Supplementary Items Summary

Supplementary Figures

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- Supplementary Figure 2. Clonogenic assays confirmed the synergy of temozolomide with olaparib and nilotinib with MEK inhibitors. (Panels a-b, PDF file)
- Supplementary Figure 3. Identification of the association between AXL expression and synergy between trametinib and nilotinib. (Panels a-l, PDF file)
- Supplementary Figure 4. Identification of trametinib and nilotinib/trametinib drug resistance genes by CRISPR/Cas9 genome-wide library screening. (Panels a-q, PDF file).

Supplementary Tables

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- Supplementary Table 2. Somatic single nucleotide mutation in the collection of 22 melanoma cell lines. (Sheets a-c, Excel file)
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- Supplementary Table 6. Validation of somatic single nucleotide mutation by RNA sequencing data analysis. (one sheet, Excel file)
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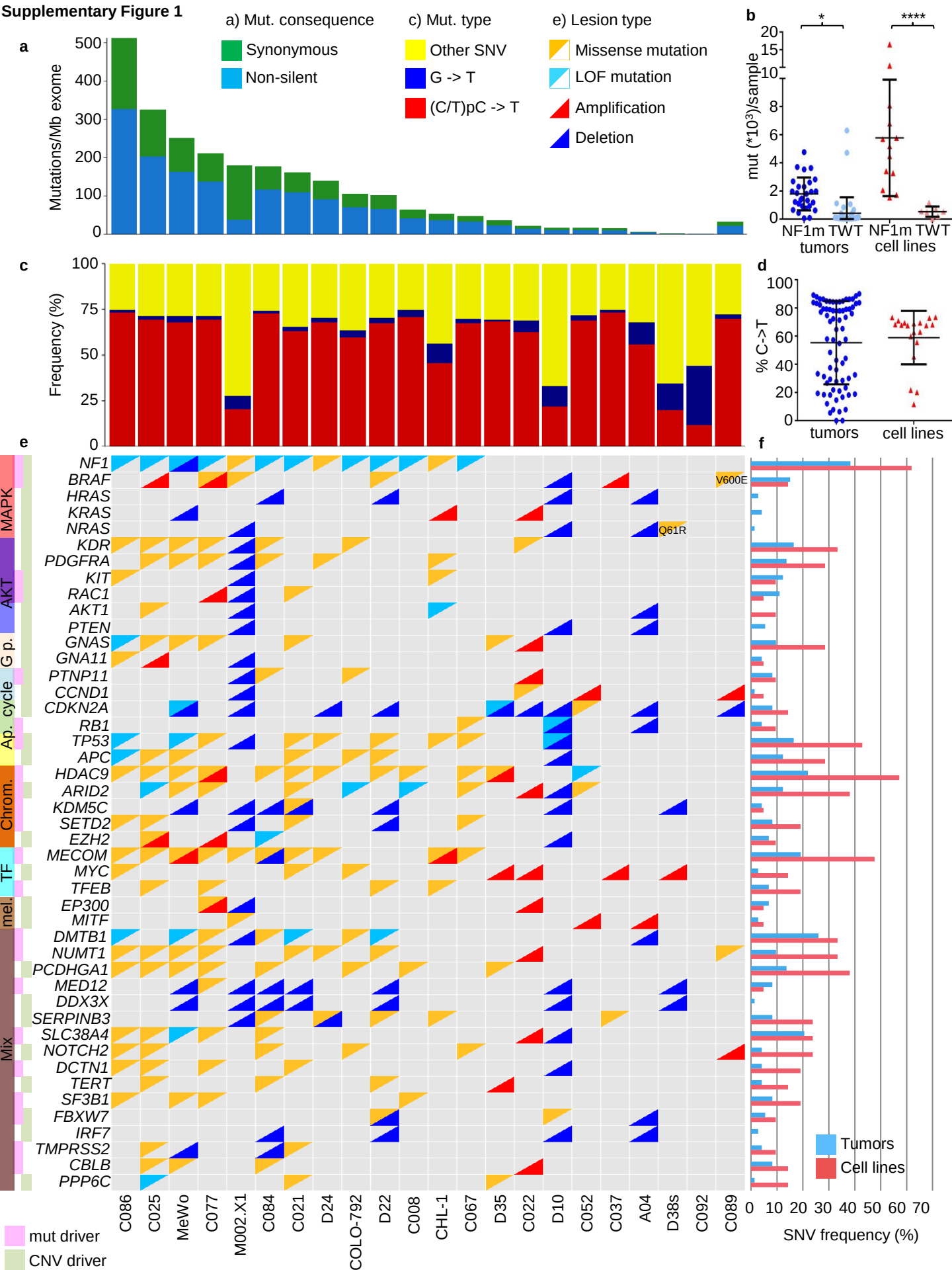
- Supplementary Table 16. Effects of AXL overexpression and knockdown on the synergy between nilotinib and trametinib. (one sheet; Excel file)

- Supplementary Table 17. Phosphoproteome analysis of a sensitive and a non-sensitive cell line treated with nilotinib, trametinib or nilotinib/trametinib combination. (Sheets A-c, Excel file)
- Supplementary Table 18. Nilotinib/trametinib combination resistance genes identified by CRISPR/Cas9 screens. (Sheets a-k, Excel file)
- Supplementary Table 19. Trametinib resistance genes identified by CRISPR/Cas9 screens. (Sheets a-j, Excel file)
- Supplementary Table 20. Pathway enrichment analysis for the genes whose loss confer resistance to trametinib or nilotinib/trametinib combination in each cell line. (Sheets a-h, Excel file)
- Supplementary Table 21. Pathway enrichment analysis for the genes that confer resistance to trametinib or nilotinib/trametinib combination in 2 or more cell lines. (Sheets a-b, Excel file)

Supplementary Figure 1



Supplementary Figure 1

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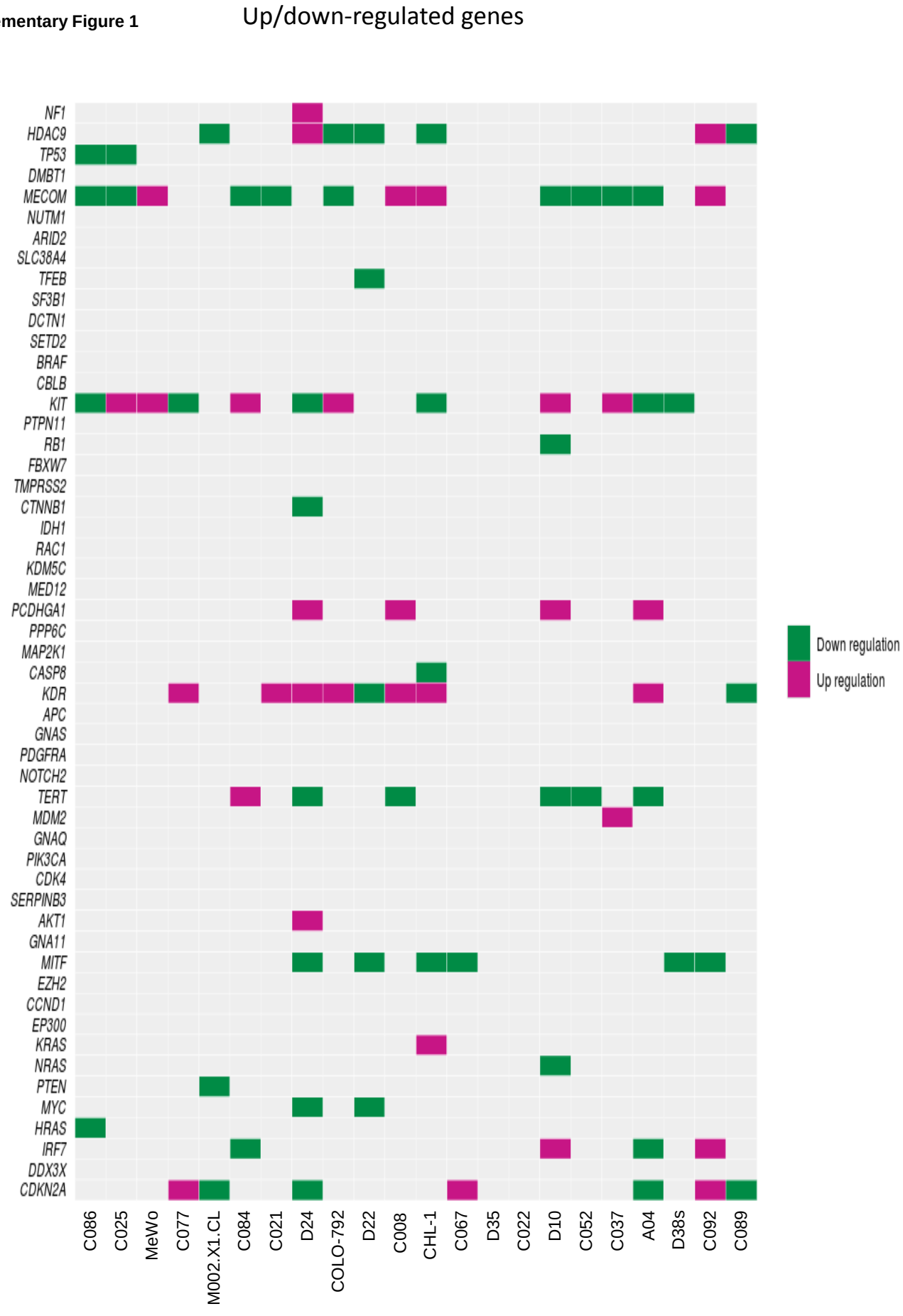

Supplementary Figure 1

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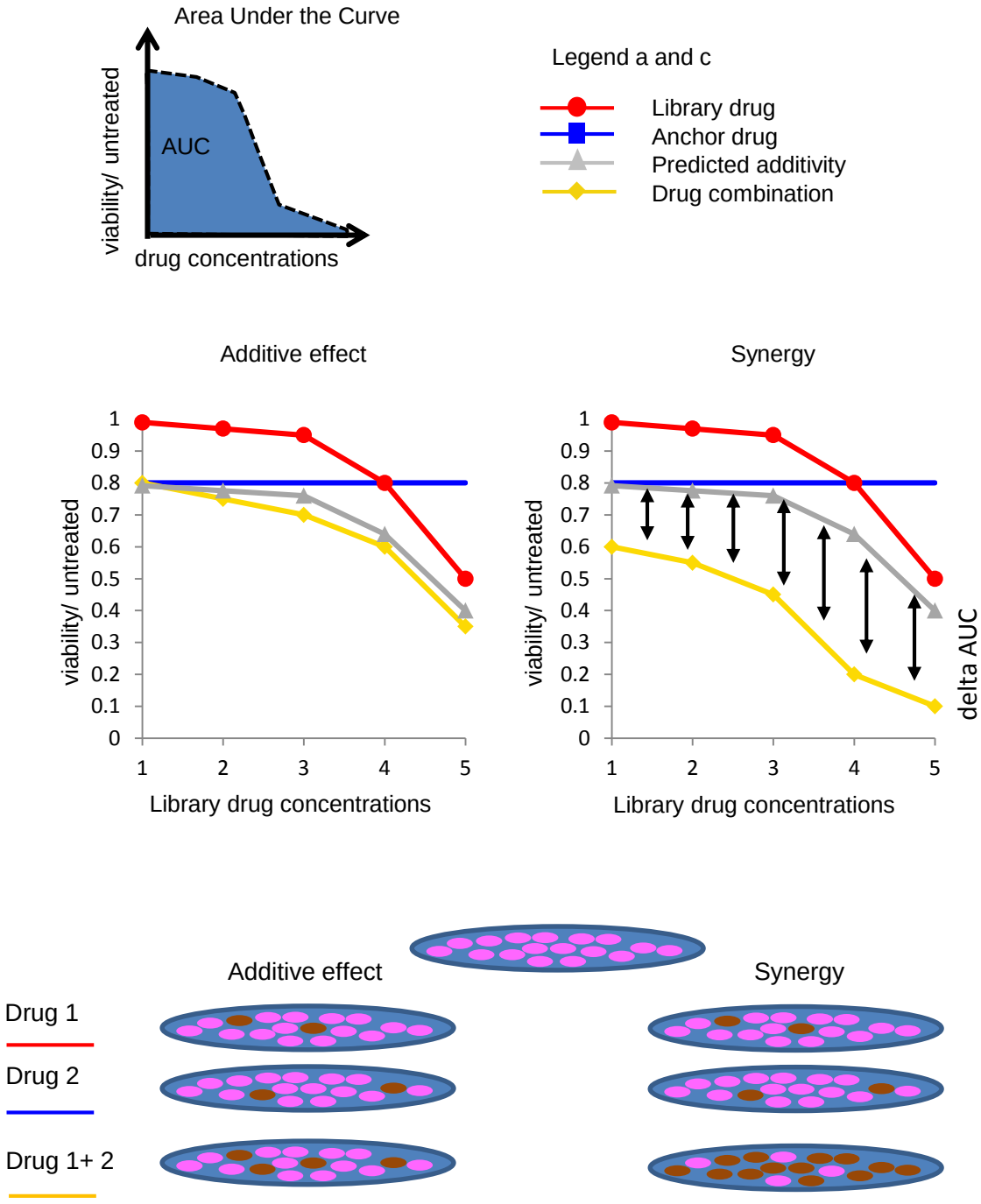
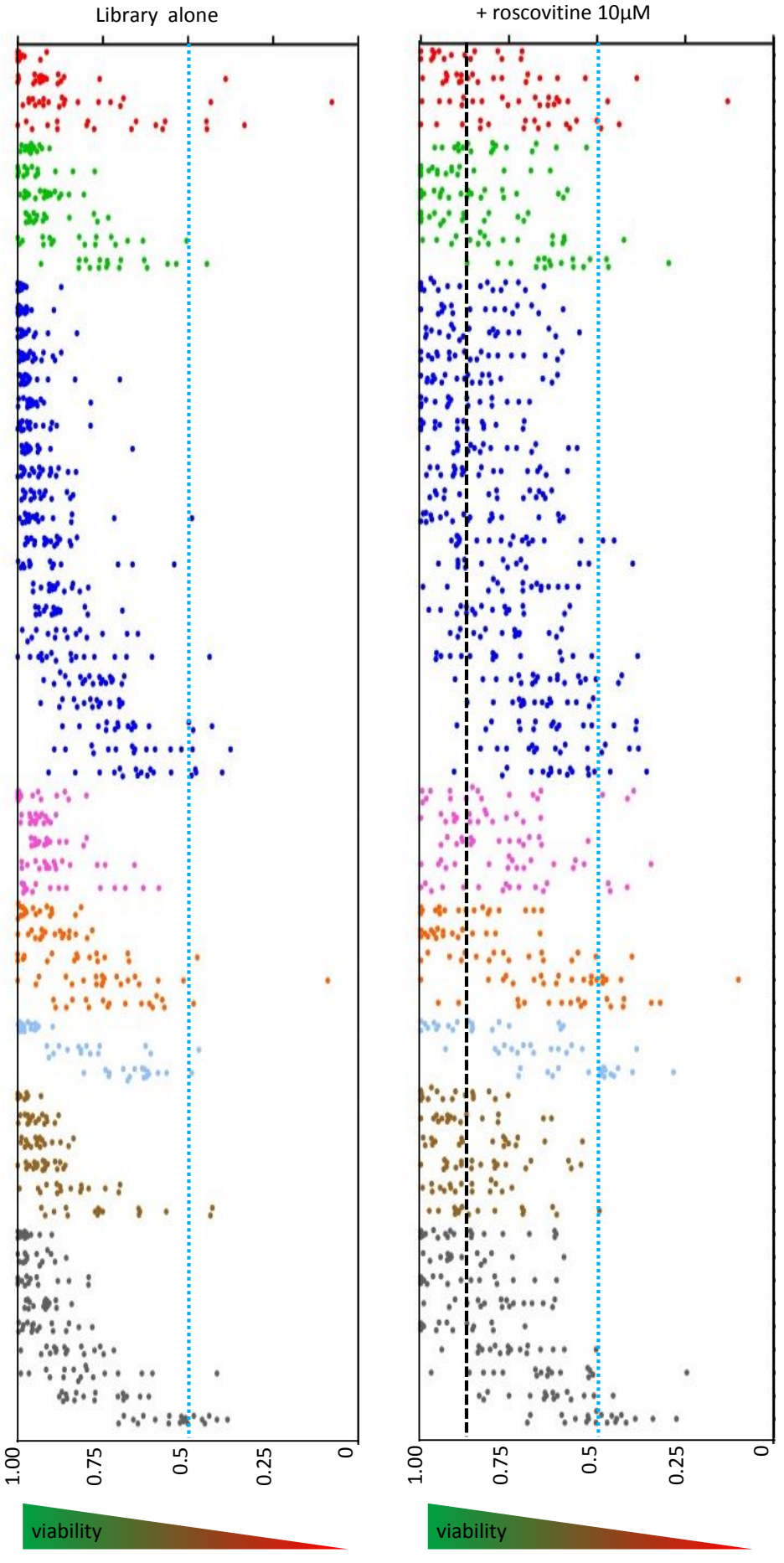


Supplementary Figure 1

i

drug	target(s)
BIRB 0796	p38, JNK2
SCH772984	ERK1/2
GSK2118436	BRAF
PD-0325901	MEK1/2
PF-4708671	p70 S6KA
MK-2206	AKT1/2
BYL719	PI3Ka
AZD6482	PI3Kb
GDC0941	PI3K (class 1)
AZD8055	mTORC1/2
AMG-706	VEGFR, RET, c-KIT, PDGFR
PF-02341066	MET, ALK
INCB-18424	JAK1, JAK2, TYK2
OSI-906	IGFR-1
Lapatinib	ERBB2, EGFR
Gefitinib	EGFR
GW 441756	NTRK1
Axitinib	PDGFR, KIT, VEGFR
LY317615	PKC beta
PD-173074	FGFR1/3
AZD8931	ERBB1, ERBB2, ERRB3
BMS-345541	IKK-beta
GSK269962A	ROCK1/2
BI-D1870	RSK1/2/3/5, PLK1, AURKB
BX-795	TBK1, PDK1, IKK, AURKB/C
SB-505124	TGFbetaR-I (ALK5)
Dasatanib	ABL, Src-family, BMX-pan Y
PF-562271	FAK
GSK2334470	PDK1
BMS-754807	IGF-1R/IR
(5Z)-7-Oxozeaenol	TAK1 (MAP3K7)
CEP-701	FLT3, JAK2, NTRK1, RET
LCL161	(SMAC) mimetic and inhibitor of IAP
Embelin	XIAP
JNJ-26854165	MDM2
ABT-263	BCL2, BCL-XL, BCL-W
Nutlin-3a	MDM2
ZM-447439	AURKB
RO-3306	CDK1
MLN8237	Aurora A
PD-0332991	CDK4/6
Docetaxel	Microtubules
EPZ004777	DOT1L
JQ1	BRD2, BRD3, BRD4
Vorinostat	HDAC inhibitor Class I, IIa, IIb, IV
KU-55933	ATM
RI-1	RAD51
NU-7441	DNAPK
AZD2281	PARP1/2
681640	WEE1, CHK1
AZD7762	CHK1/2
XAV 939	Tankyrase (PARP5a)
CHIR-99021	GSK3B
BMS-708163	gamma-secretase
SB 216763	GSK3A/B
GDC-0449	SMO
Leflunomide	DHODH; AhR
AICAR	AMPK agonist
Bortezomib	Proteasome
NVP-AUY922	HSP90



Supplementary Figure 1

j

drug	target(s)
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Docetaxel	Microtubules
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JQ1	BRD2, BRD3, BRD4
Vorinostat	HDAC inhibitor Class I, IIa, IIb, IV
KU-55933	ATM
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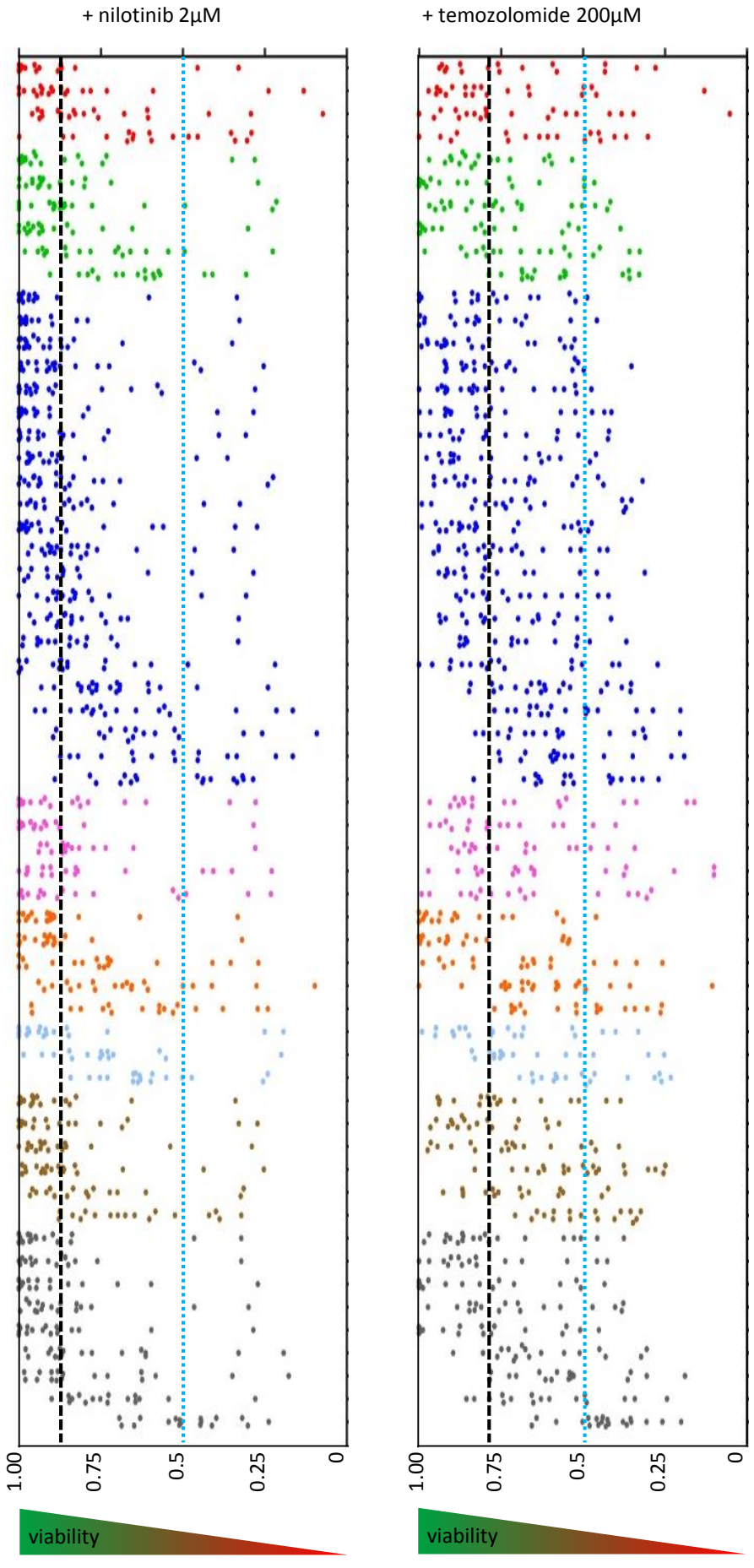
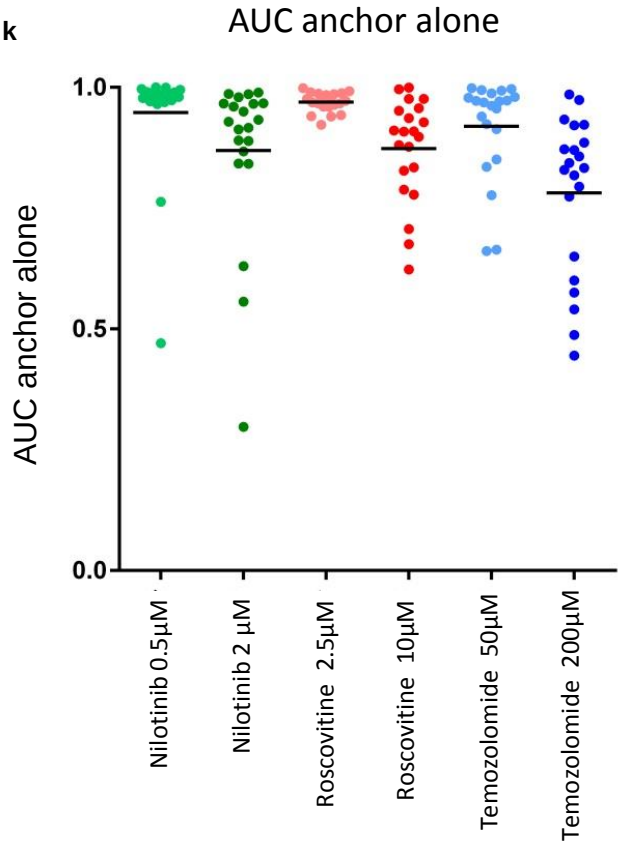
681640	WEE1, CHK1
AZD7762	CHK1/2
XAV 939	Tankyrase (PARP5a)
CHIR-99021	GSK3B
BMS-708163	gamma-secretase
SB 216763	GSK3A/B
GDC-0449	SMO
Leflunomide	DHODH; AhR
AICAR	AMPK agonist
Bortezomib	Proteasome
NVP-AUY922	HSP90



Supplementary Figure 1



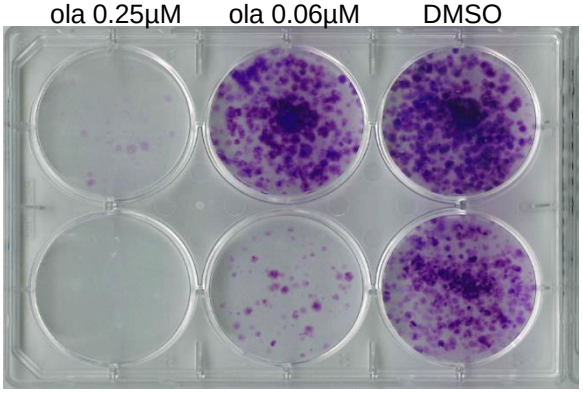
Supplementary Figure 1. Landscape of the alterations and drug sensitivity in the collection of 22 melanoma cell lines.

a) Frequency of somatic mutation (see **Methods**) per Mb of exome in each of the 22 melanoma cell lines; cell line ID at the bottom of the page**. b)** Frequency of mutations in *NF1* mutant (NF1m) and triple wild type (TWT) cell lines (right 2 columns) and human tumors (left 2 columns). Y axis represents the number (in thousands) of mutations per sample, mean and standard deviation are shown. Significance by one way Anova and Tukey's multiple comparison test. * =P<0.05; **** P<0.0001. **c)** Frequency (%) of mutation spectra, legend at the top of the page. **d)** C to T at dipyrimidines frequency in *BRAF/NRAS* wild type melanoma cell lines (red) and tumors (blue); mean and standard deviation are shown. **e)** Status of the melanoma drivers, legend at the top of the page. If both missense and LOF mutation occurs in a gene, LOF was displayed. Gene symbol is displayed on the left; grey and pink indicate if the gene is a copy number variation driver (CNV driver) or a *BRAF*/*NRAS* WT melanoma mutation driver, respectively (see **Methods**). Genes were grouped in main functional families with different color code, from top: MAPK, AKT, G protein (G p.), cell cycle (cycle), apoptosis (Ap.), Chromatin remodelling (Chrom.), transcription factors (TF), melanoma pigmentation (Mel), mixed function (Mix). Only genes mutated in 3 or more cell lines are displayed. C089 and D38s carry hotspot mutations in *BRAF* and *NRAS* as indicated by the amino acid code. **f)** 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. **g)** Approach to estimate drug synergy (see **Methods**). We calculated the area under the viability curve (AUC) for each treatment (top left), then the predicted additivity as arithmetic product of the viability of the cells treated with anchor and the library drug alone, and then calculated the delta AUC as AUC of the predicted additivity minus the AUC of the drug combination. An additive combination (yellow line, left panel) results in a growth inhibition that is the sum of the 2 single drugs (grey line, left panel), while a synergistic combination (yellow line, right panel) displays a growth inhibition much higher than the predicted additivity (grey line, right panel), thus scoring a positive delta AUC (double arrows area). An outline of the growth inhibition effects is displayed in the bottom panel, where pink circles represent viable cells, brown circles represent dead cells. **h-i)** Viability of the cell lines treated with 60 library drugs (left panel in i) or 180 drug combinations (right panel in i, left and right panel in j). Each dot represents the 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 row represents a library drug in colour code according to the molecular pathway/function of the main drug target, from top: MAPK, AKT, other kinases, apoptosis, cell cycle, chromatin remodelling, DNA replication, other function. The dotted blue line highlights 50% growth inhibition, the dashed black line shows the AUC of the anchor drug alone. The table on the left describes each library drug and its putative target(s). **j)** AUC (Y axis) of the cell line treated with 2 concentrations the anchor drugs alone (X axis). The black line shows the mean.

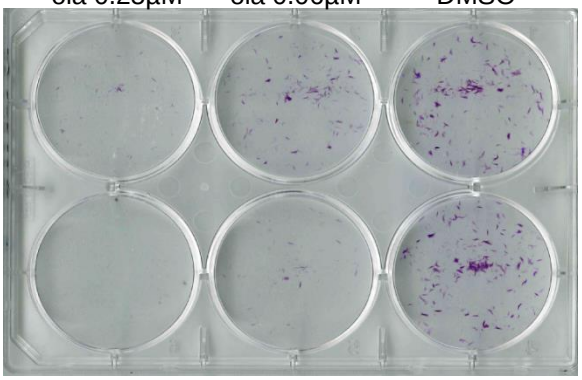
Supplementary Figure 2

a

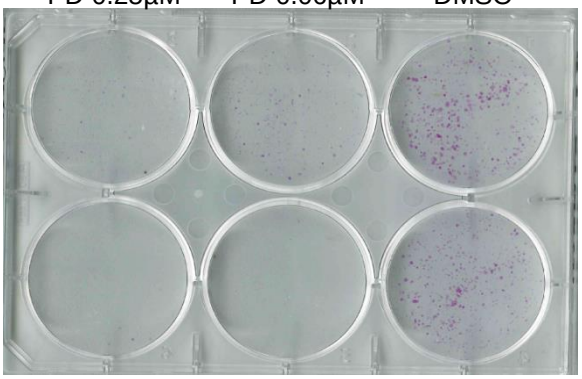
Temozolomide + olaparib in CHL-1



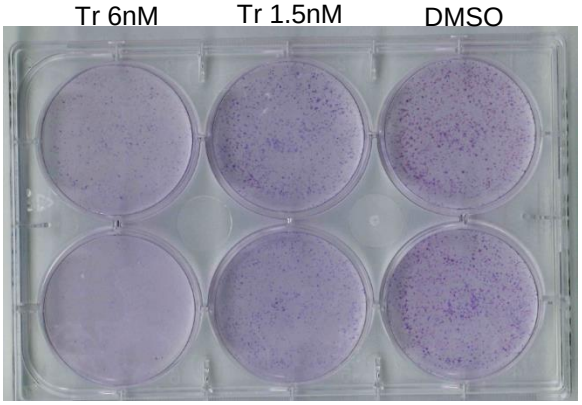
Temozolomide + olaparib in MeWo



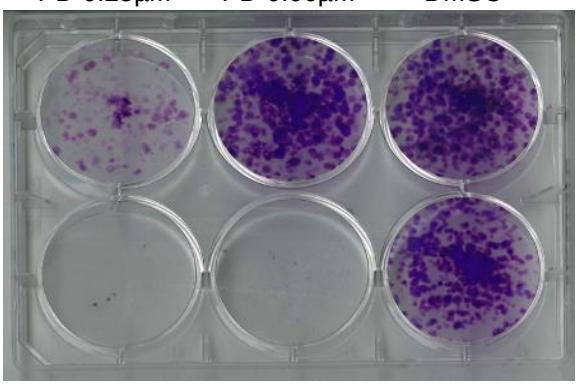
Nilotinib + PD-0325901 in C077



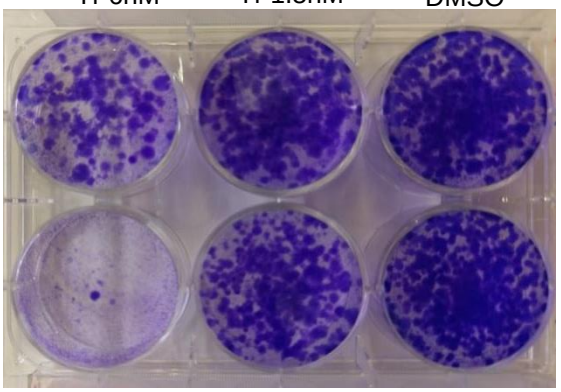
Nilotinib + trametinib in C077



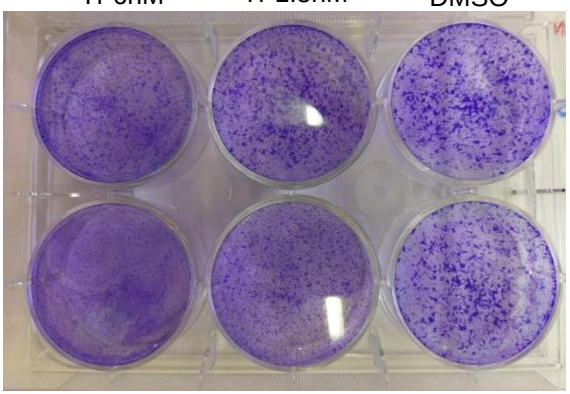
Nilotinib + PD-0325901 in CHL-1



Nilotinib + trametinib in CHL-1



Nilotinib + trametinib in MeWo



Supplementary Figure 2

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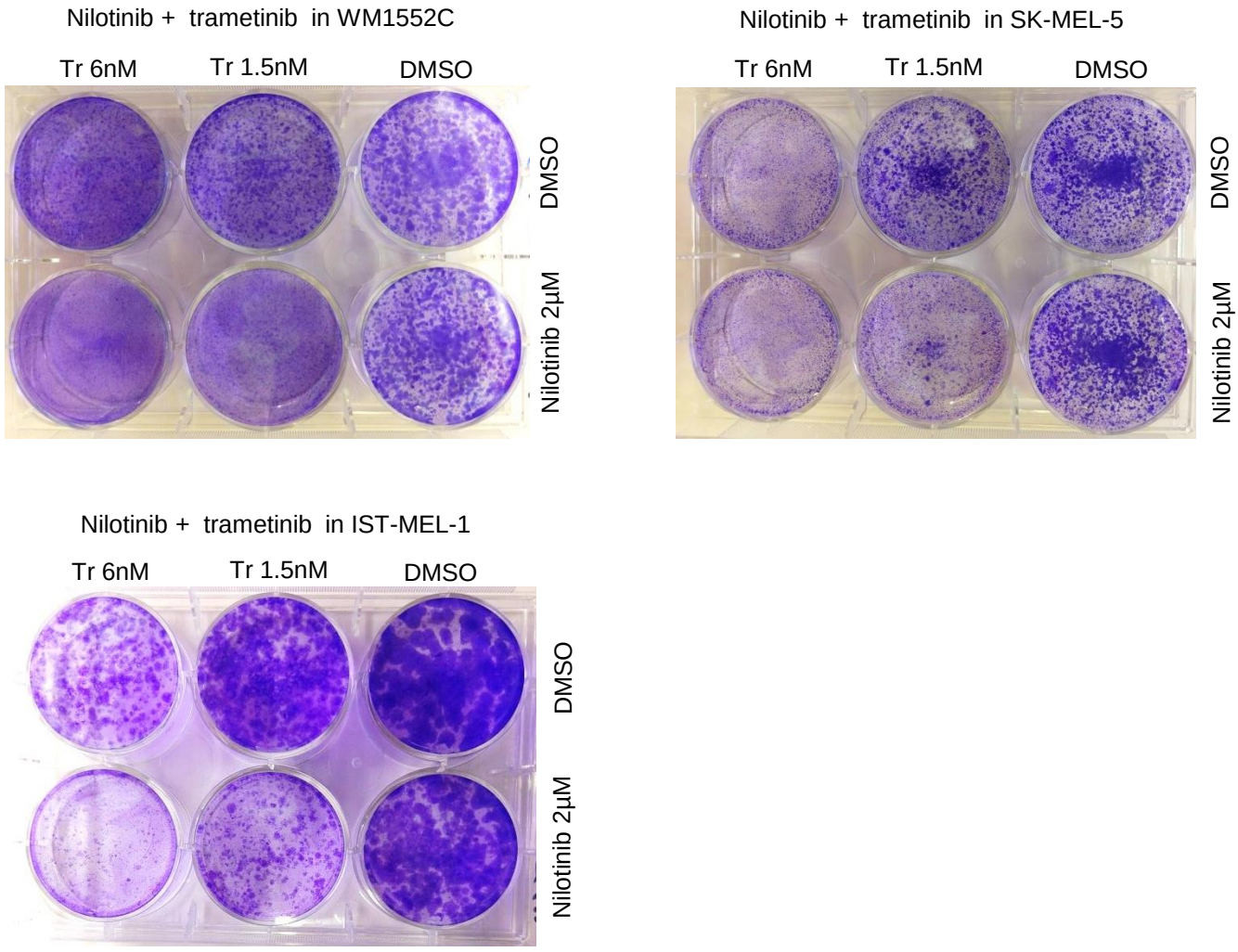
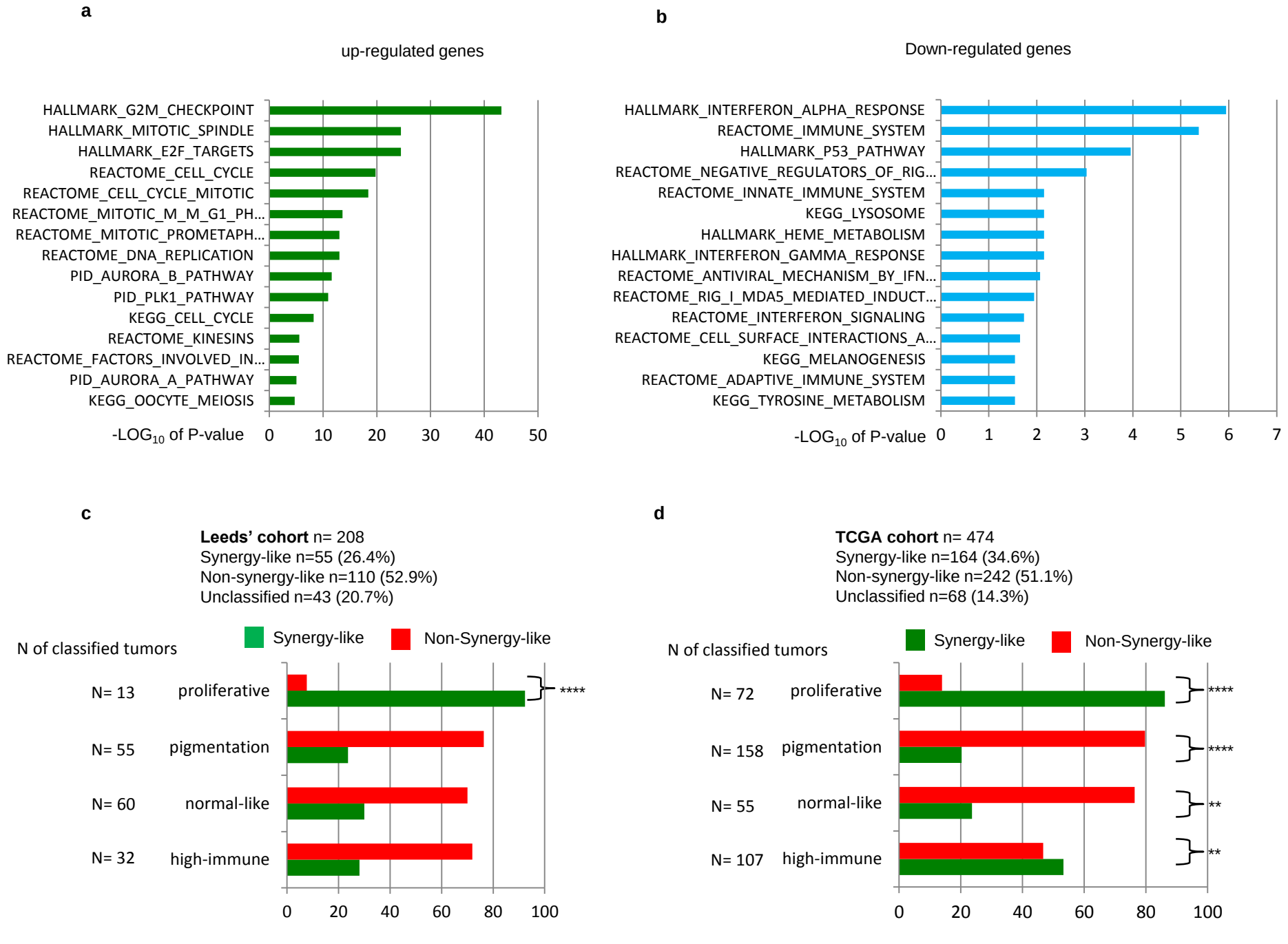


Supplementary Figure 2. Clonogenic assays confirmed the synergy of temozolomide with olaparib and nilotinib with MEK inhibitors.

a) Clonogenic assays (2 weeks) of representative *BRAF*/*NRAS* wild type melanoma cell lines. **b)** Clonogenic assays of representative *BRAF*^{V600} -mutant cell lines. The concentrations of the library and anchor drugs are indicated on the top or on the right, respectively.

Supplementary Figure 3



Supplementary Figure 3

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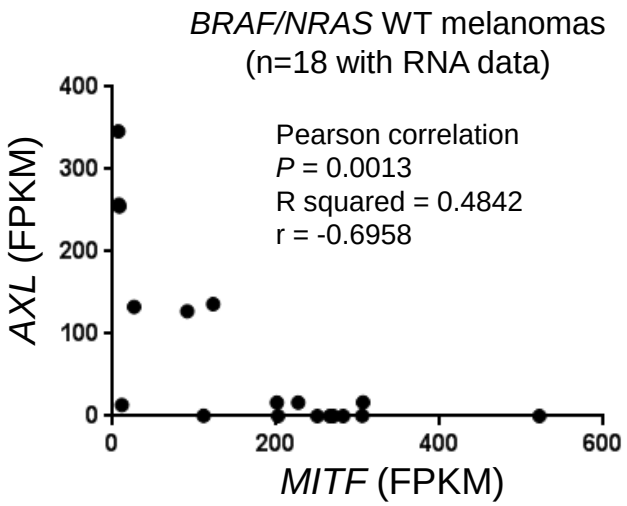

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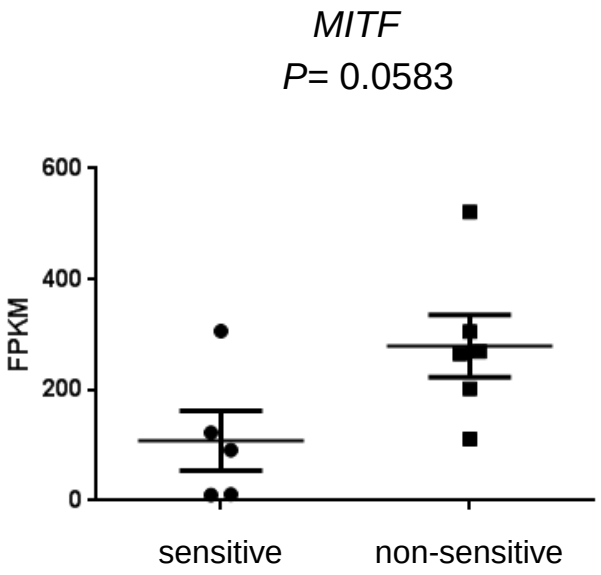

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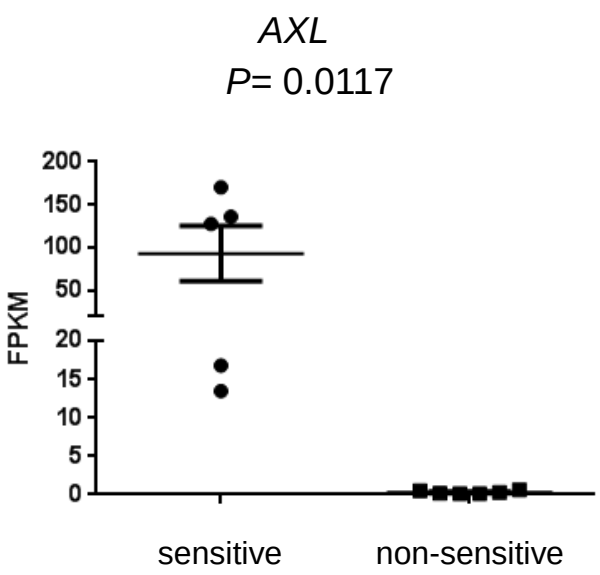

Supplementary Figure 3

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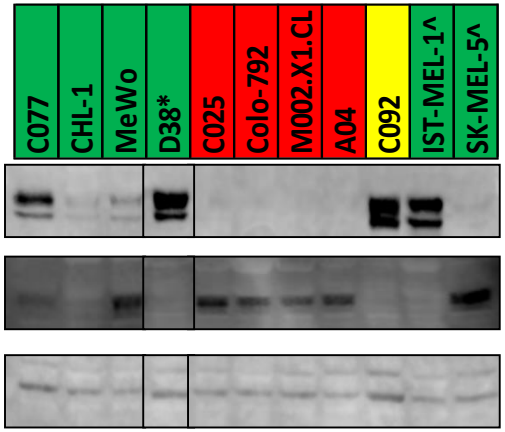

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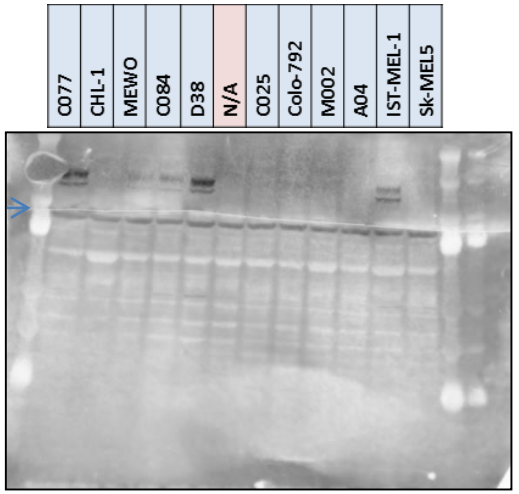

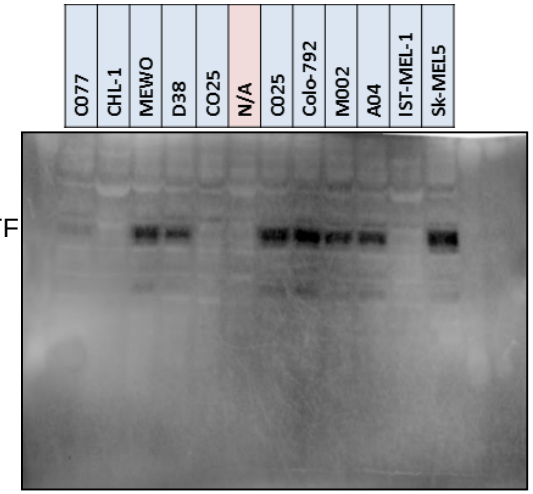

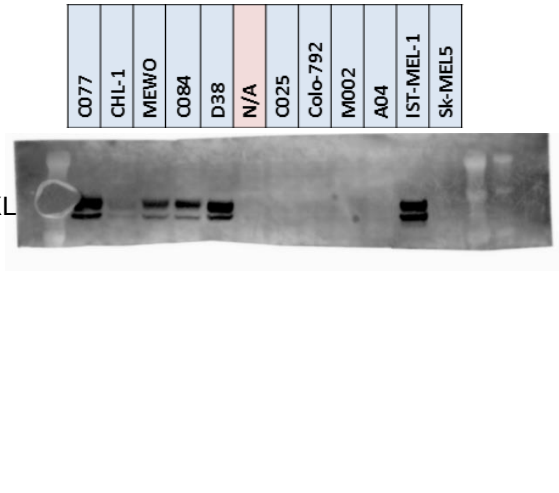

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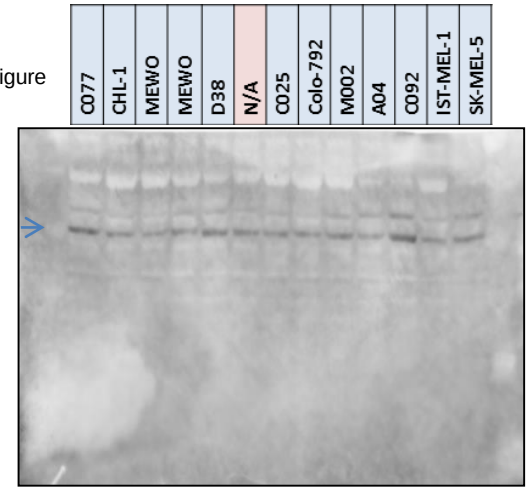

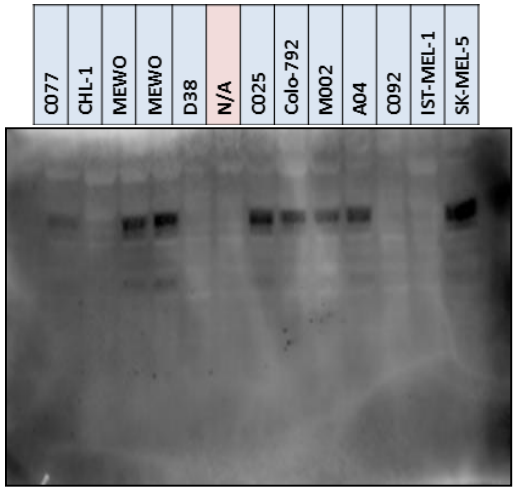

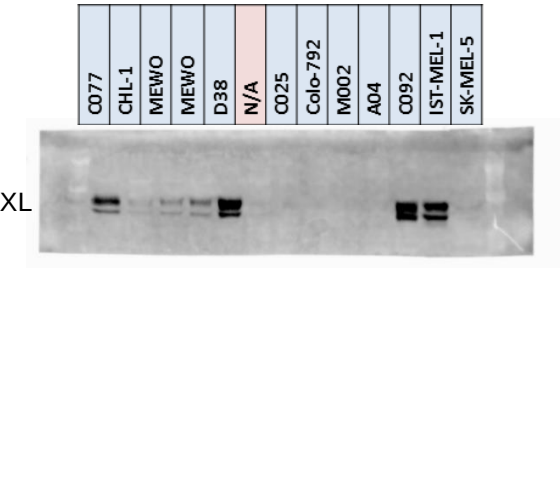

Supplementary Figure 3

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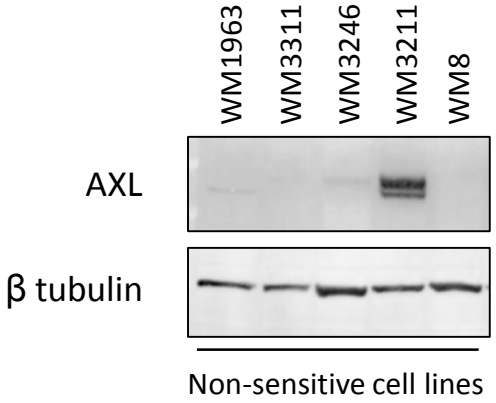

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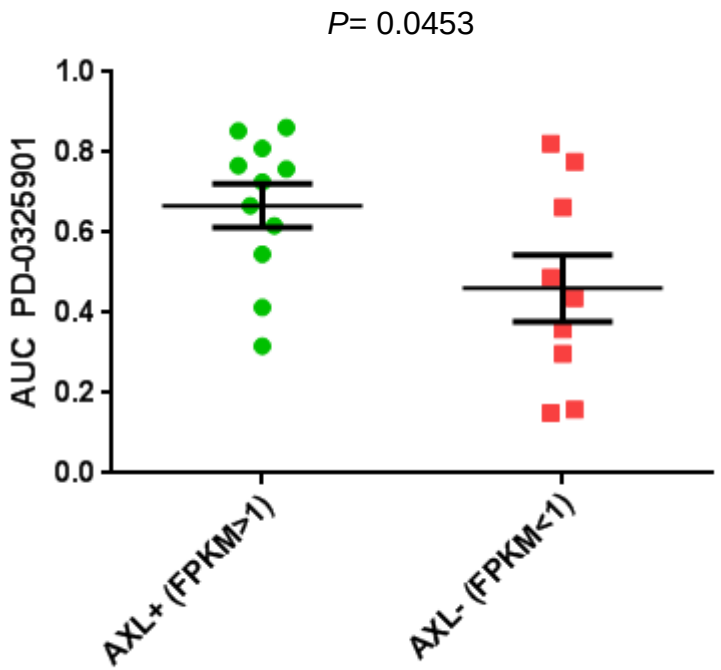

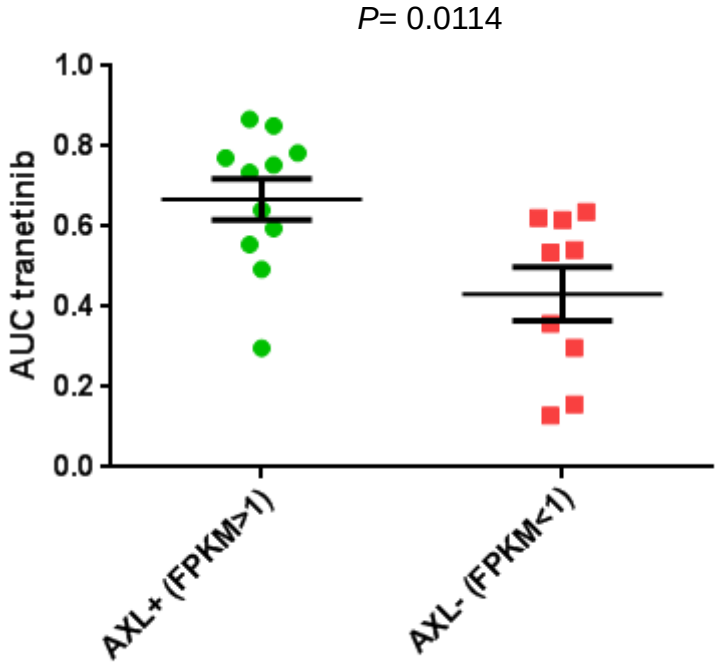

Supplementary Figure 3

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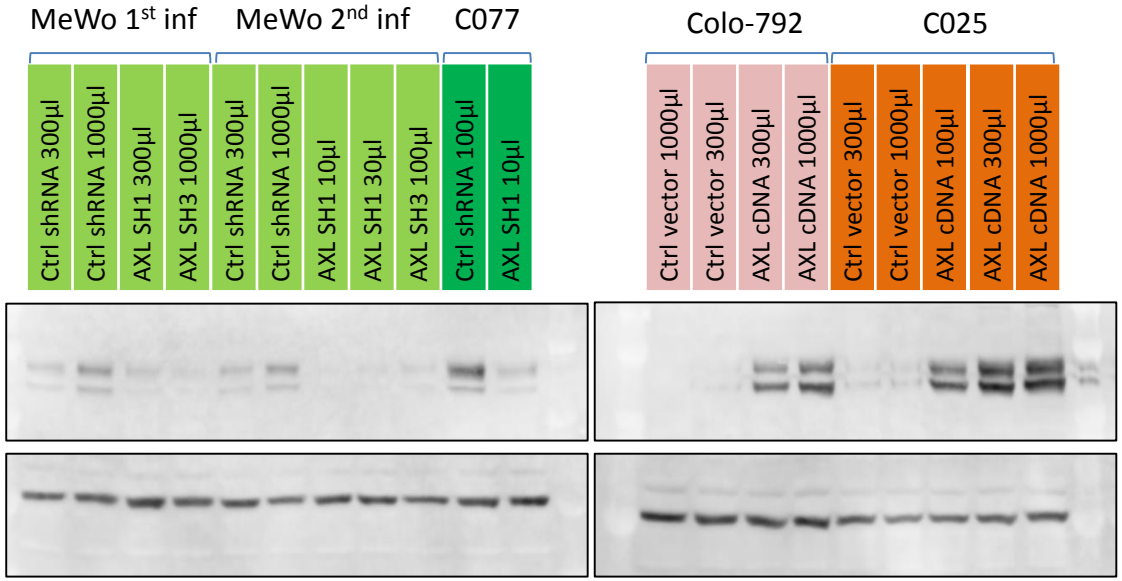

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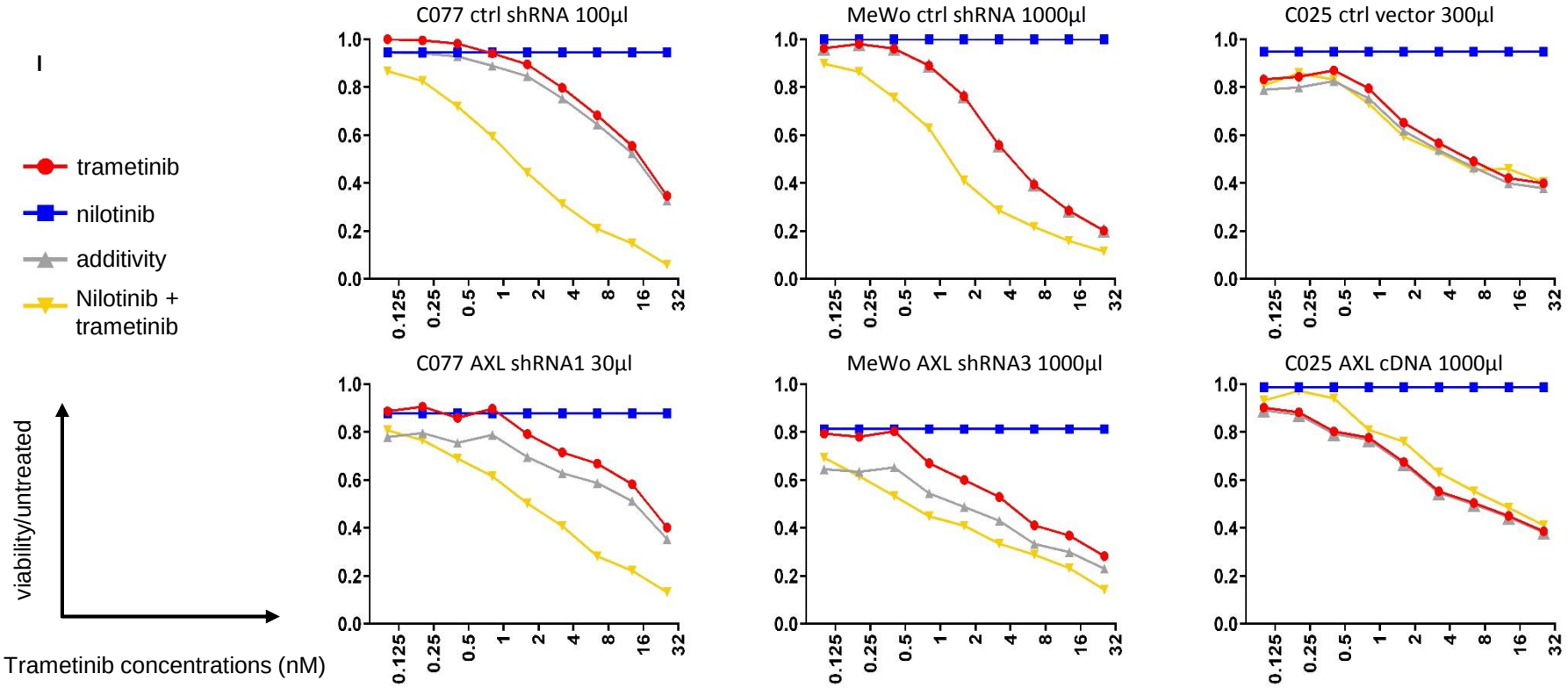

Supplementary Figure 3

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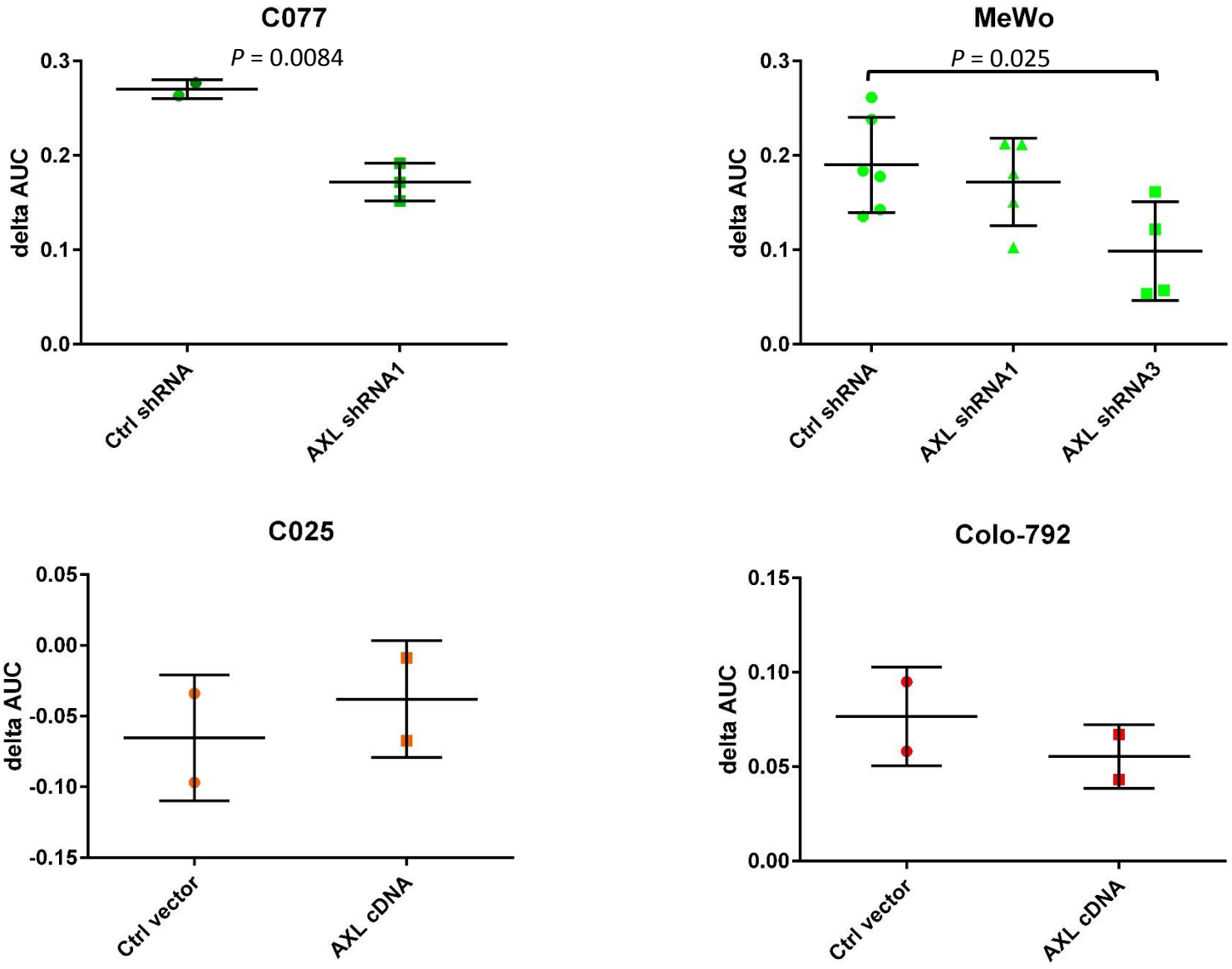

Supplementary Figure 3

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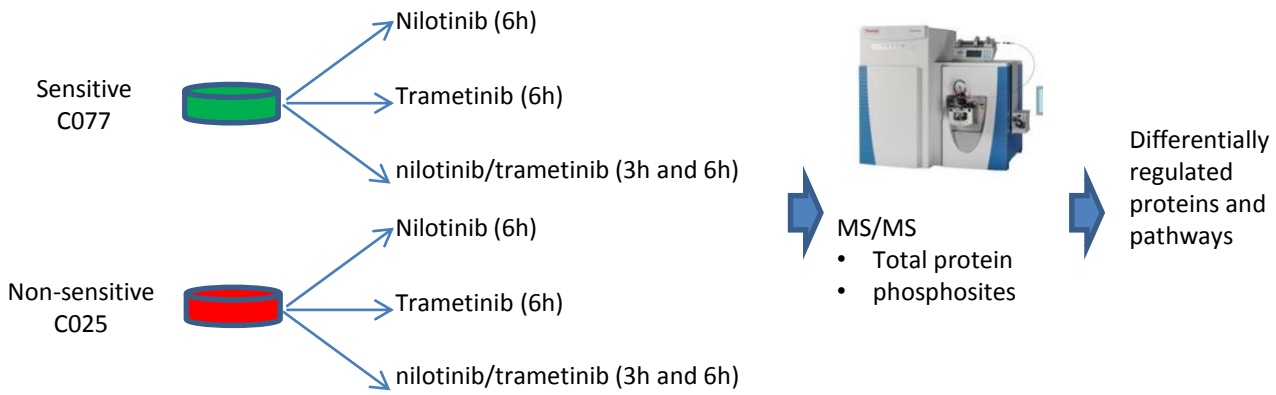

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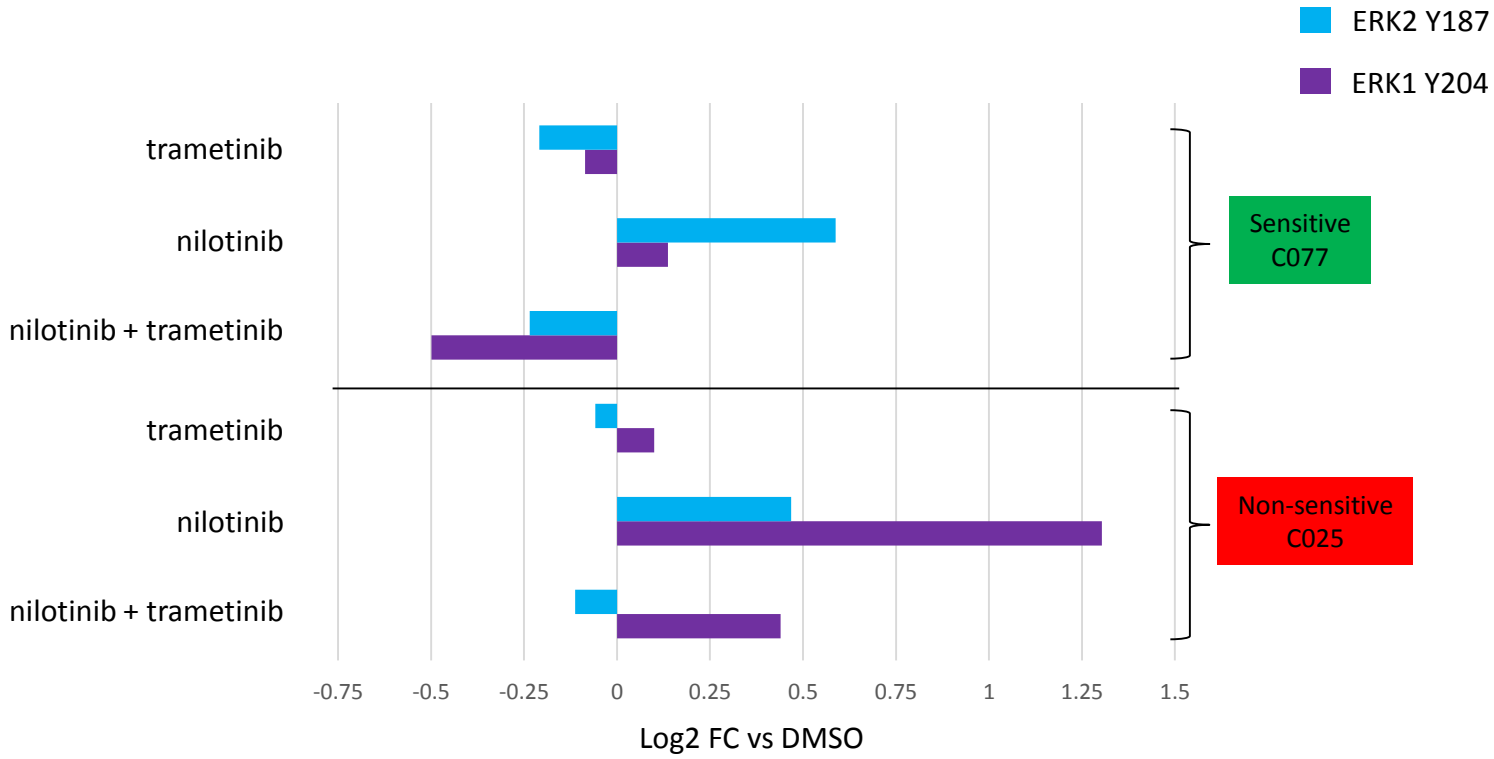
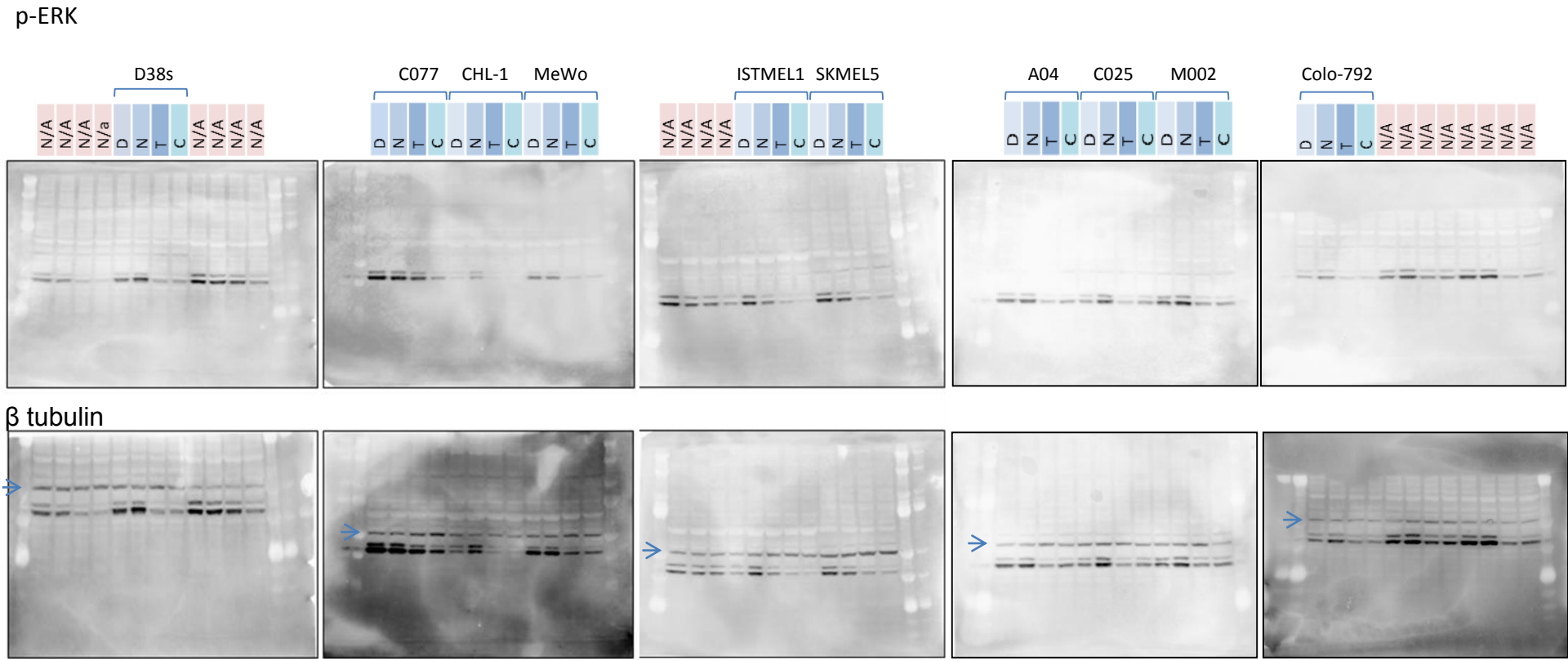


Supplementary Figure 3

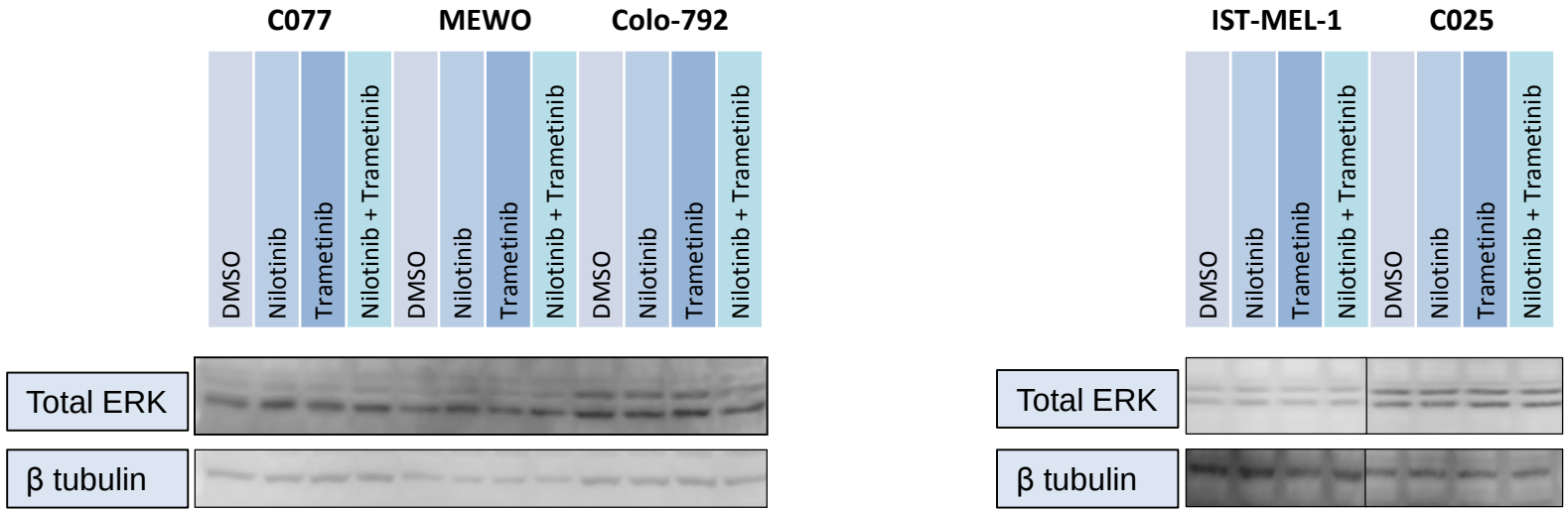
p

Full picture for Figure 3 b-c



Supplementary Figure 3

q



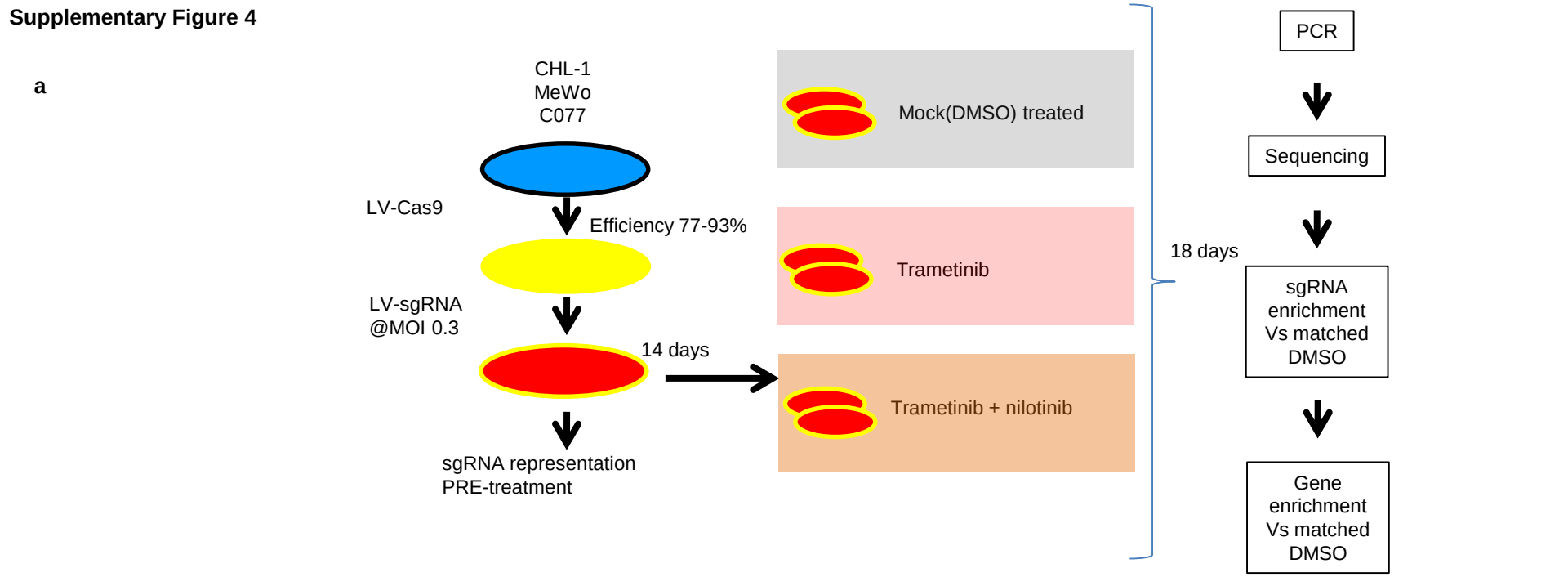
Supplementary Figure 3. Identification of the association between AXL expression and nilotinib/trametinib synergy.

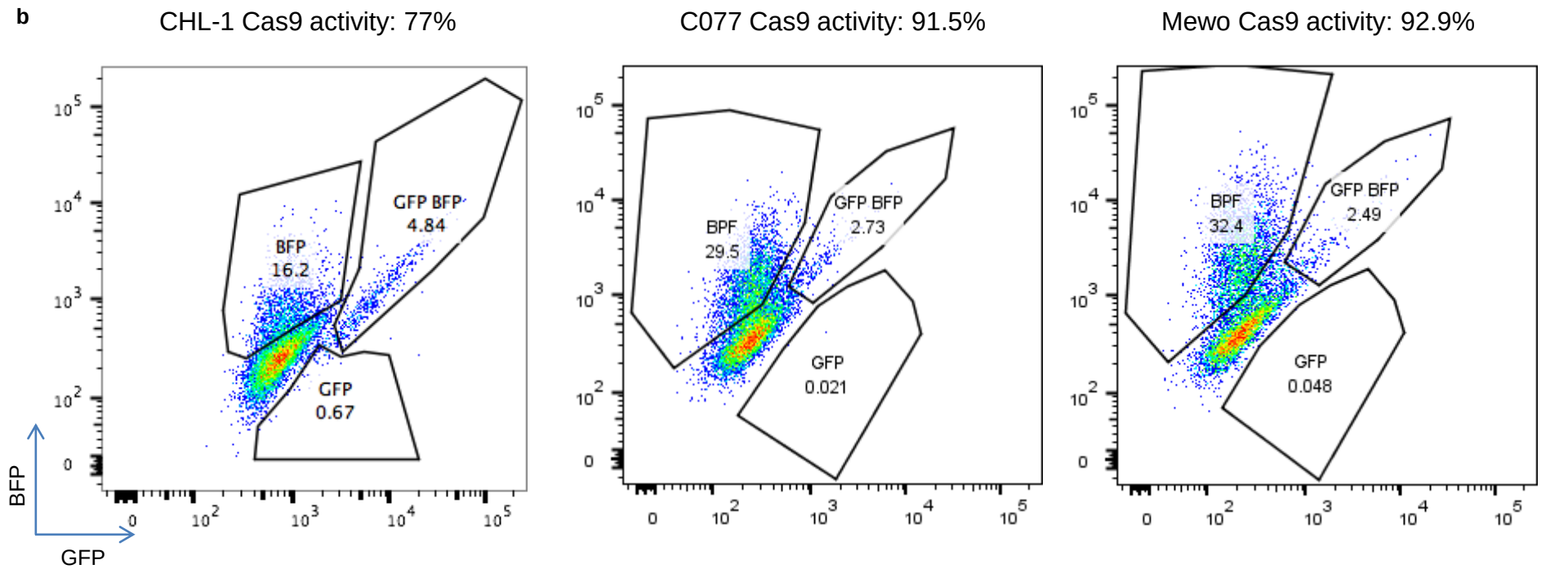
a-b) Pathway enrichment analysis for the genes up or downregulated, respectively, in sensitive vs non-sensitive cell lines. The enrichment analysis was run in MsigDB website considering Canonical Pathways and Hallmarks datasets. Minus LOG_{10} of the FDR corrected *P*-value is displayed in the histogram for the top 15 enriched pathways. **c-d)** Human tumors from Leeds (c) and TCGA (d) cohort that match the gene expression pattern associated with synergy or non-synergy and their classification into Jonsson's gene expression subclasses. The % of tumors significantly matching the gene expression pattern associated to synergy (synergy-like) or matching the opposite of the signature (non-synergy-like) are displayed in the histogram (see **Methods**). The total number (and %) of tumors classified as synergy-like and non-synergy-like in each cohort is indicated above the graph. The total number of tumors classified into each of the 4 Jonson classes (excluding the ones that failed to be classified into either synergy-like or non-synergy-like) is indicated on the left. *P*-value was calculated by two tailed Fisher's exact test comparing the number of synergy-like and non-synergy-like samples within a Jonsson's class vs the remaining 3 classes: * $P<0.05$, ** $P<0.01$, *** $P<0.001$, **** $P<0.0001$. **e)** Expression (in FPKM) of *MITF* (X axis) and *AXL* (Y axis) in the *BRAF*/*NRAS* WT cell lines. Pearson correlation *P*-value, R squared and r values are indicated. **f-g)** Expression of *MITF* (f) and *AXL* (g) (in FPKM, Y axis) in the cell lines that displayed synergy for nilotinib plus MEK inhibitors (sensitive) and in the cell lines that did not (non-sensitive). *P* value by unpaired Student's t-test. **h)** Top panel: Western blot for AXL (above) and MITF (below) in sensitive and non-sensitive cell lines (green and red background, respectively; "intermediate" C092 cell line in yellow background). *=$NRAS^{Q61R}$-mutant cell line, ^=$BRAF^{V600E}$-mutant cell lines. β-tubulin is shown as loading control. The eight samples on the left are biological replicates of the ones presented in Figure 3a. The black line separates different membranes. Bottom panels: Full pictures of western blot membranes presented in Fig.3a and above in the top panel. **i)** Western blot for AXL in the 5 *BRAF*/*NRAS* WT non-sensitive cell lines of the second melanoma collection. β-tubulin is shown as loading control. **j)** Sensitivity (expressed as AUC in Y axis) to PD-0325901 (left) and trametinib (right) in cell lines that express (FPKM>1, green), or not (FPKM<1, red) *AXL*. *P* value by unpaired Student's t-test, mean and standard error mean are shown. **k**) Western blot for AXL in sensitive cell lines transduced with lentiviral vectors expressing shRNA targeting AXL (left) and in non-sensitive cell lines transduced with a lentiviral vector expressing AXL cDNA (right). β tubulin is shown as loading control, volume of virus used for the infection is indicated in the legend. **l)** Survival curves of representative cell lines transduced with control vectors (top panels) or lentiviral vector expressing shRNA targeting AXL (left and middle bottom panel) or overexpressing AXL cDNA (right bottom panel). The Y axis shows viability vs vehicle treated control, X axis the concentration of trametinib (nM). The red line shows the viability of the cells treated with trametinib, the blue line the viability with nilotinib 2μM, the yellow line the viability with nilotinib/trametinib combination, the grey line the predicted additivity (see **Methods**). Each point is the average value of a technical triplicate. **m)** Delta AUC (Y axis) for nilotinib/trametinib combination in sensitive cell lines with knockdown of AXL (top panels) and in non-sensitive cell lines with AXL overexpression (bottom panels). *P*-values by unpaired Student's t-test; mean and standard error mean are shown. **n)** Experimental outline of the samples analysed for proteome and phosphoproteome. **o**) Variation of the detected ERK phosphopeptides (X axis, expressed as log_2 of the fold change vs DMSO-treated matched controls) in a sensitive (top part) and a non-sensitive (bottom part) cell line treated with trametinib (1nM), nilotinib (2μM) or nilotinib/trametinib

Supplementary Figure 3

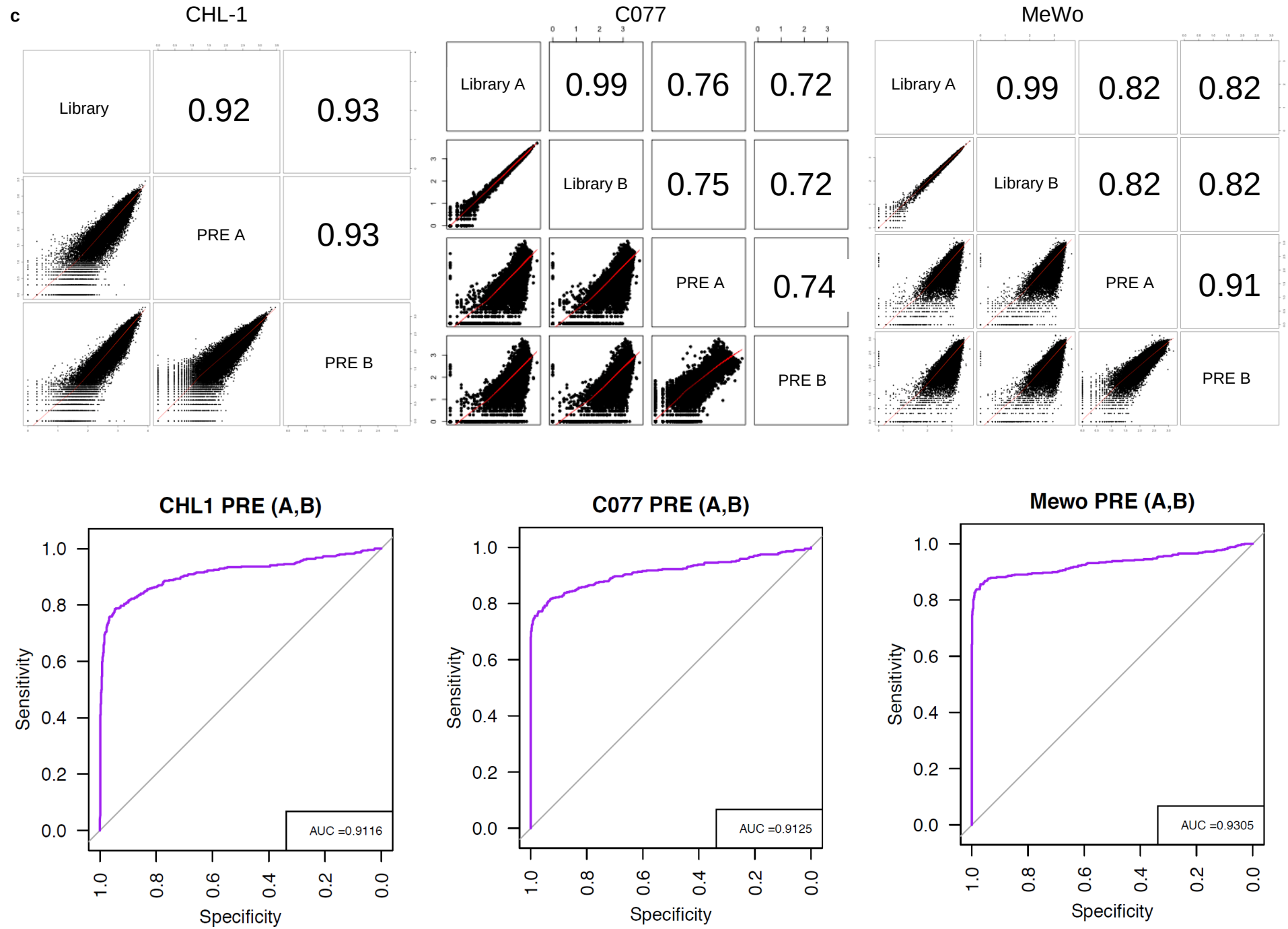
combination for 6 hours. The legend indicates the specific phosphorylation site. **p)** Full pictures of the membrane presented in Fig. 3b-c. N/A indicates samples not analysed for the scope of the manuscript. **q)** Western blot for total ERK upon treatment with DMSO vehicle, nilotinib (2μM), trametinib (1nM) or combination for 6h in representative cell lines.

Supplementary Figure 4



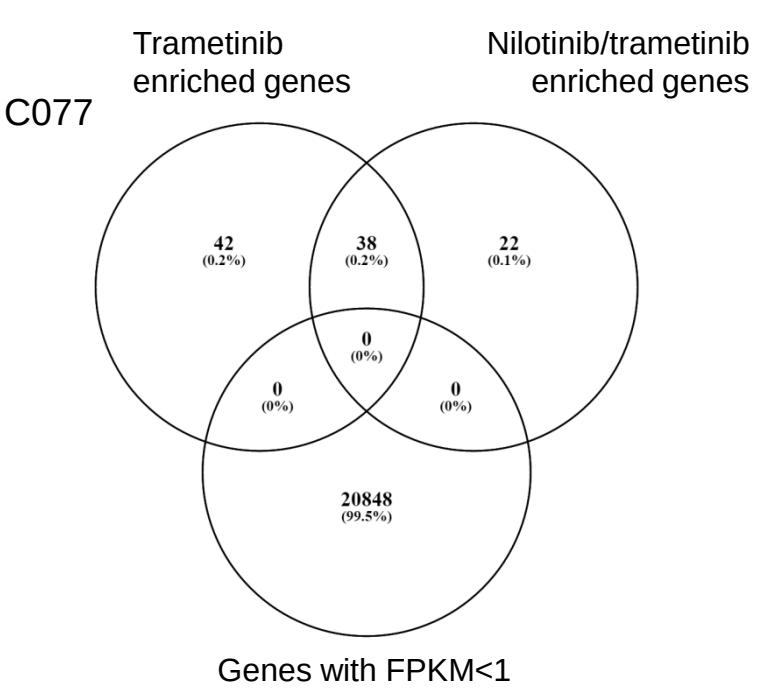


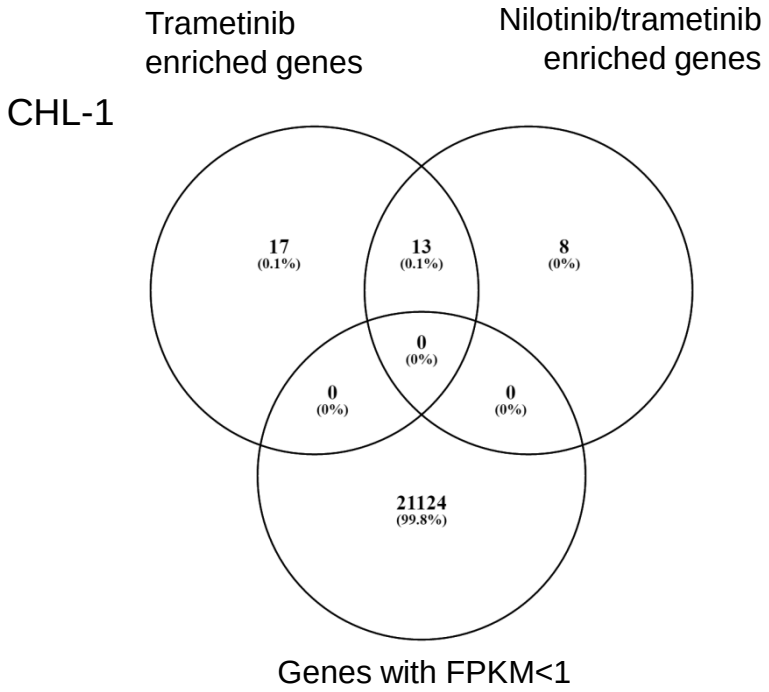
Supplementary Figure 4

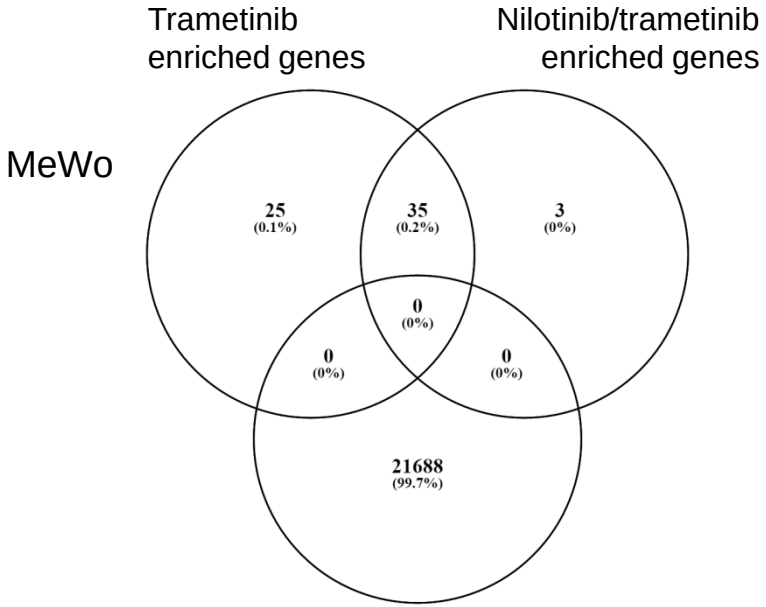


Supplementary Figure 4

d



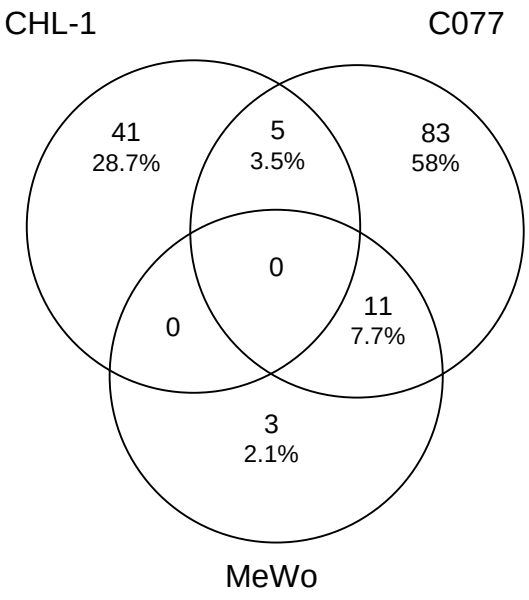




Supplementary Figure 4

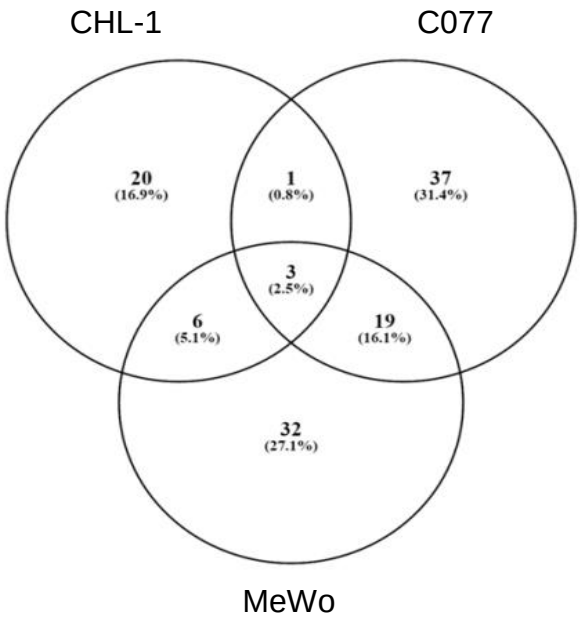
e

Pathway enriched in combination resistance genes



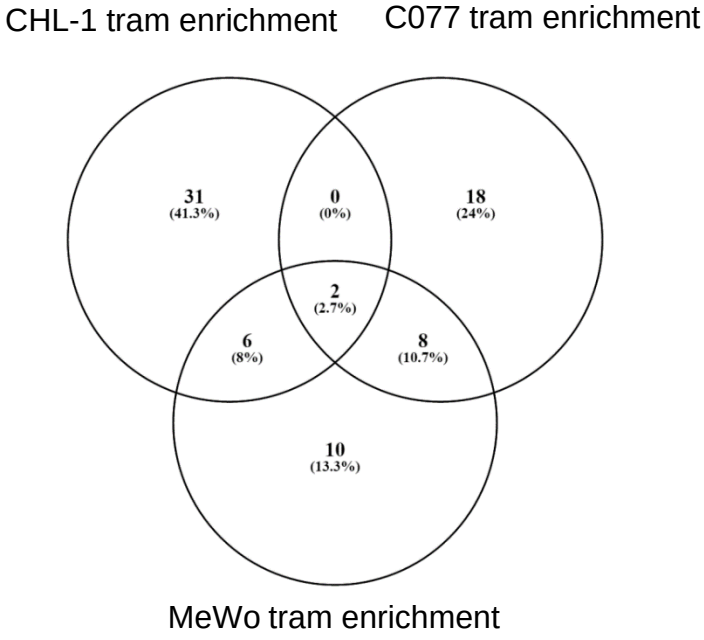
f

Trametinib resistance genes



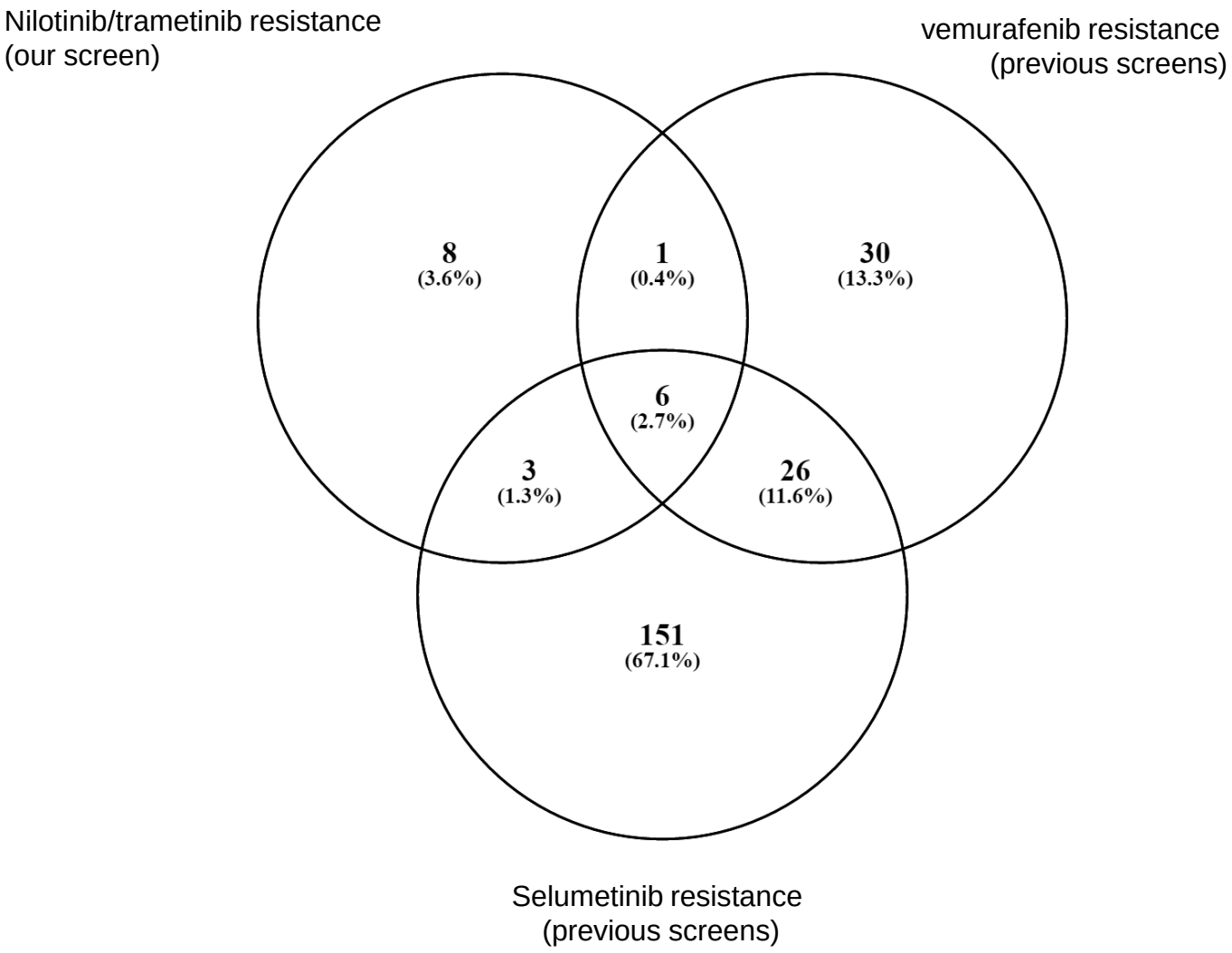
g

Pathway enriched in trametinib resistance genes

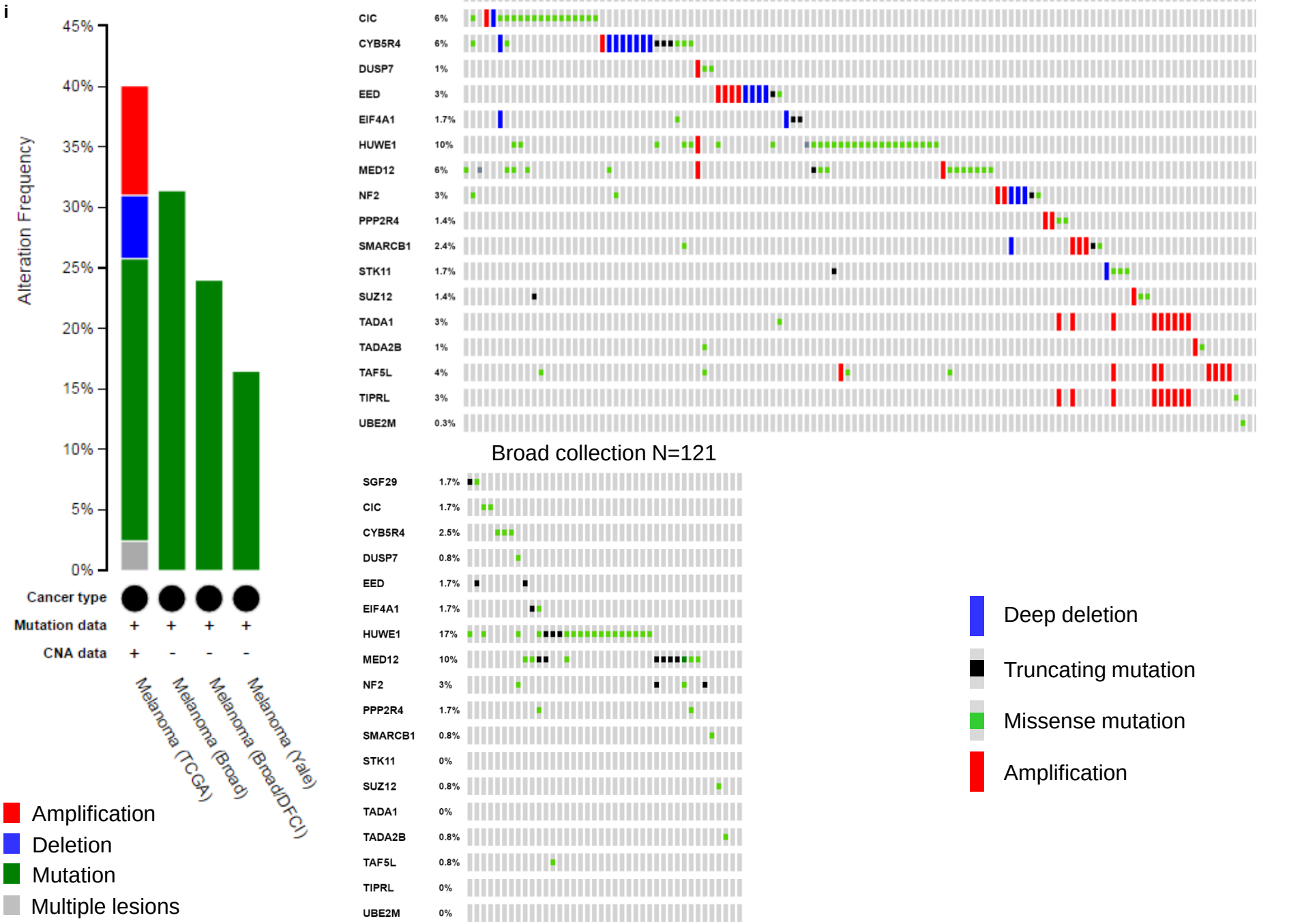


Supplementary Figure 4

h



Supplementary Figure 4



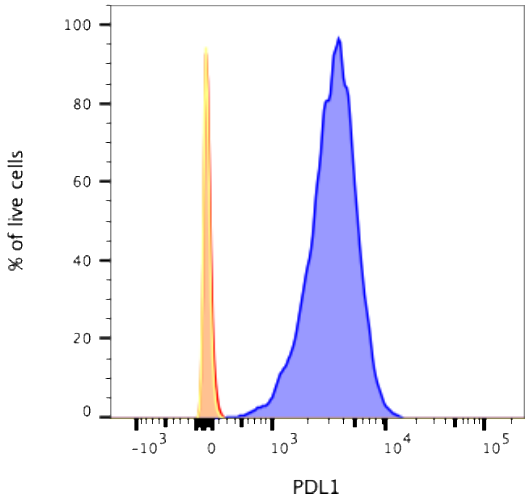
Supplementary Figure 4. Identification of trametinib and nilotinib/trametinib drug resistance genes by CRISPR/Cas9 genome wide library screening.

a) Experimental outline of the CRISPR/Cas9 screening. Briefly, we generated for each of the 3 cell lines a derivative that expresses efficiently Cas9 and transduced it in duplicate with the sgRNA library. Fourteen days after the library infection, we performed drug selection for each replicate with vehicle, trametinib or nilotinib/trametinib combination and collected them after 18 days of drug treatment to estimate by PCR and sequencing the representation of each sgRNA. **b)** Efficiency of the Cas9 cut in BFP/GFP reporter assay. The cells that stably express Cas9 were infected with a lentiviral vector (LV) that express BFP, GFP and a sgRNA targeting GFP (see **Methods**). BFP and GFP fluorescent intensity by FACS analysis (Log scale) are displayed in X and Y axis, respectively. Above the graph the Cas9 activity (ratio between $BFP^{pos}GFP^{neg}$ and total BFP^{pos}) is indicated. The gates for the analysis were defined using negative and single fluorescent controls. **c)** Quality control of the Cas9 screening experiment. The top panels show pair-wise Pearson correlation coefficients (upper right quadrants) and scatter plots (lower left quadrants) between sgRNA counts in replicates of infection prior to drug treatment (PRE A and B, 2 weeks after the library infection), and between each individual replicate and the reference counts from the plasmid library (2 replicate of sequencing for the library plasmid for C077 and MeWo). The bottom panel show receiver operating characteristic (ROC) curves quantifying classification performances of the gene essentiality scores derived applying MAGeCK to the pooled PRE A and B population vs the library. Two *a priori* defined gene sets of known essential and non-essential genes (as specified in the **Methods**) were considered as positive and negative cases, respectively. The performances assessment was restricted only to genes in these two sets. One plot per cell line is shown and the inset contains the extent of the area under each curve. Grey diagonal lines indicate performance expectations. **d)** Venn diagram among the Cas9 screening hits (FDR<0.1 in both duplicates) for trametinib, trametinib plus nilotinib, and poorly expressed genes (FPKM<1) for C077 (top left), CHL-1 (top right) and MeWo (bottom) cell lines. All the significant hits are expressed genes. **e)** Venn diagram of the significantly enriched pathways in the list of combination resistance genes for each cell line. The enrichment was performed on genes with FDR<0.1 in both replicates (see **Methods**). **f)** Venn diagram of the trametinib resistance genes (FDR<0.1 in both duplicates) among the 3 cell lines**. g)** Venn diagram of the significantly enriched pathways in the list of trametinib resistance genes for each cell line. The enrichment analysis was performed as in (e). **h)** Venn diagram showing the overlap between combination resistance genes identified in our screenings and vemurafenib and selumetinib resistance genes identified previously (see **Methods**). **i)** Status of the nilotinib/trametinib resistance genes in 4 collections of human tumors (by cBioportal, see **Methods**). Left panel: frequency of tumors carrying a mutation (red amplification, blue deep deletion, green missense mutation, grey multiple mutation type) in one of the 18 nilotinib/trametinib resistance genes. Top right panel: mutation status of the combination resistance genes in each of the melanoma from the TCGA cohort (n=287 samples analysed by cBioportal website); bottom right panel: mutation status of the combination resistance genes in melanoma from the Broad cohort (n=121 samples by cBioportal website). Each grey square represents a sample; only tumors with a mutation in the interrogated genes were displayed; frequency of mutation across the cohort per each gene is displayed on the right. Amplification/deletion data are available only for the TCGA cohort.

Supplementary Figure 5

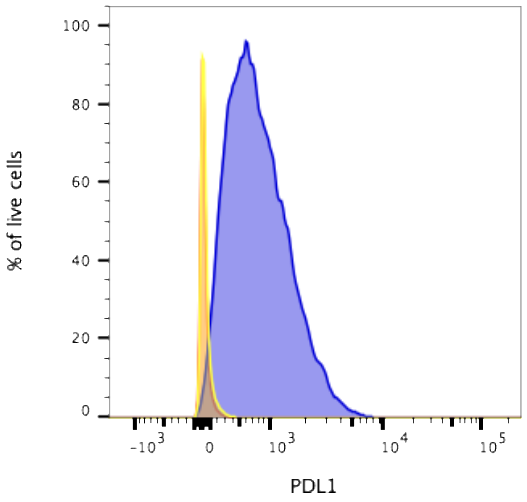
Sensitive cell lines

CHL-1



Sample Name	% PDL1 positive
Nilotinib + Trametinib	0.071
IFNg (pos ctrl)	99.9
DMSO (neg ctrl)	0.14

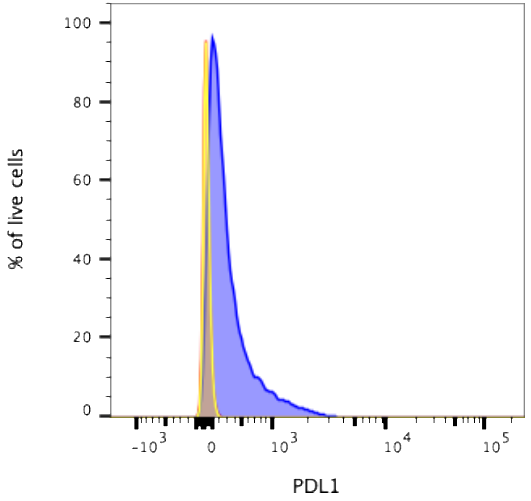
C077



Sample Name	% PDL1 positive
Nilotinib + Trametinib	3.63
IFNg (pos ctrl)	93.5
DMSO (neg ctrl)	2.13

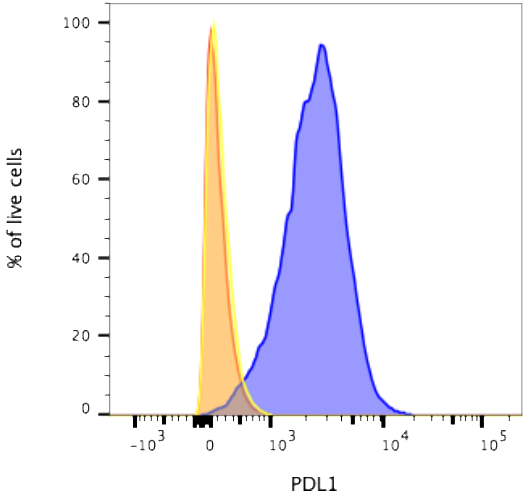
Non-sensitive cell lines

Colo-792



Sample Name	% PDL1 positive
Nilotinib + Trametinib	0.38
IFNg (pos ctrl)	61.0
DMSO (neg ctrl)	0.59

C025



Sample Name	% PDL1 positive
Nilotinib + Trametinib	2.35
IFNg (pos ctrl)	97.1
DMSO (neg ctrl)	1.91

Supplementary Figure 5. The nilotinib/trametinib combination treatment did not induce PD-L1 expression in *BRAF/NRAS* WT melanoma cell lines.

PD-L1 expression by flow cytometry, shown as % of live cells (Y axis), in 2 sensitive (top panel) and 2 non-sensitive (bottom panel) cell lines. Cells were treated for 72h with DMSO (vehicle, in red), 50ng/ml IFNγ (positive control of PD-L1 induction, in blue) or 2μM nilotinib + 1nM trametinib (drug combination, in yellow). PD-L1 expression of all cell lines was induced by IFNγ treatment, however treatment with the nilotinib/trametinib combination did not alter the PD-L1 expression of any of the cell lines.