Supplementary material

Immunofluorescence (IF) assay

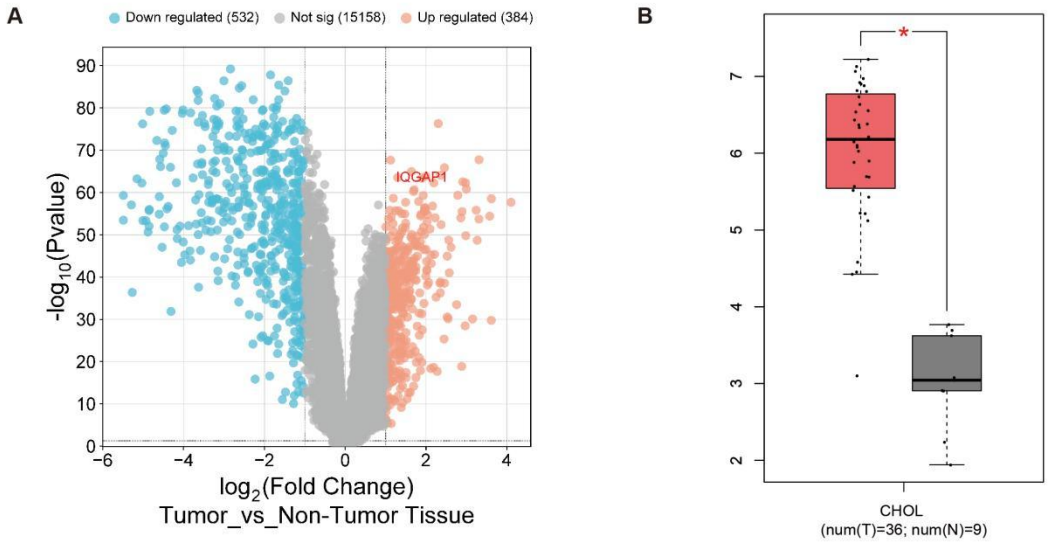
For immunofluorescence, cells attached to slides were fixed with 4% paraformaldehyde and permeabilized with Immunostaining Permeabilization Buffer with Saponin (Beyotime, China). After washing, the sliders were blocked with 5% BSA in PBS for 1 hour at room temperature and then incubated with appropriate primary antibody overnight at 4°C. The cells were washed three times and incubated with the corresponding secondary antibody for 1 hour at room temperature. Nuclei were stained with DAPI. The slides were photographed under a fluorescence microscope (Olympus, Japan).

Cell culture

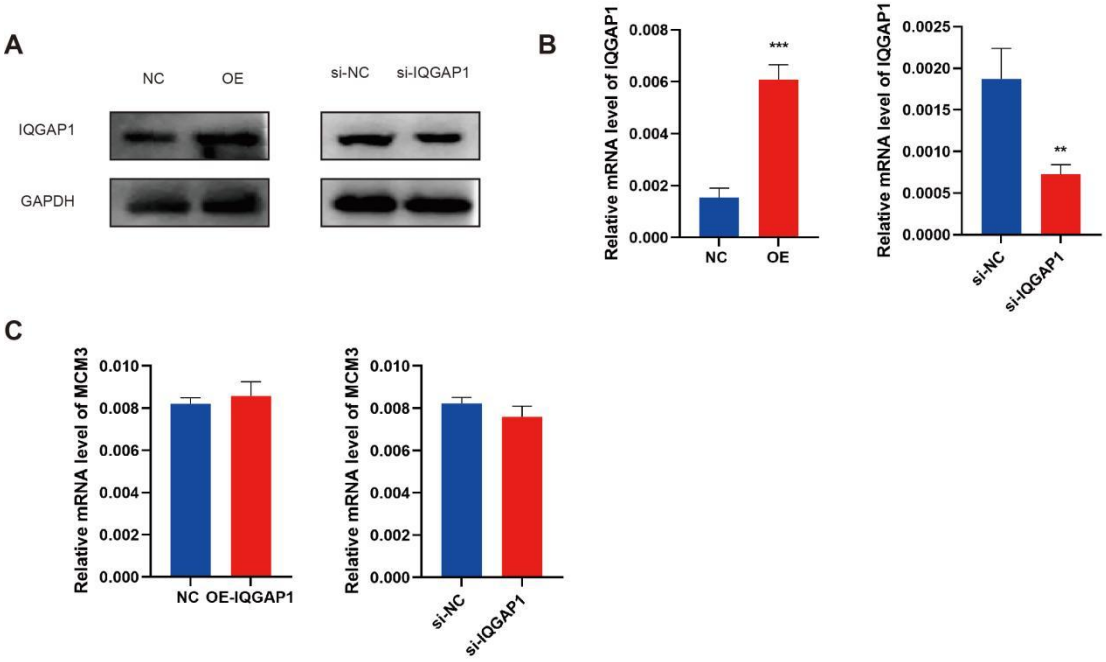
Human CCA cell lines HUCCT1 and QBC939 were purchased from the Chinese National Human Genome Center (Shanghai, China). The CCA cells were cultured in Dulbecco's modified Eagle's medium (Gibco, USA) supplemented with 10% fetal bovine serum (Gibco, USA) and 1% antibiotics (penicillin/streptomycin; Gibco, USA). Cell lines were maintained in a humidified atmosphere at 37 °C in 5% CO2 incubator.

Cell transfection

Lentiviral vectors of shMCM3 and IQGAP1 was designed and generated. si-IQGAP1/MCM3 and other overexpression plasmids were purchased from Gene Pharma (Shanghai, China). Transfection was performed by using Lipofectamine 3000 (Invitrogen, USA) under the instruction of the instruction manual.

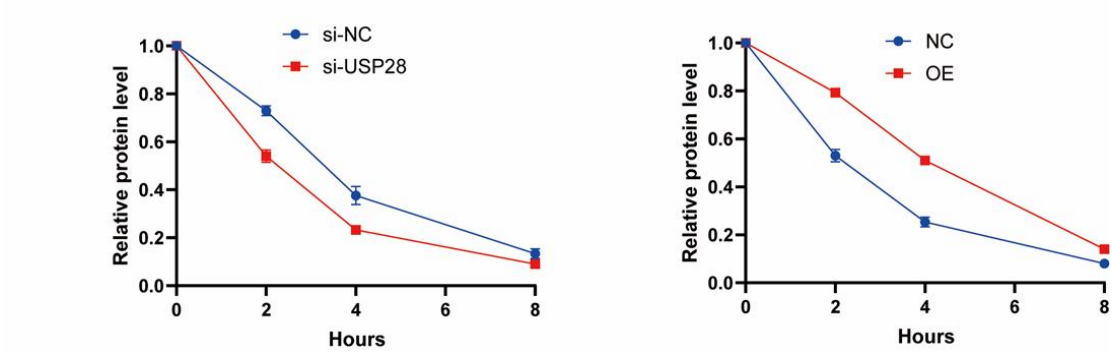


Supplementary Figure 1 Public database gene expression of IQGAP1. A: The expression of IQGAP1 in the volcano map shown in the GSE76297 database; B: IQGAP1 expression in TCGA database ($P < 0.05$).

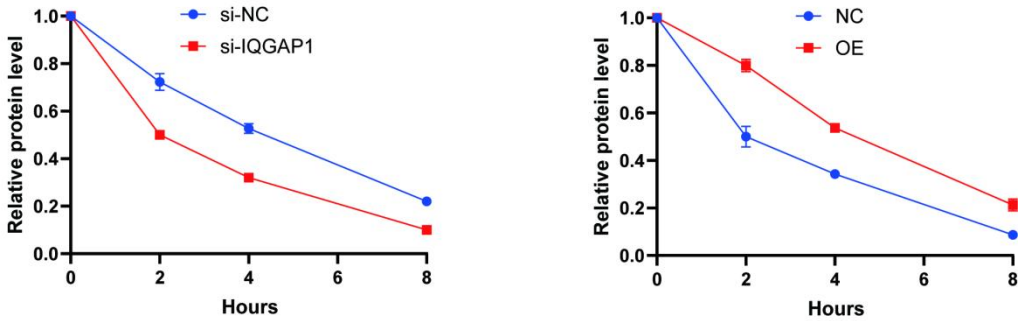


Supplementary Figure 2 Verification of transfection efficiency. A and B: Knockdown and overexpression efficiency of IQGAP1; C: The mRNA level of MCM3 was not affected by IQGAP1 knockdown and overexpression ($P < 0.01$,

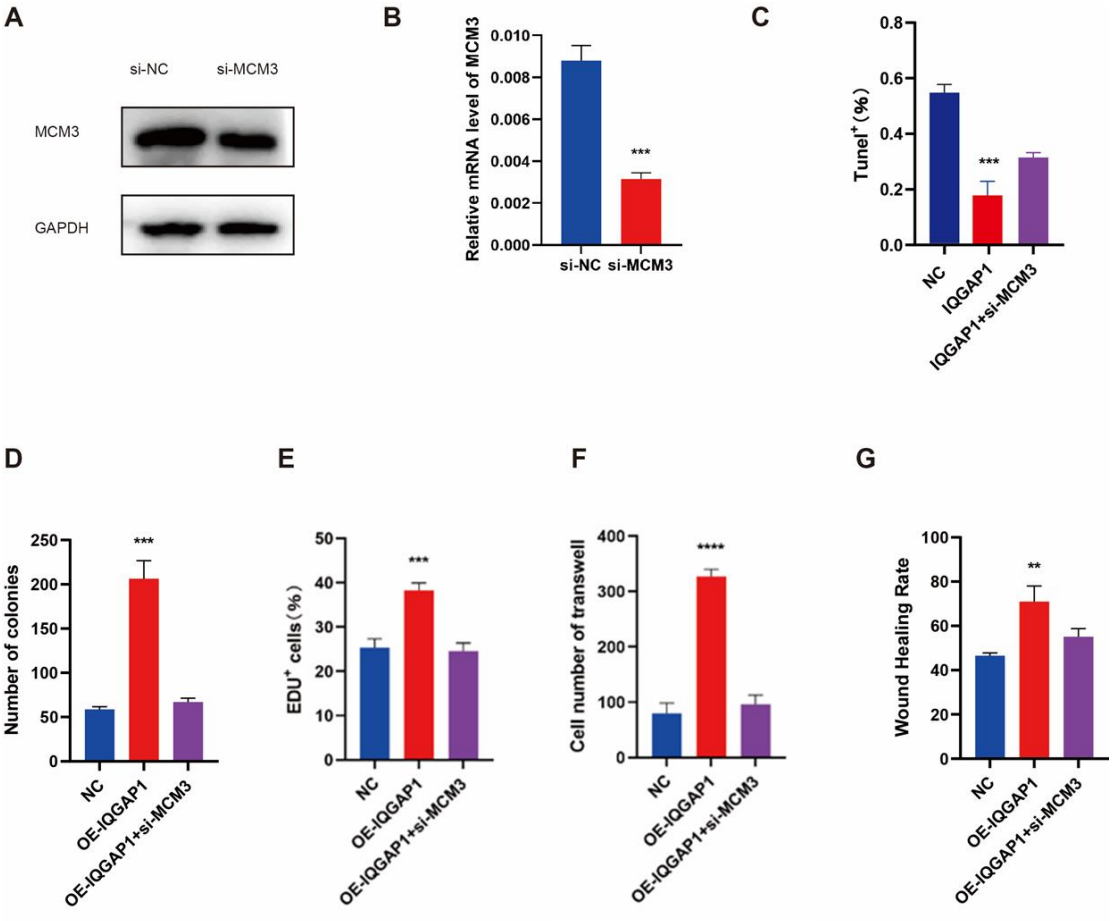
$P < 0.001$).



Supplementary Figure 3 Quantitative analysis of the degradation rate of the MCM3 protein.



Supplementary Figure 4 Quantitative analysis of the degradation rate of the MCM3 protein.



Supplementary Figure 5 Statistics of cell function results. A and B: Knockdown and overexpression efficiency of MCM3; C-F: Quantification of cell function experiments of EDU, Plate cloning, Transwell, Wound healing tests ($P < 0.01$, $P < 0.001$, $P < 0.0001$).