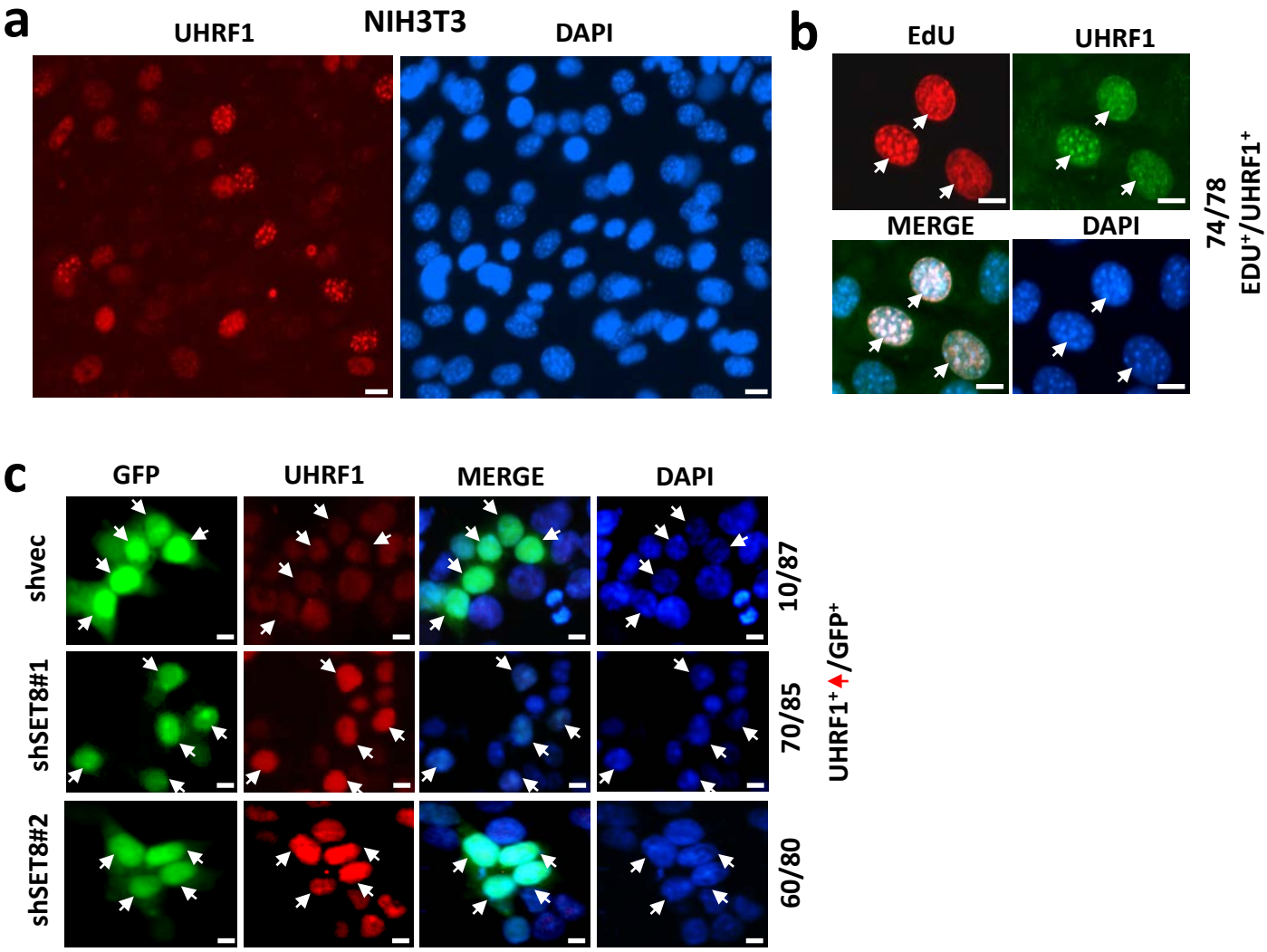
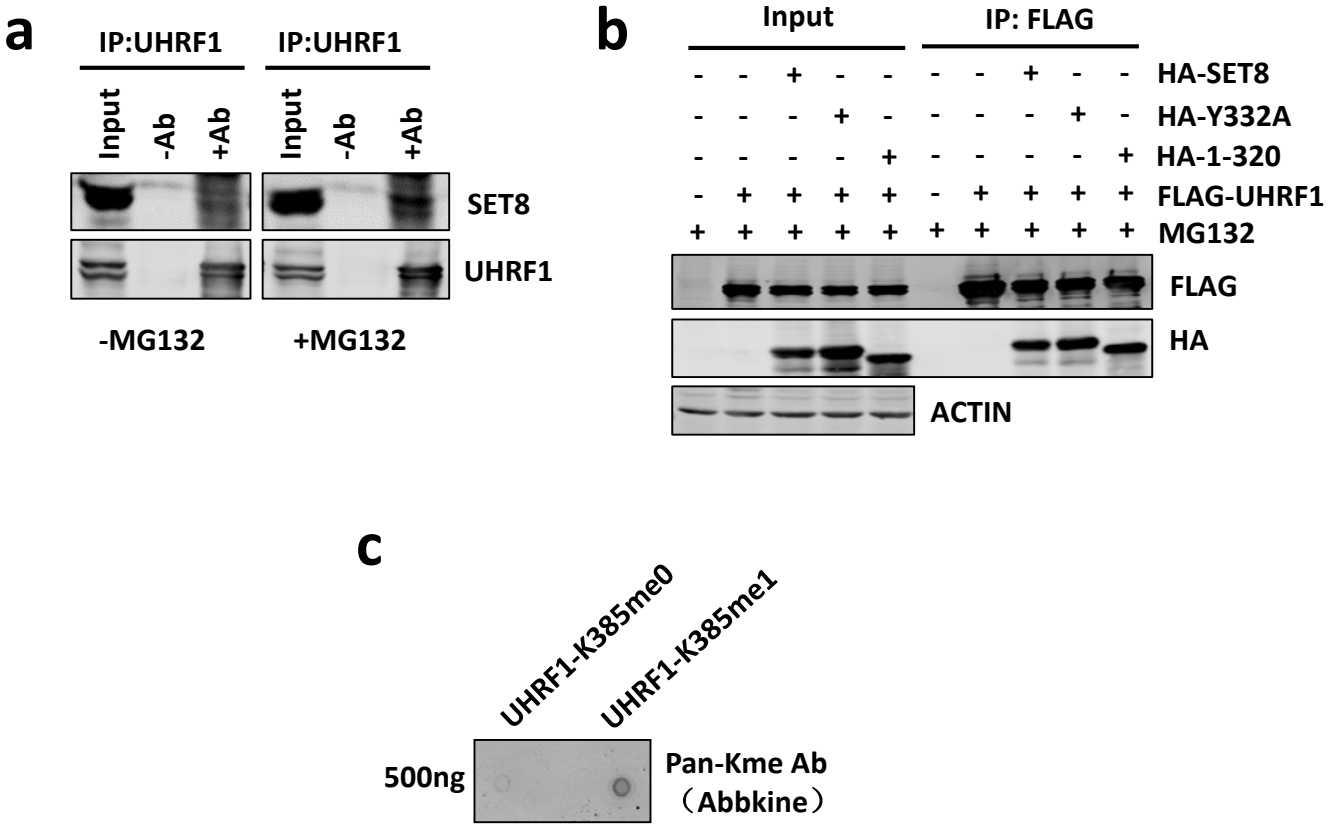
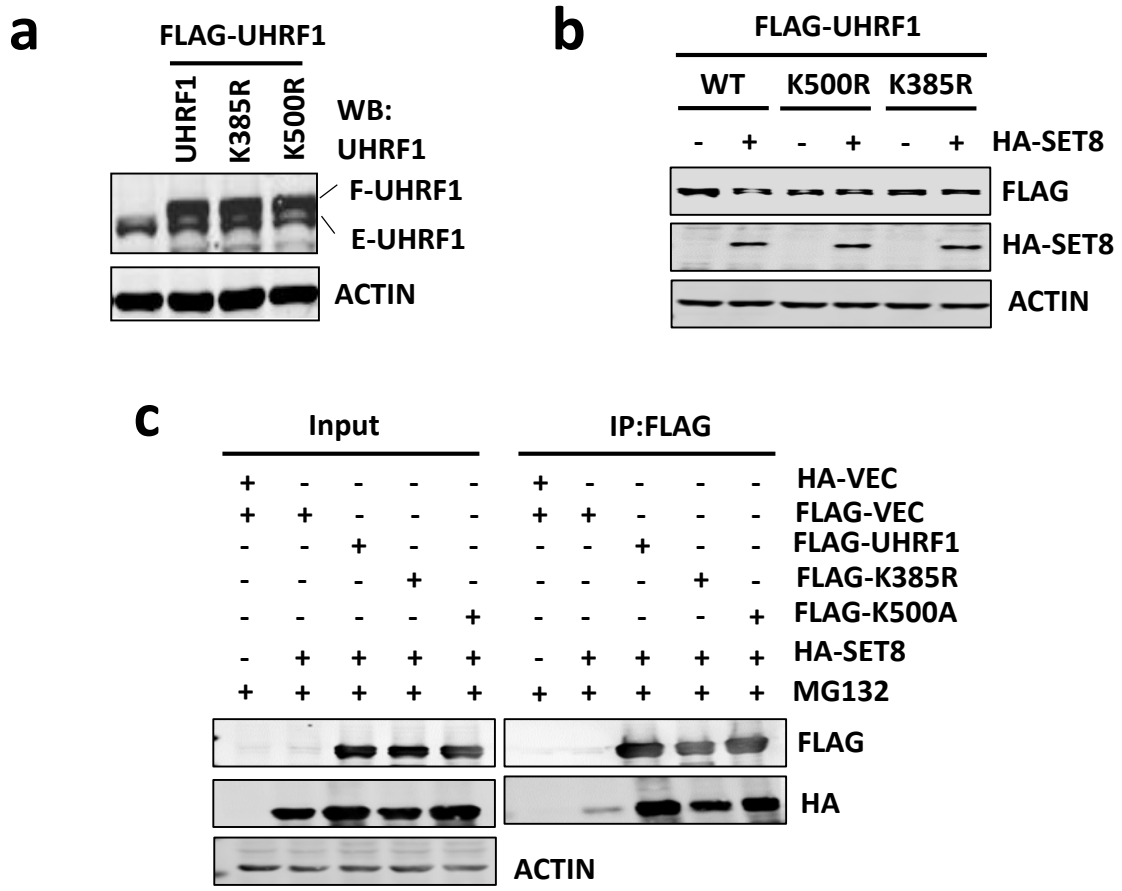


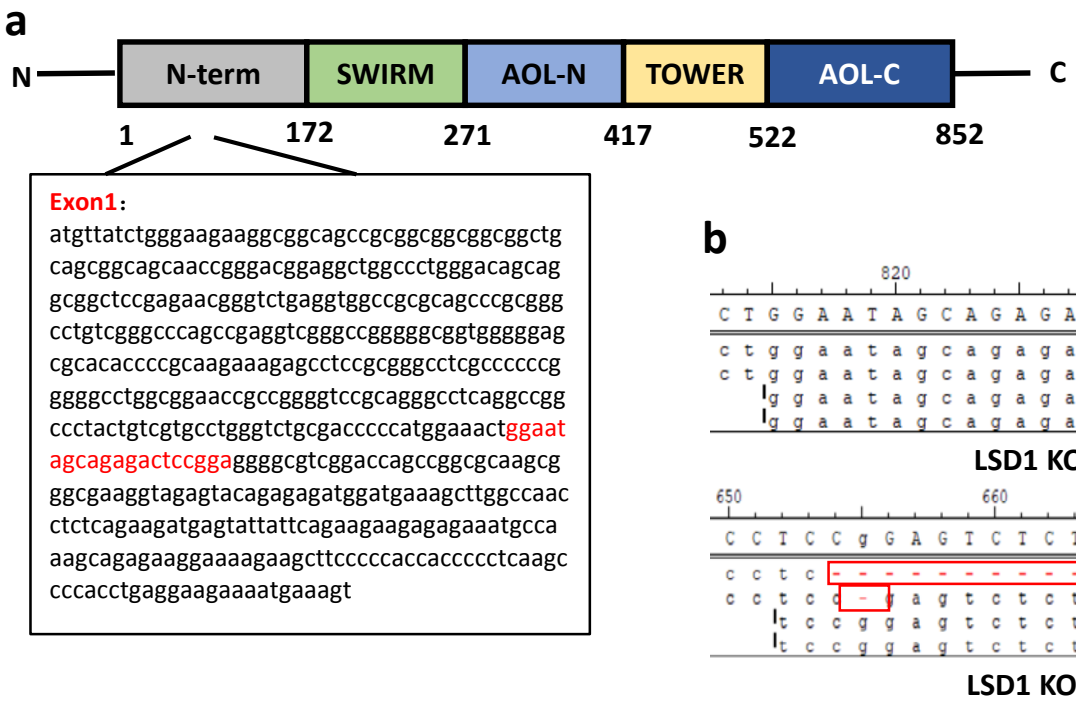
Supplementary Figure S1. In NIH3T3 cells the UHRF1 protein is highly expressed in S phase of cell cycle. **a**) Immunostaining showing a highly variable level of UHRF1 protein in unsynchronized NIH3T3 cells. Scale bar, 20 µm. **b**) The high UHRF1-expressing NIH3T3 cells were also EdU labeled in S phase. NIH3T3 cells were cultured in the presence of EdU for 2hs before processed for fluorescent imaging of EdU by a click chemistry reaction and UHRF1 by immunostaining. Scale bar, 20 µm. **c**) Immunostaining showing that knockdown of SET8 by transient transfection of SET8-specific shRNAs (green) resulted in an elevated level of UHRF1 (red) protein in HeLa cells. Scale bar, 20 µm.



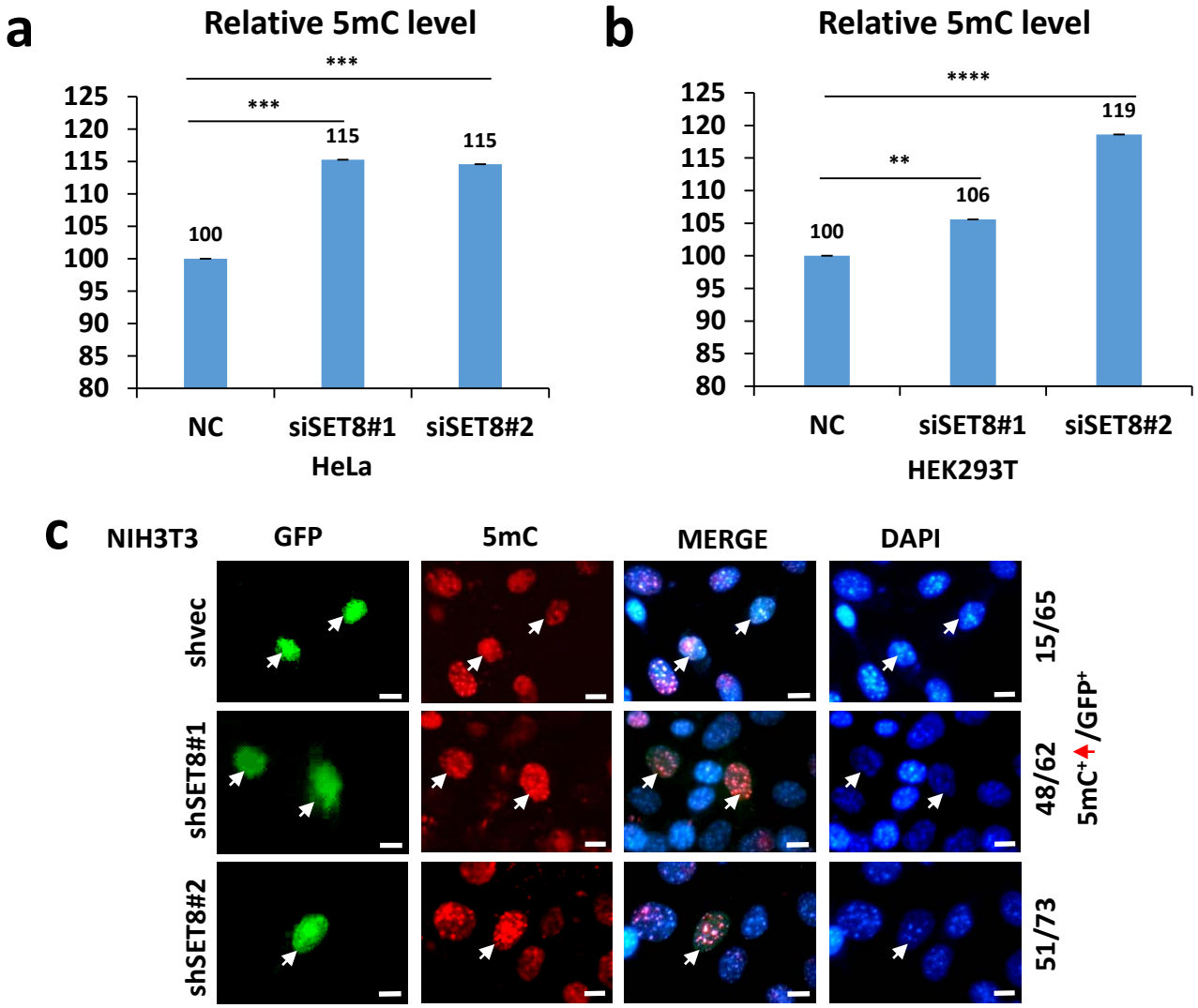
Supplementary Figure S2. SET8 interacts with UHRF1 in co-IP assay. a) Analysis of endogenous UHRF1 and SET8 interaction by co-IP. HEK293T cells were cultured with or without addition of MG132 for 8 hours before harvested for preparation of nuclear extracts. The resulting nuclear extracts were used for IP-Western Blot analysis using antibodies as indicated. **b**) IP-Western blot analysis for the interaction between ectopically expressed UHRF1 and SET8 or SET8 mutants as indicated. **c**) Dot blot assay for validation of Pan-Kme antibody. 500 ng of synthetic control and K385me1 peptides were spotted onto nitrocellulose membrane and blotted with a Pan-Kme antibody purchased from Abbkine.



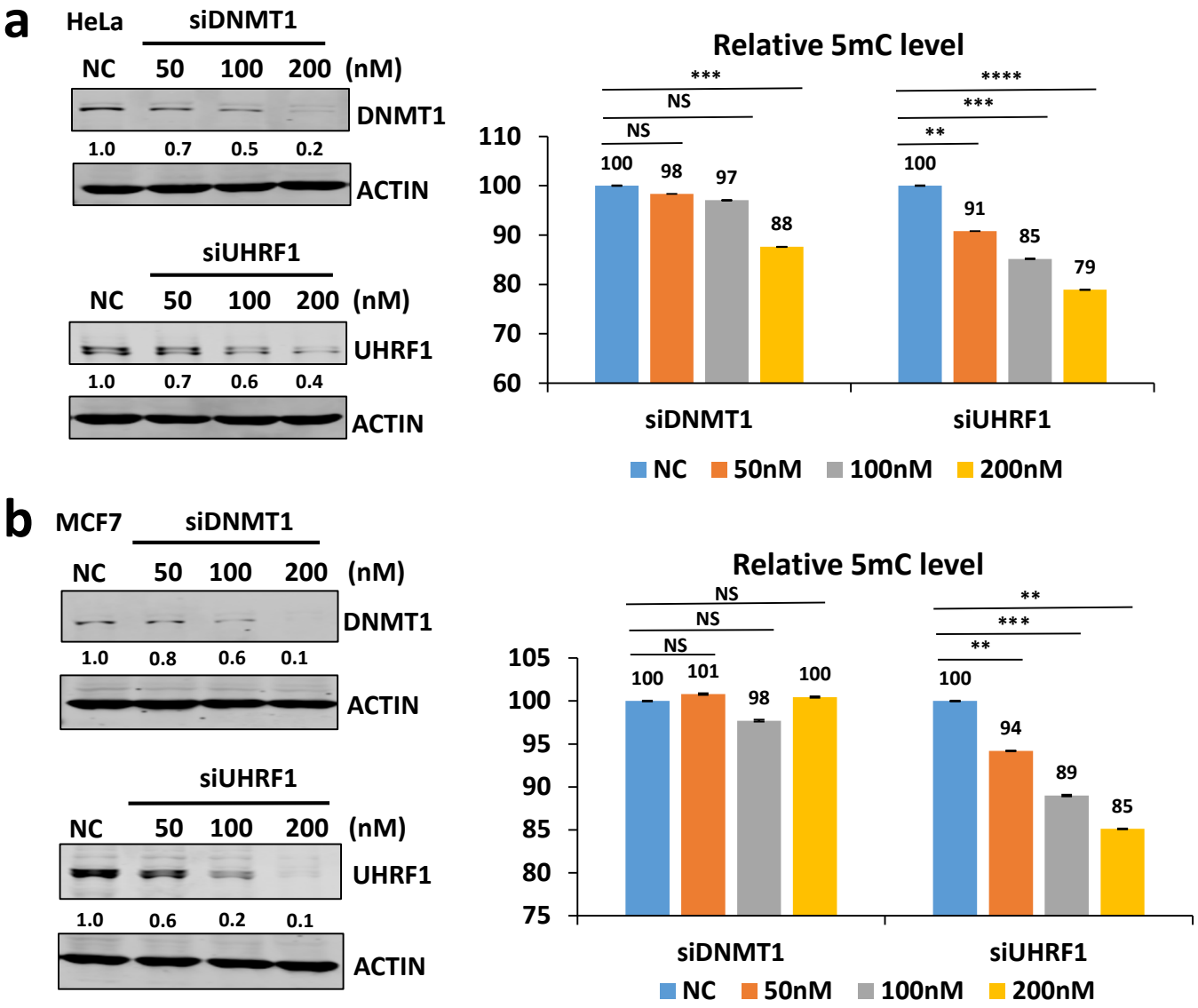
Supplementary Figure S3. Characterization of UHRF1 K385R and K500R mutants. a) Western blot analysis of cellular extracts derived from control and FLAG-UHRF1 or mutant UHRF1-expressing stable cell lines. The stable cell lines were established by transfection of HeLa cells with pPYCAGIP-based UHRF1 –expressing plasmids and selected with puromycin. **b**) Western blot analysis showing that both K385R and K500R mutant UHRF1 proteins were resistant to SET8-induced downregulation. The above stable cell lines were transfected with or without SET8 and the effect on UHRF1 protein was assayed by Western Blot analysis. **c**) IP-Western blot analysis for the interaction between ectopically expressed SET8 and UHRF1 or UHRF1 mutants as indicated.



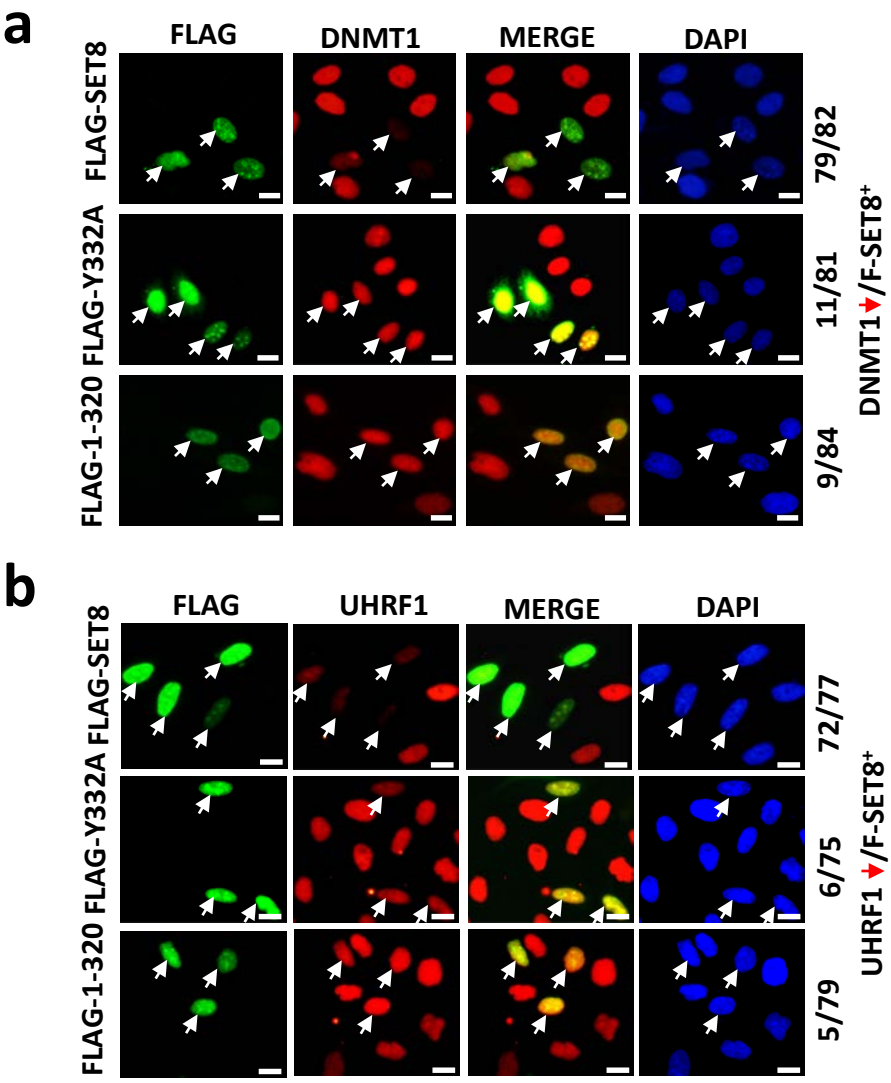
Supplementary Figure S4. Knockout of LSD1 by CRISPR-Cas9. a) sgRNA was designed at the first exon of LSD1. **b**) Sequencing results of TA clones of the two LSD1 knockout HeLa cells. **c**) qRT-PCR analysis showing that knockout of LSD1 by the CRISPR-Cas9 system resulted in increased transcription levels of UHRF1 and DNMT1 in LSD1-knockout HeLa cells.***,P<0.001 ;****,P<0.0001 .



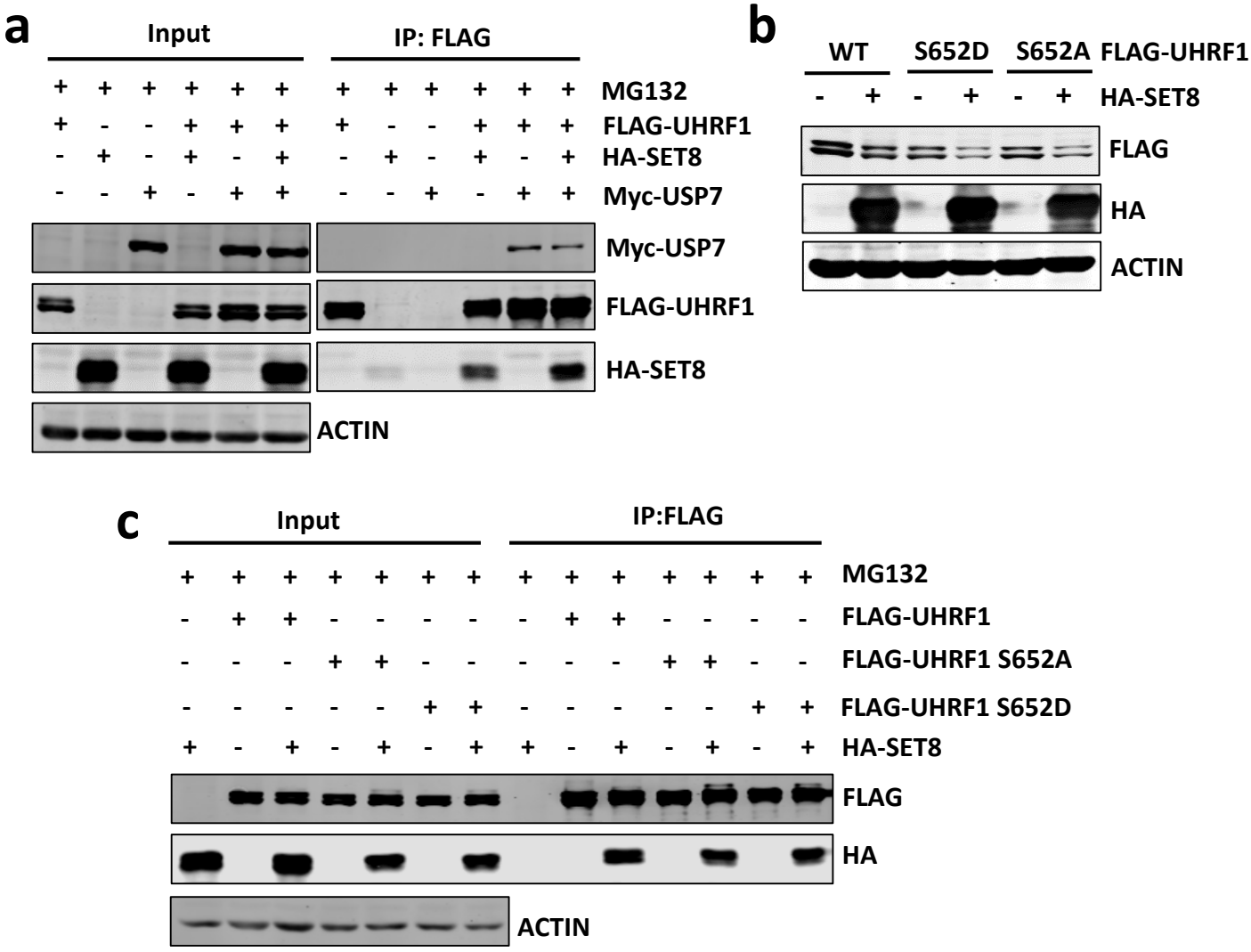
Supplementary Figure S5. SET8 has a role in suppressing of global DNA methylation. **a**) LC-MS analysis showing that knockdown of SET8 by transient transfection of SET8-specific siRNAs resulted in elevated levels of DNA methylation in HeLa cells. .***,P<0.001. **b**) LC-MS analysis showing that knockdown of SET8 by transient transfection of SET8-specific siRNAs resulted in elevated levels of DNA methylation in HEK293T cells. **,P<0.01;****,P<0.0001. **c**) Immunostaining showing that knockdown of SET8 by transient transfection of SET8-specific shRNAs (green) led to increased DNA methylation (red) in NIH3T3 cells. Scale bar, 20 μm.



Supplementary Figure S6. DNA methylation is more sensitive to reduction of UHRF1 than DNMT1. **a**) Increased knockdown of DNMT1 and UHRF1 in HeLa cells was associated with a progressively reduced level of DNA methylation. **,P<0.01;***,P<0.001. **b**) Increased knockdown of DNMT1 and UHRF1 in MCF7 cells was associated with a progressively reduced level of DNA methylation. **, P<0.01; ***, P<0.001.



Supplementary Figure S7. SET8 activity regulates the stability of UHRF1 and DNMT1 independent of DNMT3A/3B. **a**) Immunostaining showing that overexpression of a FLAG-tagged SET8 (green) in DNMT3A/3B DKO cells diminished the level of endogenous DNMT1 (red) proteins . But the mutants Y332A (green) and 1-320 (green) could not. Scale bar, 20 µm. **b**) Immunostaining showing that overexpression of a FLAG-tagged SET8 (green) in DNMT3A/3B DKO cells diminished the level of endogenous UHRF1 (red) proteins . But the mutants Y332A (green) and 1-320 (green) could not. Scale bar, 20 µm.



Supplementary Figure S8. SET8 regulates UHRF1 stability independent of USP7 and S652 phosphorylation. **a**) IP-Western blot analysis showing that SET8 did not compete with USP7 for binding of UHRF1. **b**) Western blot analysis showing that ectopic overexpression of SET8 down-regulated wild-type and S652A and S652D mutants of UHRF1. HeLa cells stably expressing FLAG-UHRF1 or S652A or S652D UHRF1 mutant were established first and then transfected with a SET8-expressing plasmid. **c**) IP-Western blot analysis showing that S652A or S652D mutation did not affect the interaction between ectopically expressed SET8 and UHRF1.