Willcocks et al., http://www.jem.org/cgi/content/full/jem.20072413/DC1

SUPPLEMENTAL MATERIALS AND METHODS

SLE and AASV cohorts

The UK SLE cohort (n = 171) was obtained from the MRC/Kidney Research UK National DNA Bank for Glomerulonephritis. All individuals were between the ages of 18 and 50 with a definite diagnosis of lupus nephritis based on biopsy and on the clinical and serological features defined by the American College of Rheumatology (1). The Hong Kong SLE cohort (n = 159) was recruited from the Queen Mary Hospital, Hong Kong. All patients satisfied the revised American College of Rheumatology criteria for systemic lupus. Hong Kong controls (n = 150) were obtained from the Hong Kong Red Cross.

The UK vasculitis cohort 1 (n = 347) was obtained from the MRC/Kidney Research UK National DNA Bank for Glomerulonephritis. Individuals were between the ages of 18 and 70, were antineutrophil cytoplasmic antibody (ANCA) seropositive, and had biopsy-proven necrotizing glomerulonephritis. The UK vasculitis cohort 2 (n = 136) was recruited from the Norwich Health Authority, and comprised patients seropositive for ANCA and/or with histological evidence of small vessel vasculitis. The UK vasculitis cohort 3 (n = 73) was recruited from the University of Birmingham. All individuals were ANCA seropositive with firm clinical and/or histological evidence of vasculitis. More than 97% of all patients were Caucasian. Ethnically matched UK controls (n = 286) were obtained from the UK Glomerulonephritis DNA bank.

FCGR3B copy number determination for Fig. S5

Individuals from Fig. S5 were genotyped using a novel *FCGR3B* CN assay (unpublished data).

SUPPLEMENTAL CLINICAL INFORMATION

FCGR3B-deficient family

Mrs. P presented to Addenbrooke's Hospital in 2004 at the age of 62 with a 4-yr history of SLE characterized by rash, arthritis, Raynaud's phenomenon, and nephritis. She had hematuria, proteinuria, a creatinine of 213 μmol/liter (normal range 53–124), urea 22.7 mmol/liter (normal range 2.5–7.0), and Banff grade IV proliferative lupus nephritis on renal biopsy, with immunofluorescence showing immune complexes containing IgG, IgM, C1q, and C4. Her antinuclear antibody titer was 4.1 U by ELISA (normal range 0–0.9), C3 0.7 g/liter (0.8–2.14), and C4 0.07 g/liter (0.13–0.6). Her disease had worsened despite treatment with corticosteroids and hydroxychloroquine, and she had become profoundly leukopenic after azathioprine. She was therefore treated with B cell depletion therapy (rituximab) (2), after which her symptoms improved, her renal function stabilized, and complement normalized. 6 mo later, her symptoms flared, but responded to retreatment with rituximab. She has since received one further course of B cell depletion therapy, and remains in remission. The functional studies shown in Figs. 2 and 3 were performed when she had no clinical evidence of active disease and was not on corticosteroid therapy. Her family members have no symptoms of SLE or other systemic disease, and those whose FcγRIIIb expression and gene copy numbers are shown in Fig. 1 all have antinuclear antibody titers ≤0.6.

SLE patients from Fig. 2 A and Fig. S8

The clinical details of 15 SLE patients enrolled into the Cambridge Hinxton Centre for Translational Research in Autoimmune Disease program are shown in Table S3.

The microarray expression pattern for these patients shown in Fig. 2 A was performed on neutrophils and monocytes from blood taken when patients presented with SLE (T_0). At this time, their mean British Isles Lupus Advisory Group scores were high, which is consistent with active disease. 3 mo after treatment, the scores were much improved.

AASV patients from Fig. S6

The clinical details of 43 AASV patients enrolled into the Cambridge Hinxton Centre for Translational Research in Autoimmune Disease program are also shown in Table S3.

REFERENCES

1. Hochberg, M.C. 1997. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum.* 40:1725. PubMed doi:10.1002/art.1780400928
2. Smith, K.G.C., R.B. Jones, S.M. Burns, and D.R. Jayne. 2006. Long-term comparison of rituximab treatment for refractory systemic lupus erythematosus and vasculitis: remission, relapse, and re-treatment. *Arthritis Rheum.* 54:2970–2982. PubMed doi:10.1002/art.22046

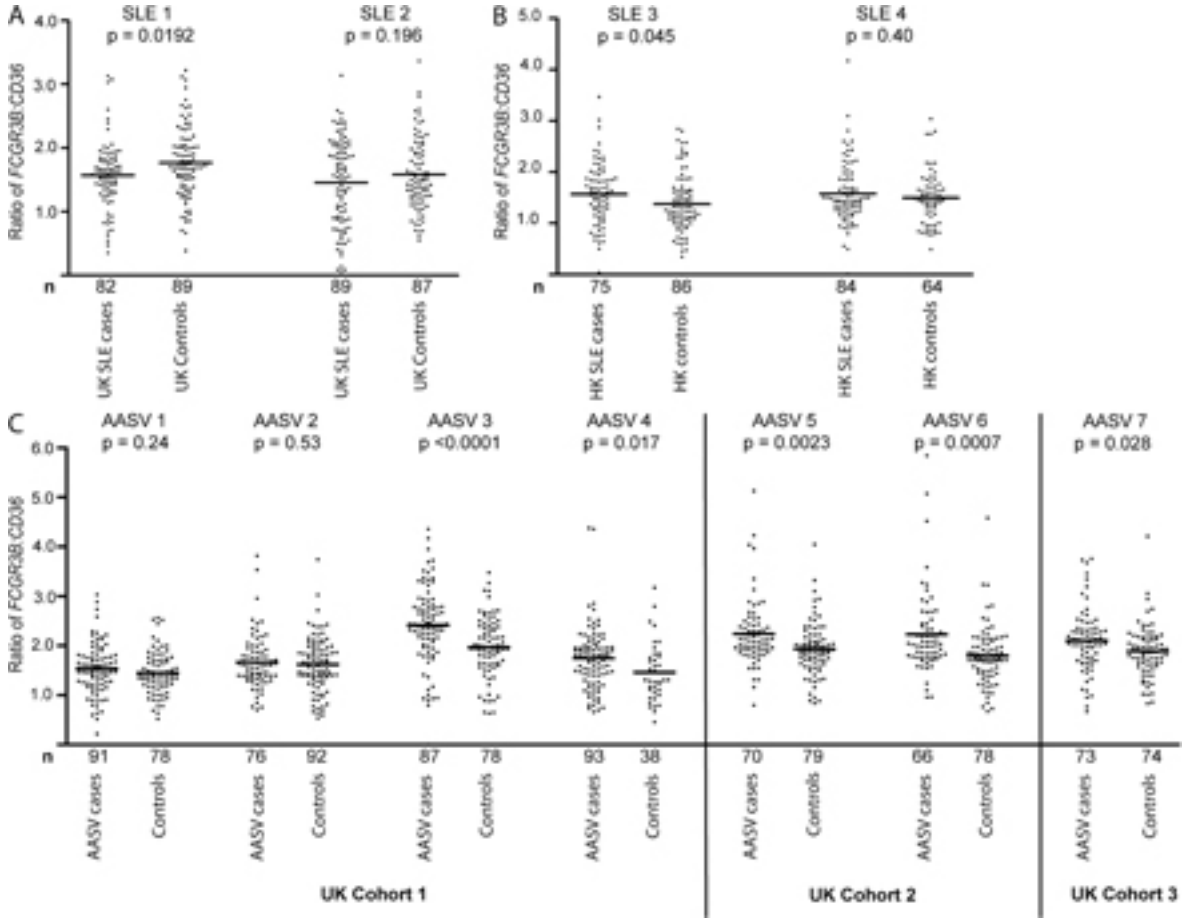

Figure S1. qPCR raw data for all plates of samples analyzed. *FCGR3B/CD36* ratio data for individual plates of UK SLE (n = 171) and control samples (n = 176; A), Hong Kong SLE (n = 159) and Hong Kong controls (n = 150; B), and UK AASV (cohort 1, n = 347; cohort 2, n = 136; and cohort 3, n = 73) and control samples (n = 286; C). The actual numbers of cases and controls genotyped on each plate are as indicated. P values indicate Student's t test results for each individual plate comparison, overall P values for each case versus control comparison using a Student's t test stratified by plate are shown in Figs. 1 and 5.

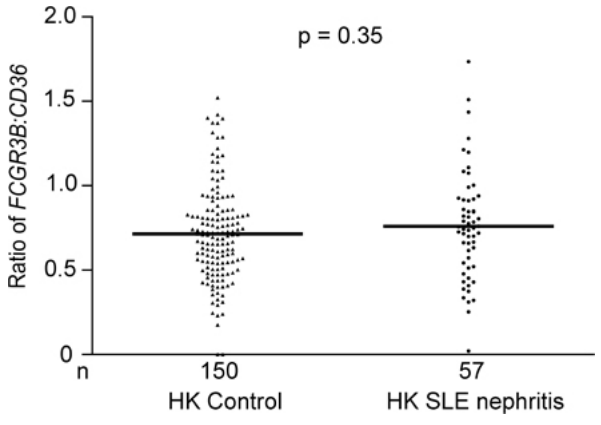

Figure S2. In a Chinese population from Hong Kong, *FCGR3B* CN does not differ significantly in patients with SLE nephritis compared with healthy controls. Number of samples in each group is shown, and P value represents unpaired Student's t test with Welch's correction.

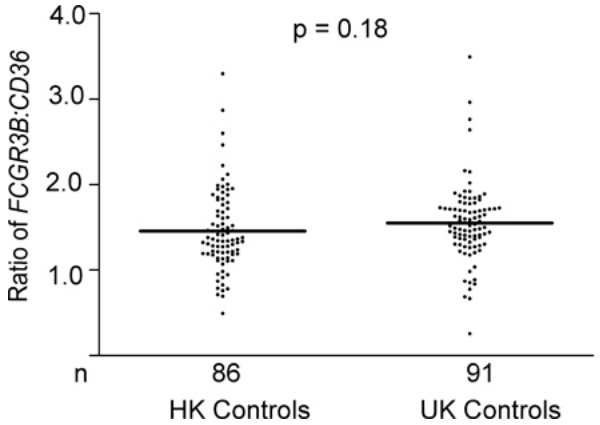

Figure S3. *FCGR3B* CN does not differ significantly in healthy Chinese individuals from Hong Kong compared with UK Caucasians. Number of samples in each group is shown, and P value represents unpaired Student's t test with Welch's correction.

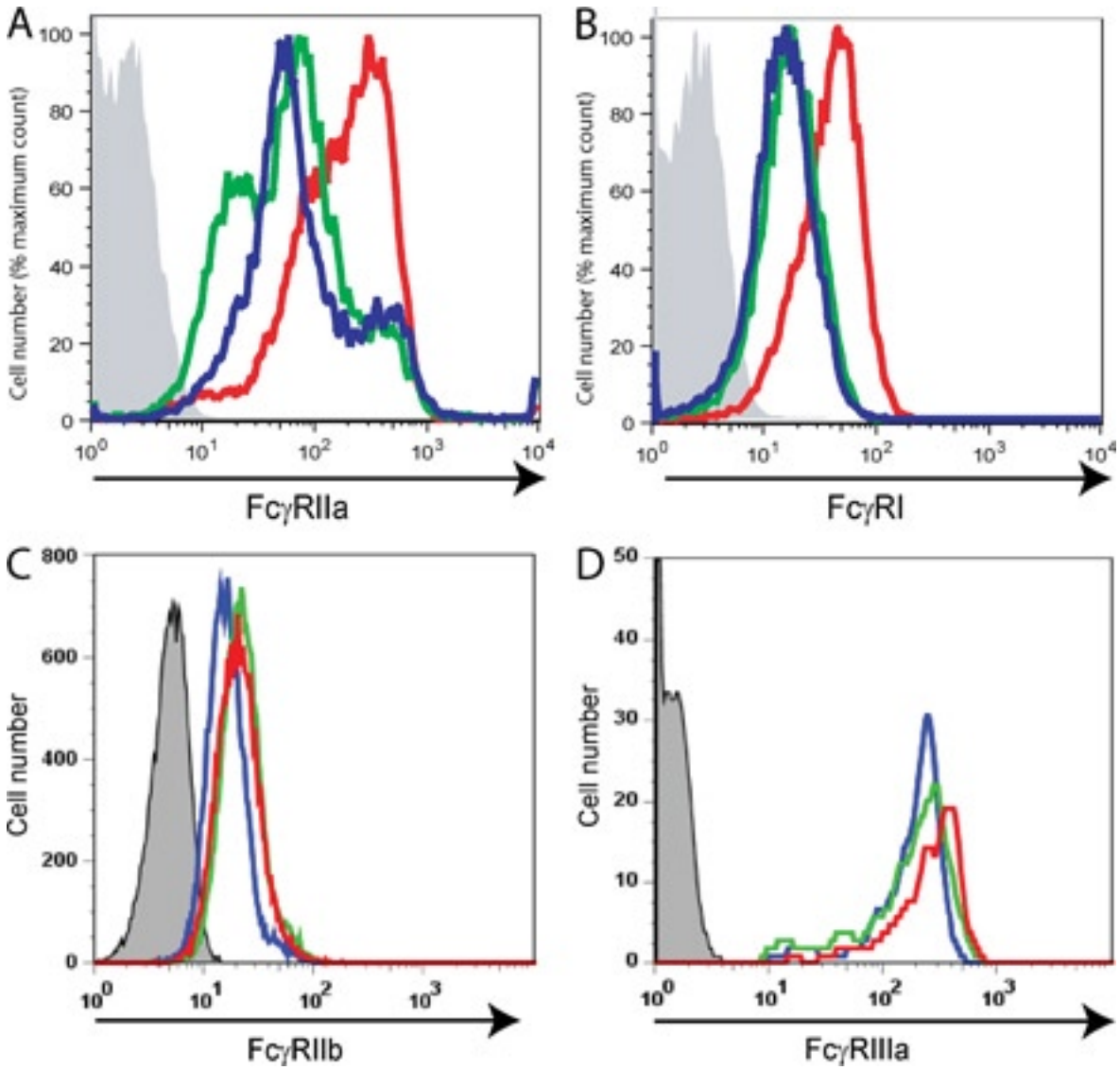

Figure S4. Cell surface expression by flow cytometry. Cell surface expression by flow cytometry of FcγRIIa (A), FcγRI (B), and FcγRIIb (C) on neutrophils, and FcγRIIIa (D) on monocytes of patient A (in red) and two individuals known to have *FCGR3B*.

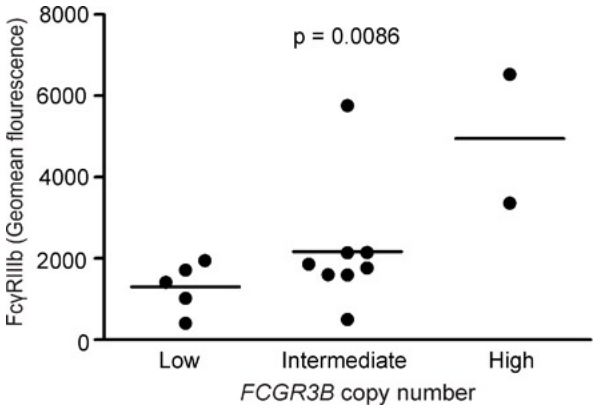

Figure S5. The finding that *FCGR3B* copy number is proportional to gene expression is reproducible. Surface expression of FcγRIIIb (clone 3G8, geometric mean fluorescence) correlated with gene dosage of *FCGR3B* in a separate population to that shown in Fig. 4 (comprising 15 healthy individuals from Leeds). P value represents one way ANOVA with a posttest for linear trend.

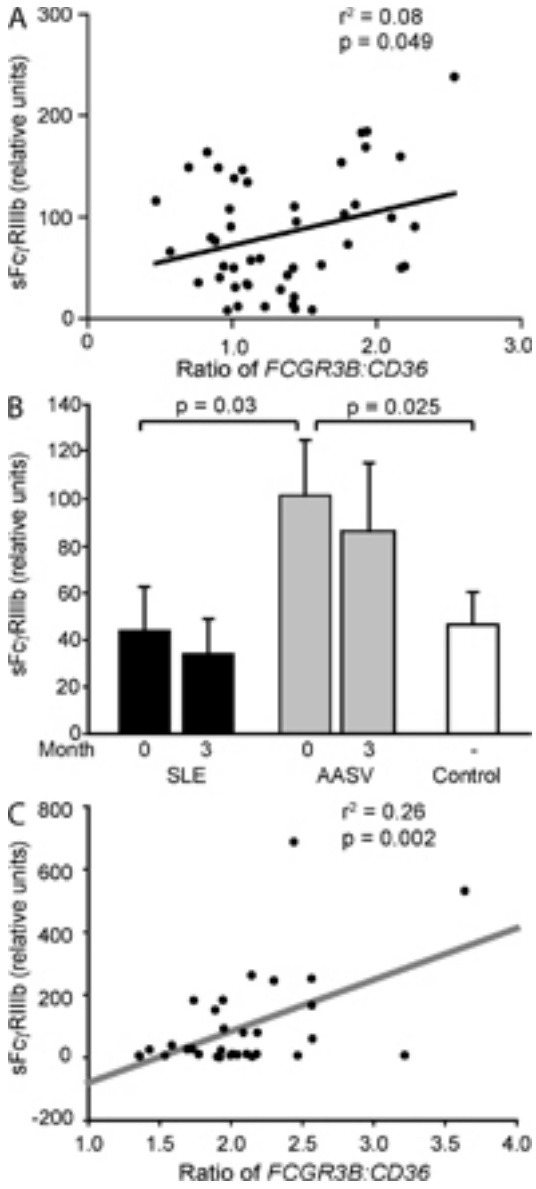

Figure S6. Correlation of *FCGR3B* CN and soluble FcγRIIIb. (A) Soluble FcγRIIIb levels correlate with *FCGR3B*/*CD36* in healthy volunteers. (B) Soluble FcγRIIIb levels in serum from control individuals and patients with SLE or AASV at presentation or 3 mo after treatment. Error bars represent the SEM. P values refer to unpaired Student's *t* test with Welch's correction. (C) Soluble FcγRIIIb levels correlate with *FCGR3B*/*CD36* in an AASV disease population. P and r^2 values in A and C represent linear regression analysis using GraphPad Prism software.

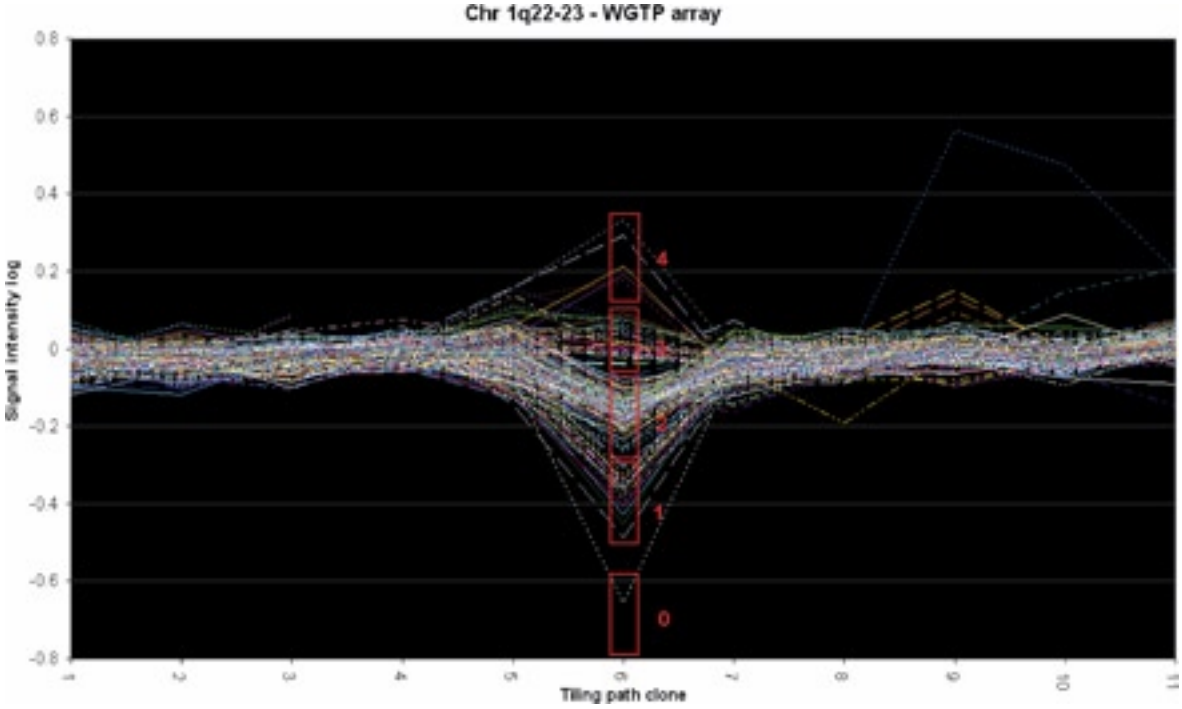

Figure S7. Array comparative genomic hybridization data across the *FCGR3B* locus on chromosome 1q22–23. Log intensity data for 11 probes from the Whole Genome Tiling Path Array3 that encompass the *FCGR3B* locus were downloaded from http://www.sanger.ac.uk/humgen/cnv/data for 270 HapMap individuals. *FCGR3B* CN, indicated by boxes, was inferred by clustering intensity values for probe Chr1tp-8H4, which contains the *FCGR3B* locus.

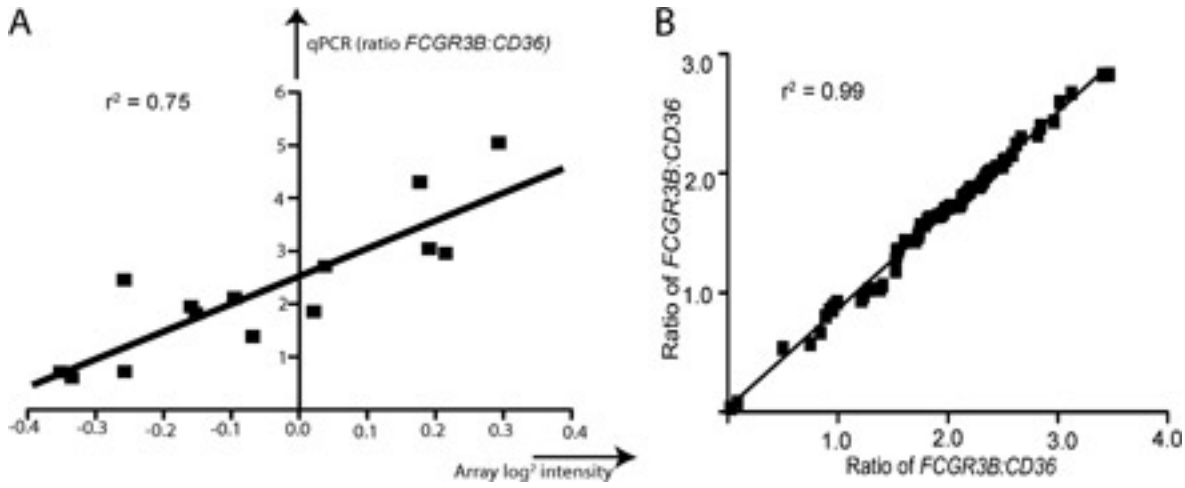

Figure S8. *FCGR3B*/*CD36* ratio detected by qPCR correlates with aCGH data and is reproducible. (A) We downloaded Array Comparative Genomic Hybridization (aCGH) data from probe Chr1tp-8H4, which contains the *FCGR3B* locus, for 14 HapMap individuals from http://www.sanger.ac.uk/humgen/cnv/data. DNA from these individuals was then genotyped using the qPCR method. The plot shows the close correlation between CN as measured by aCGH (x axis) and qPCR (y axis). (B) *FCGR3B/CD36* ratios were determined in 79 SLE patients by qPCR on separate occasions. Correlation of the *FCGR3B/CD36* ratios obtained is shown. r^2 values represent linear regression analysis using GraphPad Prism software.

Table S1. Percentage of cells that undergo spreading after adhesion is unrelated to *FCGR3B* CN

FCGR3B copy number	Spreading[a] Mean ± SD
0	91
Low	96 ± 1.41
Intermediate	98.3 ± 1.16
High	98.3 ± 1.16

[a]Percentage of stationary, adherent cells that underwent transformation to a flattened, phase-dark appearance after 4 min of flow at a shear stress of 0.1 Pa.

Table S2. Gene-specific primer sequences

Primer	Sequence (5′-3′)	AT[a]	Amplicon size	Target location[b]
		°C	*bp*	
FCGR2A F	dGGAGAAACCATCATGCTGAG	57	369	chr1:159,746,275-
FCGR2A R	dTCAATACTTAGCCAGGCT			159,746,643
HSPA6 F	dGAAGGTGCGGGAAGGTGCGGAAA	64	140	chr1:159,760,855-
HSPA6 R	dCTCTCCCTGCGGTTTCTCTGCA			159,760,994
FCGR3A F	dTCACATATTTACAGAATGGCAATGG	52	199	chr1:159,781,114-
FCGR3A R	dCAGGAAACAGCTATGACCCTTGAGTGATGGTGATGTTCA			159,781,276
FCGR2C F	dTTGGGCTTCCTCTTCCTCAC	57	455	chr1:159,825,931-
FCGR2C R	dGACGCAAAGCAACCACTGAC			159,826,385
HSPA7 F	dGCTGATTATCTCTGGCCATTCCT	62	345	chr1:159,842,018-
HSPA7 R	dTCAGGGCTGCTGAAAGAAAC			159,842,362
FCGR3B F	dGATGAAGTTTCAAGAAAAGGAAACTGGCA	58	591	chr1:159,867,065-
FCGR3B R	dGTACAGGTTGAATTTCCCTAAACCAAGCC			159,867,655
FCGR2B F	dGGAGGACAGGGAGATGCTGCAGT	60	337	chr1:159,914,048-
FCGR2B R	dCCCAGAAACAATCACTTTTAATGTGCTGG			159,914,384

Primer sequences for *FCGR3A* and *FCGR2A* were adapted from previously published sequences (Morgan, A.W., B. Griffiths, B.M. Montague, et al. 2000. *Arthritis Rheum.* 43:2328–2334; Morgan, A.W., J.H. Barrett, B. Griffiths, et al. 2006. *Arthritis Res. Ther.* 8:R5).

[a]AT, annealing temperature.

[b]Target location refers to March 2006 human genome build (hg18) in the University of California at Santa Cruz genome browser (http://genome.ucsc.edu/)

Table S3. Clinical details of SLE and AASV patients

	SLE	AASV
Number	15	43
Age (mean and range)	42 (21–59)	55 (19–81)
Sex (M:F)	1:14	18:25
T_0 BILAG (mean ± SD)[a]	16 ± 7	na
T_3 BILAG (mean ± SD)[b]	5 ± 4	na
T_0 BVAS (mean ± SD)[c]	na	10 ± 9
T_3 BVAS (mean ± SD)[d]	na	4 ± 4

na, not applicable.

[a]British Isles Lupus Advisory Group (BILAG) score at presentation.

[b]BILAG score 3 mo after treatment.

[c]Birmingham Vasculitis Activity Score (BVAS) at presentation.

[d]BVAS 3 mo after treatment.