

NATIVE PAGE

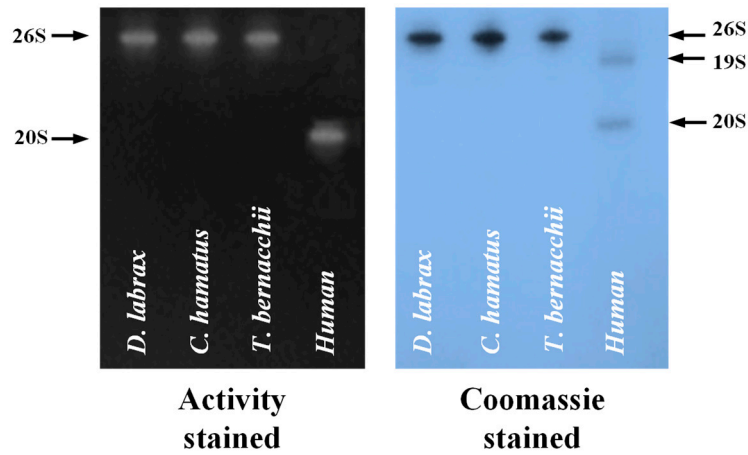


Figure S1. Native-PAGE analysis of purified 26S proteasomes from *D. labrax* and *C. hamatus* in comparison to those from *T. bernacchii* and human. In-gel CT-like activity ($\beta 5$ subunit) of 26S proteasomes was detected by using the fluorogenic substrate LLVY in the presence of 0.02% SDS. The Coomassie blue stained Native-PAGE was also shown. 26S proteasome from *T. bernacchii* and human were used as positive controls. The main proteasome species are indicated: doubly capped 26S (19S-20S-19S); 19S (regulatory complex) and 20S (catalytic core), free particles.

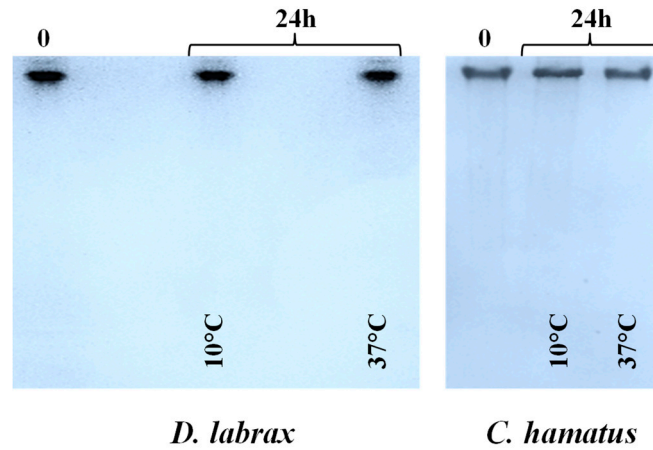
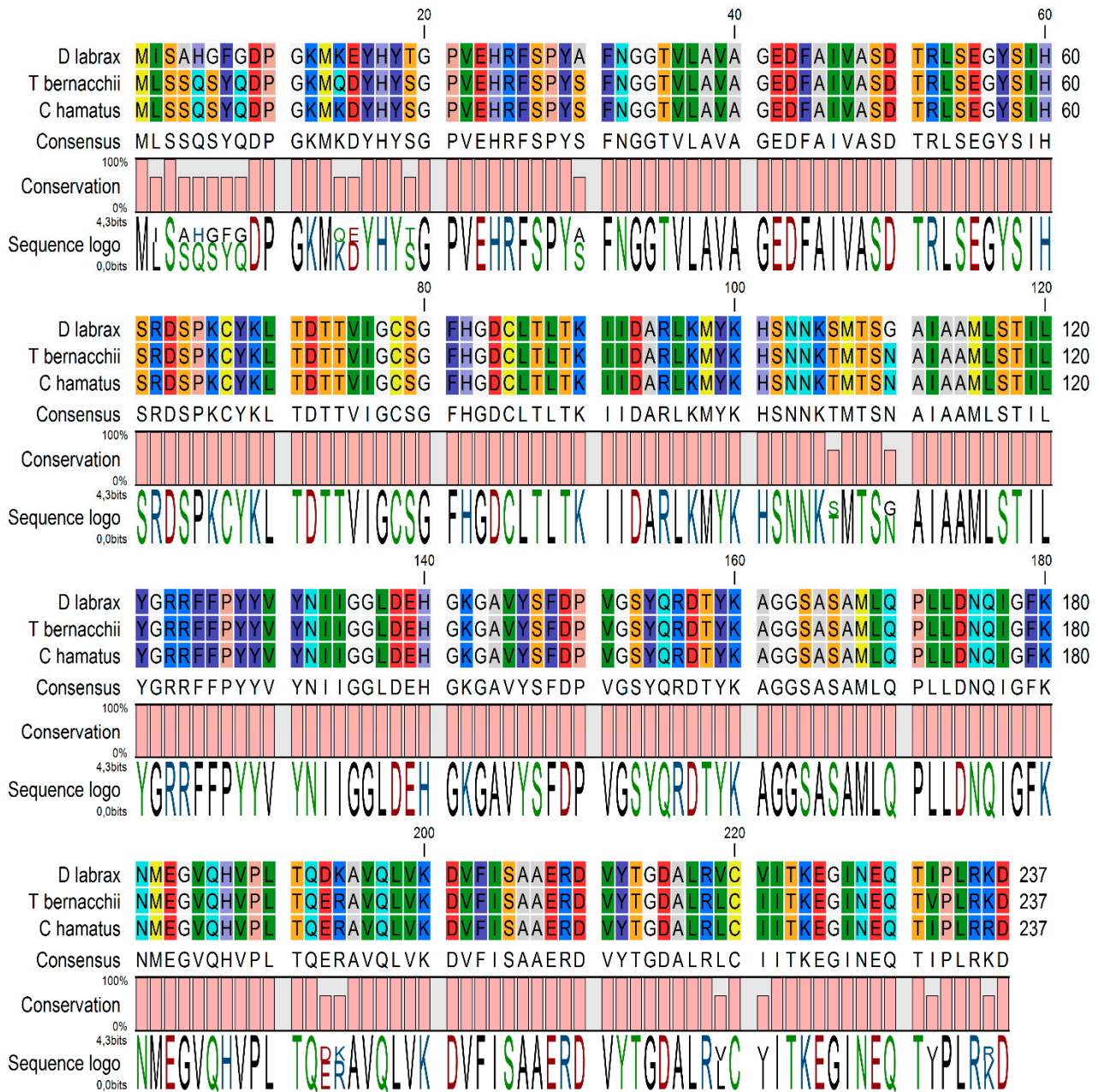
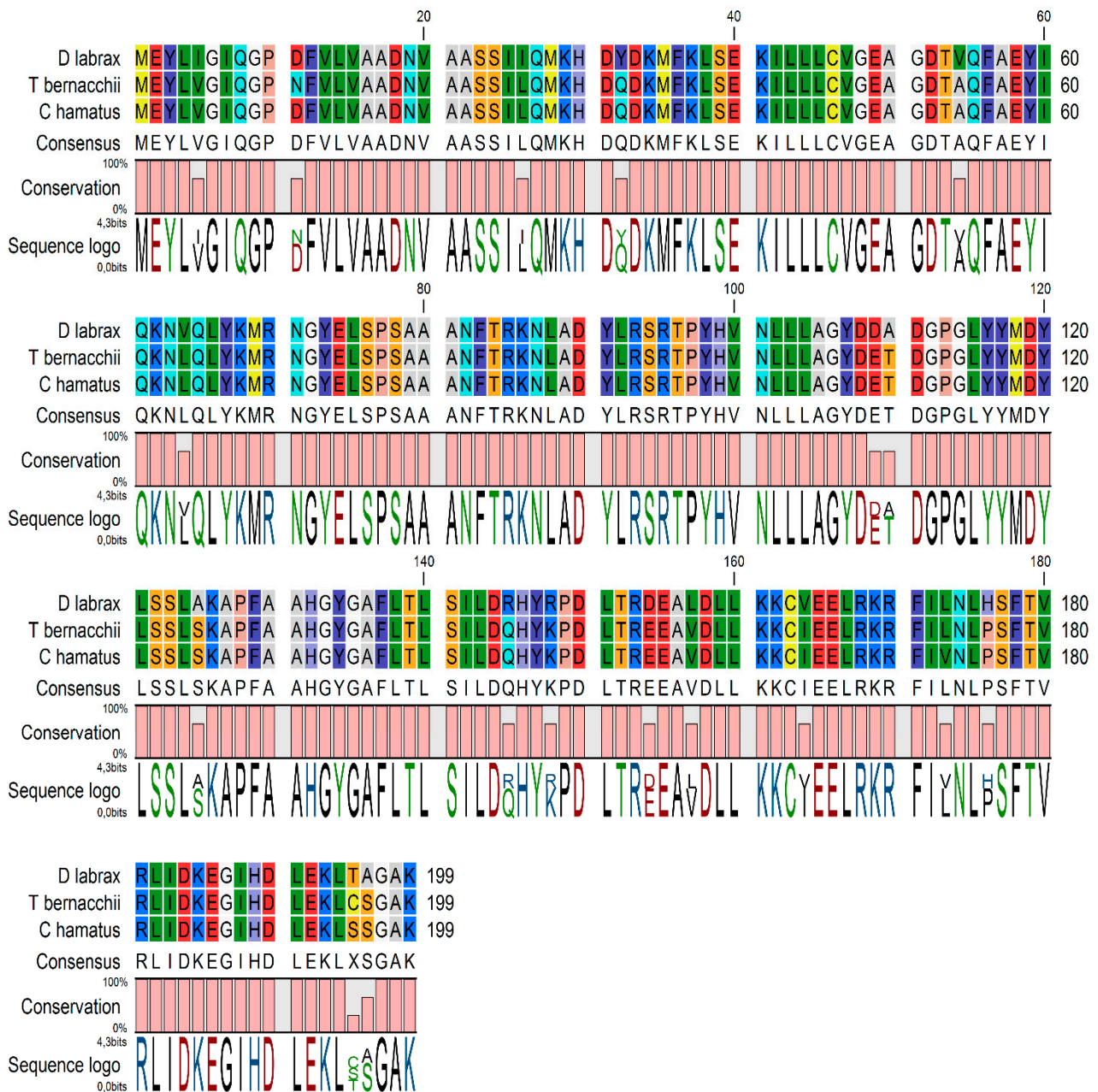


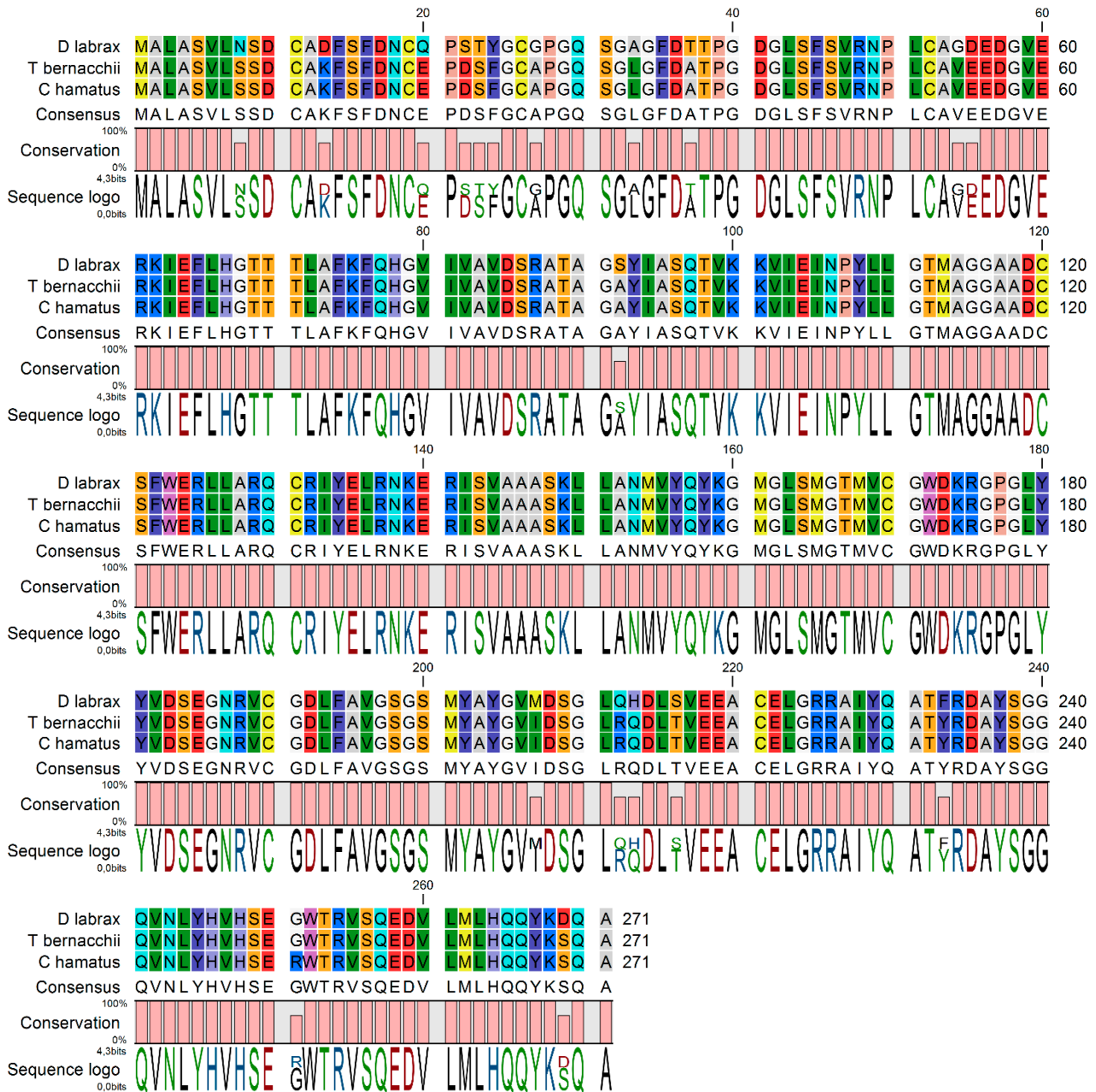
Figure S2. Effects of temperature on *D. labrax* and *C. hamatus* 26S proteasomes. Native-PAGE analyses of purified 26S proteasomes after incubation at 10 °C and 37 °C for 24 h are shown. The bands were visualized by Coomassie blue staining. The experiments were performed in duplicate on two different protein preparations loading equal amounts of 26S proteasomes.



A



B



C

Figure S3. MUSCLE alignments of the amino acid sequences of β_1 , β_2 , and β_5 proteasome catalytic subunits. Alignments of the amino acid translations of *C. hamatus* and *D. labrax* proteasome catalytic β subunits cDNAs with the homologues sequences from *T. bernacchii*. **A**, β_1 subunits; **B**, β_2 subunits; **C**, β_5 subunits. The amino acids are indicated observing the IUPAC codes, where the residues with negative polarity are in shades of red, and those with positive polarity are in blue shades. The consensus sequence, the conservation histogram and the sequence logo are shown at the bottom of the alignments.

Table S1. Purification steps of 26S proteasome from *D. labrax* erythrocytes.

Purification step	Total activity (U)	Total protein (mg)	Specific activity (U/mg)	U/mL blood	Purification fold	Yield (%)
Extract	5.16×10^5	69.00	7.47×10^3	1.84×10^5	1	100
DEAE	2.27×10^5	24.00	9.45×10^3	-	1.3	44
Phenyl Sepharose	6.50×10^4	0.90	7.22×10^4	-	9.7	12
Superose 6	6.50×10^3	0.08	8.10×10^4	-	10.8	1.2

The proteasome active fractions, recovered after each purification step, were detected by measuring the chymotrypsin (CT)-like activity using the specific fluorogenic substrate N-succinyl-Leu-Leu-Val-Tyr-7-amido-4-methylcoumarin and expressed in arbitrary unit.

Table S2. Purification steps of 26S proteasome from *C. hamatus* erythrocytes-like cells.

Purification step	Total activity (U)	Total protein (mg)	Specific activity (U/mg)	U/mL blood	Purification fold	Yield (%)
Extract	1.52×10^6	638.0	2.38×10^3	3.80×10^5	1	100
DEAE	2.89×10^5	68.0	4.25×10^3	-	1.80	19
Phenyl Sepharose	3.59×10^4	10.0	3.6×10^3	-	1.70	2.4
Superose 6	2.69×10^4	3.0	9.0×10^3	-	3.80	1.8

The proteasome active fractions, recovered after each purification step, were detected by measuring the chymotrypsin (CT)-like activity using the specific fluorogenic substrate N-succinyl-Leu-Leu-Val-Tyr-7-amido-4-methylcoumarin and expressed in arbitrary unit.