Supplementary material belonging to the manuscript:

Uveal Melanoma zebrafish xenograft models illustrate the mutation status dependent-effect of compound synergism or antagonism

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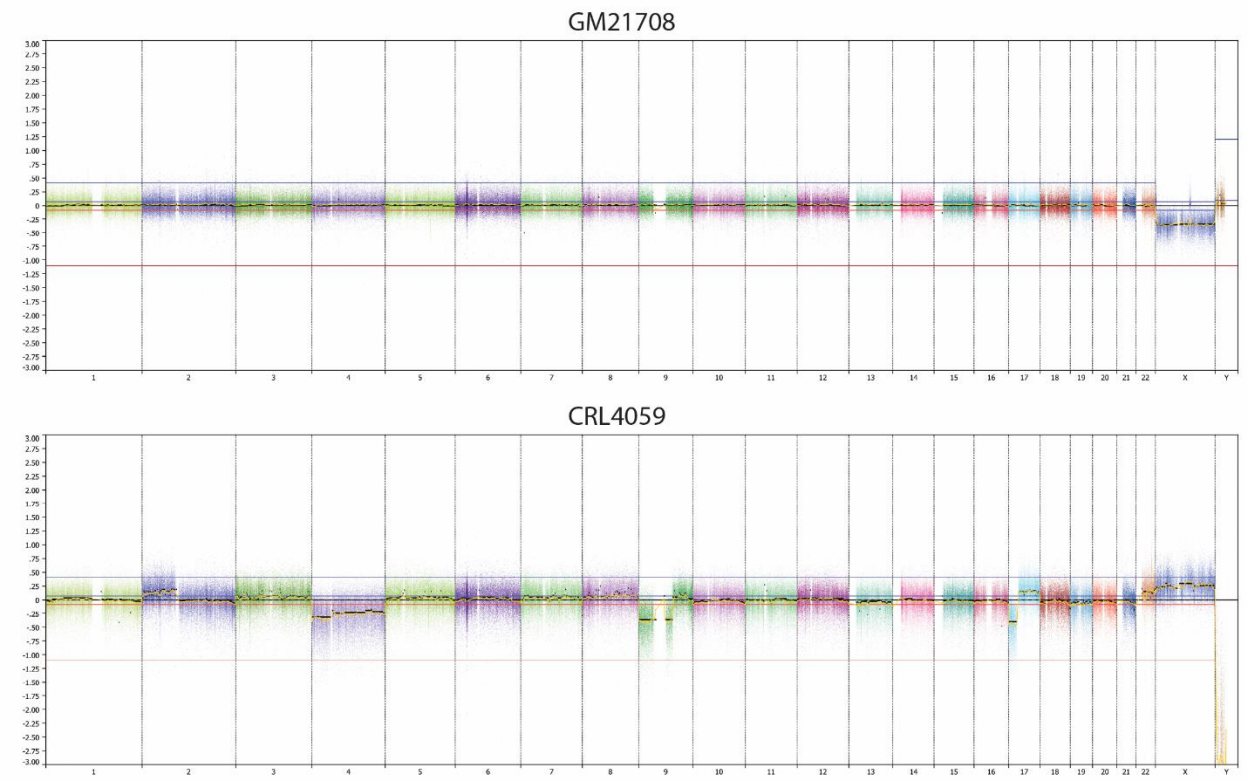
Contents:

Copy number variations and mutation status of cell lines used in this manuscript.

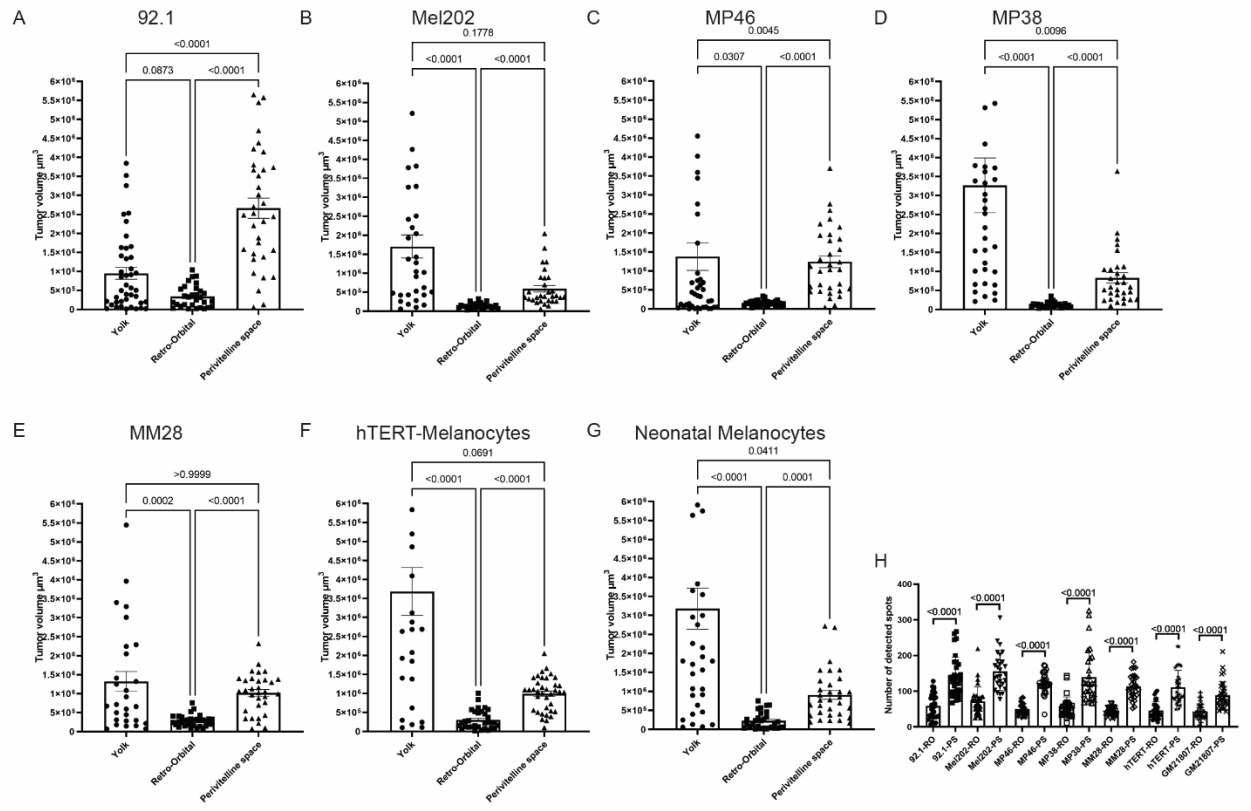
Cell line	Origin	Driver Mutation	Secondary Mutation	Chr. 3	Chr. 6	Chr. 8	BAP1 protein	Ref	RRID
GM21807	Infant Skin	-	-	Disomy 3	Disomy 6	Disomy 8	-	-	CVCL_H988
CRL4059	Adult Skin	-	-	Disomy 3	Disomy 6	Disomy 8	-	-	N/A
92.1	Primary	GNAQ Q209L c.626A>T	EIF1AX c. 17G/A	Disomy 3	Gain 6p	Gain 8q	Yes	[1]	CVCL_8607
Mel202	Primary	GNAQ Q209L c.626A>T	SF3B1 c.1793 C>T	Disomy 3	Gain 6p, Loss 6q	Gain 8q	Yes	[1]	CVCL_C301
MP38	Primary	GNAQ Q209L c.626A>T	BAP1 c.68-9_72del	Monosomy 3	Gain 6p, Loss 6q	Gain 8q, Loss 8p	No	[2]	CVCL_4D11
MP46	Primary	GNAQ Q209L c.626A>T	WT	Disomy 3, LOH	Gain 6p, Loss 6q	Gain 8q, Loss 8p	No	[2]	CVCL_4D13
MM28	Liver Metastasis	GNA11 Q209L c.626 A>T	BAP1 c.1881 C>A	Loss 3q	Gain 6p, Loss 6q	Gain 8q, Loss 8p	No	[2]	CVCL_4D15

Supplementary Table 1: Overview of molecular status of the used cell lines in this manuscript.



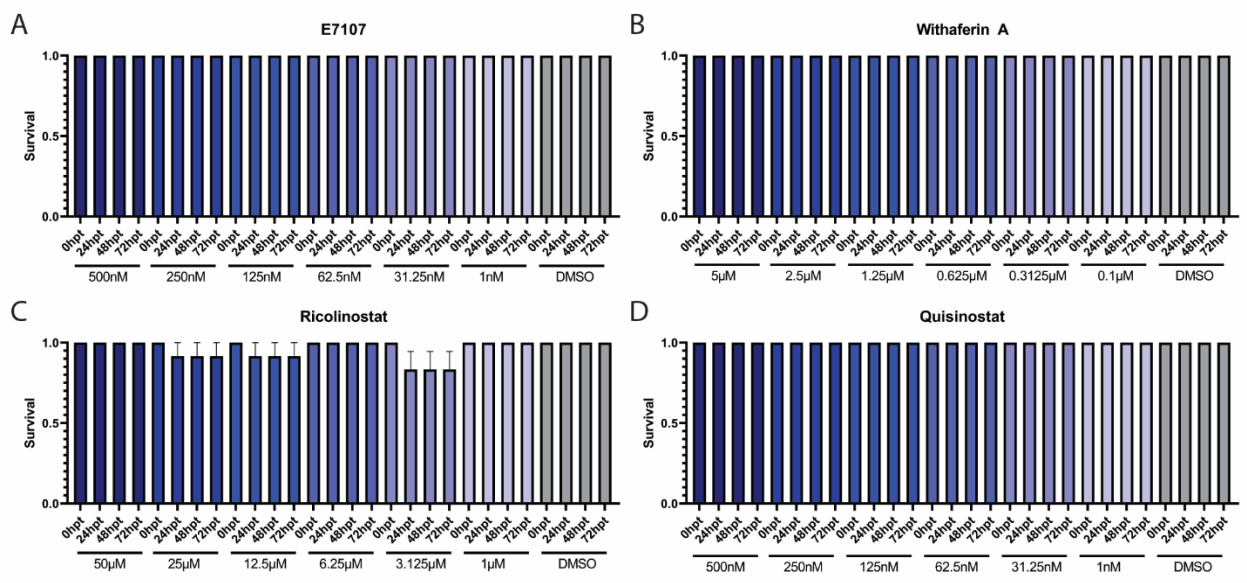
Supplementary Figure 1: *Single nucleotide polymorphism array of dermal melanocytes*

Neonatal melanocytes (GM21708) illustrate a normal chromosomal pattern; whereas hTERT-dermal melanocytes (CRL4059) demonstrate chromosomal aberration in chr.2, chr. 4, chr. 9 and chr.17



Supplementary Figure 2: *Overview of tumor volume and detected disseminated cells per inoculation site for each cell line*

Comparison of tumor volumes between yolk sac, retro-orbital and perivitilline space injections illustrated the differences between injection site (A-G). H) comparison of the detected number of spots between retro-orbital and perivitilline space inoculations.



Supplementary Figure 3: *Toxicity tolerance of zebrafish larvae*

Each compound was evaluated for toxicity based on the effective concentrations found by in vitro screenings of UM cell lines.

Supplementary Methods: *Scripts to quantify zebrafish xenografts.*

Script to quantify tumor volume (Can be copy and pasted into the macro-manager of FIJI)

```
//splits all channels.
run("Split Channels");
//Closes brightfield image. Adjust 'C2-*' to whatever channel you want to remove from analysis
close('C2-*');
//transform image to get the right orientation. This might differ depending on how the zebrafish is positioned under the microscope.
run("Rotate 90 Degrees Right")
//measure tumor spots in cubic micron.
run("3D Objects Counter", "threshold=90 slice=1 min.=10 max.=10000000 exclude_on_edges surfaces_numbered statistics");
//Saves measurements in text file.
saveAs("text");
```

```
//Closes analysed image.
close("C1-*");
//Closes measurements.
close("*.txt");
```

Script to quantify number of disseminated cells and their migration distance *(Can be copy and pasted into the macro-manager of FIJI)*

```
// Splits all channels
run("Split Channels");
// Close channel 2
close("*C2*");
// Rotate image 90 degrees to the right. This might differ depending on how the zebrafish is positioned under the microscope.
run("Rotate 90 Degrees Right");
// Max-intensity Z projection without keeping the stack
run("Z Project...", "projection=[Max Intensity]");
zProjID = getImageID();
saveAs("Tiff", "zProj.tif");
// Threshold image and obtain mask.
setThreshold(90, 255);
run("Create Mask");
maskID = getImageID();
saveAs("Tiff", "mask.tif");
// Multiply z-projected image with mask using image calculator to obtain binary thresholded image.
imageCalculator("Multiply", "zProj.tif", "mask.tif");
//Selects image which will be analysed.
selectImage("zProj.tif");
```

```
//Identify x and y coordinates of injection spot
waitForUser("Hover over the reference point and remember the coordinates.");
// Manually enter the x and y coordinates of the reference point
xRef = getNumber("Enter x-coordinate of the reference point:", 0);
yRef = getNumber("Enter y-coordinate of the reference point:", 0);
// Set parameters for spot detection.
minSize = 20; // minimum spot size in pixels
maxSize = 5000; // maximum spot size in pixels
threshold = 20; // detection threshold
// Find spots using the Find Maxima function
run("Find Maxima...", "noise=" + threshold + " output=[Point Selection] exclude");
// Filter spots by size
run("Analyze Particles...", "size=" + minSize + "-" + maxSize + " circularity=0.00-1.00 show=Masks add");
 // Get the number of spots and their x and y coordinates
nSpots = nResults();
for (i = 0; i < nSpots; i++) {
    x = getResult("X", i);
    y = getResult("Y", i);
// Calculate the distance between the spot and the reference point
    distance = sqrt(pow(x - xRef, 2) + pow(y - yRef, 2)); // convert distance to microns
// Write the spot's x and y coordinates and the distance to the reference point to the output file
   print("Spot #" + (i+1) + ": X = " + x + ", Y = " + y + ", Distance to reference point = " + distance + "
microns\n");
}
//Selects the measurements window
selectWindow("Log");
//Saves measurements as text file.
saveAs("Text");
```

```
//String of commands to close and reset all parameters and images used to obtain measurements without closing stacks that are still to be analyzed.
print("\\Clear");
run("Clear Results");
roiManager("Reset");
run("Close");
close("C*");
close("zProj.tif");
close("mask.tif");
close("Mask of zProj.tif");
```

References

1. Jager MJ, Magner JA, Ksander BR, Dubovy SR. Uveal Melanoma Cell Lines: Where do they come from? (An American Ophthalmological Society Thesis). *Trans Am Ophthalmol Soc* 2016;114:T5.
2. Amirouchene-Angelozzi N, Nemati F, Gentien D, et al. Establishment of novel cell lines recapitulating the genetic landscape of uveal melanoma and preclinical validation of mTOR as a therapeutic target. *Mol Oncol* 2014;8:1508-1520.