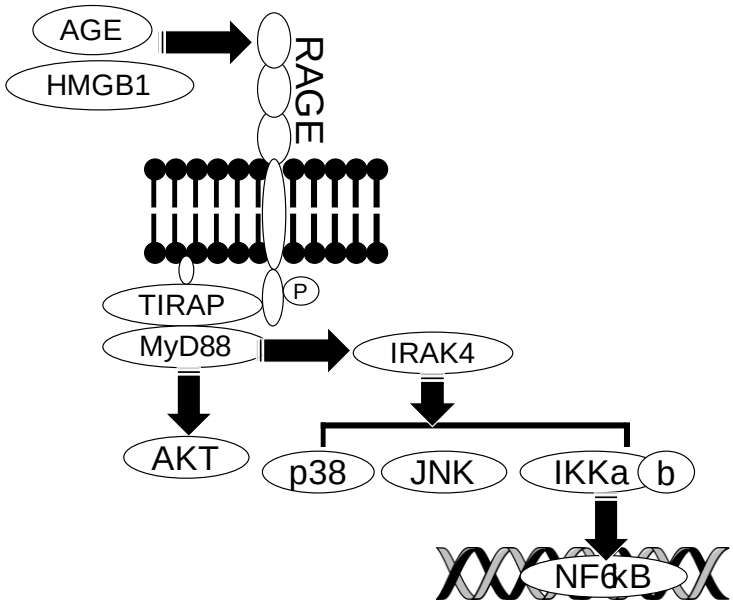
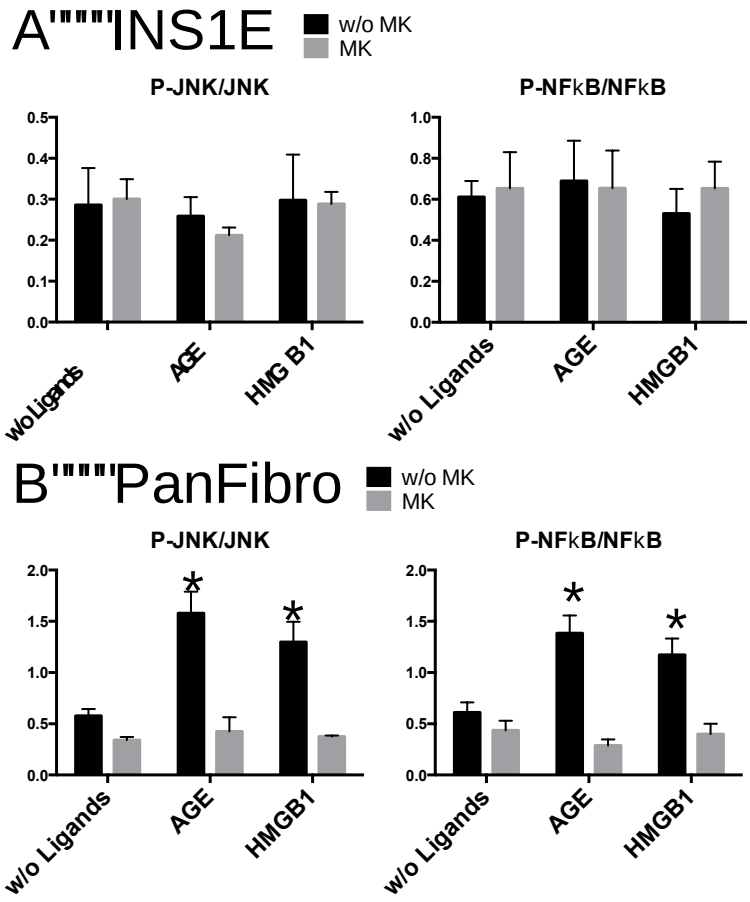


Supplementary Figure1

Schematic representation of the RAGE signaling cascades

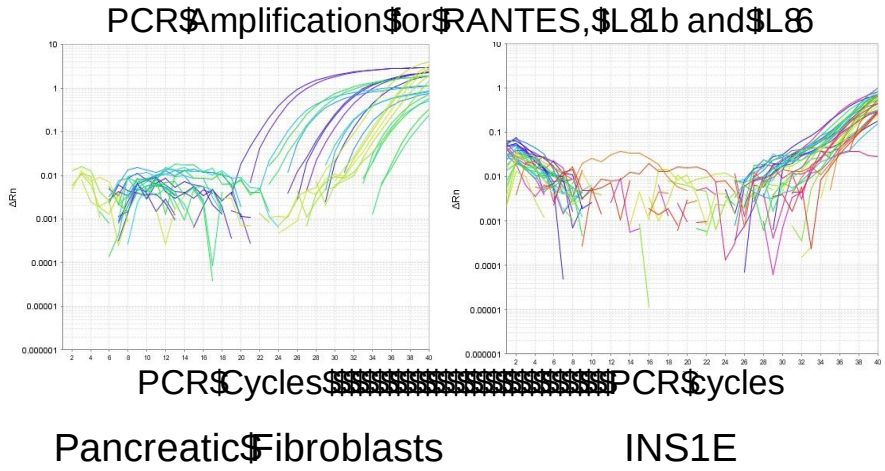
RAGE is a pattern-recognition receptor with several known ligands, including AGE and HMGB1. The intracellular domain of RAGE transmits a signal to the downstream effector proteins TIRAP and MyD88. MyD88 subsequently splits the signal to AKT and IRAK4, and the latter then further splits the signal to p38, JNK and the IKK complex. The IKK signal culminates in nuclear translocation of transcription factor NF-κB, upregulating a battery of inflammatory cytokine genes.



Supplementary Figure2

JNK and NFκB are phosphorylated by RAGE ligands in pancreatic fibroblasts, but not in INS1E

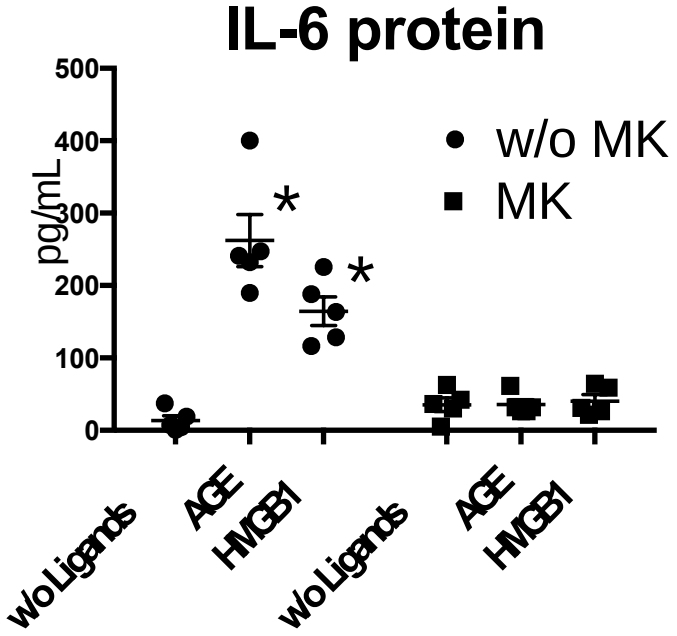
Amount of proteins and their phosphorylation were quantified by western blotting (Figure2) and the ratios between phosphorylated and protein *per se* were calculated. Each bar represents the mean ± standard error (n=3, from different passage points). Asterisks: statistically significant difference ($p<0.05$ by Dunnett)



Supplementary Figure3

cDNAs of cytokine genes are poorly amplified in INS1E cells

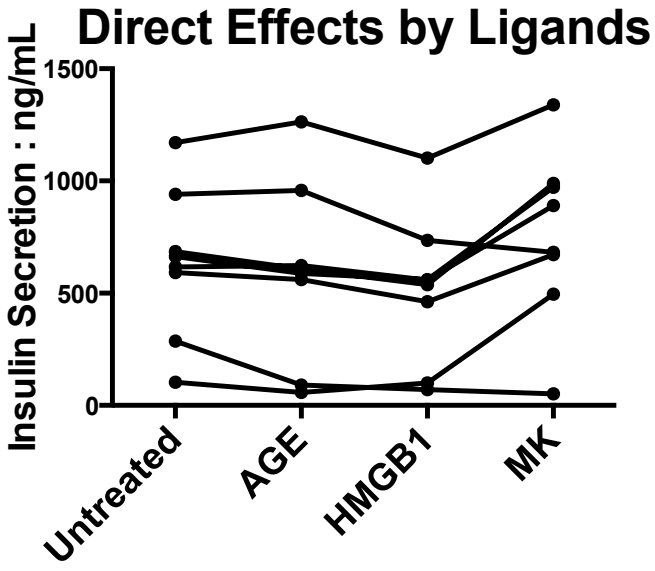
Right: Amplification plot of the PCR product for each condition in INS1E cells. Neither AGE, HMGB1 nor MK615 affected the PCR amplification for RANTES, IL-1β and IL-6. Left: In contrast, AGE or HMGB1 shifted the amplification plots markedly to the left (normalized C_T values are presented in Figure3), indicating an increase of each cDNA.



Supplementary Figure4

IL-6 protein secreted from pancreatic fibroblasts

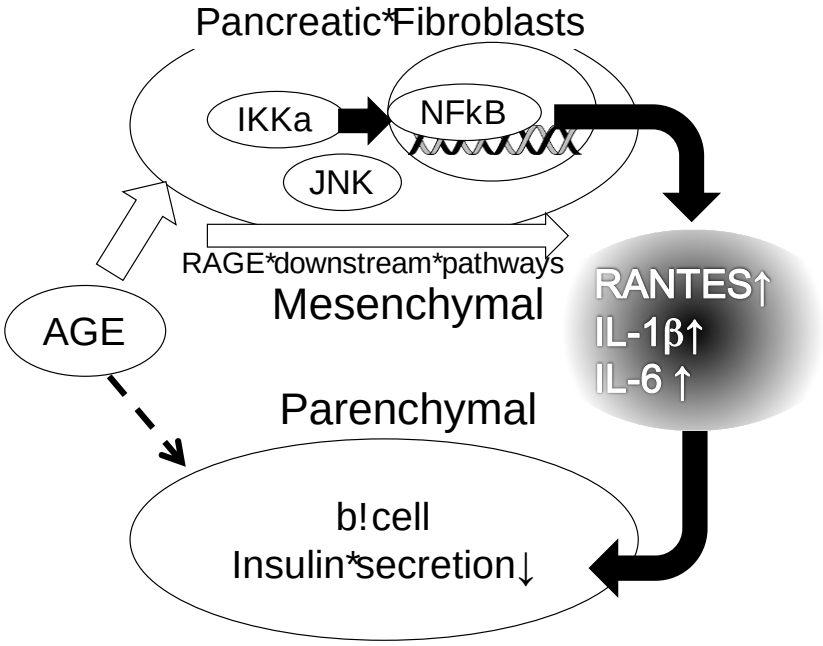
After 24 hours of treatment with each drug (AGE: 0.1 mg/ml, HMGB1: 100 ng/ml, MK615: diluted with 100-fold), conditioned medium were collected and IL-6 secretion in the absence of MK (circle). In contrast, AGE or HMGB1 did not change the secretion in the presence of MK (square), suggesting that MK615 nullified the effects of AGE/HMGB1. Scatter plot represents each experimental data (n=5: different numbers of passages, from two independently established fibroblast cultures) Asterisks: statistically significant difference ($p<0.05$ by Dunnett)



Supplementary Figure5

Insulin secretion from INS1E cells was not influenced by direct administration of neither RAGE ligand nor MK615

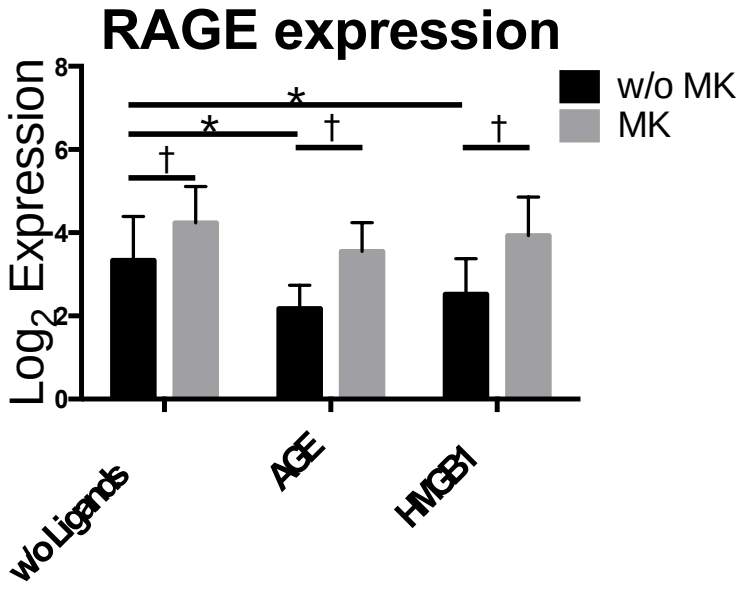
Insulin secretion from INS1E cells incubated with AGE1, HMGB1 or MK615 for 24 hours and accumulated insulin was measured. Line graph represents each experimental data (n=8: different numbers of INS1E passages).



Supplementary Figure6

Putative model of RAGE action in pancreatic islets.

AGE or HMGB1 activates the RAGE downstream pathways, (i.e. phosphorylation of JNK and IKKα/NF-κB) in mesenchymal fibroblasts. However, the activation of the same pathways could be lower or minimal in parenchymal β-cells. Phosphorylation of IKKα/NF-κB results in enhanced production of RANTES, IL-1β, and IL-6 in mesenchymal fibroblasts. Paracrine effects of inflammatory cytokines could be responsible for AGE1/AGE2 suppression of insulin secretion from parenchymal β-cells. A similar mechanism of natural immunity has been observed in heart cardiomyocytes/fibroblasts.



Supplementary Figure7

Expression of mRNAs for RAGE in rat pancreatic fibroblasts.

After 24 hours of treatment with each drug (AGE: 0.1 mg/ml, HMGB1: 100 ng/ml, MK615: diluted with 100-fold), mRNA was purified and reverse-transcribed for quantitative polymerase chain reaction. Data are represented as mean ± standard deviation (n=4: different numbers of passages, from two independently established fibroblast cultures). AGE or HMGB1 decreased the expression of RAGE (Log scale, black bar). On the other hand, MK615 increased the expression of RAGE (grey bar). Asterisks: statistically significant difference ($p<0.05$, by paired-*t*) between untreated and RAGE ligand. Dagger: statistically significant difference between with and without MK615.