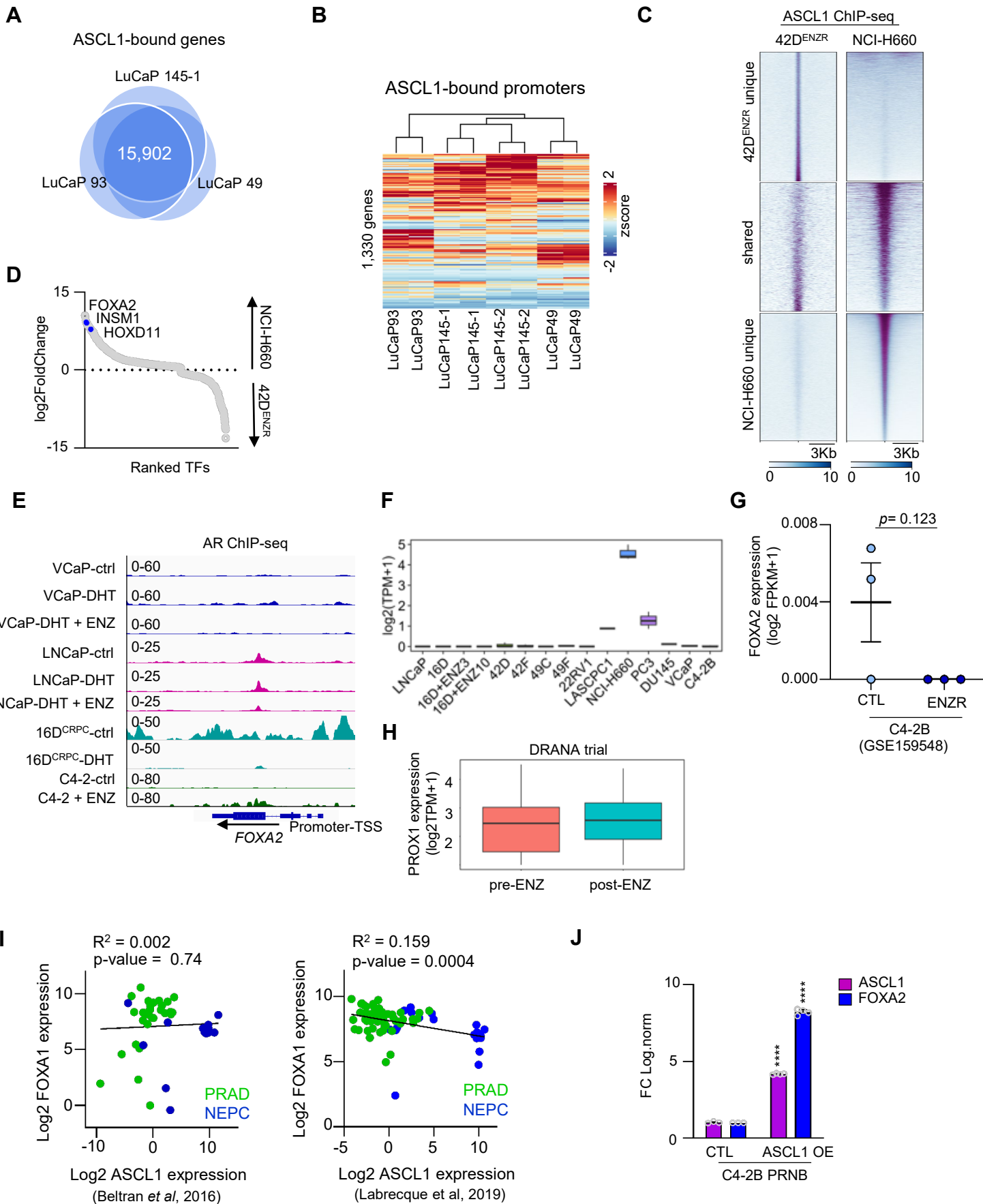


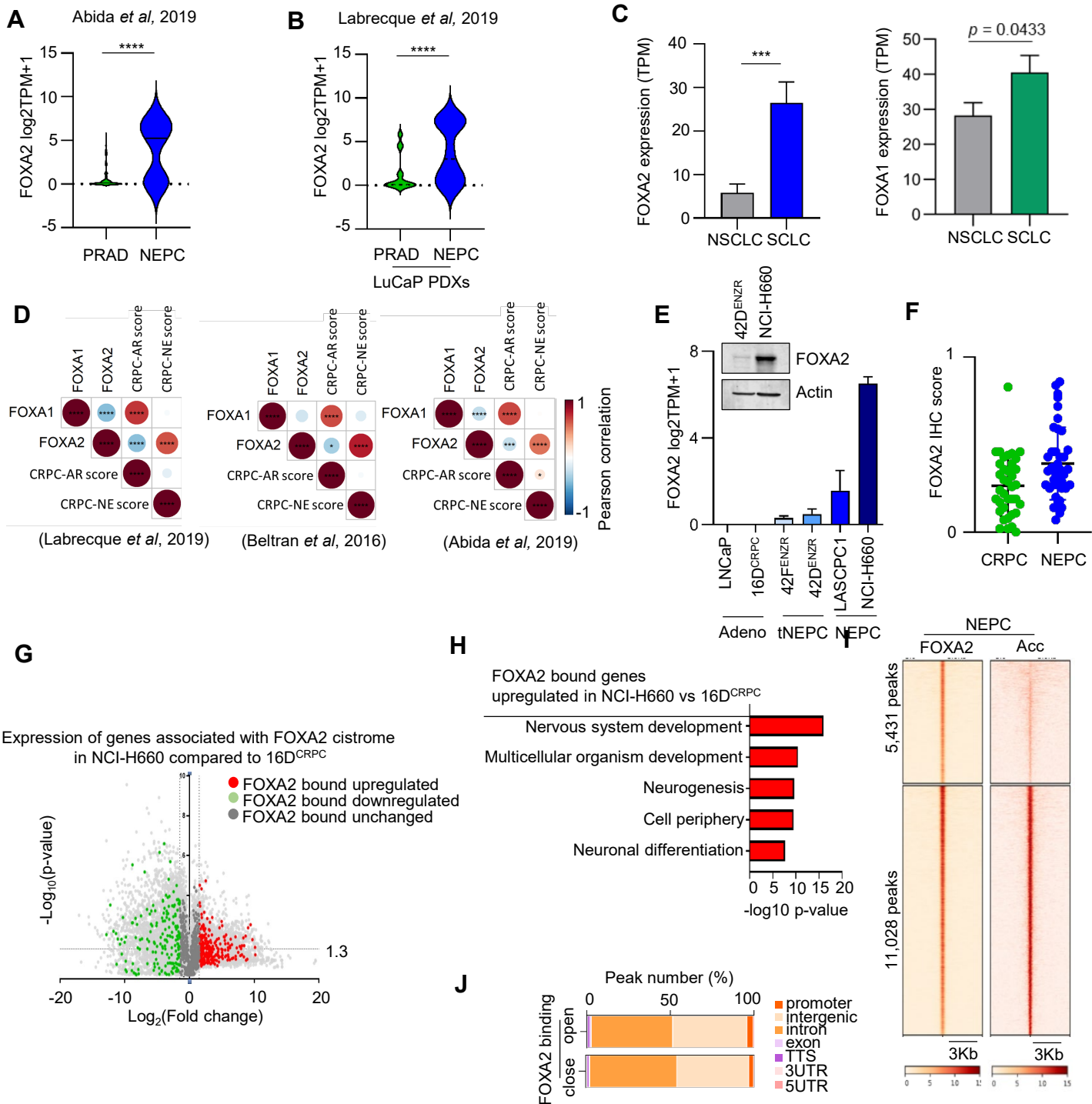
**Figure S1. ASCL1 regulates FOXA2 in terminal NEPC**



## Figure S1. ASCL1 regulates FOXA2 in terminal NEPC

- A. Genes co-bound by ASCL1 in three LuCaP PDXs.
- B. Heatmap shows expression of all (1,330) ASCL1-bound promoters in ASCL1-driven LuCaP patient-derived xenografts. Data are represented as z-score.
- C. Heatmap of unique ASCL1 binding intensity in tNEPC cell line 42D<sup>ENZ<sup>R</sup></sup> and de novo NEPC cell line NCI-H660. Data is presented as fold change over input, with each horizontal line representing a 3 kb locus.
- D. Transcription factors (TFs) expression enriched in de novo NEPC cell line NCI-H660 compared to tNEPC cell line 42D<sup>ENZ<sup>R</sup></sup>. TFs are ranked based on log<sub>2</sub> fold change.
- E. AR binding at FOXA2 promoter region shown using IGV tracks in a panel of prostate cancer cell lines treated with DHT or ENZ.
- F. Expression of FOXA2 shown as log<sub>2</sub> TPM+1 in a panel of prostate cancer cell lines as well as in CRPC (16D<sup>CRPC</sup>) following 3 and 10 days of enzalutamide (ENZ) treatment.
- G. Expression of FOXA2 shown as log<sub>2</sub> FPKM+1 in C4-2B CTL and after ENZ treatment.
- H. Expression of FOXA2 shown as log<sub>2</sub> TPM+1 in the patient dataset (DRANA trial) (41) pre- and post-enzalutamide treatment.
- I. Correlation between ASCL1 and FOXA1 expression in patient datasets (Beltran et al) (9) (left), and (Labrecque et al) (3) (right).
- J. Expression of ASCL1 and FOXA2 in C4-2B PRNB following overexpression ASCL1 (ASCL1OE) shown as fold change of logged normalized count (FC log<sub>2</sub> norm). Data are reported as mean ± SD;  $p < 0.05$  (CTL  $n = 4$  and ASCL1 OE  $n = 4$  biologically independent samples) (ASCL1 and FOXA2  $p < 0.00001$ ).

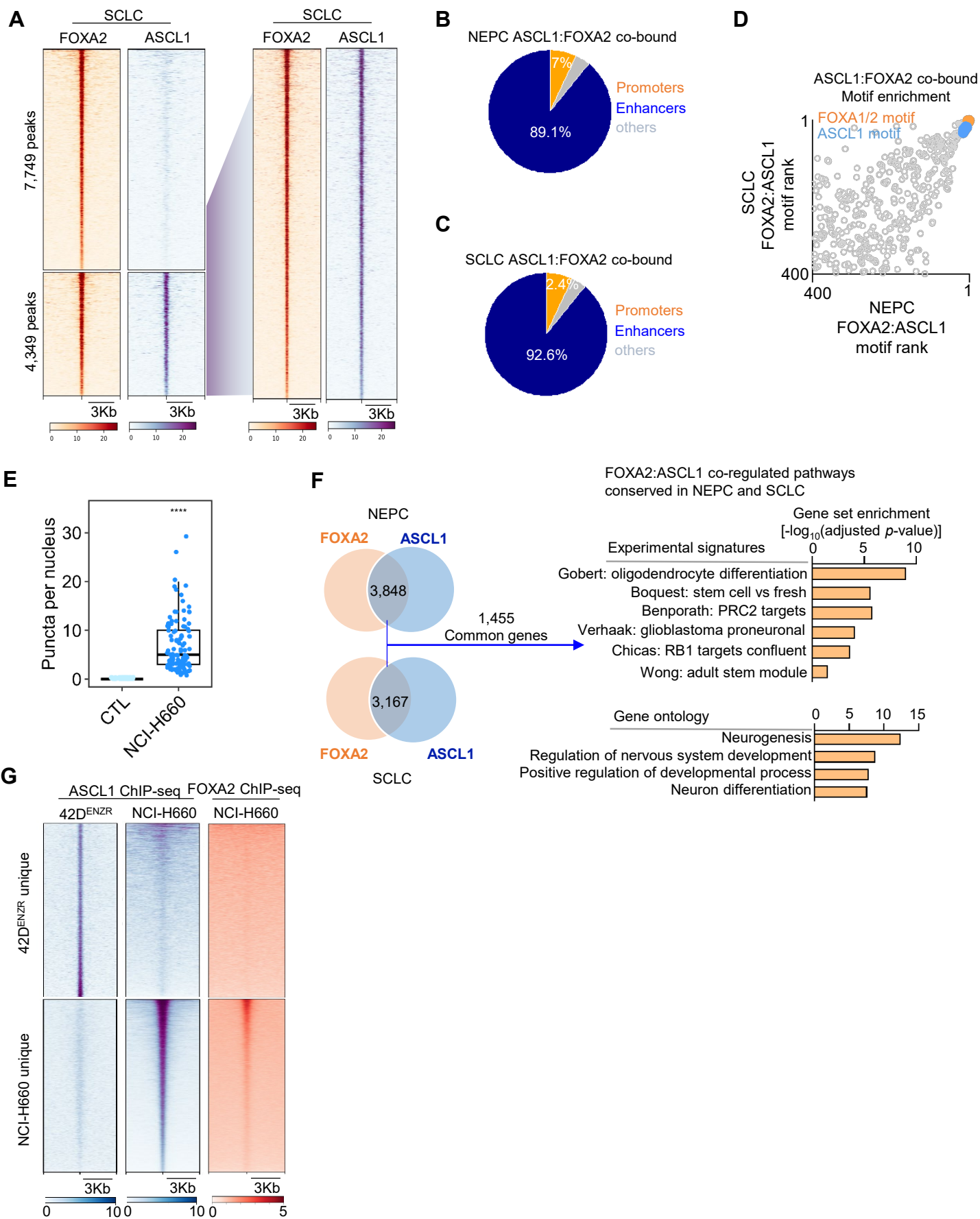
**Figure S2. FOXA2 in NEPC and SCLC**



## Figure S2. ASCL1 regulates FOXA2 in terminal NEPC

- A. FOXA2 gene expression ( $\log_2\text{TPM}+1$ ) in patient datasets (46). Violin plots show median (middle solid line), quartiles as dotted lines and interquartile range, with significance assessed using a two-tailed unpaired t-test.
- B. FOXA2 gene expression ( $\log_2\text{TPM}+1$ ) in LuCaP PDXs (3). Violin plots show median (middle solid line), quartiles as dotted lines and interquartile range, with significance assessed using a two-tailed unpaired t-test.
- C. FOXA1 and FOXA2 mRNA expression (TPM) in small cell (SCLC) and non-small-cell lung cancers (NSCLC) cell lines (NSCLC  $n = 71$  and SCLC  $n = 50$ ) (47). Each dot represents an individual patient. Data is shown as mean  $\pm$  SD, with significance assessed using a two-tailed unpaired t-test. (\* $p < 0.05$ , \*\* $p < 0.001$ , \*\*\* $p < 0.0001$ ).
- D. The correlation between FOXA1 and FOXA2 mRNA expression with AR or NE score (9) in patient datasets (3, 9, 61) using Pearson correlation.
- E. FOXA2 gene expression ( $\log_2\text{TPM}+1$ ) in Adeno (LNCaP and 16D<sup>CRPC</sup>), tNEPC (42D<sup>ENZR</sup> and 42F<sup>ENZR</sup>), and NEPC (LASCPC1 and NCI-H660) cell lines.
- F. FOXA2 staining intensity was quantified, mean  $\pm$  SD, CRPC ( $n = 40$ ) and NEPC ( $n = 26$ ); two-tailed unpaired t-test ( $p$ -value = 0.0058).
- G. Volcano plot shows differentially regulated FOXA2 bound genes in NCI-H660 compared to 16D<sup>CRPC</sup>. Red shows up-regulated ( $\log_2$  fold change  $>1$ ), green shows down-regulated ( $\log_2$  fold change  $<-1$ ), and gray shows unchanged genes.
- H. Pathways associated with upregulated FOXA2 bound genes in NCI-H660 compared to 16D<sup>CRPC</sup> were identified using gProfiler.  $p < 0.05$ .
- I. Heatmap represents the FOXA2 ChIP-seq binding intensity and chromatin accessibility in the NCI-H660 cell line. Each horizontal line represents a 3-kb locus.
- J. Genomic annotation is shown as a percentage of all peaks showing FOXA2 binding in open or close chromatin.

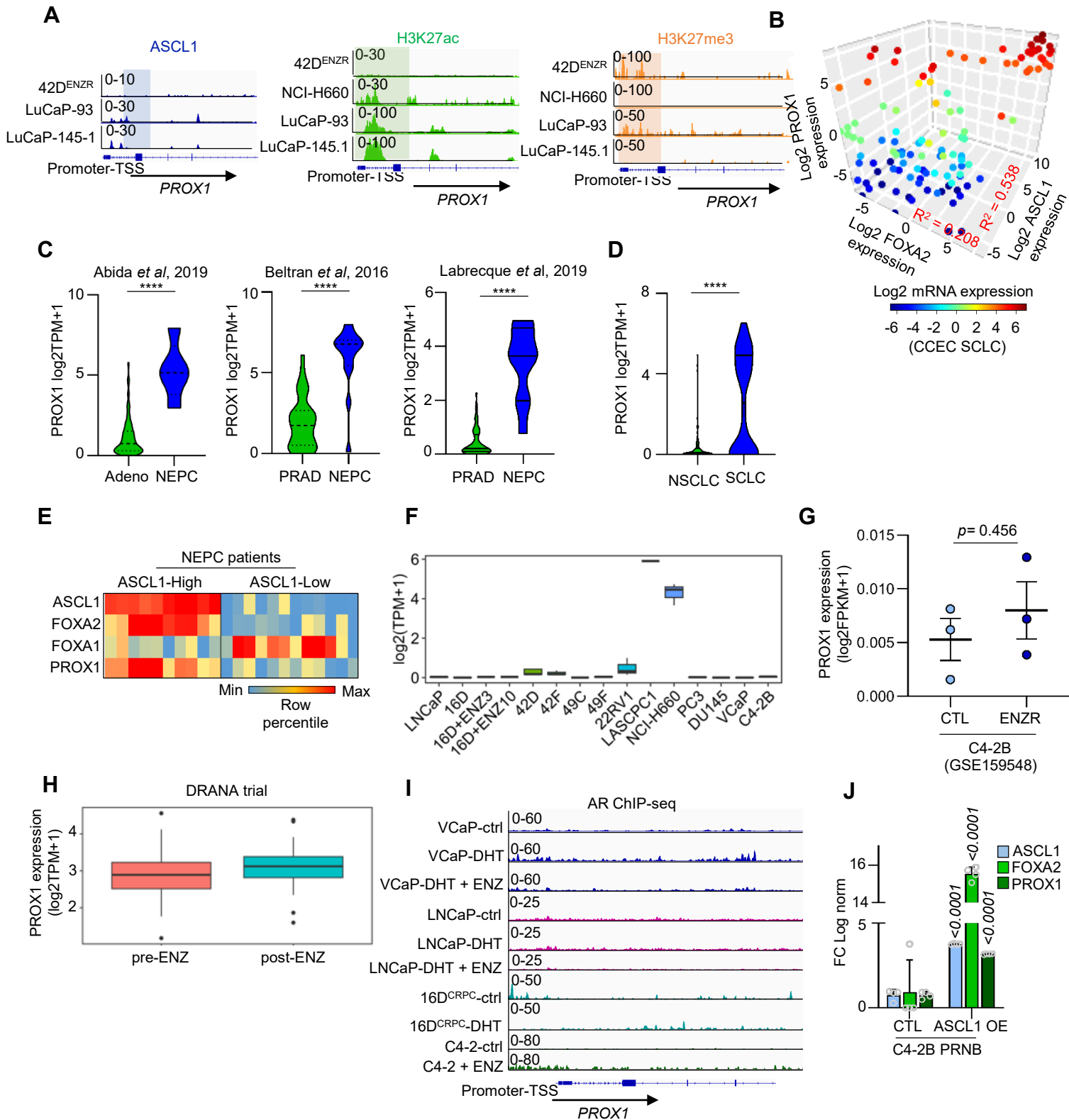
**Figure S3. FOXA2 cooperates with ASCL1 in small-cell cancers**



### Figure S3. FOXA2 cooperates with ASCL1 in small-cell lung cancer

- A. Heatmap of FOXA2 and ASCL1 ChIP-seq binding intensity in NCI-H889 cells. Each horizontal line represents a 3-kb locus. The heatmap represents the regions co-occupied by FOXA2 alone or FOXA2 and ASCL1.
- B. Genomic annotation of ASCL1:FOXA2 co-bound regions in NEPC presented as a percentage of total peaks. Intron and intergenic regions were called enhancers.
- C. Genomic annotation of ASCL1:FOXA2 co-bound regions in SCLC presented as a percentage of total peaks. Intron and intergenic regions were called enhancers.
- D. TF binding motif enrichment surrounding ASCL1:FOXA2 co-bound regions in SCLC and NEPC. Motifs were ranked based on differential p-value. Each dot represents a motif. Orange shows the FOXA motif family (FOXA1 and FOXA2), and blue shows the ASCL1 motif.
- E. PLA quantification showing ASCL1 and FOXA2 interaction as puncta per nuclei in control (CTL) and NCI-H660 cell line.
- F. Genes co-bound by ASCL1 and FOXA2 in NEPC and SCLC. Pathway analysis was performed using gProfiler on common genes between SCLC and NEPC.  $p < 0.5$ .
- G. Heatmap of unique ASCL1 binding intensity overlaid with FOXA2 ChIP-seq in regions unique to 42D<sup>ENZ<sup>R</sup></sup> and NCI-H660 cell lines. Data is presented as fold change over input, with each horizontal line representing a 3 kb locus.

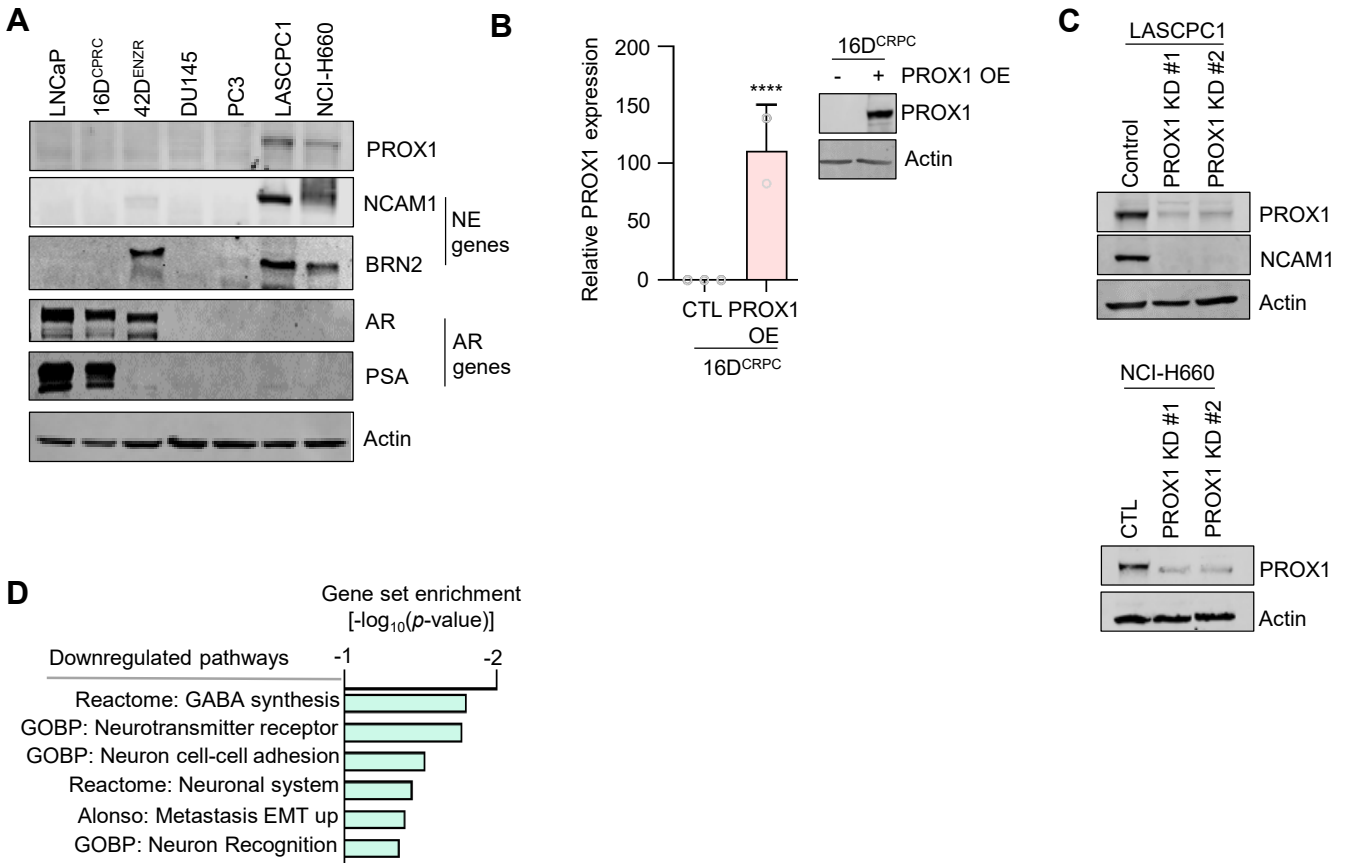
**Figure S4. ASCL1 and FOXA2 co-regulate PROX1**



### Figure S4. ASCL1 and FOXA2 co-regulate PROX1

- A. IGV tracks show ASCL1 binding (left), H3K27ac signal (middle), and H3K27me3 signal (right) on PROX promoter in 42D<sup>ENZ<sup>R</sup></sup>, NCI-H660, and LuCaPs 93 and 145-1.
- B. The correlation between PROX1 mRNA expression with FOXA2 and ASCL1 in the SCLC dataset (47), with each dot representing a patient tumor and the colour reflecting PROX1 mRNA expression (log<sub>2</sub> TPM); p-value < 0.05, with significance assessed by two-tailed unpaired t-test.
- C. PROX1 expression presented as log<sub>2</sub>TPM in prostate cancer patient datasets (3, 9, 61).
- D. PROX1 expression presented as log<sub>2</sub>TPM in lung cancer datasets (47).
- E. Heatmap shows the expression of ASCL1, FOXA1/2, and PROX1 in Labrecque et al dataset (3), presented as log<sub>2</sub>TPM with each row showing the minimum and maximum row percentile.
- F. Expression of PROX1 shown as log<sub>2</sub> TPM+1 in a panel of prostate cancer cell lines as well as in CRPC (16D<sup>CRPC</sup>) following 3 and 10 days of enzalutamide (ENZ) treatment.
- G. Expression of PROX1 shown as log<sub>2</sub> FPKM+1 in C4-2B CTL and after ENZ treatment.
- H. Expression of PROX1 shown as log<sub>2</sub> TPM+1 in the patient dataset (DRANA trial) (41) pre- and post-enzalutamide treatment.
- I. AR binding at PROX1 promoter region shown using IGV tracks in a panel of prostate cancer cell lines treated with DHT or ENZ.
- J. ASCL1, FOXA2 and PROX1 gene expression in C4-2B PRNB cell line (CTL) and following overexpression of ASCL1 (ASCL1 OE). Data are reported as fold change of log<sub>2</sub> normalized count, and significance is assessed using a two-tailed unpaired t-test (CTL and ASCL1 OE n = 3 biologically independent samples).

# Figure S5. PROX1 is required for NE phenotype characteristics and proliferation



**Figure S5. PROX1 is required for NE phenotype characteristics and proliferation**

- A) Western blot shows the expression of PROX1, NE markers (BRN2 and NCAM1), AR and AR marker (PSA) in a panel of prostate cancer cell lines, with actin used as a loading control.
- B) PROX1 expression shown as relative mRNA (left) and protein expression (right) in 16D<sup>CRPC</sup> following PROX1 overexpression (OE). mRNA data was normalized to GAPDH. Actin is used as a loading control.
- C) Western blot shows the expression of PROX1 and NE marker NCAM1 in the LASCPC1 cell line (top) and NCI-H660 cell line (bottom) following knockdown of PROX1 using two different shRNA.
- D) Pathways downregulated in LASCPC1 following knockdown of PROX1 (shPROX1) shown as  $-\log_{10}$  p-value with  $p < 0.05$ .