Supplementary Materials

*In vitro* prebiotic and anti-colon cancer activity of agar-derived sugars from red seaweeds

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Figure S1. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis analysis of the purified recombinant proteins of Aga16B, Aga50D, and SdNABH. Lanes: M, protein markers; 1–3, (1) Aga16B, (2) Aga50D, and (3) SdNABH purified by His-tag affinity chromatography.
**Figure S2.** Stability test of AgaDP3 in the presence of simulated gastric fluid. (A) AgaDP3 was incubated with simulated gastric fluid comprising 0.2% (w/v) sodium chloride in 0.7% (v/v) hydrochloric acid at 37°C for 3 h. The concentration of AgaDP3 was monitored using HPLC. (B) Overlaid HPLC chromatograms profiling the partial degradation of AgaDP3 during incubation.
Figure S3. Calibration curves of purified agar-derived sugars produced from agarose by the enzymatic reactions of Aga16B, Aga50D, and ScNABH. (A–C) Calibration curves of AHG, NeoDP2, and AgaDP3 for quantitative analyses by HPLC. (D–F) Calibration curves for cellotetraose, cellopentaose, and cellohexaose for quantitative analyses of NeoDP4, AgaDP5, and NeoDP6, respectively, by HPAEC-PAD.