Supplementary Figure S1: AR-E1 and AR-CE3 RISH assays are specific. (A) AR-E1 is undetectable in AR-negative, untransfected M12 cells. Transfection with AR-FL construct is detectable by AR\_E1, but not by AR\_CE3 RISH as expected in M12 cells. (B) Untransfected HeLa cells have low to undetectable levels of AR-E1. Transfection with AR-E1-containing construct leads to highly detectable AR\_E1 by RISH. (C) RISH with probes detected human AR\_E1 sequence is negative in murine prostate. As a positive control for RNA quality, RISH for c-myc is positive in these transgenic mice which express the c-myc transgene.

Supplementary Figure S2: AR-E1 RISH signals show greater nuclear localization compared to other genes and are decreased with RNase pre-treatment of slides prior to RISH assay. (A) AR-E1 RISH signal shows dramatic nuclear localization (arrows) with some cytoplasmic signal (arrowheads) across benign and malignant primary prostate tumors, including some that have been treated with androgen deprivation therapy. In contrast, other genes such as positive control gene PPIB (cyclophilin B) show only cytoplasmic signal. (B) Both intranuclear and cytoplasmic signals are markedly decreased and nearly undetectable after RNase, confirming both signals correspond to RNA. (C) qRT-PCR analysis of LNCaP and LAPC4 cells performed on nuclear and cytosolic fractions of RNA (accompanying immunoblot confirms appropriate laminin and GAPDH enrichment in each fraction) reveals abundance of unspliced AR RNA in the nuclear compartment of both cell lines, while spliced AR message is present in both compartments.

Supplementary Figure S3: Correlation of relative quantified AR-E1 and AR-CE3 RISH labeling with one another and with relative AR-FL and AR-V7 expression by RT-PCR in metastases from Figure 3. AR-E1 by RISH and AR-V7 by RT-PCR are significantly correlated (A), as are AR-FL vs AR-V7 by RT-PCR (B) and AR-E1 vs AR-CE3 by RISH (C).

Supplementary Figure S4: AR-E1 and AR-CE3 RISH assay digital image quantification shows minimal inter-observer variability. To examine inter-observer variability in scoring, a second, independent operator (Observer 2) circled tumor areas and performed the scoring for AR-E1 and AR-CE3 in Frida across a subset of the autopsy samples and compared with the original values created by Observer 1 for Supplementary Figure S3C. In one experiment, the second operator made their own thresholds for brown and blue intensity scoring and in another experiment, the second operator used the intensity thresholds set by the first operator. In both cases there was negligible variability between operators, with highly correlated values across all specimens.

Supplementary Figure S5: Correlation of AR protein by IHC and AR-E1 RISH labeling in high grade primary tumors and benign prostate epithelium sampled on needle biopsy (Figure 5). (A) AR IHC quantified as brown/(brown+blue) pixels vs AR-E1 quantification shows a weak but significant correlation among primary tumors. (B) Quantified AR-IHC from (A) is higher in tumor compared to paired benign epithelium. (C) AR-IHC quantified as a modified H-score (see Methods) shows an insignificant correlation with quantified AR-E1 in the same samples. (D) There is a significant trend towards increased AR IHC H-scores in tumor compared to paired benign epithelium.

Supplementary Figure S6: Comparison of AR RISH in bone and soft tissue metastases from the same patient.