**Supplement Materials and Methods**

**Tissue Microarray analysis of TOP2A and EZH2 (DFCI cohort)**

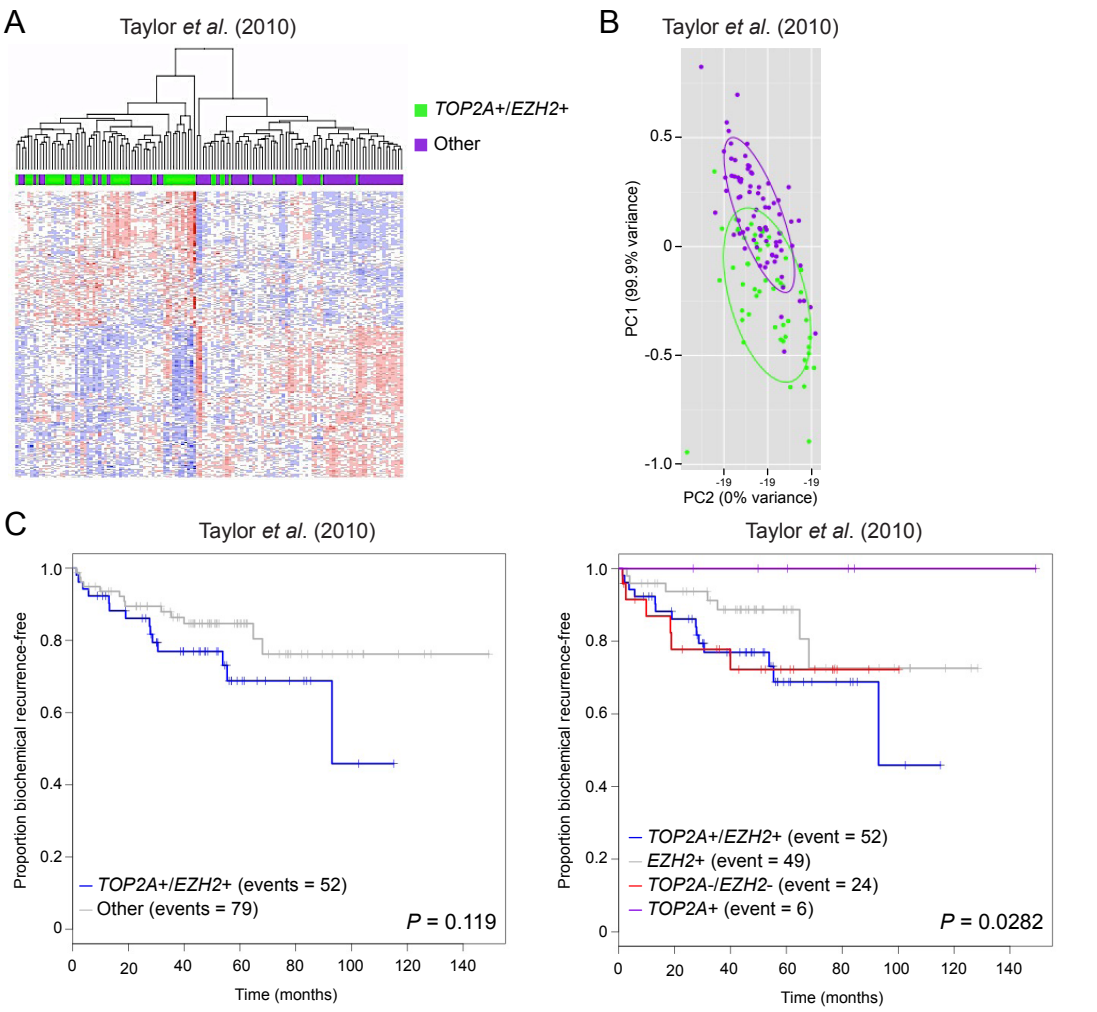
*TSA-plus Fluorescence Immunohistochemistry*

A total of 131 formalin-fixed, paraffin-embedded prostate cancer tissues were obtained from consented patients at Brigham and Women's Hospital (Supplement Figure 5) and utilized to construct the 3 TMA's, each sample is represented by up to 3 tissue microarray cores for tumor. A multiplexed tyramide signal amplification method was performed on 4-μm sections of the TMA for detection of EZH2 and TOP2A protein. Alpha-methylacyl-CoA racemase (AMACR) staining was also implemented to mask for tumor epithelial gland. The staining approach consists of a multi-step protocol of sequential TSA-amplified immunofluorescence labels for EZH2, TOP2A and AMACR, and a 4',6-diamidino-2-phenylindole (DAPI) counterstain. Briefly the sections were deparaffinized and hydrated; prior to each immunofluorescence labeling, EZH2, TOP2A and AMACR antigens are retrieved with a single microwave step. Each labeling cycle consists of application of a primary antibody, and a secondary antibody conjugated to horse radish peroxidase (HRP), and TSA conjugated to a fluorophore. The slides were consecutively incubated with the antibody against EZH2 (Clone 6A10, Leica NCL-L-EZH2) at a dilution of 1:200, TOP2A (Clone 3F6, Leica, NCL-TOPOIIA) at a dilution of 1:100 and AMACR (P504S, Clone 13H4, ZETA Corporation #Z2001) at a dilution of 1:250 for 30 mins respectively. TSA reagents were obtained from PerkinElmer, Inc. TSA conjugated fluorescein was used FITC for EZH2; CY3 for TOP2A and CY5 for AMACR, respectively. Prostate cancer tissue from RP specimens was used as positive controls for EZH2, TOP2A and AMACR; Omission of the primary antibody was utilized as a negative control. Additionally, tonsil was used as a positive control for EZH2 and TOP2A, skeletal muscle as negative/weak control for EZH2 and adipose tissue as negative/weak control for TOP2A.

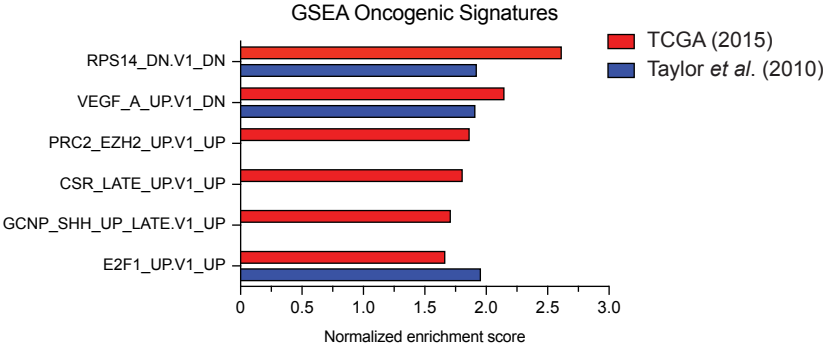
Single staining of each antibody and DAPI only counterstained slides were used for spectral library construction.

*Spectral Imaging*

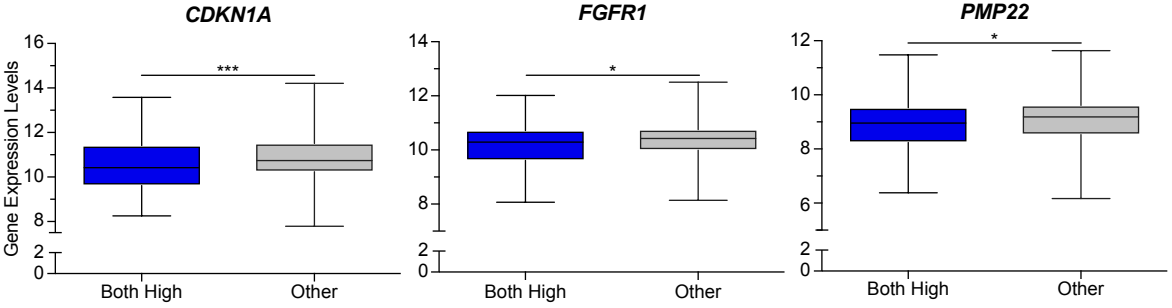
Each TSA-FIHC stained TMA was scanned on a Vectra 2 multispectral slide analysis system from Perkin Elmer using the 20x objective to obtain high power field images of individual cores. Exposure times for the mercury-halide light source at 10% power output were optimized for each target-dye combination; nuclei-DAPI, EZH2-FITC, TOP2A-CY3, AMACR-CY5. Using DAPI filter for autofocus, the full slide TMA was surveyed at 4x to identify and align cores that were accepted for automated 20x image acquisition. Single stained control slides were imaged with the multiplex filters for use in generating the spectral library and blank control for autofluorescence. Using inForm image analysis software from Perkin Elmer, the spectral library was built and applied to unmix the fluorescence spectra. An inForm algorithm was configured for trainable tissue segmentation, DAPI based cell segmentation and AMACR based Tumor phenotyping. Tissue segmentation was implemented to distinguish glands from stroma. Gland tissue was classified and binned into "tumor" and "other" based on pathology review and use of AMACR positive expression as a tumor-specific mask, employed for phenotyping with the Nuance software tool. The normalized total expression intensity was recorded on a per pixel basis. The reported mean for a given cell was the average intensity of all the normalized total pixel values in each nucleus. The algorithm was then applied to all the images contained within the TMA. The processed batch was reviewed and edited for the quality of tissue segmentation and tumor phenotyping, and status of each core matched to the definition from the TMA map. Case level statistics for each patient across the 3 TMA's were calculated nuclear EZH2 were compared to nuclear TOP2A mean expression levels in all cases that were AMACR positive.



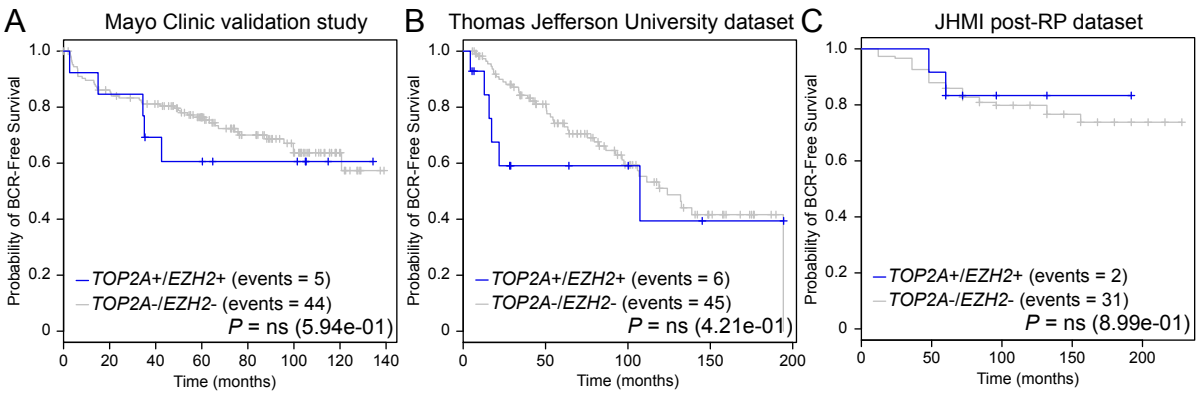

**Supplement Figure 1:** Taylor *et al*. (2010) data indicate that concurrent *TOP2A* and *EZH2* expression identify primary PCa patients with a more aggressive disease. **(A)** Unsupervised clustering analysis of Taylor *et al*. (2010) data demonstrates *TOP2A*+/*EZH2*+ patients (green) tightly cluster apart from other patients (purple) based on their differentially expressed genes (DEGs). **(B)** Unique DEGs between *TOP2A*+/*EZH2*+ and other patients was validated by PCA. **(C)** Kaplan-Meier analysis reveals that patients with concurrent high *TOP2A* and *EZH2* expression (*TOP2A*+/*EZH2*+) have a faster progression to biochemical recurrence when compared to other conditions (*right*).



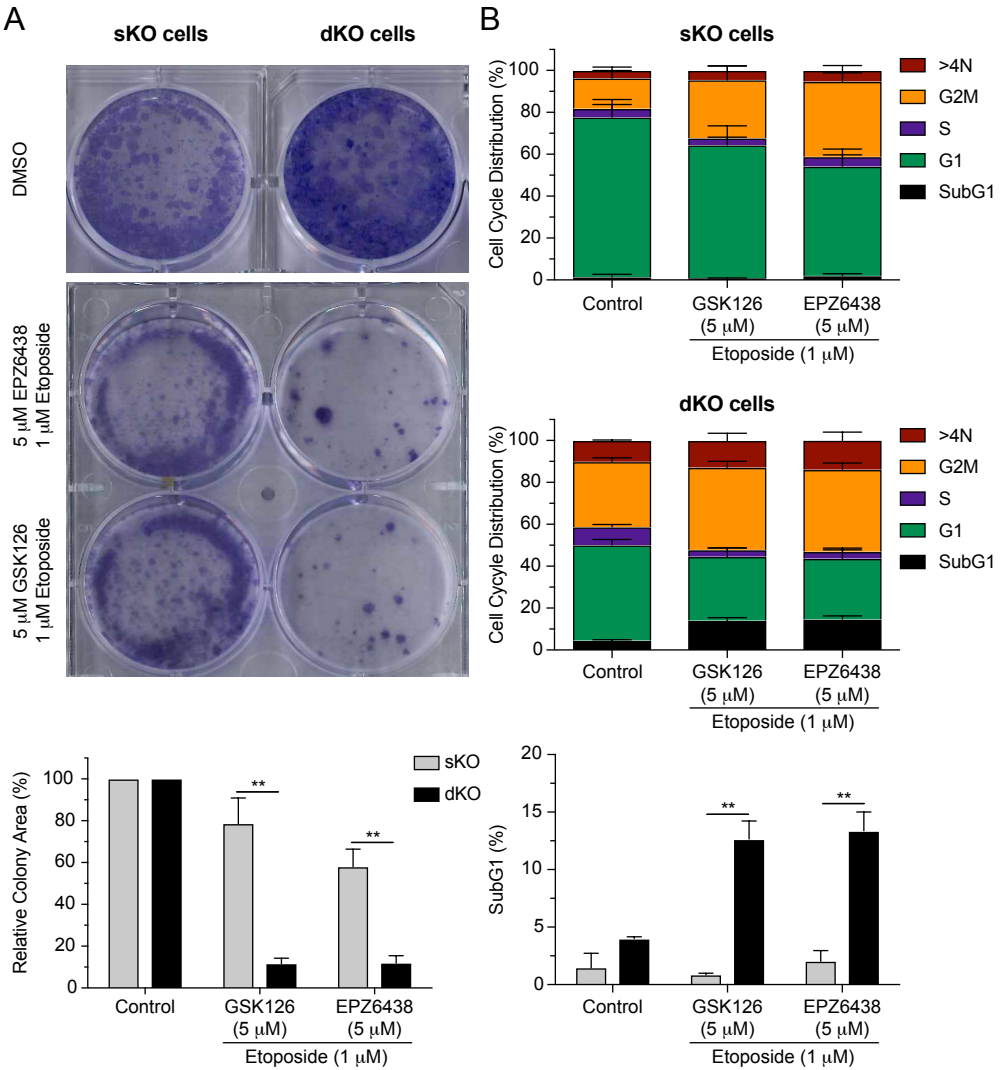

**Supplement Figure 2:** GSEA for oncogenic signatures in primary human PCa revealed statistically significant overlapping for gene signatures involving VEGF and E2F1 signaling from the TCGA (2015) and Taylor *et al*. (2010) datasets. ($P < 0.05$ and FDR < 0.15; Supplement Table 3).



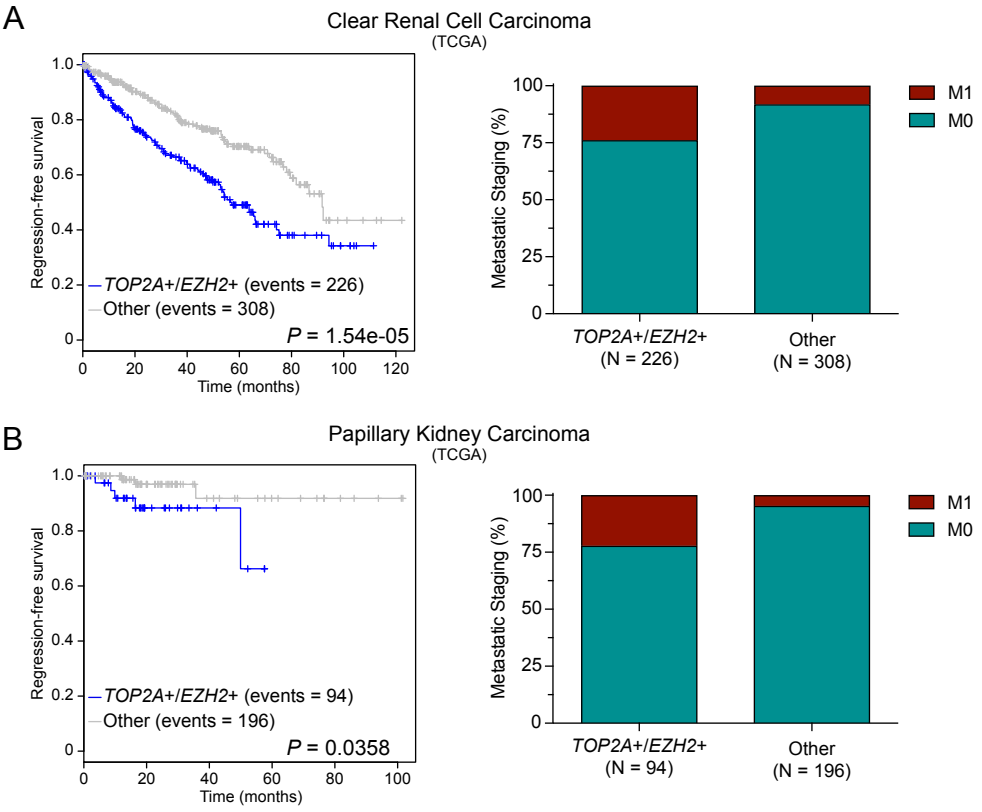

**Supplement Figure 3:** Genes that have been demonstrated to be up-regulated in indolent primary PCa are down regulated in TCGA (2015) patients with concurrent up-regulation of *TOP2A* and *EZH2* (*TOP2A*+/*EZH2*+) (Mann-Whitney; * $P < 0.05$; *** $P < 0.001$).




**Supplement Figure 4:** Concurrent high *TOP2A* and *EZH2* expression in primary prostatic tumors (*TOP2A*+/*EZH2*+) is not associated to a shorter time to biochemical recurrence when compared to *TOP2A*-/EZH2- patients included in the **(A)** Mayo Clinic Validation study, **(B)** Thomas Jefferson University dataset or the **(C)** Johns Hopkins University post-RP dataset.




**Supplement Figure 5: (A)** Combination therapy induces loss of clonogenicity specific to dKO cells (*top*: representative experiment; unpaired *t* test; ** $P < 0.01$, triplicate, mean ±SEM). **(B)** Cell cycle analysis indicates that combination therapy targeting both EZH2 (GSK126 or EPZ6438, 5 μM) and TOP2A (Etoposide, 1 μM) induces G2M arrest in both sKO and dKO cell lines but results in a significant increase in apoptosis as indicated by SubG1 accumulation only in dKO (unpaired *t* test; ** $P < 0.01$, triplicate, mean ±SEM).




**Supplement Figure 6:** *TOP2A* and *EZH2* co-expression can select for metastatic disease progression and shorter regression-free survival in the TCGA's **(A)** Clear Renal Cell Carcinoma and the **(B)** Papillary Kidney Carcinoma datasets. (Abbreviations: no distant metastasis, M0; distant metastasis, M1)