

# Insulin can delay neutrophil extracellular trap formation *in vitro* - Implication for diabetic wound care?

Caren Linnemann<sup>1</sup>, Filiz Sahin<sup>1</sup>, Ningna Li<sup>2</sup>, Andreas K. Nussler<sup>1</sup>, Stefan Pscherer<sup>1,3</sup>, Friedrich Götz<sup>2</sup>, Tina Histing<sup>1</sup>, Sabrina Ehnert<sup>1,\*</sup>

<sup>1</sup>Siegfried Weller Institute for trauma research, BG Unfallklinik Tübingen, Eberhard Karls Universität Tuebingen, Tuebingen, Germany

<sup>2</sup>Microbial Genetics, Interfaculty Institute of Microbiology and Infection Medicine Tübingen, Eberhard Karls Universität Tuebingen, Germany

<sup>3</sup>Department of Internal Medicine III, Sophien- and Hufeland-Hospital, Weimar, Germany;

\*Correspondence: [sabrina.ehnert@gmail.com](mailto:sabrina.ehnert@gmail.com); Tel.: Tel.: +49 7071 606 1065, fax.: +49 7071 606 1978

**\* Corresponding author: Sabrina Ehnert**

Siegfried Weller Institute for trauma research at BG Unfallklinik Tuebingen,

Eberhard Karls Universität Tuebingen

Schnarrenbergstr. 95, 72076 Tuebingen

Tel.: +49 7071 606 1065, fax.: +49 7071 606 1978

E-mail: [sabrina.ehnert@gmail.com](mailto:sabrina.ehnert@gmail.com)

Running title: Insulin delays NET formation

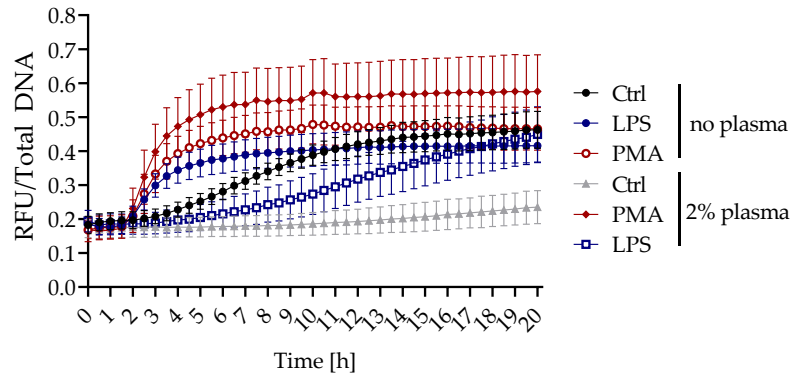
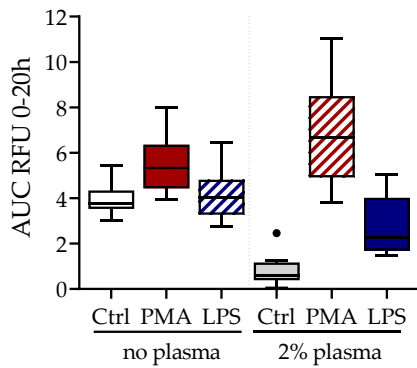
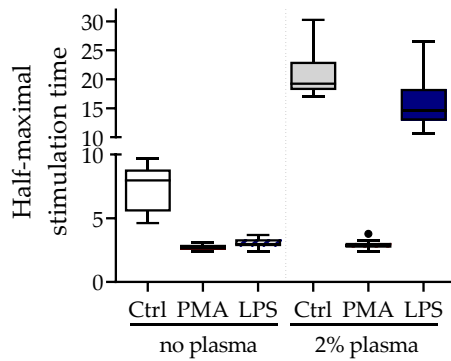
**A****Plasma effect****B****Total amount of released DNA****C****Activation time**

Figure S1: Addition of 2% autologous plasma prevents NET release by LPS **(A)** Time course of Sytox Green Assay measurement 0-20 h, values were normalized to total DNA. **(B)** Total amount of released DNA determined by Sytox Green Assay after stimulation with LPS or PMA  $\pm$  2% autologous plasma **(C)** Half-maximal stimulation time determined from Sytox Green Assay. N=4, n=4. LPS 25  $\mu$ g/mL. PMA: 100 nM.

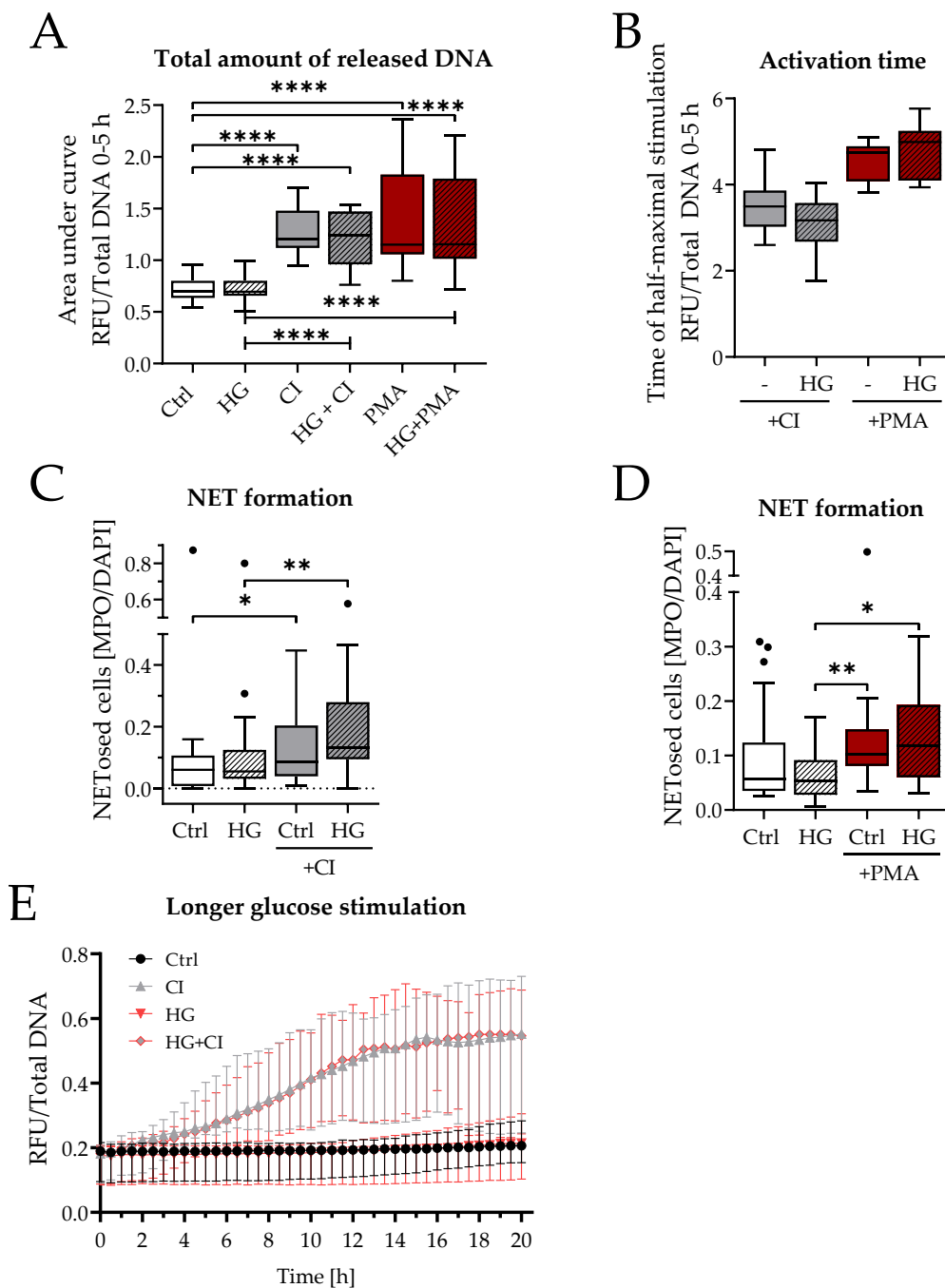


Figure S2: HG does not prime neutrophils for NET release **(A)** Total amount of released DNA determined by Sytox Green Assay after 1 h pre-stimulation with 25 mM glucose and then addition of 100 nM PMA or 4  $\mu$ M CI **(B)** Half-maximal stimulation time determined from Sytox Green Assay **(C)** NET formation determined by immunofluorescence analysis after 2 h stimulation (1 h pre-stimulation + 1 h CI) **(D)** NET formation determined by immunofluorescence analysis after 3 h stimulation time (1 h pre-stimulation + 2 h PMA) or 3 h (PMA) stimulation time. N=4, n=5. **(E)** Time course of Sytox Green Assay measurement 0-20 h, values were normalized to total DNA. \* $p$ <0.05, \*\* $p$ <0.01, \*\*\*\* $p$ <0.0001 as determined by Kruskal-Wallis test.

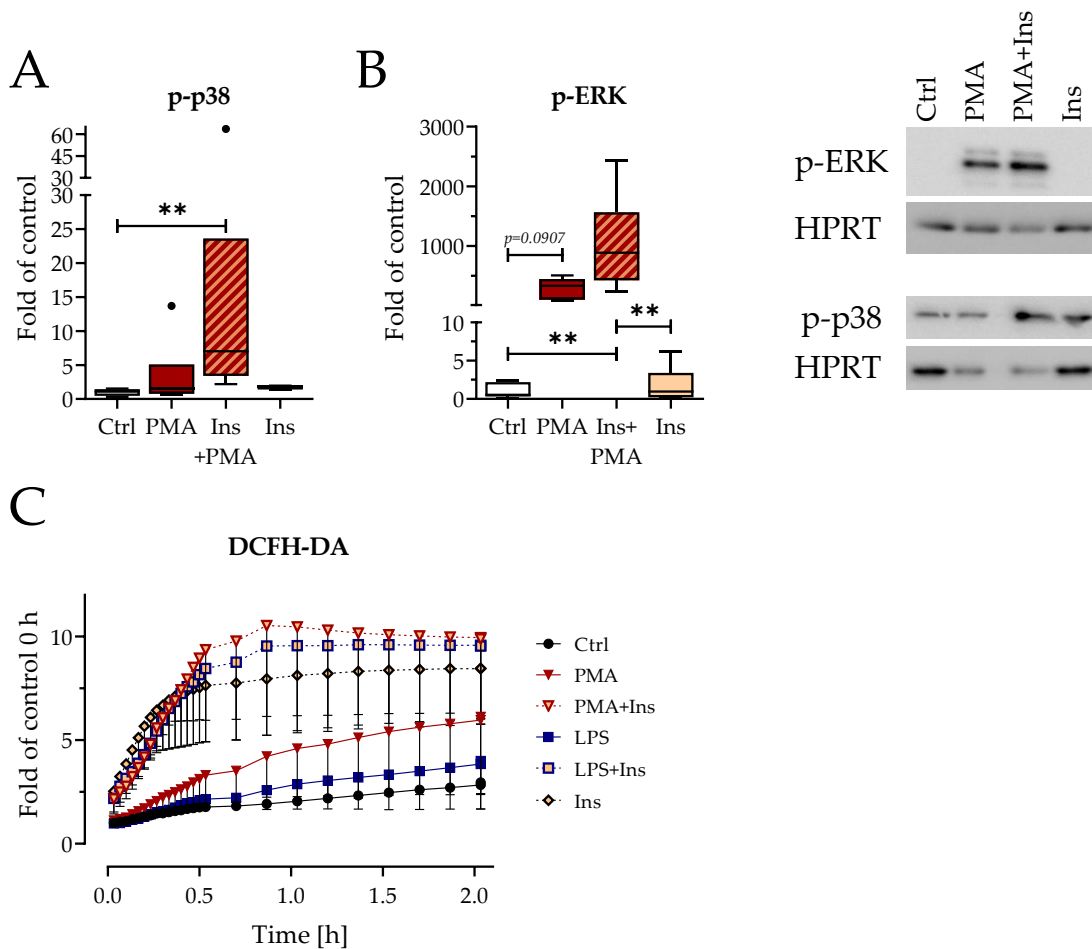


Figure S3: MAPK activation after 1 h stimulation with PMA  $\pm$  Ins. Western Blot of stimulated neutrophils (1 h). **(A)** p-p38 **(B)** p-ERK. Exemplary blot images are shown on the right. N=4, n=1-2. **(C)** Time course analysis of ROS production in stimulated neutrophils (DCFH-DA staining). N=4, n=3. \*\* $p < 0.01$  as determined by Kruskal-Wallis test.