Supplementary Materials

Single cell RNA sequencing and bioinformatics analysis

Experimental protocol and library generation

Single cell suspensions, achieved as described above, were loaded onto a 10 × Genomics Chromium instrument to generate single-cell gel beads in emulsion.

Sequencing and data processing

The single cell libraries were sequenced on an Illumina NextSeq 500 sequencer according to the manufacturer's protocol. We used the 10 × Genomics Cell Ranger (version 3.1.0) pipeline to process the raw data from the sequencer and to generate the count matrices, including the filtered count matrices by following the manufacture's standard workflow for 10 × Genomics Single Cell Expression data. The 10 × Genomics Loupe Browser software was used to visualize and interactively explore the results from the Cell Ranger pipeline.

Raw RNASeq transcripts were aligned to the GRCh38 human genome and the mm10 mouse genome using CellRanger v7.1.0 [1]. Count matrices for samples were subsequently imported into the R computing environment (v4.2.2) using the Seurat package (v4.3) [2, 3]. Cells were initially filtered using a 15% upper threshold in read count fraction or unique gene fraction of the minor species, then classified as murine or human based on the majority of aligned reads. Subsequent low quality cells were identified and removed using the miQC tool within Seurat [4]. Single cell read counts were then normalized and variance-stabilized using SCTransform [5]. Doublets were identified and removed using the DoubletFinder tool [6]. Cell type annotation was performed using the SingleR tool, with the Human Primary Cell Atlas as reference, as retrieved via the celldex package [7]. Samples were then combined into a single dataset using the Harmony batch correction tool [8]. Visualization using the UMAP projection was generated through the Seurat package [9].

Differential expression was conducted through the Seurat package using the MAST algorithm [10]. Gene scores were calculated using the following equation:

$$Score = ([-\log_{10}(p) * sign(FC)] + \log_2(FC)) * \max(pct.1, pct.2)$$

In the event that the *p*-value was indicated as zero (i.e. smaller than machine error), the value 500 was substituted for -log10(p). *pct.1* and *pct.2* are calculated through the differential expression as the percentage of cells in each contrast condition that express the gene in question. Based on these gene scores, a preranked gene list was then analyzed using Gene Set Enrichment Analysis on the Hallmark gene sets [11, 12].

References

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Supplementary Table 1. Antibodies used in the analysis

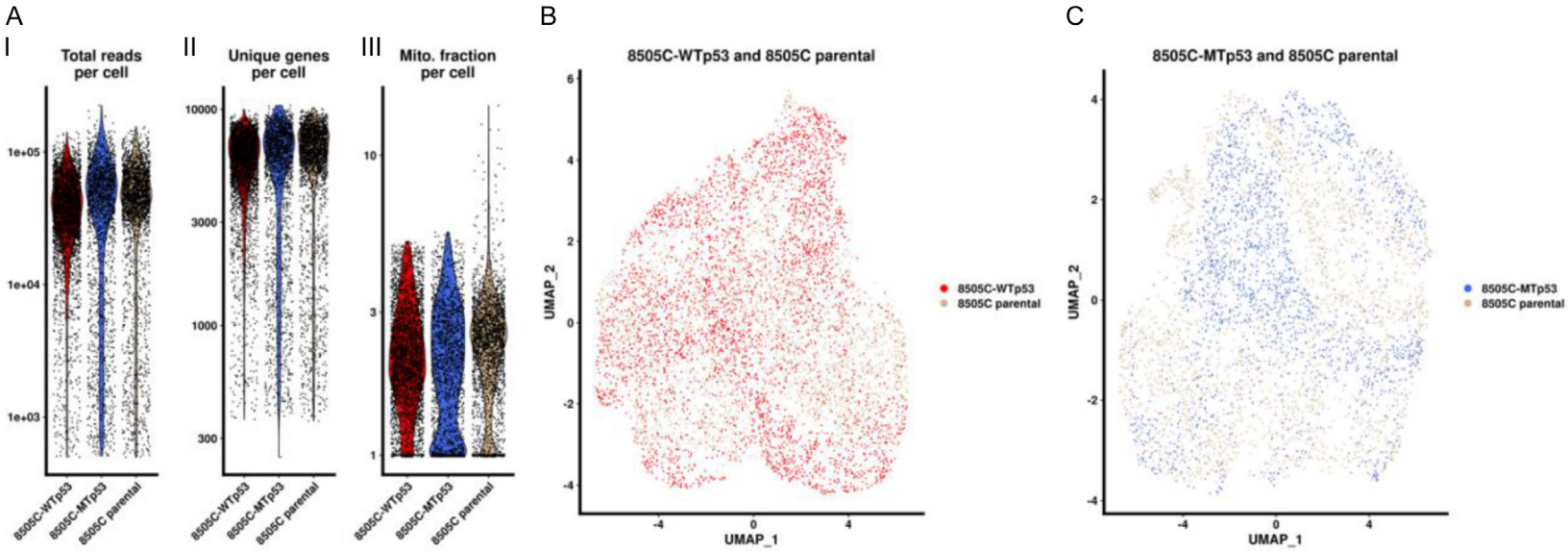
Reagent	Source	Identifier
Antibodies		
Mouse anti β-actin	Santa Cruz	Cat# sc-47778
Rabbit Anti p53	Abcam	Cat# ab131442
Mouse Anti p53	Proteintech	Cat# 60283-1-Ig
Rabbit anti Cleaved caspase-3	Cell Signaling Technology	Cat# 9664
Rabbit anti Ki-67	Cell Signaling Technology	Cat# 44092
Mouse Anti PUMA	Abcam	Cat# ab9645
Rabbit Anti BAX	Abcam	Cat# ab32503
Mouse p21	Proteintech	Cat# 10355-1-AP
Rabbit anti NIS	Gifts From Dr. Jhiang	
Donkey anti-rabbit IgG	GE Healthcare Life	Cat# NA9340V
Peroxidase AffiniPure Goat Anti-Rabbit IgG (H+L)	Jackson ImmunoResearch	Cat# 111-035-144
Goat anti-Rabbit IgG Alexa Flor 488	Thermo Fisher	Cat# A11034
Goat anti-mouse IgG Alexa Flor 568	Thermo Fisher	Cat# A11004
MemBrite® Fix Cell Surface Staining Kits	Biotium	Cat# 30097-T

Supplementary Table 2. Primer sequences used in the q/PCR analysis

Gene name	Forward sequence	Reverse sequence
PUMA	ACGACCTCAACGCACAGTACGA	CCTAATTGGGCTCCATCTCGGG
BAX	TCAGGATGCGTCCACCAAGAAG	TGTGTCCACGGCGGCAATCATC
p21	AGGTGGACCTGGAGACTCTCAG	TCCTCTTGGAGAAGATCAGCCG
CEBPD	TCCGGCAGTTCTTCAAGCAGCT	GAGGTATGGGTCGTTGCTGAGT
NFkBIA	TCCACTCCATCCTGAAGGCTAC	CAAGGACACCAAAAGCTCCACG
TNFAIP3	CTCAACTGGTGTCGAGAAGTCC	TTCCTTGAGCGTGCTGAACAGC
CXCL2	GGCAGAAAGCTTGTCTCAACCC	CTCCTTCAGGAACAGCCACCAA
CXCL3	TTCACCTCAAGAACATCCAAAGTG	TTCTTCCCATTCTTGAGTGTGGC
IL6	AGACAGCCACTCACCTCTTCAG	TTCTGCCAGTGCCTCTTTGCTG
TNF	CTCTTCTGCCTGCTGCACTTTG	ATGGGCTACAGGCTTGTCACTC
ID2	TTGTCAGCCTGCATCACCAGAG	AGCCACACAGTGCTTTGCTGTC
CXCL1	AGCTTGCCTCAATCCTGCATCC	TCCTTCAGGAACAGCCACCAGT
TNFAIP8	CGTGGTCAGTTTCCATCAGGTG	CGTCCATGTGACTTGGCAGTGA
cMYC	CCTGGTGCTCCATGAGGAGAC	CAGACTCTGACCTTTTGCCAGG
SLC5A5	CTCTGCTGGTGCTGGACATCTT	GAGGTCTTCTACAGTGACTGCAG
GAPDH	GTCTCCTCTGACTTCAACAGCG	ACCACCCTGTTGCTGTAGCCAA

Supplementary Table 3. Total cell counts before filtering

Sample	Mouse	Human	Total
8505C-WTp53	1422	3684	4286
8505C-MTp53	1222	2043	3265
8505C parental	931	2551	3482



Supplementary Figure 1. Transcriptomic analysis of single cells from tumors induced by 8505C, 8505C-WTp53 or 8505C-MTp53 cells. (A) Total read counts (I), unique gene counts (II), and mitochondrial fraction in the human cells (III) show reasonably consistent quality (all values shown in logarithmic scale). (B) UMAP profiles to compare the distribution of cells between 8505C-WTp53- and 8505C parental cells-induced tumors. (C) UMAP profiles to compare the distribution of cells between 8505C-MTp53- and 8505C parental cells-induced tumors.

Supplementary Table 4. Top 20 enriched gene sets in WTp53 cells

Rank	Geneset	Size	Enrichment score	Normalized enrichment score	*P*-val	FDR
1	Hallmark TNFA Signaling Via NFKB	190	-0.28	-4.48	<1E-03	<1E-03
2	Hallmark Adipogenesis	189	-0.25	-4.04	<1E-03	<1E-03
3	Hallmark IL6 Jak STAT3 Signaling	70	-0.38	-3.76	<1E-03	<1E-03
4	Hallmark Fatty Acid Metabolism	141	-0.27	-3.71	<1E-03	<1E-03
5	Hallmark Mitotic Spindle	199	-0.23	-3.7	<1E-03	<1E-03
6	Hallmark UV Response DN	143	-0.25	-3.48	<1E-03	<1E-03
7	Hallmark Interferon Gamma Response	174	-0.21	-3.16	<1E-03	<1E-03
8	Hallmark Peroxisome	97	-0.28	-3.16	<1E-03	<1E-03
9	Hallmark Complement	173	-0.19	-2.98	<1E-03	<1E-03
10	Hallmark Xenobiotic Metabolism	163	-0.2	-2.96	<1E-03	<1E-03
11	Hallmark PI3K AKT mTOR Signaling	99	-0.23	-2.7	<1E-03	<1E-03
12	Hallmark Interferon Alpha Response	92	-0.24	-2.66	<1E-03	<1E-03
13	Hallmark Inflammatory Response	165	-0.17	-2.54	0.002	<1E-03
14	Hallmark KRAS Signaling UP	167	-0.17	-2.46	<1E-03	0.001
15	Hallmark Apical Junction	180	-0.15	-2.44	0.002	0.001
16	Hallmark Allograft Rejection	144	-0.15	-2.08	0.002	0.004
17	Hallmark BILE Acid Metabolism	93	-0.15	-1.68	0.034	0.036
18	Hallmark Wnt Beta Catenin Signaling	38	-0.19	-1.37	0.112	0.139
19	Hallmark Hedgehog Signaling	33	-0.2	-1.35	0.117	0.142
20	Hallmark KRAS Signaling DN	124	-0.1	-1.26	0.179	0.192