

Supplementary Figure Legends

Supplementary Figure S1. A-B, mRNA expression of *AADC* and *MAOA* was detected by real-time PCR in human sourced normal colorectal epithelial cell line (NCM460) and four CRC cell lines (SW480, SW620, HCT116 and HT29). **C-D**, quantitative analysis of Western blotting for TPH1 expression in NCM460, SW480, SW620, HCT116, HT29 and THP-1 cells, as well as CEC, CT26 and iBMDM. **E, F**, quantitative analysis of Western blotting for cleaved-caspase-1 (C-casp1) and cleaved-IL1 β (C-IL1 β) expression in cell lysates from THP-1 cells after activated by LPS (100 ng/mL, 4 hours) and ATP (5 mM, 1 hour) with/without SW620 or HT29 conditional medium (CM) incubation for 24 hours. **G-H**, quantitative analysis of Western blotting for C-casp1 and C-IL1 β expression in cell lysates from iBMDM cells after activated by LPS (100 ng/mL, 4 hours) and ATP (5 mM, 1 hour) with/without CT26 CM incubation for 24 hours. **I-J**, quantitative analysis of Western blotting for C-casp1 and C-IL1 β expression in cell lysates from THP-1 cells after activated by LPS (100 ng/mL, 4 hours) and ATP (5 mM, 1 hour) with incubation of the CM from SW620/HT29 cells or LP-533401 (0.5 μ M) treated SW620/HT29 cells for 24 hours. **K**, verification for TPH1 silence in SW620, HT29 cells after shRNA lentivirus transfection by Western blotting. **L**, quantitative analysis of Western blotting for C-casp1 expression in cell lysates from THP-1 cells after activated by LPS (100 ng/mL, 4 hours) and ATP (5 mM, 1 hour) with/without incubation of CM from TPH1 silenced (by anti-TPH1 shRNA) SW620 cells for 24 hours. Data represent mean \pm SD for at least three independent experiments; one-way ANOVA; N.D.: not detected. * p <0.05, ** p <0.01.

Supplementary Figure S2. A-C, quantitative analysis of Western blotting for C-casp1 and C-IL1 β expression in cell lysates from THP-1 cells after activated by LPS (100 ng/mL, 4 hours) and ATP (5 mM, 1 hour) with/without incubation of CM from TPH1 silenced (by anti-TPH1 shRNA) SW620 or HT29 cells for 24 hours. **D-E**, quantitative analysis of Western blotting for C-casp1 and C-IL1 β expression in cell lysates from iBMDM cells after activated by LPS (100 ng/mL, 4 hours) and ATP (5 mM, 1 hour) with incubation of the CM from CT26 cells or LP-533401 (0.5 μ M) treated CT26 cells for 24 hours. **F**, protein expression bands and **G-J**, quantitative analysis of C-casp1 and C-IL1 β in cell lysates detected by Western blotting, as well as **K-L**, IL1 β concentration in supernatants from THP-1 and iBMDM cells detected by ELISA after activated by LPS (100 ng/mL, 4 hours) and ATP (5 mM, 1 hour) with/without 5-HT treatment (10 μ M, 5 hours). **M**, protein expression bands of TPH1, NLRP3, phospho-NLRP3 (Ser194), C-casp1 and C-IL1 β in tumor tissues from each group of the CT26 and iBMDM co-implanted subcutaneous allograft CRC mouse model. For **A-E** and **G-L**: data represent mean \pm SD for three independent experiments; one-way ANOVA; Ctrl, control; WT: wild-type; sh3A: shHTR3A; shN3: shNLRP3; N.D.: not detected. * p <0.05, ** p <0.01.

Supplementary Figure S3. A-B, quantitative analysis of Western blotting for C-casp1 and C-IL1 β expression in tumor tissues from each group of the CT26 and iBMDM co-implanted subcutaneous allograft CRC mouse model. **C-F**, quantitative analysis of Western blotting for C-casp1 and C-IL1 β in cell lysates from THP-1 and iBMDM cells after activated by LPS (100 ng/mL, 4 hours) and ATP (5 mM, 1 hour) with 5-HT (10 μ M, 5 hours) and SGR (10 μ M), or TPS (10 μ M), or SB-269970 (10 μ M) treatments for 5 hours. **G**, verification for HTR3A silence in THP-1 cells by Western blotting after shRNA lentivirus stable transfection. **H-K**, quantitative analysis of Western blotting for C-casp1 and C-IL1 β in cell lysates from HTR3A silenced (by anti-HTR3A shRNA) THP-1 and iBMDM cells after activated by LPS (100 ng/mL, 4 hours) and ATP (5 mM, 1 hour) with 5-HT (10 μ M, 5 hours)

treatment. **L**, quantitative analysis of Western blotting for C-casp1 in cell lysates from THP-1 cells after activated by LPS (100 ng/mL, 4 hours) and ATP (5 mM, 1 hour) with/without 10, 50, 100 nM 2Met5HT treatments for 5 hours. For **A-B**: data represent mean±SD for each group; for **C-F** and **H-L**: data represent mean±SD for three independent experiments; one-way ANOVA. WT: wild-type; sh3A: shHTR3A; shN3: shNLRP3; Ctrl, control; n.s.: no significant differences; N.D.: not detected. * $p < 0.05$, ** $p < 0.01$.

Supplementary Figure S4. A, quantitative analysis of Western blotting for C-IL1 β in cell lysates from THP-1 cells after activated by LPS (100 ng/mL, 4 hours) and ATP (5 mM, 1 hour) with/without 10, 50, 100 nM 2Met5HT treatments for 5 hours. **B-C**, quantitative analysis of Western blotting for C-casp1 and C-IL1 β in cell lysates from THP-1 cells after activated by LPS (100 ng/mL, 4 hours) and ATP (5 mM, 1 hour) with/without 5-HT (10 μ M) and 10, 50, 100 μ M TPS treatments for 5 hours. **D**, protein expression bands and **E-H**, quantitative analysis of C-casp1 and C-IL1 β in cell lysates detected by Western blotting, and **I-J**, IL1 β concentration in supernatants from THP-1 cells detected by ELISA after activated by LPS (100 ng/mL, 4 hours) and MSU (500 μ g/mL, 1 hour) or Nig (10 μ M, 1 hour) and 5-HT (10 μ M, 5 hours) with 10, 50, 100 μ M TPS treatments for 5 hours. **K**, protein expression bands and **L-O**, quantitative analysis of C-casp1 and C-IL1 β in cell lysates from THP-1 cells detected by Western blotting after activated by LPS (100 ng/mL, 4 hours) and MSU (500 μ g/mL, 1 hour) or Nig (10 μ M, 1 hour) with/without 10, 50, 100 nM 2Met5HT treatments for 5 hours. For **A-C**, **E-J** and **L-O**: data represent mean±SD for three independent experiments; one-way ANOVA; N.D.: not detected. * $p < 0.05$, ** $p < 0.01$.

Supplementary Figure S5. A-B, IL1 β concentration of supernatants was detected by ELISA from THP-1 cells after activated by LPS (100 ng/mL, 4 hours) and MSU (500 μ g/mL, 1 hour) or Nig (10 μ M, 1 hour) with/without 10, 50, 100 nM 2Met5HT treatments for 5 hours. **C**, protein expression bands and **E-F**, quantitative analysis of C-casp1 and C-IL1 β in cell lysates detected by Western blotting, and **I**, IL1 β concentration of supernatants from iBMDM cells detected by ELISA after activated by LPS (100 ng/mL, 4 hours) and ATP (5 mM, 1 hour) with/without 5-HT (10 μ M) and TPS (100 μ M) treatment for 5 hours. **D**, protein expression bands and **G-H**, quantitative analysis of C-casp1 and C-IL1 β in cell lysates detected by Western blotting, and **J**, IL1 β concentration of supernatants from iBMDM cells detected by ELISA after activated by LPS (100 ng/mL, 4 hours) and ATP (5 mM, 1 hour) with/without 2Met5HT (100 nM) treatment for 5 hours. **K-M**, concentrations of IL18, IL33 and IL1 α in supernatants from THP-1 cells detected by ELISA after activated by LPS (100 ng/mL, 4 hours) and ATP (5 mM, 1 hour) with/without 5-HT (10 μ M, 5 hours) and TPS (100 μ M) treatment for 5 hours. **N**, expression bands and **O-R**, quantitative analysis of NLRP3, ASC, pro-caspase-1 (P-casp1), and pro-IL1 β in cell lysates of THP-1 cells detected by Western blotting after activated by LPS (100 ng/mL, 4 hours) and ATP (5 mM, 1 hour) with/without TPS (100 μ M) or 2Met5HT (100 nM) treatment for 5 hours. For **A-B**, **E-M** and **O-R**: data represent mean±SD for three independent experiments; one-way ANOVA; N.D.: not detected; n.s.: no significant differences. * $p < 0.05$, ** $p < 0.01$.

Supplementary Figure S6. A, mRNA expression of *NLRP3*, *IL1B*, and *IL18* in THP-1 cells after incubation with CM from SW620 or HT29 cells; **B**, mRNA expression of *Nlrp3*, *Il1b*, and *Il18* in iBMDM cells after incubation with CM from CT26 cells detected by real-time PCR analysis. **C**,

quantitative analysis for ASC oligomerization assay in THP-1 cells (with/without anti-HTR3A shRNA transfected) after activated by LPS (100 ng/mL, 4 hours) and ATP (5 mM, 1 hour) with 5-HT (10 μ M, 5 hours) treatment. **D**, quantitative analysis for ASC oligomerization assay in THP-1 cells after activated by LPS (100 ng/mL, 4 hours) and ATP (5 mM, 1 hour) with/without 5-HT (10 μ M) and TPS (100 μ M) treatments for 5 hours. **E**, quantitative analysis for ASC oligomerization assay in THP-1 cells after activated by LPS (100 ng/mL, 4 hours) and ATP (5 mM, 1 hour) with/without 2Met5HT (100 nM) treatment for 5 hours. **F-I**, ASC oligomerization analysis for iBMDM cells after activated by LPS (100 ng/mL, 4 hours) and ATP (5 mM, 1 hour) and 5-HT (10 μ M, 5 hours) with/without TPS (100 μ M, 5 hours) treatment; and iBMDM cells after activated by LPS (100 ng/mL, 4 hours) and ATP (5 mM, 1 hour) with/without 2Met5HT (100 nM, 5 hours) treatment. **J-K**, CaMKII α phosphorylation quantitative analysis of Western blotting in THP-1 and iBMDM cells after primed by LPS (100 ng/mL, 4 hours), and then treated with ATP (5 mM), 2Met5HT (100 nM) and TPS (100 μ M) for 0, 30, 60 minutes. **L**, mRNA expression of *CAMK2A* in THP-1 cells was detected by real-time PCR after activated by LPS (100 ng/mL, 4 hours) and ATP (5 mM, 1 hour) with/without TPS (100 μ M) or 2Met5HT (100 nM) treatment for 5 hours. **M**, protein expression bands and **N-Q**, quantitative analysis of C-casp1 and C-IL1 β in cell lysates of THP-1 and iBMDM cells detected by Western blotting after activated by LPS (100 ng/mL, 4 hours) and ATP (5 mM, 1 hour) with/without 2Met5HT (100 nM) or KN-62 (1 μ M) treatments for 5 hours. **R-S**, quantitative analysis for ASC oligomerization assay in THP-1 and iBMDM cells after activated by LPS (100 ng/mL, 4 hours) and ATP (5 mM, 1 hour) with/without 2Met5HT (100 nM) or KN-62 (1 μ M) treatments for 5 hours. **T**, protein expression bands of NLRP3, ASC, P-casp1 and pro-IL1 β , and **U**, quantitative analysis of NLRP3 and ASC in cell lysates of THP-1 cells detected by Western blotting after activated by LPS (100 ng/mL, 4 hours) and ATP (5 mM, 1 hour) with/without 2Met5HT (100 nM) or KN-62 (1 μ M) treatments for 5 hours. For **A-E**, **H-L**, **N-S** and **U**: data represent mean \pm SD for at least three independent experiments; one-way ANOVA. Ctrl, control; n.s.: no significant differences; N.D.: not detected. * p <0.05, ** p <0.01.

Supplementary Figure S7. A, quantitative analysis of Western blotting for P-casp1 and pro-IL1 β in cell lysates from THP-1 cells after activated by LPS (100 ng/mL, 4 hours) and ATP (5 mM, 1 hour) with/without 2Met5HT (100 nM) or KN-62 (1 μ M) treatments for 5 hours. **B**, protein expression bands and **C-F**, quantitative analysis of NLRP3, ASC, P-casp1, and pro-IL1 β in cell lysates from iBMDM cells detected by Western blotting after activated by LPS (100 ng/mL, 4 hours) and ATP (5 mM, 1 hour) with/without 2Met5HT (100 nM) or KN-62 (1 μ M) treatments for 5 hours. **G**, quantitative analysis of Western blotting for NLRP3 phosphorylation levels in tumor tissues from each group of the CT26 and iBMDM co-implanted subcutaneous allograft CRC mouse model. **H**, representative immunohistochemical photos for NLRP3, phospho-NLRP3 (Ser194) in tumor tissues from the CT26 and iBMDM co-implanted subcutaneous allograft CRC mouse model. Scale bar: 50 μ m. **I**, quantitative analysis of Western blotting for TPH1 in cell lysates from SW620, HT29 and CT26 cells after incubation with CM from activated macrophages (A- Φ CM, THP-1 for SW620/HT29, and iBMDM for CT26) with/without Dia (10 μ M) treatment. **J**, protein expression bands and **K-L**, quantitative analysis of TPH1 in SW620, HT29, and CT26 cells detected by Western blotting after incubation with CM from activated (A-THP-1, A-iBMDM) or inactivated THP-1, iBMDM cells for 24 hours (THP-1 and iBMDM cells activation were induced by 100 ng/mL LPS for 4 hours and 5 mM ATP for 1 hour). **M**, protein expression bands and **N**, quantitative analysis of TPH1 in cell lysates (SW620) after SW620 and HT29 cells were treated with/without rhIL1 β (10 μ g/mL) and TLR1 (100

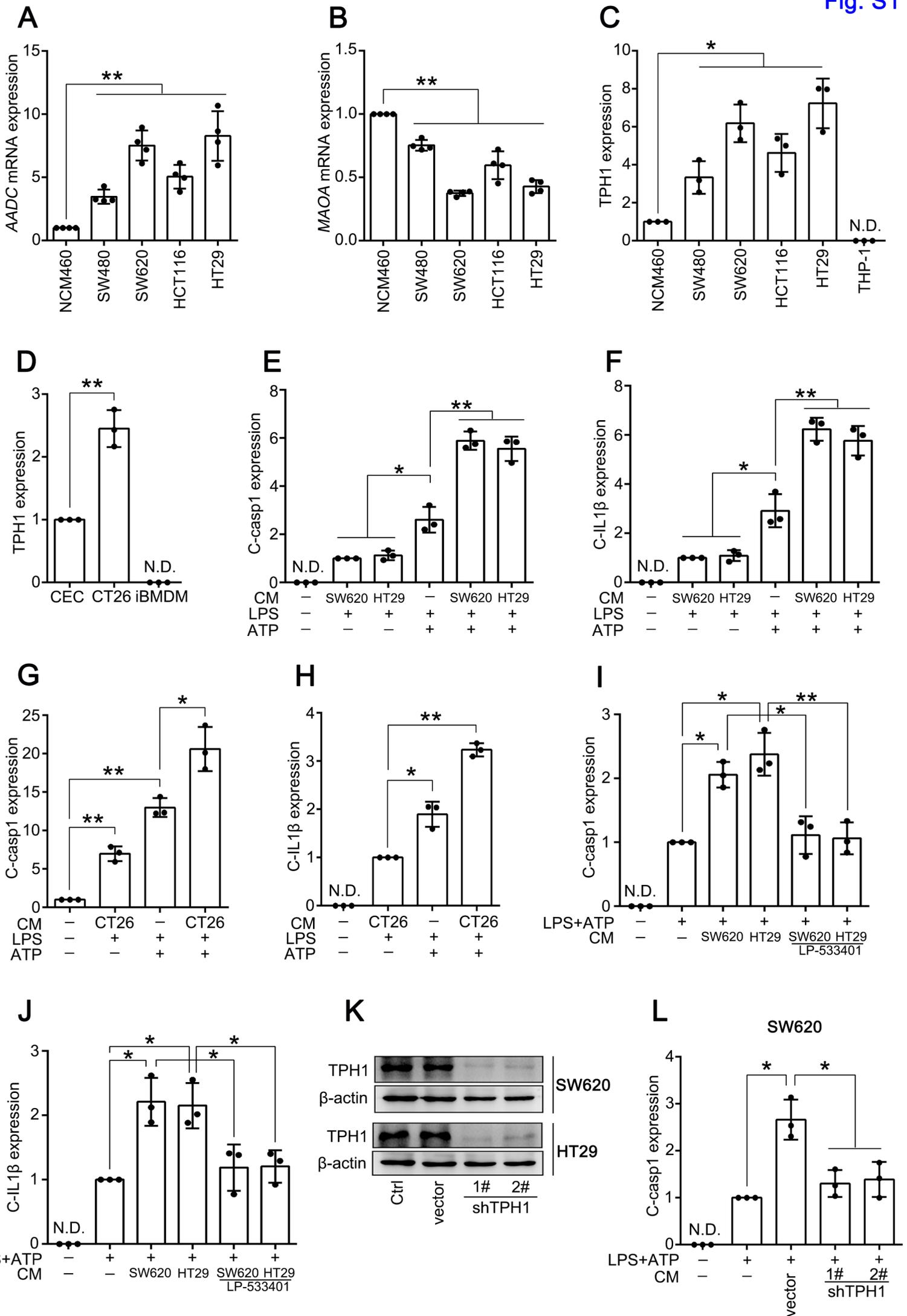
μM) for 24 hours. For **A**, **C-F**, **I**, **K-L** and **N**: data represent mean±SD for three independent experiments; for **G**: data represent mean±SD for each group; one-way ANOVA. WT, wild-type; sh3A, shHTR3A; shN3, shNLRP3; n.s.: no significant differences. * $p < 0.05$, ** $p < 0.01$.

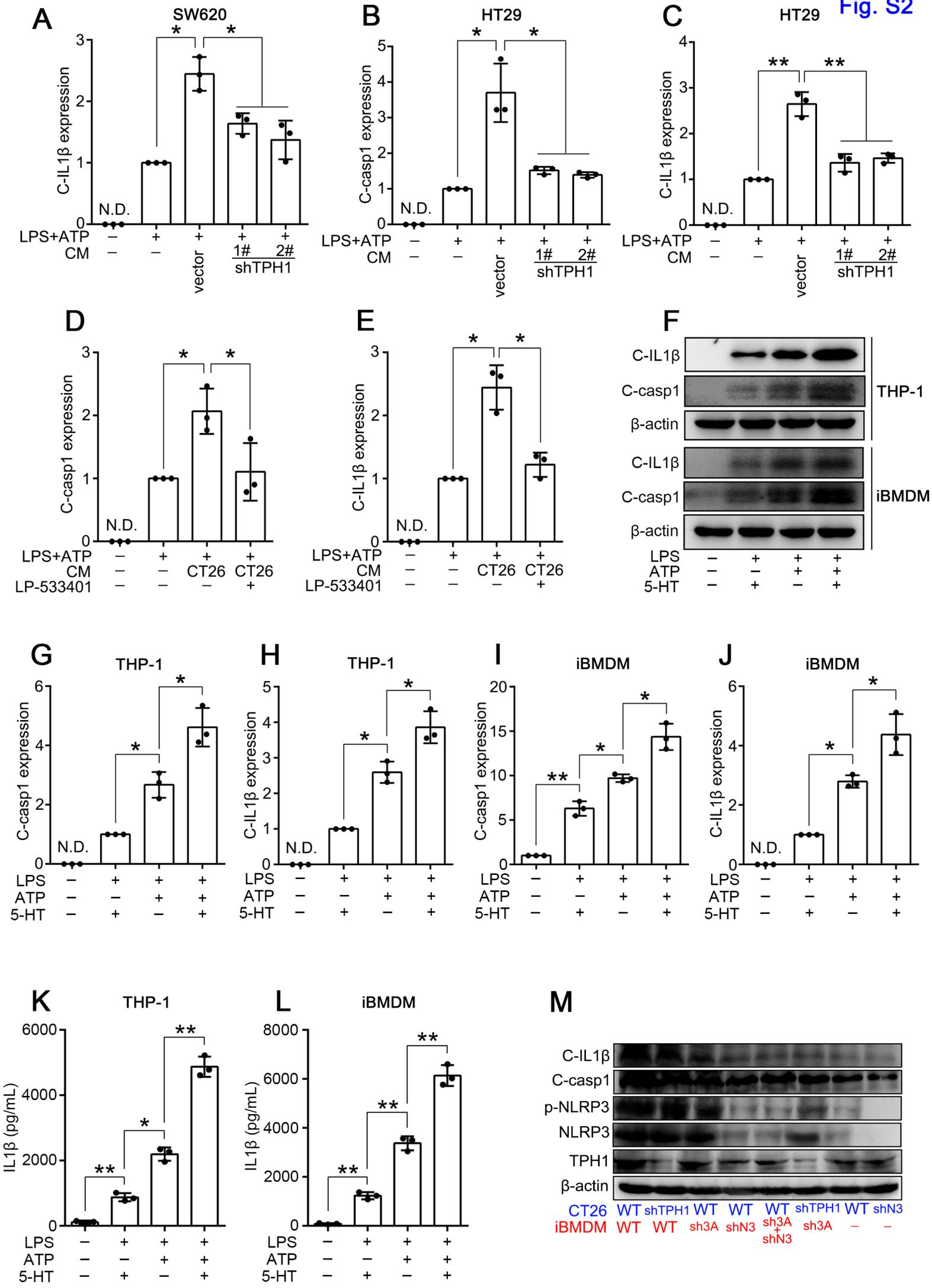
Supplementary Figure S8. A, quantitative analysis of TPH1 in cell lysates (HT29) detected by Western blotting, **B-C**, 5-HT concentration in supernatants detected by ELISA from SW620 and HT29 cells after treated with/without rhIL1β (10 μg/mL) and TLR1 (100 μM) for 24 hours. **D-F**, quantitative analysis of IKKβ (Tyr 188) and NF-κB p65 (Ser 536) phosphorylation levels in cell lysates from SW620, HT29 and CT26 cells after incubation with CM from activated macrophages (A-Φ CM: THP-1 for SW620/HT29, and iBMDM for CT26) with/without Dia (10 μM) treatment. **G**, protein expression bands and **H**, quantitative analysis of TPH1 in cell lysates of SW620, and HT29 cells detected by Western blotting after incubation with CM from activated THP-1 cells (A-THP-1), and treated with/without IL1RA (0.1 μg/mL) or TLR1 (100 μM) or BMS-345541 (4 μM) for 24 hours. **I**, protein expression bands and **J**, quantitative analysis of TPH1 expression in cell lysates from CT26 cells detected by Western blotting after incubation with CM from activated iBMDM cells (A-iBMDM), and treated with/without TLR1 (100 μM) or BMS-345541 (4 μM) for 24 hours. **K**, quantitative analysis of Western blotting for TPH1, **L**, mRNA expression of *Tph1* detected by real-time PCR, **M**, 5-HT concentrations detected by ELISA in tumor tissues from each group of the CT26 and iBMDM co-implanted subcutaneous allograft CRC mouse model. **N**, verification for TPH1 and NLRP3 silence in CT26 cells after shRNA lentivirus transfection by Western blotting. **O**, verification for HTR3A and NLRP3 silence in iBMDM cells by Western blotting after shRNA lentivirus stable transfection. Since both shRNA sequences 1# and 2# had similar silence rates on expression of TPH1, NLRP3 in CT26 cells, as well as expression of HTR3A, NLRP3 in iBMDM cells, shRNA 1# for each gene was selected as stable transfected CT26, iBMDM cells for further co-implanted subcutaneous allograft experiment. For **A-F**, **H**, and **J**: data represent mean±SD for three independent experiments, for **K-M**: data represent mean±SD for each group; one-way ANOVA. WT, wild-type; sh3A, shHTR3A; shN3, shNLRP3; n.s.: no significantly differences. * $p < 0.05$, ** $p < 0.01$.

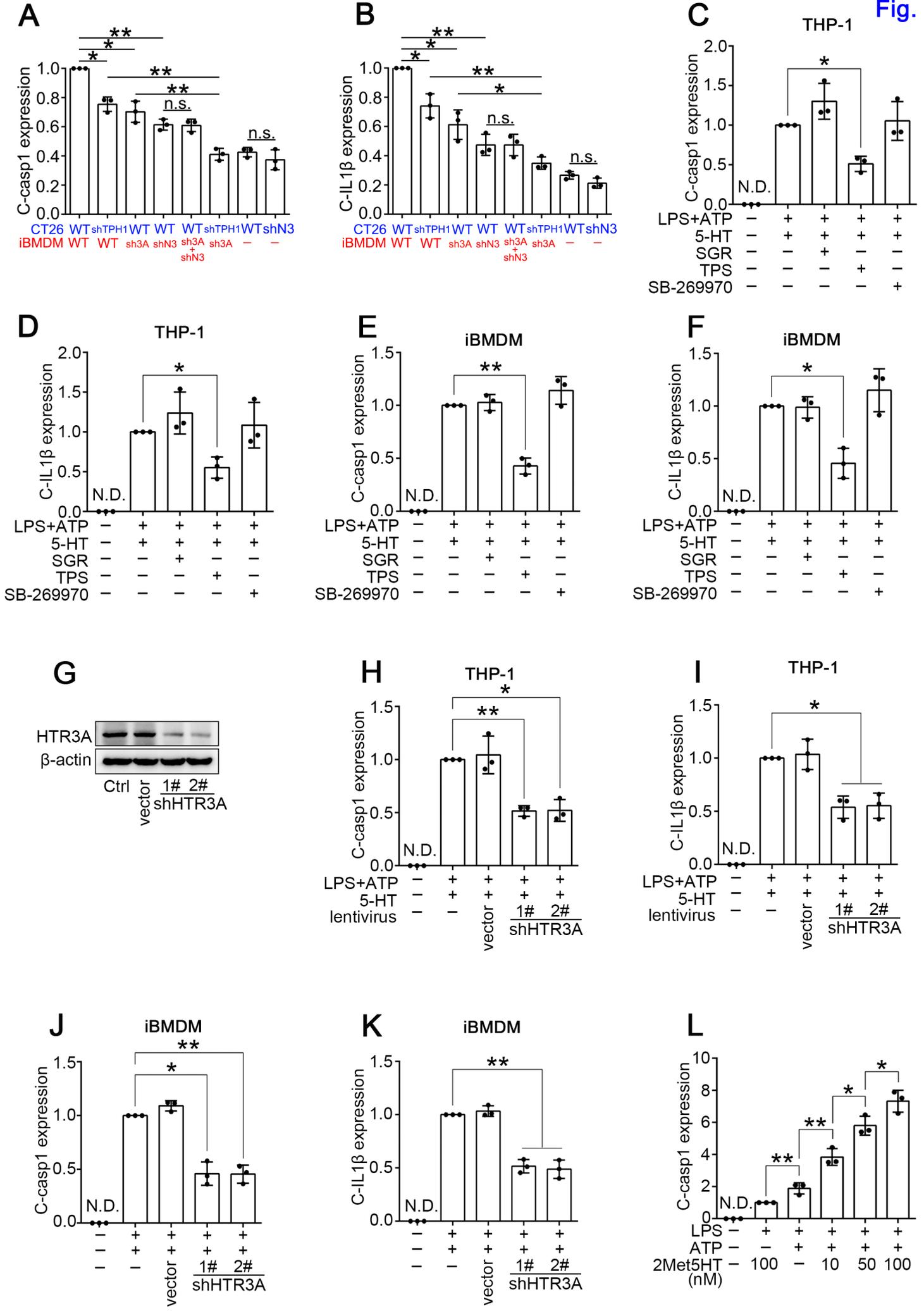
Supplementary Figure S9. A, representative immunohistochemical photos for Ki67, F4/80, and GP70 in tumor tissues from each group of the CT26 and iBMDM co-implanted subcutaneous allograft CRC mouse model. Scale bar: 50 μm. **B**, protein expression bands and **C-D**, quantitative analysis of NLRP3 detected by Western blotting in human sourced cell lines (NCM460, SW480, SW620, HCT116, HT29, and THP-1) and mouse sourced cell lines (CT26 and iBMDM). **E**, protein expression bands of TPH1, NLRP3, phospho-NLRP3 (Ser194), C-casp1 and C-IL1β and **F-I**, quantitative analysis of TPH1, C-casp1 and C-IL1β, as well as phosphorylation levels of NLRP3 in colorectal tumor or normal tissues (in Ctrl group) detected by Western blotting. **J**, 5-HT concentrations of colorectal tumor and normal tissues detected by ELISA from each group of AOM/DSS induced CRC mouse model. For **C-D**: data represent mean±SD for three independent experiments, for **F-J**: data represent mean±SD for each group; one-way ANOVA for **C**, **F-J**, and unpaired student's t test for **D**. WT, wild-type; sh3A, shHTR3A; shN3, shNLRP3; Ctrl, control; n.s.: no significantly differences. * $p < 0.05$, ** $p < 0.01$.

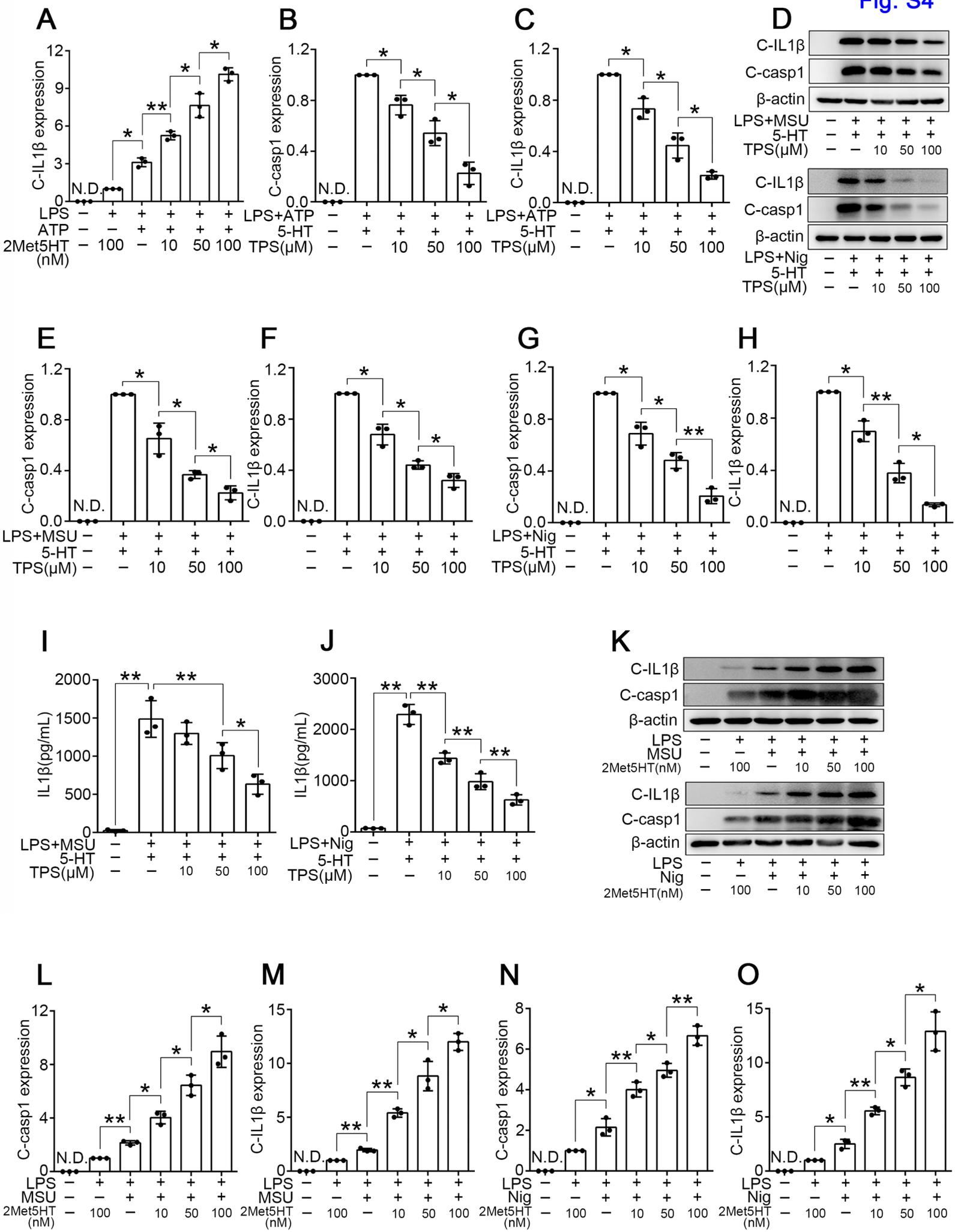
Supplementary Figure S10. 5-HT-NLRP3 positive feedback loop in CRC microenvironment. In CRC microenvironment, CRC cell-secreted 5-HT binds to HTR3A receptor on macrophages, which

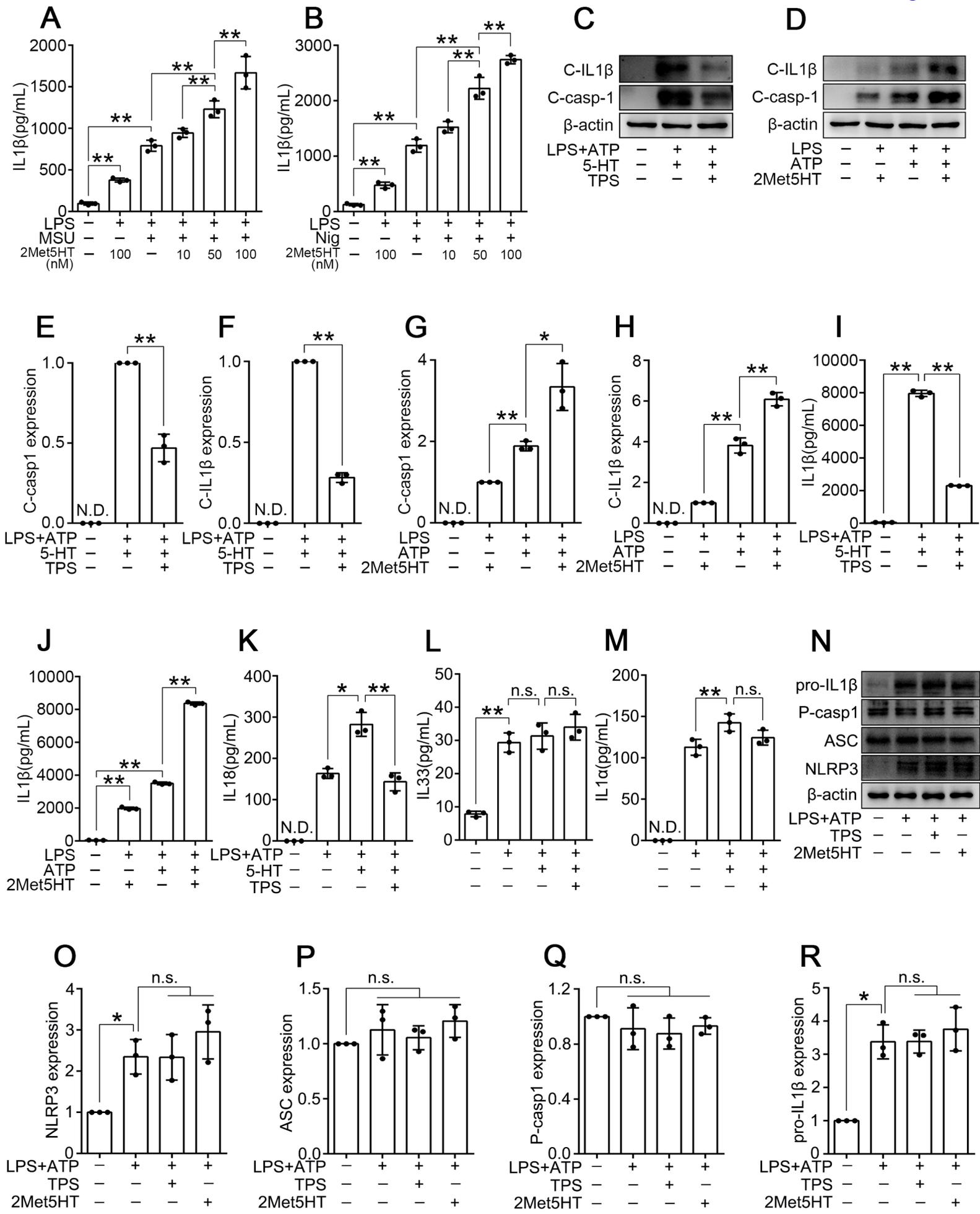
induces Ca^{2+} influx, and follows with phosphorylation and activation of CaMKII α . Activated CaMKII α induces NLRP3 phosphorylation (human: Ser198, and mouse: Ser194), an essential step for NLRP3 activation, which enhances NLRP3 inflammasome assembling and activation under DAMPs or PAMPs challenges, resulted in excessive IL1 β maturation and secretion into CRC microenvironment. On the other hand, IL1 β activates NF- κ B signaling via IL1R in CRC cells, which induces transcription of *TPH1*, resulted in excessive 5-HT biosynthesis and secretion from CRC cells.

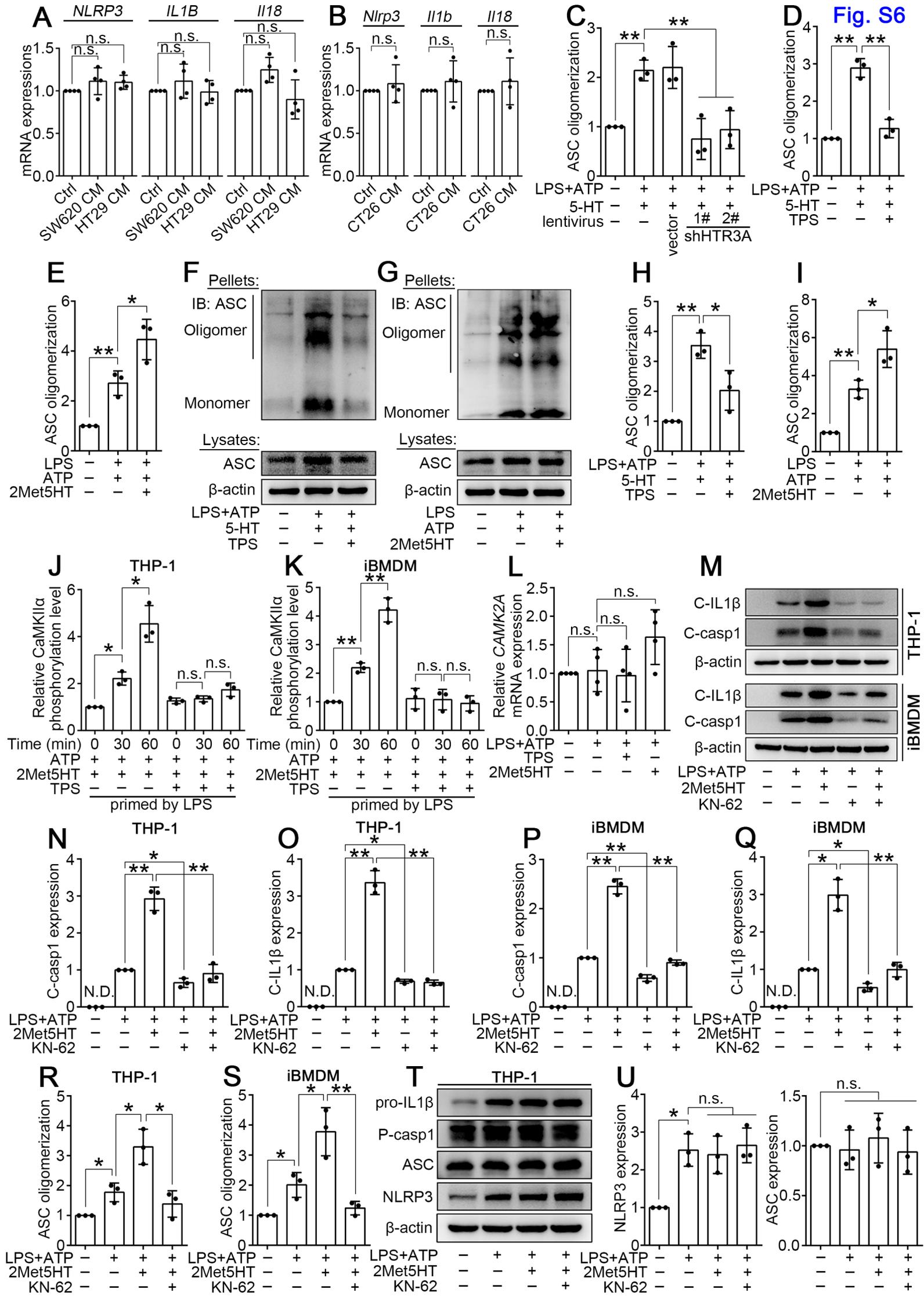


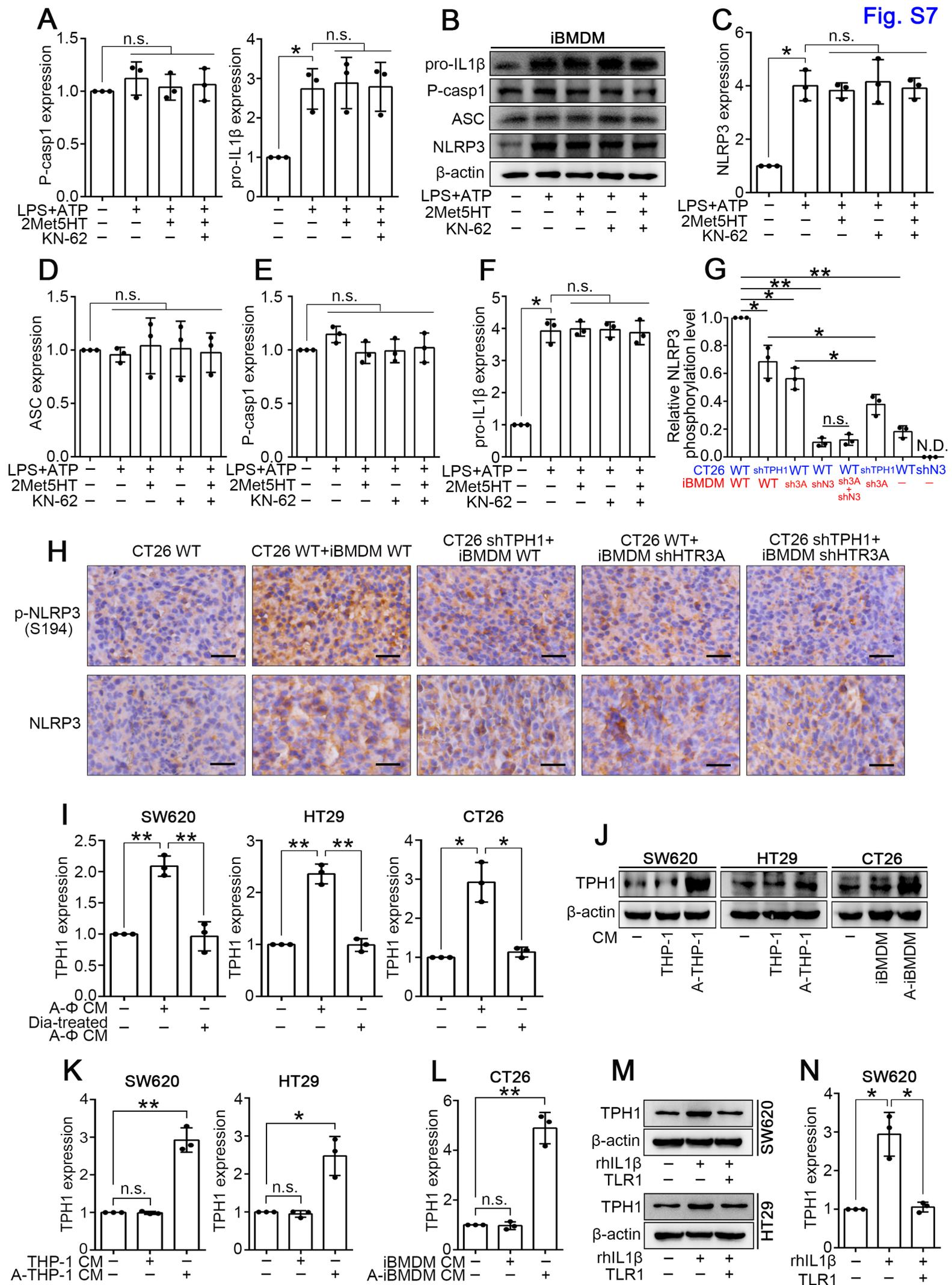


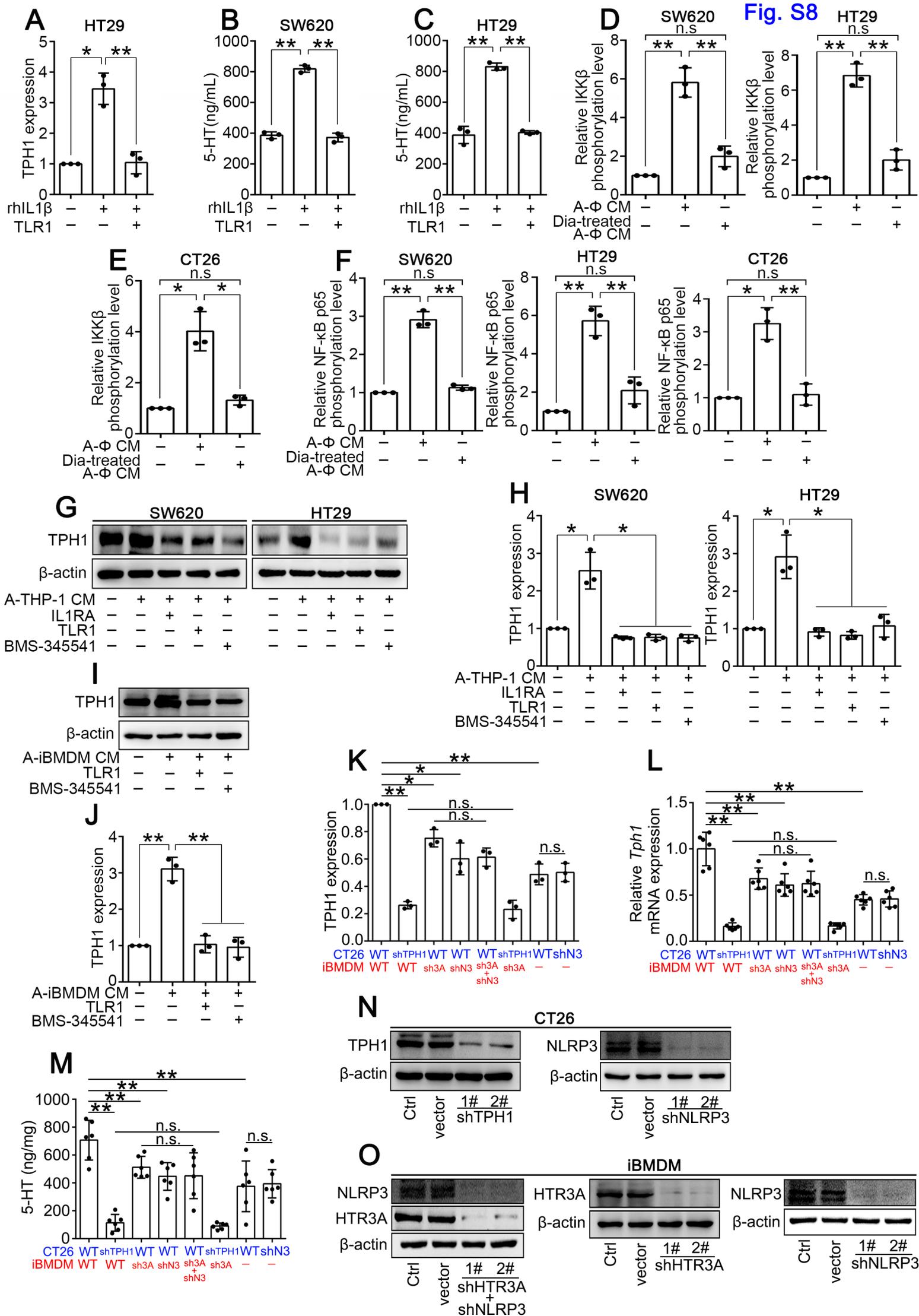












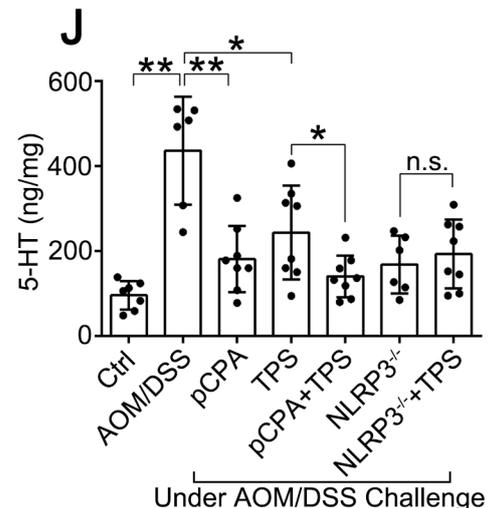
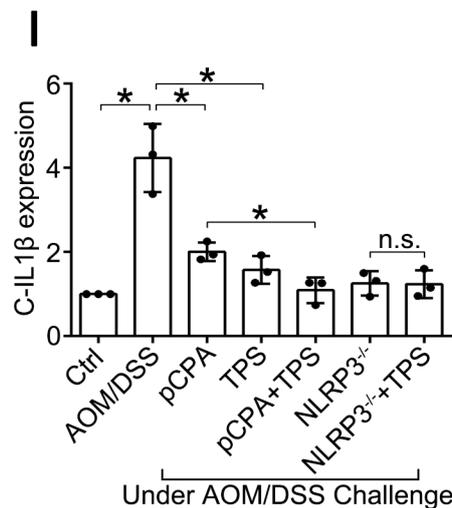
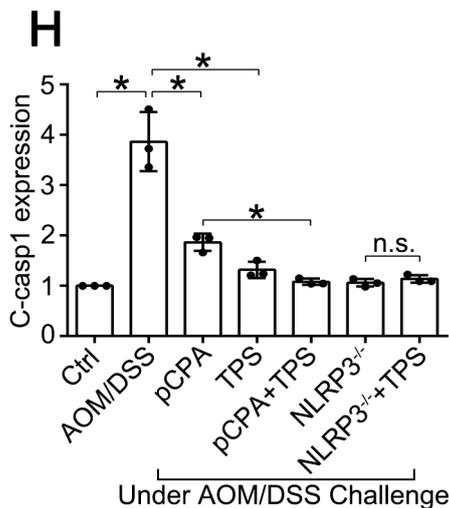
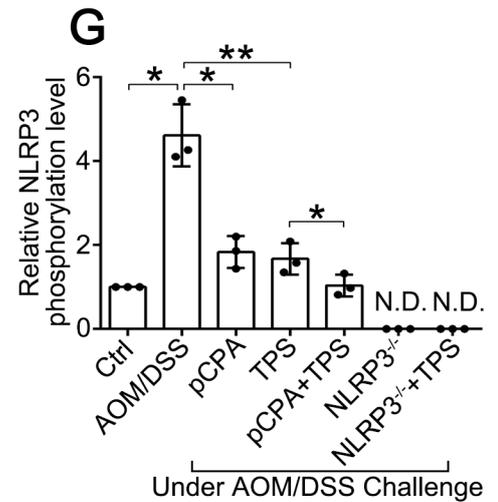
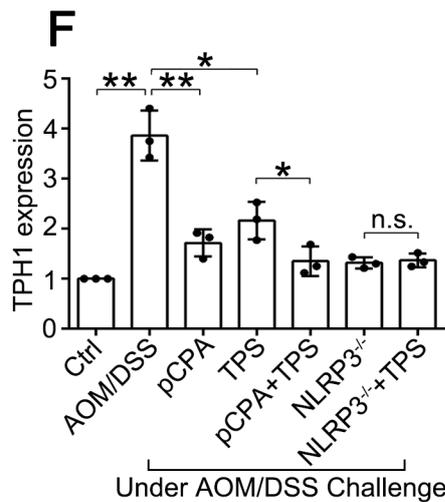
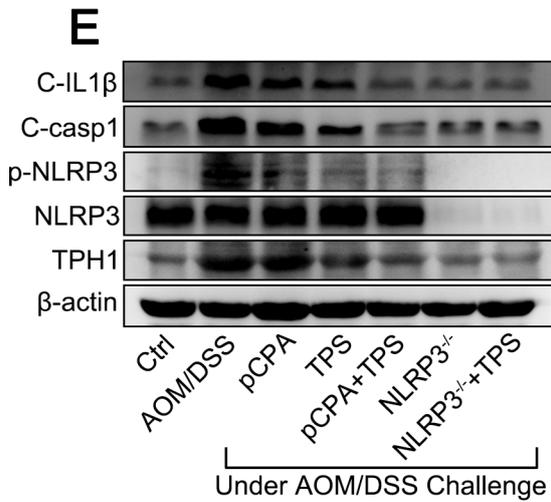
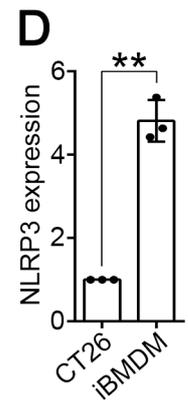
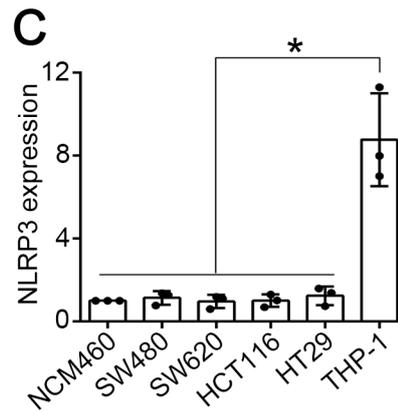
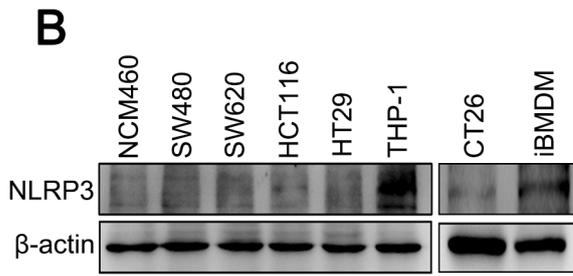
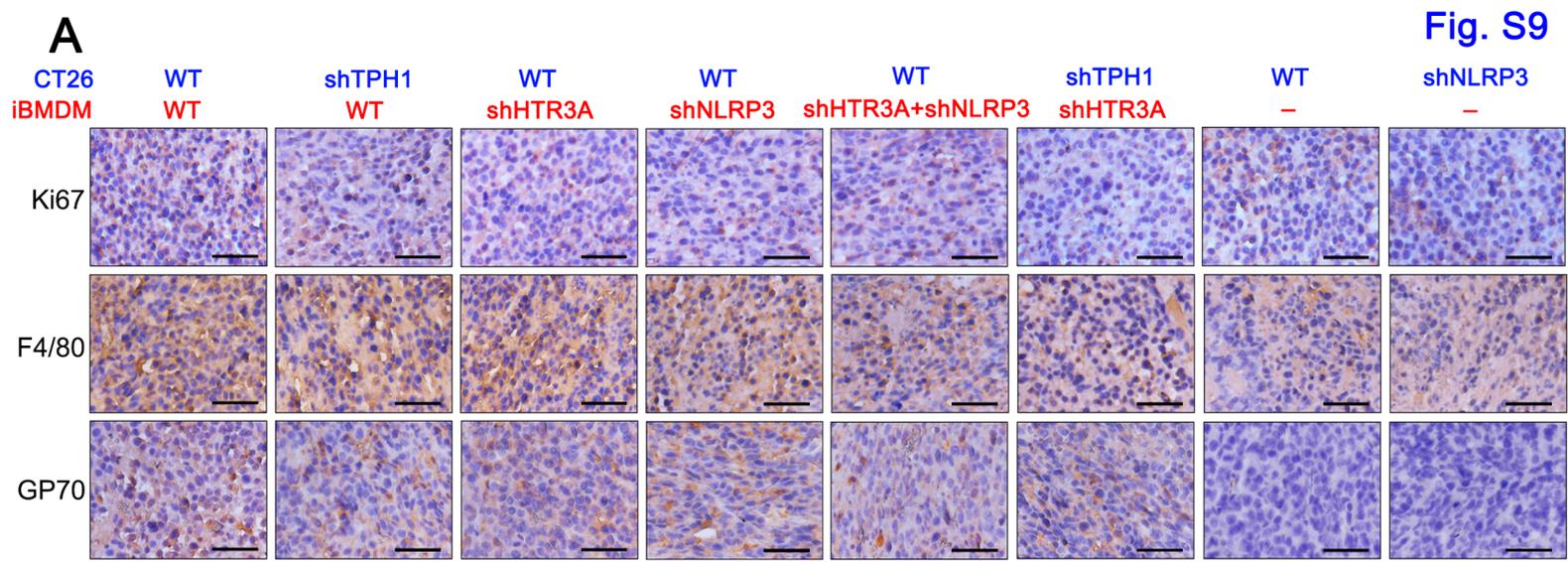


Fig. S10

IL1 β
5-HT
Ca²⁺

