Supplemental experimental procedures

T. brucei cell lines

427-derived bloodstream form *T. brucei* single marker-221 cells (1) were cultivated in HMI-9 with 10% fetal bovine serum at 37°C and 5% CO_2. *T. brucei* transfection and drug selection were performed as described (2). *TbBBS-HA/-* and *TbBBS4-PTP/-* lines (used in experiments of Figures 1, 2 and 4) were created by ablating one endogenous allele and epitope tagging the other allele with the triple hemagglutinin (HA) tag and protein C-TEV-protein A (PTP) tag (3), respectively. The resulting fusion proteins have a C-terminus tag. PCR primers for tagging were: BBS1-HA-ORF-For: CGATACGGGCGCGAAGATGC; BBS1-HA-ORF-Rev: TGAATTGTCTTCTATGAATCCAGCGTCAG; BBS1-HA-UTR-For: AGGTGGACAACTGATCGAACGT; BBS1-HA-UTR-Rev: TCGAGCTAGAGCTCCATACCCT; BBS4-HA-ORF-For: CGTCCGACTCTAGGGCAACCA; BBS4-HA-ORF-Rev: TTTGCGTAGCTTTGCCGTAAGGA; BBS4-HA-UTR-For: TGAACGTAGGTGACTGCAAGTACAG; BBS4-HA-UTR-Rev: GCCTTTCGTTGTTCCCTATCCCT; BBS4-PTP-ORF-For: GCACTCGCGCGCTATTAGTTTGC ; BBS4-PTP-ORF-Rev: TTGCGTAGCTTTGCCGTAAGGAG; BBS5-HA-ORF-For: ACGCGTAAAGGGCGTGAGCA; BBS5-HA-ORF-Rev: TGTCACGACATTCCACAAATCCTGCA; BBS5-HA-UTR-For: GTCGGGGGAAAGTGGAAAGAGAG; BBS5-HA-UTR-Rev: ACACCTCGGTGGAATTTGATGTCGT; BBS7-HA-ORF-For: GCTGCGGGAACACATGTACG; BBS7-HA-ORF-Rev: ACAACGAAAAATACGCCGAAGCTC; BBS7-HA-UTR-For: GCTGGAGGTATGTGTGGCTGG; BBS7-HA-UTR-Rev: AGGGGTAAAACAAGGAGAGGAAGAGA. PCR-amplified fragments were cloned in pMOT-2H vector (4) with the exception of BBS4-PTP amplicon which was cloned into pC-Puro-PTP (3). *TbBBS -/-* lines were created by ablating both alleles using two different drug markers. PCR primers for genetic knockouts were: BBS1-KO-For-upstream: TCCGGAGAACACCATGTC ; BBS1-KO-Rev-upstream: ATAAAACGCTAATAATAACAATC; BBS1-KO-For-downstream: AGGTGGACAACTGATCGAAC; BBS1-KO-Rev-downstream: AAAGTCTTTTGCCGCGTCGC; BBS4-KO-For-upstream: TTCCGCGATTTCTAGTTTCCTTCACGATATTCTGAAAATAC; BBS4-KO-Rev-upstream: ACCTATTGAGGGTTAGGGTTAGGGAAGTCCCAC; BBS4-KO-For-downstream: ATGGAGCCACTGGAAAAGCTACAGAGTGAAATCG; BBS4-KO-Rev-downstream: CGTCCACGCGCTTGTCCTCCTCTAG; BBS5-KO-For-upstream: TGCGGGGAAACACGACACGA ; BBS5-KO-Rev-upstream: TGTTGTACACACAGGCCGGAAC ; BBS5-KO-For-downstream: GTCGGGGGAAAGTGGAAAGAGAG; BBS5-KO-Rev-downstream: CCATTCCAGCTCTAAACCGCCA; BBS7-KO-For-upstream:

ATCAGGAGTAGTCCATGCCTTATCT; BBS7-KO-Rev-upstream:
CCTCAGCTCACTCTCCCCTTC; BBS7-KO-For-downstream:
GCTGGAGGTATGTGTGGCTGG; BBS7-KO-Rev-downstream:
CACATGTCGGGGTCACTCCTCT; BBS9-KO-For-upstream:
AGATAGTGAGGTGAAAGAGGCTGAG; BBS9-KO-Rev-upstream:
GGCTCGTGAAGCGCTATTATAACAG; BBS9-KO-For-downstream:
GTATAAATGACGCTACCACCTGCGTTG; BBS9-KO-Rev-downstream:
ATATATACACGTGGCGTGAAGCAACG. Amplified fragments representing flanking sequences of the targeted gene were cloned in pTub-Blast and pTub-Phleo vectors (gift of R. Sabatini) (5). *TbBBSx-HA/+ in TbBBSy-/-* lines (used in experiments of Figure 3) were created by epitope tagging one *TbBBS* allele with the HA tag in KO background of a different *TbBBS* gene; as control, one *TbBBS* allele was tagged with HA in WT background. *TbBBS1* add-back was created by introducing an ectopic copy of *TbBBS1* ORF in *TbBBS1 -/-*. *TbBBS1* ORF was amplified with BBS1-ORF-For: ATGCGTCGGGAGCAGTTAG; BBS1-ORF-Rev:
CTATGAATTGTCTTCTATGAATCCAGCGTC and cloned in the pHD496 vector (6).

Western blotting

Cell lysates were run on standard SDS-PAGE gels and blotted on nitrocellulose membranes. For Blue Native electrophoresis (BN-PAGE) (7), cells were extracted with 20 mM Tris-HCl (pH=7.7), 150 mM KCl, 0.5% Igepal CA-630 (US Biological), 3 mM $MgCl_2$ and SIGMA*FAST* EDTA-free protease inhibitors (Sigma). Extracts were cleared by 18,000g centrifugation, ran on 4-16% Bis-Tris NativePAGE gels (Life Technologies), and blotted on 0.2µm PVDF membranes (Bio-Rad). Equal loading in BN-PAGE was confirmed by internal Coomassie staining or Western blot of VSG 221 from the same sample. Membranes were incubated with the following primary antibodies; mouse anti-HA (1:3,000, BioLegend clone 16B12); mouse anti-beta-Tubulin (1:5,000, DSHB); rabbit peroxidase-anti-peroxidase (PAP) for protein A detection (1:3,000, Sigma); mouse anti-protein C (1:2,000, Genscript clone HPC4); rabbit anti-VSG 221 (8) (1;100,000, gift of J. Bangs); rabbit anti-clathrin heavy chain (9) (1:5,000, gift of M. Field) and anti-ESAG7 (10) (1:5,000, gift of P. Borst). For detection, membranes were incubated with HRP-conjugated goat anti-mouse (1:5,000; Bio-Rad) and goat anti-rabbit (1:10,000; Bio-Rad). To allow detection the following cell equivalents were typically loaded: 5-10x10^6 for tagged BBS proteins, 1x10^6 for clathrin heavy chain, 2x10^6 for ESAG7, 5x10^5 for tubulin and 5x10^5 for VSG 221. Predicted sizes are: TbBBS1-HA= 69.2 kDa, TbBBS4-HA=55.4 kDa, TbBBS5-HA=44.5 kDa, TbBBS7-HA=86 kDa, TbBBS4-PTP=71 kDa.

Glycerol gradient centrifugation

Linear 10-30% glycerol gradients were prepared in SW 41 centrifuge tubes using a Biocomp gradient maker (Biocomp Instruments, Inc.) by mixing equal volumes of 10% and 30% glycerol in 25 mM HEPES (pH 7.4), 60 mM KCl, 10 mM $MgCl_2$ and SIGMA*FAST* EDTA-free protease inhibitors (Sigma). Cells were harvested and washed twice in vPBS (137 mM NaCl, 3 mM KCl, 16 mM Na_2HPO_4, 3 mM KH_2PO_4, 46 mM sucrose, 10 mM glucose, pH 7.6) (11), then incubated for 10 min at room temperature in extraction buffer (100 mM PIPES, 2 mM EGTA, 1 mM $MgSO_4$, 0.1 mM EDTA, pH 6.9) containing 0.5% Igepal CA-630 and SIGMA*FAST* protease inhibitors. Samples were pelleted at 14,000 rpm for 5 min. Supernatants were loaded onto glycerol gradients. HMW native marker (GE) standards were loaded onto an identical gradient. Gradients were centrifuged at 30,000 rpm for 20 hr at 4ºC in a Beckman SW 41 rotor. 16 fractions were collected using a density gradient fractionator (Instrument Specialties Co). Samples were analyzed by SDS-PAGE, followed by Sypro Ruby staining of molecular weight standards or Western blotting for HA-tagged BBS proteins as described above.

Affinity purification and mass spectrometry analysis of TbBBS4-PTP associated protein complexes

Single step and tandem immunoprecipitation of TbBBS4-PTP were performed as described (12). Cells were extracted in 50 mM Tris-HCl (pH=7.7), 150 mM KCl, 0.5% Igepal CA-630 (US Biological), 3 mM $MgCl_2$ and SIGMA*FAST* EDTA-free protease inhibitors (Sigma) on ice for 10 min. Cell extract was centrifuged at 25,000g, 4°C and incubated with Pierce control agarose resin (Life Technologies) for 1h at 4°C. Pre-cleared extract was then incubated with IgG sepharose (GE Healthcare) for 2h at 4°C and washed 3x with 20 mM Tris-HCl (pH=7.7), 150 mM KCl, 0.1% Tween-20 (Calbiochem), 3 mM $MgCl_2$, SIGMA*FAST* EDTA-free protease inhibitors (Sigma) and 2x with 20 mM Tris-HCl (pH=7.7), 150 mM KCl, 0.1% Tween-20 (Calbiochem), 3 mM $MgCl_2$, 1 mM Dithiothreitol (Sigma) and 0.5 mM Ethylenediaminetetraacetic acid (Promega). Elution was performed by adding 200 units ProTEV Plus protease (Promega) in 20 mM Tris-HCl (pH=7.7), 150 mM KCl, 0.1% Tween-20 (Calbiochem), 3 mM $MgCl_2$, 1 mM Dithiothreitol (Sigma), 0.5 mM Ethylenediaminetetraacetic acid (Promega) and incubating overnight at 4°C. For one-step protein A purifications, the eluate was collected, concentrated with Amicon Ultra filters (10 kDa, Millipore) and boiled with SDS sample buffer. For tandem purifications (or one-step protein C purifications), the eluate (or pre-cleared cell extract) was collected and $CaCl_2$ was added to final concentration of 1 mM. Sample was then incubated with

anti-Protein C affinity matrix (Roche) for 2h at 4°C and washed 5x with 20 mM Tris-HCl (pH=7.7), 150 mM KCl, 0.1% Tween-20 (Calbiochem), 3 mM $MgCl_2$, 0.5 mM Dithiothreitol (Sigma), 1 mM $CaCl_2$. Elution was performed by adding 3x 5 mM Tris-Cl, pH 7.7, 5 mM Ethylenediaminetetraacetic acid (Promega), 10 mM Ethylene glycol tetraacetic acid (Bioworld), 0.1% Tween-20 (Calbiochem) and incubating at room temp for 10 min. Eluate was then concentrated with Amicon Ultra filters (10 kDa, Millipore) and boiled with SDS sample buffer. Tween-20 was omitted in final washes of samples destined for shotgun proteomics. These samples were precipitated with trichloroacetic acid (TCA, Sigma) and resuspended in digestion buffer (100 mM Tris-HCl, pH 8.5, 8M urea), reduced, alkylated and digested by sequential addition of lys-C and trypsin proteases (13, 14). The digested peptide mixture was desalted and fractionated online using a 75 µm inner diameter fritted fused silica capillary column with a 5 µm pulled electrospray tip and packed with 15 cm of Luna C18(2) 3 µM reversed phase particles in house. The gradient was delivered via an easy-nLC 1000 ultra high-pressure liquid chromatography (UHPLC) system (Thermo Scientific) (15, 16). MS/MS spectra were collected on a Q-Exactive mass spectrometer (Thermo Scientific). Data analysis was performed using the ProLuCID and DTASelect2 implemented in the Integrated Proteomics Pipeline - IP2 (Integrated Proteomics Applications, Inc., San Diego, CA) (17-19). Protein and peptide identifications were filtered using DTASelect and required at least two unique peptides per protein at a peptide-level false positive rate of 5% as estimated by a decoy database strategy (20). NSAF (Normalized spectral abundance factor) values were obtained as described (21).

Immunofluorescence microscopy

Cells were washed once in PBS + 1% glucose (PBS-G) and fixed on ice for 10 min in PBS-G + 3% paraformaldehyde (Electron Microscopy Sciences) at a concentration of 1e7 cells/ml. Fixed cells were washed once in PBS and air-dried onto glass cover slips. After 15 min rehydration in PBS, cover slips were incubated for 10 min in PBS + 0.1% Triton X-100 (EMD Millipore) to permeabilize cells. Cover slips were washed 3 times with PBS and then blocked in PBS + 20% fetal bovine serum (Life Technologies), followed by incubation with the following primary antibodies diluted in blocking solution: mouse anti-HA (1:200, Covance), rabbit anti-PFR2 (1:1000) (22) . After 3 PBS washes, cover slips were incubated with donkey anti-mouse Alexa 488 (1:1500, Life Technologies) and donkey anti-rabbit Alexa 594 (1:1500, Life Technologies) secondary antibodies. Cover slips were washed 3 times with PBS and mounted in Vectashield antifade mounting medium with DAPI (Vector Labs). Images were acquired using a 100x objective and Axiovision HRc camera on a Zeiss Axioskop II compound microscope with

Axiovision software (Zeiss) and processed using Adobe Photoshop (Adobe Systems).

Immunoelectron microscopy

Immunoelectron microscopy was performed as described (23). Parasites were fixed in 4% paraformaldehyde, 0.05% glutaraldehyde in 100 mM sodium cacodylate, pH 7.2 for 1 hr at 4°C. Samples were then embedded in 10% gelatin and infiltrated overnight with 2.3M sucrose, 20% polyvinyl pyrrolidone in PIPES/$MgCl_2$ at 4°C. Samples were trimmed, frozen in liquid nitrogen, and sectioned with a Leica Ultracut UCT7 cryo-ultramicrotome (Leica Microsystems Inc., Bannockburn, IL). 60 nm sections were blocked with 5% fetal bovine serum, 5% normal goat serum for 30 min and subsequently incubated with mouse anti-HA (BioLegend clone 16B12) followed by secondary antibody conjugated to 18nm colloidal gold (Jackson ImmunoResearch Laboratories, Inc., West Grove PA). Sections were washed in PIPES buffer followed by a water rinse, and stained with 0.3% uranyl acetate/2% methyl cellulose. Samples were viewed with a JEOL 1200EX transmission electron microscope (JEOL USA Inc., Peabody, MA) equipped with an AMT 8 megapixel digital camera (Advanced Microscopy Techniques, Woburn, MA). All labeling experiments were conducted in parallel with controls cells which did not express HA-tagged proteins. Localization to membranes and basal body was scored for gold particles that were observed on the mentioned structures.

Transmission electron microscopy

Parasites were fixed in 3% paraformaldehyde, 3% glutaraldehyde, 1% tannic acid in 100 mM sodium cacodylate, pH 7.2 for 1 hr at room temperature. Fixed cells were washed in fixative without tannic acid and then rinsed and postfixed with 1% osmium tetroxide plus 1.5% potassium ferrocyanide. Dehydration through an acetone gradient was performed, followed by infiltration and embedment in Eponate 12 (Ted Pella, Co.). Sections were cut on a Reichert Ultracut E ultramicrotome, poststained with uranyl acetate and lead citrate, and imaged using a Hitachi H-7000 or a 20 Philips CM 120 transmission electron microscope.

Immunoprecipitation of human BBSome proteins

Human Embryonic Kidney (HEK) 293 cells were reverse transfected with Lipofectamine 2000 (Life Technologies) for plasmids encoding Myc-tagged BBSome subunits. After 48hr, cells were harvested and lysed with 50 mM Tris (pH 7.4), 150 mM NaCl, 1% Triton X-100, and protease inhibitors. Lysates were clarified and incubated with mouse C-Myc monoclonal 9E10 for 1 hour. The antibody was then captured with Protein G Sepharose 4 fast flow beads (GE),

washed 20x with Wash buffer (25 mM Tris pH 7.4, 300 mM NaCl, 1% Triton X-100), and then boiled off beads in SDS sample buffer. Clathrin Heavy Chain (CHC) was detected by western with mouse clone 23 monoclonal (BD Biosciences, #610499).

Mouse infections

All animal experiments strictly complied with the Institutional Animal Care and Use Committee of University of California, Los Angeles (Approved protocol permit: ARC # 2001-065). BALB/c female mice (The Jackson Laboratory, Bar Harbor, ME), 6 to 10 weeks old, were injected intraperitoneally with 1000 parasites in 0.2 ml cold phosphate buffered saline (pH 7.4). Parasitemia was monitored daily beginning 4 days post infection using an improved Neubaeur hemocytometer.

Southern blotting

Southern blotting was performed as described (22). Genomic DNA was isolated with a PureLink genomic DNA kit (Invitrogen), digested for 8 hours with restriction site enzymes (New England BioLabs) and ran on a 0.8% agarose gel. Gels were treated in depurination buffer (0.25 M HCl), denaturation buffer (0.5 M NaOH, 1.0 M NaCl), and neutralization buffer (1 M Tris-HCl [pH 7.5], 3 M NaCl) for 15 min each and then rinsed in distilled water. DNA was transferred to a Hybond-XL membrane (GE Healthcare) overnight in SSC buffer (3 M NaCl, 300 mM $Na_3C_6H_5O_7$) and cross-linked to the membrane by 1,500 J at a UV Stratalinker cross linker (Stratagene). Digoxigenin-labeled probes were PCR generated using primers: BlastProbe_F: CCTCATTGAAAGAGCAACGG; BlastProbe_R: CTCGAATTCTTAGCCCTCCC; PhleoProbe_F: GCCATCACGAGATTTCGATT; and PhleoProbe_R: CACACTCATGAGATGCCTGC. Hybridization, washes, and detection were performed using DIG EasyHyb buffer, CDP-Star substrate, and a PCR DIG-probe synthesis kit (Roche).

Sedimentation assay

Cells were resuspended to 3×10^6 cells/ml in fresh HMI-9 with 50% fetal bovine serum. Each culture was aliquoted into six samples (1 ml per cuvette) and incubated for 7.5 hours at 37°C and 5% CO_2. The optical density at 600 nm (OD_{600}) was measured every 2.5 hours. At each time point, three samples from each culture were left undisturbed to monitor sedimentation, while the other three samples were resuspended to monitor growth. The change in the OD_{600} (ΔOD_{600}) was calculated by subtracting the OD_{600} of resuspended samples from that of

undisturbed samples. Statistical analysis was performed with GraphPad Prism 5 (GraphPad Software).

Growth curve

Cells were resuspended to 1.5×10^5 cells/ml in fresh HMI-9 with 20% fetal bovine serum and incubated at 37°C and 5% CO_2. Every 24 hours, cultures were counted (Coulter counter) and split to the same seeding density. Statistical analysis was performed with GraphPad Prism 5 (GraphPad Software).

VSG clearance assay

VSG clearance was performed as described previously (24). Cells were washed with ice-cold 20% fetal bovine serum in HMI-9, resuspended at 10^7 cells/ml in ice-cold 50% fetal bovine serum in HMI-9 and kept on ice for 15 min. Rabbit anti-VSG 221 (gift of J. Bangs) was then added at 1:3000; to stain dead cells Propidium Iodide (PI, Life Technologies) was added at 1 µg/ml final concentration and cells were kept on ice for 10 min. An aliquot was then transferred to 1% Glucose in ice-cold Phosphate Buffer Saline (PBS-G) and used as 0 min sample. Cells were then transferred to 37°C for 5 min to allow endocytic anti-VSG clearance. Clearance was stopped by adding ice-cold PBS-G and cells were fixed with ice-cold 4% paraformaldehyde (PFA) in PBS on ice for 5 min. Fixative was washed out with ice-cold 1% BSA in PBS (PBS-B), cells were resuspended in ice-cold PBS-B with anti-rabbit Alexa Fluor 488 (1:3000, Life Technologies) and kept on ice for 10 min. Secondary antibody was washed out with ice-cold PBS-B and cells were resuspended in 10 µg/ml DAPI (4',6-diamidino-2-phenylindole, Sigma) in PBS-B and passed through a 40 µM filter (BD Biosciences) to ensure single-cell separation. Flow cytometry analysis was performed using LSR II (Becton Dickinson) and FlowJo software (Tree Star) to report mean Alexa Fluor 488 intensity of PI-negative/DAPI-positive cells at 5 min as percentage of 0 min clearance. Statistical analysis (one-way ANOVA with Dunnett post-test) was performed with GraphPad Prism 5 (GraphPad Software).

Uptake assays

Uptake assays were performed as described previously (24, 25). Cells were washed with warm 1% Bovine Serum Albumin (BSA) in HMI-9, resuspended at 10^7 cells/ml in 1% BSA-HMI-9 and incubated at 37°C for 30 min. An aliquot was then transferred to 1% Glucose in ice-cold Phosphate Buffer Saline (PBS-G) and used as unstained control sample). Transferrin-Alexa Fluor 594 (Life Technologies) or Dextran-Alexa Fluor 594 (10,000 MW, Life Technologies) were

added to the remaining sample to final concentration of 50 µg/ml and 500 µg/ml respectively. Samples were incubated at 37°C for 15 min and uptake was stopped by adding ice-cold PBS-G. Cells were centrifuged, washed with ice-cold PBS-G and fixed with ice-cold 4% paraformaldehyde (PFA) in PBS on ice for 5 min. Fixative was washed out with ice-cold 1% BSA in PBS (PBS-B) and cells were resuspended in 10 µg/ml DAPI (4',6-diamidino-2-phenylindole, Sigma) in PBS-B and passed through a 40 µM filter (BD Biosciences) to ensure single-cell separation. Flow cytometry analysis was performed using LSR II (Becton Dickinson) and FlowJo software (Tree Star) to report mean Alexa Fluor 594 intensity of DAPI-positive cells at 15 min uptake. The transferrin binding assay was done in similar fashion but all steps were performed on ice to inhibit endocytosis. Statistical analysis (one-way ANOVA with Dunnett post-test) was performed with GraphPad Prism 5 (GraphPad Software).

SILAC proteomic analysis

HMI-9 media was prepared as described (2), using IMDM lacking L-lysine and L-arginine (Caisson Labs) but supplemented with either normal L-lysine (Sigma) and L-arginine (Sigma) or stable heavy isotopes $^{13}C_6$ L-lysine and $^{13}C_6$ L-arginine (Cambridge Isotope Laboratories). 8% dialyzed heat-inactivated fetal bovine serum (Atlanta Biologicals) and 2% heat-inactivated fetal bovine serum (Life Technologies) were added. Wild-type (WT) and *BBS*-KO cells were washed in heavy or light media, respectively, and harvested after 3 days of growth in their respective medias (>9 generations for wild-type, >98% $^{13}C_6$ incorporation). Each *BBS*-KO was mixed 1:1 with an equal number of WT cells. Surface proteins were biotinylated and purified from each *BBS*-KO:*WT* cell mixture following VSG depletion, as previously described (26). Proteomic analyses were performed essentially as described (27). On-bead samples were mixed with digestion buffer (100 mM Tris-HCl, pH 8.5, 8 M urea). The samples were reduced and alkylated by sequential treatment with 5 mM tris(2-carboxyethyl) phosphine (TCEP) and 10 mM iodoacetamide as described earlier (13, 14). Afterward, samples were sequentially digested with Lys-C and trypsin proteases as previously described (14). The digestion was stopped by addition of formic acid to 5% and peptide digests analyzed by LC-MS/MS on a Thermofisher Q-Exactive. Peptide digests were desalted offline by C18 tip (Pierce) and separated online using reversed-phase chromatography on a 75 µM inner diameter fritted fused silica capillary column with a 5 µM pulled electrospray tip that was packed in-house with 15 cm of Luna C18(2) 3 µM reversed phase particles. An EASY-nLC 1000 ultra-high pressure liquid chromatography (UHPLC) system (Thermo Scientific) was used to deliver a linear acetonitrile gradient from 3% to 30% solvent B (Buffer A: 0.1% formic acid, Buffer B: Acetonitrile/ 0.1% formic acid) at a flow

rate of 200 nl/min. MS/MS spectra were collected on a Q-Exactive mass spectrometer (Thermo Scientific) as described (15, 16). Raw data files were converted to MS2 files using RawExtractor v.1.9.9.2 (Yates Lab, http://fields.scripps.edu/downloads.php). Data analysis was performed using ProLuCID for database searching, and DTASelect2 for probabilistic filtering as implemented in the Integrated Proteomics Pipeline v.2-IP2 (Integrated Proteomics Applications, Inc.) (17-19). MS/MS spectra were searched against a protein FASTA database obtained from TriTrypDB (downloaded from tritrypdb.org on January 31, 2014) appended with sequences for ESAGs from the 221 VSG expression site (GI numbers 189094616–189094632) plus ESAG13 (28) and predicted short ORFs (29) and concatenated to a decoy database in which the amino acid sequence of each entry was reversed (23,172 total entries). The search parameters (13) were as follows: (1) precursor ion mass tolerance of +/-15 ppm, (2) fragment ion mass tolerance of +/-15 ppm, (3) only peptides with fully tryptic ends and unlimited missed cleavages were considered as candidates, and (4) a static modification of +57.02156 Da on cysteine residues resulting from carbamidomethylation. Protein and peptide identifications were filtered using DTASelect with mass accuracy statistics enabled (--mass) and required at least two unique peptides per protein and a spectra-level false positive rate of less than 1% as estimated by a reverse decoy database strategy (20). Intensity based quantitative interrogation of SILAC datasets was performed within the Skyline software package (30). Within Skyline, only uniquely mapping, fully tryptic peptides with no missed cleavages were considered for inquiry. Heavy-Light peak sets were annotated with q-values based on a target-decoy peak-picking approach provided by the mProphet algorithm, generating a randomly mass shifted decoy for each peptide (31). SILAC isotopic peak area ratios for the mProphet annotated peak boundaries were exported from Skyline and analyzed within R v3.2 (32). For subsequent analysis, we considered only proteins which were detected in wild type and all five TbBBS mutants by at least three peptides passing the mProphet q-value threshold of 0.01. Mutant/WT ratios were Log2-transformed to derive Log Fold Changes (LogFC). LogFC were then standardized to Z-scores to facilitate comparisons across mutants. Individual Z-scores are shown in heat maps with hierarchical clustering (Euclidean distance and Ward's linkage), while Z-scores averaged over TbBBS mutants are shown in dot plots.

Ubiquitin pulldown assay

Cells were extracted in 50 mM Tris-HCl (pH=7.7), 150 mM KCl, 0.5% Igepal CA-630 (US Biological), 100 μM N-ethylmaleimide (NEM) (Calbiochem), 3 mM $MgCl_2$ and SIGMA*FAST* EDTA-free protease inhibitors (Sigma) on ice for 10 min. Cell extract was centrifuged at

25,000g, 4°C and incubated with Pierce control agarose resin (Life Technologies) for 1h at 4°C. Pre-cleared extract was then split in two and each half was incubated with Pierce control agarose resin (Life Technologies) and ubiquitin resin (Boston Biochem) respectively for 2h at 4°C. Beads were washed 5x with 20 mM Tris-HCl (pH=7.7), 150 mM KCl, 0.1% Tween-20 (Calbiochem), 3 mM $MgCl_2$, 100 μM NEM and SIGMA*FAST* EDTA-free protease inhibitors (Sigma) and then boiled in SDS sample buffer.

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Supplemental Figure legends

Figure S1. The TbBBSome is dispensable *in vitro*. (A) Southern blotting to confirm correct integration of drug resistance cassettes. Genomic DNA from each cell line was digested with three different restriction enzymes and probed with blasticidin and phleomycin resistance gene probes. Enzymes and expected sizes for blasticidin and phleomycin blots respectively are: 1) MfeI (none), 2) ApaLI (none), 3) MluI (none), 4) Mfe1 (6025, 6087 bp), 5) ApaLI (5203, 5265 bp), 6) SspI (6809, 6871 bp), 7) MfeI (6301, 6346 bp), 8) ApaLI (8481, 8526 bp), 9) AgeI (5023, 5068 bp), 10) AgeI (5796, 5858 bp), 11) ApaLI (3118, 3180 bp), 12) MluI (6877, 6939 bp), 13) MfeI (4589, 4651 bp), 14) SspI (7448, 7510 bp), 15) MluI (9358, 9420 bp). (B) Sedimentation assay for TbBBS mutants. All TbBBS-KOs sedimentation slope is similar to *wild type* (WT); ns, not significant. (C) Cumulative growth curve for TbBBS mutants. TbBBS9-KO growth slope is different than wild type (WT); *$p<0.05$. (D) Transmission electron micrographs of flagellar cross-sections in wild type (WT) and TbBBS mutants.

Figure S2. **The TbBBSome is required for transferrin uptake**. (A) Western blot on lysates of *wild type* (WT) and *TbBBS* mutants. Tubulin (Tub) blot was used as loading control to compare levels of clathrin heavy chain (CHC) and expression site-associated gene 7 (ESAG7). (B) Transferrin binding in conditions that block endocytosis as % of wild type (WT). (C) Transferrin uptake as % of *TbBBS4 +/-* (parental cell line used as control). In B-C, data are represented as mean ± SEM; ns, not significant; *$p < 0.05$ and ***$p < 0.001$ via one-way analysis of variance with Dunnett post test.

Figure S3. **Cell surface SILAC proteomics of TbBBS mutants**. All mutants were compared to wild type (WT*)*. Proteins were compared via Z-scores of Log2-transformed Fold Change (Mutant/WT). (A) Distribution of Z-scores in TbBBS mutants. (B) Heat map of Z-scores in TbBBS mutants. The inset at the bottom shows the number of proteins within each bin of the heat map.

Figure S4. **Localization of TbBBS proteins**. Immunofluorescence for TbBBS-HA proteins; TbBBS-HA (green), PFR2 (red), DAPI (blue). Wild type (WT) is shown as negative control for anti-HA staining. Scale bar is 5 µm.

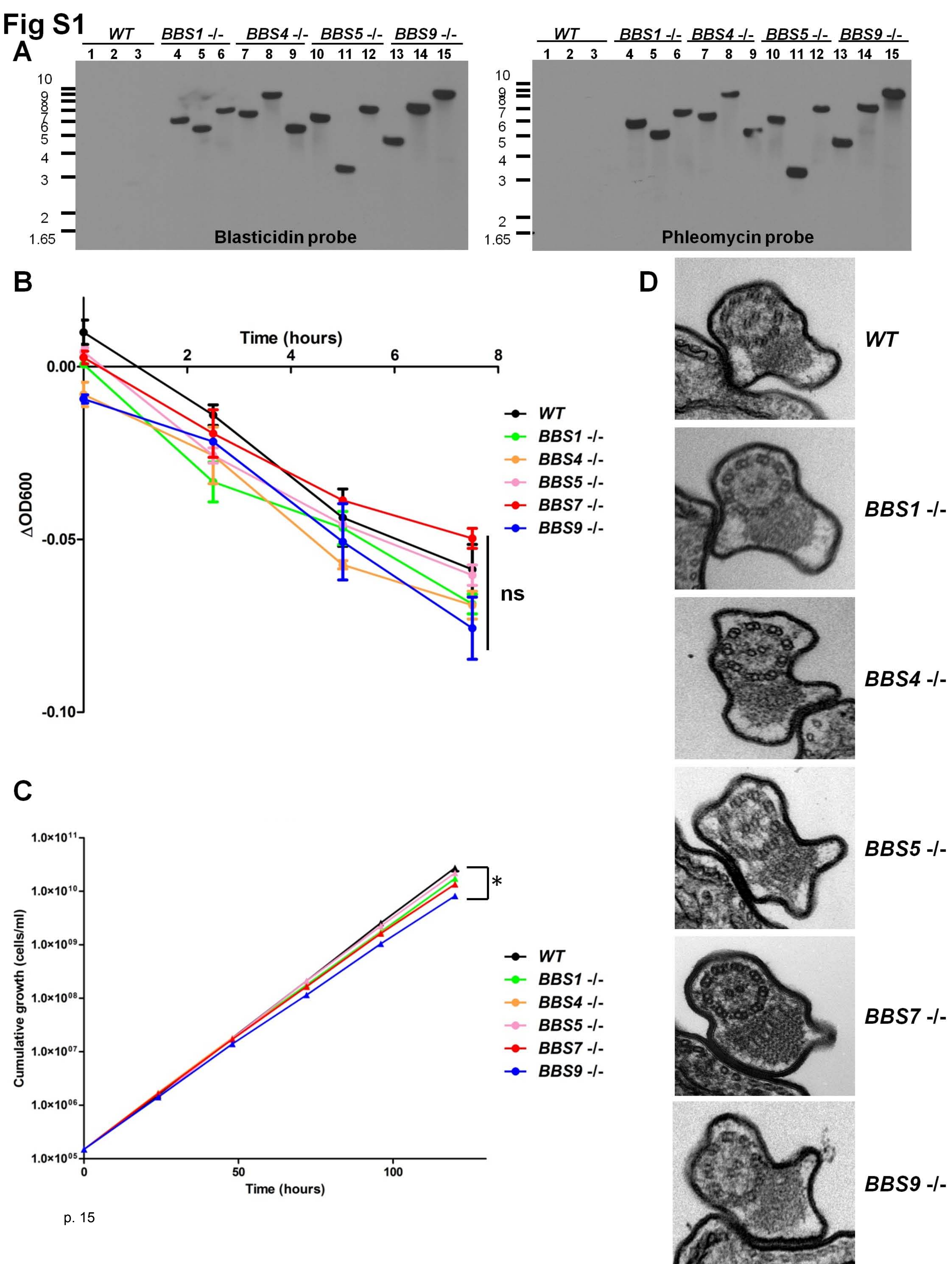
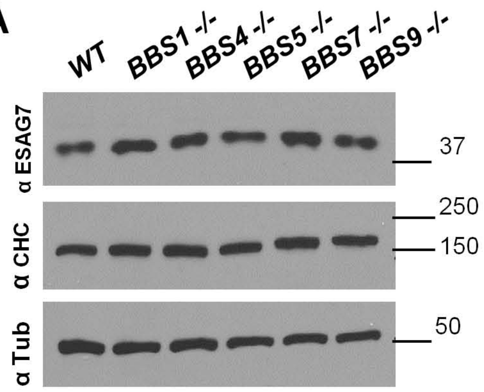
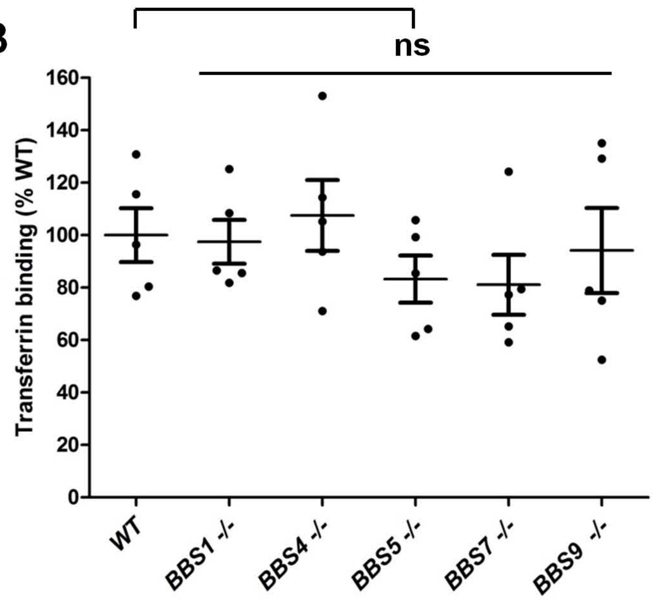


Fig S1
A
WT
BBS1 -/-
BBS4 -/-
BBS5 -/-
BBS9 -/-
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
10
9
8
7
6
5
4
3
2
1.65
Blasticidin probe
Phleomycin probe
B
Time (hours)
2
4
6
8
0.00
-0.05
-0.10
ΔOD600
ns
WT
BBS1 -/-
BBS4 -/-
BBS5 -/-
BBS7 -/-
BBS9 -/-
C
1.0×10¹¹
1.0×10¹⁰
1.0×10⁰⁹
1.0×10⁰⁸
1.0×10⁰⁷
1.0×10⁰⁶
1.0×10⁰⁵
Cumulative growth (cells/ml)
0
50
100
Time (hours)
*
D
WT
BBS1 -/-
BBS4 -/-
BBS5 -/-
BBS7 -/-
BBS9 -/-

Fig S2





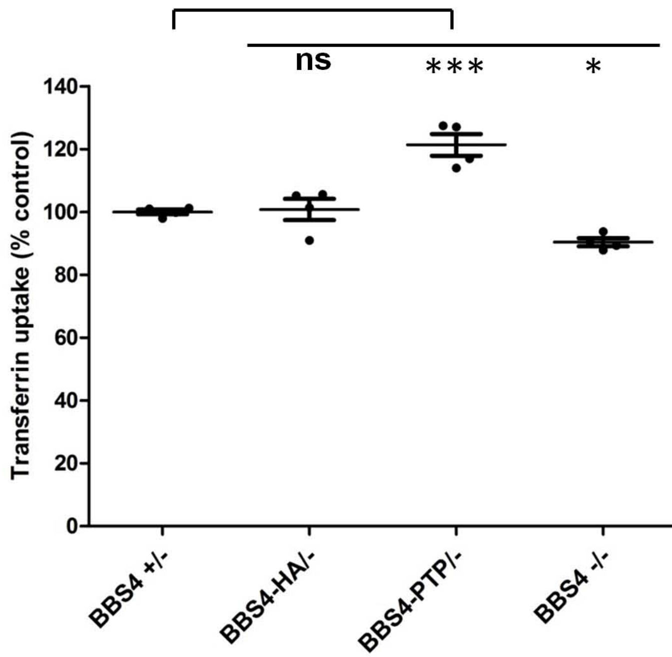


Fig S3

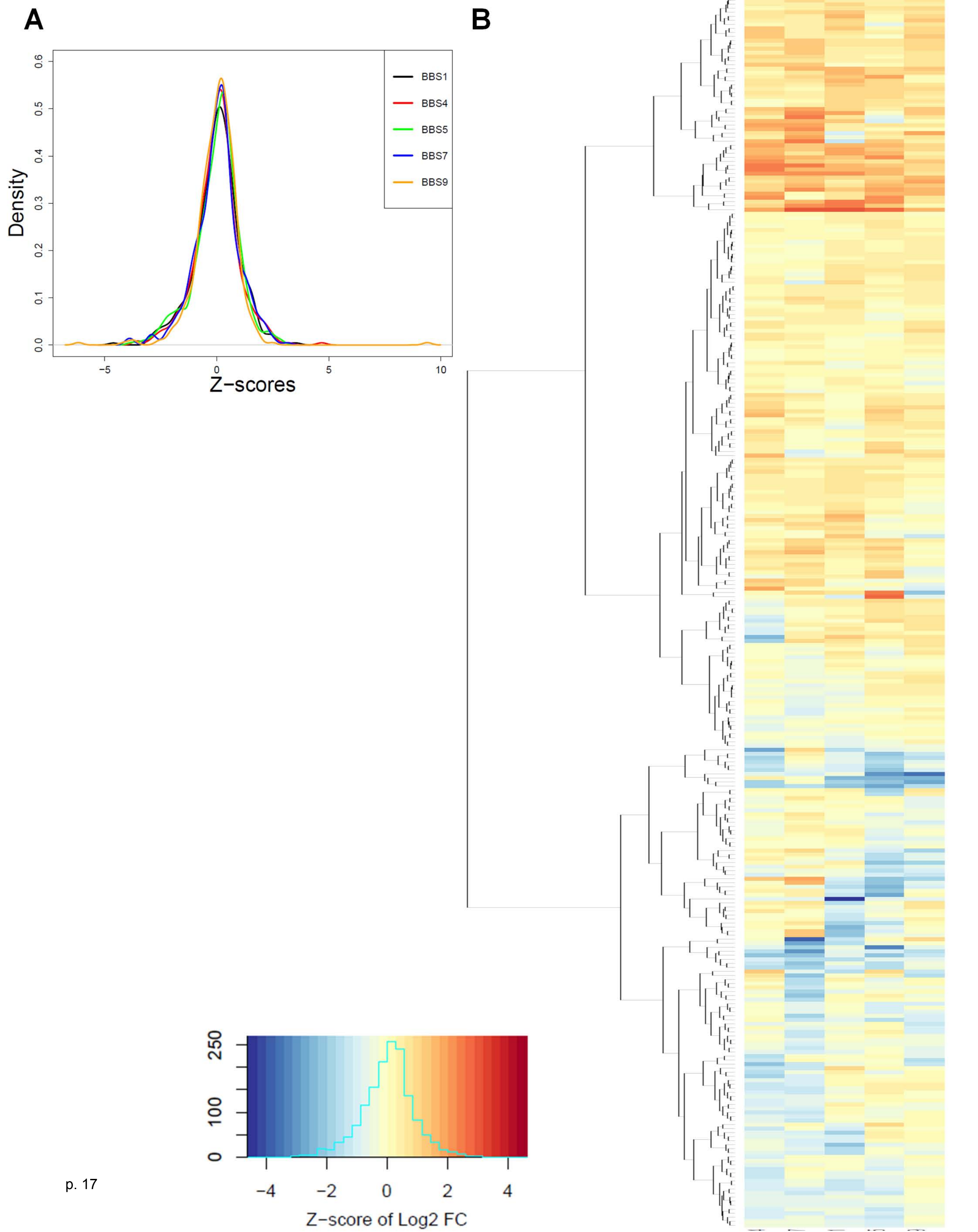


Fig S4

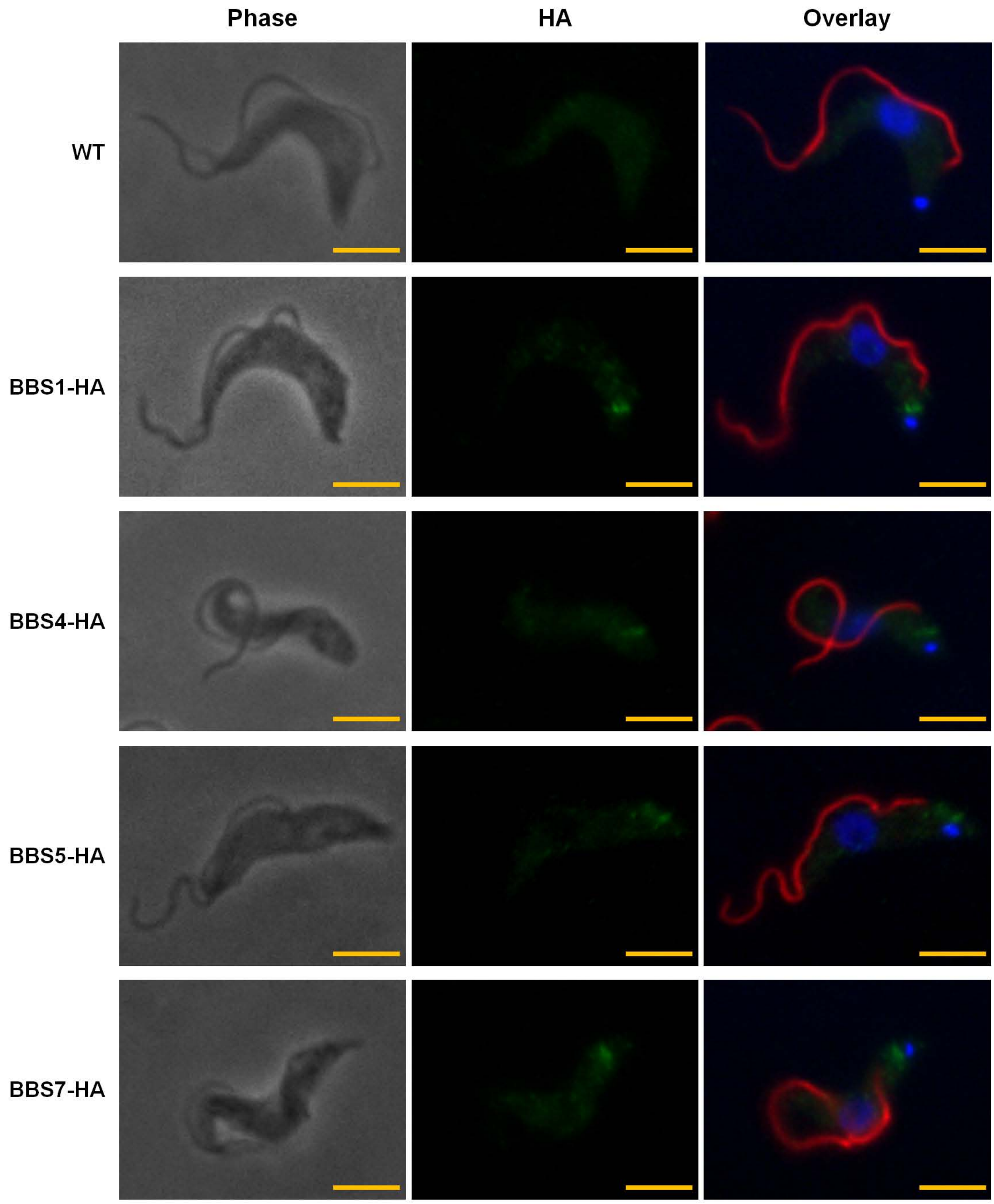


Table S1. The top 10 proteins detected specifically in TbBBS4-PTP tandem purification by Normalized Spectral Abundance Factor (NSAF)

NSAF (e^5)	Spectra	Protein	Tritryp ID	Predicted size (Da)
7901	97	TbBBS5	Tb927.1.4140	41005
7298	203	TbBBS9	Tb927.11.14470	92379
7288	113	TbBBS4	Tb927.10.6830	51895
6779	166	TbBBS2	Tb927.6.2020	80173
6382	118	TbBBS8	Tb927.11.12450.1 Tb927.11.12450.2	59842 62629
5483	137	TbBBS7	Tb927.3.3640	82529
5165	105	TbBBS1	Tb927.9.10670	65643
3757	12	TbBBS18	Tb7.NT.49_2 [see (29)]	10949
1043	11	TbMCP11	Tb927.9.10310	34296
661	4	TbARF1	Tb927.9.13650 Tb927.9.13740 Tb927.9.13710 Tb927.9.13680	20652 20624 20624 20624

Table S2. Quantification of gold particles in immuno-electron micrographs

Cell line	Micrographs	Total gold particles	Flagellar pocket membrane and vesicles (% of total)	Basal body (% of total)
WT	37	33	3 (9.1%)	1 (3%)
TbBBS1-HA	21	94	53 (56.4%)	24 (25.5%)
TbBBS4-HA	21	100	42 (42%)	7 (7%)
TbBBS5-HA	29	115	57 (49.6%)	1 (0.87%)

Table S3. The top 20 over-represented proteins in TbBBS mutants by cell surface SILAC proteomics Z-score

Protein ID	Description	Z score	Standard Deviation	Peptide number
Tb927.7.180	VSG-related	2.50389	0.704726	9.4
Tb927.11.13130	MBAP1	1.726392	0.687796	9.8
CAQ57290.1	ESAG2	1.721865	0.660893	5.6
Tb927.7.2160	Hypothetical	1.686534	0.541925	4.2
Tb927.8.2070	Hypothetical	1.638948	0.410412	11.6
Tb927.11.4100	VSG-related	1.574393	0.875002	6.6
Tb927.11.12240	Ubiquitin hydrolase	1.533892	0.51237	9.4
Tb927.9.11480	Hypothetical	1.477354	0.305009	8.4
Tb927.5.630	ISG-related	1.424376	0.316022	20.6
Tb927.3.4720	Dynamin	1.418338	0.898791	15.2
Tb927.11.7410	MSP-A	1.363737	0.480541	4.6
Tb927.5.360	ISG75	1.268022	0.593007	28.4
Tb927.11.5290	MCP9	1.258433	0.340361	8.6
Tb927.5.1390	ISG64	1.188723	0.706698	14.2
Tb927.11.7780	ATOM46	1.180361	0.515534	3.8
Tb927.8.6730	Hypothetical	1.159344	0.837454	6.6
Tb927.10.4770	PI4P-5 kinase	1.134434	0.230585	5.6
Tb927.8.4060	Flagellum adhesion glycoprotein	1.134293	0.48578	4
Tb927.6.1520	Aquaglyceroporin 1	1.114121	0.797565	4
Tb927.11.2430	Cytoplasmic dynein HC	1.102358	1.052828	10.6

Table S4. The top 20 under-represented proteins in TbBBS mutants by cell surface SILAC proteomics Z-score

Protein ID	Description	Z score	Standard Deviation	Peptide number
Tb927.10.14740	Hypothetical	-2.02642	0.47777	4.4
Tb927.5.1810	Lysosomal/endosomal p67	-1.78907	1.513292	5.8
Tb927.10.6810	Guanylate kinase	-1.75093	0.975107	6.8
Tb927.11.10760	Kinesin-like	-1.53084	0.911641	7.2
Tb927.8.7100	Acetyl coA carboxylase	-1.50758	1.737859	10.2
Tb11.v5.0208	ISG-related	-1.49328	1.411813	6.6
Tb927.10.15850	PEX12	-1.40036	1.199963	3.6
Tb927.5.1210	Short chain dehydrogenase	-1.31935	0.542058	7.8
Tb927.4.5010	Calreticulin	-1.26346	0.604535	11
Tb927.5.890	Oligosaccharyl transferase subunit	-1.1723	0.779902	8
Tb927.2.3270	ISG65	-1.13676	0.473674	4.4
Tb927.11.6280	Pyruvate phosphate dikinase	-1.13587	0.846185	5.4
Tb927.7.480	Hypothetical	-1.12429	1.133975	5.6
Tb927.9.8950	Prenyl protease	-1.08662	0.640498	8
Tb927.6.930	Metacaspase 3	-1.08325	0.734215	3.8
Tb927.11.1070	Serine peptidase	-1.07245	1.020696	5.8
Tb927.3.4230	Hypothetical	-1.0577	0.96336	5.2
Tb927.7.900	Hypothetical	-0.97279	0.65166	6
Tb927.7.2390	Hypothetical	-0.90254	0.618136	7.2
Tb927.7.1420	Deoxyribose-phosphate aldolase	-0.88926	0.55567	6.8

Table S5. Proteins detected in TbBBS4-PTP tandem purification but absent in control one-step and tandem purifications from WT cells lacking the PTP tag.

Locus	Spectra	NSAF (x10^5)	BBS protein	Description
Tb927.1.4140; Tb11.v5.0758;	97	7900.67	TbBBS5	| organism=Trypanosoma_brucei_TREU927 | product=hypothetical protein, conserved | location=Tb927_11_bin_v5.1:3959461-3960570(-) | length=369 | sequence_SO=random_sequence | SO=protein_coding ;
Tb927.11.14470;	203	7298.08	TbBBS9	| organism=Trypanosoma_brucei_TREU927 | product=hypothetical protein, conserved | location=Tb927_11_v5.1:3845532-3848042(+) | length=836 | sequence_SO=chromosome | SO=protein_coding ;
Tb927.10.6830; Tb11.v5.0539	113	7288.05	TbBBS4	| organism=Trypanosoma_brucei_TREU927 | product=hypothetical protein, conserved | location=Tb927_11_bin_v5.1:2352623-2354023(-) | length=466 | sequence_SO=random_sequence | SO=protein_coding ;| organism=Trypanosoma_brucei_TREU927 | product=Bardet-Biedl syndrome 4 protein homolog (BBS4-like protein 4), putative | location=Tb927_10_v5.1:1720306-1721706(+) | length=466 | sequence_SO=chromosome | SO=protein_coding ;
Tb927.6.2020;	166	6778.74	TbBBS2	| organism=Trypanosoma_brucei_TREU927 | product=intergrin alpha chain protein, putative | location=Tb927_06_v5.1:656186-658396(-) | length=736 | sequence_SO=chromosome | SO=protein_coding ;
Tb927.11.12450.1;Tb927.11.12450.2;	118	6381.58	TbBBS8	| organism=Trypanosoma_brucei_TREU927 | product=unspecified product | location=Tb927_11_v5.1:3332809-3335153(+) | length=544 | sequence_SO=chromosome | SO=protein_coding ;| organism=Trypanosoma_brucei_TREU927 | product=unspecified product | location=Tb927_11_v5.1:3333447-3335153(+) | length=568 | sequence_SO=chromosome | SO=protein_coding ;
Tb927.3.3640;	137	5482.76	TbBBS7	| organism=Trypanosoma_brucei_TREU927 | product=intergrin alpha chain protein, putative | location=Tb927_03_v5.1:1019968-1022621(+) | length=751 | sequence_SO=chromosome | SO=protein_coding ;
Tb927.9.10670;	105	5164.96	TbBBS1	| organism=Trypanosoma_brucei_TREU927 | product=Bardet-Biedl syndrome 1 protein homolog (BBS1-like protein 1), putative | location=Tb927_09_v5.1:1688915-1690750(-) | length=611 | sequence_SO=chromosome | SO=protein_coding ;
Tb7.NT.49_2;	12	3756.89	TbBBS18	no description;
Tb927.9.10310;	11	1042.92		| organism=Trypanosoma_brucei_TREU927 | product=mitochondrial carrier protein (MCP11) | location=Tb927_09_v5.1:1619646 1621538(-) | length=317 | sequence_SO=chromosome | SO=protein_coding ;
Tb927.9.13650;Tb927.9.13740;Tb927.9.13710;Tb927.9.13680;	4	660.55		| organism=Trypanosoma_brucei_TREU927 | product=ADP-ribosylation factor, putative | location=Tb927_09_v5.1:2171988-2172536(-) | length=182 | sequence_SO=chromosome | SO=protein_coding ;| organism=Trypanosoma_brucei_TREU927 | product=ADP-ribosylation factor, putative | location=Tb927_09_v5.1:2174469-2175017(-) | length=182 | sequence_SO=chromosome | SO=protein_coding ;| organism=Trypanosoma_brucei_TREU927 | product=ADP-ribosylation factor, putative | location=Tb927_09_v5.1:2173645-2174193(-) | length=182 | sequence_SO=chromosome | SO=protein_coding ;| organism=Trypanosoma_brucei_TREU927 | product=ADP-ribosylation factor, putative | location=Tb927_09_v5.1:2172112-2172660(-) | length=182 | sequence_SO=chromosome | SO=protein_coding ;
Tb927.9.5690;	2	531.95		| organism=Trypanosoma_brucei_TREU927 | product=60S acidic ribosomal protein, putative | location=Tb927_09_v5.1:976021-976362(+) | length=113 | sequence_SO=chromosome | SO=protein_coding ;
Tb927.10.12960;	4	522.70		| organism=Trypanosoma_brucei_TREU927 | product=ras-related protein rab-5,small GTPase, putative (RAB5A) | location=Tb927_10_v5.1:3150211-3150903(-) | length=230 | sequence_SO=chromosome | SO=protein_coding ;
Tb927.5.2540;	6	439.83		| organism=Trypanosoma_brucei_TREU927 | product=hypothetical protein, conserved | location=Tb927_11_bin_v5.1:2616298-2617530(-) | length=410 | sequence_SO=random_sequence | SO=protein_coding ;| organism=Trypanosoma_brucei_TREU927 | product=hypothetical protein, conserved | location=Tb927_05_v5.1:878309-879541(+) | length=410 | sequence_SO=chromosome | SO=protein_coding ;
Tb927.8.890;	3	433.49		| organism=Trypanosoma_brucei_TREU927 | product=small GTP-binding protein Rab1, putative | location=Tb927_08_v5.1:265034-266020(-) | length=208 | sequence_SO=chromosome | SO=protein_coding ;
Tb927.3.3760;Tb927.3.3780;	2	417.43		| organism=Trypanosoma_brucei_TREU927 | product=tryparedoxin (TRYP1) | location=Tb927_03_v5.1:1049840-1050898(+) | length=144 | sequence_SO=chromosome | SO=protein_coding ;| organism=Trypanosoma_brucei_TREU927 | product=tryparedoxin | location=Tb927_03_v5.1:1053583-1054017(+) | length=144 | sequence_SO=chromosome | SO=protein_coding ;
Tb927.11.16800;	11	389.41		| organism=Trypanosoma_brucei_TREU927 | product=hypothetical protein, conserved | location=Tb927_11_v5.1:4470731-4473280(-) | length=849 | sequence_SO=chromosome | SO=protein_coding ;

Table S5. Proteins detected in TbBBS4-PTP tandem purification but absent in control one-step and tandem purifications from WT cells lacking the PTP tag.

Locus	Spectra	NSAF (x10^5)	BBS protein	Description
Tb927.10.14800;	5	388.31		| organism=Trypanosoma_brucei_TREU927 | product=protein kinase, putative | location=Tb927_11_bin_v5.1:3017415-3018578(+) | length=387 | sequence_SO=random_sequence | SO=protein_coding ;| organism=Trypanosoma_brucei_TREU927 | product=protein kinase, putative,mitogen-activated protein kinase 9, putative | location=Tb927_10_v5.1:3608595-3609758(-) | length=387 | sequence_SO=chromosome | SO=protein_coding ;
Tb927.2.5980;	11	380.01		| organism=Trypanosoma_brucei_TREU927 | product=ATP-dependent Clp protease subunit, heat shock protein 104 (HSP104), putative,atp-dependent chaperone (HSP104) | location=Tb927_02_v5.1:1084458-1087070(+) | length=870 | sequence_SO=chromosome | SO=protein_coding ;
Tb927.11.9300;	7	375.69		| organism=Trypanosoma_brucei_TREU927 | product=hypothetical protein, conserved
Tb927.8.6110;	6	361.38		| organism=Trypanosoma_brucei_TREU927 | product=hypothetical protein, conserved | location=Tb927_08_v5.1:1785314-1786813(+) | length=499 | sequence_SO=chromosome | SO=protein_coding ;
Tb927.11.690;	7	296.32		| organism=Trypanosoma_brucei_TREU927 | product=hypothetical protein,chrXI additional, unordered contigs | location=tryp_XI-147f01.q1ca:7725-9797(+) | length=690 | sequence_SO=contig | SO=protein_coding ;| organism=Trypanosoma_brucei_TREU927 | product=hypothetical protein, conserved | location=Tb927_11_v5.1:166308-168440(-) | length=710 | sequence_SO=chromosome | SO=protein_coding ;
Tb927.7.4180;	3	295.62		| organism=Trypanosoma_brucei_TREU927 | product=fatty acid elongase, putative | location=Tb927_07_v5.1:1114698-1115615(+) | length=305 | sequence_SO=chromosome | SO=protein_coding ;
Tb927.11.13230;	2	278.29		| organism=Trypanosoma_brucei_TREU927 | product=hypothetical protein, conserved | location=Tb927_11_v5.1:3548093-3548743(-) | length=216 | sequence_SO=chromosome | SO=protein_coding ;
Tb927.5.3810;	4	262.49		| organism=Trypanosoma_brucei_TREU927 | product=orotidine-5-phosphate decarboxylase/orotate phosphoribosyltransferase, putative,OMPDCase-OPRTase, putative | location=Tb927_05_v5.1:1246263-1247652(+) | length=458 | sequence_SO=chromosome | SO=protein_coding ;
Tb927.1.120;	7	245.78		| organism=Trypanosoma_brucei_TREU927 | product=retrotransposon hot spot protein 4 (RHS4), putative | location=Tb927_01_v5.1:60266-62836(-) | length=856 | sequence_SO=chromosome | SO=protein_coding ;
Tb927.7.710;	5	235.17		| organism=Trypanosoma_brucei_TREU927 | product=heat shock 70 kDa protein, putative (HSP70) | location=Tb927_07_v5.1:155358-157286(-) | length=639 | sequence_SO=chromosome | SO=protein_coding ;
Tb927.2.2970;	2	192.66		| organism=Trypanosoma_brucei_TREU927 | product=mitochondrial carrier protein (MCP13) | location=Tb927_02_v5.1:564310-565248(+) | length=312 | sequence_SO=chromosome | SO=protein_coding ;
Tb11.01.1680.v5;Tb927.11.9920;Tb11.v5.0537;Tb11.NT.155_1;Tb11.NT.154_1;	10	192.57 (1054.77)*		| organism=Trypanosoma_brucei_TREU927 | product=polyubiquitin, putative | location=Tb927_11_bin_v5.1:1116876-1120727(+) | length=1283 | sequence_SO=random_sequence | SO=protein_coding ;| organism=Trypanosoma_brucei_TREU927 | product=polyubiquitin, putative | location=Tb927_11_v5.1:2675201-2677258(+) | length=685 | sequence_SO=chromosome | SO=protein_coding ;| organism=Trypanosoma_brucei_TREU927 | product=polyubiquitin, putative | location=Tb927_11_bin_v5.1:2333410-2335281(+) | length=623 | sequence_SO=random_sequence | SO=protein_coding ;no description;no description;
CAQ57292.1;	2	182.71		expression site-associated gene 1 (ESAG1) protein Tb427.BES40.20 [Trypanosoma brucei brucei] ;
Tb927.9.10770;	3	162.46		| organism=Trypanosoma_brucei_TREU927 | product=Polyadenylate-binding protein 2 (Poly(A)-binding protein 2) (Poly(A)-binding protein II) (PABII) (Polyadenylate-binding nuclear protein 1) (Nuclear poly(A)-binding protein 1) (PABP2), putative (PABP2) | location=Tb927_09_v5.1:1699911-1701578(-) | length=555 | sequence_SO=chromosome | SO=protein_coding ;
Tb927.6.4840;Tb927.6.4920;Tb927.6.4910;Tb927.6.4900;Tb927.6.4890;Tb927.6.4880;Tb927.6.4870;Tb927.6.4860;Tb927.6.4850;	2	151.41		| organism=Trypanosoma_brucei_TREU927 | product=S-adenosylmethionine synthetase, putative (METK1)
Tb927.5.3400;	5	148.64		| organism=Trypanosoma_brucei_TREU927 | product=calcium-translocating P-type ATPase,calcium pump | location=Tb927_05_v5.1:1131431-1134466(-) | length=1011 | sequence_SO=chromosome | SO=protein_coding ;

Table S5. Proteins detected in TbBBS4-PTP tandem purification but absent in control one-step and tandem purifications from WT cells lacking the PTP tag.

Locus	Spectra	NSAF (x10^5)	BBS protein	Description
Tb927.11.4210;	3	143.12		| organism=Trypanosoma_brucei_TREU927 | product=hypothetical protein, conserved | location=Tb927_11_v5.1:1233113-1235005(-) | length=630 | sequence_SO=chromosome | SO=protein_coding ;
Tb927.8.4890;	2	136.00		| organism=Trypanosoma_brucei_TREU927 | product=endoplasmic reticulum oxidoreductin, putative,pol-associated gene 1 | location=Tb927_08_v5.1:1441680-1443008(-) | length=442 | sequence_SO=chromosome | SO=protein_coding ;
Tb11.v5.0713;	3	131.05		| organism=Trypanosoma_brucei_TREU927 | product=retrotransposon hot spot (RHS) protein, putative | location=Tb927_11_bin_v5.1:3629729-3631795(+) | length=688 | sequence_SO=random_sequence | SO=protein_coding ;
Tb927.2.5080;	2	128.17		| organism=Trypanosoma_brucei_TREU927 | product=hypothetical protein, conserved | location=Tb927_02_v5.1:897372-898781(-) | length=469 | sequence_SO=chromosome | SO=protein_coding ;
Tb927.4.4820;	2	122.67		| organism=Trypanosoma_brucei_TREU927 | product=amino acid transporter 8, putative | location=Tb927_11_bin_v5.1:2354708-2356180(-) | length=490 | sequence_SO=random_sequence | SO=protein_coding ;| organism=Trypanosoma_brucei_TREU927 | product=amino acid transporter 10, putative (AATP10) | location=Tb927_04_v5.1:1317708-1319180(-) | length=490 | sequence_SO=chromosome | SO=protein_coding ;
Tb927.3.1380;	2	115.82		| organism=Trypanosoma_brucei_TREU927 | product=ATP synthase beta chain, mitochondrial precursor,ATP synthase F1, beta subunit |
Tb927.6.2640;	2	112.78		| organism=Trypanosoma_brucei_TREU927 | product=importin alpha subunit, putative (TbKap60) | location=Tb927_06_v5.1:793568-795169(+) | length=533 | sequence_SO=chromosome | SO=protein_coding ;
Tb927.2.3030;	2	74.03		| organism=Trypanosoma_brucei_TREU927 | product=ATP-dependent Clp protease subunit, heat shock protein 78 (HSP78), putative | location=Tb927_02_v5.1:575019-577457(+) | length=812 | sequence_SO=chromosome | SO=protein_coding ;
Tb927.1.180;Tb927.2.370;Tb927.1.220;	2	72.13		| organism=Trypanosoma_brucei_TREU927 | product=retrotransposon hot spot protein 1 (RHS1), putative | location=Tb927_01_v5.1:69904-72405(-) | length=833 | sequence_SO=chromosome | SO=protein_coding ;| organism=Trypanosoma_brucei_TREU927 | product=retrotransposon hot spot protein 1 (RHS1), putative | location=Tb927_02_v5.1:55836-58340(-) | length=834 | sequence_SO=chromosome | SO=protein_coding ;| organism=Trypanosoma_brucei_TREU927 | product=retrotransposon hot spot protein 1 (RHS1), putative | location=Tb927_01_v5.1:85854-88355(-) | length=833 | sequence_SO=chromosome | SO=protein_coding ;
Tb927.11.9150;	3	52.18		| organism=Trypanosoma_brucei_TREU927 | product=hypothetical protein, conserved | location=Tb927_11_v5.1:2505165-2510351(+) | length=1728 | sequence_SO=chromosome | SO=protein_coding ;
Tb927.8.1590;	2	13.97		| organism=Trypanosoma_brucei_TREU927 | product=ubiquitin-protein ligase, putative (upl3) | location=Tb927_08_v5.1:516817-529731(+) | length=4304 | sequence_SO=chromosome | SO=protein_coding ;

(if small ORFs Tb11.NT.155_1 and Tb11.NT.154_1 [reference 118] are included in NSAF calculation)*