Supporting Information

Global Profiling and Inhibition of Protein Lipidation in Vector and Host Stages of the Sleeping Sickness Parasite *Trypanosoma brucei*

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This document contains Supporting Data, in the form of Figures (14) and a list of supporting Tables (7, available as separate Excel files), and Supporting Methods. A separate Supporting Data File contains MS/MS spectra of YnMyr modified peptides.

Supporting Data

Supporting Figures

Fig. S1: Structure of capture reagents AzTB, YnTB, AzRB, AzRTB

Fig. S2: Full gels Figs. 1 & 2

Fig. S3: Pictures of YnMyr vs Myr-treated BSF parasites

Fig. S4: NaOH optimisation BSF; +/- base treatment timecourse YnMyr; +/- base-treatment timecourse YnPal vs YnMyr

Fig. S5: Example pull-down

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Fig. S12: Heat map and hierarchical clustering of hits from inhibition experiment

Fig. S13: Profile plots of enrichment in response to inhibitor **2** and for 'medium confidence' hits

Fig. S14: Prediction of *N*-myristoylation of high confidence NMT substrates with bioinformatics tools

Supporting Tables

Supporting tables are available as separate Excel spreadsheets. Each contains a key describing the contents of the file.

Table S1: BSF YnMyr data

Table S2: PCF YnMyr data

Table S3: Modified peptide comparisons

Table S4: BSF vs PCF comparison

Table S5: YnPal and comparison with YnMyr

Table S6: Inhibition data

Table S7: Prediction of *N*-myristoylation for all MG proteins, cross-referenced to current study

Supporting Data File

Separate ZIP file. MS/MS spectra of YnMyr modified peptides. Best spectra shown.

Contents

Supporting Figures

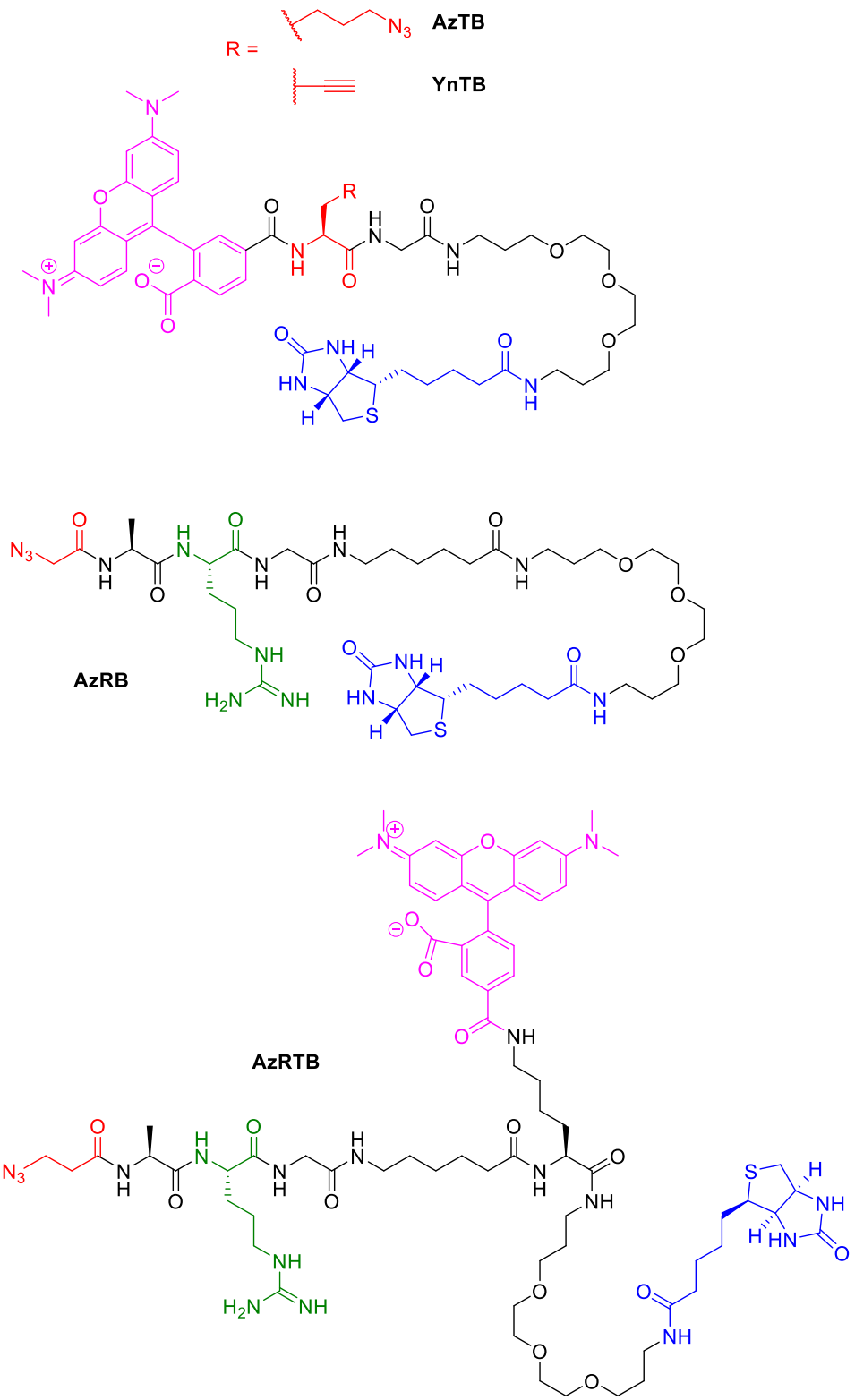


Figure S1. Structure of reagents for CuAAC.

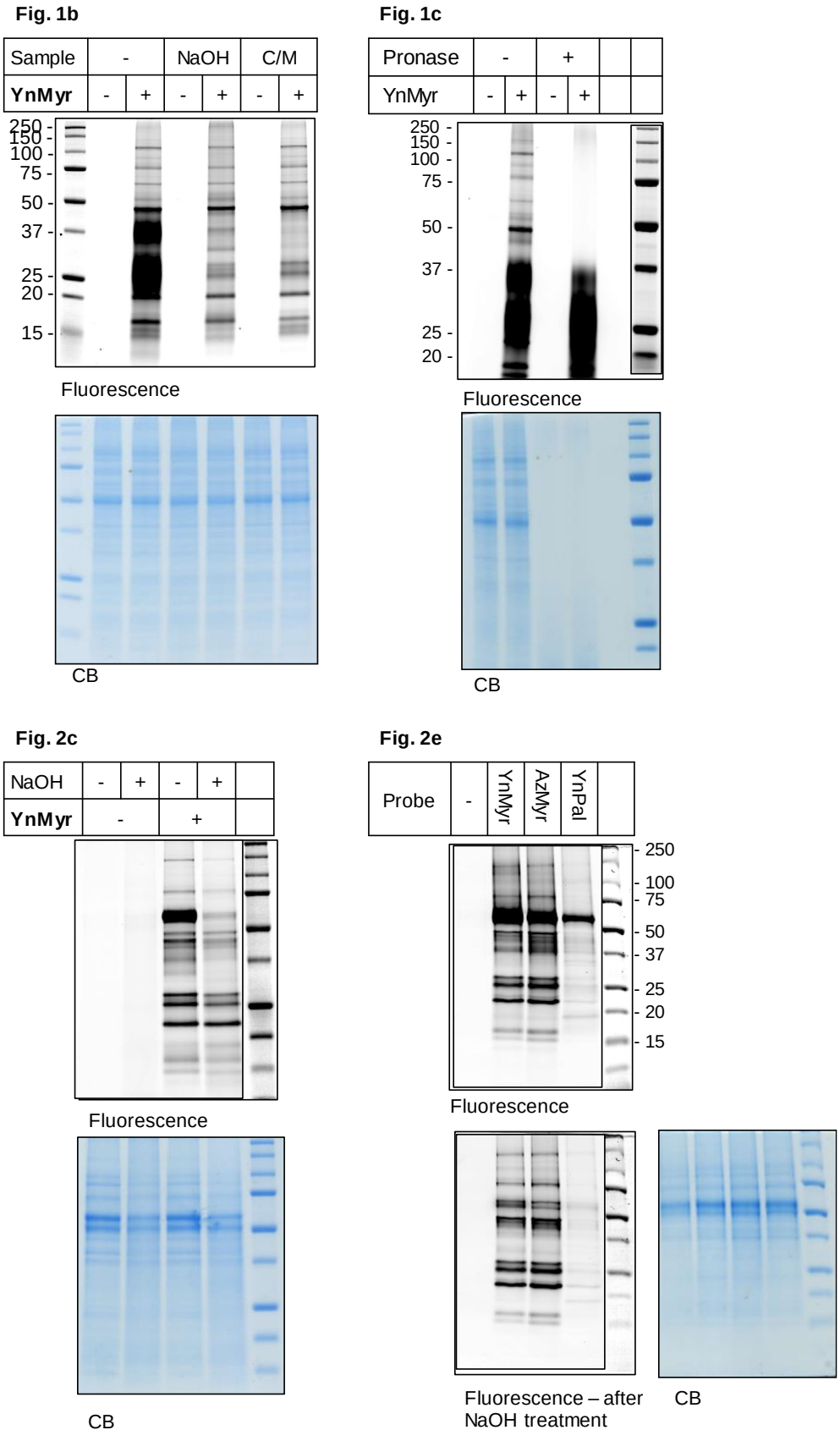
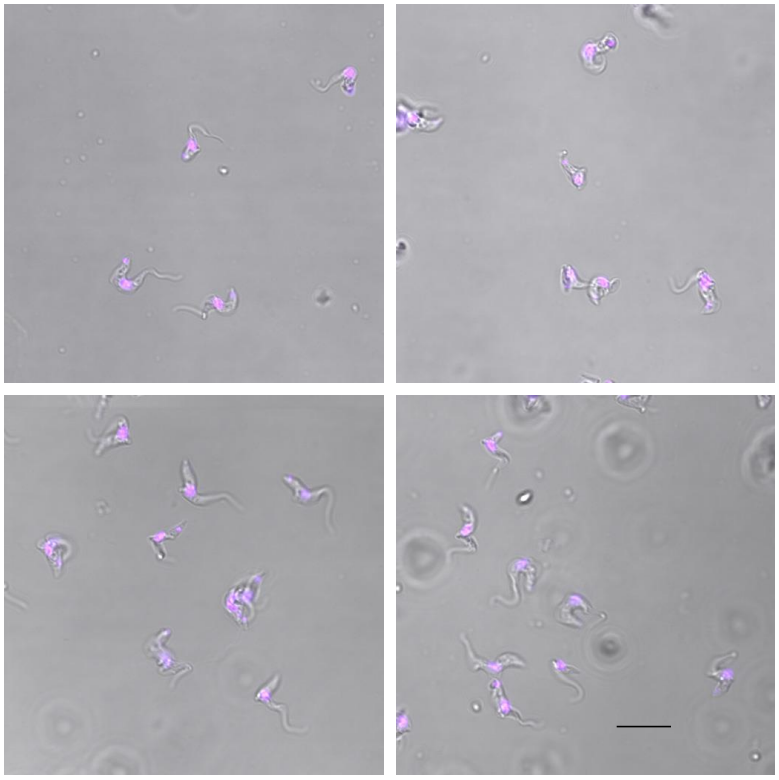
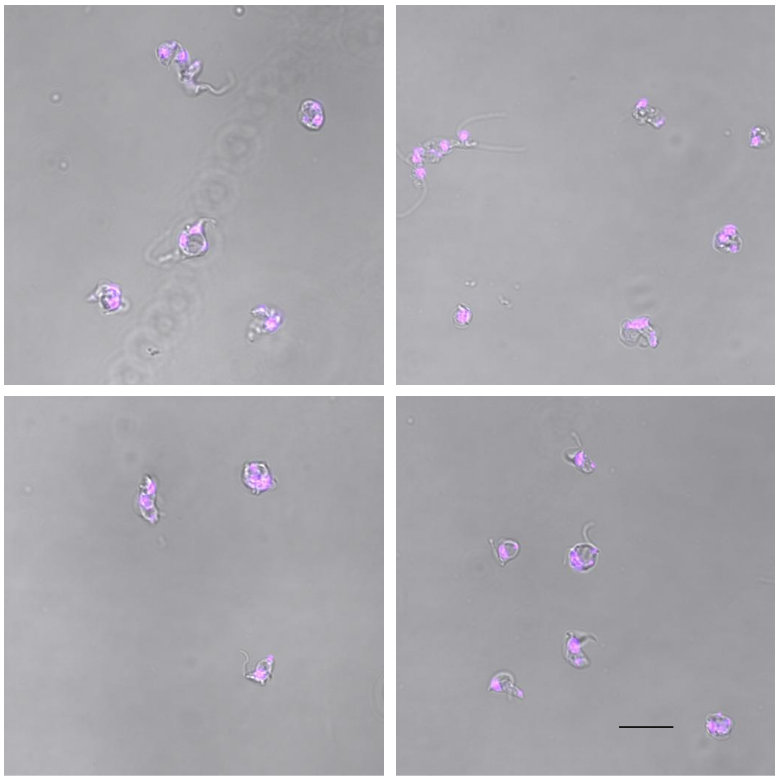


Figure S2. Full gels from main text Figures 1 & 2. CB = Coomassie blue. Mw markers in kDa.



***T. brucei* BSF**
+ Myr 18h



***T. brucei* BSF**
+ YnMyr 18h

Figure S3. Related to Figure 2. Images of *T. brucei* BSF parasites treated with myristic acid (Myr) or YnMyr for 18 hours. YnMyr treated parasites exhibit an enlarged flagellar pocket. Scale bar: 10 µm.

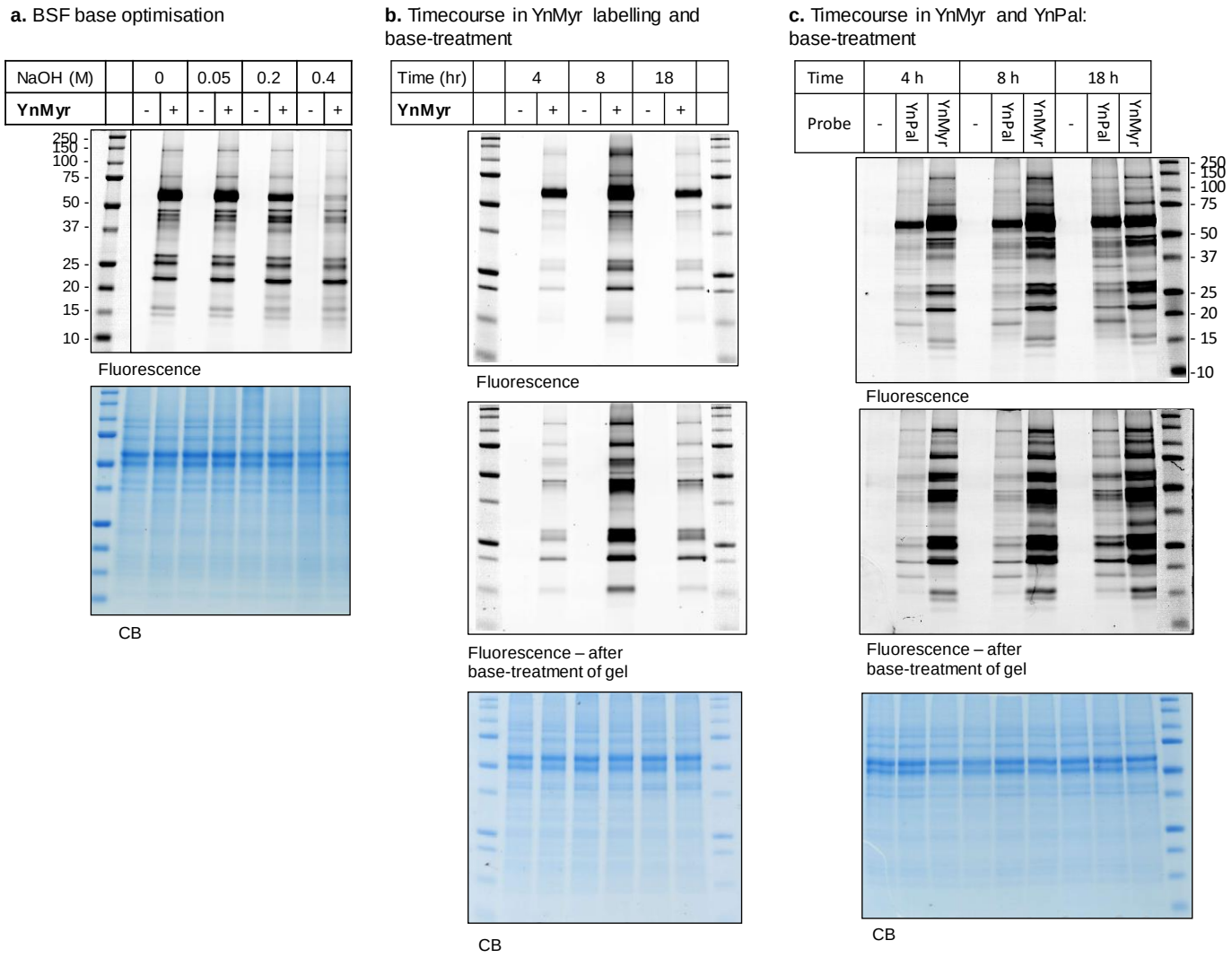


Figure S4. Optimisation of metabolic tagging in BSF parasites. **a.** Treatment of lysates from YnMyr or Myr (-) tagged parasites with different concentrations of base (NaOH) for removal of GPI-anchor labelling. **b.** YnMyr labelling for different time periods. **c.** Comparison of YnMyr and YnPal labelling at different timepoints. CB = Coomassie blue. Mw markers in kDa.

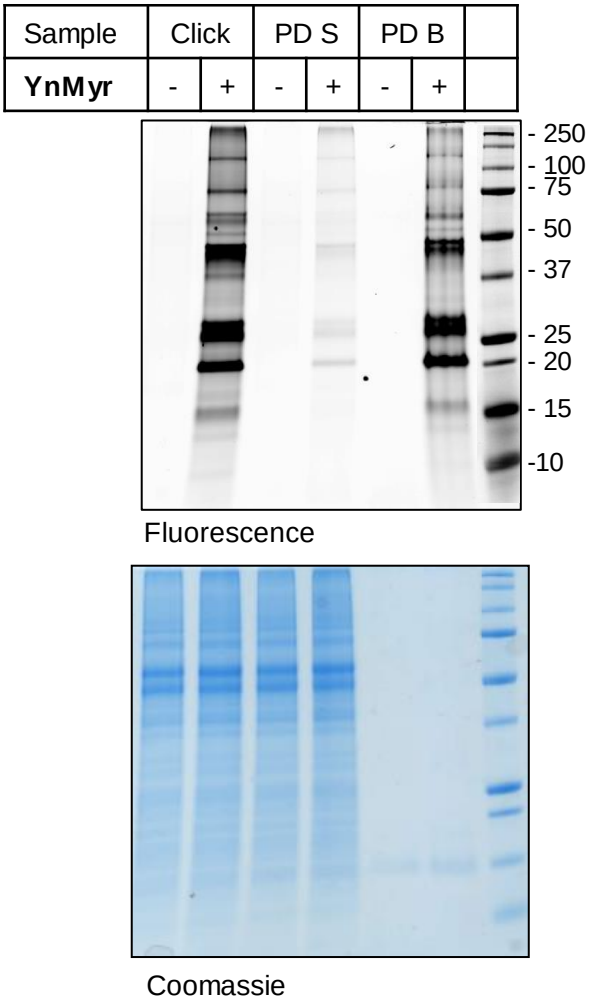
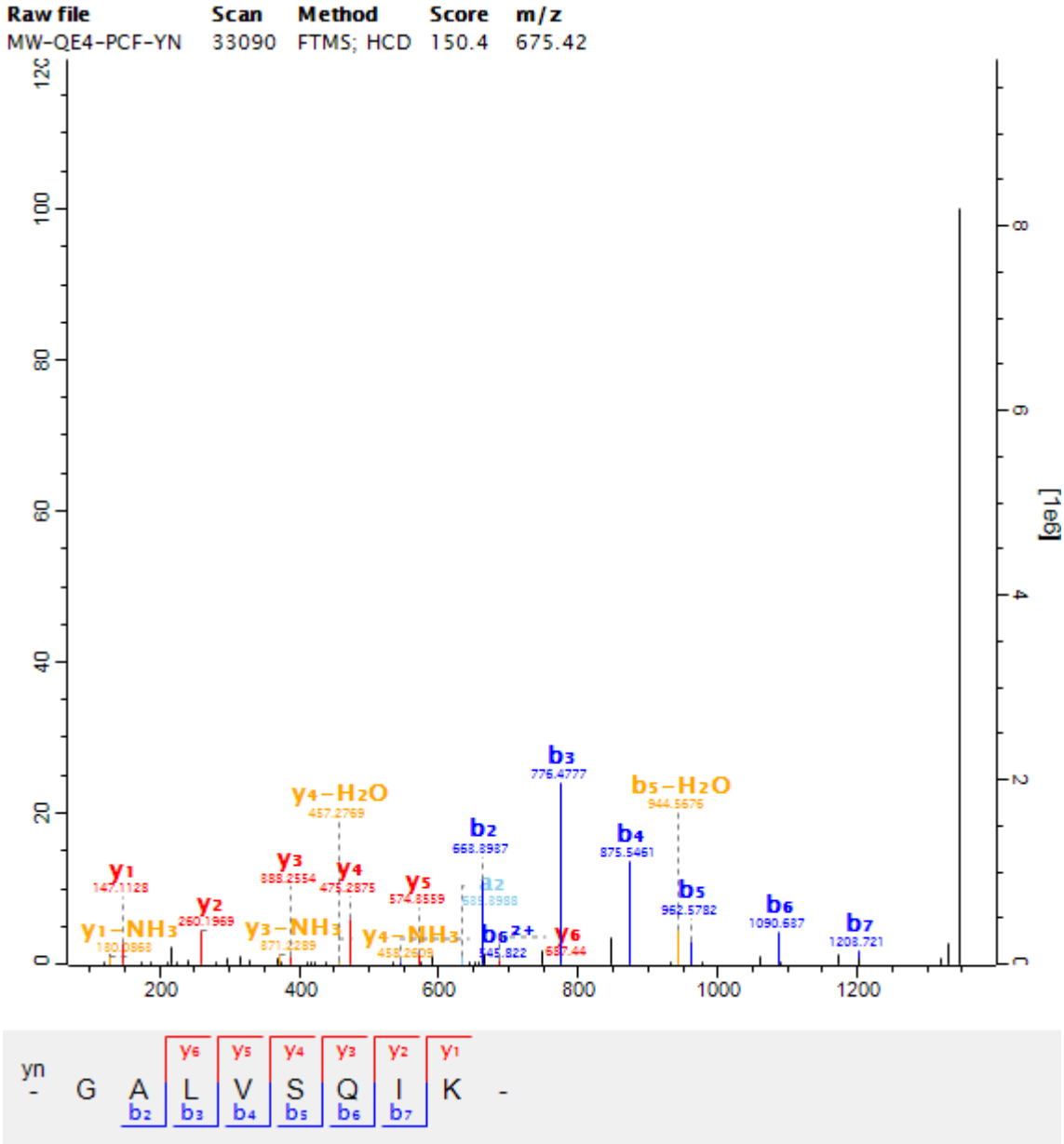


Figure S5. Example of enrichment experiment. Samples from BSF parasites treated with Myr (-) or YnMyr were subject to CuAAC (click), then pull-down (PD) onto streptavidin-coated beads. Beads were boiled to release bound proteins. Click = aliquot taken after CuAAC but before pull-down; PD S = aliquot taken from supernatant after pull-down; PD B = aliquot of elution from beads after pull-down.

Figure S6. Examples of spectra from proteomic identification of YnMyr modified peptides (see also Table S3 and Supporting Data File). For each spectrum: Protein ID_raw file_scan number.

Tb927.7.6230_P6_YnMyr_33090

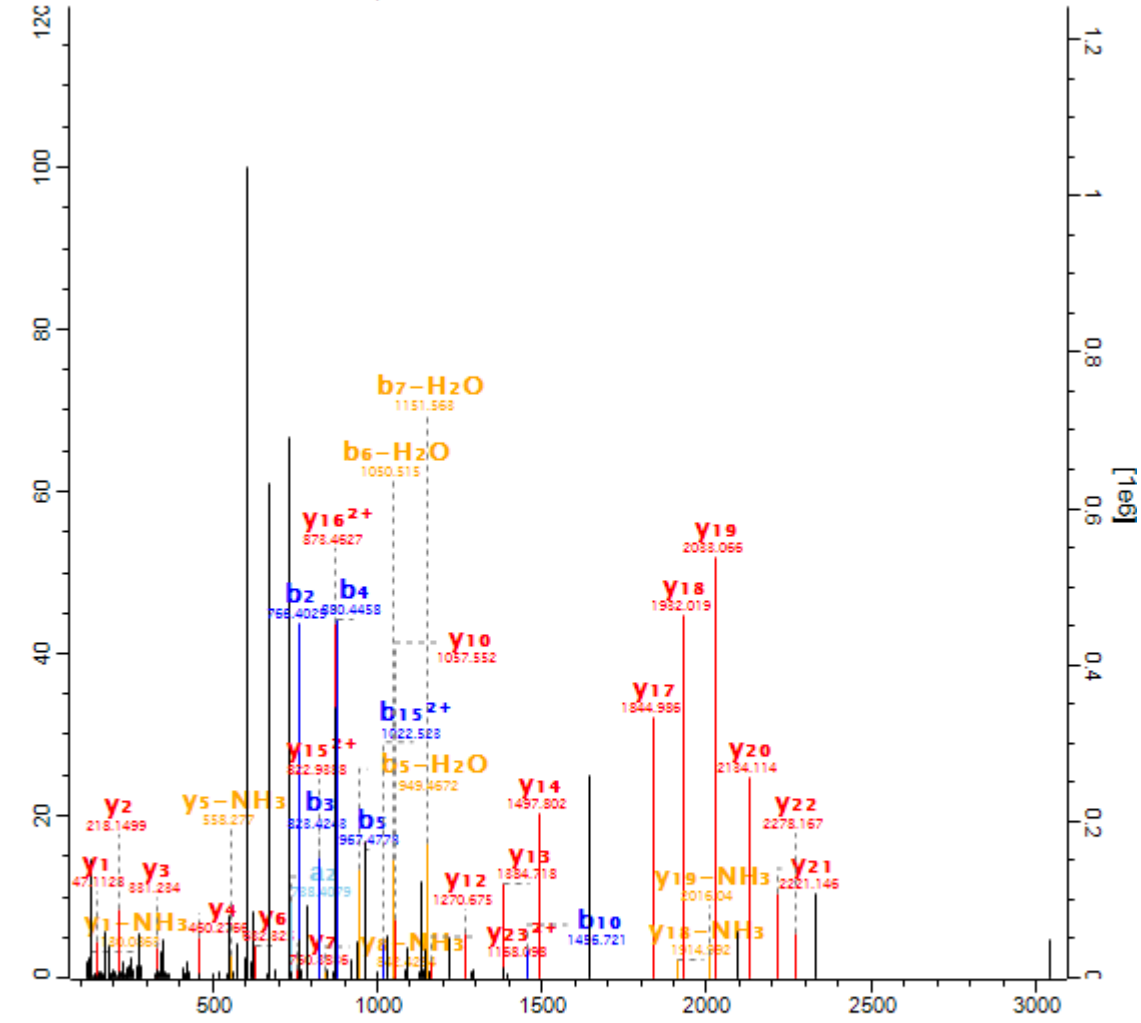
Protein name: ARF3



Tb927.1.2260_P5_YnMyr_24329

Protein name: Calpain-like protein fragment (SMP homologue)

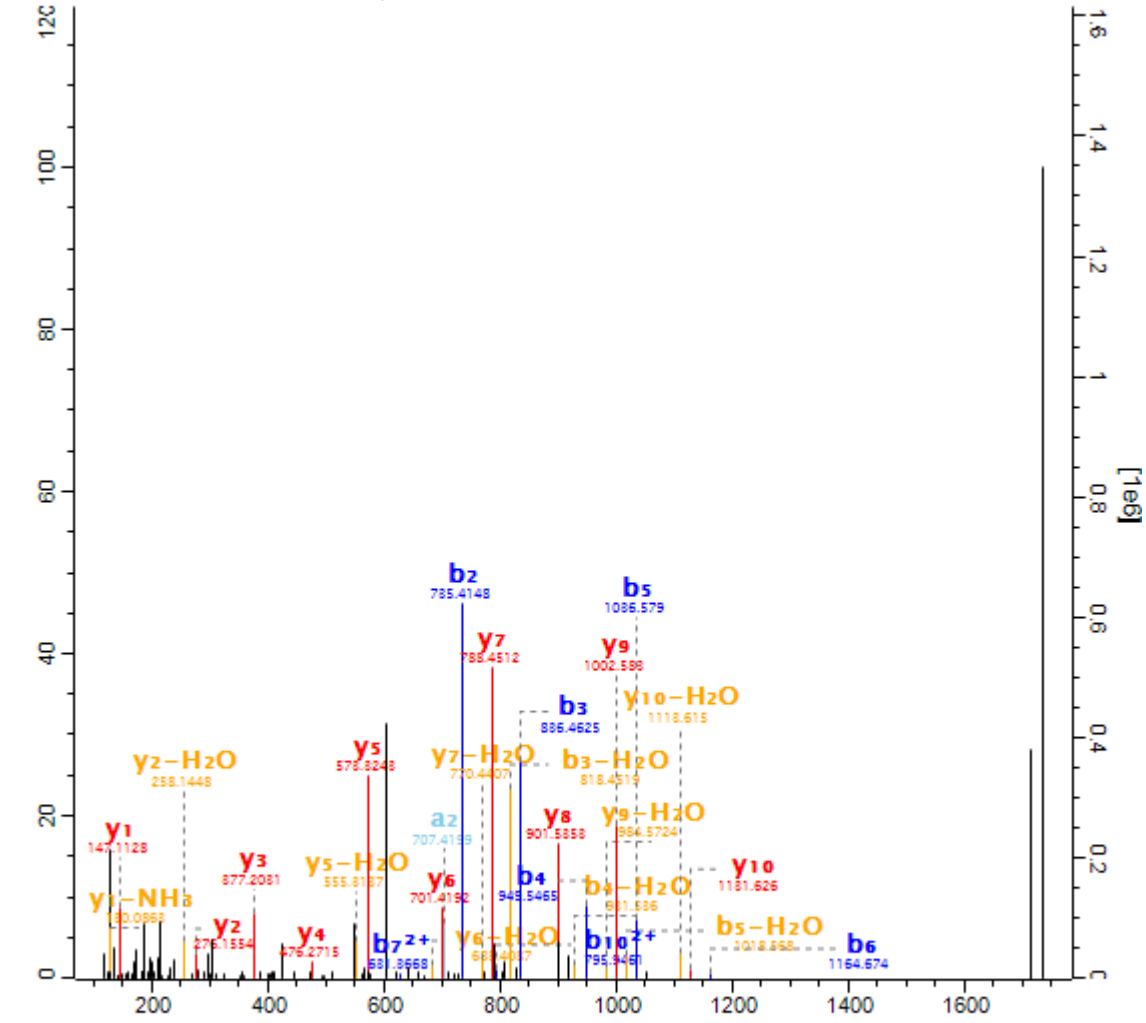
Raw file	Scan	Method	Score	m/z
MW-QE3-RTB-YN	24329	FTMS; HCD	99.08	776.15

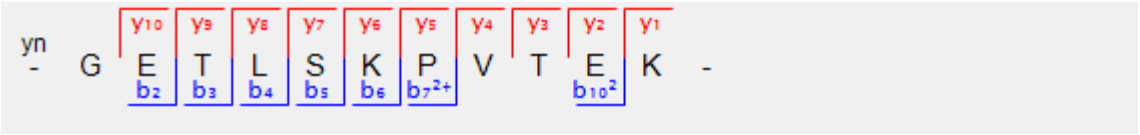


Tb927.10.4930_P5_YnMyr_21563 (AzRTB reagent)

Protein name: PP2C

Raw file	Scan	Method	Score	m/z
MW-QE3-RTB-YN	21563	FTMS; HCD	164.9	579.67

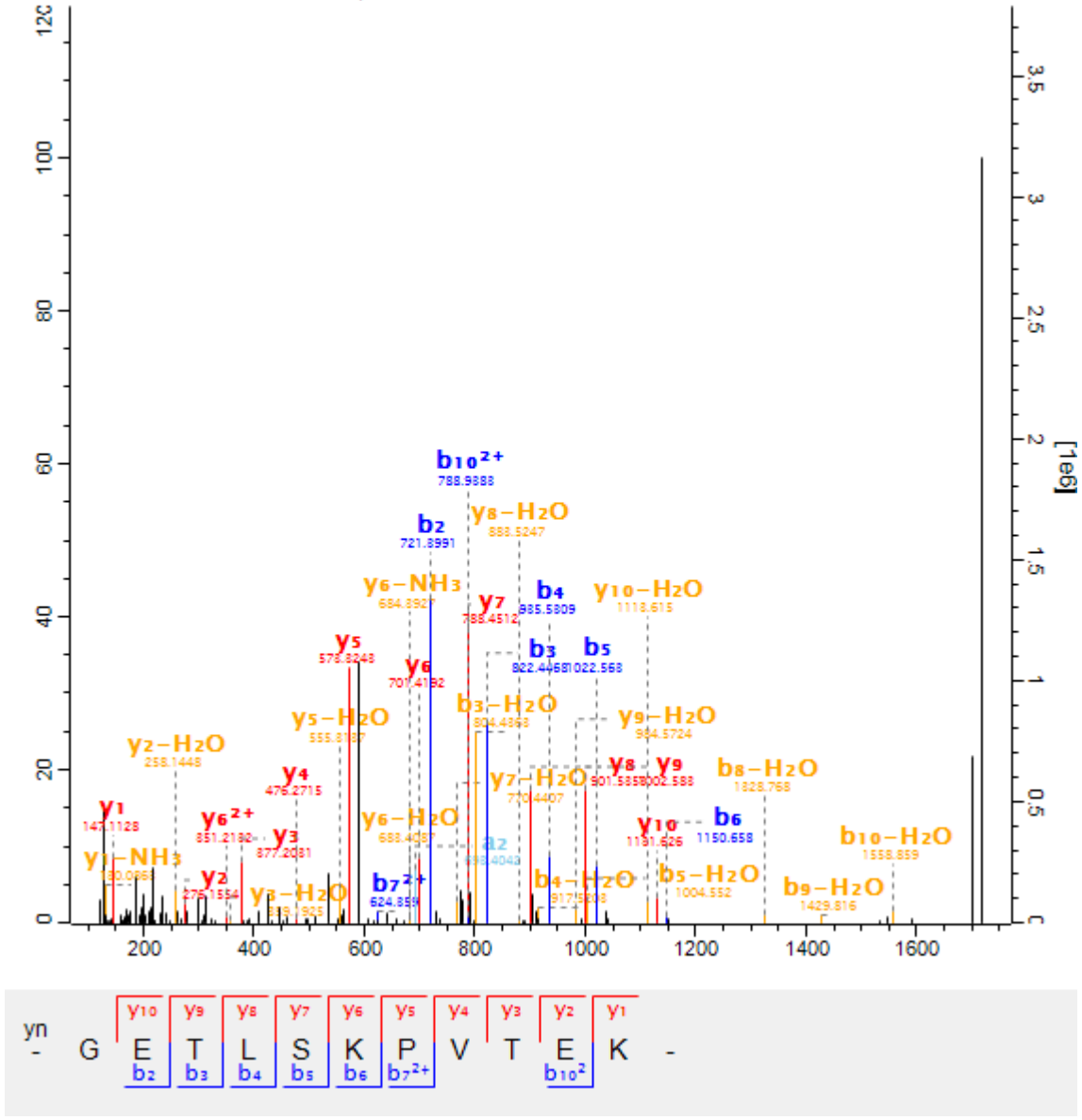




Tb927.10.4930_P6_YnMyr_23100 (AzRB reagent)

Protein name: PP2C

Raw file	Scan	Method	Score	m/z
MW-QE4-PCF-YN	23100	FTMS; HCD	179.72	575.33



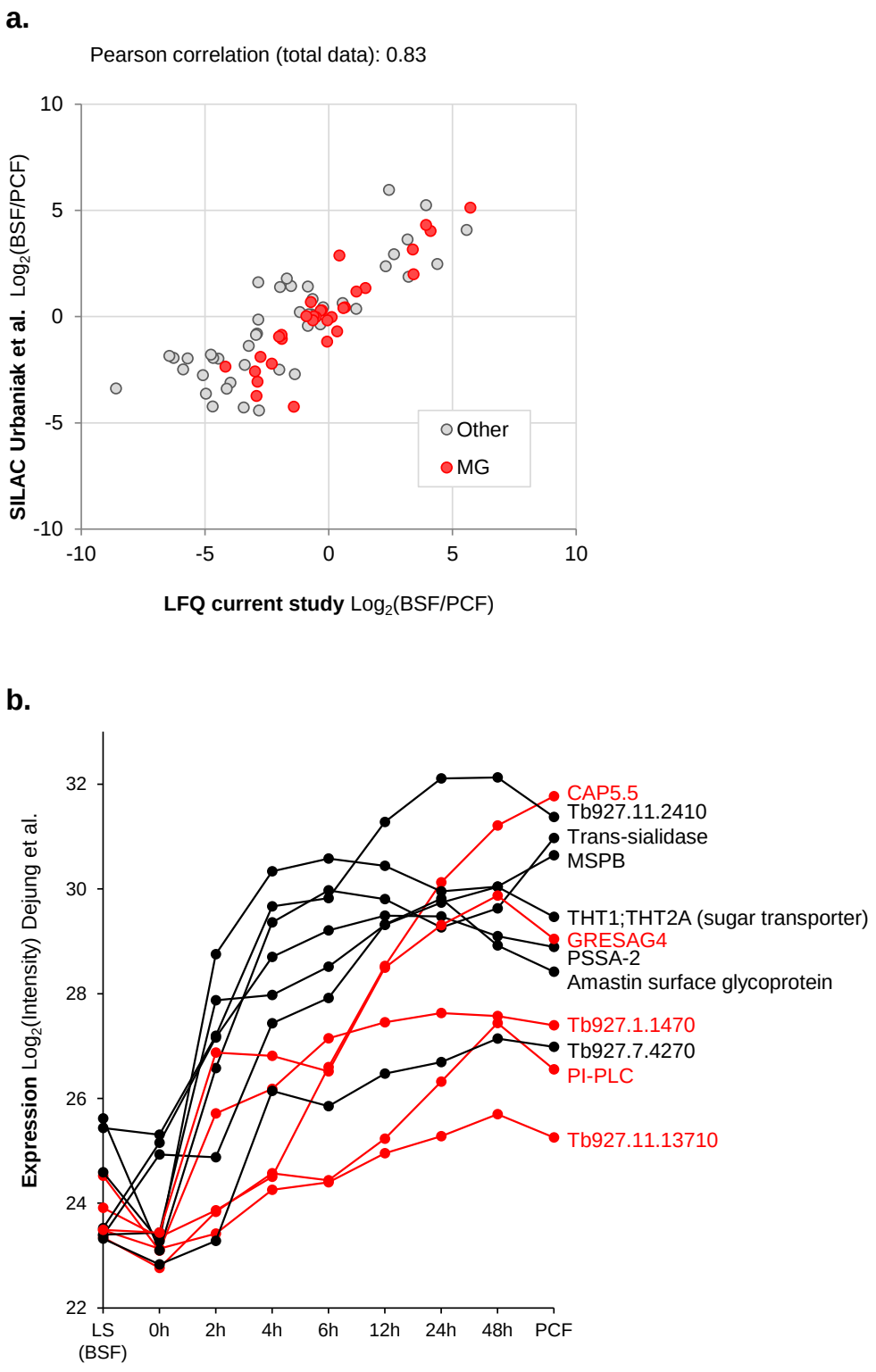
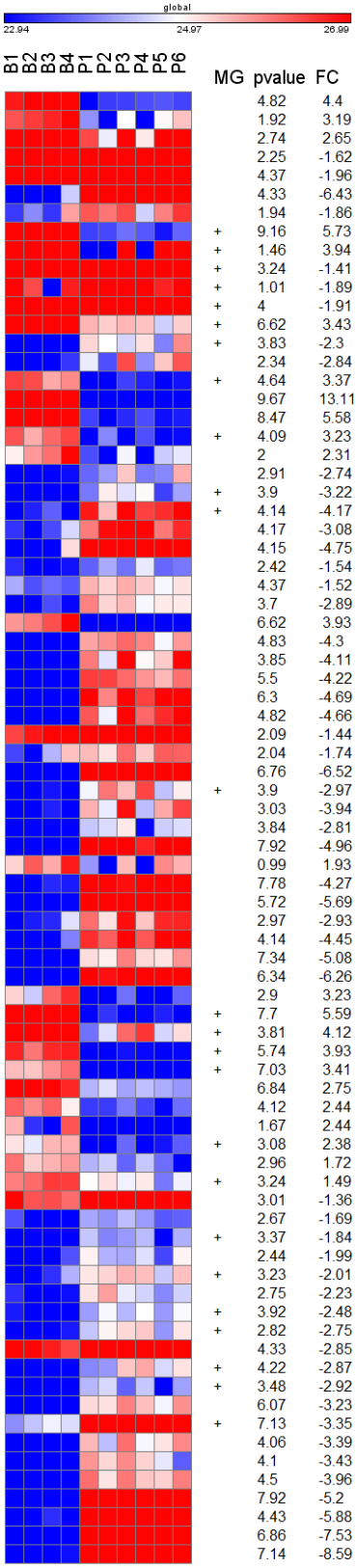


Figure S7. **a.** Comparison of relative abundance of protein hits (defined as significantly enriched in YnMyr over Myr samples) in BSF and PCF parasites in the current study (LFQ quantification; ratio of YnMyr intensities is plotted; see also Table S4) with the study of Urbaniak *et al.* (quantification via SILAC).[1] MG = proteins containing an N-terminal glycine, in red. **b.** Expression profiles of select lipidated proteins during differentiation from short stumpy BSF parasites (0h) to PCF, and expression level in long slender (LS) BSF, using data from Dejung *et al.*[2] Long slender forms were used in the current study. Proteins shown were enriched in PCF in the current study were also upregulated early during differentiation.



MG	pvalue	FC	Protein ID	Protein description	Key word
	4.82	4.4	Tb927.10.2020	hexokinase (HK2)	carbohydrate metabolic process
	1.92	3.19	Tb927.3.3270	ATP-dependent phosphofructokinase (PFK)	carbohydrate metabolic process
	2.74	2.65	Tb927.10.5620	fructose-bisphosphate aldolase (glycosomal)	carbohydrate metabolic process
	2.25	-1.62	Tb927.9.12630	glycerol kinase (glycosomal)	carbohydrate metabolic process
	4.37	-1.96	Tb927.8.3530	glycerol-3-phosphate dehydrogenase [NAD+] (glycosomal)	carbohydrate metabolic process
	4.33	-6.43	Tb927.2.4210	glycosomal phosphoenolpyruvate carboxykinase (PEPCK)	carbohydrate metabolic process
	1.94	-1.86	Tb927.9.2470	nucleolar protein (NOP66)	cell cycle
+	9.16	5.73	Tb927.10.2440	Metacaspase-4 (MCA4)	cysteine peptidase (like)
+	1.46	3.94	Tb927.8.8330	calpain cysteine peptidase	cysteine peptidase (like)
+	3.24	-1.41	Tb927.4.3950	cytoskeleton-associated protein CAP5.5	cysteine peptidase (like)
+	1.01	-1.89	Tb927.1.2230	calpain-like protein fragment	cysteine peptidase (like)
+	4	-1.91	Tb927.1.2260	calpain-like protein fragment	cysteine peptidase (like)
+	6.62	3.43	Tb927.6.1800	protein phosphatase 2C	dephosphorylation
+	3.83	-2.3	Tb927.3.2150	protein phosphatase 2C	dephosphorylation
	2.34	-2.84	Tb927.6.4630	kinetoplastid-specific phospho-protein phosphatase	dephosphorylation
+	4.64	3.37	Tb927.9.4210	fatty acyl CoA synthetase	fatty acid metabolic process
	9.67	13.11	Tb427.BES40.22	Variant surface glycoprotein (VSG)	GPI process
	8.47	5.58	Tb927.2.6000	glycosylphosphatidylinositol-specific phospholipase C (GPI-PLC)	GPI process
+	4.09	3.23	Tb927.9.7230	ADP-ribosylation factor-like protein (ARL1B)	intracellular protein transport
	2	2.31	Tb927.10.6050	clathrin heavy chain	intracellular protein transport
	2.91	-2.74	Tb927.5.330	receptor-type adenylate cyclase GRESAG 4	intracellular signal transduction
+	3.9	-3.22	Tb927.11.5970	phosphoinositide-specific phospholipase C	intracellular signal transduction
+	4.14	-4.17	Tb927.7.7470	receptor-type adenylate cyclase GRESAG 4	intracellular signal transduction
	4.17	-3.08	Tb927.9.15460	calcium motive p-type ATPase	ion transport
	4.15	-4.75	Tb927.2.2520	voltage-dependent anion-selective channel 1 (VDAC1)	ion transport
	2.42	-1.54	Tb927.10.13680	lipase domain protein	lipid metabolic process
	4.37	-1.52	Tb927.5.1810	lysosomal/endosomal membrane protein p67	lysosomal process
	3.7	-2.89	Tb927.7.3900	vacuolar transporter chaperone	lysosomal process
	6.62	3.93	Tb927.10.14890	C-terminal motor kinesin	motor activity
	4.83	-4.3	Tb927.11.10760	kinesin-like protein	motor activity
	3.85	-4.11	Tb927.7.6850	trans-sialidase (TS)	organic substance transport
	5.5	-4.22	Tb927.8.7650	amino acid transporter	organic substance transport
	6.3	-4.69	Tb927.10.8530	THT1 - hexose transporter	organic substance transport
	4.82	-4.66	Tb927.5.930	NADH-dependent fumarate reductase	oxidation-reduction process
	2.09	-1.44	Tb927.9.11600	glycosomal membrane protein (gim5B)	peroxisome fission
	2.04	-1.74	Tb927.11.11520	glycosomal membrane protein (PEX11)	peroxisome fission
	6.76	-6.52	Tb927.11.6280	pyruvate phosphate dikinase (PPDK)	phosphorylation
+	3.9	-2.97	Tb927.10.7290	Phytochelatin synthase	phytochelatin biosynthetic process
	3.03	-3.94	Tb927.11.12220	catalytic subunit of the vacuolar transporter chaperone 4	polyphosphate biosynthetic process
	3.84	-2.81	Tb927.7.5990	protein associated with differentiation 5 (PAD5)	protein associated with differentiation
	7.92	-4.96	Tb927.7.5940	Protein Associated with Differentiation (PAD2)	protein associated with differentiation
	0.99	1.93	Tb927.11.7510	glucose-regulated protein 78 (BiP)	protein folding
	7.78	-4.27	Tb927.9.8160	chaperone protein DNAj	protein folding
	5.72	-5.69	Tb927.8.1630	major surface protease gp63	proteolysis
	2.97	-2.93	Tb927.8.7980	Vacuolar proton pyrophosphatase 2 (VP2)	proton transport
	4.14	-4.45	Tb927.10.14840	Mitochondrial ADP/ATP carrier protein 5c (MCP5c)	transport
	7.34	-5.08	Tb927.10.7700	ABC transporter	transport
	6.34	-6.26	Tb927.8.2160	p-glycoprotein (PGPA)	transport
	2.9	3.23	Tb927.8.2080	Fungal tRNA ligase phosphodiesterase domain containing protein	tRNA splicing
+	7.7	5.59	Tb927.11.2400	unknown protein	unknown
+	3.81	4.12	Tb927.8.2070	unknown protein (POMP39A)	unknown
+	5.74	3.93	Tb927.9.6530	unknown protein	unknown
+	7.03	3.41	Tb927.1.1500	conserved protein	unknown
	6.84	2.75	Tb927.8.3890	unknown protein	unknown
	4.12	2.44	Tb927.2.3340	unknown protein	unknown
	1.67	2.44	Tb927.10.6720	Plasma-membrane choline transporter	unknown
+	3.08	2.38	Tb927.4.4580	unknown protein	unknown
	2.96	1.72	Tb927.5.2390	unknown protein	unknown
+	3.24	1.49	Tb927.10.12940	predicted zinc finger protein	unknown
	3.01	-1.36	Tb927.10.6730	Plasma-membrane choline transporter	unknown
	2.67	-1.69	Tb927.10.390	DUF2407 ubiquitin-like domain containing protein	unknown
+	3.37	-1.84	Tb927.11.1850	unknown protein	unknown
	2.44	-1.99	Tb927.4.5340	unknown protein	unknown
+	3.23	-2.01	Tb927.8.4940	unknown protein	unknown
	2.75	-2.23	Tb927.10.10720	Tetraspanin family	unknown
+	3.92	-2.48	Tb927.11.13710	unknown protein	unknown
+	2.82	-2.75	Tb927.1.1470	unknown protein	unknown
	4.33	-2.85	Tb927.7.3440	I/6 autoantigen	unknown
+	4.22	-2.87	Tb927.10.9810	unknown protein	unknown
+	3.48	-2.92	Tb927.7.5260	Cytochrome c oxidase biogenesis protein Cmc1 like	unknown
	6.07	-3.23	Tb927.5.1250	GAF domain/TIP41-like family	unknown
+	7.13	-3.35	Tb927.11.13610	rhodanese-like domain containing protein	unknown
	4.06	-3.39	Tb927.6.5090	unknown protein	unknown
	4.1	-3.43	Tb927.5.4020	unknown protein	unknown
	4.5	-3.96	Tb927.7.4270	unknown protein	unknown
	7.92	-5.2	Tb11.1390	unknown protein	unknown
	4.43	-5.88	Tb927.4.3500	Amastin surface glycoprotein	unknown
	6.86	-7.53	Tb927.11.2410	unknown protein	unknown
	7.14	-8.59	Tb927.10.11220	procyclic form surface phosphoprotein (PSSA-2)	unknown

Figure S8. Heatmap of YnMyr intensities of all proteins showing significant differences (by t-test) between BSF (B1-B4) and PCF (P1-P6). Colour-coding globally across all rows in the matrix (note that in Fig. 4c colour-coding is within a row). FC = fold-change (Log_2(BSF/PCF). pvalue = -log_{10}(p-value t-test). MG = protein contains N-terminal glycine. One representative ID and protein name per ProteinGroup is shown (see Supp. Table S4 for complete data). Keyword based on GO annotation from TriTrypDB. Heatmap created with Gene-E (http://www.broadinstitute.org/cancer/software/GENE-E/index.html).

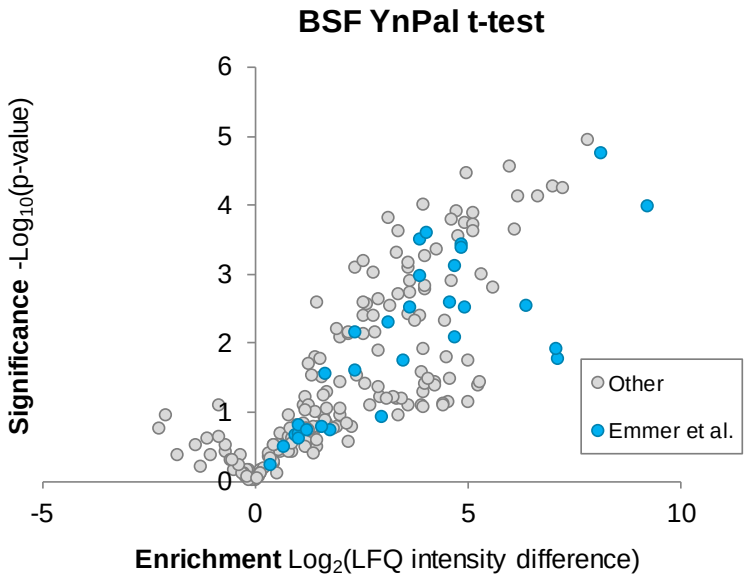


Figure S9. Volcano plot comparing LFQ intensities (after imputation of missing values) of YnPal or Pal (palmitic acid) treated BSF parasites (n=3). T-test: 250 permutations; FDR 0.05, s0 1. Proteins also identified in palmitoylation studies by Emmer *et al.*[3] are indicated in blue. See also Supp. Table S5.

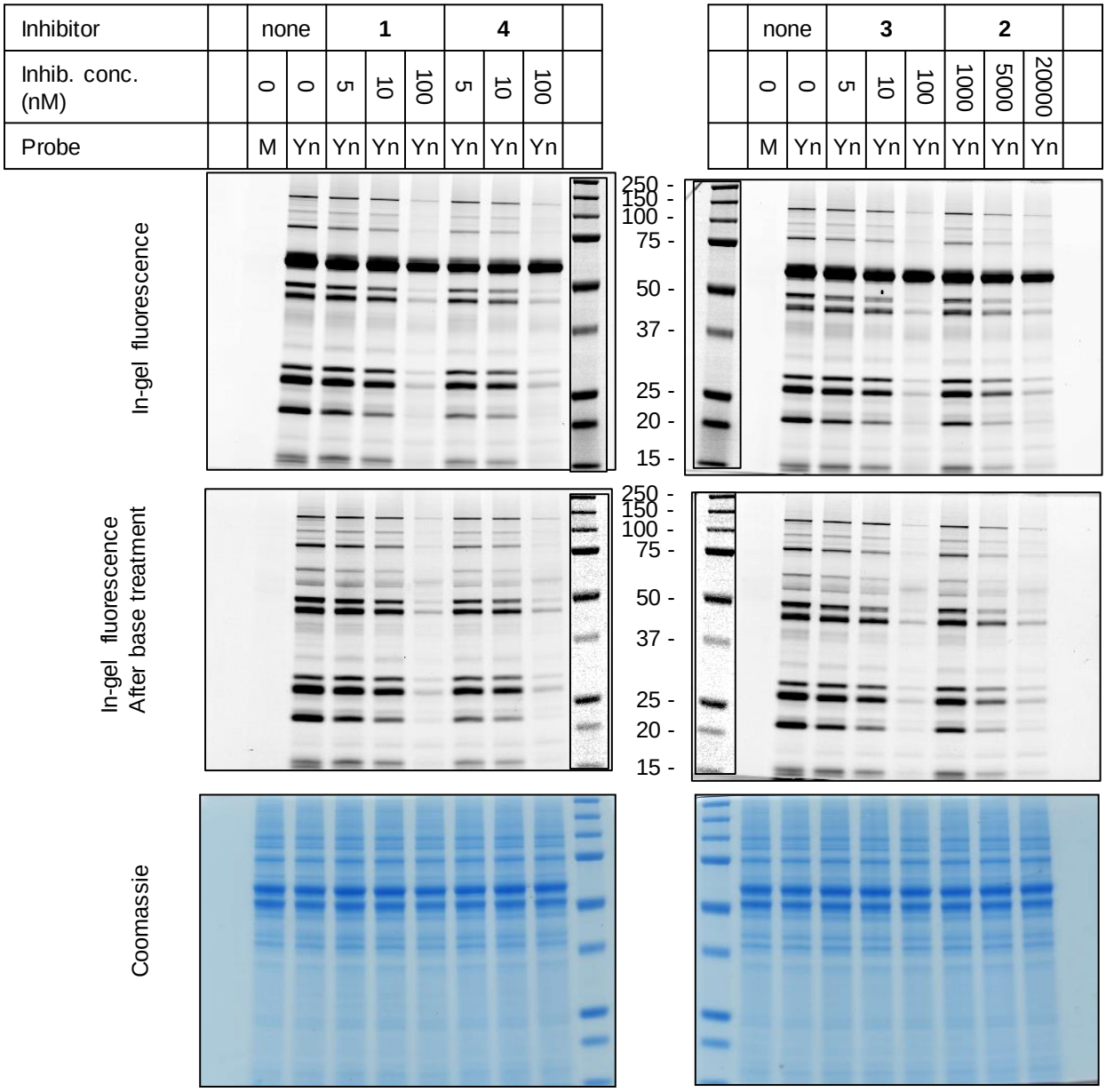


Figure S10. Fluorescence-based analysis of samples from *T. brucei* BSF parasites tagged with YnMyr in the presence of NMT inhibitors **1**-**4** (Fig. 6) at different concentrations. Probe: M = myristic acid; Yn = YnMyr.

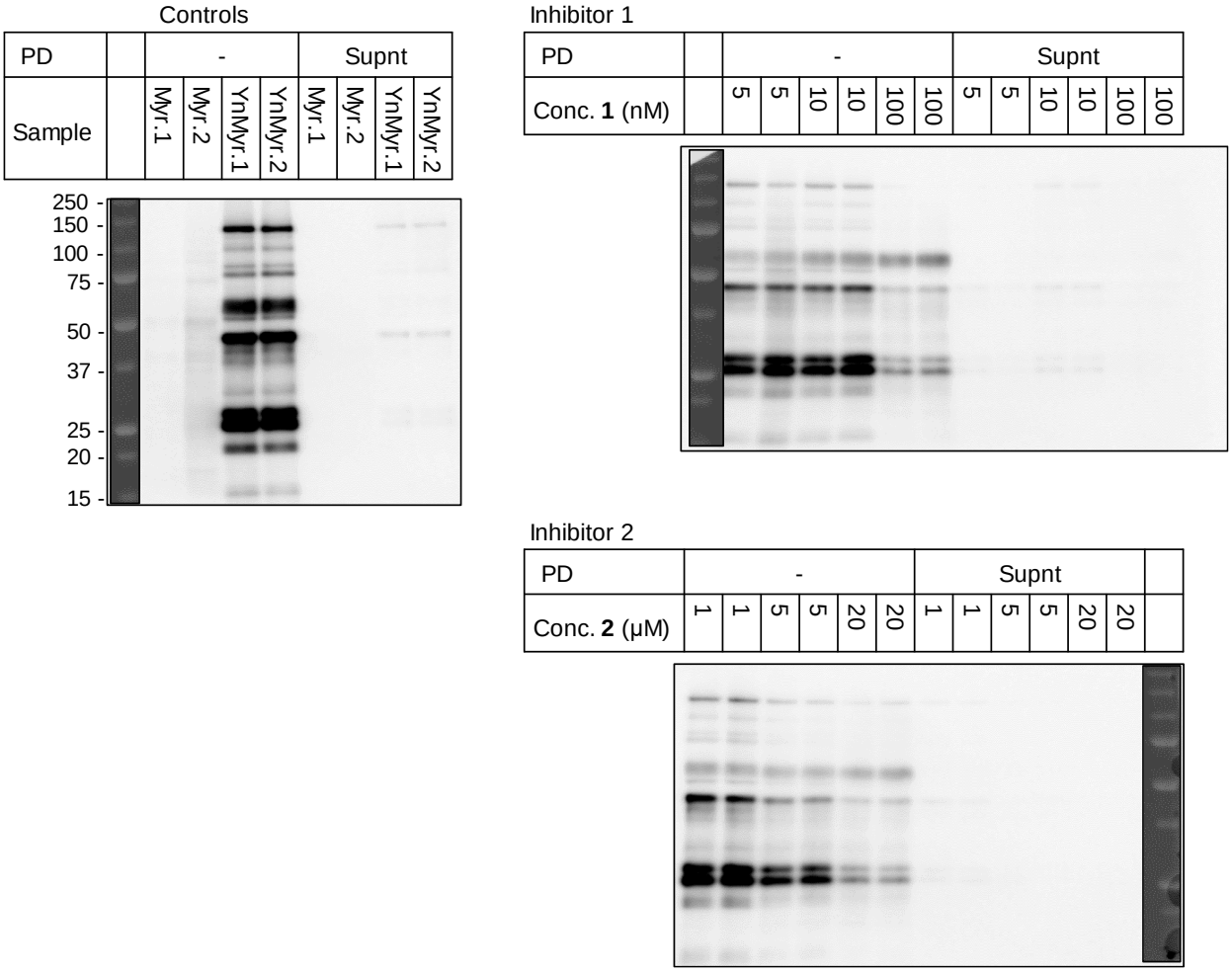


Figure S11. NeutrAvidin-HRP Western blots for detection of biotin for inhibition samples prepared for proteomic analysis. *T. brucei* BSF parasites were treated with YnMyr in the presence of inhibitors **1** or **2** at indicated concentrations. Technical duplicate samples were reacted with AzRB by CuAAC, treated with NaOH to remove the majority of GPI-anchor labelling, and enriched by pull-down (PD) onto NeutrAvidin agarose resin. Aliquots were taken before pull-down and of the supernatant (Supnt.) to ensure efficient enrichment onto the beads. Proteins attached to the beads were digested with trypsin for MS analysis.

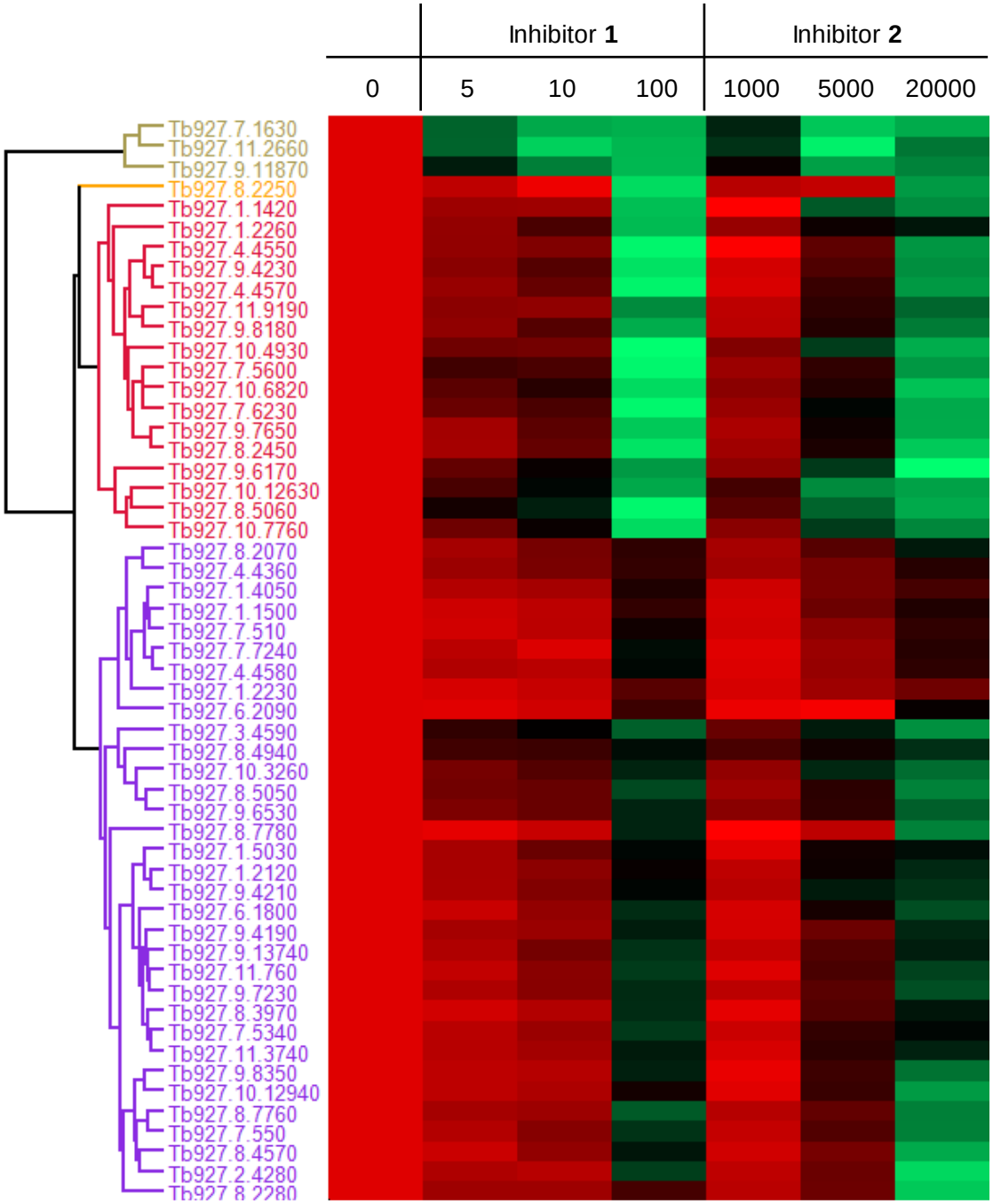


Figure S12. Hierarchical clustering of 54 proteins that respond robustly to the highest concentrations of inhibitors **1** and **2**. Performed in Perseus. The four clusters are indicated by different colours at the left (protein IDs). The heat map is coloured from high (red) to low (green) intensity. Data used: normalised enrichment ratios (YnMyr/Myr) – see Table S6.

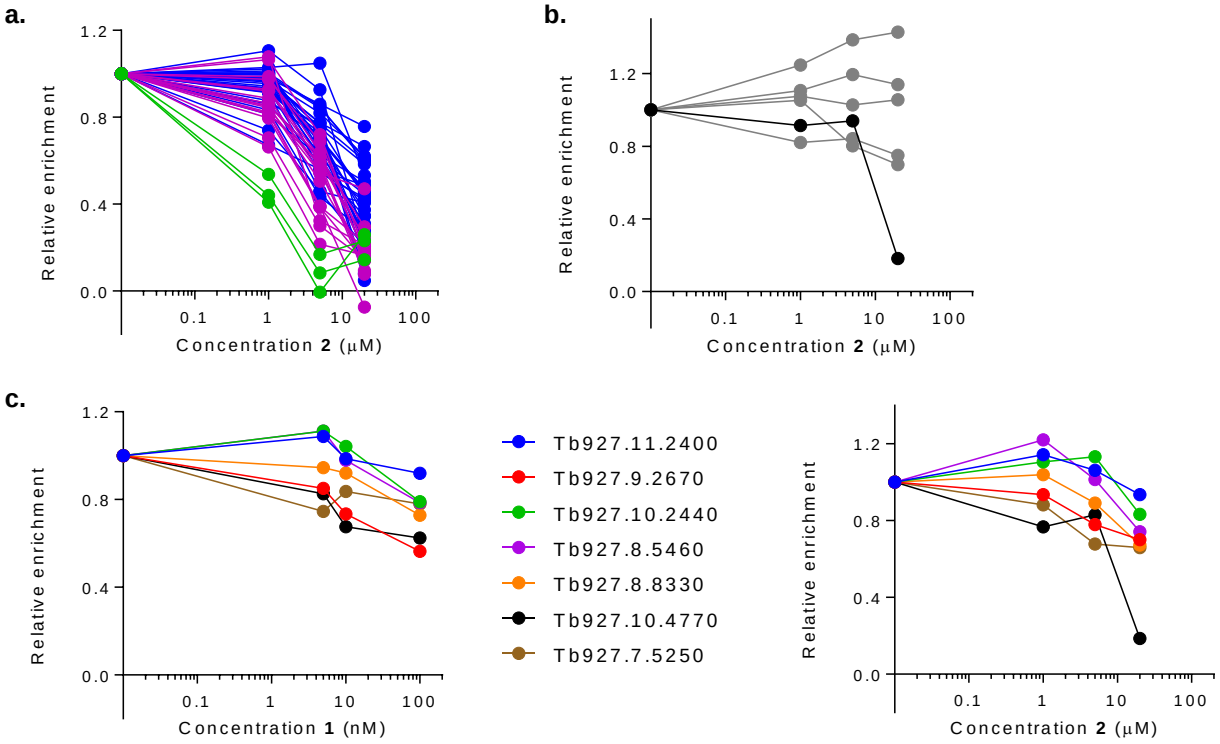


Figure S13. Related to Figure 7. **a.** Dose-response plots (treatment with **2**) for protein hits (high confidence hits – see Table S6; colour-coded by clustering). **b.** Dose-response plots (treatment with **2**) for other MG proteins not assigned as hits (grey) and for outlier non-MG protein (Tb927.8.2250, black) that is decreased only at high concentrations of inhibitors. **c.** Dose-response plots for 7 proteins that show only a weak response to NMT inhibitors **1** & **2** but where the YnMyr modified peptide was identified (see Tables S3 and S6).

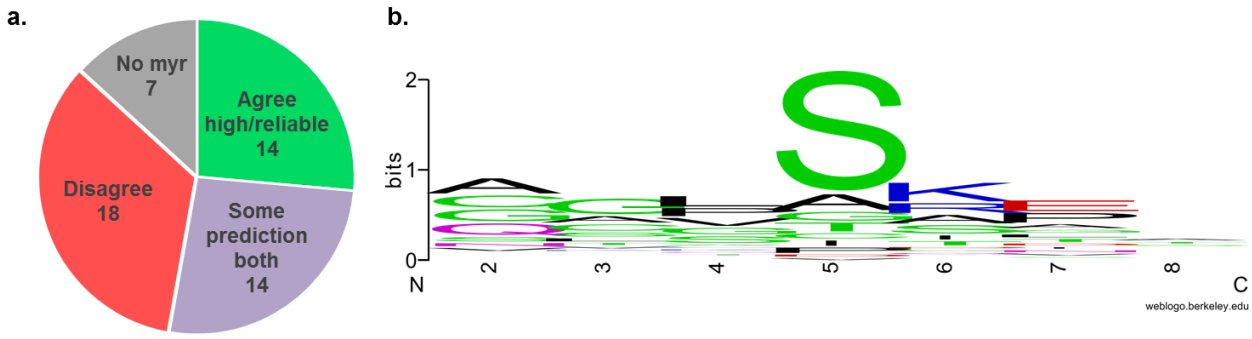


Figure S14. **a.** Prediction of *N*-myristoylation by two bioinformatics tools[4] for the proteins identified as high confidence NMT hits in the current study. Related to Supp. Tables S6 and S7. Agree high/reliable = both tools identify protein as high confidence NMT substrate; some prediction both = both tools predict some degree of *N*-myristoylation; disagree = one tool predicts *N*-myristoylation (to some degree) but the other tool does not; no myr = no myristoylation site predicted. **b.** Sequence logo analysis using the N-terminal 8 residues of high confidence NMT hits. Created using Weblogo (http://weblogo.berkeley.edu/).

Supporting Methods

Enzyme inhibition assays

Assessment of compound inhibition of NMT was carried out as described previously.[5] Data were fitted to a back-corrected IC_{50} function using GraFit 7.0 (Erithacus Software Ltd, UK).

CuAAC labelling and pull-down

CuAAC. Proteins were precipitated with chloroform/methanol (MeOH:$CHCl_3$:ddH_2O 4:1:3), or acetone (4 vol. -20 °C 1 h) and then resuspended at 1 mg/mL in 1 % SDS in PBS. This precipitation step was found to increase labeling intensity after CuAAC, likely due to the presence of probe-incorporating glycolipids in the lysates (see main text). Premixed click reagents (100 µM AzTB, 1 mM $CuSO_4$, 1 mM TCEP, 100 µM TBTA, final concentrations) were added as described previously [6] and samples vortexed for 1 h RT, then quenched by the addition of 10 mM EDTA. Proteins were precipitated again with MeOH/$CHCl_3$, washed with ice-cold MeOH, air-dried for ~15 min, and then resuspended in 2 % SDS, 10 mM EDTA in PBS. For direct gel analysis, 4 x sample loading buffer (SLB, NuPAGE LDS sample buffer) with 2-mercaptoethanol (4 % final) was added and proteins heated for 3 min at 95 °C prior to SDS-PAGE.

NaOH treatment. Samples were resuspended after CuAAC as above, then 0.2M NaOH added and samples incubated at RT for 1 hour. Samples were quenched by addition of 4 x SLB.

Pronase treatment of proteins. Following CuAAC and resuspension (1% SDS, PBS, without EDTA), samples were split in two and treated with pronase (Sigma) at 1 mg/mL or water (control) from a 10x stock and incubated at 37 °C for 4 h. 4 x SLB was added and proteins heated for 5 min at 90 °C prior to SDS-PAGE.

Pull-down for gel analysis. Protein was resuspended following CuAAC at 10 mg/mL in 2 % SDS, 10 mM EDTA in PBS, and then diluted to 1 mg/mL with PBS. DTT (from a fresh 100 × stock in water) was added to give a final concentration of 1 mM. Proteins were incubated with Dynabeads® MyOne™ Streptavidin C1 (pre-washed 3 × 0.2 % SDS in PBS) for 1.5-2 h at RT with rotation. Following removal of the supernatant, beads were washed with 3 × 1 % SDS in PBS, then boiled for 10 mins in SLB to elute bound proteins.

Gel and Western blot analysis

Samples were separated by SDS-PAGE and the gel soaked in fixing solution (10 % AcOH, 40 % MeOH), then rinsed in water for in-gel fluorescent imaging: gels were scanned with Cy3 filters to detect the TAMRA fluorophore using an Ettan DIGE scanner, GE Healthcare. Molecular weight markers (Precision Plus All Blue Standards, Bio-Rad) were detected with Cy5 filters. For base treatment of gels: the gel was first fixed by treatment with gel soaking solution (10 % AcOH, 40 % MeOH) for 20 min, then washed briefly with water. A solution of 0.5 M NaOH in 50 % MeOH was added and the gel agitated gently for 1 hr at RT. The gel was washed briefly in water and then treated again with gel soaking solution for 20 min. The gel was washed again briefly with water before reimaging.

ImageJ[7] was used for quantification of fluorescent bands. A thin rectangle was dropped down the length of the lane and the 'gel analyzer' function used to plot the profile of intensity down the lane (with averaging across horizontally). The signal was measured by integrating the area under each band of interest and normalizing relative to no inhibition (**YnMyr** only). Any background (from Myr treated sample) was subtracted. Total protein loading was checked by Coomassie. TC_{50} was calculated by fitting data to a back-corrected IC_{50} function using GraFit 7.0 (Erithacus Software Ltd, UK).

For Western blot detection of biotinylated proteins, proteins were transferred from gels to a PVDF membrane (Immobilon-PSQ, Millipore) using a semi-dry Western blot system (Invitrogen). Tris-glycine transfer buffer (NuPAGE, Invitrogen) was used to soak the blotting paper (2.5 mm paper, Invitrogen) and membrane prior to transfer. PVDF membrane was soaked first in MeOH (~30 sec), then water (2 min) before soaking in transfer buffer (at least 5 min). Transfer took 27 min at 20 V. Membranes were blocked for 1 hr RT with BSA (3 % w/v in 10 mL TBST) and then washed with TBST (3 x 10 mL for 5 min). Membranes were then incubated with NeutrAvidin-HRP (1:10000, Invitrogen) in TBST for one hour and washed again with TBST (3 x 10 mL for 10 min). Detection was carried out using Luminata Crescendo Western HRP substrate (Millipore) according to the manufactures instructions and on a Fujifilm LAS 3000 imager.

Proteomics experiments

Pull-down and preparation of peptides for MS. 0.25-0.5 mg lysate was prepared for proteomic analysis. Proteins were precipitated with acetone (4 volumes added and sample left at -20 °C for at least 1 h) or chloroform/methanol and resuspended at 1 mg/mL in 1% SDS in PBS. Chloroform/methanol precipitation: 4 volumes of MeOH were added, followed by 1 volume of $CHCl_3$ and 3 volumes of H_2O, with vortexing between each addition. Samples were vortexed vigorously and centrifuged at 17,000 ×g for 5 min at RT. The top aqueous-MeOH layer was removed and 4 volumes of MeOH added. Samples were gently mixed and centrifuged again. The supernatant was removed and the pellet washed 2x with MeOH.

Proteins were captured by CuAAC as before with the following modifications: CuAAC reaction was carried out for 2 hours and, for some samples, with AzRB or AzRTB in place of AzTB. When AzRB/RTB was used, proteins were precipitated following CuAAC via a modified chloroform/methanol precipitation procedure: 4 volumes of MeOH, 1 vol. $CHCl_3$, 3 vol. H2O were added to the sample, which was centrifuged at 17,000 ×g for 5 min to pellet proteins at the interface. Both layers were then removed simultaneously, the pellet resuspended in 0.2 % SDS/PBS to the original volume and the precipitation procedure repeated. The pellet was then washed 2x with MeOH. Proteins were finally resuspended at 10 mg/mL in 2 % SDS, 10 mM EDTA in PBS, and then diluted to 1 mg/mL with PBS. DTT (from a fresh 100 × stock in water) was added to give a final concentration of 1 mM.

For base-treatment (inhibition samples), proteins were resuspended in 50 µL 2 % SDS, 10 mM EDTA in PBS; to this was added 50 µL PBS and 25 µL 2M NaOH. Samples were shaken for 1 hr RT then 25 µL 2M HCl added to neutralise; next 350 µL PBS was added and 5 µL 0.1M DTT (giving 1 mM DTT final concentration). Samples were centrifuged at 17000xg, 10 min RT to pellet any undissolved protein.

Affinity enrichment were performed for all samples as before with the following modifications: NeutrAvidin agarose resin (Thermo Scientific, pre-washed 3 × 0.2 % SDS in PBS; typically 50 µL of bead slurry was used for 0.5 mg of lysate) was used in place of the magnetic Dynabeads. Beads were stringently washed following pull-down: 3 × 1 % SDS in PBS, 3 × 4M Urea in 50 mM PBS, 5 × AMBIC (50 mM ammonium bicarbonate). For a 50 µL bed of beads resuspended in 50 µL AMBIC, samples were reduced (5 µL of 100 mM DTT in 50 mM AMBIC) at 60 °C for 30 minutes and allowed to cool to room temperature. The beads were washed with 2 x AMBIC. Cysteines were alkylated (5 µL of 100 mM iodoacetamide in AMBIC) at room temperature for 30 min in the dark. The beads were washed with 2 x AMBIC. Trypsin (1 µg Sequencing Grade Modified Trypsin (Promega) dissolved at 0.2 µg/µL in AMBIC per mg starting lysate) was added to the beads and samples were placed on a shaker and digested overnight at 37 °C. The samples were centrifuged and the supernatant was transferred into clean tubes. The beads were washed twice with 0.1% aqueous formic acid, and these washes were combined with the

first supernatant. The solutions were stage-tipped according to a published protocol [8]. Elution from the sorbent (SDC-XC from 3M) with 70 % acetonitrile in water was followed by speed-vac-assisted solvent removal, reconstitution of peptides in 0.5 % TFA, 2 % acetonitrile in water, and transferred into LC-MS sample vials.

LC-MS/MS analysis. The analysis was performed using an Acclaim PepMap RSLC column 50 cm × 75 µm inner diameter (Thermo Fisher Scientific) using a 2 h acetonitrile gradient in 0.1 % aqueous formic acid at a flow rate of 250 nL/min. Easy nLC-1000 was coupled to a Q Exactive mass spectrometer via an easy-spray source (all Thermo Fisher Scientific). The Q Exactive was operated in data-dependent mode with survey scans acquired at a resolution of 75,000 at m/z 200 (transient time 256 ms). Up to 10 of the most abundant isotope patterns with charge +2 or higher from the survey scan were selected with an isolation window of 3.0 m/z and fragmented by HCD with normalized collision energies of 25. The maximum ion injection times for the survey scan and the MS/MS scans (acquired with a resolution of 17 500 at m/z 200) were 20 and 120 ms, respectively. The ion target value for MS was set to 10^6 and for MS/MS to 10^5, and the intensity threshold was set to 8.3×10^2.

Further notes on data processing. An error in database redundancy was noted: ARF proteins Tb927.9.13740, Tb927.9.13710 and Tb927.9.13680 have identical sequences; Tb927.9.13650 differs by just one amino acid.

Bioinformatics and comparisons with literature datasets. Prediction of myristoylation was performed using the Myristoylator (http://web.expasy.org/myristoylator/)[4a] and the NMT Predictor (http://mendel.imp.ac.at/myristate/SUPLpredictor.htm).[4b] Prediction of *S*-palmitoylation was carried out using CSS-Palm 3.0 (downloaded from http://csspalm.biocuckoo.org/) with threshold set to 'high'.[9]

For analysis of hits during differentiation of *T. brucei* from BSF to PCF, the dataset of Dejung *et al.* was used.[2] Briefly, the LFQ intensity data (ProteinGroups file) was downloaded from PRIDE (PXD003319), intensities logarithmized (base 2) and missing intensities imputed with random numbers from a normal distribution, whose mean and standard deviation were chosen to simulate low abundance values close to noise level (impute criteria: width 0.1 and down shift 2.1; imputation for each sample individually). Intensities were averaged (mean) across replicates and cross-referenced with the current dataset to generate profile plots of select proteins during the differentiation process (Fig. S7).

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