

Figure S1. mRNA levels of METTL3 in METTL3-KD or METTL3-OV HCT-8 cells. (A) METTL3 mRNA levels in control and METTL3-KD HCT-8 cells. (B) METTL3 mRNA levels in control and METTL3-OV HCT-8 cells. (C and D) Dot blot analysis of the m<sup>6</sup>A level of total RNAs isolated from sh-METTL3 or METTL3-OV HCT-8 and control cells. Methylene blue staining served as a loading control. \*\*P<0.01 and \*\*\*P<0.001. METTL3, methyltransferase-like 3; KD, knockdown; OV, overexpressing; m<sup>6</sup>A, N<sup>6</sup>-methyladenosine; sh-, small hairpin; CN or Con, control.

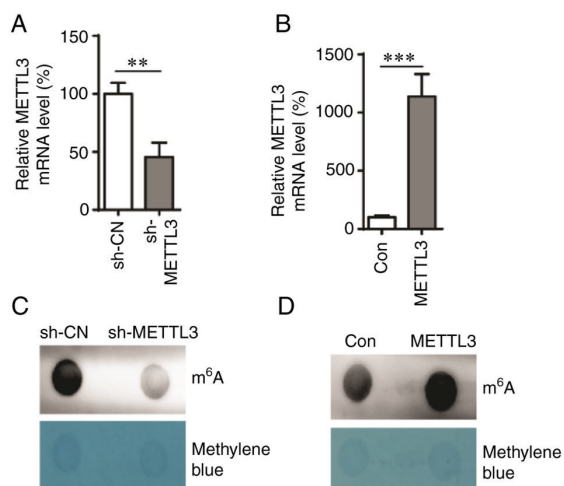


Figure S2. METTL3 regulates DNA damage in HCT-8 cells with 5-FU treatment. (A) Immunofluorescence staining of 53BP1 foci in different cells treated with 5-FU for 4 h. (B) Overexpression of METTL3 decreased the sensitivity of HCT-8 cells to 5-FU as determined using a CCK-8 assay. (C) Overexpression of METTL3 reduced the expression of pro-apoptotic proteins caspase-3 and Bax in HCT-8 cells treated with 5-FU. The quantification of the western blotting bands are on the right. (D) Western blotting to determine  $\gamma$ -H2AX levels in control and METTL3-OV HCT-8 cells following treatment with various doses (0, 2 and 4  $\mu$ g/ml) of 5-FU treatments for 4 h. The quantification of the western blotting bands is shown under the panel. (E) The foci of  $\gamma$ -H2AX in HCT-8 cells following treatment with various doses (0, 2 and 4  $\mu$ g/ml) of 5-FU. The quantification of the foci numbers per cells are shown on the right. Data are presented as means  $\pm$  SD. \* $P$ <0.05, \*\* $P$ <0.01 and \*\*\* $P$ <0.001. METTL3, methyltransferase-like 3; 5-FU, 5-fluorouracil; CCK-8, Cell Counting Kit-8; OV, overexpressing; sh-, small hairpin; CN or Con, control.

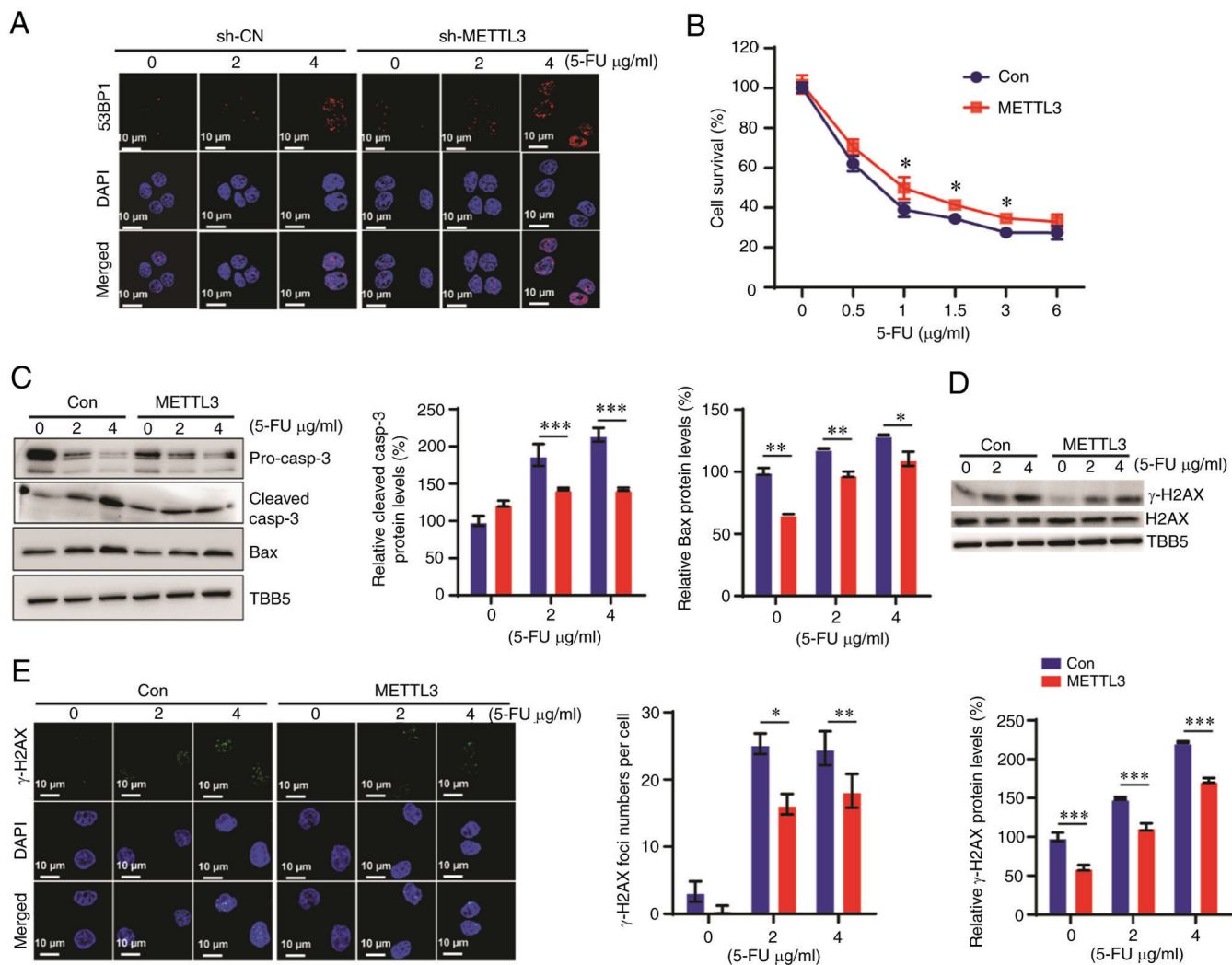


Figure S3. mRNA levels of METTL3 determined by reverse-transcription-quantitative PCR to confirm the knockdown of METTL3 in HCT-8R cells. Data are presented as the means  $\pm$  SD. \* $P$ <0.05. METTL3, methyltransferase-like 3; HCT-8R, 5-FU-resistant HCT-8 cells; sh-, small hairpin; CN, control.

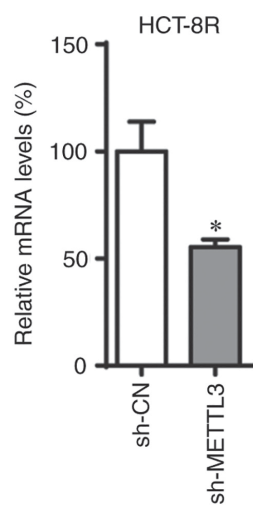


Figure S4. Screening of METTL3 targets among HR in HCT-8R cells. (A) HR genes regulated in HCT-8R and HCT-8 cells. (B) HR genes regulated by METTL3 in HCT-8 cells. (C) HR genes regulated by METTL3 in HCT-8R cells. Data are presented as the means  $\pm$  SD. \* $P$ <0.05, \*\* $P$ <0.01 and \*\*\* $P$ <0.001. METTL3, methyltransferase-like 3; HR, homologous recombination; HCT-8R, 5-FU-resistant HCT-8 cells; sh-, small hairpin; CN, control.

