Supplementary information

Human adipose tissue-derived mesenchymal stem cells secrete functional neprilysin-bound exosomes

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Supplementary Table 1. Donor information for used adipose tissue-derived mesenchymal stem cells.

Supplementary Table 2. Donor information for used bone marrow-derived mesenchymal stem cells.

Supplementary figure legends

Fig. S1. Surface marker profiles of ADSCs and BM-MSCs.

Both cells showed similar surface marker profiles that are characteristic of MSCs (positive for CD105, CD73, CD90, and CD44; negative for CD45, CD31, and CD34).

Fig. S2. NEP enzyme activity of ADSC lysates.

Enzyme activity was measured using a fluorogenic substrate for ADSCs #1–3 (A, B) and ADSCs #4–6 (C, D) in the absence or presence of the NEP inhibitor thiorphan. Data are the mean ± S.D. (n = 3). In each experiment, the enzyme activity rate was determined as the gradient of the fluorescence intensity versus time plots in the linear region (where $R^2 > 0.97$). Enzyme activity was also measured for rhNEP dilution series to produce a standard curve (E, G). Good standard curves were obtained in each experiment ($R^2 > 0.99$) (F, H) and were used to determine the NEP activity indices.

Fig. S3. NEP enzyme activity of BM-MSC lysates.

Enzyme activity was measured using a fluorogenic substrate for BM-MSCs #1-4 and ADSC #4 for comparison in the absence (A) or presence (B) of the NEP inhibitor thiorphan. The specific NEP activity was calculated by subtracting residual fluorescent intensity after incubation with the NEP inhibitor thiorphan. Data are the mean ± S.D. (n = 3).

Fig. S4. Calculation of NEP activity index of ADSC-derived exosomes.

Enzyme activity was measured using a fluorogenic substrate for exosomes from ADSCs

#1–4 in the presence and absence of the NEP inhibitor thiorphan (Fig 4B-D). In each experiment, the enzyme activity rate was determined as the gradient of the fluorescence intensity versus time plots in the linear region (where $R^2 > 0.97$). Enzyme activity was also measured for rhNEP dilution series (A) to produce a standard curve (B). ($R^2 > 0.99$). Using the produced standard curve, NEP activity indices was calculated (Fig. 4F).

Fig. S5. Effect of ADSC-derived exosomes on the intracellular Aβ42 level of N2a cells.

(a) The schematic representation of the experiment. (b) ADSC-derived exosomes decrease slightly, but significantly, the intracellular Aβ42 level in the recipient N2a cells. Data are the mean ± S.D. (n = 4).

Fig. S6. NEP contribution ratio for BM-MSC-derived exosomes

Shown are the NEP contribution ratios for exosomes derived from BM-MSCs #1 and #4 (right 2 columns) in comparison with that for ADSC #2-derived exosomes (left column). Data are the mean ± S.D. (n = 3).

Table S1. Donor information for used adipose tissue-derived mesenchymal stem cells.

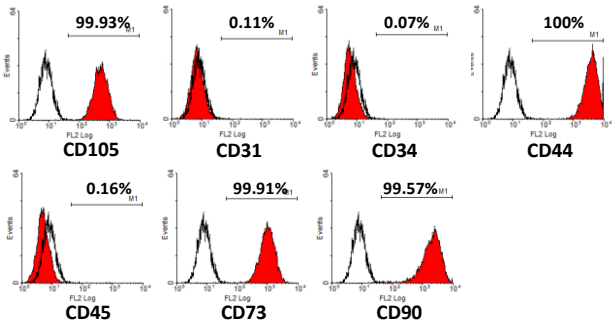
ID	#1	#2	#3	#4	#5	#6
Sex	Female	Female	Female	Female	Female	Female
Age	42	22	45	22	49	23
Site	Flanks, Hips, Thighs (Subcutaneous)	Flanks, Hips, Thighs (Subcutaneous)	Flanks, Hips, Thighs (Subcutaneous)	Abdomen, Flanks (Subcutaneous)	Abdomen, Flanks (Subcutaneous)	Hips, Thighs (Subcutaneous)

Table S2. Donor information for used bone marrow-derived mesenchymal stem cells.

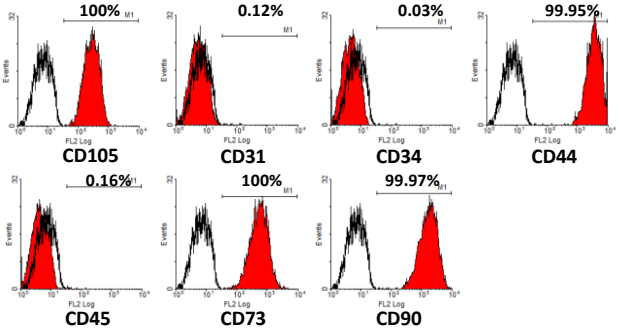
ID	#1	#2	#3	#4
Sex	Male	Male	Male	Male
Age	55	30	64	21
Site	Ilium	Ilium	Ilium	No information

Fig. S1

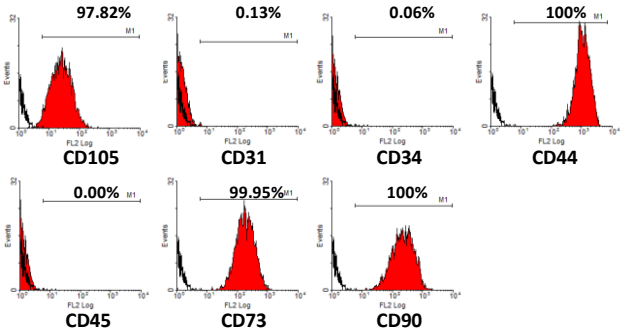
BM-MSC #1



BM-MSC #4



ADSC #1



ADSC #4

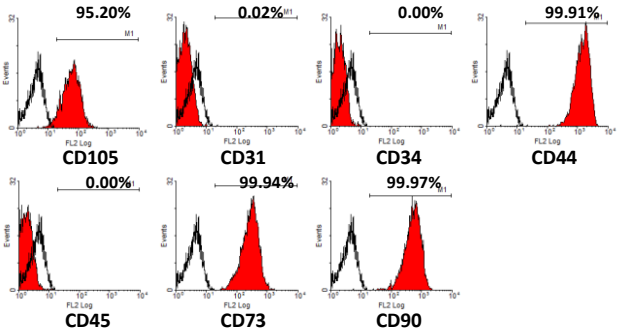


Fig. S2

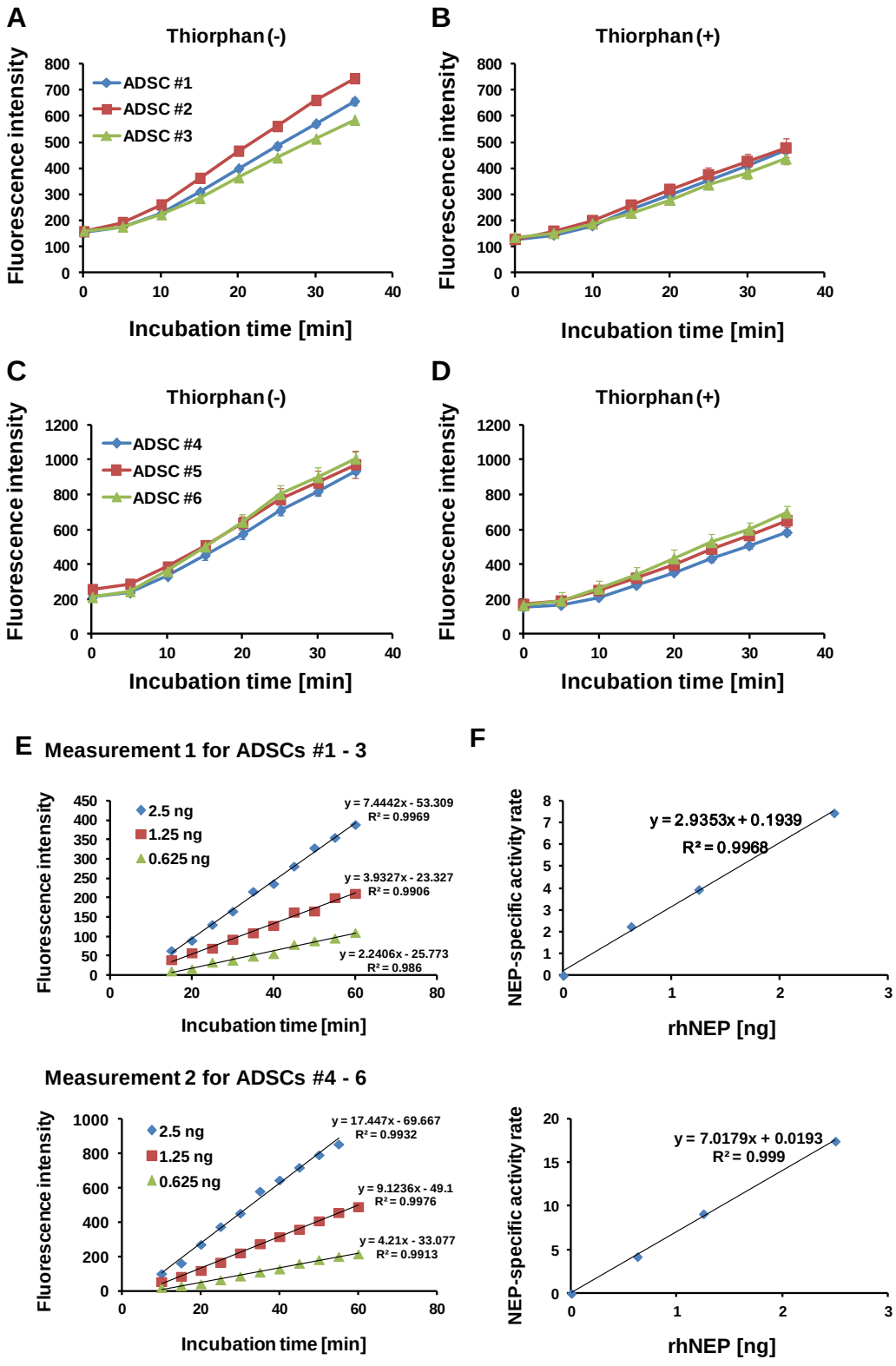

A
Thiorphan (-)
Fluorescence intensity
ADSC #1
ADSC #2
ADSC #3
Incubation time [min]
B
Thiorphan (+)
Fluorescence intensity
Incubation time [min]
C
Thiorphan (-)
Fluorescence intensity
ADSC #4
ADSC #5
ADSC #6
Incubation time [min]
D
Thiorphan (+)
Fluorescence intensity
Incubation time [min]
E
Measurement 1 for ADSCs #1 - 3
Fluorescence intensity
2.5 ng
1.25 ng
0.625 ng
y = 7.4442x - 53.309
R² = 0.9969
y = 3.9327x - 23.327
R² = 0.9906
y = 2.2406x - 25.773
R² = 0.986
Incubation time [min]
F
NEP-specific activity rate
y = 2.9353x + 0.1939
R² = 0.9968
rhNEP [ng]
Measurement 2 for ADSCs #4 - 6
Fluorescence intensity
2.5 ng
1.25 ng
0.625 ng
y = 17.447x - 69.667
R² = 0.9932
y = 9.1236x - 49.1
R² = 0.9976
y = 4.21x - 33.077
R² = 0.9913
Incubation time [min]
NEP-specific activity rate
y = 7.0179x + 0.0193
R² = 0.999
rhNEP [ng]

Fig. S3

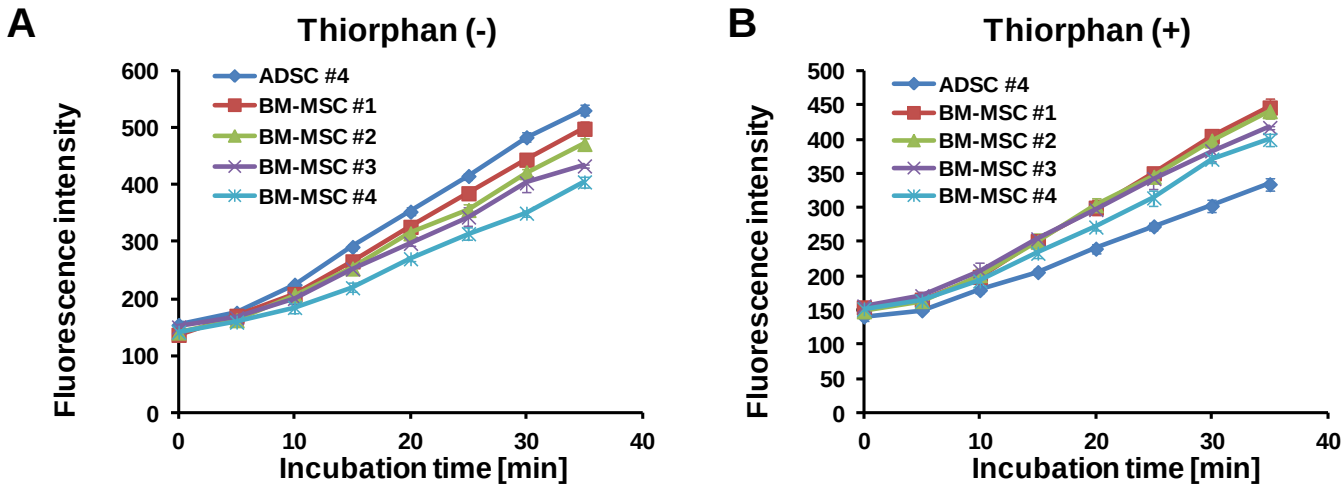

A
Thiorphan (-)
Fluorescence intensity
Incubation time [min]
ADSC #4
BM-MSC #1
BM-MSC #2
BM-MSC #3
BM-MSC #4
0
100
200
300
400
500
600
0
10
20
30
40
B
Thiorphan (+)
Fluorescence intensity
Incubation time [min]
ADSC #4
BM-MSC #1
BM-MSC #2
BM-MSC #3
BM-MSC #4
0
50
100
150
200
250
300
350
400
450
500
0
10
20
30
40

Fig. S4

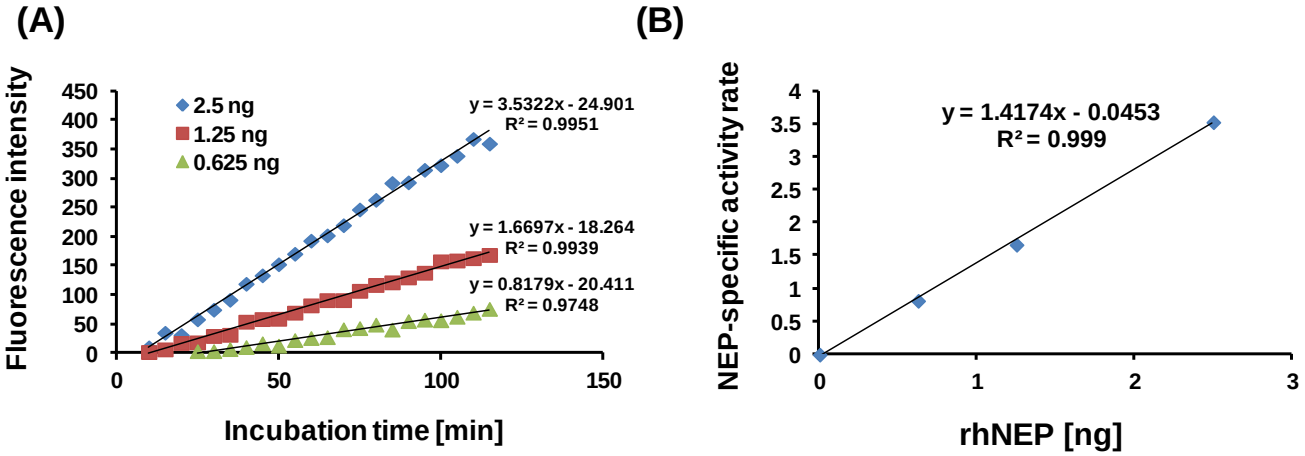

(A)
(B)
Fluorescence intensity
Incubation time [min]
2.5 ng
1.25 ng
0.625 ng
y = 3.5322x - 24.901
R² = 0.9951
y = 1.6697x - 18.264
R² = 0.9939
y = 0.8179x - 20.411
R² = 0.9748
NEP-specific activity rate
rhNEP [ng]
y = 1.4174x - 0.0453
R² = 0.999

Fig. S5

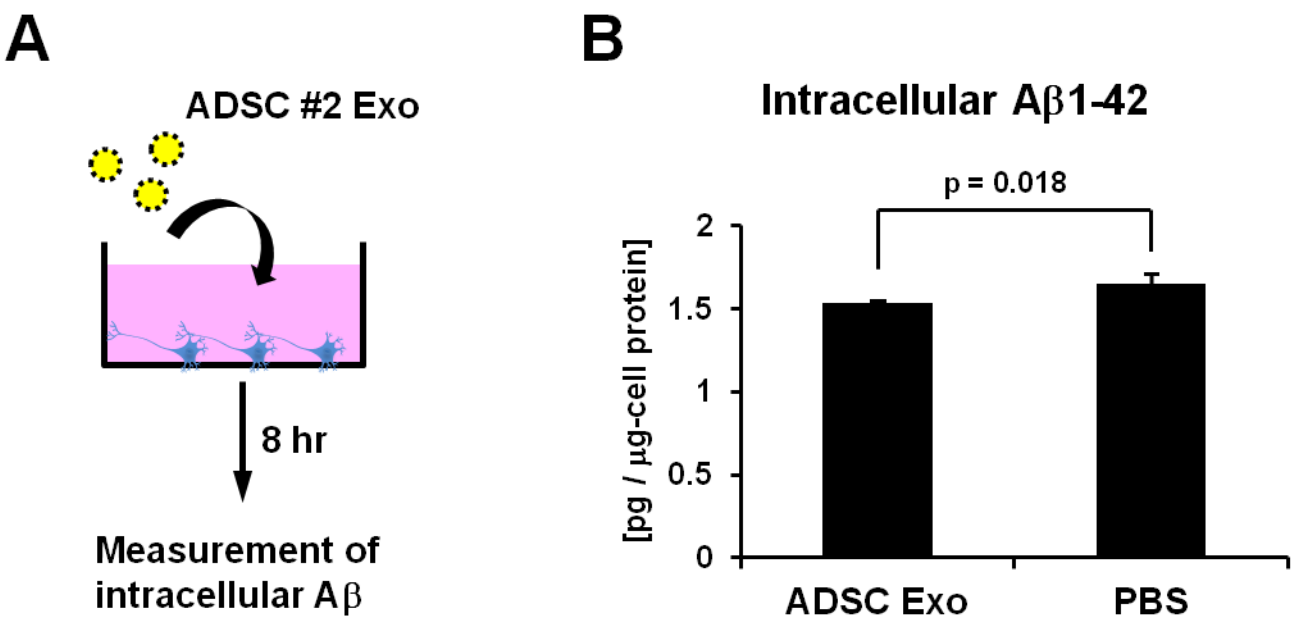
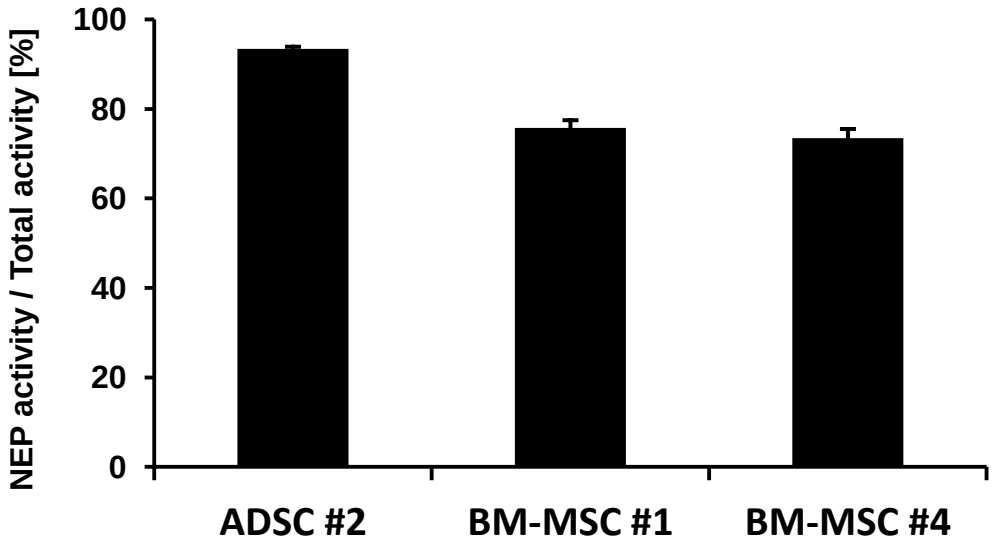

A
ADSC #2 Exo
8 hr
Measurement of
intracellular Aβ
B
Intracellular Aβ1-42
p = 0.018
[pg / μg-cell protein]
2
1.5
1
0.5
0
ADSC Exo
PBS

Fig. S6


NEP activity / Total activity [%]
100
80
60
40
20
0
ADSC #2
BM-MSC #1
BM-MSC #4