Supplemental Figure Legends

Supplemental Figure 1. Copy number alterations of AAPC exome set. A)
GISTIC2.0 cytoband copy number calls were arranged by chromosome.
Unsupervised hierarchical clustering of global copy number changes across the
AAPC WES cohort (n=102). B) Comparison of frequencies of copy number
alterations at loci that are frequently affected between the AAPC cohort and TCGA
(n=487). Fisher's exact test was performed for each copy number alteration and p-
values reported, * indicates p <0.05.

Supplemental Figure 2. Copy number analysis grouped by Gleason scores
Comparison of frequencies of copy number alterations at loci that are frequently
affected between the AAPC cohort and TCGA grouped by a lower SCNA threshold
(log2 copy ratio > 0.1 and < 0.-1) in the AAPC cohort and Gleason score categories 6,
7 (3+4) and (4+3) and 8+. Fisher's exact test was performed for each copy number
alteration and p-values reported.

**Supplemental Figure 3. Recurrent somatic copy number alterations in the
AAPC cohort.** GISTIC2.0 plots of significant focal amplifications (left and red) and
deletions (right and blue) across the AAPC exome cohort (Supplemental Table 6).

**Supplemental Figure 4. Analysis of fraction of copy number altered genome in
the AAPC exome and TCGA cohorts.** Boxplots showing fraction of genome with
copy number alterations. The center line of the box represents the median. Mean
fraction of genome altered and the sample size of the dataset are annotated on top
of each boxplot. Each filled circle represents an individual tumor within a dataset.
For the AAPC cohort, boxplots for a lower threshold (log2 copy ratio > 0.1 and < -
0.1) and higher threshold (log2 copy ratio > 0.3 and < -0.3) for SCNA are shown.

**Supplemental Figure 5. Prostatic adenocarcinomas with unique *ERF* mutations
demonstrate decreased RNA expression by RNA ISH.** Case STID17668 harbors
ERF Y89C missense mutation. The benign glands (left) show visible signals. In
contrast, visible signals are scarce in tumor glands (right). Case STID21576 harbors
ERF in-frame deletion (p.371_372SS>S). Similar to case STID17668, benign glands
(left) show detectable signals but these are not visible in the tumor glands (right).
RNA expression analysis of these tumors was included in Fig. 2 A-C of the main text.
Smaller squared areas of each panel are seen at higher magnification in the right
upper corner. (Original magnification: 100x under oil immersion).

Supplemental Figure 6. IGV screenshots of validated *ERF* mutations. Fluidigm
Access Array sequencing reads of designated mutations at the *ERF* locus.

**Supplemental Figure 7. Frequencies of mutations in *ERF* across several
published prostate cancer sequencing cohorts.**

Supplemental Figure 8. Visualization of deletions at chr19q13.2 in the TCGA cohort and analysis of SU2C/CRPC dataset for *ERF* mutations A) IGV screen shot of TCGA prostate cancer samples with focal deletions at chr19q13.2 harboring the genes *ERF* and *CIC*. B) Stickplot of mutations in *ERF* identified in the TCGA, SU2C and AAPC cohorts. C) Mutations and copy number deletions of *ERF* in the Stand Up 2 Cancer Castration-Resistant Prostate Cancer (CRPC) cohort (n=269)

Supplemental Figure 9. Association ERF deletion status with pathologic features. Deletions at the ERF locus are correlated with higher Gleason scores.

Supplemental Figure 10. Knockdown of ERF mRNA in prostate cancer cell lines and an immortalized prostate epithelial cell line. qRT-PCR results of ERF knockdown using lentiviral shRNAs in prostate cancer cell lines (VCaP, PC-3, and LNCaP) and an immortalized prostate epithelial cell line (LHS-AR) with a series of shRNAs directed against ERF. Samples were measured in quadruplicate.

Supplemental Figure 11. Knockdown of ERF in PC-3 cell line augments invasion and tumor xenograft growth. A) Cell invasion assays showed increased invasion in ERF knockdown cells vs control knockdown (shERF 1 vs control, shERF 2 vs control, *p <0.05, student's T test). Samples were measured in triplicate. B) PC-3 cells expressing hairpins against ERF or control were cultured for 9-10 days followed by crystal violet staining. Quantitation of crystal violet uptake in ERF knockdown cells vs. control (* p <0.05, student's T test) Samples were measured in triplicate. C) Tumor xenograft studies of PC-3 cells stably expressing shRNAs against ERF vs control. Average-fold change of tumor volume is depicted over time.

Supplemental Figure 12. Overexpression of ERF in PC-3 cell line is associated with a growth inhibitory effect. A) Focus formation assay of PC-3 cell line overexpressing ERF vs LacZ control and ERF Y89C mutant (ERF vs LacZ, ERF-Y89C vs ERF, *p<0.05; student's T test) Mean and standard error of three replicates is shown B) Soft agar assay of PC-3 cell line over-expressing ERF. (ERF vs LacZ, ERF-Y89C vs ERF, * p<0.05; student's T test) Mean and standard error of three replicates is shown. C. Western blot analysis of ERF and ERF mutant Y89C overexpression.

Supplemental Figure 13. Knockdown of ERF in RWPE-1 and LNCaP cell lines contributes to growth proliferation. A) Focus formation assay of ERF knockdown in RWPE-1 cells vs control shRNA. Cells expressing hairpins against ERF or control were cultured for 9 days followed by crystal violet uptake and quantitation. (* p<0.05; student's T test) Mean and standard error of three replicates is shown B) Short-term (5-day) growth proliferation assay (CellTiter-Glo) of ERF knockdown in RWPE-1 cells vs control shRNA cells. (* p<0.05; student's T test) C) Cell invasion assay of ERF knockdown vs control (shERF 1 vs control, * p<0.05; shERF 2 vs control, p=0.15). D) Western blot analysis of ERF knockdown in RWPE-1 cells. E)

Focus formation assay of ERF knockdown in LNCaP cells vs control shRNA. LNCaP cells expressing hairpins against ERF or control were cultured for 8 days followed by crystal violet uptake and quantitation. (* p<0.05; student's T test) Mean and standard error of three replicates is shown. F) Short-term (5-day) growth proliferation assay of ERF knockdown in LNCaP cells vs control shRNA cells. (* p<0.05; student's T test) G) Cell invasion assay of ERF knockdown vs control (shERF 1 vs control, p=0.85; shERF 2 vs control, p=0.37). H) Western blot analysis of ERF knockdown in LNCaP cells.

Supplemental Figure 14. Overexpression of ERF in DU-145 cell line. A) Overexpression of ERF is associated with growth inhibition in a low-attachment assay (GILA). (* p<0.05; student's T test) Samples were measured in six replicates. B) Overexpression of ERF is associated with a reduction in cell invasion (* p<0.05; student's T test) Samples were performed in triplicate C) Overexpression of ERF is not associated with a change in growth proliferation in a focus formation assay (ERF vs LacZ control, p=0.78; student's T test) Mean and standard error of three replicates is shown. D) Western blot analysis of ERF overexpression in the DU-145 cell line.

Supplemental Figure 15.   Association of LHS-AR ERF KD signature in the CCLE and CRPC datasets. The ERF KD signature profile generated from the LHS-AR cell line is significantly associated with an ERG expression signature and IPA ETV1_UP signature in the CCLE data set. The ERF KD signature profile generated from the LHS-AR cell line is also significantly associated with the ERG expression signature profile in the CRPC data set.

Supplemental Figure 16. *ERF* **mutation and deletions are mutually exclusive from ERG rearrangement events in the published TCGA cohort (n=333) (cbioportal.org).**

Supplemental Figure 17.   ERF KD signature in setting of ERF overexpression and correlation with Gleason score. A) Overexpression of ERF is associated with a diminished ERF KD signature. The bar plots in this figure show: the increase of expression of ERF in the DU-145 ERF WT samples with respect to DU-145 Cntrl (LacZ); and the relative change of ssGSEA enrichment scores between the DU-145 Cntrl (LacZ) and ERF WT samples. The introduction of ERF WT in DU-145 increases the expression of ERF and reduces the enrichment of the shRNA ERF and ERG signatures shown in Figure 4. B) Association of ERF signature with Gleason score in the TCGA cohort. An ERF KD signature is correlated with higher Gleason scores.

Supplemental Figure 18. Association of LHS-AR ERF KD signature projected across the CCLE. The shERF_LnCAP_UP_VCAP_UP_signature profile generated from the LHS-AR cell line was projected across the CCLE and is significantly associated with the NELSON_RESPONSE_TO_ANDROGEN_UP signature and was the top hit among the signature profiles interrogated.

Supplemental Figure 19. Mutation plot of combined AAPC (n=102) + TCGA (n=457) analysis. Significantly mutated genes as determined by MutSigCV (FDR q < 0.1) are shown with alteration type and frequency.

Supplemental Figure 20. *ERF* **nucleotide sequence map**. Colored nucleotides indicating unique target probe oligonucleotides used for RNA ISH assay.