Supplemental Methods:

Antigen-C3 capture ELISA assay: Antigen-specific monoclonal antibodies (mouse anti human PF4/heparin; KKO or mouse anti-protamine/heparin; ADA at 2 μg/mL)[28-30] were incubated overnight on a microtiter plate (in phosphate buffered saline, PBS) followed by washing and blocking with 1% bovine serum albumin (BSA) in PBS for 2 hours. To activate complement, plasma was incubated with buffer or heparin alone or PF4 ± heparin or PRT ± heparin at 37°C for 1 hour (hr) followed by addition of 10 mM ethylenediaminetetraacetic acid (EDTA) to inhibit further complement generation. Unless specified antigen concentrations were 25 μg/mL (PF4) and 0.25 U/mL (heparin) for KKO coated plates and 125 μg/mL (PRT) and 6 U/mL (heparin) for ADA coated plates. Next, plasma containing antigen fixed by complement activation fragments was added to the capture plate for 1hr followed by serial washes. Complement-coated antigen was detected using a biotinylated anti-C3c antibody (recognizes C3 and all C3c-containing fragments of C3, including iC3b; Quidel Corporation, San Diego, CA) followed by colorimetric detection as previously described.[6] To circumvent day to day variation in longitudinal or cohort assays involving the antigen-C3 capture ELISA assay, we used fresh plasma from one healthy donor (Donor 1) as an internal control in each assay and normalized data to readings obtained from this donor.

Supplemental Table: 1

Supplemental Table 1: Table shows the fold changes in the levels of various complement and complement regulatory proteins in the high (n=3)/ intermediate (n=2) Vs low (n=3) complement responders.

Primary Protein Name	Protein Description	# quantified peptides	Foldchange High/ Intermediate vs. Low	p-value (t-test) High/ Intermediate vs. Low
IGHM_HUMAN	Ig mu chain C region	40	**4.08**	**0.001**
C1QB_HUMAN	Complement C1q subcomponent subunit B	10	**1.13**	**0.01**
FHR4_HUMAN	Complement factor H-related protein 4	1	**2.07**	**0.031**
C1QA_HUMAN	Complement C1q subcomponent subunit A	4	**1.12**	**0.033**
CFAD_HUMAN	Complement factor D	4	**-1.19**	**0.034**
MBL2_HUMAN	Mannose-binding protein C	4	-2.13	0.054
CO9_HUMAN	Complement component C9	22	1.49	0.131
C1RL_HUMAN	Complement C1r subcomponent-like protein	6	1.53	0.133
FHR1_HUMAN	Complement factor H-related protein 1	7	2.69	0.134
FCN3_HUMAN	Ficolin-3	8	-1.29	0.139
IC1_HUMAN	Plasma protease C1 inhibitor	29	1.16	0.153
C1QC_HUMAN	Complement C1q subcomponent subunit C	12	1.08	0.165
CO4A_HUMAN	Complement C4-A	4	2.25	0.208
CO7_HUMAN	Complement component C7	29	-1.13	0.21
CO2_HUMAN	Complement C2	23	-1.13	0.229
CFAI_HUMAN	Complement factor I	25	-1.15	0.24
CO4B_HUMAN	Complement C4-B	138	1.18	0.269
C1S_HUMAN	Complement C1s subcomponent	24	1.05	0.36
CFAH_HUMAN	Complement factor H	108	-1.07	0.392
C1R_HUMAN	Complement C1r subcomponent	33	1.06	0.438
CO8G_HUMAN	Complement component C8 gamma chain	12	-1.11	0.438
CO8B_HUMAN	Complement component C8 beta chain	25	-1.06	0.442
MASP1_HUMAN	Mannan-binding lectin serine protease 1	7	-1.10	0.542
CO3_HUMAN	Complement C3	235	-1.07	0.603
FHR5_HUMAN	Complement factor H-related protein 5	3	-1.11	0.603
FHR2_HUMAN	Complement factor H-related protein 2	3	1.14	0.606
MASP2_HUMAN	Mannan-binding lectin serine protease 2	1	-1.17	0.647
CLUS_HUMAN	Clusterin	24	1.05	0.664
VTNC_HUMAN	Vitronectin	24	-1.06	0.692
FCN2_HUMAN	Ficolin-2	4	-1.11	0.73
CO8A_HUMAN	Complement component C8 alpha chain	23	-1.03	0.82
CFAB_HUMAN	Complement factor B	49	1.03	0.873
CO5_HUMAN	Complement C5	88	-1.00	1
CO6_HUMAN	Complement component C6	34	1.01	1

Supplemental Figure 1

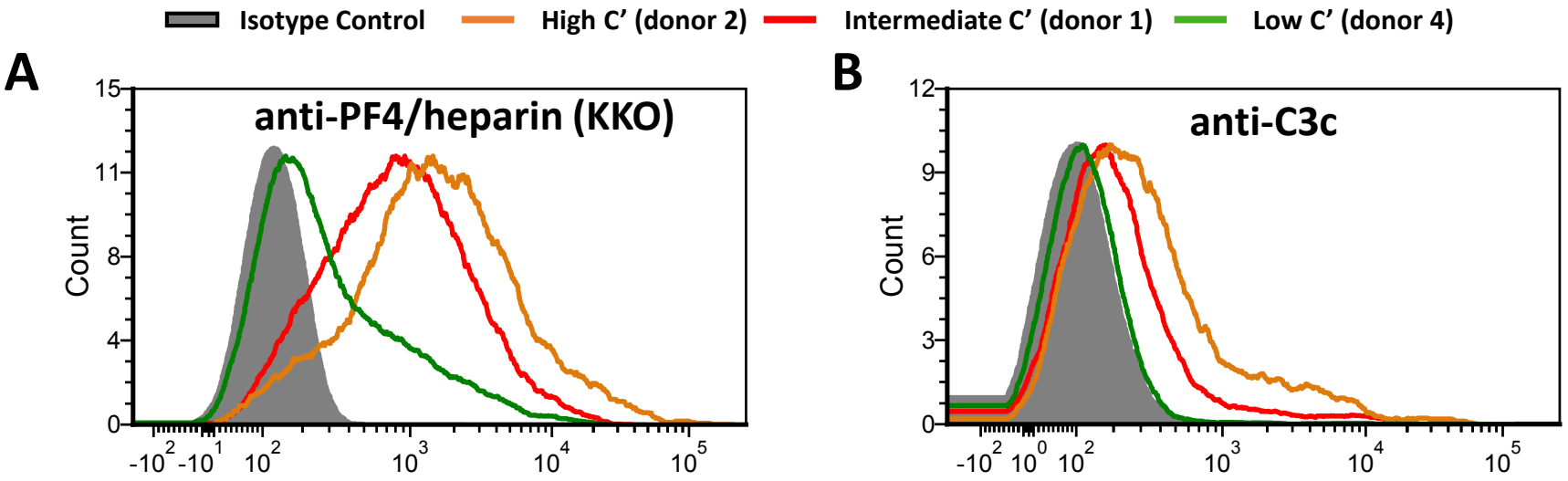
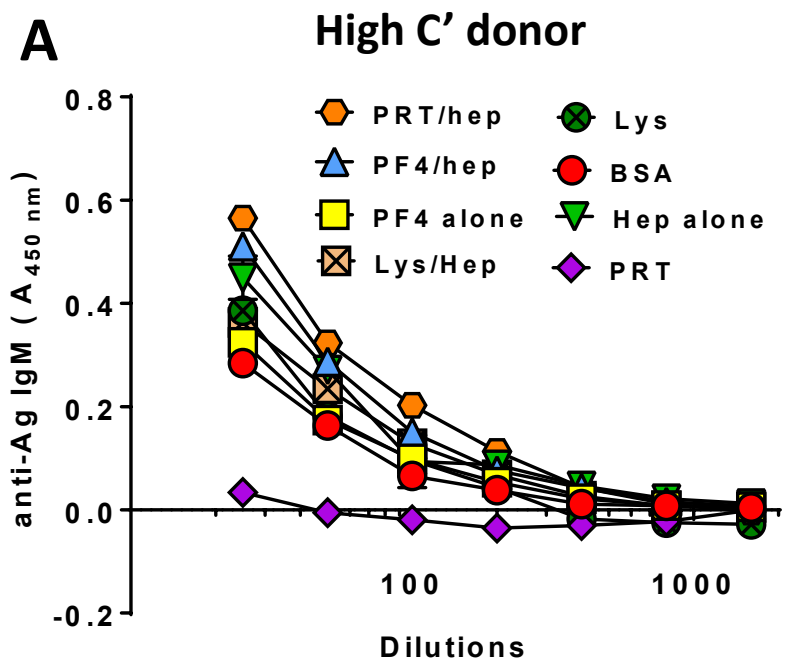
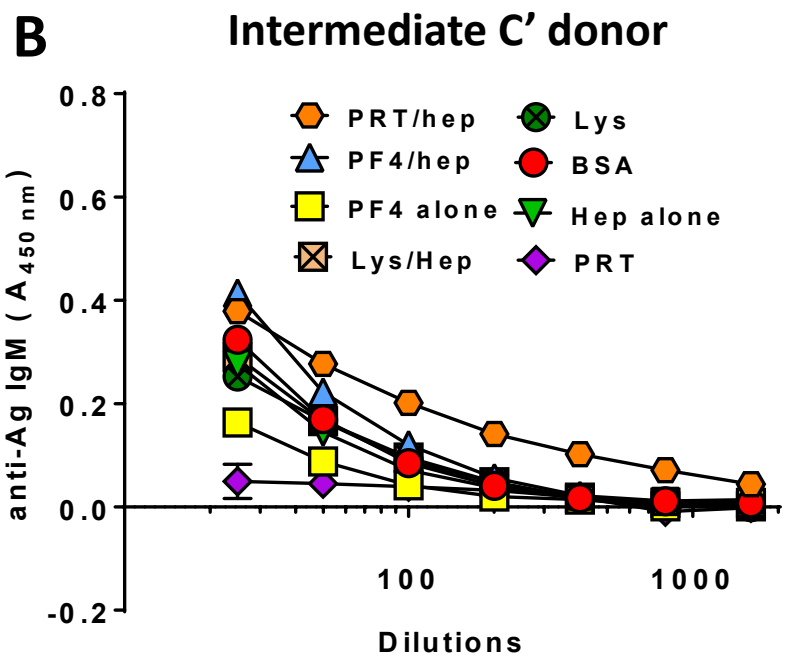


Figure S1: Complement activation and PF4/heparin binding to B cells in healthy donors. whole blood from "high", "intermediate" and "low" complement (C') response type healthy donors from Figure 1A/B was incubated with PF4 and heparin and binding of PF4/heparin and C3c to B cells was determined by flow cytometry as described in the methods section. Histogram shows the binding of (A) anti-PF4/heparin (KKO) or (B) C3c on the B cells for the three donors. Filled histogram show the isotype staining.

Supplemental Figure 2





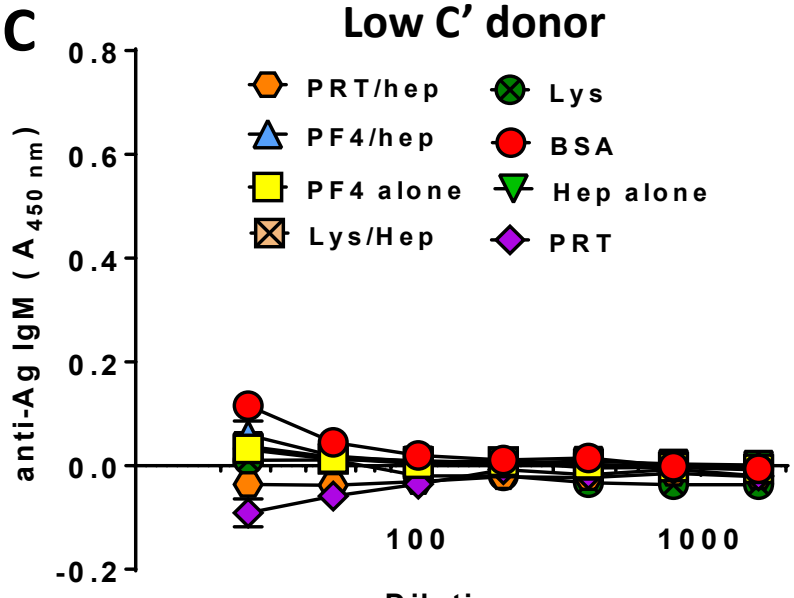


Figure S2: Antigen specificity of IgM in the plasma of high, intermediate and low complement (C') donors. Antigen-specificity of IgM in the plasma of (A) high (B) intermediate (C) low complement response donor was determined using microtiter plates coated with various antigens. Graph show the binding IgM (y- axis) to various antigens at different plasma dilutions.

Supplemental Figure 3

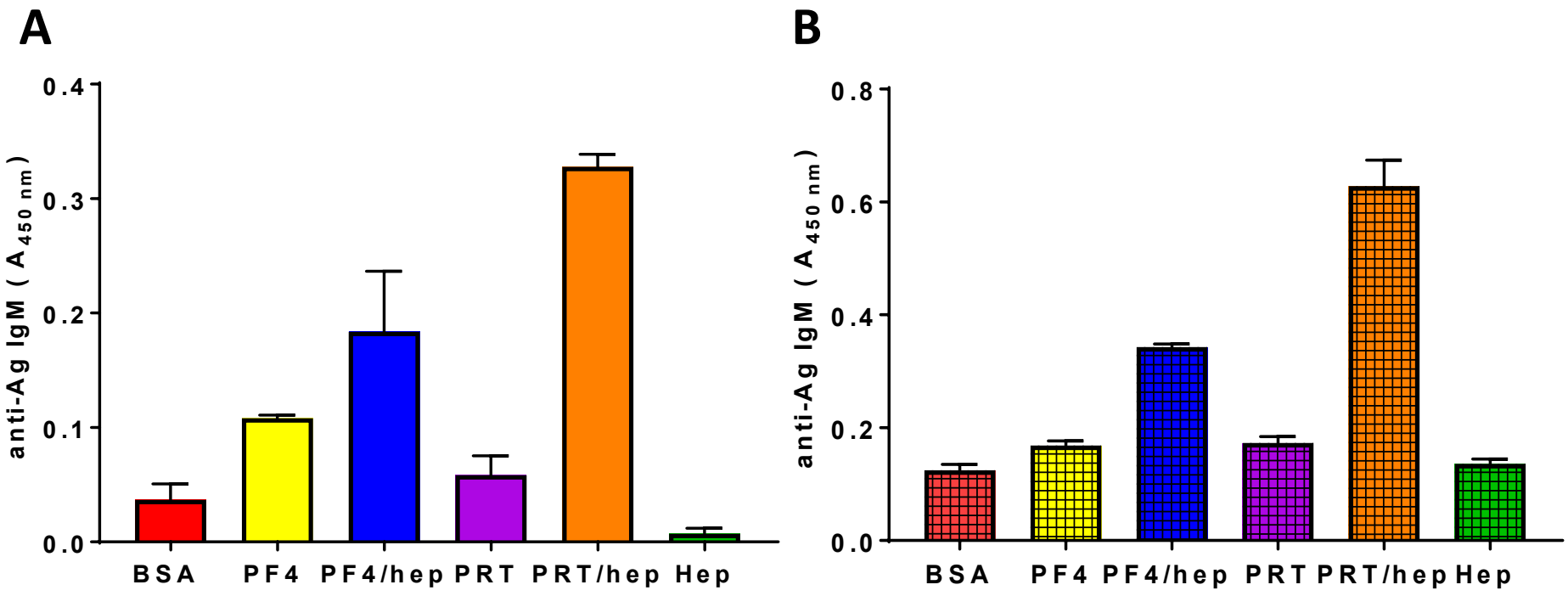


Figure S3: Polyreactivity of PF4/heparin binding IgM. (A) PF4/heparin binding IgM were isolated from the pooled healthy donor IgM by using a PF4/heparin column. Polyreactivity of these isolated PF4/heparin binding IgM (2 μg/mL) was determined by using microtiter plates coated with various antigens. Graph show the binding of IgM (y- axis) to various antigens. (B) Antigen binding specificity of unfractionated IgM (pooled healthy donor IgM; 20 μg/mL) was determined by using microtiter plates coated with various antigens. Graph show the binding of IgM (y- axis) to various antigens.

Supplemental Figure 4

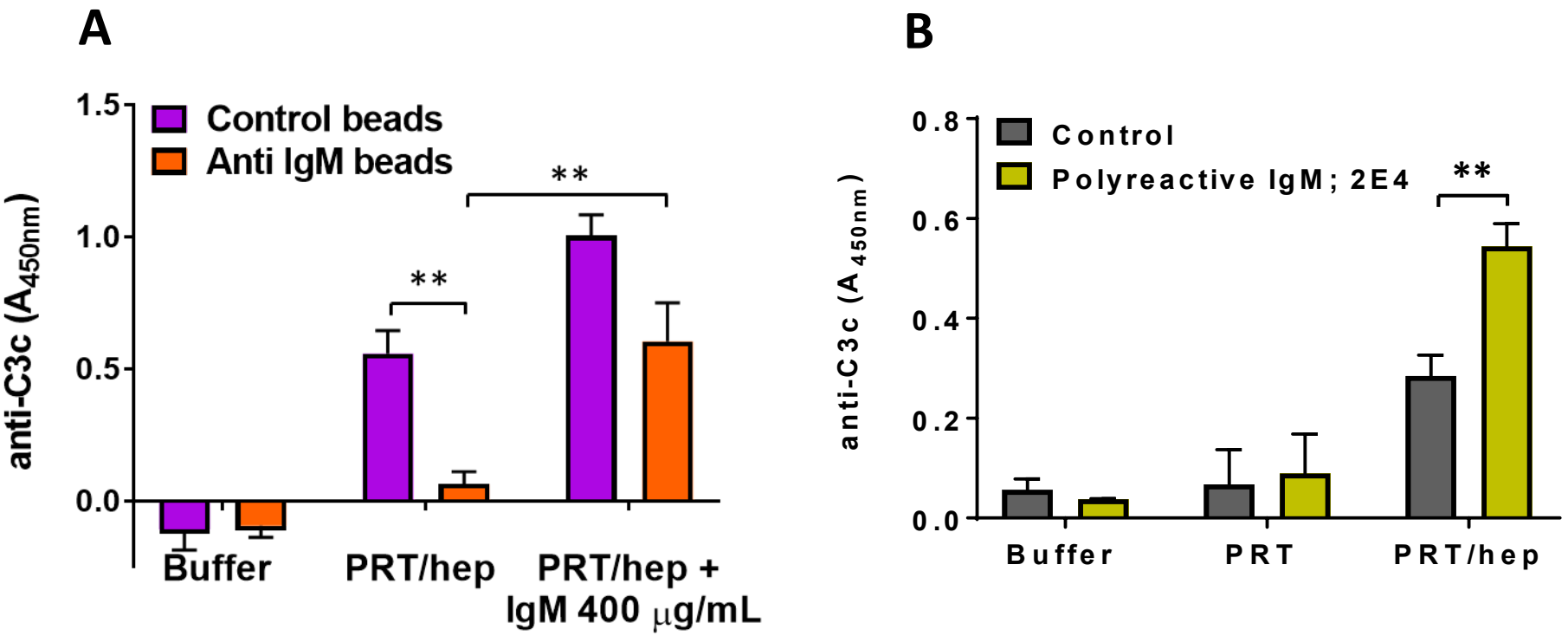


Figure S4: Polyreactive IgM mediates complement activation by PRT/heparin complexes. (A) Plasma of an "intermediate" donor phenotype was treated with anti IgM or control beads, followed by addition of buffer, PRT(125 μg/mL)/heparin (6 U/mL) or PRT/heparin + 400 μg/mL IgM and complement activation was measured by antigen-C3c capture ELISA assay on a mouse anti PRT/heparin antibody (ADA) coated plate. Graph shows complement activation (y-axis) in control or IgM depleted plasma (x-axis) ** $p<0.0001$ (B) Complement activation by polyreactive monoclonal IgM, 2E4, in the plasma of an "intermediate" donor phenotype in response to buffer and PRT (125 μg/mL) $\pm$ heparin (6 U/mL) was measured by antigen-C3c capture ELISA assay. Graph shows complement activation (y-axis) in different incubation conditions with and without added polyreactive monoclonal IgM (10 μg/mL, 2E4). ** $p<0.0001$, relative to no polyreactive IgM added.

Supplemental Figure 5

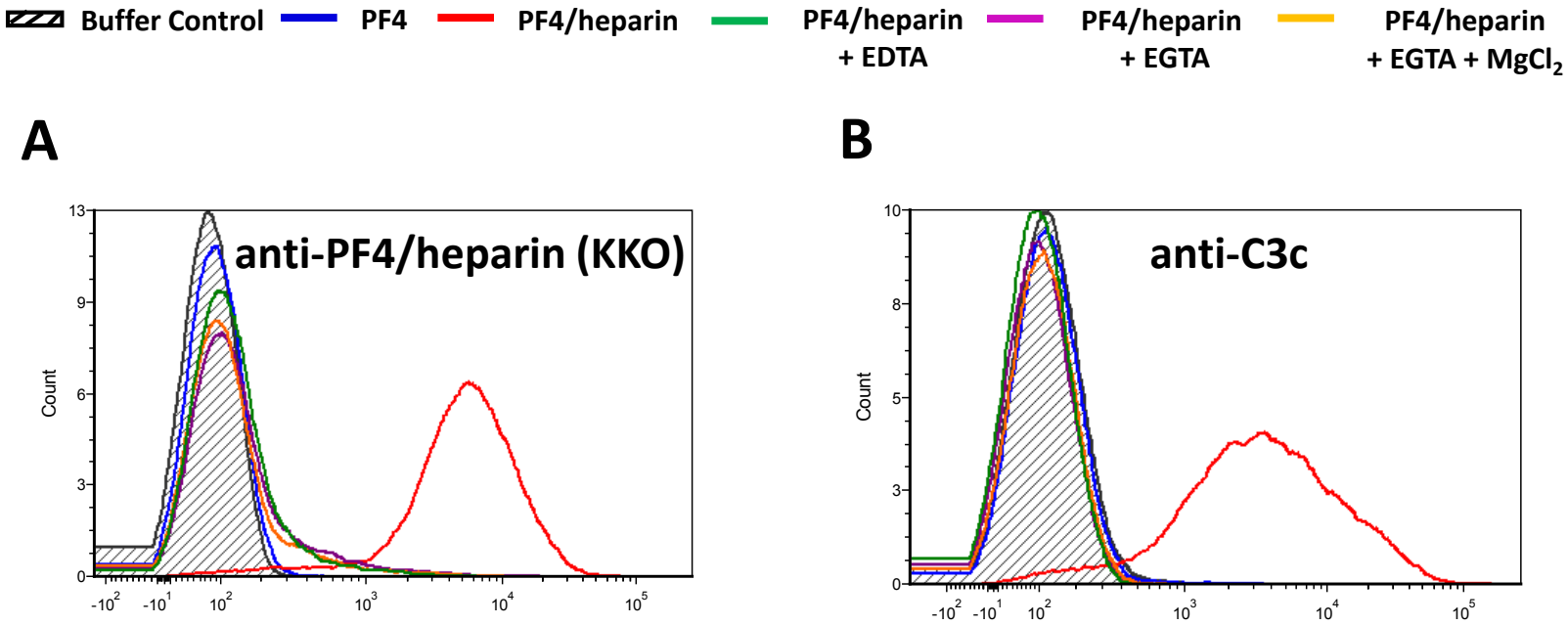


Figure S5: PF4/heparin complexes do not activate complement via alternative pathway: Whole blood from a healthy donor was incubated with or without EDTA (10 mM) or EGTA (10 mM) ± $MgCl_2$ (10 mM) before incubating with buffer or antigen (PF4; 25µg/mL ± heparin; 0.25 U/mL) and binding of PF4/heparin and C3c to B cells was determined by flow cytometry as described in the methods section. Histogram shows the binding of (A) anti-PF4/heparin (KKO) or (B) anti-C3c to B cells in various incubation conditions.

Supplemental Figure 6

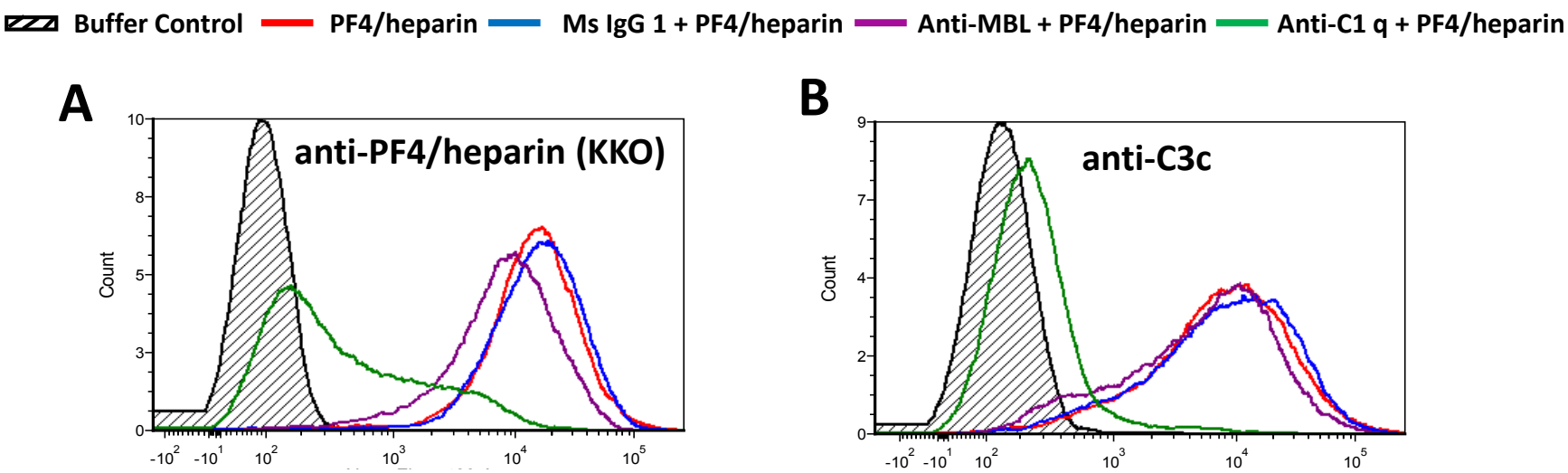


Figure S6: PF4/heparin complexes activate complement by classical pathway . Whole blood from a healthy donor was incubated with 100 μg/mL of mouse IgG1 or anti-MBL antibody or anti-C1q antibody before incubating with PF4/heparin. Binding of PF4/heparin and C3c to B cells was determined by flow cytometry as described in the methods section. Histogram shows the binding of (A) anti- PF4/heparin (KKO) or (B) anti-C3c to B cells in various incubation conditions.