# Antisense Oligonucleotides Targeting Parasite Inositol 1,4,5-Trisphosphate Receptor Inhibits Mammalian Host Cell Invasion by *Trypanosoma cruzi*

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## Supplementary information

## Supplementary Figure Legends

**Figure S1 | Life span of TcIP$_3$R protein in *Trypanosoma cruzi* epimastigotes.**

Full-length image of western blots of epimastigotes probed with anti-$TcIP_3R$ antibody (A) and with anti-tubulin antibody (B). *T. cruzi* epimastigotes were incubated in LIT medium supplemented with 200 μg/mL cycloheximide (CHX) for the indicated time, collected by centrifugation at 1,500 x g for 10 min at 4ºC, and lysed by sonication in PBS supplemented with protease inhibitor cocktail (cOmplete Mini, Roche Diagnostics K.K., Tokyo, Japan). The cell extracts were separated by SDS-PAGE and transferred to a PVDF membrane (Immobilon®-P, EMD Millipore Corporation, Billerica, MA). Western blots were probed with anti-$TcIP_3R$ antibody or with anti-tubulin antibody, reacted with AP-conjugated secondary antibody, and developed using Immobilon® Western AP substrate (Milipore). Imaging was processed using ChemiDoc™ XRS+ system (Bio-Rad Laboratories, Inc., Tokyo, Japan). M; molecular weight marker. A dashed line indicates a cropping area in Figure 1A. High background signals in panel A are due to the enhanced exposure for detection of the native, full-length $TcIP_3R$ and likely due to the cross-reaction of anti-$TcIP_3R$ antibody with degraded $TcIP_3R$.

**Figure S2 | Life span of EGFP-$TcIP_3R$ and EGFP in the transgenic *T. cruzi* epimastigotes.** Full-length image of western blots of epimastigotes overexpressing EGFP-$TcIP_3R$ fusion protein (A and B) or EGFP (C and D). Epimastigotes overexpressing EGFP-$TcIP_3R$ were incubated with 200 μg/mL CHX for the indicated time, collected by centrifugation, and lysed by sonication. The cell extracts were separated by SDS-PAGE and transferred to a PVDF membrane. The resulting membrane was cut horizontally into 2 pieces; one with higher molecular weight was probed with anti-$TcIP_3R$ antibody (A) and the other with low molecular weight was reacted with anti-tubulin antibody (B), followed by chemiluminescent detection using

SuperSignal® West Dura Extended Duration Substrate (Thermo Fisher Scientific) and Immobilon® Western AP substrate (Milipore), respectively. Imaging was performed using LAS4000 (GE Healthcare Japan Corporation, Tokyo, Japan). Likewise, epimastigotes overexpressing EGFP were processed as above, and western blots were probed with anti-EGFP antibody (C) and anti-tubulin antibody (D) and developed colorimetrically. M; molecular weight marker. See also legends for Supplementary Figure S1.

**Figure S3 | Expression levels of TcIP$_3$R in *T. cruzi* trypomastigotes.** Full-length image of western blots of trypomastigotes probed with anti-TcIP$_3$R antibody (A) and with anti-tubulin antibody (B). Western blots were processed essentially as in Figure S1. Note that TcIP$_3$R was undetectable. High background signals in panel A are due to the enhanced exposure for detection of the native, full-length TcIP$_3$R and likely due to the cross-reaction of anti-TcIP$_3$R antibody with degraded TcIP$_3$R. M; molecular weight marker. See also legends for Supplementary Figure S1.

**Figure S4 | Inhibition of EGFP-TcIP$_3$R expression in the transgenic *T. cruzi* trypomastigotes by treatment with phosphorothioate antisense TcIP$_3$R oligonucleotide.** Trypomastigotes overexpressing EGFP-TcIP$_3$R were treated with either Antisense 5995 or the complementary Sense 5995S oligonucleotide for the indicated time. Western blots were processed essentially as in Figure S2 and probed with anti-EGFP antibody (A) or anti-tubulin antibody (B). The lysate of EGFP-TcIP$_3$R-expressing epimastigotes without treatment was loaded as expression control. M; molecular weight marker.

Figure S1

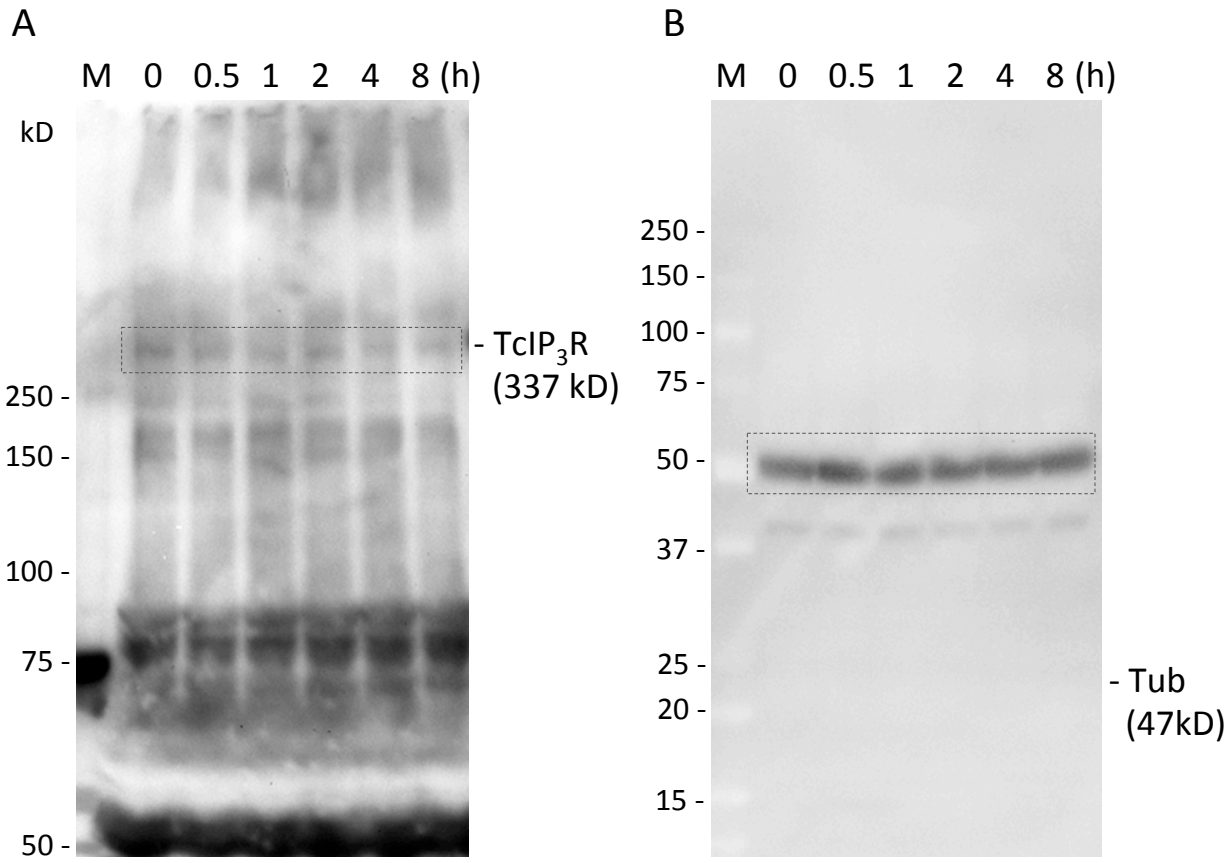

A
M 0 0.5 1 2 4 8 (h)
kD
250 -
150 -
100 -
75 -
50 -
- TcIP3R
(337 kD)
B
M 0 0.5 1 2 4 8 (h)
250 -
150 -
100 -
75 -
50 -
37 -
25 -
20 -
15 -
- Tub
(47kD)

Figure S2

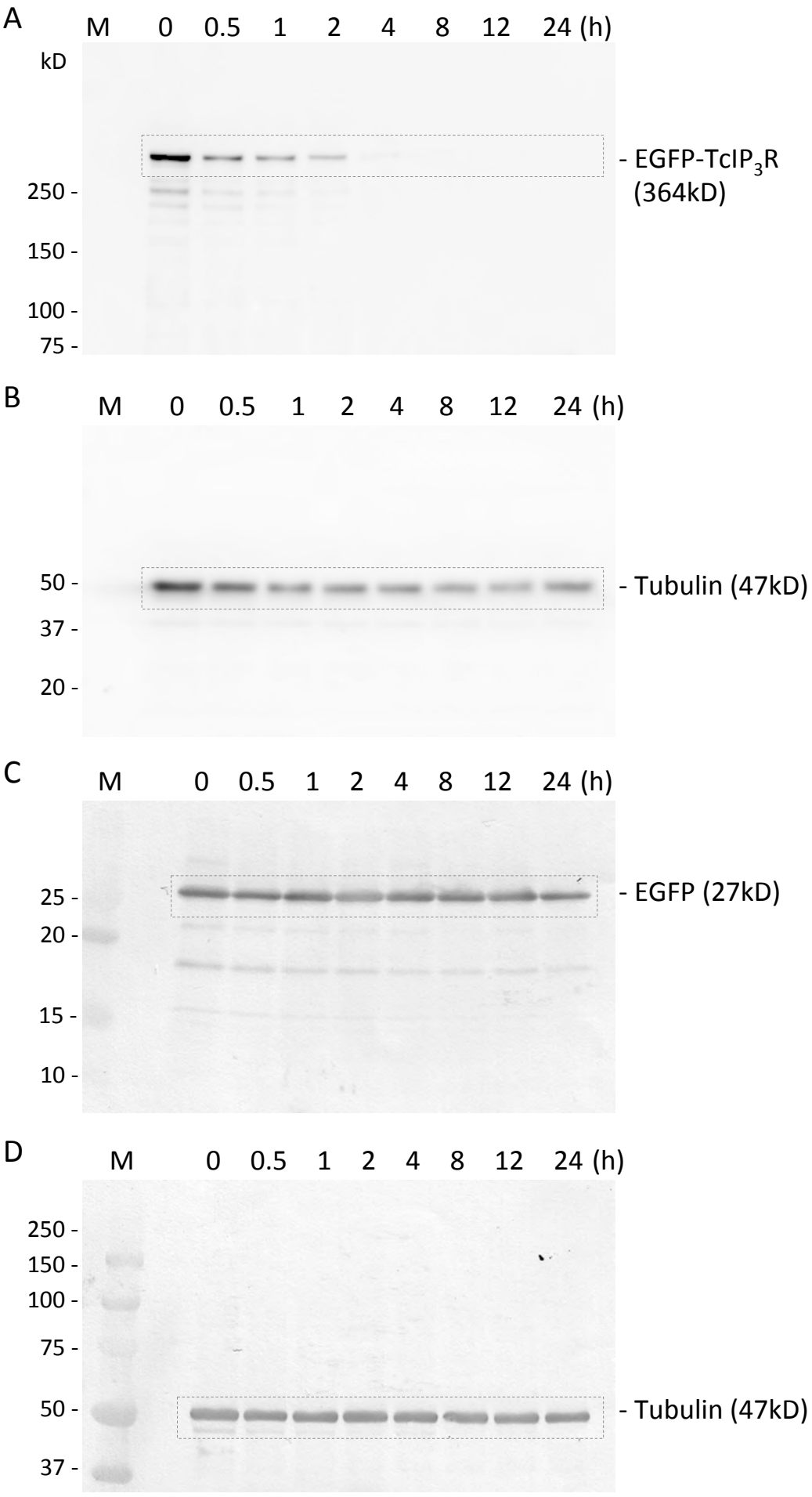

A
M 0 0.5 1 2 4 8 12 24 (h)
kD
250 -
150 -
100 -
75 -
- EGFP-TcIP3R (364kD)
B
M 0 0.5 1 2 4 8 12 24 (h)
50 -
37 -
20 -
- Tubulin (47kD)
C
M 0 0.5 1 2 4 8 12 24 (h)
25 -
20 -
15 -
10 -
- EGFP (27kD)
D
M 0 0.5 1 2 4 8 12 24 (h)
250 -
150 -
100 -
75 -
50 -
37 -
- Tubulin (47kD)

Figure S3

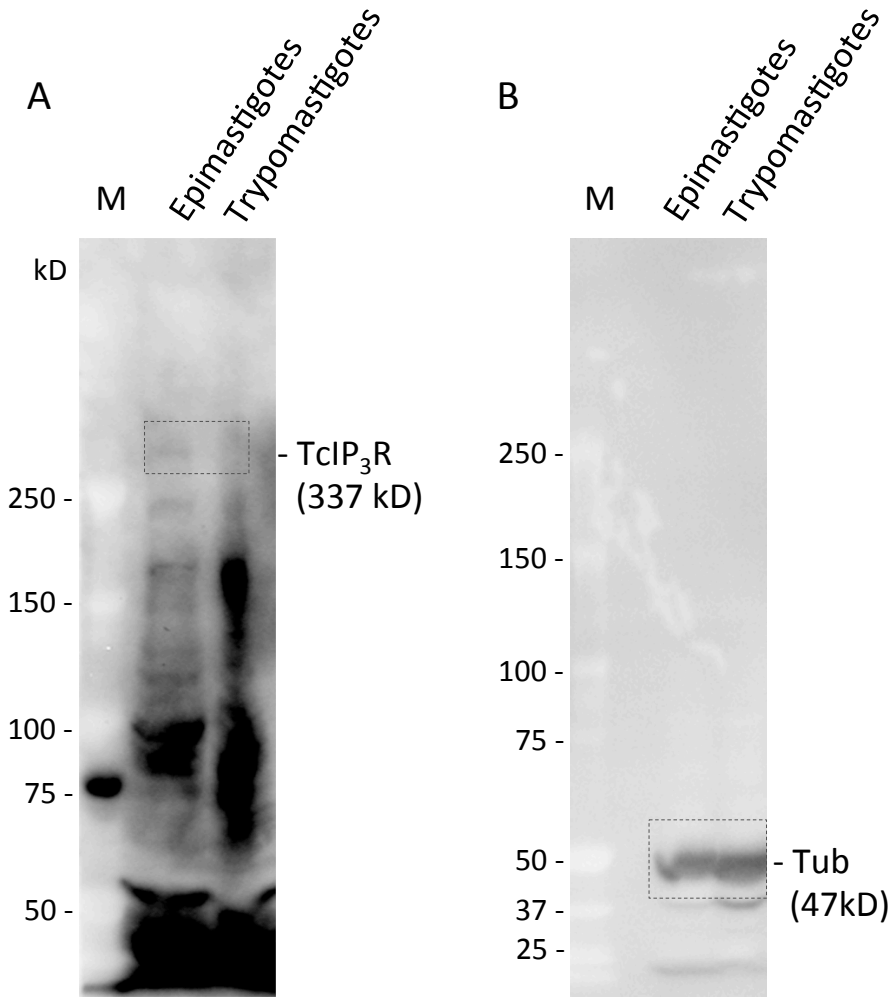
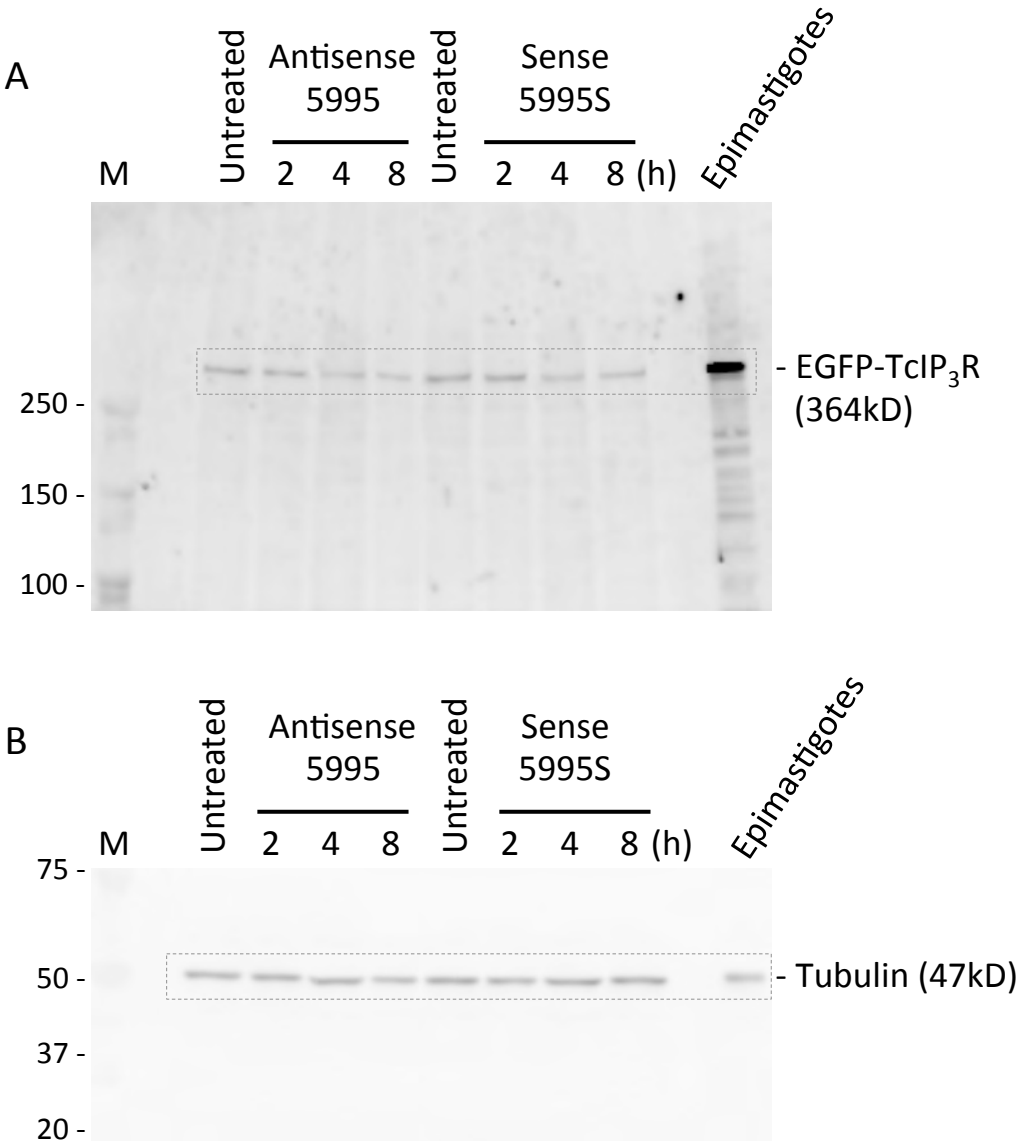

A
M
Epimastigotes
Trypomastigotes
kD
250 -
150 -
100 -
75 -
50 -
- TcIP3R
(337 kD)
B
M
Epimastigotes
Trypomastigotes
250 -
150 -
100 -
75 -
50 -
37 -
25 -
- Tub
(47kD)

Figure S4


A
M
Untreated
Antisense 5995
2 4 8
Untreated
Sense 5995S
2 4 8 (h)
Epimastigotes
250 -
150 -
100 -
- EGFP-TcIP3R (364kD)
B
M
Untreated
Antisense 5995
2 4 8
Untreated
Sense 5995S
2 4 8 (h)
Epimastigotes
75 -
50 -
37 -
20 -
- Tubulin (47kD)