

Supplementary Table S1. Genes tested for Prostate CTC digital PCR Assay

| Gene name | # Primer pairs tested | # primers specific by qPCR | # primers specific by ddPCR | ddPCR signal in mCRPC patients | Status |
|-----------------|-----------------------|----------------------------|-----------------------------|--------------------------------|-------------------------------|
| AGR2 | 3 | 2 | 1 | Yes | Good primer |
| ALDH1A3 | 1 | 1 | 1 | Yes (not robust) | Good primer |
| AMACR | 6 | 1 | - | - | failed ddPCR specificity test |
| AR | 6 | 0 | - | - | failed qPCR specificity test |
| ARG2 | 3 | 0 | - | - | failed qPCR specificity test |
| ARNTL (BMAL1) | 2 | 0 | - | - | failed qPCR specificity test |
| BCL2 | 1 | 0 | - | - | failed qPCR specificity test |
| CDKN2B-AS1 | 4 | 1 | 0 | - | no ddPCR signal in cells |
| DDX11 | 2 | 0 | - | - | failed qPCR specificity test |
| ECT2 | 2 | 0 | - | - | failed qPCR specificity test |
| ENO2 | 4 | 0 | - | - | failed qPCR specificity test |
| EPCAM (TACSTD1) | 3 | 1 | 0 | - | failed ddPCR specificity test |
| ERG | 2 | 0 | - | - | failed qPCR specificity test |
| ETV1 | 2 | 0 | - | - | failed qPCR specificity test |
| FAT1 | 1 | 1 | 1 | Yes | Good primer |
| FOLH1 | 3 | 1 | 1 | Yes | Good primer |
| GOLM1 | 3 | 0 | - | - | failed qPCR specificity test |
| HAL | 2 | 0 | - | - | failed qPCR specificity test |
| HOXB13 | 1 | 1 | 1 | Yes | Good primer |
| IGFBP5 | 2 | 2 | 0 | - | failed ddPCR specificity test |
| JUP | 2 | 0 | - | - | failed qPCR specificity test |
| KLK2 | 4 | 4 | 1 | Yes | Good primer |
| KLK3 | 4 | 1 | 1 | Yes | Good primer |
| KRT18 | 3 | 0 | - | - | failed qPCR specificity test |
| KRT19 | 2 | 1 | 0 | - | failed ddPCR specificity test |
| KRT8 | 5 | 0 | - | - | failed qPCR specificity test |
| PCA3 | 1 | 1 | 0 | - | no ddPCR signal in cells |
| PCAT1 | 1 | 1 | 1 | No | Good primer |
| PCGEM1 | 2 | 1 | 0 | - | no ddPCR signal in cells |
| PDLIM5 | 2 | 0 | - | - | failed qPCR specificity test |
| PDPK1 | 2 | 0 | - | - | failed qPCR specificity test |
| PLA2G2A | 1 | 1 | - | - | failed ddPCR specificity test |
| SCHLAP1 | 5 | 4 | 0 | - | failed ddPCR specificity test |
| SELENBP1 | 2 | 1 | 0 | - | failed ddPCR specificity test |
| SLC45A3 | 2 | 0 | - | - | failed qPCR specificity test |
| STEAP2 | 2 | 2 | 1 | Yes | Good primer |
| SUPT7L | 2 | 0 | - | - | failed qPCR specificity test |
| TGM4 | 2 | 2 | 1 | Yes (not robust) | Good primer |
| TMPRSS2 | 2 | 2 | 1 | Yes | Good primer |
| UGT2B15 | 7 | 2 | - | - | failed ddPCR specificity test |
| TOTAL | 106 | 34 | 11 | 8 | |

Supplementary Table S2. Clinical information for prostate cancer patients and healthy donor subjects

Localized Prostate Cancer Patients

| ID | Sex | Age | Initial PSA | cT Stage | Gleason Score (bx) | pT stage | Gleason Score (RP) | Margin status (RP) |
|-----|------|-----|-------------|----------|--------------------|----------|--------------------|--------------------|
| Pr1 | Male | 66 | | 5.4 T2a | 3+4 | T2cN0 | 3+4 | - |
| Pr2 | Male | 53 | | 4.4 T1c | 4+3 | T2cN0 | 3+4 | - |
| Pr3 | Male | 57 | | 9.7 T1c | 4+3 | T2aNO | 3+4 | + |
| Pr4 | Male | 70 | | 8.3 T2a | 4+3 | T3aNO | 4+3 | - |
| Pr5 | Male | 65 | | 17.0 T1c | 3+4 | T3aNO | 4+3 | - |
| Pr6 | Male | 55 | | 5.5 T1c | 3+3 | T2cNx | 3+4 | - |
| Pr7 | Male | 55 | | 3.4 T1c | 3+4 | T2cNO | 3+4 | - |
| Pr8 | Male | 58 | | 6.2 T1c | 3+3 | T3aNO | 3+4 | + |

Metastatic Prostate Cancer Patients

| ID | Sex | Age | PSA (at time of CTC draw) | Sites of disease | Prior therapies |
|------|------|-----|---------------------------|------------------|--|
| Pr9 | Male | 74 | 391.6 | bone | RT, ADT, bicalutamide, cabozantinib, abiraterone, radium, docetaxel |
| Pr10 | Male | 79 | 6149.0 | bone | RP, RT/bicalutamide, ADT, bicalutamide, abiraterone, radium, docetaxel, enzalutamide |
| Pr11 | Male | 55 | 4272.0 | bone, LNs | ADT, RP, bicalutamide, RT, ARN509, sipuleucel-T, abiraterone, docetaxel, cabazitaxel, enzalutamide, mitoxantrone |
| Pr12 | Male | 73 | 861.9 | bone | RT/ADT, ADT, bicalutamide, sipuleucel-T, abiraterone, docetaxel, enzalutamide, cabazitaxel |
| Pr13 | Male | 64 | 6.8 | bone | ADT, abiraterone, docetaxel, cabazitaxel |
| Pr14 | Male | 67 | 15.3 | bone | bicalutamide, ADT, abiraterone, docetaxel, radium |
| Pr15 | Male | 89 | 17.7 | bone, LNs | ADT, bicalutamide, abiraterone, enzalutamide, radium |
| Pr16 | Male | 68 | 5.6 | bone | RP, RT/ADT, ADT, abiraterone |
| Pr17 | Male | 65 | 41.5 | bone, LNs | ADT/RT, abiraterone, enzalutamide |
| Pr18 | Male | 64 | 104.6 | bone, LNs | ADT, bicalutamide, abiraterone, enzalutamide, radium |
| Pr19 | Male | 70 | 772.3 | bone | none |
| Pr20 | Male | 67 | 13.2 | bone, LNs | ADT |
| Pr21 | Male | 85 | 1.85 | bone | ADT, abiraterone |
| Pr22 | Male | 68 | 43.27 | bone, LNs | ADT, RT, docetaxel, abiraterone |
| Pr23 | Male | 68 | 474.20 | bone, LNs | ADT, bicalutamide, sipuleucel-T, abiraterone, enzalutamide, RT |
| Pr24 | Male | 85 | 140.50 | bone | ADT, bicalutamide, denosumab, abiraterone, enzalutamide |
| Pr25 | Male | 74 | 98.58 | bone | ADT, abiraterone, galaterone, docetaxel, radium, RT, cabazitaxel |
| Pr26 | Male | 61 | 0.59 | bone | ADT, abiraterone |
| Pr27 | Male | 67 | 1.81 | bone | ADT, abiraterone |
| Pr28 | Male | 57 | <0.1 | bone | ADT, abiraterone |
| Pr29 | Male | 65 | 99.27 | bone, LNs | ADT, abiraterone |

Healthy Donor Subjects

| ID | Sex | Age |
|--------|--------|-----|
| HDF1 | Female | 53 |
| HDF2 | Female | 54 |
| HDF3 | Female | 54 |
| HDF4 | Female | 62 |
| HDF5 | Female | 73 |
| HDMY1 | Male | 37 |
| HDMY2 | Male | 41 |
| HDMY3 | Male | 43 |
| HDMY4 | Male | 44 |
| HDMY5 | Male | 45 |
| HDMY6 | Male | 29 |
| HDMY7 | Male | 40 |
| HDMY8 | Male | 24 |
| HDMY9 | Male | 32 |
| HDMY10 | Male | 41 |
| HDMY11 | Male | 25 |
| HDMY12 | Male | 43 |
| HDMY13 | Male | 23 |
| HDMY14 | Male | 23 |
| HDMY15 | Male | 31 |
| HDMO1 | Male | 53 |
| HDMO2 | Male | 53 |
| HDMO3 | Male | 54 |
| HDMO4 | Male | 54 |
| HDMO5 | Male | 62 |
| HDMO6 | Male | 63 |
| HDMO7 | Male | 63 |
| HDMO8 | Male | 63 |
| HDMO9 | Male | 68 |
| HDMO10 | Male | 68 |
| HDMO11 | Male | 68 |
| HDMO12 | Male | 69 |
| HDMO13 | Male | 69 |
| HDMO14 | Male | 72 |
| HDMO15 | Male | 72 |
| HDMO16 | Male | 73 |
| HDMO17 | Male | 73 |
| HDMO18 | Male | 58 |
| HDMO19 | Male | 53 |

Supplementary Table S3. Clinical information for patients enrolled on prospective trial of first-line abiraterone for mCRPC

| ID | Sex | Age | Sites of disease | High Disease Burden* | PSA at C1D1 | PSA at C4D1 | PSA nadir | Prior therapies |
|-------|------|-----|--------------------------|----------------------|-------------|-------------|-----------|-------------------|
| PrA1 | Male | 67 | bone | Y | 3.52 | 4.39 | 2.70 | ADT |
| PrA2 | Male | 67 | LN | N | 41.73 | 2.66 | 0.20 | ADT, metformin |
| PrA3 | Male | 62 | bone | Y | 194.50 | 2.26 | 1.89 | ADT |
| PrA4 | Male | 68 | bone | Y | 163.20 | 98.34 | 89.35 | ADT |
| PrA5 | Male | 48 | bone | N | 3.91 | 24.80 | NA | ADT |
| PrA6 | Male | 78 | bone, LN | Y | 43.59 | 43.59 | 36.54 | ADT |
| PrA7 | Male | 71 | LN | N | 3.39 | 0.87 | 0.37 | ADT, bicalutamide |
| PrA8 | Male | 67 | LN | N | 45.27 | 3.45 | 3.91 | ADT |
| PrA9 | Male | 63 | bone | Y | 50.77 | 0.34 | 0.28 | ADT |
| PrA10 | Male | 85 | bone | Y | 108.60 | 428.60 | 104.90 | ADT |
| PrA11 | Male | 80 | bone | N | 20.35 | 5.41 | 0.98 | ADT |
| PrA12 | Male | 78 | bone, LN, adrenal nodule | Y | 34.03 | 101.10 | NA | ADT |
| PrA13 | Male | 68 | bone | N | 4.69 | 2.67 | 2.49 | ADT |
| PrA14 | Male | 85 | bone | Y | 21.03 | 20.45 | 14.22 | ADT, bicalutamide |
| PrA15 | Male | 83 | bone | Y | 172.30 | 30.64 | 8.26 | ADT |
| PrA16 | Male | 87 | bone, LN | Y | 5.23 | 1.73 | 1.59 | ADT |
| PrA17 | Male | 72 | bone, local | N | 14.28 | 9.23 | 3.74 | ADT |
| PrA18 | Male | 75 | bone, LN | Y | 151.20 | 451.10 | 120.6 | ADT, bicalutamide |
| PrA19 | Male | 67 | LN | N | 31.48 | 0.25 | <0.10 | ADT |
| PrA20 | Male | 85 | bone | Y | 5.77 | 8.78 | 5.96 | ADT |
| PrA21 | Male | 78 | bone, LN | Y | 37.40 | 23.35 | 6.74 | ADT |
| PrA22 | Male | 78 | bone | N | 3.19 | 1.89 | 0.67 | ADT, bicalutamide |
| PrA23 | Male | 71 | LN | N | 9.04 | 0.56 | 0.56 | ADT, bicalutamide |
| PrA24 | Male | 66 | LN | N | 8.77 | 5.48 | 3.24 | ADT, bicalutamide |
| PrA25 | Male | 73 | bone | Y | 26.62 | 10.24 | 7.66 | ADT+docetaxel |
| PrA26 | Male | 76 | bone | N | 15.17 | 6.75 | 2.42 | ADT, bicalutamide |
| PrA27 | Male | 58 | bone | N | 9.00 | 0.38 | 0.38 | ADT |

*High disease burden defined as >4 bone metastases, at least one outside pelvis/spine (per CHARTED trial), or visceral metastases.

Supplementary Table S4. Clinical information for patients with localized prostate cancer in radical prostatectomy study

| ID | Sex | Age | D'Amico Risk Group | UCSF-CAPRA | cT Stage | WHO Grade Group (bx) | Gleason Score (bx) | %Cores Involved | Initial PSA | Pre-op Imaging | pT Stage | pN Stage | WHO Grade Group (RP) | Gleason Score (RP) | Margin Status (RP) |
|--------|------|-----|--------------------|------------|----------|----------------------|--------------------|-----------------|-------------|--------------------|----------|----------|----------------------|--------------------|--------------------|
| PrRP1 | Male | 49 | Low | 0 | T1c | 1 | 6 | 17% | 4.76 | None | T2c | Nx | 1 | 6 | Negative |
| PrRP2 | Male | 51 | Low | 1 | T1c | 1 | 6 | 33% | 3.21 | None | T2c | Nx | 1 | 6 | Negative |
| PrRP3 | Male | 56 | Low | 1 | T2a | 1 | 6 | 25% | 3.47 | MRI | T3a | N0 | 1 | 6 | Negative |
| PrRP4 | Male | 57 | Low | 1 | T2c | 1 | 6 | 17% | 4.25 | MRI | T2c | N0 | 1 | 3+3 (+4) | Negative |
| PrRP5 | Male | 55 | Low | 2 | T1c | 1 | 6 | 75% | 5.17 | None | T2b | Nx | 2 | 3+4 | Negative |
| PrRP6 | Male | 51 | Low | 2 | T1c | 1 | 6 | 42% | 5.1 | None | T2c | Nx | 1 | 6 | Negative |
| PrRP7 | Male | 61 | Low | 2 | T1c | 1 | 6 | 33% | 6.58 | MRI | T3a | Nx | 2 | 3+4 | Positive |
| PrRP8 | Male | 67 | Low | 2 | T1c | 1 | 6 | 67% | 3.92 | MRI | T3a | N0 | 2 | 3+4 | Positive |
| PrRP9 | Male | 61 | Low | 2 | T2b | 1 | 6 | 58% | 4.36 | None | T2c | N0 | 1 | 6 | Negative |
| PrRP10 | Male | 52 | Low | 3 | T1c | 1 | 6 | 67% | 6.15 | MRI | T2c | N0 | 2 | 3+4 | Negative |
| PrRP11 | Male | 69 | Intermediate | 2 | T1c | 2 | 3+4 | 8% | 4.38 | MRI | T2a | N0 | 2 | 3+4 | Negative |
| PrRP12 | Male | 52 | Intermediate | 2 | T2a | 2 | 3+4 | 17% | 5.29 | MRI | T3a | N0 | 5 | 4+5 | Negative |
| PrRP13 | Male | 57 | Intermediate | 3 | T1c | 2 | 3+4 | 50% | 4.5 | MRI | T2c | N0 | 1 | 6 | Negative |
| PrRP14 | Male | 52 | Intermediate | 3 | T1c | 2 | 3+4 | 100% | 2.25 | MRI, Bone scan | T2c | N0 | 1 | 6 | Negative |
| PrRP15 | Male | 65 | Intermediate | 3 | T1c | 2 | 3+4 | 50% | 5.23 | MRI | T2c | N0 | 2 | 3+4 | Negative |
| PrRP16 | Male | 63 | Intermediate | 3 | T1c | 2 | 3+4 | 67% | 5.32 | None | T2c | N0 | 2 | 3+4 | Negative |
| PrRP17 | Male | 63 | Intermediate | 3 | T2a | 2 | 3+4 | 42% | 4.85 | MRI | T3a | N0 | 2 | 3+4 | Negative |
| PrRP18 | Male | 80 | Intermediate | 4 | T2b | 1 | 6 | 50% | 17.71 | MRI | T3a | N0 | 2 | 3+4 | Negative |
| PrRP19 | Male | 68 | Intermediate | 4 | T1c | 2 | 3+4 | 8% | 10.5 | MRI | T2c | N0 | 1 | 3+3 (+4) | Negative |
| PrRP20 | Male | 57 | Intermediate | 4 | T1c | 2 | 3+4 | 50% | 6.82 | MRI, CT, Bone scan | T3a | N0 | 2 | 3+4 | Positive |
| PrRP21 | Male | 55 | Intermediate | 4 | T2b | 2 | 3+4 | 8% | 12.21 | MRI, CT, Bone scan | T3b | N1 | 3 | 4+3 | Negative |
| PrRP22 | Male | 63 | Intermediate | 5 | T1c | 3 | 4+3 | 42% | 5.38 | MRI | T3a | N1 | 2 | 3+4 | Negative |
| PrRP23 | Male | 73 | Intermediate | 5 | T2a | 3 | 4+3 | 58% | 4.97 | MRI, CT, Bone scan | T3b | N0 | 5 | 4+5 | Negative |
| PrRP24 | Male | 68 | Intermediate | 6 | T1c | 3 | 4+3 | 33% | 15.1 | MRI, CT, Bone scan | T2c | N0 | 3 | 4+3 | Negative |
| PrRP25 | Male | 74 | Intermediate | 6 | T1c | 3 | 4+3 | 33% | 13.3 | CT, Bone scan | T2c | N0 | 2 | 3+4 | Negative |
| PrRP26 | Male | 70 | High | 2 | T1c | 4 | 3+5 | 33% | 5.35 | MRI, Bone scan | T3a | N0 | 2 | 3+4 (+5) | Negative |
| PrRP27 | Male | 53 | High | 4 | T1c | 1 | 6 | 17% | 26.23 | None | T2c | N0 | 2 | 3+4 | Negative |
| PrRP28 | Male | 66 | High | 5 | T1c | 1 | 6 | 8% | 65.25 | MRI, Bone scan | T2c | N0 | 2 | 3+4 | Negative |
| PrRP29 | Male | 68 | High | 5 | T2a | 4 | 4+4 | 33% | 1.27 | MRI, CT, Bone scan | T2a | N0 | 4 | 4+4 | Negative |
| PrRP30 | Male | 64 | High | 6 | T1c | 4 | 4+4 | 42% | 2.55 | MRI | T2a | N0 | 3 | 4+3 | Negative |
| PrRP31 | Male | 54 | High | 6 | T1c | 4 | 4+4 | 42% | 5.02 | MRI, CT, Bone scan | T2c | N0 | 5 | 4+5 | Negative |
| PrRP32 | Male | 72 | High | 7 | T3b | 5 | 4+5 | 42% | 3.67 | MRI, CT, Bone scan | T3b | N0 | 5 | 4+5 | Positive |
| PrRP33 | Male | 69 | High | 8 | T2b | 3 | 4+3 | 83% | 25.31 | MRI, CT, Bone scan | T3b | N1 | 5 | 5+4 | Positive |
| PrRP34 | Male | 50 | High | 8 | T2b | 4 | 4+4 | 92% | 11.27 | MRI, CT, Bone scan | T3b | N1 | 5 | 4+5 | Positive |

Supplementary Table S5. Droplet digital PCR primers used in this study.

| Gene | Forward 5'→3' | Reverse 5'→3' | Probe 5'→3' |
|----------------------------|---------------------------------------|--------------------------------------|--|
| TMPRSS2 | TCA ATG AGA AGC ACC TTG GC | CCC AAC CCA GGC ATG ATG | ACC CGG AAA CC AGC AGA GCT |
| FAT1 | ATC AGC AGA GTC AAT CAG TGA G | GAT CCT TAT GCC ATC ACC GT | TCT TGT CAG CAG CGT TCC CGG |
| KLK2 | GTC TTC AGG CTC AAA CAG GT | GCT GTG TAC AGT CAT GGA TGG | TGG CTA TTC TTC TTT AGG CAA TGG GCA |
| STEAP2 | TCT CCA AAC TTC TTC CTC ATT CC | CAT GTT GCC TAC AGC CTC T | ACA TGG CTT ATC AGC AGG TTC ATG CA |
| KLK3 | GTG TGC TGG ACG CTG GA | GTG ATA CCT TGA AGC ACA CCA TTA C | AAA GCA CCT GCT CGG GTG ATT CT |
| HOXB13 | CTG TAC GGA ATG CGT TTC TTG | CAG CCA GAT GTG TTG CCA | CAG CAT TTG CAG ACT CCA GCG G |
| AGR2 | CAA TTC AGT CTT CAG CAA CTT GAG | CTG ACA GTT AGA GCC GAT ATC AC | ATG CTT ACG AAC CTG CAG ATA CAG CTC |
| FOLH1 | TGT TCC AAA GCT CCT CAC AA | CAA TGT GAT AGG TAC TCT CAG AGG | ATG AAC AAC AGC TGC TCC ACT CTG A |
| AR-V7 | CTT GTC GTC TTC GGA AAT GTT ATG | CTT TCT TCA GGG TCT GGT CAT T | AAG CAG GGA TGA CTC TGG GAG AAA |
| TMPRSS2:ERG (ddPCR) | GAG TAG GCG CGA GCT AAG | CGT AGG CAC ACT CAA ACA AC | CGG CAG GAA GCC TTA TCA GTT GTG A |
| TMPRSS:ERG (nested STA) | GAG TAG GCG CGA GCT AAG | GGC TCA TCT TGG AAG TCT GT | None |