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Article

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# Programmable protein expression using a genetically encoded m$^6$A sensor

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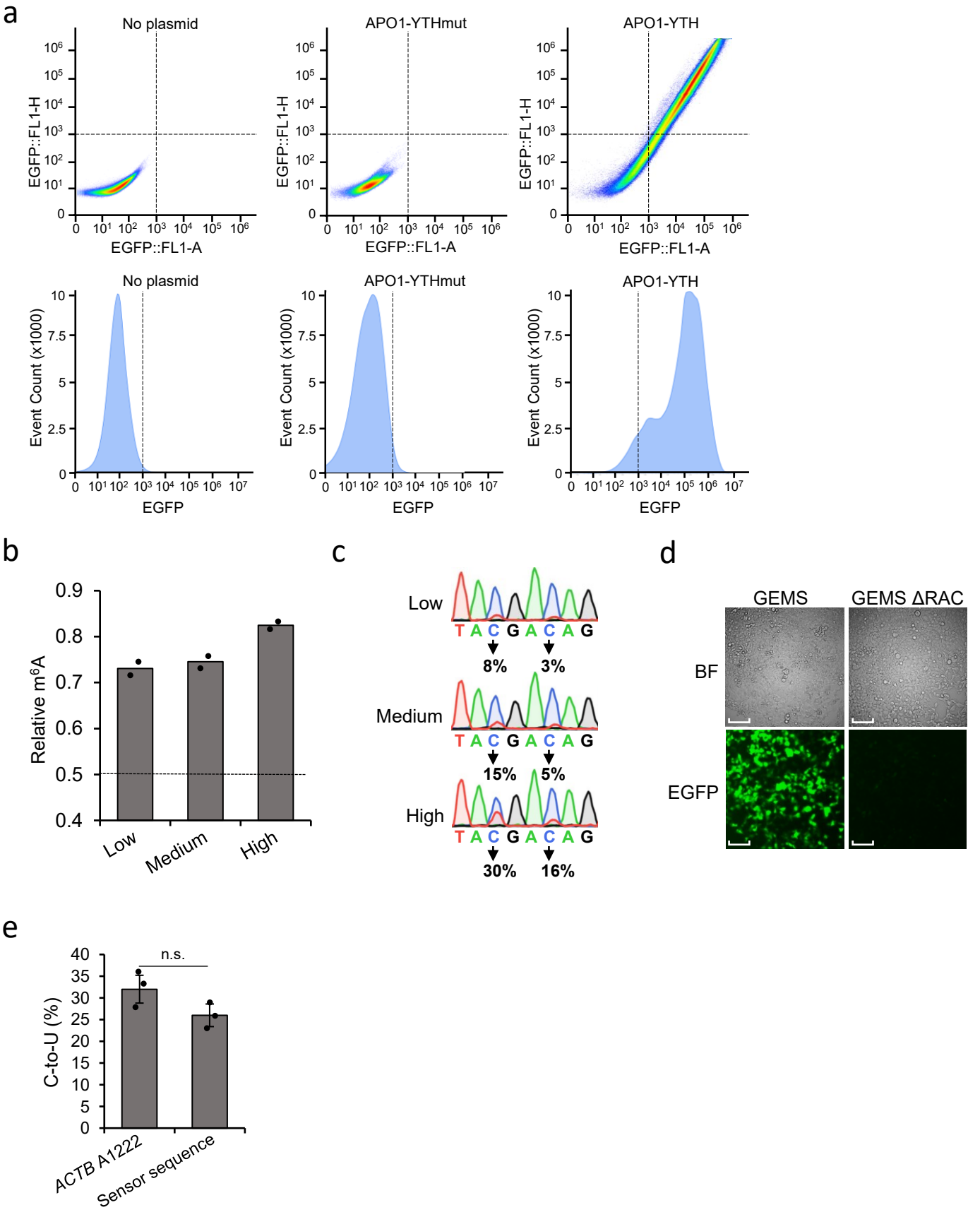
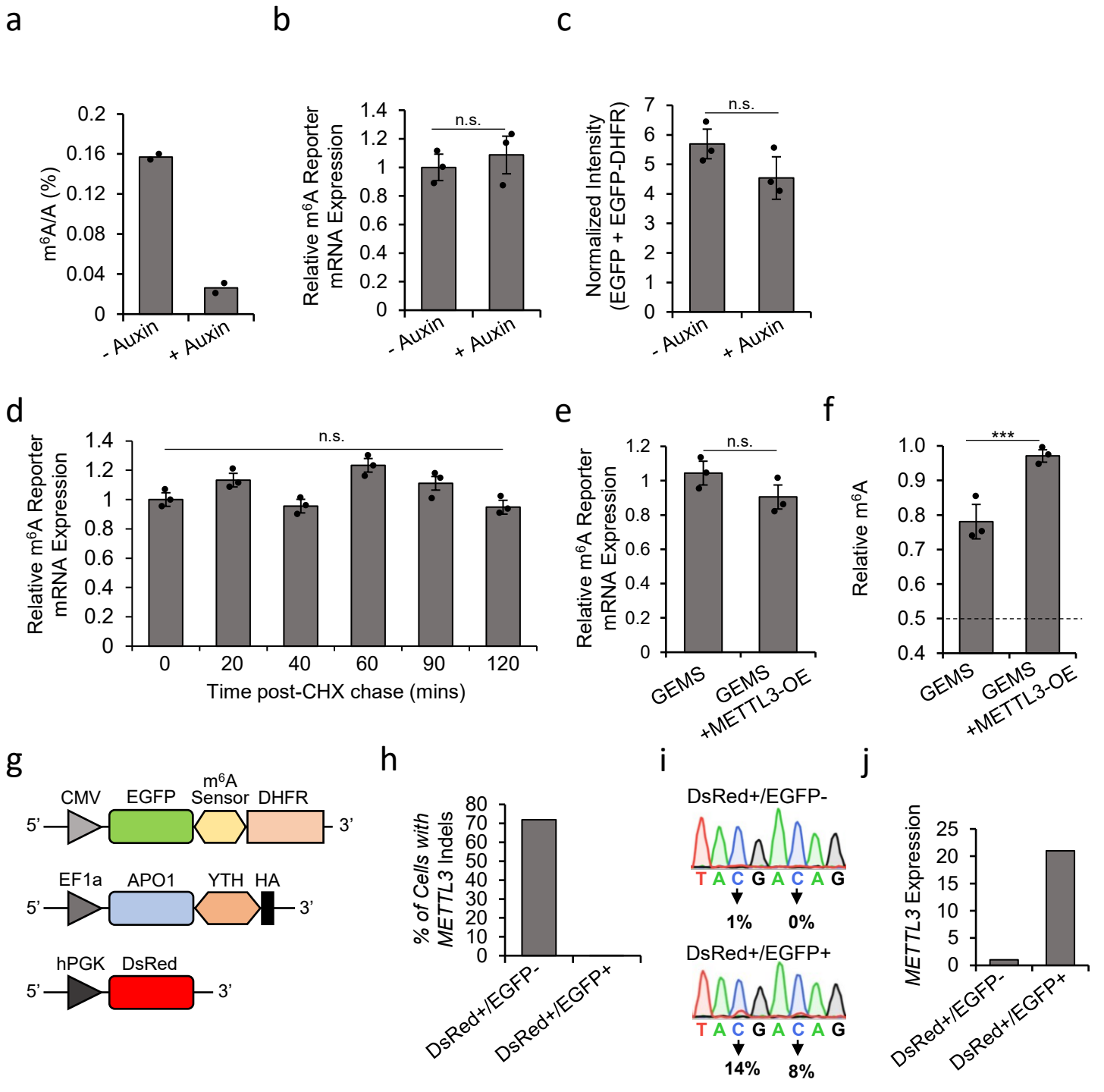
**Supplementary Figure 1**



**Supplementary Figure 1. GEMS mRNA methylation mirrors endogenous mRNA methylation.**

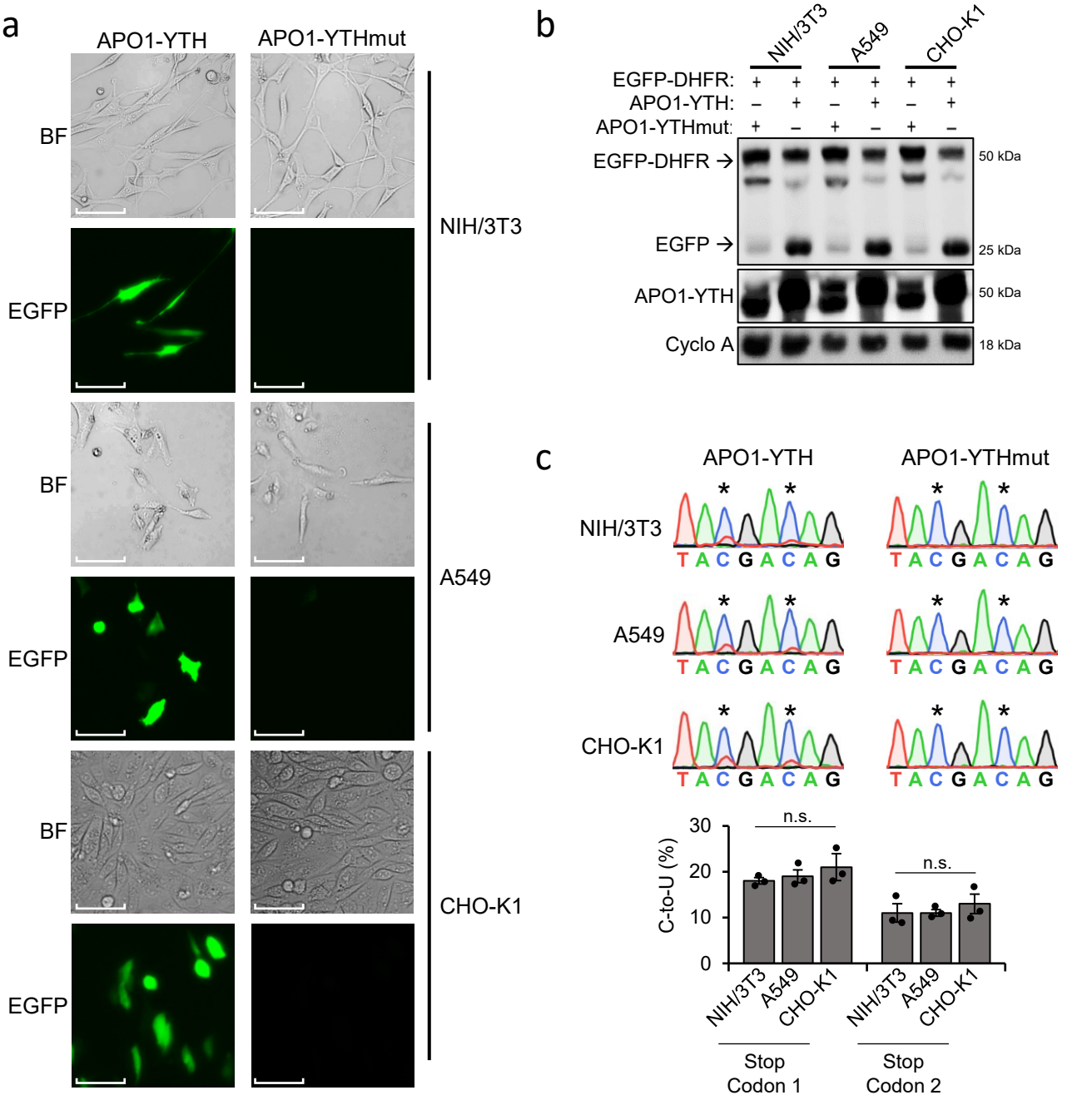
**a.** HEK293T cells were co-transfected with the m$^6$A reporter mRNA and APO1-YTH or APO1-YTHmut, then subjected to flow cytometry. Robust EGFP fluorescence is detected only in cells expressing the reporter mRNA and APO1-YTH. **b.** HEK293T cells were transfected with the GEMS system, and flow cytometry was used to sort cells into three populations based on EGFP fluorescence intensity. RT-qPCR-based m$^6$A quantification of the sensor sequence shows an increase in m$^6$A with increasing EGFP fluorescence. *n* = 2 biological replicates. **c.** RNA was isolated from sorted cell populations in (b) and analyzed by RT-PCR/Sanger sequencing. C-to-U editing of the m$^6$A sensor sequence is increased in cells with higher levels of EGFP fluorescence. **d.** HEK293T cells were transfected with GEMS containing the full length m$^6$A sensor sequence or a version with RAC motifs mutated (GEMS ΔRAC). EGFP fluorescence is abolished in cells expressing GEMS ΔRAC. *n* = 3 biological replicates. Scale bar: 100 μm. **e.** RNA was isolated from HEK293T cells expressing the GEMS system for 24 h, followed by RT-PCR and Sanger sequencing. Sequencing traces show C-to-U editing of site A1222 in endogenous *ACTB* and the second consensus A in the m$^6$A sensor sequence. n.s. = no significant difference by two-sided Student's t-test; *n* = 3 biological replicates. All data are presented as mean values ± SEM.

**Supplementary Figure 2**



**Supplementary Figure 2. The GEMS system responds to changes in METTL3 levels.**
**a.** Mass spectrometry analysis of purified mRNA from *METTL3* degron cells shows a decrease in $m^6A$ in auxin-treated cells. *n* = 2 biological replicates. **b.** RT-qPCR quantification of $m^6A$ reporter mRNA expression was performed on RNA samples extracted from *METTL3* degron cells with and without auxin treatment. n.s. = no significant difference by two-sided Student's t-test; *n* = 3 biological replicates. All data are presented as mean values ± SEM. **c.** Densitometry analysis of western blot data was used to quantify total reporter mRNA protein production (EGFP+EGFP-DHFR) relative to cyclophilin A in *METTL3* degron cells expressing the GEMS system. n.s. = no significant difference by two-sided Student's t-test; *n* = 3 biological replicates. All data are presented as mean values ± SEM. **d.** HEK293T cells were transfected with GEMS for 24 hours and then treated with cycloheximide (CHX) and collected after 0, 20, 40, 60, 90, and 120 minutes of treatment. RNA was extracted at each time point and $m^6A$ reporter mRNA abundance was measured by RT-qPCR. n.s. = no significant difference by two-sided Student's t-test; *n* = 3 biological replicates. All data are presented as mean values ± SEM. **e.** RNA was extracted from cells transfected with the GEMS system with or without simultaneous METTL3 overexpression and subjected to RT-qPCR to measure the expression of the $m^6A$ reporter mRNA. n.s. = no significant difference by two-sided Student's t-test; *n* = 3 biological replicates. All data are presented as mean values ± SEM. **f.** RNA was extracted from HEK293T cells transfected with GEMS with or without simultaneous overexpression of METTL3, followed by RT-qPCR-based $m^6A$ quantification of the $m^6A$ sensor sequence. ***$p < 0.001$ by two-sided Student's t-test; *n* = 3 biological replicates. All data are presented as mean values ± SEM. **g.** Schematic showing the main components of the GEMS plasmid with the addition of DsRed under the control of a separate promoter. **h.** HEK293T cells infected with Cas9 and either *METTL3* sgRNA or *AAVS1* sgRNA (control) were transfected with the GEMS system and subjected to flow cytometry based on EGFP and DsRed fluorescence. The proportion of cells in the indicated flow-sorted populations that contain *METTL3* indels is shown. **i.** RNA samples were prepared from cell populations sorted in (i) and subjected to RT-PCR/Sanger sequencing of the $m^6A$ sensor sequence. C-to-U editing is only detected in the EGFP+ population. **j.** RNA was isolated from sorted cell populations and analyzed by RT-qPCR for *METTL3* expression. The DsRed+/EGFP- population shows significantly lower *METTL3* expression. *n* = 2 biological replicates.

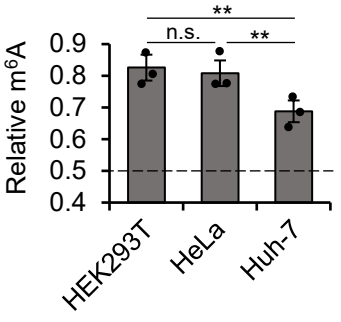
**Supplementary Figure 3**



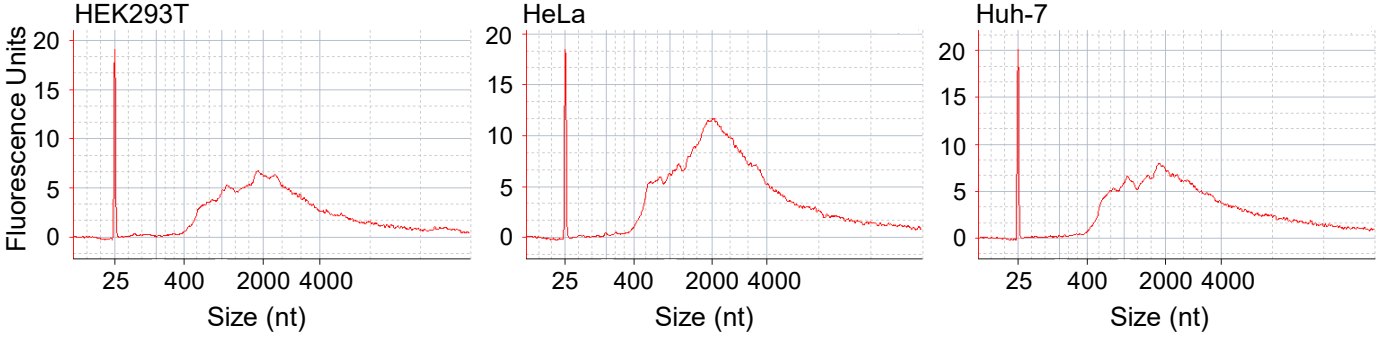
**Supplementary Figure 3. GEMS senses m$^6$A in diverse cell types.**
**a.** The GEMS system expressing APO1-YTH or APO1-YTHmut was transfected into the indicated cell types followed by fluorescence microscopy 24 h later. In all cell types, EGFP fluorescence was detected in the presence of APO1-YTH but not APO1-YTHmut, indicating m$^6$A-dependent activity of the GEMS system. Scale bar: 10 μm. **b.** Cell lysates were prepared from cells transfected as in (a) and analyzed by western blot. All cell types show decreased EGFP protein production in the presence of APO1-YTHmut. **c.** RT-PCR/Sanger sequencing analysis of the m$^6$A sensor sequence shows C-to-U editing in all cell types tested which is absent in the presence of APO1-YTHmut. Quantification of editing is shown below. n.s. = not statistically significant by two-sided Student's t-test; $n$ = 3 biological replicates. All data are presented as mean values ± SEM.

**Supplementary Figure 4**

a



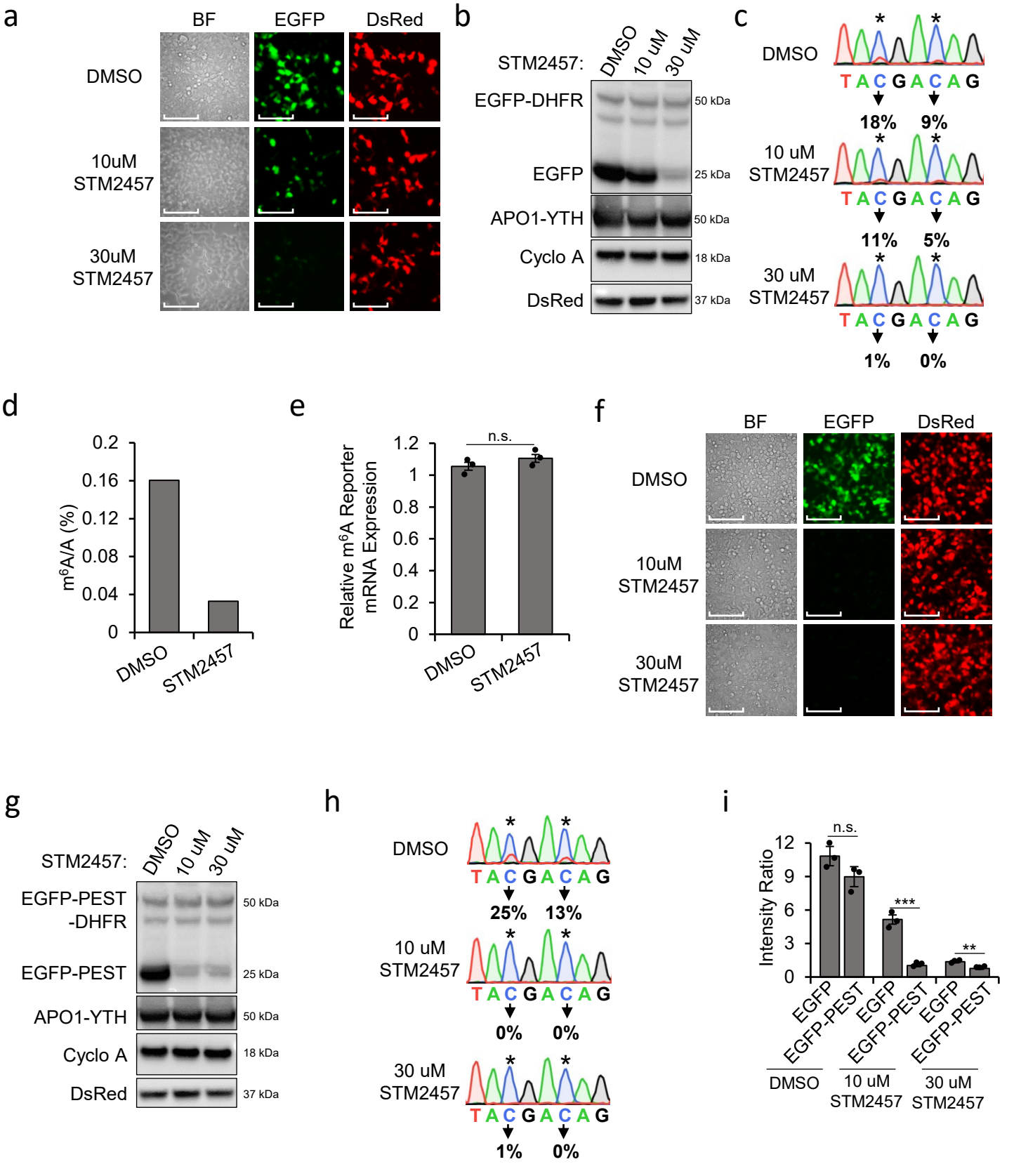

b



**Supplementary Figure 4. Differences in GEMS methylation across cell lines and confirmation of mRNA purity.**

**a.** RT-qPCR-based $m^6A$ quantification shows reduced $m^6A$ in the $m^6A$ sensor sequence in Huh-7 cells compared to HEK293T and HeLa cells. Dotted line indicates minimum $m^6A$ detection threshold. n.s. = no significant difference, $^{**}p < 0.01$ by two-sided Student's t-test; $n = 3$ biological replicates. All data are presented as mean values ± SEM. **b.** Bioanalyzer traces are shown for purified mRNA samples from HEK293T, HeLa, and Huh-7 cells that were subsequently analyzed by mass spectrometry to quantify cellular $m^6A$. The traces confirm removal of rRNA in each sample.

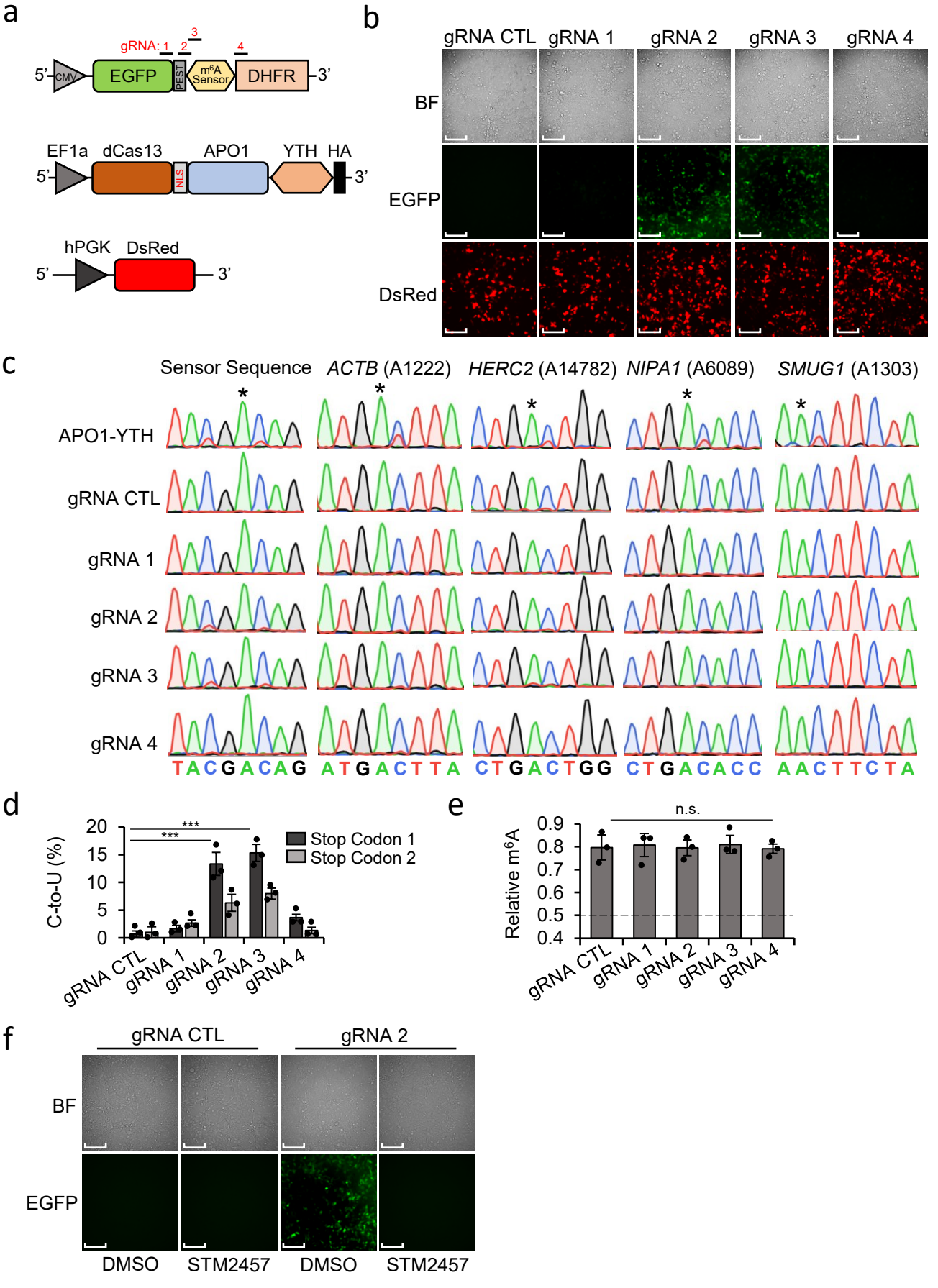
**Supplementary Figure 5**



**Supplementary Figure 5. GEMS shows a dose-dependent response to the METTL3 inhibitor STM2457.**

**a.** HEK293T cells were treated with 10 or 30 μM of STM2457 for 24 hours followed by transfection with the GEMS system containing DsRed as an $m^6A$-uncoupled internal control. Increasing amounts of STM2457 lead to increased depletion of $m^6A$-coupled EGFP fluorescence. Scale bar: 100 μm. **b.** Western blot from cells in (a) shows decreased production of EGFP protein with increasing doses of STM2457. **c.** Sanger sequencing traces (top) and quantification (bottom) of the $m^6A$ sensor sequence from cells in (a) indicates decreased editing with increasing amounts of STM2457. $n$ = 3 biological replicates. **d.** Mass spectrometry analysis of purified mRNA from cells in (a) indicates depletion of $m^6A$ following STM2457 treatment. $n$ = 2 biological replicates. **e.** RNA was extracted from cells in (a) that were treated with 30 μM STM2457 or DMSO and subjected to RT-qPCR to measure abundance of the $m^6A$ reporter mRNA. n.s. = no significant difference by two-sided Student's t-test; $n$ = 3 biological replicates. All data are presented as mean values ± SEM. **f.** HEK293T cells were treated as in (a) but transfected with a version of GEMS containing EGFP fused to a PEST destabilization domain. The EGFP signal shows greater depletion at lower doses of STM2457 compared to (a), indicating improved sensitivity of GEMS-EGFP-PEST as a readout for changes in $m^6A$ compared to GEMS-EGFP. **g.** Western blot from cells in (e) shows decreased production of EGFP protein with increasing doses of STM2457. **h.** Sanger sequencing traces of the $m^6A$ sensor sequence from cells in (e) indicates decreased editing with increasing amounts of STM2457. $n$ = 3 biological replicates. **i.** Quantification of EGFP/EGFP-DHFR ratio following STM2457 treatment of HEK293T cells expressing GEMS with EGFP or EGFP-PEST. The EGFP-PEST version shows an improved response at low doses of STM2457 compared to EGFP. ***$p < 0.001$, **$p < 0.01$ by two-sided Student's t-test; $n$ = 3 biological replicates. All data are presented as mean values ± SEM.

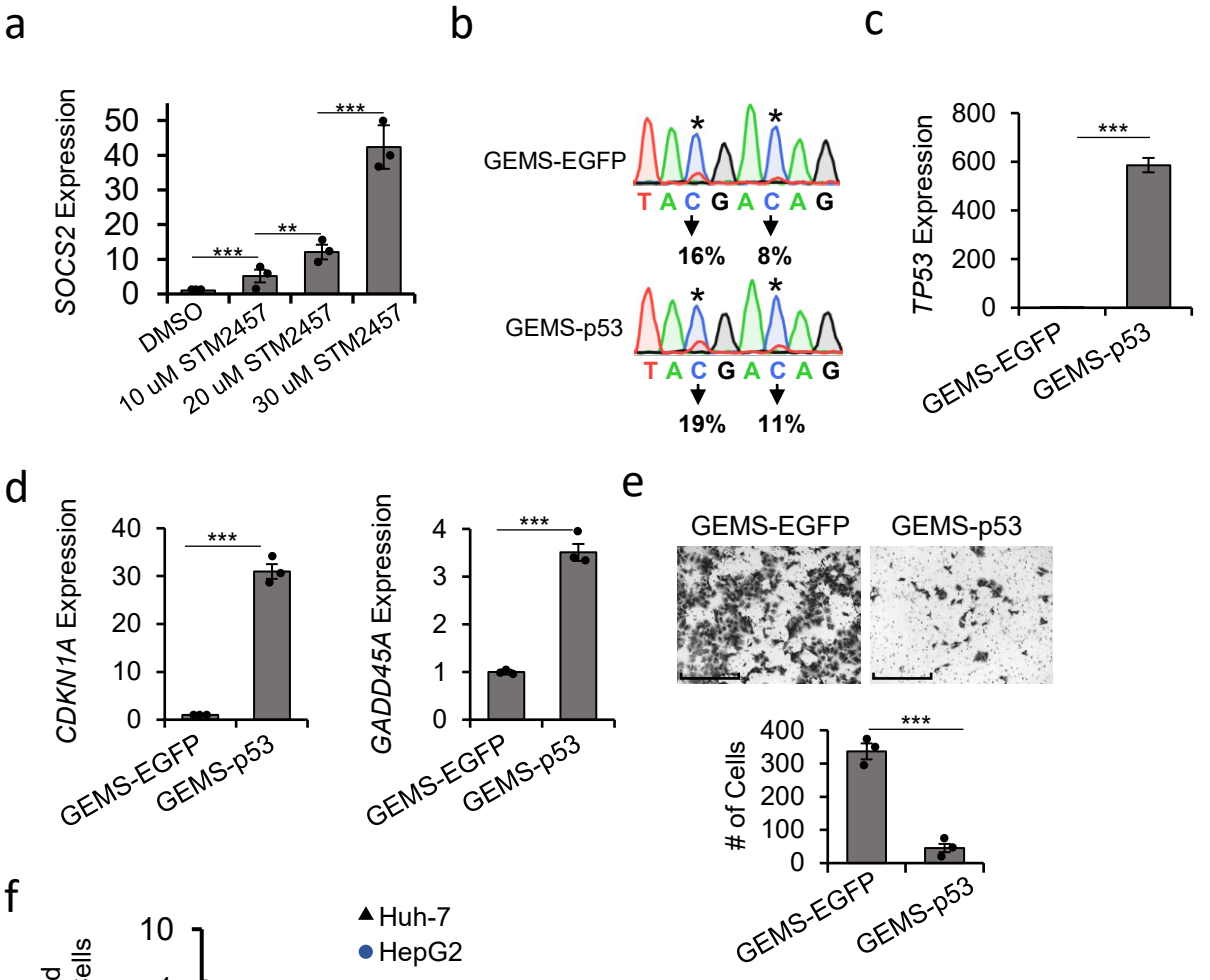
**Supplementary Figure 6**



**Supplementary Figure 6. dCas13-tethered APO1-YTH enables targeted m$^6$A sensor sequence editing.**

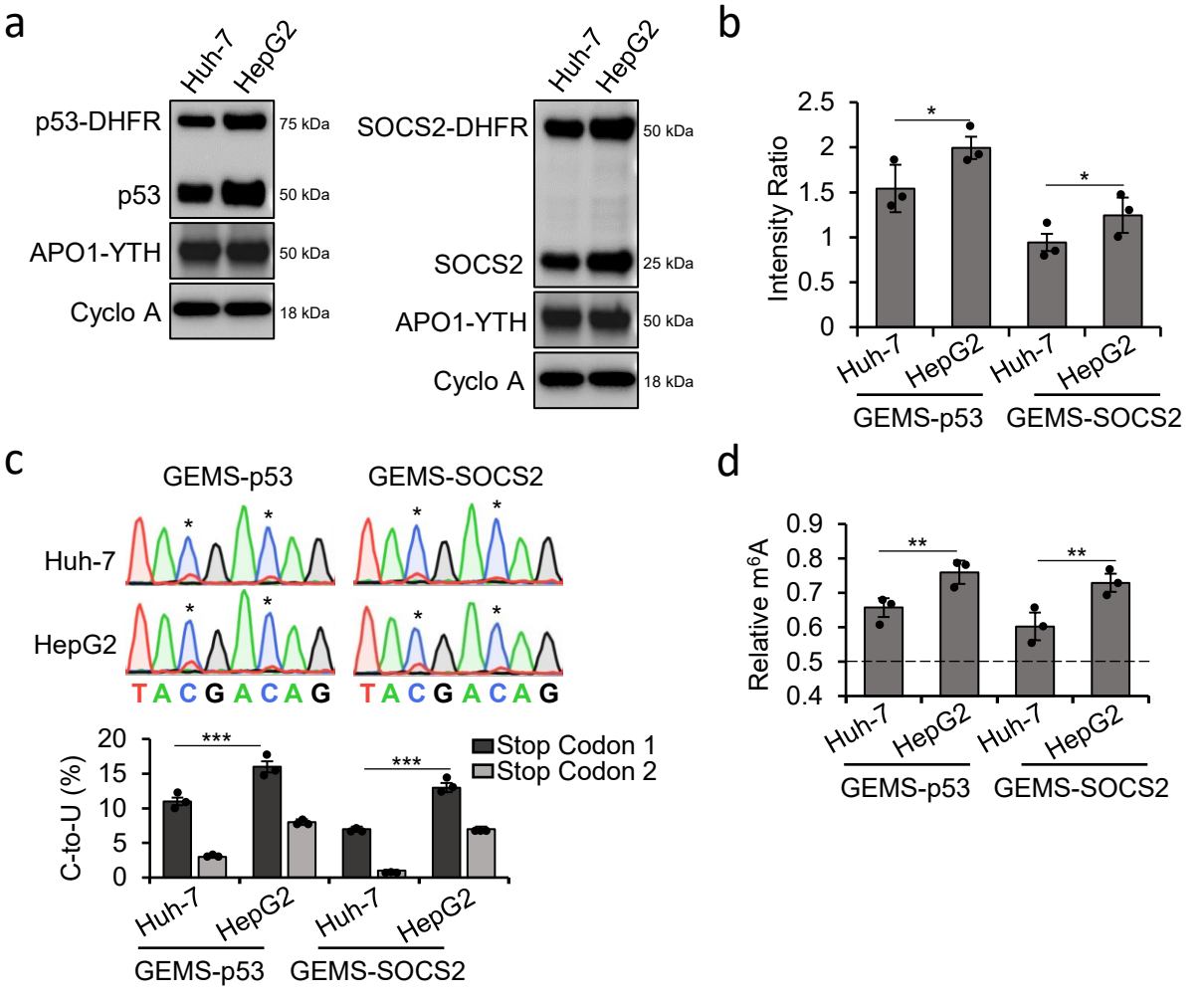
**a.** Schematic showing the main components of the GEMS system with dCas13-APO1-YTH (dCas13-GEMS). Location of regions in the m$^6$A reporter mRNA targeted by the indicated gRNAs is shown. **b.** HEK293T cells were co-transfected with dCas13-GEMS and the indicated gRNA. Only gRNAs targeting within the m$^6$A sensor sequence enable GEMS activity (EGFP fluorescence). gRNA CTL = scrambled gRNA control. Scale bar: 100 μm. **c.** RNA from cells in (b) was subjected to RT-PCR/Sanger sequencing targeting the m$^6$A sensor sequence and known m$^6$A sites in four cellular mRNAs (*ACTB* A1222, *HERC2* A14782, *NIPA1* A6089, and *SMUG1* A1303). In cells expressing dCas13-GEMS and a gRNA targeting the sensor sequence, C-to-U editing is only detected in the m$^6$A sensor sequence and not in cellular mRNAs. In contrast, cells expressing the APO1-YTH version of GEMS have editing of both the sensor sequence and cellular mRNAs. Asterisks denote m$^6$A sites. **d.** Quantification of editing at the 2 convertible stop codons within the m$^6$A sensor sequence from (c). n.s. = no significant difference by two-sided Student's t-test; $n$ = 3 biological replicates. All data are presented as mean values ± SEM. **e.** RNA was extracted from cells in (b) and RT-qPCR-based m$^6$A quantification was used to measure m$^6$A in the m$^6$A sensor sequence. n.s. = no significant difference by two-sided Student's t-test; $n$ = 3 biological replicates. All data are presented as mean values ± SEM. **f.** HEK293T cells were treated with STM2457 for 16 hours and then co-transfected with dCas13-GEMS and the indicated gRNAs. EGFP fluorescence activated by dCas13-GEMS is decreased in response to STM2457 treatment. $n$ = 2 biological replicates. Scale bar: 100 μm.

**Supplementary Figure 7**



**Supplementary Figure 7. GEMS achieves m$^6$A-coupled p53 expression in cancer cells.** **a.** Huh-7 cells treated with increasing amounts of STM2457 show a dose-dependent increase in *SOCS2* mRNA expression as measured by RT-qPCR. These data are consistent with m$^6$A-mediated inhibition of *SOCS2* abundance. ***$p < 0.001$, **$p < 0.01$ by two-sided Student's t-test; $n = 3$ biological replicates. All data are presented as mean values ± SEM. **b.** Huh-7 cells expressing GEMS-EGFP or GEMS-p53 were subjected to RT-PCR and Sanger sequencing of the m$^6$A sensor sequence. Similar C-to-U editing of target cytidines is achieved with GEMS-EGFP and GEMS-p53. **c.** RT-qPCR shows *TP53* coding sequence expression in Huh-7 cells transfected with GEMS-p53. **$p < 0.001$ by two-sided Student's t-test; $n = 3$ biological replicates. All data are presented as mean values ± SEM. **d.** RT-qPCR shows reduced expression of p53 target mRNAs *CDKN1A* and *GADD45A* in Huh-7 cells expressing GEMS-p53. ***$p < 0.001$ by two-sided Student's t-test; $n = 3$ biological replicates. All data are presented as mean values ± SEM. **e.** Brightfield images (top) and quantification (bottom) of Huh-7 cell migration measured 24 h after transfection with GEMS-EGFP or GEMS-p53. ***$p < 0.001$ by two-sided Student's t-test; $n = 3$ biological replicates. All data are presented as mean values ± SEM. Scale bar: 100 μm. **f.** Comparison of the effects of GEMS-p53 delivery into Huh-7 cells (which express mutant p53) and HepG2 cells (which express wild type p53). The number of cells following GEMS-p53 transfection relative to GEMS-EGFP transfection for each cell type across 5 days is shown. ***$p < 0.001$ by two-sided Student's t-test; $n = 3$ biological replicates. All data are presented as mean values ± SEM.

**Supplementary Figure 8**



**Supplementary Figure 8. GEMS enables tunable protein expression with m$^6$A levels.**
**a.** Huh-7 and HepG2 cells were transfected with either GEMS-p53 or GEMS-SOCS2. Western blot shows increased production of SOCS2 and p53 proteins in HepG2 cells. **b.** Quantification of p53/p53-DHFR and SOCS2/SOCS2-DHFR ratios in Huh-7 and HepG2 cells shows increased ratios in HepG2 cells compared to Huh-7 cells. GEMS-p53 *p = 0.0073; GEMS-SOCS2 *p = 0.009 by two-sided Student's t-test; $n$ = 3 biological replicates. All data are presented as mean values ± SEM. **c.** Top: Sanger sequencing traces of the m$^6$A sensor sequence from cells in (a) indicates increased editing of convertible stop codons in HepG2 cells compared to Huh-7 cells. Bottom: quantification of C-to-U editing. ***$p$ < 0.001 by two-sided Student's t-test; $n$ = 3 biological replicates. All data are presented as mean values ± SEM. **d.** RT-qPCR-based m$^6$A quantification shows increased m$^6$A in the m$^6$A sensor sequence in HepG2 cells compared to Huh-7 cells. Dotted line indicates minimum m$^6$A detection threshold. **$p$ < 0.01, *$p$ < 0.05 by two-sided Student's t-test; $n$ = 3 biological replicates. All data are presented as mean values ± SEM.