Supplementary Information

The inositol pyrophosphate synthesis pathway in *Trypanosoma brucei* is linked to polyphosphate synthesis in acidocalcisomes

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Running title: Inositol pyrophosphates in T. brucei

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Table S1

Table S2

Figure S1

Table S1. Soluble inositol phosphate kinases identified in the human genome and orthologs from *S. cerevisiae* and *T. brucei*. Listed below the enzyme definition is the primary, but not exclusive, enzymatic reaction.

Inositol phosphate kinase	***H. sapiens*** Gene ID	***S. cerevisiae*** SGD	***T. brucei*** TriTrypDB
Inositol-trisphosphate 3-kinase I(1,4,5)P_3 => I(1,3,4,5)P_4	**ITPKA,B,C** 3706 3707 80271		
Inositol polyphosphate multikinase I(1,4,5)P_3 => IP_4 => I(1,3,4,5,6)P_5	**IPMK** 253430	**Arg82** YDR173C	**TbIPMK** Tb427tmp.211.3460
Inositol-tetrakisphosphate 1-kinase I(1,3,4)P_3 => IP_4 => I(1,3,4,5,6)P_5	**ITPK1** 3705		
Inositol pentakisphosphate 2-kinase I(1,3,4,5,6)P_5 => IP_6	**IPPK** 64768	**Ipk1** YDR315C	**TbIP5K** Tb427.04.1050
Inositol hexakisphosphate kinase IP_6 => 5PP-IP_5	**IP6K1,2,3** 9807 51447 117283	**Kcs1** YDR017C	**TbIP6K** Tb427.07.4400
Diphosphoinositol pentakisphosphate kinase 5PP-IP_5 => PP_2-IP_4	**PPIP5K1,2** 9677 23262	**Vip1** YLR410W	

Table S2. *S. cerevisiae* strains, plasmids and primers used in this study.

Strain	Genotype	Source
DDY1810	*MATa leu2-3,112 trp1-Δ901 ura3-52 prb1-1122 pep4-3 prc1-407*	[1]
kcs1Δ	DDY1810 kcs1:Leu2	[1]
ipk1Δ	DDY1810 ipk1::Leu2	this study
arg82Δ	DDY1810 arg82::Leu2	this study
BY4741	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0	[2]
Ipk1Δkcs1Δ	BY4741 ipk1:kanMX4 kcs1:kanMX4	[2]

Plasmids	Source
pET-32 Ek/LIC	Novagen
pADH:GST	[3]
pMOTag4H	[4]
pMOTag33M	[4]

Primer	Sequence	Use
1	GACGACGACAAGATGTTAAATATTTGCCAAAAC	(fwd); cloning of *TbIPMK* in pET32
2	GAGGAGAAGCCCGGTTCATGAAAGAAGAAAAATAATT	(rev); cloning of *TbIPMK* in pET32
3	GACGACGACAAGATGTTGTCGGAAGAGGAGGCACG	(fwd); cloning of *TbIP5K* in pET32
4	GAGGAGAAGCCCGGTCTAACAATGGAACTCAGGTGCG	(rev); cloning of *TbIP5K* in pET32
5	GACGACGACAAGATGGGGGAAGAGGAGAATTTAC	(fwd); cloning of *TbIP6K* in pET32
6	GAGGAGAAGCCCGGTTATGTGAGCATGTCAAGTACA	(rev); cloning of *TbIP6K* in pET32
7	TCAGGTGACAGGCGGTTACACTTTCCTGAGGAGGTGGTTGGGTTTGTTCAAGGTTTGGAAAAAATTATTTTTCTTCTTTCAGGTACCGGGCCCCCCCTCGAG	(fwd); tagging *TbIPMK*; template pMOTag4H
8	AAAAGAGAGTGAGATCGAATAAATATAAGACACCATGTCATACTACCAAATTTAAAACAACCGAAATACCGAAGATCGCCGGTTCTCATGGCGGCCGCTCTAGAACTAGTGGAT	(rev); tagging *TbIPMK*; template pMOTag4H
9	CTGAGCCGCTATTTTGAGCTTGACCGTGAAGTCCTTGCAGCGTGGGAGGATTATAAAGTTGTAAGCGCACCTGAGTTCCATTGTGGTACCGGGCCCCCCCTCGAG	(fwd); tagging *TbIP5K*; template pMOTag4H or pMOTag33M
10	GATACACACAAACAAACAAACGAACGTTTACGCAACTTCGTCTCACATGGACTAAACCTAAAGAGGTGATCACACCCCCACAAAAAGTGGCGGCCGCTCTAGAACTAGTGGAT	(rev); tagging *TbIP5K*; template pMOTag4H or pMOTag33M
11	AGCTGCTACGAGGTGGCGATGCAGACGCGGAACAGGATAAGGACGTGGGATATATTGAGGCCCTGAAAACTGTACTTGACATGCTCACAGGTACCGGGCCCCCCCTCGAG	(fwd); tagging *TbIP6K*; template pMOTag4H
12	TCCCGCACAAAACTCTGCTGCTTATGCTCCATTCATGCGTCCTGAAACGAACGTCCGCGATCATTTGGGAAGACACACTGTTGAAGGTATGGCGGCCGCTCTAGAACTAGTGGAT	(rev); tagging *TbIP6K*; template pMOTag4H
13	ACGCGTCGACAATGTTAAATATTTGCCAAAACTTGTCTTCCGTTG	(fwd); cloning of *TbIPMK* in pADH:GST
14	ATAAGAATGCGGCCGCTCATGAAAGAAGAAAAATAATTTTTCCAAACCTTGAAC	(rev); cloning of *TbIPMK* in pADH:GST
15	ACGCGTCGACAATGCGCTTCCTCGGTGC	(fwd); cloning of *TbIP5K* in pADH:GST

16	ATAAGAATGCGGCCGCCTAACAATGGAACTCAGGTGCGC	(rev); cloning of *TbIP5K* in pADH:GST
17	ACGCGTCGACAATGGGGGAAGAGGAGAATTTACGTAGAA	(fwd); cloning of *TbIP6K* in pADH:GST
18	ATAAGAATGCGGCCGCTTATGTGAGCATGTCAAGTACAGTTTTCAGG	(rev); cloning of *TbIP6K* in pADH:GST
19	ACATGGATATGTGCATACGTGTGCCTAAGTAGAAATTTTTTTCACATGCAGCTGAAGCTTCGTACGC3	(fwd); generation of *arg82Δ*
20	TGTACCATATACCATAAACAAGGTAAACTTCACCTCTCAATATATCTAGCATAGGCCACTAGTGGTACTG	(rev); generation of *arg82Δ*
21	TCGAAAATTGTCAGAGATAAGTTCCTTTTTTGAAAAGAAAGATCGATGCAGCTGAAGCTTCGTACGC3	(fwd); generation of ipk1*Δ*
22	TATGTGCATCTGCCAGTACCAAAGGTGGAAAGAAAAGTATACAGTTTAGCATAGGCCACTAGTGGTACTG	(rev); generation of *ipk1Δ*
23	TGACTTCTCTCGCTCAGGTG	(fwd); qRT-PCR *TbIPMK*
24	TCATGAAAGAAGAAAAATAATTTTTTCCAAACC	(rev); qRT-PCR *TbIPMK*
25	GTATAGCGTGTGGATTGGCGG	(fwd); qRT-PCR *Actin*
26	TGCTGTGTACGATGCTGGG	(rev); qRT-PCR *Actin*

References

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Figure Legend

Figure S1. TbIPMK conditional KO does not change labeling or distribution of acidocalcisomes. Immunofluorescence of TbIPMK conditional KO with (A) or without (B) tetracycline shows acidocalcisome marker TbVP1 (*green*) and DAPI-stained DNA (*blue*) in four different cells for each condition. The Zen software was used to combine all super-resolution Z-stacks into a single projection. Bar = 5 µm.

Figure S1