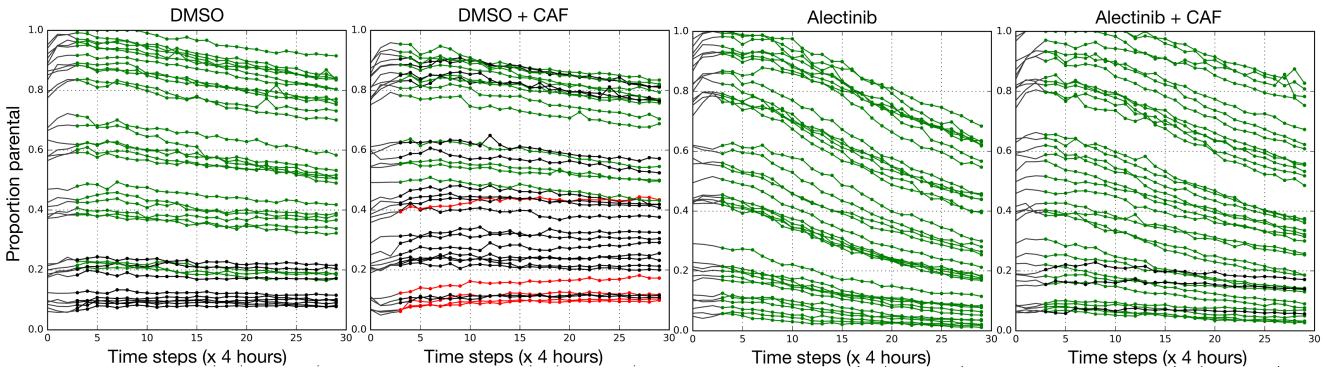


Supplementary Figure 5: Proportion of parental cells versus time for competition of parental vs. resistant NSCLC. Each line corresponds to the time dynamics of a separate well. A line is coloured green if proportion of resistant cells increased from start to end; red if proportion of parental cells increased; black if statistically indistinguishable proportions at start and end.

Methods and Materials

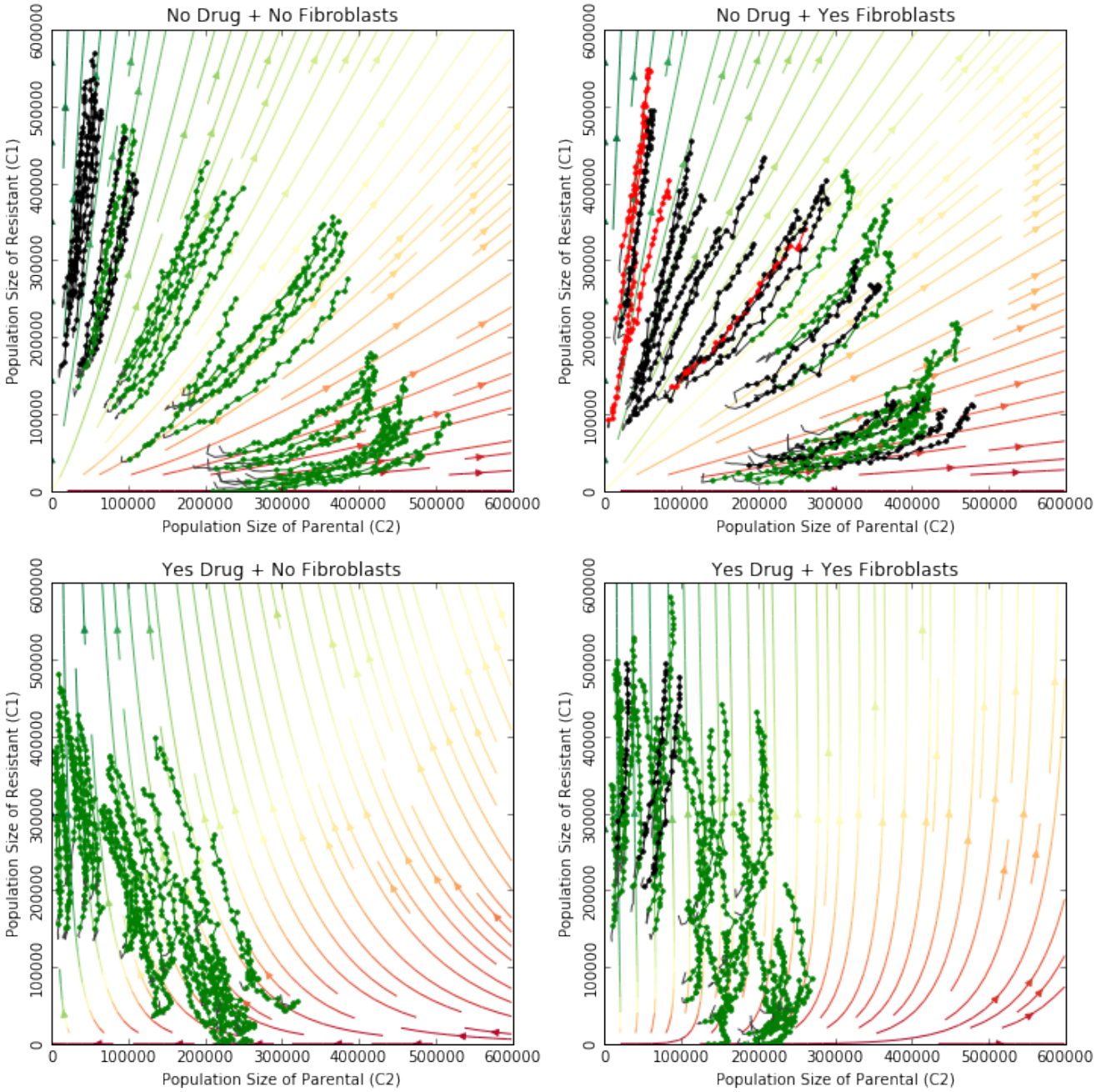
H3122 cell line was purchased from ATCC (Manassas, Virginia). Identity of cell lines was confirmed by short tandem repeats (STR) analysis, performed at Moffitt Cancer Center Molecular Genetics core facility. Primary lung cancer associated fibroblasts were obtained from S. Antonia lab (Moffitt Cancer Center). The cells were isolated as previously described (*31*) and expanded for 3-10 passages prior to the experiments. All human tissue was collected using protocols approved by the USF Institutional Review Board. Alectinib resistant derivative cell line was obtained through escalating inhibitor concentration protocol, as described in Dhawan *et al.* (*12*). Stable GFP and mCherry expressing derivative cell H3122 cell lines were obtained through lentiviral transduction with pLVX-AcGFP (Clontech) and mCherry (obtained from K. Mitsiades, DFCI) respectively. Both H3122 cells and CAFs were cultured in RPMI media (Gibco brand from Thermo Scientific), supplemented with 10% FBS, (purchased from Serum Source, Charlotte, NC). Regular tests for mycoplasma contamination were performed with MycoScope PCR based kit from GenLantis, San Diego, CA.

The cells were harvested upon reaching 70% confluence and counted using Countess II automatic cell counter (Invitrogen). For the determination of competitive growth rates, 2,000 H3122 cells were seeded with or without 500 CAF cells in 50 μL RPMI media per well into 384 well plates (Corning, catalogueue #7200655), with different proportions of differentially labelled parental and alectinib resistant variants. 20 hours after seeding, alectinib – that was purchased from ChemieTek (Indianapolis, IN) – or DMSO vehicle control, diluted in 20 μL RPMI were added to each well, to achieve final alectinib concentration of 500 nM/L (*13*). Time lapse microscopy measurements were performed every 4 hours in white light, as well as green and red fluorescent channels using Incucyte Zoom system from Essen Bioscience.

Units of size for populations was fluorescent area, measured from timelapse images via python code using the OpenCV package. We cleaned images by renormalizing them (GFP and mCherry intensities vary over different orders of magnitude), removing vignetting with CLAHE, and thresholding to identify fluorescent regions. We eliminate salt-and-pepper noise from the thresholded images with the opening morphological transform. The resultant area is then taken as measure of population size for the purposes of computing fitnesses. In order to minimise the impact of growth inhibition by confluency, we analyzed the competitive dynamics during the first 5 days of culture, when the cell population was expanding exponentially. We use growth rate as our measure of fitness. We learn growth rate along with a confidence interval from the time-series of population size in each well using the Theil-Sen estimator. The above is summarised in Figure 2.

Since raw population sizes have different units (GFP Fluorescent Area (GFA) vs mCherry Fluorescent Area (CFA)), we converted them to common cell-number units by learning the linear transform that scales GFA and CFA into cell-number. In Figure 3, to measure the fitness functions we plotted in fitness of each cell-type in each well vs seeding proportion (p) of parental cells – computed from the first time-point. We estimated the line of best-fit and error on parameters for this data using least-squares. The $p = 0$ and $p = 1$ intercepts of the fitness functions serve as the entries of the game matrices in Figure 4b. The game point are calculated from the matrices, and the error is propagated from the error estimates on fitness function's parameters.

Code and data are available on request.



Supplementary Figure 6: Dynamics of population sizes of resistant cells versus parental cells in four different conditions. Clockwise from top left: no alectinib, no fibroblasts; no alectinib but co-cultured with fibroblasts; in 0.5μM of alectinib, no fibroblasts; yes drug, yes fibroblasts. Foreground: raw data. Each line corresponds to the time dynamics of a separate well. A line is coloured green if proportion of resistant cells increased from start to end; red if proportion of parental cells increased; black if statistically indistinguishable proportions at start and end. Background: flow diagram for model from Figure 4a. Each coloured point shows the proportion of parental-resistant (red-green) at that time point. So if arrow goes from red to green then parental proportion increased, if from green to red then resistant proportion increased.