A
Supplementary Information

Mathematical Supplement

Non-cell autonomous tumor-growth driving supports sub-clonal heterogeneity

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Overview

In order to derive a mathematical framework our goal was to construct a hierarchy of growth models and then identify the model that best described the experimental data; this was done in three major steps. First, we determined the best-fitting growth law and estimated individual growth rates for the 18 isogenic lines, from experiments in which each single clone grew in competition with solely the parental clone. Second, we described a set of nested mathematical models of polyclonal tumors based on these growth rates. Depending on the complexity of each model, we calculated additional parameters that govern the influence of an individual clonal sub-population on the other clones, and hence on total tumor growth. The prime candidates of driver clones to be tested were IL11 and CCL5 (see main text, Fig. 2). In the simplest model, one interaction parameter was used. We independently measured two different quantities in each of the 12 *in vivo* tumor growth experiments used: (i) clonal growth in terms of frequency change, and (ii) tumor growth in terms of tumor size change. Thus, third, we used our mathematical models to predict tumor sizes for each independent polyclonal tumor growth experiments. We then compared predicted and observed final tumor sizes, using a concordance correlation coefficient (*CCC*)[1]. In addition to correlation, *CCC* respects how well two data sets fit the 45 degree line (which is the concordance part). Based on the *CCC*, we were able to make a quantitative statement about which model best described overall tumor growth.

In the following we discuss how we identified tumor growth patterns, calculated monoclonal growth rates, formulated a minimalistic mathematical modeling framework for tumor growth under non-cell autonomous interactions, and compared prediction accuracy of different instances of that model. Along with this, we discuss tumor density, calculate clonal diversity and describe how to estimate tumor cell numbers from diameter measures. In addition, we discuss conditions for extinction of a driver sub-clone and how clonal growth dynamics and diversity/heterogeneity changed under influence of a therapeutic agent.

Identification of tumor growth patterns

To investigate the dynamics of tumor growth, we compared several different options of describing the change of tumor size over time. These options were constrained by the structure of the data from tumor growth experiments. Two kinds of experiments were performed: (i) individual clones competing against a population of parental cells, and (ii) individual clones competing against one another within polyclonal tumors. The 18 different clones (compare with Extended Data Table 1) used in the experiments were assigned different indices, as shown in Table M1. There are several options of describing tumor growth mathematically, for

instance using an exponential or logistic growth law[2]. By comparison of the linear regression performances, we found that an exponential growth model provided the best fit to the data. As two alternatives to the exponential growth law, we investigated the Gompertzian growth law[3,4], and the classical Verhulst equation typically used in mathematical ecology[5,6]. To test the performance of an exponential growth model, we performed a linear regression (in R or Wolfram Mathematica) on the log-linear-transformed data; to test the Gompertz law, we performed a linear regression on log-transformed logarithmic differences in size over time (e.g. the logarithm of Log[N(t+1)]- Log[N(t)]), which typically smoothens the data significantly. To test the Verhulst equation, we performed a nonlinear regression. The P-values of a two-sided t-statistic were lowest for simple exponential growth (P<0.001). Exponential tumor growth is discussed also in the Extended Data Figure 3.

Based on the analyses outlined above, we described the tumor size dynamics by an exponential growth law over time *t*,

$$\text{tumor size} \sim exp^{\text{growth rate} \times t} \qquad (1)$$

This growth law is the simplest choice of deterministic population expansion. Then, for each point in time, the size of the entire tumor population is the sum of its subpopulations, which are assumed to each grow exponentially as well,

$$N_t = \sum_{j=1}^{18} n_j(t). \qquad (2)$$

The quantities N_t and $n_j(t)$ represent the total cell count in the tumor population and cell count of clone j, respectively, both at time *t*. The initial size of each clone is equal to the product of its initial frequency and the total size: $n_j(0) = x_j(0)N_0$.

For cell numbers *N*, volumes *V* and masses *m*, we assume identity of the ratios, $N_t/N_0 = V_t/V_0 = m_t/m_0$. We convinced ourselves that tumor density (mass per volume) did not correlate with time of extraction, and that volume and mass are in linear relation to each other (see Extended Data Figure 3).

If N_0 is the total initial size and $x_j(0)$ are the initial clonal frequencies, then the fold-change in size follows

$$\frac{N_t}{N_0} = \sum_{j=1}^{18} x_j(0)\, R_j(t). \qquad (3)$$

The function $R_j(t)$ describes the growth function of clone j in polyclonal tumors, which might be different from the growth rate observed when that same clone grows in a parental background. We thus aimed to model the context-dependent growth of individual clones using this function $R_j(t)$. Equation (3) states that the growth of the polyclonal population, measured in fold-change, is the sum of individual clonal expansions, where the functions $R_j(t)$ may account for clonal interactions. The resulting mathematical model can be used to predict the size of the total tumor population from the growth dynamics of individual clones.

Different mathematical model assumptions about the context-dependent growth dynamics lead to different predictions. These different model predictions can be compared in terms of predictive power using a concordance correlation coefficient, comparing a set of predictions and a set of measurements. With each variant of a tumor growth model we predicted a set of tumor sizes $\widehat{N}^{(j)}$, where the superscript index represents a particular experiment. Predictions could then be compared to the set of observed size measurements, which

were independently performed on the same tumors and are denoted by $N^{(j)}$. With respective averages and variances of tumor size predictions and measurements given by $\hat{\mu}$, μ, and $\hat{s}^2, s^2$, the concordance correlation coefficient for k experiments [1] can be written as

$$CCC_{Model\ X} = \frac{2}{k}\frac{\sum_{j=1}^{k}\left(N_t{}^{(j)} - \mu\right)\left(\hat{N}_t{}^{(j)} - \hat{\mu}\right)}{s^2 + \hat{s}^2 + (\mu - \hat{\mu})^2} \qquad (4)$$

Such a statistical measure allowed us to assess and compare the performance of individual models in terms of their predictive accuracy, where different models are characterized by different functions R_j. The value of *CCC* always lies between -1 (perfect anti-correlation and no concordance) and 1 (perfect correlation and full concordance).

Individual clonal growth against parental background (monoclonal experiments)

We first analyzed experimental data of each of the 18 individual clones growing against parental cells at a frequency of 1/18 (clone i) versus 17/18 (parental cells) (see Table M1). These 18 different clone-vs-parental experiments were performed starting with a fixed size of 10^6 cells. For each independent experiment, after varying times, tumor volume and mass were recorded and the frequency of the clone was detected. The growth rate of each clonal line was determined in the following way. If the time from first to last measurement is denoted as T, initial total tumor size and clonal frequency are given by $x_i(0)$ and N_0, and the final size and frequency are given by N_T, $x_i(T)$, we can estimate the growth rate of clone *i* as

$$r_i = \frac{\ln\left[N_{T_0}\, x_i(T_0)\right] - \ln\left[N_T\, x_i(T)\right]}{T - T_0}. \qquad (5)$$

All average growth rates are summarized in Table M1. For N_0, a standard measured value of 10^6 cell was used. The first volume measurements were taken several (4-12) days after tumor transplantation. Hence, when volumes were chosen to estimate r_i, we re-set T_0 to 4-12 days (depending on the individual experiment) and assumed that frequencies had not changed significantly during initiation. For initial tumor sizes used in this calculation see Table M2.

Polyclonal experiments

Twelve polyclonal tumor growth experiments were performed by initializing each tumor with equal frequencies of all 18 clones, i.e. with initial frequency of each clone of 1/18 = 5.56%. All tumors were initiated with 10^6 cells, i.e. containing about 55,500 cells of each clone. Examples for initial tumor masses and volumes of polyclonal tumors are provided in Table M2. For each of the 12 independent experiments of polyclonal tumors, two measurements were taken at the final time point: (i) size (diameter/volume, and weight) and (ii) the 18 clonal frequencies (qPCR, see Figure 2 and Extended Data Figure 5). From the frequency data, the individual expansions of each clonal sub-population were estimated by maximizing the concordance correlation coefficient between an array of predicted sizes of clonal sub-populations and the actually measured clonal sizes. One could also minimize a mean squared error varying the interaction parameters of the model. IN the following we describe a hierarchy of nested growth models.

Using a given mathematical model, the change in total tumor size was predicted, which then allowed comparison of different models. The simplest model assumes linear and independent clonal growth in polyclonal tumors, i.e., $\dot{n}_i = n_i\, r_i$, This leads to the prediction *(Model 0*):

$$\frac{\widehat{N}_t}{N_0} = \sum_{j=1}^{18} x_j(0)\, e^{r_i\, t}. \qquad (6)$$

Here, a mixed tumor would only be influenced by the independent growth of its clones as observed in the respective clone-vs-parental context. Calculating the concordance correlation coefficient between tumor size prediction and measurement, Eq. (4), for *Model 0* gave a value of 0.019, corresponding to very weak positive correlation and concordance between predicted and measured total tumor sizes across the 12 polyclonal experiments (see Table M3).

Context-dependent clonal growth

We next extended our mathematical framework to include a growth effect on polyclonal tumors induced by IL11, quantified by a single additional parameter ρ. The clonal growth law was thus extended to

$$\dot{n}_i = n_i\, r_i + \rho\, f(n_1), \qquad (7)$$

where f(n_1) represents a function of the frequency of the IL11 clone. We considered two distinct choices for this function $f(n_1)$. First, $f_A(n_1) = \theta[n_1 - \nu_{IL11}]$ (*Model A*), where $\theta[n_1 - \nu_{IL11}]$ is one if the frequency of the IL11 clone is above a threshold ν_{IL11}, and zero if IL11 is not present. Second, we used the linear form $f_B(n_1) = n_1$ (*Model B*). These choices were made to design a linear extension of *Model* 0, either by a constant addition in growth only modulated by the existence of IL11 (*Model A*), independent of its frequency, or by assuming that the growth advantage is proportional to the amount of IL11 present in the tumor population and distributed onto the beneficiary clone (*Model B*). In particular, the system of equations

$$\dot{n}_1 = n_1\, r_1, \dot{n}_i = n_i\, r_i + \rho\, \theta[n_1 - \nu_{IL11}] \qquad (8)$$

for $i = 2,3, \ldots, 18$, leads to the clonal growth laws of *Model A:*

$$n_1(t) = n_1(0) e^{r_1\, t} \qquad (9)$$

for the driving (IL11, or CCL5) clone, and

$$n_i(t) = n_i(0) e^{r_i\, t} + \rho\, e^{r_i t} \frac{1 - e^{-r_i t}}{r_i} \qquad (10)$$

for all other clones. In addition we chose to describe non-cell autonomously driven clonal expansions by the set of differential equations

$$\dot{n}_1 = n_1\, r_1, \dot{n}_i = n_i\, r_i + \rho\, n_1. \qquad (11)$$

The effect tuned by the parameter ρ is also proportional to the number of IL11 cells and not only to their presence above threshold, i.e. the clonal dynamics of *Model B* are governed by the following equations:

$$n_1(t) = n_1(0) e^{r_1\, t} \qquad (12)$$

$$n_i(t) = n_i(0)e^{r_i t} + \rho\, n_1(0)e^{r_1 t}\, \frac{e^{(r_i - r_1)t} - 1}{r_i - r_1} \qquad (13)$$

Note that the growth rate-enhancing factor is assumed to be the same across all clones. This is a deliberate choice of minimal complexity, constrained by the data.

In the next step, we designed a more complex model in order to evaluate whether in our framework, the influence of IL11 on tumor growth would be sufficient to describe the observed tumor sizes, or whether additional clones needed to be considered. In this more complex model, a second cell type affects growth according to the following system of differential equations:

$$\dot{n}_1 = n_1\, r_1 + \sigma\, n_2, \dot{n}_\mathrm{k} = n_\mathrm{k}\, r_\mathrm{k} + \rho\, n_{1,i}\, \dot{n} = n_i\, r_i + \rho\, n_1 + \sigma\, n_2 \qquad (14)$$

where $i = 2,3,\ldots,k-1,k+1,\ldots,18$. This assumption quickly led to a solution of complicated form, nonlinear in orders of ρ, σ, and $\rho\sigma$, but generally solvable using standard methods such as variation of parameters. We performed a further linearization, omitting terms of higher than linear order for small values of ρ and σ, which led to the following system of clonal growth equations, *Model C:*

$$n_1(t) \approx n_1(0)e^{r_1 t} + \sigma\, n_k(0)e^{r_k t}\, \frac{e^{(r_i - r_k)t} - 1}{r_i - r_k} \qquad (15)$$

$$n_k(t) \approx n_k(0)e^{r_k t} + \rho\, n_1(0)\, e^{r_1 t}\, \frac{e^{(r_i - r_1)t} - 1}{r_i - r_1} \qquad (16)$$

$$n_i(t) \approx n_i(0)e^{r_i t} + \rho\, n_1(0)e^{r_1 t}\, \frac{e^{(r_i - r_1)t} - 1}{r_i - r_1} + \sigma\, n_k(0)e^{r_k t}\, \frac{e^{(r_i - r_k)t} - 1}{r_i - r_k} \qquad (17)$$

Using this model, we sought to test whether including any other additional clone would lead to a significantly improved description of context-dependent growth of polyclonal tumors.

Comparing different models of clonal interdependence

A step-by-step increase in complexity outlined above led to a set of different tumor size predictions. These predictions were then evaluated in their fit to experimental data, using the *CCC,* Eq. (4), see Table M3. *Model 0* did not lead to a satisfying outcome in predictive power (*CCC*=0.02). The linear effect of a driver clone in *Models A* and *B* demonstrated a drastic improvement of the predictions (Table M3). In the case of CCL5 as the additional driver clone, including a second driver (*Model C*) did not improve the predictive power. In the case of IL11, however, the prediction improved when including CCL5 as the second driver of tumor growth.

In summary, our models are linear in all growth rate parameters after log-transformation. The search for the model parameters ρ was performed by maximizing the correlation between the predicted sizes of all 18 clones in the tumor within each of the 12 experiments. This approach yielded 12 different sets of parameters; in the case of *Models A* and *B,* we obtained 12 different ρ, while in the case of *Model C*, we obtained 12 different pairs of ρ and σ. These individual estimates show variability of the parameters across experiments. The choice of the drivers IL11 and CCL5 was motivated by the experimental observation that both were consistently associated with an increase in the tumor size without a significant increase in frequency. The 12 different sets of parameters were then used to predict a total tumor size for each model by summing up all individual

expansions of the 18 clonal sub-populations. This approach led to a set of size predictions per model, provided in Table M3.

Parameter variability across polyclonal experiments

We used the clonal frequency data in mixed experiments to optimize predicted clonal frequencies compared to measured clonal frequencies, as a function of the independent parameters of our model, i.e. as a function of ρ in the cases of *Models A* and *B*, or as a function of ρ *and* σ in the case of *Model C*. We decided to maximize the concordance correlation coefficient between 18 pairs of measured and predicted clonal frequencies, separately for each individual experiment. In this way, the model parameters exhibited variability across experiments. Within each experiment, this led to a prediction of total tumor size. A limitation of our linear modeling approach is that in principle, the mathematical framework can predict a negative tumor size. Negative predicted tumor size occurred in several experiments; these cases were omitted from further analysis of model comparison.

Measuring clonal heterogeneity: Shannon index

A classical function in ecological modeling that measures heterogeneity, or diversity, is Shannon index $h(t)$. This index quantifies the degree of diversity or "information content" in a sample, i.e. the uncertainty of picking a cell of a particular clone at random. If there are 18 different clones and their frequencies at time t are denoted by $x_i(t)$, Shannon's diversity index[7] is given by

$$h(t) = -\sum_{i=1}^{18} x_i(t) \ln [x_i(t)] \qquad (18)$$

Here, $\ln[x]$ is the natural logarithm of x. For *Model 0* (no effect of IL11) and *Model B* (linear effect of IL11), we provide a qualitative example for the temporal evolution of Shannon index using values of growth rates estimated from Tables M1 and M3, see Extended Data Figure 3. In both cases, clonal heterogeneity increases at first, reaching a maximum after 40 to 50 days. After further time of tumor growth, clonal heterogeneity decreases to zero in *Model 0*, but reaches a plateau in *Model B,* indicating a possible maintenance of clonal interference mediated by the presence of a single driver clone. Our mathematical model promotes stable clonal diversity that cannot be found without interaction of the sub-clones (main text, Figure 4).

Illustrative example discussed in the main text using estimated parameters and *Model B*

The polyclonal growth data, with only two time points, is rather simple in structure. Hence, the actual value of the additional growth effect as modeled by the parameter ρ might not be very informative. However, in the main text, Fig. 4, as well as Extended Data Fig. 3, we present an example to illustrate the effect of non-cell autonomous driving by IL11 on sub-clonal diversity. In this example, we use the average additional growth rate measured across polyclonal experiments, $\rho \approx 0.012/day$, as well as four values of linear autonomous growth, calculated using Equation (5), from the data presented in Figure 2. We modeled a hierarchy of four clones with monoclonal net growth rates of 0.07, 0.06, 0.05, and 0.03 per day, in a tumor of 10^6 cells initially. The lowest growing sub-clone was initially present with frequency 15/18. The other three sub-clones were initiated with

frequency 1/18, respectively. Growth was calculated according to *Model B*, Equations (12), (13). In the main text, Figure 4, we then compared how sub-clonal diversity, measured by Equation (18), changed over time, with ($\rho = 0.012/day$) or without ($\rho = 0.000/day$) non-cell autonomous driving. Note that diversity peaks in both cases, which is simply due to the initial condition. However, diversity was lost without non-cell autonomous growth support, but could be maintained otherwise. Extended Data Figure 3 shows the growth of each clone and the total tumor, as well as the respective frequency changes under non-cell autonomous driving to the effect described above.

Estimation of cell numbers

To estimate the number of cells in a given tumor volume, we measured cell densities in two dimensional tumor biopsies. Tumors also showed necrosis, which lead to estimates of the necrotic core area in percent of tumor volume. We first estimated the number of cells in a volume unit. We assumed homogeneous cell density in non-necrotic regions irrespective of the clonal sub-type. If there are m cells counted in an area of a tumor slice A, the linear cell density is $m^{\frac{1}{2}}$ per unit of length. Thus

$$V[l] \sim m^{\frac{3}{2}}[cells] \qquad (19)$$

gives the relation between a densely populated unit of tumor volume V and the number of cells in it.

Estimates of necrotic cores were also based on area percentages. Then, if the tumor had overall diameter D, and necrotic percentage x, the diameter of the necrotic core was estimated to be $d = D\sqrt{x}$. The actual volume containing non-necrotic tumor cells could be calculated as

$$\Delta V = \frac{\pi}{6} D^3 \left(1 - x^{\frac{3}{2}}\right) \qquad (20)$$

This is an approximation as we assumed spherical symmetry and confined necrosis. A sketch of how we estimated cell numbers in a tumor sample is given in the Extended Data Figure 3.

Non-cell autonomous driving by LOXL3 can drive IL11 below detection threshold

The context dependent tumor growth advantage provided by IL11 can lead to a significant decrease of IL11 cells in relative abundance when LOXL3-driven cells are present because the latter are a second strongly autonomous growing population. In the main text (Figure 4), we discuss how LOXL3, driven by a co-growing IL11 population, brought ILL11 below detection threshold. This effect depends on the initial condition. We can calculate the frequency of IL11 when co-growing with LOXL3 using Equations (12) and (13), as a function of its initial frequency $x_{IL11}(0)$,

$$x_{IL11}(t) = \frac{1}{1 + \frac{1 - x_{IL11}(0)}{x_{IL11}(0)}\, e^{r_{LoxL3} t} + \rho e^{r_{IL11} t}\, \frac{e^{(r_{LoxL3} - r_{IL11})t} - 1}{r_{LoxL3} - r_{IL11}}} \qquad (21)$$

Here, r_{LoxL3} and r_{IL11} are the growth rates according to Table M5. In two setups of 1:18 and 1:1 initial mixture of LOXL3 and IL11, the IL11 population was below detection threshold in the former ($x_{IL11}(0) = 1/18$), but not in the latter ($x_{IL11}(0) = 1/2$) scenario. In the first case, IL11 was driven to frequencies below 0.01. In the second

case IL11 was detectable at values between 0.02 and 0.1. Equation (21) can explain this observation under variability of the non-cell autonomous driving factor ρ, which has to be significantly larger in the LOXL3-IL11 cell mixture with $x_{IL11}(0) = 1/18$. The non-cell autonomous effect seemed to be optimized when IL11 cells are rare, but eventually lead to dominance of the beneficiary cell line.

Treatment dynamics

A different batch of tumor populations was used to estimate the change of clonal diversity under the influence of the therapeutic agent Doxorubicin. This treatment effectively slowed down the *in vivo* tumor growth dynamics. In addition, a significant reduction in tumor size variability, as well as clonal diversity measured by Shannon's index could be estimated. Exponential tumor growth was observed in a control and in a treatment cohort. Doxorubicin was given twice: between days 8 and 14, and between days 14 and 21 (see Extended Data Figure 7). Median tumor growth did not change significantly (signed rank test comparing control and treatment groups of polyclonal tumors, P-value 0.47). From the final frequencies of the 18 clones, Shannon indices were calculated. Treatment reduced the clonal diversity significantly (comparing Shannon index distributions of control and treatment with Doxorubicin using a two-sample Kolmogorov-Smirnov test, P-Value 0.03), see Table M6 and Extended Data Figure 7.

Supplementary Information: References

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Supplementary Information: Tables

Table M1: List of clones with estimated growth rates. Shown are monoclonal growth rates according to the exponential growth model (averages, taken over multiple experiments).

Clone	*Index j*	*Monoclonal growth rate (per day),* r_j
IL11	1	0.064
SPP1	2	0.040
VEGFC	3	0.033
HGF	4	-0.013
CCL5	5	0.129
VEGFB	6	0.006
FGF	7	0.016
VCAN	8	-0.001
SHH	9	0.018
VEGFA	10	0.028
CXCL14	11	0.020
LOXL3	12	0.060
ANGPTL12	13	0.033
LACZ	14	0.024
GFP	15	0.016
IHH	16	0.027
CXCL12	17	0.034
LOXL1	18	0.008

Table M2: Calculating mean and median for initial tumor size and mass (polyclonal experiments). All tumors in mixed (polyclonal) experiments were initiated with $\mathbf{10^6}$ cells, for which we assumed the here given initial tumor size distribution after 12 days.

Tumor mass (mg)	*Tumor volume (mm^3)*
42.84	14.14
1.59	0.52
42.84	14.14
62.36	20.60
62.36	20.60
74.03	24.43
31.23	10.31
47.27	15.60
Mean tumor mass (mg)	*Mean tumor volume (mm^3)*
45.56	15.04
Median tumor mass (mg)	*Median tumor volume (mm^3)*
45.05	14.87

Table M3: Predictive power of the different models according to the CCC-value comparing a set of predicted tumor sizes with a set of measured tumor sizes. *Model 0* describes independent growth, i.e. independent exponential clonal expansions according to the average growth rates given in Table M1. *Models A* and *B* describe tumor growth influenced by IL11 or CCL5, the former independent of density, the latter proportional to density of the driver. *Model C* is a linear extension of *Model B*, emerging by adding a second clone to provide an additional influence on tumor growth. Here, we use IL11 *and* CCL5. A limitation of our model hierarchy emerges: for some experiments, a tumor size prediction may turn out to be a negative number. These cases are not considered in the calculation of the *CCC*-value.

	Model 0	*Model A* (IL11)	*Model A* (CCL5)	*Model B* (IL11)	*Model B* (CCL5)	*Model C* (CCL5+IL11)
CCC	0.02	0.92	0.71	0.93	0.72	0.91

Table M4: Measurements of Cells per area from tumor slices. Cell counts were performed in in non-necrotic areas of tumor slices. Cells per volume (cubic cm) were calculated.

% necrotic core	*Cells per $10^4 \mu m^2$ (measured)*	*Cells per cm^3 (calculated)*
46.6	53	0.385 x10^9
17.3	59	0.453 x10^9
52.1	56	0.419 x10^9
47.1	61	0.476 x10^9
52.5	69	0.573 x10^9
12.0	38	0.234 x10^9
33.9	41	0.262 x10^9
74.7	52	0.374 x10^9
50.2	54	0.396 x10^9
19.2	60	0.464 x10^9
63.2	62	0.488 x10^9
54.7	58	0.441 x10^9

Table M5: Sub-clonal growth in competition of IL11 with LOXL3. When competing against the parental cell line, IL11 and LOXL3 show very similar clonal growth rates, but total tumor growth is significantly enhanced when IL11 is present (see also Figure 4 of the main text).

IL11 vs. P final frequency	IL11 vs. P final weight (mg) [days post transplant]	LOXL3 vs. P final frequency	LOXL3 vs. P final weight (mg) [days post transplant]
0.246	820 [60d]	0.437	78 [65d]
0.039	917 [60d]	0.449	55 [65d]
0.336	170 [60d]	0.361	56 [53d]
0.672	767 [60d]	0.424	46 [53d]
0.112	764 [60d]	0.071	370 [53d]
0.616	696 [60d]	0.566	30 [53d]
0.280	701 [60d]	0.437	110 [60d]
0.952	956 [60d]	0.442	210 [60d]
0.504	6989 [60d]	0.476	153 [60d]
0.291	107 [53d]	0.672	95 [60d]
0.340	160 [53d]	0.521	119 [60d]
0.378	200 [53d]	0.532	154 [60d]
0.286	350 [53d]	0.616	216 [60d]
0.365	950 [53d]	0.554	115 [60d]
0.007	240 [53d]	0.342	127 [60d]
0.165	140 [67d]		
0.309	650 [67d]		
0.563	30 [67d]		
0.317	60 [67d]		
0.115	720 [67d]		
0.364	440 [67d]		
Median clonal growth rate: (see Eq 5)	0.1586/day	Median clonal growth rate: (see Eq 5)	0.1625/day

Table M6: Diversity after 41 days of tumor growth. We measured clonal frequencies polyclonal tumors after 41 days in 9 control tumors and 8 tumors treated with Doxorubicin. Diversity is calculated using Shannon's index, Equation (18). A two-sample Kolmogorov-Smirnov test revealed that diversity significantly decreased (P-value=0.03).

Simpson's Index Control	Simpson's Index Treatment
2.5312	2.4632
2.5684	2.5177
2.5169	2.4229
2.4793	2.511
2.514	2.4704
2.553	2.4801
2.6356	2.4052
2.6681	2.4804
2.481	