SUPPLEMENTARY INFORMATION:

Glycophagy is an ancient bilaterian pathway supporting metabolic adaptation through STBD1 structural evolution

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Supplemental information list:

This PDF file includes:

Figures S1 to S7

Other supporting information for this manuscript include the following:

Tables S1 and S2 (provided as a separate Excel file)

Data S1-S3 (provided as a separate Excel file)

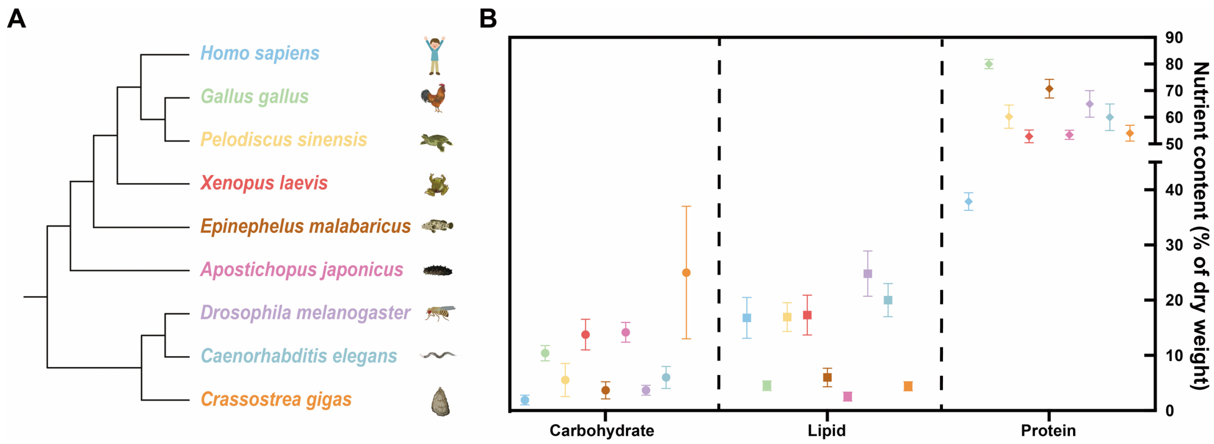

Fig. S1. Carbohydrate, lipid and protein storage across the Metazoa. A Phylogenetic tree for representative species. **B** Dry weight composition of carbohydrates, lipids, and proteins in *Homo sapiens* (adult man) was 1.91 ± 0.87%, 16.8 ± 3.7%, and 37.88 ± 1.6%[1-3]; *Gallus gallus* was 10.4 ± 1.39%, 4.50 ± 0.57% , and 79.98 ± 1.69%[4]; *Pelodiscus sinensis* was 5.52 ± 3%, 16.94 ± 2.6%, and 60.22 ± 4.37%[5]; *Xenopus laevis* (male) was 13.7 ± 2.77%, 17.3 ± 3.6%, and 52.8 ± 2.4%[6]; *Epinephelus malabaricus* was 3.67 ± 1.55%, 6.00 ± 1.67%, and 70.74 ± 2.52%[7]; *Apostichopus japonicus* was 14.17 ± 1.79%, 2.54 ± 0.21%, and 53.42 ± 1.22%[8]; *Drosophila melanogaster* was 3.69 ± 0.9%, 24.8 ± 4.1%, and 65 ± 5%[9,10]; *Caenorhabditis elegans* was 6 ± 2%, 20 ± 3%, and 60 ± 5%[11], and *Crassostrea gigas* was 25 ± 12%, 4.4 ± 0.8%, and 54 ± 3%[12].

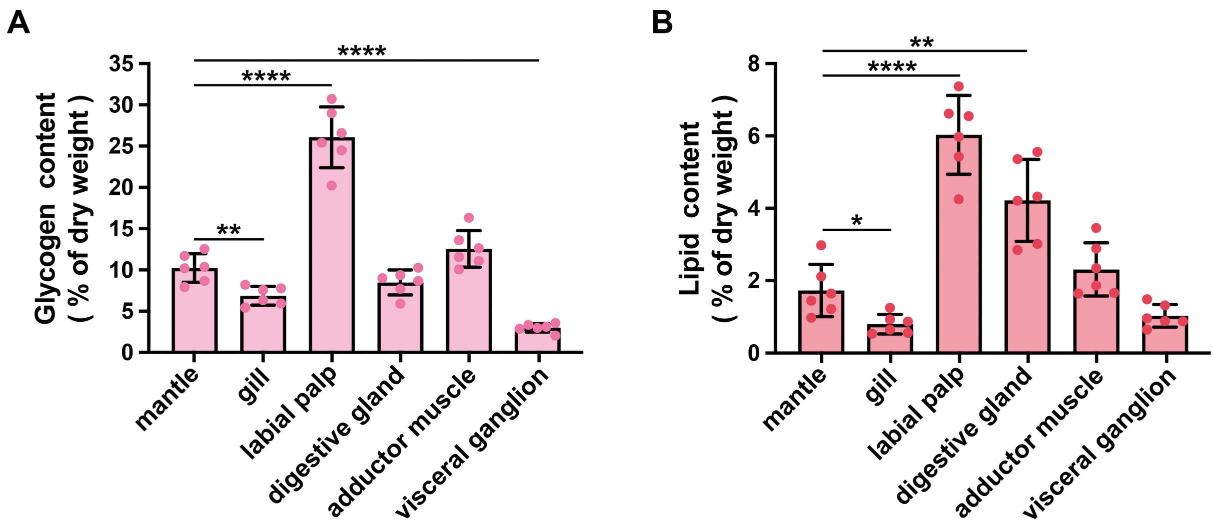

Fig. S2. Glycogen and lipid content in the mantle, gill, labial palp, digestive gland, adductor muscle and visceral ganglion. $n = 6$. Data are represented as mean ± SD in (**A, B**), where statistical significance was calculated using one-way ANOVA. *$p < 0.05$, **$p < 0.01$, ****$p < 0.0001$.

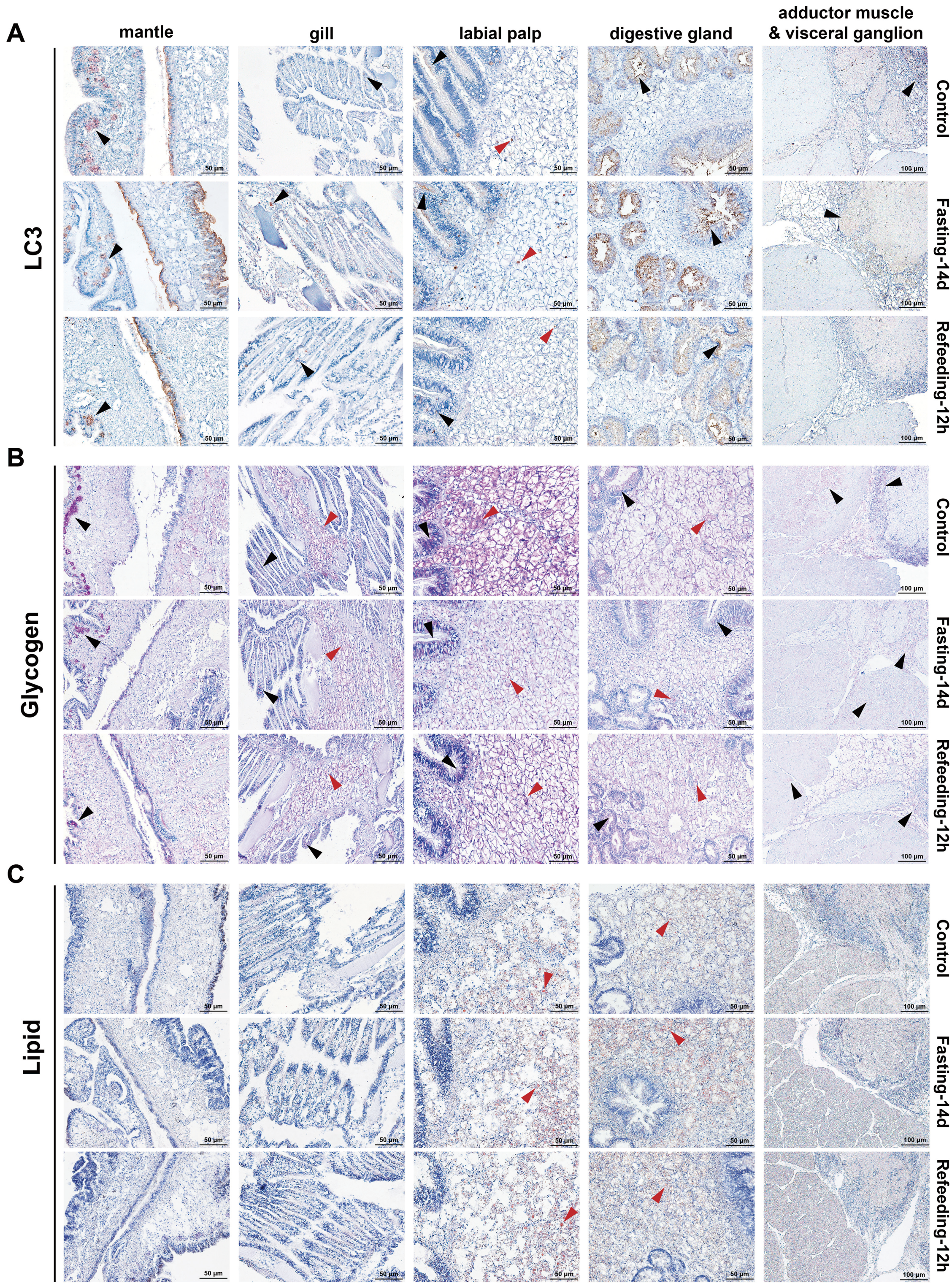

Fig. S3. Signals for the autophagy marker (A) LC3, (B) glycogen, (C) lipid in mantle, gill, labial palp, gonad, digestive gland, adductor muscle and visceral ganglion were observed after 14 d of fasting followed by 12 h of refeeding. Arrows indicate dense distribution of signals in the CE (black) and VCT (red) cells. Scale bars: 50 μm.

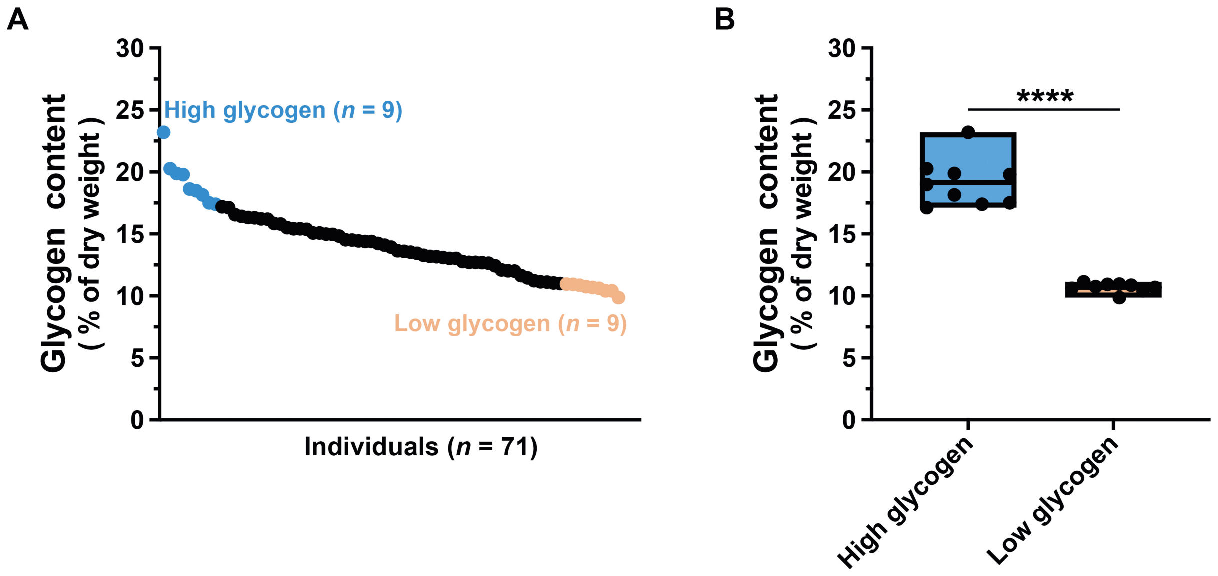

Fig. S4. Glycogen content in high glycogen and low glycogen groups. A Glycogen content in the labial palp dissected from 71 oyster individuals. Blue and pink points represent oyster samples with high and low glycogen, respectively. **B** The highest (average 19.15%) and lowest (average 10.7%) glycogen contents of 9 individuals sampled from 71 individuals, defining the high and low glycogen groups, respectively. Data are represented as mean ± SD in (**B**), where statistical significance was calculated using Student's *t* test. ****$p < 0.0001$.

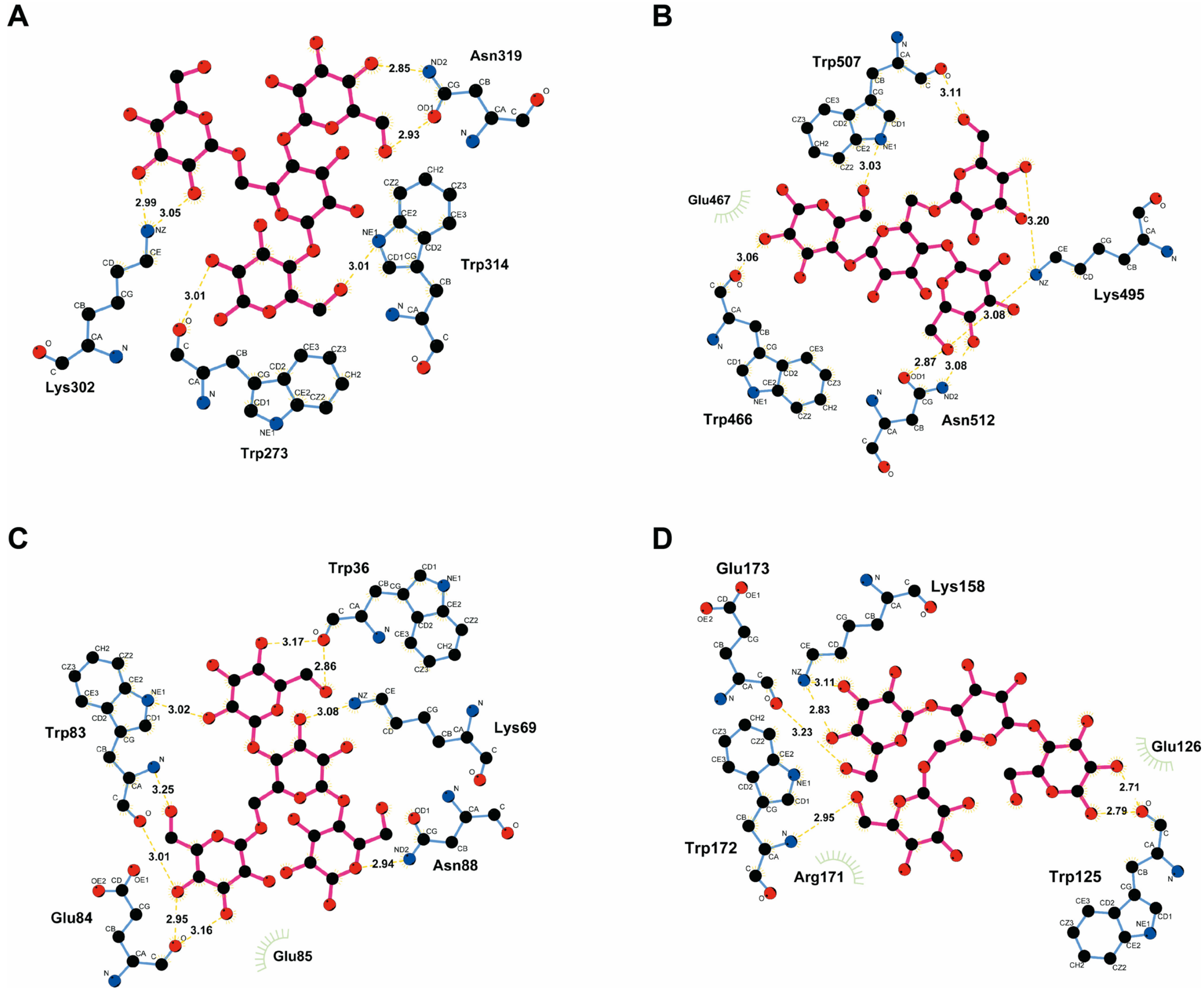

Fig. S5. Two dimensional views of docking models of glycogen with STBD1 from (A) mouse, (B) zebrafish, (C) *C. gigas*, and (D) a *C. gigas* STBD1 *in silico* mutant with CBM20 moved to the C-terminus. Hydrophobic residues are indicated by green radiations.

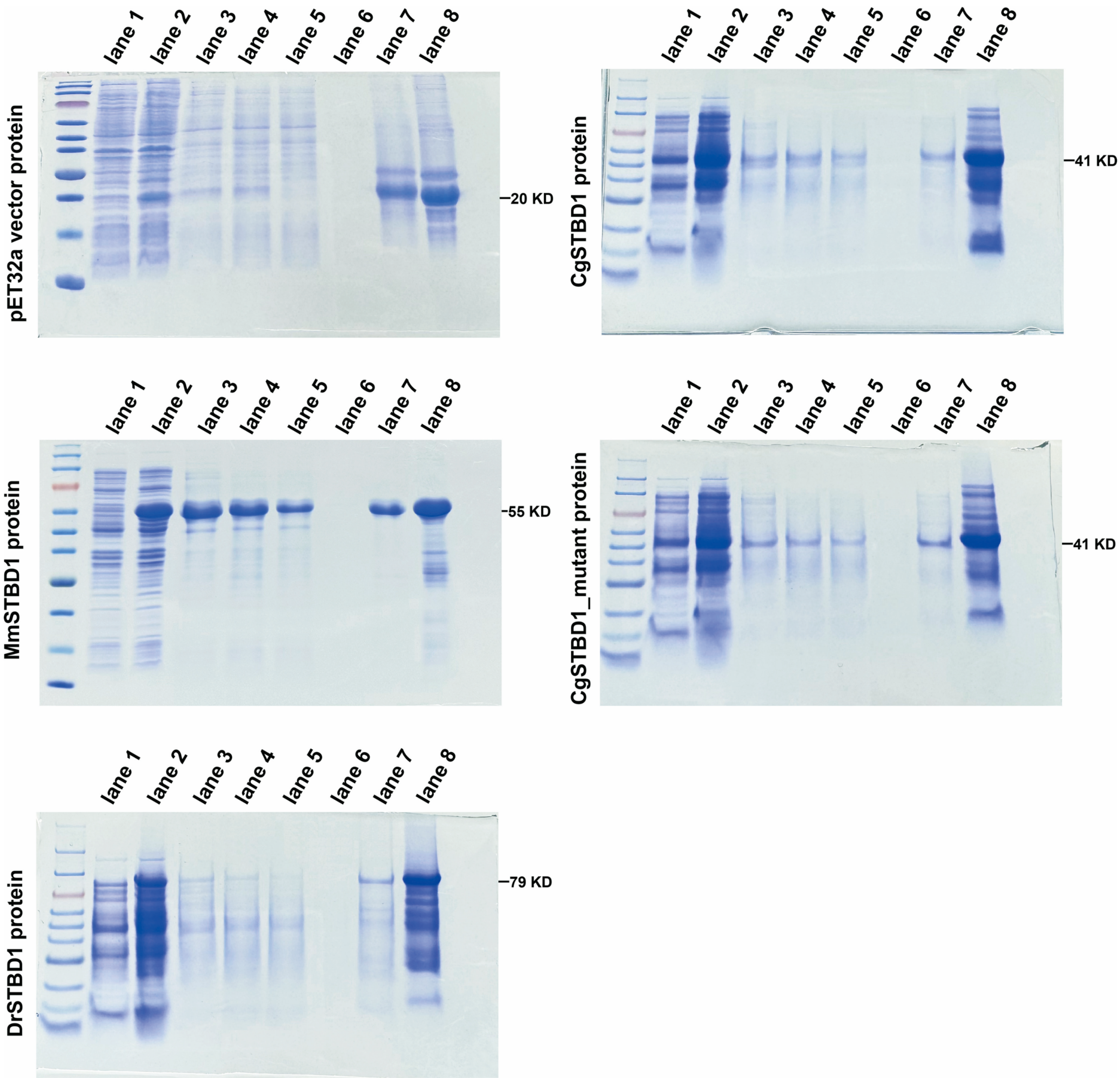

Fig. S6. SDS-PAGE was used to verify the molecular weights of pET32a vector recombinant proteins for STBD1 from mouse, zebrafish, wide-type *C. gigas* and a mutant *C. gigas* STBD1 with CBM20 moved to the C-terminus. Lane 1: prior to induction; lane 2: post induction; lane 3: bacterial lysate; lane 4: post-lysis supernatant; lane 5: flow-through fraction; lane 6: washing buffer; lane 7: elution buffer; lane 8: concentration buffer. Abbreviations used: Mm, *Mus musculus*; Dr, *Danio rerio*; Cg, *Crassostrea gigas.*

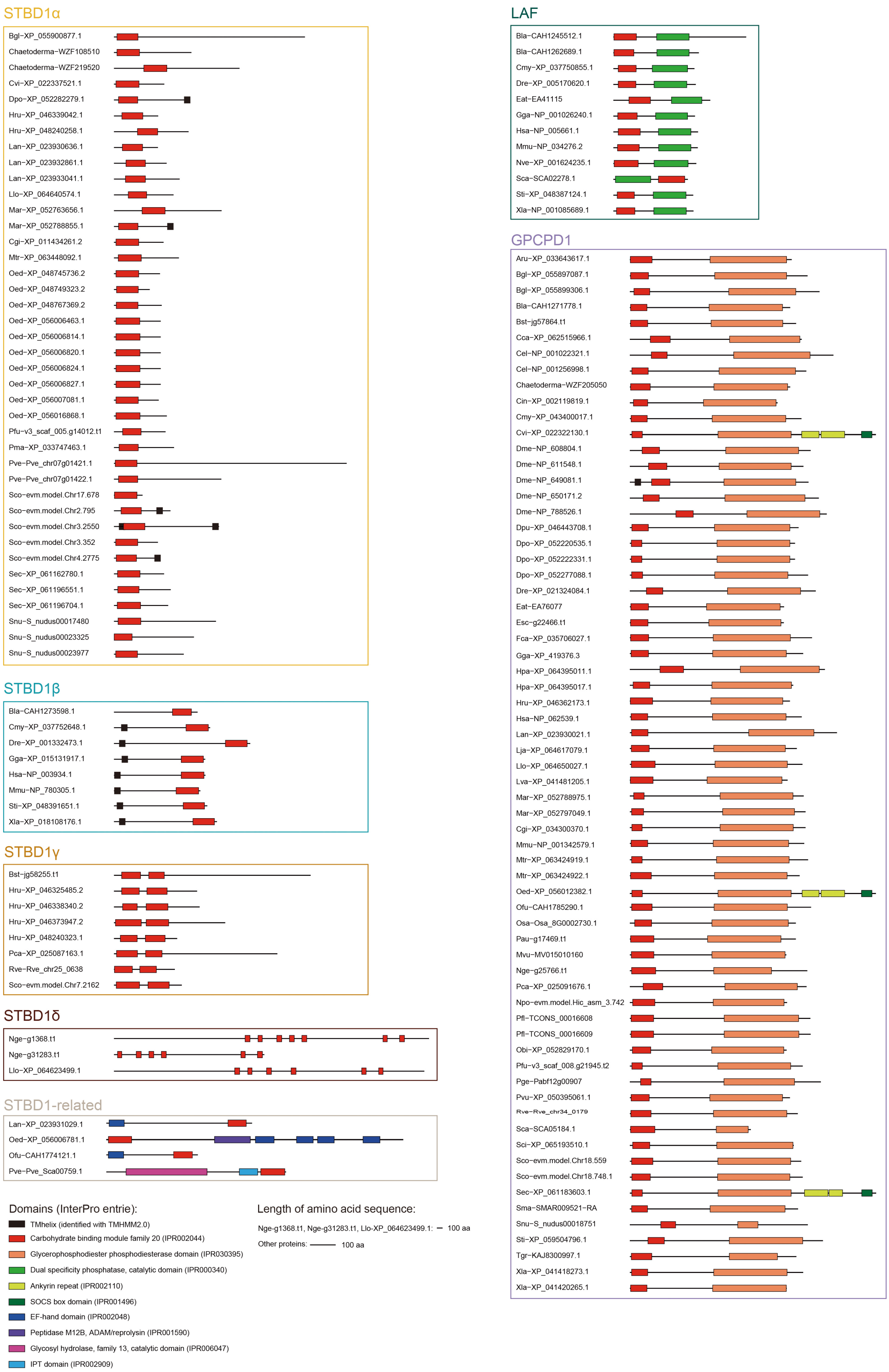

Fig. S7. Domain arrangements of CBM20 containing proteins. The proteins containing

CBM20 domain(s) were classified into seven types based on domain architectures. Briefly, among all identified proteins, those with CBM20 and dual specificity phosphatase domains were considered as LAF; those with CBM20 and glycerophosphodiester phosphodiesterase domains as GPCPD1; those with a single N-terminal CBM20 domain as STBD1α; those with one C-terminal CBM20 domain as STBD1β; those with two N-terminal CBM20 domains as STBD1γ; those with more than two N-terminal CBM20 domains as STBD1δ; and those with other domains such as EF-hand, peptidase M12B, IPT or glycosyl hydrolase family 13 as STBD1-related proteins. The corresponding abbreviations of species names are shown in Supplementary Table S1.

Supplemental references

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