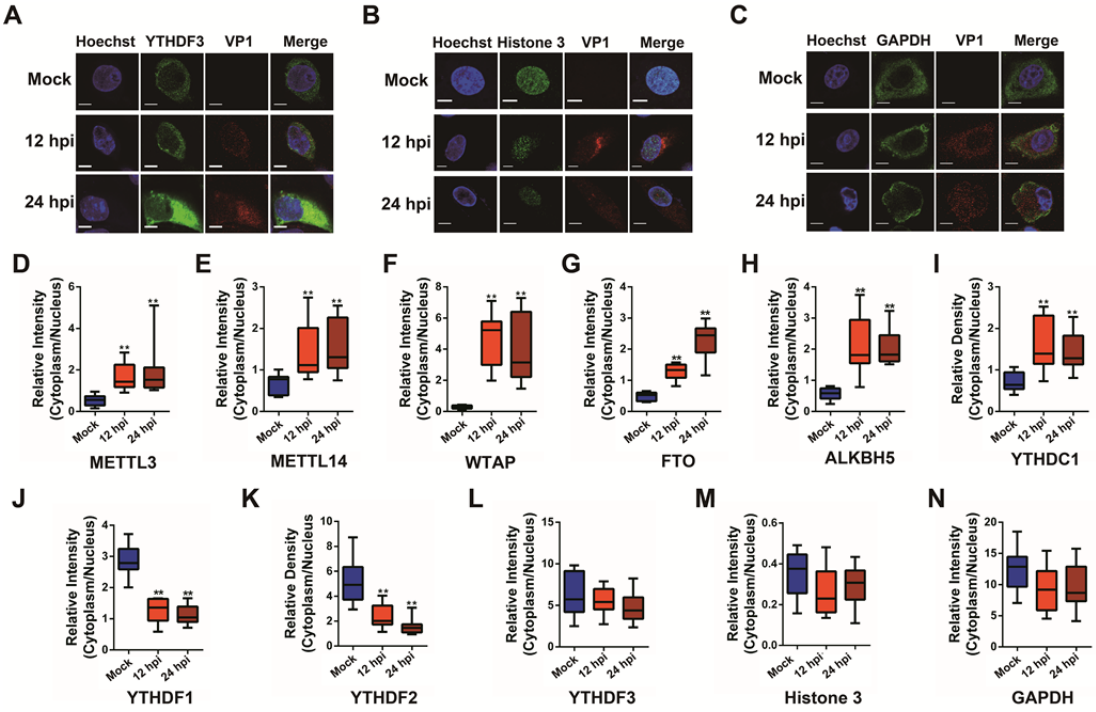

Figure S1. EV71 infection influences subcellular localization of m^{6}A methyltransferases, demethylases, and YTH proteins. (**A** – **C**) Confocal microscopy images of EV71- or mock-infected Vero cells. The nucleus (blue) and virus protein (red) were labeled with Hoechst and VP1-specific antibody, respectively. YTHDF3 (A), Histone 3 (B) and GAPDH (C) were stained with antibodies as indicated. Scale bars, 5 μm. (**D** – **N**) The ratio of methyltransferases, demethylases, YTH proteins, Histone 3, and GAPDH in cytoplasm versus nucleus was quantified by using the ImageJ program and graphed in box-and-whisker plots, representing the minimum, first quartile, median, third quartile, and maximum. Unpaired Student's *t*-test was performed ($n \geq 10$). $^{**}P \leq 0.01$.

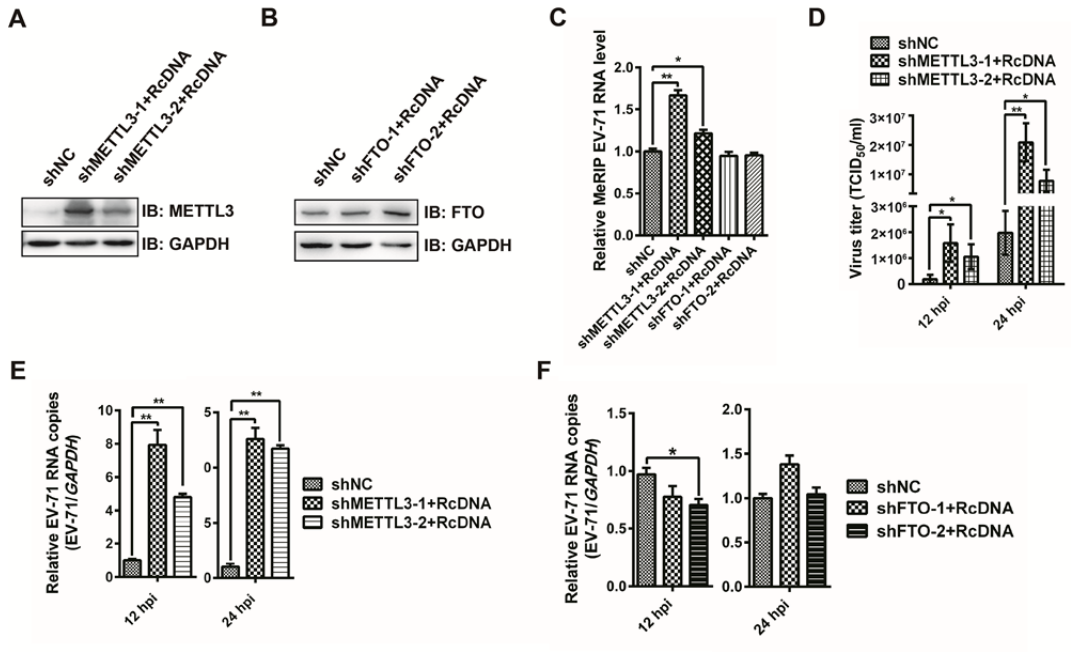

Figure S2. Restoring the expression of methyltransferases and demethylases in knockdown cells influenced the replication of EV71. (**A** & **B**) Western blotting. shMETTL3 and shFTO-resistant cDNAs were expressed in METTL3 and FTO knockdown Vero cells. Western blotting was carried out to check the expression of METTL3 and FTO. shNC was used as a control. (**C**) MeRIP-qPCR. RNA was extracted from EV71-infected METTL3 or FTO knockdown cells in which the expression of METTL3 or FTO was restored using shRNA-resistant cDNAs, Me-RIP was performed, and m^6A antibody bound RNA was quantified by qRT-PCR. Unpaired Student's t-test was performed, and data are presented as the means ± SEM ($n = 3$). *$P \leq 0.05$, **$P \leq 0.01$. (**D**) Viral titers ($TCID_{50}$/ml) at 12 and 24 h post infection. shRNA resistant METTL3 was transfected to METTL3 knockdown cells, followed by EV71 infection. The virus was titrated at 12 and 24 hpi. Significant differences were determined by using the Student *t* test (*$P \leq 0.05$, **$P \leq 0.01$). (**E** & **F**) qRT-PCR. Total RNA was extracted at the indicated times from EV71-infected knockdown cell lines in which METTL3 or FTO was restored by shRNA-resistant cDNAs. Quantification of EV71 RNA was performed by qRT-PCR, with *GAPDH* used as a control. Unpaired Student's *t*-test was performed *$P \leq 0.05$, **$P \leq 0.01$.

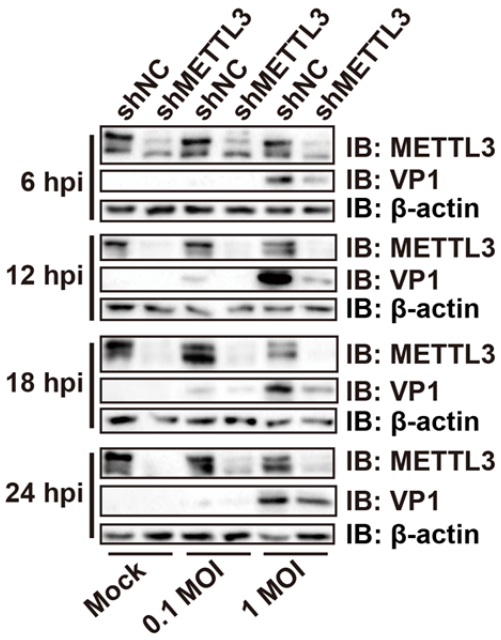

Figure S3. METTL3 regulates the expression of EV71 VP1. Proteins were extracted at the indicated times from EV71-infected Vero cells in which METTL3 was knocked down by shMETTL3. shNC was used as a control. The expression of METTL3 and VP1 was detected by Western blotting using anti-METTL3 and anti-VP1 antibodies, respectively.

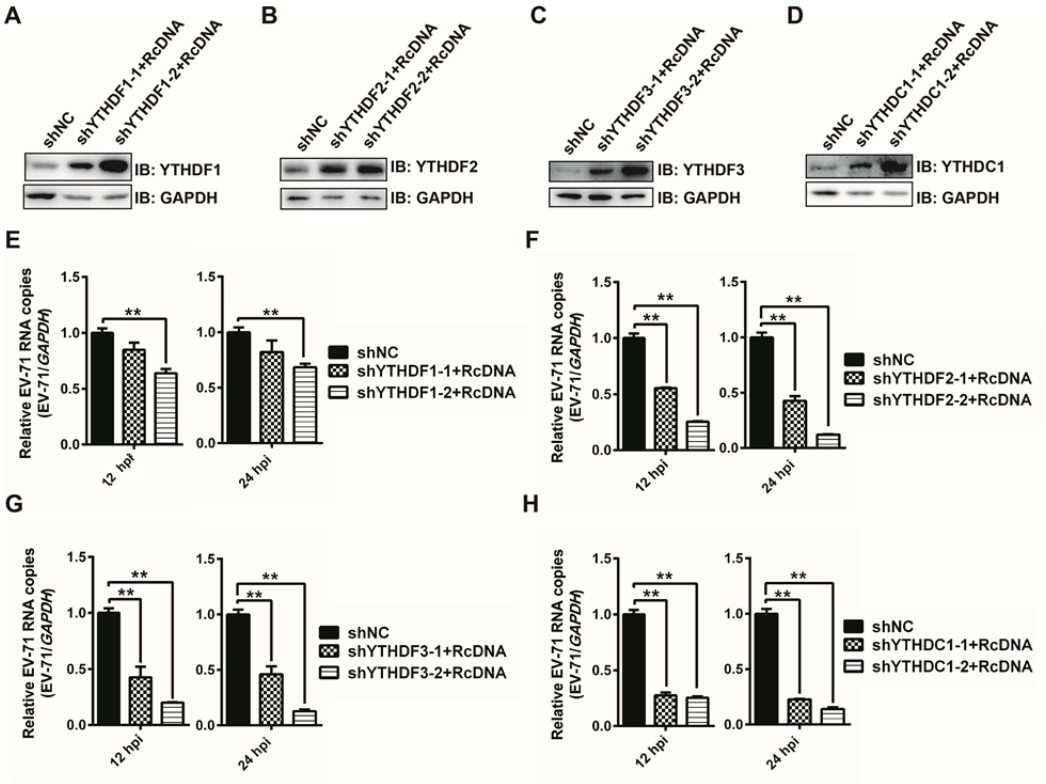

Figure S4. Restoring the expression of YTH proteins in RD cells decreased viral replication. (**A** – **D**) Western blotting. shRNA-resistant cDNAs of YTH proteins were transfected into RD cells in which YTH proteins were knocked down. The expression of YTH proteins was detected by Western blotting with the corresponding antibodies. (**E** – **H**) qRT-PCR. Total RNA was extracted at indicated times from EV71-infected RD cells with knockdown of YTH proteins, in which the expression of YTH proteins was restored by expression of shRNA-resistant cDNAs. Quantification of EV71 RNA was performed by qRT-PCR, with *GAPDH* used as a control. Unpaired Student's *t*-test was performed. **P* ≤ 0.05, ***P* ≤ 0.01.

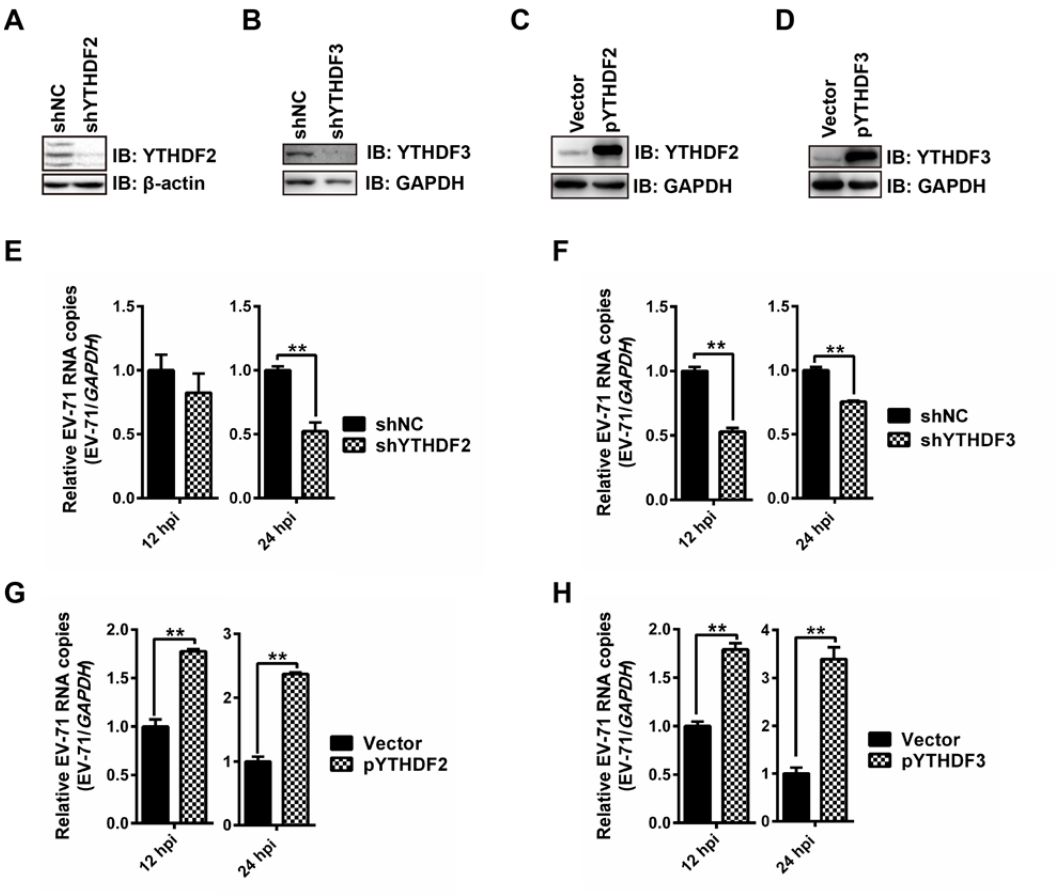

Figure S5. YTH proteins positively regulate EV71 replication in Vero cell. (**A** – **D**) Western blotting. YTHDF2 and YTHDF3 were knocked down by shRNA lentivirus or overexpressed by pYTHDF2 and pYTHDF3 transfection. YTH protein expression was detected by Western blotting with indicated antibodies. (**E** – **H**) qRT-PCR. Total RNA was extracted at indicated times from EV71-infected Vero cells in which YTHDF2 or YTHDF3 was knocked down or overexpressed. EV71 RNA was quantified by qRT-PCR, with *GAPDH* used as a control. Unpaired Student's *t*-test was performed. *$P \leq 0.05$, **$P \leq 0.01$.

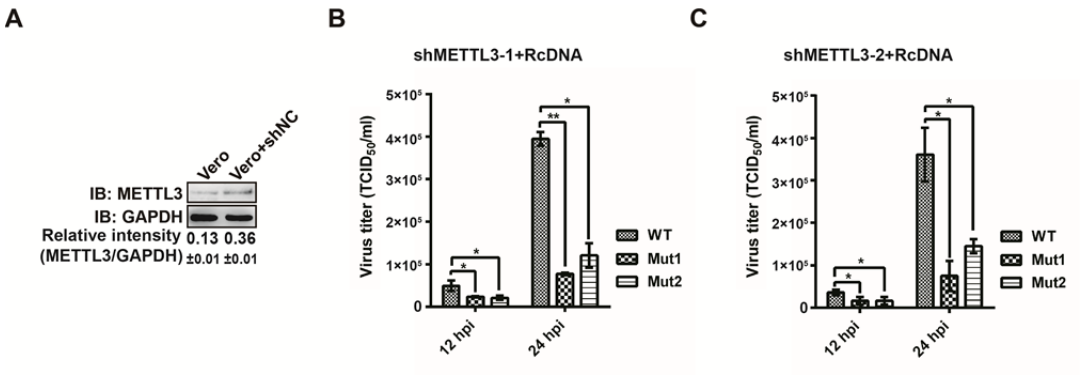

Figure S6. Titers of m^{6}A site mutated virus are significantly decreased in METTL3 expression restored Vero cells. (**A**) Western blotting. The expression of METTL3 in Vero cells and Vero cells infected by shNC lentivirus was detected by western blotting with the respective antibodies. Relative gray intensity of METTL3 in METTL3 versus GAPDH was quantified using the ImageJ program. The data are shown as the means ± SD (n = 3). (**B** & **C**) Viral titers ($TCID_{50}$/ml) at 12 and 24 h post infection. METTL3 knockdown Vero cells were transfected by shMETTL3-resistant cDNAs and subjected to be infected by EV71 WT, Mut1 or Mut2, and the supernatants were collected at indicated times post infection to determine virus titers as $TCID_{50}$/ml. Data presented are the mean viral titers and SDs from three independent experiments. Significant differences were determined using the Student t test (*$P \leq 0.05$, **$P \leq 0.01$).

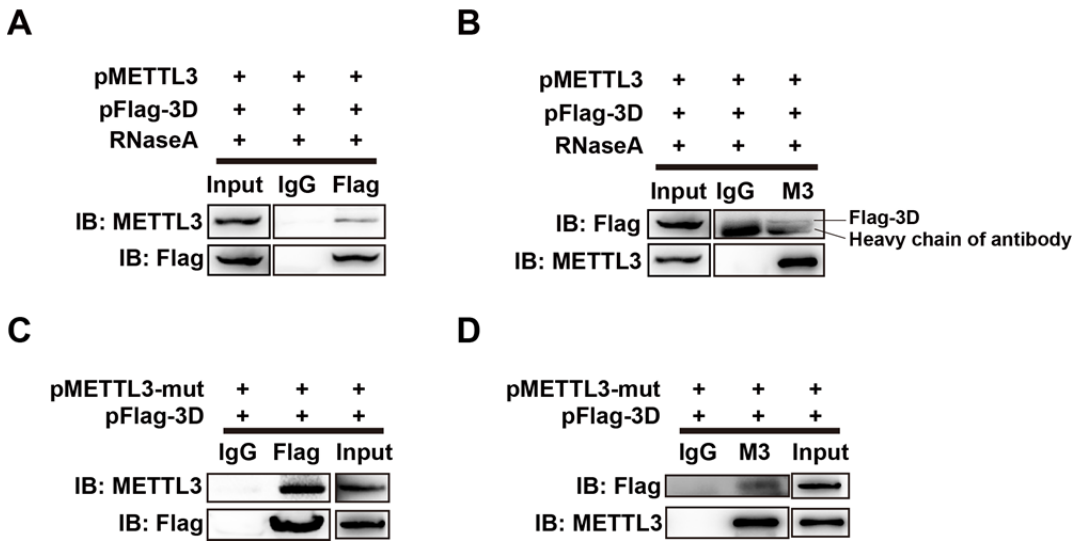

Figure S7. The interaction between METTL3 and 3D polymerase is independent of RNA and the catalytic center of METTL3. (**A** & **B**) Western blotting. HEK293T cells were transfected with pMETTL3 and pFlag-3D. Extracts were digested with RNase A for 15min at 37 ˚C, followed by IP with anti-Flag (A) or anti-METTL (B) antibodies. The immunoblots were probed with indicated antibodies. (**C** & **D**) Western blotting. pMETTL3-mut (catalytic center mutant) and pFlag-3D were transfected into HEK293T cells. IP was performed with anti-Flag (C) or anti-METTL3 (D) antibodies. The immunoblots were probed with anti-METTL3 and anti-Flag antibodies, respectively.

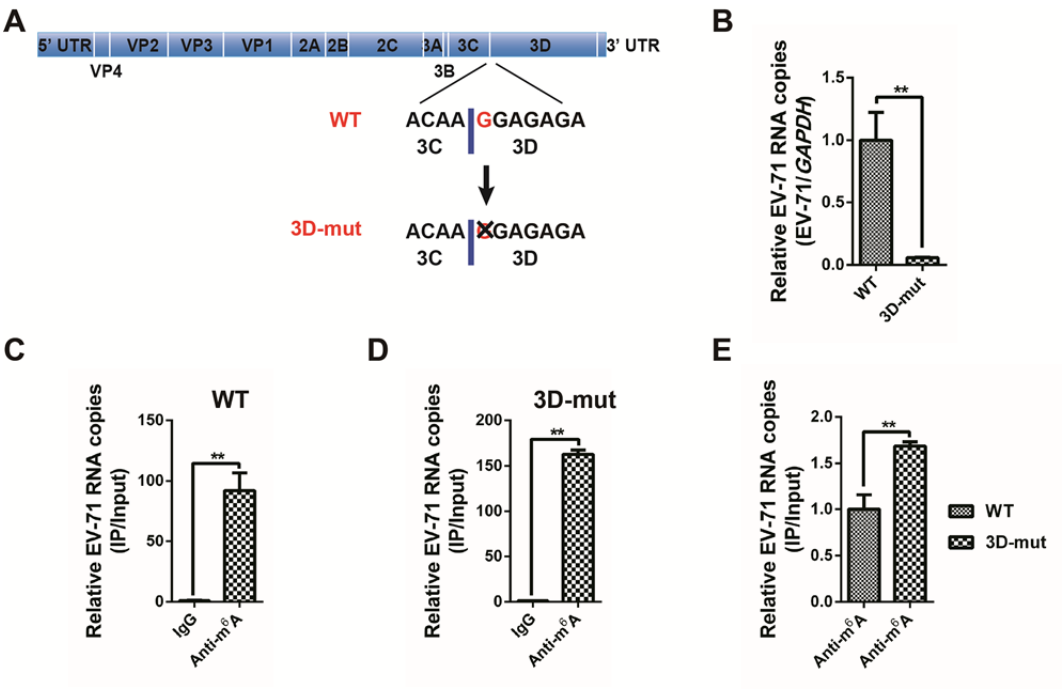

Figure S8. The presence of m^{6}A within EV71 RNA is independent on the interaction between 3D and METTL3. (**A**) A diagram of EV71 3D mutants. G 5937 was deleted in 3D-mut. (**B**) qRT-PCR. Total RNA was extracted at 24 hpi from HEK293T cells that were transfected with *in vitro* T7 transcribed EV71 WT or EV71 3D-mut RNA. EV71 RNA was quantified by qRT-PCR, with *GAPDH* used as a control. Unpaired Student's *t*-test was performed. ***P* ≤ 0.01. (**C** – **E**) MeRIP-qPCR. RNA was extracted from EV71 WT or EV71 3D-mut RNA transfected HEK293T cells, followed by Me-RIP with m^{6}A antibody. Isolated RNA was quantified by qRT-PCR. Unpaired Student's *t*-test was performed and data are presented as the means ± SEM (*n* = 3). ***P* ≤ 0.01.

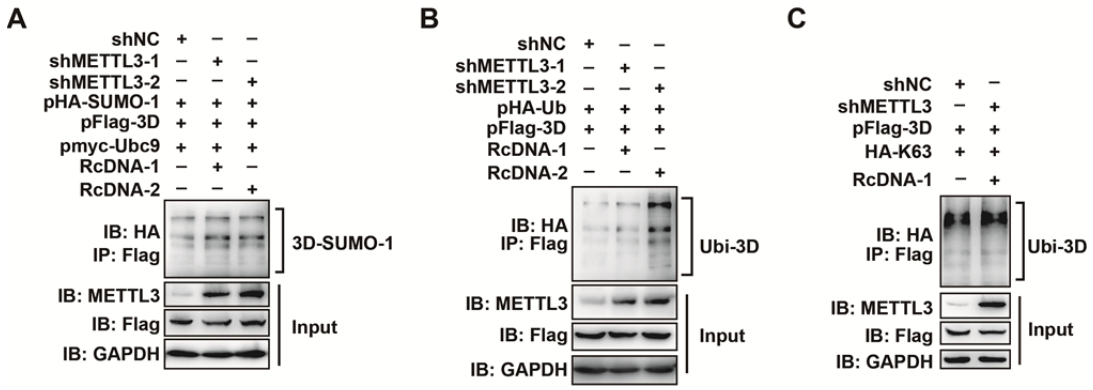

Figure S9. Sumoylation and ubiquitination level of 3D are increased when the expression of METTL3 is complemented. (**A**) Sumoylation assay. METTL3 was complemented in knockdown HEK293T cells by transfecting an shRNA-resistant cDNA together with pFlag-3D, pHA-SUMO-1, and pMyc-Ubc9. IP and immunoblot analysis were performed with the indicated antibodies for the sumoylation assay. (**B** & **C**) Ubiquitination assay. METTL3 knockdown HEK293T cells were transfected with indicated plasmids. IP and immunoblot analysis were performed with the indicated antibodies.