Expanded View Figures

Figure EV1. BAZ2A-bromodomain is an epigenetic reader of H3K14ac.

A Schematic representation of the strategy to generate PC3 cell line with FLAG-HA (F/H) tag insertion at *BAZ2A* locus by CRISPR/Cas9 method. Lower panel. PCR genotyping to measure the insertion of F/H sequences into *BAZ2A* locus. Arrows represent the primers used for PCR genotyping.

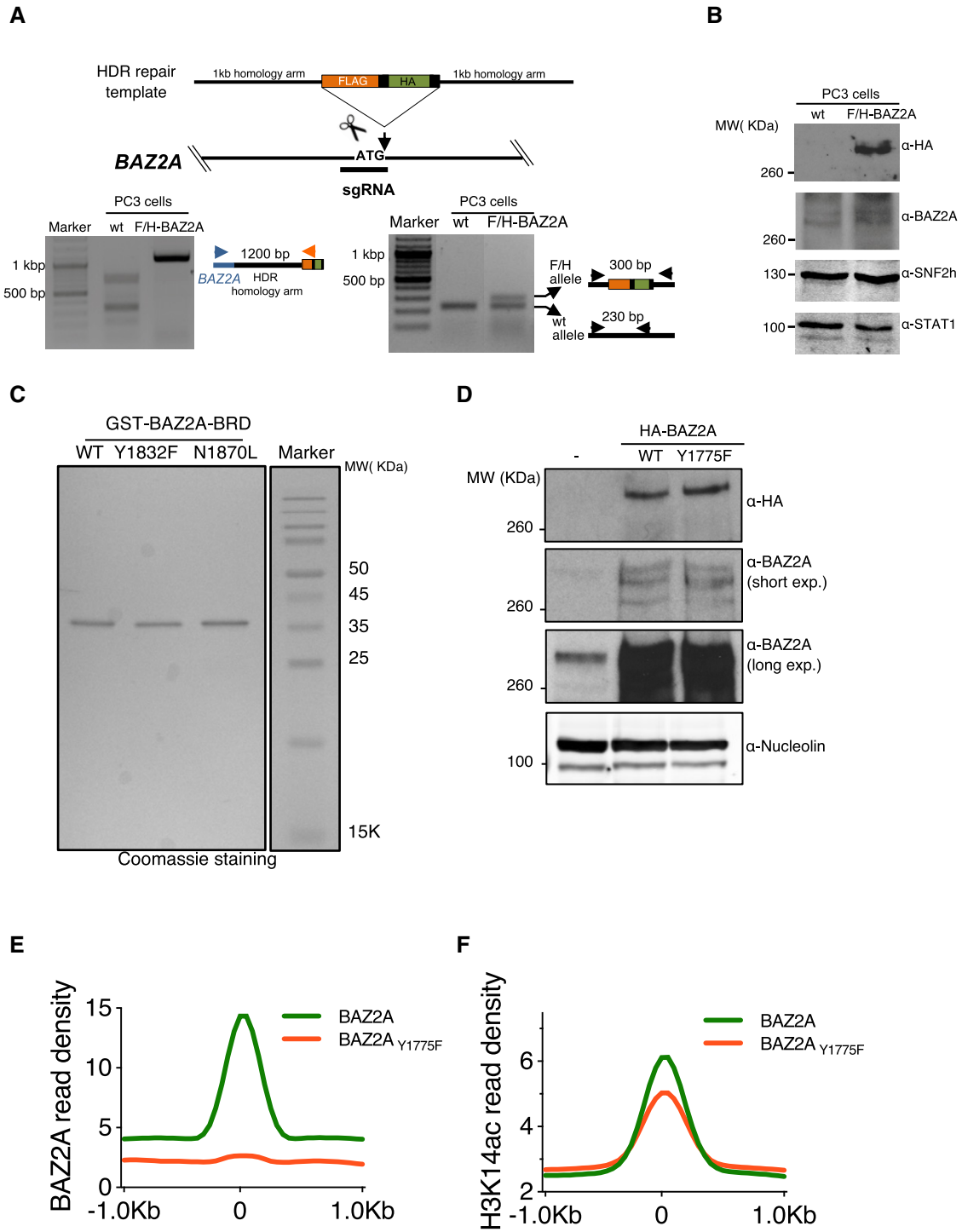
B Immunoblot of PC3 wild-type (wt) cells and the PC3 cell line expressing endogenous BAZ2A with F/H tag showing comparable levels of BAZ2A. SNF2h and STAT1 serve as protein loading control.

C Representative protein gel showing the purity of recombinant GST-BAZ2A-BRD wild-type [WT]) and mutants (GST-BAZ2A-BRD_{Y1830F} or GST-BAZ2A-BRD_{N1873L}). Proteins are visualized by Coomassie staining.

D Western blot showing equal expression of ectopically expressed HA-BAZ2Awt and HA-BAZ2A-BRD mutant (Y1775F) in transfected PC3 cells and endogenous levels of BAZ2A in untransfected PC3 cells. Whole cell lysates from equivalent amounts of cells were analyzed. Nucleolin is shown as a protein loading control.

E, F Average density plots of ChIPseq read counts of BAZ2A (BAZ2Awt and BAZ2A-BRD mutant, Y1775F) and H3K14ac at $\pm$ 1 Kb from BAZ2A peak summits in PC3 cells.

Source data are available online for this figure.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HA
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500 bp
1200 bp
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Marker
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500 bp
F/H allele
300 bp
wt allele
230 bp
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α-SNF2h
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α-STAT1
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C
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WT
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N1870L
Marker
MW(KDa)
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45
35
25
15K
Coomassie staining
D
HA-BAZ2A
-
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MW (KDa)
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α-BAZ2A (long exp.)
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α-Nucleolin
100
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5
0
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BAZ2A Y1775F
-1.0Kb
0
1.0Kb
F
H3K14ac read density
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2
BAZ2A
BAZ2A Y1775F
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1.0Kb

Figure EV1.

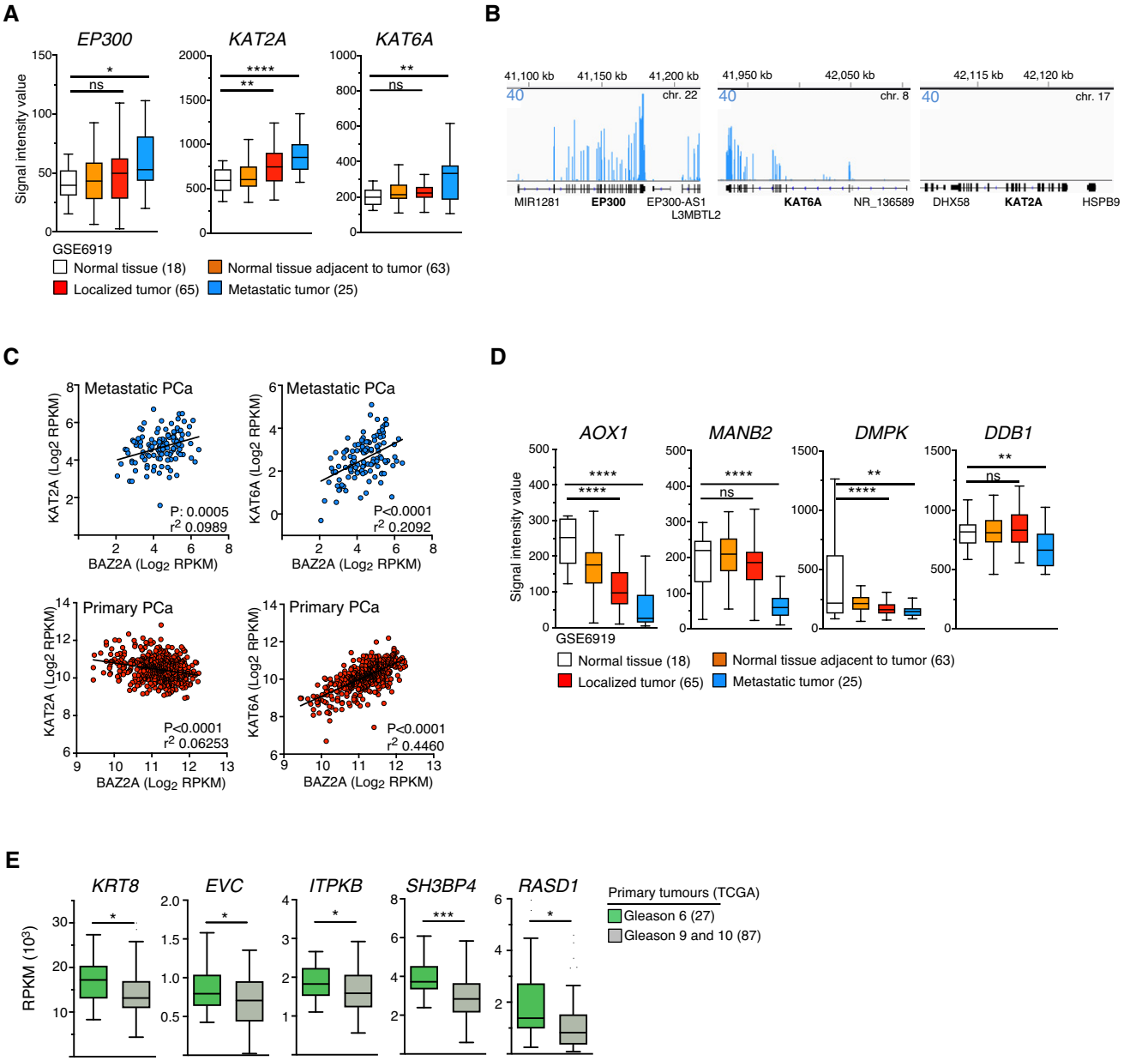

Figure EV2. BAZ2A binds a class of inactive enhancers marked by H3K14ac and represses genes implicated in aggressive PCa.

A The H3K14ac histone acetyltransferases *EP300*, *KAT2A*, and *KAT6A* are highly expressed in metastatic prostate cancer tissues. Boxplots showing expression profiles from Gene Expression Omnibus (GEO) data sets GSE6919 (gene expression microarray data) (Yu *et al*, 2004; Chandran *et al*, 2007). *< 0.05, **< 0.01, ****$P < 0.0001$, two-tailed Student's *t*-test; ns, not significant. Boxplots show the median and quartiles with the whiskers extending to the most extreme data point within 1.5 times the interquartile range.

B Wiggle tracks showing RNAseq expression profiles of *EP300*, *KAT2A*, and *KAT6A* in PC3 cells. *KAT2A* is not expressed in PC3 cells due to deletion of the entire locus.

C Scatter plot showing the expression of *KAT2A* and *KAT6A* vs BAZ2A in two large cohorts of metastatic and primary PCa. Data were from (Cancer Genome Atlas Research Network, 2015; Robinson *et al*, 2015).

D Boxplots showing expression profiles of *AOX1*, *MANB2*, *DMPK*, and *DDB1* from GEO data sets GSE6919 (gene expression microarray data) (Yu *et al*, 2004; Chandran *et al*, 2007). **< 0.01, ****$P < 0.0001$, two-tailed Student's *t*-test; ns, not significant. Boxplots show the median and quartiles with the whiskers extending to the most extreme data point within 1.5 times the interquartile range.

E Boxplots showing expression of BAZ2A-bound C2-enhancer genes KRT8, *EVC*, *ITPKB*, *SH3BP4*, and *RASD1* in dedifferentiated and aggressive primary tumors scored with Gleason 9 and 10 and indolent tumors assigned with Gleason 6. Data are from TCGA_PRAD (Cancer Genome Atlas Research Network, 2015). Statistical significance (*P*-values) was calculated using two-tailed *t*-test (*< 0.05, ***< 0.001). Boxplots show the median and quartiles with the whiskers extending to the most extreme data point within 1.5 times the interquartile range.

Figure EV3. PC3-CSCs display the molecular signature of a cancer stem cell-like phenotype.

A Representative images showing PC3 cells, the derived tumorspheres PC3-CSCs and PC3 cells generated from PC3-CSCs under adherent cell culture conditions.

B Genes upregulated in PC3-CSCs are enriched in stem cell-like signature. GSEA analysis was obtained by comparing expression values of tumorspheres vs PC3 cells of genes upregulated in PC3-CSCs ($P < 0.05$, $\log_2 FC > 1$). P was calculated over 100,000 permutations based on the phenotype, and FDR was < 25%. NES: normalized enrichment score.

C Representative wiggle tracks from RNAseq of PC3 and PC3-CSCs of cancer stem cell markers that are upregulated in PC3-CSCs compared with the parental PC3 cells.

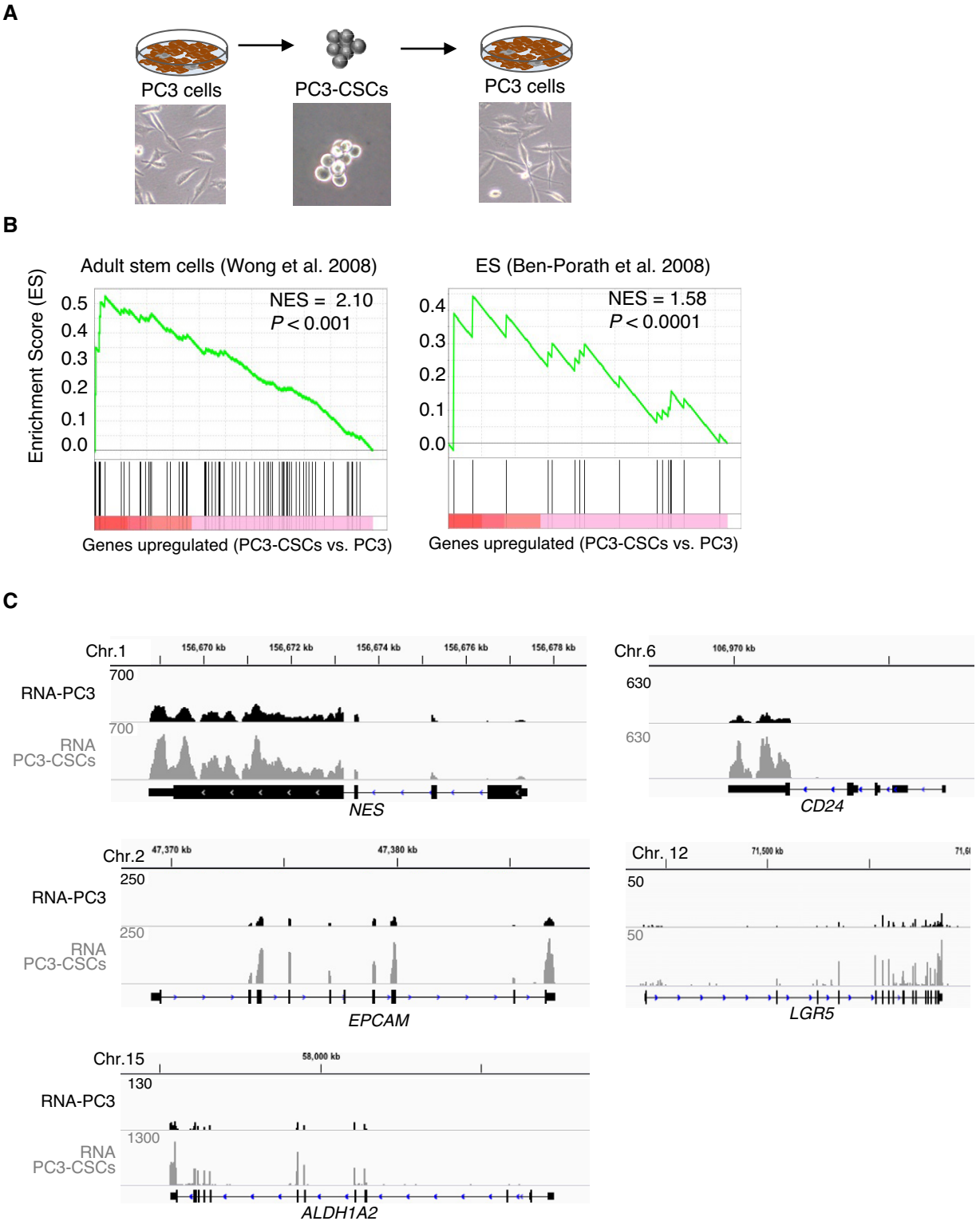

Figure EV3.

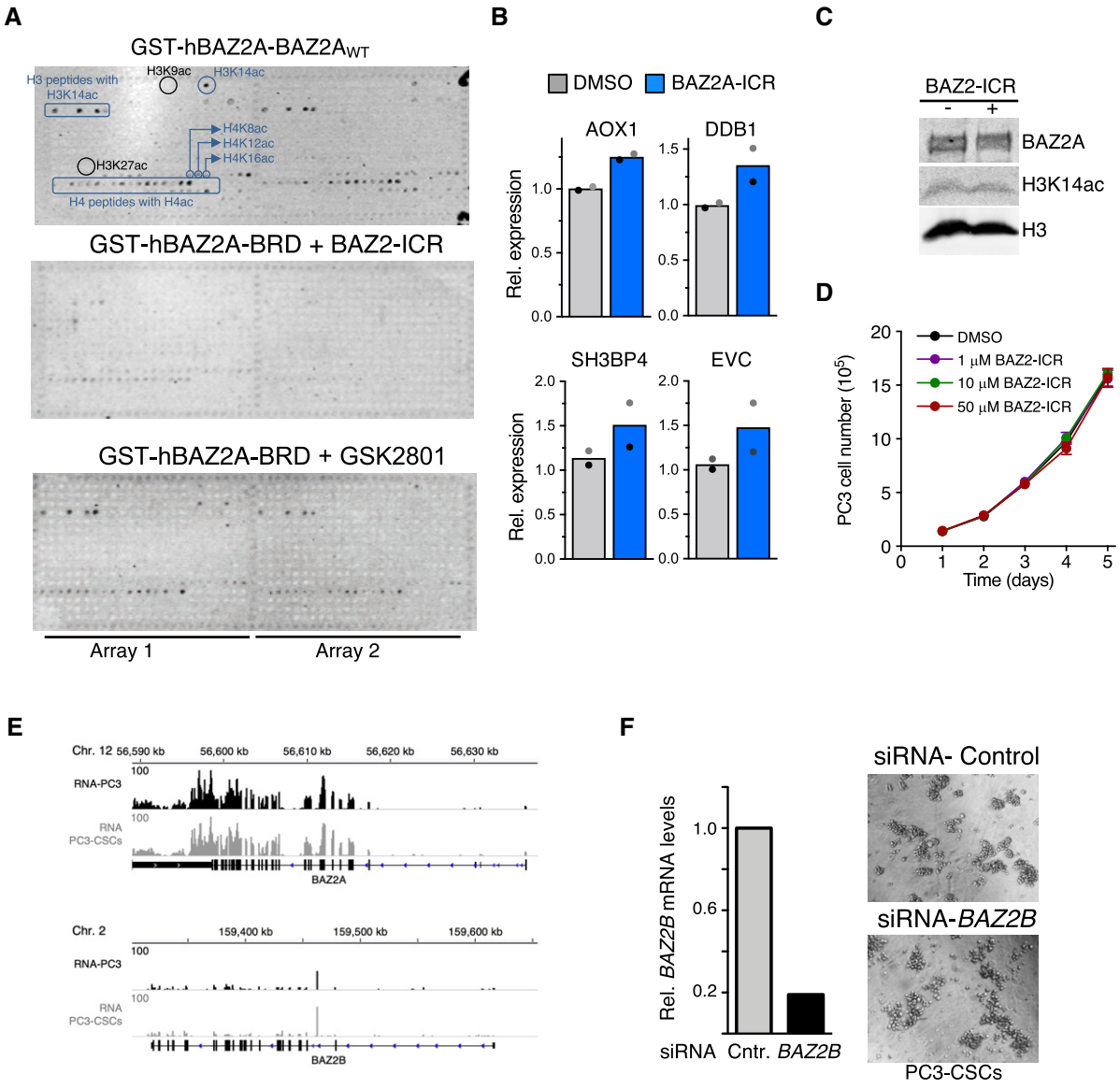

Figure EV4. BAZ2A-bromodomain inhibitors impair PCa cells with a cancer stem-like state.

A Images of histone peptide arrays incubated with 10 nM of recombinant GST-BAZ2A-BRD$_{wt}$ in the presence of 50 nM of BAZ2A-BRDi, BAZ2-ICR, or GSK2801. Visualization of binding was performed by incubation with anti-GST antibodies and imaged on Odyssey Infrared Imaging System. For a better visualization of the data, the image of GST-BAZ2A-BRD$_{wt}$ without BAZ2A-BRDi shown in Fig 1D has been included. Blue circles mark peptides recognized by BAZ2A-BRD (i.e., H3K14ac), whereas black circles show some of the acetylated peptides not recognized by BAZ2A-BRD (H3K27ac and H3K9ac).

B qRT–PCR showing increased expression levels of BAZ2A-regulated genes in PC3 cells treated with DMSO or 1 μM BAZ2-ICR for 5 days. Values were normalized to *GAPDH* mRNA levels. Average values of two independent experiments. Gray and black dots represent the values obtained from each single experiment.

C Western blot showing similar levels of BAZ2A and H3K14ac in PC3 cells treated without or with 1 μM BAZ2-ICR for 5 days. Histone H3 was used to ensure similar protein levels.

D Cell proliferation curves of PC3 cells treated with 5, 10, and 50 μM BAZ2-ICR. Error bars represent the standard deviation from three independent experiments.

E Representative wiggle tracks from RNAseq of PC3 and PC3-CSCs of *BAZ2A* and *BAZ2B*.

F *BAZ2B* depletion in PC3 cells does not affect the dedifferentiation into PC3-CSCs. Left panel: *BAZ2B* mRNA levels were measured by qRT–PCR in PC3-CSCs and normalized to *GAPDH* mRNA. Right panel: Representative figures showing PC3-CSCs generated from PC3 cells transfected with siRNA-control or siRNA-*BAZ2B*.

Source data are available online for this figure.

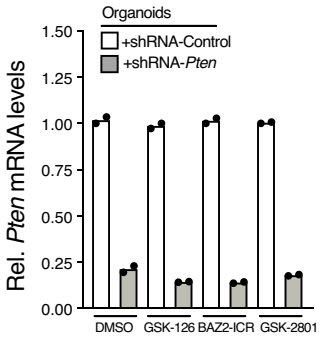

Figure EV5. BAZ2A-bromodomain inhibitors impair the initiation of PCa driven by *Pten*-loss in a model of PCa organoids.

qRT–PCR showing *Pten* mRNA levels in mouse prostate organoids expressing shRNA-control and shRNA-*Pten* and treated with DMSO, GSK126 (5 μM), BAZ2-ICR (5 μM), or GSK2801 (5 μM). Values are from two experiments. Data were normalized to *Hprt* mRNA and organoids treated with siRNA-Control.