SUPPLEMENTARY INFORMATION FOR:

**Antibodies to human serum amyloid P component eliminate visceral amyloid deposits**

Karl Bodin, Stephan Ellmerich, Melvyn C. Kahan, Glenys A. Tennent, Andrzej Loesch, Janet A. Gilbertson, Winston L. Hutchinson, Palma P. Mangione, J. Ruth Gallimore, David J. Millar, Shane Minogue, Amar P. Dhillon, Graham W. Taylor, Arthur R. Bradwell, Aviva Petrie, Julian D. Gillmore, Vittorio Bellotti, Marina Botto, Philip N. Hawkins & Mark B. Pepys
1. Schematic summarising main findings

**Background**
- Amyloid deposits, composed of amyloid fibrils and serum amyloid P component (SAP), damage tissues and cause fatal systemic amyloidosis. Deposits are usually extensive at diagnosis. No treatment exists which directly eliminates amyloid.
- The liver both produces and clears from the blood, 50-100 mg of SAP daily. Normal subjects have 50-100 mg of SAP in the blood and extracellular fluid.
- Plasma SAP concentration and turnover are normal in systemic amyloidosis but SAP binds avidly to amyloid fibrils deposited in the tissues and accumulates there. The amyloid-associated SAP pool may be as much as 20,000 mg.
- CPHPC treatment causes rapid clearance of SAP by the liver, specifically depleting circulating SAP by >90%, but some SAP persists in the amyloid deposits.

**Findings**
- After SAP depletion by CPHPC, anti-SAP antibodies can be administered safely and without being consumed by binding to free circulating SAP. Anti-SAP antibodies can then target SAP in the amyloid deposits.
- Antibody binding to SAP on amyloid fibrils triggers local complement activation which opsonises the deposits and attracts macrophages. Macrophages and multinucleate giant cells then efficiently eliminate amyloid with no adverse effects, restoring normal tissue architecture.
- Mouse monoclonal anti-SAP antibodies are potent and effective in mice.
- Humanised anti-SAP antibodies should be effective in clearing established systemic amyloid deposits in patients.
Snap frozen sections of kidney from a C57BL/6 SAP deficient mouse transgenically expressing human SAP were stained by the immunoperoxidase technique using monospecific rabbit anti-human SAP antiserum. a, Positive staining in all glomeruli and some inter-tubular interstitial structures. b, Negative control using the same antiserum after it had been absorbed with isolated pure human SAP covalently immobilised on Sepharose beads, confirming the immunological specificity of the staining in a. c, Higher magnification of a
single glomerulus positively stained for human SAP in the characteristic pattern of the
glomerular basement membrane, as in humans\textsuperscript{1}.

3. Localisation of human SAP in mouse splenic AA amyloid deposits

![Image of stained tissue sample]

Formalin fixed wax embedded spleen from an AA amyloidotic human SAP transgenic mouse
stained by the immunoperoxidase technique with monospecific rabbit anti-human SAP
antiserum. Strong positive staining is seen in all the marginal zone areas characteristically
occupied by the AA amyloid deposits.

4. Circulating human SAP concentrations and tissue content, and amyloid scores in
mice in the experiment shown in Fig. 1 of the paper

Serum concentrations of human SAP were the same in all groups at the first bleed taken after
induction of amyloidosis and before any other treatment, with significantly higher values
among the female than the male mice (Table 1), as we have previously observed in this
strain. At the second bleed, taken 4 days after CPHPC treatment had been started in groups
1 and 2, and before administration of anti-SAP antibody or control sheep IgG (from
anti-human oncostatin M antiserum which did not cross react with any murine antigen), there
was greater than 90% depletion of circulating human SAP (Table 1). Estimates of human
SAP concentration were not possible in sera from animals in groups 1 and 2 at days 14 and
28 after injection of sheep anti-SAP antibody or control IgG, because persistent circulating
sheep immunoglobulin interfered with the assay. In group 3, which received no other
treatment, human SAP values were unchanged at day 14 but were lower at day 28 (Table 1), possibly due to liver damage by amyloid.

The quantities of human SAP present in the spleens removed at the end of the experiment are shown in Table 2. The virtual absence of SAP in group 1 is consistent with the elimination of amyloid shown histologically after CPHPC plus anti-SAP treatment. The variable but generally high SAP content in group 3 reflects the variable but mostly extensive splenic amyloid deposits in the control untreated mice. The reduced SAP values in group 2 demonstrate the partial depletion of SAP from amyloid produced by CPHPC treatment. Interpretation of the human SAP content in the livers at the end of the experiment is complicated by the fact that the liver is the site of SAP synthesis. Some SAP is thus inevitably present as a result of its production by hepatocytes and not just due to the binding of circulating SAP to amyloid deposits within the liver, if these are present. Also the females have consistently significantly higher circulating (Table 1) and liver concentrations of human SAP than males (Table 3). Nevertheless, the human SAP content of the livers in the different groups ranked in the same order as the unequivocal values for human SAP in spleen.

Among 31 mice in group 1, no amyloid was detected in 22 (71%), 8 (26%) had only occasional microscopic specks and just one mouse had a small residual amyloid load (paper Fig. 1). In contrast there was no animal among the 62 individuals in the two control groups not receiving anti-SAP which had no amyloid at all in both spleen and liver (P<0.001 by Fisher's exact test comparing the anti-SAP treated group with all the control mice). Among the 62 control mice in groups 2 and 3, only 12 (19%) had trace or small amounts of amyloid, the deposits in all the rest being moderate or heavy (paper Fig. 1).
**Table 1 | Human SAP concentrations in serum (mean (SD) mg/l)**

|                | Males                      | Females                   |
|----------------|----------------------------|----------------------------|
|                | 1 (n=21)                   | 2 (n=20)                  | 3 (n=20)                  | 1 (n=10)                  | 2 (n=10)                  | 3 (n=12)                  |
| Treatment      | CPHPC + anti-SAP           | CPHPC                     | none                      | CPHPC + anti-SAP          | CPHPC                     | none                      |
| Pre-bleed day -2| 37.1 (14.1)                | 35.1 (7.9)                | 35.8 (12.0)               | 72.0 (14.3)               | 82.6 (20.2)               | 78.2 (21.3)               |
| Post CPHPC before antibody day 0 | 2.6 (2.5)                | 2.9 (1.3)                | 35.4 (14.6)               | 3.1 (1.9)                | 4.0 (1.4)                | 74.3 (17.3)               |
| Post antibody day 14 | NI                      | trace                     | 33.2 (11.1)               | NI                       | trace                     | 67.9 (20.9)               |
| Post antibody day 28 | NI                      | trace                     | 24.0 (6.4)                | NI                       | trace                     | 54.0 (12.6)               |

NI, not interpretable due to persistence of anti-SAP antibody in serum; trace, very low concentration but not quantifiable due to interference by persistence of sheep IgG in serum.
Table 2 | Human SAP content of spleen at end of experiment (µg/whole organ)

| Group | 1 (n=31) | 2 (n=30) | 3 (n=32) |
|-------|----------|----------|----------|
| Treatment | CPHPC + anti-SAP | CPHPC + control IgG | none |
| Median | 0 | 15 | 69 |
| IQ range | 0-0 | 1-18 | 38-113 |
| Range | 0-4 | 0-35 | 3-304 |

SAP is not expressed in the spleen and there was no difference between the males and females with respect to either the quantity of spleen amyloid or the amount of human SAP within any of the three groups. SAP content of the spleens is therefore shown here for the whole of each group. Kruskal-Wallis test: P<0.0001. Dunn's multiple comparison test: group 1 vs group 2, P<0.001; group 1 vs group 3, P<0.001; group 2 vs group 3, P<0.001.

Table 3 | Human SAP content of liver at end of experiment (µg/whole organ)

| Group | 1 (n=20) | 2 (n=20) | 3 (n=20) | 1 (n=10*) | 2 (n=10) | 3 (n-12) |
|-------|----------|----------|----------|-----------|----------|----------|
| Treatment | CPHPC + anti-SAP | CPHPC + control IgG | none | CPHPC + anti-SAP | CPHPC + control IgG | none |
| Median | 0 | 59 | 82 | 58 | 78 | 142 |
| IQ range | 0-0 | 42-75 | 56-117 | 40-76 | 60-112 | 76-289 |
| Range | 0-56 | 0-100 | 7-150 | 24-87 | 52-148 | 35-735 |

* Sample from one mouse was not available for assay. Kruskal-Wallis test: males, P<0.0001; females, P<0.0. Dunn's multiple comparison test: male group 1 vs group 2 or group 3, P<0.001; group 2 vs group 3, not significant P>0.05; female group 1 vs group 2, not significant P>0.05; group 1 vs group 3, P<0.01; group 2 vs group 3, not significant P>0.05.
5. Body weight during treatment with CPHPC and anti-SAP antibody

There was no significant difference between group means in the experiment shown in Fig. 1 of the paper, either on day -20, shortly after amyloid induction and just before $^{125}$I-SAP tracer injection for estimation of whole body amyloid load, or at day 23 after anti-SAP antibody treatment shortly before the end of the study at day 28.

| Group | Day -20   | Day 23   |
|-------|-----------|----------|
| 1     | 28.0 (2.7)| 28.5 (2.8)|
| 2     | 27.7 (3.2)| 28.4 (3.6)|
| 3     | 27.3 (3.1)| 27.8 (3.3)|

6. Co-localization of amyloid and phagolysosomes within macrophages, movie SV1

QTVR interactive movie, using data from Figs 2j and 2k in the paper.

7. Complement dependence of amyloid elimination

AA amyloidosis was induced in wild type and complement deficient mice, followed by confirmation of comparable amyloid loads in each group and then administration of human SAP followed 3 days later by the standard dose of sheep IgG anti-SAP antibodies. The median (range) spleen amyloid scores 14 days thereafter were: wild type mice, 1.17 (0.0-1.5), n=15; C3 deficient mice, 1.92 (1.17-4.33), n=12; C1q deficient mice, 1.25 (1.17-3.5), n=10 (Kruskal-Wallis test, P=0.0005). The differences in the median scores between the wild type controls and each of the complement deficient groups were significant: P<0.001 for the C3 knockouts and P=0.036 for the C1q knockouts, but the difference between the C3 and C1q knockouts was not significant, P>0.05 (Dunn’s multiple comparison test).

8. Efficacy of F(ab)$_2$ and intact IgG anti-SAP antibody in clearing amyloid deposits

The role of the IgG Fc region in mediating amyloid elimination was tested in wild type AA amyloidotic mice loaded with human SAP. The molar dose of F(ab)$_2$, 73 nmol (7.28 mg) per mouse, was substantially greater than the dose of intact IgG antibody, 47 nmol (7.0 mg) per mouse. When the mice were killed 14 days later, clearance of amyloid deposits was, as
usual, almost complete in mice receiving IgG anti-SAP antibody compared to the massive amyloid deposits in control mice receiving vehicle alone. The mice receiving F(ab)\textsubscript{2} antibody had less amyloid than untreated controls but more than the mice treated with whole IgG.

| Group (treatment, group size) | Amyloid score median, range |
|------------------------------|-----------------------------|
|                              | Spleen                      | Liver                        |
| 1 (no antibody, n=10)        | 4.0, 4.0-4.33               | 3.5, 2.67-4.67               |
| 2 (IgG anti-SAP antibody, n=8)| 1.0, 1.0-3.67*              | 1.25, 1.0-1.5                |
| 3 (F(ab)\textsubscript{2} anti-SAP antibody, n=5)| 2.17, 1.33-3.0 | 1.67, 1.33-1.67 |

Kruskal-Wallis test: spleen, P<0.001; liver P<0.001.
Dunn’s multiple comparison test: 1 vs 2, spleen & liver, both P<0.001; 1 vs 3, spleen, P<0.05; liver, not significant P>0.05 (but the Mann-Whitney test on these same scores gives P=0.002 after applying the Bonferroni adjustment for multiple testing); 2* vs 3, spleen and liver, both not significant, P>0.05.
* Single outlier in group 2 with heavy spleen amyloid despite IgG anti-SAP treatment. Excluding this animal gives a significant difference between efficacy of IgG and F(ab)\textsubscript{2} anti-SAP antibody treatment.

9. Essential role of macrophages
AA amyloidotic wild type C57BL/6 mice were loaded with injected human SAP and then given liposomal clodronate 2, 7 and 14 days afterwards (http://tinyurl.com/7naksg). Test and control groups received the standard dose of the IgG fraction of sheep anti-human SAP antiserum on day 3 after the human SAP injection. A second control group received neither clodronate nor anti-SAP. Fourteen days later there was the usual almost complete clearance of amyloid deposits in the antibody alone group but in mice in which macrophage function had been ablated by liposomal clodronate, the amyloid load was the same as in the untreated controls.
| Group                              | Amyloid score |          |          |
|-----------------------------------|---------------|----------|----------|
|                                   | Spleen        | Liver    |
| (treatment, group size)           |               |          |
| 1 (clodronate plus anti-SAP, n=13)| 4.83, 2.0-5.0 | 3.17, 2.0-3.5 |
| 2 (anti-SAP only, n=12)           | 0.42, 0.22-1.17 | 1.0, 0.67-2.5 |
| 3 (none, n=12)                    | 4.0, 3.5-4.5 | 2.83, 1.33-3.17 |

Kruskal-Wallis test: spleen, P<0.0001; liver P<0.0001.
Dunn’s multiple comparison test: 1 vs 2: spleen & liver, both P<0.001; 1 vs 3: spleen & liver, both not significant P>0.05; 2 vs 3, spleen, P<0.01, liver, P<0.001.

10. Characterisation of monoclonal mouse IgG2a anti-human SAP antibodies

The two different monoclonals, SAP-5 and Abp1, showed comparable binding to human SAP which had been covalently immobilised on a Biacore® sensor chip: \( k_\text{on} \ 2-3 \times 10^4 \text{M}^{-1}\text{sec}^{-1} \), \( k_\text{off} \ 2-6 \times 10^{-5}\text{sec}^{-1} \), \( K_D \ 1-5 \times 10^{-9}\text{M}^{-1} \). Both antibodies precipitated SAP from solution, reflecting the homopolymeric structure of SAP, and both bound to SAP which had been immobilised by its typical calcium dependent binding to amyloid fibrils. The epitope recognised by SAP-5 mapped to residues 140-158 of the SAP sequence and evidently comprised some denaturation resistant secondary structure because SAP-5 bound well to reduced denatured human SAP and was only partly inhibited by the 140-158 peptide in solution, consistent with the kinetic stability and resistance to denaturation of human SAP\(^2\). Abp1 did not bind at all to denatured SAP and thus recognised a conformational epitope. Mapping by Pepscan Presto BV CLIPS® technology (http://tinyurl.com/2ejekrc) identified the epitope as the exposed peripheral loop, residues 121-131, at the circumference of the disc like pentameric native SAP molecule.
Complete amino acid sequence of human SAP showing the points at which it is cleaved by CNBr in 70% TFA (room temperature for 18 h) (residue 159M) and by chymotrypsin (1:20 enzyme:SAP w/w, 18 h at 37°C) without prior reduction/carbamidomethylation, in ammonium bicarbonate in the absence of calcium, (residues 140Y and 144F).

SDS-PAGE analysis of SAP cleaved with CNBr. Left panel: Coomassie blue stain; lane 1, untreated control SAP; lane 2, SAP after CNBr cleavage, showing trace residual uncleaved intact protomer and the expected fragments at approximately 20kD (residues 1-159) and 5kD (160-204) respectively. These were precisely confirmed by electrospray mass spectrometry (Quatro II triple quadrupole). Right panel: Western blot with SAP-5 showing intense staining of intact untreated SAP in lanes 1 (100 ng loaded) and 2 (10 ng), and also residual intact SAP and the larger residue 1-159 fragment in CNBr cleaved SAP in lanes 3 (600 ng), 4 (130 ng) and 5 (64 ng). Lane 6 contained isolated pure human CRP with which the SAP-5 did not react at all.
SDS-PAGE analysis of SAP digested with chymotrypsin. Left panel: Coomassie blue stain; lane 1, untreated control SAP; lane 2, SAP after chymotrypsin digestion, showing the expected major fragments corresponding to residues 1-140 and 145-204. These were precisely confirmed by mass spectrometry. Right panel: Western blot with SAP-5 showing intense staining of intact untreated SAP in lanes 1 (500 ng loaded) and 2 (100 ng), and also residual intact SAP in lane 3 which contained the chymotrypsin digested SAP. Very weak binding of SAP-5 to the residue 1-140 fragment is seen in lane 3.
Sequence comparison between human SAP (h) and mouse SAP (m) for residues 136-147.

Top panel, differences indicated above by residues shown in black in the murine sequence.

Bottom panel, position of this extended loop with residue 140Y at its apex shown in white in the 3D subunit structure of human SAP. The different residues in the murine sequence are shown in black. The grey spheres represent the calcium atoms bound in the ligand binding pocket.

The structure comprising the epitope recognised by SAP-5 is shown in white.
The conformational epitope recognised by Abp1 is highlighted in black.

11. Efficacy of monoclonal mouse IgG2a anti-human SAP antibody

AA amyloidotic wild type mice loaded with human SAP were used and amyloid scored 17 days after injection of anti-SAP antibodies.

| Group (treatment, group size) | Spleen       | Liver        |
|------------------------------|--------------|--------------|
| 1 (negative control mouse IgG2a, n=8) | 4.08, 1.5-4.50 | 2.42, 2.0-2.67 |
| 2 (7 mg sheep polyclonal IgG anti-human SAP antibody, n=5) | 1.17, 1.0-1.5 | 1.0, 0.67-1.17 |
| 3 (1 mg monoclonal mouse IgG2a anti-human SAP antibody, Abp1, n=10) | 3.5, 2.83-4.5 | 1.83, 1.0-2.83 |
| 4 (5 mg monoclonal mouse IgG2a anti-human SAP antibody, Abp1, n=10) | 1.25, 1.0-2.0 | 1.0, 1.0-1.33 |

Kruskal-Wallis test: spleen, P<0.001; liver P<0.001.

Dunn's multiple comparison test: 1 vs 2, spleen, P<0.01; liver, P<0.01; 1 vs 3, spleen, not significant P>0.05; liver, not significant P>0.05; 1 vs 4, spleen, P<0.001; liver, P<0.001; 2 vs 3, spleen, P<0.05; liver, not significant P>0.05; 2 vs 4, spleen, not significant P>0.05; liver, not significant P>0.05; 3 vs 4, spleen, P<0.01; liver, P<0.05.
Comparison of two different monoclonal mouse IgG2a anti-human SAP antibodies

AA amyloidotic wild type mice loaded with human SAP were used and amyloid scored 21 days after injection of anti-SAP antibodies. The sum of the scores for spleen and liver were analysed.

| Group                  | Spleen plus liver amyloid score |
|------------------------|---------------------------------|
|                        | (treatment, no. of mice)         | median, range          |
| C (negative control, PBS only) | 6.81, 4.25-8.0                  |
| A5 (Abp1 5 mg, n=5)     | 2.25, 2.25-2.5                   |
| A3 (Abp1 3 mg, n=10)    | 2.81, 2.0-4.25                   |
| A1 (Abp1 1 mg, n=10)    | 5.63, 4.0-6.5                    |
| S5 (SAP-5 5 mg, n=5)    | 2.0, 1.5-2.38                    |
| S3 (SAP-5 3 mg, n=10)   | 2.5, 2.0-5.0                     |
| S1 (SAP-5 1 mg, n=10)   | 3.38, 2.5-5.63                   |

Kruskal-Wallis test: P<0.0001.

Dunn's multiple comparison test: A5 vs S5, P>0.05; A3 vs S3, P>0.05; A1 vs S1, P>0.05; A5 vs A3, P>0.05; A5 vs A1, P<0.05; A3 vs A1, P<0.05; S5 vs S3, P>0.05; S5 vs S1, P>0.05; S3 vs S1, P>0.05; A5 vs C, P>0.05; S5 vs C, P=0.015; A3 vs C, P<0.015; A1 vs C, P>0.05.

Mann-Whitney tests, with Bonferroni correction for multiple comparisons, give some notably different results: A5 vs S5, P>0.5; A3 vs S3, P>0.5; A1 vs S1, P=0.015; A5 vs A3, P>0.5; A5 vs A1, P=0.015; A3 vs A1, P<0.015; S5 vs S3, P>0.5; S5 vs S1, P=0.015; S3 vs S1, P=0.06; A5 vs C, P=0.015; S5 vs C, P=0.015; A3 vs C, P<0.015; S3 vs C, P<0.015; A1 vs C, P>0.5; S1 vs C, P<0.015.

12. Comparison of mouse monoclonal IgG1 and IgG2a anti-human SAP antibodies

Circulating human SAP was depleted by giving CHPDC to AA amyloidotic human SAP transgenic mouse SAP deficient mice and they then received a single intraperitoneal dose of the anti-human SAP antibodies shown in the Table below. The sheep antibody dose was
50 mg of the whole IgG fraction containing 7 mg of anti-SAP activity; the monoclonal antibodies were 5 mg of pure anti-SAP antibody each. The different numbers of mice in the various groups given monoclonal antibodies reflect availability of the respective antibodies. Splenic amyloid deposits were then quantified histologically by a single observer 21 days after the antibody injection. Only one of the monoclonal antibodies in this panel, the IgG2a SAP-5, was effective in eliminating amyloid deposits, despite several of the IgG1 antibodies having similar affinity and avidity. The ineffective IgG2a antibody was less avid than SAP-5.

| Anti-human SAP antibody treatment | Antibody isotype | Amyloid score median, range |
|----------------------------------|-----------------|-----------------------------|
| None (n=15)                      |                 | 3, 3-5                      |
| Sheep polyclonal (n=10)          | NA              | 1, 1-1                      |
| Monoclonal SAP-1 (n=7)           | IgG1            | 3, 3-4                      |
| Monoclonal SAP-2 (n=6)           | IgG2a           | 3, 2-4                      |
| Monoclonal SAP-3 (n=7)           | IgG1            | 4, 2-4                      |
| Monoclonal SAP-4 (n=1)           | IgG1            | 4                           |
| Monoclonal SAP-5 (n=5)           | IgG2a           | 1, 1-1                      |
| Monoclonal SAP-6 (n=1)           | IgG1            | 2                           |
| Monoclonal SAP-7 (n=7)           | IgG1            | 3, 2-4                      |

13. Lack of toxicity of anti-SAP antibodies in human SAP transgenic mice

Circulating human SAP was depleted by giving CPHPC at 1 mg/ml in the drinking water to C57BL/6 SAP deficient mice transgenically expressing human SAP, which then received a single intraperitoneal dose of either Abp1 mouse monoclonal anti-human SAP antibody or an unrelated mouse IgG2a monoclonal antibody. Two animals from each group were killed 2 days later and 3 more were killed on day 10. Plasma was assayed for sodium, potassium, chloride, urea, creatinine, calcium, phosphate, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, total protein, albumin, total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, glucose, total bilirubin, lactate dehydrogenase,
amyrase and creatine kinase. All values were within normal limits in all animals and there were no differences between mice receiving the anti-SAP or control IgG. Histology of H&E stained sections of heart, lung, liver, kidney, spleen, skeletal muscle, small bowel and colon from all animals was normal apart from occasional inflammatory foci and rare reactive lymphoid follicles in the small bowel, with no difference in number or intensity between the two treatment groups. Presumably the SAP in normal extracellular matrix structures is not accessible to the antibody in vivo and/or is not sufficiently abundant to support adverse events.

Materials and Methods

Binding of monoclonal antibodies to human SAP covalently immobilised on NHS activated microtitre plates (Costar, Corning, NY) or captured by calcium dependent binding to Aβ amyloid fibrils immobilised on hydrophobic microtitre plates (Greiner bio-one, Germany) was characterised using 125I-labelled goat anti-mouse IgG (Dako, Denmark) or anti-mouse IgG2a (AutogenBioclear, UK). Binding of monoclonal antibodies to human SAP immobilized on the CM5 sensorchip by standard amine coupling was measured in the BIAcoreX (Pharmacia Biosensor AB) at 6 different concentrations injected at 5μl/min in 10 mM HEPES, 150mM NaCl, 3mM EDTA, pH 7.4 at 25°C. Sheep F(ab)2 was produced by pepsin digestion of IgG at 1:50 for 3 h at 37°C and pH 4.0. Serial 6μ cryosections of spleen and liver were post-fixed, permeabilized, quenched for endogenous peroxidase/tissue biotin activity, and immunostained with primary and secondary antibodies using either avidin-biotin-peroxidase (VectaStain ABC/Elite; Vector Laboratories Ltd, UK) or peroxidase-anti peroxidase (Dako UK Ltd). Peroxidase activity was visualised with metal enhanced diaminobenzidine (Thermo Fisher Scientific UK) and sections counterstained with haematoxylin. Primary antibodies were monoclonal anti-murine CD68 and F4/80 (AbD Serotec) and monospecific polyclonal sheep anti-mouse C3, anti-human SAP and anti-mouse SAA raised by immunization with the respective isolated pure antigens. Secondary antibodies were polyclonal anti-rat IgG (Jackson Immunoresearch Europe), anti-goat IgG (Sigma-Aldrich) or anti-rabbit IgG (Dako). No staining was observed in control sections processed with omission of either the primary or secondary antibody. Separate adjacent cryo sections were stained with alkaline alcoholic
Congo red. Sections were viewed using the DMR XA2 microscopy system controlled via QWIN Pro (Leica Microsystems UK Ltd) and representative images captured using a DFC 300FX digital camera (Leica). For laser scanning confocal microscopy, unfixed frozen sections were processed as described above but were stained with Alexa 488 labelled anti-rabbit IgG and Alexa 546 labelled anti-rat IgG secondary antibodies (Invitrogen, Paisely, UK). Z-series data were acquired using a Zeiss LSM 510 Meta confocal microscope with a 63 x 1.4 NA oil immersion objective. Individual confocal sections were collected to peak fluorescence intensity using separate excitation with 405, 488 and 543 nm laser lines. Data was rendered into 3D using Volocity 3.0 (Improvision, UK). Except where otherwise indicated, amyloid load was scored independently in each Congo red stained section of formalin fixed tissue by 3 expert observers blinded to treatments given and identities of the mice. The scales described in Fig. 1 of the paper were used and results for each section were extremely consistent between observers, never differing by more than one grade. The mean of the 3 values for each section were then used for analysis.

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