Share, but unequally: A plausible mechanism for emergence and maintenance of intratumor heterogeneity - Supplementary Information

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In the Supplementary Information (SI) we provide a number of different aspects of the results that are relevant to the results in the main text. Because the SI covers diverse topics we provide a roadmap. In section 1, we describes the procedure used to extract the parameters using the non-linear feedback function used to analyze the experimental data given in [1]. In sections (2-4) we provide all the details needed to not only quantitatively describe the experiments on Glioblastoma using the same theory for the role of IGF-II in maintaining heterogeneity in neuroendocrine pancreatic cancer but also makes testable predictions.

1 Qualitative results for maintenance of ITH do not depend on the precise form of non-linear fitness function

Insulin-like growth factor II (IGF-II), overexpressed in many cancers, stimulates cell proliferation, and prevents apoptosis[5, 6]. The growth rate of -/- cells (tumor cells with the deletion of the IGF-II gene) as a function of the exogenous IGF-II concentration has been measured systematically[1]. There is a nonlinear relation between the growth rate and the concentration of IGF-II (see the open squares in Fig. S2), which is perfectly described by the Hill function defined in Eq. (10) (with $f_+ = 0$) in the main text (see the blue dashed line in the same figure). The growth rate of -/- cells is strongly influenced by the exogenous IGF-II concentration. In contrast, the proliferation rate of +/+ cells, derived from the Eq. (12) (with $f_+ = 1$, $a = 80$, and $p_0 = 4.65$) in the main text, is quite insensitive to this parameter and only small changes are observed at very high IGF-II concentrations (see the red solid line in Fig. S2). This too is consistent

with experimental observations[1]. The reason is that +/+ cells can produce the IGF-II proteins by themselves to sustain and promote their growth. This also explains the slower growth rate for -/- cells compared with +/+ cells in Fig. S2 in the absence of exogenous IGF-II. However, -/- cells grow faster than +/+ cell at very high IGF-II concentrations, which is a consequence of +/+ cells having to pay a cost for the production of IGF-II. The results in the main text were obtained using the Hill function for the dependence of the growth rate on the concentration of IGF-II. In order to assess the robustness of our conclusions we repeated the calculations using the logistic function (see Eq. (S.1) below).

It may seem that a different nonlinear relation (the logistic function),

$$w_- = \frac{a_1}{1 + a_2 e^{-\gamma_1 c}}\,, \tag{S.1}$$

could fit the growth curve of -/- cells as a function IGF-II concentration. The best fit yields $a_1 = 19.97$, $a_2 = 6.07$, and $\gamma_1 = 0.28$ (see the red solid line in Fig. S3). However, the logistic function does not give as good a fit to the measured IGF-II dependent growth rate of -/- cells as the Hill function used in Eq. (10) in the main text. Nevertheless, Eq. (S.1) also captures the nonlinear growth profile of -/- cells. To demonstrate that this is indeed the case, we followed the same procedure described in the main text, and assumed that the public goods allocation strategy for -/- and +/+ cells are given by Eq. (11) and (13), respectively, except that w_- is given by Eq. (S.1). Reassuringly, we found qualitatively the same behavior as observed in Fig. 2 (see Fig. S4). Figs. S4A and S4B show that equal ($b/a = 1$) or no (b/a=0) share of public goods (generated by producer cells) cannot maintain a stable heterogeneous state. A stable coexisting state with both +/+ and -/- cells can only be reached when +/+ cells are allocated more resources than -/- cells ($0 < b/a < 1$) as illustrated in Fig. S4C. Therefore, the exact form of the growth curve is not critical in arriving qualitatively at the same conclusions that establish the presence of a stable heterogeneous system, as long as it is a nonlinear function of the resources, and a suitable allocation strategy for the public goods is used.

2 The evolution of glioblastoma tumor size containing a mixture of wt and Δ cells

Here, we provide the details needed to establish the conditions for coexistence of different cell types (Δ and wt) in glioblastoma using the same theoretical framework to obtain the results for neuroendocrine pancreatic cancer. The present application is intended to show the generality of the theory by analyzing the experiments described elsewhere[7]. In the absence of Δ cells, the wt cells alone cannot induce the tumor growth in mouse[7] (see the pink down-triangles in Fig. 6 in the main text). In the experiments, consisting of wt and Δ cells, the authors did not consider the consequences of exogenous public goods (IL-6). Therefore, we take a simple non-linear fitness function (w_-) for wt cells similar

to the one in Eq. (10) ($\alpha = 1$) in the main text which leads to,

$$w_- = bf_+/(1 + bf_+)\,. \tag{S.2}$$

Note that the fitness function w_- is zero if the fraction of Δ cells, $f_+ = 0$, and, hence the constant c_0 in Eq. (11) is no longer required (no exogenous public goods). Similarly, the fitness function (w_+) for Δ cells can be written as

$$w_+ = af_+/(1 + af_+) - p_0\,, \tag{S.3}$$

where p_0 is the price paid by the Δ cells. We take different parameters a and b ($a \neq b$, in general) in the above equations to illustrate the consequences of unequal sharing of public goods (produced by the Δ cells) between the two cell types. The evolution of the tumor size is described by Eq. (4) in the main text. Given $f_+ = 1$, the average fitness of the system is given by $\langle w\rangle = w_+$ (see Eq. (3)). Therefore, the tumor size N grows exponentially with $N = N_0 e^{w_+ t}$. By fitting the growth curve (blue) shown in the upper panel of Fig. 6 in the main text to an exponential function, we obtain,

$$\frac{a}{(1 + a)} - p_0 \approx 0.335\,. \tag{S.4}$$

Thus, we obtain $w_+ \approx 0.335$ from the fit. It was noted in the experiments[7] that these two types of cells grow at the same rate ($f_+ = f_-$), which leads to

$$\frac{0.5a}{(1 + 0.5a)} - p_0 = \frac{0.5b}{(1 + 0.5b)}\,. \tag{S.5}$$

The average fitness $\langle w\rangle = 0.5(w_+ + w_-) = w_+ = w_-$ given $f_+ = f_-$ and the evolution of the system size N can be described by $N = N_0 e^{w- t}$. Similarly, the tumor growth curve (green line) in the upper panel of Fig. 6, results in,

$$\frac{0.5b}{(1 + 0.5b)} \approx 0.321\,, \tag{S.6}$$

with the constant 0.321 obtained from the exponential fit. Thus, the three parameters a, b, and p_0 can be calculated from Eqs. (S.4)-(S.6), which yield to $a = 68.4$, $b = 0.946$, and $p_0 = 0.651$. The relation $a \gg b$ derived here using the experimental data shows that more public goods are allocated to producers than non-producers, which is the prerequisite for the maintenance of a stable heterogeneous system predicted from the theory.

After obtaining the values for all the three free parameters in Eqs. (S.2) and (S.3), the evolution of the tumor size for different initial fractions of Δ cells can be predicted. First, the evolution of f_+ and f_- can be calculated from Eqs. (1) and (2) in the main text given the initial fraction $f_+(0)$. Then, the evolution of the tumor size can be derived from Eq. (4) directly with $\langle w\rangle$ given by Eq. (3) in the main text. Two examples are shown in the lower panel of Fig. 6 with the fraction of Δ cells given by 10% and 90% separately. The theoretical predictions (see the dash-dotted and solid lines) are in excellent agreement with experimental results (purple and green symbols, respectively). Additional experiments can be carried out to further test the predictions of our theory.

3 The growth rate of tumors at different fractions of a subpopulation

It is frequently found in the experiments that the mean growth rate of tumors is a non-monotonic function of the fraction of one cell type. A maximum growth rate is often observed in the middle fraction of producer cells[1, 7]. Generally speaking, the growth rate of a tumor at certain fractions of producer cells is difficult to measure accurately because the tumor evolves with time, and the fraction of different cells also changes. Therefore, the growth rate as a function of fraction of a cell type has to be measured over relatively short time interval because otherwise large fluctuation would be expected.

In contrast, it is relatively easy to calculate this quantity theoretically. The mean growth rate is just the average fitness as given by Eq. (3). One example is shown in Fig. 7 in the main text for the system composed of wt and Δ cells. The dotted and dashed lines correspond to the growth rate of wt and Δ cells described by Eqs. (S.2) and (S.3), respectively. The mean velocity as demonstrated by the solid line (lying between the dotted and dashed lines) reaches a maximum value at the fraction 0.77 of Δ cells (see also the inset in Fig. 7). The parameter values for a, b, and p_0 used in this figure are the same as discussed in the last section. Therefore, the relation observed here provides a direct explanation for the surprising finding shown in Fig. 6 that the tumor grows faster as the fraction of Δ cell is 90% compared with the case with 100% of Δ cell.

4 The evolution of the fraction of Δ cells with and without exogenous resources.

From the Eqs. (S.2), (S.3), combined with Eqs. (1) and (2), the evolution of the fraction $f_+(t)$ of Δ cells (without any supply of exogenous cytokines) can be calculated as shown in Fig. S5A. We found a very stable heterogeneous state (composed of both wt and Δ cells) as $f_+(0)$ is varied from 0.1 to 0.9. A poor prognosis for recovery would be expected for patients with such a stable heterogeneous tumor as long as a small fraction of Δ cells is present in the tumor.

Adding exogenous cytokines (c_0), such as IL-6 or LIF into the tumor, the Eqs. (S.2), and (S.3) change to the following forms,

$$w_- = (bf_+ + c_0)/(1 + bf_+ + c_0)\,, \tag{S.7}$$

$$w_+ = (af_+ + c_0)/(1 + af_+ + c_0) - p_0\,. \tag{S.8}$$

A stable homogeneous tumor consisting only of wt cells can be obtained rapidly irrespective of $f_+(0)$ (see the evolution of $f_+(t)$ in Fig. S5B). It should not go unnoticed that the predictions in Fig. S5 are amenable to experimental tests, along the lines conducted in [1].

References

[1] Archetti M, Ferraro D A, & Christofori G. Heterogeneity for IGF-II production maintained by public goods dynamics in neuroendocrine pancreatic cancer. *Proc. Natl. Acad. Sci. U.S.A.*, 2015; 112, 1833-1838. (DOI: 10.1073/pnas.1414653112).

[2] Blythe, R. A., & McKane, A. J. Stochastic models of evolution in genetics, ecology and linguistics. *Journal of Statistical Mechanics: Theory and Experiment*, 2007; 2007(07), P07018. (DOI: 10.1088/1742-5468/2007/07/P07018).

[3] Melbinger, A., Cremer, J., & Frey, E. Evolutionary game theory in growing populations. *Physical review letters*, 2010; 105(17), 178101. (DOI: 10.1103/PhysRevLett.105.178101).

[4] Hardin, G. The Tragedy of the Commons. *Science*. 1968; 162 (3859): 1243-1248. (DOI: 10.1126/science.162.3859.1243).

[5] Christofori G, Naik P, & Hanahan D. A second signal supplied by insulin-like growth factor II in oncogene-induced tumorigenesis. *Nature*, 1994; 369(6479): 414–418. (DOI: 10.1038/369414a0).

[6] Pollak, M. Insulin and insulin-like growth factor signalling in neoplasia. *Nature Reviews Cancer*, 2008; 8(12), 915-928. (DOI: 10.1038/nrc2536).

[7] Inda Maria-del Mar, Rudy Bonavia, Akitake Mukasa, Yoshitaka Narita, Dinah WY Sah, et al. Tumor heterogeneity is an active process maintained by a mutant EGFR-induced cytokine circuit in glioblastoma. *Genes and development*, 2010; 24(16): 1731-1745. (DOI: 10.1101/gad.1890510).

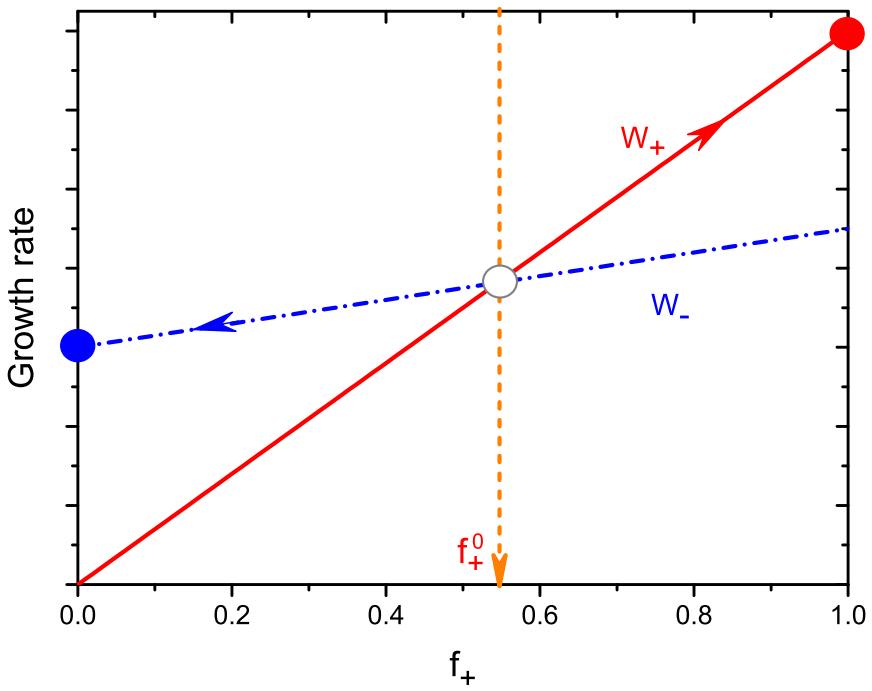

Figure S1: A schematic figure for the growth rate of the producer and non-producer as a function of the fraction (f_+) of the producer with a linear fitness function. The red solid line shows the the growth rate (w_+) of the producer described by Eq. (7) and the blue dash-dotted line represents the growth rate (w_-) of the non-producer given by Eq. (8) in the main text. The parameter $k_+ > k_-$ and the internal unstable state is indicated by the open circle with $f_+ = f_+^0$. The blue and red filled circles show the homogeneous state consisting of only non-producers and producers, respectively. The figure illustrates that upon an infinitesimal perturbation the flow from the internal state (open circle) to the stable states (filled circles) occurs depending on the value of f_+.

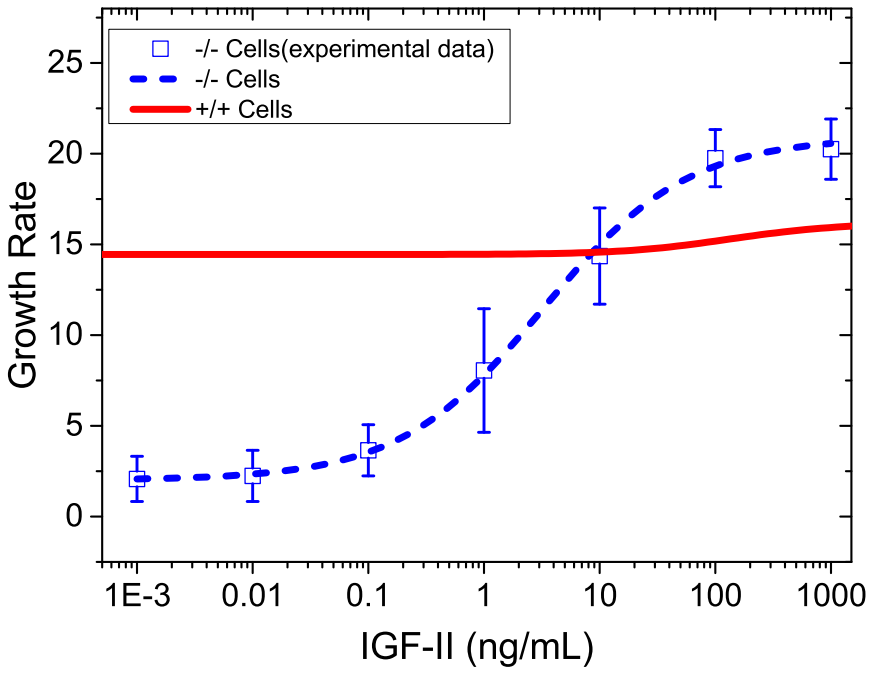

Figure S2: Growth rate of the +/+ and -/- cells as a function of IGF-II concentration. Experimental results[1] for -/- cells are represented by open squares which can be perfectly fitted by a Hill function as described by the Eq. (10) in the main text (see the blue dashed line). The red solid red line shows the growth of +/+ cells derived from the Eq. (12) in the main text. The error bars represent the standard deviation. The growth rate is defined the same as in Fig. 2 in the main text.

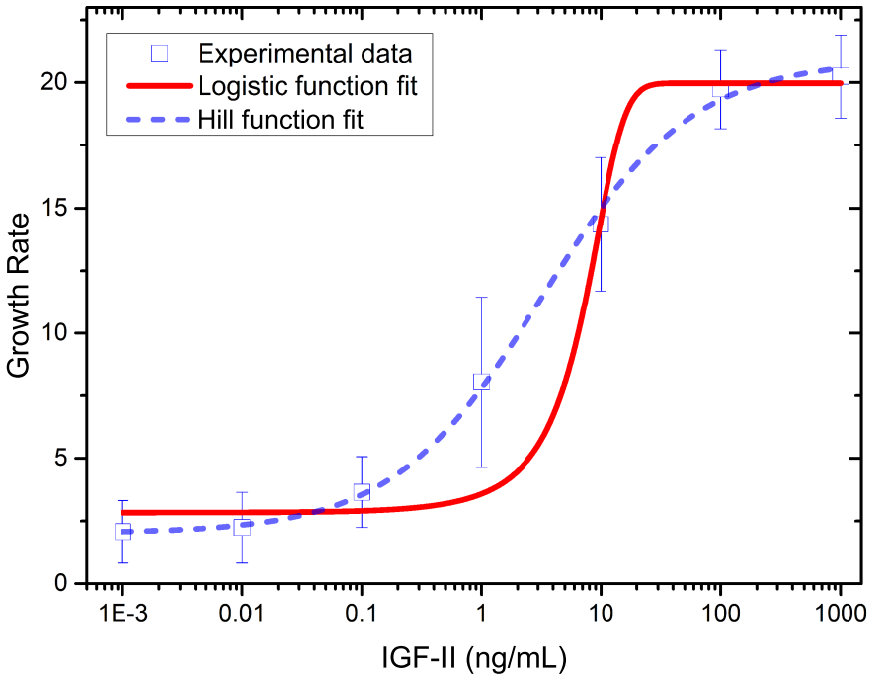

Figure S3: Growth rate of the -/- cells as a function of IGF-II concentration. Experimental results[1] for -/- cells are represented by open squares. The red solid red line shows the fit using a logistic function given Eq. (S.1). For comparison, we also show the blue dashed line, is obtained by fitting to the Hill function (see Fig. S1). The growth rate is defined the same as in Fig. 2 in the main text.

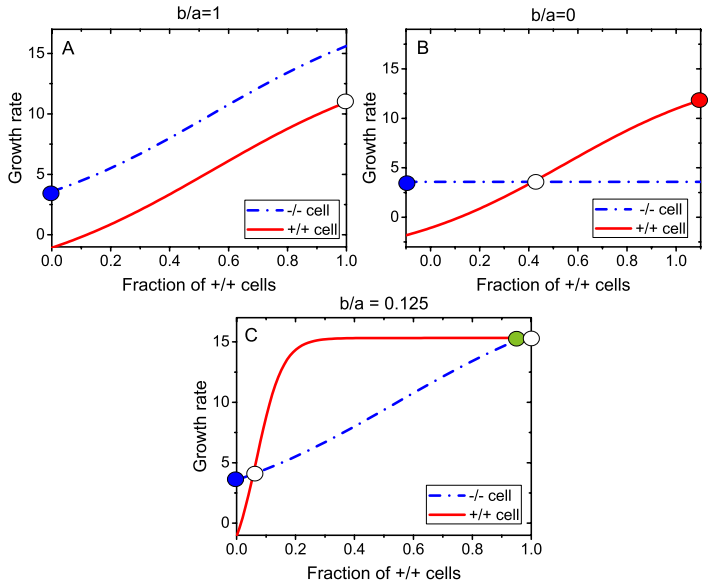

Figure S4: Growth rate of the +/+ and -/- cells as a function of the fraction of +/+ cells under different allocation strategies of IGF-II produced by the +/+ cells. (A) Equal share of IGF-II ($b = a = 10$), (B) no share ($b = 0$, and $a = 10$), (C) a small portion ($b = 10$, and $a = 80$) is allocated to -/- cells. The value of $c_0 = 1$, $p_0 = 4.65$ (same as used in Fig. 2 in the main text) in Eqs. (11) and (13) in the main text. The growth rate of +/+ cells are shown in solid red lines while dot-dashed blue lines describe the growth rate of -/- cells. The filled and empty circles are used to indicate a stable or unstable state, respectively. A stable state consisting of only +/+ (-/-) cells is indicated in red (blue) color. The green filled circle shows a stable heterogeneous state. The growth rate is defined the same as in Fig. 2 in the main text.

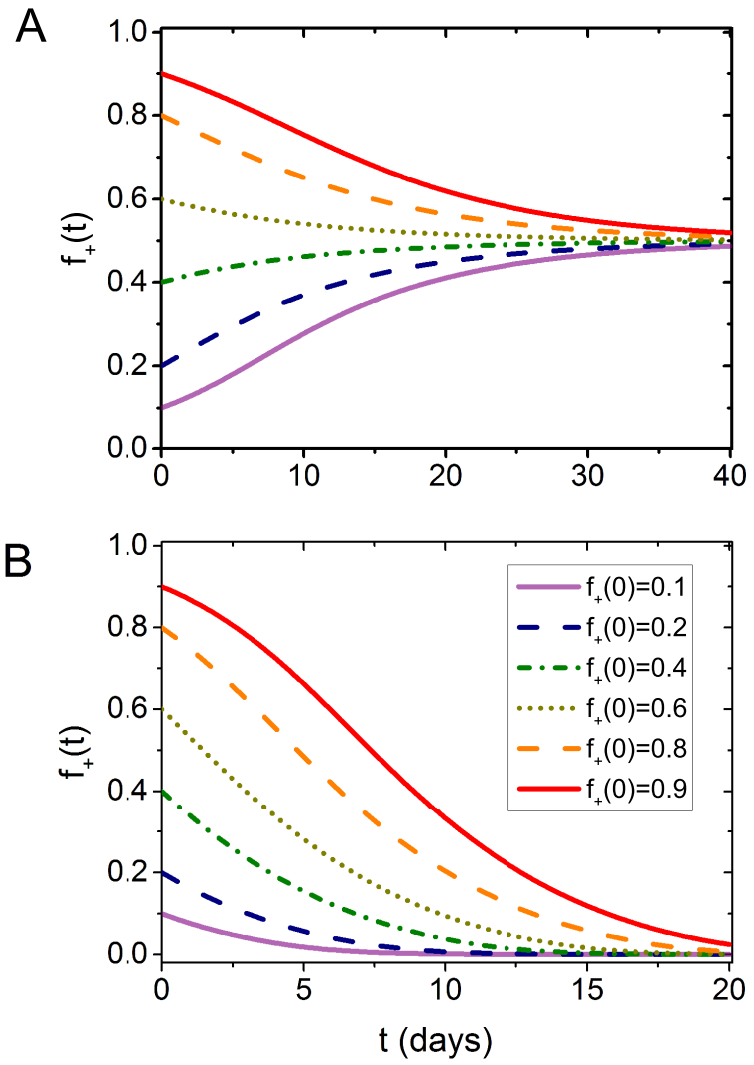

Figure S5: The evolution of the fraction $f_+(t)$ of Δ cells in GBM tumor as a function of time for distinct initial conditions specified by $f_+(0)$. (A) Without supply of exogenous public goods w_- and w_+ are given by Eqs. (S.2) and (S.3); (B) With supply of exogenous public goods w_- and w_+ are described by Eqs. (S.7) and (S.8) with $c_0 = 1.0$. Other parameters are the same as used in Fig. 6 in the main text. The labels for different $f_+(0)$ are the same in (A) and (B).