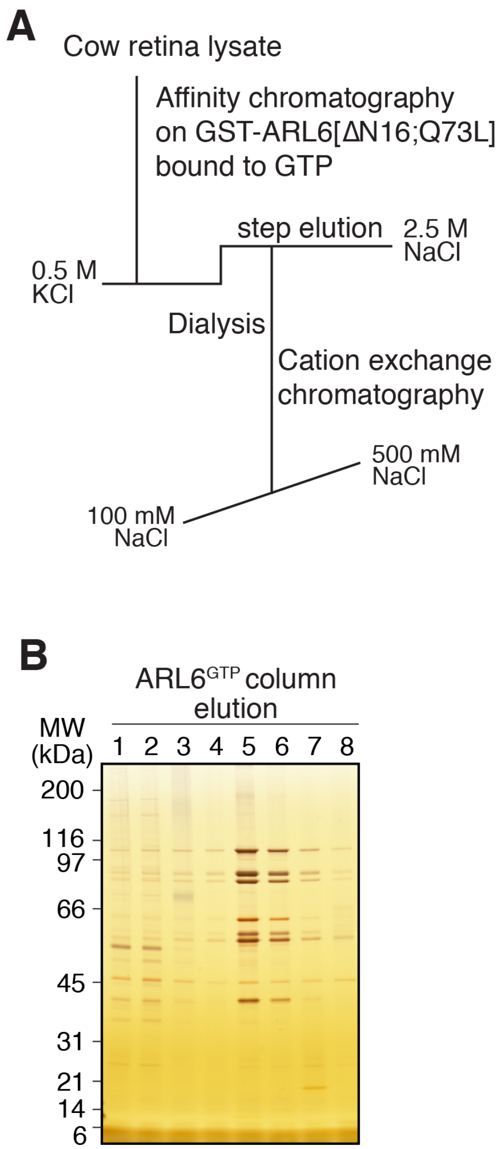

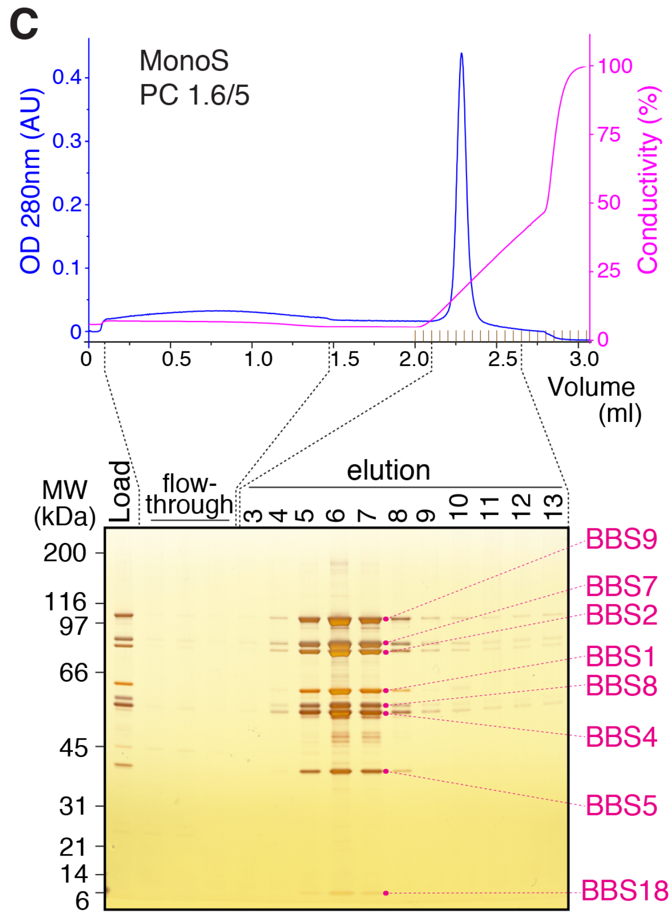

D

Protein	NSAF (spectra/100aa)	Coverage
BBS8	50.2	76.1%
BBS1	49.1	84.3%
BBS7	47.8	72.7%
BBS9	43.5	68.4%
BBS5	39.9	79.8%
BBS2	39.8	64.9%
BBS4	37.8	72.1%
BBS18	12.8	27.6%

Figure S1, related to STAR Methods and Figure 1. Purification of native BBSome. A. Workflow for the purification of native BBSome from cow retina. **B.** Silver-stained 4-12% SDS-PAGE gel of fractions eluting from the ARL6^{GTP}-affinity column. **C.** Chromatogram (top) and silver-stained 4-12% SDS-PAGE gel of elution fractions (bottom) from the MonoS column. **D.** Mass spectrometric identification of the eight BBSome subunits in the peak fraction. NSAF, normalized spectral abundance factor (Zybailov et al., 2006).

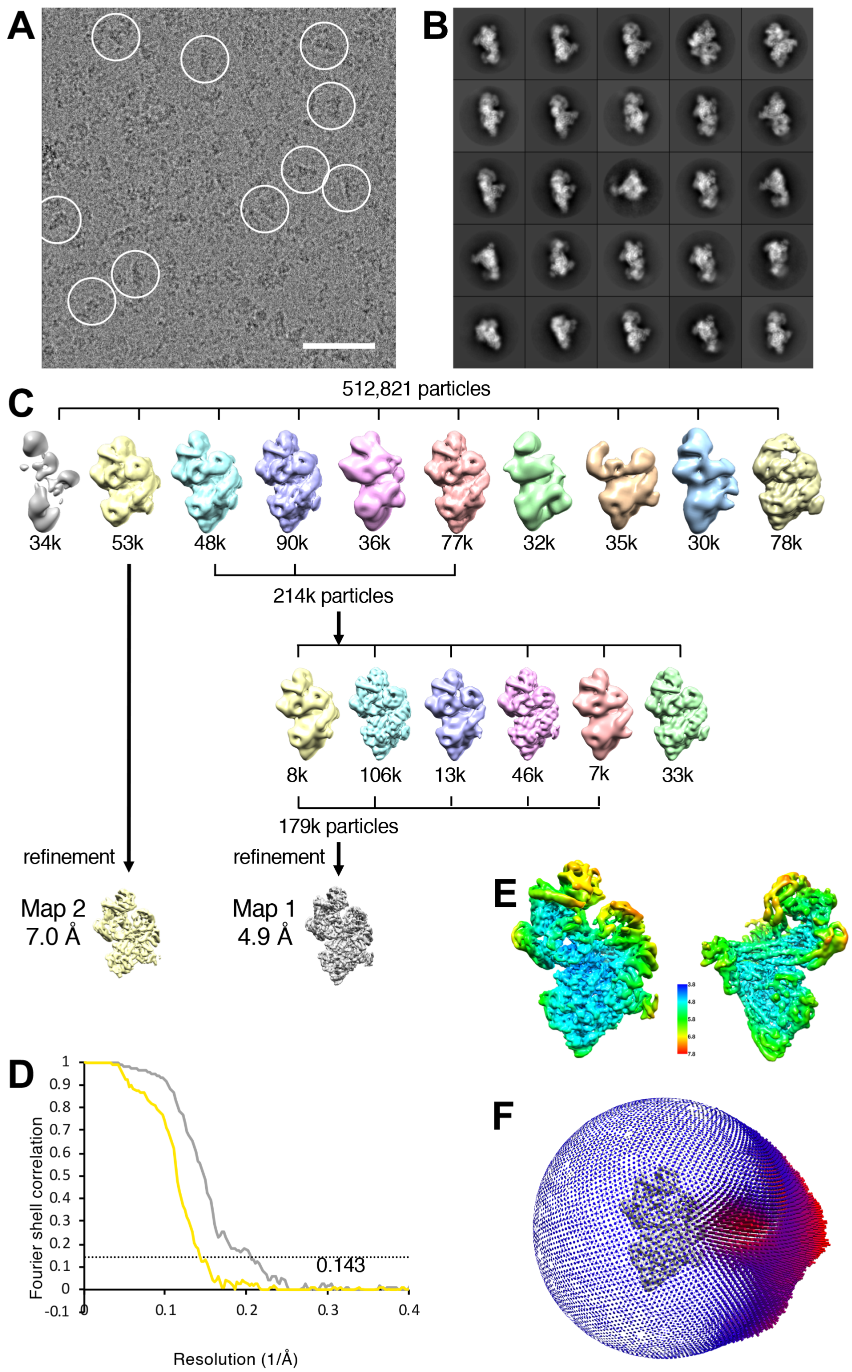

Figure S2, related to STAR Methods and Figures 1 and 2. Cryo-EM analysis of native BBSome. A. Cryo-EM image of vitrified BBSome processed with MotionCor2. Some particles are circled. Scale bar: 50 nm. **B.** Selected 2D class averages obtained with Relion 1.3. Side length of individual averages: 20 nm. **C.** Image-processing workflow for 3D classifications and refinement in Relion 1.3 that yielded the four density maps discussed in the main text. See Methods section for details. **D.** Fourier shell correlation curves calculated between independently refined half maps for the four BBSome density maps colored as in C. **E.** Local resolution for Map 1 as determined by using the ResMap algorithm included in Relion.

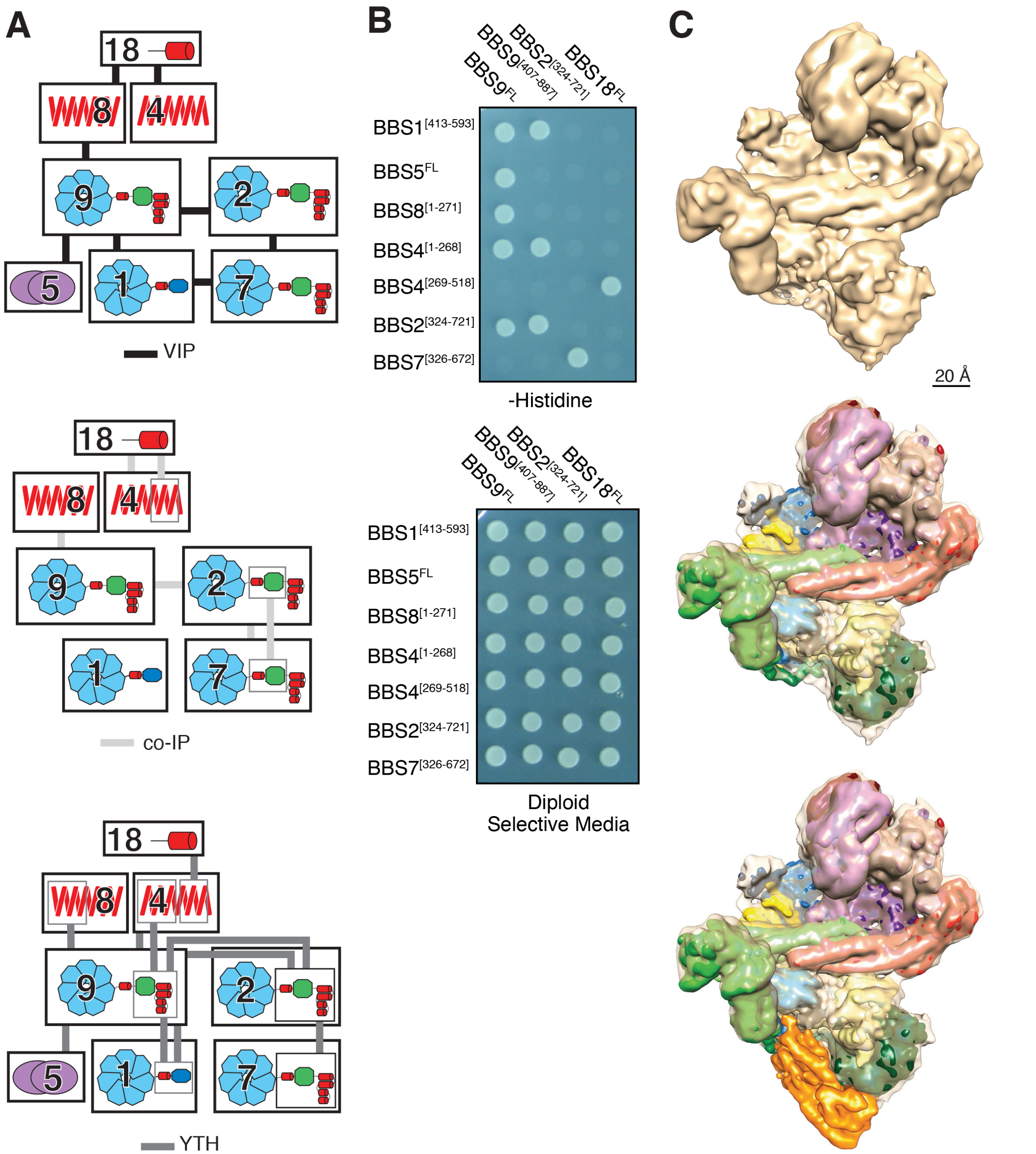

Figure S3, related to Figure 2. Interactions between domains of BBSome subunits and map differences. A. The three interaction datasets used to assign subunits to density in the cryo-EM map: VIP, visual immunoprecipitation; co-IP, co-immunoprecipitation; YTH, yeast-two hybrid. **B.** YTH array. *Upper panel:* Binary interactions between BBS Y2H constructs (column: bait, row: prey) as indicated by yeast colony growth on medium lacking histidine, tryptophan and leucine. Lower panel: Growth control for yeast mating by diploid selection on medium lacking tryptophan and leucine. **C.** *Top panel:* Map 2 at 7.0-Å resolution in opaque sand color. *Middle and bottom panels:* Comparison of Map 2 (transparent sand) with segmented Map 1 (colored segments). The orange region is removed in the middle panel and included in the bottom panel. Note that the orange segment makes extensive contacts with the β-propeller in the base (dark green).

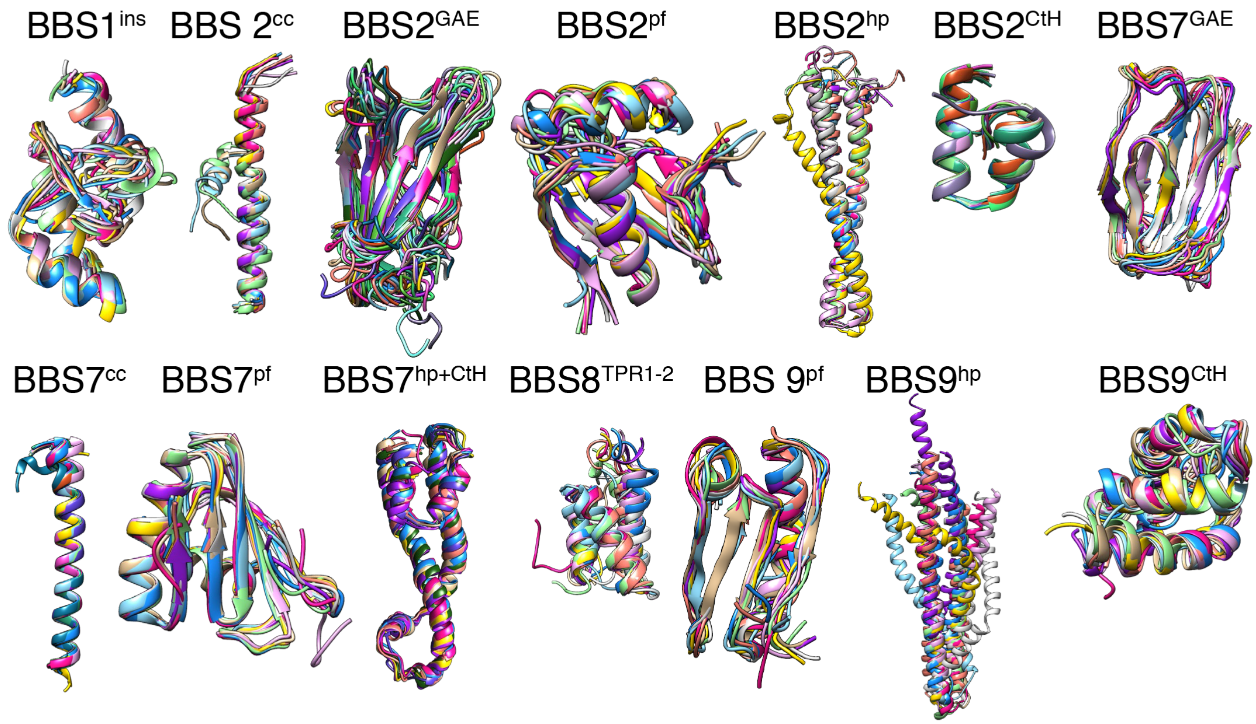

Figure S4, related to STAR Methods. Details of Rosetta modeling. Top 10 scoring models for the 15 domains that could be modeled with the Rosetta *ab initio* protocol. For BBS7^{hp} and BBS9^{CtH}, the *ab initio* modeling only converged for a core segment. The top 10 models shown for these two domains were obtained after the consistent core segments were placed into the cryo-EM map and the unassigned segments were modeled in the context of the density with Rosetta CM.

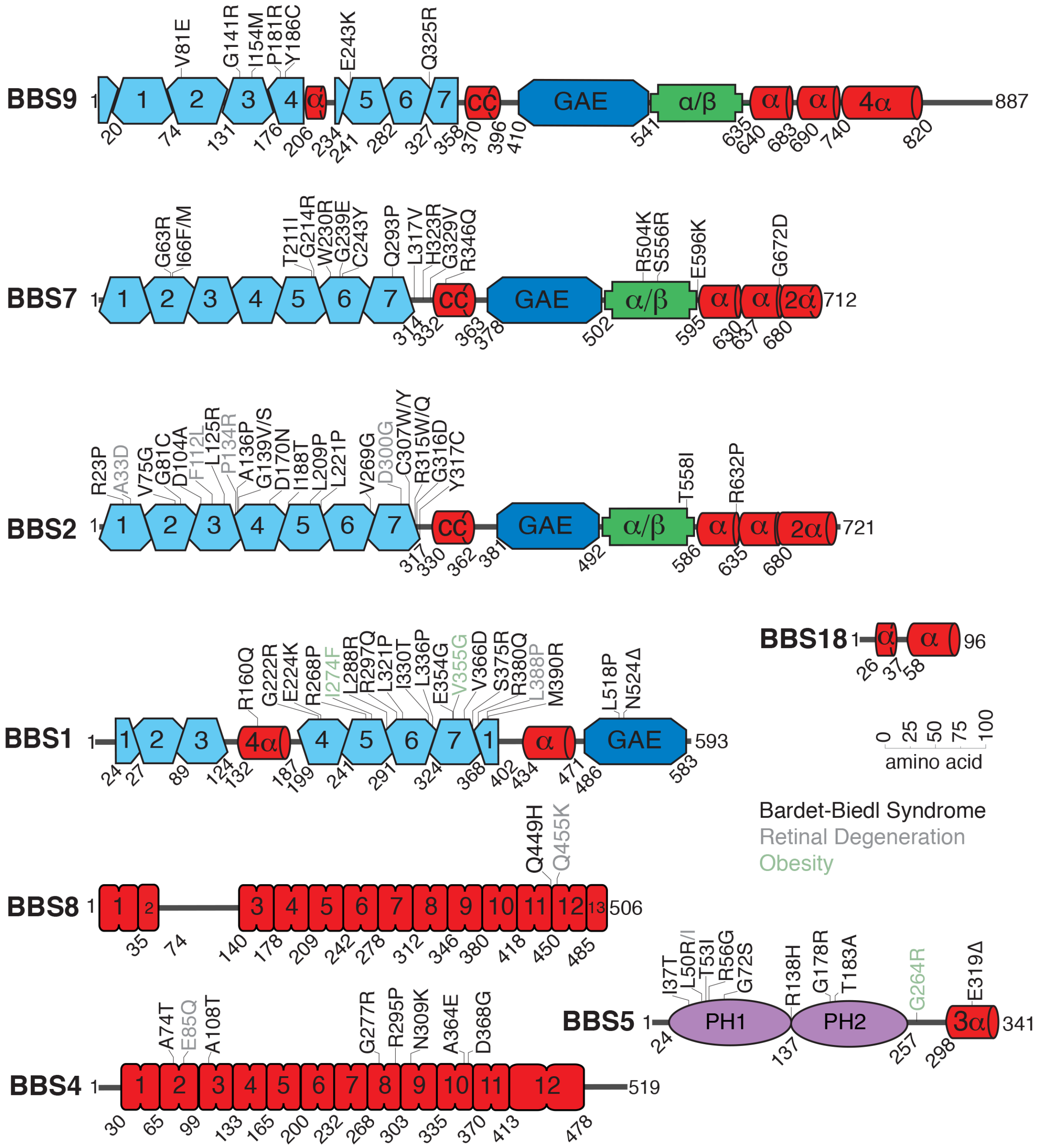

Figure S5, related to Figure 4. Disease-causing variants in BBSome subunits. BBSome subunits with positions of known disease-causing human variants. Variants causing Bardet-Biedl syndrome are labeled in black, variants causing retinal degeneration in gray, and variants causing obesity in green. Numbers below the subunits denote domain boundaries.

Table S1, related to Figure 2. Binary interactions between BBSome subunits reported by VIP (Katoh et al., 2015), co-IP (Nachury et al., 2007; Zhang et al., 2012a, 2013) and YTH (Woodsmith et al., 2017).

Bait		Prey		Method	Reference
BBS5	FL	**BBS9**	FL	**VIP**	Katoh et al., 2015
BBS9	FL	**BBS5**	FL	**VIP**	Katoh et al., 2015
BBS9	FL	**BBS8**	FL	**Co-IP**	Nachury et al., 2007
BBS8	FL	**BBS9**	FL	**VIP**	Katoh et al., 2015
BBS9	FL	**BBS8**	FL	**VIP**	Katoh et al., 2015
BBS1	FL	**BBS9**	FL	**VIP**	Katoh et al., 2015
BBS9	FL	**BBS1**	FL	**VIP**	Katoh et al., 2015
BBS9	FL	**BBS2**	FL	**CoIP**	Zhang et al., 2012a
BBS2	FL	**BBS9**	FL	**CoIP**	Nachury et al., 2007
BBS2	FL	**BBS9**	FL	**VIP**	Katoh et al., 2015
BBS9	FL	**BBS2**	FL	**VIP**	Katoh et al., 2015
BBS2	FL	**BBS7**	FL	**Co-IP**	Nachury et al., 2007
BBS2	FL	**BBS7**	FL	**VIP**	Katoh et al., 2015
BBS7	FL	**BBS2**	FL	**VIP**	Katoh et al., 2015
BBS7	FL	**BBS2**	325-721	**CoIP**	Zhang et al., 2013
BBS7	FL	**BBS2**	1-621	**CoIP**	Zhang et al., 2013
BBS7	301-615	**BBS2**	FL	**CoIP**	Zhang et al., 2013
BBS4	236-439	**BBS18**	FL	**CoIP**	Zhang et al., 2012a
BBS4	FL	**BBS18**	FL	**VIP**	Katoh et al., 2015
BBS8	FL	**BBS18**	FL	**VIP**	Katoh et al., 2015
BBS18	FL	**BBS8**	FL	**VIP**	Katoh et al., 2015
BBS1	FL	**BBS7**	FL	**VIP**	Katoh et al., 2015
BBS7	FL	**BBS1**	FL	**VIP**	Katoh et al., 2015

Table S2, related to STAR Methods. Crosslinks between and within BBSome subunits identified by mass spectrometry. The e-values for each peptide pair come from three independent experiments, one using the crosslinker BS3 and the other two using the crosslinker DSSeb. Only peptide pairs with e-values < 0.001 are included.

Experiment	BS3	DSE_1	DSE_2
BBS4-BBS1			
BBS1(80)-BBS4(25)		1.71E-006	
BBS1(80)-BBS4(5)			1.55E-004
BBS1(143)-BBS4(5)			1.49E-004
BBS4(25)-BBS1(192)	4.91E-05	1.30E-004	1.35E-004
BBS4(20)-BBS1(192)		7.14E-006	
BBS4(24)-BBS1(192)	2.50E-04		8.12E-007
BBS1(123)-BBS4(25)	1.35E-06	6.28E-006	2.32E-013
BBS1(116)-BBS4(5)	2.51E-06	2.32E-004	
BBS1(123)-BBS4(20)			1.95E-005
BBS1(143)-BBS4(25)			1.57E-010
BBS1(123)-BBS4(5)			3.18E-005
BBS1(424)-BBS4(285)	5.73E-14	3.98E-009	1.27E-006
BBS4(129)-BBS1(162)		2.67E-012	
BBS2-BBS7			
BBS2(360)-BBS7(359)		5.57E-010	4.91E-014
BBS2(360)-BBS7(352)		4.60E-006	7.74E-012
BBS7(330)-BBS2(345)			4.79E-005
BBS7(338)-BBS2(345)		3.99E-007	
BBS7(352)-BBS2(345)	4.55E-10	2.99E-010	8.80E-012
BBS2(360)-BBS7(370)			4.12E-004
BBS7(56)-BBS2(345)		1.39E-005	1.73E-006
BBS7(658)-BBS2(13)		1.87E-010	4.93E-008
BBS4-BBS7			
BBS7(604)-BBS4(5)		1.04E-006	
BBS7(222)-BBS4(5)		3.58E-007	1.80E-011
BBS7(659)-BBS4(5)	2.52E-08	5.24E-005	6.09E-007
BBS4(20)-BBS7(222)			2.74E-005
BBS4(20)-BBS7(619)		7.21E-010	7.50E-010
BBS4(20)-BBS7(659)		7.17E-011	3.21E-010

BBS9-BBS8			
BBS9(226)-BBS8(179)		2.39E-004	7.49E-004
BBS9(226)-BBS8(181)	1.99E-06		1.11E-004
BBS8(181)-BBS9(231)		3.71E-005	1.49E-006
BBS9(218)-BBS8(181)	5.94E-06	9.94E-008	
BBS4-BBS9			
BBS4(116)-BBS9(789)	4.99E-05		
BBS4(118)-BBS9(786)		6.27E-007	3.97E-005
BBS9(810)-BBS4(116)		6.75E-004	7.43E-004
BBS9(789)-BBS4(118)		7.85E-007	
BBS9(803)-BBS4(116)			5.09E-004
BBS1-BBS18			
BBS1(553)-BBS18(93)		7.52E-005	1.40E-004
BBS1(553)-BBS18(90)			2.51E-008
BBS1-BBS2			
BBS2(9)-BBS1(69)		3.28E-004	
BBS1(553)-BBS2(638)			1.91E-004
BBS1-BBS9			
BBS9(789)-BBS1(162)		6.16E-008	3.67E-012
BBS5-BBS18			
BBS18(90)-BBS5(87)			1.79E-005
BBS4-BBS4			
BBS4(20)-BBS4(25)	4.13E-07		
BBS4(5)-BBS4(25)	1.09E-05		
BBS4(24)-BBS4(5)			1.16E-09
BBS4(153)-BBS4(118)			4.64E-07
BBS4(438)-BBS4(446)		2.16E-05	
BBS4(441)-BBS4(446)		2.72E-04	1.17E-09
BBS4(480)-BBS4(441)		1.18E-04	
BBS4(480)-BBS4(446)		4.21E-13	1.07E-05

BBS1-BBS1			
BBS1(72)-BBS1(119)			2.60E-05
BBS1(80)-BBS1(119)		9.32E-12	6.54E-15
BBS1(123)-BBS1(80)		1.99E-12	1.13E-13
BBS1(226)-BBS1(162)		1.80E-13	
BBS9-BBS9			
BBS9(218)-BBS9(226)	4.99E-05	2.16E-09	1.65E-05
BBS9(397)-BBS9(386)		9.08E-06	3.37E-06
BBS9(568)-BBS9(532)		2.02E-13	3.33E-12
BBS9(568)-BBS9(542)		2.19E-06	
BBS9(594)-BBS9(542)		2.24E-05	4.08E-07
BBS9(594)-BBS9(547)		1.22E-04	5.19E-06
BBS9(810)-BBS9(786)		8.17E-07	1.94E-05
BBS9(786)-BBS9(821)		4.70E-04	7.34E-04
BBS9(789)-BBS9(810)		3.17E-04	
BBS7-BBS7			
BBS7(31)-BBS7(48)		5.69E-09	5.34E-07
BBS7(48)-BBS7(25)		1.63E-04	4.47E-04
BBS7(25)-BBS7(92)		4.78E-04	
BBS7(78)-BBS7(95)		4.56E-18	2.17E-18
BBS7(352)-BBS7(56)		1.28E-10	
BBS7(574)-BBS7(682)		1.29E-09	1.17E-04
BBS7(574)-BBS7(687)		5.86E-06	3.74E-12
BBS7(352)-BBS7(359)			3.72E-11
BBS7(609)-BBS7(659)		3.64E-09	1.08E-06
BBS7(678)-BBS7(574)			8.79E-04
BBS7(680)-BBS7(687)		9.63E-06	5.17E-05
BBS2-BBS2			
BBS2(9)-BBS2(13)			7.92E-08
BBS8-BBS8			
BBS8(34)-BBS8(1)		1.21E-04	

Table S3, related to Figure 4. Summary of missense variants in BBSome subunit genes identified in patients with BBS (black font), non-syndromic retinitis pigmentosa (RP, red font) or Leber Congenital Amaurosis (LCA, red font) or non-syndromic obesity (cyan font).

Gene	Nucleotide	Protein	Phenotype	Reference
BBS1	c.479G>A	p.R160Q	BBS, RP	Deveault (2011) Hum Mutat 32, 610
				Sharon (2015) Mol Vis 21, 783
BBS1	c.664G>C	p.G222R	BBS	Esposito (2017) BMC Med Genet 18,
BBS1	c.670G>A	p.E224K	BBS	Redin (2012) J Med Genet 49, 502
BBS1	c.803G>C	p.R268P	BBS	Estrada-Cuzcano (2012) Arch Ophthalmol 130, 1425
BBS1	c.820A>T	p.I274F	Obesity	Gerhard (2013) Hum Hered 75, 144
BBS1	c.863T>G	p.L288R	BBS	Muller (2010) Hum Genet 127, 583
BBS1	c.890G>A	p.R297Q	BBS	Denniston (2014) Retina 34, 2282
BBS1	c.962T>C	p.L321P	BBS	Jean Muller, unpublished
BBS1	c.989T>C	p.I330T	BBS	Deveault (2011) Hum Mutat 32, 610
BBS1	c.1007T>C	p.L336P	BBS	Jean Muller, unpublished
BBS1	c.1061A>G	p.E354G	BBS	Kim (2015) Diabetes Metab J 39, 439
BBS1	c.1064T>G	p.V355G	Obesity	Gerhard (2013) Hum Hered 75, 144
BBS1	c.1097T>A	p.V366D	BBS	Alvarez-Satta (2014) Clin Genet 86, 601
BBS1	c.1125C>G	p.S375R	BBS	Jean Muller, unpublished
BBS1	c.1139G>A	p.R380Q	BBS	Suzuki (2016) Clin Genet 90, 526
BBS1	c.1163T>C	p.L388P	RP	Estrada-Cuzcano (2012) Arch Ophthalmol 130, 1425
BBS1	c.1169T>G	p.M390R	BBS	Mykytyn (2002) Nat Genet 31, 435
BBS1	c.1553T>C	p.L518P	BBS	Mykytyn (2003) Am J Hum Genet 72, 429
BBS1	c.1570_1572delAAC	p.N524del	BBS	Deveault (2011) Hum Mutat 32, 610

Gene	Nucleotide	Protein	Phenotype	Reference
BBS2	c.68G>C	p.R23P	BBS	Harville (2010) J Med Genet 47, 262
				Karmous-Benailly (2005) Am J Hum Genet 76, 493
BBS2	c.98C>A	p.A33D	RP	Shevach (2015) JAMA Ophthalmol 133, 312
BBS2	c.224T>G	p.V75G	BBS	Nishimura (2001) Hum Mol Genet 10, 865
BBS2	c.241G>T	p.G81C	BBS	Deveault (2011) Hum Mutat 32, 610
BBS2	c.311A>C	p.D104A	BBS	Katsanis (2001) Science 293, 2256
				Shevach (2015) JAMA Ophthalmol 133: 312
BBS2	c.334T>C	p.F112L	RP	de Castro-Miró (2016) PLoS One 11, e0168966
BBS2	c.374T>G	p.L125R	BBS	Billingsley (2010) J Med Genet 47, 453
BBS2	c.401C>G	p.P134R	RP	Shevach (2015) JAMA Ophthalmol 133, 312
BBS2	c.406G>C	p.A136P	BBS	Deveault (2011) Hum Mutat 32, 610
BBS2	c.416G>T	p.G139V	BBS	Laurier (2006) Eur J Hum Genet 14, 1195
BBS2	c.415G>A	p.G139S	BBS	Jean Muller, unpublished
BBS2	c.508G>A	p.D170N	BBS	Patel (2016) Genet Med 18, 554

BBS2	c.563T>C	p.I188T	BBS	Jean Muller, unpublished
BBS2	c.626T>C	p.L209P	BBS	Redin (2012) J Med Genet 49, 502
BBS2	c.662T>C	p.L221P	BBS	Muller (2010) Hum Genet 127, 583
BBS2	c.806T>G	p.V269G	BBS	Shaheen (2016) Genome Biol 17, 242
BBS2	c.899A>G	p.D300G	RP	Xu (2015) Mol Vis 21, 477
BBS2	c.921C>G	p.C307W	BBS	Deveault (2011) Hum Mutat 32, 610
BBS2	c.920G>A	p.C307Y	BBS	Muller (2010) Hum Genet 127, 583
BBS2	c.944G>A	p.R315Q	BBS	Katsanis (2001) Science 293, 2256
BBS2	c.943C>T	p.R315W	BBS	Katsanis (2001) Science 293, 2256
BBS2	c.947G>A	p.G316D	BBS	Janssen (2011) Hum Genet 129, 79
BBS2	c.950A>G	p.Y317C	BBS	Deveault (2011) Hum Mutat 32, 610
BBS2	c.1673C>T	p.T558I	BBS	Katsanis (2001) Science 293, 2256
BBS2	c.1895G>C	p.R632P	BBS, RP	Katsanis (2001) Science 293, 2256
				Bin (2009) Hum Mutat 30: E737
				Consugar (2015) Genet Med 17: 253
				Shevach (2015) JAMA Ophthalmol 133, 312

Gene	Nucleotide	Protein	Phenotype	Reference
BBS4	c.220G>A	p.A74T	BBS	Jean Muller, unpublished
BBS4	c.253G>C	p.E85Q	LCA	Wang (2011) Mol Vis 17, 3529
BBS4	c.322G>A	p.A108T	BBS	Glöckle (2014) Eur J Hum Genet 22, 99
BBS4	c.829G>A	p.G277R	BBS	Fattahi (2014) J Hum Genet 59, 368
BBS4	c.884G>C	p.R295P	BBS	Mykytyn (2001) Nat Genet 28, 188
BBS4	c.927T>G	p.N309K	BBS	Muller (2010) Hum Genet 127, 583
BBS4	c.1091C>A	p.A364E	BBS	Katsanis (2002) Am J Hum Genet 71, 22
BBS4	c.1103A>G	p.D368G	BBS	Karmous-Benailly (2005) Am J Hum Genet 76, 493

Gene	Nucleotide	Protein	Phenotype	Reference
BBS5	c.110T>C	p.I37T	BBS	Jean Muller, unpublished
BBS5	c.149T>G	p.L50R	BBS	Chen (2011) Invest Ophthalmol Vis Sci 52, 5317
BBS5				Redin (2012) J Med Genet 49: 502
BBS5	c.148C>A	p.L50I	RP	Wang (2014) Hum Genet 133, 331
BBS5	c.158C>T	p.T53I	BBS	Abu-Safieh (2012) Eur J Hum Genet 20, 420
BBS5	c.166A>G	p.R56G	BBS	Muller (2010) Hum Genet 127, 583
BBS5	c.214G>A	p.G72S	BBS	Hjortshoj (2008) Am J Med Genet A 146A, 517
BBS5	c.413G>A	p.R138H	BBS	Redin (2012) J Med Genet 49, 502
BBS5	c.532G>A	p.G178R	BBS	Shaheen (2016) Genome Biol 17, 242
BBS5	c.547A>G	p.T183A	BBS	Hjortshoj (2008) Am J Med Genet A 146A, 517
BBS5	c.790G>A	p.G264R	Obesity	Weisschuh (2016) PLoS One 11, e0145951
BBS5	c.955_957delGAA	p.E319del	BBS	Patel (2016) Genet Med 18, 554

Gene	Nucleotide	Protein	Phenotype	Reference
BBS7	c.187G>A	p.G63R	BBS	Bin (2009) Hum Mutat 30, E737
BBS7	c.198T>G	p.I66M	BBS	Harville (2010) J Med Genet 47, 262
BBS7	c.196A>T	p.I66F	BBS	Hichri (2005) Eur J Hum Genet 13, 607
BBS7	c.632C>T	p.T211I	BBS	Badano (2003) Am J Hum Genet 72, 650
BBS7	c.640G>A	p.G214R	BBS	Feuillan (2011) J Clin Endocrinol Metab 96, E528
BBS7	c.688T>C	p.W230R	BBS	Harville (2010) J Med Genet 47, 262
BBS7	c.716G>A	p.G239E	BBS	Jean Muller, unpublished
BBS7	c.728G>A	p.C243Y	RP	Wang (2013) J Med Genet 50, 674
BBS7			BBS	Shin (2015) Ann Lab Med 35: 181
BBS7	c.878A>C	p.Q293P	BBS	Janssen (2011) Hum Genet 129, 79
BBS7	c.949C>G	p.L317V	BBS	Ece Solmaz (2015) Eur J Med Genet 58, 689
BBS7	c.968A>G	p.H323R	BBS	Badano (2003) Am J Hum Genet 72, 650
BBS7	c.986G>T	p.G329V	BBS	Jean Muller, unpublished
BBS7	c.1037G>A	p.R346Q	BBS	Muller (2010) Hum Genet 127, 583
BBS7	c.1511G>A	p.R504K	BBS	de Pontual (2009) Proc Natl Acad Sci U S A 106, 13921
BBS7	c.1666A>C	p.S556R	BBS	Yang (2008) Mol Vis 14, 2304
BBS7	c.1786G>A	p.E596K	BBS	Harville (2010) J Med Genet 47, 262
BBS7	c.2015G>A	p.G672D	BBS	Jean Muller, unpublished

Gene	Nucleotide	Protein	Phenotype	Reference
BBS8	c.1317G>C	p.Q439H	RP	Goyal (2015) Clin Genet 10.1111/cge.12644
			BBS	Ullah (2017) Mol Vis 23:482
BBS8	c.1333C>A	p.Q445K	RP	van Huet (2015) Mol Vis 21, 461

Gene	Nucleotide	Protein	Phenotype	Reference
BBS9	c.242T>A	p.V81E	BBS	Muller (2010) Hum Genet 127, 583
BBS9	c.421G>A	p.G141R	BBS	Nishimura (2005) Am J Hum Genet 77, 1021
BBS9	c.462C>G	p.I154M	BBS	Muller (2010) Hum Genet 127, 583
BBS9	c.542C>G	p.P181R	BBS	Carrigan (2016) Sci Rep 6, 33248
BBS9	c.557A>G	p.Y186C	BBS	Jean Muller, unpublished
BBS9	c.727G>A	p.E243K	BBS	Jean Muller, unpublished
BBS9	c.974A>G	p.Q325R	BBS	Jean Muller, unpublished