

S1: Gating strategy of human splenocytes. **A)** Macrophages (green) were characterized as autofluorescent cells within the life gate and checked for CD163 positivity. Neutrophils (red), monocytes (blue) and lymphocytes (yellow) were gated within the non-fluorescent population based on forward and side scatter. **B)** Control stainings of the macrophage gate using anti-CD163 and of the monocyte gate using anti-CD14. When sorting cells with flow cytometry we obtain a pure population of **A)** neutrophils, **B)** macrophages or **C)** monocytes when analyzing with cytospin. These images were blindly chosen and are representative for 3 different experiments.

S2: Antibodies used for opsonization in phagocytosis assays. Table showing the origin, poly- vs. monoclonality of the antibodies and subclass specificity of the antibodies used for opsonization in our phagocytosis assays.

S3: IgG-opsonization of RBCs induces phagocytosis neutrophils and monocytes. The percentage of splenocytes that take up RBCs per cell type. Neutrophils are the primary phagocytes in case of anti-GPA opsonization (n=5 to 12). Error bars denote the standard error of the mean. Stars represent highly significant differences ($**P < .01$; $***P < .001$; $****P < .0001$). ns, nonsignificant differences.

S4: Neutrophils and monocytes have a different threshold for RBC phagocytosis. **A)** Phagocytosis by neutrophils or monocytes depends on the degree of opsonization. The threshold for RBC phagocytosis is higher for neutrophils while monocytes readily take up RBCs at low opsonization conditions (n=4). **B)** Magnetic RBCs were unopsonized, opsonized

with an IgG1 isotype control or opsonized with anti-GPA. Neutrophils and RBCs were incubated at 1:10 ratio for an hour after which RBCs were lysed using an isotonic ammoniumchloride buffer for 10 minutes at 4°C followed by a second lysis step of 5 minutes at 4°C. Next the phagocytic fraction was obtained using magnetic activated cell sorting. The absolute number of neutrophils that have taken up a RBC are shown (n=4).

S5: A) and B) Antibodies directed against various RBC antigens show different levels of opsonization and this correlates with the ability to induce phagocytosis by neutrophils. **C)** The IgG subclass does not influence RBC phagocytosis by neutrophils. Using polyclonal or monoclonal anti-RhD of IgG1, IgG2 or IgG3 subclass does not show differences in level of phagocytosis by neutrophils. As a positive control high opsonization with anti-GPA (monoclonal IgG1) shows high levels of phagocytosis. Error bars denote the standard error of the mean. **D) and E)** Monoclonal mouse IgG1 antibodies show different levels of opsonization and this correlates with the ability to induce phagocytosis by neutrophils.

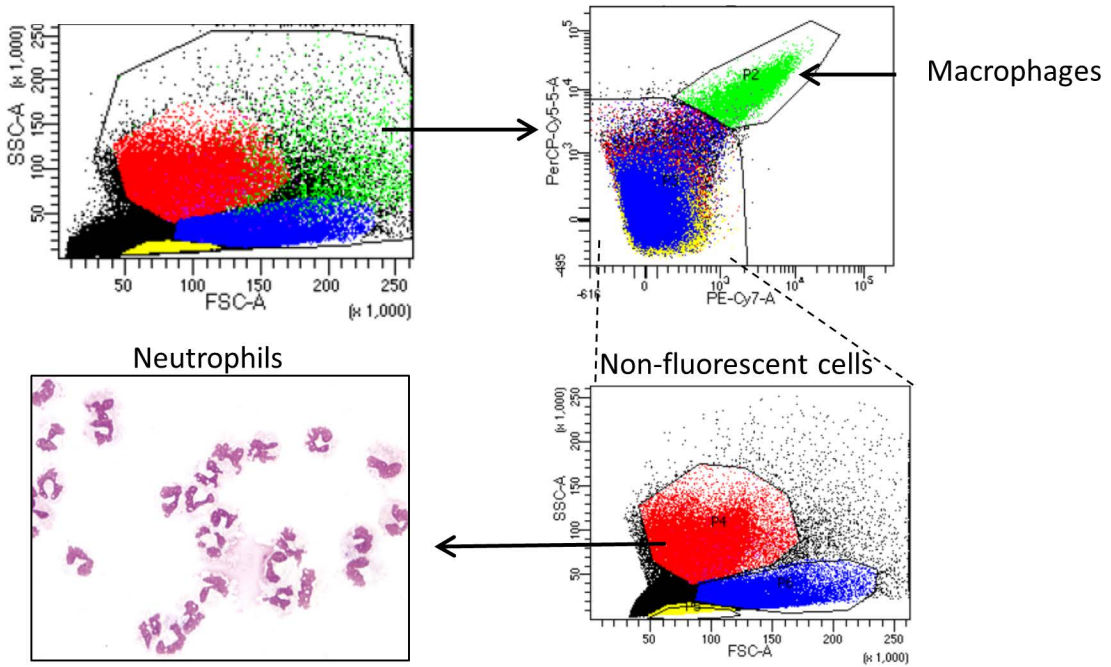
S6: CD47 blocking enhances phagocytosis of IgG-opsonized RBCs by neutrophils of the blood. **A)** Neutrophils from the blood show increased phagocytosis of RBC when anti-RhD opsonization is combined with CD47 blocking. **B)** Phagocytosis of anti-GPA opsonized RBCs can also be augmented by blocking CD47. Error bars denote the standard error of the mean. Stars represent highly significant differences (** $P < .01$, *** $P < .001$, **** $P < .0001$). ns, nonsignificant differences.

S7: Gating strategy for phagocytosis experiments in mice in vivo. **A)** Neutrophils were gated based on forward and side scatter followed by a selection for CD11b+, Gr-1+ cells. Transfused RBCs were labeled with the fluorescent dye PKH26 before opsonization and transfusion. **B)** Cytospins showing RBC phagocytosis by murine neutrophils. Cells were spun onto glass slides before and after lysis of RBC. These images were blindly chosen and are representative for 4 different experiments.

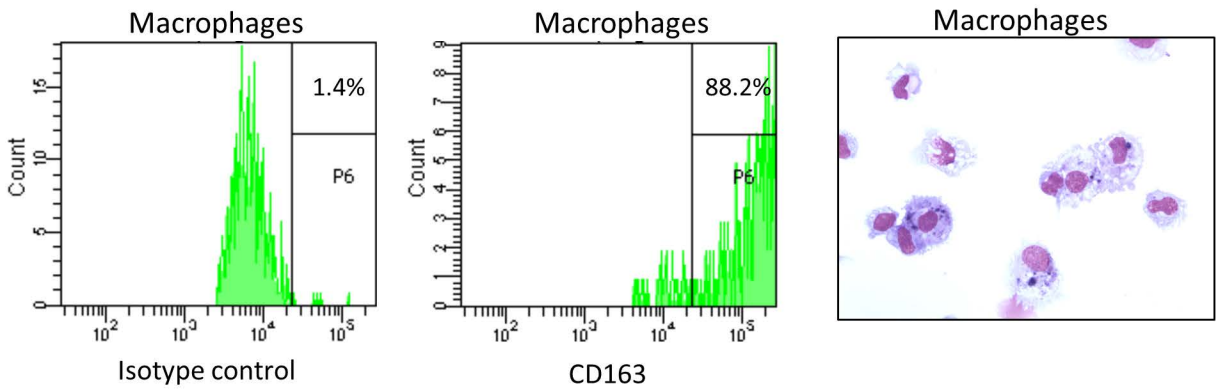
S8: Opsonization of RBC from AIHA patients. **A)** Antibody opsonization of patient RBCs measured with flow cytometry using a goat-anti-human antibody. **B and C)** Complement deposition on patient RBCs measured using an antibody directed against **B)** iC3b or **C)** C3-19. **D)** Bilirubin, lactate dehydrogenase (LDH) and hemoglobin (Hb) levels of the AIHA patients. **E)** CD47 blocking increased phagocytosis of patient II and III RBCs but not of RBCs from a healthy control. CD47 blocking did not further increase phagocytosis of patient IV. Error bars denote the standard error of the mean. Stars represent highly significant differences ($*P < .05$, $**P < .01$, $***P < .001$, $****P < .0001$). ns, nonsignificant differences. **C)** Results of the Coombs test (direct antiglobulin test). All patients were tested positive for IgG and negative for IgM and IgA in the Coombs test performed by diagnostics. Patient II and IV showed low positivity for complement and remaining patients were tested negative for complement. (+ : positive, - : negative, (+) : low positivity). Error bars denote the standard error of the mean.

S1.

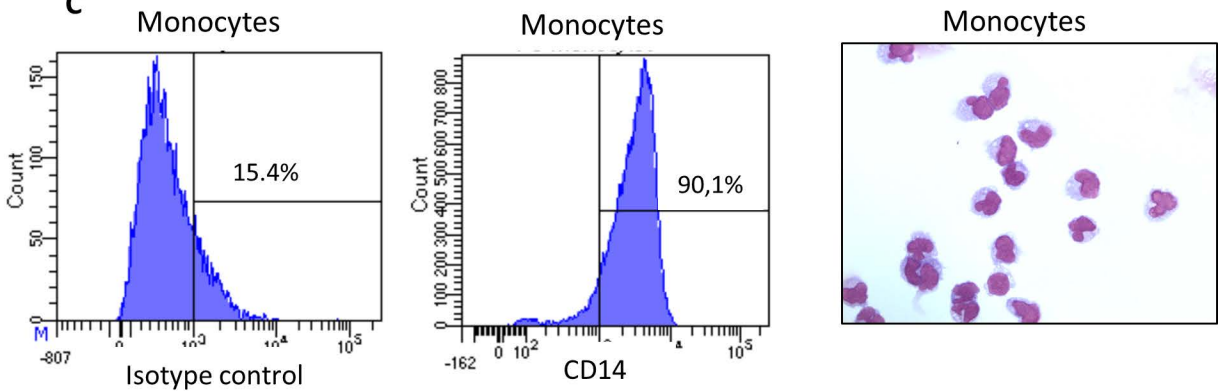
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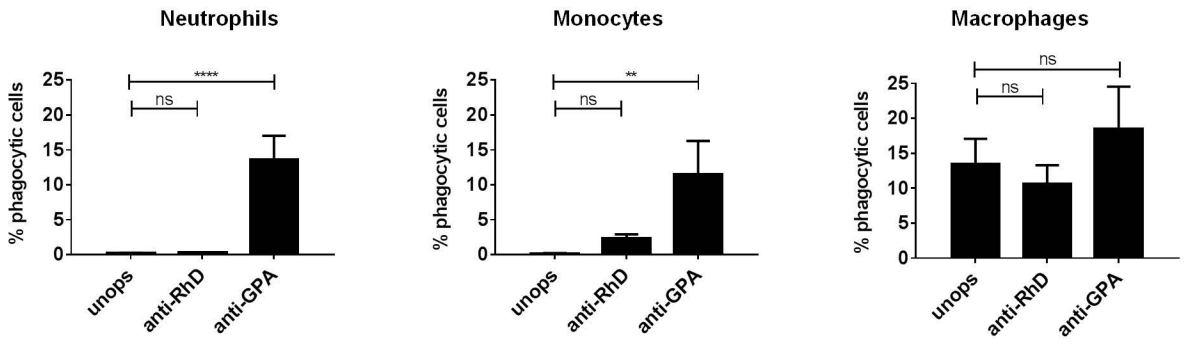
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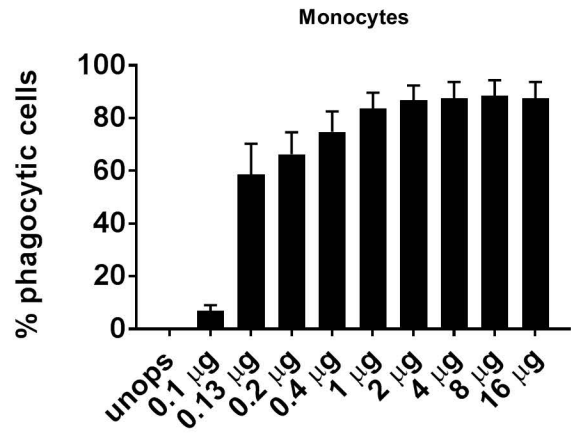
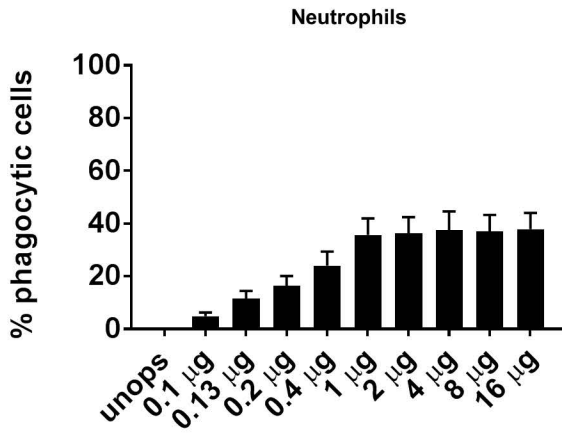
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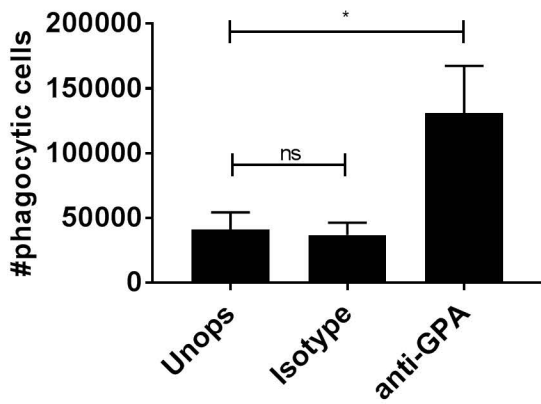
Antibody	Origin	Poly/mono-clonal	subclass
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Anti-RhD	Human	Monoclonal	IgG2
Anti-RhD	Human	Monoclonal	IgG3
Anti-GPA	Mouse	Monoclonal	IgG1
Anti-CD59	Mouse	Monoclonal	IgG2a
Anti-CR1	Rabbit	Polyclonal	
Anti-CD147	Mouse	Monoclonal	IgG2a
Anti-CD55	Mouse	Monoclonal	IgG1

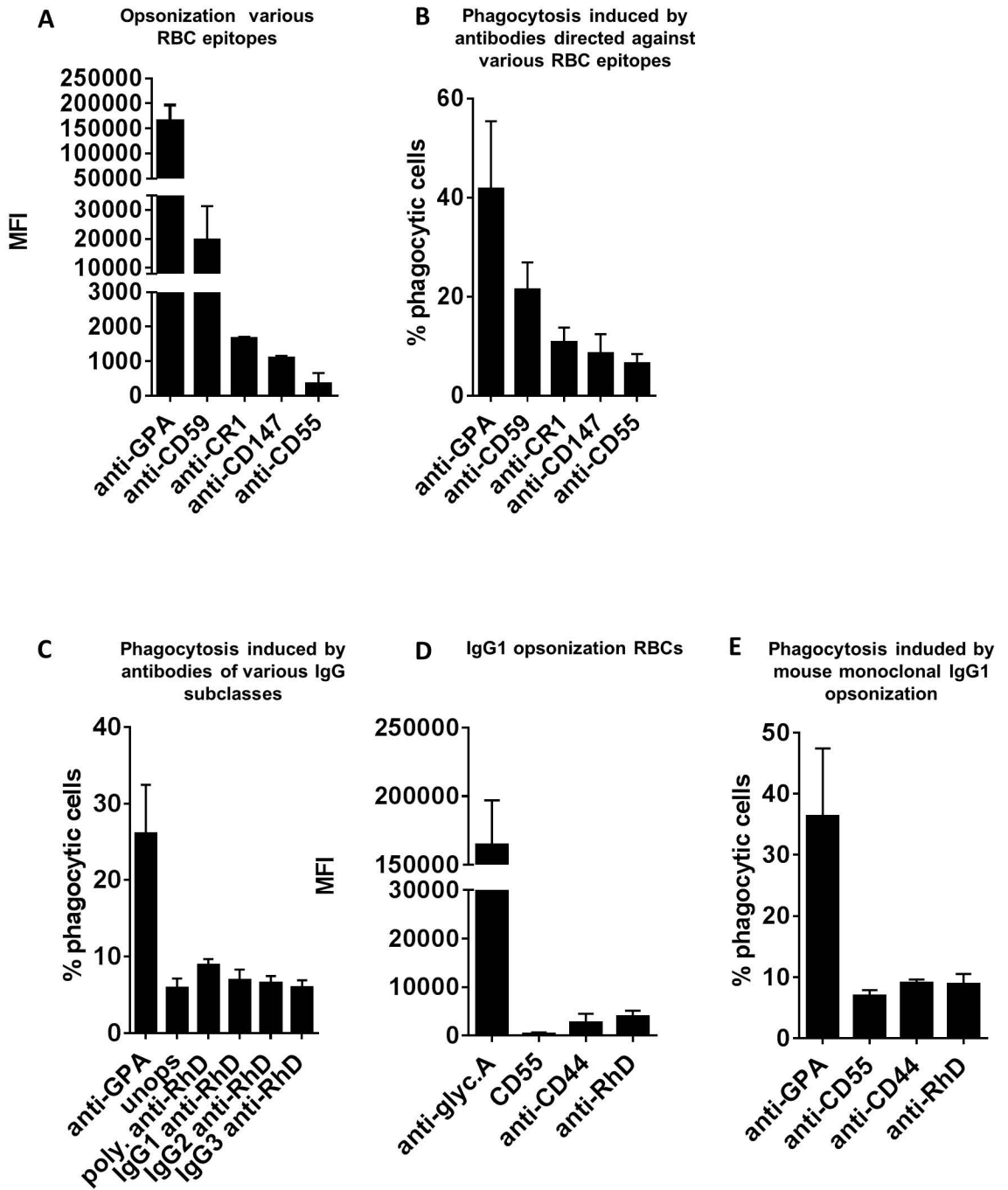


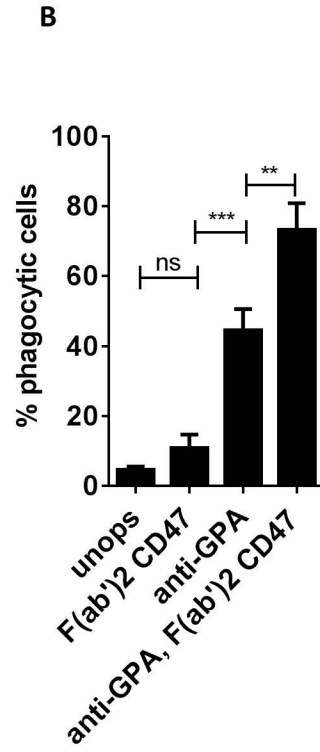
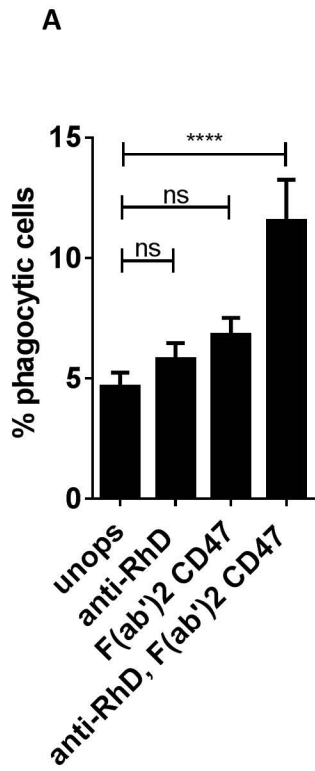
A



B

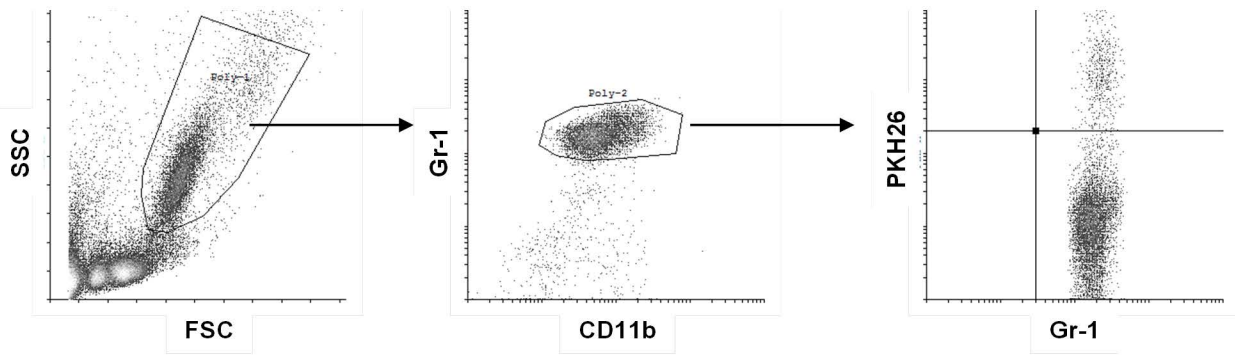




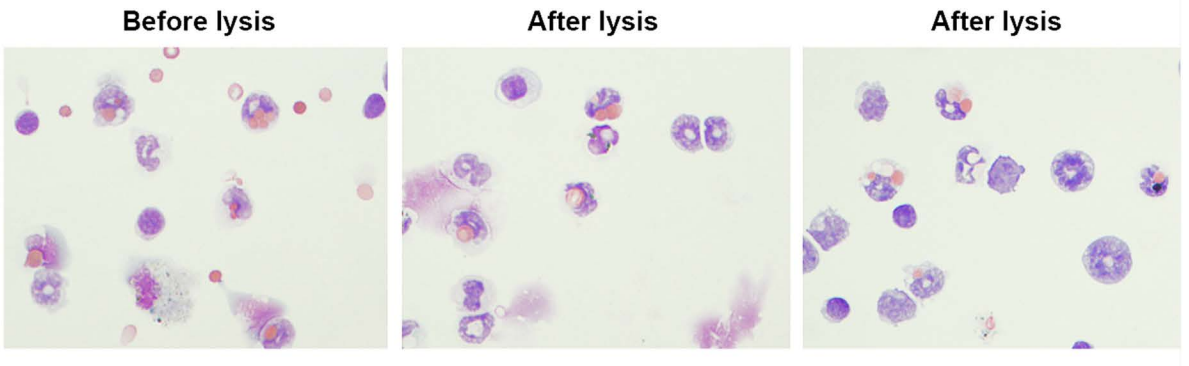


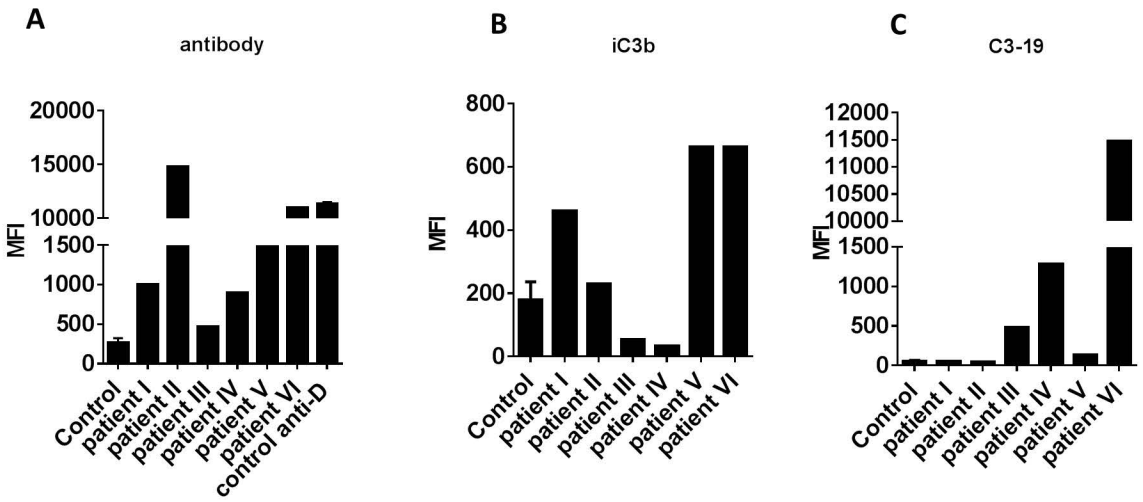
S7

A



B





E

	Sex	Bilirubin ($\mu\text{mol/L}$)	LDH (512 U/L)	Hb (mmol/L)
Patient I	M	120	743	4
Patient II	F	10	220	5,6
Patient III	F	6	188	8,5
Patient IV	F	16	277	4,6
Patient V	F	114	334	4,8
Patient VI	M	74	512	5,7
Reference value male		< 7	< 248	8.5 - 10.5
Reference value female		< 7	< 247	7.5 - 10.0

F

