Figure S1

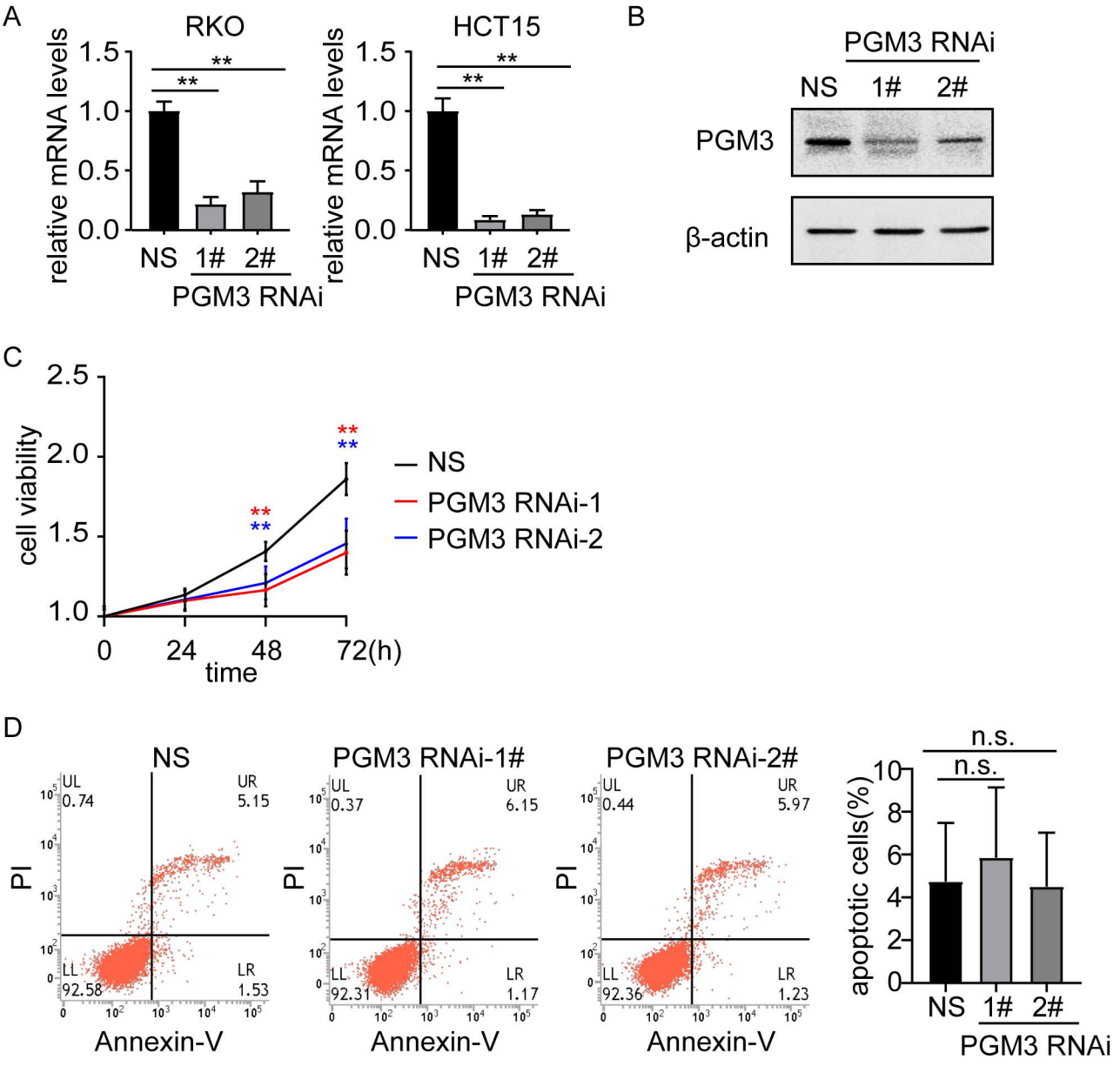


Figure S1. PGM3 is necessary for CRC proliferation and migration. Related to Figure 2.

A. RKO and HCT15 cells were transfected with a non-specific siRNA or PGM3 siRNA. 48h after transfection, RNA was extracted and analyzed with real-time PCR.

B. RKO cells were transfected with a non-specific siRNA or PGM3 siRNA. 48h after transfection, cells were harvested, and western blotting was performed to detect PGM3 protein levels.

C. Cell proliferation after PGM3 silencing in RKO cell lines was determined by CCK-8 assay.

D. HCT15 cells were transfected with a non-specific siRNA or PGM3 siRNA. 48h after transfection, the effect of PGM3 on apoptosis was analyzed using flow cytometry after PI and annexin V-FITC double staining.

Figure S2

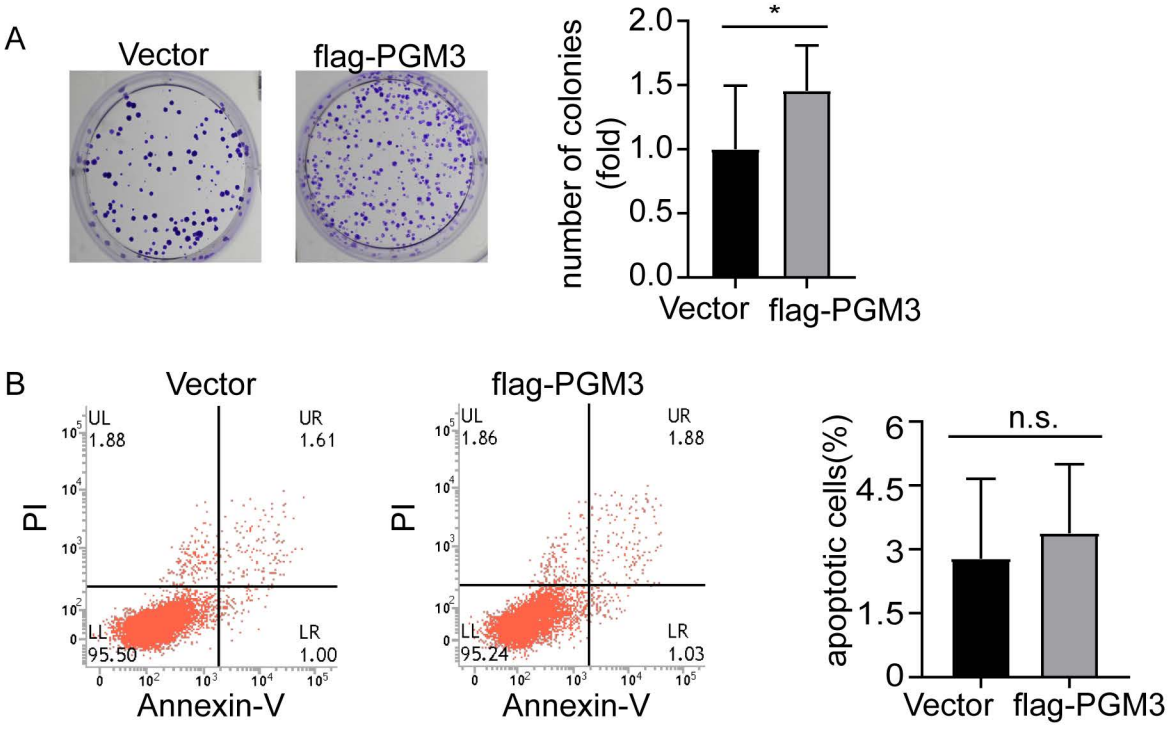
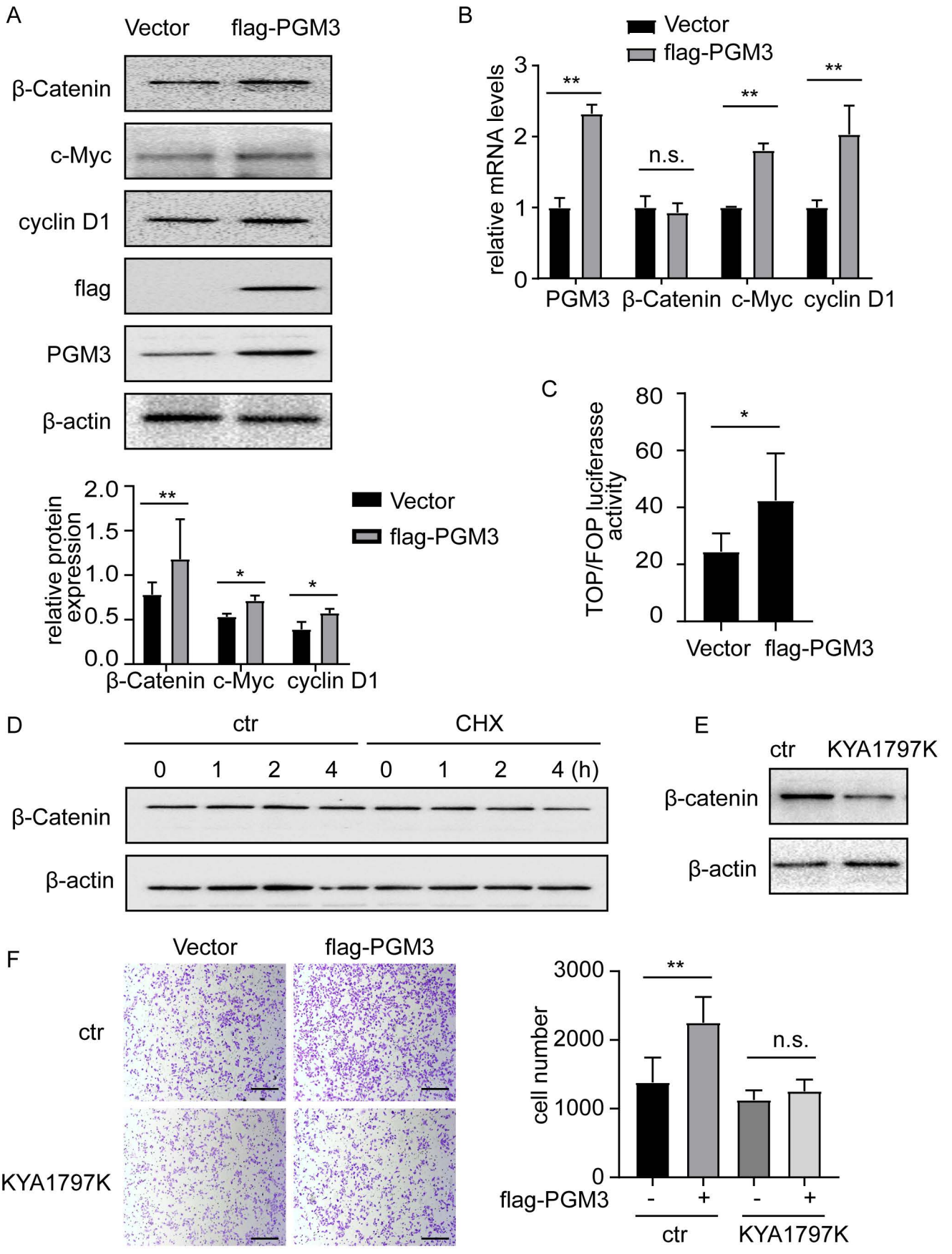


Figure S2. PGM3 promotes progression of CRC. Related to Figure 4.

A. SW480 cells were transfected with an empty vector or flag-PGM3 plasmid. After 2 weeks, the colony formation was analyzed with crystal violet staining.

B. SW480 cells were transfected with an empty vector or flag-PGM3 plasmid. 24h after transfection, the effect of PGM3 on apoptosis was analyzed using flow cytometry after PI and annexin V-FITC double staining.

Figure S3


A
Vector
flag-PGM3
β-Catenin
c-Myc
cyclin D1
flag
PGM3
β-actin
relative protein expression
2.0
1.5
1.0
0.5
0.0
**
*
*
β-Catenin
c-Myc
cyclin D1
Vector
flag-PGM3
B
Vector
flag-PGM3
relative mRNA levels
3
2
1
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n.s.
**
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PGM3
β-Catenin
c-Myc
cyclin D1
C
TOP/FOP luciferasse activity
80
60
40
20
0
*
Vector
flag-PGM3
D
ctr
CHX
0
1
2
4
0
1
2
4 (h)
β-Catenin
β-actin
E
ctr
KYA1797K
β-catenin
β-actin
F
Vector
flag-PGM3
ctr
KYA1797K
cell number
3000
2000
1000
0
**
n.s.
flag-PGM3
-
+
-
+
ctr
KYA1797K

Figure S3. Wnt/β-catenin signaling pathway is activated by PGM3. Related to Figure 5.

A. SW480 cells were transfected with an empty vector or flag-PGM3 plasmid. 24h after transfection, cells were harvested, and western blotting was performed to detect β-catenin, c-Myc, and cyclin D1 protein levels.

B. SW480 cells were transfected with an empty vector or flag-PGM3 plasmid. 24h after transfection, RNA was extracted and analyzed with real-time PCR.

C. SW480 cells were cotransfected with an empty vector or flag-PGM3 plasmid and TOP/ FOP Flash reporter plasmid. 24h after transfection, luciferase activity was measured.

D. SW480 cells were treated with or without 50μM CHX, expression of β-catenin was analyzed by western blotting.

E. SW480 cells were treated with or without 25μM KYA1797K for 24h, expression of β-catenin was analyzed by western blotting.

F. SW480 cells were transfected with PGM3 plasmids and treated with or without 25μM KYA1797K for 24h. Transwell assays were performed to examine cell migration.

Figure S4

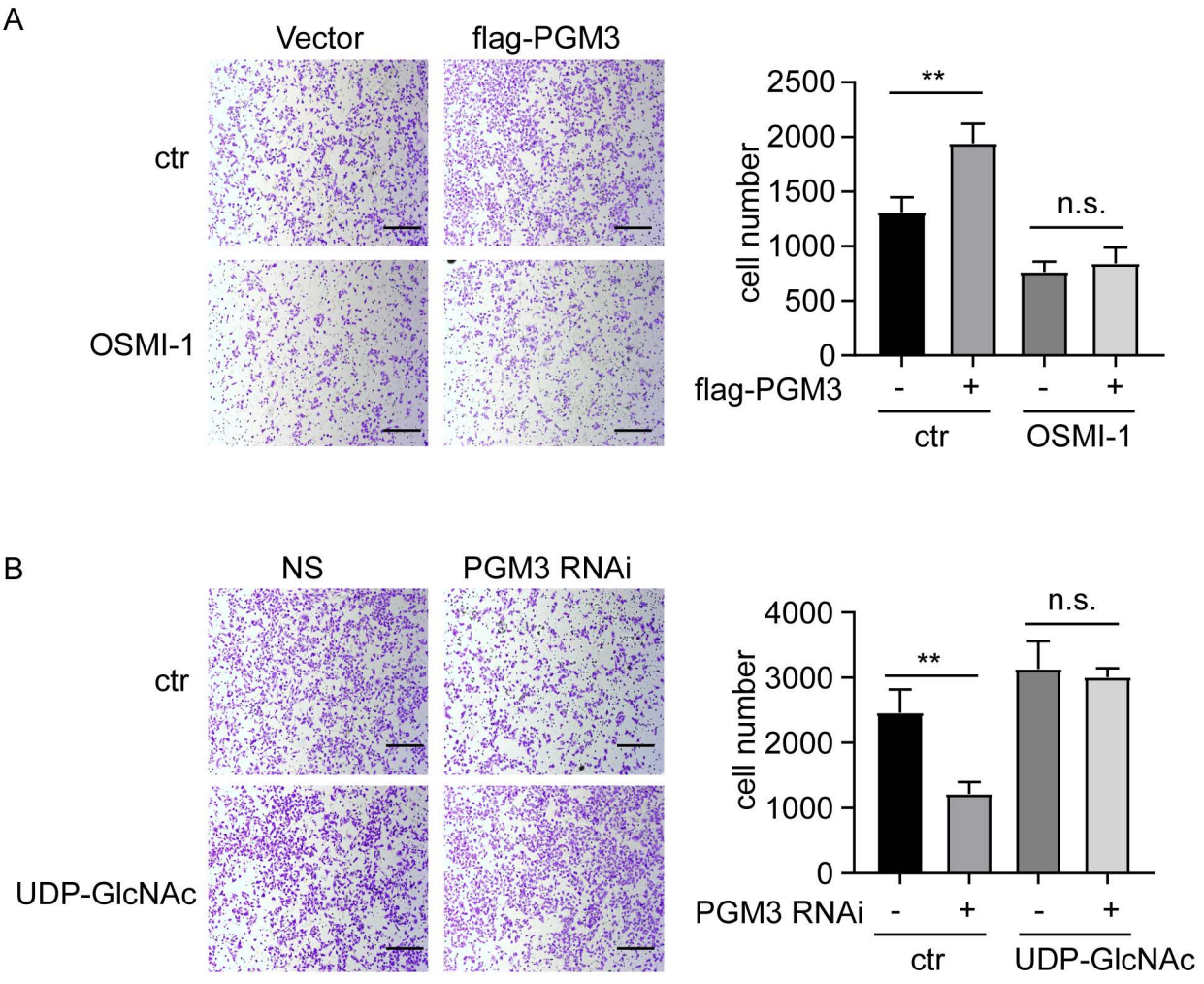

Figure S4. PGM3 promotes cell migration by elevating O-GlcNAcylation levels.

A. SW480 cells were transfected with PGM3 plasmids and treated with or without 30μM OMSI-1 for 24h. Transwell assays were performed to examine cell migration.

B. HCT15 cells were transfected with PGM3 siRNA and treated with or without 50mM UDP-GlcNAc for 48h. Transwell assays were performed to examine cell migration.