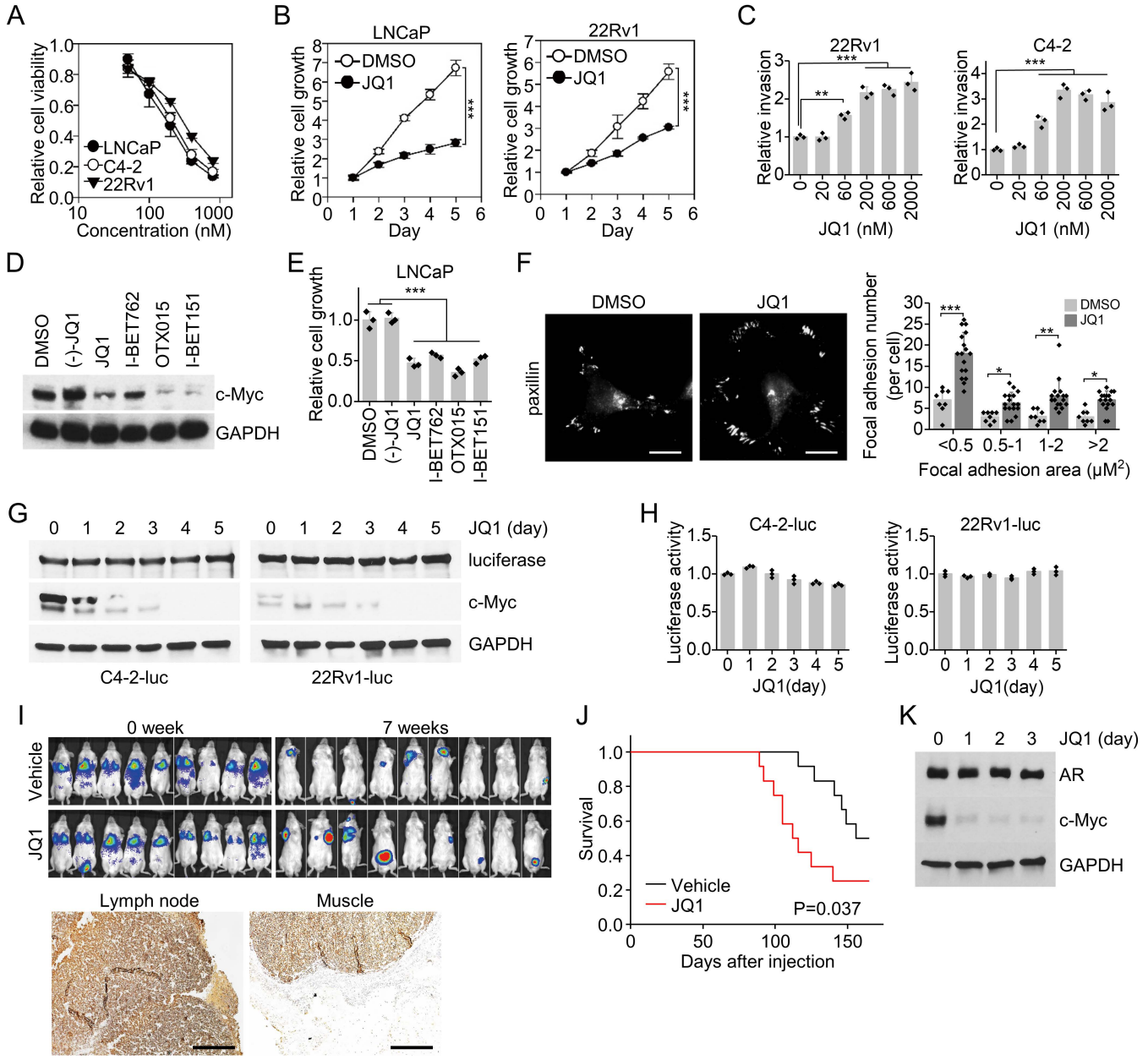
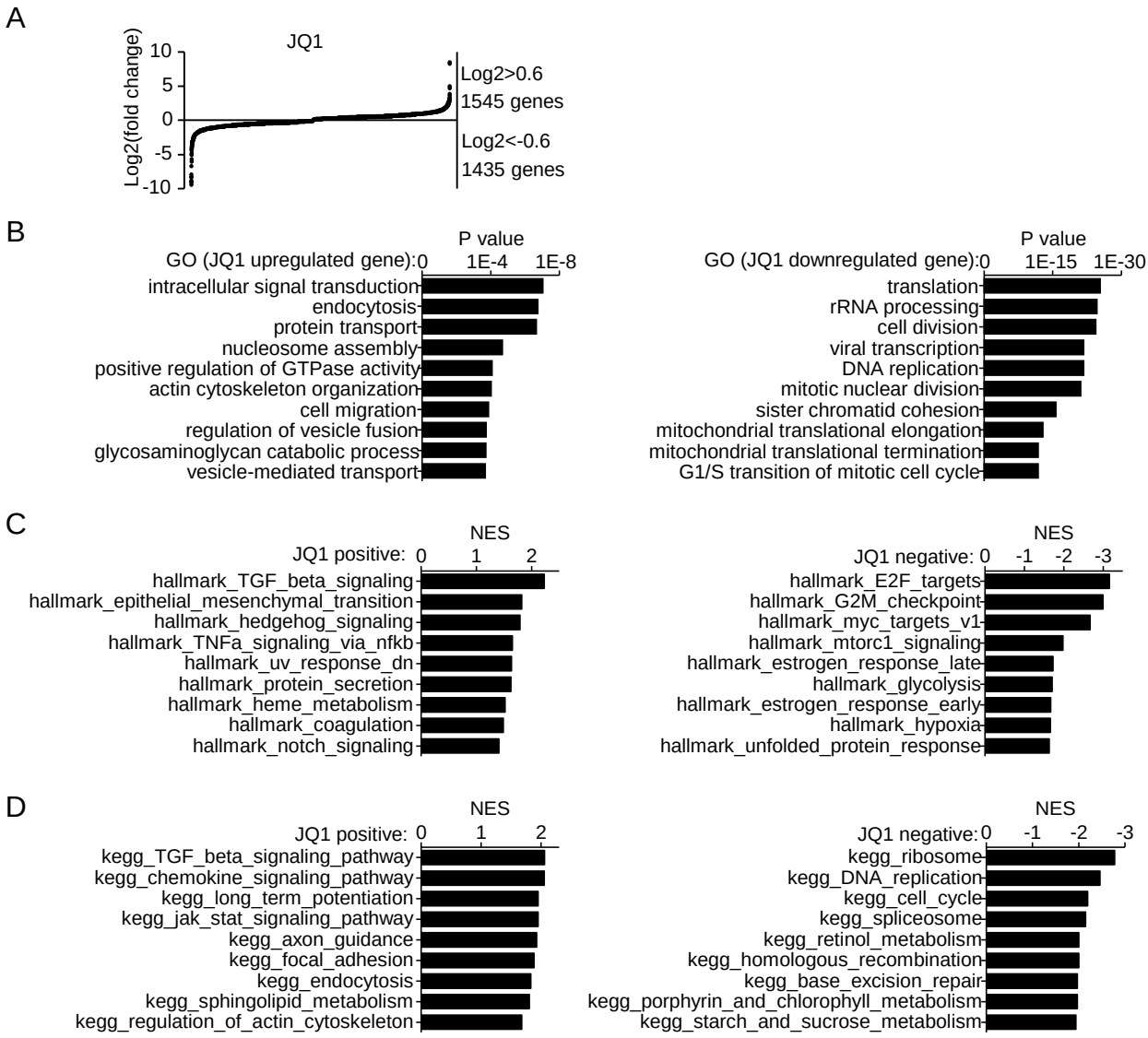
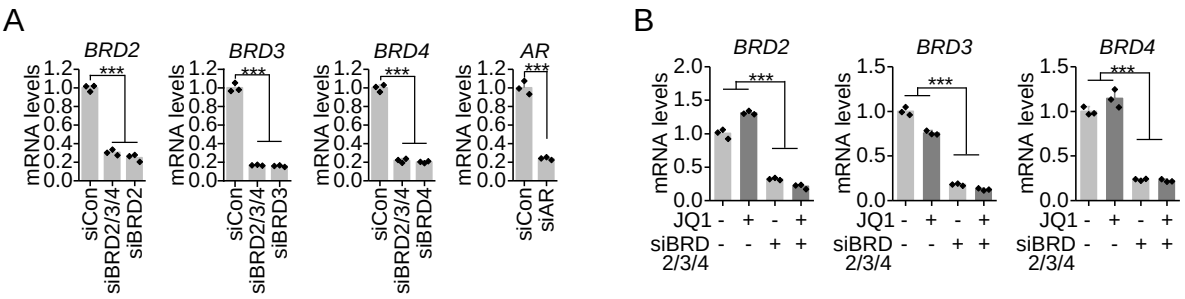


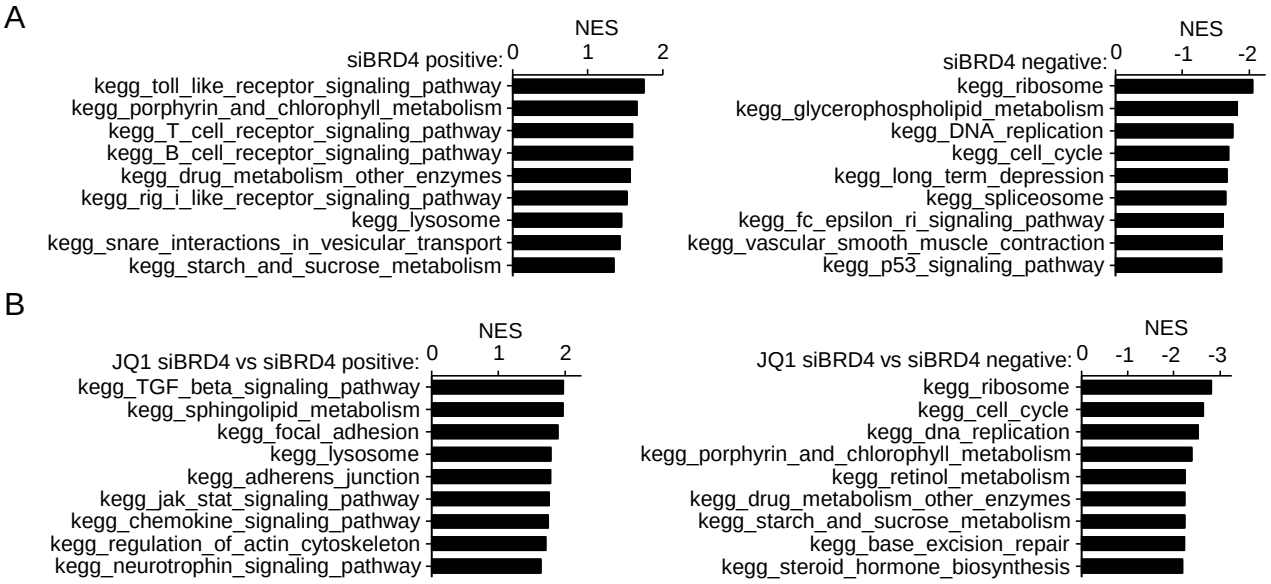
Supplemental Figure 1. JQ1 promotes prostate cancer cell invasion. (A) Cell viability was measured 96 hours after JQ1 treatment. (B) Cell growth was measured after 200 nM JQ1 treatment. n=3 per group, two-way ANOVA, ***P<0.001. (C) Cell invasion was measured 3 days after JQ1 treatment. n=3 per group, one-way ANOVA, **P<0.01, ***P<0.001. (D) c-Myc protein levels were measured 3 days after 200 nM inhibitor treatment. (E) Cell growth was measured 3 days after 200 nM inhibitor treatment. n=3 per group, one-way ANOVA, ***P<0.001. (F) Focal adhesion was stained with paxillin antibody 3 days after JQ1 treatment. Representative images were shown. Scale bar: 10 μm. Focal adhesion number and area were measured. n=8-17 per group, two-way ANOVA, *P<0.05, **P<0.01, ***P<0.001. (G) C4-2-Luc and 22Rv1-luc cells were treated with 200 nM JQ1 for indicated days. Protein levels were measured by western blot. (H) Luciferase activity was measured. (I) C4-2-Luc cells were injected into SCID mice through tail vein. 10 mg/kg JQ1 was given daily by intraperitoneal injection. Luciferase signal image was taken 10 minutes after cell injection and 7 weeks after JQ1 treatment. Metastatic sites with luciferase signal in different tissues were stained with AR antibody. Representative images of AR staining were shown. Scale bar: 400 μm. (J) Kaplan-Meier survival curve of C4-2-Luc cells injected mice treated with JQ1. 10 mg/kg JQ1 was given daily for 7 weeks and then every other day by intraperitoneal injection. n= 12, P value was determined using the log-rank test. (K) LNCaP cells were treated with 200 nM JQ1 for indicated days. Protein levels were measured by western blot.



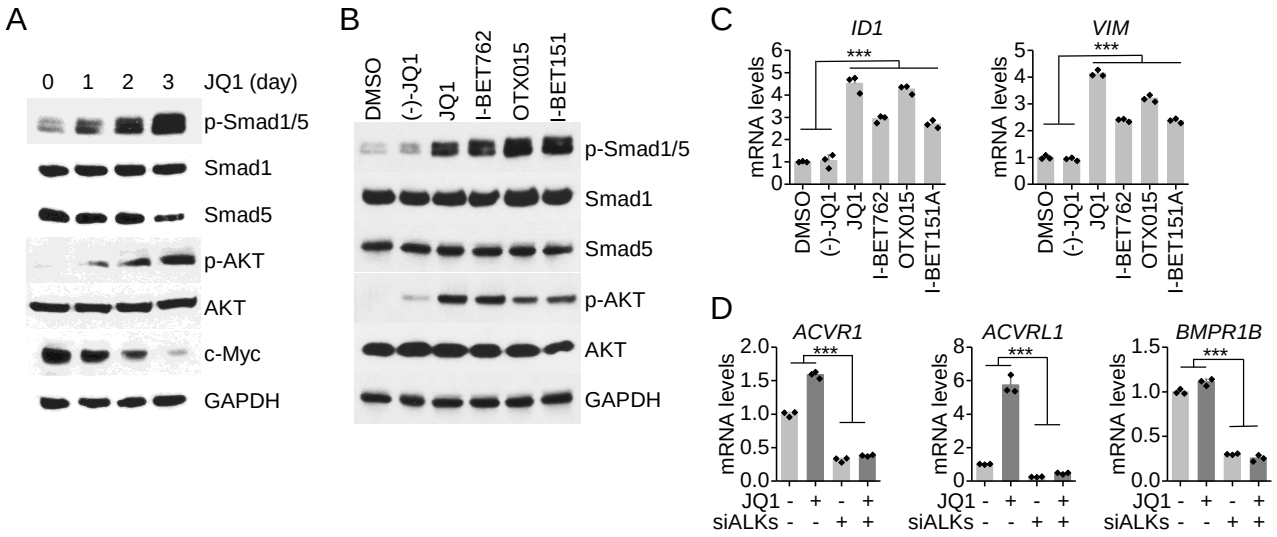
Supplemental Figure 2. JQ1 activates invasion pathways. (A) RNA-sequencing was performed in LNCaP cells 3 days after 200 nM JQ1 treatment. Log2FC (fold change) was shown. (B) GO analysis was done with David program for JQ1 upregulated genes (Log2FC>0.6) and downregulated genes (Log2FC<-0.6). (C) GSEA showed the top positive and negative enriched Hallmark signatures after JQ1 treatment. NES: normalized enrichment score. (D) GSEA showed the top positive and negative enriched KEGG pathways after JQ1 treatment. NES: normalized enrichment score.



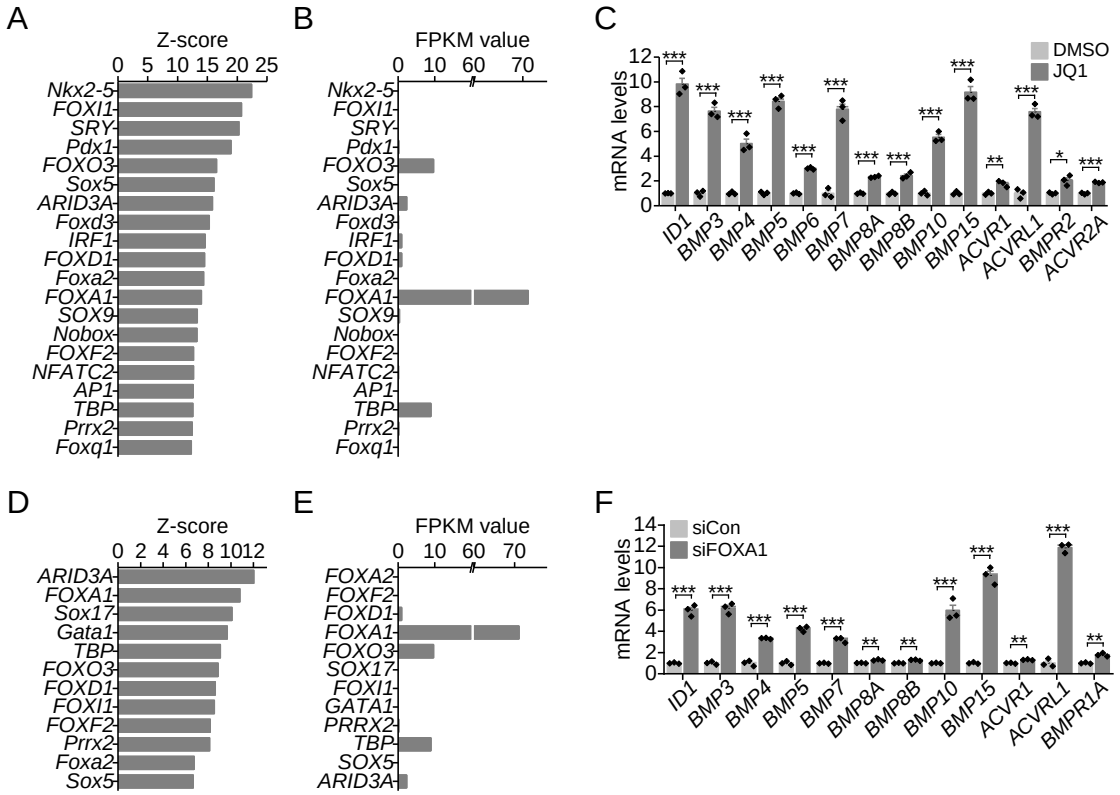
Supplemental Figure 3. (A) Gene silencing efficiency validated for Figure 2C. n=3 per group, one-way ANOVA, ***P<0.001. (B) Gene silencing efficiency validated for Figure 2E. n=3 per group, two-way ANOVA, ***P<0.001.



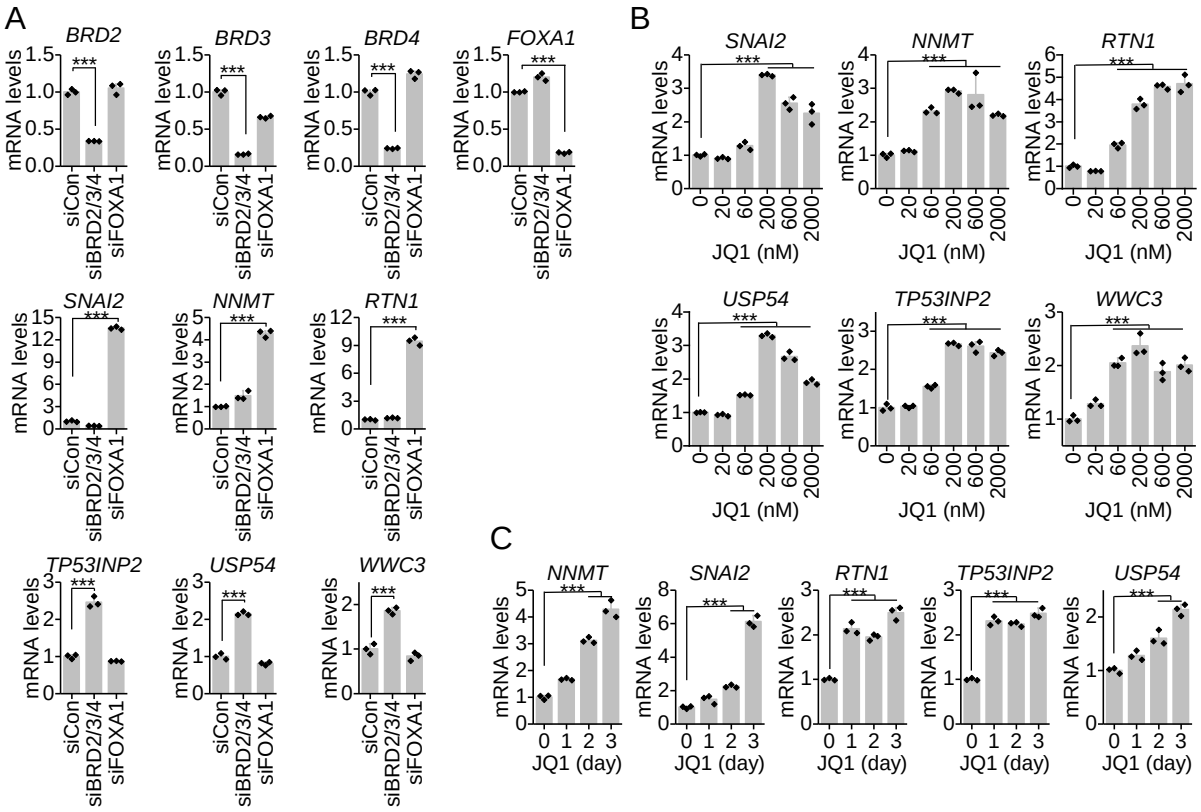
Supplemental Figure 4. JQ1 and BRD4 knockdown regulated pathways. (A) GSEA showed the top positive and negative enriched KEGG pathways by BRD4 knockdown. NES: normalized enrichment score. (B) GSEA showed the top positive and negative enriched KEGG pathways by JQ1 treatment in the absence of BRD4. NES: normalized enrichment score.



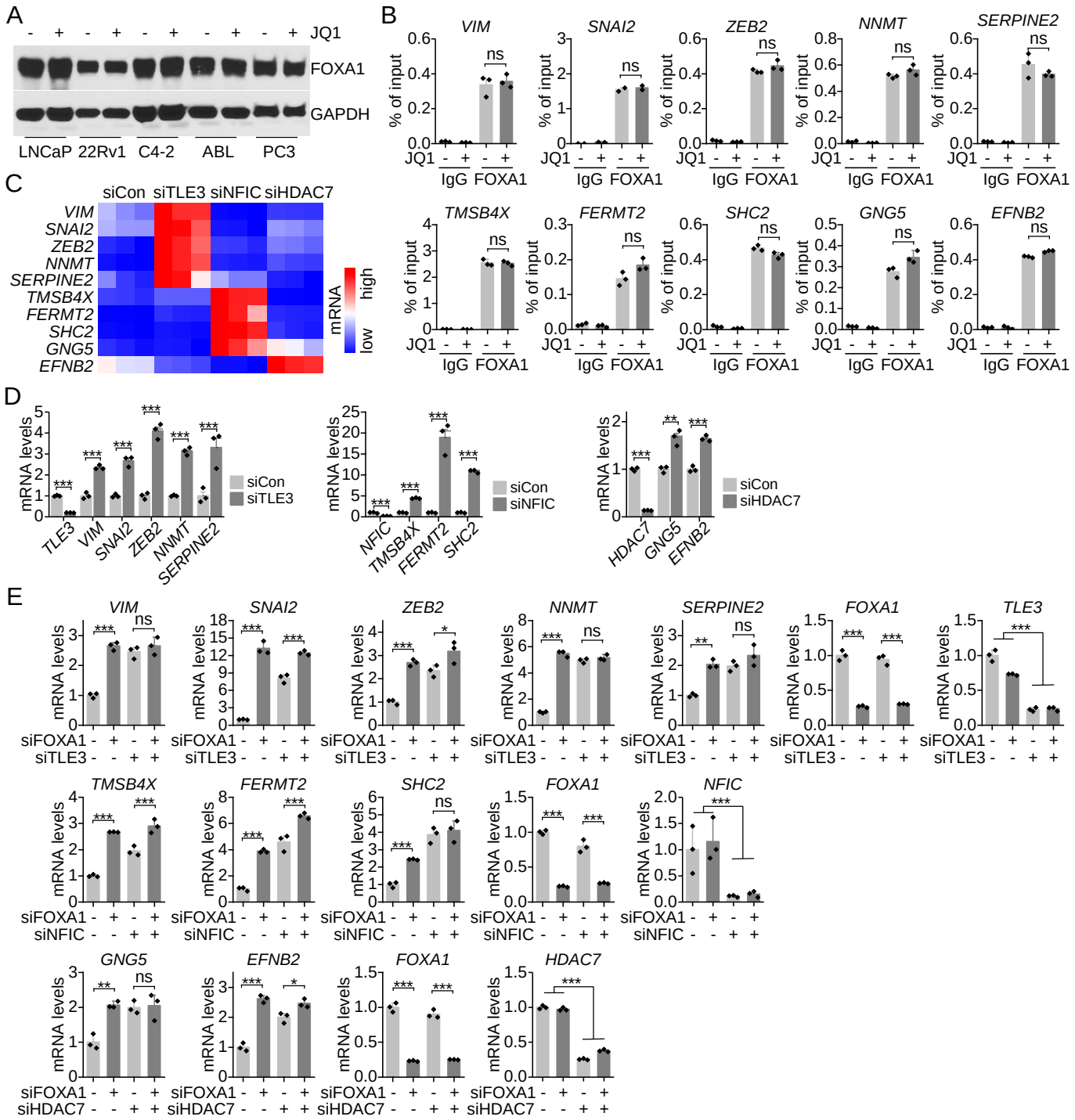
Supplemental Figure 5. JQ1 activates BMP signaling. (A) Time response of JQ1 treatment on BMP signaling. (B) Smad1/5 phosphorylation activated by JQ1 derived inhibitors. (C) BMP signaling marker ID1 and EMT marker VIM were upregulated by JQ1 derived inhibitors. mRNA was measured in LNCaP cells 3 days after 200 nM inhibitor treatment. n=3 per group, one-way ANOVA, ***P<0.001. (D) Gene silencing efficiency validated for Figure 3H. n=3 per group, two-way ANOVA, ***P<0.001.



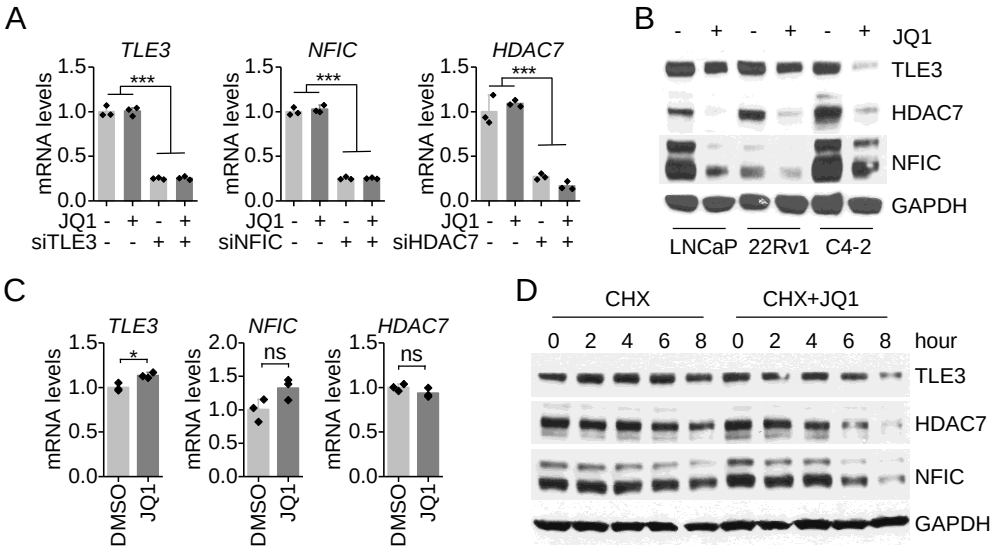
Supplemental Figure 6. FOXA1 is involved in JQ1 activated invasion genes and BMP genes. (A) Predicted transcription factors that have potential binding sites on the promoter of JQ1 activated invasion genes. Prediction was done by oPOSSUM 3.0. (B) Expression of transcription factors in LNCaP cells by RNA-sequencing. FPKM: fragments per kilobase of exon per million reads. (C) BMP signaling genes activated by JQ1. qPCR assay was done for JQ1 treated LNCaP cells. n=3 per group, t test, *P<0.05, **P<0.01, ***P<0.001. (D) Predicted transcription factors that have potential binding sites on the promoter of BMP signaling genes. Prediction was done by oPOSSUM 3.0. (E) Expression of transcription factors in LNCaP cells by RNA-sequencing. FPKM: fragments per kilobase of exon per million reads. (F) Upregulated BMP signaling genes after FOXA1 knockdown. n=3 per group, t test, **P<0.01, ***P<0.001.



Supplemental Figure 7. JQ1 regulates activity of BET proteins and FOXA1. (A) Specific target genes regulated by BET proteins or FOXA1. n=3 per group, one-way ANOVA, ***P<0.001. (B) Dose dependent regulation of BET protein and FOXA1 specific target genes by JQ1. n=3 per group, two-way ANOVA, ***P<0.001. (C) Time dependent regulation of BET protein and FOXA1 specific target genes by JQ1. n=3 per group, two-way ANOVA, ***P<0.001.



Supplemental Figure 8. FOXA1 associated repressors play important role in suppression of invasion gene expression. (A) FOXA1 protein levels after JQ1 treatment for 3 days. (B) ChIP-qPCR analysis of FOXA1 binding sites on target invasion genes after JQ1 treatment. n=3 per group, t test, ns=P>0.05. (C) FOXA1 associated repressors regulated their respective invasion genes. mRNA in LNCaP cell was measured 3 days after siRNA transfection. (D) Expression of FOXA1 repressed invasion genes after silencing of repressors. Gene expression in LNCaP cells was measured 3 days after siRNA transfection. n=3 per group, t test, **P<0.01, ***P<0.001. (E) FOXA1 repressed invasion gene expression through binding repressors. Gene expression in LNCaP cells was measured 3 days after siRNA transfection. n=3 per group, two-way ANOVA, ns=P>0.05, *P<0.05, **P<0.01, ***P<0.001.



Supplemental Figure 9. JQ1 affects FOXA1 interacting repressors. (A) Gene silencing efficiency validated for Figure 5G. n=3 per group, two-way ANOVA, ***P<0.001. (B) Protein levels of FOXA1 binding repressors 3 days after JQ1 treatment. (C) mRNA levels of FOXA1 interacting repressors 3 days after JQ1 treatment. n=3 per group, t test, ns=P>0.05, *P<0.05. (D) Enhanced degradation of FOXA1 associated repressors after JQ1 treatment. LNCaP cells were treated with 50 μg/ml CHX and 10 μM JQ1 for indicated times.

Supplemental Table 1. siRNAs

siRNA:
universal negative control siRNA: Sigma (SIC001)
siBRD4: Dharmacon Smartpool (L-004937-00-0005)
siBRD2-1: Sigma (SASI_Hs01_00018114)
siBRD2-2: Sigma (SASI_Hs01_00018116)
siBRD3-1: Sigma (SASI_Hs01_00086811)
siBRD3-2: Sigma (SASI_Hs01_00086814)
siHDAC7-1: Sigma (SASI_Hs01_00318889)
siHDAC7-2: Sigma (SASI_Hs01_00318894)
siNFIC-1: Sigma (SASI_Hs01_00048020)
siNFIC-2: Sigma (SASI_Hs01_00048021)
siFOXA1-1: GAGAGAAAAAAUCAACAGC
siFOXA1-2: CCAGACGGGUUUCAUUAUU
siTLE3-1: GUCAUCUACUAAACAAGAA
siTLE3-2: GGAUGAGAAGAACCACCAU
siAR-1: GACCUACCGAGGAGCUUUC
siAR-2: CAAGGGAGGUUACACCAAA
siALK1: GGGAUGAACAGUCCUGGUU
siALK2: CCAUCCAGGUGGAUUGUUU
siALK3: GGAUACAAGCUGGGAACUU

Supplemental Table 2. q-PCR primers

q-PCR primers:
ACTB-F: TGGCATTGCCGACAGGAT
ACTB-R: GCTCAGGAGGAGCAATGATCT
VIM-F: CATCAACACCGAGTTCAAG
VIM-R: ATCTTATTCTGCTGCTCCA
SNAI2-F: CGAACTGGACACACATACAGTG
SNAI2-R: CTGAGGATCTCTGGTTGTGGT
ZEB2-F: CGGTAGTGAGTCATAATGGT
ZEB2-R: GTCTCCTTGAGTCAGTAGTC
NNMT-F: TGGCCCTCCCTCTACCATAC
NNMT-R: TCCTTCCATCAGTTCAGCCC
SERPINE2-F: TCAGTGAAGATGGAACCAA
SERPINE2-R: TGTAGGATTATGTCGGATGA
FERMT2-F: AGAACAAGCAGCCAGGCTAT
FERMT2-R: TTTTGCCCCCTTGGAACCTT
SHC2-F: GGACTTCCTTGTGCGAGACA
SHC2-R: CAGGTGGCTGATGCTCTCAA
TMSB4X-F: GAATCGTAATGAGGCGTGCG
TMSB4X-R: CGGCCTTCGTTGTCAGTAGT
GNG5-F: CCACAAACTGGGAGGAAA
GNG5-R: CGCGAAGAACTAAGAGGG
QSOX1-F: ATGGCTGACCTGGAATCTGC
QSOX1-R: CACGGAGTGCAGGAAGTTCT
LAMC1-F: GAGGCAAGATATCGCCGTGA
LAMC1-R: GTATCTCGCCTGTCCACTCG
APLP1-F: CTGCCTAAAGCCGACAGACA
APLP1-R: TGGTCGTTGATAAGGGCGAT
GNAI2-F: AAGTGACTCCGTGCCTTGA
GNAI2-R: GAACAGCCCTTGGAACCC
EFNB2-F: ACTGTGCCAAACCAGACCAA
EFNB2-R: ACCCCTCCCTCCTGGTTATC
GSN-F: GCTCTACAAGGTCTCCAAT
GSN-R: GTCCAGGATGAAGCAGTC
SEMA3C-F: CGGCCCAGAAACACACTTTG
SEMA3C-R: TACACACACACGGCTGATCC
PLXNA3-F: TCTTTAAGCTGGCCCCCAAC
PLXNA3-R: GAGCAGCAGCTTGTTGATGT
FSTL1-F: TGGCAAGACCTACCTCAA
FSTL1-R: GCAGATGGACTTACGGATT
BRD2-F: GAGGACGAGGAGGAAGAA
BRD2-R: GAGCAGCCAGTTGTTCAT
BRD3-F: CAGGCTCAGCAGAAGAAG
BRD3-R: GAGTCGTAGGAGGCAGAT
BRD4-F: GGAGGAAGAGGACAAGTG
BRD4-R: CCGTGACTGGATGATGTG
AR-F: CAGTGGATGGGCTGAAAAAT
AR-R: GGAGCTTGGTGAGCTGGTAG

ACVR1-F: CTCTCCGAGTACCCCAGTGA
ACVR1-R: ACCATTGTACAACTTAATGCAGGC
ACVRL1-F: ATTGTGCAAGGCTCGGAAGA
ACVRL1-R: CCAGAGGACGTGCAAACTCT
BMPR1B-F: GAACTTGCTGTATTGCTGAC
BMPR1B-R: AACACTTCTGGAGGCATATAG
ID1-F: TGTTACTCACGCCTCAAG
ID1-R: TGAAGGTCCCTGATGTAGT
BMP3-F: AGAGCCAGACGCTCCAATTT
BMP3-R: TCCAGCCAATATCTGCAAAGTC
BMP4-F: AGCGTAGCCCTAAGCATCAC
BMP4-R: AGTCATTCCAGCCCACATCG
BMP5-F: TGGTAGTACGCTCATGTGGC
BMP5-R: AGGAAATTCCCCGTTTGTCTGA
BMP6-F: CTTTCTGCGAGCGGGTTTG
BMP6-R: CCGTCGCTCCCACTCAAAAT
BMP7-F: GACTTCAGCCTGGACAACGA
BMP7-R: GCAAGCCCAAAATGGAGAGG
BMP8A-F: CTCAGCCACTGACTTGGTGT
BMP8A-R: TCTCTGGGTTGGAAGAGCCT
BMP8B-F: GTGGCTTCCTTATCTGCG
BMP8B-R: GTTATGAGGCTGCTTCTGG
BMP10-F: GGGAGTGCAGAGAGCAACAT
BMP10-R: TGGGAGAACTCCTCTACGGG
BMP15-F: TTCCAAATCAGCTTCCGCCA
BMP15-R: TCGCGTAGTACTCGGAGACA
BMPR1A-F: AAAAAGTGGCGGTGAAAGT
BMPR1A-R: TGTCTGCCGCTATGAAAC
BMPR2-F: AGATCCTGGGCCATCAAAGC
BMPR2-R: ACTCACCTATCTGTATACTGCTGC
ACVR2A-F: ACAAGGTTGTTGGCTGGATGA
ACVR2A-R: ACACATATTGCCCTCACAGCA
FOXA1-F: CTACTACGCAGACACGCAGG
FOXA1-R: CCGCTCGTAGTCATGGTGTT
HDAC7-F: GTCACTGACCTCGCCTTCAA
HDAC7-R: GTGCACGTCCCAGTCTACAA
TLE3-F: TGGTGAGCTTTGGAGCTGTT
TLE3-R: ATCTGCCCATCAGCACTCAC
NFIC-F: GCAGTACCAGCAGGACTTGT
NFIC-R: GGATCCCCTTCCTCCTTCCT
RTN1-F: AAGCACCAGGCACAGATTGA
RTN1-R: CGCCTGGGATTTTAGCCTGA
TP53INP2-F: TGACCATGTGACGTGTCAAC
TP53INP2-R: ACACACATACACAGGCATGC
USP54-F: AAAATTAGCCAGGCGTGACG
USP54-R: TCACTCTATTGCCAAGCTGGAG
WWC3-F: AGCCTGCAAGAAACAGATGC
WWC3-R: TTGTCATGGCTGCATTGAGC

Supplemental Table 3. ChIP-qPCR primers

ChIP-PCR primers:
FOXA1-VIM-F: TAGGCCACTGGGCAAATACCT
FOXA1-VIM-R: CTCATGTCACTTGAAAGAGCCC
FOXA1-SNAI2-F: CAGGAGAAAATGCCTTTGGA
FOXA1-SNAI2-R: ATGAGGAATCTGGCTGCTGT
FOXA1-ZEB2-F: GAGGCATCGGTCACTTCCAC
FOXA1-ZEB2-R: CCAGATCGGCTGTTGCATAGT
FOXA1-NNMT-F: AGGCTTTCAGGAAAAGGGTGT
FOXA1-NNMT-R: CTCCAGAGGTGCTACACCAA
FOXA1-SERPINE2-F: TGCAGCTTTGGTCCATCCAT
FOXA1-SERPINE2-R: ACCCACCTCTTGTACCCAGT
FOXA1-TMSB4X-F: CAGGTGCCTACGTTTCCGT
FOXA1-TMSB4X-R: TCATTCCTCTCACGCTGGTG
FOXA1-FERMT2-F: AGCCTACTTGTCTGGCTTGTC
FOXA1-FERMT2-R: ACAGGGGTATGTAGGTTTGCC
FOXA1-SHC2-F: CTCTCTCAGAAGACGGGGGA
FOXA1-SHC2-R: GCCTCCACCTAGGAGGGTAA
FOXA1-GNG5-F: AAGCAGGATGGGAAATGCCT
FOXA1-GNG5-R: TCAGAGTGGTTGTGAAAATTAAGGG
FOXA1-EFNB2-F: AGGCTCTACTCCGCTACACA
FOXA1-EFNB2-R: ATTCGAGATCCCAGCGTGAC

Full unedited gel for Figure 3

Figure 3A

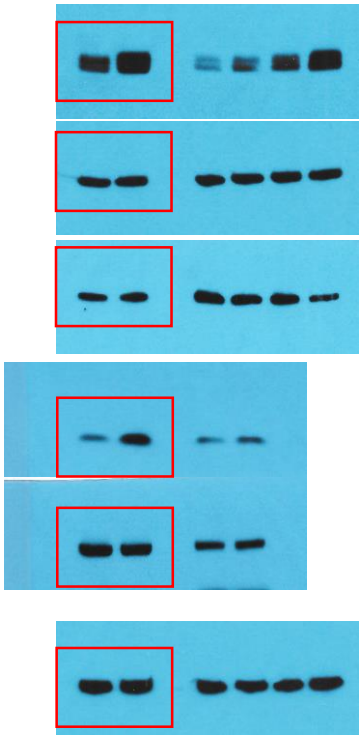


Figure 3B

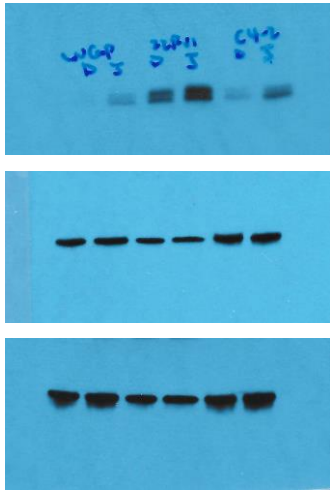
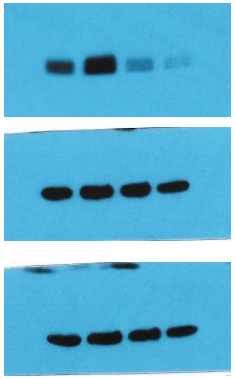


Figure 3E



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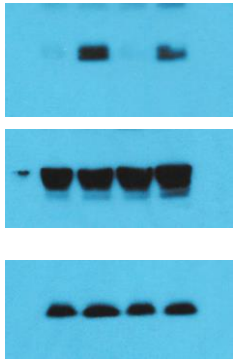


Full unedited gel for Figure 5

Figure 5A

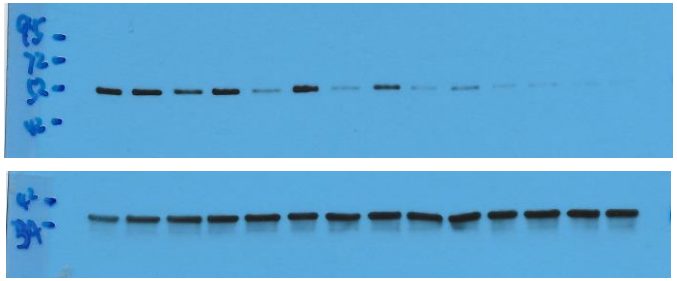


Figure 5B

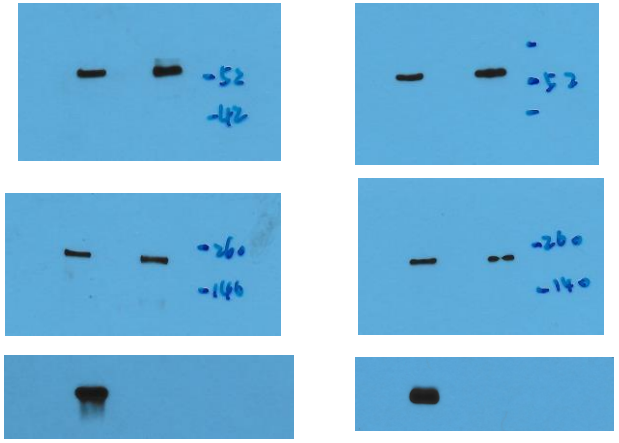


Figure 5C

Figure 5D

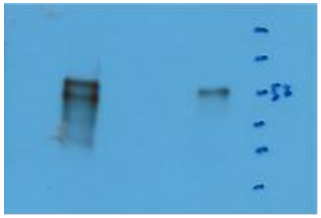
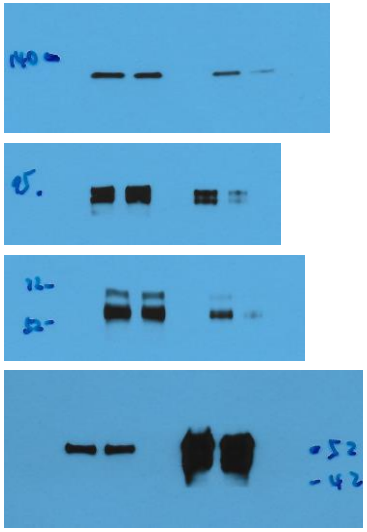
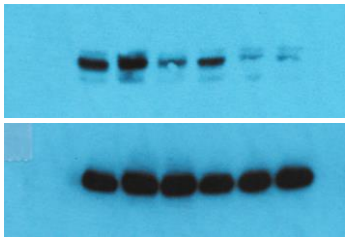


Figure 5E

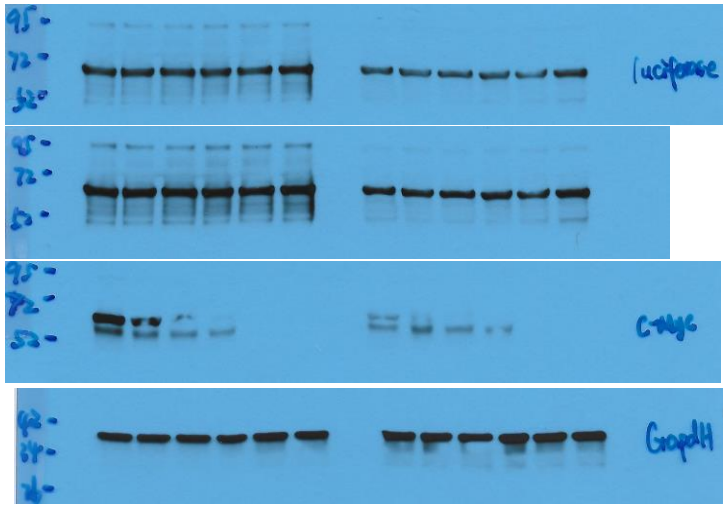


Full unedited gel for Supplemental Figure 1

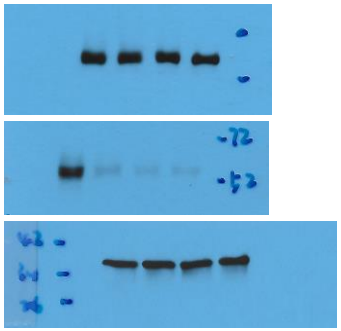
Supplemental Figure 1D



Supplemental Figure 1G

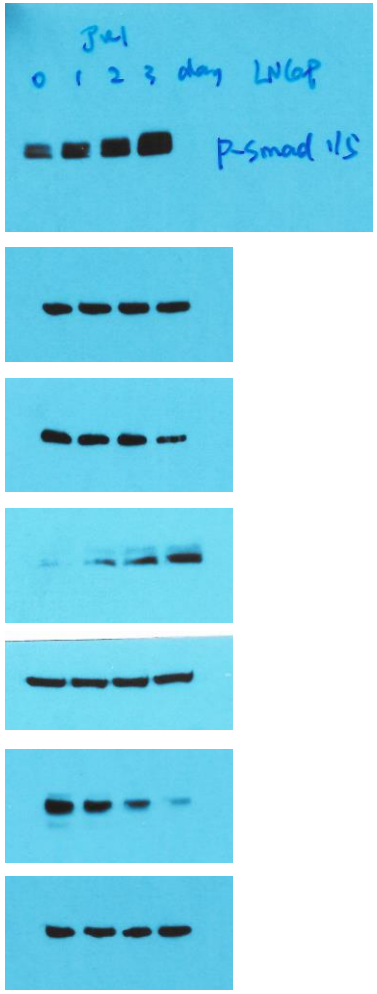


Supplemental Figure 1K

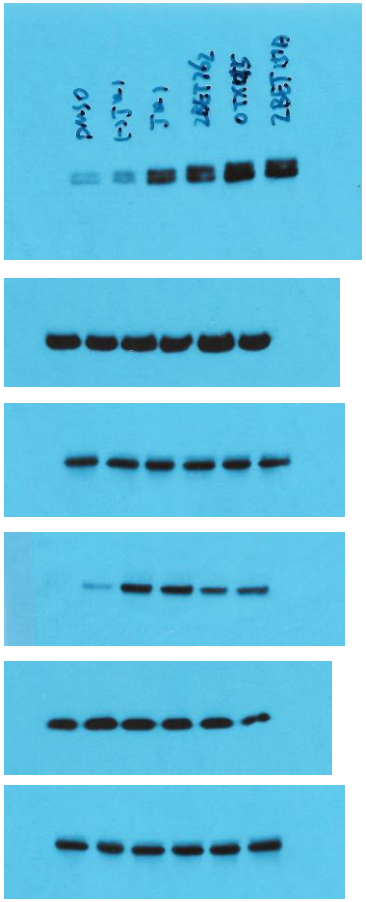


Full unedited gel for Supplemental Figure 5

Supplemental Figure 5A

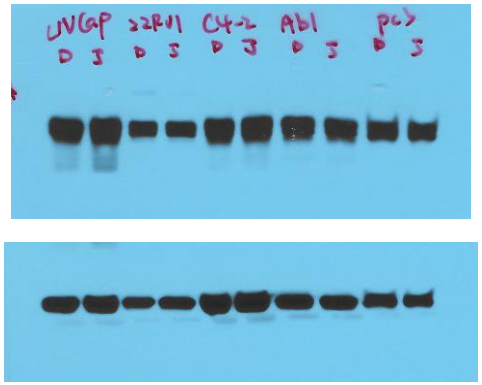


Supplemental Figure 5B

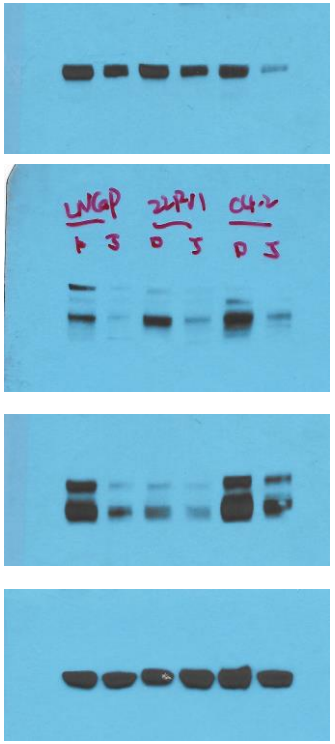


Full unedited gel for Supplemental Figure 8

Supplemental Figure 8A



Supplemental Figure 9A



Supplemental Figure 9D