

Supplementary information to: Development of Novel Epigenetic Anti-Cancer Therapy Targeting TET Proteins

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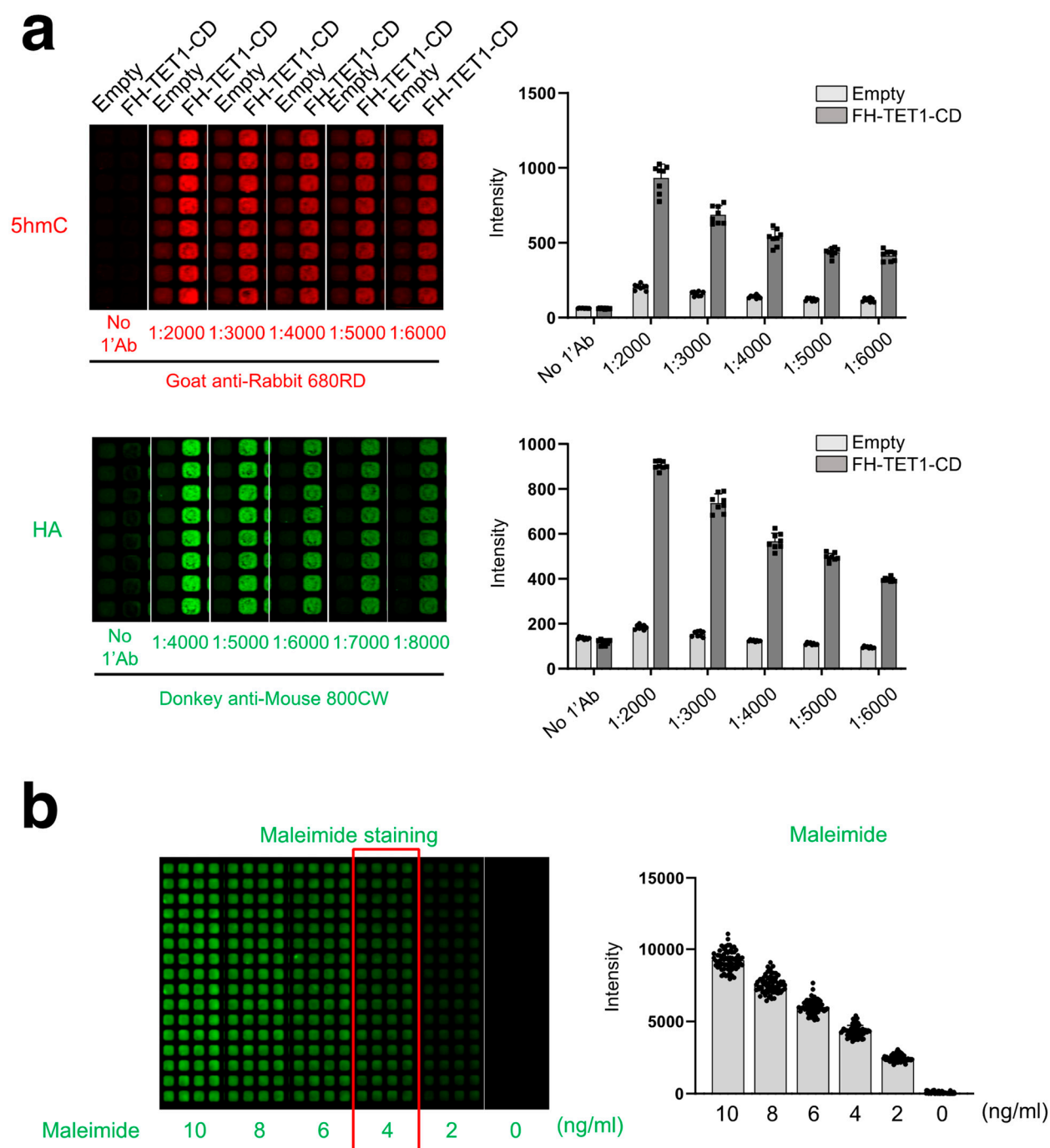


Figure S1. Optimization of the TET agonist screening system. **(a)** To optimize primary antibody titers, HEK293 cells transfected with empty vector or FH-TET1-CD were co-stained with varying concentrations of antibodies specific for 5hmC (red) and HA (green). A summary of intensities is shown on the right. **(b)** Determination of optimal concentration of maleimide. Cells were stained with sequentially diluted maleimide.

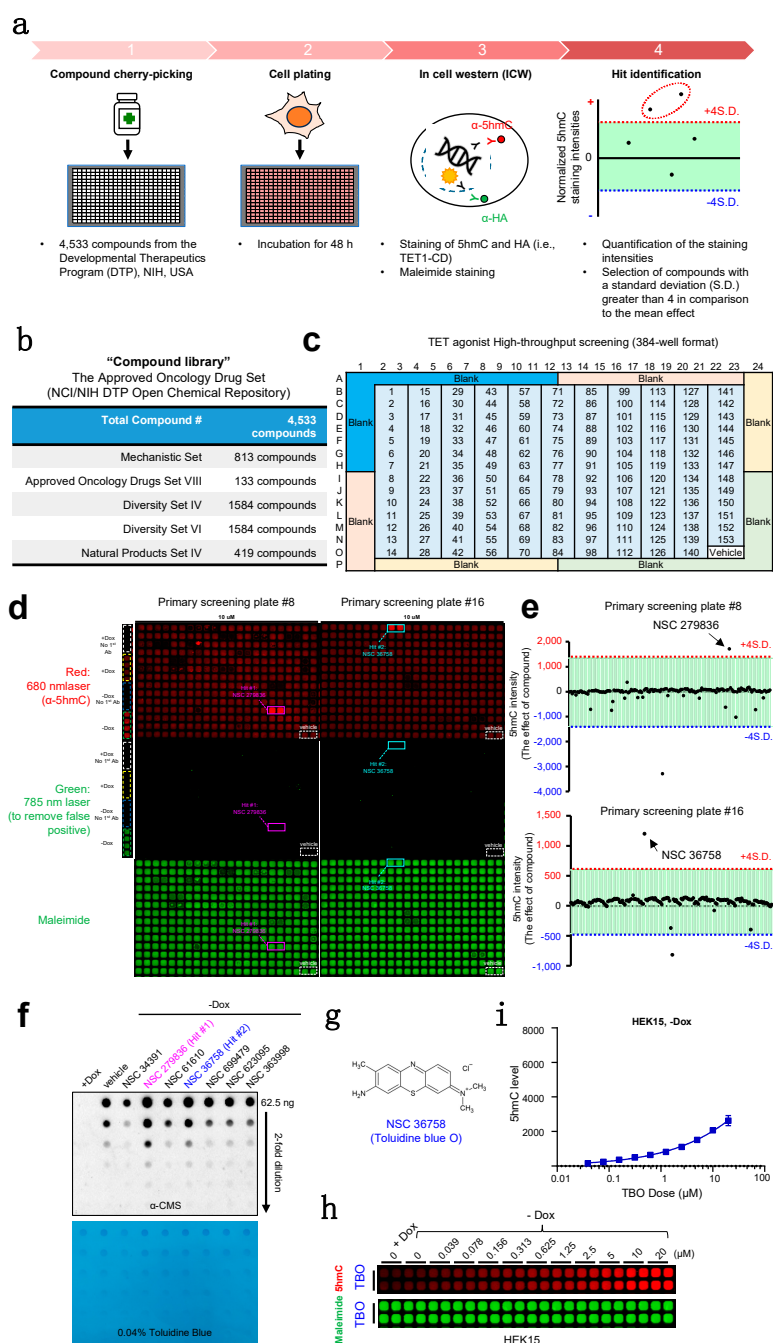


Figure S2. Identification of potential TET agonists through primary screening. **(a)** Flowchart of the screening process. **(b)** Composition of the small molecule libraries used in this study. **(c)** A representative layout in a 384-well plate. **(d)** A representative example of staining results. Two identified hits were shown in the square. **(e)** Identification of NSC 279836 and NSC36758 as potential TET agonists based on the standard deviation (S.D.) greater than 4 in comparison to the mean effect induced by the compounds in each plate. **(f)** HEK#15 cells, cultured without Dox, were treated with indicated compounds for 48 hr, followed by 5hmC quantification using anti-CMS dot blot. Toluidine blue staining was used to monitor equivalent DNA loading. **(g)** Molecular structure of NSC36758, also known as Toluidine Blue O (TBO). **(h)** Dose-dependent increase in 5hmC intensities in HEK15 cells upon treatment with NSC36758. Maleimide staining was used to monitor compound-induced cytotoxicity. **(i)** Summary of the 5hmC signal intensities from **(h)**. The 5hmC signal intensity was calculated by subtracting the mean of the +Dox group from that of drug-treated groups, which were then normalized to the mean of maleimide staining intensities.

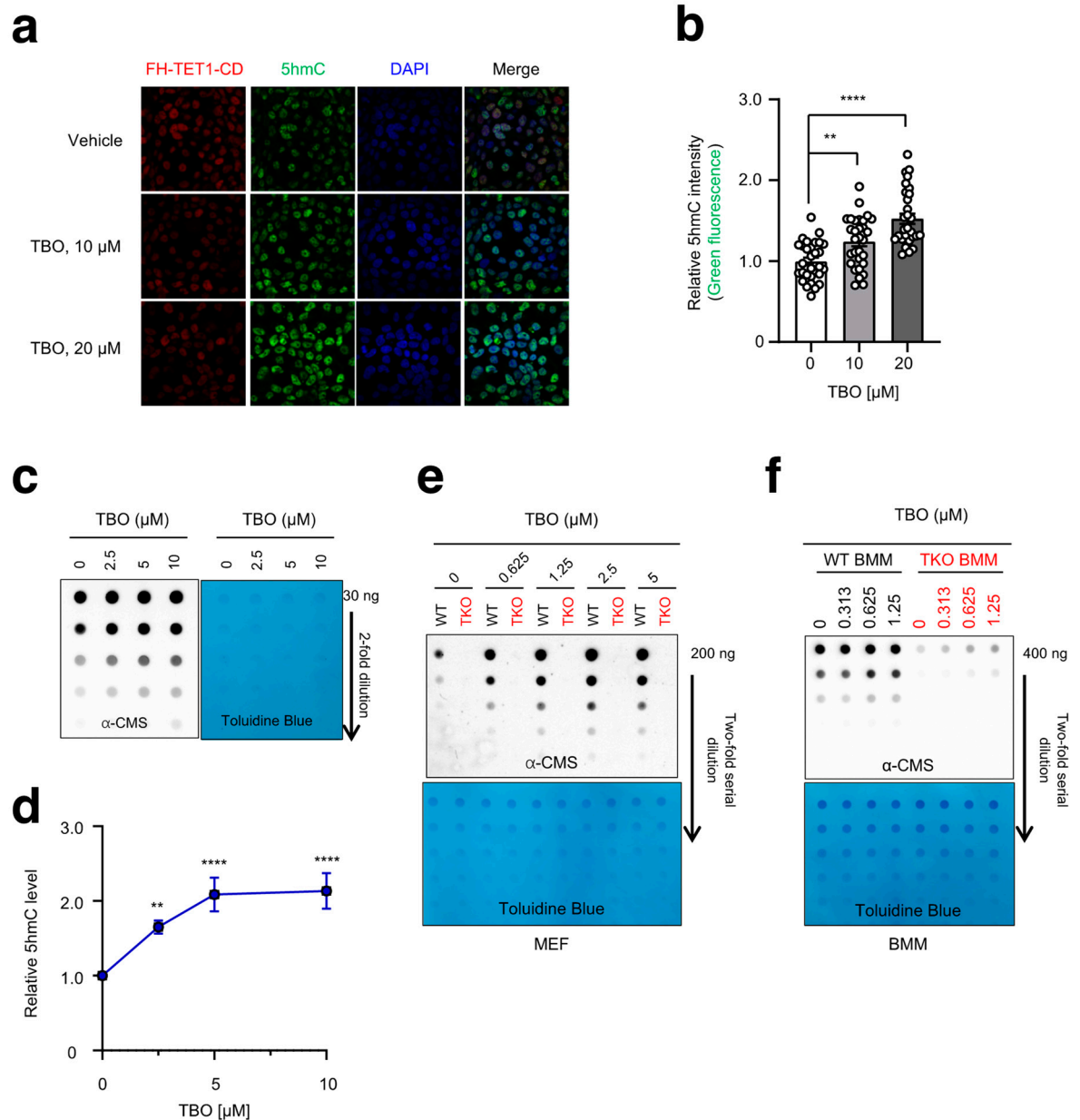


Figure S3. Toluidine Blue O (TBO) augments 5hmC levels in a TET-dependent manner. (a) Toluidine Blue O increases 5hmC by immunocytochemistry. HEK#15 cells, cultured without Dox, were treated with TBO at the indicated concentration and co-stained with antibodies specific for the HA epitope (red) and 5hmC (green). DAPI (blue) indicates nuclear staining. (b) Quantification of 5hmC intensities shown in (a). (c) Genomic DNA purified from HEK#15 cells treated with TBO as indicated was treated with bisulfite to convert 5hmC to CMS (cytosine 5-methylenesulfonate). CMS was quantified by dot blot assay using anti-CMS antibody. Toluidine blue staining was used to monitor equivalent DNA loading. (d) Summary of the 5hmC intensities shown in (c). (e, f) WT and TET TKO MEFs (e) or BMMs (f) were treated with increasing concentrations of TBO for 48 hr, followed by quantification of 5hmC by anti-CMS dot blot. Toluidine blue staining was used to monitor equivalent DNA loading. All data are presented as the mean \pm s.d. The *P*-values were determined by unpaired Student's *t*-test. ***P* \leq 0.01, ****P* \leq 0.001, and *****P* \leq 0.0001.

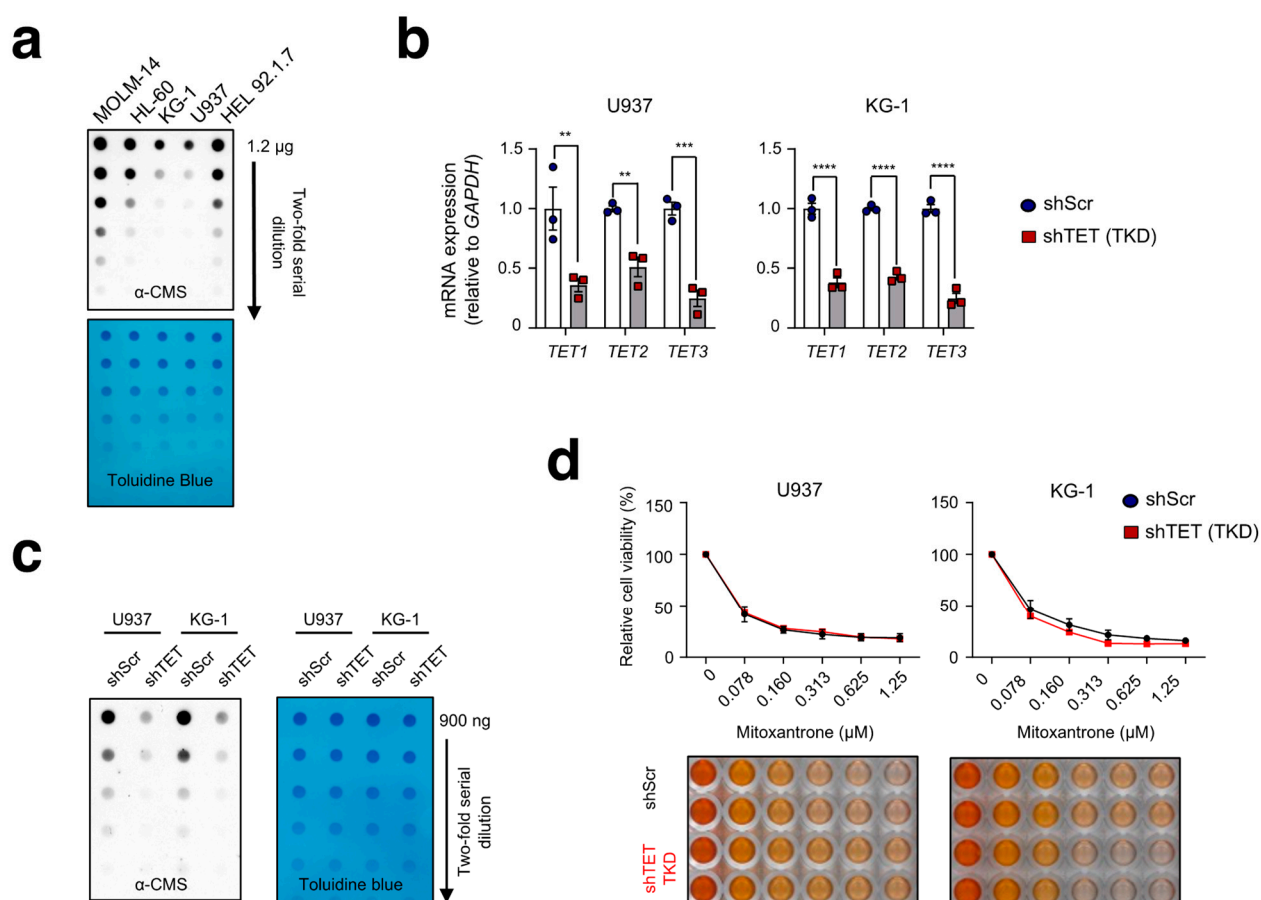


Figure S4. Mitoxantrone does not increase 5hmC levels in leukemic cell lines with low basal 5hmC levels. **(a)** Quantification of 5hmC levels in different leukemic cell lines by anti-CMS dot blot. Toluidine blue staining was used to monitor equivalent DNA loading. **(b)** Quantitative RT-PCR was performed to assess the levels of *TET1*, *TET2*, and *TET3* mRNAs relative to *GAPDH* in control and TET triple knockdown (TKD) U937 and KG-1 cells. $n = 3$. **(c)** Quantification of 5hmC by anti-CMS dot blot in cells shown in **(b)**. Toluidine blue staining was used to monitor equivalent DNA loading. **(d)** No significant effect of mitoxantrone on cell viability of U937 and KG-1 cells. Control and TET TKD U937 (left) and KG-1 (right) cells were treated with mitoxantrone at the indicated concentrations. After 48 hr, cell viability was measured with WST-8 assay. All data are presented as the mean \pm s.d. The P -values were determined by unpaired Student's t -test. $***P \leq 0.01$, $***P \leq 0.001$, and $****P \leq 0.0001$.

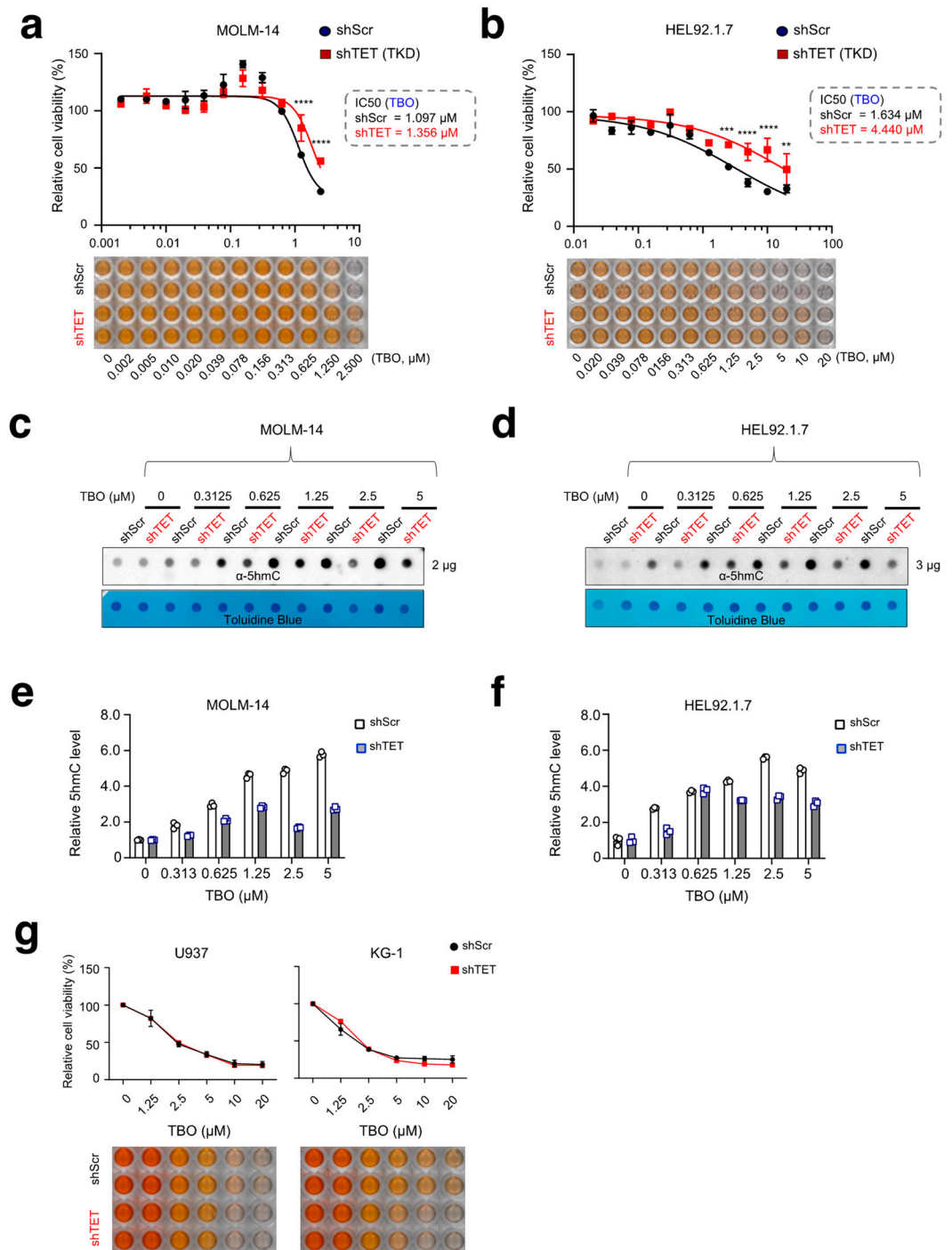


Figure S5. Toluidine Blue O (TBO) induces cancer cell death via TET activation. **(a,b)** The effect of TBO on cancer cell viability. Control and TET TKD MOLM-14 **(a)** and HEL92.1.7 **(b)** cells were treated with TBO at the indicated concentrations. After 48 hr, cell viability was measured with WST-8 assay. **(c,d)** Dose-dependent increase in 5hmC levels upon TBO treatment. Control and TET TKD MOLM-14 **(c)** and HEL92.1.7 **(d)** cells were treated with TBO at the indicated concentrations for 48 hr, followed by 5hmC quantification by anti-CMS dot blot. Toluidine blue staining was used to monitor equivalent DNA loading. **(e,f)** Summary of 5hmC levels shown in **(c)** and **(d)**. **(g)** No significant effect of TBO on cell viability of U937 and KG-1 cells. Control and TET TKD U937 (left) and KG-1 (right) cells were treated with TBO at the indicated concentrations. After 48 hr, cell viability was measured with WST-8 assay.