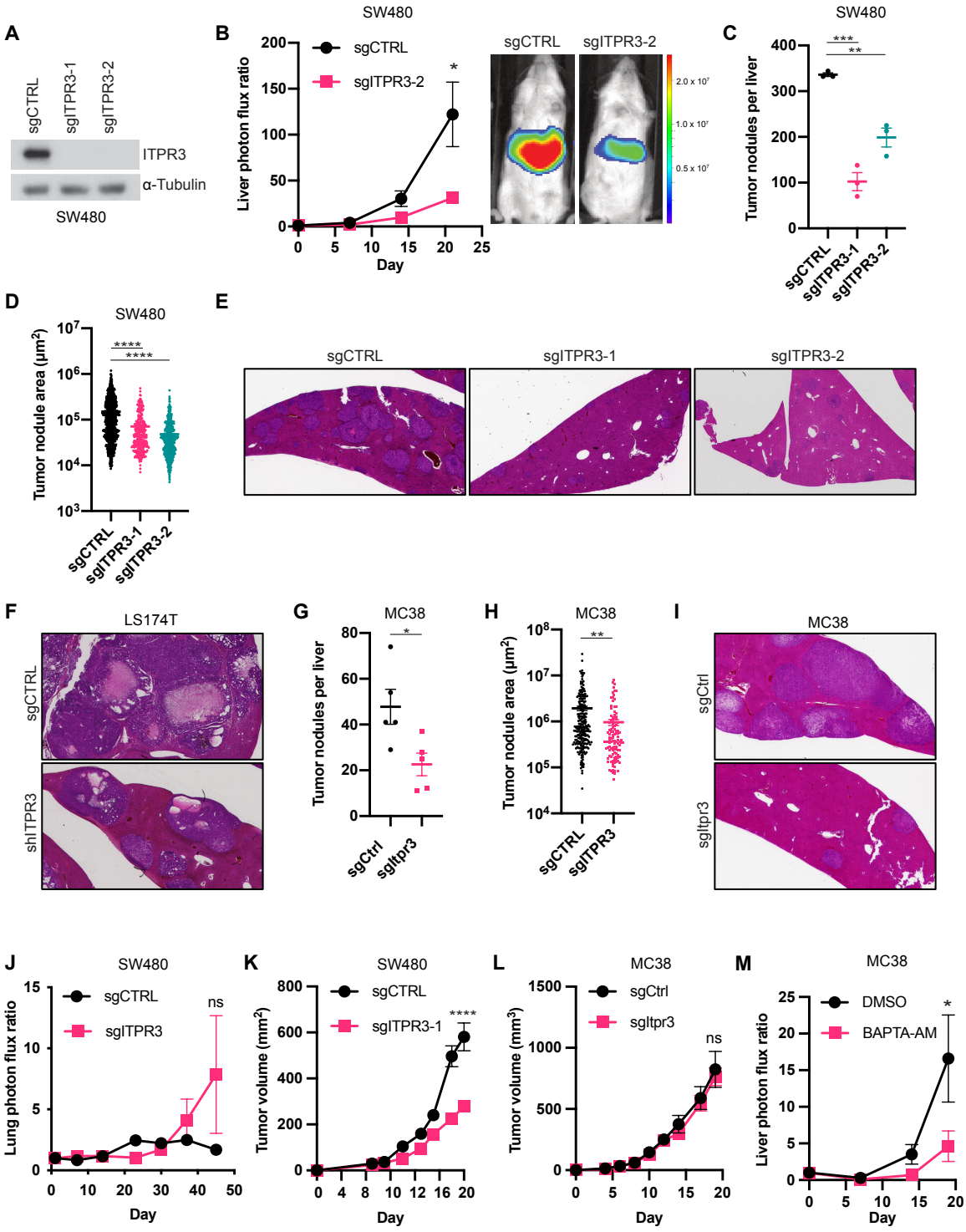


**Figure S1. ITPR3 facilitates metastatic CRC liver colonization, related to Figure 2. (A)** ITPR3 expression by immunoblot in CRISPR-edited sgCTRL or sgITPR3 SW480 cells (two independent

guide RNAs). **(B)** 5 x $10^5$ SW480 sgCTRL or sgITPR3-2 cells were inoculated by portal circulation injection, and metastatic colonization was measured by liver bioluminescence at the indicated timepoints (n=4 mice). **(C-E)** Quantification of tumor nodules per liver (C), tumor nodule area (D), and representative H&E staining (E) of livers with sgCTRL or sgITPR3 SW480 metastases. **(F)** Representative H&E staining of livers with shCTRL or shITPR3 LS174T metastases. **(G-I)** Quantification of tumor nodules per liver (G), tumor nodule area (H), and representative H&E staining (I) of livers with sgCtrl or sgItpr3 MC38 metastases. **(J)** 2 x $10^5$ SW480 sgCTRL or sgITPR3-1 cells were inoculated by tail vein injection, and lung metastatic burden was monitored by bioluminescence. **(K)** 5 x $10^5$ SW480 sgCTRL or sgITPR3-1 cells were injected into the flanks of mice and subcutaneous xenograft volume was monitored. **(L)** 5 x $10^5$ SW480 sgCtrl or sgItpr3 MC38 cells were injected into the flanks of mice and subcutaneous xenograft volume was monitored. **(M)** MC38 cells were treated with 20 μM BAPTA-AM or DMSO control for two hours priors to portal circulation injection, and metastatic colonization was measured by liver bioluminescence at the indicated timepoints (n = 8 mice). Mean ± SEM; *p < 0.05, **p < 0.01, ***p < 0.001, **** p < 0.0001, Student's t-test.

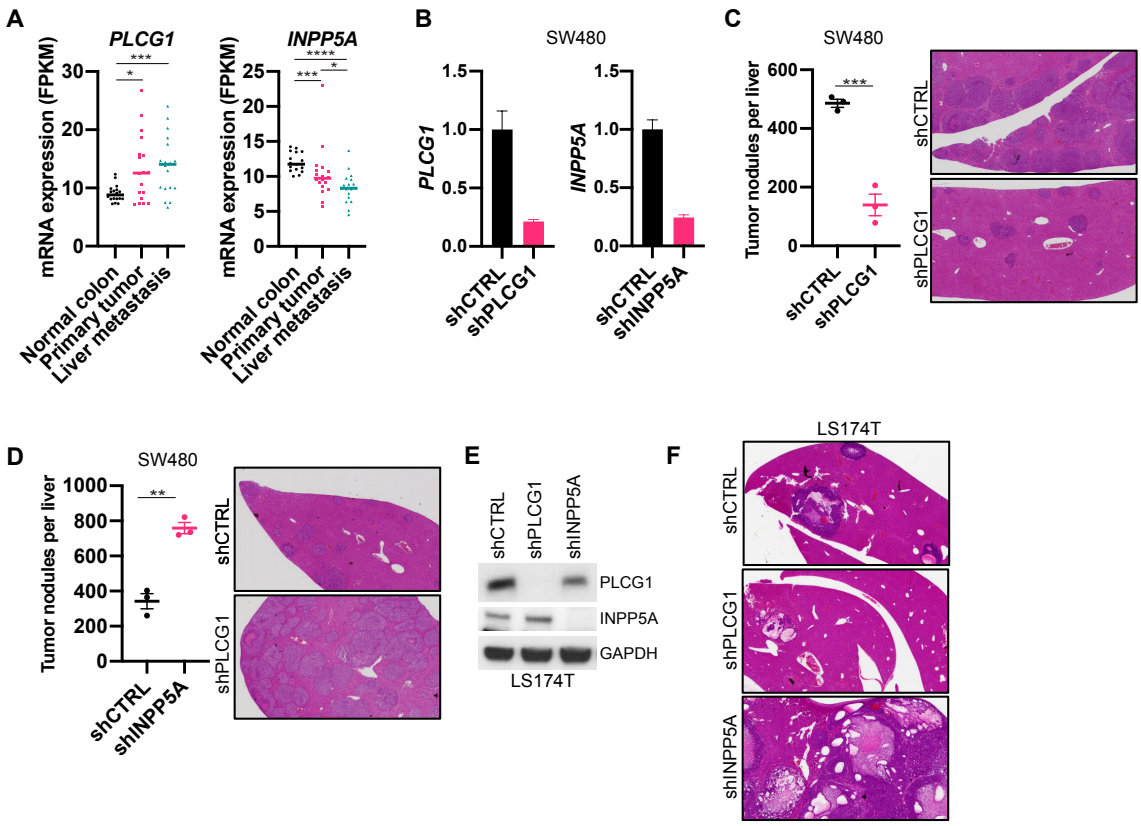


**Figure S2. Expression of genes involved in IP3 signaling and metabolism are modulated in CRC liver metastases, related to Figure 3. (A)** *PLCG1* and *INPP5A* expression as measured by RNA sequencing from normal colon, primary tumors or liver metastases (GSE50760). **(B)** RNA transcript levels of *PLCG1* and *INPP5A* from control or shRNA-expressing SW480 cells as measured by qRT-PCR. **(C)** $5x10^5$ SW480 cells expressing control or PLCG1 shRNA were inoculated by portal circulation injection, and metastatic tumor nodules were quantified by H&E staining. **(D)** $5x10^5$ SW480 cells expressing control or INPP5A shRNA were inoculated by portal circulation injection, and metastatic tumor nodules were quantified by H&E staining. **(E)** Immunoblot for PLCG1 and INPP5A protein in LS174T cells expressing the indicated shRNAs. **(F)** $5x10^5$ LS174T cells expressing control, PLCG1, or INPP5A shRNA were inoculated by portal circulation injection, and metastatic tumor nodules were quantified by H&E staining. Median (A) or Mean ± SEM (C-D); *p < 0.05, ** p < 0.01, *** p < 0.001, **** p < 0.0001, Mann-Whitney test (A) or Student's t-test (C-D)

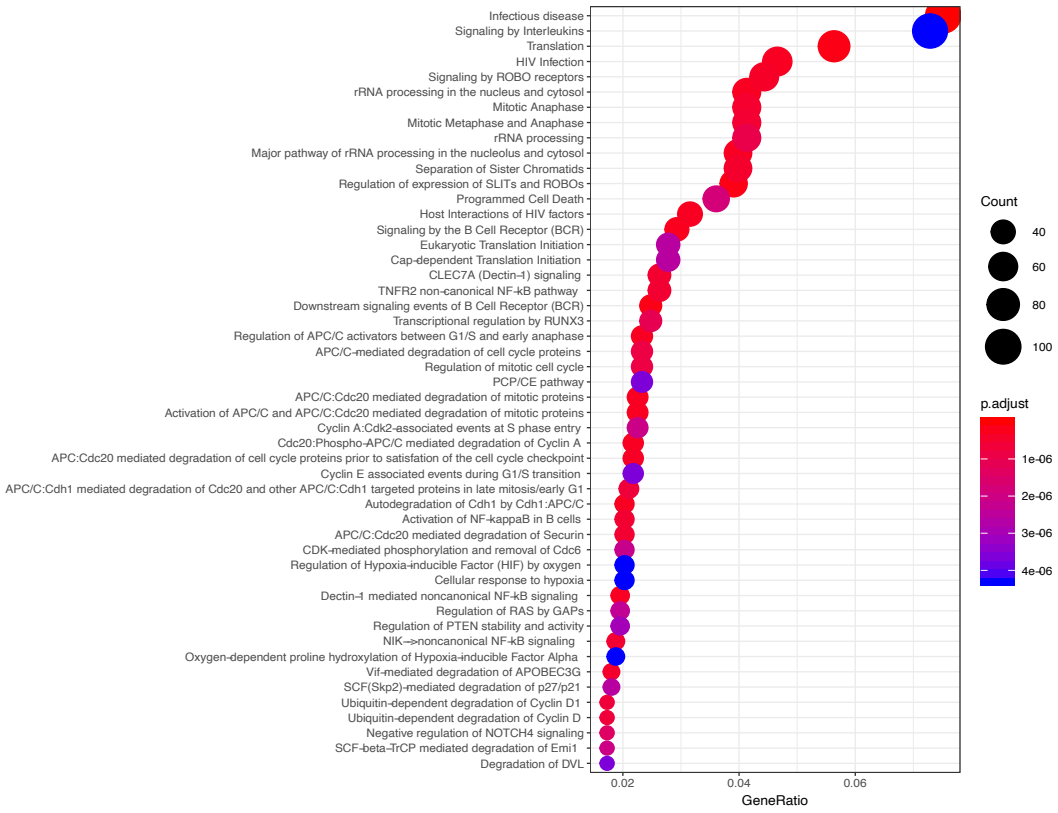


**Figure S3. Transcriptomic analysis of ITPR3-regulated genes in established CRC liver metastases, related to Figure 5.** 5 x $10^5$ SW480 sgCTRL or sgITPR3 cells were inoculated by portal circulation injection, and cells were isolated from liver metastases harvested at day 27 prior to RNA-seq. Enrichment of biological pathways among down-regulated genes in *ex vivo* SW480 sgITPR3 cells by Reactome pathway analysis.

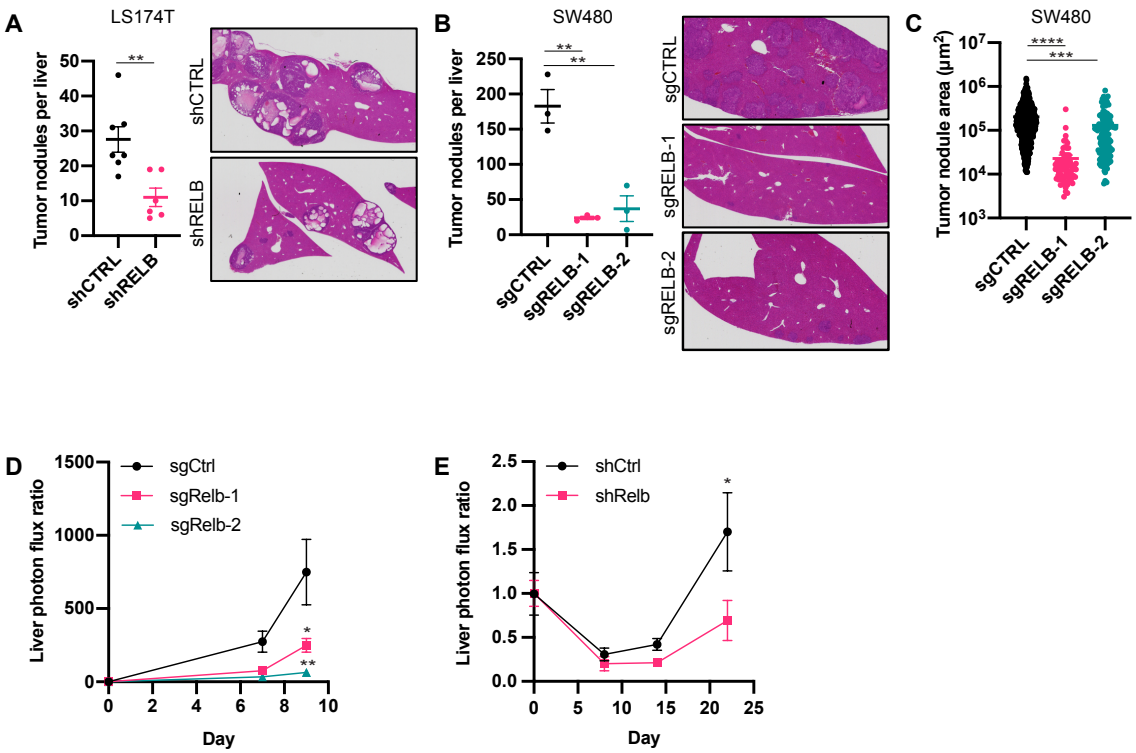


**Figure S4. RELB facilitates metastatic liver colonization, related to Figure 5. (A)** $5x10^5$ LS174T cells expressing control or RELB shRNA were inoculated by portal circulation injection, and metastatic tumor nodules were quantified by H&E staining. **(B)** $5x10^5$ SW480 sgCTRL or sgRELB cells were inoculated by portal circulation injection, and metastatic tumor nodules were quantified by H&E staining. **(C)** Tumor nodule area of SW480 sgCTRL or sgRELB liver metastases. **(D)** $5x10^5$ CT26 cells were inoculated by portal circulation injection, and metastatic liver burden was monitored by bioluminescence. **(E)** $5x10^5$ MC38 cells were inoculated by portal circulation injection, and metastatic liver burden was monitored by bioluminescence. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001, Student's t-test.

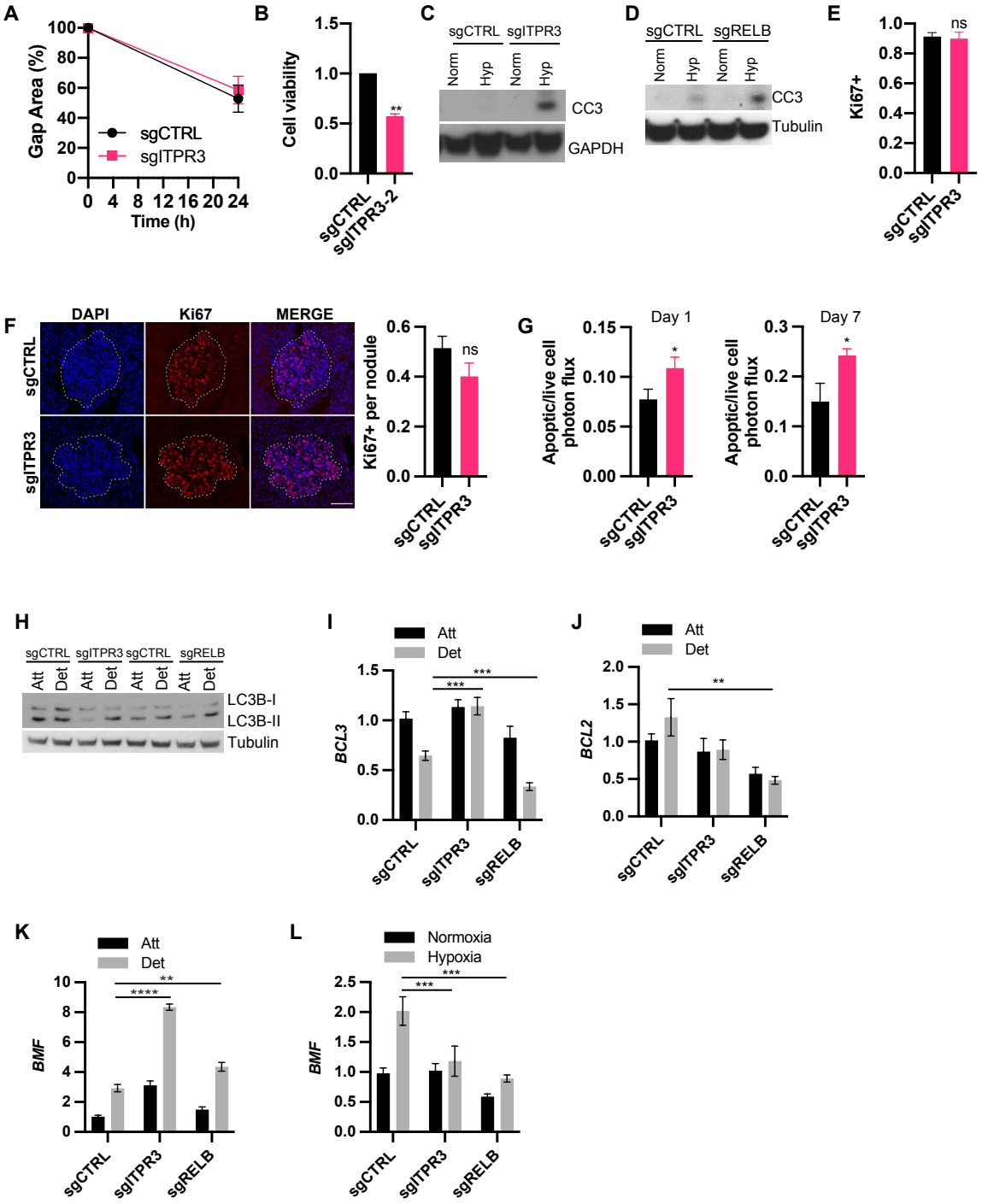


**Figure S5. Effect of ITPR3 deficiency on migration and CRC growth, related to Figure 6. (A)**

A scratch was made through a confluent monolayer of SW480 cells, and migration as measured

by gap area closure was monitored. **(B)** Viability of SW480 cells cultured in ULA plates for 5 days as assessed by ATP-based luminescent cell viability assays (n=3 independent experiments). **(C)** Immunoblot for cleaved caspase-3 levels in sgCTRL or sgITPR3 cells cultured under normoxia or hypoxia (0.5% $O_2$) for 48H. **(D)** Immunoblot for cleaved caspase-3 levels in sgCTRL or sgRELB cells cultured under normoxia or hypoxia (0.5% $O_2$) for 48H. **(E)** SW480 cells labeled with CellTracker Red (sgCTRL) or Green (sgITPR3) were mixed 1:1, and 1x $10^6$ cells were introduced into the portal circulation by splenic injection (n = 5 mice). Livers were harvested at 2H post-inoculation and Ki67+ cells were monitored by immunofluorescence. Fraction of Ki67+ cells among sgCTRL and sgITPR3 cells is shown. **(F)** Representative immunofluorescence images and fraction of Ki67+ cells among sgCTRL and sgITPR3 cells in metastatic tumor nodules. **(G)** 5x$10^5$ SW480 sgCTRL or sgRELB cells were inoculated by portal circulation injection. Apoptotic cell burden was monitored using DEVD-luciferin bioluminescence relative to live cell bioluminescence over time. **(H)** Immunoblot for LC3 in sgCTRL, sgITPR3, or sgRELB cells cultured under attached or detached conditions for 48H. **(I)** *BCL3* RNA expression by qRT-PCR in cells cultured under attached or detached conditions for 24H. **(J)** *BCL2* RNA expression by qRT-PCR in cells cultured under attached or detached conditions for 24H. **(K)** *BMF* RNA expression by qRT-PCR in cells cultured under attached or detached conditions for 24H. **(L)** *BMF* RNA expression by qRT-PCR in cells cultured under normoxia or hypoxia for 72H. Mean ± SEM; *p < 0.05; **p < 0.01; ***p < 0.001, ****p < 0.0001, Student's t-test.

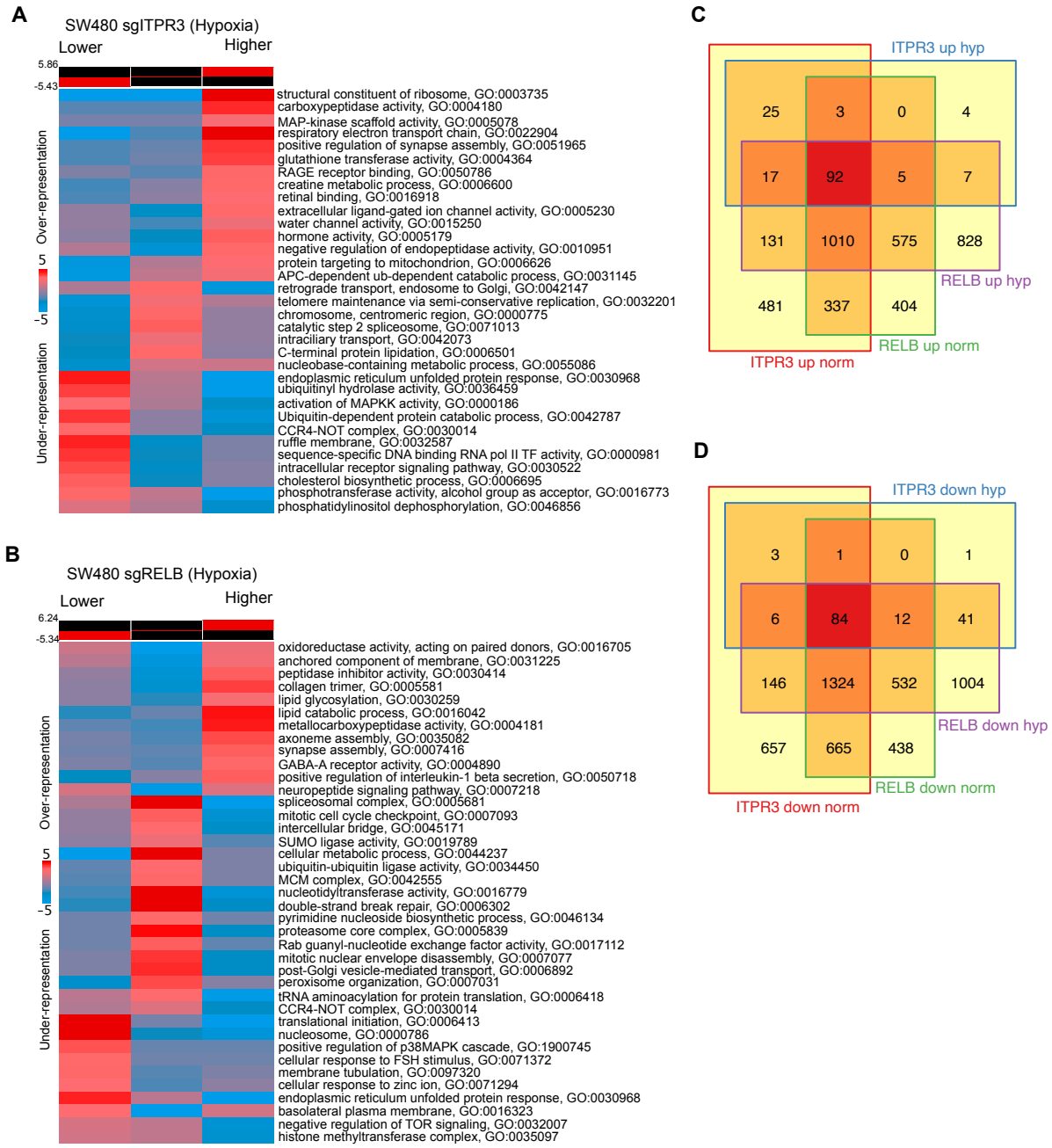


**Figure S6. iPAGE analysis of RNA-seq from ITPR3 and RELB KO cells exposed to hypoxia, related to Figure 6. (A)** iPAGE analysis of SW480 sgITPR3 cells cultured for 72 hours in hypoxia (0.5% $O_2$) compared to control cells. **(B)** iPAGE analysis of SW480 sgRELB cells cultured for 72 hours in hypoxia (0.5% $O_2$) relative to control cells. **(C-D)** Venn diagram showing number of

overlapping differentially expressed up-regulated (C) and down-regulated (D) genes under normoxia or hypoxia (0.5% $O_2$ for 72 hours) in ITPR3 and RELB KO SW480 cells relative to control cells.

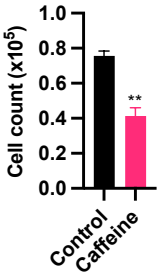


**Figure S7. Caffeine treatment of CRC cells inhibits cell growth under detached conditions, related to Figure 7.** SW480 cells were cultured on ULA plates for 4 days in the absence or presence of caffeine 5 mM. **p < 0.01, Student's t-test.

**Table S5. shRNA and sgRNA sequences, related to the STAR Methods.**

| Name | shRNA or sgRNA target sequence (5'-3') |
|---|---|
| shCTRL | CAACAAGATGAAGAGCACCAA |
| shITPR3-1 (Human) | CGTGAAGAACAAGACCGACTA |
| shITPR3-2 (Human) | GATGACAAGAAGAACAAGTTT |
| shRELB-1 (Human) | AGCCCGTCTATGACAAGAAAT |
| shRELB-2 (Human) | CATGCTTCTGAAGTGGACATA |
| shPLCG1 (Human) | AGAAGTTCCTTCAGTACAATC |
| shITPKA (Human) | CCTTGTGTGCTCGACTGCAAA |
| shINPP5A (Human) | GCGATTCGAGAAGGTTTCCTA |
| shRelb (Mouse) | CGGTTCTCTTTGAGCCCATTT |
| sgCTRL | GCGAGGTATTCGGCTCCGCG |
| sgITPR3-1 (Human) | GTCCAGCTTTCTTCACATCG |
| sgITPR3-2 (Human) | GCTGGTGGATGACCGCTGTG |
| sgRELB-1 (Human) | GGGGACACTAGTCGGCCCAG |
| sgRELB-2 (Human) | GTGGGGAAAGACTGCACCGA |
| sgItpr3 (Mouse) | CTGGAAGCTTCGGAGCAATG |
| sgRelb-1 (Mouse) | CCTACCAGAGGACATATCCG |
| sgRelb-2 (Mouse) | GTTCAAAACGCCACCCTACG |

**Table S6. siRNAs, related to the STAR Methods.**

| Gene Symbol | Dharmacon Catalog Number |
|---|---|
| PLK4 | L-005036-00 |
| DDR2 | L-003112-00 |
| ADGRG1 | L-004552-00 |
| RELB | L-004767-00 |
| MEF2D | L-009884-00 |
| CCL5 | L-007844-00 |
| CORO1B | L-010493-01 |
| ITPR3 | L-006209-00 |
| PLG | L-006001-00 |
| INSR | L-003014-00 |
| UBR4 | L-014021-01 |
| MST1R | L-003157-00 |
| COL6A1 | L-011620-00 |
| OR10A5 | L-008750-00 |
| TMEM164 | L-014805-02 |
| MYH7 | L-011086-00 |
| GPR173 | L-005727-00 |
| NOB1 | L-020594-01 |
| MRPL24 | L-017442-02 |
| ODAM | L-016965-02 |
| IGF1R | L-003012-00 |
| CMTM6 | L-010711-00 |
| FGF8 | L-013693-00 |
| SPACA1 | L-013464-02 |
| MTMR9 | L-019244-00 |
| PRSS37 | L-025360-00 |
| ON-TARGETplus Non-targeting Control | D-001810-10 |

**Table S7. Primers for qRT-PCR, related to the STAR Methods.**

| Name | Sequence (5'-3') |
|---|---|
| BCL2_F | GGAGGATTGTGGCCTTCTTT |
| BCL2_R | CATCCCAGCCTCCGTTATC |
| BCL3_F | GAACACCGAGTGCCAAGAAACC |
| BCL3_R | GCTAAGGCTGTTGTTTTCCACGG |
| BMF_F | GAGGTACAGATTGCCCGAAA |
| BMF_R | CCCCGTTCCTGTTCTCTTCT |
| GAPDH_F | AGCCACATCGCTCAGACAC |
| GAPDH_R | GCCCAATACGACCAAATCC |
| HPRT_F | GACCAGTCAACAGGGGACAT |
| HPRT_R | CCTGACCAAGGAAAGCAAAG |
| ITPR3_F | TATGCAGTTTCGGGACCACC |
| ITPR3_R | TGCCCTTGTACTCGTCACAC |
| PLCG1_F | CATCTGCCAAAGAATGGCCG |
| PLCG1_R | AGTCCATTGTCCACCACAAACT |
| ITPKA_F | CTTCGACGGACCTTGTGTG |
| ITPKA_R | TACATGTCCTTCCGCAGCTT |
| INPP5A_F | TTCGACGACCCAGAAAACCT |
| INPP5A_R | GCCTCGTAGTTCTTCCCTCC |
| TNF_F | CTCTTCTGCCTGCTGCACTTTG |
| TNF_R | ATGGGCTACAGGCTTGTCACTC |
| RELB_F | AAGAAAAAGCCGGCCATC |
| RELB_R | CACGGTGCCAGAGAAGAAGT |