WD repeat domain 5 promotes chemoresistance and Programmed Death-Ligand 1 expression in prostate cancer

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Figure S1. An integrated analysis of the histone methylation modification regulators expression in TCGA data. A. A heat map showing the expression of 25 histone methylation modifiers in adjacent normal (left) and PCa (right) tissues. B. 9 histone methylation modifiers were overexpressed in PCa, compared with adjacent normal tissues. C, D. Kaplan-Meier curves for Progression free survival of PCa patients with high vs. low expression of EZH2 (C) or WDR5 (D) in TCGA Cohort. *p < 0.05, **p < 0.01
Figure S2. The expression of WDR5 in PCa tissues with or without LN metastasis in Cohort 1 (A) and TCGA cohort (B).

Figure S3. Knockdown WDR5 or OICR-9429 increase the apoptotic proportion in PCa cells. A, B. Representative images (A) and quantification (B) of cell apoptosis in DU145 and PC-3 cells transfected with WDR5 or Ctrl siRNA, analyzed by flow cytometry analysis. C, D. Representative images (C) and quantification (D) of cell apoptosis in DU145 and PC-3 cells treated with OICR-9429 or DMSO, analyzed by flow cytometry analysis. The error bars represent standard deviations of three independent experiments. *p < 0.05, **p < 0.01
Figure S4. Knockdown WDR5 increases the chemosensitivity to cisplatin but not docetaxel in PCa cells. A-D. The cell viability of knockdown WDR5 by siRNA combined with docetaxel (A-B) or cisplatin (C-D) in DU145 and PC-3 cells by MTT assay. E. The quantification of IC\textsubscript{50} of cisplatin in DU145 and PC-3 cells transfected with WDR5 or Ctrl siRNA. The error bars represent standard deviations of three independent experiments. ** \( p < 0.01 \).
Figure S5. A. The protein level of WDR5 in WPMY-1, PC-3 and DU-145 cells. B. Knockdown of WDR5 reduced OICR-9429 sensitivity in DU145 and PC-3 cells.
Figure S6. OICR-9429 inhibits proliferation of PCa cells in vitro. **A.** The images of formation assay of DU145 and PC-3 cells treated with OICR-9429 or DMSO. **B.** Representative images of cell cycle in DU145 and PC-3 cells treated with OICR-9429 or DMSO, analyzed by flow cytometry analysis. **C.** The images of EdU assay of DU145 and PC-3 transfected with WDR5 or Ctrl siRNA. Scale bars: red, 50 μm.

Figure S7. Synergistic effects of OICR-9429 and Cisplatin against PCa cells. DU145 and PC-3 cells treated with OICR-9429 and Cisplatin alone or in combination at indicated concentrations for 48 h. Cell viabilities were measured and normalized to DMSO control values (top). CI was calculated by using CalcuSyn software (bottom). CI less than 0.9 demonstrates synergy between two drugs.
Figure S8. OICR-9429 enhances the efficacy of cisplatin in PCa cells in vitro and in vivo.

A. Representative images of cell apoptosis in the indicated cells treated with OICR-9429, Cisplatin or a combination of both for 48 h. B. Representative images of Comet assay in PCa cells treated with OICR-9429, Cisplatin or a combination of both for 48 h. C. Images of tumors...
in indicated groups. D. Representative images of Ki67 expression in tumors of indicated groups, examined by IHC. E. Images of apoptosis in tumors of indicated groups, detected by TUNEL assay. The scale bars in IHC and TUNEL images represent 50 μm.

Figure S9. H&E staining of kidney, liver, lung, and heart from the mice in indicated groups. Scale bars: red, 50 μm.
Figure S10. The target genes of WDR5 are identified in PCa cells. A, B. Validation of candidate down-regulated genes by qRT-PCR (A) and Western blotting (B) in DU145 and PC-3. GAPDH and H3 were used as internal controls. C-E. ChIP analysis of IgG, WDR5, H3K4me3, and RNA polymerase-II status of candidate WDR5 target genes in DU145 and PC-3 cells,
transfected with WDR5 or Ctrl siRNA. The values are normalized to input and presented as the means ± SD. The error bars represent standard deviations of three independent experiments. *p < 0.05, **p < 0.01.

Figure S11. Pearson correlations between the expression of WDR5 and CDK1, PLK1, CCNB1, AURKA, E2F1, TopBP1, MCM2, BIRC5 and XRCC2 in TCGA cohort.
Figure S12. The WDR5 target genes are regulated by MLL1 and c-Myc. A, B. MLL1 silencing down-regulated the mRNA level of WDR5 target genes in DU145 (A) and PC-3 (B) cells. C, D. c-Myc silencing down-regulated the mRNA level of WDR5 target genes in DU145 (C) and PC-3 (D) cells. The values are normalized to input and presented as the means ± SD. The error bars represent standard deviations of three independent experiments. *p < 0.05, **p < 0.01.

Figure S13. OICR-9429 did not change the expression of both c-Myc and MLL1 in DU145 and PC-3 cells.
Figure S14. Both OICR-9429 and WDR5 knockdown reduced the recruitment of c-Myc on the promoter of WDR5 target genes. A. ChIP analysis of IgG, c-Myc status of candidate WDR5 target genes in Du145 and PC-3 cells, treated with OICR-9429 or DMSO. B. ChIP analysis of IgG, c-Myc status of candidate WDR5 target genes in DU145 and PC-3 cells, treated with WDR5 siRNA or control. The values are normalized to input and presented as the means ± SD. *p < 0.05; **p < 0.01.
Figure 15. Knockdown of MLL1 (A), but not c-Myc (B) decreased the mRNA level of IFNy-induced PD-L1 in DU145 and PC-3 cells. The values are normalized to input and presented as the means ± SD. *p < 0.05; **p < 0.01.

Figure S16. The representative images of different WDR5 expression in PCa by IHC. The level of WDR5 expression was graded as intensity of IHC staining: 0, absent staining; 1, weak staining; 2, moderate staining; 3, strong staining.
## Tables

### Table S1. Univariate and multivariate analyses of PFS in patients of Cohort 1 and TCGA

| Variables            | Cohort 1                  | TCGA                     |
|----------------------|----------------------------|--------------------------|
|                      | Univariate | Multivariate | Univariate | Multivariate | Univariate | Multivariate |
|                      | HR (95%CI) | p-value    | HR (95%CI) | p-value    | HR (95%CI) | p-value    |
| Age (y)              |            |             |            |             |            |             |
| ≥60/60               | 0.87       | 0.690       | -          | -          | 0.88       | 0.681       |
|                      | 0.43-1.75  |             | 0.46-1.65  |             |             |             |
| Gleason score        | 2.34       | 0.010       | 1.19       | 0.659      | 2.49       | 0.017       |
| 7(4+3)-10/6-7(3+4)   | 1.22-4.49  |             | 0.55-2.56  |             | 1.18-5.27  |             |
|                      |             |             | 0.65-3.38  |             |             |             |
| Tumor stage          | 2.02       | 0.030       | 1.12       | 0.744      | 2.47       | 0.019       |
|                      | 1.07-3.81  |             | 0.57-2.21  |             | 1.16-5.22  |             |
|                      |             |             | 0.83-4.33  |             |             |             |
| Nodal metastasis     | 4.28       | <0.001      | 3.37       | 0.003      | 2.74       | 0.005       |
|                      | 1.29-8.33  |             | 1.52-7.46  |             | 1.35-5.57  |             |
|                      | 0.32-18.54 |             | 0.61-14.05 |             | 0.83-3.77  |             |
| WDR5                 | 3.26       | 0.010       | 2.81       | 0.006      | 3.00       | 0.002       |
| high/low             | 1.59-6.69  |             | 1.34-5.91  |             | 1.51-5.95  |             |
|                      |             |             | 1.36-5.49  |             |             |             |

### Table S2. Univariate and multivariate analyses of OS in patients of Cohort 1 and Cohort 2

| Variables            | Cohort 1                  | Cohort 2                  |
|----------------------|----------------------------|----------------------------|
|                      | Univariate | Multivariate | Univariate | Multivariate | Univariate | Multivariate |
|                      | HR (95%CI) | p-value    | HR (95%CI) | p-value    | HR (95%CI) | p-value    |
| Age (y)              |            |             |            |             |            |             |
| ≥60/60               | 1.39       | 0.420       | -          | -          | 2.50       | 0.369       |
|                      | 0.62-3.10  |             | 0.34-18.54 |             |             |             |
| Gleason score        | 2.50       | 0.010       | 1.29       | 0.535      | 8.35       | 0.001       |
| 7(4+3)-10/6-7(3+4)   | 1.25-5.01  |             | 0.58-2.84  |             | 2.49-28.09 |             |
|                      |             |             | 1.33-14.05 |             |             |             |
| Tumor stage          | 2.09       | 0.032       | 1.26       | 0.541      | 6.39       | <0.001      |
|                      | 1.06-4.09  |             | 0.61-2.61  |             | 2.73-14.97 |             |
|                      |             |             | 1.86-10.85 |             |             |             |
| Nodal metastasis     | 4.28       | <0.001      | 3.28       | 0.004      | 8.42       | 0.001       |
|                      | 2.11-8.66  |             | 1.45-7.40  |             | 2.40-29.53 |             |
|                      | 0.30       | 0.004       | 2.41       | 0.029      | 3.41       | 0.003       |
|                      | 1.41-6.40  |             | 1.09-5.29  |             | 1.52-7.64  |             |
|                      |             |             | 0.69-4.55  |             |             |             |
Table S3. The clinicopathological characteristics of patients in present study

| Variables              | Cohort 1 | Cohort 2 | TCGA |
|------------------------|----------|----------|------|
|                        | Number of cases (%) | Number of cases (%) | Number of cases (%) |
| Age(y)                 |          |          |      |
| <60                    | 32 (23.5%) | 12 (9.5%) | 113 (44.3%) |
| ≥60                    | 104 (76.5%) | 114 (90.5%) | 142 (55.7%) |
| Gleason score          |          |          |      |
| 6-7(3+4)               | 73 (53.7%) | 62 (49.2%) | 110 (43.1%) |
| 7(4+3)-10              | 63 (46.3%) | 64 (50.8%) | 145 (56.9%) |
| T stage                |          |          |      |
| T2                     | 78 (57.4%) | 90 (71.4%) | 105 (41.2%) |
| T3                     | 36 (26.5%) | 30 (23.8%) | 146 (57.3%) |
| T4                     | 22 (16.1%) | 6 (4.8%) | 4 (1.5%) |
| N status               |          |          |      |
| N0                     | 118 (86.8%) | 122 (96.8%) | 222 (87.1%) |
| N1                     | 18 (13.2%) | 4 (3.2%) | 33 (12.9%) |
| WDR5                   |          |          |      |
| Low                    | 67 (49.3%) | 88 (69.8%) | 141 (55.3%) |
| High                   | 69 (50.7%) | 38 (30.2%) | 114 (44.7%) |

Patients without available clinical data, including age, Gleason score, T stage and N status, were excluded for the univariate and multivariate analyses in TCGA.

Table S4. The siRNA and shRNA sequences of WDR5 and negative control are listed as follows.

| Primer Name     | Sequence 5’-3’          |
|-----------------|-------------------------|
| WDR5-si1-sence  | GCUCAGAGGAUAACCUUGUTT   |
| WDR5-si1-antisence | ACAAGGUUAUCCUCUGAGCTT |
| WDR5-si2-sence  | CCCAGUCCAACCUUAUUGUTT   |
| WDR5-si2-antisence | ACAAAUAGGUUGGACUGGTT   |
| MLL1-si1-sence  | GCACUGUUAACAUCUUCACUUTT |
| MLL1-si1-antisence | AAGUGGAAUGUUAACAGUGCTT |
| MLL1-si2-sence  | CCACAGCAGAACCAGAAGUUAUTT|
| MLL1-si2-antisence | UACUCUCUGGUUCUGGAUGGGTT|
| c-Myc-si1-sence  | CCAGGUAGUUAACCUAAATT    |
| c-Myc-si1-antisence | UCCUGAGACAGGAUCGCAAAATT |
| c-Myc-si2-sence  | CCAGGUAGUUAACCUAAATT    |
| c-Myc-si2-antisence | UCCCCGACACGUGUCAGGATT  |
| Negative control-sence | UCCUCGAACGUGUCAGGUTT |
| Negative control-antisence | ACGUGACACGCUUGAGAATT |

Table S5. The primers used in real time qPCR are listed as follows.

| Primer Name   | Sequence 5’-3’           |
|---------------|---------------------------|
| WDR5 Forward  | AATTCAGCCCGAATGGAGAGT     |
| Primer Name      | Sequence 5’-3’                  |
|-----------------|---------------------------------|
| WDR5 Reverse    | AGGCTACATCGGATATTCGCCAG         |
| AURKA Forward   | CAAATGCCCCTGTCTTACTGTC          |
| AURKA Reverse   | ATGGAGCATGTACTGACCACC           |
| BIRC5 Forward   | CCACTGAGAACGAGCCAGACCTT         |
| BIRC5 Reverse   | GTATTACAGGGCGTAAGCCACCG         |
| CCNB1 Forward   | TAAGGCGAAGATCAACATGG            |
| CCNB1 Reverse   | TTACCAATGTCCCCAAAGAGC           |
| CDK1 Forward    | GGAACCAGGAAGCCTAGCATC           |
| CDK1 Reverse    | GGATGATTCACTGCCATTTTGCC         |
| E2F1 Forward    | GGACCTGGAAACTGACCATCAG          |
| E2F1 Reverse    | CAGTGAGGTCTCATAGGCTGAC          |
| PLK1 Forward    | CGGCCAACCATTAACGAGCT            |
| PLK1 Reverse    | AACTTGTTGGAAATGGTCAGGC          |
| MCM2 Forward    | TGCCAGCATTTGCTCTTCCATC          |
| MCM2 Reverse    | AAACTGCGACTTCGCTGTGCCA          |
| TopBP1 Forward  | TGTGACCCTTTTAGTGCGGT            |
| TopBP1 Reverse  | CTCTTGGGACACATCGCTGG            |
| XRCC2 Forward   | TCTGTGGTTCGTAAGGAGTTCACC       |
| XRCC2 Reverse   | CATCGTGCTGTGTTAGGTAAGGCG        |
| MLL Forward     | TAGTGAGCCCAAGAAAAAGCA           |
| MLL Reverse     | TGGAGAGAGTGCTGGAGATGT           |
| c-Myc Forward   | CCACACATCAGCACAACACTACG         |
| c-Myc Reverse   | AAGCTCCGTTTTTAGCTGCTTTC         |
| GPADH Forward   | CAAGGCTGAGAAGGGAAG              |
| GPADH Reverse   | TGAAGACGCCAGTGAGCT              |

**Table S6.** The primers used in ChIP-real time qPCR are listed as follows.
| Primer          | Sequence                  |
|----------------|---------------------------|
| BIRC5-P Reverse| AGCATCACTTGAGTCTGGAG      |
| CDK1-P Forward | AGAAGAACGGAGCGAACAGTA     |
| CDK1-P Reverse | TAGAGCGCGAAAGAAAGAGGA     |
| XRCC2-P Forward| GGCTAACTGTGAGAAGCATGT     |
| XRCC2-P Reverse| TACCCCGAGATAACTTTGCCCA    |
| CD274-P Forward| AAACTGGATTTGCTGCTTTGG     |
| CD274-P Reverse| GGAACAACGCTCCCTACCT       |
| Negative control F | GTAATCAGGAAACTGCATAC   |
| Negative control R | CTCAAGACTCAATAGTGATC   |