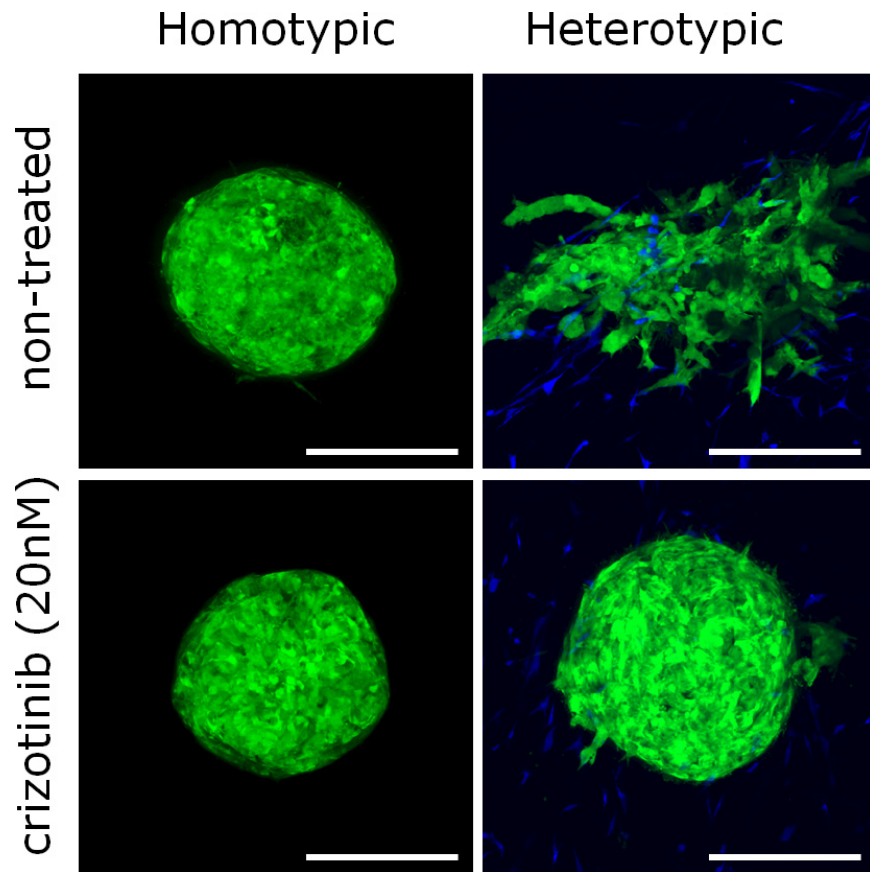
## Cisplatin



**Supplementary Figure 4: Efficacy of cisplatin in 2D and 3D cultures.** HCC827, NCI-H1975, NCI-H1437 and Calu-1 cells were treated with increasing concentrations of cisplatin for 72 hours. Cell viability, relative to untreated controls, was measured after 72 hours using alamarBlue® assay according to manufacturer's protocol for 6 hours at 37°C. Each data point represents the mean ± s.d. of at least four independent experiments.



**Supplementary Figure 5: Efficacy of paclitaxel in 2D and 3D cultures.** HCC827, NCI-H1975, NCI-H1437 and Calu-1 cells were treated with increasing concentrations of paclitaxel for 72 hours. Cell viability, relative to untreated controls, was measured after 72 hours using alamarBlue<sup>®</sup> assay according to manufacturer's protocol for 6 hours at 37°C. Each data point represents the mean  $\pm$  s.d. of at least four independent experiments.



**Supplementary Figure 6: Crizotinib inhibits cancer cell invasion in heterotypic cocultures.** Homo- and heterotypic organoid cultures of NCI-H1975 cancer cells (EGFP, green) and cancer-associated lung fibroblasts (mCFP, blue) were treated with the multikinase inhibitor crizotinib (20 nM) for 96 hours. Scale bars: 300  $\mu$ m.