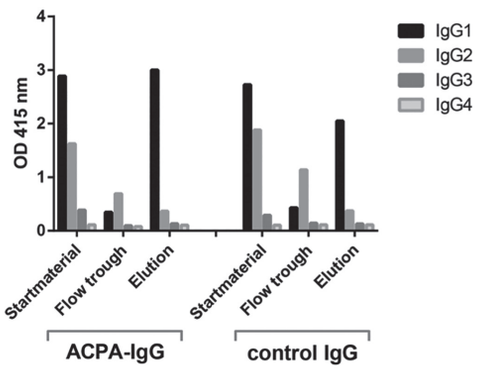
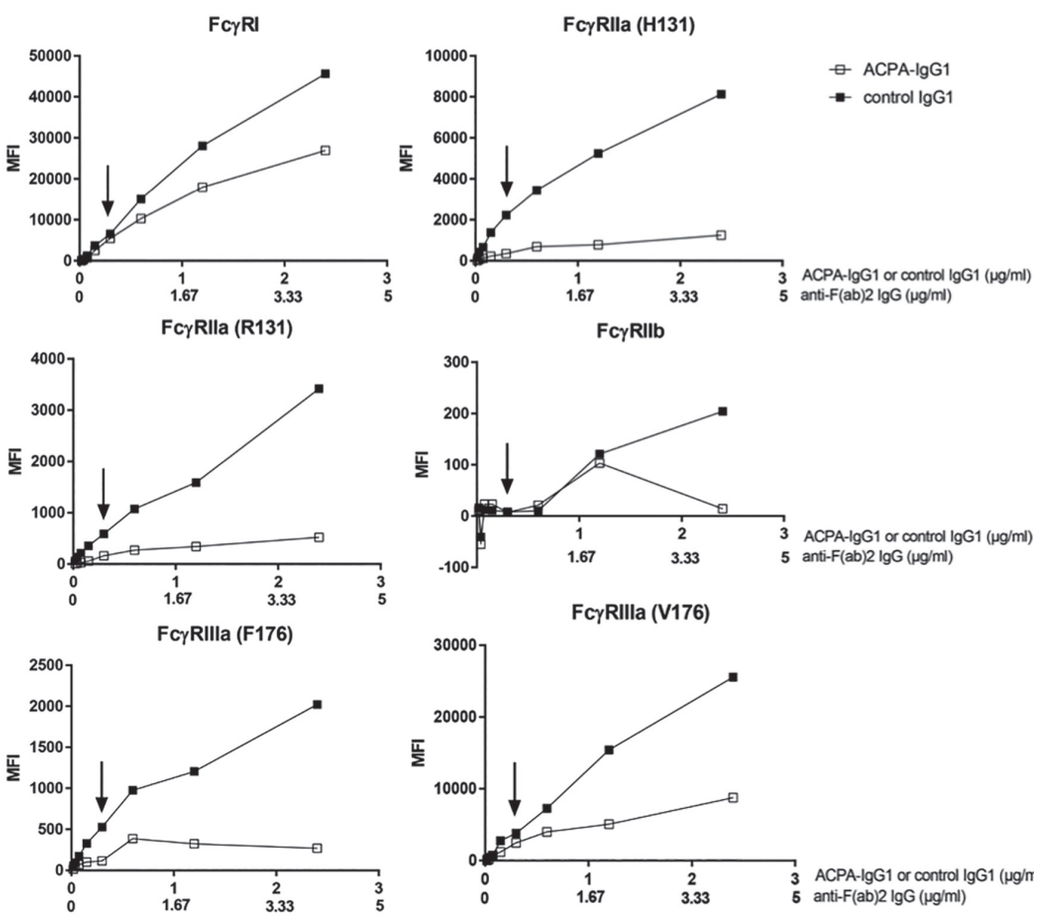
Supplementary methods

Immune complex size analysis (AF4)

In order to control for potential differences in size of ACPA-IgG1 IC and control IgG1 IC, asymmetrical flow field-flow fractionation (AF4) was used. ACPA-IgG1 IC and control IgG1 IC (70 μg/ml) as well as F(ab')$_2$ anti-human IgG F(ab')2 (43.8 µg/ml), monomeric ACPA-IgG1 (26.2 µg/ml), monomeric control IgG1 (26.2 µg/ml) and a standard control IgG (0.1 mg/ml) were used for the analysis. AF4 measurements were performed on an Agilent 1200 system (Agilent Technologies, Palo Alto, California) combined with Wyatt Eclipse (Wyatt Technology Europe GmbH, Dernbach, Germany) detectors for refractive index, UV (280 nm), fluorescence (λ_{ex} 280 nm, λ_{em} 350 nm) and multiangle laser light scattering (MALLS, 658 nm laser). For the separation, a channel equipped with a 350 μm spacer of medium width and a regenerated cellulose membrane with a cut-off of 10 kDa was used. The injection volume was 120 µL and the mobile phase was a solution of 50 mM $NaNO_3$ that was supplemented with 0.25 mg/ml of sodium azide to prevent any microbial growth. The detector flow was 0.9 ml/min. The cross-flow was initially set to 1.5 ml/min (for 21 min) which was gradually decreased to 0.3 (from 21 to 26 min) and 0 ml/min (at 36 min) over time. The dn/dc value and the molar extinction coefficient were assumed to be 0.185 (ml/g) and 1.44 (ml/mg.cm), respectively. The molecular weights of the species were estimated by relating the MALLS signal to UV signals according to the built-in functions in the Astra software version 5.3.2.22 (Wyatt Technology Europe GmbH, Dernbach, Germany).

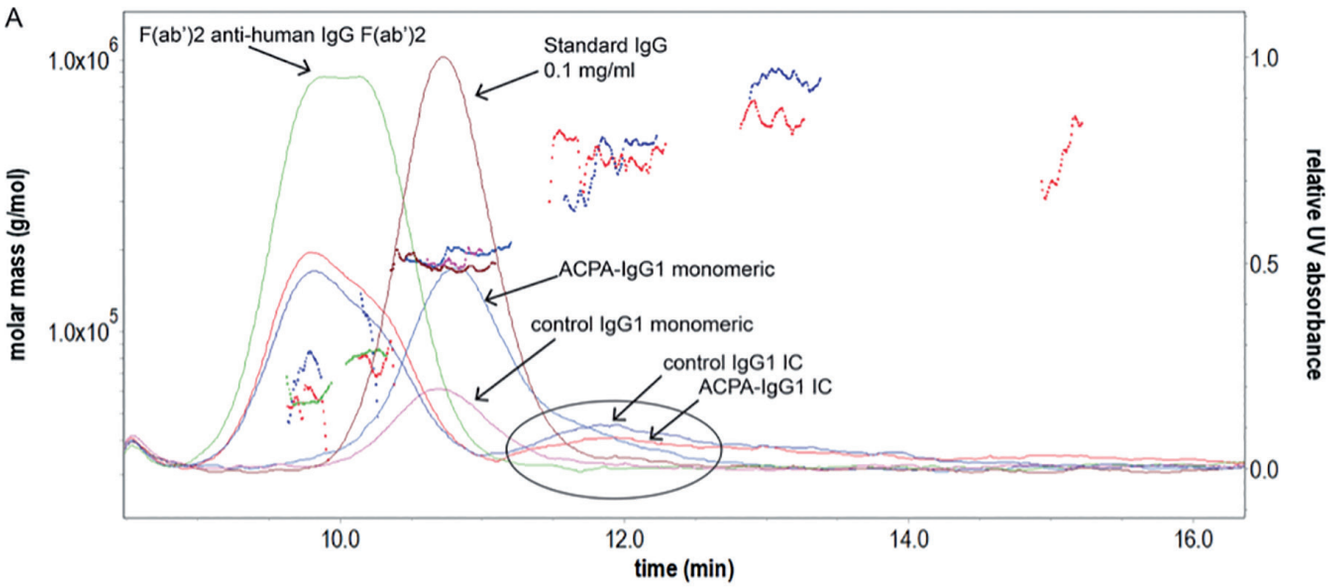


Suppl. Fig. 1. ACPA-IgG1 and control IgG1 consist predominantly of IgG1. ACPA-IgG and control IgG isolated from RA patient fluids were further purified over an IgG1 column. Presence of IgG subclasses was measured in the starting material, IgG1 elution and flow through of both ACPA-IgG and control IgG by using the PeliClass human IgG subclass ELISA kit (Sanquin).



Suppl. Fig. 2. ACPA-IgG1 and control IgG1 IC titrations. Different concentrations of ACPA-IgG1 (white squares) and control IgG1 (black squares) were combined with several concentrations of anti-F(ab')$_2$ IgG as indicated, and tested for binding to FcγR-transfected CHO cell lines by flow cytometry. The arrow indicates the concentration used in our subsequent experiments, which was chosen to be below saturation levels.

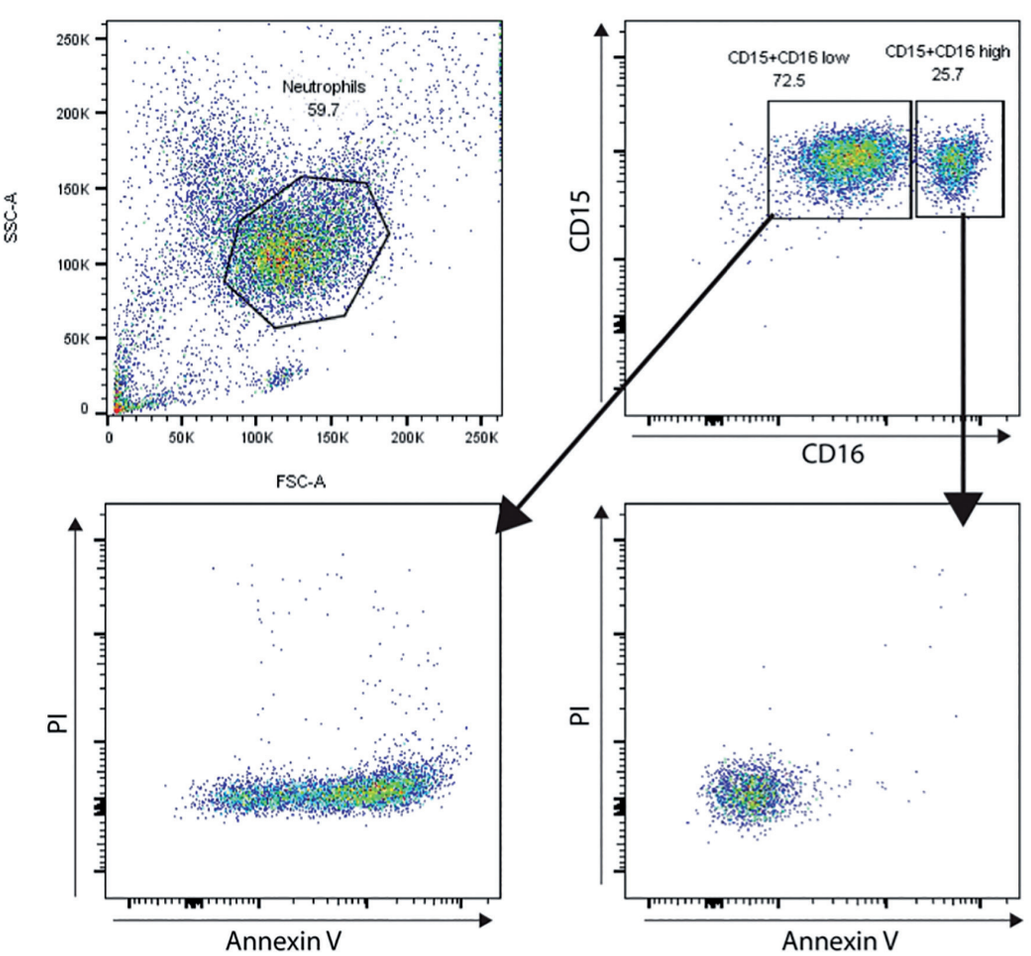
A



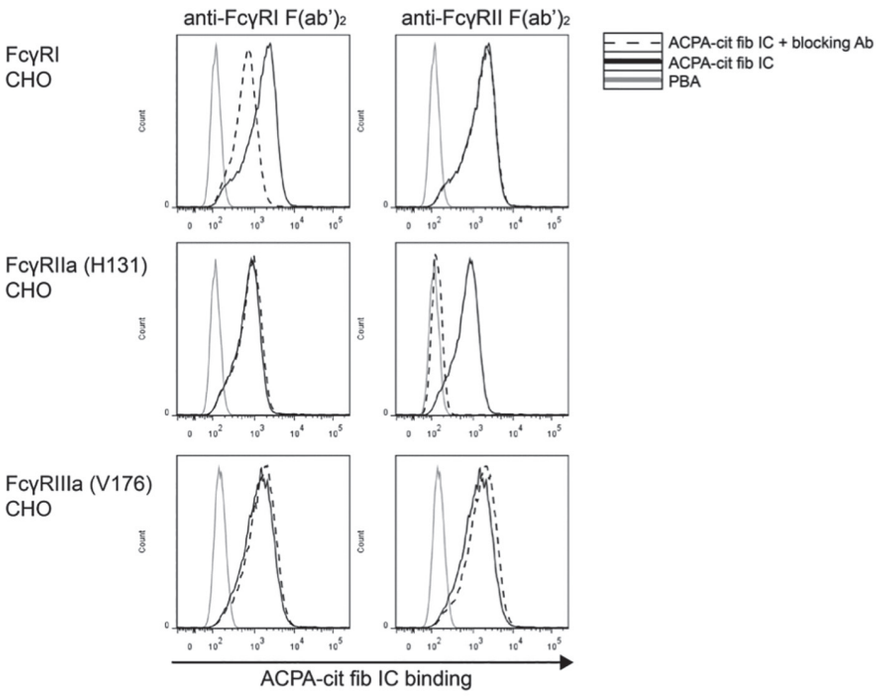
B

Samples	MW (kDa)
Standard IgG	175
$F(ab')_2$ anti-human IgG $F(ab')_2$	82
ACPA-IgG1 monomeric	191
control IgG1 monomeric	182
ACPA-IgG1 IC	420
control IgG1 IC	429

Suppl. Fig. 3. ACPA-IgG1 IC and control IgG1 IC have comparable sizes. (**A**) AF4 measurement of $F(ab')_2$ anti-human IgG $F(ab')_2$ (green peak), standard IgG (brown peak), monomeric ACPA-IgG1 (blue peak), monomeric control IgG1 (purple peak), control IgG1 IC (dark blue peak) and ACPA-IgG1 IC (red peak). Peaks represent the UV signal which associates with the protein concentration. The dotted lines correspond with the molecular weight (MW) of the peaks, as calculated from the UV and MALLS signals. (**B**) Summary of the estimated MW of the peaks.



Suppl. Fig. 4. $CD15^+$ neutrophil population expressing lower CD16 shows the presence of (pre-)apoptotic cells. After overnight stimulation with IFNγ, neutrophils from one healthy donor were stained with Annexin V or Propidium Iodide (PI) and analysed by flow cytometry.



Suppl. Fig. 5. FcγR blocking antibody specificity tested on FcγR-transfected CHO cell lines. FcγRI, FcγRIIA (H131) and FcγRIIIA (V176) transfected CHO cells were incubated with (dotted line) or without (black line) anti-FcγRI $F(ab')_2$ or anti-FcγRII $F(ab')_2$ blocking antibodies before staining with ACPA-cit fib IC. Depicted are histograms of the flow cytometry analysis of the ACPA-cit fib IC binding.