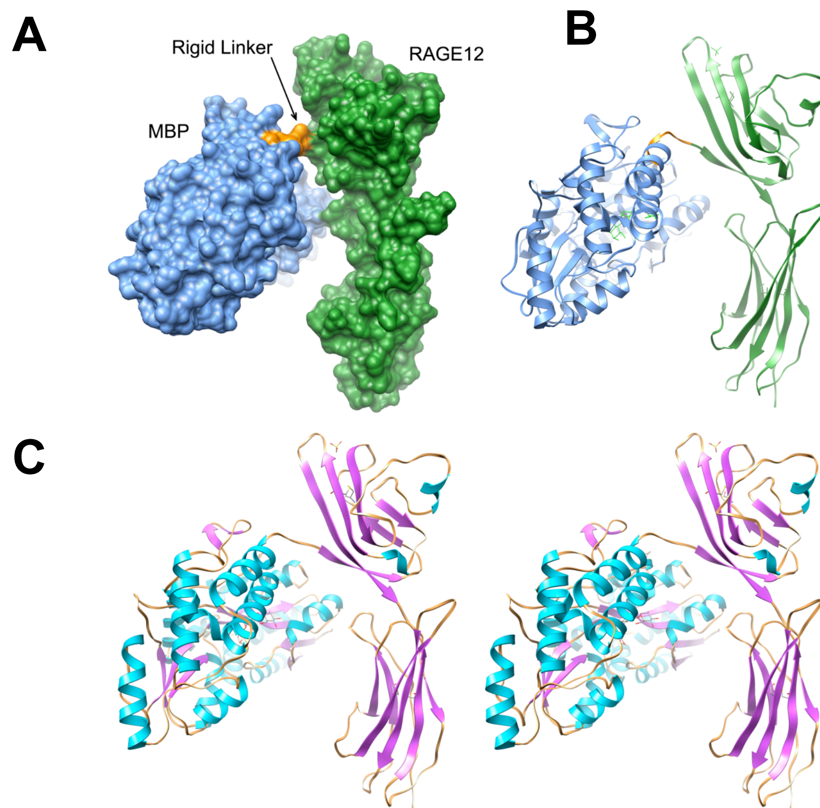
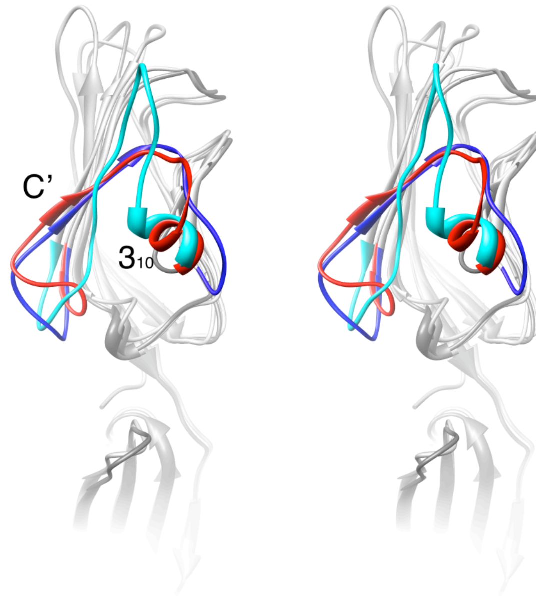
Supplementary Figure 1



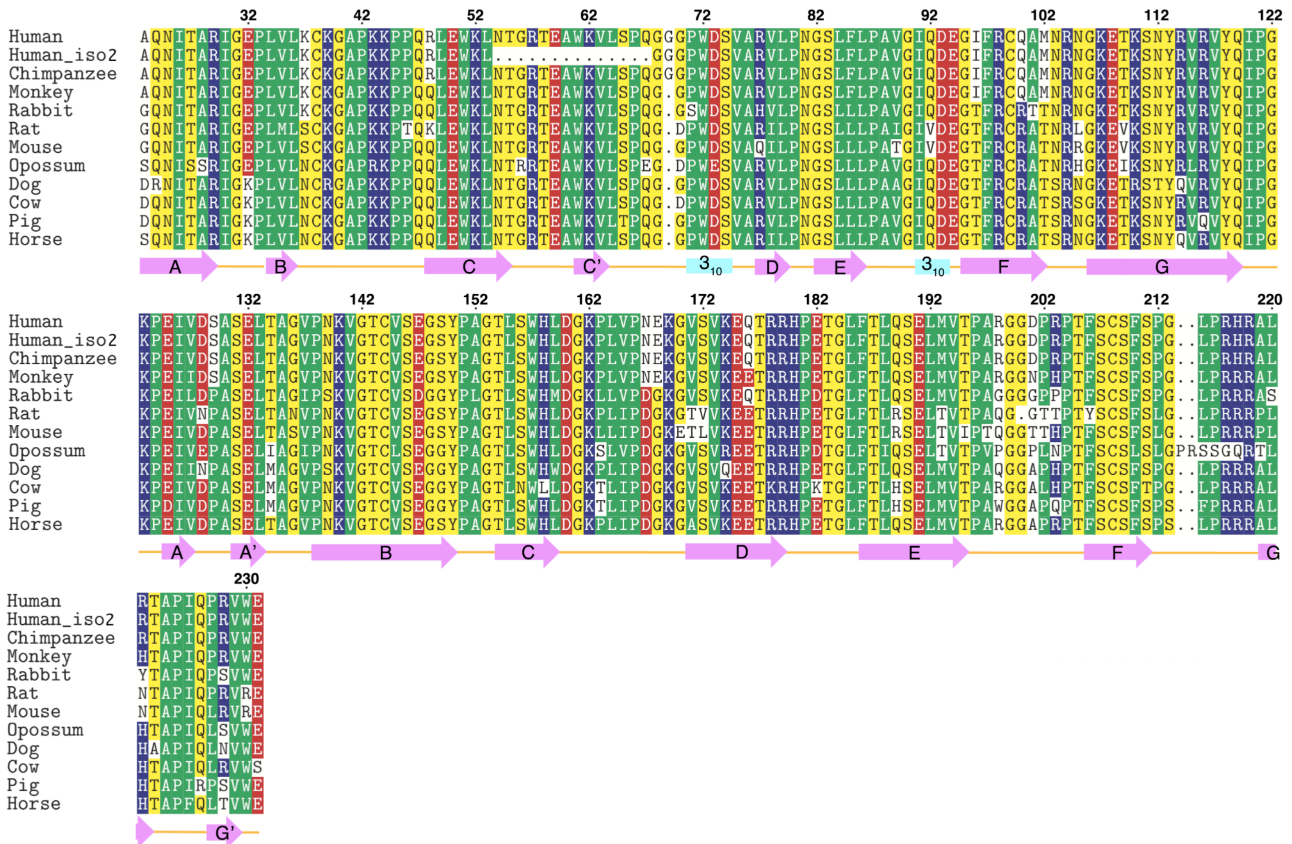
Supplementary Figure 1 Overall structure of MBP-RAGE12 fusion protein in (A) surface model and (B) ribbon diagram. MBP is colored blue, RAGE12 is colored green, and the linker region is colored orange. (C) Stereo view showing ribbon digram of MBP-RAGE12. All views are in same orientation. Strands, helices, and loops are colored magenta, cyan, and orange, respectively.

Supplementary Figure 2



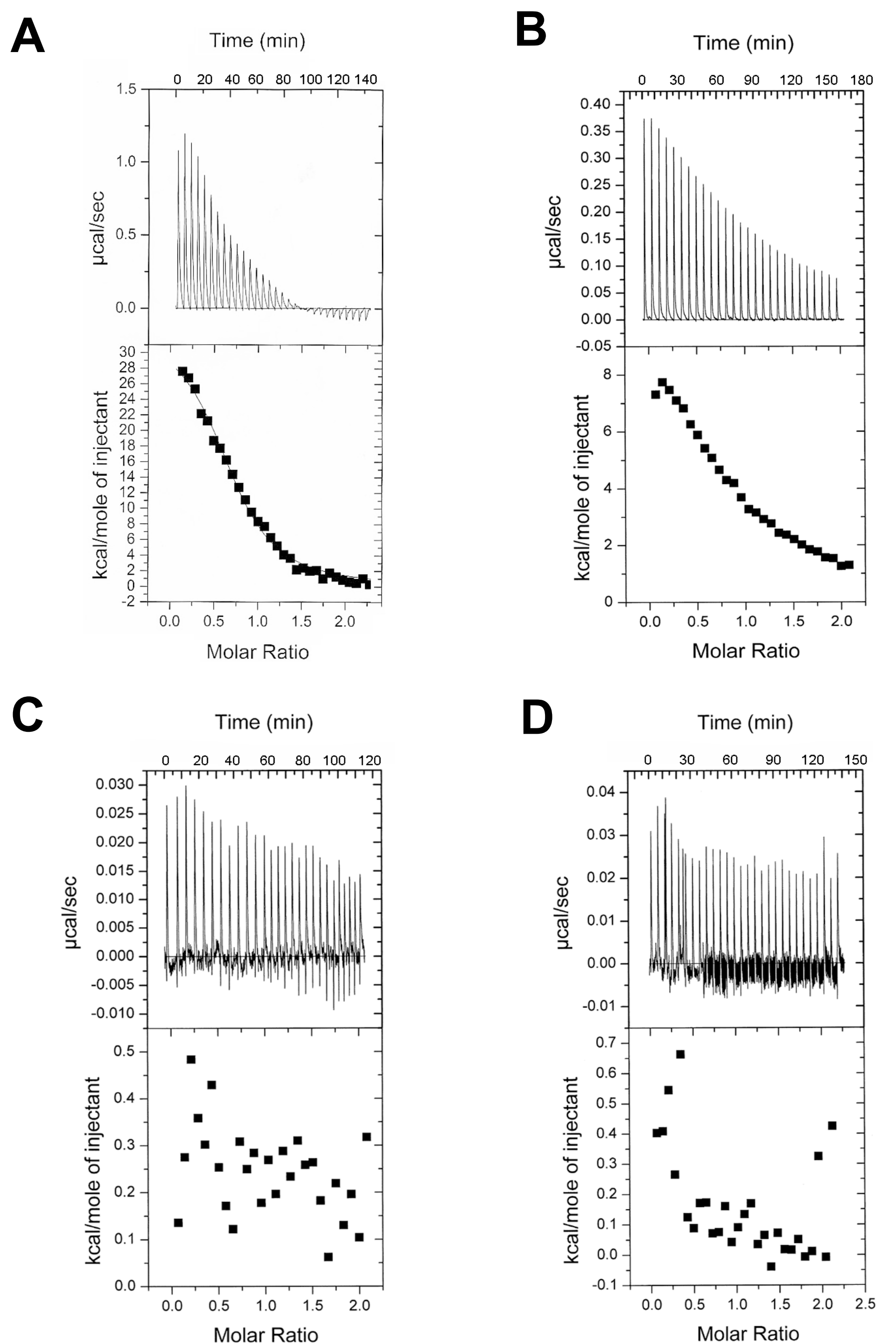
Supplementary Figure 2 Stereoview showing ribbon diagrams of superposed models of RAGE domain 1. RAGE12 is in red, PDB entry 3CJJ is in blue, and PDB entry 2E5E is in cyan. Only residues 54 to 74 are highlighted and the rest of the molecules are colored grey. The view is in the same orientation as shown in right panel of Figure 1.

Supplementary Figure 3



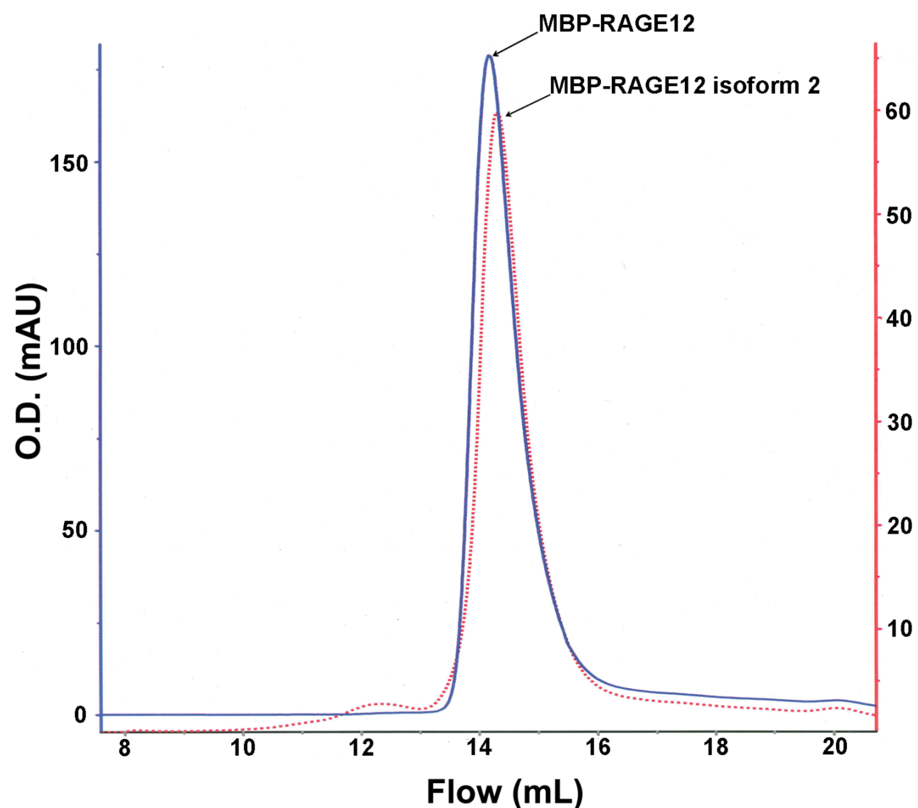
Supplementary Figure 3. Multiple amino acid sequence alignment of all known RAGE orthologs. Regions equivalent to human RAGE12 (amino acid 23 to 231) are shown and the residue numbers of human RAGE is labeled. Conserved acidic, basic, uncharged polar, and hydrophobic residues are colored red, blue, yellow, and green, respectively. The sequence alignment and the figure production were performed in Biology WorkBench (http://workbench.sdsc.edu/). The secondary structure is indicated below each line with pink arrows representing b-strands and light blue rectangles representing helices.

Supplementary Figure 4



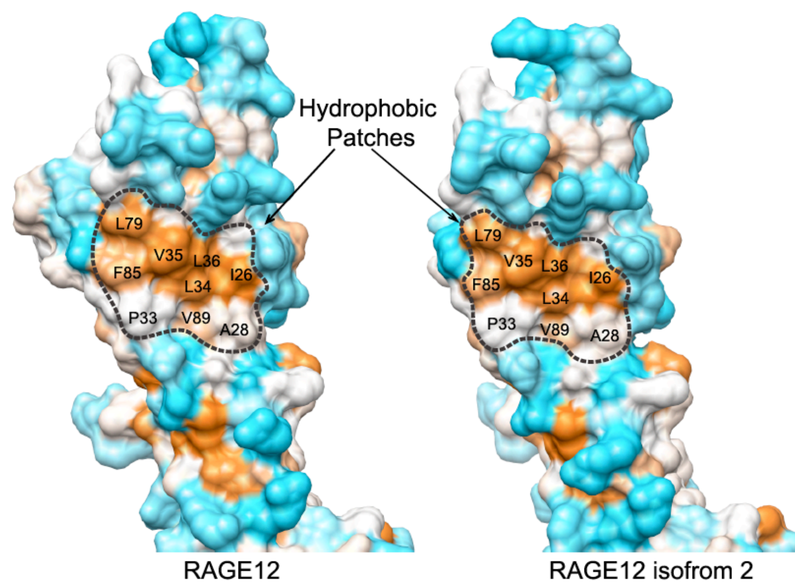
Supplementary Figure 4. The ITC binding curves measured for RAGE12+Ca^{++} (A), MBP-RAGE12+Ca^{++} (B), MBP-RAGE12 isoform 2+Ca^{++}(C), MBP-RAGE12+EDTA (D). Top panels: peak shows the heat absorbed by injection. Bottom panels: binding isotherm generated from the peak integration with the respect of time with dilution correction.

Supplementary Figure 5



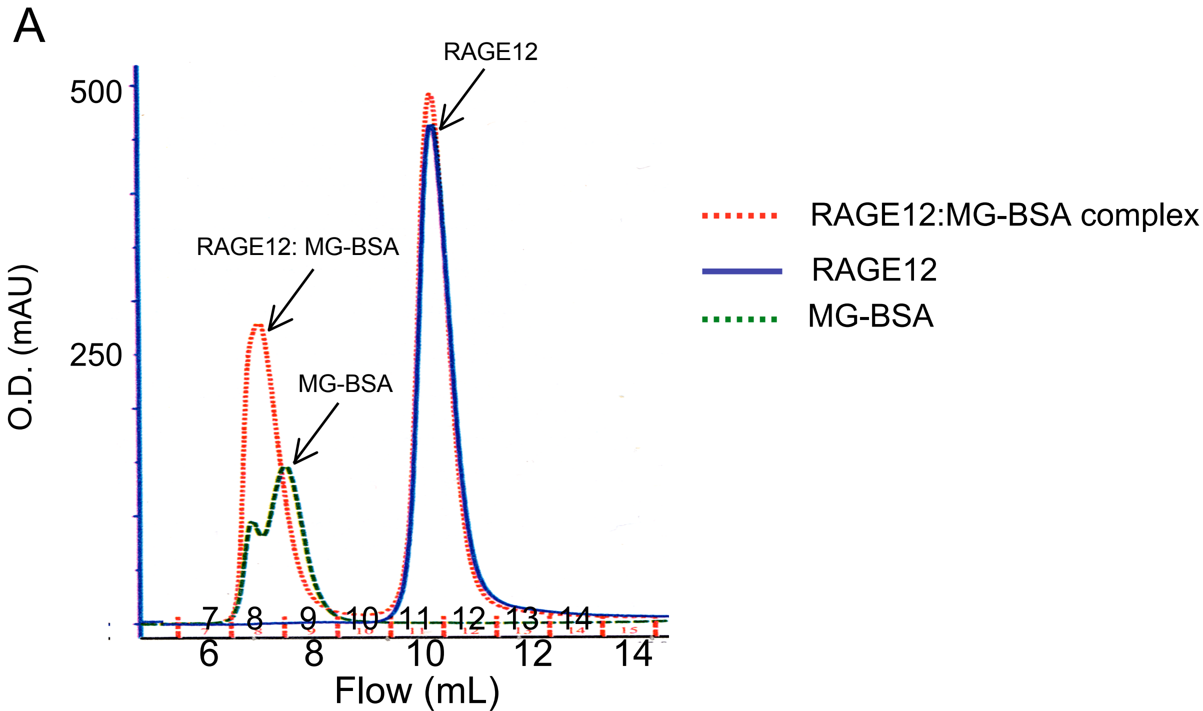
Supplementary Figure 5. Size exclusion chromatography profile comparison between MBP-RAGE12 (blue) and MBP-RAGE12 isoform 2 (dotted red). Purified protein samples were run on a Superdex 200 10/300 GL (GE Healthcare) column that was pre-equilibrated with 150 mM NaCl, 10 mM TrisHCl pH 7.5. The peaks correspond to apparent molecular weights of approximately 64 kDa (Ve = 14.2 mL). The x-axis represents flow that passed through the column and the y-axis represents absorbance at 280 nm for MBP RAGE12 (left) and MBR-RAGE12 isoform 2 (right)

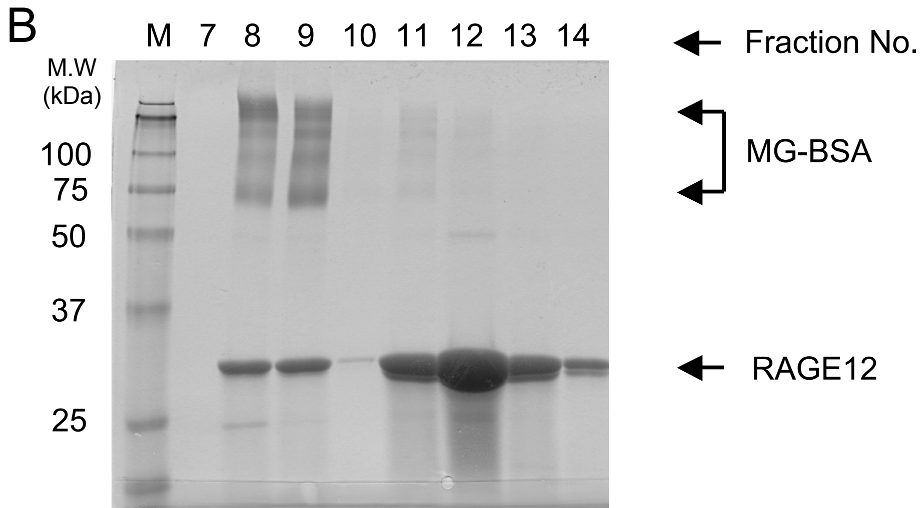
Supplementary Figure 6



Supplementary Figure 6. The hydrophobic patch on the DEB face of domain 1 in wild type RAGE (left) and isoform 2 (right). The coloring scheme of hydrophobicity is same as Figure 2. Dotted circles on each molecular surface define the hydrophobic patches generated on DEB face. Select hydrophobic residues are labeled. The hydrophobic patch is well preserved in the model structure of RAGE isoform 2. Only the core region of the hydrophobic patch is encircled for comparison purpose.

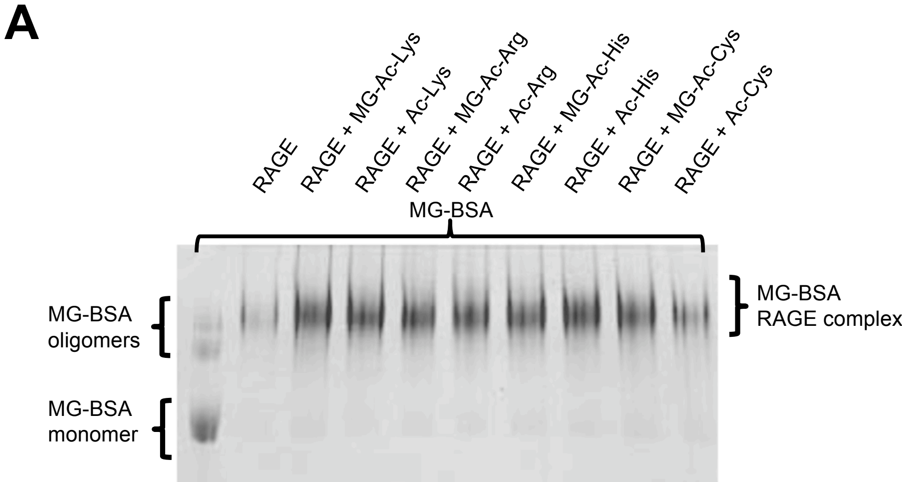
Supplementary Figure 7

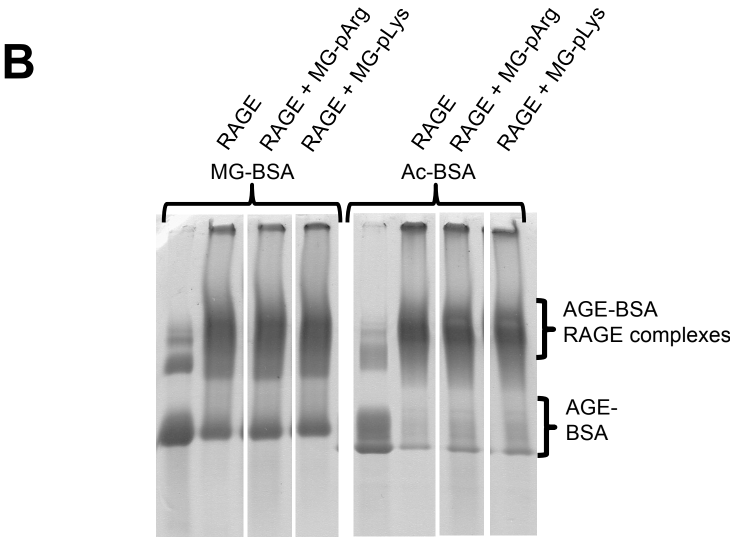


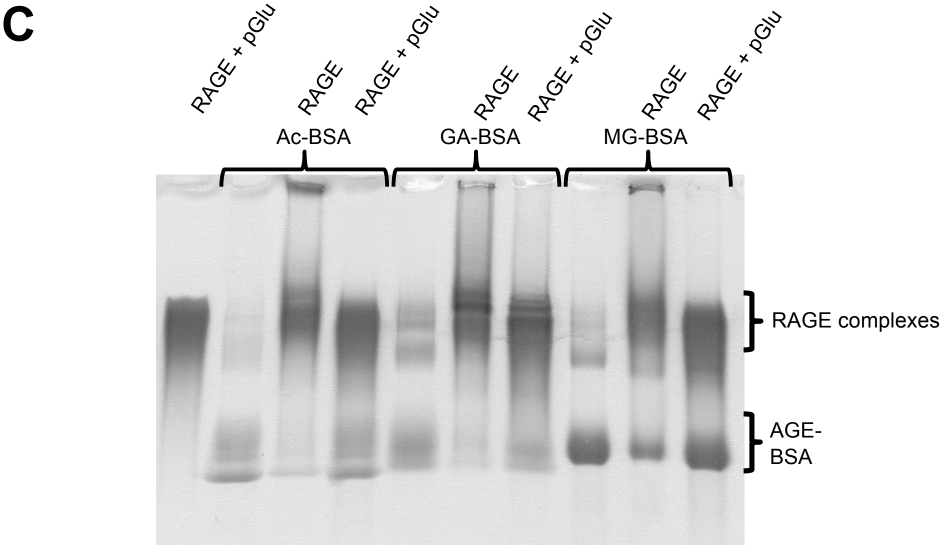


Supplementary Figure 7. RAGE recognition of MB-BSA demonstrated by size exclusion chromatography. (A) Purified RAGE12 (350 µM) was incubated with (red dotted line) and without (blue line) 35 µM of MG-BSA and run on a Superdex75 10/300 column (GE Healthcare) which was equilibrated with 150 mM NaCl, 10 mM Tris-HCl, pH 7.5. Total injection volume was 100 uL. The chromatogram for MG-BSA on the same column is shown in green. (B) The column elution from the injection of RAGE/MG-BSA complex was collected in 500 µl fractions and analyzed by SDS-PAGE followed by Coomassie staining. The presence of MG-BSA shifted the elution of both RAGE12 and BSA into the void volume, indicating a complex had formed. On the SDS-PAGE gel, M depicts the molecular weight marker lane and the rest of the lanes are labeled according to the fraction numbers from the size exclusion chromatography.

Supplementary Figure 8

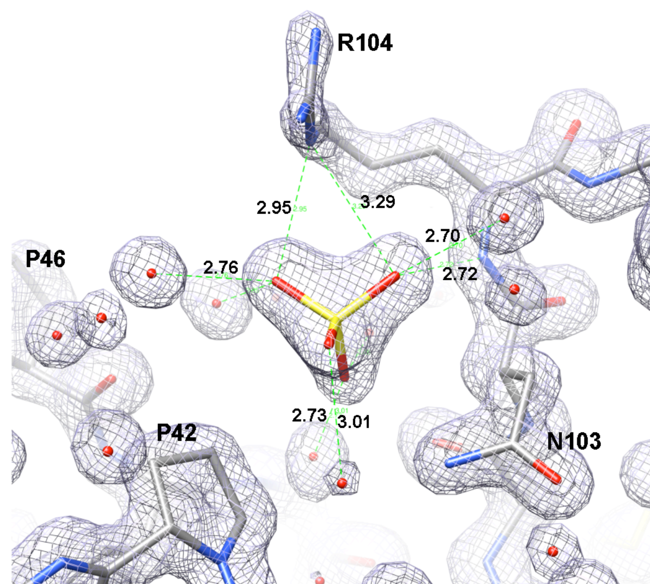






Supplementary Figure 8. Displacement of AGE-BSA from RAGE12 in native PAGE band-shift assays. (A) Effect of MG-modified amino acids on MG-BSA interaction with RAGE12. Each lane contains 2.5 uL of MG-BSA at 0.08 mM, 5 uL of RAGE12 at 0.1 mM, and 5 uL of modified amino acids at 20 mM as labeled on the gel. Considering all the target residues on BSA are modified, the molar excesses of individual modified amino acids on BSA become 8-fold, 22-fold, 29-fold, and 14-fold for Lys, Arg, His, and Cys, respectively, to modified free amino acids. (B) Effect of MG modified poly-L-Arginine or poly-L-Lysine on RAGE12 interaction with MG-BSA or Ac-BSA. Respective lanes contain 47uM RAGE12 and 15uM modified BSA. MG modified Poly-L-Arginine (MG-pArg) and MG modified poly-L-Lysine (MG-pLys) are each 62 ug/ml. (C) Effect of poly-L-Glutamate in RAGE interaction with Ac-BSA, GA-BSA or MG-BSA. Respective lanes contain 47uM RAGE12 and 15uM modified BSA. The concentration of poly-L-Glutamate (pGlu) is approximately 83 uM. Unliganded RAGE is not visible because since it is a basic protein and forced out of the top of the gel by the applied voltage.

Supplementary Figure 9



Supplementary Figure 9. 2Fo-Fc electron density map of a RAGE domain 1 region where sulphate ion is bound. Electron density is contoured at 1.6 σ. Dotted lines indicates hydrogen bond between sulfur ion and RAGE or water molecules. Bond distances and the select residues are labeled.