m^{6}A modification regulates cell proliferation via reprogramming the balance between glycolysis and pentose phosphate pathway

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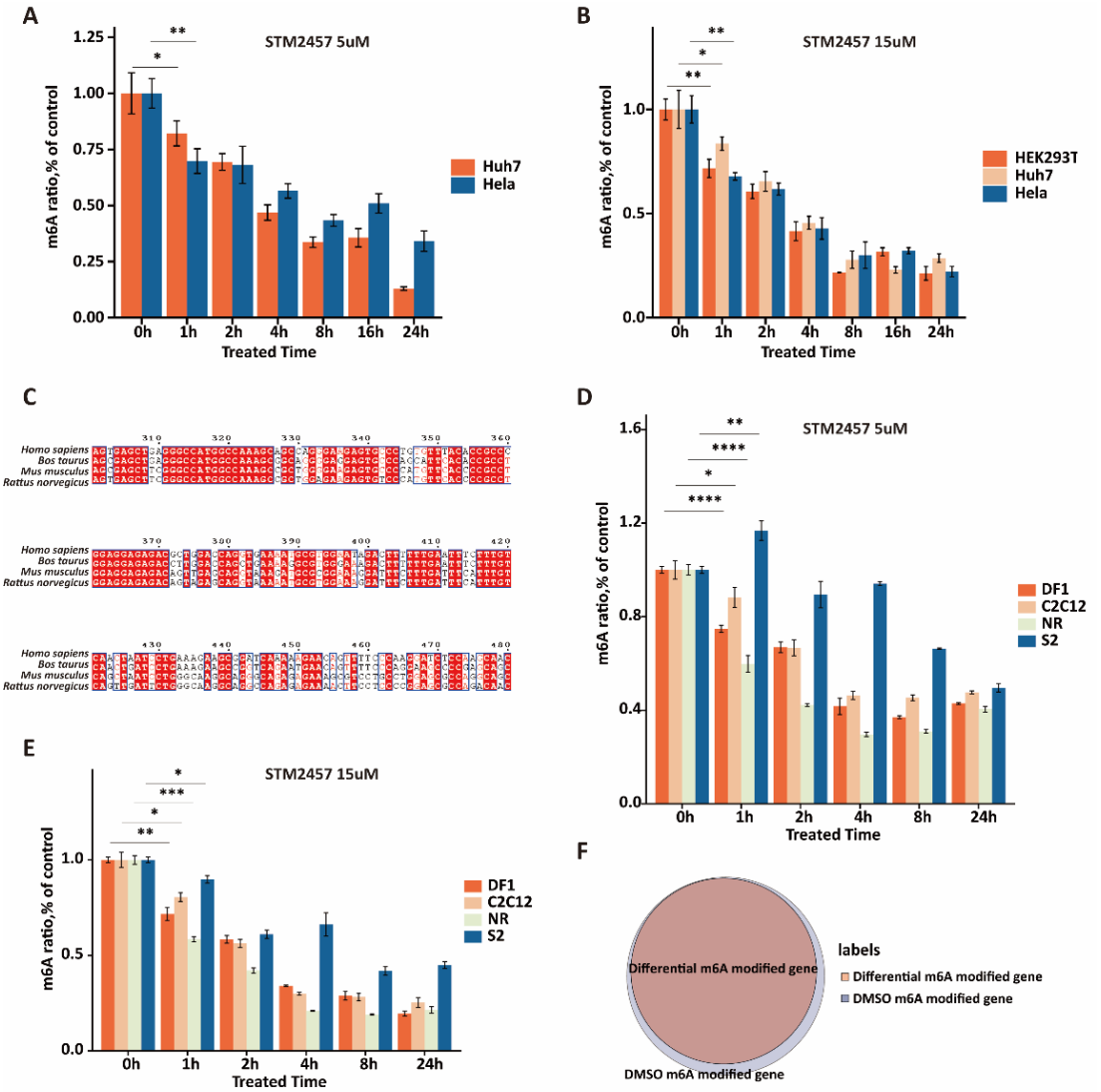
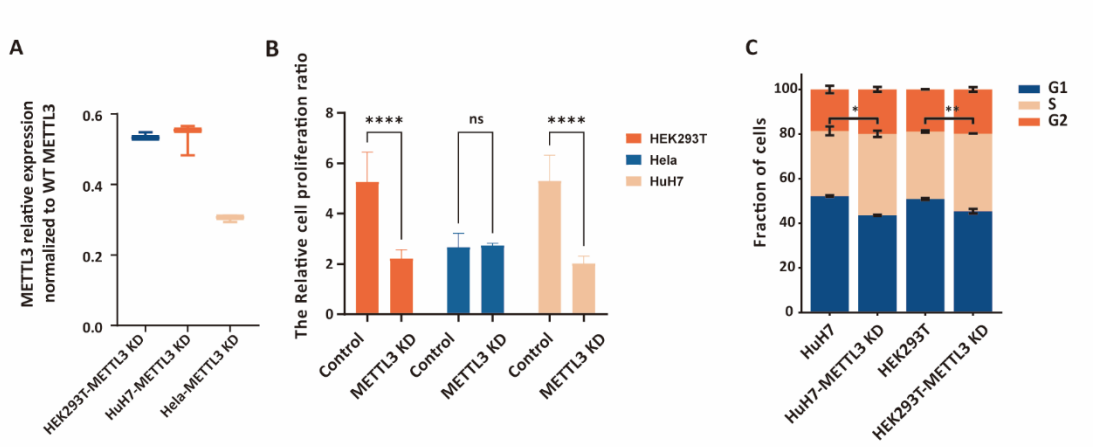
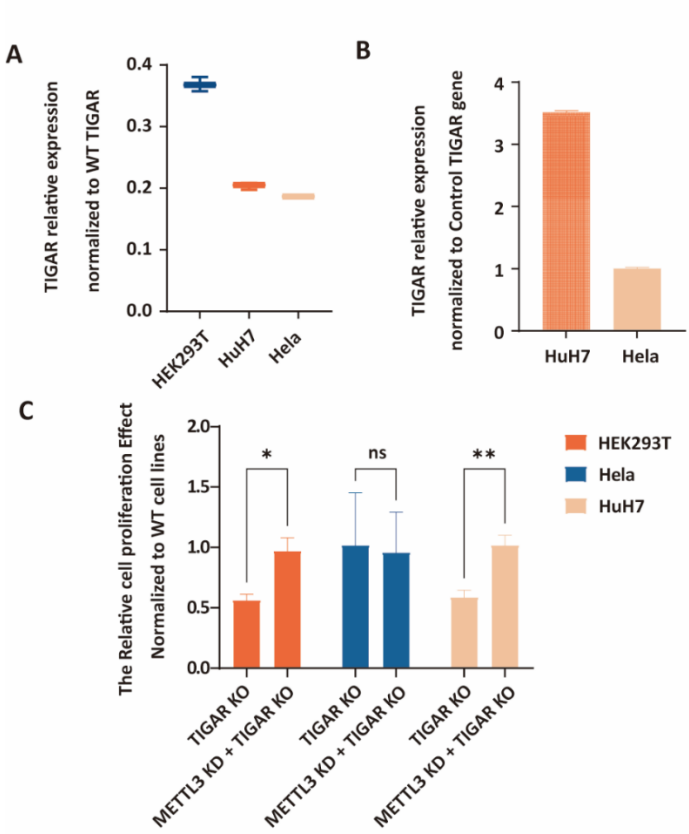


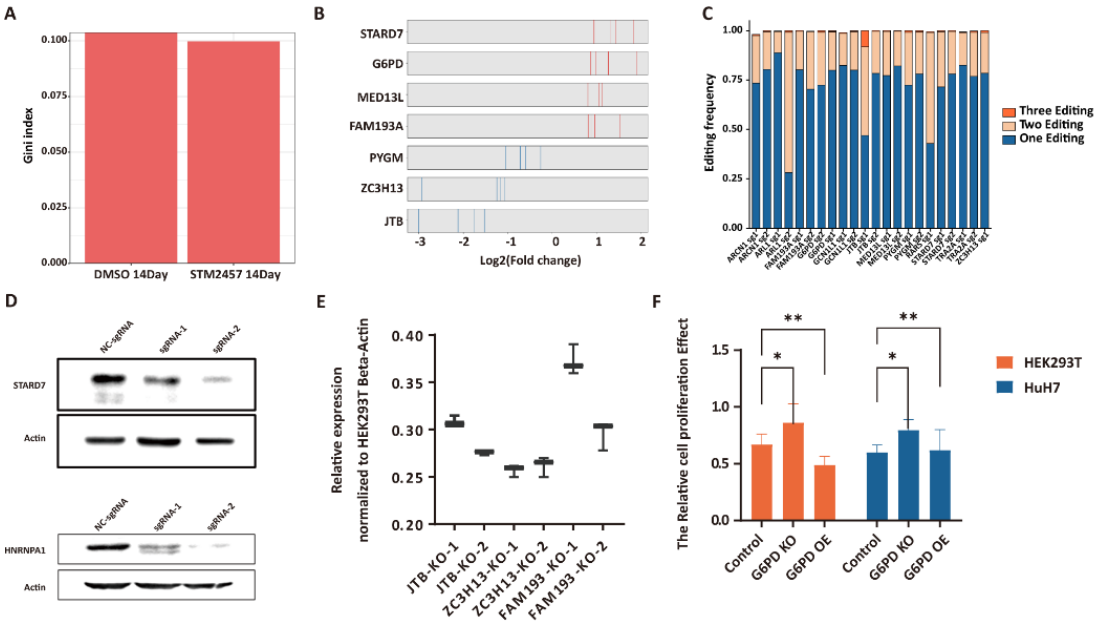
Supplementary Figure 1. STM2457 broadly inhibits m^{6}A modification across diverse cell types and species. **A.** LC-MS/MS analyses of mRNA m^{6}A levels in Hela and HuH7 cells treated with 5 μM STM2457 for the indicated times. *p < 0.01(two-tailed t test). **B.** LC-MS/MS analyses of mRNA m^{6}A levels in cells treated with 15 μM STM2457 for the indicated times. **C.** Sequence alignment of METTL3 protein sequences from human, bovine (*Bos*), mouse (*Mus*), and rat (*Rattus*). The alignment was performed using the CLUSTAL program. **D.** LC-MS/MS analyses of mRNA m^{6}A levels in cells treated with 5 μM STM2457 for the indicated times. **E.** LC-MS/MS analyses of mRNA m^{6}A levels in cells treated with 15 μM STM2457 for the indicated times. **F.** Venn diagram showing the overlap between genes with altered m^{6}A levels after STM2457 treatment and all genes with detectable m^{6}A modifications in the control.



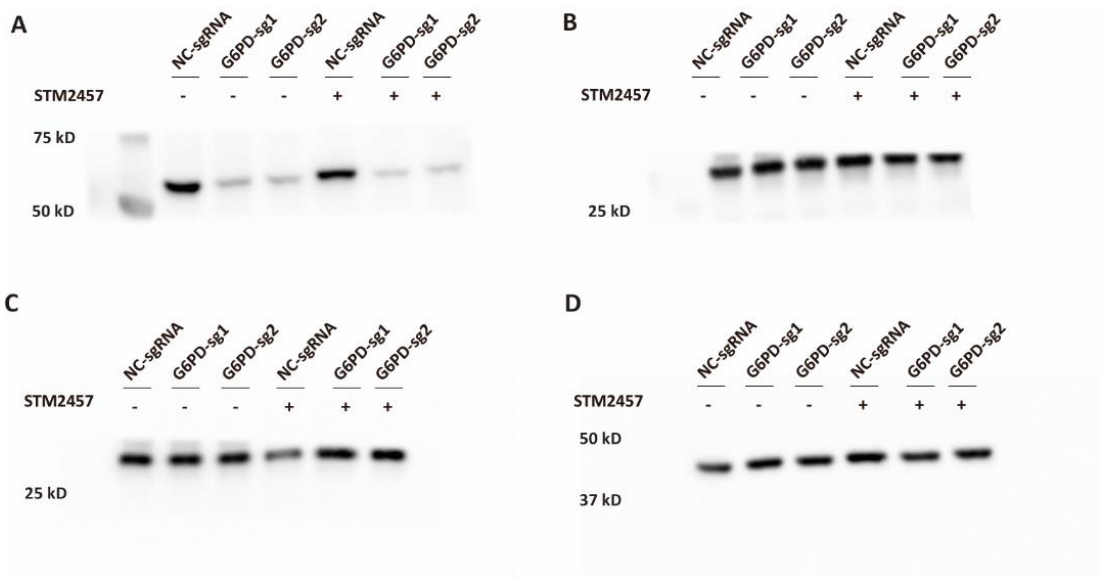
Supplementary Figure 2. **Inhibition of cell proliferation by METTL3 knockdown in multiple cell lines. A.** *METTL3* shRNA knockdown efficiency in HEK293T, HuH7, and HeLa Cell Lines**. B.** Relative cell proliferation ratio after *METTL3* knockdown. **C.** Cell cycle analysis in *METTL3* knockdown HEK293T and HuH7 cells.



Supplementary Figure 3. TIGAR mediates the anti-proliferative effects of m^{6}A reduction. A. *TIGAR* mRNA expression after CRISPR-Cas9 KO in three cell lines. **B.** Effect of *METTL3* shRNA KD on *TIGAR* gene expression in HuH7 and HeLa cells. Knockdown of *METTL3* leads to an increase in *TIGAR* gene expression in HuH7 cell lines, as indicated by quantitative RT-qPCR analysis. **C.** Impact of *TIGAR* KO and *METTL3* KD on cell proliferation. Comparative analysis of relative cell proliferation ratios in HEK293T, HuH7, and HeLa cell lines following *TIGAR* KO and *METTL3* KD.



Supplementary Figure 4. Quality control and validation of CRISPR Screen results. A. Gini index of sgRNA distribution in STM2457-treated and DMSO-treated control cells. **B.** Distribution of sgRNA counts for representative genes. Each vertical line represents a different sgRNA targeting the indicated gene. **C.** Editing efficiency of candidate gene confirmed by PCR amplification of editing sites and NGS sequencing. **D.** Western blot analysis confirming protein knockdown for the indicated candidate genes. **E.** RT-qPCR confirmation of KO efficiency for the indicated candidate genes. **F.** The effects of *G6PD* KO and overexpression for cell proliferation in HEK293T and HuH7 *METTL3* KD cells.



Supplementary Figure 5. Uncropped western blot images corresponding to Figure 5E. A. G6PD protein expression in G6PD-KO and wild-type cells treated with DMSO or STM2457. **B.** CDK2 protein expression in G6PD-KO and wild-type cells treated with DMSO or STM2457. **C.** CDK2 (T160) phosphorylation status in G6PD-KO and wild-type cells treated with DMSO or STM2457. **D.** Actin loading control for western blot analysis in G6PD-KO and wild-type cells.