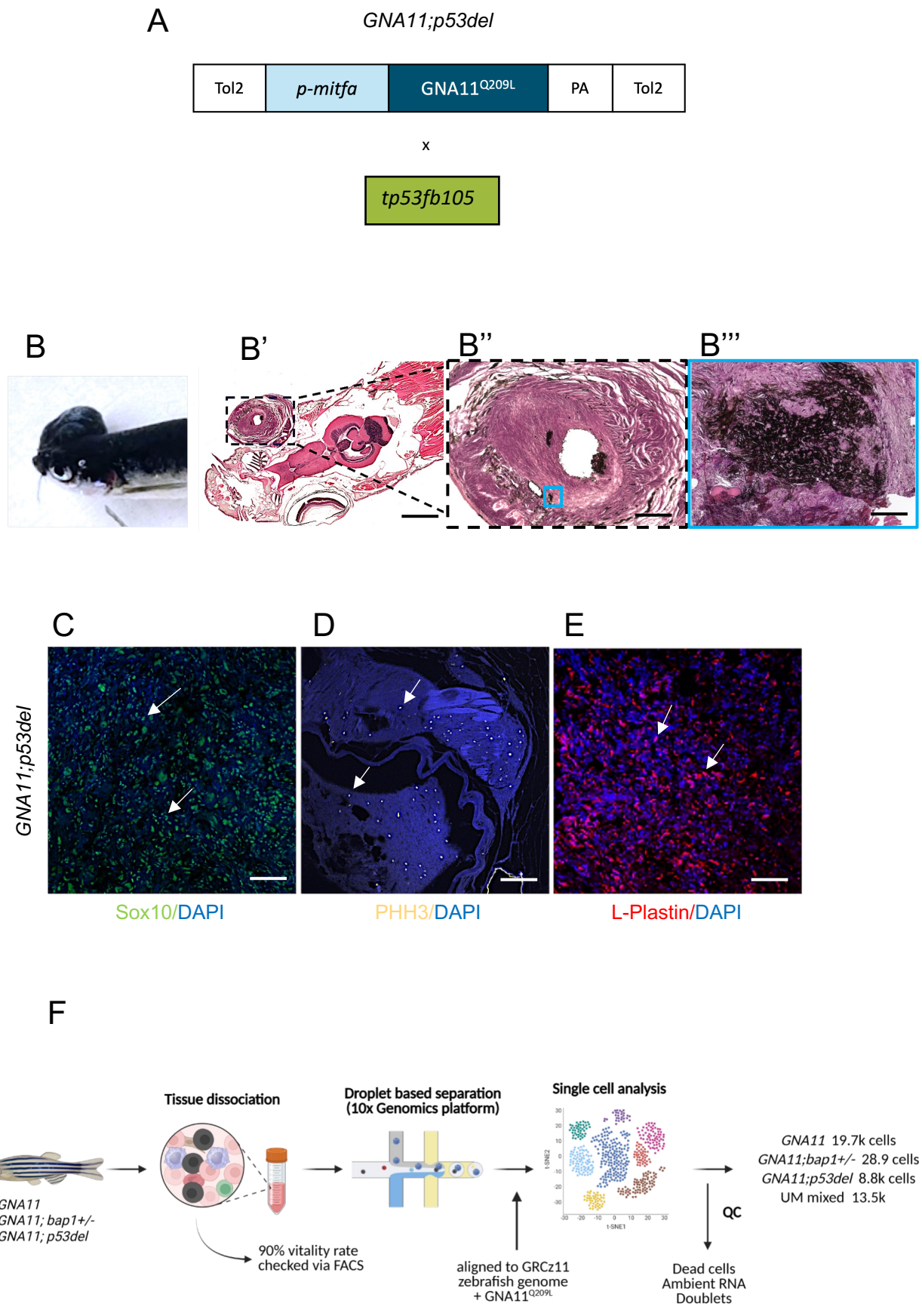
Supplementary figure 1



Supplementary Figure 1: Transgenic *GNA11* fish are crossed with *tp53del (tp53^{fb105})* mutant fish to generate *GNA11;p53del* UM model. (related to fig. 1).

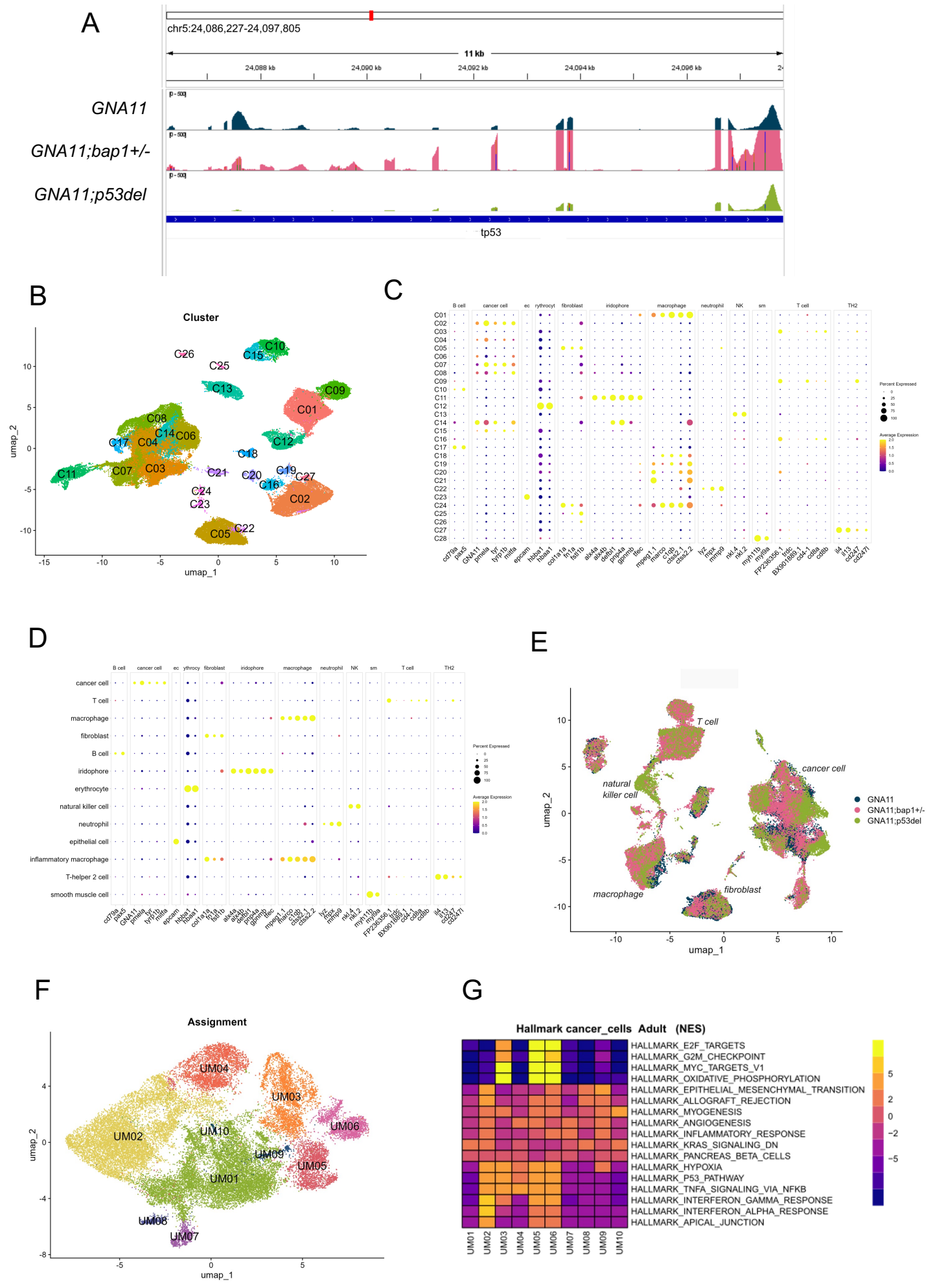
A: Schematic representation of the gene constructs used to generate the GNA11 transgenic *line*, expressing a mutant form of human *GNA11* gene (encoding for the AA substitution Q209L) under the control of the zebrafish melanocyte-specific *mitfa* promoter.

B: Representative H&E- stained sections of a tumour developing in a *GNA11;p53del* zebrafish. Boxed regions are shown progressively at higher magnification in B', B'' and B'''. B': Scale bar = 500 μm. B'': Scale bar = 100 μm. B''': Scale bar = 20 μm.

C-E: Immunofluorescence staining of sections from *GNA11;p53del* tumour. **(C):** Sox10 in green and DAPI in blue. (**D)**: phosphohistone 3 (PHH3) in yellow and DAPI in blue. **(E):** L-plastin in red and DAPI in blue. Arrows point to positive cells. Scale bars = 20 μm.

F: Schematic overview of the single-cell RNA sequencing experimental pipeline used in this study.

Supplementary figure 2



Supplementary Figure 2: Single cell RNA sequencing of zebrafish uveal melanoma tissue.

A: Reads coverage across the *tp53* locus (chr5:24,086,227-24,097,805), showing a markedly reduced coverage in the *GNA11;p53del* sample, consistent with successful genetic deletion.

B: UMAP of 70,900 aggregated single cells derived from the three different UM models, showing the clusters identified by unsupervised clustering.

C: Dot plot showing the expression of marker genes (x-axis) used for manuaannotation the of the transcriptionally distict clusters (y-axis). Dot size indicates the percentage of cells within each cluster expressing the gene, while colour represents average expression (high expression in yellow and low expression in blue).

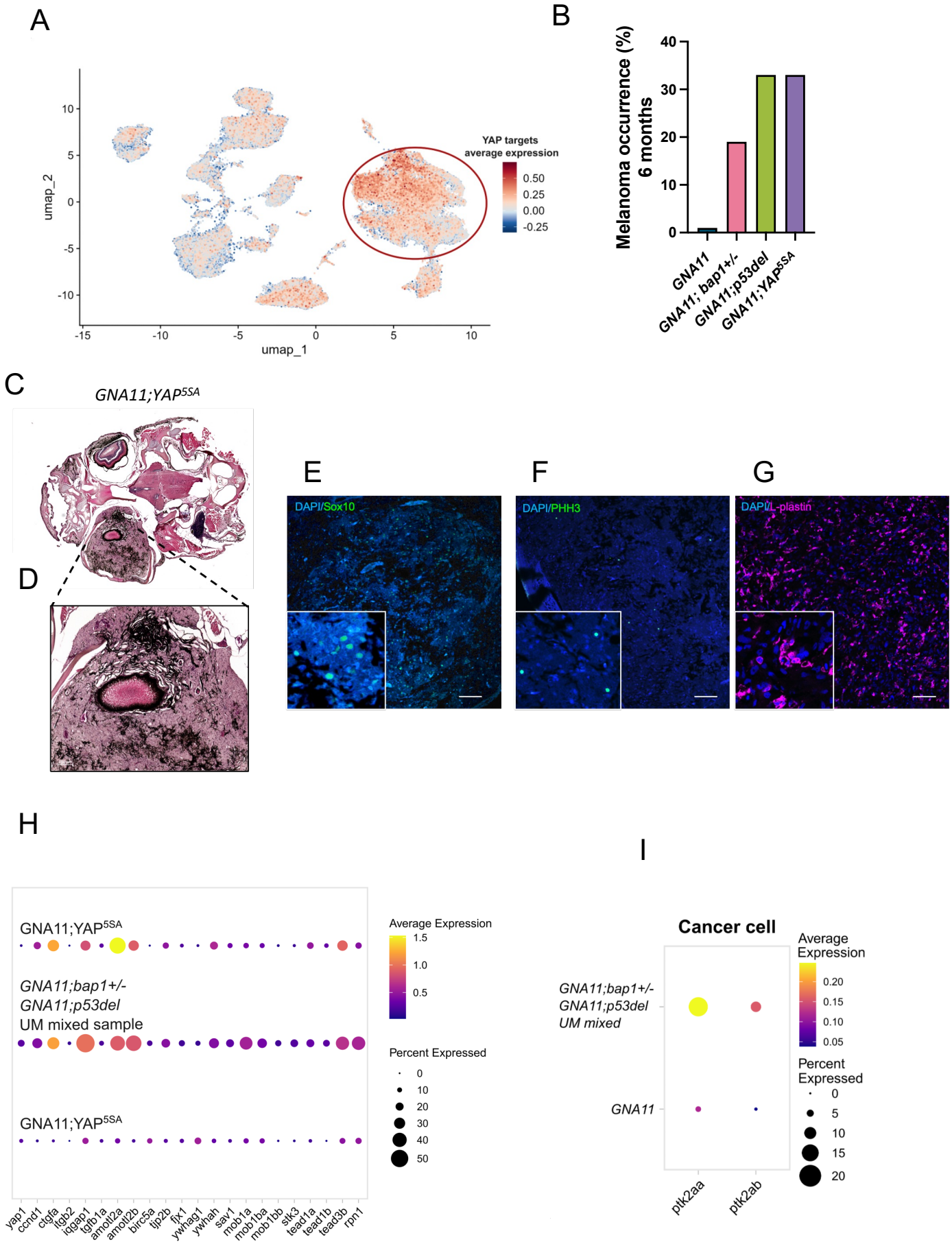
D: Dot plot showing the expression of marker genes used to manually annotate the major cell types identified in the dataset. Dot size indicates the percentage of cells of that cell type expressing the gene, and colour indicates average expression (high expression in yellow and low expression in blue).

E: UMAP projections of all 70,900 single cells, coloured by model, showing the relative contributions of each model to the different clusters.

F: UMAP projection of *de novo* clustering of the cancer cells from the three different UM models, showing the number of clusters generated by unsupervised clustering.

G: Gene set enrichment analysis (GSEA) using Hallmark Pathway gene sets across the ten transcriptionally distinct cancer cell clusters. The heatmap displays normalized enrichment score (NES) of the top two enriched pathways per cluster: positive enrichment is shown (in yellow.

Supplementary figure 3



Supplementary Figure 3: Transgenic *GNA11;YAP^{5SA}* histologically resemble the other transgenic UM fish but differ for YAP targets expression. (related to fig. 3).

A: UMAP showing a YAP target gene signature score, calculated as the average expression of 21 yap target genes across all cell types and samples. Colour scale indicates high score in red and low score in blue. Cancer cells display a higher YAP target score (coloured circle).

B: Graph shows the percentage of UM development in the indicated transgenic lines over a period of 6 months. *GNA11* n=12; *GNA11;bap1+/-* n = 53; *GNA11;p53del* n=70; *GNA11;YAP^{5SA}* n=92.

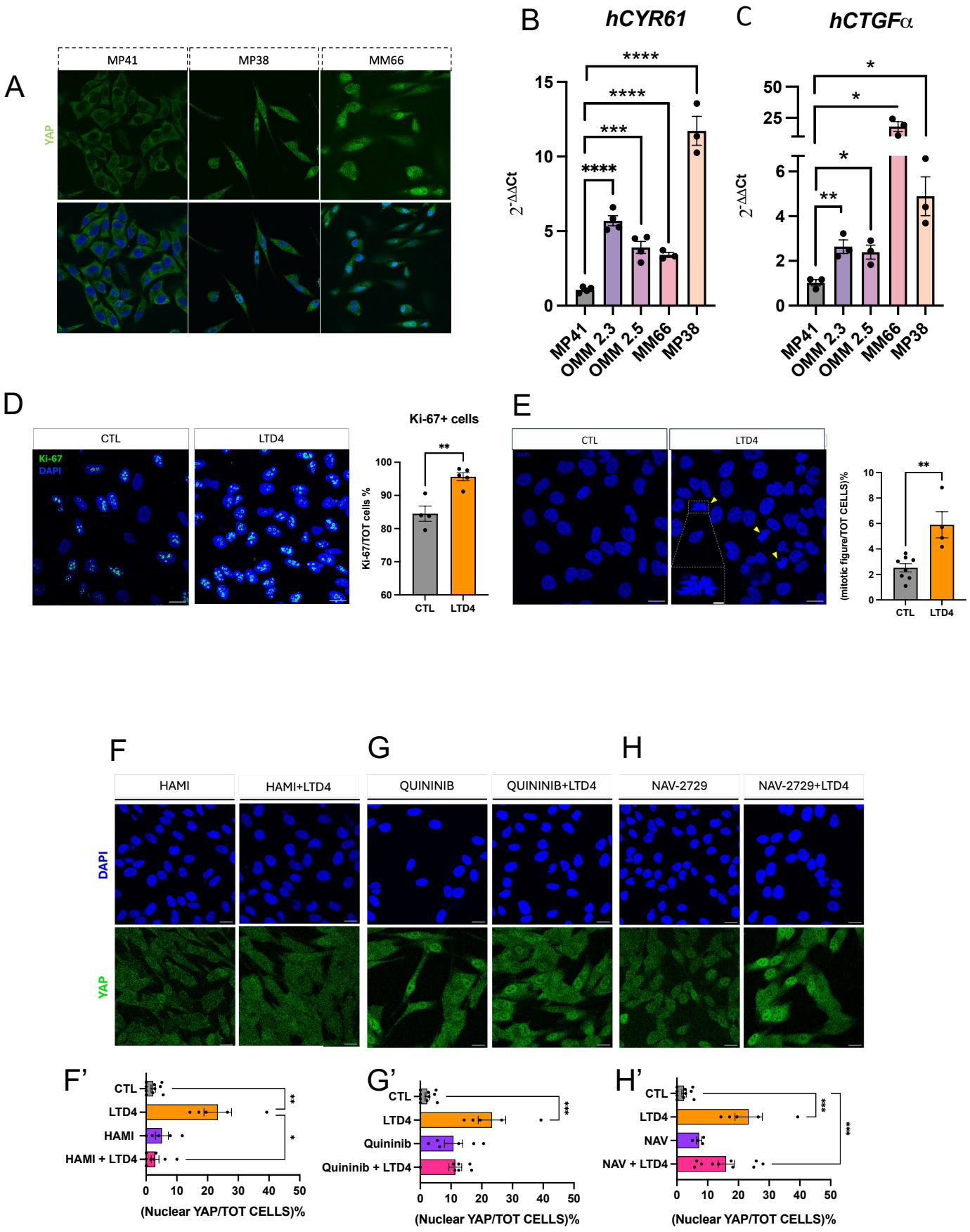
C-D: Representative H&E-stained sections of a tumour from a *GNA11;YAP^{5SA}* zebrafish. Image shows a section of the head of the transgenic fish bearing an ocular melanoma. Scale bar = 500 μm. In the high magnification image, the disruption of eye tissue is shown. Scale bar = 100 μm.

E-G: Immunofluorescence staining of paraffin sections of *GNA11;YAP^{5SA}* UM tissue. Immunofluorescence staining was performed for (E) Sox10 (green) and DNA (blue, DAPI); **(F)** phospho histone 3 (PHH3, green) and DNA (DAPI, blue); **(G)** L-plastin (magenta) and DNA (DAPI, blue). Scale bar = 20 μm.

H: Dot plot showing the average expression of YAP target genes in the cancer cells across the indicated UM models. Colour indicates average expression (high expression in yellow and low expression in blue), and dot size represents the percentage of cancer cells expressing each gene.

I: Dot plot showing the average expression of zebrafish *ptk2aa* and *ptk2ab* (orthologs of PTK2/FAK) genes in cancer cells of the three indicated models, compared with the cancer cells of the GNA11 model, which do not display YAP signalling activation (see panel H). Colour indicates average expression (high expression in yellow and low expression in blue), and dot size represents the percentage of cancer cells expressing each gene.

Supplementary figure 4



Supplementary Figure 4: Leukotrienes activate YAP signaling through CYSLTR2, thereby promoting cancer cell proliferation (related to fig. 4).

A: Immunofluorescence staining for YAP (green) in human UM cell lines MP41, MP38, MM66. Merged images with nuclear DAPI staining (blue) are shown in A'. Scale bar = 10 μm.

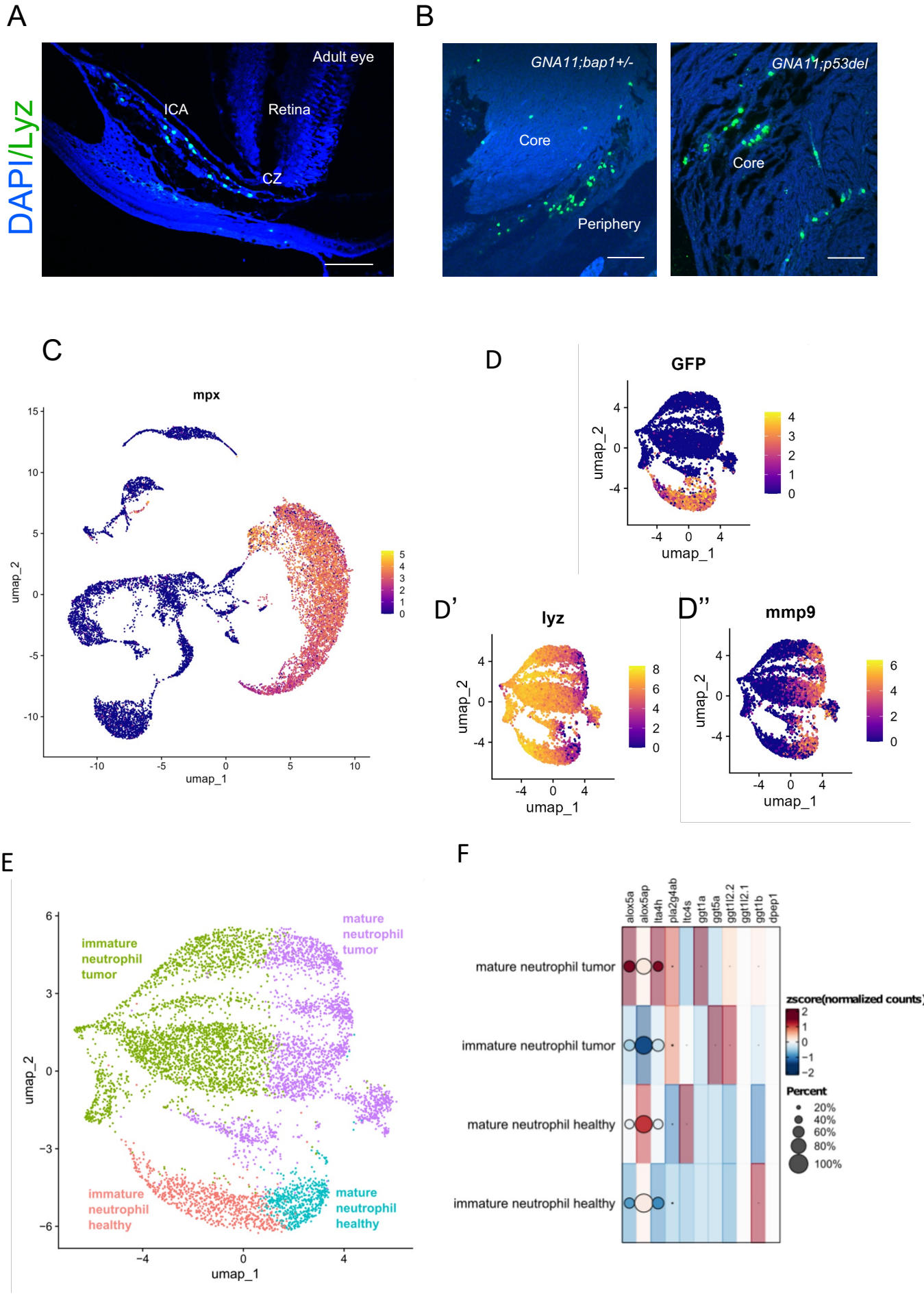
B-C: qPCR analysis of the expression of human YAP target genes *CYR61* and *CTGFa* in MP41, MP38, MM66, OMM2.3 and OMM2.5 UM cells, as indicated. Gene expression levels are shown as fold change (2^-ΔΔCq) relative to MP41 cells (CTL). Results are expressed as mean ±SEM. n=3-4. An ordinary one-way ANOVA was performed to evaluate significance. ** P-value < 0.05; ** P-value < 0.01; *** P-value < 0.001; **** P-value < 0.0001.*

D: Representative confocal images of immunofluorescence staining for Ki67 (green) and DNA (DAPI, blue) in MP41 human UM cells, treated or not with LTD4, as indicated. Scale bar = 20μm. The accompanying graph shows quantification of Ki67-positive MP41 cells treated or not (CTL) with LTD4, expressed as a percentage of the total number of cell counted. A minimum of 25 cell per field was analysed. Results are expressed as mean ±SEM. Number of fields n=4-5. A two tailed Unpaired Student's t-test was performed to evaluate significance. Arrows point to positive cells. *** P-value = 0.0026*.

E: Representative confocal images of immunofluorescence staining for DNA (DAPI, blue) in MP41 human UM cells, treated or not with LTD4, as indicated. Yellow arrowheads indicate mitotic figures. Scale bar = 20 μm. The accompanying graph shows quantification of mitotic figures in MP41 cells treated or not with LTD4, expressed as a percentage of the total number of cell counted. A minimum of 18 cell per field was analysed. Results are expressed as mean ±SEM. Number of fields = 4-8. A two tailed Unpaired Student's t-test was performed to evaluate significance. *** P-value = 0.0022.*

F-H: Immunofluorescence staining of MP41 human UM cells, treated with HAMI or HAMI+ LTD4 (**G**), Quininib or Quininib + LTD4 (**H**), NAV-2729 or NAV-2729 + LTD4 (**I**). Cells were stained for YAP (green) and nuclei were counterstained with (blue). Scale bar = 20 μm. Immunofluorescence images are accompanied by graphs showing quantification of nuclear YAP in MP41 cells before or after treatment with HAMI, HAMI+ LTD4 (**F**), Quininib and Quininib + LTD4 (**G**), NAV-2729 and NAV-2729 + LTD4 (**H**), as indicated. At least 16 cells were counted per field. Number of fields= 4-10. Results are expressed as mean ±SEM. A kruskal-Wallis test was performed to evaluate significance. ** P-value < 0.05; ** P-value < 0.01; *** P-value < 0.001*.

Supplementary figure 5



Supplementary Figure 5: Neutrophils of UM-bearing fish produce leukotrienes before leaving the hematopoietic niches.

A-B: Immunofluorescence staining for Lyz (neutrophils, green) and DNA (DAPI, blue) in paraffin sections of adult eye (A), *GNA11;bap1+/-* and *GNA11;p53del* uveal melanomas. ICA: iridocorneal angle; CZ: ciliary zone; Core: central zone of the tumour; Periphery: peripheral zone of the tumour. Scale bar = 20 μm.

C: UMAP projection of 14,232 single cells coming from kidney marrow of wt or UM-bearing fish showing the expression of mpx+ cells, indicating neutrophils. Colour scale indicates high expression in yellow and low expression in blue.

D: UMAP projection of neutrophils showing the expression of GFP (wt neutrophils). Colour scale indicates high expression in yellow and low expression in blue.

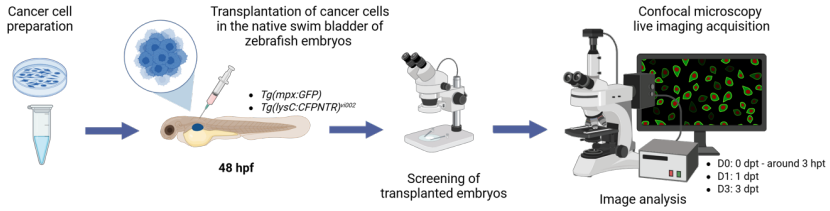
D': UMAP showing the expression of *lyz* in neutrophils of the kidney marrow, marking immature neutrophils. Colour scale indicates high expression in yellow and low expression in blue.

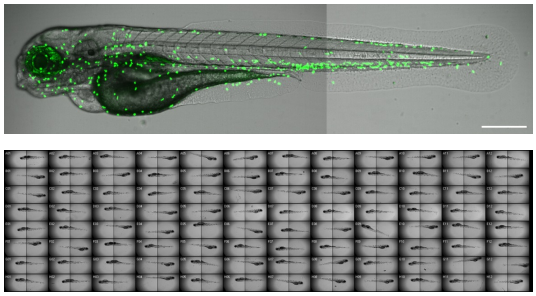
D": UMAP showing the expression of *mmp9* in neutrophils of the kidney marrow, marking mature neutrophils. Colour scale indicates high expression in yellow and low expression in blue.

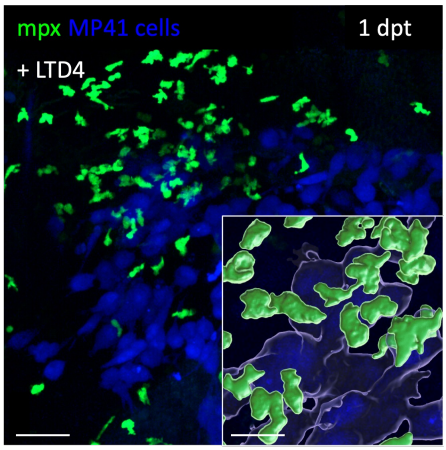
E: UMAP projection of 6,753 neutrophils derived from kidney marrows of wt or UM-bearing fish. Annotation is based on neutrophil maturation stages.

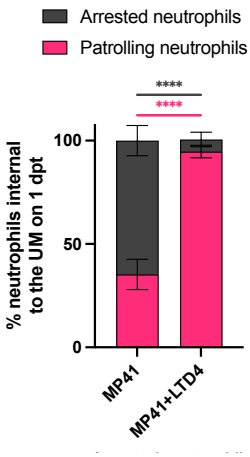
F: Heatmap showing the z-score of normalised counts of the expression of enzymes involved in leukotriene production in immature and mature neutrophils in the kidney marrows of wt and tumour-bearing fish. Higher expression is indicated in dark red and low expression in blue. Dot size represents the percentage of cells expressing the gene of interest over the total number of neutrophils.

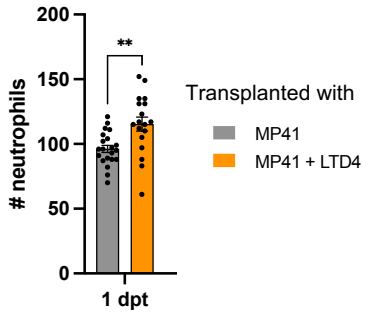
Supplementary figure 6

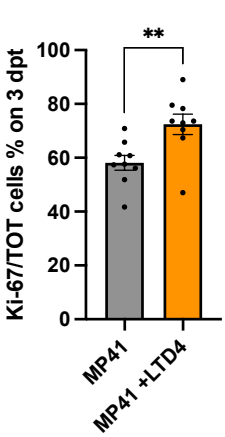


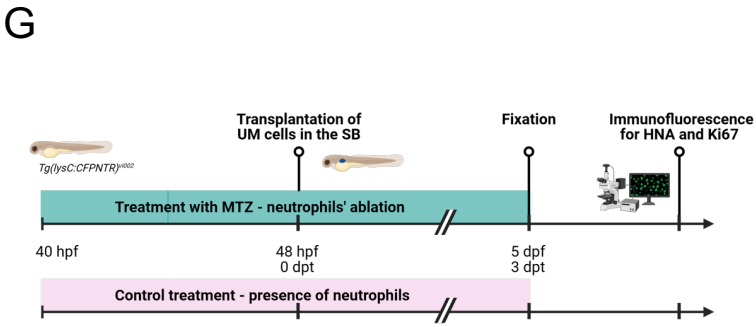


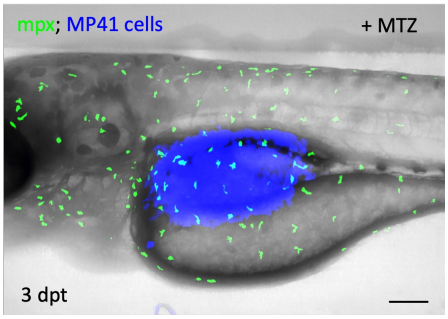


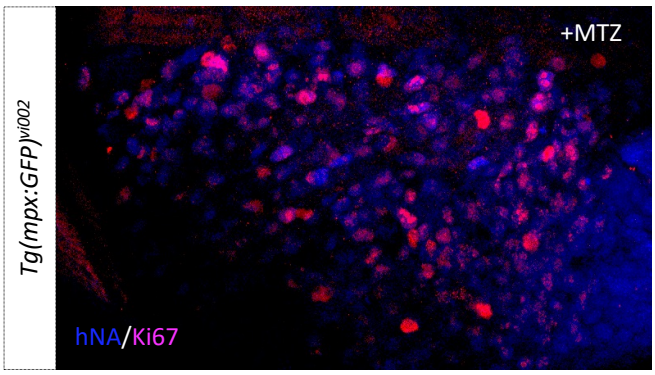


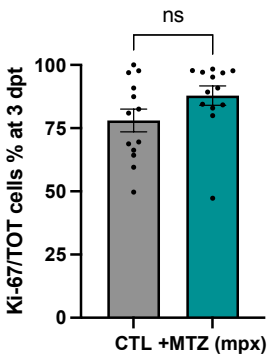












Supplementary Figure 6: Neutrophils exhibit altered behaviour in the presence of LTD4, supporting cancer cell proliferation. (related to fig. 6).

A: Schematic representation of zebrafish transplantation pipeline: transplantation, image acquisition and analysis (prepared with Biorender).

B: Representative image of a *tg(mpx:GFP)^{i114}* transgenic zebrafish larva at 60 hpf, bearing GFP+ neutrophils (green cells), acquired with IXM-C. Scale bar: 400 mm. Overview of the 96 well plate used to automatically acquire images and perform neutrophils counting with the ImageXpress® Micro Confocal system (IXM-C), containing transplanted larvae.

C: Representative fluorescent image of a transplanted *tg(mpx:GFP)^{I114}* zebrafish larva, bearing green neutrophils and transplanted with MP41 cells (blue), treated with LTD4 and imaged at 1 dpt. Scale bar = 50 μm. In the zoomed area, a magnified view of the interaction between neutrophils and MP41 cells. Scale bar = 75 μm.

D: Graph representing the percentage of arrested (magenta) and patrolling (black) neutrophils inside the tumour mass formed by transplanted MP1 cells (blue), pre-treated or not with LTD4 at 1 dpt. Related to fig. 6D. Results are expressed as mean ±SEM. n=8-9. A two-way ANOVA test was performed to evaluate significance. **** *P-value < 0.0001.*

E: Quantification of neutrophils number infiltrating the mass formed by transplanted MP41 cells at 1 dpt, pretreated or not with LTD4, as indicated. Results are expressed as mean ±SEM. n=9-21. A two-tailed unpaired Student's t-test was performed to evaluate significance. ** *P-value = 0.0024.*

F: Quantification of Ki67-positive MP41 cells treated or not with LTD4, expressed as a percentage of the total number of cells in the transplants, at 3 3 dpt. Results are expressed as mean ±SEM. n=9-21. A two-tailed unpaired Student's t-test was performed to evaluate significance. ** *P-value = 0.0076.*

G: Schematic representation of the pipeline used to perform transplantation in zebrafish larvae with or without neutrophils depletion (prepared with Biorender).

H: Representative confocal image of a *tg(mpx:GFP)^{i114}* zebrafish, with neutrophils in green, transplanted with MP41 cells (blue) and treated with metronidazole (+MTZ). Scale bar=50 mm**.**

I: Whole-mount immunofluorescence staining of transplanted *tg(mpx:GFP)^{i114}* zebrafish larvae treated with MTZ to ablate neutrophils. Scale bar = 10 μm.

J: Quantification of Ki67-positive MP41 cells in *tg(mpx:GFP)^{I114}* larvae treated or not with MTZ, expressed as a percentage of the total cell counted. Results are expressed as mean ±SEM. n=13. A two-tailed unpaired Student's t-test was performed to evaluate significance. *P-value=0.11*.

Suppl. video 1

Confocal time-lapse video of transplanted MP41 cells (blue, plastic transparent rendering generated with IMARIS) in *tg(mpx:GFP)^{i114}* zebrafish at 1 dpt, showing neutrophils (green) and their behaviour inside and outside the tumour mass, related to Figure 6D. Few neutrophils displaying an arrested phenotype are visualized in the tumour mass. Visualization at 8 frames per second. Scale bar = 50 μm.

Suppl. video 2

Confocal time-lapse video of transplanted MP41 cells (blue, plastic transparent rendering generated with IMARIS) in *tg(mpx:GFP)^{i114}* zebrafish at 3 dpt, showing neutrophils (green) and their behaviour inside and outside the tumour mass. Many neutrophils displaying a patrolling phenotype are visualized in the tumour mass. Visualization at 8 frames per second. Scale bar = 50 μm.

Suppl. video 3

Confocal time-lapse video of transplanted MP41 cells (blue, plastic transparent rendering generated with IMARIS) in *tg(mpx:GFP)^{i114}* zebrafish at 1 dpt, showing neutrophils (green), their behaviour and morphology inside and outside the tumour mass. An example of neutrophils displaying an arrested phenotype and a round morphology within the tumour mass. Visualization at 8 frames per second. Scale bar = 50 μm.

Suppl. video 4

Confocal time-lapse video of transplanted MP41 cells (blue, plastic transparent rendering generated with IMARIS) in *tg(mpx:GFP)^{i114}* zebrafish at 3 dpt, showing neutrophils (green), their behaviour and morphology inside and outside the tumour mass. Many neutrophils displaying a patrolling phenotype and an elongated and ramified morphology are observed in the tumour mass. Visualization at 8 frames per second. Scale bar = 50 μm.

Suppl. video 5

Confocal time-lapse video of transplanted MP41 cells (blue, plastic transparent rendering generated with IMARIS) exposed to 1 μM LTD4 in *tg(mpx:GFP)^{i114}* zebrafish at 3 dpt, showing neutrophils (green) and their behaviour inside and outside the tumour mass, related to suppl. Fig. 6C. Many neutrophils displaying a patrolling phenotype are visualized in the tumour mass. Visualization at 8 frames per second. Scale bar = 50 μm.