# Advanced glycation end-product (AGE)-albumin from activated macrophage is critical in human mesenchymal stem cells survival and post-ischemic reperfusion injury

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## Supplementary figures

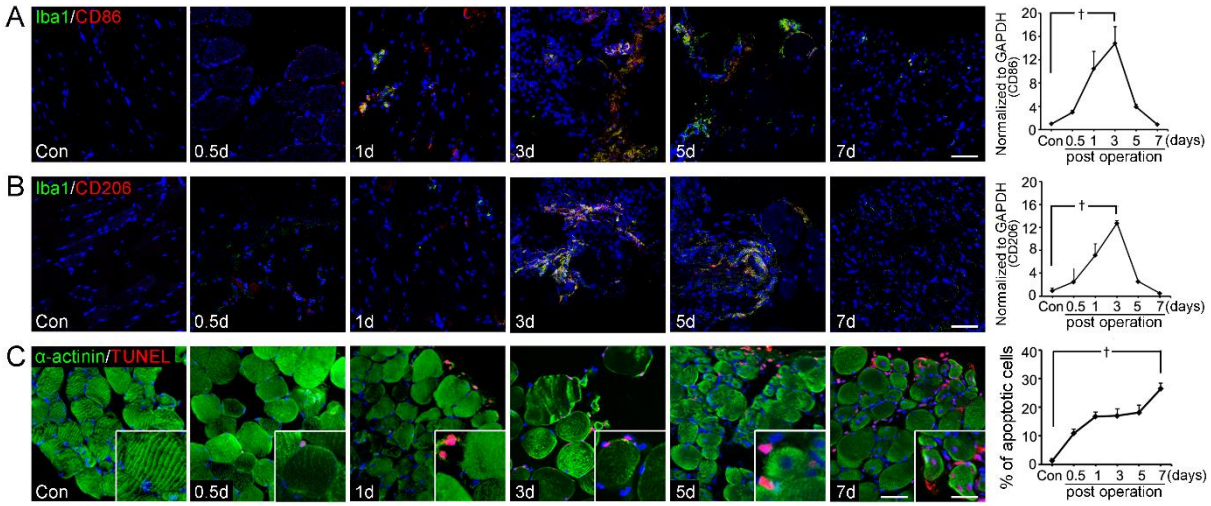


**Figure S1. Confirmation of relationship between M1/M2 macrophage activation time and skeletal muscle cell death**

(A) Confocal microscopic images illustrate activated M1 macrophage marker (Iba1/CD86, yellow) and activated M2 macrophage marker (Iba1/CD206, yellow) or nucleus (DAPI, blue) until 7 days after reperfusion. (B) mRNA expression levels of M1 (CD86), and M2 (CD206) macrophage were confirmed by qRT-PCR. (C) Apoptotic skeletal muscle cells were illustrated by TUNEL (red) in a time dependent manner. Scale bar = 50 ㎛. mean ± s.d. †, ($P<0.01$) vs. control mice.

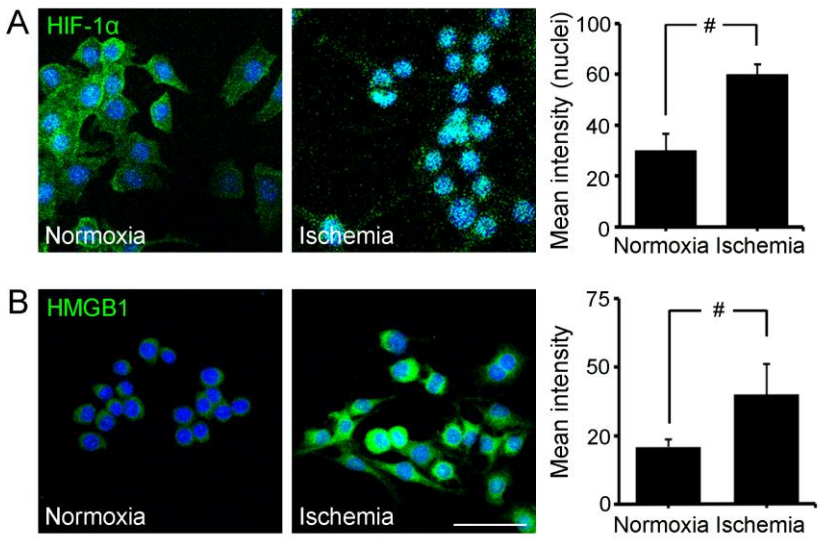


**Figure S2. Validation of hypoxia and serum deprivation treated L6 cells**

(A, B) Double-labeled confocal microscopic images show fluorescence levels of HIF-1α (green, top), HMGB1 (green, bottom) and DAPI (blue) in L6 cells. Graph is processed from fluorescence intensity and it indicates differences level of hypoxia related proteins after hypoxia and serum deprivation treatment (ischemia) Scale bar = 50 ㎛. mean ± s.d. #, ($P < 0.001$)

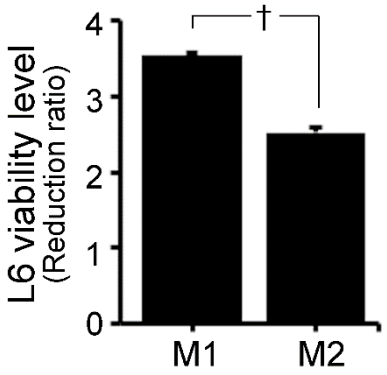


**Figure S3. Skeletal muscle cell viability after supernatant treatment from M1 or M2 macrophage**

This graph shows M1 or M2 extracellular secretion after conditioned medium (C.M.) treatment for 48 hrs decreased L6 cell number as determined by cell viability assay. Especially, the number of L6 cells after M1 C.M. treatment was significantly decreased compared to M2 C.M treatment. mean ± s.d. †, ($P<0.01$)

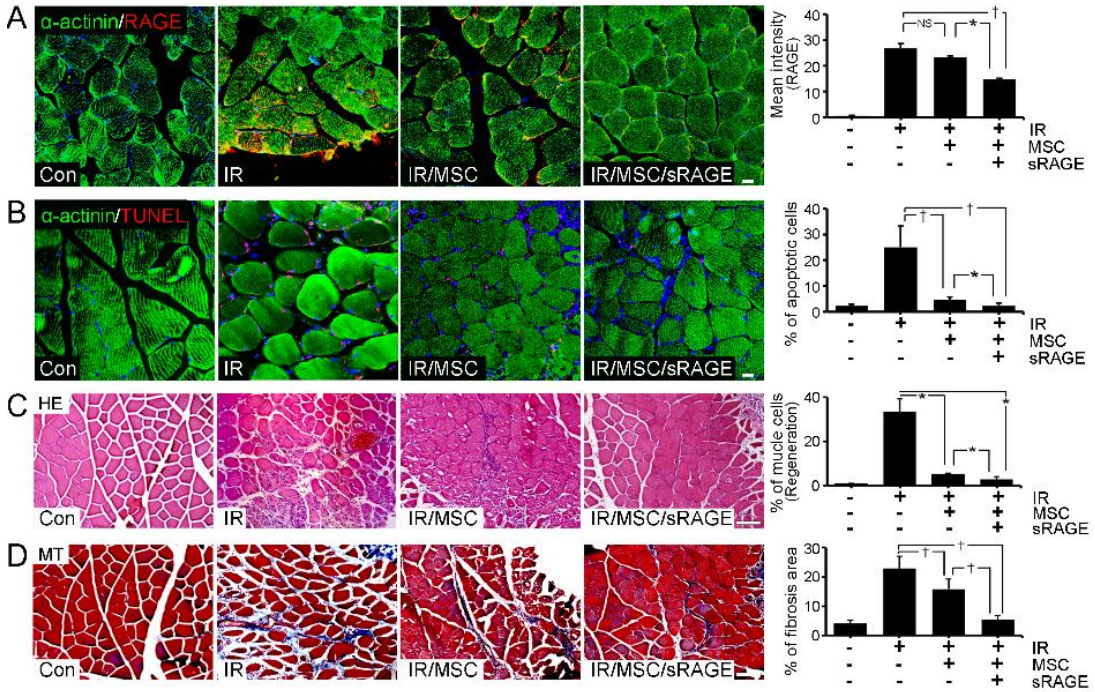


**Figure S4. Reduced RAGE expression by sRAGE treatment in PIRI-CLI on 7 days**

(A) The relative levels of RAGE (red) in skeletal muscle cells (α-actinin, green), after hBD-MSCs with (IR/MSC/sRAGE) or without sRAGE treatment (IR/MSC) in PIRI-CLI mice were evaluated by triple confocal microscopic analyses. (B) Apoptotic cells were illustrated by TUNEL (red) in skeletal muscle cells (green), after hBD-MSC treatment with or without sRAGE treatment. Scale bar = 20 ㎛

(C,D) The damaged skeletal muscles were measured by hematoxylin and eosin staining (HE) and fibrotic area was evaluated by Masson's trichrome (MT) staining in PIRI-CLI mice on 7 days. Scale bar = 50 ㎛. mean ± s.d. *, ($P<0.05$) and †, ($P<0.01$)

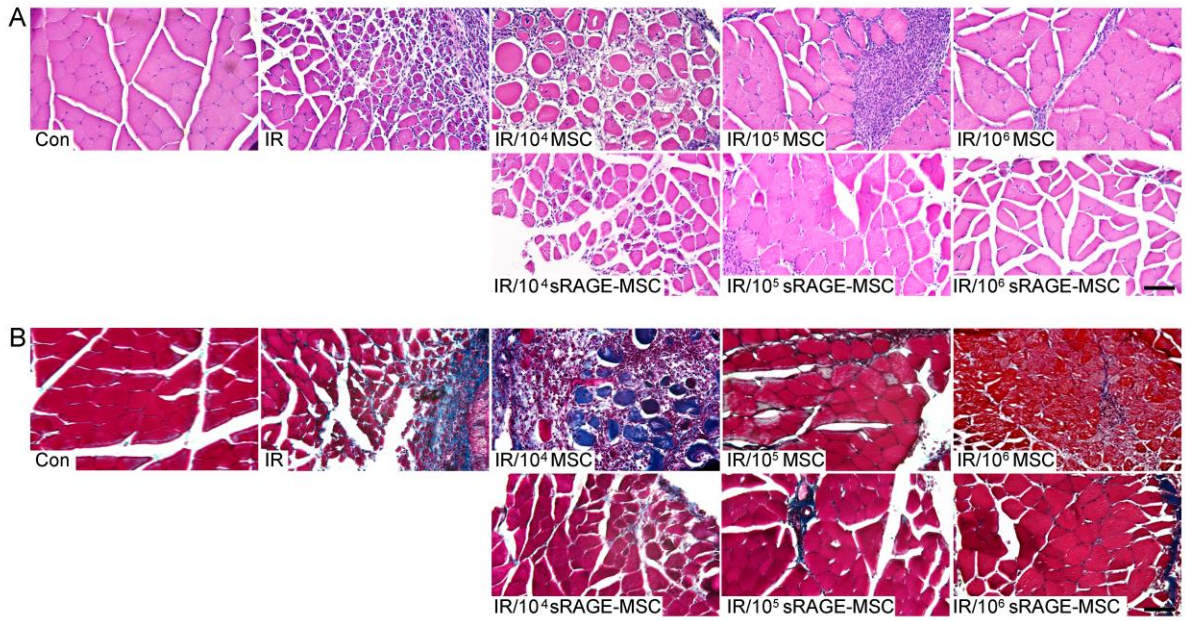


**Figure S5. sRAGE improves the protective effect of hBD-MSC in low dosage cell number**

(A, B) The damaged skeletal muscles were visualized by hematoxylin and eosin staining (HE) and fibrotic area was evaluated by Masson's trichrome (MT) staining in PIRI-CLI mice with the dose-dependent treatment of hBD-MSCs. Scale bar = 50 ㎛

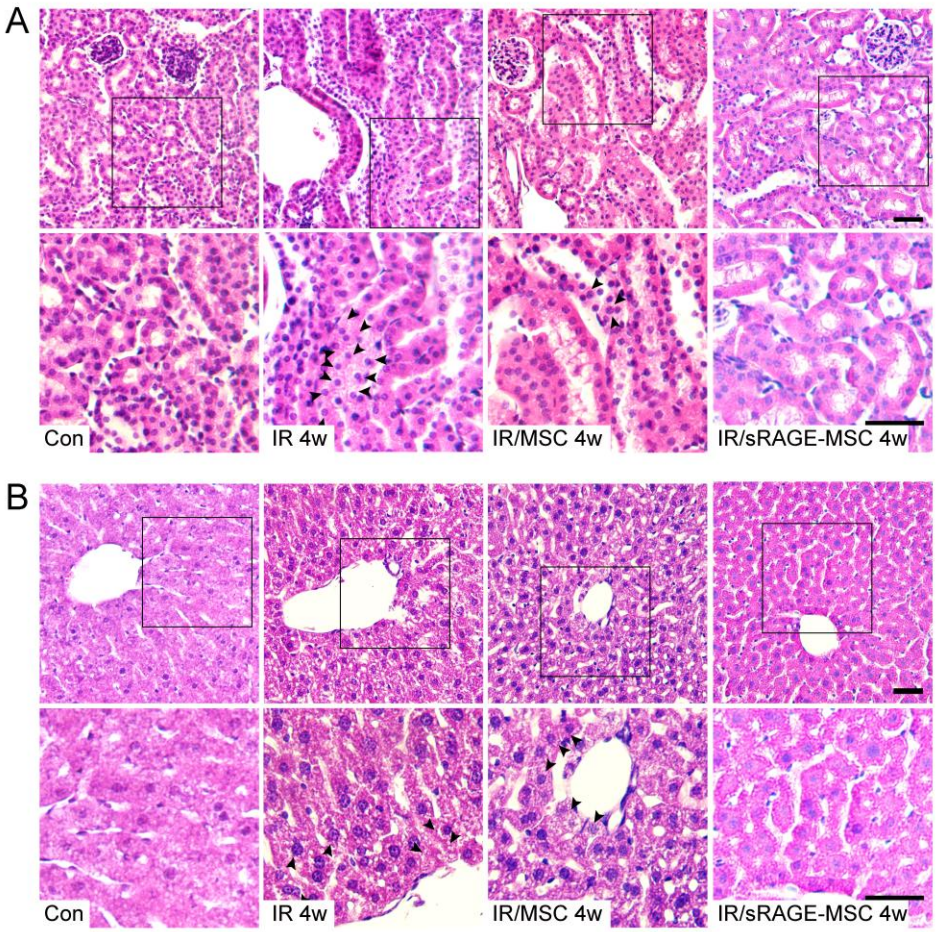


**Figure S6. sRAGE-MSC has systemic protective effects on kidney and liver of PIRI-CLI model**

The histological images show sRAGE-MSC has systemic protective effects in PIRI-CLI damaged kidney (A) and liver (B). Swollen tubular cells (arrow head) were observed in PIRI-CLI damaged kidney and hepatocellular necrosis (arrow head) in PIRI-CLI damaged liver. The sRAGE-MSC treatment showed the improved systemic protective effects in PIRI-CLI.

## Supplemental Tables

**Table S1. List of antibodies for immunoblotting and immunostaining**

| Antigen (host) | Company | Cat. No | Application | |
|---|---|---|---|---|
| | | | ICC/IHC | WB |
| β-actin (mouse) | Sigma-Aldrich | A5316 | - | 1:15,000 |
| Erk1/2 (rabbit) | Cell signaling | 9102S | - | 1:1,000 |
| pErk1/2 (rabbit) | Cell signaling | 4377S | 1:100 | 1:1,000 |
| p38 (rabbit) | Cell signaling | 9212L | - | 1:1,000 |
| pp38 (rabbit) | Cell signaling | 9211S | 1:100 | 1:1,000 |
| SAPK/ JNK (rabbit) | Cell signaling | 9252S | - | 1:1,000 |
| pSAPK, JNK (rabbit) | Cell signaling | 9251S | 1:100 | 1:1,000 |
| Albumin (mouse) | Abcam | ab10241 | 1:100 | - |
| AGE (rabbit) | Abcam | ab23722 | 1:200 | - |
| Iba-1 (goat) | Abcam | ab5076 | 1:100 | - |
| B7-2 (CD86, mouse) | Santa Cruz | sc-19617 | 1:100 | 1:100 |
| CD206 (mouse) | Santa Cruz | sc-58987 | 1:100 | 1:100 |
| α-actinin (mouse) | Sigma-Aldrich | A7732 | 1:800 | - |
| CD44 (rabbit) | Abcam | Ab51037 | 1:50 | |
| α-SMA (rabbit) | Abcam | Ab5694 | 1:200 | |
| vWF (mouse) | Dako | A0082 | 1:200 | |
| HIF-1α (mouse) | Abcam | ab463 | 1:200 | - |
| HMGB1 (rabbit) | Abcam | ab18256 | 1:200 | - |
| RAGE (goat) | Abcam | ab7764 | 1:400 | 1:4000 |
| Peroxidase labeled anti-mouse IgG | Vector | PI2000 | - | 1:5000 |
| Peroxidase labeled anti-rabbit IgG | Vector | PI1000 | - | 1:5000 |
| Peroxidase labeled anti-goat IgG | Vector | PI9500 | - | 1:5000 |
| Alexa Fluor 555 donkey anti rabbit IgG | Invitrogen | A31572 | 1:500 | - |
| Alexa Fluor 633 goat anti rabbit IgG | Invitrogen | A21070 | 1:500 | - |
| Alexa Fluor 555 donkey anti goat IgG | Invitrogen | A21432 | 1:500 | - |
| Alexa Fluor 488 donkey anti mouse IgG | Invitrogen | A11001 | 1:500 | - |

**Table S2. List of primers for Quantitative polymerase chain reaction (qPCR)**

| Gene | | Primers |
|---|---|---|
| GAPDH | Forward | 5'-CGT CTT CAC CAC CAT GGA AGA-3’ |
| | Reverse | 5'-CGG CCA TCA CGC CAC AGT TT-3’ |
| CD86 | Forward | 5'-TCA GTG ATC GCC AAC TTC AG-3’ |
| | Reverse | 5'-TTA GGT TTC GGG TGA CCT TG-3’ |
| CD206 | Forward | 5'-CCT CTG GTG AAC GGA ATG AT-3’ |
| | Reverse | 5'-CTT CCT TTG GTC AGC TTT GG-3’ |

## Supplemental methods

### PIRI-CLI animal group

The mice were anesthetized with a mixture of 2% xylazine hydrochloride (Rompun, 5-10 mg/kg; Bayer) and tiletamine/zolazepam (Zoletil 50, 25 mg/kg, Virbac Animal Health) through intra-peritoneal injection. After induction of anesthesia, surgical procedures were performed by following protocol. Briefly, longitudinal incision of left thigh skin was made. Proximal and distal ends of the femoral artery was ligated. Complete femoral artery occlusion was achieved and continued for 1 hrs. The ligation was removed and blood reperfusion was allowed into the ischemic muscle. After recovery of blood supply to the damaged muscles, the following 2 arms were established: 1) dose dependent hBD-MSC treatment: $1 \times 10^4$, $1 \times 10^5$ and $1 \times 10^6$ hBD-MSC or hBD-MSC with sRAGE, 2) validation of sRAGE protective effects: incubate for 7 days and 4 weeks after PIRI-CLI model generation.