**Figure W1.** Endothelial cells and lymphocytes express endogenous ERG protein by IHC but not *ERG* rearrangements. The endothelial cells of small vessels cut in cross section (A) and longitudinally (B) exhibit ERG protein expression. (C and D) Lymphocytes surrounding benign glands also exhibit positive ERG staining. Representative FISH images of an endothelial cell (C) and a lymphocyte (D) showing lack of *ERG* rearrangement (IHC 40×, FISH 60×).
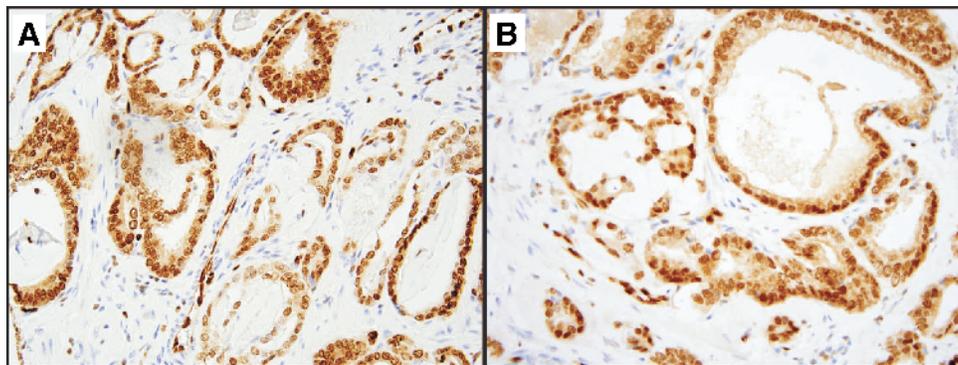


**Figure W2.** ERG expression in rare cases without a detectable *ERG* rearrangement. Examples of two discrepant cases (A and B) with ERG protein expression without *ERG* rearrangement by FISH.
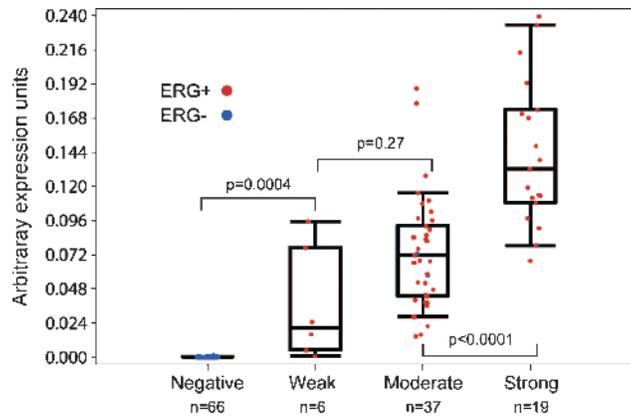
**Figure W3.** Significant association between interpretation of ERG protein expression by manual and automated image analyses. Cases from the WCMC TMA were identified as *ERG* rearranged (ERG+) or wild type (ERG−) by FISH, and ERG protein was assessed manually by study pathologists as negative, weak, moderate, or strong in neoplastic cells. Automated image analysis was also performed for ERG expression, and results are plotted stratified on manual staining intensity. No significant difference was seen between weak and moderate expressions after Bonferroni correction. Subjective evaluation correctly classified all ERG-negative cases. Boxes indicate the first, median, and third quartiles, and whiskers indicate the 10th and 90th percentiles.
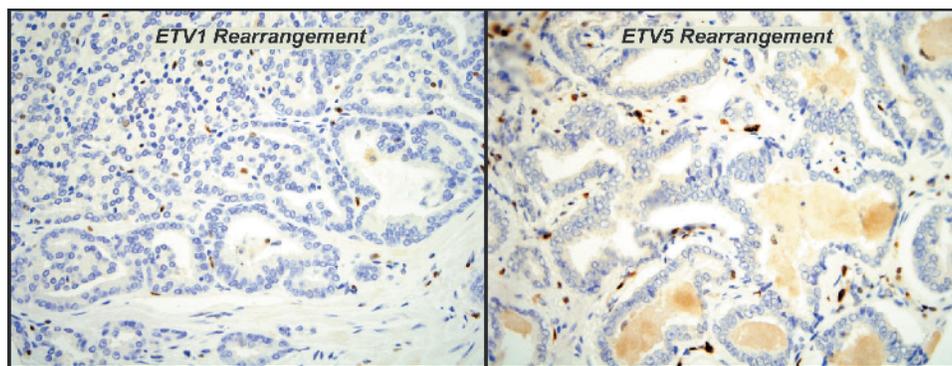


**Figure W4.** Anti-ERG antibody does not cross-react with other ETS rearrangements in prostate cancer. Representative examples of other ETS rearrangement prostate cancers without ERG protein expression. The first case harbors an *ETV1* rearrangement (unknown 5' partner) and the second case harbors an *SLC45A3-ETV5* gene fusion. In the study, we identified four cases with ETV1, one case with ETV5, and one case with ETV4 rearrangements, all of which lacked ERG expression.
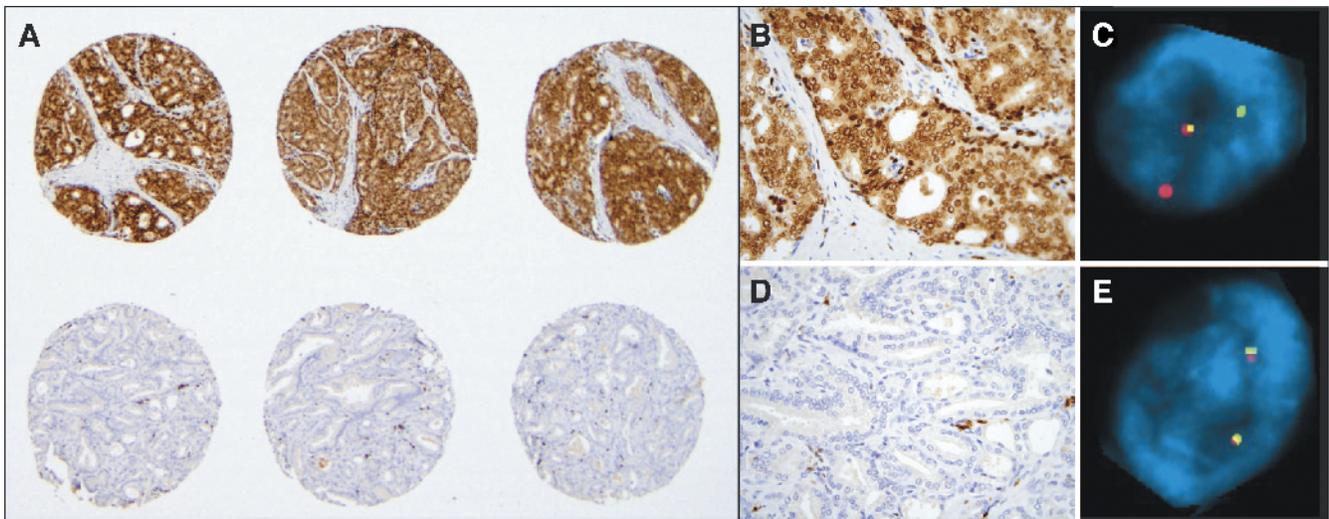
**Figure W5.** Molecular heterogeneity of multifocal localized prostate cancer demonstrated by ERG protein expression. (A) Two sets of three TMA cores sampled from two discrete tumors from the same patient show distinct patterns of ERG protein expression. The three cores on the top exhibit intense ERG protein expression (B), and the three on the bottom are negative for ERG protein expression (D) with the corresponding FISH assays demonstrating *ERG* rearrangement through insertion (C) and the absence of *ERG* rearrangement (E), respectively. IHC images were taken at ×20 objective magnification (A) and at ×40 magnification (B, D). FISH images (C, E) were taken at ×60 magnification.