SUPPLEMENTARY DATA

Supplementary Figure Legends

Figure S1. PMVs from THP-1 cells expose phosphatidylserine and carry actin. A) Flow cytometry analysis of PMVs labelled with annexin-V-PE (Guava technologies) or annexin-V-PE with 5 mM EGTA (as indicated). PMVs without annexin-V-PE were used as control (gray). EGTA was used to inhibit annexin-V-PE binding to phosphatidylserine since their interaction has been shown to be Ca^{2+}-dependent. B) Logarithmic forward and sited scatter of the PMVs labelled with annexin-V-PE. C) Western blotting of PMVs from THP-1 cells with monoclonal antibodies against actin. D) *T. cruzi* protein extract (corresponding to $5.0x10^6$ parasites) from epimastigotes (Epi) or metacyclic trypomastigotes (Meta) were obtained by cycles of freezing and thawing and incubated with THP-1 cells ($1.0x10^6$) for 60 minutes at 37ºC for PMV induction. Afterwards, cell supernatants were analysed by flow cytometry for PMV quantification. NI = non-induced. E) Flow cytometry analysis of PMVs from mouse plasma. a) Histogram of the logarithmic forward and reverse scatter of PMVs obtained from mouse plasma (by differential centrifugation). The Gate R1 shows the diversity on size and granularity of the PMVs. b) Histogram of the Gate R1 (from a), analysed by forward scatter and FITC log. Note the absence of positive PMVs for FITC in this sample (unlabelled, negative control). c) The same Gate R1 (from a) analysed with a sample labelled with annexin-V-FITC. In this case there is a positive population to annexin-V-FITC (see Gate R2). The Gate R2 contains annexin-V positive PMVs, and it is used to other analysis with cell surface markers. d) Histogram of the sample labelled with annexin-V, inside of the Gate

R2, analysed for forward scatter and APC log. The sample is a negative control (isotype IgG-APC). e) Another sample labelled with annexin-V, as the one inside of the Gate R2 (demonstrated in c), but now labelled with CD184-APC. Note that the sample now is positive for APC. This sample correspond to annexin-V-FITC and CD184-APC PMVs. f) Histogram showing the overlay of the sample annexin-V-FITC positive CD184-APC negative (red, from d), and the sample Annexin-V-FITC and CD184-APC positive (Green, from e).

Figure S2. THP-1 cells release PMVs upon contact with *T. cruzi*. A) Flow cytometry analysis of THP-1 cells and *T. cruzi* metacyclic trypomastigotes supernatant reveals that PMVs originate from THP-1 cells. *a*) PKH26 (green fluorescent dye)-labeled THP-1 cells were incubated for 1h at 37°C in PBS and the supernatant was collected and analysed by flow cytometry. b) PKH26-labelled THP-1 cells were incubated in PBS with 5% NHS for 1h at 37°C (for induction of PMVs release) and the supernatant was collected and analysed. c) *T. cruzi* was labelled with PKH67 (red fluorescent dye) and the supernatant collected after 1h incubation in PBS at 37°C for flow cytometry analysis. d) PKH67-labelled *T. cruzi* were incubated in PBS with 5% NHS for 1 h at 37°C and the supernatant analysed. Note the increase in green fluorescence (*in b*) indicating PMVs release from THP-1 cells. For *T. cruzi* no significant increase in red fluorescence was detected (*in d*). e) *T. cruzi* (PKH67 labelled) and THP-1 cells (PKH26 labelled) were incubated together at 37°C for 1h and the supernatant analysed by flow cytometry. Note that there is a predominant increase in green fluorescence (indicating the release of THP-1 cells-derived PMVs) and negligible detection of red fluorescence. For the above experiments THP-1

cells ($1x10^6$/well) were labelled with 4 μM PKH26 (green fluorescence dye), while *T. cruzi* metacyclic trypomastigotes were labelled with 4 μM PKH67 (red fluorescence dye) at RT for 5 min with shaking. They were washed 5 times with PBS to remove excess of dyes. B) Vero cells were pre-incubated with annexin-V AlexaFluor 488 (green) prior to *T. cruzi* metacyclic trypomastigotes (ratio = 5:1, parasites to cells) addition and incubation for 30 minutes at 37°C (activated cells). As control cells were incubated without parasites (resting cells). Cells were analysed by fluorescence microscopy. DAPI was used to stain nucleus (blue). Note the increase in green fluorescence in activated cells indicating transient exposition of phosphatidylserine (detected by annexin-V AlexaFluor 488) and the release of PMVs (also in green) as indicated by arrowheads. C) Transmission electron microscopy (*a-d*) of *T. cruzi* incubated in 50% NHS for 1h at 37°C. Note the intact parasite surface and that no vesicles can be detected on the cell surface. D) Sucrose gradient to analysis PMVs and exosomes. PMVs and exosomes migrate in different fractions in a sucrose gradient confirming their different characteristics. 20 ml of THP-1 cells supernatant were ultracentrifuged at 100.000xg for 16h to collect PMVs and exosomes. They were resuspendend in 500μl of PBS buffer and added to the top of 10-40% continuous sucrose gradient. They were centrifuged for 100.000xg for 1h, 1 ml fractions were collected and 100 μl of each fraction were analysed by flow cytometry. For PMV labelling it was used annexin-V-FITC and for exosome labelling it was used LAMP-1-FITC. An IgG-FITC was used for labelling control. Note that fractions 1-4 were positive for LAMP-1 while fractions 8-10 were positive for annexin-V-FITC. E) Electron microscopy to analyse fractions separated by sucrose gradient (from D). Fractions were pooled in two groups: Positive for LAMP-1-

FITC (1-4) and positive for annexin-V-FITC (7-10). Fractions positive for annexin-V-FITC (*upper panel*) containing vesicles with size between 250-500 nm (bar = 250 nm), which correspond to PMVs. While fractions positive for LAMP-1-FITC (1-4, *lower panel*) presented vesicles with size between 40-100 nm (bar = 100 nm), which correspond to exosomes. Note the *cup-like* shape of exosomes.

Figure S3. A) PMVs bind to complement factors on the *T. cruzi* surface and inhibit complement-mediated lysis. A) PMVs inhibit metacyclic trypomastigotes lysis in conditions nearly fisiological: 50% NHS at 37ºC. *Left,* PMVs were obtained from PBMCs after *T. cruzi* induction and used in complement-mediated lysis assay. Incubations were performed for 60 minutes and with $1.5x10^5$ PMVs/ml. *Right,* similar experiment was performed with PMVs obtained from Jurkat cells. B) PMVs bind to complement factors deposited on the *T. cruzi* surface. Metacyclic trypomastigotes adsorbed on ELISA plates were incubated for 30 minutes at 37 ºC with either 10% NHS or Hepes buffer. After washing, biotinylated PMVs were added (as indicated) and incubated for 2 hours at 37 ºC. Streptavidin-HRP was added to detect PMVs, and reactions were developed with ABTS peroxidase solution prior to spectrophotometric measurements at 405 nm. C) Binding of PMVs to complement factors on the *T. cruzi* surface is inhibited by polyclonal anti-C4 antibodies. Metacyclic trypomastigotes adsorbed on ELISA plates were incubated with 10% NHS or buffer (as in A). After washing, polyclonal anti-C2, anti-C4 and anti-egg-ovalbumin (as control) antibodies were added and incubated for 2 hours at RT. After that, biotinylated PMVs were added and incubated for 2 hours at 37 ºC. Detection was performed as in A. D) *T. cruzi*

metacyclic trypomastigotes ($1.0x10^5$) were incubated with biotinylated PMVs at ratios 1:1, 1:5 and 1:10 (parasites : PMVs) for 30 minutes at 37º C. Afterwards, they were washed with PBS and incubated for 5, 15 and 60 minutes at 37º C or non-incubated (time 0). After that, they were fixed with ice-cold paraformaldehyde 4%, washed with PBS and bound to ELISA plates. PMVs bound to the parasites surface were detected using streptavidin-HRP and developed with ABTS peroxidase. Data from A-D represent the mean ± SD from at least three experiments performed in triplicate. * $p<0.05$, *** $p<0.001$.

Figure S4. TGF-ß-bearing PMVs induce *T. cruzi* invasion of eukaryotic cells. A) PBMCs-derived PMVs induce increased *T. cruzi* invasion of Vero cells. Ratio=10:1, parasites to cells; PMVs = $1.0x10^5$/ml. B) PMVs induce *T. cruzi* invasion in a dose-dependent fashion. Metacyclic trypomastigotes (strain Silvio X10/6) were incubated with Vero cells (r=10:1 parasites to cells) in the presence of increasing concentrations of THP-1-derived PMVs ($1.0\text{-}10x10^5$/ml) for 3 hours at 37ºC. C) PMVs induce *T. cruzi* invasion of HeLa cells. Metacyclic trypomastigotes (strain Silvio X10/6) were incubated with HeLa cells (r=5:1, parasites to cells) in the presence of THP-1-derived PMVs ($1.0x10^5$ PMVs/ml) for 3 hous at 37ºC. D) PMVs induce *T. cruzi* invasion of eukaryotic cells independent of the parasite strain. Metacyclic trypomastigotes (strain Cl Brener) were incubated with Vero cells (r=5:1, parasites to cells) in the presence of $1.0x10^5$ PMVs/ml (from THP-1 cells) for 3 hours at 37ºC. E) PMVs effect on invasion depends on the parasite infective stage. Invasion was performed with metacyclic trypomastigotes and epimastigotes in the presence of PMVs ($1.0x10^5$ PMVs/ml) from THP-1 cells for 3 hours

at 37ºC. F) *T. cruzi* carry PMVs bound to its surface to invade the cells. Invasion assays were performed as follows: 1) Vero cells pre-incubated and maintained with PMVs ($1.0x10^5$/ml) during the invasion (-), 2) cells pre-incubated with PMVs ($1.0x10^5$/ml, for 30 minutes), which were removed prior cell invasion (PMVs-R), and 3) metacyclic trypomastigotes were pre-incubated (30 minutes at 37ºC) with PMVs ($1.0x10^5$/ml) and washed to remove unbound PMVs prior cell invasion (PMVs-PI). G) PMVs effect on invasion is inhibited by anti-TGF-β antibodies. PMVs ($1.0x10^5$/ml) were pre-treated with polyclonal anti-TGF-β antibodies before invasion assay. H) The TGF-β receptor antagonist SB431542 inhibits PMV enhancement of *T. cruzi* invasion. 10μM of SB431542 were added to the cells for 30 minutes prior invasion assays. As control was performed cell invasion without PMVs (Control). For all experiments were quantified the number of intracellular parasites. Data from A-H represent the mean ± SD from at least three experiments performed in triplicate. I) PMVs carry TGF-ß associated to their surface. *Left,* ELISA measurements of TGF-ß levels in intact or lysed PMVs ($1.0x10^5$, PMVs were lysed with Triton-X100 1% in PBS). Note that the amount of TGF-ß does not change after PMVs lysis indicating that PMVs do not carry extra TGF-ß in the intravesicular space. *Right,* ELISA measurements of MIF (macrophage migration inhibitory factor) levels in intact or lysed PMVs ($1.0x10^5$). Note that the amount of MIF increase after PMVs lysis indicating that PMVs carry MIF mainly in the intravesicular space. J) *Upper panel,* Flow cytometry analysis of TGF-ß (*left*) and MIF (*right*) in THP-1 cells. *Lower panel,* Flow cytometry analysis of TGF-ß (*left*) and MIF (*right*) in PMVs. Note that TGF-ß is detected on PMVs surface, while MIF are not detected. K) THP-1 cells were labelled with anti-TGF-ß antibodies (followed by FITC-labelled secondary

antibodies for TGF-ß detection) and cells induced for PMVs release. The PMVs-containing supernatant was analysed by fluorescence microscopy. *Insert,* THP-1 cells labelled with anti-TGF-ß showing the presence of TGF-ß on the cell surface. F) As in E, but with anti-MIF antibodies (*Insert,* THP-1 cells labelled with anti-MIF antibodies showing a low detection of surface associated MIF). Note that there is a low number of anti-MIF-labelled PMVs (green) in the supernatant compared with anti-TGF-ß-labelled PMVs (green, panel E), confirming that the PMVs carry the cell-derived TGF-ß mainly on their surface instead of carrying it in the intravesicular space (as occur for MIF).

Figure S1

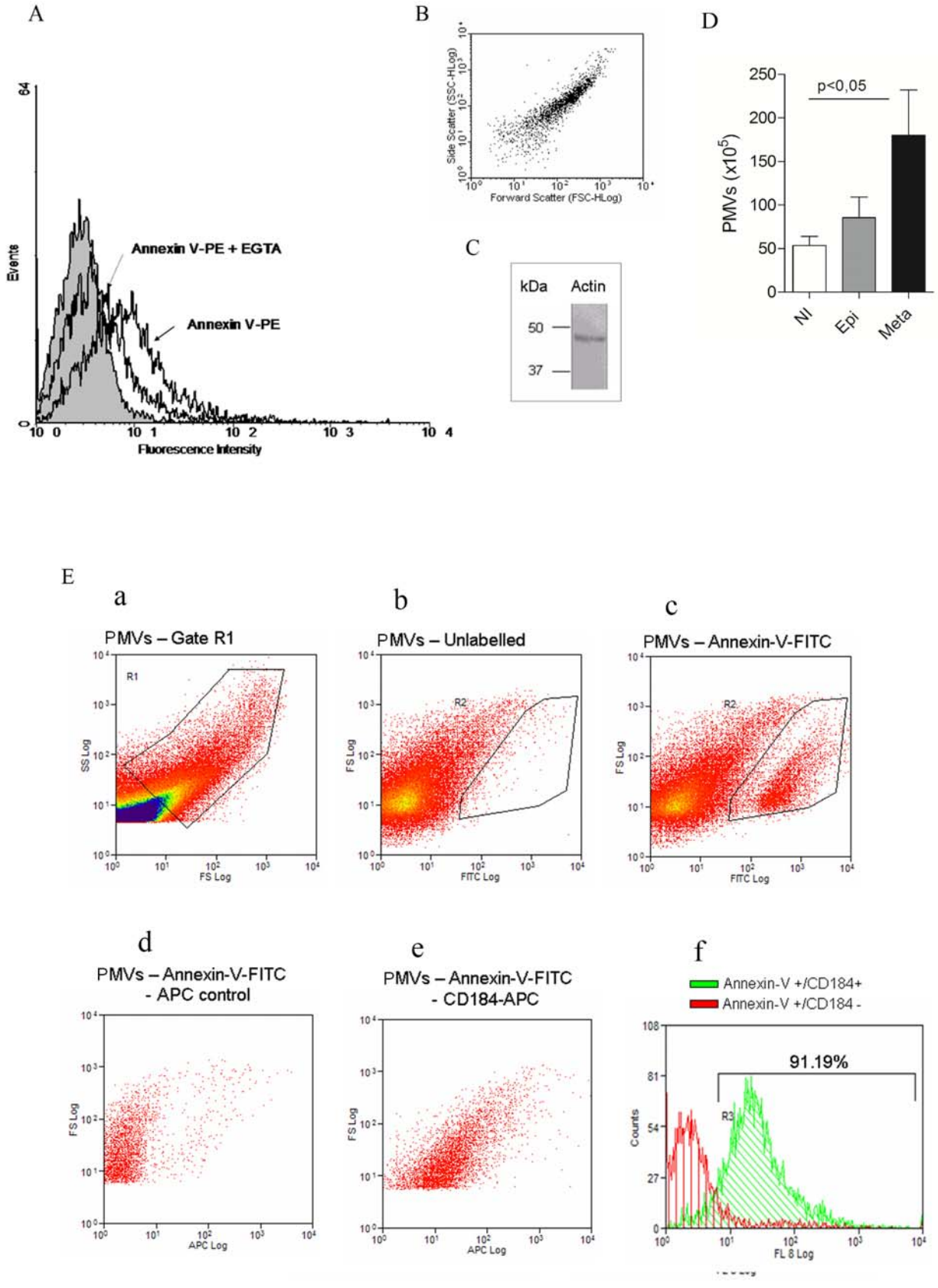
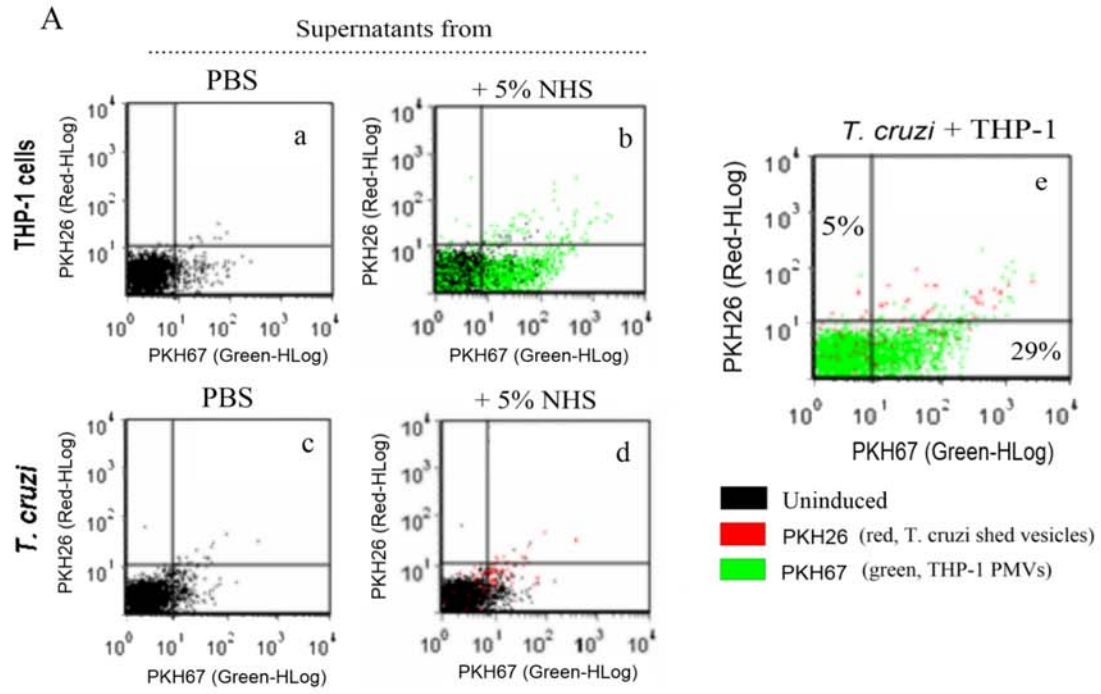
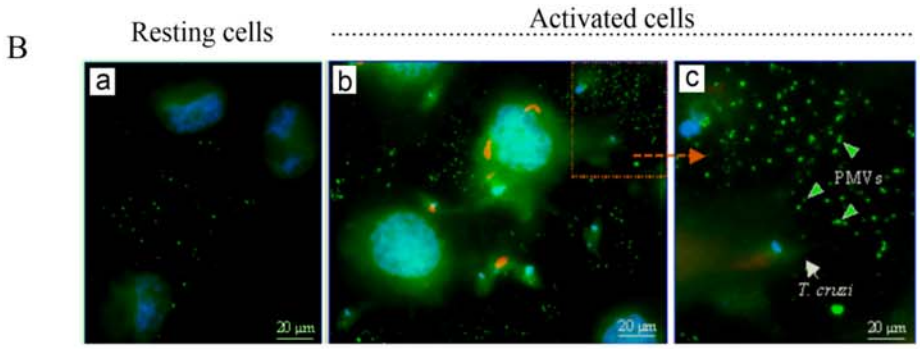
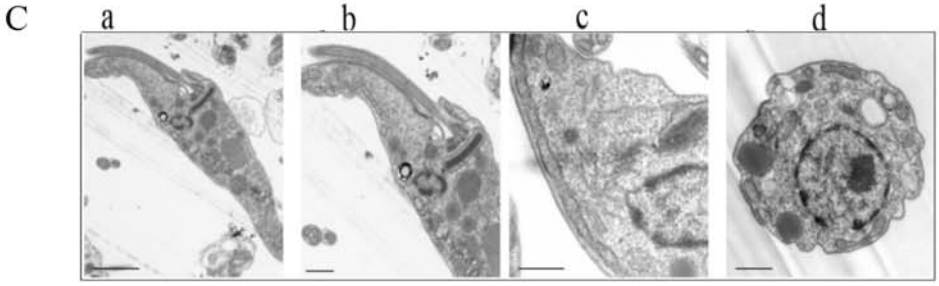
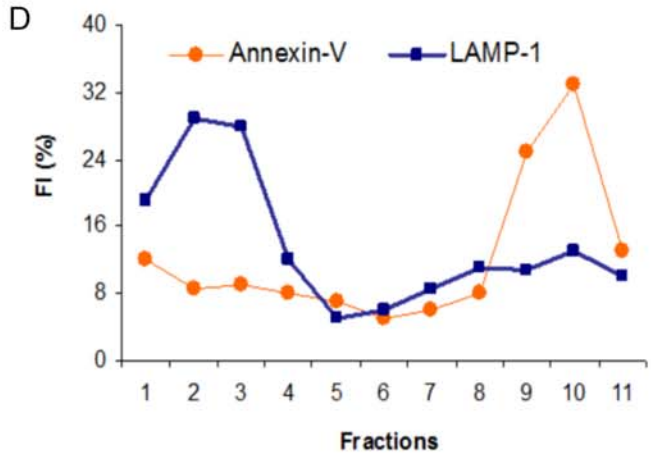

A
Annexin V-PE + EGTA
Annexin V-PE
Events
Fluorescence Intensity
B
Side Scatter (SSC-HLog)
Forward Scatter (FSC-HLog)
C
kDa
Actin
50
37
D
PMVs (x10^5)
p<0,05
NI
Epi
Meta
E
a
PMVs – Gate R1
R1
SS Log
FS Log
b
PMVs – Unlabelled
R2
FS Log
FITC Log
c
PMVs – Annexin-V-FITC
R2
FS Log
FITC Log
d
PMVs – Annexin-V-FITC
- APC control
FS Log
APC Log
e
PMVs – Annexin-V-FITC
- CD184-APC
FS Log
APC Log
f
Annexin-V +/CD184+
Annexin-V +/CD184 -
91.19%
R3
Counts
FL 8 Log

Figure S2


A
Supernatants from
PBS
+ 5% NHS
THP-1 cells
T. cruzi
PKH26 (Red-HLog)
PKH67 (Green-HLog)
a
b
c
d
T. cruzi + THP-1
e
5%
29%
Uninduced
PKH26 (red, T. cruzi shed vesicles)
PKH67 (green, THP-1 PMVs)


B
Resting cells
Activated cells
a
b
c
PMVs
T. cruzi
20 µm


C
a
b
c
d


D
Annexin-V
LAMP-1
FI (%)
Fractions

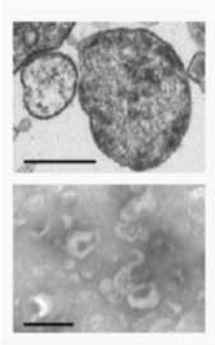

E

Figure S3

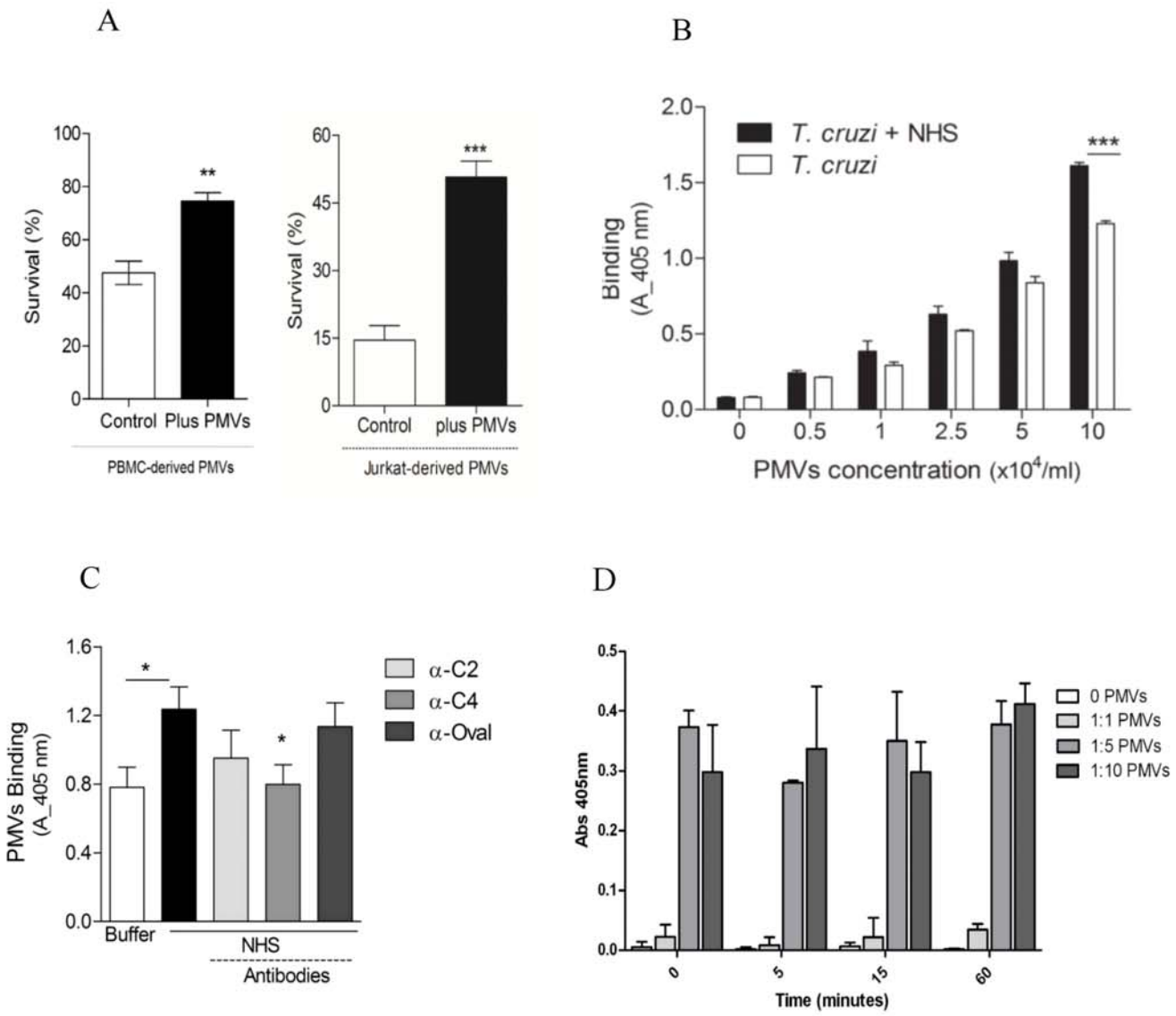
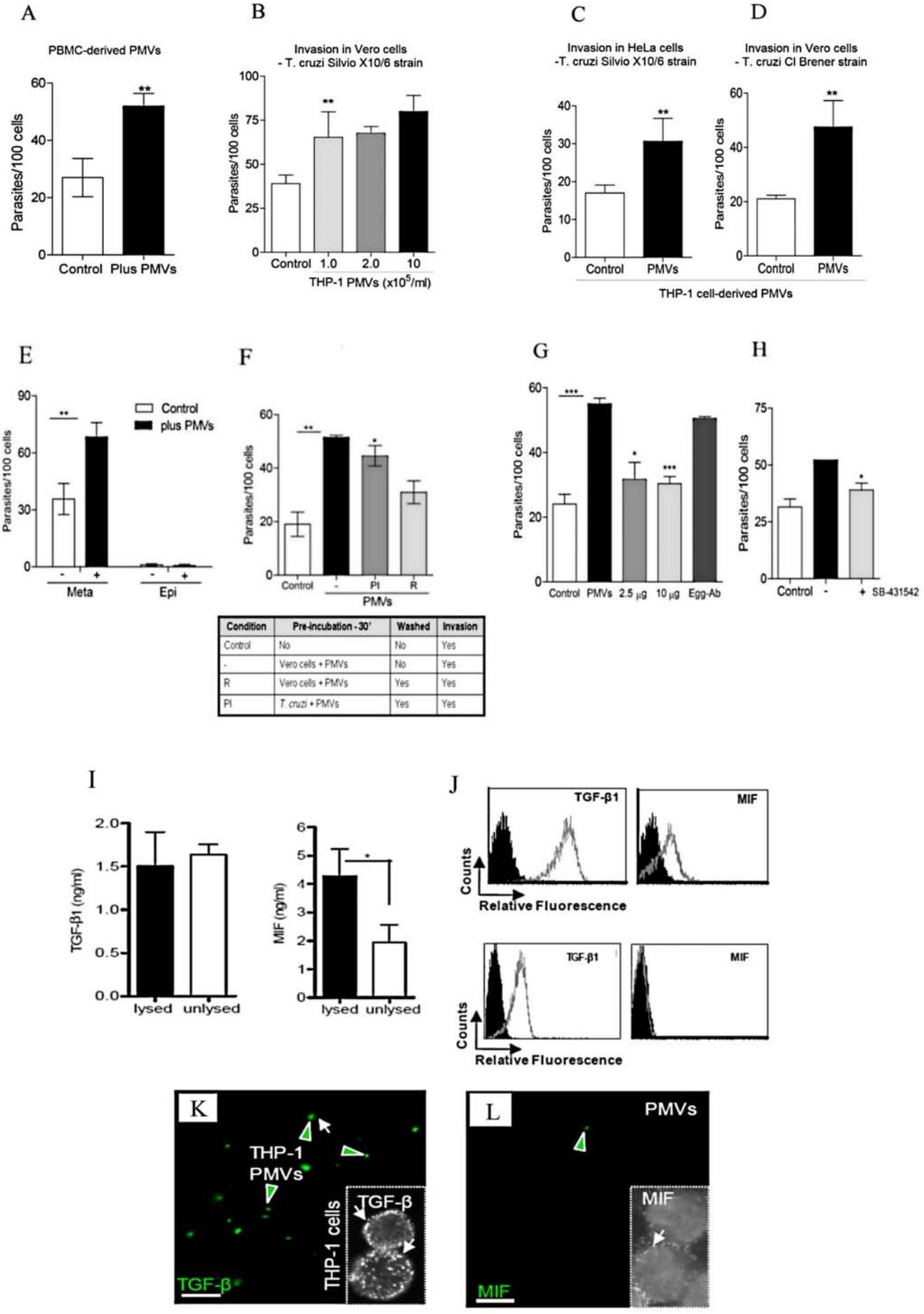

A
Survival (%)
100
80
60
40
20
0
**
Control
Plus PMVs
PBMC-derived PMVs
Survival (%)
60
45
30
15
0
***
Control
plus PMVs
Jurkat-derived PMVs
B
Binding (A_405 nm)
2.0
1.5
1.0
0.5
0.0
T. cruzi + NHS
T. cruzi
***
0
0.5
1
2.5
5
10
PMVs concentration (x10^4/ml)
C
PMVs Binding (A_405 nm)
1.6
1.2
0.8
0.4
0.0
*
*
α-C2
α-C4
α-Oval
Buffer
NHS
Antibodies
D
Abs 405nm
0.5
0.4
0.3
0.2
0.1
0.0
0 PMVs
1:1 PMVs
1:5 PMVs
1:10 PMVs
0
5
15
60
Time (minutes)

Figure S4



Condition	Pre-incubation - 30'	Washed	Invasion
Control	No	No	Yes
-	Vero cells + PMVs	No	Yes
R	Vero cells + PMVs	Yes	Yes
PI	*T. cruzi* + PMVs	Yes	Yes