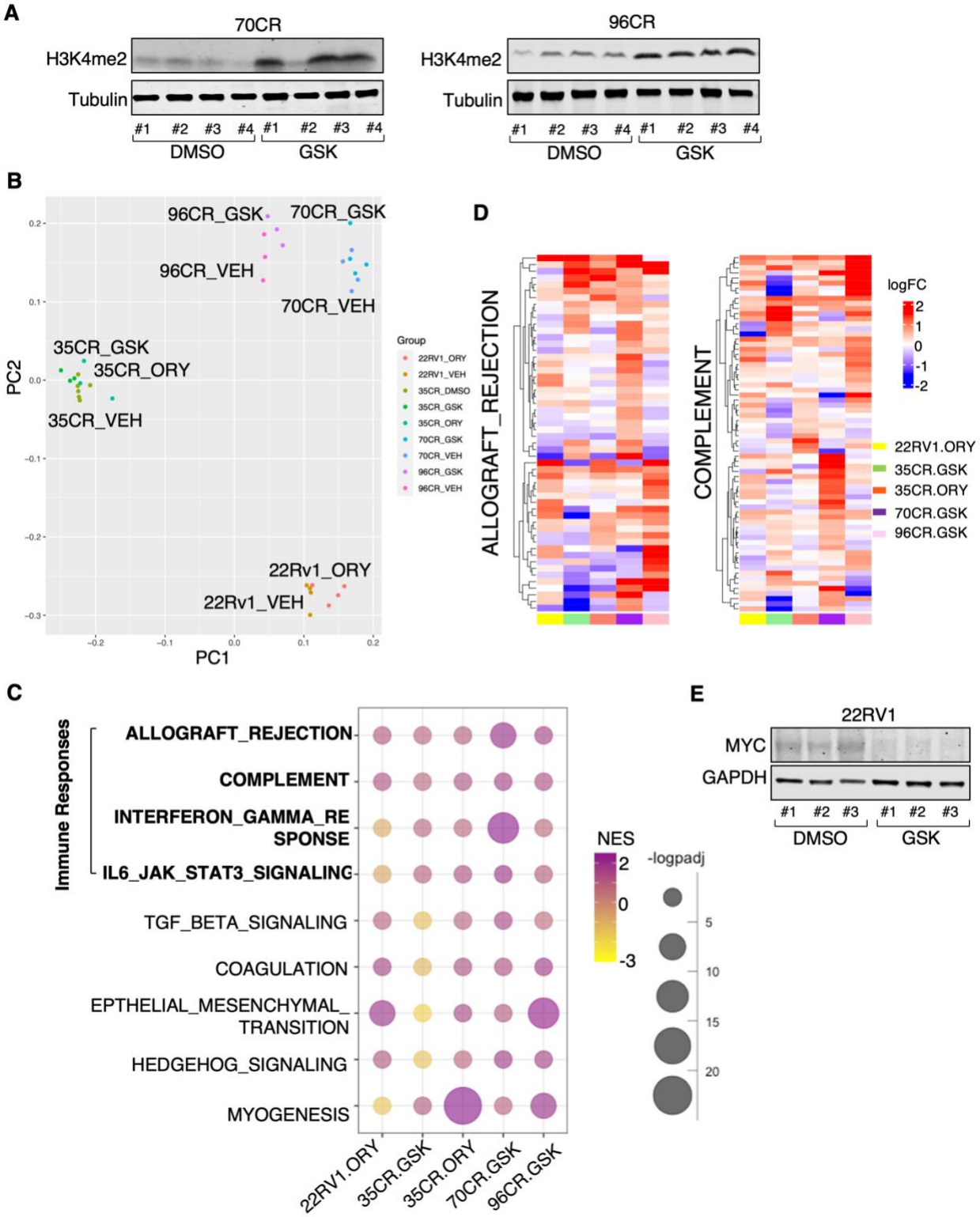
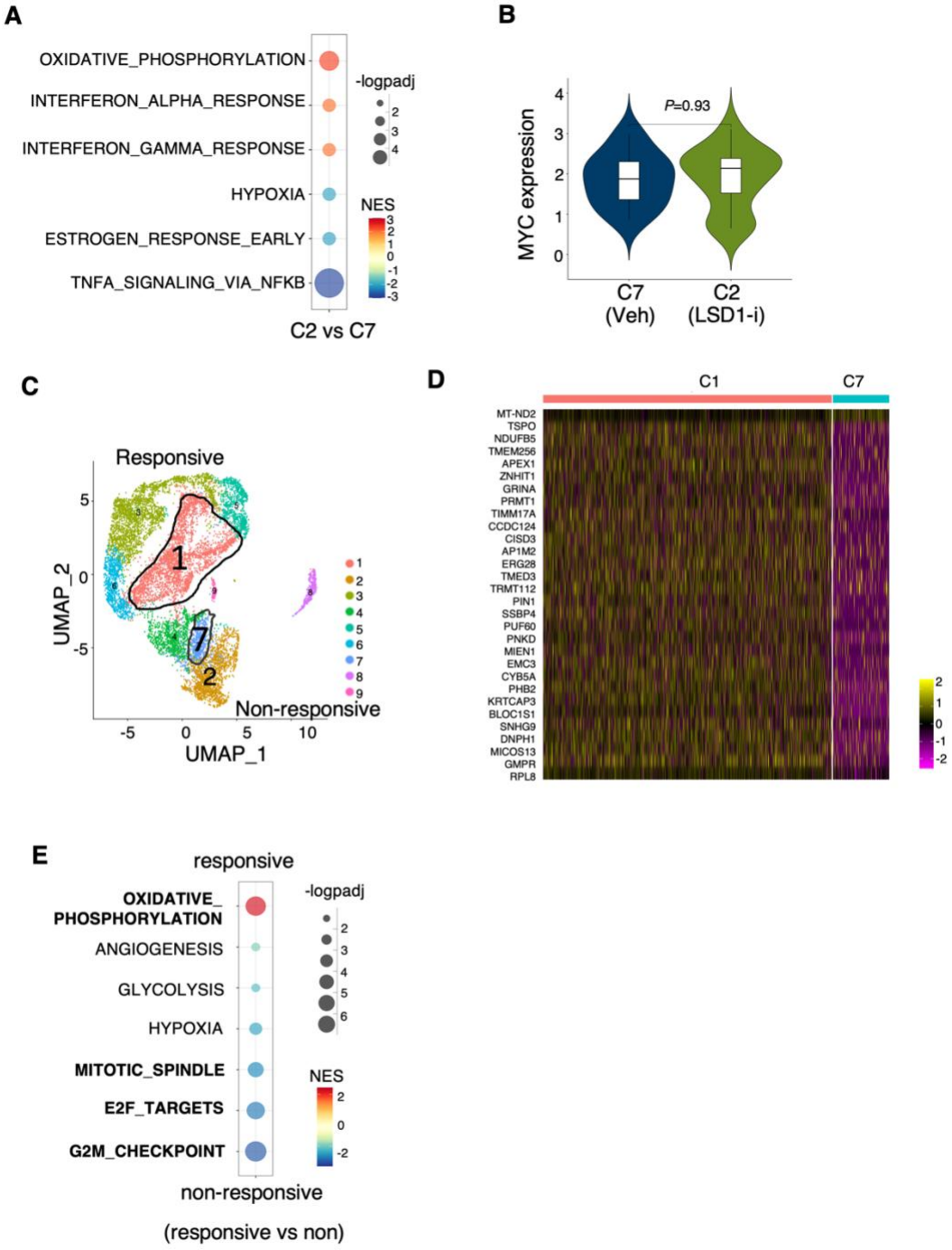
Supplementary Information Inventory:


A
70CR
H3K4me2
Tubulin
#1 #2 #3 #4 #1 #2 #3 #4
DMSO GSK
96CR
H3K4me2
Tubulin
#1 #2 #3 #4 #1 #2 #3 #4
DMSO GSK
B
96CR_GSK
70CR_GSK
96CR_VEH
70CR_VEH
35CR_GSK
35CR_ORY
35CR_VEH
22Rv1_ORY
22Rv1_VEH
PC2
PC1
Group
22RV1_ORY
22RV1_VEH
35CR_DMSO
35CR_GSK
35CR_ORY
70CR_GSK
70CR_VEH
96CR_GSK
96CR_VEH
D
ALLOGRAFT_REJECTION
COMPLEMENT
logFC
22RV1.ORY
35CR.GSK
35CR.ORY
70CR.GSK
96CR.GSK
C
Immune Responses
ALLOGRAFT_REJECTION
COMPLEMENT
INTERFERON_GAMMA_RESPONSE
IL6_JAK_STAT3_SIGNALING
TGF_BETA_SIGNALING
COAGULATION
EPTHELIAL_MESENCHYMAL_TRANSITION
HEDGEHOG_SIGNALING
MYOGENESIS
22RV1.ORY
35CR.GSK
35CR.ORY
70CR.GSK
96CR.GSK
NES
-logpadj
E
22RV1
MYC
GAPDH
#1 #2 #3 #1 #2 #3
DMSO GSK

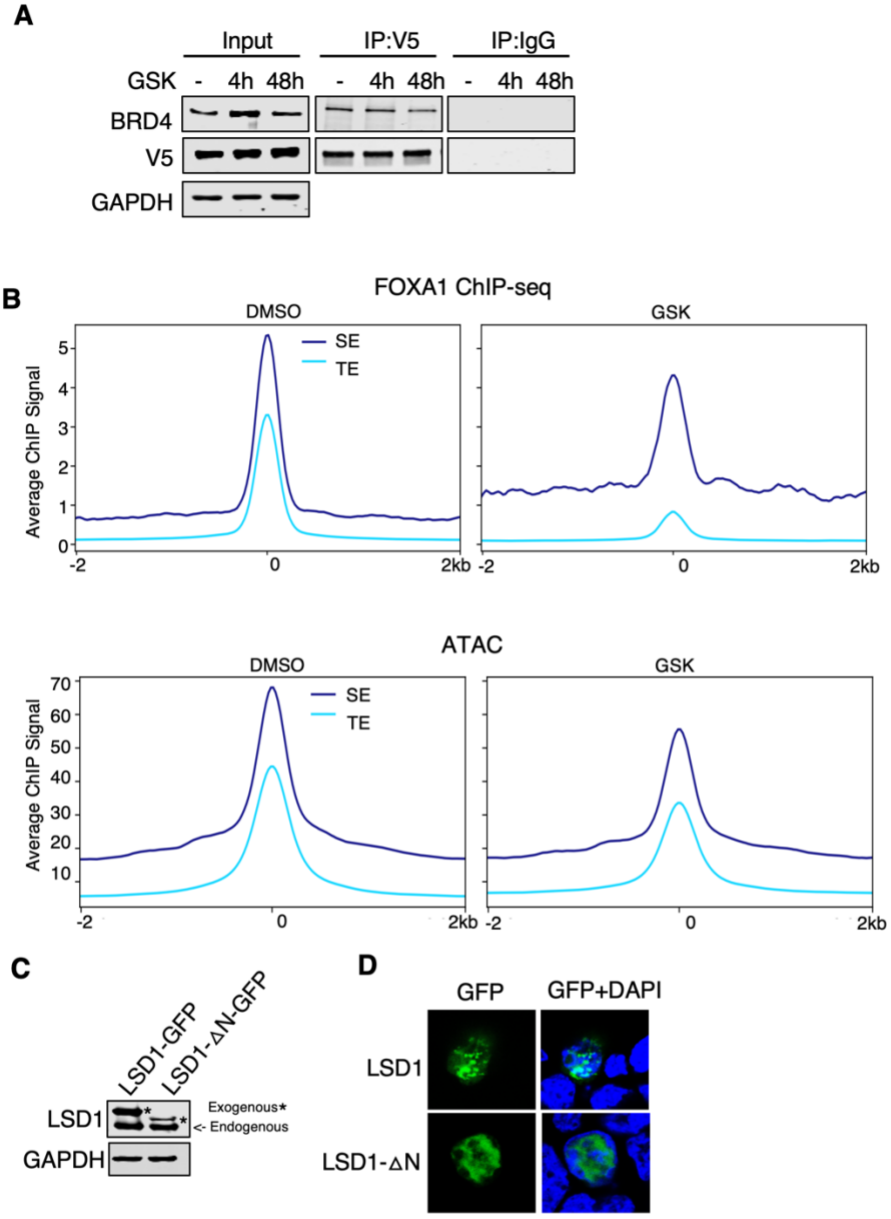
Supplementary Figure S1. LSD1-i activates immune response pathways

(**A**) Immunoblotting for H3K4me2 in LuCaP70CR and 96CR xenograft tumor samples. (**B**) The PCA plot of the RNA-seq data from all xenograft studies. (**C**) GSEA for the top-ranked LSD1-i-upregulated pathways that are common among models. (**D**) The heatmap view of the genes in Allograft Rejection and Complement pathways (immune-related). (**E**) Immunoblotting for MYC in 22RV1 xenograft tissues treated with LSD1 inhibitor.


A
OXIDATIVE_PHOSPHORYLATION
INTERFERON_ALPHA_RESPONSE
INTERFERON_GAMMA_RESPONSE
HYPOXIA
ESTROGEN_RESPONSE_EARLY
TNFA_SIGNALING_VIA_NFKB
-logpadj
NES
C2 vs C7
B
MYC expression
P=0.93
C7 (Veh)
C2 (LSD1-i)
C
Responsive
Non-responsive
UMAP_1
UMAP_2
D
C1
C7
MT-ND2
TSPO
NDUFB5
TMEM256
APEX1
ZNHIT1
GRINA
PRMT1
TIMM17A
CCDC124
CISD3
AP1M2
ERG28
TMED3
TRMT112
PIN1
SSBP4
PUF60
PNKD
MIEN1
EMC3
CYB5A
PHB2
KRTCAP3
BLOC1S1
SNHG9
DNPH1
MICOS13
GMPR
RPL8
E
responsive
OXIDATIVE_ PHOSPHORYLATION
ANGIOGENESIS
GLYCOLYSIS
HYPOXIA
MITOTIC_SPINDLE
E2F_TARGETS
G2M_CHECKPOINT
-logpadj
NES
non-responsive
(responsive vs non)

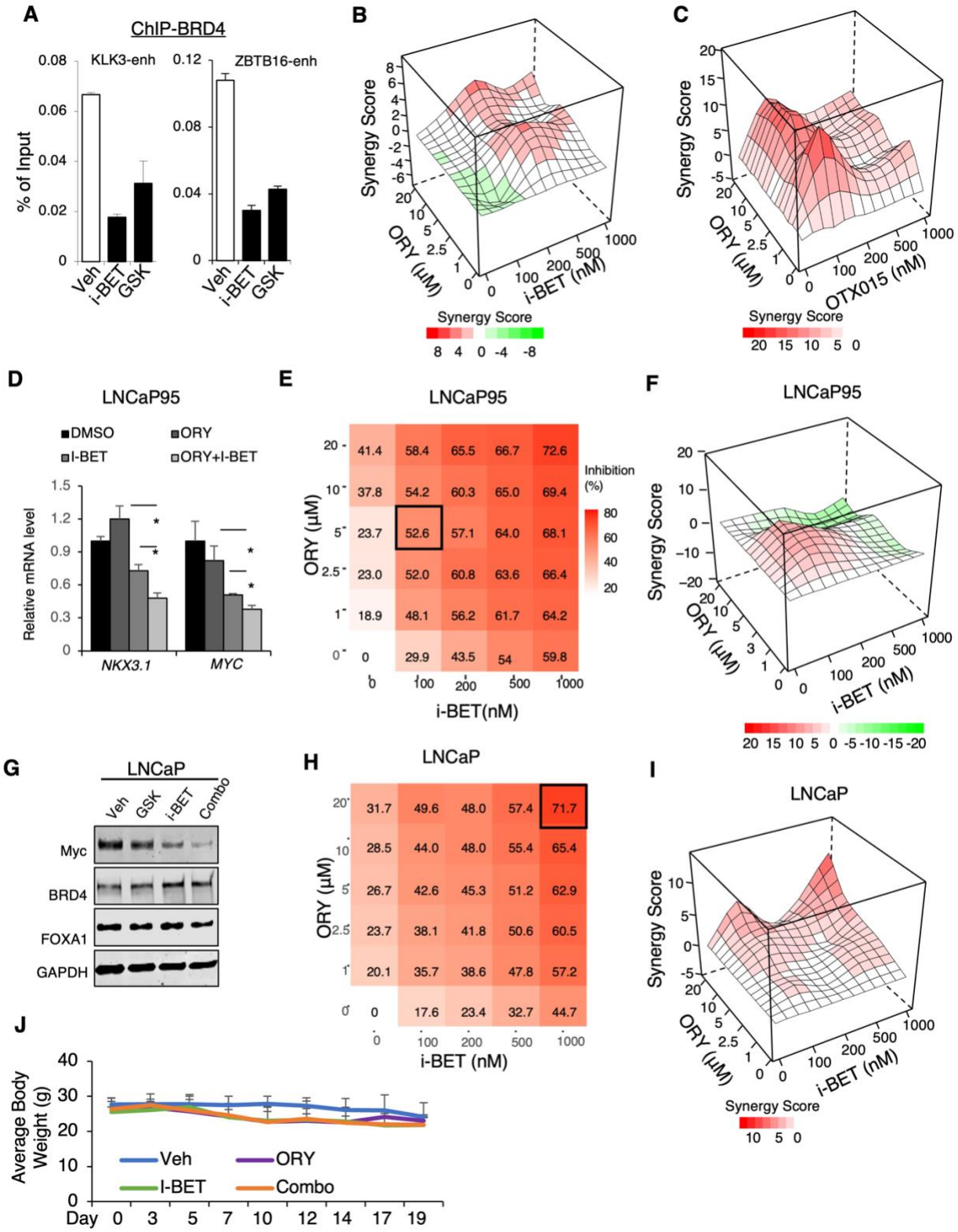
Supplementary Figure S2. Comparing gene expressions in responsive versus non-responsive populations in LuCaP 96CR model

(**A**) The GSEA showing the hallmark pathways that were significantly changed by GSK2879552 treatment in cluster 7 versus cluster 2 (cutoff: *P*-adj<0.05). (**B**) The violin plot of *MYC* gene expression in Vehicle (cluster 7) vs. GSK2879552 (cluster 2). (**C**) Cluster 1 (Responsive, vehicle-treated) and cluster 7 (Non-responsive, vehicle-treated) are highlighted. (**D**) Top-ranked genes that are differentially expressed between C1 and C7. (**E**) The GSEA showing the hallmark pathways that were enriched and de-enriched in the non-responsive populations (cutoff: *P*-adj<0.05).


A
Input
IP:V5
IP:IgG
GSK - 4h 48h - 4h 48h - 4h 48h
BRD4
V5
GAPDH
B
FOXA1 ChIP-seq
DMSO
GSK
SE
TE
Average ChIP Signal
0
1
2
3
4
5
-2
0
2kb
ATAC
10
20
30
40
50
60
70
C
LSD1-GFP
LSD1-ΔN-GFP
LSD1
GAPDH
Exogenous*
<- Endogenous
D
GFP
GFP+DAPI
LSD1
LSD1-ΔN

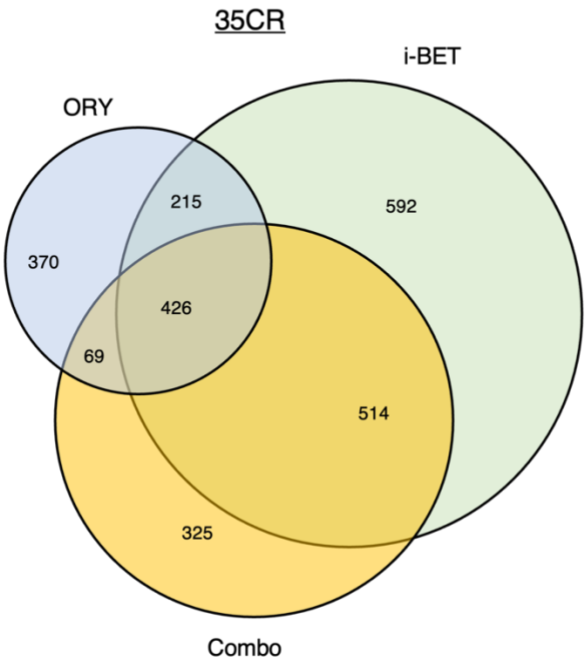
Supplementary Figure S3. LSD1 enriches at SEs and forms phase separation

(**A**) LNCaP cells stably expressing V5-FOXA1 were treated with LSD1 inhibitor GSK2879552 (50 μM) for 4 hours and 48 hours, and subjected to V5 pull down. Immunoblotting for BRD4 and V5 were shown. (**B**) FOXA1 binding (ChIP-seq of FOXA1) and ATAC signals on both SEs and TEs in LNCaP cells (grown in CSS) treated with GSK2879552 (50 μM, 4 hours). (**C, D**) 293T cells transfected with LSD1-GFP and LSD1-ΔN-GFP (1-100 amino acids deletion) were subjected to immunoblotting for LSD1 (C) and confocal microscopy of GFP (D).


A
ChIP-BRD4
KLK3-enh
ZBTB16-enh
% of Input
Veh
i-BET
GSK
B
Synergy Score
ORY (μM)
i-BET (nM)
C
OTX015 (nM)
D
LNCaP95
DMSO
ORY
I-BET
ORY+I-BET
Relative mRNA level
NKX3.1
MYC
E
Inhibition (%)
i-BET(nM)
F
G
LNCaP
Myc
BRD4
FOXA1
GAPDH
H
I
J
Average Body Weight (g)
Day
Veh
ORY
I-BET
Combo

Supplementary Figure S4. LSD1-i synergizes with BET-i to suppress CRPC growth

(**A**) 22RV1 cells grown in hormone-depleted medium were treated with i-BET762 (1 μM) or GSK2879552 (1 μM) for 4 hours. BRD4 binding on two AR-activated enhancers was measured by ChIP-qPCR. (**B**, **C**) 3-D plot of synergy scores of the combination treatments – ORY-1001+i-BET762 (B) and ORY-1001+OTX015 (C). (**D**) LNCaP95 cells grown in hormone-depleted medium were treated with 5μM ORY-1001, 100 nM i-BET 762, or the combination for 1 day, and *NKX3*.1 and *MYC* expression was examined by RT-qPCR. (**E**) The heatmap view of the percentage of inhibition of LNCaP95 cells treated with the combination of indicated doses of ORY-1001 and i-BET762 for 4 days. (**F**) 3-D plot of synergy scores of the combination treatments – ORY-1001+i-BET762 in LNCaP95 cells. (**G**) LNCaP cells grown in full serum were treated with 1μM ORY-1001, 1μM i-BET 762, or the combination for 1 day, and MYC protein was examined by immunoblotting. (**H**) The heatmap view of the percentage of inhibition of LNCaP cells treated with the combination of indicated doses of ORY-1001 and i-BET762 for 4 days. (**I**) 3-D plot of synergy scores of the combination treatments – ORY-1001+i-BET762 in LNCaP cells. (**J**) Body weight of castrated SCID mice receiving the indicated treatment.



Supplementary Figure S5. Co-targeting LSD1 and BRD4 in 35CR model

RNA-seq analyses were performed using the tissue samples at the end of the treatment in 35CR model. The Venn diagram for ORY-1001-repressed genes, i-BET762-repressed genes, and the combination treatment-repressed genes is shown.

SUPPLEMENTRAY METHODS

Plasmids construction:

The mutant LSD1-del(1-100) was generated using QuickChange Lightning Site-Directed Mutagenesis Kit (Agilent Technologies, cat# 210518) from wild-type LSD1. The mutagenesis PCR primers are: 5'-ATGTTATCTGGGAAGAAGGGAATAGCAGAGACTCCG-3'; 5'-CGGAGTCTCTGCTATTCCCTTCTTCCCAGATAACAT-3'. Then wildtype (WT) and the mutant LSD1-del100 was cloned into the pDEST-CMV-N-EGFP vector (Addgene 122842) using the Gateway Technology with Clonase II (Invitrogen, Cat# 12535-029) to generate EGFP-tagged LSD1 at its N terminus.