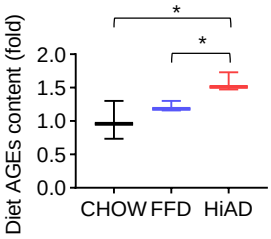
Supplementary Figure 1.



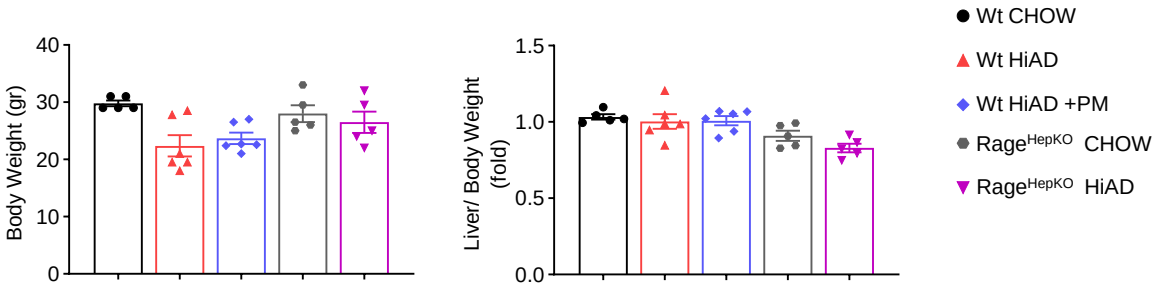
Supplementary Figure 1. Diet composition and AGEs content of regular chow, fast food diet (FFD), and high AGE diet (HiAD). Heating FFD at 121°C for 20 minutes significantly increased the amount of AGEs (3 different batches of each diet, *p<0.05, Unpaired two tail t.test, Bar: 25-75 percentile, line: Median, whiskers: Min/ Max). Diet compositions of each diet are listed in Supplementary Table 1.

Supplementary Table 1

	Regular CHOW [#]	Fast food diet (FFD) [$]	High AGEs content diet (HiAD) [&]
Calories	310 kcal/100g	449 kcal/100g	441 kcal/100g
Protein	18.6%	17.4%	16.7%
Carbohydrates	44.2%	49.9%	56.8%
Fiber	14.7%	5.0%	5.6%
Fat	6.2%	20.0%	16.3%
Sucrose	-	34.0%	32.4%
Cholesterol	-	2027 ppm	2030 ppm
Total Saturated Fatty Acids	0.9%	12.09%	10.21%
Polyunsaturated Fatty Acids	3.4%	0.58%	0.97%
Monounsaturated Fatty Acids	1.3%	4.61%	3.85%

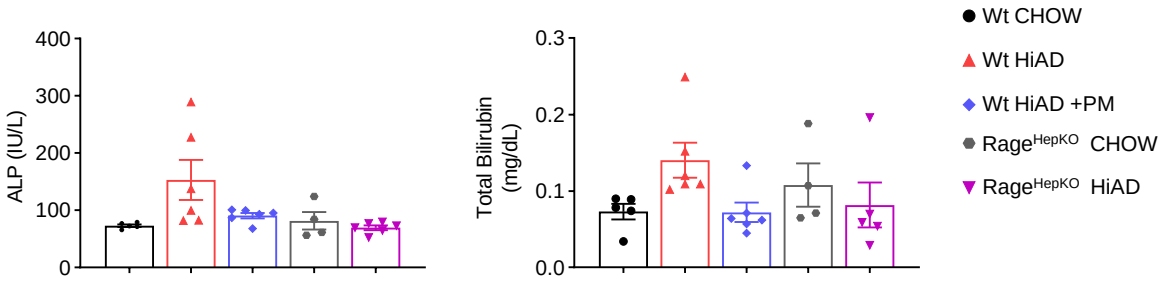
Supplementary Table 1. Diet composition and AGEs content of regular chow, fast food diet (FFD), and high AGE diet (HiAD). [#] Teklad Global 18% Protein Rodent Diet (Cat# 2918, data provided by the vendor Envigo), [$] AIN-76A Western Diet (Cat#1810060, data provided by the vendor TestDiet), [&] Prepared by heating FFD at 121° C for 20 minutes (data generated by Eurofins Scientific Testing laboratories company).

Supplementary Figure 2.



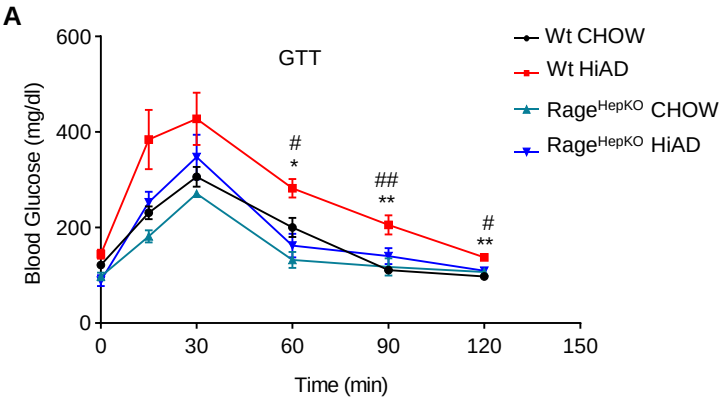
Supplementary Figure 2. Body weights and liver/body weight ratios do not change significantly in the experimental groups. *Fl/fl* and *Rage^{HepKO}* mice were placed on chow or high AGE diet (HiAD) for 14 weeks with daily Pyridoxamine, i.p. (PM, 60 mg/kg) or vehicle injection. Body and liver weights were recorded at the end of the experiments. (Data are represented as mean±SEM; One-way ANOVA.)

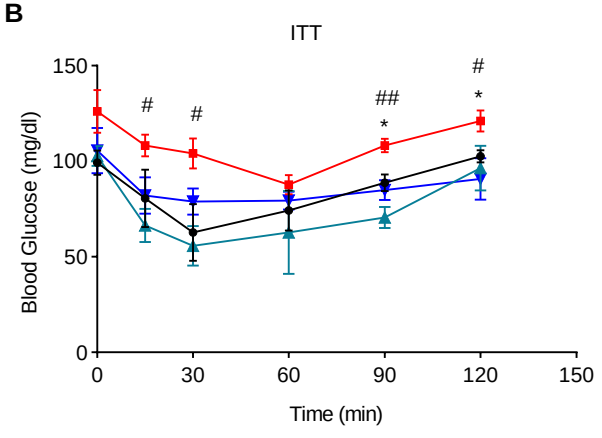
Supplementary Figure 3.



Supplementary Figure 3. Alkaline phosphatase (ALP) and bilirubin showed trends in increase in HiAD fed mice. *Fl/fl* and *RageE*^{HepKO} mice were placed on chow or high AGE diet (HiAD) for 14 weeks with daily Pyridoxamine, i.p. (PM, 60 mg/kg) or vehicle injection. Serum ALP and serum total bilirubin were measured. (Data are represented as mean±SEM; One-way ANOVA).

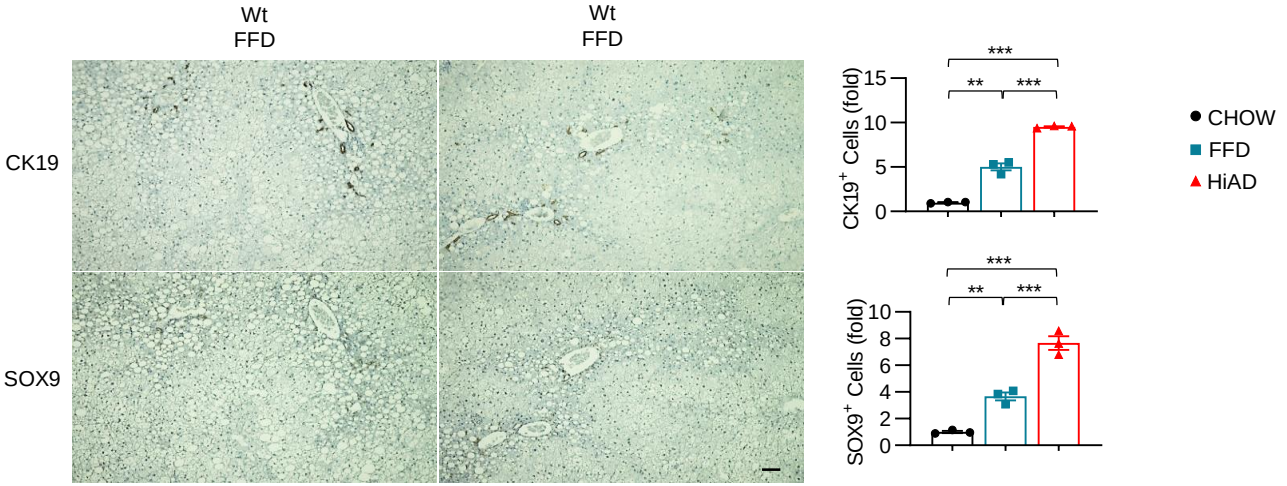
Supplementary Figure 4.





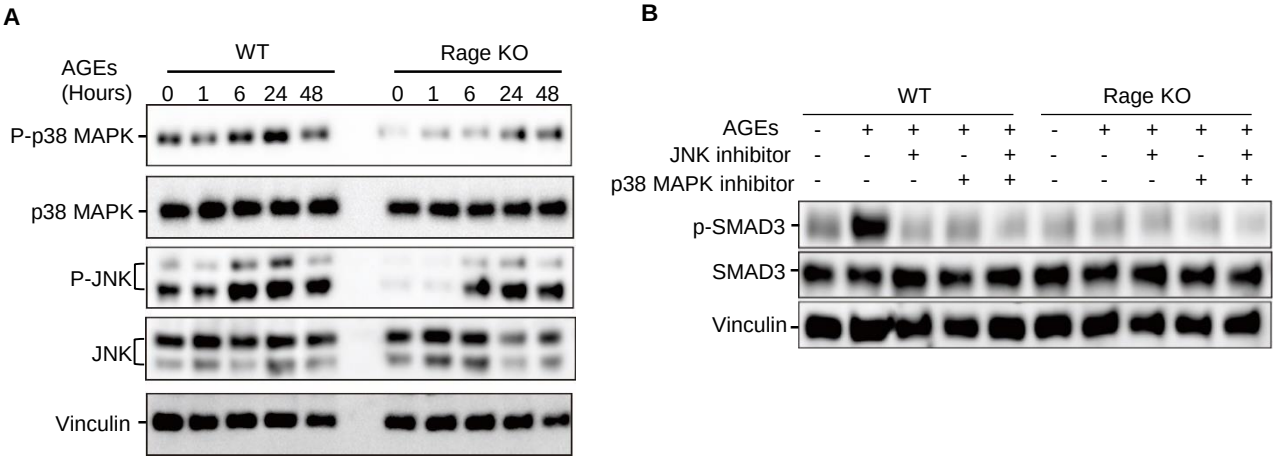
Supplementary Figure 4. Glucose tolerance and insulin sensitivity are improved in *Rage*^{HepKO} mice on HiAD. (A) Glucose tolerance test (GTT) was performed in WT and *Rage*^{HepKO} mice fed chow or HiAD for 14 weeks. Following overnight fasting, mice were injected i.p. with 2mg/g glucose, and glucose levels were measured at the indicated times. There was an improvement in glucose tolerance in *Rage*^{HepKO} mice. **(B)** Insulin tolerance test (ITT) was performed after 4 hours fasting (1 mU/g, i.p. Insulin). *Rage* deletion in hepatocytes significantly improved insulin sensitivity in mice on HiAD (Mean ± SEM, *p<0.05 WT CHOW vs HiAD, **p<0.01 WT CHOW vs HiAD, #p<0.05 WT vs. *Rage*^{HepKO} HiAD, ##p<0.01 WT vs. *Rage*^{HepKO} HiAD, One way ANOVA, followed by post hoc Tukey test. *n*= WT CHOW:4, WT HiAD: 6, *Rage*^{HepKO} CHOW: 3, *Rage*^{HepKO} HiAD: 6).

Supplementary Figure 5.



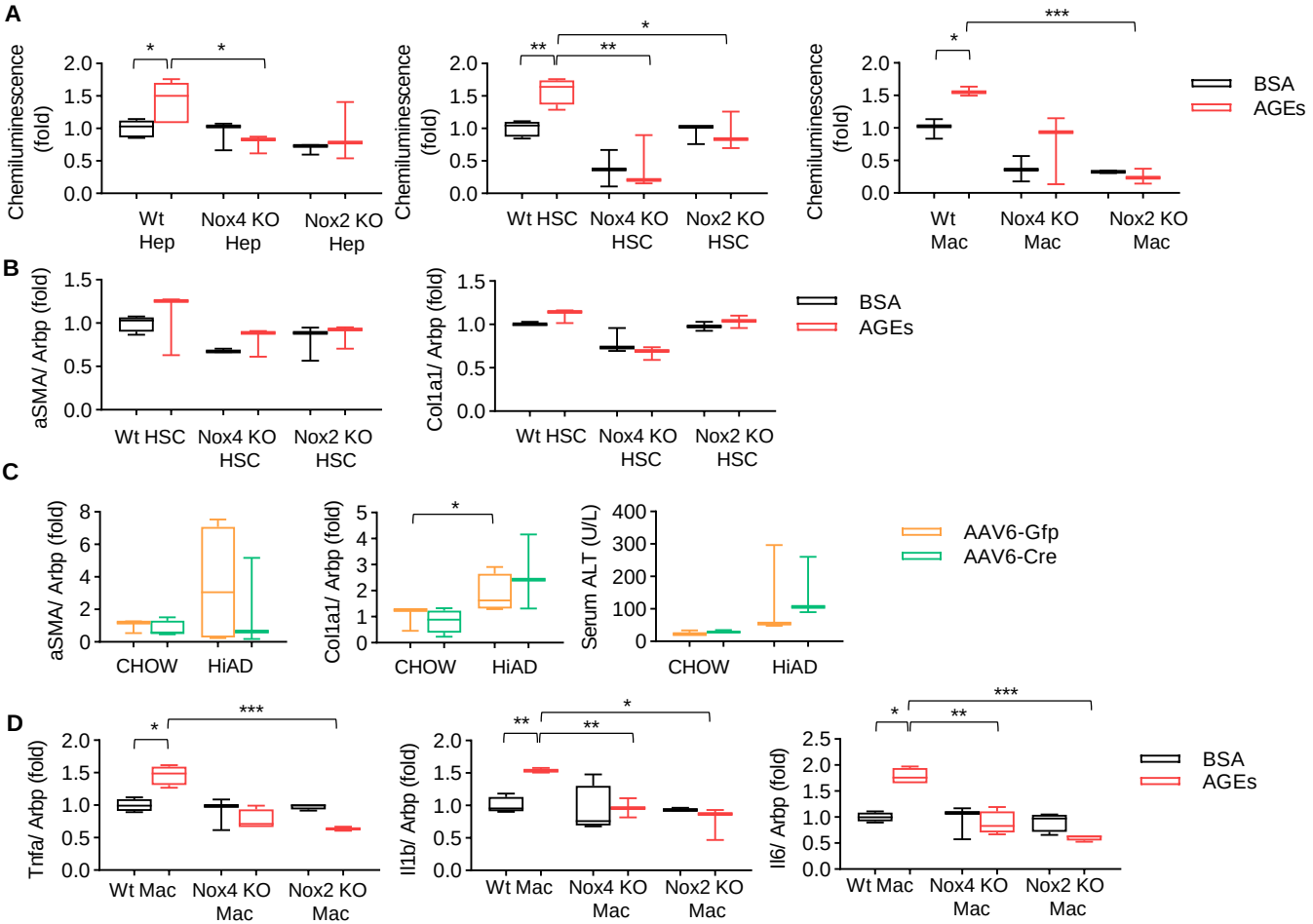
Supplementary Figure 5. Representative images of CK19 and SOX9 immunohistochemistry and calculation of positive cells in *fl/fl* mice on fast food diet (FFD). Immunohistochemistry of CK19 and SOX9 were performed on paraffin-embedded slides (For HiAD images, please see Figure 1F). The number of CK19/SOX9 positive cells was counted in 5 different fields in 3-4 mice/group. (Scale bar: 100μm, Data are represented as mean ± SEM; one way ANOVA followed by post-hoc Tukey test. **p<0.01, ***p<0.001).

Supplementary Figure 6.



Supplementary Figure 6. AGEs-mediated p38MAPK and JNK1/2 signals are involved in SMAD3 phosphorylation. (A) Primary, *fl/fl* (WT) or *Rage* KO hepatocytes were treated with 100μg/ml AGEs-BSA for the indicated times. P38MAPK and JNK1/2 phosphorylation were induced in WT hepatocytes by AGEs, and this was attenuated in *Rage* KO cells. **(B)** Primary, WT or *Rage* KO hepatocytes were treated with 100μg/ml AGEs-BSA, in the presence or absence of JNK (10μM, SP600125) or p38MAPK inhibitor (10μM, SB203580). SMAD3 phosphorylation in WT cells was attenuated by the inhibitors.

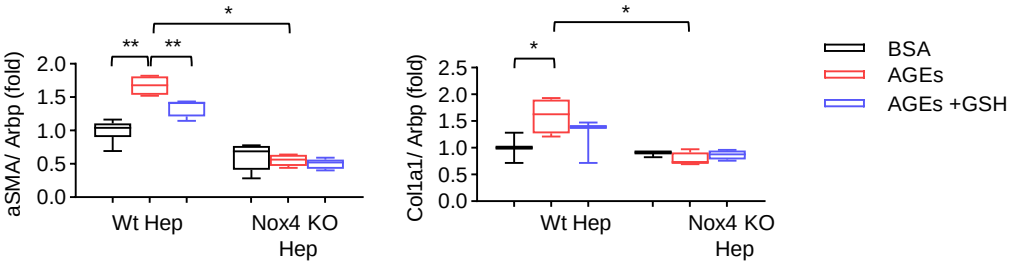
Supplementary Figure 7.



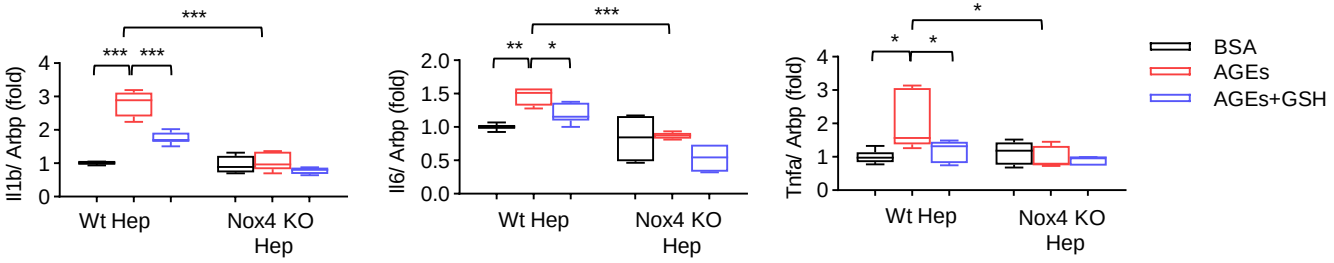
Supplementary Figure 7. NOX4 and NOX2 are differentially involved in AGEs mediated ROS production in primary hepatocytes, hepatic stellate cells, and macrophages. Primary hepatocytes (Hep), hepatic stellate cells (HSCs), and macrophages (Mac) were isolated from wild type (WT), *Nox4* KO, and *Nox2* KO mice and treated with 100ug/ml AGEs, or control BSA for 24 hours. **(A)** *Nox4* KO primary hepatocytes had significant reduction in ROS production. Both *Nox4* and *Nox2* KO HSC exhibited significant decrease in ROS production, whereas only *Nox2* KO macrophages had significantly less ROS. **(B)** RT-qPCR analysis of HSCs stimulated with AGEs did not show significant induction in *aSMA* and *Col1a1* expression. **(C)** *Rage fl/fl* mice were fed normal chow or HiAD diet for 14 weeks. To delete *Rage* in HSCs, after 7 weeks of feeding mice were injected with AAV6-cre (5x10e11 GC) or control AAV6-Gfp. Compared to AAV6-Gfp injected mice there was no reduction in *aSMA, Col1a1* or serum ALT levels. (D) Exposure to AGEs induced *Tnfa*, *Il1b*, and *Il6* expression in primary macrophages from WT mice. No induction was observed in *Nox4* KO and *Nox2* KO macrophages. (A, B, D: Data represent 3 independent experiments. *p<0.05, **p<0.01, ***p<0.001, Unpaired two-tailed *t.* test. C: n=3-4 mice in each group, *p<0.05, **p<0.01, ***p<0.001, Unpaired two-tailed *t.* test. Bar: 25-75 percentile, line: Median, whiskers: Min/ Max).

Supplementary Figure 8.

A. Hepatocyte-HSC co-culture

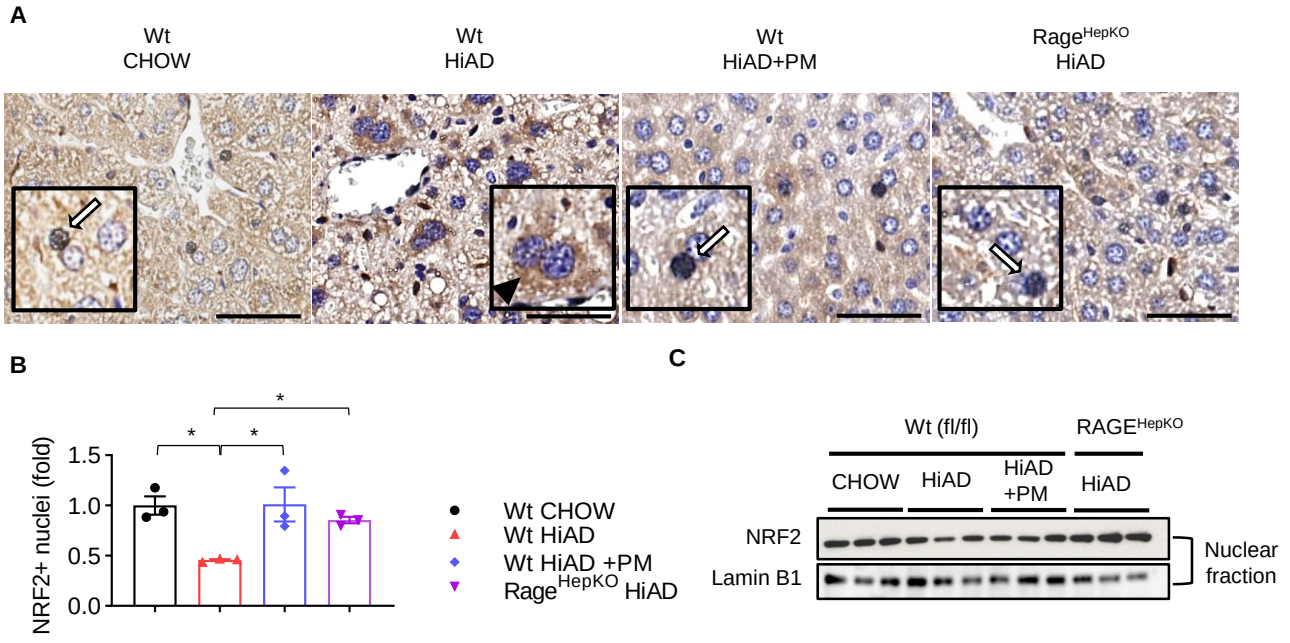


B. Hepatocyte-macrophage co-culture



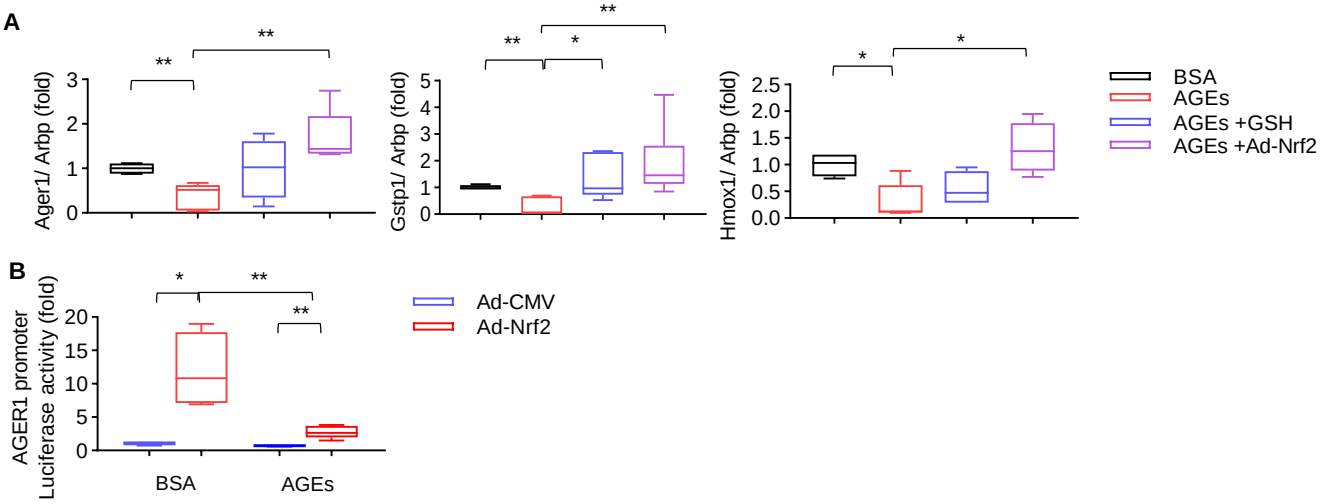
Supplementary Figure 8. AGEs-treated hepatocytes induce hepatic stellate cell transdifferentiation and macrophage polarization in co-cultures. Primary hepatocytes from WT or *Nox4* KO mice were isolated and treated with 100ug/ml AGEs with or without 10mM GSH. After 24 hours, medium was changed, and hepatic stellate cells (HSCs) or macrophages from WT mice were co-cultured with the hepatocytes using a transwell co-culture system. **(A)** AGEs-exposed WT but not *Nox4* KO hepatocytes significantly induced *aSMA* and *Col1a1* in primary HSCs. Scavenging ROS with GSH reduced HSC activation. **(B)** Macrophages exhibited significant induction of *Il1b*, *Il6*, and *Tnfa*, when co-cultured with AGEs-exposed WT but not *Nox4* KO hepatocytes. GSH treatment of hepatocytes significantly reduced *Il1b*, *Il6*, and *Tnfa* (data represent 3 independent experiments, * p< 0.05, **p< 0.01, ***p< 0.001. One way ANOVA followed by post hoc Tukey. Bar: 25-75 percentile, line: Median, whiskers: Min/ Max).

Supplementary Figure 9.



Supplementary Figure 9. Nuclear NRF2 decreased in *fl/fl* (WT) mice on HiAD, nuclear translocation improved by PM and in *Rage^{HepKO}* mice. **(A)** Immunohistochemistry and **(B)** counting nuclear NRF2 in eight random fields/mouse (40X) demonstrates decreased nuclear NRF2 in WT mice on HiAD, and improved nuclear translocation in mice exposed to PM and in *Rage^{HepKO}* mice. **(C)** Western blot analyses revealed lower nuclear NRF2 levels in WT mice on HiAD and increased nuclear NRF2 in *RAGE^{HepKO}* mice on HiAD. (Mean ± SEM, *p<0.05, one way ANOVA and post hoc Tukey test).

Supplementary Figure 10.



Supplementary Figure 10. NRF2 reversed the effects of AGEs treatment in hepatocytes. (A) In primary hepatocytes, NRF2 transduction by adeno-*Nrf2* (Ad-*Nrf2*) or treatment with reduced GSH reversed *Ager1* downregulation by AGEs, and improved the expression of NRF2 targets *Gstp1*, and *Hmox1*. **(B)** Human *AGER1* promoter was induced by Ad-*Nrf2*. (N=3, *p<0.05, **p<0.01. One-way ANOVA, and post hoc Tukey test. Bar: 25-75 percentile, line: Median, whiskers: Min/ Max).

Supplementary Table 2. Sequence of the primers used in this study

Mouse *Mcp1*	Forward: 5'-CTTCTGGGCCTGCTGTTCA-3' Reverse: 5'-CCAGCCTACTCATTGGGATCA-3'
Mouse *Tnfa*	Forward: 5'-TCCCAGGTTCTCTTCAAGGGA-3' Reverse: 5'-GGTGAGGAGCACGTAGTCGG-3'
Mouse *Il1b*	Forward: 5'-CAACCAACAAGTGATATTCTCCATG-3' Reverse: 5'-GATCCACACTCTCCAGCTGCA-3'
Mouse *Il6*	Forward: 5'- CCCAATTTCCAATGCTCTC-3' Reverse: 5'- TGAATTGGATGGTCTTGGTC-3'
Mouse *Col1a1*	Forward: 5'- AGAGGCGAAGGCAACAGTCG-3' Reverse: 5'- GCAGGGCCAATGTCTAGTCC-3'
Mouse *Tgfb*	Forward: 5'- TGGAGCAACATGTGGAACTC-3' Reverse: 5'- CAGCAGCCGGTTACCAAG-3'
Mouse *Mmp2*	Forward: 5'- GGACAGTGACACCACGTGACA-3' Reverse: 5'- ACTCATTCCCTGCGAAGAACA-3'
Mouse *Nox4*	Forward: 5'-TTGCCTGGAAGAACCCAAGT-3' Reverse: 5'-TCCGCACAATAAAGGCACAA-3'
Mouse *Nox2*	Forward: 5'- CCCTTTGGTACAGCCAGTGAAGAT-3' Reverse: 5'- CAATCCCGGCTCCCACTAACATCA-3'
Mouse *Ager1*	Forward: 5'- TGGCAAGTCATCTCTGAACC-3' Reverse: 5'-TGGGTGATTGGTTTATCTGGG-3'
Mouse *Gstp1*	Forward: 5'- CCATAGATACCTGGATGCAAGG-3' Reverse: 5'- TCCCATAAAGCCCCAAAGAG-3'
Mouse *Hmox1*	Forward: 5'- GTTCAAACAGCTCTATCGTGC-3' Reverse: 5'- TCTTTGTGTTCCTCTGTCAGC-3'
Mouse *Nqo1*	Forward: 5'- CAAGTTTGGCCTCTCTGTGG-3' Reverse: 5'- GATCTGGTTGTCAGCTGGAAT-3'
Mouse *Nrf2*	Forward: 5'- GCCCAGCACATCCAGACAGA-3' Reverse: 5'- GGGATATCCAGGGCAAGCGA-3'
Mouse *Nae1*	Forward: 5'- ACGTTGCCAAATTGCTTCAG-3' Reverse: 5'- ATCCAAGCCATACTCTTCAGC-3'
Mouse *Den1*	Forward: 5'- AGCAAAGGAGACAAACTGGTC-3' Reverse: 5'- CGTACATCCCACAGTCATAGC-3'
Mouse *Arbp*	Forward: 5'-CAAAGCTGAAGCAAAGGAAGAG-3' Reverse: 5'-AATTAAGCAGGCTGACTTGGTTG-3'
Human *RAGE*	Forward: 5'- GCCAGAAGGTGGAGCAGTAG-3' Reverse: 5'- CCAGTGGATTTGAGGAGAGG-3'
Human *AGER1*	Forward: 5'- GTGTTCAAGGAGGAGGGTGT-3' Reverse: 5'- GTGACAGTGTAGGCATTGGG-3'
Human *B2M*	Forward: 5'-TTCTGGCCTGGAGGCTATC-3' Reverse: 5'-TCAGGAAATTTGACTTTCCATTC-3'

Supplementary Table 3. Antibodies used in this study

Immunohistochemistry				
Antibody	Manufacturer	Cat#	Concentration	Description
CK19	Abcam	ab133496	1:1000	Rabbit monoclonal
SOX9	EMDmillipore	AB5535	1:1000	Rabbit polyclonal
RAGE	Abcam	ab7764	1:100	Goat polyclonal
4-Hydroxynonenal (4-HNE)	Abcam	ab46545	1:500	Rabbit polyclonal
NRF2	Abcam	ab62352	1:500	Rabbit monoclonal
Immunoprecipitation				
SMAD3	Abcam	ab28379	10μg/ml	Rabbit monoclonal
Western Blot				
CUL3	Cells Signaling Technology	2759	1:1000	Rabbit polyclonal
NRF2	Abcam	ab31163	1:500	Rabbit polyclonal
GAPDH	R&D Systems	2275-PC-100	1:2000	Rabbit polyclonal
Vinculin	Abcam	Ab129002	1:20000	Rabbit monoclonal
SMAD3	Abcam	ab28379	1:1000	Rabbit monoclonal
Phospho-SMAD3	Abcam	ab52903	1:2000	Rabbit monoclonal
JNK	Cells Signaling Technology	9252S	1:1000	Rabbit polyclonal
Phospho-JNK	Cells Signaling Technology	9251S	1:1000	Rabbit polyclonal
Phospho-p38 MAPK	Cells Signaling Technology	4631S	1:1000	Rabbit monoclonal
p38 MAPK	Cells Signaling Technology	8690S	1:1000	Rabbit monoclonal
Lamin B1	Abcam	ab133741	1:1000	Rabbit monoclonal