

# **Ubiquitylation of the Bardet-Biedl syndrome protein complex is essential for cilium assembly and signaling**

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Appendix Figures

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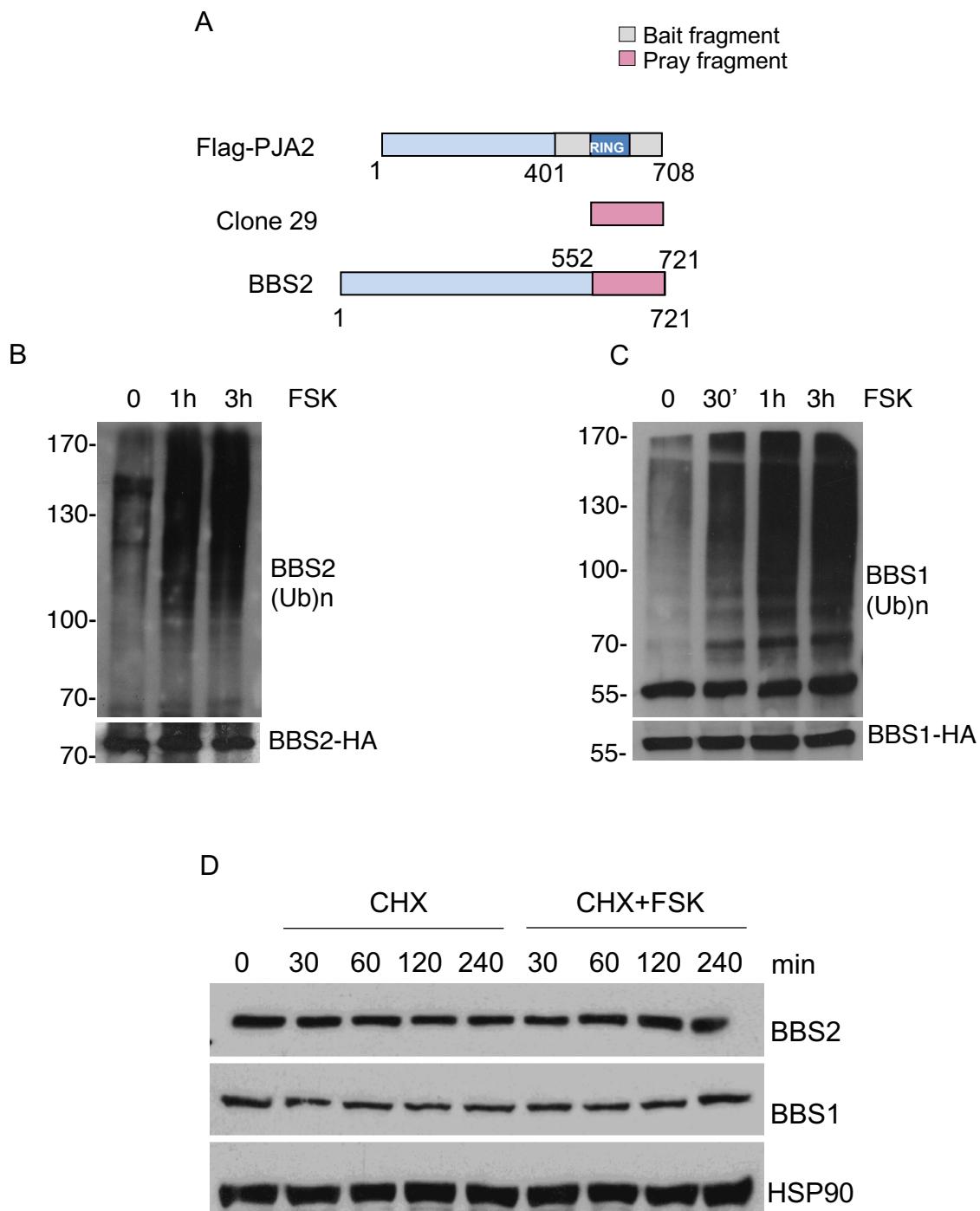
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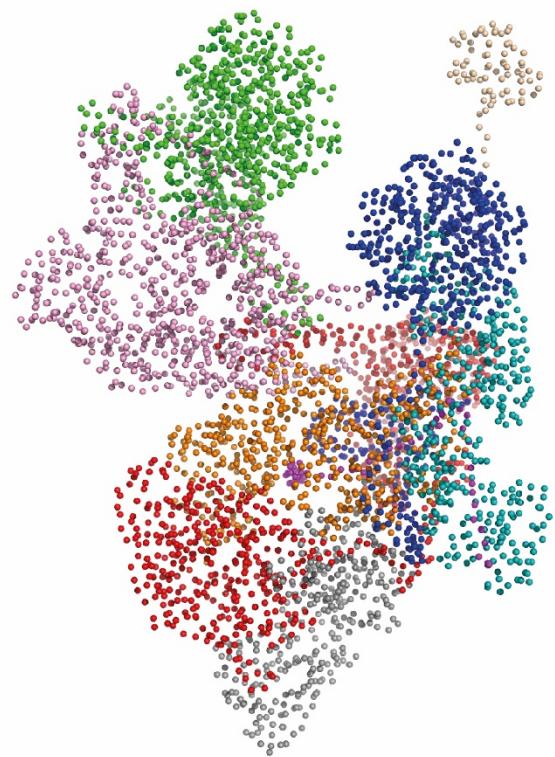
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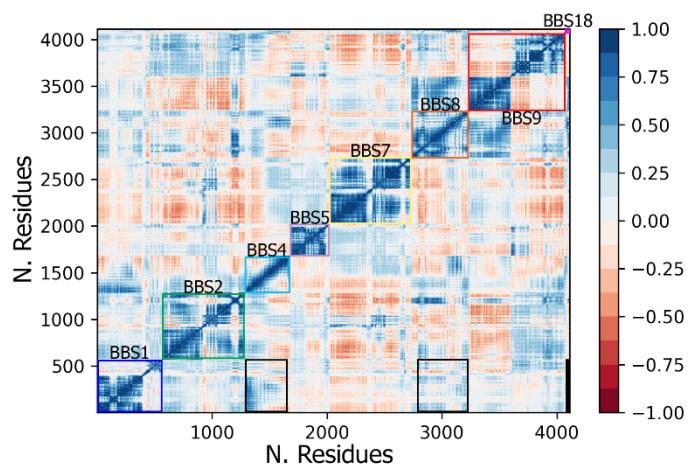


**Appendix Figure S1. A.** Schematic representation of human PJA2. The cysteine-rich region (RING) and the C-terminal rat clone 33 isolated by the yeast two-hybrid system are shown. Bait and pray fragment were indicated. **B,C** Immunoprecipitation of BBS2 (**B**) or BBS1 (**C**) from HEK293 cell lysates expressing HA-tagged BBS1 or BBS2 and ubiquitin-myc. Serum-deprived cells were left untreated or stimulated with FSK for 1 and 3 hours. Ubiquitinated BBS1 and BBS2 proteins and HA-tagged were detected. The precipitates were immunoblotted with anti-myc (ubiquitinated BBS1 or BBS2) and anti-HA antibodies. **D.** HEK293 cells were serum deprived for 24h, pretreated with cycloheximide (10 μM) and stimulated with foscarnet (40 μM) at indicated time. BBS1 and BBS2 protein levels were detected with endogenous antibody as indicated.

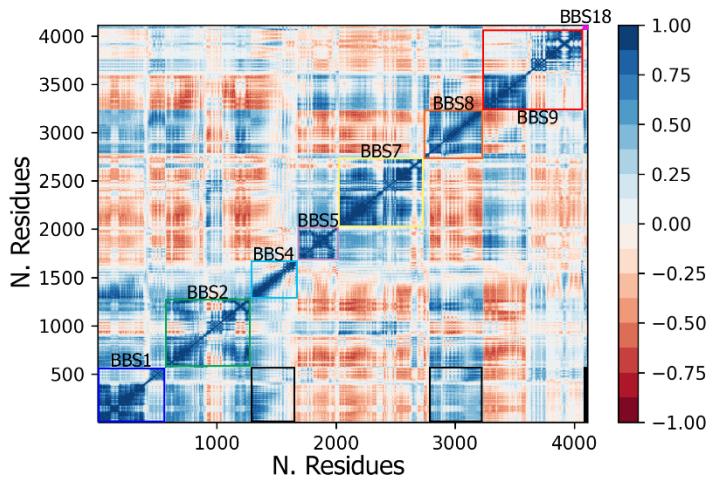


**Appendix Figure S2:** Coarse –Grained model of the Ub-hBBSome in an open conformation. Beads (BB) are shown as spheres.

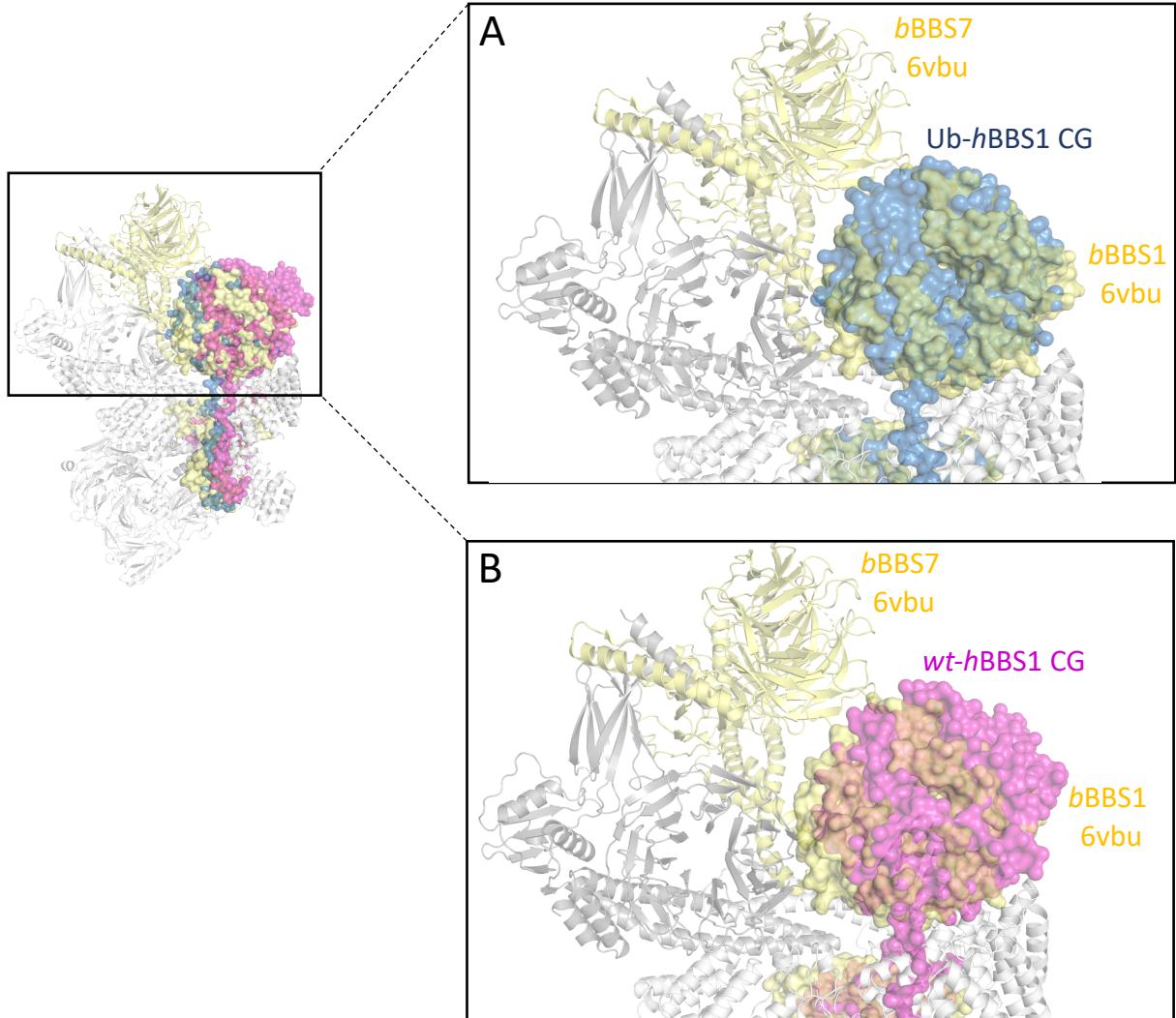
A



B



**Appendix Figure S3:** Atomic position Pearson Correlation Coefficient (aPCC) matrices calculated on **A** wt-hBBSome and **B** Ub-hBBSome after 5 $\mu$ s of CG-MD. Residues of each subunit are numbered as follows: BBS1 (S2-S568, blue rectangle), BBS2 (M570-V1285, green rectangle), BBS4 (F1288-L1683, cyan rectangle), BBS5 (M1685-S2025, light pink rectangle), BBS7 (M2026-A2740, yellow rectangle), BBS8 (M2742-F3239, orange rectangle), BBS9 (S3242-S4068, red rectangle), BBS18 (M4071-Q4122, purple rectangle). In particular, BBS18 presents a relatively high correlation ( $> 0.5$ ) with BBS4 and BBS8, which is observed only in the Ub-hBBSome model. BBS18 also has high aPCC values with BBS1 and BBS2, which correlate with each other, too. BBS1 and BBS2 anticorrelate with BBS7 (aPCC  $< -0.5$ ). The black rectangles highlight the zones showing high correlation between BBS1, BBS4, BBS8 and BBS18 in Ub-hBBSome. The same areas are also highlighted in wt-hBBSome for the sake of comparison.



**Appendix Figure S4:** Superimposition between the bovine *bBBS1* subunit of the apo (closed) form of the Cryo-EM *bBBSome* structure (PDB ID: 6vbu) (light-yellow surface) and the last CG-MD frame of: **A** CG model of the K143 Ubiquitinated *hBBS1* (Ub-*hBBS1* CG) (light-blue surface) and **B** CG model of the wild-type *hBBS1* (wt-*hBBS1* CG) (light-magenta surface). After 5  $\mu$ s of CG-MD, a more closed conformation of the Ub-*hBBS1* CG can be observed, compared to the wt-*hBBS1* CG.

A

		2		41
hBBS1	-----	SKWLDAHYDPMANIHTFSACLA LADLHGDG EYKLVVGDLGP		
6vbu_BBS1	MAATSSSDGGKGESEAN SKWLDSLS DLSMANIHTFSACLA LADLHGDG EYKLAMGDLGP	20		60
		61	82	103
hBBS1	GGQQPRLKVLGPLVMTE SPLPA AAAATFLMEQHEP RTPALASG PCVYVYKNL R PY			
6vbu_BBS1	DGRQPRLKVLKGHTLV SQKPLPD AAVTFLMASHEP RTPALIASG PCVYVYKNL PY	80	100	121
		124	142	162
hBBS1	FKFSLPQLPPNP LEQD LNQAKEDR IDPLTL KEMLESIRE TAEEPLSI QSLRFL QLESE			
6vbu_BBS1	FKFSLP LPTN PLEQD LNQAKED QIDPLTL KEMLEG IREKA EVPLSV QSLRFL PLESE	143	160	180
		182	202	222
hBBS1	MEAFVNQHK NSIKRQTV ITMTLKK NLAD EAVSCL VLGTEN KELL LVDPEA FTILAK			
6vbu_BBS1	MEAFVNQHK KSIRRQTV ITMTLKK NLAD EAVSCL VLGTEN KELL LVDPEA FTILAK	200	220	240
		242	262	282
hBBS1	MSLPSVP VFL EVSGQFD VEFRL AACRN GNIYIL RRDSK HPKYCIEL SAQPV GLIRV HVK			
6vbu_BBS1	MSLPSVP AF LEASGQFD VEFRL AACRN GSIYIL RRDSK HPKYCIEL GAQPV GLVG HVK	260	280	300
		302	322	342
hBBS1	L VVGST QDSLHGF THKGK LLWTV QMPAA ILT TMNL LEQHSR GLQAVM AGLANGE EVRIY RDK			
6vbu_BBS1	L VVGS NQDSLHGF TYKGK RLWTV QMPAA ILAM NLLEQHSR GLQAVM AALANEE EVRIY HDK	320	340	360
		362	382	402
hBBS1	ALLNVI HTPDAV TSLCF GFRY GREDNT LIMTT TRGG GLI KILK RTAVF VEGG SEVG PPPAQ			
6vbu_BBS1	VLLN VIRT PEAV TSLCF GFRY GREDNT LIMTT LGG GLI KILK RTAVF AEGG GEAG PPPSQ	380	400	420
		422	442	462
hBBS1	AMKLNV PRKTRLY VDQTL REREAG TAMHRA FQT DLY LLRL RAARAY LQA LESS LSP LT			
6vbu_BBS1	AIKLN VPRKTRLY VDQTL REREAG TAMHRT FQAD LY LLRL RAARAY VQA LESS LSP VLT	440	460	480
		482	502	522
hBBS1	AREPLKL HAVVQGL GPTFK LTLH LQNT STR PVL G L V C FLY NE AL Y S L P R A F F K V P L V			
6vbu_BBS1	AREPLKL HAVVQGL GPTFK LTLH LQNT STAR PIL GL V C FLY NE LY A L P R A F F K V P L V	500	520	540
		542	562	568
hBBS1	PGLN YPLETF VESLS NKG ISDI KVL LREG QS AP L SAH VN MP GS -----			
6vbu_BBS1	PGLN YPLETF VK SLSDK GISDI KVL LREG QST PL SAH IN MP MSEG LAAD	560	580	592

B

	570	589	609	629
hBBS2	MLLPVFTL KLRHK ISPRMVA I	GRYDGTH PCLA AAATQ	TGKVFI HNPH TRNQH VSASR VQS	
6vbu_BBS2	MLQPVFTL KLRHK ISPRMVA AV	GRYDGTH PCLA AAATQ	AGKVF IHNP HSRSQ HLGAP RVLQS	1
		20	40	60
		649	669	689
hBBS2	PLESDV SLLN INQAV SCLTAG VLNPE LGYD ALLV GTQTN LLAYD V YNSD LFY REVAD GA			
6vbu_BBS2	PLESDV SLLN INQTV SCLTAG VLNPE LGYD ALLV GTQTN LLAYD V YNSD LFY REVAD GA	80	100	120
		709	729	749
hBBS2	NAIVL GTLGDI SSPLAI GGNCAL QGFN HEG SDLF W VTG DN NS L S A L C D F D G K K E L L			
6vbu_BBS2	SAIVL GTLGDT TSPLAI GGNCAL QGFN HEG NDLF W VTG DN H S A L C D F D G K K E L L	140	160	180
		769	789	809
hBBS2	VGSEDF DIRV FKEDE I VA M SET E T E I V T S L C P M Y G S R F G Y A L S N G T V G V Y D K T A R Y W R I K S			
6vbu_BBS2	VGSEDF DIRV FKEDE I VA M SET E T E I V T S L C P M Y G S R F G Y A L S N G T V G V Y D K T A R Y W R I K S	200	220	240
		829	849	869
hBBS2	KNHAM SIHAF DLNSD GV EL IT GWS NGK VDAR SDRT GE V IF KD NF SSAI AGV VEG DYR M D			
6vbu_BBS2	KNQAM SIHAF DLNSD GV EL IT GWS NGK VDAR SDRT GE V IF KD NF SSAI AGV VEG DYR M D	260	280	300
		889	909	929
hBBS2	GHIQL ICCS VDGE EIRG YLP GTA EMRG NLMD TSAEQ DLIRE LSQKK QNL LELR NYE ENAK ...			
6vbu_BBS2	GCGQL ICCS VDGE EIRG YLP GTA EMRG NLMD ISVE QD LIRE LSQKK QNL LELR NYE ENAK ...	320	340	360

hBBS2	930	...AELASPLNEADGHRG <b>I</b> IPANTRLHTT <b>L</b> SVSLGNETQTAH <b>E</b> RLISTSND <b>T</b> IIRAVL <b>F</b> AE	949	969	969
6vbu_BBS2	361	...AEL <b>S</b> PLNEADGHRG <b>V</b> IPANTRKH <b>T</b> ALSVLG <b>S</b> EA <b>Q</b> AA <b>H</b> AE <b>C</b> ISTSND <b>T</b> IIRAVL <b>F</b> AE	380	400	420
hBBS2	989	G <b>I</b> FTGESHVVP <i>H</i> <b>N</b> LSS <b>S</b> <b>I</b> C <b>I</b> PIVPPKD <b>V</b> PVDL <b>H</b> L <b>K</b> A <b>F</b> V <b>G</b> Y <b>R</b> <b>S</b> <b>S</b> TQF <b>H</b> V <b>F</b> <b>E</b> <b>S</b> TRQLPR	1029	1049	
6vbu_BBS2	440	GV <b>F</b> AGESHVVP <i>H</i> <b>S</b> <b>S</b> <b>S</b> <b>V</b> R <b>I</b> P <b>T</b> PPKD <b>I</b> PV <b>D</b> L <b>H</b> L <b>K</b> <b>T</b> <b>F</b> V <b>G</b> Y <b>R</b> <b>S</b> <b>S</b> TQF <b>H</b> V <b>F</b> <b>E</b> <b>L</b> TRQLPR	460	480	
hBBS2	1069	FSMYALTSLDPASEP <b>I</b> SYVN <b>F</b> <b>I</b> TIAERAQRVV <b>V</b> WL <b>G</b> Q <b>N</b> F <b>L</b> LPED <b>H</b> IQ <b>N</b> APF <b>Q</b> VCFTSLRN	1089	1109	
6vbu_BBS2	500	FSMYALTSPD <b>P</b> ASEP <b>I</b> SYVN <b>F</b> <b>I</b> TIAERAQRVV <b>V</b> WL <b>G</b> Q <b>N</b> F <b>L</b> LPED <b>T</b> NI <b>Q</b> APF <b>Q</b> VCFTSLRN	520	540	
hBBS2	1129	GG <b>H</b> L <b>H</b> IKIKL <b>S</b> GEIT <b>I</b> NT <b>D</b> D <b>I</b> LAG <b>D</b> I <b>I</b> QS <b>M</b> AS <b>F</b> FA <b>E</b> DL <b>Q</b> VE <b>A</b> DF <b>P</b> V <b>Y</b> FE <b>E</b> LR <b>K</b> V <b>L</b> V <b>K</b>	1149	1169	
6vbu_BBS2	560	GG <b>Q</b> <b>Y</b> IKIKL <b>S</b> GEIT <b>V</b> NT <b>D</b> D <b>I</b> LAG <b>D</b> I <b>I</b> QS <b>M</b> AS <b>F</b> FA <b>E</b> DL <b>Q</b> VE <b>A</b> DF <b>P</b> V <b>Y</b> FE <b>E</b> LR <b>K</b> V <b>L</b> V <b>K</b>	580	600	
hBBS2	1189	DEYHSVHQ <b>K</b> L <b>S</b> ADM <b>D</b> H <b>S</b> N <b>I</b> R <b>S</b> L <b>L</b> <b>V</b> GA <b>E</b> DA <b>R</b> L <b>M</b> R <b>D</b> <b>K</b> T <b>M</b> <b>K</b> <b>S</b> RY <b>M</b> E <b>L</b> Y <b>D</b> L <b>N</b> R <b>D</b> <b>L</b> LNG <b>Y</b> <b>K</b>	1209	1229	
6vbu_BBS2	620	DEYHSVHQ <b>K</b> L <b>S</b> ADM <b>D</b> <b>N</b> <b>S</b> N <b>I</b> R <b>S</b> L <b>L</b> <b>V</b> QA <b>E</b> DA <b>R</b> L <b>M</b> R <b>D</b> <b>K</b> T <b>M</b> <b>K</b> <b>N</b> RY <b>K</b> E <b>L</b> Y <b>D</b> L <b>N</b> K <b>D</b> <b>L</b> LNG <b>Y</b> <b>K</b>	640	660	
hBBS2	1249	RCNNHTEL <b>L</b> <b>G</b> N <b>L</b> KAVN <b>Q</b> A <b>I</b> Q <b>R</b> A <b>G</b> <b>R</b> <b>L</b> RV <b>G</b> K <b>P</b> K <b>N</b> Q <b>V</b> ITACR <b>D</b> A <b>I</b> R <b>S</b> NN <b>I</b> N <b>T</b> <b>L</b> <b>F</b> <b>K</b> <b>I</b> <b>M</b> <b>R</b> <b>V</b> <b>G</b> ---	1269	1286	
6vbu_BBS2	680	RCNNHTEL <b>L</b> <b>G</b> <b>S</b> LKAVN <b>Q</b> A <b>I</b> Q <b>R</b> A <b>G</b> <b>H</b> <b>L</b> RV <b>G</b> K <b>P</b> K <b>N</b> Q <b>V</b> ITACR <b>D</b> A <b>I</b> R <b>S</b> NN <b>I</b> N <b>M</b> <b>L</b> <b>F</b> <b>I</b> <b>M</b> <b>R</b> <b>V</b> <b>G</b> TASS	700	717	721

C

hBBS4	1288	-F <b>P</b> ILE <b>K</b> QN <b>W</b> LI <b>H</b> L <b>H</b> <b>I</b> Y <b>R</b> K <b>D</b> <b>Y</b> E <b>A</b> CK <b>A</b> VI <b>K</b> E <b>Q</b> <b>L</b> <b>Q</b>	1319		
6vbu_BBS4	29	MAEE <b>K</b> LSART <b>Q</b> LP <b>V</b> SA <b>E</b> SQ <b>K</b> P <b>V</b> L <b>K</b> KA <b>P</b> E <b>F</b> ILE <b>K</b> QN <b>W</b> LI <b>H</b> <b>Y</b> <b>I</b> Q <b>K</b> D <b>Y</b> E <b>A</b> CK <b>A</b> VI <b>K</b> E <b>Q</b> <b>L</b> <b>Q</b>	60		
hBBS4	1339	ET <b>Q</b> GLCEY <b>A</b> I <b>Y</b> V <b>Q</b> AL <b>I</b> F <b>R</b> LEG <b>N</b> Q <b>E</b> <b>S</b> <b>L</b> E <b>F</b> QT <b>C</b> AV <b>L</b> <b>S</b> <b>P</b> <b>Q</b> <b>S</b> <b>A</b> <b>D</b> <b>N</b> <b>L</b> <b>K</b> Q <b>V</b> <b>A</b> <b>R</b> <b>S</b> <b>L</b> <b>F</b> <b>L</b> <b>G</b> <b>K</b> <b>H</b> <b>K</b> <b>A</b>	1359	1379	
6vbu_BBS4	80	ET <b>H</b> GLCEY <b>A</b> I <b>Y</b> V <b>Q</b> AL <b>I</b> F <b>R</b> LEG <b>N</b> Q <b>E</b> <b>S</b> <b>L</b> R <b>F</b> QM <b>C</b> AF <b>L</b> <b>S</b> <b>P</b> <b>Q</b> <b>C</b> <b>A</b> <b>D</b> <b>N</b> <b>L</b> <b>K</b> Q <b>V</b> <b>A</b> <b>R</b> <b>S</b> <b>L</b> <b>F</b> <b>L</b> <b>G</b> <b>K</b> <b>H</b> <b>K</b> <b>A</b>	100	120	
hBBS4	1399	IEV <b>Y</b> NE <b>A</b> AK <b>L</b> <b>N</b> Q <b>K</b> D <b>W</b> E <b>I</b> SH <b>N</b> LG <b>C</b> Y <b>I</b> Y <b>L</b> <b>K</b> <b>Q</b> <b>F</b> <b>N</b> <b>K</b> A <b>Q</b> D <b>Q</b> <b>L</b> <b>H</b> <b>N</b> <b>A</b> <b>L</b> <b>N</b> <b>L</b> <b>N</b> R <b>H</b> <b>D</b> <b>L</b> <b>T</b> <b>Y</b> <b>I</b> <b>M</b> <b>L</b> <b>G</b> <b>K</b> <b>I</b> <b>H</b> <b>L</b>	1419	1439	
6vbu_BBS4	140	IEV <b>Y</b> NE <b>A</b> AK <b>L</b> <b>N</b> Q <b>K</b> D <b>W</b> E <b>I</b> CH <b>N</b> LG <b>C</b> Y <b>I</b> Y <b>L</b> <b>K</b> <b>Q</b> <b>F</b> <b>D</b> <b>K</b> A <b>Q</b> D <b>Q</b> <b>L</b> <b>H</b> <b>N</b> <b>A</b> <b>L</b> <b>N</b> <b>L</b> <b>N</b> R <b>H</b> <b>D</b> <b>L</b> <b>T</b> <b>Y</b> <b>I</b> <b>M</b> <b>L</b> <b>G</b> <b>K</b> <b>I</b> <b>F</b> <b>L</b> <b>L</b>	160	180	
hBBS4	1459	EG <b>D</b> LD <b>K</b> A <b>I</b> E <b>V</b> Y <b>K</b> K <b>A</b> E <b>F</b> SP <b>E</b> NT <b>E</b> LL <b>T</b> <b>L</b> <b>G</b> <b>L</b> <b>L</b> <b>L</b> <b>L</b> <b>Q</b> <b>L</b> <b>G</b> <b>I</b> <b>Y</b> <b>Q</b> <b>K</b> A <b>F</b> E <b>H</b> <b>L</b> <b>G</b> <b>N</b> <b>A</b> <b>L</b> <b>T</b> <b>D</b> <b>P</b> <b>T</b> <b>N</b> <b>Y</b> <b>K</b> <b>A</b> <b>I</b> <b>L</b>	1479	1499	
6vbu_BBS4	200	KG <b>D</b> LD <b>K</b> A <b>I</b> E <b>V</b> Y <b>K</b> K <b>A</b> E <b>F</b> SP <b>E</b> NT <b>E</b> LL <b>T</b> <b>L</b> <b>G</b> <b>L</b> <b>L</b> <b>L</b> <b>Q</b> <b>L</b> <b>G</b> <b>I</b> <b>Y</b> <b>Q</b> <b>K</b> A <b>F</b> E <b>H</b> <b>L</b> <b>G</b> <b>N</b> <b>T</b> <b>L</b> <b>D</b> <b>P</b> <b>T</b> <b>N</b> <b>Y</b> <b>K</b> <b>A</b> <b>I</b> <b>L</b>	220	240	
hBBS4	1519	AAG <b>S</b> MM <b>Q</b> TH <b>G</b> DF <b>D</b> VAL <b>T</b> <b>K</b> <b>Y</b> <b>R</b> V <b>V</b> AC <b>V</b> P <b>E</b> <b>S</b> <b>P</b> <b>PL</b> W <b>N</b> N <b>I</b> G <b>C</b> FF <b>G</b> KK <b>K</b> <b>Y</b> <b>V</b> <b>A</b> <b>I</b> <b>S</b> <b>C</b> <b>L</b> <b>K</b> <b>R</b> <b>A</b>	1539	1559	
6vbu_BBS4	260	AAG <b>S</b> MM <b>Q</b> TH <b>G</b> DF <b>D</b> VAL <b>T</b> <b>K</b> <b>Y</b> <b>K</b> V <b>V</b> AC <b>V</b> I <b>E</b> <b>S</b> <b>P</b> <b>PL</b> W <b>N</b> N <b>I</b> G <b>C</b> FF <b>G</b> KK <b>K</b> <b>Y</b> <b>V</b> <b>A</b> <b>I</b> <b>S</b> <b>C</b> <b>L</b> <b>K</b> <b>R</b> <b>A</b>	280	300	
hBBS4	1579	PF <b>D</b> WK <b>I</b> L <b>Y</b> <b>N</b> LG <b>V</b> <b>H</b> <b>L</b> <b>T</b> <b>M</b> <b>Q</b> <b>Y</b> <b>A</b> <b>S</b> <b>F</b> <b>H</b> <b>L</b> <b>S</b> <b>A</b> <b>I</b> <b>N</b> <b>F</b> <b>Q</b> <b>P</b> <b>K</b> <b>M</b> <b>E</b> <b>L</b> <b>Y</b> <b>M</b> <b>L</b> <b>A</b> <b>V</b> <b>A</b> <b>L</b> <b>T</b> <b>N</b> <b>L</b> <b>E</b> <b>I</b> <b>D</b> <b>E</b> <b>N</b> <b>A</b> <b>R</b> <b>A</b>	1599	1619	
6vbu_BBS4	320	PL <b>D</b> WK <b>I</b> L <b>Y</b> <b>N</b> LG <b>V</b> <b>H</b> <b>L</b> <b>T</b> <b>M</b> <b>Q</b> <b>Y</b> <b>A</b> <b>S</b> <b>F</b> <b>H</b> <b>L</b> <b>S</b> <b>A</b> <b>I</b> <b>N</b> <b>F</b> <b>Q</b> <b>P</b> <b>K</b> <b>M</b> <b>E</b> <b>L</b> <b>Y</b> <b>M</b> <b>L</b> <b>A</b> <b>V</b> <b>A</b> <b>L</b> <b>T</b> <b>N</b> <b>L</b> <b>E</b> <b>D</b> <b>S</b> <b>E</b> <b>N</b> <b>A</b> <b>R</b> <b>A</b>	340	360	
hBBS4	1639	YAE <b>A</b> V <b>H</b> LD <b>K</b> C <b>N</b> P <b>L</b> V <b>N</b> L <b>N</b> Y <b>A</b> V <b>L</b> <b>L</b> <b>Y</b> <b>N</b> Q <b>G</b> E <b>K</b> <b>N</b> <b>A</b> <b>L</b> <b>Q</b> <b>Y</b> <b>Q</b> <b>E</b> <b>M</b> <b>E</b> <b>K</b> <b>V</b> <b>N</b> <b>L</b> <b>K</b> <b>Y</b> <b>S</b> <b>S</b> <b>L</b> <b>E</b> <b>F</b> <b>D</b> <b>P</b> <b>E</b> <b>M</b> <b>V</b> <b>E</b>	1659	1679	
6vbu_BBS4	380	YEE <b>A</b> V <b>R</b> LD <b>K</b> C <b>N</b> P <b>L</b> V <b>N</b> L <b>N</b> Y <b>A</b> V <b>L</b> <b>L</b> <b>Y</b> <b>N</b> Q <b>G</b> E <b>K</b> <b>R</b> <b>D</b> <b>A</b> <b>L</b> <b>Q</b> <b>Y</b> <b>Q</b> <b>E</b> <b>M</b> <b>E</b> <b>K</b> <b>V</b> <b>N</b> <b>L</b> <b>K</b> <b>Y</b> <b>S</b> <b>S</b> <b>L</b> <b>E</b> <b>F</b> <b>D</b> <b>P</b> <b>E</b> <b>M</b> <b>V</b> <b>E</b>	400	420	
hBBS4	1683	AQ <b>K</b> L-----			
6vbu_BBS4	424	AQ <b>K</b> L <b>G</b> A <b>A</b> L <b>Q</b> V <b>G</b> E <b>A</b> L <b>V</b> W <b>T</b> P <b>V</b> K <b>D</b> P <b>K</b> S <b>H</b> <b>Q</b> <b>T</b> <b>A</b> <b>S</b> <b>K</b> A <b>G</b> F <b>Q</b> <b>Q</b> <b>P</b> <b>L</b> <b>G</b> <b>S</b> <b>N</b> <b>Q</b> <b>A</b> <b>G</b> <b>S</b> <b>A</b> <b>T</b> <b>C</b> <b>R</b> <b>K</b>			
hBBS4		-----LSS <b>G</b> AG <b>G</b> TSQL <b>K</b> PP <b>S</b> L <b>P</b> E <b>P</b> T <b>V</b> EA <b>Q</b> P <b>T</b> EA <b>S</b> A <b>Q</b> T <b>R</b> <b>E</b> <b>K</b>			
6vbu_BBS4					

D

6VBU_5	MSVLDALWEDRDVRFDVSSQQMKTRPGEVLIDCLDSV	20	EDTKGNNGDRGRLLVTNLRI	40	WH
hBBS5	MSVLDALWEDRDVRFDLSAQQMKTTRPGEVLIDCLDSI	1704	EDTKGNNGDRGRLLVTNLRI	1724	WH
6VBU_5	SLALPRVNLSIGYNCILNITRTANSKLRGQTEALYV	80	LTKCNSTRFEFIFTNLVPGSPRL	100	120
hBBS5	SLALSRVNVSGYNCILNITRTANSKLRGQTEALYI	1764	LTKCNSTRFEFIFTNLVPGSPRL	1784	1804
6VBU_5	YTSLLIAVHRAYETSKMYRDFKLRSLALIQNKQLRLLPQE	140	NVVKINGVWNLSSDQGNLGTF	160	180
hBBS5	FTSVMMAVHRAYETSKMYRDFKLRSLALIQNKQLRLLPQE	1824	HVYD KINGVWNLSSDQGNLGTF	1844	1864
6VBU_5	FITNVRIWCHANMNDSFNVSIPIYLQIRSVKIRDSDKFG	200	GLALVIESSQQSGGYVLGFKIDPV	220	240
hBBS5	FITNVRIWCHANMNDSFNVSIPIYLQIRSTKIRDSDKFG	1884	GLALVIESSQQSGGYVLGFKIDPV	1904	1924
6VBU_5	EKLQESVKEINSLHKVYSANPIFGVDYEMEEKPQPLEALT	260	VKQIQDDVEIDSDDHTDAFV	280	300
hBBS5	EKLQESVKEINSLHKVYSAPIFGVD-----	1944	-----YTDAFV	1984	
6VBU_5	AYFADGNKQQDREPVFSEELGLAIEKLKDGT	320	LQGLWEVMN	341	
hBBS5	AYFADGNKQQDREPVFSEELGLAIEKLKDGT	2004	LQGLWEVS-	2025	

E

6VBU_7	MDLNLRADYLQVGVTSQ	20	KMKLIPASKHRATQKV	40	GDHDGIVMCFGMKKGEAVTVFKT	60
hBBS7	MDLIINRMDYLOVGVTSOK	2045	MKLIPASRHRATOKV	2065	GDHDGIVMCFGMKKGEAAAVFKT	2085
6VBU_7	LPGQKIA	80	RELGGALNTPQE	100	KIFIAAGSEIRGFTKRGKF	120
hBBS7	LPGPKIA	2105	RELGGVINTPQE	2125	KIFIAASEIRGFTKRGKF	2145
6VBU_7	LFLSASYIYNH	140	YCDCKDQH	160	YLSGDKINDVICL	180
hBBS7	LFLSASYIYNH	2165	YCDCKDQHY	2185	LPVERLLREV	2205
6VBU_7	TYEIEVPGPPTVLA	200	LHNGNGDGEDLL	220	HPGKLIQITTSKPIHKWEIRNEKKRG	240
hBBS7	MYAVEVPGPPTVLA	2225	LHNGNGDGEDLL	2245	HPGKLIQITTSKPVRKWEIQNEKKRG	2265
6VBU_7	ILCVDSFDIVGDGV	260	KDLLVGRDDGMVEVY	280	GFDNANEPEVLRFDH	300
hBBS7	ILCIDSFDIVGDGV	2285	KDLLVGRDDGMVEVY	2305	HTLSESVTSIQGGCVKD	2325
6VBU_7	GYDEIVVSTYSGWIT	320	TGLTTEPVHKESGPGEELKF	340	QFMQNKESSLRSELEQLQYKV	360
hBBS7	SYDEIVVSTYSGWIT	2345	TGLTTEPVHKESGPGEELKF	2365	QFMQNKESSLRNELEHLQYKV	2385
6VBU_7	EKYQQSSQSSAKSAPSF	380	VNDKFTLNKDDASYSLILEV	400	QTAIDNVLIQSDVPIDL	420
hBBS7	ENYQQSSQSSAKSAPSF	2405	VNDKFTLNKDDASYSLILEV	2425	QTAIDNVLIQSDVPIDL	2445
6VBU_7	DKNSAVVSFSSCDSE	440	NFLLATYRCQANTTR	460	LELKIRSIEGQYGT	480
hBBS7	DKNSAVVSFSSCDSE	2465	NFLLATYRCQADTR	2485	LELKIRSIEGQYGT	2505
6VBU_7	CQRQYHIKPLSLH	500	QRTFHIDHDPMTNTLTGQFS	520	LSFELHSWVFC	540
hBBS7	CQRQYHIKPLSLH	2525	QRTFHIDHDPMTNTLTGQFS	2545	AEVHSWVFC	2565
6VBU_7	VTFYFQNTFLDTQLE	560	ESTYRKGEGVFKSDN	580	IISTISILKDVLSKEATKR	600
hBBS7	VTFYFQNTFLDTQLE	2585	ESTYRKGEGVFKSDN	2605	IISTISILKDVLSKEATKR	2625

6VBU_7 hBBS7	<b>620</b> <b>SVKHTLKLHPKLEYQLLLAKKVQLIDALKELQVHEGNTNLIPEYRCILEEADHLQEY</b> <b>2645</b> <b>SVKHTLKLHPKLEYQLLLAKKVQLIDALKELQIHEGNTNLIPEYHCILEEADHLQEY</b> <b>2665</b> <b>2685</b>	<b>640</b> <b>660</b>
6VBU_7 hBBS7	<b>680</b> <b>KKQPAHLERLYGMITDLFIDKFKFGTNVKVPLLEILDSYDQNALIAFFDA</b> <b>2705</b> <b>KKQPAHLERLYGMITDLFIDKFKFGTNVKVPLLEILDSYDQNALISFFDA</b> <b>2725</b> <b>2740</b>	<b>700</b> <b>715</b>

F

6VBU_BBS8 hBBS8	<b>20</b> <b>MEPLL LAWSYFRRRFQLCADLCTQMЛЕKSPCDQAAWILKARALTEMVVDEIDVDEEGI</b> <b>2760</b> <b>MEPLL LAWSYFRRRKFOQLCADLCTQMЛЕKSPYDQAAWILKARALTEMVYIDEIDVDQEGI</b> <b>2780</b> <b>2800</b>	<b>40</b> <b>60</b>
6VBU_BBS8 hBBS8	<b>80</b> <b>AEMILDENAIAQVPRPGTSKLKPGTNQTGGPSPAVRPVQTQAGRPITGFLRPSTQSGRP GT</b> <b>2820</b> <b>AEMMLDENAIAQVPRPGTSKLKPGTNQTGGPSQAVRPQTQAGRPITGFLRPSTQSGRP GT</b> <b>2840</b> <b>2860</b>	<b>100</b> <b>120</b>
6VBU_BBS8 hBBS8	<b>140</b> <b>IEQAIKTPRTAYTARPPISSSGRFVRLGTASMLTSPDGPFINLSRLNLAKYAQKPKLAKA</b> <b>2880</b> <b>MEQAIRTPRTAYTARPITSSSGRFVRLGTASMLTSPDGPFINLSRLNLTKYSQPKLAKA</b> <b>2900</b> <b>2920</b>	<b>160</b> <b>180</b>
6VBU_BBS8 hBBS8	<b>200</b> <b>LFEYIFHHENDVKTALDLAALSTEHSQYKDWWWKVQIGKCYYRLGLYREAEKQFKSALKQ</b> <b>2940</b> <b>LFEYIFHHENDVKTALDLAALSTEHSQYKDWWWKVQIGKCYYRLGMYREAEKQFKSALKQ</b> <b>2960</b> <b>2980</b>	<b>220</b> <b>240</b>
6VBU_BBS8 hBBS8	<b>260</b> <b>QEMVDTFLYLAKVYISLDQPLTALNLFQGLDKFPGEVTLLCGIARIYEEMNNISSATEY</b> <b>3000</b> <b>QEMVDTFLYLAKVYVSLDQPVTAALNLFQGLDKFPGEVTLLCGIARIYEEMNNMSSAAEY</b> <b>3020</b> <b>3040</b>	<b>280</b> <b>300</b>
6VBU_BBS8 hBBS8	<b>320</b> <b>YKEVLKQDNTHVEAIACIGSNHFYTQDPEVALRFYRLLQMGVYNQCLFNNLGLCCFYAQ</b> <b>3060</b> <b>YKEVLKQDNTHVEAIACIGSNHFYSDQPEIALRFYRLLQMGVYNQCLFNNLGLCCFYAQ</b> <b>3080</b> <b>3100</b>	<b>340</b> <b>360</b>
6VBU_BBS8 hBBS8	<b>380</b> <b>QYDMTLTSFERALS LAENEVEVADWVYNLGHVAVGTGDTNLAHQCFRLALVSNNQHAEAY</b> <b>3120</b> <b>QYDMTLTSFERALS LAENEVEAADWVYNLGHVAVGIGDTNLAHQCFRLALVNNNNHAEAY</b> <b>3140</b> <b>3160</b>	<b>400</b> <b>420</b>
6VBU_BBS8 hBBS8	<b>440</b> <b>NNLAVLEMRRGHVEQAKALLQTASSLAPHMYEPHFNFATISDKIGDLQRSYAAAKSEAA</b> <b>3180</b> <b>NNLAVLEMRKGHVEQARALLQTASSLAPHMYEPHFNFATISDKIGDLQRSYVAAQKSEAA</b> <b>3200</b> <b>3220</b>	<b>460</b> <b>480</b>
6VBU_BBS8 hBBS8	<b>500</b> <b>FPDHVDTQHLIKQLEQHFA</b> <b>3240</b>	<b>500</b>

G

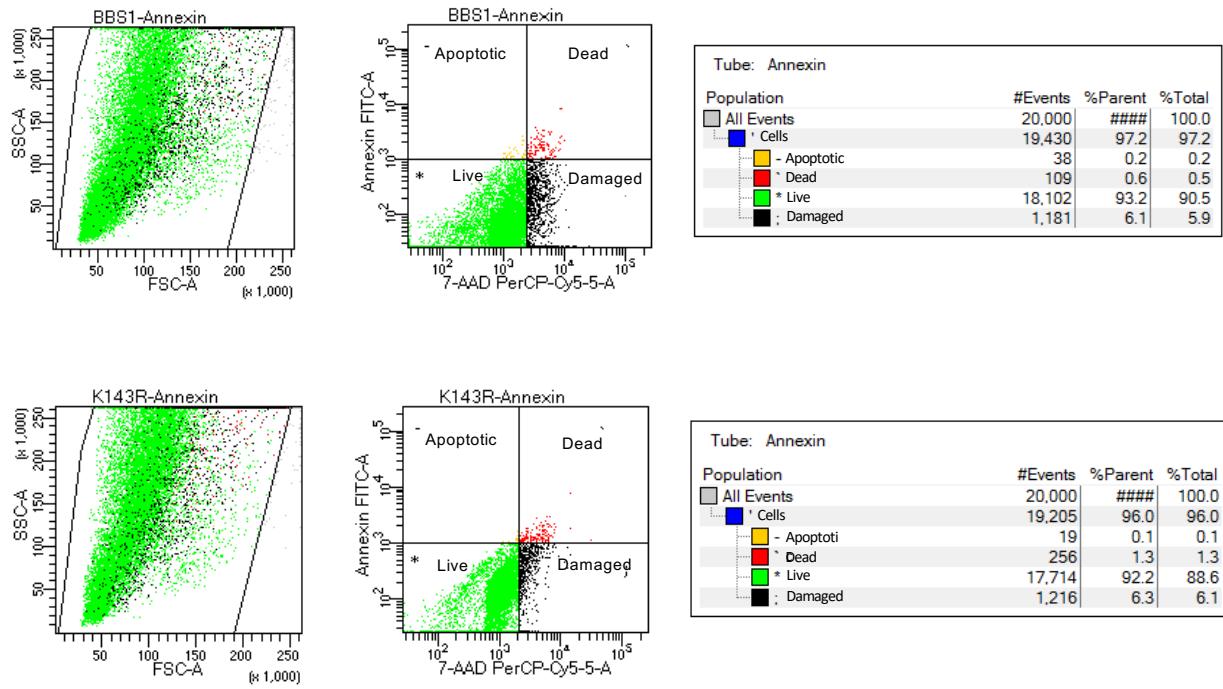
6VBU_BBS9 hBBS9	<b>20</b> <b>MSLFKARDWWSTVLDKEEFQGCLCLADVDNTNGQDKIIIVGSFMGYLRIFNPHPVKTG</b> <b>3260</b> <b>-SLFKARDWWSTVLDKEEFQGCLCLANVDNSNGODKIIIVGSFMGYLRIFSPHPAKTG</b> <b>3280</b> <b>3300</b>	<b>40</b> <b>60</b>
6VBU_BBS9 hBBS9	<b>80</b> <b>DGAQAEDLLLLEVHLRDPVILQEVVGKFVSGTEMLHLAVLHSRKLCVYSVSGTLGNVEHGNQ</b> <b>3320</b> <b>DGAQAEDLLLLEVHLRDPVVLQEVVGKFVSGTEMLHLAVLHSRKLCVYSVSGTLGNVEHGNQ</b> <b>3340</b> <b>3360</b>	<b>100</b> <b>120</b>
6VBU_BBS9 hBBS9	<b>140</b> <b>YQIKLMYEHNLRQRTACNMITYGSFGGVKGDRDLICIQSVQDGMLMVFEQESYAFGRFLPGSLL</b> <b>3380</b> <b>CQMKLMYEHNLRQRTACNMITYGSFGGVKGDRDLICIQSMQDGMLMVFEQESYAFGRFLPGFLL</b> <b>3400</b> <b>3420</b>	<b>160</b> <b>180</b>
6VBU_BBS9 hBBS9	<b>200</b> <b>PGPLAYSSRTDSFITVSSCHQVESYKYQVLAFATDADKRQETEQQQKHGSGKRLVVDWTLN</b> <b>3440</b> <b>PGPLAYSSRTDSFTVSSCQVESYKYQVLAFATDADKRQETEQQQKLHGSGKRLVVDWTLN</b> <b>3460</b> <b>3480</b>	<b>220</b> <b>240</b>
6VBU_BBS9 hBBS9	<b>260</b> <b>IGEQAIIDICIVSFIQSASSVFVLGERNFFCLKDNGQIQFMKKLDYSPSCFLPYCSVSEG</b> <b>3500</b> <b>IGEQALDICIVSFNQASSVFVLGERNFFCLKDNGQIRFMKKLDWSPSCFLPYCSVSEG</b> <b>3520</b> <b>3540</b>	<b>280</b> <b>300</b>
6VBU_BBS9 hBBS9	<b>320</b> <b>INTLIGNHNNMLHIYQDVTLKWATQLPHVPVAVRVGCLHDLKGVI</b> <b>3560</b> <b>INTLIGNHNNMLHIYQDVTLKWATQLPHIPVAVRVGCLHDLKGVI</b> <b>3580</b> <b>3600</b>	<b>340</b> <b>360</b>

6VBU_BBS9	TDPSLFQAPKVESRELNYDELDME <b>L</b> KELQ <b>K</b> VIK <b>N</b> VNKSQDVWPLTEREDDL <b>K</b> VS <b>A</b> MVSPN
hBBS9	TDPSLFQAPN <b>V</b> SRELNYDELD <b>V</b> E <b>M</b> KELQ <b>K</b> I <b>K</b> DVNKSQGVWPMTEREDDL <b>N</b> VS <b>V</b> VSPN
	3620 3640 3660
6VBU_BBS9	FDSVSQATDVEVG <b>A</b> D <b>L</b> VPSVTVKVTL <b>K</b> NRVALQ <b>K</b> IKL <b>S</b> I <b>V</b> VQPPVL <b>T</b> GDQFTFEF <b>M</b> AP <b>E</b>
hBBS9	FDSVSQATDVEVG <b>T</b> DLVPSVTVKVTL <b>Q</b> NRV <b>I</b> LQ <b>K</b> AKL <b>S</b> V <b>V</b> VQPP <b>E</b> LT <b>C</b> DQFTFEF <b>M</b> TPD
	3680 3700 3720
6VBU_BBS9	<b>M</b> TRTV <b>G</b> FSVYL <b>K</b> GSY <b>S</b> PPELEGNAVV <b>S</b> Y <b>R</b> PT <b>E</b> RNP <b>D</b> GIP <b>R</b> V <b>S</b> Q <b>C</b> K <b>F</b> RL <b>P</b> L <b>K</b> <b>L</b> <b>V</b> CLPGQP
hBBS9	<b>L</b> TRTV <b>S</b> FSVYL <b>K</b> RSY <b>T</b> PS <b>E</b> LEGNAVV <b>S</b> Y <b>R</b> PT <b>D</b> RNP <b>D</b> GIP <b>R</b> V <b>I</b> Q <b>C</b> K <b>F</b> RL <b>P</b> L <b>K</b> <b>L</b> <b>I</b> CLPGQP
	3740 3760 3780
6VBU_BBS9	SKTASH <b>K</b> L <b>T</b> IDTNKSPV <b>S</b> LL <b>S</b> LPFGFA <b>K</b> Q <b>S</b> EDD <b>Q</b> V <b>N</b> MG <b>F</b> R <b>L</b> GG <b>S</b> Q <b>V</b> T <b>L</b> AS <b>K</b> T <b>S</b> QR <b>Y</b> R
hBBS9	SKTASH <b>K</b> I <b>T</b> IDTNKSPV <b>S</b> LL <b>S</b> LPFGFA <b>S</b> Q <b>S</b> DD <b>Q</b> V <b>N</b> MG <b>H</b> F <b>L</b> GG <b>A</b> R <b>I</b> T <b>V</b> LA <b>S</b> K <b>T</b> S <b>Q</b> RYR
	3800 3820 3840
6VBU_BBS9	I <b>Q</b> SEQ <b>F</b> ED <b>L</b> WL <b>I</b> T <b>N</b> EL <b>I</b> <b>L</b> <b>R</b> Q <b>E</b> Y <b>F</b> E <b>K</b> Q <b>G</b> <b>I</b> K <b>D</b> <b>F</b> <b>T</b> <b>C</b> SG <b>S</b> <b>V</b> <b>P</b> <b>L</b> <b>E</b> <b>Y</b> <b>F</b> E <b>L</b> <b>I</b> D <b>H</b> <b>H</b> <b>F</b> E <b>L</b> R <b>I</b> <b>N</b> <b>G</b> <b>E</b>
hBBS9	I <b>Q</b> SEQ <b>F</b> ED <b>L</b> WL <b>I</b> T <b>N</b> EL <b>I</b> <b>L</b> <b>R</b> Q <b>E</b> Y <b>F</b> E <b>K</b> Q <b>G</b> <b>V</b> K <b>D</b> <b>F</b> <b>A</b> <b>C</b> SG <b>S</b> <b>I</b> <b>P</b> <b>L</b> <b>Q</b> <b>E</b> <b>Y</b> <b>F</b> E <b>L</b> <b>I</b> D <b>H</b> <b>H</b> <b>F</b> E <b>L</b> R <b>I</b> <b>N</b> <b>G</b> <b>E</b>
	3860 3880 3900
6VBU_BBS9	K <b>L</b> ELL <b>S</b> ERAV <b>Q</b> F <b>R</b> A <b>I</b> Q <b>R</b> LL <b>T</b> RF <b>K</b> D <b>K</b> T <b>P</b> <b>A</b> <b>P</b> <b>L</b> <b>Q</b> <b>H</b> LD <b>T</b> <b>L</b> <b>L</b> <b>D</b> <b>G</b> <b>T</b> <b>Y</b> <b>K</b> Q <b>V</b> IAL <b>A</b> DA <b>V</b> E <b>E</b> <b>N</b> <b>Q</b> <b>D</b> <b>N</b> <b>L</b>
hBBS9	K <b>L</b> ELL <b>S</b> ERAV <b>Q</b> F <b>R</b> A <b>I</b> Q <b>R</b> LL <b>A</b> R <b>F</b> K <b>D</b> <b>K</b> T <b>P</b> <b>A</b> <b>P</b> <b>L</b> <b>Q</b> <b>H</b> LD <b>T</b> <b>L</b> <b>L</b> <b>D</b> <b>G</b> <b>T</b> <b>Y</b> <b>K</b> Q <b>V</b> IAL <b>A</b> DA <b>V</b> E <b>E</b> <b>N</b> <b>Q</b> <b>G</b> <b>N</b> <b>L</b>
	3920 3940 3960
6VBU_BBS9	FQS <b>F</b> TRL <b>K</b> SATH <b>L</b> V <b>I</b> LL <b>I</b> <b>G</b> W <b>Q</b> KL <b>S</b> AD <b>Q</b> <b>T</b> A <b>I</b> LE <b>A</b> AF <b>L</b> <b>P</b> <b>L</b> <b>Q</b> <b>D</b> <b>T</b> <b>Q</b> <b>E</b> <b>L</b> WE <b>E</b> <b>T</b> <b>V</b> DA <b>A</b> <b>L</b> <b>S</b> <b>H</b> <b>L</b> <b>L</b>
hBBS9	FQS <b>F</b> TRL <b>K</b> SATH <b>L</b> V <b>I</b> LL <b>I</b> <b>A</b> W <b>Q</b> KL <b>S</b> AD <b>Q</b> <b>V</b> A <b>I</b> LE <b>A</b> AF <b>L</b> <b>P</b> <b>L</b> <b>Q</b> <b>E</b> <b>D</b> <b>T</b> <b>Q</b> <b>E</b> <b>L</b> WE <b>E</b> <b>T</b> <b>V</b> DA <b>A</b> <b>I</b> <b>S</b> <b>H</b> <b>L</b> <b>L</b>
	3980 4000 4020
6VBU_BBS9	K <b>T</b> CS <b>K</b> S <b>S</b> KE <b>Q</b> AL <b>N</b> LN <b>S</b> QL <b>G</b> <b>I</b> P <b>K</b> D <b>T</b> <b>S</b> QL <b>K</b> K <b>H</b> IT <b>L</b> <b>F</b> <b>C</b> DR <b>L</b> <b>A</b> <b>K</b> <b>G</b> <b>R</b> <b>L</b> <b>C</b> <b>L</b> <b>S</b> <b>T</b> <b>D</b> <b>A</b> <b>A</b> <b>P</b> <b>Q</b> <b>T</b> <b>M</b> <b>V</b> <b>M</b> KTCLS <b>K</b> S <b>S</b> KE <b>Q</b> AL <b>N</b> LN <b>S</b> QL <b>N</b> <b>I</b> P <b>K</b> D <b>T</b> <b>S</b> QL <b>K</b> K <b>H</b> IT <b>L</b> <b>C</b> DR <b>L</b> <b>S</b> <b>K</b> <b>G</b> <b>R</b> <b>L</b> <b>C</b> <b>L</b> <b>S</b> <b>-</b> <b>-</b> <b>-</b> <b>-</b>
hBBS9	
	4040 4060 4068
6VBU_BBS9	GGCAT <b>I</b> P <b>E</b> SD <b>L</b> GR <b>S</b> <b>I</b> D <b>Q</b> <b>D</b> <b>S</b> <b>S</b> <b>E</b> <b>L</b> FT <b>N</b> HK <b>H</b> LM <b>V</b> ET <b>P</b> <b>V</b> <b>E</b> <b>S</b> <b>P</b> <b>L</b> <b>Q</b> <b>G</b> <b>V</b> <b>T</b> -----
hBBS9	

H

6VBU_BBS18	MAETKSM <b>F</b> REV <b>L</b> PK <b>Q</b> <b>G</b> <b>Q</b> <b>L</b> <b>Y</b> <b>V</b> <b>E</b> <b>D</b> <b>I</b> <b>T</b> <b>T</b> TMV <b>L</b> CK <b>P</b> <b>K</b> <b>L</b> <b>L</b> <b>P</b> <b>K</b> <b>S</b> <b>L</b> <b>T</b> <b>L</b> <b>E</b> <b>K</b> <b>L</b> <b>E</b> <b>K</b> <b>M</b> <b>Q</b> <b>Q</b> <b>A</b> <b>Q</b> <b>D</b> <b>T</b> <b>I</b> <b>H</b> <b>Q</b> <b>E</b>
hBBS18	----- <b>M</b> <b>F</b> REV <b>L</b> PK <b>Q</b> <b>G</b> <b>Q</b> <b>P</b> <b>L</b> <b>F</b> <b>V</b> <b>E</b> <b>D</b> <b>I</b> <b>M</b> <b>T</b> <b>M</b> <b>V</b> <b>L</b> CK <b>P</b> <b>K</b> <b>L</b> <b>L</b> <b>P</b> <b>K</b> <b>S</b> <b>L</b> <b>T</b> <b>L</b> <b>E</b> <b>K</b> <b>L</b> <b>E</b> <b>K</b> <b>M</b> <b>H</b> <b>O</b> <b>A</b> <b>A</b> <b>Q</b> <b>N</b> <b>T</b> <b>I</b> <b>R</b> <b>O</b> <b>Q</b> <b>Q</b> <b>E</b>
	4084 4104 4122
6VBU_BBS18	TEKEQKITH -----
hBBS18	

**Appendix Figure S5:** BBSome bovine and human sequence alignments, highlighting the different residue numbers between the experimental (PDB ID: 6vbu) and the human homology model used during CG-MD simulations. **A.** bBBS1/hBBS1; **B.** bBBS2/hBBS2; **C.** bBBS4/hBBS4; **D.** bBBS5/hBBS5; **E.** bBBS7/hBBS7; **F.** bBBS8/hBBS8; **G.** bBBS9/hBBS9; **H.** bBBS18/hBBS18.



**Appendix Figure S6:** ARPE-19 cells were transiently transfected with Myc-tagged BBS1 variants or Myc-tagged BBS1- K143R. After 24h from transfection, cells were harvested and analyzed using a fluorescein isothiocyanate (FITC) Annexin V Apoptosis Detection Kit and 7-AAD staining solution, according to the manufacturer's protocol.