Supplementary Information

Bioassay for monitoring the anti-aging effect of cord blood treatment

Sang-Hun Bae[1#], Ala Jo[1,2#], Jae Hyun Park[1], Chul-Woo Lim[1], Yuri Choi[1], Juhyun Oh[2], Ji-Min Park[1], TaeHo Kong[1], Ralph Weissleder[2,3], Hakho Lee[2*], Jisook Moon[1*]

1. Department of Biotechnology, College of Life Science, CHA University, Gyeonggi-do 13488, Republic of Korea

2. Center for Systems Biology, Massachusetts General Hospital, Harvard Medical School, Boston, MA 02114, USA

3. Department of Systems Biology, Harvard Medical School, Boston, MA 02115, USA

#These authors contributed equally.

Supplementary Figures

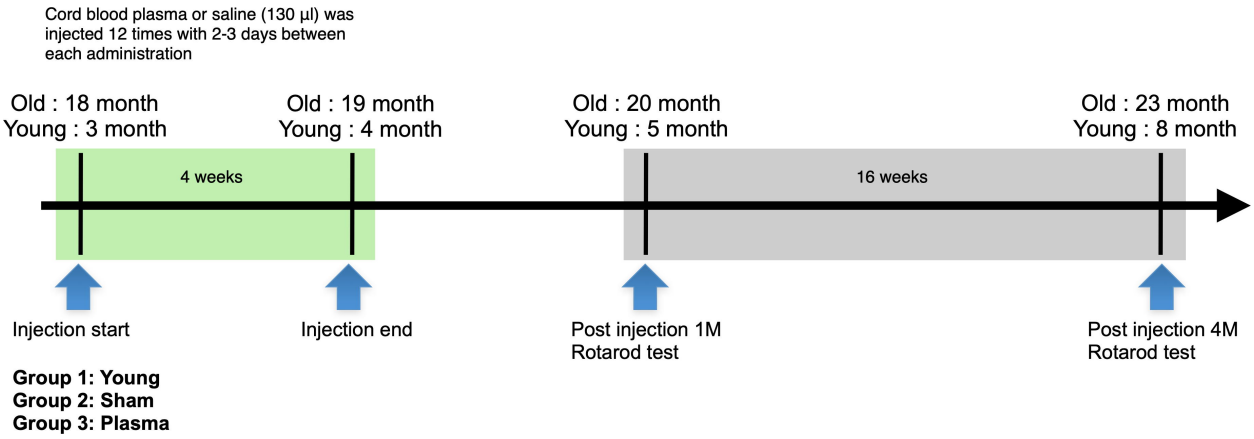

Figure S1. Scheme of animal experiment design. Experimental animal groups were separated into three groups:1) young group was used as a control, 2) sham group received saline, and 3) Plasma group was given cord plasma. Cord blood plasma or saline (130 µl) was administered to the old (18-month-old) group 12 times at an interval of 2 to 3 days during 4 weeks, and the 12th or last injection was conducted at 4 weeks after the first injection (shaded in green). Rotarod test was performed 1 month and 4 months (shaded in gray) after the 12th injection.

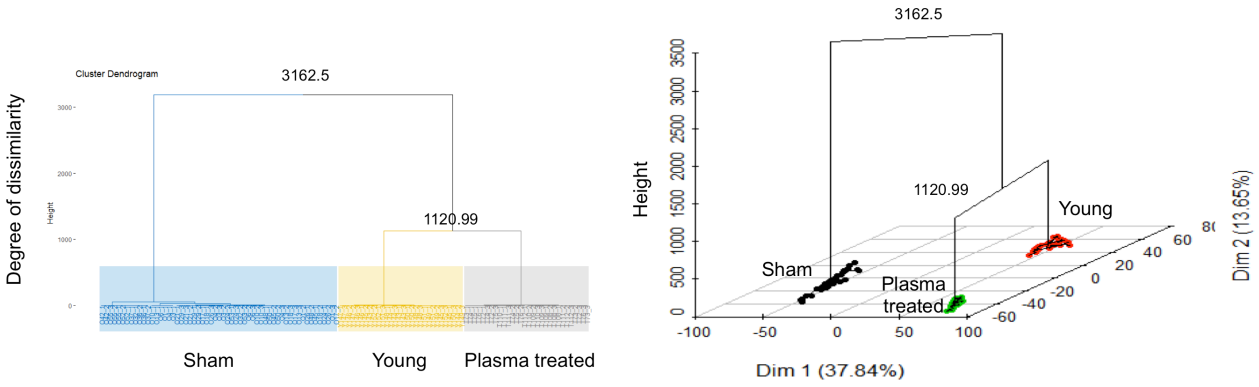

Figure S2. Hierarchical clustering on PC 1 and 2 from PCA with dendrogram. Height means the degree of dissimilarity between the corresponding clusters. The dissimilarity (3162.5) between the sham group and the rest of groups is larger than that between the young and plasma-treated groups (1120.99).

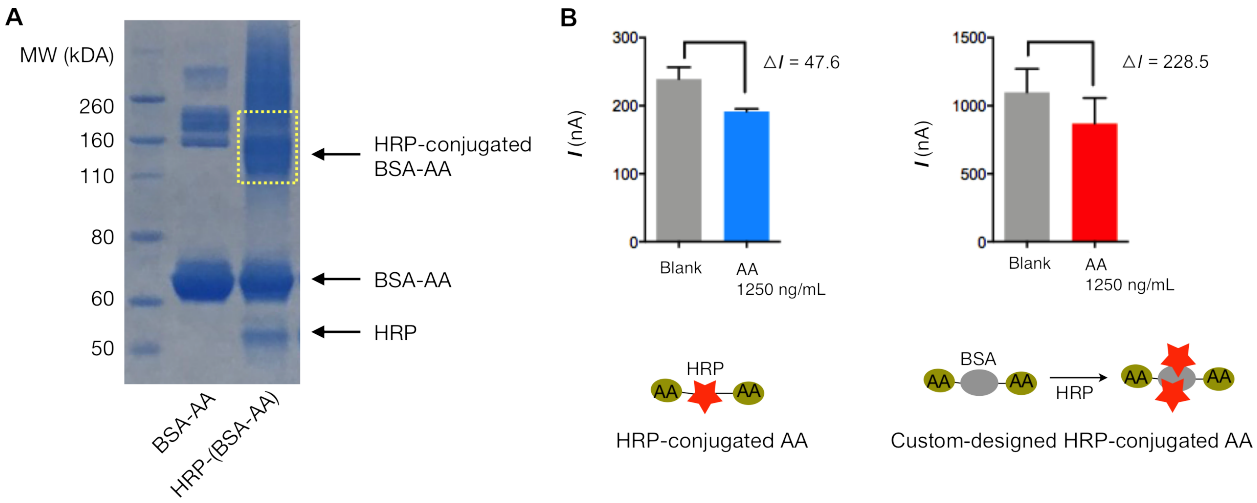

Figure S3. Preparation and validation of in-house HRP-conjugated AA. We used BSA-conjugated AA (BSA-AA; Biomatik, Cat. #RPU51089) as a starting material and linked HRP to BSA using reductive amination chemistry. (A) Gel electrophoresis and protein band analysis. The starting material, BSA-AA, showed a strong band at 66 kDa. With HRP (~44.5 kDa) conjugation, new bands appeared at the molecular weight of 110 kDa and 160 kDa. The results indicated about 1.5:1 binding ratio between HRP and BSA-AA. (B) Comparison between commercially available HRP-conjugated AA (Biomatik; left) and custom-made AA-HRP (right). Test samples were prepared by spiking in the same concentration of AA (1250 ng/mL). The electrical current was about 5-fold higher when custom-made AA-HRP was used.

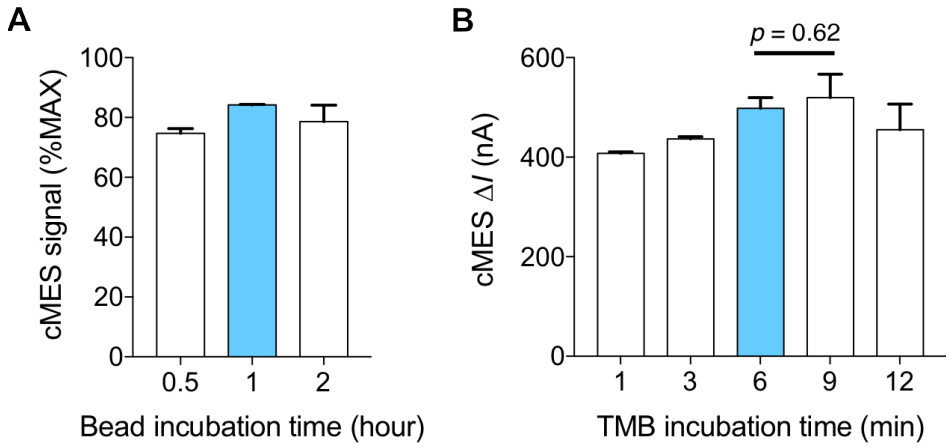

Figure S4. Assay optimization. (A) AA capture time. AA samples and magnetic beads (MBs) were mixed and incubated for different durations. Control samples had AA-HPR only and were used to define the maximum signal. One hour incubation with MBs yielded the largest signal. (B) The reaction time between AA-captured MBs and TMB was varied. Six minutes after initiating the reaction, the signal level reached its maximum.