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Supplementary information

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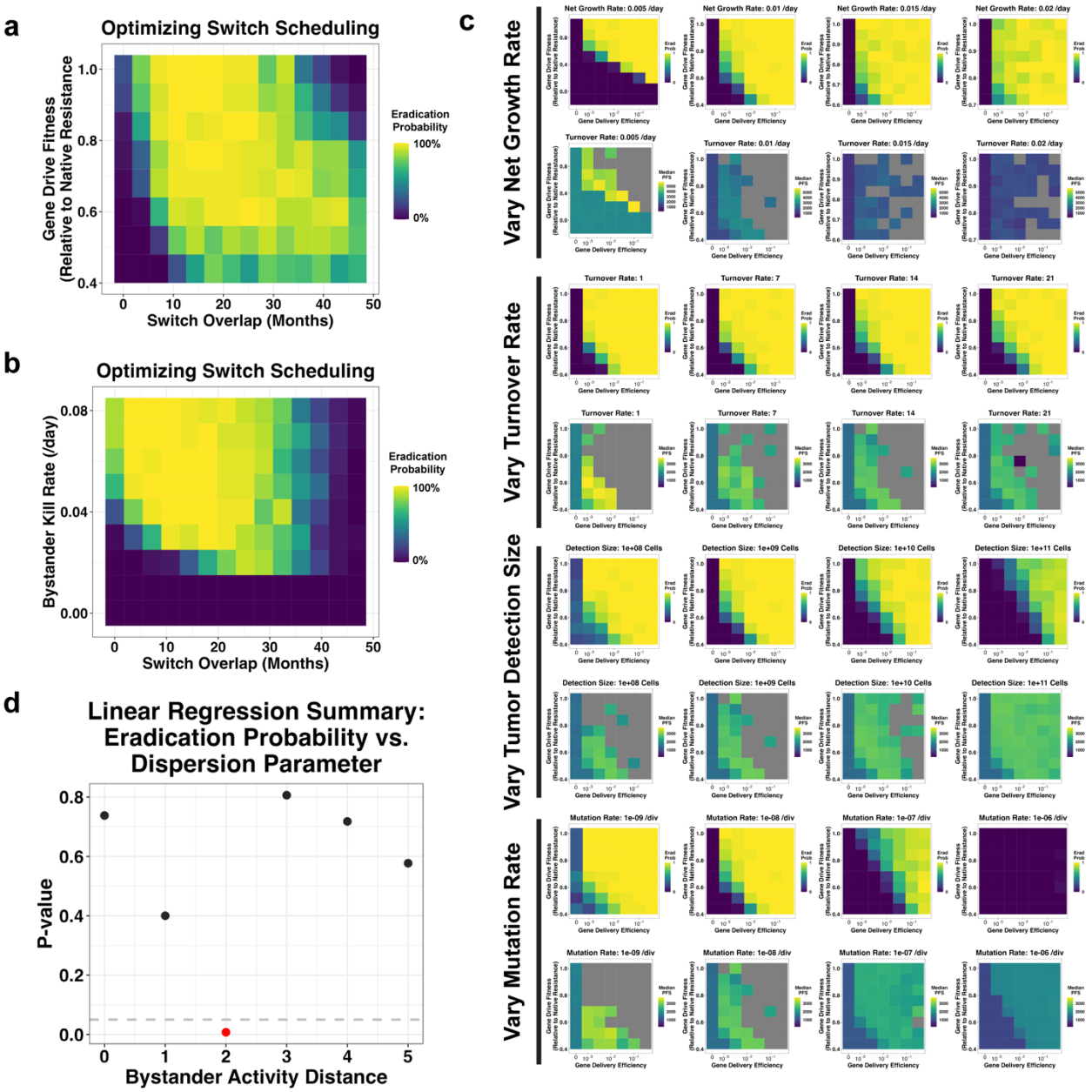
Programming tumor evolution with selection gene drives to proactively combat drug resistance

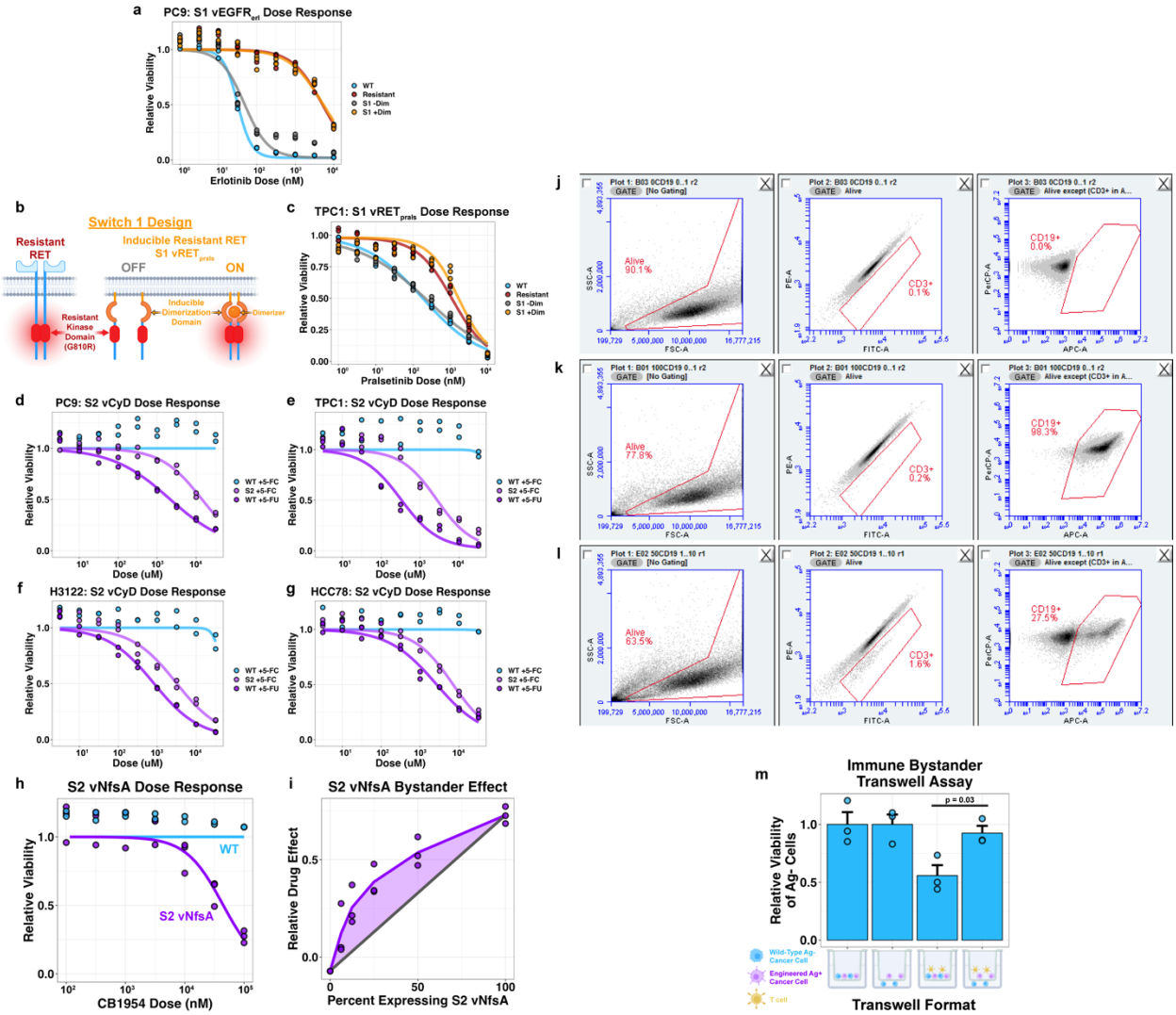
In the format provided by the authors and unedited



Supplemental Figure 1

(a) Compartmental model outcomes as the "switch delay" parameter varies. The switch delay parameter dictates the time between the beginning of Switch 2 treatment and the end of Switch 1 treatment, thus allowing for some overlap. While Switch 2 always begins when the gene drive population reaches the predetermined detection size, there may be benefit to maintaining Switch 1 for some period of time after Switch 2 initiation, as shown here. This is because Switch 1 activity is predicted to slow the collapse of the gene drive population, thus maximizing the Switch 2 bystander effect. However, if Switch 1 is maintained for too long, the gene drive population may serve as a reservoir for the development of cross-resistance. The probability of eradication is shown for ranges of switch delay and gene drive fitness values.

(b) Compartmental model outcomes as in **(a)** as switch delay and bystander kill rate vary.
(c) Sensitivity analysis of the compartmental model. For each parameter that is allowed to vary, probability of eradication (top heatmaps in each row) and median progression-free-survival (bottom heatmaps in each row) are shown. Parameter sweeps through net growth rate (top row) and turnover rate (second row) indicate that the model is relatively robust to growth kinetics. However, predicted outcomes worsen as tumor detection size (third row) and mutation rate (fourth row) increase.
(d) Summary for linear regression analyses of eradication probability as predicted by gene drive cell dispersion. Results represent the p-value (y-axis) of the cell dispersion metric ($1/\theta$) in a model to predict eradication probability, for a range of bystander activity distances (ρ) along the x-axis. Gene drive dispersion is only significantly correlated with eradication probability for $\rho = 2$. P-values calculated using the *lm* function in base R.



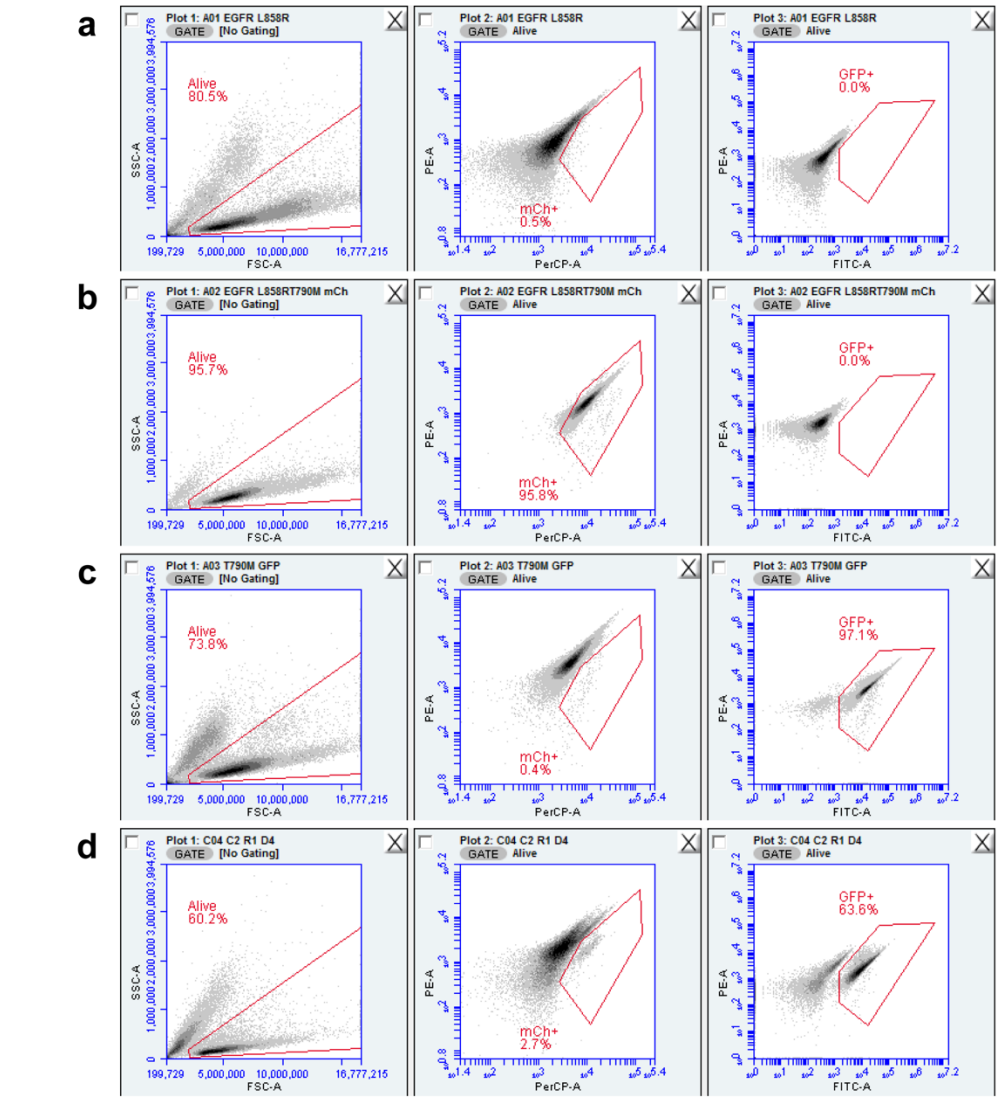
Supplemental Figure 2

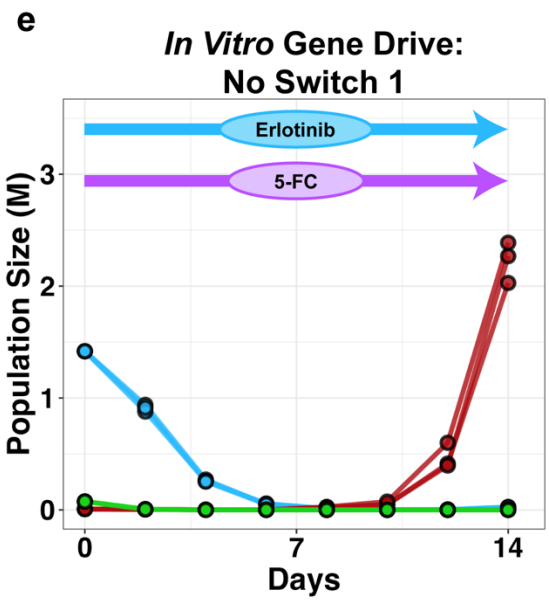
(a) Switch 1 confers inducible resistance in S1 vEGFR$_{erl}$ PC9 cells. EGFR+ PC9 cells were transduced with the S1 vEGFR$_{erl}$ gene and treated with a range of erlotinib concentrations in the presence (orange) or absence (gray) of dimerizer. Data for sensitive (wild-type; blue) and resistant (EGFR L858R/T790M; red) PC9 cells are also shown. N = 3 technical replicates per condition.

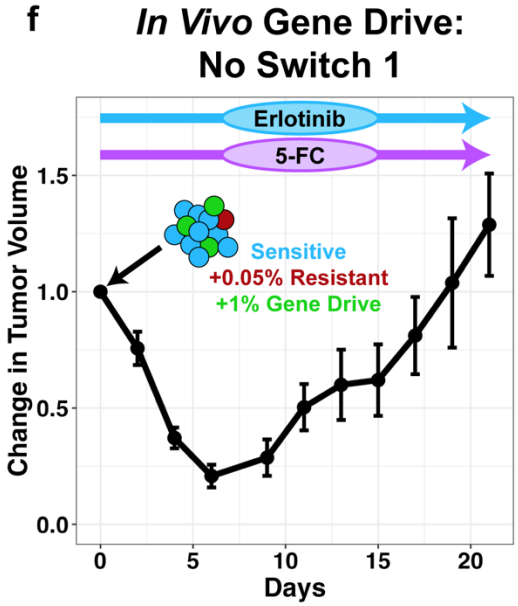
(b) Schematic of Switch 1 vRET$_{prals}$ design. A G810R mutation in the RET kinase domain confers resistance to pralsetinib activity (*left*). An inducible FKBP12-RET fusion protein controllably induces RET signaling (*right*). A G810R resistance mutation in the RET kinase domain (S1 vRET$_{prals}$) rescues signaling in the presence of pralsetinib.

(c) Switch 1 confers inducible resistance in S1 vRET$_{prals}$ TPC1 cells. RET+ TPC1 cells were transduced with S1 vRET$_{prals}$ and treated with a range of pralsetinib concentrations in the presence (orange) or absence (gray) of dimerizer. Data for sensitive (wild-type; blue) and

resistant (CCDC6-RET G810R; red) TPC1 cells are also shown. N = 3 technical replicates per condition.
(d-g) S2 vCyD activity across a panel of cancer cell lines. EGFR+ PC9 **(d)**, RET+ TPC1 **(e)**, ALK+ H3122 **(f)**, and ROS1+ HCC78 **(g)** cells were transduced with S2 vCyD and treated with a range of 5-FC concentrations (light purple). Data for wild-type cells treated with 5-FC (blue) and 5-FU (the activated form of the drug; dark purple) are also shown. N = 3 technical replicates per condition.
(h) S2 vNfsA activity in 293T cells. The enzyme NfsA converts CB1954 into an active nitrogen mustard. Wild-type (blue) and S2 vNfsA (purple) 293T cells were treated with a range of CB1954 concentrations. N = 3 technical replicates per condition.
(i) Switch 2 bystander activity for S2 vNfsA 293T cells treated with 100 μM CB1954, as in Figure 2i. N = 3 technical replicates per mixed population; purple line indicates the mean.
(j-l) Gating strategy for immune bystander experiment. Gating for viable (left panel), CD3+ (middle panel, subset on viable gate), and CD19+ (right panel, subset on viable gate) are shown for pure CD19- **(j)** and CD19+ **(k)** PC9 populations, as well as a representative sample from a mixed 50% CD19+ population treated with T cells and blinatumomab **(l)**.
(m) Immune bystander activity in transwell plates. T cells (gold) were cocultured with CD19+ (purple) and CD19- (blue) PC9 cells with blinatumomab in the formats shown. After 48 hours, cells were stained and analyzed by flow cytometry to measure CD19- counts. N = 3 technical replicates per condition; bar plots are mean +/- SEM. P-value calculated by two-sided t-test in base R.


a
Plot 1: A01 EGFR L858R
GATE [No Gating]
Alive
80.5%
Plot 2: A01 EGFR L858R
GATE Alive
mCh+
0.5%
Plot 3: A01 EGFR L858R
GATE Alive
GFP+
0.0%
b
Plot 1: A02 EGFR L858RT790M mCh
GATE [No Gating]
Alive
95.7%
Plot 2: A02 EGFR L858RT790M mCh
GATE Alive
mCh+
95.8%
Plot 3: A02 EGFR L858RT790M mCh
GATE Alive
GFP+
0.0%
c
Plot 1: A03 T790M GFP
GATE [No Gating]
Alive
73.8%
Plot 2: A03 T790M GFP
GATE Alive
mCh+
0.4%
Plot 3: A03 T790M GFP
GATE Alive
GFP+
97.1%
d
Plot 1: C04 C2 R1 D4
GATE [No Gating]
Alive
60.2%
Plot 2: C04 C2 R1 D4
GATE Alive
mCh+
2.7%
Plot 3: C04 C2 R1 D4
GATE Alive
GFP+
63.6%
SSC-A
FSC-A
PE-A
PerCP-A
FITC-A


e
In Vitro Gene Drive:
No Switch 1
Erlotinib
5-FC
Population Size (M)
Days

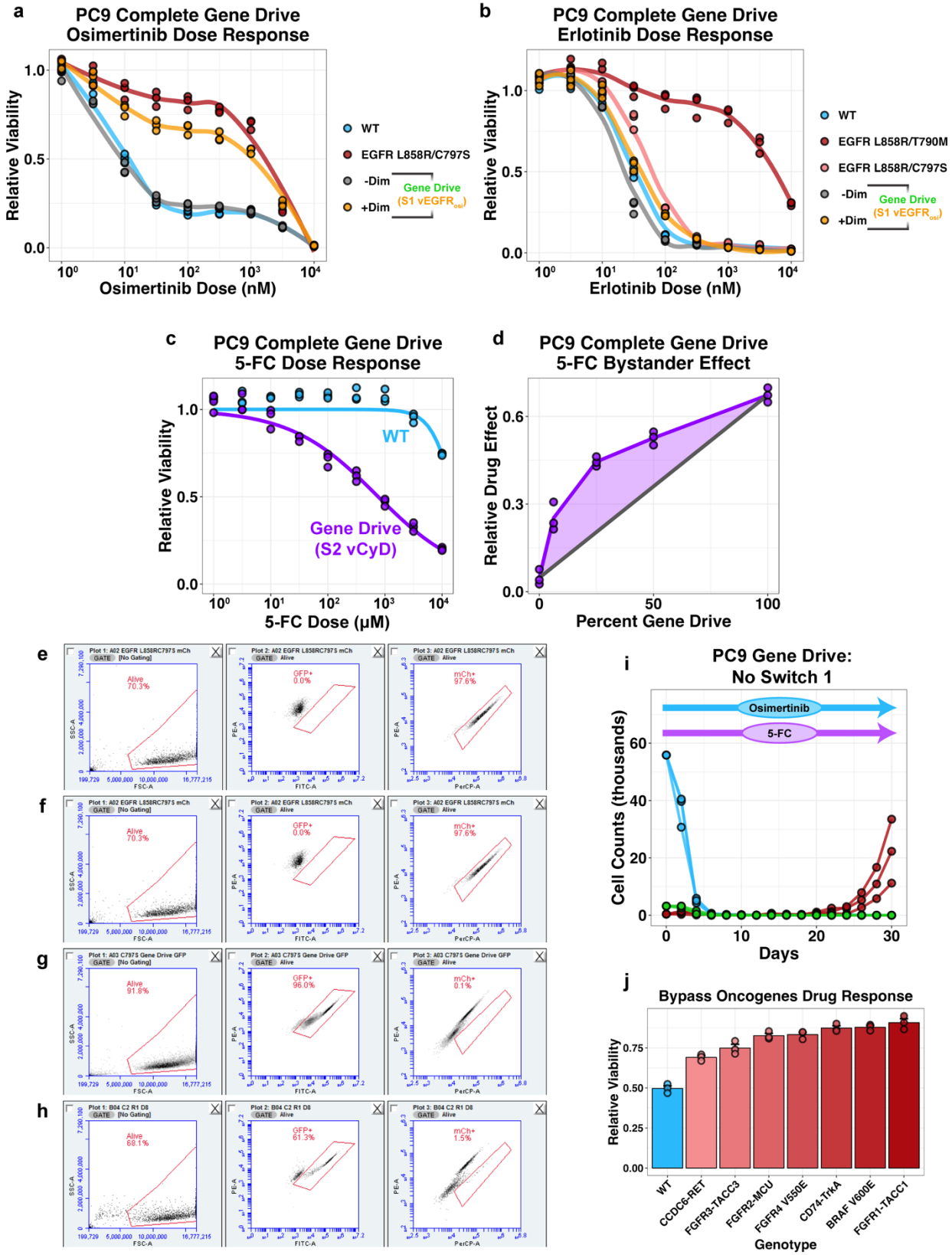

f
In Vivo Gene Drive:
No Switch 1
Erlotinib
5-FC
Sensitive
+0.05% Resistant
+1% Gene Drive
Change in Tumor Volume
Days

Supplemental Figure 3

(a-d) Gating strategy for BaF3 growth tracking experiments. Gating for viable (left panel), mCherry+ EGFR L858R/T790M (middle panel, subset on viable gate), and GFP+ gene drive cells (right panel, subset on viable gate) are shown for pure EGFR L858R **(a)**, EGFR L858R/T790M **(b)** and gene drive **(c)** BaF3 populations, as well as a representative sample from a mixed population at day 4 of treatment **(d)**.

(e) Population dynamics for *in vitro* experiment with gene drive cell spike-in (as in Figure 3d) but without initial Switch 1 selection. Cells were treated with erlotinib and 5-FC from D0. N = 3 technical replicates.

(f) Tumor growth dynamics for *in vivo* experiment with gene drive cell spike-in (as in Figure 3g) but without initial Switch 1 selection. Mice were treated with erlotinib and 5-FC from D0. N = 10 tumors (5 mice) per condition; data are presented as mean +/- SEM.


a
PC9 Complete Gene Drive
Osimertinib Dose Response
Relative Viability
Osimertinib Dose (nM)
WT
EGFR L858R/C797S
-Dim
+Dim
Gene Drive
(S1 vEGFR$_{osi}$)
b
PC9 Complete Gene Drive
Erlotinib Dose Response
Relative Viability
Erlotinib Dose (nM)
WT
EGFR L858R/T790M
EGFR L858R/C797S
-Dim
+Dim
Gene Drive
(S1 vEGFR$_{osi}$)
c
PC9 Complete Gene Drive
5-FC Dose Response
Relative Viability
5-FC Dose (μM)
WT
Gene Drive
(S2 vCyD)
d
PC9 Complete Gene Drive
5-FC Bystander Effect
Relative Drug Effect
Percent Gene Drive
e
f
g
h
i
PC9 Gene Drive:
No Switch 1
Osimertinib
5-FC
Cell Counts (thousands)
Days
j
Bypass Oncogenes Drug Response
Relative Viability
WT
CCDC6-RET
FGFR3-TACC3
FGFR2-MCU
FGFR4 V550E
CD74-TrkA
BRAF V600E
FGFR1-TACC1
Genotype

Supplemental Figure 4

(a) Switch 1 activity in complete gene drive S1vEGFR$_{osi}$-S2vCyD PC9 cells. Cells were treated with a range of osimertinib concentrations in the presence (orange) or absence (gray) of dimerizer. Data for sensitive (wild-type; blue) and resistant (EGFR L858R/C797S; red) PC9 cells are also shown. N = 3 technical replicates per condition.

(b) Osimertinib-gene drive PC9 cells retain sensitivity to erlotinib. S1vEGFR$_{osi}$-S2vCyD PC9 cells were treated with a range of erlotinib concentrations in the presence (orange) or absence (gray) of dimerizer. Data for sensitive (wild-type; blue), osimertinib-resistant (EGFR L858R/C797S; light red), and erlotinib-resistant (EGFR L858R/T790M; dark red) PC9 cells are also shown. N = 3 technical replicates per condition.

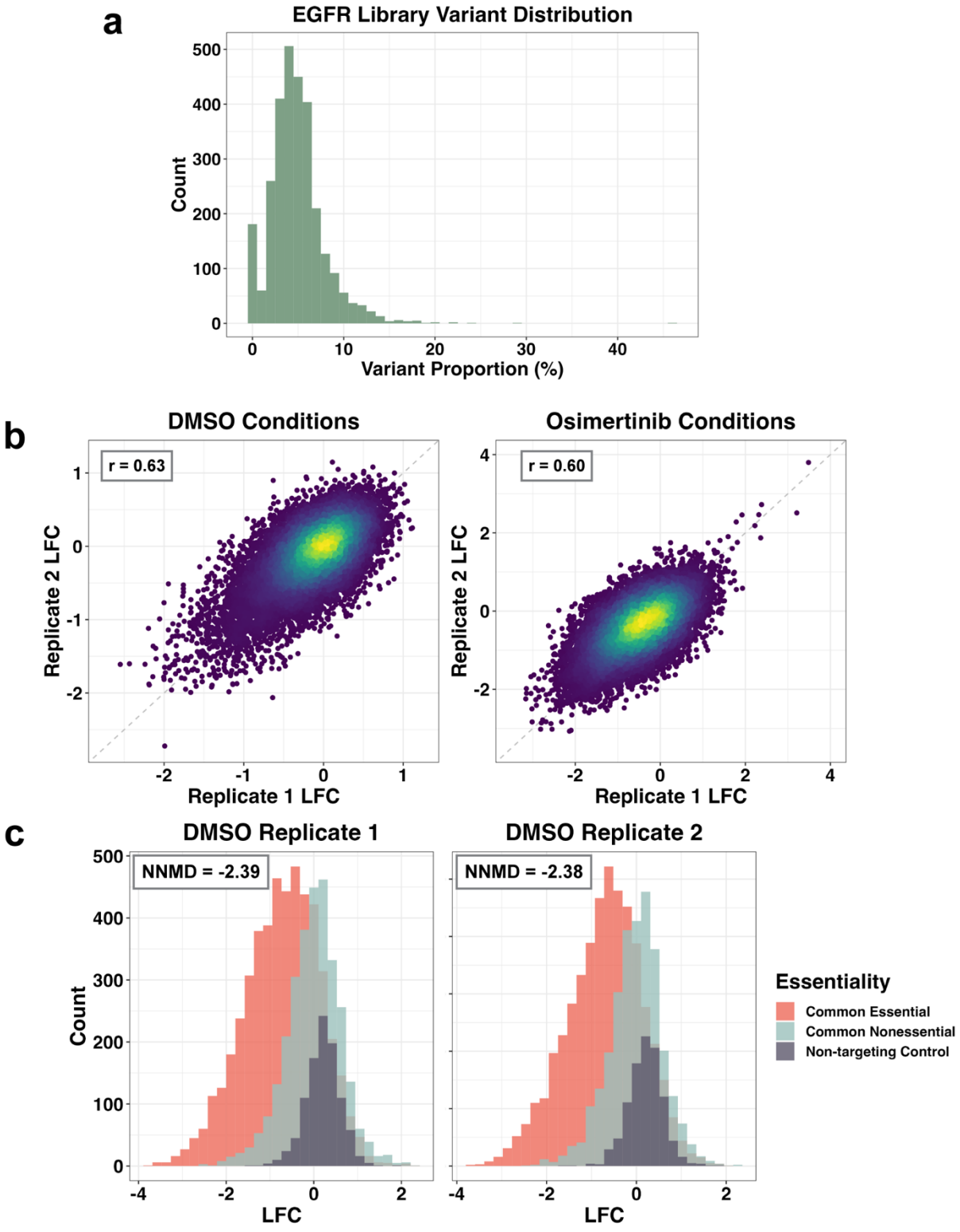
(c) Switch 2 activity in complete gene drive S1vEGFR$_{osi}$-S2vCyD PC9 cells. Wild-type (blue) and gene drive cells (purple) were treated with a range of 5-FC concentrations. N = 3 technical replicates per condition.

(d) Bystander Switch 2 activity in complete gene drive S1vEGFR$_{osi}$-S2vCyD PC9 cells. Mixtures of cells were pooled and treated with 1 mM 5-FC as in Figure 2i. N = 3 technical replicates per mixed population; purple line indicates the mean.

(e-h) Gating strategy for PC9 growth tracking experiments. Gating for viable (left panel), GFP+ gene drive (middle panel, subset on viable gate), and mCherry+ C797S cells (right panel, subset on viable gate) are shown for pure wild-type **(e)**, C797S **(f)** and gene drive **(g)** PC9 populations, as well as a representative sample from a mixed population at day 8 of treatment **(h)**.

(i) Population dynamics for experiment with PC9 gene drive cell spike-in (as in Figure 3k) but without initial Switch 1 selection. Cells were treated with osimertinib and 5-FC from D0. N = 3 technical replicates.

(j) Resistance conferred by activated bypass oncogenes in PC9 cells. PC9 cells were transduced with a panel of parallel and downstream effectors and treated with 100 nM osimertinib. N = 3 technical replicates per population; bar plots are mean +/- SEM.

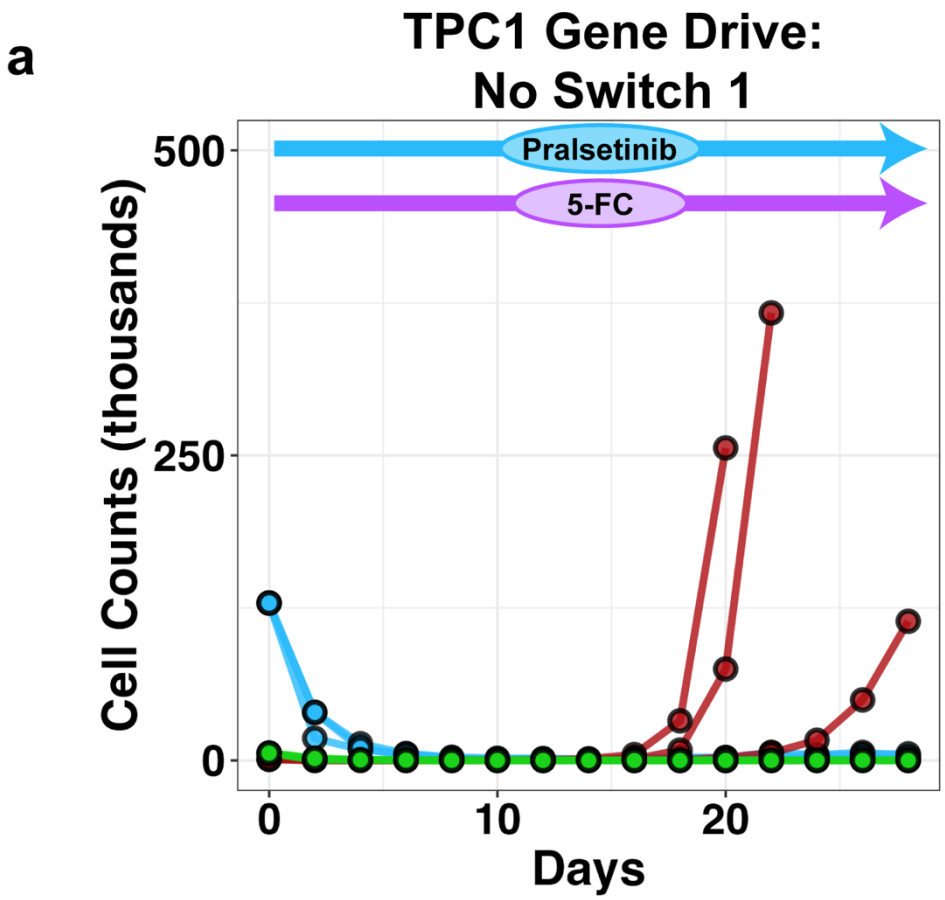

a
EGFR Library Variant Distribution
Count
Variant Proportion (%)
b
DMSO Conditions
r = 0.63
Replicate 2 LFC
Replicate 1 LFC
Osimertinib Conditions
r = 0.60
Replicate 2 LFC
Replicate 1 LFC
c
DMSO Replicate 1
NNMD = -2.39
Count
LFC
DMSO Replicate 2
NNMD = -2.38
LFC
Essentiality
Common Essential
Common Nonessential
Non-targeting Control

Supplemental Figure 5

(a) Distribution of variant proportion in the EGFR variant library. Values represent the proportion of each variant with respect to all other variants at that residue.

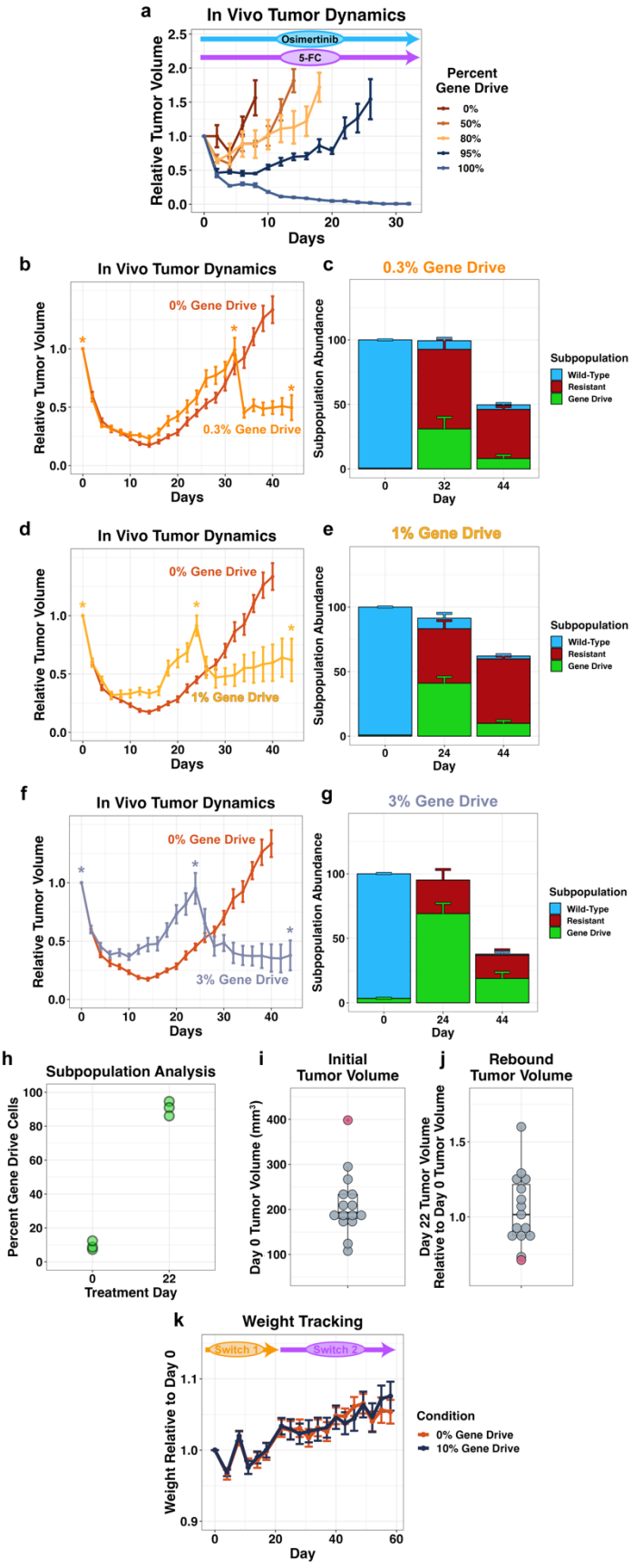
(b) Replicate correlations for gene-level LFC values in genome-wide CRISPR screen for DMSO (*left*) and osimertinib (*right*) conditions. Pearson correlations were calculated in base R.

(c) Separation of essential and nonessential/non-targeting control guides for replicates of the DMSO condition. NNMD quality control metric from[55] is shown. NNMD values below a threshold of -1 are generally thought to exhibit good separation between controls.



Supplemental Figure 6

(a) Population dynamics for experiment with RET gene drive cell spike-in (as in Figure 5d) but without initial Switch 1 selection. Cells were treated with pralsetinib and 5-FC from D0. N = 3 technical replicates.


a
In Vivo Tumor Dynamics
Osimertinib
5-FC
Relative Tumor Volume
Days
Percent Gene Drive
0%
50%
80%
95%
100%
b
In Vivo Tumor Dynamics
0% Gene Drive
0.3% Gene Drive
Relative Tumor Volume
Days
c
0.3% Gene Drive
Subpopulation Abundance
Day
Subpopulation
Wild-Type
Resistant
Gene Drive
d
In Vivo Tumor Dynamics
0% Gene Drive
1% Gene Drive
Relative Tumor Volume
Days
e
1% Gene Drive
Subpopulation Abundance
Day
Subpopulation
Wild-Type
Resistant
Gene Drive
f
In Vivo Tumor Dynamics
0% Gene Drive
3% Gene Drive
Relative Tumor Volume
Days
g
3% Gene Drive
Subpopulation Abundance
Day
Subpopulation
Wild-Type
Resistant
Gene Drive
h
Subpopulation Analysis
Percent Gene Drive Cells
Treatment Day
i
Initial Tumor Volume
Day 0 Tumor Volume (mm³)
j
Rebound Tumor Volume
Day 22 Tumor Volume Relative to Day 0 Tumor Volume
k
Weight Tracking
Switch 1
Switch 2
Weight Relative to Day 0
Day
Condition
0% Gene Drive
10% Gene Drive

Supplemental Figure 7

(a) Tumor dynamics for a range of initial gene drive frequencies under Switch 2 treatment. Mixed populations of PC9 cells reflecting potential population structures at the end of Switch 1 treatment were grafted in mice. Mice were treated once daily with osimertinib and 5-FC. N = 6 tumors (3 mice) per cohort; data are presented as mean +/- SEM.

(b) Tumor dynamics for the complete gene drive system in PC9 cells, as in Figure 6e. Tumor volumes for 0% gene drive (dark orange) and 0.3% gene drive (light orange) are shown. Asterisks indicate timepoints for tumor harvesting and analysis. The second asterisk also indicates the initiation of Switch 2 treatment. N = 6 tumors (3 mice) per condition; data are presented as mean +/- SEM.

(c) Subpopulation analysis of tumors undergoing gene drive therapy (as in Figures 6f-g) for 0.3% gene drive populations. N = 6 tumors (3 mice) per condition and time point; data are presented as mean +/- SEM.

(d) Tumor dynamics as in **(b)** for 1% gene drive populations.

(e) Subpopulation analysis as in **(c)** for 1% gene drive populations.

(f) Tumor dynamics as in **(b)** for 3% gene drive populations.

(g) Subpopulation analysis as in **(c)** for 3% gene drive populations.

(h) Gene drive fraction for treatment arm of long-term survival experiment. Mice were subcutaneously grafted with 10% gene drive cells. At treatment days 0 and 22 (beginning of Switch 2) a subset of tumors (N = 3 for each timepoint) were harvested and analyzed by flow cytometry.

(i) Dotplot of initial (day 0 of treatment) tumor volumes of mice in gene drive cohort of long-term survival study. The pink dot indicates an outlier tumor that progressed on treatment. N = 15 tumors (15 mice). The boxplot indicates median; hinges indicate first and third quartiles; whiskers indicate range up to 1.5 IQR from the hinge.

(j) Dotplot of rebound (day 22 of treatment, beginning of Switch 2) tumor volumes relative to day 0 tumor volumes for mice in gene drive cohort of long-term survival study. The pink dot indicates the same tumor that progressed on treatment. N = 15 tumors (15 mice). The boxplot indicates summary statistics as in **(i)**.

(k) Mouse weight (relative to day 0 of treatment) for control (orange) and gene drive (dark blue) arms of the long-term survival study. Arrows indicate phase of treatment. N = 10 tumors (10 mice) in 0% gene drive cohort; N = 12 tumors (12 mice) in 10% gene drive cohort; data are presented as mean +/- SEM.

Supplemental Appendix 1
Compartmental Model:
Mutational Risk

1 Introduction

We develop a theoretical framework for selection gene drive therapy to assess the mutational risks of the system. In the model, cancer evolution is simulated stochastically as a continuous birth-death-mutation process. Subpopulations resistant to therapeutic action, either by targeted therapy or activated prodrug, are included to evaluate sources of potential treatment failure. The system is solved for a range of parameter values to derive selection gene drive design criteria and identify tumor characteristics where success is predicted to be most likely.

2 Compartmental Model

2.1 Model Description

The model considers an initial population of 100 drug-sensitive cells that expand in the absence of drug treatment. Mutation events spawn subclones resistant to therapy at a rate μ. Once the cell population reaches a predefined detection size M, a fraction (q) of cells are modified with the gene drive construct and assigned appropriate parameters. At the same time, targeted therapy is initiated and Switch 1 is engaged. During the Switch 1 phase of treatment, gene drive cells retain a positive net growth rate until they reach population size M, whereupon Switch 2 is engaged. During the Switch 2 phase of treatment, gene drive cells manufacture a diffusible therapeutic capable of killing both modified and unmodified tumor cells. The end of Switch 1 engagement may extend beyond the initiation of the Switch 2 phase (switch overlap), such that gene drive cells continue to be rescued from targeted therapy action while also producing a toxic agent.

2.2 Modeled Populations

In total, eight tumor cell subpopulations are modeled . These include sensitive wild-type cells, cells resistant to targeted therapy, cells resistant to Switch 2

bystander activity, and cross-resistant cells insensitive to both forms of therapy. Additionally, the model considers gene drive cells, as well as those with acquired resistance to either or both forms of therapy. Populations can seed resistance subclones according to the mutational map in **Figure 1d**. In brief, sensitive and gene drive cells can acquire resistance to either form of therapy. Additionally, gene drive cells can lose expression of the gene drive system.

3 Simulation

3.1 Parameter Description

For all genotypes, birth (β) and death (δ) rates are 0.14 and 0.13 /day, respectively, as in Bozic et al.[**1**] Among subpopulations sensitive to targeted therapy or bystander activity, the drug kill rates (α_T and α_B) are 0.04 /day.[**1**] Resistant populations are completely insensitive to drug effect (drug kill rate of 0). The model also considers a fitness cost among gene drive cells relative to native resistant populations. The bystander effect of Switch 2 is modeled by scaling the drug kill rate α_B by the proportion of tumor cells that express the Switch 2 gene.

Key parameters relating to gene drive function were allowed to vary: gene delivery/modification efficiency (q) from 10^{-3} to $10^{-0.5}$; and gene drive net growth (γ_{gd}) from 0.004 to 0.01 /day (corresponding to 40% and 100% of native resistance net growth, respectively). For the sensitivity analysis, additional parameter sweeps were conducted. In general, one parameter varied at a time while all other values were fixed at a base value. These parameters are described in the table below:

Appendix Table 1.1: Compartmental Model Parameters

Description	Parameter	Base	Min	Max	Unit
Net growth rate	γ	0.01	0.005	0.025	[/day]
Turnover rate	τ	14	1	21	[]
Tumor detection size	M	10^9	10^8	10^{11}	[cells]
Mutation rate	μ	10^{-8}	10^{-9}	10^{-6}	[/division]
Switch delay	–	365	0	1200	[days]

where $\gamma = \beta - \delta$ and $\tau = \beta/\gamma$, i.e. $\beta = \gamma\tau$ and $\delta = \gamma(\tau - 1)$.

3.2 Simulation Details

The described system was solved stochastically using a custom Gillespie algorithm[**2**] with adaptive tau leaping[**3**] using MATLAB. The abbreviated propensity func-

tion is:

$$a(x) = \frac{1}{a_0} \begin{bmatrix} \beta x_S \\ \delta x_S \\ \mu_R \beta x_S \\ \mu_M \beta x_S \\ \alpha_{T,S} x_S \\ \alpha_{A,S} x_S \\ \phi \alpha_{B,S} x_S \\ \beta x_R \\ \delta x_R \\ \mu_D \beta x_R \\ \alpha_{T,R} x_R \\ \alpha_{A,R} x_R \\ \phi \alpha_{B,R} x_R \\ \vdots \end{bmatrix} \tag{1}$$

where S, R, etc. denote cellular genotype. $\alpha_{T,X}$ is the genotype-specific targeted therapy drug kill rate, $\alpha_{A,X}$ is the genotype-specific activated prodrug drug kill rate by direct action (0 for populations not expressing the gene drive system), and $\alpha_{B,X}$ is the genotype-specific activated prodrug drug kill rate by bystander action. The scaling factor ϕ is the proportion of cells expressing the gene drive system. a_0 is the sum of the rates of the propensity vector.

The abbreviated state change matrix is:

$$\nu = \begin{bmatrix} 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 \\ -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & -1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 \\ 0 & -1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & -1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & -1 & 0 & 0 & 0 & 0 & 0 & 0 \\ \vdots & \vdots & \vdots & \vdots & \vdots & \vdots & \vdots & \vdots \end{bmatrix}$$

3.3 Simulation Conditions

Each combination of parameters was simulated independently 48 times using MATLAB's *parfor* function. The simulation code is available on GitHub: `github.com/pritchardlabatpsu/SelectionGeneDrives/Figure1/CompartmentalModel`.

References

[1] Bozic I, Reiter JG, Allen B, Antal T, Chatterjee K, Shah P, Moon YS, Yaqubie A, Kelly N, Le DT, Lipson EJ, Chapman PB, Diaz LA Jr, Vogelstein B, Nowak MA. Evolutionary dynamics of cancer in response to targeted combination therapy. Elife. 2013 Jun 25;2:e00747. doi: 10.7554/eLife.00747.

[2] Gillespie DT. Exact Stochastic Simulation of Coupled Chemical Reactions. J Phys Chem. 1977 Dec 1;81, 25, 2340–2361. doi: 10.1021/j100540a008

[3] Cao Y, Gillespie DT, Petzold LR. Efficient step size selection for the tau-leaping simulation method. J Chem Phys. 2006 Jan 28;124(4):044109. doi: 10.1063/1.2159468.

Supplemental Appendix 2
Agent-Based Model:
Spatial Risk

1 Introduction

We develop a stochastic agent-based model of tumor dynamics to assess the spatial risks of selection gene therapy. In the model, a virtual tumor is initiated with gene drive cells seeded according to a dispersion parameter. Tumor dynamics are solved using an exact birth-death process. Under the Switch 2 phase of treatment, unmodified cells within a predefined radius of gene drive cells are subject to bystander activity. The system is solved for a range of parameter values to determine the effects of spatial heterogeneity and bystander action distance on the predicted success of selection gene drive therapy.

2 ABM Description

2.1 Initial Cell Seeding

In the model, a mixed population of 10^4 tumor cells (0.5% resistant and 5% gene drive) is initiated on a 3D lattice. Cell positions are assigned according to a random distribution weighted by the exponential of the negative of the distance from the origin, to establish an approximate spherical geometry. Resistant cells are sampled uniformly from this population and assigned appropriate parameters. Gene drive cells, however, are centered around a "focus of delivery." This focus is randomly determined for each simulation. The positions of gene drive cells are drawn from a distribution weighted according to distance of site x from the focus:

$$P(X = x) = \frac{1}{\sum_{x \in N} e^{-\theta r(x)}} e^{-\theta r(x)} \tag{1}$$

where N is the set of all sites occupied by cells, $r(x)$ is the distance between site x and the focus, and θ is the gene drive dispersion parameter. Thus, when the value of θ is large, gene drive cells are seeded tightly around the focus. Conversely, when the value of θ is small, positioning is effectively random and gene drive cells are seeded uniformly. Cells not assigned a resistant or gene drive phenotype are designated sensitive.

2.2 Population Dynamics

After seeding, tumor treatment is simulated according to an exact birth-death process using a custom agent-based algorithm. For each step of the simulation, the next event is drawn from a vector with weights according to birth (β), death (δ), and drug kill (α) rates. Then, a cell from the appropriate population is randomly sampled. In the case of a birth event, the model repositions nearby cells (described in **Secion 2.3**) as necessary and then a neighboring cell is added. In the case of a death event, the cell is removed.

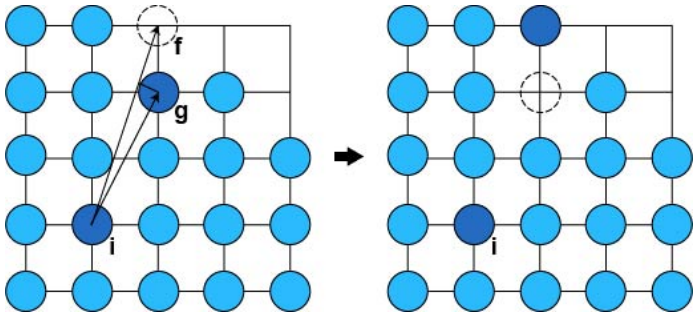
Sensitive and resistant cells exhibit a net negative and positive growth rate, respectively, under targeted therapy treatment. Gene drive cells retain a resistant phenotype under Switch 1 treatment, but are sensitive otherwise.

Switch 2 is initiated when the number of gene drive cells exceeds 80% the initial total number of cells. Under Switch 2 treatment, gene drive cells and their neighbors (up to ρ units away) are subject to the bystander activity of the activated prodrug (drug kill rate α_B).

As the activated prodrug is expected to remain in the immediate environment of a gene drive cell following its death, the model maintains bystander activity of the dying gene drive cell for a period of time. That is, if an unmodified (i.e. sensitive or resistant) cell is subject to bystander activity from a nearby gene drive cell (within ρ units) and that gene drive cell dies (and no other gene drive cells are acting on the unmodified cell), the unmodified cell remains subject to bystander activity. This residual bystander activity is cleared according to a decay parameter τ.

2.3 Cell Budging

When a cell divides, the new cell is randomly assigned the position of an unoccupied adjacent site, if any exist. If all adjacent sites are occupied, the nearby cells "budge" to provide space for the new daughter cell. The algorithm identifies the nearest unoccupied site by first searching for sites within $k = 1$ units of the dividing cell i. If no available sites are found, sites within $k = 2$ units are evaluated, and so on. Once the nearest unoccupied site (site f) is determined, the cells between cell i and site f are moved to make room for the new daughter cell. This is done by identifying the cell g adjacent to site f that is closest to the line between i and f (distance to the line is calculated using the parallelogram definition of the cross-product, i.e. $||\vec{gf} \times \vec{if}||/||\vec{if}||$; **Supplemental Figure 2.1**). Cell g is moved to site f and the process is repeated with the new vacant site g until the vacant site is adjacent to cell i. Then the new daughter cell is assigned the adjacent position.



Supplemental Appendix Figure 2.1: Diagram of the "budging" algorithm for division events. The vacant site f nearest to dividing cell i is identified, and the cell g adjacent to f and nearest the vector from i to f is moved to occupy site f. The process is repeated until the vacant site is adjacent to i.

2.4 Pseudocode

Algorithm 1: Spatial Agent-Based Model Algorithm

```
initialize tumor cells centered at the origin (build position matrix);
assign gene drive cells according to dispersion parameter (build state
 vector);
assign resistant cells according to uniform distribution (update state
 vector);
identify sensitive and resistant cells within ρ units of gene drive cells
 (termed "bystander" cells; update state vector);
while viable cells remain and tumor has not relapsed on treatment do
    if number of gene drive cells exceeds 80% initial tumor size then
        begin Switch 2 (update drug kill terms);
    draw time to next event from exponential distribution;
    sample next event from distribution weighted by cell dynamics rates
     and state matrix;
    if next event is division then
        draw cell i from appropriate subpopulation;
        scan lattice units near cell i for unoccupied site;
        while nearest unoccupied site not adjacent to cell i do
            move intermediate cell g into unoccupied site;
        add new cell to position matrix;
        assign genotype to match cell in state vector i;
    else if next event is cell death then
        draw cell i from appropriate subpopulation;
        remove cell i from position matrix and state vector;
    else if next event is bystander activity clearance then
        reclassify cells subject to residual bystander effect from a dead
         gene drive cell as not "bystander" cells
    determine new sensitive and resistant cells within ρ units of gene
     drive cells and classify as "bystander" cells update position matrix
     and state vector;
    update index variable;
if tumor relapsed on treatment then
    record final tumor population structure
```

3 Simulation

3.1 Parameter Description

For all genotypes, birth (β) and (δ) rates are 0.14 and 0.13 /day, respectively, as in Bozic et al.[**1**] Among subpopulations sensitive to targeted therapy (α_T) or bystander activity (α_B), the drug kill rates are 0.04/day.[**1**] Resistant cells are completely insensitive to drug effect (drug kill rate of 0). All cells within the

bystander effect radius ρ of a gene drive cell are subject to killing by the activated prodrug. Residual bystander activity following the death of a gene drive cell is cleared according to the decay rate $\tau = 0.3$ /day. Key spatial parameters relating to gene drive function were allowed to vary: bystander effect radius (ρ) from 0 to 5 cell lengths; and gene drive dispersion parameter (θ) from 0 to 1, corresponding to 100% and $\sim$0% theoretical maximal dispersion, respectively.

3.2 Simulation Conditions

Each combination of parameters was simulated independently 25 times using MATLAB's *parfor* function. The simulation code is available on GitHub: `github.com/pritchardlabatpsu/SelectionGeneDrives/Figure1/SpatialABM`.

References

[1] Bozic I, Reiter JG, Allen B, Antal T, Chatterjee K, Shah P, Moon YS, Yaqubie A, Kelly N, Le DT, Lipson EJ, Chapman PB, Diaz LA Jr, Vogelstein B, Nowak MA. Evolutionary dynamics of cancer in response to targeted combination therapy. Elife. 2013 Jun 25;2:e00747. doi: 10.7554/eLife.00747.