Figure 1 AST significantly alleviates LPS-induced ALI *in vivo*.

Fig.1(A): Molecular formula of AST

Fig.1(B)： A flowchart for the treatments of mice.

Fig.1(C): The raw data of Fig.1(C) is detailed in excel (lung injury score).

Fig.1(D): The raw data of Fig.1(D) is detailed in excel (Wet Dry Weight Ratio).

Fig.1(E): Hematoxylin and eosin (HE) and Masson staining images of the lung tissues.
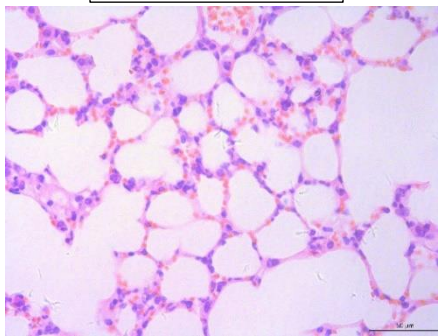
Fer-1+LPS

AST(1μM)+LPS

AST(10μM)+LPS

Masson staining images of the lung tissues.

Control

LPS

Fer-1+LPS

AST(1μM)+LPS
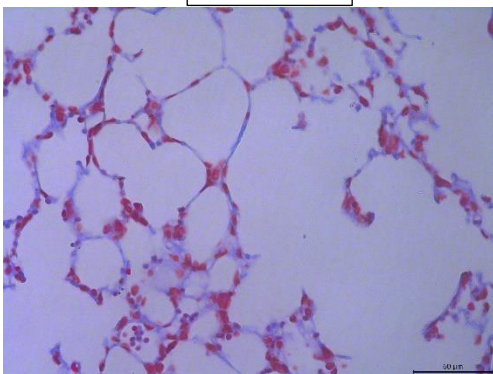
AST(10μM)+LPS
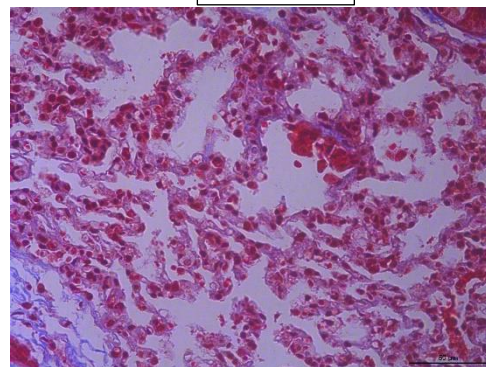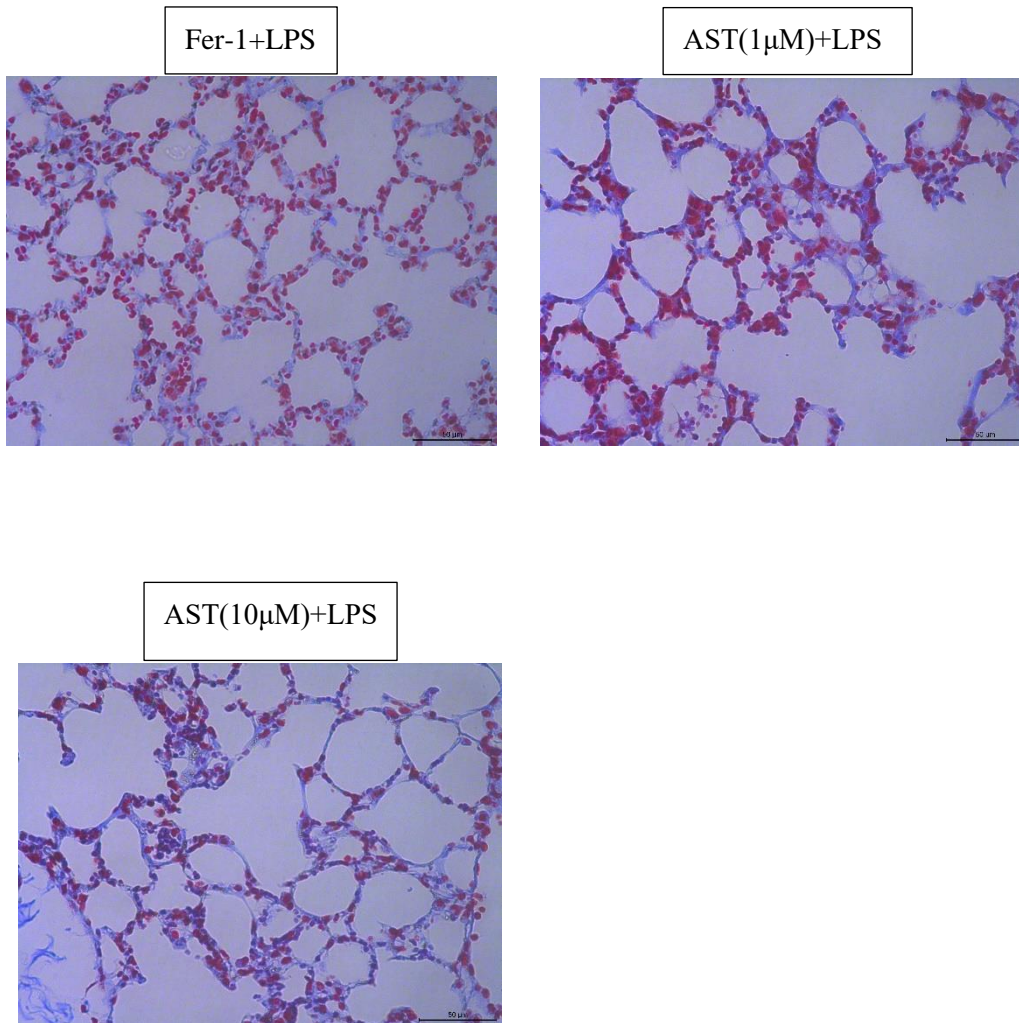
Fig.1(F): The raw data of Fig.1(F) is detailed in excel (Elisa， IL-1β).

Fig.1(G): The raw data of Fig.1(G) is detailed in excel (Elisa， IL-6).

Fig.1(H): The raw data of Fig.1(H) is detailed in excel (Elisa， TNF-α).

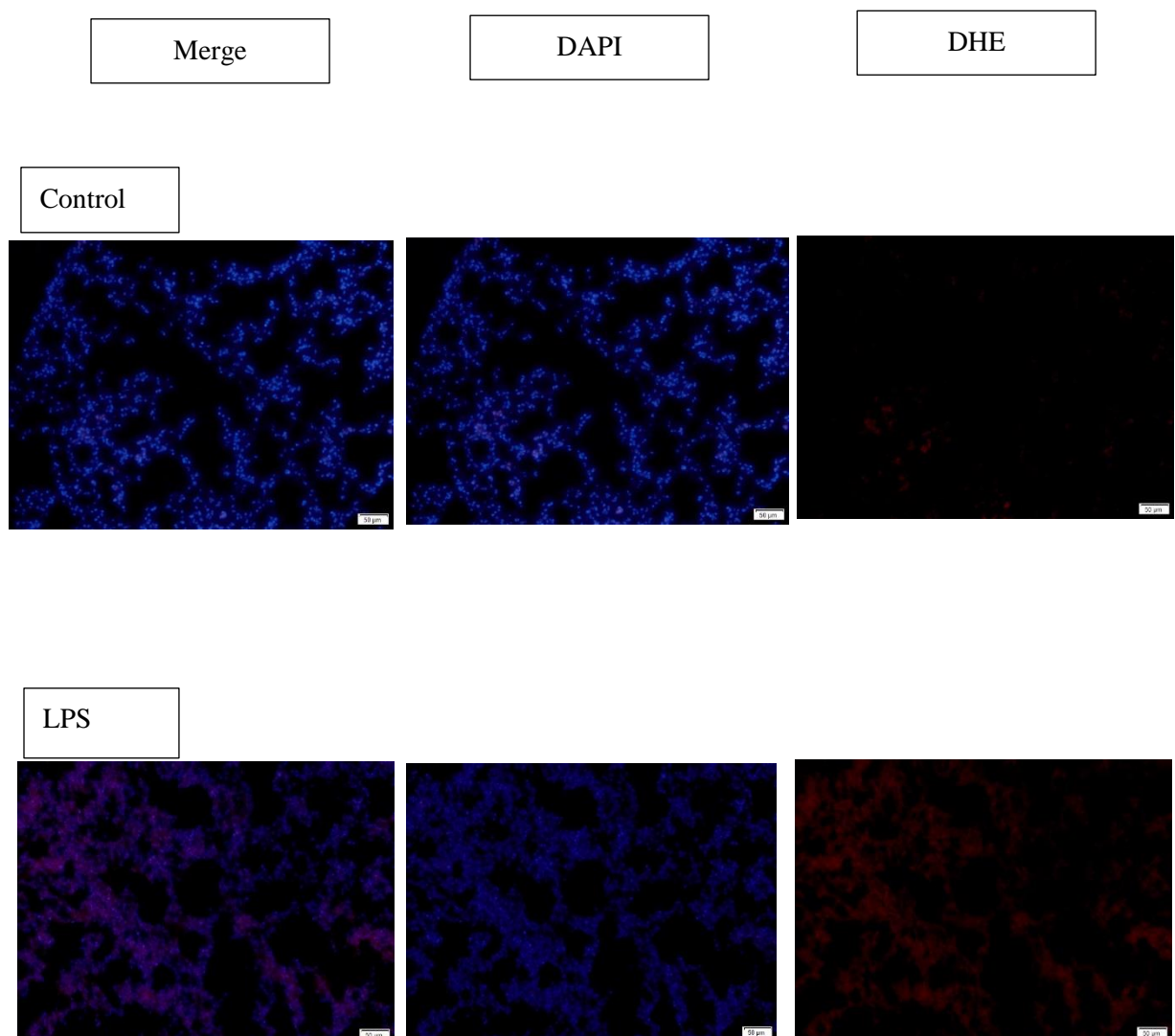**Figure 2 AST reduced oxidative stress during LPS-induced ALI.**

Fig.2(A): The raw data of Fig.2(A) is detailed in excel (Serum GSH).

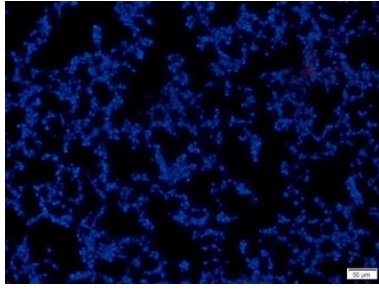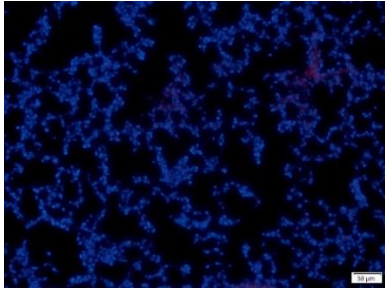**Fig.2(B):** The raw data of Fig.2(B) is detailed in excel (Tissue GSH).

Fig.2(C): The raw data of Fig.2(C) is detailed in excel (Serum MDA).

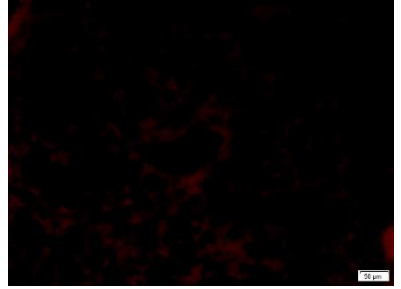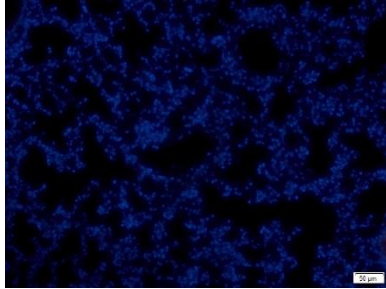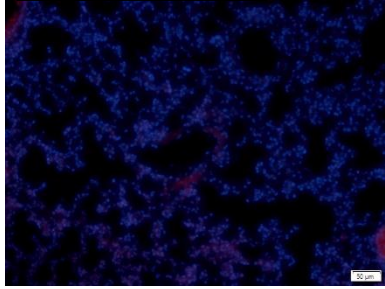**Fig.2(D):** The raw data of Fig.2(D) is detailed in excel (Tissue MDA).

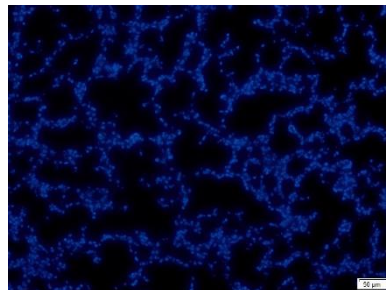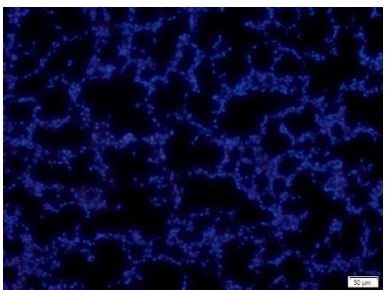**Fig.2(E):** DHE fluorescence images of the mice lung tissues.
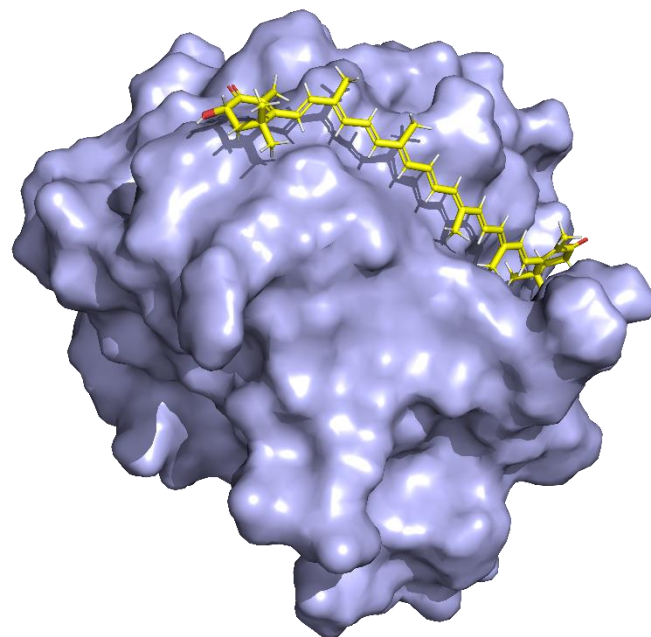
Fer-1+LPS

AST(1μM)+LPS

AST(10μM)+LPS

Figure 3 AST inhibits LPS-induced ALI and ferroptosis *in vivo*.

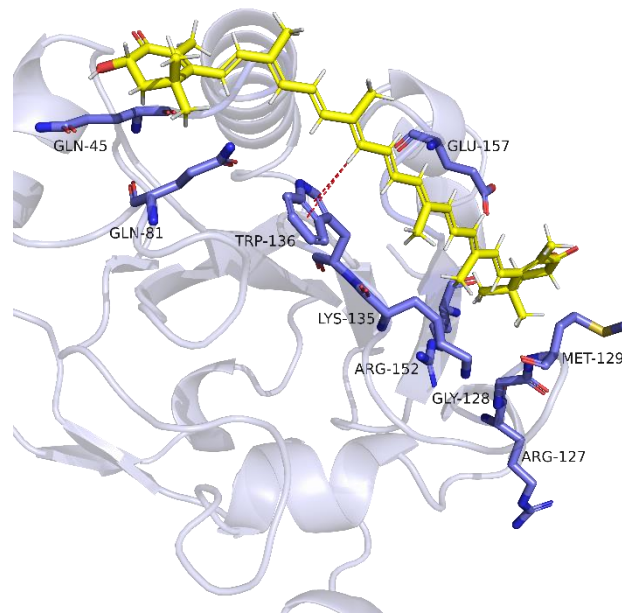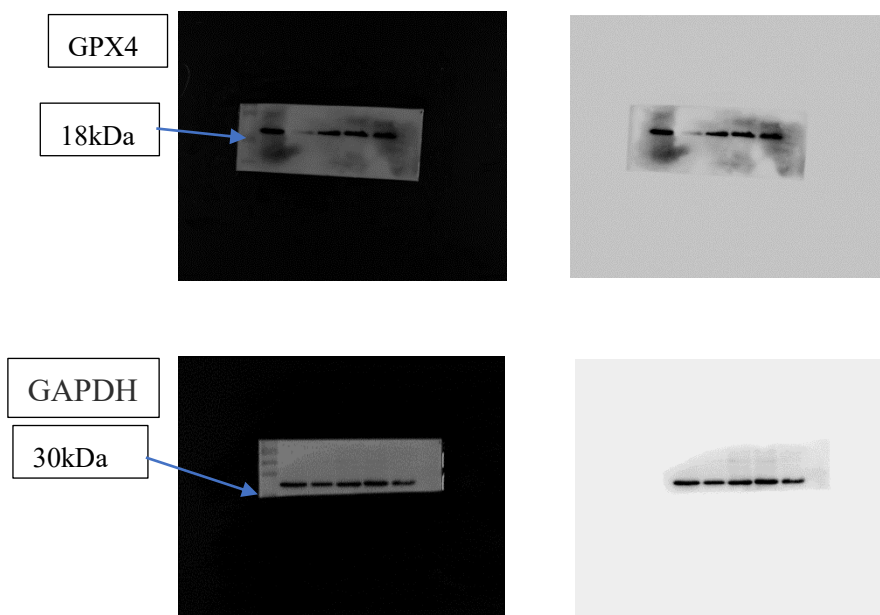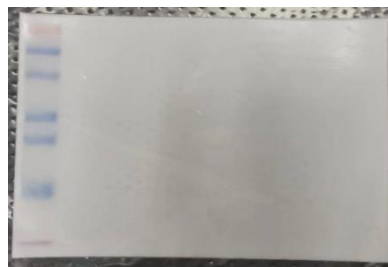**Fig.3(A-C):**. Molecular docking of AST and Gpx4.
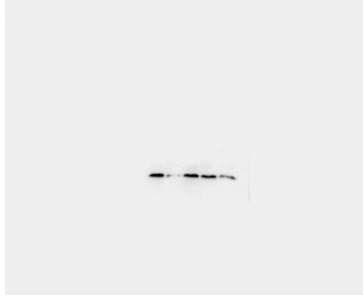
Fig.3(A)：
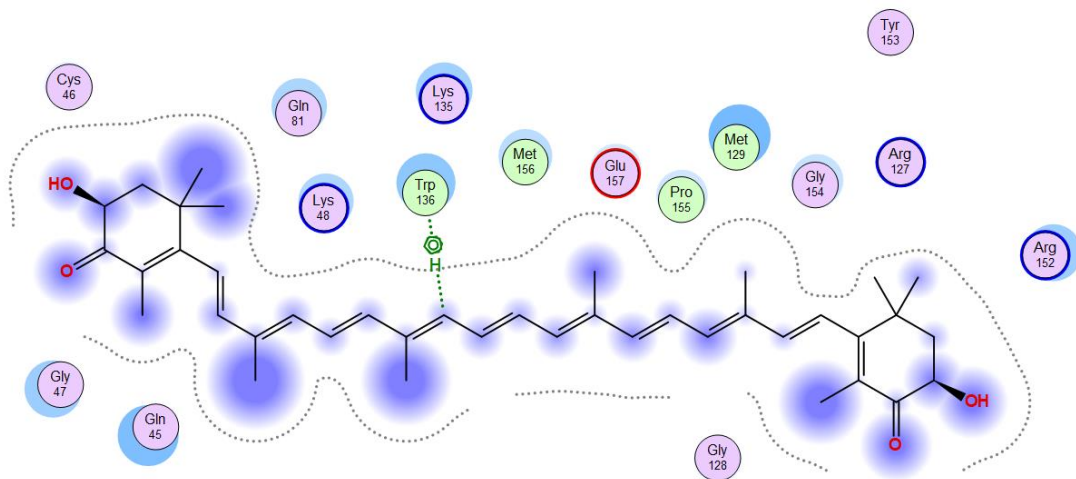
Fig.3(B):

Fig.3(C):
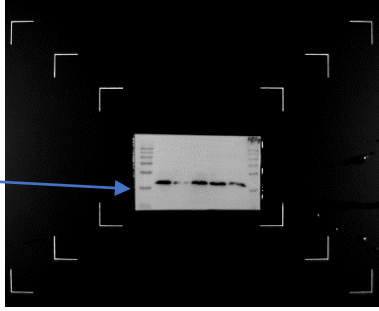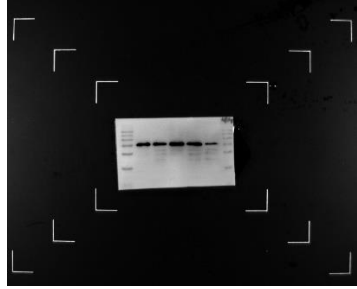

Fig.3(D):
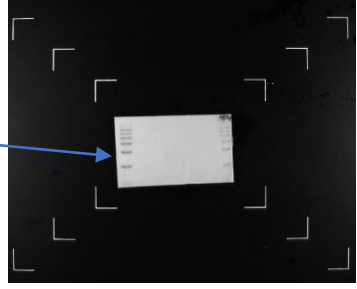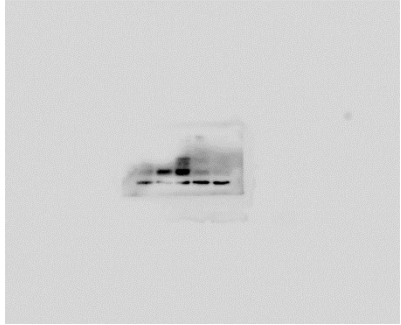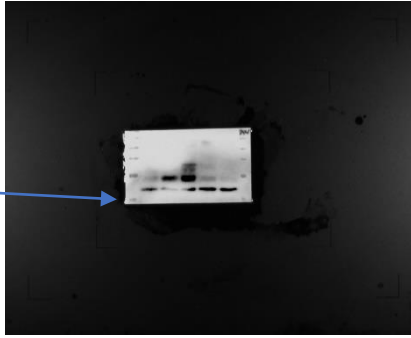
Western blots for GPX4 in mice lung tissues.

GPX4

18kDa

GAPDH

30kDa

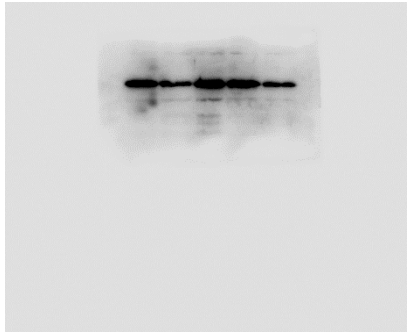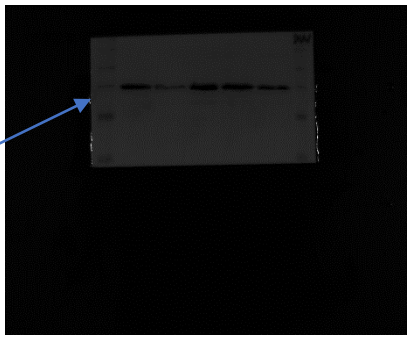GPX4

18kDa

GAPDH

30kDa

Fig.3(E): The raw data of Fig.3(E) is detailed in excel (Relative protein expression，mouse- GPX4/GAPDH).

Fig.3(F): The raw data of Fig.3(F) is detailed in excel (Serum iron).
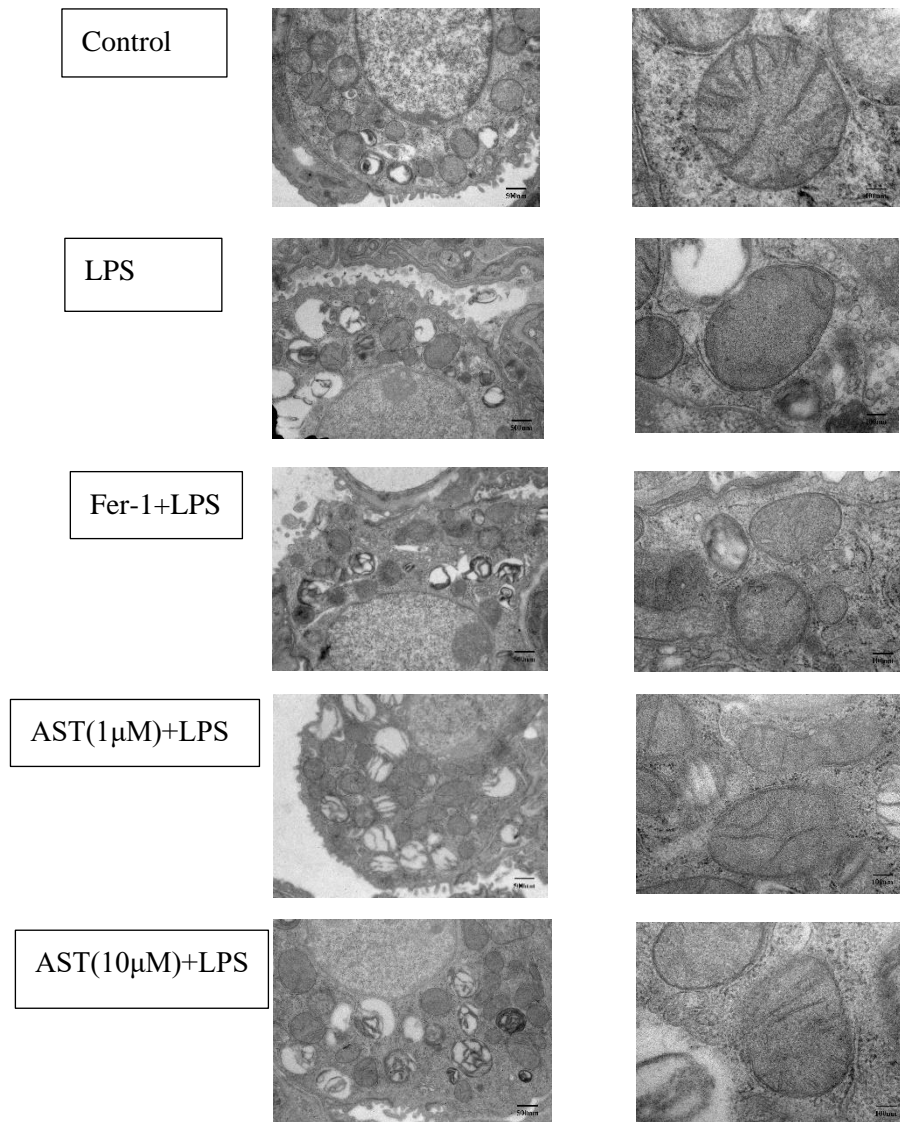
Fig.3(G): The raw data of Fig.3(G) is detailed in excel (Tissue iron).

Fig.3(H): Immunohistochemistry scores for GPX4 in mice lung tissues.

Fig.3(I): Immunohistochemistry staining for GPX4 in mice lung tissues.

Control

LPS

Fer-1+LPS

AST(1μM)+LPS

AST(10μM)+LPS
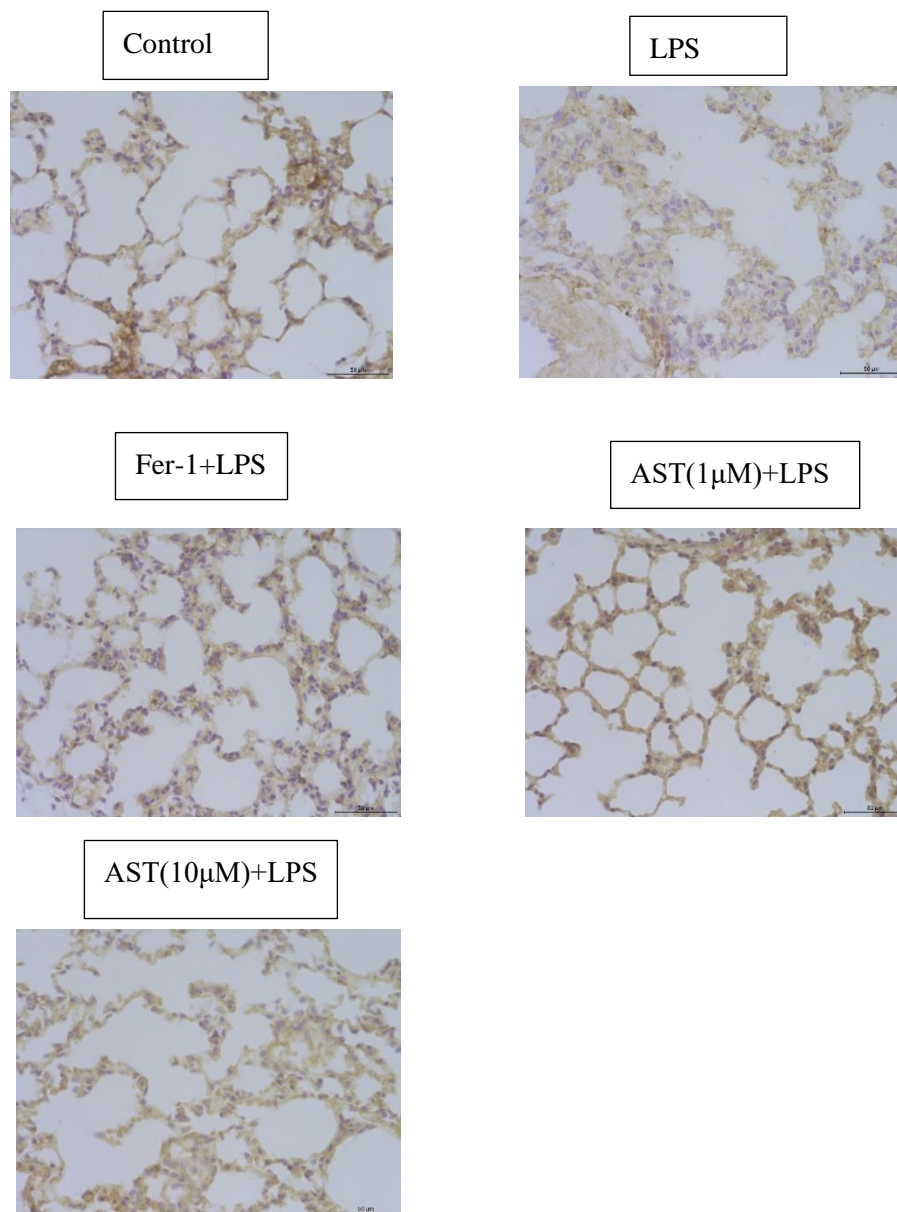
Fig.3(J): The TEM images of mice

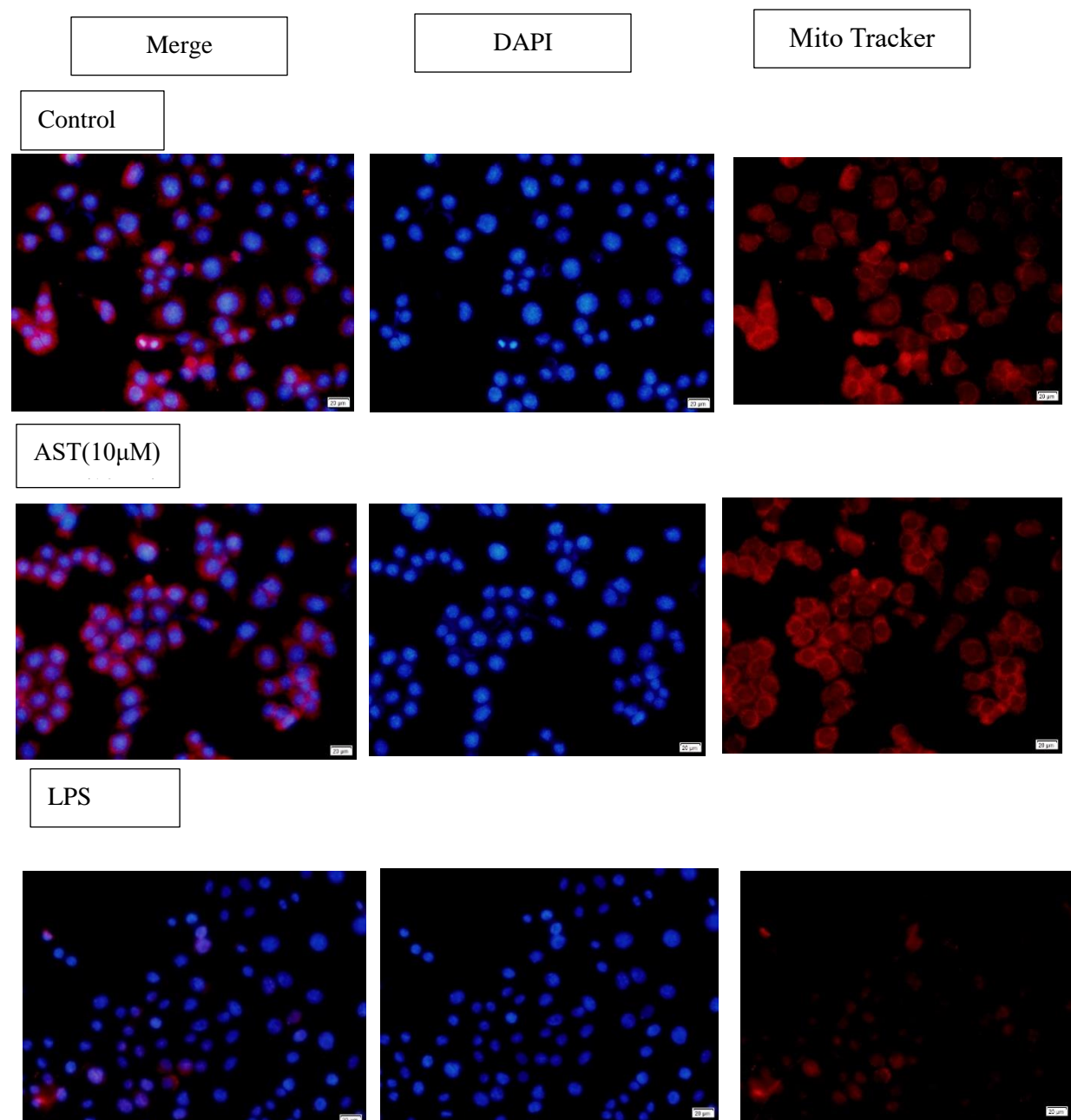**Figure 4 AST reduced LPS-induced lipid peroxidation and ferroptosis in MLE-12 cells.**
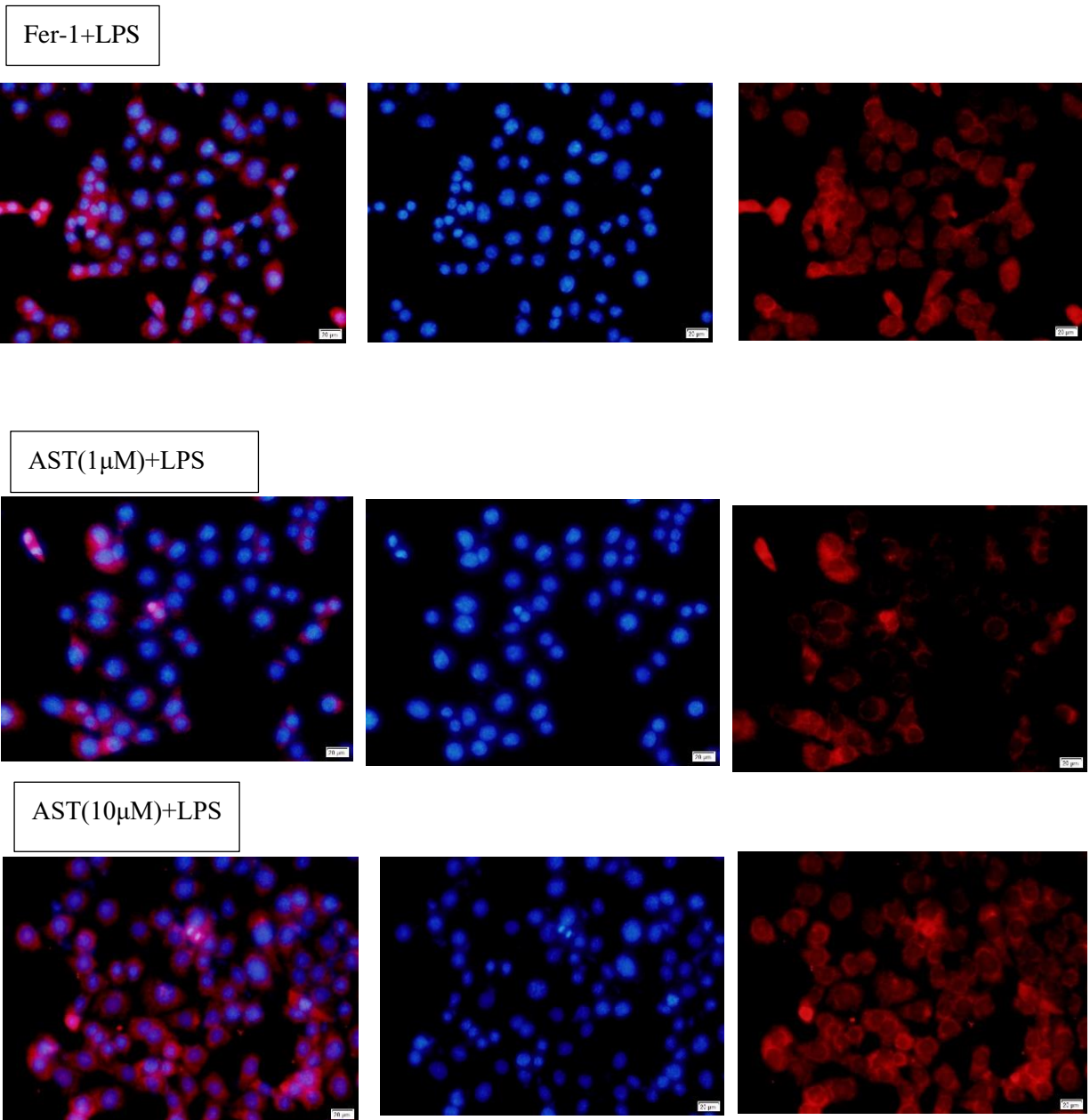
Fig.4(A)： The raw data of Fig.4(A) is detailed in excel (Cell viability).

Fig.4(B)： The raw data of Fig.4(B) is detailed in excel (Cell MDA).
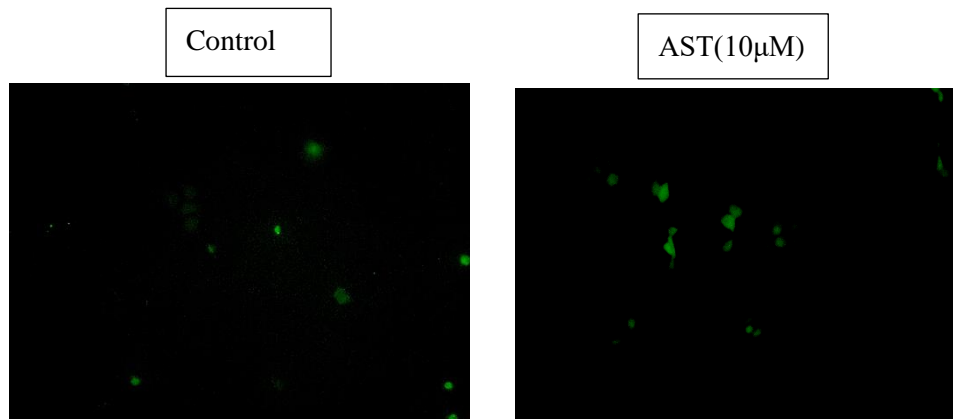
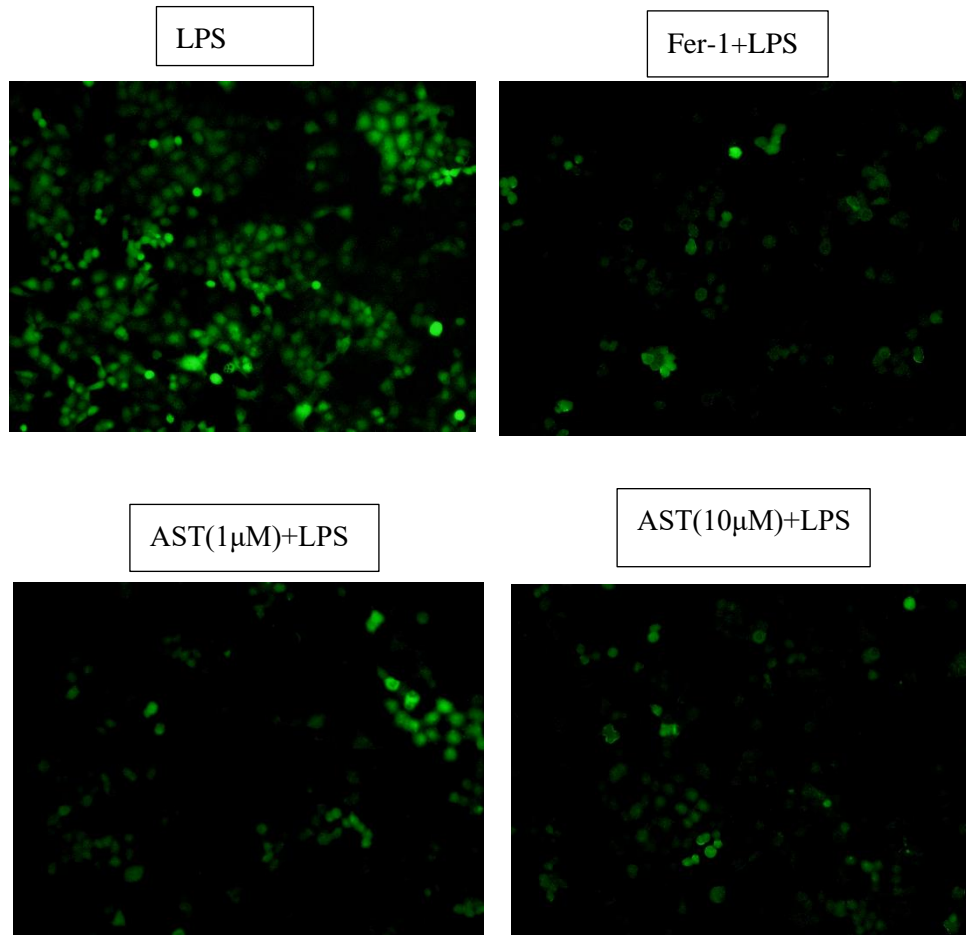**Fig.4(C)：** The raw data of Fig.4(C) is detailed in excel (Cell GSH).
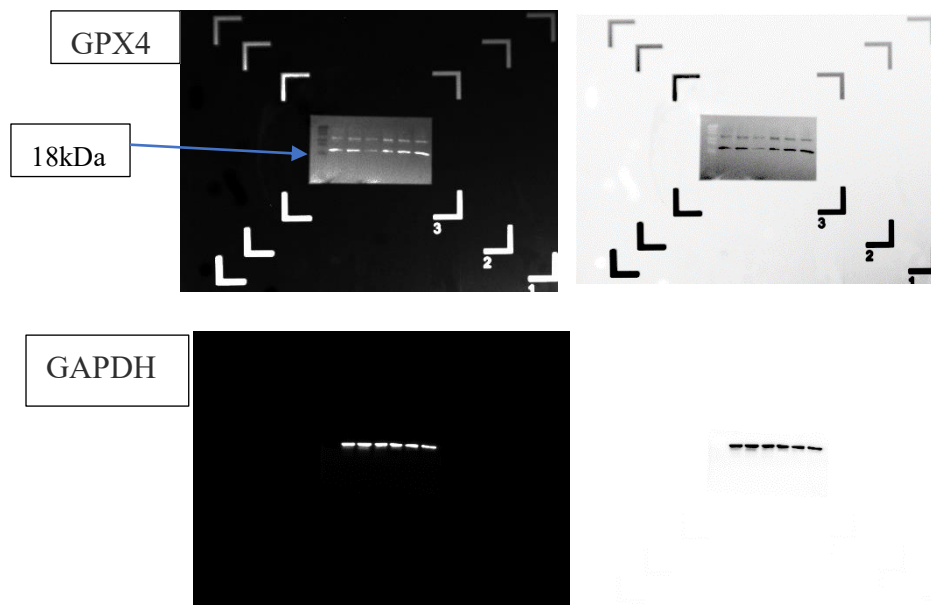
Fig.4(D)： Staining of Mito Tracker

Fer-1+LPS

AST(1μM)+LPS

AST(10μM)+LPS

Fig.4(E)： Representative images of fluorescence probe for ROS in MLE-12 cells

Control

AST(10μM)

LPS

Fer-1+LPS

AST(1µM)+LPS

AST(10µM)+LPS

Fig.4(F)： The raw data of Fig.4(F) is detailed in excel (Relative mRNA expression of GPX4).

Fig.4(G)： The protein level of GPX4 in MLE-12 cells

GPX4

18kDa
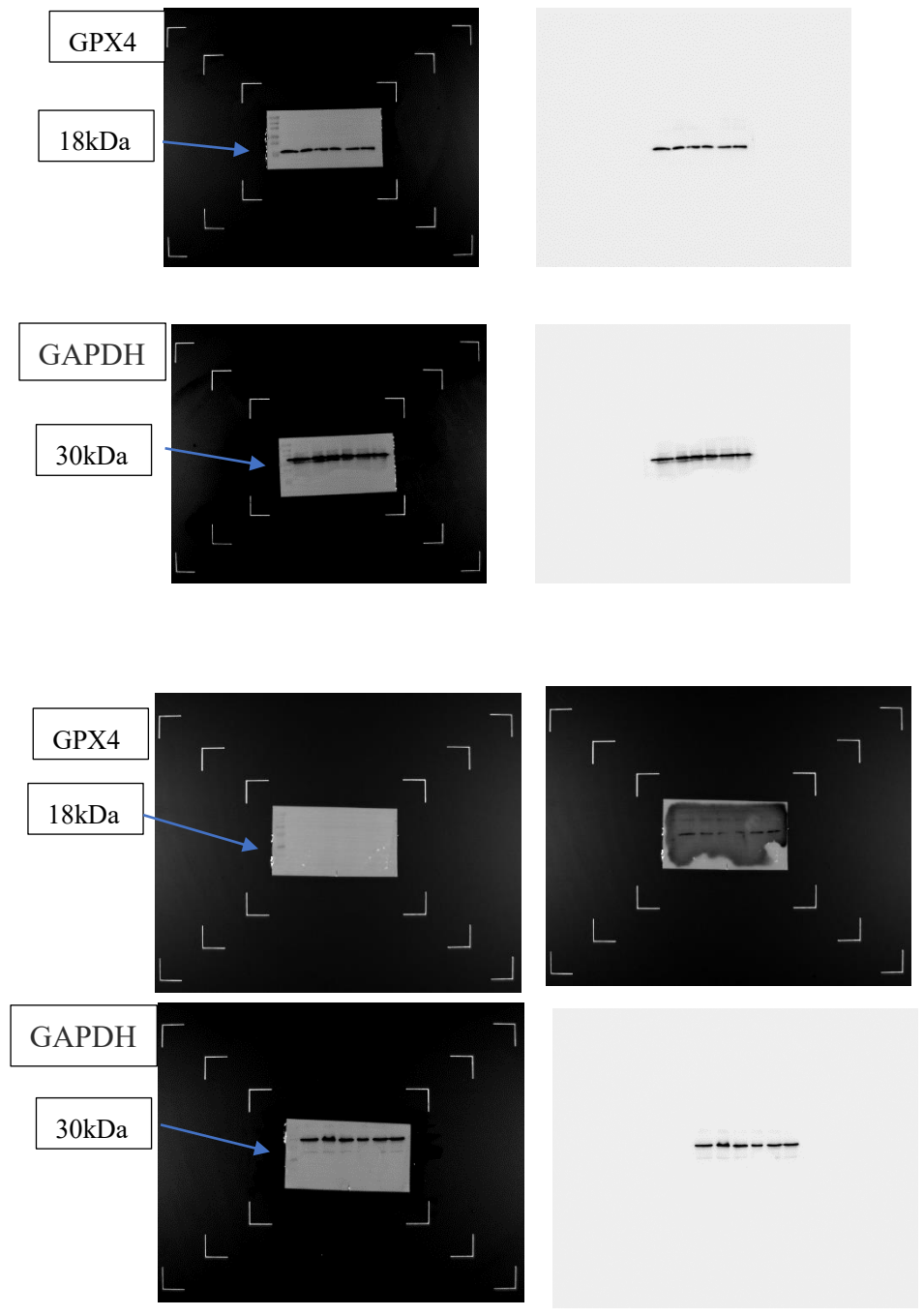
GAPDH

Fig.4(H)： The raw data of Fig.4(H) is detailed in excel (Relative protein expression, Cell- GPX4/GAPDH).

Fig.4(I)： The TEM images of Cell

Control

AST(10μM)

LPS

Fer-1+LPS

AST(1μM)+LPS

AST(10μM)+LPS
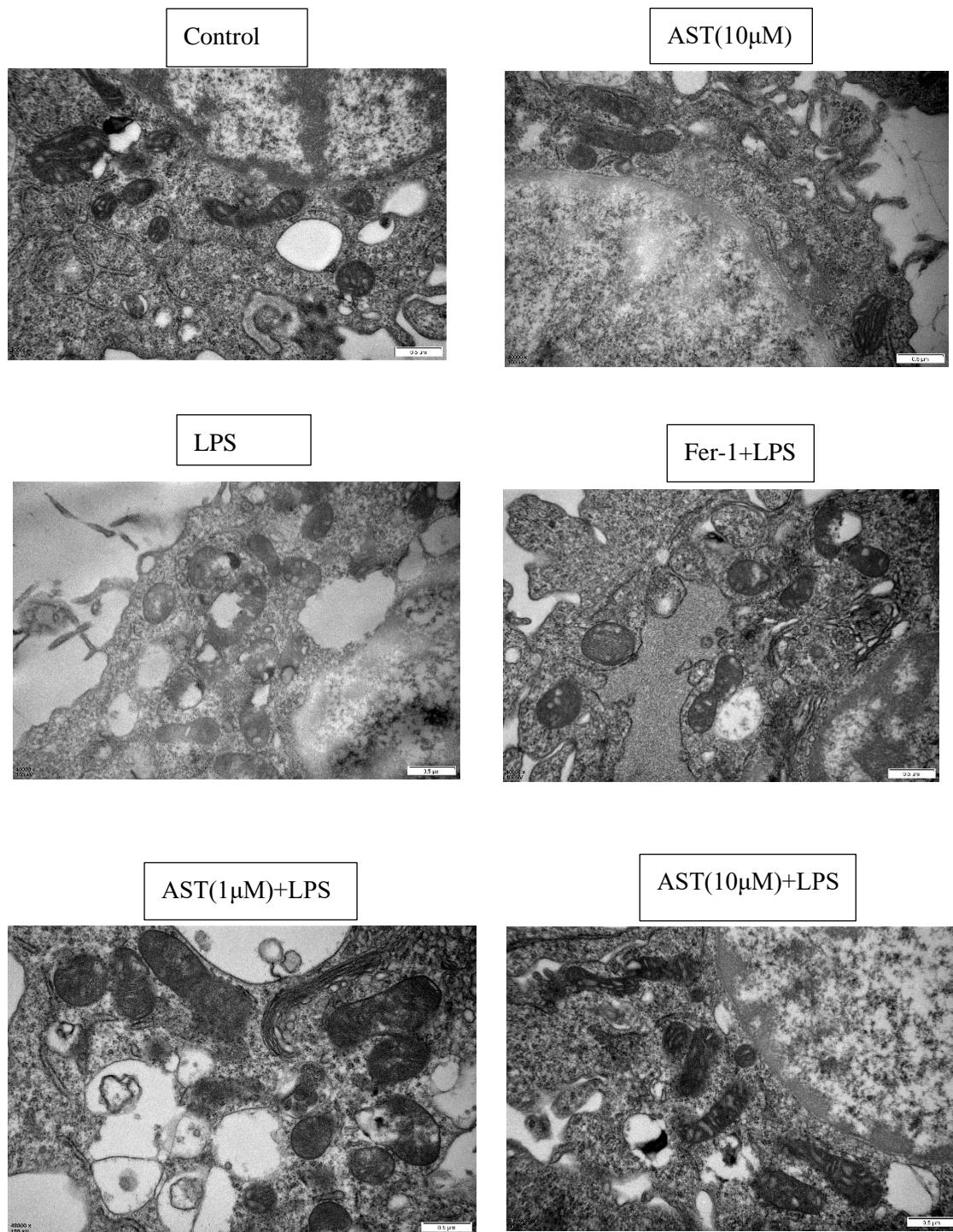
Figure 5 AST suppressed ferroptosis in MLE-12 cells *via* inhibiting ferritinophagy.

Fig.5(A)： The protein level of Ferritin in MLE-12 cells and the raw data of Fig.5(A) is detailed in excel (Relative protein expression, Ferritin/GAPDH).
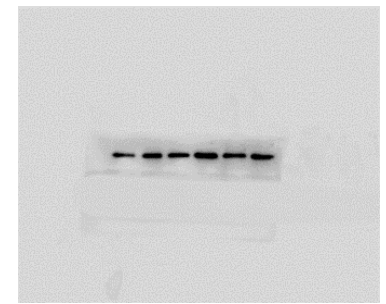
Ferritin

23kDa

18kDa
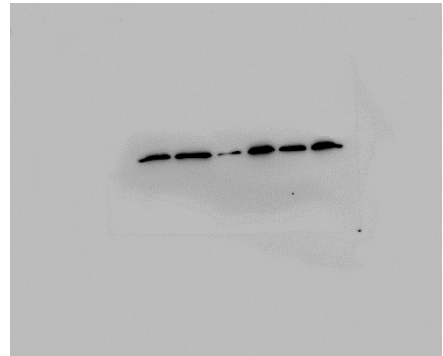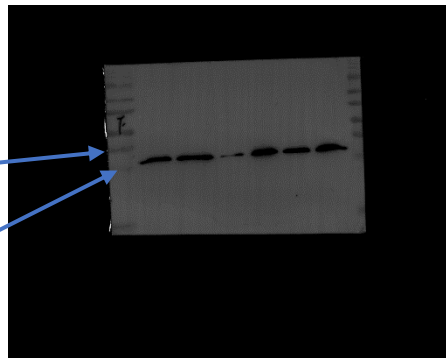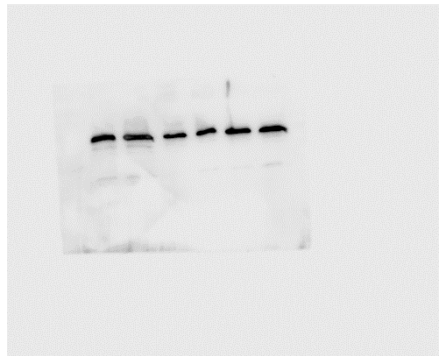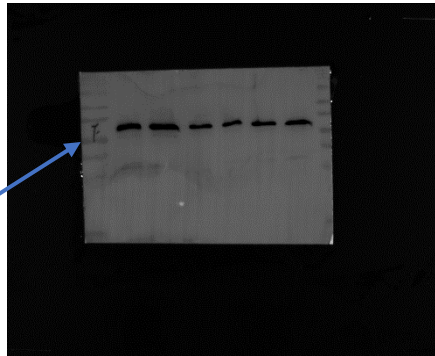
GAPDH

30kDa

Ferritin
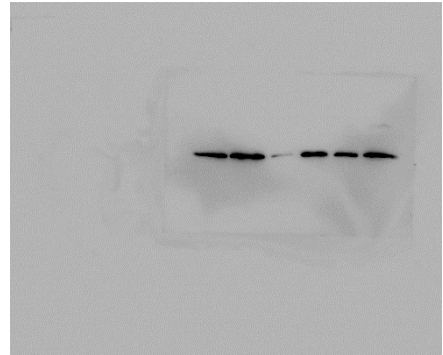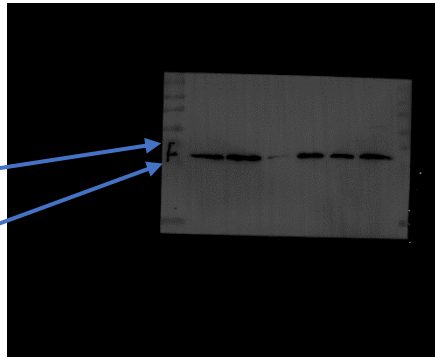
23kDa
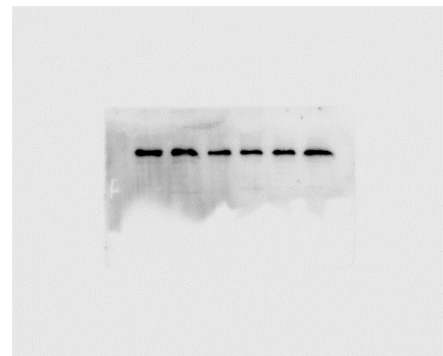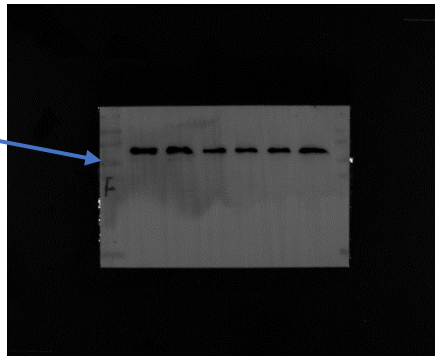
18kDa

GAPDH

30kDa

Ferritin
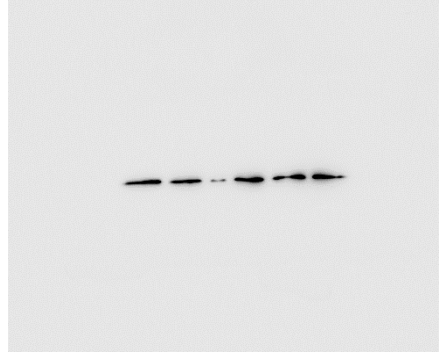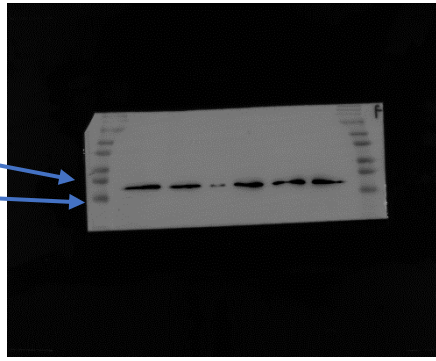
23kDa
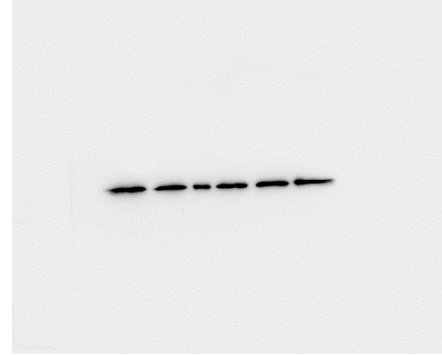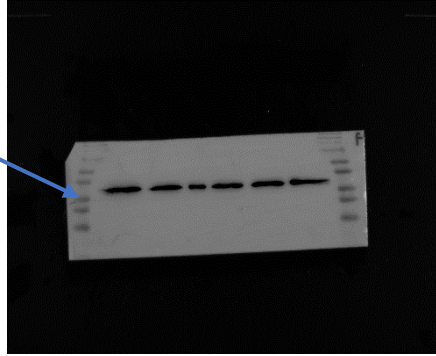
18kDa

GAPDH

30kDa

Ferritin
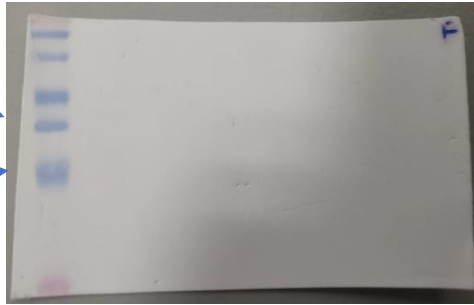
23kDa

18kDa

23kDa

18kDa
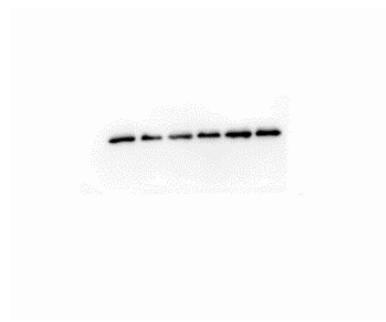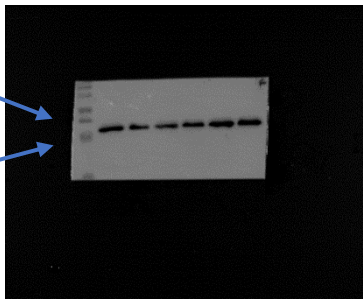
GAPDH

30kDa
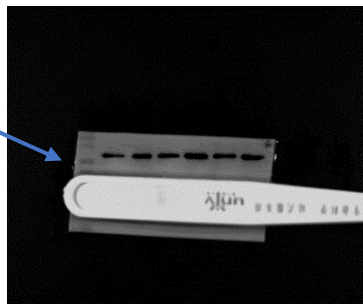
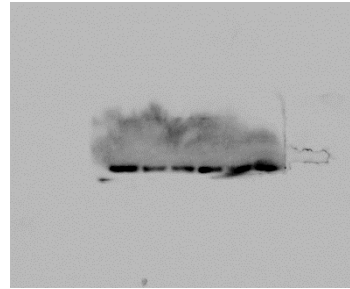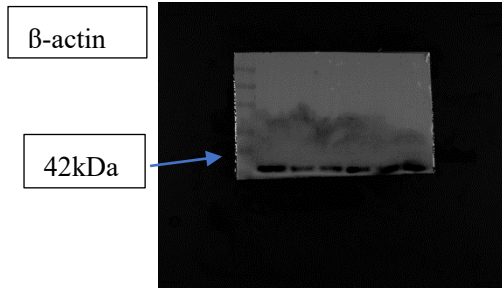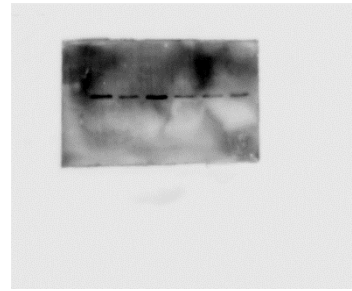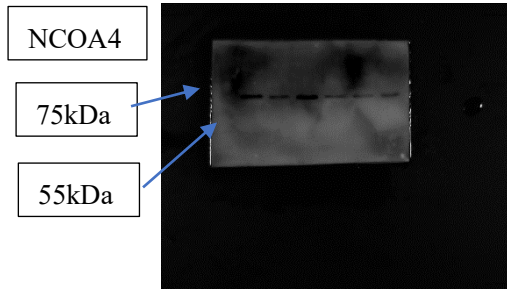**Fig.5(B)：** The protein level of NCOA4 in MLE-12 cells and the raw data of Fig.5(B) is detailed in excel (Relative protein expression, NCOA4 β-Actin)
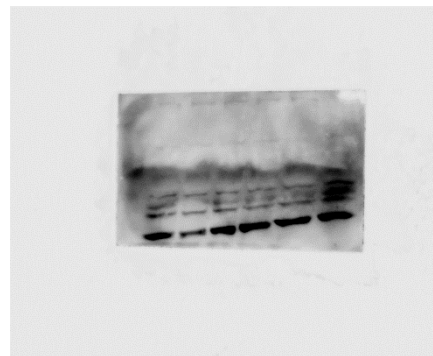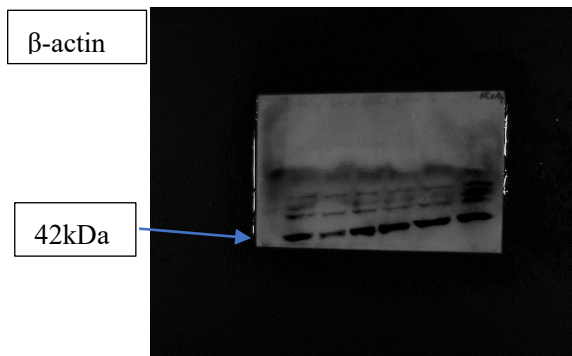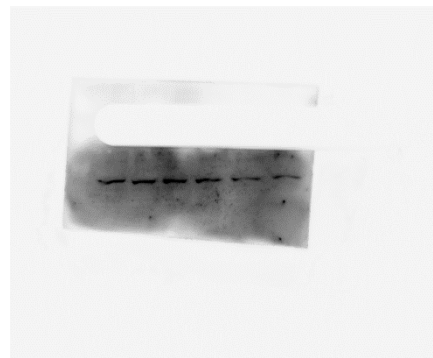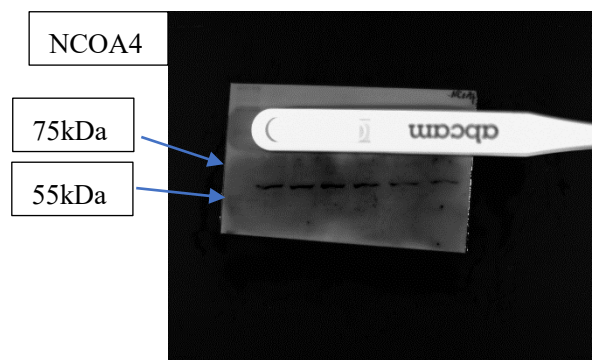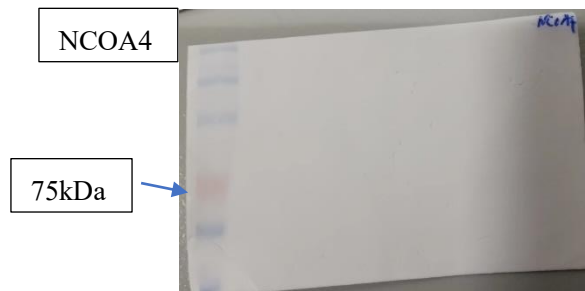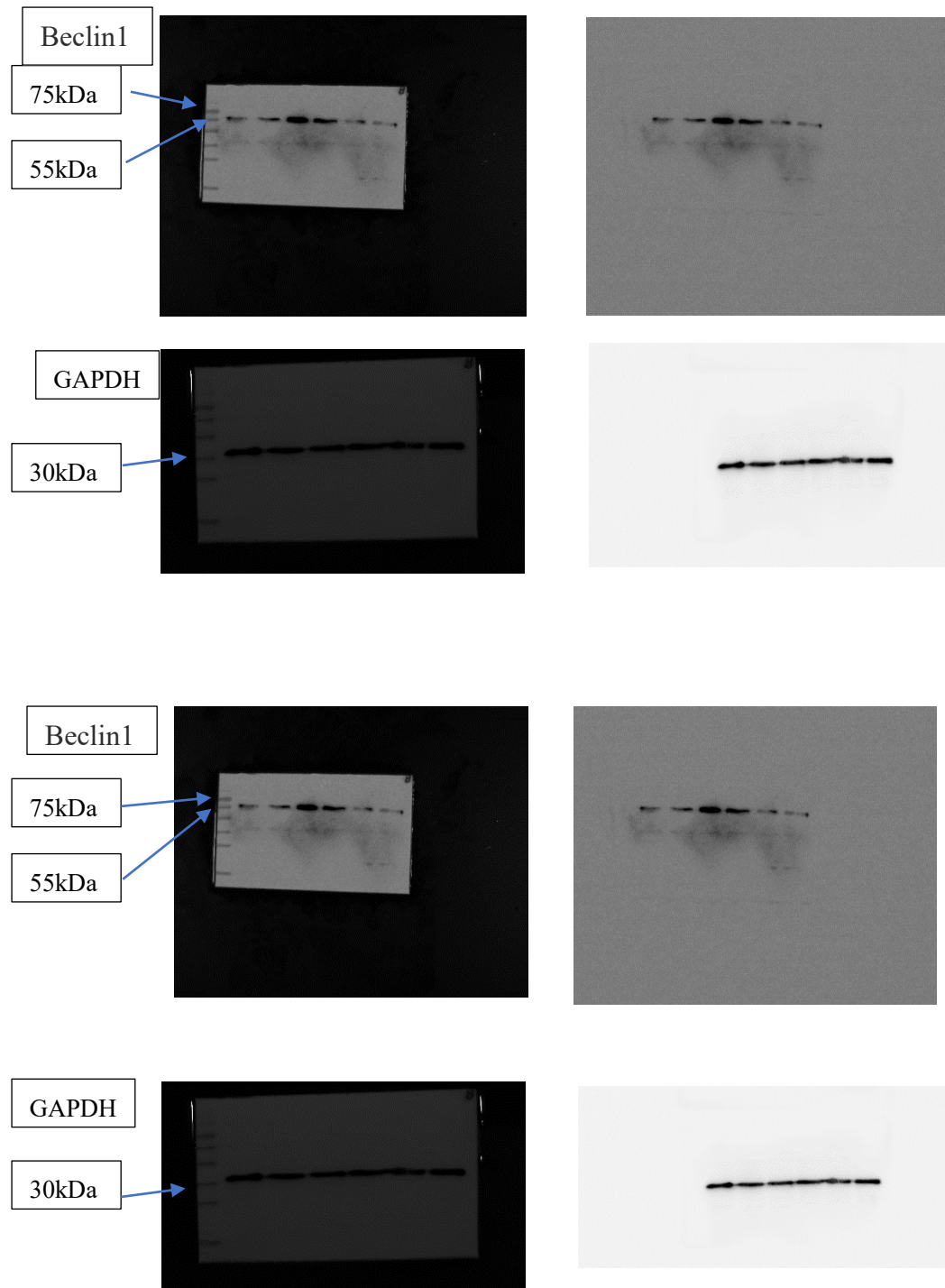

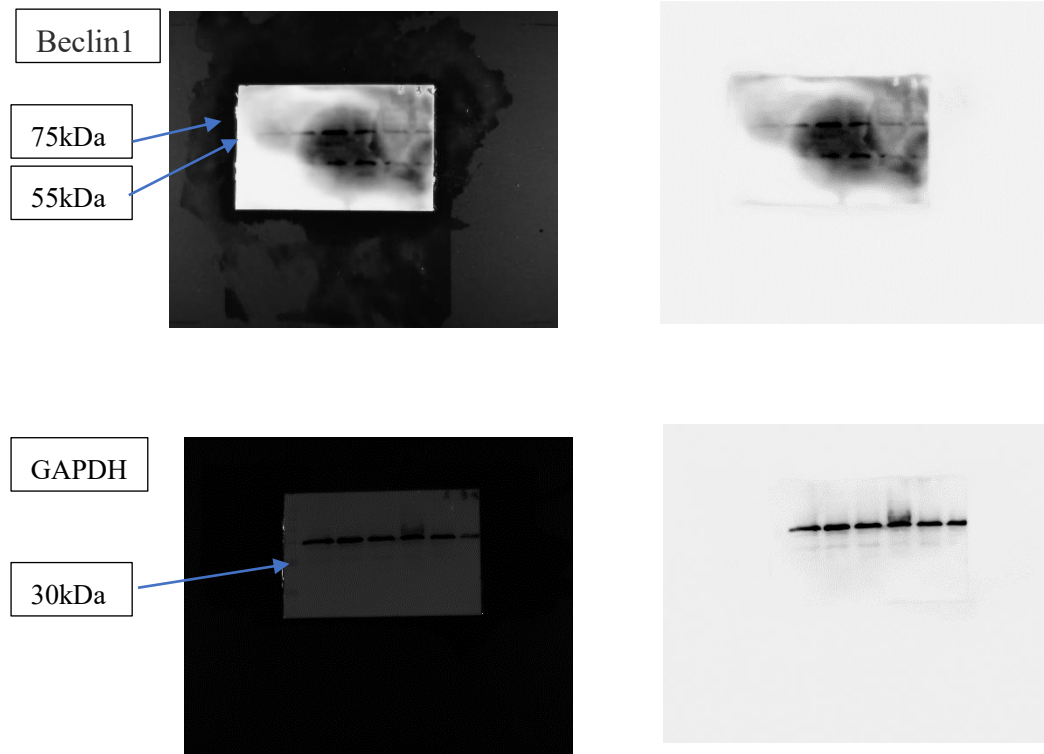We used an object to block part of the image since the background of the membrane is a bit dirty, which influences how NCOA4 forms.

NCOA4

75kDa

55kDa

β-actin

42kDa

NCOA4

75kDa

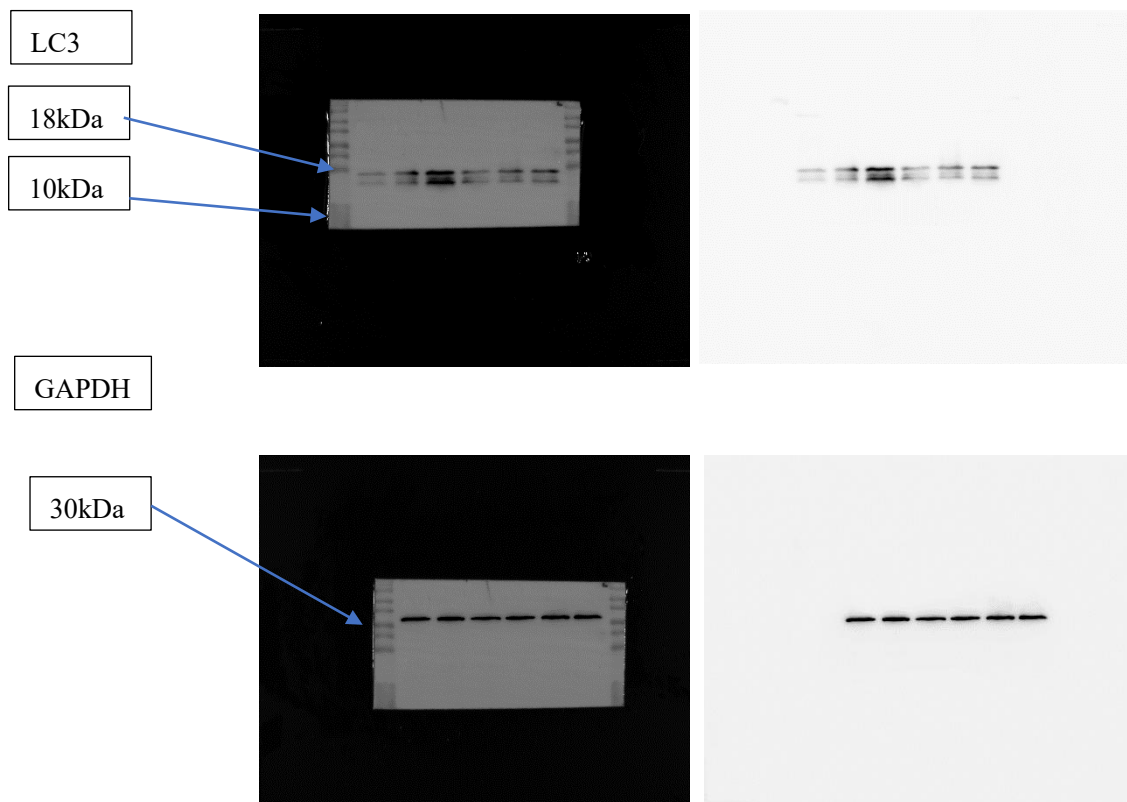55kDa

β-actin

42kDa

NCOA4

75kDa

55kDa

ß-actin

42kDa
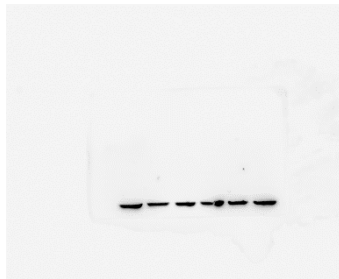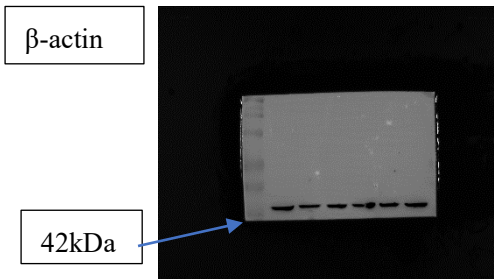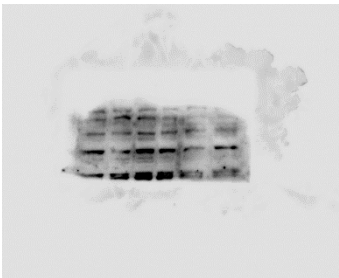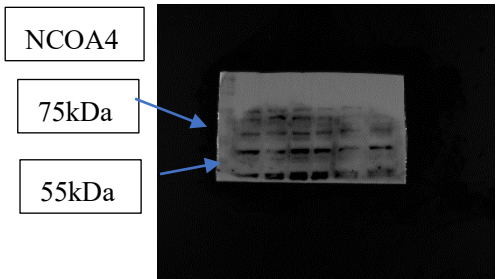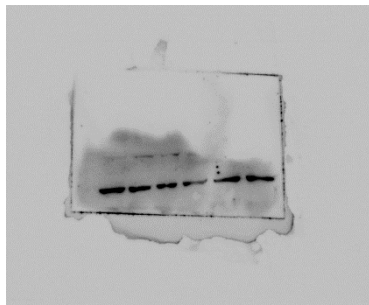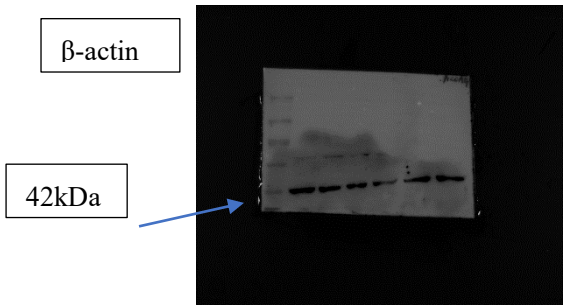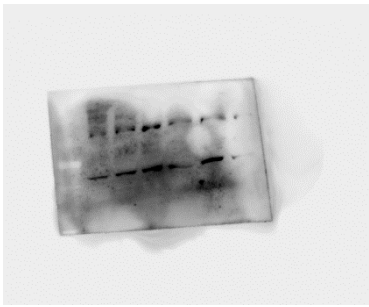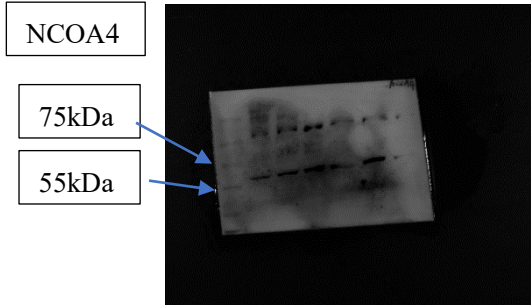
Fig.5(C)：The protein level of Beclin1 in MLE-12 cells and the raw data of Fig.5(C) is detailed in excel (Relative protein expression, Beclin-1/GAPDH)
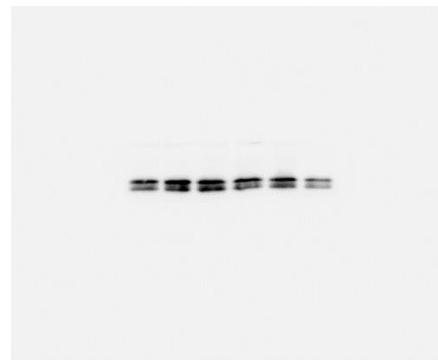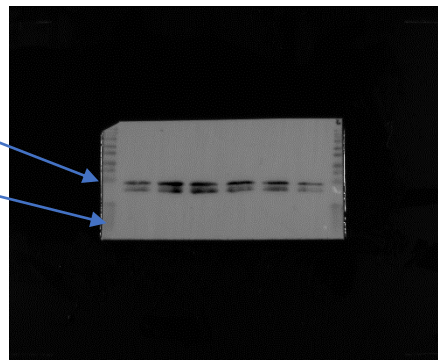
Fig.5(D)： The protein level of LC3 in MLE-12 cells and the raw data of Fig.5(D) is detailed in excel (Relative protein expression, LC3B/GAPDH).
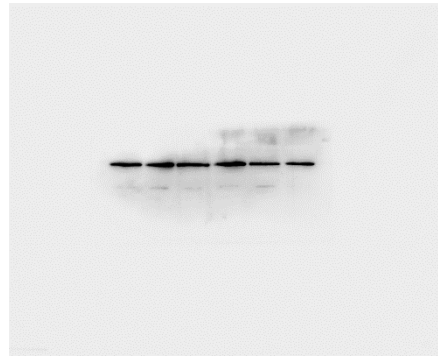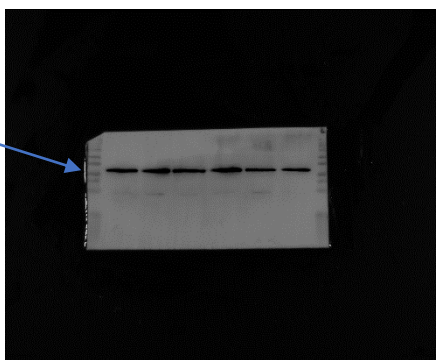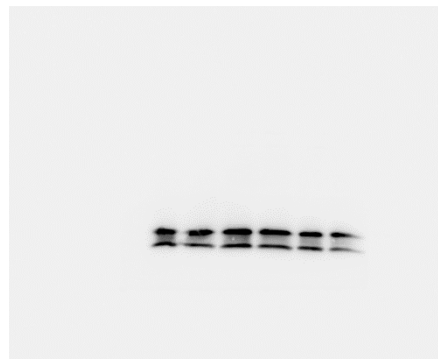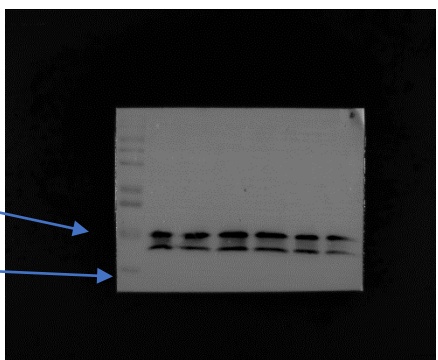
LC3

18kDa

10kDa

GAPDH

30kD

LC3

18kDa

10kDa

GAPDH

30kDa