# Supplementary Information

# BET Bromodomain Inhibition as a Therapeutic Strategy in Ovarian Cancer by Downregulating FoxM1

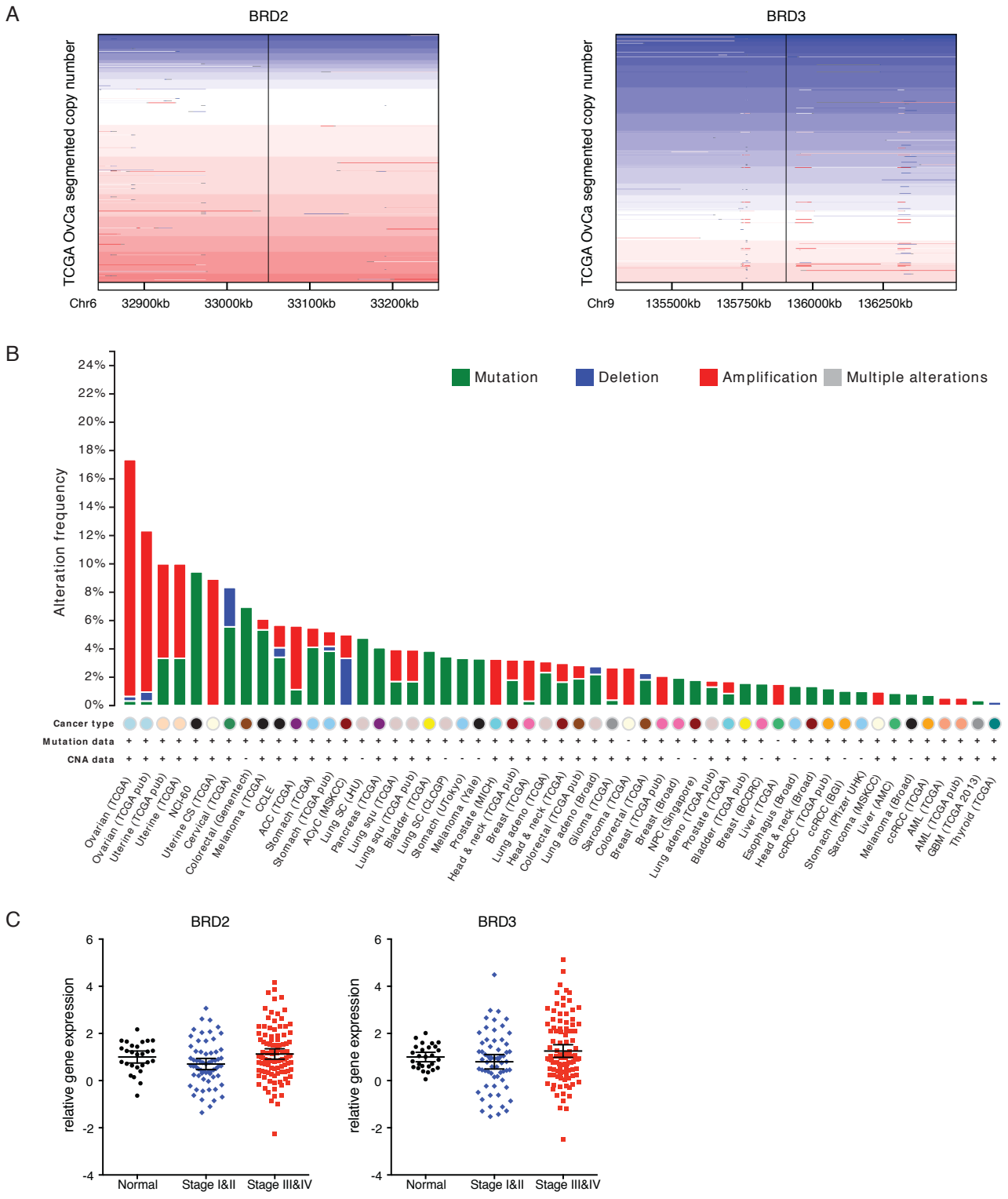
Zhenfeng Zhang[1,2,*], Pengfei Ma[1,*], Ying Jing[1,*], Ying Yan[3], Mei-Chun Cai[4], Meiying Zhang[5,6], Shengzhe Zhang[7], Huixin Peng[1], Zhi-Liang Ji[4], Wen Di[5,6], Zhenyu Gu[3], Wei-Qiang Gao[1,7], Guanglei Zhuang[1,6]

[1]State Key Laboratory of Oncogenes and Related Genes, Renji-Med X Clinical Stem Cell Research Center, Ren Ji Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China
[2]State Key Laboratory of Oncogenes and Related Genes, Shanghai Cancer Institute, Ren Ji Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China
[3]GenenDesign Co., Ltd, Shanghai, China
[4]State Key Laboratory of Cellular Stress Biology, School of Life Sciences, Xiamen University, Xiamen, Fujian, China
[5]Department of Obstetrics and Gynecology, Ren Ji Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China
[6]Shanghai Key Laboratory of Gynecologic Oncology, Ren Ji Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China
[7]School of Biomedical Engineering & Med-X Research Institute, Shanghai Jiao Tong University, Shanghai, China

Corresponding author:
Guanglei Zhuang
State Key Laboratory of Oncogenes and Related Genes, Renji-Med X Clinical Stem Cell Research Center, Ren Ji Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China
Email: zhuangguanglei@gmail.com

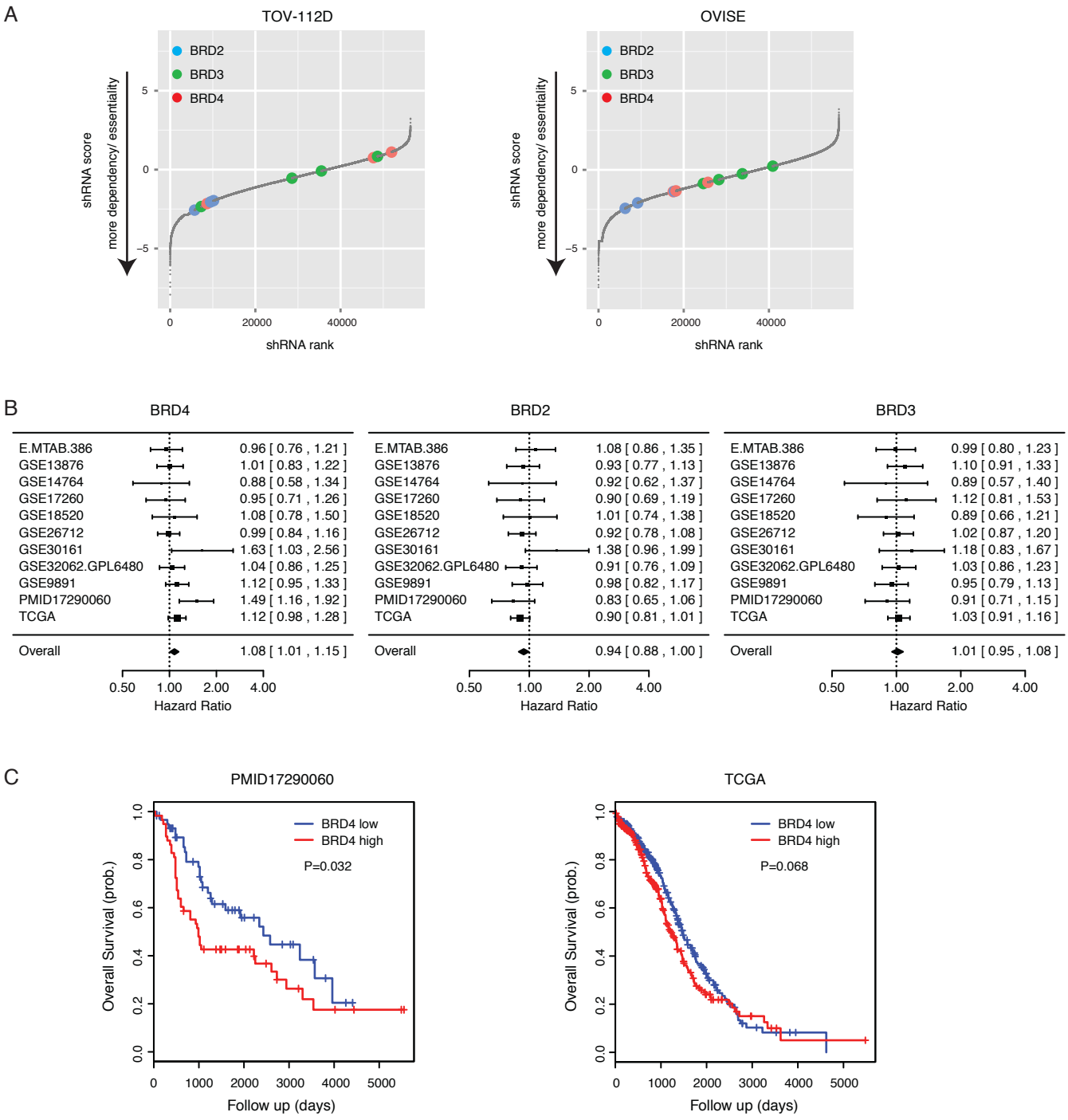
[*]These authors contributed equally to this work.

Supplementary Figure 1.



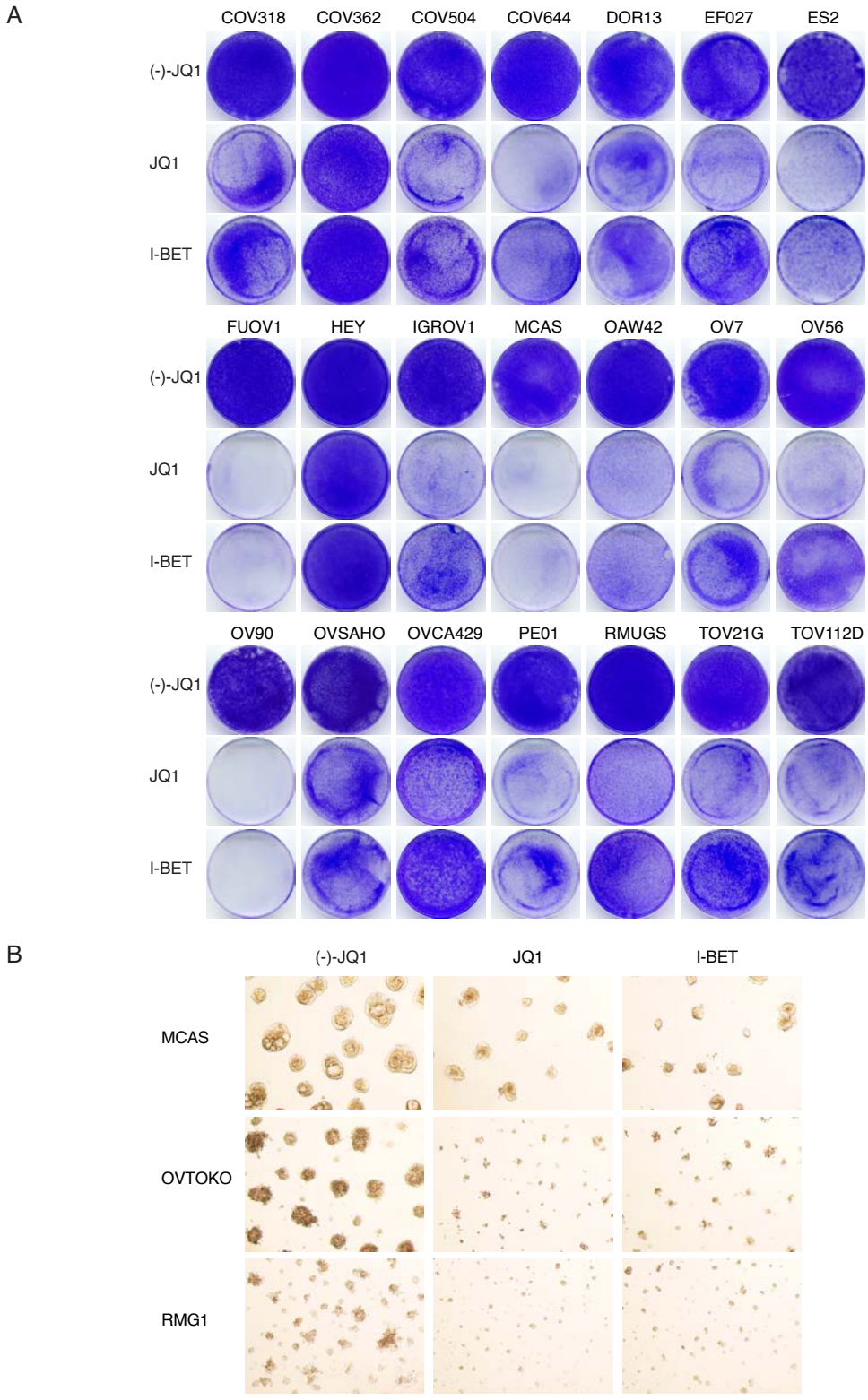

Supplementary Figure 1. A. Copy number analysis of BRD2 and BRD3 in TCGA ovarian cancer samples. Color scale: amplification in red and deletion in blue. B. Pan-cancer analysis of BRD4 genomic alterations in cBioportal database. C. Quantitative PCR of BRD2 and BRD3 in an ovarian cancer tissue cDNA array containing 192 clinical samples.

Supplementary Figure 2.



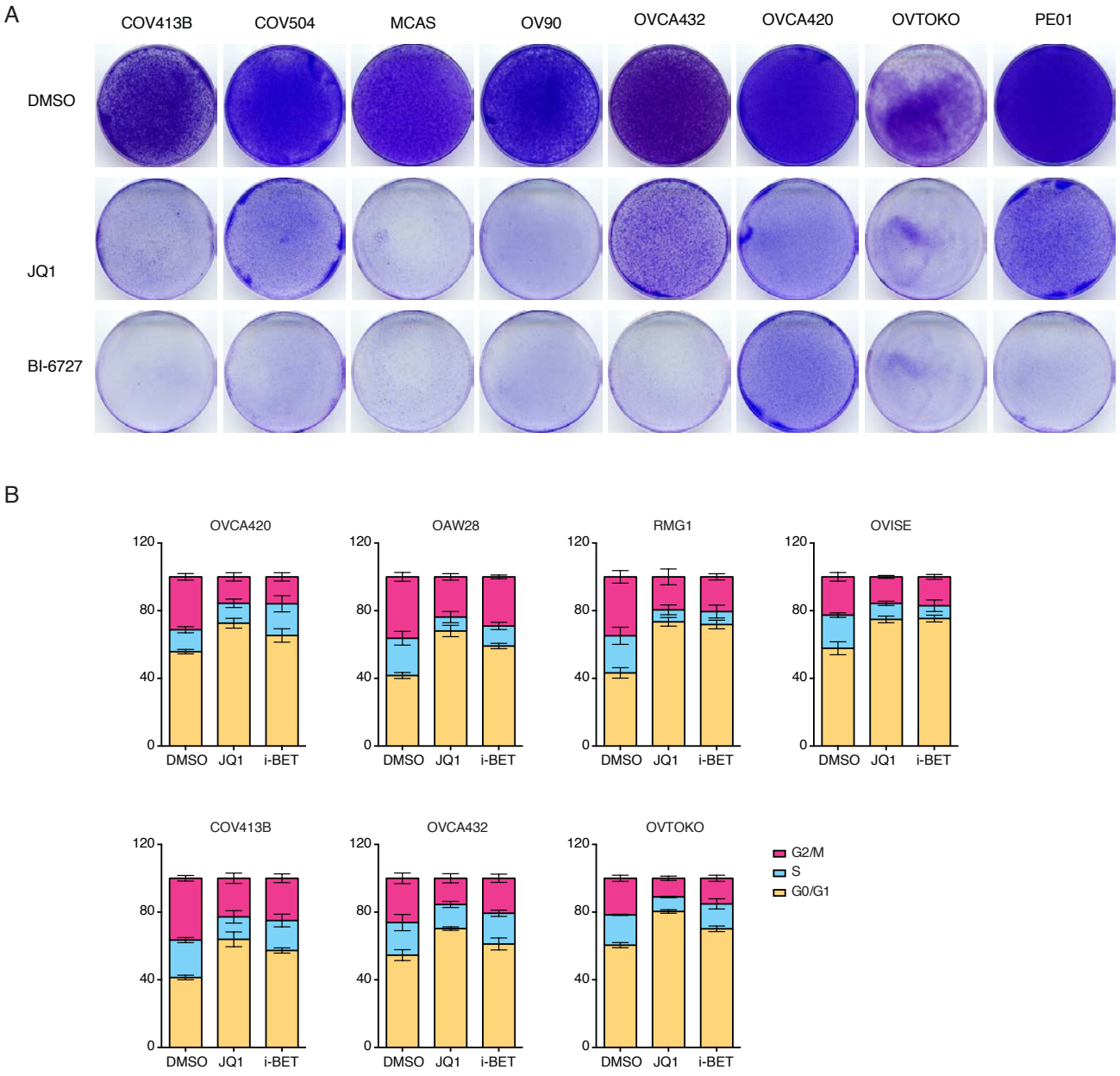

Supplementary Figure 2. A. Results of shRNA lentiviral screen in TOV-112D and OVISE cells were presented in rank order of ascending shRNA scores. The effect of shRNAs targeting BET proteins was highlighted by colored dots. Gray dots represent results for non-BET shRNAs. B. Forest plot of the expression of BRD2, BRD3 or BRD4 as a univariate predictor of overall survival in multiple ovarian cancer data sets. C. Kaplan-Meier plot of overall survival in ovarian cancer patients with high or low BRD4 gene expression.

Supplementary Figure 3.



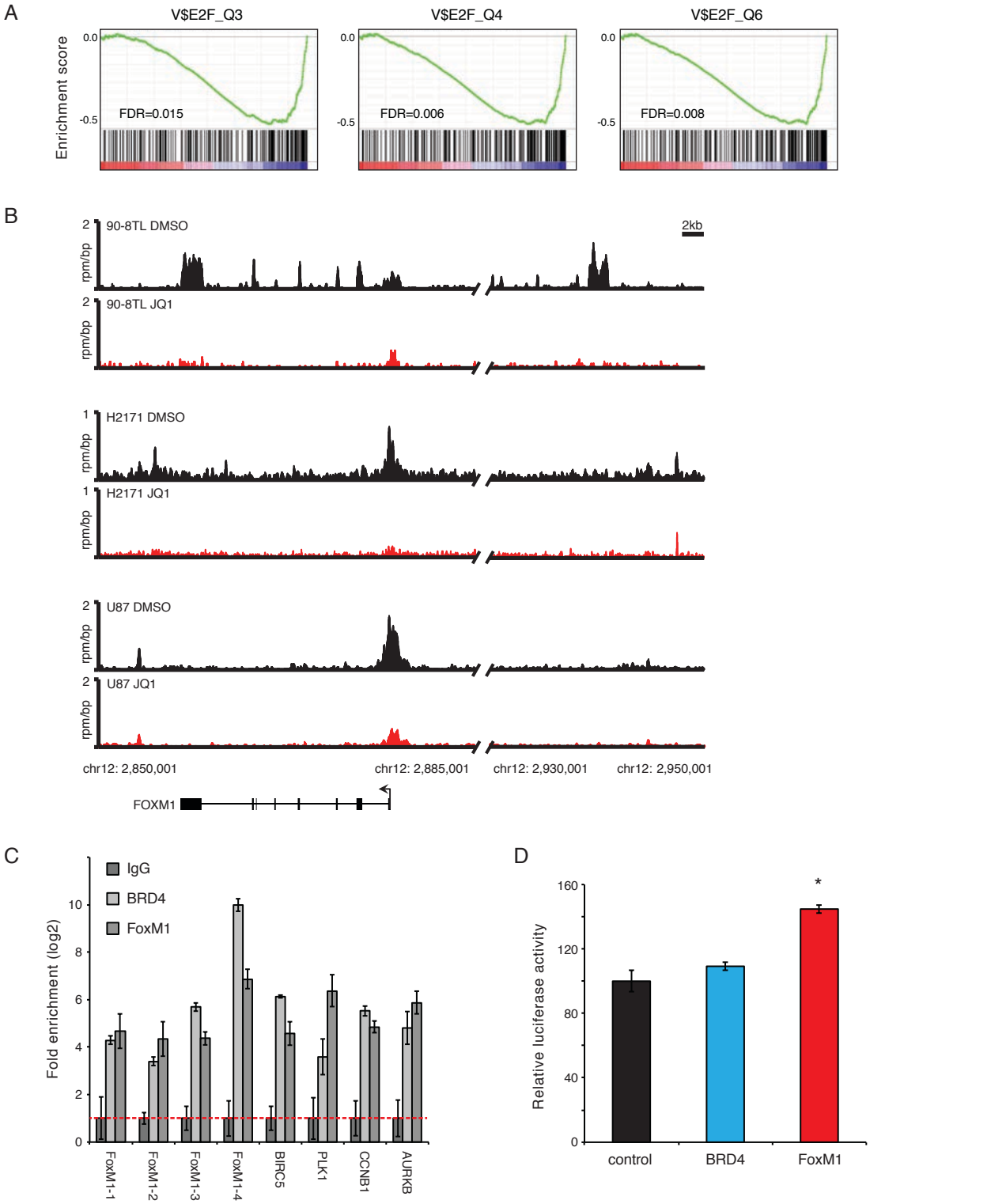

Supplementary Figure 3. A. Cells were treated with (-)-JQ1, JQ1 (1 µM) or I-BET151 (1 µM) for 10 days. The remaining cells were stained with crystal violet. B. Cells were cultured on Matrigel and treated with (-)-JQ1, JQ1 (1 µM) or I-BET151 (1 µM) for 10 days.

Supplementary Figure 4.



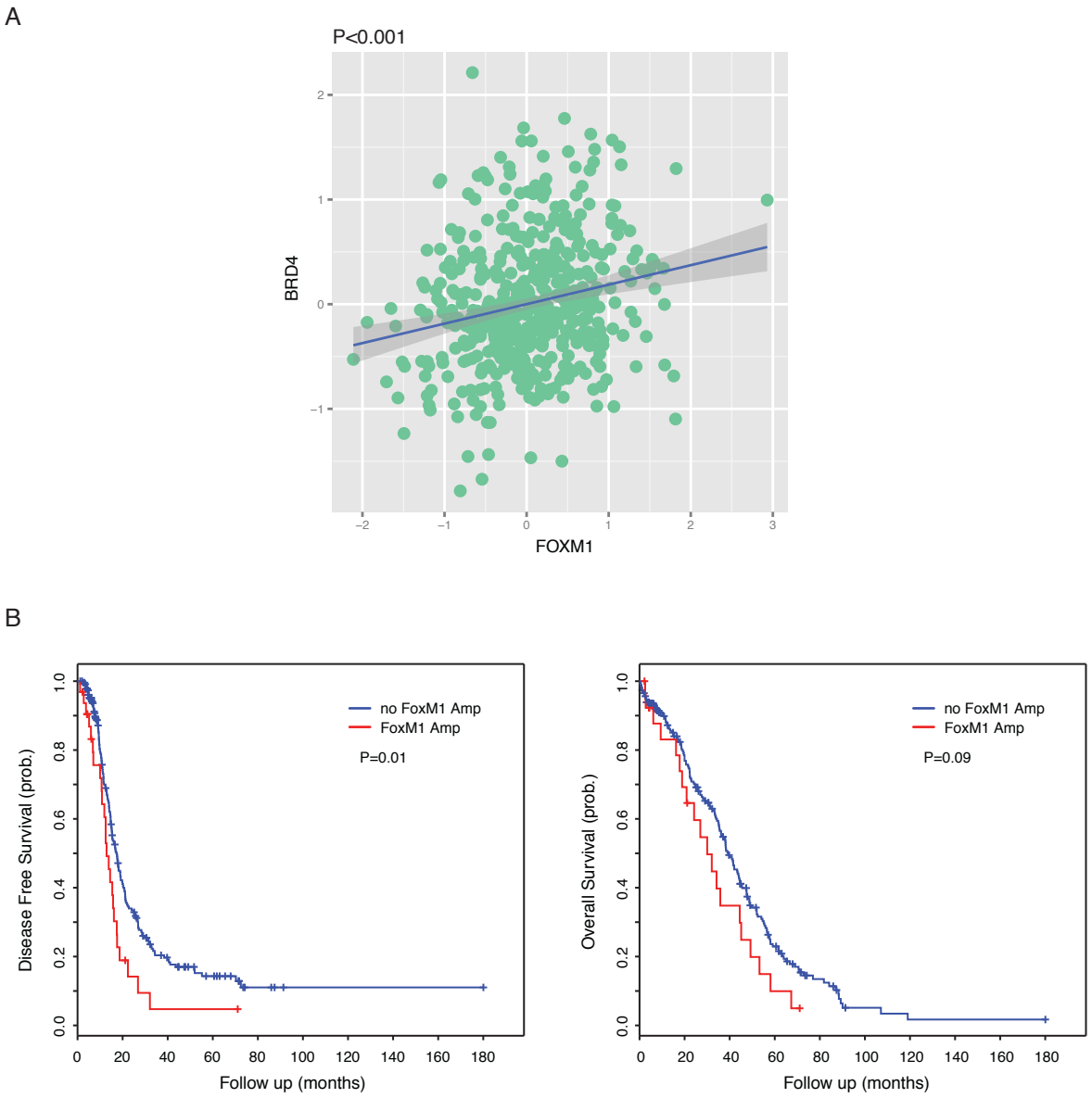

Supplementary Figure 4. A. Cells were treated with DMSO, JQ1 (1 μM) or BI-6727 (1 μM) for 10 days. The remaining cells were stained with crystal violet. B. Quantification of cell cycle analysis.

Supplementary Figure 5.



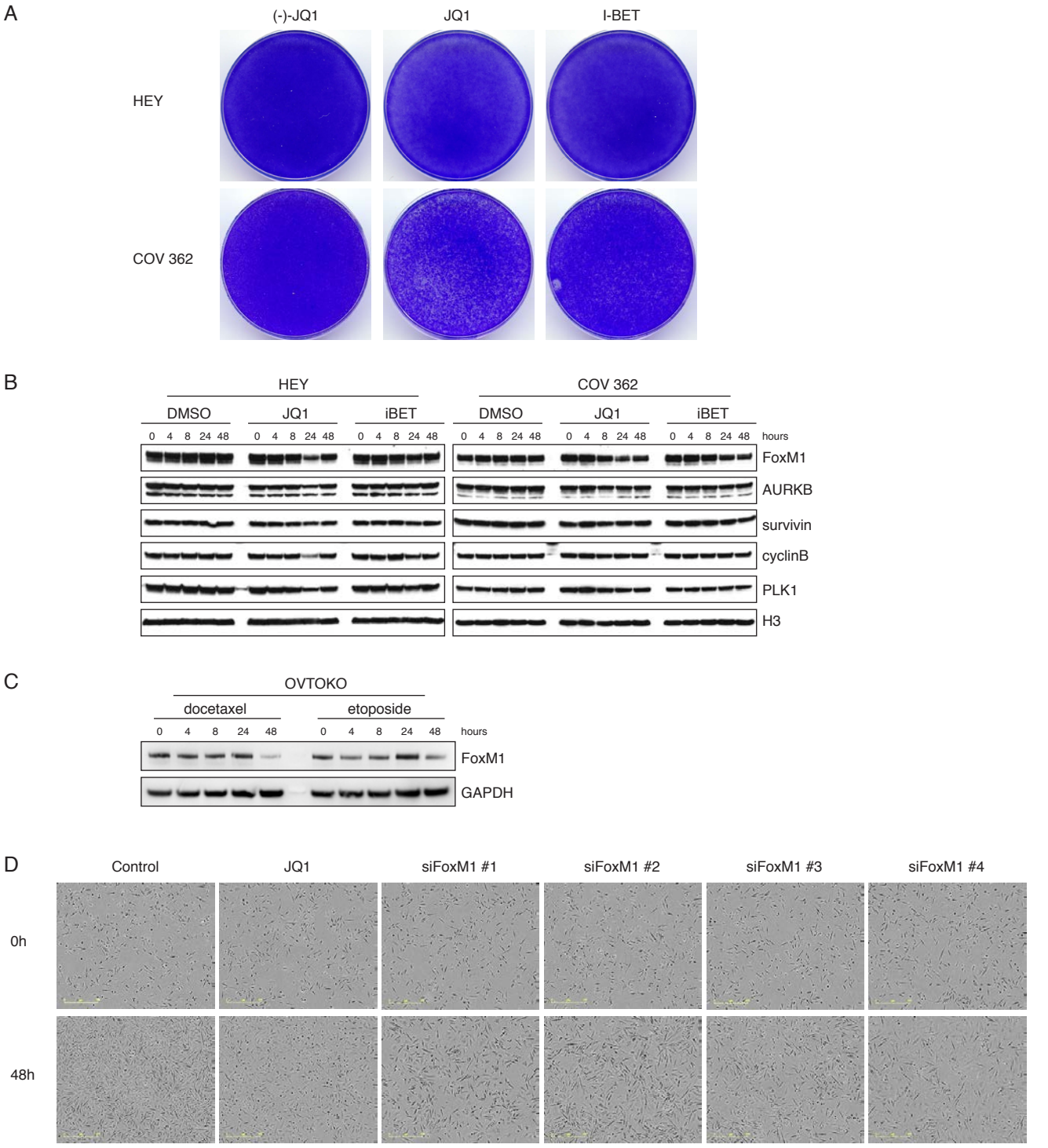

Supplementary Figure 5. A. GSEA plots of indicated functionally defined gene sets in DMSO versus JQ1 treated OVTOKO cells at 24 hour. B. ChIP-seq binding density for BRD4 at the enhancer and promoter of FoxM1 following DMSO or JQ1 treatment. C. CHIP-qPCR analysis indicated BRD4 and FoxM1 occupancy at promoters of FoxM1 and FoxM1 target genes. D. Luciferase assay of FoxM1 promoter in HEK293T cells transfected with control, BRD4 or FoxM1. *P<0.05.

Supplementary Figure 6.




Supplementary Figure 6. A. Correlation of FoxM1 and BRD4 gene expression in TCGA ovarian cancer patients. B. Kaplan-Meier plots of disease free survival and overall survival in ovarian cancer patients with or without FoxM1 gene amplification.

Supplementary Figure 7.




Supplementary Figure 7. A. Cells were treated with (-)-JQ1, JQ1 (1 μM) or I-BET151 (1 μM) for 10 days. The remaining cells were stained with crystal violet. B. HEY and COV362 cells were treated with DMSO, JQ1 (1 μM) or I-BET151 (1 μM). Cell lysates were immunoblotted with indicated antibodies. C. Images of OVTOKO cell growth upon FoxM1 knockdown or JQ1 treatment.

## Supplementary Table 1: IC50 of compounds (μM)

| Cell line | IC50 of (-)-JQ1 | IC50 of JQ1 | IC50 of I-BET |
|---|---|---|---|
| DOV 13 | 10 | 0.59 | 0.98 |
| HEY | 10 | 1.00 | 10 |
| COV 318 | 10 | 0.26 | 0.97 |
| COV 413B | 10 | 0.19 | 0.61 |
| COV 504 | 10 | 0.50 | 1.51 |
| FU-OV-1 | 10 | 0.10 | 0.24 |
| OAW28 | 10 | 0.10 | 0.35 |
| OAW42 | 10 | 0.36 | 1.36 |
| OV7 | 10 | 0.57 | 3.73 |
| OV56 | 10 | 0.91 | 3.85 |
| OV90 | 10 | 0.10 | 0.22 |
| OVCA420 | 10 | 0.11 | 0.35 |
| OVCA429 | 10 | 10 | 10 |
| OVCA432 | 10 | 0.27 | 0.54 |
| OVSAHO | 10 | 2.19 | 0.79 |
| PE01 | 10 | 0.30 | 0.75 |
| ES-2 | 10 | 1.04 | 1.95 |
| OVISE | 10 | 0.10 | 0.10 |
| OVTOKO | 10 | 0.10 | 0.65 |
| RMG-1 | 10 | 0.10 | 0.18 |
| TOV-21G | 10 | 1.63 | 4.14 |
| COV 644 | 10 | 0.74 | 2.47 |
| EFO 27 | 10 | 0.30 | 0.93 |
| MCAS | 10 | 0.12 | 0.89 |
| RMUG-S | 10 | 0.10 | 0.52 |
| COV 362 | 10 | 1.86 | 3.10 |
| IGROV1 | 10 | 1.40 | 10 |
| TOV-112D | 10 | 1.19 | 2.37 |