

## Background

Folate (vitamin B9) is an essential vitamin required to generate S-adenosylmethionine (SAM), deoxynucleotides (dNTPs), and polyamines for the regulation of methylation status, DNA synthesis, and other cellular processes. Luminal prostatic epithelial cells are unique in that they secrete high levels of acetylated polyamines into the lumen resulting in the upregulation of polyamine metabolism (1). As a consequence, prostatic epithelial cells require relatively high levels of folate to maintain SAM and dNTP pools due to increased flux through the polyamine biosynthetic pathway(2). This unique characteristic of prostatic epithelial places stress on one-carbon metabolism and the methionine cycle in order to maintain these pools. More importantly, this stress is increased in PCA due to increased polyamine biosynthesis, DNA synthesis, and proliferation. Therefore, these metabolic pathways are attractive targets for development of therapeutic strategies. **We hypothesize that adding more stress to an already strained system by targeting two different aspects of these metabolic pathways will result in the development of novel therapeutic strategies to complement existing therapies with the goal of enhancing the extent and/or duration of clinical benefit.**

The first target is spermine-spermidine acetyltransferase (SSAT), which regulates polyamine catabolism and, as a consequence, biosynthesis. Polyamine biosynthesis can be upregulated by increasing polyamine export through the upregulation of SSAT. Upregulating polyamine biosynthesis will draw SAM away from the methionine cycle, potentially causing deficits in methionine, SAM and dNTP pools, which homeostatic mechanisms will attempt to correct, thereby placing further stress on the system. The polyamine analogue, BENSpm, increases the activity of SSAT and therefore polyamine export and catabolism, resulting in increased use of SAM for the production of polyamines.

The second target is methylthioadenosine phosphorylase (MTAP), which is the rate-limiting enzyme involved in the methionine salvage pathway (MSP). This pathway is critical to reclaim the one-carbon unit consumed by polyamine biosynthesis back into the methionine cycle in order to replenish methionine and SAM pools thereby helping to maintain homeostatic control. This enzyme is often deleted in other cancers, but is highly protected in PCA. We have demonstrated that shRNA knockdown of MTAP blocks PCA cell line growth *in vitro* and *in vivo*. Current studies are focused on the use of a pharmacological inhibitor of MTAP (MTDIA), in cell lines, xenografts, and mouse models of PCA.

## Hypothesis

The combination of BENSpm (to increase metabolic stress) and MTDIA (to prevent mitigation of that stress) will lead to crisis and apoptosis in prostate cancer cells. Our long term goal is to take advantage of this in a clinical setting to **enhance the extent and/or duration of clinical benefit of current therapies such as androgen deprivation therapy.**

## Folate Metabolism and Our Key Targets

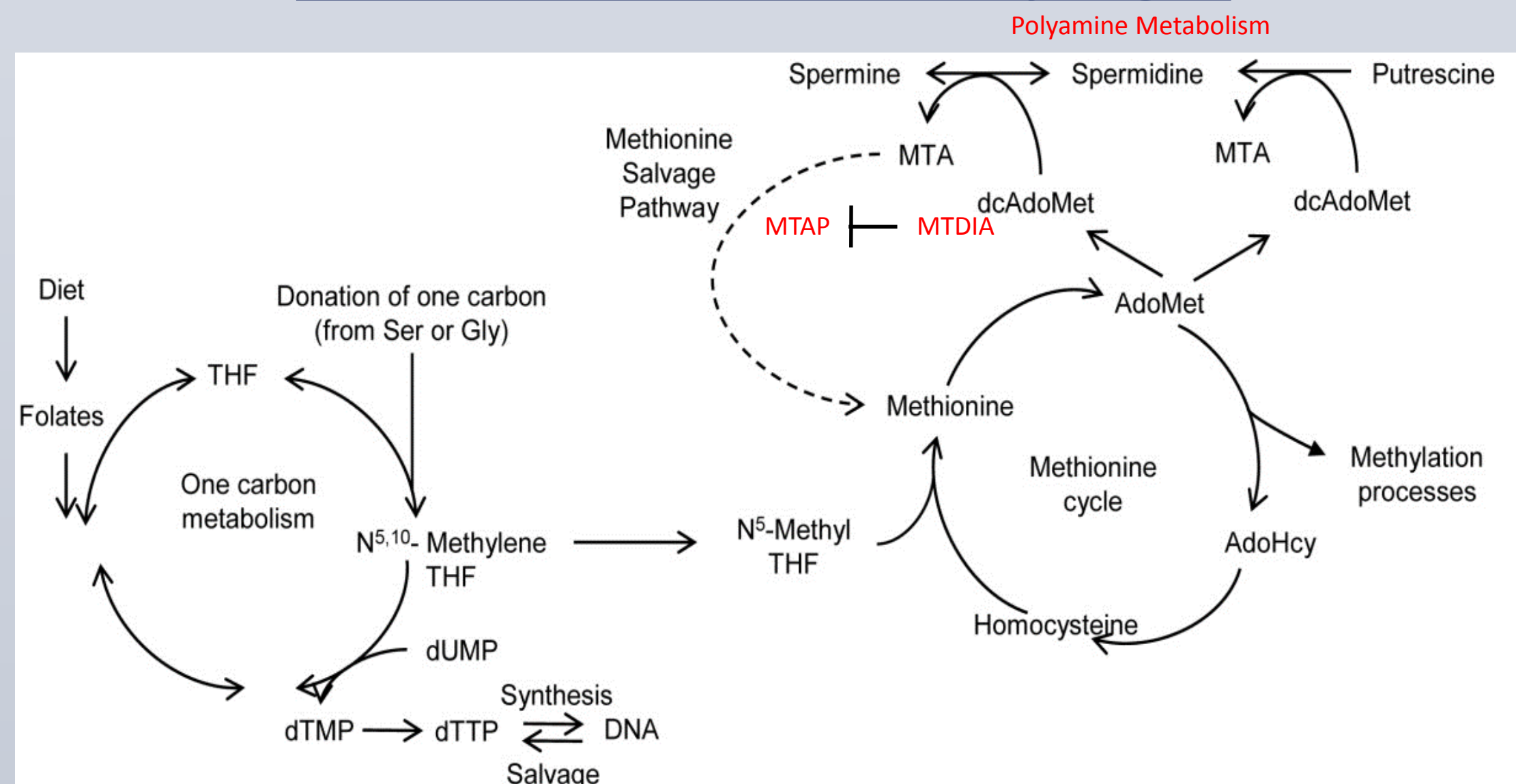


Figure 1a: The folate metabolism pathway, highlighting the two key aspects we propose to interrogate; inhibition of MTAP with MTDIA and the polyamine metabolism.

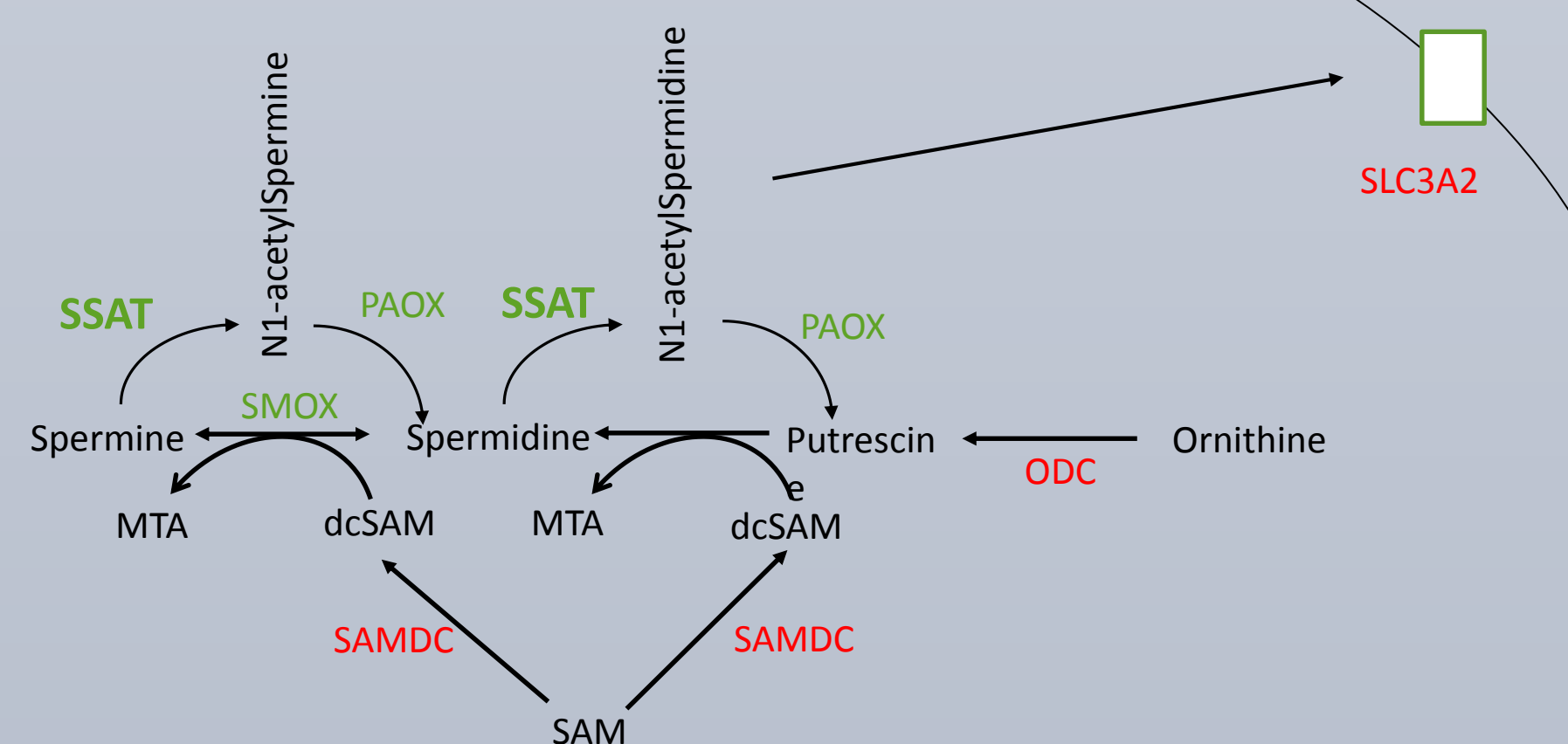


Figure 1b: Polyamine Metabolism pathway and the proposed effect addition of BENSpm would have on the enzymes involved. green = upregulation, red = downregulation

## Preliminary Data for targeting MTAP

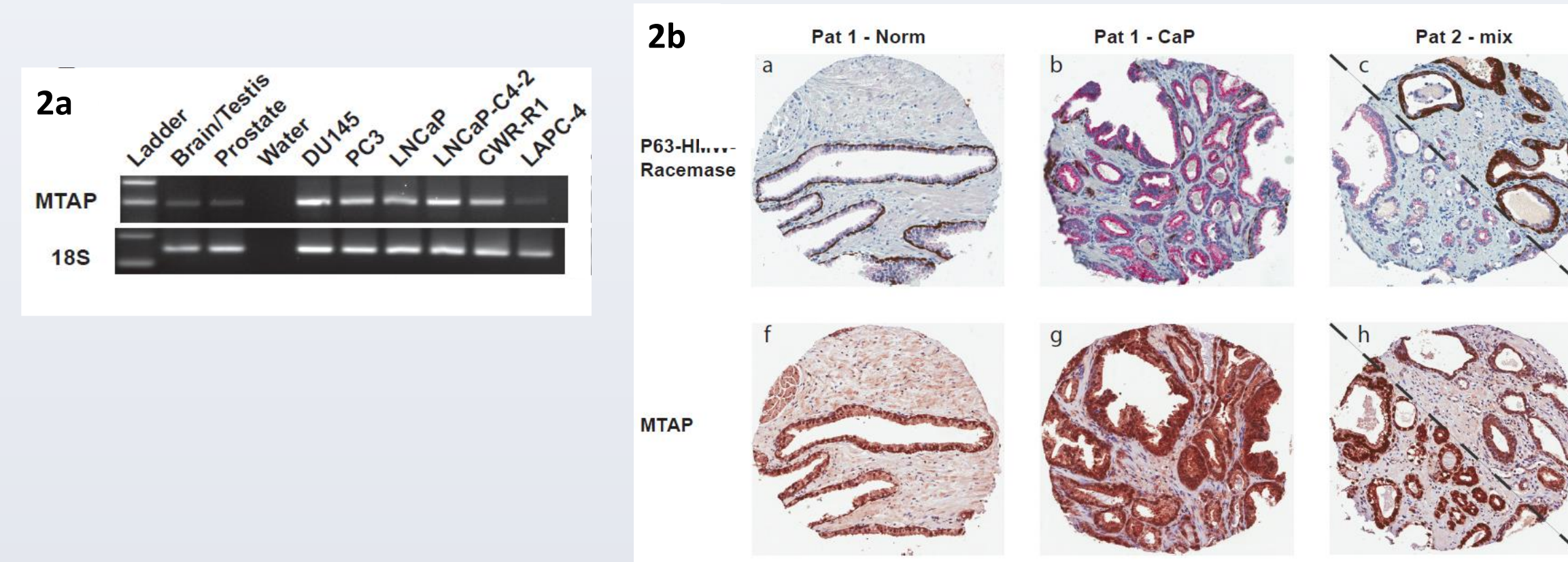


Figure 2: (A) qRT-PCR results measuring 18S and MTAP in human brain/testis, human prostate, and 6 human prostate cancer cell lines. (B) Immunohistochemistry measuring P63-HMW-Racemase and MTAP from 67 human prostate cancers and normal prostate tissue. In the top panels, brown staining indicates normal ducts of PIN; red staining indicates adenocarcinoma. MTAP staining is shown as brown staining in the bottom panels. Patient 2 displays a mix of normal ducts/PIN and adenocarcinoma on either side of the diagonal.

## Preliminary Data for targeting Polyamine Metabolism

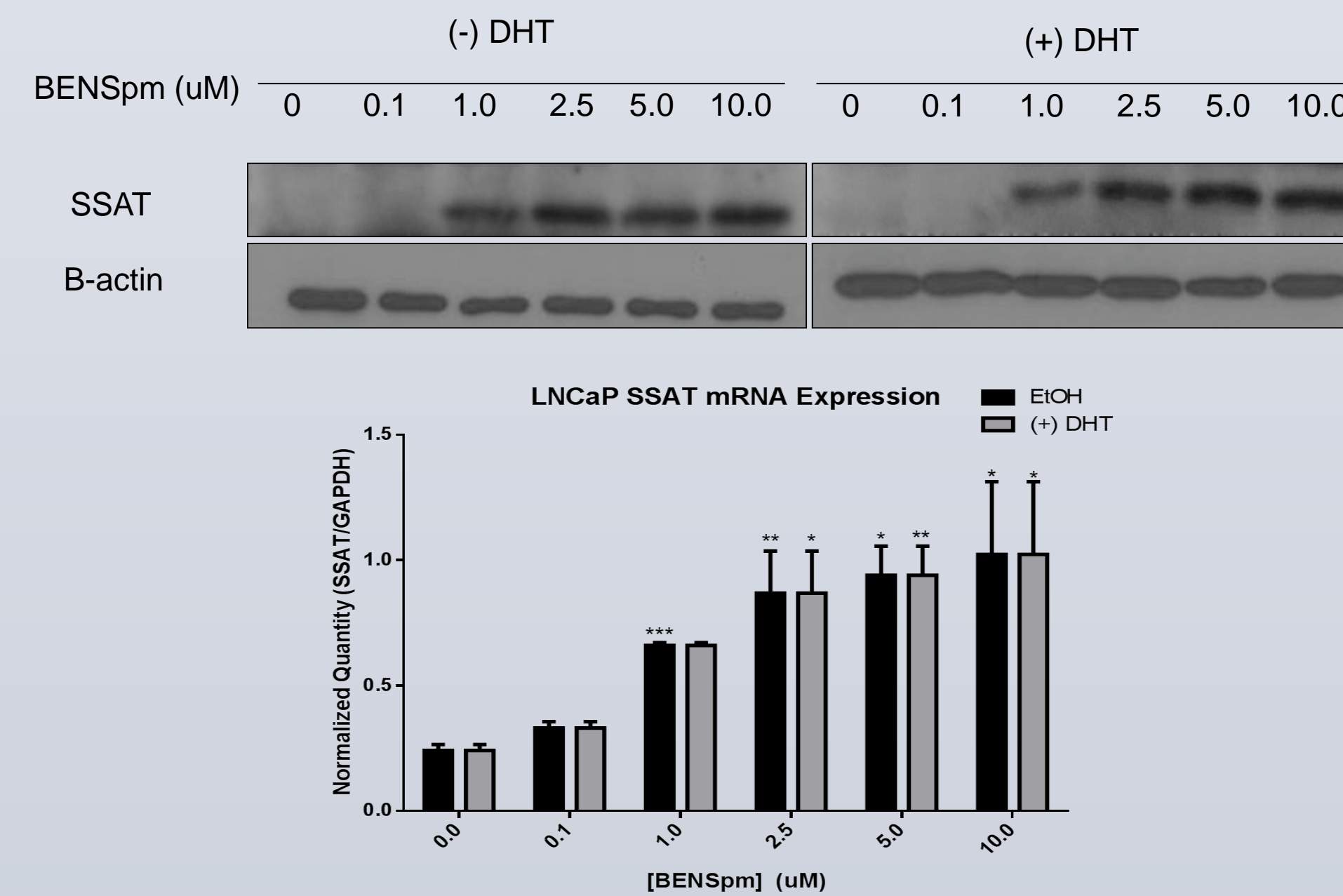


Figure 3. SSAT was conditionally overexpressed in LNCaP prostate carcinoma cells via a tetracycline-regulatable (Tet-off) system. Tetracycline removal resulted in a rapid increase in SSAT protein expression.

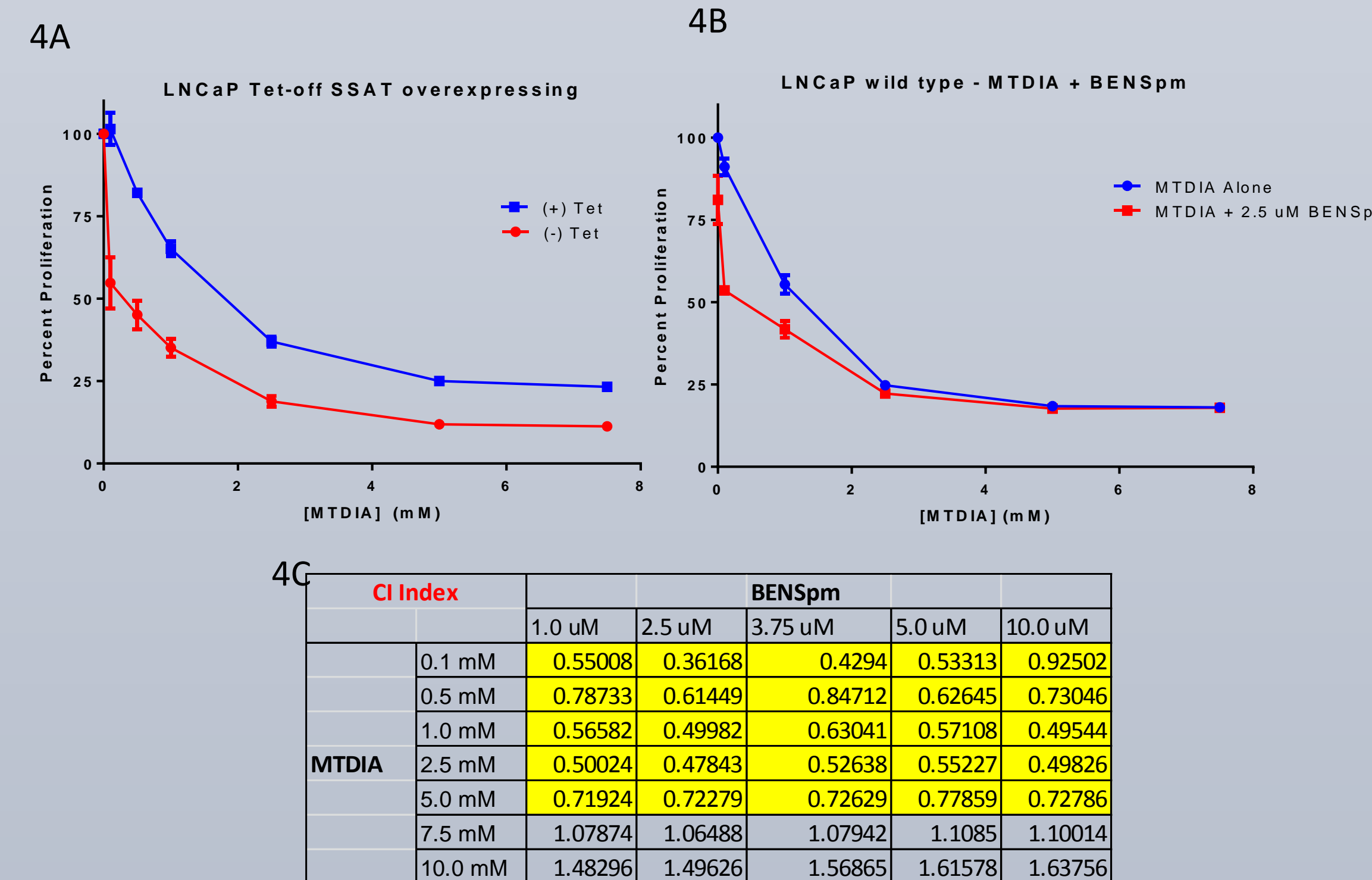


Figure 4: (A) LNCaP tet off overexpressing cells treated with MTDIA in the presence or absence of tetracycline. Tet was removed 24 hours prior to 96 hour MTDIA treatment (p<0.001). (B) LNCaP wild type cells treated with MTDIA alone or MTDIA + 2.5 uM BENSpm. BENSpm was added 24 hours prior to 96 hour MTDIA treatment (p<0.001). (C) Combination Index (CI) for treatment with BENSpm and MTDIA (CI<1.0 = synergism, CI>1.0 = antagonism, CI=1.0 shows additive relationship)

## Results

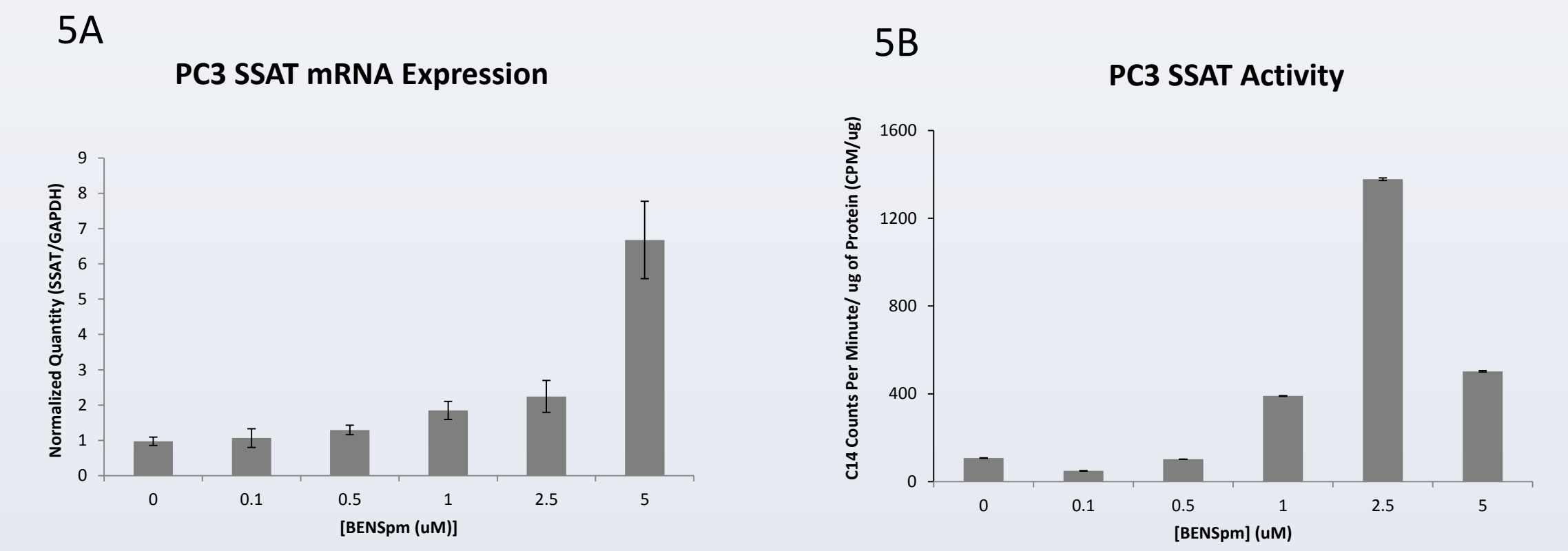


Figure 5. (A) BENSpm upregulates SSAT mRNA expression and (B) SSAT enzyme activity in PC3 cells, human prostate carcinoma cells which are androgen insensitive.

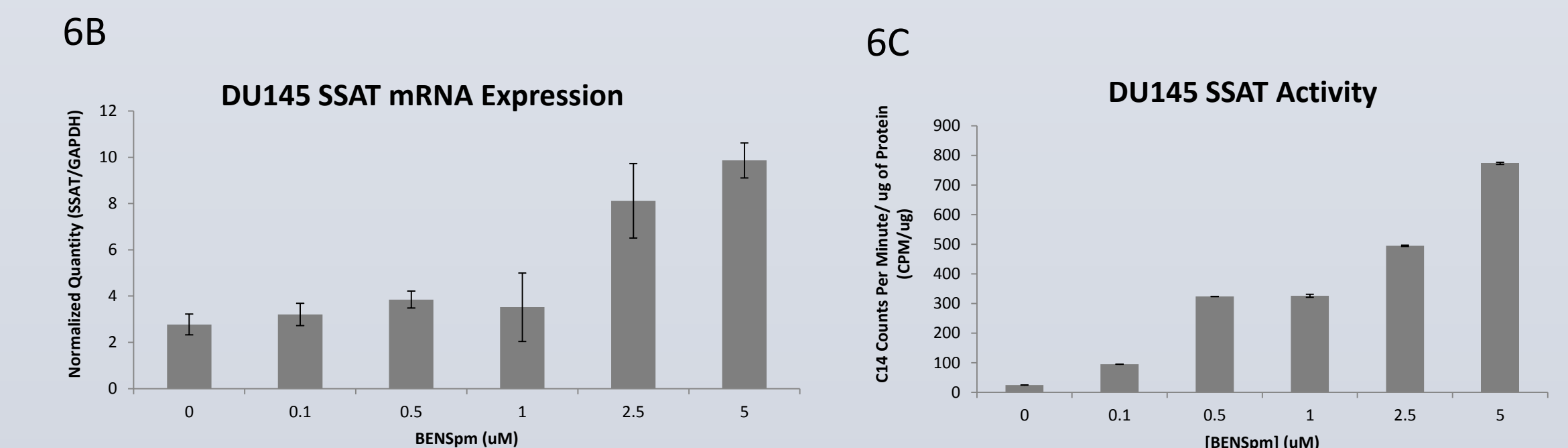


Figure 6. (A) BENSpm upregulates SSAT protein expression, (B) SSAT mRNA expression and (C) SSAT enzyme activity in DU145 cells, prostate carcinoma cells which are androgen insensitive.

## Conclusions and Future Directions

- Genetic and pharmacological interference of the methionine salvage pathway profoundly blocks xenograft growth.
- Overexpression of SSAT significantly increases sensitivity to MTAP inhibition, providing critical proof of principal.
- Treatment with BENSpm in the androgen sensitive LNCaP cell line causes upregulation of SSAT in (+) and (-) DHT. This results in increased sensitivity to MTAP inhibition.
- Combination Index demonstrated treatment with MTDIA and BENSpm is synergistic. In the future, we hope to further evaluate the effects BENSpm has *in vitro*, as well as the effects of treatment with BENSpm and/or MTDIA have *in vivo* in androgen independent xenografts and recurrent prostate cancer models. Our long-term goal is to take advantage of this in a clinical setting to enhance the extent and/or duration of clinical benefit of current therapies.
- Treatment with BENSpm in androgen insensitive PC3 and DU145 cell lines causes upregulation of SSAT.

## Acknowledgements

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## References

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- Bistulfi G, Diegelman P, Foster BA, Kramer DL, Porter CW, Smiraglia DJ. Polyamine biosynthesis impacts cellular folate requirements necessary to maintain S-adenosylmethionine and nucleotide pools. *FASEB J.* 2009 Sep; 23 (9): 2888-2897.