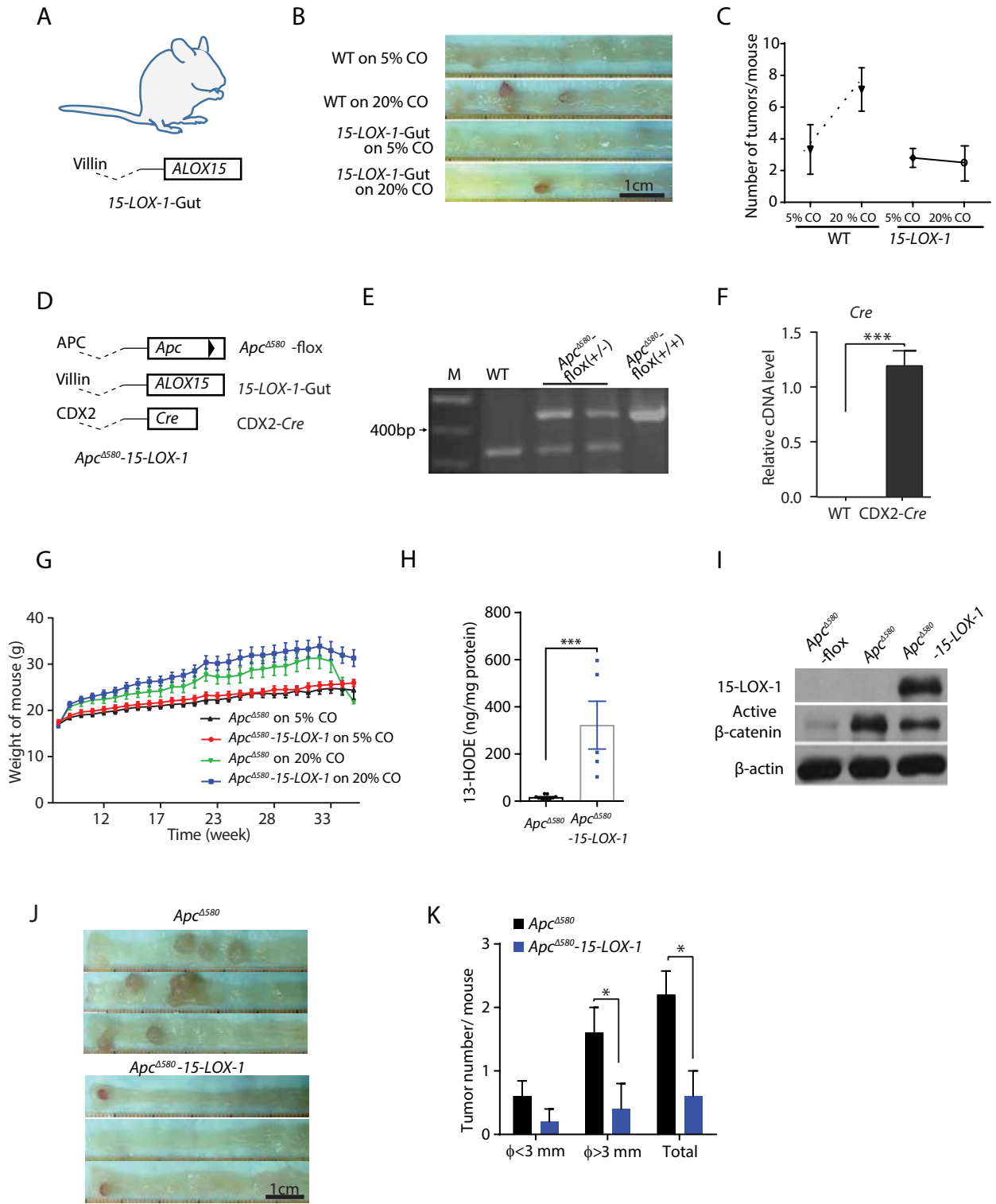


Supplementary Figure 1



Supplementary Figure 1. 15-LOX-1 suppresses linoleic acid (LA) promotion of AOM-induced colorectal cancer (CRC) and *Apc* mutation–induced CRC in mice.

(A) Schematic diagram of villin-15-LOX-1 (*15-LOX-1-Gut*) mice. Dashed lines indicate the promoter region, followed by the 15-LOX-1 gene coding region.

(B, C) *15-LOX-1-Gut* homozygotes and sex- and age-matched wild-type littermates (WT) were fed either 5% or 20% corn oil diet and then treated with 7.5 mg/kg AOM weekly via intraperitoneal injection for 6 consecutive weeks. Mice were killed 20 weeks after the last AOM injection and examined for tumor formation (n= 10 mice per group). Representative colonic images **(B)** and colonic tumor numbers per mouse **(C)** of the indicated mice groups.

(D) Schematic diagram of *Apc*^{Δ580}–*15-LOX-1* mice. *Apc*^{Δ580} mice were bred with *15-LOX-1-Gut* mice to produce *Apc*^{Δ580};*15-LOX-1-Gut*, designated as *Apc*^{Δ580}–*15-LOX-1* mice.

(E) Genotyping of mice carrying *Apc*^{Δ580}-flox alleles, in which *Apc* exon 14 is flanked with *loxP* sites by regular polymerase chain reaction (PCR). *Apc* WT band: 320 bp. *Apc*^{Δ580} flox band: 430 bp. M represents DNA ladder marker.

(F) *Cre* genotyping for CDX2-*Cre* mice. Genomic *Cre*-recombinase cDNA levels were measured by quantitative PCR. WT indicates no detectable genomic *Cre*-recombinase cDNA.

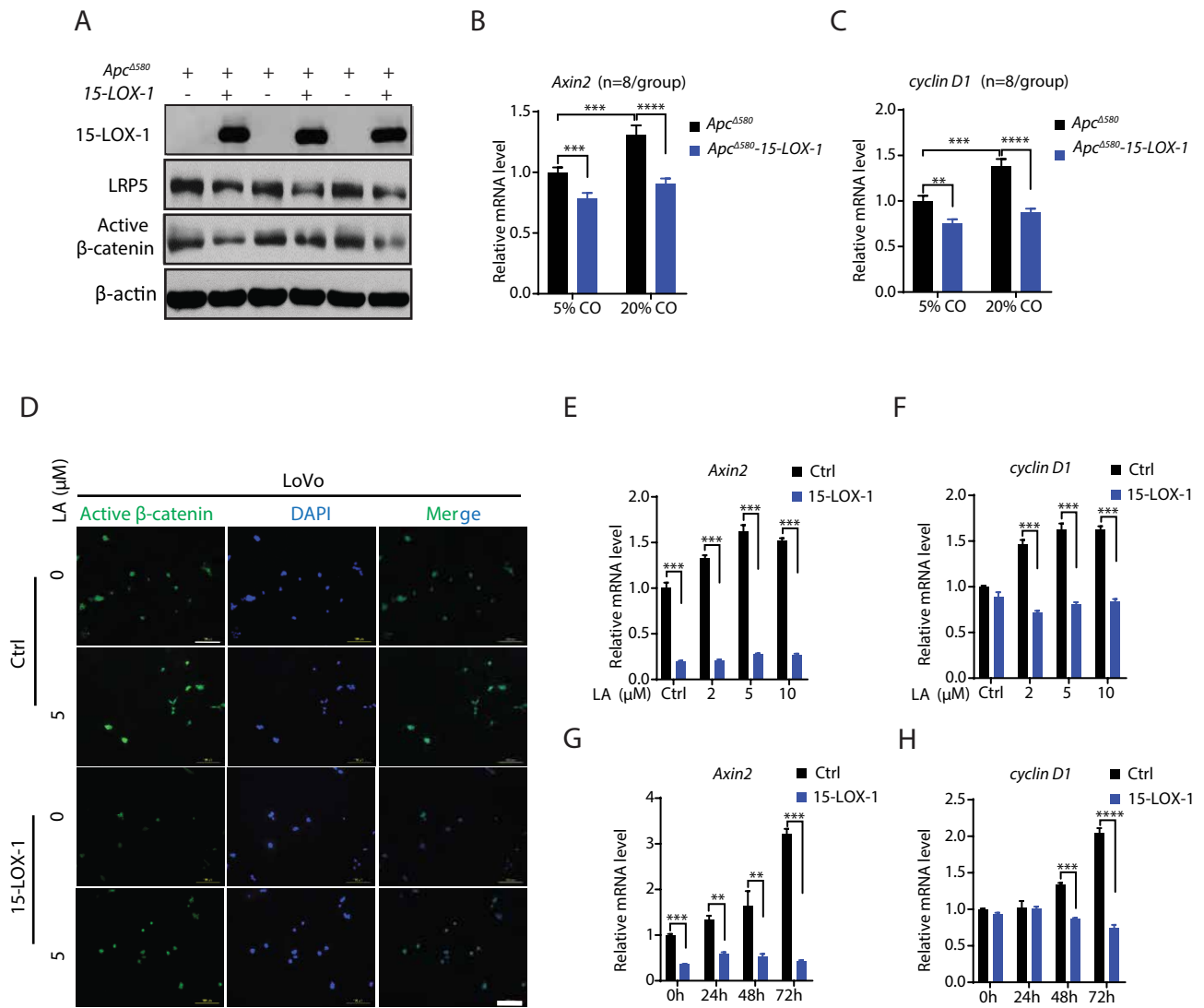
(G) *Apc*^{Δ580} and *Apc*^{Δ580}–*15-LOX-1* mice at 4 weeks were fed 5% or 20% corn oil diets, and the body weights of the mice were assessed every week until age 35 weeks. The body weight curves of the four mouse groups are shown (n=14-20 per group).

(H, I) Normal colonic epithelial cells of *Apc*^{Δ580}-flox, *Apc*^{Δ580}, and *Apc*^{Δ580}–*15-LOX-1* mouse groups were scraped and harvested for measuring 13-HODE levels by LC-MS/MS **(H)** and 15-LOX-1 and active β-catenin levels by Western blot **(I)**.

(J, K) 15-LOX-1 expression inhibited CRC tumorigenesis in *Apc*^{Δ580} mice. *Apc*^{Δ580} mice and *Apc*^{Δ580}–*15-LOX-1* mice fed a standardized diet with fixed LA content (7% corn oil) were followed for 25 weeks and evaluated for tumor formation (n=5 per group). Representative colon photographs **(J)** and the colonic tumor numbers per mouse with tumor size distributions **(K)** of *Apc*^{Δ580} and *Apc*^{Δ580}–*15-LOX-1* mice.

Scale bars: 1 cm (B and J). ∅: diameter.

Supplementary Figure 2



Supplementary Figure 2. 15-LOX-1 suppresses LRP5 and active β -catenin expression in IECs of mice and human CRC cells.

(A) LRP5 and active β -catenin protein levels in normal IECs of *Apc* ^{Δ 580} and *Apc* ^{Δ 580}-15-LOX-1 littermates at age 6-8 weeks were measured by Western blot.

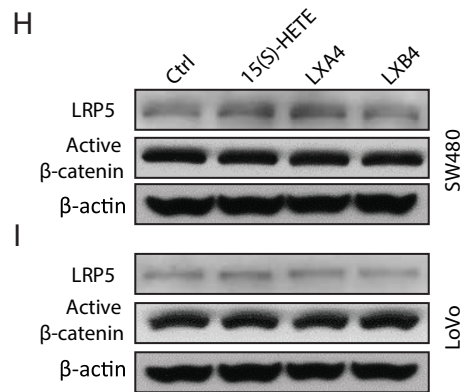
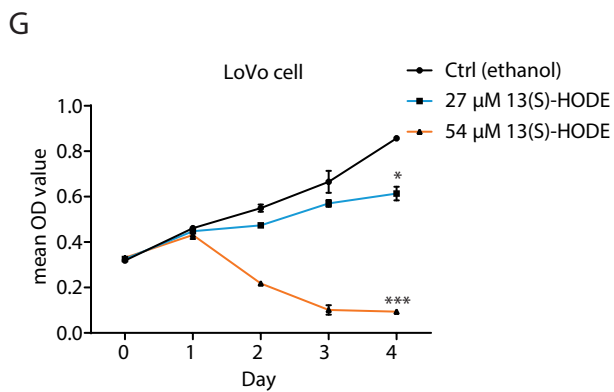
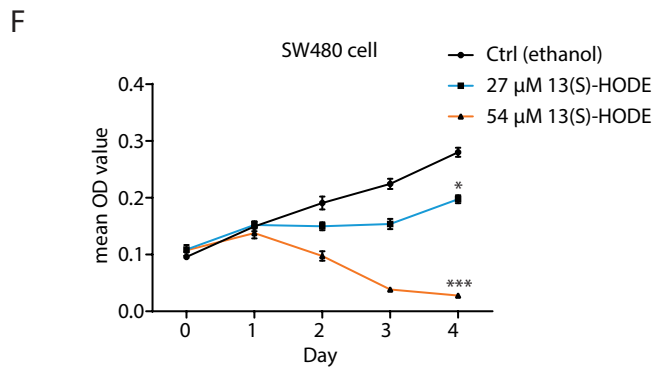
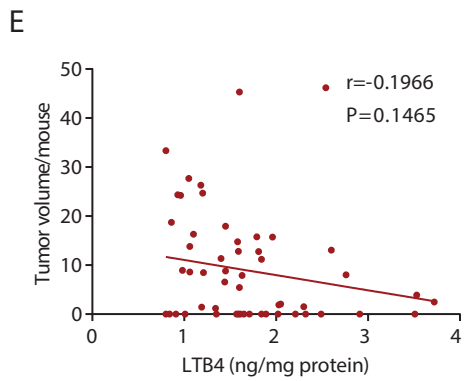
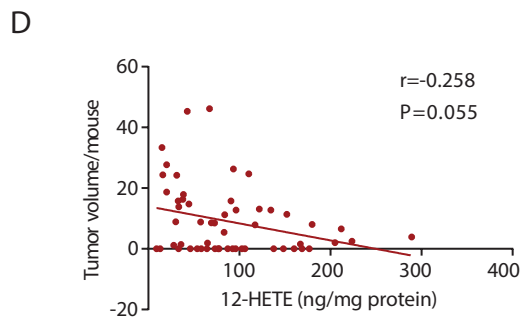
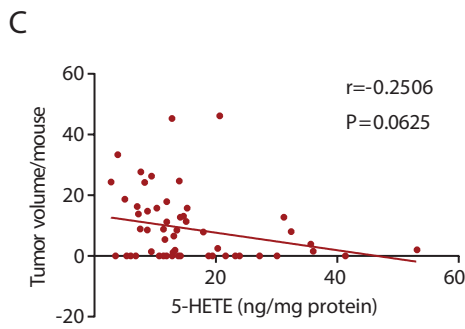
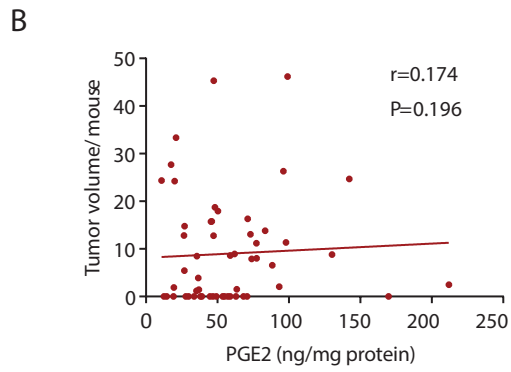
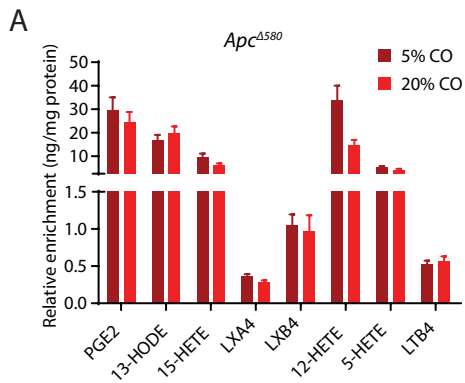
(B, C) *Apc* ^{Δ 580} and *Apc* ^{Δ 580}-15-LOX-1 mice at 4 weeks were fed 5% or 20% corn oil (CO) diets for 10 weeks. The mRNA expression levels of *Axin2* **(B)** and *cyclin D1* **(C)** in the IECs of the mice were measured by qRT-PCR.

(D) Immunofluorescence staining of active β -catenin in LoVo stably transduced with either control (Ctrl) or 15-LOX-1 lentivirus and treated with 5 μ M LA for 48 hours. Representative immunofluorescence images are shown.

(E-H) *Axin2* and *cyclin D1* mRNA expression in LoVo cells stably transduced with either control (Ctrl) or 15-LOX-1 lentivirus and treated with LA at different concentrations in culture medium supplemented with 5% dialyzed FBS for 48 hours **(E,F)** or treated with 5 μ M LA at different time points **(G, H)**, measured by qRT-PCR.

Scale bars: 100 μ m (F).

Supplementary Figure 3



Supplementary Figure 3. The profile analysis of 15-LOX-1-mediated eicosanoid metabolites in *Apc*^{Δ580} mice and 13(S)-HODE suppression of human CRC proliferation.

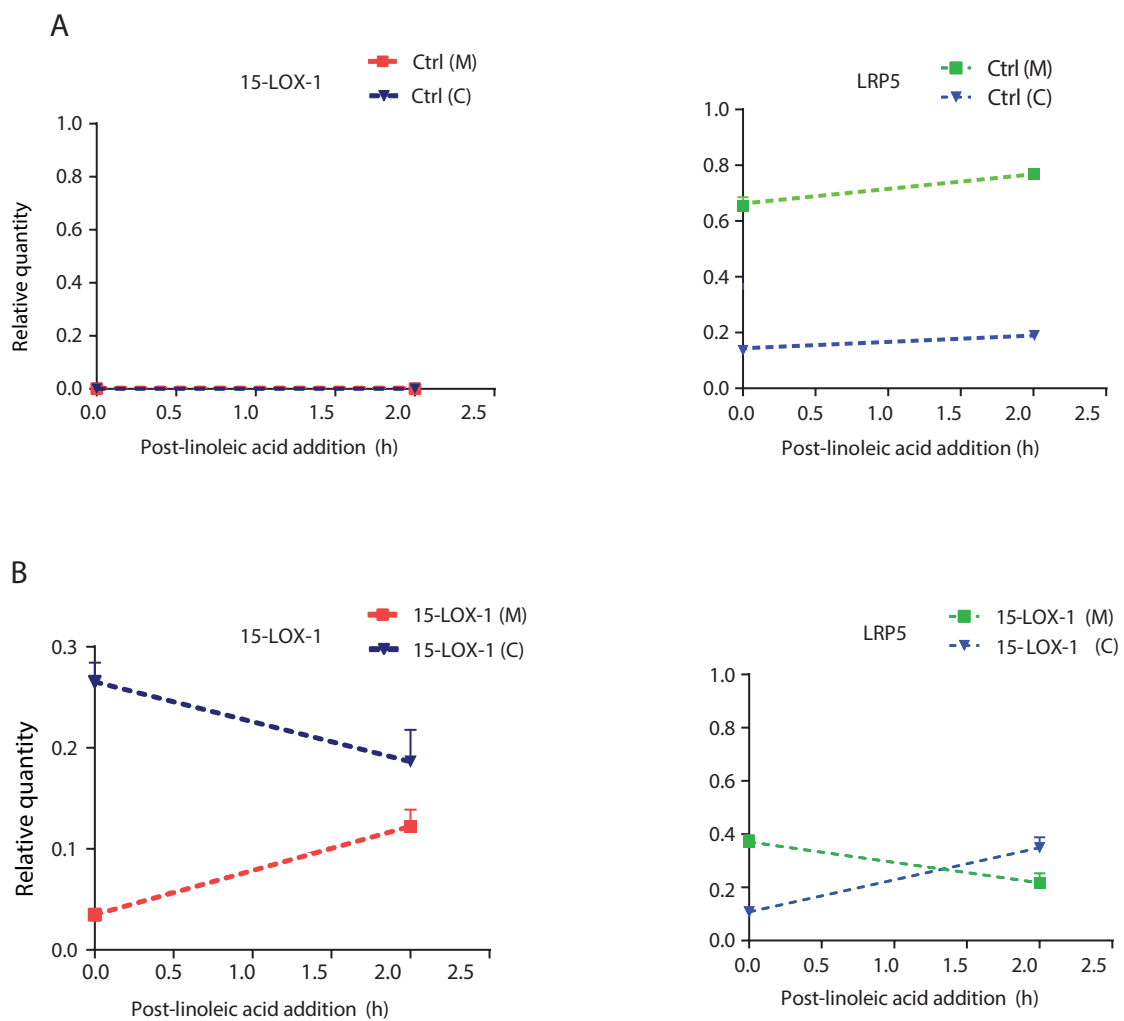
(A-E) *Apc*^{Δ580} and *Apc*^{Δ580}-15-LOX-1 littermates at 4 weeks were fed either 5% or 20% corn oil diet for 10 weeks, killed at age 14 weeks, and examined for tumor formation (n=14 mice per group).

(A) Eicosanoid metabolite profile of the IECs of the indicated *Apc*^{Δ580} mouse groups were measured by liquid chromatography-tandem mass spectrometry (LC-MS/MS). **(B-E)** Spearman correlation analysis of colonic tumor volumes with levels of PGE2 **(B)**, 5-HETE **(C)**, 12-HETE **(D)**, or LTB4 **(E)** in IECs of all the examined *Apc*^{Δ580} and *Apc*^{Δ580}-15-LOX-1 mice (n=56).

(F, G) 13(S)-HODE suppresses human CRC proliferation. SW480 **(F)** and LoVo **(G)** cells were treated with 0, 27, and 54 μM 13(S)-HODE for 0, 1, 2, 3, and 4 days. The cell viability and proliferation were measured by MTT assay.

(H, I) SW480 **(H)** and LoVo **(I)** were treated with 20 μM 15(S)-HETE, 100 nM LXA4, and 200 nM LXB4 for 48 hours, and the cells were harvested and examined for LRP5 and active β-catenin expression by Western blot.

Supplement Figure 4

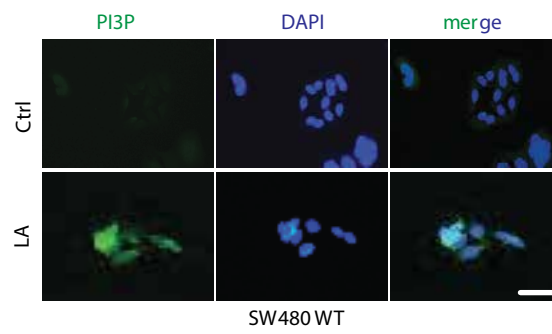


Supplementary Figure 4. 15-LOX-1 decreases cell membranous LRP5 levels.

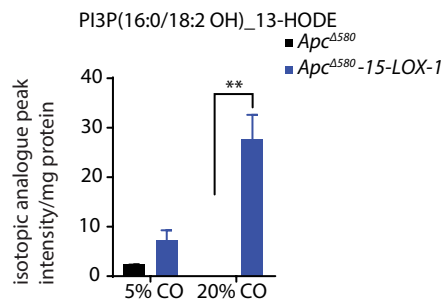
(A, B) LoVo cells stably transduced with control (Ctrl) or 15-LOX-1 lentivirus treated with 10 μ M LA for 2 hours were processed for cytoplasmic (C) and membranous (M) protein fractions, and then analyzed for LRP5 and 15-LOX-1 protein expression by Western blot. The ratios of protein band densities of cytoplasmic 15-LOX-1 or LRP5 over β -actin and membranous 15-LOX-1 or LRP5 over Na-K-ATPase in Ctrl **(A)** or 15-LOX-1 **(B)** lentivirus transduced LoVo cells corresponding to **Figure 5D**.

Supplement Figure 5

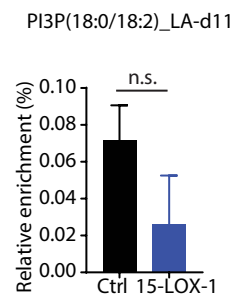
A



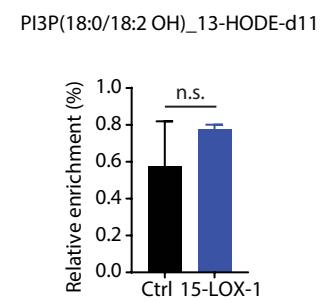
B



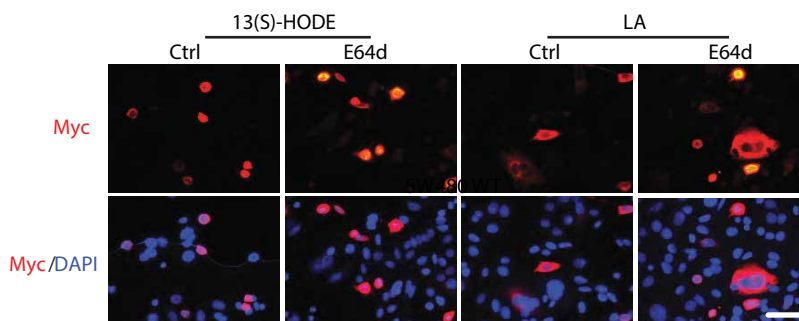
C



D



E



Supplementary Figure 5. LA increases phosphatidylinositol 3-phosphate (PI3P) production and 15-LOX-1 increases PI3P_13-HODE production.

(A) Immunofluorescence staining of PI3P in SW480 WT cells treated with 5 μ M LA for 48 hours. Representative images are shown.

(B) *Apc* ^{Δ 580} and *Apc* ^{Δ 580}-15-LOX-1 littermates at age 4 weeks fed either 5% or 20% corn oil diet for 10 weeks were killed at age 14 weeks. The IECs of these mice were scraped and examined for patterns of PI3P incorporated with (16:0/18:2 OH)_13-HODE by LC-HRMS (n=3-4 mice per group).

(C, D) SW480 cells stably transduced with control (Ctrl) or 15-LOX-1 lentivirus were treated with 100 μ M LA-d11 for 36 hours in culture medium containing 5% dialyzed FBS. PI3P profile was traced with d11-labeled LA by LC-HRMS. The percentage of d11-labeled PI3P incorporated with (18:0/18:2)_LA over PI3P incorporated with total (18:0/18:2)_LA **(C)**, or d11-labeled PI3P incorporated with (18:0/18:2 OH)_13-HODE over PI3P incorporated with total (18:0/18:2 OH)_13-HODE **(D)**, was calculated and presented.

(E) SW480 cells transfected with Myc-tagged LRP5 expression vector for 48 hours, then treated with E-64d or solvent for 1 hour, followed by treatment of LA (100 μ M) or 13(S)-HODE (27 μ M) for an additional 4 hours. The cells were harvested and processed for immunofluorescence staining using anti-c-Myc antibody. Representative images are shown.

n.s. means no significance difference. Scale bars: 100 μ m (A and E).