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Supplemental Experimental Procedures.

Reagents and Chemicals

Sodium 2-mercaptoethanesulfonate (MESNA) was from Sigma-Aldrich Co. Restriction enzymes and *Taq* polymerase were from NEB. Cross-linking reagents bis-sulfosuccinimidyl suberate (BS3), Sulfo-SMCC and SM(PEG)$_6$ NHS-PEG-Maleimide Crosslinkers, Trypsin and Glu-C were from Thermo Scientific. All other chemicals used were reagent grade or better.

Cloning, Expression and Purification of free VC1 domain

DNA coding for VC1 fragments (amino acids 23-243) were PCR amplified using oligonucleotides 5'-TTTCATATGGCTCAAAACATCACAGCCCGGATTGG and 5'-GACCTCCTCCAGGTTAACCACCACCTCGAGTTT-3' containing 5'-*Nde*I and 3'-*Xho*I restriction sites. DNA fragments were subcloned into the *Nde*I and *Xho*I sites of pET15b (Novagen), which confers ampicillin resistance. The resulting plasmid, pET15b-VC1, expresses an N terminal His-tagged VC1 domain.

E. coli strain Origami B(DE3) (Novagen) was transformed with pET15b-VC1, grown at 37 °C to an OD_{600} of ~0.8, re-equilibrated to 20 °C for 30 min, induced with 1 mM IPTG, and allowed to express for 4-6 hours. Cells were lysed at 4 °C in lysis buffer (20 mM Tris-HCl [pH 8.0], 20 mM imidazole, 300 mM NaCl) in the presence of lysozyme (5 mg/mL), followed by sonication (5 min with a 50% duty cycle). Clarified lysate was initially purified on a Ni-NTA (Qiagen) column equilibrated with lysis buffer and eluted with 20 mM Tris-HCl [pH 8.0], 250 mM imidazole, 300 mM NaCl. The His-tag was cleaved by thrombin (Novagen) at room temperature for 1 h before gel filtration chromatography on a SE-75 column (Amersham Biosciences). Fractions containing the eluted protein were concentrated by using Amicon-Ultra-Centricones (Millipore). Residual endotoxin was removed from the sample by repetitive use of EndoTrap Red (Lonza). The endotoxin level of the final protein solution was determined by Gel Clot LAL Assay (Lonza) to be less than 0.1 EU/mL. Purity was estimated to be >95% by Coomassie-stained SDS-PAGE.

Cloning, Expression and Purification of N-terminal Thiol-containing C2 domain

Oligonucleotides 5'-GTTGACGTCCGAGACACCCTACTCAGCTGTTT-3' and 5'-TTTTTTCCATGGGCAGCAGCCATCATCATCATCATCATCACAGCAGCGGCCTGGTGC CGCGCTGCAGC-3', which contain 5'-*Nco*I and 3'-*Sal*I restriction sites, were used to clone an N-terminal His-tag and thrombin cleavage site, LVPRC, into pET28-C2[29] to yield pCL-sRAGE C2. pCL-sRAGE C2 expresses the C2 domain which, when cleaved with thrombin, yields an N-terminal thiol group suitable for chemical ligation (**Figure 1a**).

E.coli strain BL21(DE3) Codon+ (Novagen)was transformed with pET28-CL-sRAGE C2 , and grown in LB medium at 37 °C for 16 hours. The cells were transferred into a secondary medium, induced with 1 mM isopropyl β-D-thiogalactoside (IPTG) for another 16 hours at 37 °C, harvested and stored at -80 °C. For [*U*-^{15}N] labeled proteins, the secondary medium was replaced with M9 minimal medium, supplemented with 0.1% (w/v) [^{15}N]-NH_4Cl. Frozen cells were re-suspended in 20 mM HEPES, pH 7.0, containing 8 M urea and heat shocked at 100 °C for 10 min. The resulting lysate was clarified by centrifugation and the supernatant was loaded onto a Ni-NTA column. The column was washed with 20 mM HEPES, pH 7.0, 8 M urea. The protein was re-natured on the column by washing with 5 column volumes of 20 mM HEPES buffer, pH7.0, and the protein was eluted with 20 mM HEPES, pH 7.0, 250 mM imidazole. Fractions containing the eluted protein were pooled and dialyzed into chemical ligation buffer, 20 mM potassium phosphate, pH 7.2, 500 mM NaCl.

Cloning, Expression and Purification of VC1 domain containing C-terminal Thioester-

DNAs coding for the VC1 domain was PCR amplified from pET15b-VC1[15] using oligonucleotides 5'-TTTCATATGGCTCAAAACATCACAGCCCGGATTGG-3' and 5'-GGGGCACAGACCCTCGGACCCACGTAGTGCCCTCTACGTGATCATTTT-3' containing 5'-*Nde*I and 3'-*Spe*I restriction sites. The resulting PCR products were cloned into pTXB1 to yield pTXB1-VC1. pTXB1 encodes the GyrA intein with a single mutation, N198A, which prevents cleavage of the intein–C-extein peptide bond. pTXB1-VC1 was used as a template to introduce a K52A mutation into the VC1 domain by using the QuikChange site-directed mutagenesis kit (Stratagene). pTXB1-VC1-K52A, expresses a K52A mutant VC1 domain-GyrA intein-chitin binding domain (CBD) C-terminal fusion (**Figure 1A**).

E. coli Origami B(DE3) cells were transformed with pTXB1--VC1-K52A , grown in LB medium at 37 °C overnight to an OD_{600} of ~0.8, re-equilibrated to 20 °C for 30 min, induced with 1 mM IPTG, and allowed to express for 5 hours.. For [*U*-^{13}C, ^{15}N] labeling, cells were grown and induced in M9 minimal medium, supplemented with 0.1% (w/v) [^{15}N]-NH_4Cl and 0.2% (w/v) [^{13}C]-glucose. Following over-expression cells were harvested and pressure lysed by using a French pressure cell. Fusion proteins were loaded onto an affinity chromatography column of chitin resin (NEBioLabs), washed with 20 mM sodium phosphate buffer, pH 7.2, 500 mM NaCl, and cleaved on the column in 20 mM sodium phosphate buffer, pH 7.2, 500 mM NaCl, containing 40 mM sodium 2-sulfanylethanesulfonate, MESNA, at 30 °C for 20 h, eluted, and dialyzed into chemical ligation buffer, 20 mM sodium phosphate, pH 7.2, 500 mM NaCl.

Expression and Purification of S100B

One liter of *E. coli* strain N99/pSS2 was grown in LB medium supplemented with 40 mg/L of carbenicillin in a 2.8 L Fernbach flask at 37 °C with vigorous shaking to an OD_{600} of 0.7. 1 mM IPTG was added, and growth was continued for 3-4 h. The cells were harvested by centrifugation, washed once with 50 mM Tris-HCl, pH 8.0, 5 mM $MgCl_2$, and stored at -80 °C. All fractionation steps were performed at 4 °C. Cells were re-suspended in a volume of 50 mM Tris-HCl, pH 8.0, 5 mM $MgCl_2$ equal to 10 times their packed weight, and disrupted by one passage through a French pressure cell at 20,000 psi. Cell debris was removed by centrifugation for 15 min at 12,000 rcf. The supernatant solution was centrifuged for 90 min at 150,000 rcf. Ammonium sulfate was added to the supernatant to 90% of saturation to precipitate unwanted protein. After the supernatant was collected, the pH was adjusted to 4 by adding 10% (v/v) formic acid and stirred at 4 °C for an additional 30 min. The precipitate was collected by centrifugation at 12,000 rcf for 15 min, re-dissolved in 25 mM Tris-HCl, pH 8.0, 1 mM EDTA, 1 mM DTT, and dialyzed overnight against the same buffer.

The dialyzed sample was applied to a HiTrapTM Q FF column (GE Healthcare). The column was equilibrated at 1 mL/min in 100% buffer A, 25 mM Tris-HCl, pH 8.0, 1 mM EDTA, 1mM DTT, and elution was monitored at 280 nm. The protein was eluted using a gradient of 0-1 M NaCl over 30 minutes. S100B eluted at 0.43 M NaCl and appeared as a single band on SDS-PAGE. The purity of the protein was confirmed by electrospray mass spectrometry analysis.

In-gel Digestion

Cross-linked CL-sRAGE bands were cut from Coomassie-stained SDS polyacrylamide gels and divided into pieces ~1 mm^3 in volume. The pieces were destained for 3 h in 0.5 mL of 50% methanol and 5% acetic acid. The solution was refreshed once after 2 hours. After destaining, three 200 µL aliquots of acetonitrile were used to dehydrate the gel fragments. Acetonitrile was discarded and the fragments were dried. The fragments were reduced by adding 30 µL of 10 mM DTT, and chemically modified by adding 30 µL of 50 mM iodoacetamide at room temperature for 30 min, followed by a 100 µL wash of 100 mM ammonium bicarbonate for 10 min and dehydration with 200 µL of acetonitrile for 5 min. After rehydration with 200 µL of 100 mM ammonium bicarbonate and dehydrating two more times, the gel fragments were dried in a speed vacuum for 2-3 min. Digestion was accomplished by adding 50 µL of 20 ng/µL endoproteinase Trypsin and 100 ng/µL of endoproteinase GluC (*Staphylococcus aureus* Protease V8) and incubating on ice for 30 min. Excess enzyme solution was removed and an additional 20 µL aliquot of 50 mM ammonium bicarbonate was added and incubated overnight at 37 °C. The digested peptides were extracted by using 10 µL of 5% formic acid followed by 20 µL of 5% formic acid and 50% acetonitrile. The peptide solution was lyophilized, and re-dissolved in 50 µL of 1% acetic acid.

Enzyme-Linked Immunosorbent Assay (ELISA)

To estimate the binding affinity of CL-sRAGE for S100B, we used purified CL-sRAGE and Bovine brain S100B protein (Calbiochem). 96-well plates (Falcon ProBind, Becton Dickinson) were coated with CL-sRAGE at 20 ng/well in 0.1 M sodium carbonate buffer, pH 9.6, overnight at 4 °C. Wells were blocked with wash buffer (20 mM Tris-HCl, pH 7.0, 100 mM Na_2SO_4, 1 mM $CaCl_2$) containing 1% BSA (Fraction V, Calbiochem), and incubated at room temperature for 2 hours. S100B, in wash buffer, at concentrations ranging from 0-10 µM, was added to the wells and the samples incubated for 2 h at room temperature. Mouse monoclonal [6G1] anti-S100B HRP conjugate antibody (Abcam) diluted 1:1000-1:3000 in wash buffer containing 1% BSA was added to each well and incubated for 3 h at room temperature or overnight at 4 °C. After adding chromogenic TMB substrate solution (Thermo Scientific), plates were read on a scanner. All washes between steps were performed 4-6 times using wash buffer. Data was analyzed by using GraphPad Prism 5 software.

Sample preparation for NMR Spectroscopy.

Protein samples of free [*U*- ^{15}N]-C2, free [*U*- ^{2}D, ^{15}N]-VC1 and CL-sRAGE constructs, [*U*-^{15}N, ^{2}H]-VC1-C2 and VC1-[*U*-^{15}N]-C2, were dissolved in NMR buffer (20 mM sodium phosphate pH 7.0, 100 mM Na_2SO_4, 0.5 mM DTT, 1 mM EDTA, 90%/10% H_2O/D_2O) at concentrations ranging from 40 μM to 100 μM. To create the CL-sRAGE-Ca^{2+}-S100B complex, 0.5 mM of either unlabeled or [*U*-^{15}N]-S100B was added to samples of either 60 mM VC1-[*U*-^{15}N]-C2 or 80 mM [*U*-^{15}N, ^{2}H]-VC1-C2, dissolved in 20 mM Tris-HCl (D_{11}, 98% enrichment) pH 7.0, 100 mM Na_2SO_4, 0.5 mM DTT, 1 mM Ca_2Cl, 90%/10% H_2O/D_2O. DTT was added to 0.5 mM to maintain the reduced state of the cysteines introduced into the CL-sRAGE linker during chemical ligation. Previously, we observed that reducing disulfide bonds within either VC1 or C2 domains results in sRAGE unfolding (Xie et al., 2007; Xie et al., 2008; Xue et al., 2011). Since the NMR spectra of CL-sRAGEs indicate that the protein is folded, we concluded that the CL-sRAGE constructs contain intact disulfide bonds.

Hydrodynamic Calculations

^{15}N-NMR relaxation rates for monomers and dimers were calculated by using the program HydroNMR (Garcia de la Torre et al., 2000). The atomic radius was assigned to be 2 Å and the (N–H) atomic distance 1.02 Å. The temperature was set to 25 °C, the viscosity to $8.1*10^{-3}$ poise and the NMR field strength to 16.45 T. For CL-sRAGE monomers, a rotational correlation time of τ_{cM} = 16.5 ns ($<R_2/R_1>$ = 40) was calculated, with an anisotropy of D_{par}/D_{per} = 1.3. The dimer had a τ_{cD} value of 32.33 ns ($<R_2/R_1>$ = 135) and D_{par}/D_{per} = 0.83, consistent with it being the most anisotropic species.

^{15}N-NMR Relaxation Data Analysis

^{15}N-NMR relaxation data were filtered for fitting as described elsewhere(Blobel et al., 2009; Blobel et al., 2007; Fushman, 2012). Briefly, data from individual residues were not used when any of the following three situations were encountered: (a) Heteronuclear Overhauser Effect < 0.6, (b) large (> 25%) experimental errors when compared with the relaxation rates R_2/R_1, where R_1 and R_2 correspond to longitudinal and transverse relaxation, respectively, and (c) large disagreement (> 25%) between the experimental and simulated R_2/R_1 values using the relevant model, filtering for residues affected by chemical exchange. A total of 71 experimental R_2/R_1

values were extracted from CL-sRAGE at two concentrations, 40 μM and 80 μM. The average relative relaxation rates ($<R_2/R_1>$) were calculated for each protein concentration.

Experimental R_1 and R_2 values represent the concentration weighted average of the relaxation rates of all participating species. Equation (1) shows the calculation of the relaxation rates for the monomer–dimer model, where M is the molar fraction of monomer, D is the molar fraction of dimer, P is the population of monomer, P_M, or dimer, P_D.

$$R_n = p_M R_{n,M} + p_D R_{n,D} \quad , \tag{1}$$

The relaxation rate R_n of each species is denoted with the subscript n, which is equal to 1 and 2 in the case of longitudinal and transverse relaxation, respectively. The equilibrium parameters were determined by minimizing the error function:

$$X^2 = \frac{1}{N} \times \left(\sum_i \sum_j \left[\left(\frac{R_2}{R_1}\right)_{ij}^{exp} - \left(\frac{R_2}{R_1}\right)_{ij}^{theo} \right]^2 \div \left[E\left(\frac{R_2}{R_1}\right)_{ij}^{exp} \right]^2 \right) \quad , \tag{2}$$

where i and j denote different residues (i) at varying protein concentrations (j), $E(R_2/R_1)^{exp}$ is the corresponding experimental error, and N is the number of experimental data sets.

Fitting theoretical relaxation rates of two species to experimental values using a monomer-dimer model requires that only the dissociation constant, K_d, be adjusted.

$$K_d = \frac{[M]^2}{[D]} = \frac{2P_M^2}{P_D} c \quad , \tag{3}$$

where c is the total protein concentration and ($P_M + P_D = 1$). The minimization protocol consists of a grid search for each variable using the function "fmincon" as implemented in Matlab (MathWork Inc).

hRAGE and DIAPH1 Fusions with Fluorescent Proteins

Construction of human RAGE-mYFP was described previously (Xie et al., 2008). A fragment containing human *Diaphanous-1* cDNA was PCR amplified using source clone #401258087 (Open Biosystems) as a template, and inserted into a modified Clontech vector pmCFP-N1(Xie et al., 2008) at *Xho*I and *Age*I sites, upstream of a fluorescent protein sequence, to obtain DIAPH1-mCFP. Deletion of the 3'-terminus of *DIAPH1* containing the *DAD domain* (aa 1194-1272) was performed using the QuikChange site-directed mutagenesis II-E kit (Stratagene) resulting in construct DIAPH1ΔDAD-mCFP.

Sample preparation for confocal microscopy.

HEK293 cells were plated on 15 mm glass coverslips from Ted Pella, Inc (Redding,CA) and treated with collagen type IV from human placenta (Sigma). Cells were grown at 37 ºC, with 5% CO_2 in low glucose DMEM medium (Thermo Scientific) supplemented with 10% FBS, to 50-70% confluency. DIAPH1-mCFP, DIAPH1ΔDAD-mCFP (donor) or hRAGE-mYFP (acceptor) plasmids were introduced into the cells separately or simultaneously with the help of TransIT-293 transfection reagent (MirusBio LLC, Madison,WI). Prior to transfection the medium was exchanged with Opti-MEM (Invitrogen) supplemented with 5% FBS. Cells were incubated with transfection mix for 16 hours, the medium was then replaced with Opti-MEM/0.1% FBS and incubated for additional 6 hours. Appropriate wells were treated with RAGE ligand S100B (EMD Millipore) at a final concentration of 0.5 μM for 0, 10 or 60 minutes. Cells were fixed by adding equal volume of 4% formaldehyde in 1X PBS for 20-30 minutes at 37 ºC, washed 3X with 1 mL of 1X PBS and mounted on slides with Fluoromount G (Electron Microscopy Sciences, Hatfield, PA).

Cell Lines.

Wild-type (WT) and DIAPH1 knockout (DIAPH1 KO) primary murine aortic vascular smooth muscle cells (SMC) were isolated and employed through passages 5 to 7. Vascular SMCs were grown in 10% FBS containing DMEM medium (Invitrogen)(Toure et al., 2012).

Stimulation assay.

SMCs were seeded at 1×10^6 cells/100 mm dish in complete medium and grown for 24 h before subjecting them to overnight starvation in serum-free medium. The next day cells were stimulated with 10 μ g/mL of S100B-BSA for five minutes at 37 °C. Cells were rinsed with ice-cold PBS and lysed using lysis buffer (Cell Signaling Technology) containing 1 mM phenylmethylsulfonyl fluoride and Complete Protease Inhibitors (Roche Applied Science).

Western Blotting.

Total SMC lysates were immunoblotted and probed with AKT-specific antibody, and phospho-AKT-specific antibody. Antibody to DIAPH1 was obtained from Santa Cruz Biotechnology. After probing with the primary antibodies, membranes were stripped and reprobed for relative total AKT protein. Donkey anti-rabbit HRP-conjugated-IgG (Amersham Pharmacia Biotechnology) or sheep anti-mouse HRP-conjugated-IgG (Amersham Pharmacia

Biotechnology) was used to identify sites of binding of the primary antibody where indicated. Blots were scanned by using an AlfaImager TM 2200 scanner with AlfaEase (AlfaImager) FC 2200 software. Results are reported as the relative absorbance of test antigen to relative total protein. In all Western blot studies, at least triplicate cell lysates per group were used; results of representative experiments are shown.

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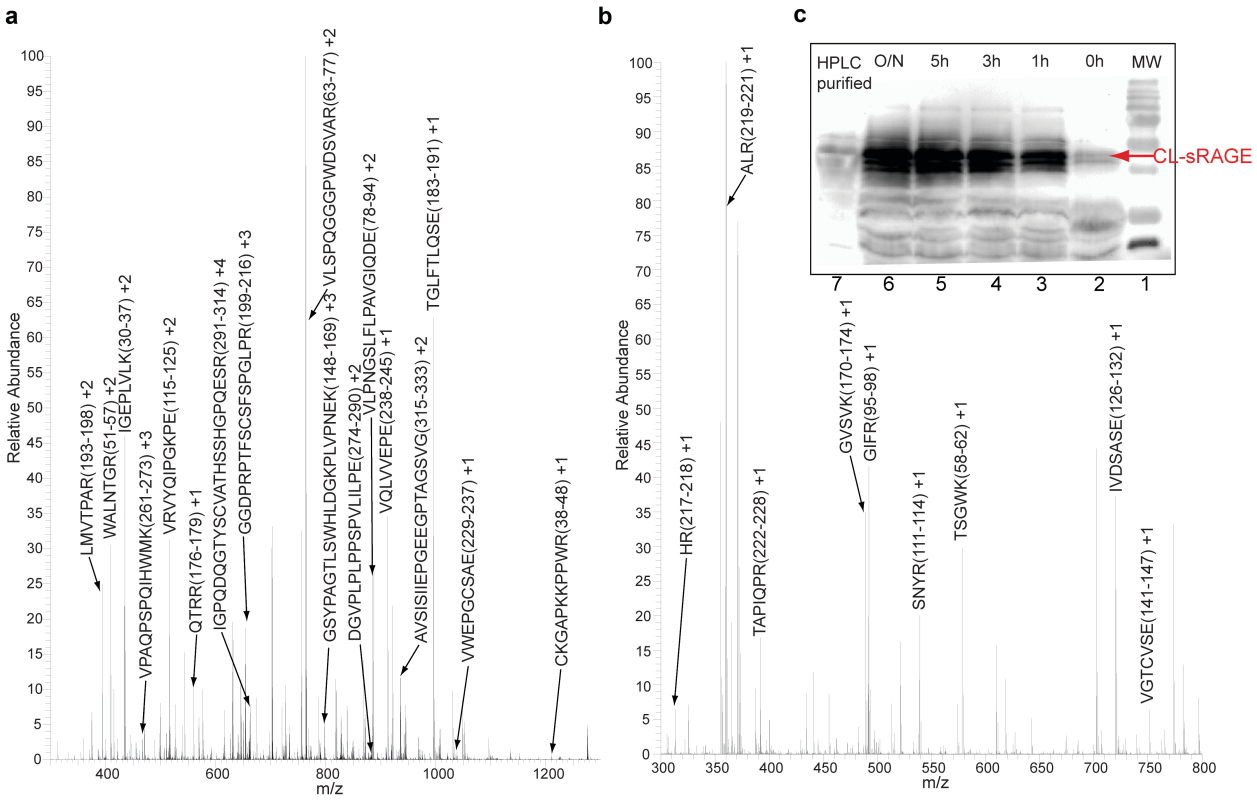

Figure S1. Related to Figure 1. Identification of CL-sRAGE. (**a,b**) CL-sRAGE was digested with Trypsin and Glu-C at room temperature for 16 hours. Peptide coverage reached 90% by mass spectrometry analysis. Peptides in *A* and *B* were separately isolated from a C-18 column using 70% and 0% acetonitrile, respectively. (**c**) Western analysis of the chemical ligation reaction time-course. From right to left, lane 1 is molecular weight markers; lanes 2, 3, 4, 5 and 6 are time points taken at 0 h, 1 h, 3 h, 5 h and overnight; lane 7 is CL-sRAGE purified by using HPLC chromatography. CL-sRAGE is indicated by the red arrow.

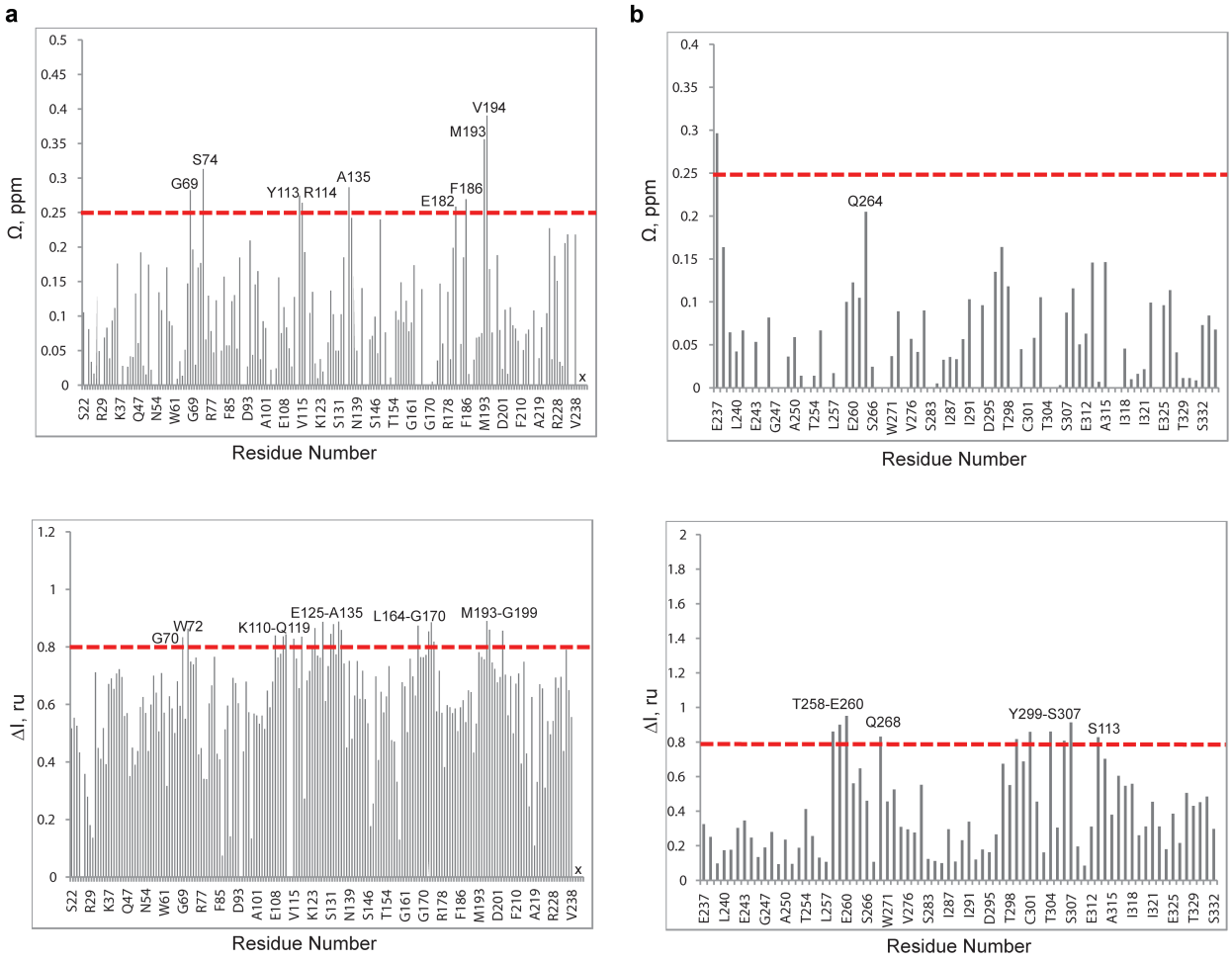

Figure S2. Related to Figure 2. Changes in chemical shifts and intensities of VC1 and C2 domain peaks due to chemical ligation and dimerization. (**a**) Changes in the ^{1}H,^{15}N-HSQC NMR chemical shifts (top) and signal intensities (bottom) of [*U*- ^{2}H, ^{15}N]-VC1 upon ligation with the C2 domain. (**b**) Changes in the ^{1}H,^{15}N-HSQC NMR chemical shifts (top) and signal intensities (bottom) of [*U*- ^{15}N]-C2 upon ligation with the VC1 domain.

Chemical shift changes (Ω) were calculated by $\Omega = \sqrt{\Delta\delta_H{}^2 + (\frac{\Delta\delta_N}{4})^2}$, where $\Delta\delta_H$ and $\Delta\delta_N$ are the changes in amide proton and nitrogen chemical shifts, respectively. Residues that exhibited chemical shift changes above 0.25 ppm were considered to constitute the interaction surfaces. Cut-offs for selecting residues involved in the interaction are indicated by red dashed lines.

NMR signal intensity changes were calculated by using $\Delta I = (I_f - I_l)/I_f$, where $I_{f(l)}$ is the NMR signal intensity. Most of the VC1 residues exhibited uniform broadening upon complex formation. Residues that exhibited signal broadening above 80% were considered to constitute the VC1 interaction surface.

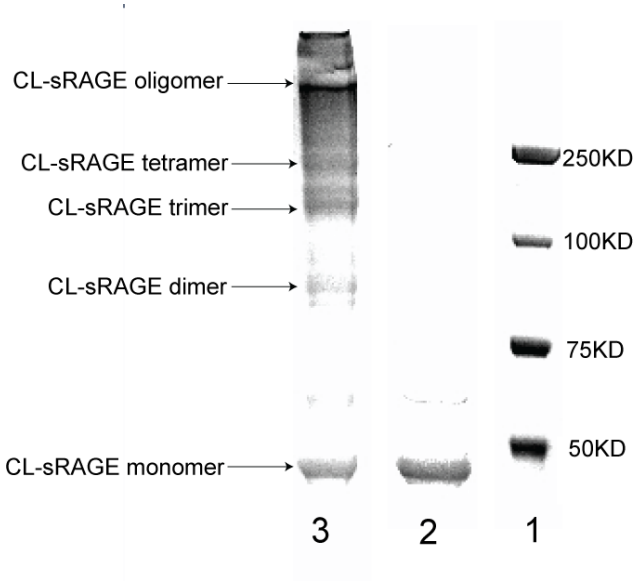

Figure S3. Related to Figure 3. CL-sRAGE is an oligomer. SDS-PAGE of CL-sRAGE crosslinking using BS_3. Lane 1 is molecular weight markers; lane 2 is purified CL-sRAGE monomer; lane 3 is crosslinked CL-sRAGE. Labeled CL-sRAGE dimers, trimers, and tetramers are located at ~90 kDa, 125 kDa and 200 kDa. At the very top an oligomer greater than 250 kDa is evident.

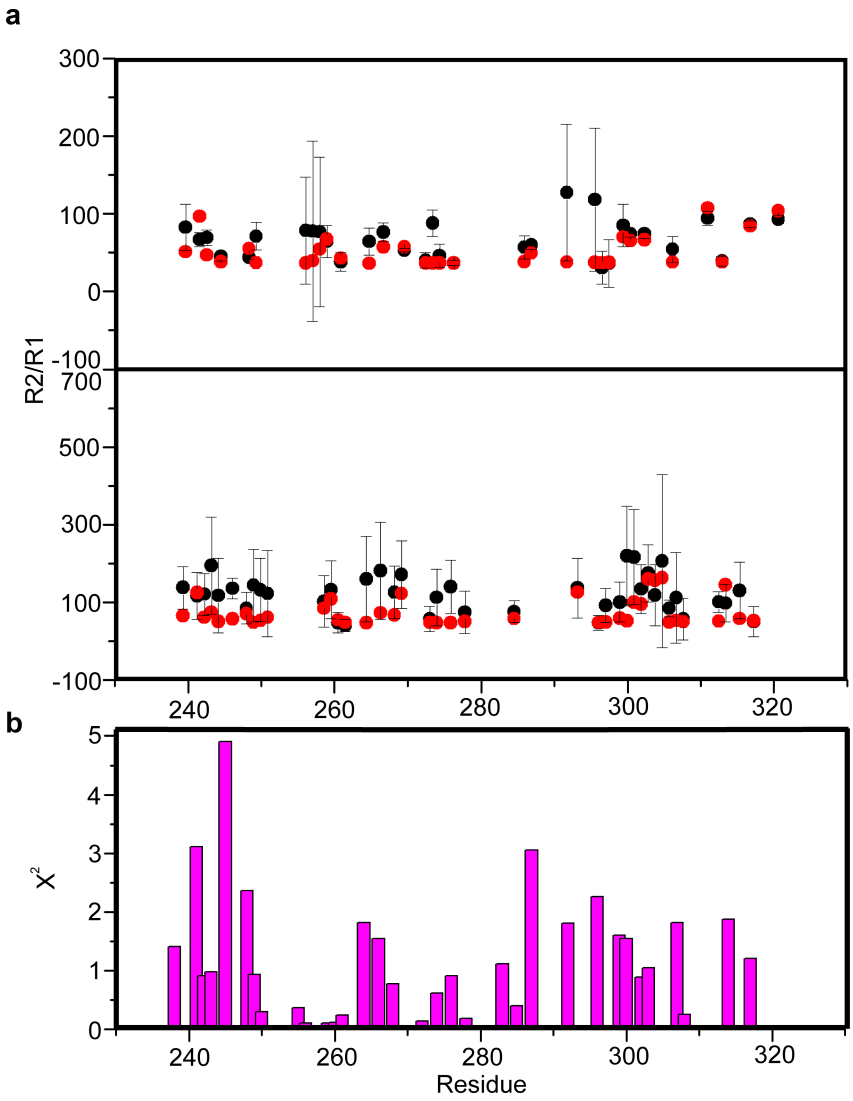

Figure S4. Related to Figure 4. The model homo-dimer structure of sRAGE is consistent with CL-sRAGE NMR relaxation data. (**a**) R_2/R_1 values of individual residues on the C2 domain in CL-sRAGE (black) were measured at two concentrations: 20 μM (upper panel) and 80 μM (lower panel). Values calculated using the best-fit parameters for a monomer–dimer model are shown in red. (**b**) The average contribution of both experiments to the fitting error of individual residues for the monomer–dimer model.

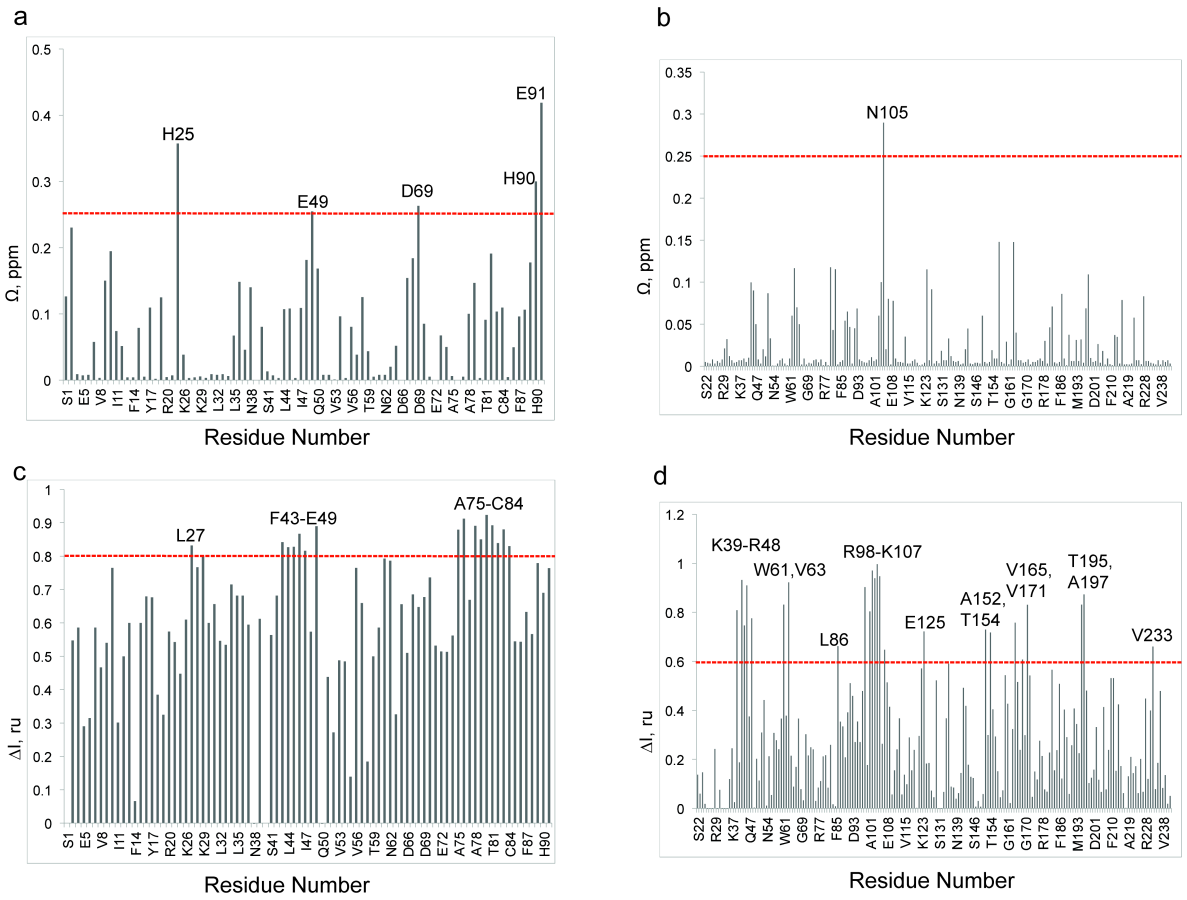

Figure S5. Related to Figure 5. Changes in the chemical shifts and intensities of the VC1 domain in CL-RAGE and S100B peaks due to the binding interaction. (**a**) Changes in the ^{1}H,^{15}N-HSQC NMR chemical shifts (top) and signal intensities (bottom) of [*U*- ^{15}N]-Ca^{2+}-S100B due to interaction with the VC1 domain. (**b**) Changes in the ^{1}H,^{15}N-HSQC NMR chemical shifts (top) and signal intensities (bottom) of [*U*- ^{2}H, ^{15}N]-VC1 due to interaction with S100B.

Chemical shift changes (Ω) were calculated by $\Omega = \sqrt{\Delta\delta_H{}^2 + (\frac{\Delta\delta_N}{4})^2}$, where $\Delta\delta_H$ and $\Delta\delta_N$ are the changes in amide proton and nitrogen chemical shifts, respectively. Residues that exhibited chemical shift changes above 0.25 ppm, were considered to constitute the CL-sRAGE-Ca^{2+}-S100B interaction surfaces. Cut-offs for selecting residues involved in the interaction are indicated by red dashed lines.

NMR signal intensity changes were calculated by $\Delta I = (I_f - I_l)/I_f$, where $I_{f(l)}$ is the NMR signal intensity. Most of the VC1 and Ca^{2+}-S100B residues exhibited uniform broadening upon

complex formation. Residues that exhibited signal broadening above 80% were considered to constitute the CL-sRAGE-Ca^{2+}-S100B interaction surface.

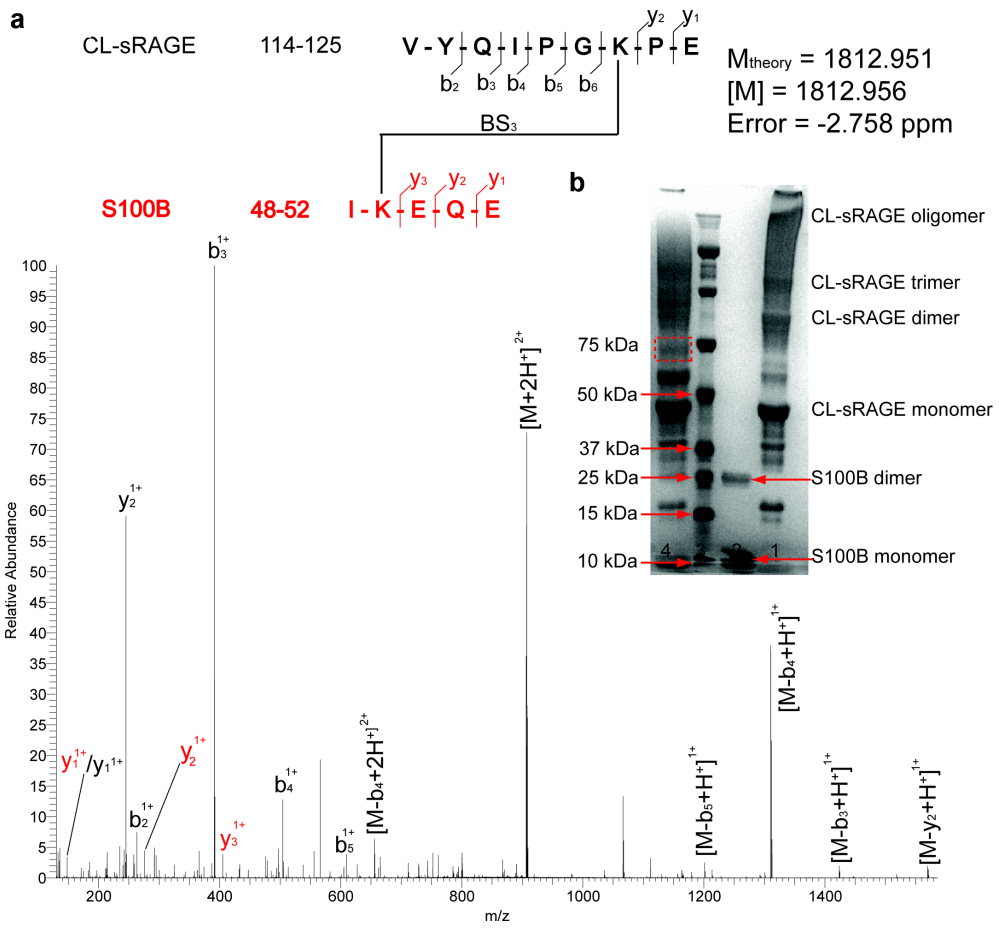

Figure S6. Related to Figure 5. MS analysis of the Ca^{2+}-S100B-CL-sRAGE-complex (**a**) Peptide composition and residue detected ion fragments of the cross-linked peptide. The HCD spectrum of the cross-linked product, at m/z value of 905.487^{2+}, is consistent with the theoretical monoisotropic mass of 1812.951. Observed fragment ions and their charge states are labeled according to standard nomenclature. Black and red colors, respectively, represent each peptide in the cross-linked product. Coverage of almost all ion fragments (y and b fragment ions) and the involvement of BS3 in many peptide pieces supports the identity of this cross-linked product, and providing an upper distance constraint between the cross-linked residues equal to the BS_3 spacer arm length of 11.4 Å. (**b**) Gradient (4% - 12%) SDS-PAGE gel of CL-sRAGE BS3 crosslinks with S100B. Lane 1 is CL-sRAGE self-crosslinks; lane 2 is S100B self-crosslinks with BS_3, displaying both monomers and dimers; lane 3 is the molecular marker; lane 4 is CL-sRAGE crosslinks with S100B, and the red circle indicates a new band distinguishable from CL-sRAGE and S100B self-crosslinking.

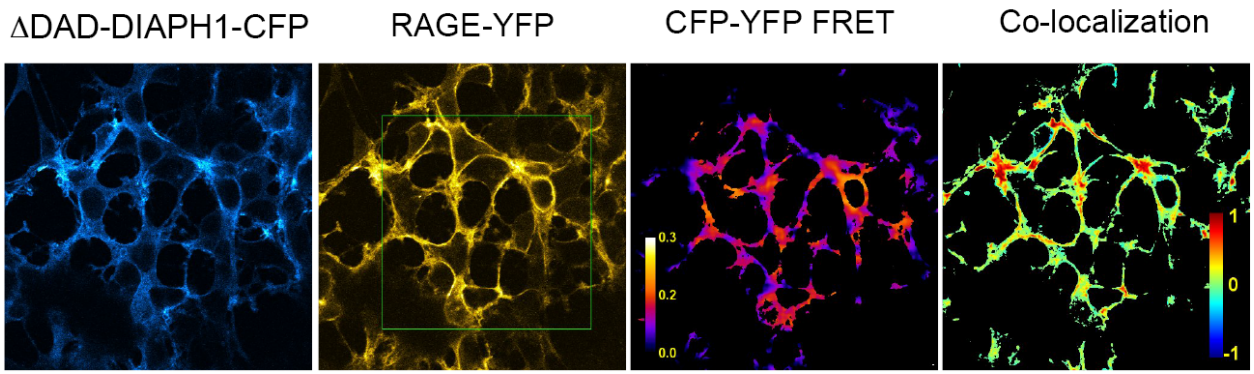

Figure S7. Related to Figure 6. Active DIAPH1, ΔDAD-DIAPH1, interacts with RAGE in the absence of RAGE ligands. Constitutively active ΔDAD-DIAPH1-CFP, in which DAD is removed, readily co-localizes with RAGE, nMDP=0.091 +/- 0.001. nMDP=-1 signifies no co-localization and nMDP=1 is 100% co-localization between CFP and YFP. Forster resonance transfer, FRET, experiments were used to assess the strength of the molecular interaction between fluorescently labeled RAGE-YFP and ΔDAD-DIAPH1-CFP. The increase in donor, RAGE-CFP, emission due to acceptor, ΔDAD-DIAPH1-CFP, photobleaching in the region shown by the green rectangle is shown in the CFP-YFP FRET channel. FRET efficiency between RAGE-YFP and ΔDAD-DIAPH1-CFP was 10 ± 3%, whereas acceptor photobleaching typically results in over a 90% decrease in fluorescence.

Table S1. Related to Figure 4 and 5. AIRs and unambiguous distance constraints used in the docking experiments.

Interaction Surfaces	Docked Domains	Active and passive AIRs		Unambiguous distance constraints
		Active AIRs	Passive AIRs	
	Chemical shift perturbations			Chemical Cross-linking
Between V and C1 domains	V domain	K110, R114, R116	auto	K107-K123 11.4Å K62-K123 11.4Å K110-C234 11.4-32.5Å P323-P323 50Å*
	C1 domain	E125, D128, S131, E132	auto	
Between C1 and C2 domains	C1 domain	L164, V165, E168, K169	auto	
	C2 domain	Q268, T304, H305, S306, S307	auto	
Between V domain and Ca^{2+}-S100B	V domain	K43, K44, A101, M102, N103, R104, N105, G106, K107	auto	K110-K48 11.4Å
	Ca^{2+}-S100B	E43, L44, E45, E49, A78, T81, A83, H85, E86, F87, F88, E89, H90	auto	

* This constraint is based on the observation of FRET between RAGE-CFP and RAGE-YFP expressed in HEK293 (Xie, J., *et al.* (2008) Structural basis for pattern recognition by the receptor for advanced glycation end products (RAGE). *J. Biol.Chem.* **283:** 27255-27269).

Table S2. Related to Figure 4 and 5. Statistics of the docked complexes.

Parameter	sRAGE dimer [a]	Ca^{2+}-S100B-sRAGE [b]
No. of clusters	1	4
Best cluster [c]	1	3
No. of structures	200	196
iRMSD [d]	2.7	6.2
lRMSD [e]	6.6	17.2
fnat [f]	0.56	0.25
HADDOCK score	-87.6 +/- 1.5	-132.8 +/- 2.6

[a]: sRAGE homo-dimer structure.

[b]: S100B dimer binding to sRAGE .

[c]: The cluster with the lowest average HADDOCK score. The number refers to the rank in cluster size.

[d]: Interface RMSD, iRMSD, (within 10 Å of the interface) calculated from the backbone atoms of all residues involved in intermolecular contact.

[e]: Ligand RMSD, lRMSD, calculated from the backbone atoms of all molecules after fitting to the backbone atoms of the first molecule.

[f]: The fraction of native contacts is calculated by counting all residue contacts between each interacting protein in the reference structure and comparing how many are used in the average structure calculation for each cluster.