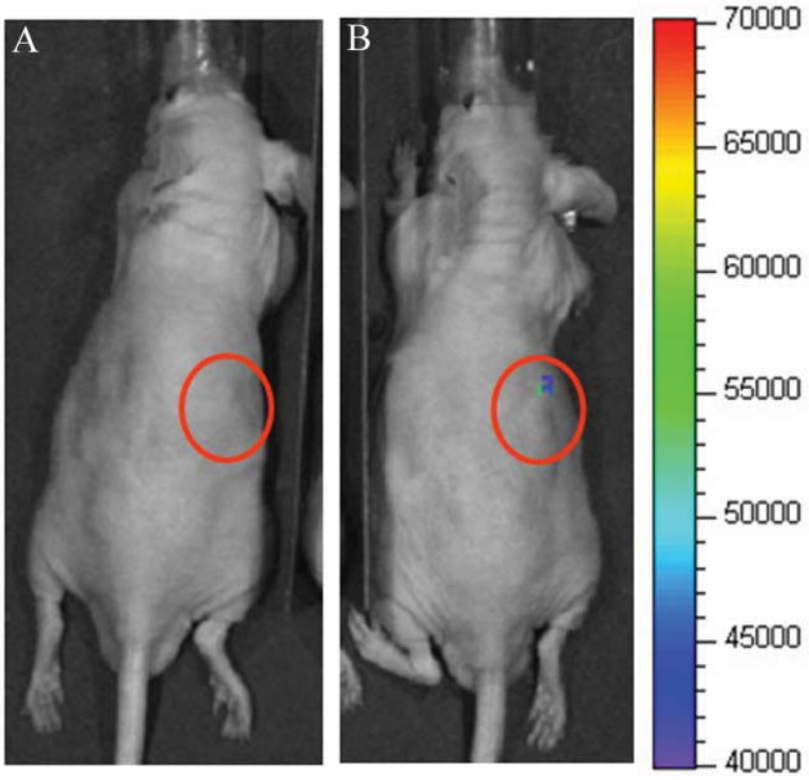
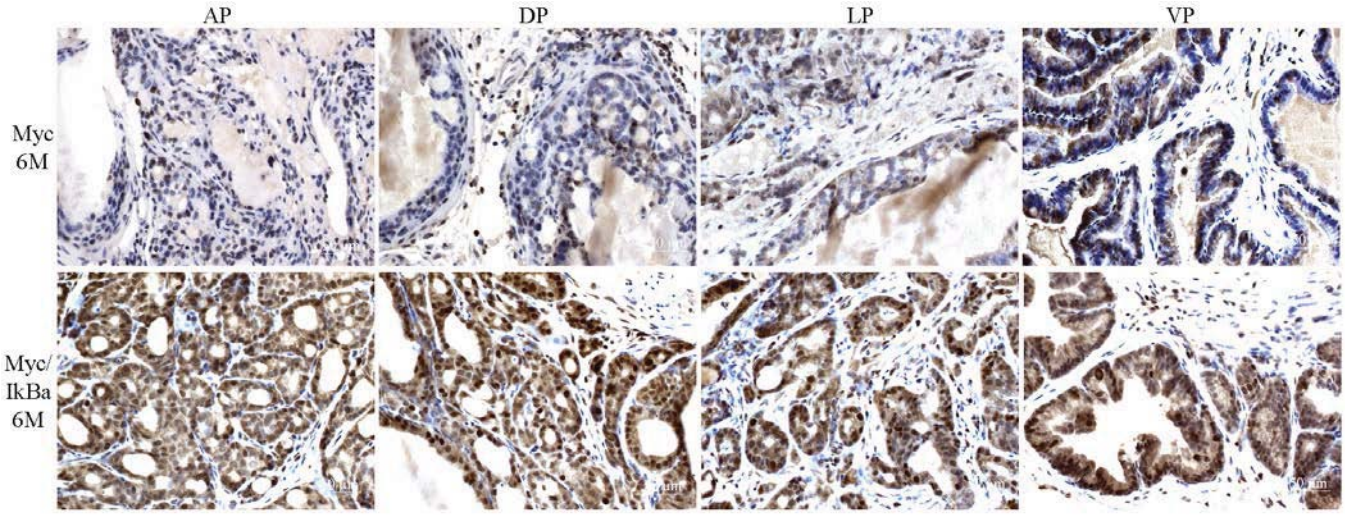
SUPPLEMENTARY DATA:

Supplementary Figure 1. NF-κB signaling is continuously activated in the prostate of IκBα+/- mouse. In order to determine the NF-κB activity in the prostate of IκBα+/- mouse, we crossed the IκBα+/- mice with NGL, a NF-κB reporter mouse. NGL transgenic mice are engineered to express a GFP/luciferase fusion protein under the control of a promoter containing multiple NF-κB consensus binding sites (1). Since the NF-κB signaling in the IκBα+/- NGL mouse is activated in the whole body, the relatively high level of background activation does not allow detection of NF-κB activity in the prostate. Therefore, in order to determine the NF-κB activity in the prostate of the IκBα+/- mouse, we grafted the prostates from new born IκBα/NGL mice into the kidney capsule of male nude mice using a tissue rescue technique. NF-κB activity was measured at 7 weeks after grafting. The bioluminescence imaging shows NF-κB signaling is activated (green) in the kidney, where the grafted prostate from IκBα/NGL mouse resides (**B**). In panel (**A**), the control mouse (grafted with the prostate from NGL mouse) has no bioluminescence, illustrating that in the absence of IκBα+/-, there is not activation of NF-κB. The circles indicate kidney areas.

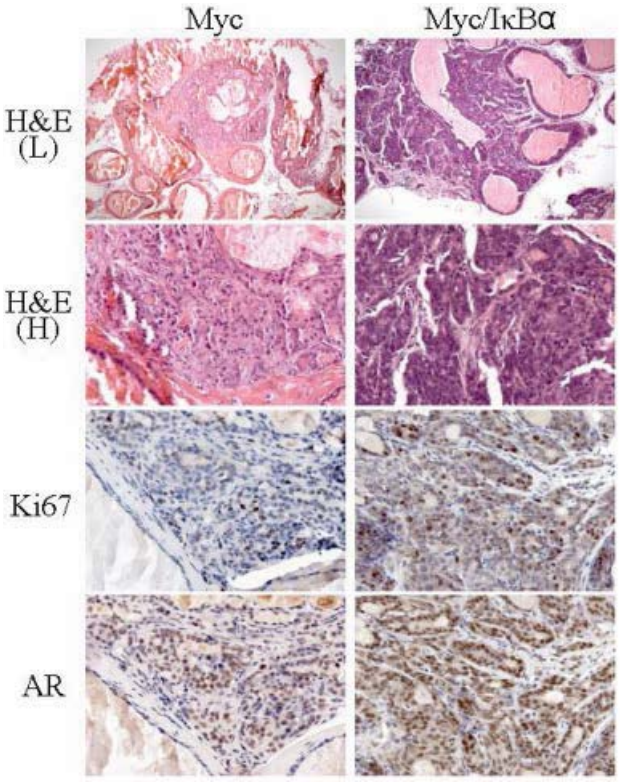


Supplementary Figure 2. NF-κB signaling activated in the prostate of Myc/IκBα bigenic mouse. The prostates from Myc alone (Myc) and bigenic (Myc/IκBα) mice were harvested at 6 months of age. Activation of NF-κB signaling in the prostate was determined by IHC staining of p65-pho antibody.

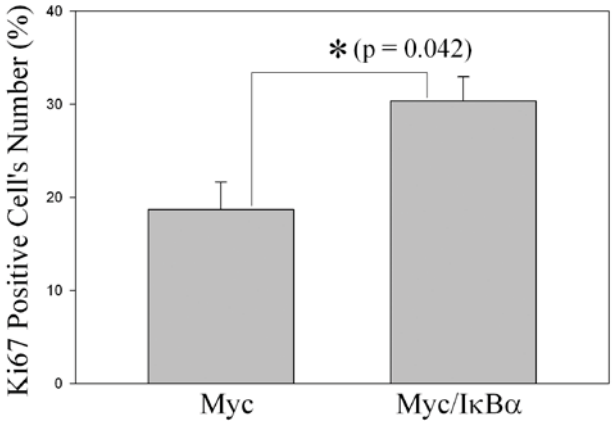


Supplementary Figure 3. Continuous activation of NF-κB signaling promotes PCa progression in the Hi-Myc transgenic mouse. The prostates from Myc alone (Myc) and bigeneic (Myc/IκBα) mice were harvested at 6 months of age. (**A**) Histological analysis was performed by H&E staining. IHC analyses were performed to determine AR expression and proliferation (Ki67) of the prostates. (**B**) Cells positive for Ki67 were counted by monitoring at least 200 luminal epithelial cells from 3-5 different fields of each sample and plotted as a percentage of total counted. The results are reported as mean value (%); *bars*, ± SEM. * $P = 0.042$ by Student's *t* test (*t* test).

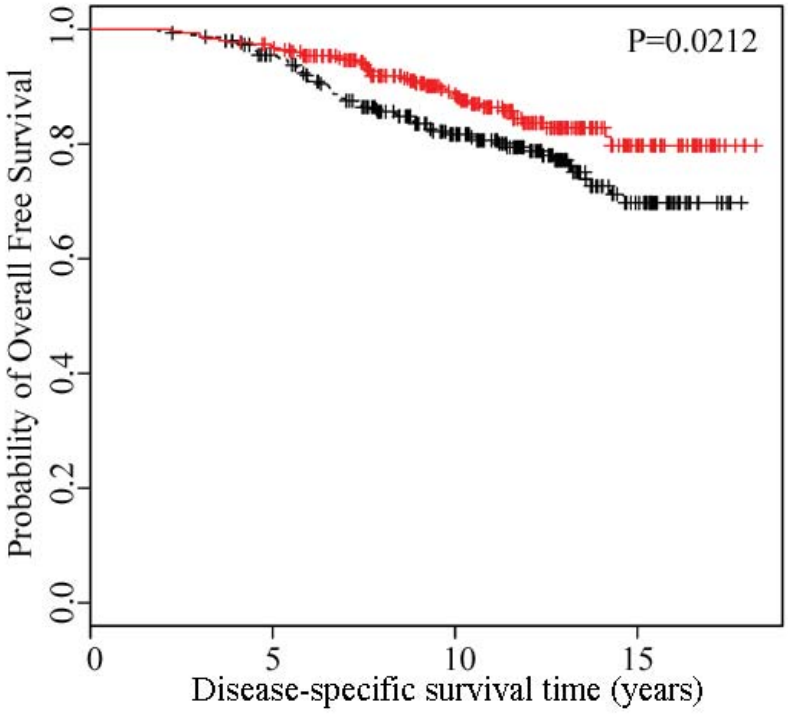
A



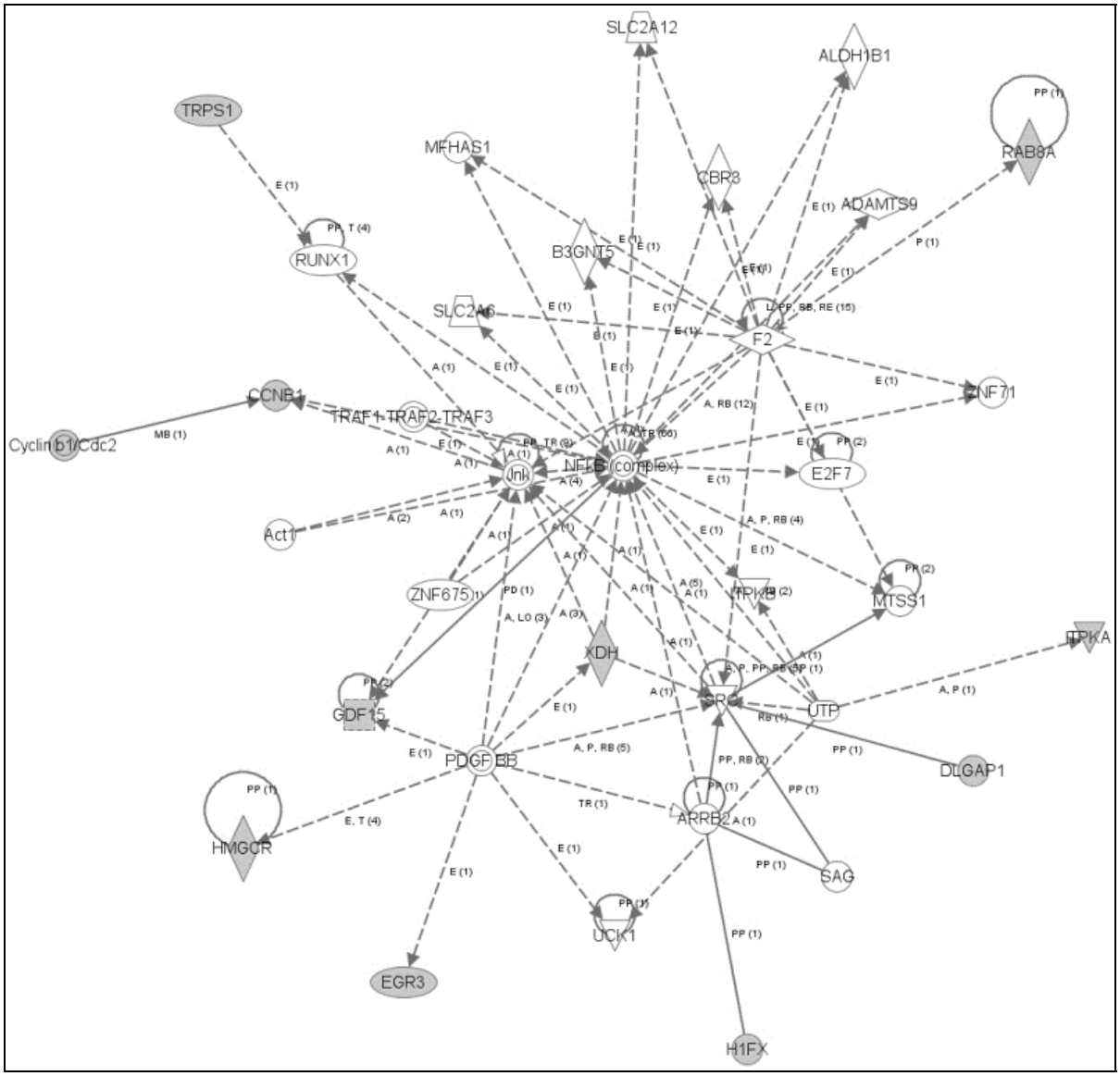
B.



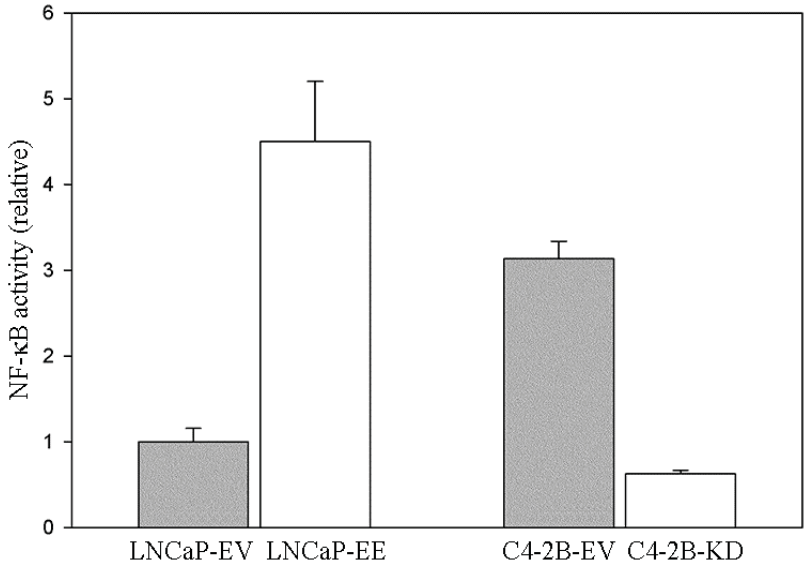
Supplementary Figure 4. The NF24 gene signature predicts significant differences in the overall cancer-specific survival of the PCa patients. Kaplan-Meier (KM) analyses were used to examine whether there was a significant association between overall cancer-specific survival prediction and the signature generated from NF-κB activated intact (no castration) mouse prostate (NF24). Two types of overall cancer-specific survival outcomes were compared in the plot: a poor-prognosis group (black dashed line) and a favorable-prognosis group (red solid line). The disease-specific survival time in years is displayed on the X-axis, and the Y-axis shows the probability of overall cancer-specific survival. *P* value is by log-rank test.



Supplemental Figure 5. Molecular network analysis using Ingenuity Pathway Analysis (IPA). Network associated with the NARP21 gene signature genes (21 genes) derived from NF-κB activated androgen depleted mouse prostate. The network was generated using the NARP21 gene set. Molecules are represented as nodes, and the biological relationship between two nodes is represented as an edge (line). Direct relationships are indicated by solid lines and indirect through dashed lines. Line beginnings and endings illustrate the direction of the relationship (e.g. arrow head indicates gene A influences gene B).

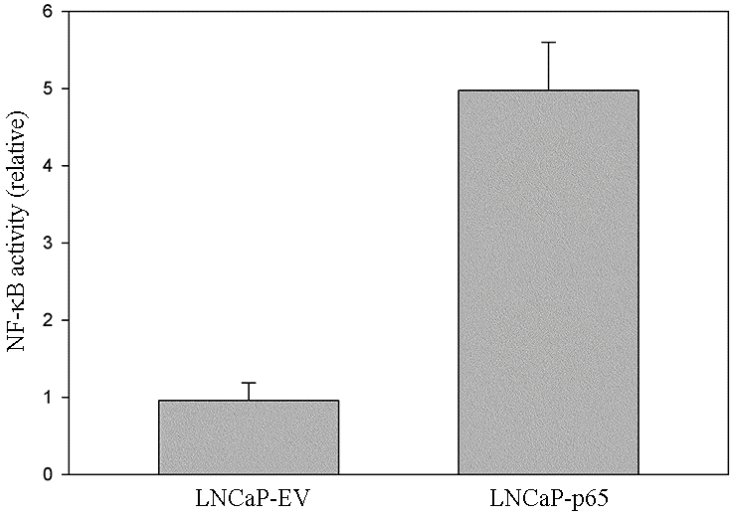


Supplemental Figure 6. NF-κB signaling is activated/inactivated in PCa cells by infecting with IKK2-EE or IKK2-KD retroviral vectors. NF-κB signaling was activated in LNCaP PCa cells by infecting with IKK2-EE retroviral vector, in which NF-κB activity was activated with a constitutively active (EE) mutants of IKK2 (2, 3); while NF-κB signaling was inactivated in C4-2B PCa cells by infecting with IKK2-KD retroviral vector, in which NF-κB activity was inhibited with a kinase dead (KD) IKK2 mutant (2, 3). The cells infected with empty vector were used as controls. NF-κB activity in PCa cells were measured by using a NF-κB responsive NGL reporter (1). The values plotted represent the mean of at least three individual samples ± SEM.



Supplemental Figure 7. Activation of NF-κB signaling increases JNK phosphorylation in PCa cells. In order to further confirm that JNK phosphorylation is directly caused by activation of NF-κB signaling in PCa cells, NF-κB signaling was activated in LNCaP PCa cells by directly infecting with p65 expression adenoviral vector (LNCaP-p65). The cells infected with empty vector (LNCaP-EV) were used as controls. (**A**) NF-κB activity in PCa cells were measured by using a NF-κB responsive NGL reporter(1). The values plotted represent the mean of at least three individual samples ± SEM. (**B**) Total and phosphorylated JNK levels in NF-κB activated (LNCaP-p65) or inactivated (LNCaP-EV) PCa cells were evaluated by Western blot.

A



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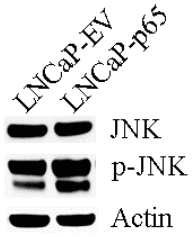
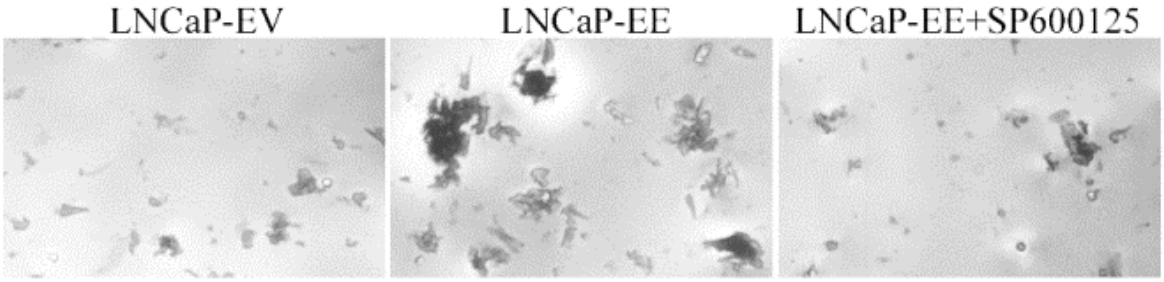


Supplemental Figure 8. Blocking JNK signaling inhibits NF-κB induced invasive ability efficiently in PCa cells. NF-κB activated LNCaP cells (LNCaP-EE: infected with IKK2-EE retroviral vector) were used to perform invasive assay. LNCaP cells infected with IKK2-EV retroviral vector (empty vector) (LNCaP-EV) were used as control. SP600125 (10^{-5}M) was used as JNK signaling inhibitor. Invasive ability was measured by Boyden chamber cell migration assay at 48 hour after treatment.



SUPPLEMENTARY TABLES:

Supplementary Table 1. Matched human homolog genes list from NF-κB activated intact (no castration) mouse prostate (24 genes).

Gene Symbol	Gene Title
ABCC5	"ATP-binding cassette, sub-family C (CFTR/MRP), member 5"
ANK3	"ankyrin 3, epithelial"
CD38	CD38 antigen
CD69	CD69 antigen
CTSB	cathepsin B
F5	coagulation factor V
GLUD1	glutamate dehydrogenase 1
IGFBP6	insulin-like growth factor binding protein 6
LOX	lysyl oxidase
MAPRE2	"microtubule-associated protein, RP/EB family, member 2"
MEF2C	myocyte enhancer factor 2C
MPP7	"membrane protein, palmitoylated 7 (MAGUK p55 subfamily member 7)"
MSMB	beta-microseminoprotein
NOV	nephroblastoma overexpressed gene
PDGFRA	"platelet derived growth factor receptor, alpha polypeptide"
PRIC285	CDNA sequence BC006779
RAD21	RAD21 homolog (S. pombe)
RAP2B	"RAP2B, member of RAS oncogene family"
RNASEL	"ribonuclease L (2', 5'-oligoisoadenylate synthetase-dependent)"
SFRP4	secreted frizzled-related sequence protein 4
ST6GAL1	"beta galactoside alpha 2,6 sialyltransferase 1"
TNFRSF11B	"tumor necrosis factor receptor superfamily, member 11b (osteoprotegerin)"
TRPS1	Trichorhinophalangeal syndrome I (human)
WISP1	WNT1 inducible signaling pathway protein 1

Supplementary Table 2. Matched human homolog genes list from wild-type androgen depleted (castrated) mouse prostate (228 genes).

Gene Symbol	Description
ABL1	c-abl oncogene 1, receptor tyrosine kinase
ADH1C	alcohol dehydrogenase 1C (class I), gamma polypeptide
AKR1B1	aldo-keto reductase family 1, member B1 (aldose reductase)
ALX3	ALX homeobox 3
ANG	angiogenin, ribonuclease, RNase A family, 5
ATP12A	ATPase, H+/K+ transporting, nongastric, alpha polypeptide
BCL2L2	BCL2-like 2
TNFRSF17	tumor necrosis factor receptor superfamily, member 17
BMP7	bone morphogenetic protein 7
CAPN1	calpain 1, (mu/I) large subunit
CCBL1	cysteine conjugate-beta lyase, cytoplasmic
MS4A3	membrane-spanning 4-domains, subfamily A, member 3 (hematopoietic cell-specific)
CHRM1	cholinergic receptor, muscarinic 1
COL4A3	collagen, type IV, alpha 3 (Goodpasture antigen)
COL4A4	collagen, type IV, alpha 4
CPB2	carboxypeptidase B2 (plasma)
CLDN4	claudin 4
CRYGD	crystallin, gamma D
CSRP1	cysteine and glycine-rich protein 1
DPP4	dipeptidyl-peptidase 4
SLC26A3	solute carrier family 26, member 3
DUSP9	dual specificity phosphatase 9
EIF4EBP2	eukaryotic translation initiation factor 4E binding protein 2
EIF4G2	eukaryotic translation initiation factor 4 gamma, 2
EN2	engrailed homeobox 2
ERN1	endoplasmic reticulum to nucleus signaling 1
EXTL1	exostoses (multiple)-like 1
FABP6	fatty acid binding protein 6, ileal
FGL1	fibrinogen-like 1
FPGS	folylpolyglutamate synthase
FUCA1	fucosidase, alpha-L- 1, tissue
GAD2	glutamate decarboxylase 2 (pancreatic islets and brain, 65kDa)
GATA3	GATA binding protein 3
GLRB	glycine receptor, beta
GRIN2B	glutamate receptor, ionotropic, N-methyl D-aspartate 2B
GRM8	glutamate receptor, metabotropic 8
GYS1	glycogen synthase 1 (muscle)
CFHR2	complement factor H-related 2

HLA-B	major histocompatibility complex, class I, B
HSD17B2	hydroxysteroid (17-beta) dehydrogenase 2
IGJ	immunoglobulin J polypeptide, linker protein for immunoglobulin alpha and mu polypeptides
ITPA	inosine triphosphatase (nucleoside triphosphate pyrophosphatase)
LRCH4	leucine-rich repeats and calponin homology (CH) domain containing 4
MAN1A1	mannosidase, alpha, class 1A, member 1
MLLT6	myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, Drosophila); translocated to, 6
MMP7	matrix metallopeptidase 7 (matrilysin, uterine)
MSMB	microseminoprotein, beta-
MT1E	metallothionein 1E
MT1F	metallothionein 1F
MYCL1	v-myc myelocytomatosis viral oncogene homolog 1, lung carcinoma derived (avian)
NINJ1	ninjurin 1
NKX3-1	NK3 homeobox 1
NODAL	nodal homolog (mouse)
SERPINA5	serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 5
PCK1	phosphoenolpyruvate carboxykinase 1 (soluble)
PDE6H	phosphodiesterase 6H, cGMP-specific, cone, gamma
PITX1	paired-like homeodomain 1
PKP2	plakophilin 2
PLXNB3	plexin B3
PNLIPRP2	pancreatic lipase-related protein 2
POU2AF1	POU class 2 associating factor 1
PSEN1	presenilin 1
QSOX1	quiescin Q6 sulfhydryl oxidase 1
RABIF	RAB interacting factor
RNASE1	ribonuclease, RNase A family, 1 (pancreatic)
RPS9	ribosomal protein S9
SORT1	sortilin 1
SFRP5	secreted frizzled-related protein 5
SH3GL2	SH3-domain GRB2-like 2
SLC4A1	solute carrier family 4, anion exchanger, member 1 (erythrocyte membrane protein band 3, Diego blood group)
SLC7A4	solute carrier family 7 (cationic amino acid transporter, y+ system), member 4
SLC9A2	solute carrier family 9 (sodium/hydrogen exchanger), member 2
SLC18A1	solute carrier family 18 (vesicular monoamine), member 1
SORD	sorbitol dehydrogenase
STAT5A	signal transducer and activator of transcription 5A
SULT1E1	sulfotransferase family 1E, estrogen-preferring, member 1
TACR3	tachykinin receptor 3
TAF4B	TAF4b RNA polymerase II, TATA box binding protein (TBP)-associated factor, 105kDa
TCF15	transcription factor 15 (basic helix-loop-helix)

TIMP4	TIMP metallopeptidase inhibitor 4
TP53	tumor protein p53
TRPC1	transient receptor potential cation channel, subfamily C, member 1
TST	thiosulfate sulfurtransferase (rhodanese)
ZIC3	Zic family member 3 (odd-paired homolog, Drosophila)
ZBTB16	zinc finger and BTB domain containing 16
SLC30A2	solute carrier family 30 (zinc transporter), member 2
GLRA3	glycine receptor, alpha 3
DGCR6	DiGeorge syndrome critical region gene 6
FZD7	frizzled homolog 7 (Drosophila)
PLA2G6	phospholipase A2, group VI (cytosolic, calcium-independent)
ADAM7	ADAM metallopeptidase domain 7
TRIM24	tripartite motif-containing 24
BRSK2	BR serine/threonine kinase 2
SYT7	synaptotagmin VII
ANGPTL1	angiopoietin-like 1
SYNGR3	synaptogyrin 3
FIBP	fibroblast growth factor (acidic) intracellular binding protein
SLC4A8	solute carrier family 4, sodium bicarbonate cotransporter, member 8
BAG4	BCL2-associated athanogene 4
PTGES	prostaglandin E synthase
PRDX6	peroxiredoxin 6
NUP155	nucleoporin 155kDa
SH3PXD2A	SH3 and PX domains 2A
KIAA0232	KIAA0232
TRIM14	tripartite motif-containing 14
SETDB1	SET domain, bifurcated 1
TELO2	TEL2, telomere maintenance 2, homolog (S. cerevisiae)
HS3ST2	heparan sulfate (glucosamine) 3-O-sulfotransferase 2
REC8	REC8 homolog (yeast)
PREB	prolactin regulatory element binding
KCNMB2	potassium large conductance calcium-activated channel, subfamily M, beta member 2
TESK2	testis-specific kinase 2
OLFM4	olfactomedin 4
SORBS1	sorbin and SH3 domain containing 1
FUT9	fucosyltransferase 9 (alpha (1,3) fucosyltransferase)
IQGAP2	IQ motif containing GTPase activating protein 2
C1QL1	complement component 1, q subcomponent-like 1
KDELR1	KDEL (Lys-Asp-Glu-Leu) endoplasmic reticulum protein retention receptor 1
OS9	osteosarcoma amplified 9, endoplasmic reticulum lectin
WWP2	WW domain containing E3 ubiquitin protein ligase 2

CAPN10	calpain 10
NISCH	nischarin
SBNO2	strawberry notch homolog 2 (Drosophila)
HABP4	hyaluronan binding protein 4
DKK1	dickkopf homolog 1 (Xenopus laevis)
TRIM2	tripartite motif-containing 2
GSPT2	G1 to S phase transition 2
C9orf5	chromosome 9 open reading frame 5
ZNF473	zinc finger protein 473
PNKD	paroxysmal nonkinesigenic dyskinesia
ACOT11	acyl-CoA thioesterase 11
CCDC9	coiled-coil domain containing 9
EHF	ets homologous factor
SRPK3	SRSF protein kinase 3
ATP2C1	ATPase, Ca++ transporting, type 2C, member 1
CACNG4	calcium channel, voltage-dependent, gamma subunit 4
CCDC22	coiled-coil domain containing 22
COMMD9	COMM domain containing 9
SEC61A1	Sec61 alpha 1 subunit (S. cerevisiae)
BOLA1	bolA homolog 1 (E. coli)
DERA	deoxyribose-phosphate aldolase (putative)
TFB1M	transcription factor B1, mitochondrial
HSD17B14	hydroxysteroid (17-beta) dehydrogenase 14
USP53	ubiquitin specific peptidase 53
SDK2	sidekick homolog 2 (chicken)
AFTPH	aftiphilin
QPCTL	glutaminyl-peptide cyclotransferase-like
PDPR	pyruvate dehydrogenase phosphatase regulatory subunit
ACOXL	acyl-CoA oxidase-like
ELAC1	elaC homolog 1 (E. coli)
MYO5C	myosin VC
AJAP1	adherens junctions associated protein 1
NXF2	nuclear RNA export factor 2
TDRD1	tudor domain containing 1
C16orf61	chromosome 16 open reading frame 61
BBX	bobby sox homolog (Drosophila)
PLEKHH1	pleckstrin homology domain containing, family H (with MyTH4 domain) member 1
KIAA1549	KIAA1549
RINT1	RAD50 interactor 1
CELA2A	chymotrypsin-like elastase family, member 2A
MRPL17	mitochondrial ribosomal protein L17

NEUROG2	neurogenin 2
SLC28A3	solute carrier family 28 (sodium-coupled nucleoside transporter), member 3
MCCC2	methylcrotonoyl-CoA carboxylase 2 (beta)
HIF3A	hypoxia inducible factor 3, alpha subunit
NDST4	N-deacetylase/N-sulfotransferase (heparan glucosaminyl) 4
NUCKS1	nuclear casein kinase and cyclin-dependent kinase substrate 1
AACS	acetoacetyl-CoA synthetase
ZBTB10	zinc finger and BTB domain containing 10
YIPF2	Yip1 domain family, member 2
ADIPOR2	adiponectin receptor 2
MORN1	MORN repeat containing 1
GRHL2	grainyhead-like 2 (Drosophila)
TRABD	TraB domain containing
LRRC27	leucine rich repeat containing 27
STARD5	StAR-related lipid transfer (START) domain containing 5
CYB5B	cytochrome b5 type B (outer mitochondrial membrane)
TRIM7	tripartite motif-containing 7
ZNF503	zinc finger protein 503
ATAD1	ATPase family, AAA domain containing 1
KNDC1	kinase non-catalytic C-lobe domain (KIND) containing 1
SCRT2	scratch homolog 2, zinc finger protein (Drosophila)
CEACAM21	carcinoembryonic antigen-related cell adhesion molecule 21
OTOP2	otopetrin 2
PEX11G	peroxisomal biogenesis factor 11 gamma
B3GNT7	UDP-GlcNAc:betaGal beta-1,3-N-acetylglucosaminyltransferase 7
CACNA2D4	calcium channel, voltage-dependent, alpha 2/delta subunit 4
ARHGAP18	Rho GTPase activating protein 18
RP1L1	retinitis pigmentosa 1-like 1
ERI2	ERI1 exoribonuclease family member 2
GLB1L3	galactosidase, beta 1-like 3
OSBPL6	oxysterol binding protein-like 6
RAB39B	RAB39B, member RAS oncogene family
RFFL	ring finger and FYVE-like domain containing 1
SP7	Sp7 transcription factor
C13orf26	chromosome 13 open reading frame 26
DEGS2	degenerative spermatocyte homolog 2, lipid desaturase (Drosophila)
MSI2	musashi homolog 2 (Drosophila)
TATDN3	TatD DNase domain containing 3
LYPD6	LY6/PLAUR domain containing 6
TMEM42	transmembrane protein 42
UROC1	urocanase domain containing 1

IL17RE	interleukin 17 receptor E
ADHFE1	alcohol dehydrogenase, iron containing, 1
TMEM56	transmembrane protein 56
MBOAT1	membrane bound O-acyltransferase domain containing 1
WBP2NL	WBP2 N-terminal like
SGMS2	sphingomyelin synthase 2
C6orf81	chromosome 6 open reading frame 81
C7orf47	chromosome 7 open reading frame 47
ATP6V1C2	ATPase, H+ transporting, lysosomal 42kDa, V1 subunit C2
SLC25A30	solute carrier family 25, member 30
WDR27	WD repeat domain 27
TTLL10	tubulin tyrosine ligase-like family, member 10
SERPINA11	serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 11
THEM5	thioesterase superfamily member 5
C1orf185	chromosome 1 open reading frame 185
RNF149	ring finger protein 149
C14orf39	chromosome 14 open reading frame 39
KRTAP19-3	keratin associated protein 19-3
NLRP10	NLR family, pyrin domain containing 10
TSPAN33	tetraspanin 33
KIAA2022	KIAA2022
BARHL2	BarH-like homeobox 2
PTAR1	protein prenyltransferase alpha subunit repeat containing 1
WIPF3	WAS/WASL interacting protein family, member 3
SPINK8	serine peptidase inhibitor, Kazal type 8 (putative)
NXF2B	nuclear RNA export factor 2B

Supplementary Table 3. Stratification of 77 PCa patients who had lymph node metastasis at the time of RRP surgery based on the NARP21 gene signature and clinical outcome (systemic metastasis).

Years (post surgery)	3	5	7	10	More then 10
Favorable-prognosis Group (10/23 cases)(%)	3 (30 %)	5 (20%)	6 (17.6%)	8 (18.6%)	10 (21.3%)
Poor-prognosis Group (37/54 cases)(%)	7 (70%)	20 (80%)	28 (82.4%)	35 (81.4%)	37 (78.7%)
Total (47/77)	10 (13%)	25 (32.5%)	34 (44.2%)	43 (55.8%)	47 (61%)

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