

Supplemental Figure Legends

Supplemental Figure 1. Quantitative RT-PCR (a) and semi-quantitative RT-PCR (b) for detection of Nox1 in 786-0, RCC4 or LNCaP prostate cancer cells using two published primer pairs (14, 17). Error bar represents +/- SE.

Supplemental Figure 2. Characterization of 786-0 cells stably transfected with the human, full-length Nox4 cDNA. (a) Morphology of 786-0 cells following exogenous expression of Nox4 reveals smaller, denser, more-rounded appearance relative to parental 786-0 cells. (b) Cell viability assay. Equal numbers of untransfected cells or 786-0 Nox4 cells were plated in 96-well plates. After attachment, assay reagent was added, incubated at 37°C, and the fluorescent signal read at 590 nm.

Supplemental Figure 3. Lucigenin chemiluminescence assay for superoxide detection performed as described in materials and methods on a) isolated membrane fractions from parental 786-0, RCC4 and Caki-1 cells or isolated cell fractions from 786-0 (b) or RCC4 (c) cells. RLU: relative light units.

Supplemental Figure 4. Cytotoxicity was measured by CellTiter-Blue (Promega, Madison, WI). 786-0 NS cells were plated at 1×10^4 cells per well in 96-well plates. After 24 hours, dithiothreitol was added as indicated in 5 replicate wells. 20 μ l of the CellTiter-Blue reagent was added to each well and viability quantitated by spectrofluorometer (Spectra Max Plus384, Molecular Devices) and expressed as percentage of viability of media-only controls. Error bars indicate +/- S.E. mean

Supplemental Figure 5 Cellular distribution of HIF-2 α under normal oxygen conditions. (a) Representative confocal microscopy images (magnification 400x) showing HIF-2 α or Nox4 immunofluorescence, DAPI nuclear staining, or merged images. b) Control (NS) or Nox4 shRNA (KD) cells were imaged following exposure to TEMPOL (0.25 mM), DTT (1mM). c) 786-0 NS cells imaged following transduction with Ad-GFP or Ad-MnSOD. d) 786-0 cells expressing pcDNA-Nox4 imaged with or without treatment with TEMPOL (0.25 mM).

Supplemental Figure 6 Immunohistochemistry for Nox4 and HIF-2 α in 786-0 NS and KD xenograft explants confirms re-expression of Nox4 in KD tumors with corresponding elevated HIF-2 α expression. Magnification 200X.