Supplementary Materials and Methods

Initial tree

The full alignment of the members of the PFAM Vps51 (PF08700, http://pfam.sanger.ac.uk/family?entry=PF08700) family was retrieved and a neighbour-joining (NJ) phylogenetic tree reconstructed. The *Caenorhabditis elegans* Vps51 (FFR_CAEEL), together with the human functional ortholog (FFR_HUMAN), were found to be present in a monophyletic clade, albeit without bootstrap support (A). All other sequences annotated as FFR (fat-free homolog) were also present in that clade. To reduce the dataset to the functional Vps51 ortholog group, all member sequences from the monophyletic Vps51 clade were selected, except if they represented redundant versions of the same sequence (identical sequences from different organisms were kept). To provide a suitable outgroup, all sequences annotated as EXO (exocyst complex component) were also selected (A).

Detailed phylogenetic analyses

Based on this refined dataset, in-depth phylogenetic inference was carried out. Using ProtTest (Abascal et al., 2005), the most suitable model was found to be JTT+G+F. Phylogenetic trees were inferred by NJ using quicktree_sd (Howe et al., 2002; Frickenhaus and Beszteri, 2008) with 1000 bootstrap resamplings, maximum likelihood (ML, Phyml as implemented in ProtTest) and Bayesian inference using MrBayes (Ronquist and Huelsenbeck, 2003) with eight gamma distributed rate categories, the JTT+G+F model and five million generations (converging at a standard deviation of 0.07). Trees were displayed using Figtree v1.2.2 (http://tree.bio.ed.ac.uk/software/figtree/).

In both the NJ (B) and BI tree, the majority of EXO sequences is present in a long-branched clade that was used to root the tree. In both cases, The *C. elegans* and *Dictyostelium discoideum* sequences are not part of that clade, but branch of basal within the Vps51 clade.

The majority of nodes is not well supported. The metazoan FFR clade has low bootstrap support in the NJ tree (B) and contains some sequences from protists. In the BI analysis, the metazoan clade is well supported and does not contain protist sequences. However, it lacks the *C. elegans* and *C. briggsae* sequences. The high divergence of the domain makes confident phylogenetic inference impossible. The lack of support for the majority of branches is most probably due to long branch effects.

In order to overcome these problems and to analyse the metazoan FFR clade in mode detail, a full length alignment of these sequences was constructed using M.A.F.F.T. (Katoh et al., 2005), using the *D. discoideum* FFR sequence as an outgroup. Visual inspection of the alignment using Jalview (Clamp et al., 2004) demonstrated a high quality alignment with no need for manual refinement. The BI tree fulfilled the convergence criterion (average standard deviation of split frequencies < 0.01) after only 101,000 generations; the support for the majority of nodes is good (C). In general, the tree resolves the taxonomy; the flies (Diptera) are very well supported, as are the bony vertebrates (Euteleostomi, including the mammals, frogs, birds and fishes). The clade that is monophyletic with these both groups contains Coelomata, Cnidaria and Placozoa; the *Ixodes scapularis* (Black-legged tick) sequence is apparently difficult to place, probably due to its long branch. The two basal branches contain the Pseudocoelomata (the two *Caenorhabditis* sequences) and the Acoelomata. The somewhat lesser amount of support for the lower branches is probably due to the fact that most taxonomic groups are represented by a single sequence only.

Sequence length and domain structure

The average length of the metazoan FFR/Vsp51 proteins is 729 residues (shortest sequence 261, longest sequence 917). It should be noted that a large proportion of the sequences are predicted (C), lacking transcript or protein evidence, so that parts might be missing; in addition, there are two sequences annotated as being fragmentary. Prime candidates for

incomplete sequences are those from *Branchiostoma floridae* (261), *Trichoplax adhaerens* (495) and *Ixodes scapularis* (545), especially as the sequences from the Acoelomata and Pseudocoelomata are longer. The longest sequence is from the outgroup, the Mycetozoan *D. discoideum*.

Motif discovery on the dataset was carried out using MEME v4.3.0 (Bailey et al., 2009). Settings (distribution of motif occurrences: zero or one per sequence, number of different motifs: ten, minimum motif width: six, maximum motif width: thirty) were chosen based on visual inspection of the alignment. The detected motif 1 (D) covers positions 38-67 of the PFAM Vps51 HMM, representing the most highly conserved core of the domain. The presence of the motifs 1, 7 and 8 (E, F) in the N-terminal part of all proteins is the unifying factor, this region represents the Vps51 core domain. Motif 9, N-terminal of this arrangement, is present in all but two sequences (*Nematostella vectensis* and *T. adhaerens*). Motif 4, C-terminal of the core block, is present in all sequences but the *C. elegans* Vps51. It is apparent from the motif arrangement that the exceptional length of the *D. discoideum* sequence is due to an N-terminal extension.

The middle region of the proteins contains some occurences of motifs out of their canonic place. Specifically, motif 7 (in two *Drosophila* sequences and in *D. discoideum*) and motifs 1 (*Caenorhabditis*), 2 (chicken), 6 (mouse) and 9 (*Aedes aegypti*) appear as duplicates within the same sequence. The obviously secondarily derived arrangements make a certain plasticity of the genes evident. Located C-terminal of the middle region are motifs 10 (present in all but four sequences) and 3 (present in all but three sequences). The pattern of presence and absence suggests that these motifs were secondarily lost from the different lineages, including a common ancestor of the genus Caenorhabditis. Motif 7, immediately behind 3, is present in this place as an additional copy in mammals and amphibians only. Given the dissipate pattern described for this motif above, it appears plausible that it was transferred to this location in the last common ancestor of tetrapods and secondarily lost in birds.

The immediate C-terminus consists of the motifs 5, 2 and 6. These motifs are present in all sequences but three (*T. adhaerens*, *Ixodes scapularis* and *B. floridae*), resp. motif 6 lacking from *Schistosoma mansonii*. Again, the presence/absence pattern in light of the taxonomy suggests secondary losses of these motifs from the respective lineages. However, given that the three sequences lacking all these motifs are the shortest ones present, it might also be possible that the gene prediction is incomplete.

Taken together, the presence of the conserved core domain located in the N-terminal part of the protein is expected to be crucial for ist function. The additonal motifs might be necessary for interactions or functions that are obligatory. The C-terminal set of motifs might also be important, pending closer study of the genomic loci within those organisms that seem to lack them.

Literature cited

Abascal F, Zardoya R, Posada D (2005) ProtTest: selection of best-fit models of protein evolution. Bioinformatics **21:** 2104-2105

Bailey TL, Boden M, Buske FA, Frith M, Grant CE, Clementi L, Ren J, Li WW, Noble WS (2009) MEME SUITE: tools for motif discovery and searching. Nucleic Acids Res **37:** W202-208

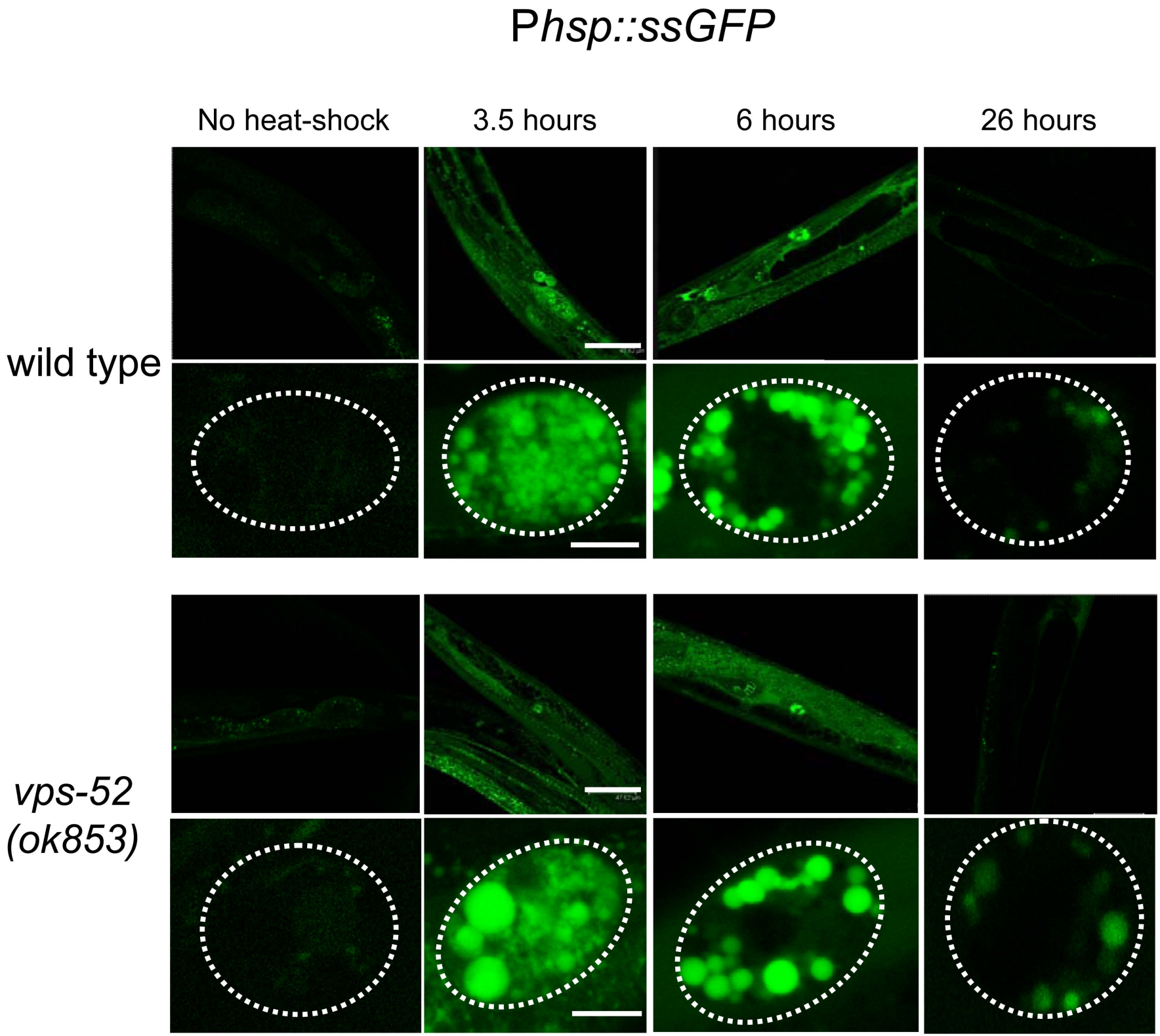
Clamp M, Cuff J, Searle SM, Barton GJ (2004) The Jalview Java alignment editor. Bioinformatics **20:** 426-427

Frickenhaus S, Beszteri B (2008) Quicktree-SD, Software developed by AWI-Bioinformatics. *In*,

Howe K, Bateman A, Durbin R (2002) QuickTree: building huge Neighbour-Joining trees of protein sequences. Bioinformatics **18:** 1546-1547

Katoh K, Kuma K, Toh H, Miyata T (2005) MAFFT version 5: improvement in accuracy of multiple sequence alignment. Nucleic Acids Res **33:** 511-518

Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics **19:** 1572-1574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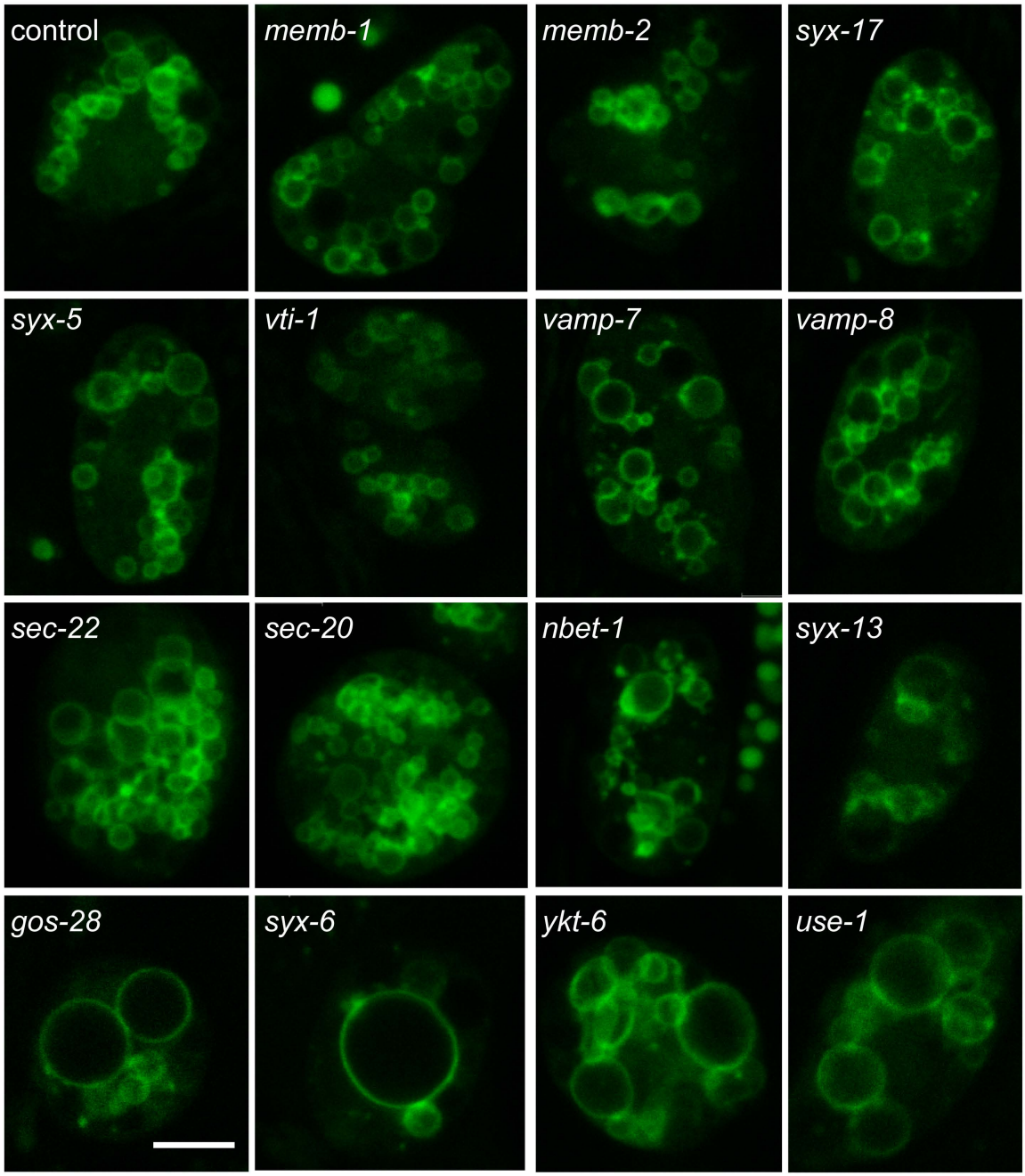
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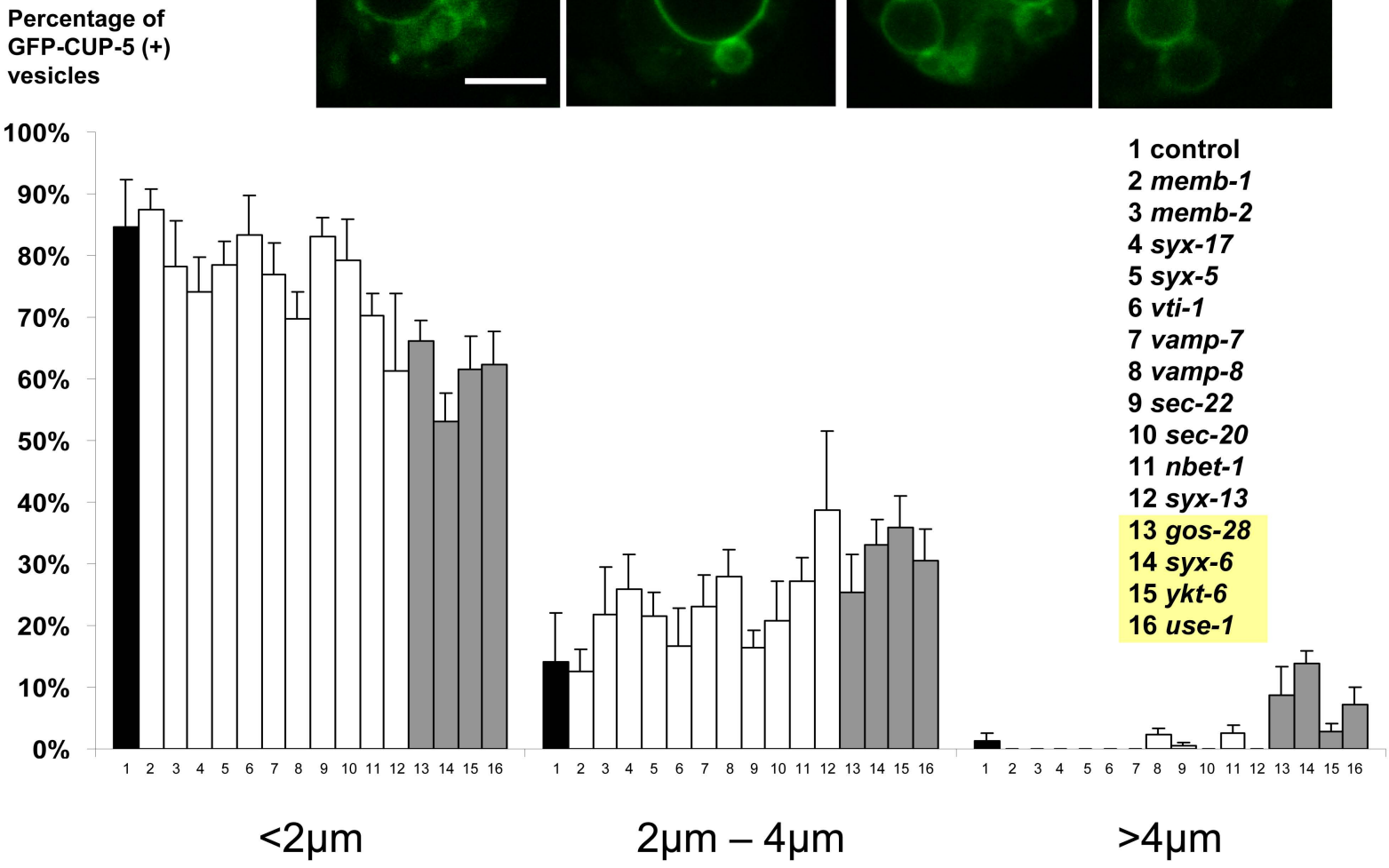
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2 memb-1
3 memb-2
4 syx-17
5 syx-5
6 vti-1
7 vamp-7
8 vamp-8
9 sec-22
10 sec-20
11 nbet-1
12 syx-13
13 gos-28
14 syx-6
15 ykt-6
16 use-1
<2µm
2µm – 4µm
>4µm

Table S1. RNAi phenotype of SNAREs in GARP mutants background

Gene of RNAi	Background		
	eri-1 (mg366)	*vps-52(ok853);eri-1(mg366)*	*vps-54(ok1463);eri-1(mg366)*
syx-5	Let	Let	Let
syx-6	NG	NG	NG
syx-13	NG	NG	NG
syx-17	NG	NG	NG
vamp-7	NG	NG	NG
vamp-8	NG	NG	NG
ykt-6	Slo	Slo	Slo
vti-1	NG	NG	NG
nbet-1	Let	Let	Let
gos-28	NG	NG	NG
memb-1	NG	NG	NG
memb-2	NG	NG	NG
sec-20	Slo	Slo	Slo
sec-22	NG	NG	NG
use-1	Slo	Slo	Slo

Let: lethal

Slo: slow growth

NG: normal growth

Fig. 1SA

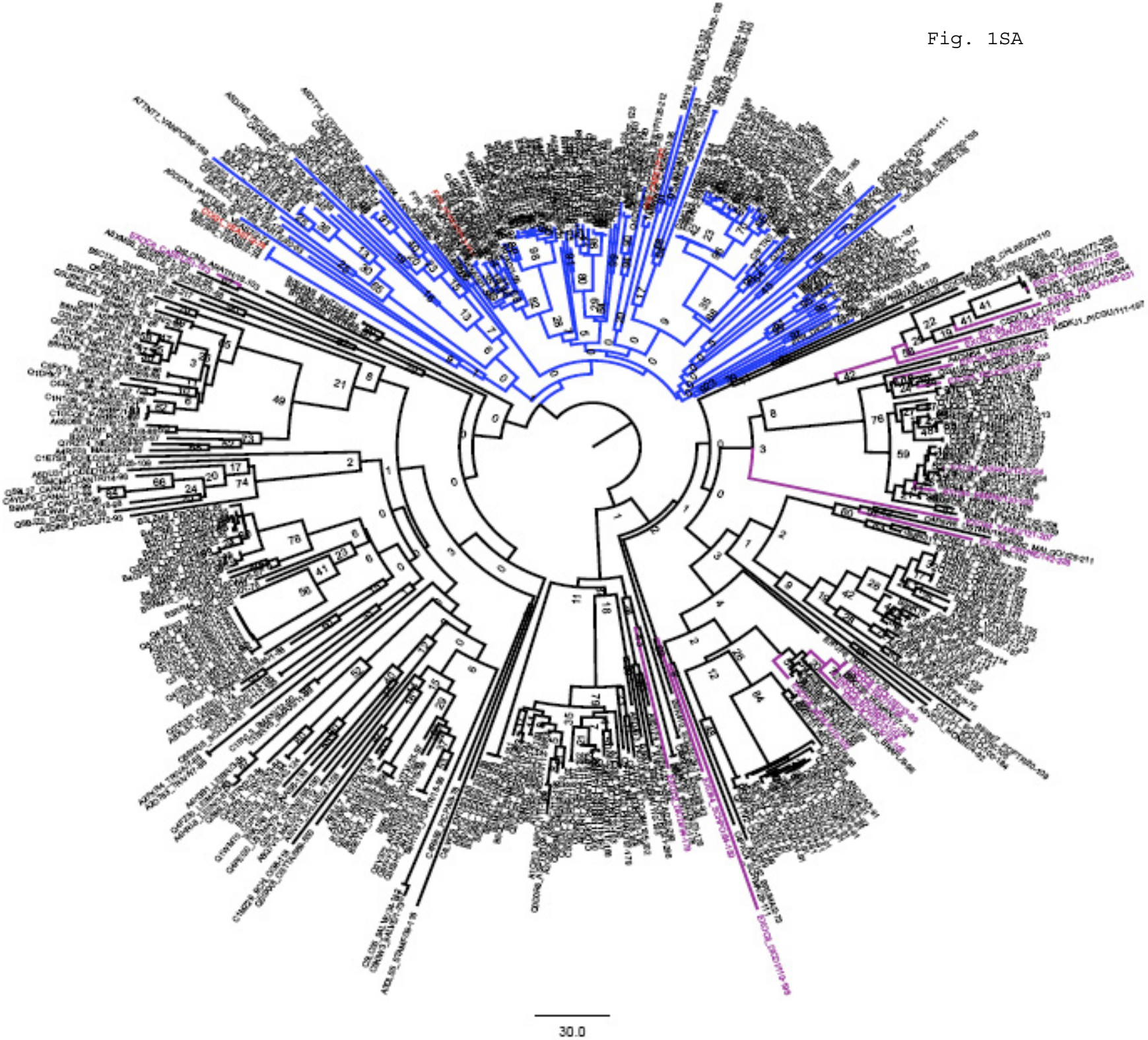

Fig. 1SB

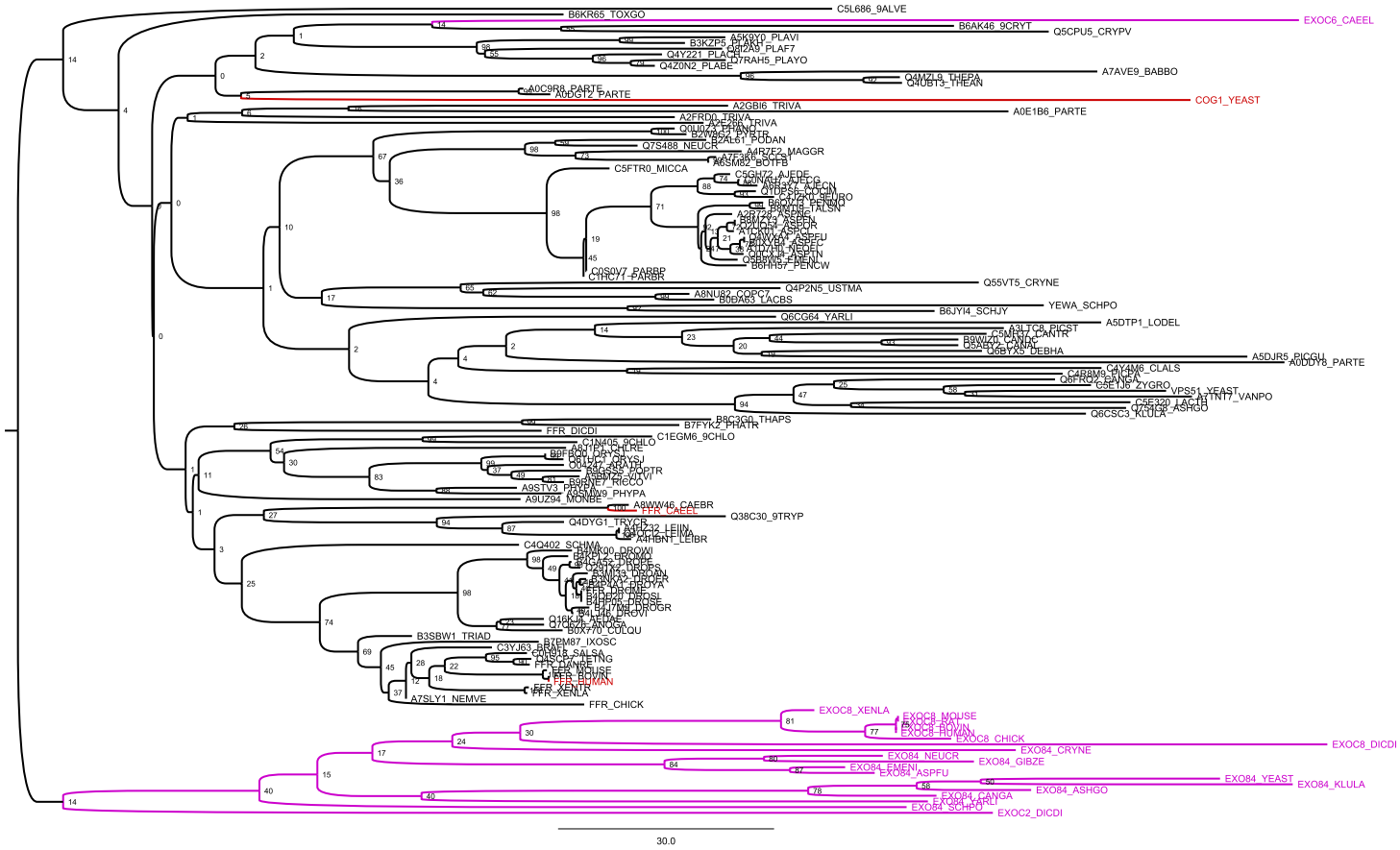
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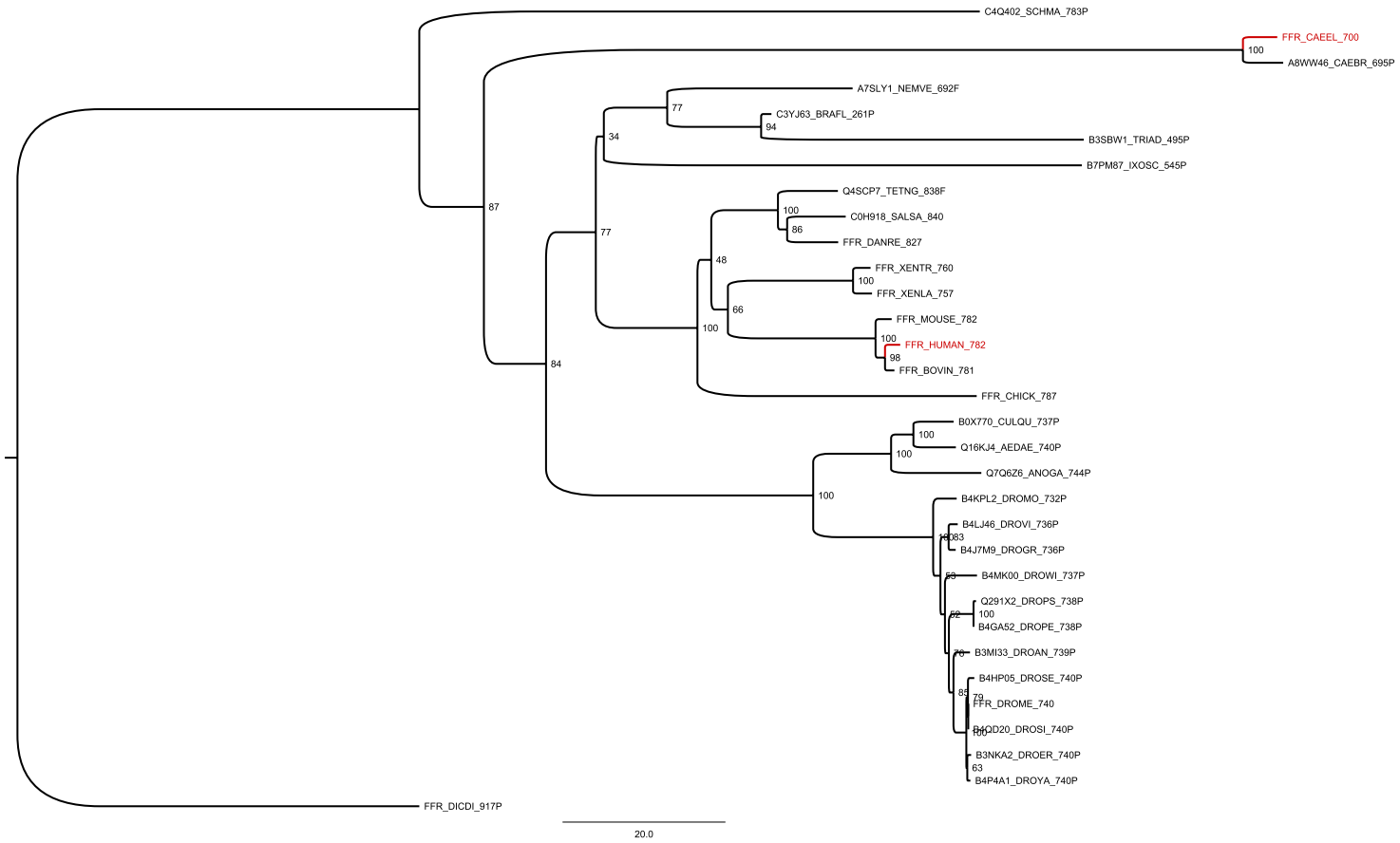
Fig. 1SC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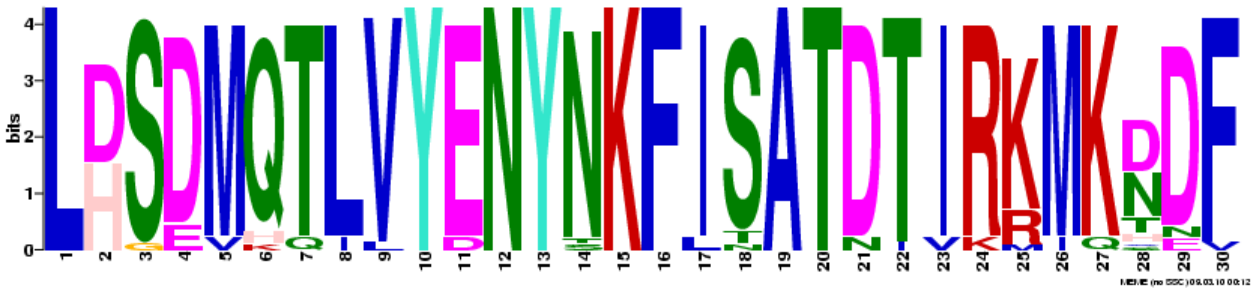
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Fig. S1E

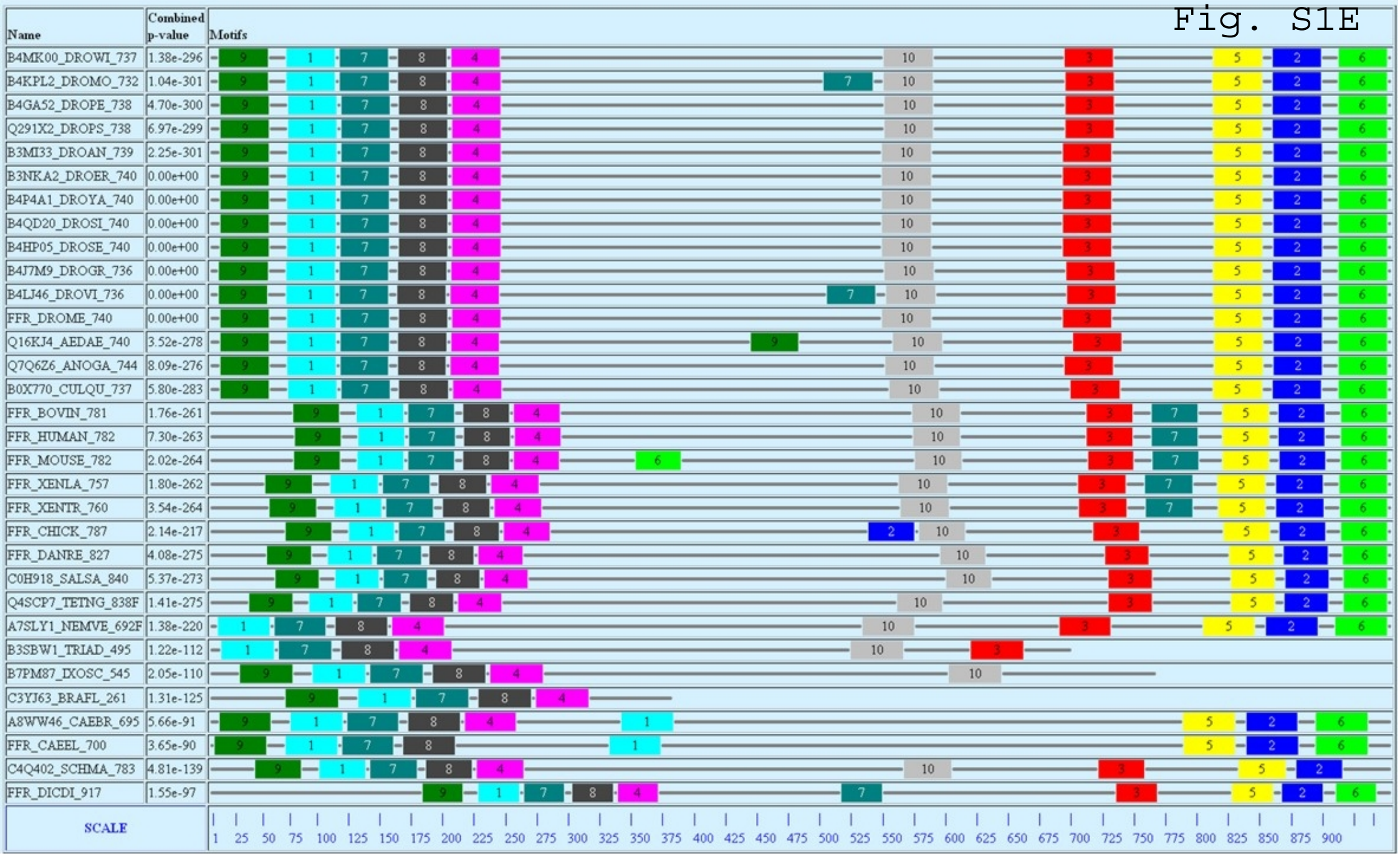
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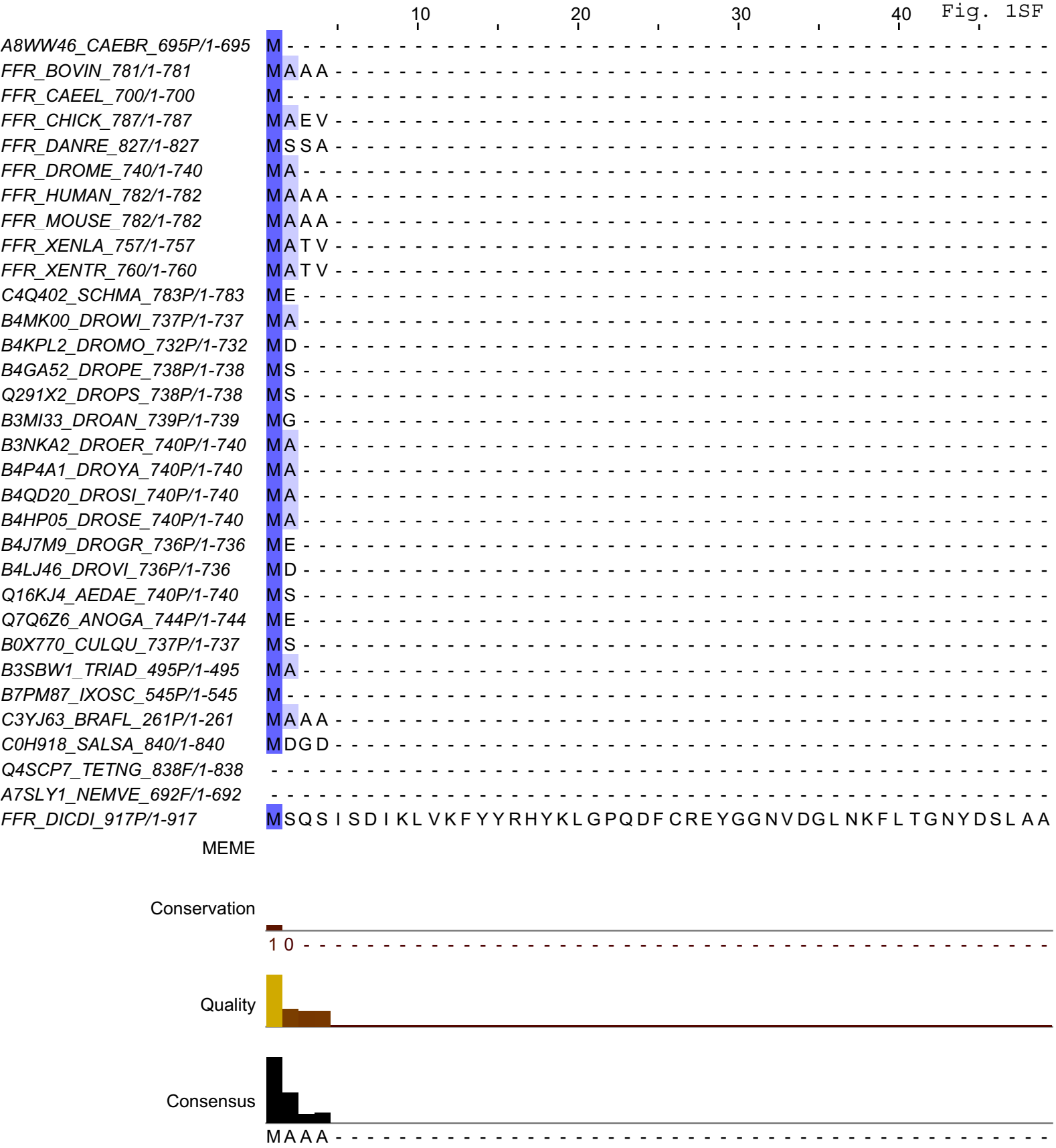
Fig. 1SF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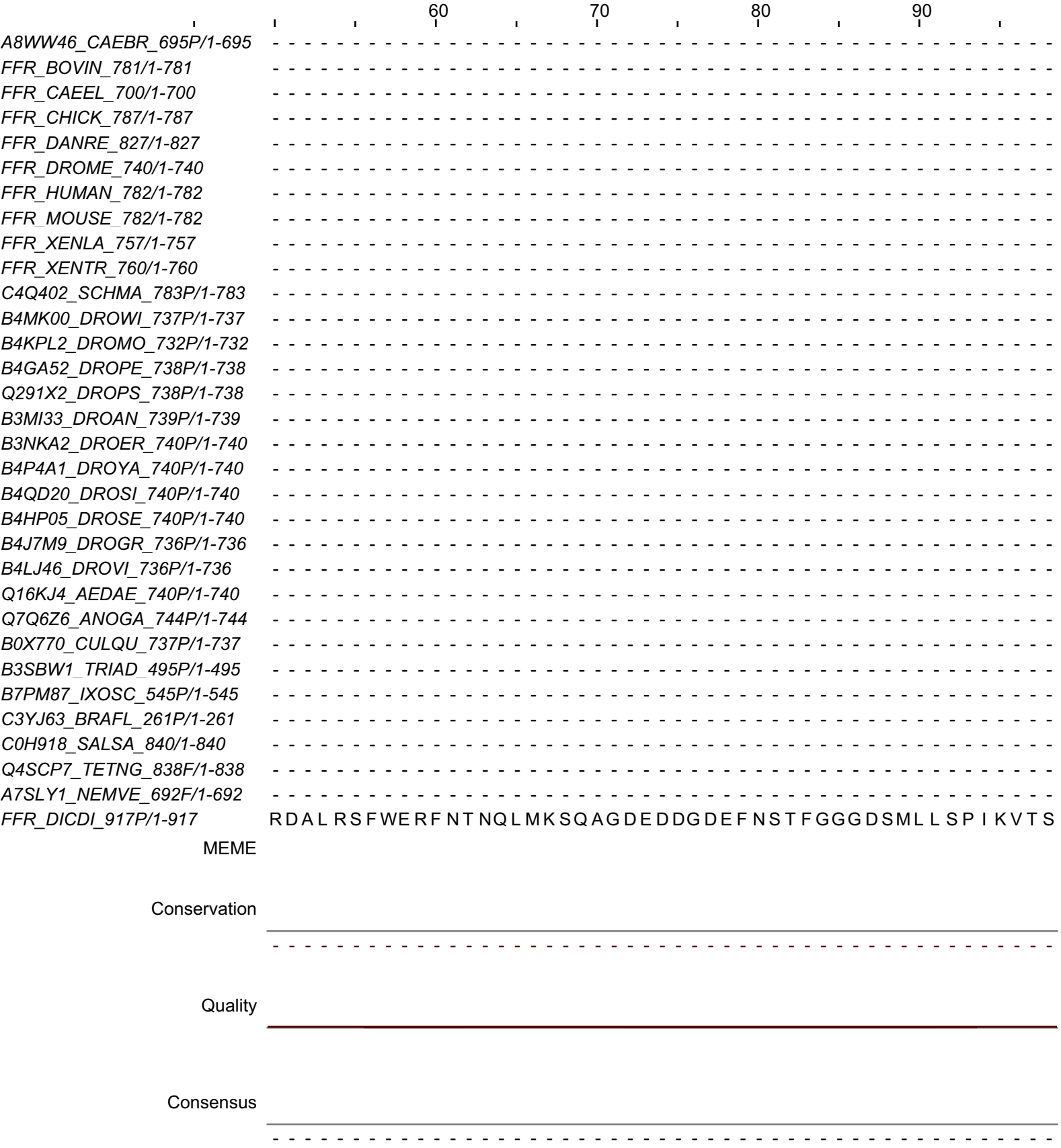
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Quality
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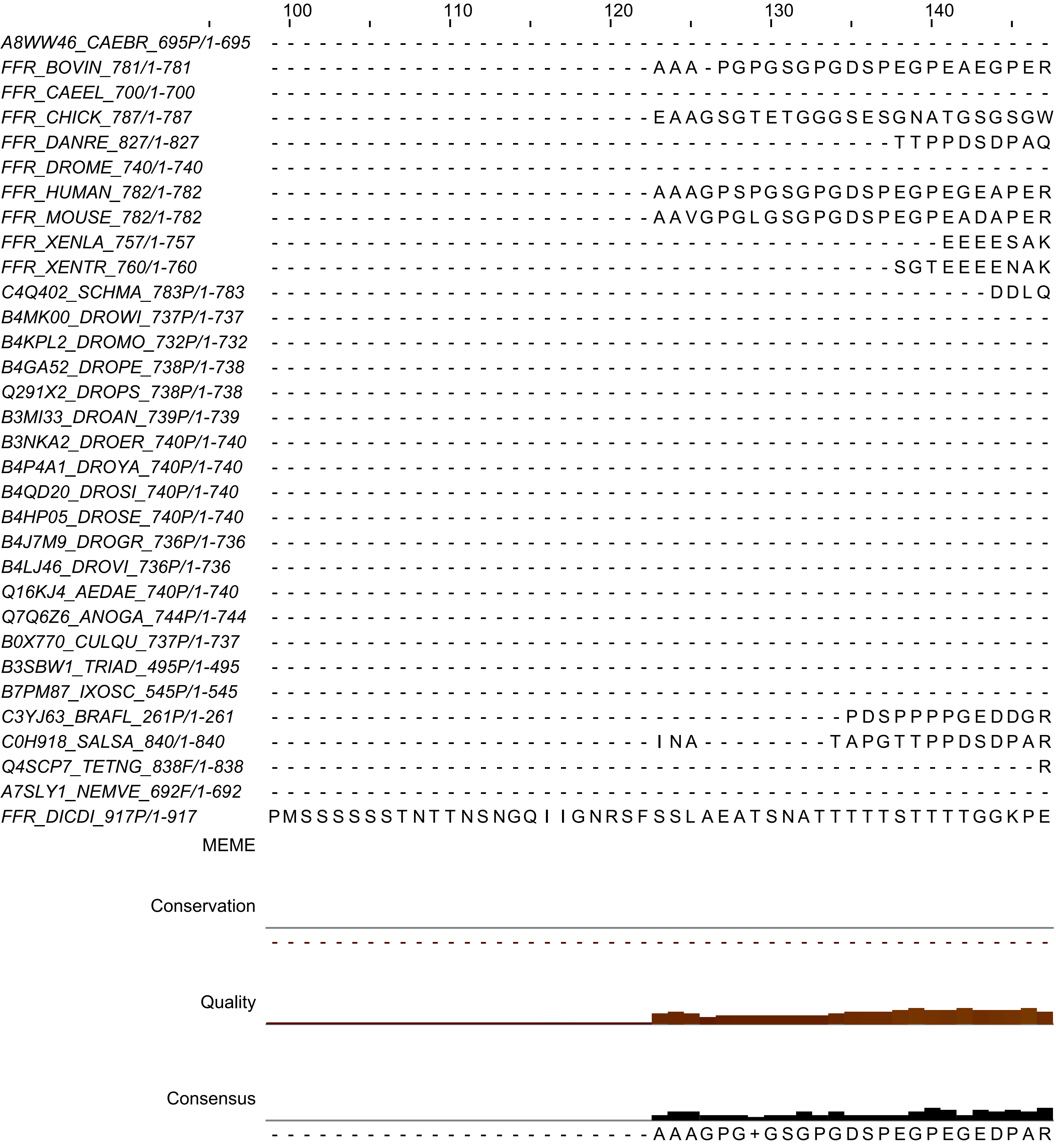

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Q291X2_DROPS_738P/1-738   -------------------------------------------------
B3MI33_DROAN_739P/1-739   -------------------------------------------------
B3NKA2_DROER_740P/1-740   -------------------------------------------------
B4P4A1_DROYA_740P/1-740   -------------------------------------------------
B4QD20_DROSI_740P/1-740   -------------------------------------------------
B4HP05_DROSE_740P/1-740   -------------------------------------------------
B4J7M9_DROGR_736P/1-736   -------------------------------------------------
B4LJ46_DROVI_736P/1-736   -------------------------------------------------
Q16KJ4_AEDAE_740P/1-740   -------------------------------------------------
Q7Q6Z6_ANOGA_744P/1-744   -------------------------------------------------
B0X770_CULQU_737P/1-737   -------------------------------------------------
B3SBW1_TRIAD_495P/1-495   -------------------------------------------------
B7PM87_IXOSC_545P/1-545   -------------------------------------------------
C3YJ63_BRAFL_261P/1-261   ------------------------------------PDSPPPPGEDDGR
C0H918_SALSA_840/1-840    ------------------------INA--------TAPGTTPPDSDPAR
Q4SCP7_TETNG_838F/1-838   ------------------------------------------------R
A7SLY1_NEMVE_692F/1-692   -------------------------------------------------
FFR_DICDI_917P/1-917      PMSSSSSSTNTTNSNGQIIGNRSFSSLAEATSNATTTTTSTTTTGGKPE
MEME
Conservation              -------------------------------------------------
Quality
Consensus                 ------------------------AAAGPG+GSGPGDSPEGPEGEDPAR
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FFR_CAEEL_700/1-700        ------------------------------SSVLDVTKPDFDVEAFVVKL
FFR_CHICK_787/1-787        RRP-HGPLQRYYGPSAAEAA------EATPDPADINGPHFDPEVFLTKV
FFR_DANRE_827/1-827        RRRVHSMLKLYYGLNEEGKATEQ---AESLDPCDINGPHFDPEIYLNKL
FFR_DROME_740/1-740        ------------------------------ETKANPFDMDSSSFDAEKYLERL
FFR_HUMAN_782/1-782        RRKAHGMLKLYYGLS-EGEAAGRPAGPDPLDPTDLNGAHFDPEVYLDKL
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FFR_XENLA_757/1-757        KKKSHGILKLYYGMTEEGKAA-----ERHLEPTDVDGVHFNPELYLTKL
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B0X770_CULQU_737P/1-737    ------------------------------EKSTNPYDMDSPCFDPDRYLQKL
B3SBW1_TRIAD_495P/1-495    ------------------------------------------------------
B7PM87_IXOSC_545P/1-545    -------LKQFYQGSSEAS-------DIGFDPYNINGPDFNPDLYLNKL
C3YJ63_BRAFL_261P/1-261    RRKRHGMLKLYYGLQAESGNKGT---TATTDPCDINGAHFKPEVYLQRL
C0H918_SALSA_840/1-840     KRRVHGMLKLYYGLNEEGKTVDQ---AESMDPCDINGPHFDPEVYLNKL
Q4SCP7_TETNG_838F/1-838    RRRVHGMLKLYYGLNEEGKADEQ---PRSLDPCDINGPHFDPELYLNKL
A7SLY1_NEMVE_692F/1-692    ------------------------------------------------------
FFR_DICDI_917P/1-917       SKRVRNLLKTYYGPGIGEGSDS----SSMNDPLNIDGPSFNLNSYFDSI
MEME                                                     9999999999999999999
```

Conservation

```
- - - - - - - - - - - - - - - - - - - - - - - - - - - - - - 0 0 0 0 1 1 0 2 1 2 0 1 0 2 1 0 1 0 2 1 0 1 0
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Quality

Consensus

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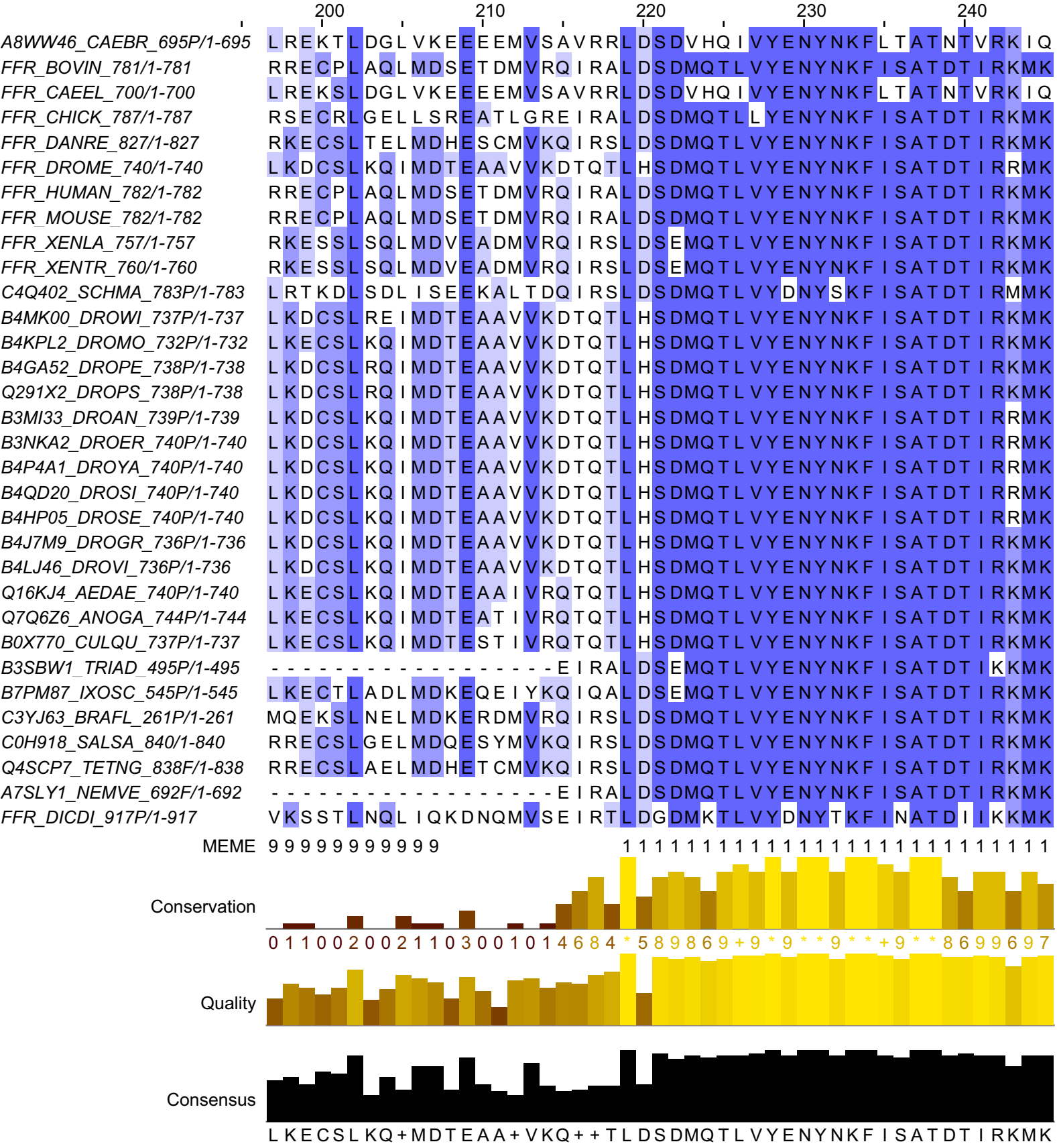
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FFR_CAEEL_700/1-700 LREKSLDGLVKEEEEMVSAVRRLDSDVHQIVYENYNKFLTATNTVRKIQ
FFR_CHICK_787/1-787 RSECRLGELLSREATLGREIRALDSDMQTLLYENYNKFISATDTIRKMK
FFR_DANRE_827/1-827 RKECSLTELMDHESCMVKQIRSLDSDMQTLVYENYNKFISATDTIRKMK
FFR_DROME_740/1-740 LKDCSLKQIMDTEAAVVKDTQTLHSDMQTLVYENYNKFISATDTIRRMK
FFR_HUMAN_782/1-782 RRECPLAQLMDSETDMVRQIRALDSDMQTLVYENYNKFISATDTIRKMK
FFR_MOUSE_782/1-782 RRECPLAQLMDSETDMVRQIRALDSDMQTLVYENYNKFISATDTIRKMK
FFR_XENLA_757/1-757 RKESSLSQLMDVEADMVRQIRSLDSEMQTLVYENYNKFISATDTIRKMK
FFR_XENTR_760/1-760 RKESSLSQLMDVEADMVRQIRSLDSEMQTLVYENYNKFISATDTIRKMK
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C3YJ63_BRAFL_261P/1-261 MQEKSLNELMDKERDMVRQIRSLDSDMQTLVYENYNKFISATDTIRKMK
C0H918_SALSA_840/1-840 RRECSLGELMDQESYMVKQIRSLDSDMQTLVYENYNKFISATDTIRKMK
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A7SLY1_NEMVE_692F/1-692 ----------EIRALDSDMQTLVYENYNKFISATDTIRKMK
FFR_DICDI_917P/1-917 VKSSTLNQLIQKDNQMVSEIRTLDGDMKTLVYDNYTKFINATDIIKKMK
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Conservation
0110020021103001014684*589869+9*9**9**+9**8699697
Quality
Consensus
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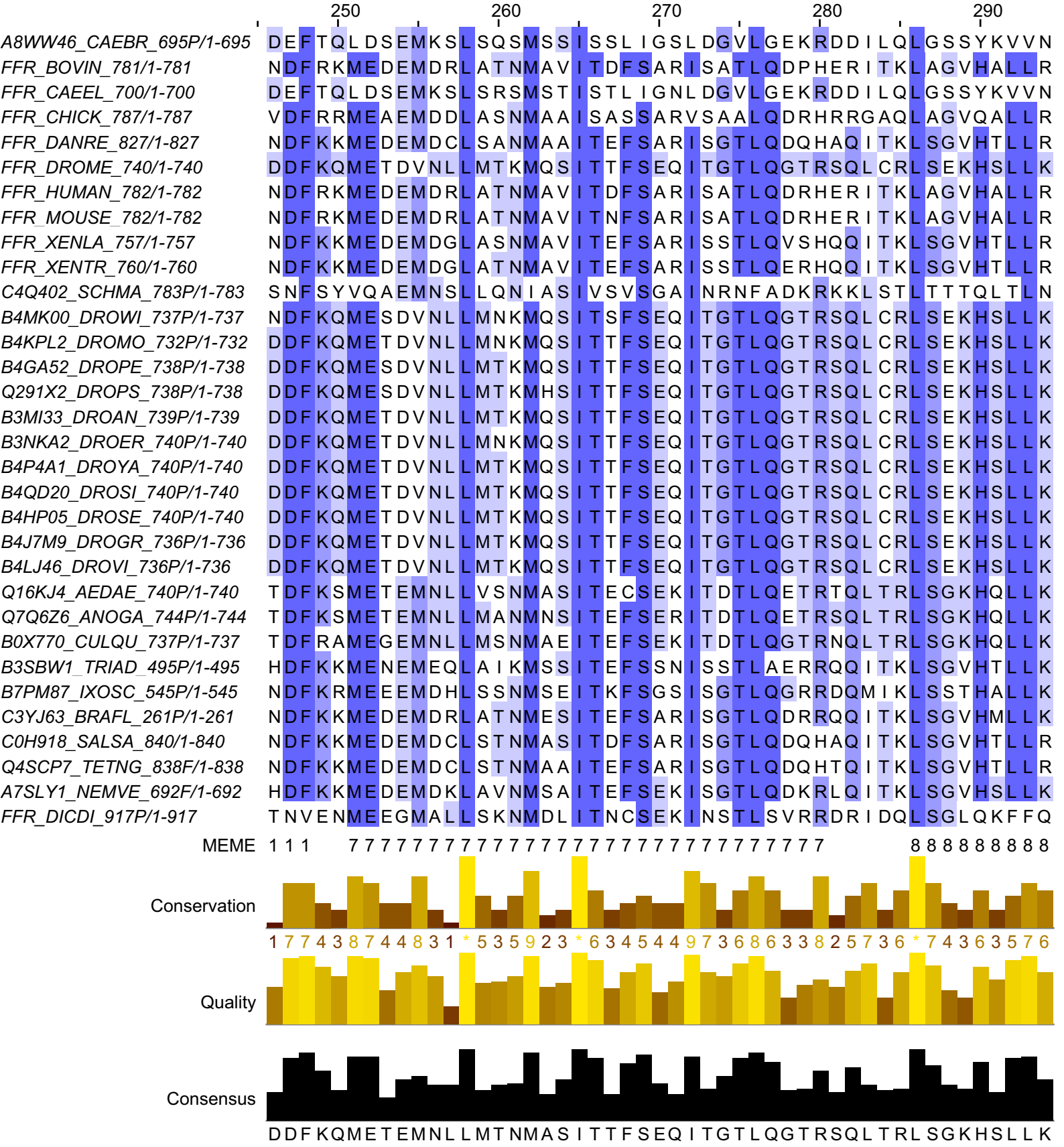
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FFR_CAEEL_700/1-700 DEFTQLDSEMKSLSRSMSTISTLIGNLDGVLGEKRDDILQLGSSYKVVN
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Quality
Consensus
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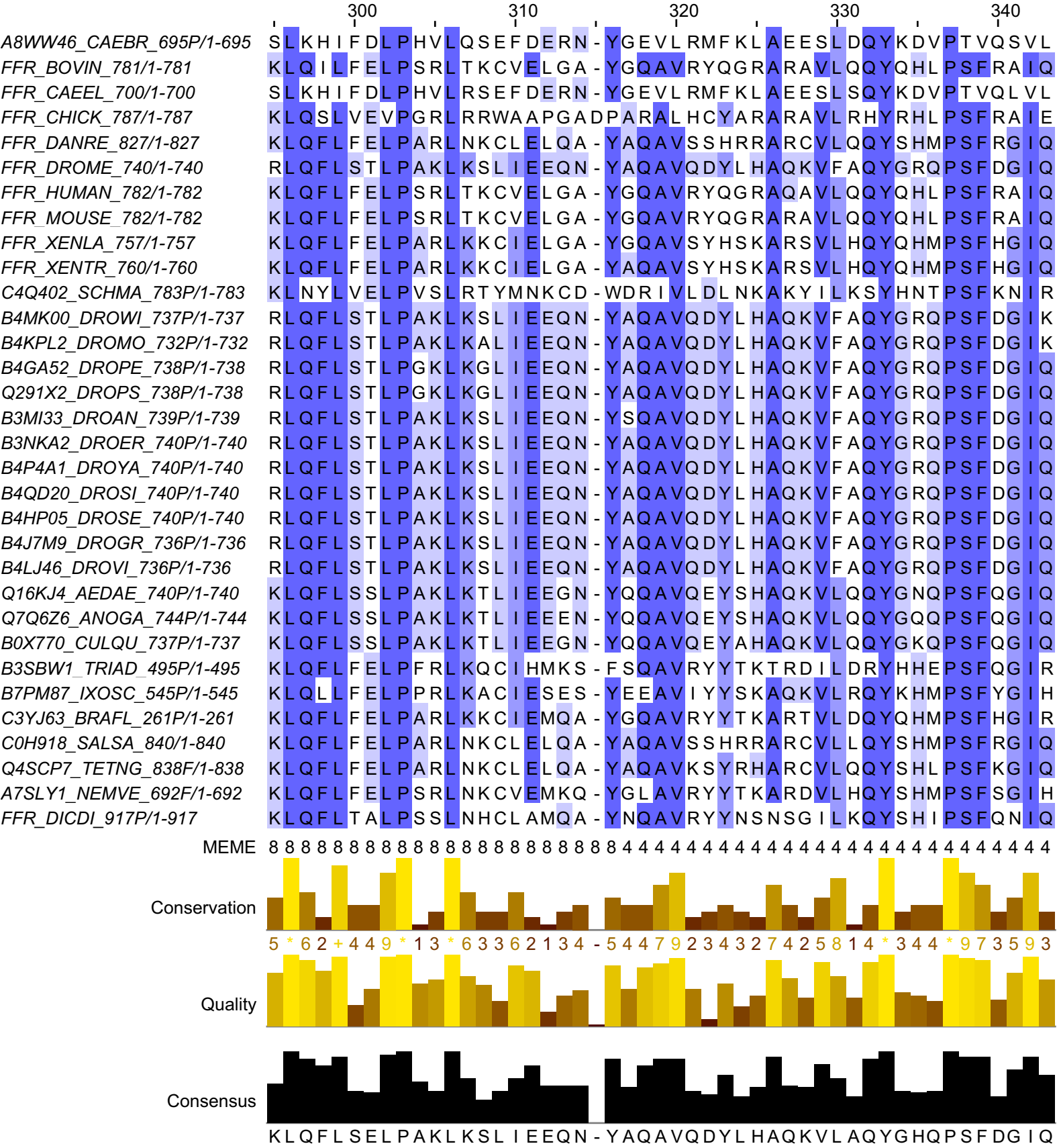
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Conservation
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Quality
Consensus
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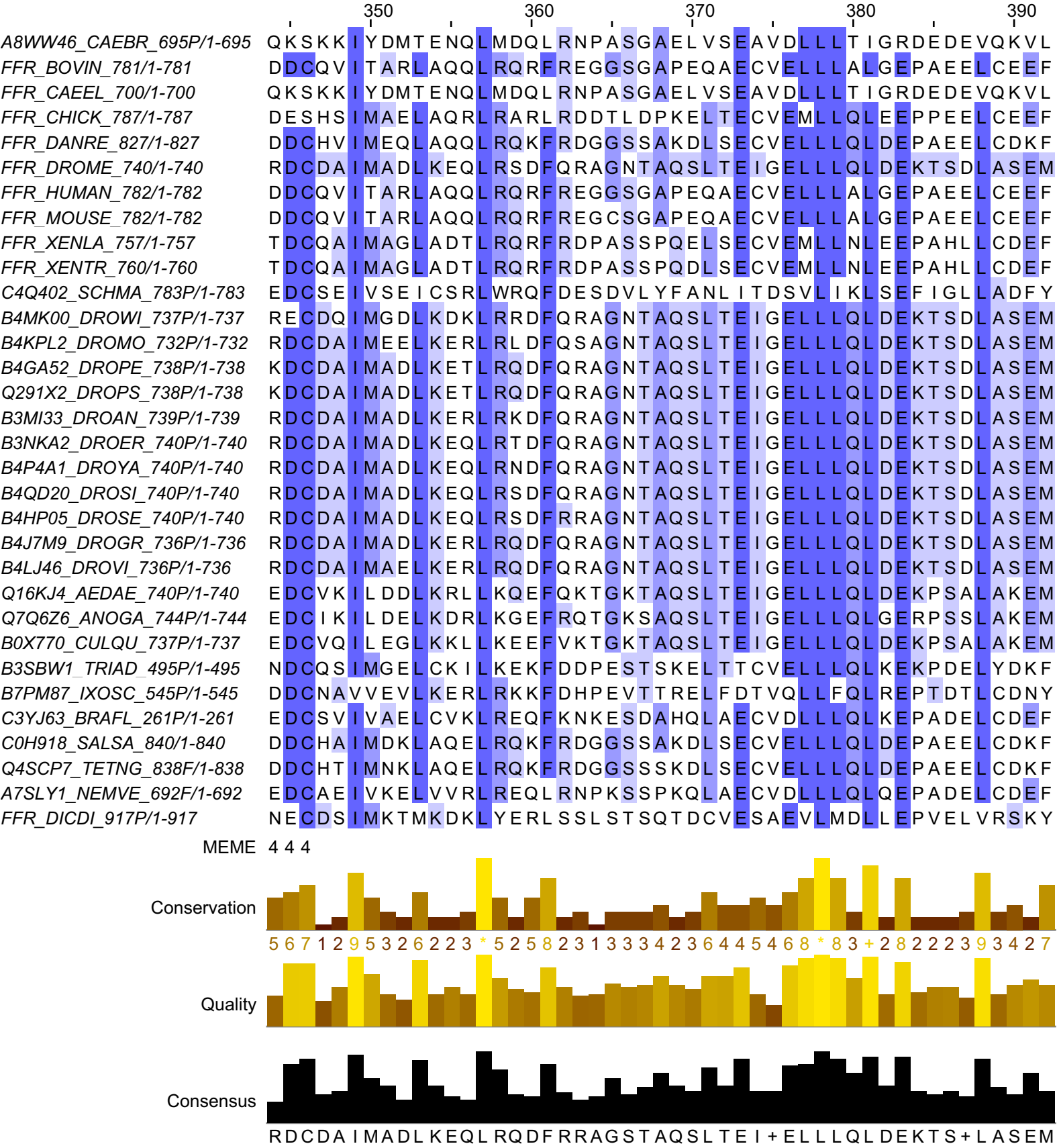
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B3SBW1_TRIAD_495P/1-495 NDCQSIMGELCKILKEKFDDPESTSKELTTCVELLLQLKEKPDELYDKF
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MEME 4 4 4
Conservation
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Quality
Consensus
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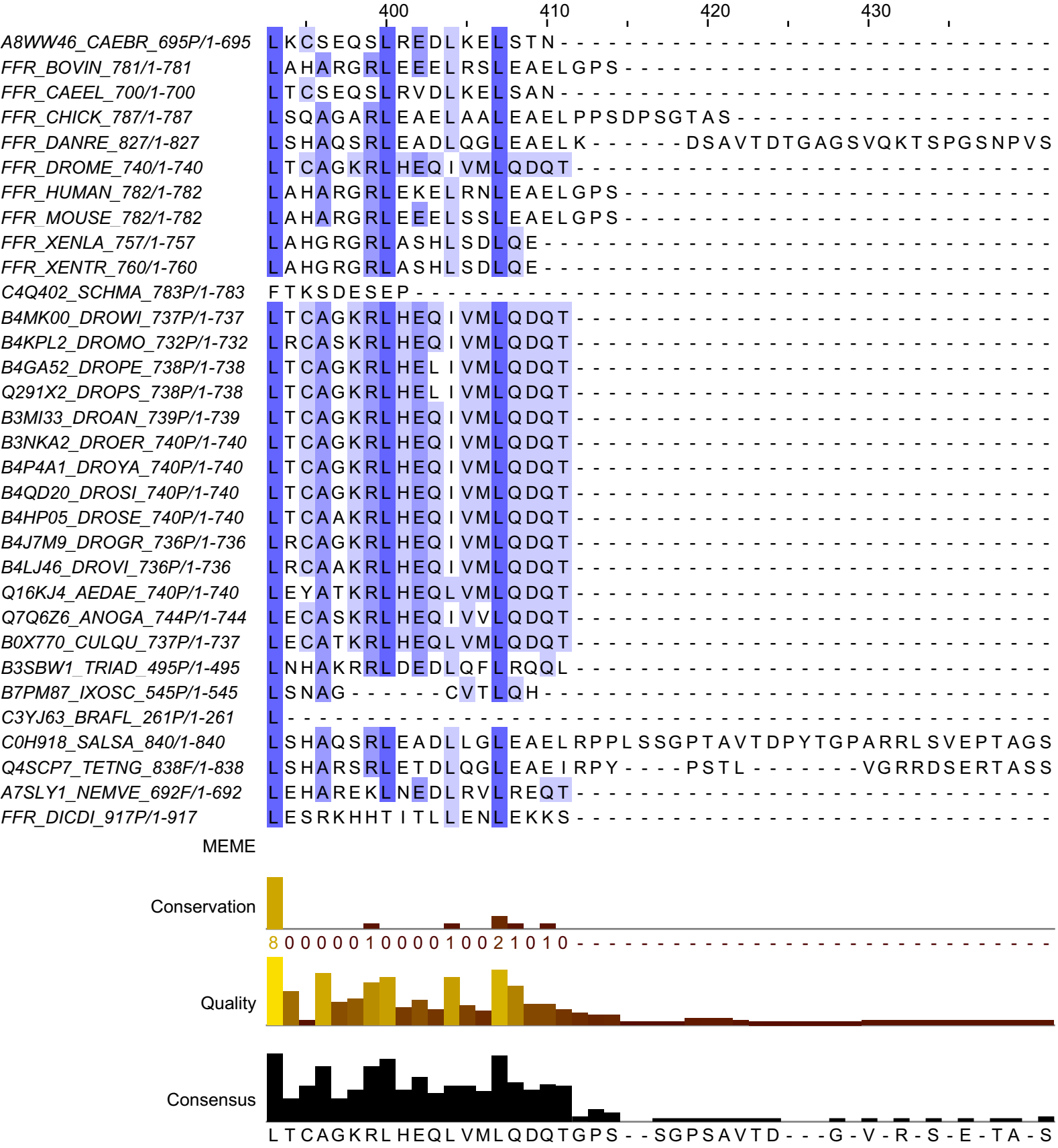
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MEME
Conservation
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Quality
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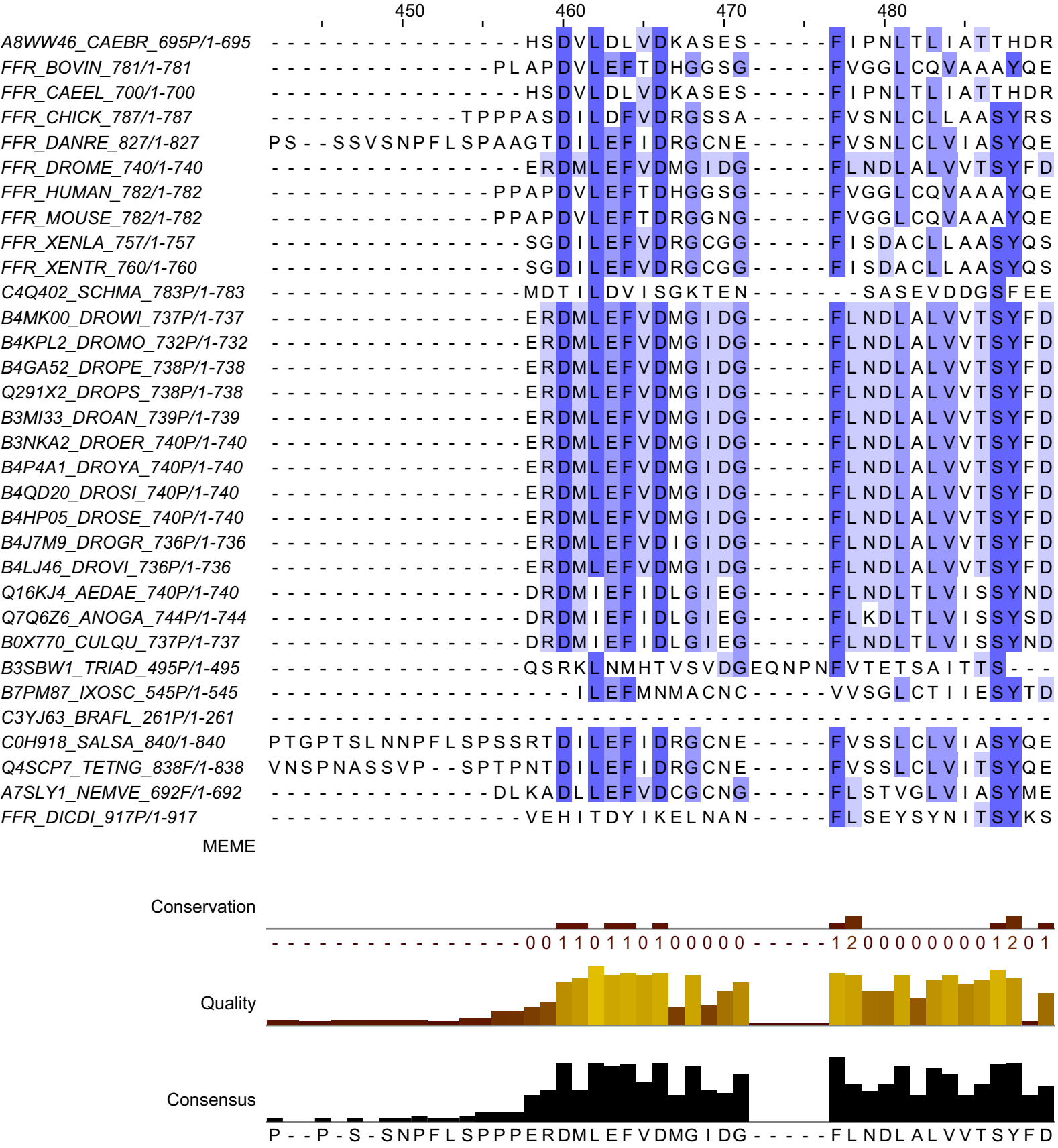
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C3YJ63_BRAFL_261P/1-261
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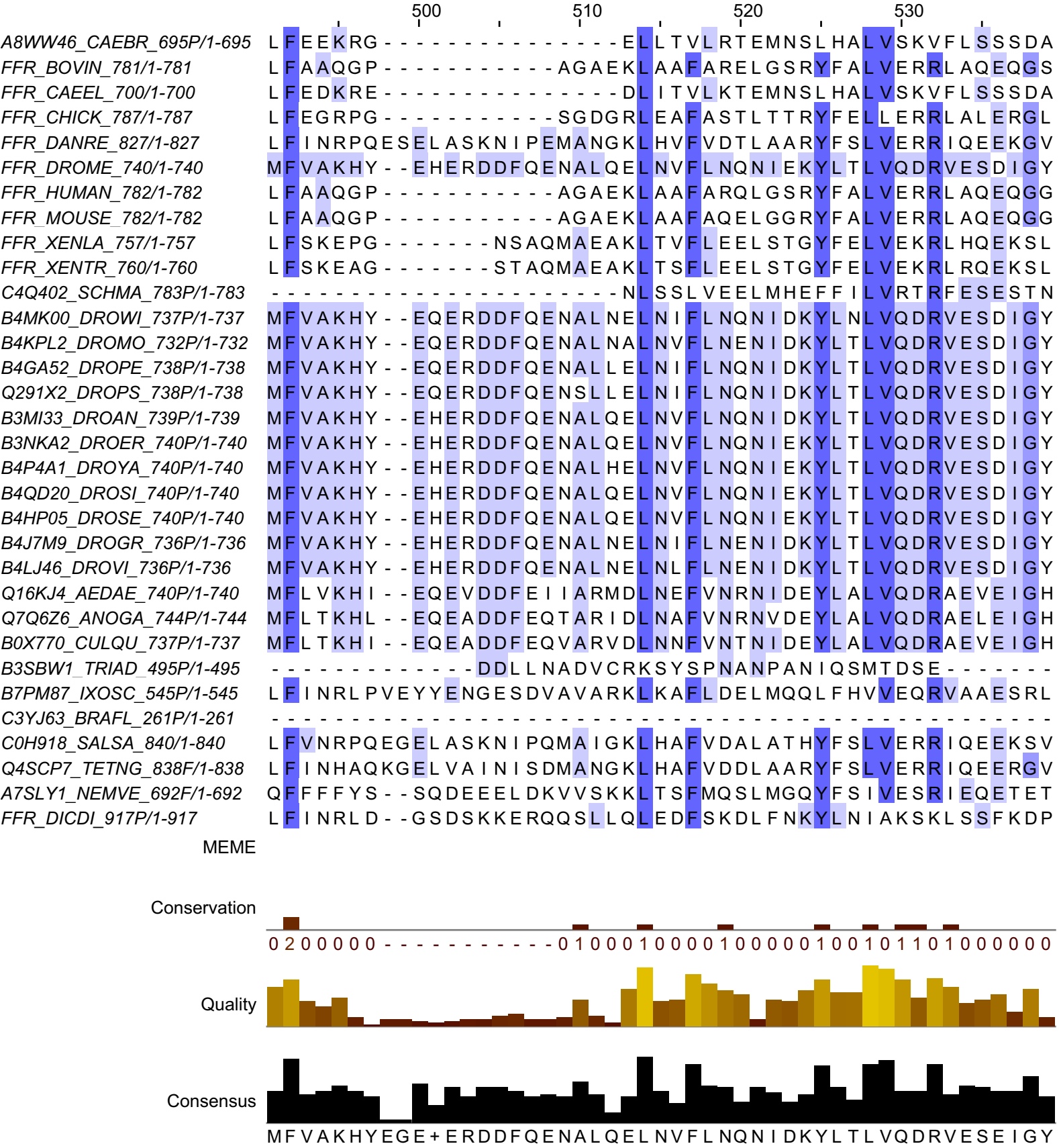
Conservation
Quality
Consensus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FFR_CHICK_787/1-787 LFEGRPG-----SGDGRLEAFASTLTTRYFELLERRLALERGL
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FFR_MOUSE_782/1-782 LFAAQGP-----AGAEKLAAFAQELGGRYFALVERRLAQEQGG
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Quality
Consensus
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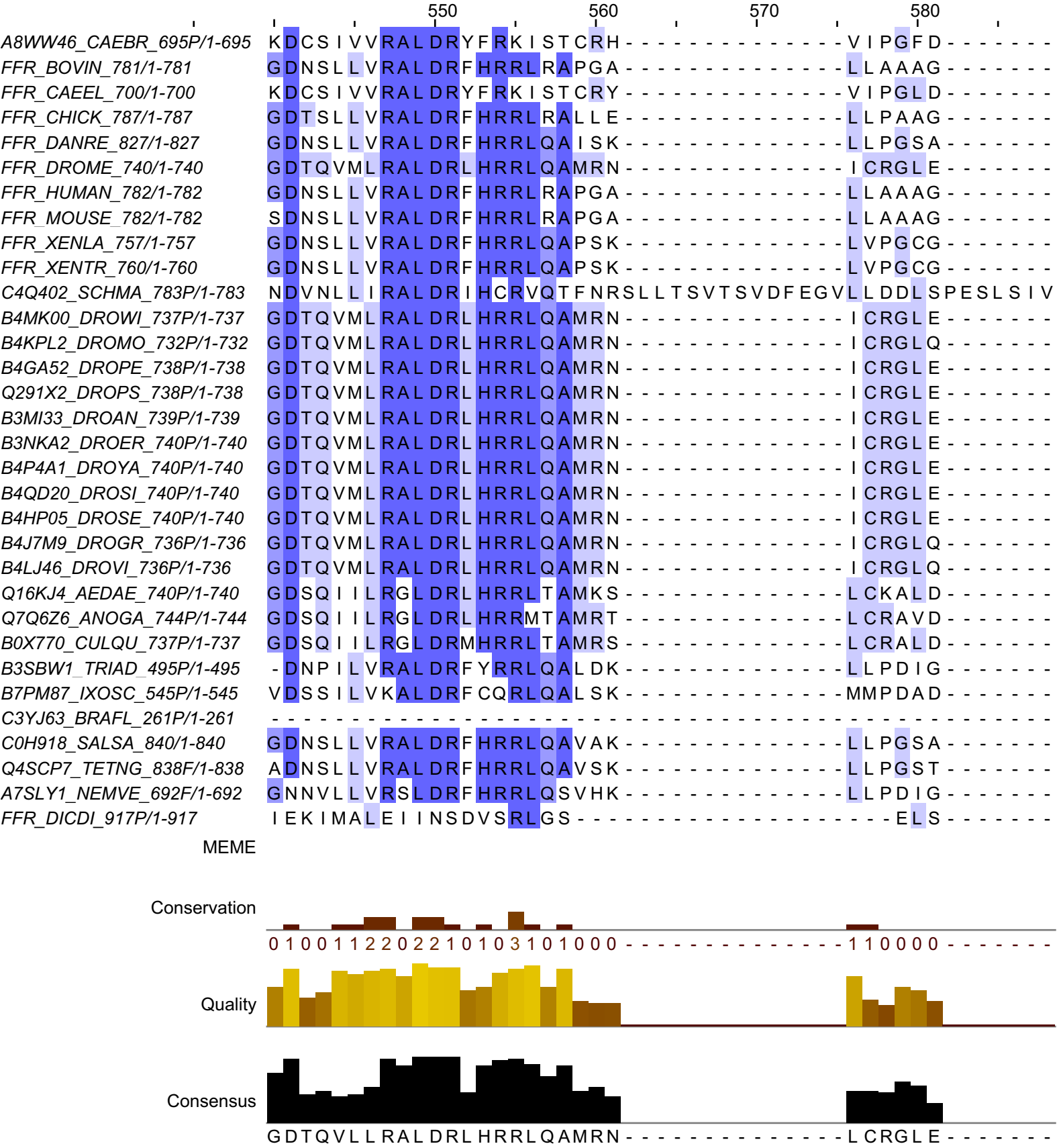
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FFR_HUMAN_782/1-782 GDNSLLVRALDRFHRRLRAPGA----------LLAAAG------
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Q7Q6Z6_ANOGA_744P/1-744 GDSQIILRGLDRLHRRMTAMRT----------LCRAVD------
B0X770_CULQU_737P/1-737 GDSQIILRGLDRMHRRLTAMRS----------LCRALD------
B3SBW1_TRIAD_495P/1-495 -DNPILVRALDRFYRRLQALDK----------LLPDIG------
B7PM87_IXOSC_545P/1-545 VDSSILVKALDRFCQRLQALSK----------MMPDAD------
C3YJ63_BRAFL_261P/1-261 ----------
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A7SLY1_NEMVE_692F/1-692 GNNVLLVRSLDRFHRRLQSVHK----------LLPDIG------
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Conservation
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Consensus
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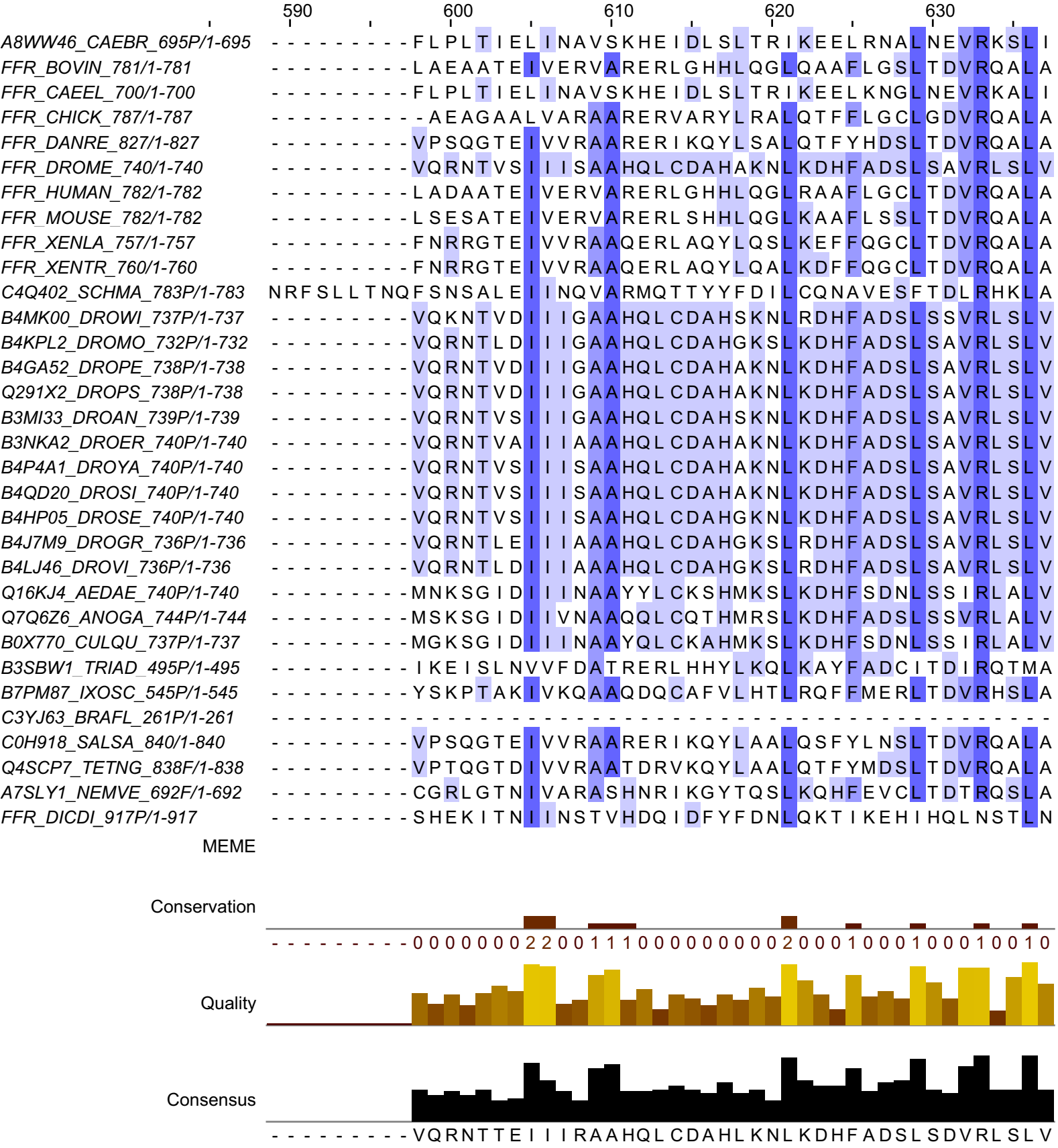

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FFR_CAEEL_700/1-700       ---------FLPLTIELINAVSKHEIDLSLTRIKEELKNGLNEVRKALI
FFR_CHICK_787/1-787       ----------AEAGAALVARAARERVARYLRALQTFFLGCLGDVRQALA
FFR_DANRE_827/1-827       ---------VPSQGTEIVVRAARERIKQYLSALQTFYHDSLTDVRQALA
FFR_DROME_740/1-740       ---------VQRNTVSIIISAAHQLCDAHAKNLKDHFADSLSAVRLSLV
FFR_HUMAN_782/1-782       ---------LADAATEIVERVARERLGHHLQGLRAAFLGCLTDVRQALA
FFR_MOUSE_782/1-782       ---------LSESATEIVERVARERLSHHLQGLKAAFLSSLTDVRQALA
FFR_XENLA_757/1-757       ---------FNRRGTEIVVRAAQERLAQYLQSLKEFFQGCLTDVRQALA
FFR_XENTR_760/1-760       ---------FNRRGTEIVVRAAQERLAQYLQALKDFFQGCLTDVRQALA
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B4KPL2_DROMO_732P/1-732   ---------VQRNTLDIIIGAAHQLCDAHGKSLKDHFADSLSAVRLSLV
B4GA52_DROPE_738P/1-738   ---------VQRNTVDIIIGAAHQLCDAHAKNLKDHFADSLSAVRLSLV
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B3NKA2_DROER_740P/1-740   ---------VQRNTVAIIIAAAHQLCDAHAKNLKDHFADSLSAVRLSLV
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C0H918_SALSA_840/1-840    ---------VPSQGTEIVVRAARERIKQYLAALQSFYLNSLTDVRQALA
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A7SLY1_NEMVE_692F/1-692   ---------CGRLGTNIVARASHNRIKGYTQSLKQHFEVCLTDTRQSLA
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Conservation              ---------0000000220011100000000020001000100010010

Consensus                 ---------VQRNTTEIIIRAAHQLCDAHLKNLKDHFADSLSDVRLSLV
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FFR_HUMAN_782/1-782       A-PRV-------------------AGKEGPG---------LAELLANVASS
FFR_MOUSE_782/1-782       A-PRL-------------------AGKEGPS---------LAELLANVASS
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MEME
Conservation
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Quality
Consensus
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FFR_CAEEL_700/1-700       FVHQVKTALANLLLFTASDV-TFANLPPDEFRQSFSFN-AHER------
FFR_CHICK_787/1-787       VLGQLKAVLAYVQLFTARDV-AFASLP--YFKGEFCVEAVREG------
FFR_DANRE_827/1-827       ILNQLKSVLASVHLFTAKDI-TFSNKP--YFKGEFCSQGVREG------
FFR_DROME_740/1-740       MVEKIKGVLQDLLIFLRTDW-SFNIKA--EHKGALCVEGIREN------
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FFR_MOUSE_782/1-782       ILSHIKTSLASVHLFTAKEV-SFSNKP--YFRGEFCSQGVREG------
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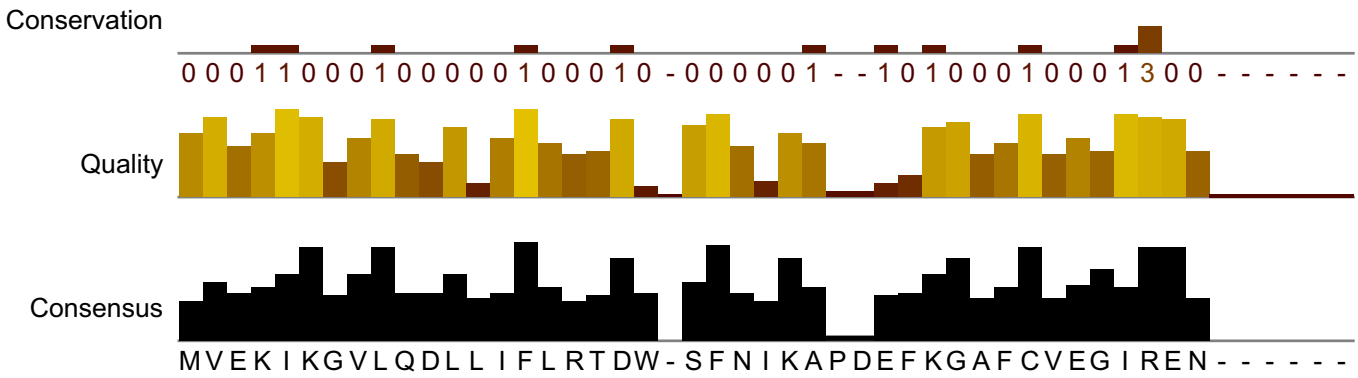

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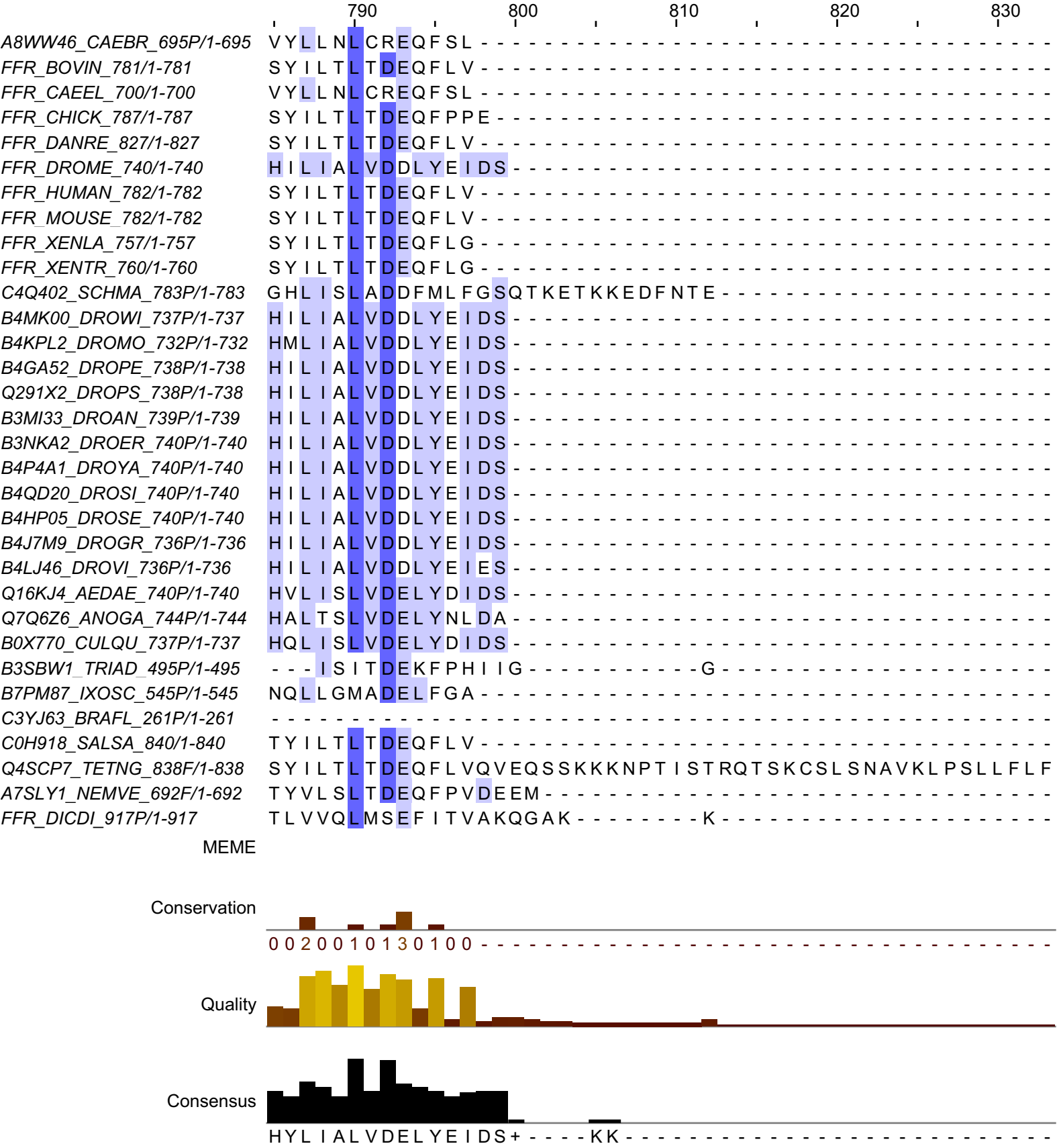
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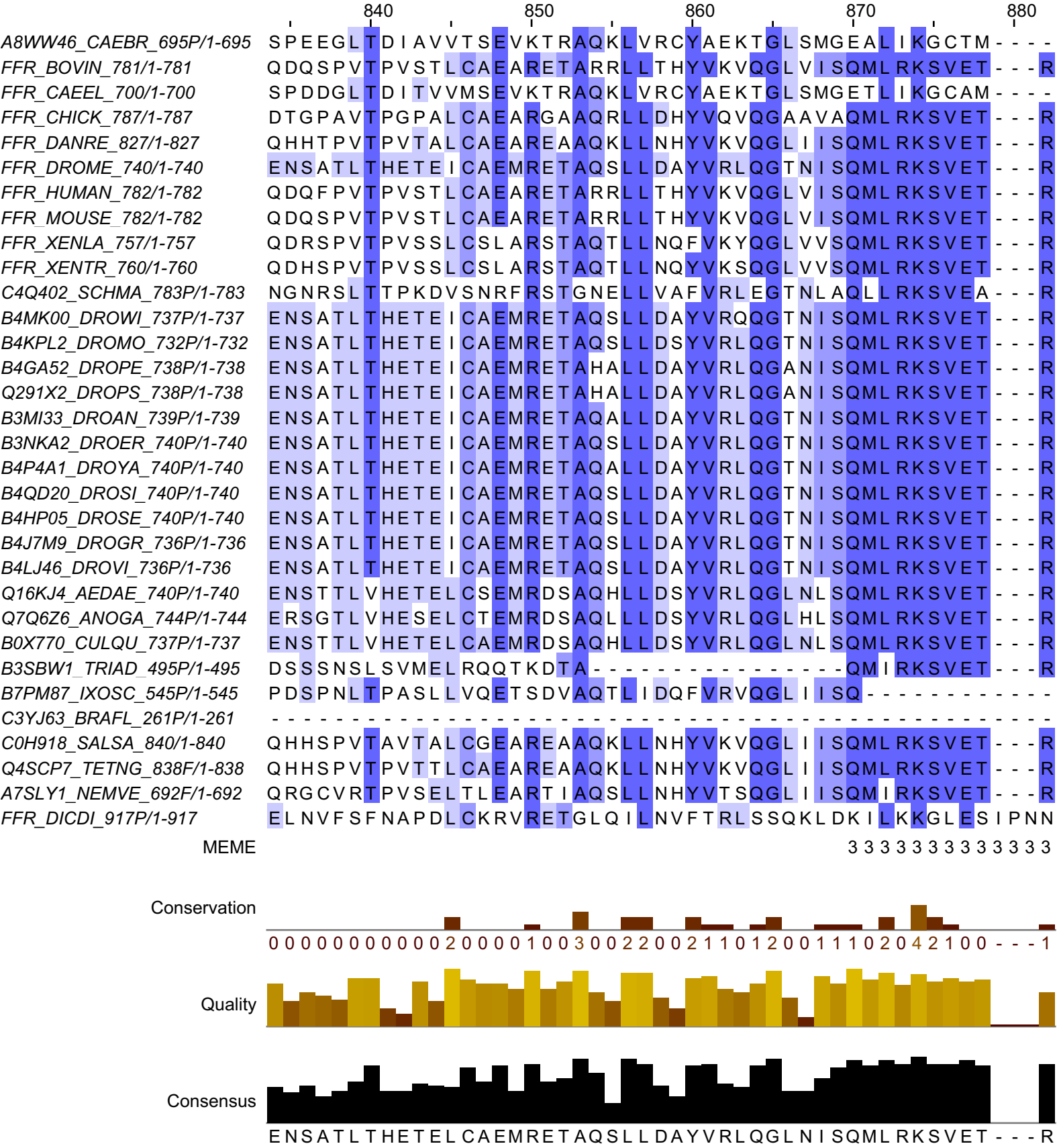
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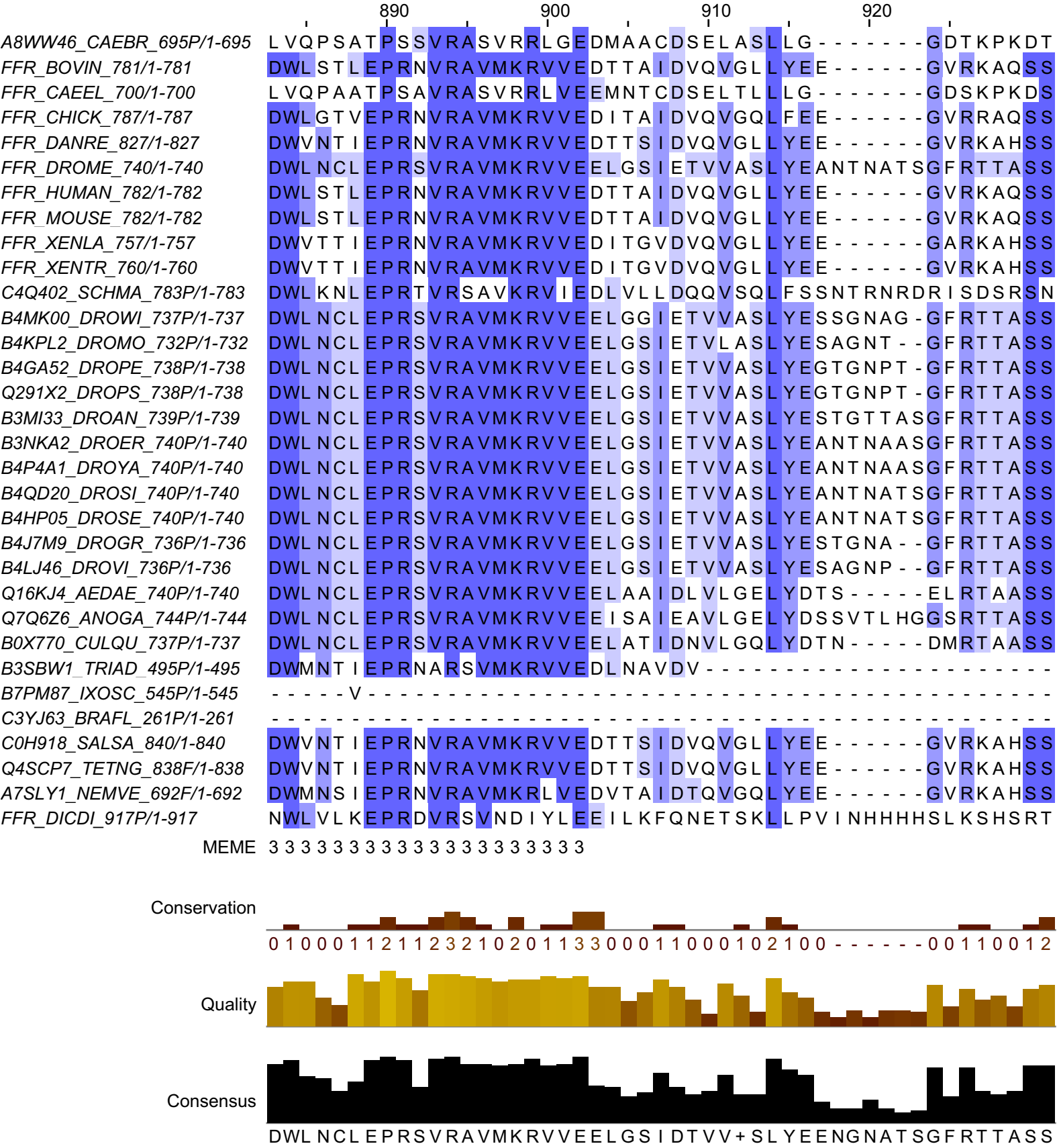
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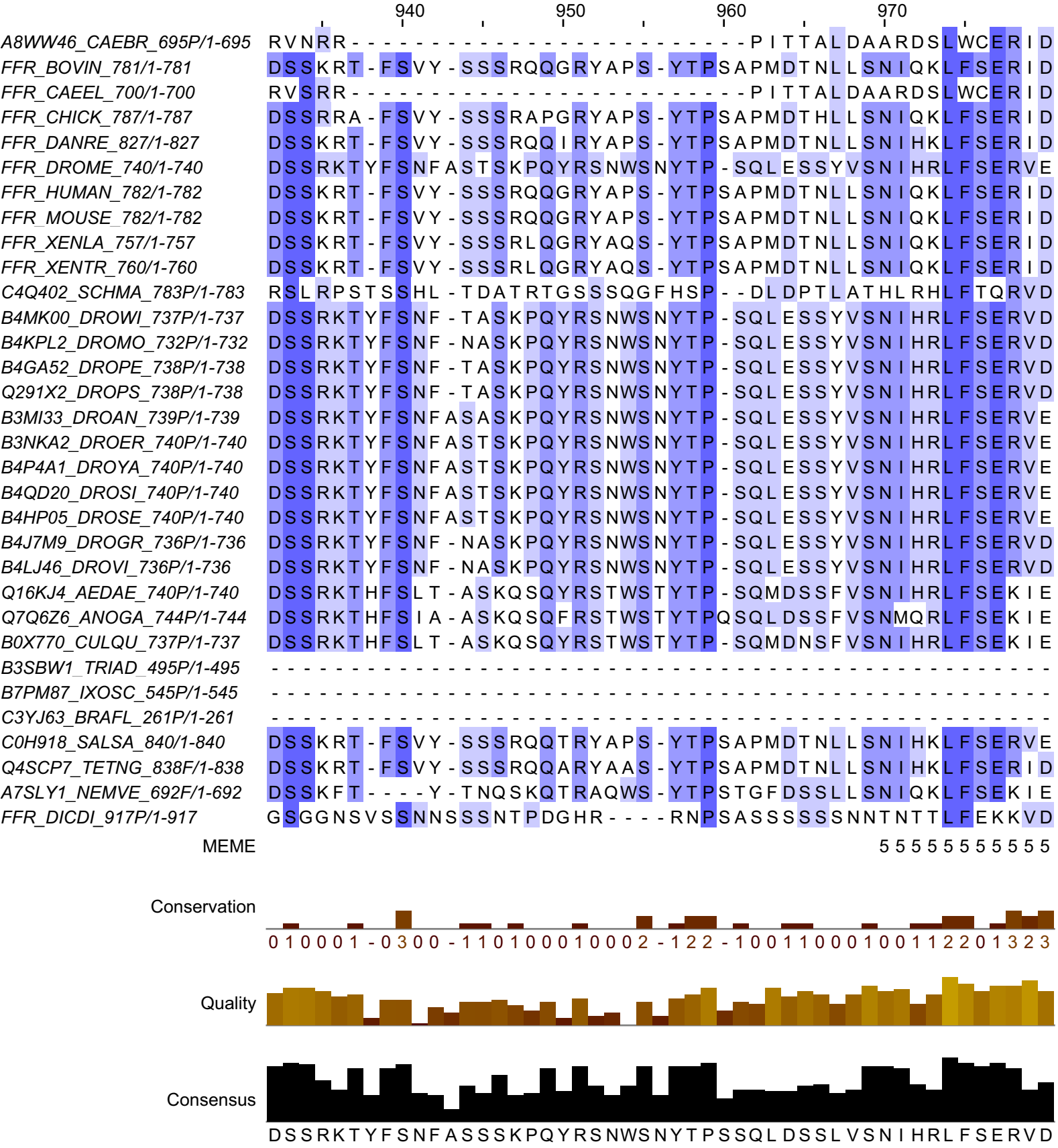
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Conservation
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Quality
Consensus
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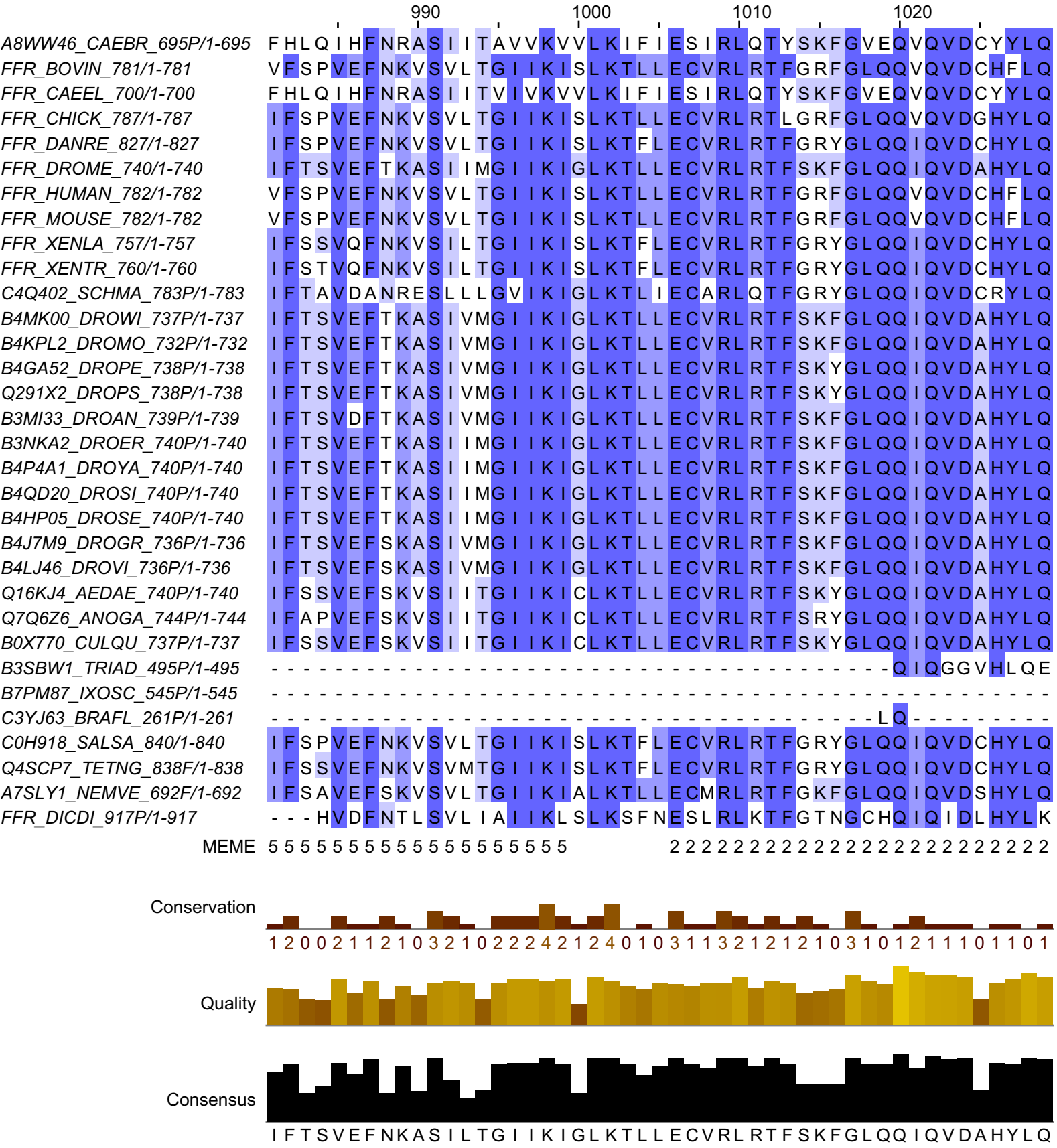
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Conservation
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Quality
Consensus
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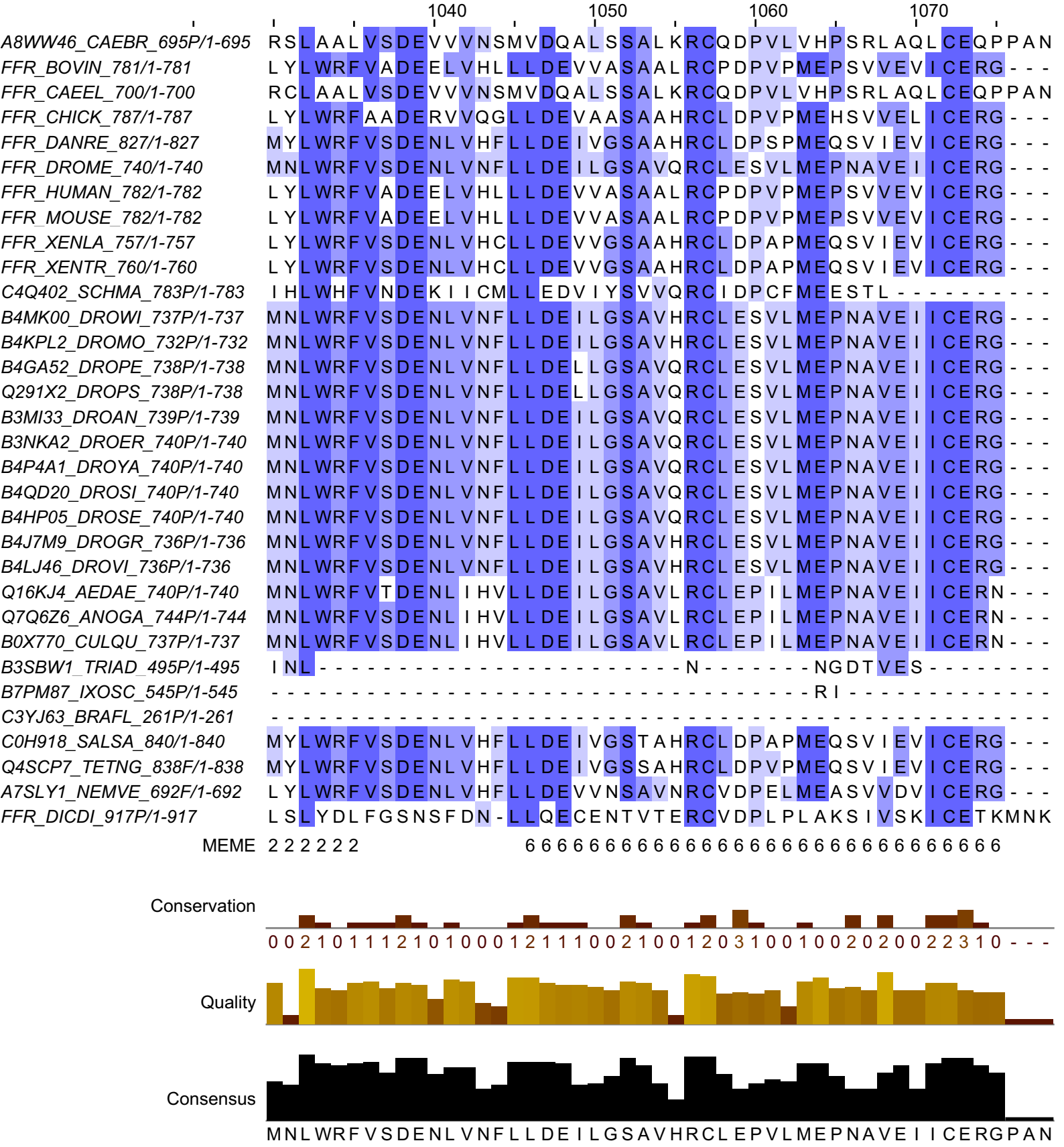
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B4GA52_DROPE_738P/1-738 MNLWRFVSDENLVNFLLDELLGSAVQRCLESVLMEPNAVEIICERG---
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Conservation
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Quality
Consensus
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FFR_CHICK_787/1-787	- - - - - - - - - - -
FFR_DANRE_827/1-827	- - - - - - - - - - -
FFR_DROME_740/1-740	- - - - - - - - - - -
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FFR_XENTR_760/1-760	- - - - - - - - - - -
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Quality	
Consensus	
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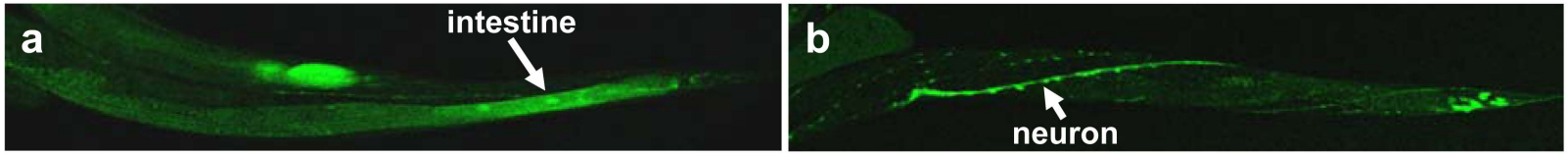

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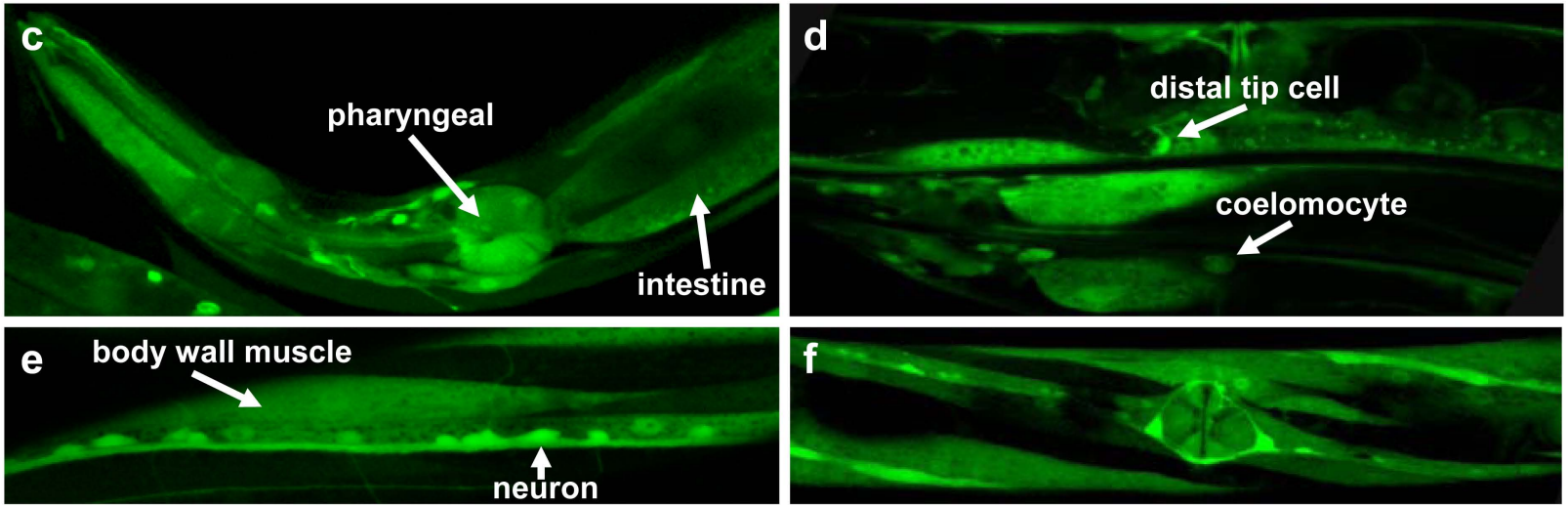

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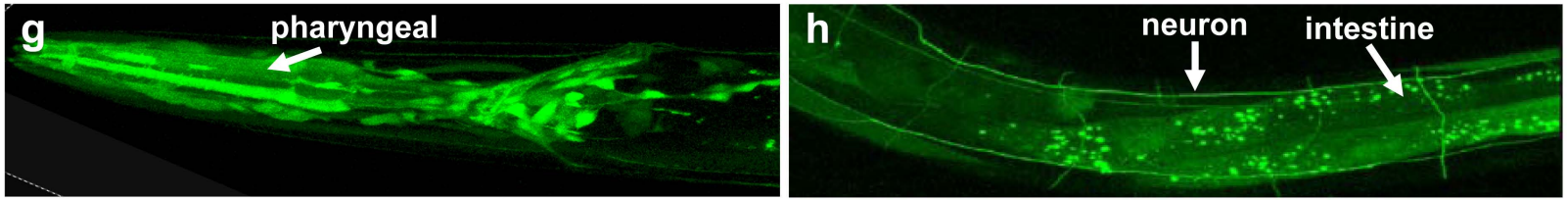

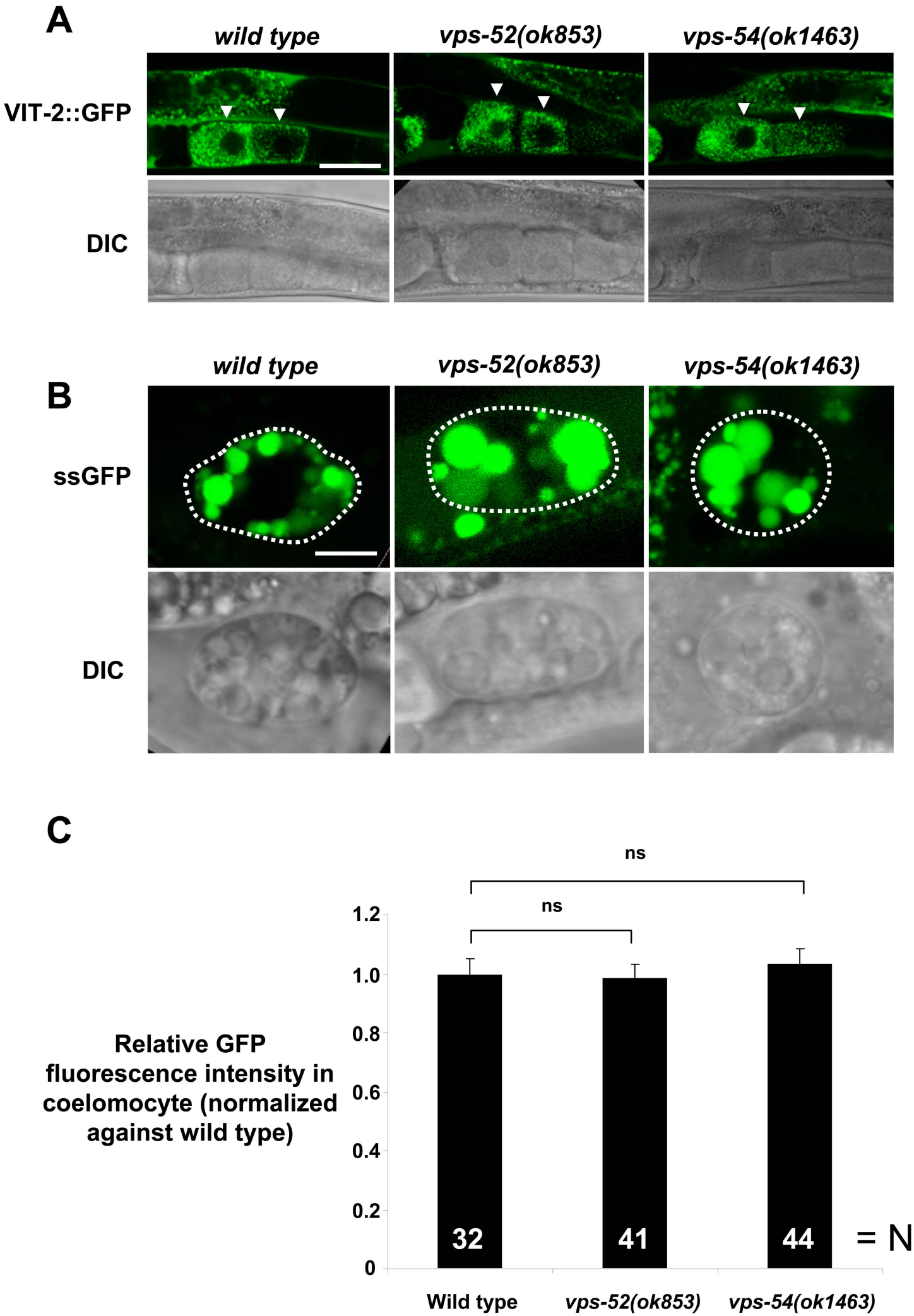
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wild type
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vps-54(ok1463)
VIT-2::GFP
DIC
B
wild type
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ssGFP
DIC
C
ns
ns
Relative GFP fluorescence intensity in coelomocyte (normalized against wild type)
1.2
1.0
0.8
0.6
0.4
0.2
0
32
41
44
= N
Wild type
vps-52(ok853)
vps-54(ok1463)