Supplemental materials and methods

Antibodies and reagents. The antibodies used in this study include anti-EGFR mAb and p-EGFR pAb (Tyr1068) (from Biosource, Camarillo, CA, and Cell Signaling Technology, Beverly, MA respectively), anti-FASN mAb (BD Transduction Laboratories, Franklin Lakes, NJ), anti-AR pAb and anti-GAPDH pAb (both from Santa Cruz Biotechnologies, Santa Cruz, CA), anti β-actin mAb AC-15 (Sigma, St. Louis, MO), anti-Akt1 mAb 2H10, anti–p-Akt (S473) pAb, anti–p-glycogen synthase kinase-3α/β (p-GSK3α/β (p-S21/9)) pAb, anti–p-Akt substrate rabbit mAb, anti-Src mAb, anti-p-FoxO1/FoxO3a pAb (p-S256) (all from Cell Signaling Technology); anti-HA tag (clone HA.11; Covance, Inc. NJ), anti-phospho-Tyr mAb (Upstate, Lake Placid, NY). The anti-DRF3 mAb was a generous gift from Dr. H. Higgs at Dartmouth University. Where indicated, EGF (R&D Systems, MN) was added from a 10,000-fold concentrated stock in 10mM acetic acid/BSA, at a final concentration of 50ng/ml. Control cultures received similar amounts of vehicle only. OptiMEM reduced serum medium was from Invitrogen Corporation (Carlsbad, CA). All other chemicals were obtained from Sigma. FuGENE6 reagent was from Roche Applied Science (Indianapolis, IN). ZD1839 (gefitinib) was a gift from AstraZeneca, Macclesfield, UK.

Preparation of whole-cell lysates and immunoblotting. Cells were washed twice in ice-cold PBS and lysed in a minimum volume of 1x cell lysis buffer (Cell Signaling Technology) supplemented with 1 mM PMSF. Protein content was determined using the Micro-BCA protein assay reagent. Protein extracts were resolved by SDS-PAGE and electrotransferred to nitrocellulose membranes. Following transfer, membranes were stained with Ponceau S to confirm equal protein

loading. Membranes were blocked with PBS/0.1% Tween 20/5% IgG-free BSA and incubated with primary antibodies overnight at 4°C. Following incubation with species-specific horseradish peroxidase–conjugated secondary antibodies, signals were detected using SuperSignal chemiluminescent reagent (Pierce Chemical, Rockford, IL) and exposure of blots to X-ray film.

Cell culture conditions. DU145 cells were cultured in Dulbecco's modified Eagle's medium (high glucose) with sodium pyruvate, L-glutamine, and 10% FBS. LNCaP cells were cultured in RPMI 1640/10% FBS. WPMY-1 cells were cultured in Dulbecco's modified Eagle's medium with 5% FBS. iPrEC cells were kept in PrEGM complete medium. All media were supplemented with 100 μg streptomycin, and 100 units/ml penicillin (Invitrogen, Carlsbad, CA). Cells were maintained in a humidified atmosphere of 5% CO_2 at 37^{o}C.

RT-PCR validating silencing of DRF3 expression in DU145 PCa cells. Total RNA was isolated from cells treated with specific DRF3 siRNA duplex or control siRNA using the RNeasy Mini Kit (QIAGEN Inc., Valencia, CA). RNA was used for one-step RT-PCR with the *DIAPH3* gene-specific primer pair (5'-TATATAGGTACCGCCAC CATGGAACGGCACCAGCCGCGGC-3'/ 5'-AAGTTGGATATCCAGGCCATC-3') using the SuperScript One-Step RT-PCR kit according to the manufacturer (Invitrogen). cDNA synthesis was performed at 55˚C. Annealing temperature for PCR amplification was 58˚C. PCR products were analyzed by agarose gel electrophoresis.

Tryptic digestion and mass spectrometry. SDS-PAGE gel slices were cut into ~1 mm^3 gel fragments and subsequently subjected to in-gel reduction, alkylation,

and tryptic digestion essentially as described (1). Briefly, proteins were reduced with 10 mM dithiothreitol (Sigma, St. Louis, MO), alkylated with 55 mM iodoacetamide (GE Healthcare, Buckinghamshire, UK), and digested with MS-grade trypsin (Promega, # V5280, Madison, WI) at 58°C for 30 min. Tryptic peptides were extracted with 50% acetonitrile followed by 100% acetronitrile, dried in vacuo, and redissolved in 15 μl 1.5% acetic acid, 7.5% acetonitrile solution. 5 μl samples were analyzed by liquid chromatography tandem mass spectrometry (LC-MS/MS). Peptides were separated at circa 400 nl/min with 60 min linear gradients from 5 to 31% acetonitrile in 0.4% formic acid and analyzed using an LTQ ProteomeX ion trap mass spectrometer (Thermo Finnigan, San Jose, CA), in which up to 6 of the most intense ions per cycle were fragmented.

Database query and spectral counting. Database searches were performed essentially as described (1). Briefly, all MS datasets were searched against the International Protein Index (IPI) human protein database (v3.36; 69012 sequences) using the MASCOT search engine (Matrix Science, v2.104, London, UK). Protein modifications were selected as carbamidomethyl (C) (fixed) and N-Acetyl (Pr) and Oxidation (M) (variable). Up to one missed cleavage was allowed. Mass tolerance was set as ±1.5 Da for MS spectra and ±1.5 Da for MS/MS spectra. Peptides were identified with an ion score no less than 40. Proteins were identified based on at least 2 unique peptides and the significance threshold was set as $p<0.01$. Likely contaminants (*e.g.*, keratins and albumin) were removed from the protein lists. Relative protein abundance was determined using a label-free spectral counting approach (2).

Pathway enrichment and network analysis. The integrated software suite MetaCore (GeneGo, St. Joseph, MI) was used to map proteins into biological networks. This tool assists in finding regulatory paths of interest using a manually curated database of human protein-protein, protein-DNA and protein-compound interactions, metabolic and signaling pathways and the effects of bioactive molecules. Assignments of proteins to biological processes and pathways were based on GeneGo ontology maps (http://www.genego.com). For network analysis, two algorithms were used: 1) the direct interaction algorithm to map direct protein-protein interaction; 2) the shortest path algorithm to map the shortest interaction pathway.

In vitro wound healing assay. DU145 cells were grown to confluence on 6-well plates in regular medium, then serum starved for 12 h. A sterile pipette tip was used to create a denuded area. Cells were washed twice with PBS and treated with or without EGF. Photographs were taken at hours 0 and 24. Mitomycin C (0.5 μM) was added to prevent the confounding event of cell proliferation.

Cell proliferation and migration assays. DU145 cells were transiently transfected with a pool of control or DRF3 silencing RNAs using nucleofection (Amaxa Inc. MD, USA). Two days later, transfected cells were serum starved for 3 h prior to treatment with 100 ng/ml EGF for 6 h. Oncosomes were then isolated as described above and used to condition unmodified DU145 cells, seeded on 6-well tissue culture plates at a density of $1x10^4$ cells/well, and serum starved for 12 h. Cell proliferation was determined by viability assay after 48 h. For migration assays, DU145 cells ($1x10^5$/well) were serum starved for 16 h, then trypsinized

and applied to cell migration chambers (Chemicon International Inc., Temecula, CA). Isolated oncosomes from transfected cells were applied to culture medium for 24 h. Cells that migrated to the bottom of the inserts were quantified by measuring absorbance at 570nm.

SNP arrays. Primary prostate cancers and lymph node metastases were derived from radical prostatectomies (3). Distant metastases were obtained through the University of Washington Tissue Acquisition Necropsy program (4) and the University of Michigan Warm Autopsy Program (5). All samples were snap frozen in OCT cryomolds and maintained at liquid nitrogen temperatures. The majority of samples were laser-capture microdissected. In a few cases (mostly primary tumors), bulk dissection was performed and the composition was verified by H&E staining of an adjacent section. In all cases only samples whose purity was sufficient to detect broad, low-level copy-number changes in the data were included. Genomic DNA was obtained from regions of the tumor determined by pathologist review to have >80% tumor purity and hybridized to 100K SNP arrays (Affymetrix, Santa Clara, CA) according to the manufacturer's instructions. Arrays were scanned with a GeneChip Scanner 3000, and genotyping was performed using Affymetrix Genotyping Tools Version 2.0. Probe-level signal intensities were normalized to a baseline array using invariant set normalization (6). SNP-level signal intensities were obtained using a model-based (PM/MM) method (6). Tumor data were normalized against data from normal tissue samples selected for similar baseline noise characteristics (7). Noise was further reduced using the segmentation algorithm Gain and Loss Analysis of DNA (8). Histogram analysis (7) was used to confirm that all tumors were sufficiently enriched to observe

copy-number changes. Samples were called deleted at the DIAPH3 locus if their segmented log2 ratios were less than -0.1, a value reached in only 0.5% of SNPs in data from normal tissue.

Fluorescence in situ hybridization (FISH). TMA slides with tissue samples from radical prostatectomy cases and a prostate cancer progression TMA were used for this study. DIAPH3 gene alteration was assessed by FISH, using a probe spanning the DIAPH3 locus (chr13q21.2) and a reference probe spanning a stable region on chromosome 21 identified by SNP data in prostate tissue (chr21q22.12). For the DIAPH3 target probe, the Biotin-14-dCTP labeled BAC clones RP11-643G22 and RP11-638B12 (eventually conjugated to produce a red signal) were applied. For the reference probe, the Digoxigenin-11-dUTP labeled BAC clone RP11-451M12 (eventually conjugated to produce a green signal) was applied. The BAC clones were obtained from the BACPAC Resource Center, Children's Hospital Oakland Research Institute (CHORI) (Oakland, CA). Nuclei which were negative for deletion displayed a randomly dispersed pattern of two red target probes and two green reference probes within each nucleus. Nuclei containing a heterozygous deletion of the DIAPH3 locus showed only one red target signal with two green reference signals.

The samples were analyzed under a 60x oil immersion objective using an Olympus BX-511 fluorescence microscope, a CCD (charge-coupled device) camera and the CytoVision FISH imaging and capturing software (Applied Imaging, San Jose, CA). Semi-quantitative evaluation of the tests was performed by two evaluators (D.D.V., C.J.L.). For each case, we attempted to analyze at least 50 nuclei.

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