

Inactivated mice IECs: *E. falciformis* sporozoites (1×10^6 : 2×10^6)

Exo-clear medium, 18hours, 37°C , $5\%\text{CO}_2$

Cell culture medium (350ml)

Serial Centrifugation

1000xg, 5min

2000xg, 10min

5000xg, 20min

10000g, 30min

Ultracentrifugation, 120,000xg, 18hr

Supernatant

0.22 μm filter

Ultra-concentration (10kDa)

SDS-PAGE

Pellet

Density gradient ultracentrifugation

(5-40% Optiprep density gradient)

0.99

1.12

1.123

1.144

1.165

1.168

1.179

g/cm^3

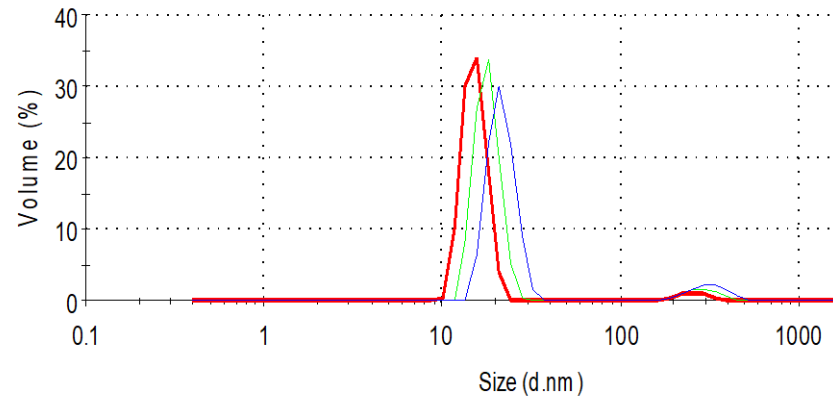
Fraction ~ 0.6ml

*Ef*SEVs

.NTA .TEM .LC-MS/MS .Protein microarray

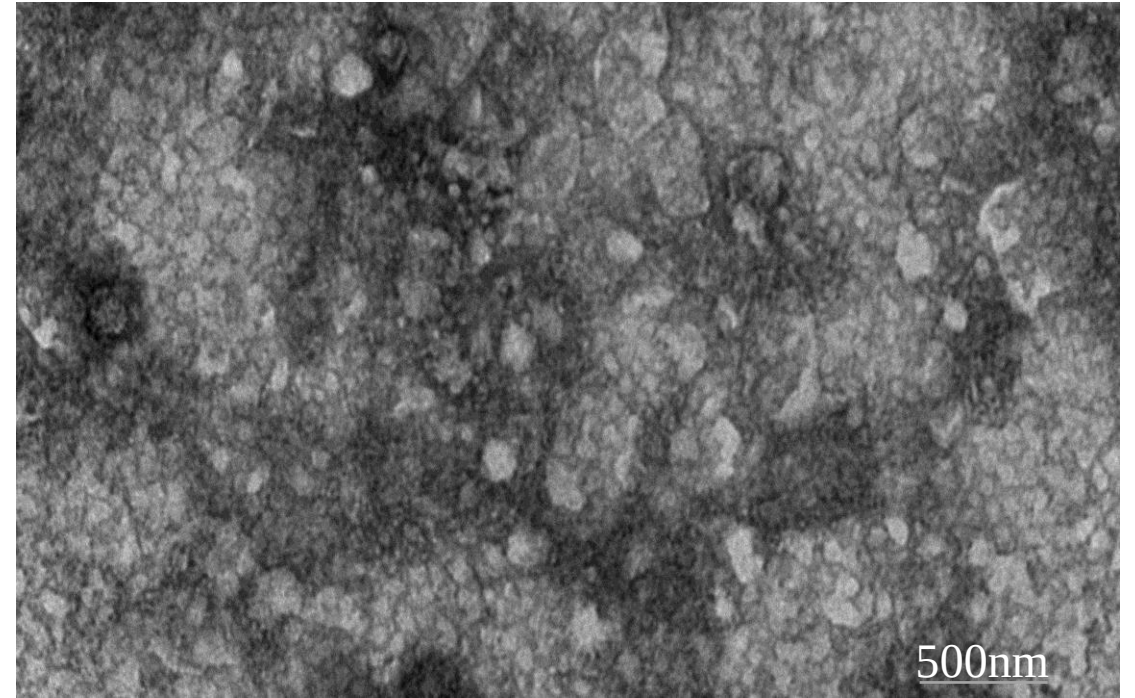
.Cytokine regulation

a



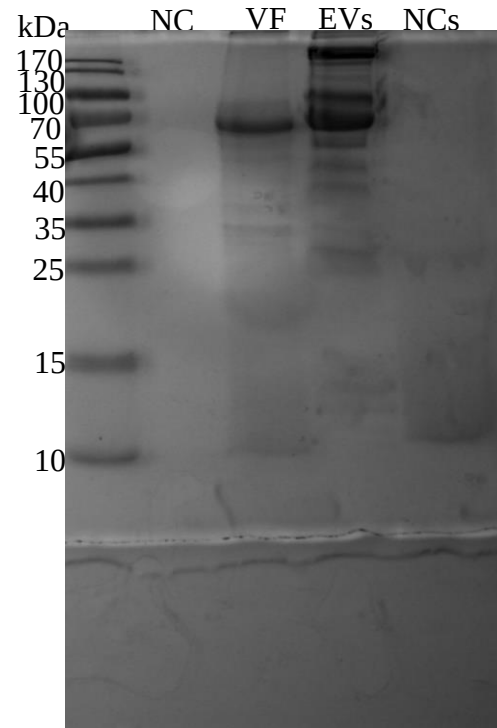
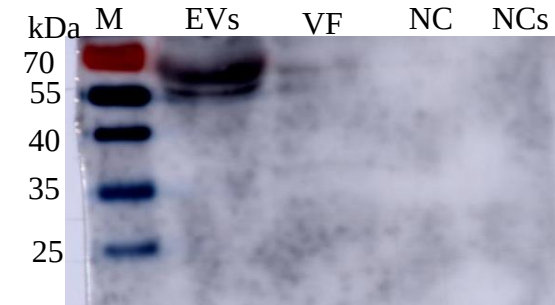
NTA pictogram of particles derived from non-treatment control experiment (NC). Size 9.70-43.36nm with density between 0.57-1.104gcm⁻³

b



TEM image of particles in non-treatment control experiment (NC)

Figure 2-2: (a) NTA measurement of the NC particles (b) TEM image of NC particles

a**b****KEY:**

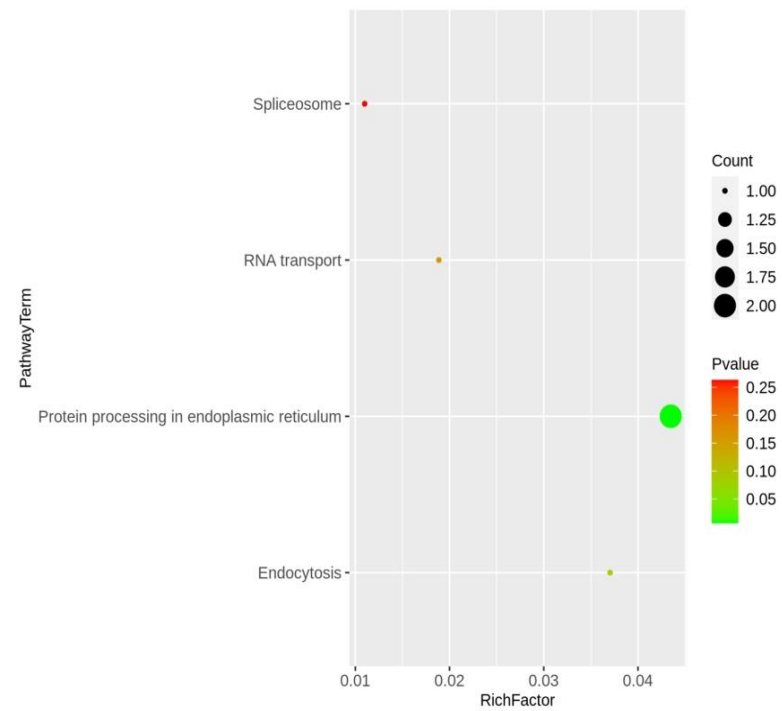
NC: ODG particles from NC

NCs: supernatant from NC

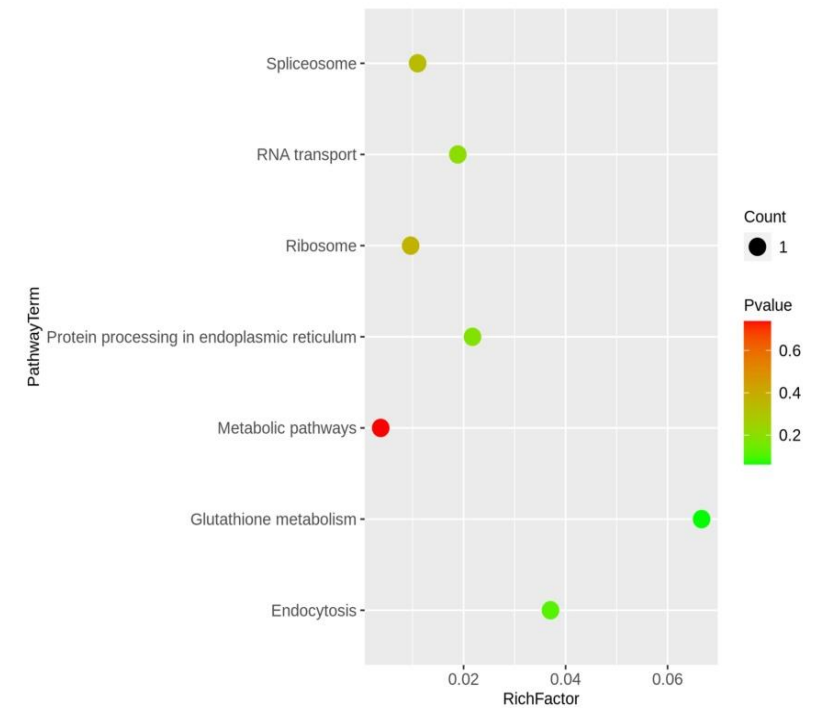
VF: vesicles free proteins from parasite culture supernatant

EVs: EVs pellets from parasite culture

Figure 2-3: (A) SDS-PAGE gels from parasite culture and control experiment (B) Western blot PVDF membrane



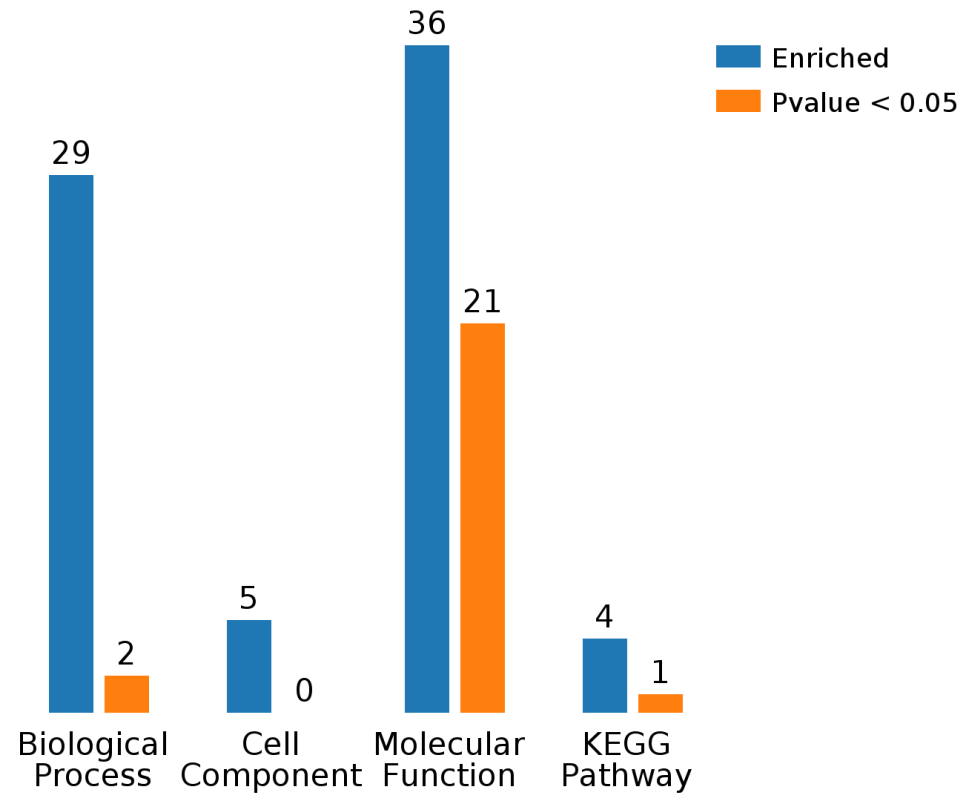
***E. falciformis* Vesicle Free Pathway**



***EfEVs* pathway**

Figure 2-4: KEGG pathway for identified proteins from EVs and vesicle free proteins

E. falciformis VF proteins



*Ef*SEV proteins

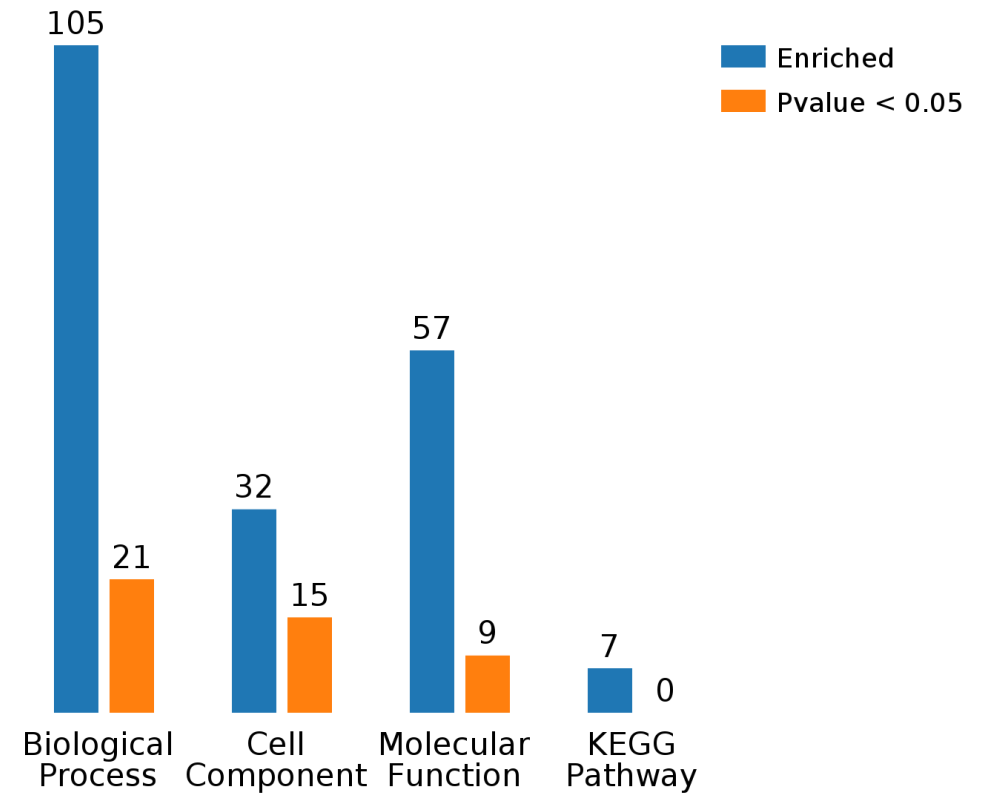


Figure 2-5: statistical summary and KEEG analysis for protein Identified from *E. falciformis* EVs and VF fraction

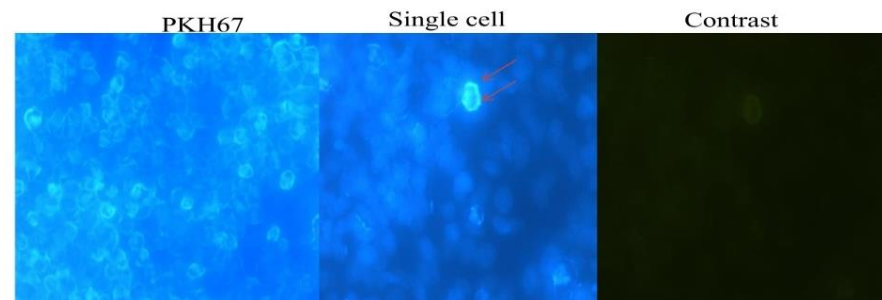
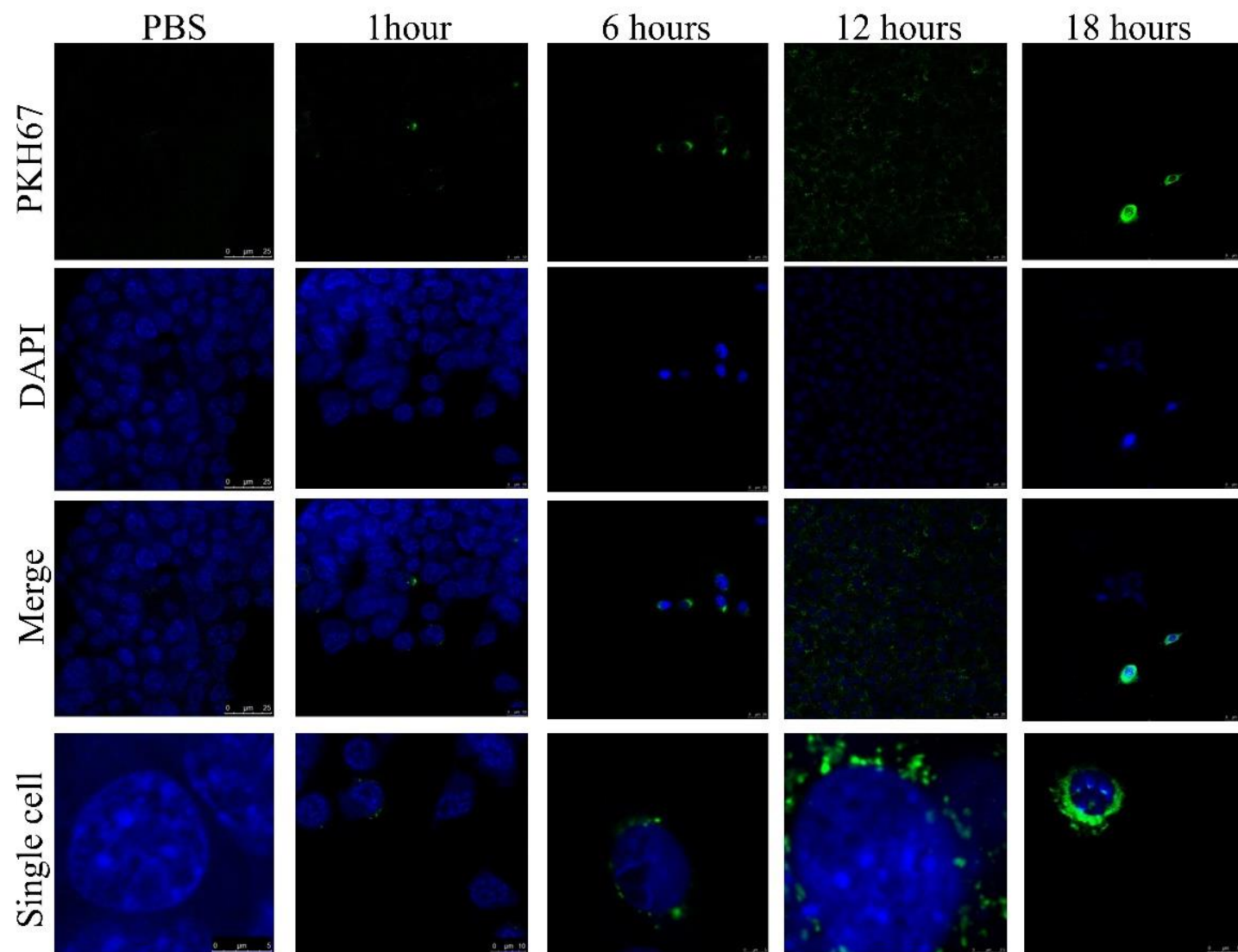


Figure 2-6: Internalization of *Ef*SEVs by murine intestinal epithelial cells. Murine IECs were incubated with green fluorescent PKH67-labelled *Ef*SEVs and processed after 0, 1, 6 and 12 hours for imaging by scanning confocal microscopy; green, labelled EVs; blue, cell nuclei. The negative control was MIECs treated with PBS-PKH67

Table 2-1: Primer sequence for cytokine, chemokine and inflammasome genes

Mouse Gene	Genebank ID	Direction	Sequence (5' to 3')	Expected size	Temp
IL-6	M24221.1	F	GCTTAATTACACATGTTCTCTGGGAAA	93	60
		R	CAAGTGCATCATCGTTGTTTCATAC		
mcp1	NM_011333.3	F	AGCCAACTCTCACTGAAG	190	60
		R	TCTCCAGCCTACTCATTG		
IL-1 β	NM_008361	F	CCAAAGAAGAAGATGGAAAAACG	113	60
		R	TCTGCTTGTGAGGTGCTGATGTA		
β -actin	NM_007393.5	F	TGGGGTGTTGAAGGTCTC	121	58-61
		R	CTACAATGAGCTGCGTGTG		
IL-18	NM_008360	F	AGGACACTTTCTTGCTTGCCA	183	60
		R	ACAGGCGAGGTCATCACAAG		
casp 11	NM_175362	F	TTTCAAGTCAAGGGGCACGA	233	60
		R	AAGGCATCAAACCCACAGCT		
IL-17	U43088.1	F	CCCTCAGACTACCTCAACCGTTC	192	61
		R	AGGCTCCCTCTTCAGGACCAG		
NLRP6	NM_133946	F	ATACGCGGCTTCTCAGACAAA	237	60
		R	GGTGATGAAGAGCAGGTACACA		