Reyes (2020)

Supplementary Figure Legends

Figure S1. A) Response to naproxen assessed by changes in PGE2 levels in normal colorectal mucosa. Changes in levels of PGE2 (ng/g) in colorectal mucosa after treatment with placebo, LD and HD naproxen. Each value corresponds to one patient and lines connect values pre- and post-treatment in every patient; **B) Response to naproxen assessed by changes in PGE-M levels in urine.** Changes in levels of PGE-M (ng/g,Cr) after treatment with Placebo, LD and HD naproxen. Each value corresponds to one patient and lines connect values pre- and post-treatment; **C) Naproxen levels in plasma and D) colorectal mucosa.** Levels of naproxen in plasma and colorectal mucosa after treatment in all patients consented for the 'Naproxen trial'. A total of 4 patients were not compliant with the intervention per protocol (less than 80% of the doses taken) or had the last dose of the study drug more than 7 days prior to the scheduled endoscopy sigmoidoscopy (both types denoted with a diamond), or were assigned to receive naproxen at LD or HD and after intervention had serum naproxen levels undetectable (denoted with a square). These patients were removed from the statistical analysis, but their levels are displayed in the graph. Comparisons were performed using a two-sample t-test (***P-value≤0.001); **E) Response to naproxen assessed by changes in other prostaglandins in colorectal mucosa.** Comparison of changes in levels of 9a11b-PGF2a, PGF2a, 6-Keto-PGF1a, PGD2, TxB2 after treatment with placebo, HD and LD naproxen using a Wilcoxon rank sum test. The graph displays absolute change calculated using [(Post) – (Baseline)], *P-value 0.05, **P-value≤0.01, ***P-value≤0.001; F) **Modulation of polyp growth in the rectosigmoid.** Swimmer plot showing all the patients from the 'Evaluable cohort' of the 'Naproxen trial' that displayed polyps in either the baseline or end-of-the-study procedures (total of 16 patients) with their pathology. The rest of the patients that did not show any polyps are not displayed (N=38). Hyperplastic polyps are displayed in blue and tubular adenomas in red.

Figure S2. A) Principal Component Analysis (PCA) plots. First (x-axis) and second (y-axis) principal components obtained from PCA output of human PL, LD and HD datasets were plotted separately as scatter plots to visualize sample distribution after dimension reduction of normalized whole transcriptome

Reyes (2020)

data. Each dot represents one sample which was colored and shaped depending on its respective pre- (blue) or post-treatment (gold) group it belongs to. The centroids of both groups were marked as squares and connected to individual samples within each group; **B) Gene pathways modulated by HD and LD naproxen with a different direction of effect and therefore considered unique to each dose level.** LD significantly downregulated signals related to cycle, and HD induced downregulation of crypt top signals thus affecting differentiation of the crypt. These pathway results confirm the annotation of genes displayed in the volcano plots. Significant pathways that met the following criteria were selected and displayed in bubble chart plot: 1. BH-adjusted *P*-value≤0.05 in LD only (Low-Dose Specific), HD only (High-Dose Specific), or both LD and HD (Common); 2. Signs of NESs are opposite in LD and HD. The sizes of bubbles were determined by BH-adjusted *P*-value. The colors of bubbles were determined by the sign and amplitude of NES, with positive NES (positively enriched in post-treatment group) in red and negative NES (negatively enriched in post-treatment group) in green.

Figure S3. A) Co-clinical trial of naproxen, aspirin and placebo in a LS intestinal tissue-specific genetically engineer mouse model. A total of 83 mice were randomized to receive naproxen, aspirin and placebo in the diet. A survival analysis rendered a significant prolongation in the lifespan of mice treated with naproxen compared to both placebo and aspirin but not when aspirin was compared to placebo; **B) Evaluation of tumor burden.** Prolongation of survival by naproxen matched the effects in the modulation of the tumor burden compared to both placebo and aspirin. Aspirin did not significantly reduce significantly the tumor burden compared to placebo.

Figure S4. A) Mouse PCA plot. First (x-axis) and second (y-axis) principal components of mice samples were obtained from PCA output and plotted as a scatter plot. Each dot represents one sample that was colored and shaped depending on the treatment assignation with control denoted in gray and naproxen in dark red. The centroids of both groups were marked as squares and connected to individual samples within each group; **B) Organoid PCA plot.** First (x-axis) and second (y-axis) principal components of

Reyes (2020)

organoid samples were obtained from PCA output and plotted as a scatter plot. Each dot represents one sample that was colored and shaped depending on treatment assignation with control denoted in grey and naproxen in dark red. The centroids of both groups were marked as squares and connected to individual samples within each group; **C) Mouse volcano plot.** Genes from the whole transcriptome in the mouse dataset were displayed in a volcano plot with log2(FoldChange) in x-axis and -log10(BH-adjusted *P*-value) in y-axis. Significantly up- and down-regulated genes with BH-adjusted *P*-value≤0.05 and absolute value of log2-Fold Change≥2 were highlighted and annotated with pathways of interest. The horizontal line represents BH-adjusted p-value=0.05. The left and right vertical lines represent log2-Fold Change=±2; **D) Organoid volcano plot.** Genes from the whole transcriptome in the organoid dataset were displayed in volcano plot with log2(FoldChange) in x-axis and -log10(BH-adjusted p-value) in y-axis. Significantly up- and down-regulated genes with BH-adjusted *P*-value≤0.05 and absolute value of log2-Fold Change≥2 were highlighted and annotated with pathways of interest in colors. The horizontal line represents BH-adjusted p-value=0.05. The left and right vertical lines represent log2-Fold Change=±2; **E) HD DEGs in mice.** Significant genes in human HD post vs pre-treatment comparison with BH-adjusted *P*-value≤0.05 and absolute value of log2-Fold Change≥0.5, which were also significant in the mice naproxen vs control comparison with BH-adjusted *P*-value≤0.1, were used for unsupervised clustering of mice samples. Their expressions were row centered and displayed in a heat map with gene symbols as row names and sample IDs as column names. Column covariate bar indicates treatment groups that samples belong to. All genes displayed were in intersection between HD and mice datasets in the corresponding Venn diagram. Dendrogram illustrates sample clustering based on distances; **D) LD DEGs in organoids.** Significant genes in human LD post vs pre-treatment comparison with BH-adjusted *P*-value≤0.05 and absolute value of log2-Fold Change≥0.5, which were also significant in the mice naproxen vs control comparison with BH-adjusted *P*-value≤0.1, were used for unsupervised clustering of mice samples. Their expressions were row centered and displayed in a heat map with gene symbols as row names and sample IDs as column names. Column covariate bar indicates treatment groups that

Reyes (2020)

samples belong to. All genes displayed were in intersection between LD and mice datasets in the corresponding Venn diagram. Dendrogram illustrates sample clustering based on distances**.**