Supplementary information

Neutrophil extracellular traps and monocyte subsets at the culprit lesion site of myocardial infarction patients

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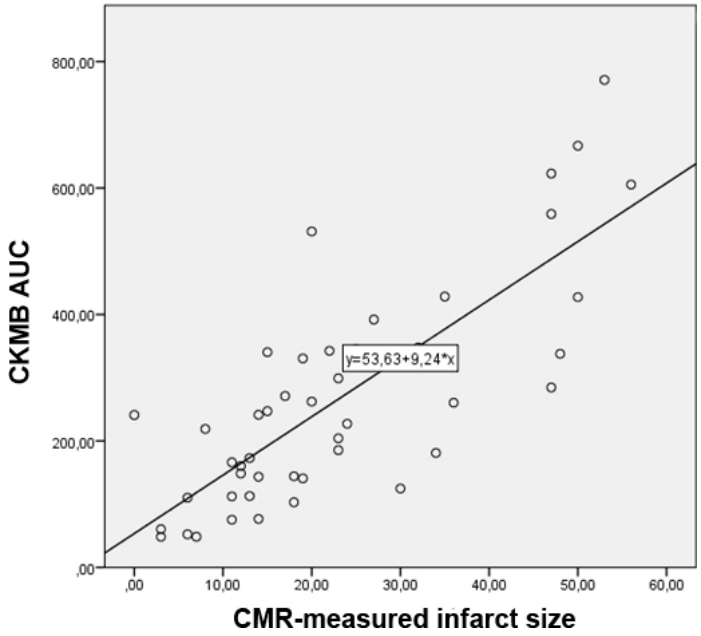
Supplementary Table 1

Monocyte subsets		femoral site	culprit site	p value
Classical monocytes	CD11a	7262.5 [5826-9772]	8243.5 [6180.5-9705]	ns
	CD11b	9894 [6207-14929]	10963 [7723-15146]	p<0.05
	CD142	81.5 [67-96]	86.5 [73-99]	ns
	HLA-DR	3409 [2595-4492]	3184 [2291-4160]	ns
	CX3CR1	2115.5 [1153.5-2919]	1138 [781-2623.5]	p<0.05
	TLR2	3126 [2406-3966]	3240 [2363-3896]	ns
	TLR4	214 [191-308]	242 [178-282]	ns
	CD192	933.5 [594.5-1410.5]	912 [542.5-1393.5]	ns
Intermediate monocytes	CD11a	8642.5 [7274.5-11607.5]	9250.5 [6920.5-11319]	ns
	CD11b	6083 [4323-9420]	6621 [4995-9641]	ns
	CD142	89 [53-139]	111 [84-209]	p<0.05
	HLA-DR	16835 [10995-18600]	9581 [6644-15867]	p<0.01
	CX3CR1	7862 [4464-12887]	5259 [2330-9323.5]	p<0.001
	TLR2	2855 [2495-4006]	3147 [2245-4155]	ns
	TLR4	282 [202-373]	288 [236-357]	ns
	CD192	549 [420.5-930]	630 [418.5-1088]	ns
Non-classical monocytes	CD11a	7795 [5771-9353]	7379 [5799-8477.5]	ns
	CD11b	1117.5 [368-2603]	1245.5 [817-1714]	ns
	CD142	23.5 [0-43]	31.5 [0-53]	ns
	HLA-DR	4398 [2626-5548]	3385 [2273-4397	ns
	CX3CR1	12463 [6092.5-17989]	7245.5 [4308-11555]	p<0.001
	TLR2	1166 [987-1544]	954 [881-1302]	ns
	TLR4	91 [35-154]	83 [33-124]	ns
	CD192	340.5 [207-538]	391 [317.5-637.5]	ns

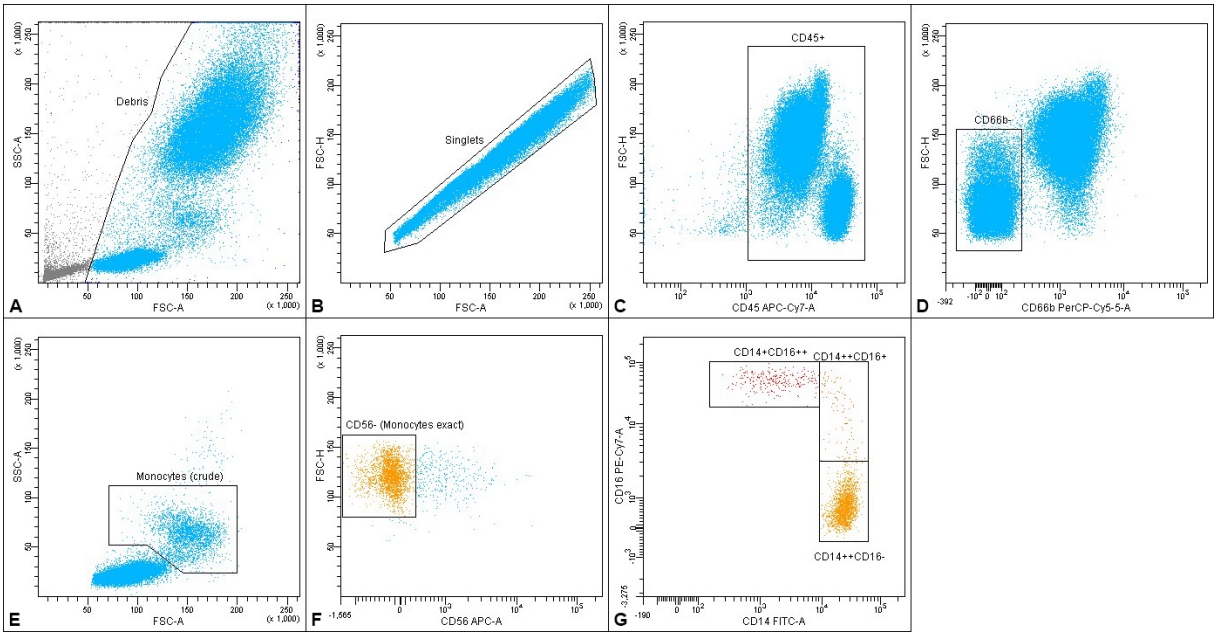
Supplementary Table 1: Activation marker expression of monocyte subsets in STEMI patients. Listed markers were measured on monocyte subsets from the femoral and the culprit site of STEMI patients (n=36) using flow cytometry to determine monocytic activation. Date are given as median [IQR]. Significance was determined by Wilcoxon‘s signed-rank test.

Supplementary Figure 1



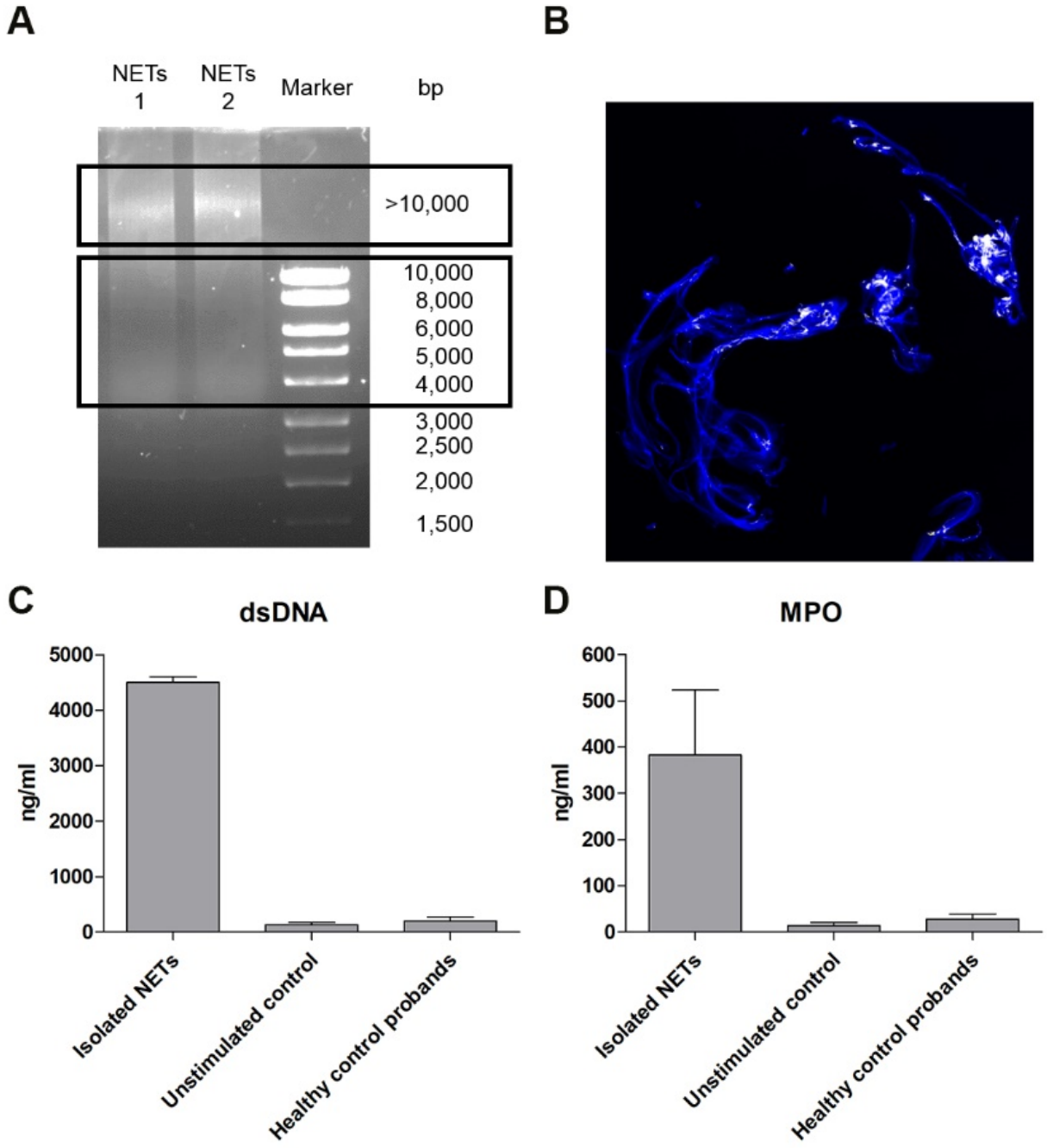
Supplementary Figure 1: Correlation of creatine phosphokinase isoform MB area under the curve (CK-MB AUC) with cardiac magnet resonance (CMR)-measured infarct size. CK-MB AUC was validated in the control group of a prospective, controlled study[1]. A published trapezoidal formula was used to calculate CK-MB AUC[2], with at least 5 consecutive values over a period of 3 days after pPCI. A strong correlation is shown (n=46, r=0.786, p<0.001).

Supplementary Figure 2



Supplementary Figure 2: Gating strategy for monocyte subsets. Cells were separated from debris (**A**) and doublets (**B**); CD45+CD66b- cells (**C**, **D**) were divided from lymphocytes by forward and sideward scatter gating (**E**), then CD56- monocytes (**F**) were divided into the subsets according to their CD14/CD16 expression profile (**G**).

Supplementary Figure 3

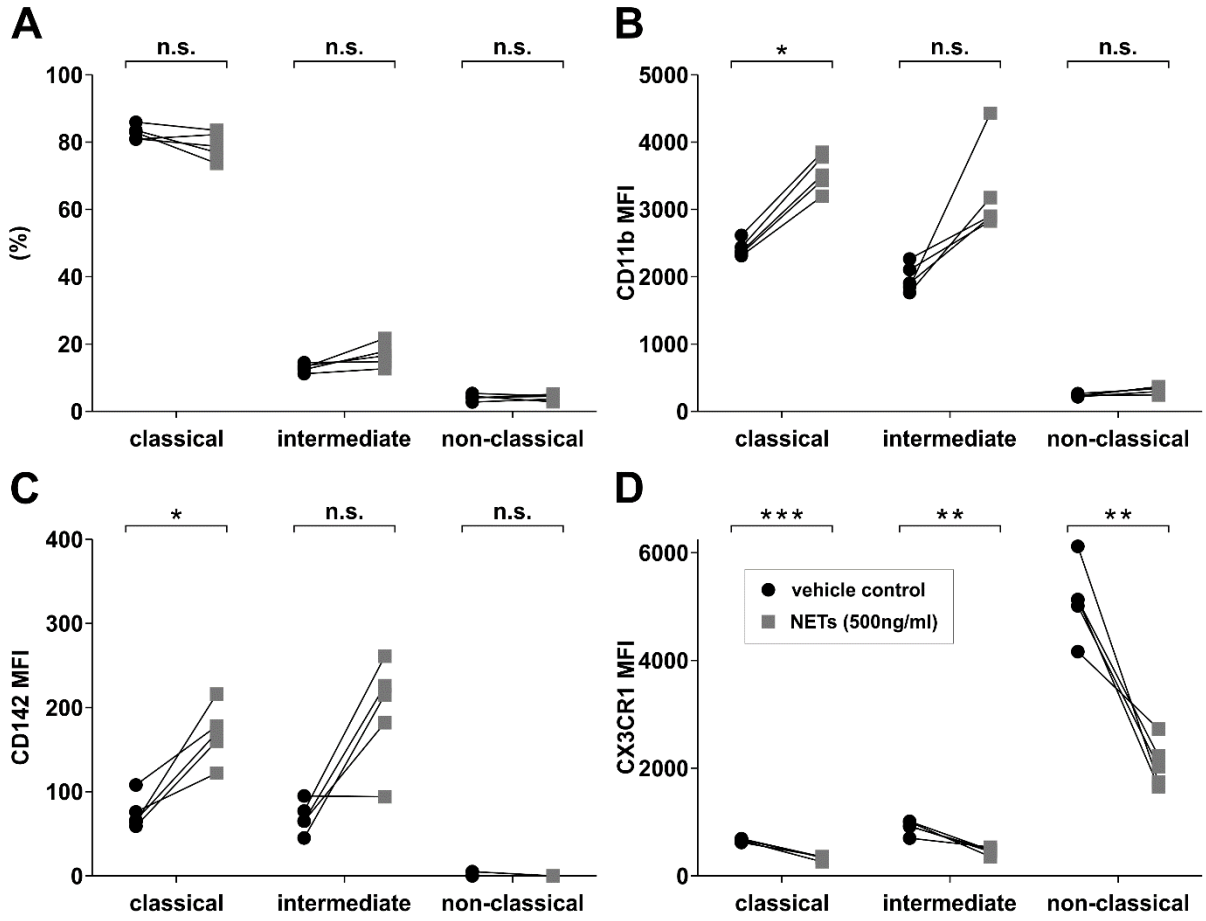


Supplementary Figure 3: Characterization of isolated neutrophil extracellular traps. Neutrophil extracellular traps (NETs) were produced, isolated and processed as described in the main document of the manuscript according to published protocols[3]. The solubilized compound was run in an electrophoresis gel (**A**). As expected, a smear of DNA over a wide range of lengths is visible.

Neutrophils were stimulated *in vitro* in chamber slides and stained utilizing immunofluorescence. Typical strand-like structures can be observed (**B**). DNA was stained using DAPI (blue), myeloperoxidase (MPO) was stained using a rabbit anti-human MPO antibody (Abcam). The slides were scanned utilizing TissueFAXS (TissueGnostics). For the analysis, TissueQuest software (Version 4.01.0128) was used.

The solubilized compound displayed high levels of double-stranded DNA (dsDNA, **C**) and MPO (**D**) compared to unstimulated *in vitro* controls as well as healthy control probands (n=200). Double-stranded DNA was measured as described in the main document of the manuscript. MPO concentration was determined utilizing a commercial Human MPO Instant ELISA (Thermo Fisher). The assay was performed according to the manufacturers' protocol.

Supplementary Figure 4



Supplementary Figure 4: Stimulation of monocytes with NETs *in vitro*. Monocytes subset shift (**A**), expression of (**B**) CD11b, (**C**) CD142 and (**D**) CX3CR1 of classical, intermediate and non-classical monocytes from a healthy donor (n=1). Mean fluorescence intensity (MFI) levels or monocyte subsets in percent (%) are displayed. Monocytes were stimulated in whole blood with NETs derived from isolated neutrophils from healthy donors (n=5) or vehicle control for 60 minutes. Significance was determined by Student's paired t-test. *p<0.05, **p<0.01, *** p<0.001.

References

1 Testori, C. *et al.* Out-of-hospital initiation of hypothermia in ST-segment elevation myocardial infarction: a randomised trial. *Heart* **105**, 531-537, doi:10.1136/heartjnl-2018-313705 (2019).

2 Crimi, G. *et al.* Remote ischemic post-conditioning of the lower limb during primary percutaneous coronary intervention safely reduces enzymatic infarct size in anterior myocardial infarction: a randomized controlled trial. *JACC. Cardiovascular interventions* **6**, 1055-1063, doi:10.1016/j.jcin.2013.05.011 (2013).

3 Silvestre-Roig, C. *et al.* Externalized histone H4 orchestrates chronic inflammation by inducing lytic cell death. *Nature* **569**, 236-240, doi:10.1038/s41586-019-1167-6 (2019).