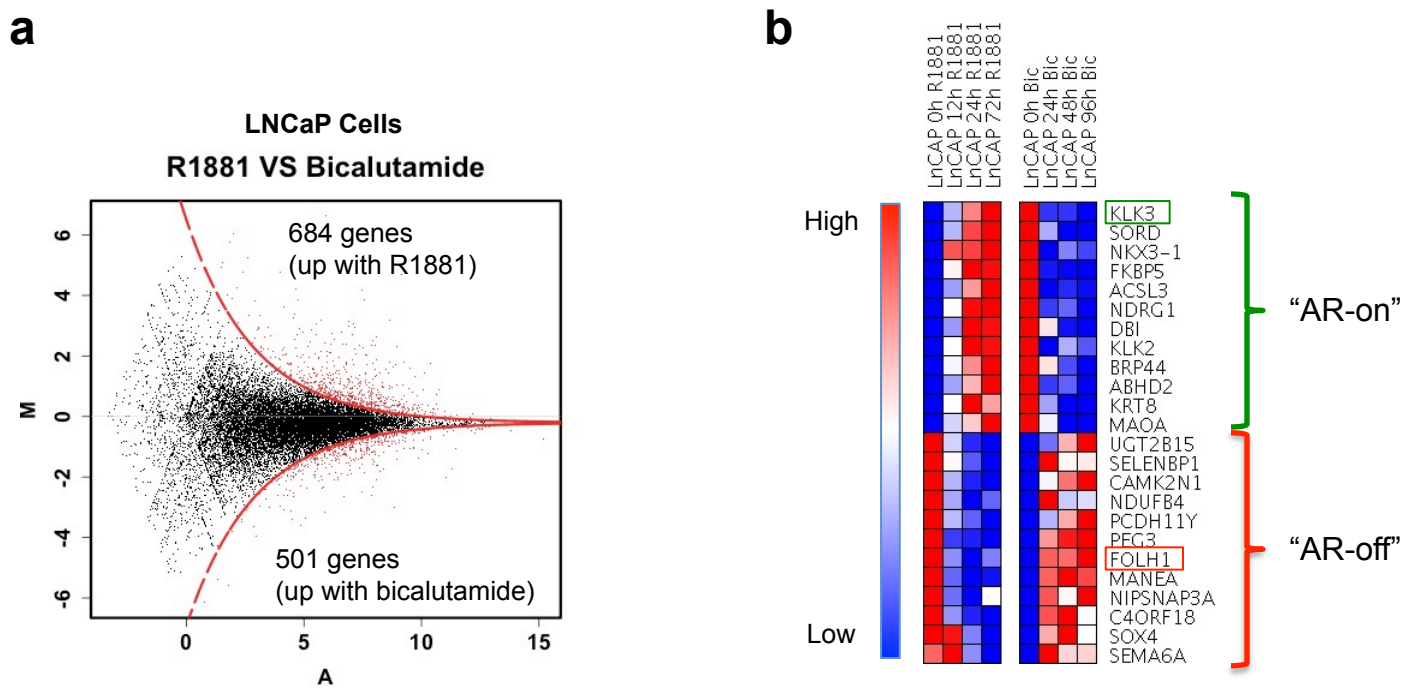
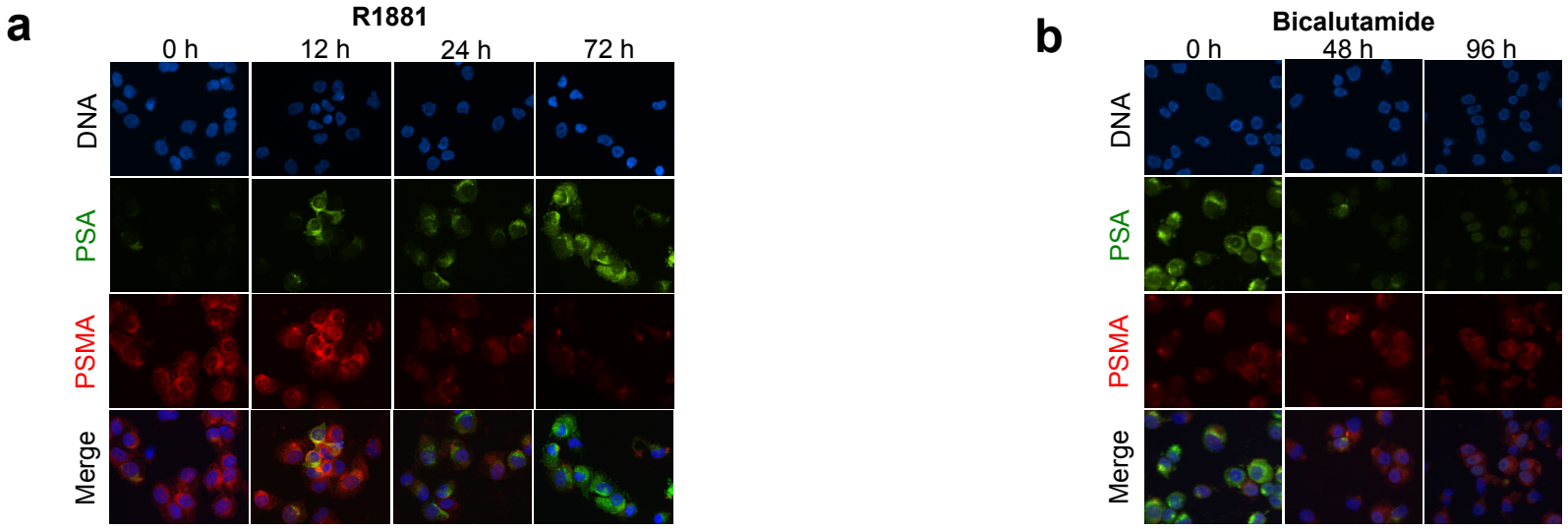
Supplemental Figure S1



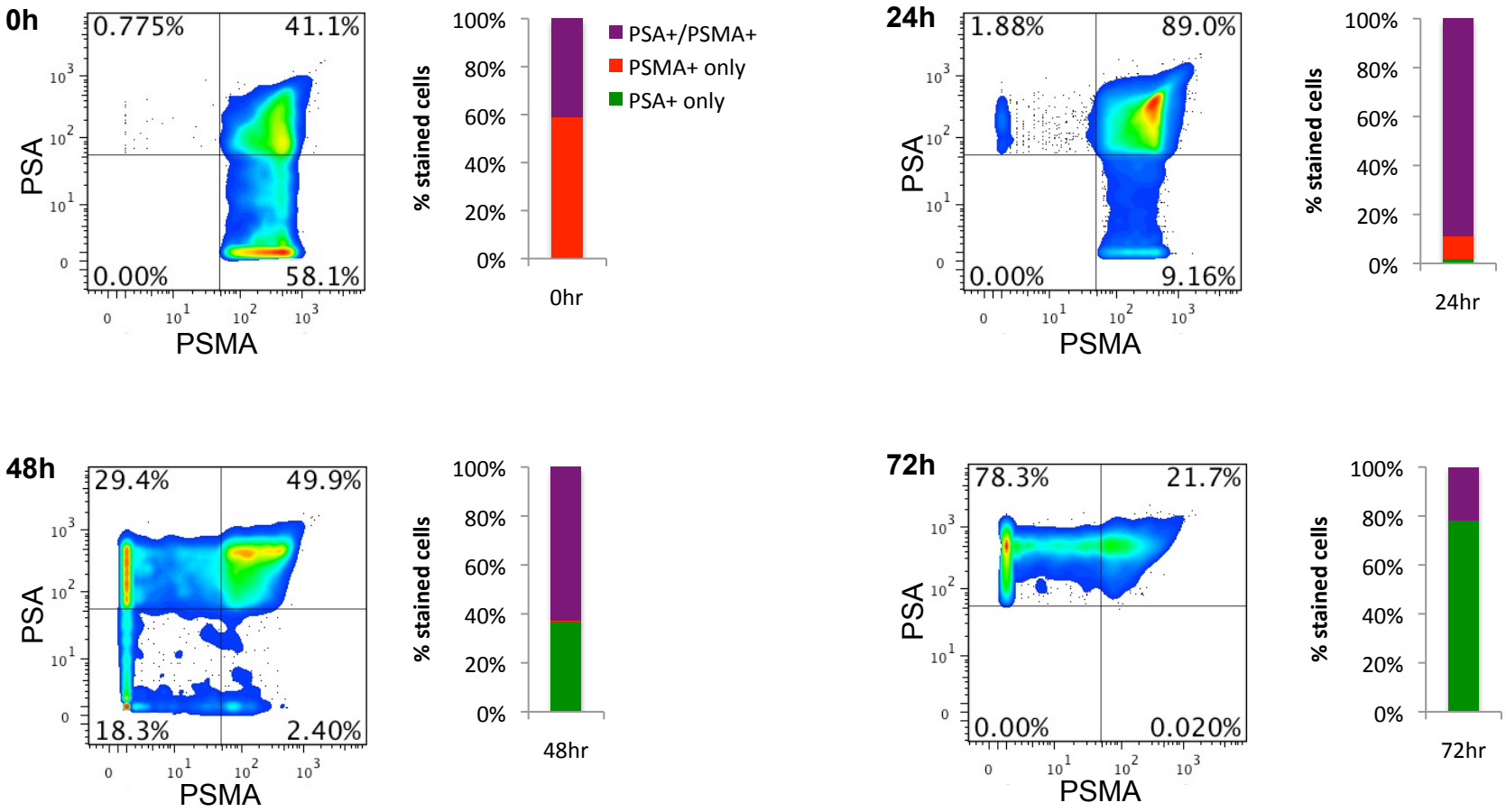
Supplemental Figure S1. AR transcriptional signature derivation and validation. (**a**) Single molecule RNA sequencing and digital gene expression (DGE) profiling reveals differentially expressed genes in LNCaP cells treated with R1881 or bicalutamide for 24 hours. Red dots on M-A plot represent individual transcripts up-regulated with R1881 or bicalutamide (FDR<0.05). The top 24 differentially expressed genes were selected to comprise the AR transcriptional signature. (**b**) The AR signature was validated using DGE profiling data generated from time course experiments of LNCaP cells treated with R1881 or bicalutamide. The heat map shows relative transcript levels, with red and blue colors representing higher and lower expression levels, respectively, normalized by row. The genes "KLK3" and "FOLH1" are highlighted with green and red boxes, respectively.

Supplemental Figure S2



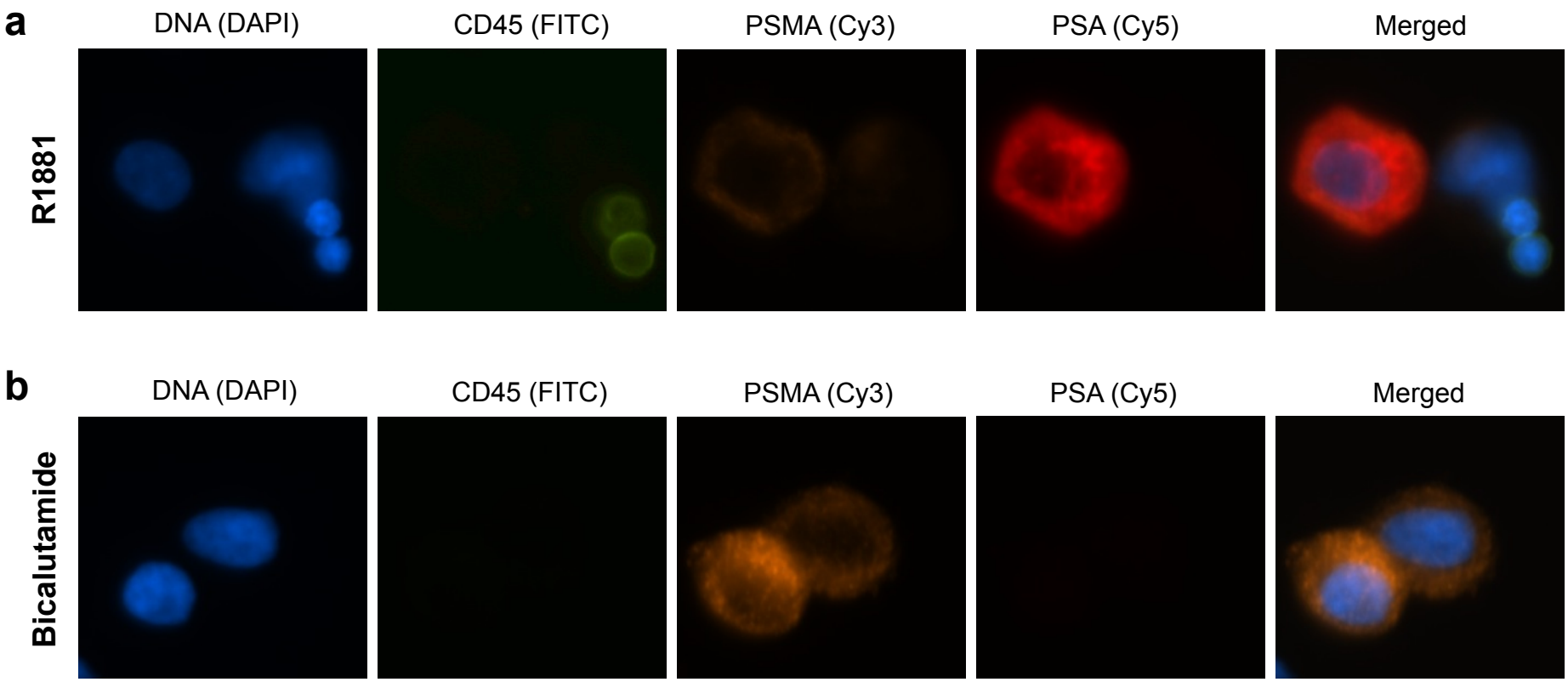
Supplemental Figure S2. Multiparameter single cell immunofluorescence assay for AR signaling to measure dynamic changes in AR activity in cultured prostate cancer cells. (**a)** Immunofluorescence images of LNCaP cells dual stained with antibodies against PSA (green) and PSMA (red) after treatment with 1 nM R1881 after 3 days culture in medium containing 10% charcoal-stripped serum. (**b**) Comparable analysis for LNCaP cells treated with 10 μM bicalutamide after being cultured under standard conditions.

Supplemental Figure S3



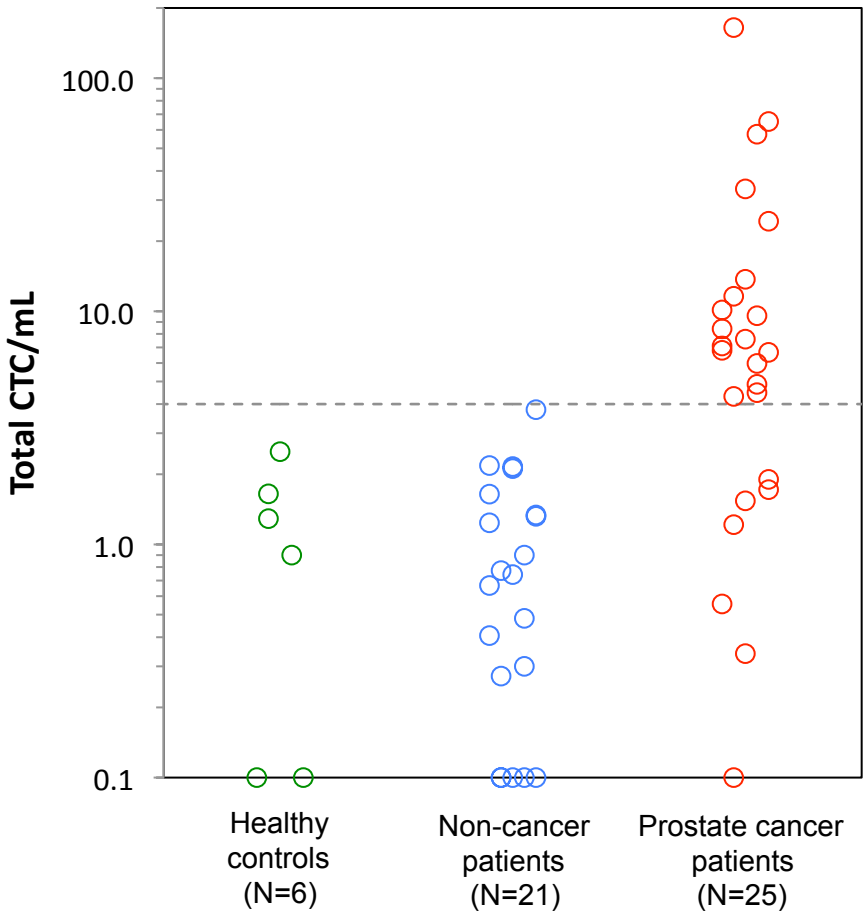
Supplemental Figure S3. Time course for VCaP prostate cancer cells treated with R1881. Multiparameter single cell immunofluorescence assay for AR signaling applied to VCaP prostate cancer cells after treatment with 1 nM R1881 after 3 days culture in medium containing 10% charcoal-stripped serum.

Supplemental Figure S4



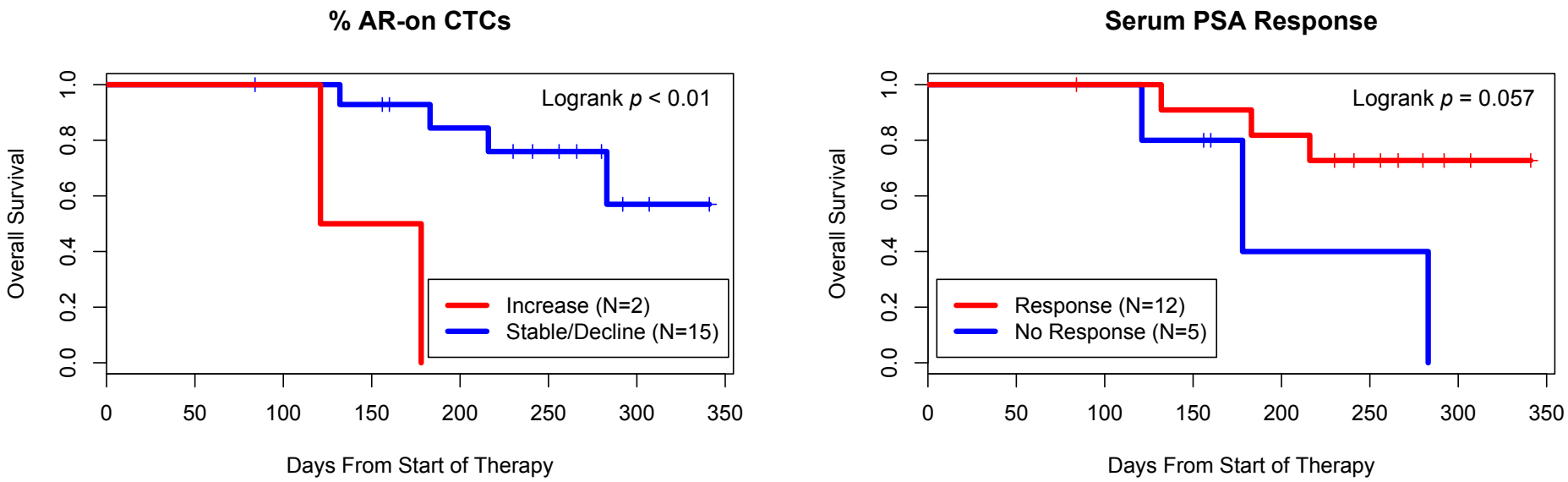
Supplemental Figure S4. Four-color immunofluorescence images of LNCaP cells treated with R1881 or bicalutamide, spiked into control blood, and captured on the ^{HB}CTC-chip. (**a)** LNCaP cells treated with1 nM R1881 for 72 hours, along with contaminating leukocytes, captured on the ^{HB}CTC-chip and stained with antibodies against PSA (Cy5), PSMA (Cy3), CD45 (FITC), and DAPI for DNA. (**b**) Comparable analysis for LNCaP cells treated with 10 μM bicalutamide.

Supplemental Figure S5



Supplemental Figure S5. Enumeration of CTCs in healthy donor controls (N=6), male patients with no known diagnosis of cancer (N=21), and metastatic prostate cancer patients (N=25) using ^{HB}CTC-chip 4-color imaging parameters. Captured cells were stained in 4 colors with antibodies against PSA (Cy5), PSMA (Cy3), CD45 (FITC), and DAPI for DNA. Total CTC/mL refers to the sum of PSA+/PSMA-/CD45- CTC count, PSA-/PSMA+/CD45- CTC count, and PSA+/PSMA+/CD45- CTC count, divided by the total volume of blood processed. Dashed line refers to the signal intensity threshold for detection (4 CTC/mL), determined by analysis of healthy donor blood samples, below which a signal is considered a false positive.

Supplemental Figure S6



Supplemental Figure S6. Kaplan-Meier survival curves for overall survival in patients with CRPC treated with abiraterone acetate, according to increase in % “AR-on” CTCs following treatment (red) versus no increase in % “AR-on” CTCs (blue) (left graph), and according to serum PSA response (red) or absence of serum PSA response (blue) (right graph). Similar analysis of changes in % “AR-off” and % “AR-mixed” CTCs showed no significant differences in overall survival.