Supplementary data:

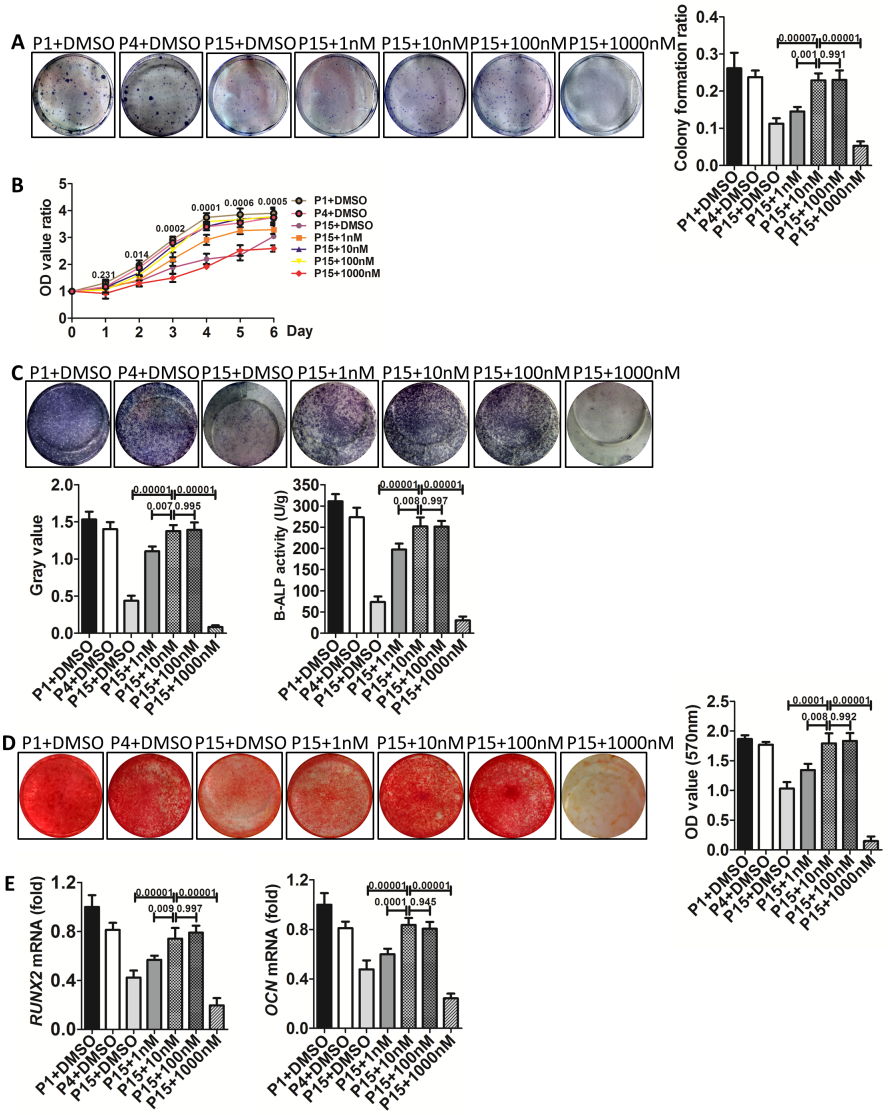

Fig.S1

Fig. S1 Optimal concentration of melatonin for stemness preservation of long-term passaged BMMSCs.

Rat BMMSCs cultured with DMSO for 1 passage (P1), 4 passages (P4), and 15 passages (P15) and with different concentration of melatonin for 15 passages were used in the following analysis. (**A**) 5×10^2 BMMSCs were seeded in 5-cm dishes for 10 days. CFU-F was analyzed by toluidine blue staining and the colony ratio was counted (n=3). (**B**) 1×10^3/well BMMSCs were seeded in 96-well plates. Proliferation of BMMSCs was detected from day 0 to day 6 (n=3). (**C**) 1×10^5/well BMMSCs seeded in 12-well plates were induced with osteogenic medium for 7 days. Activity of ALP was detected by ALP staining and quantified with Image-Pro Plus 6.0 software (n=3). Activity of B-ALP was detected by ELISA and the values were normalized to corresponding total protein concentration

(n=3). (**D**) BMMSCs were induced with osteogenic medium for 28 days. Mineralized nodules were detected by alizarin red staining and quantified with a spectrophotometer after dissolving with isopropanol (n=3). (**E**) *Runx2* and *Ocn* mRNA levels of BMMSCs were analyzed by Real-time RT-PCR. *β-actin* was used as the loading control for quantification (n=3). Data are shown as mean±SD. *P* value is presented in each graph.

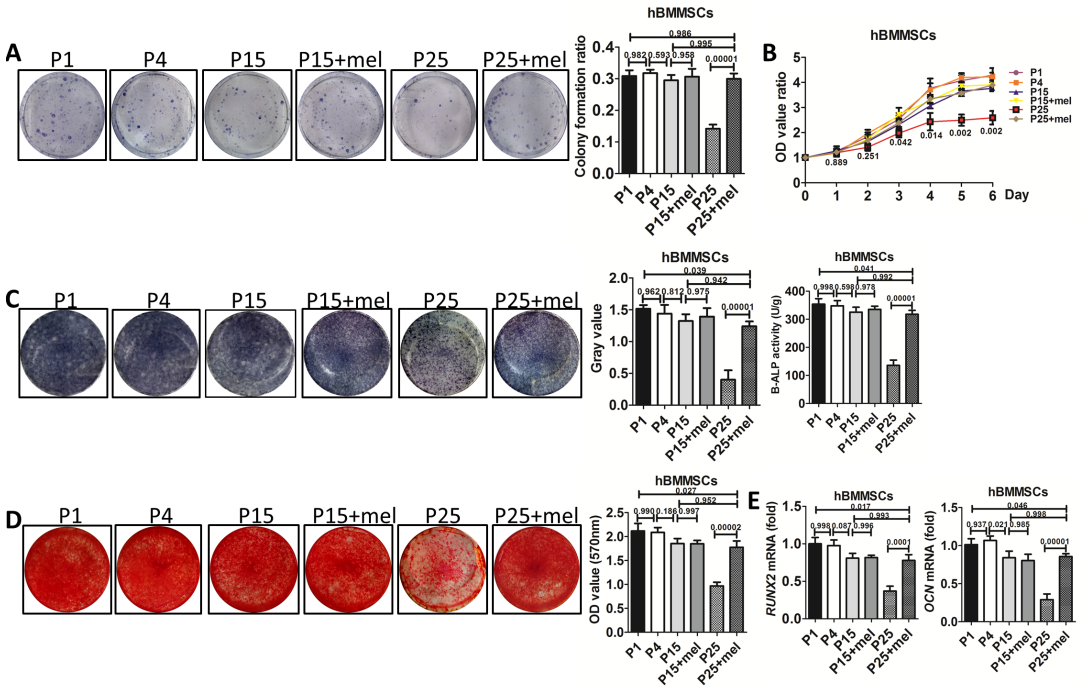

Fig.S2

Fig. S2 Melatonin treatment prevents the stemness loss of long-term passaged human BMMSCs.

Human BMMSCs cultured with DMSO for 1 passage (P1), 4 passages (P4), 15 passages (P15) and 25 passages (P25), or with melatonin for 15 passages and 25 passages were used in the following analysis. (**A**) 5×10^2 BMMSCs were seeded in 5-cm dishes for 10 days. CFU-F was analyzed by toluidine blue staining and the colony ratio was counted (n=3). (**B**) 1×10^3/well BMMSCs were seeded in 96-well plates. Proliferation of BMMSCs was detected from day 0 to day 6 (n=3). (**C**) 1×10^5/well BMMSCs seeded in 12-well plates were induced with osteogenic medium for 7 days. Activity of ALP was detected by ALP staining and quantified with Image-Pro Plus 6.0 software (n=3). Activity of B-ALP was detected by ELISA and values were normalized to corresponding total protein concentration (n=3). (**D**) BMMSCs were induced with osteogenic medium for 28 days. Mineralized nodules were detected by alizarin red staining and quantified with a spectrophotometer after dissolving with isopropanol (n=3). (**E**) *Runx2* and *Ocn* mRNA levels of BMMSCs were analyzed by Real-time RT-PCR. *β-actin* was used as the loading control for quantification (n=3). Data are shown as mean±SD. *P* value is presented in each graph.

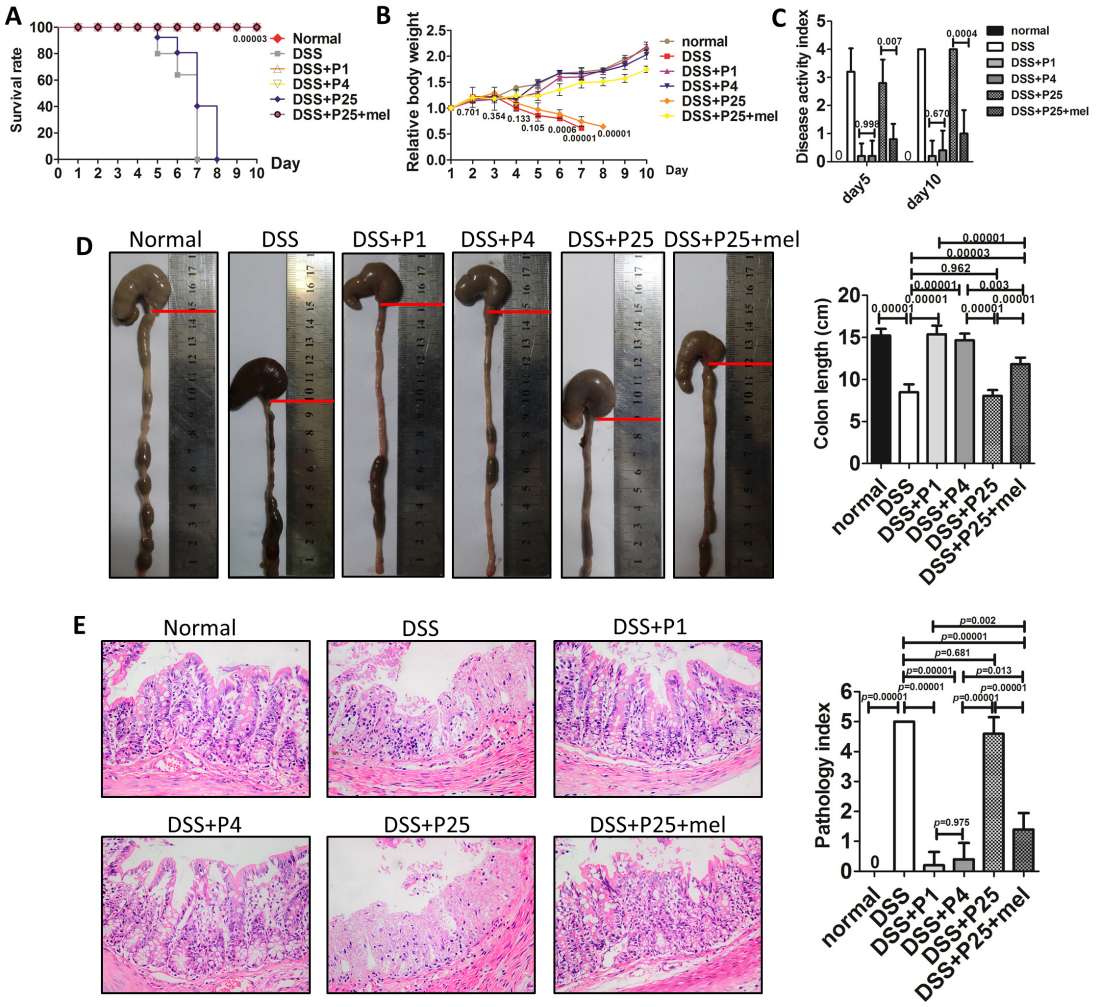

Fig.S3

Fig. S3 Melatonin treatment improves therapeutic effect of long-term passaged human BMMSCs on experimental colitis.

Rats were administrated with DMSO-treated P1, P4, P25 human BMMSCs or melatonin-treated P25 human BMMSCs *via* tail vein respectively at day 3 of DSS feeding. Normal group and DSS group were injected with equal volume of PBS. (**A**) Survival of rats was recorded every day for 10 days (n=4). (**B**) Body weights were recorded every day for 10 days. The relative change of body weight was calculated (n=4). (**C**) Disease activity index (DAI) was evaluated at day 5 and day 10 according to the symptoms (n=4). (**D**) Colon length was measured at day 10 (n=4). (**E**) H&E staining was performed to detect the histological changes of colon isolated at day 10 (n=4). The pathological index of each colon was calculated (n=4). Scale bar, 100 μm. Data are shown as mean±SD. *P* value is presented in each graph.

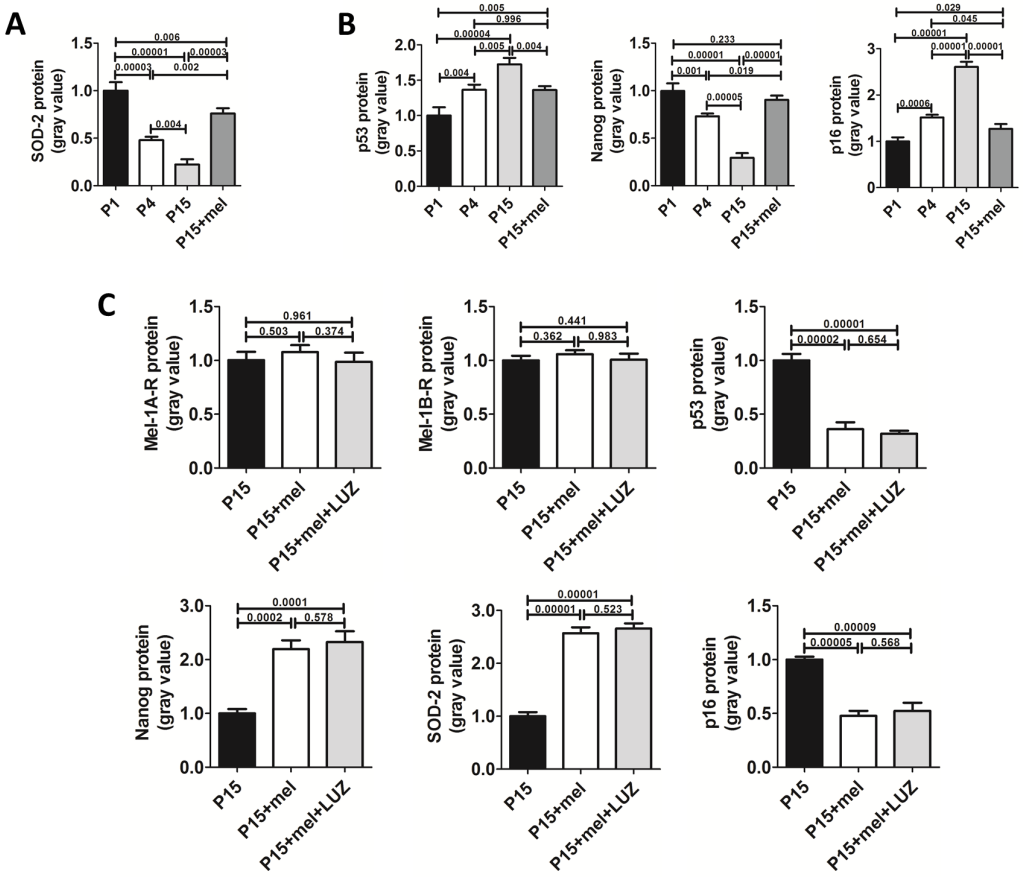

Fig.S4

Fig. S4 Quantification of western blots in Figure 8.

Expression of SOD2, p53, p16, NANOG, Mel-1A-R and Mel-1B-R protein was detected by western blot analysis. The gray values of specific protein bands were analyzed by Image J software. β-actin was used as the loading control (n=3). (**A**) Quantification of blots in Fig. 8C. (**B**) Quantification of blots in Fig. 8F. (**C**) Quantification of blots in Fig. 8P. Data are shown as mean±SD. *P* value is presented in each graph.

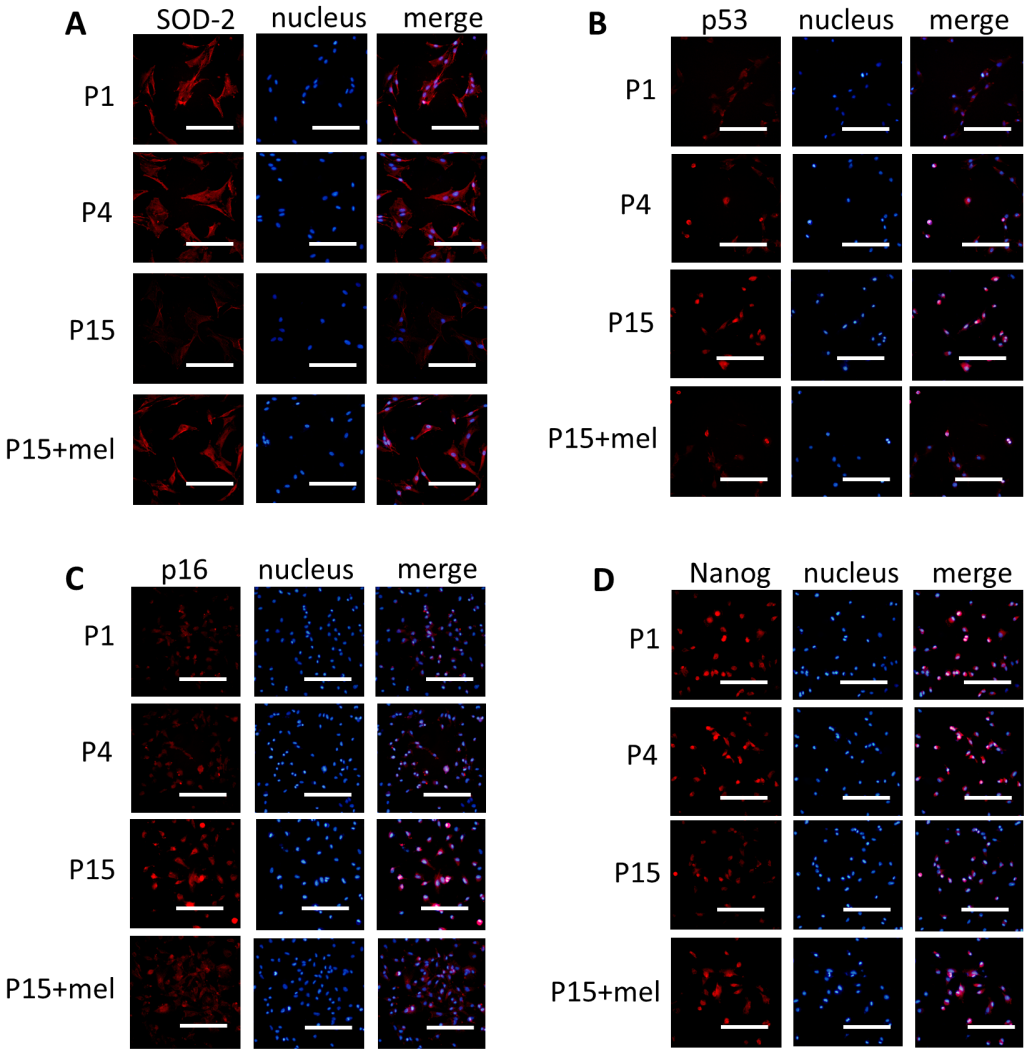

Fig.S5

Fig. S5 Melatonin treatment incresases SOD2 levels, inhibits p53 pathway, and preserves NANOG expression in long-term passaged BMMSCs.

Rat BMMSCs were treated with DMSO for 1, 4 and 15 passages or with 10 nM melatonin for 15 passages. (**A**) SOD2, (**B**) p53, (**C**) p16 and (**D**) NANOG protein accumulations in rat BMMSCs were detected by immunofluorescence. Cell nuclei were counterstained by Hoechst 33342 (n=3). Scale bar, 200 μm.

Supplementary Table:

Supplementary Table 1. Sequence of primers for realtime RT-PCR

mRNA/primer	Forward（5’-3’）	Reverse（5’-3’）
r-β-actin	GGAGATTACTGCCCTGGCTCCTA	GACTCATCGTACTCCTGCTTGCTG
r-Runx2	CATCGCCGGGAATGATGAG	TGTGAAGACCGTGATGGTCAAAGTG
r-Ocn	CCACCCGGGAGCAGTGT	GAGCTGCTGTGACATCCATACTTG
r-Sod2	CACATTAACGCGCAGATCATG	CCTTAGGGCTCAGGTTTGTC
r-Nanog	TTCTGAACCTGAGCTATAAGCAG	GAGAATAGCTGCAATGGATG
r-p16	TCCTCCGCTGGGAACGT	GGCGTGCTTGAGCAGAAGTT
r-p53	CCCATCCTTACCATCATCACG	TGCTGGTGGGCAGTGCTCTCT
r-Mel-1A-R	TTGTGGCGAGTTTAGCTGTG	TTTACCCTCCGTCTGACCTG
r-Mel-1B-R	TACATCAGCCTCATCTGGCTT	CACAAACACTGCGAACATGGT
h-β-actin	TGGCACCCAGCACAATGAA	CTAAGTCATAGTCCGCCTAGAAGCA
h-Runx2	AGATGATGACACTGCCACCTCTG	GGGATGAAATGCTTGGGAACT
h-Ocn	CCTCACACTCCTCGCCCTATT	CCCTCCTGCTTGGACACAAA