Supplementary File

Naturally-acquired adaptive immunity to *Streptococcus pneumoniae* is impaired in rheumatoid arthritis patients

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Antigen	Average MFI (% change)	SD (% change)
SP_2216	2.59	8.21
SP_0641a	2.52	3.20
SP_1174	3.32	5.19
pspA-10050-2#28	7.44	12.36
pspC-D39	11.71	15.01
SP_0107	6.52	9.96
pspC-10071-4#59	12.60	16.30
pspA-10071-4#59	13.97	20.31
SP_1518	6.50	8.43
SP_2063	3.87	17.07
SP_1732	7.37	14.37
SP_0785	13.04	16.39
pspA-TIGR4	13.68	14.60
SP_0374	5.36	28.05
SP_0641c	17.05	18.10
SP_0648c	11.60	17.58
SP_1527	17.21	15.62
SP_0641b	17.85	17.93
SP_1923	12.24	18.86
pspC-TIGR4	21.68	12.98

Table S1 Antigen response variability. Variability of IgG response for individual pneumococcal antigens is reported as the average percentage change measured by protein array in sera samples from ME/CFS patients 6 months apart. Average and standard deviation have been calculated in a cohort of 12 patients for the 20 most recognised antigens.

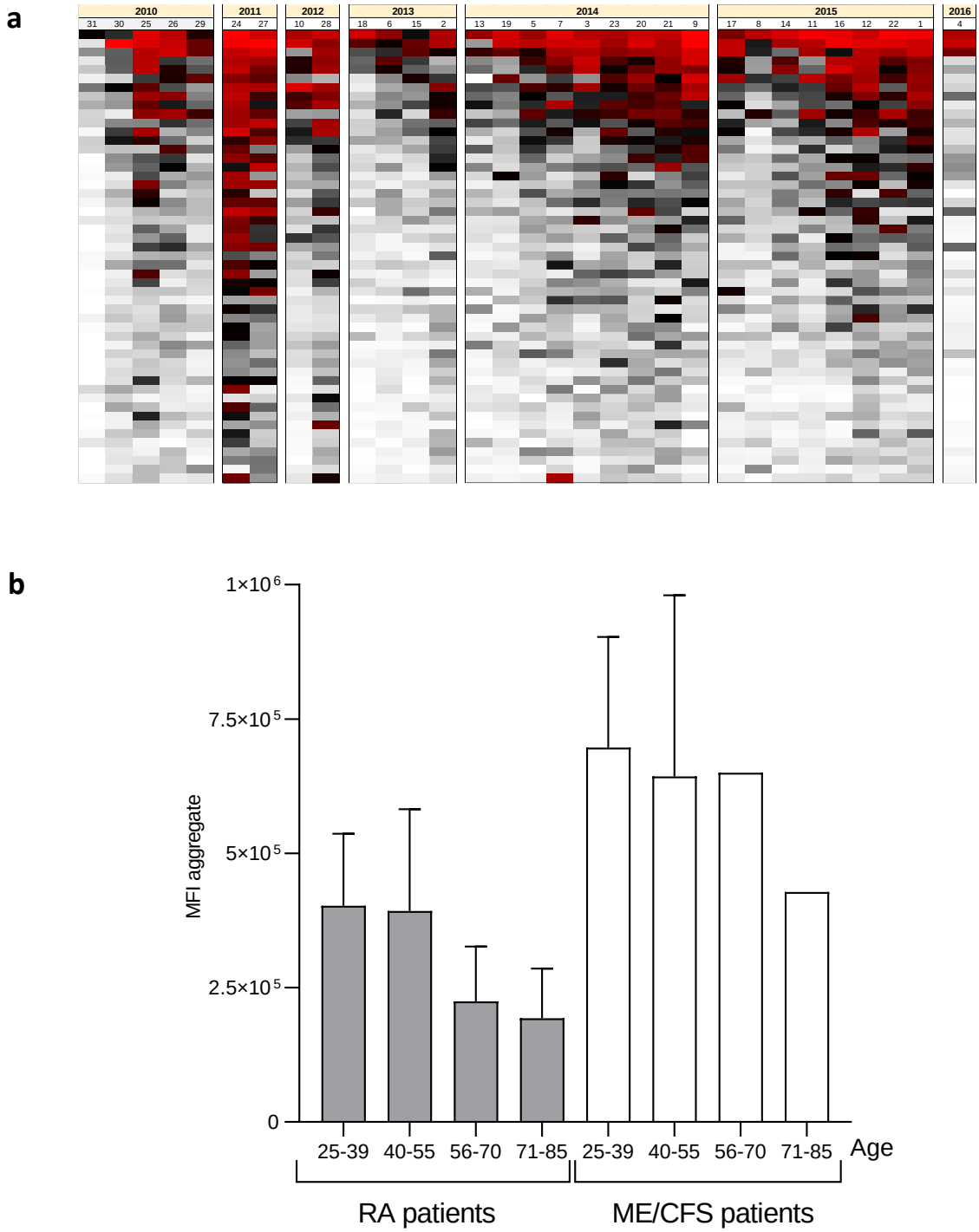

Figure S1 Time of collection and patients' age influence on array data. (A) Patients have been grouped based on their age at the time of sampling. Year of serum collection is reported for each patient in association with protein array data (top 50 ranked antigens) to show that reactivity levels are not affected by storage. Antigens are ranked from most recognised (top) to lowest (bottom) and signal strength varies from high (MFI=20000, red), medium (MFI=10000, black) and low (MFI=0, white). (B) Aggregate MFI of all antigens from each patient has been used to calculate the average antibody recognition for each cohort; same approach has been applied to ME/CFS patients.

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MEDIAN AGE ± IQR	RA	ME/CFS
Whole cohorts (22-84)	51 ± 27	36 ± 12.5
Age matched only (24-55)	40.5 ± 14.5	40 ± 13.5

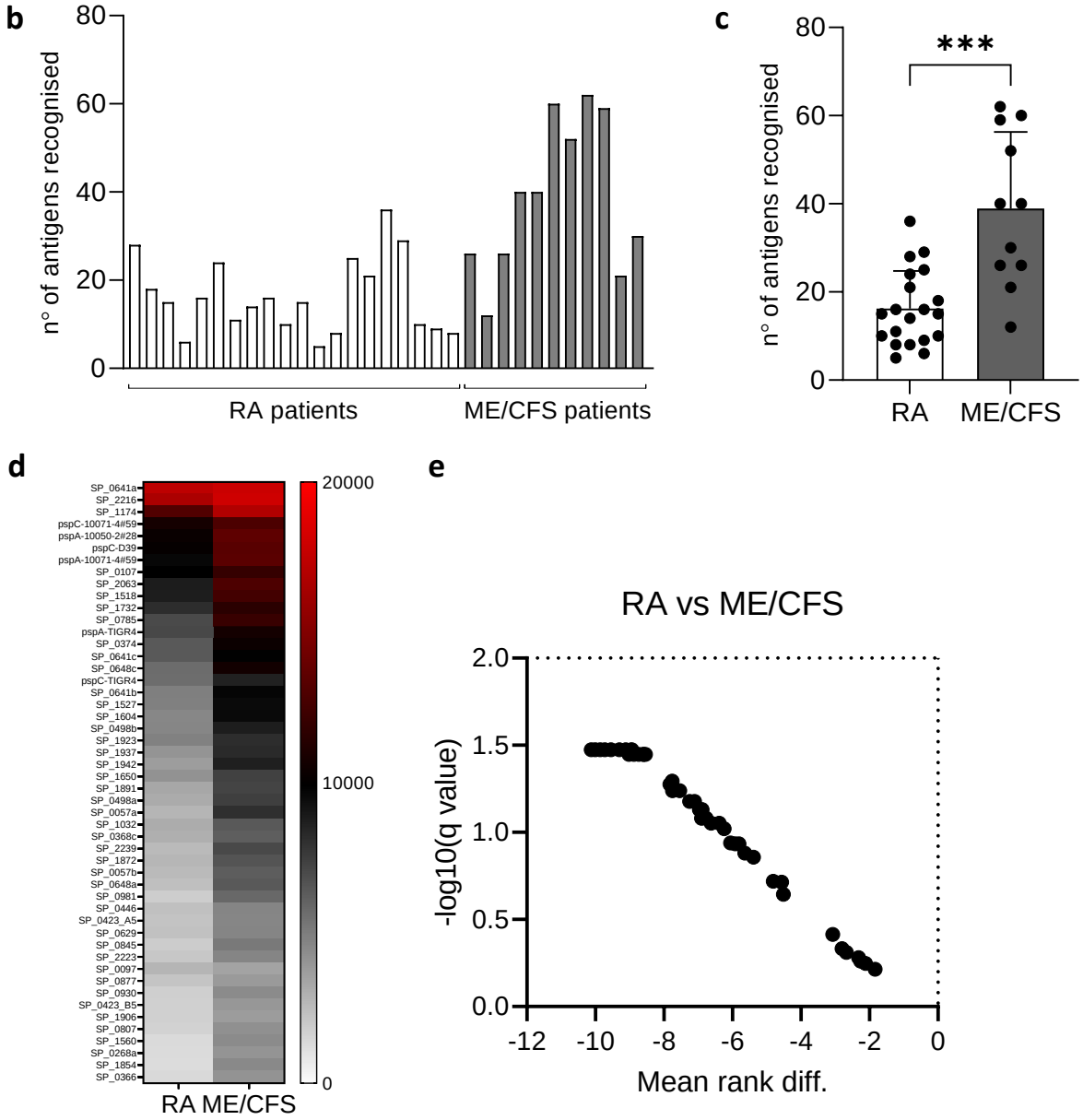

Figure S2 Analysis of array data considering age-matched cohorts. (A) Table showing the median age and IQR of the entire cohorts and the selected age-matched subjects. (B) Total number of antigens recognised by age-matched RA and ME/CFS sera. Responses to a specific antigen were considered positive when the MFI measured for the sera was higher than negative control sample (naïve mouse serum) and MFI>3000. (C) Overall mean number of antigens recognised for the age-matched RA and ME/CFS groups. Columns indicate mean values and error bars represent standard deviations; the data were analysed using a Mann Whitney U-test (**** = P<0.0001). (D) Heatmap of the mean MFI values for the top 50 recognised antigens for the age-matched RA and ME/CFS cohorts ranked from most (top) to lowest (bottom) recognised. Signal strength varies with colour from high (MFI=20000, reds), medium (MFI=10000, black through grey) and low / absent (MFI=0, pale grey to white). (E) Volcano plot comparing results for individual antigens between age-matched RA and ME/CFS subjects. Top left quarter represents antigens with higher results in ME/CSF compared to RA subjects (calculated using Mann-Whitney with false discovery rate approach).

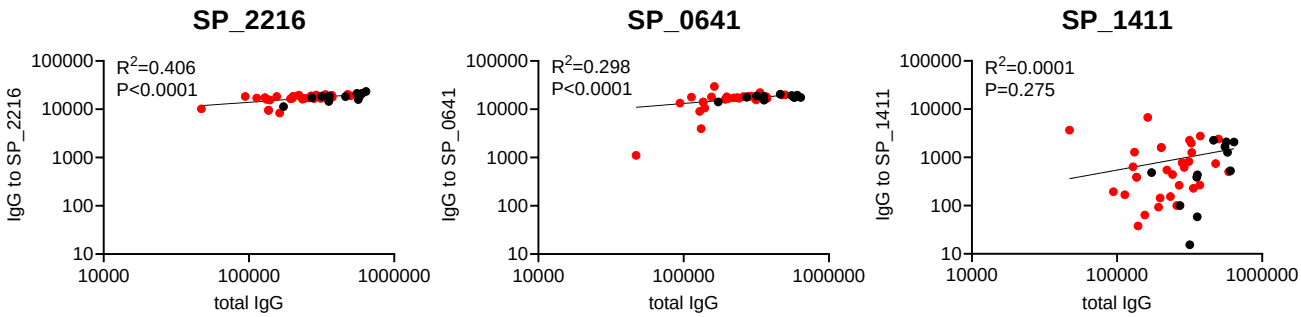

Figure S3 Anti-pneumococcal array data vs total IgG levels. Correlations between the IgG levels measured using the array for three specific antigens (SP_2216, SP_0641 and SP_1411) and flow cytometry IgG opsonisation data for RA (red symbols) and ME/CFS (black symbols). Each symbol represents an individual subject; linear regression was calculated for all correlations and shown as a black line. R-squared and P values are reported in the top right corner.

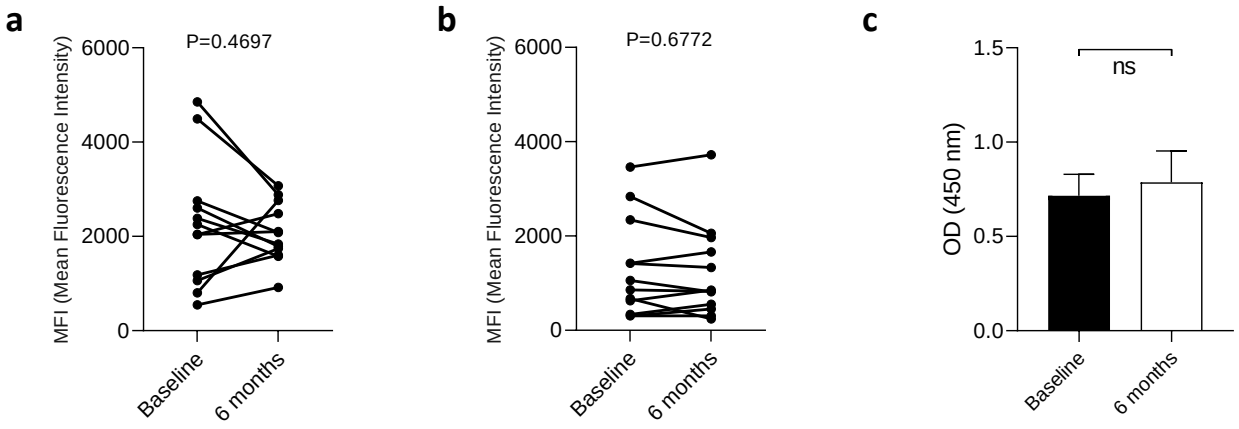

Figure S4 Level of antibodies against pneumococcus in in ME/CFS patients. Anti-pneumococcus IgG levels were measured in ME/CFS patients sera taken 6 months apart. 6B (A) and TIGR4 (B) strains were tested for IgG binding. Difference between baseline and post-6 months samples was analysed using Wilcoxon signed-rank test and P values reported in the graphs. (C) Anti-pneumococcus IgG were measured by whole cell ELISA against TIGR4 starin at the baseline and after 6 months. Wilcoxon matched-pair signed rank test has been applied to calculate statistical difference between baseline and post-6 months samples (*=P<0.05, ns=not significant).

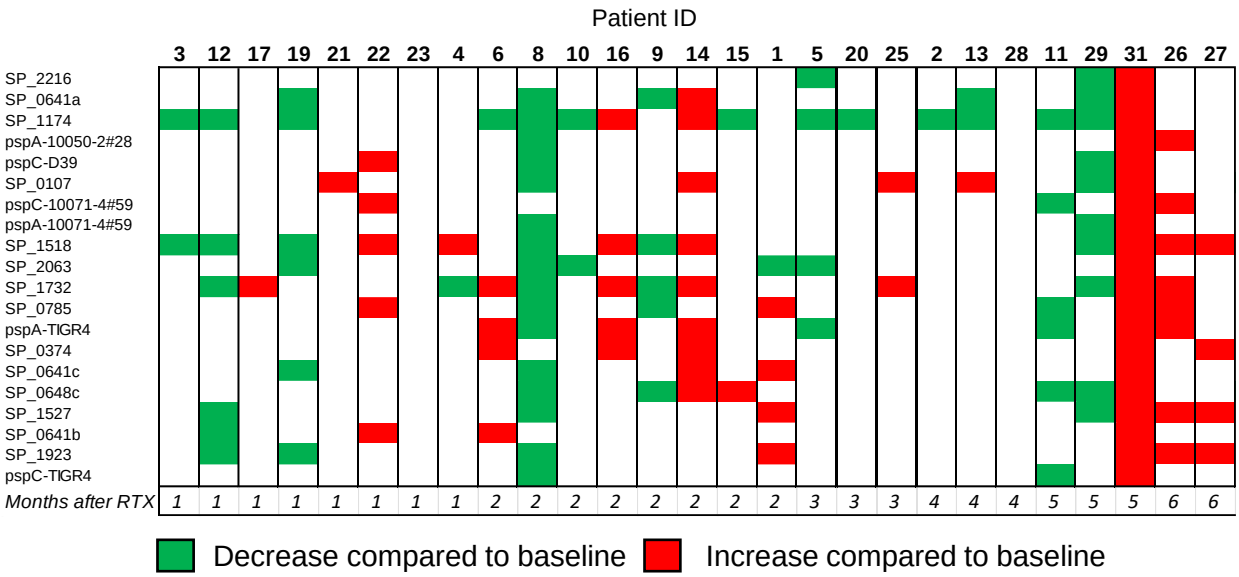

Figure S5 Variability of anti-protein response after B cell depletion in RA patients. (A) Significant changes in anti-pneumococcal protein response after B cell depletion have been reported for RA patients. Columns identify patients, rows indicate the top 20 ranked antigens in protein array. Coloured cells represent the percentage change outside the standard variability observed in the ME/CFS control cohort (> or < AVG+(2xSD), see supplementary table 1) between pre- and post-depletion samples. Green cells indicate increased IgG levels; red cells indicate decreased IgG levels for each individual antigen. The monthly interval between the pre- and post-rituximab treatment samples for each patient in also reported in the bottom row.