Supplementary Materials (*Curtiss et al.*)

Supplementary Methods

Cell Lines

Kasumi-1 (ATCC), CMK (DSMZ) and MOLM-13 (DSMZ) cells were cultured in RPMI (Gibco) supplemented with 20% fetal bovine serum (FBS, HyClone), 2 mM GlutaMAX (Gibco), 100 units/mL Penicillin, and 100 ug/mL Streptomycin (Gibco). K562 (ATCC) cells were cultured in RPMI supplemented with 10% FBS. SNKO-1 cells were obtained from DSMZ and cultured in RPMI with 10% FSB and 10 ng/ml GM-CSF (PeproTech). All cells were cultured at 5% CO_2 and 37°C. Cell lines were tested monthly for mycoplasma contamination.

Patient Samples

All patients gave informed consent to participate in this study, which had the approval and guidance of the institutional review boards at Oregon Health & Science University (OHSU), University of Utah, University of Texas Medical Center (UT Southwestern), Stanford University, University of Miami, University of Colorado, University of Florida, National Institutes of Health (NIH), Fox Chase Cancer Center and University of Kansas (KUMC). Samples were sent to the coordinating center (OHSU; IRB#9570; #4422; NCT01728402) where they were coded and processed. Mononuclear cells were isolated by Ficoll gradient centrifugation from freshly obtained bone marrow aspirates or peripheral blood draws. Clinical, prognostic, genetic, cytogenetic, and pathologic lab values as well as treatment and outcome data were manually curated from patient electronic medical records. Genetic characterization of the leukemia samples included results of a clinical deep- sequencing panel of genes commonly mutated in hematologic malignancies (Sequenome and GeneTrails (OHSU); Foundation Medicine (UTSW); Genoptix; and Illumina). Patient sample mononuclear cells were cultured in RPMI with 10% FBS and 50% HS-5 conditioned media (ATCC) or SFEMII supplemented with 1x StemSpan CD34+ Expansion Media and 1 uM UM729 (StemCell Technologies).

Mice

Wild-type C57BL/6J mice (JAX# 000664), were obtained from the Jackson Laboratories. All animals were maintained on a normal 12:12 h light:dark cycle and provided ad

libitum access to water and food (Purina rodent diet 5001; Purina Mills). Female mice, eight weeks of age, were used for experimentation. No statistical methods were used to estimate sample size. All experiments were conducted in accordance with the NIH *Guide for the Care and Use of Laboratory Animals*(45) and approved by the Institutional Animal Care and Use Committee of Oregon Health & Science University.

Histology

Prior to staining, Kasumi-1 cells were treated with avapritinib (12 nM; Selleck) and/or ORY-1001 (12 nM; Selleck) for 72 h. Cells were prepared for CytoSpin 3 Shandon as described by manufacturer's protocol (ThermoShandon). Slides were fixed and stained using May-Grunwald Giemsa. Samples were imaged using Zeiss ApoTome housed in the Advanced Light Microscopy Core (ALMC) at OHSU.

Colony Assay

Whole bone marrow was obtained (AllCells) and CD34+ cells were selected using CD34 MicroBead Kit (Miltenyi Biotec) according to manufacturer's instructions. For the colony assay, 500 CD34+ cells were used per replicate and plated in MethoCultTM H4435 Enriched (StemCell Technologies). The four groups were treated with avapritinib (12 nM), ORY-1001 (12 nM), avapritinib and ORY-1001 (12 nM/drug), or DMSO. Plates were incubated for 14 days in 5% CO2 and 37°C. Samples were imaged using STEMvision (StemCell Technologies) and blinded prior to counting by another investigator by assigning letters randomly. ImageJ (NIH) was used to count colonies after blinding.

Drug Synergy

Drug synergy was assessed using an 8 x 8 matrix of drug concentrations. Avapritinib, ORY-1001, GSK-LSD1, and Wortmannin (Selleck) were used for these studies. Cells were treated for 72 h prior to MTS assay to evaluate viability. Cell viability was used to calculate drug synergy with SynergyFinder based on the ZIP reference model(44).

Flow Cytometry

To assess apoptosis, Kasumi-1 cells were treated with 12 nM ORY-1001, 36 nM avapritinib, 12 nM ORY-1001/36 nM avapritinib or DMSO for 48 h. APC Annexin V apoptosis detection kit (Invitrogen) was used according to the manufacture's protocol. To assess myeloid differentiation, Kasumi-1 cells were treated with 12 nM ORY-1001, 12 nM avapritinib, 12 nM ORY-1001/12 nM avapritinib or DMSO for 24, 48, and 72 h. PerCP-

e710 CD86 (Invitrogen), PE-Cy7 CD11b (eBioScience), and APC CD84 (Biolegend) was used per the manufacture's protocol. For cell cycle analysis, Kasumi-1 cells were treated with 12 nM ORY-1001, 36 nM avapritinib, 12 nM ORY-1001/12 nM avapritinib or DMSO for 24, 48, and 72 h. Cells were fixed using ethyl alcohol and stained with propidium iodide (BioLegend). Strained cells were analyzed on a LSRFortessa flow cytometer (BD) housed in the OHSU Flow Cytometry Shared Resource.

Bulk RNA-sequencing

Kasumi-1 cells were treated with 12 nM GSK-LSD1, 12 nM avapritinib, 12 nM GSK-LSD1/12 nM avapritinib or DMSO for 12 h. Total RNA was isolated using a RNeasy Plus Mini Kit (Qiagen). BGI performed the library preparation and sequencing at 50 base pair (BP) paired end (PE). For the Kasumi-1 PU.1 KD experiment, cells were treated with 1 ug/ml doxycycline, 50 nM avapritinib, 1 ug/ml doxycycline/50 nM avapritinib, or DMSO for 24 h. Total RNA was isolated with RNeasy Plus Mini Kit (Qiagen) and libraries were prepared with Zymo-Seq RiboFree Total RNA Library Kit (Zymo). The OHSU Massively Parallel Sequencing Shared Resource (MPSSR) performed the sequencing at 100 BP single end.

Western Blots

Kasumi-1 cells were treated with 12 nM ORY-1001, 12 nM avapritinib, 12 nM ORY-1001/12 nM avapritinib or DMSO for 24 h. Cells were lysed in cell lysis buffer (Cell Signaling Technologies) containing complete mini protease inhibitor tablets (Roche) plus phosphatase inhibitor cocktail 2 (Sigma Aldrich) and PMSF (Sigma Aldrich) for phospho-AKT western blots. Samples were spun at 14,000 x g for 10 minutes at 4°C. Lysates were mixed with 3x SDS sample buffer (75 mmol/L Tris (pH 6.8), 3% SDS, 15% glycerol, 8% β-mercaptoethanol, and 0.1% bromophenol blue) then incubated for 5 minutes at 95°C. Samples were run on Criterion 4–15% Tris-HCl gradient gels (Bio-Rad). Gels were transferred to PVDF membranes, then blocked in Tris-buffered saline with 0.05% Tween (TBST) and 5% milk or EveryBlot Blocking Buffer (Bio-Rad). Blots were probed with MYC (#13987, CST), PU.1 (MA5-15064, Invitrogen), LSD1 (#2139, CST), KIT (#3074, CST), AKT (#9272, CST), pAKT S473 (#4060, CST), EZH2 (#5246, CST), STAT5 (#94205, CST), or β-actin (#8457, CST) antibodies at 1:1000 in TBST overnight at 4°C. Membranes were incubated for 1 hour at room temperature with HRP-

conjugated secondary antibody (#7074S, CST) at 1:5000. SuperSignalTM West Pico PLUS Chemiluminescent Substrate (ThermoFisher) was used to develop blots. Imaging was done on BioRad ChemiDoc Imaging System and analyzed using Image Lab (Bio-Rad).

Cloning

Wild type *MYC* (pDONR223_MYC_WT was a gift from Jesse Boehm & Matthew Meyerson & David Root; Addgene plasmid # 82927 ; http://n2t.net/addgene:82927 ; RRID:Addgene_82927) was cloned into a Gateway-converted pMSCV-IRES-GFP vector (a gift from Tannishtha Reya; Addgene plasmid #20672; http://n2t.net/addgene:20672; RRID:Addgene_20672). Plasmid sequences were confirmed via Sanger sequencing (Eurofins Genomics).

Retrovirus generation and transduction

HEK 293T17 cells (ATCC) were grown in DMEM (Gibco) medium with 10% FBS (HyClone), Glutamax (Gibco), and penicillin/streptomycin (Gibco). Retrovirus was generated by transfecting MYC pMSCV-IRES-GFP (pMIG) or control vector plasmids and the Eco-Pac plasmid (provided by Dr. Rick Van Etten) into the 293T17 cells using FuGENE 6 (Promega). Viral supernatants were harvested 48 and 72 h later. Kasumi-1 cells ($1x10^6$ cells/well) were spinoculated with viral supernatant, HEPES buffer, and polybrene on two subsequent days by centrifuging at 2500 rpm for 90 minutes at 30°C with no break. Transduced cells were isolated using a Symphony S6 (BD Biosciences).

CUT&Tag

Kasumi-1 cells were treated with 12 nM GSK-LSD1, 12 nM avapritinib, 12 nM GSK-LSD1/12 nM avapritinib or DMSO for 24 hr. Benchtop CUT&Tag was performed as previously described(46). Cells were counted, harvested, and centrifuged for 5 min at 300xg at room temperature. Cells were washed 2X in 1.5 mL wash buffer (20 mM HEPES pH 7.5, 150 mM NaCl, 0.5 mM Spermidine, 1× Protease inhibitor cocktail). Concanavalin A magnetic coated beads (Bangs Laboratories) were activated in binding buffer by washing 2X (20 mM HEPES pH 7.5, 10 mM KCl, 1 mM CaCl2, 1 mM MnCl2). Washed cells were separated into 100,000 cell aliquots and 10 ul of activated beads were added to each sample. Samples rotated end over end for 7 minutes at room temperature. A magnetic

stand was used to separate beads, supernatant was removed. Primary antibody was diluted 1:50 in antibody buffer (20 mM HEPES pH 7.5, 150mM NaCl, 0.5 mM Spermidine, 1× Protease inhibitor cocktail, 0.05% digitonin, 2 mM EDTA, 0.1% BSA). Cells were incubated overnight at 4°C on a nutator. Primary antibody was replaced with a guinea-pig anti rabbit secondary antibody diluted to 1:100 in wash buffer (Antibodies Online). Samples were incubated for 45 minutes at room temperature on nutator. Secondary antibody was removed and samples were washed 2X in dig-wash buffer (20 mM HEPES pH 7.5, 150 mM NaCl, 0.5 mM Spermidine, 1× Protease inhibitor cocktail, 0.05% Digitonin). pA-Tn5 transposase, prepared and loaded with adaptors, was diluted 1:100 in dig-300 buffer (20 mM HEPES pH 7.5, 300 mM NaCl, 0.5 mM Spermidine, 1× Protease inhibitor cocktail, 0.01% digitonin) and added to samples. Samples were incubated for 1 hour at room temperature on nutator. Samples were washed 2X with dig-300 buffer then resuspended in tagmentation buffer (dig-300 buffer with 1 mM MgCl2). Samples were incubated at 37°C for 1 hour. DNA was extracted with phenol:chloroform extraction. Samples were amplified by PCR using custom nextera primers at 400 nM and NEBNext HiFi 2x PCR Master Mix (New England Biolabs)(47). PCR conditions were set to: 72°C for 5 minutes, 98°C for 30 seconds, 14 cycles of 98°C for 10 sec, 63°C for 10 sec, and 72°C for 1 minute. Libraries were purified with AMPure Beads (Beckman) and sequenced on a NextSeq 500 sequencer (Illumina) using 37 BP PE sequencing by MPSSR.

Kasumi-1 PU.1 sh401 cells were treated with 1 ug/ml doxycycline, 50 nM avapritinib, 1 ug/ml doxycycline/50 nM avapritinib, or DMSO for 24 h. 14-00613 cells were treated with 12 nM ORY-1001, 350 nM avapritinib, 12 nM ORY-1001/350 nM avapritinib, or DMSO for 24 h. CUT&Tag was adapted from the benchtop version and was preformed similarly to the updated protocol(48). Nuclei were prepared by incubating cell on ice for 10 minutes with NE1 buffer (20 mM HEPES-KOH pH 7.9, 10 mM KCl, 0.5 mM spermidine, 0.1% Triton X-100, 10% glycerol, 1x Protease inhibitor cocktail). Cells were centrifuged at 1300xg for 4 minutes at 4°C and supernatant was removed. Nuclei extract was resuspended in wash buffer (20 mM HEPES pH 7.5, 150 mM NaCl, 0.5 mM Spermidine, 1× Protease inhibitor cocktail) and 10% DMSO and frozen at -80°C. Briefly, 100,000 nuclei were thawed and incubated with 5 ul activated Concanavalin A beads (Bangs Laboratory)

for 10 minutes at room temperature. Supernatant was removed and beads were resuspended in wash buffer. Samples were incubated with primary antibody diluted 1:100 in antibody buffer (wash buffer with 2 mM EDTA, 0.1% BSA) overnight at 4°C on the nutator. Primary antibody was removed, and beads incubated with guinea-pig anti rabbit secondary antibody diluted to 1:100 in wash buffer (Antibodies Online) for 30 minutes on nutator at room temperature. Beads were washed 2x with wash buffer. Beads incubated with pA-Tn5 (EpiCypher) diluted 1:20 in 300-wash buffer (20 mM HEPES pH 7.5, 300 mM NaCl, 0.5 mM Spermidine, 1× Protease inhibitor cocktail) for 1 h at room temperature on the nutator. Beads were washed 2x in 300-wash buffer and resuspended in tagmentation solution (300-wash buffer with 10 mM MgCl2). Samples incubated at 37°C for 1 hour in a PCR cycler with heated lid then were washed 1x in TAPS wash buffer (10 mM TAPS, 0.2 mM EDTA). Beads were resuspended in 0.1% SDS release solution (0.1% SDS, 10 mM TAPS) and incubated for 1 hour at 58°C in a PCR cycler with heated lid. Triton neutralization solution (0.67% triton-X100) was added to bead slurry. Samples were amplified by PCR using custom nextera primers at 400 nM and NEBNext HiFi 2x PCR Master Mix (New England Biolabs)(47). PCR conditions were set to: 58°C for 5 minutes, 72°C for 5 minutes, 98°C for 30 seconds, 12 cycles of 98°C for 10 sec, 60°C for 10 sec, and 72°C for 1 minute. Libraries were purified with HighPrep PCR Clean-up System (MagBio) and sequenced on a NextSeq 500 sequencer (Illumina) using 37 BP PE sequencing by MPSSR.

CUT&RUN

Kasumi-1 cells were treated with 12 nM GSK-LSD1, 12 nM avapritinib, 12 nM GSK-LSD1/12 nM avapritinib or DMSO for 12h or 24 h. CUT&RUN was performed as previously described(49). Briefly, concanavalin A magnetic coated beads (Bangs Laboratories) were washed 2x in binding buffer (20 mM HEPES pH 7.5, 10 mM KCl, 1 mM CaCl2, 1 mM MnCl2) to activate. 500,000 cells per replicate were washed 2x with wash buffer (20 mM HEPES pH 7.5, 150 mM NaCl, 0.5 mM Spermidine, 1× Protease inhibitor cocktail). Cells were bound to beads by nutating for 10 minutes at room temperature. Cells were permeabilized and incubated over night at 4°C on nutator with primary antibody in antibody buffer (wash buffer, 0.001% digitonin, 3 mM EDTA). Bead slurry was washed 2x with dig wash buffer (wash buffer, 0.001% dig) and resuspended with dig wash buffer and

1x pAG-MNase (Epicypher). Cell were incubated for 10 minutes on nutator at room temperature then washed 2x with dig wash buffer followed by resuspension in pAG-MNase reaction mix (dig wash buffer, 2 mM CaCl2). Bead slurry was incubated for 2 h at 4°C on nutator. STOP buffer (340 mM NaCl, 20 mM EDTA, 4 mM EGTA, 50 ug/mL RNase A, 50 ug/mL glycogen, 0.02% dig) was then added, then tubes were incubated at 37°C for 10 minutes. DNA was extracted using phenol:cholorform extraction. Libraries were prepared using NEBNext Ultra II DNA Library Prep Kit (NEB), modified for CUT&RUN as previously described(50). After adapter ligation fragments were cleaned up with 1.75x AMPure beads (Beckman). Following PCR amplification, libraries were purified 2x with 1.2x AMPure beads to rid of adaptor fragments. Libraries were quantified on the 2100 Bioanalyzer instrument (Agilent) with the High Sensitivity DNA Analysis Kit (Agilent). Libraries were pooled and sequenced by MPSSR on a NextSeq 500 sequencer (Illumina) using 37 BP PE sequencing.

RNA Interference

Two SMARTvector Inducible Human SPI1 hEF1a-TurboRFP shRNAs for SPI1/PU.1 were obtained from Horizon Discovery (230493625, 230705089). Lentivirus was produced by transfecting Lenti-X 293T cells (Clontech) with the SMARTvector transfer plasmid and packaging/pseudotyping plasmids. psPAX2 was a gift from Didier Trono (Addgene plasmid #12260; http://n2t.net/addgene:12260 ; RRID:Addgene_12260) and pMD2.G was a gift from Didier Trono (Addgene plasmid #12259 ; http://n2t.net/addgene:12259 ; RRID:Addgene_12259). The supernatants containing lentivirus was collected after 48 h of culture and filtered with a 0.45 um filter. Kasumi-1 cells were transduced with virus via spinnoculation in the presence of polybrene. Transduced cells were selected with 1 ug/ml puromycin to produce a stable cell line.

Fluorescent Biosensor Assay

Kasumi-1 cells were transduced with ERK-TB-pHAGE-Clover-H2BmiFRP670nano and AKT-TB-pHAGE-mScarlet-H2BmiRFP670nano derived from ERK-KTR (PMID: 24949979) and AKT-KTR (PMID: 27247077) respectively. Transduced cells were sorted on a BD FACSAria Fusion housed at the OHSU Flow Cytometry Shared Resource to enrich the triple positive cells (Clover+, mScarlet+, & miRFP670nano+). For time lapse imaging, biosensor-expressed cells were resuspended in FluoroBrite DMEM (Gibco), 1x

GlutaMAX (Gibco), and 20% FBS, and then seeded on a 96 well plate at density of ~10,000 cells/well. Cells were imaged on the Yokogawa CSU-X1 spinning disk confocal microscope housed in ALMC under cell culture-optimized temperature, humidity and CO2 levels. Cell images were captured every 15min for a total of 25 h after drug loading.

Reverse Phase Protein Array

Kasumi-1 cells were treated for 1 h and 24 h with 100nM avapritinib and 12 nM ORY-1001. Cells were washed 2x in PBS then flash frozen. Cell pellets were lysed and processed by the University of Texas MD Anderson Cancer Center Functional Proteomics RPPA Core Facility. Linear normalized values used for CausalPath and log normalized values used for heatmap generation.

ATAC sequencing

Patient sample 15-00807 was treated with 50 nM avapritinib, 50 nM ORY-1001, 50 nM avapritinib/50 nM ORY-1001, or DMSO for 24 h. Samples were prepared as previously described(51). In brief, cells were resuspended in cold PBS and tagmentation master mix (25 ul of 2× tagmentation buffer, 2.5 ul of TDE1 [Illumina], 0.5 ul of 1% digitonin; 2x tagmentation buffer: 66 mM Tris-Acetate, pH 7.8, 132 mM potassium acetate, 20 mM magnesium acetate, 32% v/v N,N-Dimethylformamide) was added. Samples were incubated at 37°C for 30 minutes. DNA was purified using Zymo Clean and Concentrator 5 Kit (Zymo). Transposed DNA was amplified and purified as described previously with adapted primers(34,47). Samples were quantified using Qubit dsDNA HS Assay Kit (Invitrogen), pooled, and sequenced by Genewiz with a HiSeq-X (Illumina) using 75 BP PE sequencing.

Single Cell RNA Sequencing

Patient sample 14-00613 was treated with 12 nM ORY-1001, 12 nM avapritinib, 12 nM ORY-1001/12 nM avapritinib or DMSO for 24 h then harvested. Cells were loaded onto the Chromium Controller (10X Genomics) according to the manufacturer's instructions. RNA libraries were prepared per the manufacturer's protocol, Chromium Next GEM Single Cell 3' Reagent v3.0 Kit (Biolegend, 10X Genomics). Libraries were sequenced on a HiSeq2500 (Illumina) using 100 BP PE sequencing.

Data Analysis

AML LSD1 expression analysis

Gene expression data in the form of RPKM was obtained through the BeatAML database(14,15). Patients with mutant KIT and healthy controls were included in the analysis.

CUT&Tag and CUT&RUN Analysis

CUT&Tag and CUT&RUN libraries were aligned to the human genome (hg38) using Bowtie2(52) and the following options --local --very-sensitive-local --no-unal --no-mixed --no-discordant --phred33 -I 10 -X 700. Peaks were called using GoPeaks(53). High confidence peaks were defined by presence in 2/2 or 2/3 replicates. Consensus bed files were formed by merging the high confidence peaks from DMSO, avapritinib, ORY-1001, and combination using BEDTools(54). Differential peaks were identified using DESeq2(55) with default parameters. Heatmaps were produced using the ComplexHeatmap package from Bioconductor(56). Peaks were annotated to the nearest feature using ChIPseeker(57). GO analysis was performed using Genomic Regions Enrichment of Annotations Tool (GREAT)(58). Counts tables for differential peaks were produced using multicov from BEDTools(54). Counts per million (CPM) normalized tracks, global signal heatmaps, and plot profiles at specified regions were generated using DeepTools(59). The DeepTools matrix was extracted and used to assess differences in H3K27Ac signal. Active promoters were defined by the presence of H3K4me3 and H3K27Ac within 1000 bp of a TSS. Active enhancers were defined by the presence of H3K4me1 and H3K27Ac beyond 1000 bp of a TSS. CPM normalized tracks were merged with bigWigMerge(60) and visualized using Integrative Genomics Viewer(61).

ATAC Analysis

ATAC libraries were aligned to the human genome (hg38) using BWA-MEM(62) and sorted using SAMtools(63). Duplicates were marked with Sambamba(64), duplicates and mitochondrial reads were removed. CPM normalized tracks were generated with DeepTools(59). Differential accessibility was assessed with DESeq2(55). GO analysis was performed using GREAT(58). CPM normalized tracks were merged with bigWigMerge(60) and visualized using Integrative Genomics Viewer(61).

Bulk RNA Sequencing Analysis

Raw reads were trimmed with Trimmomatic(65) and aligned with STAR(66). Differential expression analysis was performed using DESeq2(55). Raw p values were adjusted for

multiple comparisons using the Benjamini-Hochberg method. GO analysis was performed using Enrichr(67) and Gene Set Enrichment Analysis (GSEA)(68).

Single Cell RNA Sequencing Analysis

Sequencing output from 10X mRNA sequencing libraries were aligned to human genome (hg38) using CellRanger. Filtered feature matrices were analyzed using Seurat(69). Cells expressing greater than 20% mitochondrial RNA were excluded as non-viable. Data integration revealed seven transcriptional clusters. Cluster identity was established via expression of early (CD34, SOX4, ERG, MYB, and GATA2), mid (CEBPD), and late (LYZ, ITGAX, CD14) features of hematopoiesis.

Fluorescent Biosensor Analysis

Cells were tracked and subcellular fluorescence signals were measured using IMARIS software at ALMC. Heatmaps and lineplots of AKT and ERK activity were created using customized Python algorithm and Excel. Cells were separated into different AKT dynamics clusters by hierarchical clustering. Cell number was scaled to be equal across conditions and the abundance of differently treated cells per each cluster was calculated.

Quantification and Statistical Analysis

Data are shown as mean ± SEM. Prism software (version 9.1; Prism Software Corp.) or RStudio was used to perform statistical analysis, which are described in the figure legends. All data were analyzed with Kolmogorov-Smirnov (KS) test, Mann-Whitney test, or ANOVA followed by Holm-Sidak correction. For differential analysis of RNA-seq, CUT&Tag, CUT&RUN, and ATAC-seq *p*-values were adjusted for repeated testing with a false discovery rate using the Benjamini-Hochberg method(70).

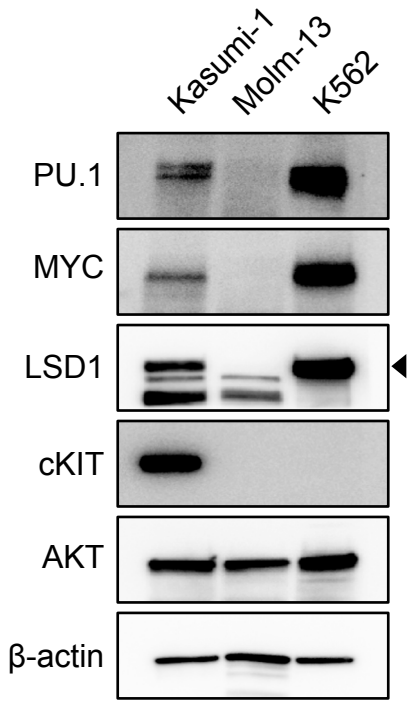
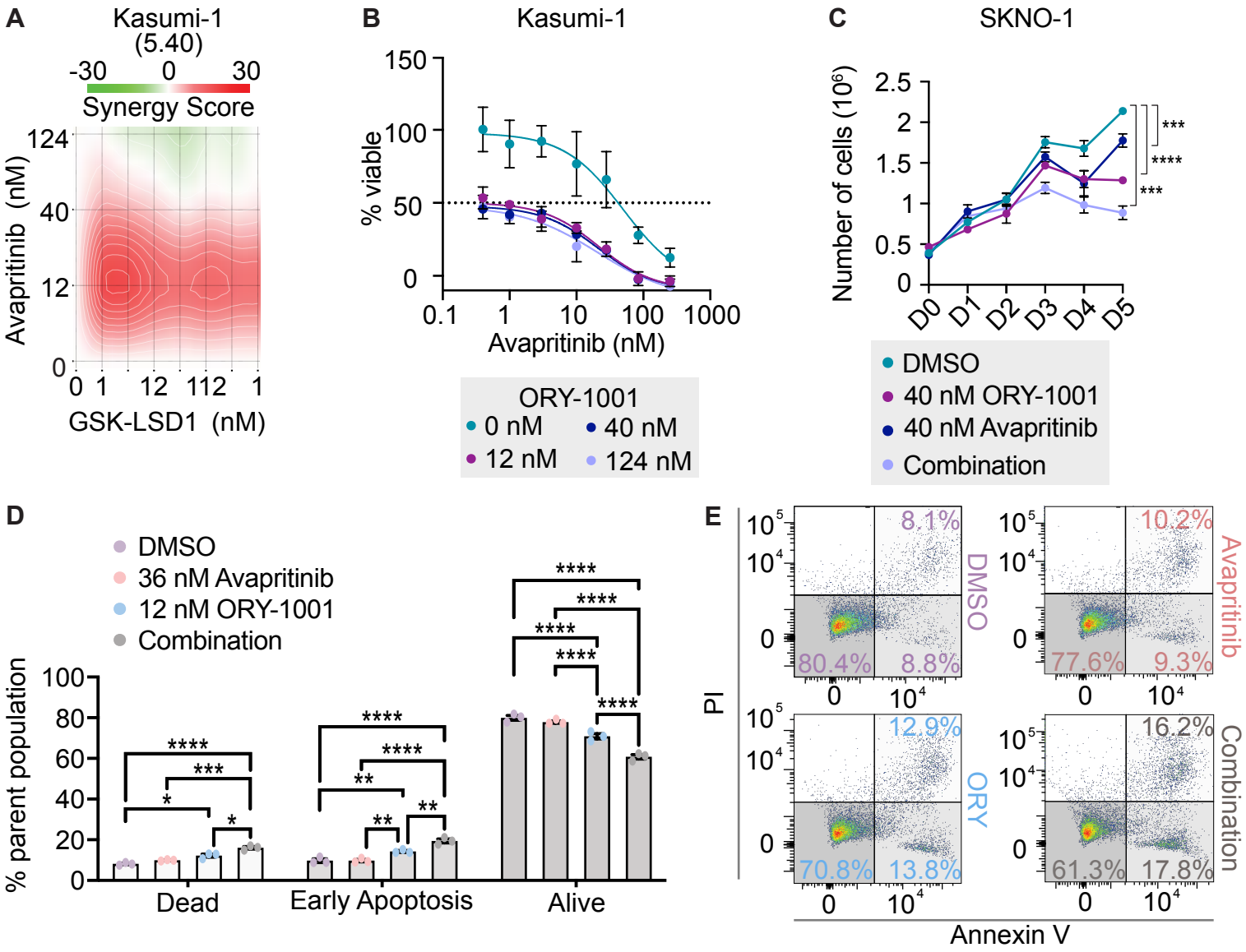


Figure S1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Kasumi-1
(5.40)
-30
0
30
Synergy Score
Avapritinib (nM)
124
40
12
0
0 1 12 112 1
GSK-LSD1 (nM)
B
Kasumi-1
% viable
150
100
50
0
0.1 1 10 100 1000
Avapritinib (nM)
ORY-1001
0 nM
40 nM
12 nM
124 nM
C
SKNO-1
Number of cells (10^6)
2.5
2
1.5
1
0.5
0
D0 D1 D2 D3 D4 D5
***
****
***
DMSO
40 nM ORY-1001
40 nM Avapritinib
Combination
D
DMSO
36 nM Avapritinib
12 nM ORY-1001
Combination
% parent population
100
80
60
40
20
0
Dead
Early Apoptosis
Alive
E
PI
Annexin V
DMSO
Avapritinib
ORY
Combination
8.1%
80.4%
8.8%
10.2%
77.6%
9.3%
12.9%
70.8%
13.8%
16.2%
61.3%
17.8%

Figure S2

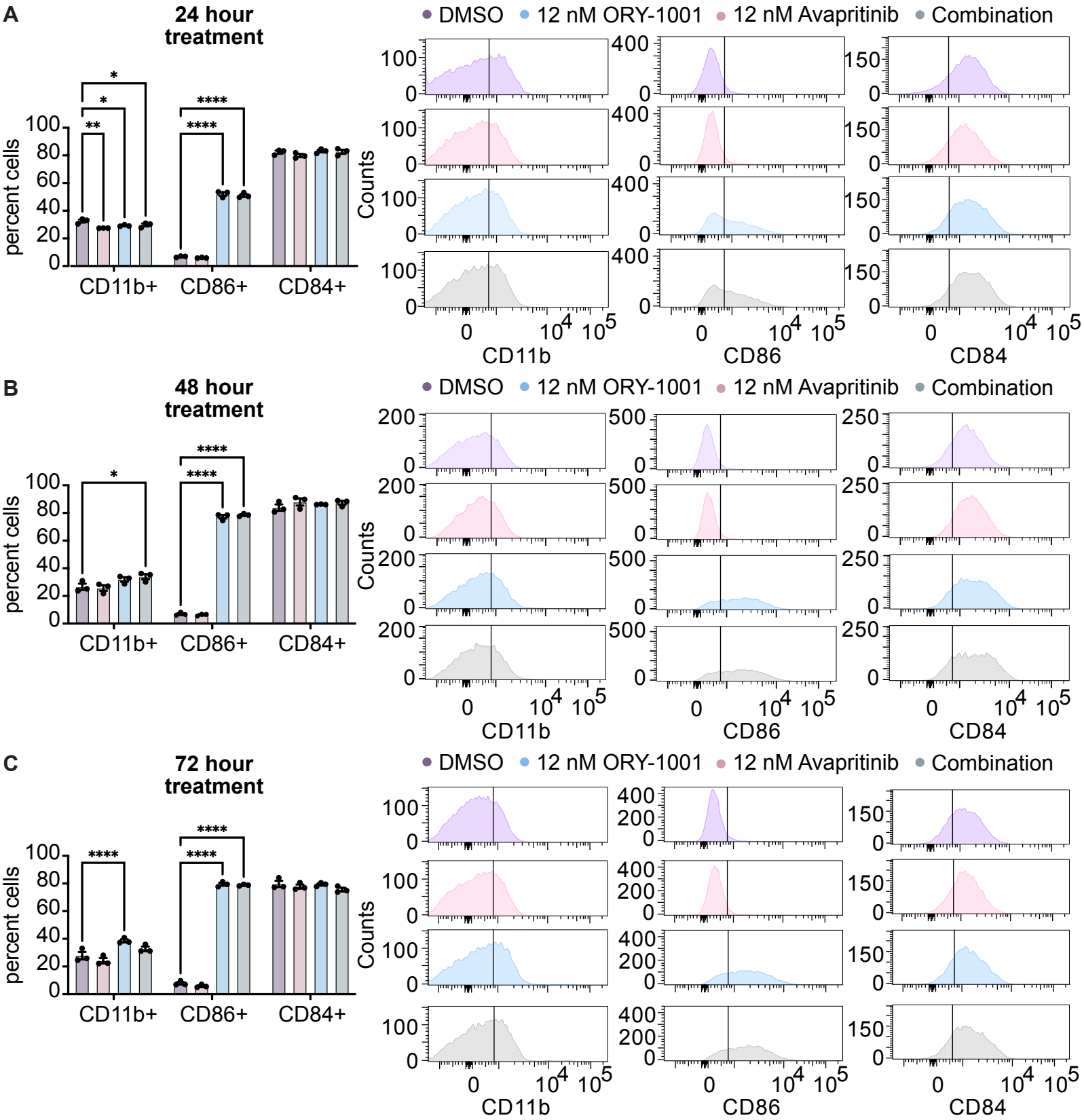

A
24 hour treatment
DMSO
12 nM ORY-1001
12 nM Avapritinib
Combination
percent cells
CD11b+
CD86+
CD84+
Counts
CD11b
CD86
CD84
B
48 hour treatment
C
72 hour treatment
D
DMSO
Avapritinib
GSK-LSD1
Avapritinib/ GSK-LSD1
ORY-1001
Avapritinib/ ORY-1001

Figure S3

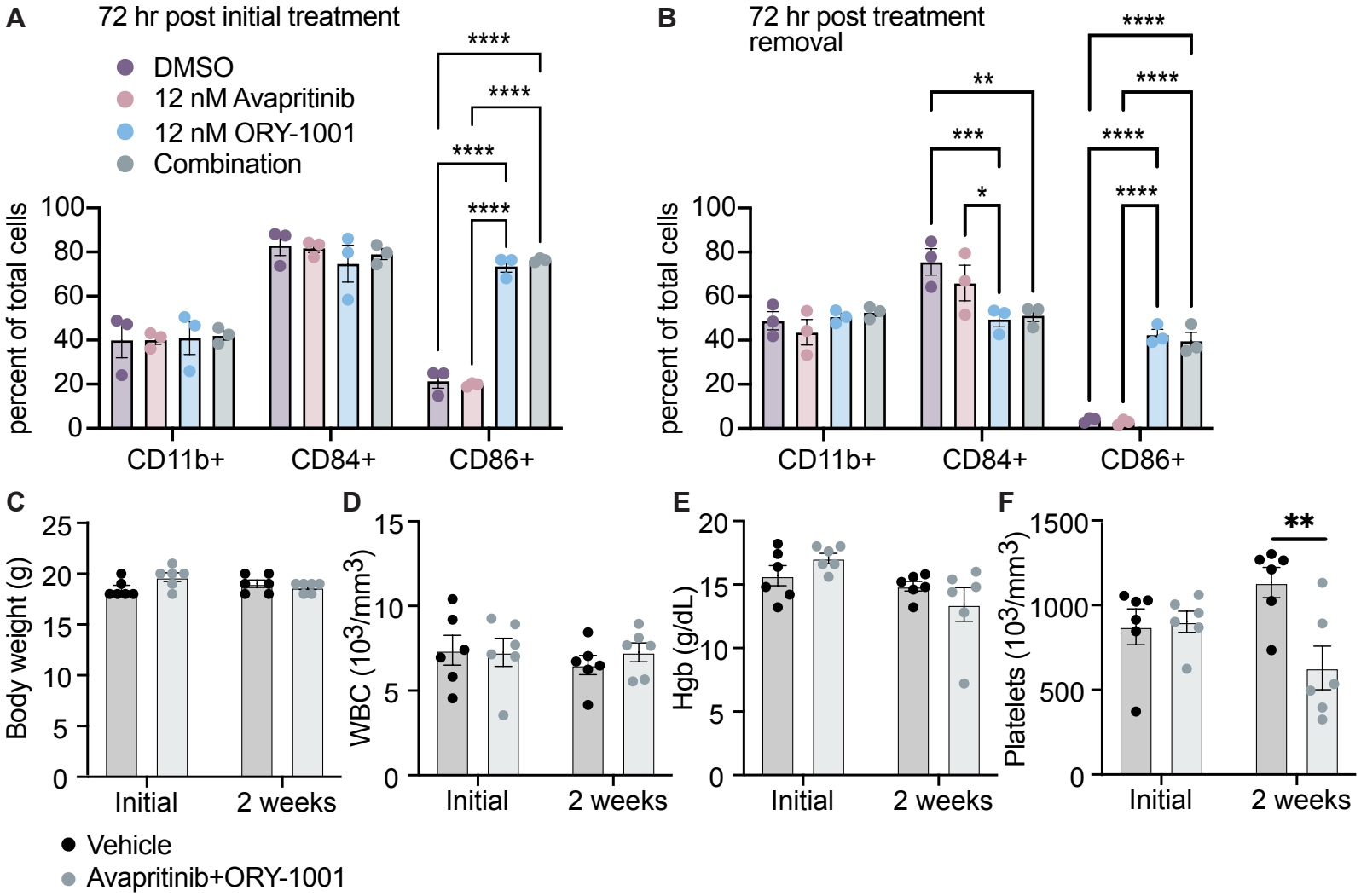

A
72 hr post initial treatment
DMSO
12 nM Avapritinib
12 nM ORY-1001
Combination
percent of total cells
CD11b+
CD84+
CD86+
B
72 hr post treatment removal
C
Body weight (g)
Initial
2 weeks
D
WBC (10^3/mm^3)
E
Hgb (g/dL)
F
Platelets (10^3/mm^3)
Vehicle
Avapritinib+ORY-1001

Figure S4

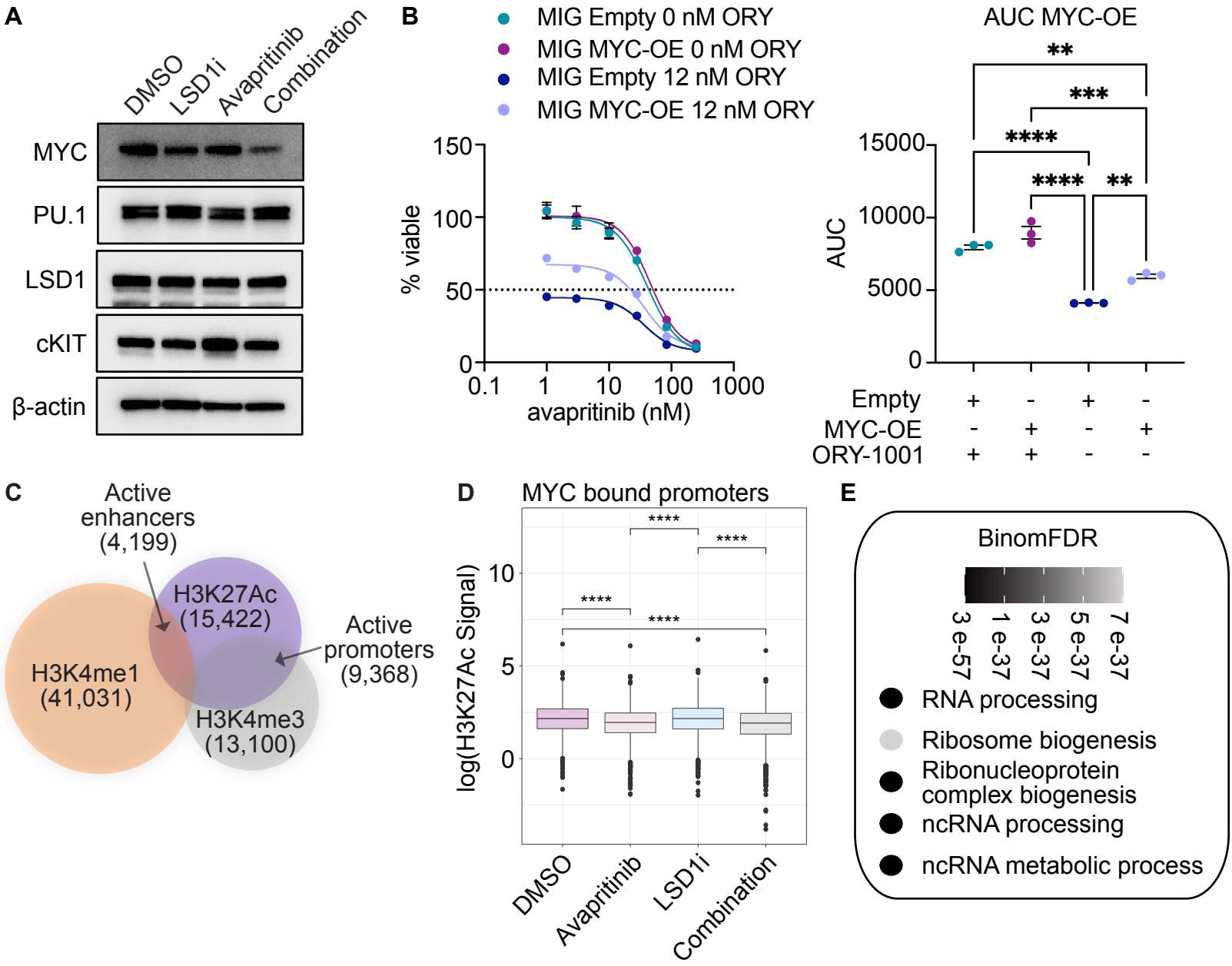

A
DMSO
LSD1i
Avapritinib
Combination
MYC
PU.1
LSD1
cKIT
β-actin
B
MIG Empty 0 nM ORY
MIG MYC-OE 0 nM ORY
MIG Empty 12 nM ORY
MIG MYC-OE 12 nM ORY
% viable
avapritinib (nM)
AUC MYC-OE
AUC
Empty
MYC-OE
ORY-1001
C
Active enhancers (4,199)
H3K27Ac (15,422)
H3K4me1 (41,031)
H3K4me3 (13,100)
Active promoters (9,368)
D
MYC bound promoters
log(H3K27Ac Signal)
DMSO
Avapritinib
LSD1i
Combination
E
BinomFDR
3 e-57
1 e-37
3 e-37
5 e-37
7 e-37
RNA processing
Ribosome biogenesis
Ribonucleoprotein complex biogenesis
ncRNA processing
ncRNA metabolic process

Figure S5

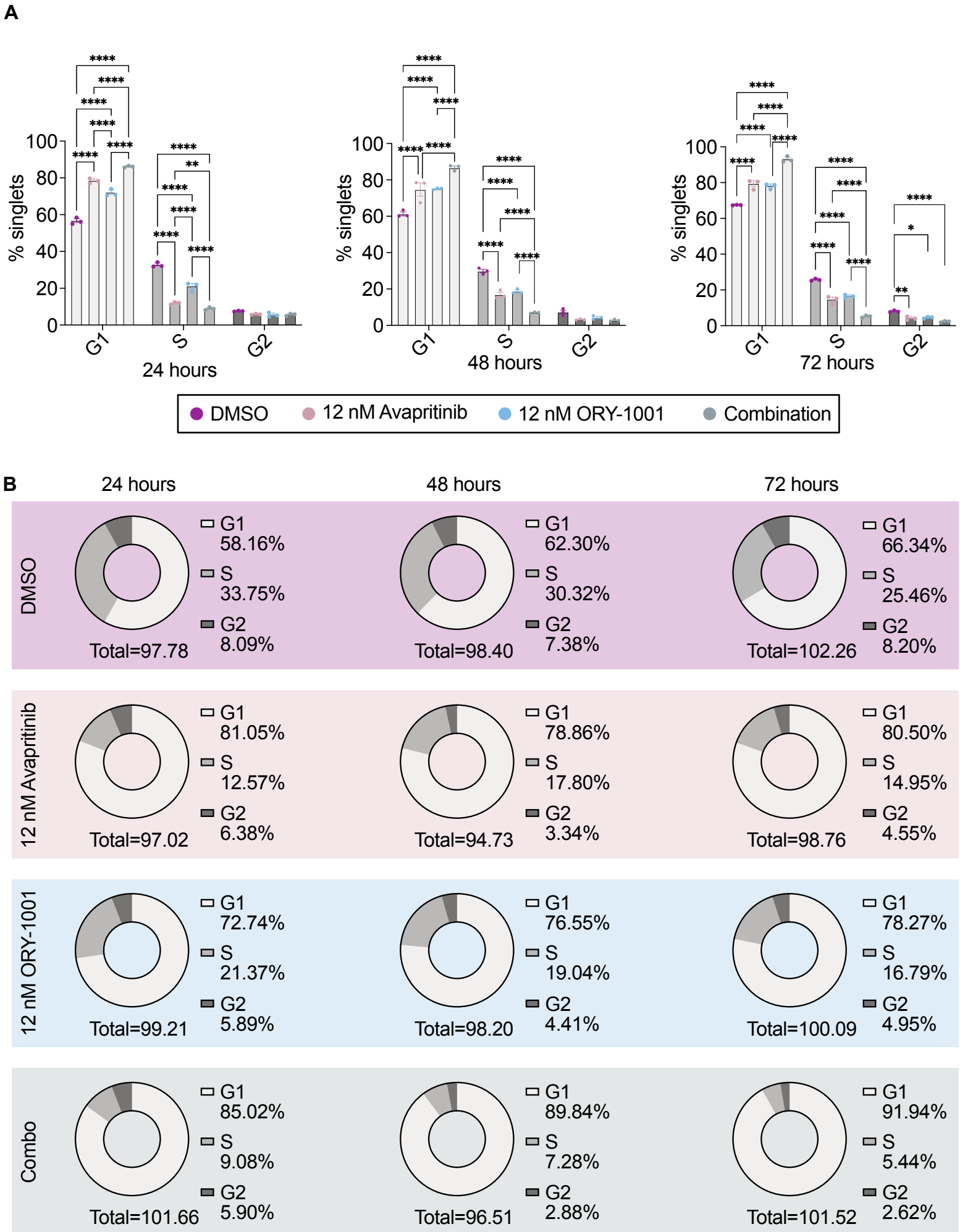
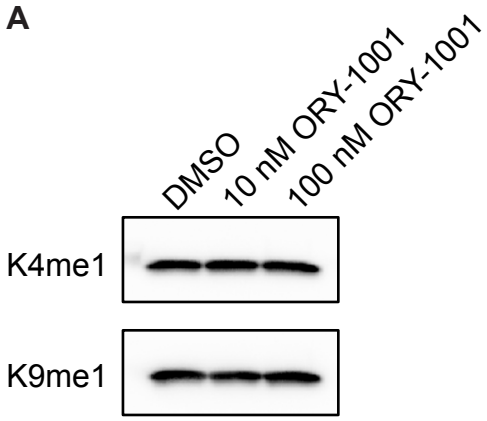
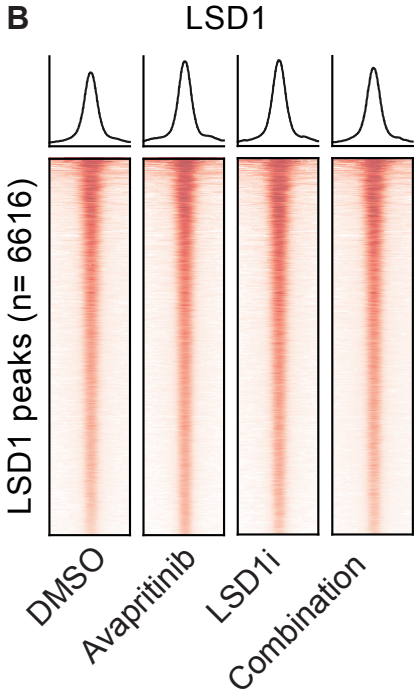
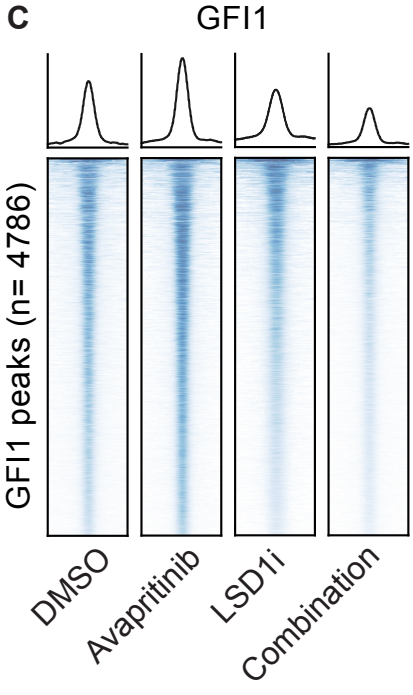

A
% singlets
100
80
60
40
20
0
G1
S
G2
24 hours
48 hours
72 hours
DMSO
12 nM Avapritinib
12 nM ORY-1001
Combination
B
24 hours
48 hours
72 hours
DMSO
G1 58.16%
S 33.75%
G2 8.09%
Total=97.78
G1 62.30%
S 30.32%
G2 7.38%
Total=98.40
G1 66.34%
S 25.46%
G2 8.20%
Total=102.26
12 nM Avapritinib
G1 81.05%
S 12.57%
G2 6.38%
Total=97.02
G1 78.86%
S 17.80%
G2 3.34%
Total=94.73
G1 80.50%
S 14.95%
G2 4.55%
Total=98.76
12 nM ORY-1001
G1 72.74%
S 21.37%
G2 5.89%
Total=99.21
G1 76.55%
S 19.04%
G2 4.41%
Total=98.20
G1 78.27%
S 16.79%
G2 4.95%
Total=100.09
Combo
G1 85.02%
S 9.08%
G2 5.90%
Total=101.66
G1 89.84%
S 7.28%
G2 2.88%
Total=96.51
G1 91.94%
S 5.44%
G2 2.62%
Total=101.52

Figure S6


A
DMSO
10 nM ORY-1001
100 nM ORY-1001
K4me1
K9me1


B
LSD1
LSD1 peaks (n= 6616)
DMSO
Avapritinib
LSD1i
Combination


C
GFI1
GFI1 peaks (n= 4786)
DMSO
Avapritinib
LSD1i
Combination

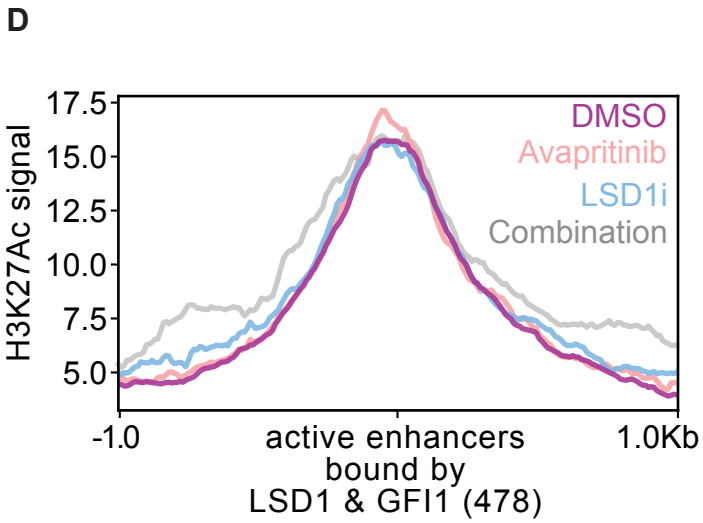

D
H3K27Ac signal
17.5
15.0
12.5
10.0
7.5
5.0
DMSO
Avapritinib
LSD1i
Combination
-1.0
active enhancers
bound by
LSD1 & GFI1 (478)
1.0Kb

Figure S7

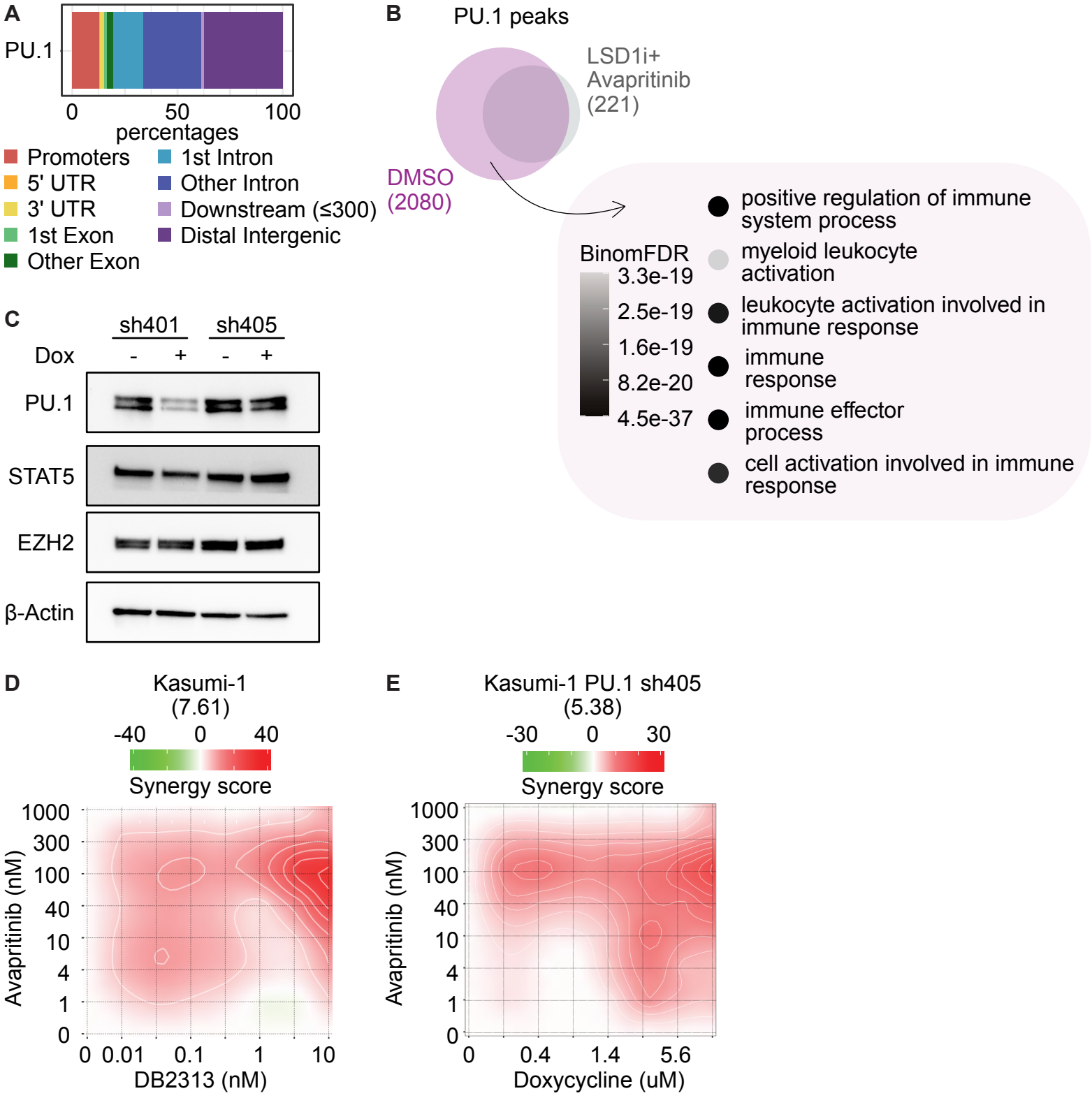

A
PU.1
0
50
100
percentages
Promoters
5' UTR
3' UTR
1st Exon
Other Exon
1st Intron
Other Intron
Downstream (≤300)
Distal Intergenic
B
PU.1 peaks
LSD1i+
Avapritinib
(221)
DMSO
(2080)
BinomFDR
3.3e-19
2.5e-19
1.6e-19
8.2e-20
4.5e-37
positive regulation of immune system process
myeloid leukocyte activation
leukocyte activation involved in immune response
immune response
immune effector process
cell activation involved in immune response
C
sh401
sh405
Dox
-
+
-
+
PU.1
STAT5
EZH2
β-Actin
D
Kasumi-1
(7.61)
-40
0
40
Synergy score
1000
300
100
40
10
4
1
0
Avapritinib (nM)
0
0.01
0.1
1
10
DB2313 (nM)
E
Kasumi-1 PU.1 sh405
(5.38)
-30
0
30
Synergy score
1000
300
100
40
10
4
1
0
Avapritinib (nM)
0
0.4
1.4
5.6
Doxycycline (uM)

Figure S8

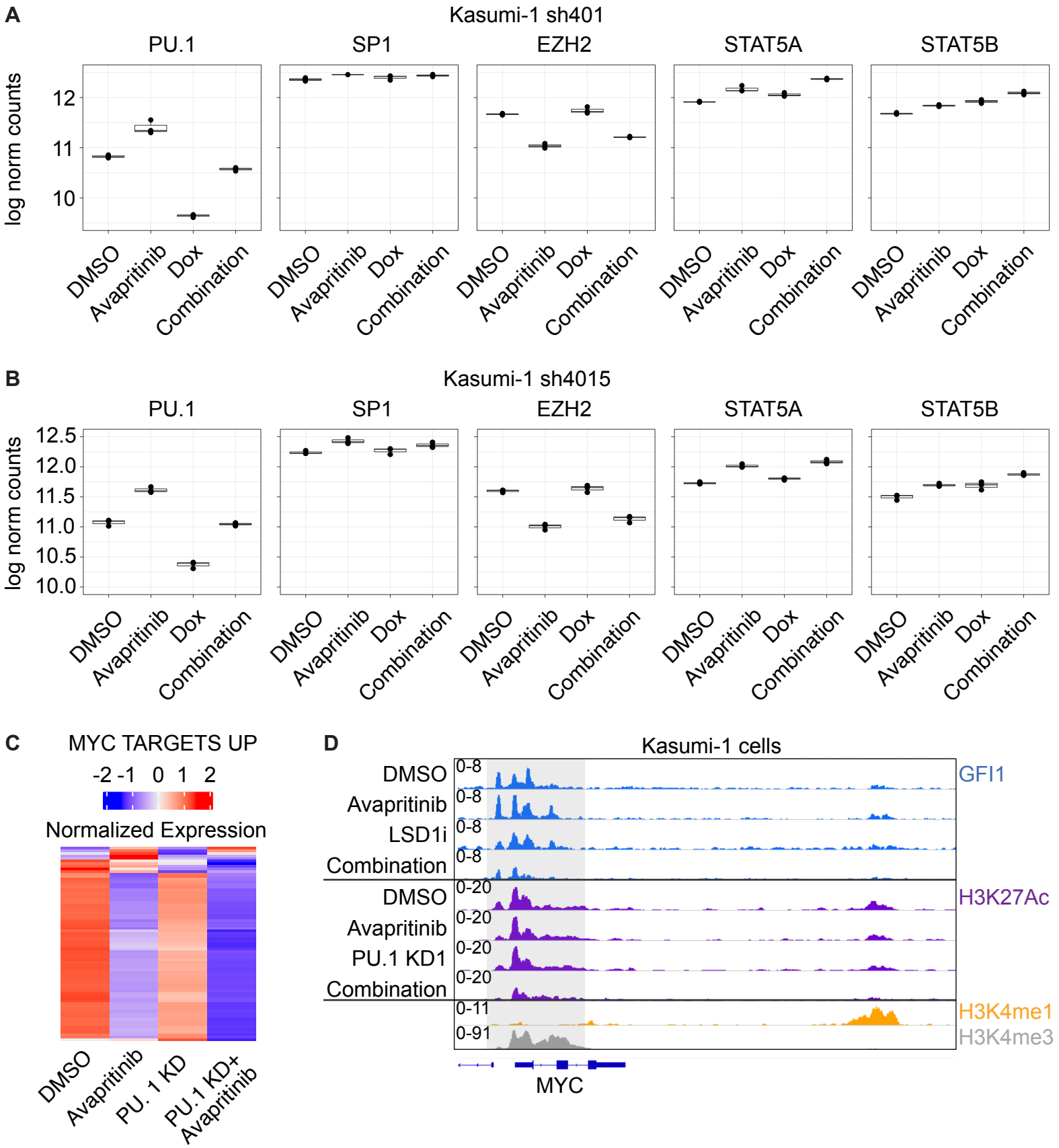

A
Kasumi-1 sh401
PU.1
SP1
EZH2
STAT5A
STAT5B
log norm counts
12
11
10
DMSO
Avapritinib
Dox
Combination
B
Kasumi-1 sh4015
12.5
12.0
11.5
11.0
10.5
10.0
C
MYC TARGETS UP
-2 -1 0 1 2
Normalized Expression
DMSO
Avapritinib
PU. 1 KD
PU.1 KD+ Avapritinib
D
Kasumi-1 cells
DMSO
Avapritinib
LSD1i
Combination
PU.1 KD1
0-8
0-20
0-11
0-91
GFI1
H3K27Ac
H3K4me1
H3K4me3
MYC

Figure S9

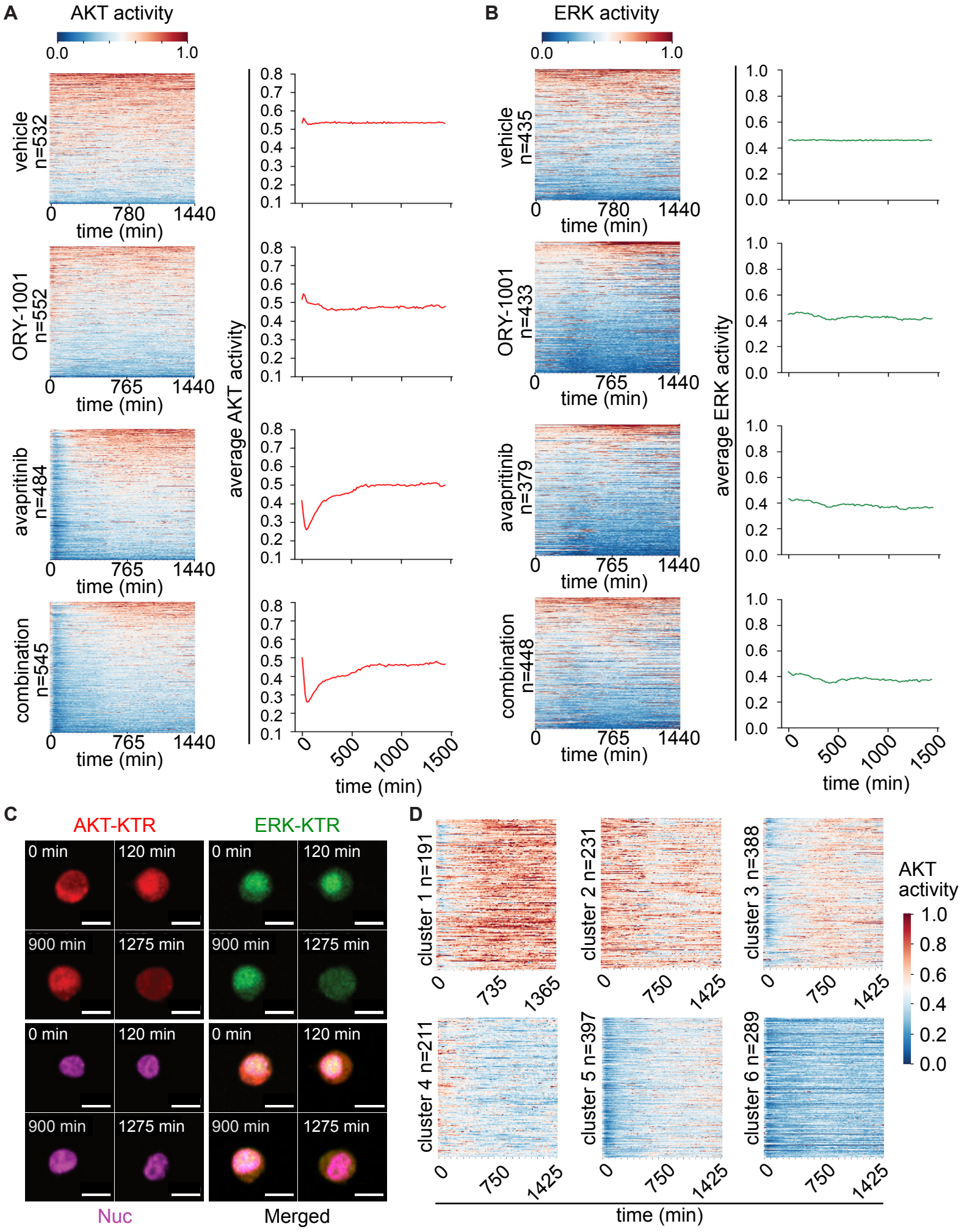

A
AKT activity
0.0
1.0
vehicle n=532
ORY-1001 n=552
avapritinib n=484
combination n=545
0
780
1440
765
time (min)
average AKT activity
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
500
1000
1500
B
ERK activity
vehicle n=435
ORY-1001 n=433
avapritinib n=379
combination n=448
average ERK activity
1.0
0.8
0.6
0.4
0.2
0.0
C
AKT-KTR
ERK-KTR
0 min
120 min
900 min
1275 min
Nuc
Merged
D
cluster 1 n=191
cluster 2 n=231
cluster 3 n=388
cluster 4 n=211
cluster 5 n=397
cluster 6 n=289
0
735
1365
750
1425
time (min)
AKT activity
1.0
0.8
0.6
0.4
0.2
0.0

Figure S10

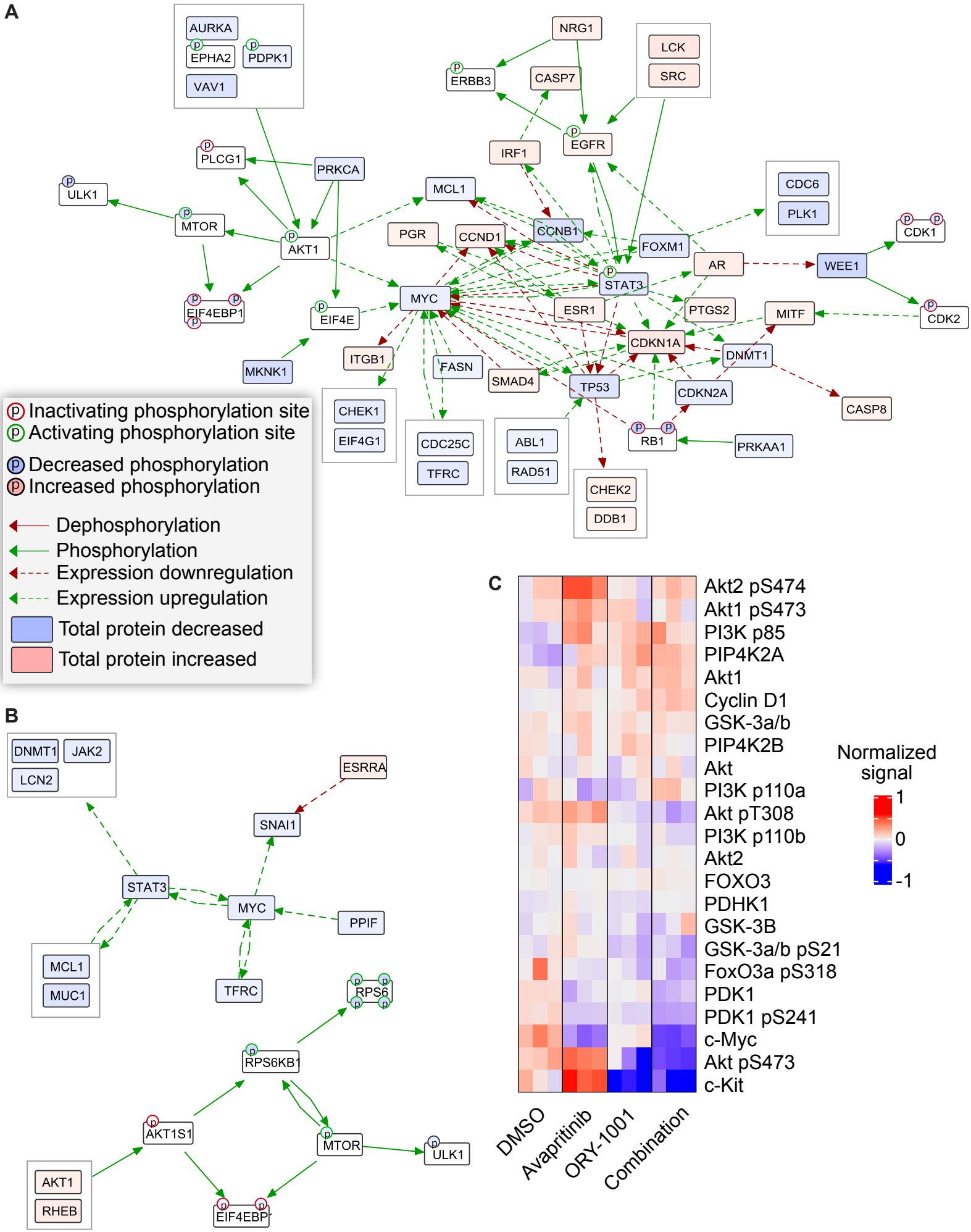

A
AURKA
EPHA2
PDPK1
VAV1
NRG1
ERBB3
CASP7
LCK
SRC
EGFR
IRF1
PLCG1
PRKCA
ULK1
MTOR
AKT1
MCL1
PGR
CCND1
CCNB1
FOXM1
CDC6
PLK1
CDK1
AR
WEE1
STAT3
EIF4EBP1
MYC
EIF4E
ESR1
PTGS2
MITF
CDK2
CDKN1A
DNMT1
MKNK1
ITGB1
FASN
SMAD4
TP53
CDKN2A
CASP8
CHEK1
EIF4G1
CDC25C
TFRC
ABL1
RAD51
RB1
PRKAA1
CHEK2
DDB1
Inactivating phosphorylation site
Activating phosphorylation site
Decreased phosphorylation
Increased phosphorylation
Dephosphorylation
Phosphorylation
Expression downregulation
Expression upregulation
Total protein decreased
Total protein increased
B
DNMT1
JAK2
LCN2
ESRRA
SNAI1
STAT3
MYC
PPIF
MCL1
MUC1
TFRC
RPS6
RPS6KB1
AKT1S1
MTOR
ULK1
AKT1
RHEB
EIF4EBP1
C
Akt2 pS474
Akt1 pS473
PI3K p85
PIP4K2A
Akt1
Cyclin D1
GSK-3a/b
PIP4K2B
Akt
PI3K p110a
Akt pT308
PI3K p110b
Akt2
FOXO3
PDHK1
GSK-3B
GSK-3a/b pS21
FoxO3a pS318
PDK1
PDK1 pS241
c-Myc
Akt pS473
c-Kit
DMSO
Avapritinib
ORY-1001
Combination
Normalized signal
1
0
-1

Figure S11

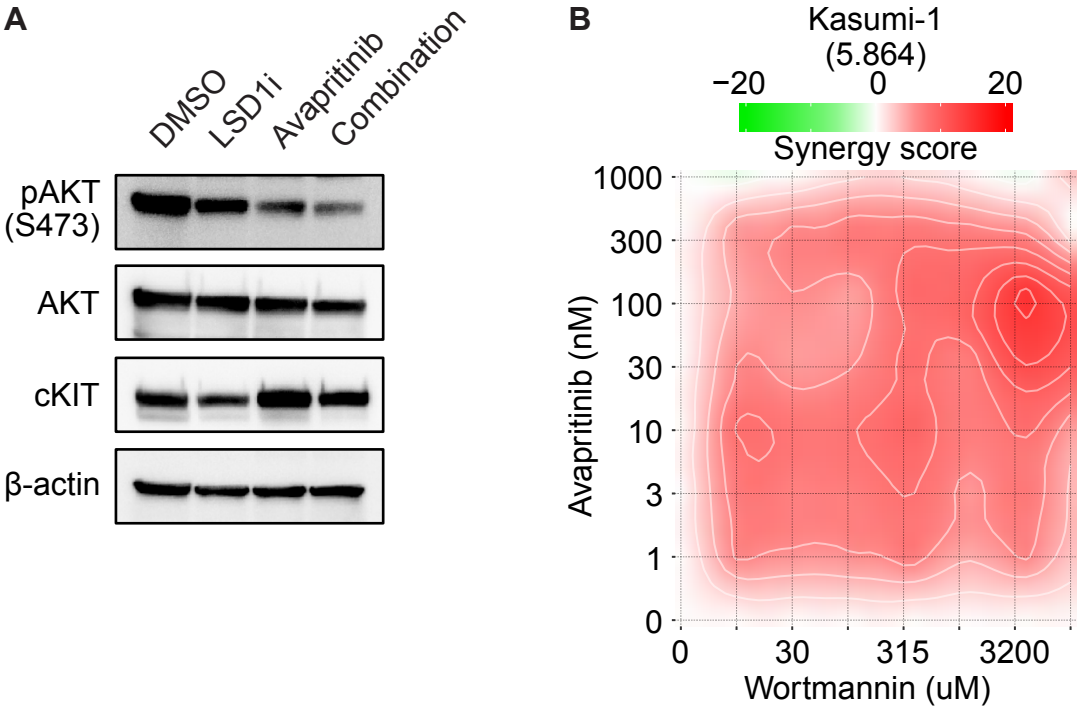

A
DMSO
LSD1i
Avapritinib
Combination
pAKT (S473)
AKT
cKIT
β-actin
B
Kasumi-1 (5.864)
−20
0
20
Synergy score
1000
300
100
30
10
3
1
0
Avapritinib (nM)
0
30
315
3200
Wortmannin (uM)

Figure S12

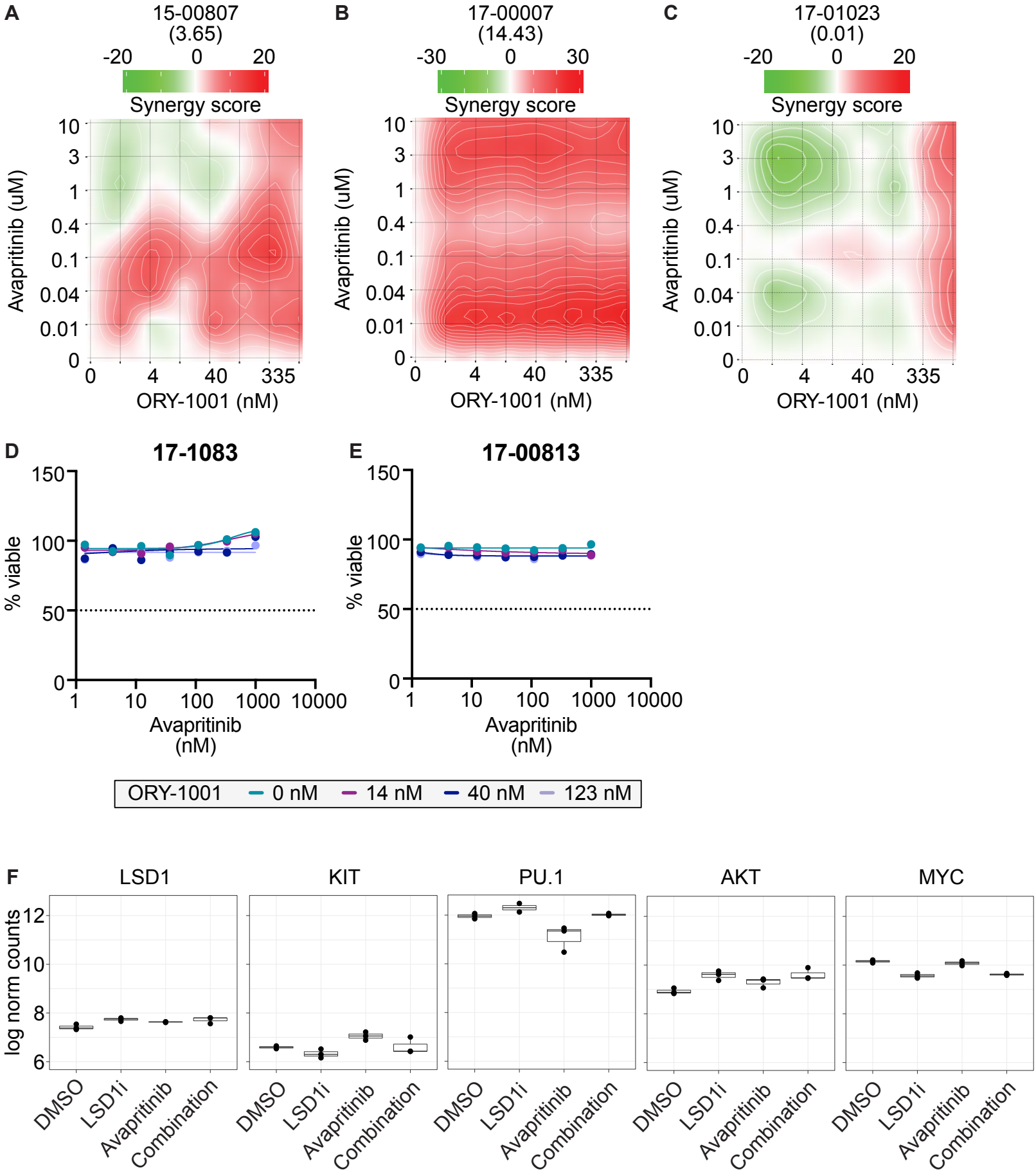

A
15-00807
(3.65)
-20
0
20
Synergy score
Avapritinib (uM)
10
3
1
0.4
0.1
0.04
0.01
0
0
4
40
335
ORY-1001 (nM)
B
17-00007
(14.43)
-30
0
30
Synergy score
Avapritinib (uM)
ORY-1001 (nM)
C
17-01023
(0.01)
-20
0
20
Synergy score
Avapritinib (uM)
ORY-1001 (nM)
D
17-1083
% viable
150
100
50
0
1
10
100
1000
10000
Avapritinib
(nM)
E
17-00813
% viable
Avapritinib
(nM)
ORY-1001
0 nM
14 nM
40 nM
123 nM
F
LSD1
KIT
PU.1
AKT
MYC
log norm counts
12
10
8
6
DMSO
LSD1i
Avapritinib
Combination

Figure S13

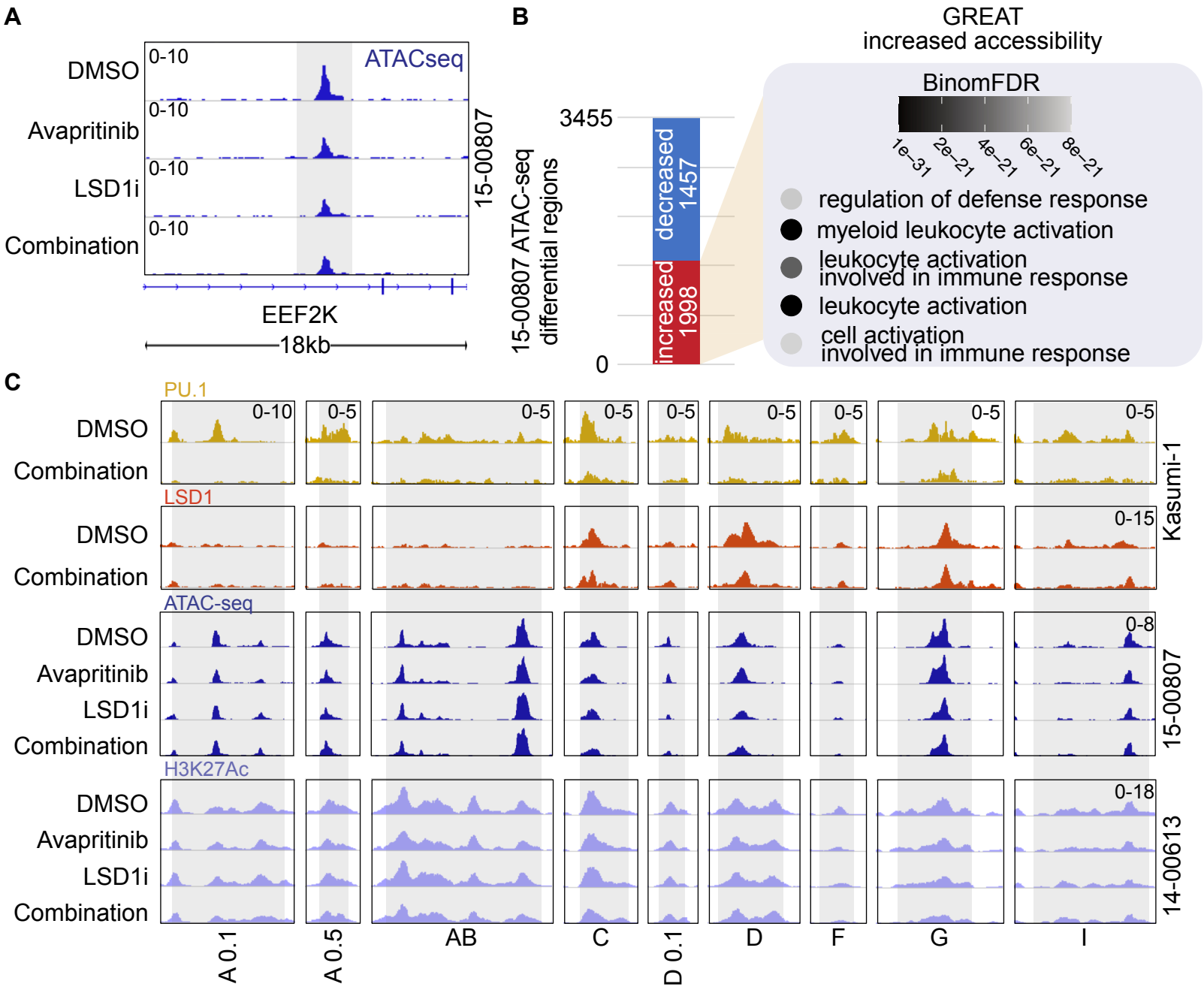

A
DMSO
Avapritinib
LSD1i
Combination
0-10
ATACseq
15-00807
EEF2K
18kb
B
GREAT
increased accessibility
BinomFDR
1e-31
2e-21
4e-21
6e-21
8e-21
3455
0
15-00807 ATAC-seq differential regions
decreased 1457
increased 1998
regulation of defense response
myeloid leukocyte activation
leukocyte activation involved in immune response
leukocyte activation
cell activation involved in immune response
C
PU.1
LSD1
ATAC-seq
H3K27Ac
0-5
0-15
0-8
0-18
Kasumi-1
15-00807
14-00613
A 0.1
A 0.5
AB
C
D 0.1
D
F
G
I

Figure S14

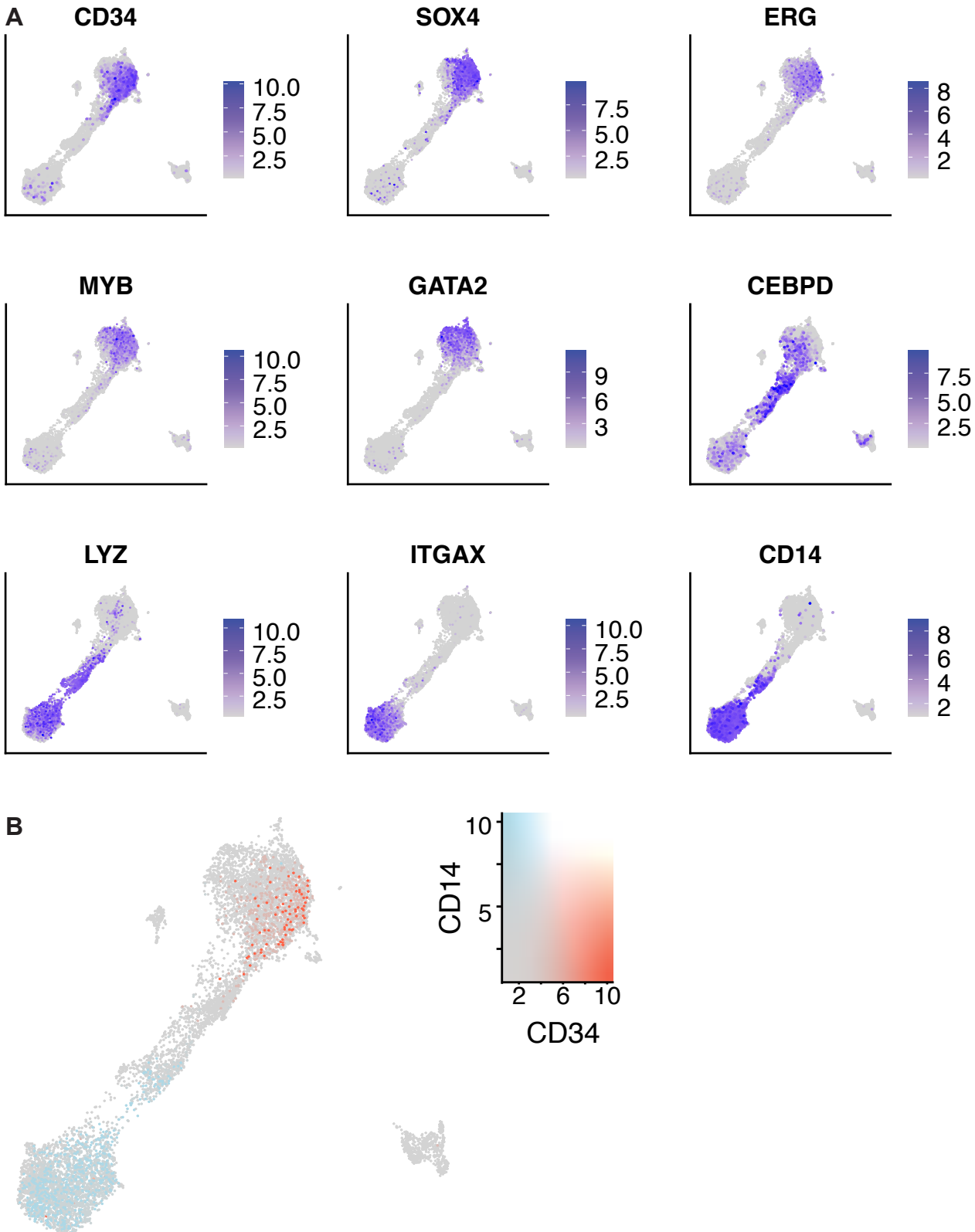

A
CD34
10.0
7.5
5.0
2.5
SOX4
7.5
5.0
2.5
ERG
8
6
4
2
MYB
10.0
7.5
5.0
2.5
GATA2
9
6
3
CEBPD
7.5
5.0
2.5
LYZ
10.0
7.5
5.0
2.5
ITGAX
10.0
7.5
5.0
2.5
CD14
8
6
4
2
B
CD14
10
5
2
6
10
CD34

Figure S15

Supplemental Figure Legends

Supplementary Figure 1. Protein expression in *KIT*-mutant AML cell line compared to *KIT* wild type cell lines.
Western blot of PU.1, MYC, LSD1, cKIT, pan-AKT, and β-actin in Kasumi-1, Molm-13, and K562 cells. Arrow corresponds to the band at the correct molecular weight for LSD1.

Supplementary Figure 2. Characterization of specificity and response of KIT and LSD1 inhibition
A. Viability assessment for Kasumi-1 cells treated for 72 h with avapritinib and ORY-1001 at different concentrations. Values normalized to average viability of cells with highest dose of both drugs. Error bars representing SEM. **B.** Drug matrix of Kasumi-1 cells treated for 72 h with avapritinib and GSK-LSD1 with synergy assessed by ZIP score (displayed in parentheses). **C**. Proliferation assay over 5 days with SKNO-1 cells treated with DMSO, ORY-1001 (40 nM), avapritinib (40 nM), or the combination; two-way ANOVA with Holm-Sidak correction. Error bars represent SEM. **D, E**. Assessment of apoptosis flow cytometry of Kasumi-1 cells treated for 48 h with avapritinib (36 nM) and/or ORY-1001 (12 nM) as a percent of the parent population and representative plots of PI and Annexin V for each condition; two-way ANOVA with Holm-Sidak correction. Error bars representing SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$

Supplementary Figure 3. Increased mature surface marker differentiation with LSD1 inhibition.
A-C. Flow cytometry assessment of CD11b, CD86, and CD84 surface markers in Kasumi-1 cells treated for 24, 48, and 72 h, respectively, with DMSO, ORY-1001 (12 nM), avapritinib (12 nM), or combination. Representative histograms of surface marker signal with solid line representing the positive cut-off and bar graph of positive cells; two-way ANOVA with Holm-Sidak correction. Error bars representing SEM. * $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$. **D.** Cytospin of Kasumi-1 cells treated for 72 h with avapritinib (12 nM), ORY-1001 (12 nM), GSK-LSD1 (12 nM), avapritinib/ORY-1001 (12 nM each), or avapritinib/GSKLSD1 (12 nM each) stained with May Grunwald-Giemsa. Scale bar represents 10 μm.

Supplementary Figure 4. LSD1 inhibitor induced differentiation is not reversible and combined LSD1/KIT inhibition is well tolerated in healthy mice.
A. Flow cytometry assessment of CD11b, CD84, and CD86 in Kasumi-1 cells treated for 72 h with DMSO, avapritinib (12 nM), ORY-1001 (12 nM), or the combination. **B.** Same flow cytometry assessment as G, 72 h after removal of treatment; two-way ANOVA with Holm-SIdak correction. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. **C.** Wild-type

C57BL/6J mice were treated with avapritinib (10 mg/kg/day) by gavage and ORY-1001 (0.0125 mg/kg/day) by intraperitoneal injection daily for 2 weeks (*n*=6/group). Body weight pre and post treatment. **D.** White blood cell (WBC) count pre and post treatment. **E.** Hemoglobin (Hgb) pre and post treatment. **F.** Platelets pre and post treatment. Two-way ANOVA with Holm-Sidak correction. Error bars represent SEM. ** $p < 0.01$

Supplementary Figure 5. Loss of total MYC protein and acetylation at MYC bound promoters

A. Western blot of total MYC, PU.1, LSD1, cKIT and loading control (β-Actin) in Kasumi-1 cells 24 h after treatment with avapritinib (12 nM) and/or ORY-1001 (12 nM; LSD1i). LSD1 and cKIT are included as controls. **B.** Viability of Kasumi-1 cells with pMIG MYC-OE (over expression) or pMIG Empty treated for 72 h with avapritinib and 0 nM or 12 nM of ORY-1001. Viability determined by MTS. Area under the curve (AUC) of the viability plot is displayed. Two-way ANOVA with Holm-Sidak correction. Error bars represent SEM. ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. **C.** Venn diagram of peaks from Kasumi-1 CUT&Tag used to define regulatory elements. Promoters defined as H3K4me3 peaks <1 kb from a TSS; enhancers defined as H3K4me1 peaks >1 kb of a TSS. Regulatory elements that overlap with H3K27Ac are defined as active (*n*=2/group). **D.** Box and whisker plot of H3K27Ac signal at MYC bound promoters. KS test used to assess statistical significance. Boxes defined by the interquartile range (IQR) split by the median, with whiskers extending no further than 1.5x IQR from the box. Outliers are individually plotted. **E.** Gene ontology term enrichment for MYC bound promoters.

Supplementary Figure 6. Decreased cell cycle progression with combined LSD1 and KIT inhibition

A, B. Cell cycle flow cytometry assay with PI of Kasumi-1 cells treated for 24, 48, or 72 h with avapritinib (12 nM) and/or ORY-1001 (12 nM). Bar chart of percentage of cells within G1, S, or G2 phase and pie chart of percent of cells within each phase of cell cycle (*n*=3/group). Two-way ANOVA with Holm-Sidak correction. Error bars represent SEM. * $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$

Supplementary Figure 7. Activation of LSD1 and GFI1 bound enhancers with KIT and LSD1 inhibition

A. Following treatment of kasumi-1 cells for 24 h with DMSO or ORY-1001, lysates were extracted and histone methylation (H3K4me1 and H3K9me1) was assessed. **B, C.** Kasumi-1 cells were treated for 12 h (LSD1) or 24 h (GFI1) with avapritinib (12 nM) and/or LSD1 inhibitor (12 nM; ORY-1001 and GSK-LSD1 respectively) then subject to CUT&RUN (*n*=2-3/group). Heatmaps of global signal for LSD1 and GFI1 at their respective high confidence consensus peak sets (peak apex ± 1 kb). **D.** Peak profile and plot

of H3K27Ac signal at active enhancers bound by LSD1 and GFI1 in Kasumi-1 cells after 24 h of treatment with avapritinib (12 nM) and/or GSK-LSD1 (12 nM; LSD1i).

Supplementary Figure 8. PU.1 inhibition and knock down synergize with KIT inhibition

A. Annotation of high confidence consensus PU.1 peaks. **B.** Gene ontology analysis of PU.1 peaks lost with LSD1 and KIT inhibition. **C.** Western blot of PU.1 in Kasumi-1 shRNA knockdown cells treated with and without 1 μg/mL doxycycline for 48 h. STAT5 and EZH2 included as controls to assess off-target effects. **D, E.** Drug matrix of Kasumi-1 cells treated for 72 h with avapritinib and DB2313 or doxycycline with synergy assessed by ZIP (reported in parentheses).

Supplementary Figure 9. PU.1 knock down with KIT inhibition results in decreased MYC target gene activation

A, B. Bulk RNA-seq of Kasumi-1 cells with PU.1 shRNA knockdown treated with DMSO, avapritinib (12 nM), PU.1 sh401 (1 μg/mL doxycycline), or both (*n*=3/group). Log2 normalized mRNA levels for PU.1, SP1, EZH2, STAT5A, and STAT5B with PU.1 knockdown via sh401 or sh405, respectively, to assess knockdown specificity. **C.** Normalized expression of MYC targets up gene set. **D.** GFI1, H3K27Ac, H3K4me1, and H3K4me3 tracks at MYC promoter and +26 kB enhancer. For GFI1 CUT&RUN, Kasumi-1 cells were treated with avapritinib (12 nM), GSK-LSD1 (LSD1i; 12 nM), or both. For H3K27Ac CUT&Tag, Kasumi-1 cells were treated with avapritinib (12 nM), PU.1 sh401 (1 μg/mL doxycycline), or both.

Supplementary Figure 10. Dual LSD1 and KIT inhibition leads to reduction of AKT and not ERK signaling

A, B. Heatmaps of AKT and ERK dynamics of individual cells (every row) in response to ~25h treatment of DMSO, ORY-1001 (12 nM), avapritinib (100 nM), and the combination. *n* represents the total number of tracked cells in each condition. Line plots represent population average of AKT and ERK dynamics over time. **C.** Example of time-series images of fluorescent cells under the combination treatment. AKT-KTR = mScarlet, ERK-KTR = Clover, and Nuc = miRFP670nano. Scale bar represents 10 μm. **D.** Heatmaps of cells grouped into different AKT dynamic clusters by hierarchical clustering.

Supplementary Figure 11. Identify AKT as prominent pathway leading to MYC repression

A. CausalPath analysis of RPPA results of Kasumi-1 cells treated for 24 h with avapritinib (100 nM) and/or ORY-1001 (12 nM) or DMSO. **B.** CausalPath analysis of

RPPA as in A after 1 h of drug exposure. **C**. Heatmap of the normalized signal for PI3K/AKT pathway members after 24 h of drug exposure.

Supplementary Figure 12. Validation of changes in pAKT due to KIT and LSD1 inhibition.
A. Kasumi-1 cells treated with DMSO, ORY-1001 (12 nM), avapritinib (500 nM), or the combination for 24 h. Western blot of pAKT (S473), pan-AKT, cKIT, and β-actin. **B.** Drug matrix of Kasumi-1 cells treated for 72 h with avapritinib and wortmannin, PI3K inhibitor, with synergy assessed by ZIP score (displayed in parentheses).

Supplementary Figure 13. Additional KIT and LSD1 inhibitor synergy plots in *KIT*-mutant AML patient samples
A-C. Drug matrices of patient samples 15-00807, 17-00007, and 17-0123 treated for 72 h with avapritinib and ORY-1001 with synergy assessed by ZIP score (displayed in parentheses). **D,E.** Viability of *KIT* wild type AML patient samples after 72 h of treatment with avapritinib and ORY-1001. **F.** Bulk RNA-seq on 14-00613 treated with DMSO, avapritinib (50 nM), ORY-1001 (12 nM), or the combination for 24 h. Log2 normalized mRNA levels of LSD1, KIT, PU.1, AKT, and MYC.

Supplementary Figure 14. Epigenetic changes in *KIT*-mutant AML patient samples following LSD1 and KIT inhibition.
A. Example of decreased accessibility at cell cycle associated locus, EEF2K, from bulk ATAC-seq in 15-00807. **B.** Differential analysis of bulk ATAC-seq on 15-00807 treated with avapritinib (50 nM) and ORY-1001 (50 nM) compared to DMSO. Enrichment of GO terms for regions with significantly increased accessibility. **C.** Visualization of Kasumi-1 PU.1 and LSD1, 15-00807 bulk ATAC-seq, and 14-00613 H3K27Ac at entire BENC locus. BENC modules were identified by previous annotation of the region(24) and intersection with H3K4me1 signal.

Supplementary Figure 15. Single cell RNA-seq markers for differentiation
A. Integrated single cell RNA-seq object showing the expression of differentiation markers. Markers were used to identify early, mid, and late stages of hematopoiesis in patient sample 14-00613. **B.** Detected levels of CD34 (early) and CD14 (late) in integrated object to show overall differentiation trajectory.