

## Supplementary Material

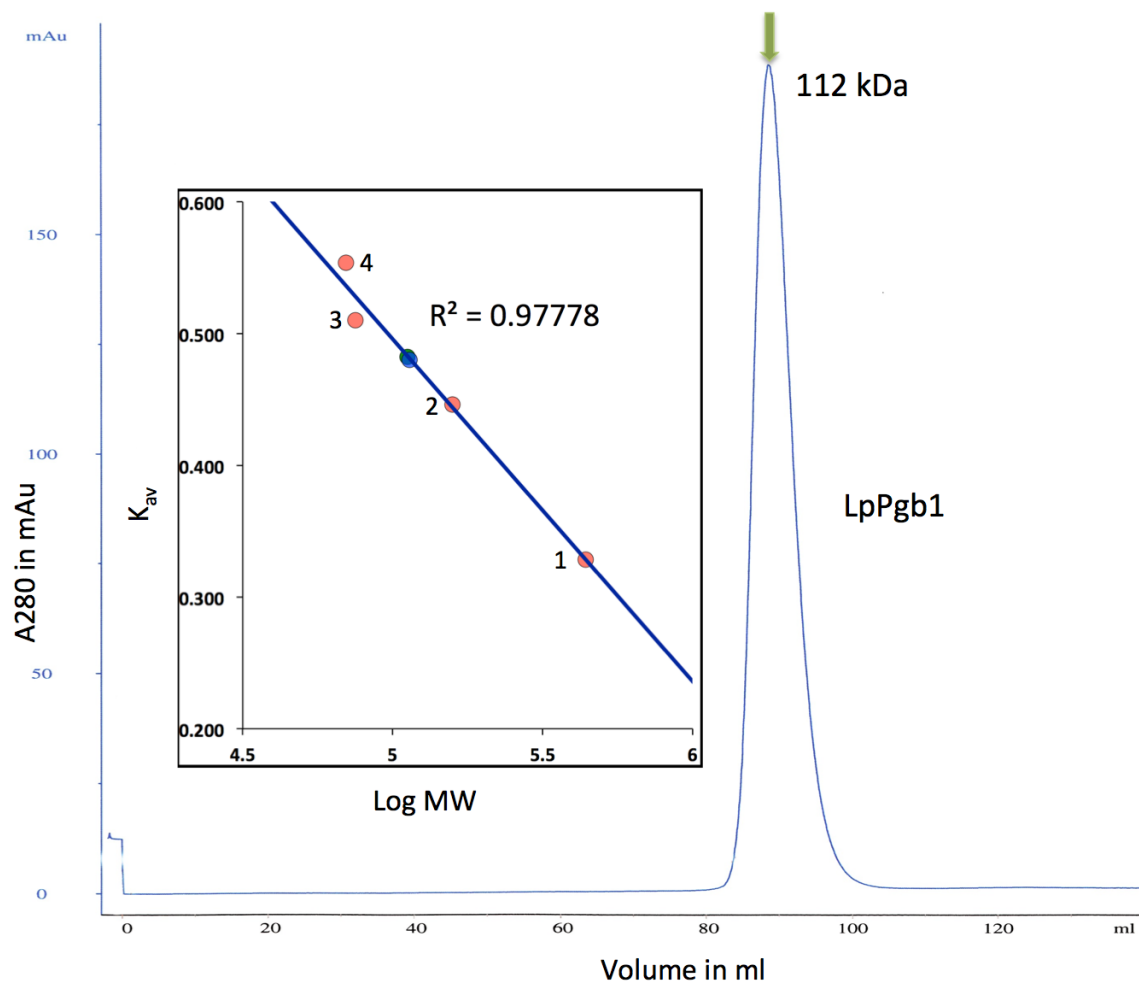
**Supplementary Table S1.** GH distribution.

| Species  | GH | GH1 | GH4 |
|--|----|-----|-----|
| <i>Enterococcus faecalis</i> V583                                      | 45 | 7   | 1   |
| <i>Lactobacillus plantarum</i> WCFS1                                   | 40 | 11  | 0   |
| <i>Lactobacillus rhamnosus</i> Lc 705                                  | 36 | 7   | 3   |
| <i>Lactobacillus plantarum</i> JDM1                                    | 35 | 9   | 2   |
| <i>Lactobacillus rhamnosus</i> GG                                      | 35 | 8   | 1   |
| <i>Streptococcus uberis</i> 0140J                                      | 34 | 12  | 1   |
| <i>Lactococcus lactis</i> subsp. <i>cremoris</i> MG1363                | 33 | 9   | 0   |
| <i>Lactobacillus acidophilus</i> NCFM                                  | 32 | 8   | 1   |
| <i>Streptococcus pneumoniae</i> 70585                                  | 30 | 6   | 0   |
| <i>Streptococcus suis</i> BM407  | 29 | 5   | 1   |
| <i>Leuconostoc mesenteroides</i> subsp. <i>mesenteroides</i> ATCC 8293 | 28 | 3   | 0   |
| <i>Lactococcus lactis</i> subsp. <i>cremoris</i> SK11                  | 28 | 7   | 0   |
| <i>Streptococcus pneumoniae</i> R6                                     | 28 | 7   | 0   |
| <i>Streptococcus pneumoniae</i> G54                                    | 28 | 6   | 0   |
| <i>Lactobacillus johnsonii</i> NCC 533                                 | 27 | 6   | 1   |
| <i>Lactobacillus casei</i> BL23  | 26 | 6   | 1   |
| <i>Lactobacillus gasseri</i> ATCC 33323                                | 26 | 7   | 0   |
| <i>Streptococcus pneumoniae</i> TIGR4                                  | 26 | 6   | 0   |
| <i>Streptococcus dysgalactiae</i> subsp. <i>equisimilis</i> GGS_124    | 25 | 8   | 0   |
| <i>Leuconostoc citreum</i> KM20  | 25 | 6   | 0   |
| <i>Streptococcus pneumoniae</i> P1031                                  | 25 | 6   | 0   |
| <i>Streptococcus pneumoniae</i> Taiwan19F-14                           | 25 | 6   | 0   |
| <i>Streptococcus gordonii</i> str. Challis substr. CH1                 | 25 | 5   | 0   |
| <i>Lactobacillus casei</i> ATCC 334                                    | 24 | 4   | 2   |

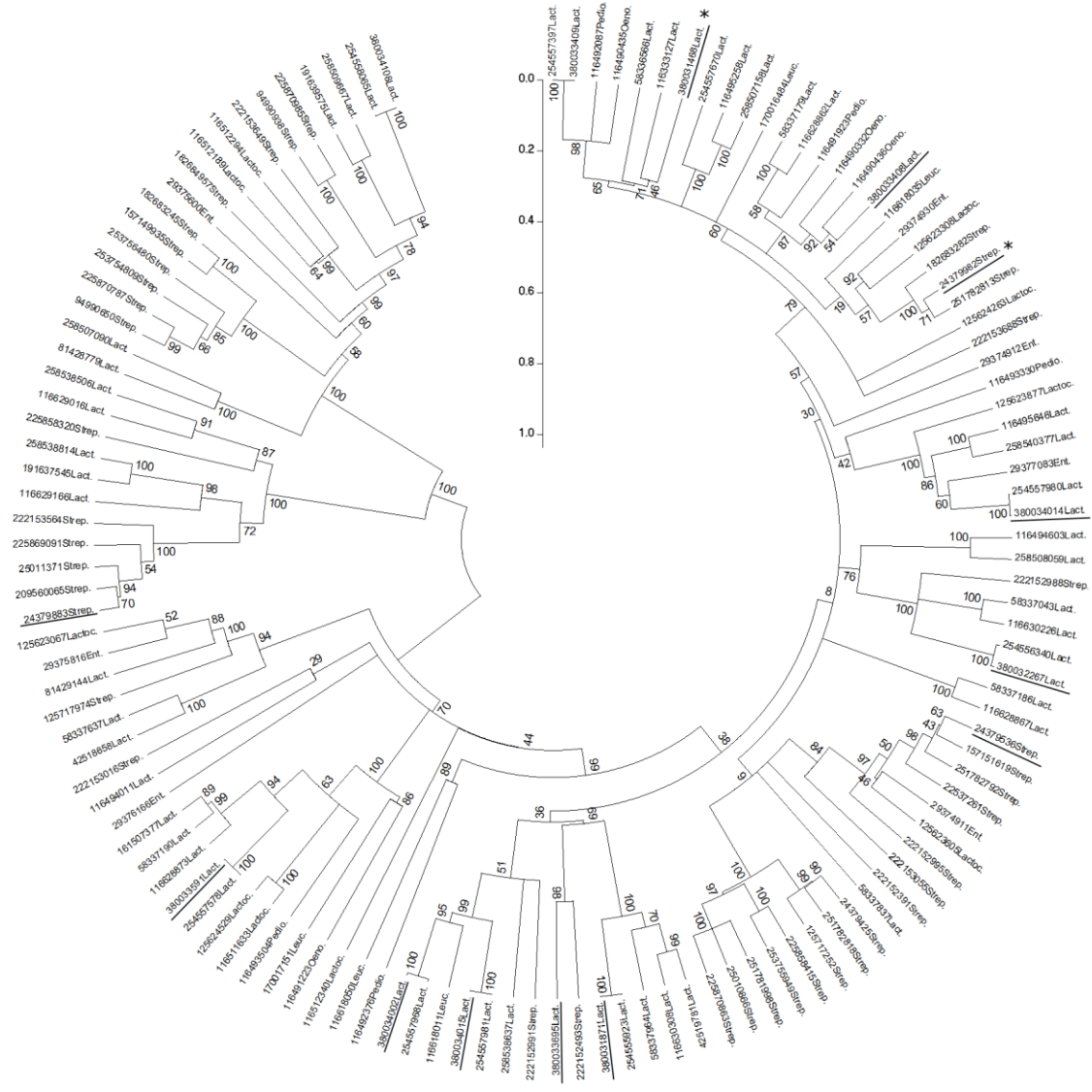
|   |    |   |   |
|---|----|---|---|
| Streptococcus pneumoniae CGSP14                   | 24 | 6 | 0 |
| Streptococcus pneumoniae JJA                      | 22 | 3 | 0 |
| Streptococcus mutans UA159                        | 21 | 4 | 0 |
| Streptococcus pneumoniae Hungary19A-6             | 21 | 4 | 0 |
| Streptococcus sanguinis SK36                      | 19 | 4 | 0 |
| Streptococcus thermophilus LMG 18311              | 19 | 6 | 0 |
| Streptococcus equi subsp. zooepidemicus           | 18 | 3 | 0 |
| Streptococcus equi subsp. equi 4047               | 17 | 3 | 0 |
| Streptococcus equi subsp. zooepidemicus MGCS10565 | 17 | 3 | 0 |
| Streptococcus pneumoniae ATCC 700669              | 17 | 3 | 0 |
| Streptococcus suis 98HAH33                        | 17 | 3 | 0 |
| Lactobacillus brevis ATCC 367                     | 17 | 1 | 0 |
| Pediococcus pentosaceus ATCC 25745                | 17 | 5 | 0 |
| Streptococcus pyogenes MGAS10270                  | 15 | 4 | 0 |
| Oenococcus oeni PSU-1                             | 14 | 4 | 0 |
| Streptococcus pyogenes MGAS2096                   | 14 | 4 | 0 |
| Streptococcus suis 05ZYH33                        | 13 | 2 | 2 |
| Streptococcus pyogenes MGAS10750                  | 13 | 4 | 0 |
| Streptococcus pyogenes MGAS315                    | 13 | 4 | 0 |
| Streptococcus pyogenes MGAS5005                   | 13 | 4 | 0 |
| Streptococcus pyogenes MGAS8232                   | 13 | 4 | 0 |
| Streptococcus agalactiae NEM316                   | 12 | 3 | 0 |
| Streptococcus pyogenes MGAS10394                  | 11 | 3 | 0 |
| Streptococcus pyogenes MGAS6180                   | 11 | 3 | 0 |
| Streptococcus pyogenes str. Manfredo              | 11 | 3 | 0 |
| Streptococcus pyogenes NZ131                      | 10 | 3 | 0 |
| Lactobacillus sakei subsp. sakei 23K              | 9  | 2 | 0 |

|  |   |   |   |
|--|---|---|---|
| Lactobacillus helveticus DPC 4571                        | 8 | 1 | 0 |
| Streptococcus agalactiae A909                            | 8 | 1 | 0 |
| Lactobacillus salivarius UCC118                          | 8 | 0 | 0 |
| Streptococcus pneumoniae D39                             | 7 | 1 | 0 |
| Lactobacillus fermentum IFO 3956                         | 7 | 0 | 0 |
| Lactobacillus reuteri JCM 1112                           | 7 | 0 | 0 |
| Streptococcus thermophilus CNRZ1066                      | 6 | 2 | 0 |
| Lactobacillus delbrueckii subsp. bulgaricus ATCC BAA-365 | 6 | 1 | 0 |
| Streptococcus suis SC84                                  | 5 | 1 | 0 |
| Streptococcus agalactiae 2603V/R                         | 4 | 1 | 0 |
| Streptococcus pyogenes MGAS9429                          | 4 | 0 | 0 |
| Lactobacillus delbrueckii subsp. bulgaricus ATCC 11842   | 3 | 0 | 0 |
| Streptococcus thermophilus LMD-9                         | 3 | 0 | 0 |
| Lactobacillus reuteri DSM 20016                          | 2 | 0 | 0 |
| Streptococcus pyogenes M1 GAS                            | 2 | 0 | 0 |
| Streptococcus pyogenes SSI-1                             | 2 | 0 | 0 |
| Streptococcus suis P1/7                                  | 2 | 0 | 0 |

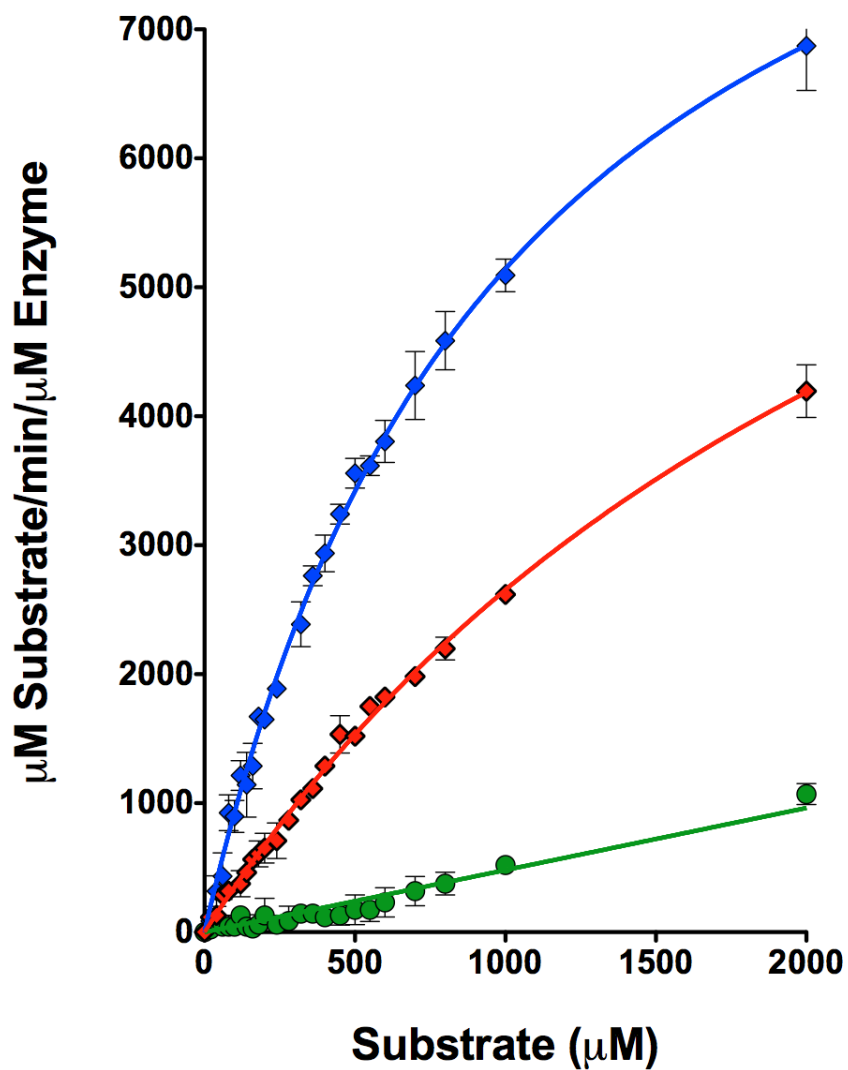
## Supplementary Figures



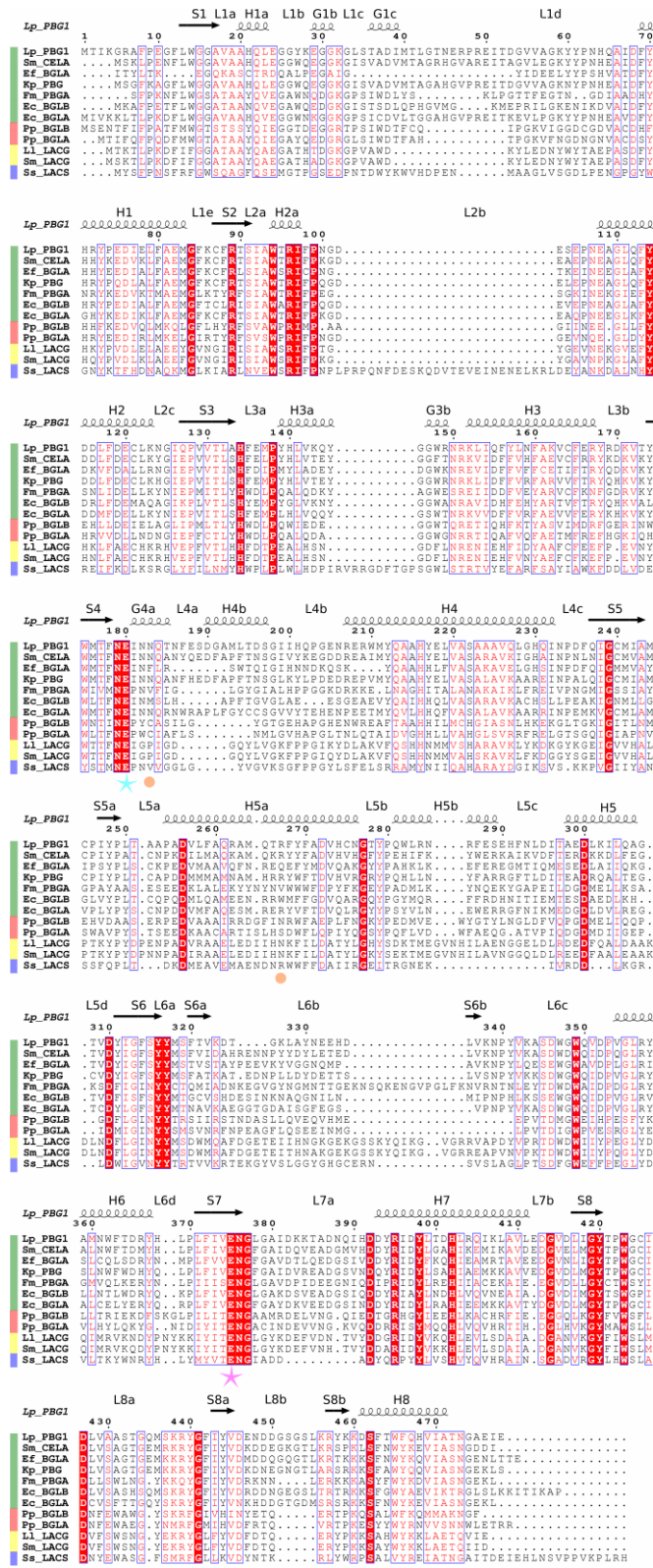
**Supplementary Figure S1.** Size exclusion chromatography. The absorbance at 280 nm is plotted in absorbance units (AU) versus retention volume in milliliters for LpPgb1. Inserted is a plot of  $K_{av}$  coefficient versus logarithm of molecular weight (red circles correspond to standard proteins: (1) ferritin (440 kDa) (2) aldolase (158 kDa), conalbumin (75 kDa), (4) ovalbumin (43 kDa), and the green circle corresponds to LpPgb1 (112 kDa) while the blue circle corresponds to SmBgl (114 kDa)). A single peak corresponding to a dimer is observed for both proteins.



**Supplementary Figure S2.** Phylogenetic analysis of LAB 6-P- $\beta$ -glucosidase family members. The tree was constructed using the aligned sequences of 126 CelA family members from 70 LABs with completed genomes. Bootstrap values are indicated. The *L. plantarum* WCFS1 and the *S. mutans* UA159 6-P- $\beta$ -glucosidase family members are underlined. Each terminal node of the tree is labeled by the Genbank Identifier (GI) number and the name of the genus. The taxonomy codes for the genus are the followings: Lact.: *Lactobacillus*, Strep.: *Streptococcus*, Leuc.: *Leuconostoc*, Pedio.: *Pediococcus*, Ent.: *Enterococcus*, Lactoc.: *Lactococcus*, and Oeno.: *Oenococcus*. The two 6-P- $\beta$ -glucosidase family members described in this manuscript are labeled with an asterisk.

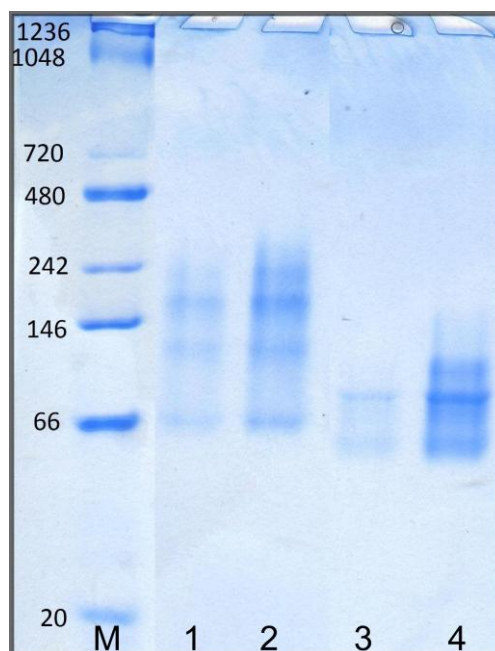


**Supplementary Figure S3.** Kinetics of 6'-P- $\beta$ -glucoside hydrolysis. Plot for substrate concentration dependent hydrolysis of 6'-P-salicin (blue), 6'-P-gentiobiose (red) and 6'-P-cellobiose (green) by SmBgl. The graph was generated using the program Prism.



**Supplementary Figure S4.** Multiple sequence alignment. 6-P-β-glucosidases (green), β-glucosidases (red), 6-P-β-galactosidases (yellow) and a β-galactosidase (blue) were aligned using ClustalW. Catalytic residues (cyan star – acid/base, magenta star – nucleophile) and phosphate-binding loop (grey) are indicated. Residues involved in the glucose binding in LpPbg1 are indicated by an orange circle. Identical residues are highlighted in red, similar

residues are shown as red letters. Secondary structure elements derived from LpPbg1 are depicted. Residues involved in aglycon binding are marked with orange dots. Lp – *L. plantarum*, Sm - *S. mutans*, Ef – *E. faecalis*, Kp – *K. pneumoniae*, Fm - *Fusobacterium mortiferum*, Ec – *E. coli*, Pp – *P. polymyxa*, Ll – *L. lactis*, Ss – *Sulfolobus solfataricus*.



**Supplementary Figure S5.** Native gel electrophoresis. M – Invitrogen Native Marker (1236 kD – IgM hexamer, 1048 kDa – IgM pentamer, 720 kDa – apoferritin band 1, 480 kDa – apoferritin band 2, 242 kDa – B-phycoerythrin, 146 kDa – lactate dehydrogenase, 66 kDa – bovine serum albumin, 20 kDa – soybean trypsin inhibitor), 1 – LpPbg1 (3.6  $\mu$ g), 2 – LpPbg1 (5.0  $\mu$ g), 3 – SmBgl (1.4  $\mu$ g), 4 – SmBgl (4.7  $\mu$ g).



