Supplementary Methods, Jiang et al. 2021

Cells

The Hox cell line was generated by transducing bone marrow cells of C57BL/6 Cas9+ GFP+ mice with an estrogen inducible retroviral construct expressing HoxB8 as described in (1,2). Hox cells are immortalized by estrogen-regulated Hoxb8 (ER-Hoxb8) and display a Granulocyte-Monocyte Progenitor (GMP) phenotype, as described in Supplementary Fig. S1A-B. Hox cells were cultured in RPMI-1640 with 10% heat-inactivated fetal bovine serum, 1% penicillin-streptomycin-glutamine, 1 μM BE, and 25 nM SCF. No cell line authentication was performed to Hox cells.

B16-F10 cell is a mouse melanoma cell line, purchased from ATCC that performs authentication and and used at a low passage number. Cas9 expressing cells were generated by transducing B16-F10 cells with lentiCas9-Blast lentiviral particles. B16 cells were cultured in RPMI-1640 with 10% serum, and 1% penicillin-streptomycin-glutamine. All cells were confirmed *Mycoplasma* negative before culture.

Trp53 KO Hox and B16-F10 cells were generated by electroporation or transfection, respectively, of a *Trp53* targeting sgRNA (Supplementary Table S2, Genomic Location 11:69479559-69479578). To limit artefacts due to selection of specific clones, cells were used as a mixed population strongly dominated by +1 and +2 insertions, and a >95% KO score.

Viral preparation and transduction

Lentiviral particles were generated by seeding 2×10^6 HEK293T cells in 10 cm plate in 10 ml of DMEM with 10% serum and 1% L-glutamine. After ~24 h of culture the medium was replaced by 5 ml of fresh media. Transfer plasmids (lentiCas9-Blast; or LentiGuide-Puro-P2A-EGFP_mRFPstuf), pMD2.G, and psPAX2 were mixed at 4:5:1 ratio, and transfected using Lyovec according to the manufacturer's protocol. After 12 h, the medium was replaced by 8 ml of DMEM with 30% serum and 1% L-glutamine. After another 36 h, the supernatant containing the virus was collected, centrifuged to remove the cell debris and used to spin infect cells.

For ER-Hoxb8 retrovirus preparation, plasmids including ER-Hoxb8 and the EcoPac gag-pol-env were mixed at 1:1 ratio for transfection following the same approach as for generating lentiviral particles.

To transduce Hox or B16 cells, the multiplicity of infection (MOI) of the viral particles was tested by infection with serial dilutions of virus particles to find a dilution resulting in a suitable MOI. Virus supernatant was added to each well of 6-well plate containing cells (4×10^5 for Hox cells, and 1×10^5 for B16 cells) with 8 μg/ml polybrene. The plate was centrifuged at 37°C, 1200g (120 min for Hox cells, and 45 min for B16 cells). After 24h, the virus-containing medium was replaced with fresh medium, and the infection rate was measured by the percentage of GFP+ cells if the vector contains GFP. Puromycin selection (10 μg/ml for HoxB8 cells, and 5ug/ml for B16 cells) or Blasticidin selection (10 μg/ml for B16 cells) was performed for 24 h to remove the non-infected cells.

sgRNA design, electroporation and transfection

sgRNAs were designed using the Green Listed software (3,4) utilizing sgRNA design from the Doench mouse library (5). 2'-O-methyl and phosphorothioate stabilized sgRNAs (Supplementary Table S2) were ordered from Sigma-Aldrich or Synthego. Off-target activity was calculated using Cas-OFFinder (http://www.rgenome.net/cas-offinder/) (6), and On-target activity was extracted from CHOPCHOP (https://chopchop.cbu.uib.no/) (7) by searching for *Ccr1*, or entering the EGFP FASTA sequence from the LentiGuide-Puro-P2A-EGFP_mRFPstuf.

For Hox cells, Neon Transfection System was used to perform the electroporation following the manufacturer's instructions (Pulse voltage: 1700 V, Pulse width: 20 ms, Pulse number: 1). 100 pmol of sgRNA or siRNA were electroporated into $2x10^5$ cells for each electroporation experiment using the Neon Transfection System 10 µL Kit. For B16 cells, Lipofectamine 2000 Transfection Reagent was used following the recommended protocol. 100pmol of sgRNA or siRNA were transfected into $1x10^5$ cells for each transfection experiment. The *Trp53* siRNA was typically delivered in the same reaction as the sgRNAs.

CRISPR KO genotyping

$1x10^5$ cells were collected for genomic DNA extraction using DNeasy Blood & Tissue Kit. Primers were designed using Primer-BLAST (Supplementary Table S2), aiming for a 400-1000 bp amplicon with the sgRNA target in the middle. Amplicons were gel purified and recovered using Zymoclean Gel DNA Recovery Kit. The PCR products were quantified using Nanodrop and sequenced by Eurofins Genomics. The Sanger sequencing data was subsequently analyzed by ICE (Synthego, https://ice.synthego.com).

Growth curve characterization

Hox cells were cultured with the following interventions: electroporation with a GFP targeting sgRNA; 0.5 µg/ml Etoposide; 3 µM AMG232. The Etoposide and AMG232 were replaced by fresh media after 8 h. The cells were cultured in 6 well plates with 3 ml medium and each day 2 ml medium was exchanged. 80 µl of cells were taken each day for flow cytometry (BD Accuri) with the existence of CountBright Absolute Counting Beads. The absolute viable cell number was calculated by comparing the events number of viable cells and counting beads in the flow cytometry data.

Cloning of sgRNAs into lentiviral transfer plasmid, and CRISPR screens

sgRNAs with overhangs for the LentiGuide transfer plasmid (Supplementary Table S3) were designed using the Green Listed software (3,4) using sgRNA design from the Doench mouse library (5) and, for intergenic controls, the Wang mouse library (8). Individual sgRNAs were ordered from Sigma-Aldrich, and the sgRNA library was ordered from CustomArray as a DNA oligo pool. Cloning was performed using BsmBI cleaved lentiGuide-Puro-P2A-EGFP_mRFPstuf plasmid and the library oligo pool, with NEBuilder HiFi DNA assembly master mix. Endura ElectroCompetent cells were subsequently transformed with the cloned plasmid pool using electroporation (1.0 mm cuvette, 10 µF, 600 Ohms, 1800 Volts). The electroporated cells were combined and seeded on ten 20 cm LB agar plates with carbenicillin and grown at 37°C overnight. The lentiGuide-Puro-P2A-EGFP_mRFPstuf plasmid makes

bacteria red if the stuffer has not been exchanged by a sgRNA, and a few red clones were removed before collecting all other white clones. Plasmids were purified using the EndoFree Plasmid Maxi Kit.

The sgRNA cloned lentiGuide-Puro-P2A-EGFP_mRFPstuf was used as transfer plasmid for lentiviral preparation and transduction. The total amount of transduced cells was calculated based on MOI (0.25 for B16 cells, and 0.05-0.1 for Hox cells), aiming for 1000 transduced cells for each sgRNA.

The CRISPR library transduced cells were exposed to GFP targeting sgRNA electroporation with or without *Trp53* siRNA, 0.5 μg/ml Etoposide 8 h pulse stimulation, or 3 μM AMG232 8 h pulse stimulation.

Cells were collected for genomic DNA extraction using the DNeasy Blood & Tissue Kit. Genomic DNA was then amplified using Q5 High-Fidelity DNA Polymerase. Sample-specific barcodes and adapters for Illumina Sequencing similar as described in (9) were introduced at the same time using primers specified in Supplementary Table S4. The final PCR products were gel purified and recovered using the Zymoclean Gel DNA Recovery Kit, and quantified with Qubit 4 Fluorometer using the Qubit dsDNA HS Assay Kit and pooled for next-generation sequencing (Illumina MiSeq v3 run, 2x75bp reads). The raw FASTQ data were analyzed by MAGeCK(10). Read counts from CRISPR screens are found in Supplementary Table S5-6.

JAK1/STAT1 signaling assay

Hox cells were cultured with or without mouse Interferon Beta and the Jak1 inhibitor Solcitinib for 7 days. Cells were taken on day 7 for flow cytometry (BD Accuri) with the existence of CountBright Absolute Counting Beads. The absolute viable cell number was calculated by comparing the events number of viable cells and counting beads in the flow cytometry data.

Competitive co-culture assay

Trp53 KO and WT cells were mixed at 1:4 ratio, and subsequently exposed to different interventions: CRISPR - electroporated with sgRNA or transduced with lentivirus and culture for 7 days; or exposed to Etoposide (0.5 μg/ml for 8h) or AMG232 (3 μM for Hox cells, 4 μM for B16 cells for 8h) and cultured for 7 days; or cultured with Cobalt(II) chloride ($CoCl_2$, 10-20 μg/ml) for 7 days. For hypoxia experiments, Hox cells were cultured in a 1% O_2 for 7 days, in Baker InvivO2 Physiological Cell Culture Workstations. For *in vivo* experiments, B16 cells were transfected with a *Ccr1* targeting sgRNA (Supplementary Table S2) or control, and directly injected (5*10^5 cells) s.c. into C57BL/6 mice, and tumors collected after 21 days. The proportion of *Trp53* KO cells was subsequently quantified by sequencing as described in CRISPR KO genotyping.

Different p53 related inhibitors were added during culture: *Trp53* ON-TARGETplus siRNA SMARTPool , KU55933 (100 ng/ml for B16, 10 ng/ml for Hox), VE821 (50 ng/ml), Pifithrin-μ (2 μg/ml for B16, 200 ng/ml for Hox), Cyclic Pifithrin-α (700 ng/ml for B16, 70 ng/ml for Hox), C646 (1 μg/ml), AZD2461 (250 ng/ml), LJI308 (2 ng/ml), Z-VAD-FMK (50 μM). siRNA was delivered to cells 1 day before CRISPR/Etoposide/AMG232 exposure (100 pmol of siRNA electroporated into $2x10^5$ Hox cells, or transfected into $1x10^5$ B16 cells), or together with sgRNA for the transfection/electroporation groups. Other inhibitors were added to cell

culture media 1 day before CRISPR/Etoposide/AMG232 exposure and cultured for 7 days. Cells were then collected for *Trp53* KO genotyping.

Flow Cytometry Analysis

Fresh bone marrow cells from C57BL/6 Cas9+ GFP+ mice and Hox cells were stained with the following antibodies (further described in Supplementary Table S1): FITC Rat anti-Mouse CD34 (1:500), PE anti-mouse CD150 (1:200), PerCP/Cyanine5.5 anti-mouse Ly-6A/E (1:200), APC anti-mouse CD117 (1:500), APC/Cyanine7 anti-mouse CD16/32 (1:500), Biotin anti-mouse Lineage Panel (1:100), BV421 Streptavidin (1:1000), LIVE/DEAD Fixable Aqua Dead Cell Stain Kit (1:2000). After 30 min of staining, the cells were washed and analyzed by flow cytometry (BD FACSVerse). FACS FCS files were analyzed by FlowJo version 10 (FlowJo, LLC).

Analysis of data from the Depmap portal

SgRNA enrichment (CRISPR (Avana) Public 20Q4 release), mutation profile (Mutation Public 20Q4 release), drug sensitivity (PRISM Repurposing Primary Screen 19Q4 release), and mRNA expression levels (Expression Public 20Q4 release) was extracted December 13th, 2020 from the Depmap portal (https://depmap.org/portal/) (11-15). Correlation analysis was performed with the Depmap data explorer tool. Connectivity maps were generated using the geneMANIA plugin for Cytoscape (16,17). tSNE plots were made with the Rtsne package (https://github.com/jkrijthe/Rtsne) to analyze the cluster and ggplot2 (https://github.com/tidyverse/ggplot2) to visualize the data, or tSNE-online (https://github.com/jefworks/tsne-online). The "ENCODE and ChEA Consensus TFs from ChIP-X" functionality of Enrichr (https://maayanlab.cloud/Enrichr/index.html) (18,19) was used to identify transcription factor binding to gene sets.

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