Supplemental data

Materials and methods

Expression and purification of recombinant COMP

For production of recombinant COMP proteins, a full-length cDNA for human COMP (IMAGE:1992316) was obtained from the IMAGE consortium (1). Although this clone was assigned as similar to human thrombospondin-4 in GenBank (AI369019), nucleotide sequencing confirmed that the cDNA is identical to nucleotides 12-2467 of the human COMP reference sequence GI:40217842, with one base difference (A replacing G2402 of GI:40217842) in the 3' UTR of the cDNA. The cDNA was PCR amplified using *Pfu* DNA polymerase and primers that introduced an in-frame *HindIII* restriction site immediately upstream of the initiating ATG codon (5'-TATAAGCTTCAGGGCCAGAGCCCG-3'), EGF repeat 1 (5'-TATAAGCTTGGGATGCAGCAGTCAG-3'), TSP3 repeat 1 (5'-TATAAGCTTCGCGACACTGACCTAGAC-3'), TSP3 repeat 8 (5'-TATAAGCTTGACAACTGCCGCCTGG-3') or the C-terminal globular domain (5'-TATAAGCTTGACGTGTGTCCGGAGAACG-3') in combination with primers that introduced a stop codon and a *XhoI* restriction site immediately downstream of the C-terminal globular domain (5'-TATCTCGAGCTAGGCTTGCCGCAGC-3'), TSP3 repeat 8 (5'-TATCTCGAGCTAGATCTTGTCTACCACC-3') or EGF repeat 4 (5'-TATCTCGAGCTAACCACAGAGGATCCC-3'). After digestion with *HindIII* and *XhoI,* the PCR products were ligated into the corresponding sites of the mammalian episomal expression plasmid pCEP4-BM40-hisEK (2) and the accuracy of sequences was verified through nucleotide sequencing. This resulted in expression constructs for the following hexahistidine tagged recombinant COMP fragments; pCOMP (full

length pentameric COMP), mCOMP (monomeric COMP, i.e. EGF repeat 1 to C-terminus), TIII1-CG (TSP3 repeat 1 to C-terminus), TIII8-CG (TSP3 repeat 8 to C-terminus), CG (C-terminal globular domain), EGF1-4 (EGF repeats 1-4), TIII1-8 (TSP3 repeats 1-8), as depicted in Fig. 4. After transfection of the expression vectors into 293-EBNA cells (Invitrogen) and selection with hygromycin B (PAA laboratories), serum-free DMEM/F12 cell culture medium with Glutamax-1 (Invitrogen) was harvested and recombinant proteins were concentrated by ultrafiltration (PM10 membrane, Amicon) and purified using HisTrap nickel chelation affinity chromatography (GE-Healthcare) followed by MonoQ ion exchange chromatography (GE Healthcare), as previously described (3). The multimeric state of pCOMP was verified through SDS-PAGE, where it migrated as a high molecular mass band just entering the separation gel under non-reducing conditions and as a sharp 120 kDa band under reducing conditions (not shown).

Inhibition of C1-assembly

C1q was coated onto microtiter plates at a concentration of 5 μg/ml in 75 mM sodium-carbonate buffer, pH 9.6 overnight at +4°C. The plates were blocked with 1% BSA in PBS for 1 h at 37°C. pCOMP or BSA diluted in $DGVB^{2+}$ were added at increasing concentrations to the plates followed by incubation for 1 h at 37°C. After washing away unbound protein, a mixture of C1r (4 μg/ml), C1s (2 μg/ml) and trace amounts of ^{125}I-C1s in $DGVB^{2+}$ was added and incubation was continued for 1 h at 37°C. The plates were washed and the amount of radioactivity bound to the wells was measured with a gamma-counter.

Dissociation of the C1-complex

Microtiter plates were coated with aggregated human IgG (4 μg/ml) in 75 mM sodium-carbonate buffer, pH 9.6 overnight at +4°C. The wells were blocked with 1% BSA in PBS to prevent unspecific interactions. The C1-complex was assembled by incubating C1r (4 μg/ml), C1s (2 μg/ml), C1q (10 μg/ml) and trace amounts of ^{125}I-C1s in $DGVB^{2+}$ for 90 min at RT after which the associated complex was allowed to attach to the IgG coated wells. After washing away any unbound complex, pCOMP, C1-inhibitor or BSA diluted in $DGVB^{2+}$ was added to the wells at increasing concentrations and incubation was continued for 1 h at RT. The wells were washed and the amount of radioactivity remaining in the wells was measured with a gamma-counter.

References

1. Lennon, G., Auffray, C., Polymeropoulos, M., & Soares, M. B. (1996) The I.M.A.G.E. Consortium: an integrated molecular analysis of genomes and their expression. *Genomics* 33**,** 151-152.
2. Bengtsson, E*., et al.* (2000) The amino-terminal part of PRELP binds to heparin and heparan sulfate. *J Biol Chem* 275**,** 40695-40702.
3. Day, J. M*., et al.* (2004) Alternative splicing in the aggrecan G3 domain influences binding interactions with tenascin-C and other extracellular matrix proteins. *J Biol Chem* 279**,** 12511-12518.

Table SI. COMP and COMP-C3b concentrations in patients and controls*.

	Serum COMP (U/l)	Synovial fluid COMP (U/l)	Serum COMP-C3b (AU)	Synovial fluid COMP-C3b (AU)	Age (years)	Sex (F:M)	ESR mm/h	SF-leukocytes
RA	10.4 (5.2-21.6)	55.6 (13.7-180.7)	5.6 (3.5-6.9)	3.1 (0.7-5.8)	64 (25-83)	10:7	42 (10-100)	11 (0.2-55)
OA	10.4 (2.7-19.8)	155.6 (41.6-315.7)	0.7 (0.5-4.2)	2.7 (2.0-3.9)	61 (38-66)	8:7	nd	nd
controls	10.5 (8.1-12.0)	nd	0.9 (0.7-3.5)	nd	60.5 (45-72)	8:6	nd	nd

* The four OA patients presented in the inset in Fig. 6C are not included in the table.

The data are presented as median and range. AU, arbitrary units; ESR, erythrocyte sedimentation rate; SF-leukocytes, synovial fluid leukocytes; nd, not determined

Supplemental Figure 1

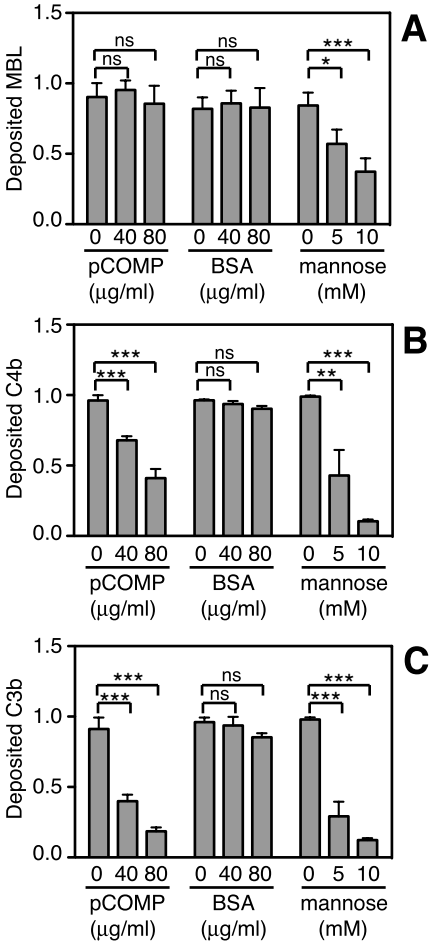

FIGURE S1. COMP inhibits the lectin pathway of complement. Microtiter plates were coated with 100 µg/ml mannan to activate the lectin pathway and NHS pre-incubated with increasing concentrations of pCOMP was added. BSA and D(+)-mannose were added to NHS as negative and positive controls, respectively. Deposition of MBL (A), C4b (B) and C3b (C) was detected with specific antibodies. The data were normalized by setting the highest obtained absorbance of each plate to 1 and are given as the mean and SD of three separate experiments. Statistical significance of differences was calculated with a two-way Anova. ns, not significant, *, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$.

Supplemental Figure 2

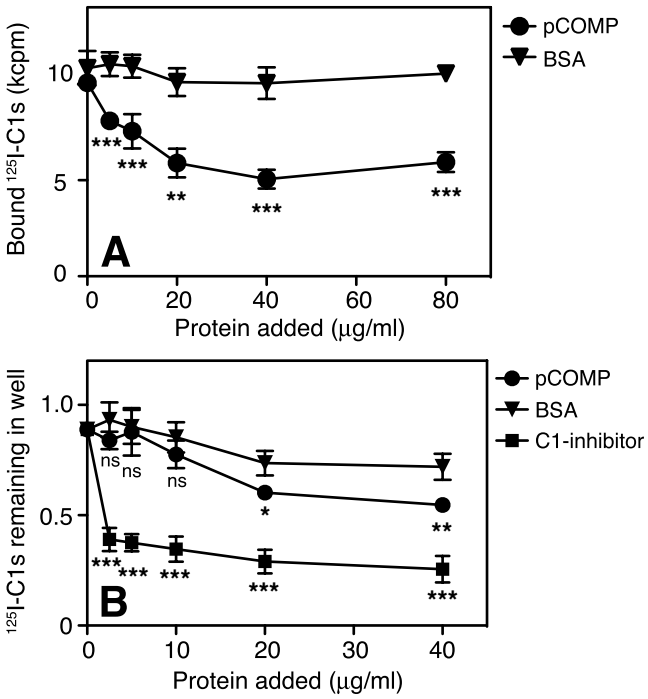

FIGURE S2. COMP inhibits the assembly of the C1-complex. C1q was immobilized on a microtiter plate at a concentration of 5 µg/ml and increasing amounts of pCOMP or BSA were allowed to attach. A mixture of pre-incubated C1r, C1s and trace amounts of ^{125}I-C1s was added to the plate and the amount of bound radioactivity was measured. (A). Aggregated human IgG was coated onto microtiter plates at 4 µg/ml. A C1-complex was formed by pre-incubating C1q, C1r, C1s and trace amounts of ^{125}I-C1s, after which the complex was added to the plate. BSA, C1-inhibitor or pCOMP was added in order to study dissociation of the complex and the radioactivity remaining in the well was measured (B). Data are given as the mean and SD of three separate experiments. The data in panel B were normalized by setting the highest obtained radioactivity count of each plate to 1. Statistical significance of differences was calculated with a two-way Anova by comparing the mean values of pCOMP or C1 inhibitor to that of BSA at a specific protein concentration. ns, not significant, *, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$.