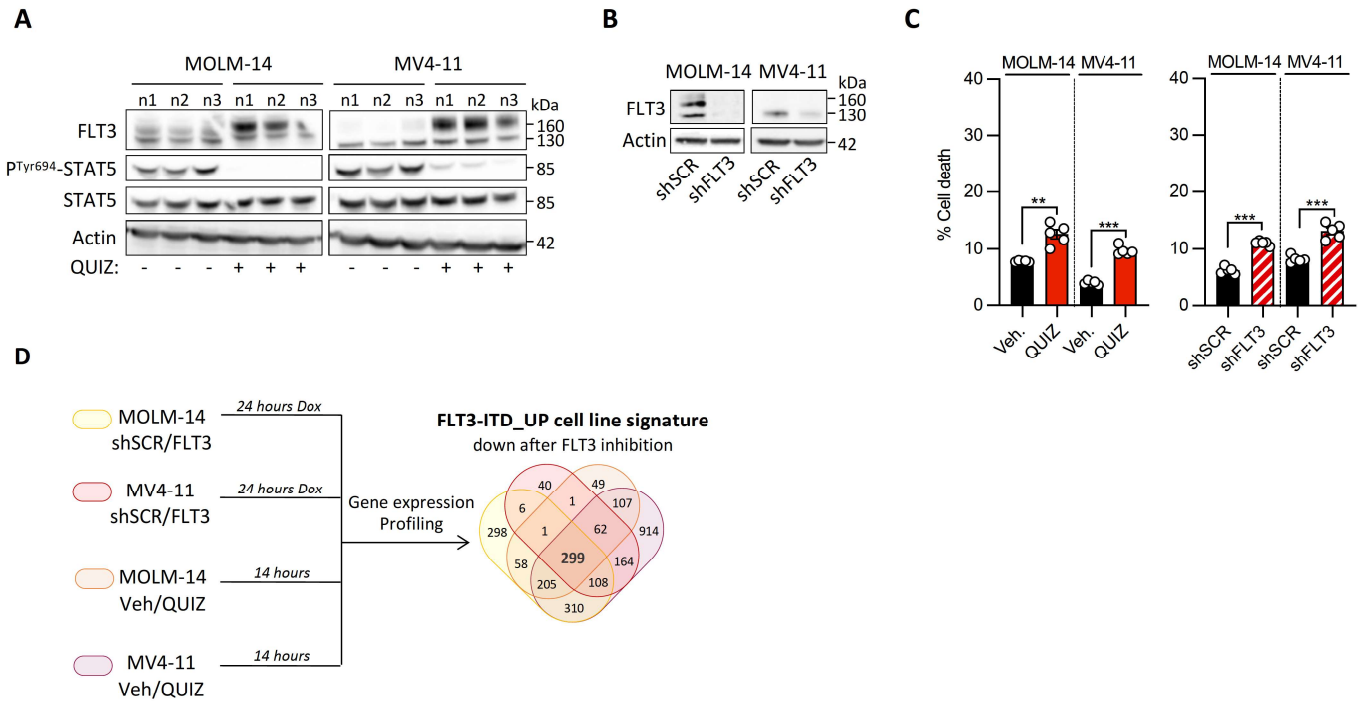

A
MOLM-14
MV4-11
n1 n2 n3 n1 n2 n3 n1 n2 n3 n1 n2 n3
kDa
FLT3
160
130
PTyr694-STAT5
85
STAT5
85
Actin
42
QUIZ: - - - + + + - - - + + +
B
MOLM-14 MV4-11
kDa
FLT3
160
130
Actin
42
shSCR shFLT3 shSCR shFLT3
C
MOLM-14 MV4-11
% Cell death
Veh. QUIZ Veh. QUIZ
shSCR shFLT3 shSCR shFLT3
D
MOLM-14 shSCR/FLT3
24 hours Dox
MV4-11 shSCR/FLT3
24 hours Dox
MOLM-14 Veh/QUIZ
14 hours
MV4-11 Veh/QUIZ
14 hours
Gene expression Profiling
FLT3-ITD_UP cell line signature
down after FLT3 inhibition
40 49 6 1 107 298 1 62 914 58 299 164 205 108 310
E
Hallmark 2020 gene set enriched in FLT3-ITD
E2F Targets
Myc Targets V2
mTORC1 Signaling
G2-M Checkpoint
DNA Repair
Cholesterol Homeostasis
Oxidative Phosphorylation
- log10 (p value)
Odds Ratio
KEGG 2021 gene set enriched in FLT3-ITD
DNA replication
Cell cycle
Arginine and proline metabolism
PPAR signaling pathway
Fatty acid biosynthesis
Biosynthesis of unsaturated fatty acids
Glutathione metabolism
Steroid biosynthesis
Terpenoid backbone biosynthesis
Cysteine and methionine metabolism
Odds Ratio
Lipid
Amino acid
Mitochondria
Redox
Signaling pathway
Cell cycle
DNA/RNA
F
KEGG 2021 gene set enriched in MLL-AF9 FLT3-ITDON (24h)
GSE163932
-log10 (pvalue)
Steroid biosynthesis
Fatty acid biosynthesis
Terpenoid backbone biosynthesis
Biosynthesis of unsaturated fatty acids
Fatty acid elongation
Odds Ratio
Lipid
Amino acid
Redox

Figure S1

Supplementary Figure S1. Lipid metabolism dependency uncovered by transcriptomics in FLT3-mutant AML. **A,** Total protein extracts from MOLM-14 and MV4-11 cells incubated with vehicle or 3nM QUIZ for 14h were immunoblotted with indicated antibodies (n=3). **B,** MOLM-14 and MV4-11 cells were transduced with Dox-inducible CTL or anti-FLT3 shRNAs. Total protein extracts were immunoblotted with indicated antibodies. **C,** Cell viability was measured by PI staining in MOLM-14 and MV4-11 cells after FLT3 inhibition using 3nM QUIZ for 14h (*left panel*) or shFLT3 induction (*right panel*). **D,** Differential gene expression (DGE) was analyzed in MOLM-14 and MV4-11 cells after FLT3 inhibition using QUIZ or shFLT3, compared to vehicle or shCTL conditions, respectively. The FLT3-ITD_UP cell line signature was generated by 299 commonly downregulated genes (FC<0.67; FDR q-value <0.05) after FLT3 inhibition. **E,** Enrichr analyses of Hallmark 2020 (*left panel*) and KEGG 2021 (*right panel*) gene signatures enriched in the FLT3-ITD_UP cell line signature (p-value<0.05). **F,** Enrichr analyses of KEGG 2021 gene signatures enriched in upregulated genes in FLT3-ITD^{ON} compared to FLT3-ITD^{OFF} MLL-AF9 murine AML cells from the GSE163932 dataset (p-value<0.05).

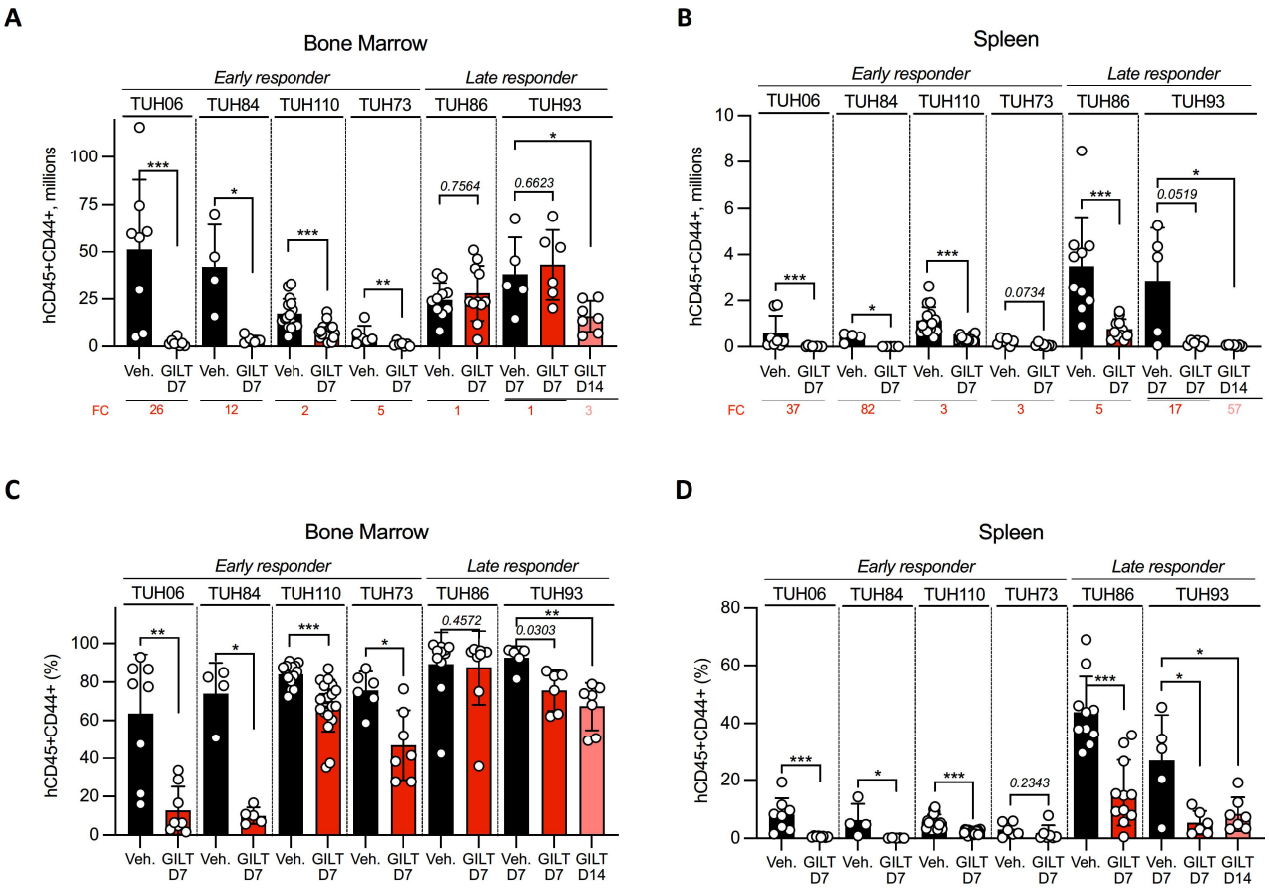

A
Bone Marrow
Early responder
Late responder
TUH06
TUH84
TUH110
TUH73
TUH86
TUH93
hCD45+CD44+, millions
Veh.
GILT D7
GILT D14
FC
26
12
2
5
1
1
3
B
Spleen
37
82
3
3
5
17
57
C
Bone Marrow
hCD45+CD44+ (%)
D
Spleen

Figure S2

Supplementary Figure S2. Anti-leukemic activity of GILT across a panel of AML PDX *ex vivo* and *in vivo*. **A-B,** Number of viable $CD33^{+}CD45^{+}$ human AML cells in bone marrow **(A)** and spleen **(B)** of six AML PDXs in vehicle and GILT groups. Fold change (FC) between the mean number of leukemic cells in vehicle and GILT groups are provided. **C-D,** Percentage of viable $CD33^{+}CD45^{+}$ human AML cells in bone marrow **(C)** and spleen **(D)** of six AML PDXs in vehicle and GILT groups. Vertical bars indicate standard deviations. *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$.

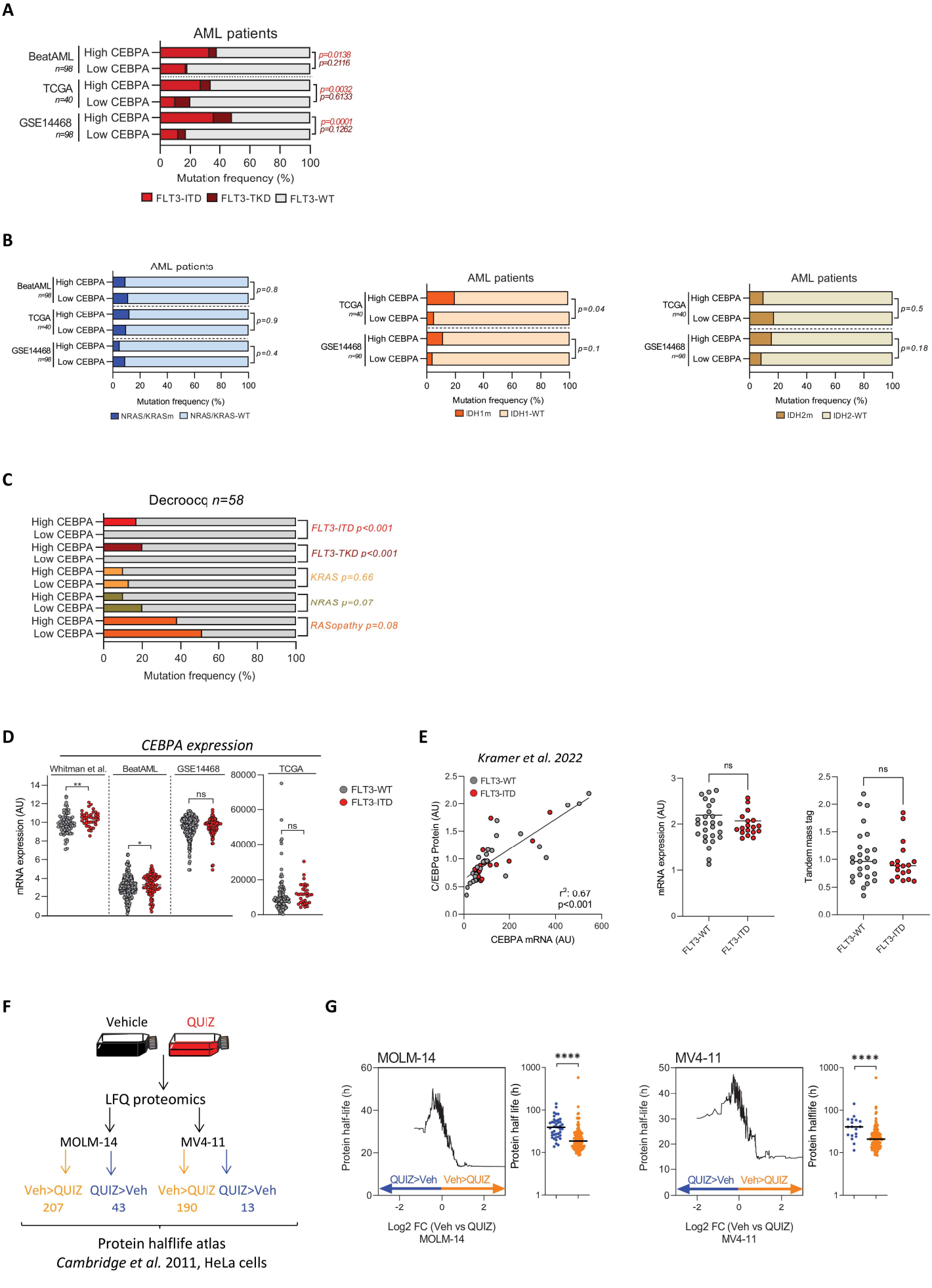

A
AML patients
BeatAML n=98
TCGA n=40
GSE14468 n=98
High CEBPA
Low CEBPA
p=0.0138
p=0.2116
p=0.0032
p=0.6133
p=0.0001
p=0.1262
Mutation frequency (%)
FLT3-ITD
FLT3-TKD
FLT3-WT
B
p=0.8
p=0.9
p=0.4
NRAS/KRASm
NRAS/KRAS-WT
p=0.04
p=0.1
IDH1m
IDH1-WT
p=0.5
p=0.18
IDH2m
IDH2-WT
C
Decrooca n=58
FLT3-ITD p<0.001
FLT3-TKD p<0.001
KRAS p=0.66
NRAS p=0.07
RASopathy p=0.08
D
CEBPA expression
Whitman et al.
BeatAML
GSE14468
TCGA
mRNA expression (AU)
FLT3-WT
FLT3-ITD
E
Kramer et al. 2022
C/EBPα Protein (AU)
CEBPA mRNA (AU)
r²: 0.67
p<0.001
miRNA expression (AU)
Tandem mass tag
ns
F
Vehicle
QUIZ
LFQ proteomics
MOLM-14
MV4-11
Veh>QUIZ
QUIZ>Veh
207
43
190
13
Protein halflife atlas
Cambridge et al. 2011, HeLa cells
G
MOLM-14
MV4-11
Protein half-life (h)
Protein half life (h)
Protein halflife (h)
QUIZ>Veh
Veh>QUIZ
****
Log2 FC (Veh vs QUIZ)
MOLM-14
MV4-11

Figure S3

Supplementary Figure S3. Post-translational regulation of C/EBPα expression. **A-B,** Publicly available datasets from three independent cohorts of AML patients (BeatAML, TCGA and GSE14468) were analyzed dependent on *CEBPA* low (lower quartile) *versus CEBPA* high (higher quartile) expression and frequencies of *FLT3* mutation (ITD in red; TKD in dark red, **A**), NRAS/KRAS and IDH1/2 (**B**). **C,** Repartition of *FLT3*, *NRAS*, *KRAS* and RASopathy mutations dependent on CEBPA expression in 58 AML patients from Decroocq RNAseq dataset published in (27). RASopathy referred to as *NRAS*, *KRAS*, *PTPN11* and *CBL* mutations, and *NF1* mutations and/or deletions. **D,** CEBPA mRNA expression in samples from patients with FLT3 wild type (WT) or FLT3-ITD AML in four independent cohorts: TCGA (28), BeatAML (29), GSE14468 (30) and Whitman (31). **E,** Data from Kramer et al. (7) on 44 patients with AML including 18 FLT3-ITD cases were analyzed for CEBPA mRNA and protein (using tandem mass tag quantification) expression dependent on FLT3 mutational status. Correlation between CEBPA mRNA and protein expression. Comparison of CEBPA mRNA *(left panel)* and protein *(right panel)* expression in FLT3 WT *versus* FLT3 ITD patients with AML. **F,** The half-life of proteins has been annotated from previous dataset (32). **G**, Curve represent a median half-life value calculated for each FC rank with a sliding window of size 100. Box-plot represent half-life of proteins significantly downregulated ((blue) with FC<0.77, FDR and q-value<0.05) and upregulated in Veh *versus* QUIZ conditions (FC>1.3, FDR and q-value<0.05).

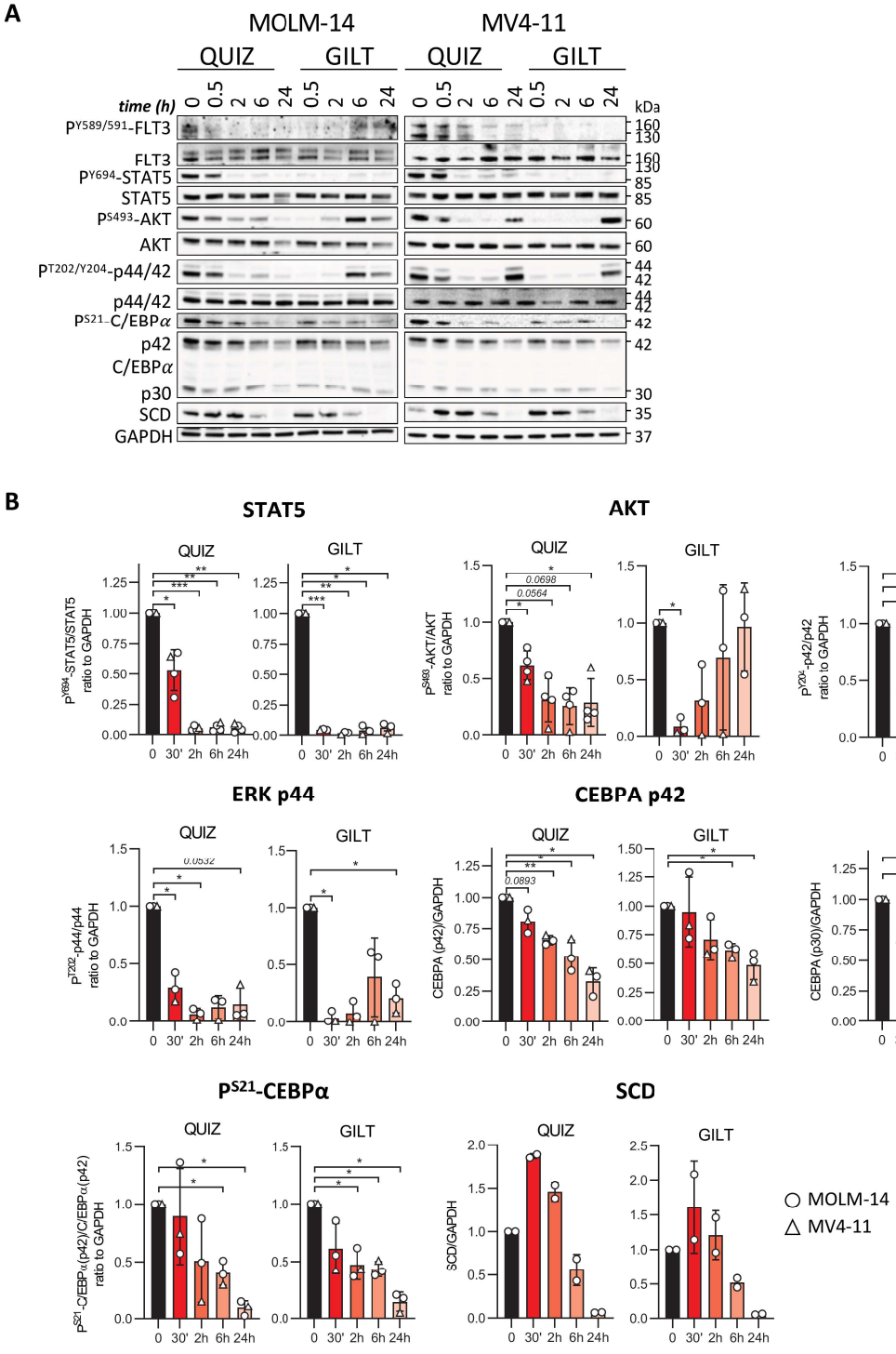

A
MOLM-14
MV4-11
QUIZ
GILT
time (h)
kDa
PY589/591-FLT3
FLT3
PY694-STAT5
STAT5
PS493-AKT
AKT
PT202/Y204-p44/42
p44/42
PS21-C/EBPα
p42
C/EBPα
p30
SCD
GAPDH
B
STAT5
AKT
ERK p42
ERK p44
CEBPA p42
CEBPA p30
PS21-CEBPα
SCD
PY694-STAT5/STAT5 ratio to GAPDH
PS493-AKT/AKT ratio to GAPDH
PY204-p42/p42 ratio to GAPDH
PT202-p44/p44 ratio to GAPDH
CEBPA (p42)/GAPDH
CEBPA (p30)/GAPDH
PS21-C/EBPα(p42)/C/EBPα(p42) ratio to GAPDH
SCD/GAPDH
0 30' 2h 6h 24h
MOLM-14
MV4-11

Figure S4

Supplementary Figure S4. Inhibition of C/EBPα phosphorylation and protein expression by FLT3i. **A-B,** MOLM-14 and MV4-11 cells were incubated with vehicle or 3nM QUIZ or 30nM GILT in time-course experiments as indicated. Western blots were done with the indicated antibodies **(A)** and densitometry analysis are provided **(B)**. Vertical bars indicate standard deviations. *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$.

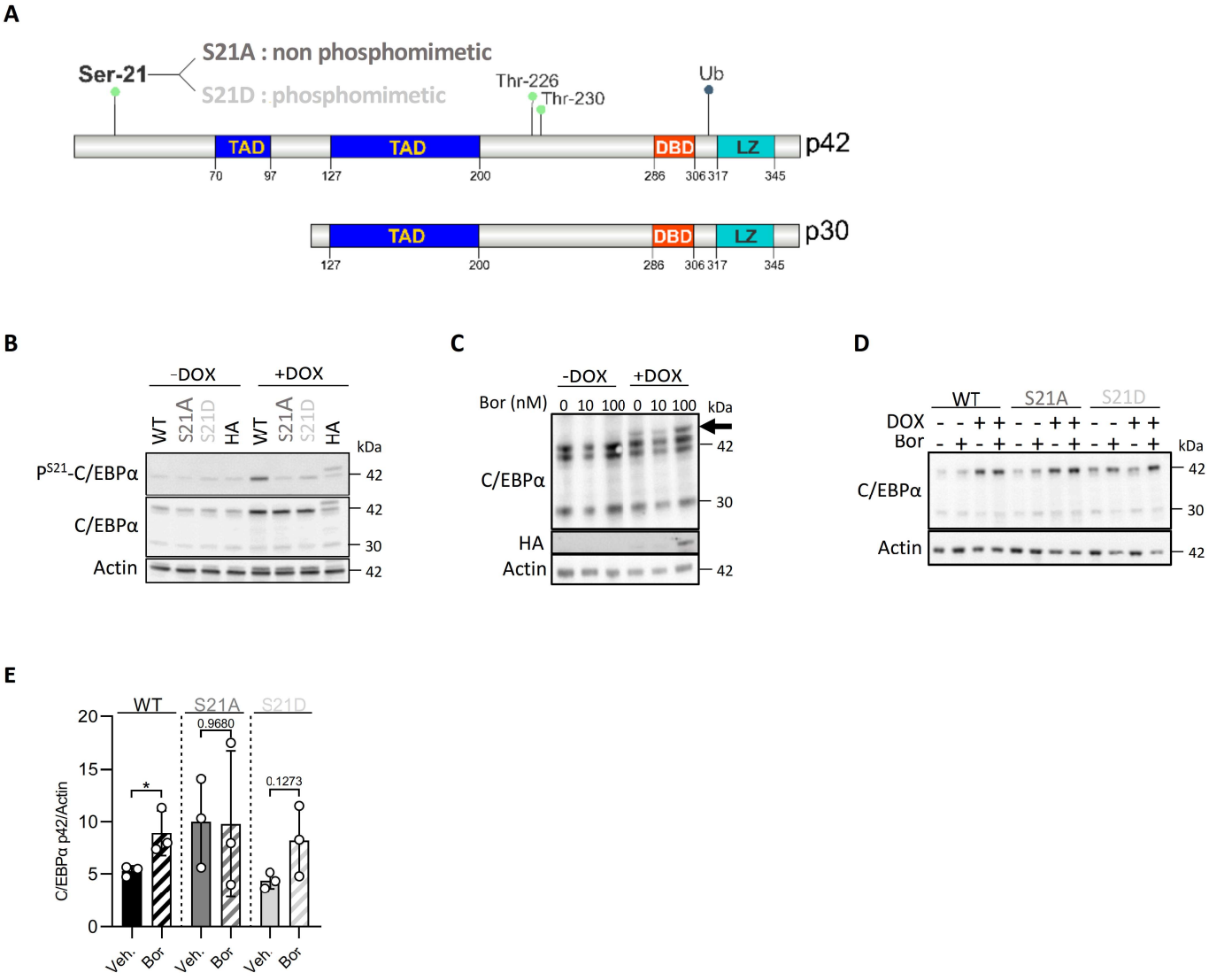
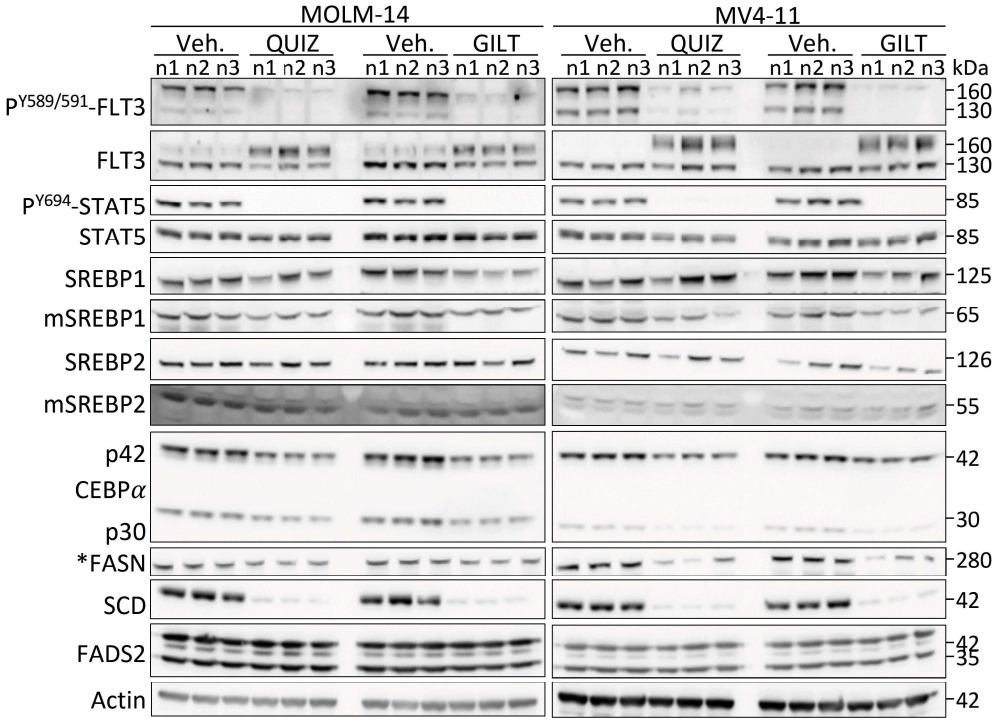

A
Ser-21
S21A : non phosphomimetic
S21D : phosphomimetic
Thr-226
Thr-230
Ub
TAD
TAD
DBD
LZ
p42
70
97
127
200
286
306
317
345
TAD
DBD
LZ
p30
127
200
286
306
317
345
B
-DOX
+DOX
WT
S21A
S21D
HA
WT
S21A
S21D
HA
kDa
P^S21-C/EBPα
C/EBPα
Actin
42
42
30
42
C
-DOX
+DOX
Bor (nM)
0 10 100 0 10 100
kDa
C/EBPα
HA
Actin
42
30
42
D
WT
S21A
S21D
DOX
Bor
kDa
C/EBPα
Actin
42
30
42
E
WT
S21A
S21D
20
15
10
5
0
C/EBPα p42/Actin
*
0.9680
0.1273
Veh.
Bor
Veh.
Bor
Veh.
Bor

Figure S5

Supplementary Figure S5. Ser-21 phosphorylation and post-translational regulation of C/EBPα expression. **A,** Schematic representation of C/EBPα p42 and p30 isoforms. Main domains including transcription activation domain (TAD), DNA binding domain (DBD) and leucine zipper domain (LZ) are shown, alongside with relevant post-translational modification sites. Amino acid substitutions at position 21 are shown: S21A (Serine to Alanine; non-phosphomimetic modification) and S21D (Serine to Aspartic acid; phosphomimetic change). **B-E,** MOLM-14 cells were transduced with dox-inducible expression vectors for wild type (HA-tagged or non-tagged), S21A or S21D CEBPA forms. **B,** Western blot after induction by dox using phospho-Ser-21 C/EBPα, C/EBPα and actin antibodies. **C,** CEBPA-HA cells were cultured without or with dox for 72h, and then incubated with 10nM or 100nM bortezomib (a proteasome inhibitor) for 4h. Western blot were done with C/EBPα, HA and actin antibodies. The black arrow indicates the electrophoretic migration of HA-tagged C/EBPα. **D-E,** Western blots were done on extracts from MOLM-14 CEBPA WT, S21A or S21D cells with anti-C/EBPα and anti-actin antibodies. **D,** Representative Western blot. **E,** Quantification of Western blots with a ratio between C/EBPα and actin signals (n=3). Vertical bars indicate standard deviations. ns: not significant, *p<0.05, **p<0.01.

A


MOLM-14
MV4-11
Veh.
QUIZ
Veh.
GILT
n1 n2 n3
kDa
P^Y589/591-FLT3
FLT3
P^Y694-STAT5
STAT5
SREBP1
mSREBP1
SREBP2
mSREBP2
p42
CEBPα
p30
*FASN
SCD
FADS2
Actin
160
130
85
125
65
126
55
42
30
280
35

B

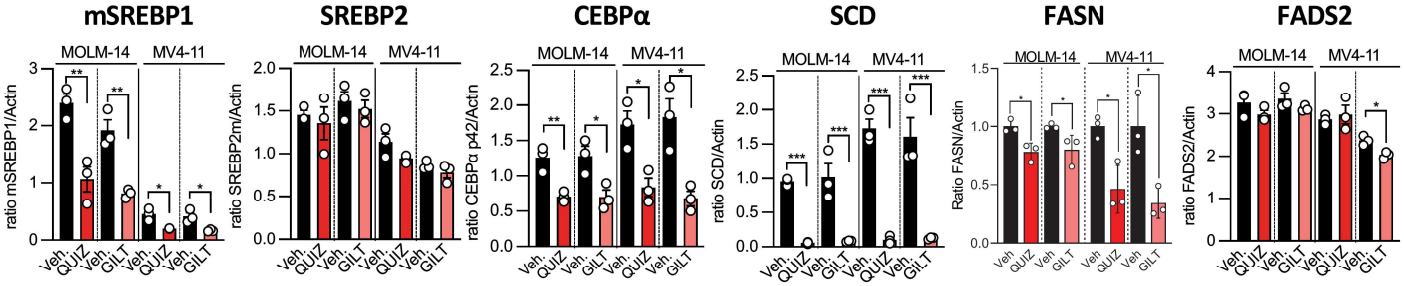

mSREBP1
SREBP2
CEBPα
SCD
FASN
FADS2
MOLM-14
MV4-11
ratio mSREBP1/Actn
ratio SREBP2m/Actn
ratio CEBPα p42/Actn
ratio SCD/Actin
Ratio FASN/Actin
ratio FADS2/Actin
Veh.
QUIZ
GILT

Figure S6

Supplementary Figure S6. FLT3-ITD regulates the expression of C/EBPα and of proteins related to lipid biosynthesis in AML cell lines. **A-B,** MOLM-14 and MV4-11 cells were incubated with 3nM QUIZ or 30nM GILT for 14h. Total protein extracts were submitted to Western blots with indicated antibodies **(A)**. Densitometry analyses are provided (n=3) **(B)**. Vertical bars indicate standard deviations. *p<0.05, **p<0.01, ***p<0.001. The *FASN symbol indicate that Western blots for FASN expression were done on different replicates (n=3) than other Western blots in this panel. FASN densitometry analysis were done relative to actin. Western blots done on these new protein extracts.

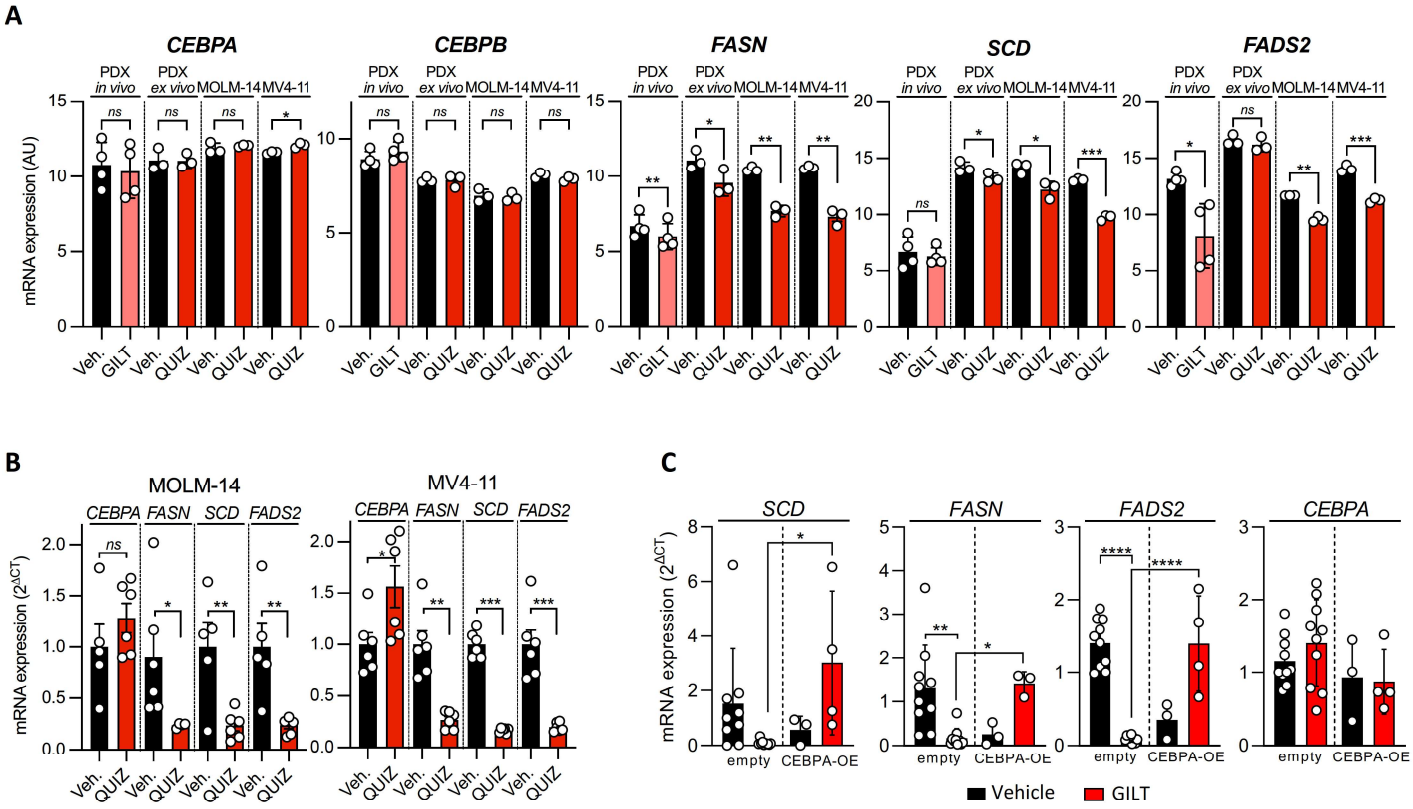

A
CEBPA
CEBPB
FASN
SCD
FADS2
PDX in vivo
PDX ex vivo
MOLM-14
MV4-11
mRNA expression (AU)
Veh.
GILT
QUIZ
B
MOLM-14
MV4-11
CEBPA
FASN
SCD
FADS2
mRNA expression (2^ΔCT)
C
SCD
FASN
FADS2
CEBPA
empty
CEBPA-OE
Vehicle
GILT
D
SCD
FASN
FADS2
CEBPA
WT
S21A
S21D

Figure S7

Supplementary Figure S7. FLT3-ITD regulates the expression of genes related to lipid biosynthesis in AML cell lines. **A,** Gene expression of *CEBPA, CEBPB, FASN, SCD* and *FADS2* upon FLT3i in *in vivo*-treated PDX, *ex* vivo-treated PDX and AML cell lines (MOLM-14 and MV4-11) from transcriptomic analysis. **B,** MOLM-14 and MV4-11 cells were incubated with 3nM QUIZ or 30nM GILT for 14h. Gene expression of *CEBPA*, *FASN*, *SCD* and *FADS2* upon QUIZ was investigated by quantitative RT-PCR. **C,** MOLM-14 cells transduced with empty vector (CTL) or CEBPA expression vector (CEBPA-OE) were incubated with vehicle or 30nM GILT for 14h, and mRNA expression of *SCD*, *FASN*, *FADS2* and *CEBPA* was investigated by quantitative RT-PCR. **D,** MOLM-14 cells transduced with dox-inducible WT, S21A or S21D CEBPA vectors were incubated with dox for 72h. *SCD*, *FASN*, *FADS2* and *CEBPA* mRNA expression was measured by quantitative RT-PCR. Vertical bars indicate standard deviations. *p<0.05, **p<0.01, ***p<0.001.

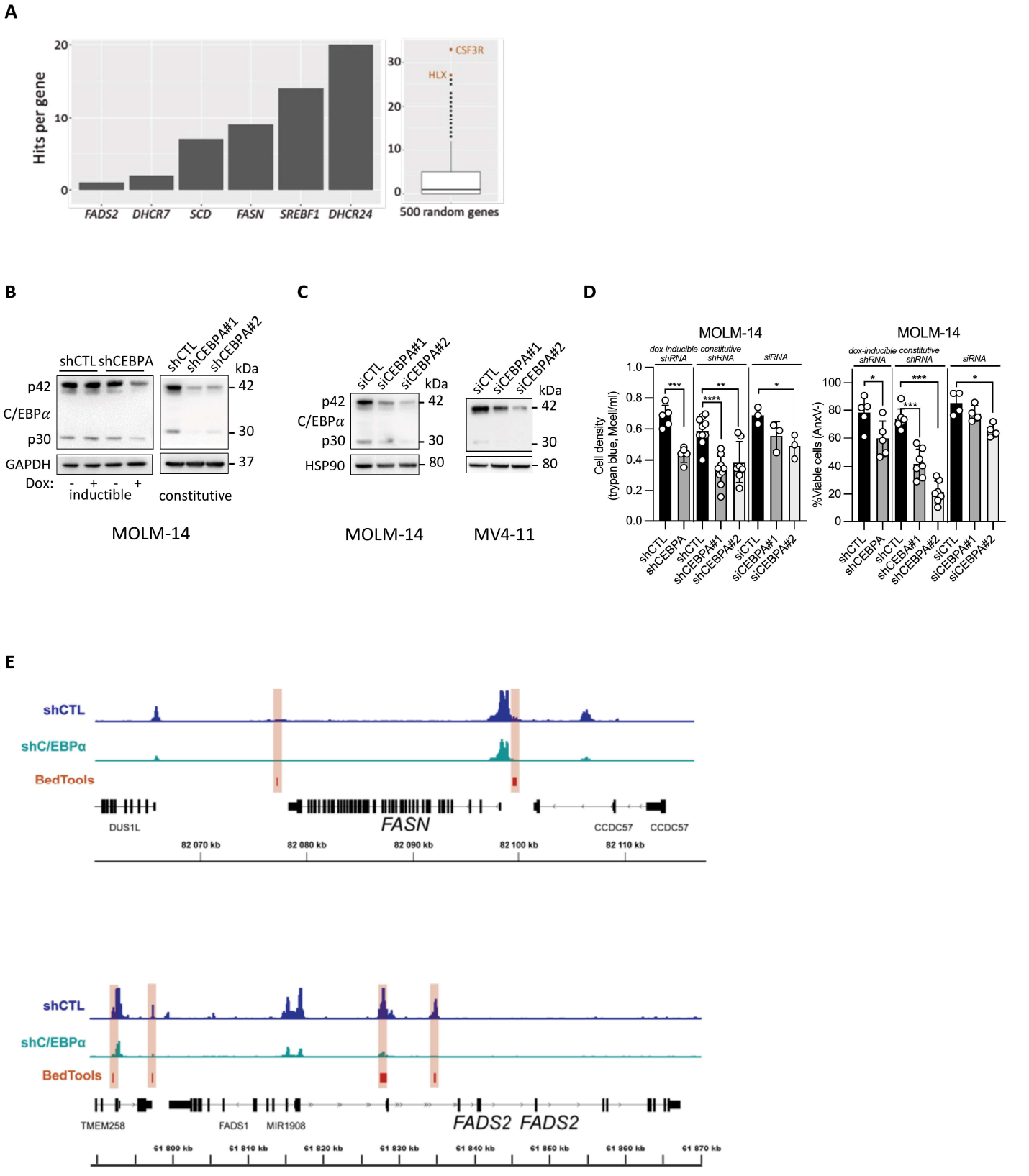

A
Hits per gene
FADS2
DHCR7
SCD
FASN
SREBF1
DHCR24
CSF3R
HLX
500 random genes
B
shCTL
shCEBPA
shCEBPA#1
shCEBPA#2
kDa
p42
C/EBPα
p30
GAPDH
Dox:
inductible
constitutive
MOLM-14
C
siCTL
siCEBPA#1
siCEBPA#2
HSP90
MOLM-14
MV4-11
D
MOLM-14
dox-inducible shRNA
constitutive shRNA
siRNA
Cell density (trypan blue, Mcell/ml)
%Viable cells (AnxV-)
shCTL
shCEBPA
shCEBA#1
shCEBPA#2
siCTL
siCEBPA#1
siCEBPA#2
E
shCTL
shC/EBPα
BedTools
DUS1L
FASN
CCDC57
82 070 kb
82 080 kb
82 090 kb
82 100 kb
82 110 kb
TMEM258
FADS1
MIR1908
FADS2
61 800 kb
61 810 kb
61 820 kb
61 830 kb
61 840 kb
61 850 kb
61 860 kb
61 870 kb

Figure S8

Supplementary Figure S8. C/EBPα directly regulates the transcription of lipid biosynthesis genes in AML. **A,** Analysis of public C/EBPα chromatin immunoprecipitation sequencing (ChIPseq) databases including CEBPA_AML, human, AML, CEBPA (published in (33), data on MV4-11, MOLM-13, U937, SKH1, Kasumi, U937 and THP1 AML cell lines), GSE102697 (published in (34), data on SKH1 and Kasumi-1 myeloid cell lines) GSM2345026 (not published, involving MV4-11) using UniBind to map direct interactions between C/EBPα and DNA (35). C/EBPα binding on the promoter region of *SCD*, *FASN*, *FADS2*, *SREBF1*, *DHCR7* and *DHCR24*, and on the promoter region of 500 randomly chosen genes was measured as number of hits per gene. Known C/EBPα targets CSF3R and HLX are highlighted. **B-C,** MOLM-14 cells were transduced with dox-inducible control (CTL) or anti-*CEBPA* shRNAs and cultured with dox for 72h or were transfected with CTL or anti-*CEBPA* siRNA for 24h. Total protein extracts were immunoblotted with indicated antibodies. **D,** Cell viability assessed by trypan blue exclusion assay (*left panel*) and by annexin V binding assays (*right panel*). **E,** Track view of the *FASN (upper part) and FADS2 (lower part)* locus showing ATAC-seq results for shCEBPα (green track) and shCTL (black track) MOLM14 AML cells, with regions of differential expression (BedTools intersect) depicted in red.

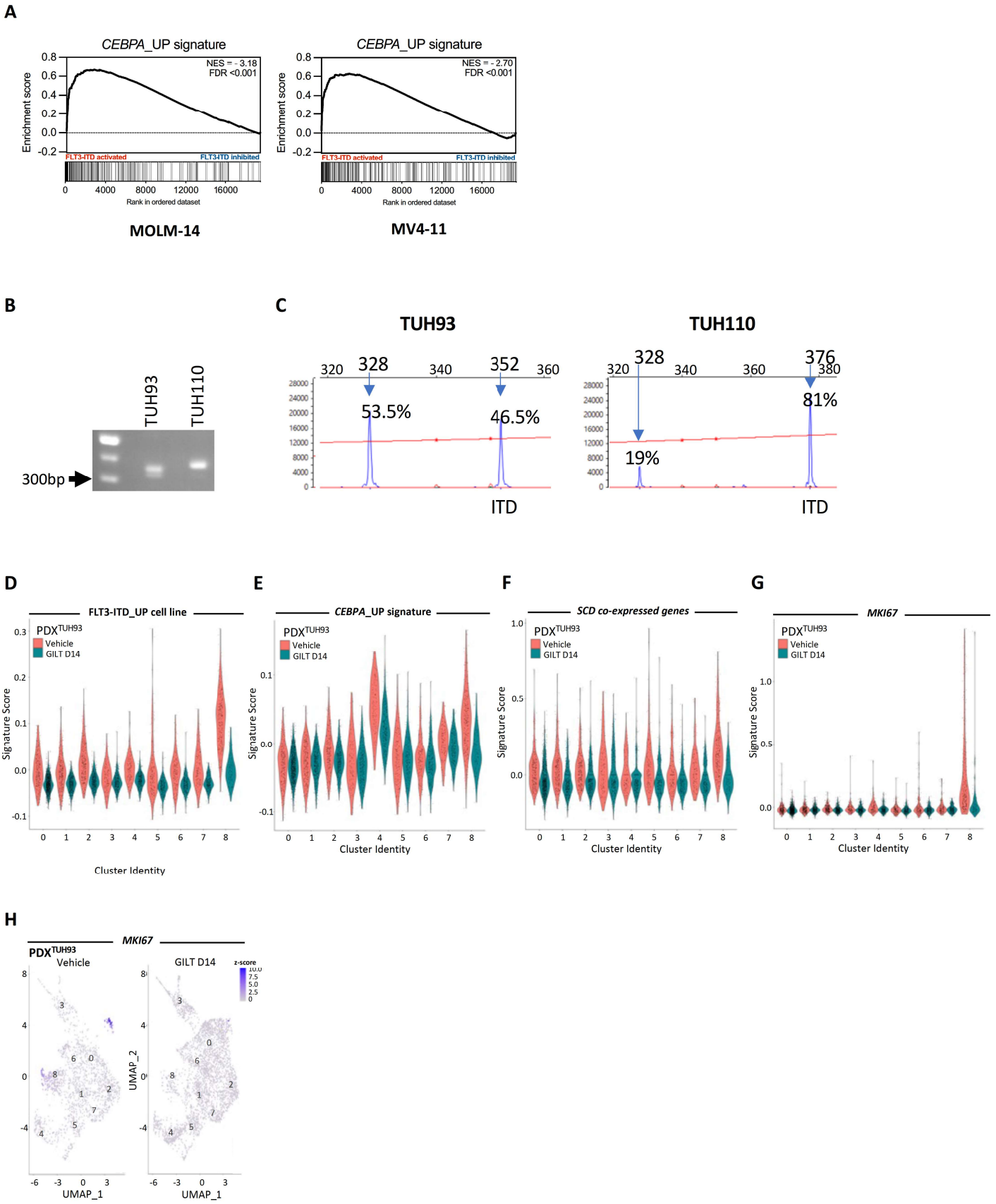

A
CEBPA_UP signature
NES = - 3.18
FDR <0.001
Enrichment score
FLT3-ITD activated
FLT3ITD inhibited
Rank in ordered dataset
MOLM-14
CEBPA_UP signature
NES = - 2.70
FDR <0.001
Enrichment score
FLT3-ITD activated
FLT3-ITD inhibited
Rank in ordered dataset
MV4-11
B
TUH93
TUH110
300bp
C
TUH93
320
328
340
352
360
53.5%
46.5%
ITD
TUH110
320
328
340
360
376
380
81%
19%
ITD
D
FLT3-ITD_UP cell line
PDXTUH93
Vehicle
GILT D14
Signature Score
Cluster Identity
E
CEBPA_UP signature
PDXTUH93
Vehicle
GILT D14
Signature Score
Cluster Identity
F
SCD co-expressed genes
PDXTUH93
Vehicle
GILT D14
Signature Score
Cluster Identity
G
MKI67
PDXTUH93
Vehicle
GILT D14
Signature Score
Cluster Identity
H
MKI67
PDXTUH93
Vehicle
GILT D14
z-score
UMAP_1
UMAP_2

Figure S9

Supplementary Figure S9. Correlation between *CEBPA* mRNA expression and *FLT3*-ITD mutations. **A,** GSEA of *CEBPA*_UP signature in MOLM-14 (*top panel*) and MV4-11 (*bottom panel*) cells incubated with vehicle (FLT3-ITD activated) or 3nM QUIZ (FLT3-ITD inhibited) for 14h. **B-C,** Variant allele frequency of the FLT3-ITD mutation in PDX AML samples. Bulk DNA samples from PDX^{TUH93} and PDX^{TUH110} were extracted from purified human leukemic cells. Then the region surrounding the ITD was amplified by PCR using the following primers: 5' 6FAM-GCAATTTAGGTATGAAAGCCAGC and 3' CTTTCAGCATTTTGACGGCAACC. **B,** Migration on a 2% agarose gel of the PCR products. **C,** Fragment analysis of the PCR products. DNA size ladders are provided in the upper X-axis (in bp) and intensity is provided in Y-axis. The FLT3-mutation is found at +24 and +48bp in TUH93 and TUH110, respectively and the VAF of each allele is indicated in %. **D-G,** Violin plot showing the enrichment of FLT3-ITD_UP cell line signature **(D)**, CEBPA_UP signature **(E)**, *SCD* co-expressed genes **(F)** and *MKI67* gene **(G)** by cluster in vehicle- compared to GILT-treated PDX^{TUH93}. **H,** Visualization on the UMAP plot of *MKI67* gene enrichment in vehicle- compared to GILT-treated PDX^{TUH93}.

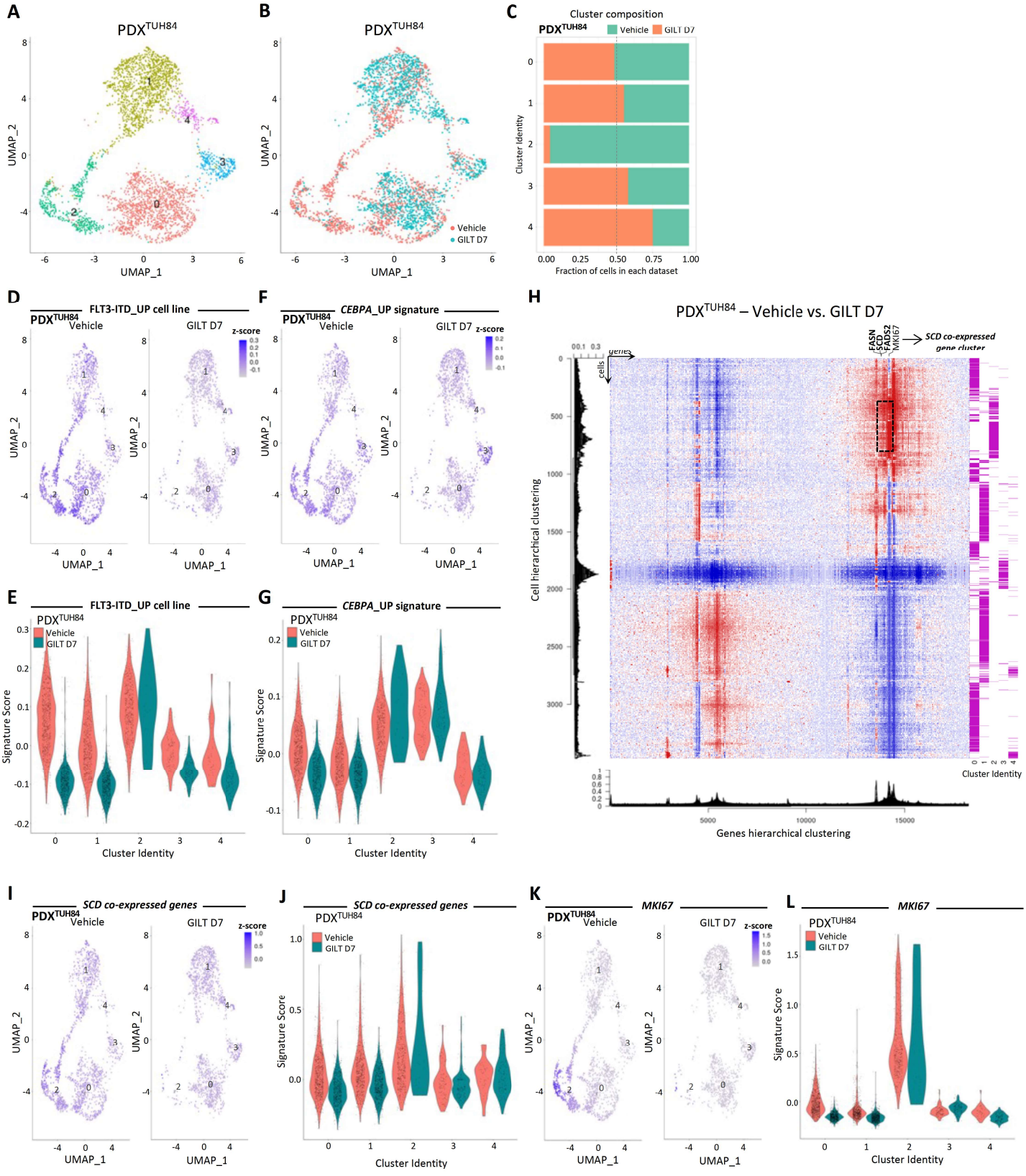

A
PDX^TUH84
UMAP_2
UMAP_1
B
PDX^TUH84
Vehicle
GILT D7
C
Cluster composition
PDX^TUH84
Vehicle
GILT D7
Cluster Identity
Fraction of cells in each dataset
D
FLT3-ITD_UP cell line
PDX^TUH84
Vehicle
GILT D7
z-score
F
CEBPA_UP signature
E
FLT3-ITD_UP cell line
Signature Score
Cluster Identity
G
CEBPA_UP signature
H
PDX^TUH84 – Vehicle vs. GILT D7
FASN
SCD
FADS2
MKI67
SCD co-expressed gene cluster
cells
genes
Cell hierarchical clustering
Cluster Identity
Genes hierarchical clustering
I
SCD co-expressed genes
J
SCD co-expressed genes
K
MKI67
L
MKI67

Figure S10

Supplementary Figure S10. scRNA-seq analysis from PDX^{TUH84}. **A,** Uniform manifold approximation and projection (UMAP) plot of 3623 cells from PDX^{TUH84} using Seurat. Colors indicate k-means clusters (k=8). **B,** UMAP plot colored by treatment condition (vehicle: n=1972 cells; GILT D7: n=1651 cells). **C,** Proportion of each condition per cluster from PDX^{TUH84}**. D,** Visualization on the UMAP plot of FLT3-ITD_UP cell line signature enrichment in vehicle- compared to GILT-treated PDX^{TUH84}. **E,** Violin plot showing the enrichment of FLT3-ITD_UP cell line signature by cluster in vehicle- compared to GILT-treated PDX^{TUH84}. **F,** Visualization on the UMAP plot of *CEBPA*_UP signature enrichment in vehicle- compared to GILT-treated PDX^{TUH84}. **G,** Violin plot showing the enrichment of *CEBPA*_UP signature by cluster in vehicle- compared to GILT-treated PDX^{TUH84}. **H,** Heatmap showing the expression of each gene per cell in vehicle- compared to GILT-treated PDX^{TUH84}. Dendrograms represents the hierarchical clustering of cells (*left*) and genes (*bottom*). Cluster identities are shown on the right of the Heatmap. **I,** Visualization on the UMAP plot of *SCD* co-expressed genes enrichment in vehicle- compared to GILT-treated PDX^{TUH84}. **J,** Violin plot showing the enrichment of *SCD* co-expressed genes by cluster in vehicle- compared to GILT-treated PDX^{TUH84}. **K,** Visualization on the UMAP plot of *MKI67* gene enrichment in vehicle- compared to GILT-treated PDX^{TUH84}. **L,** Violin plot showing the enrichment of *MKI67* gene by cluster in vehicle- compared to GILT-treated PDX^{TUH84}.

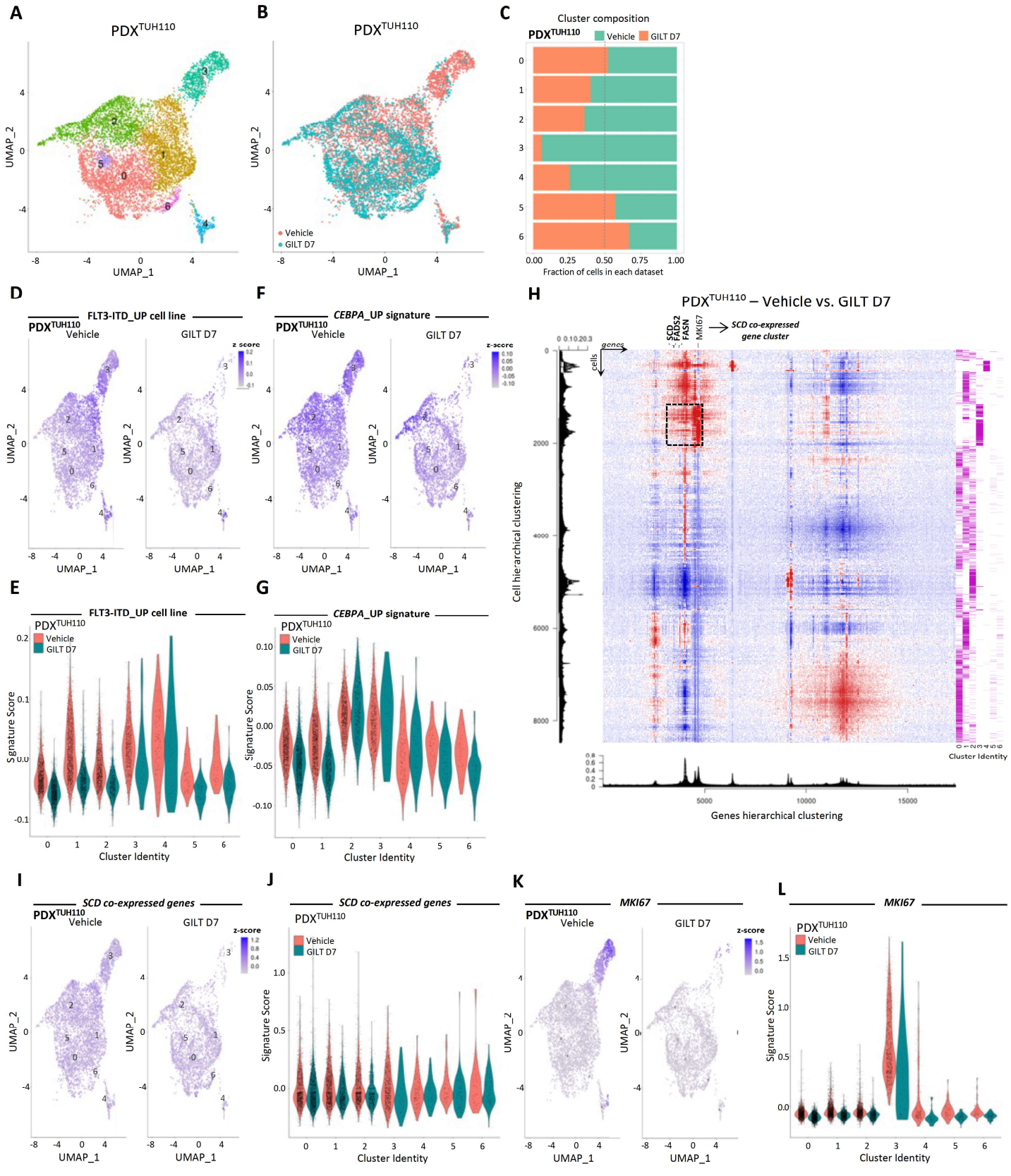

A
PDX^TUH110
UMAP_2
UMAP_1
B
PDX^TUH110
Vehicle
GILT D7
C
Cluster composition
PDX^TUH110
Vehicle
GILT D7
Fraction of cells in each dataset
D
FLT3-ITD_UP cell line
PDX^TUH110
Vehicle
GILT D7
z-score
F
CEBPA_UP signature
H
PDX^TUH110 – Vehicle vs. GILT D7
SCD
FADS2
FASN
MKI67
SCD co-expressed gene cluster
genes
cells
Cell hierarchical clustering
Cluster Identity
Genes hierarchical clustering
E
FLT3-ITD_UP cell line
Signature Score
Cluster Identity
G
CEBPA_UP signature
I
SCD co-expressed genes
J
SCD co-expressed genes
K
MKI67
L
MKI67

Figure S11

Supplementary Figure S11. scRNA-seq analysis from PDX^{TUH110}. **A,** Uniform manifold approximation and projection (UMAP) plot of 8739 cells from PDX^{TUH110} using Seurat. Colors indicate k-means clusters (k=8). **B,** UMAP plot colored by treatment condition (vehicle: n=5229 cells; GILT D7: n=3510 cells). **C,** Proportion of each condition per cluster from PDX^{TUH110}. **D,** Visualization on the UMAP plot of FLT3-ITD_UP cell line signature enrichment in vehicle- compared to GILT-treated PDX^{TUH110}. **E,** Violin plot showing the enrichment of FLT3-ITD_UP cell line signature by cluster in vehicle- compared to GILT-treated PDX^{TUH110}. **F,** Visualization on the UMAP plot of *CEBPA*_UP signature enrichment in vehicle- compared to GILT-treated PDX^{TUH110}. **G,** Violin plot showing the enrichment of *CEBPA*_UP signature by cluster in vehicle- compared to GILT-treated PDX^{TUH110}. **H,** Heatmap showing the expression of each gene per cell in vehicle- compared to GILT-treated PDX^{TUH110}. Dendrograms represent the hierarchical clustering of cells (*left*) and genes (*bottom*). Cluster identities are shown on the right of the Heatmap. **I,** Visualization on the UMAP plot of *SCD* co-expressed genes enrichment in vehicle- compared to GILT-treated PDX^{TUH110}. **J,** Violin plot showing the enrichment of *SCD* co-expressed genes by cluster in vehicle- compared to GILT-treated PDX^{TUH110}. **K,** Visualization on the UMAP plot of *MKI67* gene enrichment in vehicle- compared to GILT-treated PDX^{TUH110}. **L,** Violin plot showing the enrichment of *MKI67* gene by cluster in vehicle- compared to GILT-treated PDX^{TUH110}.

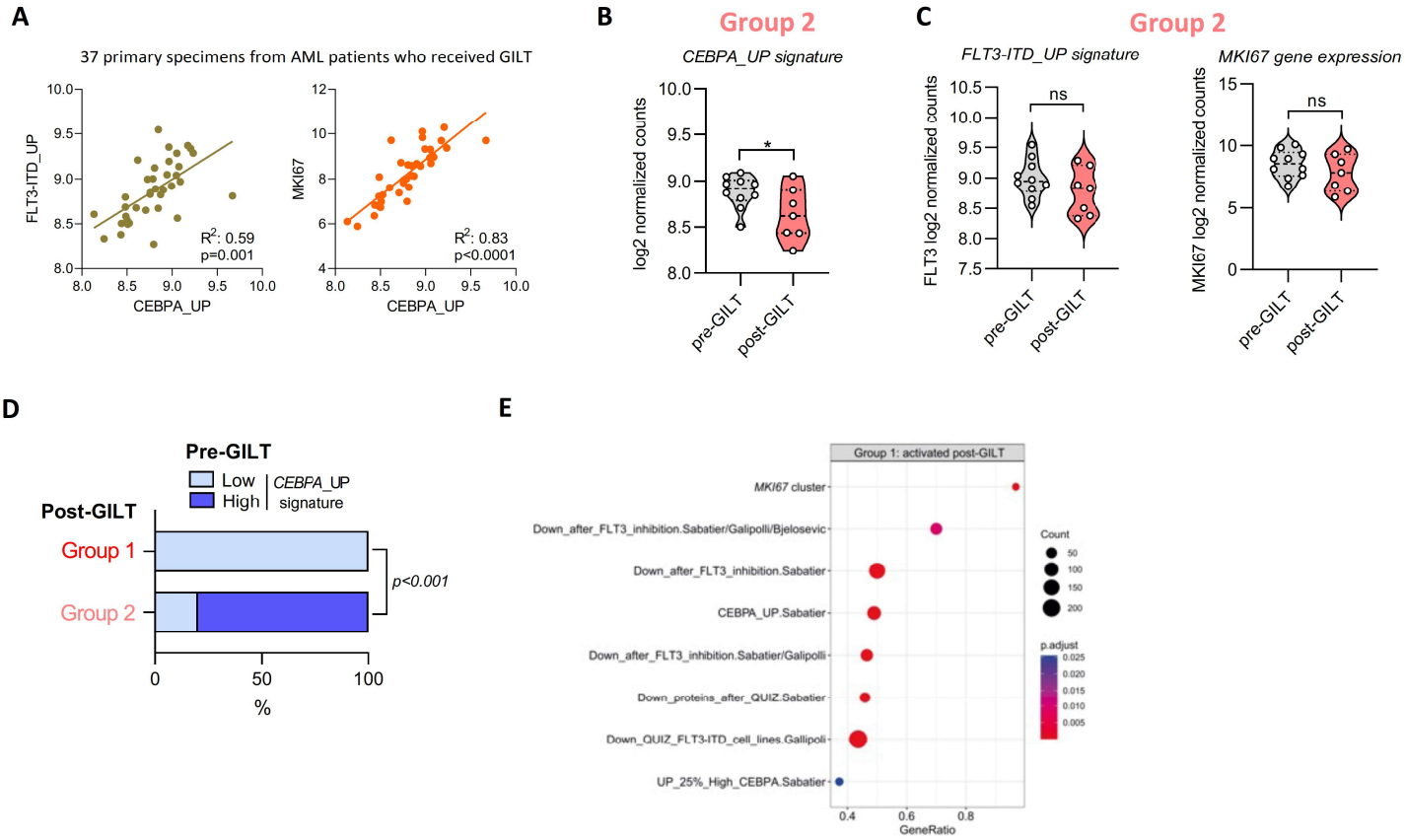

A
37 primary specimens from AML patients who received GILT
FLT3-ITD_UP
CEBPA_UP
R2: 0.59
p=0.001
MKI67
CEBPA_UP
R2: 0.83
p<0.0001
B
Group 2
CEBPA_UP signature
log2 normalized counts
*
pre-GILT
post-GILT
C
Group 2
FLT3-ITD_UP signature
FLT3 log2 normalized counts
ns
pre-GILT
post-GILT
MKI67 gene expression
MKI67 log2 normalized counts
ns
pre-GILT
post-GILT
D
Pre-GILT
Low
High
CEBPA_UP signature
Post-GILT
Group 1
Group 2
p<0.001
0
50
100
%
E
Group 1: activated post-GILT
MKI67 cluster
Down_after_FLT3_inhibition.Sabatier/Galipolli/Bjelosevic
Down_after_FLT3_inhibition.Sabatier
CEBPA_UP.Sabatier
Down_after_FLT3_inhibition.Sabatier/Galipolli
Down_proteins_after_QUIZ.Sabatier
Down_QUIZ_FLT3-ITD_cell_lines.Gallipoli
UP_25%_High_CEBPA.Sabatier
0.4
0.6
0.8
GeneRatio
Count
50
100
150
200
p.adjust
0.025
0.020
0.015
0.010
0.005

Figure S12

Supplementary Figure S12. Evolution of *CEBPA*_UP and *FLT3*-ITD_UP signatures in patients with AML treated with GILT. **A-D,** Transcriptomes from 37 samples from patients with AML (20 before and 17 after GILT treatment) were investigated. **A,** Correlation analysis for CEBPA_UP and FLT3-ITD_UP signatures and *MKI67* expression. **B-C,** Enrichment of *CEBPA*_UP, FLT3-ITD_UP and *MKI67* gene signatures during GILT therapy in the patient group 2. **C,** Evolution of FLT3-ITD_UP signature and MKI67 expression before and after GILT treatment were plotted dependent on the post-GILT CEBPA_UP signature status of group 1 and 2 of AML patients. **E,** Gene set enrichment analysis comparing after *versus* before GILT in patients with enrichment in *CEBPA* signature during GILT therapy. ns: not significant, *p<0.05, **p<0.01.

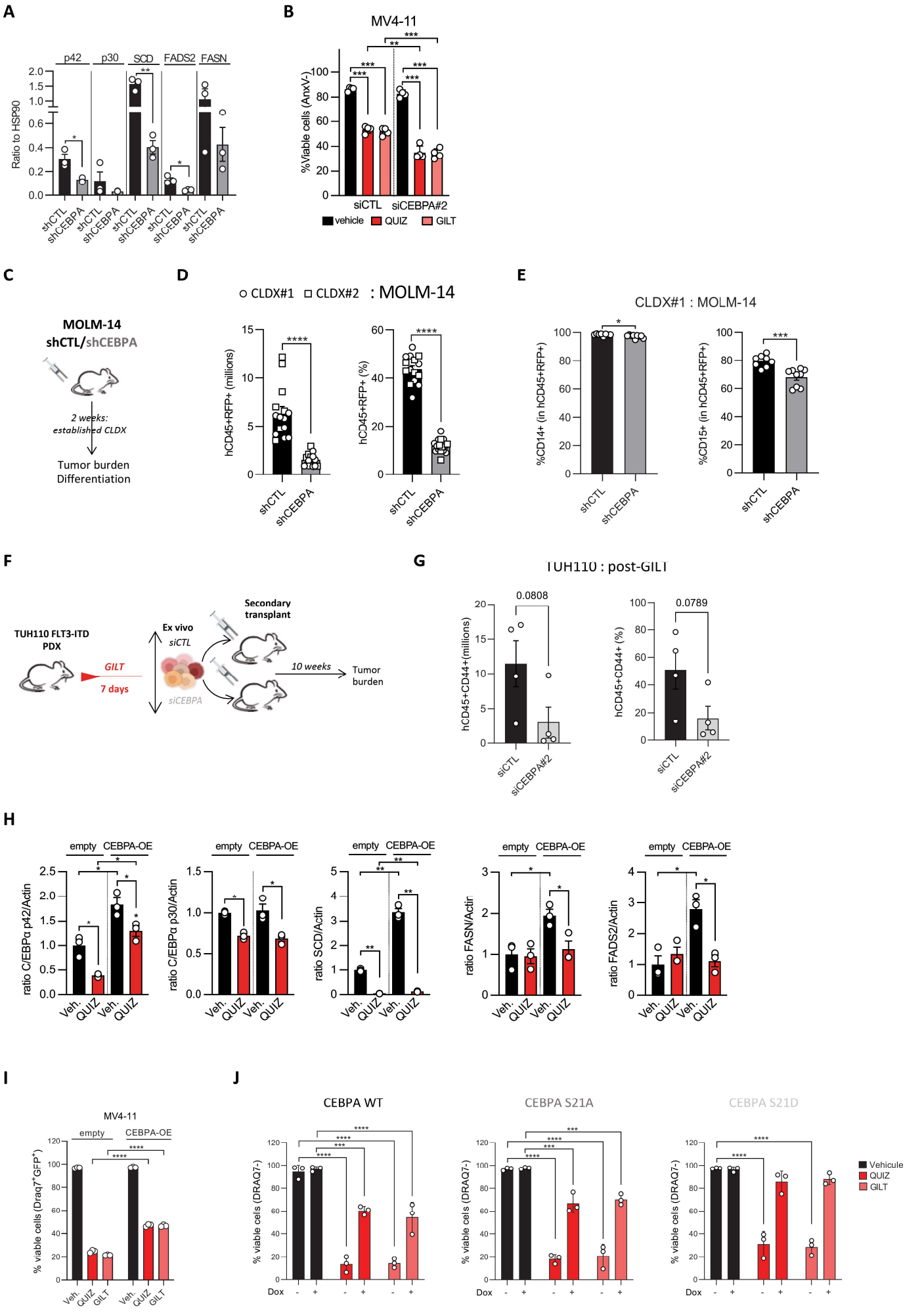

A
p42
p30
SCD
FADS2
FASN
Ratio to HSP90
shCTL
shCEBPA
B
MV4-11
%Viable cells (AnxV-)
siCTL
siCEBPA#2
vehicle
QUIZ
GILT
C
MOLM-14
shCTL/shCEBPA
2 weeks:
established CLDX
Tumor burden
Differentiation
D
CLDX#1
CLDX#2
: MOLM-14
hCD45+RFP+ (millions)
hCD45+RFP+ (%)
E
CLDX#1 : MOLM-14
%CD14+ (in hCD45+RFP+)
%CD15+ (in hCD45+RFP+)
F
TUH110 FLT3-ITD
PDX
GILT
7 days
Ex vivo
siCTL
siCEBPA
Secondary
transplant
10 weeks
Tumor
burden
G
TUH110 : post-GILT
0.0808
0.0789
hCD45+CD44+(millions)
hCD45+CD44+ (%)
siCTL
siCEBPA#2
H
empty
CEBPA-OE
ratio C/EBPα p42/Actin
ratio C/EBPα p30/Actin
ratio SCD/Actin
ratio FASN/Actir
ratio FADS2/Actin
Veh.
QUIZ
I
MV4-11
% viable cells (Draq7+GFP+)
GILT
J
CEBPA WT
CEBPA S21A
CEBPA S21D
% viable cells (DRAQ7-)
Dox
Vehicule
QUIZ
GILT

Figure S13

Supplementary Figure S13. C/EBPα regulates rate-limiting lipid biosynthetic enzymes downstream of FLT3-ITD. **A,** MOLM-14 cells transduced with dox-inducible anti-*CEBPA* and control (CTL) shRNAs were cultured with dox for 72h. Total protein extracts were immunoblotted with anti-CEBPA, anti-SCD and anti-actin antibodies. Densitometry analyses were done relative to actin. **B,** MV4-11 cells were transfected with anti-CEBPA (siCEBPA#2) and control (siCTL) siRNAs. Cell viability assessed by Annexin V staining after 14h incubation with 20nM QUIZ or 100nM GILT starting 6h after siRNAs transfection. **C-E,** MOLM-14 cells expressing RFP-tagged dox-inducible anti-CEBPA or CTL shRNAs were transplanted into NSG mice, and 10 days after transplant, 4mg/ml dox was added in the drinking water. Mice were euthanized 17 days after transplant. **C,** Experiment overview. **D,** Tumor burden was measured by hCD45 and RFP staining (two independent CLDX assays were performed with n=8 mice per group). **E,** Cell differentiation was investigated by CD14 and CD15 staining among $hCD45^+$ human AML cells (done in CLDX#1 assay). **F-G,** PDX^{TUH110} was treated *in vivo* with 30mg/kg/d GILT for 7 days. Next, residual disease was harvested, $hCD45^+$ human AML cells were sorted and transfected with CTL or anti-CEBPA siRNAs before transplantation to secondary recipient NSG mice. Tumor burden was evaluated 10 weeks after transplant. **F,** Experiment overview. **G,** Number of viable hCD45+CD44+ cells (*left panel*) and percentage of viable hCD45+hCD44+ cells (*right panel*) in bone marrow (n=4 mice per group). **H,** MOLM-14 cells transduced with a dox-inducible vector allowing CEBPA overexpression (OE), or with the empty vector were cultured with dox for 48h. Total protein extracts were immunoblotted using the indicated antibodies. Densitometry analyses were done relative to actin. **I,** MV4-11 cells transduced with CEBPA-OE or empty vector were incubated with dox for 72h, and then incubated with vehicle or 3nM QUIZ or 30nM GILT for 48h. Viability was measured by DRAQ7 staining in efficiently transduced GFP^+ cells. **J,** MOLM-14 cells were transduced with dox-inducible expression vectors for wild type (WT), S21A and S21D CEBPA forms. After 72h dox induction, cells were incubated with vehicle, 3nM QUIZ or 30nM GILT for 48h and viability was measured using DRAQ7 staining. Vertical bars indicate standard deviations. ns: not significant; *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$.

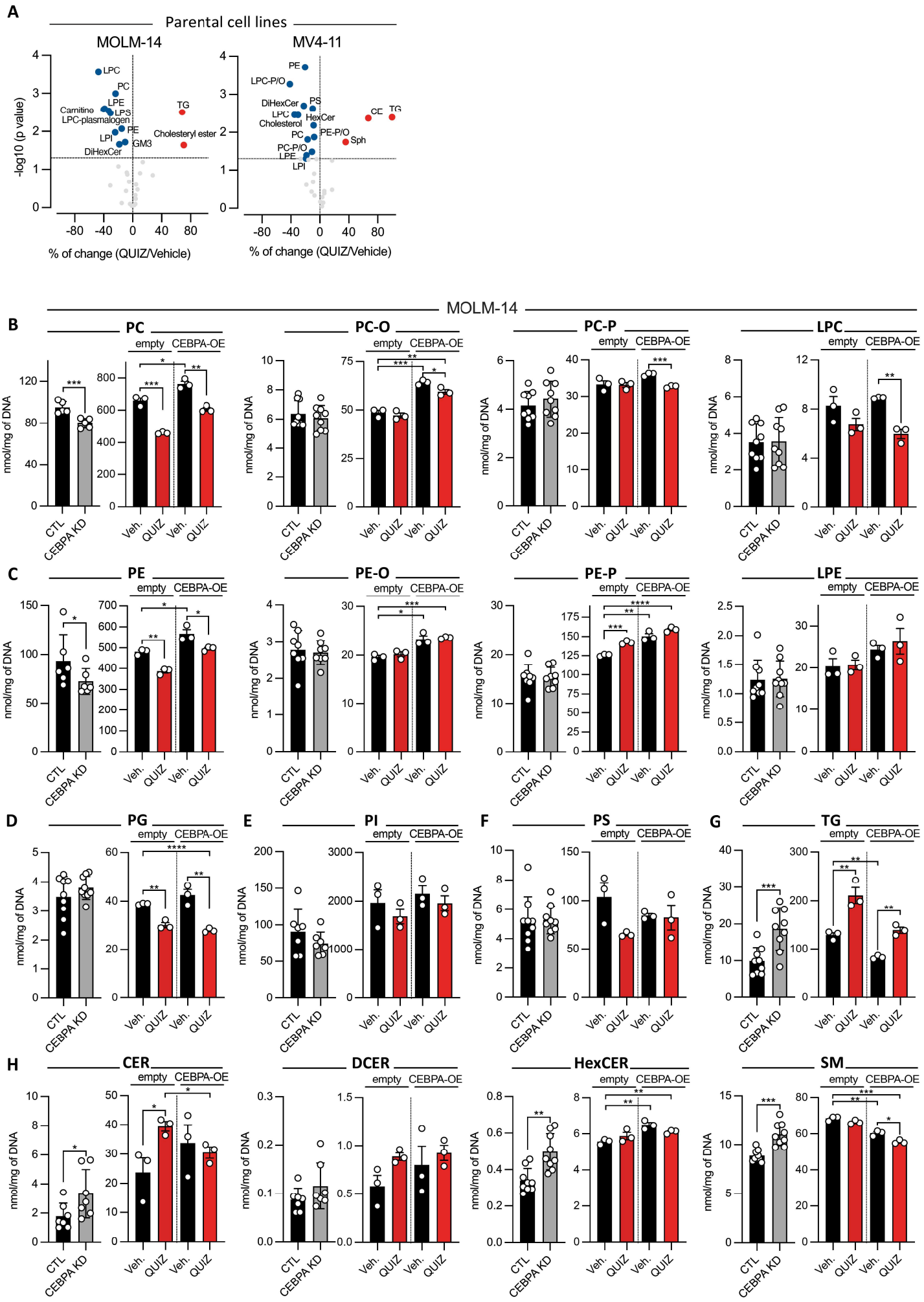

A
Parental cell lines
MOLM-14
MV4-11
-log10 (p value)
% of change (QUIZ/Vehicle)
LPC
PC
LPE
Carnitine
LPS
LPC-plasmalogen
PE
LPI
GM3
DiHexCer
TG
Cholesteryl ester
PE
LPC-P/O
DiHexCer
PS
LPC
Cholesterol
HexCer
PC
PE-P/O
PC-P/O
LPE
LPI
CE
TG
Sph
MOLM-14
B
PC
PC-O
PC-P
LPC
C
PE
PE-O
PE-P
LPE
D
PG
E
PI
F
PS
G
TG
H
CER
DCER
HexCER
SM
empty
CEBPA-OE
nmol/mg of DNA
CTL
CEBPA KD
Veh.
QUIZ

Figure S14

Supplementary Figure S14. C/EBPα controls lipid amount in FLT3-ITD cell lines. **A,** Global lipidomics in MOLM-14 (*left panel*) and MV4-11 (*right panel*) parental AML cell lines incubated with vehicle or 3nM QUIZ for 14h. LPC/PC: lyso-/phosphatidylcholine; LPE/PE: lyso-/phosphatidylethanolamine; LPI/PI: lyso-/phosphatidylinositol; LPS/PS: lyso-/phosphatidylserine; TG: triglceride; CE/Cer: ceramide; DiHexCer: dihexosylceramide; HexCER: Hexosylceramide; SM/Sp: sphingomyelin; GM3: monosiolodihexosylganglioside; -P: plasmalogen; -O: ether. **B-H,** Targeted lipidomics was performed in MOLM-14 cells were depleted from C/EBPα (siRNA 24h, dox-inducible shRNA or constitutive shRNA 72h) or overexpressing C/EBPα (dox-inducible 48h) and treated with vehicle or 3nM QUIZ for 24h. Each graph represents the quantity of each lipid species relative to DNA amount per sample. Vertical bars indicate standard deviations. *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$.

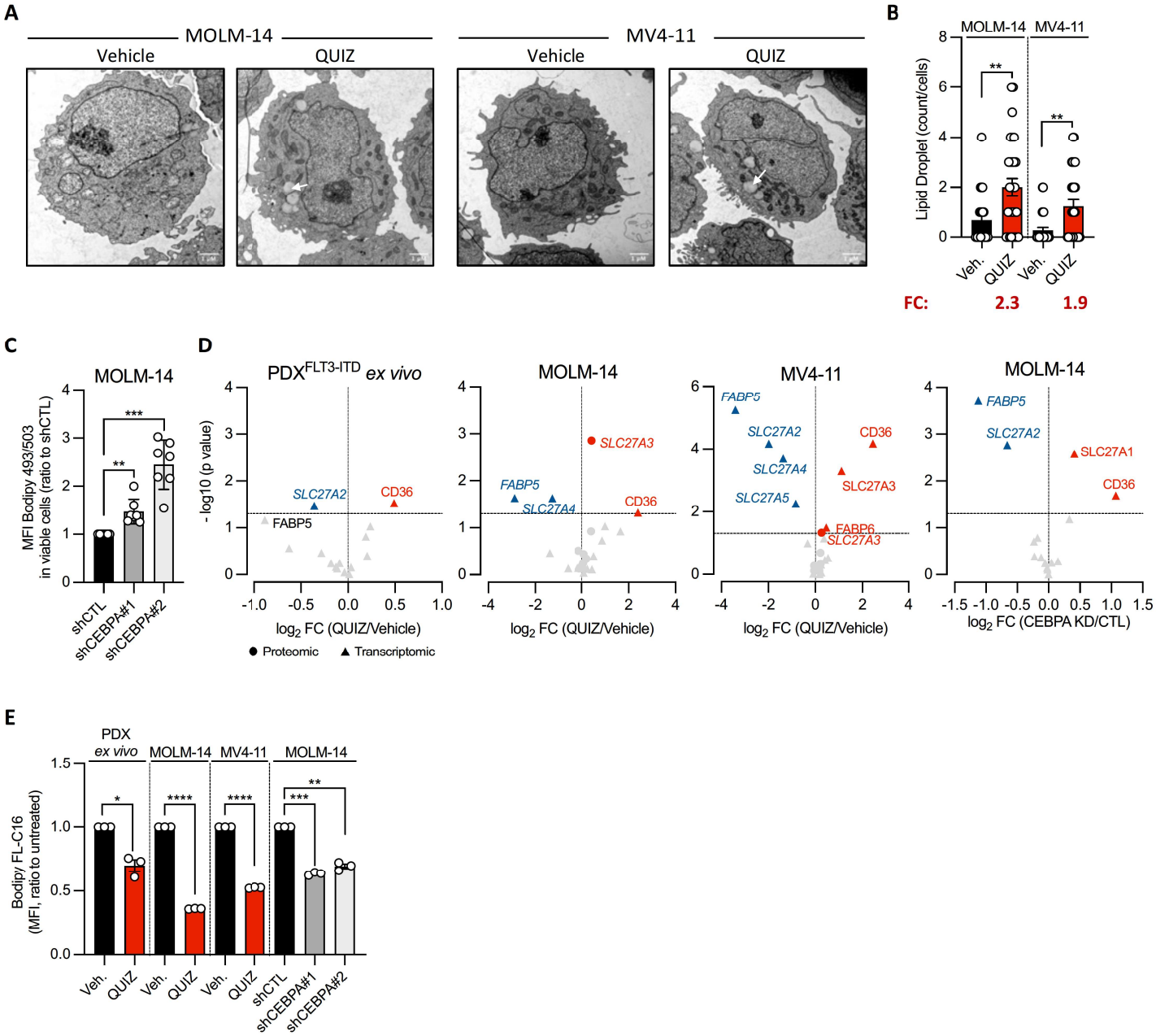

A
MOLM-14
Vehicle
QUIZ
MV4-11
Vehicle
QUIZ
B
MOLM-14
MV4-11
Lipid Droplet (count/cells)
Veh.
QUIZ
Veh.
QUIZ
FC:
2.3
1.9
C
MOLM-14
MFI Bodipy 493/503 in viable cells (ratio to shCTL)
shCTL
shCEBPA#1
shCEBPA#2
D
PDXFLT3-ITD ex vivo
MOLM-14
MV4-11
MOLM-14
- log10 (p value)
log2 FC (QUIZ/Vehicle)
log2 FC (CEBPA KD/CTL)
Proteomic
Transcriptomic
FABP5
SLC27A2
SLC27A4
SLC27A5
SLC27A3
SLC27A1
CD36
FABP6
E
PDX ex vivo
MOLM-14
MV4-11
MOLM-14
Bodipy FL-C16 (MFI, ratio to untreated)
Veh.
QUIZ
shCTL
shCEBPA#1
shCEBPA#2

Figure S15

Supplementary Figure S15. FLT3 inhibitors induce a lipid switch increasing neutral lipids dependent on C/EBPα. **A-B,** MOLM-14 and MV4-11 cells were incubated with vehicle or 3nM QUIZ for 14h. **A,** Electron microscopy (EM) morphology (scale: 5µM). **B,** EM quantification of lipid droplet content *per* cell. Each dot corresponds to a single cell. **C,** Neutral lipid content in MOLM14 silenced for C/EBPα compared to control using constitutive shRNA measured by Bodiby 493/503 staining. **D,** Gene and protein expression of FA binding protein and lipid transporter in FLT3-ITD patient-derived AML cells and cell lines incubated with vehicle or QUIZ, and in MOLM-14 cells transduced with CTL or anti-CEBPA shRNAs (decrease and increase FC<0.67 and >1.5, respectively). **E,** Patient-derived FLT3-ITD AML cells and cell lines were incubated with 3nM QUIZ for 14h, and MOLM-14 cells were transduced with CTL or two different anti-CEBPA shRNAs. Lipid transport was assessed using Bodipy FL-C16 staining. Vertical bars indicate standard deviations. *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$.

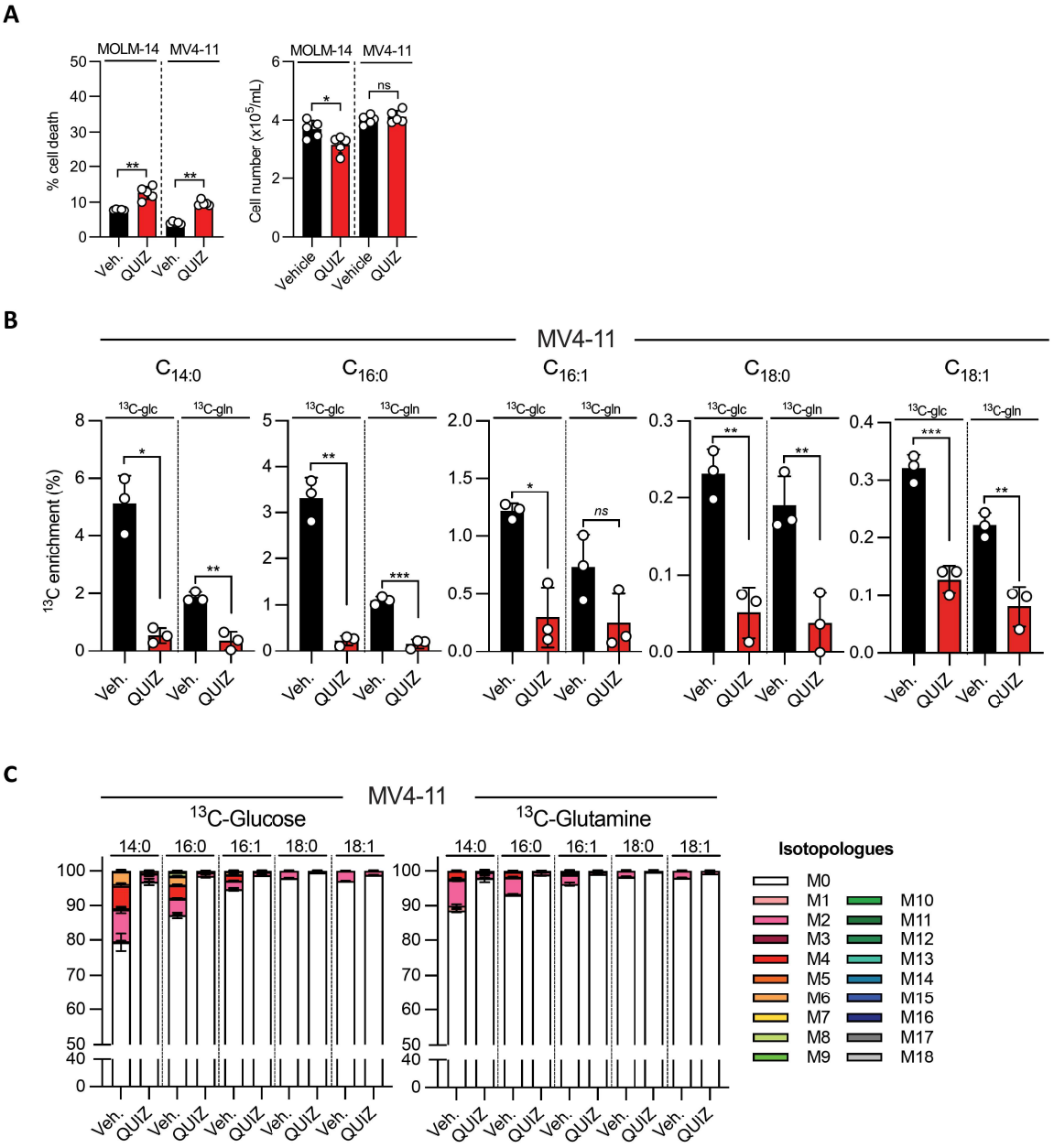

A
MOLM-14
MV4-11
% cell death
Cell number (x10^5/mL)
Veh.
QUIZ
Vehicle
ns
B
MV4-11
C14:0
C16:0
C16:1
C18:0
C18:1
13C-glc
13C-gln
13C enrichment (%)
C
MV4-11
13C-Glucose
13C-Glutamine
14:0
16:0
16:1
18:0
18:1
Isotopologues
M0
M1
M2
M3
M4
M5
M6
M7
M8
M9
M10
M11
M12
M13
M14
M15
M16
M17
M18

Figure S16

Supplementary Figure S16. FLT3 inhibitor inhibits fatty acid synthesis fueled by glucose and glutamine. **A,** MOLM-14 and MV4-11 cells were incubated with vehicle or 3nM QUIZ for 14h. Viability was measured by propidium iodine staining. Viable cells were also counted using a trypan blue exclusion assay. **B,** Percentage of ^{13}C enrichment in $C_{14:0}$, $C_{16:0}$, $C_{16:1}$, $C_{18:0}$ and $C_{18:1}$ total FA in MV4-11 cultured on [U-^{13}C]-glucose or [U-^{13}C]-glutamine and treated with vehicle or 3nM QUIZ for 24h. **C,** Isotopologues distribution in $C_{14:0}$, $C_{16:0}$, $C_{16:1}$, $C_{18:0}$ and $C_{18:1}$ total FA in MV4-11 24-cultured on [U-^{13}C]-glucose (*left* panel) or [U-^{13}C]-glutamine (*right* panel) and incubated with vehicle or 3nM QUIZ. Vertical bars indicate standard deviations. *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$.

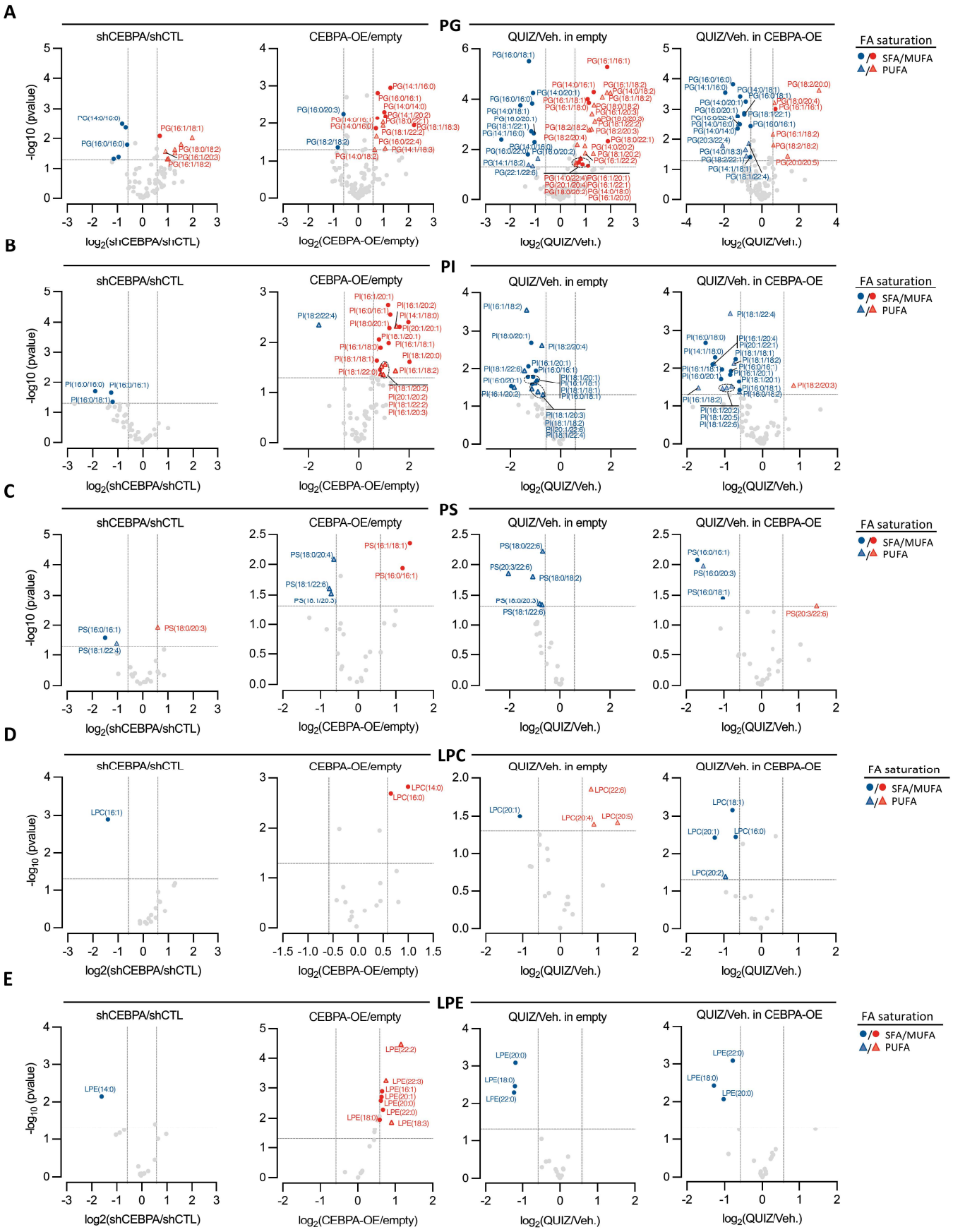

A
PG
shCEBPA/shCTL
CEBPA-OE/empty
QUIZ/Veh. in empty
QUIZ/Veh. in CEBPA-OE
-log10 (pvalue)
log2(shCEBPA/shCTL)
log2(CEBPA-OE/empty)
log2(QUIZ/Veh.)
FA saturation
SFA/MUFA
PUFA
B
PI
C
PS
D
LPC
-log10 (pvalue)
E
LPE

Figure S17

Supplementary Figure S17. FLT3 inhibition decreases monounsaturated fatty acid dependent on C/EBPα. **A-E,** From the *left* to the *right*: C/EBPα-depleted versus CTL, CEBPA-OE versus empty vector, vehicle versus 3nM QUIZ for 14h in empty vector, and vehicle versus 3nM QUIZ for 14h in CEBPA-OE in MOLM-14 cells. Targeted lipidomics showing repartition of SFAs, MUFAs and PUFAs distribution into phosphatidylglycerol (PG, **A**), phosphatidylinositol (PI, **B**), phosphatidylserine (PS, **C**), lysophosphatidylcholine (LPC, **D**) and lysophosphatidylethanolamine (LPE, **E**).

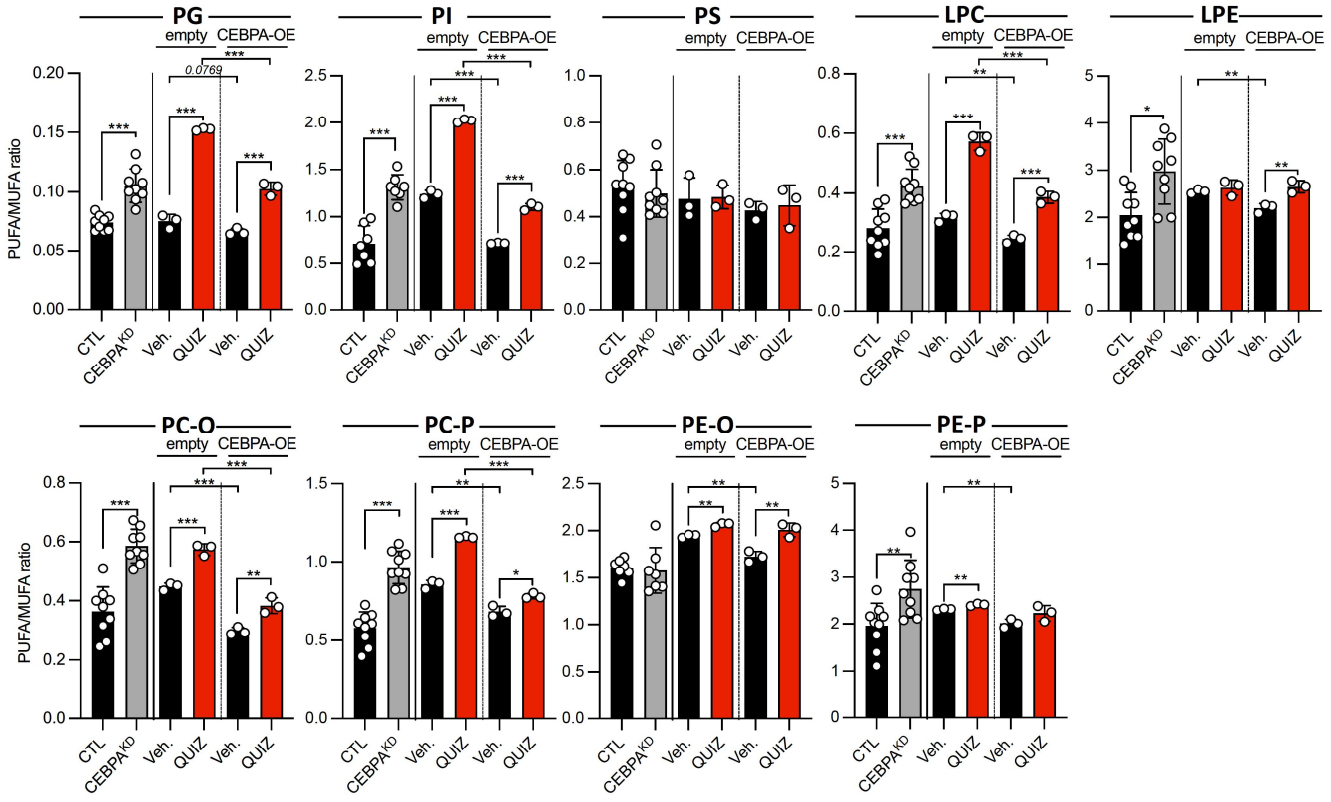

PG
PI
PS
LPC
LPE
PC-O
PC-P
PE-O
PE-P
empty
CEBPA-OE
PUFA/MUFA ratio
CTL
CEBPA^KD
Veh.
QUIZ
0.0769

Figure S18

Supplementary Figure S18. FLT3 inhibition increases PUFA/MUFA ratio dependent on C/EBPα. From the *left* to the *right*: C/EBPα-depleted versus CTL, CEBPA-OE versus empty vector, vehicle versus 3nM QUIZ for 14h in empty vector, and vehicle versus 3nM QUIZ for 14h in CEBPA-OE in MOLM-14 cells. PUFA/MUFA ratio into phosphatidylglycerol (PG), phosphatidylinositol (PI), phosphatidylserine (PS), lysophosphatidylcholine (LPC), lysophosphatidylethanolamine (LPE), phosphatidylcholine-ether/-plasmogen (PC-O/PC-P) and phosphatidylethanolamine-ether/-plasmogen (PE-O/PE-P). Vertical bars indicate standard deviations. *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$.

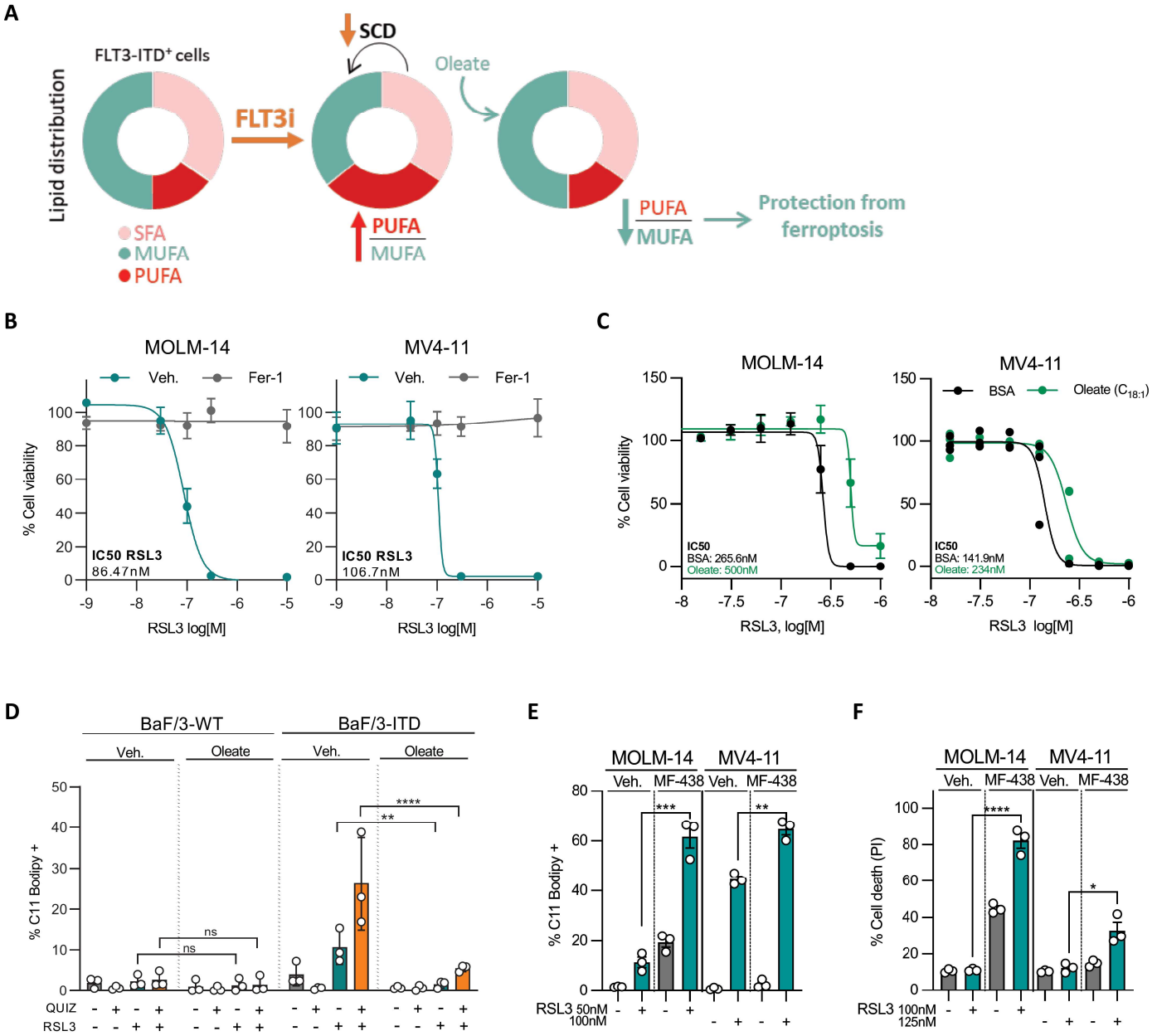

A
FLT3-ITD+ cells
Lipid distribution
SFA
MUFA
PUFA
FLT3i
SCD
Oleate
PUFA
MUFA
PUFA
MUFA
Protection from ferroptosis
B
MOLM-14
MV4-11
Veh.
Fer-1
% Cell viability
IC50 RSL3
86.47nM
106.7nM
RSL3 log[M]
C
MOLM-14
MV4-11
BSA
Oleate (C18:1)
% Cell viability
IC50
BSA: 265.6nM
Oleate: 500nM
BSA: 141.9nM
Oleate: 234nM
RSL3, log[M]
RSL3 log[M]
D
BaF/3-WT
BaF/3-ITD
Veh.
Oleate
% C11 Bodipy +
ns
**
****
QUIZ
RSL3
E
MOLM-14
MV4-11
Veh.
MF-438
% C11 Bodipy +
***
**
RSL3 50nM
100nM
F
MOLM-14
MV4-11
Veh.
MF-438
% Cell death (PI)
****
*
RSL3 100nM
125nM

Figure S19

Supplementary Figure S19. Ferroptotic cell death induced by FLT3i is mediated by inhibition of SCD-dependent mono-unsaturated FA synthesis. **A,** Decreased amount of MUFA after TKI treatment related to SCD inhibition is rescued by $C_{18:1}$ oleate supply, protecting AML cells from ferroptotic cell death. **B,** MOLM-14 (*left panel*) and MV4-11 (*right panel*) cells were exposed to dose-range RLS3 with or without 10µM Fer-1 for 48h. Cell viability assays using Alamar Blue. **C,** MOLM-14 (*left panel*) and MV4-11 (*right panel*) cells were exposed to dose-range RLS3 with vehicle (BSA) or 90µM oleate for 48h. Cell viability assays using Alamar Blue. **D,** IL3-dependent Ba/F3 murine hematopoietic cells expressing vectors for FLT3 ($FLT3^{WT}$) or FLT3-ITD-mutated ($FLT3^{ITD}$) genes were cultured with vehicle (BSA) or 90µM oleate and treated with or without 1uM RSL3 or 3nM QUIZ alone or in association for 48h. Lipid peroxidation was measured by C11-Bodipy after 48h-treatment. **E-F,** MOLM-14 and MV4-11 cells were incubated with vehicle or 1µM MF-438, combined with vehicle or 50-125nM RSL3. Lipid peroxidation was measured by C11-Bodipy after 12h-treatment **(E)** and cell viability was assessed by PI staining after 24h-treatment **(F).** Vertical bars indicate standard deviations. *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$.

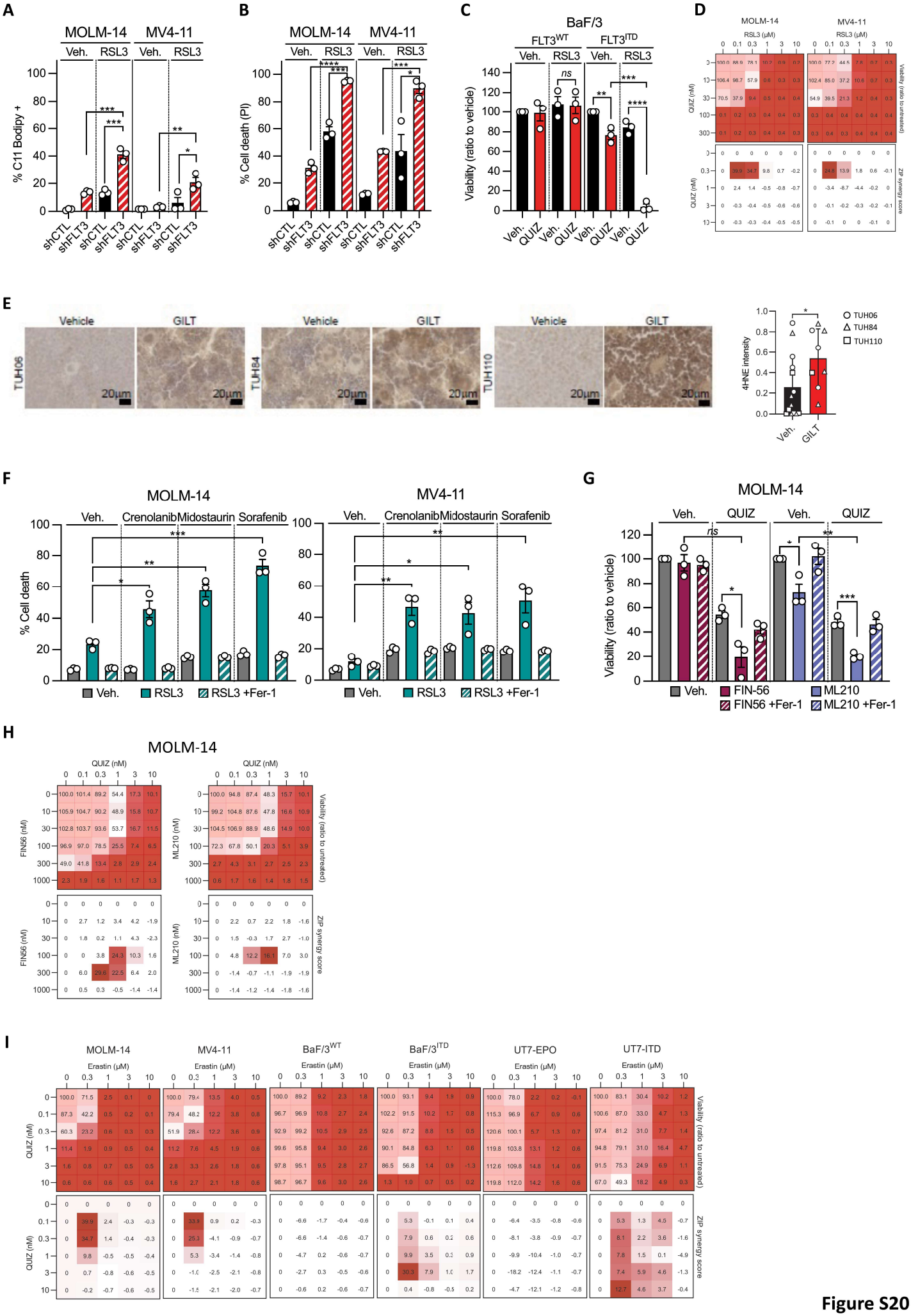

A
MOLM-14
MV4-11
Veh.
RSL3
% C11 Bodipy +
shCTL
shFLT3
B
% Cell death (PI)
C
BaF/3
FLT3WT
FLT3ITD
ns
Viability (ratio to vehicle)
Veh.
QUIZ
D
RSL3 (μM)
QUIZ (nM)
Viability (ratio to untreated)
ZIP synergy score
E
Vehicle
GILT
TUH06
TUH84
TUH110
20μm
4HNE intensity
F
Crenolanib
Midostaurin
Sorafenib
% Cell death
RSL3 +Fer-1
G
FIN-56
FIN56 +Fer-1
ML210
ML210 +Fer-1
H
FIN56 (nM)
ML210 (nM)
I
Erastin (μM)
UT7-EPO
UT7-ITD

Figure S20

Supplementary Figure S20. FLT3 inhibitors unmask a vulnerability of FLT3-mutant leukemic cells to ferroptosis. **A-B,** MOLM-14 and MV4-11 cells were transduced with CTL or FLT3-targeting shRNAs (dox-inducible, 24h), and incubated with vehicle or 100nM **(A)** or 50nM **(B)** RSL3. **A,** Lipid peroxidation measured by C11-Bodipy staining after 14h. **B,** Cell viability assessed by propidium iodine (PI) staining after 24h. **C,** IL3-dependent Ba/F3 murine hematopoietic cells were transduced with expression vectors for *FLT3* ($FLT3^{WT}$) or *FLT3*-ITD-mutated ($FLT3^{ITD}$) genes. $FLT3^{WT}$ and $FLT3^{ITD}$ Ba/F3 cells were grown with and without IL3, respectively. Cells were cultured 48h with vehicle or 3nM QUIZ, and vehicle or 1μM RSL3, and cell viability was assessed using a luminescence-based assay (Alamar Blue). Results are expressed as a ratio to the vehicle conditions. **D,** MOLM-14 and MV4-11 cell lines were incubated with crossed dose-range QUIZ and RSL3 for 48h. Cell viability was measured using a luminescence-based assay (Alamar Blue) and results are expressed as a ratio to the untreated condition for each wheel in a viability matrix (top panel) and using synergy maps using zero interaction potency (ZIP) synergy scores and a threshold ZIP>10 considered as significant (bottom panel). **E,** PDX^{TUH06}, PDH^{TUH84} and PDX^{TUH110} were treated in vivo with vehicle or 30mg/kg/d GILT for 7 days. Next, sternums were harvested and 4-HNE was detected in paraffine-embedded slices using an anti-4HNE antibody. Scales bars (20μM) are provided. **F,** MOLM-14 (*left panel*) and MV4-11(*right panel*) cells were incubated with vehicle, or 10nM and 20nM Crenolanib respectively, or 60nM and 70nM Midostaurin respectively, or 30nM Sorafenib, with vehicle or 100nM RSL3 and with or without 10μM Fer-1 for 24h as indicated. **G,** MOLM-14 cells were incubated with vehicle or 1nM QUIZ, combined without or with 100nM FIN-56 or 100nM ML-210 and with or without 10μM Fer-1 for 48h as indicated, and cell viability was assessed using a luminescence-based assay (Alamar Blue). **H,** Dose-response matrices in MOLM-14 cells incubated with crossed dose-range QUIZ and FIN56 for 48h. Cell viability was measured using a luminescence-based assay (Alamar Blue) and results are expressed as a ratio to the untreated condition for each wheel in a viability matrix (top panel) and using synergy maps using zero interaction potency (ZIP) synergy scores and a threshold ZIP>10 considered as significant (bottom panel). **I,** Dose-response matrices for QUIZ and erastin combination in MOLM-14, MV4-11, Ba/F3 transduced with FLT3 wild-type (WT) or FLT3-ITD alleles ($Ba/F3^{WT}$ and $Ba/F3^{ITD}$, respectively), parental UT7 (UT7 EPO) and UT7 transduced with FLT3-ITD (UT7-ITD) cell lines. Cell viability was measured after 48h using a luminescence-based assay (Alamar Blue) and results are expressed as a ratio to the untreated condition for each wheel in a viability matrix (top panel) and using synergy maps using zero interaction potency (ZIP) synergy scores and a threshold ZIP>10 considered as significant (bottom panel).

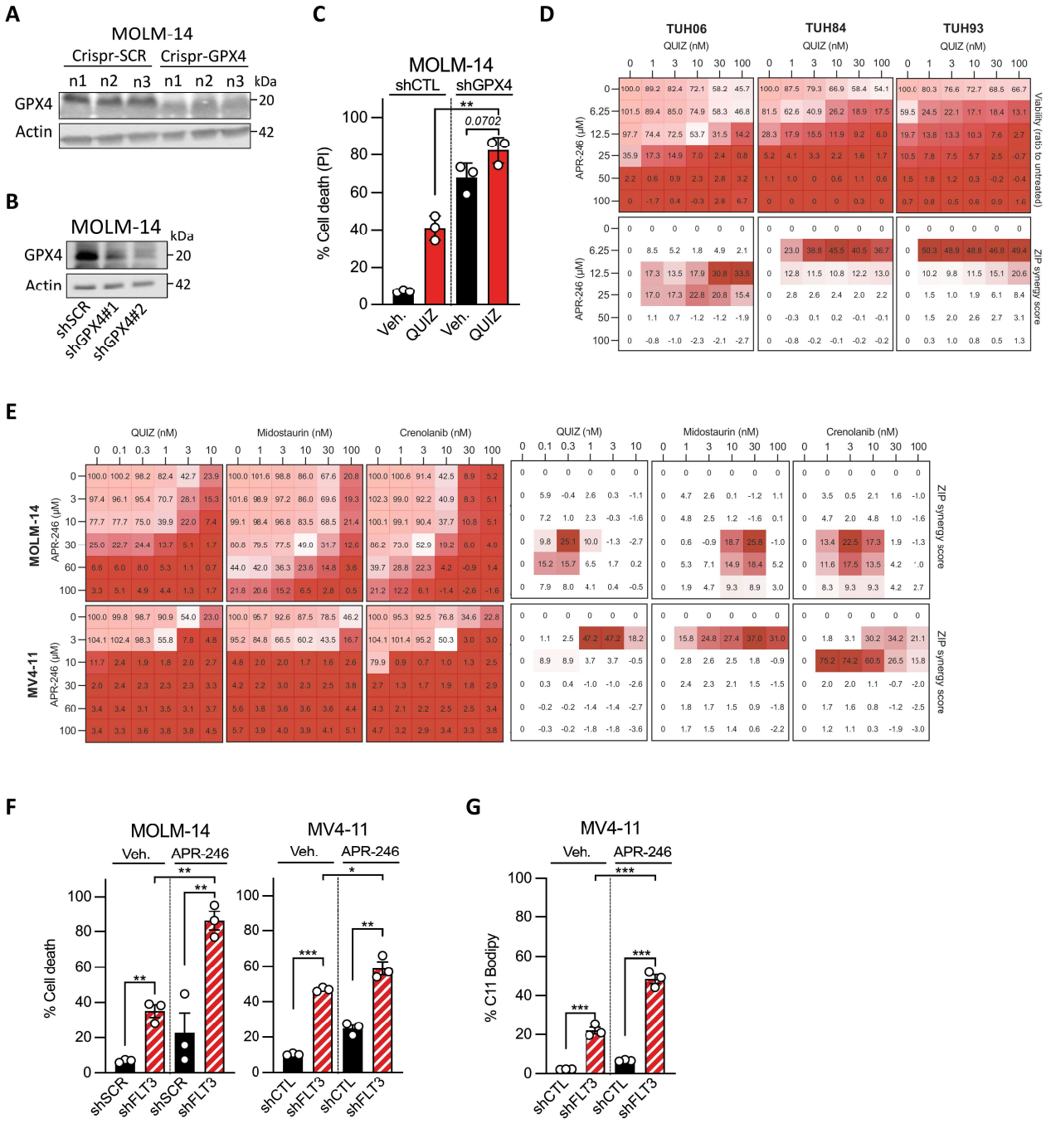

A
MOLM-14
Crispr-SCR
Crispr-GPX4
n1 n2 n3 n1 n2 n3 kDa
GPX4
Actin
20
42
B
MOLM-14
kDa
GPX4
Actin
20
42
shSCR
shGPX4#1
shGPX4#2
C
MOLM-14
shCTL
shGPX4
**
0.0702
% Cell death (PI)
Veh.
QUIZ
Veh.
QUIZ
D
TUH06
TUH84
TUH93
QUIZ (nM)
APR-246 (μM)
Viability (ratio to untreated)
ZIP synergy score
E
QUIZ (nM)
Midostaurin (nM)
Crenolanib (nM)
MOLM-14
MV4-11
APR-246 (μM)
ZIP synergy score
F
MOLM-14
MV4-11
Veh.
APR-246
% Cell death
shSCR
shFLT3
shCTL
shFLT3
G
MV4-11
Veh.
APR-246
% C11 Bodipy
shCTL
shFLT3

Figure S21

Supplementary Figure S21. Lipid redox stress induction by GPX4 inhibition primed FLT3i activity in FLT3-ITD AML cells. **A,** Representative western blot of MOLM-14 cells transduced with CTL or GPX4-targeting CRISPR-Cas9 vectors, and cultured with 10µM Fer-1 to avoid cell death induced by GPX4 ablation. **B-C**, MOLM14 cells were transduced with dox-inducible shCTL or shGPX4 and cultured with dox for 72h. **B,** Total protein extracts were immunoblotted with the indicate antibodies. **C,** Cell death was measured using PI staining in shCTL versus shGPX4 MOLM-14 cells treated with vehicle or 3nM QUIZ for 48h. **D,** Synergy matrices in cells from PDX^{TUH06}, PDH^{TUH84} and PDX^{TUH110} incubated with QUIZ and APR-246 for 48h. Cell viability was measured using a luminescence-based assay (Alamar Blue) and results are expressed as a ratio to the untreated condition for each wheel in a viability matrix (*left panels*) and using synergy maps using zero interaction potency (ZIP) synergy scores and a threshold ZIP>10 considered as significant (*right panels*). **E,** Synergy matrices in MOLM-14 and MV4-11 cells incubated with QUIZ, Midostaurin or Crenolanib and APR-246 for 48h. Cell viability was measured using a luminescence-based assay (Alamar Blue) and results are expressed as a ratio to the untreated condition for each wheel in a viability matrix (*left panels*) and using synergy maps using zero interaction potency (ZIP) synergy scores and a threshold ZIP>10 considered as significant (*right panels*). **F-G,** MOLM-14 and MV4-11 cells transduced with CTL or FLT3-targeting shRNAs were cultured with vehicle or 30-5µM APR-246, respectively, for 24h. **F,** Cell viability measured using PI staining. **G,** Lipid peroxidation measured using C11 Bodipy staining. Vertical bars indicate standard deviations. **p<0.01, ***p<0.001.

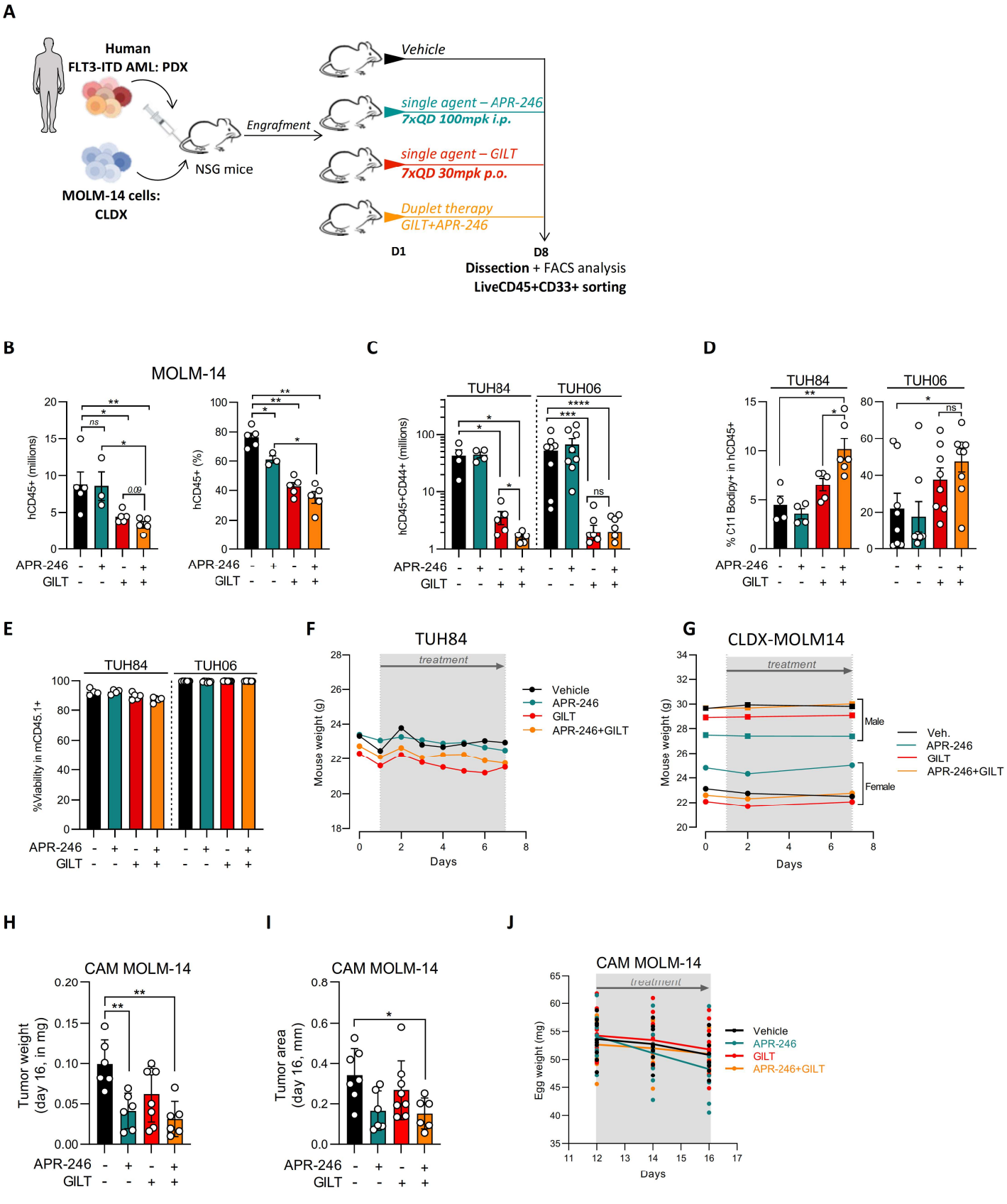

A
Human
FLT3-ITD AML: PDX
MOLM-14 cells:
CLDX
NSG mice
Engrafment
Vehicle
single agent – APR-246
7xQD 100mpk i.p.
single agent – GILT
7xQD 30mpk p.o.
Duplet therapy
GILT+APR-246
D1
D8
Dissection + FACS analysis
LiveCD45+CD33+ sorting
B
MOLM-14
hCD45+ (millions)
hCD45+ (%)
APR-246
GILT
C
TUH84
TUH06
hCD45+CD44+ (millions)
D
% C11 Bodipy+ in hCD45+
E
%Viability in mCD45.1+
F
TUH84
treatment
Mouse weight (g)
Days
Vehicle
APR-246
GILT
APR-246+GILT
G
CLDX-MOLM14
Male
Female
Veh.
H
CAM MOLM-14
Tumor weight (day 16, in mg)
I
Tumor area (day 16, mm)
J
Egg weight (mg)

Figure S22

Supplementary Figure S22. Combined treatment with GILT and APR-246 in preclinical AML models *in vivo*. **A,** PDX and cell line-derived xenograft (CLDX) assays using MOLM-14 cell line. After engraftment, mice were treated with vehicles, 100mg/kg/day APR-246 given by i.p., 30mg/kg/day GILT for PDX and 10mg/kg/day GILT for CLDX given by oral gavage, or combination of these two agents for 7 days (n= 5 to 8 mice per group). **B,** Number of viable hCD45+ cells (*left panel)* and percentage of viable hCD45+ AML cells *(right panel)* in bone marrow of CLDX MOLM14 mice. **C,** Number of viable hCD45+CD44+ AML cells in bone marrow and spleen from PDX^{TUH84} and PDX^{TUH06}. **D,** Lipid peroxidation measured by C11-bodipy staining in hCD45+ AML cells from PDX^{TUH84} and PDX^{TUH06}. **E,** Viability measurement of murine hematopoietic mCD45.1+ cells from PDX^{TUH84} *(left panel)* and PDX^{TUH06} *(right panel)* using Annexin V staining. **F,** Mice weight monitoring during treatment of PDX^{TUH84}. **G,** Mice weight monitoring during MOLM-14 CLDX assays. Weight is normalized to the initial weight by mouse before treatment start. **H-J,** Chick chorioallantoic membrane (CAM) assay of MOLM-14 transplantation treated with vehicle, 30µM APR-246, 30nM GILT or combination for 7 days. Tumor weight **(H)** and area **(I)** in eggs collected at day 16. **J,** Eggs weight monitoring during treatment.