Supplementary Figures

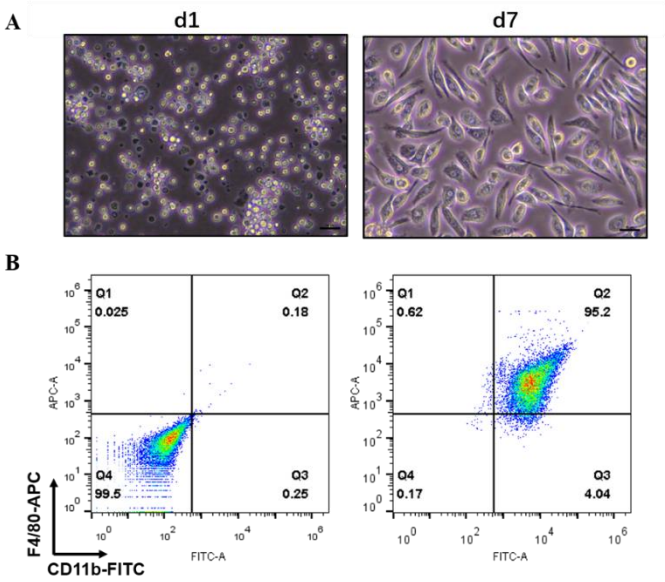


FIGURE S1 Mouse bone marrow cells differentiate into macrophages. Mouse bone marrow cells were induced by incubation in BM-medium for 7 days, and the culture medium was replaced every other day. (A) Micrographs of cells were observed using a bright-field Leica DMi8 imaging system. Scale bar, 50 μm. (B) The specific macrophage markers F4/80 and CD11b were detected by flow cytometry.

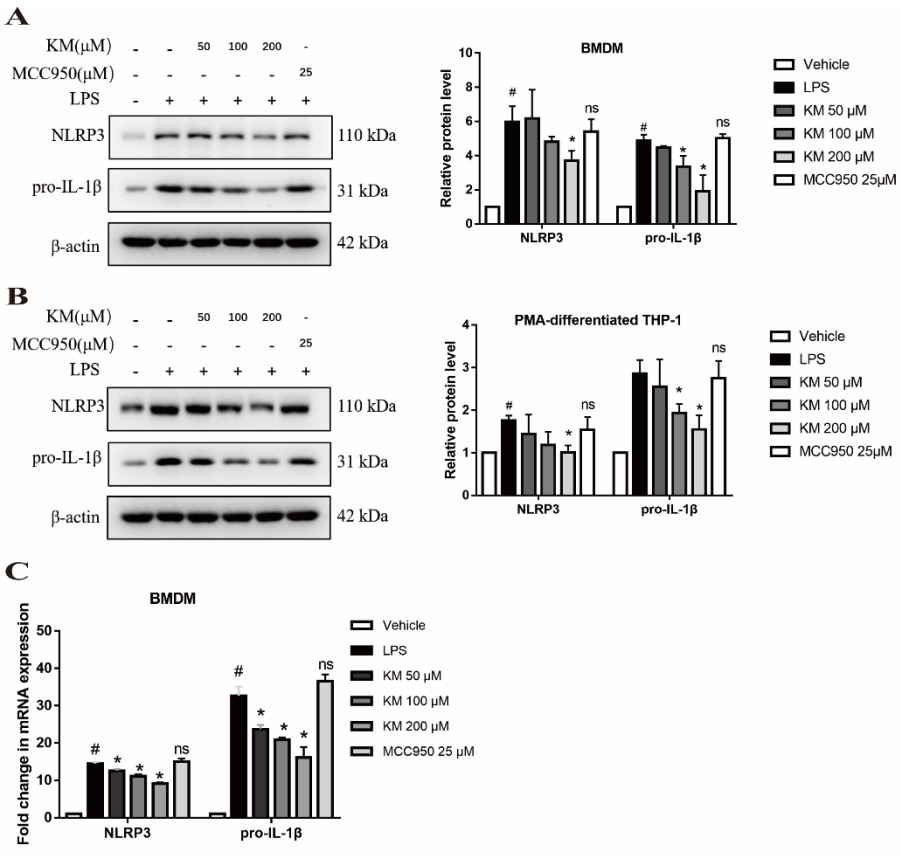


FIGURE S2 Koumine (KM) inhibits the expression of NLRP3 and pro-IL-1β stimulated by LPS in macrophages. (A) BMDMs or (B) PMA-differentiated THP-1 cells were pretreated with KM or MCC950 for 1 h and then incubated with LPS (300 ng/ml) for 3 h. Cell lyses were collected and analyzed by immunoblotting for NLRP3 and pro-IL1β. The mRNA levels of NLRP3 and pro-IL1β in (A) were analyzed by RT-qPCR. Mean ± SD of 3 independent experiments are shown. #$P < 0.05$ versus vehicle group, *$P < 0.05$ versus LPS group.

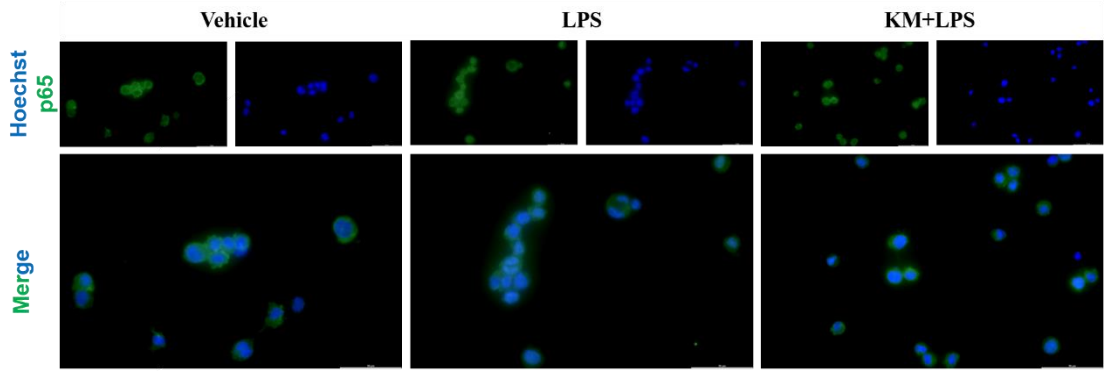


FIGURE S3 Koumine (KM) suppresses the p65 nuclear localization in PMA-differentiated THP-1 macrophages. PMA-differentiated THP-1 macrophages were pretreated with KM (200 μM) for 1 h and then incubated with LPS (300 ng/ml) for 1 h. The nuclear location of p65 in PMA-differentiated THP-1 macrophages were visualized by immunofluorescence analysis with an anti-p65 (green) antibody. The nuclei (blue) were stained with Hoechst 33324. Scale bars, 50 μm.

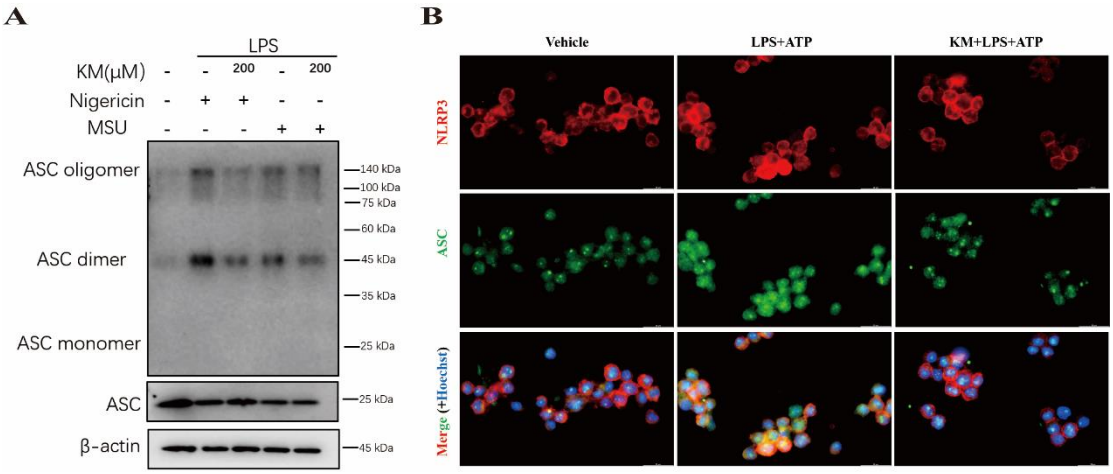


FIGURE S4 Koumine (KM) blocks NLRP3 inflammasome assembly in macrophages. (A) BMDMs were pretreated with KM (200 μM) for 1 h and then incubated with LPS (300 ng/ml) for 3 h, nigericin (10 μM) for 1 h or MSU (150 μg/ml) for 6 h. ASC oligomerization in cross-linked cytosolic pellets were analyzed by immunoblotting. (B) PMA-differentiated THP-1 macrophages were pretreated with KM (200 μM) for 1 h and then incubated with LPS (300 ng/ml) for 3 h and ATP (5 mM) for 1 h. The interaction between NLRP3 (red) and ASC (green) in cells were assayed by immunofluorescence. Scale bas, 25 μm.

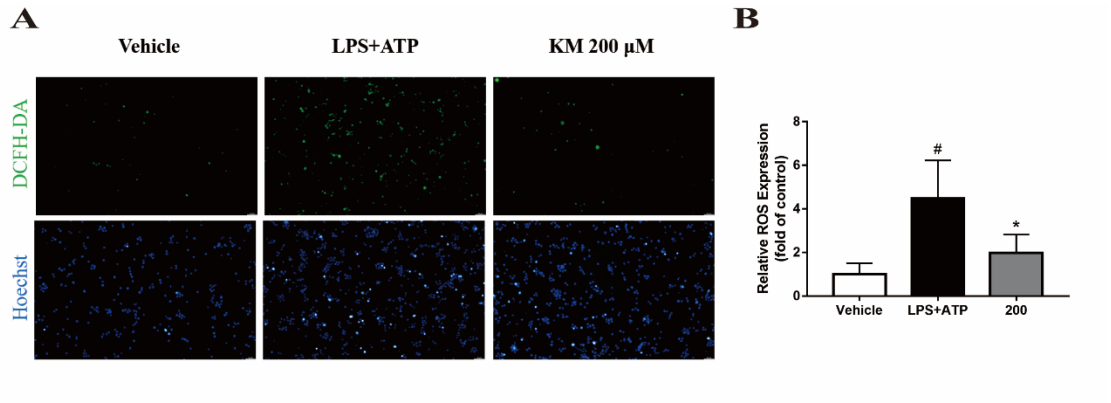
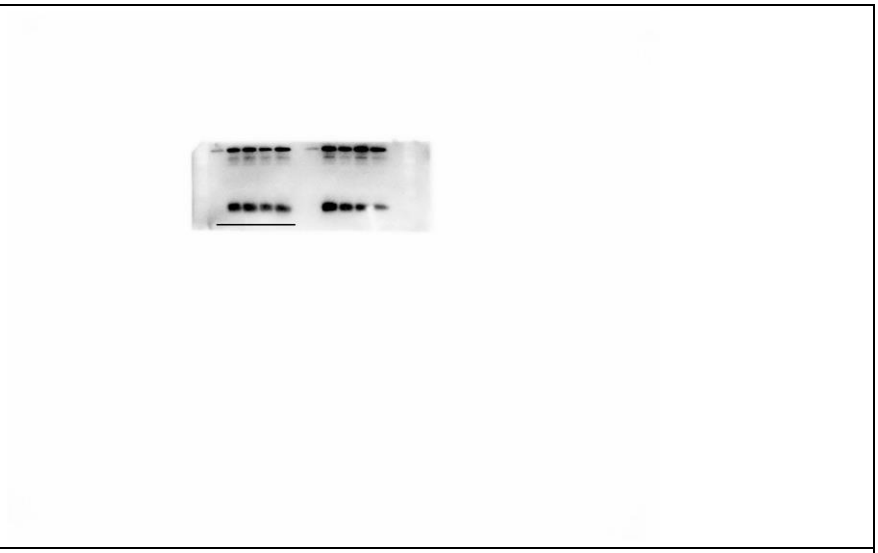
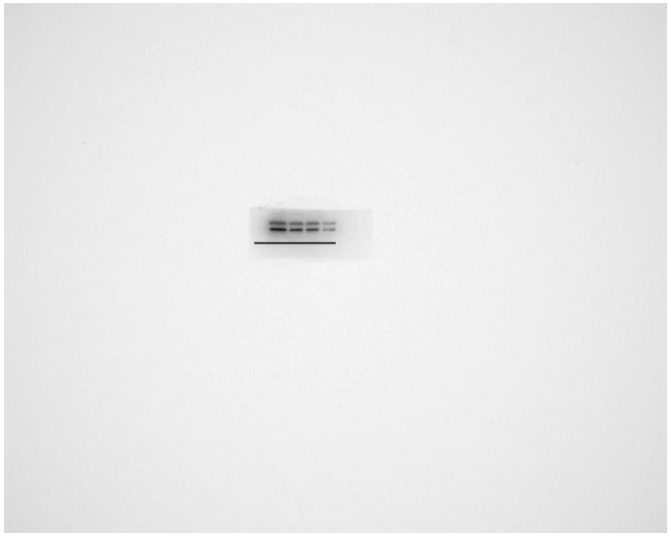
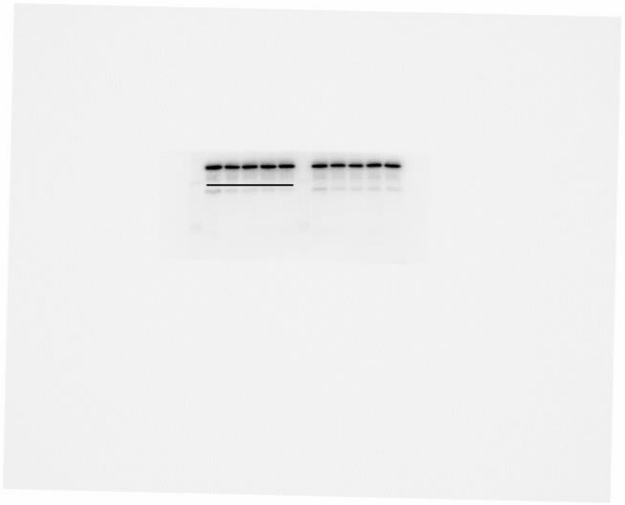


FIGURE S5 Koumine (KM) inhibits ROS generation triggered by LPS and ATP in PMA-differentiated THP-1 macrophages. PMA-differentiated THP-1 macrophages were pretreated with KM (200 μM) for 1 h and then incubated with LPS (300 ng/ml) for 3 h and ATP (5 mM) for 1 h. Intracellular ROS were labeled with the DCFH-DA probe and examined by fluorescence microscopy. Scale bas, 50 μm. Mean ± SD of 3 independent experiments are shown. #$P < 0.05$ versus vehicle group, *$P < 0.05$ versus LPS plus ATP group.

The original western blots of figure 4.

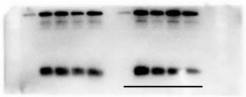
FIGURE 4A

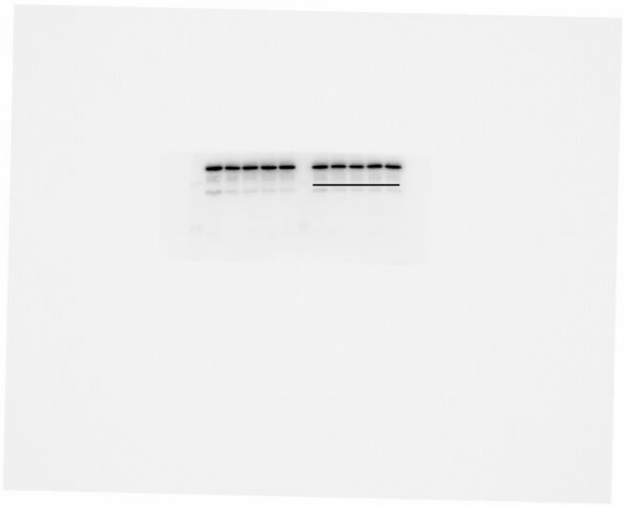
Caspase-1 p20	
IL-1β p17	
NLRP3	

Pro-caspase-1	
Pro-IL-1β	
ASC	

β-actin	

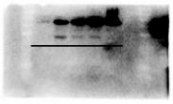
FIGURE 4C

Caspase-1 p20	
IL-1β p17	

NLRP3	
Pro-caspase-1	
Pro-IL-1β	

ASC	
β-actin	

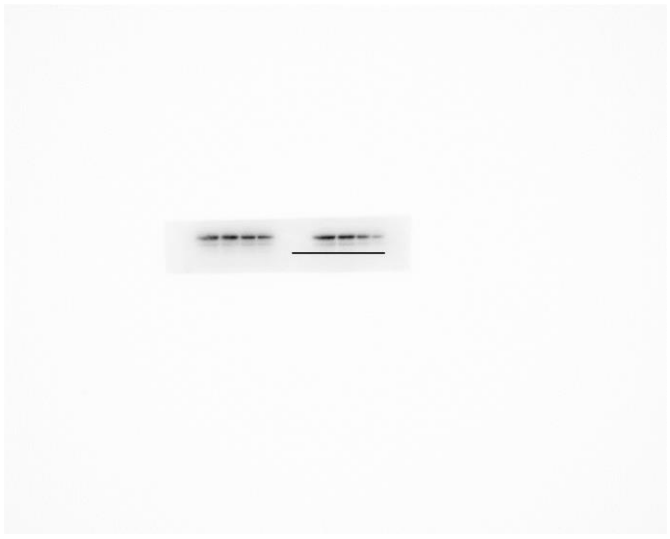
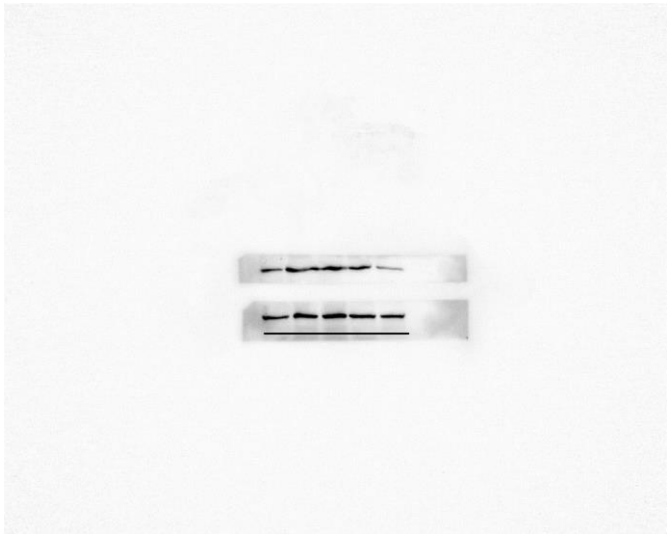
FIGURE 4E

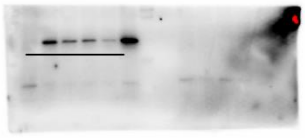
Caspase-1 p20	

IL-1β p17	
NLRP3	
Pro-caspase-1	

Pro-IL-1β	
ASC	7 
β-actin	

FIGURE 4G

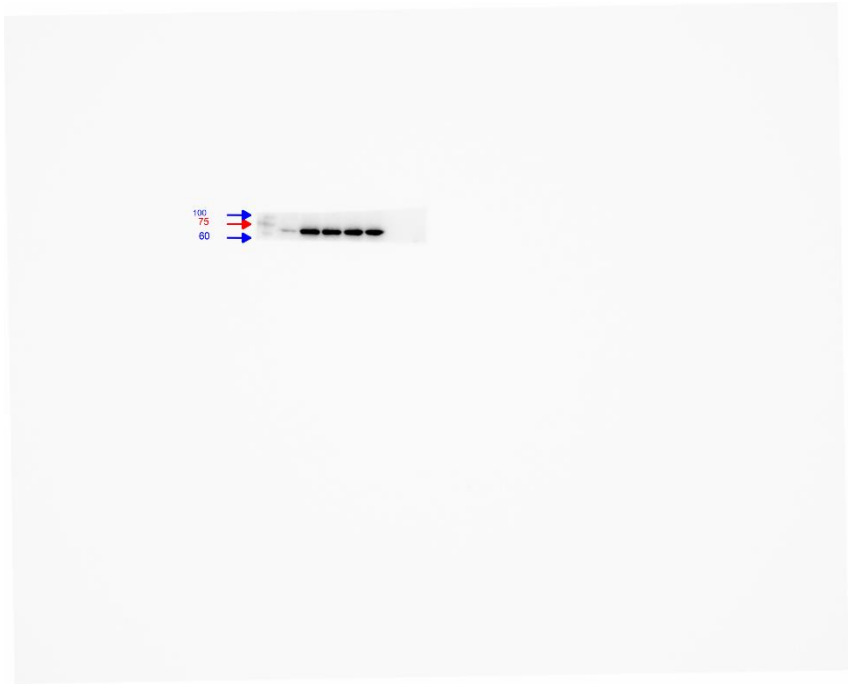
Caspase-1 p20	
IL-1β p17	
NLRP3	

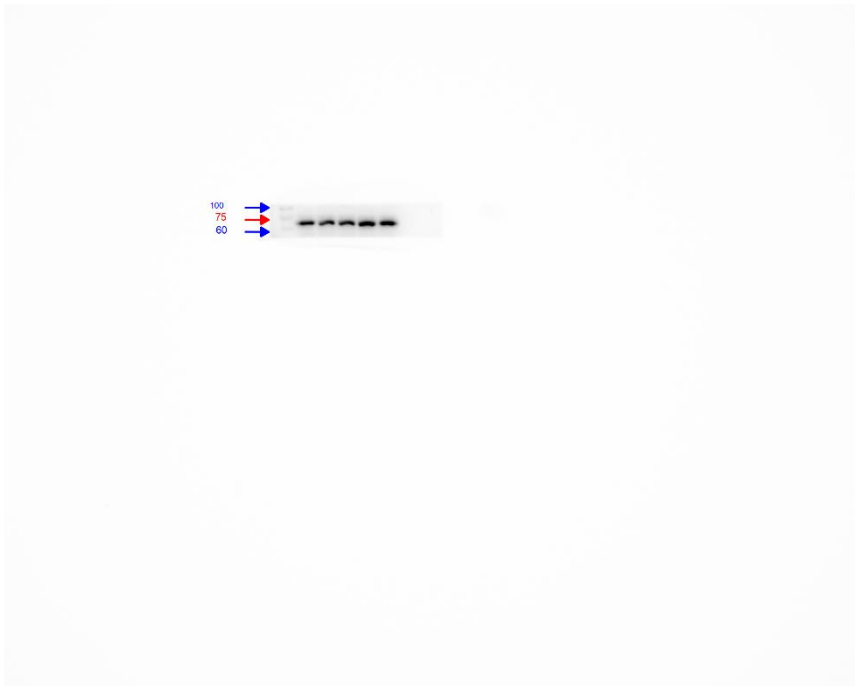
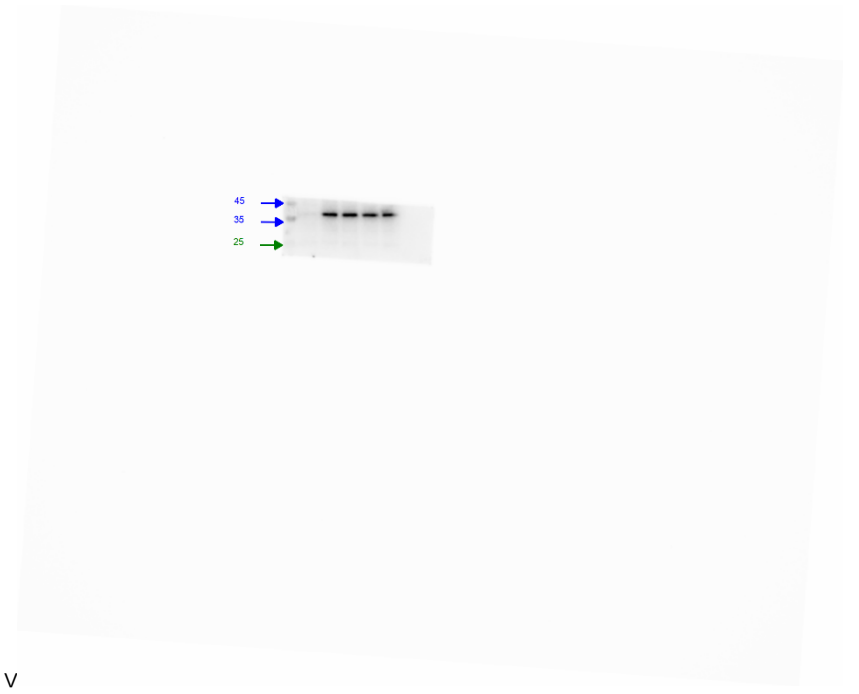
Pro-caspase-1	
Pro-IL-1β	
ASC	

β-actin	

The original western blots of figure 5.

FIGURE 5A

p-p65	

P65	
p-IκBα	 V

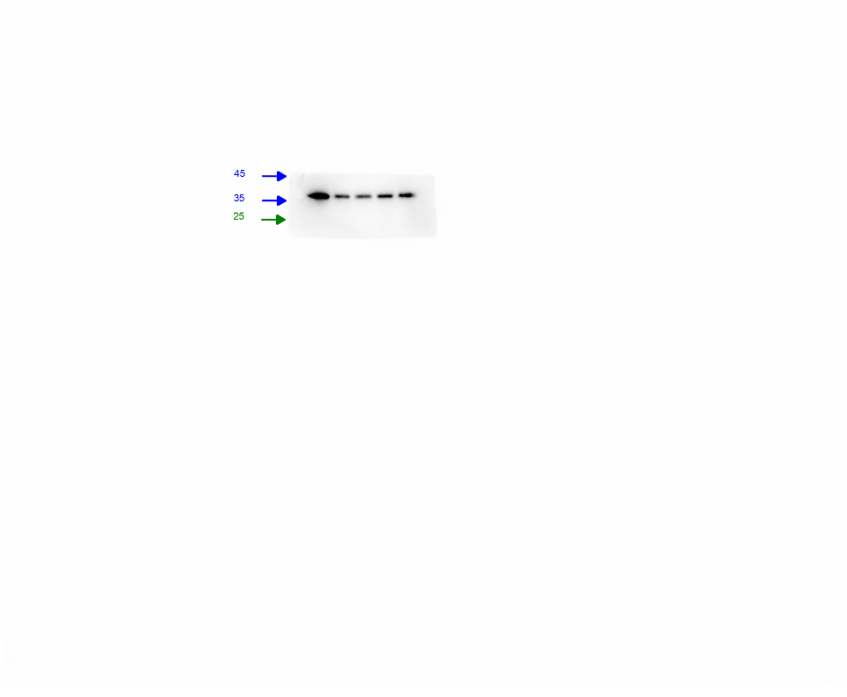
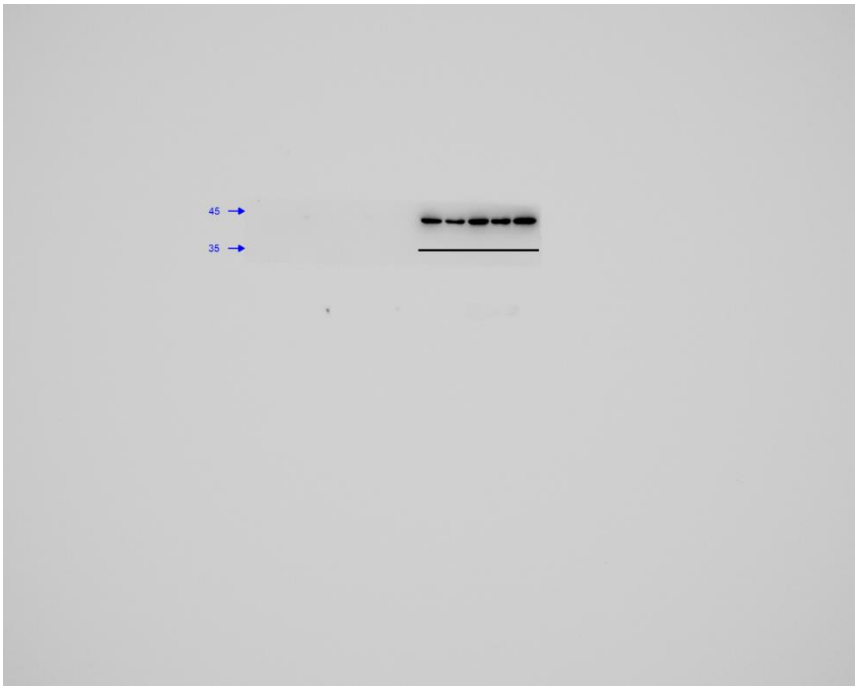
IκBα	
β-actin	

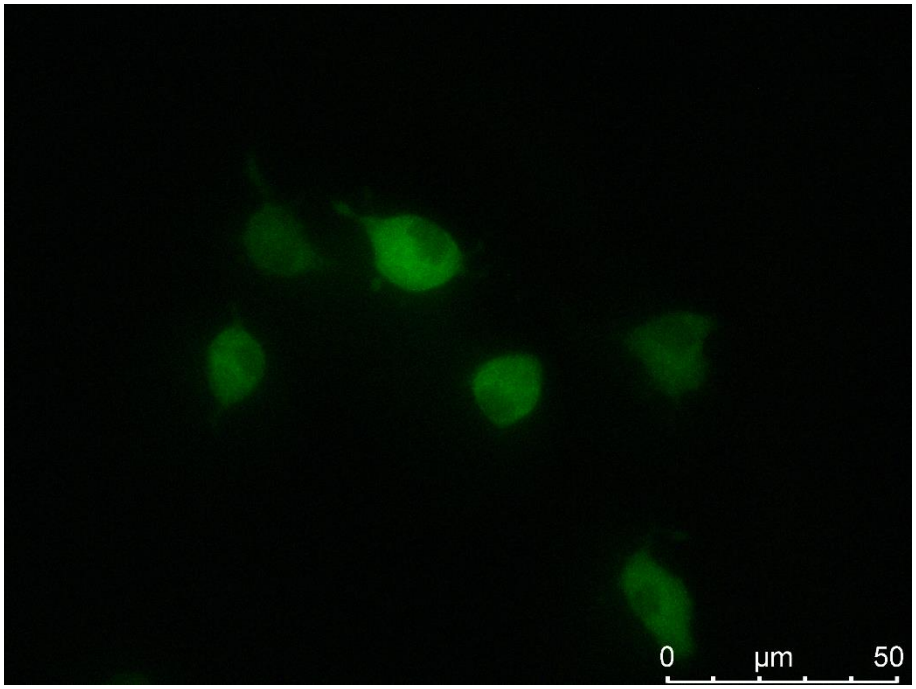
FIGURE 5C

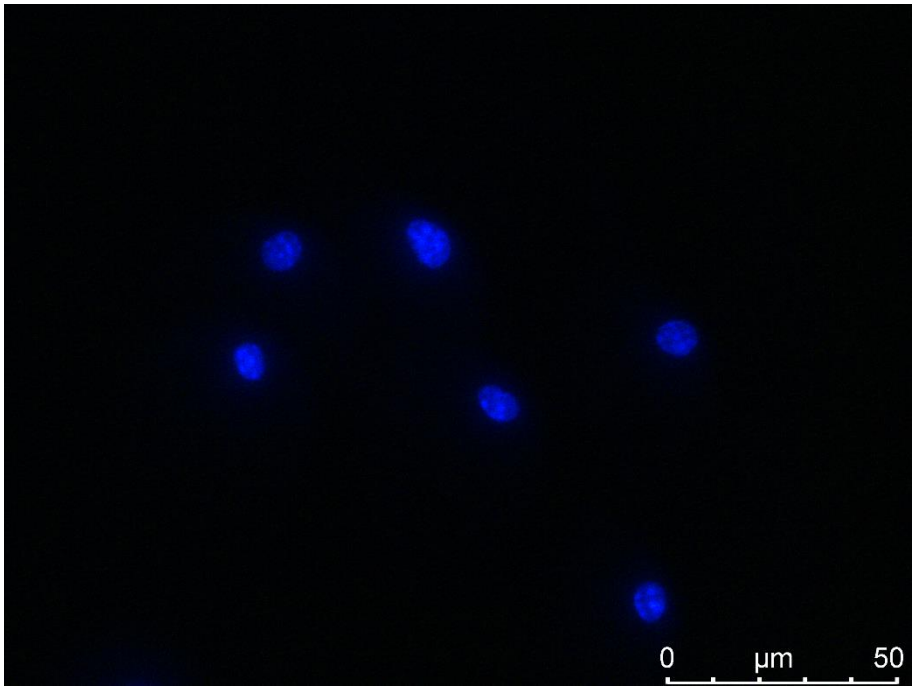
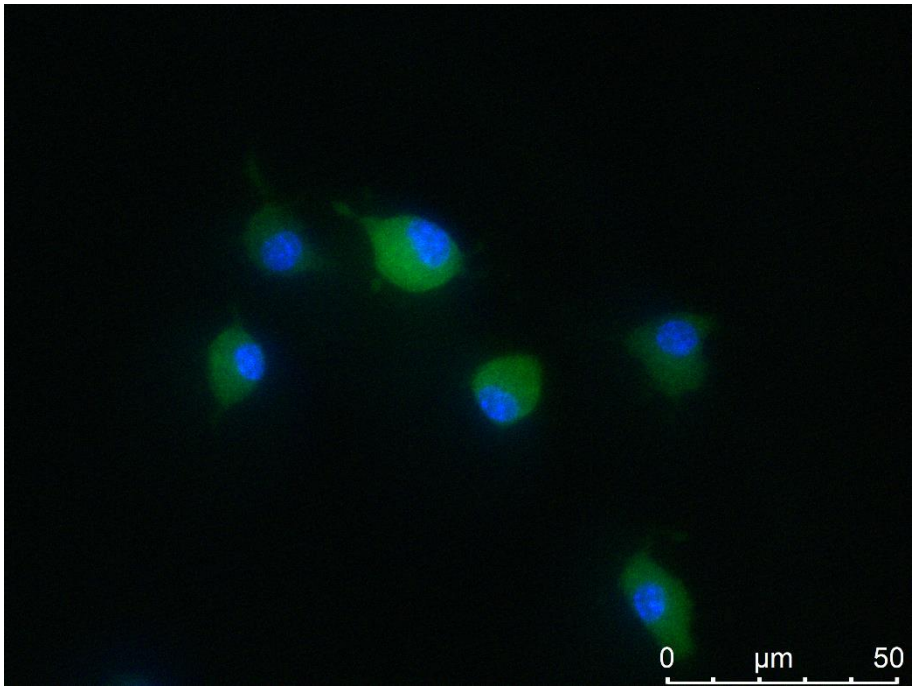
P65	
Histone H3	

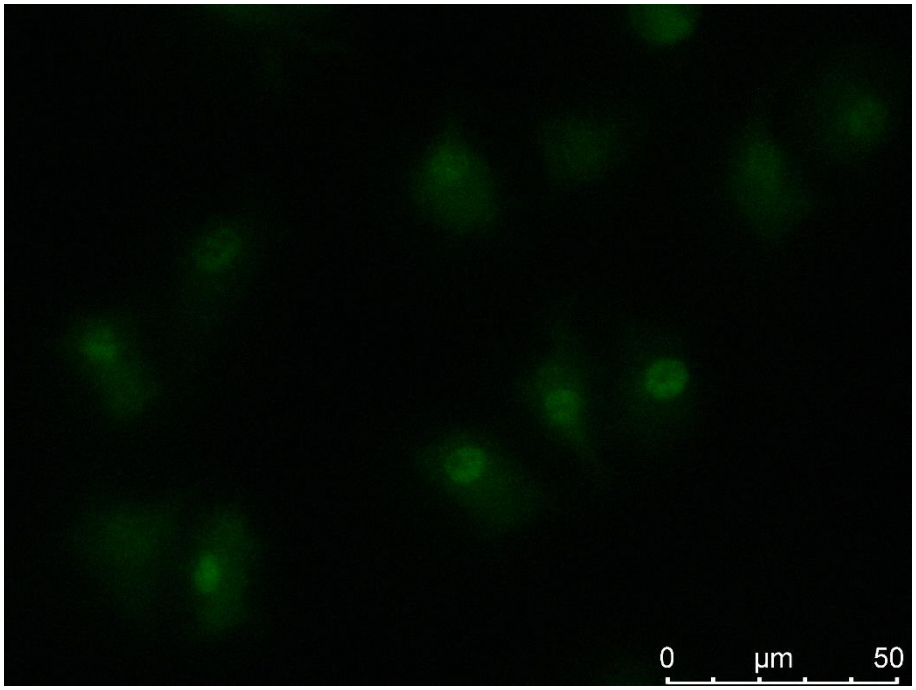
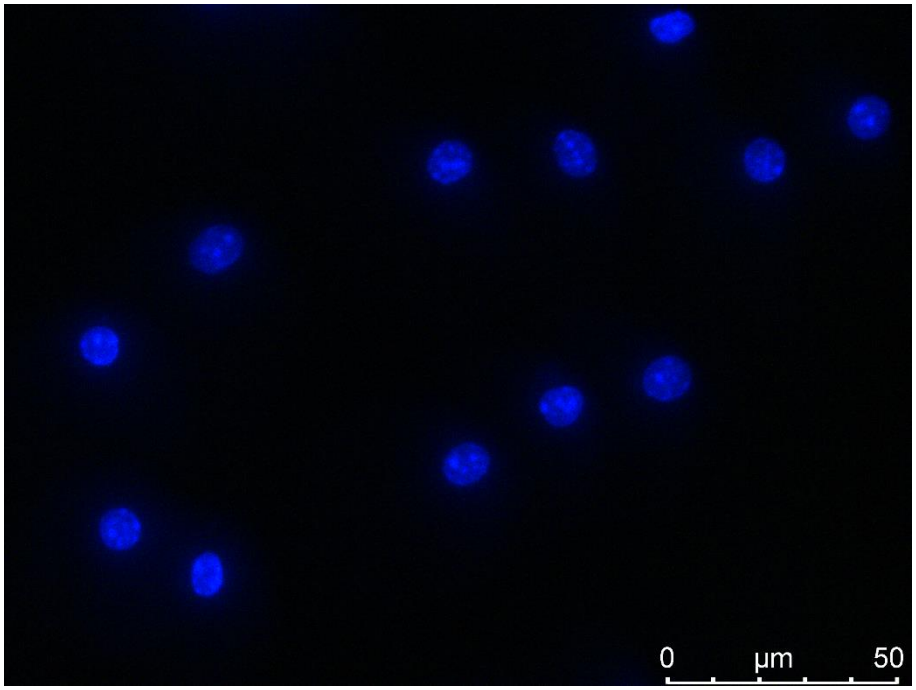
β-actin	

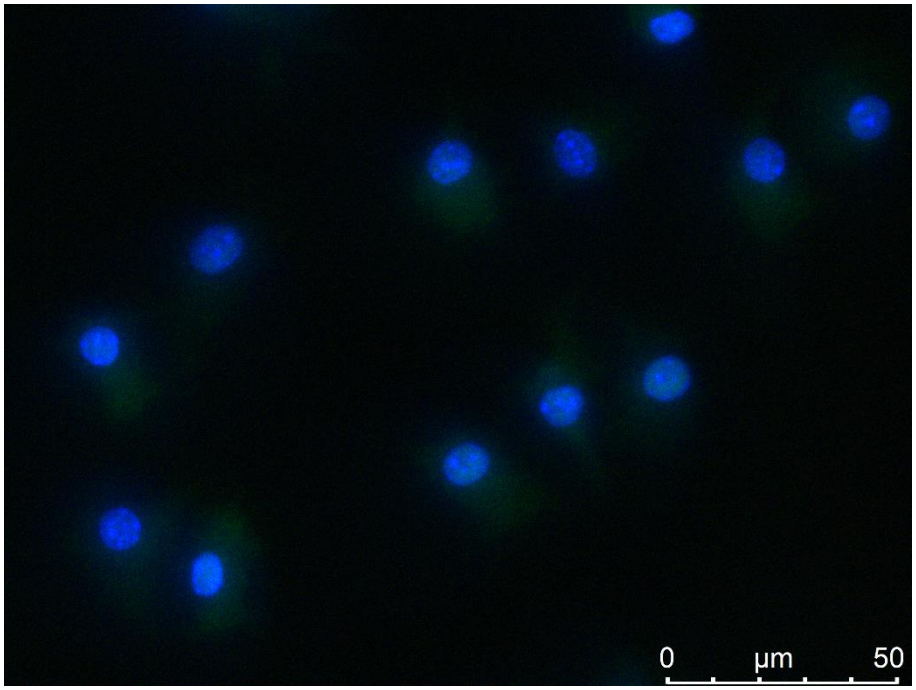
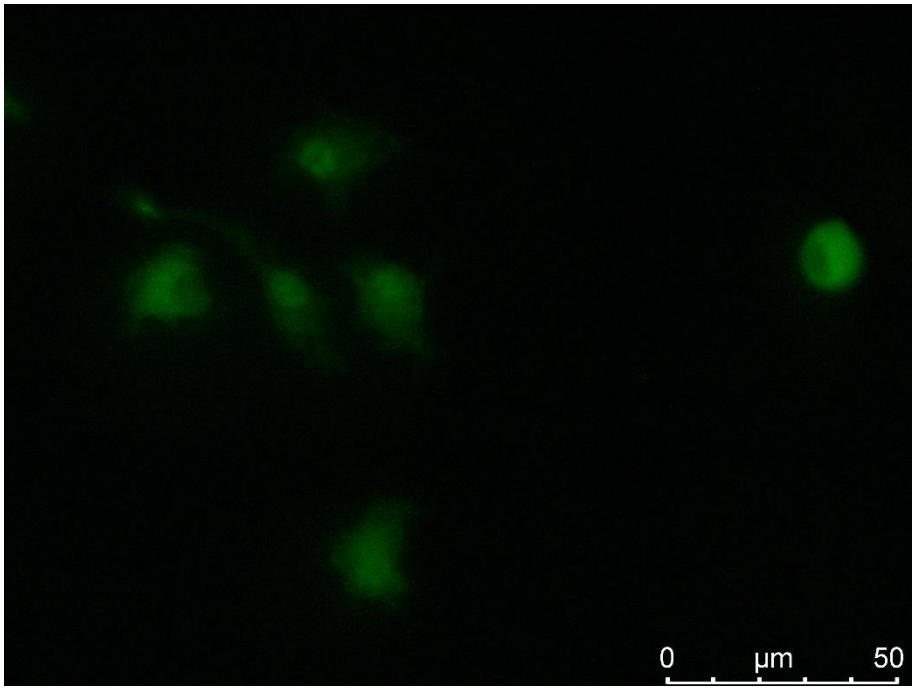
The original figures of immunofluorescent staining in figure 5.

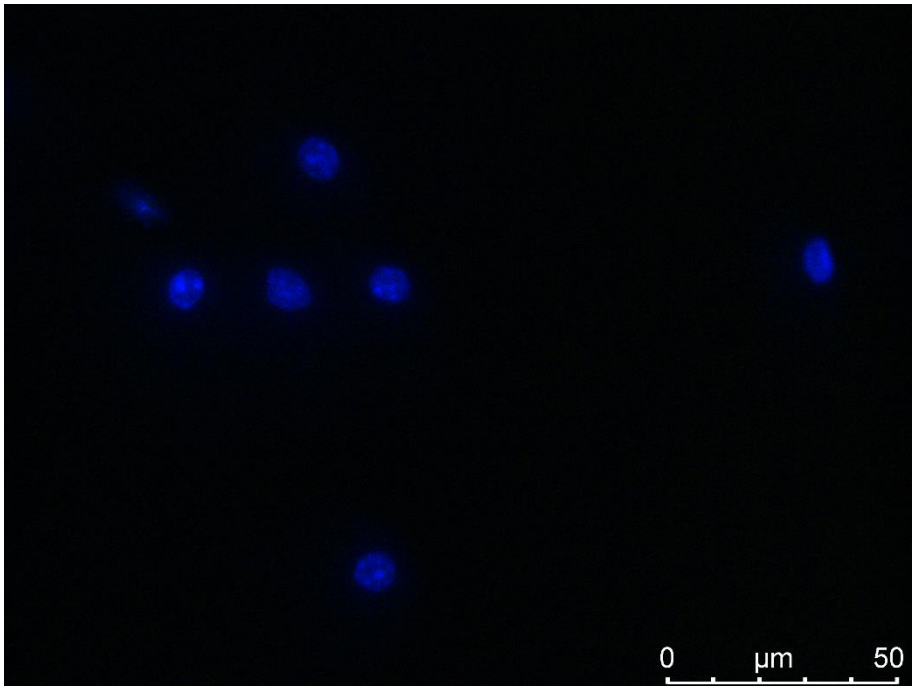
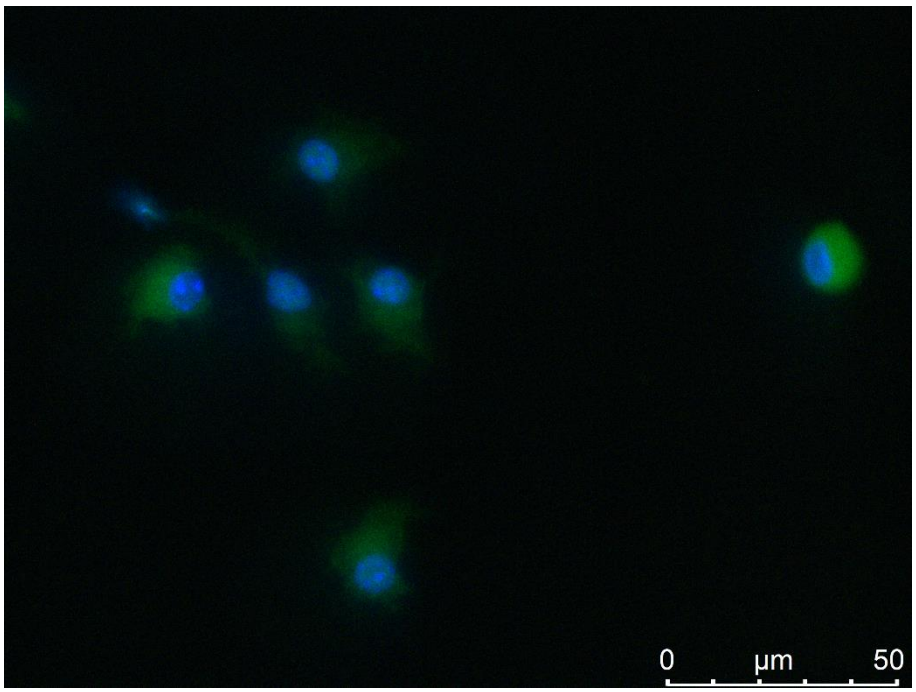
FIGURE 5E

Vehicle p65	

Vehicle Hoechst	
Vehicle Merge	

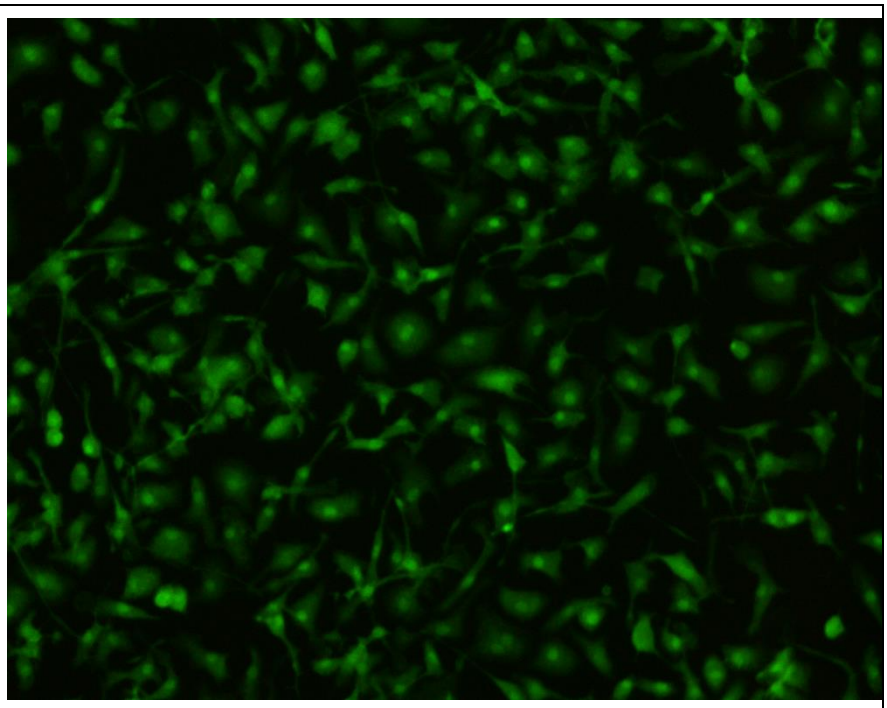
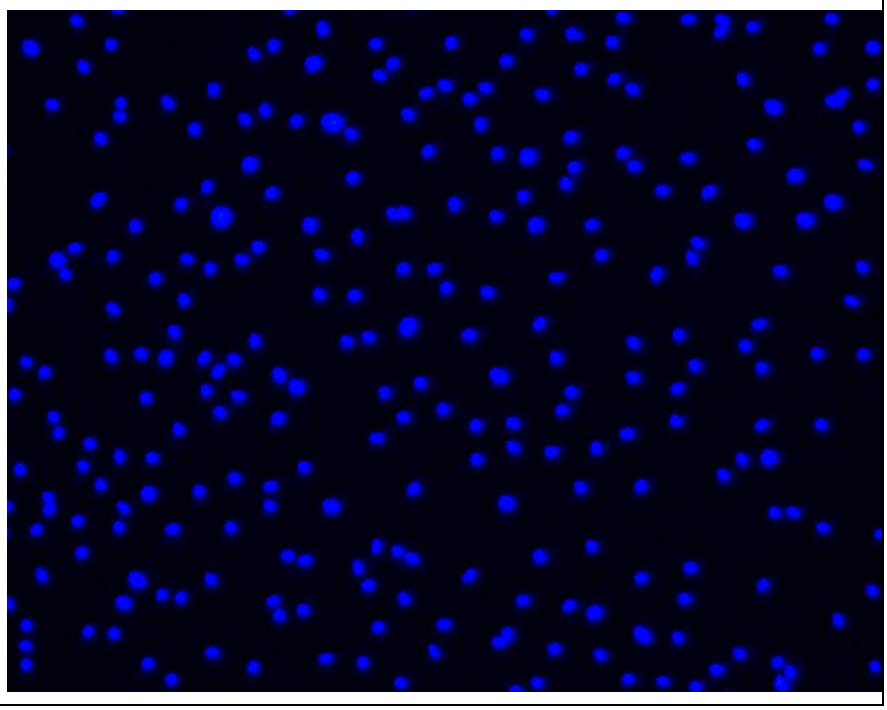
LPS P65	
LPS Hoechst	

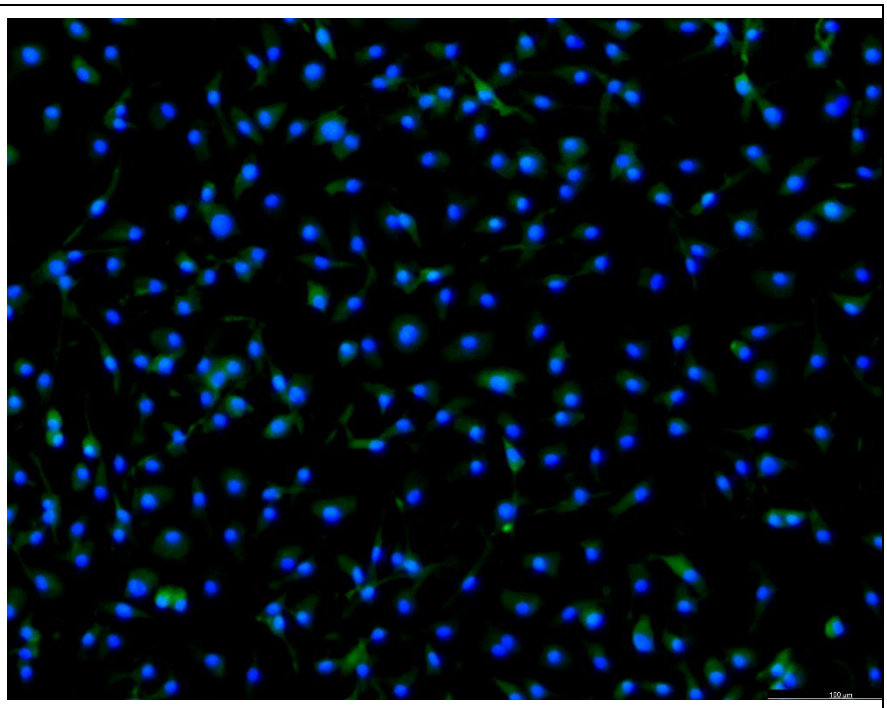
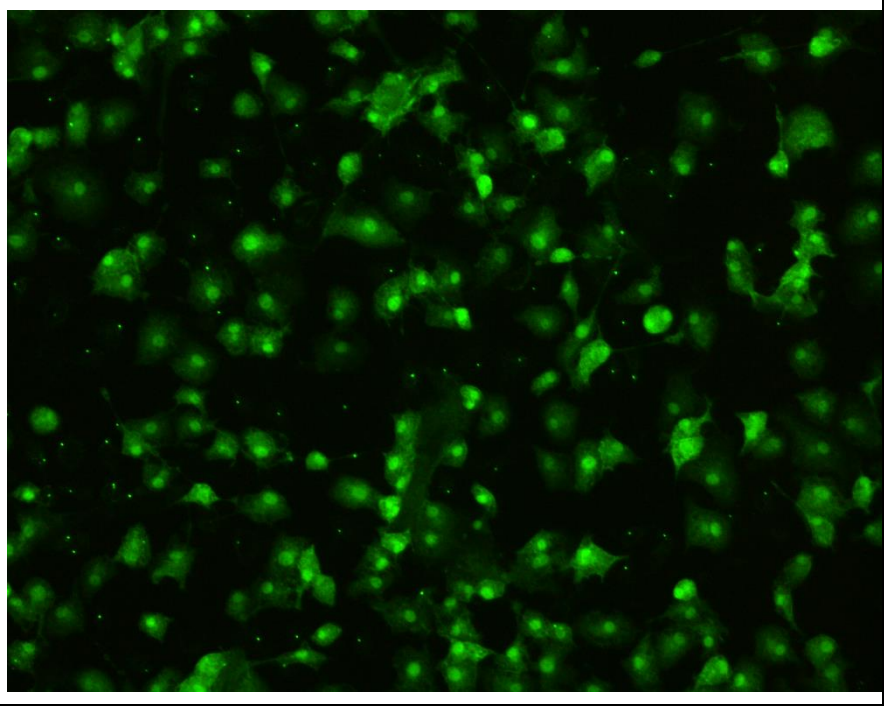
LPS Merge	
KM+LPS p65	

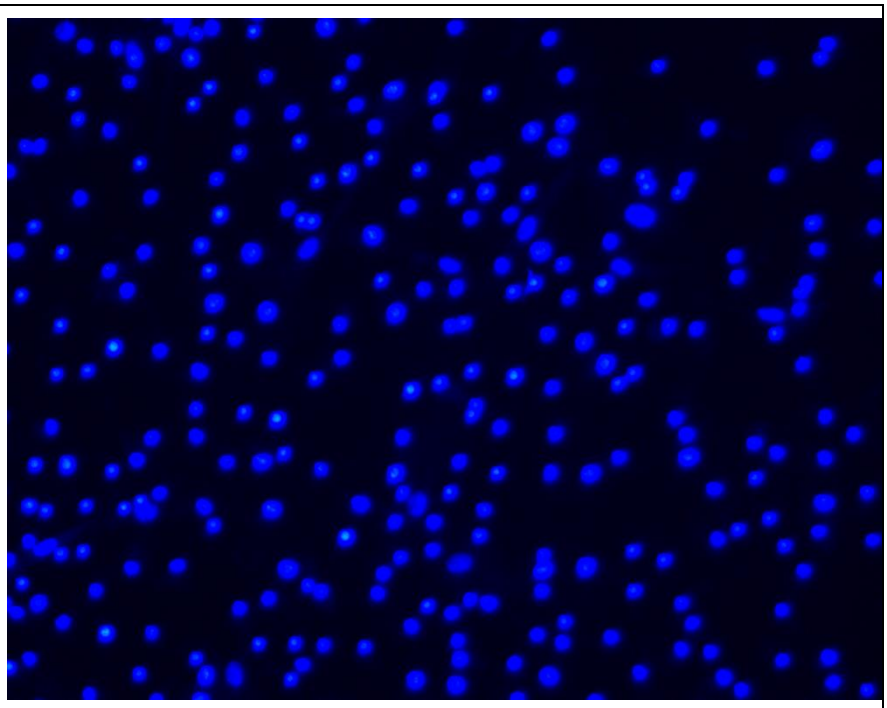
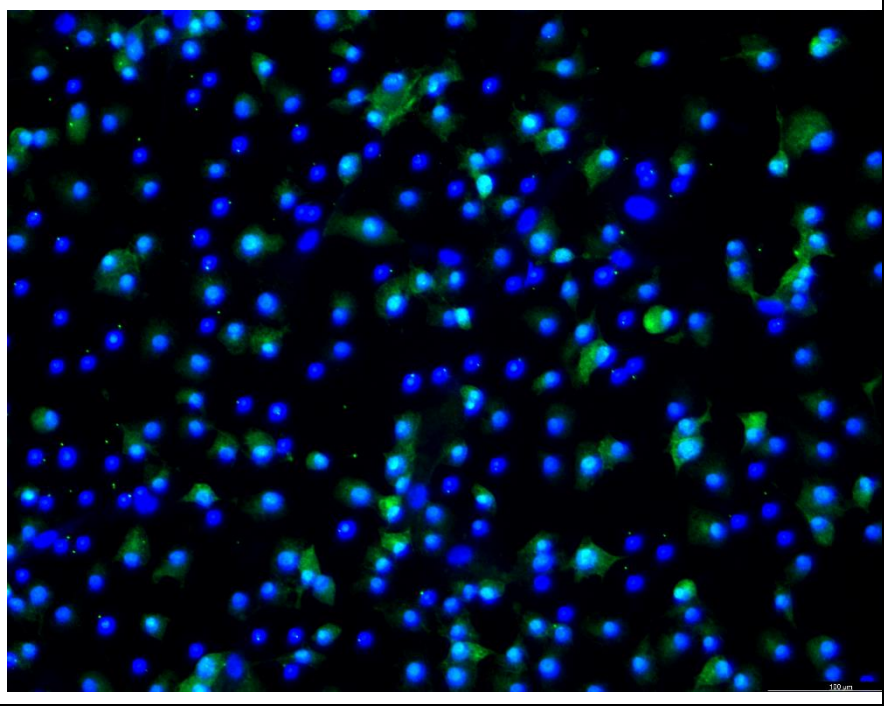
KM+LPS Hoechst	
KM+LPS Merge	

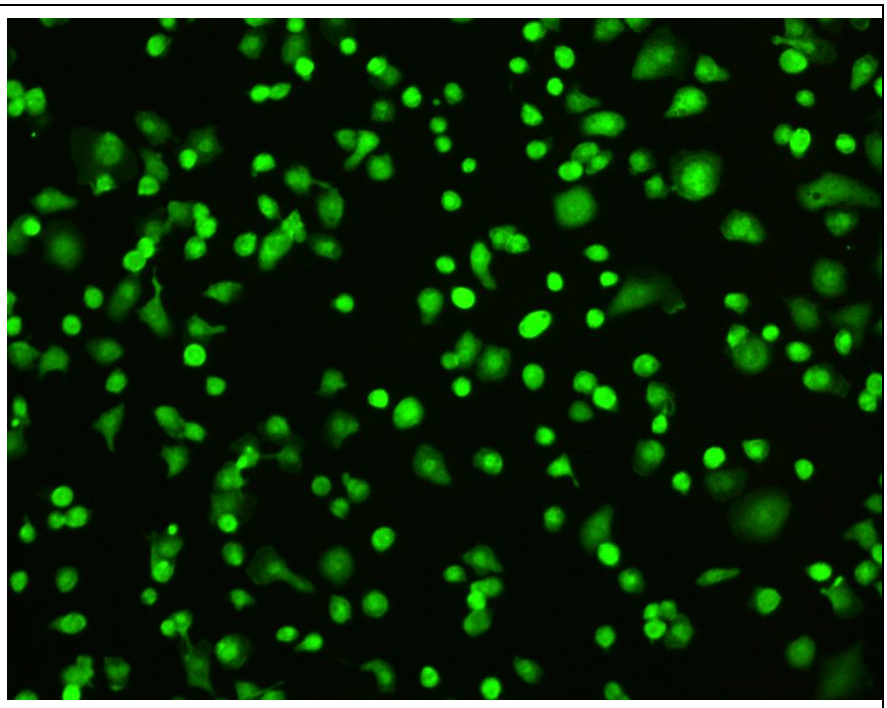
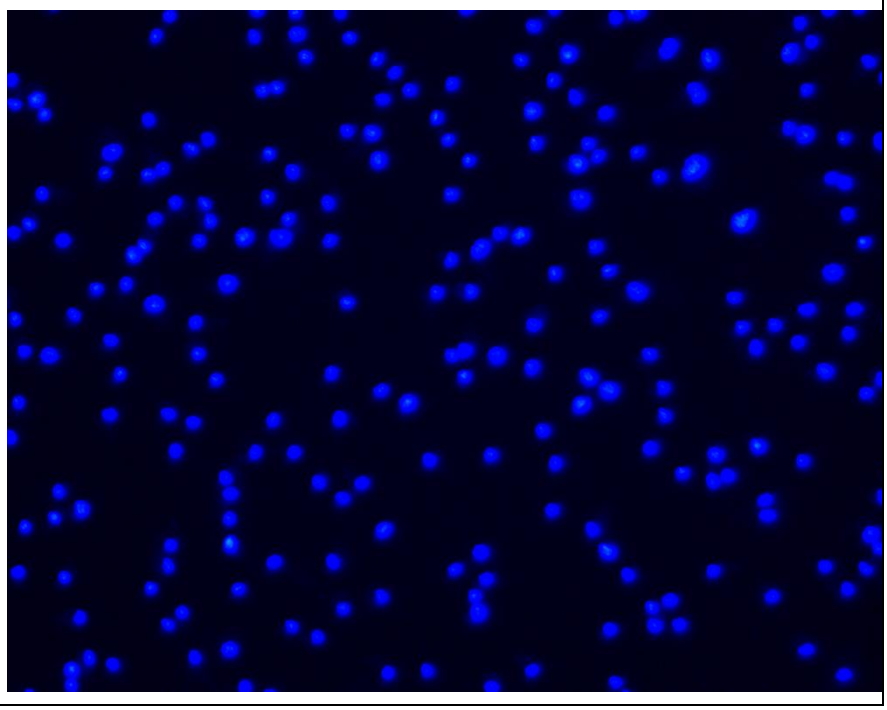
The original figures of immunofluorescent staining in figure 6.

FIGURE 6A

Vehicle ASC	
Vehicle Hoechst	

Vehicle Merge	
LPS+ATP ASC	

LPS+ATP Hoechst	
LPS+ATP Merge	

KM+LPS+ATP ASC	
KM+LPS+ATP Hoechst	

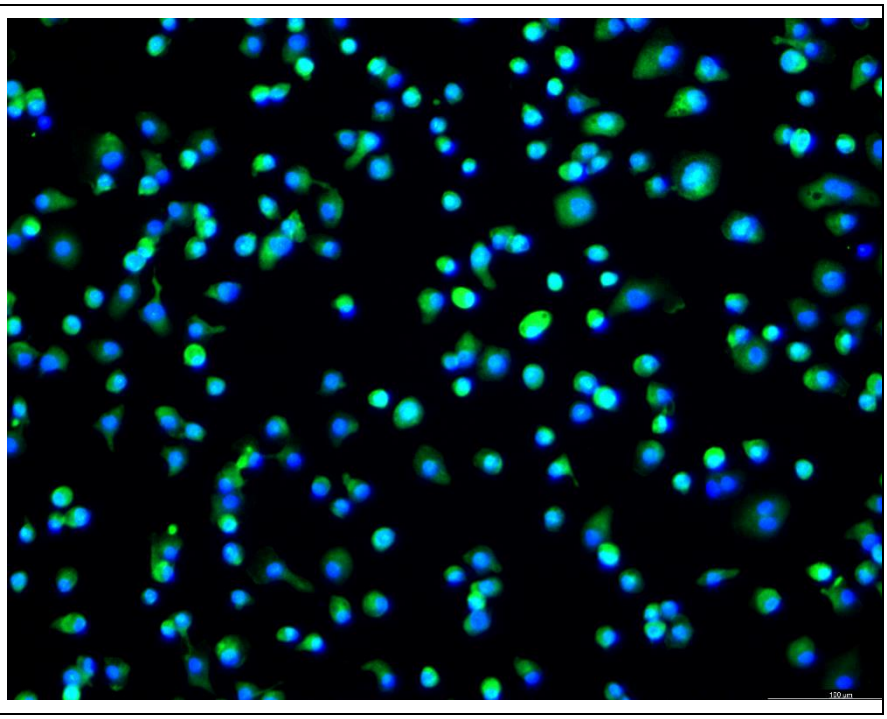
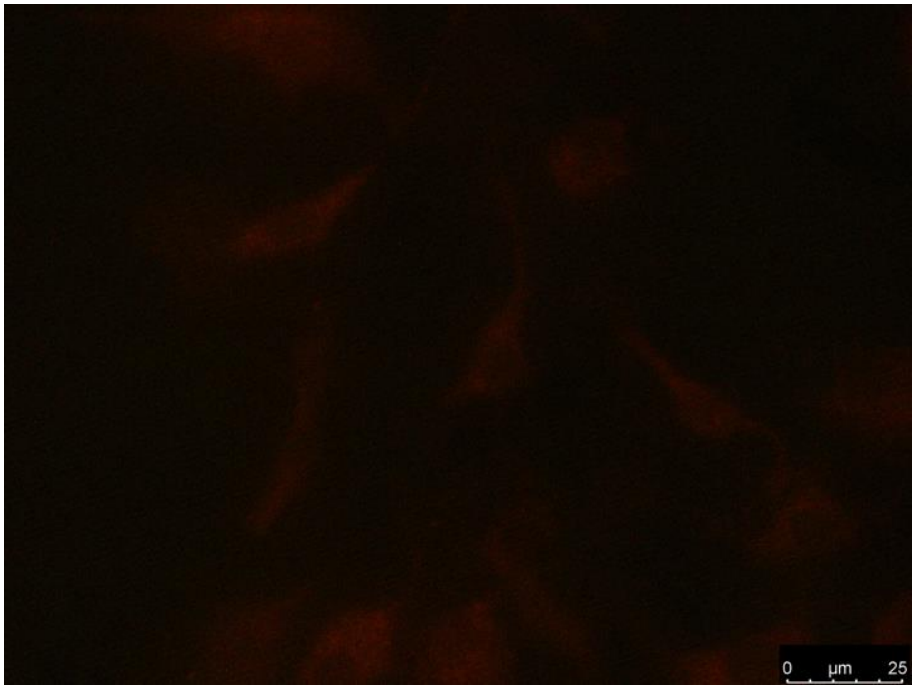
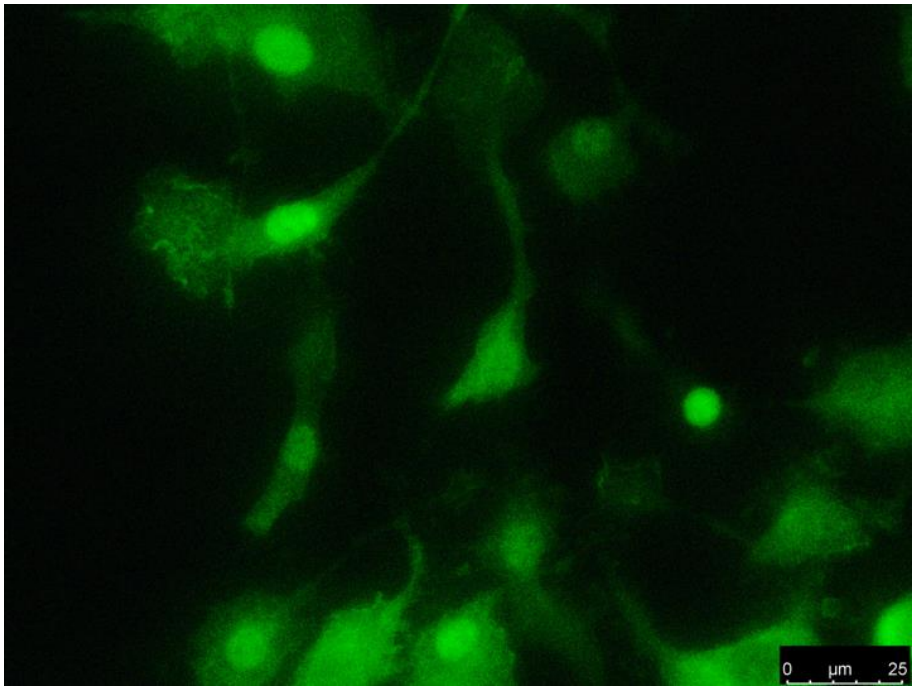
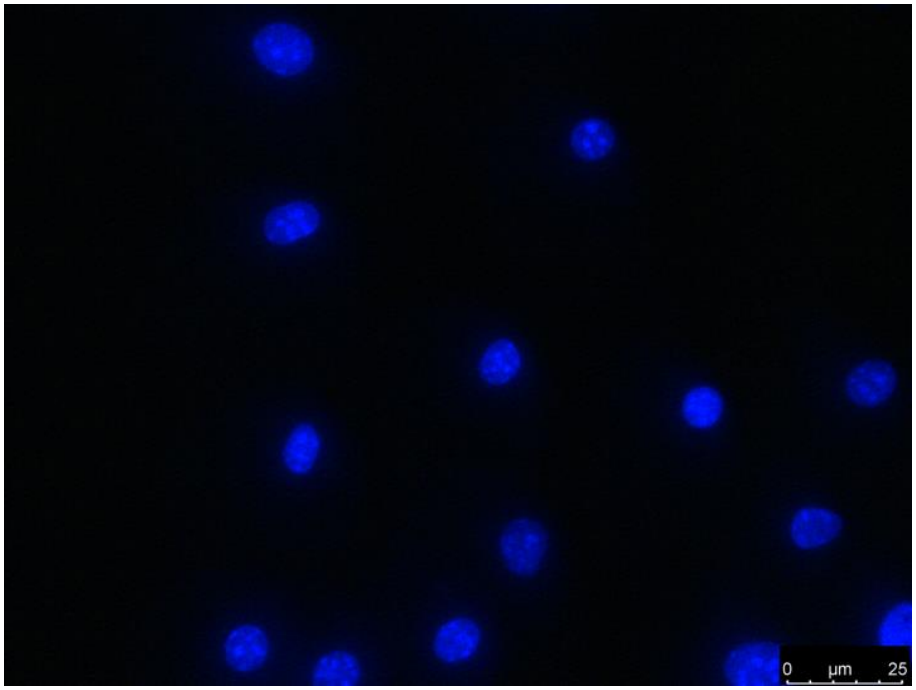
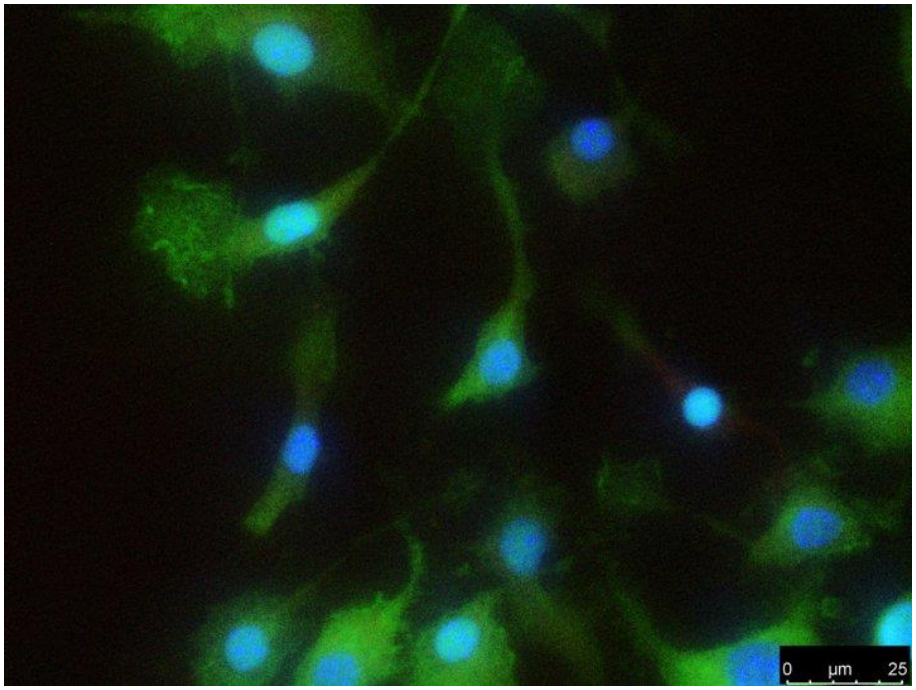
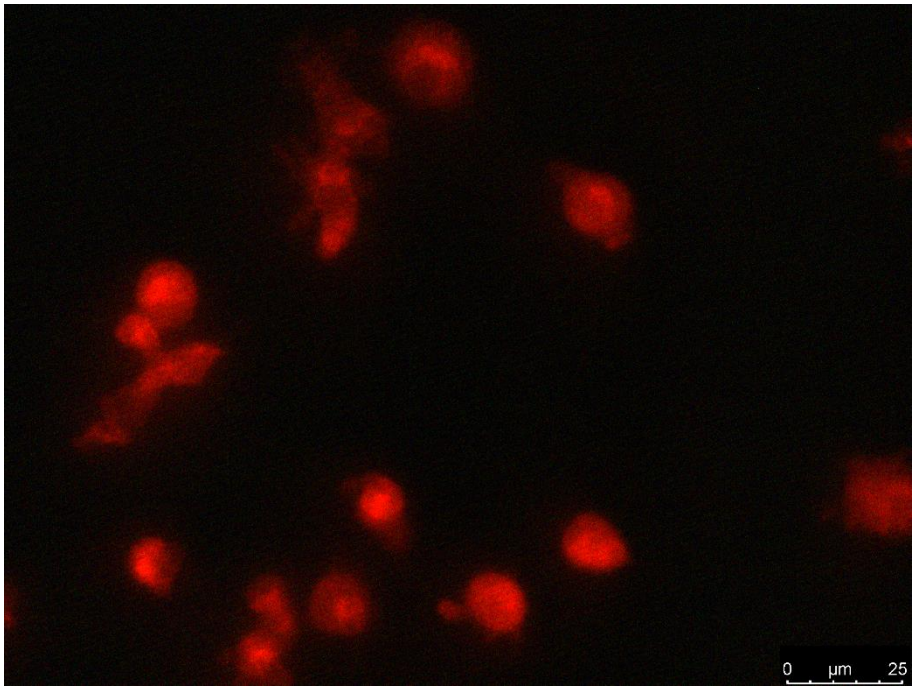
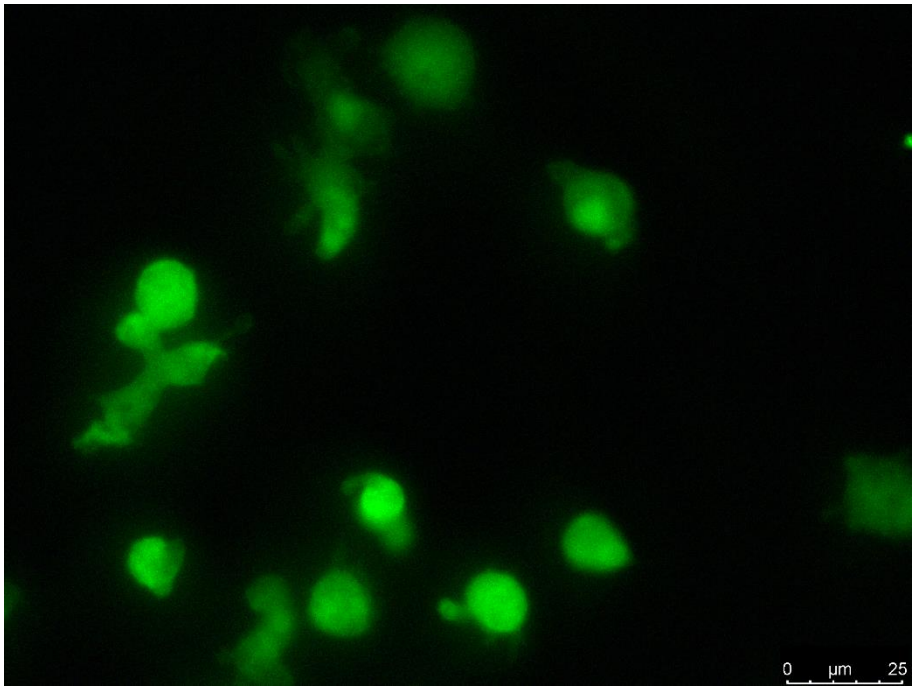
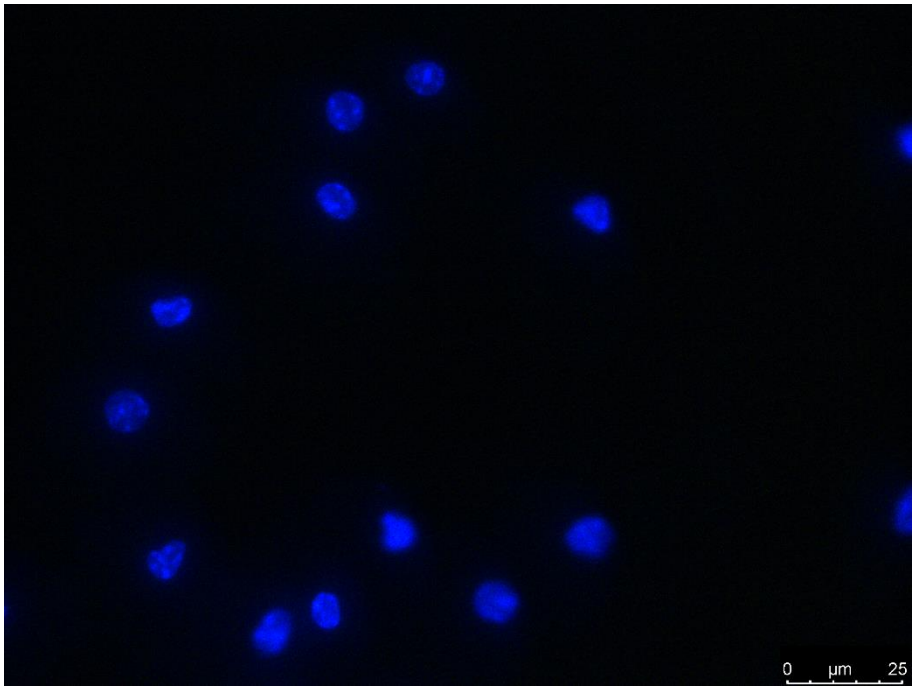
KM+LPS+ATP Merge	

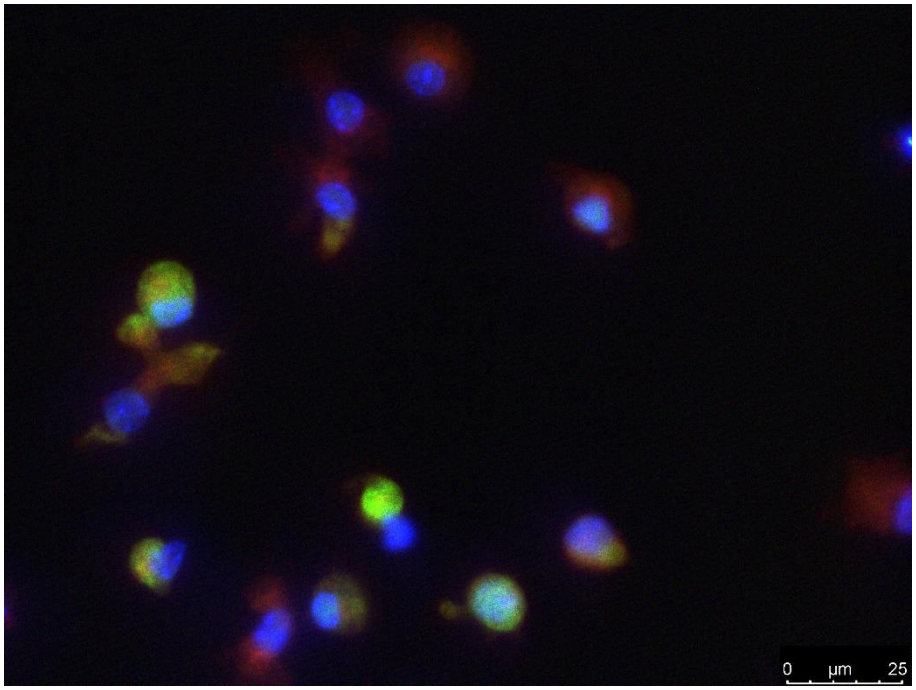
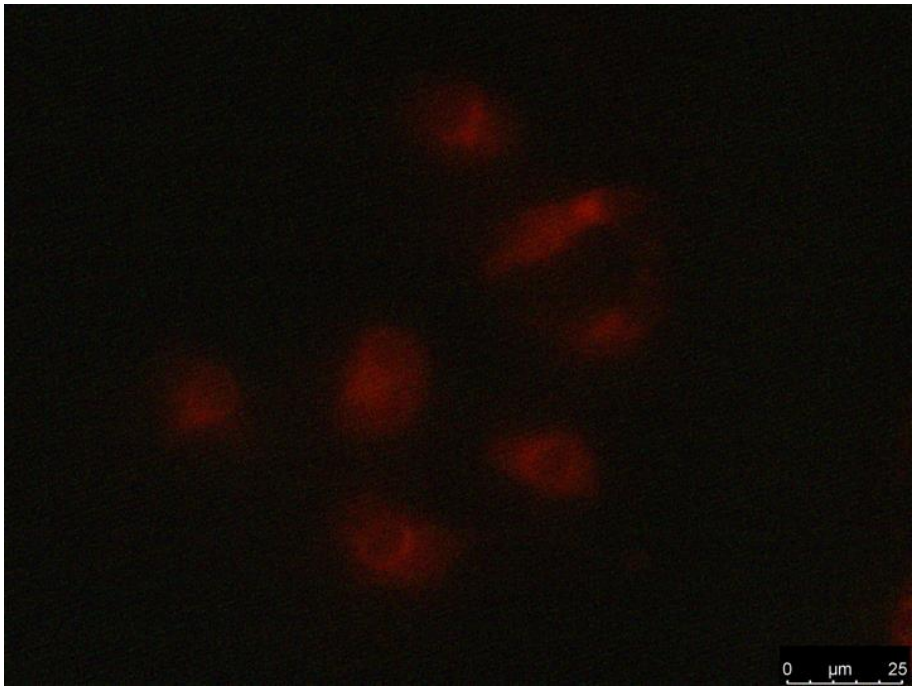
FIGURE 6D

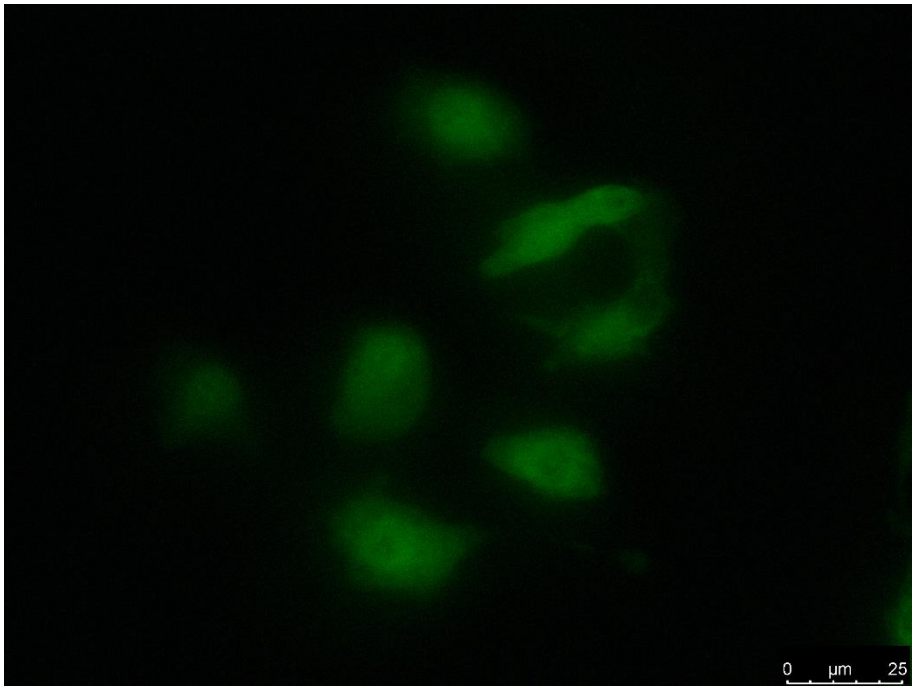
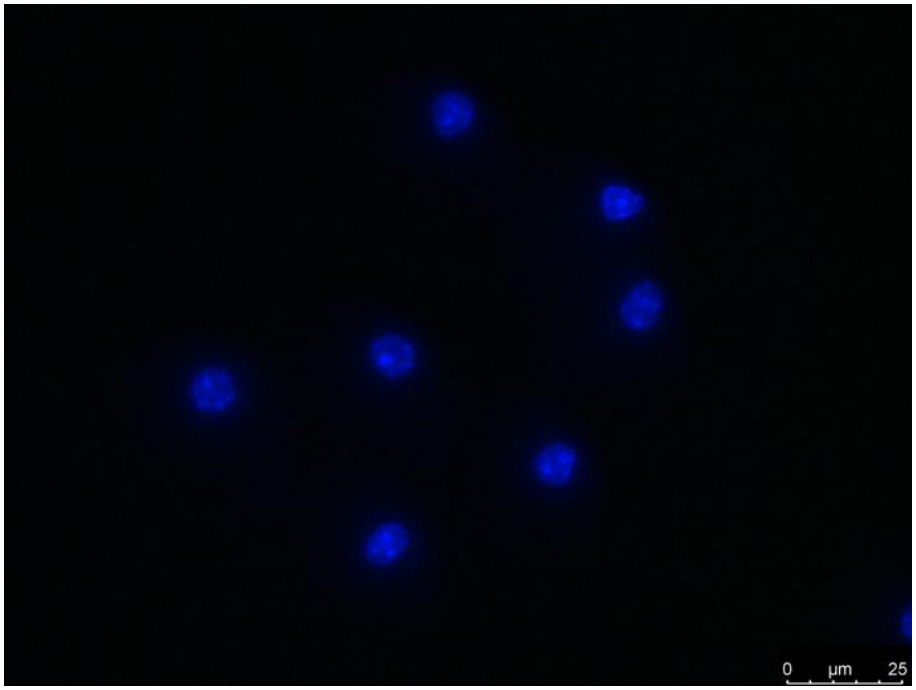
Vehicle NLRP3	

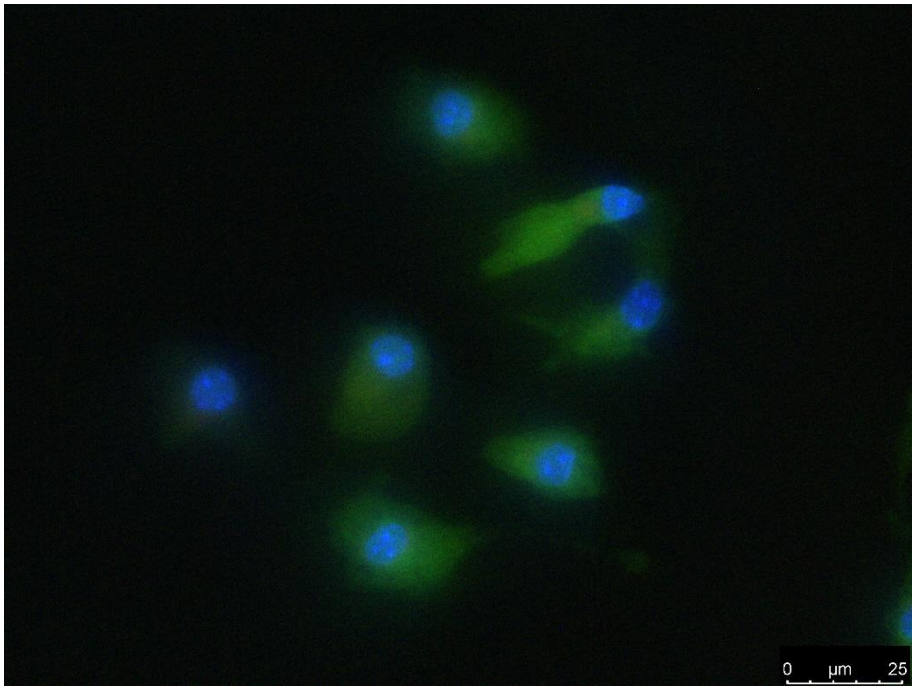
Vehicle ASC	
Vehicle Hoechst	

Vehicle Merge	
LPS+ATP NLRP3	

LPS+ATP ASC	
LPS+ATP Hoechst	

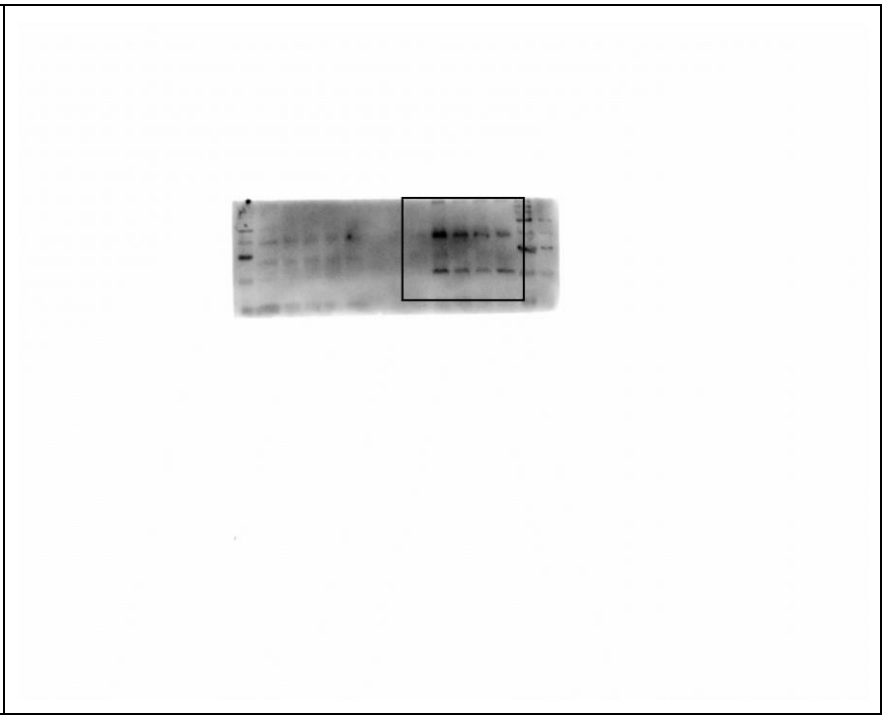
LPS+ATP Merge	
KM+ LPS+ATP NLRP3	

KM+ LPS+ATP ASC	
KM+ LPS+ATP Hoechst	

KM+ LPS+ATP Merge	

The original western blots of figure 6.

FIGURE 6C

ASC oligomerization	

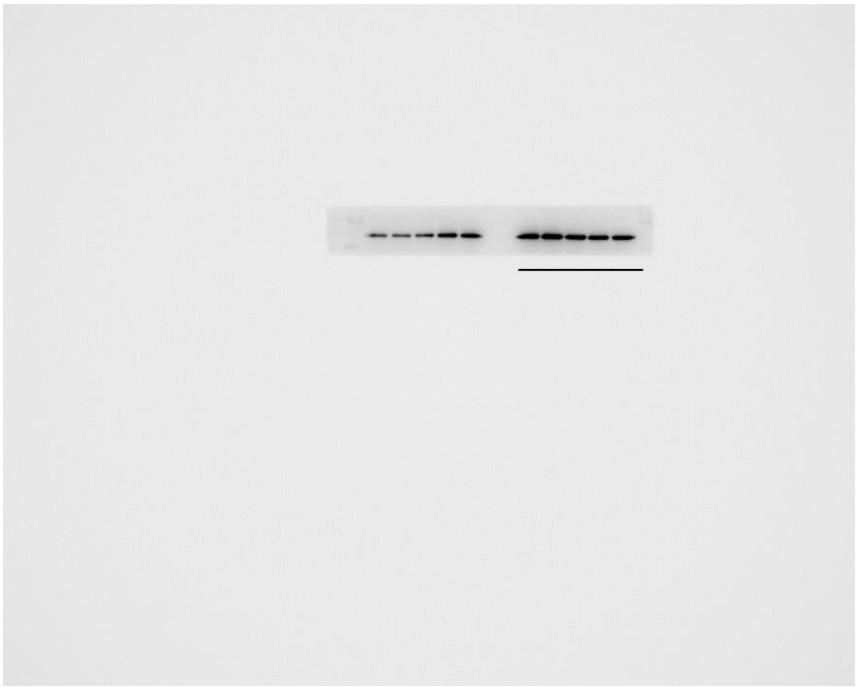
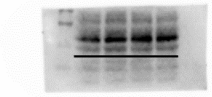
ASC	
β-actin	

FIGURE 6E

IP-NLRP3	
IP-pro-caspase-1	

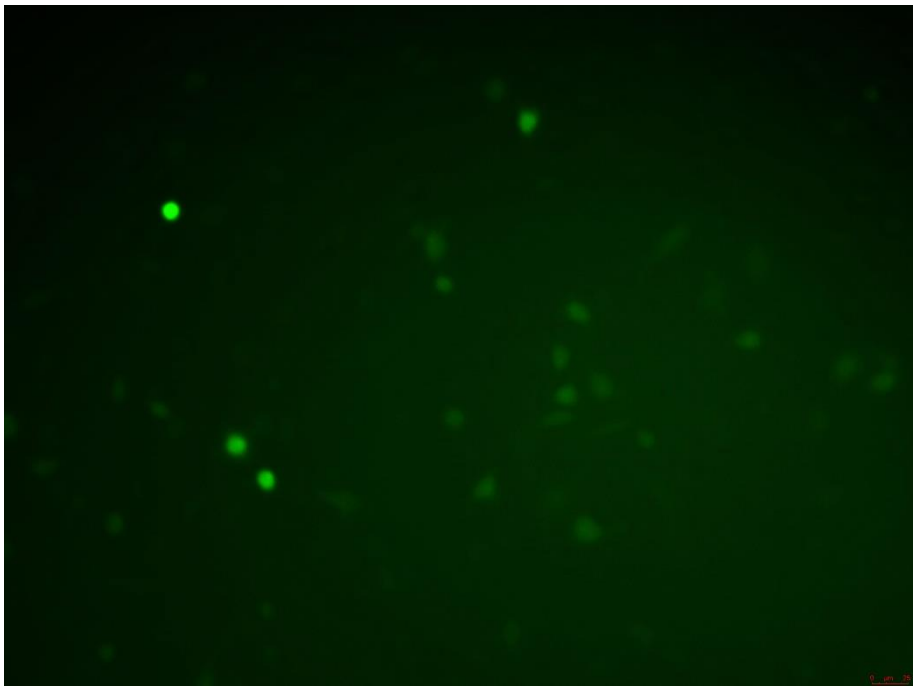
IP-ASC	
Input-NLRP3	

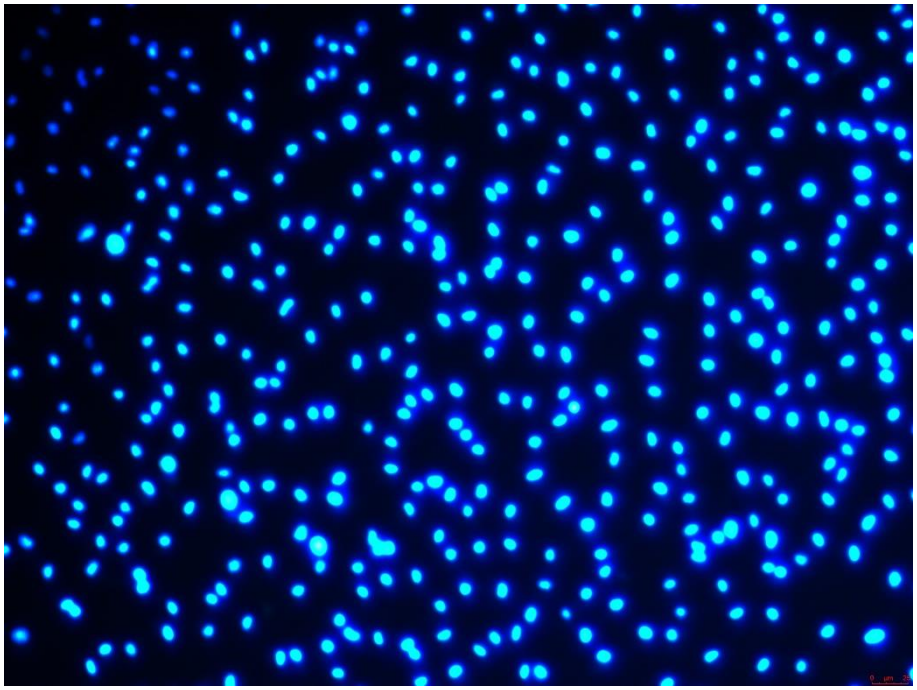
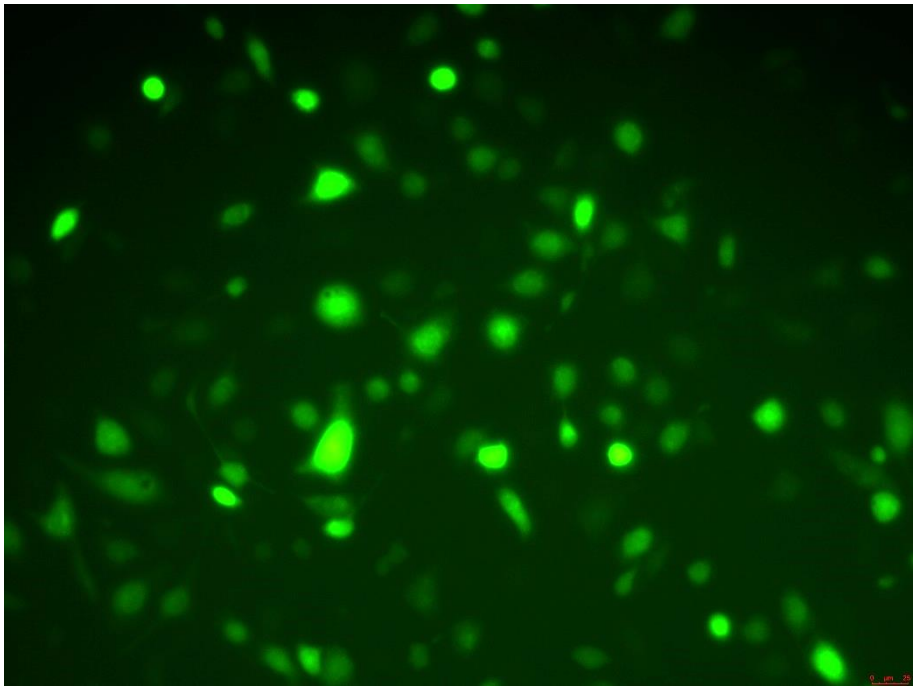
Input-pro-caspase-1	
Input-ASC	

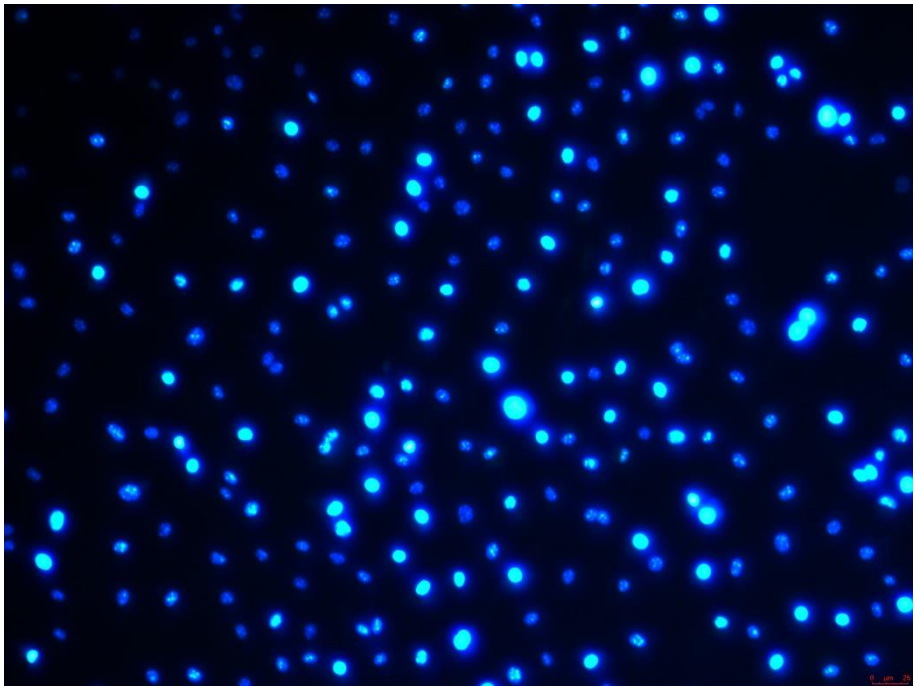
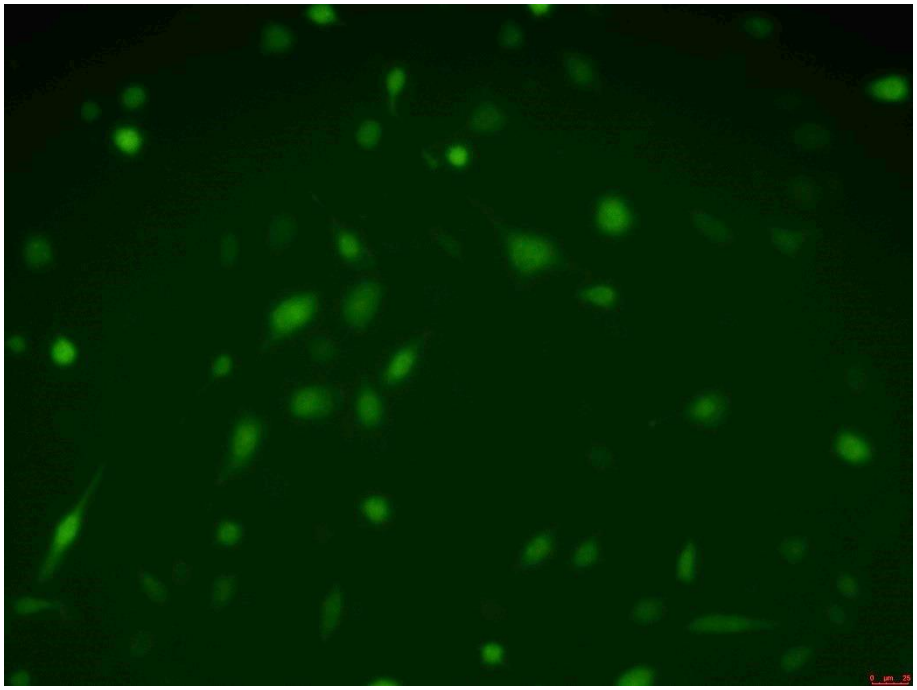
Input-β-actin	

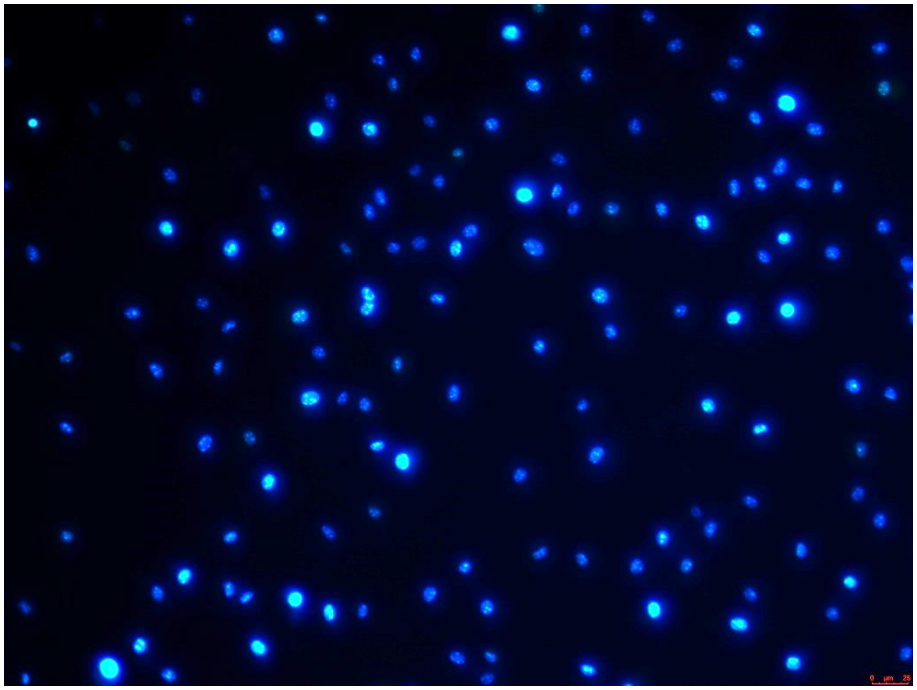
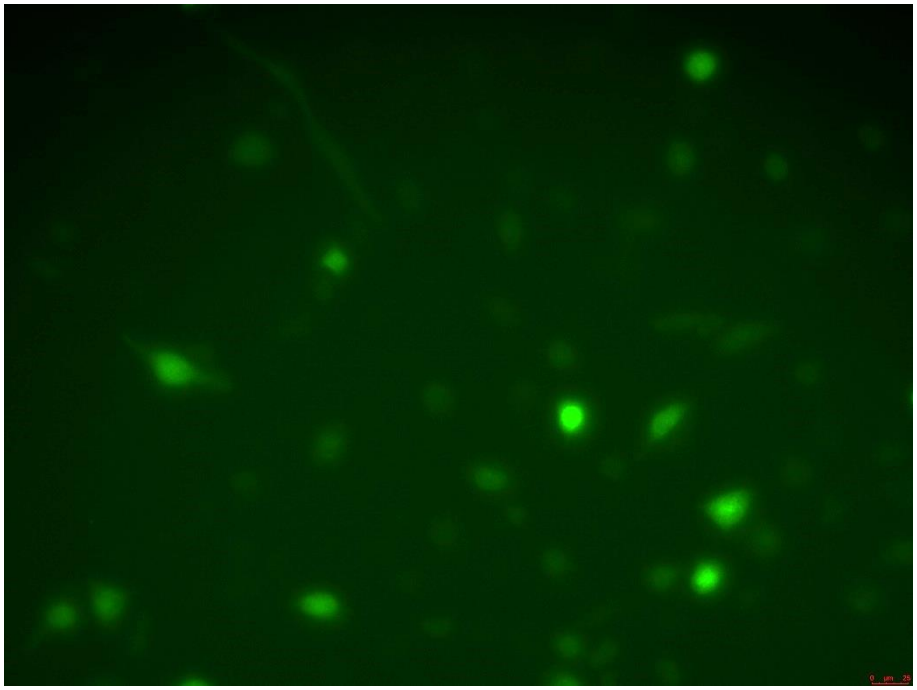
The original figures of DCFH-DA staining in figure 7.

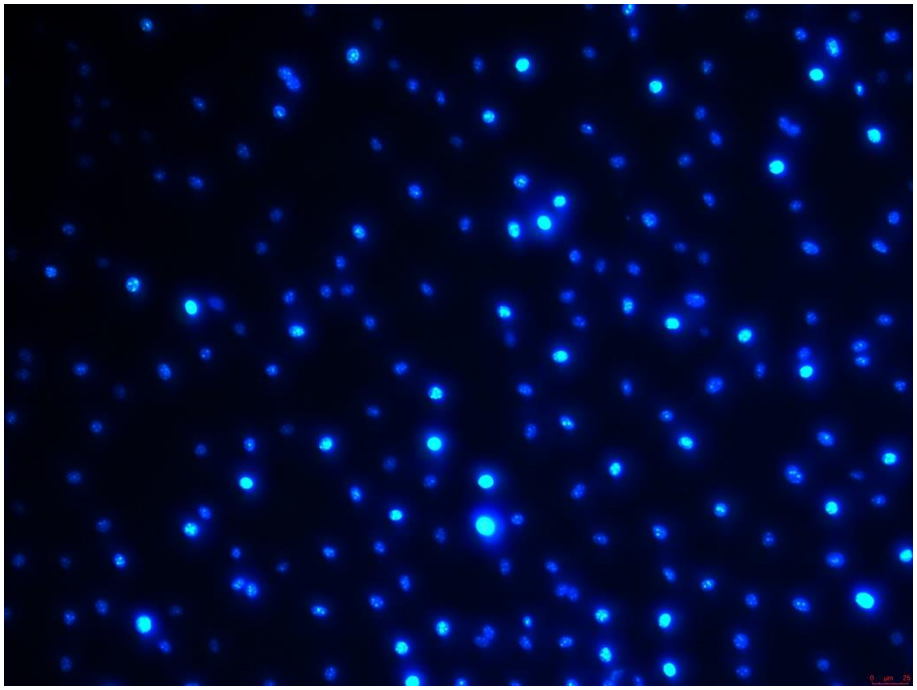
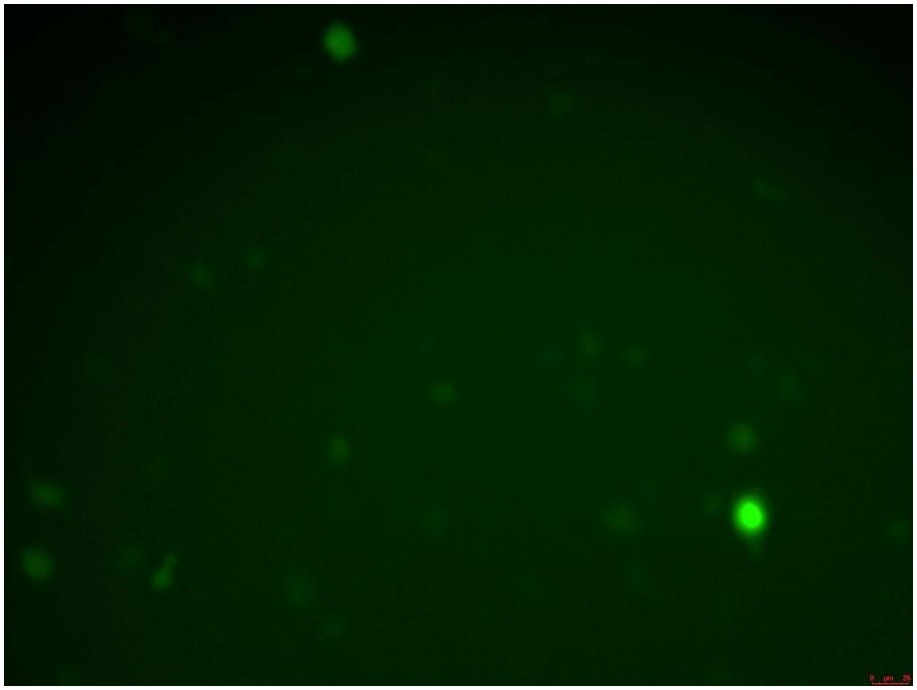
FIGURE 7A

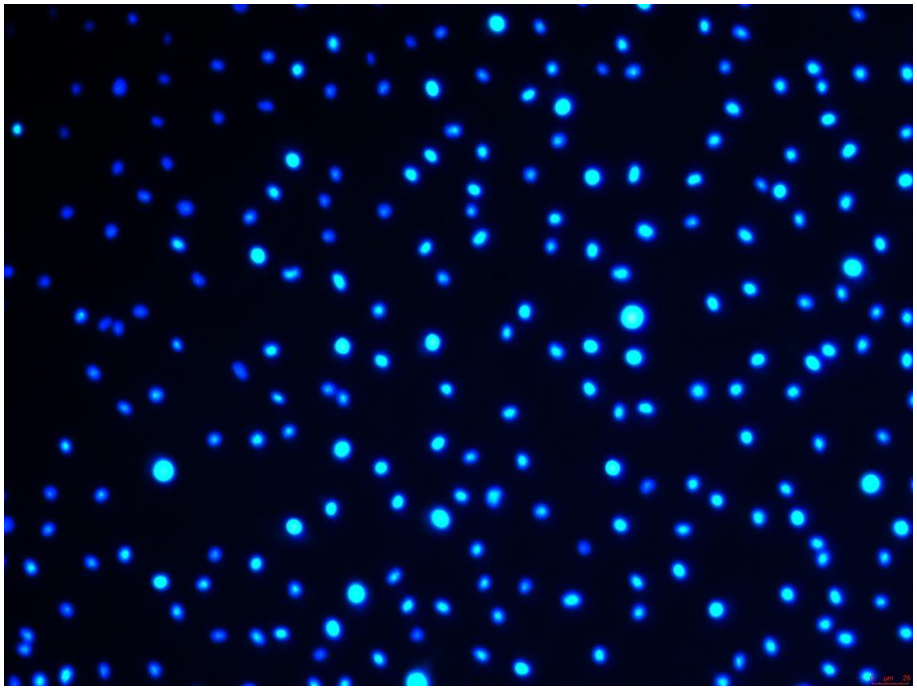
Vehicle-DCFH-DA	

Vehicel-Hoechst	
LPS+ATP-DCFH-DA	

LPS+ATP -Hoechst	
KM 50 μM-DCFH-DA	

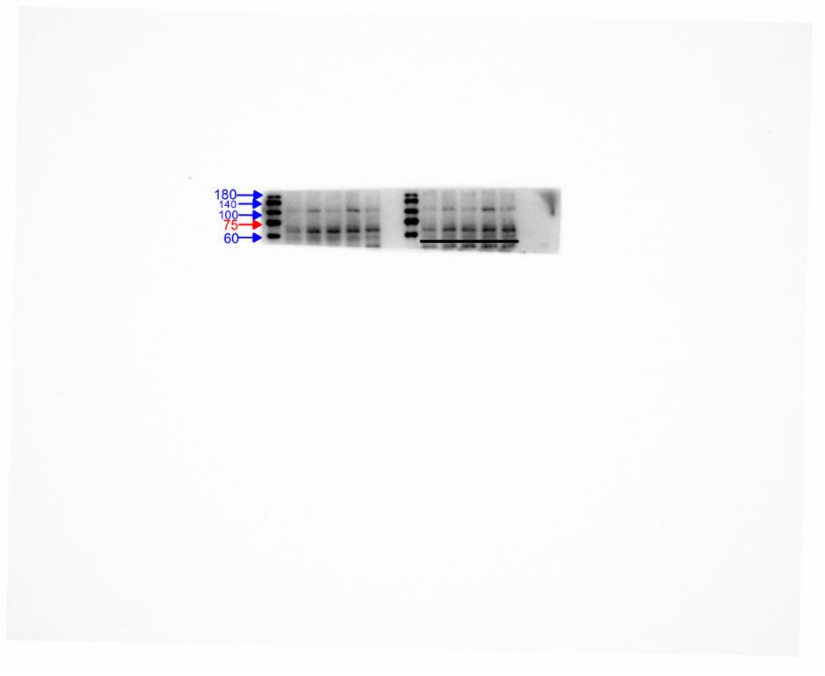
KM 50 µM-Hoechst	
KM 100 µM-DCFH-DA	

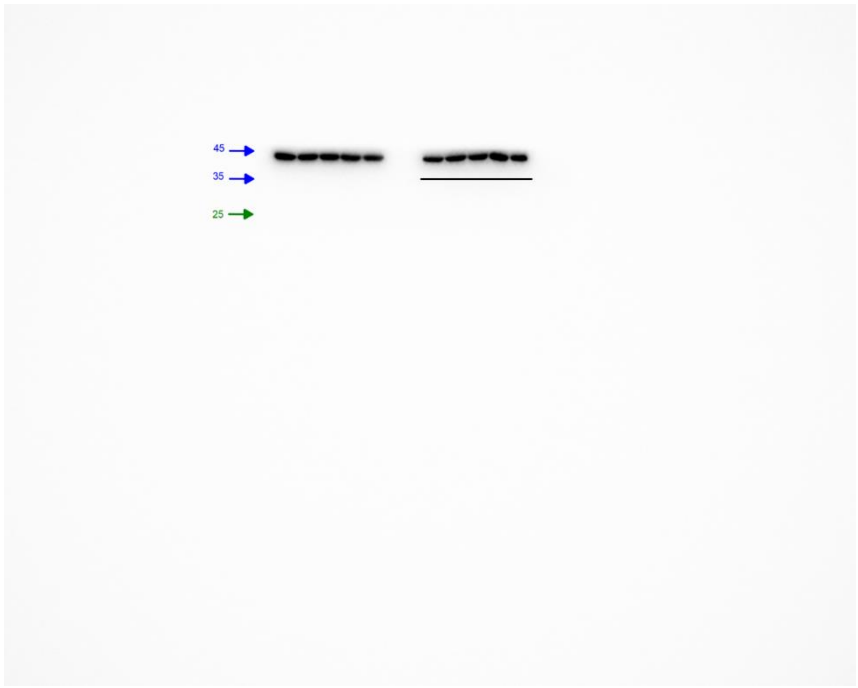
KM 100 µM-Hoechst	
KM 200 µM-DCFH-DA	

KM 200 µM-Hoechst	

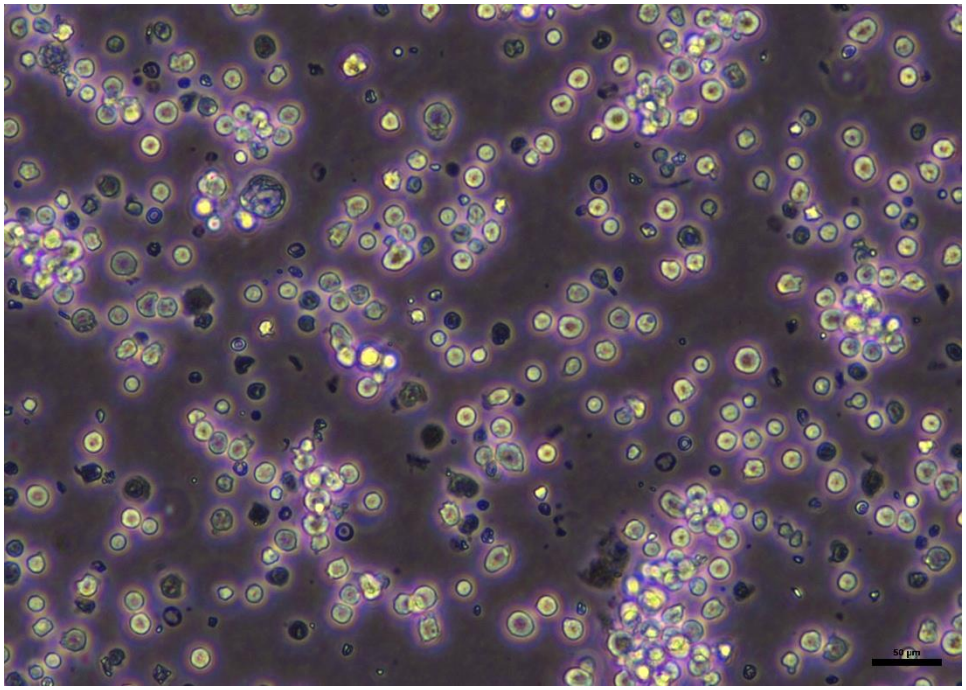
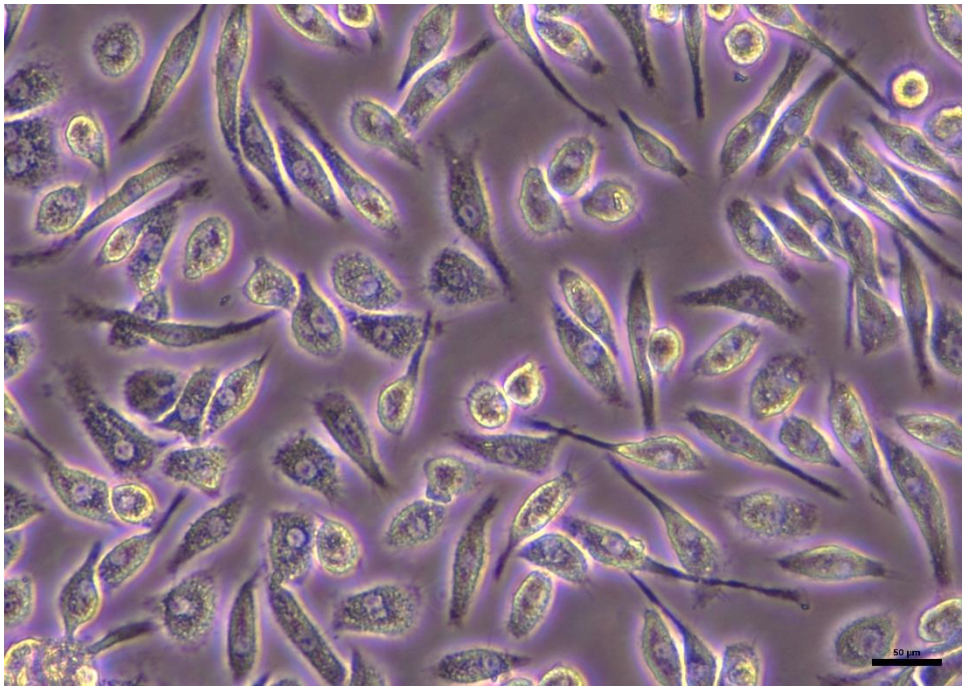
The original western blots of figure 7.

FIGURE 7E

p-AMPK	

AMPK	
β-actin	

The original figures in supplementary figure 1.

D1	
D7	

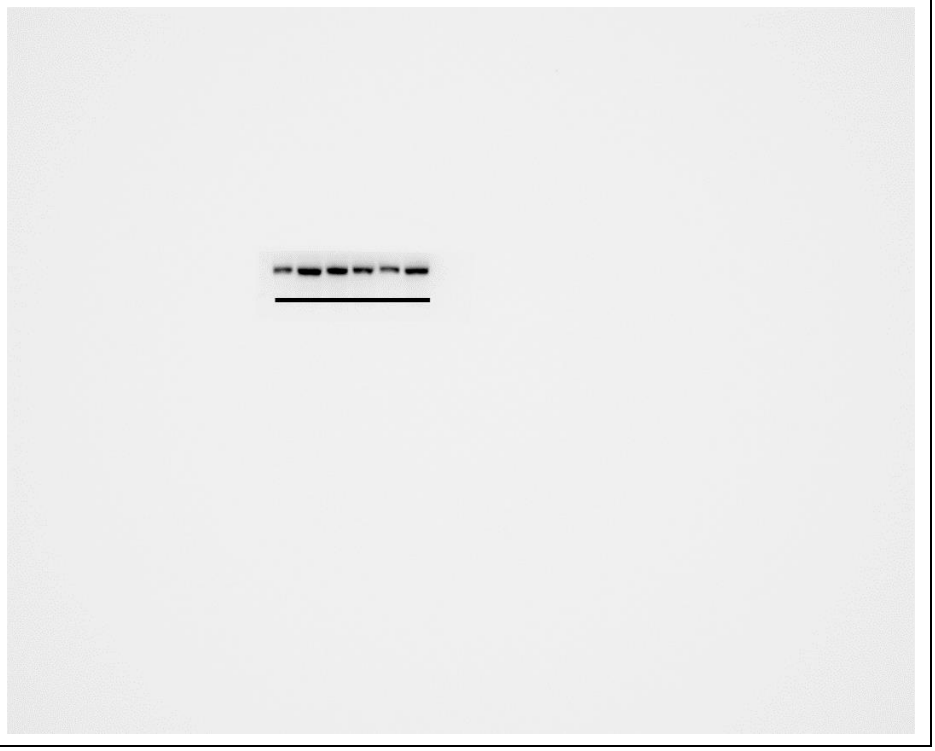
The original western blots of figure S2.

FIGURE S2A　BMDM

NLRP3	
Pro-IL-1β	

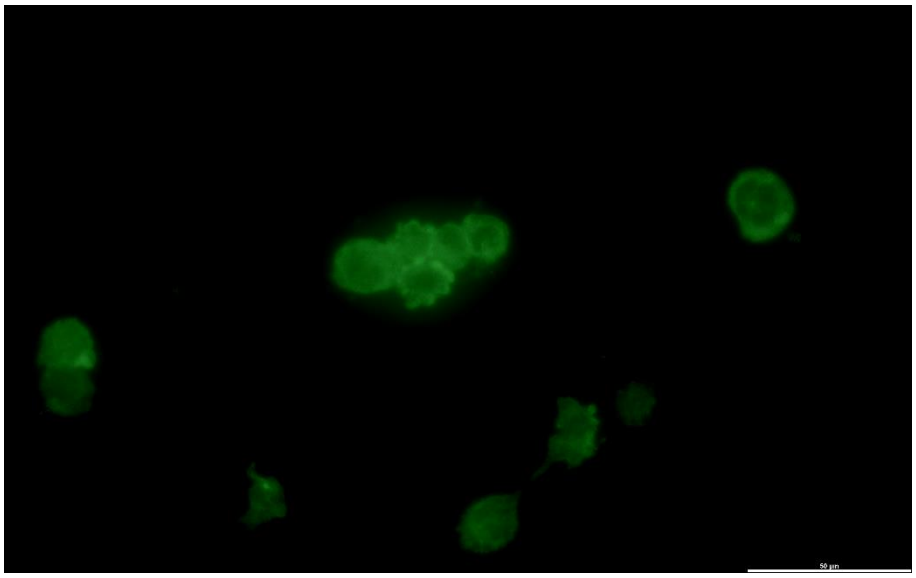
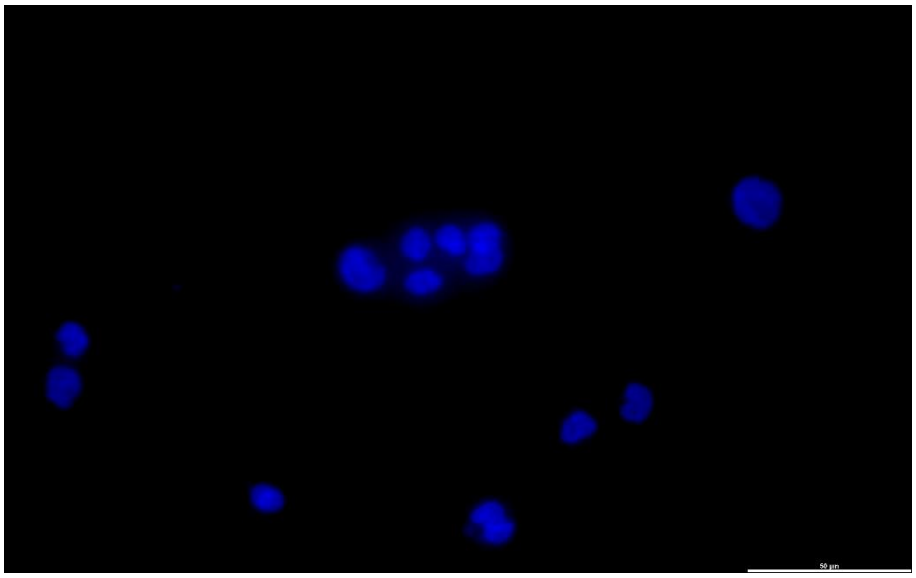
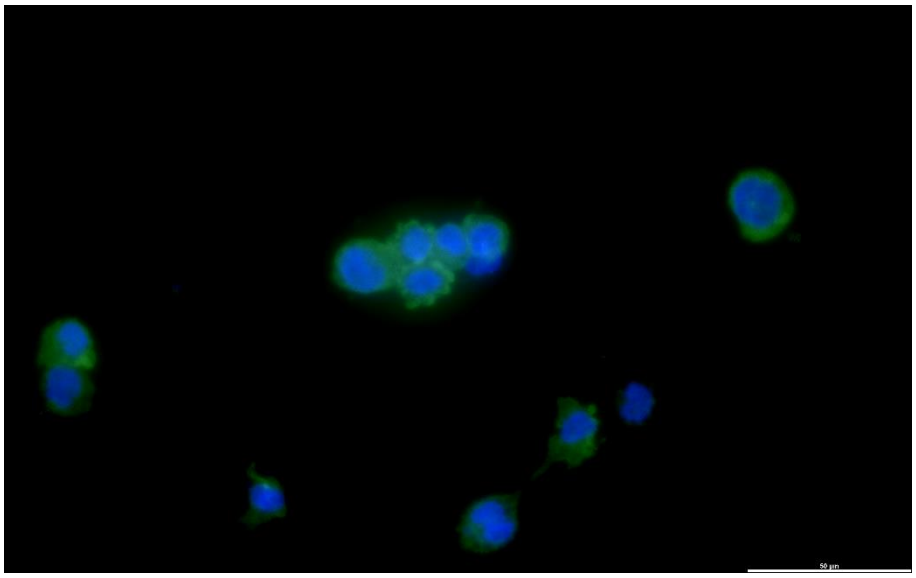
β-actin	

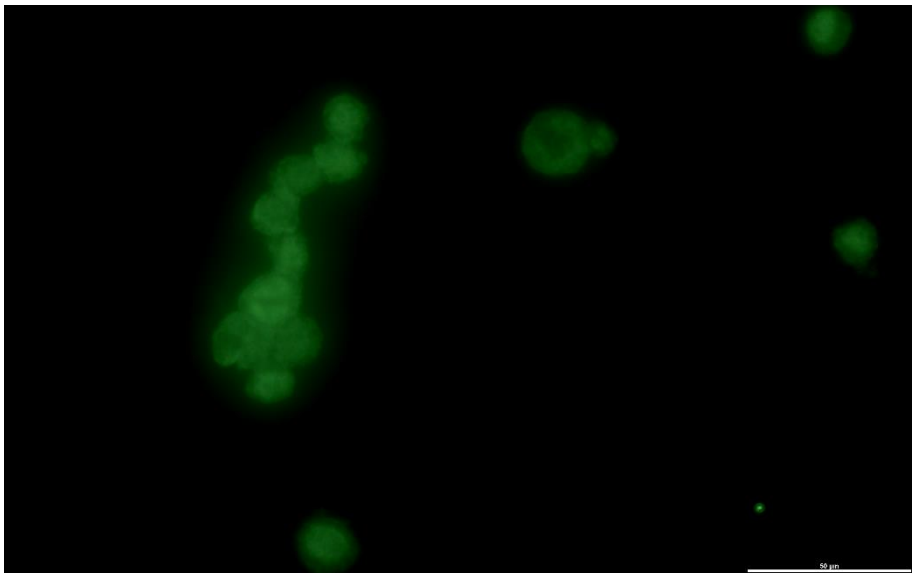
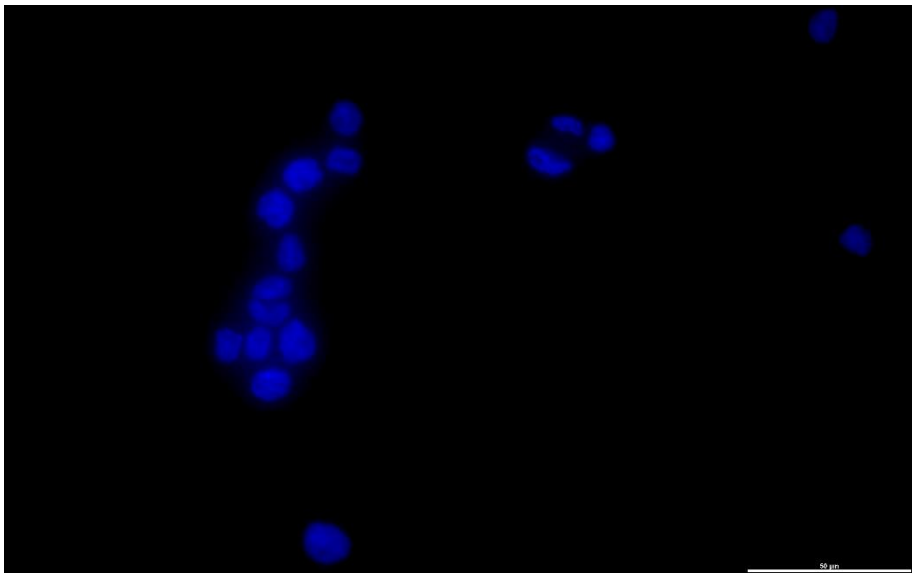
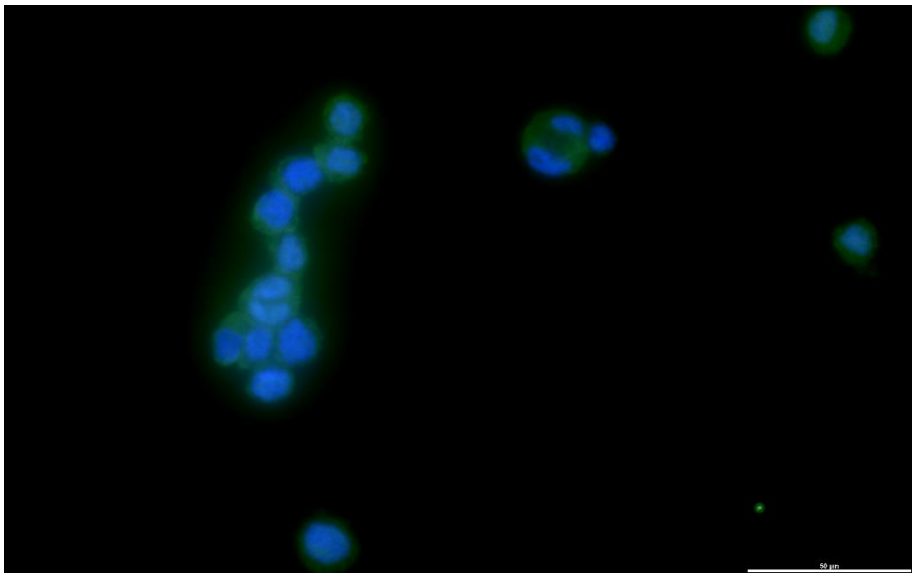
FIGURE S2B PMA-induced THP-1

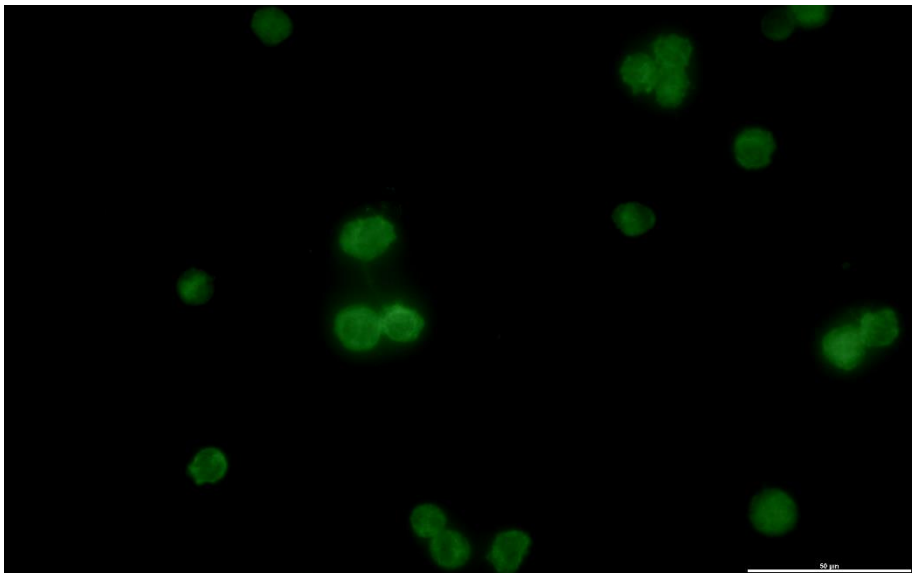
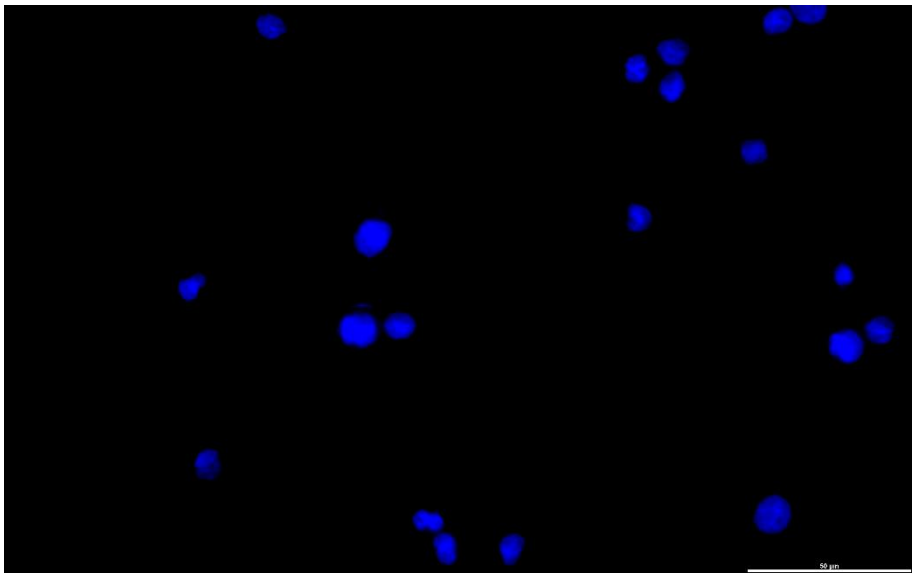
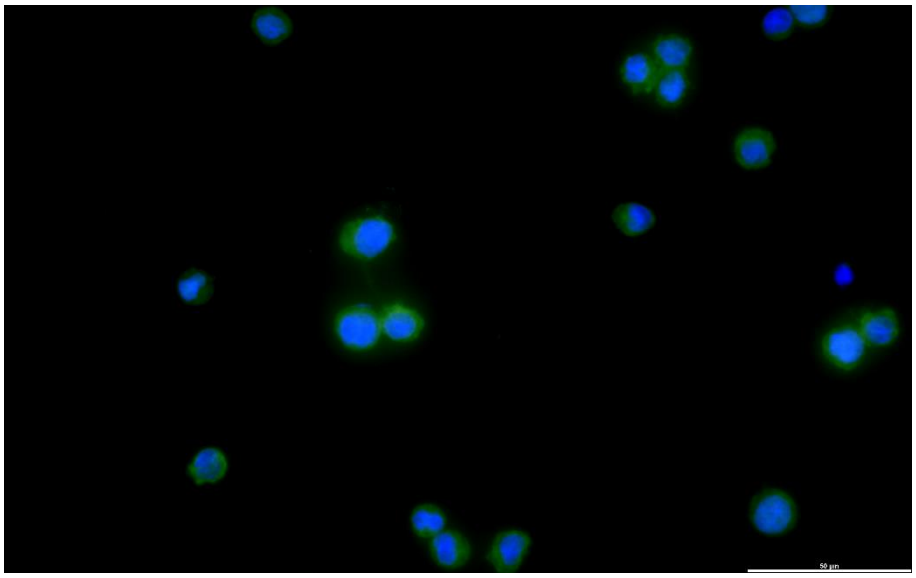
NLRP3	

Pro-IL-1β	
β-actin	

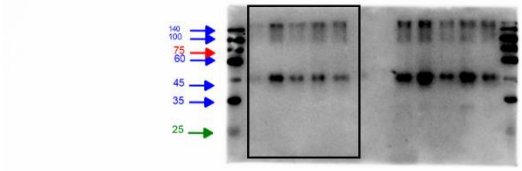
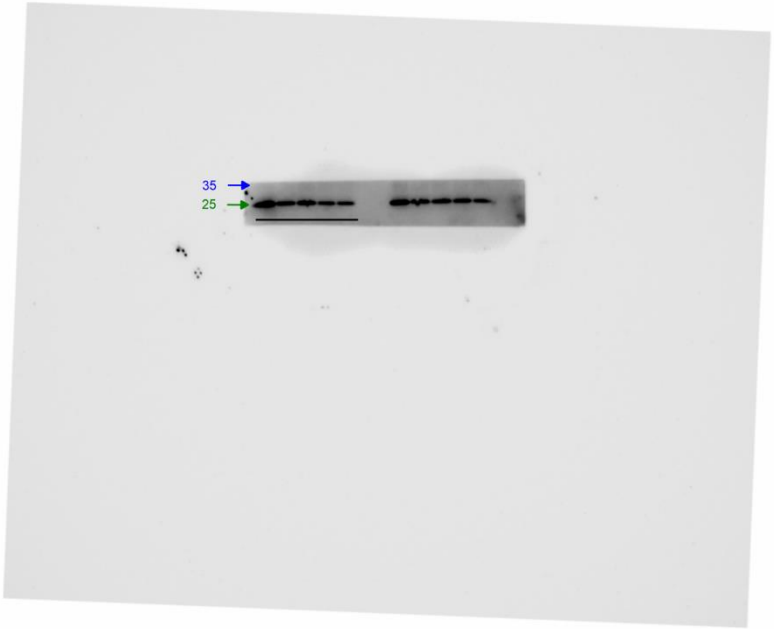
The original figures of immunofluorescent staining in figure S3.

Vehicle p65	
Vehicle Hoechst	
Vehicle Merge	

LPS P65	
LPS Hoechst	
LPS Merge	

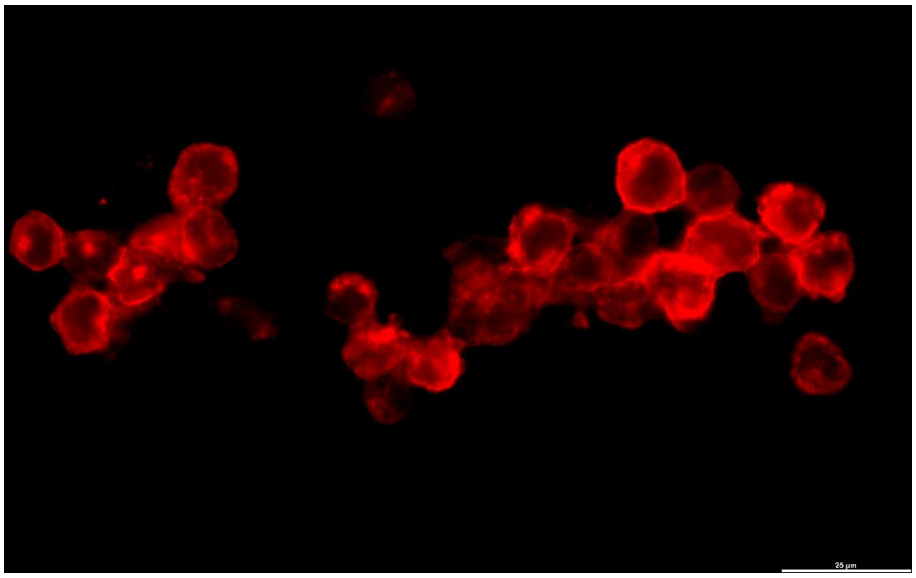
KM+LPS p65	
KM+LPS Hoechst	
KM+LPS Merge	

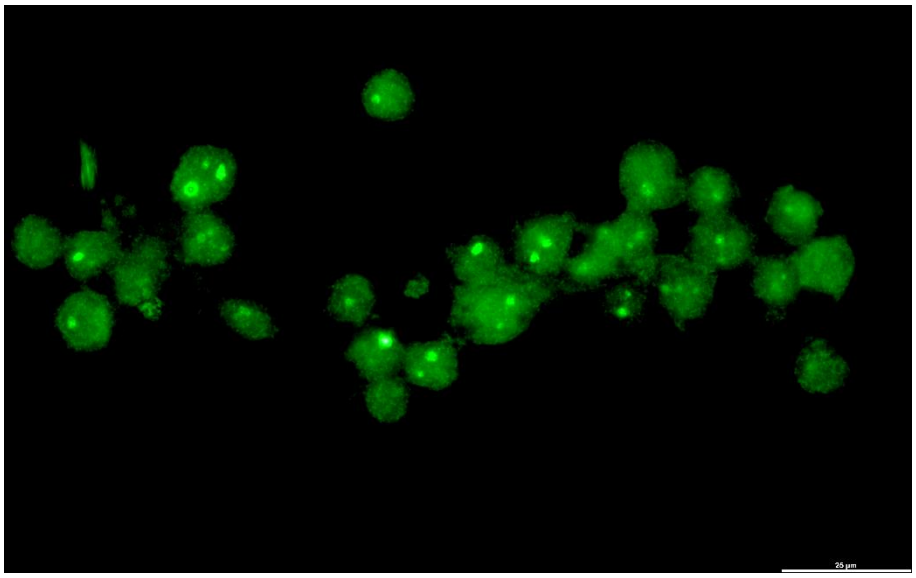
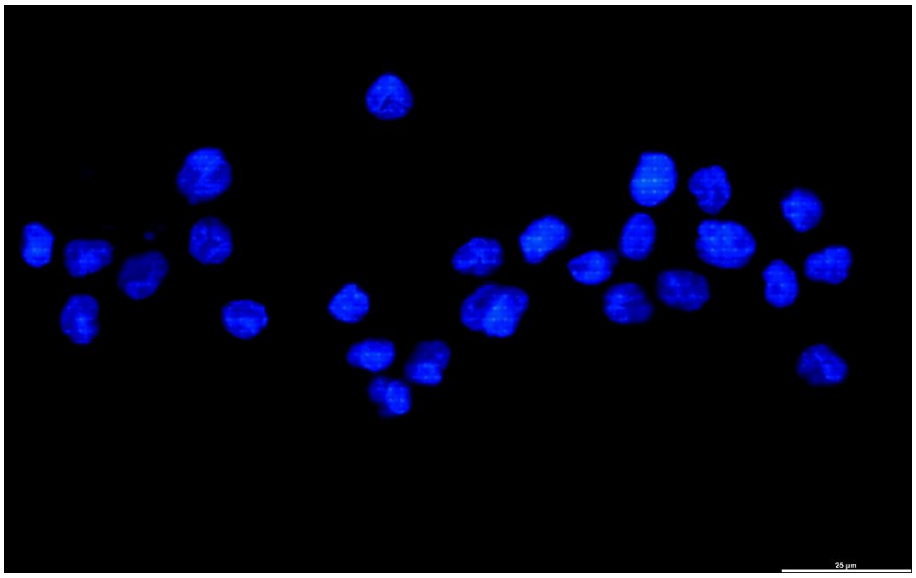
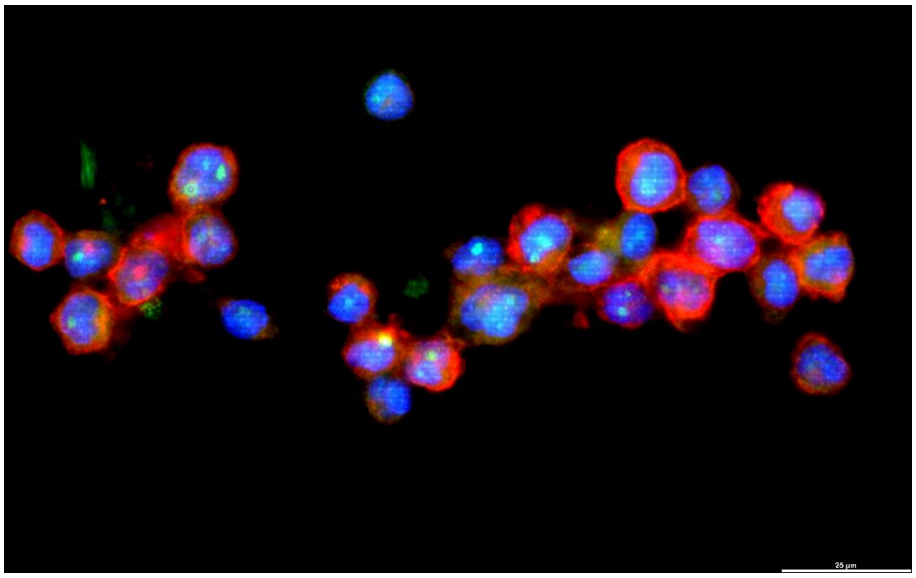
The original western blots of figure S4A.

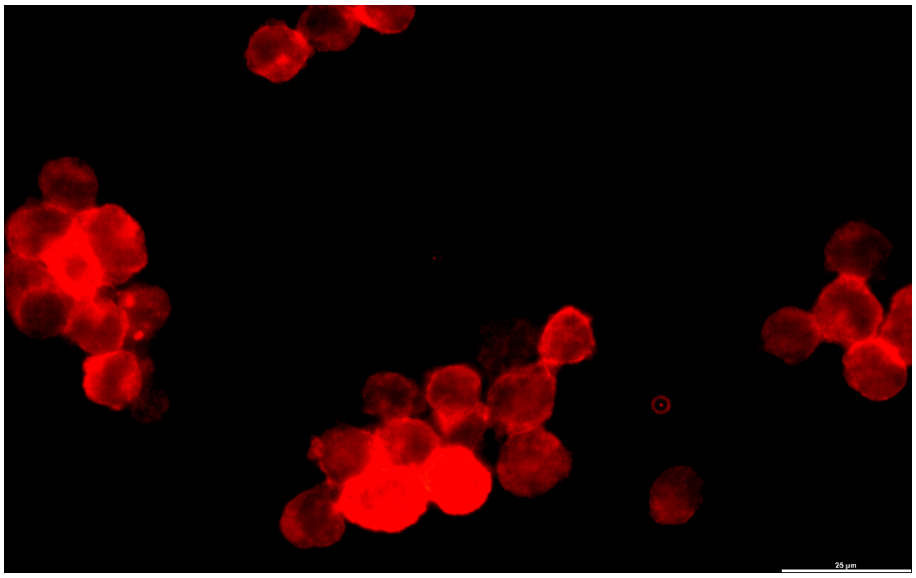
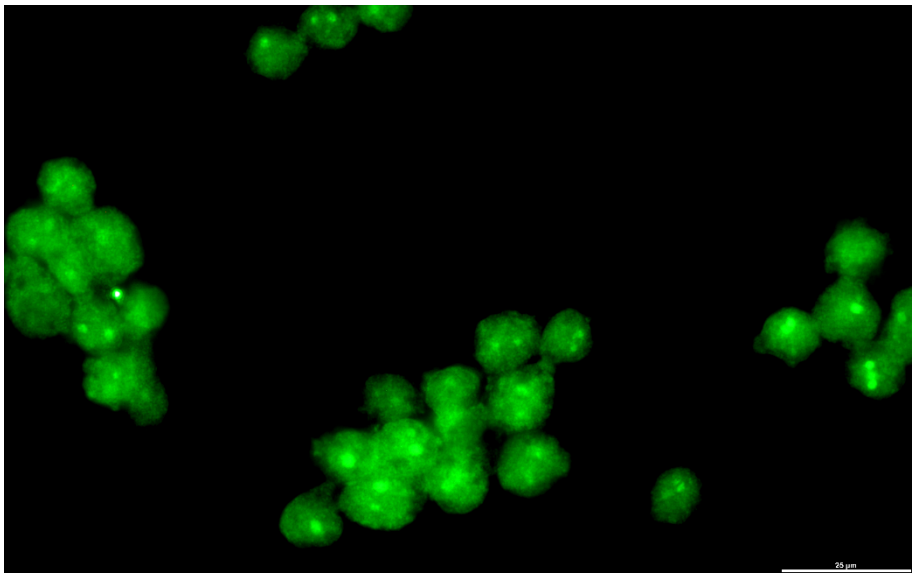
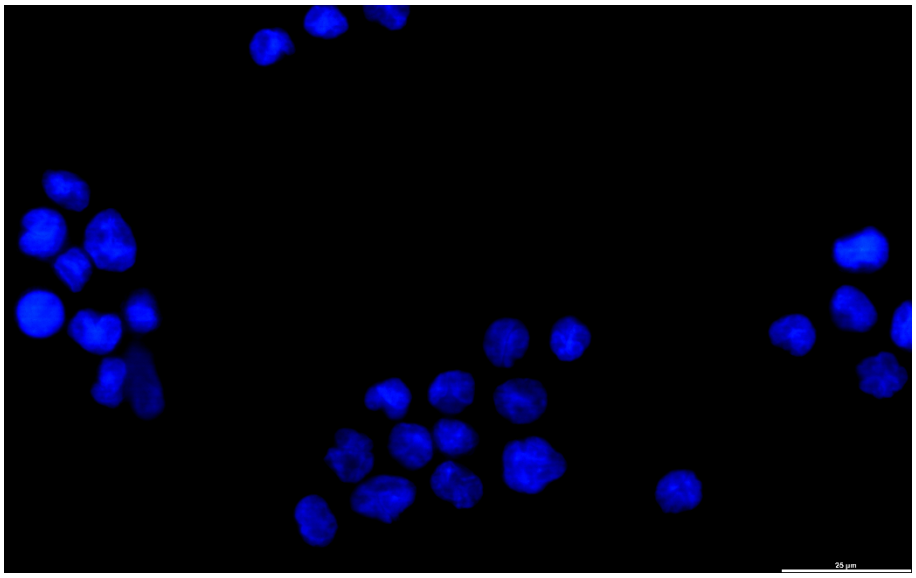
ASC oligomerization	
ASC	

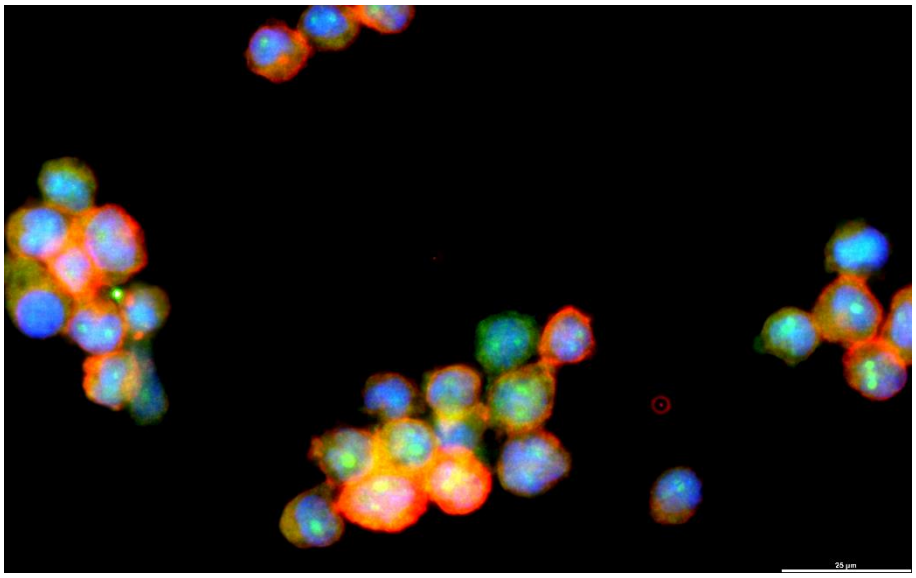
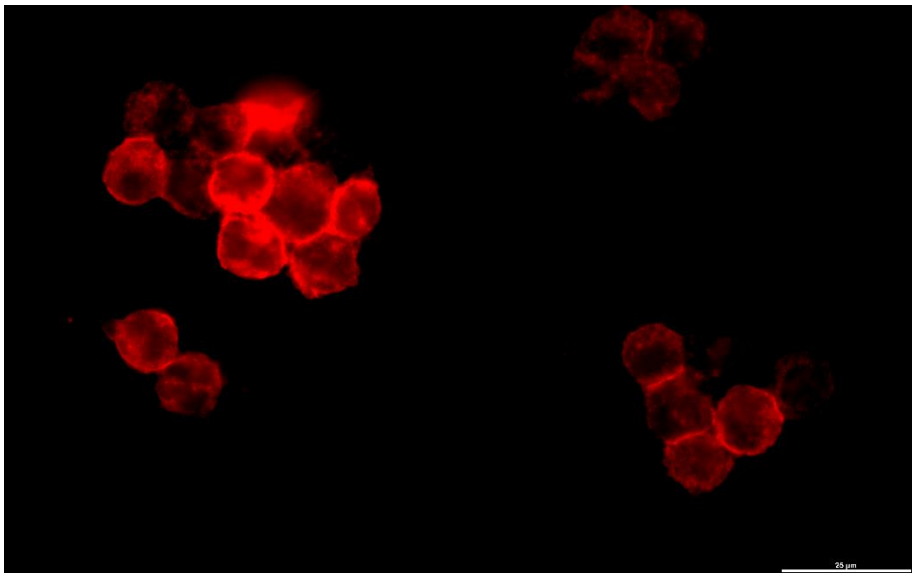
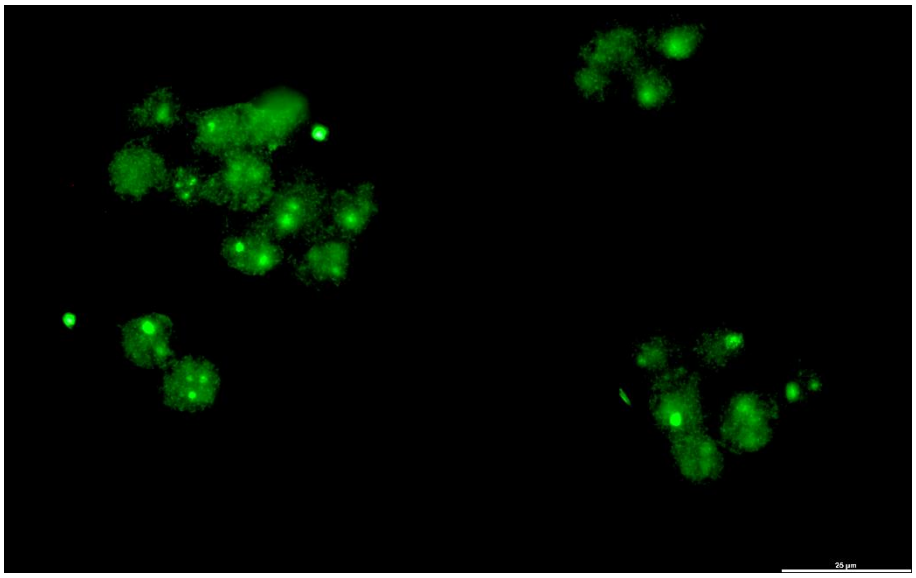
β-actin	

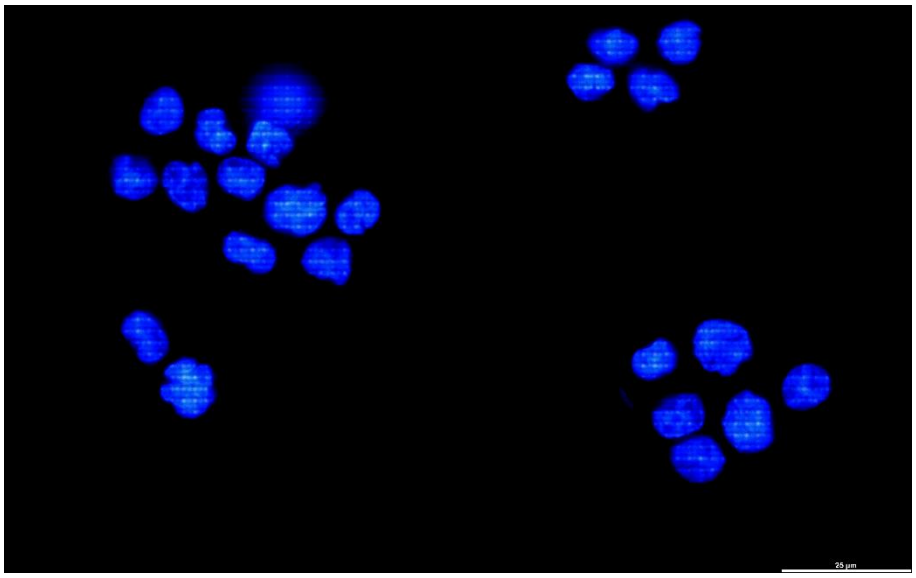
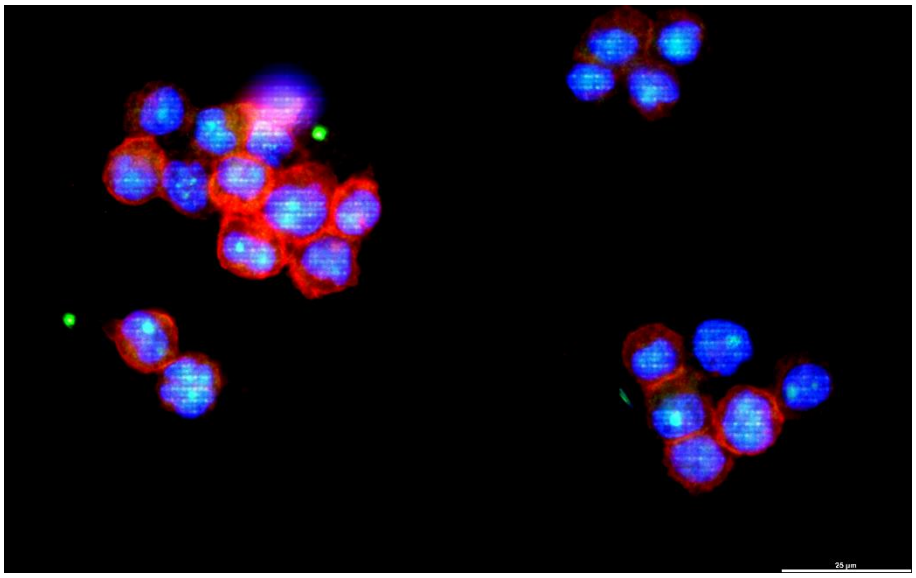
The original figures of immunofluorescent staining in figure S4B.

Vehicle NLRP3	

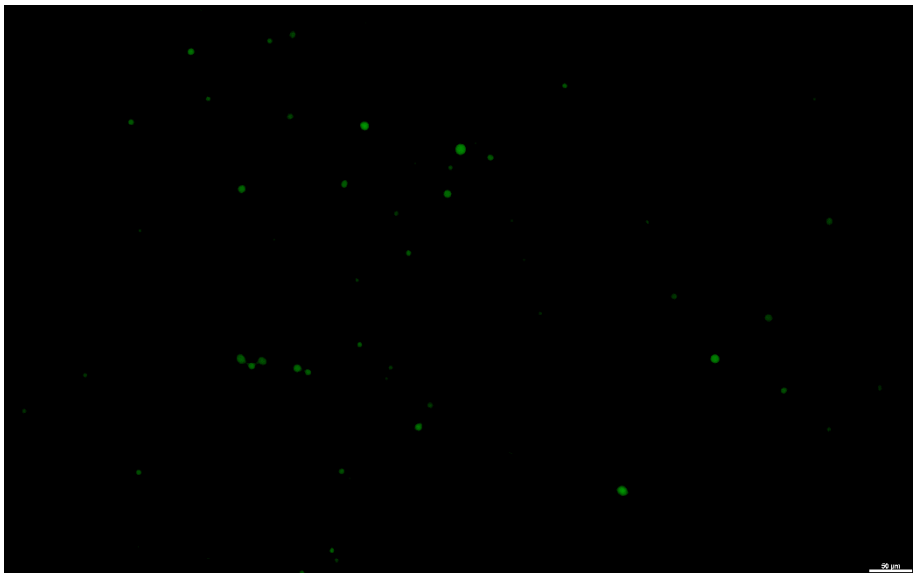
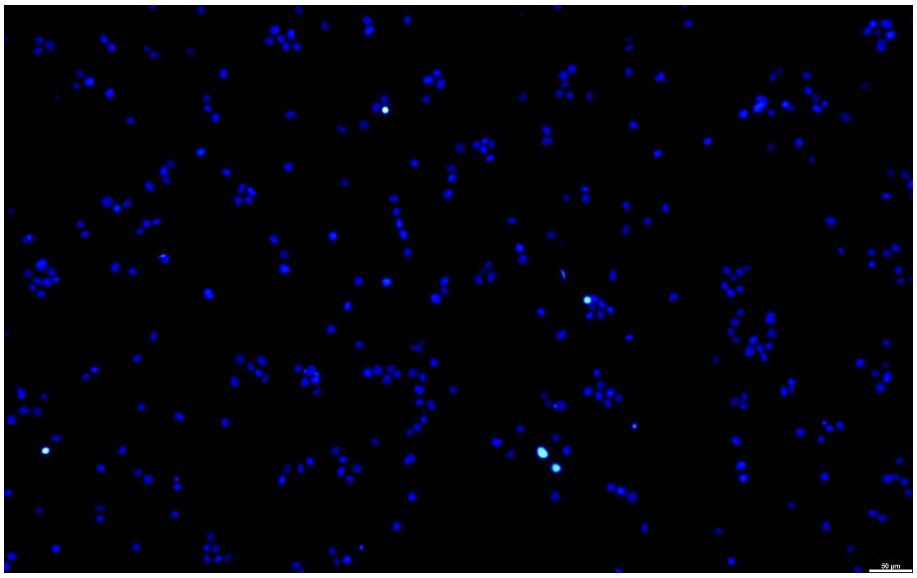
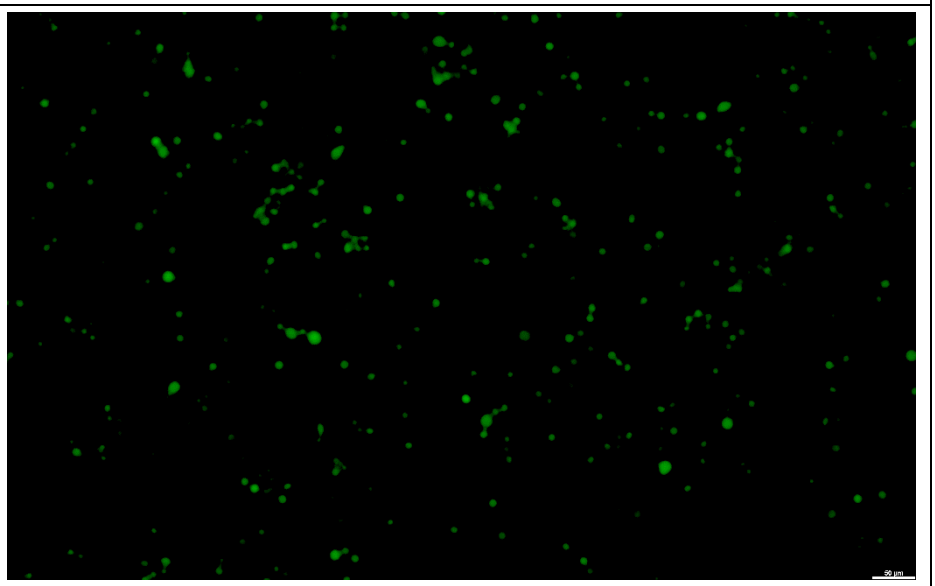
Vehicle ASC	
Vehicle Hoechst	
Vehicle Merge	

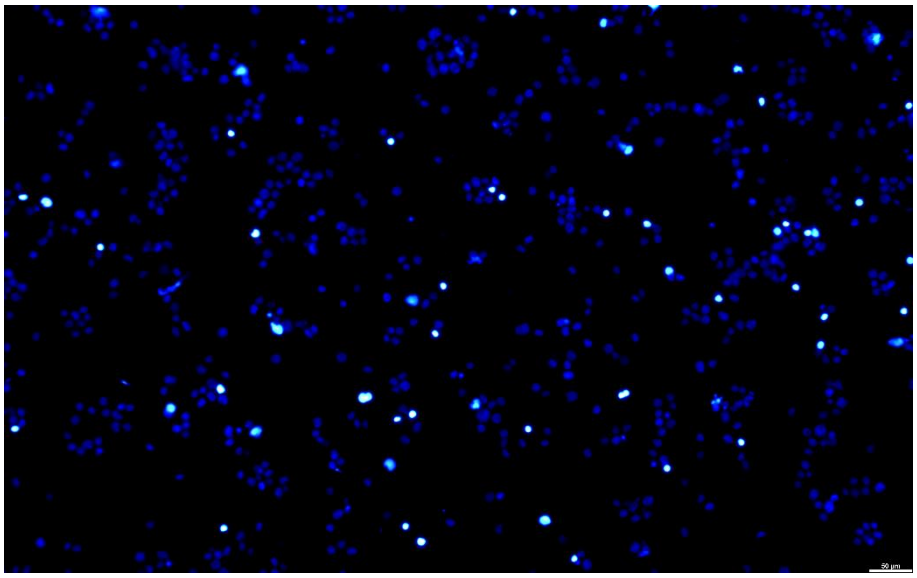
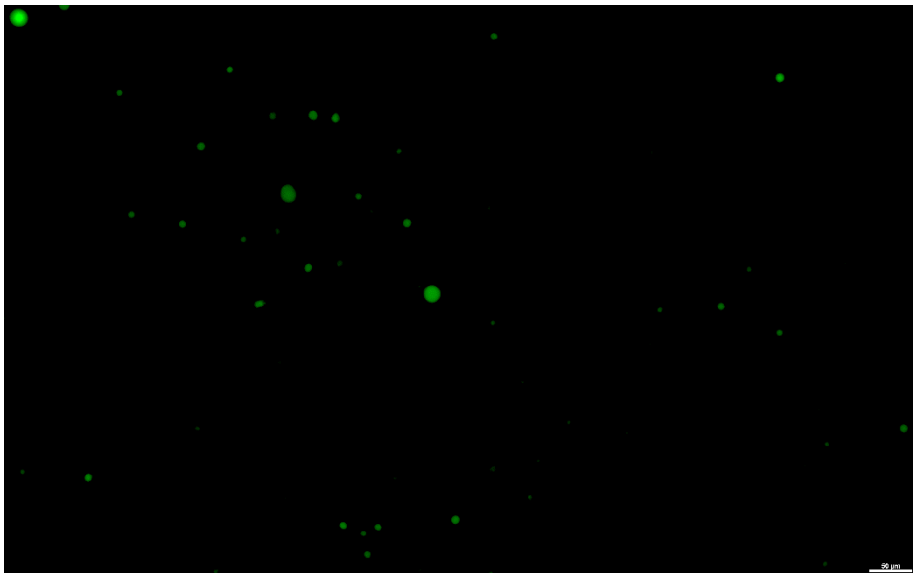
LPS+ATP NLRP3	
LPS+ATP ASC	
LPS+ATP Hoechst	

LPS+ATP Merge	
KM+ LPS+ATP NLRP3	
KM+ LPS+ATP ASC	

KM+ LPS+ATP Hoechst	
KM+ LPS+ATP Merge	

The original figures of DCFH-DA staining in figure S5.

Vehicle-DCFH-DA	
Vehicel-Hoechst	
LPS+ATP-DCFH-DA	

LPS+ATP -Hoechst	
KM 200 μM-DCFH-DA	
KM 200 μM-Hoechst