# Supplemental Materials

***Molecular Biology of the Cell***

Leshner et al.

## Supplemental Table S1. Candidate genes positioned in human prostate tissues

| Gene | Chromosomal location | BAC | Number of nuclei in PND |
|---|---|---|---|
| *AR* | Xq12 | RP11-479J1 | 752 |
| *BCL2* | 18q21.33 | RP11-299P2 | 489 |
| *BRCA2* | 13q13.1 | RP11-37E23 | 518 |
| *CCND1* | 11q13 | RP11-300I6 | 508 |
| *DCN* | 12q21.33 | RP11-644B23 | 461 |
| *EGFR* | 7p11.2 | RP11-815K24 | 612 |
| *ERG* | 21q22.2 | RP11-95I21 | 646 |
| *ESR2* | 14q23.2-q23.3 | RP11-14C21 | 641 |
| *ETV1* | 7p21.2 | RP11-692L4 | 569 |
| *FGFR1* | 8p11.23-p11.22 | RP11-100B16 | 716 |
| *FGFR2* | 10q26.13 | RP11-300A10 | 740 |
| *FLI1* | 11q24.3 | RP11-744N12 | 1047 |
| *FOXA1* | 14q21.1 | RP11-606C5 | 411 |
| *FUT4* | 11q21 | RP11-42H7 | 449 |
| *GREB1* | 2p25.1 | RP11-50E1 | 523 |
| *HOXA9* | 7p15.2 | RP11-1132K14 | 505 |
| *KLK3 (PSA)* | 19q13.33 | RP11-795B6 | 541 |
| *LMNA* | 1q22 | RP11-35P22 | 481 |
| *MATR3* | 5q31.2 | RP11-815G18 | 488 |
| *MMP1* | 11q22.2 | RP11-686G6 | 303 |
| *MMP14* | 14q11.2 | RP11-885L10 | 324 |
| *MMP2* | 16q12.2 | RP11-90H1 | 791 |
| *MMP9* | 20q13.12 | RP11-465L10 | 1128 |
| *NPM1* | 5q35.1 | RP11-117L6 | 311 |
| *NUMA1* | 11q13.4 | RP11-449G14 | 514 |
| *PADI4/6* | 1p36.13 | RP11-1119C2 | 647 |
| *PTEN* | 10q23.31 | RP11-383D9 | 391 |
| *RAF1* | 3p25.2 | RP11-148M13 | 1027 |
| *SATB1* | 3p24.3 | RP11-1021J5 | 610 |
| *SERPINB2* | 18q21.33 | RP11-75O12 | 380 |
| *SLC45A3* | 1q32.1 | RP11-6B6 | 727 |
| *SP100* | 2q37.1 | RP11-727M18 | 615 |
| *SPDEF* | 6p21.31 | RP11-375E1 | 517 |
| *TGFB1* | 19q13.2 | RP11-1012F23 | 433 |
| *THBS1* | 15q14 | RP11-590D2 | 630 |
| *TIMP2* | 17q25.3 | RP11-72M9 | 697 |
| *TIMP3* | 22q12.3 | RP11-641L14 | 760 |
| *TMPRSS2* | 21q22.3 | RP11-671L10 | 1260 |
| *VEGFA* | 6p21.1 | RP11-1152J4 | 1375 |
| *VIM* | 10p13 | RP11-1122I7 | 631 |

BAC, Bacterial artificial chromosome. Several BACs span functionally related gene clusters: The *HOXA9* BAC spans part of the *HOXA* gene cluster, including *HOXA3-7, 9-11* and *13*. The *KLK3* BAC spans the *KLK* gene cluster (*KLK1-9*, *KLK15*). The *MMP1* BAC covers part of a *MMP* gene cluster (*MMP10, MMP1*, *MMP3* and *MMP12*). The *PAD14/6* BAC spans both *PADI4* and *PADI6*. PND, pooled normal distribution.

**Supplemental Table S2. Characterization of human prostate tissues**

| Sample code | Pathology | Age (years) | Vol of cancer (cc) | Weight of prostate (g) | Stage | Gleason Score | TNM | Metastatic? | Source | Source specimen ID |
|---|---|---|---|---|---|---|---|---|---|---|
| C1 | Adenocarcinoma | 40's | 1 | 36 | | 7 | T2N0 | | L.D.T. | UW1 |
| C2 | Adenocarcinoma | 60's | 1.6 | 47 | | 9 | T2N0 | | L.D.T. | UW2 |
| C3 | Adenocarcinoma | | | | | | | | L.D.T. | UW3 |
| C4 | Adenocarcinoma | 60's | 0.6 | 40 | | 7 | T2N0 | | L.D.T. | UW4 |
| C5 | Adenocarcinoma | 50's | 0.5 | 30 | | 6 | T2N0 | | L.D.T. | UW5 |
| C6 | Adenocarcinoma | 73 | | | II | 6 (3+3) | T2N0M0 | No | Biomax | HuCAT371 |
| C7 | Adenocarcinoma | 58 | | | II | 6 (3+3) | T2N0M0 | No | Biomax | TMA: PR632, core F3 |
| C8 | Adenocarcinoma | 73 | | | II | 5 (2+3) | T2N0M0 | No | Biomax | TMA: PR632, core F5 |
| C9 | Adenocarcinoma | 62 | | | II | 7 (3+4) | T2N0M0 | No | Biomax | TMA: PR632, core G2 |
| C10 | Adenocarcinoma | 71 | | | II | 8 (4+4) | T2N0M0 | No | Biomax | TMA: PR632, core G8 |
| C11 | Adenocarcinoma | 62 | | | IV | 7 (3+4) | T3N1M1b | Yes | Biomax | TMA: T196, core A6 |
| C12 | Adenocarcinoma | 69 | | | II | 6 (3+3) | T2N0M0 | No | Biomax | TMA: T196, core B6 |
| C13 | Adenocarcinoma | 60 | | | IV | 8 (3+5) | T3N1M1b | Yes | Biomax | TMA: T196, core D2 |
| C14 | Adenocarcinoma | 64 | | | IV | 7 (3+4) | T3N0M1b | Yes | Biomax | TMA: T196, core D4 |
| C15 | Adenocarcinoma | 60's | 3.5 | 36 | | 7 | T3bN0 | | L.D.T. | UW6 |
| C16 | Adenocarcinoma | | | | | | | | L.D.T. | UW7 |
| C17 | Adenocarcinoma | | | | | | | | L.D.T. | UW8 |
| C18 | Adenocarcinoma | 50's | 0.6 | 26 | | 7 | T2N0 | | L.D.T. | UW9 |
| C19 | Adenocarcinoma | 60's | 2.5 | 30 | | 7 | T3N0 | | L.D.T. | UW10 |
| C20 | Adenocarcinoma | 60's | 1 | 33 | | 7 | T2N0 | | L.D.T. | UW11 |
| C21 | Adenocarcinoma | 50's | 4.5 | 38 | | 7 | T2N0 | | L.D.T. | UW12 |
| C22 | Adenocarcinoma | 50's | 2 | 40 | | 7 | T2N0 | | L.D.T. | UW13 |
| C23 | Adenocarcinoma | 50's | 1.2 | 32 | | 6 | T2N0 | | L.D.T. | UW14 |
| C24 | Adenocarcinoma | 50's | 3 | 31 | | 6 | T2N0 | | L.D.T. | UW15 |
| C25 | Adenocarcinoma | 40′s | 3.5 | 37 | | 7 | T2N0 | | L.D.T. | UW16 |
| C26 | Adenocarcinoma | | | | | | | | L.D.T. | UW17 |
| C27 | Adenocarcinoma | 75 | | | | | | | Biomax | HuCAT376 |
| C28 | Adenocarcinoma | 50′s | 5.5 | 36 | | 7 | T3N0 | | L.D.T. | UW18 |
| C29 | Adenocarcinoma | 72 | | | | | | | Biomax | HuCAT367 |
| C30 | Adenocarcinoma | | | | | | | | L.D.T. | UW19 |
| B1 | Hyperplasia; same individual as C3 | 50's | | | | | | | L.D.T. | UW20 |

| | | | | |
|---|---|---|---|---|
| B2 | Hyperplasia | 70 | Biomax | TMA: PR632, core D6 |
| B3 | Hyperplasia | 76 | Biomax | TMA: PR632, cores D9, D8, D7 # |
| B4 | Hyperplasia | 78 | Biomax | TMA: PR632, cores E7, E8 # |
| B5 | Hyperplasia | 74 | Biomax | TMA: PR632, core E2 |
| N1 | Normal | 28 | Biomax | TMA: PR632, core A9 |
| N2 | Normal | 35 | Biomax | TMA: PR632, core B2 |
| N3 | Normal | 35 | Biomax | TMA: PR632, core C6 |
| N4 | Normal | 38 | Biomax | TMA: PR632, core C7 |
| N5 | Normal | 28 | Biomax | HuFPT141 |
| N6 | Normal | 43/31[a] | Biomax | HuFPT142 |
| N7 | Normal | 48/35[a] | Biomax | HuFPT143 |
| N8 | Normal | | L.D.T. | UW21 |
| N9 | Normal | | L.D.T. | UW22 |
| N10 | Normal | | L.D.T. | UW23 |
| N11 | Normal | 54 | Imgenex | IMH-1035 |
| N12 | NAT (of C1) | 40's | L.D.T. | UW1 |
| N13 | NAT (of C2) | 60's | L.D.T. | UW2 |
| N14 | NAT (of C4) | 60's | L.D.T. | UW4 |
| N15 | NAT (of C5) | 50's | L.D.T. | UW5 |
| N16 | NAT (of C15) | 60's | L.D.T. | UW6 |
| N17 | NAT (of C16) | | L.D.T. | UW7 |
| N18 | NAT (of C17) | | L.D.T. | UW8 |
| N19 | NAT (of C18) | 50's | L.D.T. | UW9 |
| N20 | NAT (of C19) | 60's | L.D.T. | UW10 |
| N21 | NAT (of C20) | 60's | L.D.T. | UW11 |
| N22 | NAT (of C21) | 50's | L.D.T. | UW12 |
| N23 | NAT (of C22) | 50's | L.D.T. | UW13 |
| N24 | NAT (of C23) | 50's | L.D.T. | UW14 |
| N25 | NAT (of C24) | 50's | L.D.T. | UW15 |
| N26 | NAT (of C25) | 40’s | L.D.T. | UW16 |
| N27 | NAT (of C26) | | L.D.T. | UW17 |
| N28 | NAT (of C28) | 50s | L.D.T. | UW18 |
| N29 | NAT (of C30) | | L.D.T. | UW19 |

Biomax, US Biomax Inc; Imgenex, Imgenex corporation; L.D.T., Dr. Lawrence True; NAT, Normal Adjacent to Tumor; Normal, Normal tissue taken from cancer free prostates; TMA, Tissue microarray; Vol, volume; #, Multiple cores of the same tissue were used for analysis; [a]The individual used for these two

catalogue numbers changed with subsequent purchases, consequently it is not always tissue from the same individual used between the different genes for these sample codes.

**Supplemental Table S3. Repositioning events are not due to variations in copy number**

| Cancer | *FLI1* | *MMP9* | *MMP2* |
|---|---|---|---|
| C1 | - 58.6% | | |
| C2 | - 64.8% | | |
| C3 | - 49.2% | - 49.0% | - 58.3% |
| C4 | - 66.4% | - 50.0% | - 87.2% |
| C5 | - 77.8% | - 58.1% | - 52.7% |
| C6 | | | - 87.5% |
| C7 | + 40.8% | ++ 69.6% | + 40.3% |
| C8 | | | |
| C9 | | | |
| C10 | - 55.4% | | |
| C11 | | | |
| C12 | ++ 57.3% | + 45.1% | + 31.6% |
| C13 | + 31.9% | | |
| C14 | - 47.4% | | - 50.9% |
| C15 | | - 56.4% | - 60.6% |
| C16 | | - 50.0% | - 64.4% |
| C17 | | - 46.0% | - 61.5% |
| C18 | | + 48.4% | |
| C19 | | ++ 60.6% | |
| C20 | | - 40.3% | |
| C21 | | - 44.9% | |
| C22 | | - 46.0% | |
| C23 | | | - 62.6% |
| C24 | | | - 74.1% |

| Non-cancer | *FLI1* | *MMP9* | *MMP2* |
|---|---|---|---|
| N1 | | | |
| N2 | | | |
| N3 | | | |
| N4 | | | |
| N5 | - 40.8% | | |
| N7 | | | - 55.8% |
| N8 | | | |
| N12 | - 54.0% | | |
| N13 | - 64.5% | | |
| N14 | | - 63.6% | |
| N15 | - 52.4% | - 55.2% | - 48.5% |
| N16 | | - 59.2% | - 73.2% |
| N19 | | | |
| N20 | | | |
| N21 | | | - 66.3% |
| N22 | | | - 61.4% |
| N23 | | - 47.3% | |
| N24 | | | - 52.5% |
| N25 | | | - 64.2% |
| PND | | | - 55.1% |
| B1 | - 58.6% | - 48.0% | - 56.2% |
| B2 | | | |
| B3 | | | |
| B4 | | | |
| B5 | | | |

A comparison of the incidence of gene repositioning with the number of FISH signals detected for a gene in a given tissue. Black box: the gene was reposition compared to the pooled normal distribution ($P < 0.01$; KS test); grey box: no repositioning compared to the pooled normal ($P > 0.01$); white box: gene positioning not determined. The copy number of a gene was diploid unless otherwise stated. Copy number changes are denoted by: +, 20-49.9% of nuclei had ≥3 gene FISH signals; ++, ≥ 50% of nuclei had ≥3 gene signal; -, ≥40% of nuclei had a single FISH signal. The percentage of nuclei with the corresponding number of FISH signals (+/++/-) is also displayed. PND, Pooled normal distribution. N1-8, Normal tissue, from cancer free prostates; N12-N25 = NAT; B1-5, hyperplasia. Tissues are color-coded to indicate tissues from the same individual (e.g. N12 is the NAT from cancer C1). With the exception of C3/B1, analysis for these pairs of normal and cancer tissues was performed on the same slide (same 4-5µm tissue section), for a given gene.

## Supplemental Table S4. Gene Multiplexing

| **Gene** | *MMP9* | *MMP2* |
|---|---|---|
| *FLI1* | 100.0% (11/11) | 90.9% (10/11) |
| *MMP9* | | 71.4% (10/14) |

The percentage (and number) of cancers where at least one of the indicated pair of genes repositioned, compared to the pooled normal distribution ($P < 0.01$, KS test). Only tissues where both genes of the pair have been positioned are included.

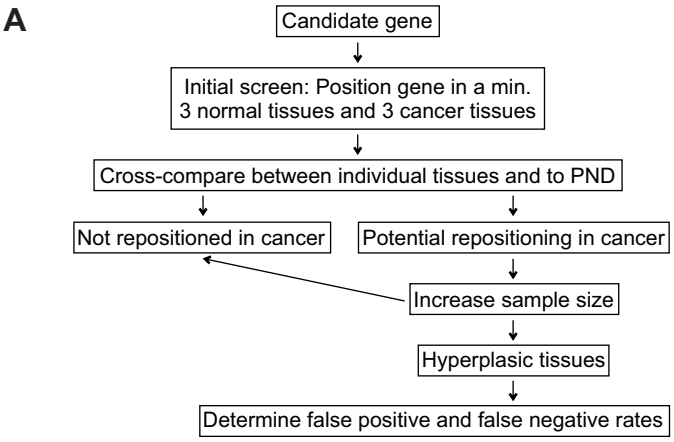
## Figure legends

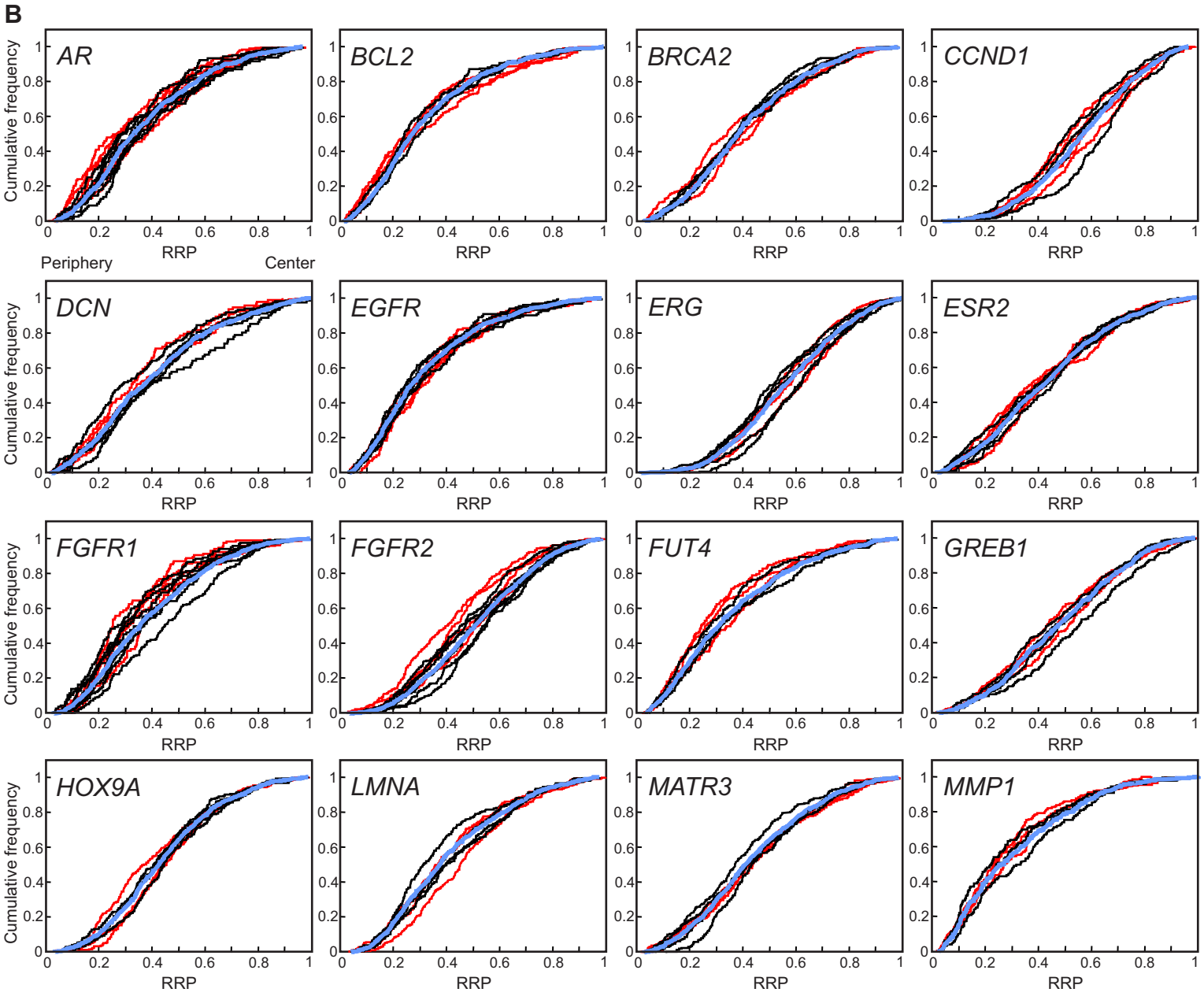
## Supplemental Figure legends

**Supplemental Figure S1.** The spatial reorganization of the genome in prostate cancer is conserved for most genes. (A) Outline of the screening strategy used to identify genes that reposition in prostate cancer. PND: Pooled normal distribution. (B) Cumulative RRDs for the indicated genes in prostate cancer (red), normal tissues (black) and the pooled normal distribution (blue). RRP, relative radial position.

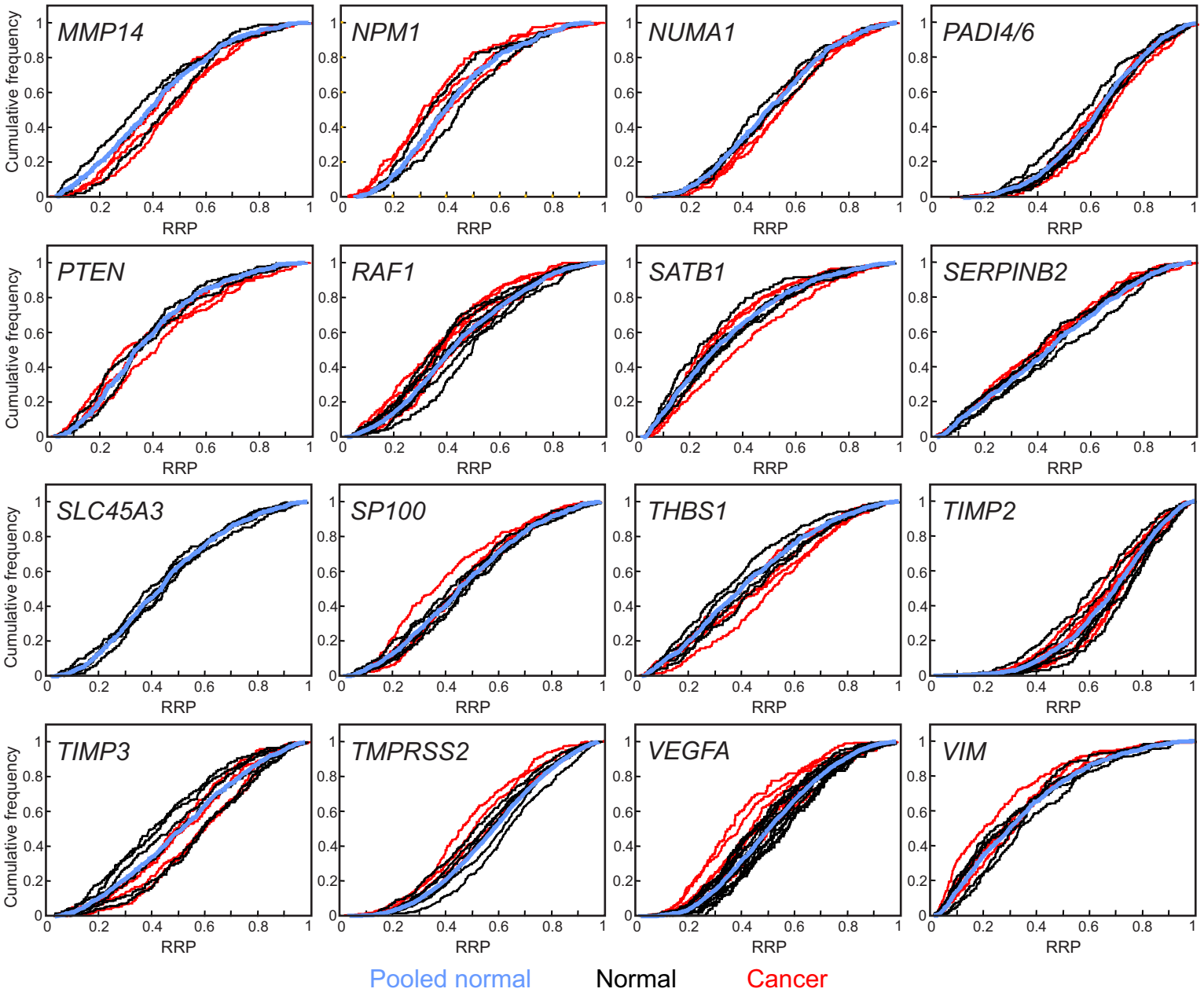
**Supplemental Figure S2.** Loci-specific genome reorganization. Heat maps representing the pair-wise statistical comparisons of positioning patterns of indicated genes between tissues, using the two-sample 1D KS test. Most genes display a limited repositioning when cancer tissues (C1-C30) are compared to normal tissues (N2-N29). However, there are some genes where the difference in spatial positioning between normal and cancer tissues is a reflection of the highly variable positioning between normal tissues (e.g. *TIMP3*). Black or white asterisks indicate a cross-comparison between a normal and cancer specimen from the same individual.

# Supplemental Figure S1

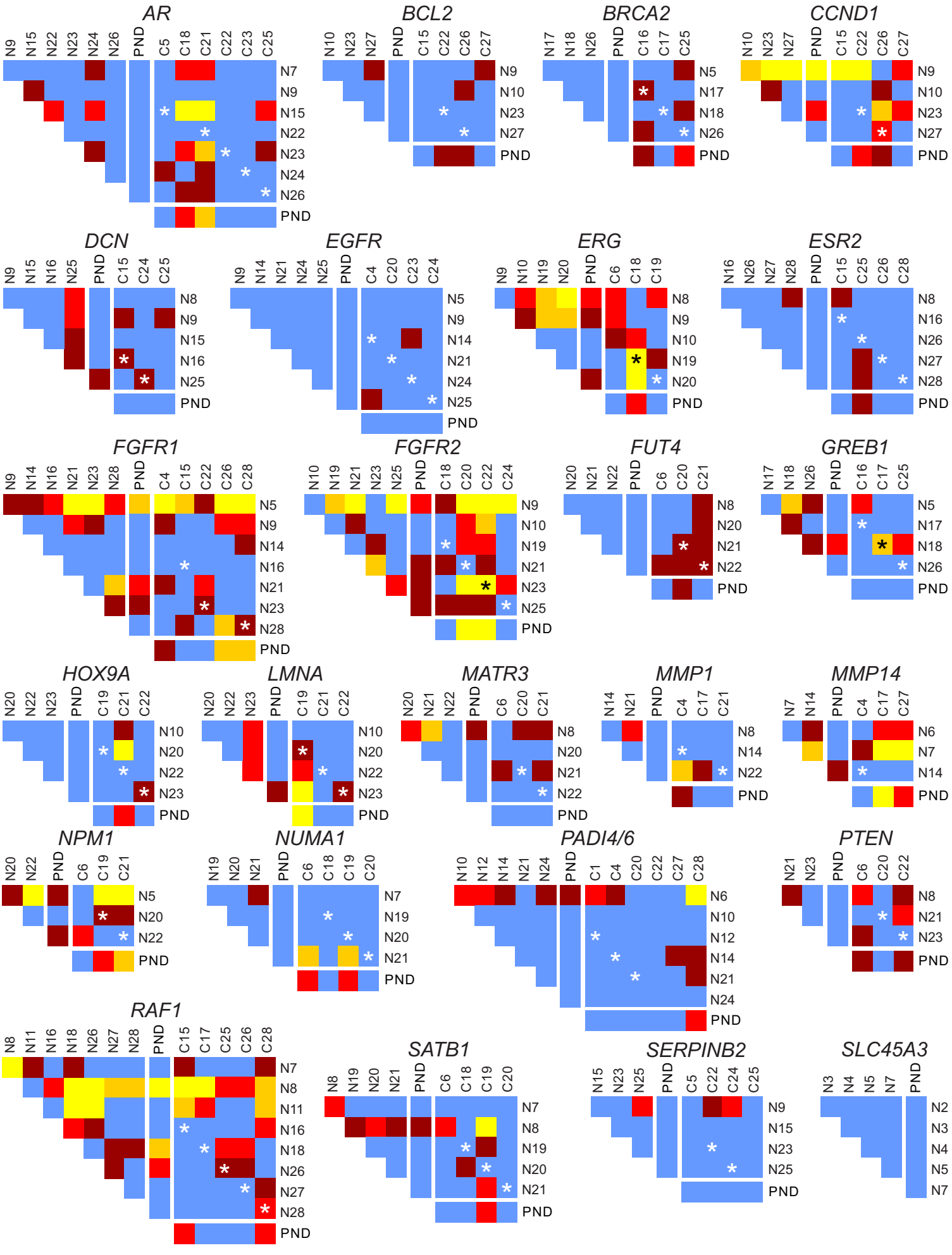


# Supplementary Figure S1 Continued



# Supplemental Figure S2



# Supplementary Figure S2 continued