

Supplementary Tables and Figures

Supplementary Tables

Supplementary Table 1. Baseline characteristics of healthy volunteers and sepsis patients for cohort 1:

	Healthy volunteers ^a n=5	Sepsis-induce ALI ^a n=5	<i>P</i> value
Age (years)	52.6 ± 5.8	54.0 ± 8.1	0.892
Gender, male, n (%)	3 (60%)	3 (60%)	
Body mass index (BMI)	20.0 ± 1.2	21.2 ± 1.0	0.460
Septic Shock (n)	/	3 (60%)	
Source of infection (n)			
Abdominal	/	3 (60%)	
Urogenital	/	2 (40%)	
Acute lung injury (n)	/	5(100%)	
SOFA score, median (IQR)	/	6.2 (4.5-8)	
APACHE II score, median (IQR)	/	18 (14-23)	

^a original samples for proteomics analysis. The values are given as Mean ±SD or n (%). APACHE II: Acute Physiology and Chronic Health Evaluation score II; SOFA: Sequential Organ Failure Assessment.

Supplementary Table 2. Baseline characteristics of healthy volunteers and sepsis patients for cohort 2, which is independent patient pool from cohort 1.

	Healthy volunteers ^b n=15	Sepsis-induce ALI ^b n=13	<i>P</i> value
Age (years)	55.7 ± 2.9	61.9 ± 5.0	0.28
Gender, male, n (%)	8 (53%)	6 (46%)	
Body mass index (BMI)	22.0 ± 0.8	22.7 ± 0.8	0.56
Septic Shock (n)	/	5 (38%)	
Source of infection (n)			
Abdominal	/	8 (61%)	
Urogenital		3 (23%)	
Blood		1 (8%)	
Wound	/	1 (8%)	
Acute lung injury (n)	/	13 (100%)	
SOFA score, median (IQR)	/	6.5 (5-8)	
APACHE II score, median (IQR)	/	18.4 (15-23)	

^b original samples for PRM. The values are given as Mean ±SD or n (%). APACHE II: Acute Physiology and Chronic Health Evaluation score II; SOFA: Sequential Organ Failure Assessment.

Supplementary Table 3. Baseline characteristics of healthy volunteers and sepsis patients for cohort 3, which is independent patient pool from cohort 1 and cohort 2.

	Healthy volunteers ^c n=36	Sepsis ^c n=16	Sepsis-induce ALI ^c n=61	P value
Age (years)	53.3 ± 2.3	53.2 ± 1.0	54.9 ± 1.6	0.79
Gender, male, n (%)	19 (53%)	11 (69%)	35 (57%)	
Body mass index (BMI)	22.7 ± 0.61	21.0 ± 0.66	21.4 ± 0.38	0.10
Septic Shock (n)	/	3 (19%)	41 (67%)	
Source of infection (n)				
Abdominal	/	10 (62%)	50 (82%)	
Urogenital		3 (19%)	6 (10%)	
Blood		2 (13%)	3 (5%)	
Wound	/	1 (6%)	2 (3%)	
Acute lung injury (n)	/	0	100 (100%)	
SOFA score, median (IQR)	/	6.1 (4-9)	8.1 (5-10)	0.03
APACHE II score, median (IQR)	/	17.1 (10-24)	20.5 (16-26)	0.08

^c confirmatory samples for ELISA. The values are given as Mean ±SD or n (%). APACHE II: Acute Physiology and Chronic Health Evaluation score II; SOFA: Sequential Organ Failure Assessment.

Supplementary Table 4. Demographic and clinical characteristics of survivors and non-survivors

	Survivors n=22	Non-survivors n=16	P value
Age (years)	55.4 ± 3.1	60.0 ± 2.9	0.31
Gender, male, n (%)	10 (45%)	10 (63%)	
Body mass index (BMI)	21.9 ± 0.62	20.4 ± 0.64	0.11
Septic Shock (n)	11 (50%)	10 (63%)	0.52
Source of infection (n)			
Abdominal	16 (73%)	12 (75%)	
Urogenital	3 (13%)	2 (13%)	
Blood	2 (9%)	1 (6%)	
Wound	1 (5%)	1 (6%)	
Acute lung injury (n)	100 (100%)	100 (100%)	
SOFA score, median (IQR)	7 (5-9)	11(9-13)	< 0.001
APACHE II score, median (IQR)	18 (12-25)	22 (19-26)	0.04

The values are given as Mean ±SD or n (%). APACHE II: Acute Physiology and Chronic Health Evaluation score II; SOFA: Sequential Organ Failure Assessment.

Supplementary Table 5. The primers used in this study

Primers name		Sequence (5'-3')
IL-6	Forward	CCACCGGGAACGAAAGAGAA
	Reverse	TCTCCTGGGGGTATTGTGGA
TNF- α	Forward	AGAACTCACTGGGGCCTACA
	Reverse	GCTCCGTGTCTCAAGGAAGT
APN/CD13	Forward	ATTGGGCAAGGTCTGGTCTG
	Reverse	GAGAAGGAGAACGAGCCACC
CHIP-APN- sitA	Forward	TCCTCAAAAGATGCCCTG
	Reverse	CCAACGTGTTCTGCCTCTT
CHIP-APN- sitB	Forward	GAGACAGGGAGCCCCACC
	Reverse	GGAGTTTTAGTAGAGACA
CHIP-APN- sitC	Forward	TGAGAAGCGCGTGTTCCTC
	Reverse	AGCCTCGCCCTGAGTGTC
c-Myc	Forward	CTGGATTTTTTTTCGGGGTAGT
	Reverse	ACGCACAAGAGTTCCGTA

Supplementary Table 6. The sequences used in this study

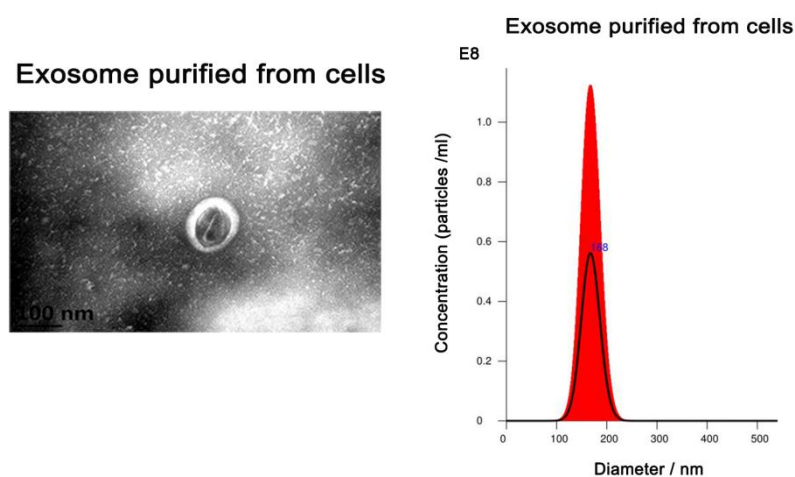
Gene		Sequence (5'-3')
Si-c-Myc1	Forward	GCUUCACCAACAGGAACUATT
	Reverse	UAGUUCCUGUUGGUGAAGCTT
Si-c-Myc2	Forward	GUGCAGCCGUUUUCUACUTT
	Reverse	AGUAGAAAUACGGCUGCACTT
Negative control	Forward	UUCUCCGAACGUGUCACGUTT
	Reverse	ACGUGACACGUUCGGAGAATT
shAPN1	Forward	CcggGCAGGACTACTGGCTGATATTCAAGAGAATTAACC TCGCTGTACTGGTTTTTTg
	Reverse	aattcaaaaaGCAGGACTACTGGCTGATATCTCTTGAAATTA ACCTCGCTGTACTGG
shAPN2	Forward	CcggCCAGTACAGCGAGGTTAATTTCAAGAGAATAGCGT TGCAGTAGACGGTTTTTTg
	Reverse	aattcaaaaaCCAGTACAGCGAGGTTAATTCTCTTGAAATAG CGTTGCAGTAGACGG
shAPN3	Forward	CcggCCGTCTACTGCAACGCTATTTCAAGAGAATAGCGTT GCAGTAGACGGTTTTTTg
	Reverse	aattcaaaaaCCGTCTACTGCAACGCTATTCTCTTGAAATAG CGTTGCAGTAGACGG
shNC	Forward	CcggCCTAAGGTTAAGTCGCCCTCGCTCGAGCGAGGGCG ACTTAACCTTAGGTTTTTTg
	Reverse	aattcaaaaaCCTAAGGTTAAGTCGCCCTCGCTCGAGCGAGG GCGACTTAACCTTAGG

Supplementary Table 7. A list of antibodies used for Western blot, IHC staining, IF, Co-IP, CHIP.

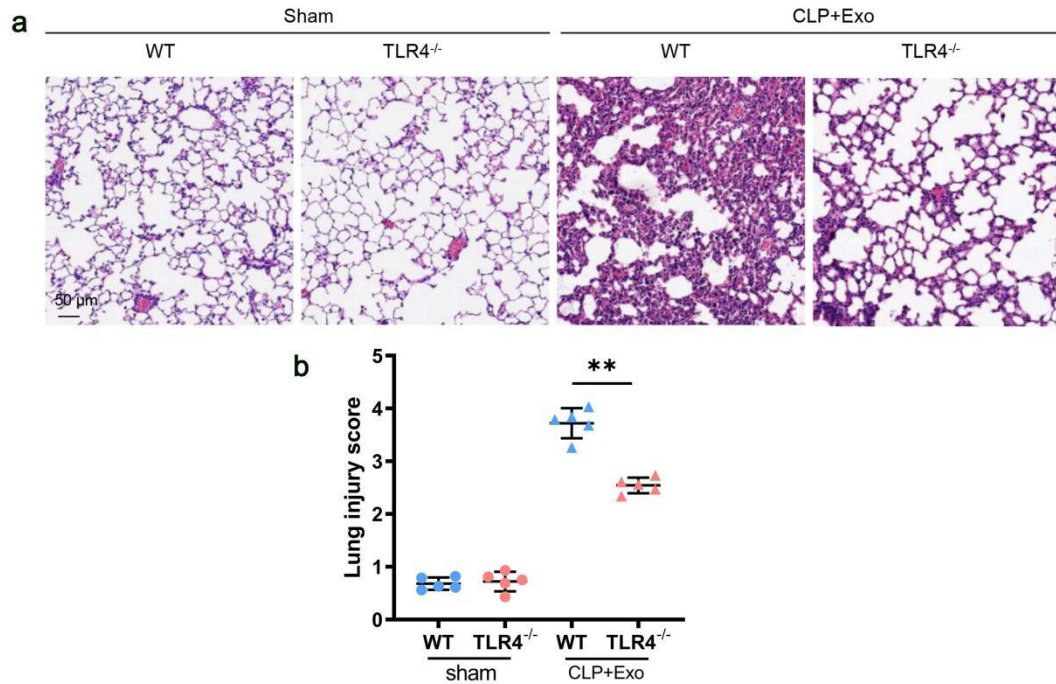
Name of antibody	Cat.No	Company	Mol weight	Dilution (WB/ IHC/ CHIP)
CD63	ab59479	Abcam	52 kDa	1:1000 (WB)
CD81	ab79559	Abcam	26 kDa	1:1000 (WB)
c-Myc F4/80	ab32072 28463-1-AP	Abcam proteintech	57 kDa	1:1000 (WB); 1:50 (CHIP) 1:100 (IF)
CD13/APN	ab108310	Abcam	150 kDa	1:1000 (WB); 1:100 (IF)
TLR4	ab13556	Abcam	96 kDa	1:1000(WB); 1:50 (IF) 1:100 (IP); 1:50 (IHC)
TLR4 (76B357.1)	sc-52962	Santa Cruz Biotechnology	96 kDa	1:1000(WB); 1:50 (IF)
NF- κ B p65	55764T	CST	65 kDa	1:1000 (WB); 1:100 (IF)
P-NF- κ B p65	55764T	CST	65 kDa	1:1000 (WB); 1:100 (IF) 1:200 (IHC)
RelB	55764T	CST	70 kDa	1:1000 (WB)
NF- κ B1 p105/p50	55764T	CST	50, 120 kDa	1:1000 (WB)
NF- κ B2 p100/p52	55764T	CST	120, 52 kDa	1:1000 (WB)
p-I κ B- α (B-9)	sc-8404	Santa Cruz Biotechnology	36 kDa	1:1000 (WB)
I κ B- α (H-4)	sc-1643	Santa Cruz Biotechnology	36 kDa	1:1000 (WB)
RIPK1	ab268944	Abcam	75 kDa	1:1000 (WB); 1:100 (IF)
RIP1	3493	CST	75 kDa	1:1000 (WB); 1:100 (IP) 1:100 (IF)
RIPK3	A5431	Abclonal	57 kDa	1:1000 (WB); 1:200 (IHC) 1:100 (IF)

Phospho-RIP (Ser166)	44590	CST	75 kDa	1:1000 (WB)
RIP3	17563-1-AP	proteintech	55 kDa	1:100 (IP); 1:1000 (WB) 1:100 (IF)
MLKL	21066-1-AP	proteintech	55 kDa	1:1000 (WB)
P-MLKL	74921S	CST	55 kDa	1:1000 (WB)
Cleaved Caspase-3	9664	CST	17 kDa	1:1000 (WB)
Caspase-3	9662	CST	37 kDa	1:1000 (WB)
Caspase-8	4790	CST	57 kDa	1:1000 (WB)
Cleaved Caspase-8	8592	CST	43 kDa	1:1000 (WB)
GAPDH	5174	CST	37 kDa	1:1000 (WB)
Tubulin	ab184966	Abcam	55 kDa	1:1000 (WB)

Supplementary Figures and Figure Legends

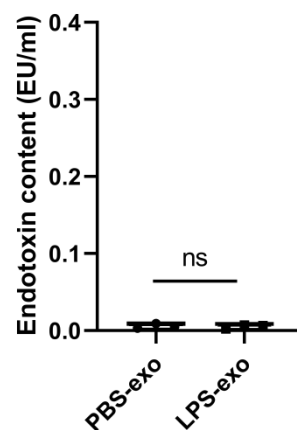


Supplementary Figure 1 Supplementary figures were related to Figure 3. Exosomes were purified from these supernatants and identified via TEM and NTA.



Supplementary Figure 2 Supplementary figures were related to Figure 6. The WT and TLR4 knockout (KO) mice were subjected to sham or CLP and injected with LPS-exo (APN in the purified exosomes). a Representative H&E staining images of mice lung tissues. Scale bar, 50 μ m. b Lung injury score was evaluated due to the pathology. All results are based on three measurements. The information is presented as the mean $\pm$ SD, * P < 0.05, ** P < 0.01.

The exosome samples were tested for endotoxin contamination. Endotoxin levels were found to be less than 0.01 EU/ml in both PBS-exo and LPS-exo.



Supplementary Figure 3 Endotoxin levels in the exosomal preparations. LPS-exo and PBS-exo represent exosomes isolated from LPS-treated BMDMs than PBS-treated BMDMs, respectively. Data are expressed as the mean $\pm$ SEM.

Supplementary Figure 4 The original uncropped blot/gel images of the main figures.

Fig 1c

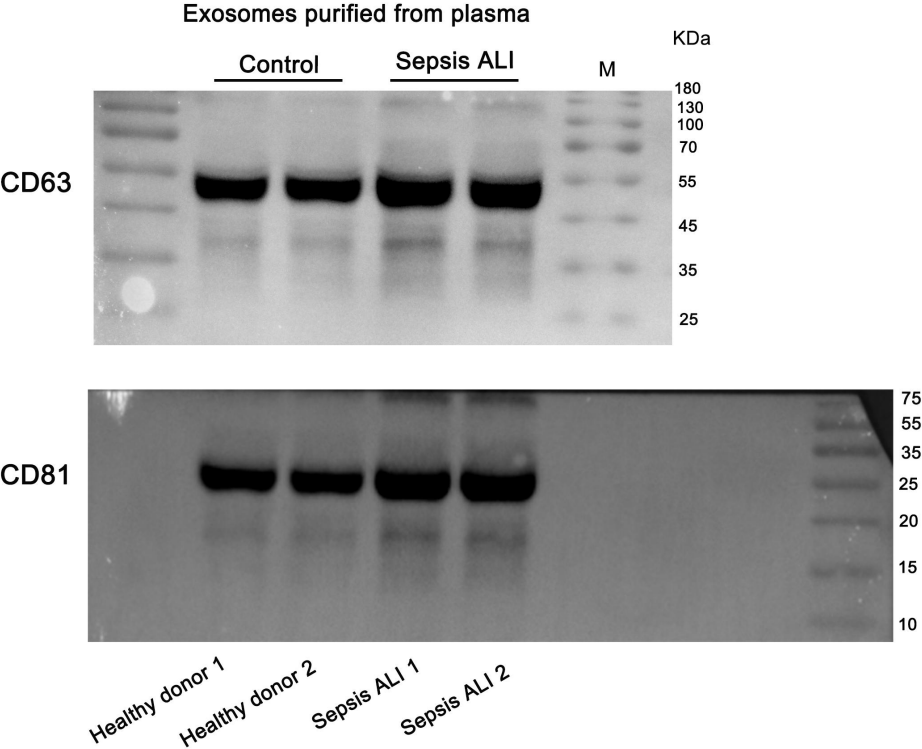


Fig 3c

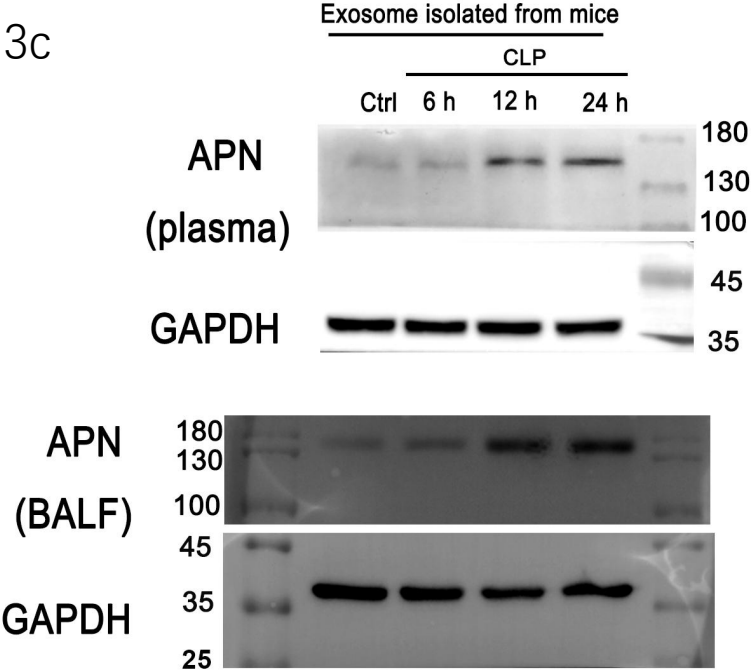


Fig 3g

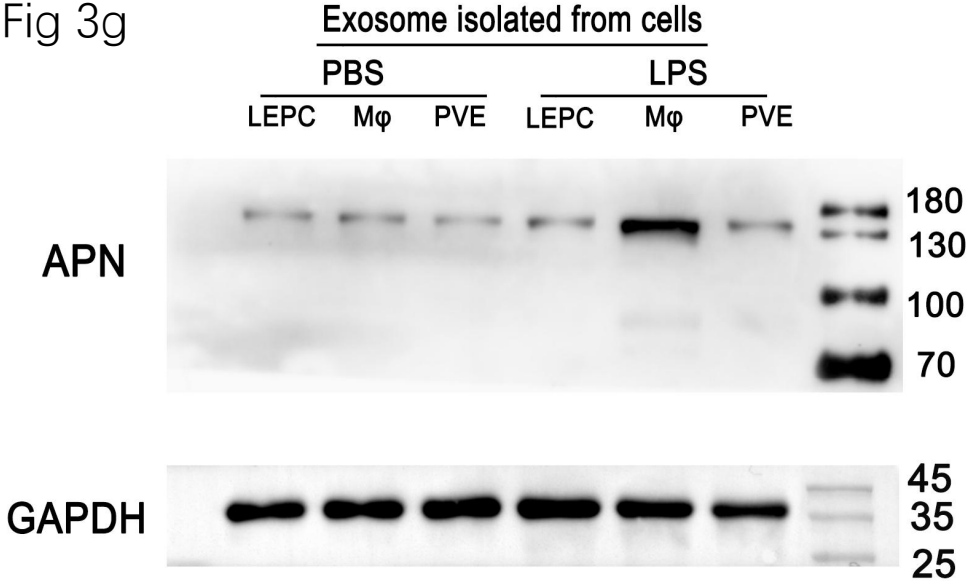


Fig 3j

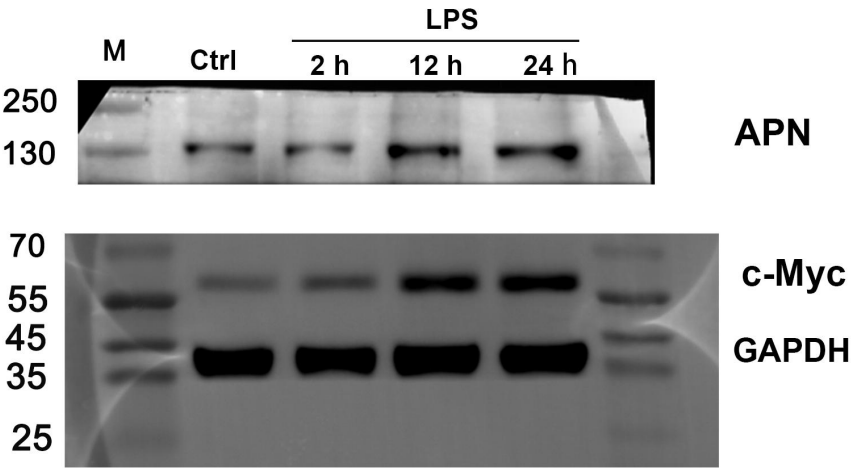


Fig 3l

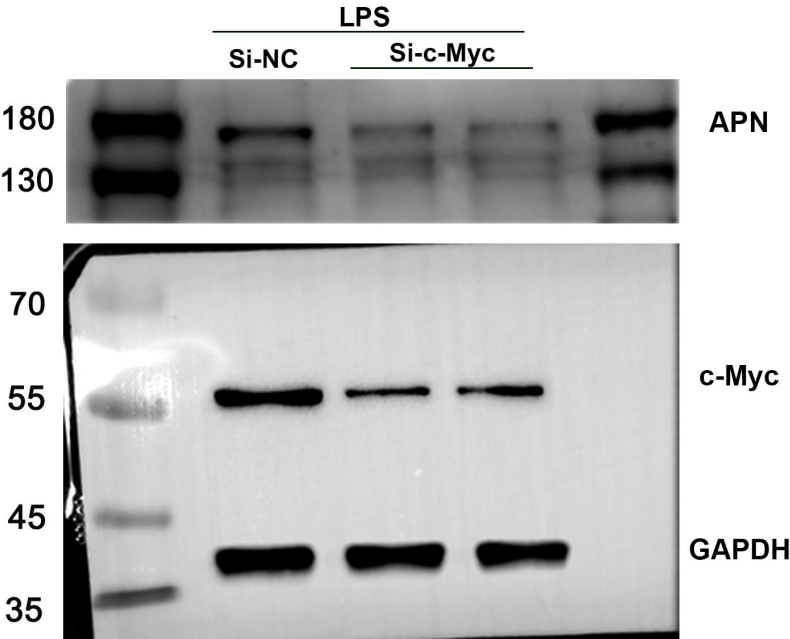


Fig 3k

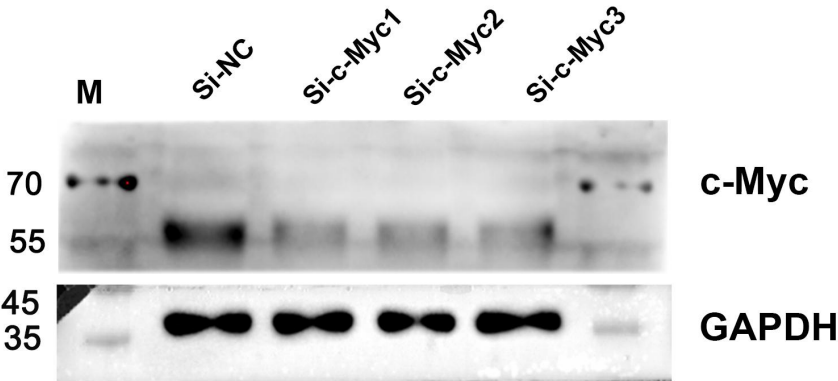


Fig 3o

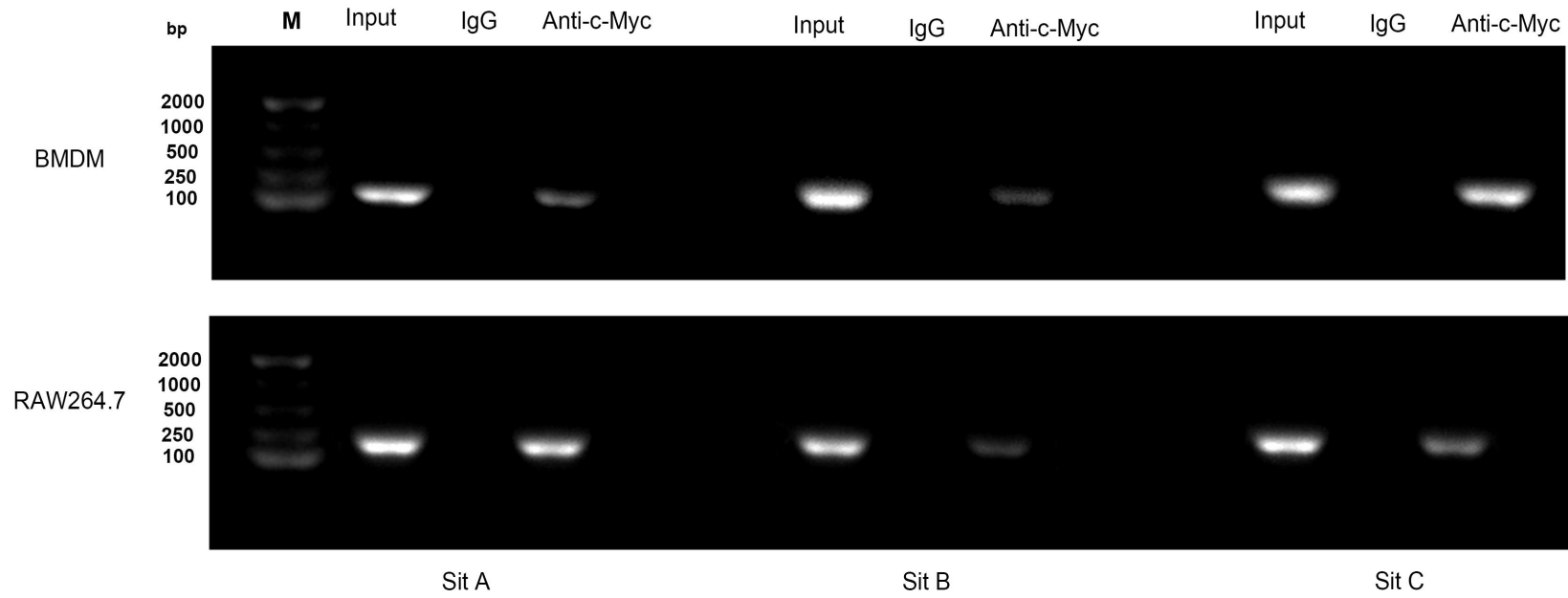


Fig 4d

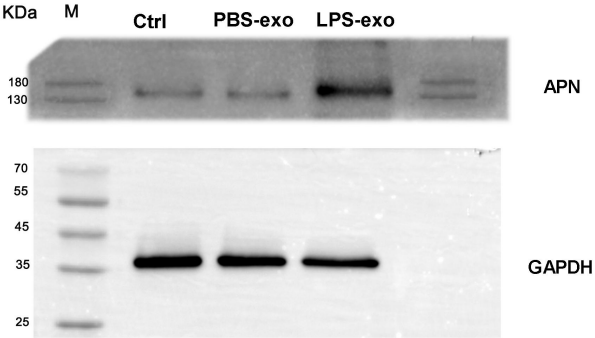


Fig 4h

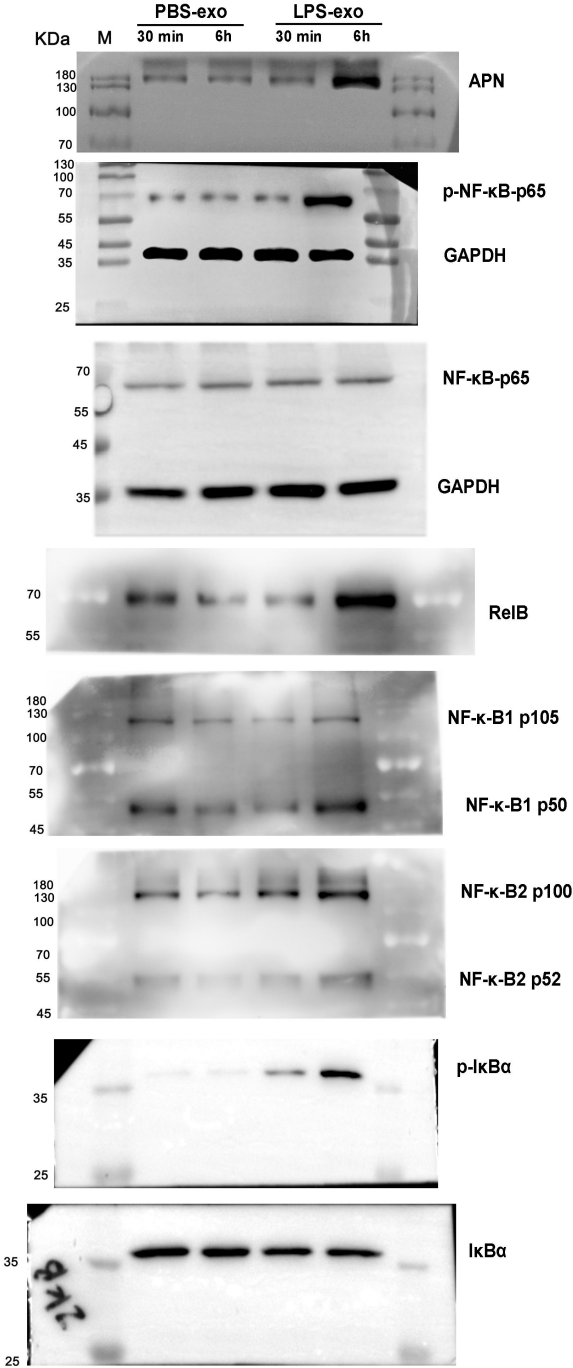


Fig 4l

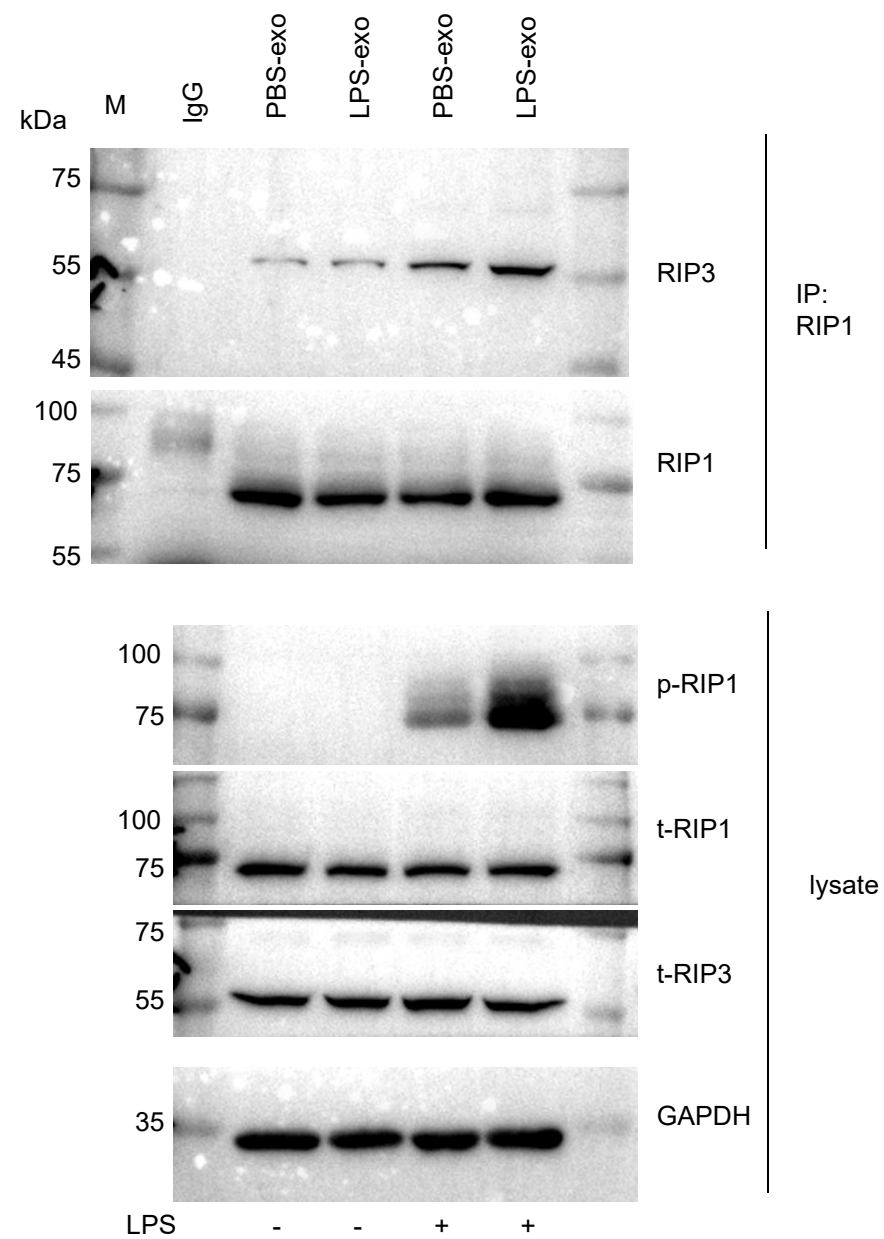


Fig 4n

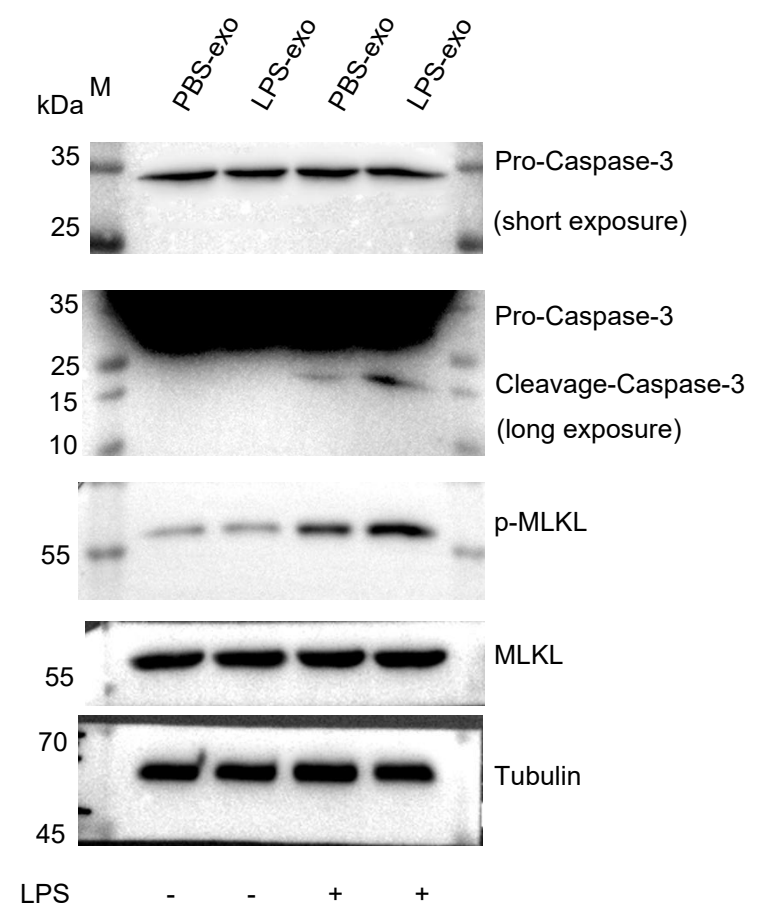


Fig 5a

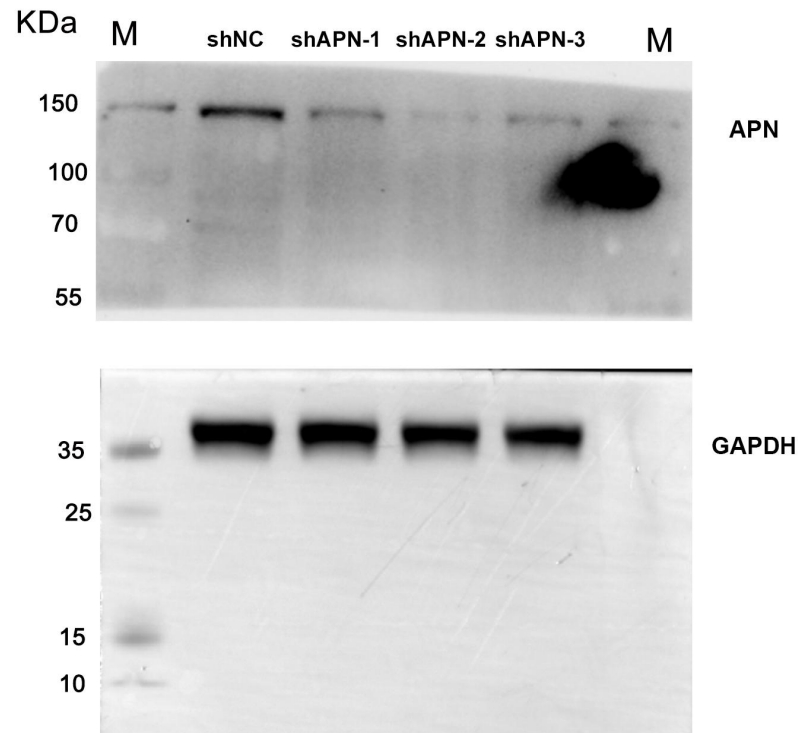


Fig 5d

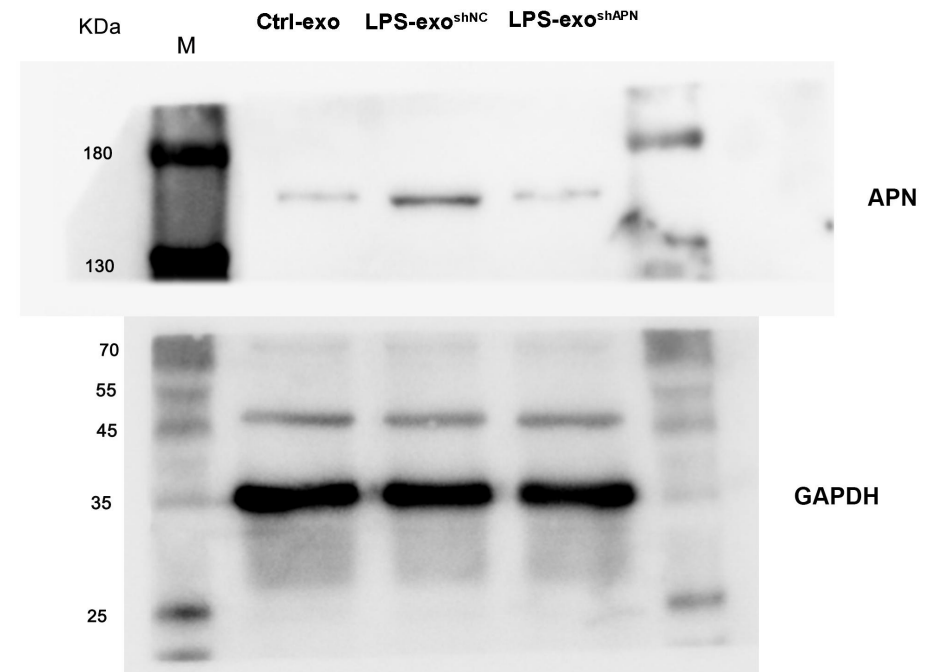


Fig 5h

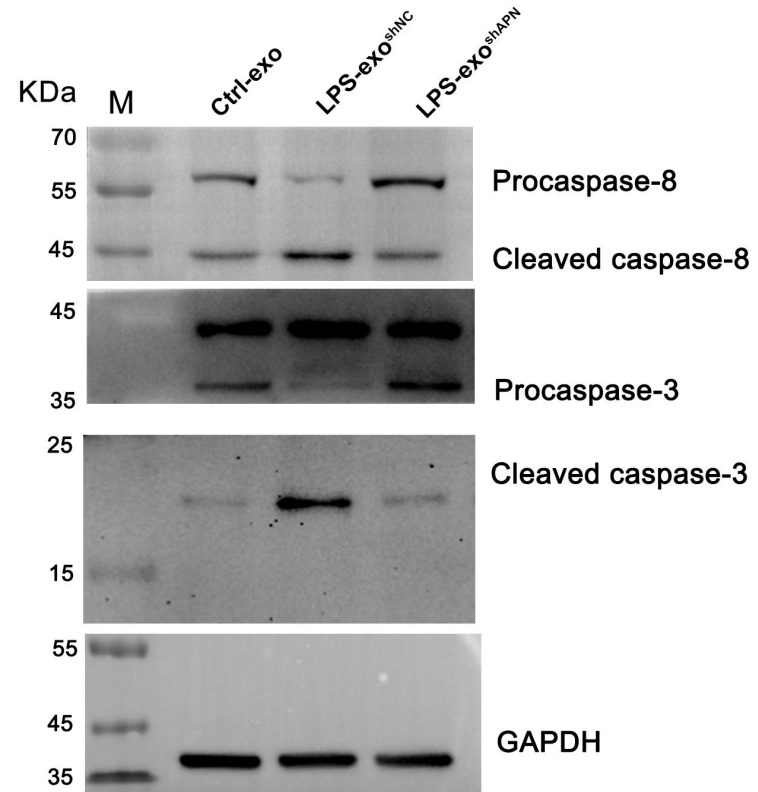


Fig 5l

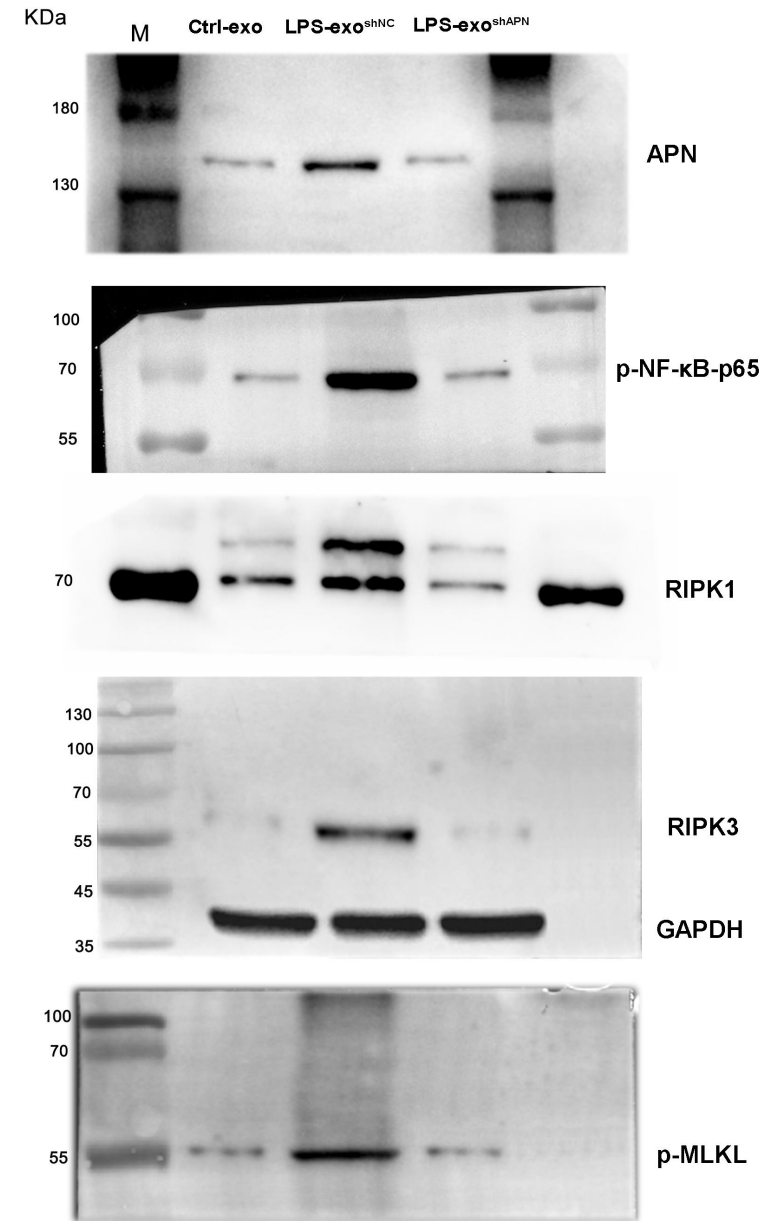


Fig 5n

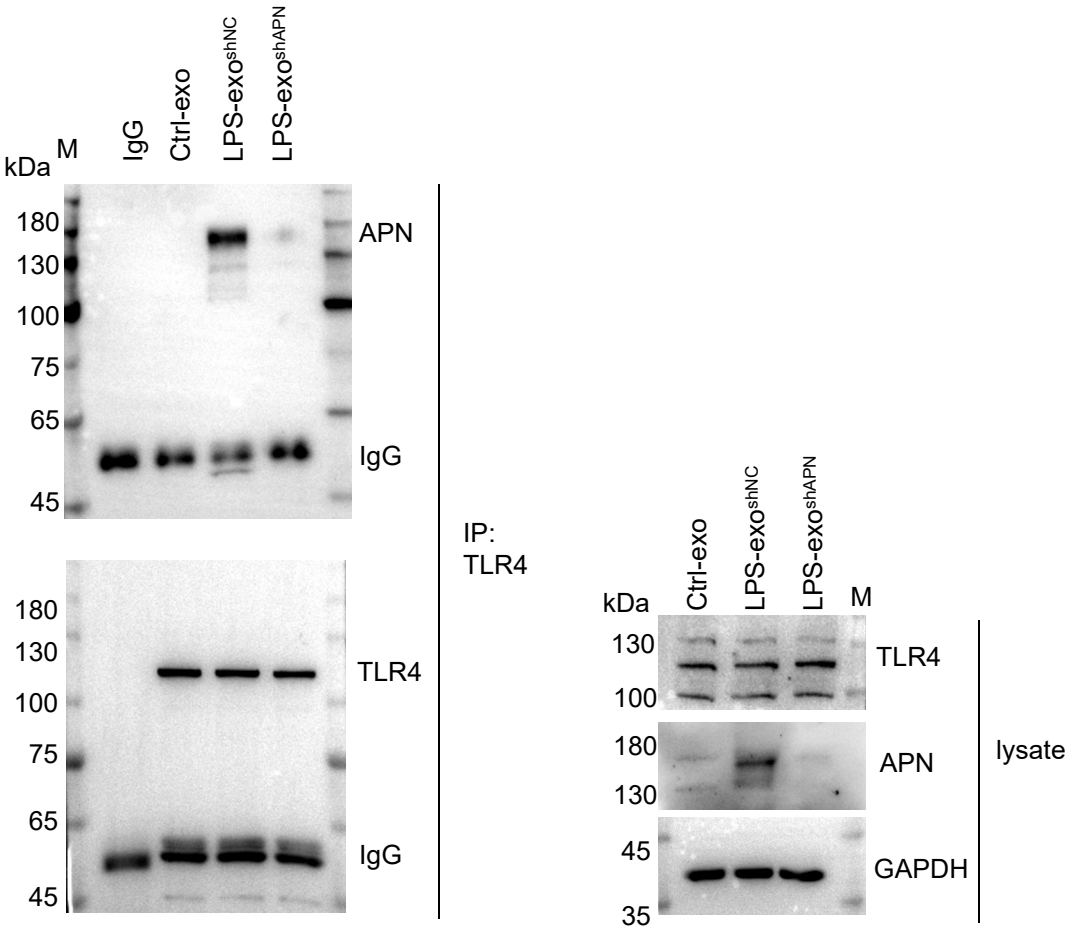


Fig 6k

