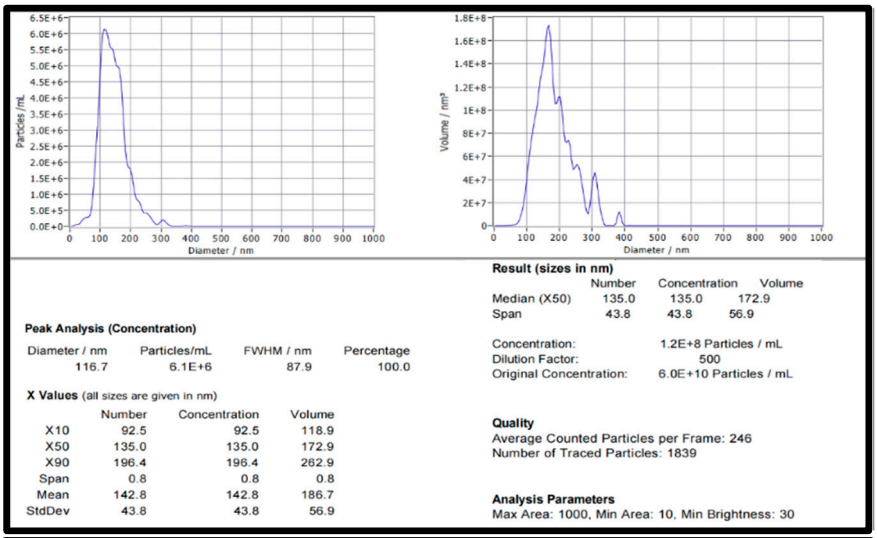
a



Result (sizes in nm)

	Number	Concentration	Volume
Median (X50)	135.0	135.0	172.9
Span	43.8	43.8	56.9

Concentration: 1.2E+8 Particles / mL
Dilution Factor: 500
Original Concentration: 6.0E+10 Particles / mL

Peak Analysis (Concentration)

Diameter / nm	Particles/mL	FWHM / nm	Percentage
116.7	6.1E+6	87.9	100.0

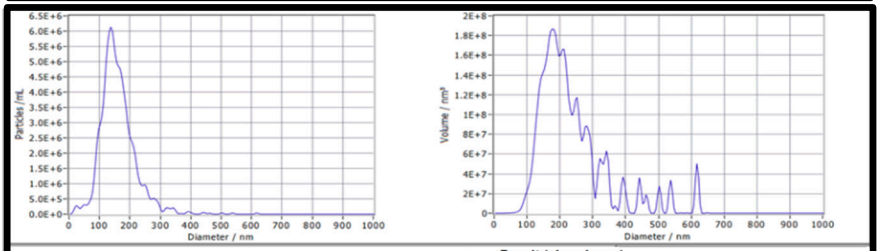
X Values (all sizes are given in nm)

	Number	Concentration	Volume
X10	92.5	92.5	118.9
X50	135.0	135.0	172.9
X90	196.4	196.4	262.9
Span	0.8	0.8	0.8
Mean	142.8	142.8	186.7
StdDev	43.8	43.8	56.9

Quality
Average Counted Particles per Frame: 246
Number of Traced Particles: 1839

Analysis Parameters
Max Area: 1000, Min Area: 10, Min Brightness: 30

b



Result (sizes in nm)

	Number	Concentration	Volume
Median (X50)	149.4	149.4	206.9
Span	56.4	56.3	104.4

Concentration: 1.3E+8 Particles / mL
Dilution Factor: 2500
Original Concentration: 3.1E+11 Particles / mL

Peak Analysis (Concentration)

Diameter / nm	Particles/mL	FWHM / nm	Percentage
137.2	6.1E+6	86.7	100.0

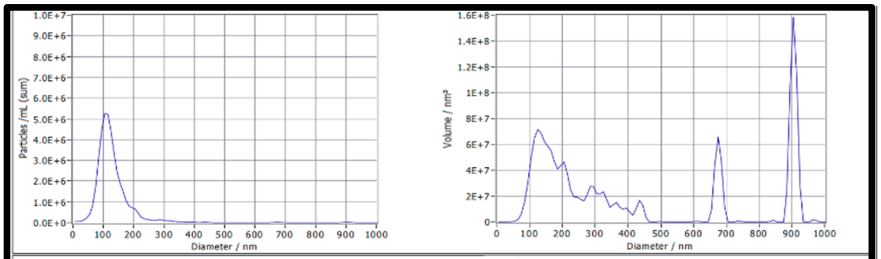
X Values (all sizes are given in nm)

	Number	Concentration	Volume
X10	97.6	97.6	136.2
X50	149.4	149.4	206.9
X90	223.9	223.9	349.7
Span	0.8	0.8	1.0
Mean	159.9	159.9	236.7
StdDev	56.4	56.3	104.4

Quality
Average Counted Particles per Frame: 259
Number of Traced Particles: 1892

Analysis Parameters
Max Area: 1000, Min Area: 10, Min Brightness: 30

c



Result (sizes in nm)

	Number	Concentration	Volume
Median (X50)	112.4	112.4	276.1
Span	61.9	61.8	312.8

Concentration: 4.1E+7 Particles / mL
Dilution Factor: 2000
Original Concentration: 8.2E+10 Particles / mL

Peak Analysis (Concentration)

Diameter / nm	Particles/mL	FWHM / nm	Percentage
110.3	5.2E+6	62.3	100.0

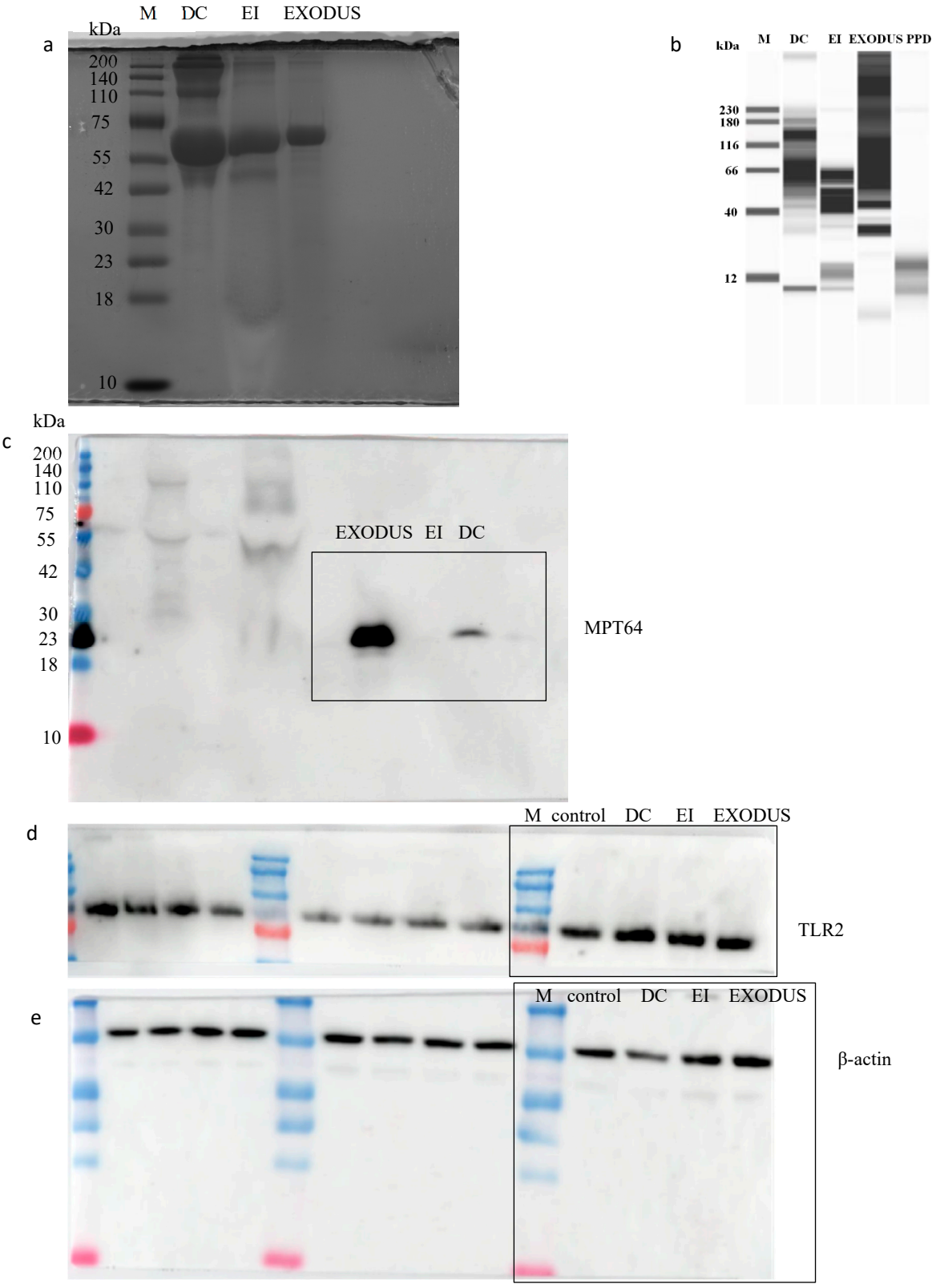
X Values (all sizes are given in nm)

	Number	Concentration	Volume
X10	77.3	77.3	113.2
X50	112.4	112.4	276.1
X90	181.9	181.9	900.8
Span	0.9	0.9	2.9
Mean	129.3	129.3	420.1
StdDev	61.9	61.8	312.8

Quality
Average Counted Particles per Frame: 104
Number of Traced Particles: 645

Analysis Parameters
Max Area: 1000, Min Area: 10, Min Brightness: 30

Figure S1: Nanoparticle Tracking Analysis report of H37Rv EVs isolated by different method. (a) DC; (b) EI; (c) EXODUS.


a
M DC EI EXODUS
kDa
200
140
110
75
55
42
30
23
18
10
b
kDa
M DC EI EXODUS PPD
230
180
116
66
40
12
c
kDa
200
140
110
75
55
42
30
23
18
10
EXODUS EI DC
MPT64
d
M control DC EI EXODUS
TLR2
e
M control DC EI EXODUS
β-actin

f

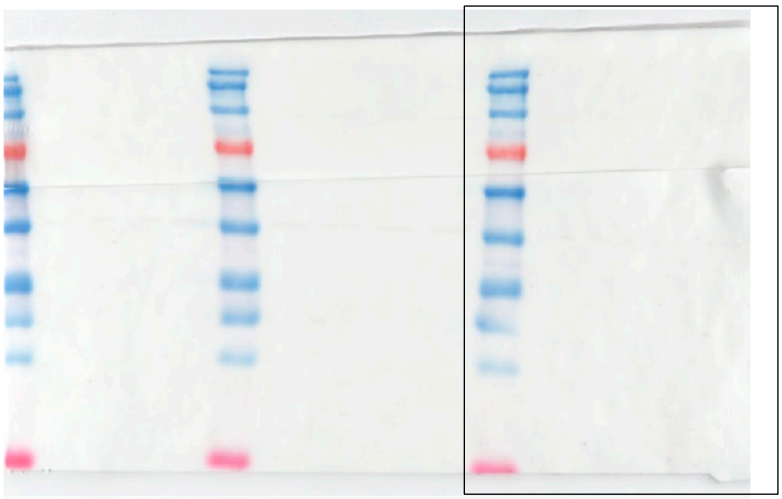


Figure S2: The original gel scans of coomassie brilliant blue staining and western blotting. (a) Coomassie brilliant blue image of three types of EVs; (b) Western blot image of PPD of three types of EVs; (c) Western blot image of MPT64 of three types of EVs; (d) Western blot image of TLR2 after three types of EVs (5 μg) infection of Raw264.7 for 4 h; (e) Western blot image of β-actin after three types of EVs (5 μg) infection of Raw264.7 for 4 h; (f) Complete western blot image of TLR2 and β-actin. M: protein marker; control: Raw264.7; DC: H37Rv EVs isolated by Differential Centrifugation; EI: H37Rv EVs isolated by Exosome Isolation kit; EXODUS: H37Rv EVs isolated by Exosome detection via the ultrafast-isolation system.

Table S1: The primer sequences used in this study

Gene name	Gene Bank accession number	Primer	Sequences (5'⟶ 3')
TLR2	NM_011905	Forward Primer	CTCTTCAGCAAACGCTGTTCT
		Reverse Primer	GGCGTCTCCCTCTATTGTATTG
IL-6	NM_031168	Forward Primer	CTGCAAGAGACTTCCATCCAG
		Reverse Primer	AGTGGTATAGACAGGTCTGTTGG
TNF-α	NM_013693	Forward Primer	CCTGTAGCCCACGTCGTAG
		Reverse Primer	GGGAGTAGACAAGGTACAACCC

Table S3: The protein content of H37Rv EVs

	Protein content (mg)		
DC	1.425	1.3	1.5
EI	8.75	8.2	8
EXODUS	1.35	1.45	1.65

Table S4. Cell viability of Raw264.7 cells after co-incubation with three types of H37Rv EVs for 24 h at 5 μg.

OD_{450}		**24h**	
RAW264.7	1.19	1.12	0.90
Differential Centrifugation	0.59	0.57	0.46
Exosome Isolation Kit	0.62	0.61	0.66
EXODUS	0.69	0.57	0.59

Table S5. Q-PCR of TLR2, IL-6, TNF-α in RAW264.7 cell infected by three types of H37Rv EVs for 4 h at 5 μg.

Types of Mtb EVs	**Compare categories**		**TLR2**			**IL-6**			**TNF-α**	
Differential Centrifugation	Ct	24.78	24.50	24.80	25.84	25.69	25.88	21.76	20.99	21.10
	$2^{-\Delta\Delta Ct}$	1.35	1.24	1.33	424.48	470.75	414.68	2.40	4.11	3.81
	P value		*P*<0.01			*P*<0.000 1			*P*<0.05	
	Up or Down		Up			Up			Up	
Exosome Isolation Kit	Ct	23.99	23.57	24.30	25.97	26.34	26.16	20.94	20.97	20.99
	$2^{-\Delta\Delta Ct}$	1.77	2.37	1.43	295.22	228.98	259.19	3.22	3.16	3.11
	P value		*P*<0.05			*P*<0.001			*P*<0.000 1	
	Up or Down		Up			Up			Up	
EXODUS	Ct	24.98	24.47	24.29	25.76	26.03	26.34	20.42	20.49	19.91
	$2^{-\Delta\Delta Ct}$	1.46	2.08	2.35	560.78	464.39	374.55	7.56	7.19	10.75
	P value		*P*<0.05			*P*≤0.001			*P*<0.01	
	Up or Down		Up			Up			Up	

Table S6. ELISA of IL-6, TNF-α in RAW264.7 cell infected by three types of H37Rv EVs for 4 h at 5 μg.

Expression of IL-6/TNF-α		**IL-6**			**TNF-α**	
Raw264.7	2.32	2.66	4.09	264.56	260.89	265.79
Differential Centrifugation	673.78	682.04	617.94	3446.43	3156.64	3231.34
Exosome Isolation Kit	427.90	433.26	459.74	3765.06	4446.41	2869.49
EXODUS	695.76	700.55	668.31	4766.22	5040.23	4892.98

Table S7: Avg Radiant Efficiency values of brain

Radiant Efficiency values	**brain (10^7)**		
1 h	1.76	1.56	1.32
4 h	2.08	1.39	1.83
8 h	2.06	1.50	1.92
12 h	1.85	1.86	2.04
24 h	1.58	1.39	1.68

Table S8: Fluorescence acquisition values of homogenised brain and liver

Fluorescence acquisition values	brain			liver		
blank	453	494	643	1062	3398	2048
8 h	528	598	674	4161	5716	3811
12 h	1065	678	729	7896	7513	4946
24 h	568	571	649	2510	4125	2518