A framework for detecting driver candidates and periods of positive growth during tumor progression

Figure 1

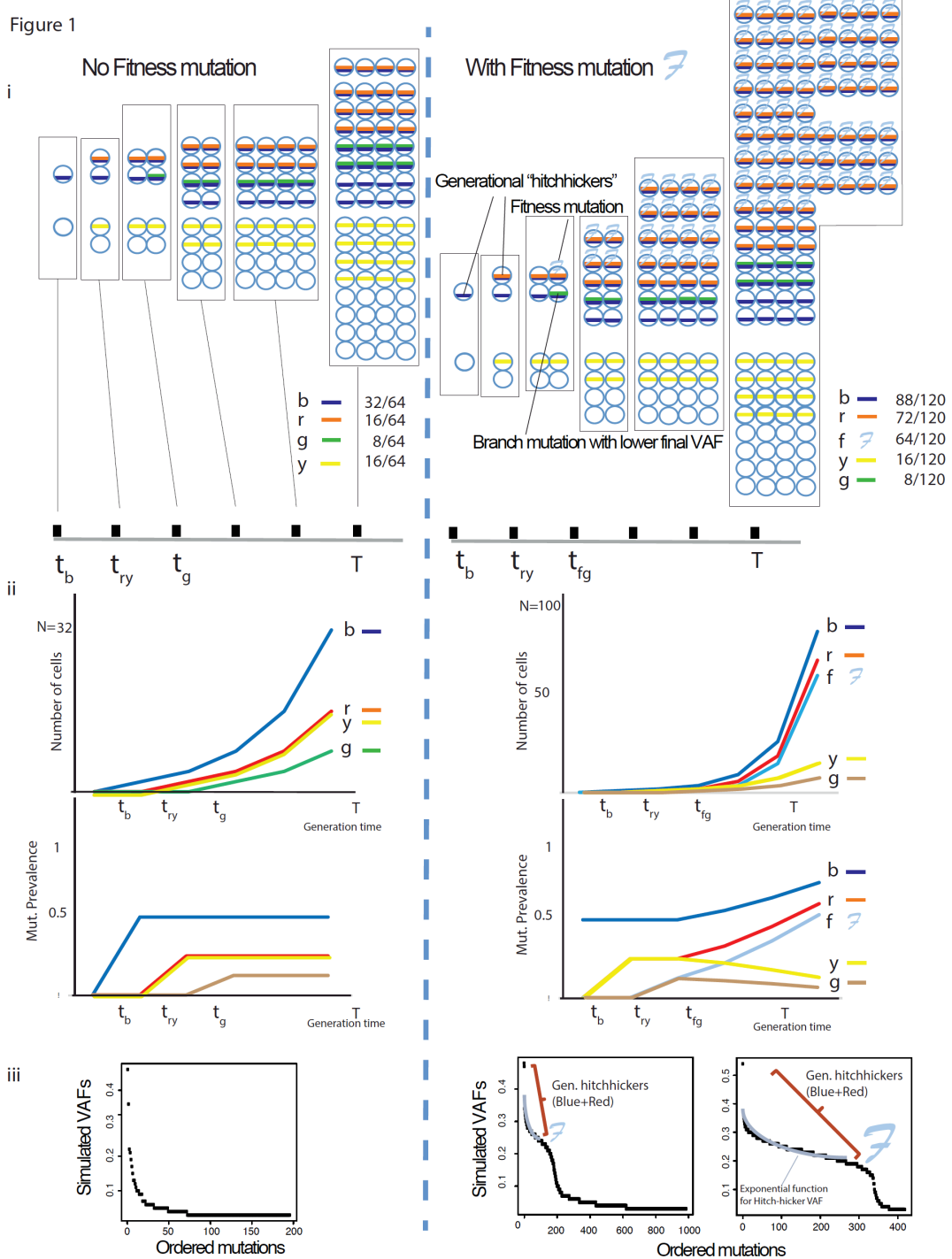
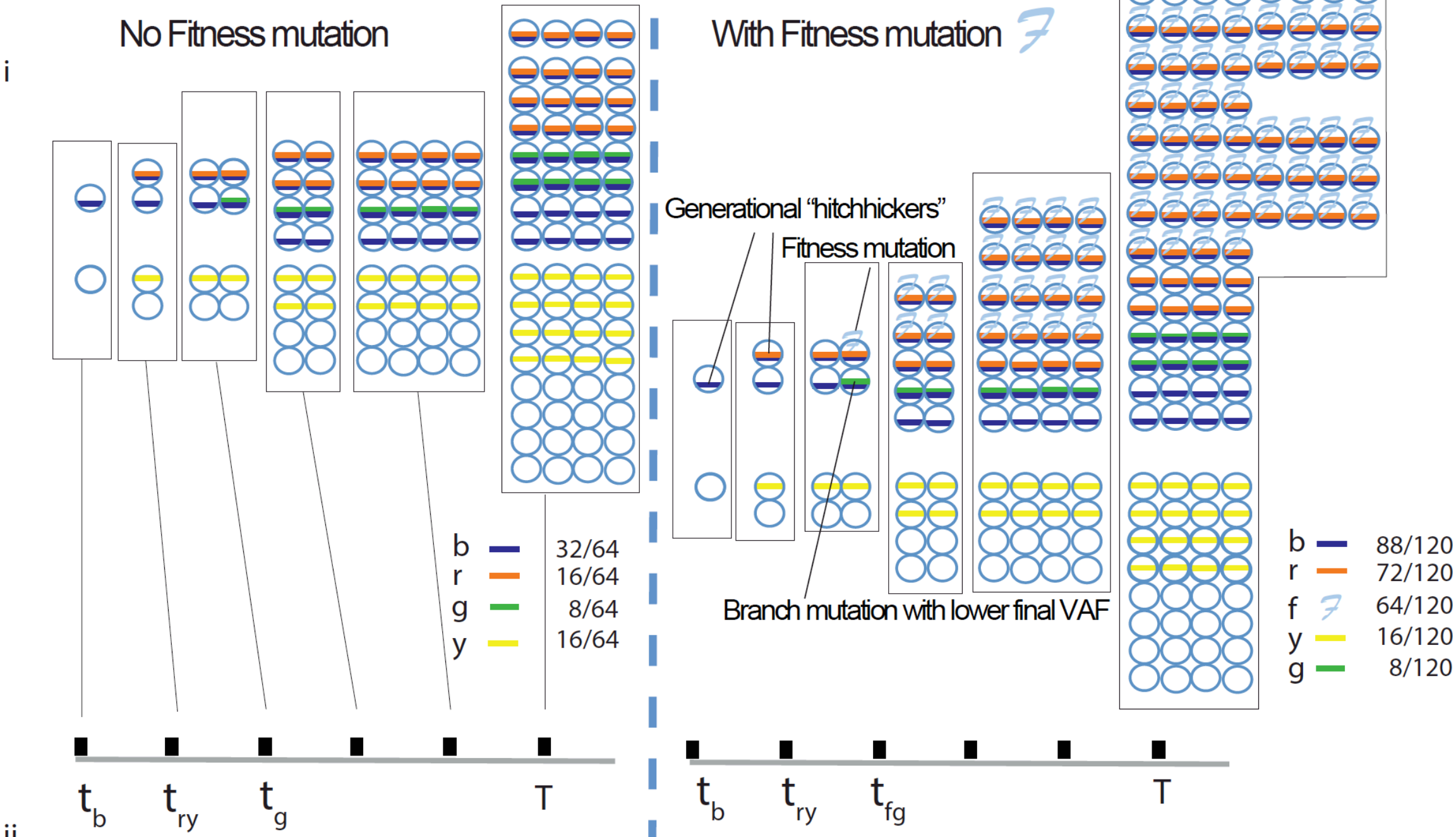
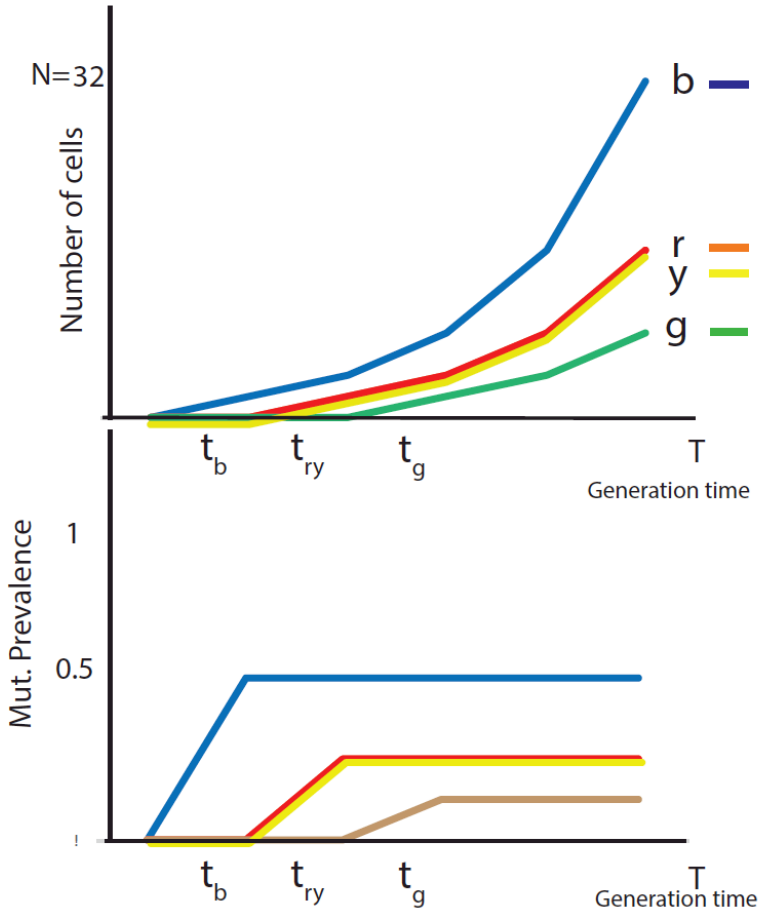
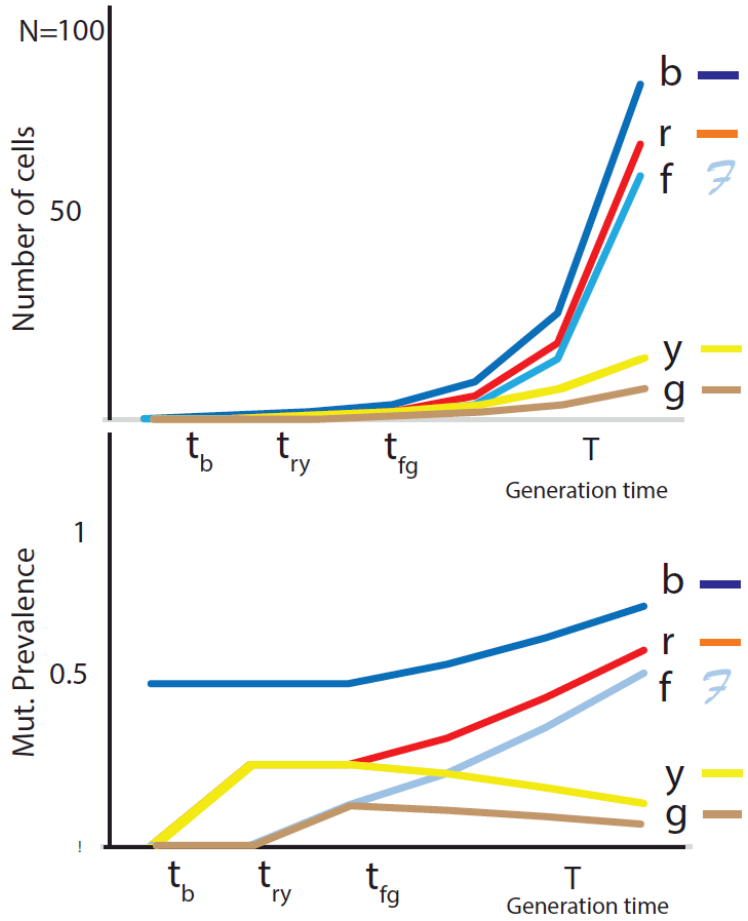

No Fitness mutation
With Fitness mutation
i
Generational "hitchhickers"
Fitness mutation
Branch mutation with lower final VAF
b 32/64
r 16/64
g 8/64
y 16/64
b 88/120
r 72/120
f 64/120
y 16/120
g 8/120
t_b t_{ry} t_g T
t_b t_{ry} t_{fg} T
ii
N=32
N=100
Number of cells
50
Generation time
Mut. Prevalence
1
0.5
iii
Simulated VAFs
Ordered mutations
Gen. hitchhickers (Blue+Red)
Exponential function for Hitch-hicker VAF

Figure 1

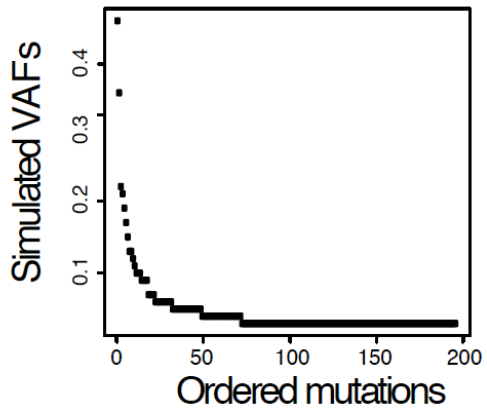

i
No Fitness mutation
With Fitness mutation
Generational "hitchhickers"
Fitness mutation
Branch mutation with lower final VAF
b 32/64
r 16/64
g 8/64
y 16/64
b 88/120
r 72/120
f 64/120
y 16/120
g 8/120
t_b
t_{ry}
t_g
T
t_b
t_{ry}
t_{fg}
T
ii

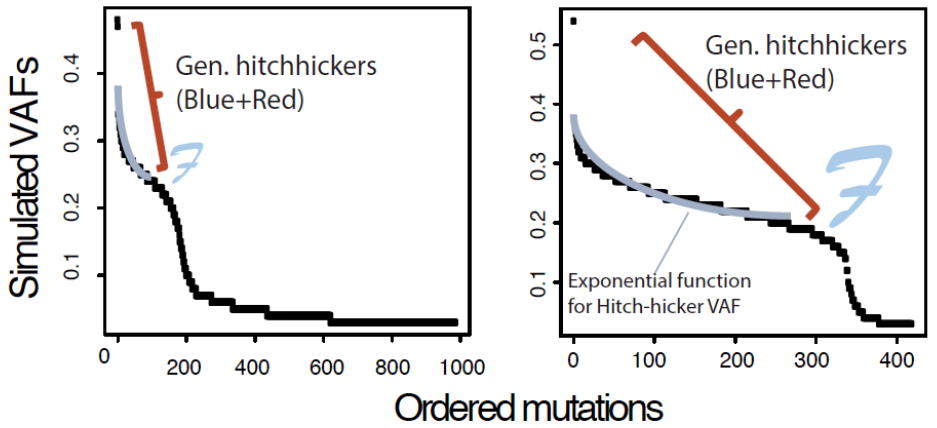
ii


N=32
Number of cells
b
r
y
g
t_b
t_{ry}
t_g
T
Generation time
Mut. Prevalence
1
0.5
0


N=100
Number of cells
50
b
r
f
y
g
t_b
t_{ry}
t_{fg}
T
Generation time
Mut. Prevalence
1
0.5
0

iii


Simulated VAFs
0.1
0.2
0.3
0.4
0
50
100
150
200
Ordered mutations


Simulated VAFs
0.1
0.2
0.3
0.4
Gen. hitchhickers
(Blue+Red)
f
0
200
400
600
800
1000
Ordered mutations
0.5
Exponential function
for Hitch-hicker VAF
100
300

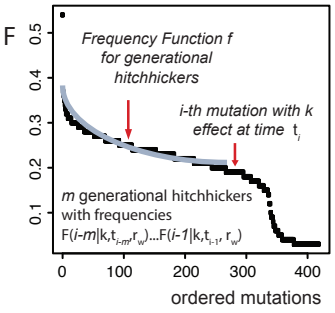
Using a single tumor

VCF file

Normalize VAF (based on ploidy, CNV and purity) to assign mutational frequency F

Use subclonal Information for each mutation to select non-overlapping subclones

Order mutations from earlier to last



For each mutation i > m using m generational hitchickers

A) estimate of growth 'r'

B) NLS optimization for effect 'k' as:

$$\mathbb{Q}(k, t_g \mid r, F, m) = \sum_m (F_m - (A * e^{-r(m+t_g)} + B))^2$$

C) Identify peaks 'K_p'

Output

$$\vec{r} = \{r_1, r_2, .. r_n\} \in \mathcal{R}^N_{+/-}$$
$$\vec{k} = \{k_1, k_2, .. k_n\} \in \mathcal{R}^N_{+}$$
$$\overrightarrow{Kp} = \{k_1, k_2, .. k_{p<n}\} \in \mathcal{R}^N_{+}, \quad where\ \overrightarrow{Kp} \subset \vec{k}$$

Glossary

Variant Allele Frequency (VAF): The fraction of sequencing reads overlapping a genomic coordinate that support the non-reference allele. This fraction can be further normalized based on the sample's ploidy and purity.

Variant Call Format (VCF) file: A text file format that includes sequencing information such as the position and frequency of every mutation in the sample.

Subclone: Cells that belong to a single lineage during population growth. Within the subclone, a higher mutational frequency is associated with an earlier time of occurence.

Linear subclones: A population growth where every subclone has at most one child subclone (e.g. subclone A -> Subclone B -> Subclone C).

Fitness mutation: A mutation that increases the growth of the population. Typically, a fitness mutation might lead to the formation of a subclone.

Generational hitchhiker (g-hitchiker): a hitchhike mutation that occurred before the fitness mutation. They have increased VAF (higher than their respective fitness mutation) and represent generational time as their respective branching mutations have typically low VAF (see figure 1).

Growth *r*: Before the **fitness mutation**, the population was growing with a rate *r*. In our model, we use the prevalence of **generational hitchhikers** to estimate **growth *r***.

Fitness effect k_i: After the **fitness mutation** *i* occurs, the population is growing with rate $k_i * r$.

Frequency F(i): The frequency for mutation *i* at time of sequencing.

Frequency Function $f(F, t_g, t_{i-m})$: The function that describes the frequency F(*i-m*) for m **g-hitchhikers** occurring before **fitness mutation** *i*.

Generational time t_g: A local re-optimized constant to nullify time for *m* respective **g-hitchhikers.**

Growth vector $\vec{r}$: For each mutation i>m in the tumor sample we estimate **growth** r_{i-1}.

Effect vector $\vec{k}$: For each mutation i>m in the tumor sample we estimate **fitness effect** k_i

Peak vector $\overrightarrow{Kp}$: Local peaks for **effect vector** $\vec{k}$ correspond to **fitness mutations** with **effect** k_i.

Optimizing function $\mathbb{Q}(k, t_g \mid r, F, m)$: Using the **Frequency F** of *m* **g-hitchhikers** occurring before mutation *i*, we use a nonlinear least square (NLS) fitting to calculate **effect** k_i and **generational time** t_g.

Positive Growth Enrichment(PGE): A type of mutation (eg. missense TP53) is assessed whether it occurs significantly more often than random during periods of positive **growth *r***.

Frequency function f() for m "hitchhickers" (with $F_m > F_i$)

$$F(T, t_{i-m}) = \frac{e^{-r_w * t_{i-m}} * [\, N_{tot} - F(t_i) * N_{tot} + \sqrt[k_i]{F(t_i) * N_{tot}}\,] + F(t_i) * N_{tot} - \sqrt[k_i]{F(t_i) * N_{tot}}}{N_{tot}}$$

r_w: growth r corresponding to window [*i-m*, *i*-1]

t_{i-m}: the time when the (*i-m*)-th mutation occured

k_i: the effect K of the i-th mutation

$F(t_i)$: the frequency of the hypothetical fitness mutation *i*

N_{tot}: the total number of mutations

Frequency function for m "hitchhickers" with local reoptimization

$$F(T, t_g, t_{i-m}) = \frac{e^{-r(t_g + t_{i-m})} * (N_{tot} - F(t_i) * N_{tot} + \sqrt[k_i]{F(t_i) * N_{tot}}) + F(t_i) * N_{tot} - \sqrt[k_i]{F(t_i) * N_{tot}}}{N_{tot}}$$

t_g: locally optimized generational time to adjust for local hitchhickers

Using M multiple tumors

A) Estimate **Positive Growth Enrichment (PGE):**

Across all M tumors,
A **type** of mutation (eg. all *missense TP53* mutations, or *all premature-stop* mutations *in Tumor Suppressor Genes*),
found w times in M tumors,
is **enriched during positive growth** if:
mutational growth $r_{mut} > 0$ more often than random

B) Estimate the range of effect k (eg. [1.2-1.4]) within a type of mutation based on enrichment

Figure 2

Simulations

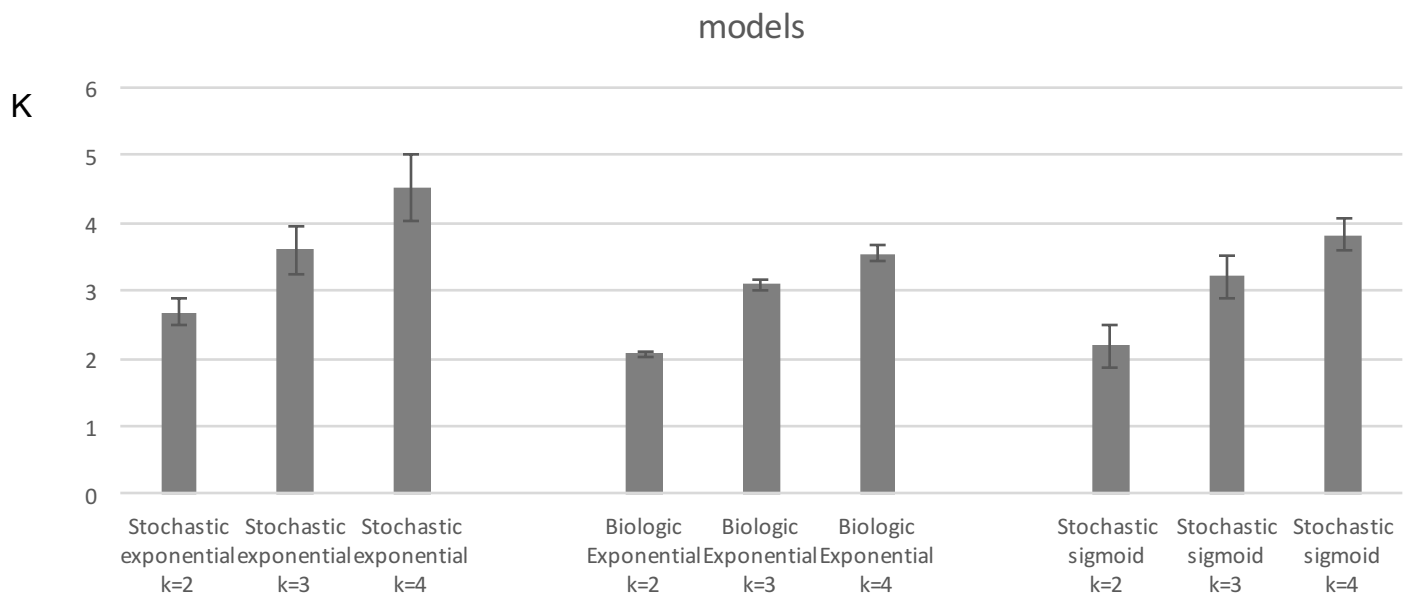
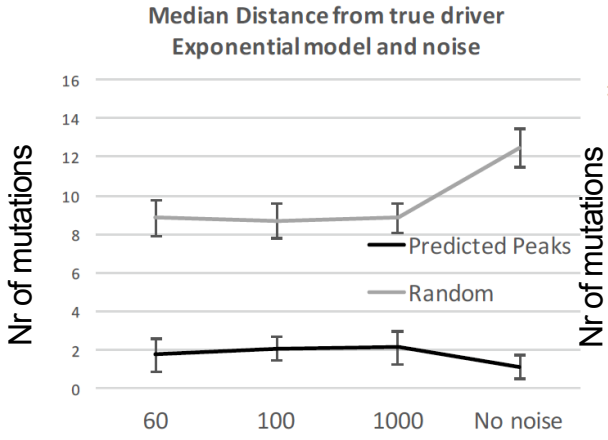
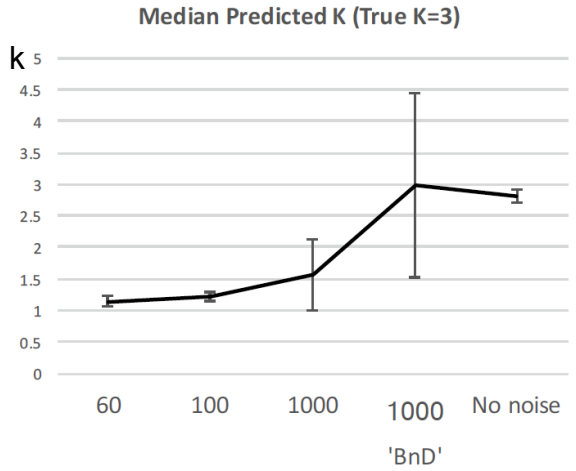
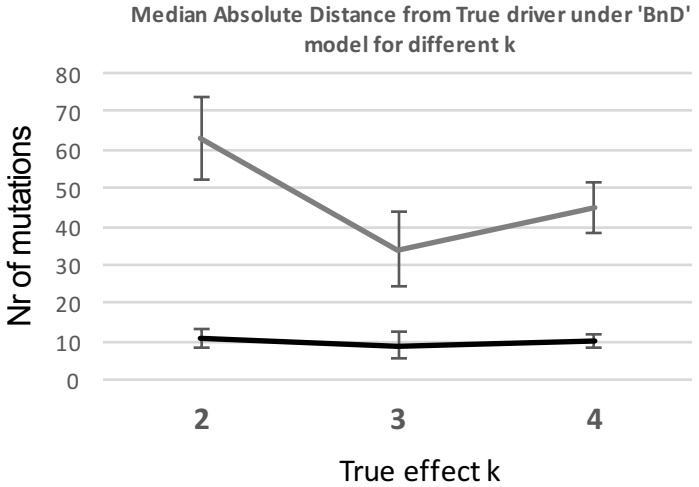


Figure 2

Driver Prediction Under Exponential and 'BnD' Model With Noise (coverage)

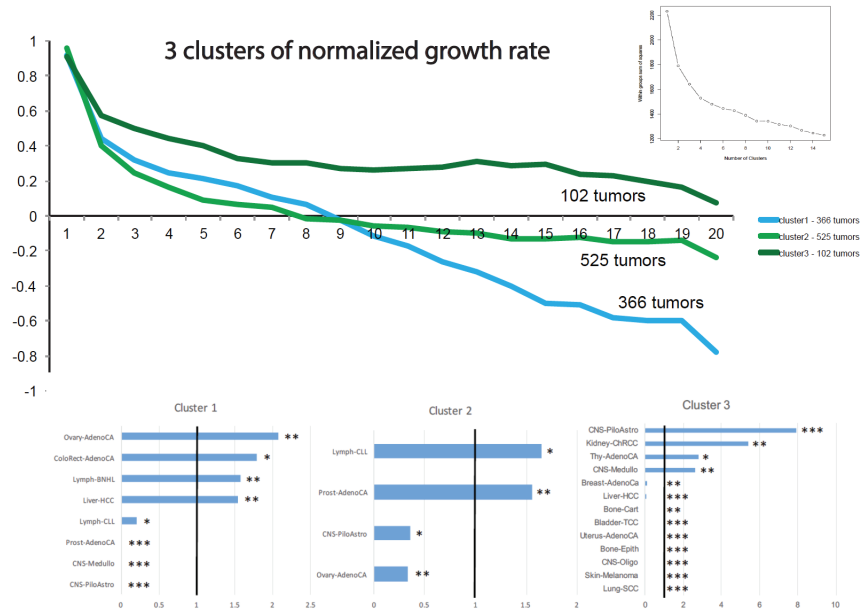




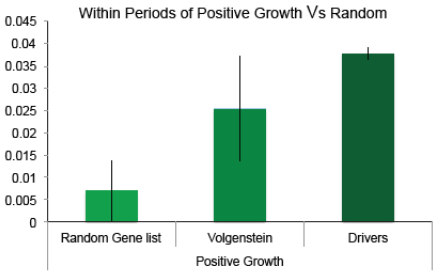


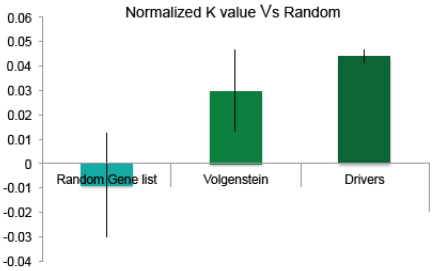
3i

Tumor growth patterns



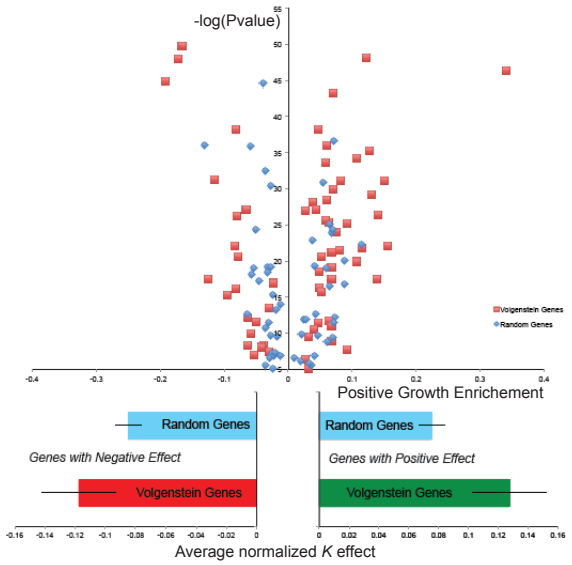
3ii





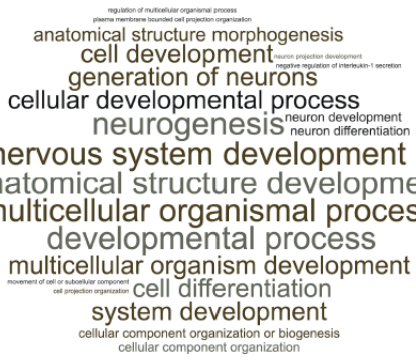
Pcawg Drivers and Volgenstein gene (VG) mutations appear enriched during periods of positive growth

3iii



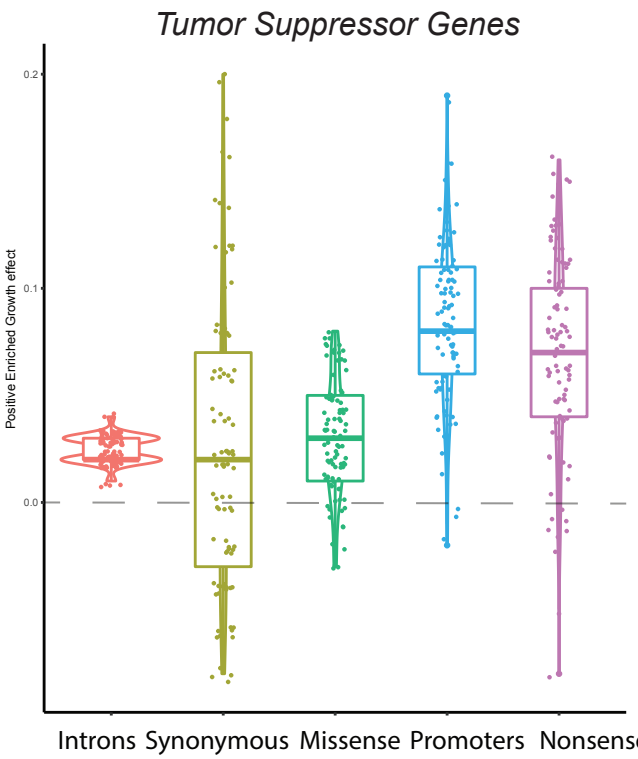
Positive and negative growth enrichment for Volgenstein genes vs similarly mutated random genes

3iv

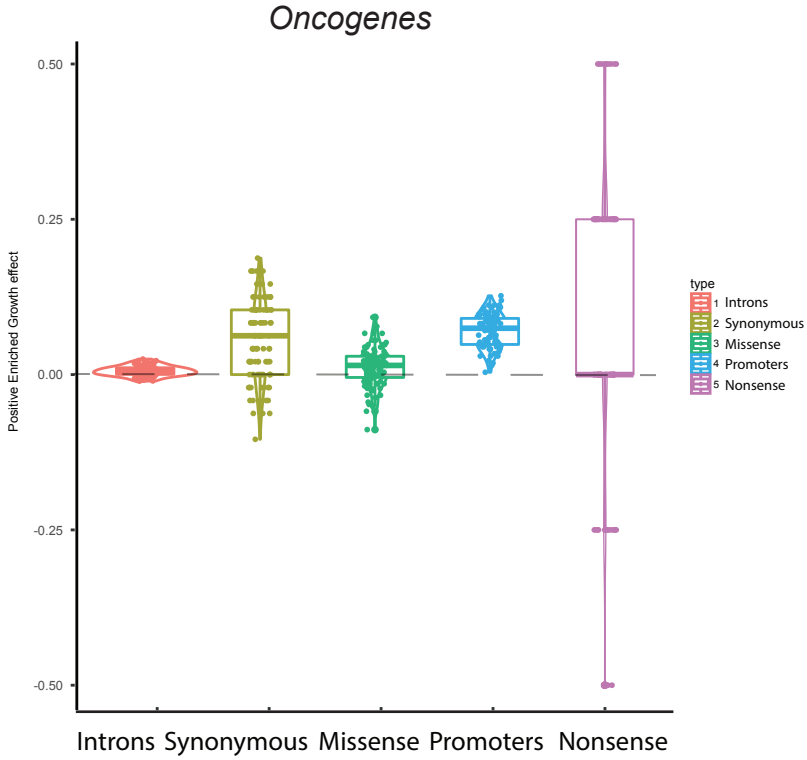


Gene Ontology enrichment analysis for the 150 most selected genes

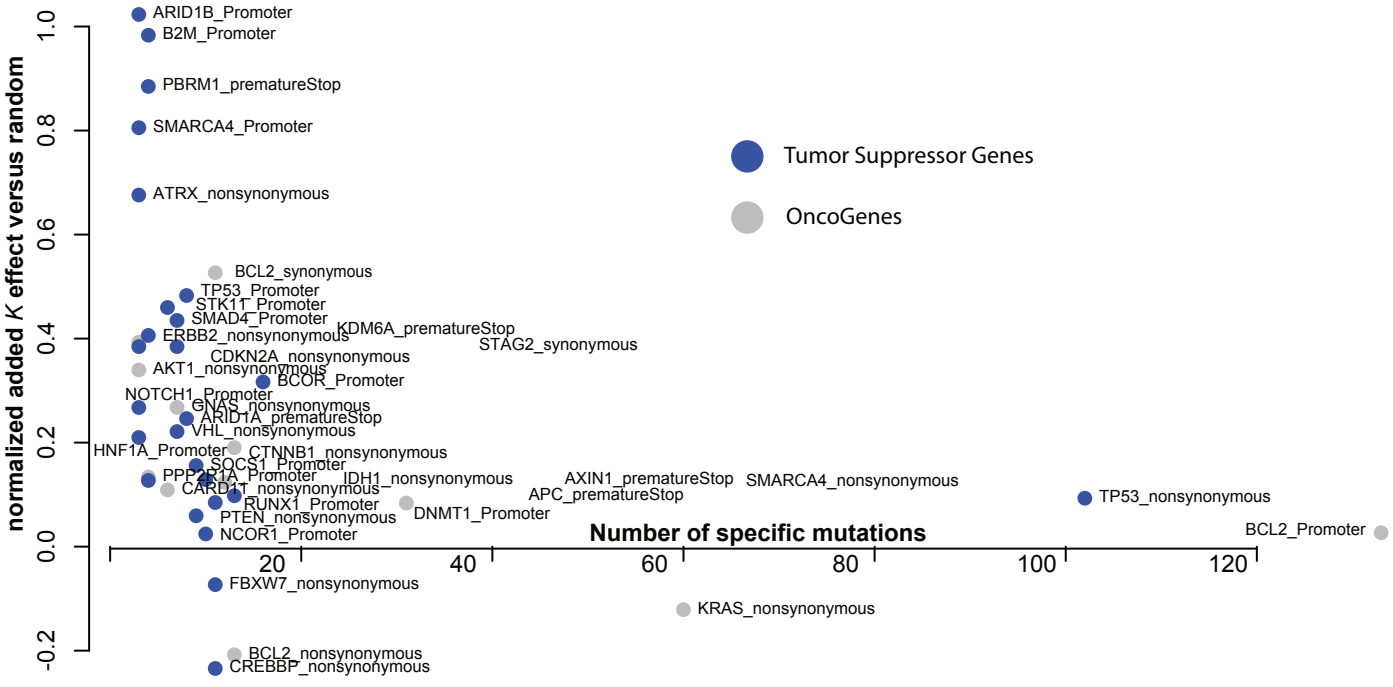
4i



4ii



4iii

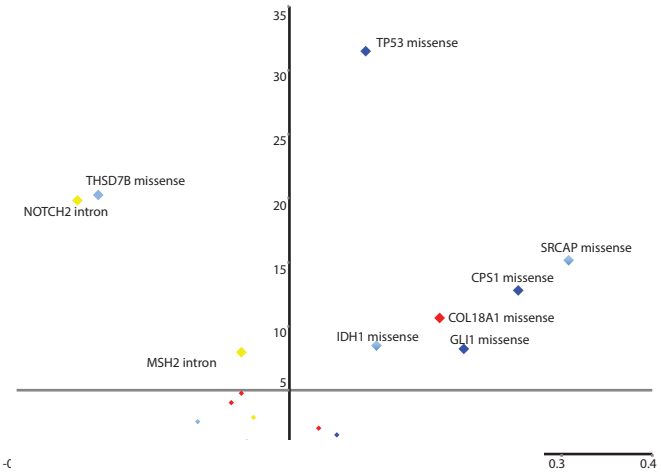


Enrichment for all significant VG mutations (categories)

Figure 5

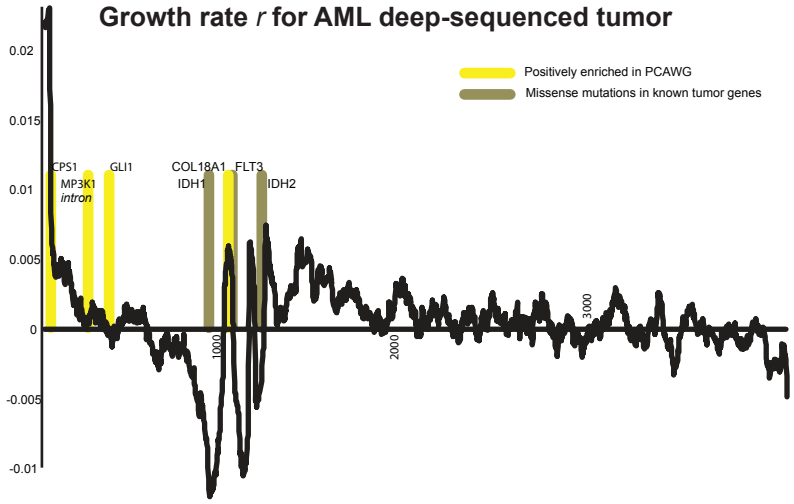
Testing all missense and VG mutations from AML300 tumor for PGE

5i

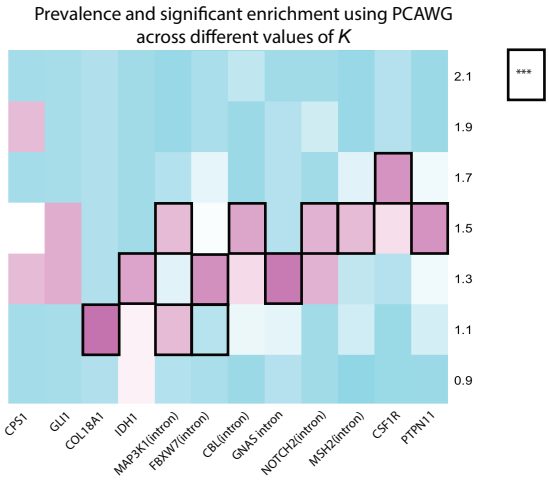


Associating with AML300's tumor growth all known tumor and Positively enriched mutations

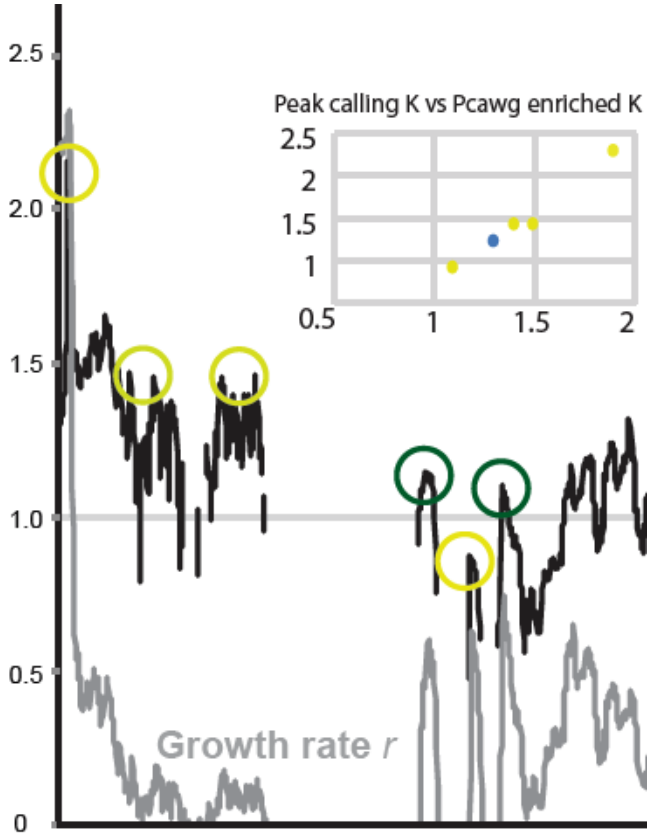
5ii



5iii



5iv



K-effect peaks for AML deep-sequenced tumor