ADAR1 controls apoptosis of stressed cells by inhibiting Staufen1-mediated mRNA decay

Masayuki Sakurai1,2, Yusuke Shiromoto1,2, Hiromitsu Ota1, Chunzi Song1, Andrew V Kossenkov1, Jayamanna Wickramasinghe1, Louise C Showe1, Emmanuel Skordalakes1, Hsin-Yao Tang1, David W Speicher1 & Kazuko Nishikura1

Both p150 and p110 isoforms of ADAR1 convert adenosine to inosine in double-stranded RNA (dsRNA). ADAR1p150 suppresses the dsRNA-sensing mechanism that activates MDA5–Mavs–IFN signaling in the cytoplasm. In contrast, the biological function of the ADAR1p110 isoform, which is usually located in the nucleus, is largely unknown. Here, we show that stress-activated phosphorylation of ADAR1p110 by MKK6–p38–MSK MAP kinases promotes its binding to Exportin-5 and its export from the nucleus. After translocating to the cytoplasm, ADAR1p110 suppresses apoptosis in stressed cells by protecting many antiapoptotic gene transcripts that contain 3′-untranslated-region dsRNA structures primarily comprising inverted Alu repeats. ADAR1p110 competitively inhibits binding of Staufen1 to the 3′-untranslated-region dsRNAs and antagonizes Staufen1-mediated mRNA decay. Our study reveals a new stress-response mechanism in which human ADAR1p110 and Staufen1 regulate surveillance of a set of mRNAs required for survival of stressed cells.

One type of RNA editing that converts adenosine residues to inosine (A-to-I RNA editing) is catalyzed by adenosine deaminases acting on RNA (ADARs). Three ADAR gene family members, ADAR1 (official symbol ADAR), ADAR2 (ADARB1), and ADAR3 (ADARB2), have been identified in humans1,2. The translation machinery reads inosine as guanosine, thus potentially resulting in recoding and diversification of gene functions3,4. Precursors of certain microRNAs (miRNAs) also undergo A-to-I RNA editing, which regulates the processing and function of mature miRNAs5,6. Furthermore, A-to-I RNA editing frequently occurs in noncoding regions containing inversely oriented repeat elements such as Alu and LINE, although its biological importance remains largely unknown7,8.

Two isoforms of ADAR1, a full-length interferon-inducible p150 and a shorter and constitutive p110, are generated through usage of different promoters and alternative splicing9. Two repeats of Z-DNA-binding domains (Zα and Zβ), three repeats of dsRNA-binding domains (dsRBD1–3), and a separate deaminase domain are present in ADAR1p150, whereas ADAR1p110 has a truncated N terminus and lacks the Zα domain (Fig. 1a). Although both isoforms are catalytically active enzymes10, their distinct localization within the cell—with p110 mainly found in the nucleoplasm and nucleoli, and p150 mainly found in the cytoplasm—suggests that their target RNAs and biological functions are different. Adar−/− (ADAR1-null) mouse embryos die at approximately embryonic day (E) 12, owing to fetal liver disintegration, defective hematopoiesis, and widespread apoptosis, thus indicating that ADAR1 is absolutely required for embryonic development11–13. The aberrantly activated dsRNA-sensing mechanism, which in turn induces interferon responses, appears to underlie the embryonic lethal Adar−/− mouse phenotype14–16. Long and unedited dsRNAs comprising inverted SINE repeats in the 3′ untranslated regions (UTRs) of certain mRNAs have been proposed as endogenous triggers of the dsRNA-sensing mechanism. A-to-I editing of those 3′-UTR dsRNAs specifically by ADAR1p150 suppresses the dsRNA sensing and consequent activation of interferon responses15,17. This particular ADAR1p150 function has been linked to Aicardi–Goutières syndrome, a severe human autoimmune disease14,15, thus establishing one important function of ADAR1p150.

In contrast, the in vivo function of ADAR1p110, which is usually much more highly expressed than ADAR1p150, is largely unknown. In the present study, we identified a new function of ADAR1p110 regulated through phosphorylation of ADAR1p110 by MKK6–p38–MSK1/2 mitogen-activated protein (MAP) kinases (in which MSK1/2 denotes MSK1 and MSK2). The MKK6–p38–MSK1/2 signaling pathway is linked to the mechanism that responds to various stresses such as UV irradiation and heat shock18,19. We show that stress-induced phosphorylation promotes ADAR1p110 binding to the nuclear-export protein Exportin-5 (Xpo5), thus resulting in a dramatic increase in cytoplasmic ADAR1p110. In the cytoplasm, ADAR1p110 protects many antiapoptotic gene transcripts containing 3′-UTR dsRNA structures, such as those comprising inverted Alu repeats, which are otherwise subjected to Staufen1-mediated mRNA decay (SMD)20–22. We demonstrate that ADAR1p110, independently of its A-to-I editing function, competes with Staufen1 for binding to 3′-UTR dsRNAs. Post-transcriptional mRNA decay mechanisms often regulate genes that must rapidly respond to environmental changes23,24. Our study reveals a new function of ADAR1p110 in the regulation of stress-induced SMD and the protection of stressed cells from apoptosis.

1 The Wistar Institute, Philadelphia, Pennsylvania, USA. 2 These authors contributed equally to this work. Correspondence should be addressed to K.N. (kazuko@wistar.org).

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RESULTS
Stress-induced cytoplasmic localization of ADAR1p110

We reasoned that the cellular location of ADAR1 might be closely linked to its functions. Accordingly, we sought to identify the mechanism regulating the cellular distribution of ADAR1. We prepared mCherry-fusion constructs of two ADAR1 isoforms and tested them in transiently transfected A172 glioblastoma cells through fluorescence microscopy. We first confirmed that mCherry-ADAR1p110 localized almost exclusively in the nucleoplasm and nucleoli (Fig. 1b, upper left and right), whereas mCherry-ADAR1p150 localized mainly in the cytoplasm (Supplementary Fig. 1a, top). We then examined various conditions and found that stress such as UV irradiation (Fig. 1b, middle left and right) and heat shock (Fig. 1c, middle left and right) significantly increased the cytoplasmic localization of ADAR1p110. The UV irradiation-induced cytoplasmic localization of ADAR1p110 was detected by 1 h and peaked at 4 h after UV irradiation. ADAR1p110 returned to the nucleus by 12 h after UV irradiation, thus indicating that its cytoplasmic localization is temporal and reversible (Fig. 1d). In contrast to dramatic effects on ADAR1p110, UV irradiation did not change the cytoplasmic localization of ADAR1p150 (Supplementary Fig. 1a, bottom). As reported previously25, however, a fraction of the cytoplasmic ADAR1p150 moved to stress granules (Supplementary Fig. 1b, bottom). The UV-irradiation-induced cytoplasmic localization of ADAR1p110 was further confirmed by

Figure 1 Stress-induced cytoplasmic localization of ADAR1p110. (a) Schematic domain structure of two ADAR1 isoforms. (b) UV-irradiation-induced cytoplasmic localization of mCherry-ADAR1p110-WT in A172 cells, which is blocked by the p38 inhibitor SB203580. (c) Heat-shock-induced cytoplasmic localization of mCherry-ADAR1p110-WT in A172 cells, which is blocked by the p38 inhibitor SB203580. Bar graphs in b and c show quantification from images of at least 180 cells (~30 cells each from ≥7 separate, independently prepared slides) examined for mCherry-ADAR1p110 distribution between the nucleus and cytoplasm. Data are shown as mean ± s.d. ***P < 0.001; NS, not significant by two-tailed Student’s t test. Source data are available in Supplementary Data Set 1. (d) Time-course analysis of mCherry-ADAR1p110-WT localization after UV irradiation. Scale bars in b–d, 20 µm.
western blotting analysis of cytoplasmic and nuclear fractions of UV-irradiated A172 cells (Supplementary Fig. 2).

**ADAR1p110 is phosphorylated by MKK6–p38–MSK MAP kinases**

Phosphorylation plays important roles in regulating the functions as well as the cellular distribution of many proteins. Furthermore, the MAP kinases p38 and JNK is activated in response to stresses such as UV irradiation and heat shock. To identify the kinase responsible for phosphorylation of ADAR1, we tested various expression constructs for MAP kinase-activating pathways by transiently transfecting mCherry-ADAR1p110-WT into A172 cells or mock transfected as a control. Images of ~200 cells (~30 cells each from ≥7 separate, independently prepared slides) were examined for the cytoplasmic localization of mCherry-ADAR1p110. Data are shown as mean ± s.d. ***P < 0.001 by two-tailed Student’s t test. Source data are available in Supplementary Data Set 1.

Finally, Phos-tag western blotting analysis revealed a slower-migrating ADAR1p110 band, which was detected only in UV-irradiated or MKK6–transfected cells but was abolished by addition of the p38 inhibitor or by knockdown of MSK1 and MSK2. Short interfering RNAs (siRNAs) are indicated with the prefix ‘si’. Scale bars in a–c, 20 μm.

**Figure 2** Cytoplasmic localization of ADAR1p110 is regulated by p38 MAP kinase signaling. (a) The indicated expression constructs for MAP kinase–activating kinase were cotransfected with mCherry-ADAR1p110-WT into A172 cells or mock transfected as a control. Images of ~200 cells (~30 cells each from ≥7 separate, independently prepared slides) were examined for mCherry-ADAR1p110 distribution between the nucleus and cytoplasm. Data are shown as mean ± s.d. ***P < 0.001 by two-tailed Student’s t test. Source data are available in Supplementary Data Set 1. (b) Phosphorylated p38 (pp38) localizes exclusively in the nucleus in UV-irradiated A172 cells. The p38 inhibitor, although it does not affect the nuclear localization of pp38, blocks the cytoplasmic localization of mCherry-ADAR1p110. (c) UV-irradiation-induced cytoplasmic localization of mCherry-ADAR1p110-WT is blocked by simultaneous knockdown of MSK1 and MSK2. Short interfering RNAs (siRNAs) are indicated with the prefix ‘si’. Scale bars in a–c, 20 μm.

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Finally, Phos-tag western blotting analysis revealed a slower-migrating ADAR1p110 band, which was detected only in UV-irradiated or MKK6–transfected cells but was abolished by addition of the p38 inhibitor or by knockdown of MSK1 and MSK2 (Fig. 3a). Treatment of the cell extract with a Lambda phosphatase abolished the slow-migrating ADAR1p110 band (marked as pADAR1p110), thus indicating that phosphorylation is responsible for this band shift (Fig. 3a). We confirmed that not only endogenous ADAR1p110 but also exogenous FLAG-tagged ADAR1p110 was phosphorylated in a UV-irradiation- and p38-dependent manner (Fig. 3b). Mass spectrometry of the phosphorylated FLAG-ADAR1p110 band revealed substantial phosphorylation of two threonines and three serines (T808, T811, and S825) in the region connecting dsRBD3 and catalytic domains (Fig. 3c, d). The effects of phosphorylation of these five sites on the cytoplasmic localization of ADAR1p110 were examined by using mCherry-fusion constructs of two isogenic ADAR1p110 mutants, a T/S-to-A phosphorylation-inhibitory mutant (with five threonine and
serine residues mutated to alanine) and a T/S-to-D phosphomimetic mutant (with five threonine and serine residues mutated to aspartate). We found that the T/S-to-A mutant, like the wild type, localized almost exclusively in the nucleoplasm and nucleoli. In contrast, markedly increased cytoplasmic localization of the T/S-to-D mutant, colocalized with EGFP-α-Tubulin, was detected (Fig. 3e), thus confirming that phosphorylation of these sites has a critical role in the cytoplasmic localization of ADAR1p110. We also investigated the effects of single-site T/S-to-D mutation at select sites and found that mutation of even a single site had an effect on cytoplasmic localization of ADAR1p110 phosphorylation-inhibitory (T/S-to-A) and phosphorylation-mimetic (T/S-to-D) mutants. Scale bar, 20 μm.

Figure 3 Phosphorylation of ADAR1p110 at five sites. (a) Phos-tag PAGE and western blotting to detect phosphorylated ADAR1p110. A172 cell extracts were prepared after UV irradiation, transient transfection with the MKK6* expression construct in the absence or presence of the p38 inhibitor SB203580, or simultaneous knockdown of MSK1 and MSK2. λPPase indicates treatment with λ-phosphatase before SDS–PAGE. β-Actin, loading control. (b) Phos-tag SDS–PAGE analysis of FLAG-ADAR1p110 recombinant proteins. Cell extracts were prepared from A172 cells transiently transfected with the FLAG-ADAR1p110 expression construct with or without UV irradiation. Uncropped images of a and b are shown in Supplementary Data Set 2. (c) Phosphorylation sites identified by LC-MS/MS are shown. The dsRBD3 region (T725–P809) is highlighted in yellow. Identified phosphorylation sites are in red. Bottom, numbered dashed lines indicate peptides identified by LC-MS/MS. (d) Model of human ADAR1 dsRBD and the downstream region in complex with dsRNA. The region of ADAR1 spanning residues 715–799 belongs to the standard dsRBD, whereas the region downstream of this domain, where T808, T811, S814, S823, S825 are located, is disordered. The lysines K777, K778, and K781 coordinating the dsRNA are indicated. (e) Localization of ADAR1p110 phosphorylation-inhibitory (T/S-to-A) and phosphorylation-mimetic (T/S-to-D) mutants. Scale bar, 20 μm.

Figure 4 Phosphorylation of ADAR1p110 promotes binding to Xpo5–Ran–GTP. (a) Binding of the phosphomimetic and phosphorylation-inhibitory mutants of FLAG-ADAR1p110 to the Xpo5–Ran–GTP complex, tested in vitro on M2 anti-FLAG monoclonal-antibody beads. An equimolar ratio of His-Xpo5 and His-Ran–GTP prey proteins to their bait proteins (FLAG-ADAR1p110) was used. On the basis of the amount of His-Xpo5 protein in the flow-through fractions, we calculated that ADAR1p110-T/S-to-D binds the Xpo5–Ran–GTP complex at ~70% efficiency, whereas ADAR1p110-T/S-to-A binding occurs at up to 10% efficiency. Data are shown as mean ± s.e.m. (n = 3 technical replicates). **P < 0.01; ***P < 0.001 by two-tailed Student’s t-test. Source data are available in Supplementary Data Set 1. (b) Binding of the phosphomimetic and phosphorylation-inhibitory mutants of FLAG-ADAR1p110 to TRN1, tested in vitro on M2 anti-FLAG monoclonal-antibody beads. Uncropped images of a and b are available online in Supplementary Data Set 2.
localization but was not as effective as mutation of the five sites (data not shown), thus indicating the importance of the multiple-site phosphorylation and its cumulative effects. The nearly exclusively cytoplasmic localization of ADAR1p150–WT was not affected by either T/S-to-A or T/S-to-D mutation (Supplementary Fig. 1c).

We also examined the effects of mutation on A-to-I editing activity in vitro by using ADAR1p110 recombinant proteins made in baculovirus-infected S99 insect cells69. We found no differences in the A-to-I RNA-editing activity among T/S-to-A, T/S-to-D, and wild-type ADAR1 (Supplementary Fig. 3a). We have previously reported that ADAR1p110 forms a complex with Dicer and promotes processing of precursor (pre)-miRNAs into mature miRNAs during mouse embryonic development53,54. Accordingly, we have also examined processing of pre-let-7a to mature let-7a in vitro by using Dicer–ADAR1p110 complexes54. In this study, we again found no differences in the pre-miRNA-cleavage activity among Dicer–ADAR1p110 complexes including the wild type, T/S-to-D, and T/S-to-A mutant (Supplementary Fig. 3b).

Finally, we examined global miRNA expression profiles in UV-irradiated and ADAR1-knockdown cells by high-throughput sequencing of small RNAs. We anticipated that ADAR1p110 newly translocated to the cytoplasm might be involved in the expression of a special set of miRNAs, perhaps those required for stress responses35,36. Unexpectedly, knockdown of ADAR1 caused very few changes in global miRNA expression in UV-irradiated cells (data not shown). Furthermore, we found very little formation of the Dicer–ADAR1p110 complex in UV-irradiated A172 cells (data not shown). These results may have been due to the presence of abundant TRBP and thus Dicer–TRBP complexes in A172 cells used in the present experiments, in contrast to the low TRBP expression in E11 and E12 mouse embryos34. ADAR1p110 appears to be exported to the cytoplasm for reasons other than its RNA-interference-promoting function34.

ADAR1p110 phosphorylation promotes binding to Xpo5

Although ADAR1p110 usually localizes in the nucleus, nuclear export of ADAR1p110 mediated by Xpo5–Ran–GTP has been reported37. Furthermore, nuclear import of ADAR1p110 is regulated by Transportin-1 (TRN1). The cellular distribution of ADAR1p110 appears to be regulated by a balance between Xpo5-mediated export and TRN1-mediated import37. We demonstrated that ADAR1p110 is phosphorylated in the nucleus by the nuclear targets of pp38, MSK1,
and MSK2 (Figs. 2c and 3a). Furthermore, the UV-irradiation-induced cytoplasmic localization of wild-type ADAR1p110 (Supplementary Fig. 4a) and the cytoplasmic localization of the ADAR1p110-T/S-to-D mutant (Supplementary Fig. 4b) were completely blocked after knockdown of Xpo5, thus indicating that the nuclear export of both phosphorylated ADAR1-WT and the ADAR1p110-T/S-to-D mutant depends on Xpo5. Finally, the nearly exclusive nuclear localization of the ADAR1p110-T/S-to-A mutant was abolished after knockdown of TRN1, thus indicating that the nuclear import of the T/S-to-A mutant depends on TRN1 (Supplementary Fig. 4c). Together, these results suggest that phosphorylation of ADAR1p110, which occurs in the nucleus, most probably affects the Xpo5-mediated nuclear-export mechanism rather than the TRN1-mediated nuclear-import mechanism.

**Figure 6** ADAR1 protects mRNAs containing 3′-UTR dsRNA from SMD. (a) Schematic domain structure of Staufen1. TBD, Tubulin-binding domain; SSM, Staufen-swapping motif. (b) RNA-seq analysis of gene expression in UV-irradiated A172 cells treated with siControl compared with siADAR1, or siADAR1 compared with siADAR1 and siStaufen1. Numbers indicate log₂ fragments per kilobase of transcript per million mapped reads (FPKM). All genes detected by RNA-seq (gray dots) and 488 selected genes (blue dots) whose expression levels decreased more than two-fold after ADAR1 knockdown and were rescued more than 1.5-fold after knockdown of ADAR1 and Staufen1 are listed in Supplementary Table 1. (c) Comparison of expression levels of genes selected from Supplementary Table 1 on the basis of the presence of 3′-UTR dsRNA structures. The 3′-UTR dsRNA structure was identified with the UCSC Genome Browser and mfold algorithm. Numbers indicate log₂ FPKM. The line shows a two-fold decrease compared with the expression after siControl treatment. Expression levels of the selected genes were also examined by qRT–PCR (Supplementary Table 3). (d) qRT–PCR of two nonstructured 3′-UTR mRNAs (labeled in blue on x axis) and four dsRNA-containing 3′-UTR mRNAs (labeled in red on x axis) after knockdown of ADAR1 only, ADAR1 and Staufen1, or ADAR1 and UPF1 in UV-irradiated A172 cells. Data are shown as mean ± s.d. (n = 3 technical replicates for other genes). ***P < 0.001; NS, not significant by two-tailed Student’s t-test. Source data are available in Supplementary Data Set 1. (e) Western blotting analysis showing that changes in mRNA levels shown in d are reflected in the protein levels. Uncropped images are shown in Supplementary Data Set 2. β-Actin, loading control. (f) GOrilla gene-ontology enrichment analysis of the selected 488 genes (described in b).
Figure 7 ADAR1 inhibits binding of Staufen1 to the 3′-UTR dsRNA structure. (a) Formaldehyde cross-linking of RNA–protein complexes and immunoprecipitation by hemagglutinin (HA)-tagged Staufen1 in siControl- or siADAR1-treated and UV-irradiated A172 cells. Input and immunoprecipitate (IP) eluate fractions were monitored by immunoblotting analysis. Unropped images are shown in Supplementary Data Set 1. (b) qRT–PCR of mRNAs without structured 3′ UTRs (blue) and mRNAs containing 3′-UTR dsRNA (red), performed on RNA purified from immunoprecipitated RNA–HA-Staufen1 complex data. Data are shown as mean ± s.d. (n = 4 technical replicates). ***P < 0.001; NS, not significant by two-tailed Student’s t-test. Source data are available in Supplementary Data Set 1.

Accordingly, we next investigated the effects of ADAR1p110 phosphorylation on binding to Xpo5 by using an in vitro binding assay, as described previously.37,38 We found that the amount of ADAR1p110-T/S-to-D bound to the Xpo5–Ran–GTP complex indeed increased significantly (approximately 10- to 20-fold) as compared with that of ADAR1p110-T/S-to-A (Fig. 4a). As expected, no differences in binding to TRN1 were detected between ADAR1p110-T/S-to-A and ADAR1p110-T/S-to-D mutants (Fig. 4b). We concluded that phosphorylation of ADAR1p110 regulated by MKK6–p38–MSK1/2 MAP kinases promotes its binding to the Xpo5–Ran–GTP complex and consequently its nuclear export to the cytoplasm.

Function of ADAR1p110 exported to the cytoplasm

The stress-activated p38 MAPK signaling pathways are involved in the regulation of death or survival of stressed cells.39,40 We therefore examined the possibility that cytoplasmic ADAR1p110 might function in the regulation of apoptosis. We found that knockdown of ADAR1p110 indeed induced apoptosis in UV-irradiated A172 cells at a significantly higher rate (approximately five- to six-fold) as compared with that of the control (Fig. 5a and Supplementary Fig. 5a), thus revealing a protective role of ADAR1 against induction of apoptosis in cells under stress. Interestingly, ADAR1p110 is the isoform specifically responsible for this antiapoptotic function, because specific knockdown of ADAR1p150 did not induce apoptosis in UV-irradiated cells (Fig. 5a). Similar results were obtained with a separate human cell line, U2OS osteosarcoma (Supplementary Fig. 5b). As expected, UV-induced apoptosis was reversed in ADAR1-knockdown cells transfected with a FLAG-tagged ADAR1p110-WT expression construct. Interestingly, ADAR1p110–E912A, a deamination-defective mutant, also rescued the ADAR1-knockdown cells from apoptosis (Fig. 5b and Supplementary Fig. 5c). These results indicated that the function of ADAR1p110—not its A-to-I editing function but most probably its dsRNA-binding function—is responsible for suppressing stress-induced apoptosis.

Finally, we found that knockdown of Xpo5 but not TRN1 also induced apoptosis in UV-irradiated cells (Fig. 5c), thus indicating that nuclear export of certain proteins such as ADAR1p110 is critical for the mechanism that suppresses stress-induced apoptosis.

Function of cytoplasmic ADAR1p110 independent of MDA5–MAVS–IFN signaling

Aberrant activation of the dsRNA-sensing mechanism mediated by melanoma differentiation associated protein-5 (MDA5)—mitochondrial antiviral signaling protein (MAVS)—interferon (IFN) signaling underlies the embryonic lethality of Adar−/− mice.14,16 ADAR1p150 suppresses this MDA5–MAVS–IFN pathway by editing its triggers—the 3′-UTR long dsRNAs arising from inverted repeats such as SINE and Alu—and consequently making them undetectable by the dsRNA-sensing mechanism.15 The 3′-UTR long dsRNAs edited extensively by ADAR1p150 may even inhibit binding of the unedited 3′-UTR dsRNAs to MDA5 (refs. 14–16). In support of this hypothesis, synthetic dsRNAs that contain multiple I–U wobble base pairs and mimic extensively A-to-I edited dsRNAs (I–U dsRNAs) have been shown to prevent activation of the MDA5–MAVS–IFN pathway.14,41

Accordingly, we investigated the relationship between the stress-induced antiapoptotic function of cytoplasmic ADAR1p110 and the MDA5–MAVS–IFN pathway. We found that transfection of I–U dsRNA did not prevent UV-induced apoptosis in ADAR1-knockdown cells (Fig. 5d). Furthermore, knockdown of neither MDA5 nor MAVS suppressed UV-induced apoptosis of ADAR1-knockdown cells (Fig. 5d). However, a known trigger of the MDA5–MAVS–IFN pathway, synthetic long dsRNA poly(I–C), induced strong IFN responses, thus indicating that the MDA5–MAVS-mediated dsRNA-sensing mechanism remained intact in A172 cells (Supplementary Fig. 5d). These results together indicated that the antiapoptotic function of ADAR1p110 translocated to the cytoplasm in response to stress is independent of its A-to-I editing activity and is also independent of the MDA5–MAVS signaling pathway.

ADAR1p110 suppresses SMD of mRNAs containing 3′-UTR dsRNAs

To gain insight into the function of ADAR1p110 exported to the cytoplasm, we investigated global mRNA expression profiles in UV-irradiated and ADAR1-knockdown cells by high-throughput sequencing analysis (RNA-seq). We found that the expression of many gene transcripts was significantly decreased in UV-irradiated and ADAR1-knockdown cells (Supplementary Table 1) but observed no significant upregulation of interferon-stimulated genes (Supplementary Table 2), in contrast to the dramatic increase in expression of interferon-stimulated genes reported in Adar−/− mouse embryos.14,16 These results confirmed that the stress-induced cytoplasmic ADAR1p110 function is independent of MDA5–MAVS–IFN signaling. Interestingly, we found that most of these ADAR1-regulated genes contained 3′-UTR dsRNAs arising primarily from inverted Alu repeats, of which some were A-to-I edited (Supplementary Fig. 6 and Supplementary Table 3).

Association of 3′-UTR Alu dsRNA with Staufen1 has been reported.42,43 Staufen1 is also a dsRNA-binding protein that, like ADAR1, contains four dsRNA-binding domains (Fig. 6a). Staufen1 binds to Staufen1-binding sites, recruits the UPF1 RNA helicase, and promotes degradation of its target mRNAs (i.e., SMD).44 Staufen1 nearly exclusively localized in the cytoplasm regardless of UV irradiation and colocalized with the ADAR1p110 newly translocated to the cytoplasm in UV-irradiated A172 cells (Supplementary Fig. 7). Accordingly, we reasoned that the cytoplasmic ADAR1p110 might protect certain mRNAs containing 3′-UTR Alu dsRNAs, which are otherwise degraded by SMD. We first confirmed that knockdown of Staufen1 and/or UPF1 did not activate apoptosis in UV-irradiated cells (Fig. 5e and Supplementary Fig. 5e). Most importantly, however, knockdown of Staufen1 and/or UPF1 indeed prevented UV-irradiation-induced apoptosis in ADAR1-knockdown cells (Fig. 5e and Supplementary Fig. 5e). RNA-seq data analysis revealed that...
ADAR1p110 protects Staufen1 from SMD degradation and inhibits binding of Staufen1 to Alu dsRNAs. Inhibition of Staufen1 binding to Alu dsRNA structures was observed after ADAR1 knockdown (Fig. 6d). We further explored the effects of Staufen1 knockdown on the expression of other genes. We selected a set of 52 genes containing 3′-UTR Alu dsRNAs and analyzed their pre-mRNA levels after ADAR1 knockdown and Staufen1 or UPF1 knockdown, respectively (Fig. 6e). In any case, the very slight changes in their mRNA levels might have reflected changes in transcriptional rates (Supplementary Fig. 8). In any case, the very slight changes in their mRNA levels may be physiologically irrelevant, unlike those of the SMD-target mRNAs, because both GAPDH and HPRT1 protein levels remained unchanged (Fig. 6e).

ADAR1p110 competes with Staufen1 and antagonizes SMD. Apoptosis induced in UV-irradiated and ADAR1-knockdown cells was rescued by the ADAR1p110-E912A catalytically defective mutant (Fig. 5b), thus indicating that the SMD-regulatory function of ADAR1p110 is independent of its A-to-I editing activity but is probably dependent on its dsRNA-binding activity. We, therefore, next conducted RNA immunoprecipitation (RIP)-qRT–PCR analysis (Fig. 7a) for selected genes whose expression was suppressed by ADAR1 knockdown but rescued by Staufen1 knockdown. We first confirmed that Staufen1 did not bind to 3′ UTRs lacking dsRNA structure, such as GAPDH and HPRT1 mRNAs (Fig. 7b) in UV-irradiated and ADAR1-knockdown cells (Supplementary Table 5). In contrast, Staufen1 bound to the 3′-UTR dsRNAs of CCNG1, CASC5, and...
ATM, and RAD51 mRNAs, and its binding was significantly increased by ADAR1 knockdown (Fig. 7b). Together, our results clearly indicated that ADAR1p110 protects only mRNAs containing a dsRNA structure in their 3′-UTRs by competitively inhibiting the binding of Staufen1 and suppressing SMD of these transcripts (Fig. 8).

**DISCUSSION**

mRNA degradation is an important mechanism that rapidly regulates the expression of many genes required for cells to respond to environmental changes or stress. In the present study, we identified ADAR1p110 as a stress-response protein and established its antiapoptotic function, which is necessary for the survival of stressed cells. To our knowledge, this is the first reported evidence that cytoplasmic ADAR1p110 is a stress-response protein. The newly identified stress-response function of ADAR1p110 is regulated through phosphorylation by MKK6–p38–MSK1/2 MAP kinases. Phosphorylation of T808, T811, S814, S823, and S825 facilitates binding of ADAR1p110 to the nuclear exporter Xpo5 and consequently its export to the cytoplasm (Fig. 8). These phosphorylation sites are highly conserved and are located in the unstructured hinge region connecting dsRBD3 and the deaminase domain (Fig. 3d). Negative charges resulting from phosphorylation probably cause substantial conformational changes and may also affect the interaction between dsRNA and dsRBD3, and consequently ADAR1p110 binding to Xpo5.

Most importantly, the newly exported ADAR1p110 then protects a set of mRNAs—those containing a 3′-UTR dsRNA structure primarily comprising inverted Alu repeats—against SMD by competitively inhibiting binding of Staufen1 to the 3′-UTR dsRNA (Fig. 8). Interestingly, mRNAs of many antiapoptotic genes contain 3′-UTR dsRNAs and are protected by the cytoplasmic ADAR1p110, thus providing a probable explanation for the apoptotic death of stressed cells in the absence of ADAR1p110. For instance, both ATM and RAD51, which are protected by ADAR1p110 (Figs. 6d and 7b) are involved in the DNA-repair and cell-cycle-checkpoint mechanisms, but their roles in protecting stressed cells against apoptosis have also been reported in several studies. For instance, ATM is required for the suppression of apoptosis and the survival of cells exposed to oxidative stress such as ionizing radiation. Similarly, the protective function of RAD51 against DNA-damage-induced apoptosis has also been reported. However, it remains unknown exactly which gene transcripts protected by ADAR1p110 are essential for survival of UV-irradiated cells. Several gene transcripts may play a critical role in promoting survival of UV-irradiated cells. Alternatively, the cumulative effects of many or all gene transcripts (488 genes) protected by ADAR1p110 may be important.

It is unclear whether ADAR1p110’s inhibition of SMD is specific to this isoform. However, the endogenous ADAR1p150 expression levels are very low (Fig. 5a) and are probably insufficient to protect all Staufen1-target mRNAs, thus potentially explaining why ADAR1p110 must be exported to the cytoplasm under stress conditions. Although a fraction of ADAR1p150 moves into stress granules, Staufen1 remains in the cytoplasm before and after stress, thus excluding the possibility of their interaction in stress granules.

ADAR1p110 appears to almost completely prevent binding of Staufen1 to its target 3′-UTR Alu dsRNAs. The reason why ADAR1p110 outcompetes Staufen1 is currently not clear, because these two dsRNA-binding proteins have been reported to have very similar dsRNA binding affinities. Interestingly, however, the interaction of Staufen1 with dsRNA is more dynamic, whereas that of ADAR1 is very static, thus possibly explaining the dominant-negative effects of ADAR1p110 on binding of Staufen1 to 3′-UTR Alu dsRNAs.

The MAP kinase p38 is involved in the regulation of death or survival of stressed cells. p38 kinase, after activation by MKK3- and/or MKK6-mediated phosphorylation, translocates to the nucleus and phosphorylates many transcription activators and suppressors as well as downstream kinases such as MSK1 and MSK2, thereby rapidly changing the expression of many crucial stress-response genes. The p38 signaling pathway also affects cellular distribution of many target-gene products. The fate of stressed cells is determined by a balance between survival and death mechanisms, both of which are regulated by p38 (refs. 19, 28, 40). Here, we demonstrated that p38 and its targets MSK1 and MSK2 regulate the nuclear export of ADAR1p110, which in turn promotes survival of stressed cells in the cytoplasm (Fig. 8).

Recent studies analyzing Adar−/− and crosses of Adar−/− and Ifih1−/− (MDA5 null) or Mavs−/− have led to the identification of a function of ADAR1p150 in suppressing the dsRNA-sensing mechanism mediated by MDA5–MAVS–IFN signaling. ADAR1p150 suppresses the dsRNA-sensing initiation step by extensively editing cytoplasmic and endogenous trigger dsRNAs such as 3′-UTR inverted Alu repeats. The activation of this pathway also results in apoptotic cell death due to strong interferon responses in Adar−/− mouse embryos. In this study, however, we found that the stress-response function of the cytoplasmic ADAR1p110 is independent of both A-to-I editing and the MDA5–MAVS pathway, thus revealing a function of ADAR1p110 distinct from that of ADAR1p150.

Although the offspring of crosses of Adar−/− and Ifih1−/− or Mavs−/− mice survive to birth, they die within 2 to 3 d, despite the suppression of the dsRNA-sensing mechanism and interferon responses in these newborn mice, thus indicating that ADAR1’s functions beyond suppression of the MDA5–MAVS–IFN pathway are required for adult life. Interestingly, the offspring of crosses of Adar−/− and Mavs−/− mice survive not only to birth but also beyond weaning. Furthermore, although AdarE861A mice carrying an editing-deficient knock-in mutation also die at E13.5 and exhibit activated interferon and dsRNA-sensing pathways, the offspring of crosses of AdarE861A and Ifih1−/− mice have normal adult lifespans. These findings together suggest that ADAR1p110’s functions independent of A-to-I editing and MDA5–MAVS–IFN signaling are required for normal postnatal development. Histological inspection of AdarP150−/−/ Mavs−/− mice and rare surviving AdarP150−/−/Mavs−/− mice has revealed the importance of the MDA5–MAVS–IFN pathway–independent functions of each ADAR1 isoform in the development and homeostasis of particular organs and tissues, for example, ADAR1p150 for ADAR1p110 for kidney patterning.

Defective development of certain organs and tissues detected in adult Adar-mutant mice probably results, at least in part, from the deficiency in the stress-response function of ADAR1p110 (independent of A-to-I editing and MDA5–MAVS-mediated dsRNA sensing) described in this study, as well as the function of ADAR1p110 in promoting Dicer activity and hence miRNA processing, both of which are also independent of A-to-I editing. Our future studies will focus on further understanding the A-to-I editing–independent functions of ADAR1p110 in postnatal development and adult life.

**METHODS**

Methods, including statements of data availability and any associated accession codes and references, are available in the online version of the paper.

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.
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Apoptosis assays. Assessment of cell apoptosis was carried out in 96-well plates with an ApoTox-Glo Triplex Assay Kit (Promega), which simultaneously measures cell viability, cytotoxicity, and apoptosis (Caspase-3/7 activity). 1 d before the assay, 1 x 10^6 cells were transfected with siRNA. One set of plates was irradiated with 40 J/m² UVC 60 h after transfection, and ApoTox-Glo Triplex assays were performed 12 h after UV irradiation. The signal intensity in relative luminescence units (RLUs) for Caspase-3/7 activity was normalized to the relative fluorescence units (RFUs) from the viability measurements (400 nm/505 nm). I-U preparations of cytoplasmic and nuclear proteins.

Preparation of cytoplasmic and nuclear proteins. After centrifugation at 6,000 x g for 10 min at 4 °C, the supernatants were collected to isolate the nuclear fractions. The cell pellets were resuspended in 300 µL of cytoplasmic-extract buffer containing 10 mM NaHPO₄, pH 7.4, 137 mM NaCl, 2.7 mM KCl, 1 mM MgCl₂, 0.5 mM dithiothreitol (DTT), 0.05% NP-40, and 0.5% sodium deoxycholate, 0.1% SDS, complete EDTA-free protease-inhibitor cocktail (Roche), and PhosStop phosphatase-inhibitor cocktail (Roche) and incubated at 4 °C for 10 min. After centrifugation at 6,000 x g for 10 min at 4 °C, the supernatants were collected to isolate the cytoplasmic fractions. Nuclear pellets were washed with cytoplasmic-extract buffer and resuspended in RIPA buffer containing 50 mM Tris, pH 8.0, 1% sodium deoxycholate, 0.1% SDS, complete EDTA-free protease-inhibitor cocktail, and PhosStop phosphatase-inhibitor cocktail. After incubation for 10 min at 4 °C and centrifugation at 20,000 x g for 30 min at 4 °C, the supernatants were collected to isolate the nuclear fractions.

Preparation of ADAR1-mutant proteins and in vitro editing assays. Preparation of the baculovirus construct HAT-ADAR1p110 was as described previously

Constructs with phosphorylation-site mutations of HAT-ADAR1p110 were prepared by PCR with mutagenesis primers (Supplementary Table 6). Recombinant HAT-ADAR1p110 proteins were purified from SF9 cell extracts as described previously.39 The A-to-I RNA-editing activity of ADAR1p110 mutants was determined as described previously.

Preparation of Dicer–ADAR1 complexes and pre-miRNA cleavage assays. FLAG-Dicer–HAT-ADAR1p110-mutant complexes were purified through two consecutive Talon and anti-Flag affinity column chromatography steps, and prelet-7a-cleavage assays were performed as described previously.

Purification of Xpo5, Ran–GTP, and TRN1 recombinant proteins. The expression constructs Exportin-5 (pQ60-Exp5)33 and RanQ69L (pET-RanQ69L)34 were purchased from Addgene. The Transportin-1 expression plasmid (pET28-His-TRN1)35 was a gift from G. Dreyfuss. Escherichia coli cells transformed with constructs for expression of histidine-fusion proteins were grown at 37 °C in LB medium supplemented with 2% (vol/vol) ethanol to an A₆₀₀ of 0.6 and induced with 1 mM IPTG for His-Transportin-1 and His-Ran(Q69L) or with 100 mM IPTG for His-Exportin-5 at 20 °C overnight. Harvested E. coli cells were sonicated, and the soluble fraction was incubated with 1 mM CaCl₂ and 400 U/mL micrococcal nuclease for 12 min at room temperature. After the NaCl concentration was adjusted to 500 mM, the extracts were applied to nickel–nitriilotriacetic acid beads (Qiagen) for 1 h at 4 °C. The beads were washed twice with buffer A (20 mM HEPES, pH 8.0, 150 mM NaCl, 5 mM imidazole, 20% glycerol, 7 mM β-mercaptoethanol, 1 mM phenylmethylsulfonylfluoride, and 1× protease-inhibitor cocktail), twice with buffer C containing 2 M NaCl, and once in buffer A containing 100 mM NaCl. Histidine-tagged proteins were eluted in buffer A containing 150 mM imidazole and then dialyzed against buffer B (20 mM HEPES, pH 8.0, 100 mM NaCl, 20% glycerol, and 7 mM β-mercaptoethanol).

Xpo5 in vitro binding assay. The VAI RNA derivative (VARdm), which lacks the short bulge in the middle of the dsRNA structure, was used for preassembly of the Xpo5–Ran–GTP–RNA ternary complex, as described previously.38 VARdm was synthesized by T7 in vitro transcription with a partial dsDNA composed of an antisense DNA oligonucleotide containing VARdm and 77 promoter sequences and a sense DNA oligonucleotide containing the T7 promoter sequence (Supplementary Table 6) with a MEGAscript T7 Transcription Kit (Thermo Fisher). The binding-assay mixture containing 80 nM of His-Xpo5, 80 nM of His-RanQ69L, 2 mM GTP, and 80 nM of the VAI RNA derivative VARdm in a total volume of 25 µl binding buffer (20 mM HEPES-KOH, pH 7.0, 50 mM NaCl, 1 mM MgCl₂, 0.01% NP-40, 5% glycerol, 1 mM EGTA, 1 mg/mL BSA, and complete EDTA-free protease-inhibitor cocktail (Roche)) was preincubated for 10 min at 37 °C. In parallel, 80 nM of FLAG-ADAR1p110 was prepared in binding buffer in a total volume of 25 µl. After preincubation, the Xpo5–Ran–GTP–VAI RNA complex was added to FLAG-ADAR1p110 and incubated further for 10 min at 37 °C. The mixture was then added to 10 µl of packed anti-FLAG M2 magnetic beads (Sigma), which had been washed and blocked with binding buffer containing 10 mg/mL BSA. After incubation for 15 min at 4 °C with gentle agitation, beads were captured with a magnetic bead separator (Thermo Fisher) and then washed with binding buffer five times. The beads were dissolved in Laemmli buffer and boiled at 95 °C for 5 min. Samples were loaded on SDS–PAGE gels, and immunoblotting was performed with anti-Xpo5, anti-Ran, and anti-ADAR1 antibodies (Supplementary Table 7).

TRN1 in vitro binding assay. The reaction mixture of 80 nM of FLAG-ADAR1p110 in a total volume of 25 µl of binding buffer containing 20 mM HEPES-KOH, pH 7.0, 50 mM NaCl, 1 mM MgCl₂, 0.05% NP-40, 5% glycerol, 1 mM EGTA, 1 mg/mL BSA, and complete EDTA-free protease-inhibitor cocktail (Roche) was preincubated for 15 min at 37 °C. In parallel, 80 nM of His-TRN1 in 25 µl of binding buffer was added and incubated for 10 min at 37 °C; this was followed by anti-FLAG M2 magnetic bead capture, washing and elution, as described above for the Xpo5 binding assay. For immunoblotting, anti-TRN1 antibody (Supplementary Table 7) was used.

Fluorescence microscopy. Transfection of fluorescent-protein-tagged plasmids or siRNAs into A172 cells was carried out as described above. After electroporation, cells were seeded on μ-Dish 35-mm high dishes (Ibidi) and incubated at...
37 °C for 24–36 h. Cells were then washed with PBS and irradiated with UV (40 J/m²). Microscopic analysis was performed in normal medium containing 1 µg/ml Hoechst 33342 (Life technologies) for nuclear staining. Microscopic images were obtained with a Leica TCS SP5 DMI6000 CS confocal microscope equipped with an ultraviolet 405 diode and argon DPPS561 and HeNe 594 lasers. Fluorescence images were captured with a 63× oil lens zoomed in 1–3× with a 1,024 × 1,024 frame and 400–Hz scanning speed. For multicolor experiments, the following wavelength settings were used: mCherry, excitation 594 nm/ emission 604–766 nm; EGFP, excitation 488 nm/emission 498–567 nm; YFP, excitation 514 nm/emission 520–590 nm; dsRed, excitation 561 nm/emission 571–723 nm; and Hoechst, excitation 405 nm/emission 414–475 nm. Images were further analyzed with Leica LAS AF software and ImageJ (NIH).

Quantitative RT–PCR. Total RNA samples were extracted with an miRNAeasy Kit (Qiagen), and quantification of select mRNAs and pre-mRNAs was performed by qRT–PCR. Reverse transcription was performed with 1 µg of total RNA with a Transcriptor First Strand cDNA Synthesis Kit (Roche), and the resultant cDNA, equivalent to 20 ng of total RNA, was used for qPCR with Power SYBR Green Master Mix (Thermo Fisher) and a 7900HT Fast Real-Time PCR system (Applied Biosystems). Each experiment was conducted in quadruplicate. Primers used are listed in Supplementary Table 6.

Combined RIP and qRT–PCR analysis. The RIP procedure was performed as described previously56 with some modifications. A172 cells were first transfected with siControl or siADAR1-1 and then with HA-Staufen1. The cells were fixed with 0.1% formaldehyde in PBS at room temperature for 5 min. The cytoplasmic fraction was sonicated, and the lysate was incubated for 6 h at 4 °C with 100 µl (1 mg) of anti-HA magnetic beads (Thermo Fisher) prewashed and blocked with 20% BSA in lysis buffer. The RNA–protein complexes captured on beads were washed five times with high-salt buffer (50 mM Tris-HCl, pH 7.6, 1 M NaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS, and 1 mM EDTA), then with suspension buffer (50 mM Tris-HCl, pH 7.6, 150 mM NaCl, and 1% NP-40). At the final washing step, beads were divided into two aliquots, one for RNA extraction and the other for protein preparation. RNA was reverse-cross-linked as described previously57 and purified with TRIzol LS (Thermo Fisher). qRT–PCR was performed with primers designed at the non-double-stranded region adjacent to the predicted dsRNA structure in each mRNA 3′ UTR (Supplementary Table 6).

Immunoblotting analysis. Cell lysates were prepared in Laemmli buffer (Boston Bioproducts) containing benzamidine nuclease (Sigma, E8263), Complete, ENDO- free protease-inhibitor cocktail (Roche), and PhosStop phosphatase-inhibitor cocktail (Roche), and fractionated by 4–15% SDS-PAGE. For detection of phosphorylated ADAR1 proteins, 6% SDS-polyacrylamide gel containing 25 µM of Phos-tag acrylamide (Wako) and 125 µM of MnCl₂ was used. For confirmation of phosphorylation, cell lysates were treated with 4 U/µl of Lambda phosphatase (NEB) at 30 °C for 30 min before Phos-tag immunoblotting analysis. Proteins were transferred to Immobilon-P nylon membranes (Millipore). Blots were blocked with 1% Blocker BSA buffer (Thermo Fisher) and incubated with primary antibodies (Supplementary Table 7) overnight at 4 °C. After incubation with secondary antibodies, membranes were developed with ECL (GE Healthcare).

LC-MS/MS analysis of phosphorylated ADAR1p110. Cell lysates were prepared from HEK293T (ATCC CRL-11268) constitutively expressing FLAG-ADAR1p110 in a buffer containing 50 mM Tris-HCl, pH 7.6, 150 mM NaCl, 0.5% NP-40, complete EDTA-free protease-inhibitor cocktail (Roche), and PhosStop phosphatase-inhibitor cocktail (Roche). FLAG-ADAR1p110 proteins were purified on anti-FLAG M2 monoclonal-antibody agarose beads (Sigma). The eluted fraction was concentrated through ethanol precipitation and stored at −80 °C overnight. The concentrated FLAG-ADAR1p110 (~30 µg from 4 × 10⁸ cells) was loaded on 7% NuPAGE Tris-acetate gels (Thermo Fisher) or 6% SDS–PAGE gel containing 25 µM of Phos-tag acrylamide and 125 µM of MnCl₂. Gels were stained with a Colloidal Blue Staining Kit (Thermo Fisher), the band corresponding to ~110 kDa was excised, and proteins in the gel were digested with trypsin (Promega). Reverse-phase liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis was performed with a Q Exactive HF mass spectrometer (Thermo Fisher) coupled with a Nano-ACQUITY UPLC system (Waters).

MS/MS spectra were screened with MaxQuant 1.5.1.2 (ref. 58) against the human UniProt protein database (July 2014) with full tryptic specificity with up to two missed cleavages, static carboxamidomethylation of cysteine, and variable oxidation of methionine, protein N-terminal acetylation, deamidation of asparagine, and phosphorylation of serine, threonine, and tyrosine. Modified peptides were required to have a minimum score of 40. Consensus identification lists were generated with false discovery rates of 1% at the protein, peptide, and site levels.

Generating the ADAR1 dsRBD3 model. To generate a model of the human ADAR1 dsRBD3 region (710–830), we used the software Protein Homology/ analogy Recognition Engine V 2.0 (Phyre 2), which produces a model of the protein of interest on the basis of sequence alignment to known structures59. Phyre 2 generated several models of this region of human ADAR1 corresponding to dsRNA-binding protein motifs (dsRBMs) with 100% confidence. From the list of models generated by Phyre 2, we selected the solution structure of the Rutters nervous Xenopus ADAR2 dsRBM in complex with dsRNA60. The two proteins have 25% sequence identity and are both involved in dsRNA binding. The model was further refined by application of geometry minimization in PhenoX61. The final model was used to generate figures in PyMOL62.

RNA-seq analysis. A172 cells were transfected with siControl, siADAR1-1, siStaufen1-1, or siADAR1-1 and siStaufen1-1, then incubated for 60 h. Cells were washed with PBS and irradiated with UV (40 J/m²) and harvested at 6 h after irradiation. Total RNA was extracted with an miRNAeasy Kit. Cultures were prepared in duplicate for each condition. Preparation of RNA-seq samples was carried out with a strand-specific SENSE mRNA Library Preparation Kit V2 (Lexogen). Paired-end 151-bp sequencing was performed with a NextSeq 500 platform (Illumina).

Raw sequencing RNA-seq reads were trimmed 10 bp at the 5′ and 30 bp at the 3′ ends and aligned with the Bowtie2 algorithm, allowing for up to two mismatches. RSEM v1.2.12 software63 was used to estimate fragment counts and FPKM values at the gene level with the transcriptome table for the hg19 genome downloaded from the UCSC Genome Browser (http://genome.ucsc.edu/; tables hg19.knownGene). EdgeR64 was used to estimate the significance of differential expression between two experimental groups. Edited sites were determined with SAMtools v0.1.19 (ref. 65). Only A-to-G editing events occurring in any sample were considered, and coordinates for all found events were used to obtain direct A/C/G/T counts across all samples. The sums of counts between replicates were calculated, and significance of differences in editing between any two conditions was tested with Fisher's exact test. Genes with at least one edited site that showed a significant decrease (P < 0.05) in a sample compared with siControl samples were considered to be edited by ADAR1.

Statistical analysis. Experiments were run in triplicate or quadruplicate and were repeated in a minimum of three independent trials. Image quantification was performed with ImageQuant image-analysis software (GE Healthcare). Data are presented as means ± s.d. or s.e.m. Two-tailed t tests were conducted, and the minimum level for significance was P < 0.05.

Data availability. RNA-seq data from this study have been deposited in the Gene Expression Omnibus database under series accession number GSE85455. The mass spectrometry proteomics data have been deposited in the ProteomeXchange Consortium via MassIVE (accession number MSV000080146) with the data set identifier PXD004959. Other data from this study are available from the corresponding author upon reasonable request.

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