

USE OF ARACHIDONIC ACID FOR AMELIORATION OF CYTOTOXIC EFFECTS FROM CHEMOTHERAPY AND RADIATION THERAPY

CROSS-REFERENCE TO RELATED APPLICATIONS

5 This application claims the benefit under 35 U.S.C. § 119(e) of U.S. Provisional Application No. 63/482,280, filed January 30, 2023, entitled “USE OF ARACHIDONIC ACID FOR AMELIORATION OF CYTOTOXIC EFFECTS FROM CHEMOTHERAPY AND RADIATION THERAPY,” the entire disclosure of which is hereby incorporated by reference.

FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

10 This invention was made with government support under grant number CA045508 awarded by the National Institutes of Health. The government has certain rights in the invention.

BACKGROUND

15 Cancer treatment ideally eliminates all cells capable of causing cancer recurrence in a patient’s lifetime. Chemotherapy and radiation therapy are two forms of treatment for several types of cancers. Chemotherapy involves administration of single or multidrug regimens to
20 kill cancer cells and shrink tumors, while radiation therapy uses high doses of radiation to do the same. Where surgery is employed as a cancer treatment, combining surgery with chemotherapy or radiotherapy may improve cure rates or allow for more limited surgery. Radiation therapy may be given before surgery or chemotherapy (neoadjuvant therapy) or after surgery or chemotherapy (adjuvant therapy). While chemotherapy and radiation therapy
25 target cancer cells, normal cells are often also affected, resulting in adverse side effects and cytotoxic effects on normal cells and tissues which severely impact a cancer patient’s quality of life.

SUMMARY

30 The present disclosure exemplifies how augmented intestinal stem cells (ISC) generation or stemness-enhancing effects of oral administration or consumption of arachidonic acid triglyceride (AA TG), described herein, provides a basis for methods and compositions for reducing, preventing or reversing the cytotoxic/adverse effects of chemotherapy or exposure to radiation, such as through radiation therapy. Among other

things, treatment of intestinal organoid-derived single cells with fatty acid (FA) members of the omega-6 family (e.g., linoleic acid (LA); gamma-linolenic acid (gamma-LA); dihomo-gamma-linolenic acid (dh-gamma-LA); and arachidonic acid (AA)) not only promotes the formation of spheroids with morphology that is correlated with an enhanced regenerative stem cell state (stemness) and reduced differentiation, but also leads to significant increase in size, compared to control organoids. Further, omega-6 FAs converge on arachidonic acid (AA) in gastrointestinal cells and omega-6 fatty acids that converge on arachidonic acid (AA) enhance the stemness of mouse and human organoids. Arachidonic acid supplementation, in the form of oral administration of arachidonic acid triglyceride (AA TG)/dietary elevation of AA in the intestine, such as through consumption of an AA TG-rich diet or other oral intake, augments ISC regeneration.

Treatment with chemotherapy or radiation therapy often results in adverse side effects and cytotoxic effects due, at least in part, to damage of normal cells that are not a target for treatment. Promoting cellular repair mechanisms is a potential avenue to prevent damage to normal cells and prevent, reduce or reverse adverse side effects or cytotoxic effects due to chemotherapy or radiation therapy. Intestinal cells, for instance, may be affected by changes in a subject's diet. One of the common features of dietary interventions to promote intestinal resilience has been increase in abundance and metabolism of fatty acids (FAs) either through dietary intake or release from adipose tissue (Novak et al., 2021). However, several clinical and epidemiological studies suggest that increasing total polyunsaturated fatty acids (PUFAs) including omega-6 fatty acids elevates cancer risk. In sum, definitive evidence for the effects of omega-6 on cancer outcomes has been lacking. PUFAs including omega-6 fatty acids such as arachidonic acid are important structural components of cell membranes that rapidly proliferating cells require for their growth. In addition, upon tissue damage, omega-6 fatty acids are released from cell membranes to give rise to inflammatory bioactive lipid mediators such as prostaglandins, which are implicated in carcinogenesis (Hanson, et al. *Br J Cancer* (2020) 122(8):1260-70; Sakai, et al. *BMC Cancer* (2012) 12:606; Liput, et al. *Int J Mol Sci* (2021) 22(13):6965; Azrad, et al. *Front Oncol* (2013) 3:224).

In some embodiments, the present disclosure provides fatty acids (FAs) (e.g., dietary FAs) to a subject in need thereof to prevent, reduce or reverse adverse side effects or cytotoxic effects due to chemotherapy or radiation therapy. In some embodiments, administration of a FA, such as arachidonic acid (AA), at least one precursor of AA (linoleic acid (LA), gamma-linolenic acid (γ -LA), dihomo- γ -linolenic acid (dh- γ -LA), LA and γ -LA, γ -LA and dh- γ -LA, or LA, γ -LA, and dh- γ -LA), or a combination of AA and at least one

precursor of AA, to a subject before the subject starts a course of chemotherapy or radiation therapy, during a course of chemotherapy or radiation therapy, or after the subject completes a course of chemotherapy or radiation therapy, prevents, reduces or reverses adverse side effects or cytotoxic effects due to chemotherapy or radiation therapy in the subject. In some
5 embodiments, providing AA, at least one precursor of AA, or AA and at least one precursor of AA prevents, reduces or reverses adverse side effects or cytotoxic effects due to chemotherapy or radiation therapy in the subject. In some embodiments, AA or at least one precursor of AA is in the form of a triglyceride (TG, AA TG, AA precursor TG).

10 In some embodiments, methods of preventing, reducing, or reversing adverse side effects due to chemotherapy or radiation therapy in a subject are disclosed.

In some embodiments, the method comprises administering orally to a subject in need thereof at least about 2 g of arachidonic acid triglyceride (AA TG) per day (2 g/d) for a sufficient time to prevent, reduce or reverse adverse side effects due to chemotherapy or radiation therapy in the subject.

15 In some embodiments, the sufficient time is at least about 7 days; and (a) administration starts no earlier than 28 days before the subject begins a course of chemotherapy or radiation therapy; (b) administration starts no later than 28 days after the subject completes a course of chemotherapy or radiation therapy; or (c) administration starts at any time during a course of chemotherapy or radiation therapy.

20 In some embodiments, the sufficient time is at least about 14 days.

In some embodiments, the sufficient time is at least about 21 days.

In some embodiments, the sufficient time is at least about 28 days.

In some embodiments, the course of chemotherapy or radiation therapy lasts for at least about 3 months.

25 In some embodiments, the course of chemotherapy or radiation therapy lasts for at least about 6 months.

In some embodiments, the course of chemotherapy or radiation therapy lasts for at least about 12 months.

30 In some embodiments, the course of chemotherapy or radiation therapy lasts from about 3 months to about 12 months.

In some embodiments, at least about 3 g of AA TG/day (3 g/d) is administered to the subject.

In some embodiments, at least about 20 g of AA TG/day (20 g/d) is administered to the subject.

In some embodiments, at least about 30 g of AA TG/day (30 g/d) is administered to the subject.

In some embodiments, at least about 60 g of AA TG/day (60 g/d) is administered to the subject.

5 In some embodiments, at least about 90 g of AA TG/day (90 g/d) is administered to the subject.

In some embodiments, at least about 100 g of AA TG/day (100 g/d) is administered to the subject.

10 In some embodiments, from about 2 g of AA TG/day (2 g/d) to about 100 g of AA TG/day (100 g/d) is administered to the subject.

In some embodiments, the AA TG is in a composition.

In some embodiments, the composition comprises at least about 2% AA TG by weight.

15 In some embodiments, the composition comprises between about 20% AA TG and about 50% AA TG by weight.

In some embodiments, the composition comprises about 40% AA TG by weight.

In some embodiments, the composition comprises no more than 5% arachidonic acid (AA) ester by weight.

In some embodiments, the composition is an oil.

20 In some embodiments, the oil is extracted from a fungus.

In some embodiments, the fungus is *Mortierella alpina*.

In some embodiments, the composition is a liquid or a powder.

In some embodiments, the composition is in a food, in a capsule or in a pill.

25 In some embodiments, the composition further comprises at least one precursor of AA. In some embodiments, the precursor of AA is in a triglyceride (TG) form. In some embodiments, the precursor of AA is linoleic acid (LA), γ -linolenic acid (γ -LA), dihomogamma-linolenic acid (dh- γ -LA), LA and γ -LA, γ -LA and dh- γ -LA, or LA, γ -LA, and dh- γ -LA.

In some embodiments, the AA TG increases an intestinal AA level in the subject that produces a beneficial effect.

30 In some embodiments, administration of AA TG increases a plasma AA level in the subject by at least 2-fold relative to a reference.

In some embodiments, the reference is an AA level in plasma or intestinal tissue from the subject before administration of AA TG, or a pre-determined AA level in plasma or intestinal tissue.

In some embodiments, the adverse side effect is a gastrointestinal side effect.

In some embodiments, the adverse side effect is nausea, vomiting, diarrhea, weight loss, intestinal tissue damage, radiation colitis, radiation mucositis, pelvic radiation disease, radiation enteritis, abdominal pain, rectal bleeding, bloating, or constipation.

5 In some embodiments, the subject is a human.

In some embodiments, methods of preventing, reducing, or reversing a cytotoxic effect due to chemotherapy or radiation therapy in a subject are disclosed.

10 In some embodiments, the method comprises administering orally to a subject in need thereof at least about 2 g of arachidonic acid triglyceride (AA TG) per day (2 g/d) for a sufficient time to prevent, reduce or reverse a cytotoxic effect due to chemotherapy or radiation therapy in the subject.

In some embodiments, the cytotoxic effect is intestinal tissue damage.

15 In some embodiments, the method comprises increasing, in a subject, a plasma arachidonic acid (AA) level to that indicative of an intestinal AA level that prevents, reduces, or reverses adverse side effects due to chemotherapy or radiation therapy.

20 In some embodiments, the method comprises (a) measuring an arachidonic acid (AA) level in a sample from a subject in need thereof and determining if the AA level is below a pre-determined AA level sufficient to prevent, reduce, or reverse adverse side effects due to chemotherapy or radiation therapy; and (b) if the AA level is below the pre-determined AA level, administering to the subject in (a) at least about 2 g of AA TG per day (2 g/d) for a sufficient time to increase the AA level to or above the pre-determined AA level.

25 In some embodiments, the method further comprises (c) measuring the AA level resulting from administering AA TG in (b) and determining the AA level; and (d) if the AA level in (b) is not at or above the pre-determined AA level, further administering to the subject a sufficient amount of AA TG per day to result in an intestinal AA level at or above the pre-determined AA level.

In some embodiments, the method further comprises repeating (c)-(d) to produce in the subject an intestinal AA level at or above the pre-determined AA level.

In some embodiments, the sample is plasma.

30 In some embodiments, the sample is intestinal tissue.

In some embodiments, methods of preventing, reducing, or reversing adverse side effects due to chemotherapy or radiation therapy in a subject are provided.

In some embodiments, AA in AA TG is substituted by at least one precursor of AA.

In some embodiments, the at least one precursor of AA is linoleic acid (LA), gamma-linolenic acid (gamma-LA), dihomo-gamma-linolenic acid (dh-gamma-LA), LA and gamma-LA, gamma-LA and dh-gamma-LA, or LA, gamma-LA, and dh-gamma-LA.

5 In some embodiments, the method comprises administering orally to a subject in need thereof at least about 2 g of at least one precursor of arachidonic acid (AA) per day (2 g/d) for a sufficient time to prevent, reduce or reverse adverse side effects due to chemotherapy or radiation therapy in the subject.

In some embodiments, the precursor of AA is in the form of a triglyceride (TG).

10 In some embodiments, the at least one precursor of AA is linoleic acid (LA), gamma-linolenic acid (gamma-LA), dihomo-gamma-linolenic acid (dh-gamma-LA), LA and gamma-LA, gamma-LA and dh-gamma-LA, or LA, gamma-LA, and dh-gamma-LA.

In some embodiments, kits for use in preventing, reducing or reversing adverse side effects due to chemotherapy or radiation therapy in a subject are provided.

15 In some embodiments, the kit comprises (a) one or more supplement units sufficient to provide to a subject in need thereof at least about 2 g of arachidonic acid triglyceride (AA TG) per day (2 g/d) for at least 7 days; and (b) instructions for preparation and consumption of the one or more supplement units.

In some embodiments, the one or more supplement units each comprise 500 mg of AA TG, 1 g of AA TG, 2 g of AA TG, or 4 g of AA TG.

20 In some embodiments, the number of supplement units to administer to a subject in need thereof is determined in consultation with a healthcare provider.

In some embodiments, the supplement units are in the form of a liquid or a powder.

In some embodiments, the supplement units are in the form of a liquid or a powder.

In some embodiments, the supplement units are in the form of pills or capsules.

25 In some embodiments, the supplement units are in one or more containers.

In some embodiments, the kit comprises (a) one or more supplement units sufficient to provide to a subject in need thereof at least about 2 g of at least one precursor of arachidonic acid (AA) per day (2 g/d) for a sufficient time; and (b) instructions for preparation and consumption of the one or more supplement units.

30 In some embodiments, the precursor of AA is in the form of a triglyceride (TG).

In some embodiments, the at least one precursor of AA is linoleic acid (LA), gamma-linolenic acid (gamma-LA), dihomo-gamma-linolenic acid (dh-gamma-LA), LA and gamma-LA, gamma-LA and dh-gamma-LA, or LA, gamma-LA, and dh-gamma-LA.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGs. 1A-1T show how fatty acid (FA) screens in mouse and human organoids identify Arachidonic acid (AA) as a regenerative FA. FIGs. 1A-1D show the FA types used in the screen (FIG. 1A), the time kinetics analysis of organoid area (FIG. 1B), the ratio of the organoid structures with spheroid morphology to budding organoid morphology (spheroid ratio) (FIG. 1C) in mouse intestinal organoids, and the time kinetics analysis of organoid area in human intestinal patient-derived organoids (PDO) (FIG. 1D). ($n=4$, t -test). FIGs. 1E-1F show the number of Ki67+ cells in vehicle- (V) or AA-treated (25 μ M) mouse organoids (FIG. 1E) and the representative images of Ki67 immunostaining in organoids (FIG. 1F) ($n=5$). FIGs. 1G-1J show the representative images of V- or AA-treated mouse organoids (FIG. 1G), the quantification of spheroid ratio (FIG. 1H), the crypt domain per organoid (FIG. 1I) and the organoid area (FIG. 1J) in V- or AA-treated organoids ($n=50$). FIGs. 1K-1N show the quantification of spheroid ratio (FIG. 1K), organoid area (FIG. 1L), the number of sub-cultured (secondary) organoids (FIG. 1M) derived from V- or AA-treated primary mouse organoids, and the representative images of secondary organoids (FIG. 1N) ($n=6$). FIGs. 1O-1Q show the quantification of organoid area (FIG. 1O), the spheroid ratio (FIG. 1P) in V- or AA-treated human PDOs (25 μ M), and the representative images of human PDOs (FIG. 1Q) ($n=7$). FIGs. 1R-1T show the quantification of organoid area (FIG. 1R), the spheroid ratio (FIG. 1S) of secondary organoids derived from primary V- or AA-treated PDO cultures, and the representative images of secondary organoids (FIG. 1T) ($n=4$). Unless otherwise indicated, the data in these figures are mean $\pm$ s.e.m. from n independent experiments. *** $P < 0.001$, **** $P < 0.0001$ (Mann-Whitney test). The scale bars represent 200 μ m (FIGs. 1F, 1G, 1N, 1Q, 1T). See also FIGs. 8A-8I, Tables 1 and 2.

FIGs. 2A-2S show how an AA-rich diet (ARD) enhances intestinal regeneration *in vivo*. FIG. 2A shows the proportion of nutrients in isocaloric (3.8 kcal/g) control diet (control) and AA-rich diet (ARD). Carbohydrate (Carb.), Protein (Prot.). FIGs. 2B-2C show metabolomics analysis by liquid chromatography coupled to mass spectrometry (LC-MS), showing AA abundance in plasma ($n=13$) (FIG. 2B) and tissue (intestine, $n=7$) (FIG. 2C) of control or ARD-fed mice. FIGs. 2D-2E show the crypt length and (FIG. 2D) the representative Hematoxylin and eosin staining (H&E) images of small intestines from control or ARD-fed mice (FIG. 2E) ($n=5$). FIGs. 2F-2G show the Ki67+ cells per crypt from control or ARD-fed mice (FIG. 2F) and the representative images of Ki67 immunostaining in small intestine (FIG. 2G) ($n=5$). FIGs. 2H-2J show the spheroid ratio (FIG. 2H), the crypt domain per organoid (FIG. 2I) in organoids-derived from control or ARD-fed mice on day 3, and the

representative images of organoids (FIG. 2J). ($n=5$, Mann-Whitney test). The scale bar represents 100 μ m. FIGs. 2K-2L show the organoid initiation capacity of sorted Epcam+ cells derived from crypts of control or ARD-fed mice (FIG. 2K) and the representative images organoids (FIG. 2L) ($n=4$). The scale bar represents 100 μ m. FIGs. 2M-2O show the intestinal length (FIG. 2M, $n=9$) and the number of surviving crypts per intestine area (N, $n=5$) in control or ARD-fed mice (three days after 15Gy γ -irradiation), and the representative H&E images of small intestine (FIG. 2O). The scale bar represents 50 μ m. FIGs. 2P-2R show EdU signal intensity per intestine area (FIG. 2P, $n=4$ 0Gy, $n=6$ 15Gy), the EdU+ cells per crypt (FIG. 2Q, $n=6$) in control or ARD-fed mice three days after 15Gy γ -irradiation and 4-hour EdU pulse, and the representative confocal microscopy images of Epcam (green) and EdU (red). DAPI (blue) stains in the intestine (FIG. 2R). The scale bar represents 50 μ m. FIG. 2S shows the intestinal length in control or ARD-fed mice three days after 10 μ M Doxorubicin (Dox) or vehicle (V) injection ($n=8$ Veh, $n=6$ Dox). Unless otherwise indicated, the data in these figures are mean $\pm$ s.e.m. from n independent experiments; ns, not significant, * $P<0.05$, ** $P<0.01$, *** $P<0.001$, **** $P<0.0001$ (ANOVA). See also FIGs. 9A-9H and Table 3.

FIGs. 3A-3L show how AA induces stem cell reprogramming gene expression signature in mouse and human organoids. FIG. 3A depicts the gene set enrichment analysis (GSEA) showing enrichment of different stem cell signatures using bulk RNA sequencing of AA versus (vs.) V-treated mouse organoids across time points ($n=3$ Day 1 (D1) and Day 3 (D3), $n=4$ Day 6 (D6)). The scale represents adjusted p -values for the enrichment analysis. FIG. 3B is a heatmap showing differentially expressed (DE) genes involved in stem cell regeneration or differentiation (rows) between AA vs. V-treated organoids across time points (columns). ($n=3$ D1 and D3, $n=4$ D6). The scale represents $\log_2$ fold change of expression between AA vs. V-treated organoids. As abbreviated in the figure, “GC” denotes Goblet cells and “EE” denotes Enteroendocrine cells. FIG. 3C is a western blot for β -catenin from cytoplasmic and nuclear fractions of V- or AA-treated organoids. ($n=5$). FIGs. 3D-3F show the organoid area (FIG. 3D, $n=5$) and the organoid count per well (FIG. 3E, $n=5$) quantification of V- or AA-treated organoids that were grown with indicated concentrations of Wnt3a, and the representative images of V- or AA-treated organoids with (100 ng/ml) or without (0 ng/ml) Wnt3a (FIG. 3F). The scale bar represents 100 μ m. FIG. 3G is a heatmap showing the DE genes involved in Egfr signaling (receptor and ligands) between AA vs. V-treated organoids across time points ($n=3$ D1 and D3, $n=4$ D6). The scale represents $\log_2$ fold change of expression between AA vs. V-treated organoids. FIGs. 3H-3I show the organoid

area (FIG. 3H, $n=5$) quantification of V- or AA-treated organoids with or without EGF supplementation (40 ng/ml) and the representative images of organoids (FIG. 3I). The scale bar represents 100 μ m. FIG. 3J. shows GSEA enrichment of different stem cell signatures using bulk RNA sequencing of AA versus (vs.) V-treated human PDOs ($n=4$). The scale
 5 represents adjusted p -values for the enrichment analysis. FIG. 3K. is a heatmap showing the DE genes involved in stemness, differentiation or proliferation between AA vs V-treated human PDOs ($n=4$). The scale represents log₂ fold change of expression between AA vs. V-treated human PDOs. FIG. 3L shows the relative expression of *CD55*, *MSLN*, *NR4A1*, *NR4A2*, *L1CAM*, *DUSP4* in V- or AA-treated human PDOs ($n=4$, t -test). Unless otherwise
 10 indicated, the data in these figures are mean $\pm$ s.e.m. from n independent experiments; NS, not significant, * $P<0.05$, ** $P<0.01$, *** $P<0.001$, **** $P<0.0001$ (ANOVA). See also FIGs. 10A-10K.

FIGs. 4A-4T show single cell analysis of AA-induced stemness *in vivo*. FIG. 4A shows Uniform Manifold Approximation and Projection (UMAP) clustering of single cell
 15 RNA sequencing (scRNA-seq) of 23,161 cells from dissociated crypts of control ($n=2$) or ARD-fed ($n=2$) mice based on the expression of known marker genes (see Example 9). The scale represents the density difference of single cell cells between ARD vs. control on UMAP ($n=2$ independent experiments). FIGs. 4B-4D are split-violin plots depicting the single cell gene expression levels of *S100a6* (FIG. 4B), *Lgr5* (FIG. 4C), *Ascl2* (FIG. 4D) in control or
 20 ARD-fed mice across different intestinal epithelial cell clusters ($n=2$, 23,161 cells, Wilcoxon Rank-Sum test). FIG. 4E shows UMAP plots from pseudotime trajectory analysis of crypt cells from control or ARD-fed mice. The arrows highlight predicted trajectories within cell clusters ($n=2$, 23,161 cells). The scale represents pseudotime. FIG. 4F is a density plots from pseudotime trajectory analysis, demonstrating density differences along pseudotime in all
 25 cells (top), cells in Stem 1 cluster (middle) and cells in Stem 2 cluster (bottom) between control and ARD-fed mice ($n=2$, 23,161 cells, Fisher test). FIG. 4G comprises of line plots, showing the expression levels of *Lgr5* (top) and *Ascl2* (bottom) in control or ARD-fed mice along the indicated pseudotime axis ($n=2$, 23,161 cells, Wilcoxon Rank-Sum test). FIGs. 4H-4P show the representative confocal microscopy images of single-molecule fluorescent *in situ*
 30 hybridization (sm-FISH) for *Lgr5* (FIG. 4H), *Ascl2* (FIG. 4K) and *S100a6* (FIG. 4N) in intestinal crypts from control or ARD-fed mice that were irradiated (15 Gy) ($n=6$ mice) or non-irradiated (0 Gy) ($n=4$ mice), the quantification of sm-FISH signal per crypt unit for *Lgr5* (FIG. 4I), *Ascl2* (FIG. 4L) and *S100a6* (FIG. 4O), and the frequency of *Lgr5*⁺ (FIG. 4J), *Ascl2*⁺ (FIG. 4M) and *S100a6*⁺ (FIG. 4P) cells in different crypt tier positions. FIGs. 4Q-4T

show *S100a6* is an AA-induced and regeneration associated gene that is regulated by PGE2-PTGER4-PKA-CREB/YAP pathway. The scale bar for FIGs. 4H, 4K, and 4N represent 10µm. Unless otherwise indicated, data in these figures are mean ± s.e.m. from *n* independent experiments; ns, not significant, **P*<0.05, ***P*<0.01, ****P*<0.001, *****P*<0.0001 (ANOVA).

5 See also FIGs. 11A-12T.

FIGs. 5A-5M show how prostaglandin E2 (PGE2) can mediate the stemness-enhancing effects of AA. FIG. 5A is a heatmap showing alterations in bioactive lipid mediators between AA- vs V-treated organoids. The scale represents log₂ fold-change in abundance of metabolites between AA- vs V-treated organoids (*n*=3). FIG. 5B shows the spheroid ratio in organoids after treatment with different AA-derived metabolites (*n*=5, Mann-Whitney test). FIGs. 5C-5D show the organoid area (FIG. 5C, *n*=5) quantification of V- or AA-treated organoids that were grown with indicated concentrations of Celecoxib, and the representative images of V- or AA-treated organoids with or without Celecoxib (15µM) (FIG. 5D). The scale bar represents 200µm. FIGs. 5E-5F show the concordance between AA-induced changes in gene expression and PGE2-induced gene expression as assessed by bulk RNA-seq in mouse (FIG. 5E, *n*=3, *R*²=0.82, *P*<0.001) and human PDOs (FIG. 5F, *n*=4, *R*²=0.64, *P*<0.001). The DE genes involved in stem cell reprogramming and differentiation are highlighted. FIG. 5G shows the UMAP clustering of single cells from V- or PGE2-treated organoids. The scale represents density difference of single cells between PGE2 vs V on the UMAP plot (*n*=2). FIG. 5H is a density UMAP plot showing the gene expression changes in fetal spheroid signature between PGE2 vs. V-treated organoids. The scale represents the density difference of gene expression for fetal spheroid signature in single cells between PGE2 vs V on the UMAP plot (*n*=2). FIGs. 5I-5J are split-violin plots depicting the gene expression levels of *S100a6* (FIG. 5I) and *Ascl2* (FIG. 5J) in V- or PGE2-treated organoids across different intestinal epithelial cell clusters (*n*=2, Wilcoxon Rank-Sum test). FIG. 5K shows UMAP plots from pseudotime trajectory analysis of cells from V or PGE2-treated organoids. The arrows highlight the predicted trajectories within cell clusters and the scale represents pseudotime. FIGs. 5L-5M are line plots showing the expression levels of *Ascl2* (top) and *S100a6* (bottom) in V- or PGE2-treated organoids along the indicated pseudotime axis (*n*=2, Wilcoxon Rank-Sum test). Unless otherwise indicated, data in these figures are mean ± s.e.m. from *n* independent experiments; ns, not significant, **P*<0.05, ***P*<0.01, ****P*<0.001, *****P*<0.0001 (ANOVA). See also FIG. 13A-13N.

FIGs. 6A-6W show how Ptger4 – cAMP – PKA signaling axis can regulate AA-induced stemness. FIGs. 6A-6C show the spheroid ratio (FIG. 6A, *n*= 10), the organoid area

(FIG. 6B, $n=10$) and the representative pictures (FIG. 6C) of V-, AA- or PGE2-treated wildtype (Ptger4^{f/f}) or Ptger4 knockout (Ptger4 KO) mouse organoids. The scale bar represents 100 μ m. FIG. 6D shows the relative expression of AA-induced signature genes (*Cd55*, *Ly6a*, *Msln*, *Nr4a1*, *S100a6*) in Ptger4 f/f or Ptger4 KO mouse organoids treated with V, AA or PGE2 ($n=5$). FIGs. 6E-6G show the spheroid ratio (FIG. 6E, $n=7$), the organoid area (FIG. 6F, $n=5$) and the representative pictures (FIG. 6G) of V- or cAMP derivative (8-Bromo)-treated (20 μ M) mouse organoids (t -test). The scale bar represents 100 μ m. FIG. 6H shows the relative expression of AA-induced signature genes (*Cd55*, *Ly6a*, *Msln*, *Nr4a1*, *S100a6*) in V- or 8-Bromo-treated mouse organoids ($n=4$, t -test). FIGs. 6I-6K show the spheroid ratio (FIG. 6I, $n=8$), the organoid area (FIG. 6J, $n=8$) and the representative pictures (FIG. 6K) of V- or AA-treated organoids with or without PKA inhibitor (H89) (20 μ M). The scale bar represents 100 μ m. FIG. 6L shows the relative expression of AA-induced signature genes (*Cd55*, *Ly6a*, *Msln*, *Nr4a1*, *S100a6*) in V- or AA-treated organoids with or without H89. ($n=4$). FIGs. 6M-6 show the organoid area (FIG. 6M, $n=8$) and the representative pictures (FIG. 6N) of V- or AA-treated human PDOs with or without Ptger4 inhibitor (Ptger4i) (10 μ M) ($n=X$). The scale bar represents 100 μ m. FIGs. 6O-6P show the organoid area (FIG. 6O, $n=5$) and representative pictures (FIG. 6P) of V- or AA-treated human PDOs with or without H89. The scale bar represents 100 μ m. FIGs. 6Q-6R show the organoid area (FIG. 6Q, $n=5$) and the representative pictures (FIG. 6R) of V- or 8-Bromo-treated human PDOs. The scale bar represents 100 μ m. FIGs. 6S-6T show the EdU+ cells per GFP+ crypts in control or ARD-fed Lgr5-CreERT2-IRES-GFP+; Ptger4 +/+ (WT) or Lgr5-CreERT2-IRES-GFP+; Ptger4 f/f (Ptger4 iKO) mice that were irradiated (15 Gy) or non-irradiated (0 Gy) (FIG. 6S, $n=5$), and the representative confocal microscopy images of intestine (FIG. 6T). FIGs. 6U-6W show that non-steroidal anti-inflammatory drugs (NSAIDs) inhibit AA-induced stemness. The scale bar represents 50 μ m. Unless otherwise indicated, the data in these figures are mean $\pm$ s.e.m. from n independent experiments; ns, not significant, * $P<0.05$, ** $P<0.01$, *** $P<0.001$, **** $P<0.0001$ (ANOVA). See also FIGs. 14A-14L.

FIGs. 7A-7I show how AA may elicit epigenetic reprogramming of stemness in a Ptger4-dependent manner. FIG. 7A shows pathway enrichment analysis of regions within 5 kb of transcription start sites (TSS), with increased or decreased chromatin accessibility in response to AA treatment in organoids as assessed by ATAC-seq. The scale represents adjusted p -value and x-axis represents normalized enrichment score (NES) from GSEA ($n=2$ independent experiments). FIG. 7B is a dot plot showing transcription factor (TF) motifs with

regulatory potential in AA-induced changes in chromatin accessibility by a multivariate analysis. The scale represents the effect size of motif presence on peak accessibility change in AA vs V-treated organoids. The dot scale represents adjusted p -value. FIG. 7C is a western blot for cyclic adenosine monophosphate response element binding protein (CREB)1, phosphorylated-CREB1 (pCREB1), YAP1, and CREB-binding protein (CBP) from cytosolic, chromatin and nuclear fractions of V- or AA-treated organoids ($n=5$). FIG. 7D is a heatmap showing the nearby genes for regions with significant chromatin accessibility gains (adjusted p -value < 0.01 by negative binomial analysis) in response to AA. The scale represents the computed z-score of normalized ATAC-seq reads in peaks. FIG. 7E depicts pathway analysis showing epigenetic reprogramming around genes that are part of regenerative stem cell signatures in AA vs V-treated organoids as assessed by Cut&Run assay for the indicated histone marks. The scale represents adjusted p -value and y-scale represents normalized enrichment score by GSEA. FIG. 7F is a ranked list of $\log_2$ fold-change of putative enhancers defined by H3K27ac peaks in AA vs V-treated organoids around proximal and distal regulatory regions of genes as annotated by Genomic Regions Enrichment of Annotations Tool (GREAT). Genes regulating intestinal stemness and differentiation are labeled. FIG. 7G is a scatter plot of genes that are both significantly gaining H3K4me3 signal around 10 kb of their TSS on Day 3 and significantly upregulated in expression on Day 6 in AA vs V-treated organoids (adjusted p -value < 0.05 and $\log_2$ fold-change > 0.58 by negative binomial test for both assays). FIG. 7H shows the ATAC-seq and Cut&Run (H3K27me3, H3K4me3, H3K4me3) tracks for the AA signature gene S100a6 locus. Below the plots are the gene structure and direction of transcription. FIG. 7I shows the profile plot of median change in H3K27ac signal in AA vs. Vehicle treated Ptger4 iKO or WT organoids for stem cell regeneration-associated gene signatures (See Example 9). *** $P < 0.001$, P -values are calculated by the effect of genotype term on a linear model of H3K27ac difference by distance to TSS. See also FIGs. 15A-15K.

FIGs. 8A-8I show the time kinetic FA screens in mouse and human organoids and are related to FIGs. 1A-1T. FIG. 8A shows a schematic of FA screen approach using live cell imaging. FIG. 8B shows the relative numbers of organoids (clonogenicity) in response to increasing doses of diverse FAs (normalized to organoid numbers in V for each FA) ($n=4$). FIG. 8C shows the representative images of mouse organoids after treatment with diverse FAs (25 μ M) for 120 hours. The scale bar represents 200 μ m. FIG. 8D shows the representative images of human PDOs after treatment with diverse FAs (25 μ M) for 120 hours. The scale bar represents 200 μ m. FIG. 8E shows a schematic of AA biosynthesis from

LA by desaturase and elongase enzymes. FIG. 8F shows the regularized $\log_2$ transformed (rlog) counts of gene expression for the enzymes regulating AA biosynthesis (*Fads1*, *Fads2*, *Elovl5*) using bulk RNA sequencing of mouse organoids ($n=3$) or human PDOs ($n=4$). Epcam was used as an abundant reference gene in intestinal epithelial cells. FIG. 8G shows the organoid area quantification after pre-treatment with the desaturase inhibitor (Sesamin) followed by indicated FA treatment ($n=4$, ANOVA). FIGs. 8H-8I show the microvillus length (FIG. 8H) and the representative transmission electron microscopy images (FIG. 8I) of V- or AA-treated organoids ($n=3$, Mann-Whitney test). The scale bar represents $10\mu\text{m}$. Unless otherwise indicated, the data in these figures are mean $\pm$ s.e.m. from n independent experiments; ns, not significant, *** $P<0.001$, **** $P<0.0001$

FIGs. 9A-9H show the characterization of the regenerative effects of AA-rich diet (ARD) *in vivo* and are related to FIGs. 2A-2S. FIGs. 9A-9B show the weight (FIG. 9A) and the blood glucose levels (FIG. 9B) of control or ARD-fed mice ($n=10$, t -test). FIG. 9C shows the polar metabolite analysis by LC-MS, using plasma from control or ARD-fed mice. The X-axis represents $\log_2$ fold-change in metabolite abundance and the Y axis represents P -values in $-\log_{10}$. ($n=5$). FIG. 9D shows the representative images of intestines from control (C) or ARD-fed mice that were irradiated (15 Gy) or non-irradiated (0 Gy) ($n=9$). FIG. 9E shows the representative confocal images of Epcam (green), EdU (red) and DAPI (blue) stains in intestines from control or ARD-fed mice three days after 15Gy γ -irradiation ($n=5$ and the scale bar represents $10\mu\text{m}$). FIG. 9F shows the representative images of intestines from control (C) or ARD-fed mice three days after $10\mu\text{M}$ Doxorubicin (Dox) or vehicle (V) injection ($n=5$). FIGs. 9G-9H show the EdU+ cells per crypt (FIG. 9G) in control or ARD-fed mice three days after $10\mu\text{M}$ Doxorubicin (Dox) or vehicle (V) injection, and the representative confocal images of Epcam (green), EdU (red) and DAPI (blue) stains in intestines ($n=5$, ANOVA). The scale bar represents $50\mu\text{m}$. Unless otherwise indicated, the data in these figures are mean $\pm$ s.e.m. from n independent experiments; ns, not significant, *** $P<0.001$, **** $P<0.0001$.

FIGs. 10A-10K show AA-mediated induction of stem cell reprogramming gene expression in mouse and human organoids and are related to FIGs. 3A-3L. FIG. 10A shows schematics for the time kinetics bulk RNA-seq experiments in organoids. FIG. 10B shows the principal component analysis (PCA) of time kinetics bulk RNA-seq data from mouse organoids after treatment with AA for one day (D1, $n=3$), three days (D3, $n=3$) or six days (D6, $n=4$). FIG. 10C comprises of upset plots (right) demonstrating the overlapping gene sets between AA-induced genes and different stem cell signatures (see Example 9). The boxplots

(left) show the log₂ fold-change of indicated gene sets in each row for each time point in AA vs. V-treated organoids. FIG. 10D depicts the assessment of the statistical significance of the AA-induced upregulation of genes that are part of different stem cell signatures, based on permutation test where 100,000 permutations were used to calculate the distribution of the difference between two average profiles. The solid line depicts empirical null model and the dotted line indicates the observed value for measured profiles. FIG. 10E shows the relative expression of AA-induced signature genes (*Cd55*, *Ly6a*, *Msln*, *Nr4a1*, *Sl00a6*) in organoids that were treated with V or AA for one day (D1) or three days (D3) (*n*=4, ANOVA). FIG. 10F shows how GSEA can enrich pathways using bulk RNA sequencing of AA vs. V-treated mouse organoids across time points (D1, D3, D6). Color scale represents adjusted *p*-values for the enrichment analysis. FIG. 10G is a heatmap showing DE genes that are targets of Wnt/ β -catenin targets between AA vs. V-treated organoids across time points (*n*=3 D1 and D3, *n*=4 D6). The scale represents log₂ fold change of expression between AA vs. V-treated organoids. FIGs. 10H-10I show the relative numbers of organoids (clonogenicity) in cultures set with Egf or other Egfr ligands Areg and Ereg (normalized to organoid numbers in Wnt3a only condition) (FIG. 10H), and the representative images of organoids (*n*=5, ANOVA). The scale bar represents 100 μ m. FIG. 10J shows the PCA analysis of bulk RNA-seq data from V- or AA-treated human PDOs (*n*=4). FIG. 10K shows how GSEA can enrich pathways using bulk RNA sequencing of AA vs. V-treated human PDOs. The scale represents adjusted *p*-values for the enrichment analysis. Unless otherwise indicated, the data in these figures are mean $\pm$ s.e.m. from *n* independent experiments; ns, not significant, **P*<0.05, ***P*<0.01, ****P*<0.001, *****P*<0.0001.

FIGs. 11A-11I show the single cell analysis of AA-induced stemness *in vivo* and are related to FIGs. 4A-4P. FIG. 11A is a bubble plot showing cluster-identifying marker gene expression levels from scRNAseq analysis of 23,161 epithelial cells in the intestinal crypts (*n*=2 independent experiments). The clusters were identified based on the expression of known marker genes. FIG. 11B depicts stacked bar plots that demonstrate proportions of different epithelial cell clusters identified by scRNAseq from crypts of C (*n*=2) or ARD-fed (*n*=2) mice. FIG. 11C depicts bar plots showing a fraction of cells in different epithelial cell clusters identified by scRNAseq from crypts of C (*n*=2) or ARD-fed (*n*=2) mice. FIG. 11D shows split-violin plots depicting the single cell gene expression levels of *Ly6a*, *Clu* and *Msi1* in control or ARD-fed mice across different intestinal epithelial cell clusters (Wilcoxon Rank-Sum test). FIG. 11E depicts line plots showing the expression levels of *Sl00a6* (top) and *Ly6a* (bottom) control or ARD-fed mice along the indicated pseudotime axis (Wilcoxon

Rank-Sum test). FIG. 11F is a density UMAP plot showing the gene expression changes in different stem cell signatures across clusters between ARD-fed vs. control mice (see Example 9). The scale represents density difference of gene expression for indicated signatures in single cells between ARD-fed mice vs. control on the UMAP plot ($n=2$). FIGs. 11G-11I present the lower magnification representative confocal microscopy images of single-molecule fluorescent *in situ* hybridization (sm-FISH) for *Lgr5* (FIG. 11G), *Ascl2* (FIG. 11H) and *Sl00a6* (FIG. 11I) in intestinal crypts from control or ARD-fed mice that were irradiated (15 Gy, $n=6$ mice) or non-irradiated (0 Gy, $n=4$ mice). FIGs. 11G-11I show the frequency of *Lgr5*⁺ (FIG. 11G), *Ascl2*⁺ (FIG. 11H) cells in different crypt tier positions and the scale bars represent 50 μ m. Unless otherwise indicated, the data in these figures are mean $\pm$ s.e.m. from n independent experiments; ns, not significant, * $P<0.05$, ** $P<0.01$, *** $P<0.001$, **** $P<0.0001$.

FIGs. 12A-12T show the single cell analysis of AA-induced stemness *in vitro* and are related to FIGs. 4A-4P. FIG. 12A is a bubble plot showing cluster-identifying marker gene expression levels from scRNAseq analysis of 23,599 single epithelial cells from V- ($n=2$) or AA-treated ($n=2$) organoids. The clusters were identified based on the expression of known marker genes (see Example 9). FIG. 12B presents stacked bar plots demonstrating proportions of different epithelial cell clusters identified by scRNAseq from V- ($n=2$) or AA-treated ($n=2$) organoids. FIG. 12C conveys bar plots showing a fraction of cells in different epithelial cell clusters identified by scRNAseq from crypts of V- ($n=2$) or AA-treated ($n=2$) organoids. FIG. 12D presents the UMAP clustering of 23,599 single cells from V- or AA-treated organoids and the scale represents density difference of single cell clusters between AA vs V treatment on the UMAP plot ($n=2$). FIGs. 12E-12J are split-violin plots depicting the gene expression levels of *Sl00a6* (FIG. 12E), *Ly6a* (FIG. 12F), *Clu* (FIG. 12G), *Ascl2* (FIG. 12H), *Lgr5* (FIG. 12I) and *Msi1* (FIG. 12J) in V- or AA-treated organoids across different intestinal epithelial cell clusters ($n=2$, Wilcoxon Rank-Sum test). FIG. 12K is a density UMAP plot showing the gene expression changes in different stem cell signatures across clusters between AA vs. V-treated organoids. The scale represents density difference of gene expression for indicated signatures in single cells between AA vs. V on the UMAP plot ($n=2$). FIG. 12L presents UMAP plots from pseudotime trajectory analysis of cells from V or AA-treated organoids. The arrows highlight the predicted trajectories within the cell clusters and the scale represents pseudotime. FIGs. 12M-12P are density plots from pseudotime trajectory analysis demonstrating density differences along pseudotime in all cells (FIG. 12M), cells in Stem 1 cluster (FIG. 12N), cells in Stem 2 cluster (FIG. 12O), and

cells in Stem 3 cluster (FIG. 12P) between AA vs. V-treated organoids (Fisher test). FIGs. 12Q-12T display line plots showing the expression levels of *S100a6* (FIG. 12Q), *Ly6a* (FIG. 12R), *Ascl2* (FIG. 12S) and *Lgr5* (FIG. 12T) in V- or AA-treated organoids along the indicated pseudotime axis (Wilcoxon Rank-Sum test). Unless otherwise indicated, the data in these figures are mean $\pm$ s.e.m. from n independent experiments; ns, not significant, $*P<0.05$, $**P<0.01$, $***P<0.001$.

FIGs. 13A-13N show AA metabolism to PGE2 leads to stem cell reprogramming, and are related to FIGs. 5A-5M. FIG. 13A is a heatmap showing DE genes that regulate AA metabolism to bioactive lipid mediators between AA vs. V-treated organoids across time points ($n=3$ D1 and D3, $n=4$ D6). The Color scale represents $\log_2$ fold change of expression between AA vs. V-treated organoids. FIG. 13B shows the abundance of bioactive lipid mediators from intestines of control or ARD-fed mice as assessed by LC-MS ($n=4$, ANOVA). FIG. 13C presents the representative images of organoids treated with indicated bioactive lipid mediators and the scale bar represents 200 μ m. FIGs. 13D-13E show the organoid area (FIG. 13D) quantification of V- or AA-treated organoids that were grown with indicated concentrations of Indomethacin, the representative images of V- or AA-treated organoids with or without Indomethacin (15 μ M) (FIG. 13E) ($n=5$, ANOVA), and the scale bar representing 200 μ m. FIG. 13F shows the PGE2 levels in sorted Epcam+ intestinal epithelial cells from crypts after treatment with V or AA as assessed by ELISA ($n=3$, ANOVA). FIG. 13G shows the relative expression of enzymes that regulate prostaglandin production (*Ptges*, *Ptgs1*, *Ptgs2*) in organoids treated with V, AA or PGE2 ($n=5$, ANOVA). FIG. 13H presents the split-violin plots depicting the single cell gene expression levels of *Ptges* in V- or AA-treated organoids across different intestinal epithelial cell clusters (Wilcoxon Rank-Sum test). FIG. 13I displays the bubble plot showing cluster-identifying marker gene expression levels from scRNAseq analysis of 23,599 single epithelial cells from V- ($n=2$) or PGE2-treated ($n=2$) organoids. The clusters were identified based on the expression of known marker genes (see Example 9). FIG. 13J conveys the stacked bar plots demonstrating proportions of different epithelial cell clusters identified by scRNAseq from V- ($n=2$) or PGE2-treated ($n=2$) organoids. FIG. 13K presents the concordance between AA-induced changes in gene expression and PGE2-induced gene expression as assessed by scRNAseq. FIG. 13L displays the density UMAP plot showing the gene expression changes in different stem cell signatures across clusters between PGE2 vs. V-treated organoids (see Example 9). Color scale represents density difference of gene expression for indicated signatures in single cells between PGE2 vs V on the UMAP plot ($n=2$). FIG. 13M conveys

the split-violin plots depicting the single cell gene expression levels of *Lgr5*, *Ly6a* and *Clu* in V- or AA-treated organoids across different intestinal epithelial cell clusters (Wilcoxon Rank-Sum test). FIG. 13N presents the line plots showing the expression levels of *Lgr5* and *Ly6a* in V- or PGE2-treated organoids along the indicated pseudotime axis (Wilcoxon Rank-Sum test). Unless otherwise indicated, the data in these figures are mean $\pm$ s.e.m. from *n* independent experiments; ns, not significant, **P*<0.05, ***P*<0.01, ****P*<0.001, *****P*<0.0001.

FIGs. 14A-14L show how AA may promote stem cell reprogramming through PGE2-Ptger4 signaling and is related to FIGs. 6A-6T. FIGs. 14A-14B present the organoid area quantification of V- or AA-treated organoids that were grown with indicated inhibitors of PGE2 receptors (Ptger1i, Ptger2i, Ptger3, Ptger4i) (FIG. 14A), the representative images of V- or AA-treated organoids with or without inhibitors (10 μ M) (FIG. 14B) (*n*=5), and the scale bar which represents 200 μ m. FIGs. 14C-14E present the organoid area (FIG. 14C, *n*=10), the spheroid ratio (FIG. 14D, *n*=10) and the representative pictures (FIG. 14E) of V-, AA- or PGE2-treated mouse organoids with or without Ptger4 inhibitor (Ptger4i) (10 μ M). The scale bar represents 200 μ m. FIG. 14F shows the relative expression of AA-induced signature genes (*Cd55*, *Ly6a*, *Msln*, *Nr4a1*, *S100a6*) in V-, AA, or PGE2-treated organoids with or without Ptger4i (*n*=4). FIGs. 14G-14H show the rlog counts of gene expression for PGE2 receptors (*Ptger1*, *Ptger2*, *Ptger3*, *Ptger4*) using bulk RNA sequencing of mouse organoids (FIG. 14G, *n*=3) or human PDOs (FIG. 14H, *n*=4). FIGs. 14I-14L present split-violin plots depicting the single cell gene expression levels of *Ptger1* (FIG. 14I), *Ptger2* (FIG. 14J), *Ptger3* (FIG. 14K) and *Ptger4* (FIG. 14L) in V- or AA-treated organoids across different intestinal epithelial cell clusters (Wilcoxon Rank-Sum test). Unless otherwise indicated, the data in these figures are mean $\pm$ s.e.m. from *n* independent experiments; ns, not significant, **P*<0.05, ***P*<0.01, ****P*<0.001.

FIGs. 15A-15K show the Ptger4-dependent epigenetic reprogramming of stemness by AA and are related to FIGs. 7A-7I. FIG. 15A presents the number of opening and closing chromatin peaks AA vs. Vehicle treated organoid ATACseq (adjusted *p* < 0.01 and log₂ fold-change > 0.58 by negative binomial tests) annotated by genic regions, FANTOM enhancers, and CpG islands. FIG. 15B conveys the differences in gene expression of differentially accessible peaks by integration of ATACseq and RNAseq for AA vs. V-treated cells (adjusted *p*-value < 0.01 and log₂ fold-change > 0.58 by negative binomial tests for ATACseq). Genes are associated to peaks within 2.5kb of their TSS. (closing vs. stable: *p*=4.9e⁻⁶, opening vs. stable: *p*<2.2e⁻¹⁶, closing vs. opening: *p*=6.4e⁻⁹, Wilcoxon Rank-Sum

test). FIG. 15C presents the number of differential peaks for indicated histone marks in AA vs. V-treated organoids (adjusted p-value < 0.01 and log₂ fold-change > 0.58 by negative binomial tests). FIGs. 15D-15F present the profile plot of median reads per genome coverage (RPGC) of H3K4me3 (FIG. 15D), H3K27ac (FIG. 15E), and H3K27me3 (FIG. 15F) histone marks near the TSSs of differentially up- or down-regulated genes in V- or AA-treated organoids. The *P*-values were calculated by finding the enrichment of the significantly up- and downregulated gene signatures within all the genes ranked by the change of the Cut&Run signal within 2.5kb of the TSS using GSEA. FIG. 15G displays a scatter plot of genes that were both significantly gaining H3K4me3 signal around 10 kb of their TSS on Day 3 and significantly upregulated in expression on Day 3 in AA vs. V-treated organoids (adjusted p-value < 0.05 and log₂ fold-change > 0.58 by negative binomial test for both assays). FIG. 15H displays a scatter plot of genes that were both significantly gaining H3K27ac signal around 25 kb of their TSS on Day 3 (left) and significantly upregulated in expression on Day 3 (left) or Day 6 (right) in AA vs. V-treated organoids (adjusted p-value < 0.05 and log₂ fold-change > 0.58 by negative binomial test for both assays). FIG. 15I shows the ATAC-seq and Cut&Run (H3K27me3, H3K4me3, H3K4me3) tracks for the regeneration-associated AA signature genes *Msln*, *Anxa10*, *Ly6a* and *Ascl2*. Below the plots are the gene structure and direction of transcription. FIG. 15J presents the profile plots for median reads per genome coverage (RPGC) of H3K27ac enrichment for AA and Vehicle treated cells near TSS ($\pm$ 5.0 kb) of differentially up- or downregulated gene list from AA- vs. V-treatment RNAseq. Two conditions are shown: WT (top) and Ptger4 iKO (bottom). The *P*-values were calculated by finding the enrichment of the significantly up- and downregulated gene signatures within all the genes ranked by the change of the Cut&Run signal within 2.5kb of the TSS using GSEA. FIG. 15K displays the profile plots of median change in H3K27ac signal in AA vs. Vehicle treated Ptger4 iKO or WT organoids for up- or downregulated genes. ****P* < 0.001, *P*-values are calculated by the effect of genotype term on a linear model of H3K27ac difference by distance to TSS.

FIGs. 16A-16E show schematics detailing potential mechanisms of stem cell reprogramming in response to AA *in vitro* and *in vivo*.

FIGs. 17A-17F show that YAP, CREB1, and CBP are necessary for the regenerative effects of dietary AA.

FIGs. 18A-18C show that loss of S100a6 blunts stem cell regeneration.

FIGs. 19A-19C show that an AA-rich diet (ARD, also referred to as FA1) does not increase the tumor burden in the small intestine (FIG. 19B), colon (FIG. 19B), or overall

(FIG. 19C) in a tumor-prone mouse model. FIG. 19A shows a summary of the experimental procedure.

FIGs. 20A-20C shows that an AA-rich diet (ARD) does not decrease survival (FIG. 20B) or increase metastasis rate (FIG. 20C) compared to a control diet (C) in a mouse model of metastatic colon cancer. FIG. 20A shows a summary of the experimental procedure.

FIGs. 21A-21B shows the kinetics of AA plasma levels in mice fed a control diet for four weeks, an AA-rich diet (ARD) for four weeks (Arasco), or an ARD for two weeks and then a control diet for two weeks (ArascoRev). FIG. 21A shows plasma lipid levels on day 3, day 7, or day 14 for mice fed a control diet or an AA-rich diet. FIG. 21B shows plasma lipid levels after 4 weeks for all three groups.

FIGs. 22A-22C show the effect of diet on the level of AA observed in a mouse model (FIG. 22B) and proliferation of intestinal cells after irradiation (FIGs. 22C-22D). FIG. 22A shows a summary of the experimental protocol.

FIGs. 23A-23B show gene expression changes in mice fed a control diet for four weeks, an AA-rich diet (ARD) for four weeks, or an ARD for two weeks and then a control diet for two weeks. FIG. 23A shows a summary of the experimental protocol. FIG. 23B shows the results of several comparisons.

FIGs. 24A-24B show the regenerative effects of AA-rich diet (ARD) *in vivo* in mice treated with different doses of 5-fluorouracil (5-FU). FIG. 24A shows a schematic of the experimental design. FIG. 24B shows the % weight loss of control or ARD-fed mice treated with 250 mg/kg 5-FU on days 1-3 (D1, D2, D3) following 5-FU treatment.

FIGs. 25A-25B the regenerative effects of AA-rich diet (ARD) *in vivo* in mice treated with a multiple dose regimen of 5-fluorouracil (5-FU) and oxaliplatin. FIG. 25A shows a schematic of the experimental procedure. FIG. 25B shows the % weight change of control or ARD-fed mice treated with a regimen of 100mg/kg 5-FU and 6mg/kg oxaliplatin once per week for two weeks, allowed for two weeks recovery, and then repeated the regimen of one dose per week for two weeks.

DETAILED DESCRIPTION

Adverse side effects of chemotherapy or radiation therapy often include gastrointestinal upset, due to damage to intestinal tissue. In some embodiments, the present disclosure relates to prevention or reduction of intestinal tissue damage by providing a subject who will be undergoing or exposed to a course of chemotherapy or radiation therapy an amount (beneficial dose) of arachidonic acid (AA), or precursors thereof, in the form of

triglycerides prior to starting a course of chemotherapy or radiation therapy. In some embodiments, AA, or precursors thereof, in the form of triglycerides (TGs), is administered during a course of chemotherapy or radiation therapy. In some embodiments, AA, or precursors thereof, in the form of TGs, is administered before and during a course of chemotherapy or radiation therapy. In some embodiments, the present disclosure relates to administration or supplementation with AA, or precursors thereof, in the form of triglycerides, for promoting tissue regeneration in a subject that has been exposed to a course of chemotherapy or radiation therapy.

Accumulating evidence posits that nutrients and metabolic pathways not only affect growth and proliferation of cells, but could also influence cellular function and fate by signaling to transcription factors and altering epigenetic landscapes (Beyaz et al., 2016; Beyaz et al., 2021b; Beyaz and Yilmaz, 2016; Chandel et al., 2016; Chen et al., 2020; Cimmino et al., 2018; Lu and Thompson, 2012). Recent studies have explored metabolic regulation of intestinal stem cell (ISC) activity through fatty acid (FA) oxidation (Chen et al., 2020; Mihaylova et al., 2018; Stine et al., 2019), ketone body signaling (Cheng et al., 2019), mitochondrial pyruvate metabolism (Rodriguez-Colman et al., 2017; Schell et al., 2017), vitamins (Jijon et al., 2018; Lukonin et al., 2020; Peregrina et al., 2015) and microbiome-derived metabolites (Kaiko et al., 2016; Lee et al., 2018).

The present disclosure provides that dietary AA and precursors of AA influence stemness and epigenetic regulation of gene expression, for instance, in the intestinal epithelium. The findings provide basis for using AA and precursors of AA, which may be in triglyceride (TG) form, to prevent, reduce or reverse adverse side effects or cytotoxic effects due to chemotherapy or radiation therapy in a subject in need thereof.

Arachidonic Acid (AA) and Precursors of AA (Precursor-AA)

In some embodiments, methods of preventing, reducing, or reversing adverse side effects due to chemotherapy or radiation therapy in a subject are disclosed. In some embodiments, the method comprises administering to a subject in need thereof arachidonic acid (AA) in the form of a triglyceride (AA TG) for a sufficient time to prevent, reduce or reverse adverse side effects due to chemotherapy or radiation therapy in the subject. In some embodiments, the method comprises administering orally to a subject in need thereof at least one precursor of arachidonic acid (precursor-AA) for a sufficient time to prevent, reduce or reverse adverse side effects due to chemotherapy or radiation therapy in the subject.

Arachidonic acid (AA) is a 20-carbon chain fatty acid with four methylene-interrupted *cis* double bonds. In some embodiments, AA is in the form of a glyceride. In some embodiments, AA is in the form of a triglyceride (AA triglyceride or AA TG). In some embodiments, AA is in the form of a free fatty acid (AA). In some embodiments, a free fatty acid AA is bound to a carrier protein (e.g., albumin). In some embodiments, AA is in the form of a phospholipid (AA phospholipid or AA PL). In some embodiments, an AA PL is used in the composition, methods and kits disclosed herein. In some embodiments, AA is not associated with triglyceride (TG) or a phospholipid (PL). In some embodiments, an AA precursor is used in compositions, methods and kits disclosed herein. In some embodiments, a precursor-AA is linoleic acid (LA), alpha-linoleic acid (ALA), gamma-linolenic acid (gamma-LA), dihomo-gamma-linolenic acid (dh-gamma-LA); LA and ALA; LA and gamma-LA; LA and dh-gamma-LA; ALA and gamma-LA; ALA and dh-gamma-LA; gamma-LA and dh-gamma-LA; LA, ALA, gamma-LA; LA, ALA, gamma-LA and dh-gamma-LA; ALA, gamma-LA and dh-gamma-LA; LA, gamma-LA, dh-gamma-LA; gamma-LA, ALA and gamma-LA; LA, gamma-LA, and dh-gamma-LA; or LA, ALA, gamma-LA and dh-gamma-LA. In some embodiments, an AA precursor is in the form of a triglyceride (TG). In some embodiments, AA or an AA precursor is in the form of a phospholipid (PL, AA PL, precursor-AA PL).

In some embodiments, a composition comprises AA TG. In some embodiments, a composition is an oil. In some embodiments, the oil is extracted from an organism (e.g., plant, fungus, etc.). In some embodiments, the organism is a microorganism (See e.g., U.S. Patent No. 8,389,808, the contents of which are incorporated by reference in their entirety). In some embodiments, the microorganism belongs to the genus *Mortierella*, *Entomophthora*, *Pythium*, or *Porphyridium*. In some embodiments, the microorganism belongs to the genus *Pythium*. In some embodiments, the microorganism is *Pythium insidiosum*. In some embodiments, the organism is a fungus. In some embodiments, the fungus belongs to the genus *Mortierella*. In some embodiments, the fungus is *Mortierella alpina*.

In some embodiments, an oil comprises about 10% or least about 10% AA TG, about or least about 15% AA TG, about 20% or least about 20% AA TG, about 25% or least about 25% AA TG, about 30% or least about 30% AA TG, about 35% or least about 35% AA TG, about 40% or least about 40% AA TG, about 45% or least about 45% AA TG, about 50% or least about 50% AA TG, about 55% or least about 55% AA TG, about 60% or least about 60% AA TG. In some embodiments, an oil comprises between about 20% AA TG and about 60% AA TG. In some embodiments, an oil comprises between about 20% AA TG and about

50% AA TG. In some embodiments, an oil comprises between about 30% AA TG and about 50% AA TG. In some embodiments, an oil comprises at least or about 40% AA TG. In some embodiments, percent AA TG is calculated as volume/volume percentage. In some embodiments, percent AA TG is calculated as a weight/volume percentage. In some
5 embodiments, percent AA TG is calculated as weight/weight percentage.

In some embodiments, methods of preventing, reducing or reversing adverse side effects due to chemotherapy or radiation therapy are disclosed. In some embodiments, the method comprises administering orally to a subject in need thereof a composition comprising (a) an oil comprising arachidonic acid triglyceride (AA TG); and (b) an oil other than the oil
10 in (a), wherein the oil of (a) and the oil of (b) are at a ratio of about 3:4, wherein the composition is administered for at least 7 days before the subject begins a course of chemotherapy or radiation therapy thereby preventing, reducing, or reversing adverse side effects in the subject due to chemotherapy or radiation therapy in the subject.

15 Administration

In some embodiments, AA TG is administered to a subject in need thereof to prevent, reduce, or reverse adverse side effects or cytotoxic effects due to chemotherapy or radiation therapy in the subject. In some embodiments, a beneficial dose of AA TG is administered. In some embodiments, a beneficial dose is a therapeutic dose, an effective dose, or a
20 therapeutically effective dose. In some embodiments, a beneficial dose is a clinically effective dose. In some embodiments, administration is or comprises supplementation. In some embodiments, administering is or comprises supplementing.

In some embodiments, an amount of AA TG is administered to a subject in need thereof per day. In some embodiments, about or at least about 2 g of AA TG/day is
25 administered to the subject. In some embodiments, about 2.5 g or at least about 2.5 g of AA TG/day is administered to the subject. In some embodiments, about 3 g or at least about 3 g of AA TG/day is administered to the subject. In some embodiments, about 4 g or at least about 4 g of AA TG/day is administered to the subject. In some embodiments, about 5 g or at least about 5 g of AA TG/day is administered to the subject. In some embodiments, about 6 g
30 or at least about 6 g of AA TG/day is administered to the subject. In some embodiments, about 7 g or at least about 7 g of AA TG/day is administered to the subject. In some embodiments, about 8 g or at least about 8 g of AA TG/day is administered to the subject. In some embodiments, about 9 g or at least about 9 g of AA TG/day is administered to the subject. In some embodiments, about 10 g or at least about 10 g of AA TG/day is

administered to the subject. In some embodiments, about 15 g or at least about 15 g of AA TG/day is administered to the subject. In some embodiments, about 20 g or at least about 20 g of AA TG/day is administered to the subject. In some embodiments, about 25 g or at least about 25 g of AA TG/day is administered to the subject. In some embodiments, about 30 g or at least about 30 g of AA TG/day is administered to the subject. In some embodiments, about 40 g or at least about 40 g of AA TG/day is administered to the subject. In some embodiments, about 50 g or at least about 50 g of AA TG/day is administered to the subject. In some embodiments, about 60 g or at least about 60 g of AA TG/day is administered to the subject. In some embodiments, about 70 g or at least about 70 g of AA TG/day is administered to the subject. In some embodiments, about 80 g or at least about 80 g of AA TG/day is administered to the subject. In some embodiments, about 90 g or at least about 90 g of AA TG/day is administered to the subject. In some embodiments, about 100 g or at least about 100 g of AA TG/day is administered to the subject.

In some embodiments, from about 2 g to about 100 g of AA TG/day is administered to the subject. In some embodiments, from about 2 g to about 90 g of AA TG/day is administered to the subject. In some embodiments, from about 2 g to about 80 g of AA TG/day is administered to the subject. In some embodiments, from about 2 g to about 70 g of AA TG/day is administered to the subject. In some embodiments, from about 2 g to about 60 g of AA TG/day is administered to the subject. In some embodiments, from about 2 g to about 50 g of AA TG/day is administered to the subject. In some embodiments, from about 2 g to about 40 g of AA TG/day is administered to the subject. In some embodiments, from about 2 g to about 30 g of AA TG/day is administered to the subject. In some embodiments, from about 2 g to about 20 g of AA TG/day is administered to the subject. In some embodiments, from about 2 g to about 10 g of AA TG/day is administered to the subject.

In some embodiments, from about 5 g to about 100 g of AA TG/day is administered to the subject. In some embodiments, from about 5 g to about 90 g of AA TG/day is administered to the subject. In some embodiments, from about 5 g to about 80 g of AA TG/day is administered to the subject. In some embodiments, from about 5 g to about 70 g of AA TG/day is administered to the subject. In some embodiments, from about 5 g to about 60 g of AA TG/day is administered to the subject. In some embodiments, from about 5 g to about 50 g of AA TG/day is administered to the subject. In some embodiments, from about 5 g to about 40 g of AA TG/day is administered to the subject. In some embodiments, from about 5 g to about 30 g of AA TG/day is administered to the subject. In some embodiments,

from about 5 g to about 20 g of AA TG/day is administered to the subject. In some embodiments, from about 5 g to about 10 g of AA TG/day is administered to the subject.

In some embodiments, AA TG is administered to a subject in need thereof based on the weight of the subject. In some embodiments, about 50 mg or at least about 50 mg of AA TG/kg of body weight, about 100 mg or at least about 100 mg of AA TG/kg of body weight, about 150 mg or at least about 150 mg of AA TG/kg of body weight, about 200 mg or at least about 200 mg of AA TG/kg of body weight, about 300 mg or at least about 300 mg of AA TG/kg of body weight, about 400 mg or at least about 400 mg of AA TG/kg of body weight, about 500 mg or at least about 500 mg of AA TG/kg of body weight, about 600 mg or at least about 600 mg of AA TG/kg of body weight, about 700 mg or at least about 700 mg of AA TG/kg of body weight, about 800 mg or at least about 800 mg of AA TG/kg of body weight, about 900 mg or at least about 900 mg of AA TG/kg of body weight, about 1 g or at least about 1 g of AA TG/kg of body weight, about 1.5 g or at least about 1.5 g of AA TG/kg of body weight, about 2 g or at least about 2 g of AA TG/kg of body weight, or any range or combination thereof.

In some embodiments, an amount of AA TG to be administered to a subject in need thereof accounts for one or more of age, sex, height, concomitant medication(s), and pre-existing conditions in the subject. In some embodiments, when a subject is a pediatric patient, the amount of AA TG to be administered is adjusted based on the pediatric patient's age and the amount of AA TG to be administered to an adult, as the amounts per day or amounts per kg of body weight disclosed herein. A non-limiting example of determining a pediatric dose is Young's Rule, according to the equation: $[\text{Age} / (\text{Age} + 12)] \times \text{Recommended Adult Dose} = \text{Pediatric Dose}$. (See e.g., ncbi.nlm.nih.gov/books/NBK554603/, which is readily available to one of ordinary skill in the art). Young's Rule may be used if the pediatric patient's age is unknown. If a pediatric patient's age is known, Clark's Rule or the Body Surface Area rule can be implemented. (See e.g., ncbi.nlm.nih.gov/books/NBK541104/, which is readily available to one of ordinary skill in the art).

In some embodiments, administration of AA TG increases an AA level in the blood or a component of blood (e.g., plasma) of the subject relative to a reference. In some embodiments, an AA level is increased in the intestine of the subject relative to a reference. In some embodiments, an AA level is increased in the plasma and intestine of the subject relative to a reference.

In some embodiments, an AA level is measured in the blood or a component of blood (e.g., plasma) of the subject. In some embodiments, the AA level is measured in the intestine of a subject. In some embodiments, the AA level is measured in the intestine of a subject and in the blood or a component of blood (e.g., plasma) of the subject.

5 In some embodiments, administration of AA TG to a subject in need thereof increases expression of a marker of stemness, such as increased expression of a gene associated with stemness, relative to a reference. In some embodiments, a gene associated with stemness is at least one of leucine rich repeat containing g protein-coupled receptor 5 (Lgr5), achaete-scute family BHLH transcription factor 2 (Ascl2), lymphocyte antigen 6 complex, locus A (Ly6a),
10 or S100 calcium binding protein A6 (S100a6), LY6/PLAUR domain containing 6 (Lypd6), connective tissue growth factor (Ctgf), annexin A13 (Anxa13), cyclin D1 (Ccnd1), Annexin A3 (Anxa3), Interleukin 33 (Il33), clusterin (Clu), amphiregulin (Areg), CD55 molecule (cromer blood group) (Cd55), epiregulin (Ereg), myoferlin (Myof), mesothelin (Msln).

In some embodiments, administration of AA TG increases expression of a gene
15 associated with stemness about 75% at least about 75%; about 100% or at least about 100%; about 125% or at least about 125%; about 150% or at least about 150%; about 175% or at least about 175%; about 200% or at least about 200%; about 300% or at least about 300%; about 400% or at least about 400%, about 500% or at least about 500%, about 600% or at least about 600%; about 700% or at least or about 700%, about 800% or at least about 800%,
20 or any ranges or combinations thereof, relative to a reference. In some embodiments, expression of a gene associated with stemness is increased in a cell (e.g., epithelial cell, intestinal cell, etc.) obtained from the subject. In some embodiments, expression of a gene associated with stemness is increased in a sample, such as blood, a component of blood (e.g., plasma, serum etc.) or a tissue (e.g., intestinal tissue) obtained from the subject.

25 In some embodiments, administration of AA TG to a subject in need thereof increases an AA level in the subject (e.g., a sample from the subject) relative to a reference. In some embodiments, administration of AA TG to a subject in need thereof increases an AA level at about 2-fold or at least about 2-fold relative to a reference. In some embodiments, administration of AA TG to a subject in need thereof increases an AA level about 2-fold or at
30 least about 2-fold, about 3-fold or at least about 3-fold, about 4-fold or at least about 4-fold, about 5-fold or at least about 5-fold, about 6-fold or at least about 6-fold, about 7-fold or at least about 7-fold, about 8-fold or at least about 8-fold, about 9-fold or at least about 9-fold, about 10-fold or at least about 10-fold, about 11-fold or at least about 11-fold, about 12-fold or at least about 12-fold, about 13-fold or at least about 13-fold, about 14-fold or at least

about 14-fold, about 15-fold or at least about 15-fold, or any ranges or combinations thereof, relative to a reference. In some embodiments, administration of AA TG increases an AA level in a subject in need thereof about 3-fold to about 20-fold relative to a reference. In some embodiments, administration of AA TG increases an AA level in a subject in need thereof about 3-fold to about 15-fold relative to a reference. In some embodiments, administration of AA TG increases an AA level in a subject in need thereof about 3-fold to about 10-fold relative to a reference. In some embodiments, administration of AA TG increases an AA level in a subject in need thereof about 1.5-fold to about 3-fold relative to a reference.

In some embodiments, AA TG, at least one of a precursor-AA TG or AA TG and at least one precursor-AA TG is administered before a course of chemotherapy, radiation therapy, or a combination of chemotherapy and radiation therapy. In some embodiments, the AA TG, at least one precursor-AA TG or AA TG and at least one precursor-AA TG is administered for a total time of 7 days, about 7 days, or at least 7 days, 10 days, about 10 days, or at least 10 days, 2 weeks or about 2 weeks, or at least 2 weeks, 3 weeks, about 3 weeks, or at least 3 weeks, 4 weeks, about 4 weeks, or at least 4 weeks, 5 weeks, about 5 weeks, or at least 5 weeks, 6 weeks, about 6 weeks, or at least 6 weeks, 7 weeks, about 7 weeks, or at least 7 weeks, 8 weeks, about 8 weeks, or at least 8 weeks, 9 weeks, about 9 weeks, or at least 9 weeks, 10 weeks, about 10 weeks, or at least 10 weeks, 11 weeks, about 11 weeks, or at least 11 weeks, 12 weeks, about 12 weeks, or at least 12 weeks before a course of chemotherapy, radiation therapy, or chemotherapy and radiation therapy. In some embodiments, the AA TG, at least one precursor-AA TG or AA TG and at least one precursor-AA TG is administered for 2 weeks to 4 weeks before a course of chemotherapy, radiation therapy, or chemotherapy and radiation therapy. In some embodiments, AA TG, at least one precursor-AA TG or AA TG and at least one precursor-AA TG is administered for 1 week to 3 weeks, 3 weeks to 5 weeks, 4 weeks to 6 weeks, or 5 weeks to 7 weeks before a course of chemotherapy, radiation therapy, or chemotherapy and radiation therapy.

A subject may complete one course of chemotherapy or one course of radiation therapy or more than one course of chemotherapy or one course of radiation therapy. The number of courses of chemotherapy or the number of courses of radiation therapy may depend on the needs of the subject.

In some embodiments, AA TG, at least one precursor-AA TG or AA TG and at least one precursor-AA TG is administered to a subject in need thereof for a sufficient time to prevent, reduce or reverse adverse side effects or cytotoxic effects due to chemotherapy or

radiation therapy in the subject. In some embodiments, a sufficient time is at least about 3 days; and (a) administration starts before the subject begins a course of chemotherapy or radiation therapy; (b) administration starts after the subject completes a course of chemotherapy or radiation therapy; or (c) administration starts at any time during a course of chemotherapy or radiation therapy. In some embodiments, a sufficient time is at least about 5 days; and (a) administration starts before the subject begins a course of chemotherapy or radiation therapy; (b) administration starts after the subject completes a course of chemotherapy or radiation therapy; or (c) administration starts at any time during a course of chemotherapy or radiation therapy. In some embodiments, a sufficient time is at least about 7 days; and (a) administration starts before the subject begins a course of chemotherapy or radiation therapy; (b) administration starts after the subject completes a course of chemotherapy or radiation therapy; or (c) administration starts at any time during a course of chemotherapy or radiation therapy. In some embodiments, a sufficient time is at least about 14 days; and (a) administration starts before the subject begins a course of chemotherapy or radiation therapy; (b) administration starts after the subject completes a course of chemotherapy or radiation therapy; or (c) administration starts at any time during a course of chemotherapy or radiation therapy. In some embodiments, a sufficient time is at least about 21 days; and (a) administration starts before the subject begins a course of chemotherapy or radiation therapy; (b) administration starts after the subject completes a course of chemotherapy or radiation therapy; or (c) administration starts at any time during a course of chemotherapy or radiation therapy. In some embodiments, a sufficient time is at least about 28 days; and (a) administration starts before the subject begins a course of chemotherapy or radiation therapy; (b) administration starts after the subject completes a course of chemotherapy or radiation therapy; or (c) administration starts at any time during a course of chemotherapy or radiation therapy. In some embodiments, a sufficient time is at least about 28 days; and (a) administration starts before the subject begins a course of chemotherapy or radiation therapy; (b) administration starts after the subject completes a course of chemotherapy or radiation therapy; or (c) administration starts at any time during a course of chemotherapy or radiation therapy.

In some embodiments, a sufficient time is about 1 month, at least 1 month, about 2 months, at least 2 months, about 3 months, at least 3 months, about 4 months, at least 4 months, about 5 months, at least 5 months, about 6 months, at least 6 months, about 7 months, at least 7 months, about 8 months, at least 8 months, about 9 months, at least 9 months, about 10 months, at least 10 months, about 11 months, at least 11 months, about 1 year, or at least 1 year; and (a) administration starts before the subject begins a course of chemotherapy or radiation therapy; (b) administration starts after the subject completes a course of chemotherapy or radiation therapy; or (c) administration starts at any time during a course of chemotherapy or radiation therapy.

In some embodiments, AA TG, at least one precursor-AA TG or AA TG and at least one precursor-AA TG is administered for one cycle which is or comprises administration of AA TG, at least one precursor-AA TG or AA TG and at least one precursor-AA TG for about 1 week or at least 1 week and followed by no administration of AA TG, at least one precursor-AA TG or AA TG and at least one precursor-AA TG for about 1 week or at least 1 week; for one cycle which is or comprises administration of AA TG, at least one precursor-AA TG or AA TG and at least one precursor-AA TG for about 2 weeks or at least 2 weeks and followed by no administration of AA TG, at least one precursor-AA TG or AA TG and at least one precursor-AA TG for about 2 weeks or at least 2 weeks; for one cycle which is or comprises administration of AA TG, at least one precursor-AA TG or AA TG and at least one precursor-AA TG for about 3 weeks or at least 3 weeks and followed by no administration of AA TG, at least one precursor-AA TG or AA TG and at least one precursor-AA TG for about 3 weeks or at least 3 weeks; for one cycle which is or comprises administration of AA TG, at least one precursor-AA TG or AA TG and at least one precursor-AA TG for about 4 weeks or at least 4 weeks and followed by no administration of AA TG, at least one precursor-AA TG or AA TG and at least one precursor-AA TG for about 4 weeks or at least 4 weeks. In some embodiments, AA TG, at least one precursor-AA TG or AA TG and at least one precursor-AA TG is administered for about 1 week or at least 1 week, about 2 weeks or at least about 2 weeks, about 3 weeks or at least about 3 weeks, about 4 weeks or at least 4 weeks, followed by no administration of AA TG, at least one precursor-AA TG or AA TG and at least one precursor-AA TG for about 1 week or at least 1 week, about 2 weeks or at least about 2 weeks, about 3 weeks or at least about 3 weeks, about 4 weeks or at least 4 weeks.

In some embodiments, AA TG, at least one precursor-AA TG or AA TG and at least one precursor-AA TG is administered daily to a subject in need thereof. In some embodiments, AA TG, at least one precursor-AA TG or AA TG and at least one precursor-AA TG is not administered daily to a subject in need thereof. In some embodiments, AA TG, at least one precursor-AA TG or AA TG and at least one precursor-AA TG is administered to a subject in need thereof every other day. In some embodiments, AA TG, at least one precursor-AA TG or AA TG and at least one precursor-AA TG is administered at least once per day to a subject in need thereof. In some embodiments, AA TG, at least one precursor-AA TG or AA TG and at least one precursor-AA TG is administered every other day to a subject in need thereof. In some embodiments, AA TG, at least one precursor-AA TG or AA

TG and at least one precursor-AA TG is administered to a subject in need thereof two, three, or four times per day.

In some embodiments, an AA level in a sample from a subject in need thereof is below a pre-determined AA level in the absence of administration of an amount of AA TG which increases the AA level in the subject in need thereof to or above a pre-determined AA level. In some embodiments, a pre-determined AA level is a two-fold increase in an AA level measured in a sample from the subject relative to an AA level measured in a sample from the subject before administering AA TG. In some embodiments, a pre-determined AA level is a 3-fold, is a 4-fold, is a 5-fold, is a 6-fold, is a 7-fold, is an 8-fold, is a 9-fold or is a 10 fold, increase in an AA level measured in a sample from the subject relative to an AA level measured in a sample from the subject before administering AA TG. In some embodiments, a pre-determined AA level is a clinically relevant AA level. In some embodiments, a pre-determined AA level is a clinically relevant plasma AA level or intestinal AA level. In some embodiments, a pre-determined AA level is an AA level sufficient to prevent, reduce, or reverse adverse side effects due to chemotherapy or radiation therapy. In some embodiments, a pre-determined AA level is the lowest AA level at which a beneficial effect is observed in the subject.

In some embodiments, a beneficial effect is increased expression of a marker of stemness, such as increased expression of a gene associated with stemness, relative to a reference. In some embodiments, a gene associated with stemness is at least one of leucine rich repeat containing g protein-coupled receptor 5 (Lgr5), achaete-scute family BHLH transcription factor 2 (Ascl2), lymphocyte antigen 6 complex, locus A (Ly6a), or S100 calcium binding protein A6 (S100a6), LY6/PLAUR domain containing 6 (Lypd6), connective tissue growth factor (Ctgf), annexin A13 (Anxa13), cyclin D1 (Ccdn1), Annexin A3 (Anxa3), Interleukin 33 (Il33), clusterin (Clu), amphiregulin (Areg), CD55 molecule (cromer blood group) (Cd55), epiregulin (Ereg), myoferlin (Myof), mesothelin (Msln).

In some embodiments, a beneficial effect is observed when expression of a gene associated with stemness is increased about 10% or at least about 10%; about 25% or at least about 25%; about 50% or at least about 50%; about 75% or at least about 75%; about 100% or at least about 100%; about 125% or at least about 125%; about 150% or at least about 150%; about 175% or at least about 175%; about 200% or at least about 200%; about 300% or at least about 300%; about 400% or at least about 400%; about 500% or at least about 500%; about 600% or at least about 600%; about 700% or at least about 700%, about 800% or at least about 800%; or any ranges or combinations thereof, relative to a reference in a

subject in need thereof. In some embodiments, expression of a gene associated with stemness is increased in a cell (e.g., epithelial cell, intestinal cell) obtained from the subject.

In some embodiments, a beneficial effect is prevention, reduction, or reversal of adverse side effects due to chemotherapy or radiation therapy in a subject thereof relative to a reference, as determined by a healthcare provider (e.g., medical doctor). For instance, a healthcare provider may determine that one or more symptomatic measures, including but not limited to frequency, volume, amount or presence of diarrhea, blood in stool, calprotectin in stool, vomiting, nausea, weight loss, intestinal tissue damage, radiation colitis, radiation mucositis, pelvic radiation disease, radiation enteritis, abdominal pain, rectal bleeding, bloating, or constipation are reduced in relative to a reference. In some embodiments, a reference is frequency, volume, amount or presence of frequency, volume, amount or presence of diarrhea, blood in stool, calprotectin in stool, vomiting, nausea, weight loss, intestinal tissue damage, radiation colitis, radiation mucositis, pelvic radiation disease, radiation enteritis, abdominal pain, rectal bleeding, bloating, or constipation due to chemotherapy or radiation therapy the subject experiences before the subject in need thereof is administered AA TG, at least one precursor-AA TG or AA TG and at least one precursor-AA TG.

In some embodiments, a beneficial effect is prevention, reduction, or reversal of a cytotoxic effect due to chemotherapy or radiation therapy relative to a reference, as determined by a healthcare provider (e.g., medical doctor). For instance, a healthcare provider may determine that relative to a reference. In some embodiments, intestinal tissue damage is prevented reduced or reversed in the subject in need thereof after administration of AA TG, at least one precursor-AA TG or AA TG and at least one precursor-AA TG relative to a reference. In some embodiments, a reference is extent of intestinal tissue damage before administering AA TG, at least one precursor-AA TG or AA TG and at least one precursor-AA TG in a subject in need thereof. Intestinal damage due to chemotherapy is discussed in Sougiannis, et al. *Am J Physiol Gastrointest Liver Physiol* (2021) 320(5):G712-G719, which is available to one of ordinary skill in the art incorporated herein by reference in its entirety. In some embodiments, a cytotoxic effect is intestinal tissue damage.

In some embodiments, a method of preventing or prevention refers to not observing, clinically, one or more adverse side effects expected in a subject who has undergone a similar course of chemotherapy or radiation therapy.

In some embodiments, a beneficial effect is assessed in a sample obtained from a subject in need thereof. In some embodiments, a sample is a cell (e.g., epithelial cell, intestinal

cell, etc.), blood, a component of blood (e.g., serum, plasma), or stool obtained from the subject in need thereof.

In some embodiments, a beneficial effect is increased AA level in a subject in need thereof relative to a reference. In some embodiments, a beneficial effect is increased AA level in a subject in need thereof of about 2-fold or at least 2-fold about relative to a reference. In some embodiments, a beneficial effect is increased AA level in a subject in need thereof of at least or about 1.5-fold, at least or about 2-fold, at least or about 3-fold, at least or about 4-fold; at least or about 6-fold, at least or about 7-fold, at least or about 8-fold, at least or about 9-fold, at least or about 10-fold, at least or about 11-fold, at least or about 12-fold, at least or about 13-fold, at least or about 14-fold, or at least or about 15-fold, or any range or combination thereof, relative to a reference. In some embodiments, a beneficial effect is increased AA level in a subject in need thereof of about 3-fold to about 15-fold relative to a reference. In some embodiments, a beneficial effect is increased AA level in a subject in need thereof of about 3-fold to about 10-fold relative to a reference. In some embodiments, a beneficial effect is increased AA level in a subject in need thereof of about 1.5-fold to about 3-fold relative to a reference.

In some embodiments, a reference is an AA level in a subject before exposure to a course of chemotherapy, radiation therapy, or both chemotherapy and radiation therapy. In some embodiments, a reference is a level of a population of AA in a sample in a subject before a course of chemotherapy, radiation therapy or both chemotherapy or radiation therapy. In some embodiments, reference is an AA level in a subject before administration to the subject of AA TG. In some embodiments, reference is an AA level in a subject not exposed to a course of chemotherapy, radiation or both chemotherapy and radiation. In some embodiments, reference is an AA level in a subject having the same condition as the subject who is to be exposed to a course of chemotherapy or radiation therapy, but the subject having the same condition is not exposed to a course of chemotherapy or radiation therapy. In some embodiments, the condition is a condition which is treated with a course of chemotherapy, radiation therapy or chemotherapy and radiation therapy. In some embodiments, the condition is cancer. In some embodiments, reference is an AA level in a cell before exposure to a course of chemotherapy or radiation therapy.

In some embodiments, AA TG is administered orally to a subject in need thereof. In some embodiments, AA TG is administered orally. In some embodiments, AA TG is administered via a nasogastric tube. In some embodiments, AA TG is administered into the stomach, such as via a gastric tube (G tube) or injection. In some embodiments, AA TG is

administered via a nasoduodenal or nasojejunal tube. In some embodiments, AA TG is administered to the small intestine via a jejunostomy (J tube). Administration can be via a variety of non-parenteral routes. In some embodiments, AA TG is not administered via intragastric injection. In some embodiments, AA TG is in a composition, wherein the composition is in the form of a liquid or a powder. In some embodiments, the composition is in the form of pills or capsules. In some embodiments, AA, as a free fatty acid bound to a carrier protein (e.g., albumin), is administered rectally, for instance, using a suppository.

Assessing the effects of administration of AA TG can be carried out by comparing the extent of one or more adverse side effects, the extent of cytotoxicity or both after administration of AA TG with the extent of the same one or more side effects, extent of cytotoxicity, or both in the subject in need thereof prior to administration of AA TG.

Similarly, the effects of administration of at least one precursor-AA TG (precursor of AA in TG form), or administration of AA TG and at least one precursor-AA TG (precursor of AA in TG form) can be assessed by comparing the extent of one or more adverse side effects, the extent of cytotoxicity or both after administration of administration of at least one precursor-AA TG (precursor of AA in TG form), or administration of AA TG and at least one precursor-AA TG (precursor of AA in TG form) with the extent of the same one or more side effects, extent of cytotoxicity, or both in the subject in need thereof prior to administration of administration of at least one precursor-AA TG (precursor of AA in TG form), or administration of AA TG and at least one precursor-AA TG (precursor of AA in TG form).

Subject

In some embodiments, a subject is a vertebrate. In some embodiments, a subject is a rodent. In some embodiments, a subject is a mouse. In some embodiments, a subject is a domestic animal (e.g., dog, cat, etc.). In some embodiments, a subject is a mammal. In some embodiments, a subject is a primate. In some embodiments, a subject is a human.

In some embodiments, a subject in need thereof is a human in need thereof. In some embodiments, a subject in need thereof is a subject before the subject begins a course of chemotherapy or radiation therapy. In some embodiments, a subject in need thereof is a subject who has been exposed to a course of chemotherapy, radiation therapy. In some embodiments, a subject in need thereof is a subject having cancer that will be exposed to or treated with a course of chemotherapy, radiation therapy, or chemotherapy and radiation therapy. In some embodiments, a subject in need thereof is administered AA TG before a course of chemotherapy, radiation therapy or chemotherapy and radiation therapy. In some

embodiments, a subject in need thereof is administered AA TG during a course of chemotherapy, radiation therapy, or chemotherapy and radiation therapy. In some embodiments, a subject in need thereof is administered AA TG before and during a course of chemotherapy, radiation therapy, or chemotherapy and radiation therapy.

5 Numerous polymorphisms in the fatty acid (FA) desaturase gene cluster associate strongly with metabolic traits and diseases including IBD (Sabatti et al. 2009; Dupuis et al., 2010; Costea et al., 2014). In some embodiments, such polymorphisms can be used to select a population of subjects for the treatments described herein.

In some embodiments, the method comprises increasing, in a subject, a plasma
10 arachidonic acid (AA) level to that indicative of an intestinal AA level that prevents, reduces, or reverses adverse side effects due to chemotherapy or radiation therapy.

In some embodiments, the method comprises (a) measuring an arachidonic acid (AA) level in a sample from a subject in need thereof and determining if the AA level is below a pre-determined AA level sufficient to prevent, reduce, or reverse adverse side effects due to
15 chemotherapy or radiation therapy; and (b) if the AA level is below the pre-determined AA level, administering to the subject in (a) at least about 2 g of AA TG per day (2 g/d) for a sufficient time to increase the AA level to or above the pre-determined AA level.

In some embodiments, the method further comprises (c) measuring the AA level resulting from administering AA TG in (b) and determining the AA level; and (d) if the AA
20 level in (b) is not at or above the pre-determined AA level, further administering to the subject a sufficient amount of AA TG per day to result in an intestinal AA level at or above the pre-determined AA level.

In some embodiments, the method further comprises repeating (c)-(d) to produce in the subject an intestinal AA level at or above the pre-determined AA level.

25 In some embodiments, methods of increasing an AA level in a subject to prevent or reduce to tissue damage due to chemotherapy or radiation therapy are disclosed. In some embodiments, a method comprises (a) measuring an AA level in a sample obtained from a subject, (b) determining if the AA level in the subject in (a) is below a pre-determined AA level; (c) administering an amount of AA TG sufficient to increase an AA level to or above a
30 pre-determined AA level, wherein the increased AA level in (c) prevents or reduces tissue damage in the subject from chemotherapy, radiation therapy, or chemotherapy and radiation therapy. In some embodiments, a method disclosed herein further comprises (d) measuring an AA level in a sample obtained from a subject after administering the AA TG in (c) and determining if the AA level in the sample in (d) is at or above the pre-determined AA level.

In some embodiments, if the AA level is below the pre-determined AA level described above, the amount of the AA TG to be administered to the subject is adjusted to an amount sufficient to increase the AA level in the subject to above the pre-determined AA level.

5 In some embodiments, measuring an AA level comprises collecting a sample from a subject in need thereof and measuring an AA level in the sample. In some embodiments, the sample is blood. In some embodiments, the sample is serum. In some embodiments, the sample is plasma. In some embodiments, the sample is or comprises tissue. In some
10 embodiments, the sample is or comprises stool. In some embodiments, the tissue is intestinal tissue. In some embodiments, an AA in the AA level is a free AA fatty acid. In some embodiments, the AA fatty acid is associated with a carrier protein (e.g., albumin). In some
15 embodiments, an AA in the AA level is an AA PL.

In some embodiments, the AA in a sample obtained from a subject is measured by detection of AA in the sample. In some embodiments, methods for measuring AA levels in a sample obtained from a subject include, but are not limited to, mass spectrometry, liquid
15 chromatography, liquid chromatography-mass spectrometry (LC-MS), gas chromatography, thin-layer chromatography, size-exclusion chromatography, enzyme-linked immunosorbent assays (ELISA), nuclear magnetic resonance (NMR).

Preventing or Reducing Tissue Damage

20 In some embodiments, methods of preventing or reducing tissue damage are disclosed. In some embodiments, the method comprises administering AA in the forms disclosed herein to a subject prior to exposure to chemotherapy, radiation therapy, or chemotherapy and radiation therapy to prevent or reduce tissue damage due to chemotherapy, radiation therapy, or chemotherapy and radiation therapy.

25 In some embodiments, preventing or reducing tissue damage comprises anticipating tissue damage in a subject and prophylactically administering AA TG to the subject before exposure to a course of chemotherapy, radiation therapy, or chemotherapy and radiation therapy. In some embodiments, preventing or reducing tissue damage further comprises observing less tissue damage in the subject relative to an extent of tissue damage that
30 occurred or existed prior to administration of AA TG or relative to a reference.

In some embodiments, preventing or reducing tissue damage comprises observing about 10% less or at least 10% less tissue damage, about 15% less or at least 15% less tissue damage, about 20% less or at least 20% less tissue damage, about 25% less or at least 25% less tissue damage, about 30% less or at least 30% less tissue damage, about 35% less or at

least 35% less tissue damage, about 40% less or at least 40% less tissue damage, about 45% less or at least 45% less tissue damage, about 50% less or at least 50% less tissue damage, about 55% less or at least 55% less tissue damage, about 60% less or at least 60% less tissue damage, about 65% less or at least 65% less tissue damage, about 70% less or at least 70% less tissue damage, about 75% less or at least 75% less tissue damage, about 80% less or at least 80% less tissue damage, about 85% less or at least 85% less tissue damage, about 90% less or at least 90% less tissue damage relative to an extent of tissue damage that occurred or existed prior to administration of AA TG or relative to a reference. In some embodiments, preventing or reducing tissue damage comprises preventing all tissue damage relative to a reference. In some embodiments, reference is a tissue damaged due to a course of chemotherapy, radiation or chemotherapy and radiation without administration (e.g., prophylactic administration) of AA TG. In some embodiments, prevention or reduction of tissue damage is determined by a healthcare provider (e.g., medical doctor). For instance, a healthcare provider may determine that symptomatic measures, including but not limited to diarrhea, blood in stool, or calprotectin in stool, are reduced in a subject relative to a reference.

In some embodiments, tissue damage is damage expected from chemotherapy, radiation therapy, or chemotherapy and radiation therapy. In some embodiments, tissue damage is measured by histology. In some embodiments, tissue damage is measured or assessed as understood by one of ordinary skill in the art. In some embodiments, tissue damage is inferred from clinical symptoms in a subject. In some embodiments, tissue damage is inferred from digestive tract symptoms, such as nausea, vomiting, diarrhea, weight loss, and the like. In some embodiments, preventing or reducing tissue damage is indicated by a reduction of clinical symptoms in a subject receiving chemotherapy, radiation therapy, or chemotherapy and radiation therapy.

Tissue Regeneration

In some embodiments, disclosed are methods of promoting tissue regeneration, comprising administering to a subject having tissue damage due to chemotherapy, radiation therapy, or chemotherapy and radiation therapy to promote tissue regeneration in the subject AA TG which increases the level of AA at least 2-fold in the subject relative to a reference. Tissue regeneration includes regrowth of tissues having experienced tissue damage. Tissue damage includes, but is not limited to, damage to the tissue of a subject from a course of chemotherapy or radiation therapy. In some embodiments, the damage is from a course of

chemotherapy and radiation therapy, or from a course including both chemotherapy and radiation therapy.

In some embodiments, promoting regeneration in a damaged tissue from a course of chemotherapy, radiation therapy or chemotherapy and radiation therapy comprises observing damage in a tissue, administering an AA TG, and observing less damage in the tissue. In some embodiments, tissue regeneration comprises 100% recovery, about 95% recovery, about 90% recovery, about 80% recovery, about 70% recovery, about 60% recovery, about 50% recovery, about 40% recovery, or about 30% recovery, in each case, from tissue damage relative to a reference. In some embodiments, reference is a tissue damaged due to a course of chemotherapy, radiation or chemotherapy and radiation without administration (e.g., prophylactic administration) of AA TG. In some embodiments, reference is a tissue damaged due to a course of chemotherapy, radiation or chemotherapy and radiation and before administration of AA TG. In some embodiments, regeneration of damaged tissue is determined by a healthcare provider (e.g., medical doctor). For instance, a healthcare provider may determine that symptomatic measures, including but not limited to diarrhea, blood in stool, or calprotectin in stool, are reduced in a subject relative to a reference.

In some embodiments, tissue damage is damage expected from chemotherapy, radiation therapy, or chemotherapy and radiation therapy. In some embodiments, tissue damage is measured by histology. In some embodiments, tissue damage is measured or assessed as understood by one of ordinary skill in the art. In some embodiments, tissue damage is inferred from clinical symptoms in a subject. In some embodiments, tissue damage is inferred from digestive tract symptoms, such as nausea, vomiting, diarrhea, weight loss, and the like. In some embodiments, regeneration of damaged tissue is indicated by a reduction of clinical symptoms in a subject receiving chemotherapy, radiation therapy, or chemotherapy and radiation therapy.

In some embodiments, regeneration in a damaged tissue comprises increased expression of a marker of stemness, such as increased expression of a gene associated with stemness, relative to a reference. In some embodiments, a gene associated with stemness is at least one of leucine rich repeat containing G protein-coupled receptor 5 (Lgr5), achaete-scute family BHLH transcription factor 2 (Ascl2), lymphocyte antigen 6 complex, locus A (Ly6a), or S100 calcium binding protein A6 (S100a6), LY6/PLAUR domain containing 6 (Lypd6), connective tissue growth factor (Ctgf), annexin A13 (Anxa13), cyclin D1 (Ccdn1), Annexin A3 (Anxa3), Interleukin 33 (Il33), clusterin (Clu), amphiregulin (Areg), CD55 molecule (cromer blood group) (Cd55), epiregulin (Ereg), myoferlin (Myof), mesothelin (Msln). In

some embodiments, expression of a gene associated with stemness is increased at least or about 75%; at least or about 100%; at least or about 125%; at least or about 150%; at least or about 175%; at least or about 200%; at least or about 300%; at least or about 400%, at least or about 500%, at least or about 600%; at least or about 700%, at least or about 800%, or any
5 ranges or combinations thereof, relative to a reference. In some embodiments, reference is a level of expression of a gene associated with stemness in a tissue damaged due to a course of chemotherapy, radiation or chemotherapy and radiation without administration (e.g., prophylactic administration) of AA TG. In some embodiments, reference is a level of expression of a gene associated with stemness in a tissue damaged due to a course of
10 chemotherapy, radiation or chemotherapy and radiation and before administration of AA TG. In some embodiments, regeneration of damaged tissue is determined by a healthcare provider (e.g., medical doctor). For instance, a healthcare provider may determine that symptomatic measures, including but not limited to diarrhea, blood in stool, or calprotectin in stool, are reduced in a subject relative to a reference.

15 In some embodiments, the present disclosure relates to a method of promoting regeneration in a damaged cell or injured cell, comprising contacting a damaged cell or injured cell having damage due to a course of chemotherapy, radiation therapy or chemotherapy and radiation therapy to promote regeneration in the damaged cell or injured cell with AA TG that increases the AA level at least 2-fold relative to a reference inside the
20 damaged cell or injured cell or milieu surrounding the damaged cell or injured cell. In some embodiments, the damaged cell or injured cell is isolated from a damaged tissue due to chemotherapy, radiation or chemotherapy and radiation.

In some embodiments, promoting regeneration in a damaged cell or injured cell comprises observing injury in a cell, administering an AA TG, and observing less injury in
25 the cell. In some embodiments, regeneration comprises 100% recovery from cellular damage, about 95% recovery, about 90% recovery, about 80% recovery, about 70% recovery, about 60% recovery, about 50% recovery, about 40% recovery, or about 30% recovery relative to a reference. In some embodiments, reference is a damaged cell or injured cell due to a course of chemotherapy, radiation or chemotherapy and radiation without administration (e.g.,
30 prophylactic administration) of AA TG. In some embodiments, prevention or reduction of cell damage or cell injury is determined by a healthcare provider.

In some embodiments, regeneration in a damaged cell comprises increased expression of a marker of stemness, such as increased expression of a gene associated with stemness, relative to a reference. In some embodiments, a gene associated with stemness is at least one

of leucine rich repeat containing G protein-coupled receptor 5 (Lgr5), achaete-scute family BHLH transcription factor 2 (Ascl2), lymphocyte antigen 6 complex, locus A (Ly6a), or S100 calcium binding protein A6 (S100a6), LY6/PLAUR domain containing 6 (Lypd6), connective tissue growth factor (Ctgf), annexin A13 (Anxa13), cyclin D1 (Ccd1), Annexin 5 A3 (Anxa3), Interleukin 33 (Il33), clusterin (Clu), amphiregulin (Areg), CD55 molecule (cromer blood group) (Cd55), epiregulin (Ereg), myoferlin (Myof), mesothelin (Msln). In some embodiments, expression of a gene associated with stemness is increased at least or about 75%; at least or about 100%; at least or about 125%; at least or about 150%; at least or about 175%; at least or about 200%; at least or about 300%; at least or about 400%, at least or about 500%, at least or about 600%; at least or about 700%, at least or about 800%, or any ranges or combinations thereof, relative to a reference. In some embodiments, reference is a level of expression of a gene associated with stemness in a tissue damaged due to a course of chemotherapy, radiation or chemotherapy and radiation without administration (e.g., prophylactic administration) of AA TG. In some embodiments, reference is a level of expression of a gene associated with stemness in a tissue damaged due to a course of chemotherapy, radiation or chemotherapy and radiation and before administration of AA TG.

In some embodiments, the damaged cell or injured cell is or comprises a damaged epithelial cell or injured epithelial cell. In some embodiments, the damaged cell or injured cell is or comprises a damaged intestinal cell or injured intestinal cell. In some embodiments, the damaged cell or injured cell is or comprises a damaged oral cell or injured oral cell. In some embodiments, the damaged cell or injured cell is or comprises a damaged skin cell or injured skin cell.

Cells

In some embodiments, the present disclosure relates to a method of preventing or reducing cell damage or cell injury, comprising contacting a cell with AA TG prior to exposure to chemotherapy, radiation therapy, or a combination of chemotherapy and radiation therapy.

In some embodiments, the cell is or comprises an epithelial cell. In some embodiments the cell is or comprises an oral cell, a skin cell, or an intestinal cell. In some embodiments, the cell is or comprises an intestinal cell. In some embodiments, the cell is or comprises a cultured cell. In some embodiments, the cell is or comprises a constituent of an organoid. In some embodiments, the cell is or comprises a human cell. In some embodiment, the cell is or comprises an animal cell. In some embodiments, the cell is or comprises a

mammalian cell. In some embodiments, the cell is or comprises part of a tissue. In some embodiments, the tissue is or comprises epithelial tissue. In some embodiments, the tissue is or comprises intestinal tissue. In some embodiments, the cell is or comprises a cell of a living multicellular organism. In some embodiments, the cell is or comprises a cell obtained from a subject.

In some embodiments, the AA level is at least or about 75%; at least or about 100%; at least or about 125%; at least or about 150%; at least or about 175%; at least or about 200%; at least or about 300%; at least or about 400%, at least or about 500%, at least or about 600%; at least or about 700%, at least or about 800%, or any ranges or combinations thereof, relative to a reference. In some embodiments, the AA level is measured inside the cell. In some embodiments, the AA level is assessed in the milieu surrounding the cell.

In some embodiments, the AA level is increased 1.5-fold, about 1.5-fold, or at least 1.5-fold; 2-fold, about 2-fold or at least 2-fold; 3-fold, about 3-fold, or at least 3-fold; 4-fold, about 4-fold, or at least 4-fold; 5-fold, about 5-fold, or at least 5-fold; 6-fold, about 6-fold, or at least 6-fold; 7-fold, about 7-fold, or at least 7-fold; 8-fold, about 8-fold, or at least 8-fold; 9-fold, about 9-fold, or at least 9-fold; 10-fold, about 10-fold, or at least 10-fold; 11-fold, about 11-fold, or at least 11-fold; 12-fold, about 12-fold, or at least 12-fold; 13-fold, about 13-fold, or at least 13-fold; 14-fold, about 14-fold, or at least 14-fold; 15-fold, about 15-fold, or at least 15-fold in a cell relative to a reference. In some embodiments, reference is the AA level in the cell or an identical cell before administration of an AA TG. In some embodiments, reference is the AA level in a control sample of cells.

Cancers

Cancer (malignant neoplasm) is a class of diseases in which a group of cells display the traits of uncontrolled growth (growth and division beyond the normal limits), invasion (intrusion on and destruction of adjacent tissues), and sometimes metastasis (spread to other locations in the body via lymph or blood). Most cancers form a tumor but some, like leukemia, do not. In some embodiments, a cancer is colon carcinoma, breast cancer, pancreatic cancer, ovarian cancer, prostate cancer, fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangoendothelia sarcoma, synovioma, mesothelioma, Ewing's sarcoma, leiomyosarcoma, rhabdomyosarcoma, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma,

- bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilms' tumor, cervical cancer, testicular tumor, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioblastomas, neuronomas, craniopharyngiomas, schwannomas, glioma, astrocytoma,
- 5 medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroama, oligodendroglioma, meningioma, melanoma, neuroblastoma, retinoblastoma, leukemias and lymphomas, acute lymphocytic leukemia and acute myelocytic polycythemia vera, multiple myeloma, Waldenstrom's macroglobulinemia, and heavy chain disease, acute nonlymphocytic leukemias, chronic lymphocytic leukemia, chronic myelogenous leukemia,
- 10 Hodgkin's Disease, non-Hodgkin's lymphomas, rectum cancer, urinary cancer, uterine cancer, oral cancer, skin cancer, stomach cancer, brain cancer, liver cancer, laryngeal cancer, esophageal cancer, mammary tumor, childhood-null acute lymphoid leukemia (ALL), thymic ALL, B-cell ALL, acute myeloid leukemia, myelomonocytoid leukemia, acute megakaryocytoid leukemia, Burkitt's lymphoma, acute myeloid leukemia, chronic myeloid
- 15 leukemia, and T cell leukemia, small and large non-small cell lung carcinoma, acute granulocytic leukemia, germ cell tumors, endometrial cancer, gastric cancer, cancer of the head and neck, chronic lymphoid leukemia, hairy cell leukemia or thyroid cancer.

Cancer Therapies

- 20 In some embodiments, AA TG is administered to a subject receiving or intended to receive chemotherapy or radiation therapy. In some embodiments, chemotherapy comprises the administration of one or more pharmaceutical compositions including, but not limited to, alkylating agents, antimetabolites, anti-tumor antibiotics, topoisomerase inhibitors, mitotic inhibitors, and corticosteroids. In some embodiments, the chemotherapy agent is one or more
- 25 agents selected from: altretamine, bendamustine, busulfan, carboplatin, carmustine, chlorambucil, cisplatin, cyclophosphamide, dacarbazine, ifosfamide, lomustine, mechlorethamine, melphalan, oxaliplatin, temozolomide, thiotepa, trabectedin, nitrosurea, azacytidine, 5-fluorouracil (5-FU), 6-mercaptopurine (6-MP), capecitabine, cladribine, clofarabine, cytarabine (Ara-C), decitabine, floxuridine, fludarabine, gemcitabine,
- 30 hydroxyurea, methotrexate, nelarabine, pemetrexed, pentostatin, pralatrexate, thioguanine, trifluridine and tipiracil combination, daunorubicin, doxorubicin, doxorubicin liposomal, epirubicin, idarubicin, valrubicin, bleomycin, dactinomycin, mitomycin-c, mitoxantrone, irinotecan, irinotecan liposomal, topotecan, etoposide (VP-16), teniposide, taxanes, cabazitaxel, docetaxel, nab-paclitaxel, paclitaxel, vinca alkaloids, vinblastine, vincristine,

vincristine liposomal, vinorelbine, prednisone, methylprednisone, dexamethasone, all-trans retinoid acid, arsenic trioxide, asparaginase, eribulin, hydroxyurea, ixabepilone, mitotane, omacetaxine, pegaspargase, procarbazine, romidepsin, or vorinostat.

In some embodiments, the subject receives or is intended to receive radiation therapy.

- 5 In some embodiments, radiation therapy comprises external-beam radiation therapy. In some embodiments, radiation therapy comprises three-dimensional conformal radiation therapy (3D-CRT). In some embodiments, radiation therapy comprises intensity modulated radiation therapy (IMRT). In some embodiments, radiation therapy comprises proton beam therapy. In some embodiments, radiation therapy comprises image-guided radiation therapy (IGRT). In
- 10 some embodiments, radiation therapy comprises stereotactic radiation therapy (SRT). In some embodiments, radiation therapy comprises internal radiation therapy. In some embodiments, internal radiation therapy comprises a permanent implant. In some embodiments, internal radiation therapy comprises temporary internal radiation therapy. In some embodiments, radiation therapy comprises intraoperative radiation therapy (IORT). In
- 15 some embodiments, radiation therapy comprises system radiation therapy. In some embodiments, radiation therapy comprises radioimmunotherapy. In some embodiments, radiation therapy comprises radiosensitizers and radioprotectors. In some embodiments radiation therapy comprises neoadjuvant radiation therapy. In some embodiments, radiation therapy comprises adjuvant radiation therapy. In some embodiments, radiation therapy
- 20 comprises palliative radiation therapy.

Kits

- In some embodiments, kits for use in preventing, reducing or reversing adverse side effects due to chemotherapy or radiation therapy in a subject are disclosed. In some
- 25 embodiments, (a) one or more supplement units sufficient to provide to a subject in need thereof at least about 2 g of arachidonic acid triglyceride (AA TG) per day (2 g/d) for at least 7 days; and (b) instructions for preparation and consumption of the one or more supplement units. In some embodiments, (a) one or more supplement units sufficient to provide to a subject in need thereof at least about 2 g of at least one precursor of arachidonic acid (AA)
- 30 per day (2 g/d) for a sufficient time; and (b) instructions for preparation and consumption of the one or more supplement units.

In some embodiments, the number of supplement units to administer to a subject in need thereof is determined in consultation with a healthcare provider.

In some embodiments of the invention, the kit can include a preparation vial, a preparation diluent vial, AA TG, at least one precursor-AA TG, or AA TG and at least one precursor-AA TG and additional agent(s). The diluent vial contains a diluent such as an edible composition for diluting what could be a solution or powder (such as a concentrated solution or lyophilized powder) of AA TG, at least one precursor-AA TG, or AA TG and at least one precursor-AA TG. In some embodiments, the edible composition is a fruit or vegetable puree. In some embodiments the edible composition is a nutritional shake or the like.

In some embodiments, the instructions include instructions for mixing a particular amount of the diluent with a particular amount of the concentrated solution or lyophilized powder, whereby a final formulation for dosing is prepared. In some embodiments, the instructions include instructions for use in a syringe or other administration device. In some embodiments, the instructions include instructions for treating a patient with an effective amount of AA TG, at least one precursor-AA TG, or AA TG and at least one precursor-AA TG and an optional additional agent or agents. It also will be understood that the containers containing the preparations, whether the container is a bottle, a vial with a septum, an ampoule with a septum, sealed bottles of edible liquid, and the like, can contain indicia such as conventional markings which change color when the preparation has been autoclaved or otherwise sterilized.

In some embodiments, the kit is provided or sold as a bundled service with guidance, instructions, or recommendations from a healthcare provider for consuming one or more supplement units. In some embodiments, a healthcare provider or advisor is a physician, a nutritionist, a registered dietician, a physician's assistant, a nurse practitioner, or a nurse. In some embodiments, the healthcare provider is an oncologist or a surgeon. In some embodiments, the guidance, instructions, or recommendations comprise oral communications with a healthcare provider. In some embodiments, the guidance, instructions, or recommendations comprise written directions.

In some embodiments, kits comprising one or more supplement units and one or more food compositions are disclosed herein. In some embodiments, the kit further comprises instructions for consuming the AA TG, at least one precursor-AA TG, or AA TG and at least one precursor-AA TG and the one or more food compositions. In some embodiments, the supplement units of AA TG, at least one precursor-AA TG, or AA TG and at least one precursor-AA TG are packaged separately from the one or more food compositions. In some embodiments, the AA TG, at least one precursor-AA TG, or AA TG and at least one

precursor-AA TG are pre-mixed with the one or more food compositions in the one or more supplement units.

Supplement Unit

5 In some embodiments, supplement unit comprising AA TG, at least one precursor of AA (e.g., in TG form), or both AA TG and at least one precursor of AA (e.g., in TG form) for administering to a subject in need thereof is disclosed. In some embodiments, a supplement unit comprises AA TG. In some embodiments, a supplement unit comprises at least one precursor of AA in TG form (precursor-AA TG). In some embodiments, a supplement unit
10 comprises AA TG and at least one precursor of AA in TG form (precursor-AA TG). In some embodiments, one supplement unit is administered per day. In some embodiments, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more than 10 supplement units are administered per day to provide a subject in need thereof at least about 2 g of AA TG per day (2 g/d). In some embodiments, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more than 10 supplement units are administered per day to provide a subject
15 in need thereof at least about 2 g of at least one precursor of AA per day (2 g/d). In some embodiments, a supplement unit comprises an oil comprising AA TG and a pharmaceutically-acceptable excipient. In some embodiments, a supplement unit comprises an oil comprising at least one precursor-AA TG and a pharmaceutically-acceptable excipient.

 In some embodiments, a supplement unit is in the form of a liquid or a powder. In
20 some embodiments, a supplement unit is in the form of a pill or a capsule. In some embodiments, the capsule comprises a soft gelatin or is a softgel capsule. In some embodiments, the capsule is an enteric capsule. In some embodiments, the capsule allows for modified release of AA TG, at least one precursor-AA TG, or both AA TG and at least one precursor-AA TG. In some embodiments, the capsule allows for timed-release of AA TG, at
25 least one precursor-AA TG, or both AA TG and at least one precursor-AA TG.

 In some embodiments, one or more supplement units are in one container (e.g., bottle, package, etc.) or more than one container. In some embodiments, one supplement unit is housed in a plastic pocket of a blister pack. In some embodiments, the blister pack is backed with a paperboard card. In some embodiments, a blister pack includes 10 plastic pockets each
30 comprising one supplement unit. In some embodiments, one blister pack houses enough supplement units to provide a subject in need thereof at least 2 g of AA TG per day (2 g/d).

 In some embodiments, AA and precursors of AA TG are commercially available to one of ordinary skill in the art. Non-limiting examples of include AA from Cargill

(cargill.com/food-bev/na/arachidonic-acid), ARASCO™ as an oil from DSM, ARASCO® powder from DSM.

In some embodiments, one or more supplement units is consumed under medical supervision and intended for dietary management of a condition in a subject in need thereof.

- 5 In some embodiments, one or more supplement units are capable of being the sole source of nourishment for a subject in need thereof. In some embodiments, one or more supplement units are intended to or supplement the general diet of a subject in need thereof.

- 10 In some embodiments, a supplement unit is in the form of a syrup, a liquid, a powder, a concentrated powder, a concentrated powder admixed with a liquid, a swallowable form, a dissolvable form, an effervescent, a granulated form, or an oral liquid solution. In some embodiments, a supplement unit is formulated in any convenient form. In some embodiments, a supplement unit is in the form of a beverage, mayonnaise, salad dressing, margarine, low fat spread, dairy product, cheese spread, processed cheese, dairy dessert, flavored milk, cream, fermented milk product, cheese, butter, condensed milk product, ice
15 cream mix, soy product, pasteurized liquid egg, bakery product, confectionary product, confectionary bar, chocolate bar, high fat bar, liquid emulsion, spray-dried powder, freeze-dried powder, ultra-high-temperature (UHT) pudding, pasteurized pudding, gel, jelly, yogurt, or a food with a fat-based or water-containing filling.

- 20 In some embodiments, a supplement unit further comprises water, sucrose, maltodextrin, milk protein concentrate, soy oil, canola oil, short chain fructooligosaccharides, soy protein isolate, com syrup, sodium caseinate, and potassium citrate.

- In some embodiments, a supplement unit comprises a flavor, such as a natural flavor or an artificial flavor. In some embodiments, a flavor is apple, banana, blueberry, caramel, cherry, chocolate, cinnamon, coffee, cranberry, grape, honey, kiwi, lemon, lime, lemon-lime,
25 mango, mint, orange, peach, pineapple, raspberry, strawberry, tangerine, vanilla, or watermelon.

- In some embodiments, a supplement unit comprises additional source or sources of fat, such as an oil which is not an oil comprising a significant amount of AA TG. In some embodiments, an oil which does not comprise a significant amount of AA TG is an oil which
30 does not comprise more than 5% AA TG per total volume of oil. In some embodiments, the source or sources of fat promote energy metabolism. In some embodiments, the supplement unit comprises fat sources comprising one or more of saturated, mono-unsaturated, and poly-unsaturated fatty acids in proportions seen in a healthy diet for a subject.

In some embodiments, a supplement unit comprises about 3% or at least about 3% of an oil comprising about 40% AA TG. In some embodiments, a supplement unit comprises about 5% or at least about 5% of an oil comprising about 40% AA TG, about 10% or at least about 10% of an oil comprising about 40% AA TG, about 15% or at least about 15% of an oil comprising about 40% AA TG, or about 20% or at least about 20% of an oil comprising about 40% AA TG, or any range or combination thereof. In some embodiments percent AA oil is calculated as a weight/volume percentage. In some embodiments, percent AA oil is calculated as a weight/weight percentage. In some embodiments, percent AA oil is calculated as a volume/volume percentage.

10 In some embodiments, one or more supplement unit comprises about 5 g or at least about 5 g of an oil comprising about 40% AA TG. In some embodiments, a supplement unit comprises about 10 g or at least about 10 g of an oil comprising about 40% AA TG, about 30 g or at least about 30 g of an oil comprising about 40% AA TG, about 40 g or at least about 40 g of an oil comprising about 40% AA TG, about 50 g or at least about 50 g of an oil comprising about 40% AA TG.

In some embodiments, one supplement unit comprises 50 mg of AA TG, 100 mg of AA TG, 200 mg of AA TG, 300 mg of AA TG, 400 mg of AA TG, 500 mg of AA TG, 1 g of AA TG, 2 g of AA TG, 4 g of AA TG, 5 g of AA TG, 10 g of AA TG, 15 g of AA TG, 20 g of AA TG, or any ranges or combinations thereof. In some embodiments, one supplement unit comprises no more than 50 mg of AA TG, 100 mg of AA TG, 200 mg of AA TG, 300 mg of AA TG, 400 mg of AA TG, 500 mg of AA TG, 1 g of AA TG, 2 g of AA TG, 4 g of AA TG, 5 g of AA TG, 10 g of AA TG, 15 g of AA TG, 20 g of AA TG. In some embodiments, one supplement unit comprises at least 50 mg of AA TG, 100 mg of AA TG, 200 mg of AA TG, 300 mg of AA TG, 400 mg of AA TG, 500 mg of AA TG, 1 g of AA TG, 2 g of AA TG, 4 g of AA TG, 5 g of AA TG, 10 g of AA TG, 15 g of AA TG, 20 g of AA TG.

In some embodiments, one supplement unit comprises 50 mg of at least one precursor-AA TG, 100 mg of at least one precursor-AA TG, 200 mg of at least one precursor-AA TG, 300 mg of at least one precursor-AA TG, 400 mg of at least one precursor-AA TG, 500 mg of at least one precursor-AA TG, 1 g of at least one precursor-AA TG, 2 g of at least one precursor-AA TG, 4 g of at least one precursor-AA TG, 5 g of at least one precursor-AA TG, 10 g of at least one precursor-AA TG, 15 g of at least one precursor-AA TG, 20 g of at least one precursor-AA TG, or any ranges or combinations thereof. In some embodiments, one supplement unit comprises no more than 50 mg of at least one precursor-AA TG, 100 mg

of at least one precursor-AA TG, 200 mg of at least one precursor-AA TG, 300 mg of at least one precursor-AA TG, 400 mg of at least one precursor-AA TG, 500 mg of at least one precursor-AA TG, 1 g of at least one precursor-AA TG, 2 g of at least one precursor-AA TG, 4 g of at least one precursor-AA TG, 5 g of at least one precursor-AA TG, 10 g of at least one precursor-AA TG, 15 g of at least one precursor-AA TG, 20 g of at least one precursor-AA TG. In some embodiments, one supplement unit comprises at least 50 mg of at least one precursor-AA TG, 100 mg of at least one precursor-AA TG, 200 mg of at least one precursor-AA TG, 300 mg of at least one precursor-AA TG, 400 mg of at least one precursor-AA TG, 500 mg of at least one precursor-AA TG, 1 g of at least one precursor-AA TG, 2 g of at least one precursor-AA TG, 4 g of at least one precursor-AA TG, 5 g of at least one precursor-AA TG, 10 g of at least one precursor-AA TG, 15 g of at least one precursor-AA TG, 20 g of at least one precursor-AA TG.

EXAMPLES

15

Example 1: Fatty acid (FA) screen in mouse and human organoids may reveal omega-6 FAs as promoters of stemness

The present disclosure provides an account of how diverse dietary fatty acids (FAs) influence intestinal stem cell (ISC) function. Through a screen of FAs in mouse and human intestinal organoids, the present disclosure characterized a subset of omega-6 family FAs, including but not limited to, arachidonic acid (AA), with robust stemness-enhancing effects. For instance, cross-species gene expression analysis revealed induction of conserved repair-associated stem cell reprogramming signatures in response to AA. Furthermore, single-cell RNA sequencing (scRNA-seq) was used to identify AA-induced *de novo* stem cell states and dedifferentiate program *in vivo* and *in vitro*. Without wishing to be bound by theory, it is believed that dietary AA (e.g., AA triglyceride) engenders production of epithelial prostaglandin E₂ (PGE₂), which activates Ptger4 – cAMP – PKA signaling axis to promote stemness in mice and humans. AA (e.g., AA triglyceride) may evoke epigenetic reprogramming around stem cell regeneration-associated genes in a Ptger4-dependent manner. The data provided herein demonstrate that dietary AA (e.g., AA triglyceride) is a conserved promoter of stem cell regeneration that mimics the repair-response to tissue injury through PGE₂-Ptger4 signaling and downstream epigenetic reprogramming.

30

ISCs may undergo frequent symmetric cell divisions to replenish the intestinal epithelium, and are one of the most regenerative tissues in mammals that is composed of

single layer of cells with absorptive, secretory and barrier functions (Barker et al., 2007; Cheng and Leblond, 1974; Leblond and Stevens, 1948; Snippert et al., 2010). The progeny of dividing ISCs gave rise to transit amplifying (TA) progenitors that proliferate and differentiate to various lineages of the intestinal epithelium including absorptive enterocytes and secretory cells such as mucus-producing goblet cells, hormone-secreting enteroendocrine (EE) cells, chemosensory tuft cells and Paneth cells (Bankaitis et al., 2018; Clevers, 2013). Self-renewal and differentiation of ISCs may be tightly regulated by niche-derived signals such as ligands, growth factors and cytokines emanating from neighboring Paneth cells (Sato et al., 2011), fibroblasts (Degirmenci et al., 2018; Greicius et al., 2018; Roulis et al., 2020; Shoshkes-Carmel et al., 2018), enteric glia (Van Landeghem et al., 2011) and immune cells (Beyaz et al., 2021a; Biton et al., 2018; Lindemans et al., 2015) around the intestinal crypt (Clevers, 2013). Homeostatic regeneration of intestinal epithelium could be sustained by ISCs expressing leucine-rich repeat, containing G protein coupled receptor 5 (*Lgr5*) both *in vivo* and in clonogenic organoid cultures *in vitro* (Barker et al., 2007; Sato et al., 2009).

When *Lgr5*+ stem cell compartment is damaged, is it possible for the plasticity and dedifferentiation of multiple epithelial lineages to enable effective regeneration and repair of the intestinal epithelium (Clevers, 2013; de Sousa and de Sauvage, 2019; Potten et al., 1978; Tian et al., 2011). Secretory progenitors, EE progenitors, TA progenitors, Paneth cells and enterocyte progenitors are among lineages that have been known to dedifferentiate and acquire stem cell potential in response to intestinal damage by irradiation, infection, chemotherapy or depletion of *Lgr5*+ ISCs using genetic models (Asfaha et al., 2015; Buczacki et al., 2013; Jadhav et al., 2017; Nusse et al., 2018; Schmitt et al., 2018; Tetteh et al., 2016; Tian et al., 2011; van Es et al., 2012; von Moltke et al., 2016; Yan et al., 2017; Yu et al., 2018). Without wishing to be bound by theory, the proposed mechanisms for stem cell reprogramming in response to tissue damage include maintenance of an accessible chromatin, induction of a fetal-like gene expression program, cytokine signaling and Notch signaling (Ayyaz et al., 2019; Gregorieff et al., 2015; Jadhav et al., 2017; Murata et al., 2020; Nusse et al., 2018; Yu et al., 2018; Yui et al., 2018).

Accumulating evidence posits that nutrients and metabolic pathways not only affect growth and proliferation, but could also significantly influence cellular function and fate by signaling to transcription factors and altering epigenetic landscapes (Beyaz et al., 2016; Beyaz et al., 2021b; Beyaz and Yilmaz, 2016; Chandel et al., 2016; Chen et al., 2020; Cimmino et al., 2018; Lu and Thompson, 2012). While recent studies started to explore metabolic regulation of ISC activity through fatty acid (FA) oxidation (Chen et al., 2020;

Mihaylova et al., 2018; Stine et al., 2019), ketone body signaling (Cheng et al., 2019), mitochondrial pyruvate metabolism (Rodriguez-Colman et al., 2017; Schell et al., 2017), vitamins (Jijon et al., 2018; Lukonin et al., 2020; Peregrina et al., 2015) and microbiome-derived metabolites (Kaiko et al., 2016; Lee et al., 2018), the present disclosure provides how nutrients and their metabolite derivatives influence ISC activity and cellular plasticity in the intestine by evoking epigenetic alterations. Different dietary interventions that perturb organismal metabolism [e.g., fasting, calorie restriction, ketogenic or pro-obesity high fat diets (HFD)] converge on the ability to enhance ISC activity through cell-intrinsic (Beyaz et al., 2016; Cheng et al., 2019; Fu et al., 2019; Mihaylova et al., 2018; Wang et al., 2018) and niche-mediated extrinsic (Igarashi and Guarente, 2016; Yilmaz et al., 2012) mechanisms. One of the common features of these stemness-enhancing dietary interventions has been that they can increase the abundance and metabolism of FAs either through dietary intake or release from adipose tissue (Novak et al., 2021). It is possible that fasting and a long-term lard-based pro-obesity HFD could partly enhance ISC function through activating FA metabolism (Beyaz et al., 2016; Beyaz et al., 2021b; Mihaylova et al., 2018).

There are several possible mechanisms that FAs can regulate stem cell fate. First, FAs or their metabolites could bind and activate FA-sensing transcription factors (TFs) such as PPAR- δ to regulate transcription directly (Beyaz et al., 2016; Beyaz et al., 2021b; Evans and Mangelsdorf, 2014; Neels and Grimaldi, 2014). Second, FA-derived metabolites such as acetyl-coA could be utilized for histone modifications and could influence epigenetic states (McDonnell et al., 2016; Schwartzman et al., 2018). Third, alterations in cellular FA abundance could perturb membrane lipid composition and influence signaling pathways (Zhu et al., 2019). Fourth, bioactive lipids generated from FAs could activate G-protein coupled receptors (GPCRs) that stimulate second messengers with ability to signal to downstream cascades and various TFs that regulate cell fate and function (Brash, 2001). Lastly, FAs could perturb microbiome and immune cells that influence ISC activity (Beyaz et al., 2021a; Biton et al., 2018). While these findings suggest that FAs and their metabolism may be linked to stem cell fate and function, it is unclear how diverse types of dietary FAs influence stemness and epigenetic regulation of gene expression in the intestinal epithelium.

It is possible for intestinal organoids to recapitulate the compositional and functional features of the mammalian intestine, including stem cell regeneration and differentiation in culture, and therefore, offer a reliable system to identify factors that promote stemness (Beyaz et al., 2016; Kaiko et al., 2016; Lukonin et al., 2020; Sato et al., 2009). To explore how diverse dietary FAs influence intestinal stemness, a live imaging screening platform that

monitored organoid formation starting from single cells and measured features that informed about stem cell activity, including organoid morphology (spheroid vs. branched), size and numbers, for five days was developed (Beyaz et al., 2016; Farin et al., 2012; Mustata et al., 2013; Schuijers et al., 2015) (Figure 8A, see Example 9). A panel of 23 FAs were assembled and stratified by saturation (polyunsaturated, monounsaturated and saturated), by double bond position (omega-3, omega-6, omega-7 and omega-9), by configuration (*cis*, *trans*), by chain length (short, medium, long) and by number of double bonds (1-6) (FIG. 1A, Table 1).

Table 1: Fatty acids that are used in screening in mouse and human organoids.

Name	Carbon Number	Position	Double Bonds	Saturation Status	Cis/Trans	Omega
Butyric	C3	n-0	0	Saturated	NA	NA
Lauric	C12	n-0	0	Saturated	NA	NA
Myristic	C14	n-0	0	Saturated	NA	NA
Pentadecanoic	C15	n-0	0	Saturated	NA	NA
Palmitic	C16	n-0	0	Saturated	NA	NA
Heptadecanoic	C17	n-0	0	Saturated	NA	NA
Stearic	C18	n-0	0	Saturated	NA	NA
Nonadecanoic	C19	n-0	0	Saturated	NA	NA
Palmitoleic	C16	n-7	1	Monounsaturated	Cis	Omega-7
Oleic	C18	n-9	1	Monounsaturated	Cis	Omega-9
Elaidic	C18	n-9	1	Monounsaturated	Trans	Omega-9
Paullinic	C20	n-7	1	Monounsaturated	Cis	Omega-7
alpha-Linoleic	C18	n-3	3	Polyunsaturated	Cis	Omega-3
Eicosapentaenoic	C20	n-3	5	Polyunsaturated	Cis	Omega-3
Linoleic	C18	n-6	2	Polyunsaturated	Cis	Omega-6
gamma-Linolenic	C18	n-6	3	Polyunsaturated	Cis	Omega-6
Dihomo-gamma-linolenic	C20	n-6	3	Polyunsaturated	Cis	Omega-6
Arachidonic	C20	n-6	4	Polyunsaturated	Cis	Omega-6
Docosatetraenoic	C22	n-6	4	Polyunsaturated	Cis	Omega-6
Linoelaidic	C18	n-6	2	Polyunsaturated	Trans	Omega-6
Eicosatetraenoic	C20	n-3	4	Polyunsaturated	Cis	Omega-3
Stearidonic	C18	n-3	4	Polyunsaturated	Cis	Omega-3
Docosahexaenoic	C22	n-3	6	Polyunsaturated	Cis	Omega-3

Since most FAs in the body are bound to serum albumin to enhance transport and solubility, poorly soluble FAs were conjugated to bovine serum albumin (BSA) (Beyaz et al., 2016; Brash, 2001; McArthur et al., 1999; Spector et al., 1969; Zhu et al., 2019) (see Example 9). Dose response experiments were performed to define FA concentrations that do not induce lipotoxicity in organoids (Alsabeeh et al., 2018; Brash, 2001) (FIG. 8B). The FA

screen in mouse intestinal organoid-derived single cells revealed that treatment with FAs (belonging to omega-6 family such as linoleic acid (LA) γ -linolenic acid (γ -LA), dihomo- γ -linolenic acid (dh- γ -LA) and arachidonic acid (AA) but not docosatetraenoic (DA) or the trans FA linoelaidic acid (LEA)) could promote the formation of spheroids that lack budding crypt-like domains (FIGs. 1B and 8C). This spheroid morphology could be correlated with an enhanced regenerative stem cell state and reduced differentiation (Beyaz et al., 2016; Beyaz et al., 2021b; Farin et al., 2012; Mustata et al., 2013; Schuijers et al., 2015). Treatment with these FAs also led to significant increase in size relative to vehicle-treated control organoids, starting around after 72 hours, which was the approximate duration for symmetry breaking in single-cell derived organoids (Serra et al., 2019) (FIGs. 1C and 8C). To assess the human relevance of these results, human patient-derived organoids (PDOs) that are generated from normal part of the colons were utilized and obtained from male or female patients, across diverse ancestries (Table 2) and performed FA screen.

Table 2: Patient information for patient-derived organoids used in study.

Patient Code	Sex	Age	BMI	
P1	Female	51	27	Asian
P2	Male	50	26	White
P3	Female	89	34	Hispanic
P4	Male	77	23	White
P5	Female	66	31	Black
P6	Female	64	31	White
P7	Male	31	23	White

Similar to the screen in mice, it is possible for the treatment of dissociated single cells from human PDOs with a subset of omega-6 family FAs including dh- γ -LA and AA to promote growth relative to control organoids (FIG. 1D and 8D). FA elongases (*Elovl5*) and desaturases (*Fads1* and *Fads2*) that regulate AA biosynthesis from essential FAs (Fan et al., 2012; Moon et al., 2009) can be expressed abundantly in both mouse and human organoids (FIGs. 8E-8F). Given DA does not promote organoid growth, it was hypothesized that stemness-enhancing effects of omega-6 FAs could converge on AA. Indeed, inhibiting FADS1, the rate limiting desaturase for AA biosynthesis (Fan et al., 2012) blunted the increase in organoid size in response to omega-6 FAs (FIG. 8G). For these reasons, AA were selected as targets for further functional assessment of stem cell regeneration. It was determined that AA treatment may lead to more proliferating cells in spheroids that lack crypt domains and have larger size relative to vehicle-treated control organoids (FIGs. 1E-1J).

Transmission electron microscopy analysis showed that these AA-induced spheroids had smaller microvilli and less Paneth cells with granules, which suggested a reduction in differentiation (Crawley et al., 2014; Miyoshi et al., 2017) (FIGs. 8H-8I). Further, subculturing experiments were performed to functionally assess stem cell activity after AA treatment (Beyaz et al., 2016). When sub-cultured, primary mouse AA-induced spheroids gave rise to more secondary organoids that were again larger in size with spheroid morphology relative to controls (FIGs. 1K-1N). Similarly, AA treatment increased the organoid size and promoted spheroid formation in human intestinal PDOs both in primary cultures and in secondary subcultures (FIGs. 1O-1T). These results indicated that omega-6 FAs that converged on AA enhanced the stemness of mouse and human organoids.

Example 2: AA-rich diet (ARD) may promote intestinal regeneration in vivo

AA is a bioactive lipid that could play essential structural and functional roles in mammalian cells and tissues, including intestinal epithelium (Brash, 2001; Fan et al., 2016; Fan et al., 2012). The functional significance of dietary AA supplementation in intestinal homeostasis was explored in Calder et al.(2019). To study the impact of AA on ISC function *in vivo*, a new isocaloric (3.8 kcal/g) AA-rich diet (ARD) model (Teklad, TD190641) was developed with a matched purified control (control) diet (Teklad, TD97184) (FIG. 2A). Oil extracted from the fungus *M. alpina* was utilized and the oil contained approximately 40% AA in the form of triglycerides (Kikukawa et al., 2018) needed to formulate a 3% AA-rich oil and 4% soybean oil containing diet (7% total fat). The ARD and its matched isocaloric control were composed of equal amounts of major nutrients (protein, carbohydrate and fat) and minor nutrients (minerals and vitamins) (Table 3).

Table 3: Composition of isocaloric AA-rich diet and its matched purified control diet.

	Matched Purified Control Diet		AA-rich Diet	
	% by weight	% kcal from	% by weight	% kcal from
Protein	17.7	18.8	17.7	18.8
Carbohydrate	60.1	63.9	60.1	63.9
Fat	7.2	17.2	7.2	17.2
Kcal/g	3.8		3.8	

Formula	Matched Purified Control Diet g/Kg	AA-rich Diet g/Kg
Casein	200	200

L-Cystine	3	3
Corn Starch	392.234	392.184
Maltodextrin	132	132
Sucrose	100	100
Soybean Oil	70	40
Arachidonic Acid Oil	0	30
Cellulose	50	50
Mineral Mix, AIN-93G-MX (94046)	35	35
Vitamin Mix-AIN-93-VX (94047)	15	15
Choline Bitartrate	2.75	2.75
Vitamin K1, phyloquinone	0.002	0.002
TBHQ, antioxidant	0.014	0.014
Orange Food Color	0	0.05

Feeding mice with ARD for four weeks did not affect their body weight or plasma glucose levels, but led to elevated abundance of AA in both plasma and intestine relative to controls (FIGs. 2B-2C and 9A-9B). Furthermore, metabolomics analysis revealed that there were no significant alterations in the quantity of other major metabolites in the intestine of mice fed with ARD (FIG. 9C). This elevation of AA abundance in the intestine resulted in increased crypt length and number of Ki67+ proliferating cells (Gerdes et al., 1984) per crypt (FIGs. 2D-2G). Intestinal crypts were isolated from mice that were fed ARD or control diet, to perform functional organoid assays. Intestinal organoid morphology and organoid forming capacity was assessed and are established proxies for stem cell activity (Beumer and Clevers, 2016; Beyaz et al., 2016; Mustata et al., 2013; Nusse et al., 2018; Sato et al., 2009; Yui et al., 2018). ARD promoted the formation of regenerative spheroid morphology and engendered a significant reduction in the crypt domains per organoid relative to controls (FIGs. 2H-2J). Sorted Epcam+ intestinal epithelial cells from crypts of ARD fed mice gave rise to more organoids than relative to controls and suggested that dietary AA promoted stem cell activity *in vivo* under homeostatic conditions (FIGs. 2K-2L).

Intestinal epithelium exhibited a rapid regenerative response upon multitude of stressors to maintain tissue function and barrier integrity (Bankaitis et al., 2018; Gehart and Clevers, 2019). Ionizing radiation is frequently used to assess stem cell regeneration after intestinal injury (Beyaz et al., 2016; Potten, 1977; Withers and Elkind, 1970). Administration of a clinically relevant 15Gy γ -irradiation to control mice induced cytotoxicity in intestinal epithelium that led to crypt loss and reduction in intestinal length (Beyaz et al., 2016; Kirsch et al., 2010) (FIGs. 2M-2O and 9D). Feeding mice with ARD reversed these effects and

enhanced crypt regeneration, as evidenced by the increase in proliferating cells that have incorporated 5-ethynyl-2'-deoxyuridine (EdU) (Salic and Mitchison, 2008) per unit area of intestine, and per crypt relative to controls (FIGs. 2P-2R and 9E). Since cancer chemotherapeutic agents elicited a similar cytotoxic effect, it was uncertain whether ARD could promote intestinal regeneration in a separate injury model that utilized doxorubicin, a commonly used anti-tumor drug with well characterized adverse effects in the intestine (Dekaney et al., 2009; Ijiri and Potten, 1987). Doxorubicin resulted in a significant decrease in intestinal length 72 hours after treatment in mice fed with control diet. ARD prevented shortening of the intestine and increased the numbers of EdU+ proliferating crypt cells in mice treated with doxorubicin (FIGs. 2S and 9F-9H). Consistent with the assessment of proliferation using Ki67 (FIG. 2F), crypts contained more EdU+ proliferating cells in uninjured ARD fed mice relative to uninjured controls for both models (FIGs. 2Q and 9G). Collectively, the data illustrated that dietary elevation of AA in the intestine may augment ISC regeneration *in vivo*.

Example 3: AA may evoke a conserved stem cell reprogramming gene expression signature

Intestinal organoids can recapitulate the regenerative features of intestinal epithelium (Beyaz et al., 2016; Sato et al., 2009; Serra et al., 2019). To elucidate the mechanisms of how AA enhances stemness, time-kinetics bulk RNA-seq analysis was performed across different mouse organoid development stages, including formation of symmetrical cyst (Day 1), symmetry breaking (Day3) and grown organoids with differentiated cells (Day 6) (Beyaz et al., 2016; Sato et al., 2009; Serra et al., 2019) (FIGs. 10A-10B). AA treatment led to strong upregulation of genes that were associated with stem cell reprogramming in response to ablation of Lgr5+ stem cells (*Lypd6*, *Ctgf*, *Anxa13*) (Murata et al., 2020), radiation injury (*Ccnd1*, *Anxa3*, *Ly6a*, *Clu*) (Ayyaz et al., 2019) and granuloma (*Il33*, *Sl00a6*, *Areg*) (Nusse et al., 2018), or a regenerative fetal-like state (fetal spheroids) (*Cd55*, *Ereg*, *Myof*, *Msln*) (Mustata et al., 2013) across time points (FIGs. 3A-3B). In contrast, organoids downregulated the markers of differentiated cells such as Paneth cells (*Defa24*, *Defa21*, *Lyz1*), tuft cells (*Dclk1*) and enteroendocrine cells (*Neurog3*) in response to AA (Haber et al., 2017) (FIG. 3B). Interestingly, the expression of *Lgr5* and homeostatic stem cell signature (Haber et al., 2017; Munoz et al., 2012) were initially suppressed on Day 1 and 3 but were restored by Day 6 in AA-treated organoids (FIGs. 3A-3B). All of the previously reported signatures of reprogrammed stem cells significantly overlapped with AA-induced genes, which suggested that AA may evoke a gene expression signature that could be central to stem cell regeneration

across different experimental models (FIGs. 10C-10D). An AA-induced signature gene list was devised and included the relevant stem cell reprogramming genes (*Ly6a*, *S100a6*, *Ccnd1*, *Cd55*, *Msln*) (Ayyaz et al., 2019; Mustata et al., 2013; Nusse et al., 2018) and the CREB target *Nr4a1* (Rodon et al., 2019), and confirmed their induction by qRT-PCR in AA-treated organoids (FIG. 10E).

Gene set enrichment analysis (GSEA) of AA-induced genes revealed enrichment for wound healing, cell proliferation, PPAR pathway and lipid metabolism and calcium signaling. In addition, GSEA revealed the key regulators of intestinal stemness during homeostasis and regeneration in response to injury, such as Wnt/ β -catenin and Egfr pathways (Beumer and Clevers, 2016) (FIG. 1F). Thus, the present disclosure assessed whether these pathways were functionally involved in AA-induced stemness phenotype. First, a subset of Wnt/ β -catenin targets (including stem cell reprogramming signature genes such as *S100a6* (Ayyaz et al., 2019; Mustata et al., 2013; Nusse et al., 2018) and *Ccnd1* (Ayyaz et al., 2019; Mustata et al., 2013)) were upregulated in AA-treated organoids (FIG. 10G) with a concomitant increase in nuclear localization of β -catenin, which can be a proxy for its activity (Molenaar et al., 1996) (FIG. 3C). Titration of exogenous Wnt3a (Beyaz et al., 2016) showed that AA treatment may have reduced Wnt dependency for organoid formation and growth (FIGs. 3D-3F). Second, AA enhanced the expression of Egf family receptor *Egfr* and its ligands such as *Areg* and *Ereg*, which are among stem cell reprogramming signature genes (Mustata et al., 2013; Nusse et al., 2018) and were predominantly produced by stromal cells to support epithelial repair after injury in a paracrine manner (Gregorieff et al., 2015; Lee et al., 2004; Monticelli et al., 2015; Shao and Sheng, 2010; Van Landeghem et al., 2011; Yang et al., 2017) (FIGs. 3B and 3G). This was the prompt to test whether AA-induced autocrine Egfr ligand expression contributed to enhanced stemness. The present disclosure found that replacing Egf, an essential component of organoid media (Basak et al., 2017; Oszvald et al., 2020; Sato et al., 2009) with Areg or Ereg could be sufficient to establish organoids from dissociated single cells (FIGs. 10H-10I). Treatment with AA significantly boosted organoid growth in the absence of Egf, which was necessary for stem cell proliferation (Basak et al., 2017; Biteau and Jasper, 2011; Jiang and Edgar, 2009) (FIGs. 3H-3I). These findings suggested that AA-induced transcriptional reprogramming may reduce dependency to niche-derived exogenous Wnt and Egf signals and may promote stemness.

To corroborate these observations in humans, human PDOs were utilized and gene expression alterations in response to AA was assessed (FIG. 10J). GSEA demonstrated that AA-induced genes may be enriched for both homeostatic and repair-associated stemness

signatures, as well as Myc pathway and cell proliferation (FIGs. 3J and 10K). Similar to mice, genes that associate with stem cell reprogramming (CD55, MYOF, MSLN, ANXA3, AREG, CCND1, LYPD6) (Ayyaz et al., 2019; Murata et al., 2020; Mustata et al., 2013; Nusse et al., 2018), Wnt/ β -catenin targets (L1CAM, TCF4, CCND1) (Beyaz et al., 2016),
 5 Egfr ligands (AREG) (Monticelli et al., 2015), and CREB targets (NR4A1, SIK1) (Rodon et al., 2019) were robustly upregulated in AA-treated human PDOs (FIGs. 3K-3L). Altogether, these results highlighted that AA may elicit a conserved stem cell regeneration program that is in part reminiscent of the repair response to injury.

10 **Example 4: Single cell analysis of AA-induced stemness *in vivo* and *in vitro***

To determine the precise cellular states that define AA-induced stemness *in vivo*, single-cell RNA sequencing was performed (scRNA-seq). 23,161 single cells from crypts were profiled after they were filtered and clustered to define the intestinal epithelial cell types (Ayyaz et al., 2019; Grun et al., 2015; Haber et al., 2017) (FIGs. 11A-11C). Dietary AA
 15 engendered a *de novo* stem-like cluster (Stem 2) *in vivo* that was not present in control crypts, and was characterized by the high expression of stem cell reprogramming-associated markers *Ly6a* and *S100a6* (Ayyaz et al., 2019; Mustata et al., 2013; Nusse et al., 2018) (FIGs. 4A-4B and 11A-11F), but not other putative stem cell markers such as *Clu* and *Msi1* (Ayyaz et al., 2019; Wang et al., 2020). *S100a6* and *Ly6a* represent the two AA-induced genes that were
 20 shared among fetal, radiation-induced and granuloma-induced repair signatures (FIG. 10C). In addition, ARD led to upregulation of the stem cell marker genes *Lgr5* and *Ascl2* in transit amplifying (TA) cells, enterocyte progenitors (EP), enteroendocrine cells (EE) and goblet cells, but not in homeostatic stem cells (Stem 1) *in vivo* (FIGs. 4C-4D). TA, EP and EE cells have been shown to dedifferentiate and regenerate intestinal crypt in response to crypt
 25 damage (Jadhav et al., 2017; Nusse et al., 2018; Tetteh et al., 2016; Tian et al., 2011; Yan et al., 2017). *Ascl2*, a stem cell restricted Wnt/ β -catenin target (Schuijers et al., 2015; van der Flier et al., 2009), has recently been shown to orchestrate stem cell regeneration upon injury through dedifferentiation of enterocyte and secretory progenitors (Murata et al., 2020). Consistent with this, the pseudotime trajectory analysis algorithm was used (Cao et al., 2019),
 30 and the present disclosure found a dedifferentiation trajectory through TA, EP and EE towards the AA-induced stem 2 cluster *in vivo* (FIGs. 4E-4F). Crypt cells from ARD mice exhibited elevated expression of *Lgr5*, *Ascl2* and reprogramming associated markers *Ly6a* and *S100a6* in the differentiation pseudotime trajectory relative to controls (FIGs. 4G and 11E). Upregulation of *Ascl2* in upper crypt cells was a hallmark of intestinal regeneration in

response to crypt damage before their dedifferentiation to stem cells (Murata et al., 2020). To assess how dietary AA affects the spatial expression of these key stemness genes, single molecule fluorescent in situ hybridization (sm-FISH) was performed. Mice fed with ARD expressed higher levels of *Lgr5* and *Ascl2* per crypt, with increased frequency of the cells expressing these markers in upper crypt tiers at steady state (FIGs. 4H-4M and 11G-11H). Consistent with the observation that ARD mice could exhibit enhanced intestinal regeneration *in vivo* after irradiation relative to controls (FIGs. 3A-3L), it was determined that dietary AA may elevate *Lgr5* and *Ascl2* expression in regenerating crypts in response to irradiation. Regenerating crypts in irradiated ARD mice contained higher percentage of cells expressing *Lgr5* and *Ascl2* in upper crypt tiers relative to irradiated controls (FIG. 4H-4M and 11G-11H). In addition, dietary AA boosted the expression of the repair-associated stem cell signature genes, such as *S100a6* in crypt cells both at steady state and in response to irradiation (FIGs. 4N-4P and 11I). Collectively, these results suggested that dietary AA may promote stemness in the intestine by inducing regeneration-associated *de novo* stem cell states and dedifferentiation program *in vivo*.

To assess whether dietary AA evokes stem cell reprogramming through an epithelial-intrinsic mechanism, 23,599 single cells from organoids by scRNA-seq were profiled (FIGs. 12A-12C). In organoid cultures, AA treatment for three days led to emergence of *de novo* stem-like states (Stem 2 and Stem 3) that were marked by stem cell reprogramming associated signature genes such as *Ly6a*, *Clu* and *S100a6* (FIGs. 12A-12G and 12K). Furthermore, pseudotime analysis of scRNA-seq from organoids unveiled a dedifferentiation trajectory towards *de novo* stem-like states and upregulation of *Ly6a* and *S100a6* across pseudotime trajectory (FIGs. 12L-12R). AA-treated organoids showed increased *Ascl2* expression in stem/progenitor cells, enterocytes and EE cells (FIG. 12H), and in pseudotime trajectory (FIG. 12S) relative to controls. Likely due to *in vitro* properties of organoid culture or temporal aspects (Lukonin et al., 2020; Sato et al., 2009; Serra et al., 2019; Yui et al., 2018), there were some different features between crypts and organoids, such as a reduction in *Lgr5* expression across stem and progenitor cell clusters and pseudotime trajectory (FIGs. 12I and 12T), consistent with the bulk RNA-seq data for day three organoids (FIG. 3B). In contrast to a recent report (Wang et al., 2020), induction of *Msi1* in response to AA was not observed *in vivo* or *in vitro* (FIGs. 11D and 12J). Overall, these results suggested that AA-treated organoids exhibited, by and large, a similar profile to crypts from ARD mice, which suggested an epithelial-intrinsic mechanism as the likely driver for the AA-induced stemness phenotype.

Example 5: AA metabolism to prostaglandin E2 (PGE2) may be necessary and sufficient to promote stem cell reprogramming

AA exerts its biological activities through several mechanisms such as by regulating
5 membrane fluidity, ion channels, levels of reactive oxygen species, lipid-sensing receptors
(such as PPARs) and producing multitude of bioactive lipids by non-enzymatic and
enzymatic breakdown, especially in response to tissue damage (Brash, 2001). The data
indicated that dietary AA may promote stemness and evoke repair-associated stem cell
10 reprogramming signatures in the absence of any apparent damage to intestinal epithelium
(FIGs. 2A-4P). Because AA and AA-derived metabolites can be conserved regulators of
wound detection and tissue repair (Fan et al., 2014; Katikaneni et al., 2020; Miyoshi et al.,
2017), the present disclosure sought to assess the alterations in AA metabolism in our
models. Metabolomics analysis revealed that AA treatment may have led to increased
production of bioactive lipid mediators in organoids *in vitro* (FIG. 5A) and crypts *in vivo*
15 (FIG. 13B). To determine whether AA-derived metabolites could be sufficient to promote
stem cell activity, a screen in mouse organoids was performed and found that prostaglandin
E₂ (PGE₂) and to lesser extent, PGD₂, recapitulated AA-induced stemness (FIGs. 5B and
13C). Inhibiting prostaglandin production using non-steroidal anti-inflammatory drug
(NSAID) celecoxib (FIGs. 5C-5D) or indomethacin (FIGs. 13D-13E) blunted AA-induced
20 stemness in organoid assays, which suggested that epithelial PGE₂ production may be
necessary for stem cell enhancing effects of AA.

Paracrine PGE₂ production in the intestine contributes to wound repair (Miyoshi et
al., 2017; Roulis et al., 2014) and carcinogenesis (Roulis et al., 2020; Wang and DuBois,
2018) but little is known about the dietary regulation of PGE₂ signaling in epithelial cells to
25 modulate stem cell function. In addition to AA-treated organoids, the present disclosure
confirmed PGE₂ production by sorted Epcam⁺ crypt cells in response to AA (FIG. 13F).
Furthermore, AA treatment resulted in an adaptive upregulation of enzymes that regulated
prostaglandin production in organoids such as *Ptges* and *Ptgs2* (FIGs. 13A and 13G). Single
cell analysis of AA-treated organoids demonstrated an adaptive induction of *Ptges* expression
30 in stem cells, progenitor cells and enterocytes, and highlighted the likely epithelial sources
for PGE₂ in response to AA (FIG. 13H). PGE₂ treatment in organoids evoked a gene
expression program that was concordant with the AA-induced stem cell reprogramming
signature in both mouse (FIG. 5E) and human (FIG. 5F) organoids. Furthermore, scRNA-seq
of mouse organoids in response to PGE₂ treatment revealed stem cell reprogramming

features similar to what was observed in AA-treated organoids, such as the emergence of *de novo* stem-like states that are marked by reprogramming-associated genes (FIGs. 5G-5I and 13I-13L), and upregulation of *Ascl2* in stem/progenitor cells (FIG. 5J). Similar to AA, PGE2 evoked a dedifferentiation trajectory with upregulation of reprogramming-associated genes
5 *Ascl2*, *S100a6*, *Ly6a*, but not *Lgr5* in organoids (FIGs. 5K-5M and 13M-13N). These data indicated that stemness enhancing effects of dietary AA may be mediated by PGE2 signaling in both mice and humans.

**Example 6: *Ptger4* – cAMP – PKA signaling axis may regulate AA-induced stemness in
10 mice and humans**

PGE2 binds to four G-protein coupled receptors (Ptger1-4) and activates diverse downstream pathways to mediate different functions (Breyer et al., 2001; Narumiya et al., 1999). To determine which PGE2 receptor subtype is necessary for AA-induced stemness, pharmacological inhibitors were screened for each receptor in organoid assays and found that
15 inhibition of Ptger4, but not other PGE2 receptors, may dampen AA-induced stemness (FIGs. 14A-14E). Ptger4 was highly expressed in both mouse and human organoids (FIGs. 14G-14H). Treatment with AA boosted Ptger4 (but did not boost other PGE2 receptor expression in secretory progenitors), TA cells and *de novo* stem cells (Stem 2 and Stem 3) (FIGs. 14I-14L). Further, Ptger4 knockout (Ptger4 KO) organoids were generated and demonstrated the
20 necessity of PGE2-Ptger4 signaling in regulating stemness enhancing effects of AA (FIGs. 6A-6C). Inhibition of Ptger4 signaling blunted the upregulation of signature genes that were associated with stem cell reprogramming in response to AA or PGE2 (FIGs. 6D and 14F).

PGE2 signaling through Ptger4 engages with diverse downstream pathways such as activating adenylyl cyclase for increased cAMP production, phosphatidylinositol 3-kinase
25 (PI3K), β -arrestin, β -catenin and extracellular signal regulated kinase (ERK) (Yokoyama et al., 2013). Using a membrane permeable and stable cAMP-derivative (8-Bromo) (Tuesta et al., 2017) in organoid assays, it was found that elevated cAMP levels could be sufficient to recapitulate the effects of AA on organoid growth and enhancing the expression of AA signature genes (FIGs. 6E-6H). Increased cAMP levels activated downstream effector
30 molecules including protein kinase A (PKA), exchange protein activated by cAMP (Epac), and cyclic nucleotide-gated ion channels (Sassone-Corsi, 2012). Inhibiting PKA using a potent antagonist (H89) (Chijiwa et al., 1990) blunted the effects of AA on organoid growth and AA-induced signature gene expression (FIGs. 6I-6L). Ptger4 was highly conserved between mouse and human (Narumiya et al., 1999). Human PDOs were utilized to assess

whether Ptger4 – cAMP – PKA signaling axis regulated AA-induced stemness in the human intestine. Similar to the observations in mouse organoids, it was found that Ptger4 and PKA may be necessary for stemness enhancing effects of AA (FIGs. 6M-6P), and for elevating cAMP levels to promote stem cell activity in human PDOs (FIGs. 6Q-6R).

5 To elucidate whether Ptger4 signaling was required for ARD-induced enhanced intestinal regeneration *in vivo*, *Lgr5-CreERT2-IRES-GFP*, *Ptger4* f/f mice were generated which allowed inducible ablation of *Ptger4* in green fluorescent protein (GFP) labeled Lgr5+ stem cells and their progeny in intestinal crypts. It was found that ARD failed to increase the numbers of EdU+ cells in *Ptger4*-deficient crypts at steady state and after irradiation, which
10 emphasized the necessity of Ptger4 signaling in mediating ARD-induced stem cell regeneration *in vivo* (FIGs. 6S-6T). Altogether, these data established that PGE2 signaling through Ptger4 – cAMP – PKA axis was a conserved mechanism in mice and humans, through which dietary AA promoted stem cell reprogramming and regeneration in the intestine.

15

Example 7: AA elicited epigenetic reprogramming around regeneration associated gene loci in a Ptger4-dependent manner

A permissive chromatin state with limited differences in epigenetic proxies, such as chromatin accessibility, histone and DNA modifications between adult stem cells and
20 differentiated cells in the intestinal epithelium, has been observed (Jadhav et al., 2016; Kaaij et al., 2013; Kazakevych et al., 2017; Kim et al., 2014; Sheaffer et al., 2014). While stem cell plasticity and dedifferentiation in response to intestinal injury are attributed in part to this low chromatin barrier between differentiated cells and stem cell states, little is known about how dietary and metabolic signals that influence stem cell regeneration affect intestinal epithelial
25 epigenome (Verzi and Shivdasani, 2020). To determine whether AA-induced stemness involves epigenetic reprogramming, the present disclosure performed assay for transposase-accessible chromatin using sequencing (ATAC-seq) that captured accessible chromatin regions in vehicle- or AA-treated organoids (Buenrostro et al., 2013). Differential analysis of accessible chromatin regions between AA-treated vs. vehicle-treated organoids revealed that
30 AA may have promoted chromatin accessibility, rather than inhibiting it around promoters, enhancers and intergenic regions (5807 opening peaks, 2132 closing peaks, $q < 0.01$ and absolute log2 fold-change > 0.58) (FIG. 15A). AA-induced reprogramming of chromatin accessibility around promoters may have led to concomitant upregulation of nearby genes with opening ATAC-seq peaks (FIG. 15B). Pathway enrichment analysis of the regions with

increased accessibility in response to AA highlighted stemness-associated signatures, including telomerase activity (Hoffmeyer et al., 2012; Montgomery et al., 2011; Schepers et al., 2011) and acetyltransferase complex activity (Sampurno et al., 2013; Yin et al., 2014). Certain pathways are crucial for stem cell proliferation and regeneration in response to injury, such as EGF (Basak et al., 2017), calcium signaling and calcium-binding S100 proteins (Bresnick et al., 2015; Deng et al., 2015), stem cell regulators such as β -catenin (Beumer and Clevers, 2016), MYB (Cheasley et al., 2011), CREB and its targets (ID1 and ID2) and partners (ATF and JUN) (Nigmatullina et al., 2017; Sampurno et al., 2013; Zhang et al., 2014), all of which were congruent with the gene expression and functional data on stemness-enhancing effects of AA (FIG. 7A). On the other hand, regions that lost accessibility by AA treatment were enriched for signatures of enteroendocrine cells, extracellular matrix, negative regulation of wound healing and PRC2 targets (FIG. 7A). To identify TFs with regulatory potential in AA-induced opening or closing chromatin regions, a multivariate linear model was built for the changing peaks with respect to TF motif presence (Doane et al., 2021). Motifs for NFIC, which was a suppressor of proliferation and *Ccdn1* expression (Eeckhoutte et al., 2006), as well as NEUROD1, an enteroendocrine cell marker (Li et al., 2019), were enriched in regions that were closed in AA-treated organoids (FIG. 7B). Enriched TF motifs that were associated with stemness and regeneration, such as NFE2L1 (NRF1) (Schell et al., 2017), AP1 family (FOS, JDP2, JUNB) (Haber et al., 2017; Nateri et al., 2005), PPAR (Beyaz et al., 2016; Beyaz et al., 2021b), YAP complex (TEAD3) (Gregorieff et al., 2015; Yui et al., 2018), Notch modulator HES1 (Pellegrinet et al., 2011; VanDussen et al., 2012), KLF5 (Nandan et al., 2015) and CREB complex (CREB1 and ATF4) (Sampurno et al., 2013), were identified in regions with gained chromatin accessibility in response to AA (FIG. 7B). Among these factors, YAP and CREB were important and conserved regulators of regeneration (Deng et al., 2015; Gregorieff et al., 2015; Li and Fan, 2017; Nusse et al., 2018; Sampurno et al., 2013; Yui et al., 2018) through their partnership with multitude of stem cell factors including AP1 family and β -catenin (Goessling et al., 2009; Shaywitz and Greenberg, 1999; Zanonato et al., 2015). Furthermore, the data demonstrated that AA-induced stemness phenotype was mediated by Ptger4-PKA signaling (FIGs. 6A-6T), which may activate and promote nuclear localization CREB1 (Sassone-Corsi, 2012; Yokoyama et al., 2013). AA-treatment may have led to enhanced CREB1 activity as assessed by its increased nuclear localization and phosphorylation (FIG. 7C) as well as the upregulation of *bona fide* CREB1 target genes, such as *Nr4a1* and *Id2*, which were linked to tissue repair and stemness (Nigmatullina et al., 2017; Wu et al., 2016) (FIGs. 10E and 10G). Similarly, AA boosted

YAP nuclear localization (FIG. 7C) and targeted gene expression such as *Ly6a* (Yui et al., 2018). Finally, the regions with most significant chromatin accessibility gains in response to AA harbored nearby genes that were known targets of CREB1 and YAP, as well as part of AA-induced signatures such as stem cell reprogramming (*Sl00a6*, *Ly6a*, *Msln*, *Anxa10*, *Il33*,
 5 *Ccnd1*, *Ascl2*), proliferation (*Myc*, *Max*, *Mki67*, *Ccnd1*), EGFR pathway (*Areg*, *Egfros*), Wnt/ β -catenin pathway (*Ascl2*, *Id2*, *Wnt4*, *Wnt7b*, *Jag1*, *Asap1*, *Plaur*, *Cd44*) and PGE2 signaling (*Ptgs2*, *Ptger4*) (Ayyaz et al., 2019; Murata et al., 2020; Mustata et al., 2013; Nusse et al., 2018) (FIG. 7D). Altogether, these data indicated that AA may reprogram chromatin accessibility around regeneration-associated gene loci, and in part through activation of
 10 CREB1 and YAP.

Covalent histone modifications have been associated with chromatin activity and transcriptional outcomes (Berger et al., 2009). To further define AA-induced alterations in epigenetic landscape, the present disclosure performed “Cleavage Under Targets and Release Using Nuclease” (Cut&Run) assay (Meers et al., 2019) in organoids and assessed genome-
 15 wide distribution of histone modifications that correlate with transcriptional activation (H3K4me3), repression (H3K27me3) or active enhancers (H3K27ac) (Beyaz et al., 2017; Das et al., 2014) (FIG. 15C). It was found that AA-induced upregulated genes may have had significant gains in activation-associated H3K4me3 and H3K27ac marks around promoters and proximal putative enhancers (FIGs. 15D-15E). In contrast, downregulated genes in AA
 20 lost these active marks and were associated with elevated repressive H3K27me3 levels (FIG. 14F). Genes that are part of regenerative fetal spheroid (Mustata et al., 2013) and repair-associated stem cell regeneration gene signatures (Ayyaz et al., 2019; Nusse et al., 2018) exhibited significant AA-induced epigenetic reprogramming with increased H3K4me3 and H3K27ac levels, and decreased H3K27me3 levels (FIG. 7E). Putative enhancers that gained
 25 H3K27ac abundance by AA included stem cell reprogramming signature genes, such as *Sl00a6*, and enhancers with H3K27ac loss in AA included differentiation genes, such as *Defa17* (FIG. 7F). Integration of gene expression data to chromatin state further corroborated these observations and revealed that AA-induced upregulated genes with most significant gains in H3K4me3 or H3K27ac abundance may be stem cell reprogramming genes (FIGs. 7G
 30 and 15G-15H). As shown in the representative genomic tracks, signature genes such as *Sl00a6*, *Msln*, *Anxa10*, *Ly6a* and *Ascl2* accumulated active histone marks with concomitant increase in chromatin accessibility around promoters or enhancers in response to AA (FIGs. 7H and 15I).

Because AA-induced upregulation of stem cell reprogramming signature genes is mediated through PGE2-Ptger4 signaling, the necessity of PGE2-Ptger4 signaling in epigenetic regulation of AA-induced stem cell reprogramming was assessed. Ptger4 KO organoids were utilized to perform Cut&Run for H3K27ac. The data suggested that Ptger4
5 may be required for AA-induced accumulation of H3K27ac around upregulated genes, as well as repair-associated stem cell regeneration gene signatures (FIGs. 7I and 15J-15K). Collectively, these results emphasized an epigenetic basis for AA-induced stem cell reprogramming that may be dependent on PGE2 – Ptger4 signaling.

10 ***Example 8: Discussion***

The intestinal epithelium is one of the most regenerative tissues in mammals owing to ISCs that reside in the crypt and replenish the tissue in approximately every 3-5 days (Clevers, 2013). However, exposure to genotoxic stressors such as radiation and chemotherapeutics have been associated with degeneration of the intestinal epithelium, and
15 there is an unmet need to develop regenerative therapeutics. Dietary intake of nutrients such as FAs influences the activity of ISCs (Beyaz et al., 2016; Beyaz et al., 2021b), but how specific FAs affect intestinal regeneration are not well understood. Several clinical and epidemiological studies suggest that increasing total polyunsaturated fatty acids (PUFAs) including omega-6 fatty acids elevates cancer risk. However, the definitive evidence for the
20 effects of omega-6 on cancer outcomes is currently lacking. PUFAs including omega-6 fatty acids such as arachidonic acid are important structural components of cell membranes that rapidly proliferating cells require for their growth. In addition, upon tissue damage, omega-6 fatty acids are released from cell membranes to give rise to inflammatory bioactive lipid mediators such as prostaglandins, which are implicated in carcinogenesis (Hanson, et al. *Br J*
25 *Cancer* (2020) 122(8):1260-70; Sakai, et al. *BMC Cancer* (2012) 12:606; Liput, et al. *Int J Mol Sci* (2021) 22(13):6965; Azrad, et al. *Front Oncol* (2013) 3:224).

Using a FA screening approach in *ex vivo* mouse and human organoid cultures coupled with *in vivo* studies that utilize isocaloric diets varying in AA abundance, the present disclosure discovered a conserved mechanism of nutrient – gene interaction that regulated
30 stemness in intestinal epithelium. The screening platform with mouse or human organoids identified regenerative dietary nutrients with beneficial (e.g., therapeutic) implications. Using this platform, causal mechanistic links between dietary AA and stem cell regeneration in the intestine was uncovered through epigenetic reprogramming that has broad implications on mitigating degeneration in the intestine. Damage to intestinal mucosa is one of the most

common debilitating side effects of cancer treatments such as radiotherapy and chemotherapy that leads to reduced quality of life and decreased survival rates in cancer patients (Kim et al., 2017; Sougiannis et al., 2021). The present disclosure showed that a regenerative dietary AA regimen (3% AA in triglyceride form) for four weeks protects against intestinal damage in mice in response to a clinically relevant abdominal 15 Gy irradiation, as well as to treatment with doxorubicin, a widely used chemotherapeutic agent that leads to intestinal mucositis in patients (Kim et al., 2017; Sougiannis et al., 2021). Based on the findings, prospective clinical studies may be conducted to validate that elevating AA abundance through dietary interventions can improve intestinal regeneration and ameliorate gastrointestinal side effects in cancer patients receiving radiotherapy or chemotherapy.

Consistent with the data on regenerative effects of dietary AA, mice that are deficient in *Fads1*, the rate limiting desaturase for AA biosynthesis, exhibit poor overall survival and impaired proliferation in intestinal epithelium unless supplemented with exogenous AA (Fan et al., 2016; Fan et al., 2012). AA is abundant in breastmilk and is considered essential for infant growth and development, but global estimates of dietary lipid intake indicate that humans obtain AA mostly through desaturating dietary LA (Calder et al., 2019; Fan et al., 2012). Numerous polymorphisms in the FA desaturase gene cluster associate strongly with metabolic traits and diseases including IBD (Sabatti et al., 2009; Dupuis et al., 2010; Costea et al., 2014). Thus, further studies are needed to ascertain the significance of dietary FAs on human physiological and disease states by accounting for human genetic variation in AA utilization and metabolism genes.

It is becoming increasingly evident that epithelial cell plasticity, rather than a reserve stem cell pool drives regeneration to recover from injury in the intestine (Ayyaz et al., 2019; de Sousa and de Sauvage, 2019; Murata et al., 2020; Nusse et al., 2018; Tian et al., 2011; Yan et al., 2017). The findings expound a new paradigm that coupled specific dietary nutrients to reprogramming of stemness in the adult intestinal epithelium. Previous studies using different experimental damage models captured distinct and divergent molecular features of epithelial cell plasticity in response to injury or ablation of ISCs (Ayyaz et al., 2019; Murata et al., 2020; Mustata et al., 2013; Nusse et al., 2018). Strikingly, in the absence of tissue damage, dietary AA mimics a conserved repair response program through epithelial PGE2 – Ptger4 signaling that shares the features of all previously defined stem cell reprogramming signatures, including targets of Wnt/ β -catenin and ligands of Egfr. AA led to upregulation of niche-derived signals in epithelial cells and reduced dependency to Wnt and Egf in organoid cultures, all of which suggested an important role for dietary nutrients in

influencing niche-mediated control of stemness in the intestine. Similarly, dietary AA-induced activation of PGE2-Ptger4 signaling in intestinal epithelium had important implications for regeneration and epithelial plasticity. Paracrine PGE2 signaling is known to promote tissue repair upon injury and PGE2 analogs have been previously characterized to have radioprotective effects in the intestine (Hanson and Ainsworth, 1985; Miyoshi et al., 2017). Pharmacologic interventions that activate PGE2 – Ptger4 – cAMP signaling have been explored in clinical studies for promoting tissue regeneration (Miyoshi et al., 2017; Taha et al., 2018; Nakase et al., 2010). It will be interesting to elucidate whether dietary AA synergizes with these therapeutic interventions to elicit regenerative stem cell plasticity in the intestine. Furthermore, lipid peroxidation and release of AA at wounds regulated injury detection and tissue repair processes (Katikaneni et al., 2020). Congruent with these observations, the aforementioned findings established that dietary AA-induced adaptive PGE2 – Ptger4 signaling engenders epithelial cell plasticity, boosts regeneration and protects intestine against damage. Thus, the regenerative effect of dietary AA through PGE2 – Ptger4 axis underscores the significance of dietary factors in influencing stem cell reprogramming and represents a new, robust and physiological model to study epithelial cell plasticity in the intestine (de Sousa and de Sauvage, 2019).

Downstream of Ptger4, cAMP – PKA signaling mediated stemness enhancing effects of dietary AA. cAMP – PKA signaling played key roles in tissue repair and resolution of inflammation. Elevating cAMP signaling in tissues may promote regeneration and block or perhaps reverse scarring after injury, yet cell type-specific mechanisms are not well understood (Insel et al., 2012).

Little is known about the effects of dietary and metabolic perturbations on intestinal epigenome (Verzi and Shivdasani, 2020). The present disclosure showed that the stemness enhancing effects of AA – Ptger4 – cAMP – PKA axis in intestinal epithelial cells are likely mediated by the activities of several TFs that are activated downstream of PKA and cooperatively orchestrate the establishment of a regenerative epigenetic program. Indeed, the data revealed significant AA-induced epigenetic reprogramming around regeneration-associated gene loci including targets of CREB1 and YAP in a Ptger4-dependent manner. It has been shown that epithelial stem cells retain functional features from past exposures (Beyaz et al., 2016; Naik et al., 2017; Ordovas-Montanes et al., 2020). An important implication of this epigenetic reprogramming was that dietary AA may elicit a regenerative memory in the intestinal epithelium which protects the tissue from subsequent damages.

The present disclosure contemplates epithelial-intrinsic mechanisms that govern the stemness enhancing effects of AA. While acute PGE2 production facilitated tissue repair in response to injury, chronic inflammation and dysregulated PGE2 signaling promoted tumorigenesis (Wang and DuBois, 2018). The short-term regenerative dietary AA regimen that was used in this study led to increased PGE2 levels in the intestine, without any apparent safety concerns in mice. However, future studies are needed to decipher the kinetics of AA-induced increase in stemness to precisely uncouple regenerative effects from the possible risk of tumor initiation. Although the data indicated robust AA-induced epigenetic reprogramming around the regeneration-associated gene loci, the epigenetic methods that were utilized only provided an average, population-based analysis and did not preserve single cell information.

Example 9: Reagents and Methods

Table 4.

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Non-phospho (Active) β -Catenin (Ser33/37/Thr41)	CST	D13A1, RRID: AB_11127203
Alpha-Tubulin	CST	DM1A, RRID: AB_1904178
Lamin B1	Abcam	Ab16048, RRID: AB_443298
EpCAM Apc, clone G8.8	Thermo Fisher Scientific	17-5791-82, RRID: AB_2716944
CD45-PE, clone 30-F11	Thermo Fisher Scientific	12-0451-83, RRID: AB_465669
CD24-PE-Cy7, clone M1/69	Thermo Fisher Scientific	25-0242-82, RRID: AB_10853806)
Ly-6A/E (Sca-1)-Pacific blue	Biolegend	108120, RRID:AB_493273
B-Actin	CST	4967, RRID:AB_330288
Anti-mouse HRP-linked	CST	7076S, RRID:AB_330924
Anti-rabbit HRP-linked	CST	7074S, RRID:AB_209923
H3K4me3	Abcam	ab8580, RRID:AB_306649
H3K27me3	Millipore	07-449, RRID:AB_310624

H3K27ac	Abcam	ab4729, RRID:AB_211829 1
Ki67	Spring Bioscience	M3062, RRID:AB_112197 41
Chemicals, peptides, and recombinant proteins		
Advance DMEM / F12	Gibco	12634010
RPMI 1640	Corning	10-040-CV
Penicillin-Streptomycin	Sigma-Aldrich	P4333
Glutamax	Thermo Fisher	35050061
B27	Thermo Fisher	17504044
N2	Thermo Fisher	17502048
Recombinant Murine EGF	PeproTech	315-09
Chiron-CHIR 99021	Tocris	4423
Recombinant Murine Noggin	PeproTech	250-38
R-Spondin	R&D Systems	3474-RS
NAC	Sigma-Aldrich	A7250
Y-27632	Tocris	1254
Penicillin-Streptomycin	Sigma-Aldrich	P4333
GlutaMAX Supplement	Gibco	35050061
TrypLE Express	Gibco	12604-013
EDTA	Invitrogen	15575-038
Amphiregulin	R&D	989-AR
Epiregulin	R&D	1068-EP
Wnt3a	Peprtech	315-20
Doxorubicin hydrochloride	Millipore (Sigma)	D1515
Tagment DNA Enzyme 1 (TDE1)	Illimuna	5027865
NEBNext High-Fidelity 2X PCR Master Mix	NEB	M0541S
Agencourt AMPure XP magnetic beads	Beckman Coulter	A63880
13z-eicosenoic acid (Paullinic Acid)	Cayman	26169
Linoelaidic acid	Cayman	90160
Palmitoleic	Cayman	10009871
Palmitic Acid	Cayman	57-10-3
Nonadecanoic acid	Cayman	19723
Gamma-linolenic acid	Cayman	90220
Dihomo-gamma-linolenic acid	Cayman	90230
Eicosapentaenoic acid	Cayman	90110
Cis-5,8,11 eicosatrienoic acid	Sigma	e6013
Elaidic acid	Sigma	e4637
Myristic acid	Sigma	m3128
Arachidonic acid	Sigma	10931
Oleic acid	Sigma	o1008
Linolenic acid	Sigma	l2376
Linoleic acid	Sigma	l1376
cis-4,7,10,13,16,19-docosaehaenoic acid	Sigma	d2534
Stearic acid	Sigma	s4751
Stearidonic Acid (All cis-6,9,12,15)	Matreya	1276

Eicosatetraenoic Acid (All <i>cis</i> -5,8,11,14)	Matreya	1042
Butyric Acid	Sigma	B103500
Docosatetraenoic Acid	Cayman	10007289
Heptadecanoic Acid	Cayman	19722
cis-4,10,13,16-Docosatetraenoic Acid	Cayman	10007289
dmPGE2	Cayman	14750
PGD2	Sigma	P5172
Celexocib	Cayman	10008672
8-Bromo	Tocris	1140
Sesamin	Sigma	SMB00705
H89	Tocris	2910
Indomethacin	Sigma	17378
Ptger1 Inhibitor	R&D	SC 51322
Ptger2 Inhibitor	R&D	PF 04418948
Ptger3 Inhibitor	R&D	L-798,106
Ptger4 Inhibitor	R&D	L-161,982
5-HETE	Cayman	34210
12-HETE	Cayman	34550
15-HETE	Cayman	34700
8(9)-EET	Cayman	50351
11(12)-EET	Cayman	50511
14(15)-EET	Cayman	50651
LTB4	Cayman	20110
TXB2	Cayman	19030
Puromycin	Thermo Fisher	A1113803
Lenti-X Concentrator	Takara	631232
Polybrene	Millipore Sigma	TR-1003-G
ProLong™ Gold Antifade Mountant	Invitrogen	P36930
Sytox Blue	Thermo Fisher Scientific	S34857
Phosstop phosphatase inhibitor tablets	Roche	04906845001
cOMplete EDTA-free Protease Inhibitor Cocktail	Millipore Sigma	11873580001
GlycoBlue Coprecipitant	Invitrogen	AM9515
Polyethylenimine, Linear, MW 25000	Polysciences	23966
Paraformaldehyde 32%	Electron Microscopy Science	15714
Tween-20	Millipore Sigma	P1379
Proliant Bovine Serum Albumin (Fatty-Acid Free)	Proliant Biologicals	68700
Glass Container	Thermo Fisher	B7999-2A
Arasco oil	DSM	5015002S02
Corn oil	Millipore Sigma	C8267
TRIzol Reagent	Thermo Fisher	15596026
Critical commercial assays		
Click-iT™ EdU Cell Proliferation Kit for Imaging, Alexa Fluor™ 647 dye	Invitrogen	C10640
Thruplex DNA-seq	Takara	R400675
NEBNext lowinput/single cell	Biolegend	E6420
NEBNext Ultra II Directional	Biolegend	E7760
DNA Clean&Concentrator-5	Zymo Research	D4013
RNAscope™ 2.5 HD Assay - RED	ACD Bio	322350

MinElute Reaction Cleanup Kit	Qiagen	28204
Agilent High Sensitivity DNA Bioanalysis Kit	Agilent	5067-4626
PGE2 Elisa Kit	Enzo	ADI-900-001
Direct-zol RNA Isolation Kit	Zymo Research	R2051
Deposited data		
Bulk RNA-seq	This study	GSE188213
Single cell RNA-seq	This study	GSE188213
ATAC-seq	This study	GSE188213
Cut&Run	This study	GSE188213
Experimental models: Cell lines		
293FT	Thermo Fisher	R70007
L-WRN	ATCC	CRL-3276
Experimental models: Organisms/strains		
C57BL/6J mice	The Jackson Laboratory	000664, RRID:IMSR_JAX:000664
Ptger4 f/f	The Jackson Laboratory	028102, RRID:IMSR_JAX:028102
Lgr5-EGFP-IRES-CreERT2	The Jackson Laboratory	008875, RRID:IMSR_JAX:008875
Lgr5-EGFP-IRES-CreERT2; Ptger4 f/f	First presented in this work	NA
Oligonucleotides		
Ccnd1	Applied Biosystems	Mm00432359_m1
Ptgs1	Applied Biosystems	Mm00477214_m1
Ptgs2	Applied Biosystems	Mm00478374_m1
Ptgs	Applied Biosystems	Mm00452105_m1
Cd55	Applied Biosystems	Mm00438377_m1
S100a6	Applied Biosystems	Mm00771682_g1
Ly6a	Applied Biosystems	Mm04337234_mH
Msln	Applied Biosystems	Mm00450773_g1
Hsp90ab1	Applied Biosystems	Mm00833431_g1
Nr4a1	Applied Biosystems	Mm01300401_m1
NR4A1	Applied Biosystems	Hs00374226_m1
NR4A2	Applied Biosystems	Hs01117527_g1
MSLN	Applied Biosystems	Hs00245879_m1
L1CAM	Applied Biosystems	Hs01109748_m1
CD55	Applied Biosystems	Hs00892618_m1
DUSP4	Applied Biosystems	Hs01027785_m1
HSP90AB	Applied Biosystems	Hs03043878_g1
Recombinant DNA		
Puro.Cre empty vector	Addgene	17408
pCMV-VSVG	Addgene	8454
psPAX2	Addgene	12260
Software and algorithms		

ImageJ	National Institutes of Health	RRID: SCR_003070
Imaris 8.3.1	Oxford Instruments	RRID: SCR_007370
Prism9	Graphpad	RRID: SCR_002798
Aperio VERSA	Leica Biosystems	RRID: SCR_021016
Mouse Diet		
AA-rich Diet	Envigo	TD.190641
Control Diet	Envigo	TD.97184

Animals, diet, and drug treatment

Mice were housed in the Cold Spring Harbor Laboratory. The following strains were obtained from the Jackson Laboratory: Ptger4 f/f (strain name: 6.129S6(D2)-

- 5 Ptger4tm1.1Matb/BreyJ, stock number: 028102), Lgr5-EGFP-IRES-CreERT2 (strain name: B6.129P2-Lgr5tm1(cre/ERT2) Cle/J, stock number 008875). Animals were housed in pathogen-free conditions and maintained at 12 hours light/dark cycles. ARD was developed by using oil extracted from fungi (*Mortierella alpina*) that contains approximately 40% AA in the form of triglycerides (Arasco oil, DSM, 5015002S02) to formulate a 3%AA-rich oil and 4% soybean oil containing diet (7% total fat) (Cat# TD.190641, Envigo) beginning at the
- 10 age of 8-12 weeks for four weeks (Table 3). Control mice were age- and sex-matched and were fed with isocaloric control diet containing equal amounts of major nutrients and minor nutrients (Cat# TD.97184, Envigo). Food and water provided *ad libitum*. Alleles crossed with Lgr5-EGFP-IRES-CreERT2 (to generate stem cell specific knockout, Lgr5-iKO) mice were
- 15 excised by administration of tamoxifen suspended in corn oil (Cat# C8267, Sigma) at a concentration of 20 mg/ml and 100 μ l per 25g of body weight, and administered by intraperitoneal injection every other day for 5 times. All animals used in this study were handled according to ethical procedures approved by The Institutional Care and Use Committee (IACUC) at Cold Spring Harbor Laboratory.

- 20 Organoid treatments were performed with following compounds; dmPGE2 (5nM, Cat# 14750, Cayman), PGD2 (5nM, Cat# P5172, Sigma), Celecoxib (1-45 μ M, Cat# 10008672, Cayman), 8-Bromo-cAMP (20 μ M, Cat# 1140, Tocris), Sesamin (20 μ M, Cat# SMB00705, Sigma), H89 (20 μ M, Cat# 2910, Tocris), Indomethacin (0-80 μ M, Cat# 17378, Sigma), Ptger1 inhibitor (50 μ M, Cat# SC51322, R&D), Ptger2 inhibitor (25 μ M, Cat#
- 25 PF04418948, R&D), Ptger3 inhibitor (50 μ M, Cat# L-798,106, R&D), Ptger4 inhibitor (50 μ M, Cat# L-161,982, R&D), 5-HETE (0.5 μ M, Cat# 34210, Cayman), 12-HETE (0.5 μ M, Cat# 34550, Cayman), 15-HETE (0.5 μ M, Cat# 34700, Cayman), 8(9)-EET (0.5 μ M, Cat#

50351, Cayman), 11(12)-EET (0.5 μ M, Cat# 50511, Cayman), 14(15)-EET (0.5 μ M, Cat# 50651, Cayman), LTB4 (0.5 μ M, Cat# 20110, Cayman), TXB2 (5 μ M, Cat# 19030, Cayman), Wnt3a (10-100ng/ml, Cat# 315-20, Peprotech), Amphiregulin (50ng/ml, Cat# 989-AR, R&D), Epiregulin (500ng/ml, Cat# 1068-EP, R&D).

5

Intestinal crypt isolation and flow cytometry

Intestinal crypt isolation was performed as previously reported (Beyaz et al., 2016). Briefly, the whole intestine was extracted and cleaned from fat, connective tissue, blood vessels and flushed with ice cold 1X PBS. After lateralizing, small intestine was cut into 3-5
10 cm small pieces and incubated in 1X PBS/EDTA (7.5 mM) with mild agitation for 30 minutes at 4°C. Crypts were mechanically dissociated from tissue, strained through 70-micron strainer to remove villus and tissue fragments. Then, the crypts were washed with ice cold PBS and centrifuged at 300g for 5 minutes.

IEC isolation was performed by dissociation of the crypt suspensions into single cells
15 with TrypLE Express (Cat# 12604-013, Invitrogen). Dissociated single cells were labeled with an antibody cocktail containing EPCAM-APC (1:400, Cat# 17-5791-82, eBioscience, G8.8), CD24-PE-Cy7 (1:400, Cat# 25-0242-82, eBioscience), and CD45-Alexa fluor 488 (1:400, Cat# 12-0451-83, eBioscience). Dead cells were excluded from the analysis with the viability dye SYTOX (Cat# S34857, Life Technologies). IECs were isolated as Epcam⁺
20 CD45⁻ SYTOX⁻ with a BD FACS Aria II SORP cell sorter into a supplemented crypt culture medium for culture or TRIzol reagent (Cat# 15596018, Thermo Fisher) to perform gene expression analysis.

Culture media for crypts and isolated cells

25 Isolated crypts were counted and embedded in Matrigel (Cat# 356231, Corning growth factor reduced) in 1:4 ratio at 5-10 crypts per μ l. Matrigel was allowed to solidify for 8-12 minutes at 37°C and solidified domes cultured in crypt media containing Advanced DMEM (Cat# 12634010, Gibco) media supplemented with recombinant murine EGF 40 ng/ml (Cat # 315-09, PeproTech), recombinant murine Noggin 50 ng/ml (Cat # 250-38,
30 PeproTech), R-spondin 62.5 ng/ml (Cat# 3474-RS, R&D Systems), N-acetyl-L-cysteine 1 μ M (Sigma-Aldrich), CHIR-99021 5 μ M (Cat# 4423, Tocris), Y-27632 20 ng/ml (Cat# 1254, Tocris), B27 1X (Cat# 17504044, Gibco), N2 1X (Cat# 17502048, Gibco), 1% GlutaMAX (Cat# 35050061, Gibco), 1% Penicillin-Streptomycin (pen/strep) (Cat# P4333, Sigma-Aldrich). The crypt media was changed every other day and maintained at 37°C in a fully

humidified chamber containing 5% CO₂. Clonogenicity (colony-forming efficiency) was determined by plating 50–300 crypts per well and assessing organoid formation after 3–7 days.

Isolated IECs cells were centrifuged at 300g for 5 minutes and suspended in the appropriate volume of crypt culture medium (500–1,000 cells/μl). Then, the cells were seeded onto Matrigel in a flat bottom plate (Cat# 3548, Corning). Crypt medium were added after the Matrigel and cells had solidified. The crypt media was replenished every other day. Organoid bodies were quantified on days 1, 3 and 6 culture, unless otherwise specified. In secondary experiments, individual primary organoids were mechanically dissociated for 6 minutes in TryPLE Express at 37°C, centrifuged and resuspended in cold crypt media, mixed with Matrigel and incubated until its solidified. Fresh crypt media was supplemented every other day and maintained at 37°C in a fully humidified chamber containing 5% CO₂.

Fatty acid (FA) BSA Conjugation

Fatty acids (FA) supplemented as powder were reconstituted in ethanol. Then, fatty acid solutions were added to 0.01M NaOH to make a 12mM solution and stirred for 30 minutes at 70°C. Then, 10% fatty acid free BSA (Cat# 68700, Proliant Biologicals) was added to the solution to have 3mM concentration and stirred for 1 hour at 37°C. BSA-conjugated FAs were filtered through 0.22μm and stored in glass containers (Cat# B7999-2A, Thermo Fisher) in -20°C.

FA Screening

10,000 dissociated cells from mouse or human intestinal organoids were seeded on 48 well plates and incubated in crypt media for 6 hours at 37°C with 5% CO₂ for recovery before proceeding to fatty acid treatment. The fatty acid screening library was composed of 23 different fatty acids, as given in Table 1. After 6 hours of incubation, media were changed to crypt media containing fatty acids at an indicated concentration (25 μM for both mouse and human organoids). After 24 hours of treatment, images (16 z-slices at 54.8 μm steps, fixed focal height at 1719 μm above plate carrier) were taken from each well with 6 hours of interval using Cytation7 and BioSpa platforms (Agilent BioTek, Winooski VT) at 37°C with 5%CO₂. Imaging was terminated at 120th hour. Then, Z-projection was obtained with a focus stacking function. Digital phase contrast was applied and images were filtered by a 100μm structuring element size. Spheroids were detected by defining low internal signal objects

gated by the new metric above ≤ 0.95 and circularity >0.2 to create a new population. This subpopulation was normalized to the total biology area.

Human study participants and crypt isolation from patient biopsies

- 5 Human colon tissue samples were obtained from patients with informed consent, undergoing surgical resection procedures at Huntington Hospital. Study protocols were reviewed and approved by the Northwell Health Biospecimen Repository (Protocol number: 1810). Tissue samples were kept in RPMI medium (Cat#, 10-040-CV, Corning) until processing. Patient metadata are provided in Table 2.
- 10 Tissue samples were first cut into small $\sim 0.5\text{cm}^2$ pieces and incubated at 4°C in an antibiotic mixture consisting of $100\mu\text{g/mL}$ Normocin (Cat# ant-nr-1, Invivogen), $50\mu\text{g/mL}$ Gentamicin (Cat# E737, Amresco), and 1X Pen/Strep (Cat# 15070063, ThermoFisher) in 1X PBS for 15 minutes. Next, the pieces were washed with 1X PBS before a 75-minute incubation in a 5mM EDTA solution at 4°C on a rocker. After incubation, the tissue samples
- 15 were washed once more with 1X PBS. Crypts were then released from the tissue by shaking the pieces in a tube with ice cold 1X PBS. Isolated crypts were transferred to a new tube and spun down at 100g for 5 minutes at 4°C .

Human Organoid Passaging and Maintenance

- 20 Isolated crypts were then embedded in Matrigel in 1:4 ratio. The Matrigel was allowed to polymerize at 37°C for 8-12 minutes before adding human crypt medium to each well, with the culture medium consisting of Advanced DMEM (Cat# 12634028, Life Technologies), 1X Glutamax (Cat# 35050061, Life Technologies), 10mM HEPES (Cat#15630080, Thermo Fisher Scientific), 50% WRN conditioned medium derived from L-
- 25 WRN cell line (ATCC, CRL-3276), 1X B27 (Cat# 12587010, Life Technologies), 1X N2 (Cat# 17502048, Life Technologies), 10mM Nicotinamide (Cat# N0636, Sigma Aldrich), 1mM N-acetyl cysteine (Cat# A9165, Sigma Aldrich), $100\mu\text{g/mL}$ Primocin (Cat# ant-pm-1, Invivogen), $10\mu\text{M}$ SB202190 (Cat# S7067, Sigma Aldrich), $10\mu\text{M}$ Y-27632 (Cat# 1254, Tocris), 10 nM Gastrin I (Cat# G9020, Sigma Aldrich), 50ng/mL EGF (Cat # AF-100-15, Peprotech), and 500nM A83-01 (Cat # SML0788, Sigma Aldrich). Culture media were
- 30 refreshed every 2-3 days and organoids were passaged roughly every 8 days. Organoids were harvested by removing Matrigel using Cell Recovery Solution (CRS) (Cat# 354253, Corning). Once the Matrigel was dissolved, the organoids were spun at 500g for 5 minutes at 4°C and incubated in TrypLE Express (Cat# 12604039, ThermoFisher) until single cells were

seen under the microscope. Cells were then centrifuged at 500g for 5 minutes at 4°C before seeding again in Matrigel as explained above. Organoids were commonly passaged in a 1:6 ratio.

5 ELISA Assay

Sorted Epcam⁺ CD45⁻ SYTOX⁻ cells from control mice plated 25,000 cells per well and incubated for 6 hours at 37°C for recovery. Then, media was replaced with vehicle or AA (25µM) supplemented crypt media. Supernatant from vehicle or AA treated organoids was collected 24 hours later and centrifuged at 300g to remove any cellular or Matrigel residuals.

- 10 Presence of PGE₂ metabolite was measured using PGE2 Elisa Kit (Cat# ADI-900-001, Enzo Life Sciences) according to manufacturer's instructions.

Nuclear Fractionation and Western Blot

- Organoids grown for 5 days in crypt media were treated with vehicle (BSA-ethanol) or 50 µM AA for 4 hours. Then, organoids were recovered from Matrigel and washed with PBS. To prepare cytosolic extract, organoids were lysed in Buffer A (10 mM HEPES pH-7.9, 10 mM KCl, 1.5 mM MgCl₂, 0.34 M Sucrose, 10% Glycerol, 0.1 mM PMSE, 1 mM DTT, 0.1% TritonX-100, PhosSTOP (Cat# 04906845001, Roche) and protease inhibitors (Cat# 11873580001, Sigma) and incubated on ice for 15 minutes with occasional gently pipetting.
- 20 Cytosolic extract was collected after centrifugation at 1300 rcf for 5:30 minutes. To prepare nuclear extract, nuclei were washed with Buffer A several times to remove any remaining cytosolic proteins and lysed in Buffer B (3 mM EDTA, 0.2 mM EGTA, 1 mM DTT, PhosSTOP protease inhibitors) followed by incubation on ice for 30-40 minutes with gently tapping every 5 minutes. Nuclear extract was collected after centrifugation at 1700 rcf for 5
- 25 minutes. The remaining insoluble chromatin was washed in Buffer B several times to eliminate nuclear protein contamination; the chromatin pellet was then resuspended in Laemmli buffer and sonicated for 30 seconds ON/15 seconds OFF. Then, the samples were run on 10% Tris-HCl gels and transferred onto nitrocellulose membranes. Membranes were blocked with 5% skim milk solution for 1 hour at room temperature, then incubated with
- 30 primary antibody in 5% skim milk overnight at 4°C. Following incubation, membranes were washed with 1X PBST (PBS, 0.1% Tween-20), then incubated with HRP-linked secondary antibody diluted with 5% skim milk for 1 hour at room temperature. Signals were detected with Mini-Med 90 (AFP Manufacturing) documentation system using Pierce ECL Western blotting substrate (Cat#32106, ThermoFisher Scientific).

Immunohistochemistry, smISH combined with immunofluorescence

Vehicle or AA-treated organoids were harvested by removing Matrigel using CRS solution and washed with 1X PBS twice. Subsequently, organoids were fixed with 4%

- 5 Paraformaldehyde (PFA) (Cat# 15714, Electron Microscopy Sciences) at room temperature for 30 minutes. Following PFA removal, organoids washed with 1X PBS and centrifuged at 400g for 3 minutes. Pelleted organoids were embedded in 2% agarose gel and sectioned at 10 μ m.

- Intestinal tissue from control or ARD-fed mice were swiss-rolled and fixed in 10% Formalin solution (Cat# HT501128, Sigma Aldrich). Formalin-fixed tissue samples were processed in Thermo Excelsior ES processor and embedded with Thermo HistoStar embedding system following the manufacturer's protocols. Paraffin embedded samples were cut into 5 μ m thick sections and mounted on positively charged slides (Cat# 48311-703, VWR superfrost plus micro slide). HE stainings were performed at the CSHL tissue imaging facility using Leica Multistainer (ST5020, Leica). Briefly, after deparaffinization and rehydration, slides were stained in hematoxylin (Hematoxylin 560 MX, Leica) for 1 minute, followed by destaining in Define MX-aq (Leica) for 30 seconds and bluing in Blue Buffer 8 (Leica) for 1 minute; then the slides were stained in eosin (EOSIN 515 LT, Leica) for 30 seconds. After dehydration, slides were coverslipped with a robotic coverslipper (Leica CV5030).

- For IHC, formalin-fixed paraffin-embedded tissue sections were deparaffinized. Antigen retrieval was performed with 0.1 mM citrate buffer (pH:6) by boiling at 96°C for 6 minutes. Following peroxidase blocking, tissues were blocked with appropriate serum and then incubated with an anti-Ki-67 antibody (1:100, Thermo Fisher, clone SP6) overnight. Biotin-conjugated secondary antibody was used from Vector Labs. Diaminobenzidine (DAB) was used for visualization and counterstaining was carried out with Haematoxylin and eosin (Vector Labs). PBST was used for washing in between each step.

- Single-molecule *in situ* hybridization (smISH) was performed for Ascl2 (Cat# 412211, ACD), Lgr5 (Cat# 312171, ACD), and S100a6 (Cat# 412981, ACD) using Advanced Cell Diagnostics RNAscope 2.5 HD Detection Kit-Red (Cat# 322350, ACD) according to manufacturer's instructions and combined with subsequent immunostaining for Epcam. For immunostaining, after performing smISH steps except DAPI staining, slides were incubated overnight at 4°C with anti-Epcam antibody (1:100, Cell Signaling, clone E6V8Y). Then slides were washed with PBST and incubated with a secondary antibody (1:500, Alexa

Fluor Plus 488, Cat# A32766, Invitrogen) for 1 hour at room temperature in the dark. Slides were washed again with PBST and stained with DAPI. Then, slides were mounted with ProLong™ Gold Antifade Mountant (Cat# P36930, Invitrogen). Images were acquired using a confocal microscope (Zeiss LSM 710, Germany) and processed via ImageJ. smISH signals were quantified by Imaris (Oxford Instruments).

EdU Incorporation

EdU (Sigma) was administered intraperitoneally at a dose of 5µg/g 4 hours before mice were euthanized. Proliferating intestinal epithelial cells was detected by EdU incorporation using Click-iT EdU Cell Proliferation Kit for Imaging, Alexa Fluor 647 (Cat# C10640, Invitrogen) following manufacturer's instructions after deparaffinization of the tissues and antigen retrieval, as described above. Then, Epcam and DAPI stainings were carried out. Images were acquired using a confocal microscope (Zeiss LSM 710, Germany) and processed via ImageJ. EdU+ cells per crypt were quantified in a blinded fashion. EdU signal per swiss roll was detected with Biotek (Agilent, USA).

Doxorubicin-induced intestinal damage

8-12-week-old mice were administered with a single intraperitoneal injection of Doxorubicin Hydrochloride (Cat# D1515, Millipore, Sigma) at a concentration of 20 mg/kg body weight (Carr et al., 2017; Cray et al., 2020). Mice were euthanized in CO₂ chamber and analyzed after 72 hours.

Irradiation-induced intestinal damage

Mice were anesthetized by intraperitoneal injection of a ketamine (100 mg/kg) and Dexdomitor (10 mg/kg) mixture. Mice were transferred into a lead shielding device and only the lower abdominal/pelvic region was exposed to 15 Gy of ionizing irradiation from a 137-cesium source (GammaCell). Mice were sacrificed after 72 hours. The number of surviving crypts was enumerated from haematoxylin and eosin-stained sections (Tustison et al., 2001).

RNA isolation, cDNA preparation and RT-qPCR

Total RNA was extracted by Direct-zol RNA Isolation Kit (Cat# R2051, Zymogen) and reverse transcription was performed with SuperScript IV Vilo (Cat# 11756050, Thermo Fisher) as described by the manufacturer. RT-qPCR was performed with probes listed in Table 5 by TaqMan Fast Advanced Master Mix (Applied Biosystems). qRT-PCR results

were analyzed by the $\Delta\Delta C_t$ method for relative quantification using Hsp90ab1 as an internal control.

Table 5. List of probes used for qRT-PCR

Name	Code	Target
Ccnd1	Mm00432359_m1	Mouse
Ptgs1	Mm00477214_m1	Mouse
Ptgs2	Mm00478374_m1	Mouse
Ptges	Mm00452105_m1	Mouse
Cd55	Mm00438377_m1	Mouse
S100a6	Mm00771682_g1	Mouse
Ly6a	Mm04337234_mH	Mouse
Msln	Mm00450773_g1	Mouse
Hsp90ab1	Mm00833431_g1	Mouse
Nr4a1	Mm01300401_m1	Mouse
NR4A1	Hs00374226_m1	Human
NR4A2	Hs01117527_g1	Human
MSLN	Hs00245879_m1	Human
L1CAM	Hs01109748_m1	Human
CD55	Hs00892618_m1	Human
DUSP4	Hs01027785_m1	Human
HSP90AB	Hs03043878_g1	Human

5

Lentiviral production and generation of Ptger4 knockout organoids

Lentiviral particles were produced in 293FT cells by co-transfection of a Puro.Cre empty vector (Addgene plasmid #17408 (Kumar et al., 2008)) and 2nd generation lentiviral system (pCMV-VSVG, Addgene plasmid #8454), psPAX2 (Addgene plasmid #12260) using transfection reagent polyethylenimine (PEI) (Cat# 23966, Polysciences) . Briefly, 293FT cells were plated on a 10 cm dish a day before transfection at 75%-80% confluency. 10 µg Puro.Cre plasmid, 7.5 µg spPAX2 and 5 µg of pCMV-VSVG mixed in DMEM-F12 media at room temperature and incubated for 15 minutes. Transfection mixture was added on to confluent 293FT cells in a dropwise fashion. Media was removed after 6 hours and fresh DMEM-F12 media supplemented with 10% FBS, 1% pen/strep. The supernatant containing the viral particles collected after 24, 48 and 72 hours and centrifuged for 5 minutes at 300g to

10

15

remove any residual cellular particles. The supernatant filtered through 0.45 µm filter and concentrated by adding (1/3 volume of the total supernatant) Lenti-X concentrator (Cat# 631232, Takara). Mixture centrifuged at 1500g for 60 minutes at 4°C, then viral pellet resuspended in mouse crypt media. For viral infection, day 4 grown Ptger4^{flf} organoids
5 rescued from Matrigel, dissociated into single cell as described above. Concentrated lentiviral particles supplemented with polybrene were mixed with 1,000,000 single cells and transferred into 48 well plate, then centrifuged with 600g for 1 hour at room temperature and incubated at 37°C for 4 hours. Infected cells resuspended in mouse crypt media and plated in 4 wells of 12 well plate until they form organoids. Infected organoids were selected after 3
10 days with puromycin (1µg/ml, Cat# A1113803, Thermo Fisher).

Electron Microscopy

Intestinal organoids grown in 12 well plates were fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate solution (pH 7.4) overnight at 4°C. Samples were washed three times
15 with 0.1 M cacodylate and post-fixed with 1% osmium tetroxide (OsO₄) solution for 1 hour at room temperature. Samples were rinsed three times with distilled water and dehydrated for 10 minutes with graded ethanol wash series (50%, 60%, 70%, 80%, 90%, 95%, 100% respectively). Following dehydration, samples were incubated with 812 EMed resin and 100% ethanol overnight. For polymerization, samples were embedded in 812 EMed resin and
20 incubated at 60°C until resin was totally polymerized. Sections of 60-90 nm were cut using 45 Diamond DiATOME Histo Knife. Sections were stained with UranyLess for two minutes, followed by intense washing with ddH₂O. H7000 Hitachi Transmission Electron Microscopy was used to visualize the samples.

25 Metabolomics analysis by liquid chromatography coupled to mass spectrometry (LC-MS)

Snap-frozen tissue specimens were cut and weighed into Precellys tubes prefilled with ceramic beads (Bertin Instruments). An exact volume of extraction solution (30% acetonitrile, 50% methanol and 20% water) was added to obtain 40 mg tissue specimen per
30 mL of extraction solution. Tissue samples were lysed using a Precellys 24 homogeniser (Bertin Instruments) and the suspension was incubated at -20°C for 60 minutes. Samples were mixed and incubated for 15 minutes at 4°C in a Thermomixer (Eppendorf, Germany), followed by centrifugation (16,000 g, 15 min at 4°C). The supernatant was collected and transferred into autosampler glass vials, which were stored at -80°C until further analysis. For

organoid experiments, media was collected three days after vehicle or AA treatment and samples were extracted for metabolomics analysis.

For the analysis of polar metabolites and arachidonic acid derivatives, samples were randomized in order to avoid bias due to machine drift and processed blindly. LC-MS analysis was performed using a Vanquish Horizon UHPLC system coupled to a Q Exactive HF mass spectrometer (both Thermo Fisher Scientific). Sample extracts (5 μ L) were injected onto a Sequant ZIC-pHILIC column (150 mm $\times$ 2.1 mm, 5 μ m) and guard column (20 mm $\times$ 2.1 mm, 5 μ m) from Merck Millipore kept at 45°C. The mobile phase was composed of 20 mM ammonium carbonate with 0.1% ammonium hydroxide in water (solvent A), and acetonitrile (solvent B). Analytes were eluted at 200 μ L/min with the previously described gradient (Mackay et al., 2015). The mass spectrometer was operated in full MS and polarity switching mode. The acquired spectra were analyzed using XCalibur Qual Browser and XCalibur Quan Browser software (Thermo Fisher Scientific) by referencing an internal library of compounds. *MetaboAnalystR* (v3.0.3, (Pang et al., 2020)) was used for quality control and normalization (with options QuantileNorm, LogNorm, and MeanCenter). Differential abundance analysis was conducted using *FC.Anal.unpaired*.

Bulk RNA Sequencing

Total RNA was isolated from day 1, 3 and 6 of vehicle and AA-treated mouse intestinal organoids and day 6 PDO using Zymo RNA isolation kit according to manufacturer's instructions. Starting from a total 250 ng RNA, rRNA depletion protocol followed according to suggested guidelines from the manufacturer. Strand specific RNA seq libraries were prepared using NebNext Ultra II kit and sequenced on Illumina NextSeq.

Reads were trimmed with *cutadapt* (v2.10) and aligned to GRCm38.p6/Genome annotation (release M24) using *STAR* (v2.7.2b, (Dobin et al., 2013)) and quantified using '--quantMode GeneCounts'. Read and alignment quality were analyzed with *rseqc* (v3.0 (Wang et al., 2012)) and summarized with *multiqc* (v1.9 (Ewels et al., 2016)). Differential gene expression between Vehicle and Arachidonic acid treated samples was assessed with DESeq2 (v1.28 (Love et al., 2014)), fitting a model with fixed effects for sequencing batch effect and treatment. Contrast for treatment were extracted and transcripts considered differentially expressed with an absolute fold change greater than log2(1.5) and adjusted p-value of less than 0.05. Differential gene expression was assessed independently at each time point. Unless specified otherwise, the union of differentially expressed genes across time points was used in all further analyses. For visualization purposes, *limma* (v3.46, (Ritchie et al., 2015)) was

used to adjust for sequencing batch effects, prior to *rlog* normalisation of the size-factor normalized read counts. Heatmaps and UpSet plots were generated with *ComplexHeatmap* (v2.6.2, (Gu et al., 2016), Volcano plots with *EnhancedVolcano* (v1.8.0).

5 Gene sets

The data was analyzed in the context of previously identified gene signatures and target genes list. The gene lists are described in brief below:

Fetal-spheroid gene signature

317 differentially upregulated genes (microarray) were assessed in regenerative spheroids versus organoids derived from embryo/mouse at different embryonic and postnatal stages (E16, E18, or P0) (Mustata et al., 2013)

Granuloma-induced gene signature

131 differentially expressed genes (bulk RNA-seq) were assessed in Granuloma (Gr) vs. Non-Granuloma (NonGr) crypt epithelium from mice infected with *H. polygyrus* for six days (Nusse et al., 2018).

Radiation-induced gene signature

50 differentially expressed genes (single-cell RNA-seq) were assessed in a cluster of regenerating intestinal cells from scRNAseq data of irradiated intestinal epithelium versus undamaged crypts (Ayyaz et al., 2019).

20 *Regeneration-induced gene signature*

316 differentially expressed genes (bulk RNA-seq) were assessed in regenerating Ascl2⁺ upper crypt cells upon DT exposure relative to uninjured (resting) ISCs at the bottom of the crypt (Murata et al., 2020).

Homeostatic stem cell gene signature

25 Differentially expressed genes were assessed in Lgr5⁺ ISCs (Haber et al., 2017).

Single Cell RNA sequencing

For single cell sequencing of the mouse intestinal organoids, organoids were collected using cell recovery solution. Organoids were then disassociated with TrypLE into single cell suspension. After dissociation of the organoids, single cells were pelleted, washed and resuspended in FACS buffer (1X PBS, 10 μ M Y-27632, 1% FBS, 0.5 mM EDTA) and passed through a 100 μ m FlowMi cell strainer (Sigma). DAPI was used for viability assessment. DAPI-negative cells were sorted by Sony SH800S sorter and single cell droplets were immediately prepared on the 10X Chromium according to manufacturer instructions at

Cold Spring Harbor Laboratory Single Cell Facility. Single cell libraries were prepared using a 10X Genomics Chromium Controller (Cat #120223, 10X Genomics) and the 10X Genomics Chromium Next GEM Single Cell 3' Gene Expression kit (Cat #1000268, 10X Genomics) according to the manufacturer's instructions. Cell suspensions were adjusted to target a yield of 8,000 cells per sample.

Single-cell RNA-Seq data analysis

Single cell datasets for each experiment were independently assessed for data quality following the guidelines described by (Amezquita et al., 2020; Luecken and Theis, 2019).

Cells with more than 15% mitochondrial transcripts as well as cells that had fewer than 2,000 feature counts or expressed fewer than 1000 genes were removed. After QC, Seurat (v3.2.1, (Butler et al., 2018) was used for normalization, graph-based clustering and differential expression analysis. Each dataset was normalized using *SCTransform* and the 3000 most variable genes were identified with *SelectIntegrationFeatures*. The organoid (Vehicle, AA and PGE2-treated) and tissue datasets (intestinal tissue from mice on control and Arasco diet) were integrated into one organoid and one tissue dataset, (Cao et al., 2019; Levine et al., 2015; Qiu et al., 2017; Stuart et al., 2019; Trapnell et al., 2014) *RunPCA* on the integrated datasets was used to identify the top 10 principle components (PCs), which were used for UMAP analysis and clustering. Louvain clustering at a resolution of 0.3 and 0.6 were used for the organoid and tissue datasets respectively. Clusters were labeled in accordance with expression levels of intestinal cell subtype signatures identified by (Haber et al., 2017); the stem 2 and stem 3 clusters were labeled using signatures identified by (Roulis et al., 2020) (see gene list descriptions above).

To determine whether AA and PGE2 treatments acted in concordance, a differential expression analysis was conducted between each treatment and the control using the *FindMarkers* function with the MAST method (Finak et al., 2015). Wilcoxon rank-sum tests to determine if gene expression was significant was conducted using the *wilcox.test* function in stats (v4.1.0, (R Core Team, 2021)). Monocle3 (v0.2.3, (Cao et al., 2019; Levine et al., 2015; Qiu et al., 2017; Trapnell et al., 2014) utilized for the trajectory analysis for both organoid and *in vivo* datasets.

Cut & Run

Sample preparation

- CutnRun was performed according to Henikoff et al.'s paper (Skene et al., 2018) with some minor changes. Briefly, intestinal organoids treated with vehicle or AA 25 μ M for 3
- 5 days or 6 days were assessed. 500,00 cells per replicate were counted and washed in 1ml of 1X PBS followed by an additional wash in 1 ml of wash buffer (20 mM HEPES pH 7.5, 150 mM NaCl, 0.5 mM Spermidine, and protease inhibitor cocktails). 10 μ l of BioMag
- 10 Concanviline A beads were resuspended and washed twice in 1 ml binding buffer (20 mM HEPES-KOH at pH 7.9, 10 mM KCl, 1 mM CaCl₂ and 1 mM MnCl₂). Pelleted cells were resuspended in 1 ml wash buffer and bead suspension added to cells and incubated on a nutator for 10 minutes at room temperature. Samples were placed on a magnetic rack until solution turned clear, then the liquid was replaced with the antibody buffer with the following
- 15 antibodies at 1:100 ratio; H3K4me₃, (Cat# ab8580, Abcam), H3K27me₃ (Cat# 07-449, Millipore), H3K27Ac (Cat# ab4729, Abcam) and incubated on a nutator at 4°C overnight.
- The next day, cells were washed with 1 ml of digitonin buffer (20 mM HEPES-NaOH pH
- 20 7.5, 150 mM NaCl, 0.5 mM Spermidine, 0.1% digitonin, and protease inhibitor cocktails) twice and resuspended in 150 μ l of digitonin buffer. In-house made pA-MNase was added at 700 ng/ml concentration, mixed gently and incubated on a nutator at 4°C for 1 hour.
- Afterwards, samples were washed twice in 1 ml digitonin buffer. Cells were resuspended in
- 25 150 μ l digitonin buffer, subsequently placed on a heat block sitting on wet ice for 5 minutes to chill down to 0°C. 3 μ l 100 mM CaCl₂ was added with gentle mixing and immediately replaced on 0°C block for incubation for 30 minutes. The digestion was stopped with 100 μ l 2X Stop buffer (340 mM NaCl, 20 mM EDTA, 4 mM EGTA, 0.02% Digitonin, 50mg/ml RNase A, 50mg/ml Glycogen, and 4 μ g/ml yeast heterologous spike-in DNA). The samples
- 30 were incubated for 10 minutes at 37°C and centrifuged for 5 minutes at 4°C at 16000 rcf. Samples were placed onto the magnetic rack and the supernatant was transferred to a fresh Eppendorf tube, without perturbing the pellet. 2 μ l of 10% SDS and 1.5 μ l of proteinase K were added to each sample and subsequently incubated for 10 minutes at 70°C. Afterward 200 μ l PCI were added and mixed at a full speed on vortex for 3 seconds followed by transfer to
- phase lock tubes and spun down at 16000 rcf for 5 minutes. Liquid above the gel was transferred into a fresh 1.5 ml eppendorf tube containing 2.5 μ l 3 mg/ml GlycoBlue (Cat# AM9515, Invitrogen) and mixed with 100% ethanol followed with 10 minutes incubation on ice and 30 minutes centrifugation at 16,000 rcf. The pellet was rinsed with 70% ethanol and spun for 10 minutes at maximum speed. After the last wash step ethanol was discarded and

all the remaining ethanol droplets removed with vacuum. The remaining pellet dissolved in 15 µl of TE Buffer (1 mM Tris-HCl pH=8, 0.1 mM EDTA).

Library preparation

Libraries were prepared using the ThruPLEX DNA-seq Kit (Cat# R400427, Takara) according to manufacturer's instructions with following PCR conditions: 72°C for 3 min, 85°C for 2 min, 98°C for 2 min, (98°C for 20s, 67°C for 20 s, 72°C for 30s) × 4 cycles, (98°C for 20s, 72°C for 20s) with 7 cycles of PCR. AMPure XP beads (Cat# A63880, Beckman Coulter) were used to select short fragments (0.5x-1.7x) to remove residual adaptor and large DNA fragment. The libraries were sequenced on a NextSeq500 using a 150 cycle v2 high output SBS kit by the CSHL The Sequencing Technologies Shared Resource. There was a 10% PhiX spiked in control sample and the sequencing was done as a paired end 76 length with an index read. Reads were demultiplexed by barcode via the bcl2fastq2 tool.

Analysis

Cut&Run libraries for H3K27me3, H3K27ac, H3K4me3, and IgG control were sequenced as paired-end with 76bp read length, with average of 13x2 million reads per sample. Sequencing data was aligned and processed by CUT&RUNtools using all read fragments without filtering for read length (Zhu et al., 2019). SEACR was used in the stringent mode to call peaks for each replicate separately, and a consensus peak set was called across replicates in different conditions by their overlap (Meers et al., 2019). Reads within peaks were quantified using featureCounts (Liao et al., 2014). Differential binding analysis between AA and Control was performed separately for each chromatin mark using a negative binomial model with DESeq2, and resulting peaks were filtered for an adjusted p-value of 0.01 (Love et al., 2014). Pathway analysis was performed by assigning peaks into genes by distance to their canonical transcription start site, and a distance threshold of 25kb, 25kb and 10kb was used for H3K27me3, H3K27ac, and H3K4me3, respectively. The many-to-one peak-to-gene annotations were collapsed into one-to-one by taking the maximally changing peak within the distance cutoff. For longer distances Genomic Regions Enrichment of Annotations Tool (GREAT) was used by basal plus extension method to annotate genes to putative distal enhancer and regulatory regions (McLean et al., 2010). The genes were ranked by the log2-fold-change value or the Wald statistic from the differential call. Pathway and gene set enrichments were calculated with GSEA and MSigDB database, as well as the gene sets from RNAseq and other published literature as described in the text (Liberzon et al., 2011; Subramanian et al., 2005). Significantly altered pathways were selected by applying an adjusted p-value cutoff of 0.05.

The profile plots for histone marks were calculated by binning the genome in 100bp bins centered around the TSS $\pm$ 5kb of the gene sets of interest and taking the normalized reads (RPGC) in those bins. Bins of specific distance from the TSS were summarized into the median value for plotting purposes. For the AA vs. Vehicle profile plots the same process was repeated for replicates of both conditions, then the difference of AA vs. Vehicle treated samples was taken before summarization by the median.

ATAC-seq

Nuclei Isolation

ATAC-seq was performed as previously described by Buenrostro et al. (Buenrostro et al., 2013) and Kaestner Lab. Briefly, 50,000 sorted alive cells from organoids were washed with 1 ml cold 1X PBS. Cells incubated with 50 μ l of lysis buffer (Tris-HCl, pH 7.5 (final 10 mM), NaCl (10 mM), MgCl₂ (3 mM), NP-40 (0.1% v/v), Tween-20 (0.1% v/v), Digitonin (0.1% v/v)) on ice for 3 minutes. 1 ml of wash buffer (Tris-HCl, pH 7.5 (final 10 mM), NaCl (10 mM), MgCl₂ (3 mM), Tween-20 (0.1% v/v) was added onto cells. Then centrifugation was done at 500g for 10 minutes at 4°C and supernatant was discarded. Pellets containing nuclei were kept for further experiments.

Library preparation

ATAC-seq libraries were generated as previously described by Buenrostro et al. and Kaestner Lab. Briefly, DNA was purified with the DNA Clean & Concentrator 5 (D4013; Zymo Research). Following the manufacturer standard protocol libraries were prepared with NEBNext High-Fidelity 2xPCR Master Mix (NEB, M0541S). DNA fragments were PCR preamplified for 5 cycles, and 5 μ l of partially-amplified library was used for qPCR amplification (20 cycles). The plot showing R vs. cycle number was generated to determine the number of cycles required to reach 1/3 of the maximum R for each sample. Then qPCR amplification was repeated with calculated additional cycles. Size selection was performed with Agencourt AMPure XP beads (A63881; Beckman Coulter) following amplification step to establish the final libraries (Ackermann AM et al). Library quality was determined with an Agilent High-Sensitivity DNA Bioanalyzer (5067- 4626; Agilent Technologies).

ATACseq analysis

DNA libraries were sequenced as pair-end with 76bp read length, with average of 46x2 million reads per sample. Sequence adapters were trimmed by Trim Galore!

(github.com/FelixKrueger/TrimGalore) and the resulting reads were aligned to the mm10 genome reference with BWA (Li and Durbin, 2009). Duplicate reads were marked by picard ("Picard Toolkit." 2019. Broad Institute, GitHub Repository. (broadinstitute.github.io/picard/)). After the alignment, mitochondrial reads, duplicate reads, reads in blacklisted regions, secondary alignments and multimapping reads, reads with insert size greater than 2kb or wrong orientation of the pairs, or orphan reads were filtered. Peaks were called using MACS2 narrow peak calling with shift of -75 basepairs and extension of 150 basepairs (Liu, 2014). Consensus peaks were called by taking the overlap of regions covered in at least 2 of the samples. Reads within peaks were quantified using featureCounts (Liao et al., 2014). Variance stabilizing transform (vst) was used for normalizing the data for visualizations and unsupervised clustering (Anders and Huber, 2010). The counts of AA treated samples were relative to control treated samples with a negative binomial comparison using DESeq2 and filtering for an absolute log2-fold-change value of 0.58 (minimum 50% change) and FDR adjusted p-value of 0.01 (Love et al., 2014).

The peaks were annotated and collapsed to genes by taking the maximally changing peak within 5kb of each transcription start site and assigning that peak to each gene by their promoter or putative proximal enhancer. The genes were ranked by the log2-fold-change value or the Wald statistic and fed into GSEA using MSigDB database and the curated gene signatures from the RNAseq experiments as well as literature. An adjusted p-value of 0.05 was used to filter the resulting gene sets (Liberzon et al., 2011; Subramanian et al., 2005). The correlation between RNAseq and ATACseq was performed by taking all the ATACseq peaks within 2.5kb of the transcription start site of every gene and binning the genes into differentially opening or closing and non-differential categories and plotting the log2-fold-change of expression differences.

Regulatory potential of transcription factors was calculated by comparing all the mammalian motifs in JASPAR (Castro-Mondragon et al., 2022) within the sequences of accessible peaks. The log₂ fold-changes of peak accessibility in AA vs Vehicle treated cells were modeled against the presence of such motifs in a multivariate manner while correcting for GC content, i.e. $\log_2\text{FC} \sim \beta_0 + \beta_1 * x_1 + \beta_2 * x_2 + \dots + \beta_N * \text{GC}$, where x_i denotes the presence of a TF motif in a peak as a binary variable and GC is the GC-content of the peak. The effect sizes and p-values for each term are taken after multivariate linear modeling using ordinary least squares regression with robust standard errors.

Statistics

Statistical analyses were performed by Graphpad Prism 9.0. The results were implemented to one of the following statistical tests; ANOVA, Wilcoxon rank-sum test, Mann-Whitney test, two-tailed t test, as indicated in figure legends. The bars are presented as the mean $\pm$ SEM. P values of <0.05 are considered statistically significant and represented as follows: *P < 0.05 , **P < 0.01 ***P < 0.005 , ****P < 0.001 .

Data and code availability

RNAseq, scRNAseq, CutnRun, Atac-seq data can be accessed from Gene Expression Omnibus (GEO) with the following accession numbers; GSE188213

Example 10: FA regulation of cell fate and the function in physiological and disease states

The present disclosure considers how members of omega-6 family fatty acids, such as Arachidonic acid (AA), may promote regeneration in the intestine and ameliorate the intestinal degenerative effects of genotoxic insults, such as radiation or chemotherapy. Dietary AA supplementation may be therapeutically significant in cancer patients receiving chemotherapy or radiotherapy, who often suffer from gastrointestinal side effects.

Example 11: The kinetics of AA-induced cellular alterations in the intestine

Mice fed with the AA-rich diet for 1, 2 or 4 weeks, are analyzed to define the kinetics of AA-induced intestinal regeneration. Approximately sixty mice (10C and 10ARD) are assessed at the three time points. Histology proxies are assessed and the metabolomics, specifically AA levels, are assessed as well.

Example 12: The optimal regimen of AA-mediated protection from genotoxic stress

The effects of diverse dietary regimens with AA supplementation, such as with continuous (AA ON), reversal (AA OFF) or cyclical (AA ON/OFF/ON/OFF) feeding in mice are assessed. Forty mice are separated into four groups of ten: C, ARD ON, ARD OFF, and ARD ON/OFF/ON/OFF. Histology is assessed. Metabolic testing assesses the AA levels and the four groups are further analyzed through eight scRNAseq libraries (n=2).

Example 13: The effect of dietary AA supplementation in response to chemotherapy against cancer

Cancer mouse models are established to assess the beneficial (e.g., therapeutic) significance of dietary AA supplementation in (1) ameliorating gastrointestinal side effects of chemotherapy and (2) improving overall survival of tumor-bearing mice. In a subset of animals, blood is assessed for impact of diet on chemotherapy-induced cytopenia. Approximately 20 mice are characterized between two groups: 10C and 10ARD. Histology proxies and metabolomics, specifically AA levels, are assessed.

Example 14: The effect of AA on human intestinal tissue in response to chemotherapy

Human intestinal organoid models are established. These models are used to assess the impact of AA treatment on damage from doxorubicin. Human PDOs (n=2, V/AA treatment +/- Doxorubicin).

Example 15: AA-rich diet does not increase colorectal cancer risk in tumor-prone mouse models

Tumor model

Tumor-prone mice (VillinCreERT2 APC L/+) were treated with tamoxifen and fed an AA-rich diet (ARD, also referred to as FA1) or a control diet as described in previous examples. After 3-4 months of the ARD or control diet, mice were sacrificed, and tumor burden was assessed. FIG. 19A shows a schematic of the experimental procedure. As shown in FIG. 19B, the ARD did not increase the number of tumors identified in the small intestine or colon. As shown in FIG. 19C, the total tumor burden of mice fed an ARD was not higher than the tumor burden of mice fed a control diet.

Metastatic cancer model

C57BL6/J mice were injected with AKPS (APC^{KO} , $KRAS^{G12D}$, $P53^{KO}$, $SMAD4^{KO}$) cells, as shown in FIG. 20A. After one week, the presence of the tumor was confirmed. Subsequently, mice were fed either an AA-rich diet or a control diet. Survival of the mice is shown in FIG. 20B. After sacrifice, the mice were dissected, and the primary tumor and metastases were assessed. As shown in FIG. 20C, the metastasis rate was the same between mice fed an AA-rich diet and mice fed a control diet. As demonstrated in this example, an

AA-rich diet does not increase the rate of metastasis or decrease survival compared to a control diet.

Example 16: Kinetics of AA-rich diet effects in mice

5

Plasma AA levels

To assess plasma levels of fatty acids over time, mice were fed i) a control diet for four weeks, ii) an AA-rich diet (Arasco) for four weeks, or iii) an AA-rich diet for two weeks and then switched to a control diet for two weeks (ArascoRev). Plasma lipid levels were
10 assessed at day 3, day 7, and day 14 for mice fed the control diet or Arasco diet; results are shown in FIG. 21A. Plasma lipid levels were assessed at four weeks for all three conditions; results are shown in FIG. 21B.

ARD-induced stem cell regeneration

15 Mice were fed a control diet (C) or an AA-rich diet (ARD) for 3 days, 1 week, or two weeks. The mice were then exposed to 15 Gy abdominal irradiation. FIG. 22A shows a schematic of the experimental protocol. Metrics, histology, and metabolomics were assessed for each mouse. The level of arachidonic acid observed under each condition are shown in FIG. 22B. FIG. 22C shows representative histological images (left) for mice fed a control diet
20 or ARD, as well as a graph showing the number of EdU+ cells/crypt. EdU (5-ethynyl-2'-deoxyuridine) is a marker of cell proliferation, and a higher number of EdU+ cells/crypt indicates increased cell proliferation in irradiated mice fed an ARD for 7 or 14 days.

Gene expression

25 Mice were fed one of three diets as described in the first section of this example. FIG. 23A shows a schematic of the experimental protocol. Subsequently, gene expression was assessed by scRNAseq and scATACseq, as described in earlier examples; results are shown in FIG. 23B.

30 ***Example 17: Protective effects of AA in response to 5-fluorouracil chemotherapy***

Mice were fed an AA-rich diet (ARD) or a control diet for two weeks as described in previous examples. The mice then were treated with vehicle control or a single dose of 5-fluorouracil (5-FU) of 50mg/kg, 250mg/kg, or 500mg/kg.

FIG. 24A shows a schematic of the experimental procedure. FIG. 24B shows that mice dosed with 250mg/kg 5-FU that were fed the ARD diet had less weight loss as compared to mice dosed with 250mg/kg 5-FU that were fed the control diet.

5 ***Example 18: Protective effects of AA in response to multidose chemotherapy***

Mice (8 weeks old) were fed either control diet or ARD for two weeks and then injected with 5-FU (100mg/kg) and Oxaliplatin (6mg/kg) in a regimen of one dose per week for two weeks, then allowed for two weeks recovery, and then repeated the regimen of one dose per week for two weeks.

10 FIG. 25A shows a schematic of the experimental procedure. FIG. 25B shows that mice fed the ARD diet had less weight loss as compared to mice that were fed the control diet.

References:

- 15 Alsabeeh, N., Chausse, B., Kakimoto, P.A., Kowaltowski, A.J., and Shirihai, O. (2018). Cell culture models of fatty acid overload: Problems and solutions. *Biochim Biophys Acta Mol Cell Biol Lipids* 1863, 143-151.
- Asfaha, S., Hayakawa, Y., Muley, A., Stokes, S., Graham, T.A., Ericksen, R.E., Westphalen, C.B., von Burstin, J., Mastracci, T.L., Worthley, D.L., et al. (2015). Krt19(+)/Lgr5(-) Cells Are Radioresistant Cancer-Initiating Stem Cells in the Colon and Intestine. *Cell Stem Cell* 20 16, 627-638.
- Ayyaz, A., Kumar, S., Sangiorgi, B., Ghoshal, B., Gosio, J., Ouladan, S., Fink, M., Barutcu, S., Trcka, D., Shen, J., et al. (2019). Single-cell transcriptomes of the regenerating intestine reveal a revival stem cell. *Nature* 569, 121-125.
- Bankaitis, E.D., Ha, A., Kuo, C.J., and Magness, S.T. (2018). Reserve Stem Cells in 25 Intestinal Homeostasis and Injury. *Gastroenterology* 155, 1348-1361.
- Barker, N., van Es, J.H., Kuipers, J., Kujala, P., van den Born, M., Cozijnsen, M., Haegbarth, A., Korving, J., Begthel, H., Peters, P.J., et al. (2007). Identification of stem cells in small intestine and colon by marker gene Lgr5. *Nature* 449, 1003-1007.
- Basak, O., Beumer, J., Wiebrands, K., Seno, H., van Oudenaarden, A., and Clevers, H. 30 (2017). Induced Quiescence of Lgr5+ Stem Cells in Intestinal Organoids Enables Differentiation of Hormone-Producing Enteroendocrine Cells. *Cell Stem Cell* 20, 177-190 e174.

- Berger, S.L., Kouzarides, T., Shiekhata, R., and Shilatifard, A. (2009). An operational definition of epigenetics. *Genes Dev* 23, 781-783.
- Beumer, J., and Clevers, H. (2016). Regulation and plasticity of intestinal stem cells during homeostasis and regeneration. *Development* 143, 3639-3649.
- 5 Beyaz, S., Chung, C., Mou, H., Bauer-Rowe, K.E., Xifaras, M.E., Ergin, I., Dohnalova, L., Biton, M., Shekhar, K., Eskiocak, O., et al. (2021a). Dietary suppression of MHC class II expression in intestinal epithelial cells enhances intestinal tumorigenesis. *Cell Stem Cell* 28, 1922-1935 e1925.
- Beyaz, S., Kim, J.H., Pinello, L., Xifaras, M.E., Hu, Y., Huang, J., Kerenyi, M.A., Das, P.P.,
10 Barnitz, R.A., Herault, A., et al. (2017). The histone demethylase UTX regulates the lineage-specific epigenetic program of invariant natural killer T cells. *Nat Immunol* 18, 184-195.
- Beyaz, S., Mana, M.D., Roper, J., Kedrin, D., Saadatpour, A., Hong, S.J., Bauer-Rowe, K.E., Xifaras, M.E., Akkad, A., Arias, E., et al. (2016). High-fat diet enhances stemness and tumorigenicity of intestinal progenitors. *Nature* 531, 53-58.
- 15 Beyaz, S., Mana, M.D., and Yilmaz, O.H. (2021b). High-fat diet activates a PPAR-delta program to enhance intestinal stem cell function. *Cell Stem Cell* 28, 598-599.
- Beyaz, S., and Yilmaz, O.H. (2016). Molecular Pathways: Dietary Regulation of Stemness and Tumor Initiation by the PPAR-delta Pathway. *Clin Cancer Res* 22, 5636-5641.
- Biteau, B., and Jasper, H. (2011). EGF signaling regulates the proliferation of intestinal stem
20 cells in *Drosophila*. *Development* 138, 1045-1055.
- Biton, M., Haber, A.L., Rogel, N., Burgin, G., Beyaz, S., Schnell, A., Ashenberg, O., Su, C.W., Smillie, C., Shekhar, K., et al. (2018). T Helper Cell Cytokines Modulate Intestinal Stem Cell Renewal and Differentiation. *Cell* 175, 1307-1320 e1322.
- Brash, A.R. (2001). Arachidonic acid as a bioactive molecule. *J Clin Invest* 107, 1339-1345.
- 25 Bresnick, A.R., Weber, D.J., and Zimmer, D.B. (2015). S100 proteins in cancer. *Nat Rev Cancer* 15, 96-109.
- Breyer, R.M., Bagdassarian, C.K., Myers, S.A., and Breyer, M.D. (2001). Prostanoid receptors: subtypes and signaling. *Annu Rev Pharmacol Toxicol* 41, 661-690.
- Buczacki, S.J., Zecchini, H.I., Nicholson, A.M., Russell, R., Vermeulen, L., Kemp, R., and
30 Winton, D.J. (2013). Intestinal label-retaining cells are secretory precursors expressing Lgr5. *Nature* 495, 65-69.

- Buenrostro, J.D., Giresi, P.G., Zaba, L.C., Chang, H.Y., and Greenleaf, W.J. (2013). Transposition of native chromatin for fast and sensitive epigenomic profiling of open chromatin, DNA-binding proteins and nucleosome position. *Nat Methods* 10, 1213-1218.
- Calder, P.C., Campoy, C., Eilander, A., Fleith, M., Forsyth, S., Larsson, P.O., Schelkle, B., Lohner, S., Szommer, A., van de Heijning, B.J.M., et al. (2019). A systematic review of the effects of increasing arachidonic acid intake on PUFA status, metabolism and health-related outcomes in humans. *Br J Nutr* 121, 1201-1214.
- Cao, J., Spielmann, M., Qiu, X., Huang, X., Ibrahim, D.M., Hill, A.J., Zhang, F., Mundlos, S., Christiansen, L., Steemers, F.J., et al. (2019). The single-cell transcriptional landscape of mammalian organogenesis. *Nature* 566, 496-502.
- Chandel, N.S., Jasper, H., Ho, T.T., and Passegue, E. (2016). Metabolic regulation of stem cell function in tissue homeostasis and organismal ageing. *Nat Cell Biol* 18, 823-832.
- Cheasley, D., Pereira, L., Lightowler, S., Vincan, E., Malaterre, J., and Ramsay, R.G. (2011). Myb controls intestinal stem cell genes and self-renewal. *Stem Cells* 29, 2042-2050.
- Chen, L., Vasoya, R.P., Toke, N.H., Parthasarathy, A., Luo, S., Chiles, E., Flores, J., Gao, N., Bonder, E.M., Su, X., et al. (2020). HNF4 Regulates Fatty Acid Oxidation and Is Required for Renewal of Intestinal Stem Cells in Mice. *Gastroenterology* 158, 985-999 e989.
- Cheng, C.W., Biton, M., Haber, A.L., Gunduz, N., Eng, G., Gaynor, L.T., Tripathi, S., Calibasi-Kocal, G., Rickelt, S., Butty, V.L., et al. (2019). Ketone Body Signaling Mediates Intestinal Stem Cell Homeostasis and Adaptation to Diet. *Cell* 178, 1115-1131 e1115.
- Cheng, H., Huang, H., Guo, Z., Chang, Y., and Li, Z. (2021). Role of prostaglandin E2 in tissue repair and regeneration. *Theranostics* 11, 8836-8854.
- Cheng, H., and Leblond, C.P. (1974). Origin, differentiation and renewal of the four main epithelial cell types in the mouse small intestine. I. Columnar cell. *Am J Anat* 141, 461-479.
- Chijiwa, T., Mishima, A., Hagiwara, M., Sano, M., Hayashi, K., Inoue, T., Naito, K., Toshioka, T., and Hidaka, H. (1990). Inhibition of forskolin-induced neurite outgrowth and protein phosphorylation by a newly synthesized selective inhibitor of cyclic AMP-dependent protein kinase, N-[2-(p-bromocinnamylamino)ethyl]-5-isoquinolinesulfonamide (H-89), of PC12D pheochromocytoma cells. *J Biol Chem* 265, 5267-5272.
- Cimmino, L., Neel, B.G., and Aifantis, I. (2018). Vitamin C in Stem Cell Reprogramming and Cancer. *Trends Cell Biol* 28, 698-708.

- Clevers, H. (2013). The intestinal crypt, a prototype stem cell compartment. *Cell* 154, 274-284.
- Costea, I., Mack, D.R., Lemaitre, R.N., Israel, D., Marcil, V., Ahmad, A., and Amre, D.K. (2014). Interactions between the dietary polyunsaturated fatty acid ratio and genetic factors determine susceptibility to pediatric Crohn's disease. *Gastroenterology* 146, 929-931.
- 5 Crawley, S.W., Mooseker, M.S., and Tyska, M.J. (2014). Shaping the intestinal brush border. *J Cell Biol* 207, 441-451.
- Das, P.P., Shao, Z., Beyaz, S., Apostolou, E., Pinello, L., De Los Angeles, A., O'Brien, K., Atsma, J.M., Fujiwara, Y., Nguyen, M., et al. (2014). Distinct and combinatorial functions of Jmjd2b/Kdm4b and Jmjd2c/Kdm4c in mouse embryonic stem cell identity. *Mol Cell* 53, 32-48.
- 10 de Sousa, E.M.F., and de Sauvage, F.J. (2019). Cellular Plasticity in Intestinal Homeostasis and Disease. *Cell Stem Cell* 24, 54-64.
- Degirmenci, B., Valenta, T., Dimitrieva, S., Hausmann, G., and Basler, K. (2018). GLI1-expressing mesenchymal cells form the essential Wnt-secreting niche for colon stem cells. *Nature* 558, 449-453.
- 15 Dekaney, C.M., Gulati, A.S., Garrison, A.P., Helmrath, M.A., and Henning, S.J. (2009). Regeneration of intestinal stem/progenitor cells following doxorubicin treatment of mice. *Am J Physiol Gastrointest Liver Physiol* 297, G461-470.
- 20 Deng, H., Gerencser, A.A., and Jasper, H. (2015). Signal integration by Ca(2+) regulates intestinal stem-cell activity. *Nature* 528, 212-217.
- Doane, A.S., Chu, C.S., Di Giammartino, D.C., Rivas, M.A., Hellmuth, J.C., Jiang, Y., Yusufova, N., Alonso, A., Roeder, R.G., Apostolou, E., et al. (2021). OCT2 pre-positioning facilitates cell fate transition and chromatin architecture changes in humoral immunity. *Nat Immunol* 22, 1327-1340.
- 25 Dupuis, J., Langenberg, C., Prokopenko, I., Saxena, R., Soranzo, N., Jackson, A.U., Wheeler, E., Glazer, N.L., Bouatia-Naji, N., Gloyn, A.L., et al. (2010). New genetic loci implicated in fasting glucose homeostasis and their impact on type 2 diabetes risk. *Nat Genet* 42, 105-116.
- Eeckhoutte, J., Carroll, J.S., Geistlinger, T.R., Torres-Arzayus, M.I., and Brown, M. (2006). A cell-type-specific transcriptional network required for estrogen regulation of cyclin D1 and cell cycle progression in breast cancer. *Genes Dev* 20, 2513-2526.
- 30

- Evans, R.M., and Mangelsdorf, D.J. (2014). Nuclear Receptors, RXR, and the Big Bang. *Cell* 157, 255-266.
- Fan, Y.Y., Callaway, E., J, M.M., J, S.G., Yang, P., Vincent, L., and R, S.C. (2016). A New Model to Study the Role of Arachidonic Acid in Colon Cancer Pathophysiology. *Cancer Prev Res (Phila)* 9, 750-757.
- Fan, Y.Y., Davidson, L.A., Callaway, E.S., Goldsby, J.S., and Chapkin, R.S. (2014). Differential effects of 2- and 3-series E-prostaglandins on in vitro expansion of Lgr5+ colonic stem cells. *Carcinogenesis* 35, 606-612.
- Fan, Y.Y., Monk, J.M., Hou, T.Y., Callway, E., Vincent, L., Weeks, B., Yang, P., and Chapkin, R.S. (2012). Characterization of an arachidonic acid-deficient (Fads1 knockout) mouse model. *J Lipid Res* 53, 1287-1295.
- Farin, H.F., Van Es, J.H., and Clevers, H. (2012). Redundant sources of Wnt regulate intestinal stem cells and promote formation of Paneth cells. *Gastroenterology* 143, 1518-1529 e1517.
- Fu, T., Coulter, S., Yoshihara, E., Oh, T.G., Fang, S., Cayabyab, F., Zhu, Q., Zhang, T., Leblanc, M., Liu, S., et al. (2019). FXR Regulates Intestinal Cancer Stem Cell Proliferation. *Cell* 176, 1098-1112 e1018.
- Gehart, H., and Clevers, H. (2019). Tales from the crypt: new insights into intestinal stem cells. *Nat Rev Gastroenterol Hepatol* 16, 19-34.
- Gerdes, J., Lemke, H., Baisch, H., Wacker, H.H., Schwab, U., and Stein, H. (1984). Cell cycle analysis of a cell proliferation-associated human nuclear antigen defined by the monoclonal antibody Ki-67. *J Immunol* 133, 1710-1715.
- Goessling, W., North, T.E., Loewer, S., Lord, A.M., Lee, S., Stoick-Cooper, C.L., Weidinger, G., Puder, M., Daley, G.Q., Moon, R.T., et al. (2009). Genetic interaction of PGE2 and Wnt signaling regulates developmental specification of stem cells and regeneration. *Cell* 136, 1136-1147.
- Gregorieff, A., Liu, Y., Inanlou, M.R., Khomchuk, Y., and Wrana, J.L. (2015). Yap-dependent reprogramming of Lgr5(+) stem cells drives intestinal regeneration and cancer. *Nature* 526, 715-718.
- Greicius, G., Kabiri, Z., Sigmundsson, K., Liang, C., Bunte, R., Singh, M.K., and Virshup, D.M. (2018). PDGFRalpha(+) pericryptal stromal cells are the critical source of Wnts and

- RSPO3 for murine intestinal stem cells in vivo. *Proc Natl Acad Sci U S A* 115, E3173-E3181.
- Grun, D., Lyubimova, A., Kester, L., Wiebrands, K., Basak, O., Sasaki, N., Clevers, H., and van Oudenaarden, A. (2015). Single-cell messenger RNA sequencing reveals rare intestinal cell types. *Nature* 525, 251-255.
- Haber, A.L., Biton, M., Rogel, N., Herbst, R.H., Shekhar, K., Smillie, C., Burgin, G., Delorey, T.M., Howitt, M.R., Katz, Y., et al. (2017). A single-cell survey of the small intestinal epithelium. *Nature* 551, 333-339.
- Hanson, W.R., and Ainsworth, E.J. (1985). 16,16-Dimethyl prostaglandin E2 induces radioprotection in murine intestinal and hematopoietic stem cells. *Radiat Res* 103, 196-203.
- Hoffmeyer, K., Raggioli, A., Rudloff, S., Anton, R., Hierholzer, A., Del Valle, I., Hein, K., Vogt, R., and Kemler, R. (2012). Wnt/beta-catenin signaling regulates telomerase in stem cells and cancer cells. *Science* 336, 1549-1554.
- Igarashi, M., and Guarente, L. (2016). mTORC1 and SIRT1 Cooperate to Foster Expansion of Gut Adult Stem Cells during Calorie Restriction. *Cell* 166, 436-450.
- Ijiri, K., and Potten, C.S. (1987). Further studies on the response of intestinal crypt cells of different hierarchical status to eighteen different cytotoxic agents. *Br J Cancer* 55, 113-123.
- Insel, P.A., Murray, F., Yokoyama, U., Romano, S., Yun, H., Brown, L., Snead, A., Lu, D., and Aroonsakool, N. (2012). cAMP and Epac in the regulation of tissue fibrosis. *Br J Pharmacol* 166, 447-456.
- Jacobi, S.K., Moeser, A.J., Corl, B.A., Harrell, R.J., Blikslager, A.T., and Odle, J. (2012). Dietary long-chain PUFA enhance acute repair of ischemia-injured intestine of suckling pigs. *J Nutr* 142, 1266-1271.
- Jadhav, U., Nalapareddy, K., Saxena, M., O'Neill, N.K., Pinello, L., Yuan, G.C., Orkin, S.H., and Shivdasani, R.A. (2016). Acquired Tissue-Specific Promoter Bivalency Is a Basis for PRC2 Necessity in Adult Cells. *Cell* 165, 1389-1400.
- Jadhav, U., Saxena, M., O'Neill, N.K., Saadatpour, A., Yuan, G.C., Herbert, Z., Murata, K., and Shivdasani, R.A. (2017). Dynamic Reorganization of Chromatin Accessibility Signatures during Dedifferentiation of Secretory Precursors into Lgr5+ Intestinal Stem Cells. *Cell Stem Cell* 21, 65-77 e65.

- Jiang, G.L., Im, W.B., Donde, Y., and Wheeler, L.A. (2009). EP4 agonist alleviates indomethacin-induced gastric lesions and promotes chronic gastric ulcer healing. *World J Gastroenterol* 15, 5149-5156.
- Jiang, H., and Edgar, B.A. (2009). EGFR signaling regulates the proliferation of *Drosophila* adult midgut progenitors. *Development* 136, 483-493.
- Jijon, H.B., Suarez-Lopez, L., Diaz, O.E., Das, S., De Calisto, J., Parada-kusz, M., Yaffe, M.B., Pittet, M.J., Mora, J.R., Belkaid, Y., et al. (2018). Intestinal epithelial cell-specific RARalpha depletion results in aberrant epithelial cell homeostasis and underdeveloped immune system. *Mucosal Immunol* 11, 703-715.
- Kaaij, L.T., van de Wetering, M., Fang, F., Decato, B., Molaro, A., van de Werken, H.J., van Es, J.H., Schuijers, J., de Wit, E., de Laat, W., et al. (2013). DNA methylation dynamics during intestinal stem cell differentiation reveals enhancers driving gene expression in the villus. *Genome Biol* 14, R50.
- Kaiko, G.E., Ryu, S.H., Koues, O.I., Collins, P.L., Solnica-Krezel, L., Pearce, E.J., Pearce, E.L., Oltz, E.M., and Stappenbeck, T.S. (2016). The Colonic Crypt Protects Stem Cells from Microbiota-Derived Metabolites. *Cell* 165, 1708-1720.
- Katikaneni, A., Jelcic, M., Gerlach, G.F., Ma, Y., Overholtzer, M., and Niethammer, P. (2020). Lipid peroxidation regulates long-range wound detection through 5-lipoxygenase in zebrafish. *Nat Cell Biol* 22, 1049-1055.
- Kazakevych, J., Sayols, S., Messner, B., Krienke, C., and Soshnikova, N. (2017). Dynamic changes in chromatin states during specification and differentiation of adult intestinal stem cells. *Nucleic Acids Res* 45, 5770-5784.
- Kikukawa, H., Sakuradani, E., Ando, A., Shimizu, S., and Ogawa, J. (2018). Arachidonic acid production by the oleaginous fungus *Mortierella alpina* 1S-4: A review. *J Adv Res* 11, 15-22.
- Kim, C.K., Yang, V.W., and Bialkowska, A.B. (2017). The Role of Intestinal Stem Cells in Epithelial Regeneration Following Radiation-Induced Gut Injury. *Curr Stem Cell Rep* 3, 320-332.
- Kim, T.H., Li, F., Ferreira-Neira, I., Ho, L.L., Luyten, A., Nalapareddy, K., Long, H., Verzi, M., and Shivdasani, R.A. (2014). Broadly permissive intestinal chromatin underlies lateral inhibition and cell plasticity. *Nature* 506, 511-515.

- Kirsch, D.G., Santiago, P.M., di Tomaso, E., Sullivan, J.M., Hou, W.S., Dayton, T., Jeffords, L.B., Sodha, P., Mercer, K.L., Cohen, R., et al. (2010). p53 controls radiation-induced gastrointestinal syndrome in mice independent of apoptosis. *Science* 327, 593-596.
- Leblond, C.P., and Stevens, C.E. (1948). The constant renewal of the intestinal epithelium in the albino rat. *Anat Rec* 100, 357-377.
- Lee, D., Pearsall, R.S., Das, S., Dey, S.K., Godfrey, V.L., and Threadgill, D.W. (2004). Epi-regulin is not essential for development of intestinal tumors but is required for protection from intestinal damage. *Mol Cell Biol* 24, 8907-8916.
- Lee, Y.S., Kim, T.Y., Kim, Y., Lee, S.H., Kim, S., Kang, S.W., Yang, J.Y., Baek, I.J., Sung, Y.H., Park, Y.Y., et al. (2018). Microbiota-Derived Lactate Accelerates Intestinal Stem-Cell-Mediated Epithelial Development. *Cell Host Microbe* 24, 833-846 e836.
- Li, H.J., Ray, S.K., Pan, N., Haigh, J., Fritzsche, B., and Leiter, A.B. (2019). Intestinal Neurod1 expression impairs paneth cell differentiation and promotes enteroendocrine lineage specification. *Sci Rep* 9, 19489.
- Li, L., and Fan, C.M. (2017). A CREB-MPP7-AMOT Regulatory Axis Controls Muscle Stem Cell Expansion and Self-Renewal Competence. *Cell Rep* 21, 1253-1266.
- Libioulle, C., Louis, E., Hansoul, S., Sandor, C., Farnir, F., Franchimont, D., Vermeire, S., Dewit, O., de Vos, M., Dixon, A., et al. (2007). Novel Crohn disease locus identified by genome-wide association maps to a gene desert on 5p13.1 and modulates expression of PTGER4. *PLoS Genet* 3, e58.
- Lin, X., Gaudino, S.J., Jang, K.K., Bahadur, T., Singh, A., Banerjee, A., Beaupre, M., Chu, T., Wong, H.T., Kim, C.K., et al. (2022). IL-17RA-signaling in Lgr5(+) intestinal stem cells induces expression of transcription factor ATOH1 to promote secretory cell lineage commitment. *Immunity* 55, 237-253 e238.
- Lindemans, C.A., Calafiore, M., Mertelsmann, A.M., O'Connor, M.H., Dudakov, J.A., Jenq, R.R., Velardi, E., Young, L.F., Smith, O.M., Lawrence, G., et al. (2015). Interleukin-22 promotes intestinal-stem-cell-mediated epithelial regeneration. *Nature* 528, 560-564.
- Lu, C., and Thompson, C.B. (2012). Metabolic regulation of epigenetics. *Cell Metab* 16, 9-17.

- Lukonin, I., Serra, D., Challet Meylan, L., Volkmann, K., Baaten, J., Zhao, R., Meeusen, S., Colman, K., Maurer, F., Stadler, M.B., et al. (2020). Phenotypic landscape of intestinal organoid regeneration. *Nature* 586, 275-280.
- Martinez, F.J., Calverley, P.M., Goehring, U.M., Brose, M., Fabbri, L.M., and Rabe, K.F. (2015). Effect of roflumilast on exacerbations in patients with severe chronic obstructive pulmonary disease uncontrolled by combination therapy (REACT): a multicentre randomised controlled trial. *Lancet* 385, 857-866.
- McArthur, M.J., Atshaves, B.P., Frolov, A., Foxworth, W.D., Kier, A.B., and Schroeder, F. (1999). Cellular uptake and intracellular trafficking of long chain fatty acids. *J Lipid Res* 40, 1371-1383.
- McDonnell, E., Crown, S.B., Fox, D.B., Kitir, B., Ilkayeva, O.R., Olsen, C.A., Grimsrud, P.A., and Hirschey, M.D. (2016). Lipids Reprogram Metabolism to Become a Major Carbon Source for Histone Acetylation. *Cell Rep* 17, 1463-1472.
- Meers, M.P., Bryson, T.D., Henikoff, J.G., and Henikoff, S. (2019). Improved CUT&RUN chromatin profiling tools. *Elife* 8.
- Mihaylova, M.M., Cheng, C.W., Cao, A.Q., Tripathi, S., Mana, M.D., Bauer-Rowe, K.E., Abu-Remaileh, M., Clavain, L., Erdemir, A., Lewis, C.A., et al. (2018). Fasting Activates Fatty Acid Oxidation to Enhance Intestinal Stem Cell Function during Homeostasis and Aging. *Cell Stem Cell* 22, 769-778 e764.
- Miyoshi, H., VanDussen, K.L., Malvin, N.P., Ryu, S.H., Wang, Y., Sonnek, N.M., Lai, C.W., and Stappenbeck, T.S. (2017). Prostaglandin E2 promotes intestinal repair through an adaptive cellular response of the epithelium. *EMBO J* 36, 5-24.
- Molenaar, M., van de Wetering, M., Oosterwegel, M., Peterson-Maduro, J., Godsave, S., Korinek, V., Roose, J., Destree, O., and Clevers, H. (1996). XTcf-3 transcription factor mediates beta-catenin-induced axis formation in *Xenopus* embryos. *Cell* 86, 391-399.
- Montgomery, R.K., Carlone, D.L., Richmond, C.A., Farilla, L., Kranendonk, M.E., Henderson, D.E., Baffour-Awuah, N.Y., Ambruzs, D.M., Fogli, L.K., Algra, S., et al. (2011). Mouse telomerase reverse transcriptase (mTert) expression marks slowly cycling intestinal stem cells. *Proc Natl Acad Sci U S A* 108, 179-184.
- Monticelli, L.A., Osborne, L.C., Noti, M., Tran, S.V., Zaiss, D.M., and Artis, D. (2015). IL-33 promotes an innate immune pathway of intestinal tissue protection dependent on amphiregulin-EGFR interactions. *Proc Natl Acad Sci U S A* 112, 10762-10767.

- Moon, Y.A., Hammer, R.E., and Horton, J.D. (2009). Deletion of ELOVL5 leads to fatty liver through activation of SREBP-1c in mice. *J Lipid Res* 50, 412-423.
- Munoz, J., Stange, D.E., Schepers, A.G., van de Wetering, M., Koo, B.K., Itzkovitz, S., Volckmann, R., Kung, K.S., Koster, J., Radulescu, S., et al. (2012). The Lgr5 intestinal stem cell signature: robust expression of proposed quiescent '+4' cell markers. *EMBO J* 31, 3079-3091.
- Murata, K., Jadhav, U., Madha, S., van Es, J., Dean, J., Cavazza, A., Wucherpennig, K., Michor, F., Clevers, H., and Shivdasani, R.A. (2020). Ascl2-Dependent Cell Dedifferentiation Drives Regeneration of Ablated Intestinal Stem Cells. *Cell Stem Cell* 26, 377-390 e376.
- Mustata, R.C., Vasile, G., Fernandez-Vallone, V., Strollo, S., Lefort, A., Libert, F., Monteyne, D., Perez-Morga, D., Vassart, G., and Garcia, M.I. (2013). Identification of Lgr5-independent spheroid-generating progenitors of the mouse fetal intestinal epithelium. *Cell Rep* 5, 421-432.
- Naik, S., Larsen, S.B., Gomez, N.C., Alaverdyan, K., Sendoel, A., Yuan, S., Polak, L., Kulukian, A., Chai, S., and Fuchs, E. (2017). Inflammatory memory sensitizes skin epithelial stem cells to tissue damage. *Nature* 550, 475-480.
- Nakase, H., Fujiyama, Y., Oshitani, N., Oga, T., Nonomura, K., Matsuoka, T., Esaki, Y., Murayama, T., Teramukai, S., Chiba, T., et al. (2010). Effect of EP4 agonist (ONO-4819CD) for patients with mild to moderate ulcerative colitis refractory to 5-aminosalicylates: a randomized phase II, placebo-controlled trial. *Inflamm Bowel Dis* 16, 731-733.
- Nandan, M.O., Ghaleb, A.M., Bialkowska, A.B., and Yang, V.W. (2015). Kruppel-like factor 5 is essential for proliferation and survival of mouse intestinal epithelial stem cells. *Stem Cell Res* 14, 10-19.
- Narumiya, S., Sugimoto, Y., and Ushikubi, F. (1999). Prostanoid receptors: structures, properties, and functions. *Physiol Rev* 79, 1193-1226.
- Nateri, A.S., Spencer-Dene, B., and Behrens, A. (2005). Interaction of phosphorylated c-Jun with TCF4 regulates intestinal cancer development. *Nature* 437, 281-285.
- Neels, J.G., and Grimaldi, P.A. (2014). Physiological functions of peroxisome proliferator-activated receptor beta. *Physiol Rev* 94, 795-858.

- Nigmatullina, L., Norkin, M., Dzama, M.M., Messner, B., Sayols, S., and Soshnikova, N. (2017). Id2 controls specification of Lgr5(+) intestinal stem cell progenitors during gut development. *EMBO J* 36, 869-885.
- Novak, J.S.S., Baksh, S.C., and Fuchs, E. (2021). Dietary interventions as regulators of stem cell behavior in homeostasis and disease. *Genes Dev* 35, 199-211.
- Nusse, Y.M., Savage, A.K., Marangoni, P., Rosendahl-Huber, A.K.M., Landman, T.A., de Sauvage, F.J., Locksley, R.M., and Klein, O.D. (2018). Parasitic helminths induce fetal-like reversion in the intestinal stem cell niche. *Nature* 559, 109-113.
- Ordovas-Montanes, J., Beyaz, S., Rakoff-Nahoum, S., and Shalek, A.K. (2020). Distribution and storage of inflammatory memory in barrier tissues. *Nat Rev Immunol* 20, 308-320.
- Oszvald, A., Szvicsek, Z., Sandor, G.O., Kelemen, A., Soos, A.A., Paloczi, K., Bursics, A., Dede, K., Tolgyes, T., Buzas, E.I., et al. (2020). Extracellular vesicles transmit epithelial growth factor activity in the intestinal stem cell niche. *Stem Cells* 38, 291-300.
- Palla, A.R., Ravichandran, M., Wang, Y.X., Alexandrova, L., Yang, A.V., Kraft, P., Holbrook, C.A., Schurch, C.M., Ho, A.T.V., and Blau, H.M. (2021). Inhibition of prostaglandin-degrading enzyme 15-PGDH rejuvenates aged muscle mass and strength. *Science* 371.
- Patankar, J.V., Muller, T.M., Kantham, S., Acera, M.G., Mascia, F., Scheibe, K., Mahapatro, M., Heichler, C., Yu, Y., Li, W., et al. (2021). E-type prostanoid receptor 4 drives resolution of intestinal inflammation by blocking epithelial necroptosis. *Nat Cell Biol* 23, 796-807.
- Pellegrinet, L., Rodilla, V., Liu, Z., Chen, S., Koch, U., Espinosa, L., Kaestner, K.H., Kopan, R., Lewis, J., and Radtke, F. (2011). Dll1- and dll4-mediated notch signaling are required for homeostasis of intestinal stem cells. *Gastroenterology* 140, 1230-1240 e1231-1237.
- Peregrina, K., Houston, M., Daroqui, C., Dhima, E., Sellers, R.S., and Augenlicht, L.H. (2015). Vitamin D is a determinant of mouse intestinal Lgr5 stem cell functions. *Carcinogenesis* 36, 25-31.
- Potten, C.S. (1977). Extreme sensitivity of some intestinal crypt cells to X and gamma irradiation. *Nature* 269, 518-521.
- Potten, C.S., Hume, W.J., Reid, P., and Cairns, J. (1978). The segregation of DNA in epithelial stem cells. *Cell* 15, 899-906.

- Rodon, L., Svensson, R.U., Wiater, E., Chun, M.G.H., Tsai, W.W., Eichner, L.J., Shaw, R.J., and Montminy, M. (2019). The CREB coactivator CRTC2 promotes oncogenesis in LKB1-mutant non-small cell lung cancer. *Sci Adv* 5, eaaw6455.
- Rodriguez-Colman, M.J., Schewe, M., Meerlo, M., Stigter, E., Gerrits, J., Pras-Raves, M.,
 5 Sacchetti, A., Hornsveld, M., Oost, K.C., Snippert, H.J., et al. (2017). Interplay between metabolic identities in the intestinal crypt supports stem cell function. *Nature* 543, 424-427.
- Roulis, M., Kaklamanos, A., Scherthanner, M., Bielecki, P., Zhao, J., Kaffe, E., Frommelt, L.S., Qu, R., Knapp, M.S., Henriques, A., et al. (2020). Paracrine orchestration of intestinal tumorigenesis by a mesenchymal niche. *Nature* 580, 524-529.
- 10 Roulis, M., Nikolaou, C., Kotsaki, E., Kaffe, E., Karagianni, N., Koliarakis, V., Salpea, K., Ragoussis, J., Aidinis, V., Martini, E., et al. (2014). Intestinal myofibroblast-specific Tpl2-Cox-2-PGE2 pathway links innate sensing to epithelial homeostasis. *Proc Natl Acad Sci U S A* 111, E4658-4667.
- Sabatti, C., Service, S.K., Hartikainen, A.L., Pouta, A., Ripatti, S., Brodsky, J., Jones, C.G.,
 15 Zaitlen, N.A., Varilo, T., Kaakinen, M., et al. (2009). Genome-wide association analysis of metabolic traits in a birth cohort from a founder population. *Nat Genet* 41, 35-46.
- Salic, A., and Mitchison, T.J. (2008). A chemical method for fast and sensitive detection of DNA synthesis in vivo. *Proc Natl Acad Sci U S A* 105, 2415-2420.
- Sampurno, S., Bijenhof, A., Cheasley, D., Xu, H., Robine, S., Hilton, D., Alexander, W.S.,
 20 Pereira, L., Mantamadiotis, T., Malaterre, J., et al. (2013). The Myb-p300-CREB axis modulates intestine homeostasis, radiosensitivity and tumorigenesis. *Cell Death Dis* 4, e605.
- Sassone-Corsi, P. (2012). The cyclic AMP pathway. *Cold Spring Harb Perspect Biol* 4.
- Sato, T., van Es, J.H., Snippert, H.J., Stange, D.E., Vries, R.G., van den Born, M., Barker, N., Shroyer, N.F., van de Wetering, M., and Clevers, H. (2011). Paneth cells constitute the niche
 25 for Lgr5 stem cells in intestinal crypts. *Nature* 469, 415-418.
- Sato, T., Vries, R.G., Snippert, H.J., van de Wetering, M., Barker, N., Stange, D.E., van Es, J.H., Abo, A., Kujala, P., Peters, P.J., et al. (2009). Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. *Nature* 459, 262-265.
- Schell, J.C., Wisidagama, D.R., Bensard, C., Zhao, H., Wei, P., Tanner, J., Flores, A.,
 30 Mohlman, J., Sorensen, L.K., Earl, C.S., et al. (2017). Control of intestinal stem cell function and proliferation by mitochondrial pyruvate metabolism. *Nat Cell Biol* 19, 1027-1036.

- Schepers, A.G., Vries, R., van den Born, M., van de Wetering, M., and Clevers, H. (2011). Lgr5 intestinal stem cells have high telomerase activity and randomly segregate their chromosomes. *EMBO J* 30, 1104-1109.
- Schmitt, M., Schewe, M., Sacchetti, A., Feijtel, D., van de Geer, W.S., Teeuwssen, M.,
5 Sleddens, H.F., Joosten, R., van Royen, M.E., van de Werken, H.J.G., et al. (2018). Paneth Cells Respond to Inflammation and Contribute to Tissue Regeneration by Acquiring Stem-like Features through SCF/c-Kit Signaling. *Cell Rep* 24, 2312-2328 e2317.
- Schuijers, J., Junker, J.P., Mokry, M., Hatzis, P., Koo, B.K., Sasselli, V., van der Flier, L.G.,
Cuppen, E., van Oudenaarden, A., and Clevers, H. (2015). Ascl2 acts as an R-spondin/Wnt-
10 responsive switch to control stemness in intestinal crypts. *Cell Stem Cell* 16, 158-170.
- Schvartzman, J.M., Thompson, C.B., and Finley, L.W.S. (2018). Metabolic regulation of chromatin modifications and gene expression. *J Cell Biol* 217, 2247-2259.
- Serra, D., Mayr, U., Boni, A., Lukonin, I., Rempfler, M., Challet Meylan, L., Stadler, M.B.,
Strnad, P., Papasaikas, P., Vischi, D., et al. (2019). Self-organization and symmetry breaking
15 in intestinal organoid development. *Nature* 569, 66-72.
- Shao, J., and Sheng, H. (2010). Amphiregulin promotes intestinal epithelial regeneration: roles of intestinal subepithelial myofibroblasts. *Endocrinology* 151, 3728-3737.
- Shaywitz, A.J., and Greenberg, M.E. (1999). CREB: a stimulus-induced transcription factor activated by a diverse array of extracellular signals. *Annu Rev Biochem* 68, 821-861.
- 20 Sheaffer, K.L., Kim, R., Aoki, R., Elliott, E.N., Schug, J., Burger, L., Schubeler, D., and Kaestner, K.H. (2014). DNA methylation is required for the control of stem cell differentiation in the small intestine. *Genes Dev* 28, 652-664.
- Shoshkes-Carmel, M., Wang, Y.J., Wangenstein, K.J., Toth, B., Kondo, A., Massasa, E.E., Itzkovitz, S., and Kaestner, K.H. (2018). Subepithelial telocytes are an important source of
25 Wnts that supports intestinal crypts. *Nature* 557, 242-246.
- Snippert, H.J., van der Flier, L.G., Sato, T., van Es, J.H., van den Born, M., Kroon-Veenboer, C., Barker, N., Klein, A.M., van Rheenen, J., Simons, B.D., et al. (2010). Intestinal crypt homeostasis results from neutral competition between symmetrically dividing Lgr5 stem cells. *Cell* 143, 134-144.

- Sougiannis, A.T., VanderVeen, B.N., Davis, J.M., Fan, D., and Murphy, E.A. (2021). Understanding chemotherapy-induced intestinal mucositis and strategies to improve gut resilience. *Am J Physiol Gastrointest Liver Physiol* 320, G712-G719.
- Spector, A.A., John, K., and Fletcher, J.E. (1969). Binding of long-chain fatty acids to bovine
5 serum albumin. *J Lipid Res* 10, 56-67.
- Stine, R.R., Sakers, A.P., TeSlaa, T., Kissig, M., Stine, Z.E., Kwon, C.W., Cheng, L., Lim, H.W., Kaestner, K.H., Rabinowitz, J.D., et al. (2019). PRDM16 Maintains Homeostasis of the Intestinal Epithelium by Controlling Region-Specific Metabolism. *Cell Stem Cell* 25, 830-845 e838.
- 10 Taha, A.S., McCloskey, C., McSkimming, P., and McConnachie, A. (2018). Misoprostol for small bowel ulcers in patients with obscure bleeding taking aspirin and non-steroidal anti-inflammatory drugs (MASTERS): a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet Gastroenterol Hepatol* 3, 469-476.
- Tetteh, P.W., Basak, O., Farin, H.F., Wiebrands, K., Kretzschmar, K., Begthel, H., van den
15 Born, M., Korving, J., de Sauvage, F., van Es, J.H., et al. (2016). Replacement of Lost Lgr5-Positive Stem Cells through Plasticity of Their Enterocyte-Lineage Daughters. *Cell Stem Cell* 18, 203-213.
- Tian, H., Biehs, B., Warming, S., Leong, K.G., Rangell, L., Klein, O.D., and de Sauvage, F.J. (2011). A reserve stem cell population in small intestine renders Lgr5-positive cells
20 dispensable. *Nature* 478, 255-259.
- Tuesta, L.M., Chen, Z., Duncan, A., Fowler, C.D., Ishikawa, M., Lee, B.R., Liu, X.A., Lu, Q., Cameron, M., Hayes, M.R., et al. (2017). GLP-1 acts on habenular avoidance circuits to control nicotine intake. *Nat Neurosci* 20, 708-716.
- van der Flier, L.G., van Gijn, M.E., Hatzis, P., Kujala, P., Haegebarth, A., Stange, D.E.,
25 Begthel, H., van den Born, M., Guryev, V., Oving, I., et al. (2009). Transcription factor achaete scute-like 2 controls intestinal stem cell fate. *Cell* 136, 903-912.
- van Es, J.H., Sato, T., van de Wetering, M., Lyubimova, A., Yee Nee, A.N., Gregorieff, A., Sasaki, N., Zeinstra, L., van den Born, M., Korving, J., et al. (2012). Dll1+ secretory progenitor cells revert to stem cells upon crypt damage. *Nat Cell Biol* 14, 1099-1104.
- 30 Van Landeghem, L., Chevalier, J., Mahe, M.M., Wedel, T., Urvil, P., Derkinderen, P., Savidge, T., and Neunlist, M. (2011). Enteric glia promote intestinal mucosal healing via

activation of focal adhesion kinase and release of proEGF. *Am J Physiol Gastrointest Liver Physiol* 300, G976-987.

- VanDussen, K.L., Carulli, A.J., Keeley, T.M., Patel, S.R., Puthoff, B.J., Magness, S.T., Tran, I.T., Maillard, I., Siebel, C., Kolterud, A., et al. (2012). Notch signaling modulates proliferation and differentiation of intestinal crypt base columnar stem cells. *Development* 139, 488-497.

Verzi, M.P., and Shivdasani, R.A. (2020). Epigenetic regulation of intestinal stem cell differentiation. *Am J Physiol Gastrointest Liver Physiol* 319, G189-G196.

- von Moltke, J., Ji, M., Liang, H.E., and Locksley, R.M. (2016). Tuft-cell-derived IL-25 regulates an intestinal ILC2-epithelial response circuit. *Nature* 529, 221-225.

Wang, B., Rong, X., Palladino, E.N.D., Wang, J., Fogelman, A.M., Martin, M.G., Alrefai, W.A., Ford, D.A., and Tontonoz, P. (2018). Phospholipid Remodeling and Cholesterol Availability Regulate Intestinal Stemness and Tumorigenesis. *Cell Stem Cell* 22, 206-220 e204.

- Wang, D., and DuBois, R.N. (2018). Role of prostanoids in gastrointestinal cancer. *J Clin Invest* 128, 2732-2742.

Wang, Q., Lin, Y., Sheng, X., Xu, J., Hou, X., Li, Y., Zhang, H., Guo, H., Yu, Z., and Ren, F. (2020). Arachidonic Acid Promotes Intestinal Regeneration by Activating WNT Signaling. *Stem Cell Reports* 15, 374-388.

- Withers, H.R., and Elkind, M.M. (1970). Microcolony survival assay for cells of mouse intestinal mucosa exposed to radiation. *Int J Radiat Biol Relat Stud Phys Chem Med* 17, 261-267.

- Wu, H., Li, X.M., Wang, J.R., Gan, W.J., Jiang, F.Q., Liu, Y., Zhang, X.D., He, X.S., Zhao, Y.Y., Lu, X.X., et al. (2016). NUR77 exerts a protective effect against inflammatory bowel disease by negatively regulating the TRAF6/TLR-IL-1R signalling axis. *J Pathol* 238, 457-469.

- Yan, K.S., Gevaert, O., Zheng, G.X.Y., Anchang, B., Probert, C.S., Larkin, K.A., Davies, P.S., Cheng, Z.F., Kaddis, J.S., Han, A., et al. (2017). Intestinal Enteroendocrine Lineage Cells Possess Homeostatic and Injury-Inducible Stem Cell Activity. *Cell Stem Cell* 21, 78-90 e76.

- Yang, Y.P., Ma, H., Starchenko, A., Huh, W.J., Li, W., Hickman, F.E., Zhang, Q., Franklin, J.L., Mortlock, D.P., Fuhrmann, S., et al. (2017). A Chimeric Egfr Protein Reporter Mouse Reveals Egfr Localization and Trafficking In Vivo. *Cell Rep* 19, 1257-1267.
- Yilmaz, O.H., Katajisto, P., Lamming, D.W., Gultekin, Y., Bauer-Rowe, K.E., Sengupta, S.,
 5 Birsoy, K., Dursun, A., Yilmaz, V.O., Selig, M., et al. (2012). mTORC1 in the Paneth cell niche couples intestinal stem-cell function to calorie intake. *Nature* 486, 490-495.
- Yin, X., Farin, H.F., van Es, J.H., Clevers, H., Langer, R., and Karp, J.M. (2014). Niche-independent high-purity cultures of Lgr5+ intestinal stem cells and their progeny. *Nat Methods* 11, 106-112.
- 10 Yokoyama, U., Iwatsubo, K., Umemura, M., Fujita, T., and Ishikawa, Y. (2013). The prostanoid EP4 receptor and its signaling pathway. *Pharmacol Rev* 65, 1010-1052.
- Yu, S., Tong, K., Zhao, Y., Balasubramanian, I., Yap, G.S., Ferraris, R.P., Bonder, E.M., Verzi, M.P., and Gao, N. (2018). Paneth Cell Multipotency Induced by Notch Activation following Injury. *Cell Stem Cell* 23, 46-59 e45.
- 15 Yui, S., Azzolin, L., Maimets, M., Pedersen, M.T., Fordham, R.P., Hansen, S.L., Larsen, H.L., Guiu, J., Alves, M.R.P., Rundsten, C.F., et al. (2018). YAP/TAZ-Dependent Reprogramming of Colonic Epithelium Links ECM Remodeling to Tissue Regeneration. *Cell Stem Cell* 22, 35-49 e37.
- Zanconato, F., Forcato, M., Battilana, G., Azzolin, L., Quaranta, E., Bodega, B., Rosato, A.,
 20 Biciato, S., Cordenonsi, M., and Piccolo, S. (2015). Genome-wide association between YAP/TAZ/TEAD and AP-1 at enhancers drives oncogenic growth. *Nat Cell Biol* 17, 1218-1227.
- Zhang, N., Yantiss, R.K., Nam, H.S., Chin, Y., Zhou, X.K., Scherl, E.J., Bosworth, B.P., Subbaramaiah, K., Dannenberg, A.J., and Benezra, R. (2014). ID1 is a functional marker for
 25 intestinal stem and progenitor cells required for normal response to injury. *Stem Cell Reports* 3, 716-724.
- Zhu, X.G., Nicholson Puthenveedu, S., Shen, Y., La, K., Ozlu, C., Wang, T., Klompstra, D., Gultekin, Y., Chi, J., Fidelin, J., et al. (2019). CHP1 Regulates Compartmentalized Glycerolipid Synthesis by Activating GPAT4. *Mol Cell* 74, 45-58 e47.

30

EQUIVALENTS AND SCOPE

Those skilled in the art will recognize or be able to ascertain using no more than routine experimentation many equivalents to the specific embodiments described herein.

The scope of the present invention is not intended to be limited to the above Description, but rather is as set forth in the appended claims.

- Claims or descriptions that include “or” between one or more members of a group are considered satisfied if one, more than one, or all of the group members are present in,
5 employed in, or otherwise relevant to a given product or process unless indicated to the contrary or otherwise evident from the context. The invention includes embodiments in which exactly one member of the group is present in, employed in, or otherwise relevant to a given product or process. The invention includes embodiments in which more than one, or all of the group members are present in, employed in, or otherwise relevant to a given product
10 or process. Furthermore, it is to be understood that the invention encompasses all variations, combinations, and permutations in which one or more limitations, elements, clauses, descriptive terms, etc., from one or more of the listed claims is introduced into another claim. For example, any claim that is dependent on another claim can be modified to include one or more limitations found in any other claim that is dependent on the same base claim.
- 15 Furthermore, where the claims recite a composition, it is to be understood that methods of using the composition for any of the purposes disclosed herein are included, and methods of making the composition according to any of the methods of making disclosed herein or other methods known in the art are included, unless otherwise indicated or unless it would be evident to one of ordinary skill in the art that a contradiction or inconsistency would arise.
- 20 Where ranges are given, endpoints are included. Furthermore, it is to be understood that unless otherwise indicated or otherwise evident from the context and understanding of one of ordinary skill in the art, values that are expressed as ranges can assume any specific value or subrange within the stated ranges in different embodiments of the invention, to the tenth of the unit of the lower limit of the range, unless the context dictates otherwise.
- 25 In addition, it is to be understood that any particular embodiment of the present invention that falls within the prior art may be explicitly excluded from any one or more of the claims. Since such embodiments are deemed to be known to one of ordinary skill in the art, they may be excluded even if the exclusion is not set forth explicitly herein. Any particular embodiment of the compositions of the invention can be excluded from any one or
30 more claims, for any reason, whether or not related to the existence of prior art.

All references cited herein, including patents, published patent applications, and non-patent publications, are incorporated by reference in their entirety.

CLAIMS

What is claimed is:

1. A method of preventing, reducing, or reversing adverse side effects due to
5 chemotherapy or radiation therapy in a subject, comprising:
administering orally to a subject in need thereof at least about 2 g of arachidonic acid
triglyceride (AA TG) per day (2 g/d) for a sufficient time to prevent, reduce or reverse
adverse side effects due to chemotherapy or radiation therapy in the subject.
- 10 2. The method of claim 1, wherein the sufficient time is at least about 7 days; and
(a) administration starts no earlier than 28 days before the subject begins a course of
chemotherapy or radiation therapy;
(b) administration starts no later than 28 days after the subject completes a course of
chemotherapy or radiation therapy; or
15 (c) administration starts at any time during a course of chemotherapy or radiation
therapy.
3. The method of claim 1 or claim 2, wherein the sufficient time is at least about 14
days.
20
4. The method of claim 1 or claim 2, wherein the sufficient time is at least about 21
days.
5. The method of claim 1 or claim 2, wherein the sufficient time is at least about 28
25 days.
6. The method of any one of claims 1-5, wherein the course of chemotherapy or
radiation therapy lasts for at least about 3 months.
- 30 7. The method of any one of claims 1-5, wherein the course of chemotherapy or
radiation therapy lasts for at least about 6 months.
8. The method of any one of claims 1-5, wherein the course of chemotherapy or
radiation therapy lasts for at least about 12 months.

9. The method of any one of claims 1-5, wherein the course of chemotherapy or radiation therapy lasts from about 3 months to about 12 months.
- 5 10. The method of any one of claims 1-9, wherein at least about 3 g of AA TG/day (3 g/d) is administered to the subject.
11. The method of any one of claims 1-9, wherein at least about 20 g of AA TG/day (20 g/d) is administered to the subject.
- 10 12. The method of any one of claims 1-9, wherein at least about 30 g of AA TG/day (30 g/d) is administered to the subject.
13. The method of any one of claims 1-9, wherein at least about 60 g of AA TG/day (60 g/d) is administered to the subject.
- 15 14. The method of any one of claims 1-9, wherein at least about 90 g of AA TG/day (90 g/d) is administered to the subject.
- 20 15. The method of any one of claims 1-9, wherein at least about 100 g of AA TG/day (100 g/d) is administered to the subject.
16. The method of any one of claims 1-15, wherein from about 2 g of AA TG/day (2 g/d) to about 100 g of AA TG/day (100 g/d) is administered to the subject.
- 25 17. The method of any one of claims 1-15, wherein the AA TG is in a composition.
18. The method of claim 17, wherein the composition comprises at least about 2% AA TG by weight.
- 30 19. The method of claim 17 or claim 18, wherein the composition comprises between about 20% AA TG and about 50% AA TG by weight.

20. The method of claim 17 or claim 18, wherein the composition comprises about 40% AA TG by weight.
21. The method of any one of claims 17-20, wherein the composition comprises no more
5 than 5% arachidonic acid (AA) ester by weight.
22. The method of any one of claims 17-21, wherein the composition is an oil.
23. The method of claim 22, wherein the oil is extracted from a fungus.
10
24. The method of claim 23, wherein the fungus is *Mortierella alpina*.
25. The method of any one of claims 17-24, wherein the composition is a liquid or a
powder.
15
26. The method of any one of claims 22-25, wherein the composition is in a food, in a
capsule or in a pill.
27. The method of any one of claims 1-26, wherein the AA TG increases an intestinal AA
20 level in the subject that produces a beneficial effect.
28. The method of any one of claims 1-27, wherein administration of AA TG increases a
plasma AA level in the subject by at least 2-fold relative to a reference.
- 25 29. The method of claim 28, wherein the reference is an AA level in plasma or intestinal
tissue from the subject before administration of AA TG, or a pre-determined AA level in
plasma or intestinal tissue.
30. The method of any one of claims 1-29, wherein the adverse side effect is a
30 gastrointestinal side effect.
31. The method of any one of claims 1-29, wherein the adverse side effect is nausea,
vomiting, diarrhea, weight loss, intestinal tissue damage, radiation colitis, radiation mucositis,

pelvic radiation disease, radiation enteritis, abdominal pain, rectal bleeding, bloating, or constipation.

32. The method of any one of claims 1-31, wherein the subject is a human.

5

33. A method of preventing, reducing, or reversing a cytotoxic effect due to chemotherapy or radiation therapy in a subject, comprising:

administering orally to a subject in need thereof at least about 2 g of arachidonic acid triglyceride (AA TG) per day (2 g/d) for a sufficient time to prevent, reduce or reverse a cytotoxic effect due to chemotherapy or radiation therapy in the subject.

10

34. The method of claim 33, wherein the cytotoxic effect is intestinal tissue damage.

35. A method of increasing, in a subject, an arachidonic acid (AA) level indicative of an intestinal AA level that prevents, reduces, or reverses adverse side effects due to chemotherapy or radiation therapy, comprising:

15

(a) measuring an arachidonic acid (AA) level in a sample from a subject in need thereof and determining if the AA level is below a pre-determined AA level sufficient to prevent, reduce, or reverse adverse side effects due to chemotherapy or radiation therapy; and
(b) if the AA level is below the pre-determined AA level, administering to the subject in (a) at least about 2 g of AA TG per day (2 g/d) for a sufficient time to increase the AA level to or above the pre-determined AA level.

20

36. The method of claim 35, further comprising:

25

(c) measuring the AA level resulting from administering AA TG in (b) and determining the AA level; and

(d) if the AA level in (b) is not at or above the pre-determined AA level, further administering to the subject a sufficient amount of AA TG per day to result in an intestinal AA level at or above the pre-determined AA level.

30

37. The method of claim 36, further comprising repeating (c)-(d) to produce in the subject an intestinal AA level at or above the pre-determined AA level.

38. The method of any one of claims 35-37, wherein the sample is plasma.

39. The method of any one of claims 35-37, wherein the sample is intestinal tissue.
40. The method of any one of claims 1-39, wherein AA in AA TG is substituted by at
5 least one precursor of AA.
41. The method of claim 40, wherein the at least one precursor of AA is linoleic acid
(LA), gamma-linolenic acid (gamma-LA), dihomo-gamma-linolenic acid (dh-gamma-LA),
LA and gamma-LA, gamma-LA and dh-gamma-LA, or LA, gamma-LA, and dh-gamma-LA.
10
42. A method of preventing, reducing, or reversing adverse side effects due to
chemotherapy or radiation therapy in a subject, comprising:
administering orally to a subject in need thereof at least about 2 g of at least one
precursor of arachidonic acid (AA) per day (2 g/d) for a sufficient time to prevent, reduce or
15 reverse adverse side effects due to chemotherapy or radiation therapy in the subject.
43. The method of claim 42, wherein the precursor of AA is in the form of a triglyceride
(TG).
- 20 44. The method of claim 43, wherein the at least one precursor of AA is linoleic acid
(LA), gamma-linolenic acid (gamma-LA), dihomo-gamma-linolenic acid (dh-gamma-LA),
LA and gamma-LA, gamma-LA and dh-gamma-LA, or LA, gamma-LA, and dh-gamma-LA.
45. A kit for use in preventing, reducing or reversing adverse side effects due to
25 chemotherapy or radiation therapy in a subject, comprising:
(a) one or more supplement units sufficient to provide to a subject in need thereof at
least about 2 g of arachidonic acid triglyceride (AA TG) per day (2 g/d) for at
least 7 days; and
(b) instructions for preparation and consumption of the one or more supplement units.
30
46. The kit of claim 45, wherein the one or more supplement units each comprise 500 mg
of AA TG, 1 g of AA TG, 2 g of AA TG, or 4 g of AA TG.

47. The kit of claim 45 or claim 46, wherein the number of supplement units to administer to a subject in need thereof is determined in consultation with a healthcare provider.
48. The kit of claim 45 or claim 46, wherein the supplement units are in the form of a liquid or a powder.
49. The kit of claim 45 or claim 46, wherein the supplement units are in the form of a liquid or a powder.
50. The kit of claim 45 or claim 46, wherein the supplement units are in the form of pills or capsules.
51. The kit of claim 50, wherein the supplement units are in one or more containers.
52. A kit for use in preventing, reducing or reversing adverse side effects due to chemotherapy or radiation therapy in a subject, comprising:
- (a) one or more supplement units sufficient to provide to a subject in need thereof at least about 2 g of at least one precursor of arachidonic acid (AA) per day (2 g/d) for a sufficient time; and
 - (b) instructions for preparation and consumption of the one or more supplement units.
53. The kit of claim 52, wherein the precursor of AA is in the form of a triglyceride (TG).
54. The kit of claim 53, wherein the at least one precursor of AA is linoleic acid (LA), gamma-linolenic acid (gamma-LA), dihomo-gamma-linolenic acid (dh-gamma-LA), LA and gamma-LA, gamma-LA and dh-gamma-LA, or LA, gamma-LA, and dh-gamma-LA.