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 *Sd*NABH. Lanes: M, protein markers; 1–3, (1) Aga16B, (2) Aga50D, and (3) *Sd*NABH 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 *Sd*NABH. (**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.