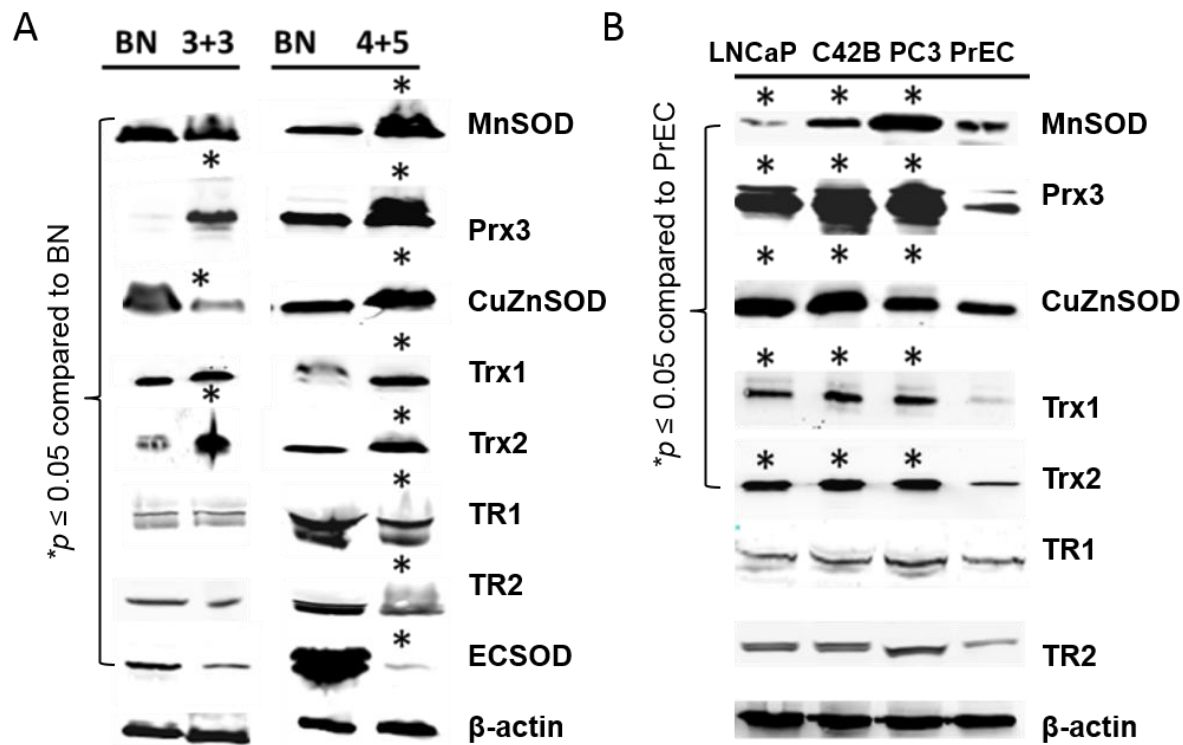
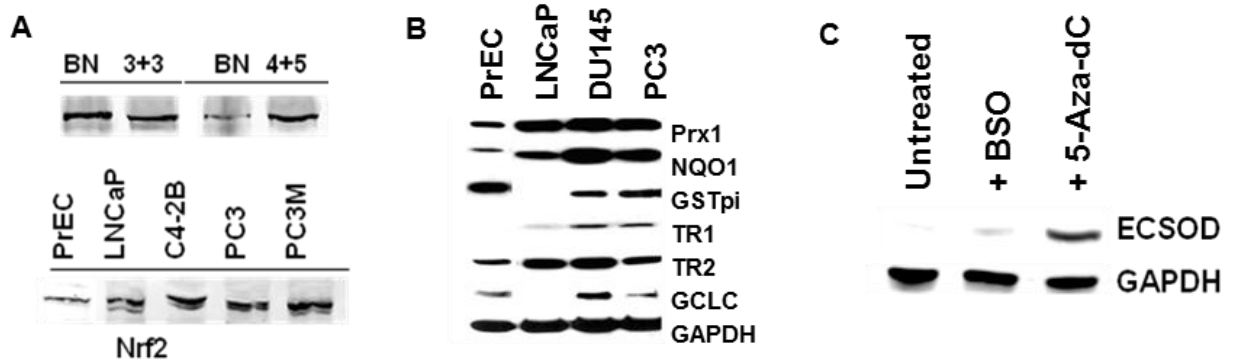


Supplementary Figure 1. GAPDH protein expression levels in human prostate tissues. Western blot analysis of GAPDH protein levels in BN tissues, and intermediate- and high grade-prostate cancer tissues that were used in Trx activity, TR protein expression, and TXNIP protein expression assays (Samples No.1-7). There were no significant differences in GAPDH expression among these tissues. BN is adjacent benign tissue; 4+4, 4+5, and 5+4 are high grade prostate cancer tissues; 3+3 and 3+4 are intermediate grade prostate cancer tissues.



Supplementary Figure 2. Alterations of selected antioxidant protein levels in human prostate tissues and cell lines. Representative western blot analysis of mitochondrial (MnSOD, Prx3), cytoplasmic (Trx1, Trx2, TR1, TR2, CuZnSOD), and extracellular (ECSOD) antioxidant proteins in prostate cancer tissues (**A**) and prostate cancer cell lines (**B**). BN, adjacent benign tissue; 4+5, high grade prostate cancer tissues with Gleason scores of 4+4 to 4+5; 3+3, intermediate grade prostate cancer tissues with Gleason scores of 3+3 to 3+4. Blots are representative of at least four independent cases for human tissues and at least three separate experiments for prostate cancer cell lines. Band intensity and statistical values were calculated.



Supplementary Figure 4. Possible regulation of Trx1 at the transcriptional level and ECSOD at the epigenetic level. Western blot analysis of (A) Nrf2 in human prostate cancer tissues and cell lines and (B) Nrf2 downstream target proteins in prostate cancer cell lines. (Prx1, peroxiredoxin 1; NQO1, quinone oxidoreductase; GSTpi, glutathione-s-transferase pi; TR, thioredoxin reductase 1 or 2; GCLC, glutamate-cysteine ligase catalytic subunit). (C) Effect of 5-Aza-dC on ECSOD expression. PC3 cells were treated with 50 μ M buthionine sulfoximine (BSO) or 5 μ M 5-aza-2-deoxycytidine (5-Aza-dC) for 96 hr. Western blot analysis of ECSOD protein expression was performed.

Supplementary Table 1. Human prostate cancer tissues used in the studies

No	Organs	Pathologic Diagnosis	Experiment
1	Prostate	Benign	Trx1 western blot, Trx1 redox western blot, TR activity, TXNIP activity, Trx activity
	Prostate	Gleason 3+3	
2	Prostate	Benign	Trx1 western blot, Trx1 redox western blot, TR activity, TXNIP activity, Trx activity
	Prostate	Gleason 3+4	
3	Prostate	Benign	Trx1 western blot, Trx1 redox western blot, TR activity, TXNIP activity, Trx activity
	Prostate	Gleason 3+4	
4	Prostate	Benign	Trx1 western blot, Trx1 redox western blot, TR activity, TXNIP activity, Trx activity
	Prostate	Gleason 4+4	
5	Prostate	Benign	Trx1 western blot, Trx1 redox western blot, TR activity, TXNIP activity, Trx activity
	Prostate	Gleason 4+4	
6	Prostate	Benign	Trx1 western blot, Trx1 redox western blot, TR activity, TXNIP activity, Trx activity MnSOD western blot and activity gel ECSOD activity gel
	Prostate	Gleason 5+4	
7	Prostate	Benign	Trx1 western blot, Trx1 redox western blot, TR activity, TXNIP activity, Trx activity
	Prostate	Gleason 5+5	
8	Prostate	Benign	Trx1 western blot
	Prostate	Gleason 4+5	
9	Prostate	Benign	Trx1 western blot, Trx1 redox western blot MnSOD western blot, IP blot, and activity gel ECSOD western blot and activity gel Prx1, 2, 3, and 4 and PrxSO3 western blot
	Prostate	Gleason 4+5	
10	Prostate	Benign	Trx1 western blot, Trx1 redox western blot MnSOD western blot, IP blot, and activity gel ECSOD activity Prx1, 2, 3, and 4 and PrxSO3 western blot
	Prostate	Gleason 4+5	
11	Prostate	Benign	Trx1 western blot, Trx1 redox western blot MnSOD western blot, IP blot, and activity gel ECSOD western blot and activity gel Prx1, 2, 3, and 4 and PrxSO3 western blot
	Prostate	Gleason 4+5	
12	Prostate	Benign	Trx1 western blot MnSOD western blot and activity gel ECSOD western blot and activity gel Prx 2 and 3 and PrxSO3 western blot
	Prostate	Gleason 4+4	
13	Prostate	Benign	Trx1 western blot, Trx1 redox western blot

	Prostate	Gleason 4+4	MnSOD western blot and activity gel ECSOD western blot and activity gel Prx1, 2, 3, and 4 and PrxSO3 western blot
14	Prostate	Benign	Trx1 western blot MnSOD western blot, IP blot, and activity gel
	Prostate	Gleason 3+4	
15	Prostate	Benign	Trx1 western blot MnSOD western blot and activity gel ECSOD activity gel
	Prostate	Gleason 3+4	
16	Prostate	Benign	Trx1 western blot, Trx1 redox western blot MnSOD western blot, IP blot, and activity gel ECSOD western blot and activity gel
	Prostate	Gleason 3+4	
17	Prostate	Benign	Trx1 western blot, Trx1 redox western blot MnSOD western blot, IP blot, and activity gel ECSOD activity
	Prostate	Gleason 3+4	
18	Prostate	Benign	Trx1 western blot ECSOD western blot and activity gel
	Prostate	Gleason 3+3	
19	Prostate	Benign	Trx1 western blot, Trx1 redox western blot MnSOD western blot and activity gels ECSOD western blot and activity gel
	Prostate	Gleason 3+3	
20	Prostate	Benign	Trx1 western blot MnSOD western blot ECSOD western blot
	Prostate	Gleason 3+3	
21	Prostate	Benign	Trx1 western blot MnSOD western blot and activity gels ECSOD western blot
	Prostate	Gleason 3+3	
22	Prostate	Benign	MnSOD western blot and activity gel, Trx1 redox western blot
	Prostate	Gleason 3+3	

Supplementary Table 2. The coefficient of Determination (R^2) of posttranslational modifications of MnSOD versus MnSOD activity in human prostate cancer tissues. R^2 indicates regression relation of nitrotyrosine or methylated-lysine in MnSOD versus MnSOD activity. High grade prostate tissues (4+4 to 4+5) demonstrate the most significant R^2 (asterisks) which indicates an increase in nitrotyrosine or methylated-lysine in MnSOD. These post-translational modifications are most likely responsible for inactivation of MnSOD in prostate cancer tissues when compare to BN tissues. BN, adjacent benign tissue; 4+4 to 4+5, high grade prostate cancer tissues with Gleason scores of 4+4 to 4+5; 3+3 to 3+4, intermediate grade prostate cancer tissues with Gleason scores of 3+3 to 3+4.

Prostate cancer		Coefficient of Determination (R^2)	
		Increasing of Nitrotyrosine MnSOD vs. decreasing of MnSOD activity	Increasing of Methylated lysine MnSOD vs. decreasing of MnSOD activity
3+3 to 3+4	BN	0.76	0.59
	PCa	0.6	0.64
4+4 to 4+5	BN	0.87	0.01
	PCa	0.99*	0.95*
3+3 to 4+5	BN	0.31	0.06
	PCa	0.47	0.64