Supplementary

Table S1 Primers for quantitative RT-PCR

Primer	Sequence
CYP1B1-forward	5'-GGCTGGATTTGGAGAACGTA-3'
CYP1B1-reverse	5'-CATAAAGGAAGGCCAGGACA-3'
GAPDH-forward	5'-ACCCACTCCTCCACCTTTG-3'
GAPDH-reverse	5'-CTGTAGCCAAATTCGTTGTCAT-3'
MCM2-forward	5'-ACCAGGACAGAACCAGCATC-3'
MCM2-reverse	5'-CAGGATGTCAAAGCGTGAGA-3'
MCM3-forward	5'-TGTGGAGGGCATTGTCACTA-3'
MCM3-reverse	5'-CAAGGGGATTGTTCTCCTCA-3'
MCM5-forward	5'-CTGGGGGAGTACTGGATTGA-3'
MCM5-reverse	5'-ATGACCTGGATGTCCTGGAG-3'
PCNA-forward	5'-CGGATACCTTGGCGCTAGTA-3'
PCNA-reverse	5'-TCACTCCGTCTTTTGCACAG-3'
FEN1-forward	5'-GACATGGACTGCCTCACCTT-3'
FEN1-reverse	5'-CCCAATACCCCGGATACTCT-3'
LIG1-forward	5'-AGGAGTGGAATGGAGTGGTG-3'
LIG1-reverse	5'-AGGTGTCAGAGAGGGAAGCA-3'
Actin-forward	5'-GGACTTCGAGCAAGAGATGG-3'
Actin-reverse	5'-AGCACTGTGTTGGCGTACAG-3'

RT-PCR, real-time polymerase chain reaction.

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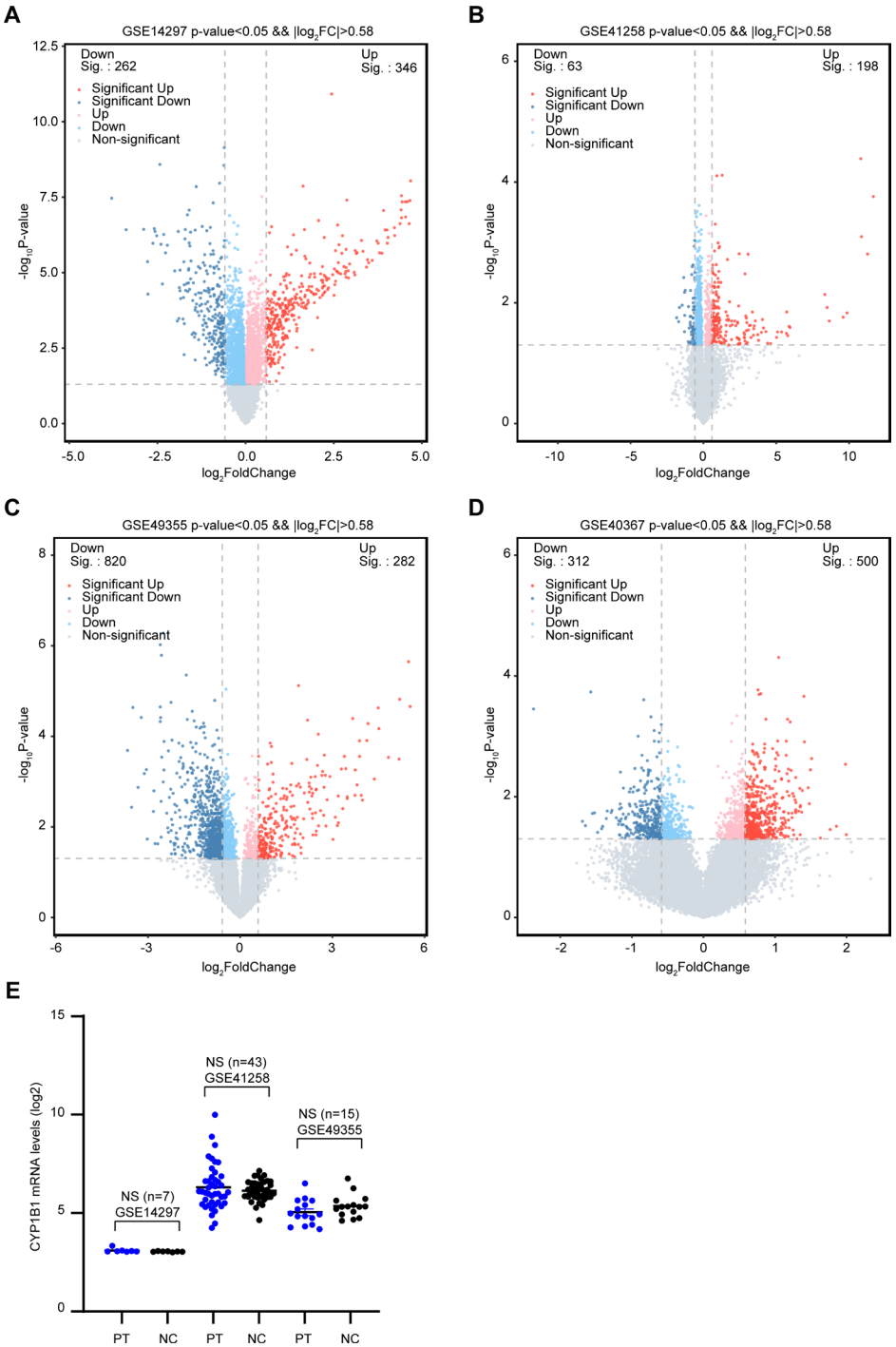

Figure S1 Genes between LM and PT from four GSE databases and CYP1B1 mRNA levels in PT and normal colon tissues (NC) from three GSE databases. (A-D) Volcano plot of mRNA profiles from 4 GSE databases. 346 up-regulated genes and 262 down-regulated genes are shown in (A) (n=18). 198 up-regulated genes and 63 down-regulated genes are shown in (B) (n=5) (B). 282 up-regulated genes and 820 down-regulated genes are shown in (C) (n=13). 500 up-regulated genes and 312 down-regulated genes are shown in (D) (n=7). (E) The CYP1B1 mRNA levels in PT and NC from three GSE databases. Each dot represents the relative mRNA level in PT or NC in each tissue sample from 3 GSE databases, data are shown as mean ± SEM. Significance was determined by a 2-tailed paired *t*-test. NS, not significant as indicated. LM, liver metastases; PT, primary tumors; GSE, GEO series; mRNA, messenger RNA; SEM, standard error of the mean.

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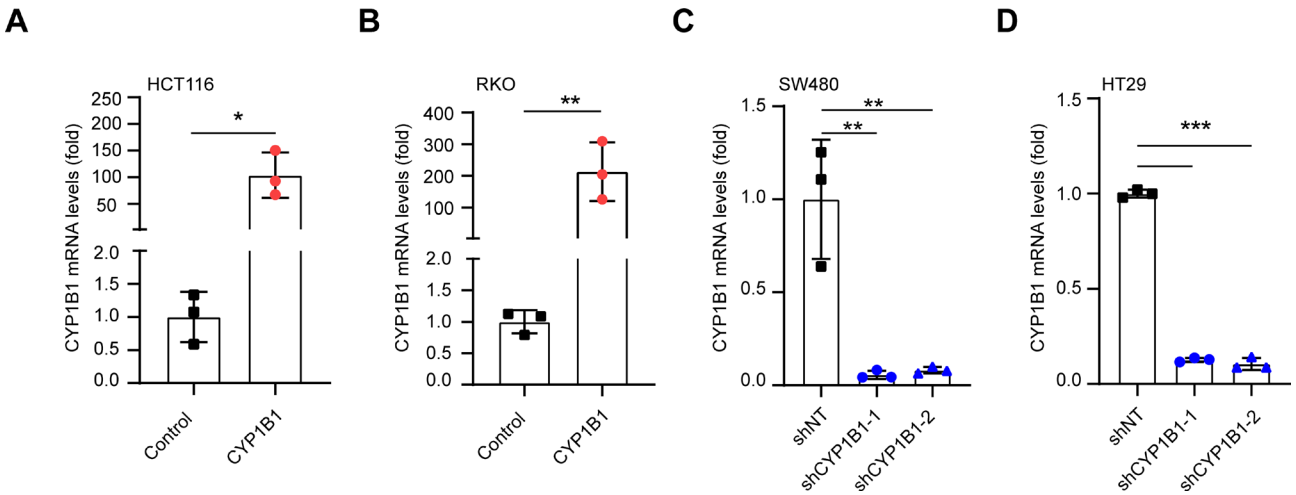

Figure S2 Confirmation of *CYP1B1* overexpression and knockdown in CRC cell lines. (A-D) Quantitative RT-PCR confirms *CYP1B1* overexpression and knockdown in CRC cell lines. The *CYP1B1* mRNA levels increased by about 100-fold in HCT116 (A) and 200-fold in RKO (B), decreased by about 80–90% in SW480 or HT29 (C,D). Representative results from at least three independent experiments are shown. Data are shown as mean ± SD. Significance was determined by Student's *t*-test (A,B) or one-way ANOVA (C,D). *, *P*<0.05; **, P<0.01; ***, *P*<0.001. shNT, non-target shRNA control; CRC, colorectal cancer; RT-PCR, real-time polymerase chain reaction; mRNA, messenger RNA; SD, standard deviation; ANOVA, analysis of variance.

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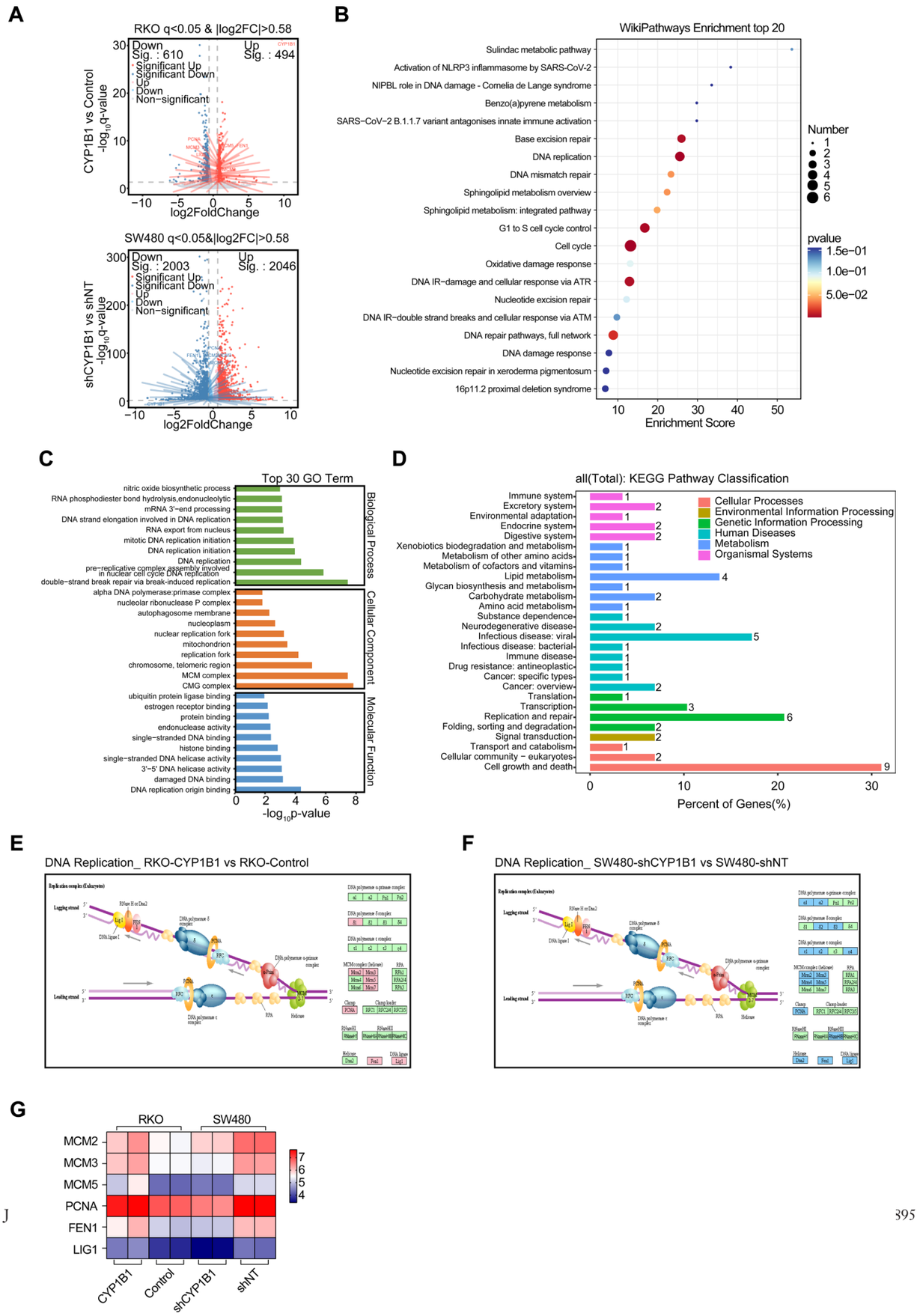

Figure S3 Enrichment analysis of potential *CYP1B1*-regulated genes. (A) Volcano plot of mRNA profiles from overexpression and knockdown cell groups. 494 up-regulated genes and 610 down-regulated genes in RKO group are shown in CYP1B1 VS control. 2,046 up-regulated genes and 2,003 down-regulated genes in SW480 group are shown in Graph shCYP1B1 VS shNT. (B-D) Enrichments of 59 genes between RKO up-regulated genes and SW480 down-regulated genes. WikiPathways enrichment shows 59 genes are mainly concentrated in cell cycle, DNA replication, etc. (B). GO analysis shows 59 genes were mainly concentrated in MCM complex, DNA replication origin binding, etc. (C). KEGG Pathway shows 59 genes are mainly concentrated in cell growth and death, replication and repair, etc. (D). (E,F) DNA replication diagram in RKO and SW480. MCM2, MCM3, MCM5, etc. are up-regulated in the DNA replication diagram of RKO group (E). FEN1, LIG1, DNA2, etc. are down-regulated in the DNA replication diagram of SW480 group (F). (G) Six growth-related differential genes from overexpression and knockdown cell groups. Data are shown in heat map with logarithm of two. GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; shNT, non-target shRNA control; mRNA, messenger RNA.

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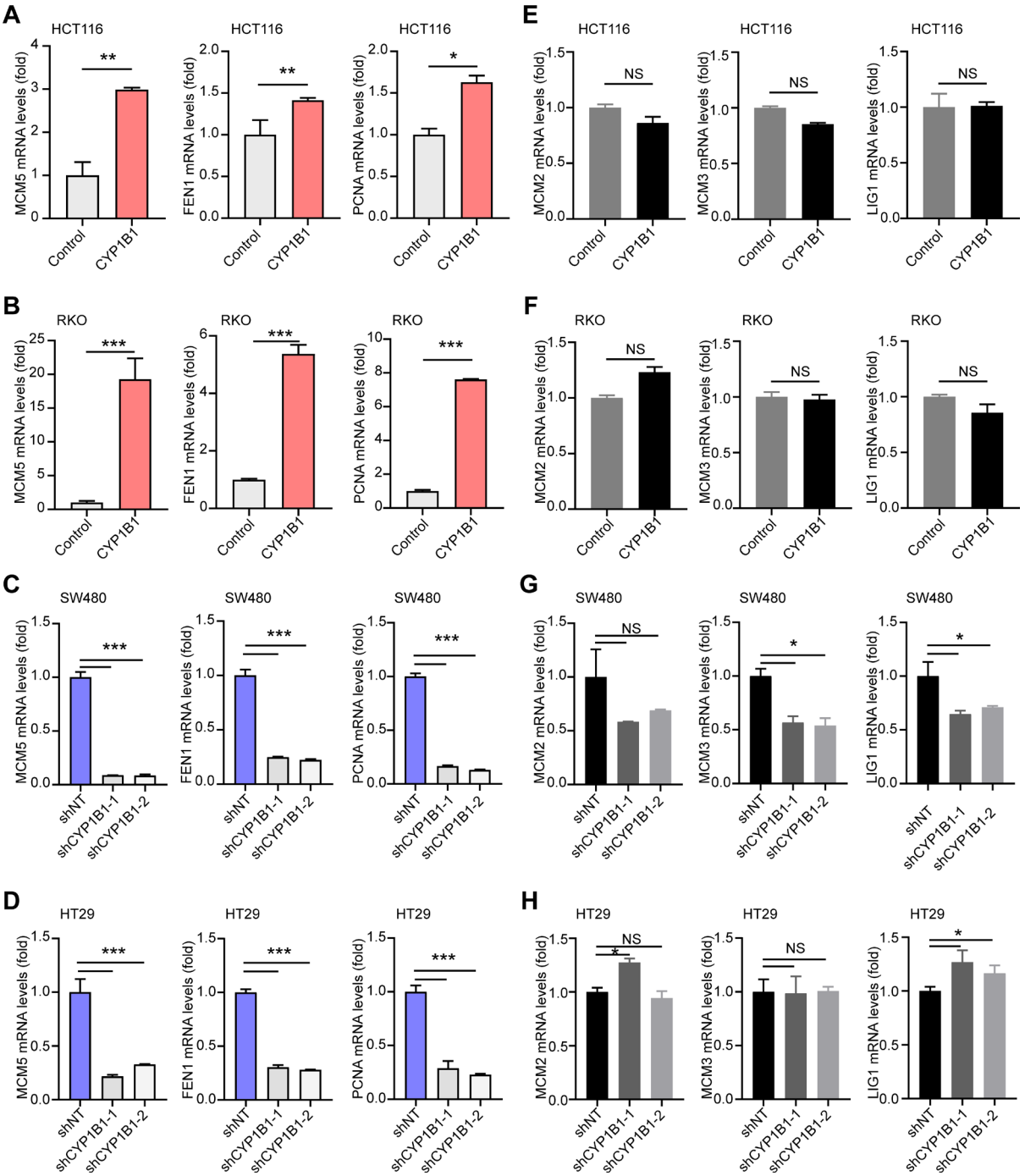

Figure S4 *CYP1B1* regulates CRC cell growth by regulating expressions of *MCM5*, *PCNA*, and *FEN1*. (A-D) Positive genes challenge were verified in CRC cell lines by quantitative RT-PCR. The mRNA levels of MCM5, FEN1 and PCNA are up-regulated after CYP1B1 overexpression in HCT116 and RKO (A,B). The mRNA levels of *MCM5*, *FEN1*, and *PCNA* are down-regulated after *CYP1B1* knockdown in SW480 and HT29 (C,D). (E-H) Negative genes challenge were verified in CRC cell lines by quantitative RT-PCR. *MCM2*, *MCM3*, or *LIG1* show unrelated tendency in CRC cells after *CYP1B1* intervention (E-H). Representative results from at least 3 independent experiments are shown. Data are shown as mean ± SD. Significance was determined by Student's *t*-test (A,B,E,F) or one-way ANOVA (C,D,G,H). *, P<0.05; **, P<0.01; ***, P<0.001. NS, not significant as indicated. shNT, non-target shRNA control; CRC, colorectal cancer; RT-PCR, real-time polymerase chain reaction; mRNA, messenger RNA; SD, standard deviation; ANOVA, analysis of variance.

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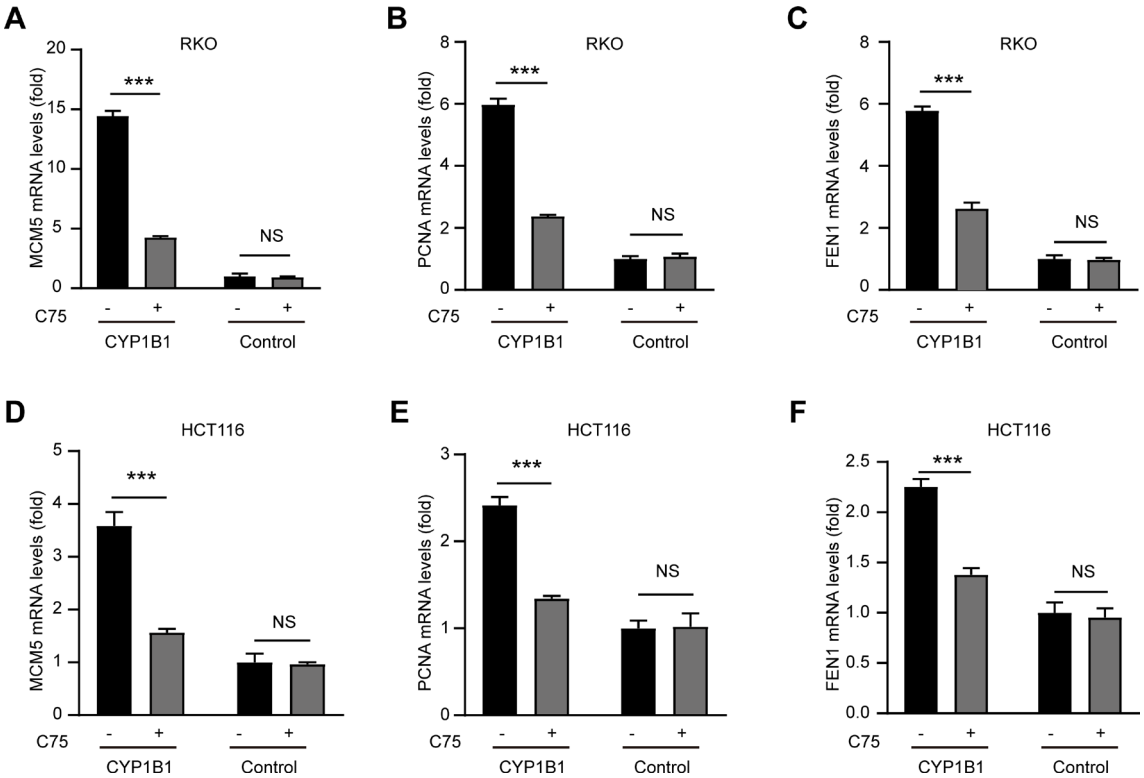

Figure S5 *CYP1B1* promotes CRC cell growth by enhancing fatty acids biosynthesis genes. (A-F) C75 suppresses growth-related genes expression in CRC cells by quantitative RT-PCR. The mRNA levels of MCM5, FEN1 and PCNA in RKO and HCT116 groups were decreased after C75 (10 μM) intervention for 48 h. Representative results from at least three independent experiments are shown. Data are shown as mean ± SD of triplicate. Significance was determined by 2-way ANOVA. ***P<0.001. NS, not significant as indicated. CRC, colorectal cancer; RT-PCR, real-time polymerase chain reaction; mRNA, messenger RNA; SD, standard deviation; ANOVA, analysis of variance.

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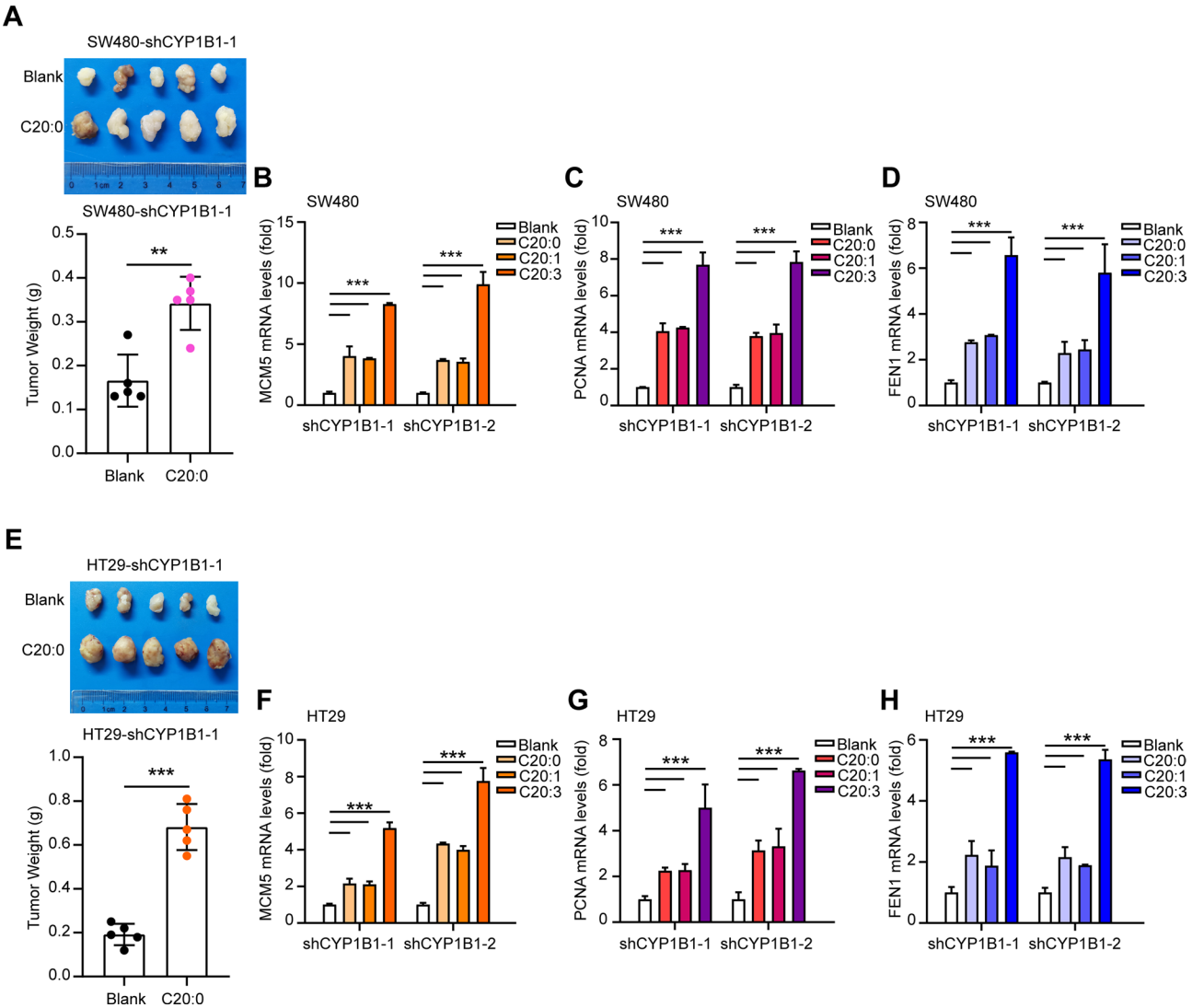

Figure S6 *CYP1B1* regulates the expression of *MCM5*, *PCNA*, and *FEN1* via LCFAs. (A) The effects of C20:0 on tumor growth of CRC xenograft tumors. After co-culture with C20:0 (50 μM, 48 h), 1×10^6 cells were subcutaneously implanted into dorsal right flank of nude mice, tumor volumes were assessed on the indicated days (4 weeks after injection) and tumors were dissected for photo in the up of (A). Data are shown as mean ± SD. (B-D) LCFAs regulate *MCM5*, *PCNA*, *FEN1* up-regulation in SW480 shCYP1B1 cells. After co-culture with LCFAs (50 μM, 48 h), mRNA levels of *MCM5*, *PCNA*, and *FEN1* were up-regulated in SW480 shCYP1B1 cells (B-D). Representative results from at least 3 independent experiments are shown. (E) The effects of C20:0 on tumor growth of CRC xenograft tumors. After co-culture with C20:0 (50 μM, 48 h), 1×10^6 cells were subcutaneously implanted into dorsal right flank of nude mice, tumor volumes were assessed on the indicated days (4 weeks after injection) and tumors were dissected for photo in the up of (E). Data are shown as mean ± SD. (F-H) LCFAs regulate *MCM5*, *PCNA*, *FEN1* up-regulation in HT29 shCYP1B1 cells. After co-culture with LCFAs (50 μM, 48 h), mRNA levels of *MCM5*, *PCNA*, and *FEN1* were up-regulated in HT29 shCYP1B1 cells (F-H). Representative results from at least 3 independent experiments are shown. Significance was determined by Student's *t*-test (A,E) or 2-way ANOVA (B-D,F-H). **, P<0.01; ***, P<0.001. NS, not significant as indicated; LCFAs, long chain fat acids; mRNA, messenger RNA; CRC, colorectal cancer; SD, standard deviation; ANOVA, analysis of variance.

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