

Selective enrichment of A-to-I edited transcripts from cellular RNA using Endonuclease V

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Abstract: Immunoprecipitation enrichment has significantly improved the sensitivity and accuracy of detecting RNA modifications in the transcriptome. However, there are no existing methods for selectively isolating adenosine-to-inosine (A-to-I) edited RNAs. Here we show that *Escherichia coli* Endonuclease V (eEndoV), an inosine-cleaving enzyme, can be repurposed to bind and isolate A-to-I edited transcripts from cellular RNA through adjustment of cationic conditions. While Mg^{2+} is required for eEndoV catalysis, it has also been shown that similar levels of Ca^{2+} instead promote binding of inosine without cleavage. Leveraging these properties, we observe that Ca^{2+} -supplemented eEndoV is highly specific for inosine in RNA and exhibits low nanomolar binding affinity. We then demonstrate EndoVIPER (Endonuclease V inosine precipitation enrichment) as a facile and robust method to isolate A-to-I edited transcripts from cellular RNA. We envision the use of this approach as a straightforward and cost-effective strategy to enrich edited RNAs and detect A-to-I sites with improved sensitivity and fidelity.

1 Introduction

2
3 Adenosine-to-inosine (A-to-I) RNA editing is an abundant post-transcriptional
4 modification found in metazoans. Catalyzed by adenosine deaminases acting on RNAs
5 (ADARs), this reaction alters both the chemical structure and hydrogen bonding patterns
6 of the nucleobase.¹ Inosines preferentially base pair with cytidine, effectively recoding
7 these sites as guanosine. A-to-I editing is ubiquitous across most RNA types, directly
8 altering amino acid sequences of protein-coding mRNAs as well as modulating the target
9 specificities and biogenesis of small-interfering RNAs (siRNAs) and microRNAs
10 (miRNAs), in turn affecting global gene expression patterns and overall cellular behavior.
11 A-to-I RNA editing is crucial for transcriptomic and proteomic diversity, and continues to
12 be implicated in a variety of biological processes, including embryogenesis, stem cell
13 differentiation, and innate cellular immunity.²⁻⁴ Dysfunctional A-to-I editing has also been
14 linked with numerous disease progressions, including autoimmune disorders,
15 neurodegenerative pathologies, and several types of cancer.⁵⁻⁶

16
17 Sensitive and accurate identification of A-to-I sites is vital to understanding these broader
18 biological roles, relationships with disease, and regulation dynamics. Contemporary
19 mapping methods typically utilize high-throughput next-generation RNA sequencing
20 (RNA-seq). Because inosine is decoded as guanosine by polymerases, raw cDNA
21 readouts can be matched to a reference genome to detect A-G transitions as inosine
22 sites.⁷ This approach has enabled a wide survey of A-to-I locations in a variety of different
23 species and tissues, and yielded substantial insights into the overall editing landscape.⁸
24 However, this technique also requires significant investment of time and materials, and is
25 further limited in both accuracy and sensitivity. In the absence of large amounts of
26 matched high-quality RNA and DNA, it can be difficult to discriminate between true A-to-
27 I editing sites and sequencing mis-calls, transcriptional errors, or single nucleotide
28 polymorphisms (SNPs), requiring further *in vitro* assays to confirm or refute editing status.
29 However, these downstream approaches are often only applicable to cultured cells, and
30 can further introduce unintended off-target cellular changes. Moreover, despite the overall
31 high number of A-to-I editing sites across the transcriptome, with millions of loci currently
32 catalogued, inosine content is relatively low in cellular RNA, requiring large quantities of
33 material and a significant number of RNA-seq reads to achieve sufficient sequencing
34 depth and transcriptome coverage. This is further complicated by the observation that
35 editing rates at individual sites can be highly variable or conditionally active, differing
36 significantly across cell and tissue types, developmental states, and disease progression
37 stages.⁸⁻¹⁰ Additionally, many key edited RNAs are only present in low abundance,
38 yielding very few actual RNA-seq reads. In these cases, identification of A-to-I sites is
39 possible, but actual editing rates cannot be quantified, as acquiring a statistically
40 significant number of reads would require impractically large amounts of RNA or
41 excessively high numbers of RNA-seq reads. Overall, while RNA-seq allows agnostic
42 transcriptome-wide analysis, it is not ideal for probing A-to-I editing, as the vast majority
43 of the data are filtered out and discarded, and thus a significant amount of throughput,
44 depth, and coverage is wasted on unwanted RNA populations. Together, the present
45 limitations in accurately and robustly characterizing A-to-I sites and RNA editing activity
46 restricts our overall understanding of epitranscriptomic dynamics and regulation.

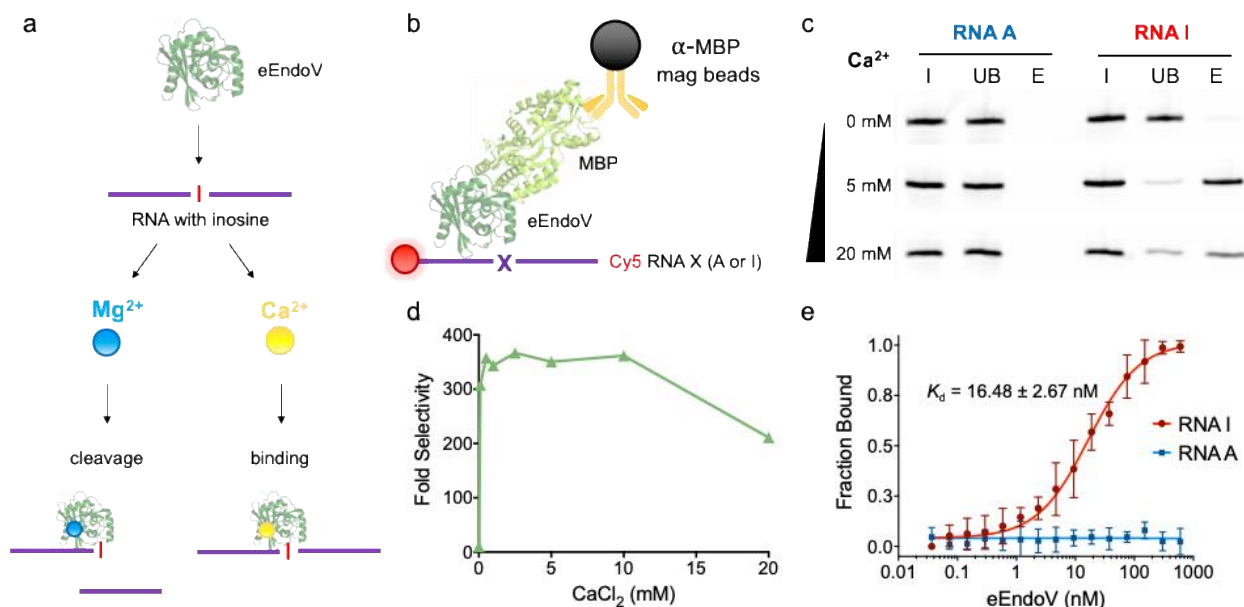
1
2 Enriching A-to-I edited transcripts from total RNA prior to analysis would largely overcome
3 these challenges by depleting unedited RNAs that otherwise lead to “wasted” sequencing
4 reads. Similar approaches for pulldown of modified bases has markedly improved the
5 throughput and reliability in detecting several other epitranscriptomic and epigenetic
6 modifications. Of note, immunoprecipitation (IP) of RNA and DNA using antibodies for *N*⁶-
7 methyladenosine and 5-methylcytosine, respectively, has significantly reduced sample
8 complexity prior to analysis, in turn drastically improving detection fidelity, sensitivity, and
9 overall throughput.¹¹⁻¹² While a previous report detailed the generation of inosine-
10 targeting polyclonal antibodies for enriching modified tRNAs, these were also found to
11 cross-react with several other nucleobases, and this method has not been reproduced.¹³
12 We previously explored chemical labeling and enrichment of inosine using an acrylamide
13 derivative, and while we demonstrated feasibility in modification and capture of inosine-
14 containing RNAs, this method also displayed off-target reactivity with pseudouridine and
15 uridine, limiting the enrichment efficiency.¹⁴ Currently, there are no robust methods for
16 the affinity purification of inosine-containing transcripts. Herein we report EndoVIPER
17 (Endonuclease V inosine precipitation enrichment) as a novel method to selectively enrich
18 A-to-I edited transcripts. EndoVIPER leverages the observation that supplementation of
19 the enzyme with Ca²⁺ promotes binding rather than cleavage of A-to-I transcripts,
20 enabling their isolation. We employ this technique in a magnetic immunoprecipitation
21 workflow, validating EndoV specificity and efficiency in binding inosine in RNA. We then
22 demonstrate EndoVIPER in enriching an A-to-I edited coding transcript from human brain
23 cellular RNA, highlighting the utility of this approach to study A-to-I RNA editing in
24 biological contexts.

25 26 **Results**

27 28 **Endonuclease V specifically recognizes inosine in RNA and exhibits robust binding** 29 **in the presence of Ca²⁺**

30
31 Due to the previously reported difficulties in developing inosine-targeting antibodies, we
32 instead searched for naturally-occurring proteins capable of recognizing and binding to
33 inosine. We identified EndoV, a highly conserved nucleic acid repair enzyme found in all
34 domains of life. In prokaryotes, EndoV is mainly responsible for detection of inosine
35 resulting from oxidative damage in DNA, and cleaves after these deamination lesions to
36 promote base excision repair.¹⁵ In humans and other metazoans, EndoV has been
37 implicated in the metabolism of A-to-I edited RNAs.¹⁶⁻¹⁷ Thus, we hypothesized that if the
38 cleavage activity could be selectively suppressed without compromising recognition and
39 binding, then EndoV could be leveraged for enriching A-to-I edited RNAs. While human
40 EndoV (hEndoV) appears to be a good candidate toward this goal, recent studies also
41 identified variable substrate preferences and possible affinity toward both unedited
42 double-stranded RNA (dsRNA) and ribosomal RNA (rRNA), properties which could be
43 problematic for use in total RNA samples.¹⁸ Interestingly, these reports also showed that
44 *Escherichia coli* EndoV (eEndoV) was both specific and highly active toward inosine in
45 single-stranded RNA (ssRNA) and exhibited minimal substrate or sequence bias.¹⁶⁻¹⁷
46 These observations, as well as the commercial availability of a purified recombinant

1 enzyme, encouraged us to explore eEndoV for the pulldown and enrichment of A-to-I
 2 edited transcripts.



3 **Figure 1. eEndoV binds inosine in RNA with high affinity and enables pulldown.** a) Mg^{2+} or
 4 Ca^{2+} supplementation modulates eEndoV activity towards inosine-containing RNA substrates. b)
 5 Schematic of EndoVIPER with a Cy5 labeled RNA using recombinant eEndoV-MBP fusion protein
 6 and anti-MBP magnetic beads. c) Representative PAGE analysis of initial (I), unbound (UB) and
 7 eluate (E) EndoVIPER fractions, illustrating the effects of Ca^{2+} supplementation on pulldown
 8 efficiency. d) Identification of optimal Ca^{2+} concentrations by comparison of pulldown efficiency
 9 for A- and I-containing RNA. e) Quantification of eEndoV binding affinity towards RNA I (red)
 10 and RNA A (blue) using MST. Values and K_d represent mean with 95% CI. (n = 3).
 11

12 Structural analyses of several orthologs have revealed that EndoV requires Mg^{2+} as a
 13 cofactor for inosine recognition and catalysis of strand scission (Fig. S1).¹⁹ Similar studies
 14 have also found that replacing Mg^{2+} with Ca^{2+} facilitates binding of EndoV to inosine
 15 substrates, yet does not support catalysis.²⁰ While the exact mechanism underlying this
 16 observation is unknown, it is likely that differences in electronics and coordination
 17 chemistry between the two metals are key factors. In any case, we hypothesized that
 18 supplementing eEndoV with Ca^{2+} would enable enrichment of inosine-containing RNAs
 19 (Fig. 1a). To test this, we synthesized a pair of Cy5-labeled oligoribonucleotides having
 20 either A or I in a defined position, and evaluated eEndoV activity in the presence of both
 21 cations. Consistent with previous reports, we observed not only specific cleavage activity
 22 towards inosine in ssRNA (RNA I) when benchmarked against a non-edited control (RNA
 23 A), but also an obligate Mg^{2+} requirement for cleavage (Fig. S2). We next sought to
 24 evaluate the effect of Ca^{2+} supplementation on the ability of eEndoV to bind and isolate
 25 inosine-containing ssRNA. Conveniently, the recombinant enzyme is genetically fused to
 26 a maltose-binding protein (MBP) tag, enabling us to design a magnetic IP workflow using
 27 anti-MBP functionalized beads, which we term EndoVIPER (Endonuclease V Inosine

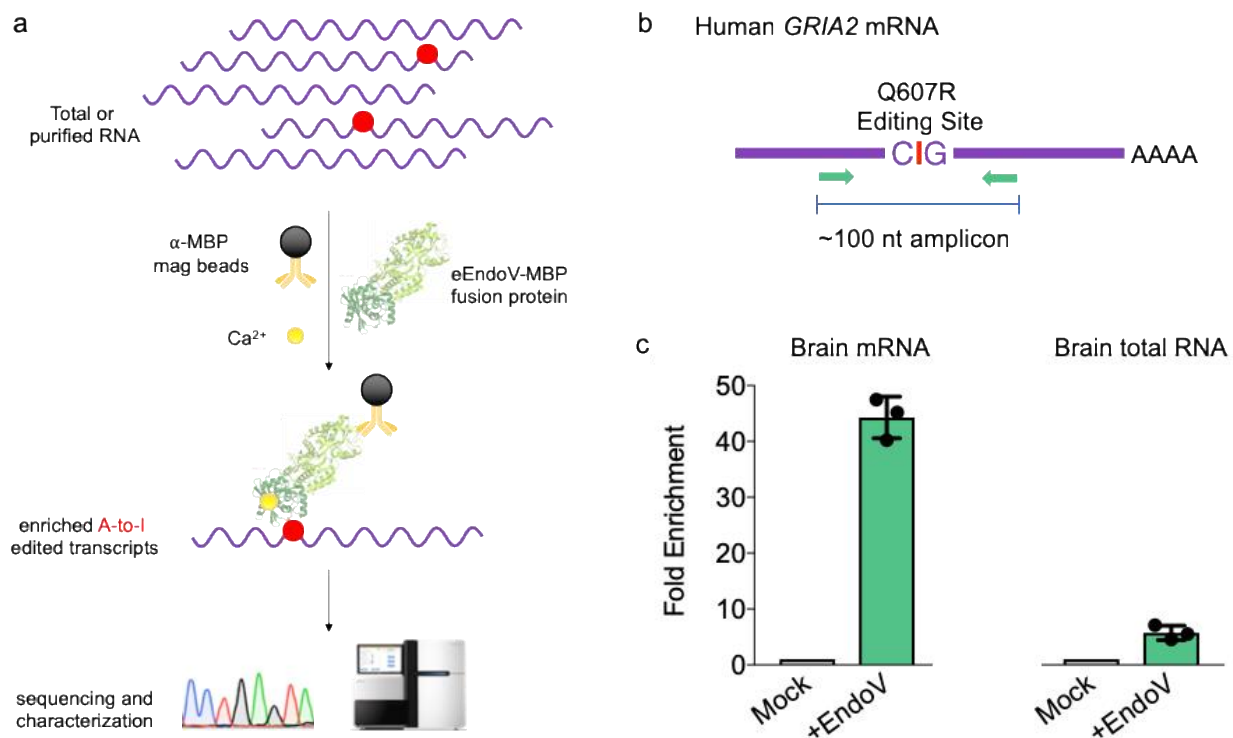
1 precipitation enrichment, Fig. 1b). Using this method, we attempted pull down of both
2 RNA A and RNA I in the presence of variable amounts of Ca^{2+} , monitoring the initial,
3 unbound (flowthrough) and elution fractions after washing (Fig. 1c, S3). Not surprisingly,
4 omitting Ca^{2+} produced little binding of either oligonucleotide, corroborating the notion
5 that Mg^{2+} aids in both recognition and cleavage of inosine substrates. Increasing amounts
6 of Ca^{2+} from 0-10 mM improved binding efficiency substantially, approaching ~80%
7 recovery with excellent selectivity (~350-fold over pulldown of RNA A). Interestingly,
8 additional supplementation beyond 10 mM Ca^{2+} quickly decreased pulldown efficiency
9 and selectivity (Fig. 1c-d, S2). While unconfirmed, these results may arise from
10 electrostatic shielding of the negative charge of the phosphodiester backbone, preventing
11 key amino acid residues from interacting with the nucleic acid substrate. Regardless, we
12 identified 5 mM Ca^{2+} as ideal for maximizing recovery and selectivity, and moved forward
13 with measuring the binding affinity of eEndoV for both RNA substrates using microscale
14 thermophoresis (MST). Consistent with our IP results, we observed low nanomolar affinity
15 for RNA I and no measurable binding to the off-target control (Fig 1e, S3).

16

17 **EndoVIPER enables enrichment of A-to-I edited transcripts from cellular RNA**

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19 Encouraged by these results, we challenged our EndoVIPER protocol to enrich a naturally
20 edited transcript from cellular RNA (Fig. 2a). To assess enrichment efficiency, we chose
21 to track the *GRIA2* mRNA, an ionotropic glutamate receptor transcript that is highly edited
22 in the brain.⁹ (Fig. 2b). We designed primers flanking the Q607R A-to-I recoding site and
23 used quantitative polymerase chain reaction (qPCR) to measure enrichment of the
24 amplicon. We observed ~45-fold enrichment of *GRIA2* from human brain mRNA
25 compared to a mock magnetic bead control. However, when we applied this method in
26 brain total RNA, we observed a nearly 10-fold drop in *GRIA2* enrichment efficiency (Fig
27 2c). We speculate this could be due to significantly higher content of other A-to-I edited
28 transcripts that were quenching our pulldown. In particular, ~7-8 human tRNAs contain
29 inosine at the wobble N34 position. Additionally, A-to-I sites have been identified in
30 ribosomal RNA (rRNA).²¹ In either case, edited tRNA and rRNA would constitute a large
31 molar proportion of our total RNA sample and potentially saturate the eEndoV binding
32 sites.²² Regardless, we still observe statistically significant enrichment (~5-fold) of *GRIA2*
33 from total RNA, demonstrating the method's compatibility and efficacy in complex RNA
34 samples. Together, these experiments show that EndoVIPER is capable of selectively
35 isolating inosine-containing transcripts with high efficiency.



1 **Figure 2. EndoVIPER enrichment of A-to-I edited transcripts from cellular RNA.** a)
2 EndoVIPER workflow for the enrichment and analysis of A-to-I edited RNAs b) *GRIA2* mRNA is
3 a naturally edited transcript containing an A-to-I recoding site, allowing detection c) Fold-
4 enrichment of *GRIA2* using endoVIPER from brain mRNA and total RNA.

7 Discussion

8
9 Herein we present EndoVIPER as a new method for the affinity pulldown of inosine-
10 containing transcripts from RNA, overcoming existing limitations in the detection and
11 characterization of A-to-I RNA editing in complex biological samples. Our approach is
12 robust, displaying high affinity and selectivity for inosine in RNA. In addition, EndoVIPER
13 exclusively utilizes low-cost, commercially available reagents with little to no modification.
14 Further, this method is compatible with both purified and total cellular RNA, and fits well
15 with existing library preparation protocols for both lower throughput analyses as well as
16 next-generation RNA-seq.

17
18 While this study establishes a novel workflow for A-to-I enrichment, there remains
19 opportunity for further improving EndoVIPER efficiency and selectivity. As described
20 previously, EndoV is present across nearly all domains of life, offering a vast array of
21 potential affinity scaffolds for use in subsequent iterations of this method. In addition, this
22 approach could be further elaborated by in-depth protein engineering and directed
23 evolution strategies to increase binding affinity and tune EndoVIPER selectivity for
24 specialized sequence contexts or sample types. Further, a wide variety of purification tags
25 and bioconjugation chemistries are available for affinity enrichment of biomolecules,

1 offering additional avenues for optimization of this workflow. We anticipate that the overall
2 ease of use and accessibility of this method will find utility in a number of empirical
3 contexts, significantly improving our understanding of the dynamics and regulation of A-
4 to-I RNA editing in a range of biological settings, in turn elucidating its role in normal
5 cellular processes and its relationship with disease pathology.

1 **Methods**

3 **RNA Oligoribonucleotides**

5 All oligonucleotides used in this study were custom designed and purchased from
6 Integrated DNA Technologies. Edited and non-edited controls were synthesized with a
7 Cyanine5 (Cy5) label at the 5' terminus as shown below.

9 RNA I 5' Cy5 AAGCAGCAGGCUIUGUUAGAACAAU 3'

11 RNA A 5' Cy5 AAGCAGCAGGCUAUGUUAGAACAAU 3'

13 **RNA Cleavage Assays**

15 10 pmol of either RNA I or RNA A was incubated in the presence or absence of both
16 Mg^{2+} at a 10 mM final concentration and/or 9pmol recombinant eEndoV (New England
17 Biolabs) in a total volume of 10 μ L. Final buffer conditions in all reactions were 10 mM
18 Tris, 125 mM NaCl, 15 μ M EDTA, 150 μ M DTT, 0.025% Triton X-100, 30 μ g/ml BSA,
19 7% glycerol, pH 7.4. Reactions were incubated for 1 hour at 25 °C, followed by a 10 min
20 heat inactivation at 85°C. Reaction products were separated using 10% denaturing
21 PAGE, and gels were imaged with a GE Amersham Typhoon RGB scanner using 635
22 nm excitation laser and the Cy5 670BP30 emission filter.

24 **EndoVIPER Magnetic IP Assays**

26 For each binding test, 10 pmol of either RNA I or RNA A was combined with 30 pmol of
27 eEndoV and variable amounts of $CaCl_2$ (0, 0.1, 0.5, 1, 2.5, 5, 10 and 20 mM) in a total
28 volume of 50 μ L. Final buffer conditions were 10 mM Tris, 125 mM NaCl, 15 μ M EDTA,
29 150 μ M DTT, 0.025% Triton X-100, 30 μ g/ml BSA, 7% glycerol, pH 7.4. Reactions were
30 incubated at 25 °C for 30 min, after which a 3 μ L sample (initial, I) was taken and set
31 aside for later analysis. Separately, 70 μ L of anti-MBP magnetic bead slurry (New
32 England Biolabs) was washed extensively with a buffer containing 10 mM Tris, 125 mM
33 NaCl, 7% glycerol, and variable amounts of $CaCl_2$ (0, 0.1, 0.5, 1, 2.5, 5, 10 and 20 mM),
34 pH 7.4. After washing, beads were resuspended in eEndoV-RNA samples and incubated
35 at 25 °C for two hours with end-over-end rotation. Magnetic field was applied to beads
36 and a 3 μ L sample (unbound, UB) of the supernatant was saved for later analysis. Beads
37 were washed extensively with respective buffers containing variable amounts of Ca^{2+} ,
38 and resuspended in 50 μ L 10 mM Tris, 125 mM NaCl, 0.5 mM EDTA, 47.5% formamide
39 0.01% SDS, pH 7.4 and heated to 95 °C for 10 min. Magnetic field was applied and a 3
40 μ L final sample (eluate, E) of the supernatant was taken of each reaction. Collected
41 fractions were analyzed using 10% denaturing PAGE, and gels were imaged with a GE
42 Amersham Typhoon RGB scanner. Densitometric quantification of bands was performed
43 using ImageJ software. % Bound is expressed as a band intensity ratio of unbound versus
44 initial fractions. % Recovered was defined as the intensity ratio of eluate versus initial
45 fractions. Fold-selectivity was calculated as the ratio of RNA I versus RNA A recovery
46 percentages.

1 **Microscale Thermophoresis (MST)**

2
3 For each binding test, varying amounts of eEndoV were combined with 6 fmol of either
4 RNA A or RNA I in a final volume of 20 μ L and allowed to incubate for 30 min at 25 $^{\circ}$ C.
5 Final buffer conditions in all samples were 10 mM Tris, 125 mM NaCl, 5 mM CaCl₂, 15
6 μ M EDTA, 150 μ M DTT, 0.025% Triton X-100, 30 μ g/ml BSA, 7% glycerol, pH 7.4. After
7 incubating, samples were loaded into NT.115 standard glass capillaries. MST
8 experiments were performed using a Nanotemper Monolith NT.115 Pico instrument. All
9 measurements were analyzed using the Pico-RED filter with 12% LED intensity and 40%
10 laser power. Data were fitted using GraphPad Prism 7 analysis software to determine K_d
11 values. Binding tests were performed in triplicate in separate trials.

12 **qPCR measurement of EndoVIPER enrichment**

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14
15 1 μ g brain mRNA (Takara Bio) or 10 μ g of brain total RNA (Thermo Fisher Scientific) was
16 briefly heated to 95 $^{\circ}$ C and incubated in the presence or absence (mock) of 30 pmol of
17 eEndoV in a volume of 50 μ L. Final buffer conditions were 10 mM Tris, 125 mM NaCl, 5
18 mM CaCl₂, 15 μ M EDTA, 150 μ M DTT, 0.025% Triton X-100, 30 μ g/ml BSA, 7% glycerol,
19 pH 7.4. eEndoV-RNA reactions were allowed to incubate at 25 $^{\circ}$ C for 30 min. Separately,
20 70 μ L of anti-MBP magnetic bead slurry (New England Biolabs) was washed extensively
21 with a buffer containing 10 mM Tris, 125 mM NaCl, 5 mM CaCl₂, and 7% glycerol, pH 7.4.
22 After washing, beads were resuspended in eEndoV-RNA samples and incubated at 25
23 $^{\circ}$ C for two hours with end-over-end rotation. Beads were then washed extensively with
24 10 mM Tris, 125 mM NaCl, 5 mM CaCl₂, and 7% glycerol, and resuspended in nuclease
25 free water. Beads were heated to 95 $^{\circ}$ C for 20 min. Magnetic field was applied and the
26 supernatant was collected, 0.2 μ m filtered, and ethanol precipitated. The RNA pellets
27 were then resuspended in 6 μ L nuclease free water, mixed with 20 pmol of GRIA2 reverse
28 primer (5' CCACACACCTCCAACAATGCG 3'), heated to 70 $^{\circ}$ C for 10 min, and cooled
29 on ice for 5 min. First strand cDNA synthesis was performed for one hour at 42 $^{\circ}$ C using
30 M-MuLV Reverse Transcriptase (New England Biolabs). Reactions were then mixed with
31 10 pmol of GRIA2 forward primer (5' GGGATTTTAAATAGTCTCTGGTTTTTCCTTGGG 3')
32 and reverse primer and 1X iTaq Universal SYBR Green Supermix (BioRad). qPCR was
33 monitored with a Roche Lightcycler 96 instrument, using the following PCR program: 94
34 $^{\circ}$ C for 3 min, followed by 45 cycles of (94 $^{\circ}$ C for 15 s, 60 $^{\circ}$ C for 30 s, 68 $^{\circ}$ C for 30 s), 68
35 $^{\circ}$ C for 5 min. Fold enrichment was determined from raw threshold cycle (C_t) values and
36 defined as $(2^{-\Delta\Delta C_t})$, where $\Delta\Delta C_t = (C_t \text{ EndoVIPER}) - (C_t \text{ mock})$. All mock and EndoVIPER
37 pulldowns were performed independently in triplicate. PCR products were purified using
38 the Monarch PCR and DNA cleanup kit (New England Biolabs), and electrophoresed on
39 1% agarose gel to verify amplicon size.

References

1. Bass, B. L., RNA editing by adenosine deaminases that act on RNA. *Annu Rev Biochem* **2002**, *71*, 817-46.
2. Nishikura, K., A-to-I editing of coding and non-coding RNAs by ADARs. *Nat Rev Mol Cell Biol* **2016**, *17* (2), 83-96.
3. Kawahara, Y. Z., Boris ; Sethupathy, Praveen ; Iizasa, Hisashi ; Hatzigeorgiou, Artemis G ; Nishikura, Kazuko, Redirection of Silencing Targets by Adenosine-to-Inosine Editing of miRNAs. *Science Vol. 315* (5815), 1137-1140.
4. Ishizuka, J. J.; Manguso, R. T.; Cheruiyot, C. K.; Bi, K.; Panda, A.; Iracheta-Vellve, A.; Miller, B. C.; Du, P. P.; Yates, K. B.; Dubrot, J., Loss of ADAR1 in tumours overcomes resistance to immune checkpoint blockade. *Nature* **2018**, *1*.
5. Slotkin, W.; Nishikura, K., Adenosine-to-inosine RNA editing and human disease. *Genome medicine* **2013**, *5* (11), 105.
6. Han, L.; Diao, L.; Yu, S.; Xu, X.; Li, J.; Zhang, R.; Yang, Y.; Werner, H. M. J.; Eterovic, A. K.; Yuan, Y.; Li, J.; Nair, N.; Minelli, R.; Tsang, Y. H.; Cheung, L. W. T.; Jeong, K. J.; Roszik, J.; Ju, Z.; Woodman, S. E.; Lu, Y.; Scott, K. L.; Li, J. B.; Mills, G. B.; Liang, H., The Genomic Landscape and Clinical Relevance of A-to-I RNA Editing in Human Cancers. *Cancer Cell* **2015**, *28* (4), 515-528.
7. Oakes, E.; Vadlamani, P.; Hundley, H. A., Methods for the Detection of Adenosine-to-Inosine Editing Events in Cellular RNA. In *mRNA Processing*, Springer: 2017; pp 103-127.
8. Tan, M. H.; Li, Q.; Shanmugam, R.; Piskol, R.; Kohler, J.; Young, A. N.; Liu, K. I.; Zhang, R.; Ramaswami, G.; Ariyoshi, K., Dynamic landscape and regulation of RNA editing in mammals. *Nature* **2017**, *550* (7675), 249.
9. Paul, M. S.; Bass, B. L., Inosine exists in mRNA at tissue-specific levels and is most abundant in brain mRNA. *The EMBO journal* **1998**, *17* (4), 1120-1127.
10. Yang, J. H.; Luo, X.; Nie, Y.; Su, Y.; Zhao, Q.; Kabir, K.; Zhang, D.; Rabinovici, R., Widespread inosine-containing mRNA in lymphocytes regulated by ADAR1 in response to inflammation. *Immunology* **2003**, *109* (1), 15-23.
11. Dominissini, D.; Moshitch-Moshkovitz, S.; Schwartz, S.; Salmon-Divon, M.; Ungar, L.; Osenberg, S.; Cesarkas, K.; Jacob-Hirsch, J.; Amariglio, N.; Kupiec, M., Topology of the human and mouse m⁶A RNA methylomes revealed by m⁶A-seq. *Nature* **2012**, *485* (7397), 201.
12. Down, T. A.; Rakyanc, V. K.; Turner, D. J.; Flicek, P.; Li, H.; Kulesha, E.; Graef, S.; Johnson, N.; Herrero, J.; Tomazou, E. M., A Bayesian deconvolution strategy for immunoprecipitation-based DNA methylome analysis. *Nature biotechnology* **2008**, *26* (7), 779.
13. Inouye, H.; Fuchs, S.; Sela, M.; Littauer, U. Z., Detection of inosine-containing transfer ribonucleic acid species by affinity chromatography on columns of anti-inosine antibodies. *Journal of Biological Chemistry* **1973**, *248* (23), 8125-8129.
14. Knutson, S. D.; Ayele, T. M.; Heemstra, J. M., Chemical Labeling and Affinity Capture of Inosine-Containing RNAs Using Acrylamidofluorescein. *Bioconjugate chemistry* **2018**, *29* (9), 2899-2903.
15. Yao, M.; Hatahet, Z.; Melamed, R. J.; Kow, Y. W., Purification and characterization of a novel deoxyinosine-specific enzyme, deoxyinosine

- 1 3'endonuclease, from Escherichia coli. *Journal of Biological Chemistry* **1994**, 269
2 (23), 16260-16268.
- 3 16. Morita, Y.; Shibutani, T.; Nakanishi, N.; Nishikura, K.; Iwai, S.; Kuraoka, I., Human
4 endonuclease V is a ribonuclease specific for inosine-containing RNA. *Nature*
5 *communications* **2013**, 4, 2273.
- 6 17. Vik, E. S.; Nawaz, M. S.; Andersen, P. S.; Fladeby, C.; Bjørås, M.; Dalhus, B.;
7 Alseth, I., Endonuclease V cleaves at inosines in RNA. *Nature communications*
8 **2013**, 4, 2271.
- 9 18. Fladeby, C.; Vik, E. S.; Laerdahl, J. K.; Neurauter, C. G.; Heggelund, J. E.;
10 Thorgaard, E.; Strøm-Andersen, P.; Bjørås, M.; Dalhus, B.; Alseth, I., The human
11 homolog of Escherichia coli endonuclease V is a nucleolar protein with affinity for
12 branched DNA structures. *PLoS One* **2012**, 7 (11), e47466.
- 13 19. Dalhus, B.; Arvai, A. S.; Rosnes, I.; Olsen, Ø. E.; Backe, P. H.; Alseth, I.; Gao, H.;
14 Cao, W.; Tainer, J. A.; Bjørås, M., Structures of endonuclease V with DNA reveal
15 initiation of deaminated adenine repair. *Nature structural & molecular biology* **2009**,
16 16 (2), 138.
- 17 20. Hitchcock, T. M.; Gao, H.; Cao, W., Cleavage of deoxyoxanosine-containing
18 oligodeoxyribonucleotides by bacterial endonuclease V. *Nucleic acids research*
19 **2004**, 32 (13), 4071-4080.
- 20 21. Cattenoz, P. B.; Taft, R. J.; Westhof, E.; Mattick, J. S., Transcriptome-wide
21 identification of A> I RNA editing sites by inosine specific cleavage. *RNA* **2013**, 19
22 (2), 257-270.
- 23 22. Torres, A. G.; Piñeyro, D.; Rodríguez-Escribà, M.; Camacho, N.; Reina, O.; Saint-
24 Léger, A.; Filonava, L.; Batlle, E.; Ribas de Pouplana, L., Inosine modifications in
25 human tRNAs are incorporated at the precursor tRNA level. *Nucleic acids research*
26 **2015**, 43 (10), 5145-5157.
- 27