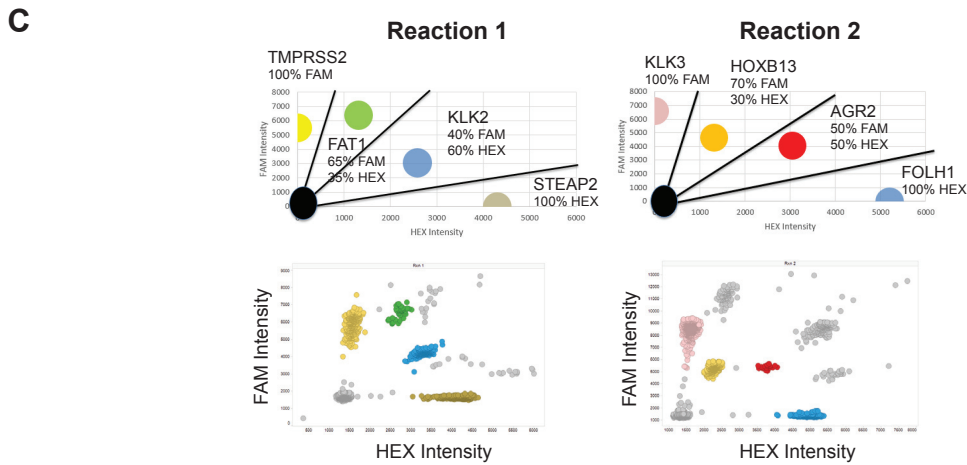# Supplementary Figure S1

A

B

C

D

Supplementary Figure S1. (A) Schematic of d-CTC assay. Whole blood is processed using the CTC-iChip, resulting in a bulk product highly enriched for intact CTCs (labeled green) but also contains WBCs (labeled red) and RBCs (unlabeled). Multiplex droplet digital PCR detects lineage-specific and cancer-specific genes expressed in CTCs. **(B)** Schematic of procedure for identification and selection of candidate gene targets and droplet digital PCR primers/ probes for d-CTC assay. **(C)** Top panels: theoretical modeling of multiplex d-CTC assay based on FAM/HEX fluorophore ratios used in probes. Bottom panels: multiplex d-CTC assay results inthe LNCaP prostate cancer cell line. **(D)** Pie charts showing relative distribution of gene signal detected using the d-CTC assay after CTC- iChip processing of varying numbers of LNCaP cells micromanipulated into healthy donor blood, as well as bulk LNCaP cell cDNA.
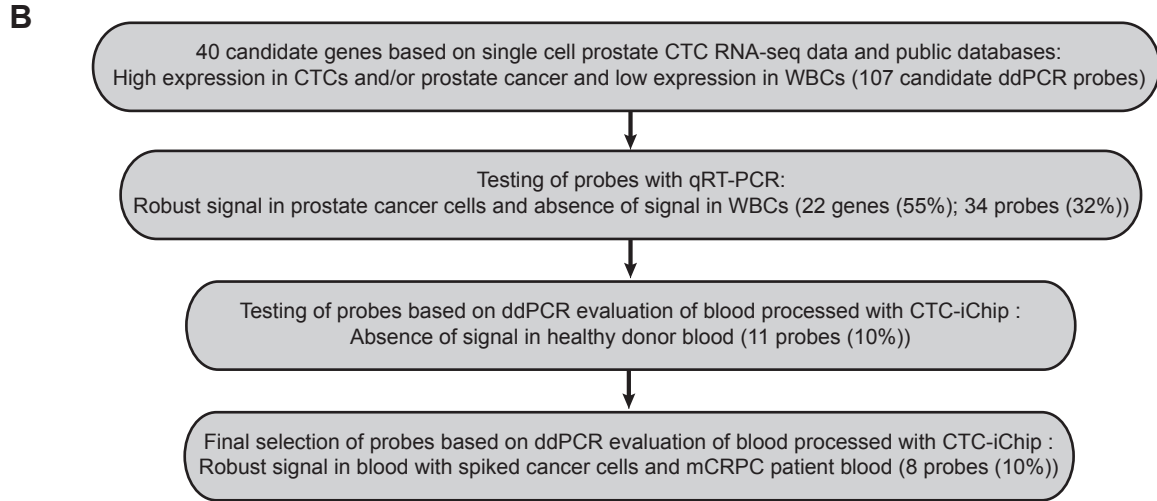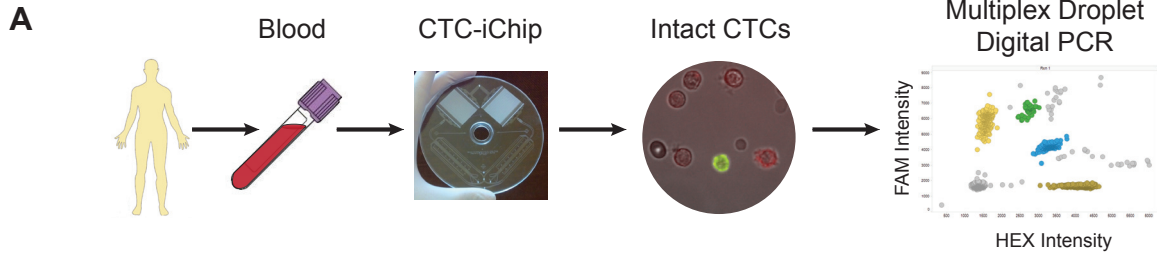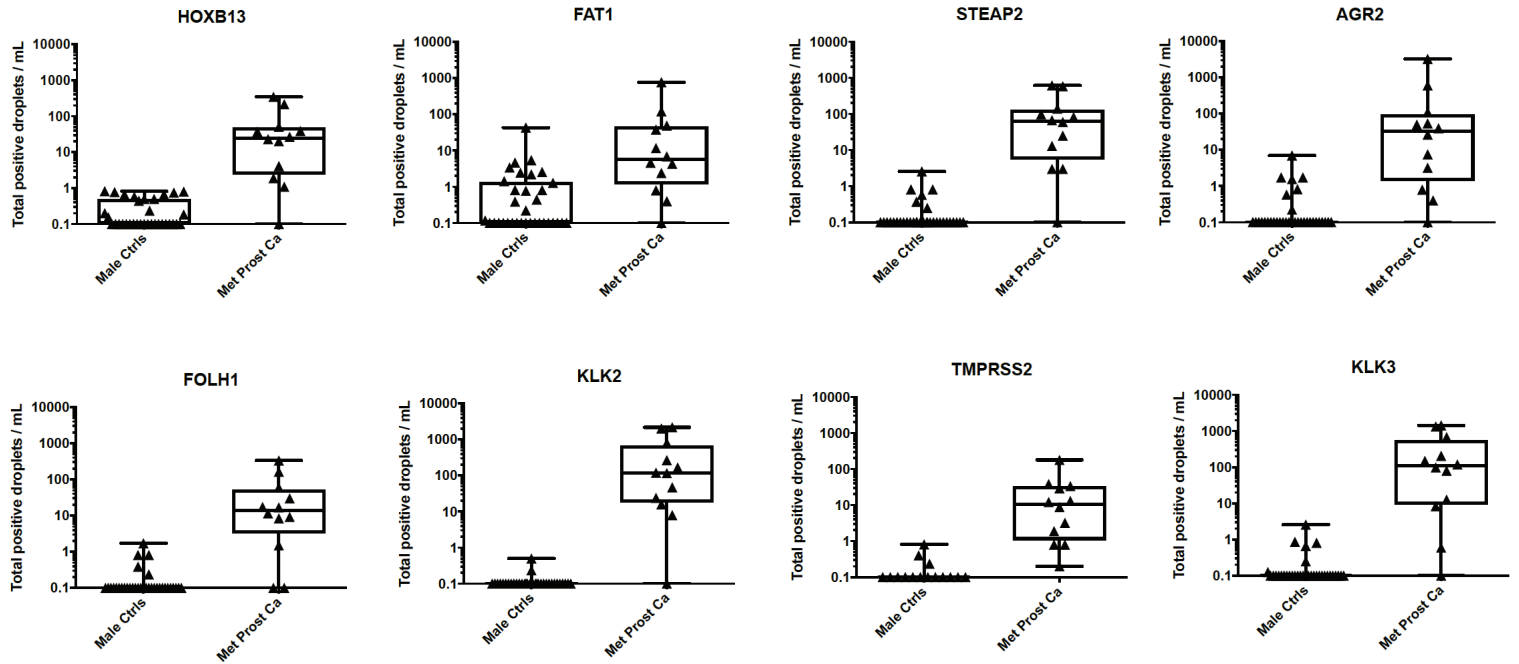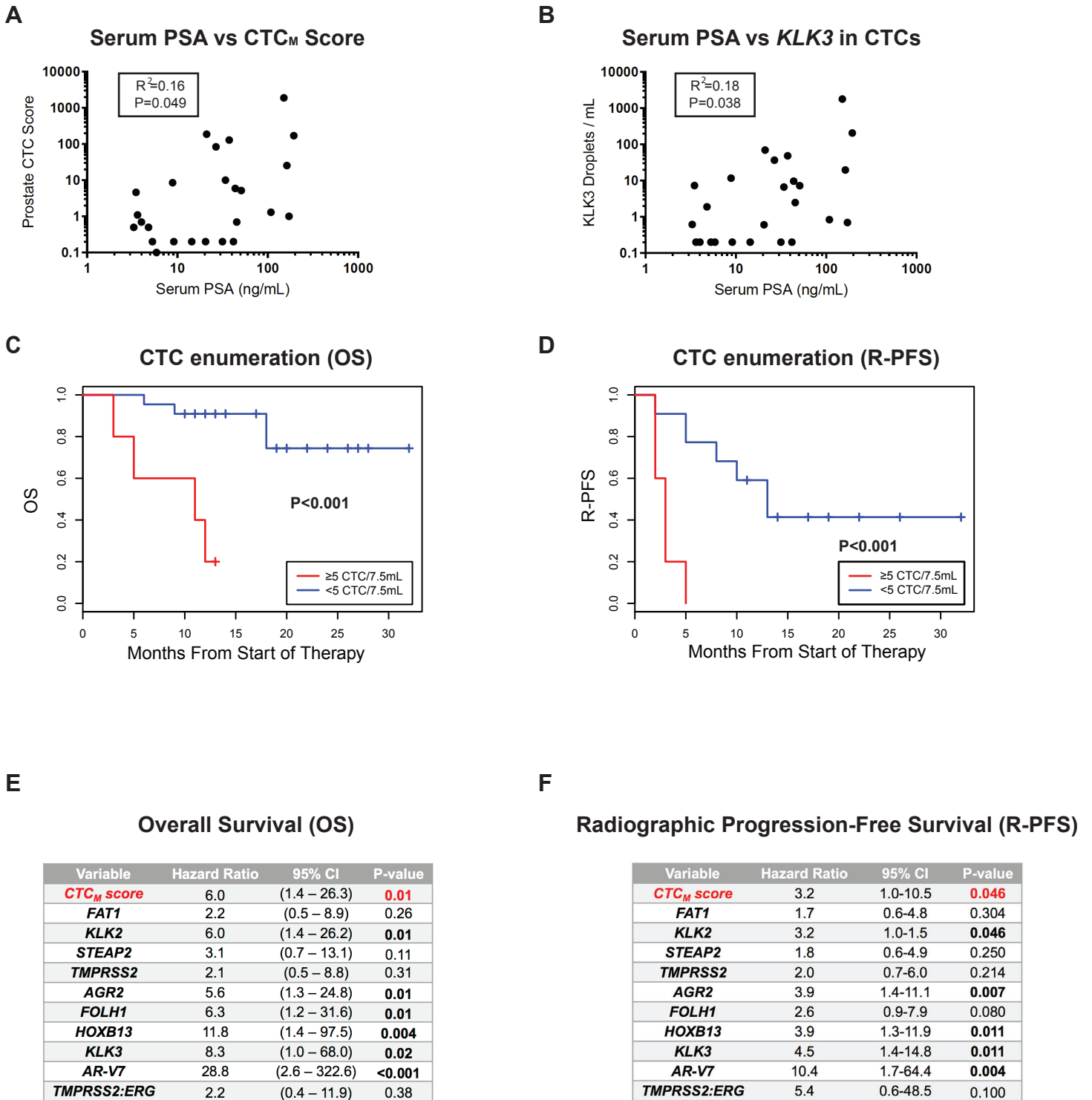
Supplementary Figure S2

Supplementary Figure S2. Graphs showing droplet digital PCR signal for each gene in 12 metastatic prostate cancer patients compared to 34 healthy male control subjects.
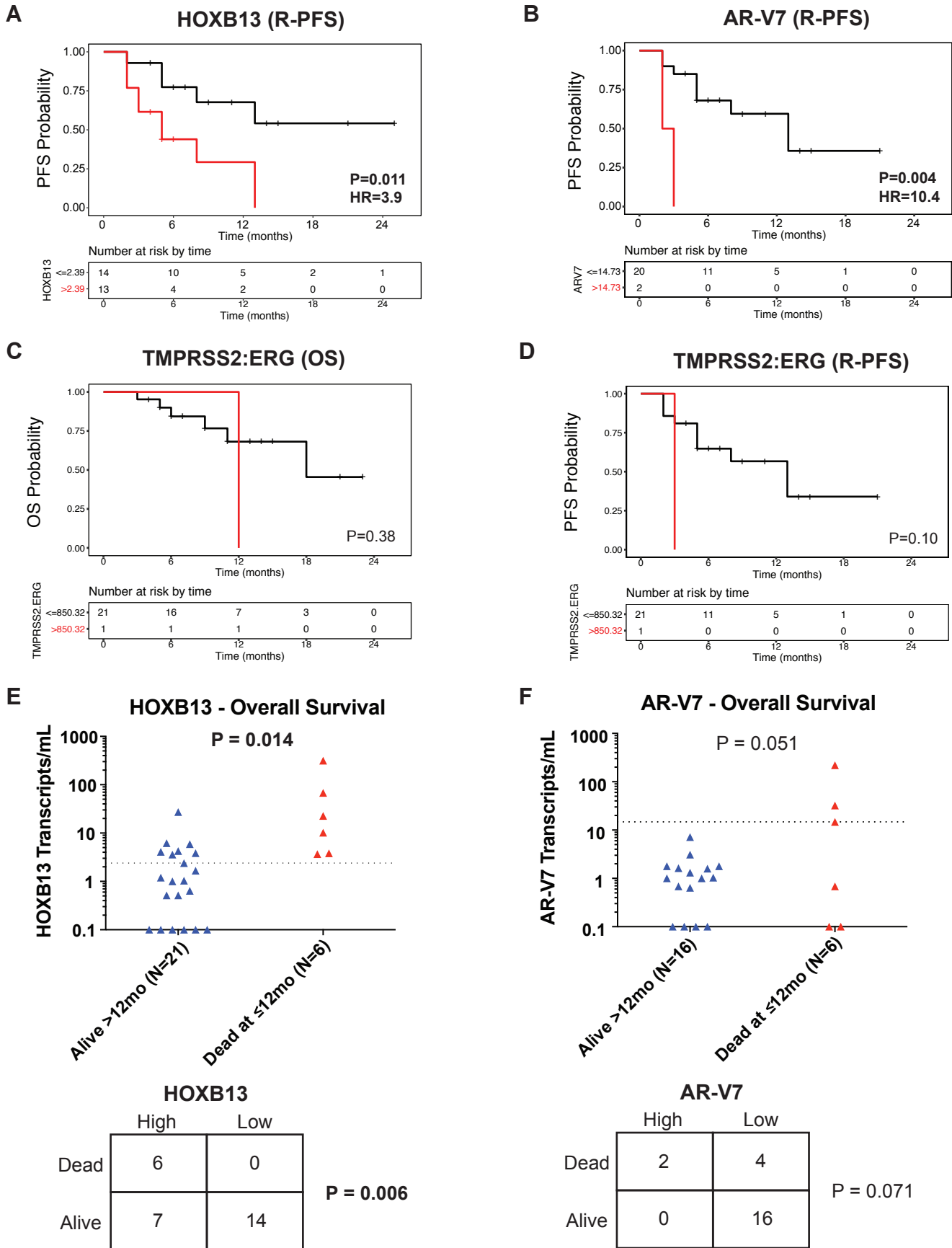
Supplementary Figure S3

A

Serum PSA vs CTC$_M$ Score

B

Serum PSA vs *KLK3* in CTCs

C

CTC enumeration (OS)

D

CTC enumeration (R-PFS)

E

Overall Survival (OS)

Variable	Hazard Ratio	95% CI	P-value
CTC$_M$ score	6.0	(1.4 – 26.3)	**0.01**
FAT1	2.2	(0.5 – 8.9)	0.26
KLK2	6.0	(1.4 – 26.2)	**0.01**
STEAP2	3.1	(0.7 – 13.1)	0.11
TMPRSS2	2.1	(0.5 – 8.8)	0.31
AGR2	5.6	(1.3 – 24.8)	**0.01**
FOLH1	6.3	(1.2 – 31.6)	**0.01**
HOXB13	11.8	(1.4 – 97.5)	**0.004**
KLK3	8.3	(1.0 – 68.0)	**0.02**
AR-V7	28.8	(2.6 – 322.6)	**<0.001**
TMPRSS2:ERG	2.2	(0.4 – 11.9)	0.38

F

Radiographic Progression-Free Survival (R-PFS)

Variable	Hazard Ratio	95% CI	P-value
CTC$_M$ score	3.2	1.0-10.5	**0.046**
FAT1	1.7	0.6-4.8	0.304
KLK2	3.2	1.0-1.5	**0.046**
STEAP2	1.8	0.6-4.9	0.250
TMPRSS2	2.0	0.7-6.0	0.214
AGR2	3.9	1.4-11.1	**0.007**
FOLH1	2.6	0.9-7.9	0.080
HOXB13	3.9	1.3-11.9	**0.011**
KLK3	4.5	1.4-14.8	**0.011**
AR-V7	10.4	1.7-64.4	**0.004**
TMPRSS2:ERG	5.4	0.6-48.5	0.100

Supplementary Figure S3. (A, B) Graphs of relationships between Prostate CTC$_M$ Score and serum PSA, and CTC droplet digital PCR KLK3 signal and serum PSA at the pre-treatment time point.
(C, D) Kaplan-Meier curves for overall survival (OS) and radiographic progression-free survival (R-PFS) by CTC enumeration at pretreatment, determined through immunofluorescence staining and microscopy.
(E, F) Tables showing univariate analysis of CTC$_M$ Score and individual genes, according to association with overall survival and radiographic progression-free survival in prospective study of mCRCPC patients receiving first-line abiraterone.
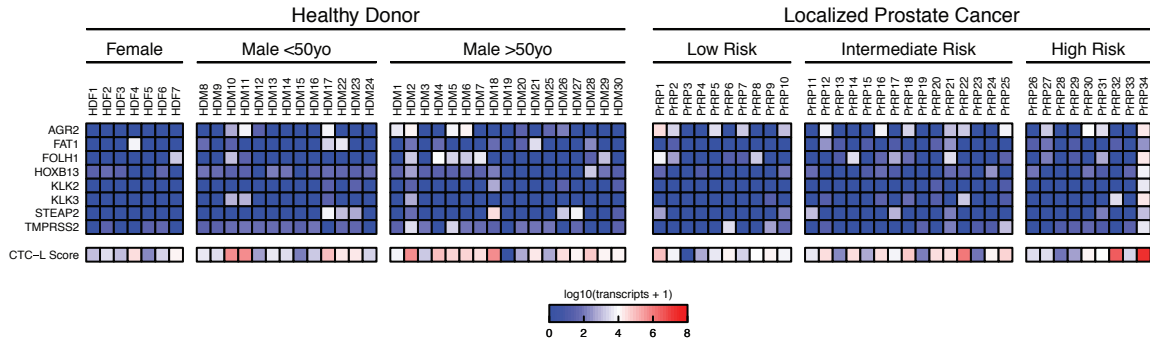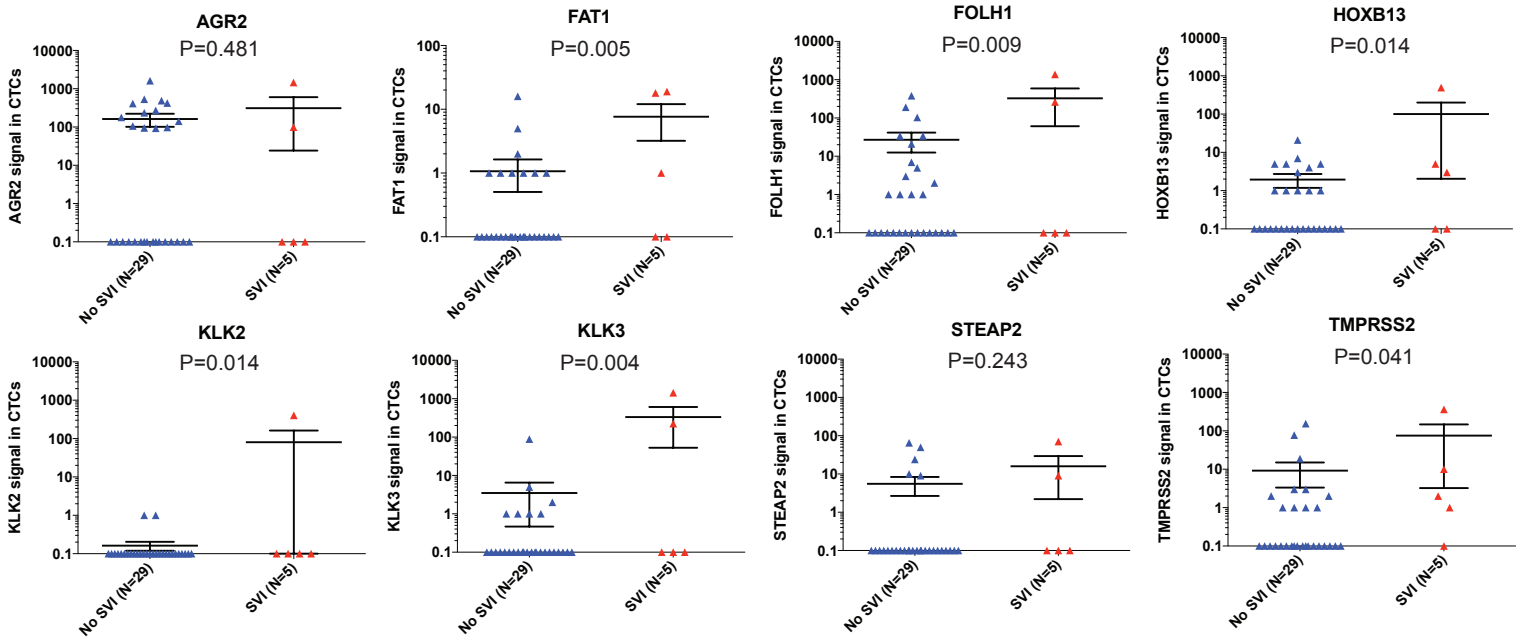
Supplementary Figure S4

A
HOXB13 (R-PFS)

P=0.011
HR=3.9

HOXB13	0	6	12	18	24
<=2.39	14	10	5	2	1
>2.39	13	4	2	0	0

B
AR-V7 (R-PFS)

P=0.004
HR=10.4

ARV7	0	6	12	18	24
<=14.73	20	11	5	1	0
>14.73	2	0	0	0	0

C
TMPRSS2:ERG (OS)

P=0.38

TMPRSS2:ERG	0	6	12	18	24
<=850.32	21	16	7	3	0
>850.32	1	1	1	0	0

D
TMPRSS2:ERG (R-PFS)

P=0.10

TMPRSS2:ERG	0	6	12	18	24
<=850.32	21	11	5	1	0
>850.32	1	0	0	0	0

E
HOXB13 - Overall Survival

P = 0.014

HOXB13

	High	Low	
Dead	6	0	
Alive	7	14	P = 0.006

F
AR-V7 - Overall Survival

P = 0.051

AR-V7

	High	Low	
Dead	2	4	
Alive	0	16	P = 0.071

Supplementary Figure S4. (A) Kaplan-Meier curves for radiographic progression-free survival (R-PFS) by CTC HOXB13 status at pretreatment. **(B)** Kaplan-Meier curves for radiographic progression-free survival (R-PFS) by CTC AR-V7 status at pretreatment. **(C, D)** Kaplan-Meier curves for overall survival (OS) and radiographic progression-free survival (R-PFS) by CTC TMPRSS2:ERG status at pretreatment. **(E, F)** Graphs showing quantitation of HOXB13 and AR-V7 CTC transcripts in mCRPC patients, according to survival status at 12 months after initiation of abiraterone therapy. 5 patients had pre-treatment data available for HOXB13 but not for AR-V7.
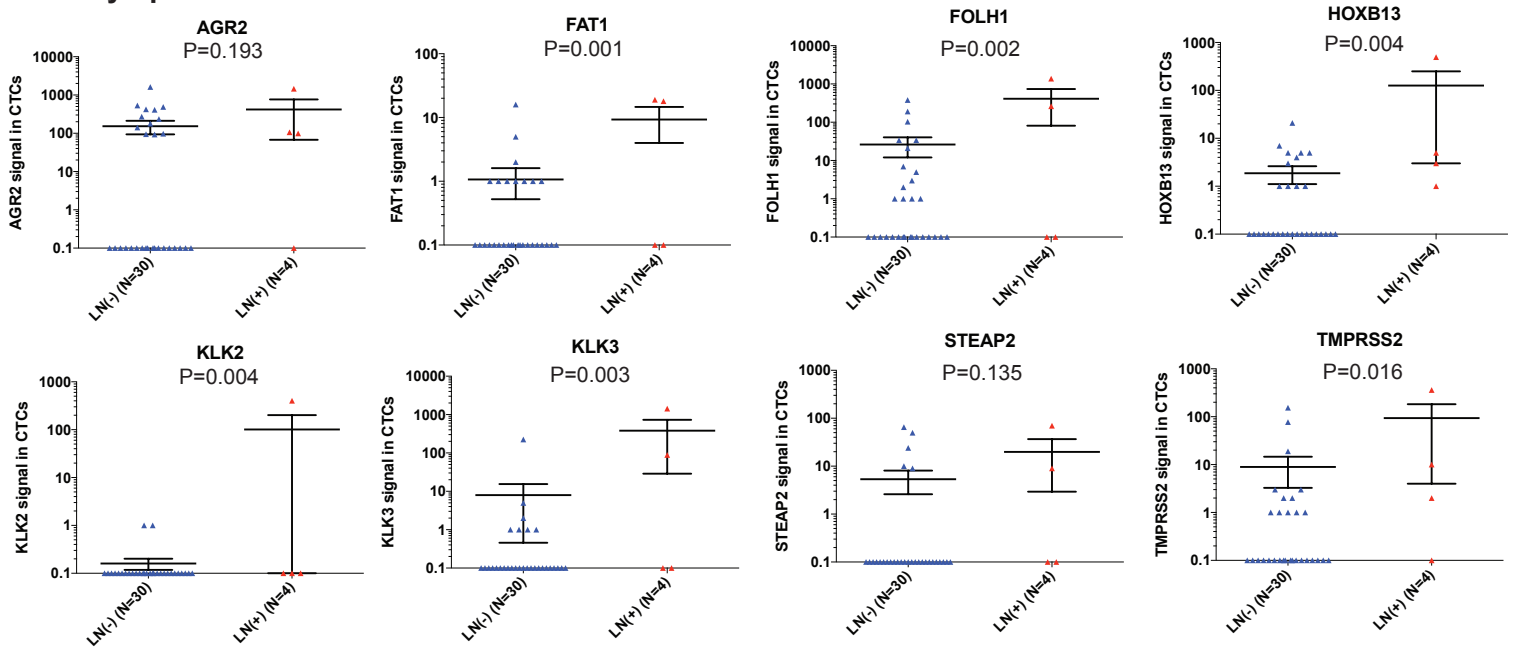
Supplementary Figure S5

A

B Seminal Vesicle Invasion

## C Lymph Node Involvement

Supplementary Figure S5. (A) Heatmap of droplet digital PCR CTC signal after whole transcriptome amplification for blood samples from healthy donor controls arranged by sex and age, and from patients with clinically localized prostate cancer, arranged by D'Amico Risk Group. **(B)** Graphs showing droplet digital PCR signal for each gene in localized prostate cancer patients with and without pathologic seminal vesicle invasion identified at radical prostatectomy. **(C)** Graphs showing droplet digital PCR signal for each gene in localized prostate cancer patients with and without pathologic lymph node involvement identified at radical prostatectomy.
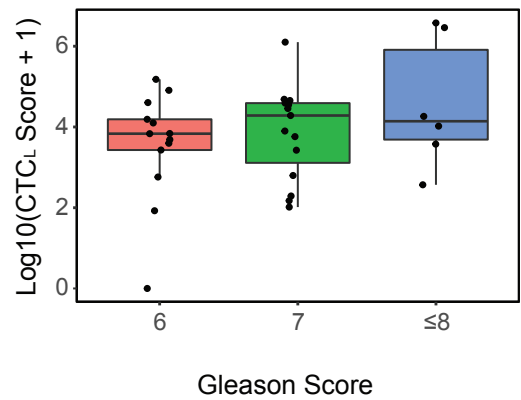
Supplementary Figure S6
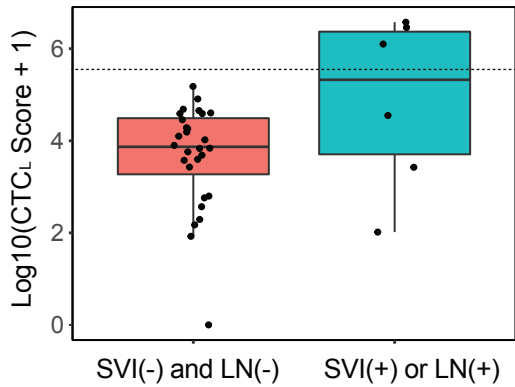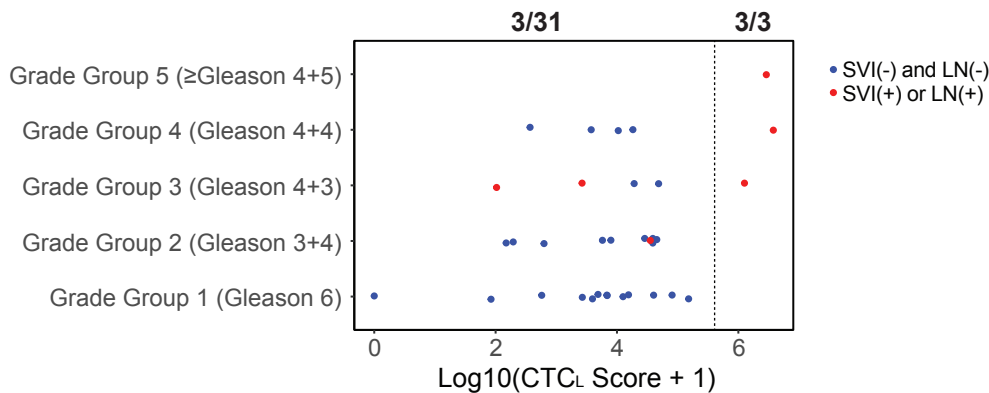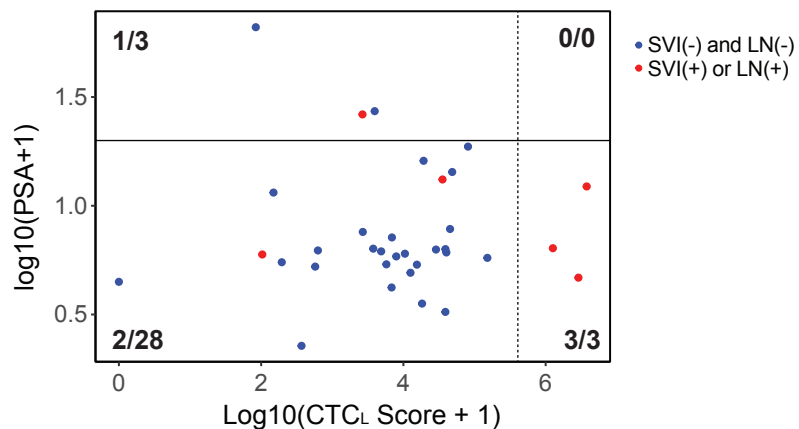
Supplementary Figure S6. (A) Box plots showing pre-operative leave-one-out cross validated (LOOCV) CTC_L Score in clinically localized prostate cancer patients according to microscopic SVI or pelvic LN involvement identified at the time of radical prostatectomy. Dashed line represents a threshold of 2 standard deviations above the average CTC_L Score signal in healthy donor controls. **(B)** Box plots showing pre-operative LOOCV CTC_L Score in clinically localized prostate cancer patients according to Gleason score of diagnostic prostate biopsies. **(C, D)** Graphs of relationship between pre-operative LOOCV CTC_L Score and Serum PSA (upper panel) or Gleason score (lower panel). Red dots represent patients who had SVI or pelvic LN involvement identified on pathologic examination of the radical prostatectomy specimen. Blue dots represent patients who did not have SVI or LN involvement. Perpendicular dashed line represents a threshold of 2 standard deviations above the average CTC_L Score signal in healthy donor controls.