Supplemental Figure Legends

Figure S1, related to Figure 1. Hyperplasia of the small intestine in Lpcat3-deficient mice.

(A) Expression of Lpcat3 in small intestine and colon of F/F and *Cre*ERT2 mice (n=6,7 mice/group).

(B) Body weight change in tamoxifen-injected F/F and *Cre*ERT2 mice (n=5,7 mice/group).

(**C**) Small intestine length of tamoxifen-injected F/F and *Cre*ERT2 mice (n=8,9 mice/group).

(D) Representative histology of Duodenum from F/F and *Cre*ERT2 mice 3 weeks after tamoxifen injection**.**

(E) Quantification of crypt number and villus/crypt length ratio in Duodenum and Jejunum from F/F and *Cre*ERT2 mice 3 weeks after tamoxifen injection (n=4 mice/group).

(F) Representative histology of Duodenum and Jejunum from female *Cre*ERT2 mice with/without tamoxifen injection for 8 weeks (n=5 mice/group).

(G) Expression of Lpcat3 in Duodenum from F/F and *Cre*ERT2 mice 14 weeks after tamoxifen injection (n=6,7 mice/group).

(H) Representative images of intestines from F/F and *Cre*ERT2 mice 14 weeks after tamoxifen injection.

(I) Small intestine length of F/F and *Cre*ERT2 mice 14 weeks after tamoxifen injection (n=6,7 mice/group).

(**J**) Representative images of Id1 immunostaining in colon of F/F and *Cre*ERT2 mice 3 weeks after tamoxifen injection.

Values are means ± SEM. Statistical analysis was performed with Student's t test (A, B, C, E, G, and I). * $P < 0.05$, ** $P < 0.01$, *** $P<0.001$, **** $P<0.0001$. Scale bars: 20 µm (J), 100 µm (F) and 200 µm (D).

Figure S2., related to Figure 1. Loss of Lpcat3 impairs ISC differentiation.

(A) Representative images of immunofluorescence (IF) staining and quantification of Lysozyme positive Paneth cells in Jejunum from F/F and *Cre*ERT2 mice 3 weeks after tamoxifen injection (~100 crypts from 4 mice/group).

(B) Representative images of Periodic Acid Schiff (PAS) staining and quantification of goblet cells in Jejunum as in **A** (~100 villi from 4 mice/group).

(C) Representative images of IF staining and quantification of Chromogranin A (ChgA) positive enteroendocrine cells in Jejunum as in **A** (~50 villus-crypt units from 4 mice/group).

(**D**) Expression of cytokines in F/F and *Cre*ERT2 intestines (n=5 mice/group).

Values are means ± SEM. Statistical analysis was performed with Student's t test. **** $P<0.0001$, n.s. not significant. Scale bars: 50 µm (A and C), and 100 µm (B).

Figure S3, related to Figure 2. Lipidomic analysis of crypts and PGE2 production in Jejunum from control and Lpcat3-deficient mice.

(**A-B**) ESI-MS/MS analysis of the abundance of PC species and total PC in crypts isolated from F/F and *Cre*ERT2 mice.

(**C**) Expression of Cox-1 and Cox-2 in crypts (n=10 F/F mice, and 9 *Cre*ERT2 mice) and intestines (n=8 F/F mice, and 6 *Cre*ERT2 mice).

(**D**) PGE2 concentration in F/F and *Cre*ERT2 Jejunums determined by ELISA assay ((n=12 F/F mice, and 10 *Cre*ERT2 mice).

Values are means ± SEM. Statistical analysis was performed with Student's t test. * $P < 0.05$, ** $P < 0.01$, *** $P<0.001$.

Figure S4, related to Figure 3. Effects of Lpcat3 deficiency on expression of the SREBP-1, Wnt, Notch, Yap and PPARδ pathways.

(A-C, E) Expression of selective genes in Wnt (A), Notch, Yap (B), PPARδ (C) and fatty acid biosynthetic pathways (E) in F/F and *Cre*ERT2 crypts analyzed by realtime RT-PCR (n=11 F/F mice, and 9 *Cre*ERT2 mice).

(**D**) Expression of selective genes in cholesterol biosynthesis in F/F and *Cre*ERT2 colons (n=6,7 mice/group)

(F) Expression of selective genes in F/F and *Cre*ERT2 organoids treated with vehicle (DMSO) or 4-hydroxytamoxifen (4-OHT, 100 nM) (n=7~8).

Values are means ± SEM. Statistical analysis was performed with Student's t test (A-E) and one-way ANOVA (F). * $P < 0.05$, ** $P < 0.01$, *** $P<0.001$.

Figure S5, related to Figure 4. Inhibition of cholesterol biosynthesis reduces Id1+ progenitor cells in Lpcat3-deficient intestine.

(A) Representative images of Ro48 treated organoids in the presence of cholesterol or epicholesterol.

(B) Quantification of villus length in Jejunum of control and Lpcat3 deficient mice treated with vehicle or Ro48 (~20-50 villi per mouse, 3 *Cre*ERT2 and 2 F/F mice/group).

(**C**) Representative images of IHC staining of Olfm4 positive ISCs in Jejunum of control mice treated with vehicle or Ro48.

(D-E) Representative images of immunostaining and quantification of Lysozyme positive Paneth cells (D) and PAS positive goblet cells (E) in Jejunum of *Cre*ERT2 mice treated with vehicle or Ro48 (n=3 mice/group, ~40 crypts and ~100 villi per mouse).

(F) Representative images of IHC of cleaved caspase 3 in Jejunum of *Cre*ERT2 mice treated with vehicle or Ro48 (n=3 mice/group).

(**G**) Representative images of immunostaining and quantification of Id1-positive progenitor cells in Jejunum of *Cre*ERT2 mice treated with vehicle or Ro48 (n=3 mice/group, ~30 crypts per mouse).

Values are means ± SEM. Statistical analysis was performed with two-way ANOVA (B) and Student's t test (G). **** $P<0.0001$. Scale bars: 20 µm (C and G), 50 µm (D and F), 100 µm (E), and 200 µm (A).

Figure S6, related to Figure 5. Overexpression of *Srebf2* increases Id1-positive progenitor cells.

(**A**) Free cholesterol content in crypts isolated from chow and cholesterol diet fed mice.

(**B**) Representative images of immunostaining and quantification of Id1 positive progenitor cells in Jejunum of WT and *Srebf2* Tg mice (n=4 mice/group, ~50 crypts per mouse).

Values are means ± SEM. Statistical analysis was performed with Student's t test. * $P < 0.05$, **** $P<0.0001$.

Figure S7, related to Figure 6. Analysis of inflammation and gene expression in *Apc*$^{min/+}$ mice.

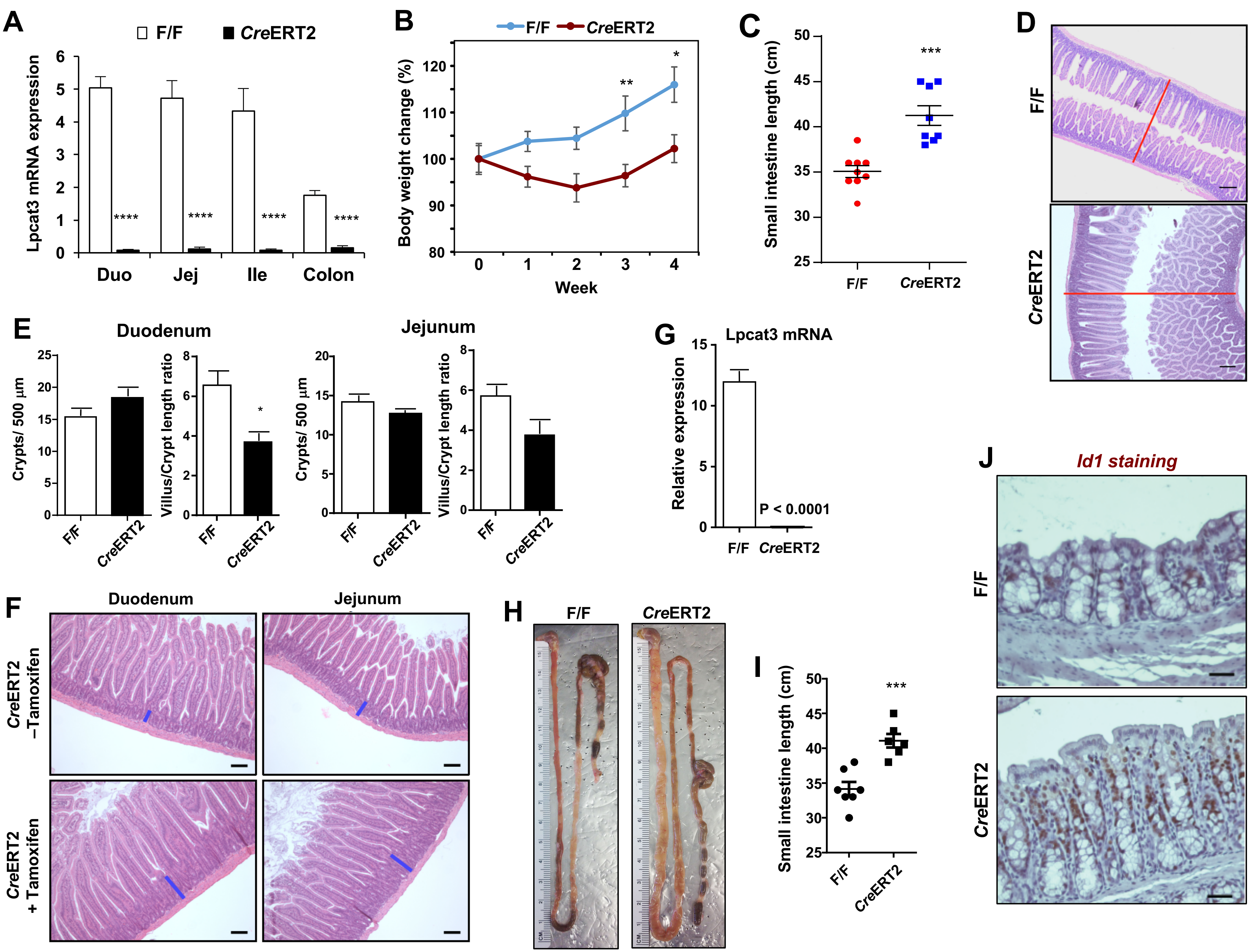
(**A-B**) Representative images of immunostaining of Ly6G and F4/80 in F/F and *Cre*ERT2, and Lpcat3$^{F/F}$, *Cre*ERT2, *Apc*$^{min/+}$ and Lpcat3$^{F/F}$, *Apc*$^{min/+}$ intestines.

(**C**) Expression of cytokines in Lpcat3$^{F/F}$, *Cre*ERT2, *Apc*$^{min/+}$ and Lpcat3$^{F/F}$, *Apc*$^{min/+}$ intestines (n=6-7 mice/group).

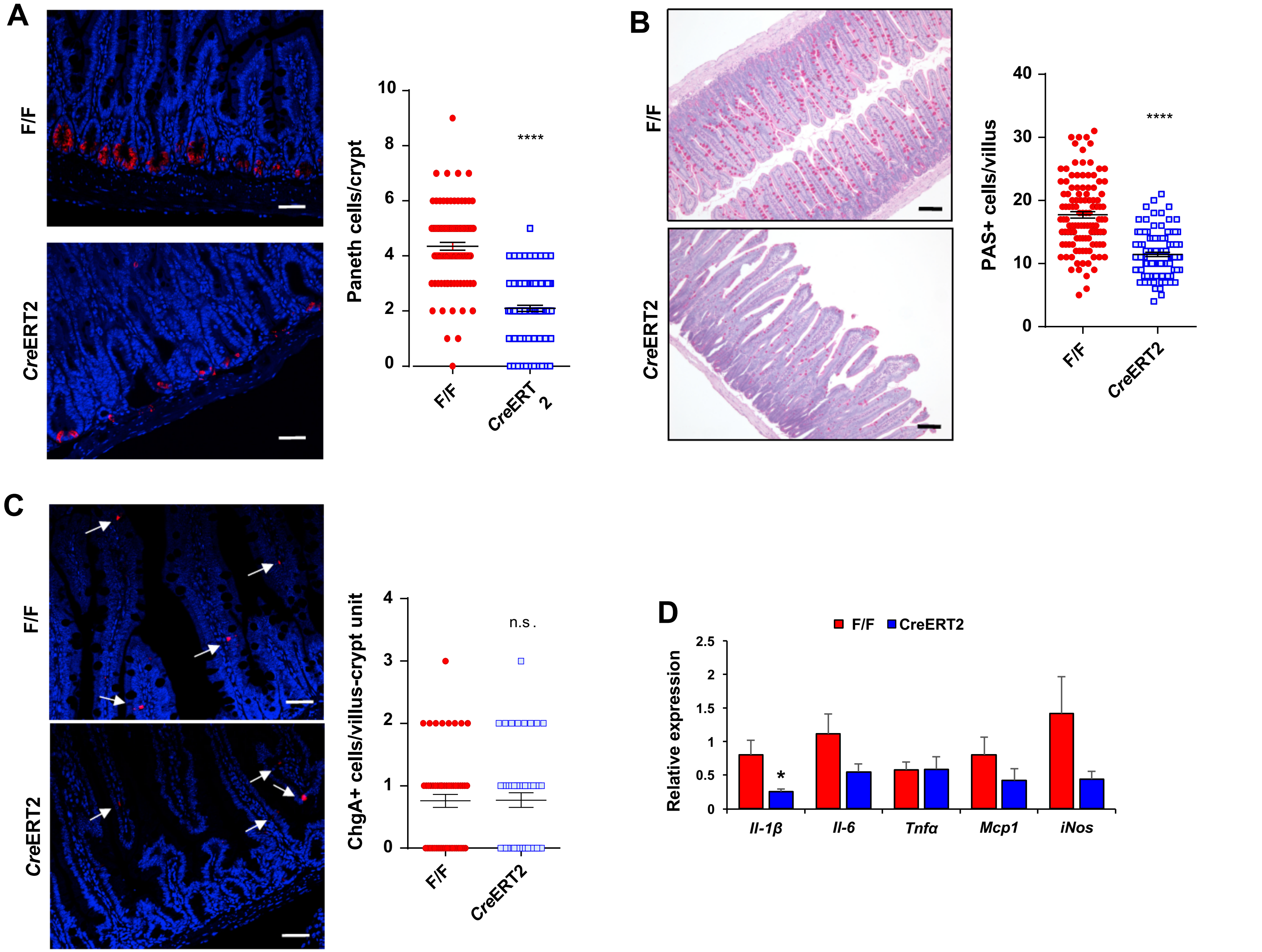
(**D**) Expression of selective genes in cholesterol biosynthetic pathway WT and *Apc*$^{min/+}$ Jejunums, analyzed by real-time RT-PCR (n=6-7 mice/group).

(**E**) Hematocrit in *Apc*$^{min/+}$ and *Srebf2* Tg, *Apc*$^{min/+}$ mice.

Values are means ± SEM. Statistical analysis was performed with Student's t test. * $P < 0.05$, ** $P < 0.01$, **** $P<0.0001$.



Wang Suppl. Fig. 1, related to Figure 1



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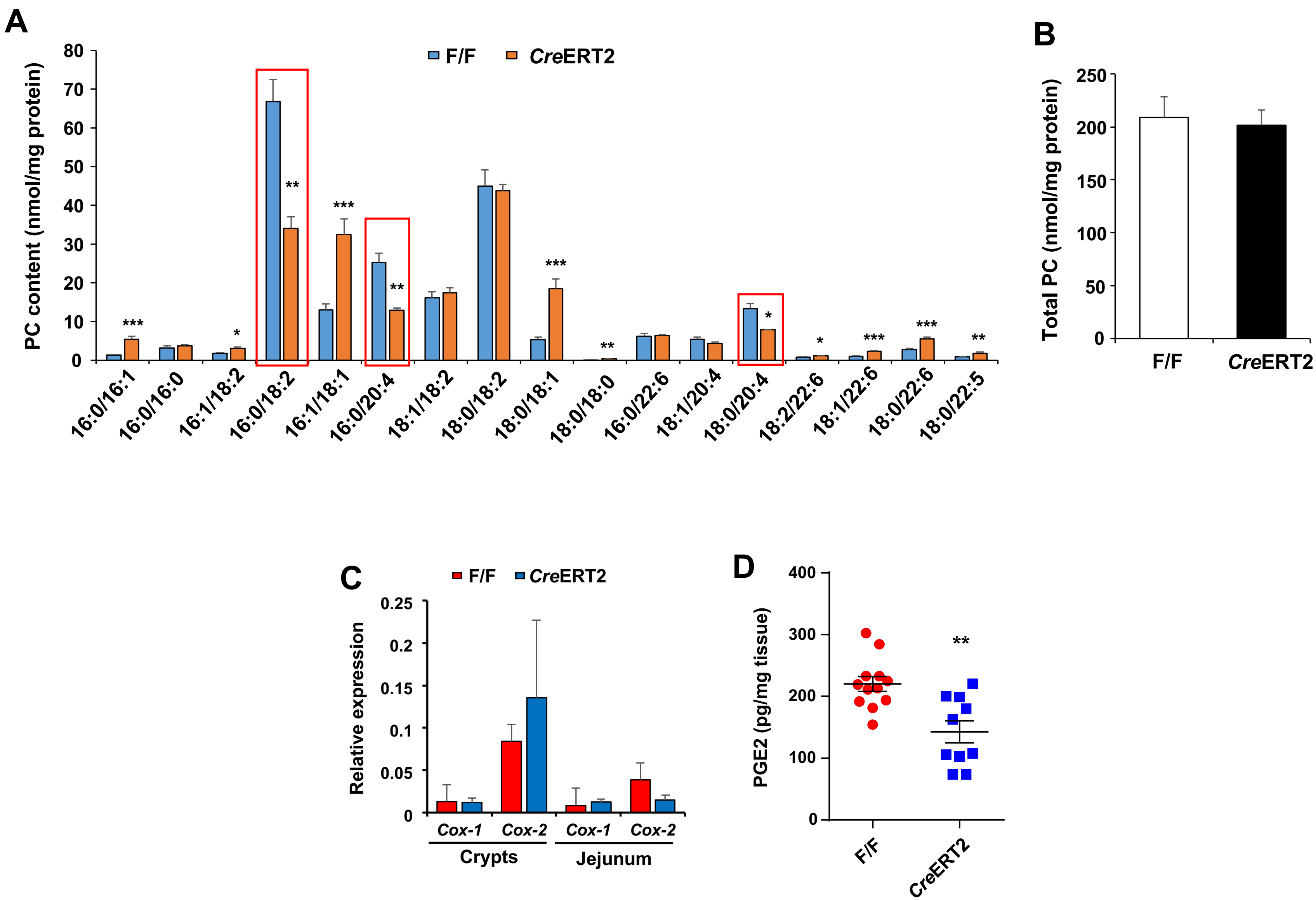

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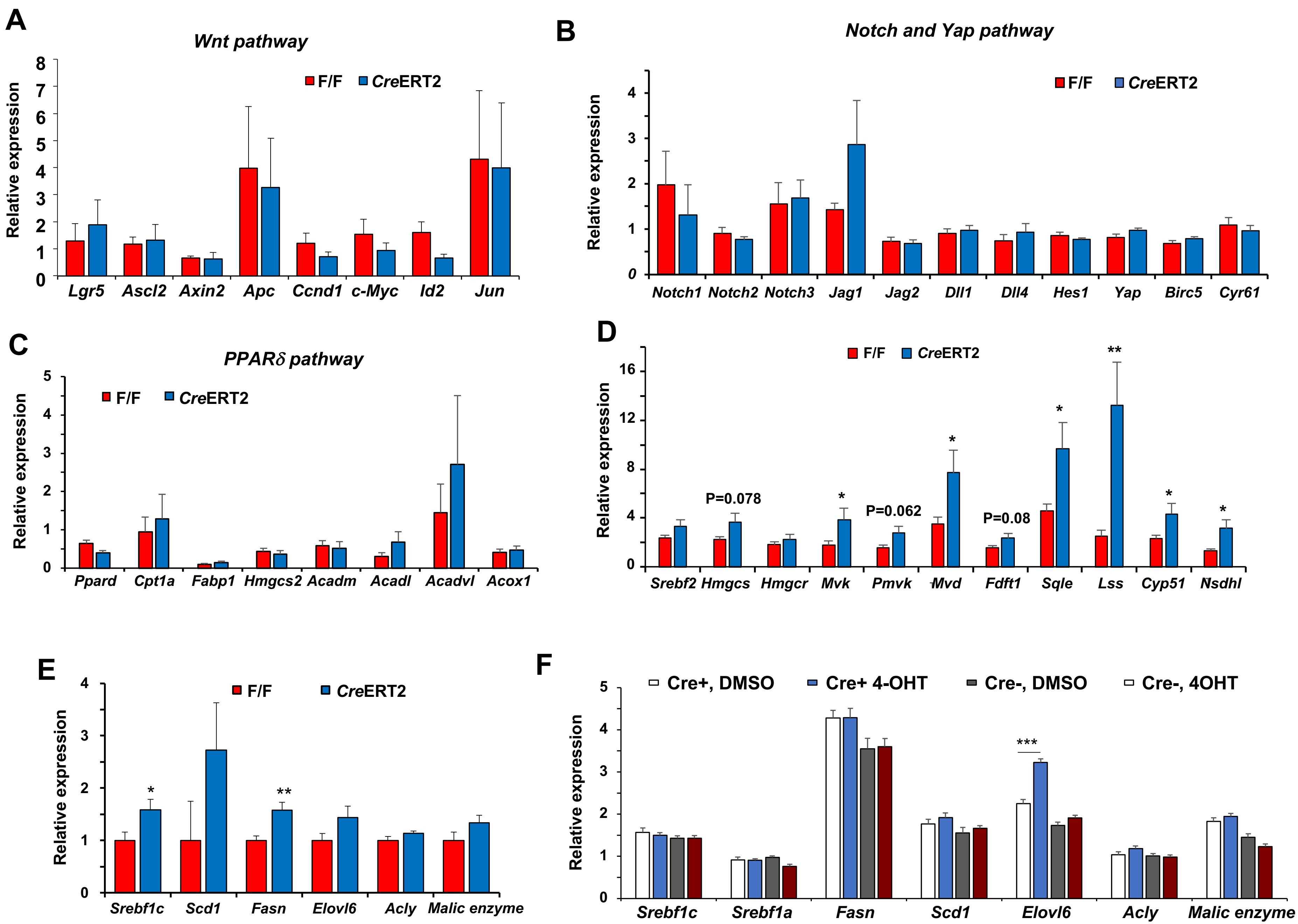

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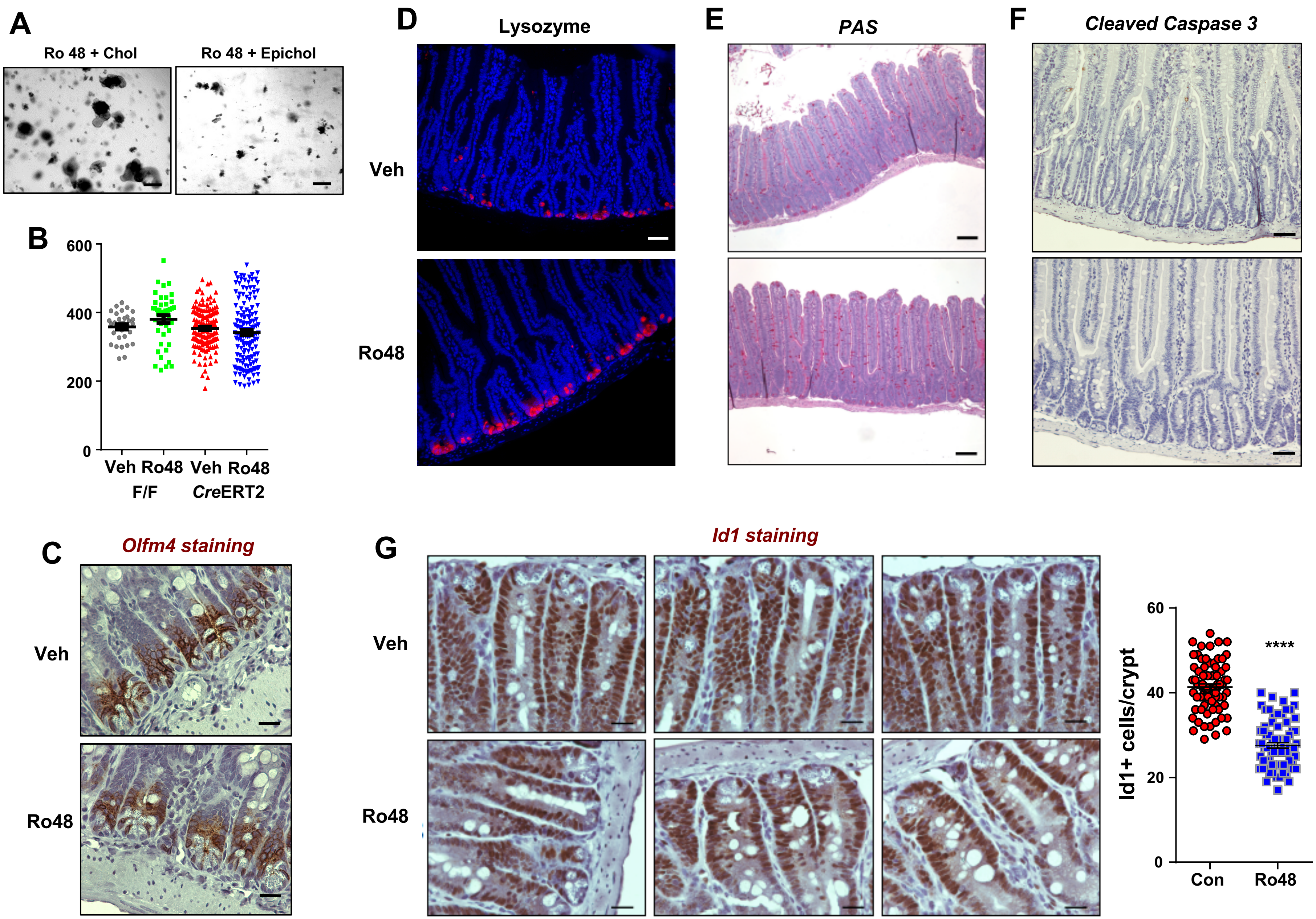
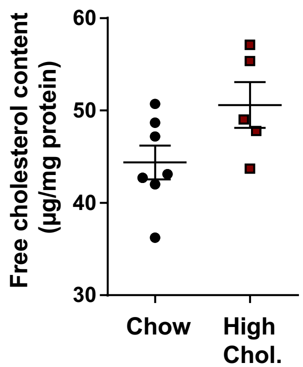


Wang Suppl. Fig. 5, related to Figure 4

A



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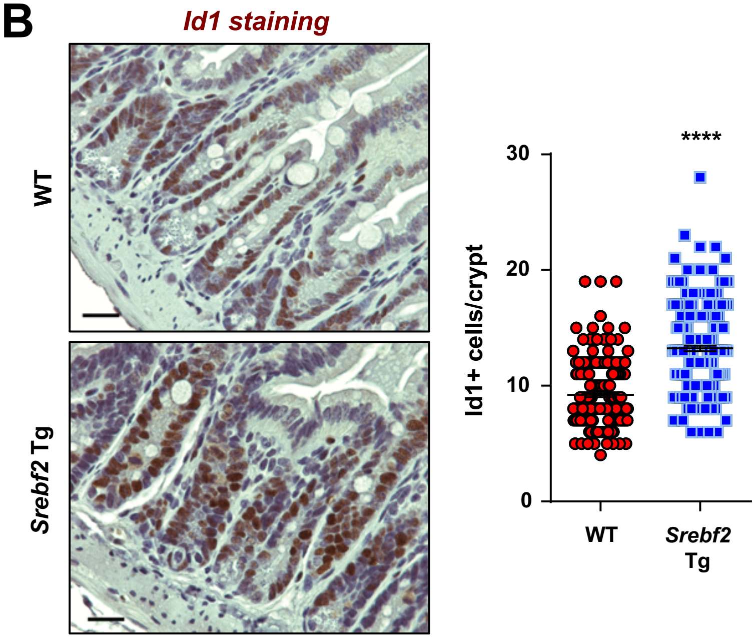

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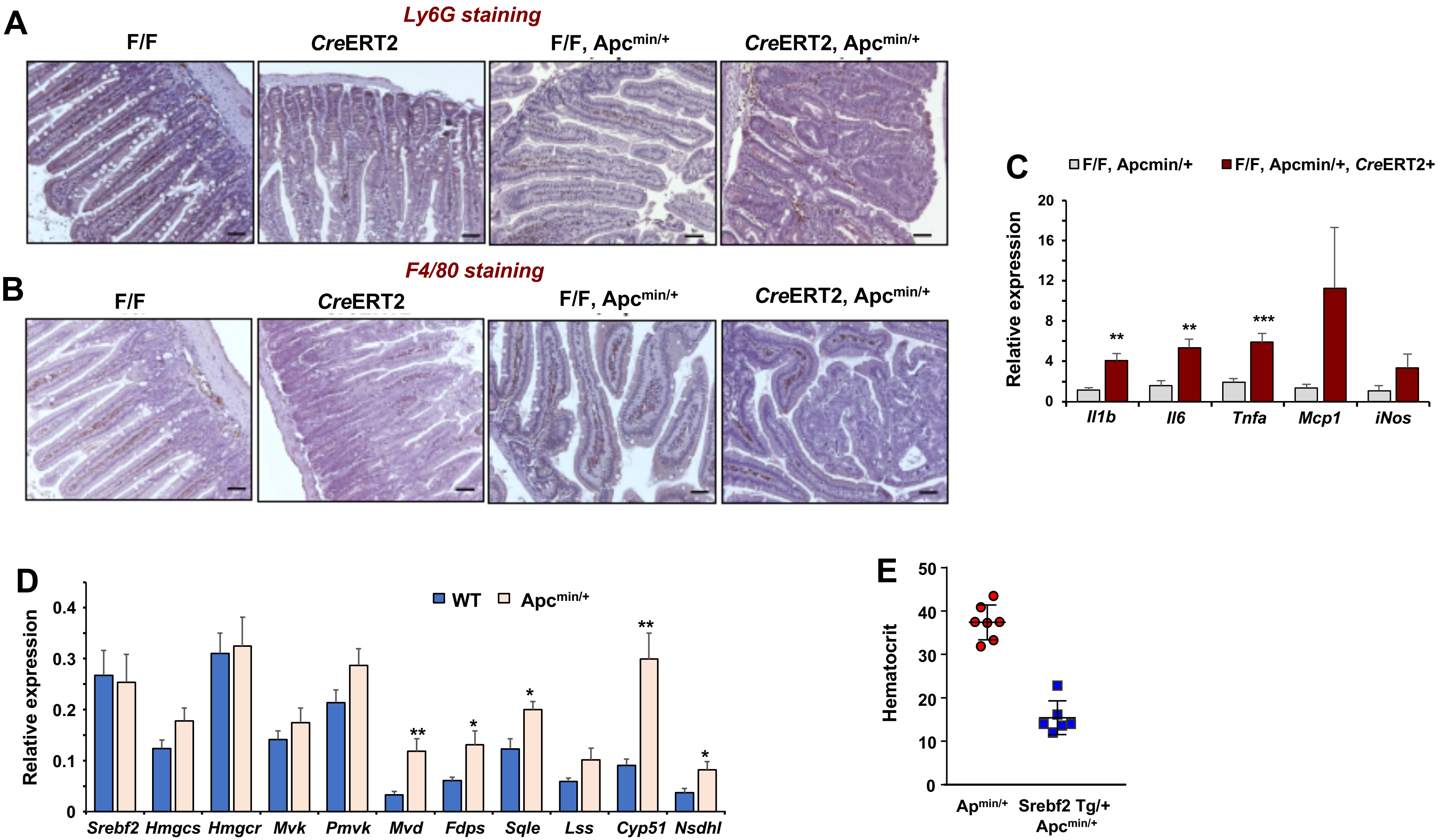

Wang Suppl. Fig. 7, related to Figure 6

Supplemental Table 1, related to STAR methods. Primer sequences used.

Primer	Sequence
36B4 F	GGCCCTGCACTCTCGCTTTC
36B4 R	TGCCAGGACGCGCTTGT
Actin F	GGCTGTATTCCCCTCCATCG
Actin R	CCAGTTGGTAACAATGCCATGT
Lpcat3 F	GGC CTC TCA ATT GCT TAT TTC A
Lpcat3 R	AGC ACG ACA CAT AGC AAG GA
Srebf2 F	ACCTAGACCTCGCCAAAGGT
Srebf2 R	GCACGGATAAGCAGGTTTGT
Hmgcr F	CTT GTG GAA TGC CTT GTG ATT G
Hmgcr R	AGC CGA AGC AGC ACA TGA T
Hmgcs1 F	GCCGTGAACTGGGTCGAA
Hmgcs1 R	GCATATATAGCAATGTCTCCT
Mvd F	ATGGCCTCAGAAAAGCCTCAG
Mvd R	TGGTCGTTTTTAGCTGGTCCT
Fdps F	GGAGGTCCTAGAGTACAATGC
Fdps R	AAGCCTGGAGCAGTTCTACAC
Lss F	TCGTGGGGGACCCTATAAAAC
Lss R	CGTCCTCCGCTTGATAATAAG
Cyp51 F	GACAGGAGGCAACTTGCTTTC
Cyp51 R	GTGGACTTTTCGCTCCAGC
Idi1 F	ACCAGCCATCTTGATGAAAAA
Idi1 R	CAGCAACTATTGGTGAAACAA
Nsdhl F	TCATGGTGAATCAAAGCGAGG
Nsdhl R	CCGGGGGTTATCAAAGCCTTG
Mvk F	GGTGTGGTCGGAACTTCCC
Mvk R	CCTTGAGCGGGTTGGAGAC
Pmvk F	AAAATCCGGGAAGGACTTCGT
Pmvk R	AGAGCACAGATGTTACCTCCA
Fdft1 F	ATGGAGTTCGTCAAGTGTCTA
Fdft1 R	CGTGCCGTATGTCCCCATC
Il-1b F	AGA AGC TGT GGC AGC TAC CTG
Il-1b R	GGA AAA GAA GGT GCT CAT GTC C
Tnf alpha F	TGC CTA TGT CTC AGC CTC TTC
Tnf alpha R	GAG GCC ATT TGG GAA CTT CT
Mcp-1 F	CATCCACGTGTTGGCTCA
Mcp-1 R	GATCATCTTGCTGGTGAATGA
iNOS F	GCAGCTGGGCTGTACAAA
iNOS R	AGCGTTTCGGGATCTGAAT
Il-6 F	GCT ACC AAA CTG GAT ATA ATC AAG A
Il-6 R	CCA GGT AGC TAT GGT ACT CCA GAA
Fasn F	TGCTCCCAGCTGCAGGC
Fasn R	GCCCGGTAGCTCTGGGTGTA
Srebp1c F	GGAGCCATGGATTGCACATT
Srebp1c R	GGCCCGGGAAGTCACTGT
Srebp1a F	GGCCGAGATGTGCGAACT
Srebp1a R	TTGTTGATGAGCTGGAGCATG
Elovl6 F	CAGCAAAGCACCCGAACTA
Elovl6 R	AGGAGCACAGTGATGTGGTG
Scd1 F	CGAAGTCCACGCTCGATCTC
Scd1 R	TGTGGGCCGGCATGAT
Lgr5 F	GGGCGTTAAGTCCACTGTGT
Lgr5 R	CGAACACCTGCGTGAATATG
Ascl2 F	AAGCACACCTTGACTGGTACG
Ascl2 R	AAGTGGACGTTTGCACCTTCA
Axin2 F	TGACTCTCCTTCCAGATCCCA
Axin2 R	TGCCCACACTAGGCTGACA
Cox-1 F	GTGCTGGGGCAGTGCTGGAG
Cox-1 R	TGGGGCCTGAGTAGCCCGTG
Cox-2 F	CAAGGGAGTCTGGAACATTG
Cox-2 R	ACCCAGGTCCTCGCTTATGA
Hes1-F	ACACCGGACAAACCAAAGAC
Hes1-R	AATGCCGGGAGCTATCTTTC
Atoh1-F	GCCTTGCCGGACTCGCTTCTC
Atoh1-R	TCTGTGCCATCATCGCTGTTAGGG
CTGF-F	AGACCTGTGCCTGCCATTAC
CTGF-R	AGCCCATGTCTCCGTACATC
Cyr61-F	AGAGGCTTCCTGTCTTTGGC
Cyr61-R	CCAAGACGTGGTCTGAACGA
Birc5-F	GAACCCGATGACAACCCGAT
Birc5-R	CTCCTTTGCAATTTTGTTCTTGGC
Yap-F	ATTTCGGCAGGCAATACGGA
Yap-R	CATCCTGCTCCAGTGTAGGC
Notch1-F	CCCTTGCTCTGCCTAACGC
Notch1-R	GGAGTCCTGGCATCGTTGG
Notch2-F	GGAATGGTGGCAGAGTTGAT
Notch2-R	TCGCCTCCACATTATTGACA
Notch3-F	GGACAAGATGCACTGGGAAT
Notch3-R	AGTCTCTTGGCCTCTGGACA
Notch4-F	TTCTCGTCCTCCAGCTCATT
Notch4-R	CCACTCCATCCTCATCCACT
Jag2-F	GCACCTGCACACATAACACC
Jag2-R	TTGACGCCATCAACACAGAT
Dll1-F	GGCTTCTCTGGCTTCAACTG
Dll1-R	CACCGGCACAGGTAAGAGTT
Dll4-F	ACCTTTGGCAATGTCTCCAC
Dll4-R	GTTTCCTGGCGAAGTCTCTG
Taz-F	GAAGGTGATGAATCAGCCTCTG
Taz-R	GTTCTGAGTCGGGTGGTTCTG
Apc F	TGAGTGCCTTATGGAACCTGT
Apc R	CTCCGGTAAGTGAGGGTGC
Ccnd1 F	GAATCTGCCCTGTGACATGAAA
Ccnd1 R	CCATGGTGTGTCAACCAGAAAT
Id2 F	GACAGAACCAGGCGTCCA
Id2 R	AGCTCAGAAGGGAATTCAGATG
Jun F	GAAAAGTAGCCCCCAACCTC
Jun R	GGGACACAGCTTTCACCCTA
Jag1 F	GCTTCGGCTCAGGGTCTAC
Jag1 R	GGCGAAACTGAAAGGCAGTA
c-Myc F	GCACAAGCTCACCTCTGAAAAGGAC
c-Myc R	CTCACGAGAGATTCCAGCTCCTCC
Acadm F	TTACCGAAGAGTTGGCGTATGG
Acadm R	TGCGGAGGGCTCTGTCAC
Acadl F	CTCCCTGCGCGTCCTGAG
Acadl R	AAAATGTCATGCTCCGAGGAAAAG
Acadvl F	GCCCAGACACACAACCTTTG
Acadvl R	CCGAGCCGACTGCATCTC
Fabp1 F	CCA TGA CTG GGG AAA AAG TC
Fabp1 R	GCC TTT GAA AGT TGT CAC CAT
Ppard F	CTAAGCACATCTACAACGCCTACCT
Ppard R	GCCTGCCACAGTGTCTCGAT
Cpt1a F	CCGCCAATTCCAAAAAGTAAC
Cpt1a R	CATTTGGTTTGTATCACTAGA
Hmgcs2 F	GCAGTGACAAACAGAACAACTTATACAA
Hmgcs2 R	GACCCCTGAAGGCCTCTAGG
Acox1 F	TCGAAGCCAGCGTTACGAG
Acox1 R	ATCTCCGTCTGGGCGTAGG