

Supplementary Information for Crystal Structure of *Borrelia turicatae* protein, BTA121, a differentially regulated gene in the tick-mammalian transmission cycle of relapsing fever spirochetes.

Zhipu Luo¹, Alan J. Kelleher², Rabih Darwiche³, Elissa H. Hudspeth², Oluwatosin K. Shittu²,
Aparna Krishnavajhala², Roger Schneider³, Job E. Lopez² and Oluwatoyin A. Asojo^{2*}

¹ Synchrotron Radiation Research Section, Macromolecular Crystallography Laboratory,
National Cancer Institute, Argonne, Illinois 60439, USA

² National School of Tropical Medicine, Baylor College of Medicine, Houston Texas, United
States of America

³ Division of Biochemistry, Department of Biology, University of Fribourg, Chemin du Musée
10, CH 1700 Fribourg, Switzerland

***Running Title:* Crystal Structure of BTA121**

Keywords: relapsing fever; neglected tropical diseases; vector adaptation; lipid binding; tick
borne diseases; structure-function relationships; bioinformatics; argasids ticks

Correspondence to: asojo@bcm.edu (OAA)

Purification of BTA121

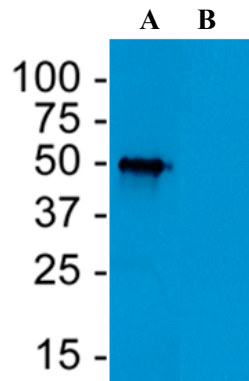


Figure S.1: Confirmation of cleavage of Histag by anti-His Western blotting. Lane A contains BTA121-His while Lane B contains BTA121 post removal of the Histag.

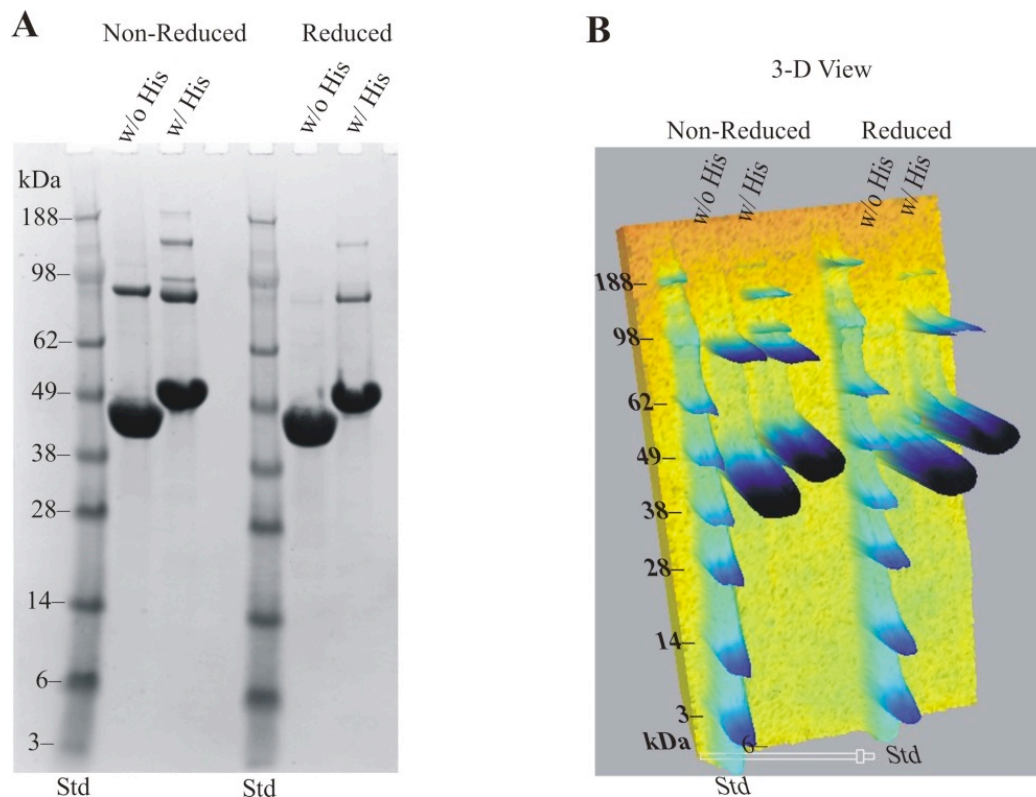


Figure S2: A) SDS-page gel showing purity of BTA121, visualized by Coomassie blue staining. Electrophoretic mobility on the reduced gel is consistent with the theoretical molecular mass for the tagged and untagged proteins. B) 3D gel of Fig S.2A visualized by false color.

Size exclusion chromatography

The molecular mass of the eluted peak is determined using a calibration curve based on known standards, not just by visualization. The standard consisted of bovine thyroglobulin (670 kDa), bovine γ -globulin (158 kDa), chicken ovalbumin (44 kDa), horse myoglobin (17 kDa), and vitamin B₁₂ (1.35 kDa), which was injected onto the column using the same method as the BTA121 samples. For samples analyzed on different days or with different column/guard cartridge set-ups, a gel filtration standard was injected on the same day and with the same set-up as the experimental sample. In addition, blank injections of the mobile phases were used to confirm a stable baseline and the absence of carry-over (prior injected sample eluting during the subsequent injection run). Blue dextran was injected to estimate the retention time of the void volume (the volume present from the injection loop to the PDA detector, excluding the pore volume of the beads). This provides the retention time at which a compound will elute that is larger than the pore size of the beads (145 μ m diameter) and has no interaction with the bead surface. A 1 M sodium chloride (NaCl) solution was injected to estimate the pore volume of the beads. The retention times of the protein standards and blue dextran were utilized to calculate the distribution coefficient (K_d) for each standard and to create a plot of the log of the molecular weight (MW) versus K_d . Normalizing the retention times to K_d removes system and column specific information (void and column volume) allowing direct comparison of the data to that obtained from other systems and columns. The slope and y-intercept of a best-fit linear line was used to calculate the molecular weight of the sample peaks.

$$K_d (\text{distribution coefficient}) = (V_e - V_o) / (V_t - V_o)$$

V_e = elution volume of sample

V_o = void volume (blue dextran)

V_t = total column volume ($3.14 \times \text{radius}^2$)

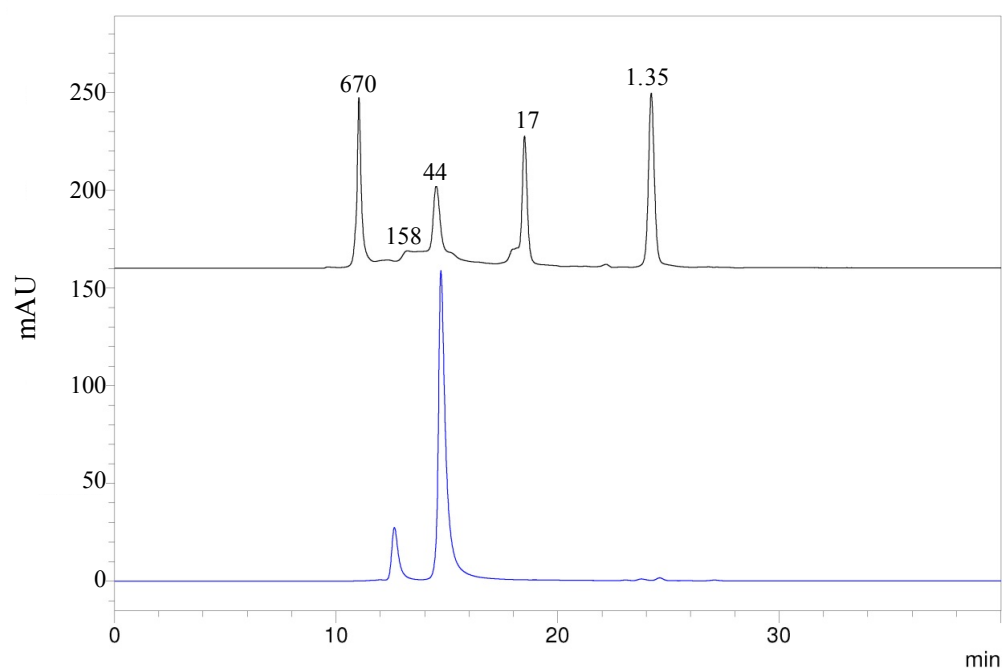


Figure S.3 Purity of freshly thawed untagged BTA121 reveals two peaks. These can be separated by gel filtration, however removing the minor peak does not improve the quality of the crystals. Similar profile was observed for the selenomethionine protein.

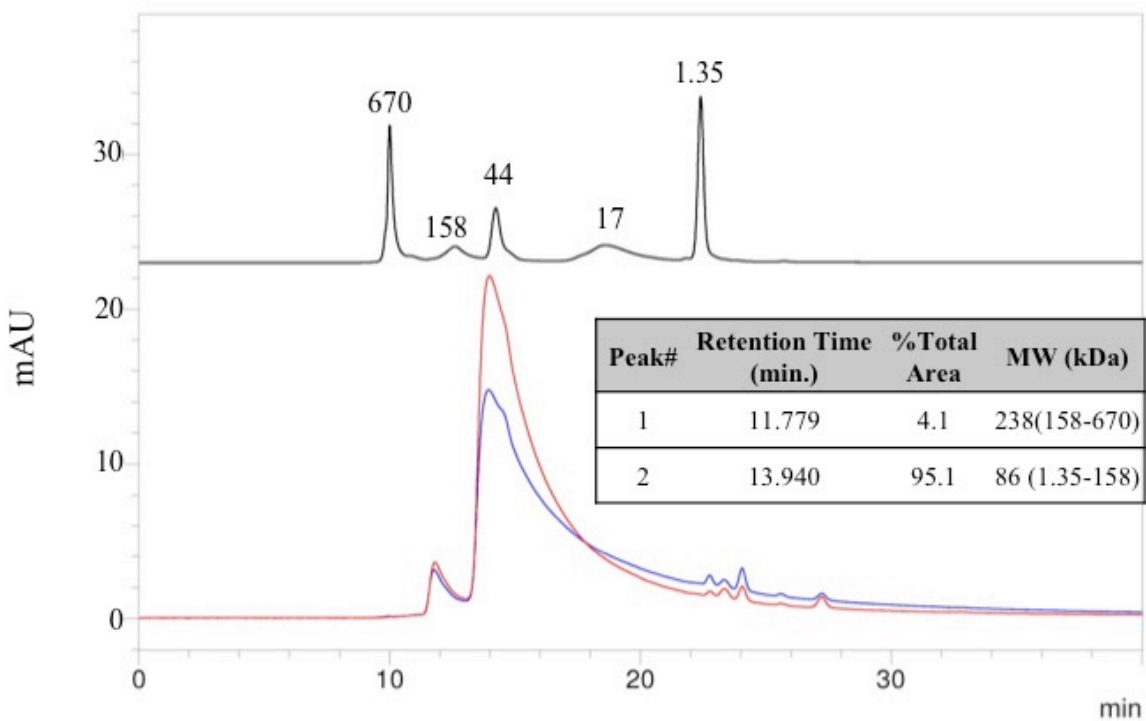


Figure S.4: Extensive peak broadening occurs after storing BTA121 at 4°C. The sample stored at 28 mg/mL for greater than 70 days (red) has a consistent profile with the sample stored at 5 mg/mL for 43 days (blue). Since 100 µg was injected of the sample stored at 28 mg/mL and 50 µg was injected of the sample stored at 5 mg/mL, the chromatograph of the 28 mg/mL sample was adjusted 2-fold for comparison.

Figure S.5. Structural and primary sequence alignment of BTA121 with other *B. turicatae* proteins from the megaplasmid. This figure was generated with ESPript¹. The different secondary structure elements shown are alpha helices as large squiggles labelled (a), 3₁₀-helices as small squiggles labelled (h), beta strands as arrows (b), and beta turns (TT). Identical residues are shown in white on red background, and conserved residues in red. The locations of the cysteine residues involved in disulfide bonds are numbered in green.

1 Gouet, P., Robert, X. & Courcelle, E. ESPript/ENDscript: Extracting and rendering sequence and 3D information from atomic structures of proteins. *Nucleic Acids Res* **31**, 3320-3323 (2003).

fBTA121

	1	10	20	30	40	50	60
fBTA121	MLKIK	CFSLLLVLT	L L L L V	I S C D	S G S K A	V A G T	D T E T E A V T N T G A G L E A G V R P G V G L R I S
BTA121
BTA121-His	HHHHHHHH
BTA116	MLKIK	CFSLLLVLT	L L L L V	I S C D	L G I K A	R T N T	R A
BTA123	MLKIK	CFSLLLVLT	L L L L V	I S C D	S G S K A	G A G T	N T G A G G G A G S G . . T N T
BTA126	MV K I K	CFSVLLVLI	L L L L V	I S C D	S G S K A	G A G T	N T R A V V K S V A G . . T N T R A V V K S
BTA127	M L N I R	H I S V L L V L I	F L L F L	I N C D	S R S K A	G A G T	N T G A G G G A G
BTA119	MV K I K	CFSLLLVLT	L L L L V	I S C D	S G S K A	G A G T	N T G I T T G K T T K . . I T T G T N T
BTA118	MV K I K	CFSLLLVLT	L L L L V	I S C D	S G S K A	G A G T	N T G I T T G K T T K . . I T T G T T T G T
BTA117	MLKIK	CFSLLLVLT	L L L L V	I S C D	S G S K A	G A G T	N T V T D T G K T T K . . I T T G T T T G T
BTA122	MLKIK	CFSLLLVLT	L L L L V	I S C D	S G S K A	G A G TG K T T G . . T N T G I T T G T
BTA124	MV K I K	CFSLLLVLT	L L L L V	I S C D	S G S K A	V T N TG K T T G . . T N T G I T T G T
BTA120	MLKIK	CFSLLLVLT	L L L L V	I S C D	S G S K A	G A G T	N T V T D T G K T T G . . T N T G
BTA115	MV K I K	CFSLLLVLT	L L L L V	I S C D	S G I K A	G T N T	K T E V K P G A G L D A V V K P G A G L D A V
BTA112	MLKVR	Y R N S L L V L I	L L L L V	M G C N F K S P K D A A	S G E N S I V K K N E
BTA129	MMKLS	H F S L F L V L I	F I L L L V	N C D L K S K G	S T G T	E E N L . T S K R E V K K

fBTA121

	70	80	90	100	110	120
fBTA121	T V P V V V I R L E S G V R P E S G V P E S G V G P E A G V G P E A G V G P E S G V G P E A G V G P E A G V G P E S G
BTA121
BTA121-His
BTA116
BTA123
BTA126
BTA127
BTA119
BTA118
BTA117
BTA122
BTA124
BTA120
BTA115	V V K P
BTA112
BTA129

fBTA121

	130	140	150	160	170	180
fBTA121	V G P E S G V G P E A G V R P E A G V R P E A G A G T D T E T E E E I	E V V G D E E A	L A Y L N E T V I D	P K L I A L
BTA121	V G P E S G V G P E A G V R P E A G V R P E A G A G T D T E T E E E I	E V V G D E E A	L A Y L N E T V I D	P K L I A L
BTA121-His	V G P E S G V G P E A G V R P E A G V R P E A G A G T D T E T E E E I	E V V G D E E A	L A Y L N E T V I D	P K L I A L
BTA116
BTA123
BTA126
BTA127
BTA119
BTA118
BTA117
BTA122
BTA124
BTA120
BTA115
BTA112
BTA129

fBTA121

	190	200	210	220
fBTA121	L D F G V S R S G R K A I S Y I Q G N L T S	D V I Y D R L N K L G A D V V I E K I I K P T
BTA121	L D F G V S R S G R K A I S Y I Q G N L T S	D V I Y D R L N K L G A D V V I E K I I K P T
BTA121-His	L D F G V S R S G R K A I S Y I Q G N L T S	D V I Y D R L N K L G A D V V I E K I I K P T
BTA116	L D F G V S G A G R K A I Y I R G G L	L G D D V Y D R L N K L G A D V V I E K I I K P T
BTA123	L D F G V S G P G R K A I G Y I G G K F R D S	G D V H K R L N A L G A D V V I E K I I K P T
BTA126	L D F G V S G A G K K T I S Y I Q R T L P A	N V V Y D Y L N Q L G A D V T I E K I I K P T
BTA127	L D F G V S E A G R K A I G Y I Q G I L R V S	Y K N G F D D K F Y N C L N Q L G A D V V I E K I I K P T
BTA119	L D F G V S G A G R K A I S Y I Q G I L S V S	Y K D G F D D K F Y N C L N K L G A D V A V E K I I K P T
BTA118	L D F G V S G A G R K A I S Y I Q G I L R V S	Y K N G F D D K F Y N S L N Q L G A D V A V E K I I K P T
BTA117	L D F G V S G A G R K A I G Y I Q E I L S F S	Y V D E F D D K F Y N S L N K L G A D V V I E K I I K P T
BTA122	L D F G V S G A G R K A I G Y I Q E I L S V S	Y K D G F D D K F Y N C L N Q L G A D V V I E K I I K P T
BTA124	L D K L G V S G A G R K A I V Y I R D N L T G	D D V D D F F Y N R L N K L R A D I V I E K I I K P T
BTA120	L D F G V S G A G R K A I G Y I Q E K L S V S	Y V D E F D D K F Y N Y L N K L G A D V V I E K I I K P T
BTA115	L D F N V S I E G K K A I G Y I R K I F I D	K N N D I E D K L Y K H L N S L E A D V V L A K I I K P T
BTA112	L N T F G I S E A G K R S I V R I K D V I T D V G I G S V E G Y R N Y T D S E L Y A L L N D L G A A K . I Q E I I K A D
BTA129	L D K F G L L D K E R E A I A Y I R R T I T D S K I G N A S Y Y K T Y T D F E F Y N L L E S L G A T R . I K E I I T F Y

$\alpha 5$ $\eta 1$ $\alpha 6$
fBTA121 230 240 250 260 270 280
fBTA121 VSL LKTKGE ALK I I EDP T NEG VKTRL Q NM CKRY DGLVKGIGY...DFFHGSIGT DRF A Q
BTA121 VSL LKTKGE ALK I I EDP T NEG VKTRL Q NM CKRY DGLVKGIGY...DFFHGSIGT DRF A Q
BTA121-His VSL LKTKGE ALK I I EDP T NEG VKTRL Q NM CKRY DGLVKGIGY...DFFHGSIGT DRF A Q
BTA116 VSL LKARGE ALRVI QDP T NEG VKVRL Q DV FHRY DNLV.DIRG...KFIYVVGSE DNF A K
BTA123 VSL LKARGE ALKVK EDP T NES IKTRL Q DMLNRY DTLV.ESVWHD...FFHNRLIEE DMF VE
BTA126 VSL LRARG KALRVI EGT T DEG IKTGL Q DMFEKY DALSFYLVWV...FNRAIKENDFVE
BTA127 VSL LRARG EALRVI EGT T DEG VKTRL Q DMLSSCDHVI.SFAWNP...DRDHILIFNE DKF AN
BTA119 VSL LIARG KALRVI QDP T NAG VKSRL Q DMLSSCDHVI.SLTWY LISGFDYFIFEE DNF A K
BTA118 VSL LRARG EALRVI QDP T NAG VKSRL Q DMLSSCDHVI.SLSWY LISGFDYFIFKE DVFETR
BTA117 VSL LRARG KALRVM QGT T DAG VKTRF Q DMLSSCDHVV.SFTWYL...DFDHL SFRE DIF EAD
BTA122 VSL LRARG KALRVM QGT T DAG VKTRL Q DMLSSCDLSV.SLAWYL...NSNPLIFHE DGFAD
BTA124 VIL LRARG EALRVI EGT T DED LKSKLR L VFHRY DSLV...NIREKFNILLER DIF E K
BTA120 VSL LIARG KALRVI QGI T DAD VKTRL Q DMLSSCDYVV.SFTWYV...NFNPLIFKE DVFETR
BTA115 VSL LRARG EALRVI QDP T NES IKSRL Q DVYDRY DALVKREFK...RYFVDRFGLI DSVVD
BTA112 LEL VKTQKT ALEAI NNV HKPKER QNLRFNSKRNEY...PLH...LKGLFNES DFN V
BTA129 LEV VNIQKS FERVI KNV KDAT SRGKIQ NE LNERKNQY...QLH...LKGLFDS DNF DD

$\alpha 7$ $\eta 2$ $\alpha 8$ $\alpha 9$ $\eta 3$ $\eta 4$ $\alpha 10$
fBTA121 290 300 310 320
fBTA121 A...V VY YAP RFR KFK EIVKNPRV MDIY G WLDADD RATINEI GKI VINA...
BTA121 A...V VY YAP RFR KFK EIVKNPRV MDIY G WLDADD RATINEI GKI VINA...
BTA121-His A...V VY YAP RFR KFK EIVKNPRV MDIY G WLDADD RATINEI GKI VINA...
BTA116 A...V TRYAS KFS KFK EMVKNPRVM.DVYAWLDADDQATIDEI ENI VSD...
BTA123 S...V TY YVP KFS KFK EMVKHPRVI.DVYAWLDADD CVIIDEI EKI VINA...
BTA126 F...I AR CDS RFR KKL KEMVNSPRVK.DVYAWLDADD RVTIDDI GKI VTSG...
BTA127 A...V NRYTP KFS KFK EMVKNPRVK.DIYVWLDADQATIDEI GNI VLS T...
BTA119 A...V TRYTP KFS KFK EMVKNPRVM.DIYVWLDADQAI IDDM EKI VINA...
BTA118 M...I TRYTP KFS KFK EMVKNPRVM.DIYVWLDADQAI IDDM EKI VINA...
BTA117 L...I TRYTS KFS KFK EMVKNPRVM.DIYVWLDADQAI IDEI EKI VINA...
BTA122 L...I TRYTS KFS KFK EMVKNPRVK.DMYAWLDADQAAIDEI EKI VINA...
BTA124 T...V TRYAP KFR KFK EMVTNPRLM.DMYAWLDADE RATIDEI EKV VNF...
BTA120 I...I TRYTS KFR KFK EMVKNPRVM.DVYVWLDADQAAIDEI EKI VINA...
BTA115 T...V TSCTS KFR KFK EMVKSPRVM.DVY G WLDVDEQATINEI EKI VINA...
BTA112 VYSNVIGDN YVNEF TAIKEE I IQVIGDF DLYKGLSSREKSVVDY I IQSVVTNANFCYANND
BTA129 IYNEIIGDN YFSELI KFK DEI TKVEGL DVYT WLSDDQ I V IDK I RGI VTDPLIG.KYKG

$\alpha 11$
fBTA121 330 340 350 360 370 380
fBTA121 . . T Y D K D K F N N V L N S V G V Y Y V V R M I D I Y R G V K I E H D E A . . . L N A I T T V P D G V V K Q D L Q A
BTA121 . . T Y D K D K F N N V L N S V G V Y Y V V R M I D I Y R G V K I E H D E A . . . L N A I T T V P D G V V K Q D L Q A
BTA121-His . . T Y D K D K F N N V L N S V G V Y Y V V R M I D I Y R G V K I E H D E A . . . L N A I T T V P D G V V K Q D L Q A
BTA116 . . T Y D K D K F N S M L N S L D D Y R I V R I I D I Y R D I K I K Q E E A . . . L K A I E G V S D D A E K Q N L K T
BTA123 . . T Y D Q D R F N M M L N S L G D A N V I A I I K I Y R D I K I E Q G E A . . . L K A I D S I S D D I V K Q A Y Q D
BTA126 . . T Y D K D R F N M M L N L L E D P L V I K I V L C Q S I K K R Q E E A . . . L K A I E G V S D D A E K Q K L Q A
BTA127 . . T Y D K D K F N N V L N S V S D Y H V V K I I S F C R L V K K M Q E E A . . . L N A I E G V S D D A E K Q K L Q V
BTA119 . . T Y D K E K F N D K L N S L S D Y R V A E I V Y N Y R D I K N G R E E A . . . L K A I D G V S D D A E K Q N L K T
BTA118 . . T Y D K E K F N D K L N S L S D Y R V A E I V C Y Y R D F K N D Q E E A . . . L K A I E G V S D D A E K Q N L K T
BTA117 . . T Y D K D K F N K K L N S L S D Y H V A T I V Y N Y R D V K I K R E E A . . . L K A I D G V S D D A E K Q N L K T
BTA122 . . T Y D R D K F N K K L N S L S D Y Y V A A I V Y S Y R D I K N D Q E E A . . . L K A V E G V S D D T E K Q N L K A
BTA124 . . T Y T K E K F D D I L D N L S S G Y I V K I I E I Y E D I K I K Q E E V . . . S K A I A T V S D D F L R Q D L Q A
BTA120 . . T Y D K E K F N D K L N S L S D Y R V A K I V C Y Y R D F K N D Q E E A . . . L K A I E G V S D D A E K Q N L K T
BTA115 . . T Y D K D K F N K K L N S I D D F N I F E L I Q A C K K V L I E K K A . . . D E A I N G V S D D A E K Q R L R N
BTA112 C K T Y T L T E F Y A L L N S L G D A K I R E M I K V N L D V F K V R E E I . . . E A D I A G L T E E K V K Q A L K T
BTA129 Y R T Y T D I E F N N L L N E L G D N K V F A M I L V Y L E E Q S N Q D A A W L E A L E A I D S V D D A D F R E Q L R V

$\alpha 12$ $\alpha 13$
fBTA121 390 400 410 420 430 440
fBTA121 R L N R F K G E Y Y S N I R G T F K . G F T D G L H F Q I M T D G D K Y R N Y F I I L K F D A Q A A R V A K A R G A T G
BTA121 R L N R F K G E Y Y S N I R G T F K . G F T D G L H F Q I M T D G D K Y R N Y F I I L K F D A Q A A R V A K A R G A T G
BTA121-His R L N R F K G E Y Y S N I R G T F K . G F T D G L H F Q I M T D G D K Y R N Y F I I L K F D A Q A A R V A K A R G A T G
BTA116 R L E R L Q G E Y N S H I R D A F N . K S E G E L S F O L T N D S N K Y R R G F D V I K S N A K A V K A S G A A G G S
BTA123 R F N T L Q G E Y D S H I R D A F N . K A S G E L Y A Q I I G N G D K Y R N D F I G S R N N A R A A K F D E E A A A A
BTA126 R F N T L Q G E Y N S H I R S A F N . Q S S V D L Y F Q I T G N G D K Y R N D F N A I E K A K A A E A A Q G A A A A V
BTA127 I F N R L K D E Y N S H I R D T F N Q . S S D K F Y A F . F I K I I K Y S N G F S I L K E D A K K A V L R A A A A A K A
BTA119 R F N T L Q G E Y Y S S I R D A F N . K S A D D L Y A Q L S Y Y P G K Y S S G F P K I R D D A K R A A A A A K E A A
BTA118 R F N T L Q G E Y Y S S I R D A F N . K S A D D L Y A Q L N H Y P D E Y S S G F P K I R D D A K R A A A A A K E A A
BTA117 R F N T L Q G E Y Y S S I R D A F N N K S A D G L Y A Q L G R D L Y E Y S S G F P K I R D D A R R A A A A A K E A A
BTA122 R F N M L K D E Y Y S H I R D A F N . R S S D D L Y F O L N H Y S Y B Y S S G F F K I K E D A K K A A A A A A A K . .
BTA124 R L K R V K D E Y N A D I R D A F D . K P L V E L V R Q I L N N R D V Y L G R L N G I K A T V A V G K S E E E A . . .
BTA120 R F N T L Q G E Y Y S H I R D A F N . K S A D D L Y S Q L N R Y P D E Y S S G F S E I K E D A K K A A A A A A A E R A
BTA115 N F N E L K G M Y Y S H I R D A F A . L S I D E L C Y A I E H Y H N N Y A D F Y G I R V E T
BTA112 R L A S Y N N E Y A F I L R G A F N . A S G A D N I Y S R F M K I . N Y I N A Y I N L K K K I R D P . R V L D V Y T W L
BTA129 K I D D E R D N Y K L H F K E L E F N . E F L S E L V Y Q K F M K N I C Y Y D K F V L I K K T A Q H I T A F K K L Y K K L

fBTA121

```
fBTA121      SG.S.....
BTA121       SG.S.....
BTA121-His   SG.S.....
BTA116       .....
BTA123       KE.AKEAEEAAAA.....A..ERAKEAKEAEAAKAAEAAKRAKEAKEAEAAAAAKR
BTA126       KS.SGA.....
BTA127       SG.AAG.....GS.....
BTA119       .....
BTA118       .....
BTA117       ...K.....
BTA122       .....
BTA124       .....
BTA120       KE.AKE.....AAVAAE...
BTA115       .....
BTA112       SDEDKGVIDKIQGIVTDSNIGHAKGYKTYTDSEFNDLLNNLGASKTRSMIT.....AFLR
BTA129       SSVQKRGLDYIRIVVTNFNIGVL..HKTYNAIEFESLLGSLGATRVERIID.....FHLN
```

fBTA121

```
fBTA121      .....
BTA121       .....
BTA121-His   .....
BTA116       .....
BTA123       AKEAKEAEAAAAAKVEESTEAGEPREGSGTDEESGATGSGS.....
BTA126       .....AGGGS.....
BTA127       .....S.....
BTA119       .....AKAAKAEEGSGTDEESGAADGGS.....
BTA118       .....AKAAEAEEGSGTDEESGAADGGS.....
BTA117       .....EAAVAEEAGEPREGSGTDEESGAADGGS.....
BTA122       .....AEEGKTDEESGTADGGS.....
BTA124       .....DKAAAAAVKAEGSTEAGEPGEESGTVEESGATGGGS.....
BTA120       .....AEESTEAGEAEESTEAGEPREGSGTDEESGAADGGS.....
BTA115       .....
BTA112       ANVLREM....ALEAINTVPAGTAKQNFQ..NRFNALSNGYALRLKGLFNSLNHDMIYDS
BTA129       VLKAKKD....AQSAISSLPESMTRHNLQ..RLFDAHSNGYELHLKELFHINFPYQVYYS
```

fBTA121

```
fBTA121      .....
BTA121       .....
BTA121-His   .....
BTA116       .....
BTA123       .....
BTA126       .....
BTA127       .....
BTA119       .....
BTA118       .....
BTA117       .....
BTA122       .....
BTA124       .....
BTA120       .....
BTA115       .....
BTA112       VVNDEYSEKFLRIKDDIRHLSSNTADSTASSN
BTA129       VARSSYVDLYFEIINYVGKIQ.....
```