Neutrophil Extracellular Traps (NETs) formation in mice with myeloid-specific ablation of *STIM1/2* genes

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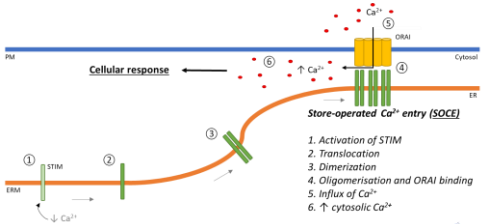
Background

SOCE promotes neutrophil antibacterial functions such as phagocytosis, transcription , chemotaxis & bacterial lysis. NETosis is a neutrophil function involving extracellular release of DNA and proteases to immobilize and degrade bacteria.

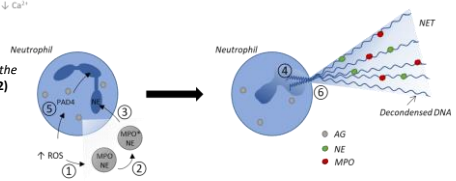
The importance of the SOCE-associated STIM proteins in NETosis has not been defined.

Measuring NETosis is tedious and subjective. An automated arrayed analysis system is required for efficient and reliable research.



NETosis

(1) ↑ *of reactive oxygen species (ROS) activates myeloperoxidase (MPO) in the azurophilic granules (AG)* **(2)** *activated MPO allows movement of neutrophil elastase (NE) from AG to nucleus* **(3)** *NE causes membrane disruption* **(4)**



(1) ↑ *of ROS activates PAD4* **(5)** *PAD4 leads to histone citrullination and chromatin decondensation* **(6)**

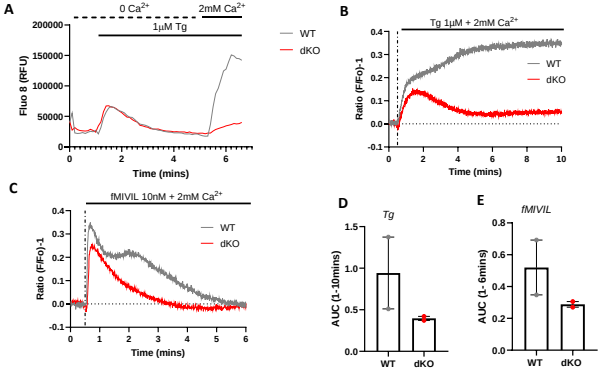
NETosis & Ca²⁺

- Ionomycin and IL8 – known to ↑ *cytosolic Ca²⁺* & produce NETs
- EGTA & BAPTA-AM – known to ↓ *cytosolic Ca²⁺* & prevents NETs

What is the role of Stim proteins and Ca²⁺ mobilisation in NETosis?

Results

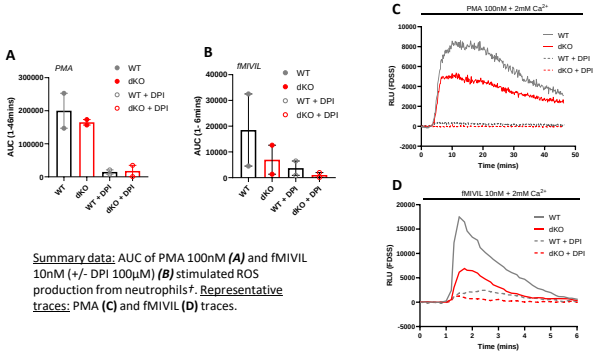
***Figure 1.* Ca²⁺ response is reduced in the *Stim* dKO genotype**



(A) Representative trace: Flowcytometry validation of animal model. ***(B-C)*** . Representative trace: Tg ***(B)*** and fMIVIL ***(C)*** of Ca^{2+} response over time. ***(D-E)*** Summary data: AUC of Tg ***(D)*** and fMIVIL ***(E)*** stimulated Ca^{2+} response from neutrophils†.

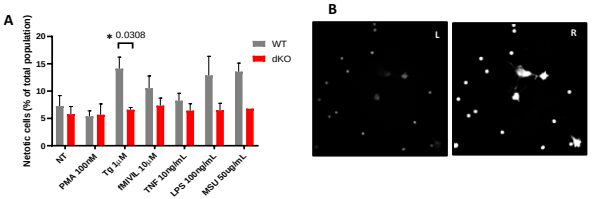
†Neutrophils isolated from mouse bone marrow of B6 and cre/lox stim1/stim2 dKO.

***Figure 2.* ROS production is reduced in the *Stim* dKO genotype**



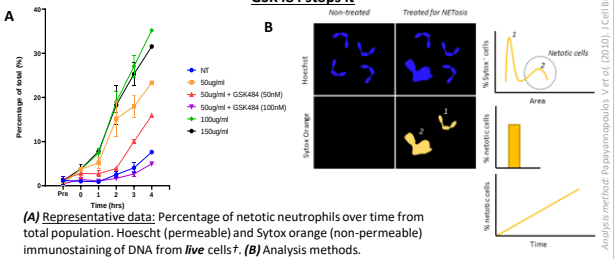
Summary data: AUC of PMA 100nM ***(A)*** and fMIVIL 10nM (+/- DPI 100µM) ***(B)*** stimulated ROS production from neutrophils†. Representative traces: PMA **(C)** and fMIVIL **(D)** traces.

***Figure 3. Stim* dKO decreases netotic activity of mouse neutrophils.**



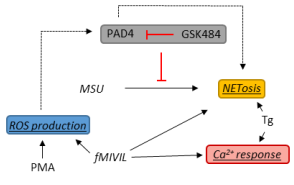
(A) Summary data: Percentage of netotic neutrophils from total population. Hoescht immunostaining of nuclei from ***fixed*** cells†. **B)** Representative image: fMIVIL treated WT neutrophils **(L)**, enhanced image **(R)**.

***Figure 4.* MSU increases the percentage of WT netotic neutrophils. PAD4 inhibitor GSK484 stops it**



(A) Representative data: Percentage of netotic neutrophils over time from total population. Hoescht (permeable) and Sytox orange (non-permeable) immunostaining of DNA from ***live*** cells†. ***(B)*** Analysis methods.

Discussion & Implications



Stim1/Stim2 myeloid ablation reduces PMN Ca^{2+} responses, ROS production and NETosis

The link between the three events implies that Ca^{2+} mobilisation via STIM proteins is involved in ROS production and NET formation.

Genetic and pharmacological approaches will be used to clarify the role of Ca^{2+} responses and ROS production in the NETosis process