Electronic Supplementary Material (ESI) for Food & Function

Supplementary Methods

Intestinal organoid culture and drug treatment.

Intestinal organoids were established and maintained as described in the Materials and Methods. For pharmacological experiments, organoids were cultured in differentiation medium and treated with propranolol or vehicle control as indicated.

Selection of propranolol concentration.

A preliminary dose-ranging experiment was conducted to guide the selection of a working concentration of propranolol that could reproducibly attenuate folic acid–induced organoid phenotypes without compromising baseline organoid growth or viability.

For the propranolol-alone experiments (Fig. S1), organoids were treated with propranolol (10, 25, 50, or 100 μM) in the absence of folic acid for the indicated duration, and organoid growth, budding, and viability were assessed to evaluate baseline effects.

For the dose–response experiments (Fig. S2), organoids were stimulated with folic acid (FA) and co-treated with propranolol at 10, 25, 50, or 100 μM. The FA + vehicle group served as the control condition and is denoted as “FA” in the figures for brevity.

Based on these preliminary experiments, lower concentrations (10–25 μM) provided weaker or less consistent modulation of folic acid–induced responses, whereas the highest concentration tested (100 μM) was less suitable due to broader effects on organoid phenotypes. Accordingly, 50 μM propranolol was selected for subsequent experiments.

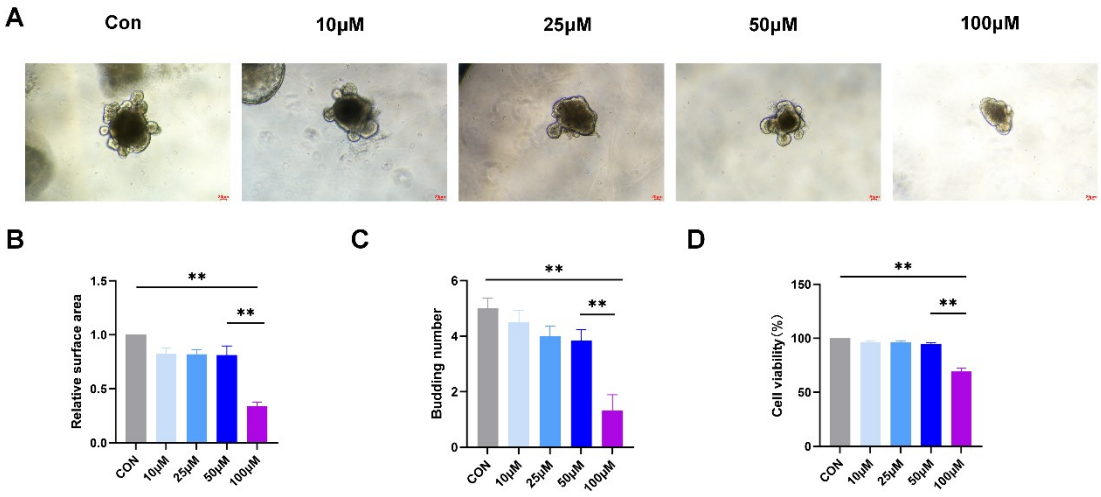

Fig. S1. Propranolol alone does not significantly affect intestinal organoid growth or viability under the conditions used in this study.

CON denotes the vehicle-treated control group.

(A) Representative bright-field images of intestinal organoids treated with vehicle or propranolol alone (scale bar, 20 μm).

(B) Quantification of organoid area after treatment with the vehicle-treated control (CON) or propranolol alone (vehicle-normalized).

(C) Budding number per organoid under the same conditions.

(D) Cell viability assay results (vehicle-normalized).

Data are presented as mean ± SEM, with n = 6 organoids per group. Statistical significance was assessed using one-way ANOVA followed by Dunnett's multiple comparisons test versus the vehicle-treated control group. *$p < 0.05$ and **$p < 0.01$.

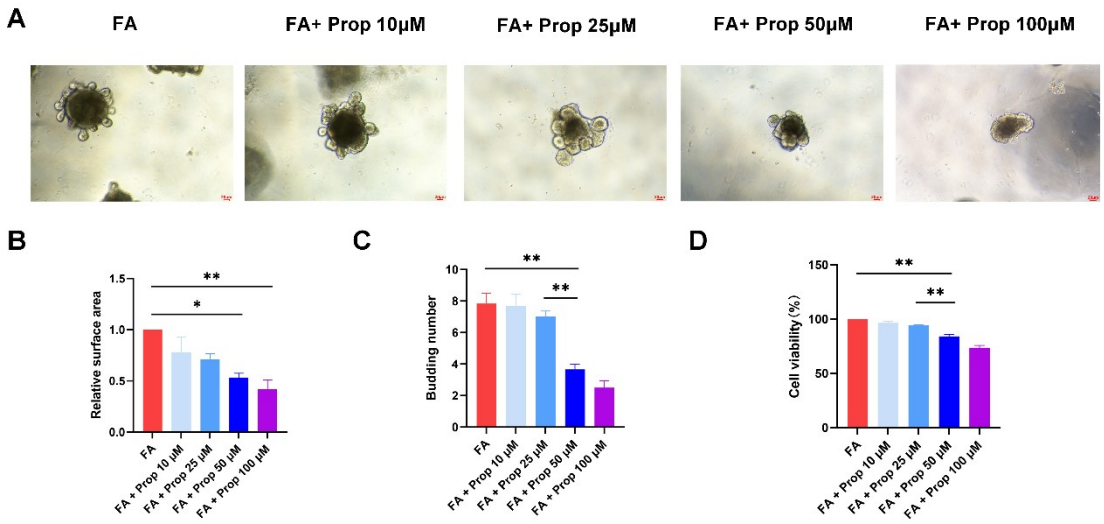

Fig. S2. Dose–response effects of propranolol on folic acid–induced organoid phenotypes.

Intestinal organoids were stimulated with folic acid (FA) and co-treated with propranolol at the indicated concentrations (10, 25, 50, or 100 μM). FA denotes the FA + vehicle group.

(A) Representative bright-field images of intestinal organoids under the indicated conditions (scale bar, 20 μm).

(B) Quantification of relative organoid area, normalized to the FA + vehicle group.

(C) Budding number per organoid under the same conditions.

(D) Cell viability assay results, normalized to the FA + vehicle group.

Data are presented as mean ± SEM, with n = 6 organoids per group. Statistical significance was assessed using one-way ANOVA followed by Dunnett's multiple comparisons test versus the FA + vehicle group. *$p < 0.05$ and **$p < 0.01$.