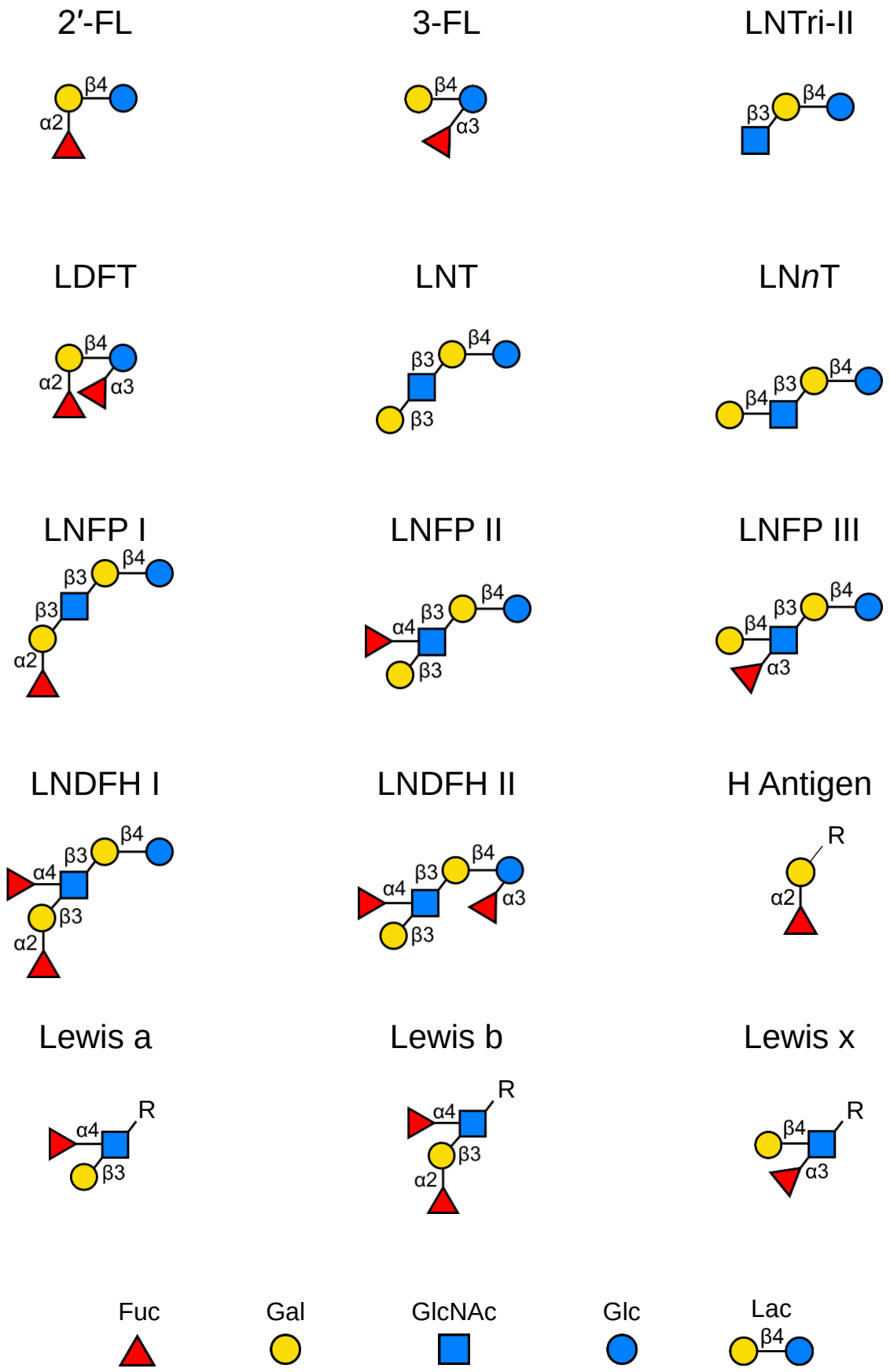


FIG S1 | Structures of glycans indicated in this study. The glycan structures are presented according to the Symbol Nomenclature for Glycans (SNFG) (https://www.ncbi.nlm.nih.gov/glycans/snfg.html).

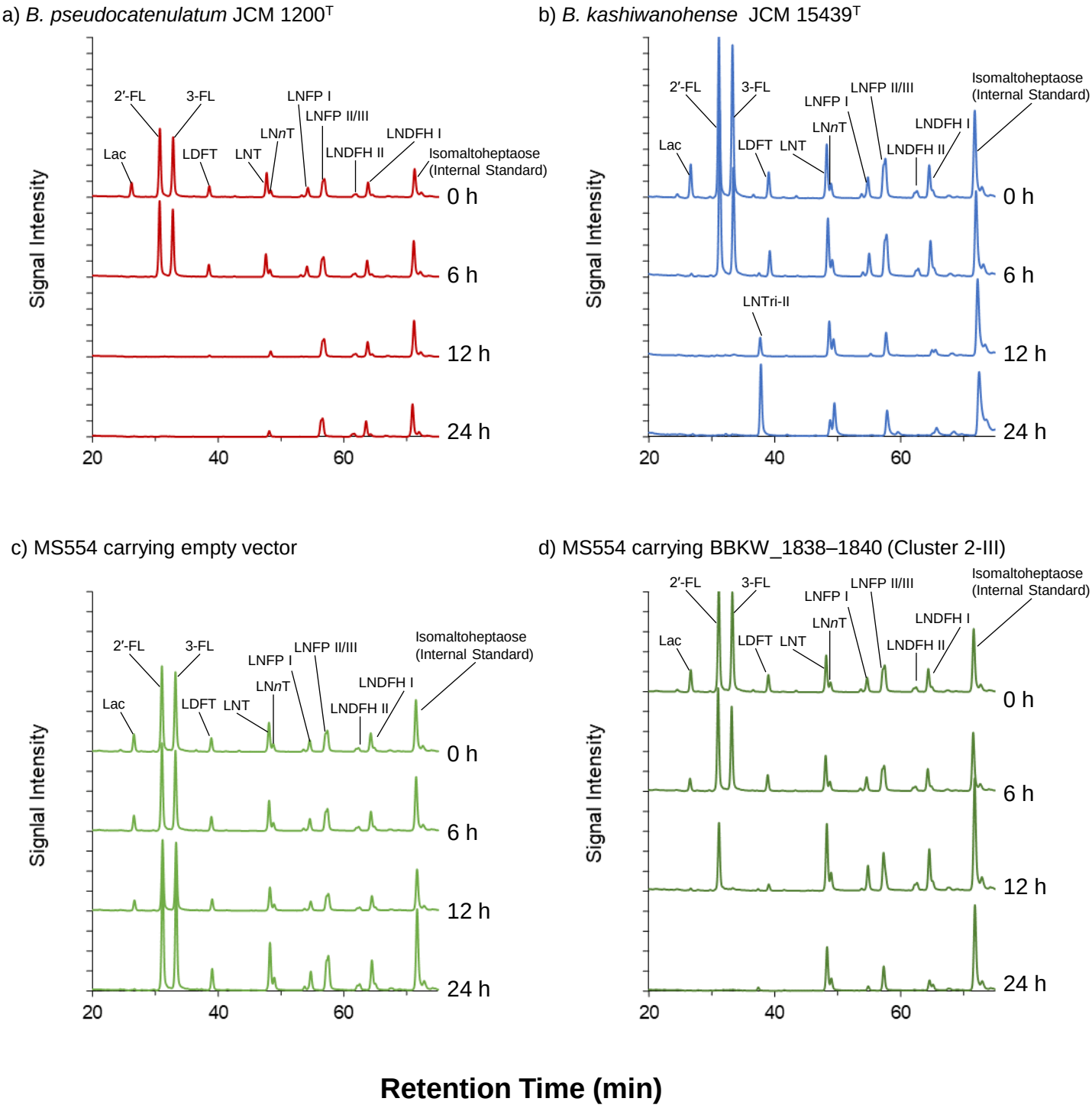
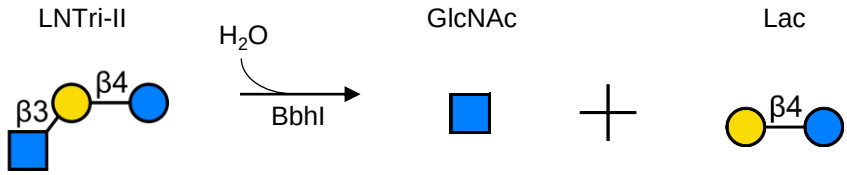


FIG S2 | HPLC profiles of HMO consumption. Culture supernatant was collected at the indicated times, and the sugars were derivatized by 2-anthranilic acid, solid phase purified, and analyzed by HPLC. a) *B. pseudocatenulatum* JCM 1200^T. b) *B. kashiwanohense* JCM 15439^T. c) MS554 carrying empty vector. d) MS554 carrying BBKW_1838–1840 [cluster 2-III]. Representative peak data of biological triplicates are shown.

a) Predicted Enzymatic Reaction with BbhI



b) HPLC Peaks for LNTri-II Fraction with BbhI Reactions

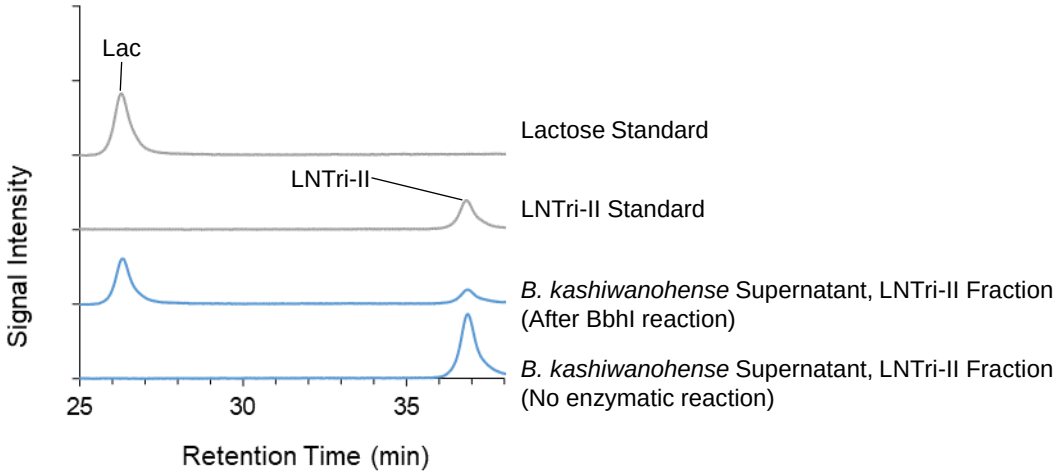
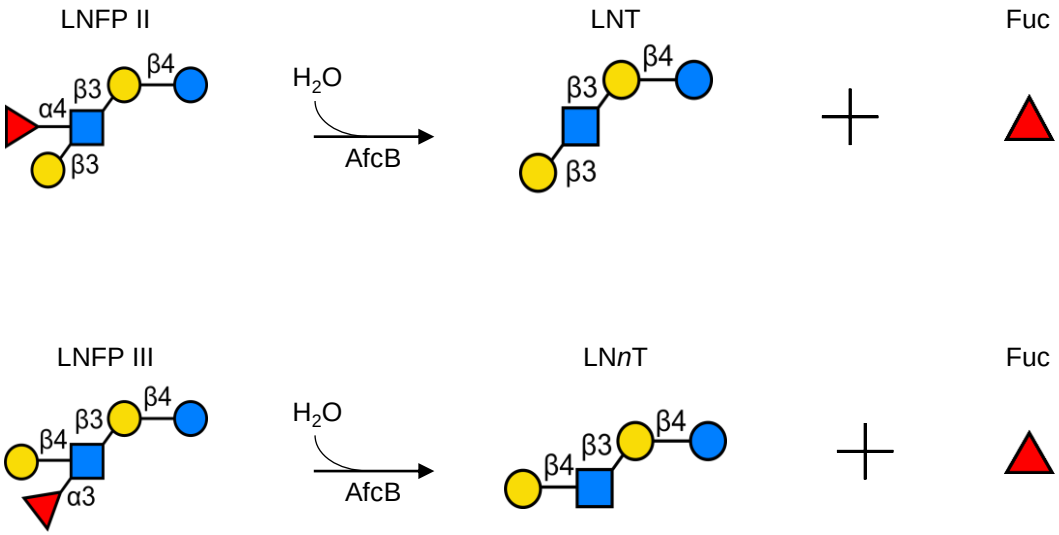


FIG S3 | Identification of LNTri-II through enzymatic assays. In order to confirm the identity of the peak found at a retention time near LNTri-II in the culture supernatant of *B. kashiwanohense* JCM 15439^T at 24 h, an enzymatic assay with BbhI was performed with the fraction containing the LNTri-II peak. a) The predicted enzymatic reaction in which BbhI would cleave the β-1,3-bond between Lac and GlcNAc of LNTri-II. b) HPLC profiles of culture supernatant before and after BbhI treatment. Data are representative of duplicate experiments.

a) Predicted Enzymatic Reaction with AfcB



b) HPLC Peaks for LNFP II/III Fraction with AfcB Reaction

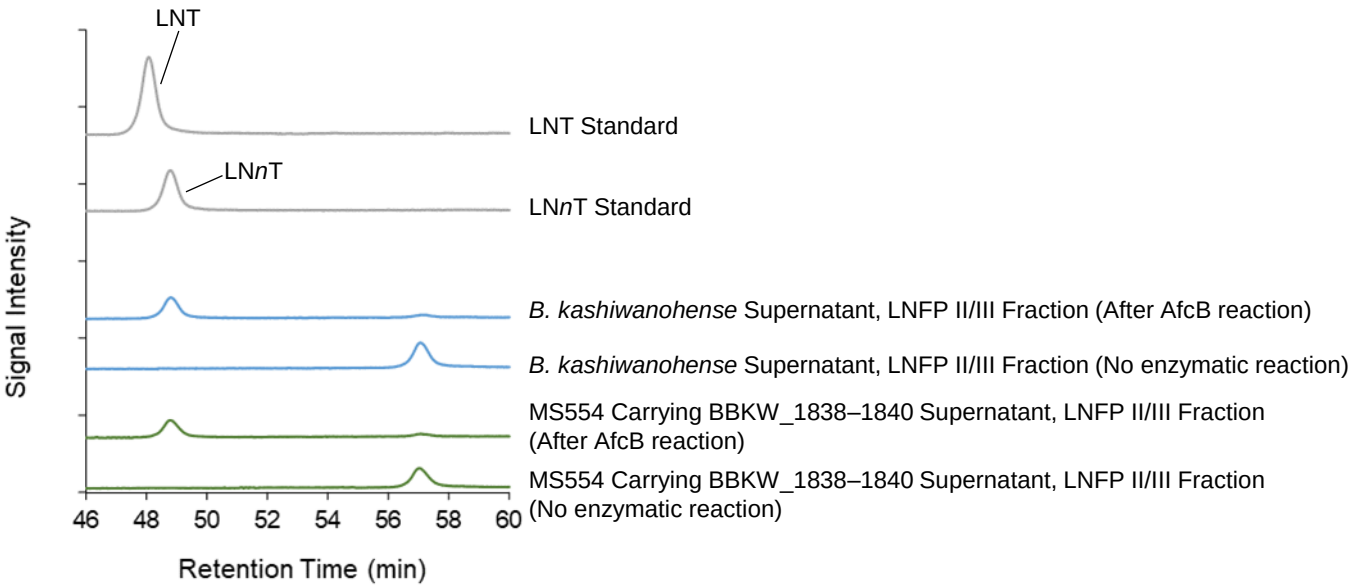


FIG S4 | Differentiation of LNFP II and LNFP III through enzymatic assays. In order to determine whether *B. kashiwanohense* JCM 15439^{T} and MS554 carrying BBKW_1838–1840 [cluster 2-III] was consuming LNFP II or LNFP III, an enzymatic assay with AfcB with the fraction containing LNFP II/III from the culture supernatant at 24 h was performed. a) The predicted enzymatic reaction of AfcB against LNFP II, in which the α-1,4-bond between Fuc and GlcNAc would be cleaved to produce LNT and Fuc, and of AfcB against LNFP III, in which the α-1,3-bond between Fuc and GlcNAc would be cleaved to produce LN*n*T and Fuc. b) HPLC profiles of culture supernatant before and after AfcB treatment. Data are representative of duplicate experiments.

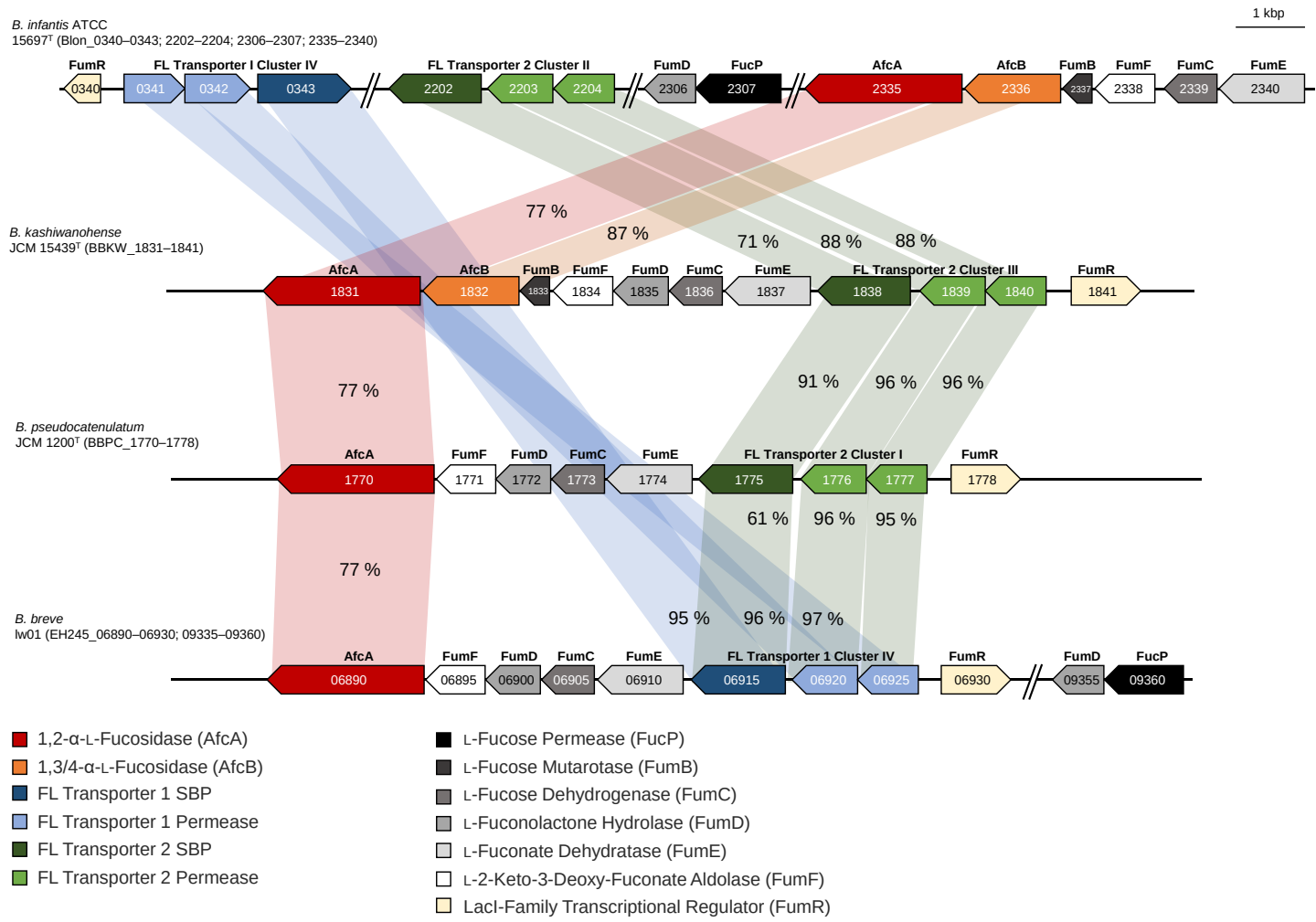


FIG S5 | FL / fucose utilization loci in representative bifidobacterial strains with FL transporter(s). Comparison of the FL transporter and fucosidase (AfcA and AfcB) gene loci in *B. infantis* ATCC 15697^{T} against *B. kashiwanohense* JCM 15439^{T}, *B. pseudocatenulatum* JCM 1200^{T}, and *B. breve* lw01. Each arrow represents an open reading frame (ORF), with lengths proportional to its size. Each ORF is labeled with its corresponding protein name. The percentages shown represent amino acid sequence identities of each protein against its corresponding protein in *B. infantis* ATCC 15697^{T}. Percentage values are shown for the FL transporter and fucosidase (AfcA and AfcB) genes, and identities are > 50 % for other proteins.