Supplement Figure 1. ***uPA attenuated inflammatory osteoclastogenesis through plasmin/PAR-1.***

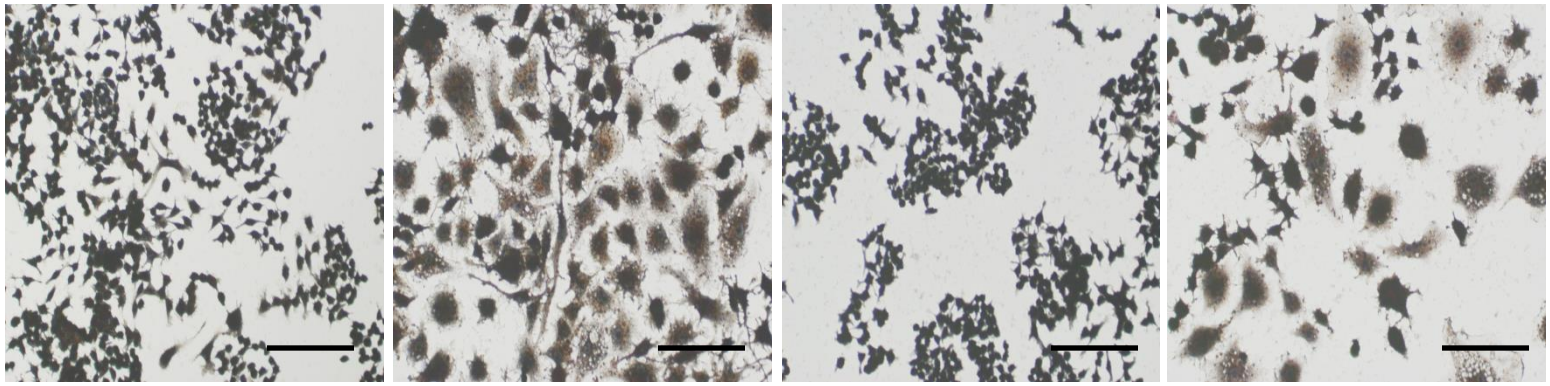
(A) RAW264.7 cells were cultured for 3 days in the absence or presence of LPS (1 μg/ml) or uPA (10 nM) as indicated. Then, TRAP-staining was performed to detect mature OCs. (B) Mature OCs were identified as multinucleated TRAP-positive cells (n=4). (C) The expression of NFATc1 in RAW264.7 cells was evaluated by Western blot analysis. The histogram on the bottom panel shows quantitative representations of NFATc1 obtained from densitometry analysis after normalization to the levels of GAPDH expression (n=3). (D) Bone marrow-derived cells from the wild-type mice were cultured for 3 days in the absence or presence of LPS (1 μg/ml), M-CSF (100 ng/ml) or uPA (10 nM). Mature OCs were identified as multinucleated TRAP-positive cells (n=3). (E) RAW264.7 cells were cultured for 3 days in the absence or presence of LPS (1 μg/ml), uPA (10 nM), α2AP (2 nM), or plasmin (1 μg/ml) as indicated. Then, TRAP-staining was performed to detect mature OCs. (F) Mature OCs were identified as multinucleated TRAP-positive cells (n=5). (G) The expression of NFATc1 in RAW264.7 cells was evaluated by Western blot analysis. The histogram on the bottom panel shows quantitative representations of NFATc1 obtained from densitometry analysis after normalization to the levels of GAPDH expression (n=3). (H) Bone marrow-derived cells from the wild-type mice were cultured for 3 days in the absence or presence of LPS (1 μg/ml), M-CSF (100 ng/ml), uPA (10 nM), α2AP (2 nM), or plasmin (1 μg/ml) as indicated. Mature OCs were identified as multinucleated TRAP-positive cells (n=3). (I) RAW264.7 cells were cultured for 3 days in the absence or presence of LPS (1 μg/ml), uPA (10 nM), or PAR-1 antagonist SCH 79797 (1 ng/ml) as indicated. Then, TRAP-staining was performed to detect mature OCs. (J) Mature OCs were identified as multinucleated TRAP-positive cells (n=4). (K) The expression of NFATc1 in RAW264.7 cells was evaluated by Western blot analysis. The histogram on the bottom panel shows quantitative representations of NFATc1 obtained from densitometry analysis after normalization to the levels of GAPDH expression (n=3). (L) Bone marrow-derived cells from the wild-type mice were cultured for 3 days in the absence or presence of LPS (1 μg/ml), M-CSF (100 ng/ml), uPA (10 nM), or PAR-1 antagonist SCH 79797 (1 ng/ml) as indicated. Mature OCs were identified as multinucleated TRAP-positive cells (n=3). (M) RAW264.7 cells were cultured for 3 days in the absence or presence of LPS (1 μg/ml) or PAR-1 agonist TFLLRN-NH_2 (1 μg/ml) as indicated. Then, TRAP-staining was performed to detect mature OCs. (N) Mature OCs were identified as multinucleated TRAP-positive cells (n=5).

(O) The expression of NFATc1 in RAW264.7 cells was evaluated by Western blot analysis. The histogram on the bottom panel shows quantitative representations of NFATc1 obtained from densitometry analysis after normalization to the levels of GAPDH expression (n=3). (P) Bone marrow-derived cells from the wild-type mice were cultured for 3 days in the absence or presence of LPS (1 μg/ml), M-CSF (100 ng/ml), or PAR-1 agonist TFLLRN-NH_2 (1 μg/ml) as indicated. Mature OCs were identified as multinucleated TRAP-positive cells (n=3). The data represent the mean ± SEM. ∗, $P<0.01$; ∗∗, $P<0.05$; NS, not significant. Scale bar = 200 μm.

Supplement Figure 2. ***The AMPK pathway activated by uPA attenuated inflammatory osteoclastogenesis.***

(A) RAW264.7 cells were cultured for 3 days in the absence or presence of LPS (1 μg/ml) or AICAR (100 μM) as indicated. Then, TRAP-staining was performed to detect mature OCs. (B) Mature OCs were identified as multinucleated TRAP-positive cells (n=4). (C) The expression of NFATc1 in RAW264.7 cells was evaluated by Western blot analysis. The histogram on the bottom panel shows quantitative representations of NFATc1 obtained from densitometry analysis after normalization to the levels of GAPDH expression (n=3). (D) Bone marrow-derived cells from the wild-type mice were cultured for 3 days in the absence or presence of LPS (1 μg/ml), M-CSF (100 ng/ml), or AICAR (100 μM) as indicated. Mature OCs were identified as multinucleated TRAP-positive cells (n=3). (E) RAW264.7 cells were stimulated with 10 nM uPA for the indicated periods. Phosphorylation of AMPK and AMPK were evaluated by a Western blot analysis. The histogram on the bottom panel shows quantitative representations of phospho-AMPK obtained from densitometry analysis after normalization to the levels of AMPK expression. (F) RAW264.7 cells were cultured for 3 days in the absence or presence of LPS (1 μg/ml), uPA (10 nM), or compound C (0.1 or 0.5 μM) as indicated. Then, TRAP-staining was performed to detect mature OCs. (G) Mature OCs were identified as multinucleated TRAP-positive cells (n=4). (H) The expression of NFATc1 in RAW264.7 cells was evaluated by Western blot analysis. The histogram on the bottom panel shows quantitative representations of NFATc1 obtained from densitometry analysis after normalization to the levels of GAPDH expression (n=3). (I) Bone marrow-derived cells from the wild-type mice were cultured for 3 days in the absence or presence of LPS (1 μg/ml), M-CSF (100 ng/ml), or compound C (0.1 μM) as indicated. Mature OCs were identified as multinucleated TRAP-positive cells (n=3). **(J)** RAW264.7 cells were pretreated with 2 nM α2AP for 30 minutes and then stimulated with 10 nM uPA for 60 minutes. Phosphorylation of AMPK and AMPK were

evaluated by a Western blot analysis. The histogram on the bottom panel shows quantitative representations of phospho-AMPK obtained from densitometry analysis after normalization to the levels of AMPK expression. (K) RAW264.7 cells were stimulated with 1 μg/ml plasmin for the indicated periods. Phosphorylation of AMPK and AMPK were evaluated by a Western blot analysis. The histogram on the bottom panel shows quantitative representations of phospho-AMPK obtained from densitometry analysis after normalization to the levels of AMPK expression. (L) RAW264.7 cells were stimulated with 1 μg/ml PAR-1 agonist TFLLRN-NH_2 for the indicated periods. Phosphorylation of AMPK and total amount of AMPK were evaluated by a Western blot analysis. The histogram on the bottom panel shows quantitative representations of phospho-AMPK obtained from densitometry analysis after normalization to the levels of AMPK expression. (M) RAW264.7 cells were pretreated with 1 μg/ml PAR-1 antagonist SCH 79797 for 60 minutes and then stimulated with 10 nM uPA for 60 minutes. Phosphorylation of AMPK and total amount of AMPK were evaluated by a Western blot analysis. The histogram on the bottom panel shows quantitative representations of phospho-AMPK obtained from densitometry analysis after normalization to the levels of AMPK expression. The data represent the mean ± SEM. ∗, $P<0.01$; ∗∗, $P<0.05$; NS, not significant. Scale bar = 200 μm.



LPS (1 μg/ml)	-	+	-	+
uPA (10 nM)	-	-	+	+

Fig. S1A

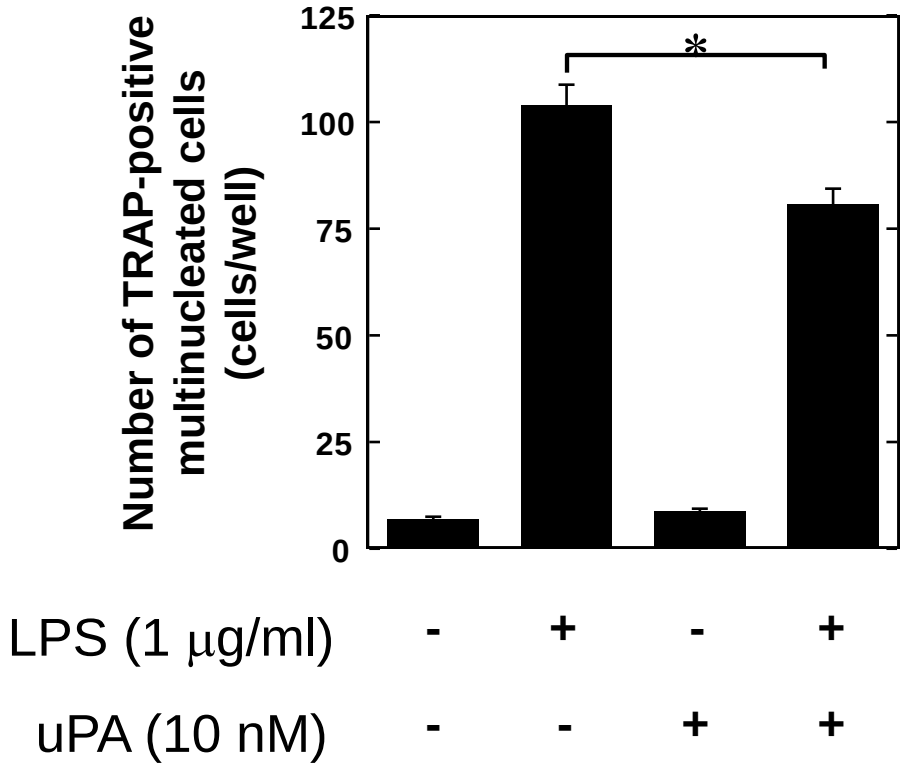

Number of TRAP-positive multinucleated cells (cells/well)
125
100
75
50
25
0
*
LPS (1 μg/ml) - + - +
uPA (10 nM) - - + +

Fig. S1B

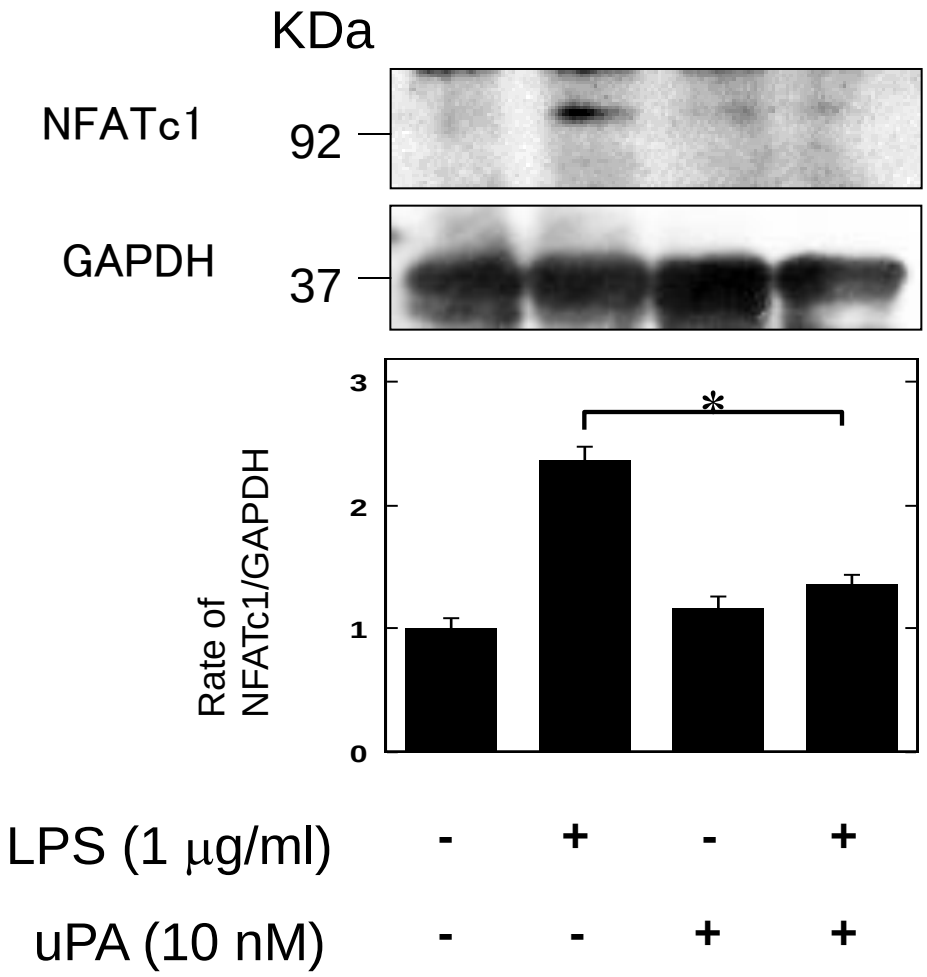

KDa
NFATc1
92
GAPDH
37
Rate of NFATc1/GAPDH
0
1
2
3
*
LPS (1 μg/ml) - + - +
uPA (10 nM) - - + +

Fig. S1C

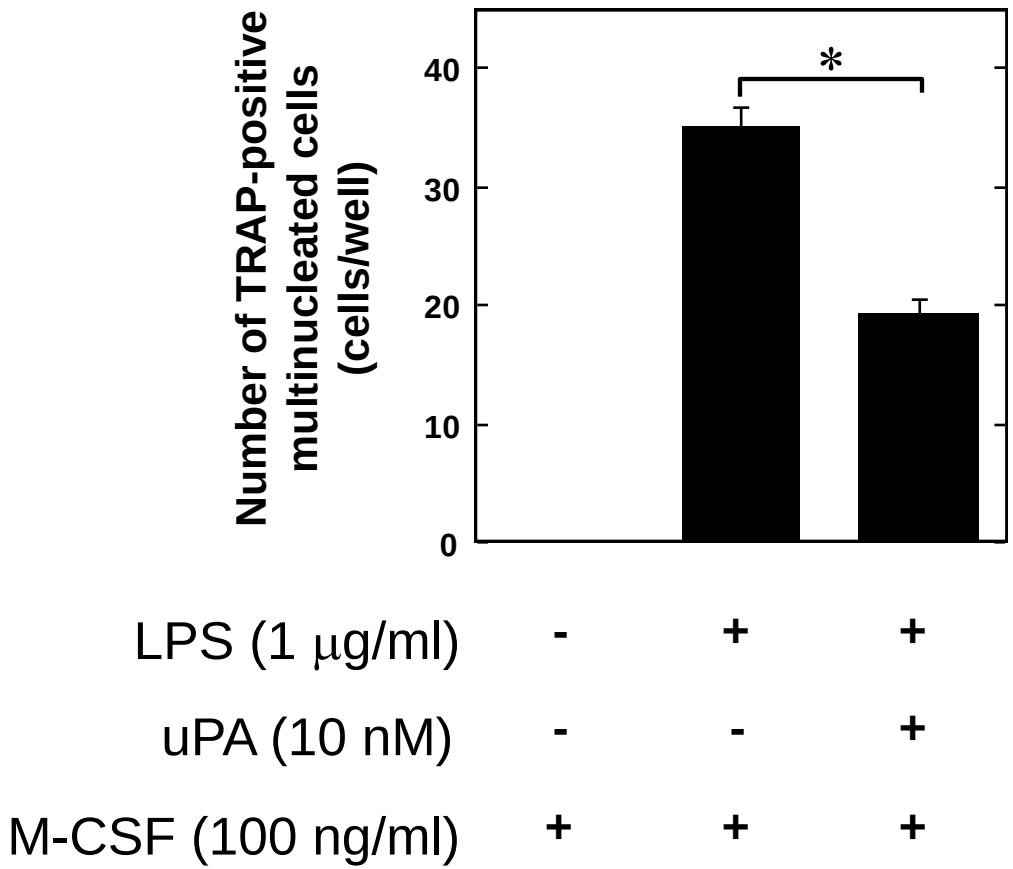
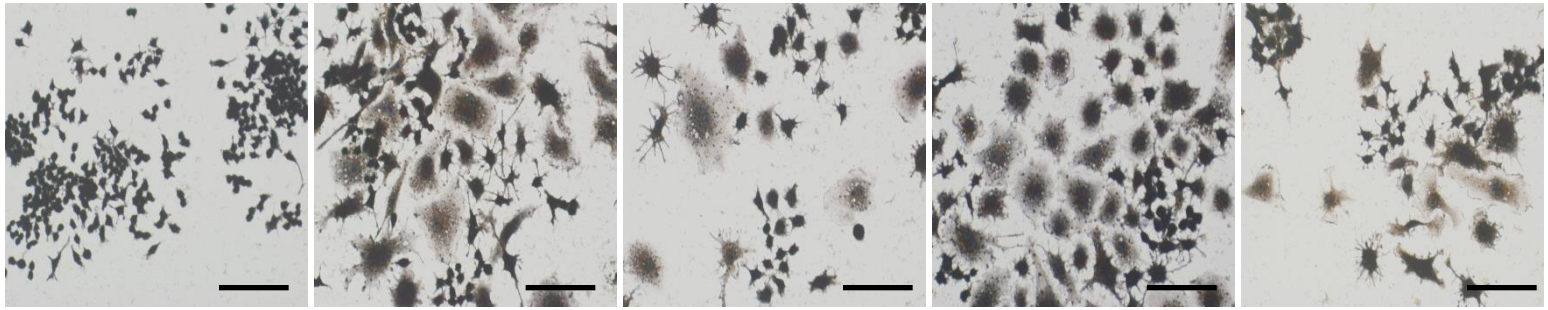

Number of TRAP-positive multinucleated cells (cells/well)
0
10
20
30
40
*
LPS (1 μg/ml) - + +
uPA (10 nM) - - +
M-CSF (100 ng/ml) + + +

Fig. S1D



LPS (1 μg/ml)	-	+	+	+	+
uPA (10 nM)	-	-	+	+	-
α2AP (2 nM)	-	-	-	+	-
Plasmin (1 μg/ml)	-	-	-	-	+

Fig. S1E

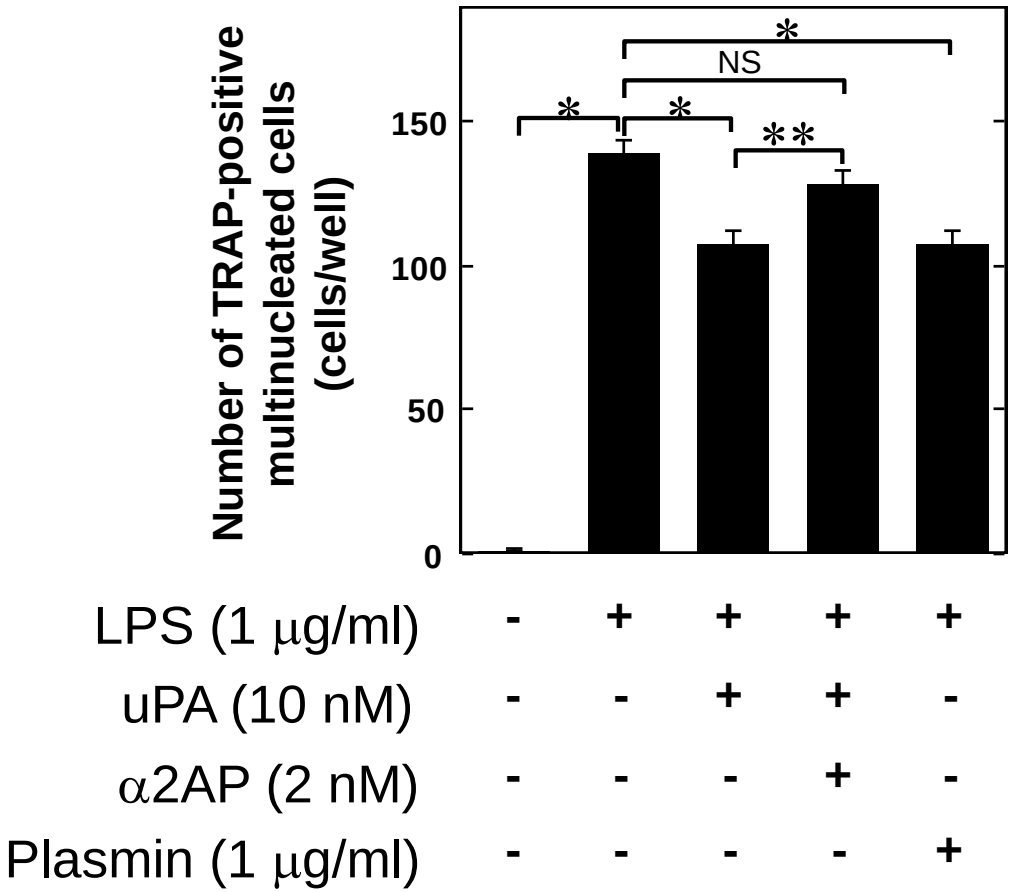

Number of TRAP-positive multinucleated cells (cells/well)
150
100
50
0
*
NS
*
*
**
LPS (1 μg/ml) - + + + +
uPA (10 nM) - - + + -
α2AP (2 nM) - - - + -
Plasmin (1 μg/ml) - - - - +

Fig. S1F

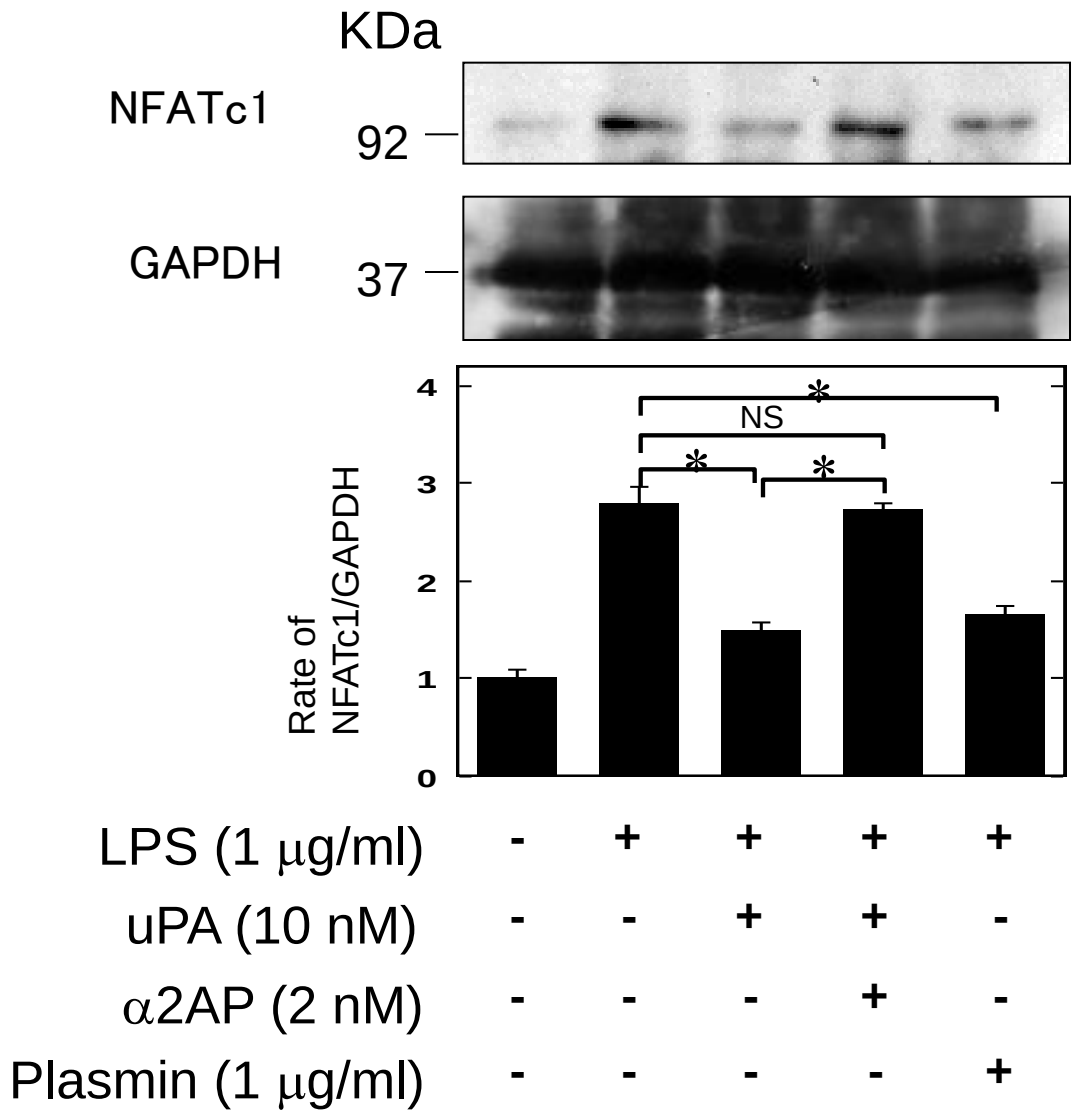

KDa
NFATc1
92
GAPDH
37
Rate of NFATc1/GAPDH
0
1
2
3
4
NS
*
*
*
LPS (1 μg/ml) - + + + +
uPA (10 nM) - - + + -
α2AP (2 nM) - - - + -
Plasmin (1 μg/ml) - - - - +

Fig. S1G

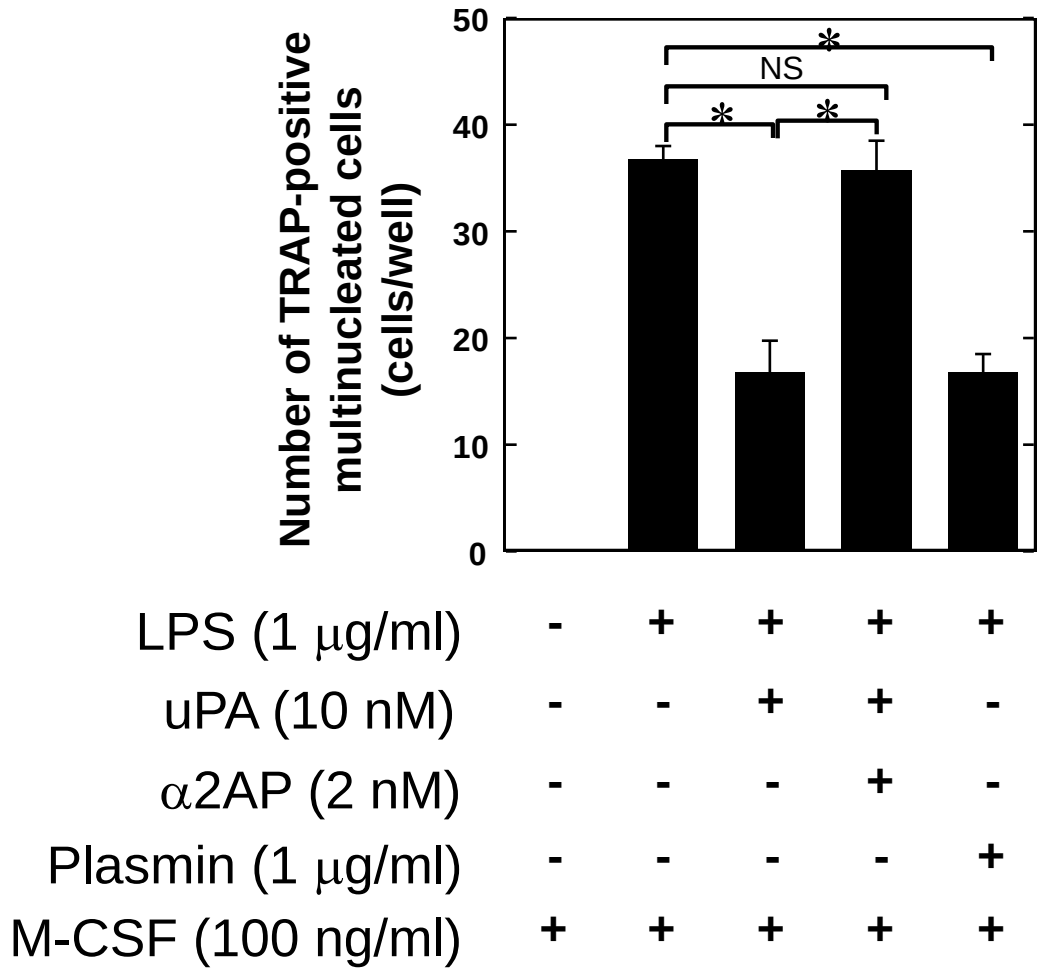
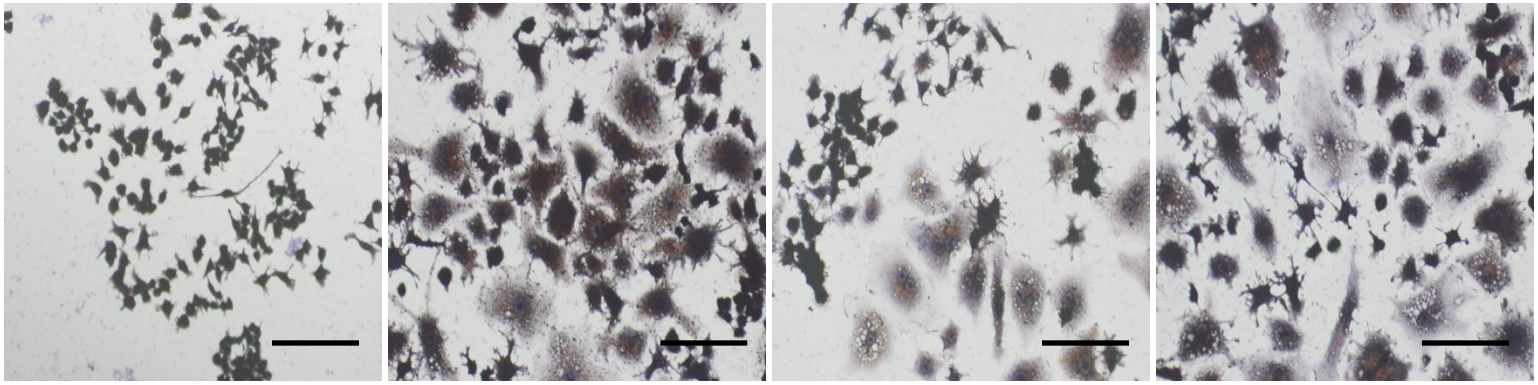

Number of TRAP-positive multinucleated cells (cells/well)
50
40
30
20
10
0
NS
*
*
*
LPS (1 μg/ml) - + + + +
uPA (10 nM) - - + + -
α2AP (2 nM) - - - + -
Plasmin (1 μg/ml) - - - - +
M-CSF (100 ng/ml) + + + + +

Fig. S1H



LPS (1 μg/ml)	-	+	+	+
uPA (10 nM)	-	-	+	+
SCH 79797 (1 ng/ml)	-	-	-	+

Fig. S1I

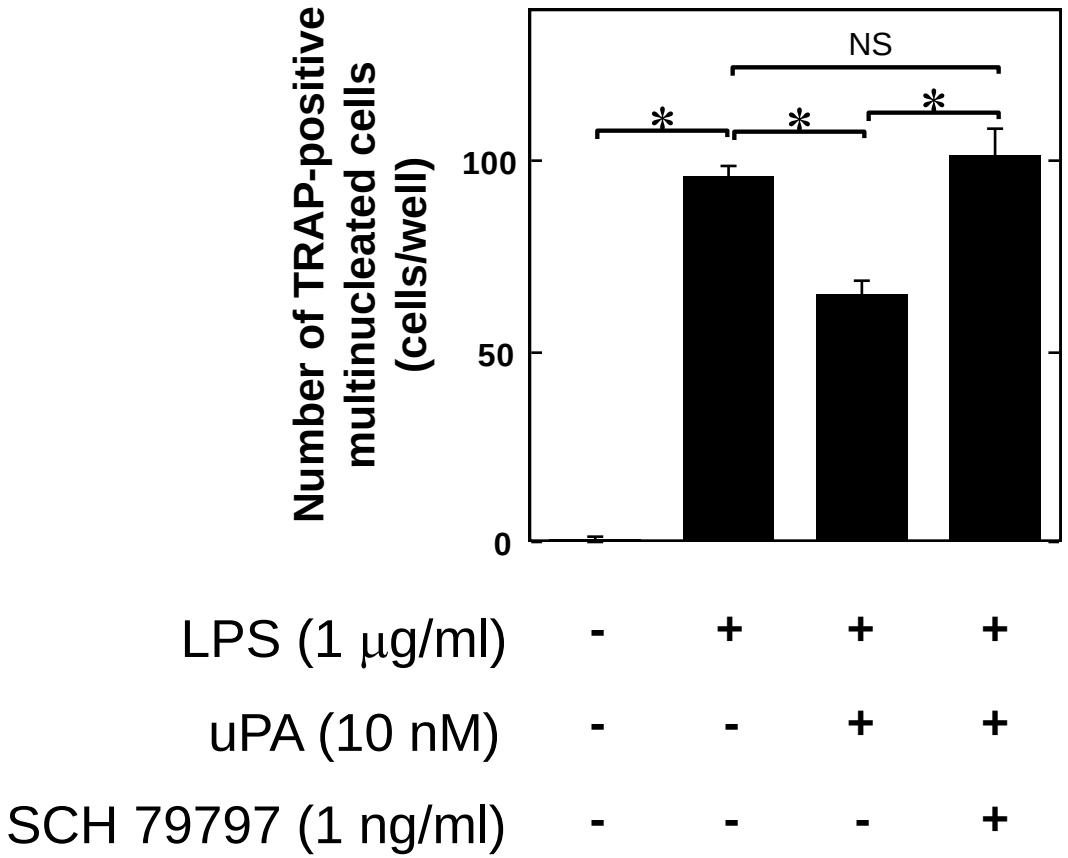

Number of TRAP-positive multinucleated cells (cells/well)
100
50
0
NS
*
*
*
LPS (1 μg/ml) - + + +
uPA (10 nM) - - + +
SCH 79797 (1 ng/ml) - - - +

Fig. S1J

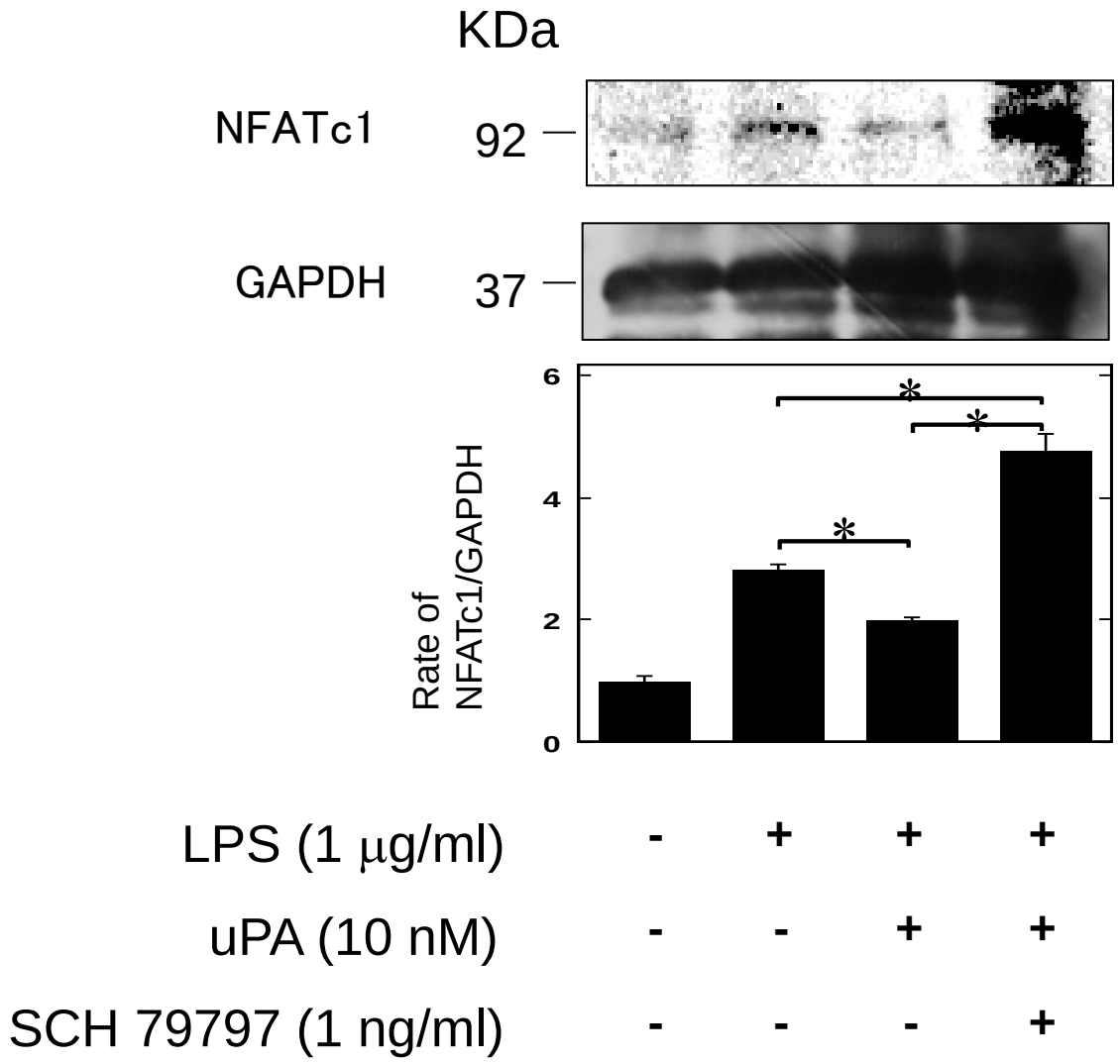

KDa
NFATc1
92
GAPDH
37
Rate of NFATc1/GAPDH
0
2
4
6
*
*
*
LPS (1 μg/ml) - + + +
uPA (10 nM) - - + +
SCH 79797 (1 ng/ml) - - - +

Fig. S1K

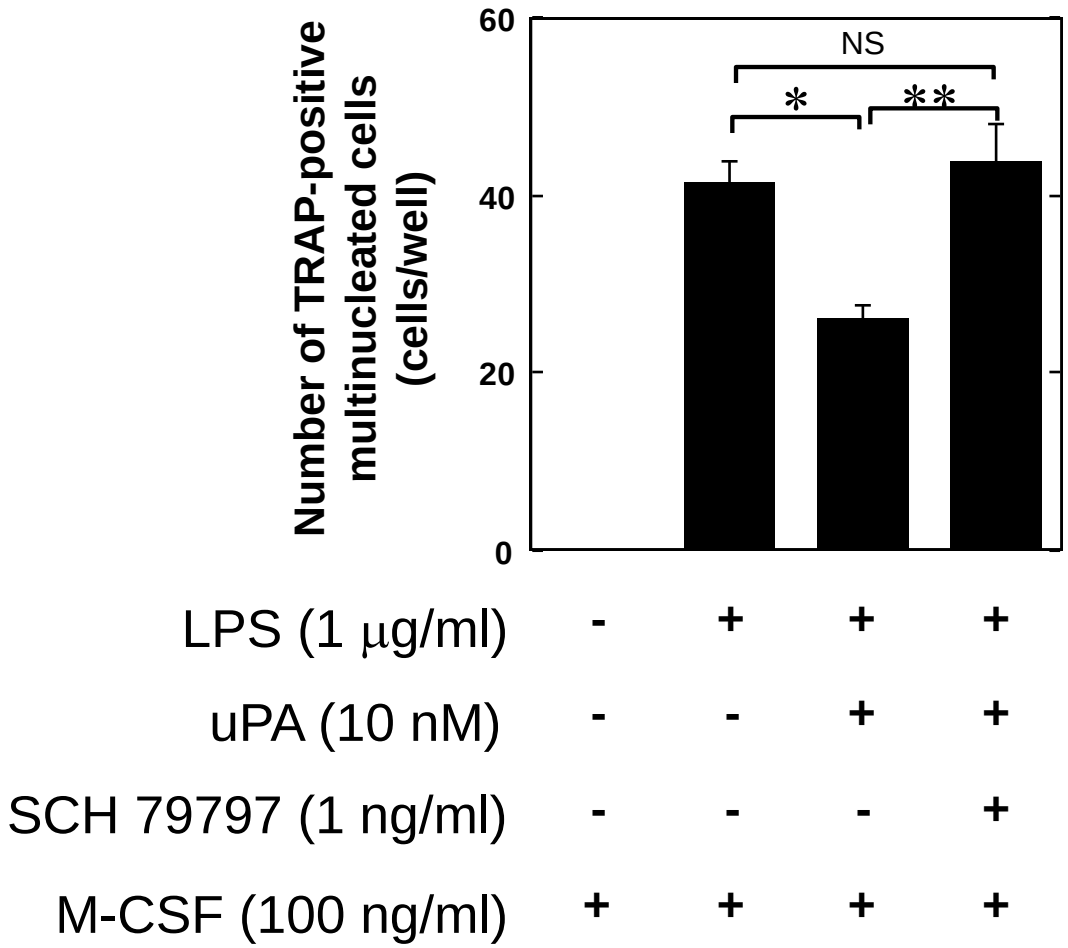
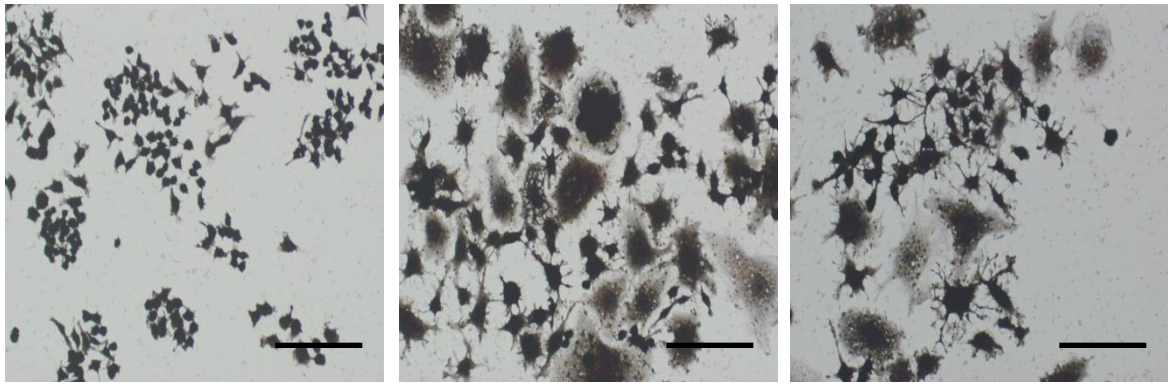

Number of TRAP-positive multinucleated cells (cells/well)
60
40
20
0
NS
*
**
LPS (1 μg/ml) - + + +
uPA (10 nM) - - + +
SCH 79797 (1 ng/ml) - - - +
M-CSF (100 ng/ml) + + + +

Fig. S1L



LPS (1 μg/ml)	-	+	+
TFLLRN-NH_2 (1 μg/ml)	-	-	+

Fig. S1M

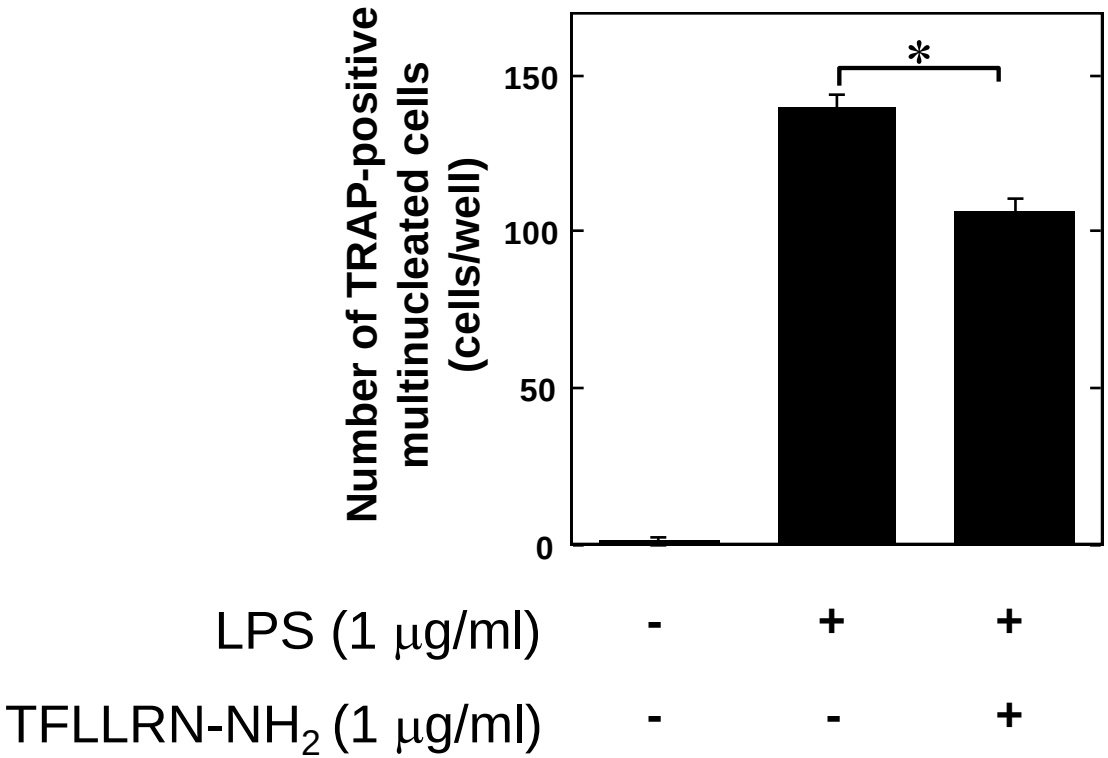

Number of TRAP-positive multinucleated cells (cells/well)
150
100
50
0
*
LPS (1 μg/ml) - + +
TFLLRN-NH2 (1 μg/ml) - - +

Fig. S1N

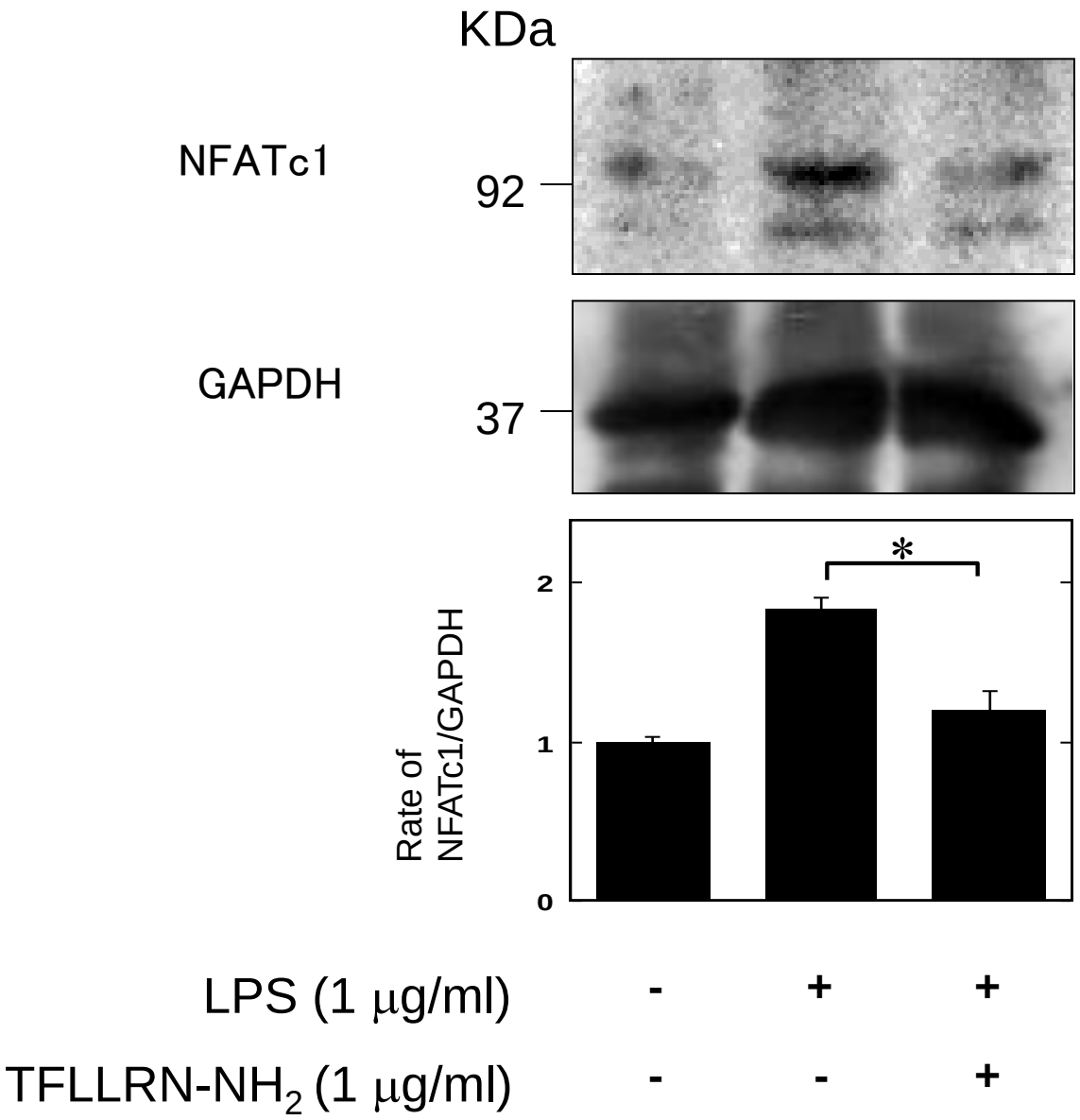

KDa
NFATc1
92
GAPDH
37
Rate of NFATc1/GAPDH
2
1
0
*
LPS (1 μg/ml) - + +
TFLLRN-NH2 (1 μg/ml) - - +

Fig. S10

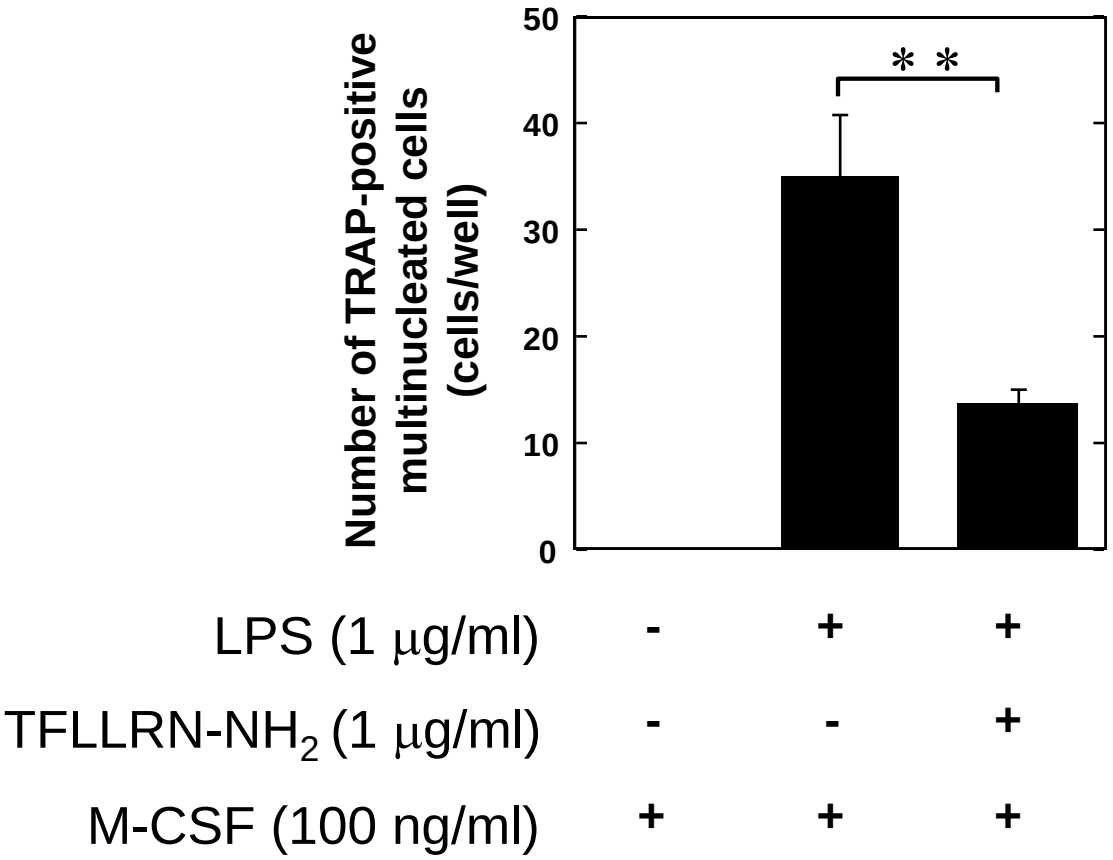
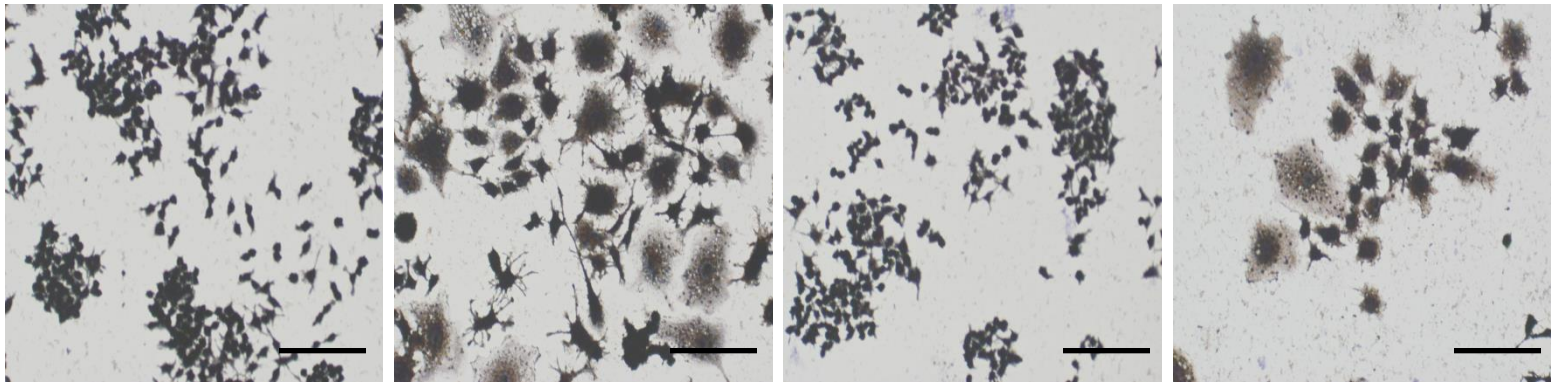

Number of TRAP-positive multinucleated cells (cells/well)
50
40
30
20
10
0
* *
LPS (1 μg/ml) - + +
TFLLRN-NH2 (1 μg/ml) - - +
M-CSF (100 ng/ml) + + +

Fig. S1P



LPS (1 μg/ml)	-	+	-	+
AICAR (100 μM)	-	-	+	+

Fig. S2A

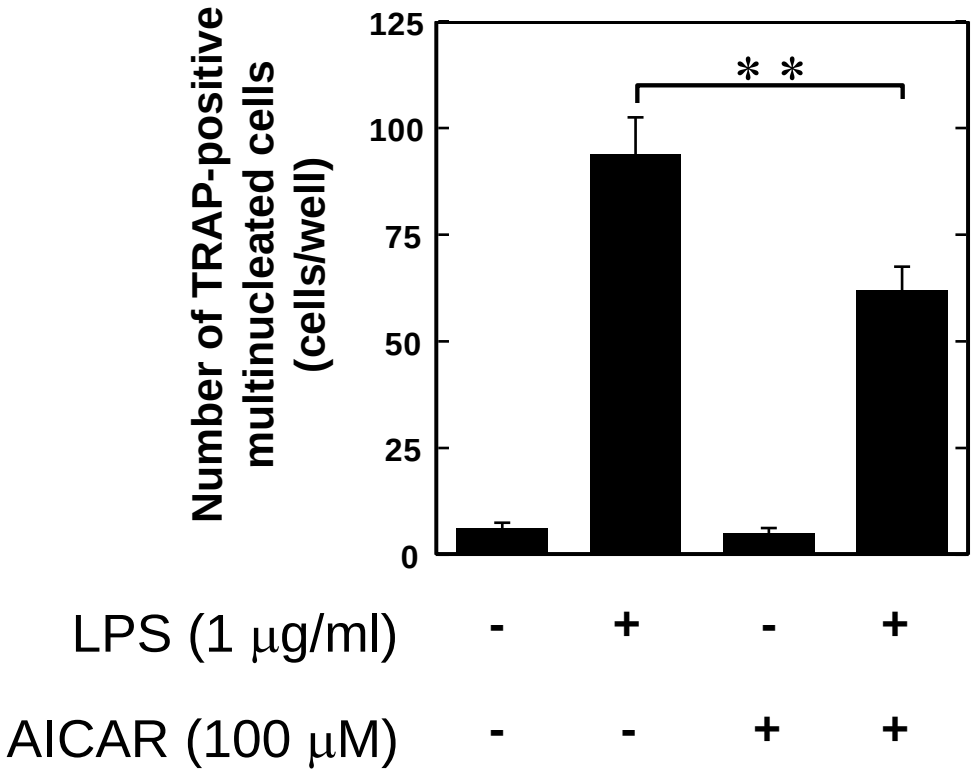

Number of TRAP-positive multinucleated cells (cells/well)
125
100
75
50
25
0
* *
LPS (1 μg/ml) - + - +
AICAR (100 μM) - - + +

Fig. S2B

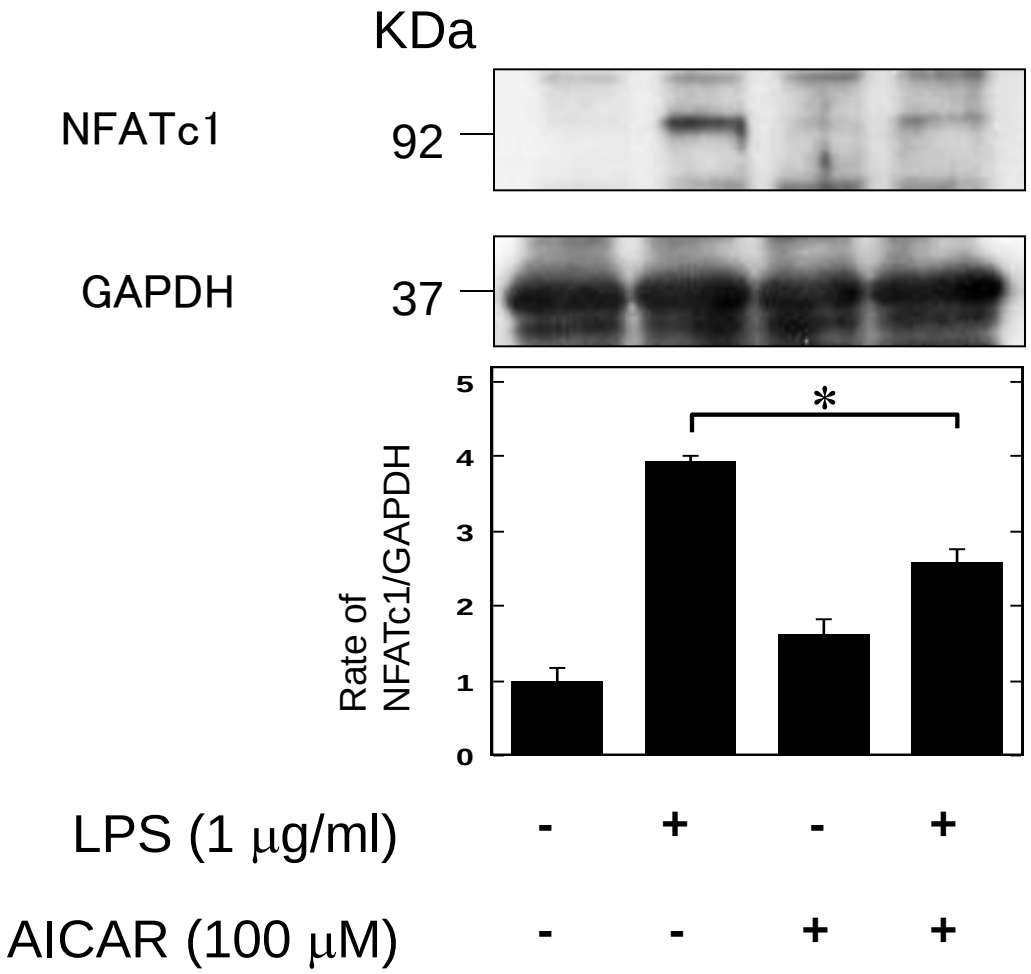
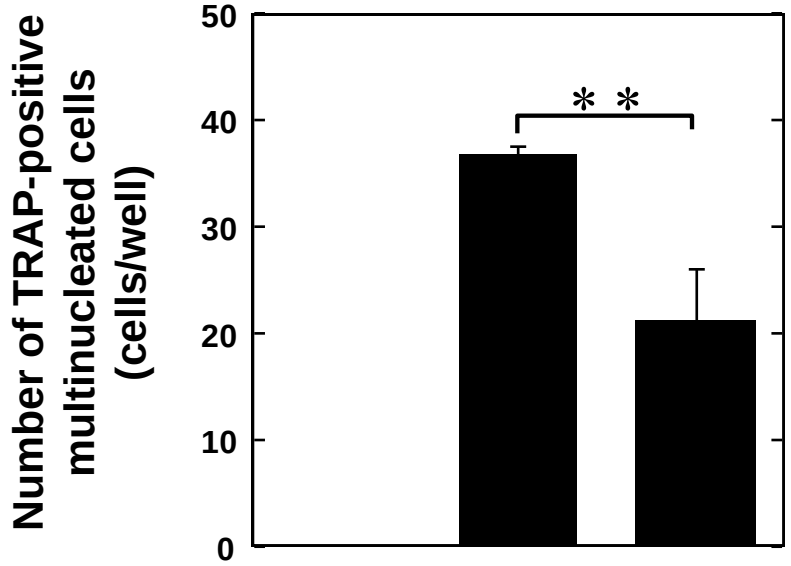

KDa
NFATc1
92
GAPDH
37
Rate of NFATc1/GAPDH
0
1
2
3
4
5
*
LPS (1 μg/ml) - + - +
AICAR (100 μM) - - + +

Fig. S2C



LPS (1 μg/ml)	-	+	+
AICAR (100 μM)	-	-	+
M-CSF (100 ng/ml)	+	+	+

Fig. S2D

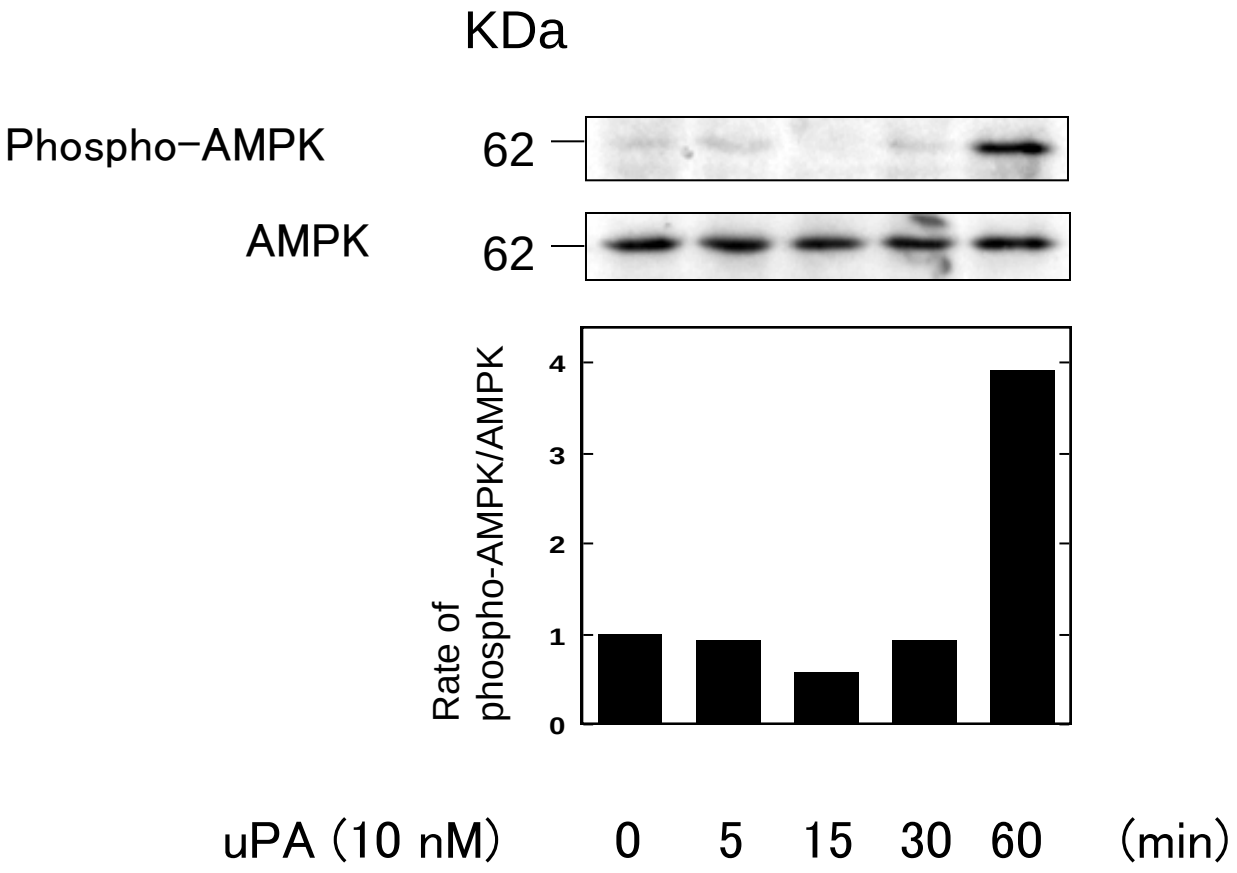
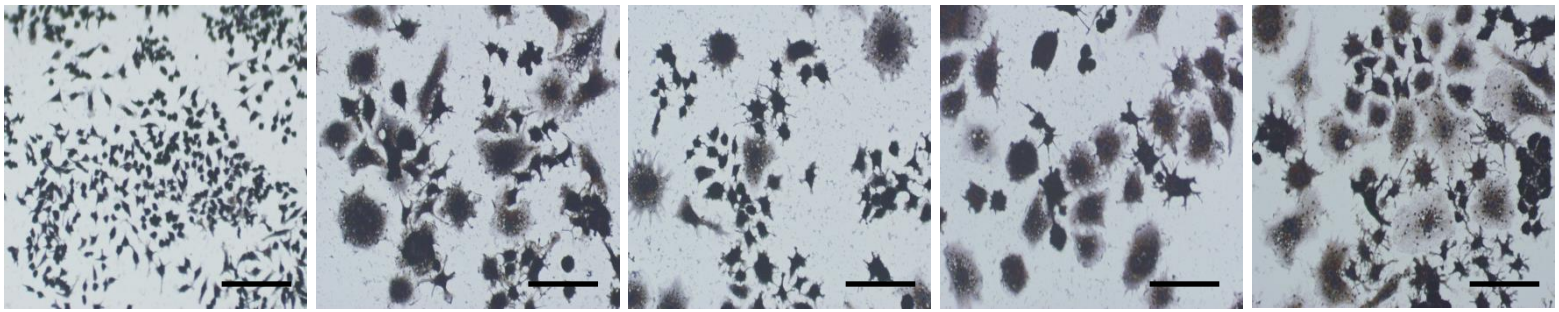
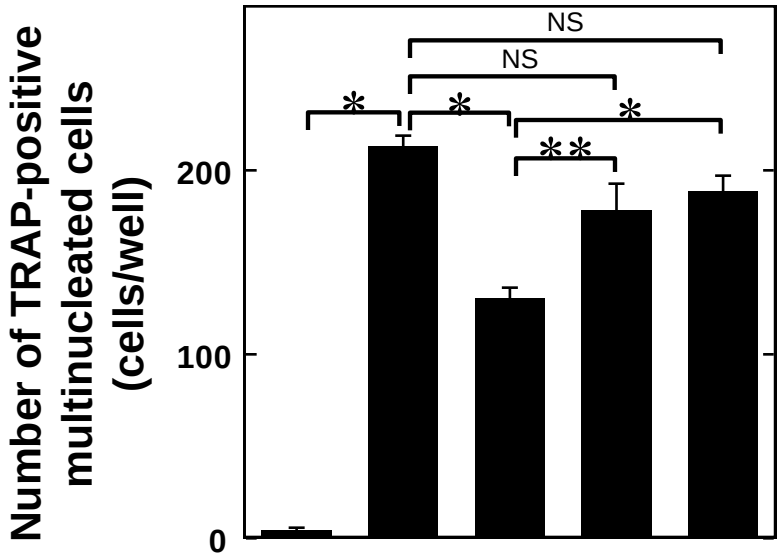

KDa
Phospho-AMPK
62
AMPK
62
Rate of phospho-AMPK/AMPK
0
1
2
3
4
uPA (10 nM)
0
5
15
30
60
(min)

Fig. S2E



LPS (1 μg/ml)	-	+	+	+	+
uPA (10 nM)	-	-	+	+	+
Compound C (μM)	-	-	-	**0.1**	**0.5**

Fig. S2F



LPS (1 μg/ml)	-	+	+	+	+
uPA (10 nM)	-	-	+	+	+
Compound C (μM)	-	-	-	**0.1**	**0.5**

Fig. S2G

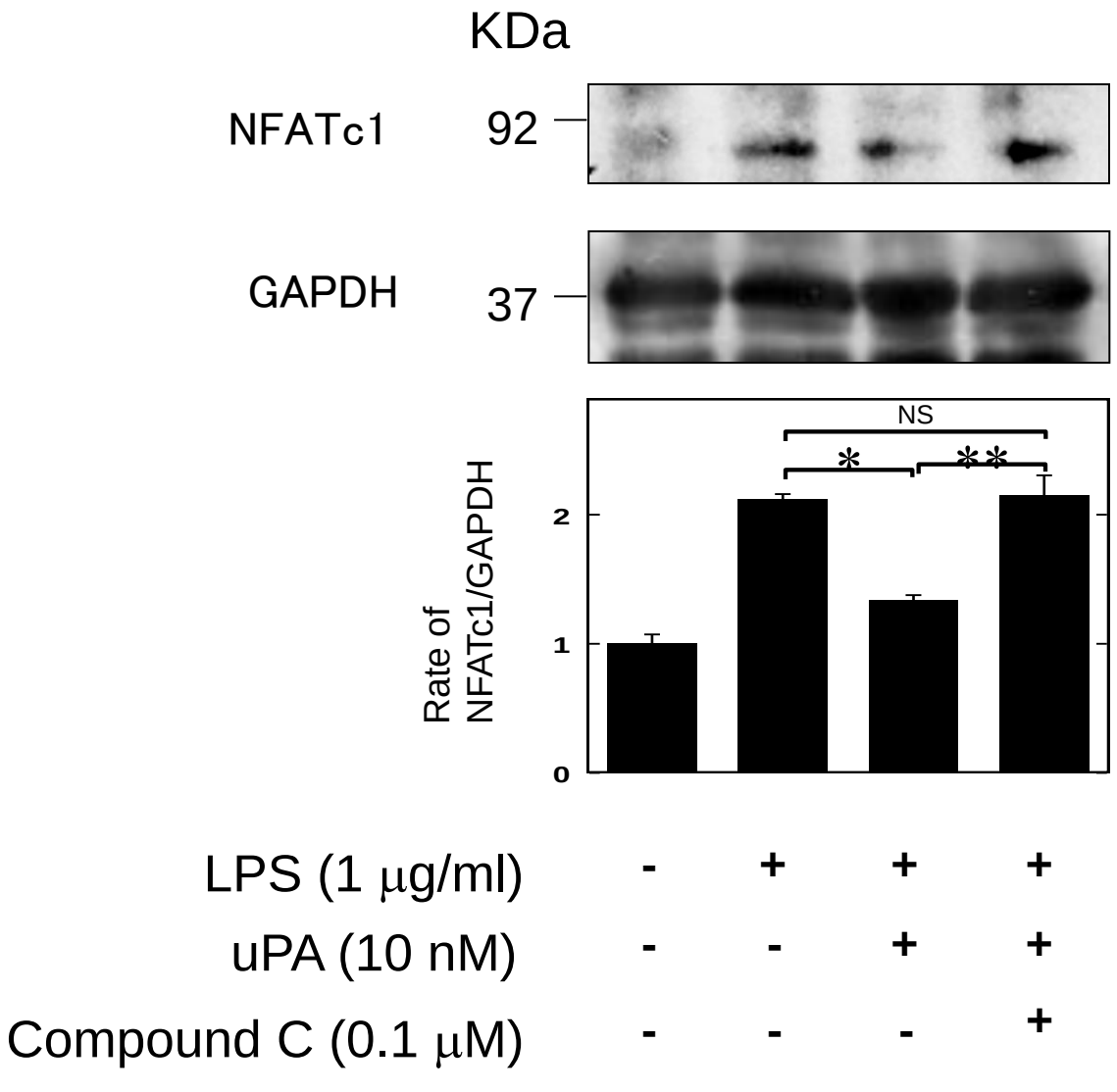
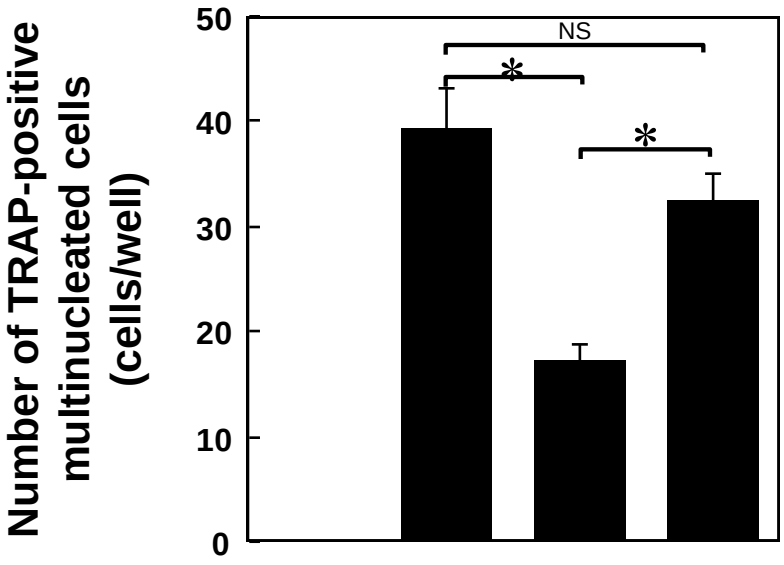

KDa
NFATc1
92
GAPDH
37
NS
*
**
Rate of NFATc1/GAPDH
2
1
0
LPS (1 μg/ml) - + + +
uPA (10 nM) - - + +
Compound C (0.1 μM) - - - +

Fig. S2H



LPS (1 μg/ml)	-	+	+	+
uPA (10 nM)	-	-	+	+
Compound C (0.1 μM)	-	-	-	+
M-CSF (100 ng/ml)	+	+	+	+

Fig. S2I

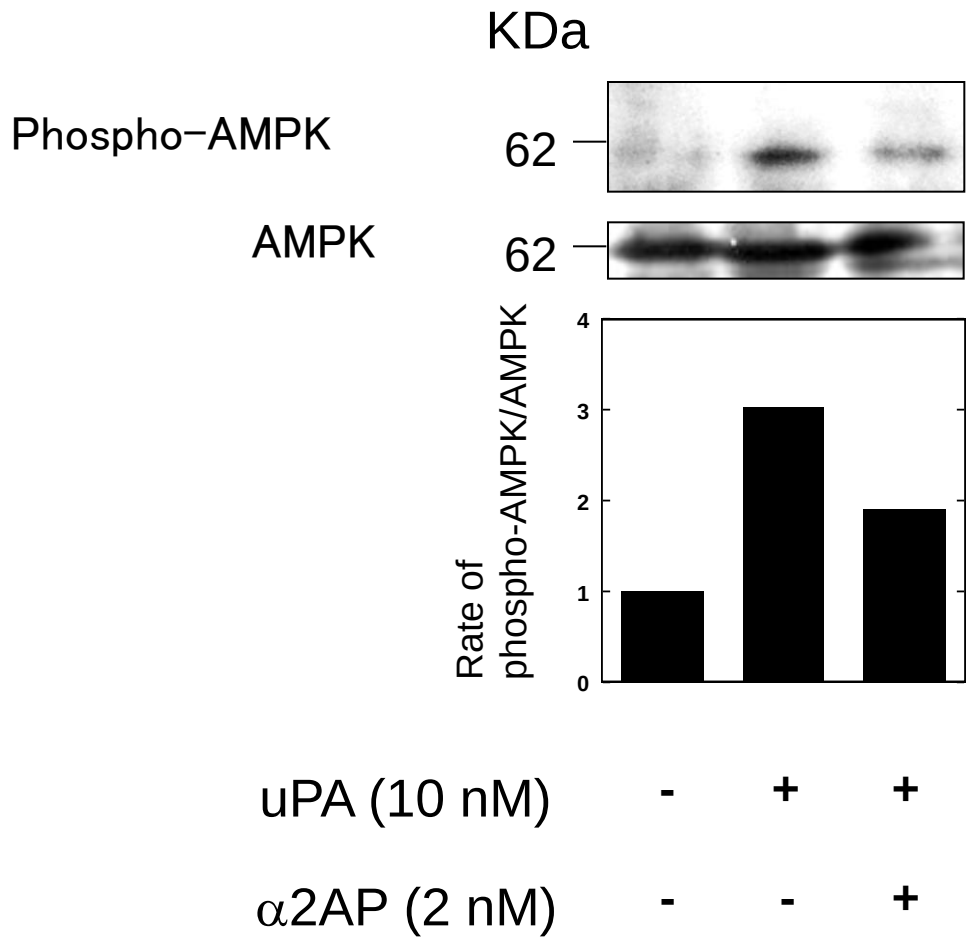

KDa
Phospho-AMPK
62
AMPK
62
Rate of phospho-AMPK/AMPK
0
1
2
3
4
uPA (10 nM) - + +
α2AP (2 nM) - - +

Fig. S2J

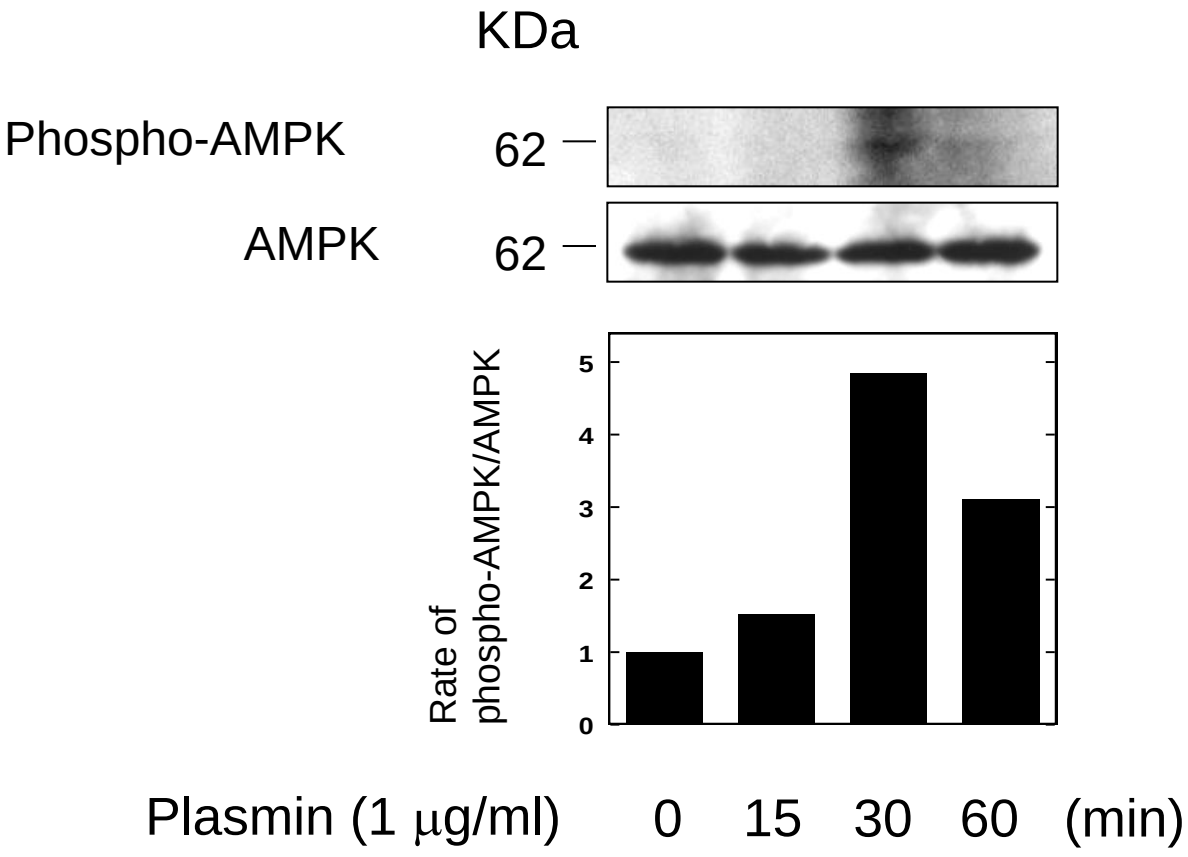

KDa
Phospho-AMPK
62
AMPK
62
Rate of phospho-AMPK/AMPK
0
1
2
3
4
5
Plasmin (1 μg/ml)
0
15
30
60
(min)

Fig. S2K

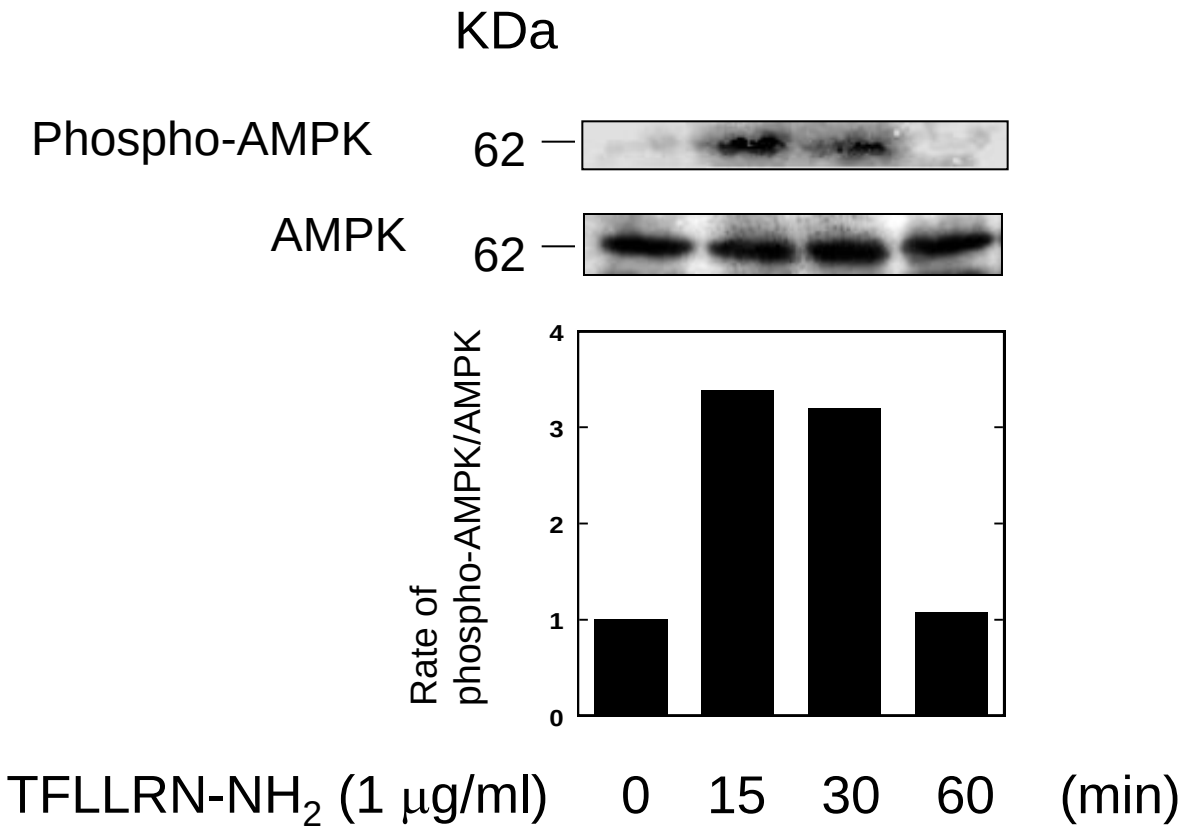

KDa
Phospho-AMPK
62
AMPK
62
Rate of phospho-AMPK/AMPK
0
1
2
3
4
TFLLRN-NH2 (1 μg/ml)
0
15
30
60
(min)

Fig. S2L

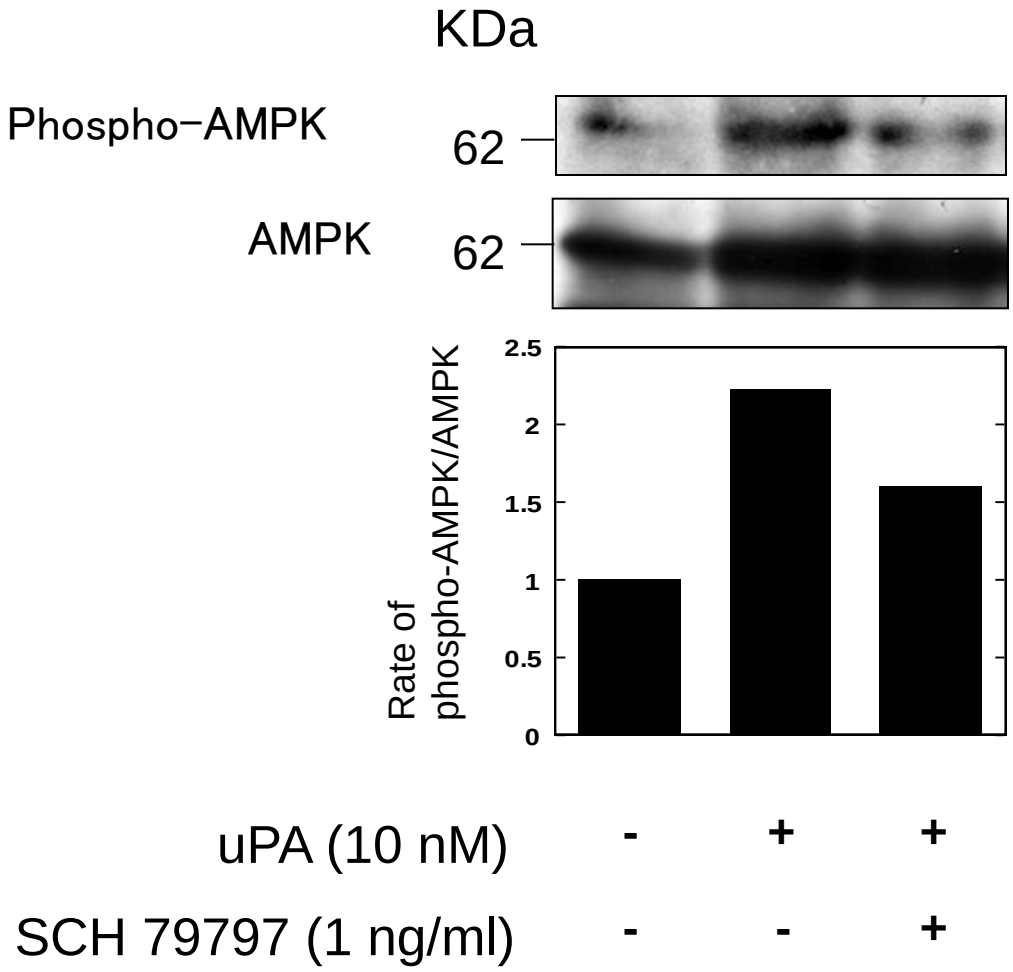

KDa
Phospho-AMPK
62
AMPK
62
Rate of phospho-AMPK/AMPK
2.5
2
1.5
1
0.5
0
uPA (10 nM) - + +
SCH 79797 (1 ng/ml) - - +

Fig. S2M