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**Supplementary information**

## 1. SUPPLEMENTARY METHODS

### Quantitative RT-PCR

RNA Plus extraction kit (Macherey-Nagel, Düren, Germany) was used to extract total RNA according to the manufacturer's protocol. Nucleospin RNA Plus kit (Ref 740990.250, Macherey-Nagel, Düren, Germany) was used when cell number was lower than $50x10^4$ cells, also according to the manufacturer's protocol. The high capacity cDNA reverse transcription kit (Cat. 4368814, Applied Biosystems, Foster City, USA) was used to perform the cDNA synthesis. Quantitative PCR was performed using SYBR Green reagent (applied Biosystems, Foster City, USA) or TaqMan (Thermo Fisher Scientific) and analysis was performed in an ABI prism 7500 sequence detection system. *Gapdh* expression was used to normalized the Ct values and relative expression was quantified using $1/\Delta Ct$. Primers are listed in Suppl. Table 1.

### *Tp53* gene quantification

Genomic DNA was isolated from total BM from diseased *TP53*$^{R248Q/+}$ mice, and used as control *Tp53*$^{+/+}$ and *TP53*$^{R248Q/-}$ total BM cells, using the Puregene Core Kit A (Qiagen). Quantitative real-time PCR was performed in duplicates with SYBR Green reagent (applied Biosystems, Foster City, USA) and analysis was performed in an ABI prims 7500 sequence detection system, using 8ng genomic DNA and the mouse wt *Tp53* allele-specific primers (Suppl. Table 1). The primers anneal to mouse exon 5 and 6 of for the mouse *Tp53*, but do not recognize the humanized *TP53*$^{R248Q}$ allele. The wt *Tp53* signal was normalized to the *Rosa26*.

### MEL cells and primary murine hematopoietic cells

Murine erythroleukemia (MEL) cells were obtained from DSMZ (cultured in suspension in DMEM (Dulbecco's Modified Eagle Medium) (Gibco, Lubio, Thermo Fisher Scientific, Reinach, Switzerland), 5% FCS (Fetal Calf Serum), 1% P/S (Penicillin-Streptomycin) (Reddy and Shen 1992). To induce differentiation, $10^5$ cells were cultured in DMEM with 2%DMSO (sigma). After 2 days, cells were washed, counted, and cultured in newly prepared medium. Differentiation was continued for 4 days in presence of DMSO. Differentiation was measured by flow cytometry ($CD71^+/TER119^+$), benzidine staining and hemoglobin expression by qRT-PCR (real-time polymerase chain reaction). To isolate adult mouse BM, long bones and spine were crushed and filtered through a 70µm cell strainer (Cat. 352350, BD, New Jersey, USA) and the red blood cells were lysed with ammonium-chloride potassium (ACK) lysis buffer (150mM

$NH_4Cl$, 10mM $KHCO_3$, and 0.1mM EDTA, pH 8.0) for 10min on ice. Mouse hematopoietic lineage depletion kit (Cat. 130-090-858, Milteny Biotech, Bergisch Gladbach, Germany) was used to enrich for HSPCs, according to manufacturer's protocol. To obtain FL cells, one male mouse and one or two females were places together overnight. 14.5 days later, pregnant females were sacrificed by $CO_2$ asphyxia, and FL cells from individual fetuses were isolated, chopped and passed through 50µm cell strainer.

**Erythroid differentiation assay**

*In vitro* erythroid differentiation of primary erythroblasts was assessed by using a previously published protocol[23]. Adult mice erythroblasts derived from BM lineage depleted cells lin(-) and fetal-liver (FL)-derived erythroblasts were cultured for more than one week and split every two days in "maintenance medium (MM)" composed of StemSpan SFEM (Stem Cell Technologies, Vancouver, Canada), supplemented with 1%Pen/Strep, 0.4%cholesterol (Gibco, Thermo Fisher Scientific, Reinach, Switzerland), 2U/ml hEpo (Eprex 4000, 9096976, Pharmacy of University Hospital Basel), 100ng/ml mScf (Peprotech, London, UK), $10^{-6}$M dexamethasone (Calbiochem, Sigma Aldrich, Buchs, Switzerland) and 50ng/ml hIGF-1 (Peprotech, London, UK). The presence of erythroblasts was confirmed by flow cytometry ($DAPI^-/Kit^+/CD71^+/Ter119^-$). Erythroid maturation of erythroblasts was induced in "differentiation medium (DM)" composed of IMDM (Iscove's Modified Dulbecco's Media) (Gibco, Thermo Fisher Scientific, Reinach, Switzerland), 5% hPDS (0.45µM filtered, Blood donation center, University Hospital Basel), monothioglycerol (Sigma Aldrich, Buchs, Switzerland), 100ng/ml mSCF and 2U/ml hEPO. Cells were also split every two days, and cultured 500.000 cells/ml.

**Colony formation assay**

To determine colony formation by erythroblasts ($CD71^+/Ter119^-$), $1x10^4$ cells were plated in methylcellulose M3434 (Methocult, Stem Cell Technologies, Vancouver, Canada). After 7 to 10 days, colonies were counted and pictures were taken on Olympus IX50 microscope with 2x and 4x magnification respectively. For propagation, cells were washed with warm PBS 2x (8min,1800rpm) counted with trypan blue followed by plating the same number of cells ($1x10^4$) into fresh methylcellulose.

**Flow cytometry**

Cells were washed with FACS buffer (0.5% BSA, 1mM EDTA in PBS, filtered) and incubated for 15 min at RT with specified antibodies in 100µl FACS buffer. After staining, cells were again washed and stained with 1µg/ml DAPI (Life Technologies, Paisley, UK) in FACS buffer. LSR Fortessa (BD, New Jersey, USA) was used to analyze the stained cells. The data was

analyzed with FlowJo software (Tree Star). For cell sorting, cells were washed with PBS and resuspended with 300µl of FACS buffer with 1µg/ml DAPI. Cells were sorted on BD FASC ARIA III (BD, New Jersey, USA) in 1.5mL Eppendorf with 300µl of medium. All antibodies used in this analysis are listed in Suppl. Table 21.

**Preparation of retroviral supernatants and transduction**

Viral supernatants were produced by co-transfection of HEK293T-LX with an ectropic packaging vector (*pIPAK6*) and the respective plasmids by using Turbofect or Jetprime transfection reagent (Life Technologies, Paisley, UK). Supernatants were harvested 48 and 72 hours after transduction and concentrated using 10x Vivaspin 20 (Sartorius, Göttingen, Germany) by centrifugating for 3500rpm for 2h at 4°C before snap freezing in liquid nitrogen and storage at -80°C until usage. To determine expression of NFIA-ETO2 deletion mutants, HeLa cells were transiently transfected with *pcDNA3* plasmid encoding the respective mutant by using the Jetprime transfection reagent. MEL cells, BM lin(-) HSPC and BM-derived erythroblasts were virally transduced in DMEM (5%FCS, 1%P/S) or in MM respectively. Cells were transduced with supernatants in presence of 5ug/ml polybrene (Sigma Aldrich, Buchs, Switzerland) 90min, 1800 rpm at 30°C. Cells were incubated overnight with the cells, and washed with PBS and plated in their respective mediums. MEL and BM lin(-) cells were spin infected twice before sorting.

**Western Blotting**

For primary cells, nuclear protein extraction was performed. All used buffers were supplemented with Complete Mini protease inhibitors (Cat. 11836153001, Roche). Nuclear protein lysates were prepared by resuspending the cell pellets in hypotonic lysis buffer (10mM HEPES pH=7.9, 10mM KCl, 0.1mM EDTA, 0.1mM EGTA, 1mM DTT) for 15 min on ice and then treated with 0.1% NP-40 with 15sec vortexing. Nuclei were then spun down at 14.000 rpm for 2min at 4°C and the supernatant which contained the cytoplasmic fraction was kept for further analysis. Pellets were well resuspended in nuclear lysis buffer (20mM HEPES, pH=7.9, 0.4M NaCl, 1mM EDTA, 1mM EGTA, 1mM DTT). Finally, pellets were sonicated for 5 cycles (30sec sonication, 30sec pause) on a Bioruptor Pico Sonicator (Diagenode, Seraing, Belgium) and kept on ice for 20min. After incubation, nuclear extraction was spin down at 14.000rpm for 15min at 4°C and the pellet which contained histones was discarded and supernatant kept for nuclear protein analysis. To quantify the proteins, Bradford assay (Biorad, München, Germany) was used. Before loading, the samples were prepared in 4x Laemmli buffer (Biorad, München, Germany) and boiled for 10min at 95°C. For whole lysate extraction of MEL and HELA cells, 1 million cells were boiled in Laemmli buffer 10min at 100°C and centrifuge 15 min at 4°C at maximum speed. Supernatant was kept for whole lysate analysis.

Nuclear extraction and whole lysate samples were loaded on a handmade 7% polyacrylamide gels. For nuclear extraction of NFIA-ETO2, 50ug of nuclear extract was loaded. Wet transfer was done for 100min on ice in 5%Methanol/0.1%SDS/Tris-Base-Bicine buffer on 0.45µM nitrocellulose membranes. Membranes were blocked in 5% non-fatty milk (NFM) in PBS-1% Tween for 2 hours at room temperature and probed overnight with the selected antibody at 4°C in PBS-1%Tween. Blots were washed three times for 15min in PBS-1% Tween and probed with a secondary antibody in PBS-1%, and again washed three times for 15min in PBS-1%. Blots were then probed with Supersignal West Femto Max substrate (Thermo Scientific, Reinach, Switzerland) and develop using Carestream Biomax Kodak films (Sigma, New York, USA). List of antibodies and respective dilutions can be found in Suppl. Table 21.

**RNA sequencing**

Total RNA was isolated using Nucleospin RNA Plus XS kit (Ref 740990.250, Macherey-Nagel). RNA was quality-checked on the Bioanalyzer instrument (Agilent Technologies, Santa Clara, CA, USA) using the RNA 6000 Nano Chip (Agilent, Cat# 5067-1511) and quantified by Fluorometry using the QuantiFluor RNA System (Cat# E3310, Promega, Madison, WI, USA). Library preparation was performed, starting from 200ng total RNA, using the TruSeq Stranded mRNA Library Kit (Cat# 20020595, Illumina, San Diego, CA, USA) and the TruSeq RNA UD Indexes (Cat# 20022371, Illumina, San Diego, CA, USA) / and the TruSeq RNA CD Index Plate (Cat# 20019792, Illumina, San Diego, CA, USA). 15 cycles of PCR were performed. Libraries were quality-checked on the Fragment Analyzer (Advanced Analytical, Ames, IA, USA) using the Standard Sensitivity NGS Fragment Analysis Kit (Cat# DNF-473, Advanced Analytical) revealing excellent quality of libraries (average concentration was 103±20 nmol/L and average library size was 381±5 base pairs). Samples were pooled to equal molarity. The pool was quantified by Fluorometry using the QuantiFluor ONE dsDNA System (Cat# E4871, Promega, Madison, WI, USA). Libraries were pooled and sequenced SR76 and PE 2x39 with the Illumina NextSeq 500 system using the High Output Kit v2.5 (75 cycles and 38) for the primary erythroblasts and MEL cells respectively. Primary data analysis was performed with the Illumina RTA version 2.4.11, bcl2fastq v2.20.0.422.

**RNA-sequencing data analysis**

RNA-seq reads were mapped to the mouse genome assembly, version mm10, with STAR (versions 2.5.2 and 2.7 for primary erythroblasts and MEL cells, respectively)[51], with default parameters except for using the multi-map settings (outFilterMultimapNmax=10 and outSAMmultNmax=1) and filtering reads without evidence in spliced junction table (outFilterType="BySJout"). The output was sorted and indexed with samtools (version 1.15)[52]. Picard markDuplicates (version 2.6.0) was used to collapse samples run on different

sequencing lanes. Coverage tracks per sample were generated by tiling the genome in 20bp windows, counting overlapping reads per window using the function bamCount from the bioconductor package bamsignals (bioconductor versions 3.6 and 3.13 for primary erythroblasts and MEL cells, respectively) and exporting these window counts in bigWig format using the export function of the bioconductor package rtracklayer[53]. To obtain one bigWig file per group, bigWig files were merged using the UCSC command line tools bigWigMerge (version 2) and bedGraphToBigWig (version 2.8)[54]. The qCount function of QuasR[55] was used to count the number of read (5'ends) overlapping with the exons of each gene assuming an exon union model. Counts were normalized in log Count per Million (CPM) and used for the gene expression plots as well as principal analysis complement (PCA). For the primary erythroblasts cells, differentially gene expression analysis was performed with the EdgeR (version 3.32) package[56]. The design to analyze the data was: ~ 0 + group, where group corresponds to the groups classified according to fusion gene expression. fGSEA[57] (version 1.16) was used to perform gene set enrichment analysis (GSEA) with the parameters: nperm=100000, minSize=8, maxSize=5000. Several databases were used for the gene signatures analysis: MsigDB[58], HALLMARK[58], Gene Ontology[59], KEGG[60] databases and erythronDB[61]. Using the functionality "build gene lists" comparing proerythroblasts and basophilic erythroblast cells, the "ERYTHROID LINEAGE DEVELOPMENT" pathway was obtained; the corresponding list can be found in Suppl. Table 22. NFIA targets were obtained using TRANSFAC[62]. The list can be found in Suppl. Table 23. Gene expression of external cohorts of AML patients was obtained from Iaccobucci *et. al* [8] and TCGA[63], using age groups and mutational state as defined in respective studies. Iaria Iaccobucci kindly provided processed mRNA expression from RNA-seq as count table format from the authors, and TCGA data was obtained raw .cel files from TCGA (project LAML HG-U133_Plus_2, Level 1120) and RMA normalised with ReadAffy[64].

For the MEL cells, gene expression analysis was performed with the EdgeR (version 3.36) package[56]. The design is the same used for the primary erythroblast cells. fGSEA[57] (version 1.20) was used to perform gene set enrichment analysis (GSEA) with the parameters: nperm=100000, minSize=8, maxSize=5000. Several databases were used for the gene signatures analysis: MsigDB[58], HALLMARK[58], Gene Ontology[59], and KEGG[60] databases.

To define DEGs with a putitive role in erythroid differentiation, we screened PubMed (https://pubmed.ncbi.nlm.nih.gov/) for "gene name" & "erythroid". We read the related abstracts and/or full paper that allowed linking a given gene/protein to erythropoiesis as listed in Suppl. Table 16.

**ATAC sequencing**

ATAC sequencing was performed using a previously published protocol optimized for blood cells[65]. Digitonin (Sigma Aldrich, Buchs, Switzerland), a very gentle detergent, was used. Same experimental setup was used as the RNA sequencing experiment. *WT* and *p53*$^{R248Q/+}$ FL erythroblasts from fetuses from three different pregnant mice were transduced with NFIA-ETO2. After sorting, cells were cultured in maintenance and differentiation medium for 24h. 75x10$^5$ cells were washed two times with cold PBS and spun down at 500g for 5 min at 4°C. Supernatant was removed using two pipetting steps. Transposase mixture was prepared (25µl 2x TD buffer, 2.5µl TDE1, 22µl $H_2O$, 0.5µl Digitonin) (FC-121-1030, Illumina; G9441, Promega) and added directly to the pellet followed by disrupting by pipetting. Samples were incubated at 37°C for 30min. Next step was to purified the transposed DNA using the QIAGEN MinElute Reaction cleanup kit (28204) following the manufacturer's protocol. Purified DNA was eluted in 12µl of elution buffer (10mM Tris-HCl, pH=8). Transposed fragments were amplified using 2X KAPA HiFi HOTSTART Ready mix (Roche) with modified primers[66]. PCR purification was performed using Qiagen PCR purification kit (Cat no. 28104) according to the manufacturer's protocol.

Libraries were quality-checked on the Fragment Analyzer (Advanced Analytical, Ames, IA, USA) using the High Sensitivity NGS Fragment Analysis Kit (Cat# DNF-474, Advanced Analytical). AMPure XP beads (Beckman Coulter) Double Size Selection (1.2-0.8x) was performed to enrich for 150-400bp library fragments before mixing library pools for sequencing. Before sequencing with the NovaSeq, the pool of libraries was treated with Free Adapter Blocking Reagent (Illumina) according to manufacturer's specifications. Library pools were sequenced PE 2x38 and PE 2x51 with the Illumina NextSeq 500 system using the High Output Kit v2.5 (75 cycles) and the Illumina NovaSeq 6000 system using the S1 Reagent Kit (100 cycles), respectively. NextSeq primary data analysis was performed with the Illumina RTA version 2.4.11, bcl2fastq v2.20.0.422, and NovaSeq primary data analysis with pipeline version v3.4.4.

**ATAC-seq analysis**

Paired-end reads were mapped to the mouse genome assembly, version mm10, with bowtie2 (version 2.3.4.2)[67] using the option '--wrapper basic-0 --maxins 2000 --no-mixed --no-discordant --local'. The output was sorted and indexed with samtools (version 1.7)[52]. Picard markDuplicates (version 2.9.2) was used to collapse samples run on different sequencing lanes. Coverage tracks per sample were generated by tiling the genome in 20bp windows, counting overlapping reads per window using the function bamCount from the bioconductor package bamsignals (bioconductor version 3.6) and exporting these window counts in bigWig format using the export function of the bioconductor package rtracklayer[53].

Moreover, only for ATAC-seq results, to create one bigWig file per group of biological replicates (CTRL or NE for the NFIA-ETO2 samples), picard MergeSamFiles was used to merge bam files belonging to the same group, and the output was converted in normalized bigWig file by using the tool bamCoverage from DeepTools[68] with the option '--normalizeUsing CPM'. The tool computeMatrix of Deeptools was used to obtain the enrichment signal of ATAC-seq around genes or their TSS (-/+ 3kb).

For each group of biological replicates in ATAC-seq (CTRL or NE for the NFIA-ETO2 samples), regions of accessible chromatin were called with macs2[69] (version 2.1.2) using the option '-f BAM -g 2652783500 --nomodel --shift -100 --extsize 200 --broad --keep-dup all --qvalue 0.05'. The resulting peak lists of ATAC-seq were cleaned from peaks called in mitochondria and ENCODE blacklist regions[70]. A log-fold-change > 1.5 and FDR<0.05 filter was applied. The filtered peak lists were converted to bigBed format using the UCSC command line tool bedToBigBed[54]. The program annotatePeaks.pl of HOMER[30] (version 4.11.1) was used to annotate the peaks. The motif analysis was performed with the program findMotifsGenome.pl from HOMER by comparing against a background bed file containing all merged peaks detected from CTRL and NE group. Moreover, NFIA-ETO2 ATAC-seq results were also compared with ETO2 ATAC-seq data[9]. Additionally, comparisons of enrichment motifs between NE vs CTRL were performed with the AME tools from the MEME suite[31] (version 5.0.5). For transcription factor prediction, a novel computational method and software package was used: Binding Analysis for Regulation of Transcription (BART)[39].

**ChIP sequencing**

Chromatin immunoprecipitation was performed as described in[71]. MEL cells were crosslinked in 1% formaldehyde (Thermo Scientific #28908) at room temperature for 10 minutes, followed by lysis in SDS lysis buffer (50mM Tris/HCl pH 8.0, 10 mM EDTA pH 8.0, 1% SDS supplemented with protease inhibitor mix (cOmplete™, EDTA-free Protease Inhibitor Cocktail, Sigma). Precipitation for all samples was performed with ChIP grade protein A/G coupled magnetic beads (Thermo (Pierce) #26162). Antibodies used for ChIP: antiHA rabbit monoclonal, Cell Signaling Technologies (#3724) 10µl per 10µg of chromatin; antiH3K27ac and anti-H3K4me rabbit monoclonal antibodies, Cell Signaling Technologies (#4353, #5326), 10µl and 5µl per 10µg chromatin, respectively. After the overnight incubation with the antibodies and beads, washing steps were performed, followed by the elution in fresh elution buffer (1%SDS, 0.1M $NaHCO_3$). Eluates were kept at 65°C overnight to reverse the cross-links. DNA purification was performed using QIAquick PCR Purification Kit (Qiagen).

Libraries were prepared following the manufacturer protocols for Chipseq sequencing with Illumina NextSeq 550DX sequencer (ILLUMINA). Briefly, 10ng of fragmented DNA was end-repaired and adapter-ligated using the KAPABiosystems® Hyper Prep Library kit for

Illumina™ (KAPABiosystems, inc) and adapter barcode KAPA for Illumina. After adapter ligation, each sample was size-selected using AMPure XP Bead (Beckman Coulter, Inc). An amplification reaction was set up in a final volume of 50µl. An SPRI cleanup with a 1X Bead:DNA ratio was performed post-amplification and final libraries were eluted in 25µl. Libraries were quantified on a Qubit fluorometer with HS DNA (Thermo Fisher Scientific/Life Technologies) and checked for size on an Agilent Bioanalyzer with an HS DNA kit (Agilent, Santa Clara, CA). Chip libraries were normalized and pooled to perform a multiplexed sequencing run. 1% PhiX control was added to the sequencing pool, to serve as a positive run control. Sequencing was performed in PE mode (2x75nt) on an Illumina NextSeq550Dx platform, generating on average 40 million PE reads per sample.

**ChIPseq analysis**

Single-end reads were mapped to the mouse genome assembly, version mm10, with bowtie2 (version 2. 4.2)[67] using the option '--wrapper basic-0 --maxins 2000 --no-mixed --no-discordant --local'. The output was sorted and indexed with samtools (version 1.11)[52]. Picard markDuplicates (version 2.23.4) was used to collapse samples run on different sequencing lanes. Coverage tracks per sample were generated by tiling the genome in 20bp windows, counting overlapping reads per window using the function bamCount from the bioconductor package bamsignals (bioconductor version 3.6) and exporting these window counts in bigWig format using the export function of the bioconductor package rtracklayer[53]. Regions of accessible chromatin were called with macs2[69] (version 2.2.7.1) using the option '--nomodel --format=BAM --keep-dup=all --bdg --SPMR -g 2652783500 --extsize 155 --qvalue 0.05'; the option --broad was used for histone marks except H3K27ac, and the input was not used as control for the HA tag. The filtered peak lists were converted to bigBed format using the UCSC command line tool bedToBigBed[54]. Motif search was performed with the program findMotifsGenome.pl of HOMER[30] (version 4.11.1). The last analyses of peaks (annotations, peaks coverage and gene set enrichment testing) was performed with the Bioconductor packages ChIPpeakAnno (version 3.28.1), and chipenrich (version 2.18)[72,73]. ChIPseq result were compared with Eto2 peak-calling result from downloaded Eric Soler's dataset[32] (GSE59859, http://www.ebi.ac.uk/ena/data/view/ERA000161). The peak-calling was done with macs2 usig the option '-g 2652783500 --keep-dup all --nomodel --extsize 112'. To remove false-positive Eto2 peaks, bed file containing the ENCODE cis-regulatory element region in mm10 was downloaded from the website https://screen.wenglab.org/, and used as positive control.

**Analysis of symptomatic mice**

Symptomatic mice were sacrificed by $CO_2$ asphyxia, and one long bone and organs (lungs, heart, liver, spleen, intestine, stomach, kidney) were removed and fixed in buffered 4% formalin solution. Liver and spleen were weighted. Blood collection of live mice was performed in the tail vein and by terminal *vena cava inferior* puncture. Blood counts were determined using an Advia120 Hematology Analyzer using Multispecies Version 5.9.0-MS software (Bayer, Leverkusen, Germany). Hematoxylin and eosin (H&E) staining was used for paraffin-embedded tissue sections and Wright-Giemsa staining (Hematology, University Hospital Basel) was used to stain blood smears. Sections and smears were analyzed on Nikon TI (Tokyo, Japan), and pictures were taken at 100, 400 and 600x magnification respectively.

## 2. LEGENDS TO SUPPLEMENTARY FIGURES

**Supplementary Figure 1**

**NFIA-ETO2 blocks *in vitro* terminal differentiation of MEL and primary murine erythroblasts**

**(A)** NFIA-ETO2 expression in primary mouse erythroblasts: experimental setup. BM, bone marrow; FL, fetal liver; EB, erythroblasts, MM, maintenance medium; DM, differentiation-inducing medium, MC, methylcellulose cultures.

**(B)** Retroviral NFIA-ETO2 expression in primary BM-derived erythroblasts at *mRNA* and protein levels assessed by qRT-PCR and immunoblotting, respectively.

**(C)** Images from cultures of primary mouse FL-derived erythroblasts expressing NFIA-ETO2, ETO2, NFIA, or the empty vector (*pMSCV-GFP*) vector after 15 days in MM.

**(D)** Representative flow cytometry panels of NFIA-ETO2- or CTRL vector-transduced fetal liver-derived erythroblasts expressing CD71 and/or Ter119 and Kit and/or CD71.

**(E)** Kit and CD71 surface expression (in %) on NFIA-ETO2- and vector-transduced control (CTRL) BM-derived erythroblasts after 6 days in DM.

**(F)** *Gata1* mRNA levels in NFIA-ETO2- and CTRL-transduced BM-derived erythroblasts grown in MM (CTRL: n=4, NFIA-ETO2: n=3) or 24h in DM (CTRL: n=4, NFIA-ETO2: n=5) assessed by qRT-PCR.

**(G)** *Hbγ* mRNA levels in NFIA-ETO2- and CTRL-transduced BM-derived erythroblasts grown in MM (CTRL: n=5, NFIA-ETO2: n=3) or 24h in DM (CTRL: n=5, NFIA-ETO2: n=4) assessed by qRT-PCR.

**(H)** *Hbβ1* mRNA levels in NFIA-ETO2- and CTRL-transduced BM-derived erythroblasts grown in MM (CTRL: n=5, NFIA-ETO2: n=3) or 24h in DM (CTRL: n=5, NFIA-ETO2: n=4) assessed by qRT-PCR.

**I)** *Epor* mRNA levels in NFIA-ETO2- and CTRL-transduced BM-derived erythroblasts grown in MM (CTRL: n=4, NFIA-ETO2: n=3) or 24h in DM (CTRL: n=4, NFIA-ETO2: n=5) assessed by qRT-PCR.

**(J)** Proliferation of NFIA-ETO2-expressing primary BM-derived erythroblasts grown for 5 days in MM with (red line) or without EPO (green line) (n=3). Values are presented as individual points, bar graphs represent the mean value of independent biological replicates (n), error bars are standard error of the mean. Statistical significances were tested with paired two-tailed t- tests.

**(K)** Images of MC (M3434) cultures (x4) taken seven days after the first and second plating of BM-derived erythroblasts expressing NFIA-ETO2 (N-E) compared to vector-transduced control (CTRL) cells.

**(L)** Colony formation in MC (M3434) by BM-derived erythroblasts expressing NFIA-ETO2 compared to vector-transduced control (CTRL) cells. Shown are absolute numbers of colonies for 6 consecutive platings (n=2).

**(M)** Kaplan Meier plot of mice transplanted with NFIA-ETO2-expressing or vector-transduced control (CTRL) BM-derived erythroblasts (NFIA-ETO2, n=9, orange line; CTRL, n=4, black dotted line). None of the mice did develop any disease within 1 year.

**(N)** Transduced $GFP^+$ BM-derived erythroblasts (in %) before transplantation and at the end of the experiment in NFIA-ETO2- (orange dots, n=9) or vector-transduced (grey dots, n=4) erythroblasts.

**(O-Q)** Peripheral WBC counts ($x10^9$/L), RBC counts ($x10^{12}$/L) and PLT counts ($x10^9$/L) in symptomatic mice transplanted with NFIA-ETO2-expressing (orange dots, n=9) vs control (CTRL) mice transplanted with vector-transduced cells (grey dots, n=4).

Values are presented as individual points, bar graphs represent the mean value of independent biological replicates (n), error bars are standard error of the mean. Statistical significances were tested with paired two-tailed t- tests.

**Supplementary Figure 2**

**The NFIA-ETO2-mediated differentiation block requires both the DNA-binding domain and the NHR domains**

**(A)** Representative flow cytometry panels showing the expression of CD71 and/or Ter119 (upper panels) and Kit and/or CD71 (lower panel) in NFIA-ETO2 transduced MEL cells upon shRNA mediated knockdown of *Myb*, *Spi1,* and control *(shRenilla)*.

**(B)** Schematic overview of the NFIA domains, DBD and MH1, and of the ETO2 domains, NHR1, NHR2, NHR3 and NHR4. Shown are single deletions of each NFIA-ETO2 domain that have been generated.

**(C)** Growth curve of BM primary erythroblasts expressing NFIA-ETO2 (red line) or single NFIA-ETO2 domain deletions (DBD, yellow line, NHR4, blue line) compared to CTRL (grey line) over 7 days in MM (n=2). Values are presented as individual points, bar graphs represent the mean value of biological replicates, error bars as standard error of the mean.

**(D)** Representative flow cytometry panels of intact NFIA-ETO2- or single NFIA-ETO2 domain deletion mutant-transduced BM-derived erythroblasts grown in MM for 7 days showing expression of CD71 and/or Ter119 (upper panels) and Kit and/or CD71 (lower panel).

**(E)** Volcano plot of DEGs between NFIA-ETO2-$^{\Delta NHR4}$ expressing FL-derived erythroblasts in MM vs DM after 24h (FDR <0.05, logFC>±1.5 black lines). Orange and red points represent statistically significant dysregulated genes (FDR < 0.05, logFC>±1.25 or 1.5 respectively), whereas purple points represent non-significantly regulated genes.

**(F)** Selected positive and negative GSEA enrichment scores of DEGs from NFIA-ETO2$^{\Delta NHR4}$ -expressing FL-derived erythroblasts grown in MM vs 24h in DM (padj < 0.05).

**(G)** NFIA-ETO2 expression in MEL and primary FL-derived erythroblasts (EBs) was associated with 4674 common DEGs (logFC>±1.5; p< 0.05). Venn diagram of RNAseq analysis.

**Supplementary Figure 3**

**NFIA-ETO2 expression alters chromatin accessibility and binds to gene loci encoding for erythroid differentiation regulators**

**(A)** p-value-based comparison of enriched specific motifs detected in ATAC-seq peaks for the vector transduced and NFIA-ETO2-expressing primary EBs.

**(B)** Pellets of mock-transduced and NFIA-ETO2-HA-expressing MEL cells grown for 48h in the presence of 2%DMSO.

**(C)** Pie chart illustrating genome wide functional annotation of detected peak locations generated from the NFIA-ETO2-HA ChiP-seq analysis.

**(D)** Motif enrichment analysis (HOMER) performed on the peaks obtained in NFIA-ETO2-HA ChIP-seq.

**(E)** Genome browser snapshots of ChIP-seq tracks showing the binding of NFIA-ETO2-HA (red), H3K27ac (green), H3K4me1 (blue), H3K9me1 (yellow) and H3K27me3 (orange) to the *Spi1* and *Lmo2* (distal) loci, and their RNA-seq tracks (dark green) showing their normalized expression in MEL cells. The known cis-regulatory element in the mm10 mouse genome from ENCODE data are indicated in the yellow track.

**(F)** Barplot representing DEGs (obtained by RNA-seq of primary erythroblasts expressing NFIA-ETO2 and NFIA-ETO2$^{\Delta NHR4}$ after 24h in DM) located in the vicinity of NFIA-ETO2-HA peaks. DEGs related to erythropoiesis were acquired through literature-based search (Supplementary Table 16).

**(G)** Venn diagrams showing common upregulated and downregulated genes from the RNAseq datasets of MEL cells and primary erythroblasts. Circle size represents the number of detected DEGs in the RNA-seq analysis.

**(H)** Bar plots representing NFIA-ETO2-HA peaks containing NF1 motifs and/or overlaping with ETO2 peaks[32], located in the vicinity of DEGs (upper plot), obtained from the RNA-seq analysis of NFIA-ETO2-expressing MEL cells. The lower plot represents NFIA-ETO2-HA peaks containing NF1 motifs and/or overlaping with ETO2 peaks[32], located in the vicinity of DEGs related to erythropoiesis selected through literature-based search (Supplementary Table 16).

**Supplementary Figure 4**

**Functional cooperation of NFIA-ETO2 with *TP53$^{R248Q/+}$* *in vitro***

**(A)** CD71 and Ter119 surface expression (in %) on NFIA-ETO2-expressing *TP53$^{R248Q/+}$* BM-derived erythroblasts grown for 6 days in MM (n=3).

**(B)** Kit and CD71 surface expression (in %) on NFIA-ETO2-expressing *TP53$^{R248Q/+}$* BM-derived erythroblasts grown for 6 days in MM (n=3).

**(C)** Representative images from MC cultures of NFIA-ETO2-expressing (bottom row) vs. vector-transduced control (upper row) *TP53$^{R248Q/+}$* erythroblasts from the 1$^{st}$, 2$^{nd}$ and 4$^{th}$ plating.

**(D)** Growth curves of BM-derived erythroblasts with different *Tp53* genotypes over 6 days in MM (n=2-3).

**(E)** Kit and CD71 surface expression (in %) on BM-derived erythroblasts with different *Tp53* genotypes grown for 6 days in MM (n=3).

**(F)** CD71 and Ter119 surface expression (in %) on BM-derived erythroblasts with different *Tp53* genotypes grown for 6 days in MM (n=3).

**(G)** Growth of BM-derived erythroblasts with different *Tp53* genotypes over 6 days in DM (n=2-3).

**(H)** Kit and CD71 surface expression (in %) on BM-derived erythroblasts with different *Tp53* genotypes grown for 6 days in DM (n=3).

**(I)** CD71 and Ter119 surface expression (in %) on BM-derived erythroblasts with different *Tp53* genotypes grown for 6 days in DM (n=3).

Values are presented as individual points, bar graphs represent the mean value of independent biological replicates (n), error bars are standard error of the mean.

**Supplementary Figure 5**

**Transplantation of NFIA-ETO2-expressing *TP53$^{R248Q/+}$* erythroblasts induces a PEL-like disease in mice**

**(A)** Images of methylcellulose (M3434) cultures showing colony formation by GFP$^+$ and GFP$^-$ erythroblasts harvested from symptomatic mice transplanted with NFIA-ETO2-expressing *TP53$^{R248Q/+}$* cells from the 1$^{st}$, 2$^{nd}$, 4$^{th}$, and 6$^{th}$ plating.

**(B)** Peripheral WBC counts (x10$^9$/L) in symptomatic secondary transplanted mice (blue dots, n=4) compared to normal controls (grey dots, n=4).

**(C)** Peripheral RBC counts (x10$^{12}$/L) in symptomatic secondary transplanted mice (blue dots, n=4) compared to normal controls (grey dots, n=4).

**(D)** Peripheral PLT counts (x10$^9$/L) in symptomatic secondary transplanted mice (blue dots, n=4) compared to normal controls (grey dots, n=4).

**(E)** Spleen and liver weight (mg) of symptomatic secondary transplanted mice with BM from diseased recipients of NFIA-ETO2-expressing *TP53*$^{R248Q/+}$ cells (blue dots, n=4) compared to normal control mice (grey dots, n=4).
**(F)** Amount of GFP$^{+}$ cells in the BM of symptomatic secondary transplanted mice (blue dots, n=4).
**(G)** CD71 and Ter119 surface expression (in %) on GFP$^{+}$ BM cells from symptomatic secondary transplanted mice (n=4).
**(H)** Kit and CD71 surface expression (in %) on GFP$^{+}$ BM cells from symptomatic secondary transplanted mice (n=4).
Values are presented as individual points, bar graphs represent the mean value of independent biological replicates (n), error bars are standard error of the mean. Statistical significances were tested with unpaired two-tailed t- test.

**Supplementary Figure 6**

Projection of the gene expression signatures of NFIA-ETO2 (wildtype and inactivate ΔNHR4 mutants) with or without a TP53R248Q mutation into a transcriptional PCA-reduced space was performed as described[9].

## 3. SUPPLEMENTARY TABLES

**Supplementary Table 1**

Primers used for cloning the NFIA-ETO2-domain mutants, RT-qPCR assays, and genotyping *TP53*$^{R248Q}$ (HUPKI) mice.

| Primers used for RT-qPCR assays | | |
|---|---|---|
| **Target** | **Forward primer (5'-3')** | **Reverse primer (5'-3')** |
| ***mGapdh*** | ATGACATCAAGAAGGTGGTG | CATACCAGGAAATGAGCTTG |
| ***mGata1*** | GTGTCCTCACCATCAGATTCCAC | TCCCTCCATACTGTTGAGCAGTG |
| ***mHb-β*** | GTCTCTTGCCTGTGGGGAAA | CAACCAGCAGCCTGCCC |
| ***mHb-α*** | TGATGTAAGCCACGGCTCTG | CAGTGGCTCAGGAGCTTGAA |
| ***mHb-γ*** | CCTCATCAATGGCCTGTGGA | CACAACAAGAAGCCTTCCCAAG |
| ***mGypa*** | TGAAGTGTCTGCTGCGTT | CCGATAATCCCTGCCATCA |
| ***mEPOR*** | GGGCTGCATCATGGACAAAC | AGGGCCGCTTTGCTCTC |
| ***NFIA-ETO2*** | TGTACATGCAGCAGATTCAAGTC | GGGCTGCGTCTTCACCTC |
| | | |
| | | |

| Primers used for genotyping *TP53*$^{R248Q}$ (HUPKI) mice | | |
|---|---|---|
| **Target** | **Forward primer (5'-3')** | **Reverse primer (5'-3')** |
| ***TP53 R248Q*** | CCCTCCAGCTCAGCCTTTGTAG | CTTGATCAAGGCTTGGAAGGC |
| ***Cre*** | CGATGCAACGAGTGATGAGG | GCATTGCTGTCACTTGGTCGT |

| shRNA oligomers cloned into LT3 (mir-E) backbones: | |
|---|---|
| *Myb* -1 | TGCTGTTGACAGTGAGCGATCGGTGTTAGATAACGGACTATAGTGAAGCCACAGATGTATAGTCCGTTATCTAACACCGAGTGCCTACTGCCTCGGA |
| *Myb* -2 | TGCTGTTGACAGTGAGCGCAAGAGACTCGGTGTTAGATAATAGTGAAGCCACAGATGTATTATCTAACACCGAGTCTCTTTTGCCTACTGCCTCGGA |
| *Spi1* -1 | TGCTGTTGACAGTGAGCGAAGGCGAGGTGAAGAAAGTCAATAGTGAAGCCACAGATGTATTGACTTTCTTCACCTCGCCTGTGCCTACTGCCTCGGA |
| *Spi1* -2 | TGCTGTTGACAGTGAGCGCCCGCAAGAAGATGACCTACCATAGTGAAGCCACAGATGTATGGTAGGTCATCTTCTTGCGGTTGCCTACTGCCTCGGA |

**Supplementary Table 2**

Differentially expressed genes in NFIA-ETO2-expressing vs. vector-transduced control MEL cells.

**Supplementary Table 3**

GSEA analysis of differentially expressed genes in NFIA-ETO2-expressing vs. vector-transduced control MEL cells.

**Supplementary Table 4**

Differentially expressed genes in primary mouse erythroblasts expressing NFIA-ETO2$^{\Delta NHR4}$ grown in maintenance (MM) vs. differentiation-inducing medium (DM).

**Supplementary Table 5**

Differentially expressed genes in primary mouse erythroblasts expressing NFIA-ETO2 vs. the NFIA-ETO2$^{\Delta NHR4}$ mutant grown for 24h in DM.

**Supplementary Table 6**

GSEA analysis of differentially expressed genes in primary mouse erythroblasts expressing NFIA-ETO2 vs. the NFIA-ETO2$^{\Delta NHr4}$ mutant grown for 24h in DM.

**Supplementary Table 7**

GSEA analysis of differentially expressed genes in primary mouse erythroblasts expressing NFIA-ETO2 vs. the NFIA-ETO2DNHr4 mutant grown for 24h in DM based on the gene list obtained in a recent study performed using ETO2 knockout cells[28].

**Supplementary Table 8**

Differentially expressed genes in primary mouse erythroblasts expressing NFIA-ETO2 vs. the NFIA-ETO2$^{\Delta NHR4}$ either grown in MM or in DM, and compared for the growth conditions.

**Supplementary Table 9**

GSEA analysis of differentially expressed genes in primary mouse erythroblasts expressing NFIA-ETO2 vs. the NFIA-ETO2$^{\Delta NHR4}$ either grown in MM or in DM, and compared for the growth conditions.

**Supplementary Table 10**

Common differentially expressed genes in NFIA-ETO2-expressing MEL and primary erythroblasts.

**Supplementary Table 11**

HOMER-based motif enrichment ranking of ATAC-seq data from primary mouse erythroblasts expressing NFIA-ETO2 vs. vector-transduced control cells.

**Supplementary Table 12**

HOMER-based motif enrichment ranking of ATAC-seq data from primary mouse erythroblasts expressing NFIA-ETO2 vs. wt ETO2[9].

**Supplementary Table 13**

Overlap between NFIA-ETO2-HA, H3K4me1, H3K27ac peaks (obtained in ChIP-seq analysis in MEL cells), and DEGs located in their vicinity (obtained in the RNA-seq analysis of NFIA-ETO2 and vector-transduced MEL cells), NF1 motifs, regulatory elements and previously identified ETO2[32] ChIP-seq peaks.

**Supplementary Table 14**

HOMER-based motif enrichment of NFIA-ETO2-HA ChIP-seq peaks from MEL cells.

**Supplementary Table 15**

HOMER-based motif enrichment of NFIA-ETO2-HA ChIP-seq peaks in vicinity of DEGs obtained from the RNA-seq comparing NFIA-ETO2-expressing vs. vector-transduced control MEL cells.

**Supplementary Table 16**

Literature links to erythroid differentiation of genes depicted from ChIPseq peaks (HA) of MEL cells expressing a NFIA-ETO2-HA fusion.

**Supplementary Table 17**

Differentially expressed genes in NFIA-ETO2-expressing *TP53*$^{R248Q/+}$ or wildtype primary mouse erythroblasts grown in MM.

**Supplementary Table 18**

Differentially expressed genes in NFIA-ETO2-expressing *TP53*$^{R248Q/+}$ or wildtype primary mouse erythroblasts grown in DM (24h).

**Supplementary Table 19**

GSEA analysis of differentially expressed genes in NFIA-ETO2-expressing *TP53*$^{R248Q/+}$ or wildtype primary mouse erythroblasts grown in DM (24h).

**Supplementary Table 20**

GSEA analysis of differentially expressed genes in primary human AEL samples with mutated p53 vs without p53 mutation[9].

**Supplementary Table 21**

Antibodies used for flow cytometry and Western Blot analyses.

| Target protein | Clone | Dilution | Supplier |
|---|---|---|---|
| **HA** | C29F4 | 1:50 | Cell signaling |
| **ETO2** | C-20/sc-9739 | 1:500 | Santa Cruz |
| **FLAG** | D6W5B | 1:1000 | Cell signaling |
| **LAMIN-A/C** | E-1/ sc-376248 | 1:5000 | Santa Cruz |
| **ACTIN** | C-11 | 1:6000 | Santa Cruz |
| **P53** | 1C12 | 1:1000 | Cell signaling |
| **H3K4me1** | Lys4 | 1:6000 | Santa Cruz |
| **H3K27Ac** | Lys27 (D5E4) | 1:50 | Cell signaling |
| **H3K27me3** | C36B11 | 1:1000 | Cell signaling |
| **H3K9me1** | D1P5R | 1:50 | Cell signaling |

**Supplementary Table 22**

Gene expression signature from ErythonDB: "ERYTHROID LINEAGE DEVELOPMENT" obtained by using the functionality "Build Gene Lists" within an erythropoietic lineage based on comparison between proerythroblasts and basophilic erythroblasts [61].

**Supplementary Table 23**

TRANSFAC-based NFIA-target gene list[62].

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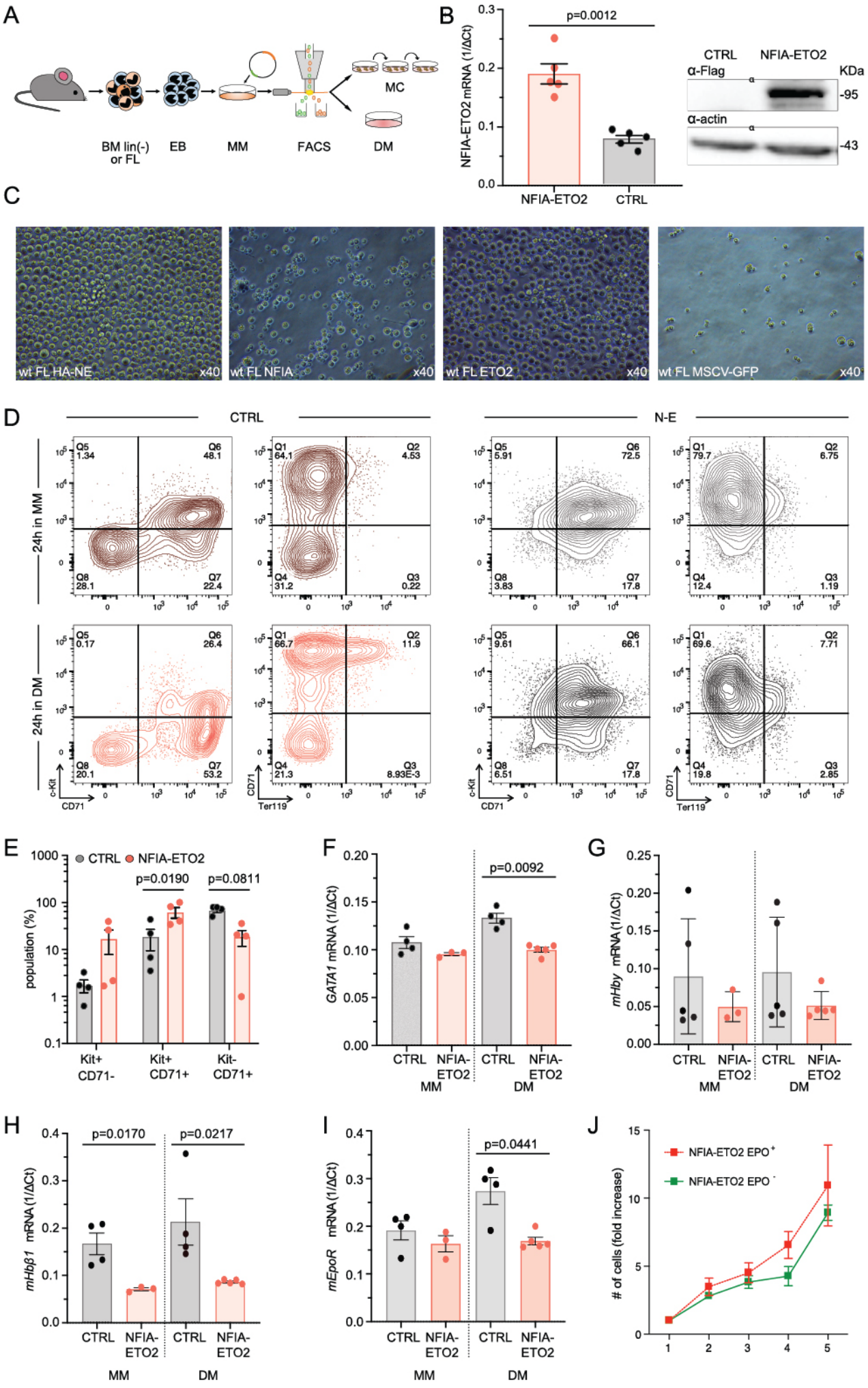


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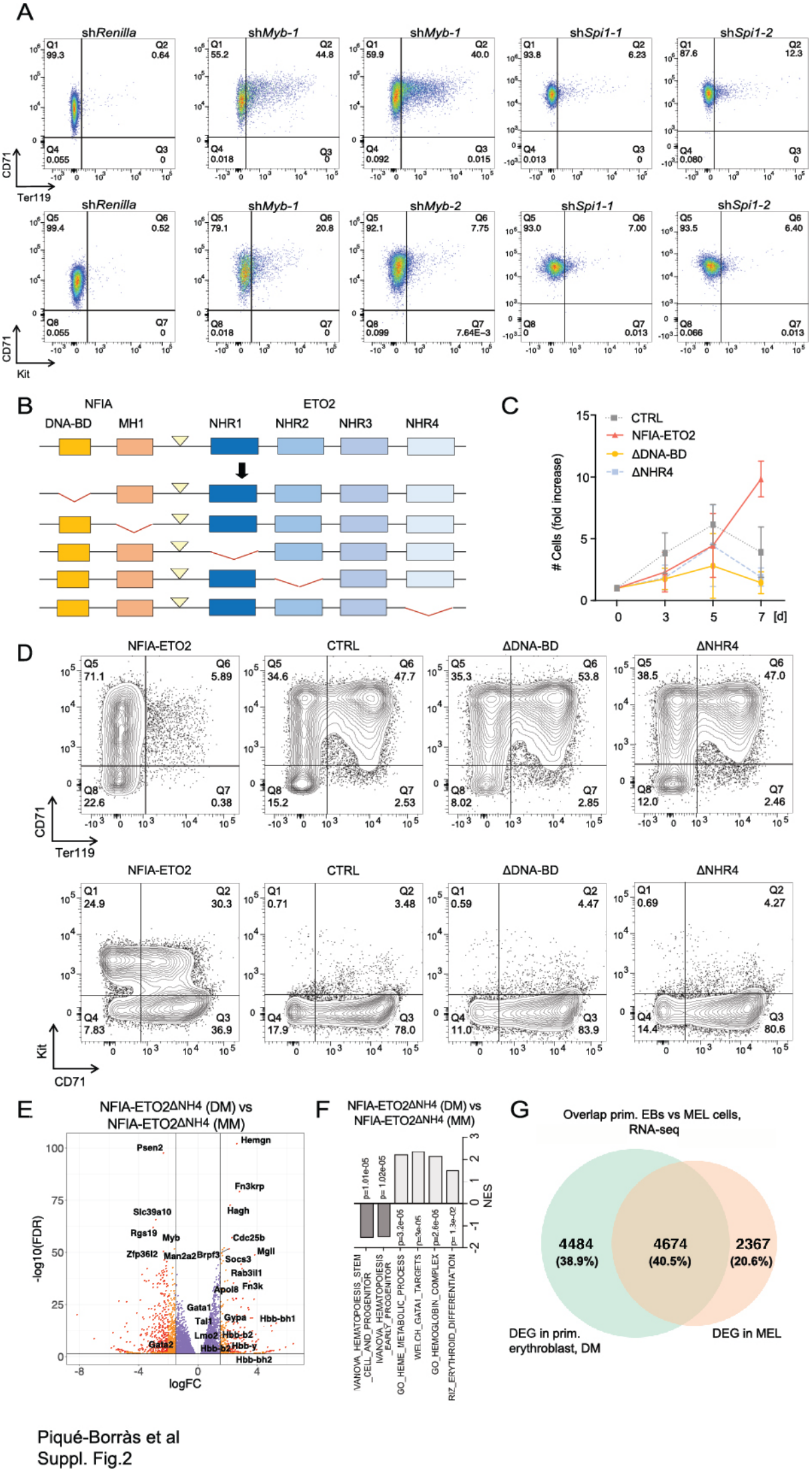


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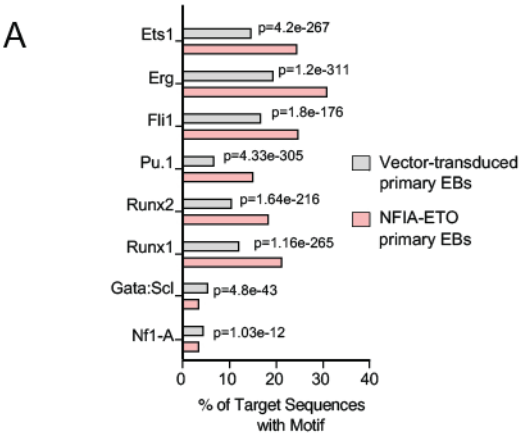


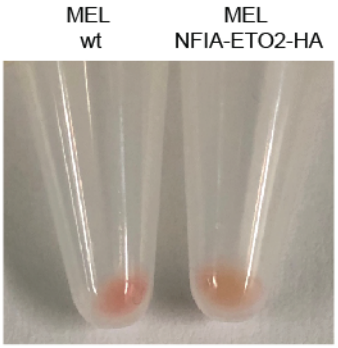


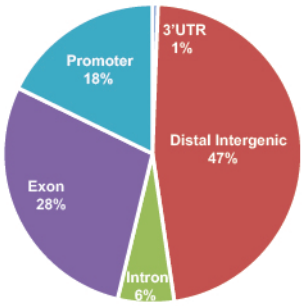


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|  | GATA:SCL | 5.49% | 1E-36 |
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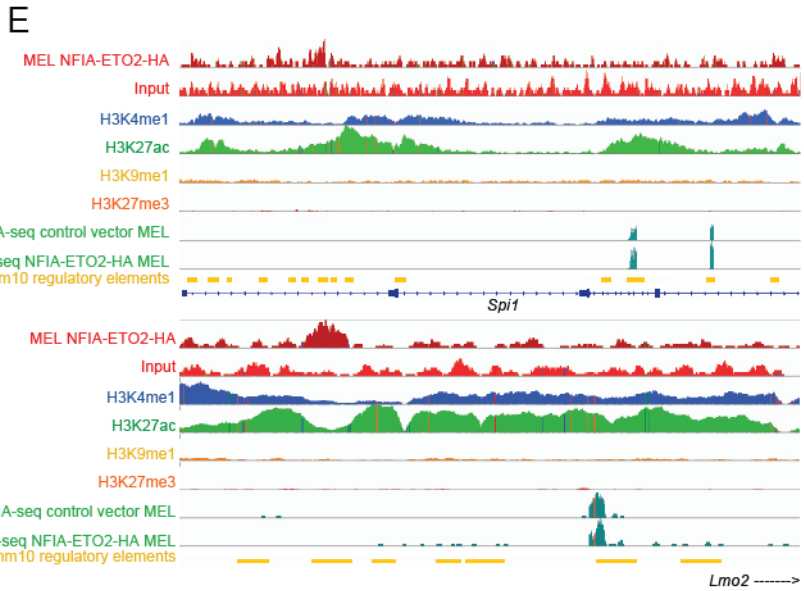


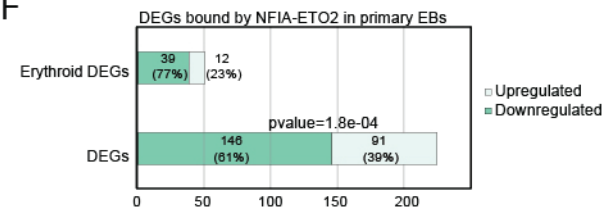


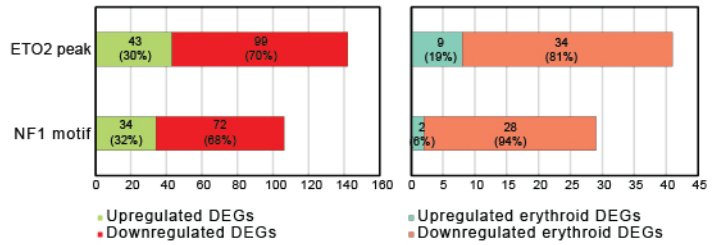


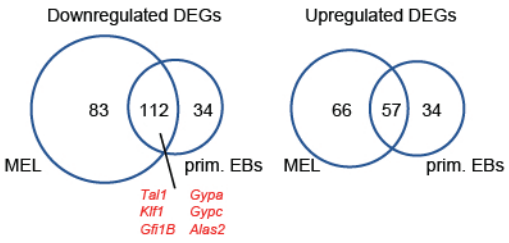


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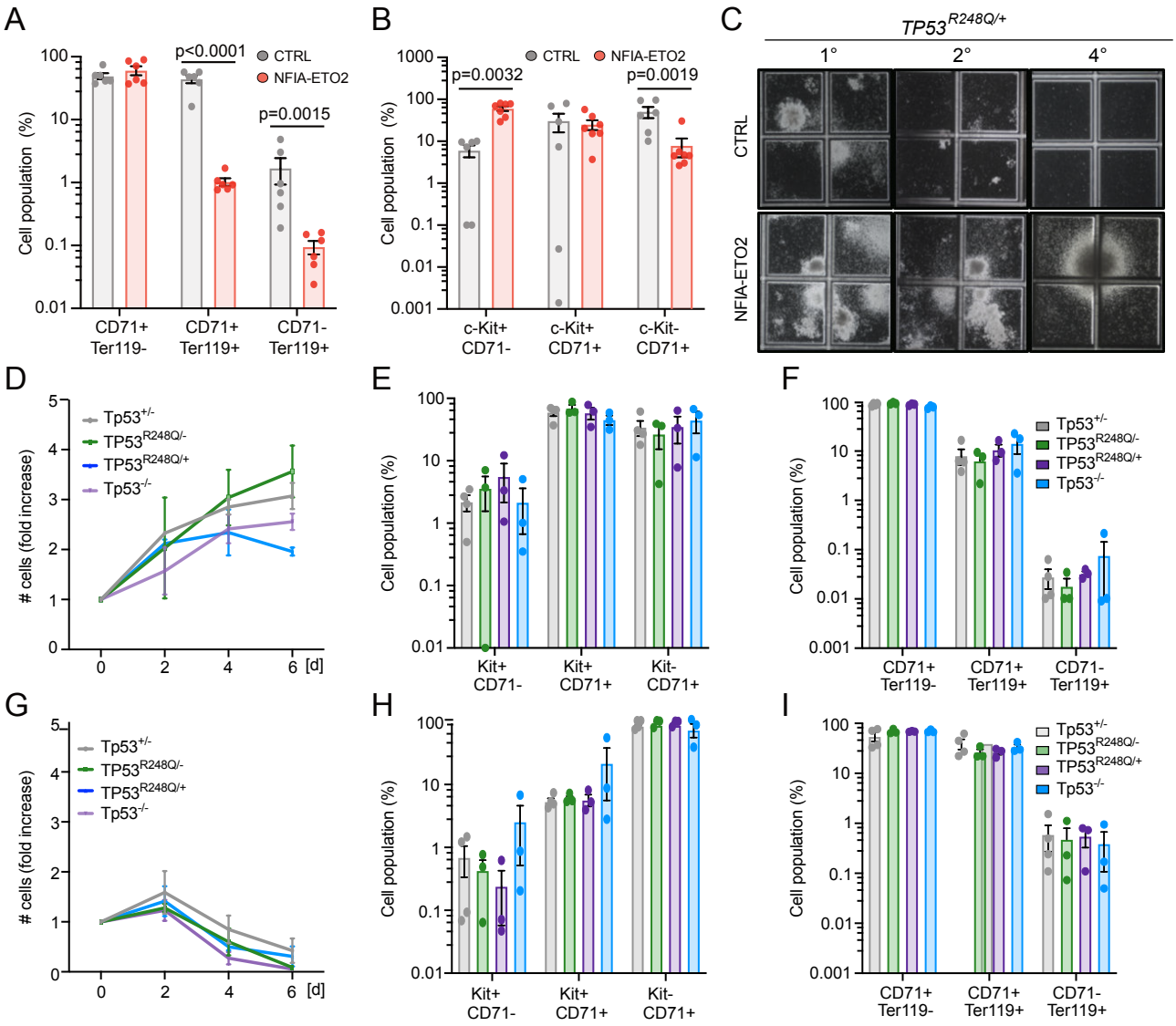


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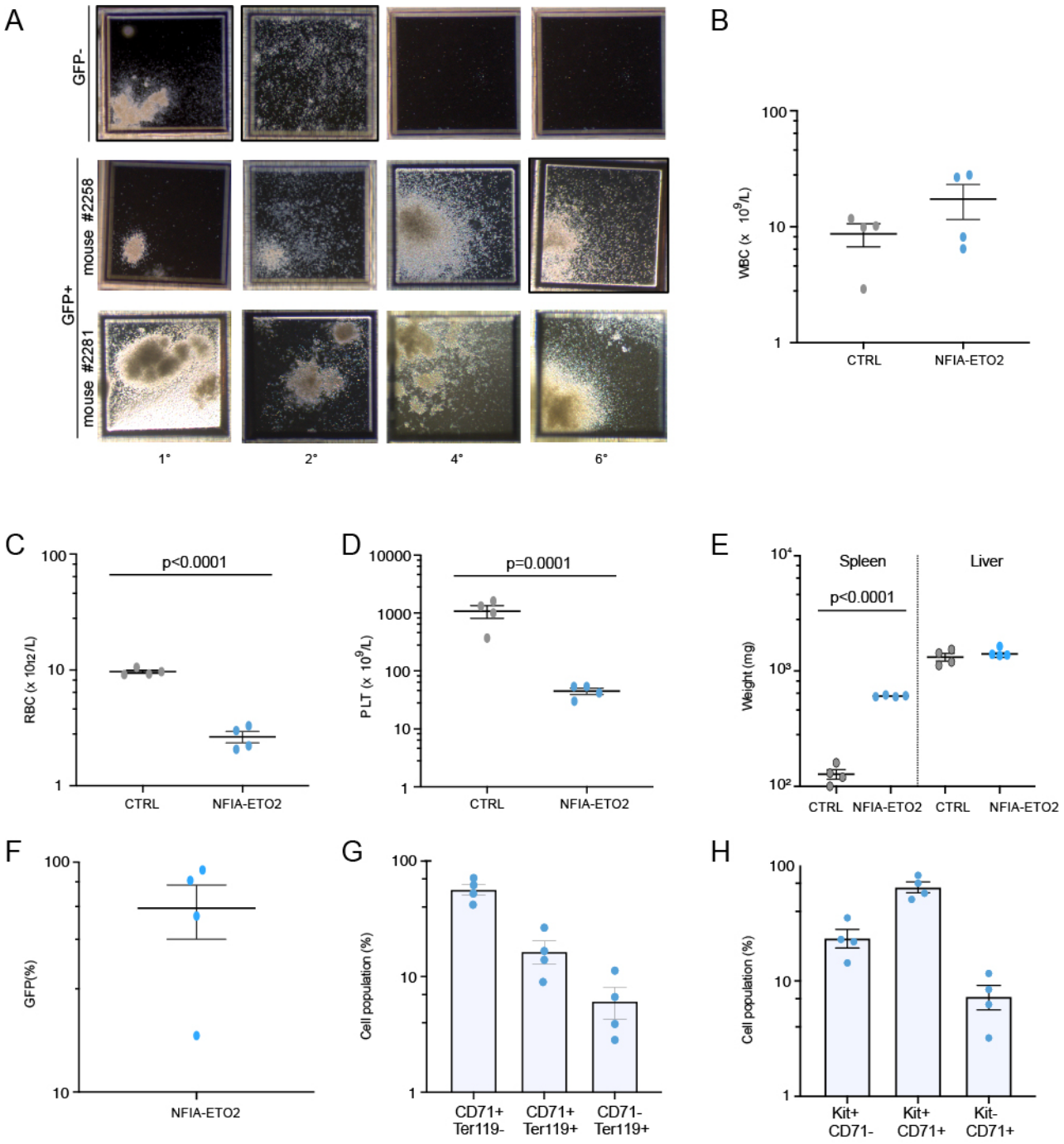


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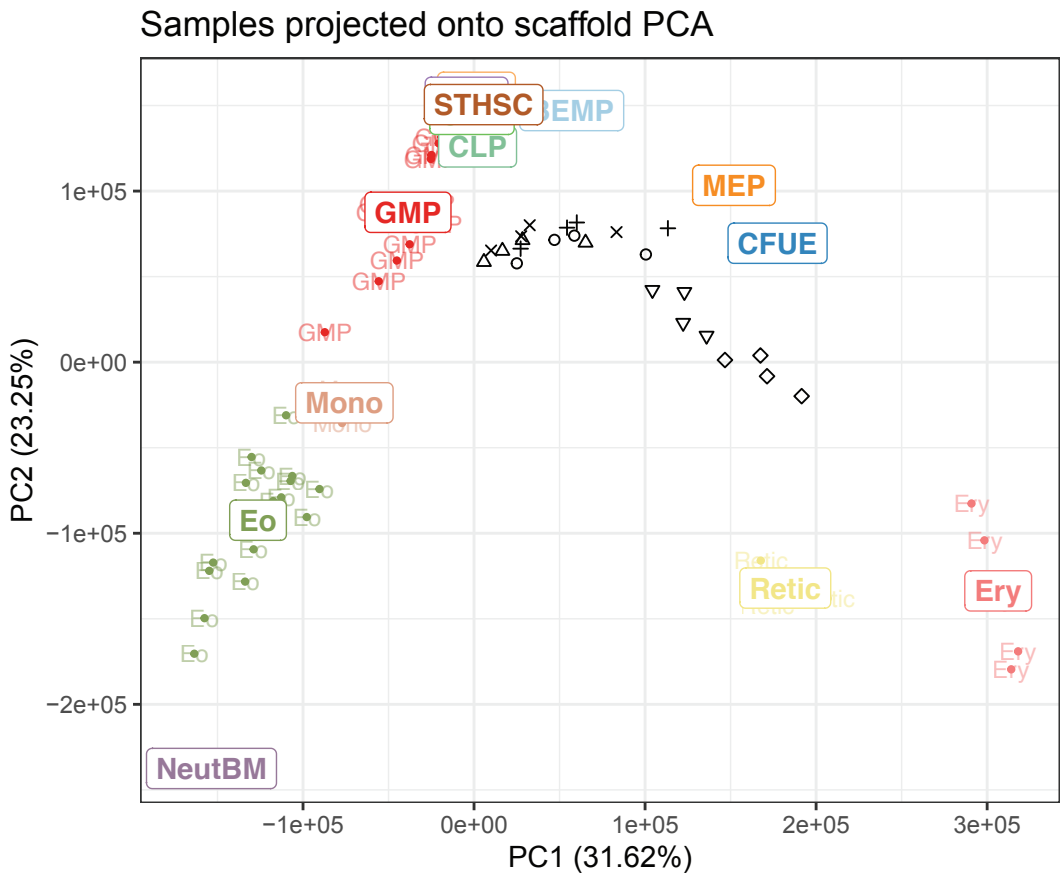


Our samples

- TP53$^{R248Q/+}$ NFIA-ETO2 (DM)
- TP53$^{R248Q/+}$ NFIA-ETO2 (MM)
- WT NFIA-ETO2 (DM)
- WT NFIA-ETO2 (MM)
- WT NFIA-ETO2$^{\Delta NHR4}$ (DM)
- WT NFIA-ETO2$^{\Delta NHR4}$ (MM)

Scaffold group

- BEMP
- CFUE
- CLP
- CMP
- Eo
- Ery
- GMP
- LSK
- MEP
- Mono
- MPP
- NeutBM
- Retic
- STHSC

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