Supplemental Data

S1. The heatmap of ID1 and ID3 in 31 patients underwent chemotherapy. Tumor epithelium was laser-capture microdissected from post-treatment radical prostatectomy samples and pre-treatment needle biopsy samples. cDNAs were synthesized as previously described (35). The expression level was expressed as post-treatment vs. pre-treatment. Rows represent genes and columns represent individual patients. Red: upregulation, Green: down-regulation, Black: no change, White: no data. The SAM score and q-value are shown.

S2. 35 patients in the clinical trial were stratified into ID1 Stable (n=20) and ID1 Upregulation (n=15) based on the ID1 mRNA level (measured by qRT-PCR) in matched-cancer epithelial cells post-chemotherapy *vs*. pre-chemotherapy.

S3. A representative western blot of c-myc using whole cell lysates harvested from LNCaP cells treated by docetaxel at the indicated doses for 48 hours.

S4. A representative western blot of c-myc using whole cell lysates harvested from LNCaP cells transduced with an ID1 overexpression vector or control.

S5. Docetaxel-induced G1 reduction in lenti-Ev and lenti-ID1 cells. Cells were treated with solvent or 10 nM docetaxel (DTX) for 48 hours. Flow cytometry was performed. The % of G1 cell population in each cell line was expressed as docetaxel-treated *minus* solvent-treated. Mean (n=3) and SD, * $P < 0.01$, t-test.

S6. ID1 overexpression reduced mitochondria content in LNCaP cells. Left: The ratio of mitochondrial DNA: nuclear DNA was determined by qRT-PCR in LNCaP-Ev and LNCaP-ID1 cells, and normalized to the Ev control. Mean (n=4) and SD, * $P < 0.05$, t-test. Right: Equal numbers of LNCaP, LNCaP-Ev and –ID1 cells were stained with DeepRed 633 and analyzed by flow cytometry to measure mitochondria mass.

S7. A representative of ID1 western blot from C42B cells. Cells were transfected with siRNA oligos for 48 hours, and followed by treatment with 0 or 10 nM of docetaxel for another 48 hours.

S8. Comparison of gene expression between LNCaP-Ev and LNCaP-ID1 cells. RNA was extracted, and gene expressions were measured by qRT-PCR, analyzed using the delta-delta Ct method.

S9. Left: ID1 overexpression down regulated p21. A representative western blot using whole cell lysates. Right: ID1 overexpression upregulated AKT and JNK signaling. LNCaP-Ev and LNCaP-ID1 cells were treated with solvent (S) or docetaxel. Whole cell lysates were used for western blots measuring phosphorylation (p) of AKT and JNK.

S10. The ID1 chemo-enhancement activity is independent of androgen / AR pathway. LNCaP-Ev and LNCaP-ID1 cells were cultured in RPMI with 10% charcoal-striped serum with or without 1nM of synthetic androgen R1881. Top: Proliferation was measured over a period of 3 days. Bottom: Cells were treated with 5 nM docetaxel for 48 hours and the viability was measured.

S11. The pre-treatment ID1 expression was lower in patients who developed biochemical relapse after the chemotherapy-radical prostatectomy. For 31 of the 35 patients involved in the current study, pre-treatment ID1 mRNA level in matched prostate cancer and adjacent benign epithelial cells were measured using qRT-PCR, and stratified by the biochemical relapse status.

S12. An analysis based on the ONCOMINE database suggests that ID1 downregulation is associated with prostate cancer progression. The data were from a study published by Dhanasekaran et al, Nature. 2001 Aug 23; 412 (6849): 822-6.