

A

XP_001682355.1	-----CDLNDTWEDVLTAFFNDCLK-GESTWSCHCLDG	31
XP_814406.1	-----MACDLDDETWEVCEMGEFKEFIGKTGNPWRCYTCEG	34
XP_844906.1	-----MCALDDTWAADCDEFKLALSTGTWKCESLAG	34
NP_001118295.1	--MQSVREDEDSSSPHIHDSKTSSSIPSGDNNSVWADVSPLLSAACSDIQLQEGE-LINGDN	57
sp Q5VZE5 NAA35_HUMAN	MVMKASVDDDSGWEL-----SMPEKMEKSNTNWVDITQDFEEACRELKLGE-LLHDKL	53
NP_579858.1	MVMKAAVDDDSGWEL-----NVPEKMEKSSTSFWVDITQDFEDACRELKLGE-LLHDKL	53
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XP_001682355.1	-----IDKEVILSAPEIMDPKTDTSGFNCEEI---YSLSHILLKTEEVPS---AATVSTEALLD	83
XP_814406.1	VEKETMLSAPEVMDAKTDPGCGYGSI---CSLNCLLKDGTTIPS---AATLTG-EALLD	85
XP_844906.1	VDKEAMLSAPEVMDAKTDPGCGYQAQI---RSLNNDLAKGDIPS---AATLQG-QELLD	85
NP_001118295.1	FNLFAAMSALEIMDPKMDGMSGMVSTFY-----IDEAIESGFAPVPISSDSTVN-VQSIID	111
sp Q5VZE5 NAA35_HUMAN	FGLFEAMSAIEMMDPKMDAGMIGNQVNKRVLNFEQAIKDGTIK----IKDLT-LPELIG	107
NP_579858.1	FGLFEAMSAIEMMDPKMDAGMIGNQVNKRVLNFEQAVKDGTIK----IKDLS-LPELIG	107
	* : *; * : * * : .. : .. : .. : .. : ..	
XP_001682355.1	VMDYIHVKELTYLQGYSLTQSYLDFPYFLRMDLLKGQNSTLHAYCRGVLRSLDCVLHAVF	143
XP_814406.1	VMDLIHLKELNYLQGFSLTSGCLEFSYFFCMDDLKEQNLTLTYTCRALARCIDLTTAVM	145
XP_844906.1	VMDLILAKELQYLGFSLTSGCLAFPYFFKMDLLKEQNPVLHAYCRGVRTVEIVLRAVM	145
NP_001118295.1	IMDHLLACEATWHMGHSLAQTVFSCIVYLRLPERTSSQ-ALLHSYCRVIRATCRAVVSVVS	170
sp Q5VZE5 NAA35_HUMAN	IMDTCFCCLITWLEGHSLAQTVFTCLYIHNPDFIEDP-AM-KAFALGILKICDIAREKVN	165
NP_579858.1	IMDTCFCCLITWLEGHSLAQTVFTCLYIHNPDFIEDP-AM-KAFALGILKICDIAREKVN	165
	: ** : * . * : .. : .. : .. : .. : .. : ..	

B

NP_010946.3	-----IDYKLRVLTQDGRVYIGQLMAFDKHMNLVLNECIEERVPKTQLDKLRPRKD	51
NP_001317040.1	-ARQQLEALLNKTMRIRMTDGRTLVGCFLCTDRDCNVILGSAQEFLKPSDSF-----	51
XP_003722006.1	MGKYTMLHNINKVLCVMLDDGRTVTGKLLVFDKHMNVVLGDAVEERPQSKKMAEE-----	55
XP_808107.1	MGRESMLHNINRTRIRIVLIDGRELTGKLLVYDRHMNVVLGDATESREETKKMKEA-----	55
XP_951644.1	MGHQNMLHNINRTLRLVTLVDGREMTGKMLLFDKFMNVVLADTVETRKETKKMKA-----	55
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NP_010946.3	SKDGTTLNKVEKRVLGLTILRGHQILSTVV-----	82
NP_001317040.1	SAGEPRVGLGLAMVPGHHIVSIEV-----	74
XP_003722006.1	-GVSSKRQLGLLILLRGEHVSVTVMKDSENGSGAVAN-----FGGAPKLAK	101
XP_808107.1	-----GISPQRSLGLVLLRGVHVVISVNVLGASENNGDGEKTKGQPANFEKAPRAKV	107
XP_951644.1	-----GISPQRKLGMILLRGHEYVVAVSVLKD-----VSEDKAQ PANFESATREKL	101
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C

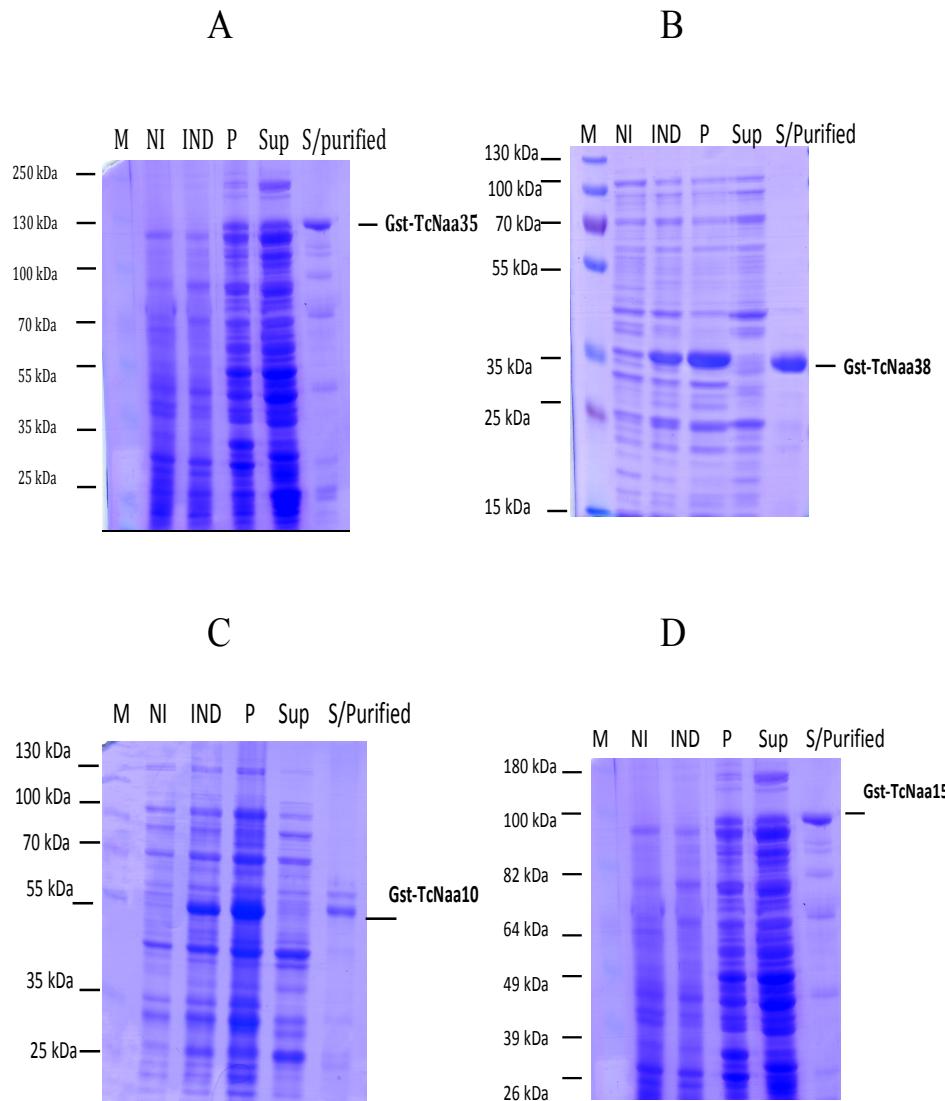
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NP_011877.1	TYRRMGLIAENLMRQALFALREVHQAEYVSLHVRQSNRAALHLYRDTLAFEVLSIEKSYYQ		180
NP_003482.1	SHRLGLAQKLMDQASRAMIENFAKYVSLHVRKSNRAALHLYSNTLNFOISEVEPKYYA		139
XP_001681770.1	SHRLGLIASRVMRATMKEMDAEYGAHYCSLHVRKTNDAALHLYQDTLGFCVGVEEKYYM		140
XP_817467.1	THRLGLIASRVMRASMKEMEKEYDANYCSLHVRKTNDAALHLYQETLGFCCANVEKGYYV		140
XP_828529.1	THRLGLIASRVMNAALHEMEHEYDANFCSLHVRKTNDAALHLYQNTLNFRCANVESKYVV		140
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NP_011877.1	DGEDAYAMKKVLIK-----		193
NP_003482.1	DGEDAYAMKR-----		149
XP_001681770.1	DEDAYAHMKSFFFHQANPGSYVDDHKRLIRK-----		170
XP_817467.1	DEDAFHMKKFFKGPNGLYVAANRQLVRQQNSAAAATGMAALQRNGGTAPPPPPSSS		200
XP_828529.1	DEEDAYHMKRFFKGTNPFGFYVTESRQLVRFQNT-----		173
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NP_011877.1	TYRRMGLIAENLMRQALFALREVHQAEYVSLHVRQSNRAALHLYRDTLAFEVLSIEKSYYQ		180
NP_003482.1	SHRLGLAQKLMDQASRAMIENFAKYVSLHVRKSNRAALHLYSNTLNFOISEVEPKYYA		139
XP_001681770.1	SHRLGLIASRVMRATMKEMDAEYGAHYCSLHVRKTNDAALHLYQDTLGFCVGVEEKYYM		140
XP_817467.1	THRLGLIASRVMRASMKEMEKEYDANYCSLHVRKTNDAALHLYQETLGFCCANVEKGYYV		140
XP_828529.1	THRLGLIASRVMNAALHEMEHEYDANFCSLHVRKTNDAALHLYQNTLNFRCANVESKYVV		140
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NP_011877.1	DGEDAYAMKKVLIK-----		193
NP_003482.1	DGEDAYAMKR-----		149
XP_001681770.1	DEDAYAHMKSFFFHQANPGSYVDDHKRLIRK-----		170
XP_817467.1	DEDAFHMKKFFKGPNGLYVAANRQLVRQQNSAAAATGMAALQRNGGTAPPPPPSSS		200
XP_828529.1	DEEDAYHMKRFFKGTNPFGFYVTESRQLVRFQNT-----		173
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D

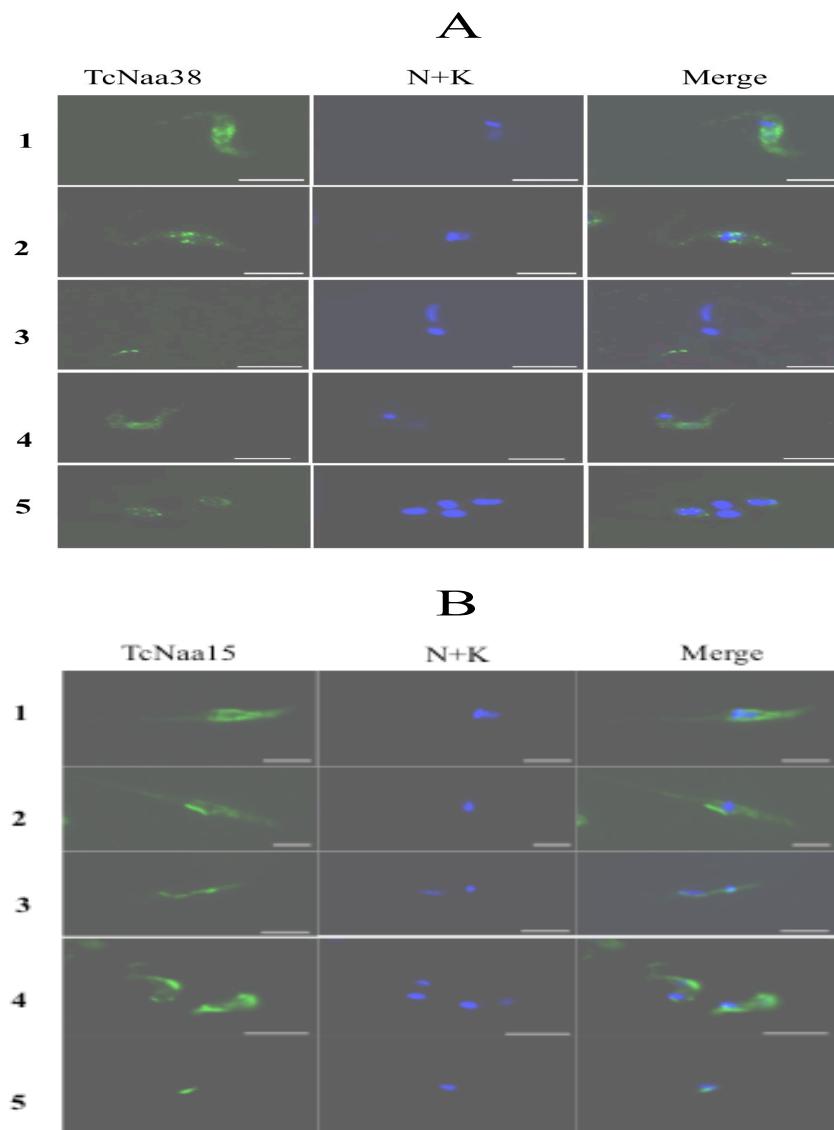
XP_001686706.1	YSNREYSKALRTSECILRVV--PDHVDTFAGRGLVLYNMERQEEGYESIKQAI---LLNP	55
XP_807954.1	FDAREYAKALRCADAILAVI--PSHADTIAMRGILTLLHILDRREEGHHLAIKEAI---ELNI	55
XP_822811.1	FDAREYAKGLRTADSILSVV--PNAHDTLALKGLTLHHMGRKEEGREIESAL---GFND	55
NE_476516.1	YEHRQYRNGLKFCQJILSNPKFAEHGETLAMKGILTINCLGKKEEAYELVRRGL---RNDL	57
EDN60314.1	YEGRKYKSKSLKLLDAILKKD--GSHVDSSLALKGLDLYSVGEKDAAASYXANAIRKIEGAS	58
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XP_001686706.1	KSMVAWHALGMQCRLDKKFGEAVKAFKRALTFDPANTEVLRDLASACIQVRDWPLFLEAR	115
XP_807954.1	NSTMAWHS LGMCHRAEK NYPE A PIKA F KKAHQ T DPT N AN V RL RD LSS VC V Q V R D W E Q F V E T R	115
XP_822811.1	TSTVWWHS LGMCHRAADDNHVEALHAFQKAHEYGP SNVN V RL DISS IC V QL RWE Q F V D V R	115
NE_476516.1	KSHWCWVHYVGLLQRSDKYYDEAIKCYRNALWKDNDLQILRLDSL L L Q T Q M R D L E G Y R E T R	117
EDN60314.1	ASPICCHVLGIYMRNTKEYKESIKWFTAAALNNGSTNKQIYRDLATLQLQSIGDFKNALVSR	118
	* : * : * : * . * : * . * : * : * : * : * : * : * : * :	
XP_001686706.1	EKMTATAKSVRANWVALSCGHRIILGH SRIA AVAM DTMTS IMDA---GDNP VEV SEA QLYR	172
XP_807954.1	QKMVT LKAGV RAN WIALSCGH RHM GMGH TELA AAV ID V M TS IM DA---GEN V V E K S E V F L Y L	172
XP_822811.1	RKMVT L R P G V R A N W I A L S C G H R M G H T E L A A V I D V M T T I M E A---GD N R A E K S E V R L Y Q	172
NE_476516.1	Y Q L L Q L R P A Q R A S W I G Y A I A X H I L E D Y E M A K I L E F R K T Q-Q T S P D K V D Y E S E L L Y Q	176
EDN60314.1	KKYWEAFLGYRANWTSLSAVAQDVNGERQQAINTLSQFEKLAEGKISDSEKYEHSECLMYK	178
	: : * : * . : * . : * . : * . : * . : * . : * : * : * :	

Supplementary Figure 1: Alignment of TcNaa35/TcNaa38 and TcNaa10/TcNaa15 with orthologous sequences from other eukaryotes. **A;** Comparison of predicted TcNaa35 with other proteins. The tick line indicates predicted Mak10 domain region. The most conserved aa are represented by a star (*). Note that only part of the alignment is displayed. Polypeptides used are: *Leishmania major* (XP_001682355.1), *T. cruzi* (XP_814406.1), *T. brucei* (XP_844906.1), Plant (NP_001118295.1), human (SP|Q5VZE5) and Yeast (NP_579858.1). **B;** TcNaa38 sequence alignment with selected proteins. * Denote the most conserved aa. Sequences included in the alignment are: Yeast (NP_010946.3), Human (NP_001317040.1), *Leishmania major* (XP_003722006.1), *T. cruzi* (XP-808107.1) and *T. brucei* (XP_951644.1)

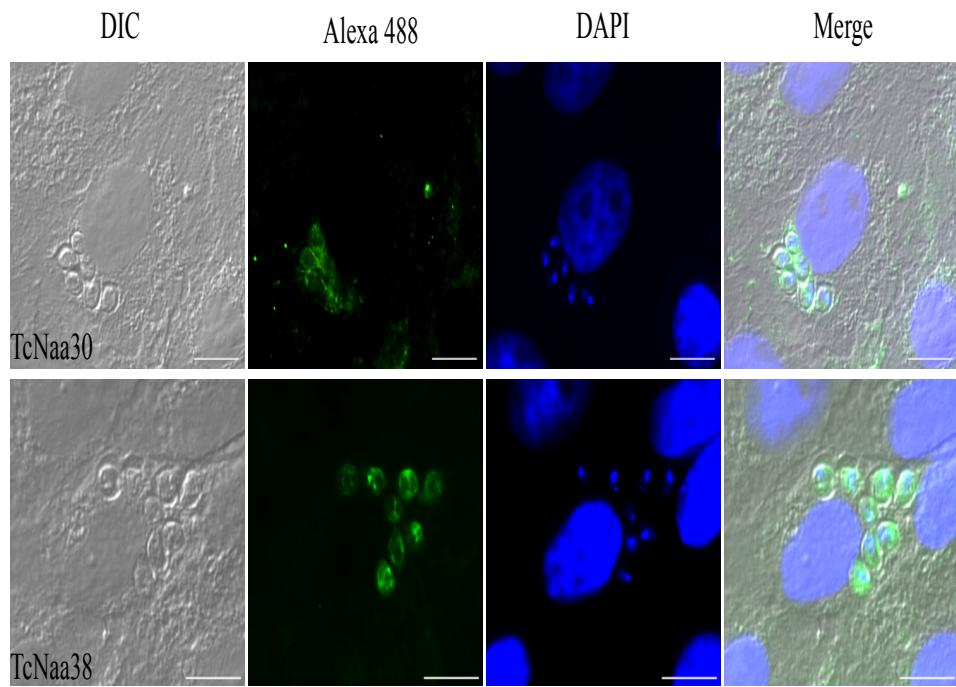
C; Comparison of TcNaa10 sequence with selected eukaryotes. The over lined indicates the consensus sequence RXXGXA for NatA, where acetyl –CoA binds. The two arrows indicate the active sites and a symbol # is the predicated possible lysine auto acetylation site. Used sequences in the alignment are: Yeast (NP_011877.1), Human (NP_003482.1), *Leishmania major* (XP_001681770.1), *T. cruzi* (XP_817467.1) and *T. brucei* (XP_828529.1). **D;** TcNaa15 sequence comparison with putative homologues from other species. Polypeptides used in the alignment are: *Leishmania major* (XP_001686706.1), *T. cruzi* (XP_807954.1), *T. brucei* (XP_822811.1), Human (NP_476516.1 and Yeast (END60314.1. * Indicates residues, which are highly conserved.



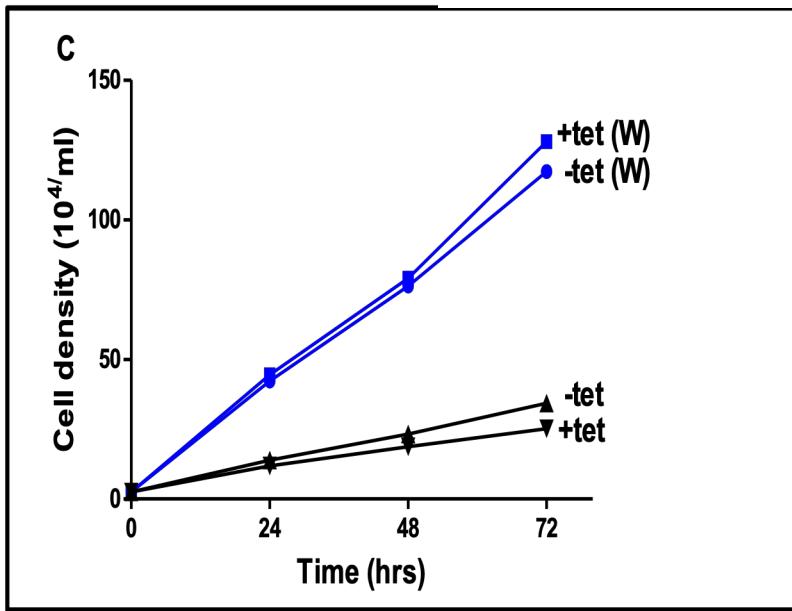
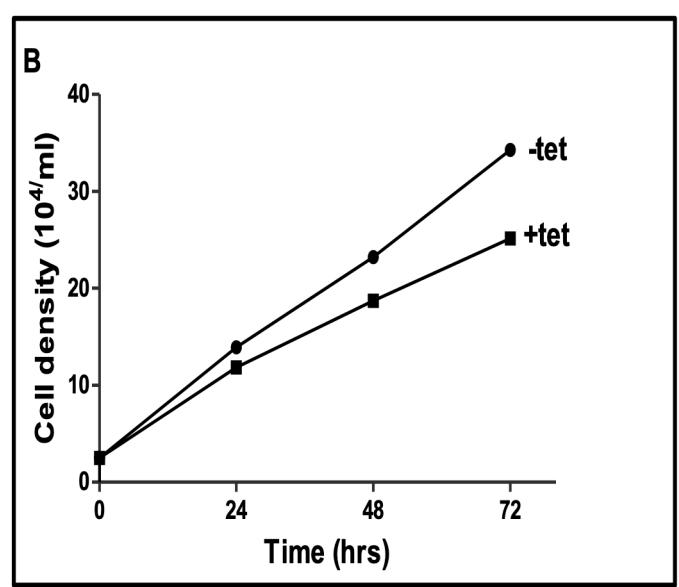
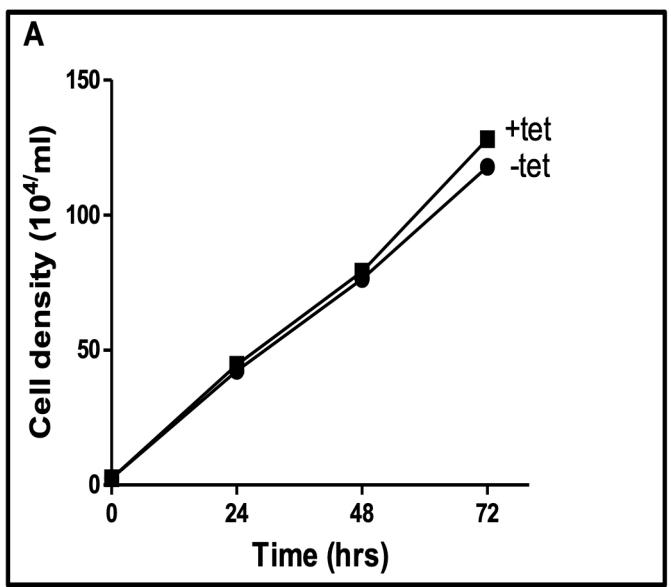
Supplementary Figure 2: Recombinant protein production and analysis on 10 % SDS-PAGE. **A;** Recombinant GST-TcNaa35. **B;** GST-TcNaa38. **C;** GST-TcNaa10 and **D;** GST-TcNaa15. Samples loaded in the different lanes are as follows, Lane1; Molecular mass in kDa. Lane 2; Non-induced (NI). Lane 3; Induced (IND), Lane 4; Pellet (P). Lane 5; Supernatant (Sup). Lane 6; Semi Purified (S/Purified).

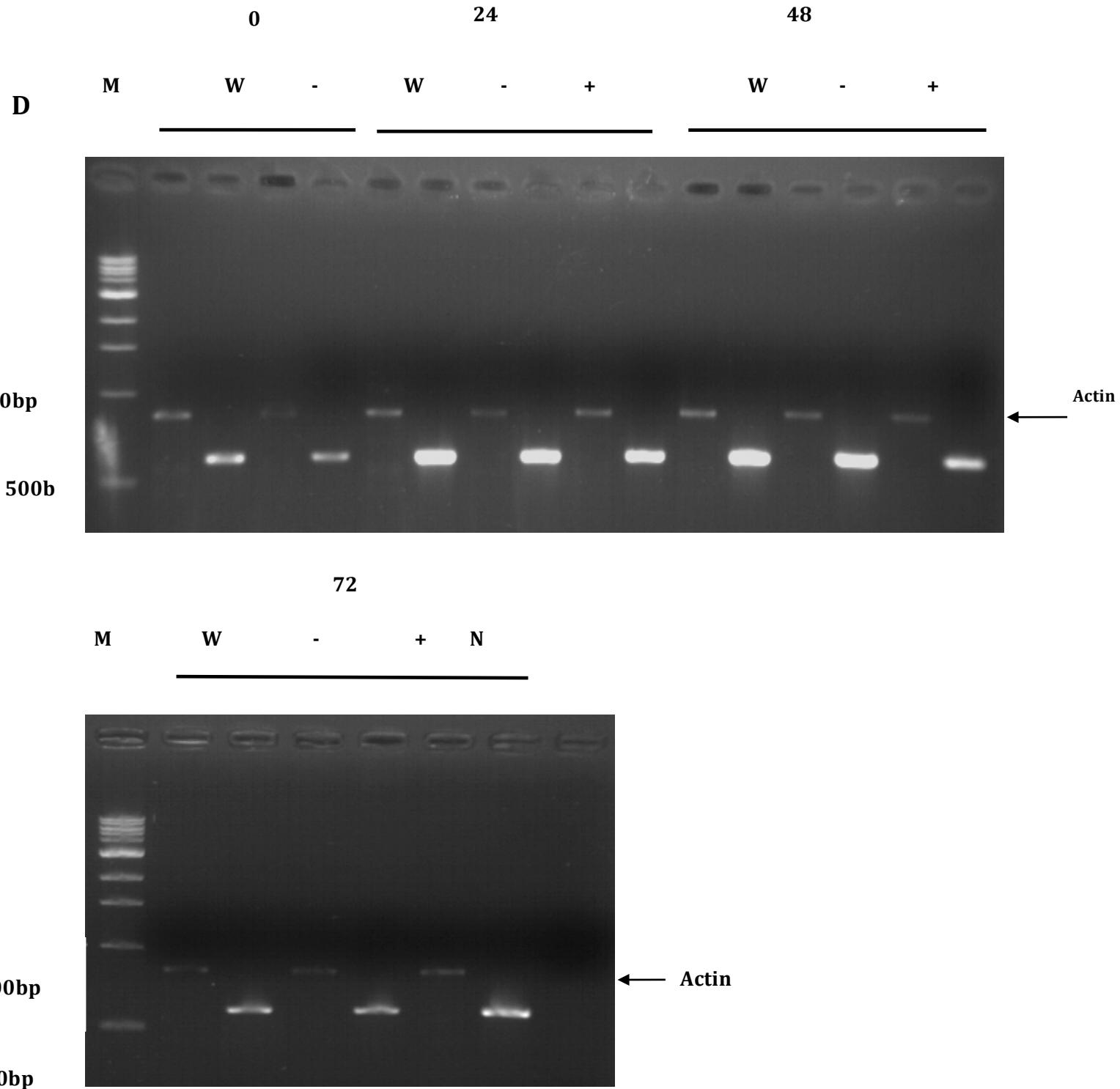


Supplementary Figure 3: Localization of TcNaa38 and TcNaa15. Number 1 to 5 denotes, Midlog epimastigotes, Stationary epimastigotes, Metacyclic trypomastigotes, Trypomastigotes, and Amastigotes, respectively. *T. cruzi* four developmental stages were immunolabelled with, **A**; anti-TcNaa38 and **B**; anti-TcNaa15. The nucleus and kinetoplast were visualized using DAPI stain (N+K), scale bars = 5 μ m.



Supplementary Figure 4: Localization of TcNaa30 and TcNaa38 in internal amastigotes. Parasites were allowed to invade Vero cells and divide. Cells were immunolabelled with anti - TcNaa30 and anti- TcNaa38 (green) and counter labeled with DAPI (blue) to visualize the nucleus and kinetoplast, scale bars = 10 μ M.





Supplementary Figure 5: Phenotype of the knock down of putative *T. brucei* Naa30 by RNAi showing growth curves and mRNA level, **A**; Wild type, **B**; Transfected, **C**; The parasite growth of the transfectants compared to that of the wild type (blue). Cells at a density of 2.5×10^4 /ml were grown in the absence (-Tet) or presence (+Tet) of tetracycline (100ng/ml) over a period of 72h. The result is a representative of the mean parasite growth of *T. b. brucei* 427 obtained from three independent experiments. **D**; Gene expression analysis of wild type (W), non-induced (-) and induced (+) cells using Reverse Transcriptase PCR. The products were separated on a 2% agarose gel alongside a 1kb DNA ladder and visualized using ethidium bromide staining. Shown is day 0-48h and day 72h post-RNAi induction. Actin (700bp) was amplified as an internal control alongside NatC catalytic subunit (573bp). The cell densities used for Day 0, 24, 48 and 72h from which RNA was isolated were 1×10^5 /ml; 2.73×10^5 /ml; 3.75×10^5 /ml and 4.7×10^5 /ml, respectively.