SUPPLEMENTAL METHODS

Cell cultures and treatments: Immortalized PCa-cells were grown in IMDM (Invitrogen) supplemented with 10% FBS (Hyclone), antibiotics and glutamine. HeLa and MCF-7 cells were cultured in DMEM (Invitrogen) supplemented with 10% FBS (Invitrogen), antibiotics and glutamine. HUVEC and renal cell carcinoma 786-O (VHL/HIF-1α deficient) were cultured according to the manufacturer's instructions (Cambrex Bio Science and American Type Culture Collection, ATCC, respectively). At least seventy-two hours prior to experimental use, the cells were switched to medium supplemented with hormone-deprived serum (1). Cells were treated with 10^{-7} M 17β-estradiol (E2; Sigma), 0.5 mM 7-nitroindazole (7N) (Biomol), 5 mM N(G)-nitro-L-arginine methyl ester (L-NAME; Alexis) and 500 μM diethylenetriamine/nitric oxide adduct (DETA-NO; Sigma) and 10 μM L-6-Hydroxymethyl-chiro-inositol-2-[(R)-2-O-methyl-3-O-octadecylcarbonate] (AKTi) (Alexis) for the times indicated in figure legends. Hypoxia condition (1% O2) was obtained as previously described (2). Briefly, cells were given fresh medium, transferred to a incubator chamber (ThermoFisher Scientific), flushed with 1% O2-5% CO_2-94 %N_2 for 20 minutes and then placed at 37° C for the times indicated in figure legends.

Antibodies. The following antibodies were used: HC-20, L-20, H-150 and Sp1 PEP2 (Santa-Cruz); 6F11, PPG5/10, 34βE12 and 28A4 (UCS Diagnostic); D7N (ZYMED laboratories Inc.); PG-21 (Upstate); AR441 βH1, 35βH1, 4A4 and OZ12 (Neomarker-LabVision Corporation); eNOS/NOS III (BD Biosciences); ab5589 and ESEE122 (Abcam); NB-100-105, NB 100-132 and NB 100-124 (Novus Biologicals); α–actin (Sigma); Phospho-Akt (Ser473), Akt and Phospho-eNOS (Ser1177) (Cell Signaling); Hsp70 (Stressgen)

Immunocytochemistry on cell cultures. Immunoperoxidase assays were performed as described (3).

Transfections, Cell extracts and Western blot. Transient transfections were performed by the Lipofectamine Plus technique (Invitrogen). Expression vectors encoding the human ERβ (4) were

gifts from J.A. Gustafsson (Huddinge, Sweden). Expression vectors encoding human HIF-1α was generated by inserting the HIF-1α cDNA (nt 1-3389) excised from pBSKhHIF-1αT7 (5) was blunted and cloned into the EcoRV site of pAdTrack-CMV (gift of B. Vogelstein, Baltimore, USA). HIF-2α expression vector was kindly provided by F. Martelli (IDI, Rome; Italy; (6)). Expression vectors encoding human EPO was gift from L.E. Huang (University of Utah, Salt Lake City, USA) (7). eNOS vector encoding S1177D was gift from S. Dimmeler (University of Frankfurt, Germany) (8). eNOS vector encoding S1177A was gift from C.M. Counter (Duke University Medical Center, Durham, USA) (9). Total extracts or nuclear and cytoplasmic fractions were obtained as previously described (10-12).

Confocal microscopy. Confocal analysis was performed as previously described (13).

Determination of nitric oxide production. Nitric oxide production was evaluated using 4,5-diaminofluorescein (DAF-2 DA; Alexis) added to the complete medium, and analyzed by flow cytometry as previously described (14).

RNA extraction and Real time PCR Analysis. RNA isolation and cDNA preparation were as previously described (3). qRT-PCR was performed with the ABI Prism 7500 and 7900HT Fast PCR instruments (Applied Biosystems). Predesigned TaqMan primers and probe (Applied Biosystems) specific for *HIF-1α*, *HIF-2α*, *HIF-1β*, *VEGF*, *AKT*, *GLUT-1*, *hKDR*, *MMP9*, β–actine, *18S* rRNA and *RNAse P* were used. qRT-PCR for *hTERT* was performed using SYBR Master mix (Applied Biosystems) with evaluation of dissociation curves as described in (3). The mRNA of each gene was quantified using the Standard Curve Method (5 log dilutions in triplicate) and expressed relative to housekeeping aldolase (1) and *GAPDH* genes. Data are represented as box plots charts (boxes show medians and upper and lower quartiles of the data and whiskers indicate minimum and maximum values) or as fold of induction (+/- treatment).

Chromatin Immunoprecipitation (ChIP) and ReChIP assay by qRT-PCR. ChIP and ReChIP assays were performed as previously described (13). DNA fragments were recovered and analyzed by qRT-PCR. Standard curves were generated by serially diluting the input (5-log dilutions in

triplicate). qRT-PCR was done in the ABI Prism 7500 and 7900HT fast PCR instruments (Applied Biosystems) using SYBR Master mix (Applied Biosystems) with evaluation of dissociation curves. The qRT-PCR analyses were performed in duplicate and the data obtained were normalized to the corresponding DNA input control. Data are represented as relative enrichment (with values for no antibody being subtracted from those with antibody, Supplemental Figure 2C) or as fold of induction over control (+/- treatment, Figure 5) .

Primers for qRT-PCR: for qRT-PCR the following primers were used**:** GAPDH 5'-TCC CTG AGC TGA ACG GGA AG-3' and 5'-GGA GGA GTG GGT GTC GCT GT-3'; hTERT1899 5'-TGGGCCCACCCACACTAA-3' and hTERT1965 5'-CAAGGGTCATGTTCGTTTCCA-3'; hTERT4149 5'-TGCAGCGGCATGATCTTG-3' and hTERT4252 5'-TGGTGGTGCATGCCTGAA-3'; hTERT5822 5'-GCGTTTGTTAGCATTTCAGTGTTT-3' and hTERT5926 5'-CGGGTTGCTCAAGTTTGGA-3'; hTERT 9691 5'-TCCTGGTCCCATCTTTAGGTATG-3' and hTERT9793 5'-TCCCCTGAACACCCACAAA-3'; hMSH2 for 5'-GCCTTGCAGCTGAGTAAACACA-3' and hMSH2 rev 5'-TAGGTCGCGCGGAAACC-3'; hVEGF for 5'-TTGTAAATGCCGGTGACAAC-3' and hVEGF rev 5'-GGGAGTGAGGGCACAGAAT-3'; hCycB1 for 5'-GCCCTGGAAACGCATTCTC-3' and hCycB1 rev 5'-CCTCCTTATTGGCCTGTTCGT-3'. hPS2 for 5'-CTAGACGGAATGGGCTTCAT-3' and hPS2 rev 5'-ATGGGAGTCTCCTCCAACCT-3'.

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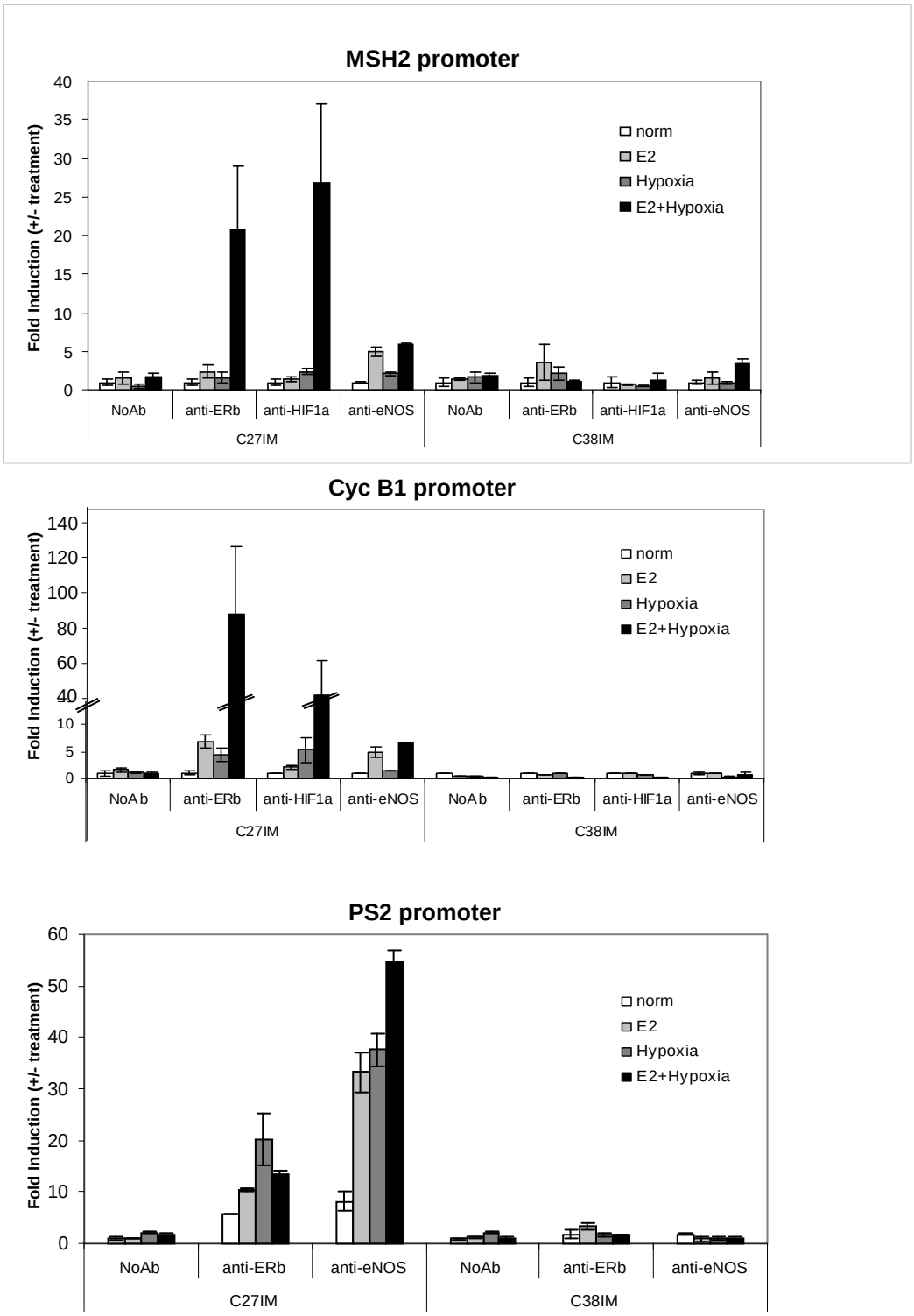
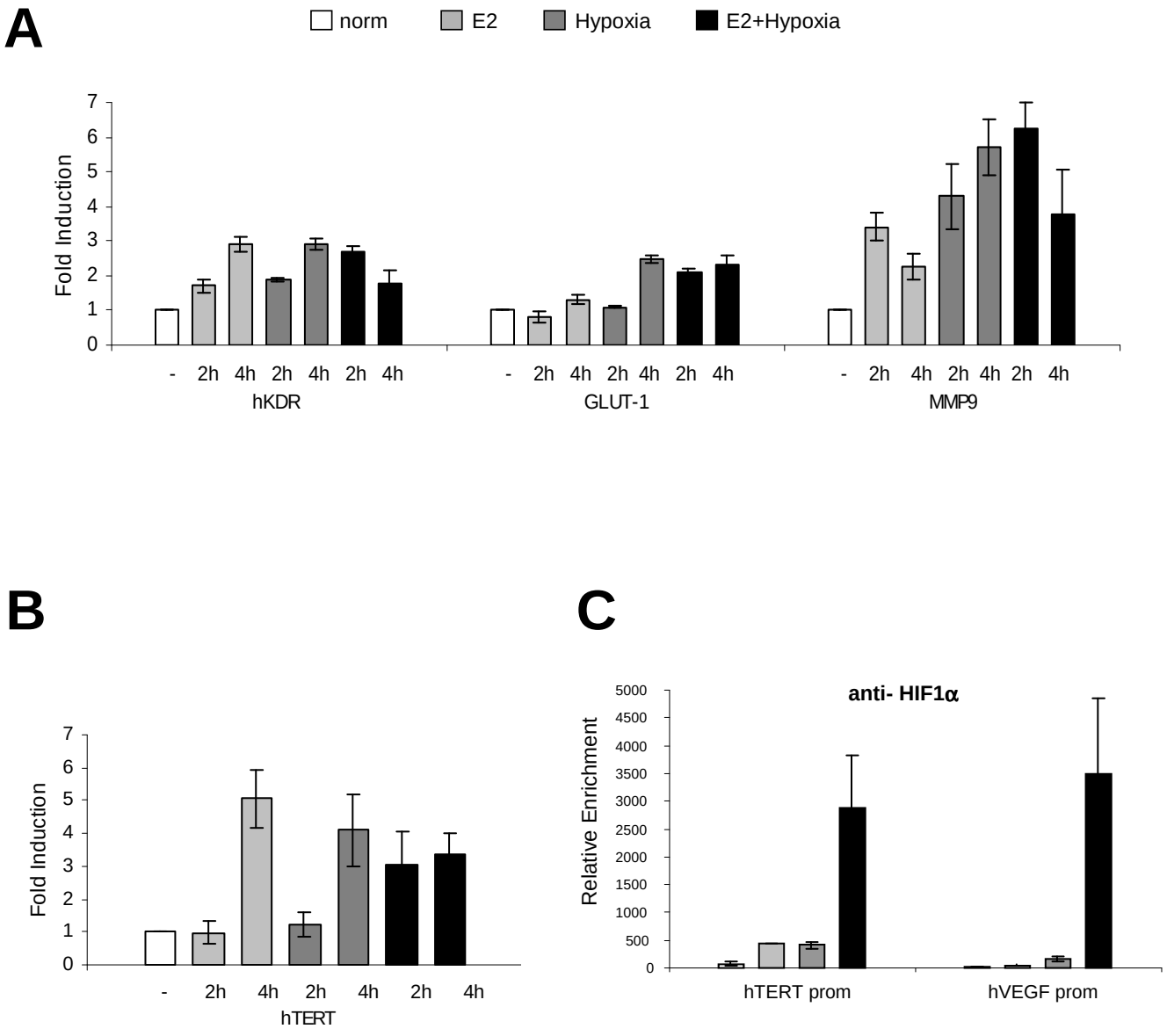
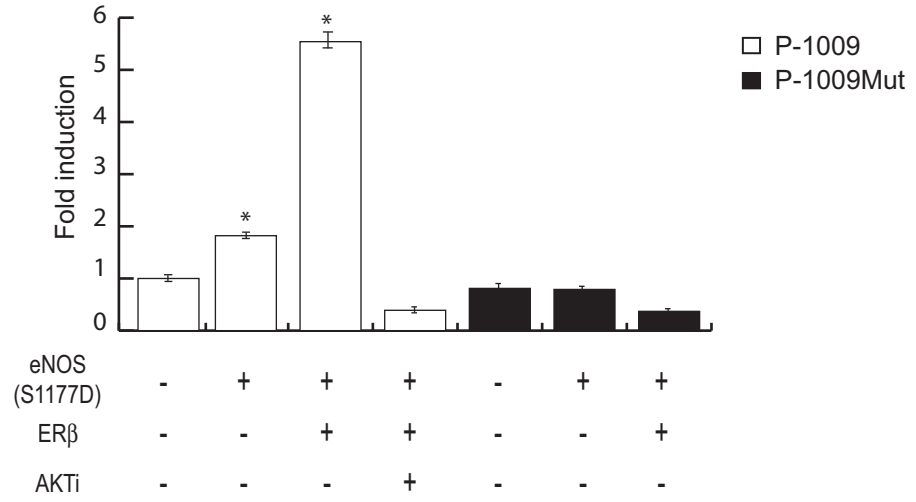


Supplemental Figure 1. Chromatin Immunoprecipitation (ChIP) assays by qRT-PCR onto promoter of genes belongings to the *prognostic signature*. ChIP experiments were performed in parallel using PCa-IM cells from the G1 and G2 prognosis groups (C27 IM and C38 IM, respectively). Cells were treated +/- E2 (10^{-7}M), in normoxia or hypoxia (1% O2), alone or in combination for 135 min. Immunoprecipitations were performed using antibodies to ERb, HIF-1a and eNOS or no antibody (No Ab) as negative control. DNA fragments were analyzed by real-time PCR using Standard Curve Method (serially diluting the input, 5-log dilutions in triplicate). qRT-PCR was done in the ABI Prism 7900HT fast PCR instruments (Applied Biosystems) using SYBR Master mix (Applied Biosystems) with evaluation of dissociation curves. The qRT-PCR analyses were performed in duplicate and the data obtained were normalized to the corresponding DNA input control. Data, represented as relative enrichment, are mean of three independent experiments ± SEM.

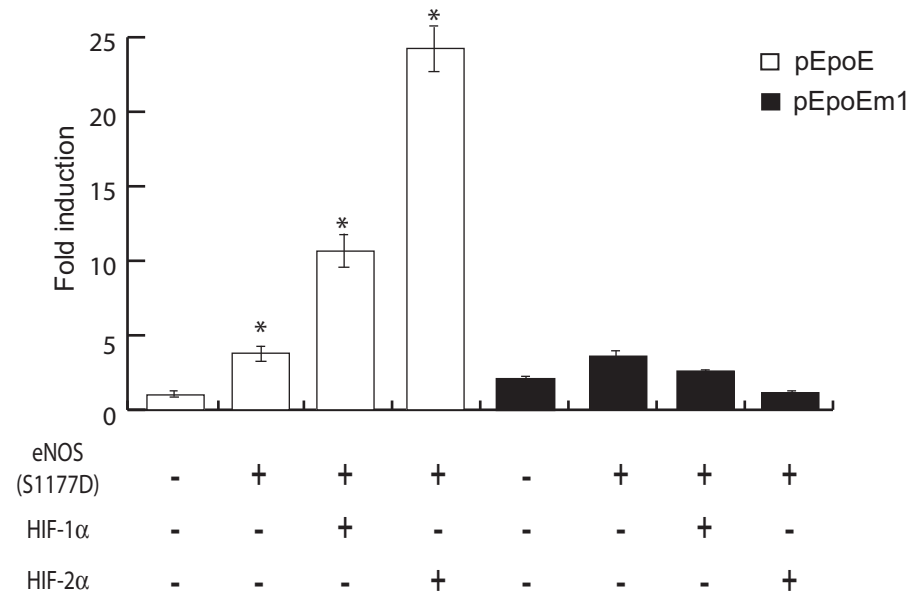


Supplemental Figure 2. Expression of Estradiol and/or Hypoxia target genes and ChIP assay in HUVEC cells. **(A)**,**(B)** mRNA level by qRT-PCR of E2 and/or Hypoxia target genes (hKDR, GLUT-1, MMP9 and hTERT) in HUVEC cells after treatment with E2 or hypoxia, alone or in combination, for the times indicated. Data are the mean ± SEM of three independent experiments and are expressed as fold induction (+/- treatment). **(C)** ChIP assay in HUVEC using the hTERT and hVEGF promoters and antibody to HIF-1α (values of no antibody, used as negative control, were subtracted in each condition). Data are the mean ± SEM of two independent experiments.

A



B



Supplemental Figure 3. eNOS activity requires intact ERE and HRE binding sites. (**A**) C19IM were transfected with the hTERT promoter-reporter, the wild type (P-1009), or with mutated ERE (P-1009Mut), in the presence of CMV-βgal, alone or in combination with ERβ and eNOS mutant at serine 1177 (S1177D). Cells were treated with vehicle, E2 (10-7M) and AKTi (10 μM) and relative activities were determined in cell lysates after 48 hrs. Results represent the average (± SEM) of two independent experiments, each performed in triplicate. Asterisks indicate $p<0.05$ vs P-1009 basal activity. (**B**) C19IM were transfected with the EPO promoter-reporter, wild type (pEpoE) or with a mutated HRE binding site (pEpoEm1), in the presence of CMV-βgal alone or in combination with eNOS S1177D and HIF-1α or HIF-2α. Relative activities were determined in cell lysates after 48 hrs and results represent the average (± SEM) of two independent experiments, each performed in triplicate. Asterisks indicate $p<0.05$ vs pEpoE basal activity.

Supplemental Table 1
Clinical, histopathologic characteristic and follow up of patients (n=88)

Age (years)	mean (range)	65.5 (41-75)
Gleason Score Combined		n (%)
	≤ 6	17 (19.3)
	=7	42 (47.7)
	≥ 8	23 (26.1)
	NV[A]	6 (6.8)
Pathologic Stage [B]		n (%)
T2-3 N0Mx	T2	34 (38.6)
	T3	45 (51.1)
	NV	9 (10.2)
Survival (years)	average	12,9
	CI [C]	12.0-13.8
Follow up (years)	median	9.9
	25^{th}, 75^{th} percentile	6.2- 11.2
Outcome [D]		n (%)
	Poor	46 (52.3)
	Good	42 (47.7)

[A] NV not valutable
[B] UICC 2002
[C] 95% Confidence Interval
[D] Poor outcome was defined by presence of recurrence (biochemical, local or metastatic), see method section, or disease-specific survival.