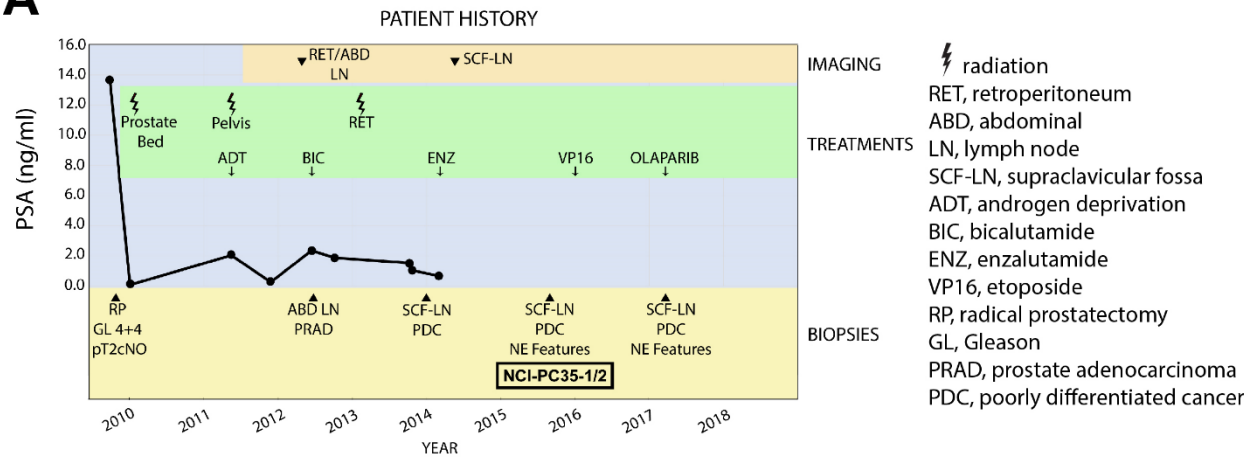
A



Primary tumor	**prostate**
Histologic type:	Adenocarcinoma
Gleason Score:	4 + 4 = 8
Tumor Quantification :	Tumor involves greater than 50% of both the right and left lobes
Margins:	Uninvolved
Extraprostatic extension:	Absent
Seminal vesicle invasion :	Absent
Number of regional lymph nodes examined:	3
Number of lymph nodes with metastasis:	0

Site of Metastasis	**subclavicular LN**
Dx	Metastatic poorly differentiated carcinoma with NE features.
Hx	goserelin, leuprolide, bicalutamide, enzalutamide
Markers	PAP, CDX2, chromogranin , synaptophysin, PSA(rare/faint)

B

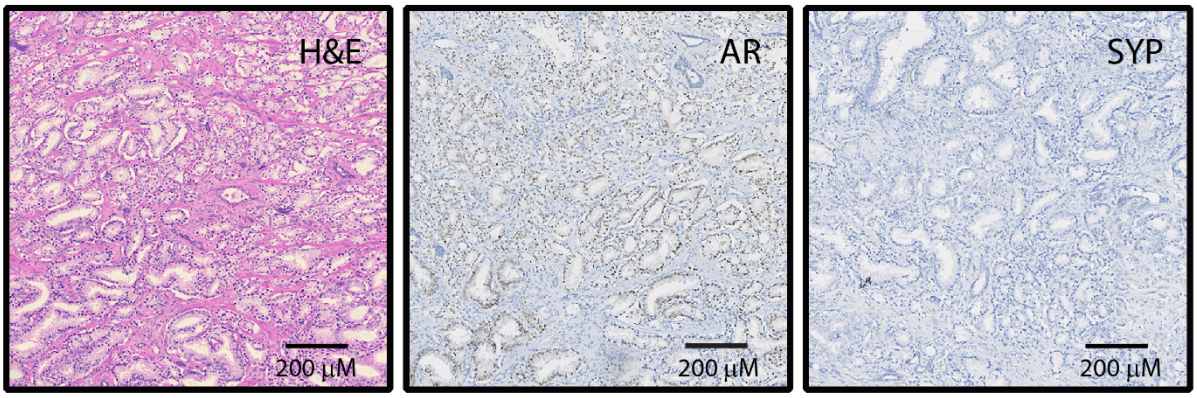


Figure S1. Patient treatment history. Related to Figure 1. (A) Indicates the course of treatment and diagnosis of the patient. The time of biopsy collection from which the NCI-PC35 organoid lines were established is indicated. (B) Primary prostate tumor sections stained with H&E or antibodies against AR or the neuroendocrine marker synaptophysin (SYP).

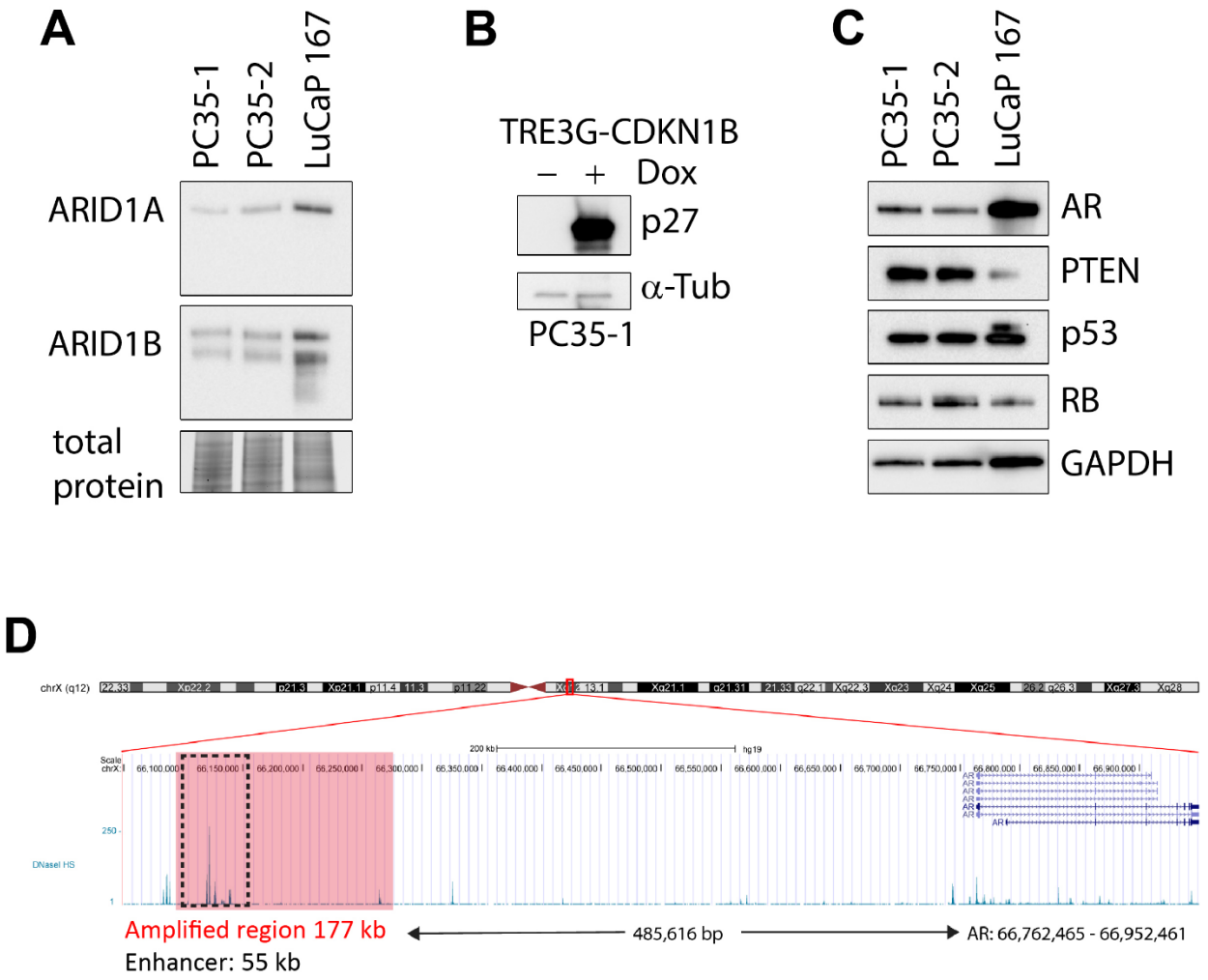

A
PC35-1
PC35-2
LuCaP 167
ARID1A
ARID1B
total protein
B
TRE3G-CDKN1B
– + Dox
p27
α-Tub
PC35-1
C
PC35-1
PC35-2
LuCaP 167
AR
PTEN
p53
RB
GAPDH
D
chrX (q12)
Amplified region 177 kb
Enhancer: 55 kb
485,616 bp
AR: 66,762,465 - 66,952,461
E
Fold Change
D0
D3
D7
D14
PC35-1
PC44
PC35-2
PC155
Fold Change
D0
D1
D3
D4
LuCaP 145.2
LuCaP 173.1

Figure S2. Genetic alterations of PC35 organoids affect protein levels. Related to Figure 1. (A) Western blots showing protein expression of ARID1A and ARID1B from lysates of PC35-1 and PC35-2 relative to WT LuCaP 167 control. Total protein is shown as a loading control. (B) Western blots showing p27 protein expression in PC35-1. Endogenous expression (-Dox) compared to expression from a positive control, doxycycline-inducible expression vector (+Dox). α-tubulin loading control. (C) Western blots showing protein expression of AR, PTEN, p53, and RB in PC35-1, PC35-2 and LuCaP 167 organoids. GAPDH loading control. (D) Region of tandem duplication that encompasses the AR enhancer. (E) Relative fold change in growth over time (D = day) for the indicated organoid lines, quantified by CellTiter Glo 3D.

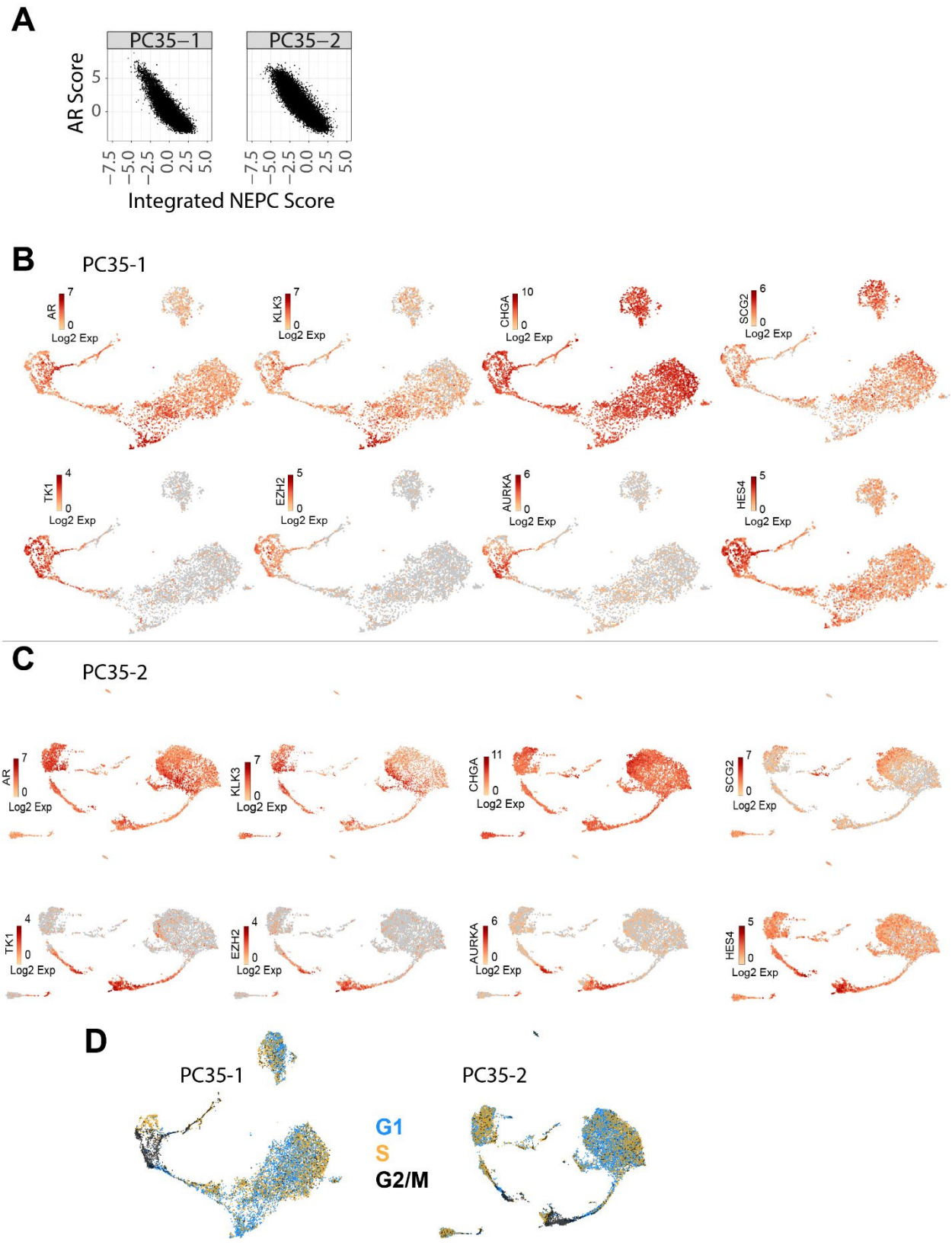

A
PC35−1
PC35−2
AR Score
5
0
−7.5
−5.0
−2.5
0.0
2.5
5.0
Integrated NEPC Score
B
PC35-1
AR
KLK3
CHGA
SCG2
TK1
EZH2
AURKA
HES4
Log2 Exp
C
PC35-2
D
PC35-1
PC35-2
G1
S
G2/M

Figure S3. Single-cell transcriptomic analysis of organoid models. Related to Figure 2. (A) Each cell from PC35-1 and PC35-2 scRNA-seq transcript data is plotted by neuroendocrine score (x axis) and AR score (y axis). (B) PC35-1 and (C) PC35-2, Log2 expression of the indicated lineage markers overlaid on the UMAPs. ACPC (AR, KLK3); NEPC (CHGA, SCG2); proliferation/stem markers (TK1, EZH2, AURKA, HES4) (D) Cell cycle phase transcriptional signature overlaid on UMAPs of PC35-1 and PC35-2.

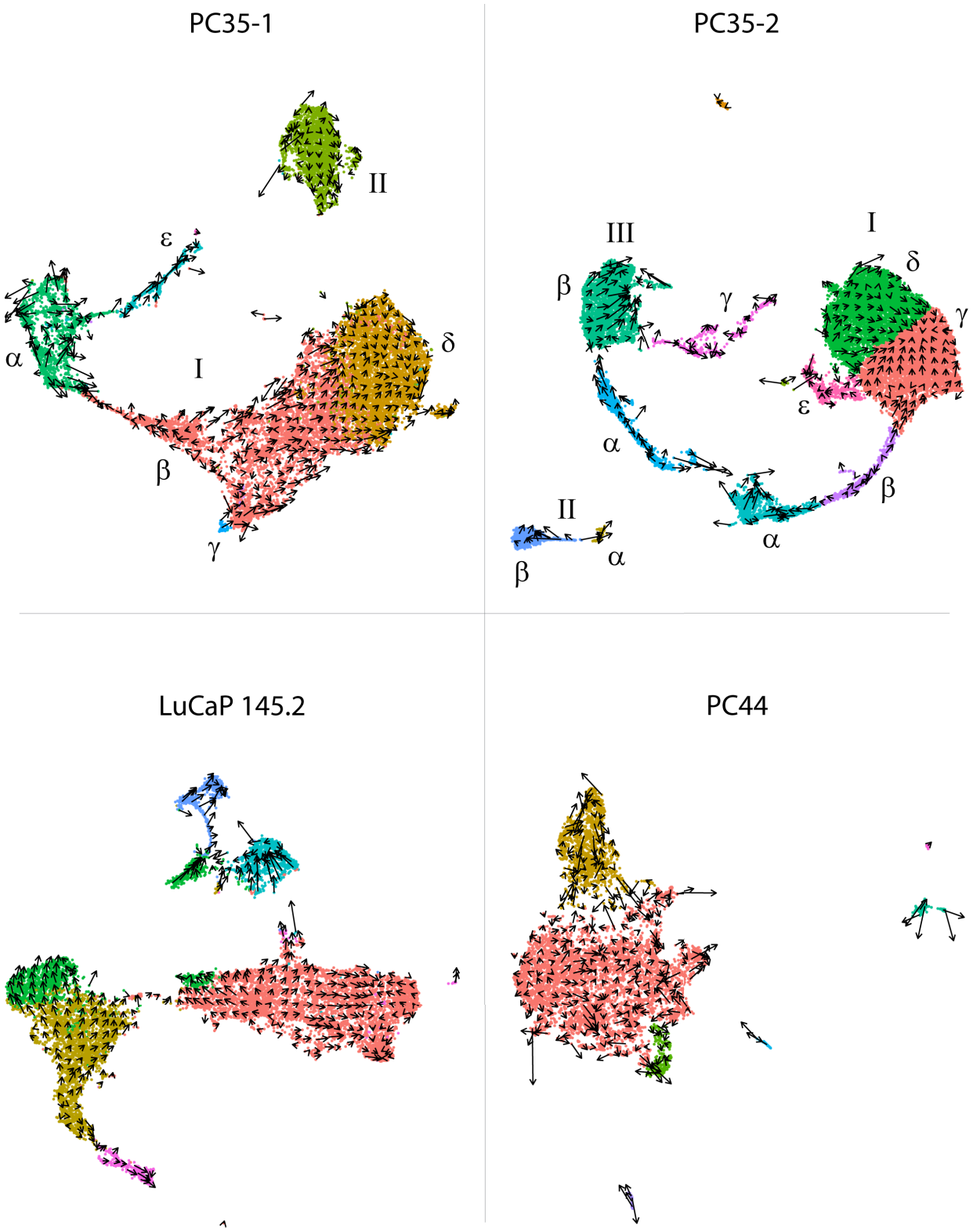
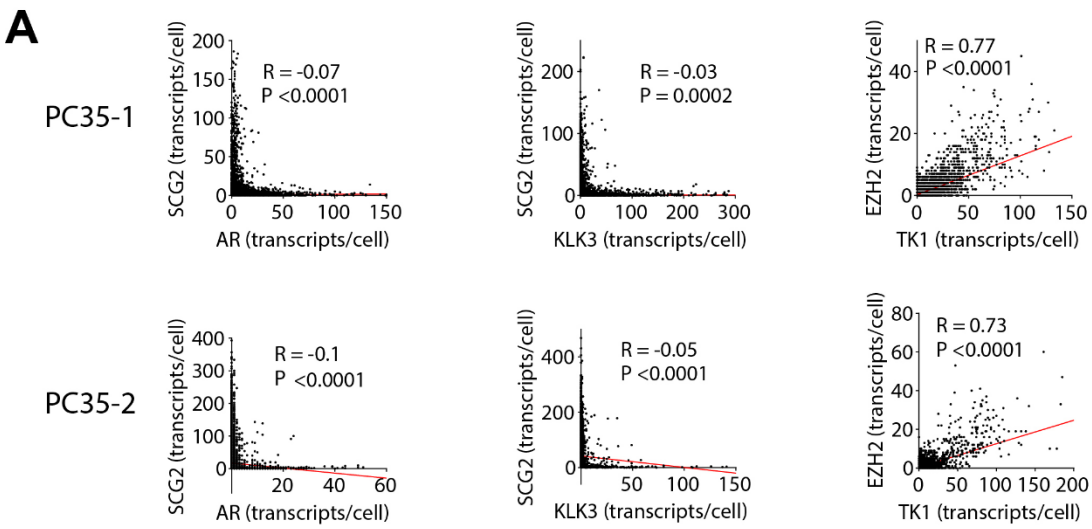
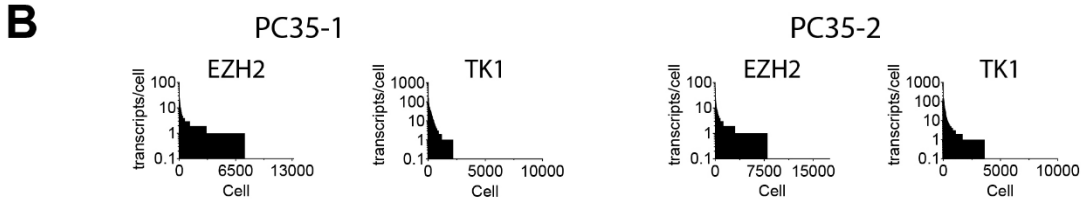
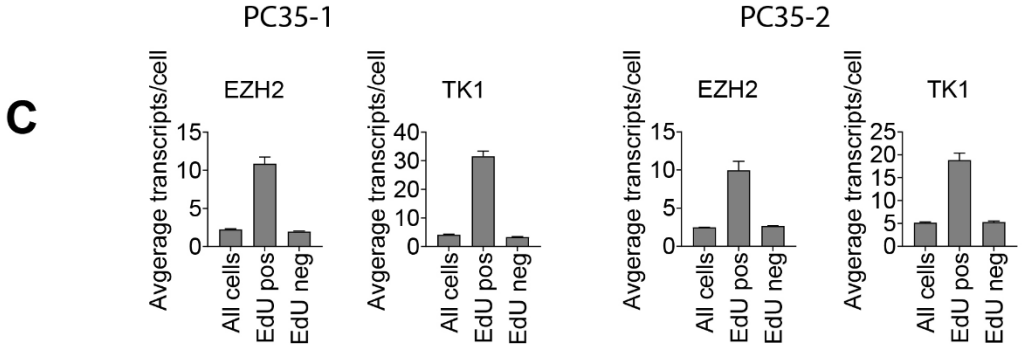

PC35-1
II
ε
α
I
δ
β
γ
PC35-2
III
I
δ
β
γ
γ
ε
α
β
II
α
α
β
LuCaP 145.2
PC44

Figure S4. RNA-velocity analysis identifies subpopulations in the PC35 organoid models with features of stem/progenitor populations. Related to Figure 2. RNA velocity vectors are embedded onto the UMAPs for each model. The direction of each velocity points toward the future state of locally averaged vector fields. The length indicates the magnitude of difference between states.


A
PC35-1
PC35-2
SCG2 (transcripts/cell)
AR (transcripts/cell)
KLK3 (transcripts/cell)
EZH2 (transcripts/cell)
TK1 (transcripts/cell)
R = -0.07
P <0.0001
R = -0.03
P = 0.0002
R = 0.77
P <0.0001
R = -0.1
P <0.0001
R = -0.05
P <0.0001
R = 0.73
P <0.0001


B
PC35-1
PC35-2
EZH2
TK1
transcripts/cell
Cell


C
PC35-1
PC35-2
EZH2
TK1
Avgerage transcripts/cell
All cells
EdU pos
EdU neg

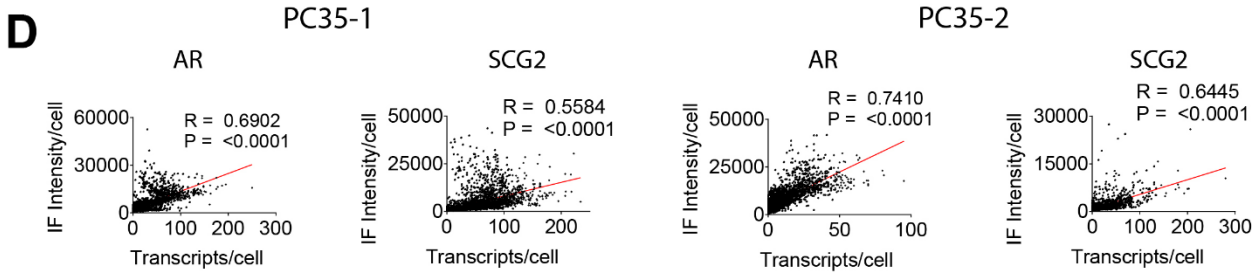

D
PC35-1
PC35-2
AR
SCG2
IF Intensity/cell
Transcripts/cell
R = 0.6902
P = <0.0001
R = 0.5584
P = <0.0001
R = 0.7410
P = <0.0001
R = 0.6445
P = <0.0001

Figure S5. Single-molecule RNA-FISH analysis of PC35-1 and PC35-2 corroborates the results of scRNA-seq analysis. Related to Figure 2. (A) Scatter plots of marker gene expression per cell. Each point represents a single cell plotted as the number of transcripts per cell for the genes indicated on X and Y. (B) Distribution plots of EZH2 and TK1 transcripts per cell quantified by RNA-FISH. Each cell is plotted on the X axis and the number of transcripts is shown on the Y axis in log scale. (C) Quantification of marker gene expression in dividing and non-dividing cells. PC35-1 and PC35-2 organoids were pulsed with EdU for 24 hours, dissociated and stained for EdU incorporation and transcript abundance. The average number of transcripts per cell for the indicated genes are shown for three populations of cells with respect to EdU-incorporation status. (D) Scatter plots of combined RNA-FISH/IF assays for PC35-1 and PC35-2. The number of transcripts per cell for the indicated gene is plotted against the immuno-fluorescence intensity per cell for the protein product of that gene (see Methods). For all scatter plots, the Pearson correlation coefficient was calculated and shown as R. The line was fit by simple linear regression. All bar plots are shown as the mean of independent experiments ± SEM.

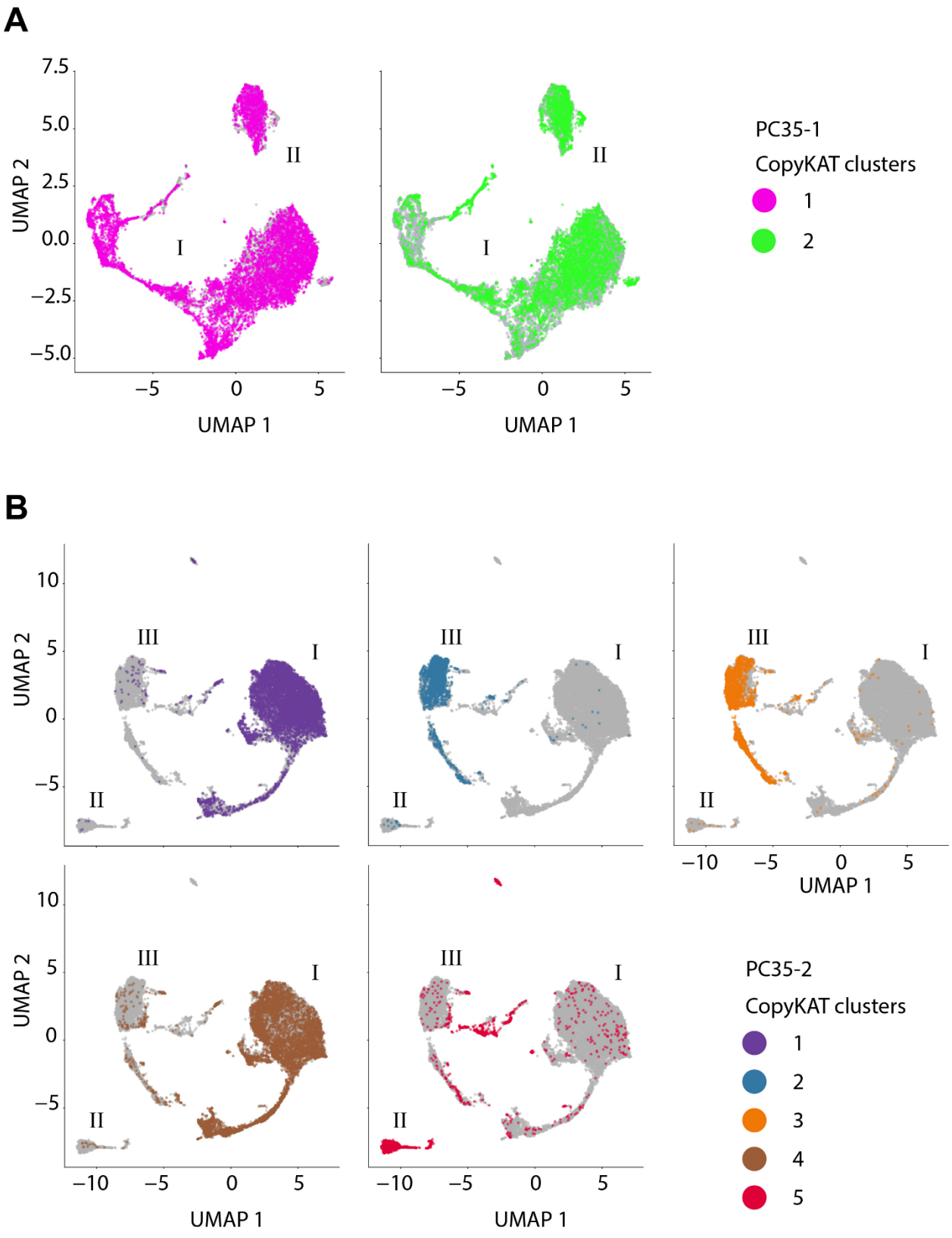

A
7.5
5.0
2.5
0.0
−2.5
−5.0
UMAP 2
I
II
−5
0
5
UMAP 1
PC35-1
CopyKAT clusters
1
2
B
10
5
0
−5
III
−10
PC35-2
3
4
5

Figure S6. PC35 major clusters defined by transcriptional profile (phenotype) are partially linked to genotype, while genotypes are not biased to specific regions within UMAP clusters. Related to Figure 4. (A) PC35-1 and (B) PC35-2 UMAPs showing major clusters (I, II, III). Each cell of the genomic CNV-determined subclonal populations (CopyKAT clusters) is indicated by color on a UMAP shown for each CopyKAT cluster.

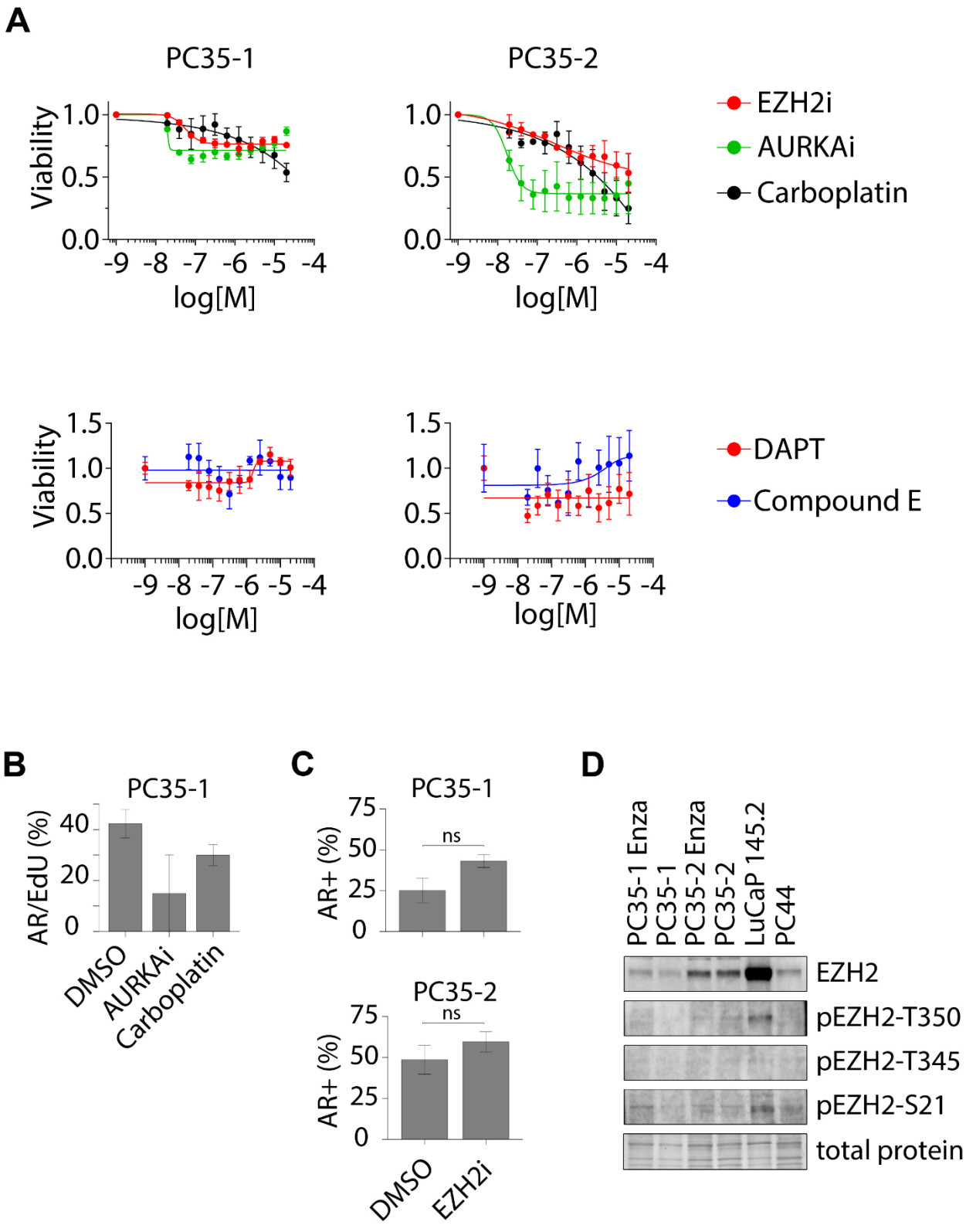

A
PC35-1
PC35-2
Viability
1.0
0.5
0.0
-9 -8 -7 -6 -5 -4
log[M]
EZH2i
AURKAi
Carboplatin
1.5
DAPT
Compound E
B
PC35-1
AR/EdU (%)
40
20
0
DMSO
AURKAi
Carboplatin
C
PC35-1
AR+ (%)
75
50
25
ns
PC35-2
DMSO
EZH2i
D
PC35-1 Enza
PC35-1
PC35-2 Enza
PC35-2
LuCaP 145.2
PC44
EZH2
pEZH2-T350
pEZH2-T345
pEZH2-S21
total protein

Figure S7. PC35-1 and PC35-2 organoids show subpopulation-specific sensitivity to drugs. Related to Figure 6. (A) Dose response assays. Organoids were treated with the indicated drugs at the indicated range of concentrations twice weekly for two weeks then quantified for viability by CellTiter Glo 3D. (B) PC35-1 organoids were treated for six weeks with AURKAi, carboplatin or DMSO. The AR expression for each cell was determined by RNA-FISH. EdU incorporation status (positive or negative) was determined for each cell. Data was plotted as the percentage of the EdU-positive cells that also expressed AR (C) PC35-1 and PC35-2 were treated with EZH2i or DMSO as described in (B). The percent AR-positive cells of the total, determined by RNA-FISH, is shown. (D) Western blots showing protein expression of EZH2 and the indicated phosphorylated forms of EZH2. Protein lysates were made from PC35-1 and PC35-2 organoids treated with or without enzalutamide for six weeks and LuCaP 145.2 and PC44 organoids. Total protein was used as a loading control. All bar and line plots are shown as the mean of three independent experiments ± SEM. Significance was determined using the student's t-test, two-tailed, unpaired.

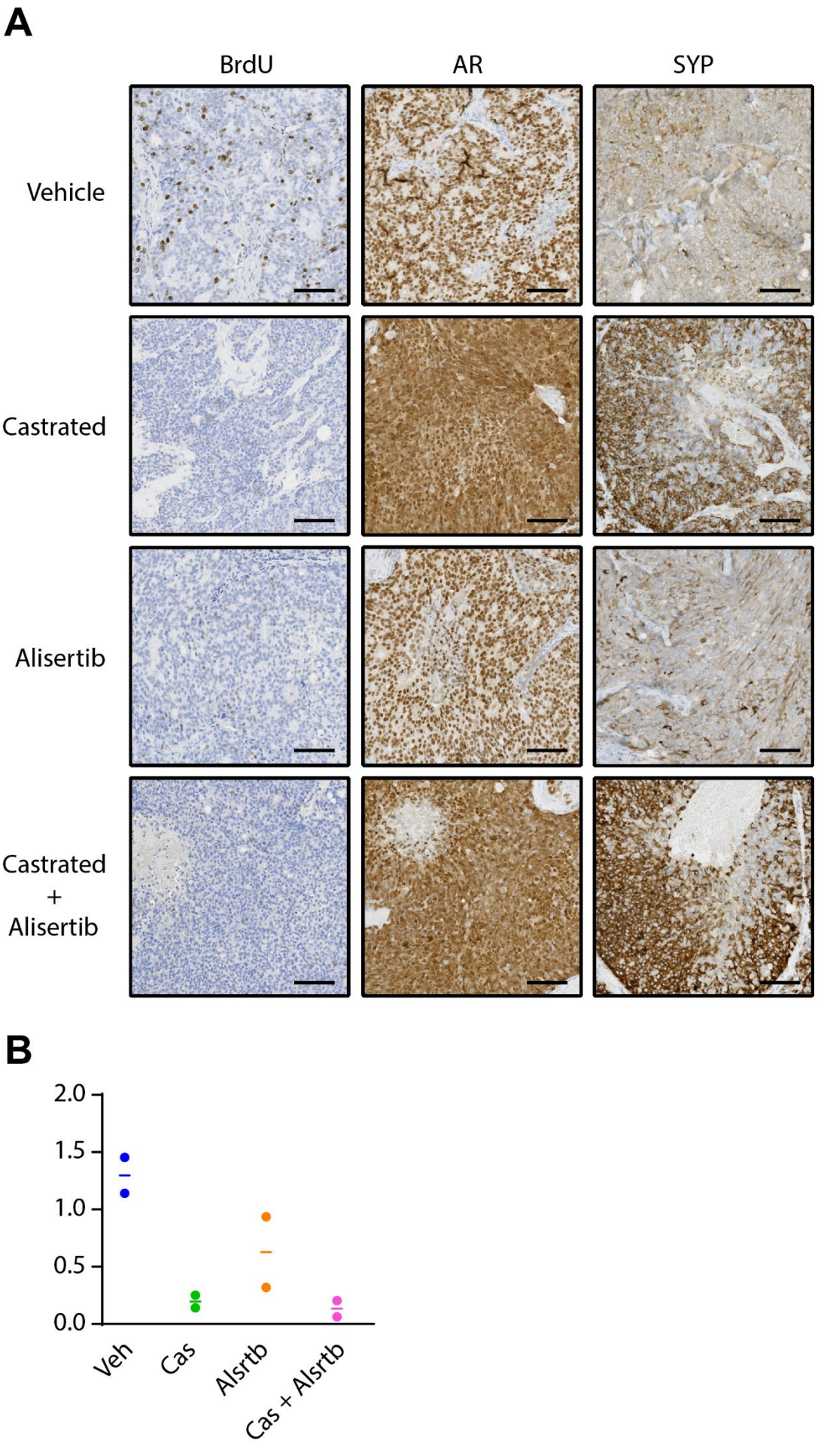

A
BrdU
AR
SYP
Vehicle
Castrated
Alisertib
Castrated
+
Alisertib
B
2.0
1.5
1.0
0.5
0.0
Veh
Cas
Alsrtb
Cas + Alsrtb

Figure S8. Organoid-derived xenograft (ODX) tumors respond to castration and treatment with the AURKA inhibitor, alisertib. Related to Figure 6. (A) Serial sections of ODX tumor tissue from treated (9 weeks) or control mice were stained with antibodies against BrdU, AR or SYP. Mice were injected with BrdU five hours prior to harvest. Scale bars, 100 μM. (B) Two stained sections from each treatment cohort were quantified for BrdU-incorporation. Positive cells classified as strong (see methods) are plotted. Horizontal line indicates the mean.