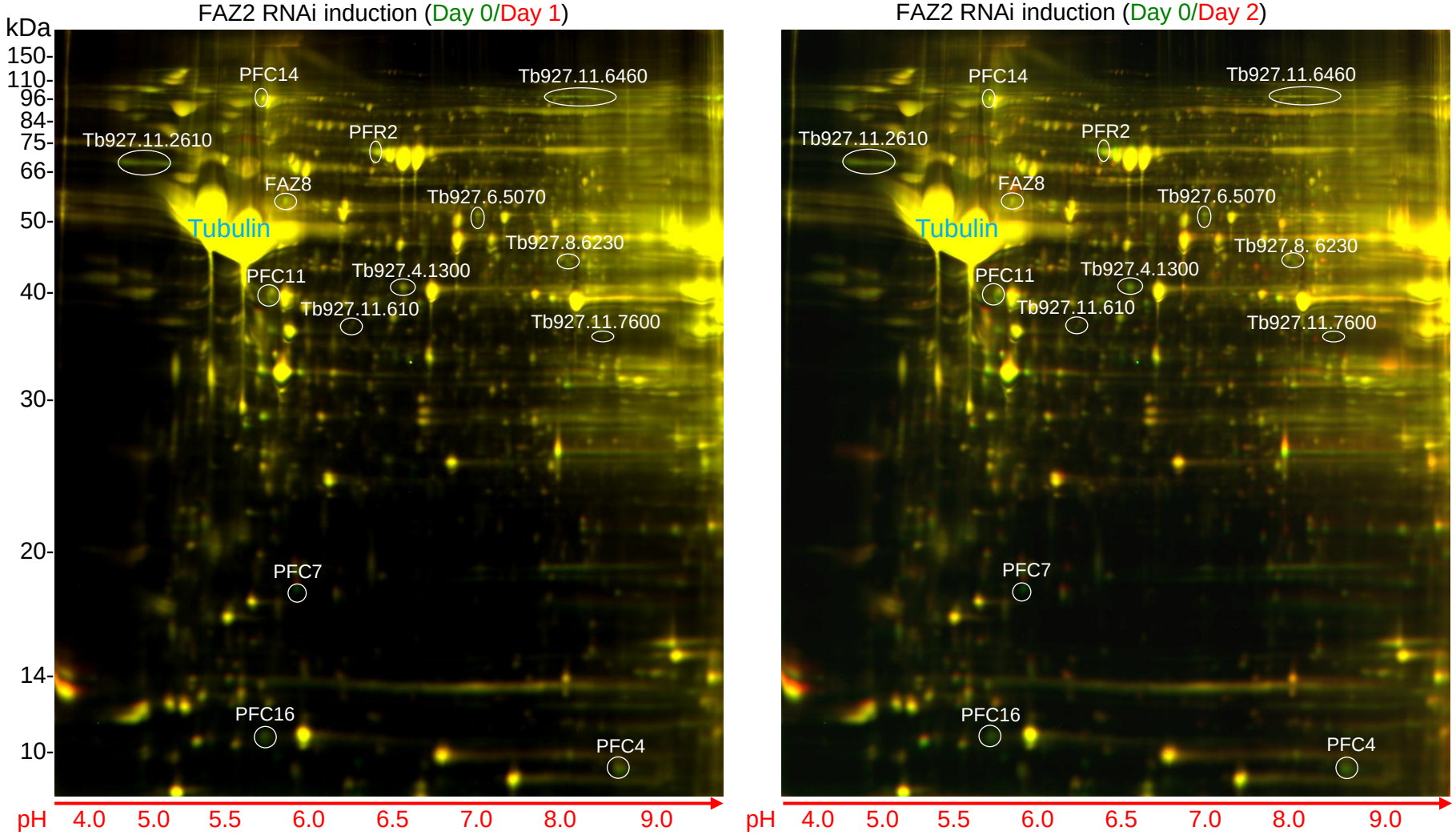
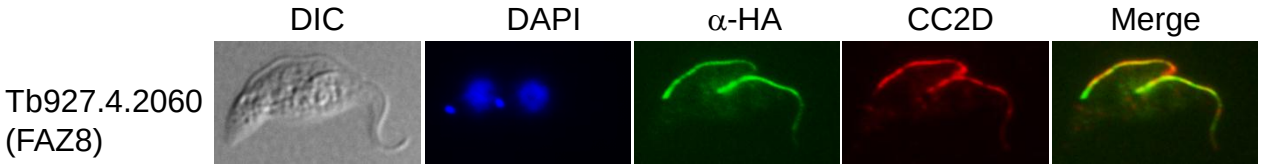
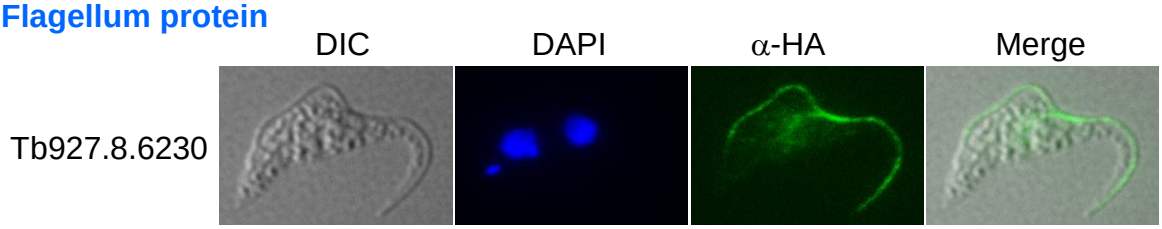


Figure S1. Two-dimensional DiGE analysis of non-induced and FAZ2 RNAi-induced cells. Non-induced and FAZ2 RNAi-induced cells (1 and 2 days) were lysed with PEME buffer containing 1% NP-40 to prepare cytoskeletons. Cytoskeletal proteins were further analyzed by 2D-DIGE. A total of 20 protein spots down-regulated from the cytoskeleton after FAZ2 RNAi were selected for mass spectrometry for protein identification. 14 out of the 20 proteins thus identified were previously reported to associate with the flagellum-associated structures. These 14 protein spots were marked in white circles, with the accession numbers or the names of the identified proteins shown above the white circles. The highly abundant tubulin protein was indicated in blue.

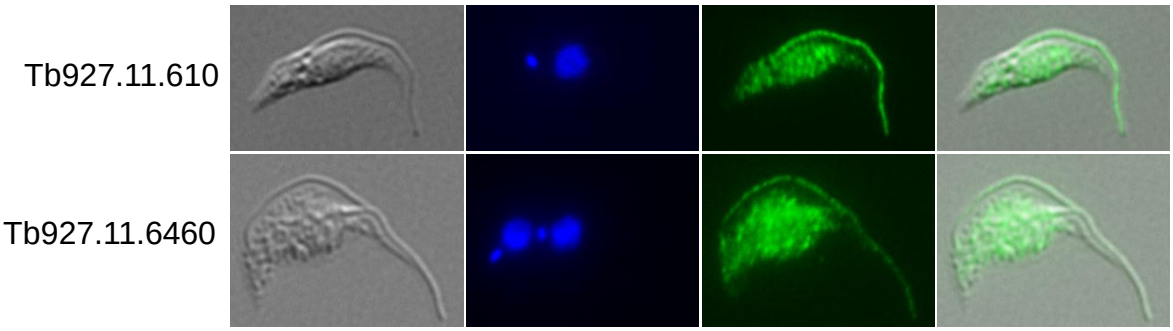
1. FAZ filament protein



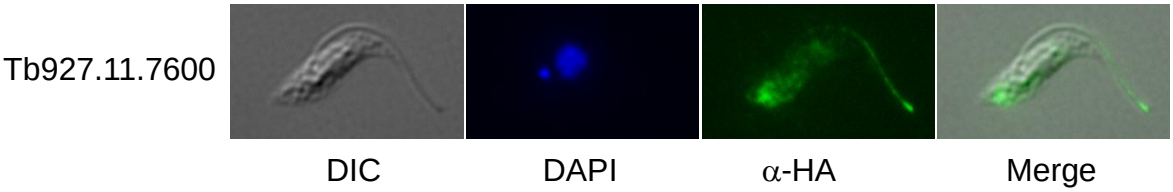
2. Flagellum protein



3. Flagellum and cytoplasm proteins



4. Flagellum and cytoskeletonprotein



5. Cytoskeleton proteins

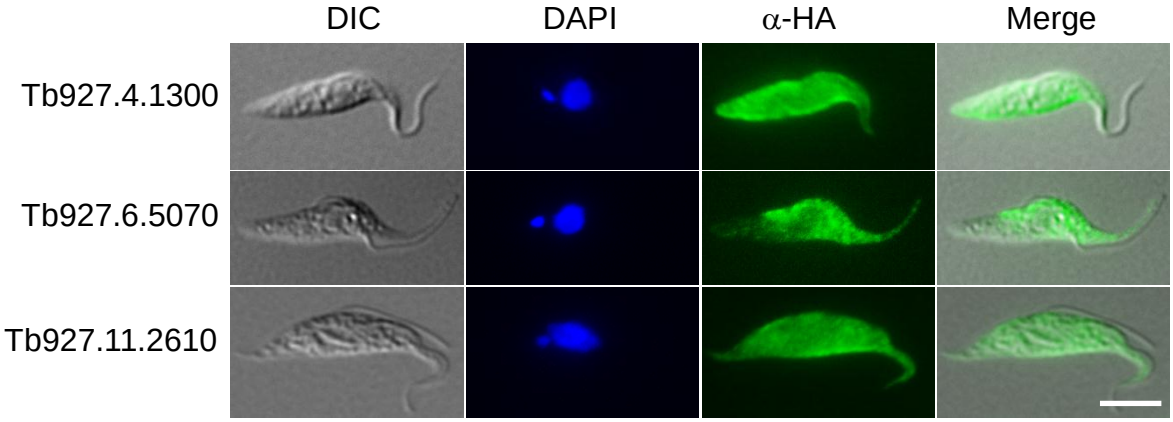
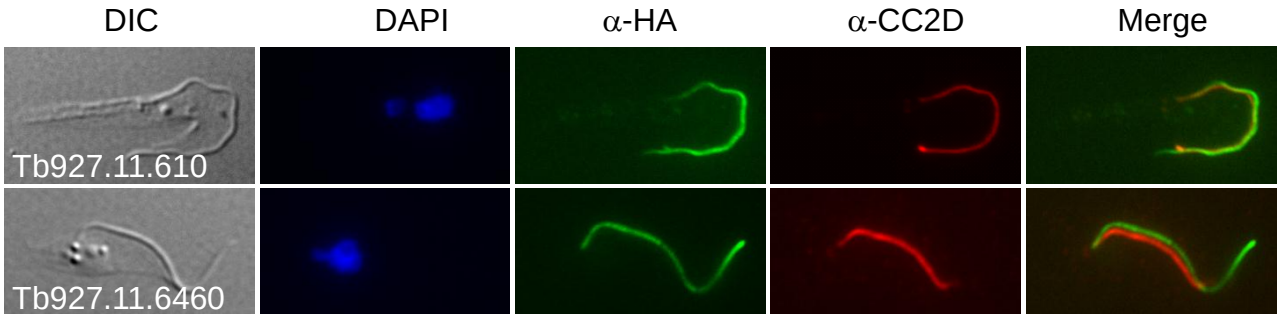


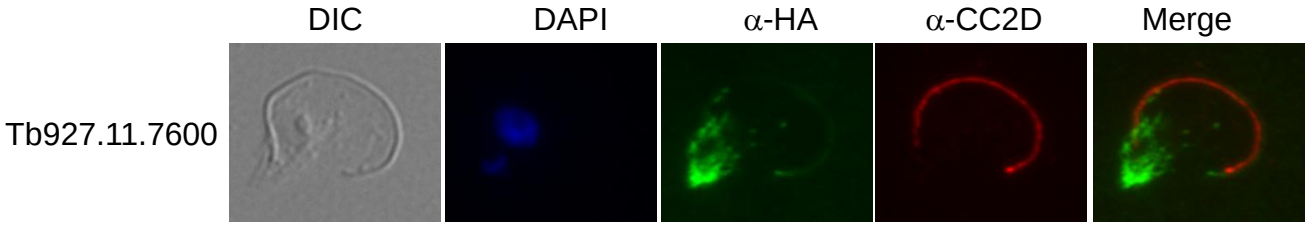
Figure S2 - continued

B. Cytoskeleton

3. Flagellum and cytoplasm proteins



4. Flagellum and cytoskeleton protein



5. Cytoskeleton proteins

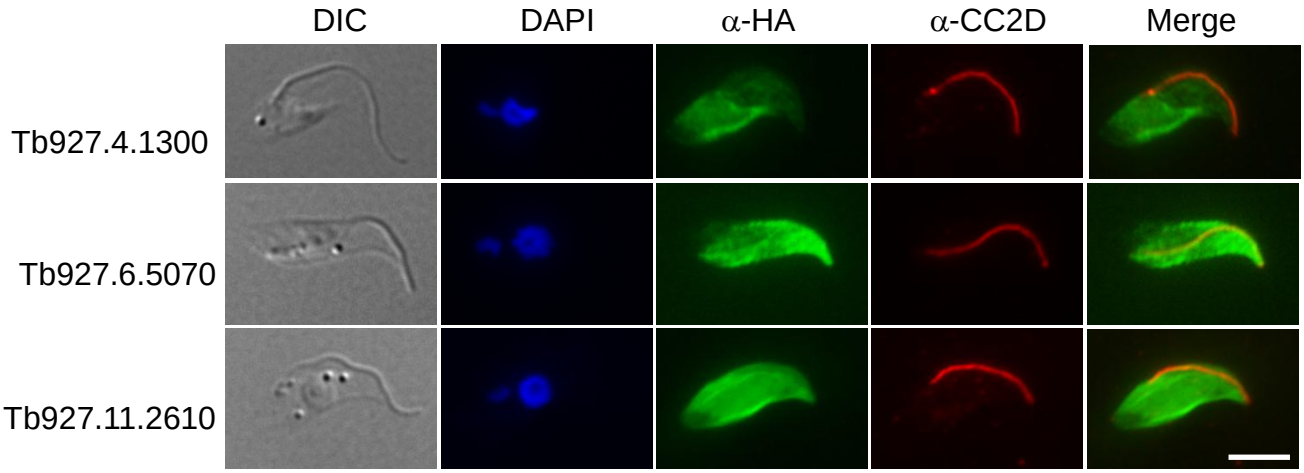


Figure S2. Subcellular localization of the proteins down-regulated from the cytoskeleton upon FAZ2 RNAi. Cells expressing endogenously 3HA-tagged proteins were immunostained with anti-HA and counterstained with DAPI. For some proteins, cells were co-immunostained with anti-CC2D to label the FAZ filament. (**A**). Intact cells were fixed with cold methanol and then immunostained with antibodies. (**B**). Cytoskeletons were prepared by treating the cells with PEME buffer containing 1% NP40 and then co-immunostainined with anti-HA mAb and anti-CC2D pAb. Scale bar: 5 µm.

Figure S3

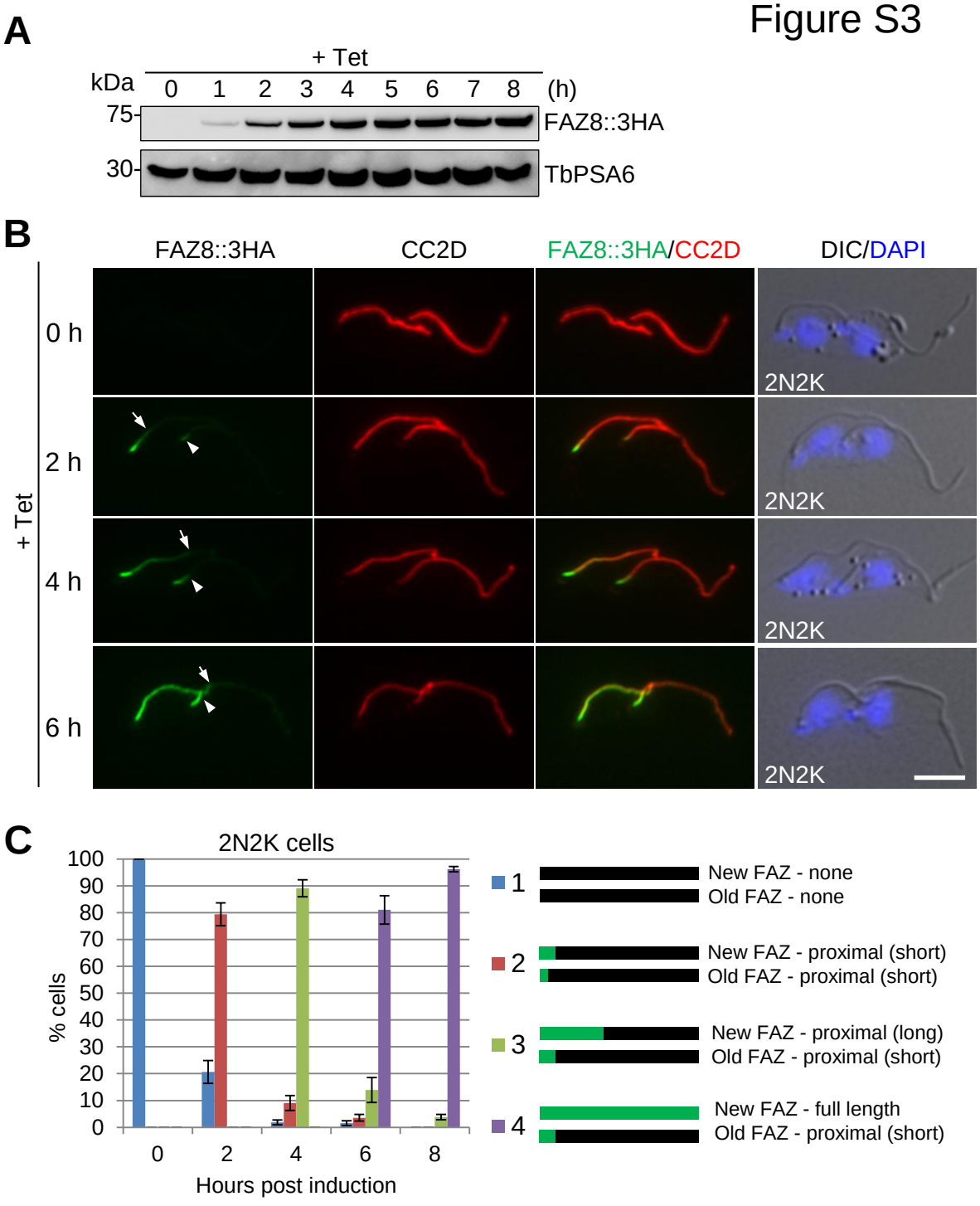


Figure S3. Incorporation of FAZ8::3HA into the FAZ filament. (**A**). Western blot to detect the overexpressed FAZ8::3HA. (**B**). Immunostaining of FAZ8::3HA. Cells induced for various times (h) were co-immunostained with FITC-conjugated anti-HA mAb and anti-CC2D pAb. Arrows and arrowheads indicate the growing tips of FAZ8::3HA fluorescence signal in the new and old FAZ filaments, respectively. Scale bar: 5 µm. (**C**). Quantitation of 2N2K cells with different types of FAZ8::3HA pattern in the new and old FAZ filaments. About 200 cells were counted, and error bars represent S.D. calculated from three independent experiments.

Table S1. List of the 14 proteins down-regulated from the cytoskeleton upon FAZ2 RNAi

GeneDB ID#	Protein	Primary function	Fold change (D1/D0)	Fold change (D2/D0)	Subcellular localization	References for the localization
Category I – Flagellum attachment zone (FAZ) proteins						
Tb927.4.2060	FAZ8	Unknown	-1.35	-1.86	FAZ	this publication
Category II – Flagellum proteins						
Tb927.8.6230	Hypothetical	Unknown	-1.51	-1.78	Flagellum	This publication
Category III – Flagellum and cytoplasm proteins						
Tb927.11.610	Hypothetical	Unknown	-1.09	-1.74	Flagellum, cytoplasm	this publication
Tb927.11.6460	Hypothetical	Unknown	-1.81	-1.26	Flagellum, cytoplasm	this publication
Category IV – Flagellum and cytoskeleton proteins						
Tb927.11.7600	Hypothetical	Unknown	-1.72	-3.3	Flagellum, cytoskeleton	this publication
Category V – cytoskeleton proteins						
Tb927.4.1300	Hypothetical	Unknown	-1.26	-1.99	Cytoskeleton	this publication
Tb927.6.5070	Hypothetical	Unknown	-1.4	-1.69	Cytoskeleton	this publication
Tb927.11.2610	Hypothetical	Unknown	-1.47	-1.57	Cytoskeleton	this publication
Category VI – Paraflagellar rod (PFR) proteins						
Tb927.6.4140	PFC4	Unknown	-1.39	-2.21	Paraflagellar rod	Portman et al., 2009
Tb927.3.3750	PFC7	Unknown	-4.33	-25.1	Paraflagellar rod	Portman et al., 2009
Tb927.2.2160	PFC11	Unknown	-1.24	-1.8	Paraflagellar rod	Portman et al., 2009
Tb927.10.9570	PFC14	Unknown	-1.17	-1.49	Paraflagellar rod	Portman et al., 2009
Tb927.10.11300	PFC16	Unknown	-1.77	-2.56	Paraflagellar rod	Portman et al., 2009
Tb927.8.4970	PFR2	PFR assembly	-1.37	-1.97	Paraflagellar rod	Portman et al., 2009

Table S2. Proteins precipitated by FAZ2 immunoprecipitation

GeneDB ID	Protein	Total Peptides	Unique peptides	Coverage (%)
Tb09.211.4511	KMP11	154	12	81.5
Tb927.4.2080	CC2D	115	59	69.4
Tb927.1.4310	FAZ2	50	37	31.4

Table S3. Primer sequences and restriction enzymes used for plasmid linearization. The restriction sites used for sub-cloning were underlined.

Download Table S3