Supplemental Information

Autophagy inhibition impairs leukemia stem cell function in FLT3-ITD AML but has antagonistic interactions with tyrosine kinase inhibition

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Inventory of Supplemental Information

Supplemental Materials and Methods

Reagents: The autophagy reporter plasmid GFP-LC3-RFP-LC3ΔG was purchased from Addgene (#84572). Lys05 (MedChem Express; Cat:HY-12855A) was dissolved in sterile PBS after sonification. AC220 (AdooQ, Cat:A10027) was dissolved in DMSO and diluted with 22% 2-hydroxypropyl-B-cyclodextrin.

Real time quantitative reverse transcription PCR (RT-QPCR): Total RNA was extracted using an RNeasy Plus Micro kit (Qiagen, Valencia, CA), first-strand cDNA synthesized using Superscript III First-Strand Synthesis Kit (Invitrogen, Grand Island, NY), and RT-QPCR analysis performed with TaqMan primers and probes for p53, p21, Bax, Puma, Noxa, GAPDH and β-actin using a HT7900 System (Applied Biosystem, Foster City, CA).[1]

Mitochondrial DNA (mtDNA) quantification: QPCR analysis was performed on genomic DNA using Murine COXII, CytB and Actin primers.

LC3B analysis: Cells were suspended in 100% methanol at -20°C, blocked in 5% serum/0.3%triton X-100/PBS, and incubated with anti-LC3b-Alexa Fluor 488 antibody overnight at 4°C, centrifuged onto slides, cover slipped with Prolong Gold AntiFade with DAPI, and examined using a Zeiss upright LSM 510 confocal microscope. For flow cytometry, cells were fixed in 4% formaldehyde, permeabilized using ice-cold 100% methanol, and incubated with anti-LC3b-Alexa Fluor 488 antibody for 1 hour.

RNA sequencing analysis. RNA was extracted from BM LSK and GMP cells using RNeasy Plus Micro kit (Qiagen, Valencia, CA) with four biological replicates per group. Libraries were

prepared using SMARTer Ultra Low Input RNA Kits for Sequencing (v4, TaKaRa Clontech) and Nextera XT DNA Library Preparation Kit (Illumina). Paired end sequencing (150bp reads) was performed by HudsonAlpha Discovery Biosciences using the Novaseq 6000 Sequencing System, S4 flow cell. RNA-Seq FASTQ reads were aligned to the mouse reference genome (Gencode Release M11) using STAR (version 2.5.3a) and reads mapping to each gene enumerated using HTSeq-count. Normalization and differential expression was calculated using DESeq2.[2] Pathway analysis was performed using gene set enrichment analysis (GSEA). R packages (ggplot2 and Pheatmap) were used to generate plots.

Table S1: Clinical characteristics of AML patients

ID	Status at collection	Mutations	BM/PB
#17	Untreated	FLT3-ITD,NPM1	PB
#62	Untreated	FLT3-ITD, DNMT3A	PB
#173	Untreated	FLT3-ITD,TET2	PB
#178	Untreated	FLT3-ITD, DNMT3A	PB
#249	Relapse	FLT3-ITD,NPM1, DNMT3A	PB
#263	Untreated	FLT3-ITD,IDH1 DNMT3A	PB
#395	Untreated	FLT3-ITD, TET2, DNMT3A, NPM1, NRAS	PB
#591	Untreated	FLT3-ITD, SRSF2, NPM1, IDH2, GATA2	PB
#699	Untreated	FLT3-ITD,NPM1,TET2	PB
#705	Untreated	FLT3-ITD,NPM1, WT1, U2AF1	PM
#777	Untreated	FLT3-ITD,NPM1, DNMT3A, IDH2	BM
#453	Untreated	NPM1, SRSF2, TET2	PB
#480	Untreated	NPM1, PTPN11, DNMT4A, CEPBA, RAD21	PB
#583	Untreated	CEBPA, CSF3R, SRSF2, TET2	BM
#775	Untreated	DNMT3A, TET2, U2AF1	PB

Table S2: Antibodies used for flow cytometry analysis

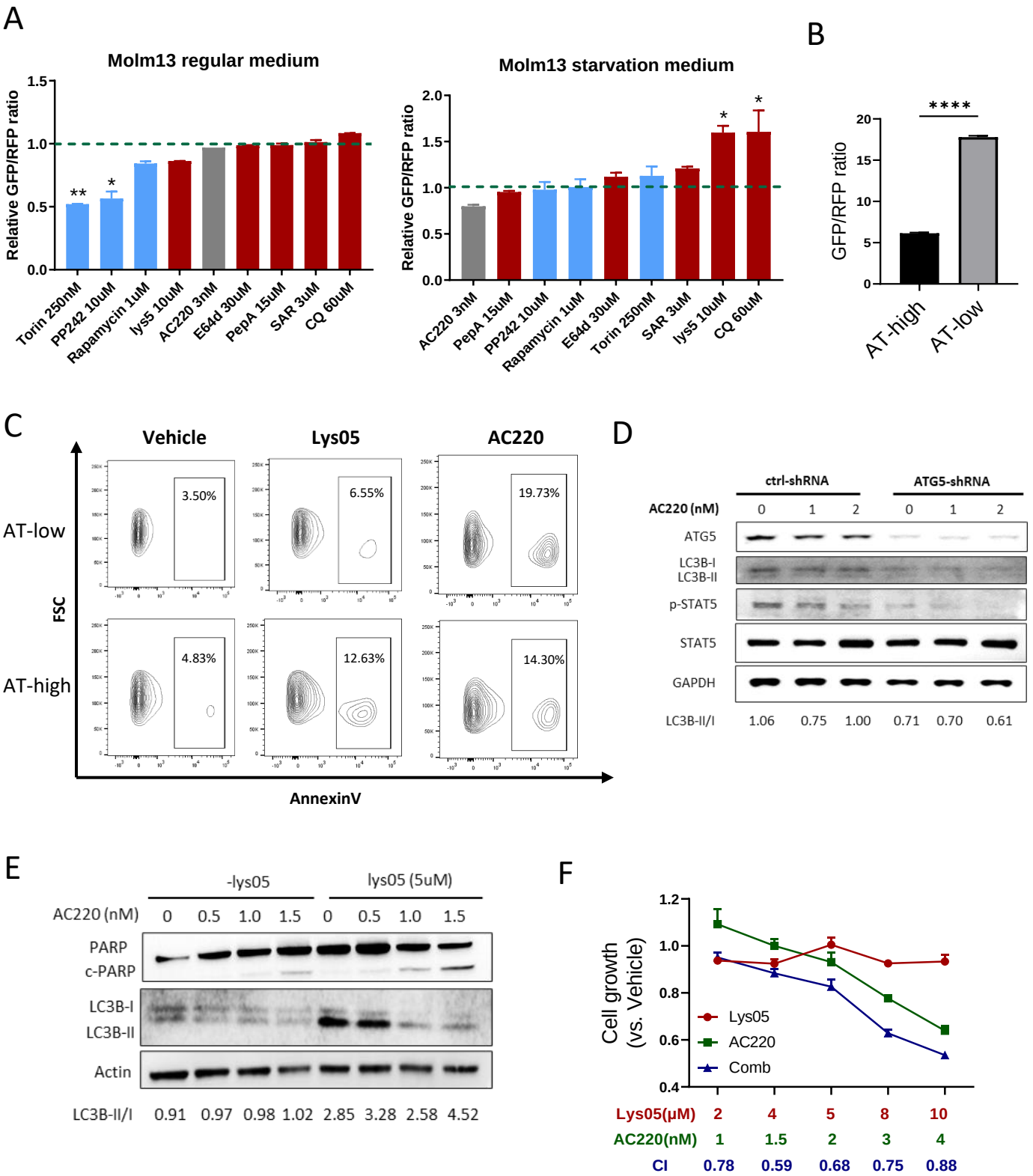
Antibody	Clone	Source	Catalog number
For Mouse Lineage cocktail			
Anti-mouse CD3e	Clone 145-2C11	eBioscience	Cat#13-0031-85; RRID:AB_466320
Anti-mouse CD4	Clone GK1.5	eBioscience	Cat#13-0041-85; RRID:AB_466326
Anti-mouse CD8a	Clone 53-6.7	eBioscience	Cat#13-0081-85; RRID: AB_466347
Anti-mouse CD8b	Clone	BioLegend	Cat#126604
Anti-mouse B220	Clone RA3-6B2	eBioscience	Cat#13-0452-85; RRID:AB_466450
Anti-mouse CD19	Clone 1D3	eBioscience	Cat#13-0193-85; RRID:AB_657658
Anti-mouse IgM	Clone II/41	eBioscience	Cat#13-5790-85; RRID:AB_466676
Anti-mouse Gr-1	Clone RB6-8C5	eBioscience	Cat#13-5931-85; RRID:AB_466801
Anti-mouse CD11b	Clone M1/70	eBioscience	Cat#13-0112-85; RRID:AB_466360
Anti-mouse NK1.1	Clone PK136	eBioscience	Cat#13-5941-85; RRID:AB_466805
Anti-mouse Ter119	Clone TER-119	eBioscience	Cat#13-5921-85; RRID:AB_466798
Streptavidin		BioLegend	Cat#405229
Mouse Stem Cell Markers			
Anti-mouse CD135 (FLT3)	Clone A2F10	eBioscience	Cat#13-1351-85 RRID: AB_466600
Anti-mouse Sca-1	Clone D7	BioLegend	Cat#108126
Anti-mouse CD117	Clone 2B8	eBioscience	Cat#47-1171-82; RRID: AB_1272177
Anti-mouse CD16/32	Clone 93	eBioscience	Cat#56-0161-82; RRID:AB_493994
Anti-mouse CD150	Clone TC15-12F12.2	BioLegend	Cat#115922
Anti-mouse CD48	Clone HM48-1	eBioscience	Cat#17-0481-82; RRID:AB_469408
Anti-mouse CD34	Clone RAM34	eBioscience	Cat#50-0341-82; RRID:AB_10596826
Mouse Mature Markers			

Anti-mouse CD45.1	Clone A20	eBioscience	Cat#25-0453-82; RRID:AB_469629
Anti-mouse CD45.2	Clone 104	eBioscience	Cat#11-0454-85; RRID:AB_465062
Anti-mouse CD45	Clone 30-F11	eBioscience	Cat#56-0451-82; RRID:AB_891454
Anti-mouse CD19	Clone 1D3	eBioscience	Cat#17-0193-82; RRID:AB_1659676
Anti-mouse B220	Clone RA3-6B2	eBioscience	Cat#56-0452-82; RRID:AB_891458
Anti-mouse CD3	Clone 17A2	BioLegend	Cat#100218
Anti-mouse CD11b	Clone M1/70	eBioscience	Cat#47-0112-82; RRID:AB_1603193
Anti-mouse Gr-1	Clone RB6-8C5	eBioscience	Cat#12-5931-83; RRID:AB_466046
Human markers on NSG/NRGS experiment and primary samples			
Anti-human CD45	Clone 2D1	BD Bioscience	Cat# 347463
Anti-human CD34	Clone 4H11	eBioscience	Cat#25-0349-42 RRID: AB_1963576
Anti-human CD38	Clone HIT2	BD Bioscience	Cat#555461
Anti-human CD33	Clone WM53	eBioscience	Cat#45-0338-42 RRID: AB_10714975
Anti-human CD3	Clone OKT3	eBioscience	Cat#56-0037-42 RRID: AB_10714978
Anti-human CD19	Clone H1B19	BD Bioscience	Cat#561742
Anti-human CD11b	Clone CBRM1/5	eBioscience	Cat#12-0113-42 RRID: AB_10717076
Anti-human CD14	Clone 61D3	eBioscience	Cat#48-0149-42 RRID: AB_1272050
Others			
Anti-Mouse Ki67	Clone SolA15	eBioscience	Cat# 11-5698-80 RRID:AB_11151689
Rat isotope control	Clone eBR2a	eBioscience	Cat# 11-4321-41 RRID: AB_10669560
Cy™ 5 Annexin V		BD Bioscience	Cat# 559934
LC3A/B Rabbit mAb		Cell signaling Technology	Cat#13082S
Rabbit IgG Isotope		Cell signaling Technology	Cat#4340S
P53 Rabbit mAb		Cell signaling Technology	Cat#5429S

Legends to Supplemental Figures

Figure S1. Autophagy inhibition reduces growth of FLT3-ITD cells and enhances sensitivity to TKI treatment in vitro.

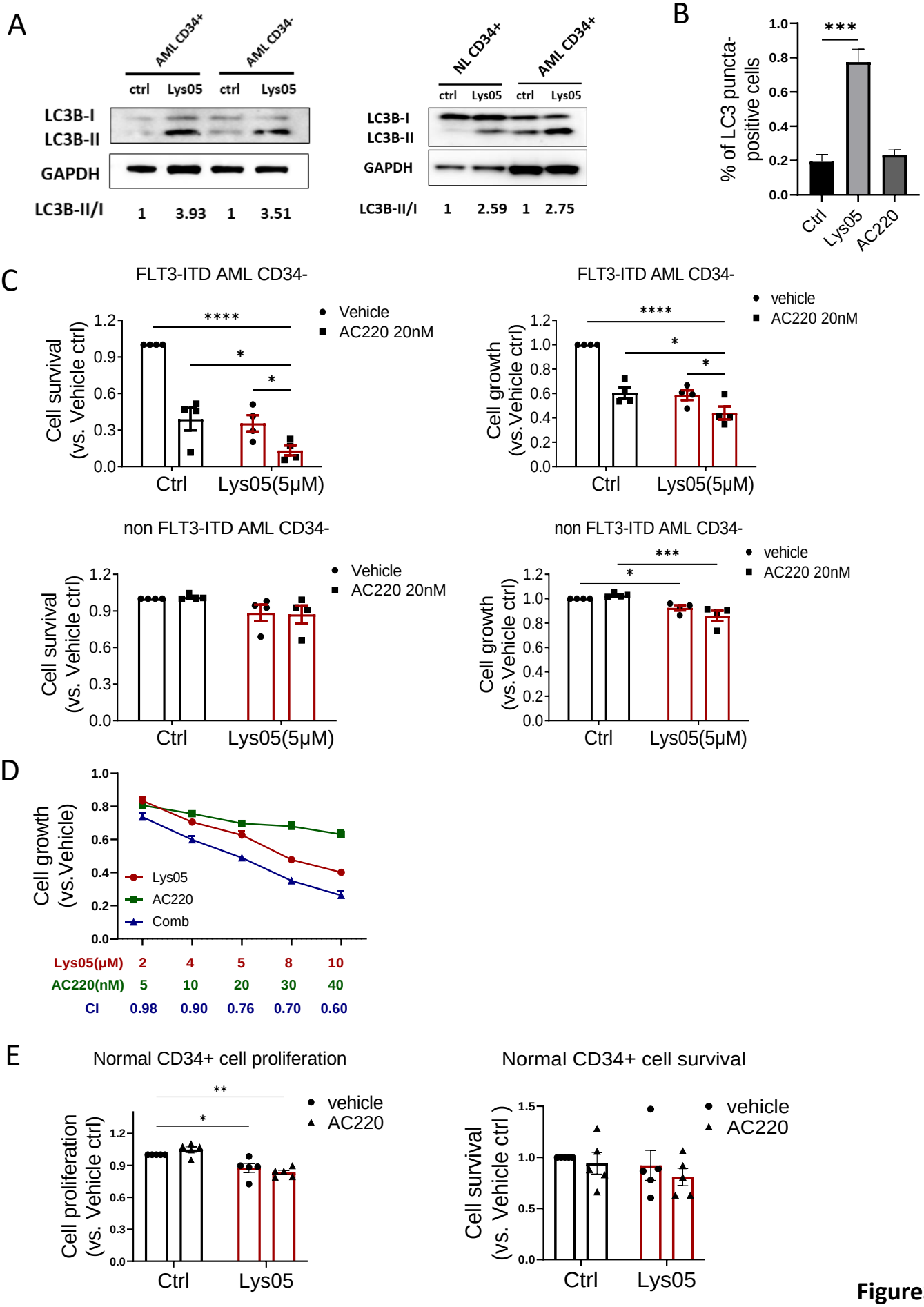
A: GFP/RFP ratio of Molm13 cells transduced with GFP-LC3-RFP-LC3ΔG plasmid and exposed to different drugs in regular condition (RPMI 1640 medium) or starvation condition (Earle's balanced salts solution (EBSS) medium).The blue columns indicate autophagy inducers and red columns indicate autophagy inhibitors. B: GFP/RFP ratio of AT-high and AT-low cells after 72 hours culture. C: Flow Histogram showing apoptosis of AT high cells and AT low cells measured by Annexin V and DAPI after exposure to Lys05 (5μM) and/or AC220 (2nM) for 48 hours as described for Fig 1A. D: Western blotting for ATG5, LC3B, p-STAT5, STAT5 and GAPDH was performed on Molm13 cells transduced with ATG5-shRNA expressing lentivirus vectors and cultured overnight with/without AC220 treatment. E: Western blotting for LC3B and PARP/c-PARP was performed on Molm13 cells after overnight exposure to Lys05 (5μM) or AC220 treatment (left). F: Combination index (CI) was measured by Compusyn software after 48h incubation of Lys05 with/without AC220 in Molm13 cells. Significance values: *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001; ns, not significant. Results represent mean ± SEM of multiple replicates.


A
Molm13 regular medium
Relative GFP/RFP ratio
Torin 250nM
PP242 10uM
Rapamycin 1uM
lys5 10uM
AC220 3nM
E64d 30uM
PepA 15uM
SAR 3uM
CQ 60uM
Molm13 starvation medium
AC220 3nM
PepA 15uM
PP242 10uM
Rapamycin 1uM
E64d 30uM
Torin 250nM
SAR 3uM
lys5 10uM
CQ 60uM
B
GFP/RFP ratio
****
AT-high
AT-low
C
Vehicle
Lys05
AC220
AT-low
AT-high
FSC
AnnexinV
3.50%
6.55%
19.73%
4.83%
12.63%
14.30%
D
ctrl-shRNA
ATG5-shRNA
AC220 (nM)
0 1 2 0 1 2
ATG5
LC3B-I
LC3B-II
p-STAT5
STAT5
GAPDH
LC3B-II/I
1.06 0.75 1.00 0.71 0.70 0.61
E
-lys05
lys05 (5uM)
AC220 (nM)
0 0.5 1.0 1.5 0 0.5 1.0 1.5
PARP
c-PARP
LC3B-I
LC3B-II
Actin
LC3B-II/I
0.91 0.97 0.98 1.02 2.85 3.28 2.58 4.52
F
Cell growth
(vs. Vehicle)
Lys05
AC220
Comb
Lys05(μM) 2 4 5 8 10
AC220(nM) 1 1.5 2 3 4
CI 0.78 0.59 0.68 0.75 0.88

FigureS1

Figure S2. Autophagy inhibition reduces human FLT3-ITD AML CD34+ cell growth and enhances TKI sensitivity in vitro

A: LC3B protein expression was measured in FLT3-ITD AML CD34+ and CD34- cells by Western blotting after 16h treatment Lys05 (5μM) (left).LC3B protein expression was measured in normal CD34+ cells and FLT3-ITD AML CD34+ cells by Western blotting after 16h treatment Lys05 (5μM) (right). B: The pecentage of LC3 puncta positive cells in each microscope image from FLT3-ITD AML CD34+ cells after 4h exposure as incidated as decribed for Fig2A. C: FLT3-ITD AML CD34- cells (n=4) and non FLT3-ITD AML CD34- cells (n=4) were treated with Lys05 with/without AC220 for 48 hours, cell survival was measured by AnnexinV and DAPI labeling (left) and cell growth was meadured using CellTiter-Glo (right) (#453, #480, #583, #775). D: FLT3-ITD AML CD34+ cells from #173 were treated with Lys05 with/without AC220 for 48 hours. Combination index (CI) was measured by Compusyn software after 48h incubation of Lys05 with/without AC220. E: Normal CD34+ cells (n=5) were cultured with Lys05 (5μM) with/without AC220(20nM) for 48h, and proliferation was measured by CFSE labeling (left), and cell survival measured by AnnexinV and DAPI labeling (right). Significance values: *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001; ns, not significant. Results represent mean ± SEM of multiple replicates.

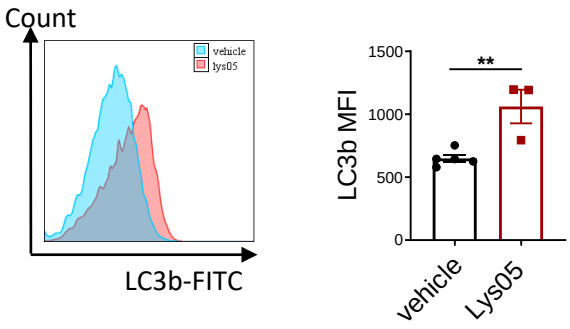

A
AML CD34+
AML CD34-
ctrl
Lys05
ctrl
Lys05
LC3B-I
LC3B-II
GAPDH
LC3B-II/I
1
3.93
1
3.51
NL CD34+
AML CD34+
ctrl
Lys05
ctrl
Lys05
LC3B-I
LC3B-II
GAPDH
LC3B-II/I
1
2.59
1
2.75
B
% of LC3 puncta-positive cells
***
Ctrl
Lys05
AC220
C
FLT3-ITD AML CD34-
Cell survival (vs. Vehicle ctrl)
Vehicle
AC220 20nM
****
*
*
Ctrl
Lys05(5μM)
FLT3-ITD AML CD34-
Cell growth (vs.Vehicle ctrl)
vehicle
AC220 20nM
****
*
*
Ctrl
Lys05(5μM)
non FLT3-ITD AML CD34-
Cell survival (vs. Vehicle ctrl)
Vehicle
AC220 20nM
Ctrl
Lys05(5μM)
non FLT3-ITD AML CD34-
Cell growth (vs.Vehicle ctrl)
vehicle
AC220 20nM
***
*
Ctrl
Lys05(5μM)
D
Cell growth (vs.Vehicle)
Lys05
AC220
Comb
Lys05(μM) 2 4 5 8 10
AC220(nM) 5 10 20 30 40
CI 0.98 0.90 0.76 0.70 0.60
E
Normal CD34+ cell proliferation
Cell proliferation (vs. Vehicle ctrl)
vehicle
AC220
**
*
Ctrl
Lys05
Normal CD34+ cell survival
Cell survival (vs. Vehicle ctrl)
vehicle
AC220
Ctrl
Lys05

FigureS2

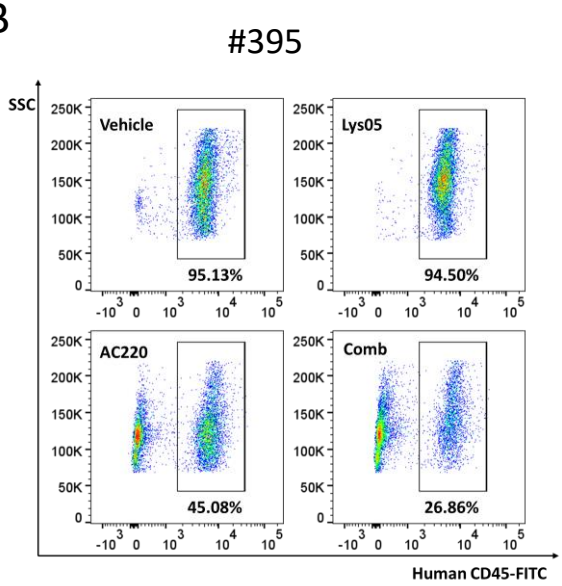
Figure S3. Autophagy inhibition enhances TKI-mediated targeting of primary human FLT3-ITD cells engrafted in immunodeficient mice

A: NSG mice engrafted with FLT3-ITD AML cells were treated with Lys05 or vehicle for 1 week, following which BM cells were obtained and labeled with anti-LC3B-FITC and analyzed by flow cytometry. Flow histogram of LC3B-FITC expression and compiled LC3B-FITC MFI data for human CD45+ cells. B: Representative flow plot of human CD45+ cells engrafted in mouse BM (#395).C: Results of cell cycle analysis of human FLT3-ITD AML CD34+CD38+ cells engrafted in NSG mice analyzed as described for Fig 3C. Significance values: *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001; ns, not significant. Results represent mean + SEM of multiple replicates.

A



B



C

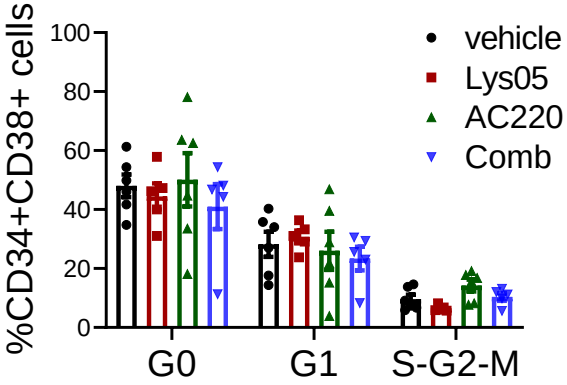


Figure S3

Figure S4. Autophagy inhibition enhances TKI-mediated inhibition of FLT3 ITD AML progenitors and increases AML stem cell cycling in a genetic mouse model

A: BM cells were labeled with anti-LC3B-FITC after incubation with vehicle or Lys05 (10μM) for 16 hours in vitro and LC3B-FITC MFI was analyzed. Results for Lineage+ cells, GMP cells and LSK cells from FLT3-ITD^{ki}/Tet2$^{f/f}$ mice are shown (n=4). B: BM cells were labeled with anti-LC3B-FITC after incubation with vehicle or Lys05 (10uM) for 16h in vitro and LC3B-FITC MFI was analyzed in LSK cells from normal C57BL/6 mice and FLT3-ITD^{ki}/Tet2$^{f/f}$ mice (n=3). C: Accumulation of GFP and RFP puncta in BM cells from C57BL/6-Tg(CAG-RFP/ EGFP/ Map1lc3b) mice (n=3) after after 5 consecutive days treatment of Lys05 (20mg/kg/d, IP) in vivo is shown in representative photomicrographs, and a graph of cumulative data. D: Total WBC and neutrophils (based on differential count) in peripheral blood are shown from the experiment described in Fig 4A. E: Photograph showing spleen size, numbers of GMP, MPP, and ST-HSC in spleens are shown. F: Total number of MPP per 2 femurs and 2 tibiae (4 bones) are shown. G: Wild-type C57BL/6 mice were treated with Lys05 for 2 weeks. Total WBC, and neutrophils in peripheral blood, and numbers of stem and progenitor populations in BM are shown. H: BM cells were labelled with DAPI, and cell cycle analysis of GMP is shown (n=6-7). I: Cell cycle analysis of LSK cells from normal C57BL/6 mice treated with Lys05 or vehicle for 2 weeks(n=6).Significance values: *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001; ns, not significant. Results represent mean + SEM of multiple replicates.

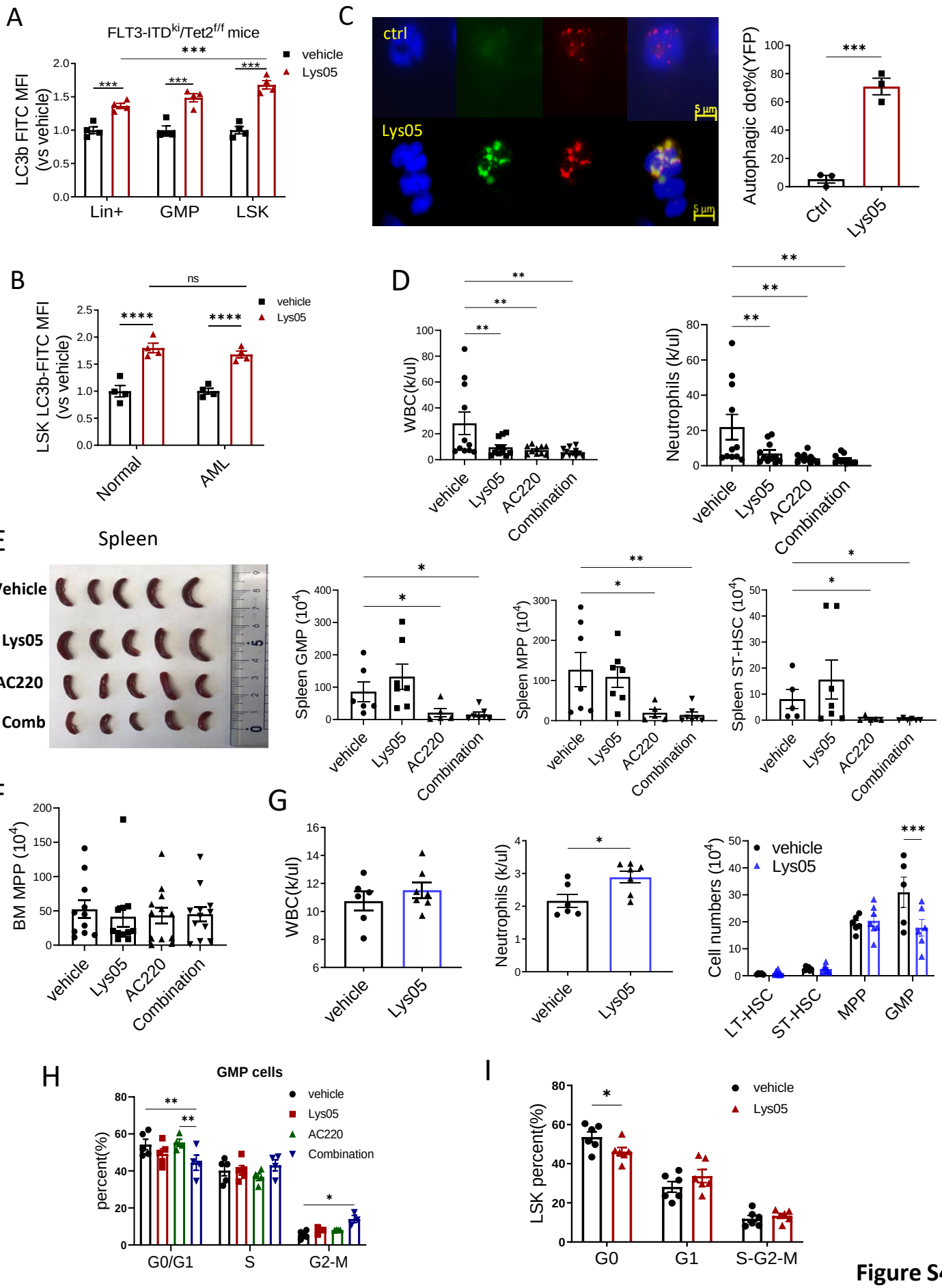

A
FLT3-ITD^{ki}/Tet2$^{f/f}$ mice
LC3b FITC MFI (vs vehicle)
vehicle
Lys05
Lin+
GMP
LSK
B
LSK LC3b-FITC MFI (vs vehicle)
Normal
AML
ns
C
ctrl
Lys05
Autophagic dot%(YFP)
Ctrl
Lys05
D
WBC(k/ul)
Neutrophils (k/ul)
vehicle
Lys05
AC220
Combination
E
Spleen
Vehicle
Lys05
AC220
Comb
Spleen GMP (10^4)
Spleen MPP (10^4)
Spleen ST-HSC (10^4)
F
BM MPP (10^4)
G
WBC(k/ul)
Neutrophils (k/ul)
Cell numbers (10^4)
LT-HSC
ST-HSC
MPP
GMP
H
GMP cells
percent(%)
G0/G1
S
G2-M
I
LSK percent(%)
G0
G1
S-G2-M

Figure S4

Figure S5. Autophagy inhibition reduces repopulating capacity of FLT3-ITD AML stem cells in a genetic mouse model

A: Total donor chimerism from peripheral blood were monitored every 4 weeks. B-D: Results from the experiment described in Fig 5A, number of total donor cells (B), donor myeloid cells, B cells, T cells (C) and MPP (D) per 2 femurs and 2 tibiae (4 bones) are shown (n=5-7). E: LSK cells were transduced with lentivirus vectors as described for Fig 5C. Reduction in ATG5 mRNA levels was confirmed by RT-Q-PCR .F: Total number of GFP+ and GFP+Myeloid cells per 2 femurs and 2 tibiae (4 bones) are shown (n=4-7). Significance values: *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001; ns, not significant. Results represent mean ± SEM of multiple replicates.

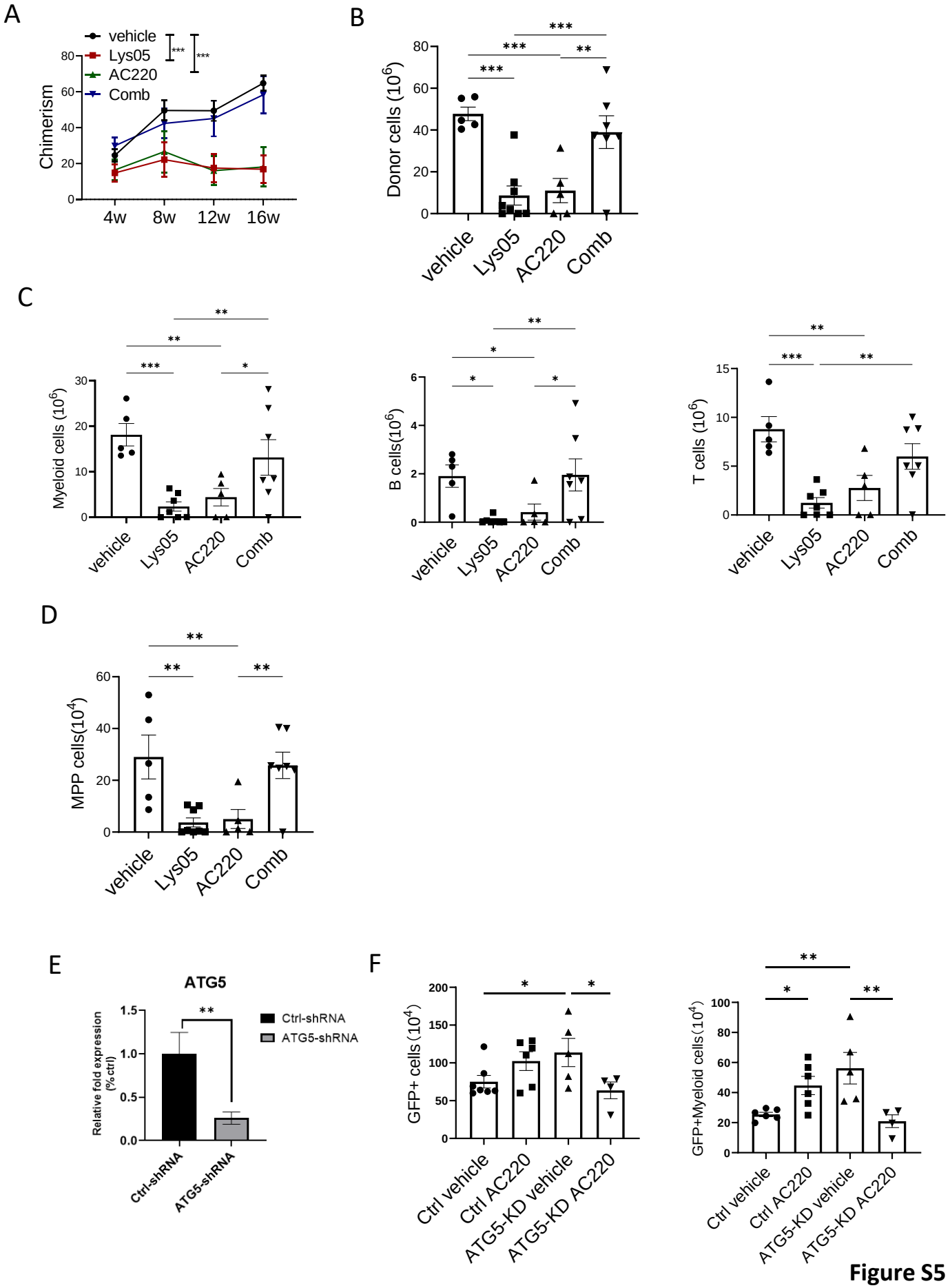

A
vehicle
Lys05
AC220
Comb
Chimerism
4w
8w
12w
16w
B
Donor cells (10^6)
C
Myeloid cells (10^6)
B cells(10^6)
T cells (10^6)
D
MPP cells(10^4)
E
ATG5
Relative fold expression (% ctrl)
Ctrl-shRNA
ATG5-shRNA
F
GFP+ cells (10^4)
GFP+Myeloid cells (10^4)
Ctrl vehicle
Ctrl AC220
ATG5-KD vehicle
ATG5-KD AC220

Figure S5

Figure S6. Enhanced mitochondrial respiration gene signatures in AML LSC following autophagy inhibition

A. Enrichment plot showing significantly increased expression of quiescence related gene sets in LSK compared with GMP cells. B: Enrichment plots showing enrichment of AML stemness and HSC-related gene sets in LSK cells compared with GMP cells. C: Enrichment plots showing enrichment of GMP and Myeloid signature gene sets in GMP compared with LSK cells. D: Enrichment plots showing unchanged quiescence signature in LSK cells treated with AC220 or the combination of AC220 and Lys05, compared to vehicle. E: Representative plots showing upregulation of mitochondrial gene sets, as indicated, in LSK from Lys05 versus vehicle treated mice. F: Enrichment plots showing enrichment of long chain FA biosynthesis, FA β-oxidation, TCA cycle and glutathione metabolism gene signatures in Lys05 treated LSK cells compared with vehicle treated LSK cells. Significance values: *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001; ns, not significant. Results represent mean $\pm$ SEM of multiple replicates.

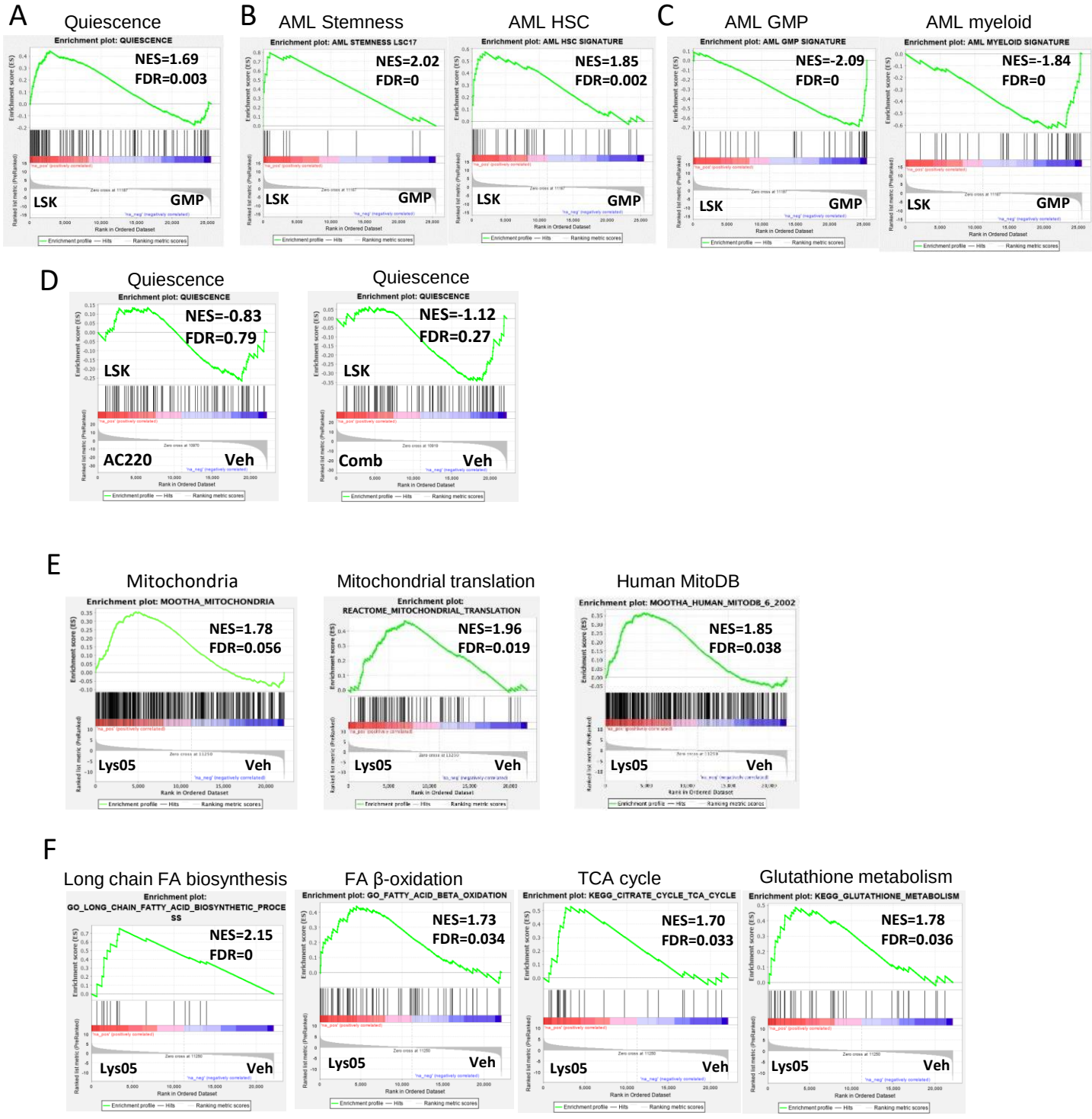

A
Quiescence
Enrichment plot: QUIESCENCE
NES=1.69
FDR=0.003
LSK
GMP
B
AML Stemness
Enrichment plot: AML STEMNESS LSC17
NES=2.02
FDR=0
LSK
GMP
AML HSC
Enrichment plot: AML HSC SIGNATURE
NES=1.85
FDR=0.002
LSK
GMP
C
AML GMP
Enrichment plot: AML GMP SIGNATURE
NES=-2.09
FDR=0
LSK
GMP
AML myeloid
Enrichment plot: AML MYELOID SIGNATURE
NES=-1.84
FDR=0
LSK
GMP
D
Quiescence
Enrichment plot: QUIESCENCE
NES=-0.83
FDR=0.79
LSK
AC220
Veh
Quiescence
Enrichment plot: QUIESCENCE
NES=-1.12
FDR=0.27
LSK
Comb
Veh
E
Mitochondria
Enrichment plot: MOOTHA_MITOCHONDRIA
NES=1.78
FDR=0.056
Lys05
Veh
Mitochondrial translation
Enrichment plot: REACTOME_MITOCHONDRIAL_TRANSLATION
NES=1.96
FDR=0.019
Lys05
Veh
Human MitoDB
Enrichment plot: MOOTHA_HUMAN_MITODB_6_2002
NES=1.85
FDR=0.038
Lys05
Veh
F
Long chain FA biosynthesis
Enrichment plot: GO_LONG_CHAIN_FATTY_ACID_BIOSYNTHETIC_PROCESS
NES=2.15
FDR=0
Lys05
Veh
FA β-oxidation
Enrichment plot: GO_FATTY_ACID_BETA_OXIDATION
NES=1.73
FDR=0.034
Lys05
Veh
TCA cycle
Enrichment plot: KEGG_CITRATE_CYCLE_TCA_CYCLE
NES=1.70
FDR=0.033
Lys05
Veh
Glutathione metabolism
Enrichment plot: KEGG_GLUTATHIONE_METABOLISM
NES=1.78
FDR=0.036
Lys05
Veh

Figure S6

Figure S7. Autophagy inhibition differentially modulates p53 activity in TKI-treated FLT3-ITD AML progenitor and stem cells

A: Western blotting for p53 and LC-3B in Molm13 cells after exposure to Torin (250nM), starvation condition (EBSS medium), or both for 4 hours. B: Western blotting for p53 and LC-3B in Molm13 cells after CQ (60μM) or SAR405 (3μM) treatment as indicated. The upper panel shows results of densitometry quantitation (p53/GAPDH). C: Western blotting of p53 expression in CHX treated Molm13 cells without or with lys05 (5μM) pretreatment for overnight or Torin(250nM). The upper panel shows results of densitometry quantitation (p53/GAPDH). D: Flow histogram for p53 protein staining on AT-low and AT-high cells as described on Fig1A. E: Cell survival (left) and growth (right) of Molm13 cells after 48h treatment of Lys05 (0, 2.5μM, 5μM, 10μM) with/without shRNA-mediated p53 knockdown.F-G: Survival of Molm13 cells with or without shRNA-mediated p53 knockdown, after 48h treatment of Lys05 (5μM), AC220 (3nM) or the combination (F), and Western blotting for p53 and LC-3B in Molm13 cells after 4h treatment of Lys05 (5μM) or/and AC220 (3nM) with/without shRNA-mediated p53 knockdown (G). H: Experimental strategy: LSK cells isolated from FLT3-ITD^{ki} /Mx1-Cre Tet2$^{f/f}$ leukemic BM were transduced with lentivirus vectors expressing P53-shRNA and GFP or ctrl-shRNA and GFP. Engrafted mice were treated with Lys05, AC220 or vehicle for 2 weeks.I: Reduction in P53 mRNA levels in LSK cells was confirmed by RT-Q-PCR. J: Total number of GFP+ cells and GFP+ LSK in BM of recipient mice (2 femurs and 2 tibias) are shown (n=3-5).Significance values: *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001; ns, not significant. Results represent mean ± SEM of multiple replicates.

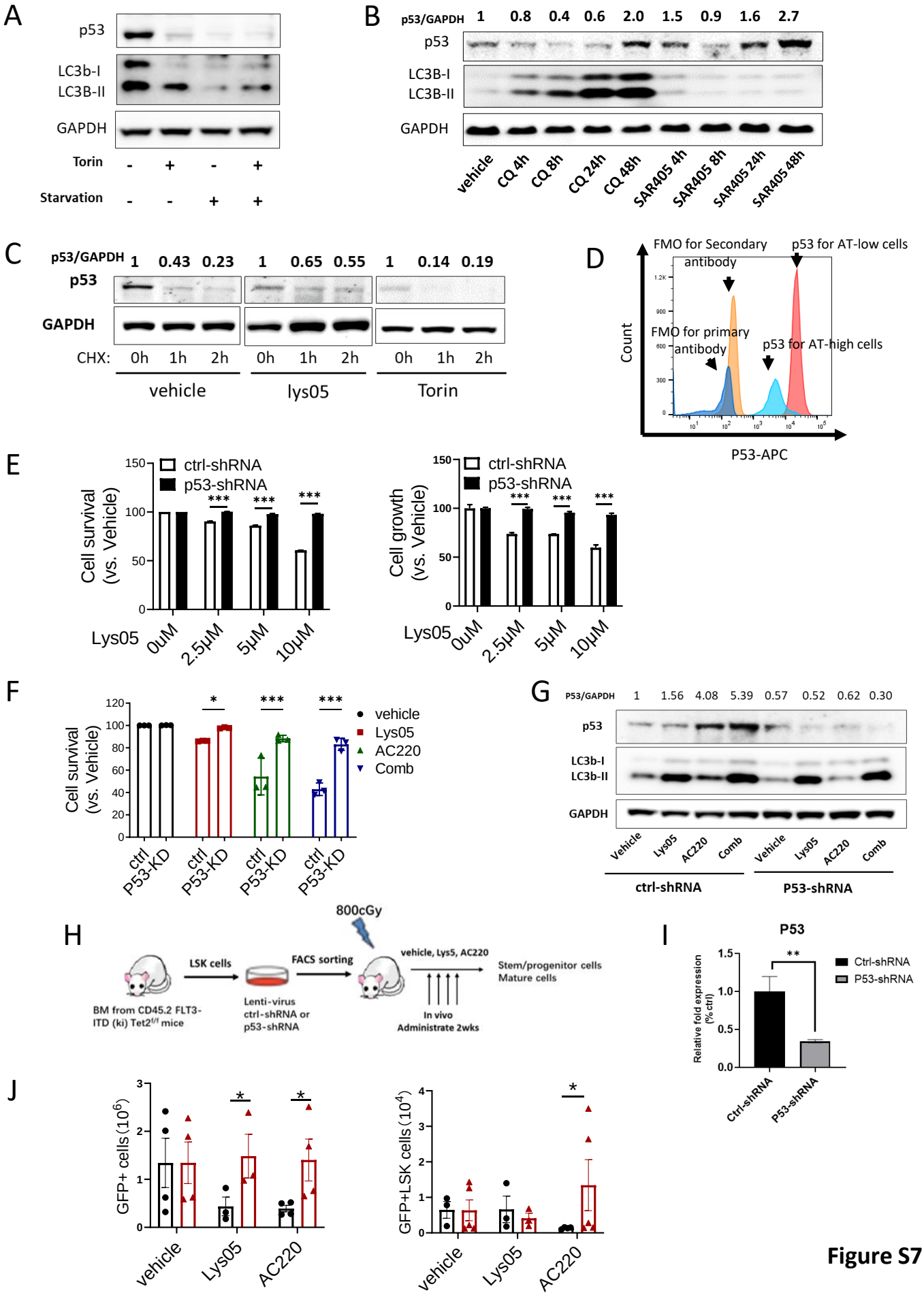

A
p53
LC3b-I
LC3B-II
GAPDH
Torin - + - +
Starvation - - + +
B
p53/GAPDH 1 0.8 0.4 0.6 2.0 1.5 0.9 1.6 2.7
p53
LC3B-I
LC3B-II
GAPDH
vehicle
CQ 4h
CQ 8h
CQ 24h
CQ 48h
SAR405 4h
SAR405 8h
SAR405 24h
SAR405 48h
C
p53/GAPDH 1 0.43 0.23 1 0.65 0.55 1 0.14 0.19
p53
GAPDH
CHX: 0h 1h 2h 0h 1h 2h 0h 1h 2h
vehicle
lys05
Torin
D
FMO for Secondary antibody
p53 for AT-low cells
FMO for primary antibody
p53 for AT-high cells
Count
P53-APC
E
ctrl-shRNA
p53-shRNA
Cell survival (vs. Vehicle)
Cell growth (vs. Vehicle)
***
Lys05 0uM 2.5μM 5μM 10μM
F
Cell survival (vs. Vehicle)
vehicle
Lys05
AC220
Comb
ctrl P53-KD
G
P53/GAPDH 1 1.56 4.08 5.39 0.57 0.52 0.62 0.30
p53
LC3b-I
LC3b-II
GAPDH
Vehicle Lys05 AC220 Comb Vehicle Lys05 AC220 Comb
ctrl-shRNA
P53-shRNA
H
800cGy
LSK cells
FACS sorting
vehicle, Lys5, AC220
Stem/progenitor cells
Mature cells
BM from CD45.2 FLT3-ITD (ki) Tet2 f/f mice
Lenti-virus ctrl-shRNA or p53-shRNA
In vivo Administrate 2wks
I
P53
Relative fold expression (% ctrl)
Ctrl-shRNA
P53-shRNA
J
GFP+ cells (10^6)
GFP+LSK cells (10^4)
vehicle
Lys05
AC220

Figure S7

Supplemental references

1. Li L, Osdal T, Ho Y, et al. SIRT1 activation by a c-MYC oncogenic network promotes the maintenance and drug resistance of human FLT3-ITD acute myeloid leukemia stem cells. *Cell Stem Cell*. 2014;15(4):431-446.
2. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol*. 2014;15(12):550.