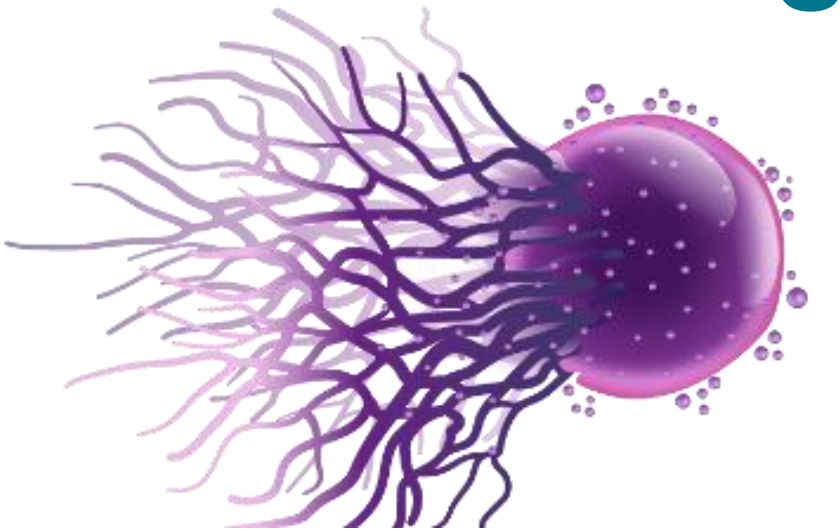
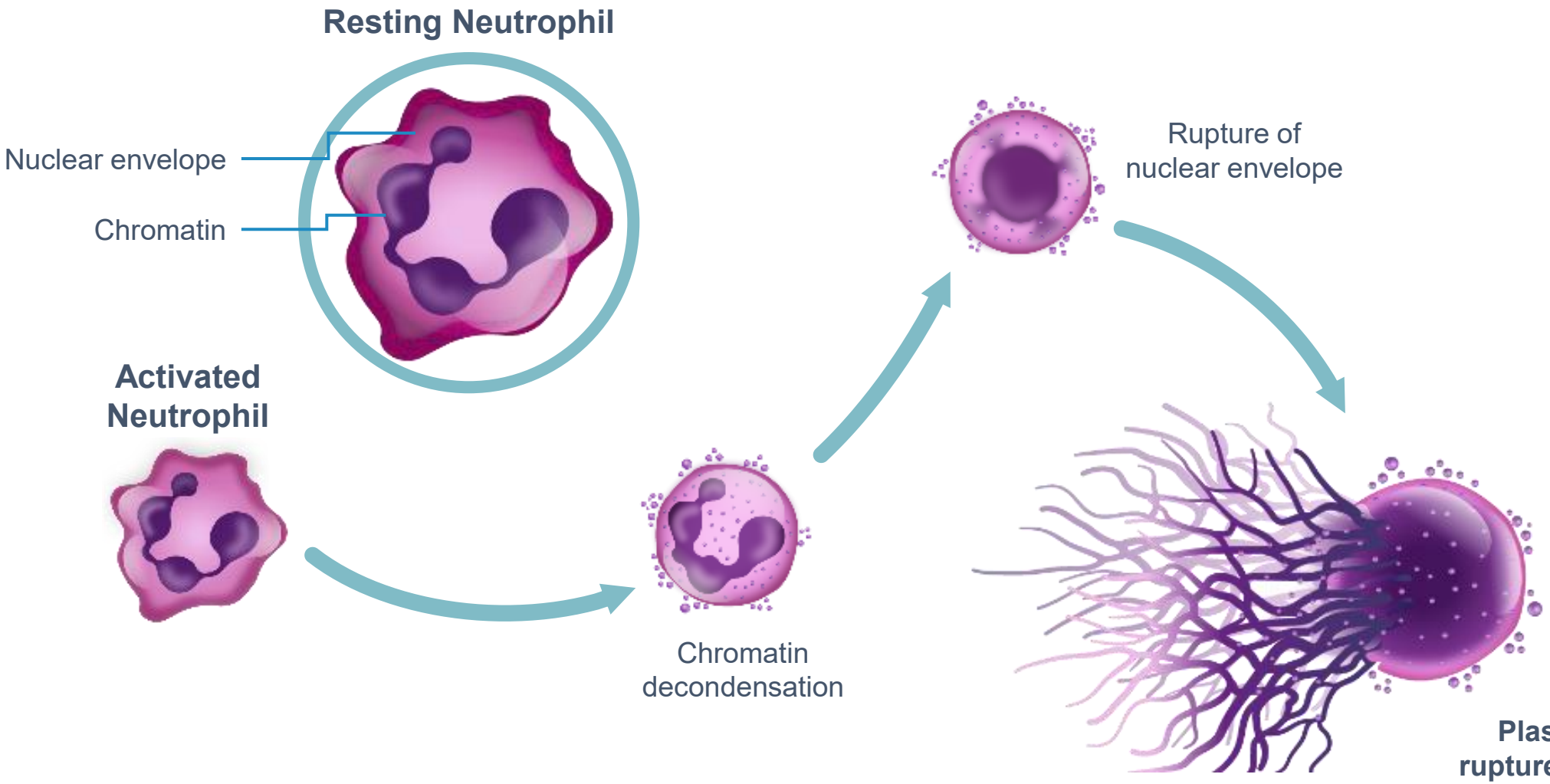
Red blood cell lysis modulates neutrophil extracellular trap release in response to physiological inflammatory stimuli



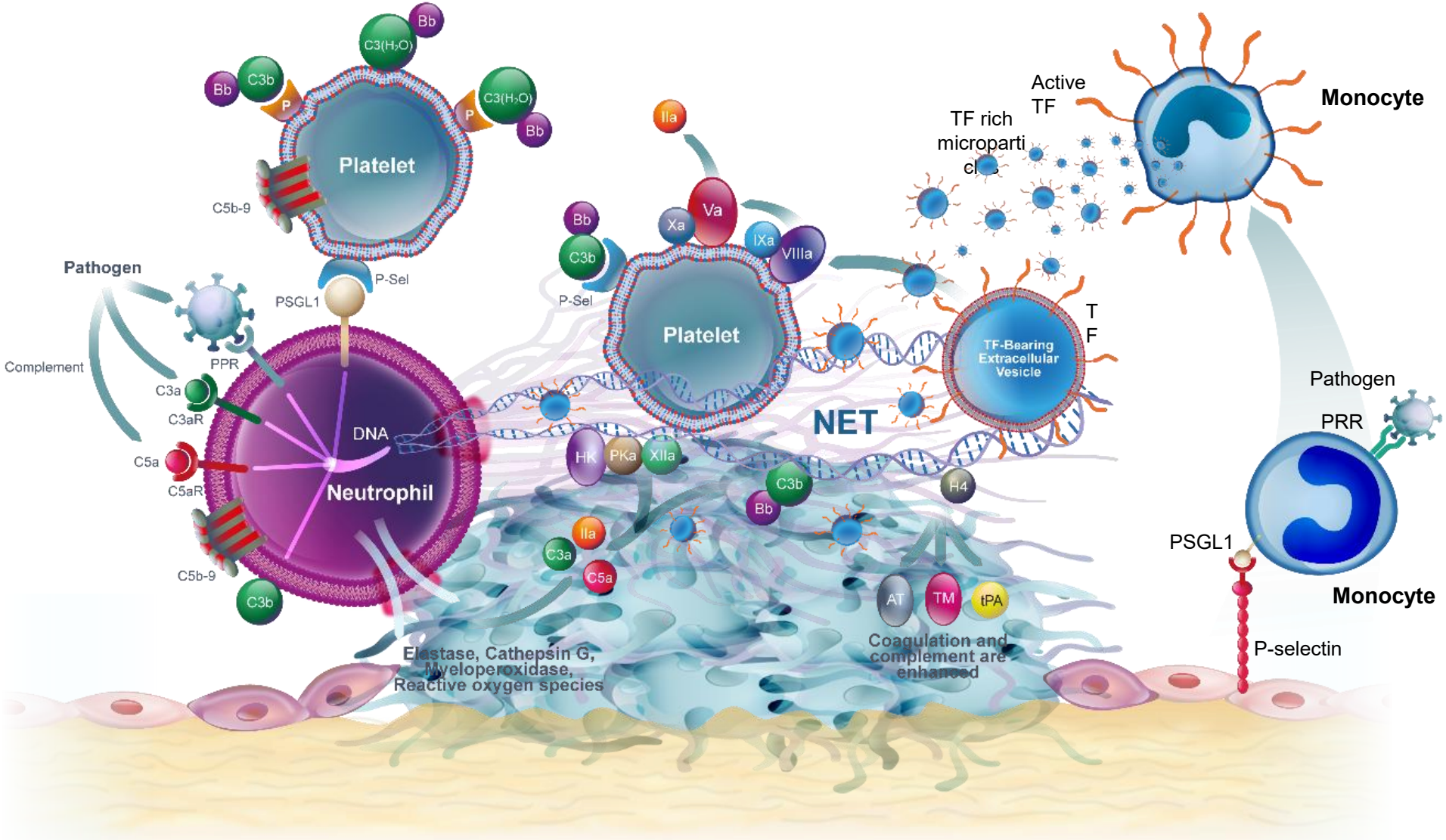
Justin Cayford, PhD

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

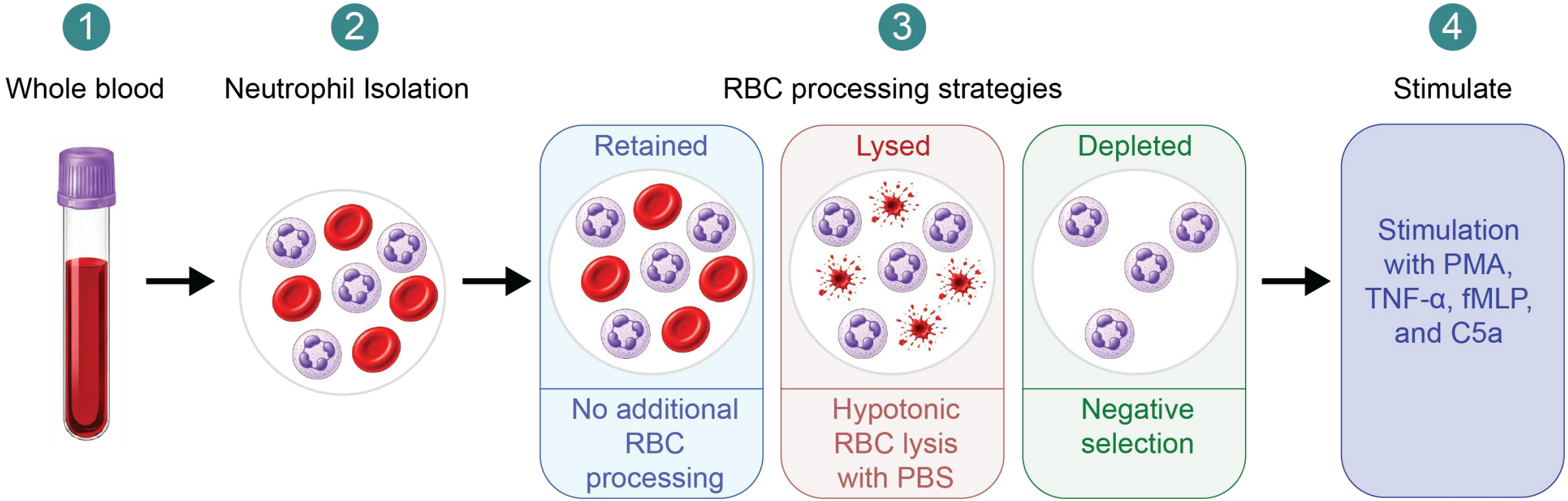
Technical Challenge

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

Study approach

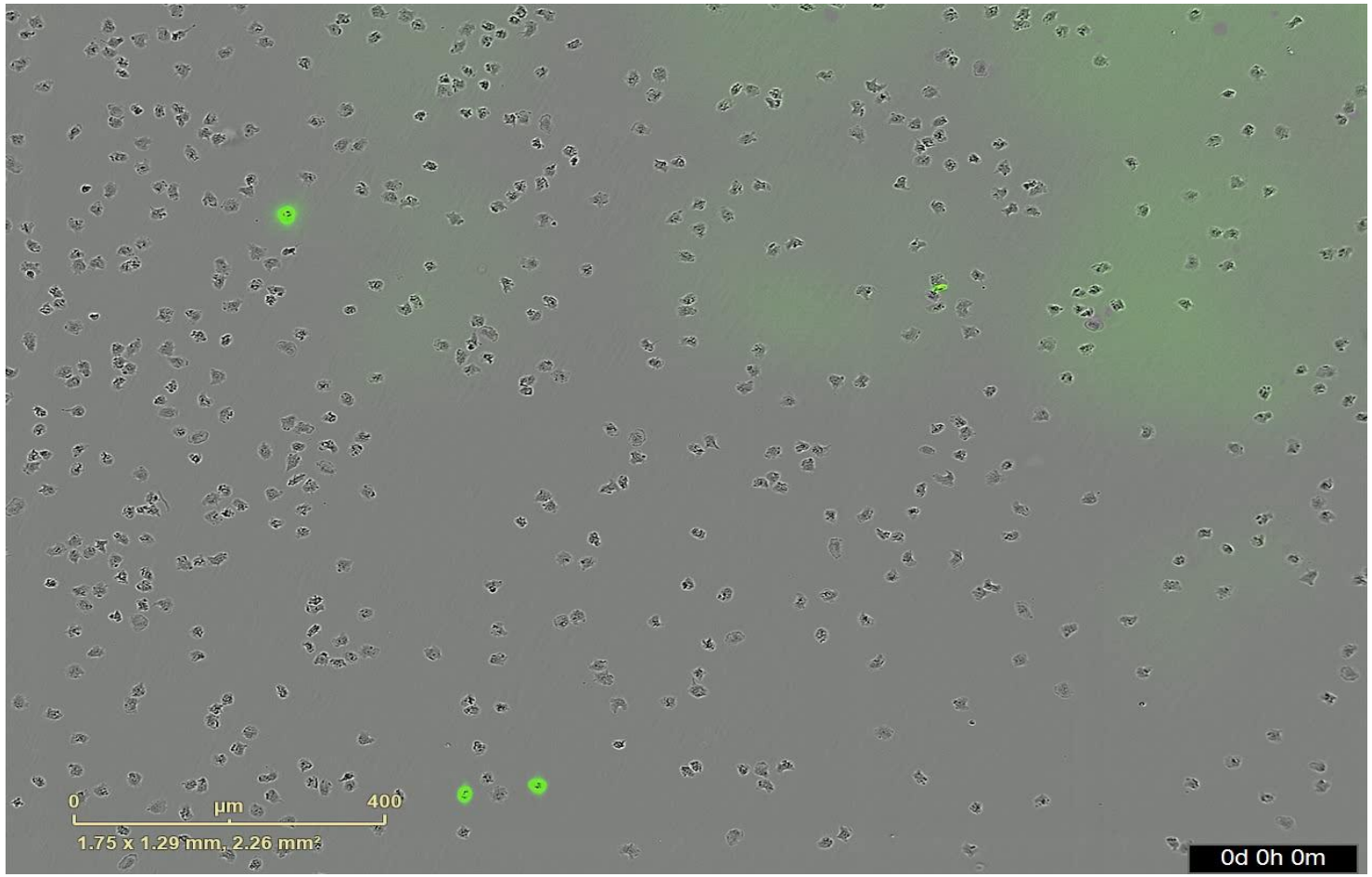
Determine how different neutrophil isolation methods affect DNA release and chromatin accessibility

After neutrophil isolation, three RBC processing strategies were compared

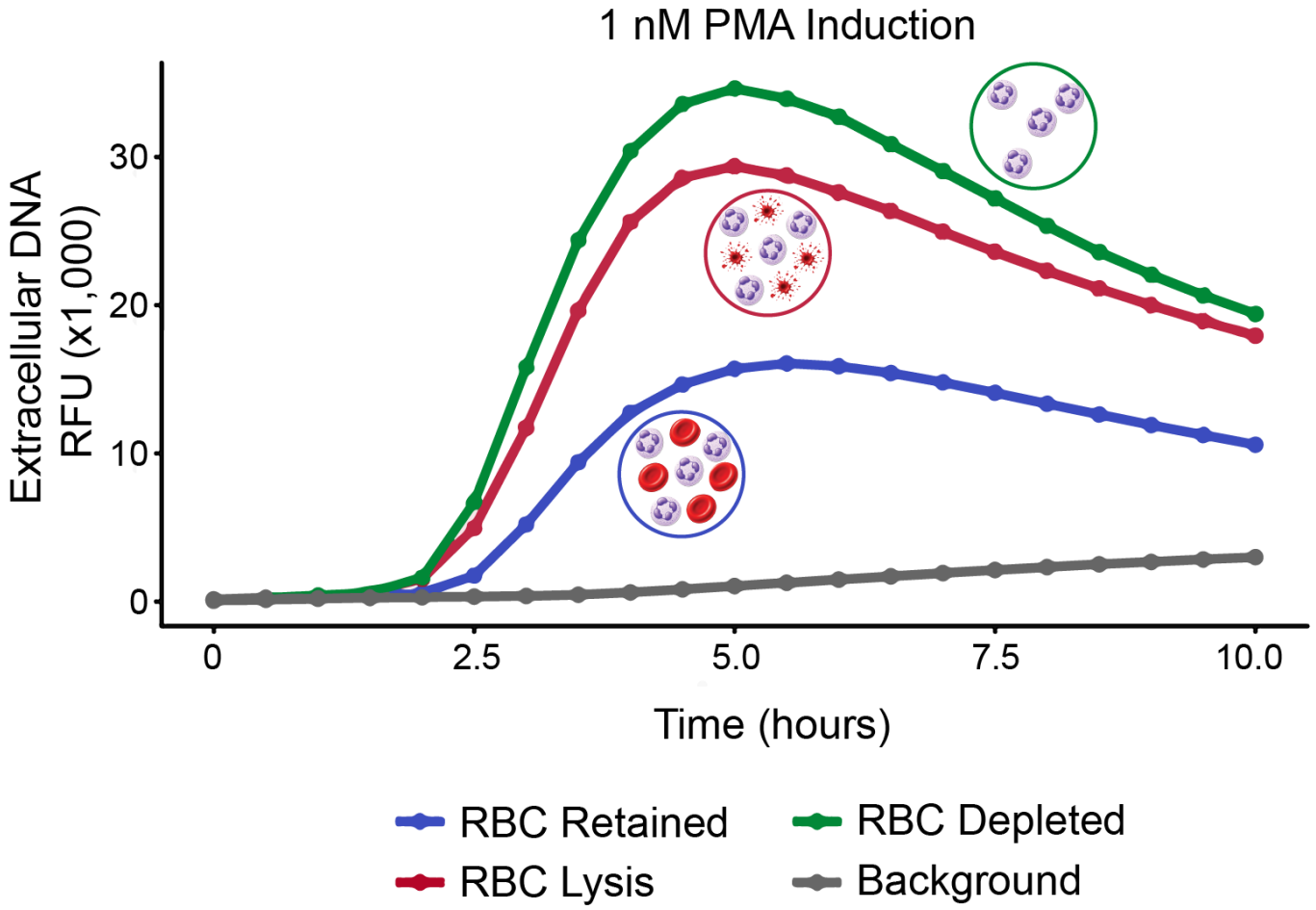


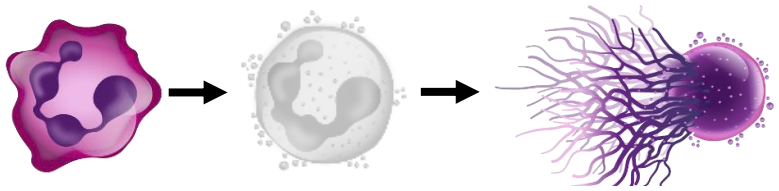
Live-cell imaging is used to show NET associated DNA release

1 nM PMA



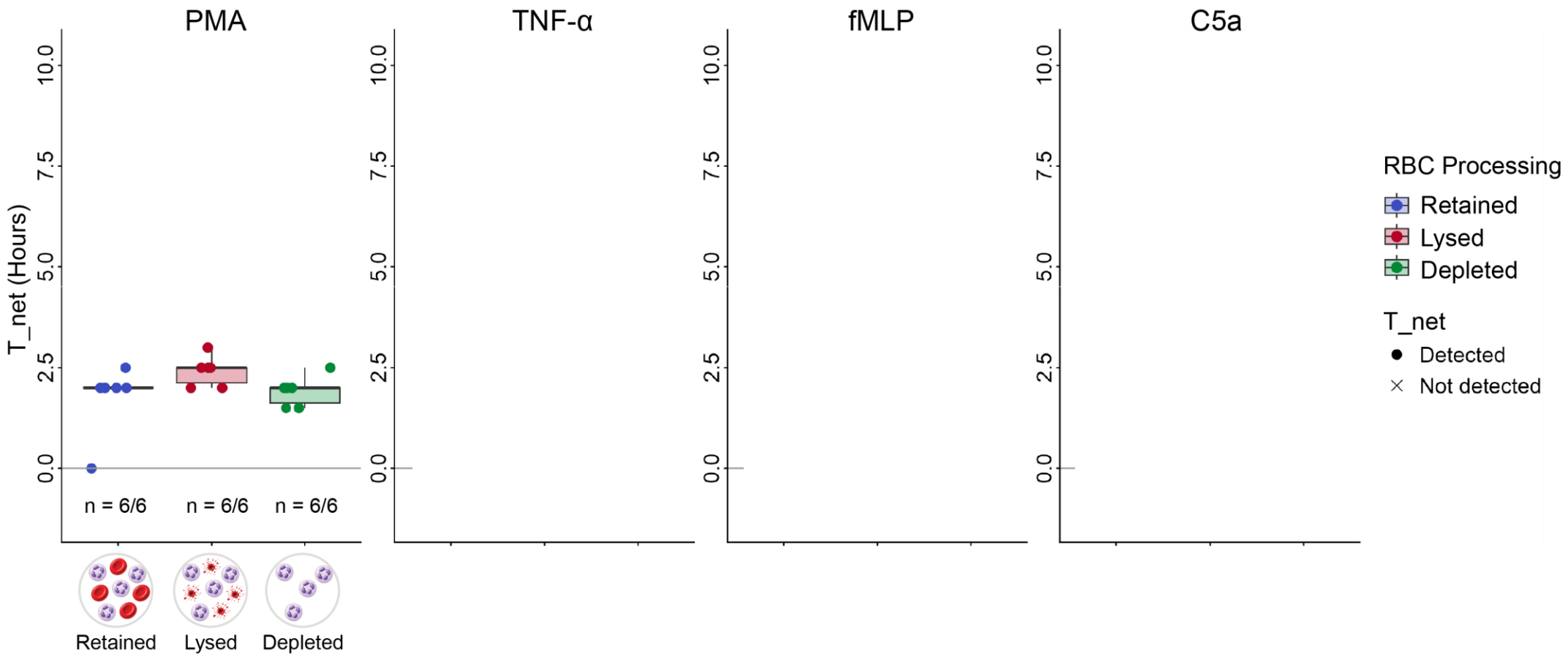
Using the live-cell imaging, timing of NET-associated DNA release was determined





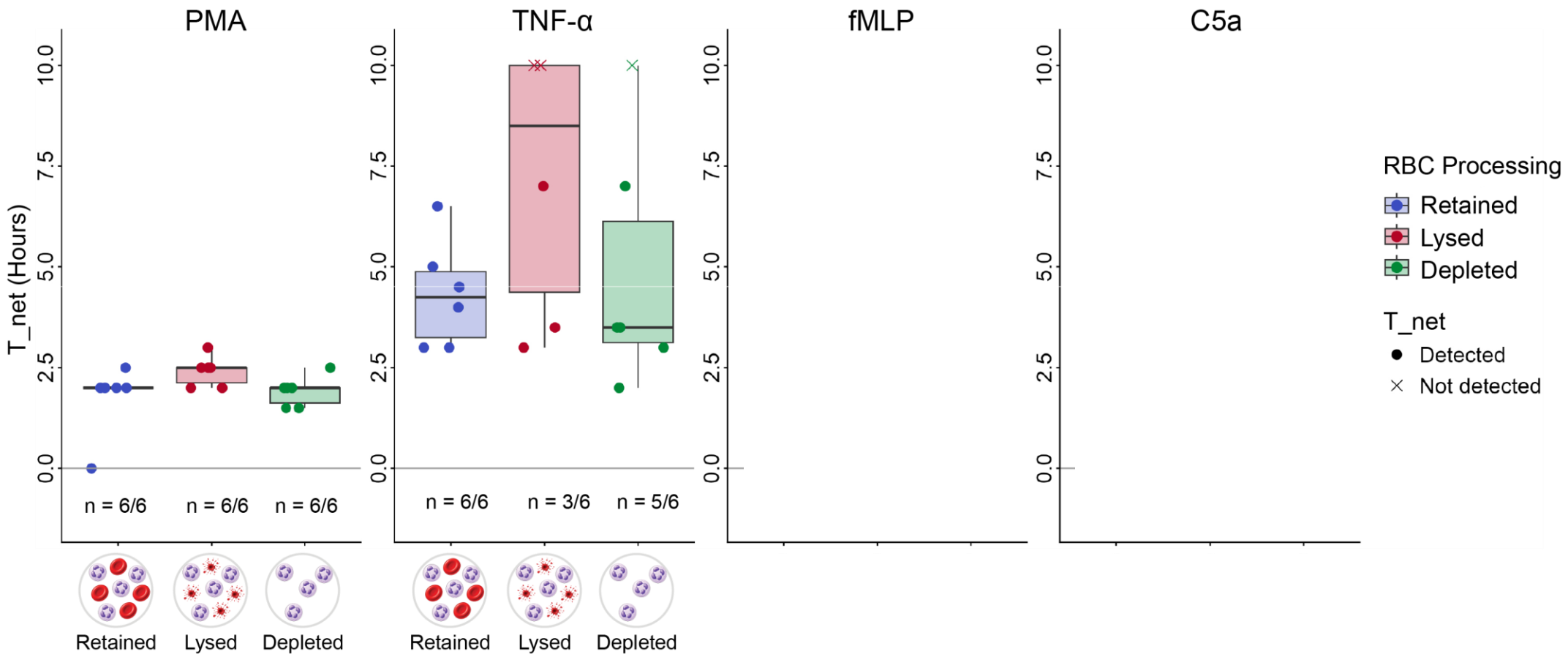
- T_net = time to detectable NET associated DNA release
- No detection by 10 hours = non-detected

PMA response timing is preserved across processing conditions



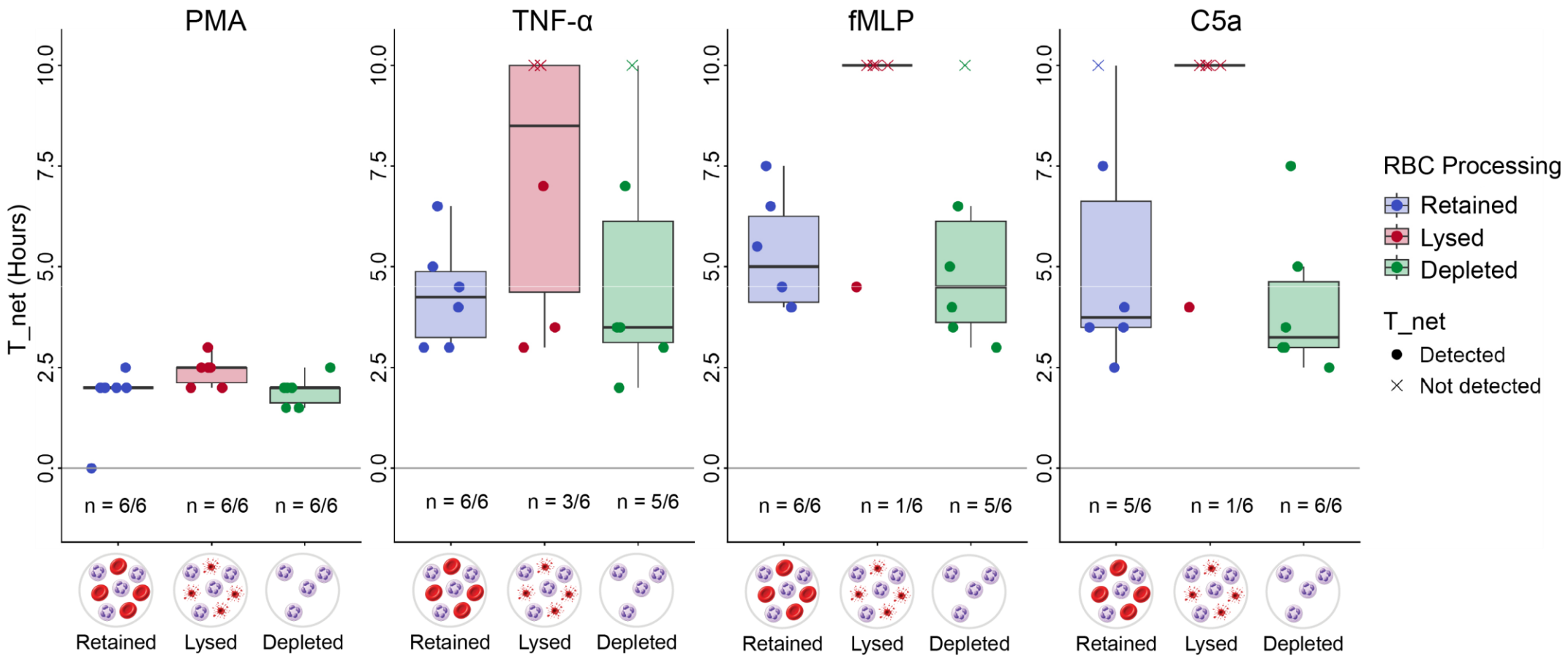
Time to detectable extracellular DNA signal; X = not detected by 10 hours

TNF-a 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



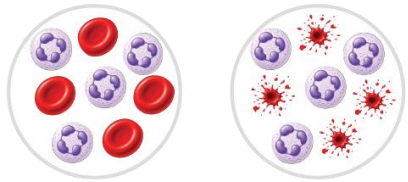
PMA response timing is preserved, while physiological stimuli reveal lysis-sensitive NET release.

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

1 Neutrophil preparation

Retained

Lysed

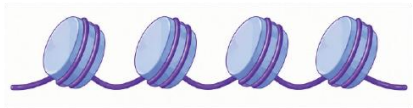


Untreated, TNF-α, fMLP, C5a

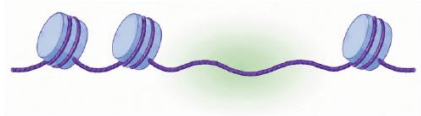
Stimulation after RBC processing

2 Chromatin accessibility

Closed (not accessible)

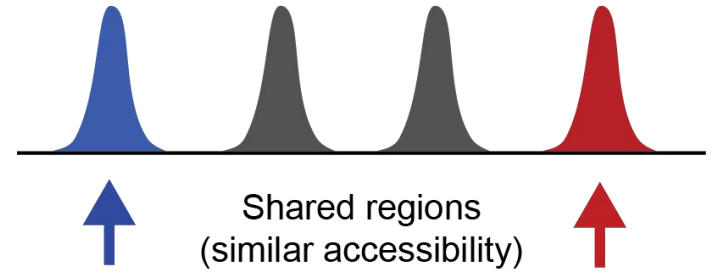


Open (accessible)

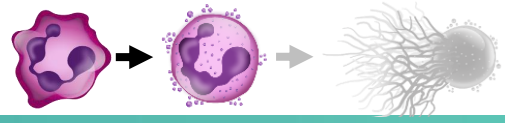


Open regulatory regions are more accessible

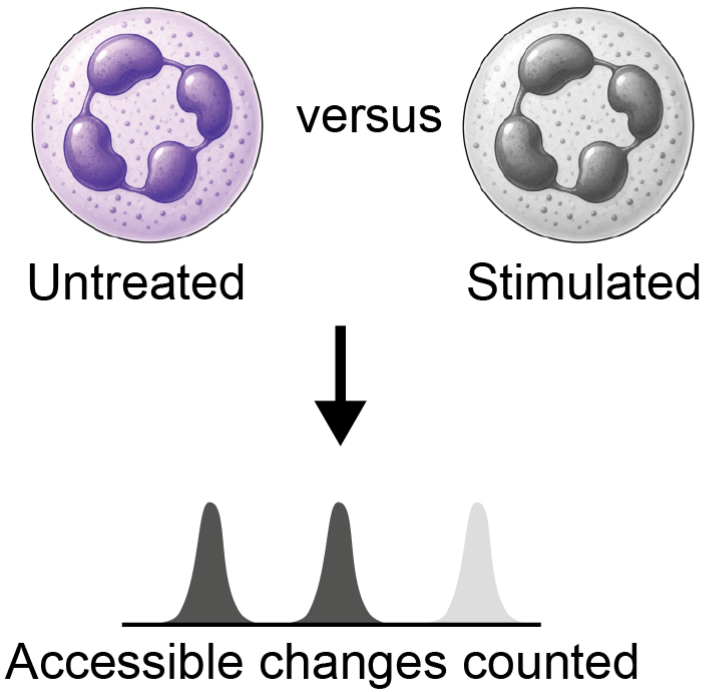
3 Compare accessibility patterns

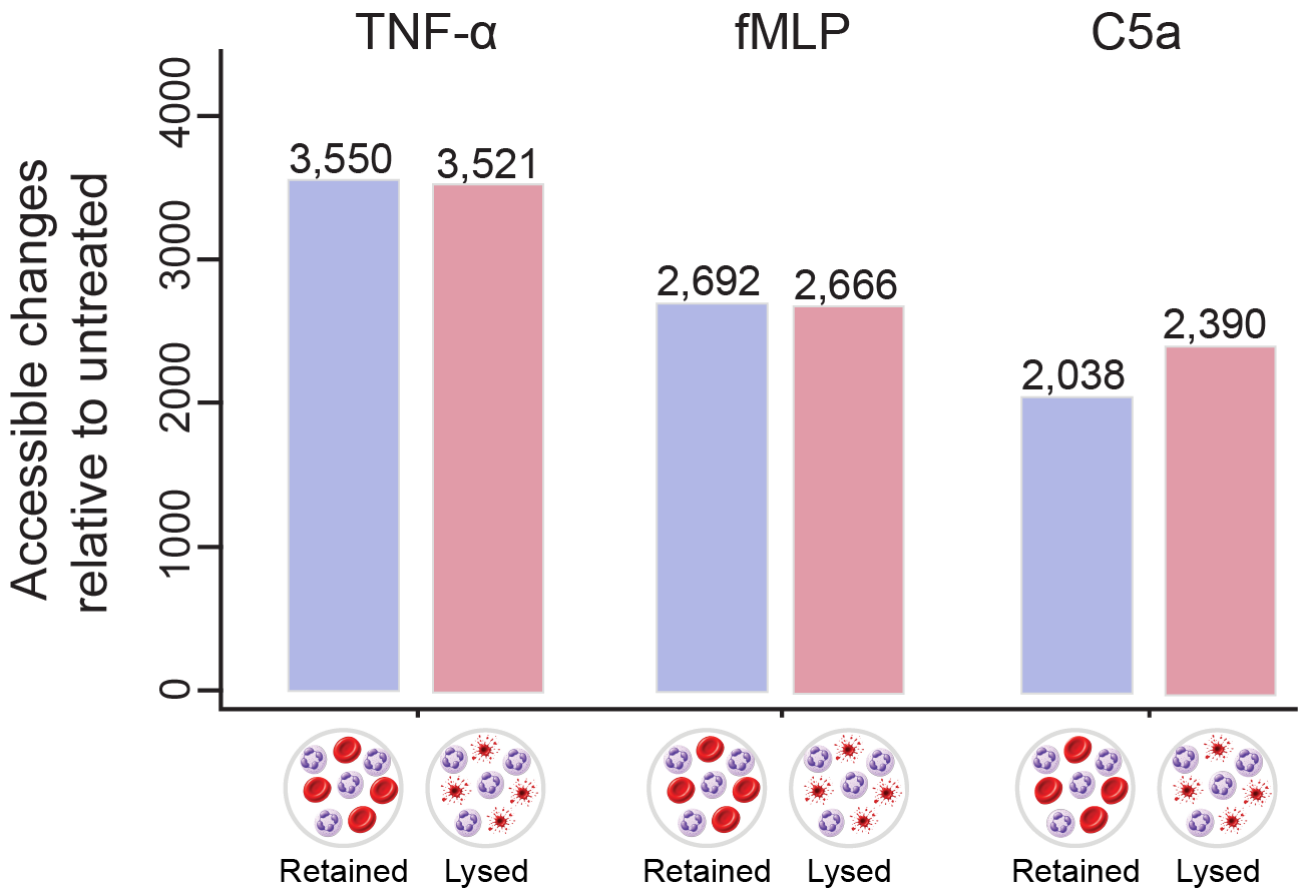


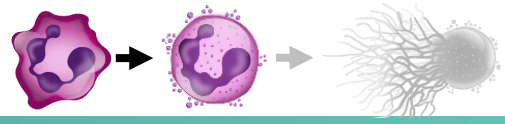
Identify regions with increased or decreased accessibility after RBC lysis



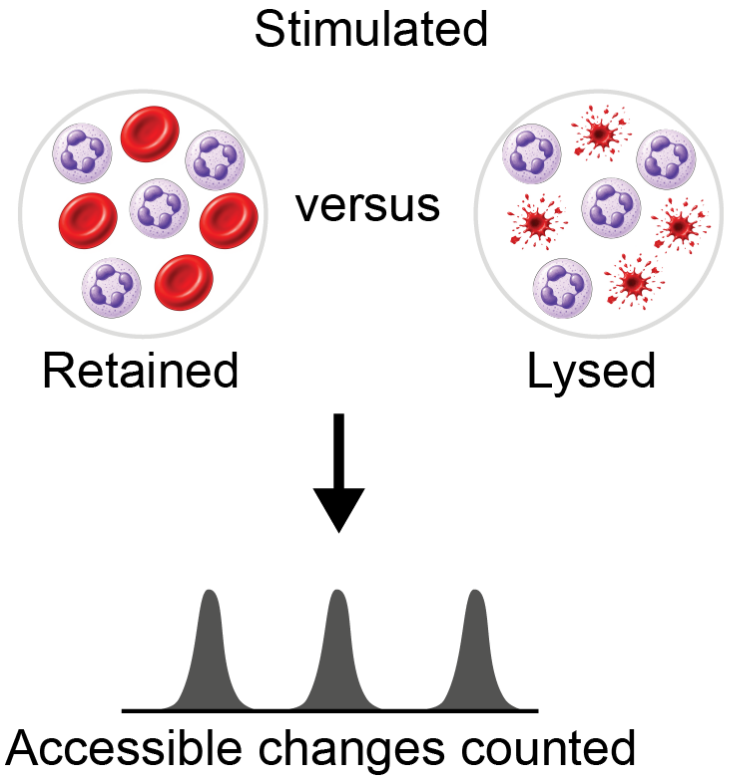
Stimulation induces a similar number of accessibility changes irrespective of RBC processing

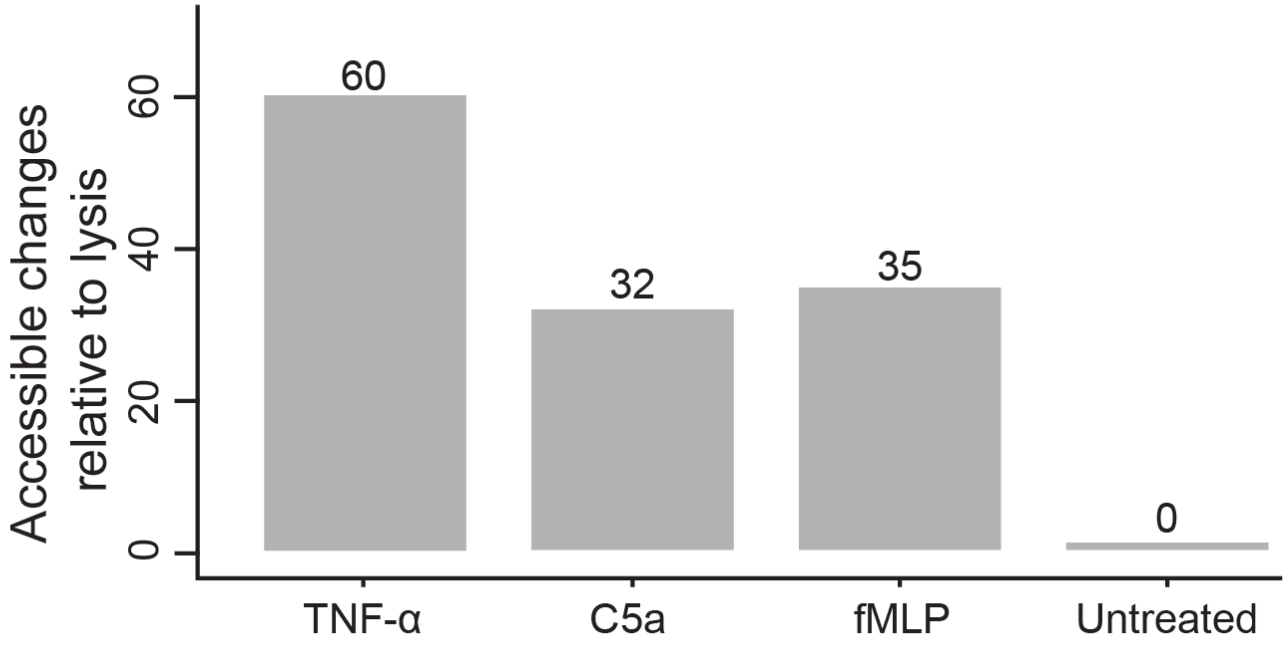




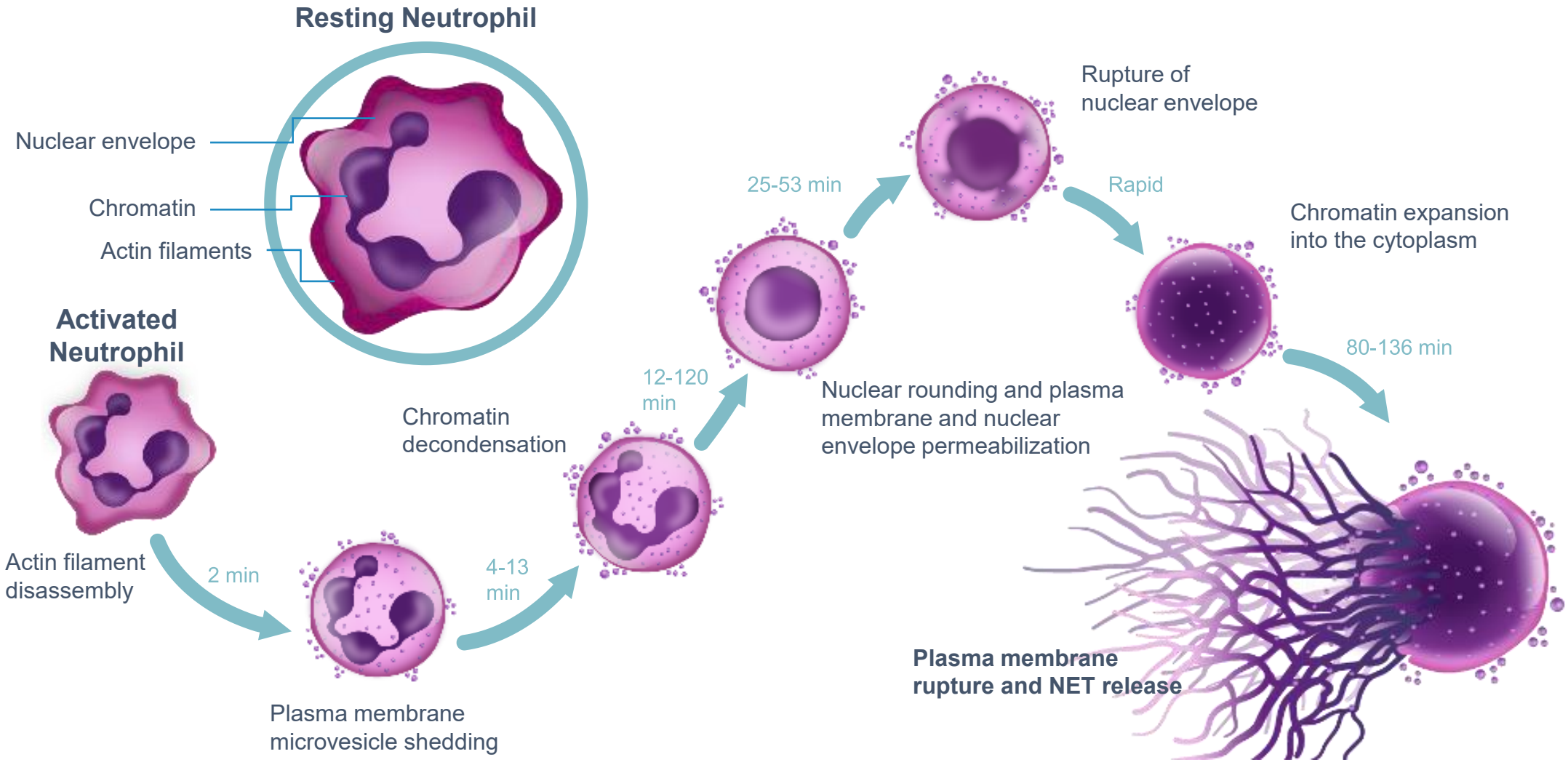


RBC processing differences resulted in few accessibility changes under the same stimulation



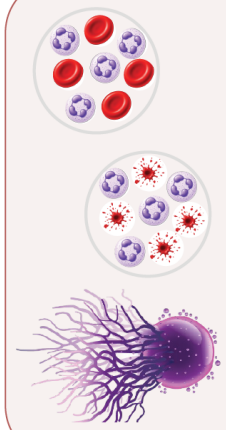


RBC lysis may impair NET release downstream of chromatin accessibility



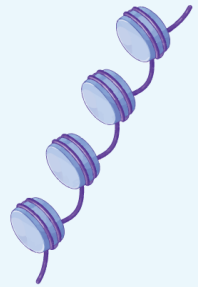
Sample processing reshapes measurable neutrophil biological responses

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



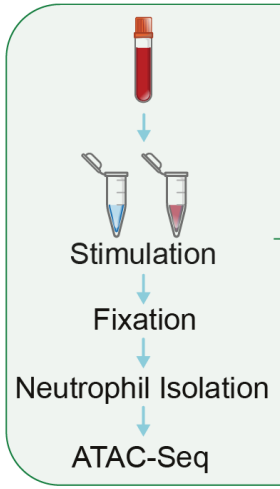
RBC lysis masks physiological NET release

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



Early chromatin accessibility is largely preserved

Only a small number of accessiblity 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

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Thank you to all the authors and 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

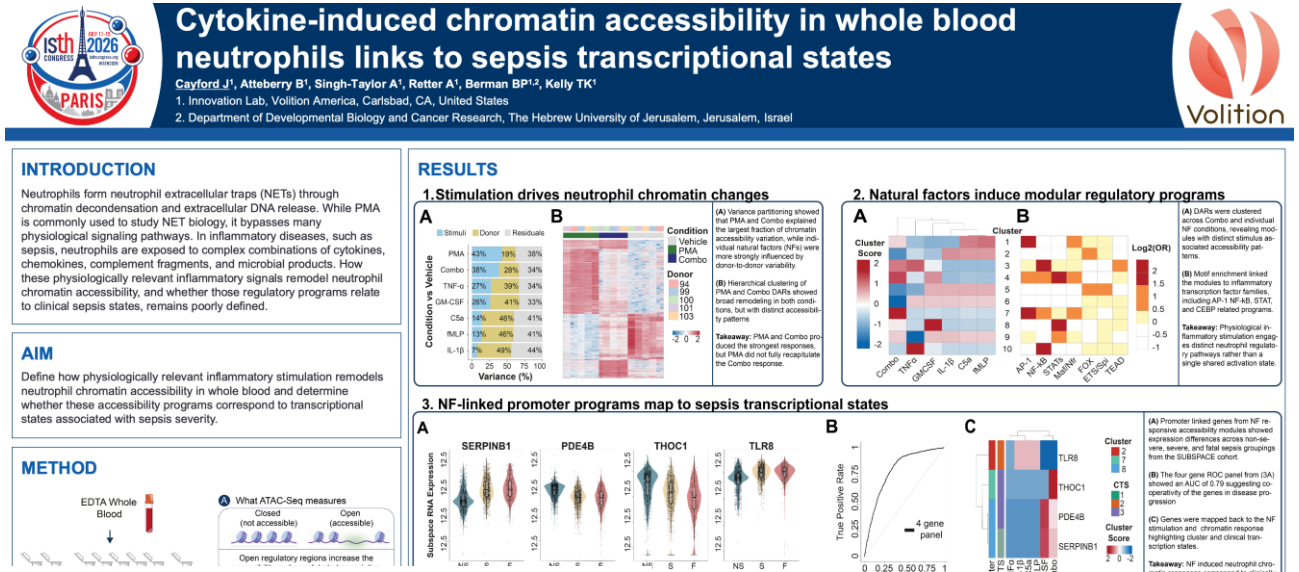
Brandi Atteberry [1] · Benjamin P. Berman [1,2] · Theresa K. Kelly [1] · Justin Cayford [1*]

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 Cayford [1] · Brandi Atteberry [1] · Akanksha Singh-Taylor [1] · Andrew Retter [1,2,3] · Benjamin P. Berman [1,4] · Theresa K. Kelly [1*]

Poster PB3254 – 14JUL 14:45



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

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