

Supplementary material for:

**The 1.7 Å Resolution Crystal Structure of AT2G44920, a Pentapeptide Repeat Protein in the Thylakoid Lumen of *Arabidopsis thaliana***

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Figure S1: Sequence analysis of AT2G44920 showing PRP motifs and secondary structure elements.

Figure S2: Electrostatic surface potential rendering of AT2G44920.

Figure S3: Multiple sequence alignment used for Consurf analysis.

Figure S4: Size exclusion chromatography analysis of AT2G44920.

Table S1: Consurf analysis results.

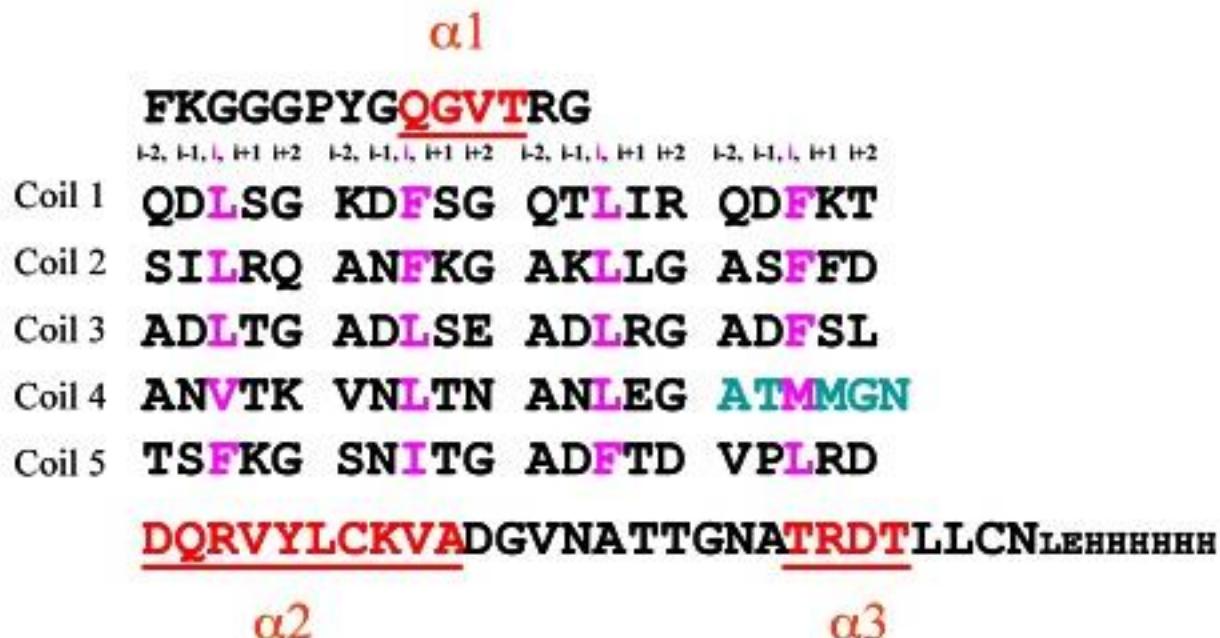


Figure S1. Amino acid sequence diagram of At2g44920.2 organized to highlight the arrangement of the pentapeptide repeat elements of the sequence in relation to the other secondary structural elements in the structure. The N-terminus begins with residue F82 of the full-length sequence just prior to  $\alpha 1$  and finishes with the residue C224 just prior to the C-terminal His tag sequence.

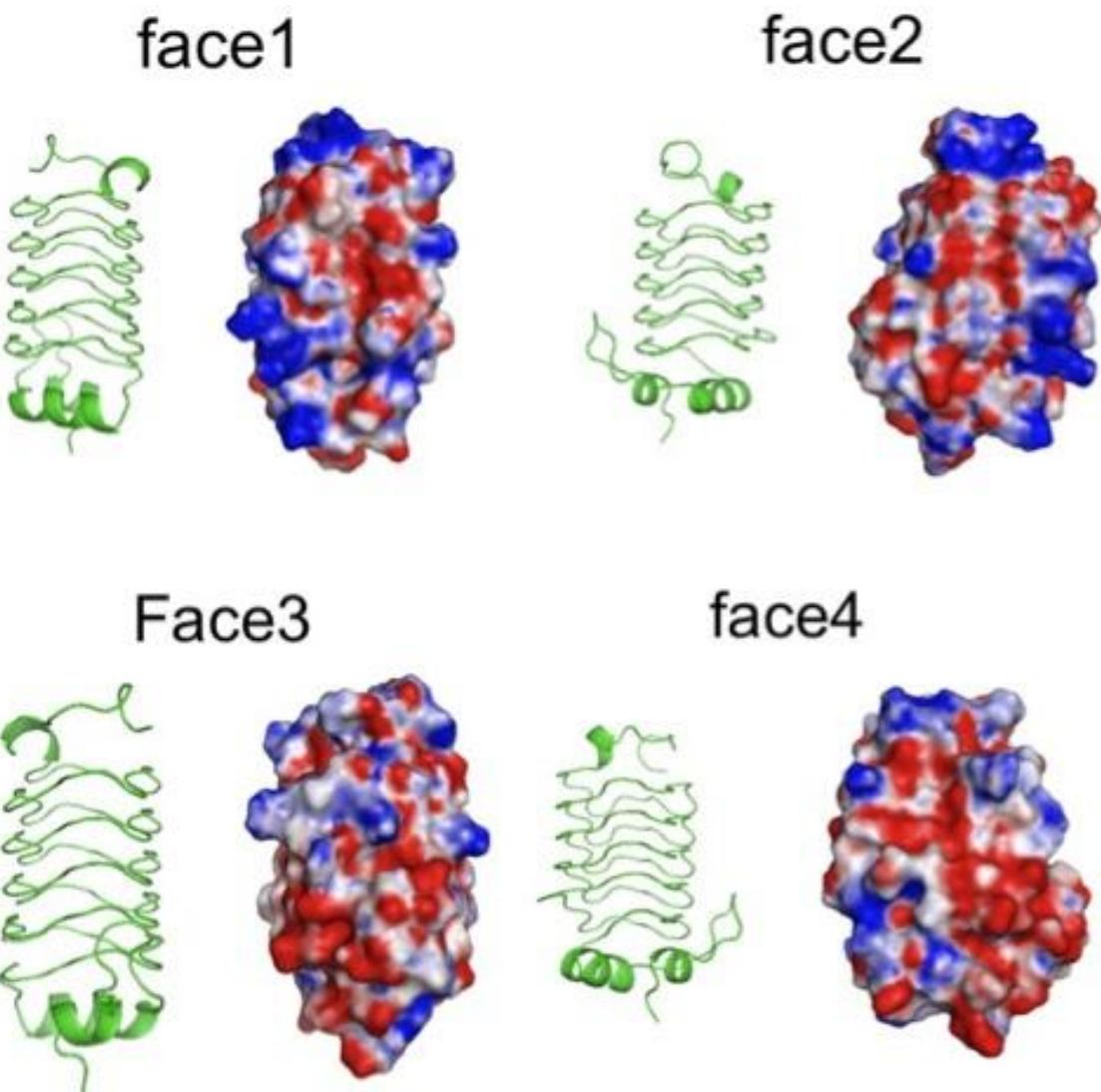
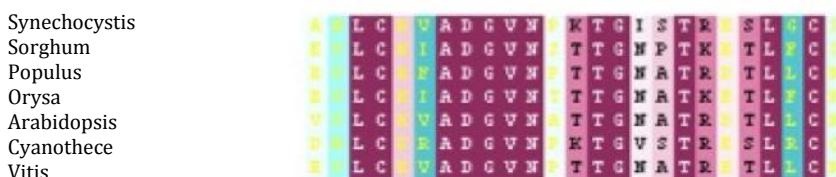


Figure S2. Electrostatic surface plots of four faces of AT2G44920. Plots were generated using the APBS (Baker et al., 2001) plug-in in Pymol (The PyMOL Molecular Graphics System, Version 1.3, Schrödinger, LLC.).

Baker NA, Sept D, Joseph S, Holst MJ, McCammon JA. Electrostatics of nanosystems: application to microtubules and the ribosome. Proc. Natl. Acad. Sci. USA 98, 10037-10041 2001.



1 2 3 4 5 6 7 8 9  
Variable Average Conserved

X - Insufficient data - the calculation for this site was performed on less than 10% of the sequences.

Figure S3. Multiple sequence alignment used for Consurf analysis. The genes and plant species included in the alignment are as follows: *Synechocystis* sp. PCC6803 (sll0577); *Sorghum bicolor* (sorghum) (SORBI 03g006310); *Populus trichocarpa* (black cottonwood) (POPTR 563930); *Oryza sativa japonica* (Japanese rice) (Os01g0144100); *Arabidopsis thaliana* (mouseear cress) (AT2G44920); *Cyanothecce* 51142 (RFR32); *Vitis vinifera* (wine grape) (Vvi 100244370).

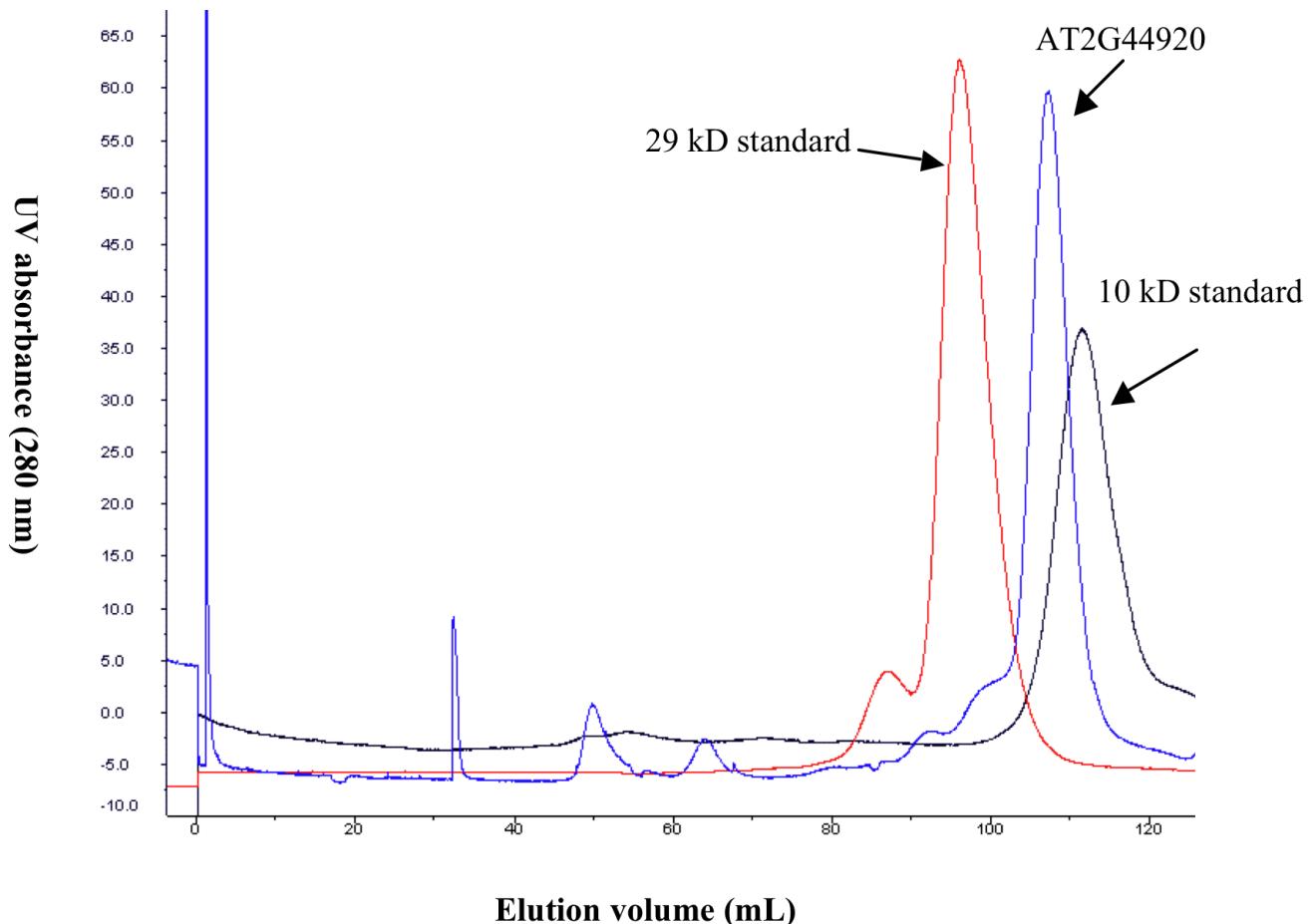


Figure S4. Overlay of SEC chromatogram of AT2G44920 with two protein standards (29 kD carbonic anhydrase and 10 kD 6XHis-tagged ubiquitin run with a flow rate of 1 ml/min on a preparative Superdex 200 column attached to AKTA (GE life science).

**Table S1.** Consurf analysis for multiple sequence alignment depicted in Figure S3.

Amino Acid Conservation Scores

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- POS: The position of the AA in the SEQRES derived sequence.
- SEQ: The SEQRES derived sequence in one letter code.
- 3LATOM: The ATOM derived sequence in three letter code, including the AA's positions as they appear in the PDB file and the chain identifier.
- SCORE: The normalized conservation scores.
- COLOR: The color scale representing the conservation scores (9 - conserved, 1 - variable).
- CONFIDENCE INTERVAL: When using the bayesian method for calculating rates, a confidence interval is assigned to each of the inferred evolutionary conservation scores.
- CONFIDENCE INTERVAL COLORS: When using the bayesian method for calculating rates. The color scale representing the lower and upper bounds of the confidence interval.
- MSA DATA: The number of aligned sequences having an amino acid (non-gapped) from the overall number of sequences at each position.
- RESIDUE VARIETY: The residues variety at each position of the multiple sequence alignment.

POS	SEQ	3LATOM	SCORE (normalized)	COLOR	CONFIDENCE INTERVAL	CONFIDENCE INTERVAL COLORS	MSA DATA	RESIDUE VARIETY
1	M	-	-0.344	6*	-1.258,-0.016	9,5	1/7	M
2	V	-	-0.344	6*	-1.258, 0.292	9,4	1/7	V
3	I	-	-0.344	6*	-1.258, 0.292	9,4	1/7	I
4	L	-	0.308	4*	-0.790, 0.704	8,2	2/7	M,L
5	S	-	1.176	1*	-0.260, 3.024	6,1	3/7	A,S,M
6	N	-	1.614	1*	0.292, 3.024	4,1	3/7	A,N,L
7	V	-	0.285	4*	-0.790, 0.704	8,2	3/7	L,V
8	S	-	0.313	4*	-0.790, 0.704	8,2	3/7	S,L
9	L	-	0.360	4*	-0.790, 1.339	8,1	3/7	N,L
10	F	-	0.234	4*	-0.925, 0.704	8,2	3/7	F,V
11	S	-	0.019	5*	-0.925, 0.704	8,2	3/7	S,Y
12	C	-	0.500	3*	-0.790, 1.339	8,1	3/7	C,L
13	C	-	0.206	4*	-0.925, 0.704	8,2	3/7	S,C
14	N	-	0.676	3*	-0.463, 1.339	7,1	3/7	S,T,N
15	I	-	0.377	4*	-0.790, 1.339	8,1	3/7	I,L
16	S	-	1.295	1*	-0.016, 3.024	5,1	3/7	S,I,L
17	Q	-	0.176	4*	-0.925, 0.704	8,2	3/7	Q,P
18	K	-	0.542	3*	-0.637, 1.339	7,1	3/7	K,P
19	P	-	1.881	1*	0.292, 3.024	4,1	3/7	F,P,K
20	S	-	0.122	5*	-0.925, 0.704	8,2	3/7	S,P
21	L	-	1.296	1*	-0.016, 3.024	5,1	3/7	H,I,L
22	F	-	0.945	2*	-0.260, 1.339	6,1	4/7	F,M,L
23	S	-	0.812	2*	-0.463, 1.339	7,1	4/7	A,S,K
24	P	-	0.644	3*	-0.637, 1.339	7,1	3/7	P,I
25	S	-	1.933	1*	0.292, 3.024	4,1	4/7	H,S,M,P
26	S	-	1.980	1*	0.292, 3.024	4,1	5/7	S,A,T,I,L
27	R	-	1.326	1*	-0.016, 3.024	5,1	5/7	S,T,R,I
28	S	-	1.867	1*	0.292, 3.024	4,1	5/7	F,S,K,L
29	S	-	0.794	2*	-0.463, 1.339	7,1	5/7	S,P,G
30	H	-	1.665	1*	0.292, 3.024	4,1	5/7	H,S,A,K
31	C	-	1.100	1*	-0.260, 3.024	6,1	5/7	C,P,L
32	P	-	1.819	1*	0.292, 3.024	4,1	5/7	H,S,P,K
33	I	-	0.786	2*	-0.463, 1.339	7,1	5/7	I,L
34	R	-	2.212	1*	0.704, 3.024	2,1	5/7	A,T,N,R,P
35	C	-	1.599	1*	0.292, 3.024	4,1	5/7	S,H,C,P
36	S	-	0.347	4*	-0.637, 0.704	7,2	5/7	S,C,L
37	Q	-	0.786	2*	-0.463, 1.339	7,1	5/7	Q,P,L
38	S	-	0.537	3*	-0.637, 1.339	7,1	5/7	F,S,P
39	Q	-	2.050	1*	0.704, 3.024	2,1	5/7	H,S,A,Q,L
40	E	-	2.151	1*	0.704, 3.024	2,1	5/7	A,S,N,E,L
41	G	-	1.938	1*	0.292, 3.024	4,1	5/7	F,A,T,G
42	K	-	1.514	1*	-0.016, 3.024	5,1	4/7	S,N,K,G
43	E	-	1.824	1*	0.292, 3.024	4,1	4/7	S,H,A,E
44	V	-	0.728	2*	-0.463, 1.339	7,1	4/7	A,C,V
45	V	-	2.003	1*	0.704, 3.024	2,1	4/7	T,D,P,V
46	T	-	1.268	1*	-0.016, 3.024	5,1	4/7	T,K,P
47	S	-	1.465	1*	-0.016, 3.024	5,1	5/7	S,A,Q,P
48	P	-	2.425	1*	0.704, 3.024	2,1	5/7	Q,T,P,G,V
49	L	-	2.055	1*	0.704, 3.024	2,1	5/7	A,S,N,R,L
50	R	-	1.457	1*	-0.016, 3.024	5,1	5/7	S,R,G,L
51	S	-	0.672	3*	-0.463, 1.339	7,1	5/7	S,I,P
52	V	-	1.561	1*	0.292, 3.024	4,1	5/7	A,N,P,V
53	V	-	2.198	1*	0.704, 3.024	2,1	5/7	F,S,N,R,V
54	W	-	1.893	1*	0.292, 3.024	4,1	5/7	S,W,D,K
55	S	-	0.442	3*	-0.637, 1.339	7,1	5/7	H,S,Q
56	L	-	0.106	5*	-0.790, 0.704	8,2	6/7	M,L,V
57	G	-	1.460	1*	-0.016, 3.024	5,1	6/7	H,G,L,V
58	E	-	2.089	1	0.704, 3.024	2,1	7/7	F,M,K,E
59	E	-	2.281	1	0.704, 3.024	2,1	7/7	S,H,D,K,E,V
60	V	-	1.594	1	0.292, 3.024	4,1	7/7	F,Y,L,V
61	S	-	1.240	1*	-0.016, 3.024	5,1	7/7	S,A,T,R,L
62	K	-	1.020	1*	-0.260, 1.339	6,1	7/7	Q,N,R,K,L
63	R	-	1.685	1	0.292, 3.024	4,1	7/7	A,S,T,R,G
64	S	-	1.492	1*	-0.016, 3.024	5,1	7/7	S,F,G,L
65	L	-	1.677	1	0.292, 3.024	4,1	7/7	F,A,T,L
66	F	-	1.052	1*	-0.260, 1.339	6,1	7/7	F,A,I,L,V
67	A	-	-0.881	8	-1.352,-0.637	9,7	7/7	A,V
68	L	-	0.119	5*	-0.790, 0.704	8,2	7/7	I,L,V
69	V	-	0.985	1*	-0.260, 1.339	6,1	7/7	F,I,L,V
70	S	-	-0.138	5*	-0.925, 0.292	8,4	7/7	S,A,V
71	A	-	-0.140	5*	-0.925, 0.292	8,4	7/7	A,L,V
72	S	-	-0.297	6*	-1.046,-0.016	9,5	7/7	S,C,L
73	L	-	-0.470	7*	-1.157,-0.016	9,5	7/7	W,L
74	F	-	0.663	3*	-0.463, 1.339	7,1	7/7	F,I,L



168	L	LEU168:A	-0.082	9	-1.520,-0.925	9.8	7/7	L
169	E	GLU169:A	-0.204	6*	-0.925, 0.292	8.4	7/7	A,T,E
170	G	GLY170:A	-0.564	7	-1.157,-0.260	9.6	7/7	G,E
171	A	ALA171:A	-1.247	9	-1.520,-1.157	9.9	7/7	A
172	T	THR172:A	0.025	5*	-0.790, 0.704	8.2	7/7	T,I,L
173	V	MET173:A	0.156	4*	-0.637, 0.704	7.2	7/7	A,M,V
174	T	MET174:A	-0.369	6*	-1.046,-0.016	9.5	7/7	M,T,L
175	G	GLY175:A	-0.640	7	-1.258,-0.260	9.6	7/7	R,G
176	N	ASN176:A	-0.994	9*	-1.520,-0.790	9.8	5/7	N
177	T	THR177:A	-1.265	9	-1.520,-1.157	9.9	7/7	T
178	S	SER178:A	-0.298	6*	-1.046,-0.016	9.5	7/7	S,T,K
179	F	PHE179:A	-1.077	9	-1.520,-0.925	9.8	7/7	F
180	K	LYS180:A	0.652	3*	-0.463, 1.339	7.1	7/7	D,R,K
181	G	GLY181:A	-0.082	5*	-0.925, 0.292	8.4	7/7	D,N,G
182	S	SER182:A	-0.560	7	-1.157,-0.260	9.6	7/7	S,A
183	N	ASN183:A	-0.382	6*	-1.046,-0.016	9.5	7/7	N,I,K
184	I	ILE184:A	-1.245	9	-1.520,-1.157	9.9	7/7	I
185	T	THR185:A	-0.248	6*	-1.046, 0.292	9.4	7/7	Q,T,Y
186	G	GLY186:A	-1.083	9	-1.520,-0.925	9.8	7/7	G
187	A	ALA187:A	-1.247	9	-1.520,-1.157	9.9	7/7	A
188	D	ASP188:A	-1.201	9	-1.520,-1.046	9.9	7/7	D
189	F	PHE189:A	-1.077	9	-1.520,-0.925	9.8	7/7	F
190	T	THR190:A	-0.942	8	-1.352,-0.790	9.8	7/7	S,T
191	D	ASP191:A	-0.354	6*	-1.046,-0.016	9.5	7/7	D,L
192	V	VAL192:A	-0.884	8	-1.352,-0.637	9.7	7/7	A,V
193	P	PRO193:A	-0.440	7*	-1.157,-0.016	9.5	7/7	P,V
194	L	LEU194:A	-1.082	9	-1.520,-0.925	9.8	7/7	L
195	R	ARG195:A	-0.465	7*	-1.157,-0.016	9.5	7/7	D,R
196	D	ASP196:A	0.877	2*	-0.260, 1.339	6.1	7/7	T,D,E,V
197	D	ASP197:A	-0.181	6*	-0.925, 0.292	8.4	7/7	D,Y,E
198	Q	GLN198:A	-0.812	8	-1.352,-0.637	9.7	7/7	Q,E
199	R	ARG199:A	-0.132	5*	-0.925, 0.292	8.4	7/7	R,I,V
200	V	VAL200:A	0.341	4*	-0.637, 0.704	7.2	7/7	A,D,E,V
201	Y	TYR201:A	0.770	2*	-0.463, 1.339	7.1	7/7	A,K,Y
202	L	LEU202:A	-1.082	9	-1.520,-0.925	9.8	7/7	L
203	C	CYS203:A	-1.052	9	-1.520,-0.790	9.8	7/7	C
204	K	LYS204:A	-0.462	7*	-1.157,-0.016	9.5	7/7	D,K
205	V	VAL205:A	1.090	1*	-0.260, 3.024	6.1	7/7	F,R,I,V
206	A	ALA206:A	-1.247	9	-1.520,-1.157	9.9	7/7	A
207	D	ASP207:A	-1.201	9	-1.520,-1.046	9.9	7/7	D
208	G	GLY208:A	-1.083	9	-1.520,-0.925	9.8	7/7	G
209	V	VAL209:A	-1.237	9	-1.520,-1.157	9.9	7/7	V
210	N	ASN210:A	-1.258	9	-1.520,-1.157	9.9	7/7	N
211	A	ALA211:A	0.213	4*	-0.637, 0.704	7.2	7/7	A,S,T,P
212	T	THR212:A	-0.792	8	-1.258,-0.463	9.7	7/7	T,K
213	T	THR213:A	-1.265	9	-1.520,-1.157	9.9	7/7	T
214	G	GLY214:A	-1.083	9	-1.520,-0.925	9.8	7/7	G
215	N	ASN215:A	-0.313	6	-0.925,-0.016	8.5	7/7	N,I,V
216	A	ALA216:A	-0.525	7	-1.157,-0.260	9.6	7/7	A,S,P
217	T	THR217:A	-1.265	9	-1.520,-1.157	9.9	7/7	T
218	R	ARG218:A	-0.801	8	-1.258,-0.463	9.7	7/7	K,R
219	D	ASP219:A	-0.250	6*	-0.925, 0.292	8.4	7/7	D,E
220	T	THR220:A	-0.942	8	-1.352,-0.790	9.8	7/7	S,T
221	L	LEU221:A	-1.082	9	-1.520,-0.925	9.8	7/7	L
222	L	LEU222:A	1.148	1*	-0.260, 3.024	6.1	7/7	F,R,G,L
223	C	CYS223:A	-1.052	9	-1.520,-0.790	9.8	7/7	C
224	N	ASN224:A	0.688	3*	-0.463, 1.339	7.1	7/7	S,Q,N,K

\*Below the confidence cut-off - The calculations for this site were performed on less than 6 non-gapped homologue sequences, or the confidence interval for the estimated score is equal to- or larger than- 4 color grades.