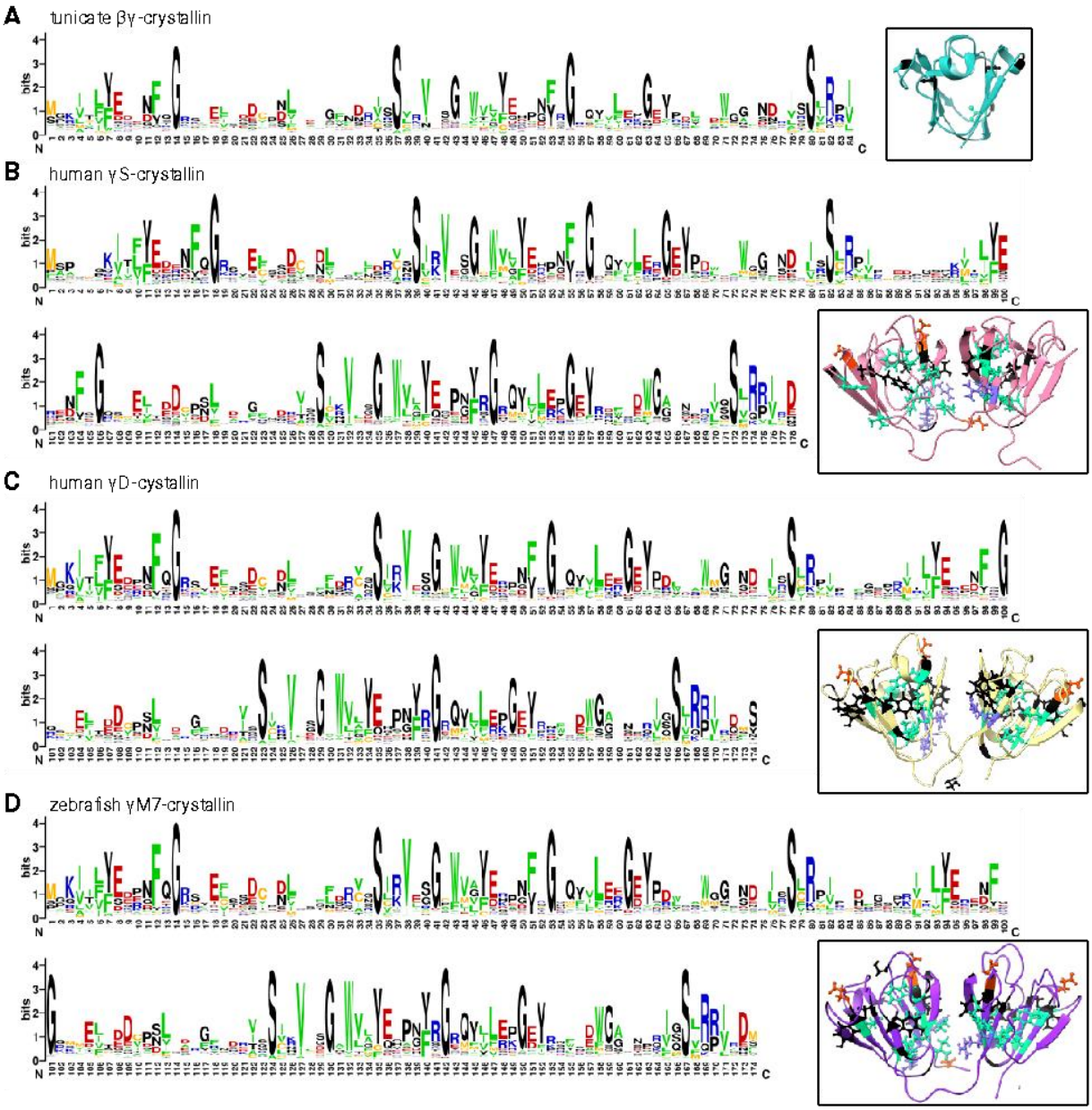

Figure S1. Multiple sequence alignment. The Deep MSA tool was used to identify conserved residues starting with **A** tunicate βγ-crystallin **B** human γS-crystallin **C** human γD-crystallin and **D** zebrafish γM7-crystallin. In all cases, the most common conserved residues are serine and glycine, along with the tryptophans commonly found in the hydrophobic core of each Greek key domain, the tyrosines associated with the tyrosine corners, and charged residues on the surface. Insets show the structure of each protein with strongly conserved residues highlighted. Cysteine and methionine are generally not among the more strongly conserved residues.

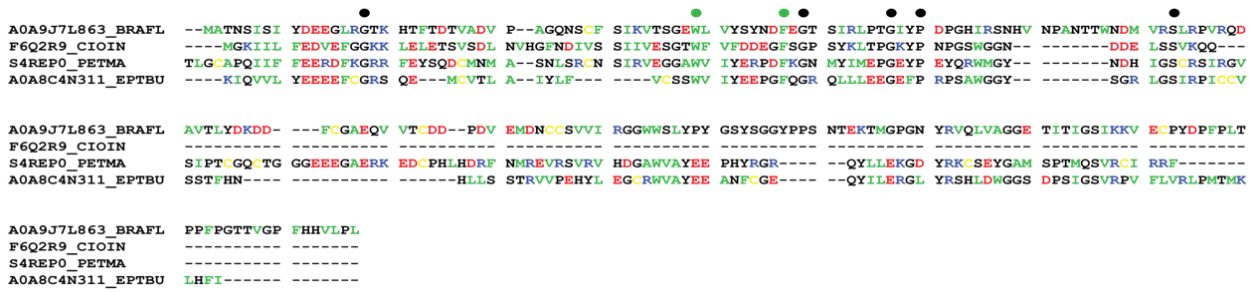

Figure S2. Sequence alignment for γ-crystallin Cluster 1 (βγ-crystallins).

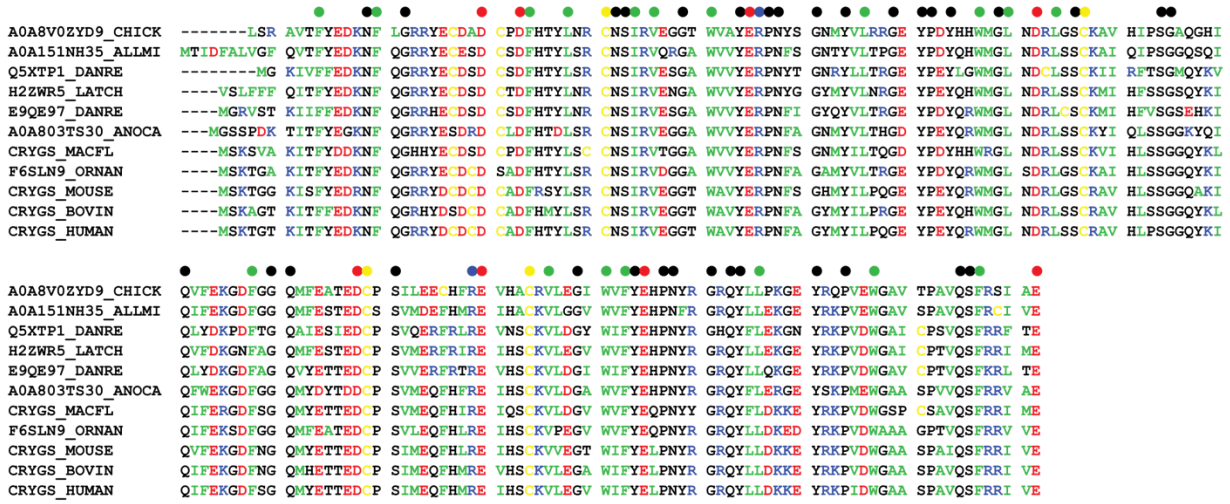

Figure S3. Sequence alignment for γ-crystallin Cluster 2 (γS-like crystallins).

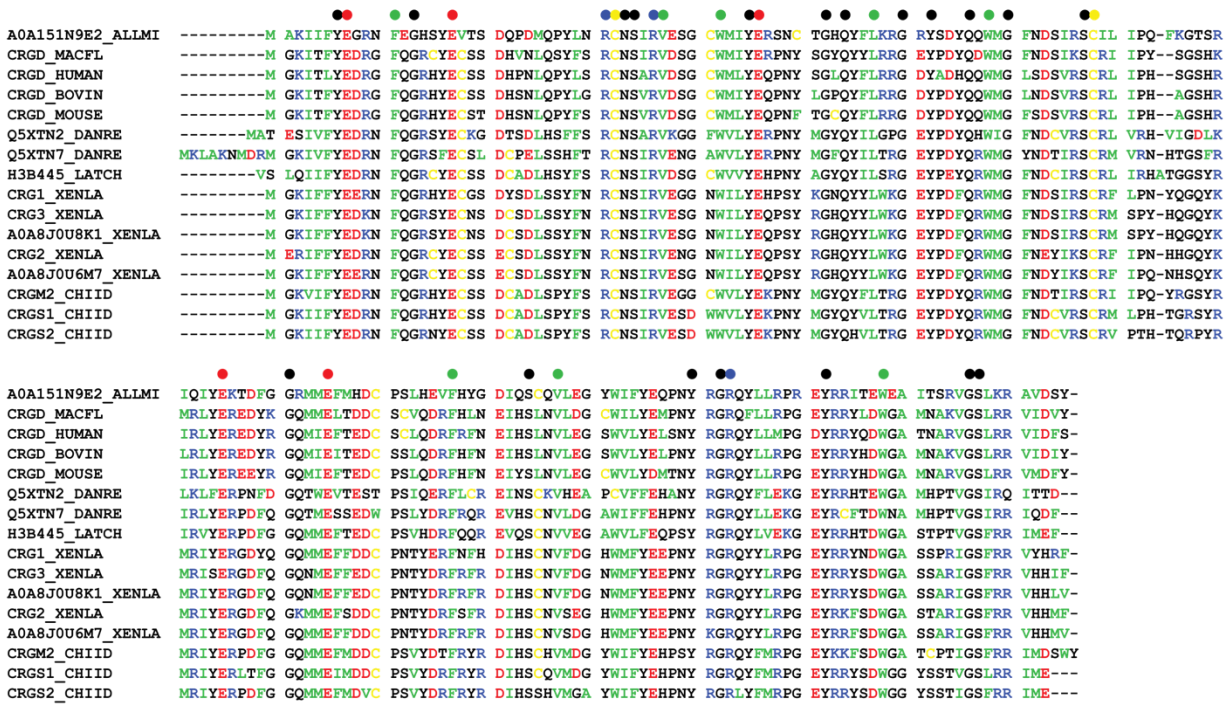

Figure S4. Sequence alignment for γ-crystallin Cluster 3 (γD-like crystallins.)

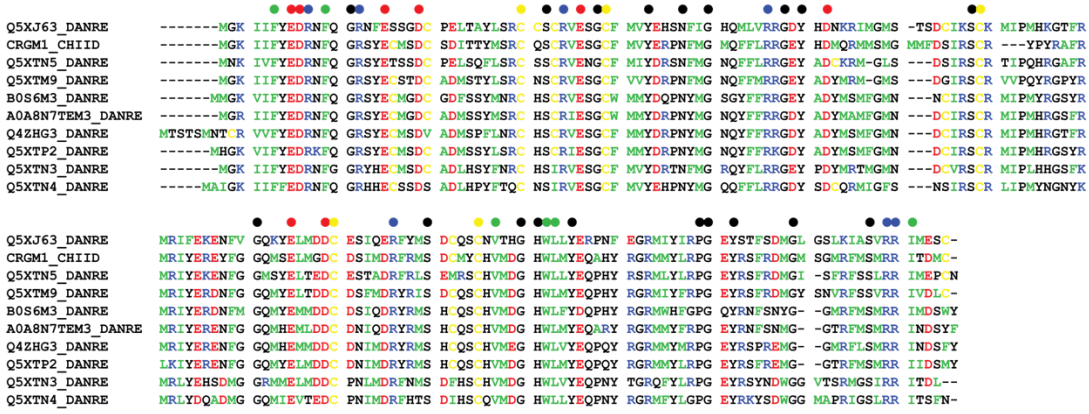

Figure S5. Sequence alignment for γ-crystallin Cluster 4 (γM-crystallins).

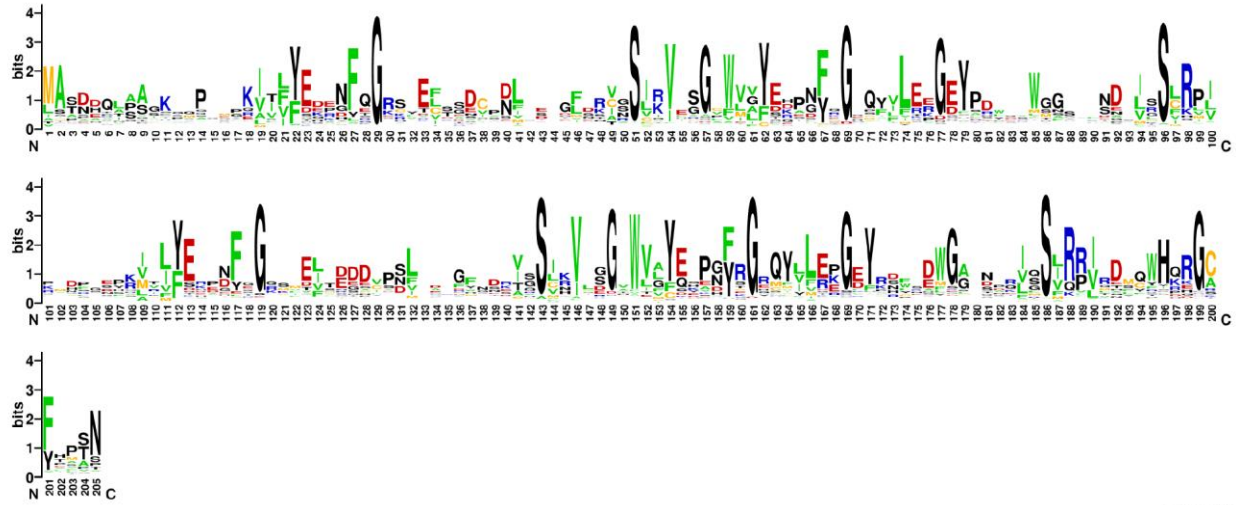

Figure S6. Multiple sequence alignment. The Deep MSA tool was used to identify conserved residues starting with human β2-crystallin. As for the γ-crystallins, the most common conserved residues are mostly serine, glycine, and aromatic residues, and not cysteine or methionine.

Table S1: Cysteine and methionine content of β-crystallins in representative vertebrates.

organism	protein	UniProt ID	method	length	Cys (no./%)	Met (no./%)
Mus musculus	βA1-crystallin	CRBA1_MOUSE	x	198	8/4.0	4/2.0
Ornithorhynchus anatinus	βA1-crystallin	F7BTY4_ORNAN	x	204	10/4.9	5/2.5
Danio rerio	βA1-crystallin	Q567D9_DANRE	x	199	11/5.5	8/4.0
Danio rerio	βA1-crystallin	Q6DH14_DANRE	x	196	7/3.6	9/4.6
Danio rerio	βA1b-crystallin	Q6DGY4_DANRE	x	198	12/6.0	12/6.0
Danio rerio	βA1c-crystallin	B5M4A7_DANRE	x	206	10/4.9	8/3.9
Latimeria chalumnae	βA1-crystallin	H3AXI2_LATCH	x	199	10/5.0	12/6.0
Latimeria chalumnae	βA1-crystallin-like	H2ZY80_LATCH	x	213	9/4.2	13/6.1
Anolis carolinensis	βA1-crystallin	H9GJE8_ANOCA	x	215	4/1.9	7/3.3
Gallus gallus	βA2-crystallin	CRBA2_CHICK	x	196	3/1.5	4/2.0
Alligator mississippiensis	βA2-crystallin	A0A151NRA2_ALLMI	x	208	4/1.9	3/1.4
Latimeria chalumnae	βA2-crystallin	H3B2W8_LATCH	x	213	7/3.3	6/2.8
Danio rerio	βA2-crystallin	Q6DGY2_DANRE	x	197	8/4.1	7/3.6
Danio rerio	βA2-crystallin	Q6IQU2_DANRE	x	197	8/4.1	9/4.6
Macropus fuliginosis	βA2-crystallin	CRBA2_MACFL	x	197	4/2.0	2/1.0
Ornithorhyncus anatinus	βA2-crystallin	A0A6I8NZG8_ORNAN	x	241	4/1.6	4/1.6
Bos taurus	βA2-crystallin	CRBA2_BOVIN	x	197	4/2.0	1/0.5
Mus musculus	βA2-crystallin	CRBA2_MOUSE	x	197	7/3.6	1/0.5
Homo sapiens	βA2-crystallin	CRBA2_HUMAN	x	197	6/3.0	1/0.5

Gallus gallus	βA3-crystallin	CRBA1_CHICK	x	215	7/3.3	7/3.3
Alligator mississippiensis	βA3-crystallin	A0A151N992_ALLMI	x	215	6/2.8	6/2.8
Homo sapiens	βA3-crystallin	CRBA1_HUMAN	x	215	5/2.3	6/2.8
Bos taurus	βA3-crystallin	CRBA1_BOVIN	x	215	8/3.7	6/2.8
Alligator mississippiensis	βA4-crystallin	A0A151MUM2_ALLMI	x	260	14/5.4	3/1.2
Gallus gallus	βA4-crystallin	CRBA4_CHICK	x	196	7/3.6	1/0.5
Homo sapiens	βA4-crystallin	CRBA4_HUMAN	x	196	5/2.5	2/1.0
Mus musculus	βA4-crystallin	CRBA4_MOUSE	x	196	4/2.0	2/1.0
Latimeria chalumnae	βA4-crystallin	H3ASM1_LATCH	x	205	9/4.4	7/3.4
Danio rerio	βA4-crystallin	Q52JI7_DANRE	x	196	11/5.6	5/2.6
Danio rerio	βB1-crystallin	A7E2K5_DANRE	x	221	9/4.1	15/6.8
Danio rerio	βB1-crystallin	E7F8M1_DANRE	x	219	9/4.1	16/7.3
Danio rerio	βB1-crystallin-like	A7E2I4_DANRE	x	208	8/3.8	12/5.8
Danio rerio	βB1-crystallin	Q6DGZ8_DANRE	x	232	9/3.9	12/5.2
Latimeria chalumnae	βB1-crystallin	H3A1B8_LATCH	x	219	10/4.6	14/6.4
Latimeria chalumnae	βB1-crystallin	H3AWX7_LATCH	x	211	10/4.7	10/4.7
Latimeria chalumnae	βB1-crystallin	H3AS10_LATCH	x	254	11/4.3	9/3.5
Gallus gallus	βB1-crystallin	CRBB1_CHICK	x	238	5/2.1	8/3.4
Alligator mississippiensis	βB1-crystallin	A0A151MUJ3_ALLMI	x	238	3/1.3	6/2.5
Bos taurus	βB1-crystallin	CRBB1_BOVIN	x	253	4/1.6	5/2.0
Mus musculus	βB1-crystallin	CRBB_MOUSE	x	250	4/1.6	5/2.0

Homo sapiens	βB1-crystallin	CRBB1_HUMAN	x	252	1/0.4	4/1.5
Latimeria chalumnae	βB2-crystallin	H3AY34_LATCH	x	205	4/2.0	1/0.5
Gallus gallus	βB2-crystallin	BRBB2_CHICK	x	219	3/1.4	2/0.9
Alligator mississippiensis	βB2-crystallin	A0A151NSD4_ALLMI	x	204	3/1.5	1/0.5
Macropus fuliginosis	βB2-crystallin	B2ZF66_MACFL	x	205	1/0.5	2/1.0
Ornithorhyncus anatinus	βB2-crystallin	F7A5Y5_ORNAN	x	187	3/1.6	1/0.5
Danio rerio	βB2-crystallin	Q52JI4_DANRE	x	208	6/2.9	1/0.5
Homo sapiens	βB2-crystallin	CRBB2_HUMAN	x	205	1/0.5	2/1.0
Bos taurus	βB2-crystallin	CRBB2_BOVIN	x	205	2/1.0	2/1.0
Mus musculus	βB2-crystallin	CRBB2_MOUSE	x	205	2/1.0	3/1.5
Danio rerio	βB3-crystallin	Q52JI3_DANRE	x	259	4/1.5	3/1.2
Latimeria chalumnae	βB3-crystallin	H3AXG5_LATCH	x	211	2/0.9	5/2.4
Gallus gallus	βB3-crystallin	CRBB3_CHICK	x	211	1/0.5	4/1.9
Ornithorhyncus anatinus	βB3-crystallin	F6Z4S4_ORNAN	x	211	3/1.4	1/0.5
Homo sapiens	βB3-crystallin	CRBB3_HUMAN	x	211	2/0.9	1/0.5
Mus musculus	βB3-crystallin	CRBB3_MOUSE	x	211	3/1.4	1/0.5
Bos taurus	βB3-crystallin	CRBB3_BOVIN	x	211	2/0.9	2/0.9

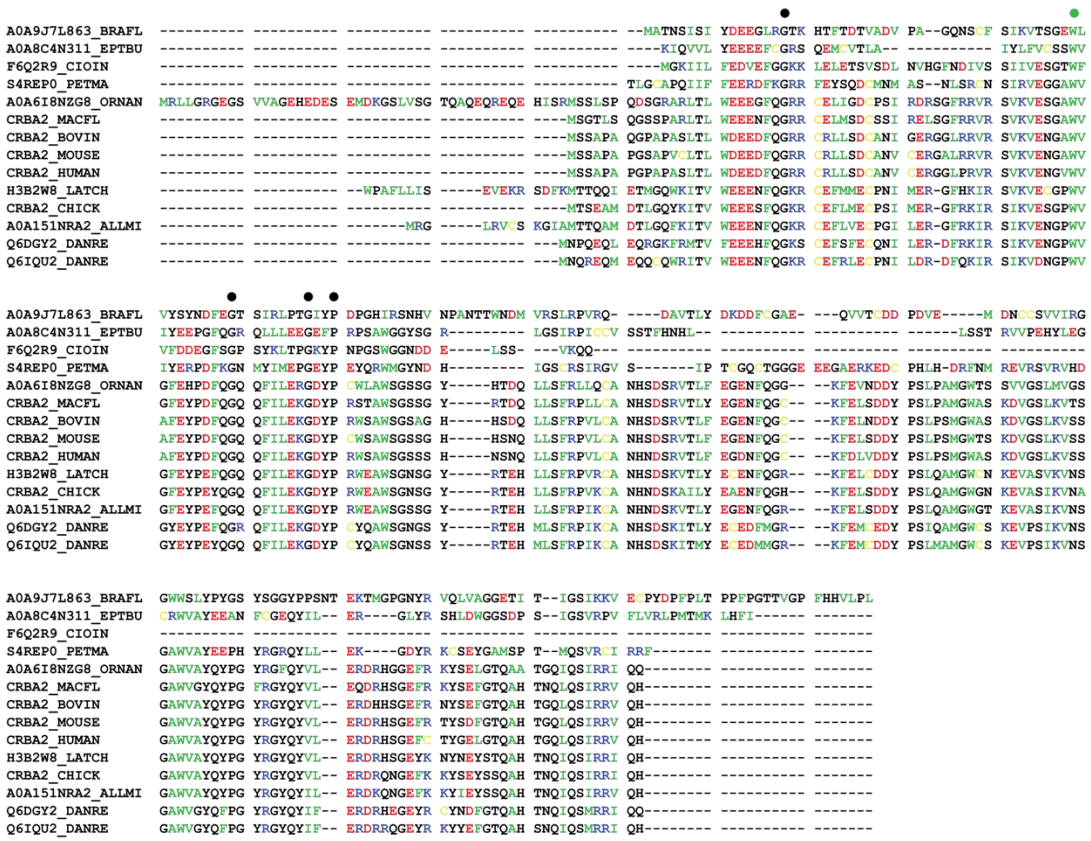

Figure S7. Sequence alignment for β-crystallin Cluster 1 (βγ and βA2-crystallins).

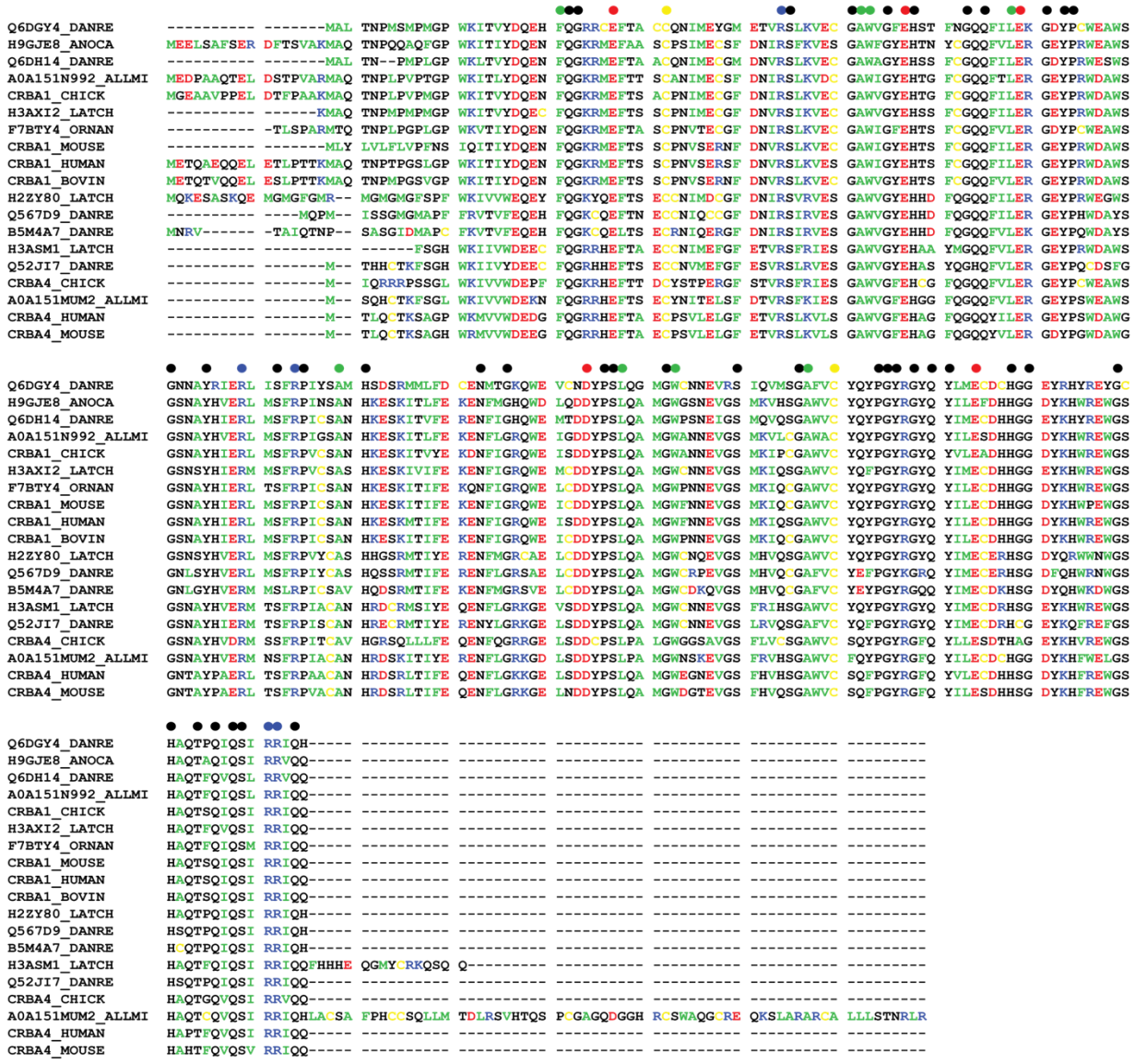

Figure S8. Sequence alignment for β-crystallin Cluster 2 (βA4-crystallins).

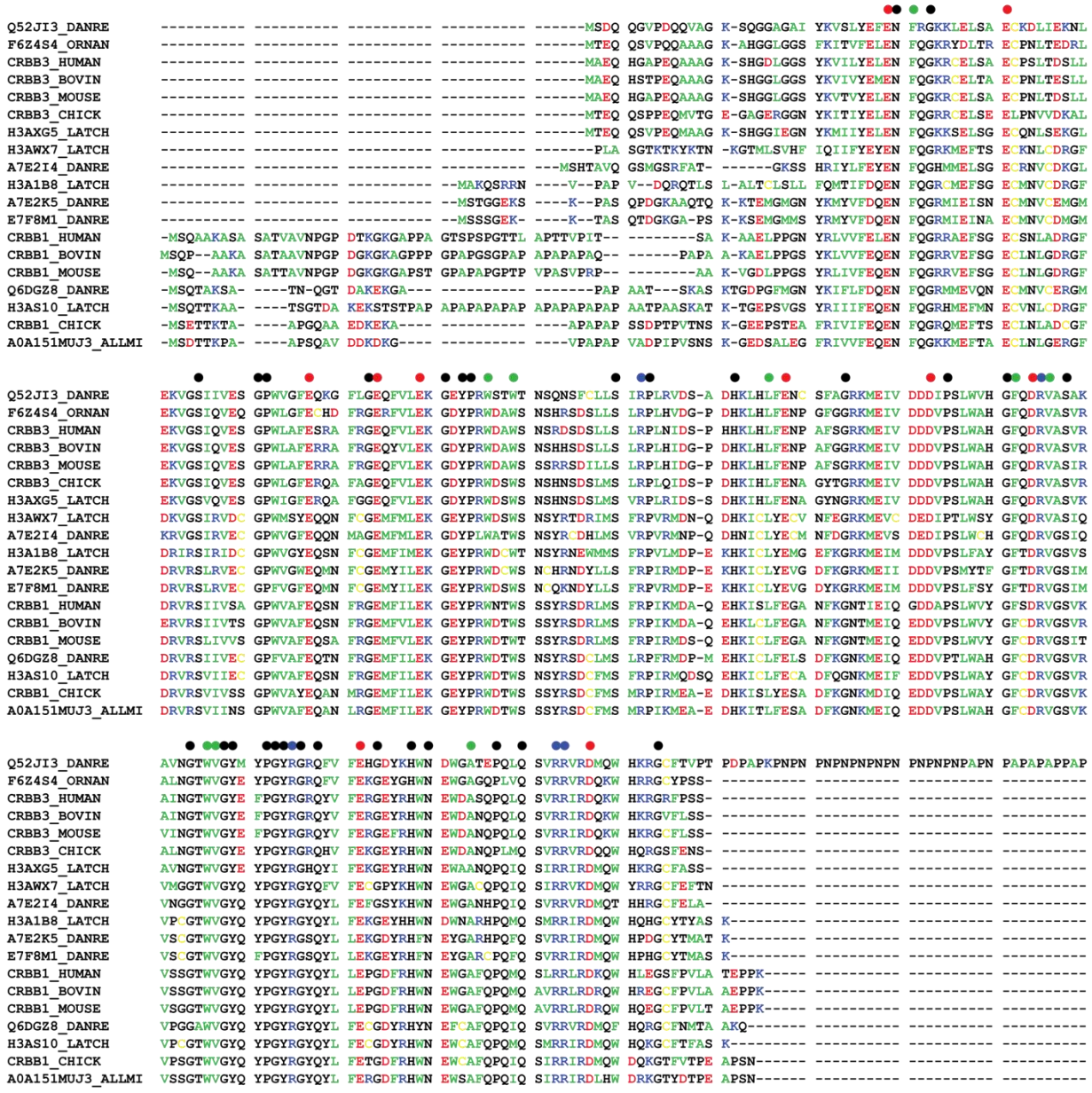

Figure S9. Sequence alignment for β-crystallin Cluster 3 (βB1- and βB3-crystallins). Q52JI3_DANRE has an additional 7 amino acids (SATAASS) that were truncated to limit the figure to one page.

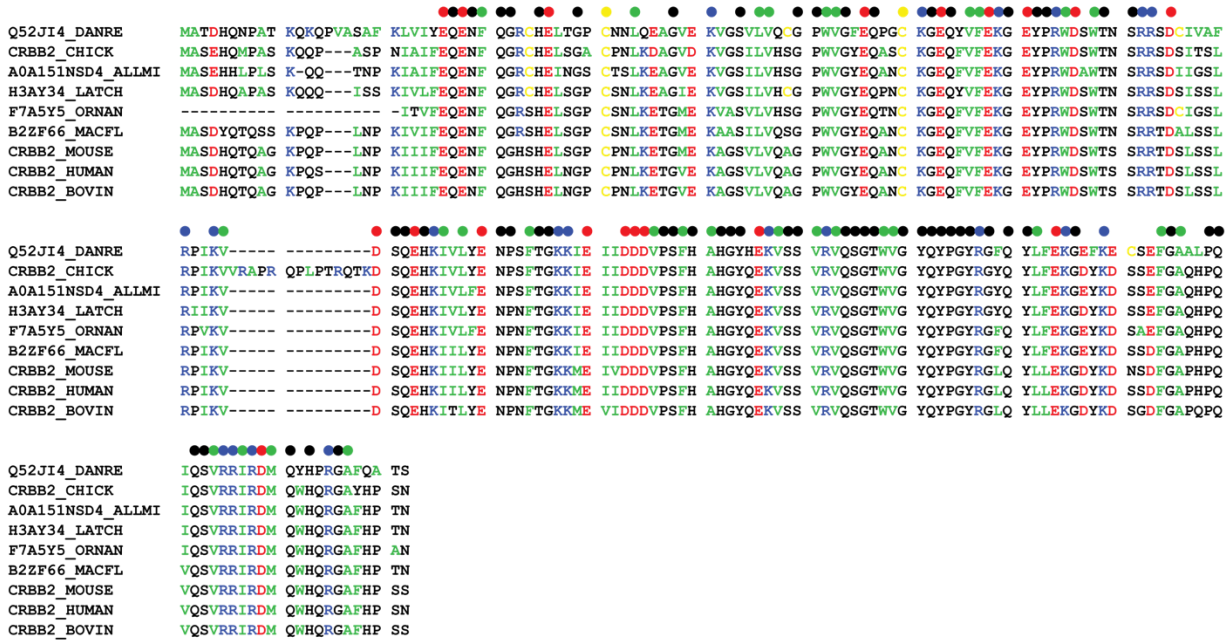

Figure S10. Sequence alignment for β-crystallin Cluster 4 (βB2-crystallins).