Supplementary Figure Legends

Supplementary Figure 1. AR-expressing prostate cancer cell lines are sensitive to CBP/P300 bromodomain inhibition. A) Western blot showing AR protein levels in the indicated cell lines, 6 days after transfection with siRNAs complementary to the C terminus or N terminus of AR. B**)** Western blots show CBP and P300 protein levels 6 days after transfection with the indicated siRNAs. * indicates non specific band. C) Summary of GNE-049 biochemical potency and selectivity by TR-FRET assay. IC50 values are mean of N=2 replicates. Assay uses isolated recombinant bromodomains. D) Cellular potency of GNE-049 on the CBP isolated bromodomain was determined by BRET assay. Data represent mean ± SD. N=4. E) The ability of GNE-049 to suppress Myc mRNA expression in MV-4-11 cells was determined after 4 h treatment with the indicated concentrations of drug. Data represent mean ± SD. N=4. F) Summary of pharmacokinetic properties of GNE-049 in mouse. GNE-049 was dosed at 30 mg/kg p.o. (suspended in 0.5% w/v methylcellulose, 0.2% w/v Tween 80).

Supplementary Figure 2. The CBP/P300 bromodomain is required for AR target gene expression. A) The indicated PC cells lines were treated for 24h with 1 µM GNE-049 or DMSO. AR expression was then determined by RT-PCR (left panel) and western blot in LNCaP cells (right panel). B) Upper left panel: The indicated PC cells lines were treated for 24h with 1 µM GNE-049 or DMSO. Myc expression was then determined by RT-PCR. Data represent the mean ± SD. N= 3. Upper right panel: Myc expression was determined by RT-PCR 3 days after

LNCaP cells were treated with CBP & P300 targeted siRNAs. Lower panels: LNCaP, VCaP and 22RV1 cells were treated for 24 h with a two-fold dilution series of the indicated compounds, starting at 1μM. Myc expression was then measured by RT-PCR. Data represent the mean ± SD. N= 3. C) LNCaP cells were treated with a matrix of enzalutamide and GNE-049 at the indicated concentrations for 6 days, before cell viability was measured by CellTiter-Glo® . Left panel values indicate % growth inhibition compared to control. Right panel values indicate synergy at that dose combination, as determined by Bliss score. D) LNCaP cells were treated with a matrix of enzalutamide and GNE-049 for 24 h before PSA expression was assessed by RT-PCR. Values indicate % reduction in mRNA compared to DMSO control. E) LNCaP cells were treated with a matrix of JQ1 and GNE-049 at the indicated concentrations for 6 days, before cell viability was measured by CellTiter-Glo® . Left panel values indicate % growth inhibition compared to control. Right panel values indicate synergy at that dose combination, as determined by Bliss score. F) Total mRNA levels were assessed after 24 h treatment with 1μM GNE-049, 500 nM actinomycin or DMSO control. Data is represented as % of total RNA, mean ± SD. N=3.

Supplementary Figure 3. Inhibition of the CBP/P300 bromodomain Prevents AR Co-activator Function. A) LNCaP and VCaP cells were treated for the indicated time with the indicated dose of GNE-049, before western blot analysis of the indicated histone modifications. B) Upper panel: summary plots showing P300 and H3K27Ac ChIP-seq peak size for all peaks, 24 h after stimulation with

R1881 ± GNE-049. Axes show normalized reads per million per base pairs in each peak. Lower panel: histograms describing P300 and H3K27Ac enrichment (average coverage) across enhancers and promoters.

Supplementary Figure 4. Cell line models of endocrine therapy resistance remain sensitive to CBP/P300 bromodomain inhibition. A) Western blot showing expression of AR and FLAG tagged exogenous AR in LNCaP stable cell lines transfected with expression constructs for FLAG tagged WT AR (LNCaP AR) or F877L LBD mutant AR (ARmut). B) Western blot showing co-immunoprecipitation of P300 with FLAG tagged AR in LNCaP cell lines expressing FLAG tagged WT AR (LNCaP AR) or F877L LBD mutant AR (ARmut). IPs were probed for the presence for P300 using anti-P300 antibodies. 4% total extract is shown as an input control.

Supplementary Table 1. Bromodomain selectivity data for GNE-049 using BROMOscan® panel. Broad bromodomain profile of GNE-049. Kd values obtained for GNE-049 across a broad panel of bromodomains as assayed with the BromoScan platform at DiscoveRx. The assay is based on competition between compound and affinity resin for binding to soluble DNA-tagged bromodomains.

Supplementary Table 2. The CBP/p300 bromodomain is required for androgen stimulated gene expression and growth. LNCaP cells were

deprived of androgen for 5 days, and then stimulated with 0.1nM R1881 for 24 h ± 1 μM Enzalutamide or GNE-049. Gene expression was then evaluated by RNA-seq, N=3. Table shows all genes up-regulated 1.5-fold or more by R1881 treatment, along with their mean expression in each treatment.

Supplementary Note 1. Synthetic Procedure for GNE-049.