

# Epigenetic Consequences of BCG-activated Leukocytes on Bladder Cancer Cells

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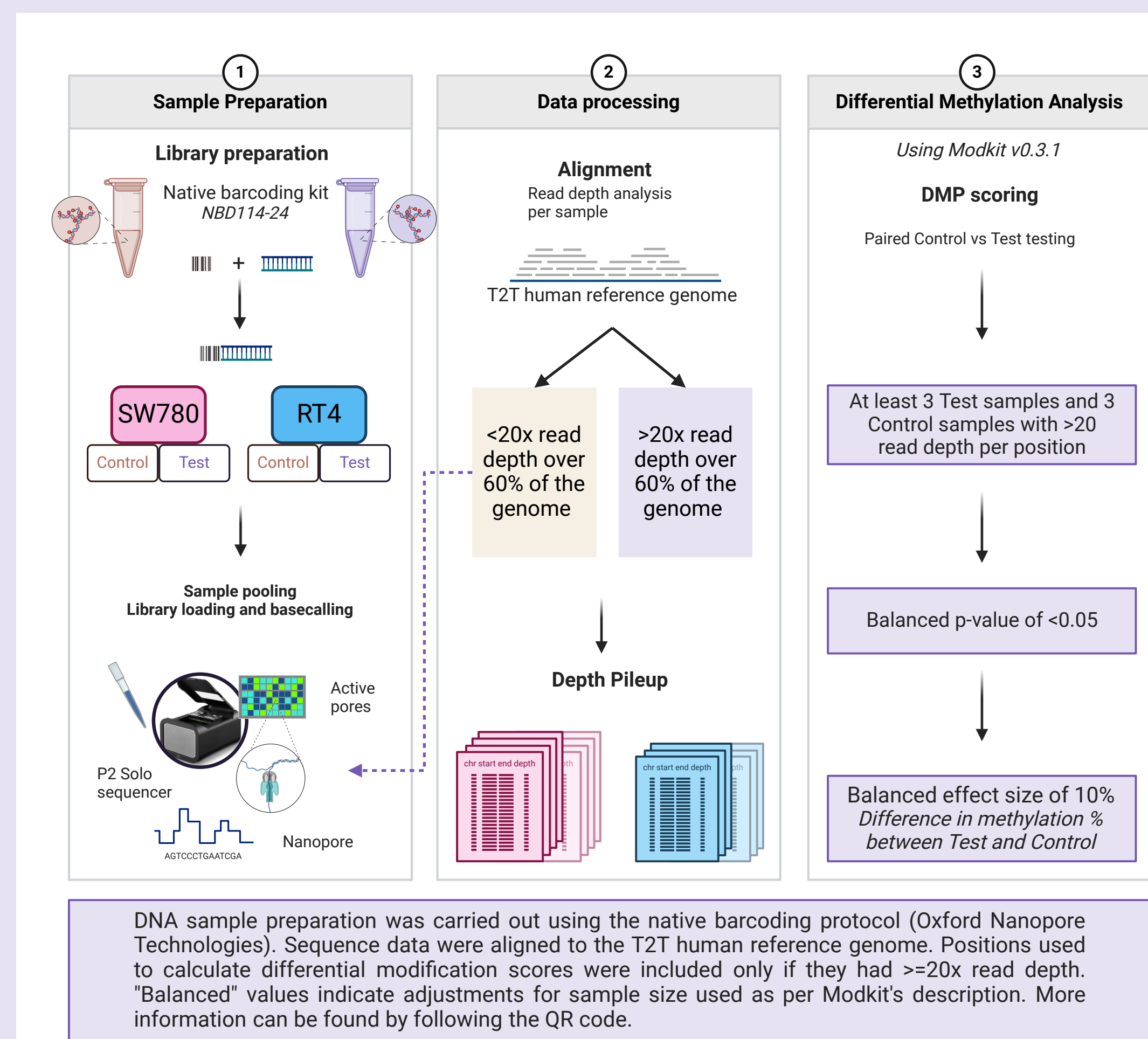
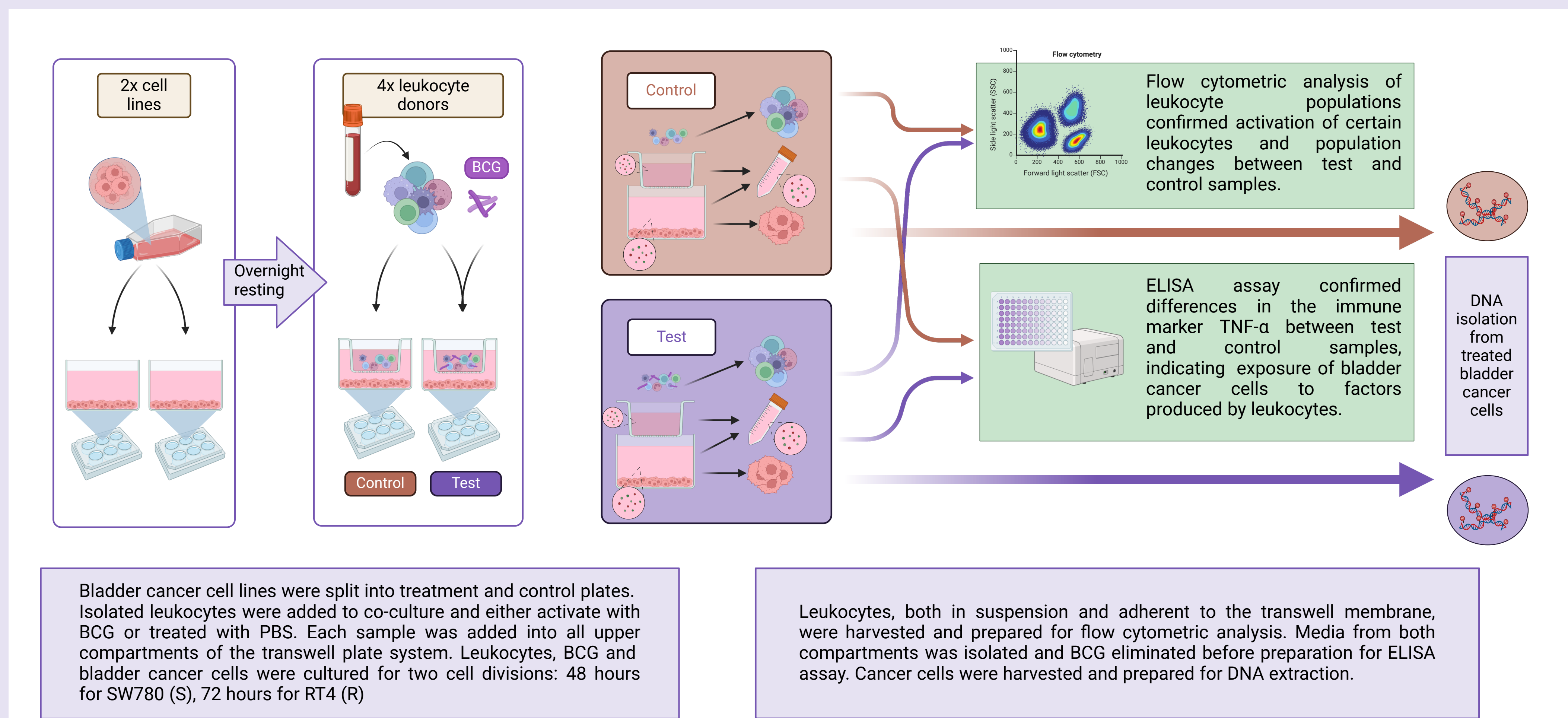
## INTRODUCTION

Immune cells, especially when activated, have the ability to change the microenvironment of cancer via cytokines and reactive oxygen species in a way that could influence epigenetic mechanisms of surrounding cells. *Bacillus Calmette-Guerin* (BCG) mycobacteria is a well established treatment of non-muscle invasive bladder cancer. Potential epigenetic consequences of this treatment were investigated in this research using an *in vitro* co-culture system, combining leukocytes, BCG, and bladder cancer cells. Two cell lines were used: SW780 (S) and RT4 (R).

## OBJECTIVE

To profile changes in cytosine and adenine DNA methylation between cancer cells co-cultured with activated vs non-activated leukocytes. Whole genome DNA sequencing and DNA base modifications were determined using the PromethION 2 Solo nanopore sequencing device.

## METHODS



## RESULTS

Flow cytometry analysis of leukocytes confirmed a change in phenotype and activation between test and control samples. The pattern of activation was slightly different between each healthy participant, and this differing response likely determines what was observed in bladder cancer cell response. The current sample size is, however, insufficient to fully understand these variations, and is outside the scope of this investigation.

Cell Line	Base with modification	
	C	A
SW780	59	28
RT4	61	17

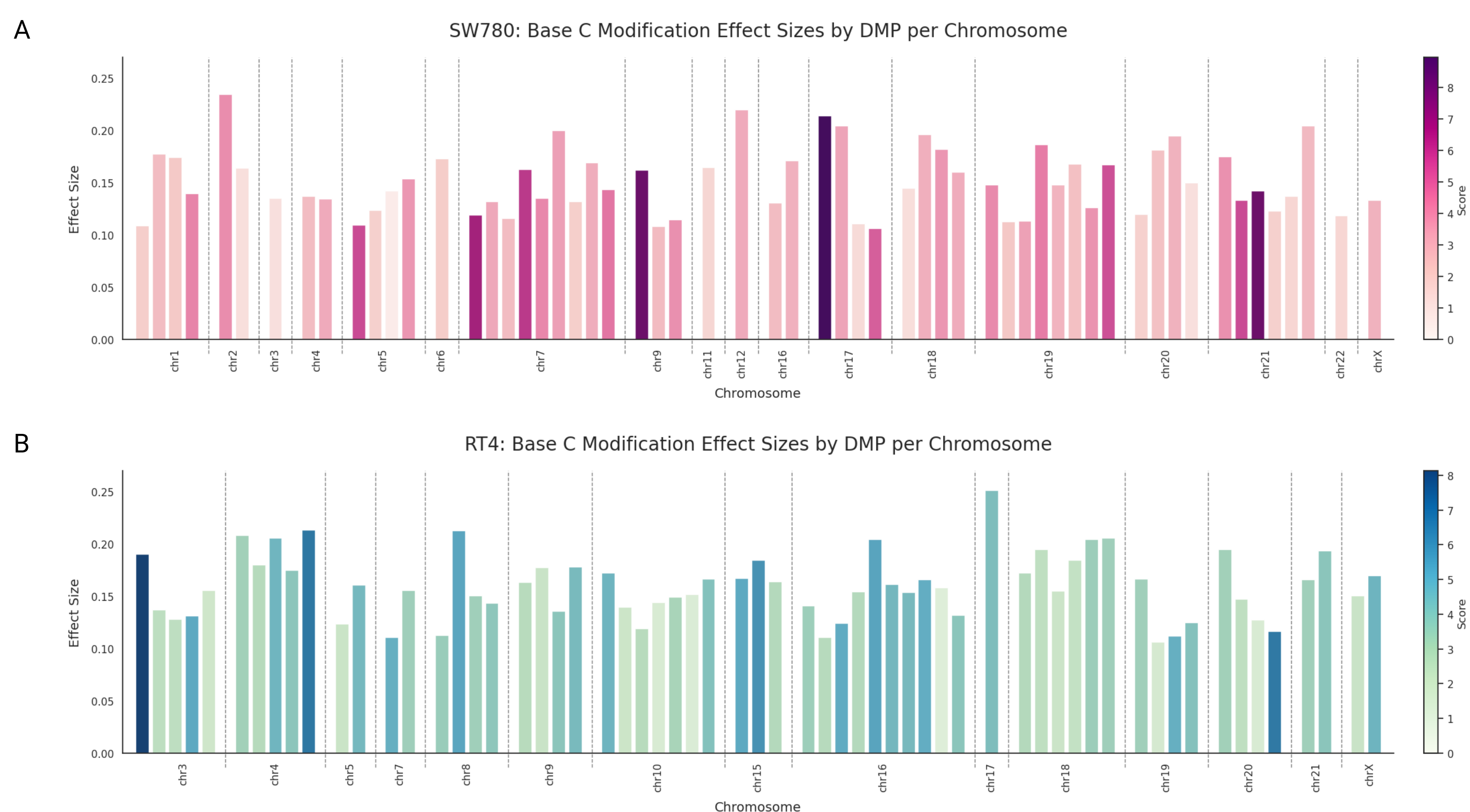


Table 1: Number of base modifications per C/A base per cell line.

Figure 1: Differentially methylated base C positions identified in cell line SW780 (A) and RT4 (B), plotted by chromosome position against effect size, and coloured by significance score (higher values mean more significant results). P-value threshold  $\geq 0.05$ , effect size threshold  $\geq 10\%$ .

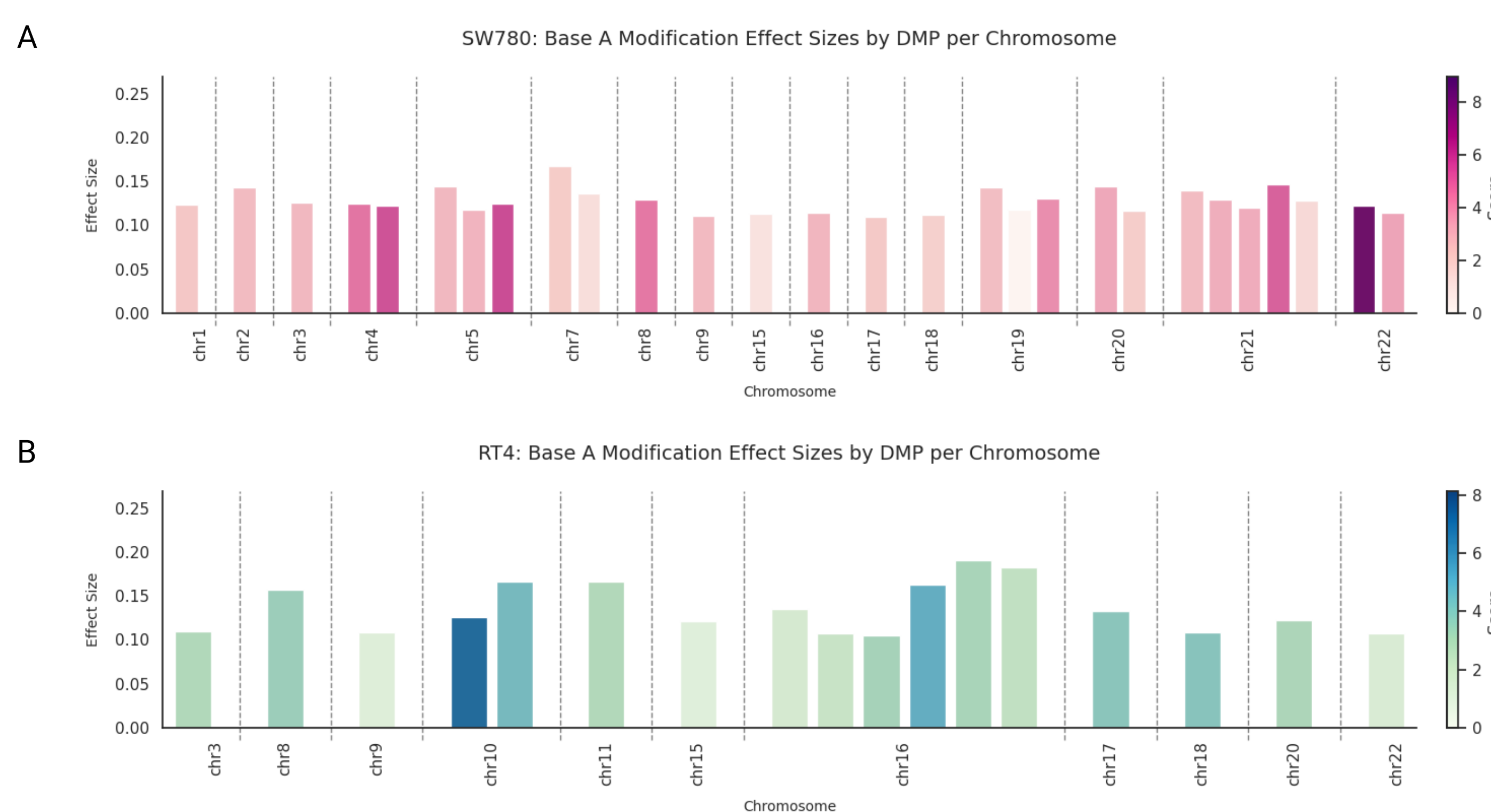


Figure 2: Differentially methylated base A positions identified in cell line SW780 (A) and RT4 (B), plotted by chromosome position against effect size, and coloured by significance score (higher values mean more significant results). P-value threshold  $\geq 0.05$ , effect size threshold  $\geq 10\%$ .

Sequencing data was aligned to the recently published Telomere-to-Telomere human genome assembly. There were 87 and 78 DMPs identified on SW780 and RT4 cell lines respectively, none of which were in common (see Table 1).

Figure 1 and figure 2 show differentially methylated positions (DMP) along the genome per cell line, at bases C and A respectively, plotted by effect size (the difference in percent methylation of positions between paired test and control samples).

Differential modification appears to be cell line specific, as there were no significant DMPs common between cell lines.

There appears to be a high concentration of DMPs occurring at centromeres (see data linked in QR code).

Further verification of these results is needed, as varying read depth may influence significance scoring. Additional investigation into functional locations of identified DMPs is ongoing, as the T2T genome lacks robust annotations at this time.

## CONCLUSION

Leukocyte response to BCG exposure is associated with changes in cytosine and adenine base modifications in adjacent cancer cells. This occurs without physical contact between immune and cancer cells. This likely has implications for understanding patient response to BCG during bladder cancer treatment and highlights the highly context dependent nature of the interactions.

Further research is required to establish the biological implications of these changes.



## ACKNOWLEDGEMENTS

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## REFERENCES

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