Figure S1. (A and B) Representative images of immunohistochemical staining of tumor tissue sections from patients with endometrial cancer. Representative images of immunohistochemical staining of (A) O-GlcNAc and (B) OGT in endometrial cancer tumor tissue sections. Patient 1, well differentiated, clinical stage I, no lymphatic metastasis; patient 2, poorly differentiated, clinical stage III, lymphatic metastasis. Scale bars, 50 µm. OGT, O-GlcNAc transferase.
Figure S2. (A and B) Results of wound healing assays for the evaluation of the migratory ability of cells. The migratory ability of (A) AN3CA and (B) HEC-1-B cells in the Con-sh and OGT-sh groups. Images were obtained at 0 and 24 h after the scraping of single molecule membranes. Scale bars, 100 µm. **P<0.01. OGT, O-GlcNAc transferase.
Figure S3. O-GlcNAcylation-mediated endometrial cancer cell malignancy is dependent on YAP. The levels of YAP in the Con-sh and YAP-sh groups of (A) AN3CA and (B) HEC-1-B cells were examined using western blot analysis. (C-F) YAP-sh cells were treated with DMSO, TMG (10 µM), or OSMI-1 (50 µM. The proliferative ability of the (C) AN3CA and (D) HEC-1-B cells was measured at the indicated time points using a Cell Counting Kit-8 assay. In addition, wound healing assays were performed to assess the migratory ability of (E) AN3CA and (F) HEC-1-B cells. Images were obtained at 0 and 24 h after the scraping of single molecule membranes. Scale bars, 100 µm. Data represent the mean ± SD (n=3). **P<0.01. YAP, Yes-associated protein; TMG, Thiamet-G.
Figure S4. O-GlcNAcylation increases YAP stability. Protein synthesis was blocked by treatment with cycloheximide for the indicated periods of time. The half-life of endogenous YAP in the Con-sh and OGT-sh groups of (A and B) AN3CA and (C and D) HEC-1-B cells, as determined using western blot analysis. The levels of YAP were normalized to those of β-actin, and the 0 h points were arbitrarily set to 100%. YAP, Yes-associated protein; OGT, O-GlcNAc transferase.
Figure S5. O-GlcNAcylation sites of YAP in HEC-1-B cells. Two different O-GlcNAcylation sites were found in YAP: (A) Serine 109 and (B) threonine 241.