SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1. Changes in the expression of pro-atherosclerotic mediators induce following RAAS activation following (A) a 4-week infusion of Ang II (1µg/kg/min) or vehicle in *ApoE* KO mice and *Ager/ApoE* DKO mice; (B) 6-weeks of a 0.05% (low) sodium diet or normal chow control in *ApoE* KO mice and *Ager/ApoE* DKO mice; (C) genetic *Ace2* deficiency in 18-week old *Ace2*/*ApoE* DKO mice and *Ager/Ace2*/*ApoE* TKO mice; or (D) following *ex vivo* exposure of aortae from *ApoE* KO and *Ager/ApoE* DKO mice to Ang II (1µM for 4 h); (E-H) shows induced changes in markers of activation of the RAAS including (E) sodium retention, (F) plasma renin activity and (G) plasma aldosterone levels following 6-weeks of a 0.05% (low) sodium diet and (H) levels of circulating Ang II in 18-week old *Ace2*/*ApoE* DKO mice and *Ace2*/*Ager/ApoE* TKO mice. Data are mean ± SEM; n=6-8 per group, *vs *ApoE* KO control, # vs *ApoE* KO + RAAS activation (respectively + Ang II *in vivo*, + low sodium diet + genetic *Ace2* deficiency or Ang II *ex vivo*), ANOVA $p<0.05$.

Supplementary Figure 2. Selective suppression of target genes using siRNA or scrambled control in cells subsequently exposed to Ang II (1µM for 2 h), as demonstrated by (A) the suppression of *Ager* expression in PMAEC; (B) the suppression of *p65* expression in PMAEC; (C) the effect of siRNA targeting *Ager, p65, PKCς* and *Dipah1* on the expression of *AT1R* and *Ager* in PMAEC; (D) the suppression of *PKCς* expression in PMAEC; (E) the suppression of *PKCς* expression in HMEC1; (F) the suppression of *Dipah1* in PMAEC; (G) the suppression of *Dipah1* in HMEC1; (H) the static adhesion of labelled monocytes to a monolayer of PMAEC exposed to Ang II (1µM for 4 h) in the presence or absence of pre-treatment with specific siRNA targeting *Ager* or *Diaph1*. Data are mean ± SEM; n=6-8 per group, *vs scrambled control + vehicle, $p<0.05$, # vs scrambled + Ang II, ANOVA $p<0.05$.

Supplementary Figure 3. The induction of gene expression following exposure to Ang II (1µg/ml for 2 h) as demonstrated by (A) the expression of early growth response gene (*Egr1*) in PMAECs from C57BL/6J and *Ager* KO mice; (B) the induction of p65 expression in AT_1R-CHO cells following exposure to the RAGE ligand, S100A8/A9 (1µM) or Ang II (1µM) in the presence or absence of expression of full length RAGE or truncated RAGE constructs; (C) the induction of p65 expression in AT_1R-CHO cells following exposure to Ang II (1µM) in the presence or absence of expression of RAGE constructs with mutations at potential phosphorylation targets; and (D) the induction of p65 expression in AT_1R-CHO cells following exposure to Ang II (1µM) in the presence or absence of expression of RAGE constructs without residues capable of sustaining phosphorylation (serines or threonines) and capable of mimicking its effects (Glutamate or aspartate; i.e. to replace the C-terminal human RAGE sequence SEEPEAGESSTGGP with PKGPQAGQGGAGGP; No STED). Data show mean ± SEM; n=6-8 per group; *vs pCIneo, $p<0.05$ Data are mean ± SEM; n=6-8 per group, * vs C57BL/6J-PMAEC, $p<0.05$, # vs CHO cells treated with Ang II, ANOVA $p<0.05$.

Supplementary Figure 4. Changes in the gene expression of leucocyte activation markers and RAGE ligands following exposure to Ang II (1 µM for 24 h) as demonstrated in (A) isolated bone-marrow derived macrophages from C57BL/6J mice and *Ager* KO mice; (B) isolated primary splenocytes from C57BL/6J mice and *Ager* KO mice; (C) the adhesion of primary splenocytes from C57BL/6J mice and *Ager* KO mice to an activated endothelial monolayer, (D-F) the increased expression of cognate ligands of RAGE including (D) circulating plasma levels of the RAGE ligand, S100A8/A9, (E) plasma AGE levels, and (F) the circulating AGE-precursor, methylglyoxal. Data are mean ± SEM; n=6-8 per group, *vs C57BL/6J, # vs C57BL/6J + Ang II, $p<0.05$. Data are mean ± SEM; n=8 per group*vs

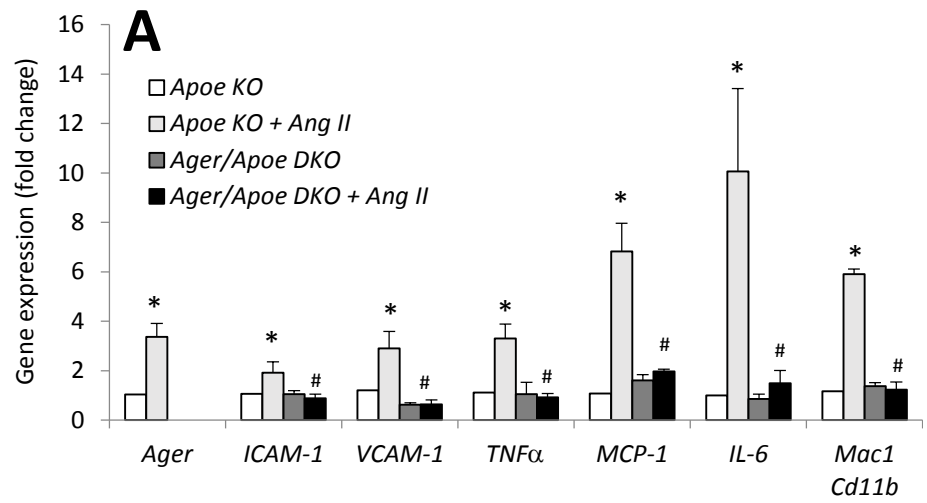
untreated *ApoE* KO mice, # vs A*poE* KO mice + RAAS activation (respectively Ang II or low sodium), ANOVA $p<0.05$.

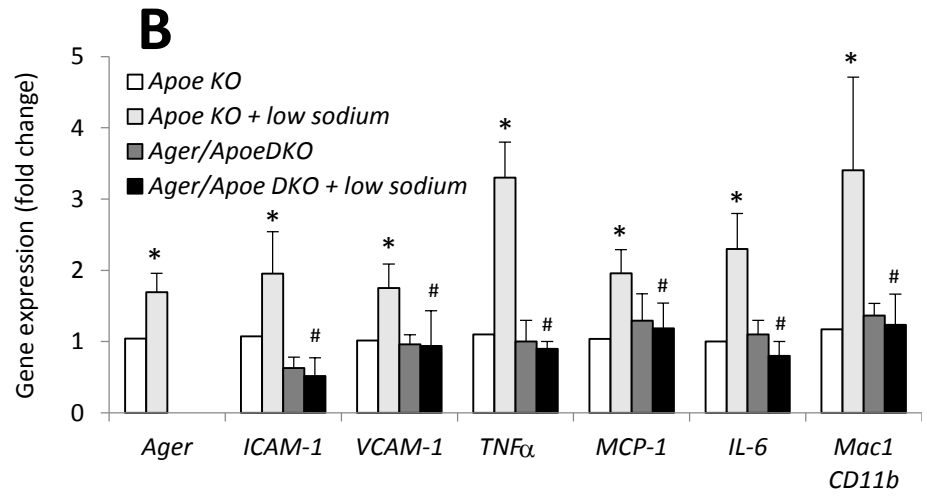
Supplementary Figure 5. Modulation of RAGE-dependent signalling, as demonstrated by (A) the induction of pro-inflammatory gene expression following exposure to the RAGE ligand S100A8/9 (2ng/mL for 2-h) in the presence and absence of a RAGE neutralising antibody (RAGEab) or soluble RAGE (sRAGE) in PMAEC; (B) the induction of pro-inflammatory gene expression following exposure to Ang II (1μM for 2 h) in the presence and absence of a RAGE neutralising antibody (RAGEab) or soluble RAGE (sRAGE) in PMAEC; (C) the suppression of Ang II-mediated induction of *MCP-1* expression by siRNA targeting *Ager, PKCζ* or *Diaph1* in HMEC1 and its rescue by mCherry-$RAGE_{362-404}$; (D) the induction of pro-inflammatory gene expression following exposure to Ang II (1μM for 2 h) in the presence and absence of mCherry-S391A-$RAGE_{362-404}$ or the AT_1R blocker, irbesartan; (E) the suppression of Ang II-mediated induction of *p65* expression in AT_1R-CHO cells by mCherry-S391A-$RAGE_{362-404}$ and its rescue by $RAGE_{370-404}$ and $RAGE_{370-392}$; (F) the suppression of Ang II-mediated induction of *p65* expression in AT_1R-CHO cells by mCherry-S391A-$RAGE_{362-404}$ and in cells expression wild type or S391Q-RAGE. Data are mean ± SEM; n=6-8 per group, *vs untreated PMAEC; # vs PMAEC + S100A8/A9; $ vs PMAEC + Ang II, ANOVA $p<0.05$.

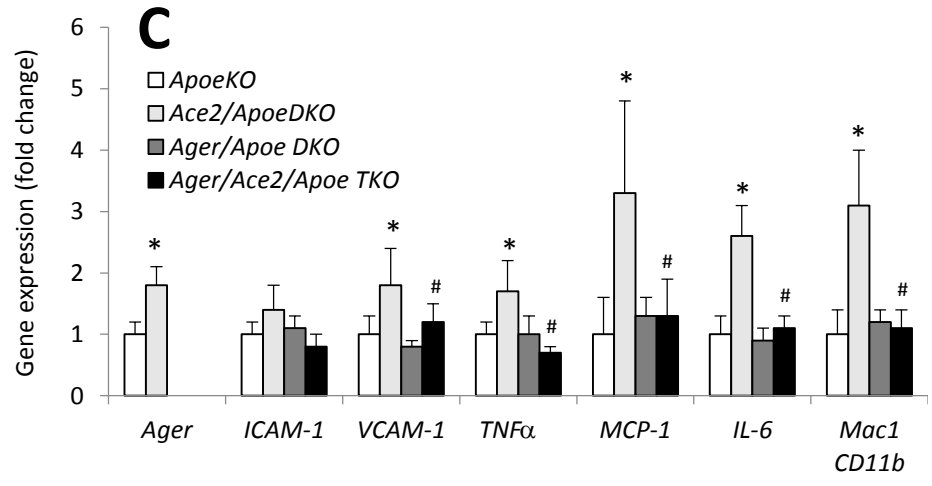
Supplementary Figure 6. The modulation of RAGE dependent signalling by mCherry-S391A-$RAGE_{362-404}$ as demonstrated by (A) the inhibition of signalling achieved by mCherry-S391A-$RAGE_{362-404}$ (0.4ng/mL) on Ang II-dependent in AT_1R-CHO cells, which is not reversed by pre-treatment with mCherry-$RAGE_{362-404}$; (B) the inhibition of signalling achieved by mCherry-S391A-$RAGE_{362-404}$ (1ng/mL) on Ang II-dependent in AT_1R-CHO cells

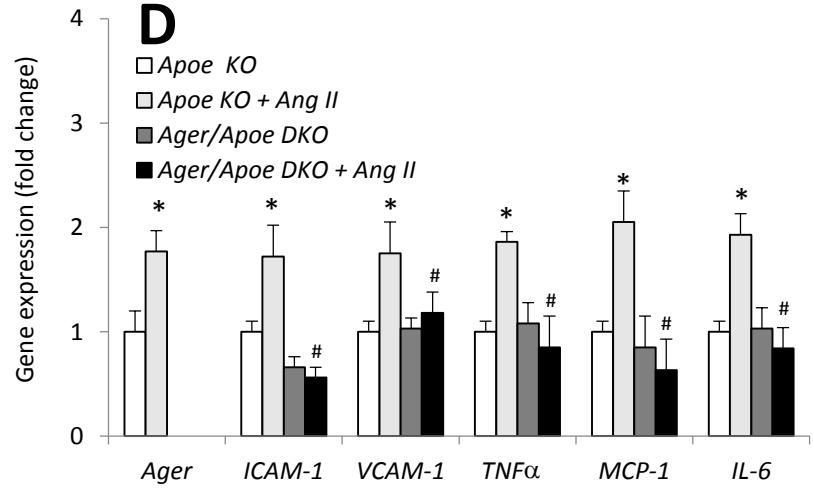
is not reversed subsequent treatment with mCherry-RAGE$_{362-404}$ (1-1000ng/mL); (C) the induction of *ICAM-1* expression in PMAEC following treatment by the RAGE ligand, S100A8/A9 (2ng/mL) or Ang II (1μM) and its inhibition by mCherry-S391A-RAGE$_{362-404}$; (D) the induction of *ICAM-1* expression in PMAEC following treatment by the prototypical NFκB-inducing cytokine, TNFα (0.1 ng/mL), or Ang II (1μM) and its inhibition by mCherry-S391A-RAGE$_{362-404}$; (E) the induction of *TNFα* expression following *ex vivo* exposure of whole aortae from *ApoE* KO mice and *Ager/ApoE* DKO mice to Ang II (1μM for 4h) in the presence and absence of pre-treatment with mCherry, mCherry-RAGE$_{362-404}$ or mCherry-S391A-RAGE$_{362-404}$ (0.4ng/mL); (F) the induction of *IL-6* expression following *ex vivo* exposure of whole aortae from *ApoE* KO mice and *Ager/ApoE* DKO mice to Ang II (1μM for 4h) in the presence and absence of pre-treatment with mCherry, mCherry-RAGE$_{362-404}$ or mCherry-S391A-RAGE$_{362-404}$ (0.4ng/mL)**.** Data are mean ± SEM; n=6-8 per group, * vs no treatment control, $p<0.05$. # vs mCherry + Ang II; ANOVA $p<0.05$.

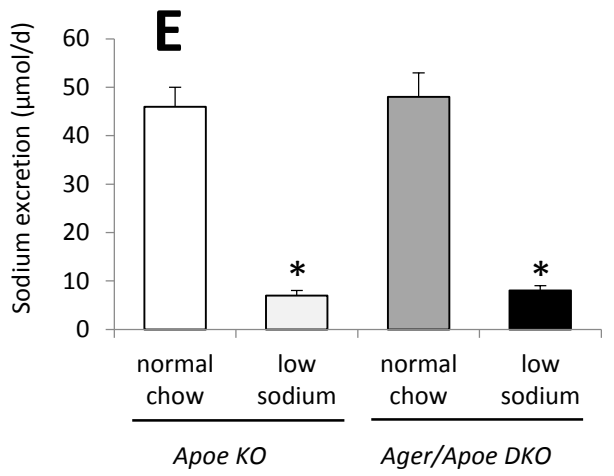
Supplementary Figure 1

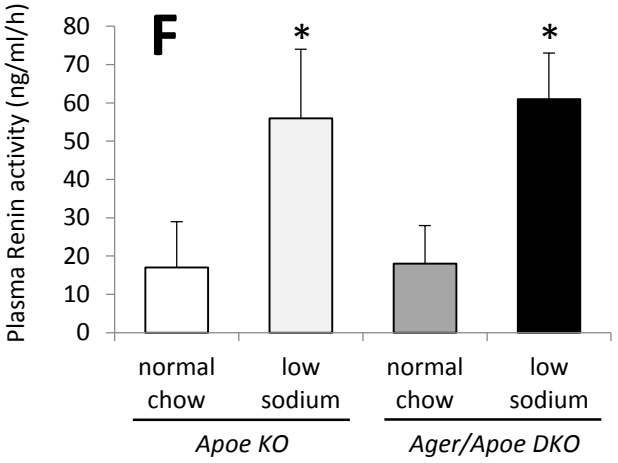


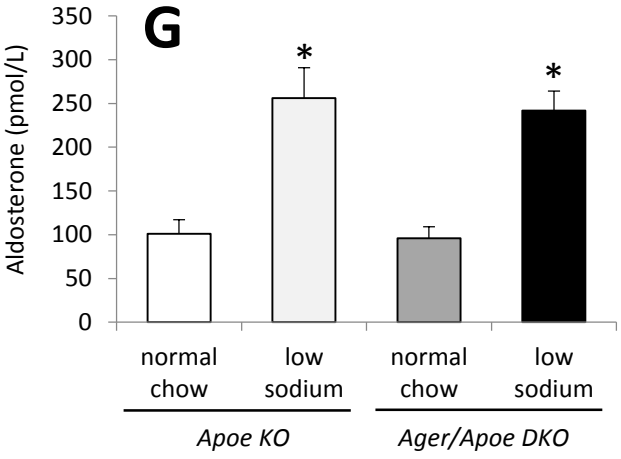


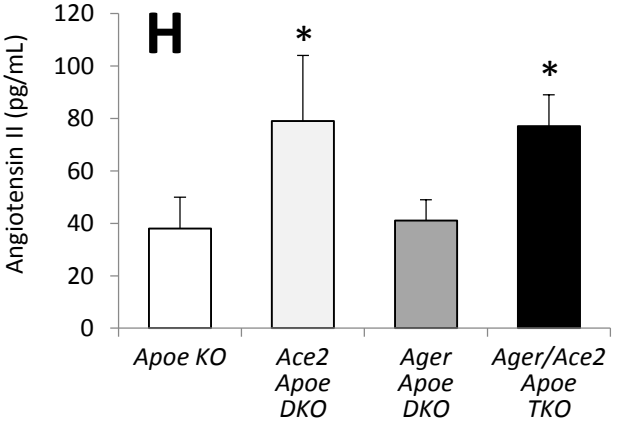












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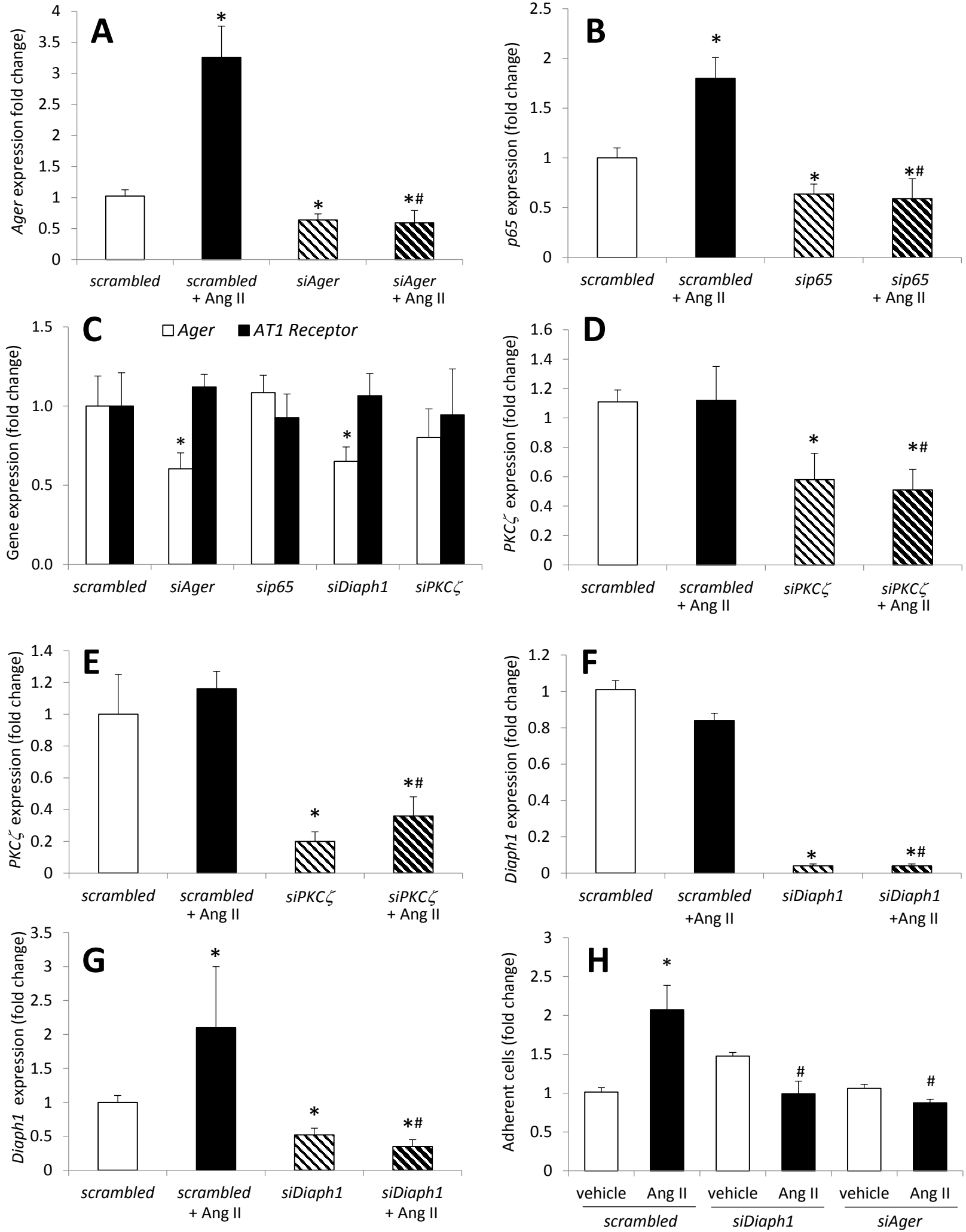
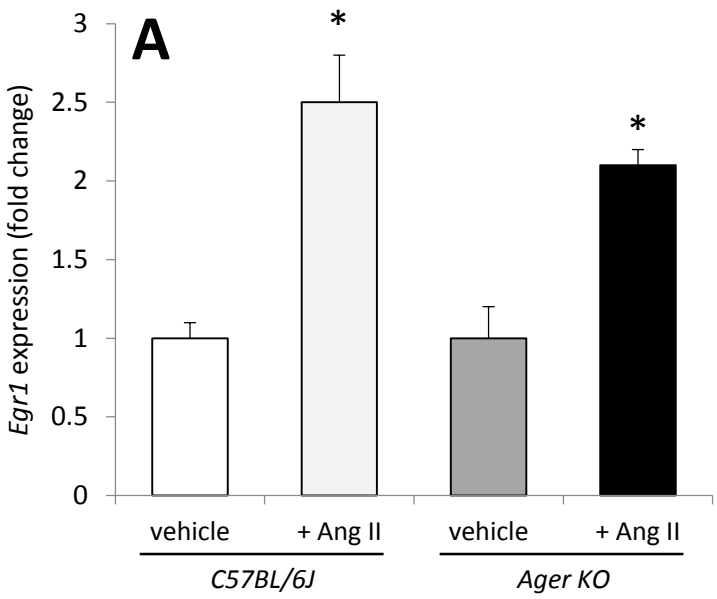
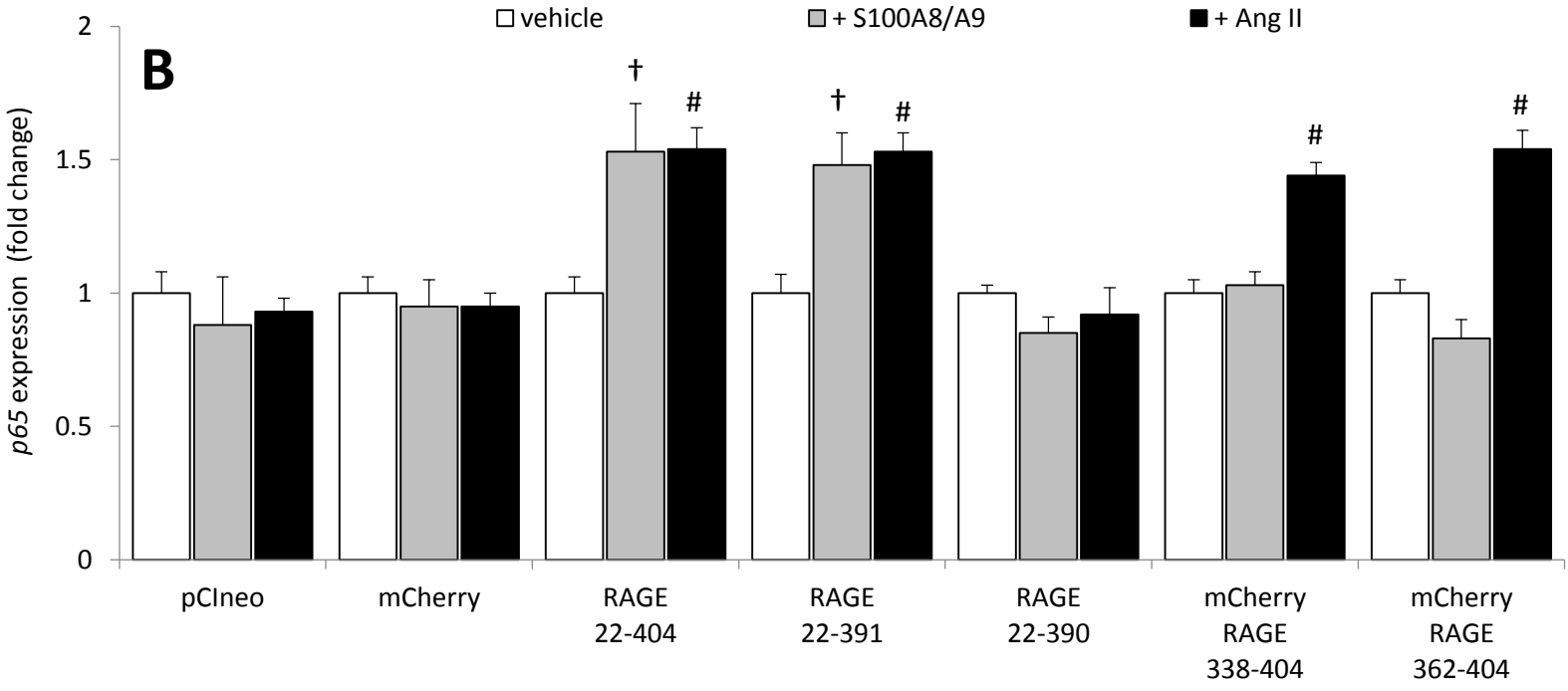
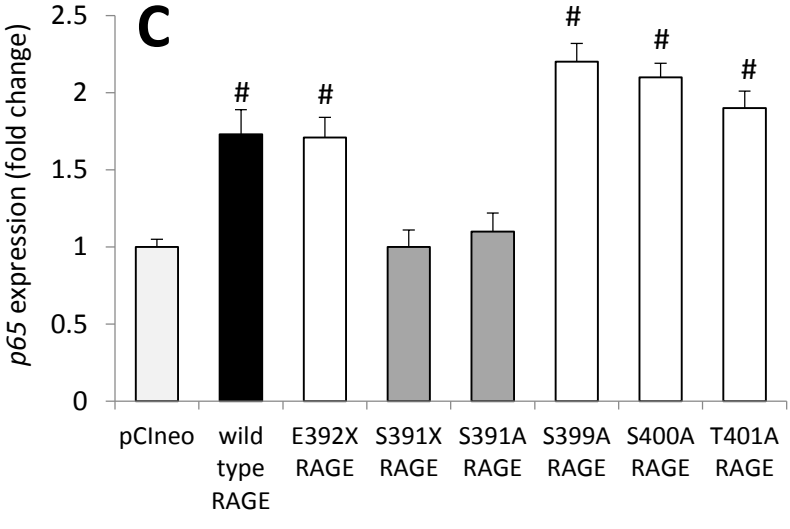
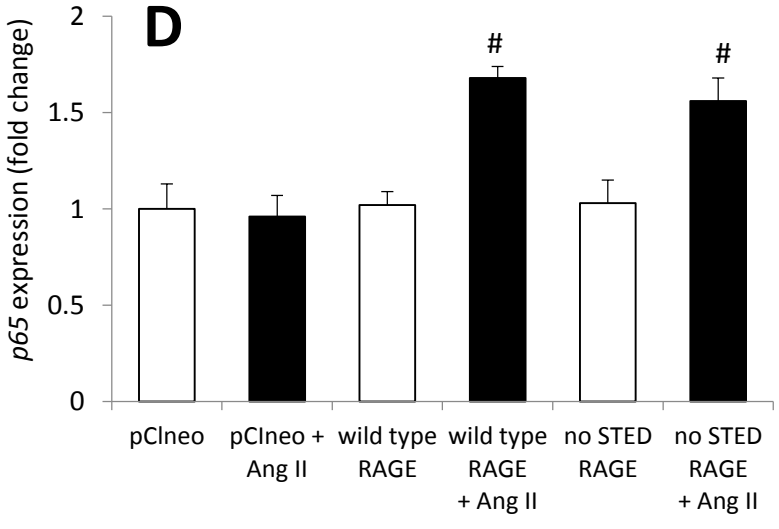


Supplementary Figure 3.

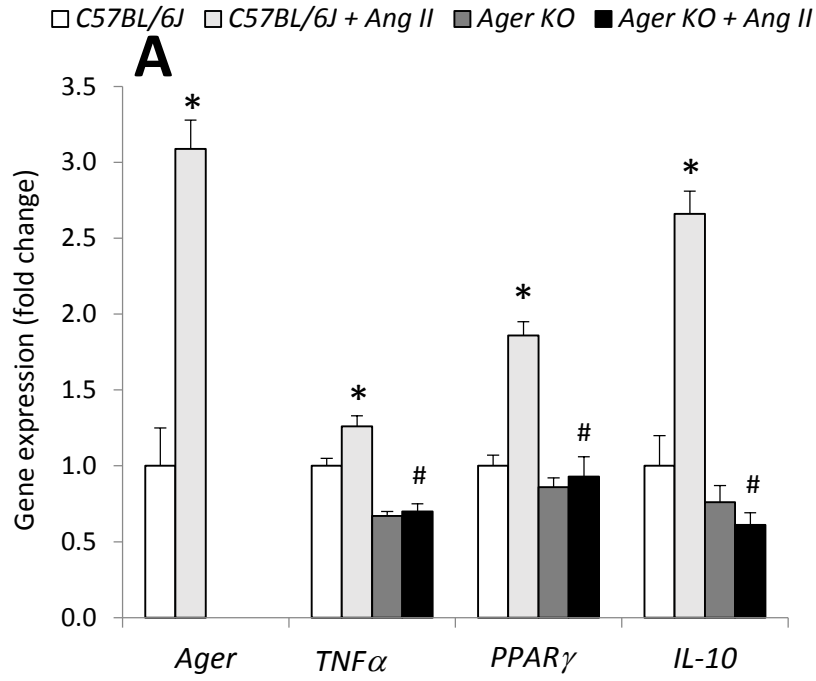


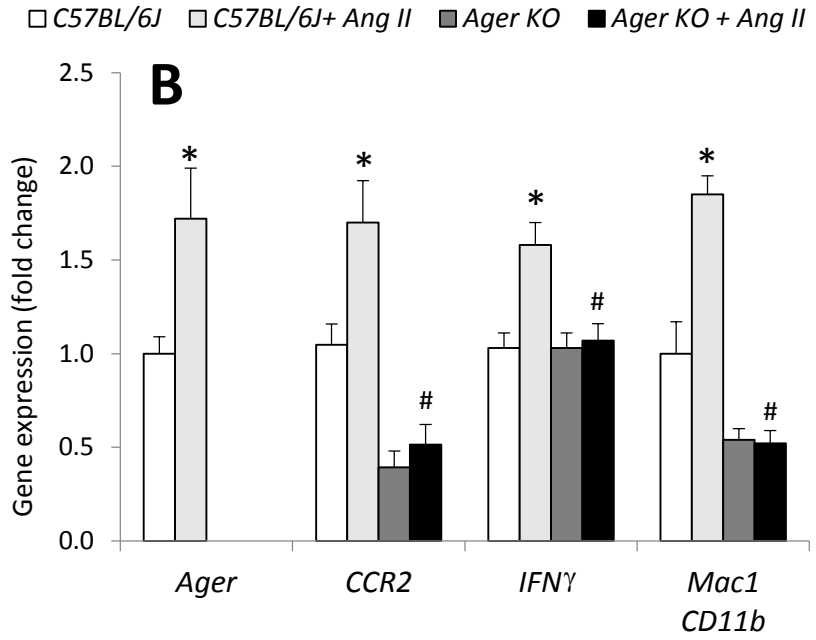


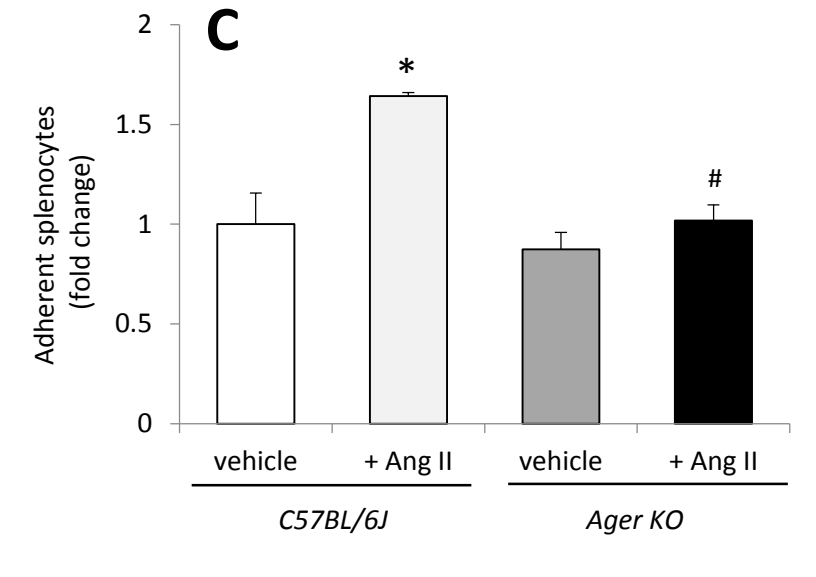


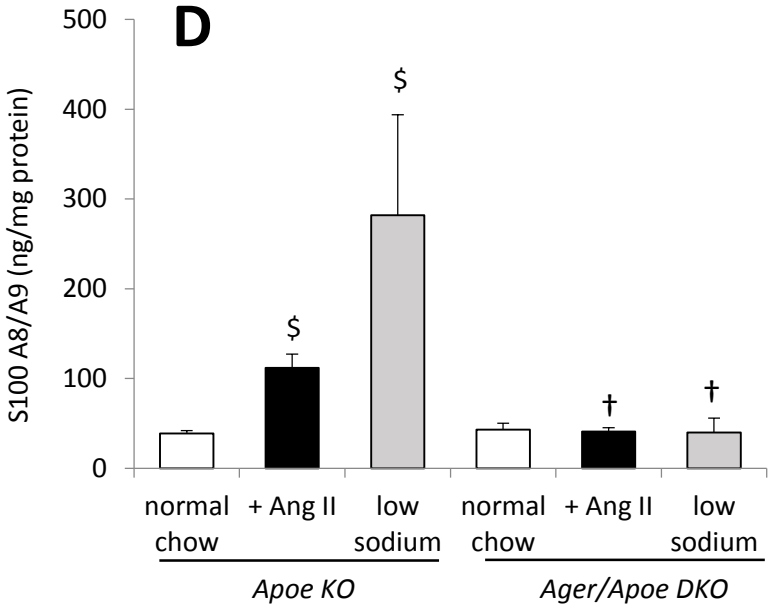


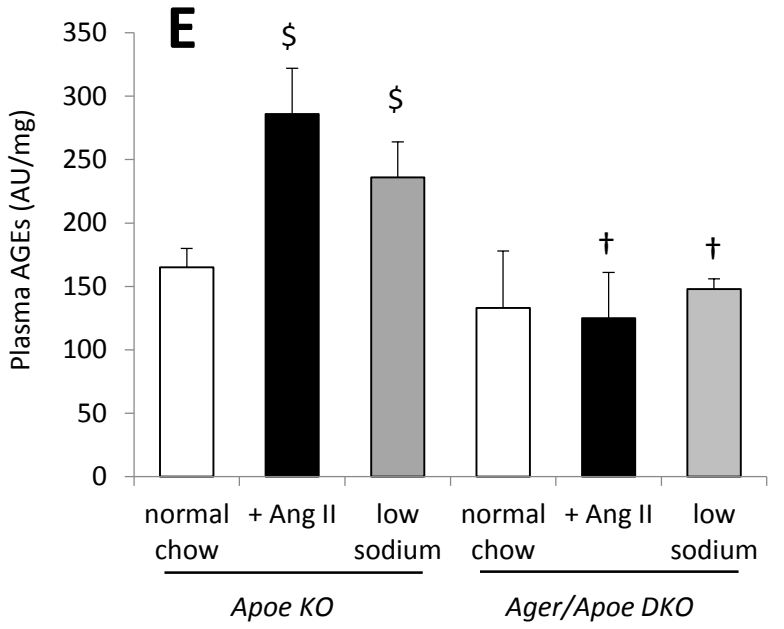
Supplementary Figure 4

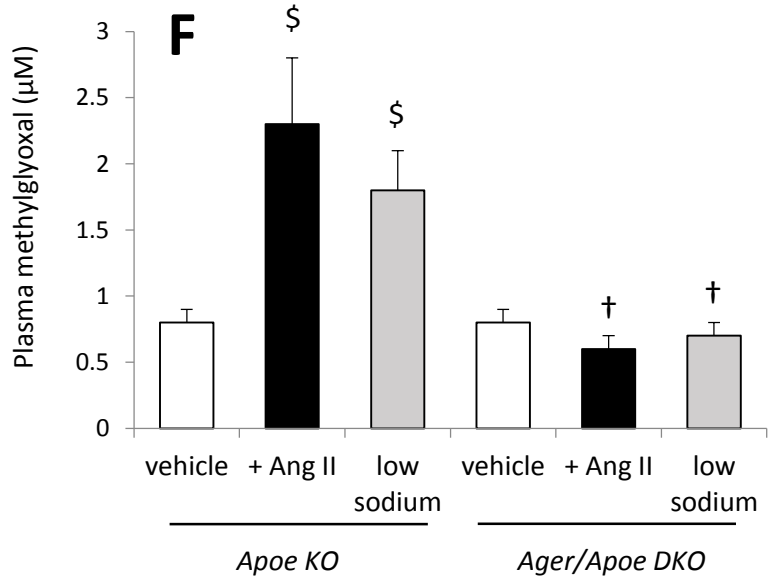




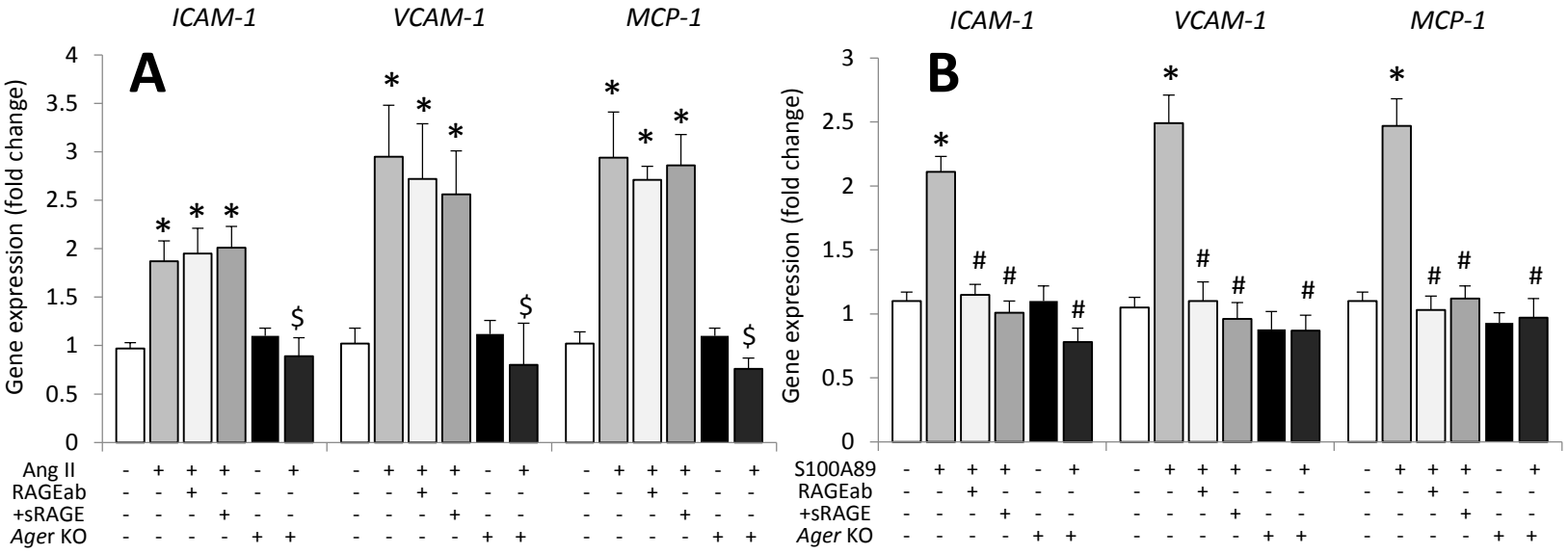


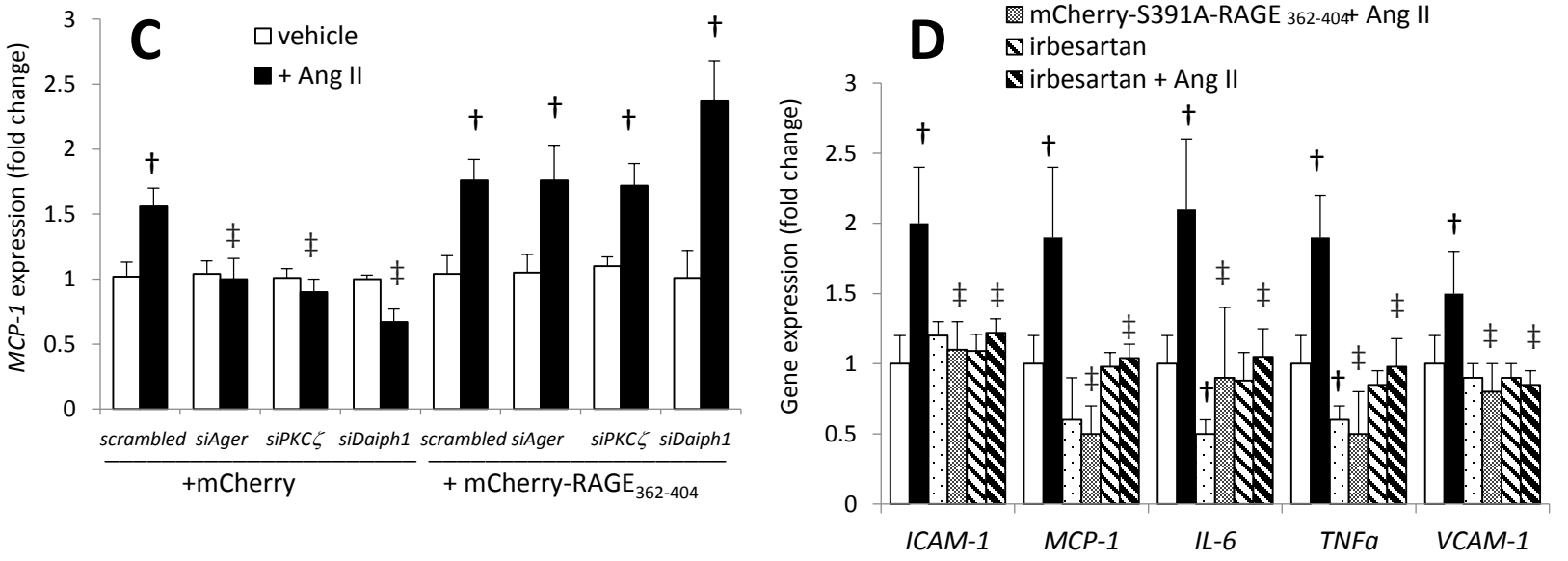


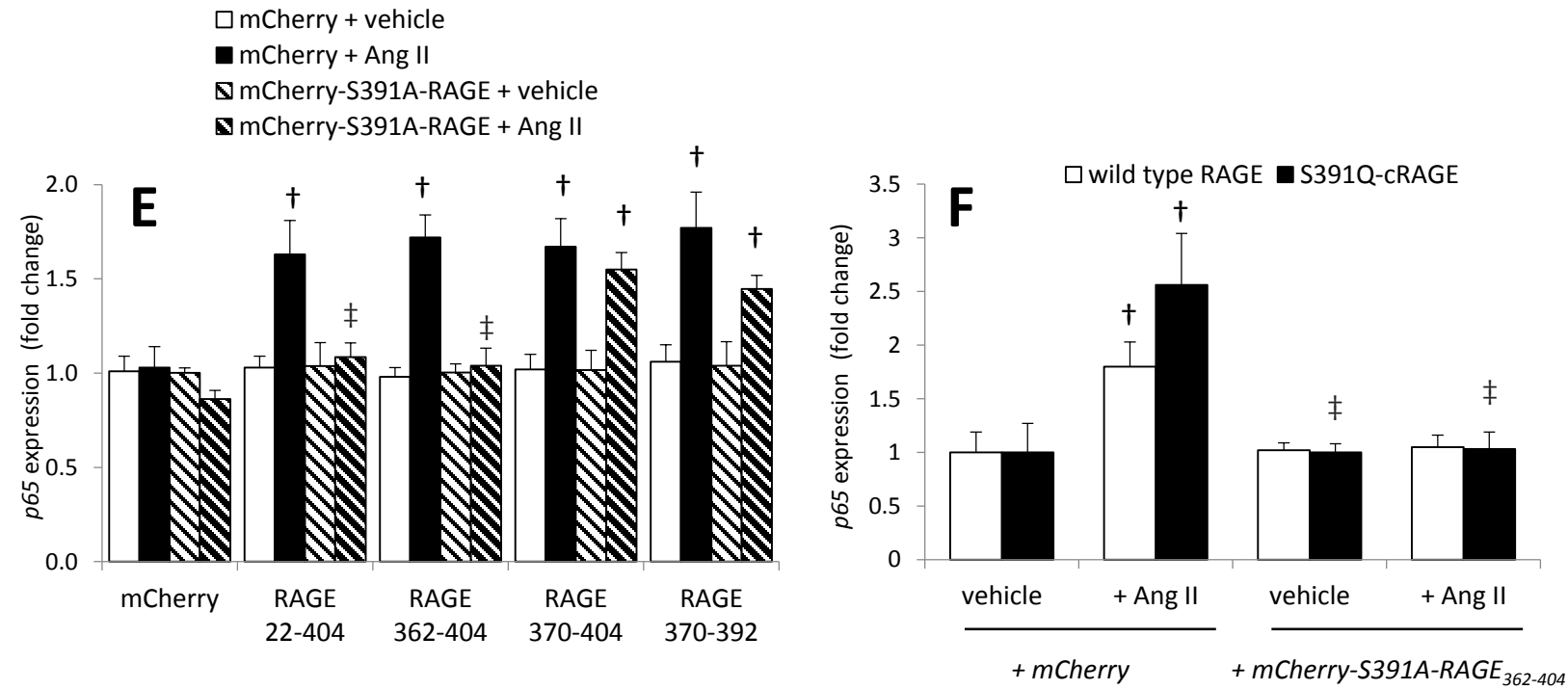




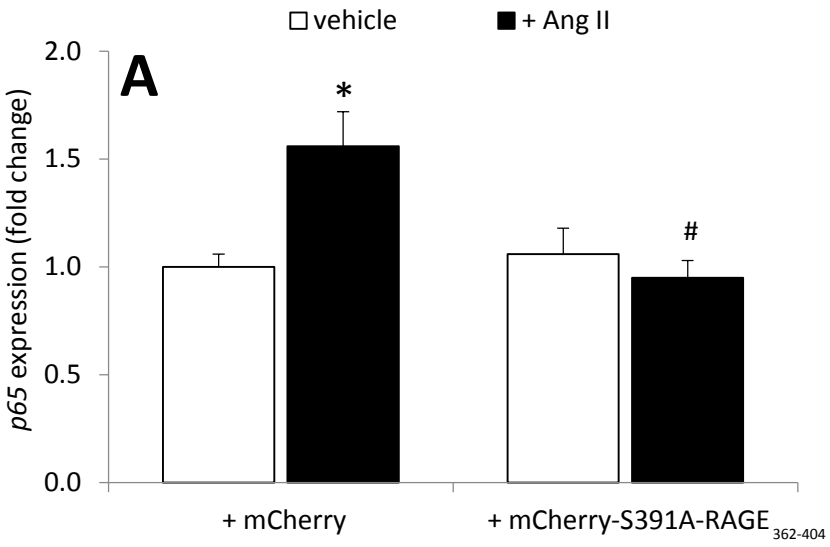
Supplementary Figure 5

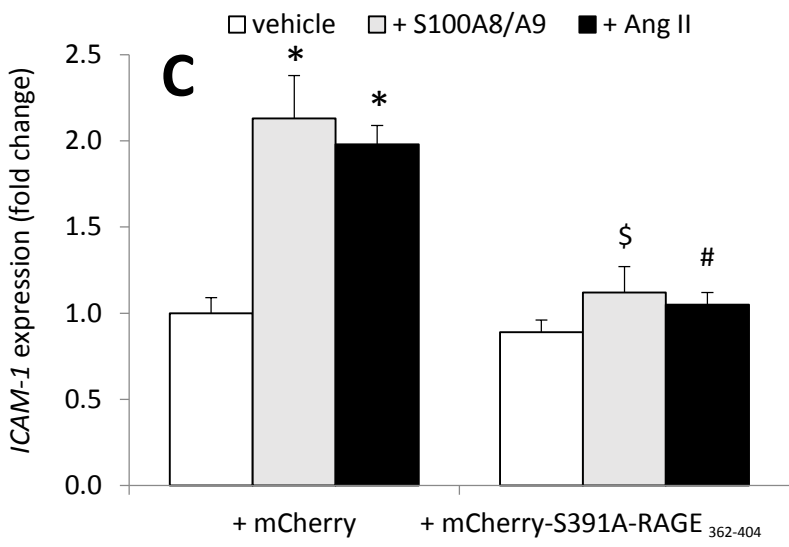


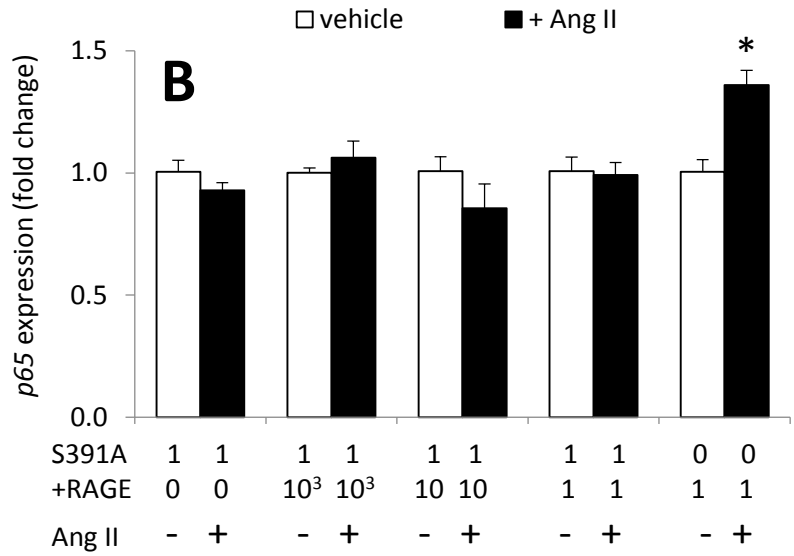


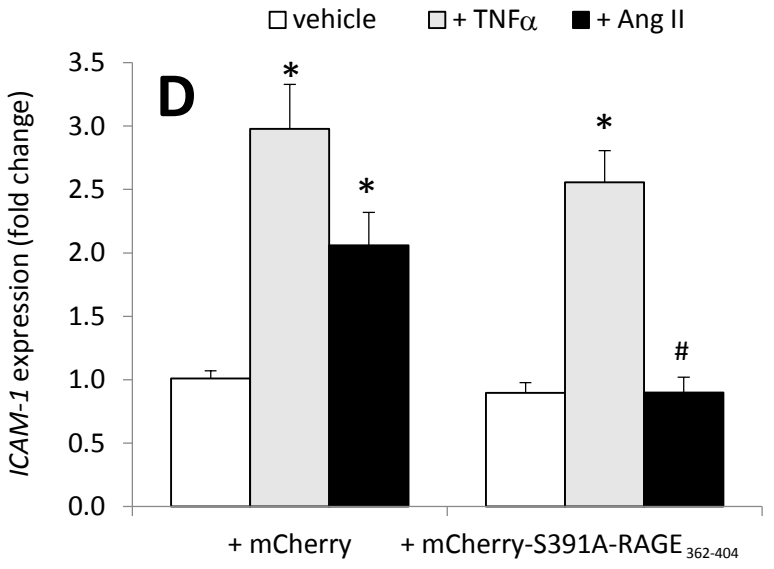


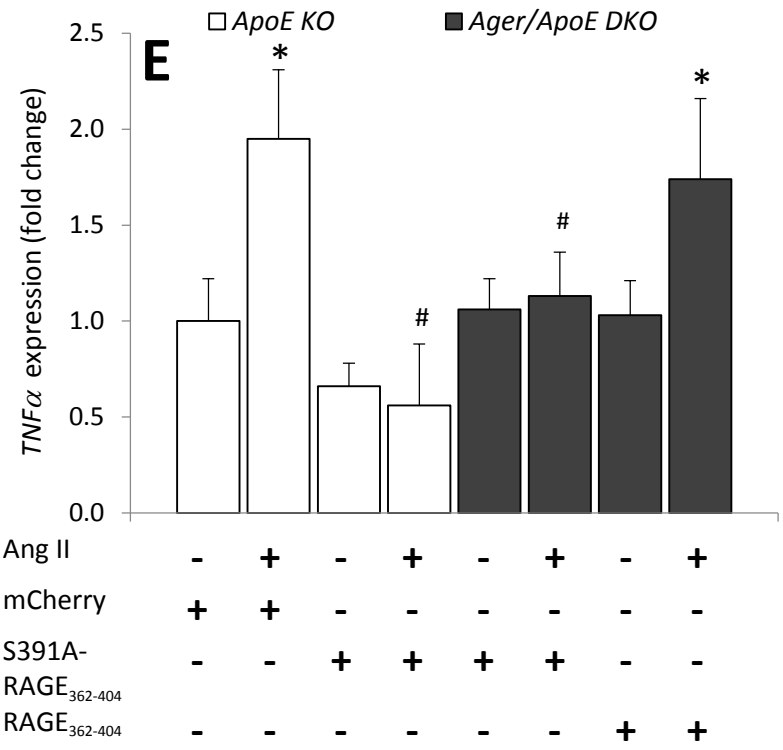
Supplementary Figure 6

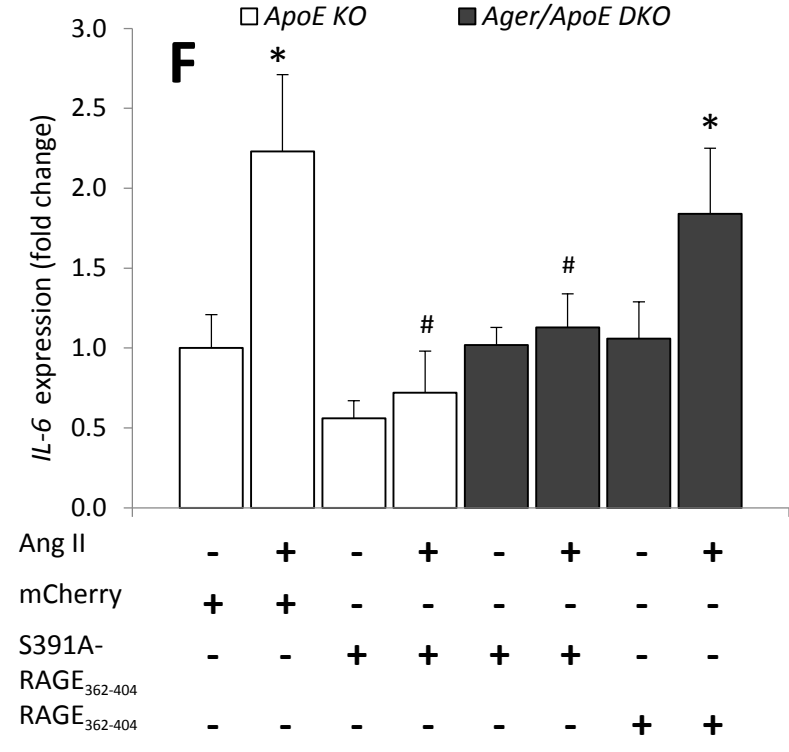












Supplementary Table 1.

Systolic blood pressure levels, as measured by tail-cuff plethysmography following a 4-week infusion of Ang II (1μg/kg/min) in *Apoe* KO mice and *Ager/Apoe* DKO mice, 6-weeks of exposure to a 0.05% (low) sodium diet in *Apoe* KO mice and *Ager/Apoe* DKO mice, associated with genetic *Ace2* deficiency in 18-week old *Ace2*/*Apoe* DKO mice and *Ace2*/*Ager/Apoe* TKO mice, and in mice receiving mCherry-S391A-RAGE$_{362-404,}$ mCherry-RAGE$_{362-404}$ or mCherry control at a dose of 10μg/kg/every second day by intraperitoneal injection for 10-weeks.

Data are mean ± SEM; n=8 per group, *vs control *Apoe* KO mice, p<0.05

	Systolic Blood Pressure
Apoe KO	95 ± 3
Apoe KO + Ang II	132 ± 6*
Ager/Apoe DKO	98 ± 4
Ager/Apoe DKO + Ang II	136 ± 10*
Apoe KO	98 ± 2
Apoe KO + Low Sodium Diet	101 ± 2
Ager/Apoe DKO	99 ± 2
Ager/Apoe DKO + Low Sodium Diet	100 ± 1
Apoe KO	97 ± 2
Ace2/Apoe DKO	108 ± 2*
Ager/Apoe DKO	101 ± 1
Ager/Ace2/Apoe TKO	106 ± 2*
Apoe KO	97 ± 2
Ace2/Apoe DKO + mCherry	112 ± 2*
Ace2/Apoe DKO + mCherry-s391A-RAGE$_{362-404}$	113 ± 1*
Ace2/Apoe DKO + mCherry-RAGE$_{362-404}$	110 ± 2*
Ager/Ace2/Apoe TKO + mCherry	112 ± 2*
Ager/Ace2/Apoe TKO + mCherry-RAGE$_{362-404}$	111 ± 1*
Apoe KO + mCherry	104 ± 2
Diabetes + *Apoe* KO + mCherry	107 ± 3
Diabetes + *Apoe* KO + mCherry-s391A-RAGE$_{362-404}$	105 ± 2
Diabetes + *Apoe* KO + mCherry-RAGE$_{362-404}$	106 ± 1
Diabetes +*Ager/Apoe* DKO + mCherry	107 ± 2
Diabetes + *Ager/Apoe* DKO + mCherry-RAGE$_{362-404}$	108 ± 2

Supplementary Table 2.

Sequence conservation of the thirty-five C-terminal residues of RAGE across different mammals. Red denotes regions of sequence homology. Blue denotes conservation around potential phosphorylation targets (serines or threonines) in the human sequence (S391, S399, S400, and T401).

	370										380										390										400				
	0	1	2	3	4	5	6	7	8	9	0	1	2			3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4
Homo sapiens	G	E	E	R	K	A	P	E	N	Q	E	E	E	.	.	E	E	R	A	E	L	N	Q	S	E	E	P	E	A	G	E	S	S	T	G	G	P
Macaca mulatta	G	E	E	R	K	A	P	E	N	Q	E	E	E	.	.	E	E	R	A	E	L	N	Q	S	E	E	P	E	A	G	E	S	S	T	G	G	P
Pan troglodytes	G	E	E	R	K	A	P	E	N	Q	E	E	E	.	.	E	E	R	A	E	L	N	Q	S	E	E	P	E	A	G	E	S	S	T	G	G	P
Gorilla gorilla gorilla	G	E	E	R	K	A	A	E	N	Q	E	E	E	.	.	E	E	C	A	E	L	N	Q	S	E	E	P	Q	A	G	E	S	S	T	G	G	P
Papio anubis	R	E	E	R	K	A	S	E	N	Q	E	E	E	.	.	E	E	R	A	E	L	N	Q	S	E	E	P	E	A	G	E	S	G	T	G	G	P
Mus musculus	R	E	E	R	K	A	P	E	S	Q	E	D	E	.	.	E	E	R	A	E	L	N	Q	S	E	E	A	E	M	P	E	N	G	A	G	G	P
Cricetulus griseus	G	E	E	R	K	A	P	E	N	P	Q	D	E	.	.	E	E	R	A	E	L	N	Q	S	E	D	A	D	A	A	E	N	G	A	G	G	P
Equus caballus	G	K	E	R	K	V	P	E	H	Q	E	E	E	.	.	E	E	R	A	E	L	N	Q	S	E	E	P	E	A	A	E	S	S	A	G	G	P
Camelus ferus	G	E	E	R	K	A	P	E	N	Q	E	E	E	E	.	E	E	R	A	E	L	N	Q	Q	E	E	P	E	A	A	E	S	S	A	G	G	P
Sus scrofa	G	Q	E	R	K	A	P	E	N	Q	E	E	D	E	.	E	E	R	A	E	L	N	Q	P	E	D	P	E	A	A	E	S	S	A	G	A	P
Rattus norvegicus	L	E	E	R	K	A	P	E	S	Q	E	D	E	.	.	E	E	R	A	E	L	N	Q	S	E	E	A	E	M	P	E	N	G	A	G	G	P
Vicugna pacos	G	E	E	R	K	A	P	E	N	Q	E	E	E	E	.	E	E	R	A	E	L	N	Q	Q	E	E	P	E	A	A	E	S	S	A	G	G	P
Panthera tigris altaica	G	E	E	R	K	T	P	E	N	Q	E	E	E	E	.	E	E	R	E	E	L	N	Q	S	E	E	P	E	A	A	E	G	S	A	A	G	P
Chinchilla lanigera	G	K	E	R	K	A	P	E	S	Q	E	E	E	.	.	E	E	R	A	E	L	N	Q	S	E	E	P	E	T	A	E	R	G	T	G	G	P
Bison bison bison	G	Q	E	R	K	V	P	E	N	Q	E	E	E	E		E	E	R	A	E	L	N	Q	P	E	E	P	E	A	A	E	S	S	T	G	G	P
Canis lupus familiaris	R	Q	E	R	K	A	P	E	N	Q	E	E	E	E	E	E	E	R	T	E	L	N	Q	S	E	E	P	E	A	A	E	S	G	A	A	G	P
Bos taurus	G	Q	E	R	K	V	P	E	N	Q	E	E	E	E	.	E	E	R	A	E	L	N	Q	P	E	E	P	E	A	A	E	S	S	T	G	G	P
Ovis aries	G	Q	E	R	K	V	P	E	N	Q	E	E	E	E	.	E	E	R	A	E	L	N	Q	P	E	E	P	E	A	A	E	S	S	T	G	G	P
Capra hircus	G	Q	E	R	K	V	P	E	N	Q	E	E	E	E	.	E	E	R	A	E	L	N	Q	P	E	E	P	E	A	A	E	S	S	T	G	G	P
Felis catus	G	E	E	R	K	A	P	E	N	Q	E	E	E	E	E	E	E	R	E	E	L	N	Q	S	G	E	P	E	A	A	E	G	S	A	A	G	P
Heterocephalus glaber	G	K	E	R	K	V	P	E	S	Q	E	E	Q	.	.	E	E	R	A	G	L	N	Q	S	E	V	P	E	T	A	G	S	S	T	G	G	P
Phascolarctos cinereus	R	E	E	R	K	V	P	E	C	P	E	E	E	.	.	E	Q	R	T	E	L	N	Q	P	E	E	Q	E	T	A	E	S	N	A	G	G	P
Castor canadensis	G	E	E	R	K	V	P	D	N	Q	E	E	E	.	.	E	E	R	T	E	L	N	Q	S	E	E	P	E	A	A	E	S	G	A	G	R	P
Sarcophilus harrisii	K	E	E	R	K	V	P	E	C	P	E	E	E	.	.	E	E	R	A	E	L	N	Q	P	E	K	E	E	T	A	E	N	N	E	G	E	P
Monodelphis domestica	K	E	E	R	K	V	P	E	C	P	E	E	E	.	.	E	E	R	A	E	L	H	Q	S	E	E	Q	E	T	A	E	S	N	A	G	G	P