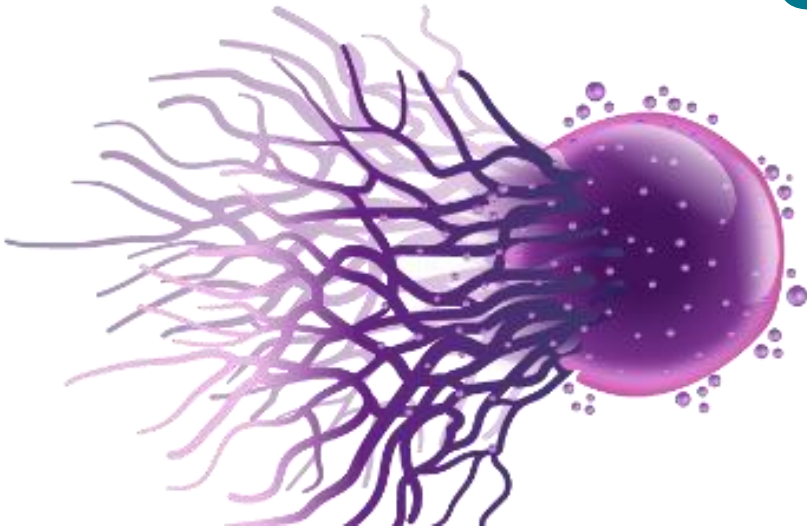
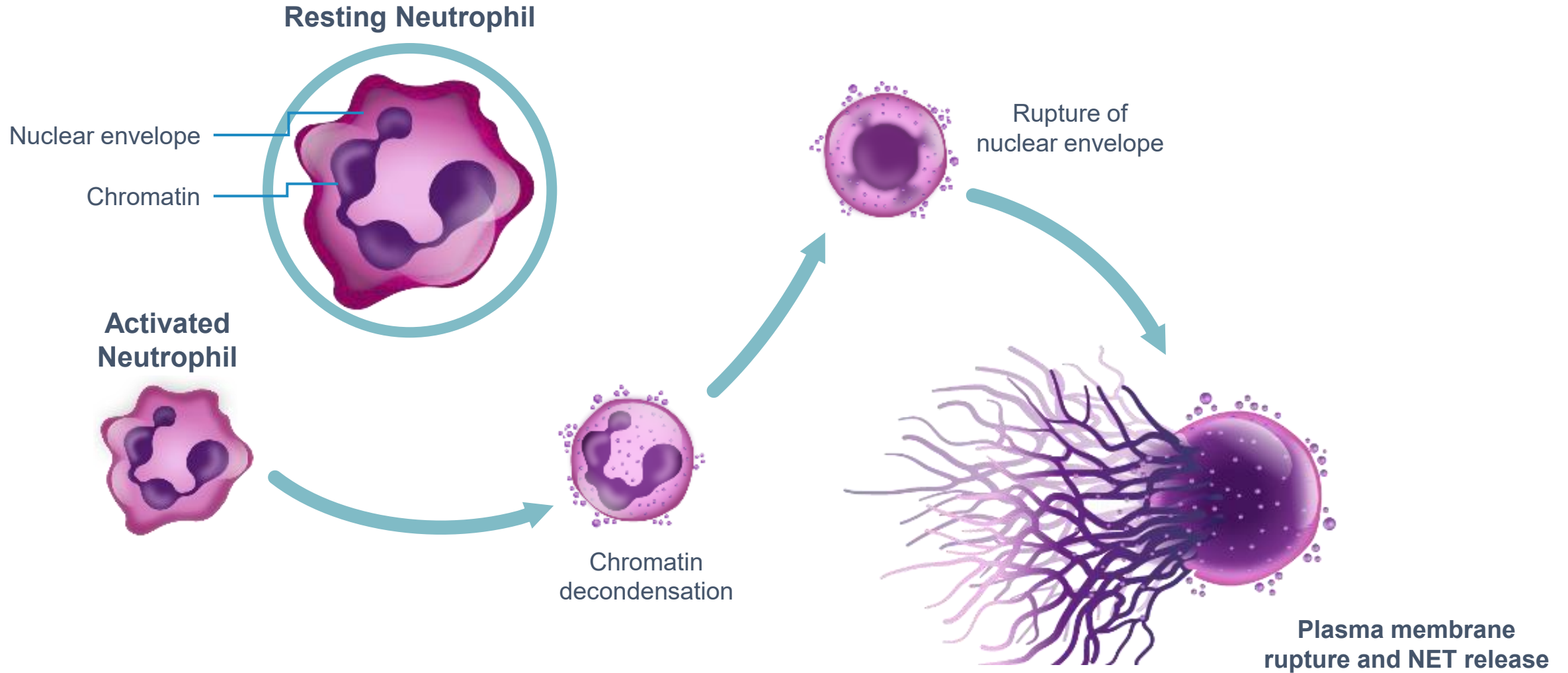


Red blood cell lysis modulates neutrophil extracellular trap release in response to physiological inflammatory stimuli

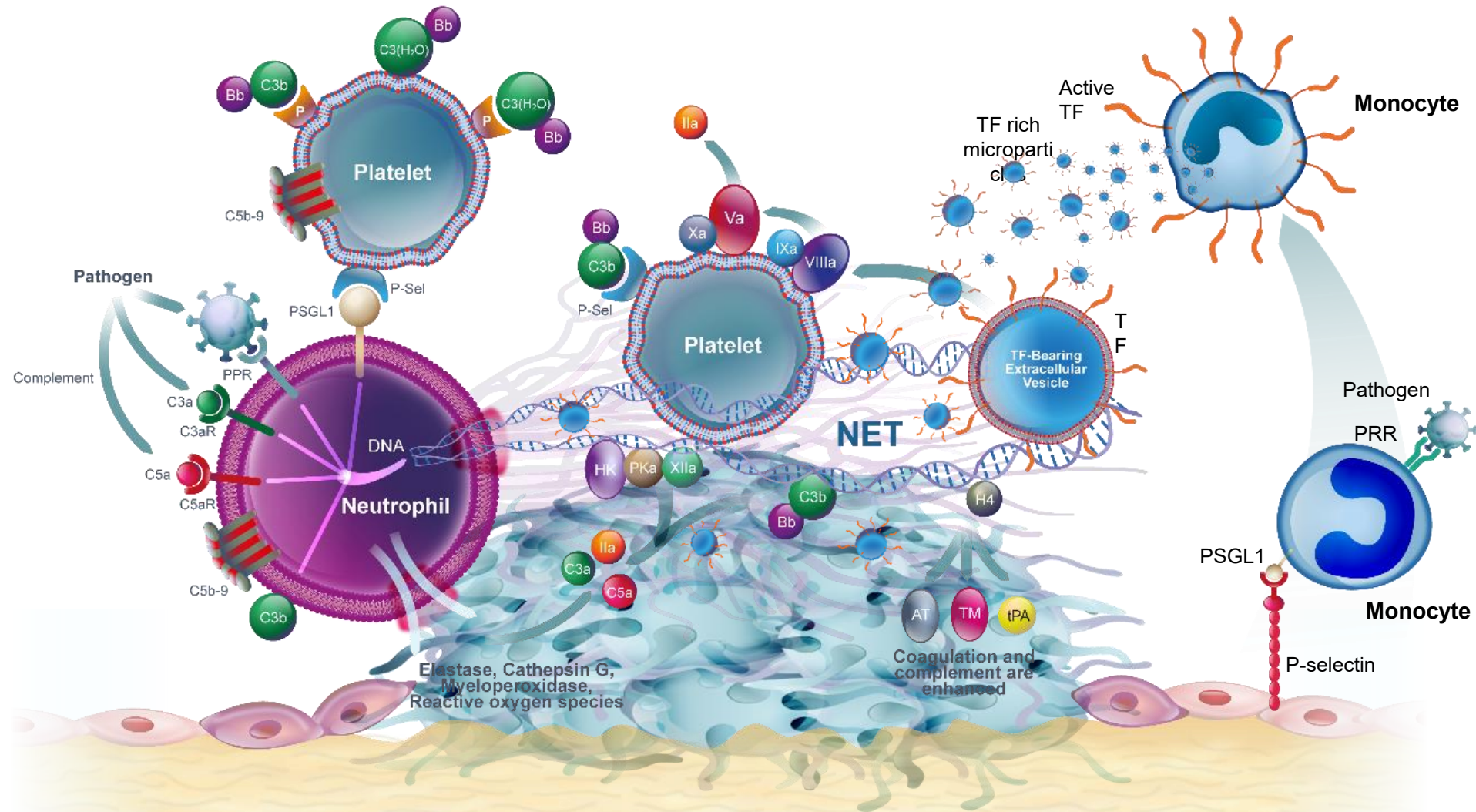


Justin Cayford, PhD
Volition

Extracellular chromatin webs are released by activated neutrophils



NETs trap microbes but can also drive thromboinflammation



NET biology reflects complex interactions among platelets, monocytes, coagulation, the vascular environment.

Clinically meaningful neutrophil biology starts with physiologically relevant models

Experimental workflows help determine how well laboratory models reflect patient biology

Clinical

Disease relevant NET regulation is difficult to reproduce in simplified experimental systems

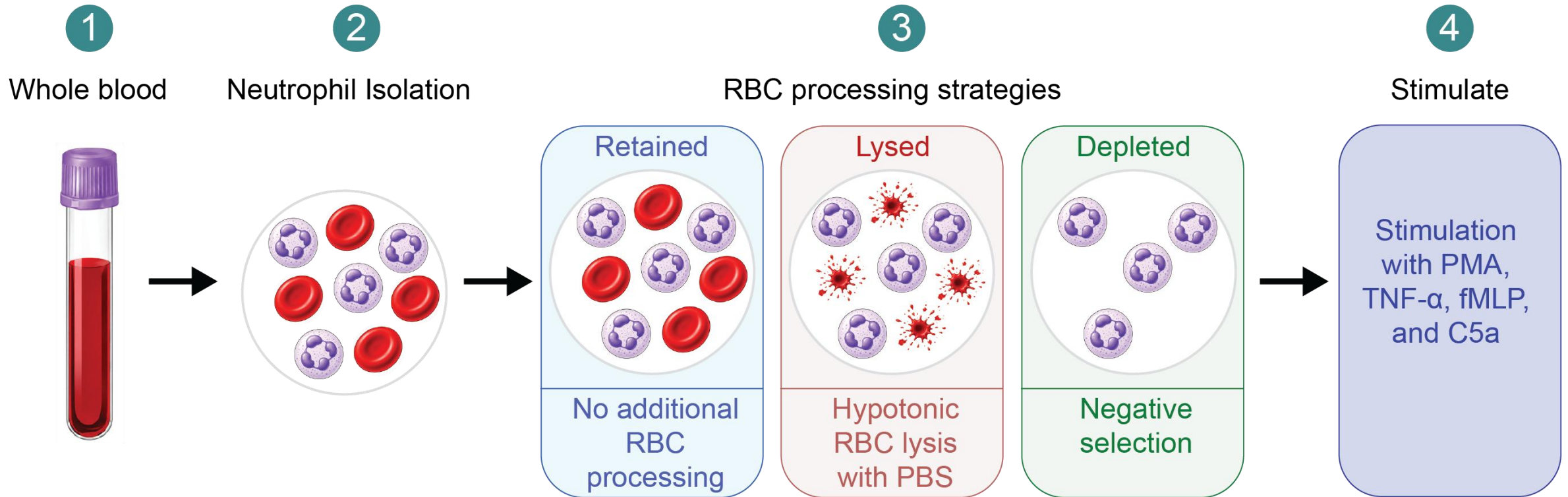
Technical Challenge

Neutrophils rapidly respond to their environment, making them very sensitive to sample handling

Study approach

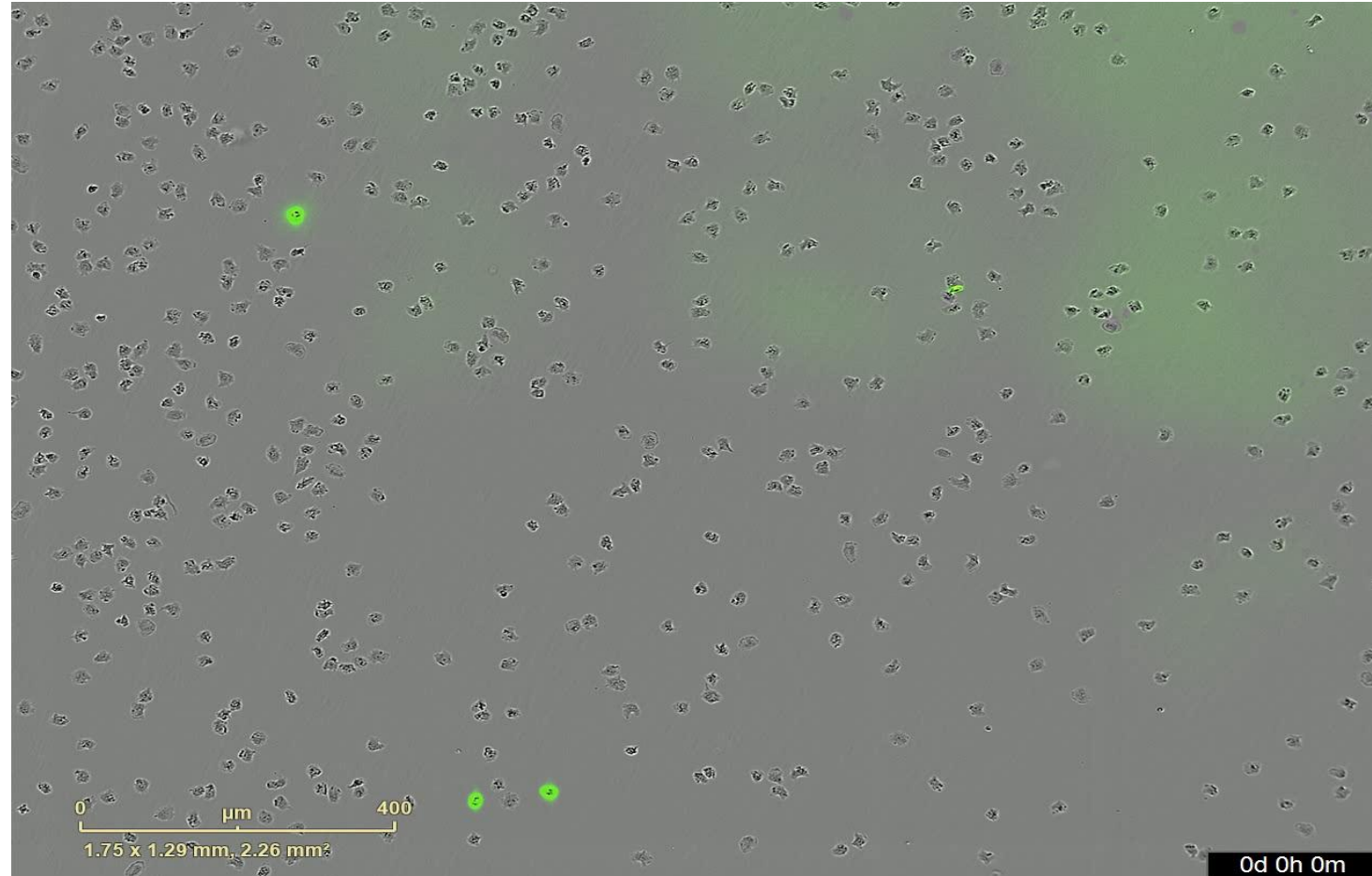
Determine how different neutrophil isolation methods affect DNA release and chromatin accessibility

After neutrophil isolation, three RBC processing strategies were compared

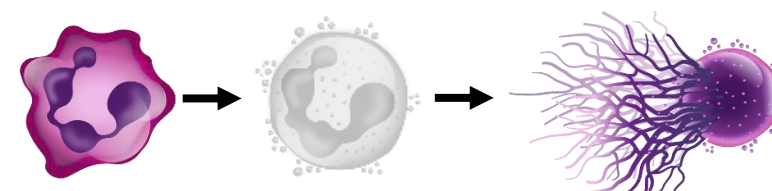
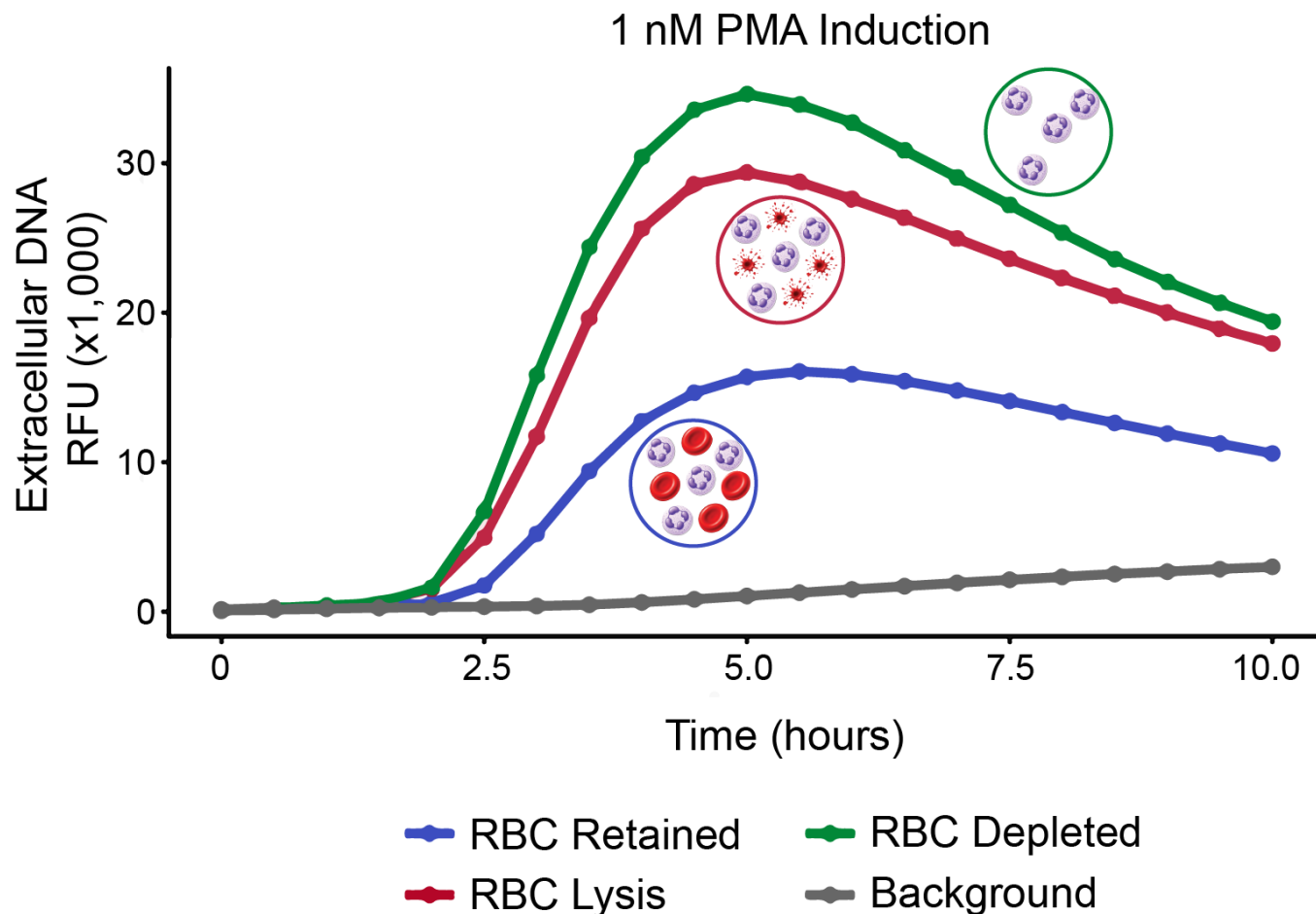


Live-cell imaging is used to show NET associated DNA release

1 nM PMA

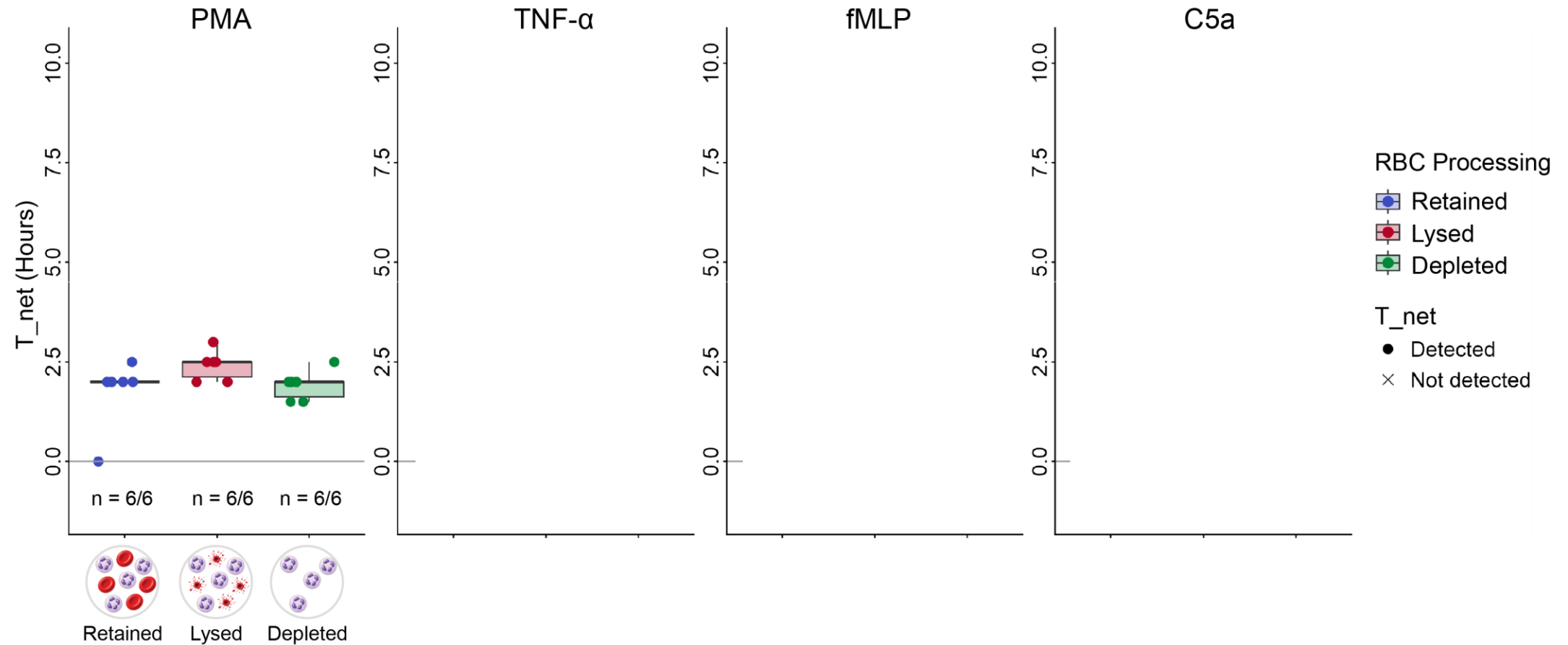


Using the live-cell imaging, timing of NET-associated DNA release was determined

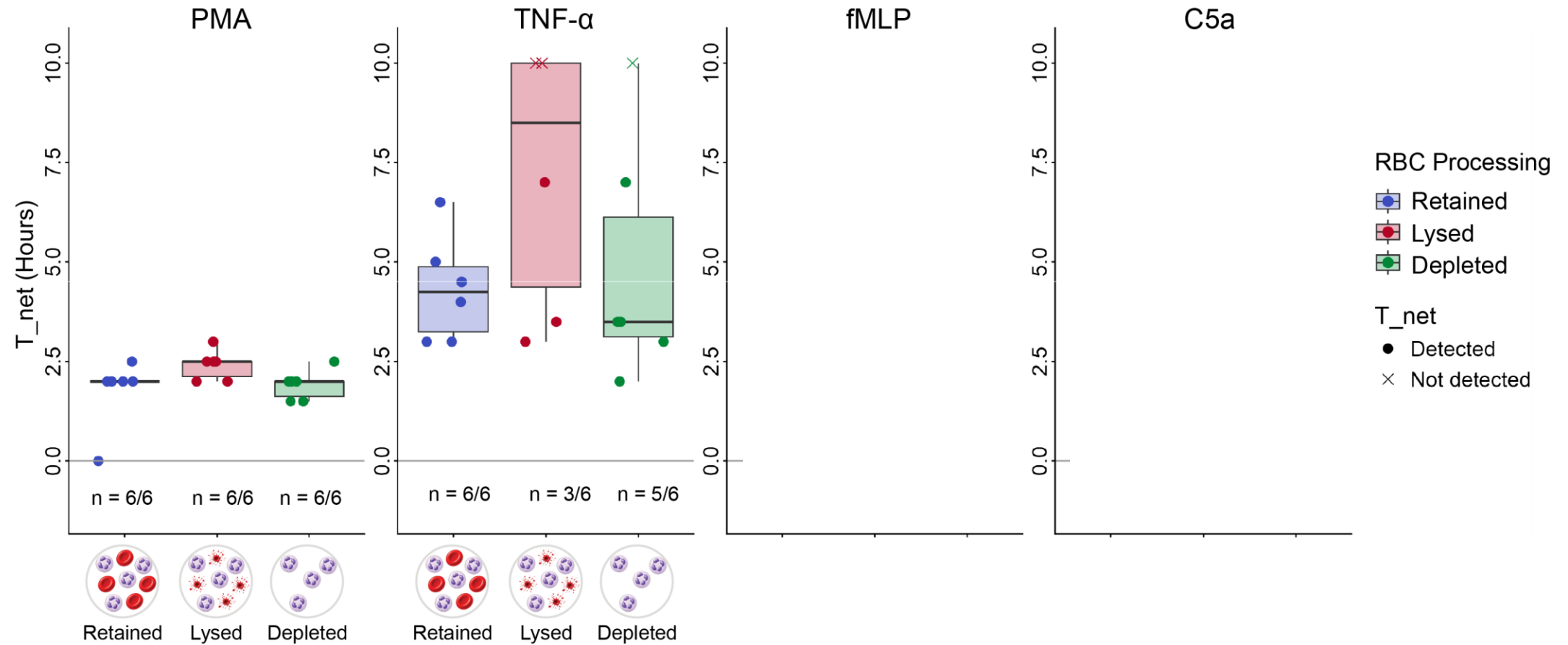


- T_{net} = time to detectable NET associated DNA release
- No detection by 10 hours = non-detected

PMA response timing is preserved across processing conditions

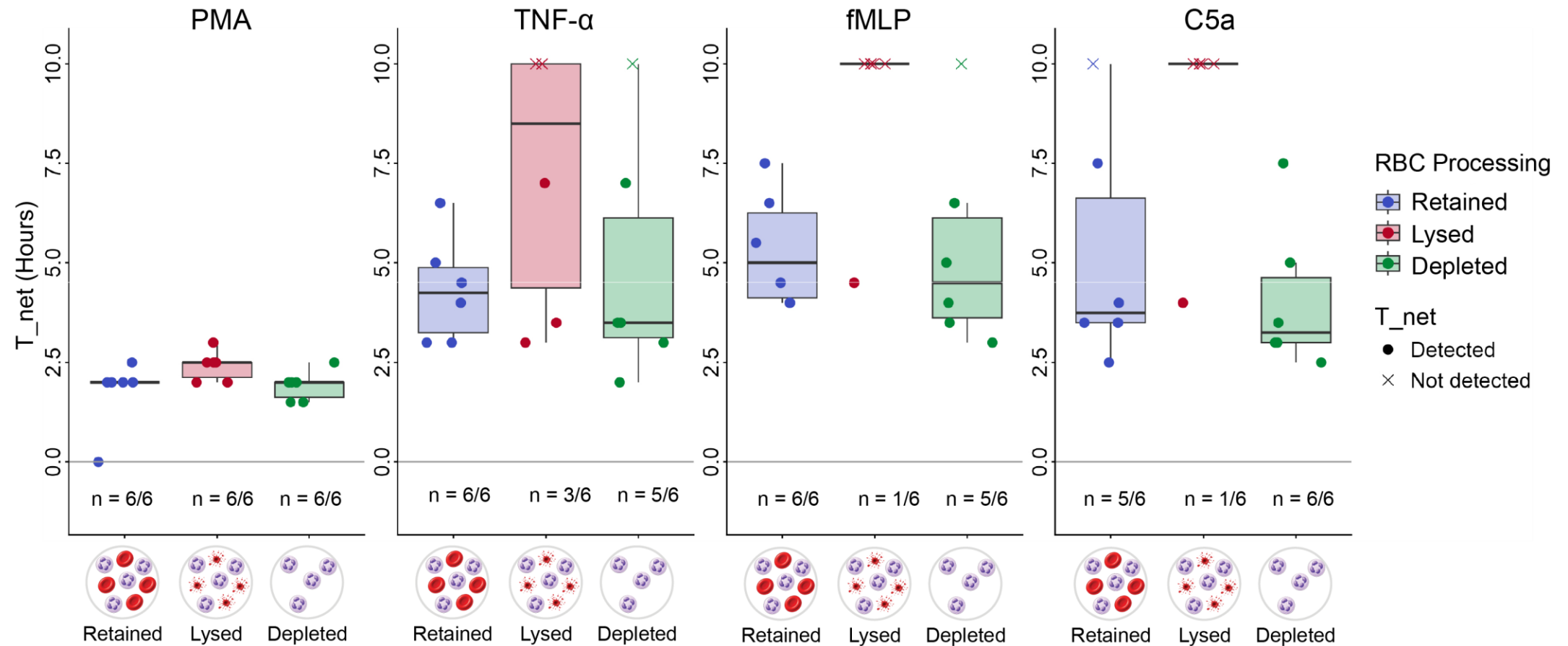


TNF- α shows NET release across all RBC processing, although only 50% of donors responded with lysis



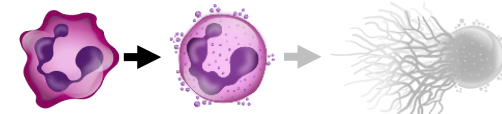
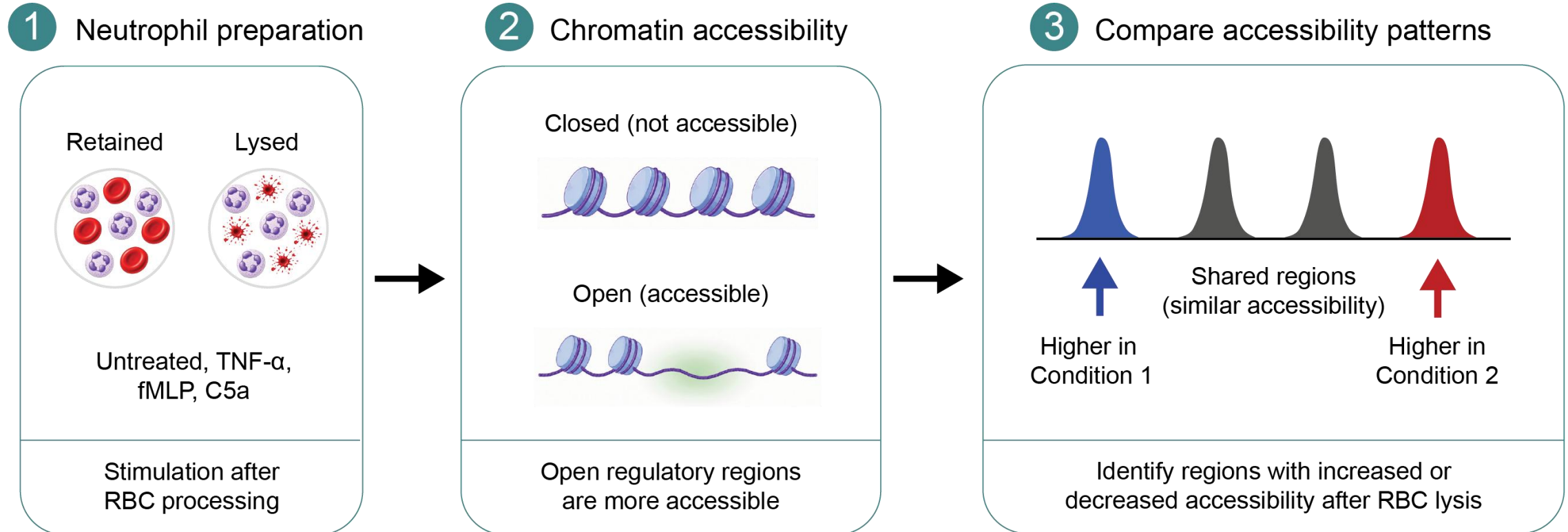
Time to detectable extracellular DNA signal; X = not detected by 10 hours

RBC lysis delays or prevents physiological NET-associated DNA release

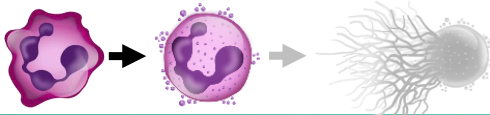
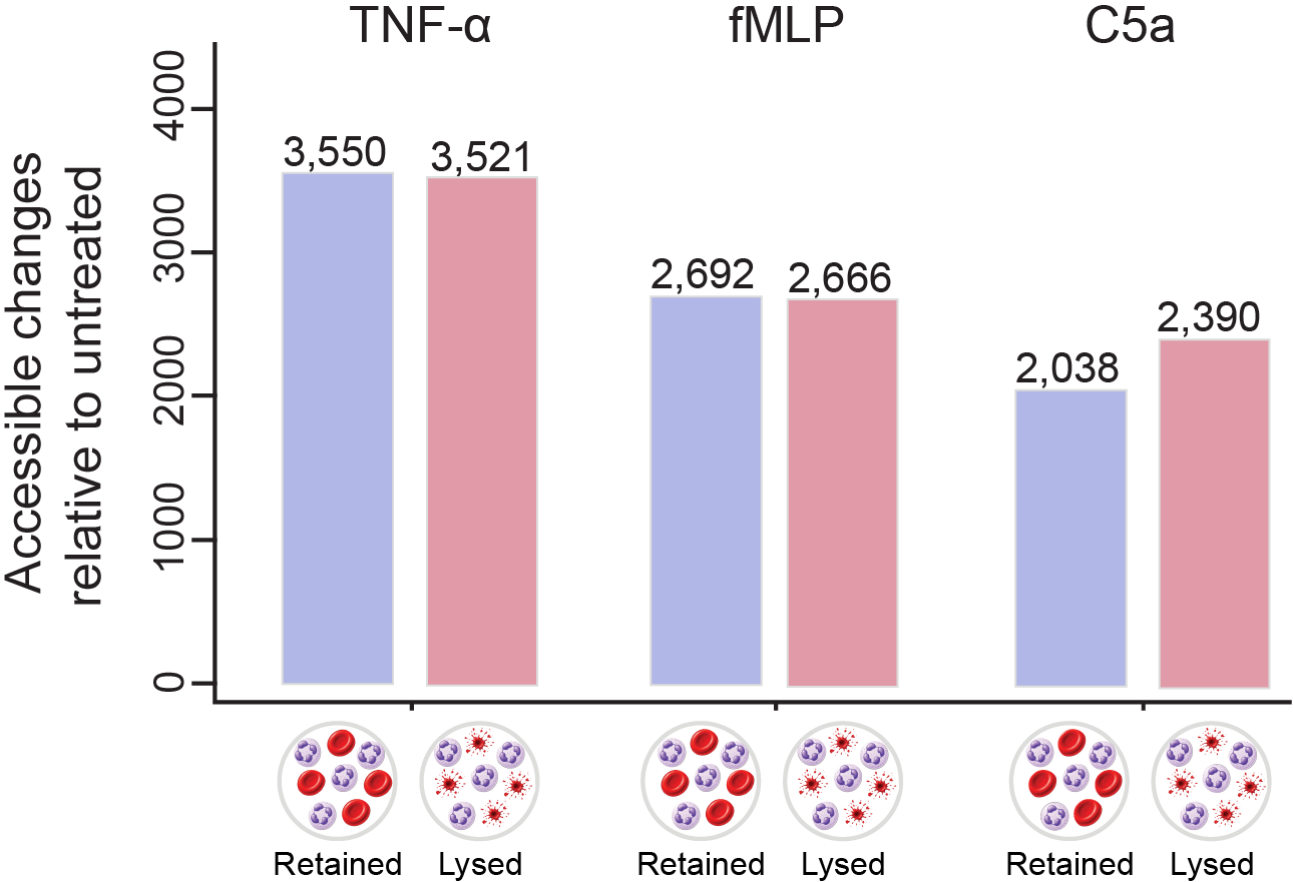
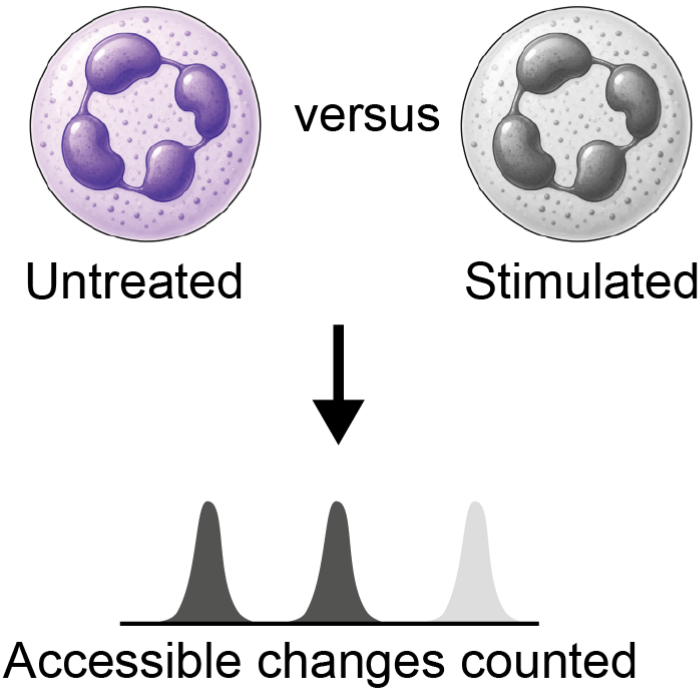


PMA response timing is preserved, while physiological stimuli reveal lysis-sensitive NET release.

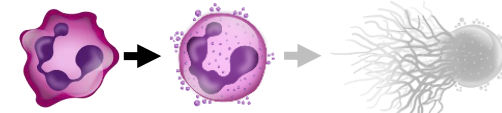
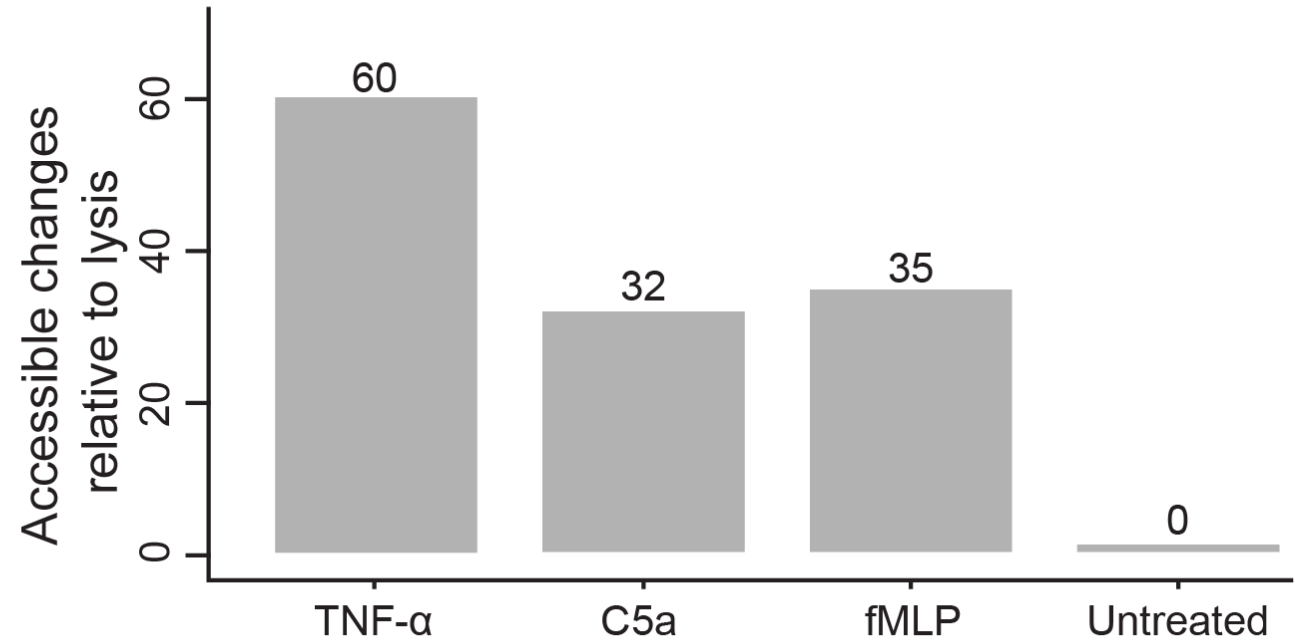
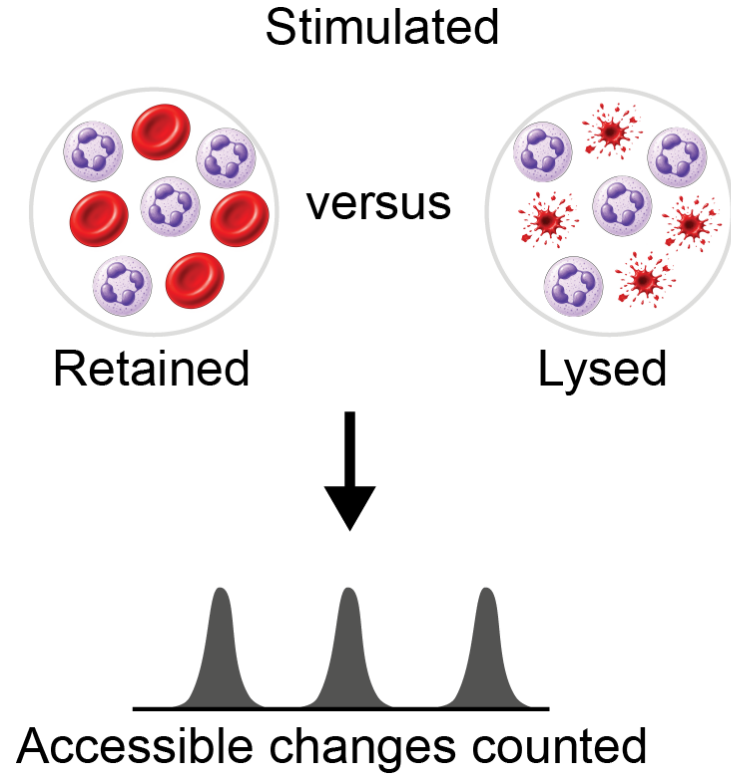
We next asked whether RBC lysis alters chromatin accessibility with ATAC-Seq



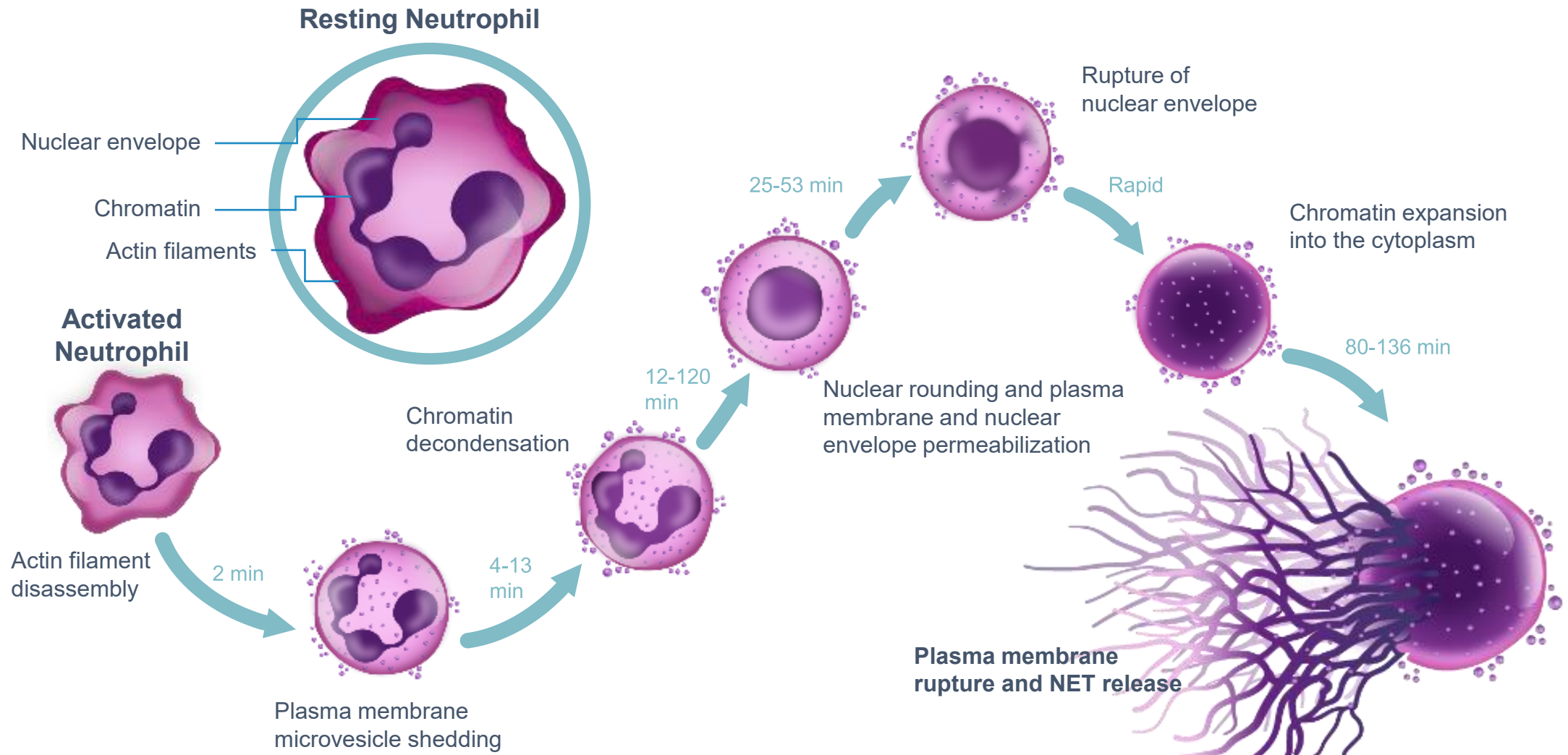
Stimulation induces a similar number of accessibility changes irrespective of RBC processing



RBC processing differences resulted in few accessibility changes under the same stimulation

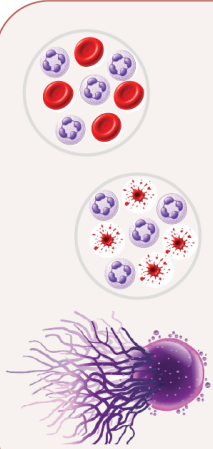


RBC lysis may impair NET release downstream of chromatin accessibility



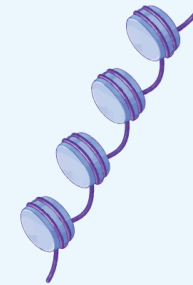
Sample processing reshapes measurable neutrophil biological responses

Assay design can preserve, mask, or misinterpret disease relevant biology



RBC lysis masks physiological NET release

Physiological stimuli showed delayed or failed NET associated DNA release after lysis.



Early chromatin accessibility is largely preserved

Only a small number of accessibility changes were detected when comparing lysed and retained.



Related work: Poster PB3254 14 Jul – 14:45

Cytokine-induced Chromatin Accessibility in Whole Blood Neutrophils Links to Sepsis Transcriptional States



Acknowledgements

Thank you to all the authors and specifically Finley Serneo, as well as the broader VolitionRx team for helpful comments and discussion. We also thank the healthy blood donors who contributed samples for this study. This work was funded by VolitionRx.

This work has been part of a sequence of understanding isolated and whole-blood derived neutrophil accessibility in response to stimuli



Front. Immunol., 24 October 2024
Sec. Molecular Innate Immunity
Volume 15 - 2024 | <https://doi.org/10.3389/fimmu.2024.1445638>

Understanding the complex chromatin dynamics in primary human neutrophils during PMA-induced NET formation

Brandi Atteberry¹ Benjamin P. Berman^{1,2} Theresa K. Kelly¹
Justin Cayford^{1*}

Front. Immunol., 03 March 2025
Sec. Molecular Innate Immunity
Volume 16 - 2025 | <https://doi.org/10.3389/fimmu.2025.1515430>

Chromatin changes associated with neutrophil extracellular trap formation in whole blood reflect complex immune signaling

Justin Cayford¹ Brandi Atteberry¹ Akanksha Singh-Taylor¹
Andrew Retter^{1,2,3} Benjamin P. Berman^{1,4} Theresa K. Kelly^{1*}

Poster PB3254 – 14JUL 14:45

Cytokine-induced chromatin accessibility in whole blood neutrophils links to sepsis transcriptional states

Cayford J¹, Atteberry B¹, Singh-Taylor A¹, Retter A¹, Berman BP^{1,2}, Kelly TK¹
1. Innovation Lab, Volition America, Carlsbad, CA, United States
2. Department of Developmental Biology and Cancer Research, The Hebrew University of Jerusalem, Jerusalem, Israel



INTRODUCTION

Neutrophils form neutrophil extracellular traps (NETs) through chromatin decondensation and extracellular DNA release. While PMA is commonly used to study NET biology, it bypasses many physiological signaling pathways. In inflammatory diseases, such as sepsis, neutrophils are exposed to complex combinations of cytokines, chemokines, complement fragments, and microbial products. How these physiologically relevant inflammatory signals remodel neutrophil chromatin accessibility, and whether those regulatory programs relate to clinical sepsis states, remains poorly defined.

AIM

Define how physiologically relevant inflammatory stimulation remodels neutrophil chromatin accessibility in whole blood and determine whether these accessibility programs correspond to transcriptional states associated with sepsis severity.

METHOD



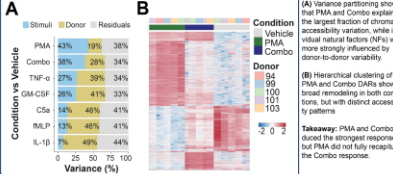
Front. Immunol., 09 June 2026
Sec. Molecular Innate Immunity
Volume 17 - 2026 | <https://doi.org/10.3389/fimmu.2026.1796580>

Cytokine-induced chromatin accessibility in whole blood neutrophils links to sepsis transcriptional states

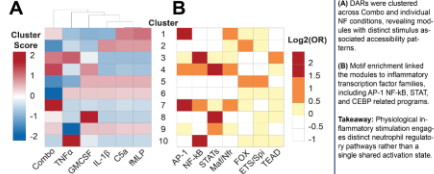
Justin Cayford¹ Brandi Atteberry¹ Akanksha Singh-Taylor¹
Andrew Retter¹ Benjamin P. Berman^{1,2} Theresa K. Kelly^{1*}

RESULTS

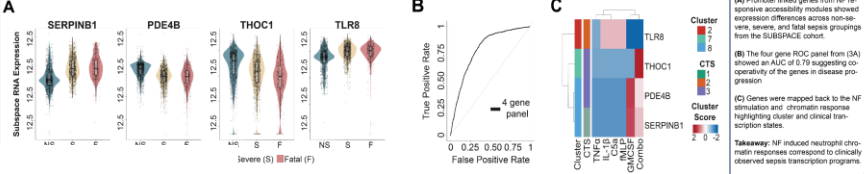
1. Stimulation drives neutrophil chromatin changes



2. Natural factors induce modular regulatory programs



3. NF-linked promoter programs map to sepsis transcriptional states



REFERENCES

- Cayford J, Atteberry B, Singh-Taylor A, Retter A, Berman BP, Kelly TK. Cytokine-induced chromatin accessibility in whole blood neutrophils links to sepsis transcriptional states. *Front Immunol*. 2026;17:1796580. doi:10.3389/fimmu.2026.1796580
- Anticicic DB, Burnham KL, Al-Bedh F, Santhakumaran S, Brett SJ, Hinds CJ, et al. Transcriptional signatures in sepsis and a differential response to steroids: from the VANISH randomized trial. *Am J Respir Crit Care Med*. 2019;199:980–986.
- Sweeney TE, Asadi TD, Donato M, Haynes WA, Pienratt TM, Henao R, et al. Unsupervised analysis of transcriptomics in bacterial sepsis across multiple datasets reveals three robust clusters. *Crit Care Med*. 2018;46:915–925.

ACKNOWLEDGEMENTS

We thank Christina Wheeler and Finley Serneo, as well as the broader VolitionRx team, for helpful review of the data and discussion. We also thank the healthy blood donors who contributed samples.



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