

Functional Characterization of HOPS Complex in *Leishmania*

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**KUSUMA SCHOOL OF BIOLOGICAL SCIENCES
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Functional Characterization of HOPS Complex in *Leishmania*

by

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KUSUMA SCHOOL OF BIOLOGICAL SCIENCES

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Dedicated to my family.....



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CERTIFICATE

This is to certify that the thesis titled “**Functional Characterization of HOPS Complex in *Leishmania***”, being submitted by **Mr. Vikas Dhar Dubey** in the Indian Institute of Technology Delhi for the award of the degree of “**Doctor of Philosophy**” is a record of the bonafide research carried out by him, which has been prepared under my supervision and guidance in conformity with rules and regulation of the Indian Institute of Technology Delhi, India. The results prescribed in it have not been submitted in part or full to any other University or Institute for the award of any Degree/Diploma.

5th June 2024

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Vikas Dhar Dubey

Abstract

Previously, we have shown that *Leishmania* endocytosed hemoglobin (Hb) by specific receptor (HbR) and traffic to lysosome via early endosomes in Rab5 and Rab7 dependent process. However, the mechanism of transition from endosomes to lysosomes is unknown. Therefore, we have characterized the role of HOPS complex in this process. Here, we have cloned and expressed all components of HOPS complex (LdVps11, LdVps16, LdVps18, LdVps33, LdVps39 and LdVps41) homologue from *Leishmania* to understand their role in endocytosis in *Leishmania*. We have found that RFP-LdVps41 co-localize with GFP-LdVps11, GFP-LdVps16, GFP-LdVps18, GFP-LdVps33 and GFP-LdVps39 in Ld-Rab7 positive and LysoTracker labelled late endosome and lysosome compartments in *Leishmania*. Using *in-vitro* protein interaction, we have found that the LdVps41 specifically interacts with LdVps11, LdVps16, LdVps18, LdVps33 and LdVps39. LdVps11 specifically binds with LdVps16, LdVps18 and LdVps39 but not with LdVps33 while LdVps16 specifically interacts with LdVps18 and LdVps33 but not with LdVps39. Thus, LdVps41 is found to be the central molecule in HOPS complex interacting with various subunits to form HOPS complex in *Leishmania*. Interestingly, we have found that LdVps41 interacts with all other HOPS complex proteins via its C-terminal domain (CTD-LdVps41⁹⁰¹⁻¹⁴⁴⁶). In contrast, we have found that N-terminal domain of LdVps41 (NTD-LdVps41¹⁻⁵⁰⁰) specifically binds with GTP form of LdRab7 and LdHbR. These results suggest that LdVps41 possibly acts as tethering molecule between LdHbR and LdRab7 to mediate the transport of Hb from endosomes to the lysosomes in *Leishmania*.

We have overexpressed LdVps41 truncated proteins in *Leishmania* to determine their role in the Hb trafficking in the parasite. We have found that transgenic parasites overexpressing GFP-NTD-LdVps41¹⁻⁵⁰⁰ inhibit the transport of Hb to the lysosomes as it does not interact with other components of HOPS complex. Similarly, trafficking of Hb to the lysosomal compartment is also blocked in GFP-CTD-LdVps41⁹⁰¹⁻¹⁴⁴⁶ overexpressed transgenic parasites as this mutant fails to recruit LdRab7 and LdHbR. As Hb is essential for the survival of parasites within macrophages, we have found that transgenic parasites overexpressing GFP-NTD-LdVps41¹⁻⁵⁰⁰ or GFP-CTD-LdVps41⁹⁰¹⁻¹⁴⁴⁶ show growth defect in macrophages as parasites fail to acquire heme from lysosomal degradation of Hb. In the present investigation, we have identified and functionally characterized the role HOPS complex in Hb trafficking to lysosomes and have found that HOPS complex function is necessary for the survival of the parasites.

सारांश

इससे पहले, हमने दिखाया है कि विशिष्ट रिसेप्टर (एच. बी. आर.) द्वारा लीशमैनिया एंडोसाइटोसिड हीमोग्लोबिन (एच. बी.) को एलडी-रैब5 और एलडी-रैब7 आश्रित प्रक्रिया में प्रारंभिक एंडोसोम के माध्यम से लाइसोसोम तक स्थानांतरित करता है। हालाँकि, एंडोसोम से लाइसोसोम में स्थानान्तरण की क्रियाविधि अज्ञात है। इसलिए हमने इस प्रक्रिया में हॉप्स कॉम्प्लेक्स की भूमिका का वर्णन किया है। यहां हमने लिशमैनिया में एंडोसाइटोसिस प्रक्रिया में भूमिका निर्वहन करने वाले सभी घटकों की भूमिका को समझने के लिए लिशमैनिया से हॉप्स कॉम्प्लेक्स (एलडीवीपीएस11, एलडीवीपीएस16, एलडीवीपीएस18, एलडीवीपीएस33, एलडीवीपीएस39, एलडीवीपीएस41) के सजातीय सभी घटकों को क्लोन और व्यक्त किया है। हमने पाया है कि आरएफपी-एलडीवीपीएस41 जीएफपी-एलडीवीपीएस11, जीएफपी-एलडीवीपीएस16, जीएफपी-एलडीवीपीएस18, जीएफपी-एलडीवीपीएस33 और जीएफपी-एलडीवीपीएस39 के साथ एलडी-रैब7 पॉजिटिव और लाइसोट्रेकर चिह्नित लेट एंडोसोम और लाइसोसोम कक्षों में लीशमैनिया में सह-स्थानीयकृत है। इन-विट्रो प्रोटीन इंटरैक्शन का उपयोग करते हुए, हमने पाया है कि एलडीवीपीएस41 विशेष रूप से एलडीवीपीएस11, एलडीवीपीएस16, एलडीवीपीएस18, एलडीवीपीएस33 और एलडीवीपीएस39 के साथ अंतःक्रिया करता है। एलडीवीपीएस11 विशेष रूप से एलडीवीपीएस16, एलडीवीपीएस18 और एलडीवीपीएस39 के साथ जुड़ता है, लेकिन एलडीवीपीएस33 के साथ नहीं, जबकि एलडीवीपीएस16 विशेष रूप से एलडीवीपीएस18 और एलडीवीपीएस33 के साथ अंतःक्रिया करता है, लेकिन एलडीवीपीएस39 के साथ नहीं। इस कारणवश, एलडीवीपीएस41 को हॉप्स कॉम्प्लेक्स का केंद्रीय अणु पाया गया है। जो विभिन्न उपइकाइयों के साथ अंतःक्रिया करके लिशमैनिया में हॉप्स कॉम्प्लेक्स का निर्माण करता है। हमने अपनी शोध में यह रोचक निष्कर्ष भी प्राप्त किया कि एलडीवीपीएस41 अपने C-टर्मिनल डोमेन (सीटीडी-एलडीवीपीएस41⁹⁰¹⁻¹⁴⁴⁶) के माध्यम से अन्य सभी HOPS कॉम्प्लेक्स प्रोटीन के साथ अंतःक्रिया करता है। इसके विपरीत, हमने पाया है कि एलडीवीपीएस41 (एनटीडी-एलडीवीपीएस41¹⁻⁵⁰⁰) का एन-टर्मिनलडोमेन विशेष रूप से एलडीएचबीआर और एलडी-रैब7 के GTP फॉर्म से जुड़ता है। प्रेक्षित परिणाम यह प्रस्तावित करते हैं कि एलडीवीपीएस41 संभवतः लिशमैनिया में एंडोसोम से लाइसोसोम तक Hb के स्थानांतरण में मध्यस्थता करने के लिए एलडीएचबीआर और एलडी-रैब7 के बीच बंधन अणु के रूप में कार्य करता है। हमने लिशमैनिया में एलडीवीपीएस41 खंडित किए गए प्रोटीन को ओवरएक्सप्रेस किया जिससे लिशमैनिया में एचबी की ट्रांस्फेकिंग में उनकी भूमिका का निर्धारण हो सके। तदनुसार यह भी पाया गया है कि जीएफपी-एनटीडी-एलडीवीपीएस41¹⁻⁵⁰⁰ को ओवरएक्सप्रेस करने वाले ट्रांसजेनिक लिशमैनिया लाइसोसोम में Hb के परिवहन को रोकते हैं क्योंकि वे HOPS कॉम्प्लेक्स के अन्य घटकों के साथ अंतःक्रिया नहीं करते हैं। तदनुसार, जीएफपी-सीटीडी-एलडीवीपीएस41⁹⁰¹⁻¹⁴⁴⁶ ओवरएक्सप्रेस्ड ट्रांसजेनिक

लीशमैनिया में भी लाइसोसोमल कक्ष में एचबी की ट्रैफ़िकिंग अवरुद्ध है क्योंकि यह उत्परिवर्ती एलडीएचबीआर और एलडी-रैब7 को रिक्रूट करने में विफल है। चूंकि एचबी मैक्रोफेज के भीतर लीशमैनिया के उत्तरजीविता के लिए आवश्यक है, हमें यह ज्ञात हुआ है कि जीएफपी-एनटीडी-एलडीवीपीएस41¹⁻⁵⁰⁰ अथवा जीएफपी-सीटीडी-एलडीवीपीएस41⁹⁰¹⁻¹⁴⁴⁶ को अधिक व्यक्त करते हुए ट्रांसजेनिक लीशमैनिया मैक्रोफेज में संख्या में वृद्धि दोष दिखाते हैं, क्योंकि लीशमैनिया एचबी के लाइसोसोमल खंडन से हीम प्राप्त करने में विफल रहते हैं। वर्तमान अध्ययन में हमने लाइसोसोम में एचबी ट्रैफ़िकिंग में हॉप्स कॉम्प्लेक्स की भूमिका की पहचान की है और कार्यात्मक रूप से इसकी विशेषता बताई है तथा पाया है कि हॉप्स कॉम्प्लेक्स प्रक्रिया लीशमैनिया के अतिजीवन के लिए आवश्यक है।

Table of Contents

Abbreviations

1.0 Introduction

Introduction.....	1
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2.0 Review of Literature

2.1. Leishmaniasis.....	3
2.1.1. Life cycle of <i>Leishmania</i>	3
2.1.2 Leishmaniasis: Treatment and control	6
2.2 Various modes of endocytosis	8
2.2.1 Phagocytosis	10
2.2.2 Macropinocytosis.....	10
2.2.3 Clathrin mediated endocytosis.....	11
2.2.4 Caveolae mediated endocytosis	12
2.2.5 Clathrin and caveolae independent endocytosis	13
2.3. Intracellular trafficking.....	14
2.3.1 Role of the Rab GTPases in intracellular trafficking.....	14
2.3.2 Role of SNAREs in intracellular trafficking.....	17
2.3.2 Role of Vacuolar Protein Sorting (Vps) in intracellular trafficking	19
2.4. CORVET Complex.....	20
2.5. Retromer Complex.....	22
2.6. HOPS Complex.....	24
2.6.1 Discovery of HOPS complex.....	24
2.6.2 Composition of HOPS Complex.....	25
2.6.3 Role of HOPS complex in vesicle fusion	28
2.6.3.1 HOPS complex mediate homotypic and heterotypic fusion.....	29
2.6.3.2 HOPS Complex facilitates endosome maturation.....	32
2.6.3.3 HOPS complex controls direct transport between Golgi apparatus and Vacuole.....	33
2.6.3.4 HOPS Complex is essential for cell cycle maintenance.....	33

2.6.3.5 HOPS complex mediate autophagosome-lysosome fusion	34
2.6.3.6 HOPS complex regulate exosome secretion.....	35
2.7. Diseases associated with HOPS complex	36
2.8. Regulation of intracellular trafficking in Protozoan parasites	38
2.8.1 <i>Trypanosomes</i>	39
2.8.2 <i>Toxoplasma</i>	40
2.8.3 <i>Plasmodium</i>	42
2.8.4 <i>Leishmania</i>	44

3.0 Chapter 1

3.1. Introduction.....	48
3.2. Materials and methods	49
3.2.1. Materials	49
3.2.2. Cells	50
3.2.3. RNA isolation and cDNA synthesis	50
3.2.4. Cloning of LdVps11 from <i>L. donovani</i>	50
3.2.5. Cloning of LdVps16 from <i>L. donovani</i>	51
3.2.6. Cloning of LdVps18 from <i>L. donovani</i>	52
3.2.7. Cloning of LdVps33 from <i>L. donovani</i>	52
3.2.8. Cloning of LdVps39 from <i>L. donovani</i>	53
3.2.9. Cloning of LdVps41 from <i>L. donovani</i>	53
3.2.10. Expression and purification of HOPS Complex as GST- tagged/His ₆ -tagged fusion proteins	54
3.2.11. Generation of polyclonal antibody against LdVps33 and LdVps41	55
3.2.12. SDS PAGE and Western Blot.....	55
3.3. Results.....	56
3.3.1. Cloning of LdVps11 homologue from <i>L. donovani</i>	56
3.3.2. Expression and purification of recombinant LdVps11 of <i>L. donovani</i>	60
3.3.3. Cloning of LdVps16 homologue from <i>L. donovani</i>	61
3.3.4. Expression and purification of recombinant LdVps16 of <i>L. donovani</i>	64
3.3.5. Cloning of LdVps18 homologue from <i>L. donovani</i>	65
3.3.6. Expression and purification of recombinant LdVps18 of <i>L. donovani</i>	69
3.3.7. Cloning of LdVps33 homologue from <i>L. donovani</i>	70

3.3.8. Expression and purification of recombinant LdVps33 of <i>L. donovani</i>	73
3.3.9. Cloning of LdVps39 homologue from <i>L. donovani</i>	74
3.3.10. Expression and purification of recombinant LdVps39 of <i>L. donovani</i>	77
3.3.11. Cloning of LdVps41 homologue from <i>L. donovani</i>	78
3.3.12. Expression and purification of recombinant LdVps41 of <i>L. donovani</i>	82
3.3.13. Generation of anti-LdVps33 and anti-LdVps41 antibodies.....	83
3.4. Discussion	85

4.0 Chapter 2

4.1. Introduction.....	87
4.2. Materials & methods.....	88
4.2.1. Materials	88
4.2.2. Cells	89
4.2.3. Overexpression of HOPS Complex proteins in <i>Leishmania</i>	89
4.2.4. Subcellular localization of LdVps41 in <i>Leishmania</i>	90
4.2.5. Subcellular localization of all other HOPS complex proteins in <i>Leishmania</i>	91
4.2.6. In vitro protein-protein interaction assay	92
4.3. Results.....	92
4.3.1. Localization of HOPS complex proteins in <i>Leishmania</i>	92
4.3.2. Identification of HOPS complex positive compartment in <i>Leishmania</i>	94
4.3.3. Interaction of His ₆ -LdVps41 with other HOPS Complex proteins.....	97
4.3.4. Interaction of His ₆ -LdVps11 with other HOPS Complex proteins.....	99
4.3.5. Interaction of His ₆ -LdVps16 with other HOPS Complex proteins.....	100
4.3.6. Interaction of His ₆ -LdVps33 with other HOPS Complex proteins.....	101
4.4. Discussion	102

5.0 Chapter 3

5.1. Introduction.....	105
5.2. Materials & methods.....	106
5.2.1. Materials	106
5.2.2. Cells	105
5.2.3. Cloning of LdVps41 truncations from <i>L. donovani</i>	107
5.2.4. Expression and purification of LdVps41 truncations as GST- tagged/His ₆ - tagged fusion proteins	108

5.2.5. In vitro protein-protein interaction assay	109
5.2.6. Overexpression of truncated LdVps41 proteins in <i>Leishmania</i>	109
5.2.7. Labeling of hemoglobin with Alexa Flour-594	110
5.2.8. Intracellular trafficking of Alexa594-Hb in <i>L. donovani</i>	110
5.2.9. Quantification of intracellular growth of <i>Leishmania</i> in macrophages	111
 5.3. Results.....	 112
5.3.1. Identification of LdVps41 domain interacting with other subunits of HOPS complex in <i>Leishmania</i>	112
5.3.2. Identification of HOPS complex subunit interacting with Rab GTPases in <i>Leishmania</i>	120
5.3.3. Characterization of interaction between LdVps41 with LdRab7	124
5.3.4. Interaction of His ₆ -LdVps41 with Hemoglobin receptor in <i>Leishmania</i>	126
5.3.5. Determination of functional role of HOPS complex in hemoglobin endocytosis in <i>Leishmania</i>	129
5.3.6. Determination of the survival of transgenic <i>Leishmania</i> in macrophages.....	136
 5.4. Discussion	 138
 6.0 Summary	
Summary	142
 7.0 Bibliography	
Bibliography	147
 8.0 Appendices	
Appendices	163
 9.0 Biodata	
Biodata	168

LIST OF FIGURES

Number	Title	Page no.
Figure 1	Life cycle of <i>L. donovani</i>	4
Figure 2	Various modes of endocytosis	9
Figure 3	Schematic representation of key regulators of endosomal pathway	14
Figure 4	Prenylation and post-prenylation reactions of RAB GTPases	15
Figure 5	Intracellular localization of Rab proteins	16
Figure 6	Intracellular localization of SNARE proteins	17
Figure 7	Steps in SNARE assembly	18
Figure 8	Mechanism of SNARE mediated membrane fusion	19
Figure 9	Localization of different tethering complexes in endosomal system	20
Figure 10	The subunit arrangement of CORVET complex	22
Figure 11	Role of retromer of in intracellular trafficking	23
Figure 12	The overall structure of HOPS complex showing the interactions of different subunits within HOPS complex	27
Figure 13	A theoretical model for HOPS-mediated membrane tethering and fusion	30

LIST OF FIGURES

Number	Title	Page no.
Figure 14	A theoretical framework of prompt-fusion intermediates	31
Figure 15	Schematic representation of endosome maturation mediated by HOPS Complex	32
Figure 16	Various steps controlled by HOPS complex in endosomal pathway	35
Figure 17	Regulation of intracellular trafficking of internalized hemoglobin in <i>Leishmania</i>	45
Figure 18	Cloning of LdVps11 from <i>L. donovani</i>	58
Figure 19	Multiple sequence alignment of LdVps11 with mammalian and yeast orthologs	60
Figure 20	Expression and purification of LdVps11 as GST and His ₆ tagged protein	61
Figure 21	Cloning of LdVps16 from <i>L. donovani</i>	63
Figure 22	Multiple sequence alignment of LdVps16 with mammalian and yeast orthologs	63
Figure 23	Expression and purification of LdVps16 as GST and His ₆ tagged protein	65
Figure 24	Cloning of LdVps18 from <i>L. donovani</i>	67
Figure 25	Multiple sequence alignment of LdVps18 with mammalian and yeast orthologs	69
Figure 26	Expression and purification of LdVps18 as GST tagged protein	70

LIST OF FIGURES

Number	Title	Page no.
Figure 27	Cloning of LdVps33 from <i>L. donovani</i>	72
Figure 28	Multiple sequence alignment of LdVps33 with mammalian and yeast orthologs	72
Figure 29	Expression and purification of LdVps33 as GST tagged and His ₆ protein	75
Figure 30	Cloning of LdVps39 from <i>L. donovani</i>	75
Figure 31	Multiple sequence alignment of LdVps39 with mammalian and yeast orthologs	77
Figure 32	Expression and purification of LdVps39 as GST tagged protein	78
Figure 33	Cloning of LdVps41 from <i>L. donovani</i>	80
Figure 34	Multiple sequence alignment of LdVps41 with mammalian and yeast orthologs	82
Figure 35	Expression and purification of LdVps41 as GST tagged and His ₆ protein	83
Figure 36	Specificity of anti-LdVps33 polyclonal sera	84
Figure 37	Specificity of anti-LdVps41 polyclonal sera	85
Figure 38	Cloning of HOPS complex proteins in <i>Leishmania</i> expression vector	93
Figure 39	Overexpression of HOPS complex proteins in <i>Leishmania</i>	94

LIST OF FIGURES

Number	Title	Page no.
Figure 40	Subcellular localization of GFP-LdVps41 in <i>Leishmania</i>	95
Figure 41	Cloning of LdVps41 in pNUS-m-RFPnD vector	96
Figure 42	Co-transfection of LdVps41 with other HOPS complex proteins	97
Figure 43	Interaction of His ₆ -LdVps41 with other HOPS complex proteins	98
Figure 44	Interaction of His ₆ -LdVps11 with other HOPS complex proteins	99
Figure 45	Interaction of His ₆ -LdVps16 with other HOPS complex proteins	100
Figure 46	Interaction of His ₆ -LdVps33 with other HOPS complex proteins	102
Figure 47	Composition and organization of HOPS complex subunits in <i>L. donovani</i>	103
Figure 48	Generation and amplification of LdVps41 truncations	112
Figure 49	Cloning of LdVps41 truncations in pGEX-4-T2 and pET28a vector	113
Figure 50	Purification of recombinant truncated LdVps41 proteins	115
Figure 51	Interaction of GST-LdVps11 with LdVps41 truncations	116

LIST OF FIGURES

Number	Title	Page no.
Figure 52	Interaction of GST-LdVps16 with LdVps41 truncations	117
Figure 53	Interaction of GST-LdVps18 with LdVps41 truncations	118
Figure 54	Interaction of GST-LdVps33 with LdVps41 truncations	119
Figure 55	Interaction of GST-LdVps39 with LdVps41 truncations	120
Figure 56	Interaction of His ₆ -LdVps11 with different Rabs	121
Figure 57	Interaction of His ₆ -LdVps16 with different Rabs	122
Figure 58	Interaction of His ₆ -LdVps33 with different Rabs	123
Figure 59	Interaction of His ₆ -LdVps41 with different Rabs	123
Figure 60	Interaction of His ₆ -LdVps41 with different forms of Rab7	124
Figure 61	Interaction of GST-LdRab7 with LdVps41 truncations	125
Figure 62	Interaction of His ₆ -LdVps41 with HbR	127
Figure 63	Interaction of His ₆ -LdHbR with HOPS complex proteins	127

LIST OF FIGURES

Number	Title	Page no.
Figure 64	Interaction of GST-LdHbR with LdVps41 truncations	128
Figure 65	Interaction of His ₆ -LdVps41 with truncated HbR proteins	129
Figure 66	Cloning of LdVps41 truncations in <i>Leishmania</i> expression vector	130
Figure 67	Overexpression of truncated LdVps41 proteins in <i>Leishmania</i>	131
Figure 68	Kinetics of Alexa594-Hb trafficking in <i>Leishmania</i> promastigotes	132
Figure 69	Kinetics of Alexa594-Hb trafficking in <i>Leishmania</i> promastigotes overexpressing GFP-LdVps41 WT	133
Figure 70	Kinetics of Alexa594-Hb trafficking in <i>Leishmania</i> promastigotes overexpressing GFP-NTD-LdVps41 ¹⁻⁵⁰⁰	134
Figure 71	Kinetics of Alexa594-Hb trafficking in <i>Leishmania</i> promastigotes overexpressing GFP-CTD-LdVps41 ⁹⁰¹⁻¹⁴⁴⁶	135
Figure 72	HOPS complex-mediated transport of Hb to lysosome is necessary for the survival of <i>Leishmania</i> in macrophages	137
Figure 73	Proposed model of role of HOPS complex in Hb endocytosis in <i>Leishmania</i>	141

LIST OF TABLES		
Number	Title	Page no.
Table 1	List of primers used for cloning HOPS complex genes in <i>Leishmania</i> expression vector	90
Table 2	List of primers used for generation of LdVps41 truncations	107
Table 3	List of primers used for cloning LdVps41 truncations in pXG-GFP+2	109

List of Abbreviations

Alexa 546-Hb	Alexa Fluor 546 conjugated hemoglobin
ARF	ADP ribosylation factor
BCA	Bicinchoninic acid
BLAST	Basic local alignment search tool
bp	Base pair
BSA	Bovine serum albumin
CaCl ₂	Calcium chloride
CCV	Clathrin coated vesicles
CCPs	Clathrin coated pits
cDNA	Complementary DNA
C-terminus	Carboxyl-terminus
CORVET	Class C core Vacuole Endosome/Tethering
CHEVI	class C Homologues in Endosome-Vesicle Interaction
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DNTPs	Deoxynucleotide Triphosphate
DTT	Dithiothreitol
ECL	Enhanced chemiluminescence
EDTA	Ethylenediamine tetraacetic acid
EE	Early endosome
ER	Endoplasmic Reticulum
FCS	Fetal calf serum
FERARI	Factor for Endosome Recycling And Retromer Interactions
GAP	GTPase activating protein
GDF	Guanine nucleotide dissociation factor
GDI	Guanine nucleotide dissociation inhibitor
GEF	Guanine nucleotide exchange factor
GFP	Green fluorescent protein

GDP	Guanosine diphosphate
GST	Glutathione-S-transferase
GTP	Guanosine triphosphate
GP63	Glycoprotein 63
Hb	Hemoglobin
HbR	Hemoglobin receptor
HCl	Hydrochloric acid
HEPES	(N-[2-Hydroxyethyl]piperazine-N'-[2ethane-sulphonic acid])
HRP	Horse radish peroxidase
HOPS	Homotypic fusion and vacuole Protein Sorting
h	Hour
IPTG	Isopropyl-beta-D-thiogalactopyranoside
KCl	Potassium Chloride
kDa	kilo Dalton
K ₂ HPO ₄	Di potassium hydrogen phosphate
LAMP	Lysosome associated membrane protein
LE	Late endosomes
LPG	Lipophosphoglycan
LPG2-HA	Hemagglutinin tagged Lipophosphoglycan-2
LPS	Lipopolysaccharide
LB	Luria Bertani
M	Molar
M199	Medium 199
MgCl ₂	Magnesium chloride
miRNA	MicroRNA
M6PR	Mannose-6-phosphate receptor
µg	microgram
µM	micromolar
µl	microliter
mg	milligram

min	minutes
ml	Milliliter
mM	Millimolar
mRNA	Messenger RNA
NaCl	Sodium Chloride
NaH ₂ PO ₄	Sodium di-hydrogen phosphate
NaOH	Sodium Hydroxide
NEM	N-ethyl maleimide
ng	nanogram
NHS	N-hydroxysuccinimide
Ni-NTA	Nickel-Nitrilotriacetic acid
nM	Nano molar
nm	Nano meter
NSF	NEM sensitive fusion protein
N-terminus	Amino-terminus
O.D. ₆₀₀	Optical density at 600nm
ORF	Open reading frame
PAMP	Pathogen associated molecular pattern
PBS	Phosphate buffered saline
PBST	Phosphate buffered saline with Tween-20
PCR	Polymerase chain reaction
PFA	Paraformaldehyde
PI	Protease Inhibitor cocktail
PI3K	Phosphoinositide-3-kinase
PI3P	Phosphoinositide-3-phosphate
PLC	Phospholipase C
PM	Plasma Membrane
PV	Parasitophorous vacuole
rpm	Revolutions per minute
REP	Rab escort protein

RFP	Red fluorescent protein
RNA	Ribonucleic acids
ROS	Reactive oxygen species
RT	Room Temperature (25°C)
RT-PCR	Reverse Transcription-PCR
SDS	Sodium dodecyl sulphate
SDS-PAGE	SDS-Polyacrylamide electrophoresis
SNARE	Soluble NSF Attachment Protein receptor
TGN	trans-golgi network
TNF	Tumor necrosis factor
Tris	Tris-hydroxy methyl- amino methyl
Tx-100	Triton X-100
V	Volt
VAMP	Vesicle associated membrane protein
WT	Wild type