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Supplementary Materials for

Suppression of adenosine-to-inosine (A-to-I) RNA editome by death associated protein 3 (DAP3) promotes cancer progression

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Supplementary Materials and Methods

Fluorescence imaging. EC109 Cells were cultured on coverslips and washed with phosphate-buffered saline (PBS; 10 mM phosphate, 137 mM NaCl and 2.7 mM potassium chloride (KCl)) before fixation with methanol for 10 minutes (mins) at room temperature. Fixed cells were washed with PBS thrice 3 mins each. Subsequently, cells were permeabilized with 0.5% Triton-X100 in PBS for 10 min, followed by three washes with 1 × PBS at every 5 min interval. Cells were blocked in 5% BSA in PBS buffer for 1 h at RT. Cells were incubated overnight at 4°C, with the following primary antibodies: rabbit anti-DAP3 (1:100; Sigma HPA023687), mouse anti-ADAR2 (1:50; Sigma SAB1405426) and mouse anti-TOMM20 (1:300, Santa Cruz). Cells were washed thrice with 0.1% Tween-20 in PBS (PBST), following secondary antibodies incubation for 1h at RT: goat anti-mouse IgG-fluorescein conjugate (1: 1,000; Invitrogen #62–6511) and goat anti-rabbit IgG-rhodamine conjugate (1: 1,000; Invitrogen #31660); followed by three washes with PBST. The coverslips were mounted onto slides using SlowFade Gold antifade mountant with DAPI (Thermo Fisher Scientific) and viewed under Zeiss Axio Imager M2 microscope.

eCLIP analysis

The eCLIP experiment was performed as previously described (*58,59*). Briefly, 20 million of EC109 cells were UV crosslinked, fragmented and immunoprecipitated using control IgG or DAP3 antibody. Then the bound protein-RNA was subjected to gel electrophoresis and membrane transfer. Bound RNA on the membrane corresponding to the size of DAP3 and 70kDa above were extracted and further processed with adaptor ligation. The cDNA library was prepared by revere transcription and sequenced by paired-end sequencing HiSeq4000 platform at Novogene. The sequencing data was processed as previously described (*58,59*). Peaks with fold-enrichment (4-fold) and significance (p-value <0.001) in immunoprecipitation versus paired size-matched input were defined as significant binding peaks. The distance between DAP3-regulated editing sites and their corresponding nearest DAP3-binding eCLIP peaks was analyzed by closest bedtools v2.29.2 (*60*). The bioinformatic analysis pipelines are accessible through CSI NGS portal (https://csibioinfo.nus.edu.sg/csingsportal/login/home.php) (*61*).

Supplementary Figures and Figure Legends

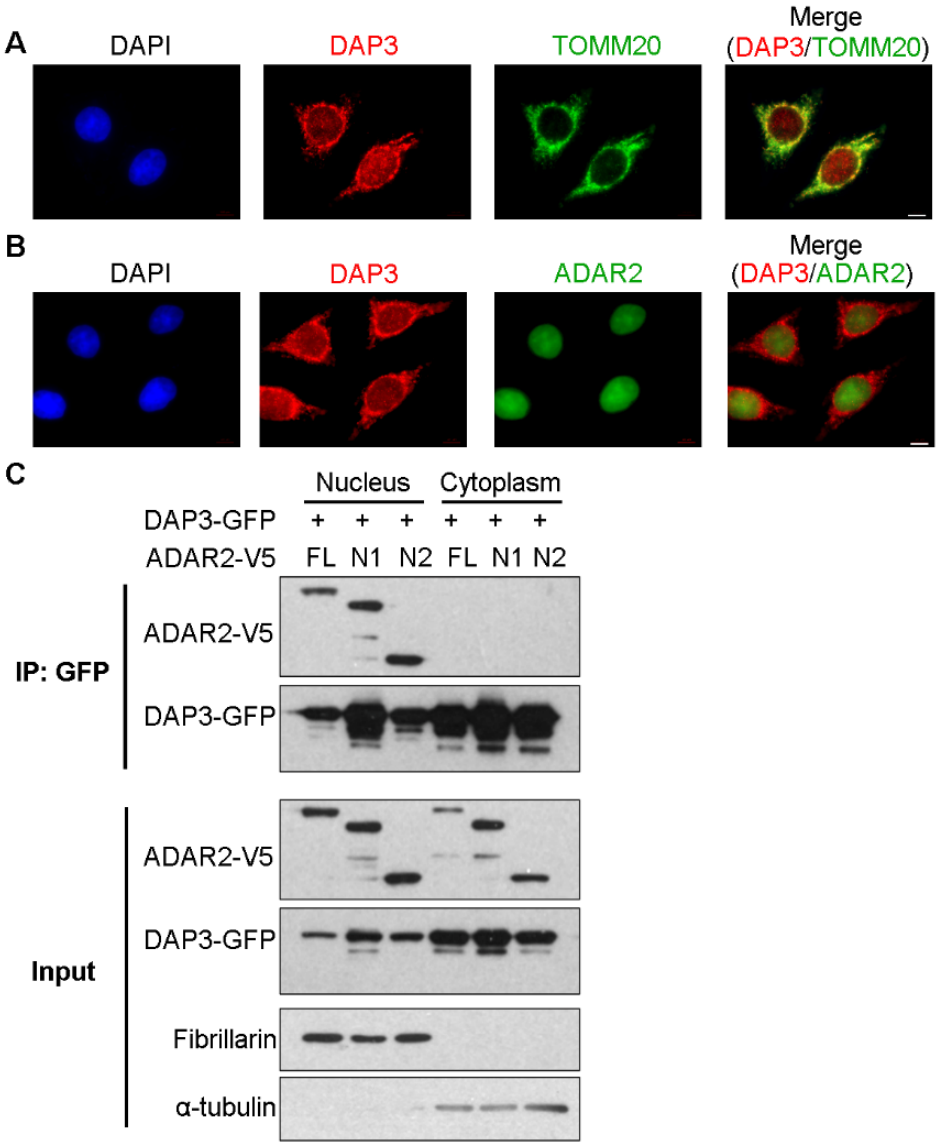

Fig. S1 DAP3 interacts with ADAR2 in the nucleus. (A, B) Immunofluorescence (IF) staining of EC109 cells by antibodies against TOMM20 (**A**) or ADAR2 (**B**) (green) and DAP3 (red). Cell nuclei were stained with DAPI (blue). Scale bar, 20 μm. **(C)** Co-IP analysis of the nuclear and cytoplasmic fractions of EC109 cells transfected with GFP-tagged DAP3 and the V5-tagged full-length (FL), N1 and N2 mutants of ADAR2. IP was conducted using a GFP-Trap system. WB analysis of GFP-pulldown products was conducted using V5 and GFP antibodies. The α-tubulin (cytoplasmic control) and Fibrillarin (nucleic control) were analyzed in the input after fractionation and 1% of the total cell lysate was loaded as an input control.

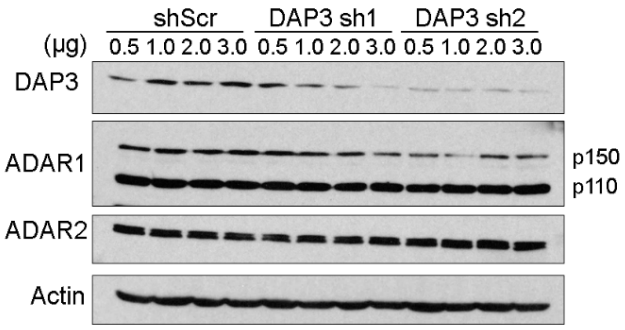

Fig. S2 Knockdown of *DAP3* does not give pronounced effects on the expression of ADARs. WB analysis of DAP3, ADAR1 (p150 and p110 isoforms) and ADAR2 expression in EC109 cells transfected with the indicated amount of *DAP3* sh1, sh2 or shScr control constructs. β-actin (Actin) was used as a loading control.

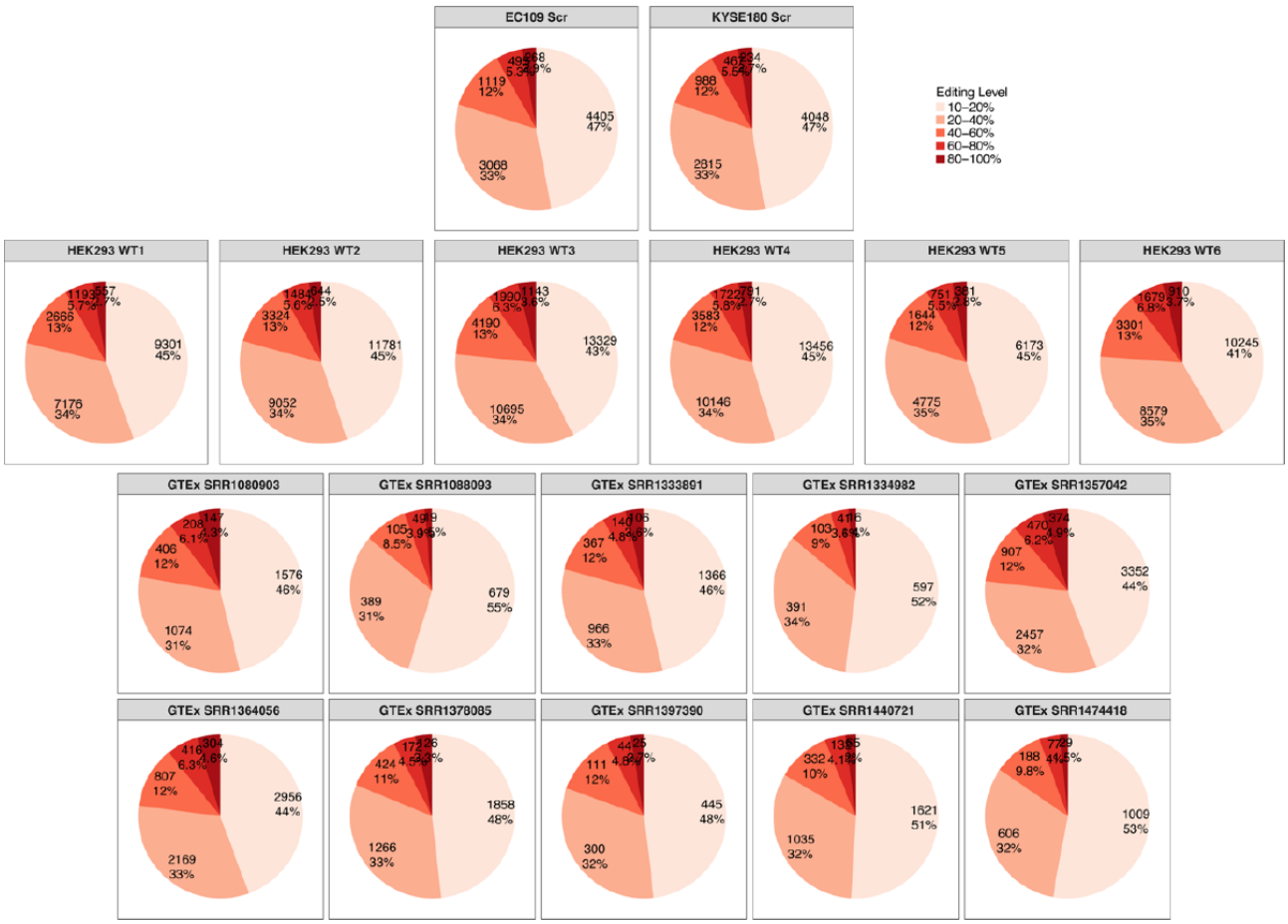

Fig. S3 Transcriptome-wide A-to-I RNA editing analysis in three RNA-Seq datasets. Pie charts showing the percentage of editing sites which are stratified into five groups based on their editing frequency in samples (10-20%, 20-40%, 40-60%, 60-80% and 80-100%) from three RNA-Seq datasets including our scramble control EC109 and KYSE180 cells (EC109 Scr and KYSE180 Scr) (n=2), HEK293 wildtype cells (HEK293 WT) (n=6), and randomly selected normal esophagus mucosa samples from the Genotype-Tissue Expression (GTEx) database (n=10), respectively.

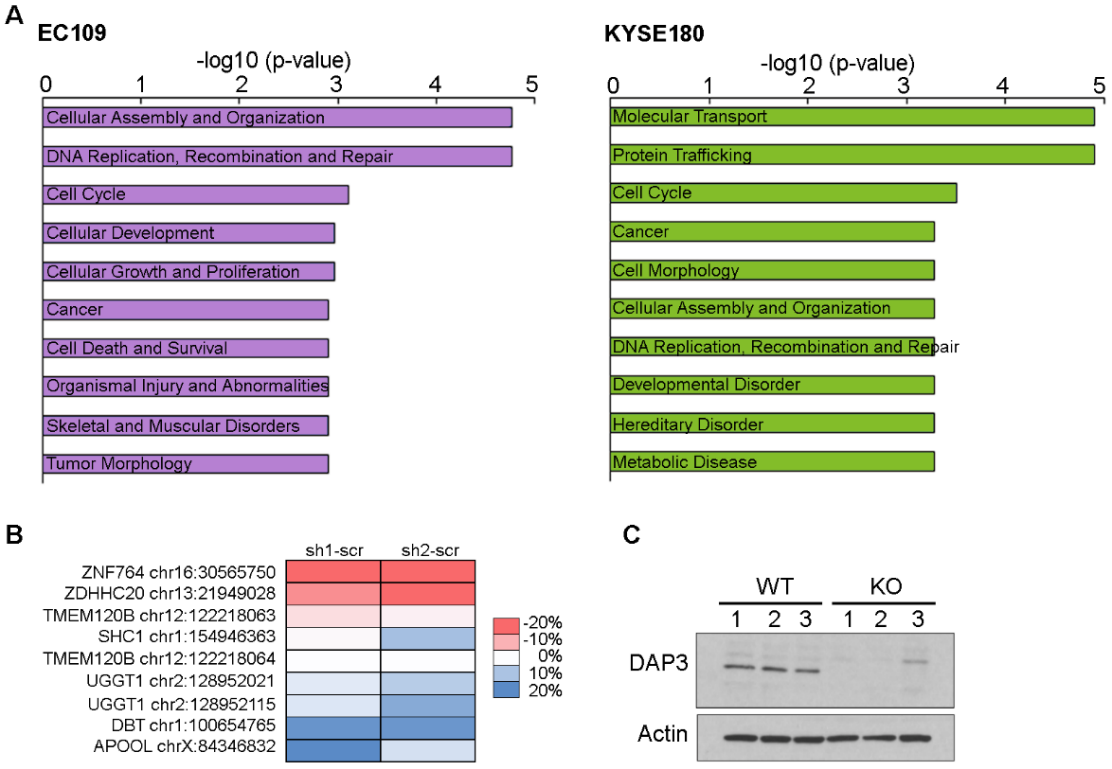

Fig. S4 Validation of DAP3-affected sites. (A) Gene ontology (GO) enrichment analysis of genes containing editing sites regulated by DAP3 in EC109 and KYSE180 cells. **(B)** Heatmap showing the Sanger sequencing validation of 9 candidate "under-edited" sites after DAP3 KD in EC109 cells. The editing frequency is calculated by taking the peak area of 'G' peak over sum of 'A' and 'G' peaks in Sanger sequencing chromatograms. **(C)** WB analysis of DAP3 expression in DAP3 WT and KO EC109 clones. β-actin (Actin) was used as a loading control.

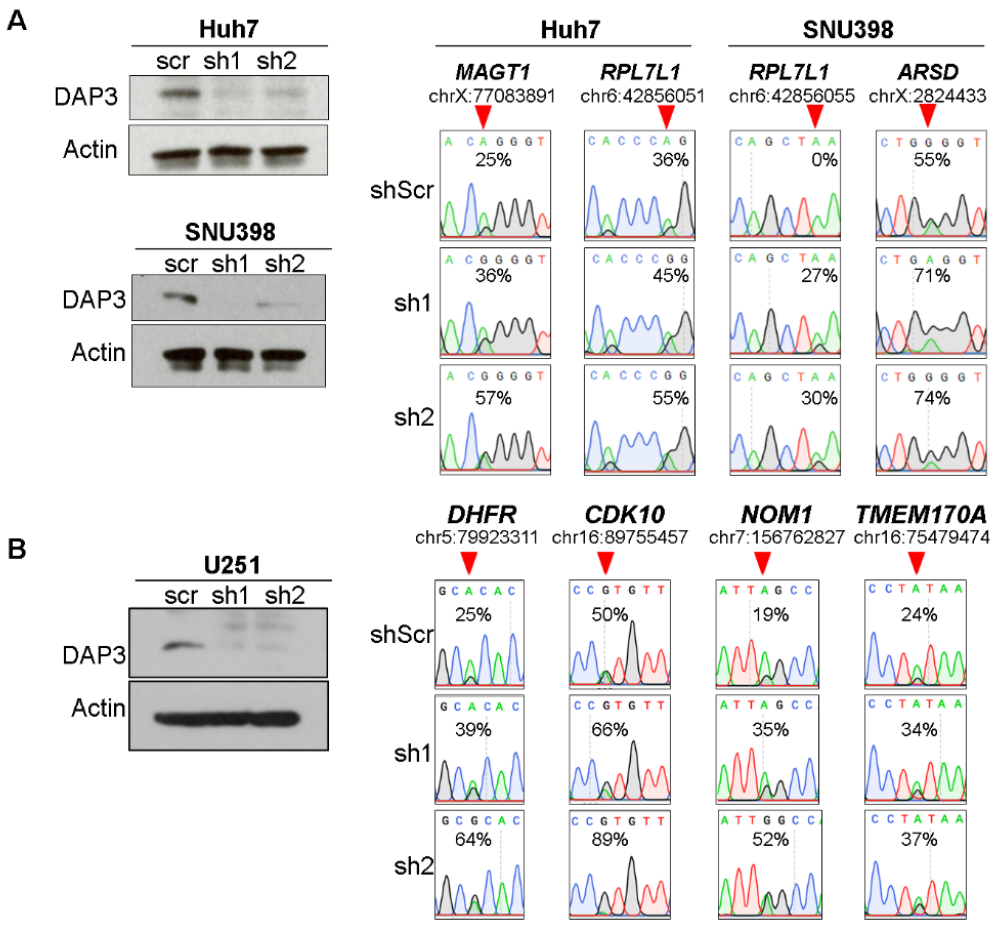

Fig. S5 DAP3 is a potent editing repressor in cancer cells.

(A) WB analysis of DAP3 expression in stable *DAP3*-knockdown Huh7 and SNU398 cells (left panel). β-actin (Actin) was used as a loading control. Sanger sequencing chromatograms illustrate editing of *MAGT1*, *RPL7L1*, and *ARSD* transcripts in the indicated stable knockdown cells (right panel). **(B)** WB analysis of DAP3 expression in stable *DAP3*-knockdown U251 cells (left panel). β-actin (Actin) was used as a loading control. Sanger sequencing chromatograms illustrate editing of *DHFR*, *CDK10*, *NOM1* and *TMEM170A* transcripts in the indicated stable knockdown cells (right panel). **(A, B)** Percentage represents the editing frequency calculated by taking the peak area of 'G' peak over sum of 'A' and 'G' peaks. Arrow indicates position of editing.

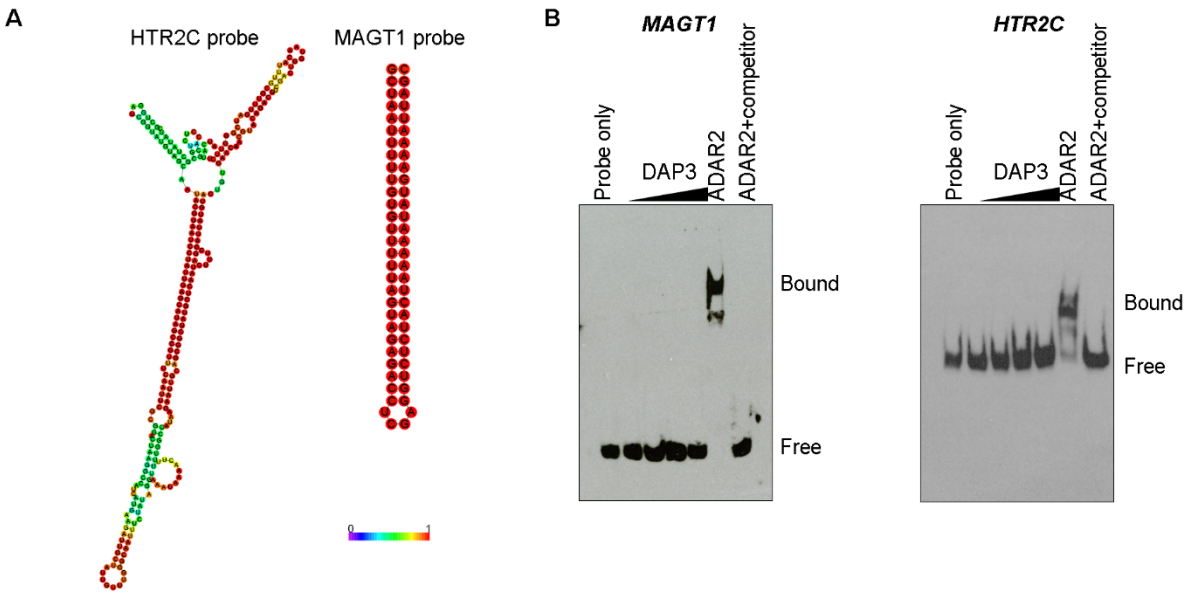

Fig. S6 DAP3 does not directly bind to *HTR2C* or *MAGT1* RNA duplexes.

(A) Predicted RNA second structures of *HTR2C* and *MAGT1* probes used in RNA electrophoresis mobility shift assay (REMSA) analysis by RNAfold. Minimum free energy (MFE) structures drawing encoding base-pair probabilities are shown. Base-pair probabilities are shown by a colour spectrum. **(B)** Biotinylated *MAGT1* and *HTR2C* RNA probes were subjected to REMSA with the addition of purified DAP3 protein in a dose-dependent manner (40, 80, 160, and 240ng). The binding of purified ADAR2 protein to RNA probes serves as a positive control. The ratio of biotin-labelled and unlabeled (competitor) RNA probes is 1:100.

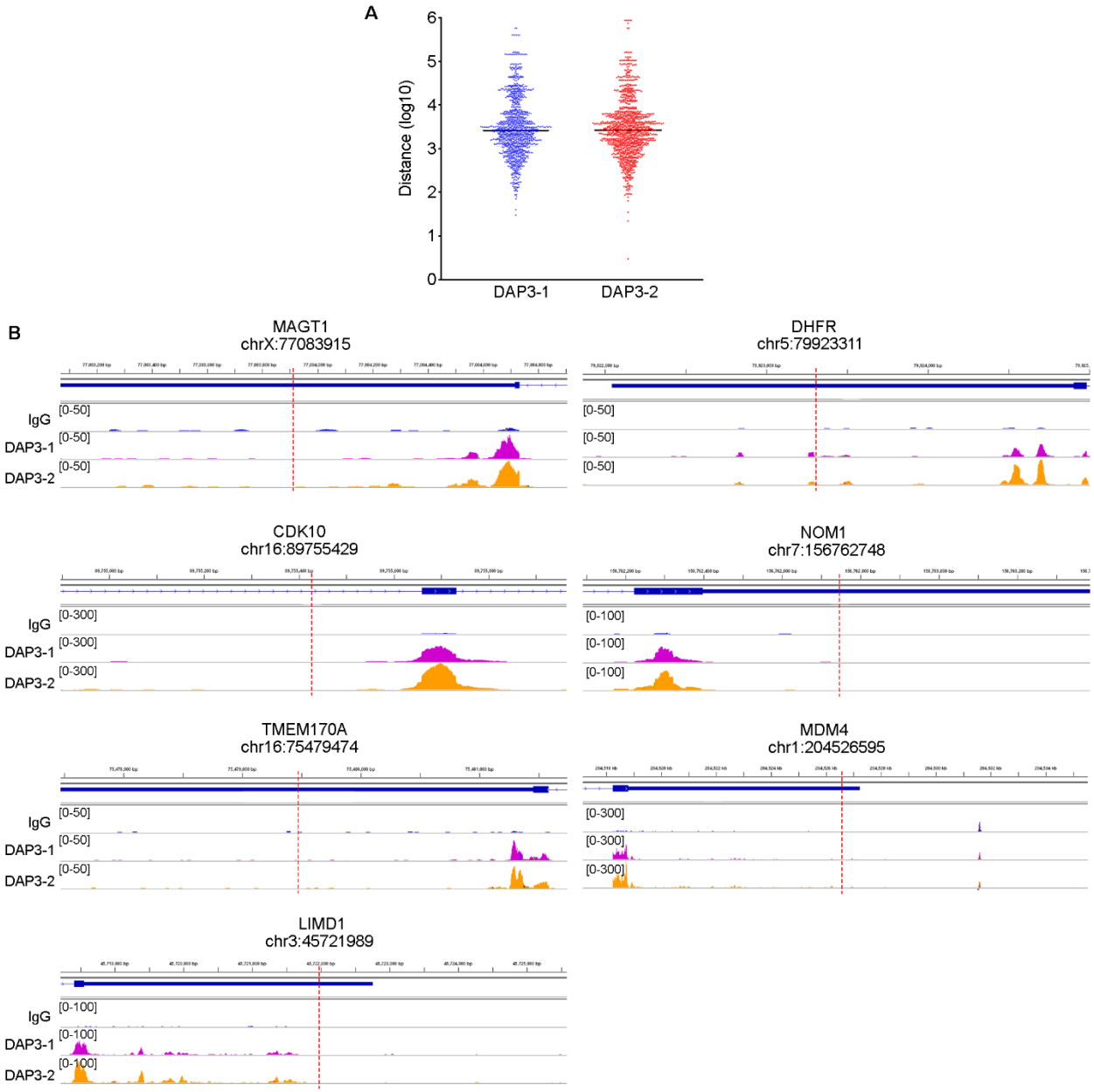

Fig. S7 eCLIP-Seq analysis of DAP3-binding sites on its target RNAs.

(A) Violin plot showing the distance between DAP3-regulated A-to-I editing sites and the nearest DAP3-binding eCLIP peaks in EC109 cells. The y-axis shows the distance (base pair) in log10 scale. **(B)** IGV tracks of normalized read density of the DAP3 eCLIP data in *MAGT1, DHFR, CDK10, NOM1, TMEM170A, MDM4* and *LIMD1* gene. IgG control and two biological replicates of DAP3 eCLIP are shown. The red dash lines indicate the position of DAP3-repressed editing sites. Read densities are shown as reads per million (RPM).

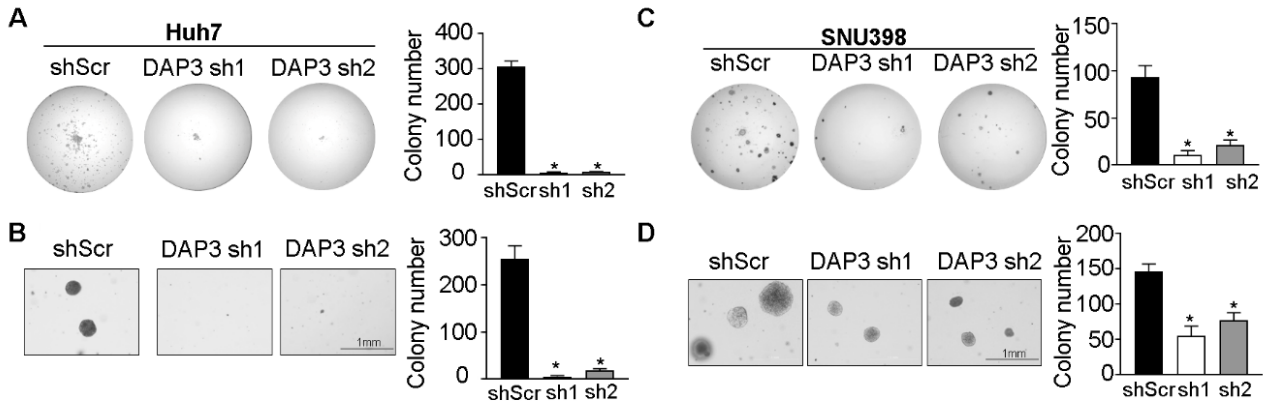

Fig. S8 DAP3 functions as an oncogene in HCC.

(A, B) Quantification of foci formation (**A**) or soft agar colony formation (**B**) induced by the indicated stable *DAP3*-knockdown (*DAP3* sh1 and sh2) and control (shScr) Huh7 cells. **(C, D)** Quantification of foci formation (**C**) or soft agar colony formation (**D**) induced by the indicated stable *DAP3*-knockdown (*DAP3* sh1 and sh2) and control (shScr) SNU398 cells. (**A-D**) Data is presented as the mean ± s.d. of duplicate wells from a representative experiment. Statistical significance is determined by unpaired, two-tailed Student's *t*-test (*, $p<0.05$).

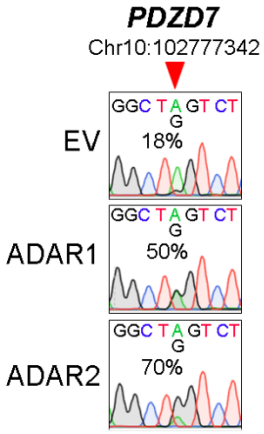

Fig. S9 Protein recoding editing of *PDZD7* gene can be regulated by ADAR1 and ADAR2. Sequence chromatograms show the editing of *PDZD7* transcripts in EC109 cells transfected with ADAR1 or ADAR2 or control expression construct. Percentage represents the editing frequency calculated by taking the peak area of 'G' peak over sum of 'A' and 'G' peaks. Arrow indicates position of editing.

Fig. S10 PDZD7 is edited in clinical samples. Sanger Sequencing chromatograms showing the editing of *PDZD7* stop codon in 46 matched ESCC tumor and non-tumor tissues. Arrow indicates position of editing.

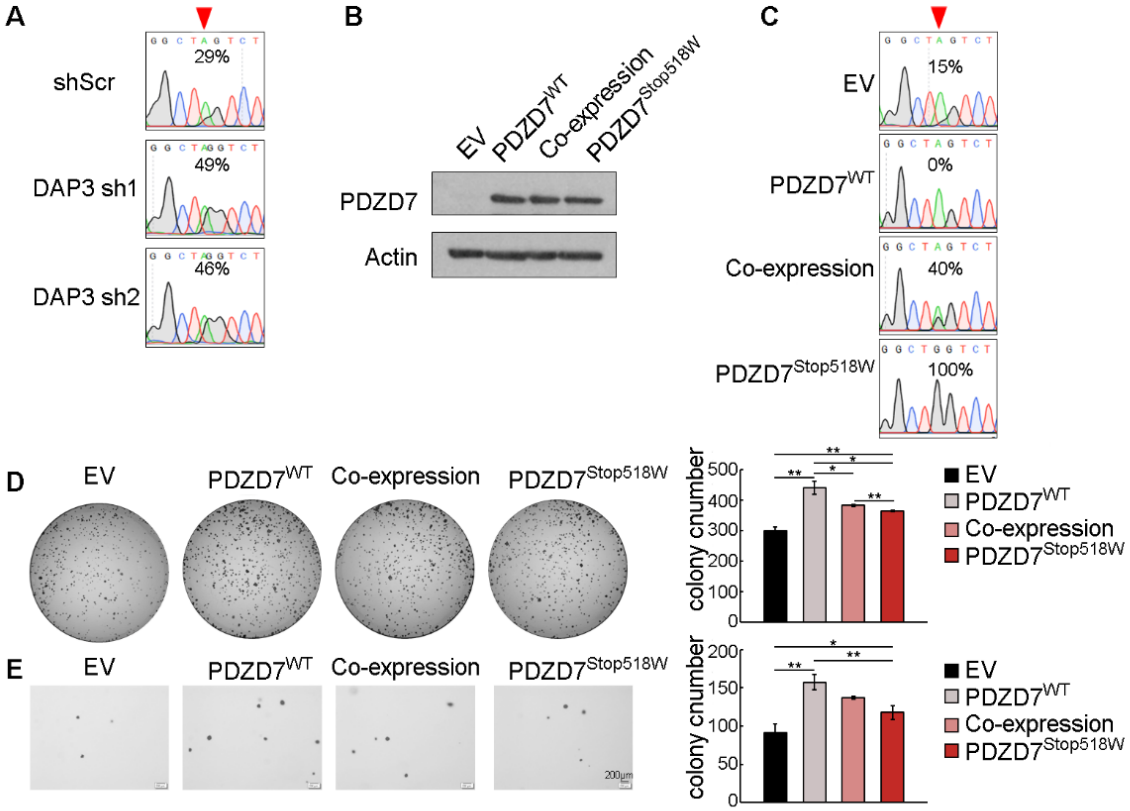

Fig. S11 Editing of *PDZD7* reduces its oncogenic ability in KYSE180 cells.

(A) Sequence chromatograms show the editing of *PDZD7* in the indicated stable cells. **(B)** PDZD7 protein level was analyzed by WB in KYSE180 cells stably expressing $PDZD7^{WT}$, $PDZD7^{Stop518W}$, or co-expressing $PDZD7^{WT}$ and $PDZD7^{Stop518W}$. Actin was used as a loading control for WB. **(C)** Sequence chromatograms show the editing of *PDZD7* in the indicated stable cells. Percentage represents the editing frequency calculated by taking the peak area of 'G' peak over sum of 'A' and 'G' peaks. Arrow indicates position of editing. **(D, E)** Quantification of foci formation (**D**) or soft agar colony formation (**E**) induced by the indicated stable cells. Data are presented as the mean ± s.d. of triplicate wells from a representative experiment. Statistical significance is determined by unpaired, two-tailed Student's *t*-test (*, $p<0.05$, **, $p<0.01$).

REFERENCES AND NOTES

1. K. Nishikura, A-to-I editing of coding and non-coding RNAs by ADARs. *Nat. Rev. Mol. Cell Biol.* **17**, 83–96 (2016).

2. C. R. Walkley, J. B. Li, Rewriting the transcriptome: Adenosine-to-inosine RNA editing by ADARs. *Genome Biol.* **18**, 205 (2017).

3. N. Paz, E. Y. Levanon, N. Amariglio, A. B. Heimberger, Z. Ram, S. Constantini, Z. S. Barbash, K. Adamsky, M. Safran, A. Hirschberg, M. Krupsky, I. Ben-Dov, S. Cazacu, T. Mikkelsen, C. Brodie, E. Eisenberg, G. Rechavi, Altered adenosine-to-inosine RNA editing in human cancer. *Genome Res.* **17**, 1586–1595 (2007).

4. H. D. Martinez, R. J. Jasavala, I. Hinkson, L. D. Fitzgerald, J. S. Trimmer, H. J. Kung, M. E. Wright, RNA editing of androgen receptor gene transcripts in prostate cancer cells. *J. Biol. Chem.* **283**, 29938–29949 (2008).

5. L. Chen, Y. Li, C. H. Lin, T. H. M. Chan, R. K. K. Chow, Y. Song, M. Liu, Y. F. Yuan, L. Fu, K. L. Kong, L. Qi, Y. Li, N. Zhang, A. H. Y. Tong, D. L. W. Kwong, K. Man, C. M. Lo, S. Lok, D. G. Tenen, X. Y. Guan, Recoding RNA editing of AZIN1 predisposes to hepatocellular carcinoma. *Nat. Med.* **19**, 209–216 (2013).

6. T. H. M. Chan, C. H. Lin, L. Qi, J. Fei, Y. Li, K. J. Yong, M. Liu, Y. Song, R. K. K. Chow, V. H. E. Ng, Y. F. Yuan, D. G. Tenen, X. Y. Guan, L. Chen, A disrupted RNA editing balance mediated by ADARs (Adenosine DeAminases that act on RNA) in human hepatocellular carcinoma. *Gut* **63**, 832–843 (2014).

7. Y.-R. Qin, J. J. Qiao, T. H. M. Chan, Y. H. Zhu, F. F. Li, H. Liu, J. Fei, Y. Li, X. Y. Guan, L. Chen, Adenosine-to-inosine RNA editing mediated by ADARs in esophageal squamous cell carcinoma. *Cancer Res.* **74**, 840–851 (2014).

8. K. Shigeyasu, Y. Okugawa, S. Toden, J. Miyoshi, Y. Toiyama, T. Nagasaka, N. Takahashi, M. Kusunoki, T. Takayama, Y. Yamada, T. Fujiwara, L. Chen, A. Goel, AZIN1 RNA editing confers cancer stemness and enhances oncogenic potential in colorectal cancer. *JCI Insight* **3**, 99976 (2018).

9. E. M. Amin, Y. Liu, S. Deng, K. S. Tan, N. Chudgar, M. W. Mayo, F. Sanchez-Vega, P. S. Adusumilli, N. Schultz, D. R. Jones, The RNA-editing enzyme ADAR promotes lung adenocarcinoma migration and invasion by stabilizing FAK. *Sci. Signal.* **10**, eaah3941 (2017).

10. M. A. Zipeto, A. C. Court, A. Sadarangani, N. P. Delos Santos, L. Balaian, H. J. Chun, G. Pineda, S. R. Morris, C. N. Mason, I. Geron, C. Barrett, D. J. Goff, R. Wall, M. Pellecchia, M. Minden, K. A. Frazer, M. A. Marra, L. A. Crews, Q. Jiang, C. H. M. Jamieson, ADAR1 activation drives leukemia stem cell self-renewal by impairing let-7 biogenesis. *Cell Stem Cell* **19**, 177–191 (2016).

11. S. N. Deffit, H. A. Hundley, To edit or not to edit: Regulation of ADAR editing specificity and efficiency. *Wiley Interdiscip. Rev. RNA* **7**, 113–127 (2016).

12. P. L. Peng, X. Zhong, W. Tu, M. M. Soundarapandian, P. Molner, D. Zhu, L. Lau, S. Liu, F. Liu, Y. M. Lu, ADAR2-dependent RNA editing of AMPA receptor subunit GluR2 determines vulnerability of neurons in forebrain ischemia. *Neuron* **49**, 719–733 (2006).

13. L. Yang, P. Huang, F. Li, L. Zhao, Y. Zhang, S. Li, Z. Gan, A. Lin, W. Li, Y. Liu, c-Jun amino-terminal kinase-1 mediates glucose-responsive upregulation of the RNA editing enzyme ADAR2 in pancreatic beta-cells. *PLOS ONE* **7**, e48611 (2012).

14. S. M. Rueter, T. R. Dawson, R. B. Emeson, Regulation of alternative splicing by RNA editing. *Nature* **399**, 75–80 (1999).

15. R. Marcucci, J. Brindle, S. Paro, A. Casadio, S. Hempel, N. Morrice, A. Bisso, L. P. Keegan, G. del Sal, M. A. O'Connell, Pin1 and WWP2 regulate GluR2 Q/R site RNA editing by ADAR2 with opposing effects. *EMBO J.* **30**, 4211–4222 (2011).

16. M. H. Tan, Q. Li, R. Shanmugam, R. Piskol, J. Kohler, A. N. Young, K. I. Liu, R. Zhang, G. Ramaswami, K. Ariyoshi, A. Gupte, L. P. Keegan, C. X. George, A. Ramu, N. Huang, E. A. Pollina, D. S. Leeman, A. Rustighi, Y. P. S. Goh; GTEx Consortium, Laboratory, Data Analysis &Coordinating Center (LDACC)—Analysis Working Group, Statistical Methods groups—Analysis Working Group, Enhancing GTEx (eGTEx) groups, NIH Common Fund, NIH/NCI, NIH/NHGRI, NIH/NIMH, NIH/NIDA, Biospecimen Collection Source Site—NDRI, Biospecimen Collection

Source Site—RPCI, Biospecimen Core Resource—VARI, Brain Bank Repository—University of Miami Brain Endowment Bank, Leidos Biomedical—Project Management, ELSI Study, Genome Browser Data Integration &Visualization—EBI, Genome Browser Data Integration &Visualization—UCSC Genomics Institute, University of California Santa Cruz, A. Chawla, G. del Sal, G. Peltz, A. Brunet, D. F. Conrad, C. E. Samuel, M. A. O'Connell, C. R. Walkley, K. Nishikura, J. B. Li, Dynamic landscape and regulation of RNA editing in mammals. *Nature* **550**, 249–254 (2017), 254.

17. J. M. P. Desterro, L. P. Keegan, E. Jaffray, R. T. Hay, M. A. O'Connell, M. Carmo-Fonseca, SUMO-1 modification alters ADAR1 editing activity. *Mol. Biol. Cell* **16**, 5115–5126 (2005).

18. J. Fritz, A. Strehblow, A. Taschner, S. Schopoff, P. Pasierbek, M. F. Jantsch, RNA-regulated interaction of transportin-1 and exportin-5 with the double-stranded RNA-binding domain regulates nucleocytoplasmic shuttling of ADAR1. *Mol. Cell. Biol.* **29**, 1487–1497 (2009).

19. D.-S. C. Cho, W. Yang, J. T. Lee, R. Shiekhattar, J. M. Murray, K. Nishikura, Requirement of dimerization for RNA editing activity of adenosine deaminases acting on RNA. *J. Biol. Chem.* **278**, 17093–17102 (2003).

20. H. Poulsen, R. Jorgensen, A. Heding, F. C. Nielsen, B. Bonven, J. Egebjerg, Dimerization of ADAR2 is mediated by the double-stranded RNA binding domain. *RNA* **12**, 1350–1360 (2006).

21. L. Valente, K. Nishikura, RNA-Binding independent dimerization of ADAR and dominant negative effects of nonfunctional subunits on dimer functions. *J. Biol. Chem.* **282**, 16054–16061 (2007).

22. R. Shanmugam, F. Zhang, H. Srinivasan, J. L. Charles Richard, K. I. Liu, X. Zhang, C. W. A. Woo, Z. H. M. Chua, J. P. Buschdorf, M. J. Meaney, M. H. Tan, SRSF9 selectively represses ADAR2-mediated editing of brain-specific sites in primates. *Nucleic Acids Res.* **46**, 7379–7395 (2018).

23. H. Hong, O. An, T. H. M. Chan, V. H. E. Ng, H. S. Kwok, J. S. Lin, L. Qi, J. Han, D. J. T. Tay, S. J. Tang, H. Yang, Y. Song, F. Bellido Molias, D. G. Tenen, L. Chen, Bidirectional regulation of adenosine-to-inosine (A-to-I) RNA editing by DEAH box helicase 9 (DHX9) in cancer. *Nucleic Acids Res.*, **46**, 7953–7969 (2018).

24. H.-R. Kim, H. J. Chae, M. Thomas, T. Miyazaki, A. Monosov, E. Monosov, M. Krajewska, S. Krajewski, J. C. Reed, Mammalian dap3 is an essential gene required for mitochondrial homeostasis in vivo and contributing to the extrinsic pathway for apoptosis. *FASEB J.* **21**, 188–196 (2006).

25. L. Xiao, H. Xian, K. Y. Lee, B. Xiao, H. Wang, F. Yu, H. M. Shen, Y. C. Liou, Death-associated protein 3 regulates mitochondrial-encoded protein synthesis and mitochondrial dynamics. *J. Biol. Chem.* **290**, 24961–24974 (2015).

26. U. Wazir, M. M. A. W. Orakzai, Z. S. Khanzada, W. G. Jiang, A. K. Sharma, A. Kasem, K. Mokbel, The role of death-associated protein 3 in apoptosis, anoikis and human cancer. *Cancer Cell Int.* **15**, 39 (2015).

27. L. Valente, K. Nishikura, RNA binding-independent dimerization of adenosine deaminases acting on RNA and dominant negative effects of nonfunctional subunits on dimer functions. *J. Biol. Chem.* **282**, 16054–16061 (2007).

28. T. H. Chan, A. Qamra, K. T. Tan, J. Guo, H. Yang, L. Qi, J. S. Lin, V. H. Ng, Y. Song, H. Hong, S. T. Tay, Y. Liu, J. Lee, S. Y. Rha, F. Zhu, J. B. So, B. T. Teh, K. G. Yeoh, S. Rozen, D. G. Tenen, P. Tan, L. Chen, ADAR-mediated RNA editing predicts progression and prognosis of gastric cancer. *Gastroenterology* **151**, 637–650.e10 (2016).

29. GTEx Consortium, Laboratory, Data Analysis &Coordinating Center (LDACC)—Analysis Working Group, Statistical Methods groups—Analysis Working Group; Enhancing GTEx (eGTEx) groups, NIH Common Fund; NIH/NCI, NIH/NHGRI, NIH/NIMH, NIH/NIDA, Biospecimen Collection Source Site—NDRI, Biospecimen Collection Source Site—RPCI, Biospecimen Core Resource—VARI, Brain Bank Repository—University of Miami Brain Endowment Bank, Leidos Biomedical—Project Management, ELSI Study, Genome Browser Data Integration &Visualization—EBI, Genome Browser Data Integration &Visualization—UCSC Genomics Institute, University of California Santa Cruz; Lead analysts, Laboratory, Data Analysis &Coordinating Center (LDACC), NIH program management, Biospecimen collection, Pathology, eQTL manuscript working group; A. Battle, C. D. Brown, B. E. Engelhardt, S. B. Montgomery, Genetic effects on gene expression across human tissues. *Nature* **550**, 204–213 (2017).

30. C. M. Burns, H. Chu, S. M. Rueter, L. K. Hutchinson, H. Canton, E. Sanders-Bush, R. B. Emeson, Regulation of serotonin-2C receptor G-protein coupling by RNA editing. *Nature* **387**, 303–308 (1997).

31. Cancer Genome Atlas Research Network, J. N. Weinstein, E. A. Collisson, G. B. Mills, K. R. Shaw, B. A. Ozenberger, K. Ellrott, I. Shmulevich, C. Sander, J. M. Stuart, The Cancer Genome Atlas Pan-Cancer analysis project. *Nat. Genet.* **45**, 1113–1120 (2013).

32. Y.-B. Chen, X. Y. Liao, J. B. Zhang, F. Wang, H. D. Qin, L. Zhang, Y. Y. Shugart, Y. X. Zeng, W. H. Jia, ADAR2 functions as a tumor suppressor via editing IGFBP7 in esophageal squamous cell carcinoma. *Int. J. Oncol.* **50**, 622–630 (2017).

33. K. Fritzell, L.-D. Xu, J. Lagergren, M. Öhman, in *Seminars in Cell & Developmental Biology* (Elsevier, 2018), vol. 79, pp. 123–130.

34. T. Hwang, C. K. Park, A. K. L. Leung, Y. Gao, T. M. Hyde, J. E. Kleinman, A. Rajpurohit, R. Tao, J. H. Shin, D. R. Weinberger, Dynamic regulation of RNA editing in human brain development and disease. *Nat. Neurosci.* **19**, 1093–1099 (2016).

35. S. Maas, S. Patt, M. Schrey, A. Rich, Underediting of glutamate receptor GluR-B mRNA in malignant gliomas. *Proc. Natl. Acad. Sci. U.S.A.* **98**, 14687–14692 (2001).

36. M. M. Matthews, J. M. Thomas, Y. Zheng, K. Tran, K. J. Phelps, A. I. Scott, J. Havel, A. J. Fisher, P. A. Beal, Structures of human ADAR2 bound to dsRNA reveal base-flipping mechanism and basis for site selectivity. *Nat. Struct. Mol. Biol.* **23**, 426–433 (2016).

37. K. J. Phelps, K. Tran, T. Eifler, A. I. Erickson, A. J. Fisher, P. A. Beal, Recognition of duplex RNA by the deaminase domain of the RNA editing enzyme ADAR2. *Nucleic Acids Res.* **43**, 1123–1132 (2015).

38. N. M. Mannion, S. M. Greenwood, R. Young, S. Cox, J. Brindle, D. Read, C. Nellåker, C. Vesely, C. P. Ponting, P. J. McLaughlin, M. F. Jantsch, J. Dorin, I. R. Adams, A. D. J. Scadden, M. Öhman, L. P. Keegan, M. A. O'Connell, The RNA-editing enzyme ADAR1 controls innate immune responses to RNA. *Cell Rep.* **9**, 1482–1494 (2014).

39. K. Pestal, C. C. Funk, J. M. Snyder, N. D. Price, P. M. Treuting, D. B. Stetson, Isoforms of RNA-editing enzyme ADAR1 independently control nucleic acid sensor MDA5-driven autoimmunity and multi-organ development. *Immunity* **43**, 933–944 (2015).

40. B. J. Liddicoat, R. Piskol, A. M. Chalk, G. Ramaswami, M. Higuchi, J. C. Hartner, J. B. Li, P. H. Seeburg, C. R. Walkley, RNA editing by ADAR1 prevents MDA5 sensing of endogenous dsRNA as nonself. *Science* **349**, 1115–1120 (2015).

41. H. Chung, J. J. A. Calis, X. Wu, T. Sun, Y. Yu, S. L. Sarbanes, V. L. Dao Thi, A. R. Shilvock, H. H. Hoffmann, B. R. Rosenberg, C. M. Rice, Human ADAR1 prevents endogenous RNA from triggering translational shutdown. *Cell **172***, 811–824. e14 (2018).

42. G. I. Rice, P. R. Kasher, G. M. A. Forte, N. M. Mannion, S. M. Greenwood, M. Szynkiewicz, J. E. Dickerson, S. S. Bhaskar, M. Zampini, T. A. Briggs, E. M. Jenkinson, C. A. Bacino, R. Battini, E. Bertini, P. A. Brogan, L. A. Brueton, M. Carpanelli, C. de Laet, P. de Lonlay, M. del Toro, I. Desguerre, E. Fazzi, À. Garcia-Cazorla, A. Heiberg, M. Kawaguchi, R. Kumar, J. P. S. M. Lin, C. M. Lourenco, A. M. Male, W. Marques Jr., C. Mignot, I. Olivieri, S. Orcesi, P. Prabhakar, M. Rasmussen, R. A. Robinson, F. Rozenberg, J. L. Schmidt, K. Steindl, T. Y. Tan, W. G. van der Merwe, A. Vanderver, G. Vassallo, E. L. Wakeling, E. Wassmer, E. Whittaker, J. H. Livingston, P. Lebon, T. Suzuki, P. J. McLaughlin, L. P. Keegan, M. A. O'Connell, S. C. Lovell, Y. J. Crow, Mutations in ADAR1 cause Aicardi-Goutières syndrome associated with a type I interferon signature. *Nat. Genet.* **44**, 1243 (2012), 1248.

43. J. L. Kissil, O. Cohen, T. Raveh, A. Kimchi, Structure–function analysis of an evolutionary conserved protein, DAP3, which mediates TNF-α-and Fas-induced cell death. *EMBO J.* **18**, 353–362 (1999).

44. J. L. Kissil, L. P. Deiss, M. Bayewitch, T. Raveh, G. Khaspekov, A. Kimchi, Isolation of DAP3, a novel mediator of interferon-γ-induced cell death. *J. Biol. Chem.* **270**, 27932–27936 (1995).

45. A. M. Chalk, S. Taylor, J. E. Heraud-Farlow, C. R. Walkley, The majority of A-to-I RNA editing is not required for mammalian homeostasis. *Genome Biol.* **20**, 1–14 (2019).

46. M. Higuchi, S. Maas, F. N. Single, J. Hartner, A. Rozov, N. Burnashev, D. Feldmeyer, R. Sprengel, P. H. Seeburg, Point mutation in an AMPA receptor gene rescues lethality in mice deficient in the RNA-editing enzyme ADAR2. *Nature* **406**, 78–81 (2000).

47. M. Horsch, P. H. Seeburg, T. Adler, J. A. Aguilar-Pimentel, L. Becker, J. Calzada-Wack, L. Garrett, A. Götz, W. Hans, M. Higuchi, S. M. Hölter, B. Naton, C. Prehn, O. Puk, I. Rácz, B. Rathkolb, J. Rozman, A. Schrewe, J. Adamski, D. H. Busch, I. Esposito, J. Graw, B. Ivandic, M. Klingenspor, T. Klopstock, M. Mempel, M. Ollert, H. Schulz, E. Wolf, W. Wurst, A. Zimmer, V. Gailus-Durner, H. Fuchs, M. H. de Angelis, J. Beckers, Requirement of the RNA-editing enzyme ADAR2 for normal physiology in mice. *J. Biol. Chem.* **286**, 18614–18622 (2011).

48. P. Bajad, F. Ebner, F. Amman, B. Szabó, U. Kapoor, G. Manjali, A. Hildebrandt, M. P. Janisiw, M. F. Jantsch, An internal deletion of ADAR rescued by MAVS deficiency leads to a minute phenotype. *Nucleic Acids Res.* **48**, 3286–3303 (2020).

49. C. Cenci, R. Barzotti, F. Galeano, S. Corbelli, R. Rota, L. Massimi, C. di Rocco, M. A. O'Connell, A. Gallo, Down-regulation of RNA editing in pediatric astrocytomas: ADAR2 editing activity inhibits cell migration and proliferation. *J. Biol. Chem.* **283**, 7251–7260 (2008).

50. A. Dobin, C. A. Davis, F. Schlesinger, J. Drenkow, C. Zaleski, S. Jha, P. Batut, M. Chaisson, T. R. Gingeras, STAR: Ultrafast universal RNA-seq aligner. *Bioinformatics* **29**, 15–21 (2013).

51. Y. Liao, G. K. Smyth, W. Shi, featureCounts: An efficient general purpose program for assigning sequence reads to genomic features. *Bioinformatics* **30**, 923–930 (2014).

52. G. Ramaswami, R. Zhang, R. Piskol, L. P. Keegan, P. Deng, M. A. O'Connell, J. B. Li, Identifying RNA editing sites using RNA sequencing data alone. *Nat. Methods* **10**, 128–132 (2013).

53. H. Li, R. Durbin, Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics* **25**, 1754–1760 (2009).

54. 1000 Genomes Project Consortium, A. Auton, L. D. Brooks, R. M. Durbin, E. P. Garrison, H. M. Kang, J. O. Korbel, J. L. Marchini, S. McCarthy, G. A. McVean, G. R. Abecasis, A global reference for human genetic variation. *Nature* **526**, 68–74 (2015).

55. S. T. Sherry, M. H. Ward, M. Kholodov, J. Baker, L. Phan, E. M. Smigielski, K. Sirotkin, dbSNP: The NCBI database of genetic variation. *Nucleic Acids Res* **29**, 308–311 (2001).

56. W. J. Kent, BLAT—The BLAST-like alignment tool. *Genome Res.* **12**, 656–664 (2002).

57. K. Wang, M. Li, H. Hakonarson, ANNOVAR: Functional annotation of genetic variants from high-throughput sequencing data. *Nucleic Acids Res.* **38**, e164 (2010).

58. E. L. Van Nostrand, T. B. Nguyen, C. Gelboin-Burkhart, R. Wang, S. M. Blue, G. A. Pratt, A. L. Louie, G. W. Yeo, Robust, Cost-Effective Profiling of RNA Binding Protein Targets with Single-end Enhanced Crosslinking and Immunoprecipitation (seCLIP), in *mRNA Processing*, Y. Shi, Ed. (Springer, 2017), pp. 177–200.

59. E. L. Van Nostrand, G. A Pratt, A. A. Shishkin, C. Gelboin-Burkhart, M. Y. Fang, B. Sundararaman, S. M. Blue, T. B. Nguyen, C. Surka, K. Elkins, R. Stanton, F. Rigo, M. Guttman, G. W. Yeo, Robust transcriptome-wide discovery of RNA-binding protein binding sites with enhanced CLIP (eCLIP). *Nat. Methods* **13**, 508–514 (2016).

60. A. R. Quinlan, I. M. Hall, BEDTools: A flexible suite of utilities for comparing genomic features. *Bioinformatics* **26**, 841–842 (2010).

61. Ö. An, K.-T. Tan, Y. Li, J. Li, C-S Wu, B. Zhang, L. Chen, H. Yang, CSI NGS Portal: An online platform for automated NGS data analysis and sharing. *Int. J. Mol. Sci.* **21**, 3828 (2020).