

Effects of PFOS on DNA methylation at cancer-relevant loci

Panteha Behboodi¹, Angelica Castano¹, Tonja Pavlovic², Chen-Hua Ma¹, Martha Salas¹, Andy Madrid¹, Mariam Tariq¹, Mauricio Rigo¹, Woo Lee², Kevin Tong¹, Marcin Iwanicki², and Benjamin Tycko^{1,3}

¹HMH Center for Discovery & Innovation, Nutley, NJ; ²Stevens Institute of Technology, Hoboken, NJ; ³Lombardi Comprehensive Cancer Center, Washington DC (USA)

Background

- Perfluoroalkyl and polyfluoroalkyl substances (PFAS), including perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA), are persistent environmental pollutants linked to increased risks of several cancers, including ovarian carcinoma as well as other diseases.
- These compounds bioaccumulate in tissues and can interfere with key metabolic and signaling pathways, potentially disrupting homeostasis.
- Our ongoing study focuses on the effects of PFOS on DNA methylation at risk loci for human cancers, with a preliminary hypothesis that such effects may be mediated, at least in part, via inflammation-driven epigenetic regulation.

Approach

Immortalized human fallopian tube epithelial (FNE) cells and de-identified patient-derived colonoids from the HMH biorepository were exposed to 25 μ M PFOS for 72 hours to evaluate epigenetic alterations induced by environmental pollutant exposure. Following treatment, DNA was extracted and subjected to whole-genome methylation profiling using Oxford Nanopore long-read sequencing to assess methylation changes.

Even at the relatively high doses used, all the methylation changes were small in magnitude. However, we observed a loss and gain of methylation in the TPM1 and ACTA1 gene regions, respectively, as well as loss of methylation in the HAGLR gene region in PFOS-treated FNE cells. Colonoids showed PFOS-induced alterations in DNA methylation patterns, including in the TSPAN11 gene region. These findings highlight epigenetic responses to PFOS exposure that may contribute to disease risks, including cancers.

Contacts: pbehbood@tyckolab.com;
amc@tyckolab.com

PFOS effects on fallopian tube non-ciliated epithelial (FNE) cell lines: a model for the precursor of high-grade serous ovarian carcinomas

In FNE cells, exposure to PFOS induced CpG methylation changes spanning candidate regulatory SNPs within the ACTA1, TPM1, and HOXD gene cluster regions. Increased CpG methylation was observed in the ACTA1 promoter–enhancer region, while PFOS treatment resulted in loss of methylation (LOM) across the TPM1/TPM1-AS locus. ACTA1 encodes a cytoskeletal actin essential for maintaining cell structure, and TPM1 functions as an actin-binding tumor suppressor involved in epithelial stability and transformation. The HOXD gene cluster, a known GWAS locus associated with ovarian cancer risk, also exhibited LOM following PFOS exposure

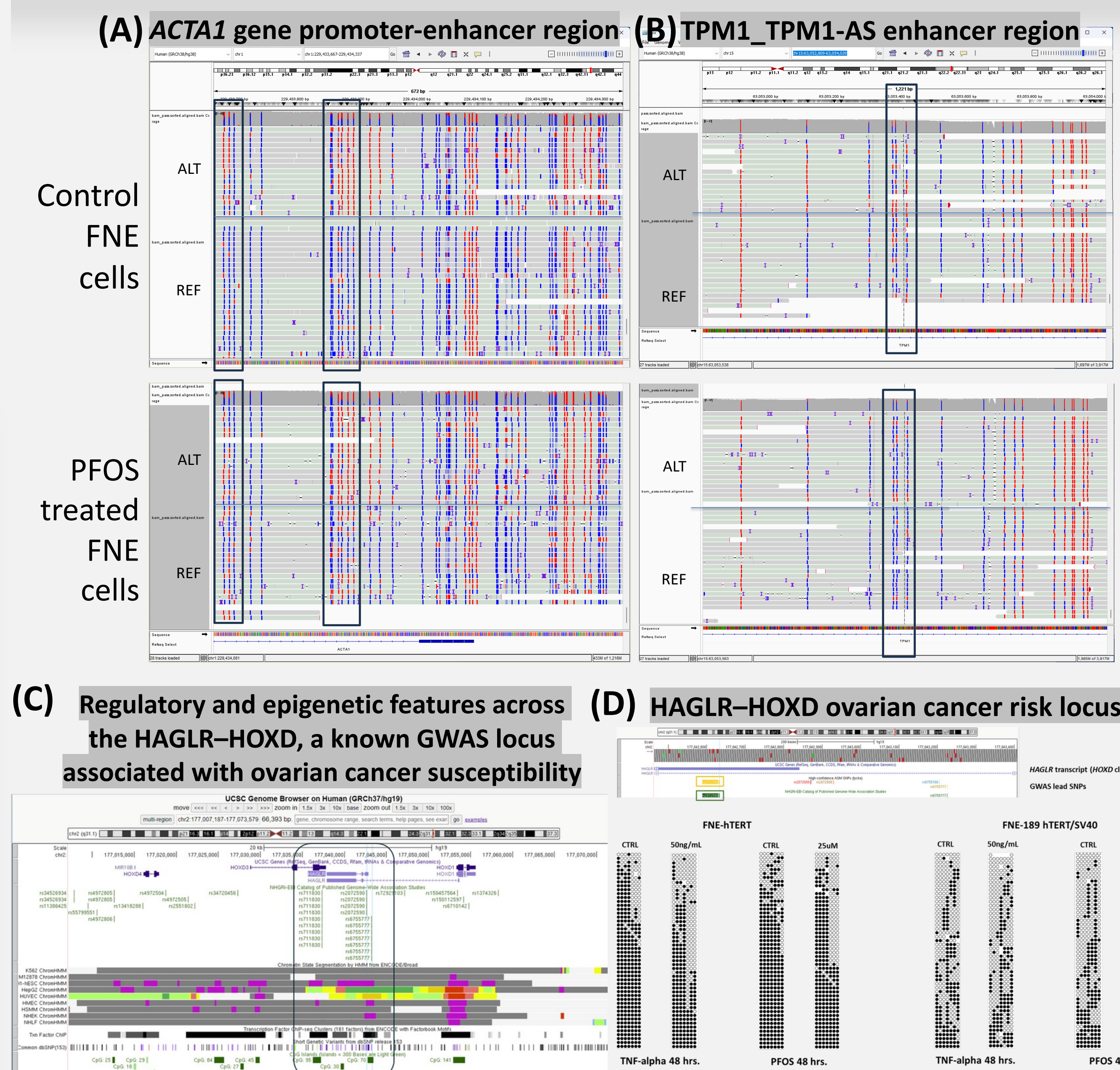


Figure 1. FNE cell line (CTRL and 25 μ M PFOS-treated) were analyzed by Nanopore long-read sequencing. **(A)** ACTA1 locus: Increased CpG methylation is observed in the ACTA1 promoter–enhancer region in FNE cells exposed PFOS. In this heterozygous cell line, reads were phased by SNP rs605428, separating alleles (REF and ALT). The REF allele, which is hypo-methylated in control cells, exhibits a clear gain of methylation upon PFOS exposure. **(B)** TPM1/TPM1-AS locus: LOM is detected in PFOS-treated cells across an enhancer region near TPM1-AS. This hypomethylation may lead to repression of the antisense transcript, suggesting PFOS-induced epigenetic deregulation in regulatory elements linked to TPM1 expression. **(C)** Map of the HOXD3-HAGLR-HOXD1 region showing GWAS lead SNPs for ovarian cancer (rs6755777 and several others) and repressive/poised chromatin states. **(D)** Targeted methyl-seq of a bisulfite PCR amplicon spanning several candidate regulatory SNPs (rs2072590 and others) shows a decrease in net methylation induced by both PFOS (25 μ M) and TNF- α (100 ng/ml) in the hTERT-immortalized FNE cells suggesting that PFOS and inflammatory signaling may converge on shared epigenetic pathways affecting ovarian cancer susceptibility genes. In a SV40-transformed FNE cell line the baseline methylation was less, and no effects of either agent were seen.

PFOS effects on patient-derived colonoids: a model for colorectal cancer

PFOS exposure induced LOM on alternating ALT and REF alleles in the upstream enhancer element of TSPAN11, a gene widely expressed in the gastrointestinal tract and implicated in colorectal and other digestive system cancers. These findings suggest that PFOS alters allele-specific methylation at regulatory elements of TSPAN11, potentially contributing to epigenetic dysregulation in intestinal epithelial cells.

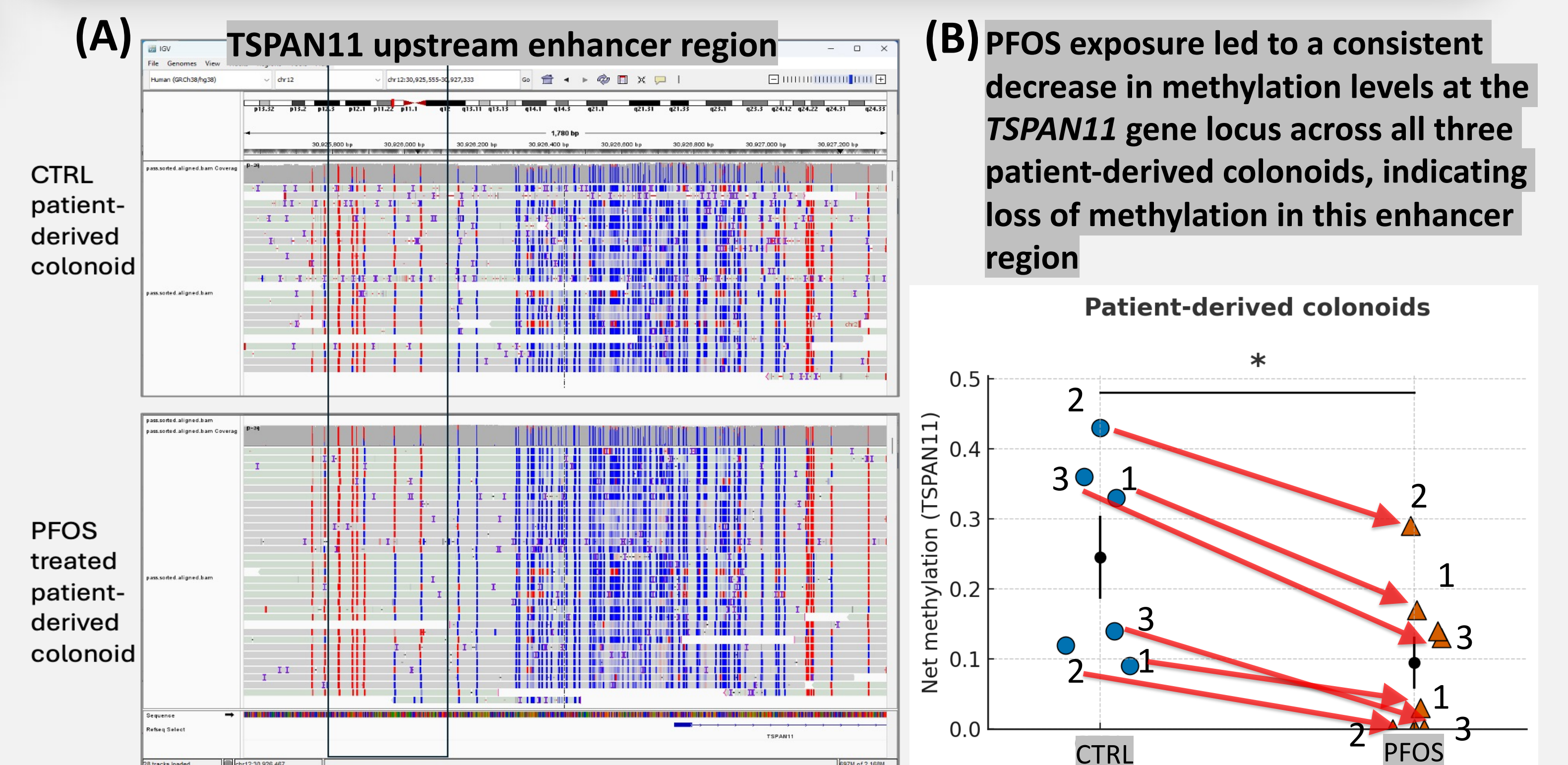


Figure 2. Three paired patient-derived colonoid samples (CTRL and 20 μ M PFOS-treated) were analyzed by Nanopore long-read sequencing. **(A)** PFOS exposure induces loss of methylation (on alternating ALT and REF alleles) in the upstream enhancer element of TSPAN11. **(B)** PFOS exposure consistently reduced net methylation levels at the TSPAN11 locus, indicating loss of methylation in this enhancer region across all three colonoids. Each pair (connected by red arrows) represents matched CTRL and PFOS-treated samples from the same patient. TSPAN11 is a tetraspanin family gene widely expressed in the gastrointestinal tract and implicated in colorectal cancer progression and immune regulation.

Conclusion

Our findings demonstrate that exposure to high concentrations of PFOS over short time periods leads to mild but recurrent changes in DNA methylation at cancer-relevant loci. Although not yet feasible given the mild changes observed, with further research methylation patterns have the potential to be developed as DNA-based biomarkers of PFAS exposures.

References

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