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DNA methylation Changes in Chromatin Released During Neutrophil Extracellular Trap (NET) Formation as Part of the Immune Response

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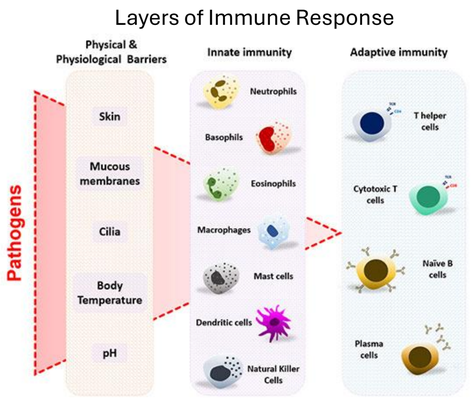
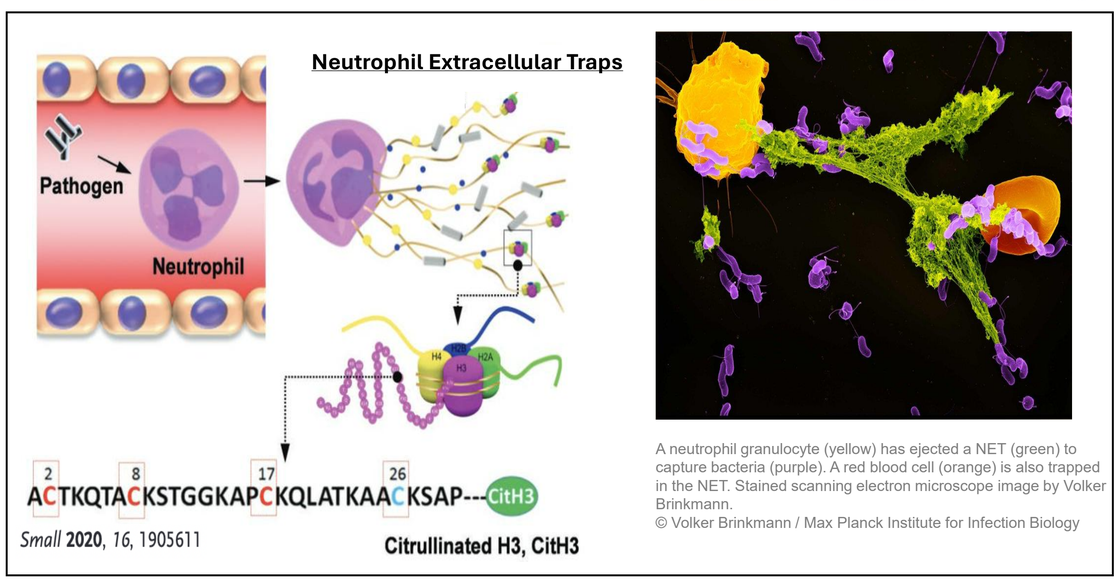
Background

- Neutrophils are the most abundant white blood cell in circulation and play a key role in the innate immune response
- There are multiple layers of chromatin organization that affect accessibility
- Upon pathogen detection neutrophils can release their chromatin in the form of a NET to trap bacteria and vital particles
- There are controlled changes within the chromatin structure of neutrophils upon NET activation





A neutrophil granulocyte (yellow) has ejected a NET (green) to capture bacteria (purple). A red blood cell (orange) is also trapped in the NET. Stained scanning electron microscope image by Volker Brinkmann.
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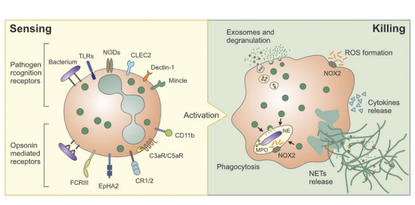
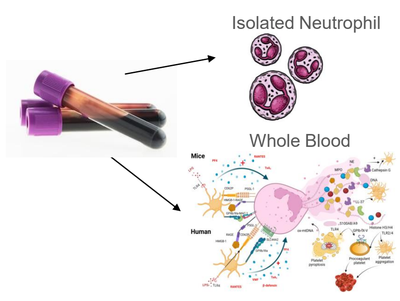
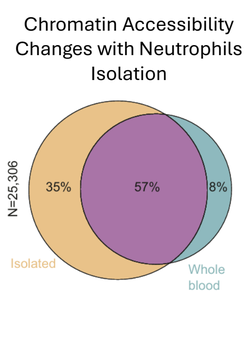
Neutrophils are key sensors of the environment through a variety of receptors on their cell surface



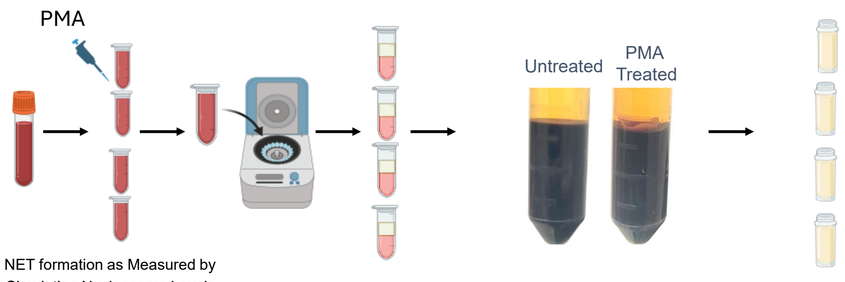




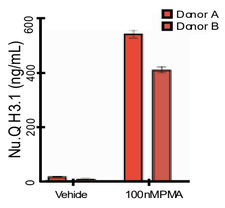
Methods

Whole Blood Ex Vivo NET Formation Model

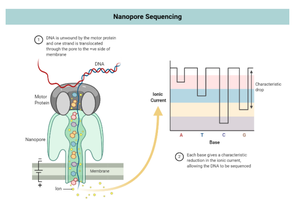
- Whole blood is collected and aliquoted into tubes, treated with various activator and/or inhibitor conditions.
- After incubation blood is centrifuged, plasma isolated and nucleosomes are measured using Nu.Q® or DNA is extracted for sequencing



NET formation as Measured by Circulating Nucleosome Levels

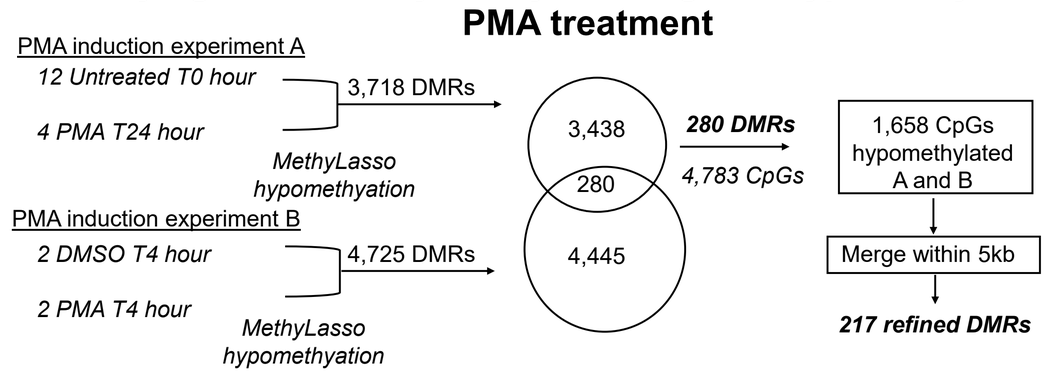


Oxford Nanopore Sequencing

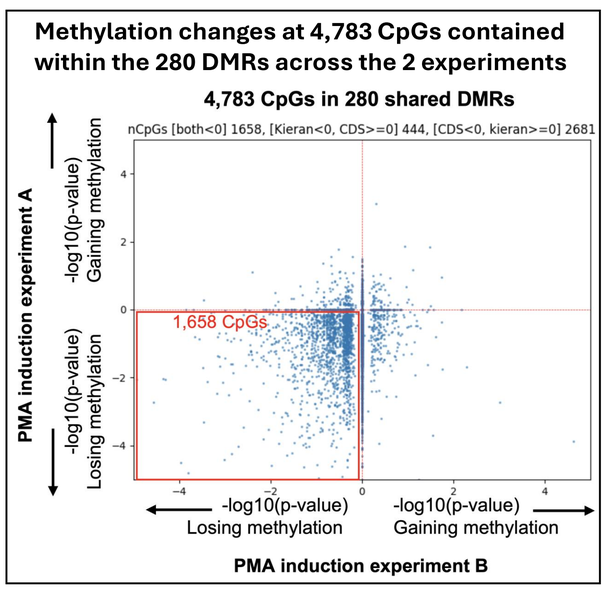


Results

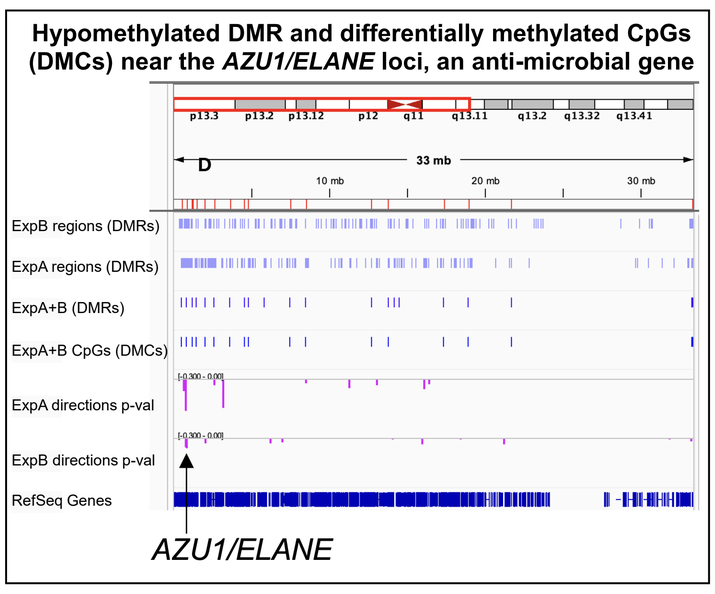
Identifying differentially methylated regions hypomethylated after PMA treatment



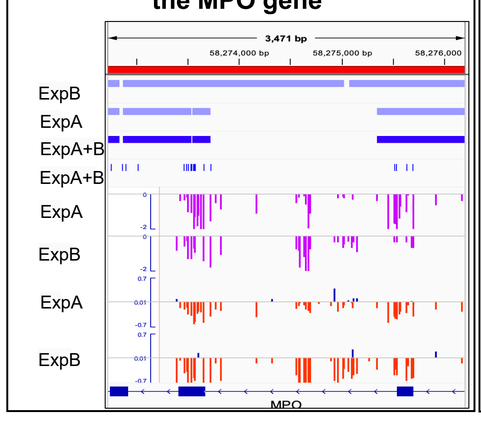
Methylation changes at 4,783 CpGs contained within the 280 DMRs across the 2 experiments



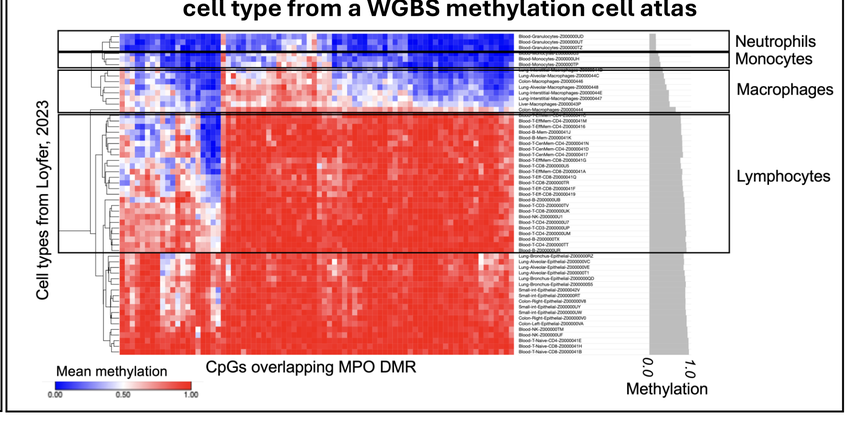
Hypomethylated DMR and differentially methylated CpGs (DMCs) near the *AZU1/ELANE* loci, an anti-microbial gene



Differentially methylated CpGs within the MPO gene



Hypomethylated CpG overlapping the MPO DMRs, across purified cell type from a WGBS methylation cell atlas



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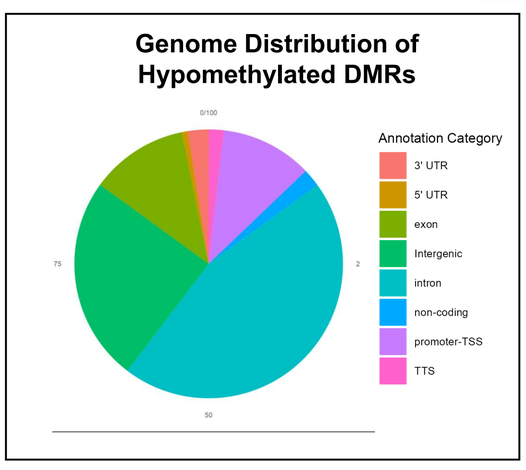
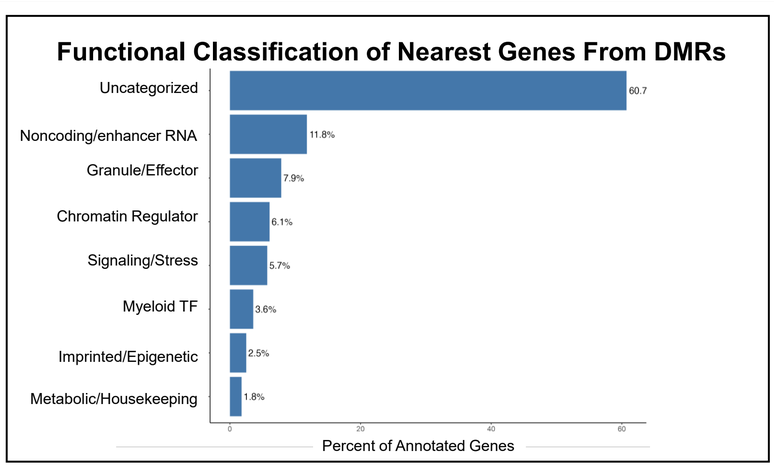
NET formation as Measured by Circulating Nucleosome Levels

Oxford Nanopore Sequencing

Genome Distribution of Hypomethylated DMRs



Functional Classification of Nearest Genes From DMRs

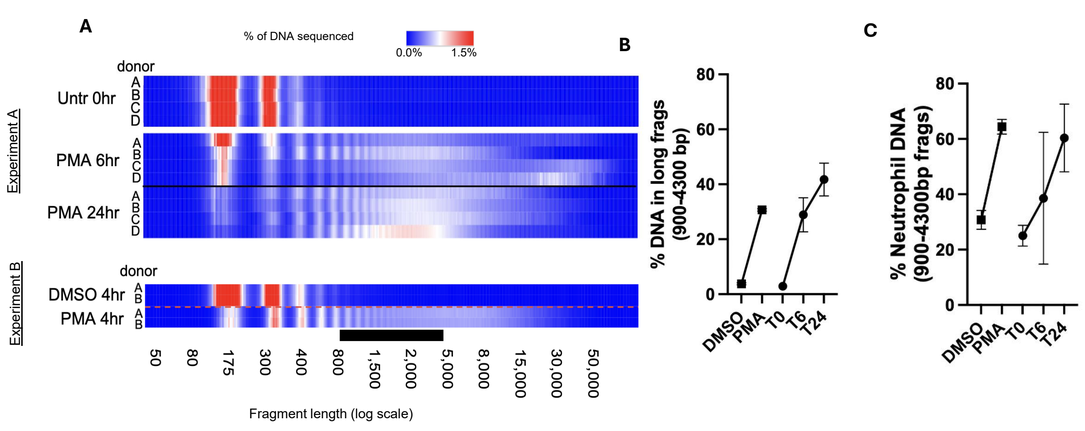


Conclusion

- NET formation can be induced in whole blood using PMA
- Cell free DNA following NET induction is a variety of lengths and preferentially derived from neutrophils
- There are DNA methylation in cfDNA following NET induction
- DNA methylation changes occur at promoters of genes with known functions in NET formation
- Hypomethylated Differentially Methylated Region (DMRs) occur across a variety of genomic features
- Hypomethylated Differentially Methylated Region (DMRs) occur in uncategorized regions as well know regions known to be associated with chromatin structure and immune cell function

Results

Cell free DNA following PMA treatment is a a variety of lengths and preferentially derived from Neutrophils



Conflicts of Interest

Authors are current or former employees and consultants of Volition Rx and some are Stock holders and authors on patents related to this work.

References

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