Supplementary Information

**We provided Figures S1, Figure S2, and their figure legends as disclosed.**

**However, the other information is provided for the reviewer only.**

**Supplementary Materials & Methods (This “Materials & Methods” is provided for the reviewer only)**

**Supplementary materials**

An anti-β-actin antibody and mouse monoclonal [6C5] anti-GA-3 phosphate dehydrogenase (anti-GAPDH) antibody were obtained from Abcam (Tokyo, Japan). WB stripping solution was purchased from Nacalai Tesque Inc. (Kyoto, Japan). An anti-mouse IgG antibody was obtained from Thermo Fisher Scientific Inc. (MA, U.S.A.).

**Supplementary methods**

**Western blotting (WB) analysis of β-actin and** **glyceraldehyde-3 phosphate dehydrogenase (anti-GAPDH)**

Cells were harvested with a radioimmunoprecipitation assay (RIPA) buffer (Thermo Fisher Scientific Inc.) solution with 10% protease inhibitor cocktail (Roche Applied Science) solution. Protein concentrations were assessed by the BCA assay kit, using BSA as a standard. Lysates (10 μg protein/lane) were mixed with sodium dodecyl sulfate (SDS) sample buffer (Bio-Rad) and 2-mercaptoethanol (Sigma-Aldrich) and heated at 95 °C for 5 min. They were separated by SDS polyacrylamide gel electrophoresis (SDS-PAGE) with 4-15% gradient polyacrylamide gels (Bio-Rad). Proteins were transferred onto PVDF membranes using the semidry electron transfer system (ATTO Co., Ltd., Tokyo, Japan). Membranes were incubated in 5% skimmed milk-PBS-T at r.t. for 30 min (blocking step). Proteins on PVDF membranes were probed with the following primary antibodies at 4 °C overnight: an anti-β-actin antibody (Abcam, ab3280, 1:1000) and anti-GAPDH antibody (Abcam; 1:10000; ab8245). PVDF membranes were washed four times with 0.5% skimmed milk-PBS-T and incubated with a secondary antibody at r.t. for 1 h. The secondary antibodies used were as follows: a HRP-conjugated goat anti-mouse IgG antibody (Thermo Fisher Scientific Inc.; 1:5000; Product Number 31458) and HRP-conjugated donkey anti-rabbit IgG antibody (Thermo Fisher Scientific Inc.; 1:2000; Product Number 31432). Proteins on PVDF membranes were incubated with the secondary antibody at r.t. for 1 h. The anti-GAPDH antibody was used after the anti-β-actin antibody was removed by the WB stripping solution

**Staining of proteins on PVDF membranes for analysis with Coomassie brilliant blue**

After removal of the antibody to target proteins, PVDF membranes were incubated in WB stripping solution at r.t. for 10 min. Membranes were washed five times with PBS-T within 1 min. Membranes were then incubated in Coomassie brilliant blue solution for 10 min at r.t., and washed seven times with water every 10 min.

**Supplementary Figure legends**

Figure S1. Images of PVDF membranes for the slot blot analysis in Figure 1b and 1d. (**a**) An image of PVDF membranes for slot blot analysis on Figure 1b. The left membrane was incubated with an anti-TAGE antibody. The right membrane was incubated with a neutralized anti-TAGE antibody. L1 and 4: TAGE-BSA (0, 1, 3, 10, 30, 60, and 100 ng of protein/lane) and a HRP-linked molecular marker were loaded onto the membranes. L2, 3, 5, and 6: Cell lysates (2.0 μg of protein/lane) of C2C12 cells treated with 0, 0.5, 1. 1.5, and 2 mM glyceraldehyde for 24 h. (**b**) An image of PVDF membranes for the slot blot analysis in Figure 1d. The left membrane was incubated with an anti-TAGE antibody. The right membrane was incubated with a neutralized anti-TAGE antibody. L1 and 4: TAGE-BSA (0, 1, 3, 10, 30, 60, and 100 ng of protein/lane) and a HRP-linked molecular marker were loaded onto the membranes. L2, 3, 5, and 6: The bands in the open box with a solid line; Cell lysates (2.0 μg of protein/lane) of C2C12 cells treated with 0, 1.5, and 2 mM glyceraldehyde without aminoguanidine for 24 h were loaded onto PVDF membranes. Bands in the open box with a broken line; Cell lysates (2.0 μg of protein/lane) of C2C12 cells pretreated with 8 mM aminoguanidine for 2 h, followed by 0, 1.5, and 2 mM glyceraldehyde were loaded onto membranes.

Figure S2. Cell viability of C2C12 cells treated with non-glycated BSA and TAGE-BSA. (**a**) Cells were treated with 0 and 20 μg/mL of non-glycated BSA and TAGE-BSA for 24 h. (**b**) Cells were treated with 0 and 50 μg/mL of non-glycated BSA and TAGE-BSA for 24 h. (**a**, **b**) Cell viability was assessed by the WST-8 assay. This assay was performed in three independent experiments. One experiment was performed using 7 wells to calculate the average. Data are shown as means ± S.D. (N=3). P-values were based on the Bonferroni test.

**(Figure S3 and S4 must be provided for only reviewer)**

Figure S3. Expression of β-actin and glycelaldehyde-3 phosphate dehydrogenase (GAPDH) and the assessment of total proteins with Coomassie brilliant blue (CBB) in C2C12 cells treated with glyceraldehyde. GA: glyceraldehyde. Cells were treated with 0, 1, 1.5, and 2 mM GA for 24 h. Cell lysates (10 μg of protein/lane) were loaded on a 4-15% polyacrylamide gradient gel. A WB analysis was performed in two independent experiments. (**a**) Proteins on PVDF membrane were probed with a β-actin antibody. (**b**) Proteins on the PVDF membrane were probed with an anti-GAPDH antibody. The arrow indicates GAPDH bands. The upper bands were β-actin, which remained after re-prove treatment of it. (**c**) Proteins on PVDF membranes were stained with CBB.

Figure S4. The CBB staining analysis of PVDF membranes in Figure S1. (**a**) PVDF membranes for the slot blot analysis in Figure S1a. L1 and 4: TAGE-BSA (0, 1, 3, 10, 30, 60, and 100 ng of protein/lane) and a HRP-linked molecular marker were loaded onto membranes. L2, 3, 5, and 6: Cell lysates (2.0 μg of protein/lane) of C2C12 cells treated with 0, 0.5, 1. 1.5, and 2 mM glyceraldehyde for 24 h were loaded onto the membranes. (**b**) PVDF membranes for the slot blot analysis in Figure S1b. L1 and 4: TAGE-BSA (0, 1, 3, 10, 30, 60, and 100 ng of protein/lane) and a HRP-linked molecular marker were loaded onto membranes. L2, 3, 5, and 6: Bands in the open box with a solid line; Cell lysates (2.0 μg of protein/lane) of C2C12 cells treated with 0, 1.5, and 2 mM glyceraldehyde without aminoguanidine for 24 h were loaded onto the membranes. Bands in the open box with a broken line; Cell lysates (2.0 μg of protein) of C2C12 cells pretreated with 8 mM aminoguanidine for 2 h, followed by 0, 1.5, and 2 mM glyceraldehyde were loaded onto membranes.