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Western blotting

Whole-cell lysates were prepared by directly lysing the cells using RIPA buffer (Thermo Scientific, Cat#PI89900) supplemented with protease inhibitor cocktail (Sigma Aldrich, Cat#P8340; 1:100) for 30 min at 4°C. After centrifugation at maximal speed for 20 min, supernatants were collected, and protein concentration was determined using Quick start Bradford 1X dye reagent (Bio-Rad). Protein extracts (25-30 µg) were mixed with 4X SDS-PAGE samples buffer (Invitrogen) and β-mercaptoethanol (2.5% final concentration), boiled for 8 min, then loaded onto a 4%–12% bis-tris protein gel (Invitrogen) and transferred onto PVDF membranes (Merck Millipore). The membranes were probed with the following primary antibodies overnight: anti-MFN2 (1:1000, Abnova Cat#H00009927-M01, RRID:AB_714775), anti-ACTIN (1:10,000, Millipore Cat#MAB1501, RRID:AB_2223041), anti-TOMM20 monoclonal (1:500, Santa Cruz, Cat# sc-17764, RRID:AB_628381), anti-LC3B (1:1,000, Cell Signaling Cat#2775, RRID:AB_915950), anti-p62 (1:1,000, MBL Cat#PM045, RRID:AB_1279301), anti-SDHA (1:2,500, Abcam Cat#ab14715, RRID:AB_301433), anti-GAPDH (1:10,000, Proteintech Cat#60004-1-Ig, RRID:AB_2107436), anti-TUBULIN (1:10,000, Sigma Aldrich Cat#T9026, RRID:AB_477593). Isotype matched secondary antibodies conjugated to horseradish peroxidase (GE Healthcare) were used at 1:5,000 and chemiluminescence was detected with ECL (Life Technologies). Band densitometric analysis was performed using ImageJ (National Institutes of Health).

Cell sorting

For human AML cell sorting from mouse BM, all samples were gated based on forward and side scatter, followed by exclusion of doublets, then gated on viable cells ($DAPI^{low}$), and then GFP^{+} human leukemic cells or $hCD45\text{-}FITC^{+}$ were sorted. Sorting was performed at a SY3200™ highly automated parallel sorting (HAPS) cell sorter (Sony).

Cell death experiments

Apoptosis analysis was determined using APC Annexin V (BD Bioscience, Cat#BDB559763) and 4',6-Diamidino-2-Phenylindole, Dihydrochloride (DAPI) for DNA content. Annexin V-stained cells were analyzed by flow cytometry and FlowJo software. Drug treatments for cell death experiments were performed for 24 hrs and the concentrations were as follows, unless otherwise stated: **MOLM-13** Ven: 6 nM, AMG176: 300 nM, Ven+AMG176: 3 nM+30 nM; **Kasumi-1** Ven: 20 nM, AMG176 (MCL1i): 800 nM, Ven + AMG176 (MCL1i): 8 nM+80 nM.

Immunofluorescence and confocal microscopy

For the Parkin translocation experiment, MOLM-13 and HeLa cells were transduced with the pBMN-mCherry-Parkin vector (Addgene, plasmid no 59419) and mCherry-Parkin expressing cells were sorted and cultured before shRNA transduction. shRNA transduced mCherry-Parkin expressing cells were selected with puromycin. After induction of shRNA and GFP by doxycycline, cells were seeded onto poly-L-lysine coated coverslips and cultured overnight. Cells were then treated with CCCP (10 µM) for 2 hrs to induce mitophagy. Cells were fixed on glass coverslips with freshly prepared, ice-cold 3.7% formaldehyde in PBS for 30 min, followed by permeabilization with 0.5% Triton X-100 in PBS for 20 min. Next, the coverslips were washed with PBS, incubated

with blocking buffer (0.5% BSA in PBS) for 30 min and then, with primary antibodies for 90 min at room temperature. We used antibodies against TOM20 (1:100; Santa Cruz - clone F10) diluted in blocking buffer. The cells were then washed with PBS and incubated with the appropriate secondary antibodies labeled with APC (1:500) for 2 hrs at room temperature. After washing, cells were stained with 1 µg/µl DAPI for 1 min and then coverslips were mounted with one drop of Fluoromount-G (Southern Biotech). Fluorescence signals were analyzed under a ZEISS 880 confocal microscope. Parkin translocation was analyzed using a macro in ImageJ calculating the ratio between Parkin fluorescence onto mitochondria (TOM20) and Parkin fluorescence in the cytosol.

For measuring autophagy puncta, MOLM-13 parental and MR cells were retrovirally transduced with the pBABEpuro GFP-LC3 vector (Addgene, plasmid no 22405) to allow overexpression of the GFP-LC3 fusion protein. Sorted cells were then treated with CCCP (Selleckchem, Cat# S6494, 10 µM) for 2 hrs to induce mitophagy. Immunofluorescence was performed as above.

MFN2 overexpression

For overexpressing MFN2 wild type (WT) and its mutant variants ($MFN2^{K109A}$ and $MFN2^{IYFFT}$), human codon-optimized DNA oligos encoding $MFN2^{WT}$, $MFN2^{K109A}$ and $MFN2^{IYFFT}$ were synthesized by Integrated DNA Technologies (IDT) and cloned into a home-made lentiviral plenti EF1a-ires-Puro-2A-ZsGreen vector[1] following the EF1a promoter. Oligo sequences and plasmid maps could be provided upon request.

To package lentivirus for transducing MFN2 and its mutant variants, HEK293T cells were transfected using polyethylenimine (PEI) reagent at 4:2:3 ratios of MFN2 constructs: pVSVG: pPax2 in OPTI-MEM solution (pVSVG; Addgene plasmid no 12259, psPAX2; Addgene plasmid no 12260). Viral supernatant was collected 36 hrs and 48 hrs post-transfection. Spin infections were performed at room temperature at 1,500 x g for 30 mins with polybrene reagent (1:2000; Fisher Scientific).

For adenoviral MFN2 overexpression, cells were infected with adenoviruses expressing cytomegalovirus-MFN2 or empty vector. For the transduction in AML cells, Viraductin Adeno Transduction Reagent (Cell Biolabs Inc. Cat#AD-201) was used according to manufacturer's protocol.

Lentiviral sgRNA and shRNA construct design and infection

sgRNAs were designed manually using the "CRISPR Guides" module on Benchling (https://benchling.com/). sgRNAs were then cloned into the plenti U6-sgRNA/EF1a-Puro-2A-ZsGreen vector. All the sgRNA transfections were performed in HEK293T cells using Lipofectamine3000 (Invitrogen) reagent at 4:2:3 ratios of sgRNA construct: pVSVG: pPax2 in OPTI-MEM solution. Viral supernatant was collected 48 hrs and 72 hrs post-transfection. Spin infections were performed at 32°C at 1,500 RCF for 30 min with polybrene reagent (1:2,000 dilution) (Fisher Scientific).

shRNAs targeting MFN2, MARCH5, PINK1, and Renilla control (**Suppl. Table 5**) were cloned into the lentiviral LT3GEPIR vector (Addgene, plasmid no 111177). Lentivirus was generated in HEK293T cells. Spin infections were performed at 32°C at 1,500 RCF for 30 min with polybrene. At day 2 post-infection, infected cells were selected with puromycin. After selection, doxycycline (1 µg/ml) treatment was initiated for 48 hrs to induce the expression of shRNAs and GFP.

RNA-Sequencing library preparation and sequencing

Parental and MR Kasumi-1 cells were harvested and washed with PBS. RNeasy Plus Mini Kit (QIAGEN, Cat#74136) were used to extract total RNA according to manufacturer's protocol. Poly(A)-tailed mRNA were enriched by magnetic isolation method (NEB) using 5~10 µg of total mRNA. NEXTflex Rapid Directional (Bioo Scientific, Cat#5138-07) protocol and NEXTflex RNA-seq barcodes (Bioo Scientific, Cat#NOVA-512912) were used to generate RNA-sequencing libraries. RNA-seq barcoded libraries were then sequenced using Hi-Seq 4000 (50 cycles single end).

Single cell RNA-Sequencing pre-processing

For demultiplexing of hashed libraries, we normalized Hashtag oligonucleotides (HTO) using a centered log ratio (CLR) transformation across cells and applied HTODemux function in Seurat. Cells positive for more than one hashtag were excluded as doublets.

Additionally, we used Souporcell[2] on every hashed library to identify cells with wrongly assigned hashtags. We excluded cells assigned as doublets and cells in which genotype and HTODemux assignment did not match.

To further exclude within patient cell doublets and from non-hashed libraries, we further filtered the data using the scDblFinder package (version 1.5.13, https://github.com/plger/scDblFinder). For the hashed libraries, we used the recoverDoublets function to identify cells similar to those identified as doublets by HTODemux. For the non-hashed libraries, we ran scDblFinder function, with trajectoryMode=TRUE, which generates cluster-based artificial doublets to identify doublets.

Droplet-based single cell assays capture any cell-free RNA present in the input solution in addition to RNA derived from a specific cell. We used SoupX[3] to reduce the amount of ambient RNA contamination. Contamination estimates varied between 1 and 9.2%. The SoupX estimated counts were used in all downstream analysis.

After doublet, quality and ambient RNA filtering, RNA expression data were normalized by total expression, multiplied by a scaling factor of 10,000 and log-transformed. We captured on average 4123.4 cells per patient with a mean and median of 1620.772 and 1581.257 genes detected per cell.

Analysis of single cell RNA-Sequencing data

Broad cell type annotation

Broad cell types included hematopoietic stem and progenitor cells (HSPC), Myeloid, B, T/NK cells, and Erythrocytes and were annotated using known cell type markers as previously described[4].

Malignant and microenvironment division

Before the more granular cell type annotation, we separated cells from AML patients into malignant and microenvironment. Only AML microenvironment and healthy donor cells were used for the granular cell type annotation.

Granular cell type annotation

Microenvironment and control cells were split into broad cell types (HSPC, B, T, NK, myeloid). Harmony integration (version 1.0) with default parameters was used to account for biological and

technical batch effects, within each broad cell type object. The individual patients were used as the integration variable. We used the first 20 dimensions of the harmony embeddings to generate UMAPs with 20 nearest neighbors defining the neighborhood size and a minimum distance of 0.3 for each of the broad cell types. We then constructed SNN graphs using the first 20 first dimensions of the harmony embeddings and clustered the graph using a range of resolutions (0.5-3). We used the following resolutions for manual cluster annotation based on cluster markers: HSPC – resolution 1, 21 clusters; Myeloid cells– resolution 1, 20 clusters; T/NK cells – resolution 3, 35 clusters; B cells – resolution 2, 22 clusters; Erythrocytes – resolution 1, 14 clusters. We utilized differential expression analysis between cells within each cluster against all other cells to identify cluster markers. Wilcoxon rank sum test with Bonferroni multiple-comparison correction (detected in at least 10% of the cluster cells, log2 fold change > 0.25 or < -0.25, adjusted $p<0.05$) was used to determine statistical significance. Clusters expressing markers of other lineages were excluded as potential doublets.

Annotation of malignant cells

To assign a cell type identity to the AML malignant cells, we used the FindTransferAnchors function and TransferData function using the first 30 principal components in Seurat[5].

Mitochondrial respiration

3.5×10^5 cells per well were seeded in Cell Tak (ThermoFisher Scientific) coated XFe24 cell culture microplates (Agilent, Cat#102340-100), as indicated by the manufacturer. The experiment was performed in XF Assay Solution (Agilent, Cat#103576-100 Seahorse XF RPMI with Hepes) supplemented with 2 g/L glucose, 1 mM sodium pyruvate and 2 mM glutamine. The assay consisted of oxygen consumption and extracellular acidification measurements during time starting with the basal conditions and followed by sequential injections of Oligomycin (VWR, Cat#80058-538) 1 µM, FCCP (Fisher Scientific, Cat#NC0904863) 1-2 µM, and Rotenone (Sigma Aldrich, Cat#R8875)/Antimycin A (Sigma Aldrich, Cat#A8674) 0.5 µM. Three measurements were performed after each compound injection in the XFe24 Extracellular Flux Analyzer. The experiment was performed simultaneously in the same plate for the parental and resistant AML cells and data were analyzed using Wave software.

mtDNA copy number

To measure mtDNA copy number, total cellular DNA was isolated using DNeasy Blood & Tissue Kit (Qiagen, Cat#69504) and then was amplified using specific oligodeoxynucleotides for tRNA-Leu(UUR) and Actin (**Suppl. Table 5**) by real-time PCR using Platinum SYBR Green qPCR Supermix (Invitrogen).

mitoTracker Green

MitoTracker Green FM (ThermoFisher Scientific, Cat#M7514) was used to quantify mitochondrial mass in a final concentration of 25 nM in HBSS buffer supplemented with 20 mM HEPES. Before staining the cells were washed in PBS and staining was performed for 10 min at 37˚C.

Supplemental Table 1: Screens results

Supplemental Table 2: Correlation score of *MFN2* expression with the *ex vivo* drug response (IC_{50}, BeatAML data)

Supplemental Table 3: AML patient samples information

Supplemental Table 4: Autophagy related genes and their ranking in NMF analysis of scRNAseq data

Supplemental Table 5: sgRNA, primers, shRNA sequences used in this study

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