SUPPLEMENTARY MATERIALS

Methods

Expression and purification of His-tagged recombinant Ag85A, B and C: Ampicillin-resistant plasmids pQE-80L_Ag85A and pQE-80L_Ag85B were transformed in either *E. coli* T7 Express (NEB) or Rosetta 2 (Merck). Positive transformants were cultivated in 2× M9 medium supplemented with 1 g/l [^{15}N]-NH_4Cl, 5 g/l glucose, 400 µl/l BME vitamins (Sigma), trace metal mix and under selection of carbenicillin (60 µg/ml) and chloramphenicol (34 µg/ml) at 37°C. Induction was initiated at an optical density of 1 at 600 nm with 1 mM isopropyl-β-D-thiogalactopyranoside and continued over night. Bacteria were resuspended in lysis buffer [50 mM Tris HCl pH 8.0, 500 mM NaCl, 5 mM Mg_2SO_4, Complete EDTA-free protease inhibitors (Roche), benzonase (Merck), according to manufacturers' instructions]. After disruption of bacteria (EmulsiFlex-C3-Avestin), lysates were cleared by centrifugation (20,000 *g*, 30 min, 4°C) and filtration (0.45 µm pore size). His-tagged protein was bound to a metal chelating column (MC-Poros, Applied Biosystems), washed with 20 mM Tris HCl, pH 8.0, 300 mM NaCl, 5 mM imidazole and eluted by a linear imidazole gradient from 5 to 500 mM in 20 mM Tris HCl, pH 8.0, 300 mM NaCl.. Protein fractions were pooled, concentrated, and purified to homogeneity by gel filtration (Superdex 75, 120 ml, GE Healthcare) using PBS (pH 7.0), 1 mM DTT as equilibration and running buffer. Fractions were pooled, concentrated and buffer was exchanged by PD10 columns, or standard dialysis if proteins

were analyzed by NMR. Yields for Ag85A and Ag85B per liter of bacterial culture were 7 mg and 15 mg, respectively.

The expression of Ag85C in *E. coli* BL21(DE3) pLysS (Merck) was done according to Scheich et al. (39) at 17°C without the detergent CHAPS in the purification buffers, yielding almost insoluble product and about 1.5 mg soluble Ag85C per liter of culture . Expression of Ag85C was then tried at 30°C in *E. coli* BL21(DE3) pG-Tf2 (Takara) allowing for anhydrotetracycline (0.2 µg/ml)-induced chaperone expression prior to induction with 1 mM isopropyl-ß-D-thiogalactopyranoside. The protocol described above was followed for culture and purification. However, lysis buffer was additionally supplemented by 1% glycerol, 0.2 mM DTT and 0.1% 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS). After affinity chromatography, Ag85C fractions were adjusted with 5 mM ATP, 20 mM Mg_2SO_4 and 5 mM DTT to release sticky chaperones. After a final gel filtration step the yield of 7 mg per liter of culture was 5 times higher then with BL21(DE3) pLysS as expression host.

Cytotoxicity assay with macrophages: Mouse primary macrophages (3×10^5/well) were plated in 48-well plates in complete Dulbecco's Modified Eagle's Medium (DMEM, Gibco) with 10% heat-inactivated fetal calf serum (Gibco), 5% horse serum (Gibco), 0.2 mM L-glutamine (Gibco), 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES, PAA), 1 mM sodium pyruvate (Biochrom AG), and incubated overnight at 37°C. Compounds were added to the wells in triplicates for each concentration, and the cells were incubated at 37°C for 48 and 96 h. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was added to each well for a final concentration of

50 μg/ml, 3 h prior to each time point. MTT is converted to purple formazan by respiratory enzymes in live cells. After 3 h of incubation at 37°C, the cells were lysed in 100 μl of 100% dimethylsulfoxide (DMSO) to allow the release of formazan. Absorbance at 550 nm was measured with a spectrophotometer. The viability is expressed in % with untreated samples taken as 100%.

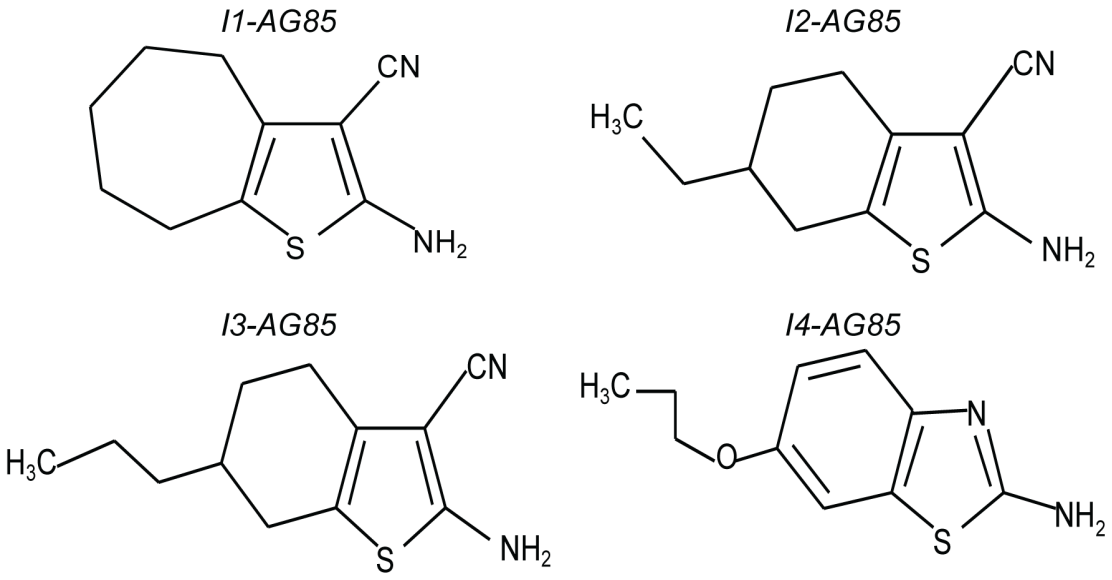

Fig. S1. Chemical structures of Ag85C antagonists.

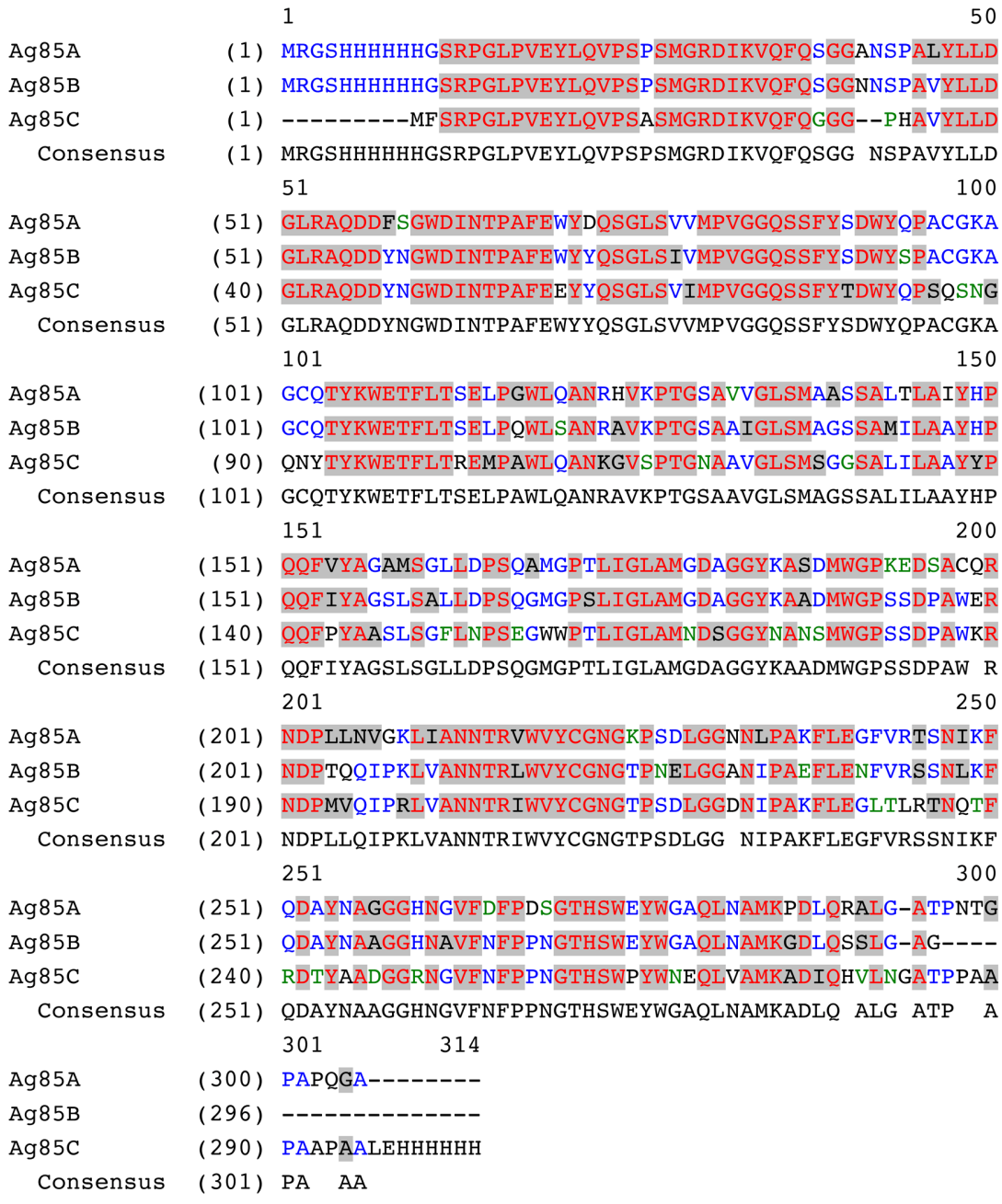

Fig. S2. Alignment of the amino acid sequences coded by the Ag85A, B, and C constructs used in this work.

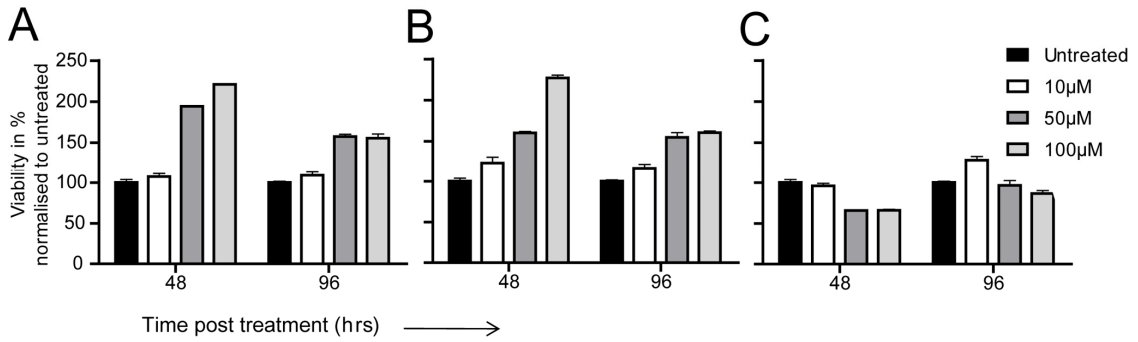

Fig. S3. ***I2-, I3- and I4-AG85*** **are non-toxic to primary macrophages.** Compounds were added at 10, 50 and 100 μM concentrations to macrophages obtained by differentiation of primary bone marrow-derived monocytes of mice. After 48 or 96 h of exposure, the MTT assay was performed as described above. Viability was normalized to untreated controls, which was taken as 100%. (A) *I2-AG85*, (B) *I3-AG85,* and (C) *I4-AG85*. Mean ± SD of three samples obtained from one representative experiment out of two, is shown.

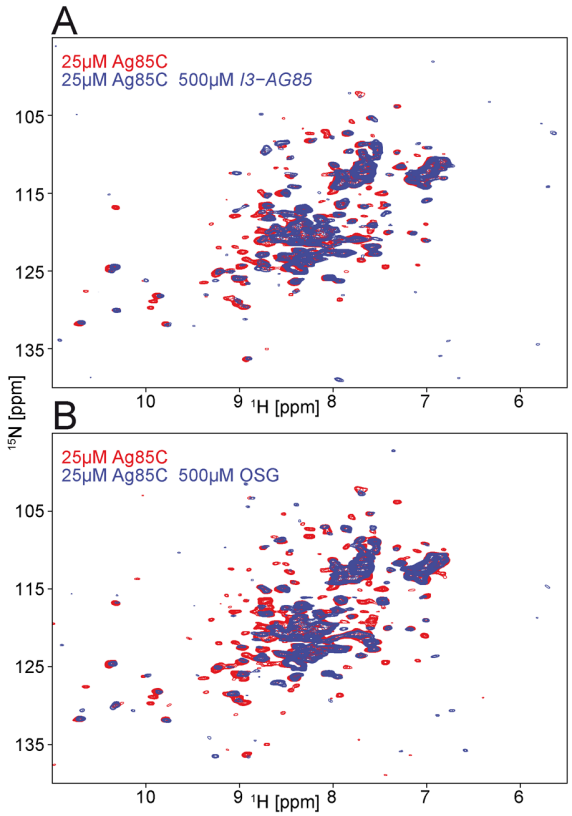

Fig. S4. *I3-AG85* and OSG bind to Ag85C in physiological condition. NMR spectra of Ag85C overlaid with (A) Ag85C and 20-fold molar concentration of *I3-AG85* and (B) Ag85C and 20-fold molar concentration of OSG in PBS at pH 7.0 with 0.5% DMSO.

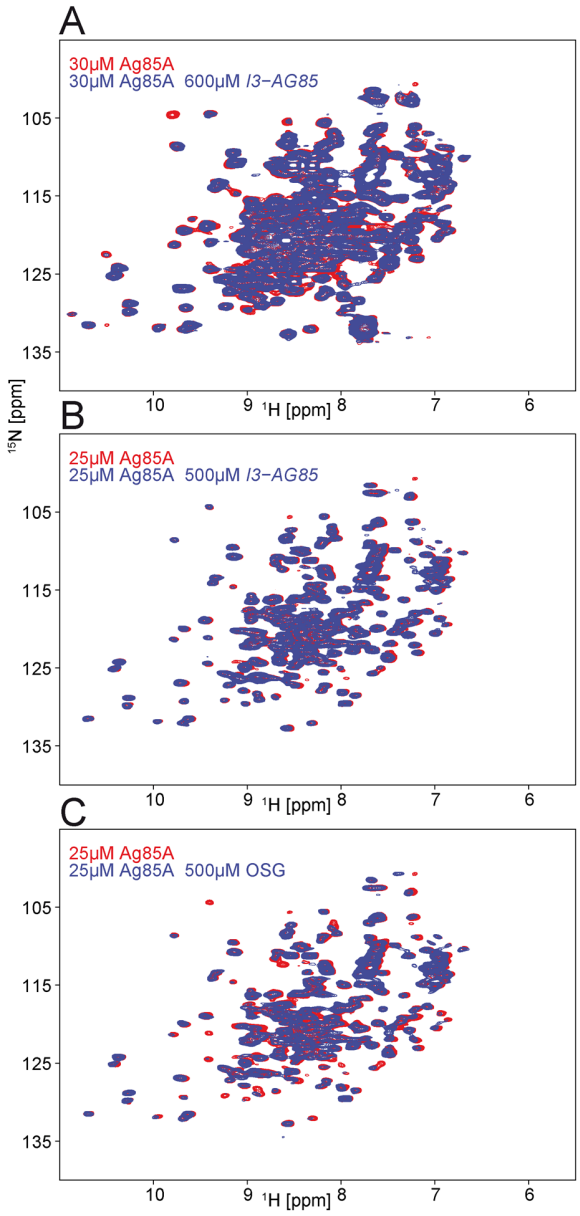

Fig. S5. *I3-AG85* and OSG binding studies with Ag85A. NMR spectra of Ag85A overlaid with (A) Ag85A and 20-fold molar concentration of *I3-AG85* in 5 mM citrate buffer pH 6.0, 1 mM DTT and 0.1% CHAPS, 5% DMSO, (B) Ag85A and 20-fold molar concentration of *I3-AG85* in PBS buffer at pH 7.0 with 0.5% DMSO, (C) Ag85A and 20-fold molar concentration of OSG in PBS at pH 7.0 with 0.5% DMSO.

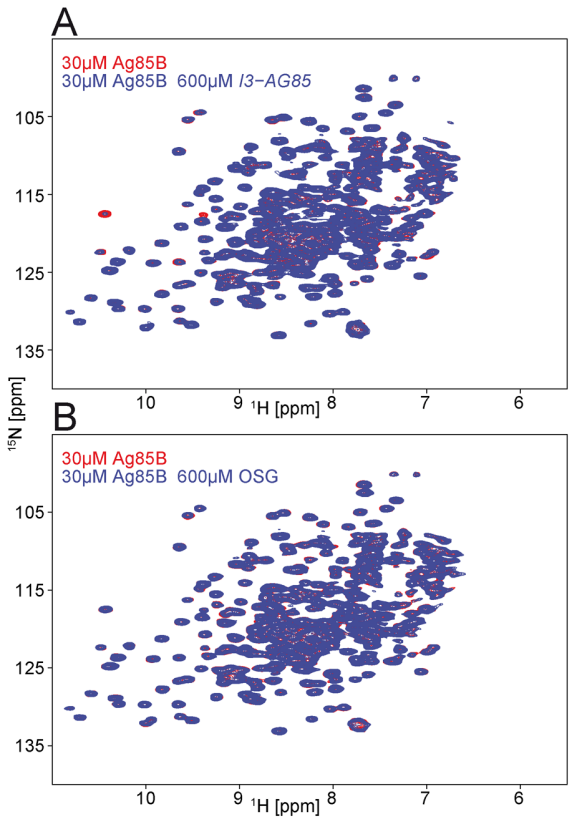

Fig. S6. Ag85B does not bind to *I3-AG85* and OSG. NMR spectra of Ag85B overlaid with (A) Ag85B and 20-fold excess of *I3-AG85* and (B) Ag85B and 20-fold excess of OSG both in 5 mM citrate buffer pH 6.0, 1 mM DTT and 0.1% CHAPS.