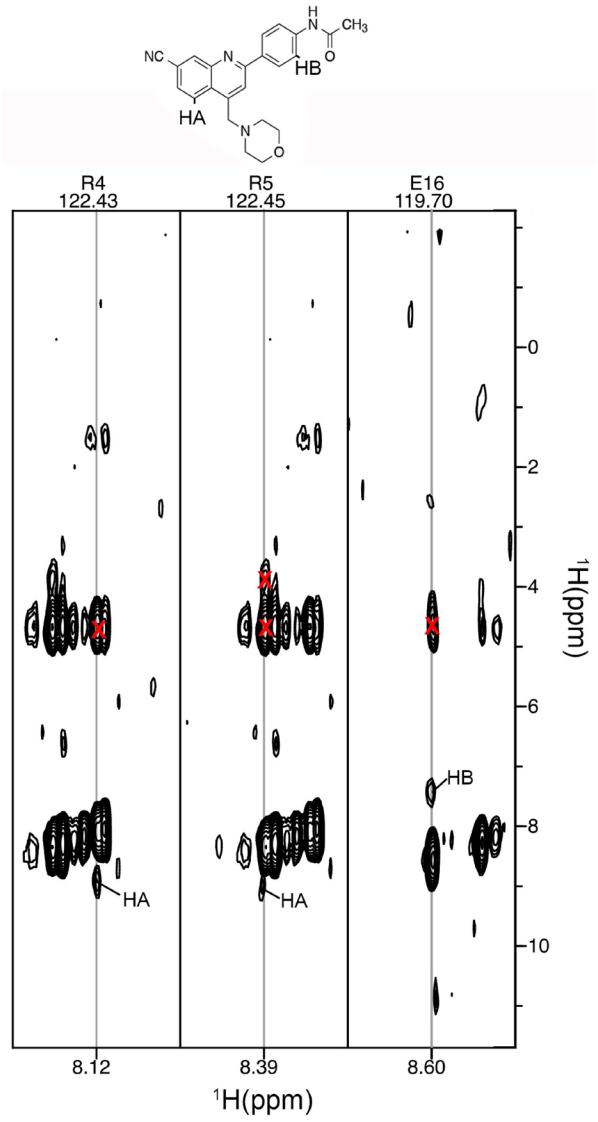
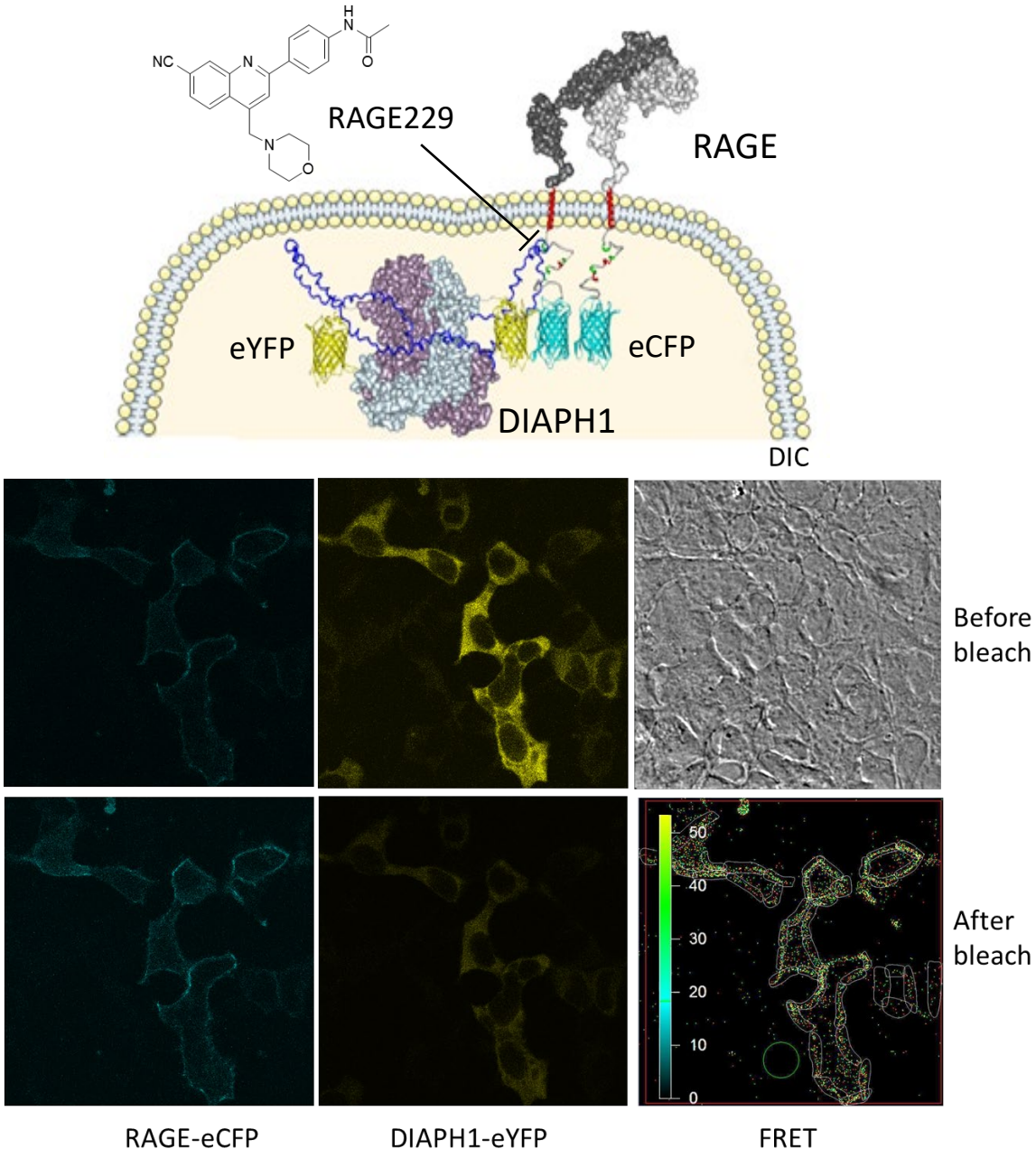
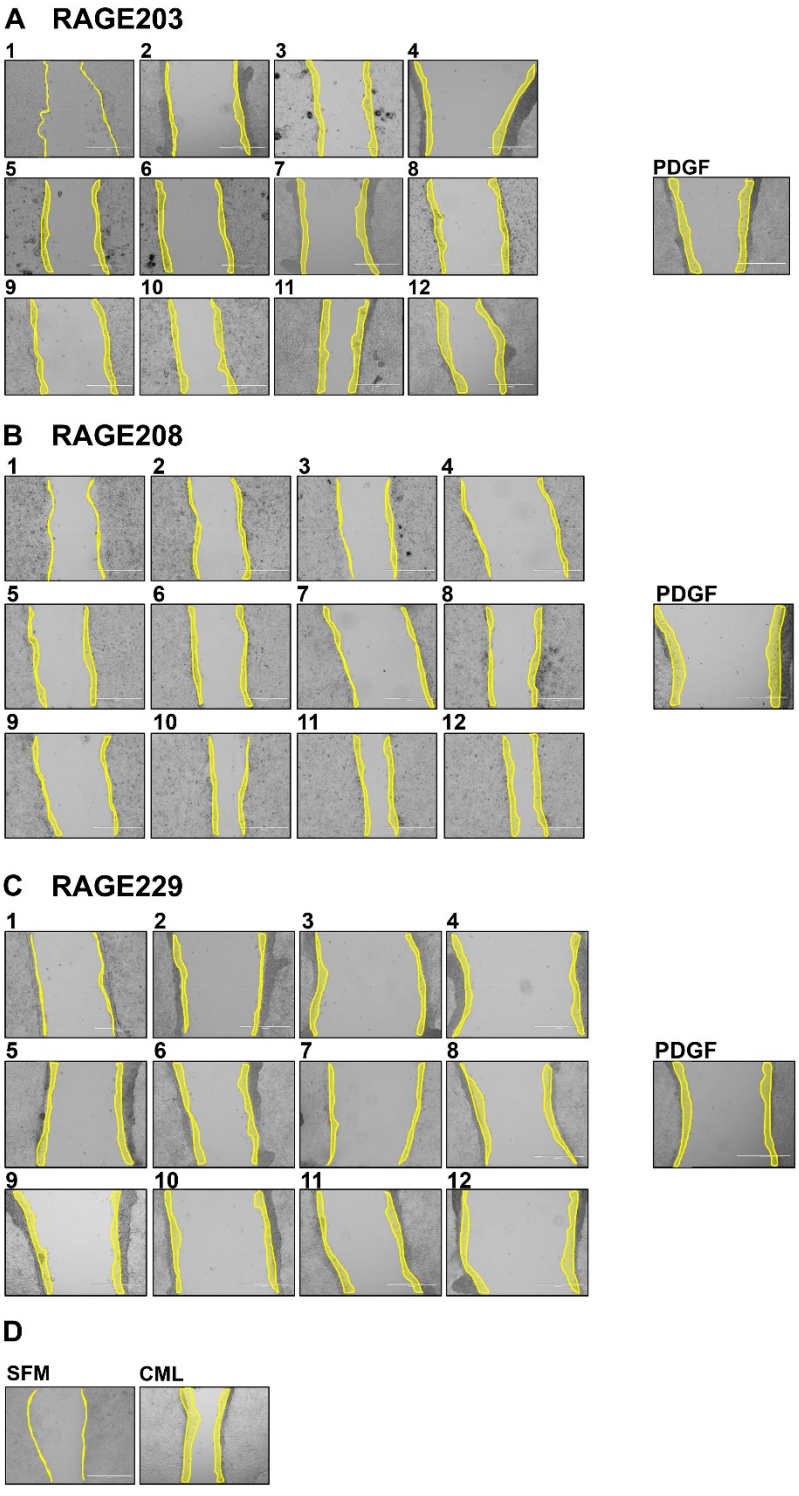
Supplemental Materials



Supplemental Figure 1. Intermolecular NOEs establishing RAGE229-ctRAGE binding mode. Selected strips from 3D ^{13}C filtered ^{15}N edited NOESY spectrum highlight intermolecular NOEs between ^{12}C bound protons of RAGE229, HA and HB (top insert) and ^{15}N bound amide protons of [*U*- ^{13}C, ^{15}N] ctRAGE. Incompletely suppressed signals from intramolecular ^{13}C-bound protons of ctRAGE are denoted by red crosses. To identify the peaks from intramolecular ^{13}C-bound protons, the NOESY spectra were acquired both with and without heteronuclear ^{13}C decoupling during the indirect proton acquisition period. Peaks that were split in the absence of decoupling were assigned to intramolecular ctRAGE NOEs. The assignments of amide protons are labeled on top of the strips. The chemical shifts of amide protons and nitrogens are indicated on the bottom of the strips, respectively.

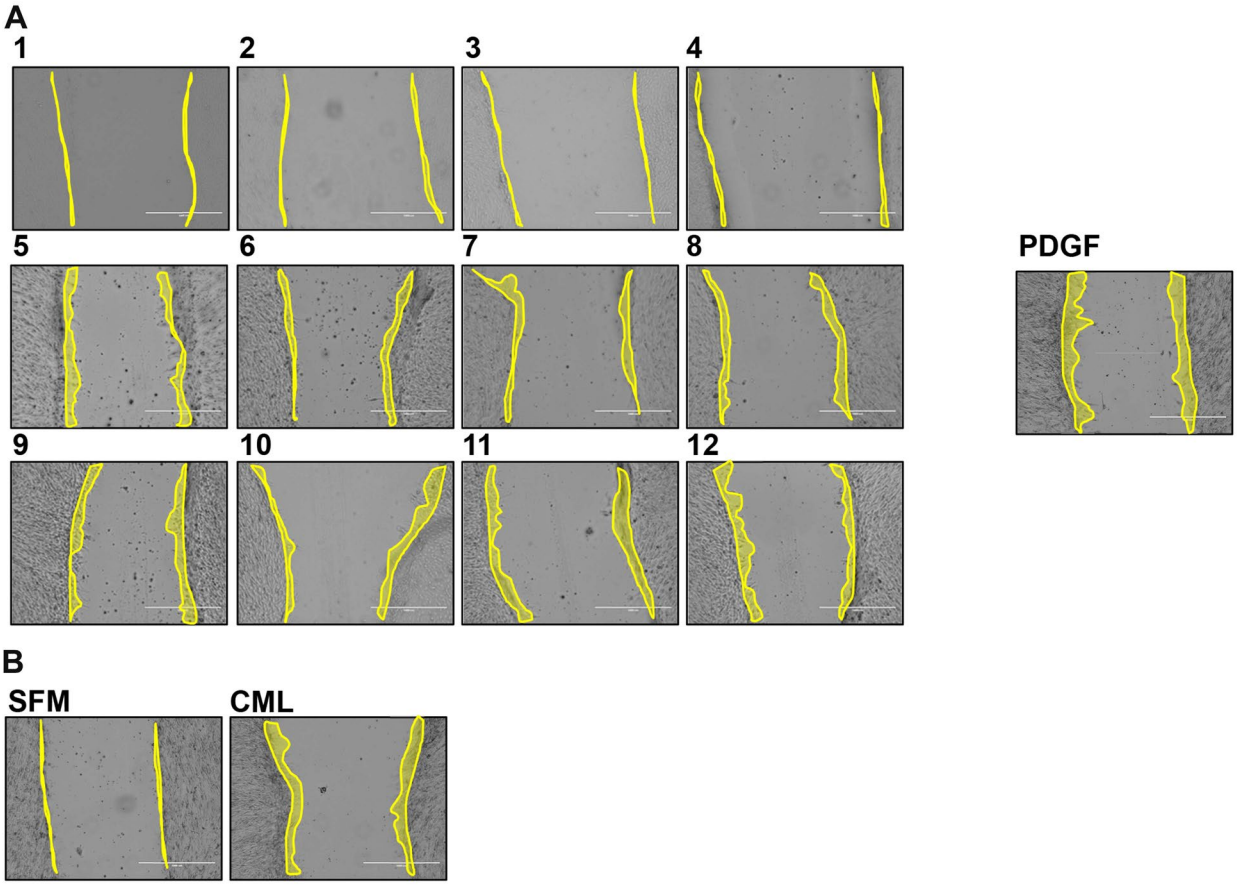


Supplemental Figure 2. **RAGE229 reduces the interaction of RAGE with constitutively active DIAPH1 mutant as revealed by FRET.** Confocal images of HEK 293T cells co-expressing RAGE-eCFP and constitutively active DIAPH1-eYFP. Top cartoon shows transmembrane location of RAGE and inner membrane-proximal localization of the **DIAPH1**. Panels show donor channel (eCFP) and acceptor channel (eYFP) images before and after bleaching (bleached areas are boxed). Membrane regions selected for FRET calculations are marked by yellow lines. DIC=differential interference contrast.

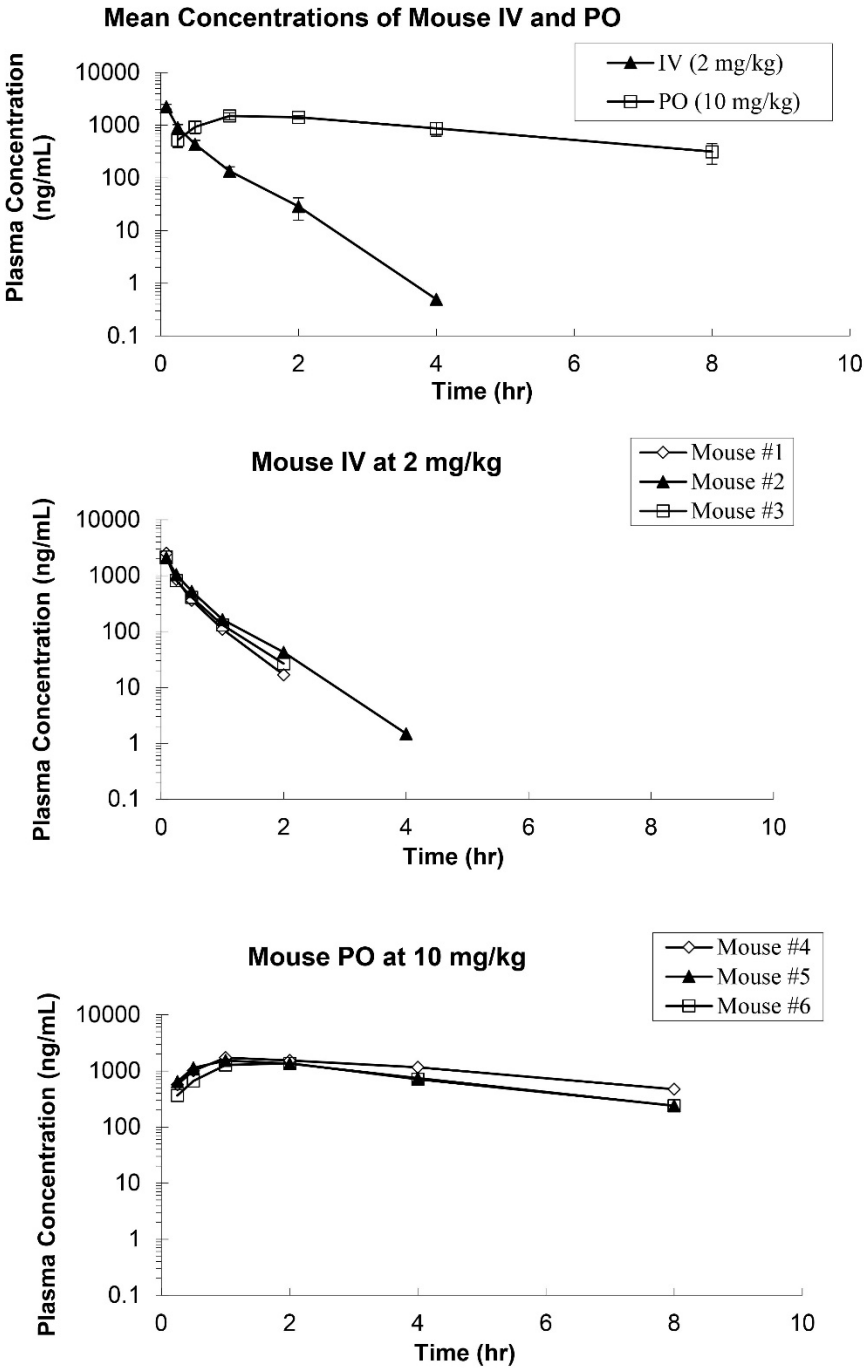


Supplemental Figure 3. Representative images of 12-point dose response of the small molecule antagonists, RAGE203, RAGE208 and RAGE229. The RAGE ligand carboxymethyl lysine (CML)-AGE was used to stimulate murine smooth muscle cells (SMCs) in the presence of

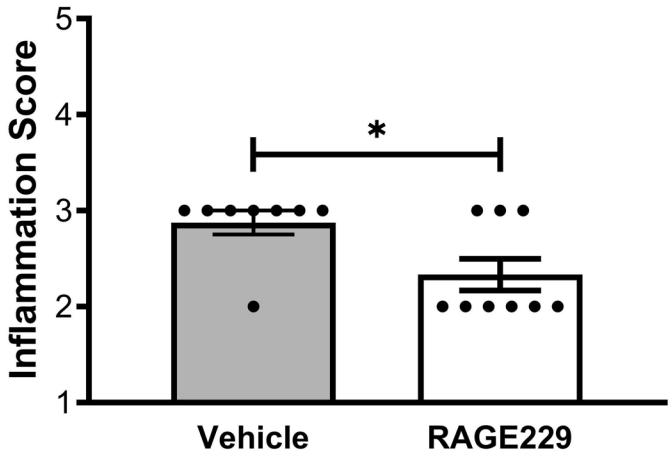
a scratch wound. The inhibitory response of the three small molecule antagonists, RAGE203 (A), RAGE208(B) and RAGE229(C), were tested in a 12-point dose-dependent manner (1=10μM, 2=3.333μM, 3=1.111μM, 4=0.370μM, 5=0.123μM, 6=0.041μM, 7=0.014μM, 8=0.005μM, 9=0.002μM, 10=0.001μM, 11=0.0002μM, 12=0.00006μM). SMCs were pre-treated with the antagonists for 1.5 hour, which were then removed and followed by addition of CML-AGE (10 μg/ml) and the scratch wounding for 4 hours, after which time the percent migration is measured at each dose. To verify specificity of the small molecules tested, the percent migration of a non-RAGE ligand, PDGF-BB (10 ng/ml), was used as a positive control. D) Serum-free medium (SFM) and CML-AGE alone (CML) were used as assay negative and positive controls. Scale bar = 1000μm.



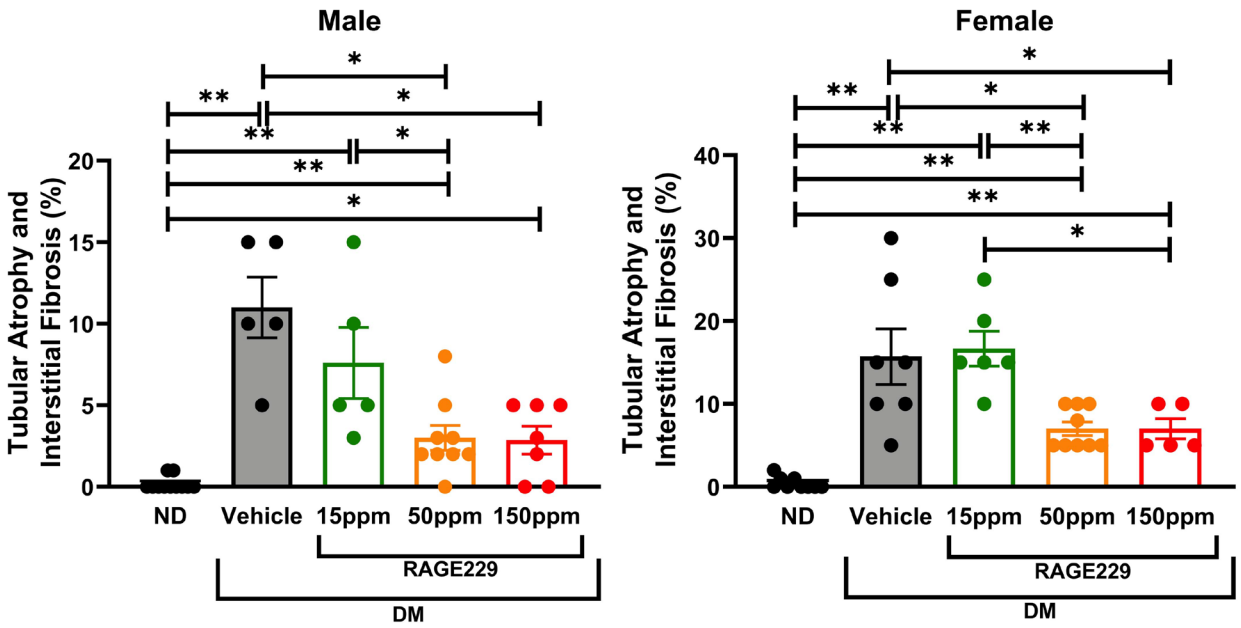
Supplemental Figure 4. Representative images of 12-point dose response of the small molecule antagonists, RAGE229, in human aortic smooth muscle cells. The RAGE ligand carboxymethyl lysine (CML)-AGE was used to stimulate human aortic smooth muscle cells (SMCs) in the presence of a scratch wound. The inhibitory response of the small molecule antagonist, RAGE229, was tested in a 12-point dose-dependent manner (1=10μM, 2=3.333μM, 3=1.111μM, 4=0.370μM, 5=0.123μM, 6=0.041μM, 7=0.014μM, 8=0.005μM, 9=0.002μM, 10=0.001μM, 11=0.0002μM, 12=0.00006μM). SMCs were pre-treated with the antagonist for 1.5 hour, which were then removed and followed by addition of CML-AGE (10 μg/ml) and the scratch wounding for 4 hours, after which time the percent migration is measured at each dose. To verify specificity of the small molecules tested, the percent migration of a non-RAGE ligand, PDGF-BB (10 ng/ml), was used as a positive control. Serum-free medium (SFM) and CML-AGE alone (CML) were used as assay negative and positive controls. Scale bar = 1000μm.



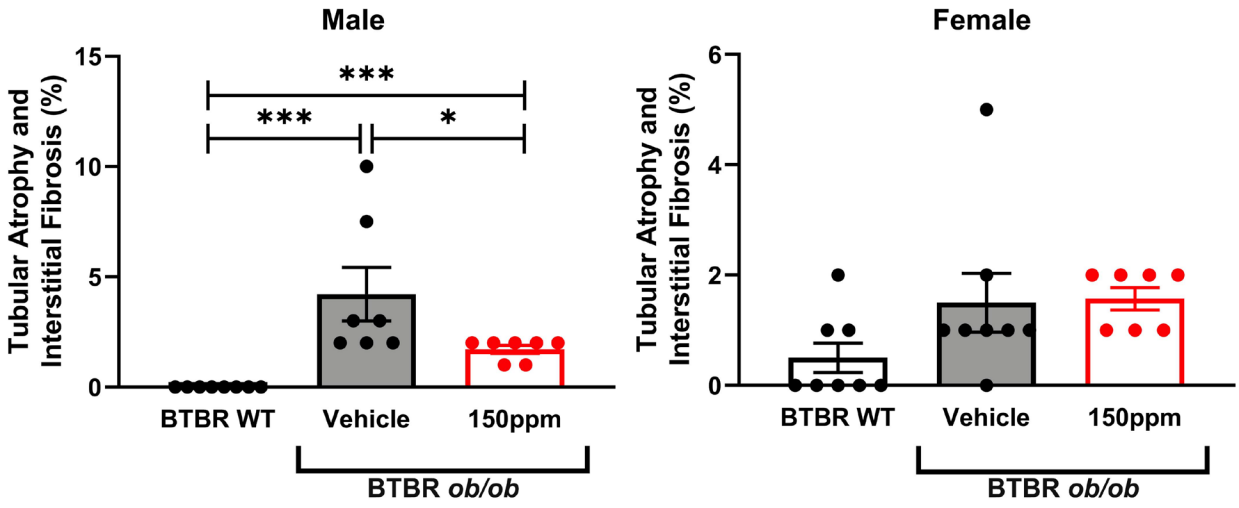
Supplemental Figure 5. Pharmacokinetic Data in Mice: RAGE229 IV and PO dosing. Mice were injected with IV or PO doses of RAGE229 and then serially assessed for determination of the mean plasma concentrations of RAGE229 after IV (2 mg/kg/mouse) or PO (10 mg/kg/mouse) administration of RAGE229. Top (Mean concentrations of Mouse IV and PO); Middle (Mean concentrations after Mouse IV RAGE229); and Bottom (Mean concentrations after Mouse PO RAGE229). N=3 male mice receiving IV RAGE229 and N=3 male mice receiving PO RAGE229.



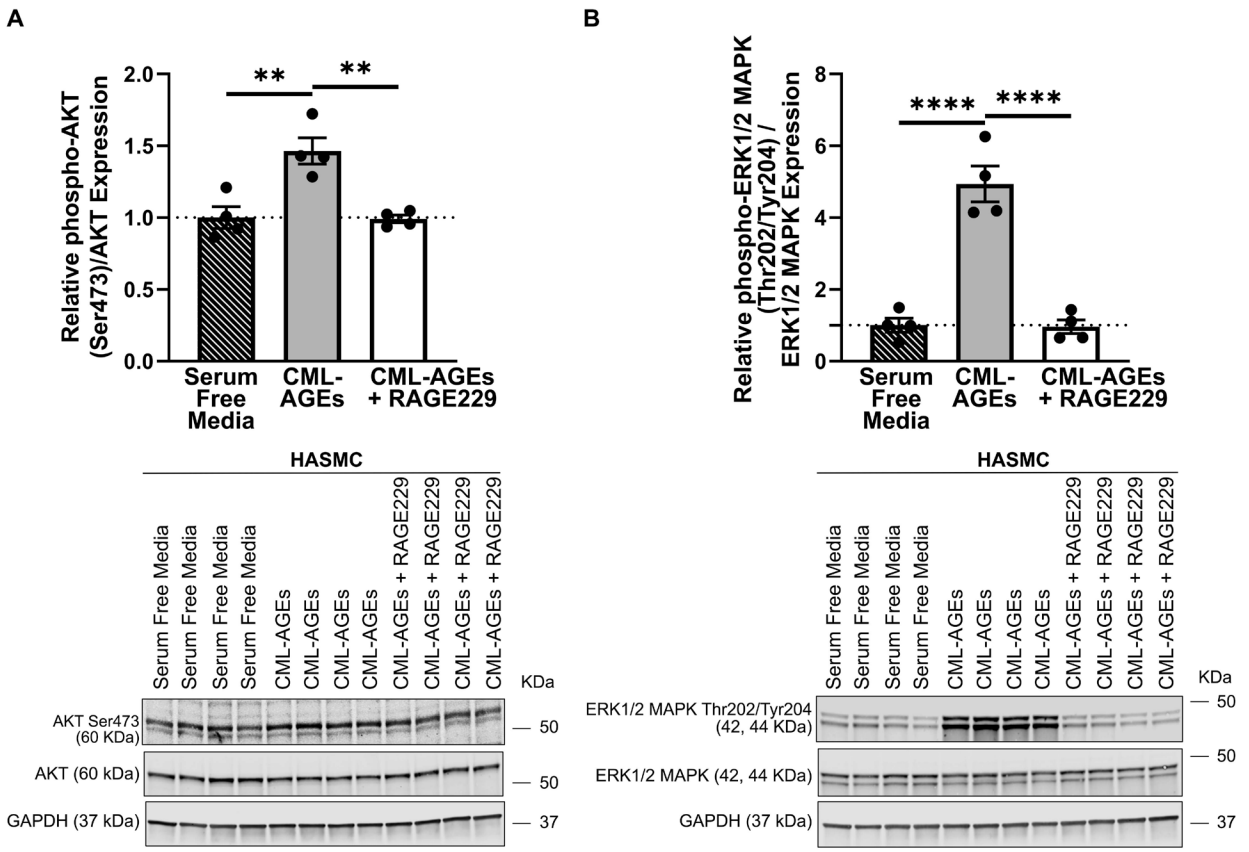
Supplemental Figure 6. Delayed-type hypersensitivity by intraperitoneal (IP) injection in female CF-1 mice. Mice were immunized and challenged with methylated (m)BSA and treated with RAGE229 or Vehicle. The small molecule antagonist or equal volume of vehicle, PBS, were administered by IP injection as described in Methods. Footpad inflammation scores are shown in mice treated with RAGE229 compared to Vehicle in female CF-1 mice. The number of mice per group is: Vehicle: N=8; RAGE229, N=9. As the data did not pass the Shapiro-Wilk test for normality, the Wilcoxon rank-sum test was employed. Statistical analysis was performed by Wilcoxon rank-sum test. Data are presented as Mean ± SEM. *indicates $P<0.05$.



Supplemental Figure 7. Treatment with RAGE229 protects from tubular atrophy and interstitial fibrosis in male and female T1D-like diabetic C57BL/6J mice. Male and female mice were randomly placed into diabetic (ND) or diabetic (DM) groups. Mice were treated with vehicle or STZ, respectively. DM animals were further randomized to either receive one of three concentrations (15ppm, 50ppm, 150ppm) of RAGE229-specially modified chow or Vehicle chow. Mice were euthanized after 6 months of STZ-induced hyperglycemia. A semi-quantitative scoring system was used to determine the percent (%) degree of tubular atrophy and interstitial fibrous by an investigator naïve to experimental conditions. The number of mice per group: Males: ND, N=11; DM Vehicle, N=5; RAGE229 15ppm, N=5; RAGE229 50ppm, N=9; and RAGE 229 150ppm, N=7. Females: ND, N=9; DM Vehicle, N=7; RAGE229 15ppm, N=6; RAGE229 50ppm, N=9; and RAGE 229 150ppm, N=5. For both male and female mice, the data did not pass the Shapiro-Wilk test for normality. Therefore, the Wilcoxon rank-sum test was employed. Data are presented as Mean ± SEM. *indicates $P<0.05$, **$P<0.01$.



Supplemental Figure 8. Treatment with RAGE229 protects from tubular atrophy and interstitial fibrosis in male and female T2D-like BTBR *ob/ob* mice. At 8 weeks of age, male and female BTBR *ob/ob* mice were randomly placed into treatment groups to receive 150ppm RAGE229-specially modified chow or Vehicle chow (Vehicle). Wild-type non-diabetic littermates (BTBR WT) were fed vehicle chow and used as control. Following 4 months of treatment, mice were euthanized. The number of mice per group: Males: WT (non-diabetic), N=8; DM Vehicle, N=7; DM RAGE229 150ppm, N=7. Females: WT (non-diabetic), N=8; DM Vehicle, N=8; DM RAGE229 150ppm, N=7. For both male and female mice, the data did not pass the Shapiro-Wilk test for normality. Therefore, the Wilcoxon rank-sum test was employed. Data are presented as Mean ± SEM. *indicates $P<0.05$, ***$P<0.001$.



Supplemental Figure 9. Effects of RAGE229 on RAGE ligand-stimulated phosphorylation of AKT and ERK1/2 MAPK in human primary aortic SMCs. Primary human SMCs were incubated with RAGE229 (1μM) for 1.5 h and then treated with RAGE-ligand CML-AGE (10 μg/ml) for 20 mins. Cells were harvested and total lysates were subjected to Western blotting with antibodies against phospho-AKT (Ser473) and phospho-ERK1/2 MAPK (Thr202/Tyr204). Blots were then stripped and re-probed with antibodies for total–AKT and total-ERK1/2 MAPK. Relative optical density (ROD) is reported. (A) Quantified amounts of phosphorylated/total AKT normalized to total AKT are shown. (B) Quantified amounts of phosphorylated/total ERK1/2 MAPK normalized to total ERK1/2 MAPK are shown. GAPDH was used only as a loading control and was not used in any measurement or for normalization. Assays shown are representative of four independent experiments. Data are presented as Mean ± SEM. *indicates $^{**}P<0.01$ and $^{****}P<0.0001$.

Table S1. Structural Statistics of the Ensemble of 20 ctRAGE-RAGE229 Conformers

Parameter	Value
NOE upper distance limits[a]	384
Intraresidual (i=j)	82
Short-range [(i-j) = 1]	191
Medium-range [1 < (i-j) ≤ 5]	92
Long-range	19
Intermolecular (between RAGE229 and ctRAGE)	13
RMM	1
Dihedral angle (ψ and ϕ) [a]	268 restraints 134 angles
Residual target function value (Å^2) [a]	3.70 ± 0.34
Residual distance restraint violations [a]	
Number ≥ 0.2 Å	5
Maximum (Å)	1.49
Residual van der Waals violations [a]	
Number ≥ 0.2 Å	3
Maximum (Å)	0.48
RMSD to the mean coordinates, residues 2-16 (Å)[b]	
Backbone	0.30
Ramachandran plot statistics (%) [b]	
Favored	64
Allowed	35
Outliers	1
All-atom clash score[b]	5

a) Given are the average values for the 20 conformers of the final ensemble with the least restraint violations, calculated using CYANA version 3.98.5 following seven cycles of simulated annealing with manually assigned NOEs.

b) Determined from the validation of the PDB deposition, with PDB ID 6VXG (http://deposit.pdb.org)

Table S2. Serum Glucose Concentrations in T1D-like Diabetic Mice Undergoing Myocardial Infarction

Treatment	Serum Glucose (mg/dl) (N/group)
Vehicle	356±31 (N=3)
RAGE203	368±50 (N=5)
RAGE208	364±56 (N=5)
RAGE229	327±21 (N=4)

The data did not pass the Shapiro-Wilk test for normality, therefore an unpaired t-test followed by Wilcoxon rank sum test was performed. There were no differences in serum glucose concentrations among the four groups of mice.

Table S3. Body Weight and Glucose Concentrations: Isolated perfused heart studies in T1D-like *Akita* mice

	Body Weight (g)	Glucose (mg/dl)
Treatment		
Vehicle (N=3)	22.0±0.5	446±12
RAGE229 (N=3)	21.1±0.3	361±42

Following confirmation that the data for weight and glucose measurements in the Vehicle- and RAGE229-treated mice passed the Shapiro-Wilk test for normality, statistical analysis was performed by unpaired t-test, and no differences were observed between treatment groups.

Table S4. Mouse Pharmacokinetic Data: RAGE229

PO: 10 mg/kg/mouse Oral (N=3/group)

$T_{1/2}$ (h)	T_{max} (h)	C_{max} (µM)	$AUC_{Inf/D}$ (hr*kg*µM/µmol)	%F
2.75±0.6	1.33±0.58	3.98±0.45	833±224	180±48

IV: 2 mg/kg/mouse IV (N=3/group)

$T_{1/2}$ (h)	AUC_{inf} (hr*µM)	V_{ss} (L/kg)	CL(ml/min/kg)	MRT_{inf}(hr)
0.389±0.048	240±0.16	0.773±0.138	36.1±2.3	0.359±0.079

Plasma Exposure (IV)

IV (5.18 µmol/kg)
Calculated concentration (µM)

Time	Mouse #1	Mouse #2	Mouse #3	Mean	SD
0.0833	6.57	5.41	5.64	5.87	0.62
0.25	2.23	2.72	2.11	2.35	0.32
0.5	0.950	1.36	1.06	1.12	0.21
1	0.285	0.427	0.339	0.350	0.072
2	0.0437	0.111	0.0691	0.0745	0.0338
4	BLOQ	0.00386	BLOQ	0.00129	NA
8	BLOQ	BLOQ	BLOQ	NA	NA
24	BLOQ	BLOQ	BLOQ	NA	NA

Plasma Exposure (PO)

PO (25.88 µmol/kg)
Calculated concentration (µM)

Time	Mouse #4	Mouse #5	Mouse #6	Mean	SD
0.25	1.48	1.66	0.939	1.36	0.38
0.5	2.61	2.92	1.71	2.42	0.63
1	4.45	3.96	3.29	3.90	0.58
2	3.98	3.49	3.54	3.67	0.27
4	3.00	1.93	1.84	2.26	0.65
8	1.22	0.618	0.621	0.820	0.347
24	BLOQ	BLOQ	BLOQ	NA	NA

BLOQ=below LLOQ, LLOQ=0.00259 µM

Table S5. Body Weight and Plasma RAGE229 Concentrations: DTH Study (Medicated Chow)

	Male		Female	
Treatment	Body Weight (g)	RAGE229 (ng/ml)	Body Weight (g)	RAGE229 (ng/ml)
Vehicle	25.0 ± 0.7	N/A	19.1 ± 0.4	N/A
RAGE229 (150 ppm)	24.7 ± 0.3	141.6 ± 51.4	18.1 ± 0	275.8 ± 76.6
RAGE229 (50 ppm)	26.5 ± 0.8	19.4 ± 3.5*	19.9 ± 0.1*	152.5 ± 36.2
RAGE229 (15 ppm)	25.4 ± 0.1	9.6 ± 1.6*	19.4 ± 0.6	10.2 ± 0.5*

*P<0.05 vs. 150 ppm

For male weight and female body weight and RAGE229 plasma concentrations, data passed Shapiro-Wilk test for normality and were analyzed using ANOVA with appropriate post-hoc test, as required.

Table S6. Body Weight and Glucose Concentrations: Wound Healing studies in T2D-like BTBR *ob/ob* mice

	Male		Female	
Treatment	Body Weight (g)	Glucose (mg/dl)	Body Weight (g)	Glucose (mg/dl)
Vehicle (N=6)	47.4 ± 2.6	412 ± 31	55.7 ± 1.1	334 ± 28
RAGE229 (N=6)	48.1 ± 3.7	413 ± 28	56.6 ± 1.8	341 ± 22

Following confirmation that the data for weight and glucose measurements in the Vehicle- and RAGE229-treated mice passed the Shapiro-Wilk test for normality, statistical analysis was performed by unpaired t-test, and no differences were observed between treatment groups.

Table S7. Body Weight, Glucose, RAGE229 Concentrations: Long-term study in T1D-like C57BL/6 mice

	Male			Female		
Treatment	Body Weight (g)	Glucose (mg/dl)	RAGE229 (ng/ml)	Body Weight (g)	Glucose (mg/dl)	RAGE229 (ng/ml)
Non-diabetes	33.62±1.35	112±7	N/A	26.44±0.75	162±18	N/A
Vehicle	25.48±1.18$^{\#\#}$	392±41$^{\#\#\#\#}$	N/A	21.78±1.64	482±16$^{\#}$	N/A
RAGE229 (150 ppm)	27.38±1.51$^{\#}$	360±36$^{\#\#\#}$	516.6±119.2	23.67±1.89	361±52$^{\#}$	354.7±25.7
RAGE229 (50 ppm)	27.67±1.70	379±33$^{\#\#\#\#}$	80.6±12.4**	21.32±1.89	408±27$^{\#}$	111.4±12.3****
RAGE229 (15 ppm)	25.35±1.28$^{\#\#}$	432±25$^{\#\#\#\#}$	16.2±3.8***	22.56±1.92	380±56$^{\#}$	31.8 ± 3.7$^{****,\wedge}$

#*P*<0.05; ##p=0.01; ###*P*<0.001; ####*P*<0.0001 vs. ND
P*<0.05; **P<0.01; ****P*<0.001; ** *P*<0.0001 vs. 150 ppm
^*P*<0.05 vs. 50ppm

For each of the analyses in male and female mice (body weight, glucose concentrations and RAGE229 concentrations), N=5 mice/group. For male weight and female weight, male glucose concentrations, and male and female plasma RAGE229 concentrations, data passed Shapiro-Wilk test for normality and were analyzed using ANOVA with appropriate post-hoc test, as required. For female glucose concentrations data did not pass the Shapiro-Wilk test for normality. Therefore, a Kruskal-Wallis test followed by the Wilcoxon rank-sum test was utilized. Note that by these statistical methods, there were no differences detected in body weight or glucose concentrations when comparing the vehicle-treated diabetic mice with any of the diabetic mice treated with RAGE229 medicated chow.

Table S8. Body Weight, Glucose, RAGE229 Concentrations: Long-term study in T2D-like BTBR *ob/ob* mice

	Male			Female		
Treatment	Body Weight (g)	Glucose (mg/dl)	RAGE229 (ng/ml)	Body Weight (g)	Glucose (mg/dl)	RAGE229 (ng/ml)
Non-diabetes	36.7±1.1	130±16	N/A	33.3±0.5	119± 21	N/A
DM, Vehicle	55.7± 3.6$^{\#\#\#}$	345±92$^{\#\#\#\#}$	N/A	66.2±4.0$^{\#\#\#\#}$	383± 90$^{\#\#}$	N/A
DM, RAGE229 (150 ppm)	43.9±2.5	474±71$^{\#\#\#\#,**}$	601.5±54.7	51.2±4.8$^{\#\#,**}$	483±28$^{\#\#}$	504.2±133.5

#*P*<0.05; ##*P*<0.01; ###*P*<0.001; ####*P*<0.0001 vs. Non-diabetes,
***P*<0.01 (RAGE229) vs. BTBR *ob/ob* (Vehicle)

The numbers of mice per group/study is as follows: for body weight and glucose concentrations in male mice: Non-diabetes, N=8; Vehicle, N=7; and 150ppm, N=7. For female mice: Non-diabetes, N=8; Vehicle, N=9; and 150ppm, N=8. For RAGE229 concentrations, Male mice: 150ppm, N=7 and Female mice: 150ppm, N=8 mice. For female body weight and male glucose concentrations data, data passed Shapiro-Wilk test for normality and were analyzed using a Welch's ANOVA with appropriate post-hoc test, as required. For male body weight and female glucose data, data did not pass the Shapiro-Wilk test for normality. Therefore, a Kruskal-Wallis test followed by the Wilcoxon rank-sum test was utilized.