

Supplementary material to:

BIO-IMPEDANCE MEASUREMENT ALLOWS DISPLAYING THE EARLY STAGES OF NEUTROPHIL EXTRACELLULAR TRAPS

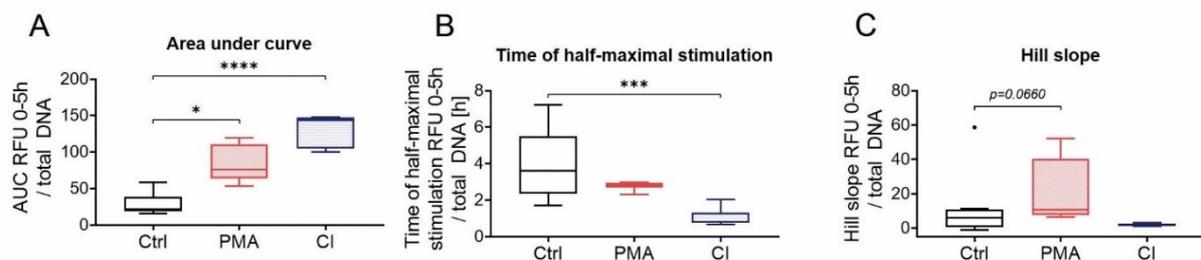
Caren Linnemann¹, Sascha Venturelli^{2,3}, Franziska Konrad⁴, Andreas K. Nussler¹, Sabrina Ehnert^{1*}

- ¹ Siegfried Weller Institute for Trauma Research, BG Unfallklinik Tuebingen, Eberhard Karls Universität Tuebingen, Tuebingen, Germany
- ² Institute of Physiology, Department of Vegetative and Clinical Physiology, University Hospital Tuebingen, Tuebingen, Germany
- ³ Institute of Nutritional Sciences, Department of Nutritional Biochemistry, University of Hohenheim, Stuttgart, Germany
- ⁴ Department of Anesthesiology and Intensive Care Medicine, University Hospital of Tuebingen, Tuebingen, Germany

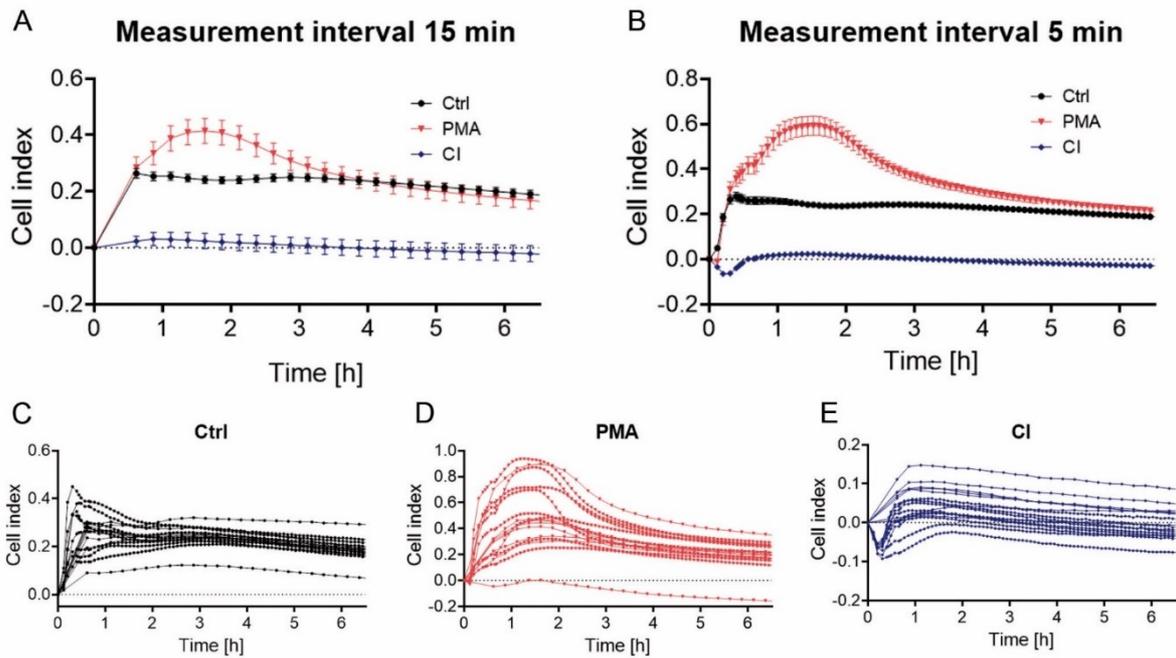
* **Corresponding author:** Dr. Sabrina Ehnert, Siegfried Weller Institute for Trauma Research at BG Unfallklinik Tuebingen, Eberhard Karls Universität Tuebingen, Schnarrenbergstr. 95, 72076 Tuebingen, Germany; Tel.: +497071 606 1065, Fax.: +49 70 71 606 1978; E-mail: sabrina.ehnert@gmail.com; ORCID: <https://orcid.org/0000-0003-4347-1702>

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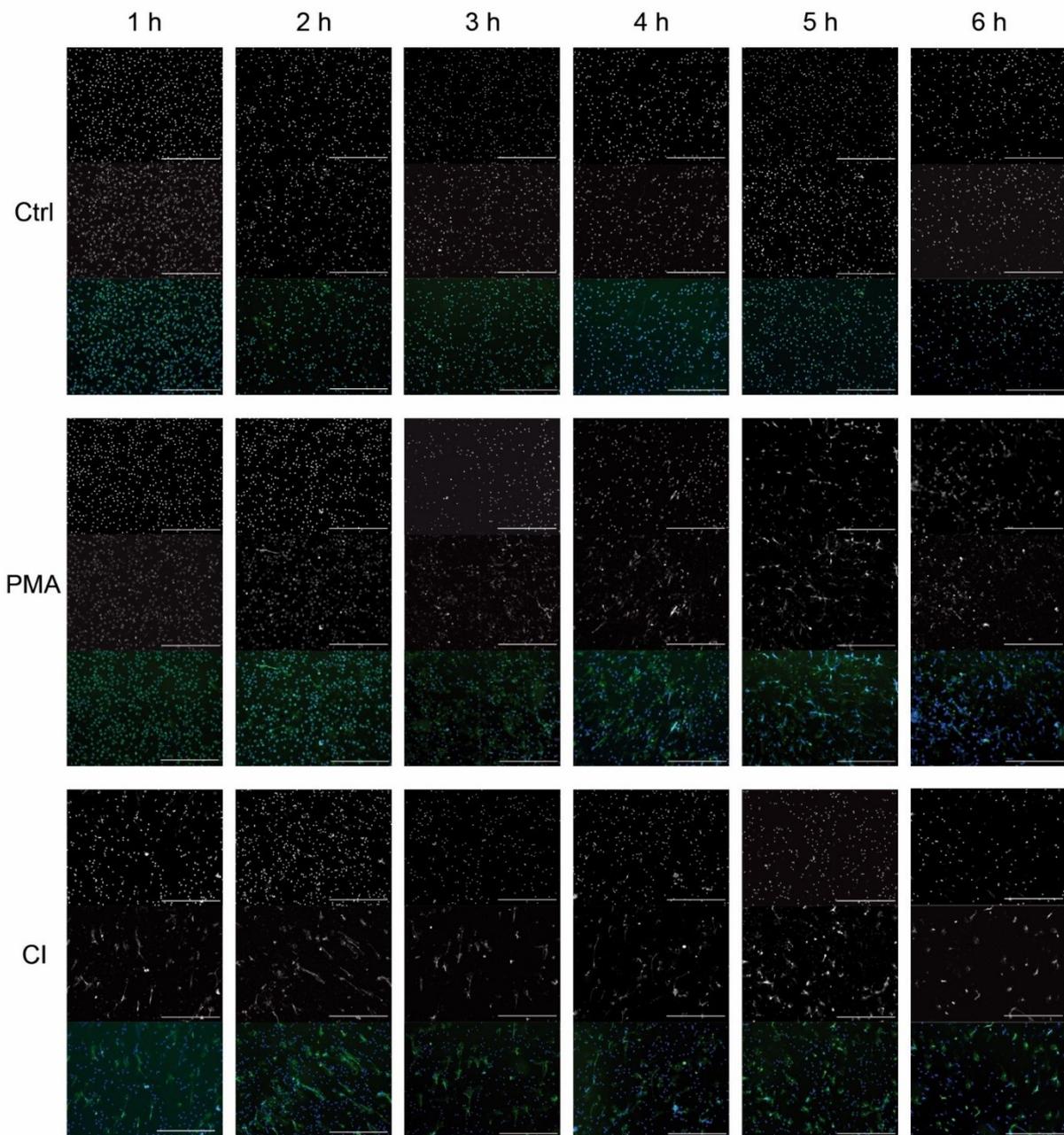
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Supplementary Figure 1: Sytox Green Assay normalized to total DNA values. Neutrophils were stained with 1 μ M Sytox Green and fluorescence measured over a time of 5 h. Different analysis methods were used to show neutrophil activation by different stimuli. **(A)** Analysis of DNA release by measurement of area under curve. **(B)** Analysis of stimulation time by determination of half-maximal stimulation time. **(C)** Analysis of strength of stimulation by measurement of hill slope. All measured fluorescence data were normalized to total DNA values determined by 1 % Triton-X-100 treated cells. N=15, n=3. Statistical analysis was done by Kruskal-Wallis test followed by Dunn's correction for multiple comparisons. Ctrl: Control, PMA: phorbol 12-myristate 13-acetate, CI: calcium ionophore A23187, AUC: Area under curve, RFU: relative fluorescence unit.



Supplementary Figure 2: Raw data of bio-impedance measurement for the activation of neutrophils. Neutrophils were seeded in eSight E-Plate VIEW 96 and bio-impedance measured for over 6 h. **(A)** Mean values of 8 donors with a measurement interval of 15 min. **(B)** Mean values of 8 donors with a measurement interval of 5 min. **(C)** Values of unstimulated neutrophils of all 15 donors. **(D)** Values of PMA-stimulated neutrophils of all 15 donors. **(E)** Values of CI-stimulated neutrophils of all 15 donors. Mean \pm SEM. Ctrl: Control, PMA: phorbol 12-myristate 13-acetate, CI: calcium ionophore A23187.



Supplementary Figure 3: Immunofluorescence images from exemplary donor over time-course of 1-6 h. Example images of one donor over the time course from 1 h to 6 h. Upper panels display nuclear staining with Hoechst 33342 in gray scale, middle panels show myeloperoxidase staining in gray scale. Lower panels display overlay images in color. Scale bar = 400 μ m. Blue = Hoechst 33342 (nuclear) staining, green = myeloperoxidase (MPO) staining. Cells were fixed with 4 % formaldehyde at defined time points. Ctrl: Control, PMA: phorbol 12-myristate 13-acetate, CI: calcium ionophore A23187.

Supplementary Table 1: Quality of curve fit analysis of PMA-peak modulation of cell index measurements

Donor	Data points	Adjusted R squared	Standard deviation of residuals
#1	36	0.6767	0.02598
#2		0.6802	0.03737
#3		0.8066	0.01908
#4		0.6311	0.05734
#5		0.8836	0.01447
#6		0.6363	0.03664
#7		0.8313	0.02138
#8		0.3688	0.02199
#9	96	0.9534	0.009359
#10		0.9261	0.02359
#11		0.9482	0.01005
#12		0.9373	0.01229
#13		0.94	0.0282
#14		0.9632	0.01951
#15		0.935	0.03064
#17		0.5936	0.03281
General formula: $Y=B_0 + B_1X + B_2X^2$			