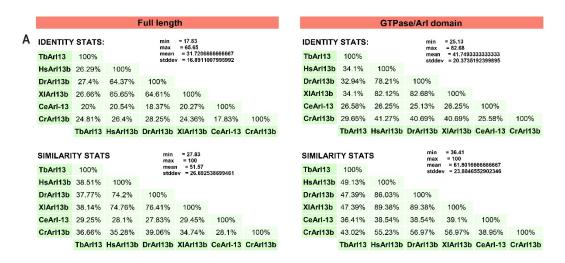
Purpose	Plasmid	Selection marker	Insert description
Endogenous	pMOTag4YH-TbArl13	Hygromycin	TbArl13-YFP-3HA
tagging	pPOTv6 3BB2-mNG-3BB2	Hygromycin	TbArl13-3BB2-mNG- 3BB2
	pPOTv6 3BB2-mNG-3BB2	Blasticidin	TbArl3A-3BB2- mNG-3BB2
	pPOTv7 TagRFPt	Hygromycin	TagRFPt-TbArl3C
Inducible	pLEW-FL-YFP (Tet)	Phleomycin	TbArl13
overexpression	pLEW-DD-YFP(Tet)	Phleomycin	TbArl13(1-186)
	pLEW-CD-YFP(Tet)	Phleomycin	TbArl13(181-270)
	pDEX-mCherry-DDiR- (Cmt)	Phleomycin	mCherry-DDiR
	pDEX-TbArl3A-Q70L-BB2 (Tet)	Phleomycin	TbArl3A(Q70L)-BB2
	pDEX-TbArl3B(Q71L)-BB2 (Tet)	Phleomycin	TbArl3B(Q71L)-BB2
	pDEX-TbArl3C(Q77L)-BB2 (Tet)	Phleomycin	TbArl3C(Q77L)-BB2
Inducible	pDEX-FLiR-YFP(Cmt)	Blasticidin	TbArl13iR (1-270)
overexpression	pDEX-DDiR-YFP(Cmt)	Blasticidin	TbArl13iR (1-186)
(for RNAi	pDEX-CDiR-YFP(Cmt)	Blasticidin	TbArl13iR (181-270)
complementation assays)	pDEX-CCtiR-YFP(Cmt)	Blasticidin	TbArl13iR (1-244)
	pDEX-R133QiR-YFP(Cmt)	Blasticidin	TbArl13(R133Q)iR
	pDEX-Tb24-CDiR-YFP(Cmt)	Blasticidin	Tb24-CDiR (187- 270)
BioID	pDEX-FLiR-MycBirA (Cmt)	Blasticidin	TbArl13iR
BiFC	pLEW-FLiR-VN(Tet)	Phleomycin	TbArl13iR
	pDEX-TbArl3A-VC (Cmt)	Blasticidin	TbArl3A
	pDEX-TbArl3B-VC (Cmt)	Blasticidin	TbArl3B
	pDEX-TbArl3C-VC (Cmt)	Blasticidin	TbArl3C
Overexpression	pXS2-TbArl3A-YFP	Blasticidin	TbArl3A
-	pXS2-TbArl3B-YFP	Blasticidin	TbArl3B
	pXS2-TbArl3C-YFP	Blasticidin	TbArl3C
	pHD1034-YFP-RSP3	Puromycin	RSP3
RNAi	p2T7-TbArl13(Tet)	Phleomycin	TbArl13 CDS (nucleotides 160-593)
Bacterial	pET-28b-TbArl13	Kanamycin	His-TbArl13
expression	pGEX-6P-1-TbArl3A	Ampicillin	GST-TbArl3A
	pGEX-6P-1-TbArl3C	Ampicillin	GST-TbArl3C

## Table S1 List of plasmids used in the study

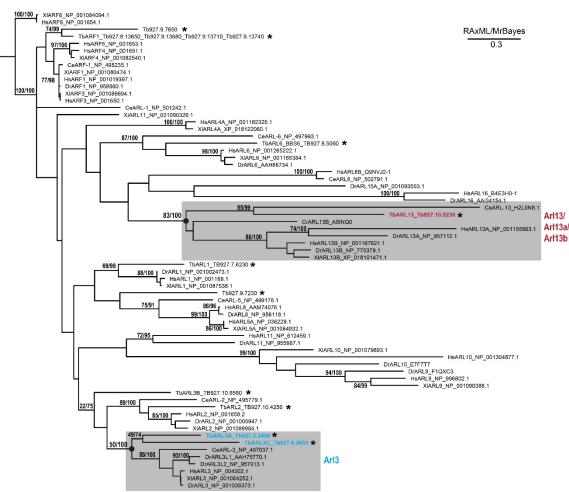
Antibodies	Antigen	Labelled Organelle/ structure	Origin/ Clonality	Reference/ Company	Used for
L3B2	FAZ1	FAZ	Mouse/	(Kohl et al., 1999)	IF
		filament	monoclonal		(1:25)
YL1/2	Tyrosinated	basal body	Rat/	Santa Cruz	IF
	α-tubulin		monoclonal	(#sc-53029)	(1:2000)
anti-PAR	PAR	PFR	Mouse/	(Ismach et al.,	IF
			monoclonal	1989)	(1:500)
anti-PFR1	PFR1	PFR	Rat/	Unpublished	IB
			polyclonal		(1:5000)
					IF
					(1:10000)
anti-TbBiP	BiP	ER	Rabbit/	(Bangs et al.,	IB
			polyclonal	1993)	(1:1000)
anti-α-tubulin	alpha-tubulin	microtubule	Mouse/	B-5-1-2/Santa	IB
			monoclonal	Cruz (#sc-23948)	(1:10000)
anti-TbArl13	TbArl13	axoneme	Rabbit/	This study	IF (1:500)
und formits	10/1113	uxoneme	polyclonal	This study	IB (1:2000)
anti-Calflagin	Calflagin	_	Mouse	(Tyler et al.,	IB
Tb24	Tb17, Tb24,		/monoclonal	2009)	(1:2000)
	Tb44			)	()
anti-YFP	YFP	-	Rabbit/	Unpublished	IB
			polyclonal	I	(1:1000)
anti-GFP	GFP	_	Mouse/	Roche	IF (1:500)
			monoclonal	#11814460001	
anti-His	His tag	-	Mouse/	GE Healthcare	IB
			monoclonal	(#27-4710-01)	(1:10000)
anti-HA	HA tag		Mouse/	Santa Cruz	IF (1:500)
			monoclonal	(#sc-7392)	
anti-BB2	BB2 tag		Mouse/	(Bastin et al.,	IB (1:500)
	(also known		monoclonal	1996)	
	as Ty1 tag)				
anti-mCherry	mCherry		Rabbit/polyclonal	Thermo Fisher	IB
				(#PA5-34974)	(1:10000)

Table S2	List of antibodies used in the	study

IF: immunofluorescence; IB: immunoblotting



В



**Fig. S1 TbArl13 is the Arl13b orthologue in** *T. brucei*. (A) Cross comparison of identity and similarity between TbArl13 and other previously published Arl13b proteins. Both full-length amino acid sequences (left) and GTPase domain sequences (right) were

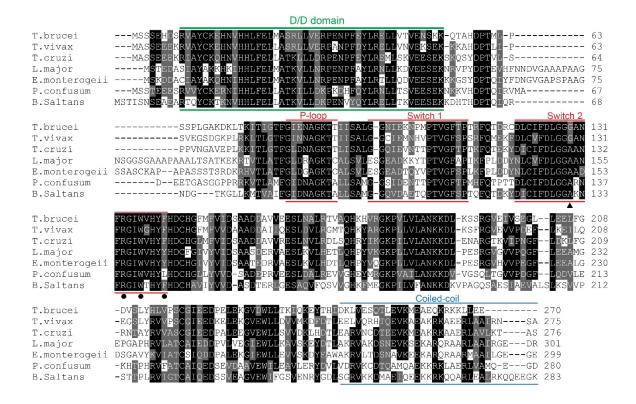
analyzed. (B) Amino acid maximum likelihood topology of all predicted Arf/Arl proteins in the *T. brucei* genome and representative Arf/Arl family members from other eukaryotic groups. TbArl13 (red) robustly forms a clade with other Arl13/Arl13a/Arl13b proteins. TbArl3A and TbArl3C (blue) also fall under the Arl3 clade (blue). ML bootstrap support values and Bayesian posterior probabilities are mapped if >=70 across both analyses. Support values for other relevant nodes are included. Major Arf/Arl members from human (H. *sapiens*, Hs), zebrafish (D. *rerio*, Dr), amphibians (X. *laevis*,Xl) and nematodes(C. *elegans*, Ce) and green algae (C. *reinhardtii*, Cr) were used for the reconstruction. Asterisks indicate *T. brucei* proteins. Branch support values calculated using Maximum-Likelihood (RAxML) and Bayesian Interefence (MrBayes) are shown.

D/D domain	
TbArll3 MSSSEHDSRVAYCKEHNVHHIEEIMMASRLLVERPENPFENFENELRELLVTVENSKKOTAHDPTMLPSSPLGAKIKITK	75
HSAr113b	21
Drar113bWENWE-NWE	21
Xlarl13b	21
TDAr113 MSSSEHDSRVAYCKEHNVHLLGEMASSCGRFK	24
Crarlisb	19
P-loop Switch 1 Switch 2	
HSAR113b VTLLMVGLDNAGKTATAKGIQGEYFEDVAPTVGFSKINLRO-GKFEVTIFDLGGGIRIRGIWKNYYAESYGVIFVVDS-S	99
DrAr113b VTLVMVGLDNAGKTATVRGIOGESPLDVAPTVGFSKVDLKO-GKFEVTIFDLGGGKRIRGIWKNYYSESYGVVFVVDS-S	99
HSAR113b VTLLMVGLDNAGKTATAKGIQGEYPEDVAPTVGFSKIN <mark>IRC-GKFEVTIFDLGGGIRIRGIWKNYYAESYG</mark> VIFVVDS-S DrAr113b VTLVMVGLDNAGKTATVRGIQGESPLDVAPTVGFSKVDLKC-GKFEVTIFDLGGGKRIRGIWKNYY <mark>S</mark> ESYGVVFVVDS-S XlAr113b VTLVMVGLDNAGKTATVKGIQGESPEDVAPTVGFSKADIKQ-GRFDITMFDLGGGKRIRGIWKNYYAESYGVVFVIDS-S	99
CeArl-13 IKLCCFCICSAGKTTFLKVLKCEDPRDLLRTNGFSTVKMEYDETFHLTIYDVGGDKGIRGIWSNYYAEVHCIIYVIDY-S	103
Crarl13b ITIALIGLDNAGKTTLLNSIQGEVDRDTTPTFGENSTTLNE-GKYKIEVFDLGGGKNIRGVWKKYLAEVHAIVYVVDA-A	97
TbArl13 DDAVVEESLNALRTVAQHKHVRGKPVLVLANKKDLKSSRGVEIVSBGL-LEELFG-VSLY-HLWP HsArl13b DEERMEETKEAMSEMIRHPRISGKPTLVLANKQDKEGALGEADVTECLSLEKLVNEHKCL-COTEPCSAI-S DrArl13b DVORIQETRTTMAEVIRHPRIAGKPVLVLANKQDQDGAMAEADIIBTISLEKLVNENKCL-COTEPCSAV-L	217
HSAR1135 DEFENDETKEAMSEMURHERISCKPILVLANKODKEGALGEADVIECUSLEKUVNEHKCL-COTEPCSAI-S	169
DrAr113b DVORIOETRDTMAEVLRHPRIAGKPVLVLANKODODGAMAEADIIETLSLEKLVNENKCL-COIEPCSAV-L	169
XIATII3b DMERMEETKETIAEVIRHPRISCKPVLVLANKODREGALSEADIIBELSLEKLVNSNKCL-COIEPCSAA-I Cearl-13 TDETFTESIEALHSLTSNPHVOKKPIFILLNNONNREFDDVEISNETKIQAGQHKIVLFSHFNKYNGYLDNIKSATLTVM	169
Cearl-13 TDETTTESTEALHSLTSNPHVOKKPIFLLINNONNREFDDVEISNETKIOAGOHKIVLFSHFNKYNGYLDNUKSATLTVM	183
Crarlish Decerereskutary from the property of the angle o	163
Coiled-coil	
Coiled-coil TbArl13 SCGIEEDPELEKGVDWLLTKICKEYTHIDKLVESDITENRAAE-CKRKKL BE	270
HSAT1135 EYEKKUDKSIKKUYWILHVIARDEDAINERICKETTEORALEE-OEKOFRAPRYRKIREERKONBOBOABLDGT-SGL-	246
Drar113b GYGKKVDKSIKNGLNWLINNTAKDYEATSERVOKDTAEOKAOEB-OBKKERAERVRTREERDROEREBAEREGRTLKE-	247
X1ar113b GHGKKTDKSTKNGINWLTRVTAKDFEALHERTLKETAEORAOEP-ODKRERAERVKRIREPREEKEKEFAERECK-OEI-	246
Cearl-13 ARAKKDRNEYOFOFVREIDSISEHYVELSEGYKTAELALRIROEEAKEORIMOMKVEHDALKADVAGLELRNO	257
Crar113b PACODVDHRLRDGLKMLVGTVDREFGRUDPRVCTBA-BEVROEBARKKKERBERLRKORBERLROCKBEBRAREVEKE-	241
TbArl13	270
HSAR113b -ABLDPEP-TNEFOR AST IENEGKERKK-NOKMEKDSDGCHRK-HKMEHEOIETOGOV	304
Drar113b -EELDDWNMFNPFOPINNWTTENODRINREKEMO-RORENGOOGSVOEOMALODEEEEE	305
X1Ar113b -VEDKSIPMVNEFOPTSAWTTENEEKIBKEKEKK-BEKMOTKONGVGTALESKEEEDOMETGSES	309
Cearl-13 PPVOPP PPDPPS PKSasVHIEESPPMSTASSTIPS IIOSTPETG	304
CrAr113b - MELHDCKAPSLLAACGGWWGAAAAGWNGVMVDEOOELRPPC@HOEAPEALGWHNGLALGLPHTIESPGK	310
TbArl13	270
HsAr113bNHNGQKNNEFGLVENYKEALTQQLKNEDET-DRPSLES	341
DrAr113bD-EES-ERQTPESTESGAV	322
X1Ar113bSQNSRTPRDNDLLESYKDALVQKLEEDDKSLDTEKEDS	347
CeArl-13 TPRDPVNFCRISQTSTKPVSPES-NSVKE	332
Crar113b FPPPPRRPLEAHPASDLRLVAPDQGVSSASGGPGLGAMPSGSHGGGGVPPQPASLPHVRAALPPLPPSAPQPSDAGV	387
TbArl13	270
HSAr113b ANGKKKTKKLRMKRNHRWEPLNIDDCARESPTPPPPPPPVGWGTPKVTRLPKLEPLGETHH	402
Drar113b DQTKKKTRKLRLKRKHRVDPLRMEEAARKSPTPPPLPVGWATPKVSRLPKLEPIGDTRH	381
XlArll3b AQSKKKPKKLKLKRNHKVEPVSIEETNEKTPSPPPPQLPVGWGTPKVTRLPKLEPIGETHH	408
CeArl-13	346
HSAT113b ANGKKKTKKLRMKRNHRWEPLNIDDCAPESPTPPPPPPVGWGTPKVTRLPKLEPLG9THHD DrAT113b DQTKKKTRKLRLKRKHRWDPLRMEEAAPKSPTPPP-LPVGWATPKVSRLPKLEPLGDTRH XlAr113b AQSKKKPKKLKLKRNHKWEPVSIEETNPKTPSPPPPQLPVGWGTPKVTRLPKLEPLGETHH CeAr1-13	445
TbArl13	270
HSAR1136NDFYRKPLPPLAVPQRPNSDNSDAHDV Drar1136SDFYGKPLPPVATRQRPNSDNSDTHDV	426
Xlar113bNGNFKNKRPILLSVFCFFACRSIST HASHRFSLTINTLRFFTNTLDDCLKAPPCCQFISSI	
CeArl-13 KAPGRQYNR	
Crar113b GGGGPGSRGSGSGMTPDARELGSGGVESGEGTFARLRAGAQQASDGGHGNSKGSFSLVHTSNKVVPVAPDLRAGIPGAPN	525
TbArl13 - 270	
HsArl13b IS 428	
DrArl13b IS 407	
XlArlib IS 472	
CeArl-13 PK 370	
CrArl13b DA 527	

Fig. S2 Multiple sequence alignment of TbArl13 and Arl13b proteins from other

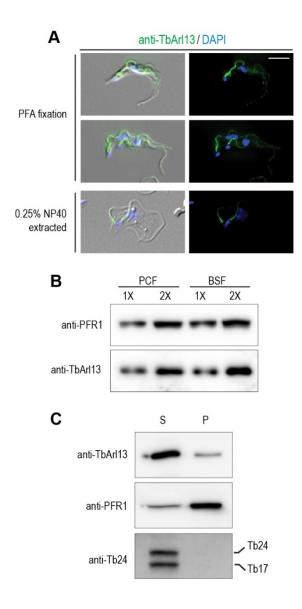
**organisms.** TbArl13 protein sequence is aligned with Arl13b from H. *sapiens* (Hs), D. *rerio* (Dr), X. *laevis*, (Xl), C. *elegans* (Ce) and C. *reinhardtii* (Cr). Black triangle indicates the missing catalytic glutamine (GXXGQ) in all Arl13b proteins. Black dots indicate residues that have been found mutated in JS patients. Highlight in brown are the

cysteine palmitoylation sites conserved in Ar113bs, but not found in CrAr113 and TbAr113. Residues conserved/similar across at least 4 out of 6 sequences are shaded in black/grey respectively.

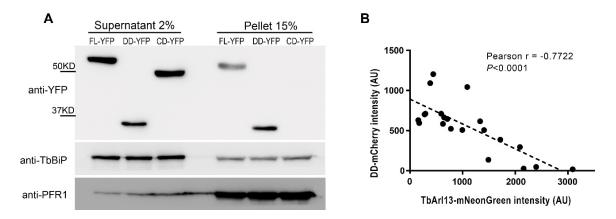


## Fig. S3 The N-terminal D/D domain is conserved in kinetoplastid Arl13 proteins.

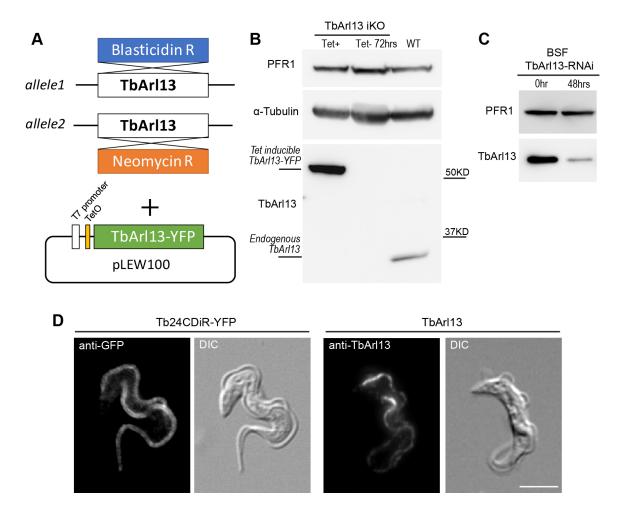
Multi-sequence alignment of Arl13 from five different kinetoplastid species. Black arrowhead indicates the missing catalytic glutamine (GXXGQ) in all known Arl13b orthologues. Black circles indicate residues that have been found mutated in JS patients. Residues conserved/similar across at least 5 out of 7 sequences are shaded in black/grey respectively.



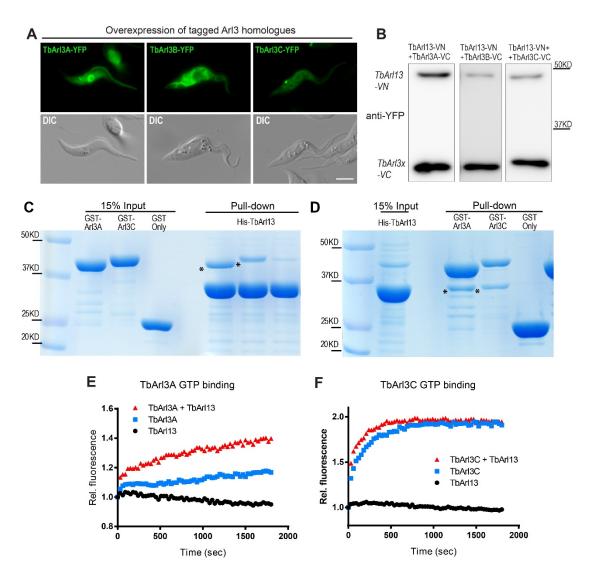
**Fig. S4 TbArl13 is expressed and localizes to the flagellum in the BSF form.** (A) BSF cells were fixed with 4% PFA either before (top) or after extraction with 0.25% NP40 (bottom) and stained with anti-TbArl13 (green) and DAPI (blue). Scale bar =  $5\mu$ m. (B) The expression level of TbArl13 in BSF is comparable to that in PCF. '2X' indicates twice the amount of cell lysate loaded in the well compared to '1X'. Anti-PFR1 was used as a loading control. (C) BSF cells were extracted using 1% Triton X-100 in PBS under 37°C to maximize removal of flagellar membrane associated proteins. Calflagin Tb24 and its paralogue Tb17 is solely found in the S (supernatant) fraction, while a portion of TbArl13 is consistently found in the P (pellet) fraction. PFR1 was used as a marker for the flagellar cytoskeleton.



**Fig. S5 D/D mediated flagellar targeting.** (A) Fractionation analysis of FL-YFP, DD-YFP and CD-YFP proteins. Both FL-YFP and DD-YFP but not CD-YFP associates with the cytoskeleton pool. For each cell type,  $2x10^8$  cells were extracted with 1% TritonX-100 in PBS before centrifugation to yield the supernatant and pellet fractions. Anti-TbBiP and anti-PFR1 were used as loading and extraction controls. (B) In relation to Fig. 2(E). In cells co-expressing TbArl13-mNeonGreen and mCherry-DD, the level of mNeonGreen measured on the axoneme is negatively correlated to the level of mCherry. Pair number n= 21, correlation coefficient = -0.7722. *P*<0.0001.



**Fig. S6 TbArl13 iKO, RNAi and Tb24CDiR-YFP localization.** (A) Schematic diagram showing the construction of TbArl13-iKO cells. Tetracycline (Tet)-inducible TbArl13b-YFP was stably introduced into cells with both endogenous TbArl13 alleles replaced with blasticidin and phleomycin resistant genes, respectively. (B) Immunoblots confirm the absence of endogenous TbArl13 in iKO cells compared to wild type (WT) cells. 72 hours after the removal of tetracycline, TbArl13-YFP expression could not be detected. (C) Immunoblots confirm the reduction of TbArl13 in BSF cells, 48hrs following the induction of TbArl13-RNAi. For all immunoblots, anti-TbArl13 was used to detect both endogenous TbArl13b and recombinant TbArl13-YFP. Anti-PFR1 and anti-alpha-tubulin were used as loading controls. (D) Comparison of the immunofluorescence-labeling of the flagellar membrane localizing chimera mutant Tb24CDiR and WT TbArl13. Tb24CDiR-YFP-expressing and wild type (WT) BSF cells were fixed and immunestained with anti-GFP and anti-TbArl13, respectively. Tb24CDiR-YFP could be seen outlining the flagella, a pattern that is typical of flagellar membrane association but is not observed with WT TbArl13. Scale bar=5μm.



**Fig. S7 GTP binding of TbArl3A and TbArl3C is accelerated by TbArl13.** (A) TbArl3A-YFP, TbArl3B-YFP and TbArl3C-YFP are expressed in PCF cells. DNA is labeled using DAPI. Scale bar = 5μm. (B) In relation to Fig. 5(B), the expression of TbArl13-VN, TbArl3A-VC, TbArl3B-VC and TbArl3C-VC were confirmed by immunoblots. Both VN and VC fragments could be recognized by anti-YFP polyclonal antibodies. (C, D) Affinity purified His-TbArl13 can pull-down affinity purified GST-TbArl3A and GST-TbArl3C (C), and vice versa (D). \* marks the pull-down band. (E, F) Addition of His-TbArl13 promotes mant-GTP binding to TbArl3A and TbArl3C. Consistent with Fig. 5(E), TbArl3C has an exceptionally high intrinsic GTP binding rate compared to TbArl3A.

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