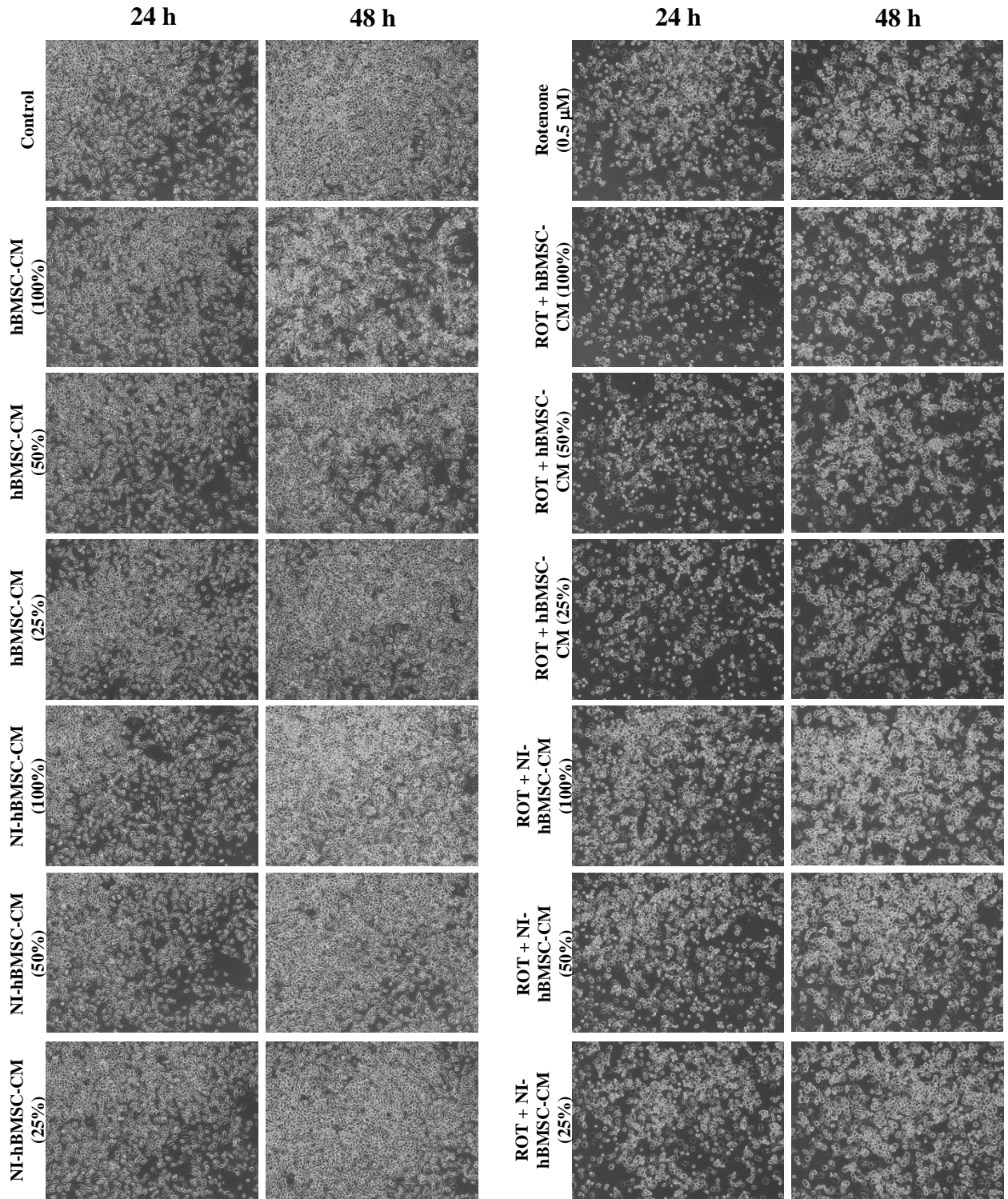
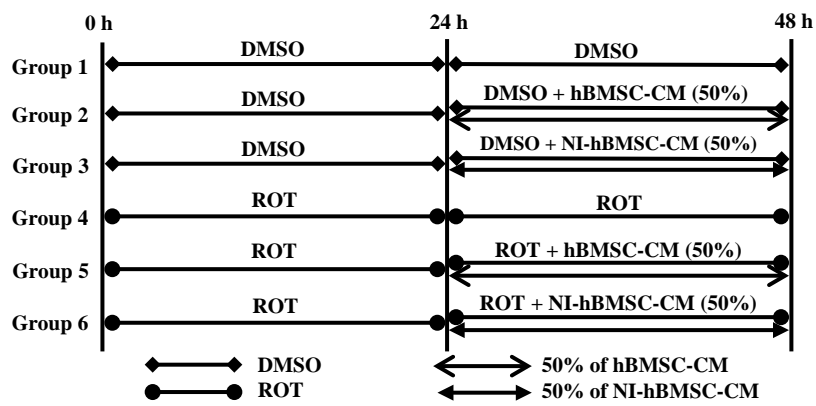


## Supplementary Materials for

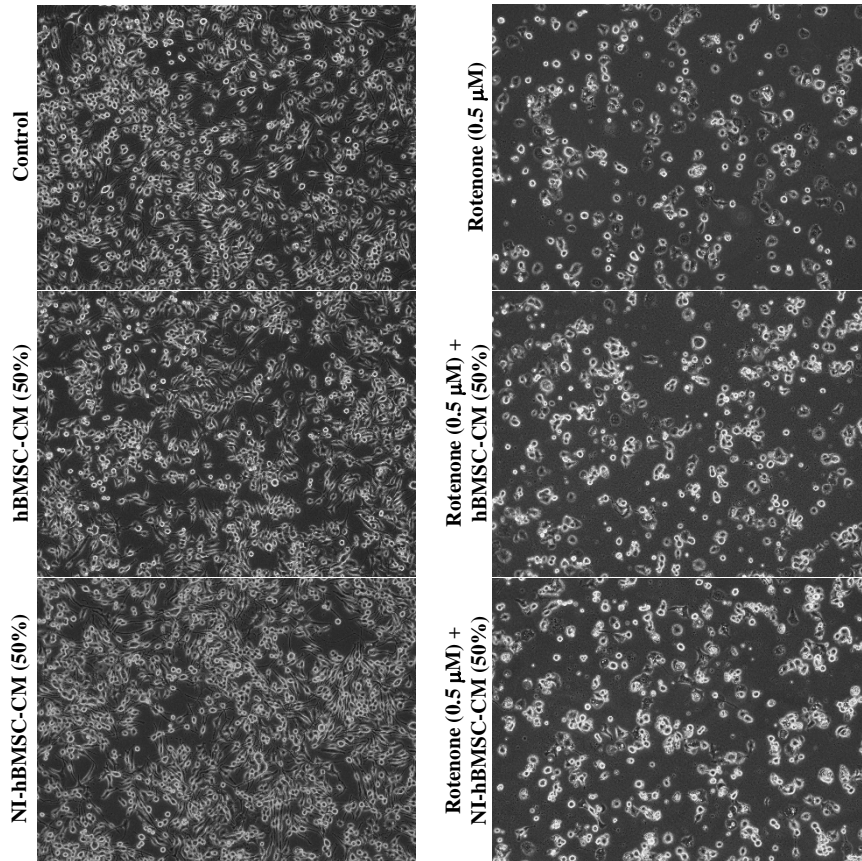
### “Therapeutic Effects of Conditioned Medium of Neural Differentiated Human Bone Marrow-Derived Stem Cells on Rotenone-Induced alpha-Synuclein Aggregation and Apoptosis”



**SUPPLEMENTARY FIGURE 1:** SH-SY5Y cells were seeded as  $5 \times 10^4$  cells/ml of DMEM containing 1% FBS and used for experiments after overnight incubation. Cells incubated with the absence or presence of ROT (0.5  $\mu$ M) for 48 h were treated with hBMSC-CM or NI-hBMSC-CM at 100 or 50 or 25% during the last 24 h and assessed for morphological changes. Each picture is representative of three independent experiments.

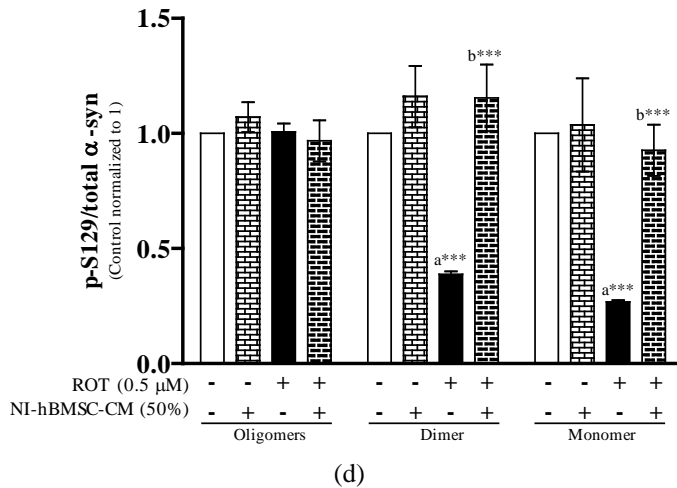
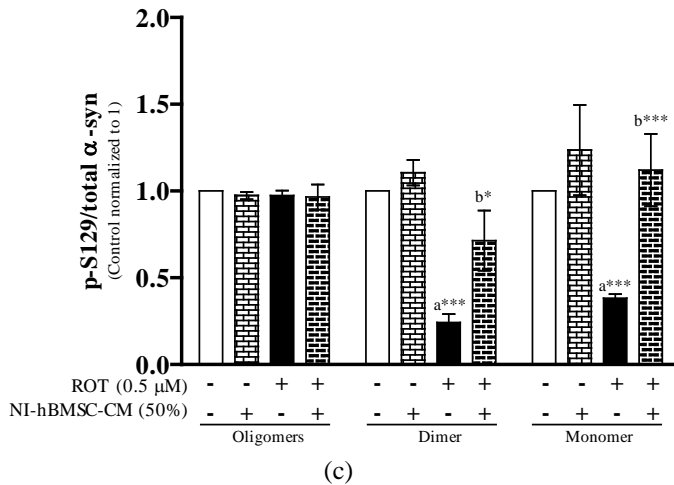
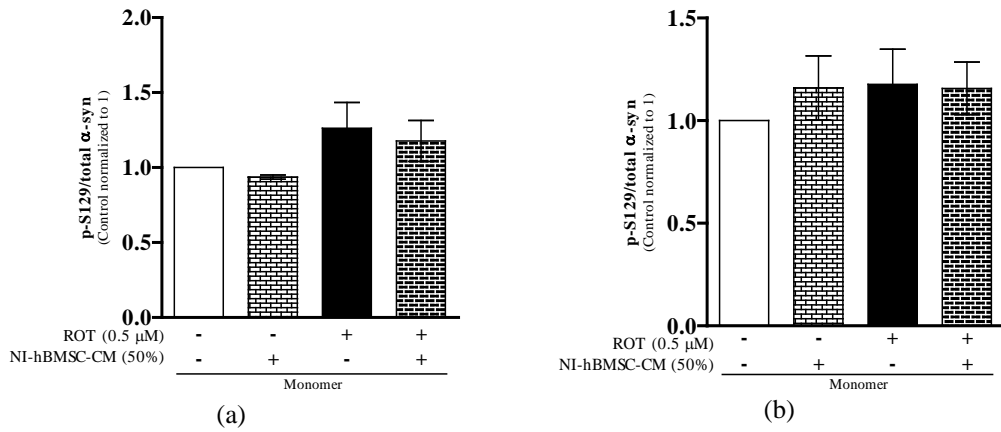


(a)

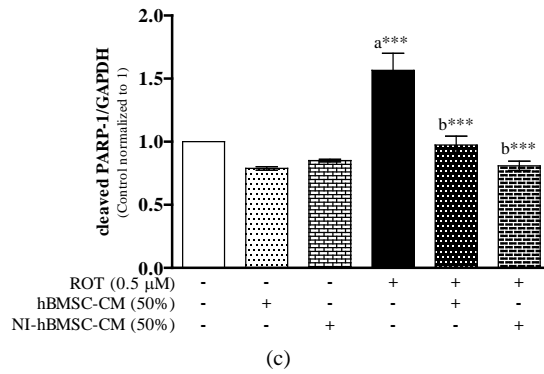
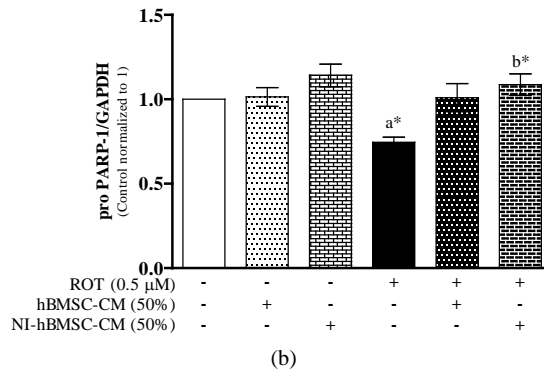
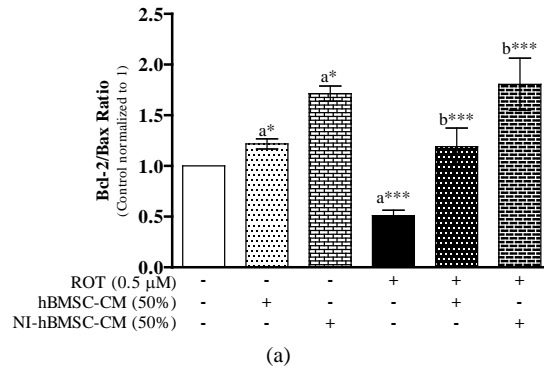


(b)

**SUPPLEMENTARY FIGURE 2:** (a) The experimental study plan. (b) SHSY5Y cells were seeded as  $5 \times 10^4$  cells/ml of DMEM containing 1% FBS and used for experiments after overnight incubation. Cells incubated with the absence or presence of ROT (0.5 μM) for 48 h were treated with hBMSC-CM (50%) or NI-hBMSC-CM (50%) during the last 24 h and assessed for morphological changes. Each picture is representative of three independent experiments.



**SUPPLEMENTARY FIGURE 3:** SH-SY5Y cells were seeded as  $5 \times 10^4$  cells/ml of DMEM containing 1% FBS and used for experiments after overnight incubation. Cells with absence or presence of ROT (0.5  $\mu$ M) for 48 h were treated with hBMSC-CM (50%) or NI-hBMSC-CM (50%) during the last 24 h and analyzed by Western blotting. Bar graphs represents fold changes in monomeric p-S129/total  $\alpha$ -syn ratios from SDS-PAGE gels of 12% (a) or 8% (b) in Triton X-100-soluble fraction. The oligomeric, dimeric and monomeric S129/total  $\alpha$ -syn ratios from SDS-PAGE gels of 12% (c) or 8% (d) in Triton X-100-insoluble fraction. Data are mean  $\pm$  SEM of three independent experiments and analyzed by one-way of variance (ANOVA) followed by Tukey's *post hoc* test. Statistical significance: <sup>a</sup>-compared with control; <sup>b</sup>-compared with ROT; \* $p < 0.05$  and \*\*\* $p < 0.001$ .



**SUPPLEMENTARY FIGURE 4:** SH-SY5Y cells were seeded as  $5 \times 10^4$  cells/ml of DMEM containing 1% FBS and used for experiments after overnight incubation. Cells with absence or presence of ROT (0.5  $\mu$ M) for 48 h were treated with hBMSC-CM (50%) or NI-hBMSC-CM (50%) during the last 24 h and analyzed by Western blotting. The bar graphs represents for Bax/Bcl-1 ratio (a), pro-PARP-1/GAPDH (b) and cleaved PARP-1/GAPDH ratio (c). Data are mean  $\pm$  SEM of three independent experiments and analyzed by one-way of variance (ANOVA) followed by Tukey's *post hoc* test. Statistical significance: <sup>a</sup>-compared with control; <sup>b</sup>-compared with ROT; \* $p < 0.05$  and \*\*\* $p < 0.001$ .

**SUPPLEMENTARY TABLE 1:** Western blotting conditions and antibodies used in this study.

Antibody Name	Host, MW Details	Company	Cat. No.	Dilution
<b>Primary Antibodies:</b>				
Tyrosine hydroxylase	Rabbit pAb, 62 kDa	Millipore	AB152	1:1,000
p-S129 $\alpha$ -synuclein	Rabbit mAb, 18 kDa	Abcam	ab51253	1:1,000
total $\alpha$ -synuclein	Rabbit mAb, 18 kDa	Abcam	ab212184	1:1,000
Neurofilament-H	Mouse mAb, 180~200 kDa	Cell Signaling	#2836	1:1,000
$\beta$ 3-tubulin	Rabbit mAb, 55 kDa	Cell Signaling	#5568	1:1,000
Neuronal Nuclei	Mouse mAb, 46~48 kDa	Millipore	MAB377	1:1,000
Synaptophysin	Mouse mAb, 38~48 kDa	Santa Cruz	sc-17750	1:2,000
Bax	Rabbit pAb, 23 kDa	Santa Cruz	sc-493	1:500
Bcl-2	Rabbit pAb, 26 kDa	Santa Cruz	sc-492	1:500
Mcl-1	Rabbit mAb, 40 kDa	Cell Signaling	#94296	1:1,000
Caspase-9	Mouse mAb, pro=47, cleaved=37,35 kDa	Cell Signaling	#9508	1:1,000
Caspase-3	Rabbit mAb, pro=35, cleaved=17,19 kDa	Cell Signaling	#9665	1:1,000
Caspase-7	Rabbit mAb, Pro=35, cleaved=20 kDa	Cell Signaling	#12827	1:1,000
PARP	Rabbit pAb, Pro=116, cleaved=89 kDa	Cell Signaling	#9542	1:1,000
GAPDH	Rabbit pAb, 37 kDa	Santa Cruz	sc-25778	1:2,000
$\beta$ -actin	Mouse mAb, 43 kDa	Santa Cruz	sc-47778	1:2,000
<b>Secondary Antibodies:</b>				
Anti-rabbit IgG, HRP-linked antibody		Cell Signaling	#7074	1:1,000 ~1:2,000
Anti-mouse IgG, HRP-linked antibody		Cell Signaling	#7076	1:1,000 ~1:2,000

**Western blotting conditions:**

SDS-PAGE Gel Percentages

- 8% = TH, p-S129  $\alpha$ -syn, total  $\alpha$ -syn, NF-H, PARP
- 12% = p-S129  $\alpha$ -syn, total  $\alpha$ -syn,  $\beta$ 3-tubulin, NeuN, SYP
- 13 or 14% = Bax, Bcl-2, Mcl-1, Cas-9, -3, -7.

SDS-PAGE Gel Running:

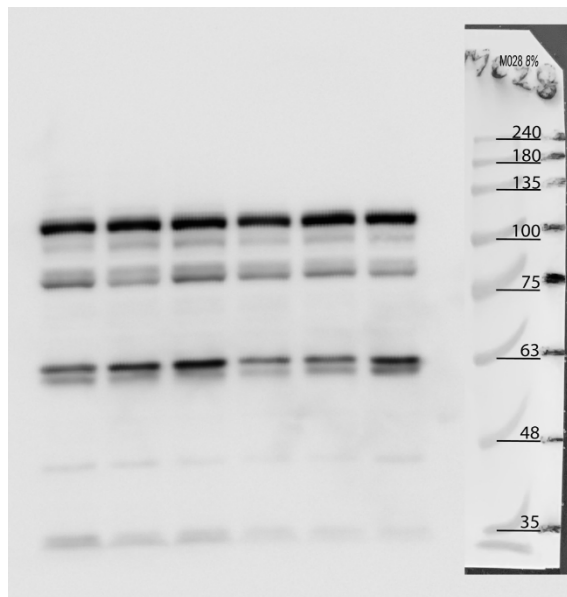
80~100 V for 100~120 min

SDS-PAGE Gel Transfer Times to Nitrocellulose Membrane:

- 8% = 250 mA for 90 min
- 12% = 200 mA for 65 min
- 13 or 14% = 200 mA for 60 min

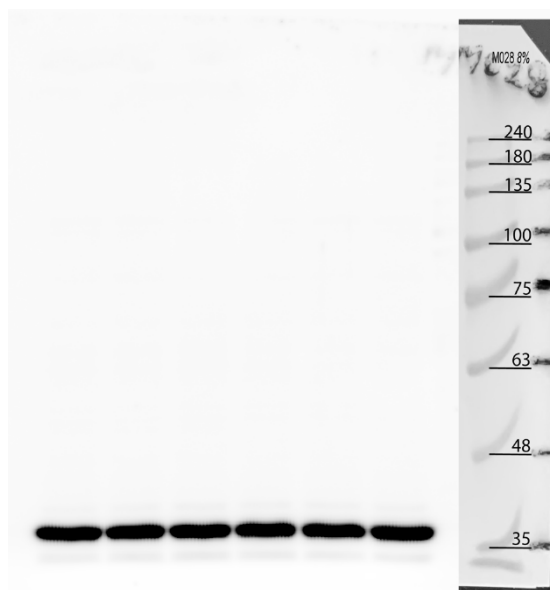
For Figure 1(b)

TH (62 kDa) →



ROT (0.5 $\mu$ M)	-	-	-	+	+	+
hBMSC-CM (50%)	-	+	-	-	+	-
NI-hBMSC-CM (50%)	-	-	+	-	-	+

GAPDH (37 kDa) →



ROT (0.5 $\mu$ M)	-	-	-	+	+	+
hBMSC-CM (50%)	-	+	-	-	+	-
NI-hBMSC-CM (50%)	-	-	+	-	-	+

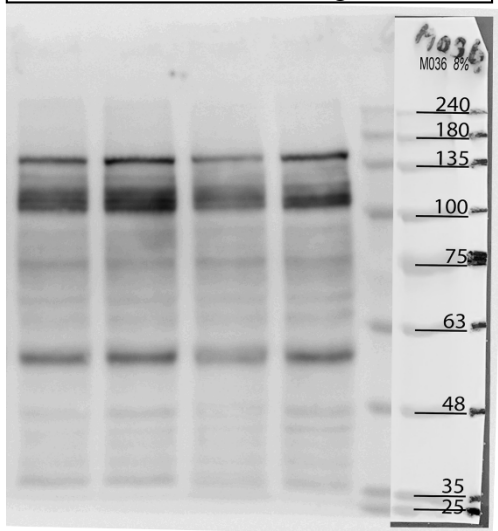
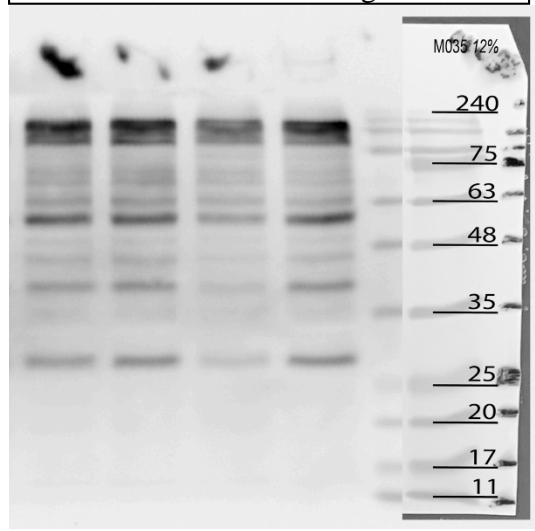
**SUPPLEMENTARY FIGURE 5:** Unedited images and their molecular weight markers for respective Western blots used in **FIGURE 1(b)** of this manuscript.

For Figure 2(a)

1% Triton X-100-soluble fraction  
12% SDS-PAGE gel

1% Triton X-100-soluble fraction  
8% SDS-PAGE gel

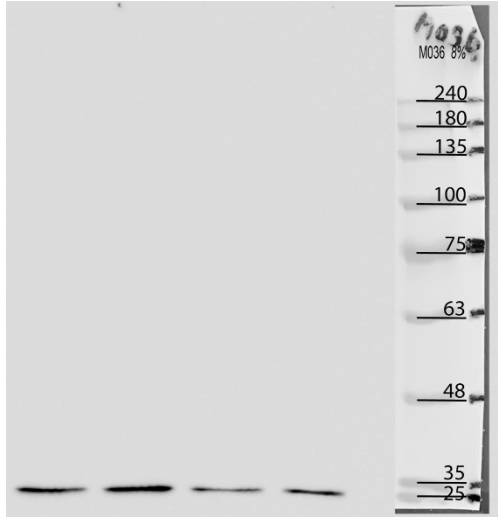
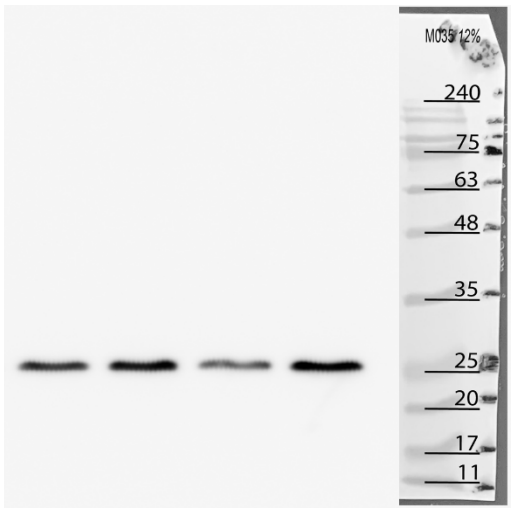
p-S129  $\alpha$ -syn



<b>ROT (0.5 <math>\mu</math>M)</b>	-	-	+	+
<b>NI-hBMSC-CM (50%)</b>	-	+	-	+

-	-	+	+
-	+	-	+

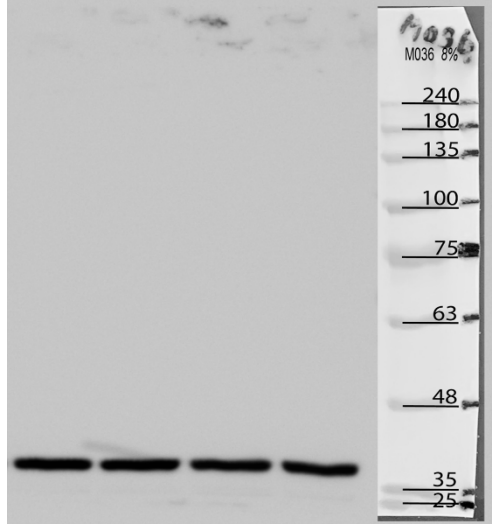
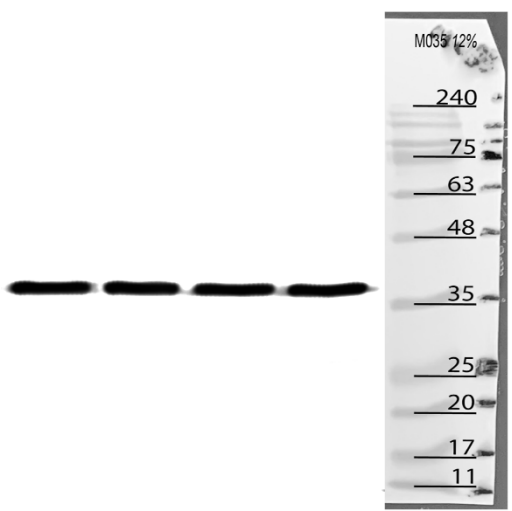
total  $\alpha$ -syn



<b>ROT (0.5 <math>\mu</math>M)</b>	-	-	+	+
<b>NI-hBMSC-CM (50%)</b>	-	+	-	+

-	-	+	+
-	+	-	+

GAPDH (37 kDa)



<b>ROT (0.5 <math>\mu</math>M)</b>	-	-	+	+
<b>NI-hBMSC-CM (50%)</b>	-	+	-	+

-	-	+	+
-	+	-	+

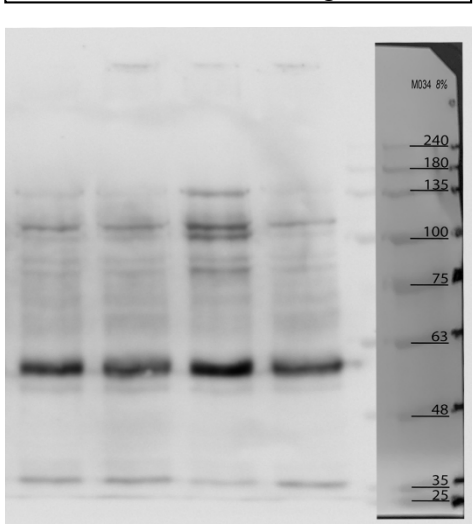
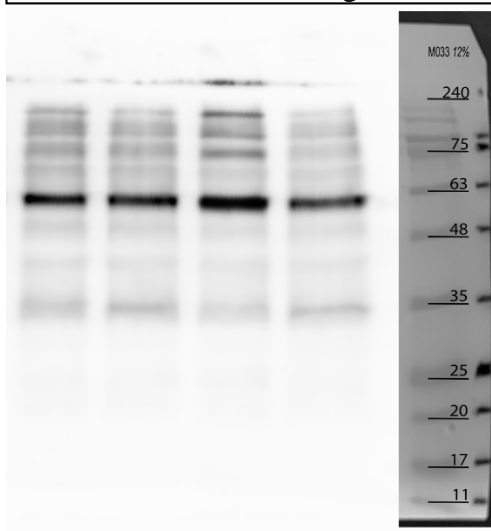
**SUPPLEMENTARY FIGURE 6:** Unedited images and their molecular weight markers for respective Western blots used in **FIGURE 2(a)** of this manuscript.

For Figure 3(a)

1% Triton X-100-insoluble fraction  
12% SDS-PAGE gel

1% Triton X-100-insoluble fraction  
8% SDS-PAGE gel

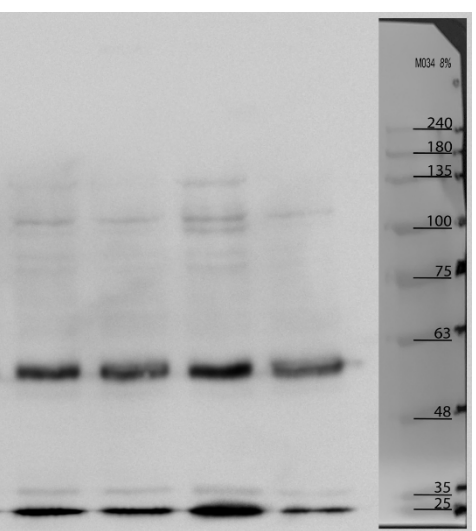
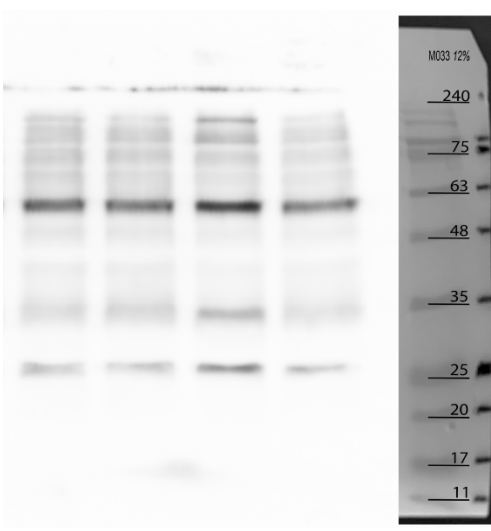
p-S129  $\alpha$ -syn



<b>ROT (0.5 <math>\mu</math>M)</b>	-	-	+	+
<b>NI-hBMSC-CM (50%)</b>	-	+	-	+

-	-	+	+
-	+	-	+

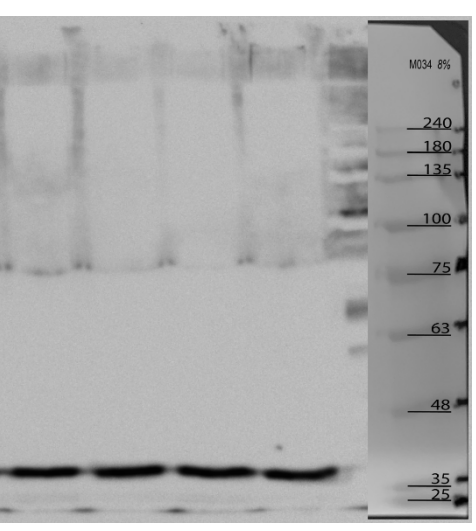
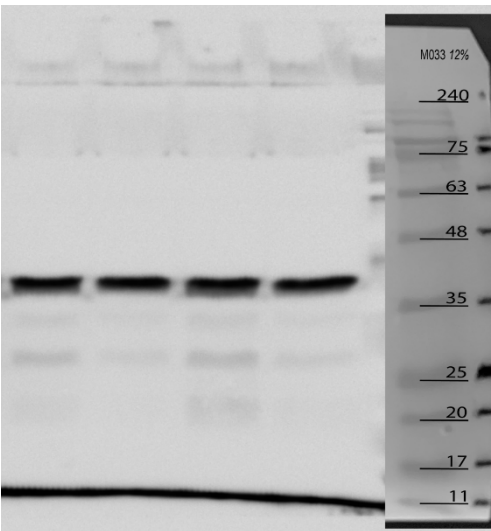
total  $\alpha$ -syn



<b>ROT (0.5 <math>\mu</math>M)</b>	-	-	+	+
<b>NI-hBMSC-CM (50%)</b>	-	+	-	+

-	-	+	+
-	+	-	+

GAPDH (37 kDa)



<b>ROT (0.5 <math>\mu</math>M)</b>	-	-	+	+
<b>NI-hBMSC-CM (50%)</b>	-	+	-	+

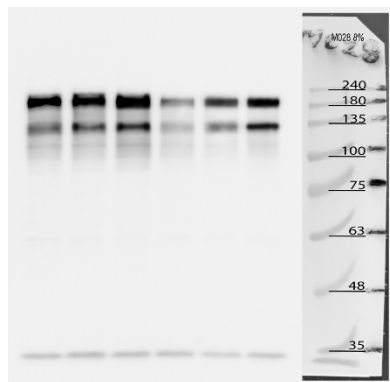
-	-	+	+
-	+	-	+

**SUPPLEMENTARY FIGURE 7:** Unedited images and their molecular weight markers for respective Western blots used in **FIGURE 3(a)** of this manuscript.

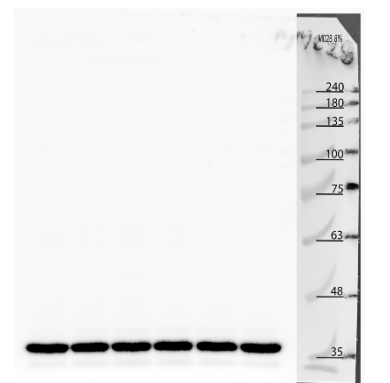


For Figure 4(a)

**NF-H**  
(180~200 kDa) →



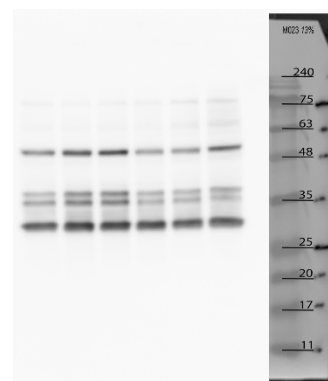
**GAPDH** (37 kDa) →



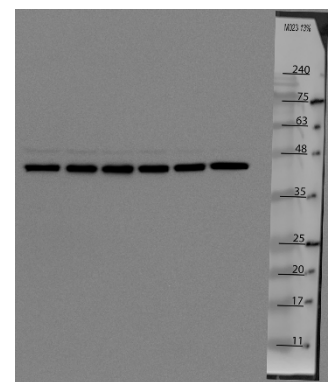
ROT (0.5 μM)	-	-	-	+	+	+
hBMSC-CM (50%)	-	+	-	-	+	-
NI-hBMSC-CM (50%)	-	-	+	-	-	+

For Figure 4(b)

**β3-Tubulin**  
(55 kDa) →



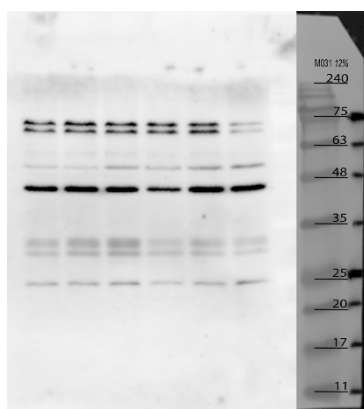
**β-actin** (43 kDa) →



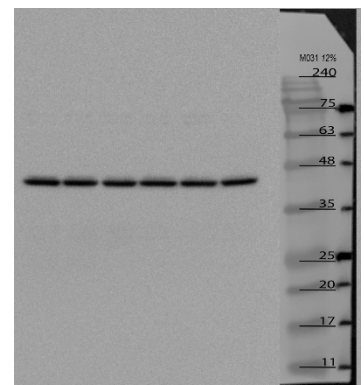
ROT (0.5 μM)	-	-	-	+	+	+
hBMSC-CM (50%)	-	+	-	-	+	-
NI-hBMSC-CM (50%)	-	-	+	-	-	+

For Figure 4(c)

**NeuN**  
(46~48 kDa) →



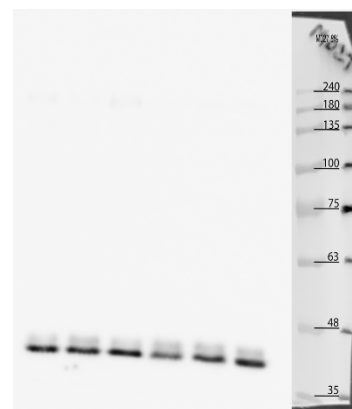
**β-actin** (43 kDa) →



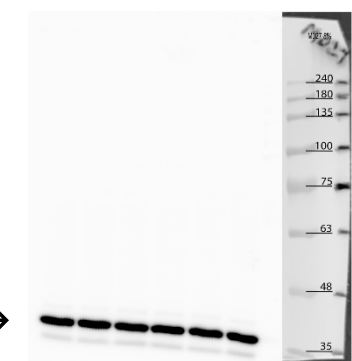
ROT (0.5 μM)	-	-	-	+	+	+
hBMSC-CM (50%)	-	+	-	-	+	-
NI-hBMSC-CM (50%)	-	-	+	-	-	+

For Figure 4(d)

**SYP**  
(38~48 kDa) →



**GAPDH** (37 kDa) →

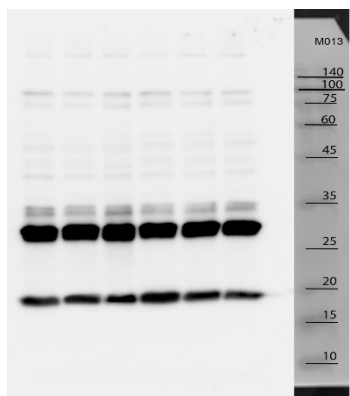


ROT (0.5 μM)	-	-	-	+	+	+
hBMSC-CM (50%)	-	+	-	-	+	-
NI-hBMSC-CM (50%)	-	-	+	-	-	+

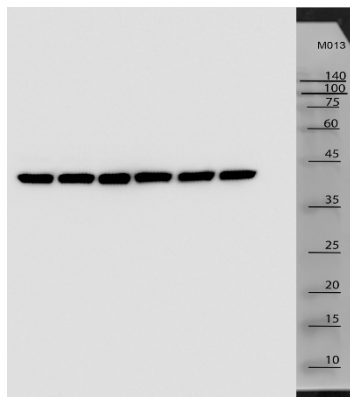
**SUPPLEMENTARY FIGURE 8:** Unedited images and their molecular weight markers for respective Western blots used in **FIGURE 4** of this manuscript.

For Figure 5(a)

Bax (20 kDa) →



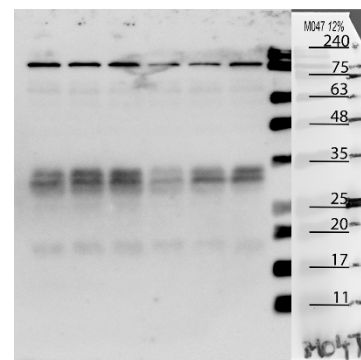
β-actin (43 kDa) →



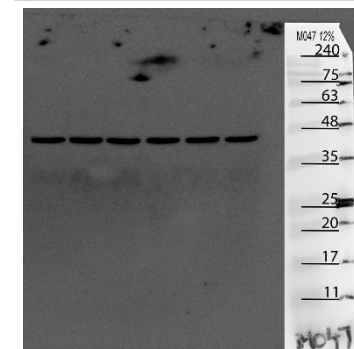
ROT (0.5 μM)	-	-	-	+	+	+
hBMSC-CM (50%)	-	+	-	-	+	-
NI-hBMSC-CM (50%)	-	-	+	-	-	+

For Figure 5(b)

Bcl-2 (26 kDa) →



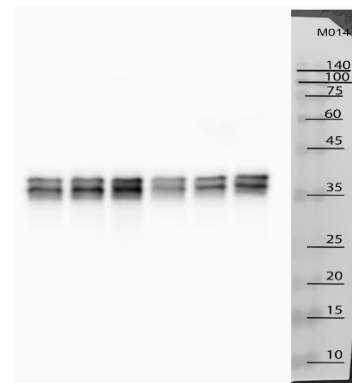
β-actin (43 kDa) →



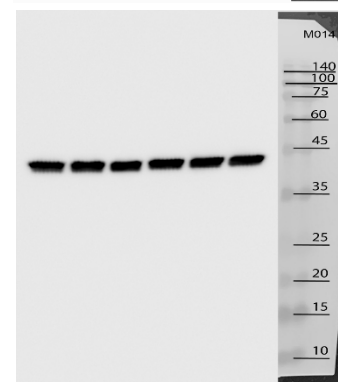
ROT (0.5 μM)	-	-	-	+	+	+
hBMSC-CM (50%)	-	+	-	-	+	-
NI-hBMSC-CM (50%)	-	-	+	-	-	+

For Figure 5(d)

Mcl-1 (40 kDa) →



β-actin (43 kDa) →

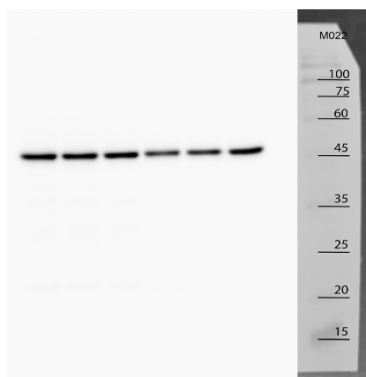


ROT (0.5 μM)	-	-	-	+	+	+
hBMSC-CM (50%)	-	+	-	-	+	-
NI-hBMSC-CM (50%)	-	-	+	-	-	+

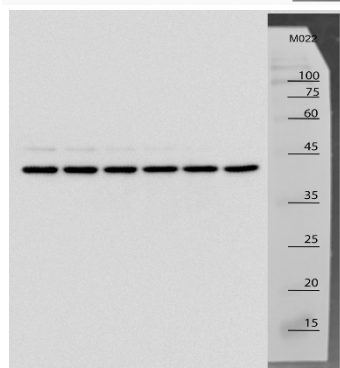
**SUPPLEMENTARY FIGURE 9:** Unedited images and their molecular weight markers for respective Western blots used in **FIGURE 5** of this manuscript.

For Figure 6(a)

pro-Cas-9  
(49 kDa) →



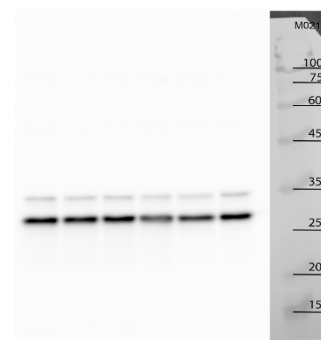
β-actin (43 kDa) →



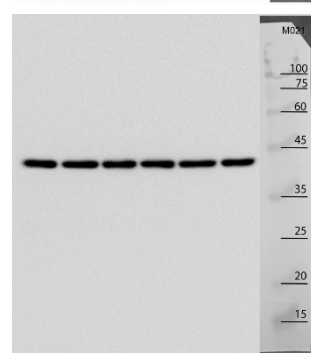
ROT (0.5 μM)	-	-	-	+	+	+
hBMSC-CM (50%)	-	+	-	-	+	-
NI-hBMSC-CM (50%)	-	-	+	-	-	+

For Figure 6(b)

pro-Cas-3  
(35 kDa) →



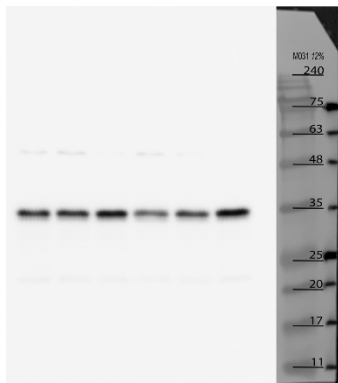
β-actin (43 kDa) →



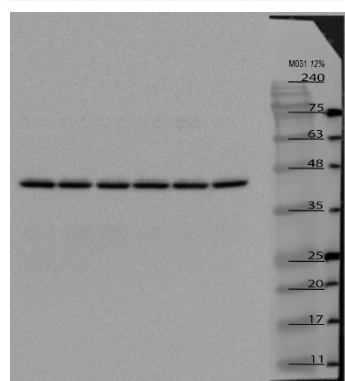
ROT (0.5 μM)	-	-	-	+	+	+
hBMSC-CM (50%)	-	+	-	-	+	-
NI-hBMSC-CM (50%)	-	-	+	-	-	+

For Figure 6(c)

pro-Cas-7  
(35 kDa) →



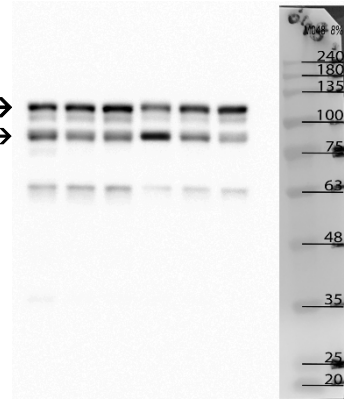
β-actin (43 kDa) →



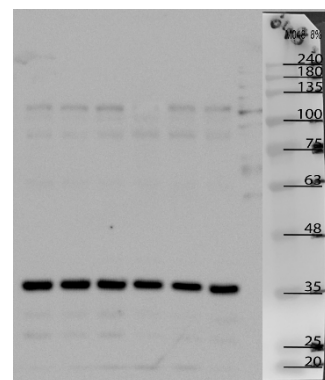
ROT (0.5 μM)	-	-	-	+	+	+
hBMSC-CM (50%)	-	+	-	-	+	-
NI-hBMSC-CM (50%)	-	-	+	-	-	+

For Figure 6(d)

pro-PARP (116 kDa) →  
cleaved-PARP (89 kDa) →



GAPDH (37 kDa) →



ROT (0.5 μM)	-	-	-	+	+	+
hBMSC-CM (50%)	-	+	-	-	+	-
NI-hBMSC-CM (50%)	-	-	+	-	-	+