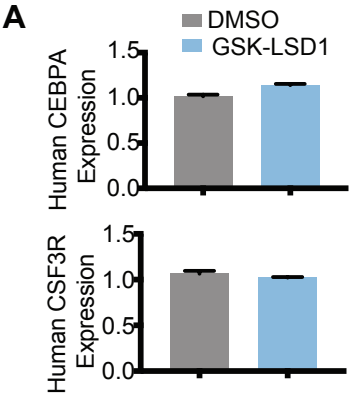

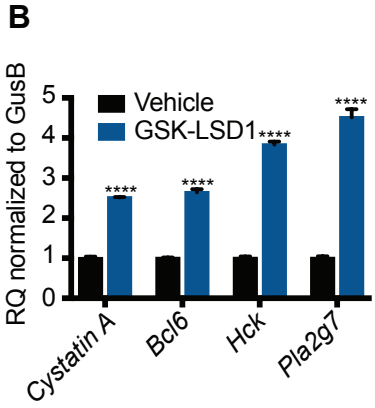

C

Overlap with Myb Peaks	P value enrichment	P value depletion
LSD1i-activated enhancers	7.9853e-69	1
LSD1i-unaffected enhancers	1	0

www.pnas.org/cgi/doi/10.1073/pnas.1918307117

Figure S1.

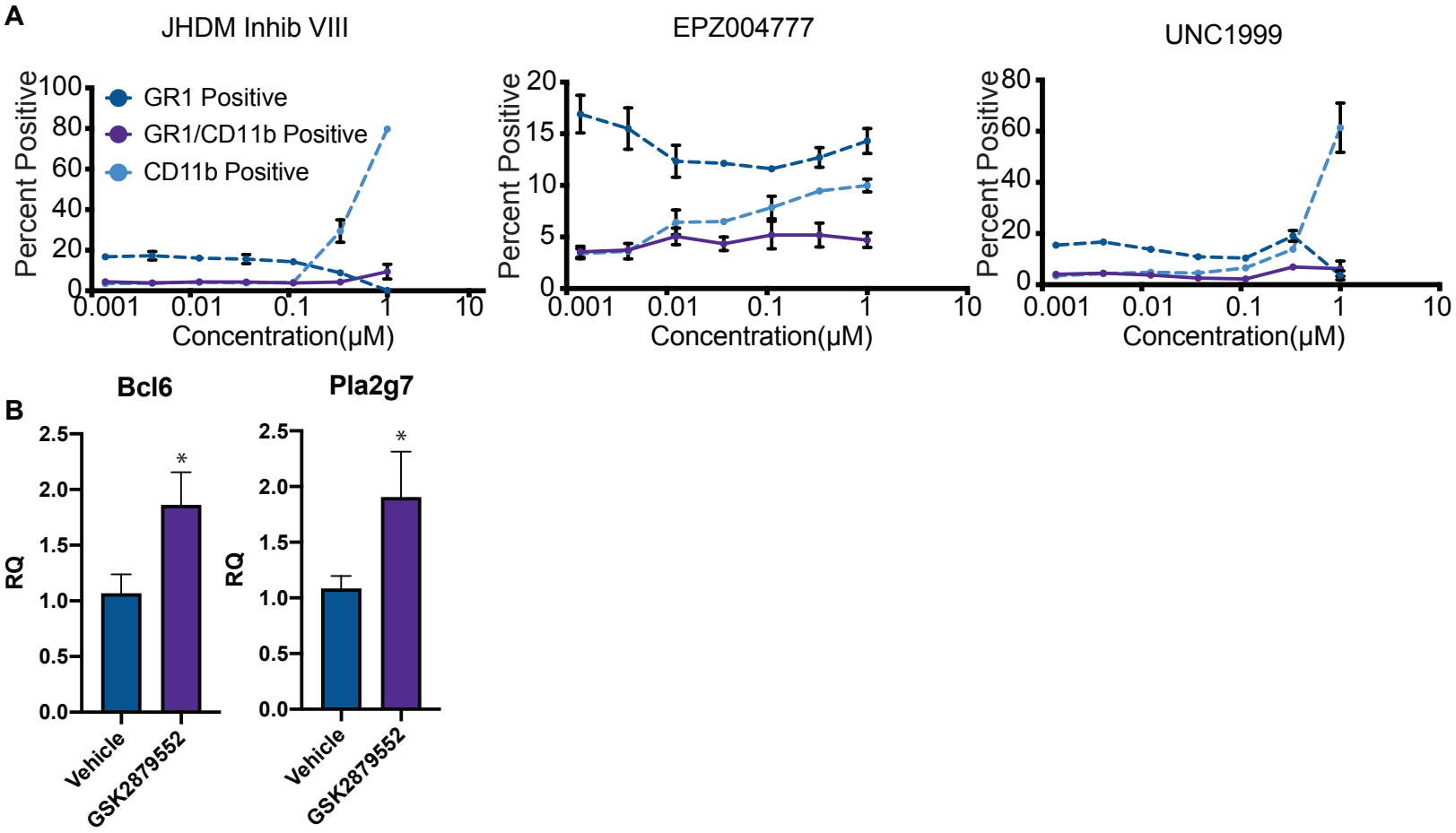
A
JHDM Inhib VIII
EPZ004777
UNC1999
Percent Positive
GR1 Positive
GR1/CD11b Positive
CD11b Positive
Concentration(μM)
0.001
0.01
0.1
1
10
B
Bcl6
Pla2g7
RQ
Vehicle
GSK2879552
*

Figure S2.

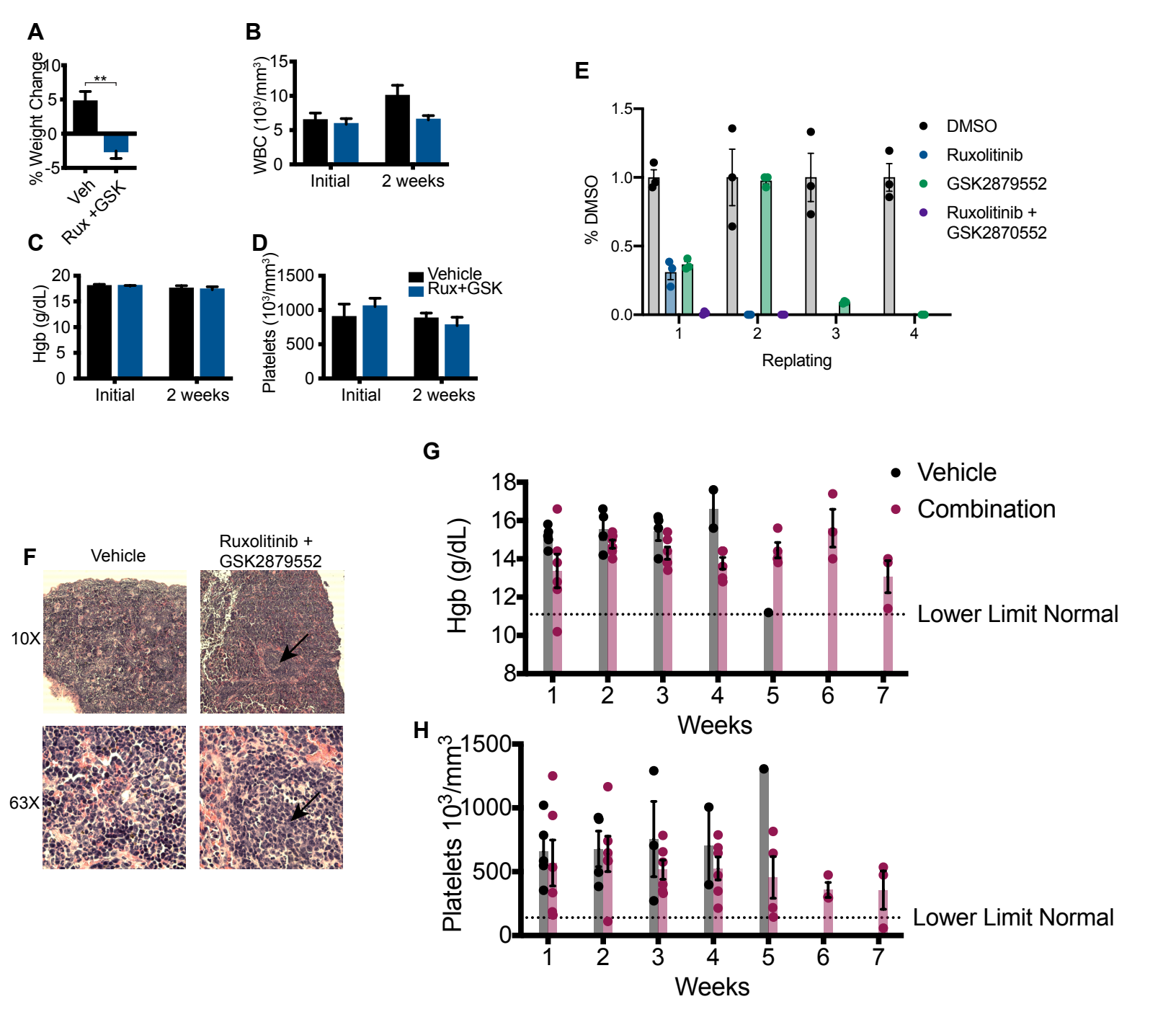
A
% Weight Change
**
Veh
Rux +GSK
B
WBC (10³/mm³)
Initial
2 weeks
C
Hgb (g/dL)
Initial
2 weeks
D
Platelets (10³/mm³)
Vehicle
Rux+GSK
Initial
2 weeks
E
% DMSO
DMSO
Ruxolitinib
GSK2879552
Ruxolitinib + GSK2870552
Replating
F
Vehicle
Ruxolitinib + GSK2879552
10X
63X
G
Hgb (g/dL)
Vehicle
Combination
Lower Limit Normal
Weeks
H
Platelets 10³/mm³
Lower Limit Normal
Weeks

Figure S3.

Supplementary Figure Legends

Supplementary Figure 1. LSD1 Inhibition Reactivates Differentiation-Associated Enhancers.
A. Human CEBPA expression in murine bone marrow cells immortalized via transduction with $CSF3R^{T618I}$ and $CEBPA^{V314VW}$ treated with GSK-LSD1 (4 nM for 48 hours) or DMSO relative to mouse *Gusb* (n=3/group). **B.** Expression of select differentiation associated genes in murine bone marrow cells immortalized through expression of $CEBPA^{V314VW}$ + $CSF3R^{T618I}$ and treated for 48 hours with 4 nM GSK-LSD1 (n=3/group). **C.** Overlap of Myb ChIP-seq peaks with LSD1-inhibitor activated enhancers assessed by bedtools fisher test.

Supplementary Figure 2. Epigenetic Agents Promote Differentiation in CEBPA/CSF3R Mutant AML with Differing Potency.
A. Myeloid differentiation in CEBPA/CSF3R AML cells treated with varying concentrations of the indicated drug for 48 hours as assessed by flow cytometry for GR1 and CD11b. **B**. Expression of Bcl6 and Pla2g7 in the bone marrow of mice harboring CSF3R/CEBPA mutant AML 24 hours after initiation of GSK2879552 treatment (0.75 mg/kg twice daily). Values are represented as mean +/-, * = $p<0.05$ by Students t-test, n=9/group.

Supplementary Figure 3. Combined Inhibition of the JAK/STAT Pathway and LSD1 is Well Tolerated and Restores Normal Splenic Architecture.
A. BALB/c mice were treated with Ruxolitinib (90 mg/kg/day) and GSK 2879552 (1.5 mg/kg/day) by twice daily oral gavage for 2 weeks (n=5/group). Weight change during treatment. **B.** WBC count pre and post treatment. **C** Hemoglobin pre and post treatment. **D.** Platelets pre and post treatment. **E.** Serial replating of CSF3R/CEBPA mutant AML cells exposed to Ruxolitinib (100 nM), GSK2879552 (100nM), the combination or DMSO. **F.** Representative images of spleens at survival endpoint. Arrow denotes restoration of follicular architecture in mice treated with Ruxolitinib + GSK2879552. **G, H** Platelets and Hbg during combined Ruxolitinib and GSK2879552 treatment in mice harboring CSF3R/CEBPA mutant AML from Figure 4G. In all cases, values are represented as mean +/- SEM. **: $p<0.01$ by Students t-test.

Supplementary Tables

Supplementary Table 1. RNA Sequencing Analysis on CEBPA/CSF3R AML Cells Treated with LSD1 Inhibitor
Supplementary Table 2. Complete GO Analysis for LSD1 Inhibitor RNA Seq
Supplementary Table 3. Enhancer Analysis on CEBPA/CSF3R AML Cells Treated with LSD1 Inhibitor
Supplementary Table 4. Complete GO Analysis for LSD1 Inhibitor Histone Mark ChIP Seq
Supplementary Table 5. RNA Sequencing Analysis on CEBPA/CSF3R AML Cells Treated with JAK/STAT and LSD1 Inhibitor

Supplementary Table 6. Complete GO Analysis from RNA Sequencing on CEBPA/CSF3R AML Cells Treated with JAK/STAT and LSD1 Inhibitor

Supplementary Table 7. Transcription Factor Overlap with DE Genes from RNA Sequencing on CEBPA/CSF3R AML Cells Treated with JAK/STAT and LSD1 Inhibitor