Figure 1

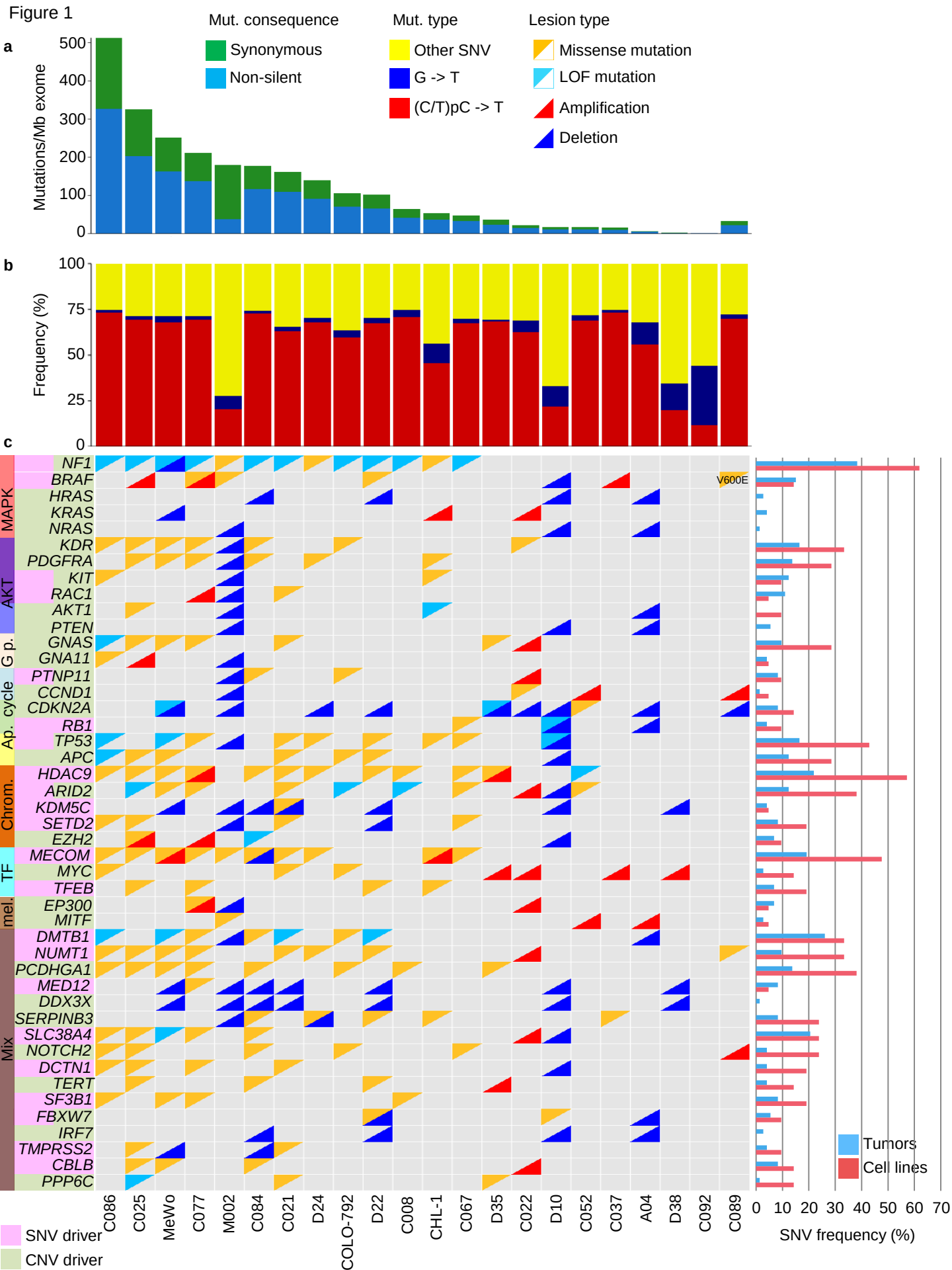
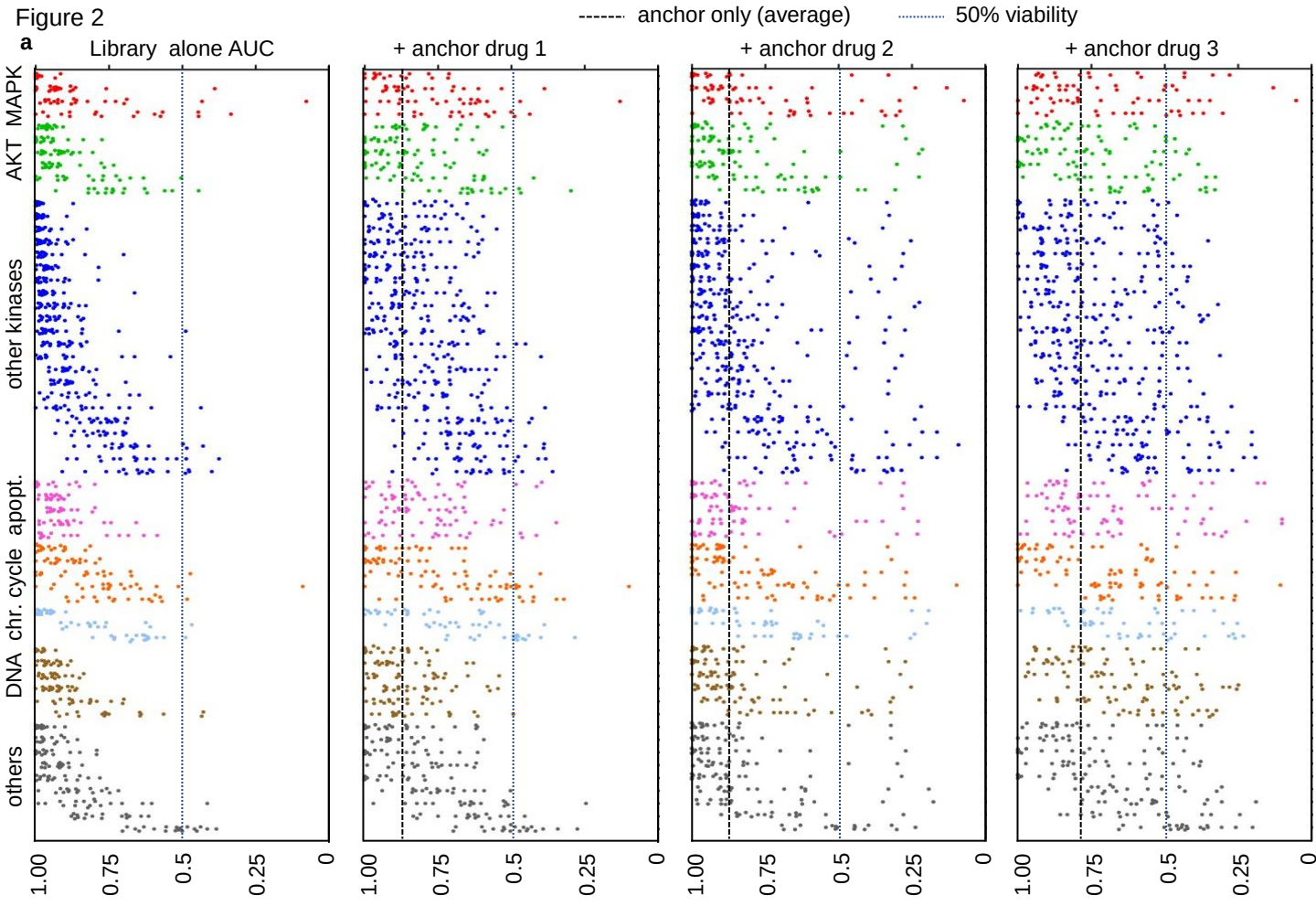
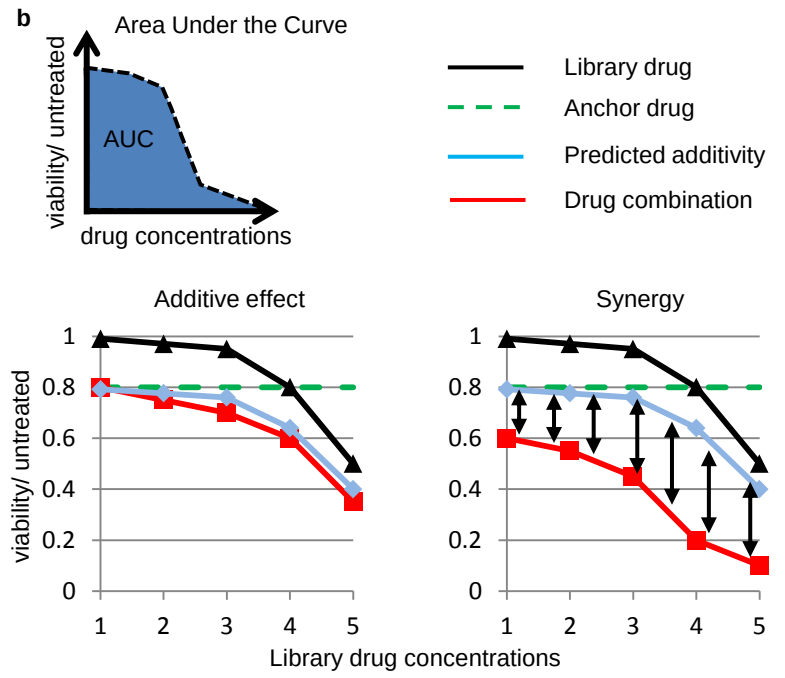
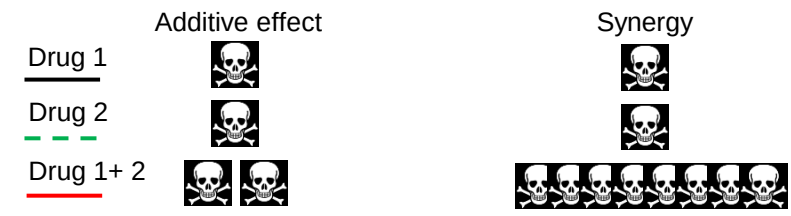
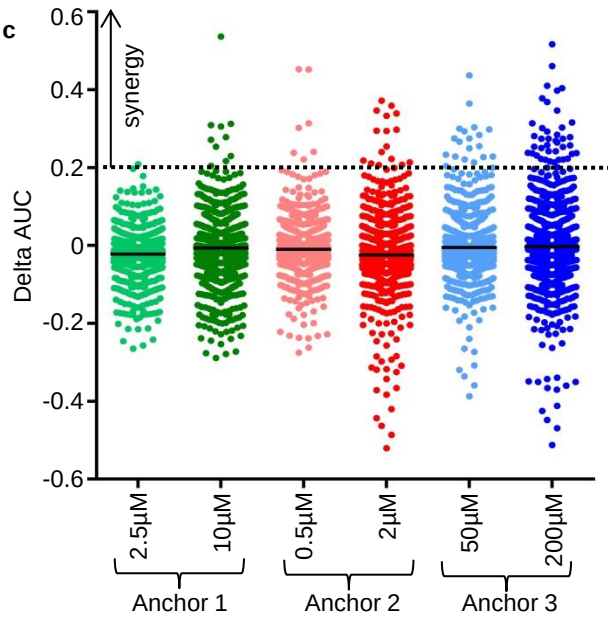

Mut. consequence
Synonymous
Non-silent
Mut. type
Other SNV
G -> T
(C/T)pC -> T
Lesion type
Missense mutation
LOF mutation
Amplification
Deletion
a
Mutations/Mb exome
500
400
300
200
100
0
b
Frequency (%)
100
75
50
25
0
c
MAPK
AKT
G p.
Ap. cycle
Chrom.
TF
mel.
Mix
NF1
BRAF
HRAS
KRAS
NRAS
KDR
PDGFRA
KIT
RAC1
AKT1
PTEN
GNAS
GNA11
PTNP11
CCND1
CDKN2A
RB1
TP53
APC
HDAC9
ARID2
KDM5C
SETD2
EZH2
MECOM
MYC
TFEB
EP300
MITF
DMTB1
NUMT1
PCDHGA1
MED12
DDX3X
SERPINB3
SLC38A4
NOTCH2
DCTN1
TERT
SF3B1
FBXW7
IRF7
TMPRSS2
CBLB
PPP6C
V600E
C086
C025
MeWo
C077
M002
C084
C021
D24
COLO-792
D22
C008
CHL-1
C067
D35
C022
D10
C052
C037
A04
D38
C092
C089
Tumors
Cell lines
0
10
20
30
40
50
60
70
SNV frequency (%)
SNV driver
CNV driver

Figure 2


a
anchor only (average)
50% viability
Library alone AUC
+ anchor drug 1
+ anchor drug 2
+ anchor drug 3
MAPK
AKT
other kinases
apopt.
chr. cycle
DNA
others
1.00
0.75
0.5
0.25
0


b
Area Under the Curve
viability/ untreated
AUC
drug concentrations
Library drug
Anchor drug
Predicted additivity
Drug combination
Additive effect
Synergy
viability/ untreated
Library drug concentrations


Additive effect
Synergy
Drug 1
Drug 2
Drug 1+ 2


c
synergy
Delta AUC
2.5μM
10μM
0.5μM
2μM
50μM
200μM
Anchor 1
Anchor 2
Anchor 3

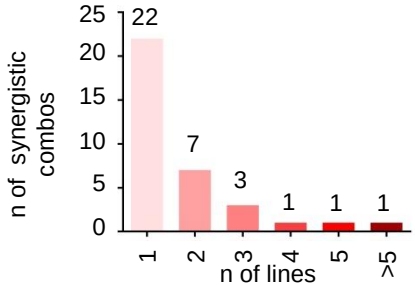
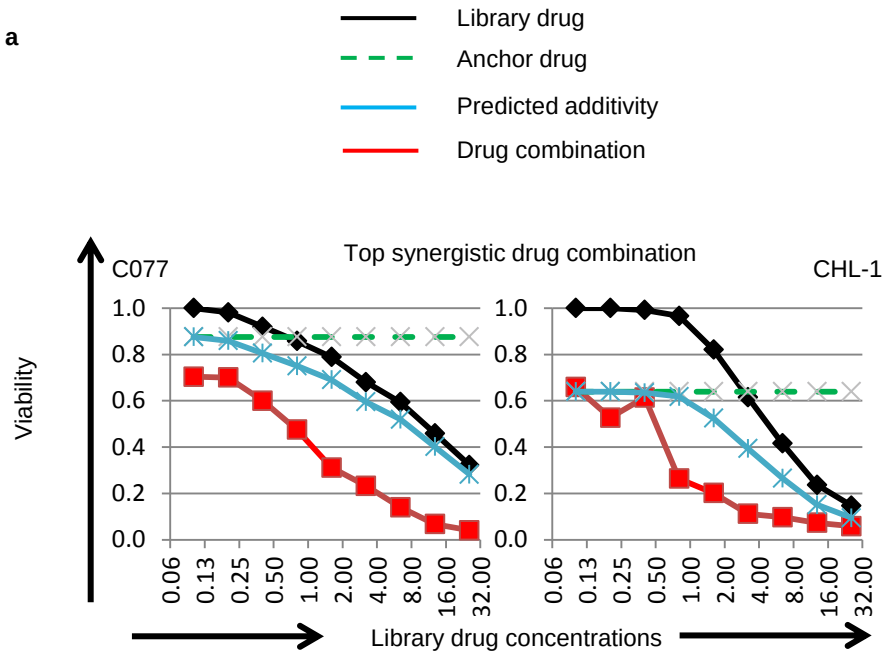
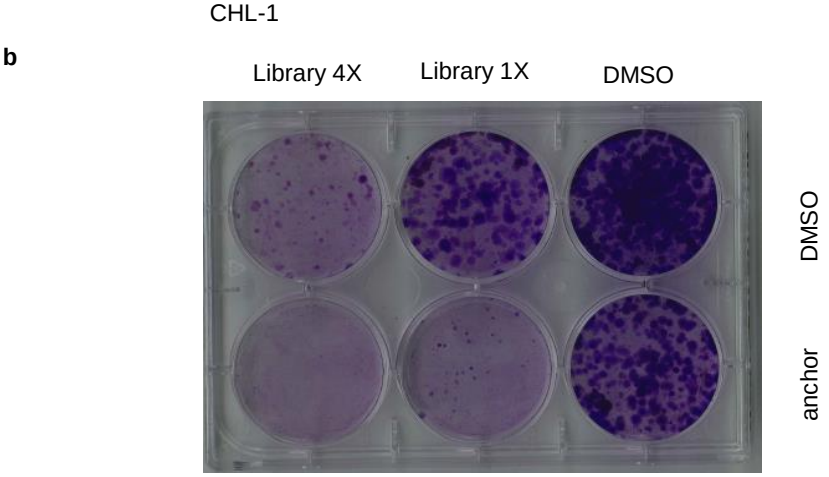

d
n of synergistic combos
n of lines

Figure 3


a
Library drug
Anchor drug
Predicted additivity
Drug combination
Top synergistic drug combination
C077
CHL-1
Viability
1.0
0.8
0.6
0.4
0.2
0.0
0.06
0.13
0.25
0.50
1.00
2.00
4.00
8.00
16.00
32.00
Library drug concentrations


b
CHL-1
Library 4X
Library 1X
DMSO
DMSO
anchor

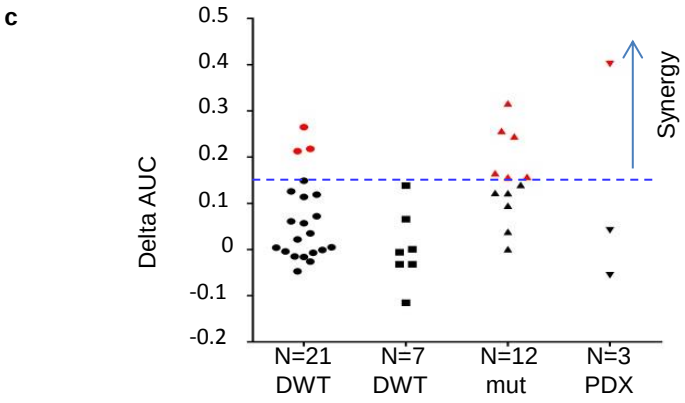

c
Delta AUC
0.5
0.4
0.3
0.2
0.1
0
-0.1
-0.2
Synergy
N=21
DWT
N=7
DWT
N=12
mut
N=3
PDX

Figure 4

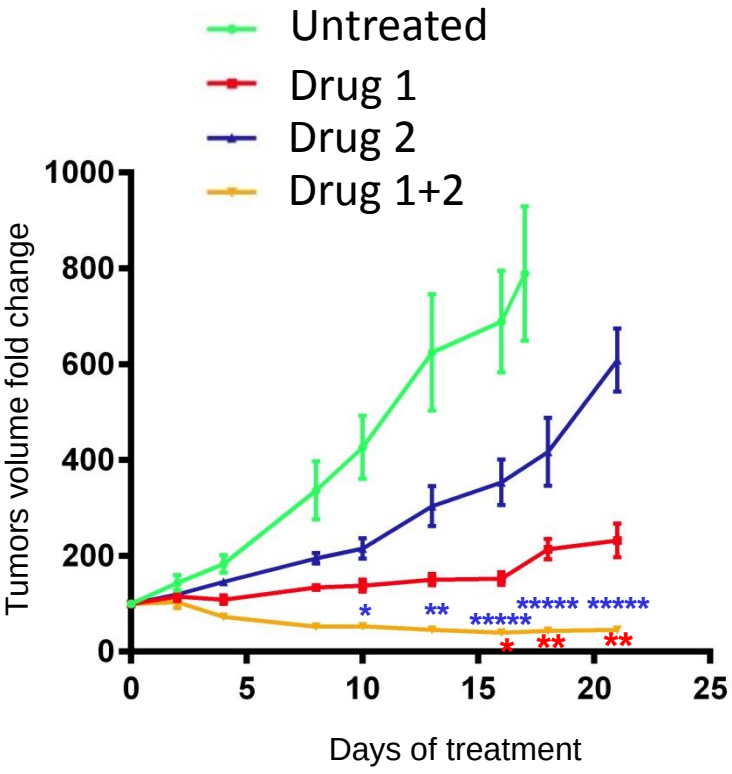

Untreated
Drug 1
Drug 2
Drug 1+2
Tumors volume fold change
1000
800
600
400
200
0
0
5
10
15
20
25
Days of treatment
*
**
*****
*****
*****
*
**
**

Figure legends

Figure 1. The genetic landscape of our melanoma cell line collection is representative of *BRAF/NRAS* wild type melanomas tumors.
a) Frequency of somatic mutation per Mb of exome in each of the 22 melanoma cell line; cell lines ID at the bottom of the page**.** **b)** Frequency (%) of mutation spectra, legend at the top of the page. **c)** status of the melanoma drivers, legend at the top of the page. If both missense and LOF mutation happens in a gene, LOF was displayed. Gene symbol is displayed on the left in grey, pink or mixed background if the gene is a melanoma copy number variation driver, a *BRAF/NRAS* wild type melanoma mutation driver or both, respectively. Gene were grouped in main functional families with the color code on the right, from top: MAPK , AKT, G protein, cell cycle, apoptosis, Chromatin remodelling, transcription factors, melanoma pigmentation, mixed function. Only genes mutated in >2 cell lines are displayed. C089 is a BRAF V600E mutant representative cell line. **d)** Frequency of *BRAF/NRAS* wild type melanoma cell lines (red) or tumors (pale blue) with a single nucleotide mutation in the melanoma driver genes displayed on the left.

Figure 2. Outline of the high-throughput drug screening data.
a) Viability of the cell lines treated with 60 library drugs (left panel) or 180 drug combinations. Each dot represents the Area Under the Curve (AUC, on the X axis) of a cell line treated with a library drug or its combination with the anchor drug (on top of the panel). Each column on the Y axis represent a library drug in color code according to the molecular function of the main drug target, from top: MAPK, AKT, other kinases, apoptosis, cell cycle, chromatin remodelling, DNA replication, other function. The dotted line highlight 50% of growth inhibition, the dashed line in the last 3 right panels shows the AUC of the anchor drug alone. **b)** Approach to estimate drug synergy. We calculated the area under the viability curve for each treatment (top left), then the predicted additivity as arithmetic product of the AUC of the 2 single drugs, and then calculated the delta AUC = AUC of the predicted addivitiy – AUC of the drug combination. An additive combination (red line, left panel) results in a growth inhibition that is the sum of the 2 single drugs (pale blue line), while a synergistic combination (red line, right panel) display a growth inhibition much higher than the predicted addivity (pale blue line), thus scoring a positive delta AUC (double arrow area). An outline of the growth inhibition effects is displayed in the bottom panel. **c)** Delta AUC (Y axis) of the library drugs combined with the different doses of the anchor drugs (X axis). Each dot represents the delta AUC of a drug combination in a cell line; the black line show the mean, the dashed line the 0.2 delta AUC value. **d)** Number of combination that achieved a delta AUC>0.2 (X axis) in recurrent (X axis) cell lines.

Figure 3. We identified a synergistic drug combination that is confirmed by 2 independent assays and in 4 independent collections of cell lines
a) Survival curves of 2 representative cell lines for the top synergistic drug combination. Y axis show viability Vs vehicle treated control, X axis the of the library drug. The black line shows the viability of the cells treated with the library drug alone, the dashed green line the viability with the anchor drug alone, the red line the viability with the drug combination, the blue line the predicted). Each dot is the average value of a technical triplicate, each of the curves is representative of a biological duplicate. **b)** Clonogenic assays confirmed the synergy between the 2 drugs. Two doses of the library drug were testet. These assays are representative of a biological duplicate. **c)** Summary of delta AUC (synergy score) of the synergistic drug combination in the 4 collections of cell lines. The dashed lines represent values of delta AUC 0.15, the top 3% (high synergy) of the values measured in our screening.

Figure 4. The newly identified drug combination is well tolerated and very effective in vivo.
Volume fold change of a patient derived xenotransplant melanoma compared to time zero of treatment. Each point is the mean of 10 tumors (5 mice); the standard error is shown. Asterisks indicate significance of the drug combination compared to drug 2 (in blue) or drug 1 (in red) by two way Anova and Holm-Sidak's multiple comparisons test. The patient derived xenotranpslant line was inoculated in mice 35 days before the drug treatment was started with the 3 drug regimens (drug 1, drug2, drug combination 1+2) by gavage.