Appendix:

a) Materials and reagents

All the chemicals used in this study were of the analytical grade. Media components for bacterial cultures were purchased either from Hi-Media Laboratories Ltd, India or Difco, USA. Media and transfection reagents for mammalian cells and Leishmania parasite cultures were from Gibco BRL / Invitrogen USA. Restriction enzymes were procured either from New England Biolabs, USA, Boerhringer Mannheim Germany or Promega, USA. Primary antibodies were procured from Sigma or ICN USA. Antibodies against Leishmania actin were raised in rabbits, and then purified from the anti-sera by affinity chromatography using the recombinant Leish-actin immobilized on Sepharose CL 6B. The antibiotics and drugs used in cultures including ampicillin, kanamycin, chloramphanicol, streptomycin, gentamycin, Latrunculin B and cytochalasin D were either from Sigma chemical company, USA or Invitrogen USA. Other chemicals and kits were from Qiagen and MBI Fermentas, USA.

b) Culture strains and cell lines

Mammalian cells BHK21 and J774A.1 were maintained in high glucose DMEM containing 40 μg/ml gentamycin supplemented with 10% (v/v) heat inactivated Fetal Bovine Serum at 37°C. L. donovani, L. major and L. tropica were maintained also in the same media but at 25°C. L. tropica was grown in Brain Heart Infusion Blood Agar plates as and when required in bulk. Bacterial strains DH5α and BL21 (DE3) were cultured in Luria Bertani medium and maintained on LB-agar plates with or without antibiotics.
c) Plasmids

For T/A cloning, InSt/A vectors were used (MBI Farmentas). Cloning for bacterial expression system was done in pET-22b, pET41b and pET42b vectors (Novagen USA) where as for mammalian cell expression, coronin was subcloned in eGFPC1 vector (Clontech). For expression of coronin in Leishmania cells, it was subcloned in pXG-GFP+21 Leishmania expression vector (Kindly provided by Prof. S.M.Beverley)

d) Oligonucleotides

All the oligonucleotides (primers) used for PCR amplification were purchased from SIGMA, Chemgene or Imperial biomedics.

e) Medium and buffer compositions

**LB (Luria- Bertani) medium**

Yeast extract powder 0.5%
Tryptone 1.0%
Sodium chloride 1.0%

(Ampicillin was added to LB to a final concentration of 100 μg/ml for the selection of the plasmids wherever needed. For preparing solid medium 2% agar was added).

**Dulbacco's Modified Eagle's Medium**

DMEM powder 9.8g
Sodium Pyruvate 120mg
Sodium Bicarbonate 1.2g
Gentamycin 40mg
FBS 100ml
Triple distilled water 900ml

(After mixing all components, media was filtered through 0.22μ membrane filter and stored at 4°C)
ATV solution

NaCl 136mM  
KCl 5.3mM  
Dextrose 5.5mM  
NaHCO₃ 6.9mM  
EDTA 0.5mM  
Trypsin (1:250) 0.05%  

TE Buffer (pH 8.0)
Tris-Cl 10 mM  
EDTA 1 mM  

TAE buffer
Tris acetate 40 mM  
EDTA (pH 8.0) 1mM  

DNA loading dye (6X)
Orange G 0.25% (w/v)  
Bromophenol Blue 0.25% (w/v)  
Xylene cyanol 0.25% (w/v)  
Glycerol 30.0% (v/v)  

Solution I
Glucose 50 mM  
Tris-Cl (pH 8.0) 25 mM  
EDTA (pH 8.0) 10 mM  
(Autoclaved for 15 min at 10 lb./sq. in. in liquid cycle and stored at 4°C).  

Solution II
NaOH 0.2 N (freshly diluted from 10 N stock)  
SDS 1.0% (w/v)  

Solution III
5 M potassium acetate 60 ml  
Glacial acetic acid 11.5 ml  
Water 28.5 ml  
(Autoclaved for 15 min at 10 lb./sq. in. in liquid cycle and stored at 4°C. The resulting solution is 3M with respect to potassium and 5 M with respect to acetate).
CaCl$_2$ 0.1 M  
(Solution was filter sterilized and stored at 4°C)

Glycerol solution 60% (v/v)  
(Sterilized by autoclaving and stored at 4°C).

10 X TE (pH-8.0)  
Tris-HCl 100 mM  
EDTA 10 mM  
(The solution was autoclaved and stored at room temperature).

Phenol : Chloroform : Isoamyl alcohol (25:24:1)  
(mixed by volumes in a amber colored bottle and stored at 4°C)

SDS-Sample buffer (5X)  
Tris-HCl (pH 6.8) 250 mM  
B-mercaptoethanol 100mM  
SDS 10% (w/v)  
Glycerol 50% (v/v)  
Bromophenol blue 0.05% (w/v)  
SDS 20% (w/v)  
(Prepared in MilliQ water and stored at room temperature)

Acrylamide solution (250 ml )  
Bis-acrylamide 1.0% (w/v)  
Acrylamide 30.0% (w/v)  
(Filtered and stored at 4°C).

Tank Buffer for SDS (1000 ml)  
Tris 3 gms  
Glycine 18.8 gms  
20%SDS 5 ml  
(Stored at room temperature).

Resolving gel (10%) SDS- PAGE (10.0ml)  
Water (autoclaved) 2.81 ml  
1.5 M Tris-HCl (pH 8.8) 3.73 ml  
Acrylamide stock (30%) 3.33 ml  
SDS (20%) 50 µl
TEMED 20 μl
APS 20 μl

**Stacking gel (5%) SDS-PAGE (5.0ml)**

- Water 3.19 ml
- 1M Tris-HCl (pH 6.8) 0.88 ml
- Acrylamide 0.84 ml
- SDS (20%) 50 μl
- TEMED 20 μl
- APS 20 μl

**Phosphate Buffered Saline (PBS)**

- NaCl 0.137M
- KCL 0.027M
- Na₂HPO₄ 0.032M
- K₂HPO₄ 0.015M
- KH₂PO₄ 0.015M

**RIPA buffer**

- Triton X-100 1.0ml
- Sodium Deoxycholate 0.5g
- SDS 0.1g
- PBS 99.0ml

**Carbonate Buffer (250ml) (pH-9.5)**

- Na₂CO₃ 0.393g
- NaHCO₃ 0.735g

**Citrate Buffer (250ml) (pH-5.5)**

- Citric Acid 0.73g
- Na₂HPO₄ 0.94g

**Electroporation buffer for transfection of Leishmania.**

- HEPES 21 mM
- NaCl 137mM
- KCI 5mM
- NaH₂PO₄ 0.7mM
- Glucose 6mM