Figure S1

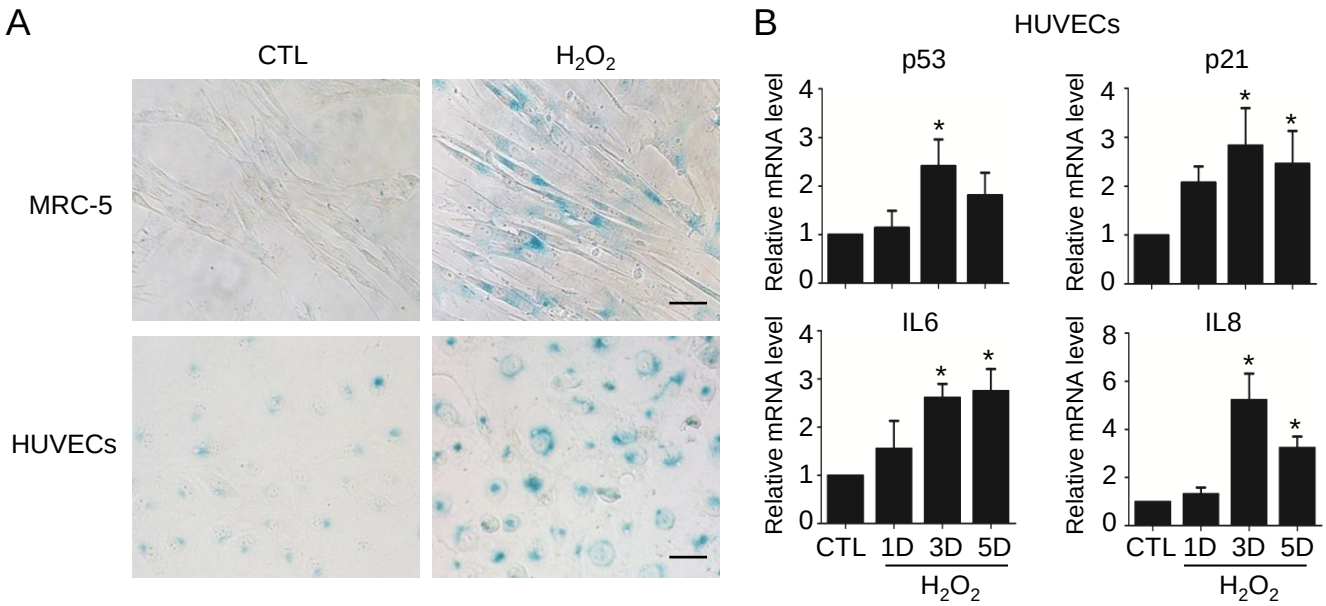

Figure S1. H_2O_2 induced senescence in MRC-5 cells and HUVECs. A and B: Cells were treated with H_2O_2 and incubated in complete medium without H_2O_2 for three days as described in the Experimental Procedures. (A) Representative images of SA-β-gal staining of MRC-5 and HUVECs. (B) Relative fold-changes in mRNA levels of senescence-associated genes, *p53*, *p21*, *IL6* and *IL8*, in HUVECs were monitored by qRT-PCR assays. * $p<0.05$ compared to the control (CTL). The bar represents 100 μm.

Figure S2

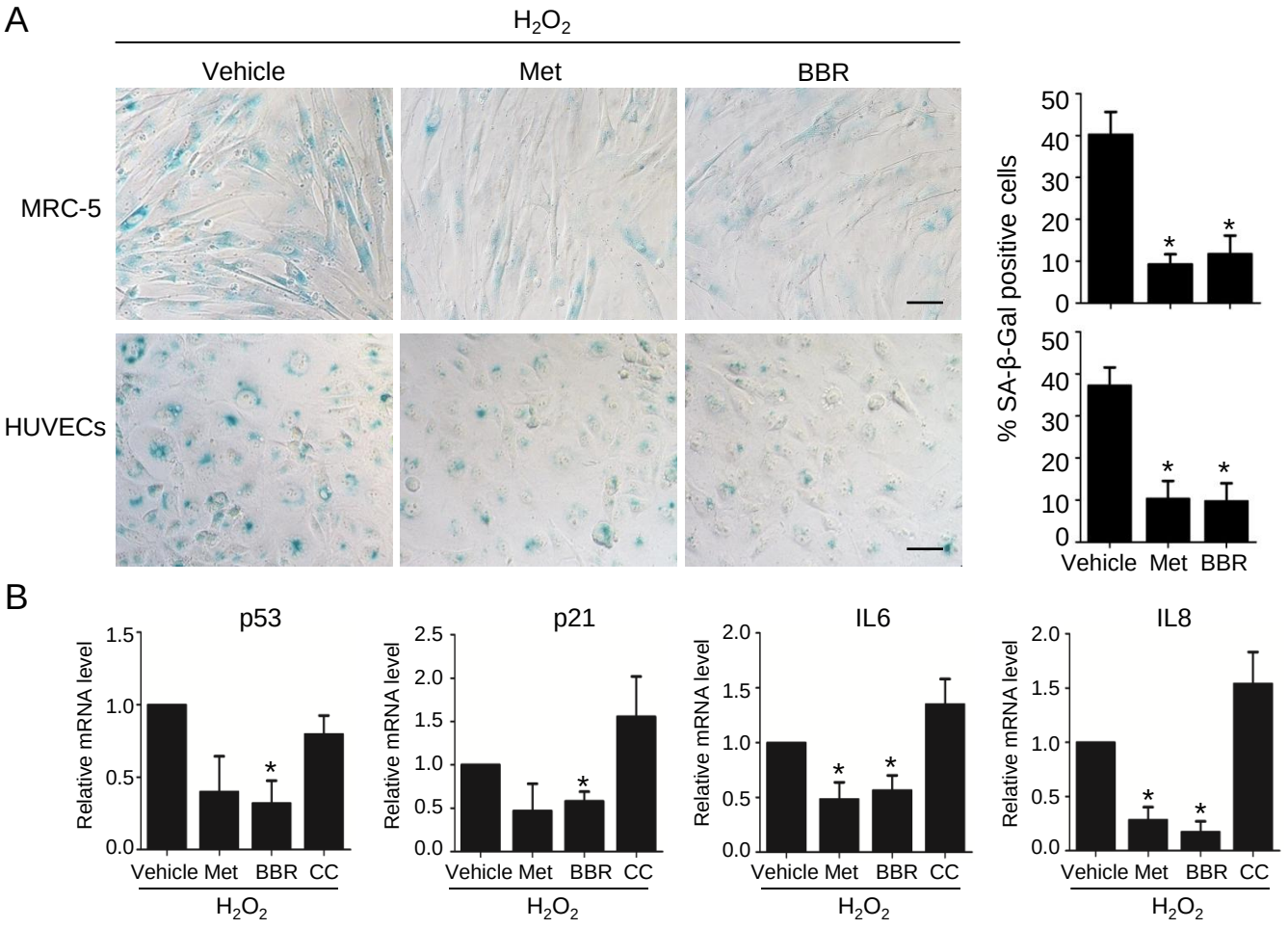

Figure S2. Activation of AMPK prevented H_2O_2-induced senescence in MRC-5 cells and HUVECs. H_2O_2-treated cells were incubated in complete medium with Met (10 mM) or BBR (10 μM) for 3 days. (A) Representative images of SA-β-Gal staining of the cells (left) and percentages of SA-β-gal-positive cells (right). The data from MRC-5 cells and HUVECs are shown in the upper and lower panels, respectively. (B) Relative fold-changes in mRNA levels of the genes encoding *p53*, *p21*, *IL6* and *IL8* in HUVECs were monitored by qRT-PCR. * $p < 0.05$ compared to the vehicle. The bar represents 100 μm.

Figure S3

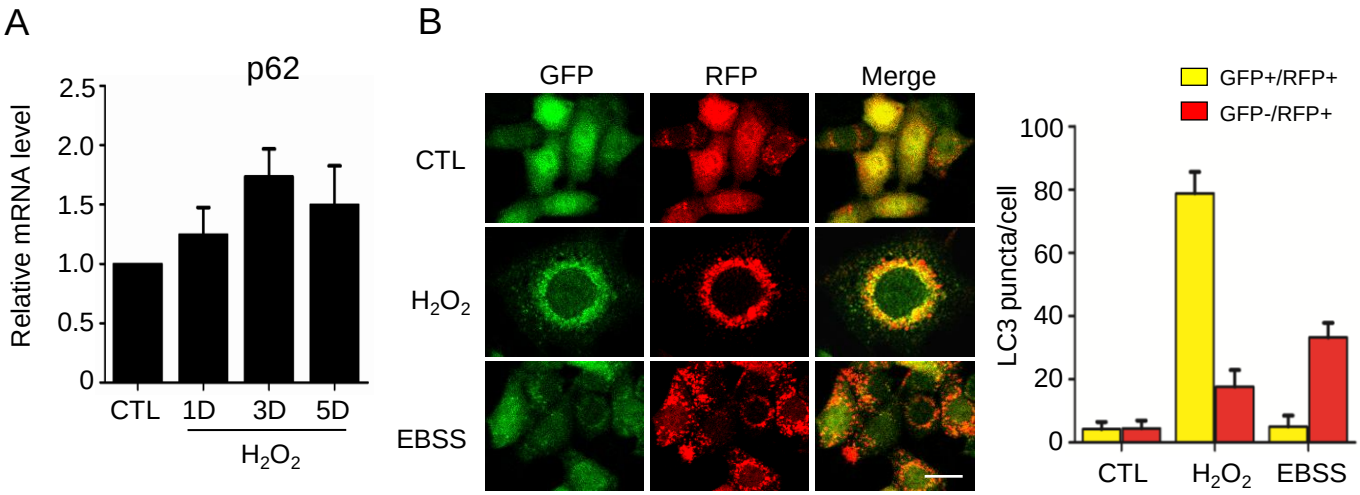

Figure S3. H_2O_2 induced decreased autophagic flux in NIH3T3 Cells. (A) NIH3T3 cells were treated with H_2O_2 and incubated in complete medium without H_2O_2 for 1-5 days. Relative fold-changes in the mRNA level of the *p62 /SQSTM1* gene were monitored by qRT-PCR. (B) Representative confocal fluorescent images of GFP-RFP-LC3-expressing cells, and the right panel shows the merged fluorescence (upper) and the percentages of cells with puncta-like LC3 (lower). The bar represents 20 μm.

Figure S4

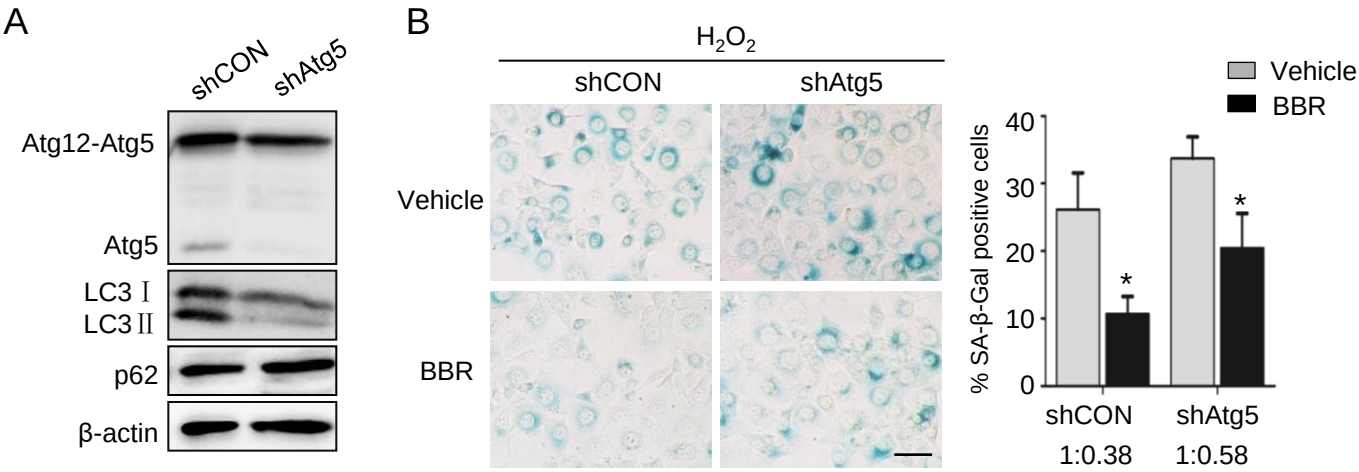

Figure S4. Atg5 knockdown attenuated the effects of BBR on protection against senescence. A to B: NIH3T3 cells infected with shAtg5 or a non-targeting shRNA (shCON). (A) Representative images from immunoblot assays against the Atg5, LC3 and p62 proteins. (B) Representative images of the SA-β-Gal staining of cells (left) and the percentages of SA-β-gal-positive cells (right). * p< 0.05 compared to the vehicle. The bar represents 100 μm.

Figure S5

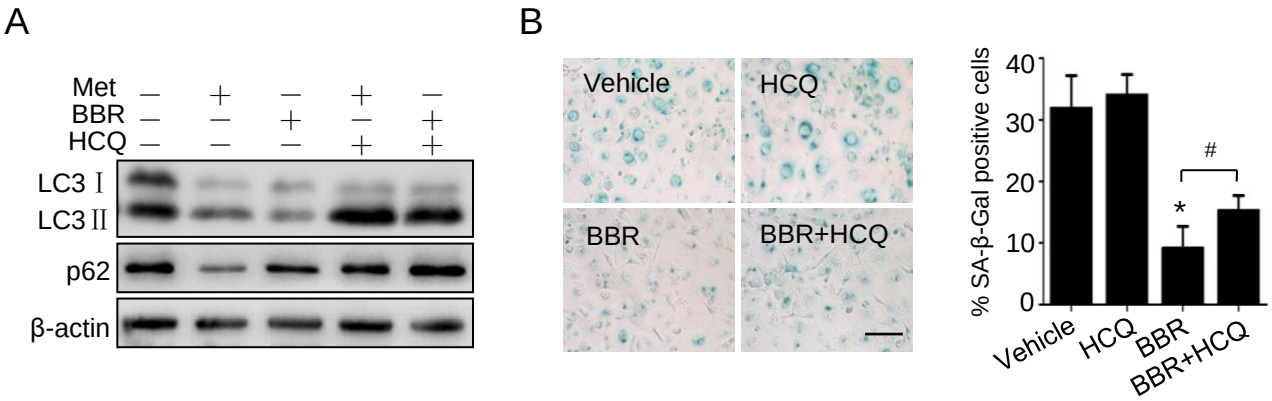

Figure S5. Activation of AMPK suppressed the impairment of H_2O_2-induced autophagic flux and decreased the senescence in HUVECs. A to C: H_2O_2-treated HUVECs were incubated with Met (10 mM), BBR (10 μM) and HCQ (2 μM) alone or in combination for 3 days. (A) Representative images from immunoblot assays against LC3 and p62 proteins. (B) Representative images of SA-β-Gal staining of the cells (left) and the percentages of SA-β-gal-positive cells (right). * $p< 0.05$ compared to the vehicle. # $p< 0.05$ compared to the indicated groups. The bar represents 100 μm.

Figure S6

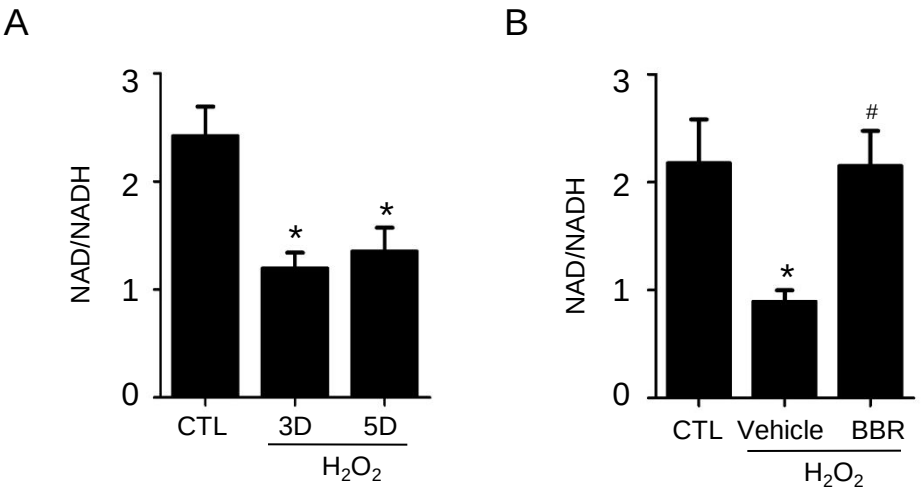

Figure S6. Activation of AMPK improved the redox status in senescent cells. (A) Cellular concentrations of NAD/NADH on day 3 or day 5 after H_2O_2 treatment in NIH3T3 cells incubated in normal medium. (B) H_2O_2-treated cells were incubated with BBR (10 μM) and CC (10 μM) for 3 days. The concentrations of NAD/NADH are shown. * $p<0.05$ compared to the CTL, # $p< 0.05$ compared to the vehicle.

Figure S7

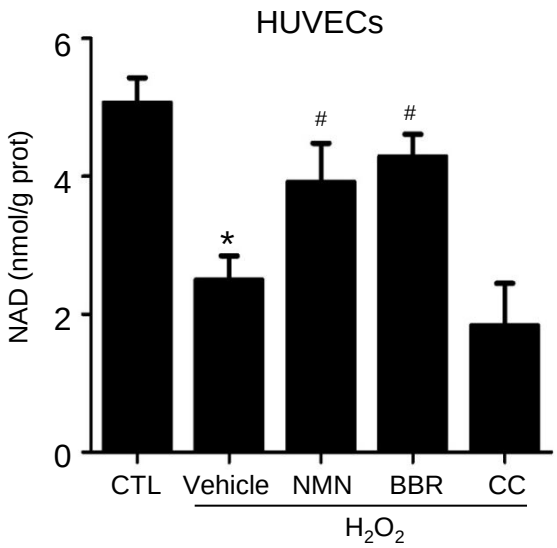

Figure S7. Activation of AMPK increased the NAD^+ level in HUVEC Cells. HUVECs were treated with H_2O_2 and incubated in complete medium with NMN (500 μM), BBR (10 μM) and CC (10 μM) for 3 days. The concentrations of NAD^+ are shown. * $p< 0.05$ compared to the control. # $p< 0.05$ compared to the vehicle.

Figure S8

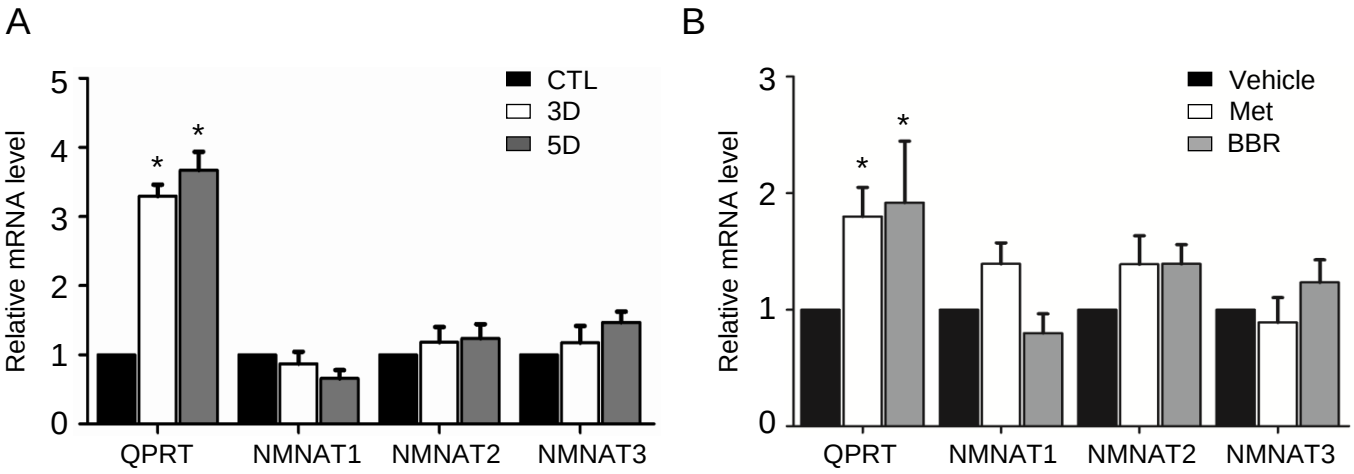

Figure S8. The mRNA level of QPRT is increased during senescence. (A) NIH3T3 cells were treated with H_2O_2 and incubated in complete medium without H_2O_2 for 3-5 days. The relative fold-changes in the mRNA level of *QPRT, NMNAT1, NMNAT2, NMNAT3* genes were monitored by qRT-PCR. (B) H_2O_2-treated cells were incubated with Met (10 mM), BBR (10 μM) for 3 days. The relative fold-changes in the mRNA level of *QPRT, NMNAT1, NMNAT2, NMNAT3* genes were monitored by qRT-PCR. * $p < 0.05$ compared to the control (A) or vehicle (B).

Figure S9

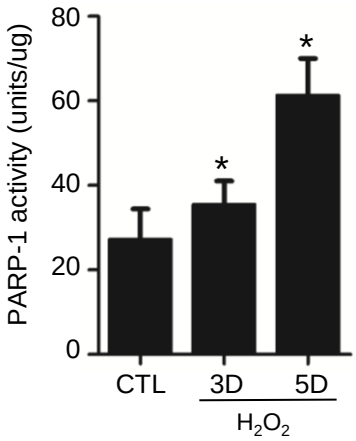

Figure. S9 The activity of PARP-1 is increased during senescence. NIH3T3 cells were treated with H_2O_2 and incubated in complete medium without H_2O_2 for 3-5 days. The activity of PARP-1 is shown. * $p < 0.05$ compared to the control.

Figure S10

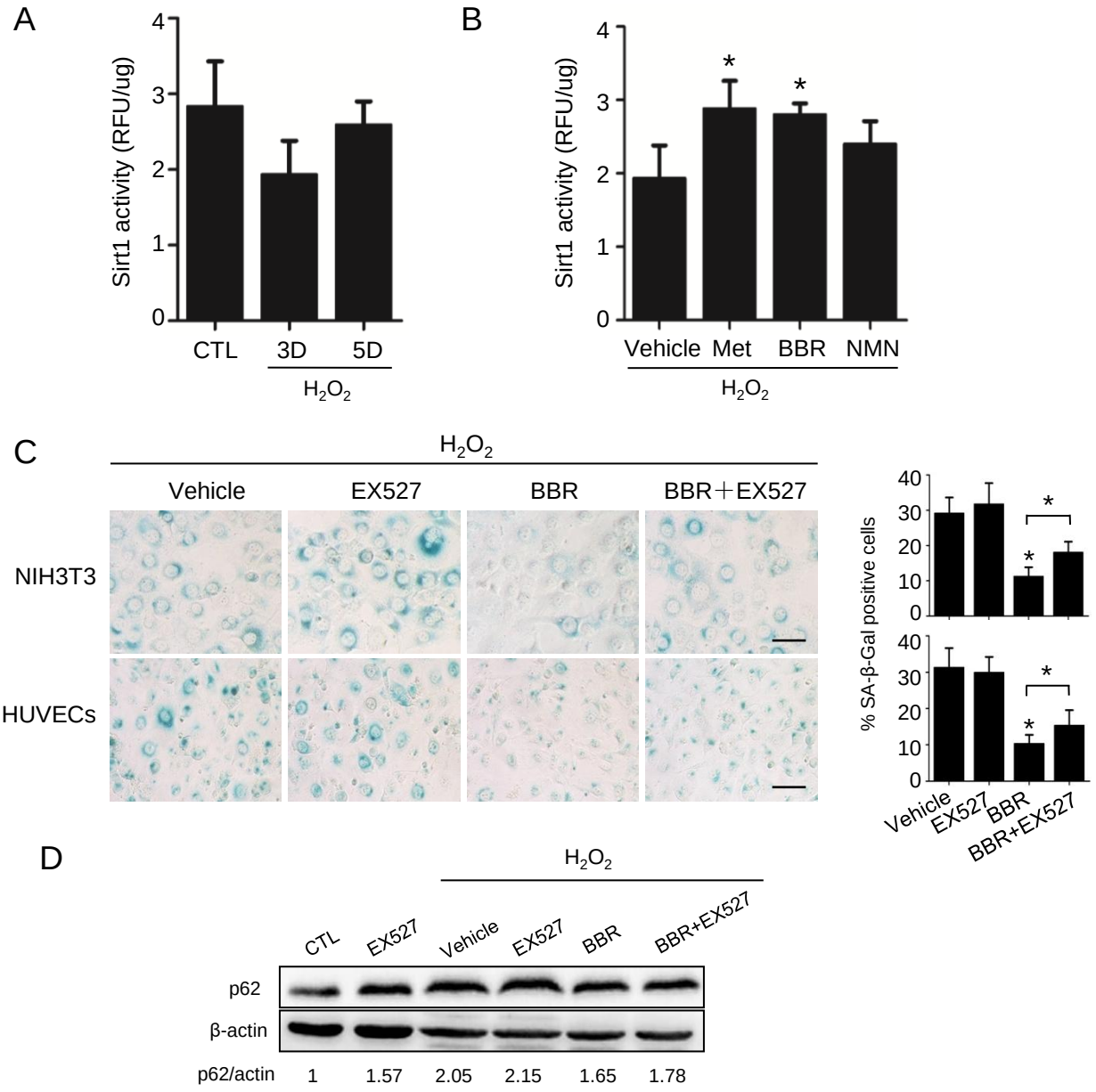

Figure S10. Sirt1 is involved in H_2O_2-induced senescence as a downstream mediator of AMPK and NAD^+ biosynthesis. (A) NIH3T3 cells were treated with H_2O_2 and incubated in complete medium without H_2O_2 for 3-5 days. The activity of Sirt1 is shown. (B) H_2O_2-treated NIH3T3 cells were incubated with Met (10 mM), BBR (10 μM) and NMN (500 μM) for 3 days. The activity of Sirt1 is shown. (C) H_2O_2-treated NIH3T3 cells and HUVECs were incubated with a Sirt1 inhibitor (EX527, 1 mM) and BBR (10 μM) alone or in combination as indicated for 3 days. Representative images of SA-β-Gal staining of cells (left) and the percentages of SA-β-gal-positive cells (right). (D) Representative images from immunoblot assays against p62 and β-actin. * p<0.05 compared to the vehicle (B) or indicated groups (C). The bar represents 100 μm.

Figure S11

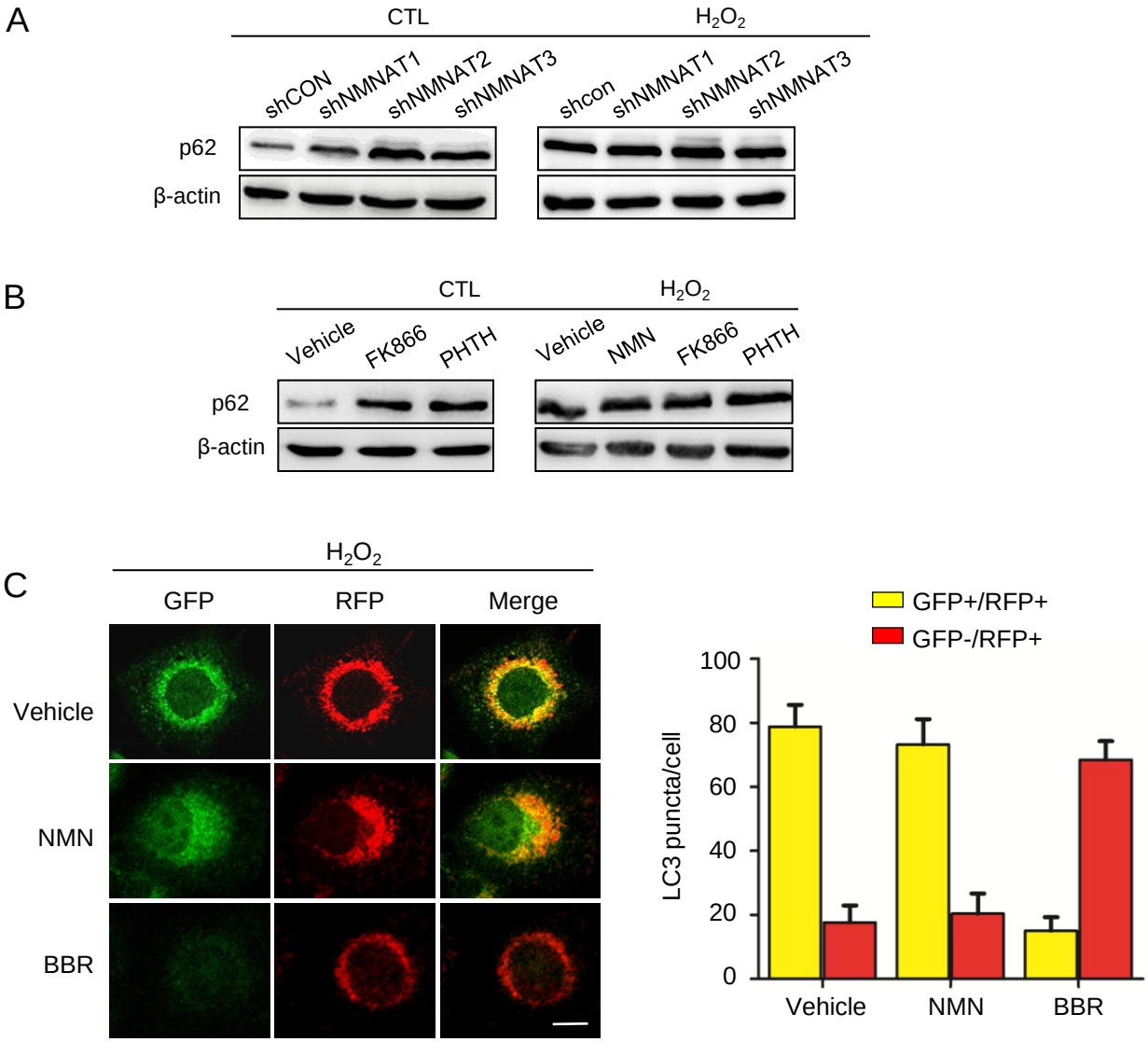

Figure S11. NAD^+ homeostasis is required for maintaining the autophagic flux in normal cells, but not in senescent cells. (A) NIH3T3 cells infected with shRNAs were treated with or without H_2O_2 and cultured for 3 days. Representative images from immunoblot assays against p62 and β-actin are shown. (B) H_2O_2-treated or untreated NIH3T3 cells were incubated with FK866 (5 nM), PHTH (1 mM), and NMN (500 μM) for 3 days. Representative images from immunoblot assays against p62 and β-actin are shown. (C) Representative confocal fluorescent images of GFP-RFP-LC3-expressing cells. The right panel showed the merged fluorescence (left), and the percentages of cells with puncta-like LC3 expression were determined (right). The bar represents 20 μm.

Figure S12

Fig 1E

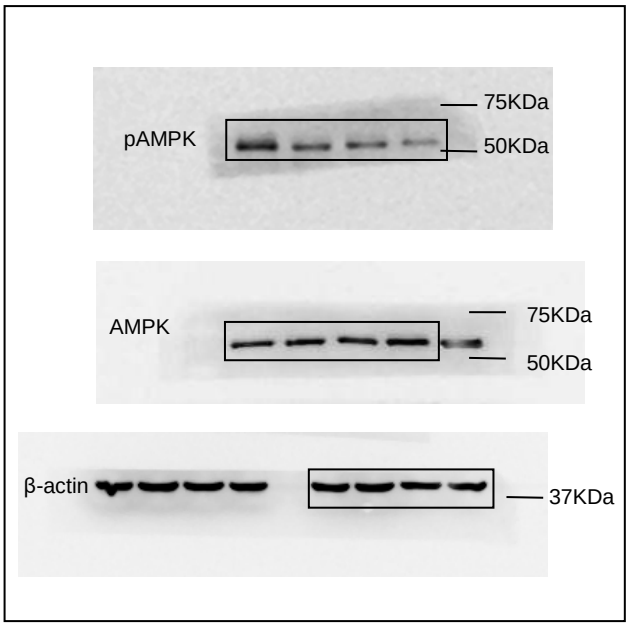

Fig 3B

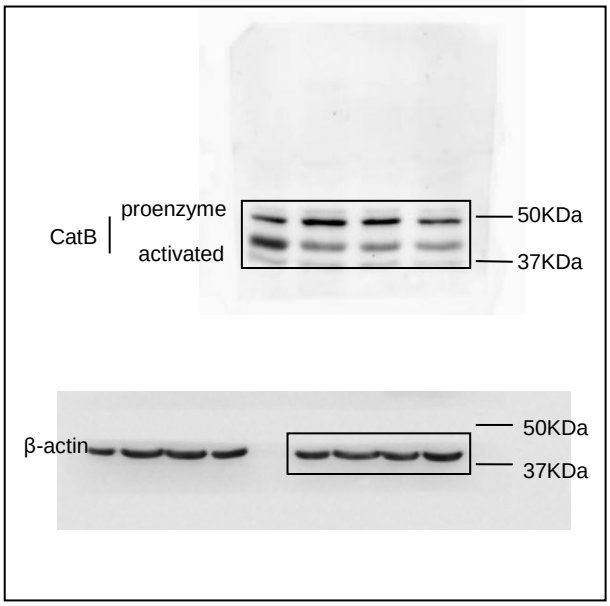

Fig 3H

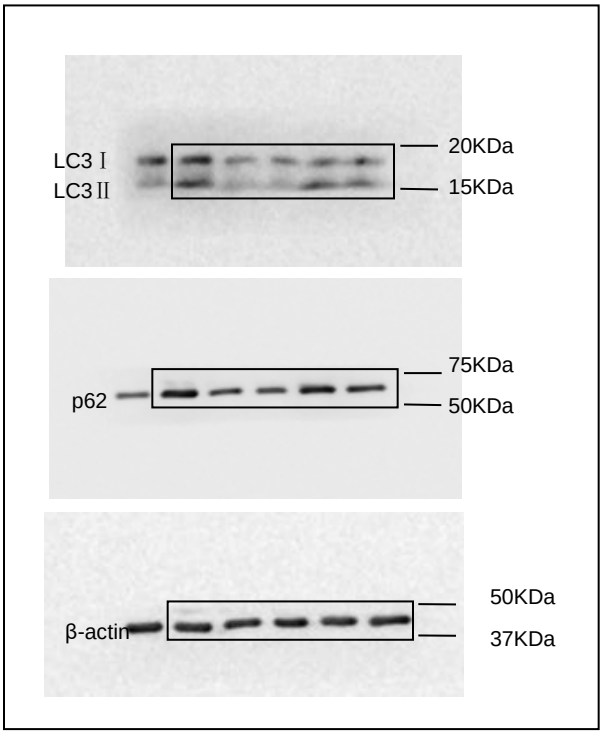

Figure S12. Unprocessed images of western-blot.