

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

Francesco Chiuso et al.

Appendix Figures

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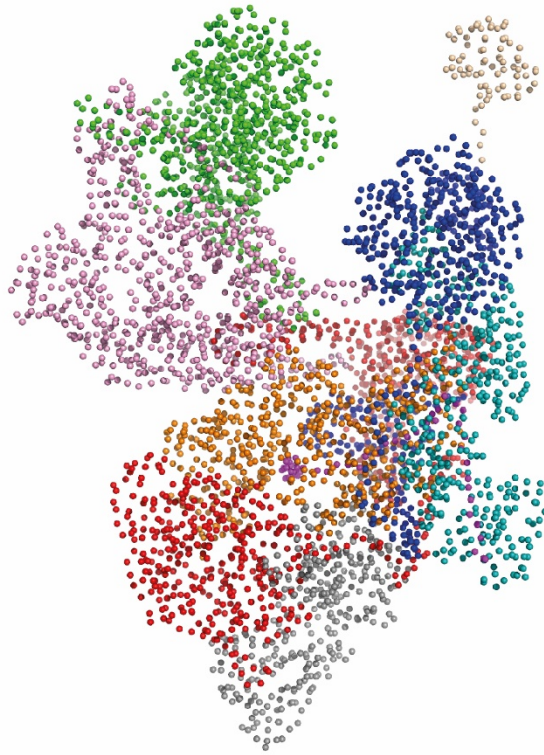
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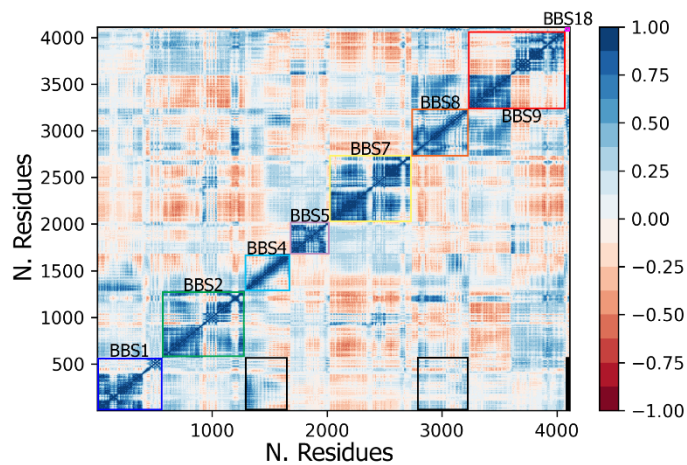
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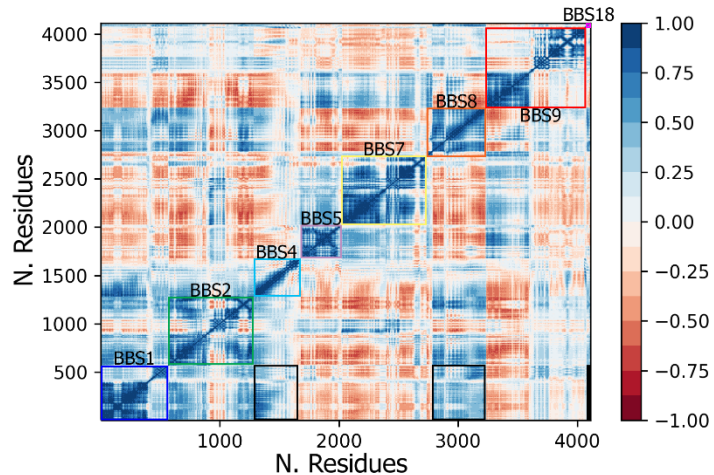


**Appendix Figure S2:** Coarse –Grained model of the Ub-hBBSome in an open conformation. Beads (BB) are shown as spheres.

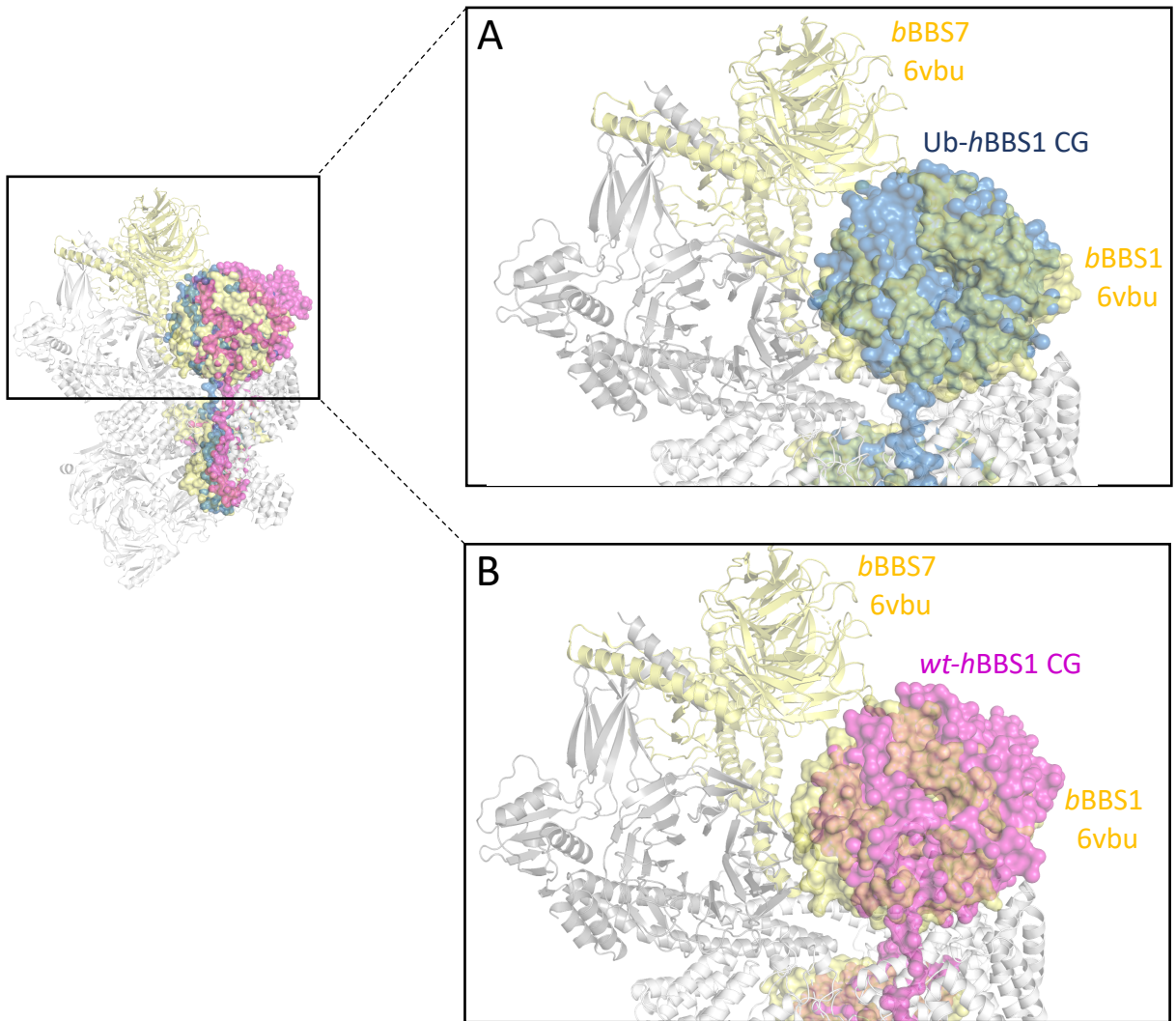
A



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  -----SKWLDAHYPMANIHTFSACLALADLHGDGEYKLVVGDGLGP
6vbu_BBS1 MAATSSSDSDGGKGESEANSKWLDSLSDSMANIHTFSACLALADLHGDGEYKLVVGDGLGP
                20                               60

                61                               82                               103
hBBS1  GGGQPRLKVLKGPLVMTESPLPALPAAAAATFLMEQHEPRTPALALASGPCVYVYKRLRPY
6vbu_BBS1 DGRQPRLKVLKGH TLVVSQKPLPDLPAAAAVTFLMASHEPRTPALALASGPCVYVYKRLRPY
                80                               100                               121

                124                               142                               162
hBBS1  FKFSLPQLPPNPLEQDLWNQAKEDRIDPLTLKEMLESIRETAEELSIQSLRFLQLELSE
6vbu_BBS1 FKFSLP SLPTNPLEQDLWNQAKEDRIDPLTLKEMLEGI REKA EVPLSVQSLRFLPLELSE
                143                               160                               180

                182                               202                               222
hBBS1  MEAFVNQHKSN SIKRQTVITMTTLKKNLADEDAVSCLVLGTENKELLVLDPEAFTILAK
6vbu_BBS1 MEAFVNQHKSKSIRRQTVITMTTLKKNLADEDAVSCLVLGTENKELLVLDPEAFTILAK
                200                               220                               240

                242                               262                               282
hBBS1  MSLPSVPVFLVSGQFDVEFRLAAACRNGNIYILRRDSKHPKYCIELSAQPVGLIRVHKV
6vbu_BBS1 MSLPSVPAFLEASGQFDVEFRLAAACRNGSIYILRRDSKRPKYCIELGAQPVGLVGVHKV
                260                               280                               300

                302                               322                               342
hBBS1  LVVGSTQDSLHGFT HKGK LWTVQMPAAIL TMN LLEQHSRGLQAVMAGLANGEVRIYRDK
6vbu_BBS1 LVVGSNQDSLHGFTYKGRRLWTVQMPAAILAMN LLEQHSRGLQAVMAALANEVRIYHDK
                320                               340                               360

                362                               382                               402
hBBS1  ALLNVIHTPDAVTS LCFGRYGRDNTLIMTTRGGGLI I KILKRTAVFVEGGSEVGP PPAQ
6vbu_BBS1 VLLNVIRTPEAVTSLCFGRYGRDNTLIMTTLGGGLI I KILKRTAVFAEGGGEAGPPPSQ
                380                               400                               420

                422                               442                               462
hBBS1  AMKLNVPKTRLYVDQTLREREAGTAMHRAFQTDLYLLR LRAARAYLQALESSLSP LSTT
6vbu_BBS1 AIKLNVPKTRLYVDQTLREREAGTAMHRTFQADLYLLR LRAARAYVQALESSLSPVSLT
                440                               460                               480

                482                               502                               522
hBBS1  AREPLKLHAVVQGLGPTFKLTLHLQNTSTTRPV LGLLVCFLYNEALYSLPRAFFKVPLLV
6vbu_BBS1 AREPLKLHAVVQGLGPTFKLTLHLQNTSTARPI LGLLVCFLYNEVLYALPRAFFKVPLLV
                500                               520                               540

                542                               562                               568
hBBS1  PGLNYPLET FVESLSNKGISDI I KVLVLRREGQSA P LLSAHVNM P GS-----
6vbu_BBS1 PGLNYPLET FVKSLSDKGISDI I KVLVLRREGQST P LLSAHINMPM SEGLAAD
                560                               580                               592

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B

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                570                               589                               609                               629
hBBS2  ML L P V F T L K L R H K I S P R M V A I G R Y D G T H P C L A A A T Q T G K V F I H N P H T R N Q H V S A S R V F Q S
6vbu_BBS2 M L Q P V F T L K L R H K I S P R M V A V G R Y D G T H P C L A A A T Q A G K V F I H N P H S R S Q H L G A P R V L Q S
                1                               20                               40                               60

                649                               669                               689
hBBS2  P L E S D V S L L N I N Q A V S C L T A G V L N P E L G Y D A L L V G T Q T N L L A Y D V Y N N S D L F Y R E V A D G A
6vbu_BBS2 P L E S D V S L L N I N Q T V S C L T A G V L N P E L G Y D A L L V G T Q T N L L A Y D V Y N N S D L F Y R E V A D G A
                80                               100                               120

                709                               729                               749
hBBS2  N A I V L G T L G D I S S P L A I I G G N C A L Q G F N H E G S D L F W T V T G D N V N S L A L C D F D G D G K K E L L
6vbu_BBS2 S A I V L G T L G D I T S P L A I I G G N C A L Q G F N H E G N D L F W T V T G D N V H S L A L C D F D G D G K K E L L
                140                               160                               180

                769                               789                               809
hBBS2  V G S E D F D I R V F K E D E I V A E M T 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 S R Y W R I K S
6vbu_BBS2 V G S E D F D I R V F K E D E I V A E M S E T E I I 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  K N H A M S I H A F D L N S D G V N E L I T G W S N G K V D A R S D R T G E V I F K D N F S S A I A G V V E G D Y R M D
6vbu_BBS2 K N Q A M S I H A F D L N S D G V C E L I T G W S N G K V D A R S D R T G E V I F K D N F S S A I A G V V E G D Y R M E
                260                               280                               300

                889                               909                               929
hBBS2  G H I Q L I C C S V D G E I R G Y L P G T A E M R G N L M D T S A E Q D L I R E L S Q K K Q N L L L E L R N Y E A N A K . . .
6vbu_BBS2 G C Q Q L I C C S V D G E I R G Y L P G T A E M R G N L M D I S V E Q D L I R E L S Q K K Q N L L L E L R N Y E A N A K . . .
                320                               340                               360

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hBBS2	930	949	969	969
6vbu_BBS2	361	380	400	420
hBBS2		989	1029	1049
6vbu_BBS2		440	460	480
hBBS2		1069	1089	1109
6vbu_BBS2		500	520	540
hBBS2		1129	1149	1169
6vbu_BBS2		560	580	600
hBBS2		1189	1209	1229
6vbu_BBS2		620	640	660
hBBS2		1249	1269	1286
6vbu_BBS2		680	700	717 721

C

hBBS4	1288	1319
6vbu_BBS4	29	60
hBBS4	1339	1359
6vbu_BBS4	80	100 120
hBBS4	1399	1419
6vbu_BBS4	140	160 180
hBBS4	1459	1479
6vbu_BBS4	200	220 240
hBBS4	1519	1539
6vbu_BBS4	260	280 300
hBBS4	1579	1599
6vbu_BBS4	320	340 360
hBBS4	1639	1659
6vbu_BBS4	380	400 420
hBBS4	1683	
6vbu_BBS4	424	
hBBS4		
6vbu_BBS4		

D

6VBU_5	MSVL	DALWEDR	DRVRF	VSSQ	QMKTRPGEVL	IDCLDS	VEDTK	GNNGDRGRL	LVTNLR	RIV	WH	20	40	60	
hBBS5	MSVL	DALWEDR	DRVRF	LSAQ	QMKTRPGEVL	IDCLDS	I	EDTK	GNNGDRGRL	LVTNLR	RIL	WH	1704	1744	
6VBU_5	SLAL	PRVNL	SIGY	NCILN	ITTRTANS	KLRGQTEAL	YVLT	KCNSTR	FEFIF	TNLV	PGSP	RL	80	100	
hBBS5	SLAL	SRVNV	SVGY	NCILN	ITTRTANS	KLRGQTEAL	YIL	KCNSTR	FEFIF	TNLV	PGSP	RL	1764	1804	
6VBU_5	YTS	LI	AVH	RAYETS	SKMYR	DFKLR	SALIQNK	QLRLL	PQEN	VY	NKING	VWNL	SSDQ	GNL	GT
hBBS5	FTS	VM	AVH	RAYETS	SKMYR	DFKLR	SALIQNK	QLRLL	PQEH	VY	DKING	VWNL	SSDQ	GNL	GT
6VBU_5	FITN	VRIV	WHAM	NDSFN	VSI	PYLQ	IRSVK	IRDSK	FGLAL	VI	ESSQ	SGGY	VL	GFK	ID
hBBS5	FITN	VRIV	WHAM	NDSFN	VSI	PYLQ	IRSIK	IRDSK	FGLAL	VI	ESSQ	SGGY	VL	GFK	ID
6VBU_5	EKL	QESV	KEIN	SLHK	VYSAN	PI	FGVD	YEM	EKPQ	P	LEAL	TVK	QIQD	VEI	DS
hBBS5	EKL	QESV	KEIN	SLHK	VYSAN	PI	FGVD	-----	-----	-----	-----	-----	-----	-----	-----
6VBU_5	AYF	ADGN	KQQD	REP	VFSE	EL	GLA	IEK	LKDG	FTL	QGL	WE	V	M	
hBBS5	AYF	ADGN	KQQD	REP	VFSE	EL	GLA	IEK	LKDG	FTL	QGL	WE	V	S	

E

6VBU_7	MDL	N	LN	R	ADYL	QVGV	T	S	Q	K	T	M	K	L	P	A	S	K	H	R	A	T	Q	K	V	V	G	D	H	G	I	V	M	C	F	G	M	K	K	G	E	A	V	T	V	F	K	20	40	60												
hBBS7	MDL	I	L	N	R	M	D	Y	L	O	V	G	V	T	S	O	K	T	M	K	L	P	A	S	R	H	R	A	T	O	K	V	V	I	G	D	H	G	V	M	C	F	G	M	K	K	G	E	A	A	V	F	K	2045	2065	2085						
6VBU_7	LPG	Q	K	I	A	R	L	E	L	G	G	A	L	N	T	P	Q	E	K	I	F	I	A	A	G	S	E	I	R	G	F	T	K	R	G	K	O	F	L	S	F	E	T	N	L	T	E	S	I	K	A	M	H	I	S	G	S	D	80	100		
hBBS7	LPG	P	K	I	A	R	L	E	L	G	G	V	I	N	T	P	Q	E	K	I	F	I	A	A	S	E	I	R	G	F	T	K	R	G	K	O	F	L	S	F	E	T	N	L	T	E	S	I	K	A	M	H	I	S	G	S	D	2105	2125	2145		
6VBU_7	L	F	L	S	A	S	I	Y	N	H	Y	C	D	K	D	Q	H	Y	L	S	G	D	K	I	N	D	V	I	C	L	P	V	E	R	L	L	R	E	V	P	V	L	A	C	Q	D	R	V	L	R	V	L	Q	G	S	D	140	160				
hBBS7	L	F	L	S	A	S	I	Y	N	H	Y	C	D	K	D	Q	H	Y	L	S	G	D	K	I	N	D	V	I	C	L	P	V	E	R	L	S	R	I	T	P	V	L	A	C	Q	D	R	V	L	R	V	L	Q	G	S	D	2165	2185	2205			
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hBBS7	M	Y	A	V	E	V	P	G	P	P	T	V	L	A	L	H	N	G	G	D	S	G	E	D	L	L	F	G	T	S	D	G	K	L	L	A	L	I	Q	I	T	T	S	K	P	V	R	K	W	E	I	Q	N	E	K	K	R	G	2225	2245	2265	
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6VBU_7	E	K	Y	Q	S	S	Q	S	S	K	A	S	A	V	P	S	F	S	V	N	D	K	F	T	L	N	K	D	A	S	Y	S	L	I	L	E	V	Q	T	A	I	D	N	V	L	I	Q	S	D	V	P	I	D	L	L	D	380	400				
hBBS7	E	N	Y	Q	S	S	Q	S	S	K	A	S	A	V	P	S	F	G	I	N	D	K	F	T	L	N	K	D	A	S	Y	S	L	I	L	E	V	Q	T	A	I	D	N	V	L	I	Q	S	D	V	P	I	D	L	D	2405	2425	2445				
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hBBS7	D	K	N	S	A	V	S	F	S	C	D	S	E	S	N	D	N	F	L	L	A	T	Y	R	C	Q	A	D	T	T	R	L	E	L	K	I	R	S	I	E	G	Q	Y	G	T	L	Q	A	Y	V	T	P	R	I	Q	P	K	2465	2485	2505		
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hBBS7	C	Q	V	R	Q	Y	H	I	K	P	L	S	L	H	Q	R	T	H	F	I	D	H	R	P	M	N	T	L	T	L	T	G	Q	F	S	F	A	E	V	H	S	W	V	V	F	C	L	P	E	V	P	E	K	P	P	A	G	E	C	2525	2545	2565
6VBU_7	V	T	F	Y	F	Q	N	T	F	L	D	T	Q	L	E	S	T	Y	R	K	G	E	G	V	F	K	S	D	N	I	S	I	S	I	L	K	D	V	L	S	K	E	A	T	K	R	K	I	N	L	N	I	S	Y	E	I	N	E	V	560	580	
hBBS7	V	T	F	Y	F	Q	N	T	F	L	D	T	Q	L	E	S	T	Y	R	K	G	E	G	V	F	K	S	D	N	I	S	I	S	I	L	K	D	V	L	S	K	E	A	T	K	R	K	I	N	L	N	I	S	Y	E	I	N	E	V	2585	2605	2625



6VBU\_7 SVKHTLKL IHPKLEYQLLAKKVQLIDALKEQVHEGNTNFLIPEYRCILEEADHLQEEY 660  
hBBS7 SVKHTLKL IHPKLEYQLLAKKVQLIDALKEQVHEGNTNFLIPEYHCILEEADHLQEEY 2685

6VBU\_7 KKQPAHLERLYGMITDLFIDKFKFKGTNVKTKVPLLEILDSYDQNALIAFFDAA 715  
hBBS7 KKQPAHLERLYGMITDLFIDKFKFKGTNVKTKVPLLEILDSYDQNALISFFDAA 2740

F

6VBU\_BBS8 MEPLLLAWSYFRRRKFQLCADLCTQMLEKSPCDQAAWILKARALTEMVYVEIDVDEEGI 60  
hBBS8 MEPLLLAWSYFRRRKFQLCADLCTQMLEKSPYDQAAWILKARALTEMVYIDEIDVDQEGIT 2800

6VBU\_BBS8 AEMILDENAIQVPRPGTSLKLPGTNQTGGPSPAVRPVTQAGRPIITGFLRPSTQSGRPGT 120  
hBBS8 AEMMLDENAIQVPRPGTSLKLPGTNQTGGPSPQAVRPITQAGRPIITGFLRPSTQSGRPGT 2860

6VBU\_BBS8 IEQAIKTPRTAYTARPIASSSGRFVRLGTASMLTSPDGPFINLSRLNLAKYAQKPKLAKA 180  
hBBS8 MEQAIKTPRTAYTARPIASSSGRFVRLGTASMLTSPDGPFINLSRLNLTKYSQKPKLAKA 2920

6VBU\_BBS8 LFEYIFHHENDVK TALDLAALSTEHSQYKDWWMKVQIGKCYRRLGLYREAEKQFKSALKQ 240  
hBBS8 LFEYIFHHENDVK TALDLAALSTEHSQYKDWWMKVQIGKCYRRLGLYREAEKQFKSALKQ 2980

6VBU\_BBS8 QEMVDTFLYLAKVYISLDQPLTALNLFKQGLDKFPGEVTL LCGIARIYEEMNMISSATEY 300  
hBBS8 QEMVDTFLYLAKVYISLDQPV TALNLFKQGLDKFPGEVTL LCGIARIYEEMNMISSAAEY 3040

6VBU\_BBS8 YKEVLKQDNTHVEAIAICIGSNHFYTDQPEVALRFYRRLQMGVYVYNQLFNNLGLCCFYAQ 360  
hBBS8 YKEVLKQDNTHVEAIAICIGSNHFYSDQPEIALRFYRRLQMGVIYVYNQLFNNLGLCCFYAQ 3100

6VBU\_BBS8 QYDMTLTSFERALS LAENEEVADVWYNLGHVAVGTGDTNLAHQCFRLALVSNNOHAEAY 420  
hBBS8 QYDMTLTSFERALS LAENEEAADVWYNLGHVAVGIGDTNLAHQCFRLALVNNNHAEAY 3160

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G

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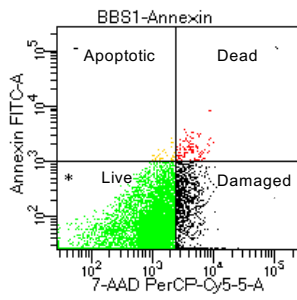
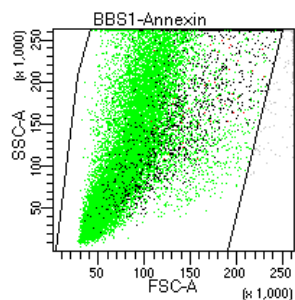
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6VBU\_BBS9 PGPLAYSSRTDSF IIVSSCHQVESYKYQVLAFATDADKROETEQQKHGSGKRLVVDWTLN 240  
hBBS9 PGPLAYSSRTDSF LTVSSCQVESYKYQVLAFATDADKROETEQQKLGSGKRLVVDWTLN 3480

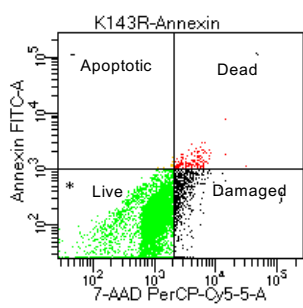
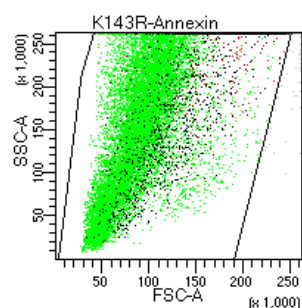
6VBU\_BBS9 IGEQAIDICIVSFIQSASSVFLGERNFFCLKDNGQIQFMKKLDYSPSCFLPYCSVSEGT 300  
hBBS9 IGEQALDICIVSFNQSSASSVFLGERNFFCLKDNGQIRFMKKLDWSPSCFLPYCSVSEGT 3540

6VBU\_BBS9 INTLIGNHNMLHIYQDVT LKQWATQLPHVPVAVRVGCLHDLKGVIVT L SDDGHLQCSYLG 360  
hBBS9 INTLIGNHNMLHIYQDVT LKQWATQLPHIPVAVRVGCLHDLKGVIVT L SDDGHLQCSYLG 3600





Tube: Annexin			
Population	#Events	%Parent	%Total
All Events	20,000	####	100.0
Cells	19,430	97.2	97.2
- Apoptotic	38	0.2	0.2
Dead	109	0.6	0.5
* Live	18,102	93.2	90.5
; Damaged	1,181	6.1	5.9



Tube: Annexin			
Population	#Events	%Parent	%Total
All Events	20,000	####	100.0
Cells	19,205	96.0	96.0
- Apoptotic	19	0.1	0.1
Dead	256	1.3	1.3
* Live	17,714	92.2	88.6
; Damaged	1,216	6.3	6.1

**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.