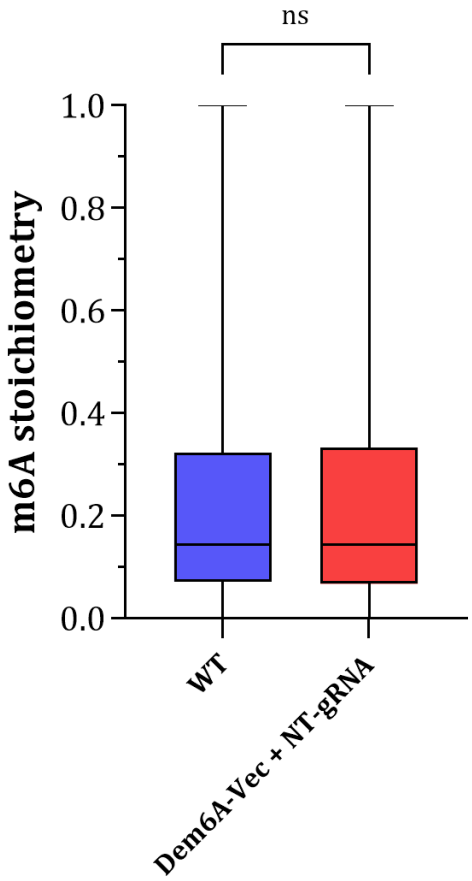
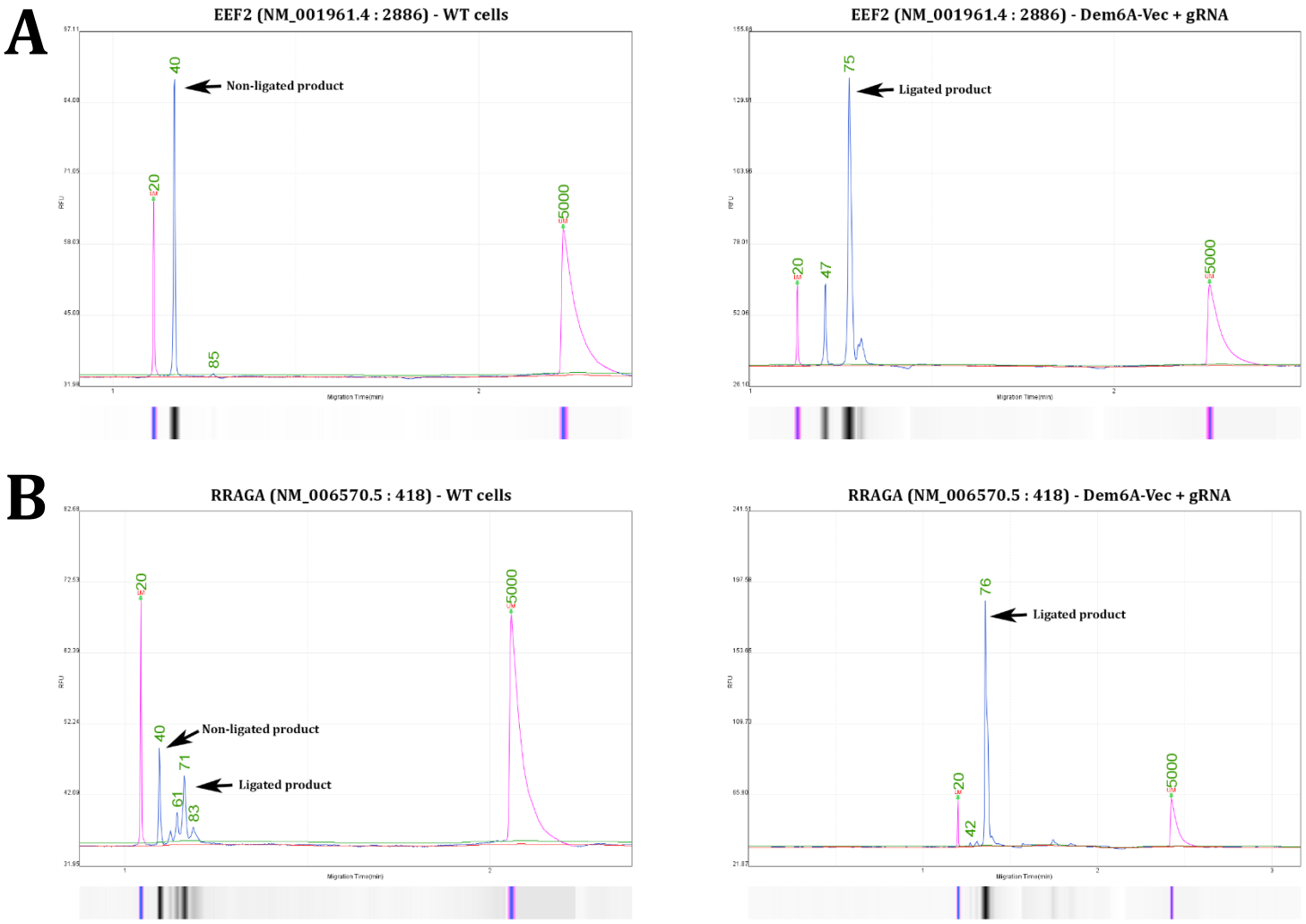
## ➢ Supplemental Figure S1



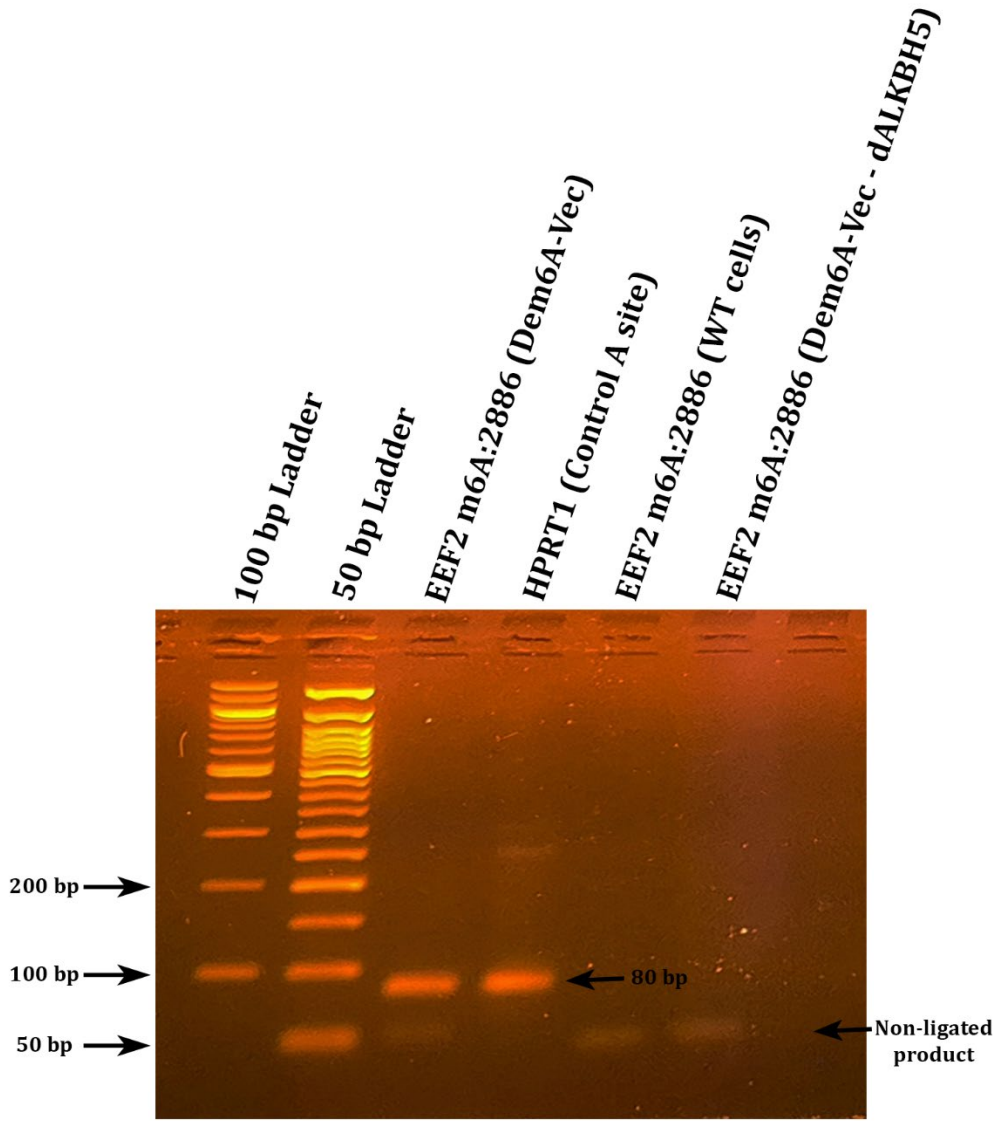

**Supplemental Figure S1**. Transcriptome-wide differential m6A analysis using CHEUI-diff between WT HeLa cells and cells transfected with Dem6A-Vec expressing a non-targeting (NT) gRNA. Direct RNA sequencing was performed on HeLa cells transfected with Dem6A-Vec carrying a non-targeting gRNA to assess potential off-target or global demethylation effects from the vector backbone. Differential methylation analysis using CHEUI-diff revealed no significant changes in m6A stoichiometry between wild-type and NT-transfected cells, indicating that the vector alone does not alter the global m6A epitranscriptome.

## ➢ Supplemental Figure S2



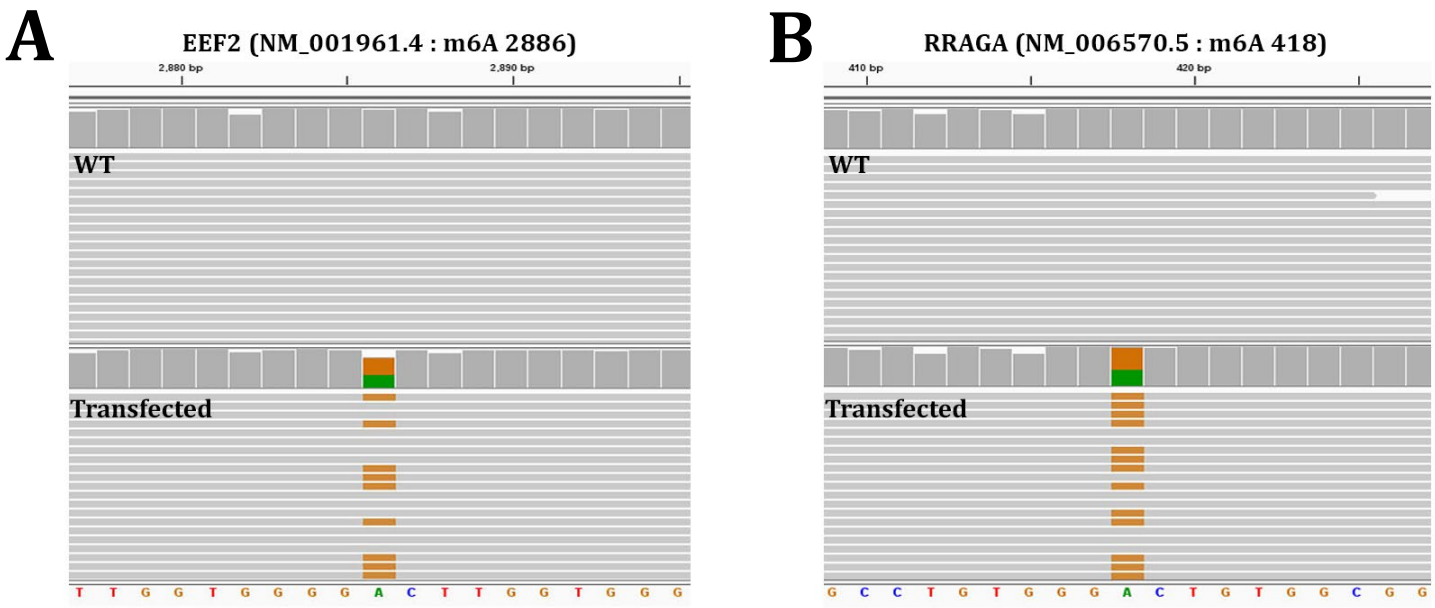

**Supplemental Figure S2**. Capillary electrophoresis of the SELECT-qPCR products targeting *EEF2* and *RRAGA* m6A sites. Capillary fragment analysis was performed on a Qsep1 automated Bio-Fragment Analyzer to validate the size and specificity of the SELECT-qPCR products for the m6A target sites of **(A)** *EEF2* (NM_001961.4 : 2886) and **(B)** *RRAGA* (NM_006570.5 : 418) mRNAs. In WT cells, only a non-ligated product was detected at ~40 bp due to the inhibitory effect of m6A on the ligation step. In contrast, HeLa cells transfected with Dem6A-Vec expressing the appropriate gRNA showed a prominent ligated product at ~75–80 bp, confirming successful demethylation and amplification.

## ➢ Supplemental Figure S3




**Supplemental Figure S3**. Agarose gel electrophoresis of SELECT-qPCR products targeting EEF2 m6A:2886. Gel electrophoresis was performed to further validate the size and specificity of the qPCR amplicons. Lanes include: 100 bp and 50 bp ladders, EEF2 m6A:2886 from Dem6A-Vec-transfected cells, HPRT1 control A site, WT HeLa cells, and cells transfected with Dem6A-Vec expressing the catalytically inactive ALKBH5 (dALKBH5). A distinct band at ~80 bp corresponding to the ligated product is observed only in Dem6A-Vec-transfected cells and the unmethylated HPRT1 control. In contrast, WT and dALKBH5 samples predominantly show a lower band (~40 bp) corresponding to non-ligated products or adapter dimers. These results confirm the demethylation-specific amplification observed in the qPCR.

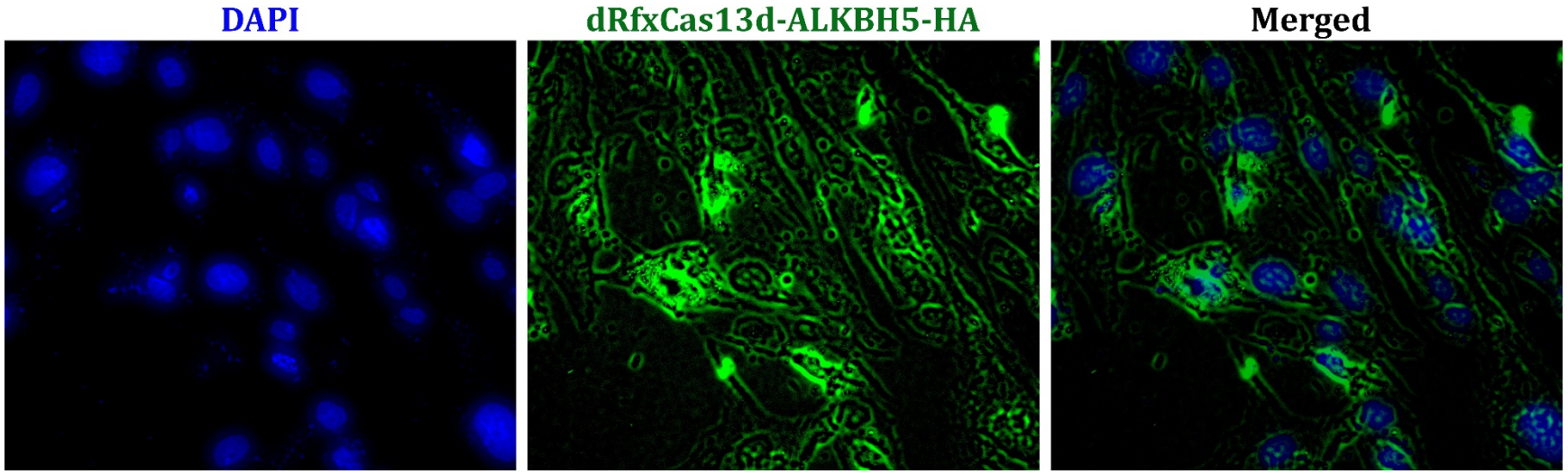
## ➢ Supplemental Figure S4



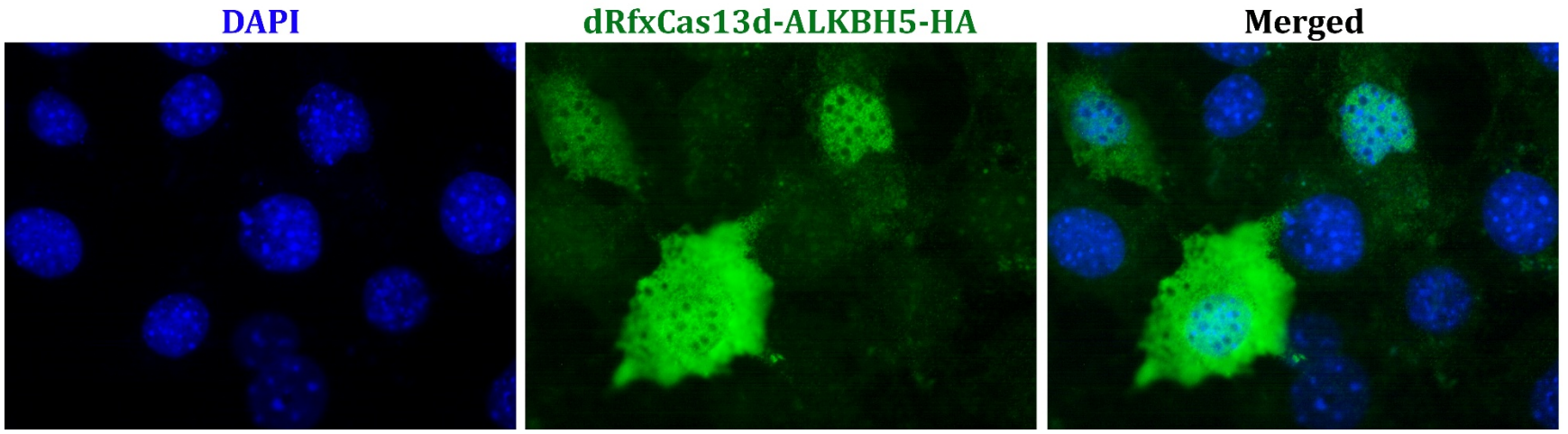

**Supplemental Figure S4**. IGV visualization of the aligned sequencing reads derived from high-throughput sequencing of GLORI-treated mRNAs in both WT and transfected HeLa cells. The mRNA samples were appropriately treated for the implementation of the targeted GLORI approach and reverse transcription was carried out with the following primer: 5' – VVVVVVVV - 3'. The generated cDNA samples were used as templates for nested PCR assays targeting a ~100bp amplicon containing the target m6A sites of **(A)** *EFF2* and **(B)** *RRAGA* mRNAs, in both WT and transfected conditions. Assessment of the RNA editing impact was carried out by high-throughput sequencing of the PCR products, alignment of the sequencing reads to the reference sequences of *EEF2* and *RRAGA* genes using minimap2 and visualization of the reads through IGV.

# Fluorescence Microscopy

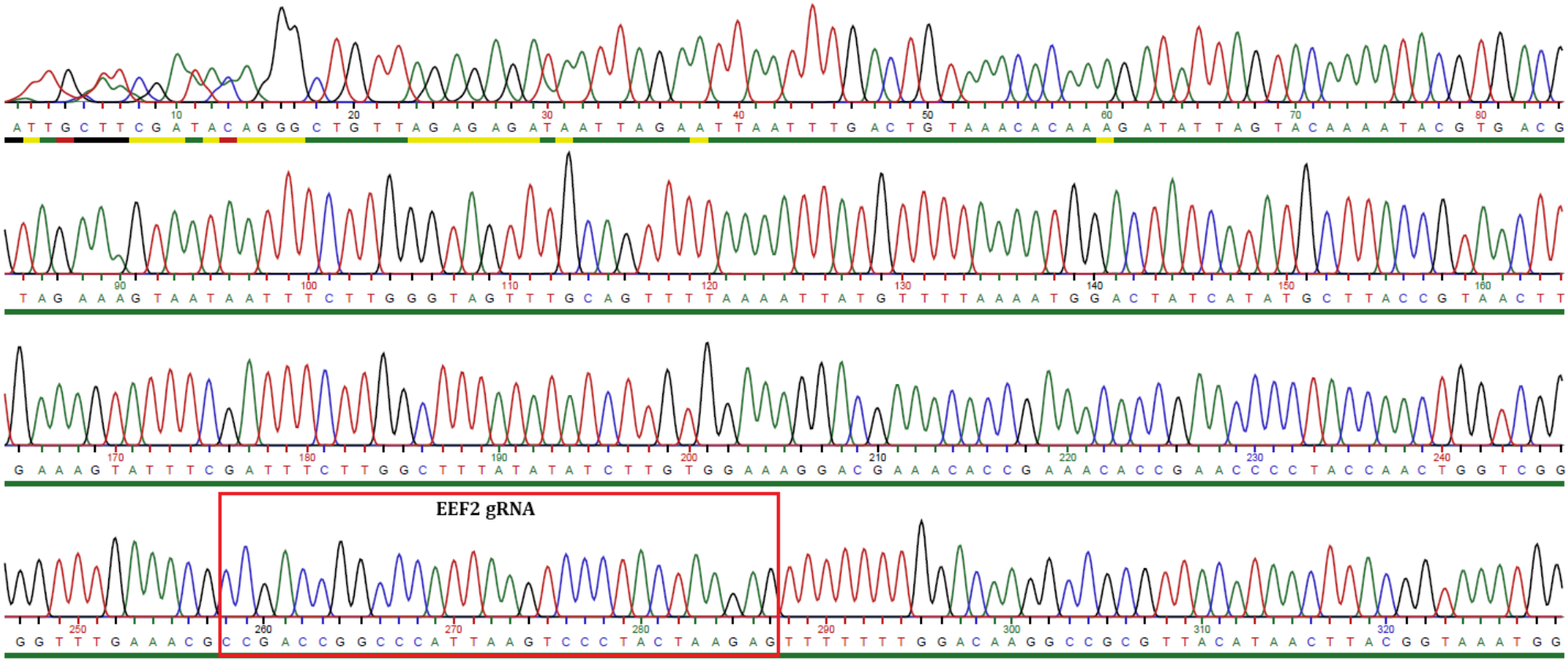
## Human BJ fibroblasts

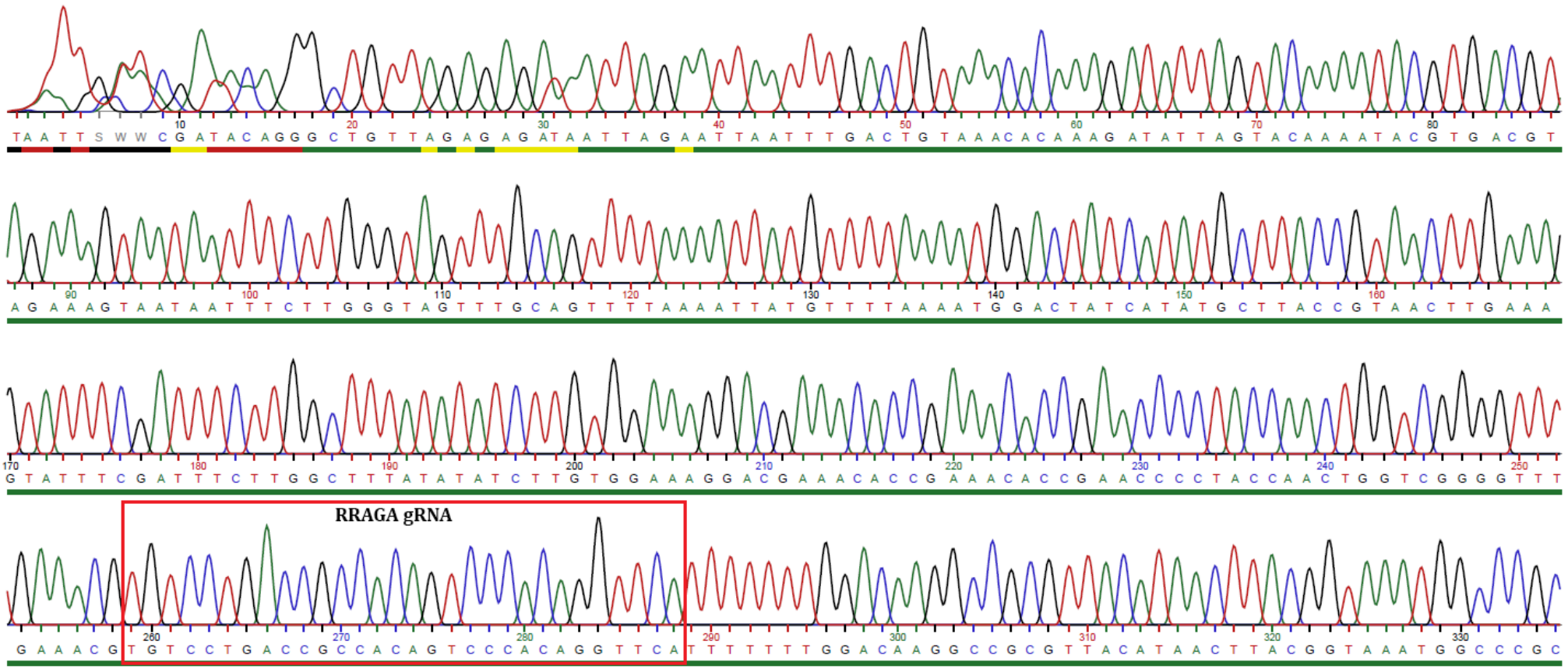



## Mouse NIH/3T3 fibroblasts



# Sanger sequencing - gRNA cloning validation




RRAGA gRNA

**Supplemental Table S2.** Primer sequences used for the gRNA cloning into Dem6A-Vec.

| Target gene Transcript:position | Primer | Primer sequence (5′→3′) |
|---|---|---|
| **EEF2** NM_001961.4:2886 | gRNA top | AAACGCCGACCGGCCCATTAAGTCCCTACTAAGAG |
| | gRNA bottom | AAAACTCTTAGTAGGGACTTAATGGGCCGGTCGGC |
| **RRAGA** NM_006570.5:418 | gRNA top | AAACGTGTCCTGACCGCCACAGTCCCACAGGTTCA |
| | gRNA bottom | AAAATGAACCTGTGGGACTGTGGCGGTCAGGACAC |
| **CDC37** NM_007065.4:1145 | gRNA top | AAACGAACAGCTTCCAGTAATGGGTCCCCAGGAC |
| | gRNA bottom | AAAAGTCCTGGGGACCCATTACTGGAAGCTGTTC |
| **LAMTOR2** NM_014017.4:332 | gRNA top | AAACGCTACACGGCCCTCCATGCAGTCCATGAGGA |
| | gRNA bottom | AAAATCCTCATGGACTGCATGGAGGGCCGTGTAGC |
| **MRPL14** NM_032111.4:476 | gRNA top | AAACGATGGGTGTCTTAATTCGTGTCCCCACAGG |
| | gRNA bottom | AAAACCTGTGGGGACACGAATTAAGACACCCATC |
| **NAGLU** NM_000263.4:2002 | gRNA top | AAACGCCAGCTGCTTGTTGGCATAGTCCAGGATGT |
| | gRNA bottom | AAAAACATCCTGGACTATGCCAACAAGCAGCTGGC |
| **PSAT1** NM_058179.4:1375 | gRNA top | AAACGAGAATTAAGGTGACATTAGGTCCCACCAGC |
| | gRNA bottom | AAAAGCTGGTGGGACCTAATGTCACCTTAATTCTC |
| **CDK2** NM_001798.5:1158 | gRNA top | AAACGTTCAGAGGGCCCACCTGAGTCCAAATAGC |
| | gRNA bottom | AAAAGCTATTTGGACTCAGGTGGGCCCTCTGAAC |

**Supplemental Table S3.** Primers used for the amplification of the *RfxCas13d* mRNA as well as the human *EEF2*, *RRAGA* and *GAPDH* genes through qPCR. Melting temperatures (Tm) were calculated based on the Primer-BLAST designing tool.

| Gene | Primer direction | Sequence (5′→3′) | Length (nt) | Tm (°C) | Amplicon size (bp) |
|---|---|---|---|---|---|
| ***EEF2*** | Forward | AGGTCGGTTCTACGCCTTTG | 20 | 60.04 | 183 |
| | Reverse | TTCCCACAAGGCACATCCTC | 20 | 59.96 | |
| ***RRAGA*** | Forward | CCAATTACATTGCTCGCGACAC | 22 | 60.79 | 171 |
| | Reverse | CCACGTTACGGAAGATATTGTCTC | 24 | 59.26 | |
| ***GAPDH*** | Forward | CCACATCGCTCAGACACCAT | 20 | 60.11 | 223 |
| | Reverse | TGACAAGCTTCCCGTTCTCA | 20 | 59.24 | |
| ***RfxCas13d*** | Forward | TCAACGCCAGGTACGTCATC | 20 | 60.11 | 121 |
| | Reverse | TTGGTGACGGAGCTGAATCC | 20 | 60.04 | |

**Supplemental Table S4.** List of the primers used for the SELECT qPCR methodology that was carried out to assess the efficiency of the targeted demethylation approach though Dem6A-Vec.

| SELECT qPCR step | Primer | Sequence (5′→3′) | Length (nt) |
|---|---|---|---|
| Ligation | EEF2_2886_up | TAGCCAGTACCGTAGTGCGTGTCCCCGACCGGCCCATTAAG | 41 |
| | EEF2_2886_down | **PHOS -** CCCTACTAAGAGGGCGTGTCCAGAGGCTGAGTCGCTGCAT | 40 |
| | EEF2_2638_up | TAGCCAGTACCGTAGTGCGTGACAATTTGTCCAGGAAGTTG | 41 |
| | EEF2_2638_down | **PHOS -** CCAGGGCAGGGATGCCTTCTCAGAGGCTGAGTCGCTGCAT | 40 |
| | RRAGA_418_up | TAGCCAGTACCGTAGTGCGTGAGGTGTCCTGACCGCCACAG | 41 |
| | RRAGA_418_down | **PHOS -** CCCACAGGTTCAGCACCAGGCAGAGGCTGAGTCGCTGCAT | 40 |
| | RRAGA_1336_up | TAGCCAGTACCGTAGTGCGTGGGGTAGCTGAGTAAATAGCG | 41 |
| | RRAGA_1336_down | **PHOS -** CCAATGTTTAAAGACTGGGGCAGAGGCTGAGTCGCTGCAT | 40 |
| | CDK2_1158_up | TAGCCAGTACCGTAGTGCGTGGTTCAGAGGGCCCACCTGAG | 41 |
| | CDK2_1158_down | **PHOS -** CCAAATAGCCCAAGGCCAAGCAGAGGCTGAGTCGCTGCAT | 40 |
| | CDC37_1145_up | TAGCCAGTACCGTAGTGCGTGGAACAGCTTCCAGTAATGGG | 41 |
| | CDC37_1145_down | **PHOS -** CCCCAGGACCTGCCTCCTCTCAGAGGCTGAGTCGCTGCAT | 40 |
| | LAMTOR2_332_up | TAGCCAGTACCGTAGTGCGTGCTACACGGCCCTCCATGCAG | 41 |
| | LAMTOR2_332_down | **PHOS -** CCATGAGGATGAATTTGAGACAGAGGCTGAGTCGCTGCAT | 40 |
| | MRPL14_476_up | TAGCCAGTACCGTAGTGCGTGGATGGGTGTCTTAATTCGTG | 41 |
| | MRPL14_476_down | **PHOS -** CCCCACAGGGTTCCCGTTGTCAGAGGCTGAGTCGCTGCAT | 40 |
| | NAGLU_2002_up | TAGCCAGTACCGTAGTGCGTGCCAGCTGCTTGTTGGCATAG | 41 |
| | NAGLU_2002_down | **PHOS -** CCAGGATGTTGCCTTCTGGCCAGAGGCTGAGTCGCTGCAT | 40 |
| | PSAT1_1375_up | TAGCCAGTACCGTAGTGCGTGAGAATTAAGGTGACATTAGG | 41 |
| | PSAT1_1375_down | **PHOS -** CCCACCAGCTTTACAGAGTTCAGAGGCTGAGTCGCTGCAT | 40 |
| | HPRT1_329_up | TAGCCAGTACCGTAGTGCGTGGCACACAGAGGGCTACAATG | 41 |
| | HPRT1_329_down | **PHOS -** GATGGCCTCCCATCTCCTTCCAGAGGCTGAGTCGCTGCAT | 40 |
| qPCR | Select F | ATGCAGCGACTCAGCCTCTG | 20 |
| | Select R | TAGCCAGTACCGTAGTGCGTG | 21 |