1 **Recognition of Z-RNA by ADAR1 limits interferon responses**

2

3 Qiannan Tang¹, Rachel E. Rigby^{1,†}, George R. Young^{2,†}, Astrid Korning-Hvidt¹,
4 Tiong Kit Tan¹, Anne Bridgeman¹, Alain R. Townsend^{1,3}, George Kassiotis^{4,5} &
5 Jan Rehwinkel^{1,*}

6

7 ¹Medical Research Council Human Immunology Unit, Medical Research
8 Council Weatherall Institute of Molecular Medicine, Radcliffe Department of
9 Medicine, University of Oxford, Oxford, OX3 9DS, UK.

10 ²Retrovirus–Host Interactions, The Francis Crick Institute, London, UK.

11 ³Centre for Translational Immunology, Chinese Academy of Medical Sciences
12 Oxford Institute, University of Oxford, Oxford, UK.

13 ⁴Retroviral Immunology, The Francis Crick Institute, London, UK.

14 ⁵Department of Infectious Disease, Faculty of Medicine, Imperial College
15 London, London, UK.

16

17 *correspondence: jan.rehwinkel@imm.ox.ac.uk

18

19 [†]These authors contributed equally.

20

21 **Keywords**

22 ADAR1, Z α domain, Z-RNA, MDA5, MAVS, interferon, influenza A virus

23

24 **Running Title**

25 Z-RNA recognition by ADAR1

26 **Abstract**

27 Nucleic acids are powerful triggers of innate immunity and can adopt the
 28 unusual Z-conformation. The p150 isoform of adenosine deaminase acting on
 29 RNA 1 (ADAR1) prevents aberrant interferon (IFN) induction and contains a Z-
 30 nucleic acid binding ($Z\alpha$) domain. We report that knock-in mice bearing two
 31 point mutations in the $Z\alpha$ domain of ADAR1, which abolish binding to Z-form
 32 nucleic acids, spontaneously induced type I IFNs and IFN-stimulated genes
 33 (ISGs) in multiple organs. This included the lung where both stromal and
 34 haematopoietic cells displayed ISG induction in *Adar1^{mZ α /mZ α}* mice.
 35 Concomitantly, *Adar1^{mZ α /mZ α}* mice showed improved control of influenza A virus.
 36 The spontaneous IFN response in *Adar1^{mZ α /mZ α}* mice required MAVS,
 37 implicating cytosolic RNA sensing. Finally, analysis of A-to-I changes revealed
 38 a specific requirement of ADAR1's $Z\alpha$ domain in editing of a subset of RNAs.
 39 In summary, our results reveal that endogenous RNAs in Z-conformation have
 40 immunostimulatory potential that is curtailed by ADAR1.

41 Introduction

42 The innate immune system monitors the intra- and extracellular
 43 environments for unusual nucleic acids (Bartok and Hartmann, 2020). This
 44 process, known as ‘nucleic acid sensing’, detects pathogen invasion and
 45 disturbances to homeostasis. It involves a large number of germline encoded
 46 nucleic acid sensors. Upon engagement by immunostimulatory DNA or RNA,
 47 these sensors signal to initiate a large spectrum of responses, including
 48 transcription of the genes encoding type I interferons (IFNs). Type I IFNs –
 49 secreted cytokines that act in paracrine and autocrine manner – induce
 50 expression of hundreds of IFN-stimulated genes (ISGs). The proteins encoded
 51 by ISGs mediate a plethora of functions and include antiviral effectors
 52 (Schoggins, 2019). Sustained type I IFN responses can have detrimental
 53 effects and cause a range of diseases including the neuroinflammatory Aicardi-
 54 Goutières Syndrome (AGS) (Uggenti et al., 2019). It is therefore important to
 55 understand the molecular mechanisms that prevent activation of nucleic acid
 56 sensors by ‘normal’ DNA and RNA present in healthy cells (Bartok and
 57 Hartmann, 2020).

58 We and others proposed that double-stranded (ds) nucleic acids adopting
 59 an unusual conformation known as Z-DNA/RNA activate innate immunity
 60 (Maelfait et al., 2017; Sridharan et al., 2017; Zhang et al., 2020b). Z-DNA was
 61 initially described by Alexander Rich (Wang et al., 1979). Unlike canonical B-
 62 DNA, a right-handed double helix, Z-DNA is a left-handed double helix with a
 63 zig-zag-shaped phosphodiester back bone (Wang et al., 1979). dsRNA can also
 64 adopt the Z-conformation (Davis et al., 1986; Hall et al., 1984). Biological
 65 functions of Z nucleic acids, in particular those of Z-RNA, are incompletely
 66 understood (Herbert, 2019). A small number of proteins, all involved in innate
 67 immunity, contain Z-DNA/RNA binding domains known as Z α domains
 68 (Athanasiadis, 2012). These domains specifically bind to and stabilise Z-

69 DNA/RNA, or induce the Z-conformation (Athanasiadis, 2012; Brown et al.,
70 2000; Kim et al., 2018; Schwartz et al., 1999).

71 There are two mammalian proteins with $Z\alpha$ domains: Z-DNA binding protein
72 1 (ZBP1) and adenosine deaminase acting on RNA 1 (ADAR1, also known as
73 DRADA1). ZBP1 contains two $Z\alpha$ domains that have been suggested to
74 recognise viral and endogenous Z-RNAs (Devos et al., 2020; Jiao et al., 2020;
75 Maelfait et al., 2017; Sridharan et al., 2017; Wang et al., 2020; Zhang et al.,
76 2020b). Binding to Z-RNA activates ZBP1 and results in the induction of
77 necroptosis, an inflammatory form of cell death (Maelfait et al., 2020).

78 ADAR1 has two splice isoforms: ADAR1-p110, which is constitutively
79 expressed and localised in the cell nucleus, and ADAR1-p150 that is IFN-
80 inducible and present in the nucleus and cytosol. Both isoforms contain a C-
81 terminal deaminase domain that converts adenosine to inosine in dsRNA, a
82 process known as A-to-I RNA editing. Conversion of adenosine to inosine in
83 protein-coding sequences can lead to incorporation of non-synonymous amino
84 acids during translation due to base pairing of inosine with cytosine. However,
85 the vast majority of A-to-I editing events occur in non-coding RNAs (Eisenberg
86 and Levanon, 2018; Reich and Bass, 2019). This includes transcripts from
87 repetitive elements (REs), particularly *Alu* elements and short interspersed
88 nuclear elements (SINEs) in human and mouse, respectively. Both ADAR1
89 isoforms further contain three dsRNA binding domains (dsRBDs) and a so-
90 called $Z\beta$ domain. $Z\beta$ adopts a fold similar to $Z\alpha$ domains but does not bind Z-
91 form nucleic acids due to substitutions of key amino acids (Athanasiadis et al.,
92 2005; Kim et al., 2003). ADAR1-p150 has an extended N-terminus harbouring
93 a $Z\alpha$ domain (Heraud-Farlow and Walkley, 2020).

94 ADAR1 deficiency results in profound inflammatory phenotypes. In human,
95 germline *ADAR1* mutations cause AGS (Rice et al., 2012). These mutations
96 predominantly map to the deaminase domain; however, one mutation encoding

p.Pro193Ala is found in the Z α domain. Pro193 contributes to Z-form nucleic acid binding (Schwartz et al., 1999) and changing it to Ala reduces RNA editing in a reporter assay (Mannion et al., 2014). *Adar1*^{-/-} mice, editing-deficient *Adar1*^{E861A/E861A} knock-in animals and *Adar1*^{p150-/p150-} mice, which only lack ADAR1-p150, all die *in utero* (Hartner et al., 2004; Liddicoat et al., 2015; Wang et al., 2004; Ward et al., 2011). Akin to spontaneous type I IFN induction in AGS patients with *ADAR1* mutation, *Adar1*^{-/-} and *Adar1*^{E861A/E861A} embryos display profound type I IFN responses prior to death (Hartner et al., 2009; Liddicoat et al., 2015). Multiple nucleic acid sensors mediate the anti-proliferative, cell death and type I IFN phenotypes observed in ADAR1-deficient settings: the oligoadenylate synthetase (OAS)–RNase L system (Li et al., 2017), protein kinase R (PKR) (Chung et al., 2018; Li et al., 2010) and melanoma differentiation-associated protein 5 (MDA5) (Liddicoat et al., 2015; Mannion et al., 2014; Pestal et al., 2015). Upon activation by dsRNA, OAS proteins synthesise 2'-5' oligoadenylate, a second messenger that in turn activates RNase L, resulting in widespread RNA degradation. PKR also detects dsRNA and represses protein translation. Both effects have been proposed to explain lethality of ADAR1-deficient cells (Chung et al., 2018; Li et al., 2017). Induction of type I IFNs in ADAR1-deficient mice and human cells is mediated by the RNA sensor MDA5, that signals via its adaptor mitochondrial antiviral-signalling protein (MAVS) (Bajad et al., 2020; Chung et al., 2018; Guallar et al., 2020; Liddicoat et al., 2015; Mannion et al., 2014; Pestal et al., 2015).

These observations suggest a model in which endogenous dsRNAs are stabilised in ADAR1-deficient cells due to the absence of RNA editing and are then recognised by RNA sensors (Dias Junior et al., 2019; Eisenberg and Levanon, 2018). Indeed, some ADAR1 substrates such as transcripts from *Alu* elements base-pair to form duplex structures, which are predicted to be destabilised by inosine : uracil mismatches introduced by RNA editing (Ahmad

et al., 2018; Chung et al., 2018; Pfaller et al., 2018; Song et al., 2020). This concept has been tested experimentally in an RNase protection assay: transcripts spanning *Alu* elements in inverted orientation are protected by recombinant MDA5 protein in RNA samples extracted from ADAR1-deficient cells (Ahmad et al., 2018; Mehdipour et al., 2020).

How the different nucleic acid binding domains in ADAR1 select and recruit RNA substrates for subsequent editing is unknown. In light of (i) emerging roles of Z-form nucleic acids in innate immunity (Kesavardhana and Kanneganti, 2020), (ii) the MAVS-dependent phenotype of *Adar1*^{p150-/p150-} mice (Pestal et al., 2015), and (iii) natural occurrence of the p.Pro193Ala mutation in AGS patients (Rice et al., 2012), we interrogated Z-form nucleic acid binding by the Z α domain in ADAR1-p150. Here, we report the generation of knock-in mice bearing two missense mutations in the Z α domain, which prevent nucleic acid binding. Although these *Adar1*^{mZ α /mZ α} mice were developmentally normal and fertile, they displayed spontaneous induction of type I IFNs and ISGs in multiple organs and cell types. This phenotype was dependent on MAVS and conferred partial protection against influenza A virus (IAV) infection. Analysis of sequencing data revealed that ~8% of RNA editing events in wild-type (WT) cells required a functional ADAR1-p150 Z α domain. Taken together, our study delineates the function of the Z α domain in ADAR1-p150 *in vivo* and suggests that recognition of Z-form RNA by ADAR1 contributes to the suppression of IFN responses.

Results

Generation of Z α domain mutated mice

To study the role of Z-form nucleic acid binding to the Z α domain in ADAR1-p150 in an *in vivo* setting, we generated knock-in mice bearing two missense mutations: p.Asn175Ala and p.Tyr179Ala. These residues are conserved and are homologous to Asn173 and Tyr177 in human ADAR1; they were chosen because of their essential role in Z-form nucleic acid binding (Feng et al., 2011; Li et al., 2009; Schade et al., 1999; Schwartz et al., 1999). Given the embryonic lethality of *Adar1*^{p150-/p150-} mice (Ward et al., 2011), we opted for a conditional knock-in strategy (Figure S1A). The Z α domain is encoded by exon 2 of the *Adar1* gene. In brief, we introduced in inverted orientation into the intron between exons 2 and 3 a mutated copy of exon 2 (designated 2*) containing four nucleotide substitutions, changing both Asn175 and Tyr179 to Ala. We flanked exons 2 and 2* with LoxP and Lox2272 sites such that Cre-mediated recombination removes exon 2 and flips exon 2* into forward orientation (Figure S1A). We designated the conditional allele '*fl-mZ α* ' and the recombined allele expressing mutant Adar1 '*mZ α* '. To determine the impact of the Z α domain mutations when present in all cells and tissues, we crossed *Adar1*^{+/*fl-mZ α*} mice with a line expressing Cre recombinase under control of the ubiquitously active *Pgk* promoter. The resulting *Adar1*^{+/*mZ α*} mice were intercrossed to generate homozygous animals. We validated the presence of the mutations by sequencing and found that *Adar1*^{*mZ α* /*mZ α*} animals were born at expected mendelian ratios (Figure S1B and S1C). Furthermore, they developed normally and were fertile. The mutations introduced into the Z α domain did not alter the expression of the two isoforms of ADAR1 in bone marrow-derived myeloid cells (BMMCs) (Figure S1D). Taken together, Z-form nucleic acid binding by ADAR1-p150 is not essential for survival at whole organism level.

Mutation of the Z α domain in ADAR1-p150 triggers a multi-organ type I IFN response

We tested whether *Adar1*^{mZ α /mZ α} mice display spontaneous activation of type I IFNs as was reported in ADAR1-deficient settings. We collected lung, liver and spleen tissues and extracted RNA for RT-qPCR analysis. Transcript levels of *Ifnb1* (encoding IFN β) were elevated in lung RNA samples from *Adar1*^{mZ α /mZ α} mice (Figure 1A). Moreover, the ISG transcripts *Ifit1* and *Ifi44* were expressed at higher levels in all three organs from Z α domain mutated animals (Figure 1A). This gene expression signature was type I IFN-specific: tissues from *Adar1*^{mZ α /mZ α} mice contained comparable mRNA levels of *Ifng*, *Tnfa* and *Il1b* (encoding IFN γ , TNF α and pro-IL-1 β), with only a minor increase of *Tnfa* mRNA in lung (Figure 1A). We validated the ISG signature at protein level by analysing expression of interferon stimulated gene 15 (ISG15) in whole lung lysates. ISG15 is a ubiquitin-like modifier that is induced by IFN and is conjugated to target proteins in a process called ISGylation (Perng and Lenschow, 2018). Western blot showed that lung lysates from *Adar1*^{mZ α /mZ α} mice contained increased amounts of monomeric ISG15 as well as increased levels of ISGylated proteins, visible as a high-molecular weight smear (Figure 1B).

We next analysed different types of cultured primary cells from WT and *Adar1*^{mZ α /mZ α} mice. We observed heightened ISG expression in Z α domain mutated lung fibroblasts (Figure 1C). In contrast, comparable levels of ISG transcripts were found in WT and *Adar1*^{mZ α /mZ α} BMMCs, as well as in mouse embryonic fibroblasts (MEFs) (Figure 1D, E). This indicates that ISG induction may be cell-type specific.

Among the organs we analysed, lung exhibited the most profound ISG signature, and spontaneous ISG induction was also observed in cultured lung

fibroblasts (Figure 1A, D). We therefore focused on the lung for the next set of experiments. We used RNA extracted from lung for RNA sequencing to obtain a global view of gene expression in *Adar1^{mZα/mZα}* mice. We found that 99 protein coding genes were differentially expressed by at least 2-fold in mutant lungs, including 89 upregulated and 10 downregulated transcripts (Figure 2A). 40% of the induced mRNAs were encoded by ISGs; this included well-known factors such as *Irf7*, *Cxcl10*, *Zbp1* and *Usp18* (Figure 2A). We used gene ontology (GO) analysis of biological processes to further classify upregulated genes. GO terms related to type I IFNs and anti-viral defence were enriched amongst genes induced in *Adar1^{mZα/mZα}* lungs (Figure 2B). The most highly enriched GO category, regulation of ribonuclease activity, included many *Oas* transcripts, which are known to be IFN-inducible (Schoggins, 2019).

We also analysed REs in our RNA sequencing data and found 540 induced and 112 repressed REs (Figure 2C and Table S1). SINEs were underrepresented amongst differentially expressed REs, whilst long terminal repeat (LTR) elements were enriched (Figure 2C). Dysregulation of REs has been reported in settings of inflammation and can be driven by IFNs (Chuong et al., 2016). Taken together, these data show that the lungs of *Zα* domain mutated animals displayed a type I IFN-driven gene signature.

Stromal and haematopoietic cells contribute to the ISG signature in the lungs of *Adar1^{mZα/mZα}* mice

To identify the type of cell(s) that display the ISG signature in the lungs of *Adar1^{mZα/mZα}* mice, we used magnetic-activated cell sorting (MACS) to isolate haematopoietic cells, marked by CD45 expression, and stromal cells lacking this marker. There was no difference in the proportions of haematopoietic and stromal cells between WT and *Adar1^{mZα/mZα}* lungs (Figure 3A). We confirmed the purity of MACS-enriched haematopoietic and stromal cells to be >97%

(Figure S2). We then extracted RNA for RT-qPCR analysis of ISGs and found that both lung haematopoietic and stromal cells displayed ISG induction (Figure 3B). For the three ISGs tested, fold inductions in *Adar1^{mZα/mZα}* samples were similar in CD45+ and CD45- cells (Figure 3B). However, it is noteworthy that baseline ISG levels appeared to be lower in haematopoietic cells compared to stromal cells (Figure 3B).

Next, we analysed ISG expression in different types of lung haematopoietic and stromal cells. Using two staining panels and fluorescence-activated cell sorting (FACS), we obtained eight different populations of CD45+ cells, including B cells, T cells, dendritic cells (DCs), monocytes, macrophages, NK cells, neutrophils and eosinophils (Figure S3). There were no differences between WT and *Adar1^{mZα/mZα}* mice in the proportions of these cell populations, with the exception of DCs, which were less abundant in the mutant lungs (Figure S3A). *Ifit1*, *Ifit2* and *Isg15* were significantly induced in neutrophils from the lungs of *Adar1^{mZα/mZα}* mice (Figure 4A, B). However, these ISGs showed no, or limited and statistically insignificant, upregulation in other types of CD45+ cells (Figure 4A, B). In contrast, the ISGs *Ifi44* and *Oas1a* were upregulated in multiple haematopoietic cell types (Figure 4A, B). Transcripts encoding type I IFNs were undetectable by RT-qPCR in all samples analysed (data not shown). Several ISGs, including *Ifit1*, *Ifit2* and *Isg15*, are not only induced by IFNAR signalling but can also be upregulated by IRF3/7, independent of type I IFN-initiated JAK-STAT activation (Ashley et al., 2019; DeFilippis et al., 2006; Goubau et al., 2009; Grandvaux et al., 2002; Lazear et al., 2013). Induction of this ISG-subset can therefore occur in a cell-autonomous manner, for example when aberrant nucleic acids are sensed by pattern recognition receptors (PRRs) that signal via IRF3/7. Other ISGs, including *Ifi44* and *Oas1a*, are only induced via IFNAR signalling (Lazear et al., 2013). Therefore, our observation that *Ifit1*, *Ifit2* and *Isg15* were primarily induced in neutrophils, while *Ifi44* and *Oas1a* were

upregulated more broadly (Figure 4A, B), suggests that *Adar1*^{mZα/mZα} neutrophils may autonomously activate IRF3. We further speculate that the ISG signature in other haematopoietic cells such as B cells may be due to paracrine type I IFN signalling via IFNAR.

We also analysed the lung stromal compartment and isolated by FACS endothelial and epithelial cells using CD31 and CD326 as markers, respectively (Figure S4). We further isolated CD45⁻ cells lacking these markers; this mixed population likely included fibroblasts and other cell types. We observed induction of *Ifit1*, *Isg15* and *Ifi44* in *Adar1*^{mZα/mZα} cells of all three populations analysed (Figure 4C). It is therefore likely that, in addition to neutrophils, multiple non-haematopoietic cells initiate type I IFN production in the lungs of *Adar1*^{mZα/mZα} mice.

Haematopoietic cells are sufficient to induce ISGs in *Adar1*^{mZα/mZα} mice

To further dissect the cellular requirements for ISG induction in *Adar1*^{mZα/mZα} mice, we generated bone marrow (BM) chimeric animals. Lethally irradiated *Cd45.1* WT recipients were reconstituted with *Cd45.2* BM from WT or *Adar1*^{mZα/mZα} animals (Figure S5A). We found reconstitution levels of the haematopoietic compartment to be about 90% by analysing cell surface levels of CD45.1 and CD45.2 on white blood cells from recipient mice (Figure S5B). Similar levels of reconstitution were observed in the lung (Figure S5C). We first obtained RNA from whole lung tissue for RT-qPCR analysis. The ISGs *Ifit1*, *Oas1a* and *Zbp1* were upregulated in *Adar1*^{mZα/mZα}→WT chimeras compared to WT→WT chimeras (Figure 4D, “Total”). This indicates that *Adar1*^{mZα/mZα} BM-derived cells triggered an IFN response in WT animals. We also isolated different cell populations from the lungs of chimeric animals by FACS (see Figure S3 for gating), extracted RNA and performed RT-qPCR. *Ifit1* and *Ifit2* mRNA levels were significantly induced in neutrophils from *Adar1*^{mZα/mZα}→WT

chimeras, but not in B cells and CD45⁻ cells (Figure 4D). In contrast, *Oas1a* and/or *Zbp1* were upregulated in multiple cell populations including CD45⁻ cells, neutrophils and B cells (Figure 4D). These observations are consistent with the results shown in Figure 4A and 4B and indicate that neutrophils may initiate type I IFN production that then results in ISGs induction in other cell types. Taken together, *Adar1*^{mZα/mZα} haematopoietic cells were sufficient to induce ISGs in the lungs of WT mice.

IAV replication is inhibited in *Adar1*^{mZα/mZα} mice

In light of the spontaneous type I IFN response in *Adar1*^{mZα/mZα} mice, we next asked whether these Zα domain mutant mice were protected against viral infection. ISGs upregulated in the lungs of *Adar1*^{mZα/mZα} mice included factors such as *Ifit1* and *Zbp1* that are known to control IAV (Pichlmair et al., 2011; Zhang et al., 2020b) (Figure 2A). We intranasally infected WT and *Adar1*^{mZα/mZα} mice with the H3N2 strain of recombinant IAV (A-X31; A/HongKong/1/1968) using a dose that caused moderate weight loss. WT animals lost about 10% body weight from day 3 until day 7 after infection, and subsequently recovered their weight (Figure 5A). In contrast, *Adar1*^{mZα/mZα} mice gained weight until day 4 after infection (Figure 5A). This was followed by weight loss on days 5-7, which slightly exceeded weight loss in WT mice, and recovery from day 8 onwards. These observations suggest that *Adar1*^{mZα/mZα} mice were protected against IAV at an early stage of the infection.

We further characterised early IAV infection in *Adar1*^{mZα/mZα} mice at day 3 after inoculation. We calculated a 'lung index' (lung weight/body weight x100; Luo et al., 2012) and found this marker of pathology and inflammation to be significantly higher in infected WT mice compared to *Adar1*^{mZα/mZα} animals (Figure 5B). Next, we analysed viral loads by determining the levels of the viral NP and M transcripts by RT-qPCR. Compared to infected WT lungs, levels of

these viral RNAs were reduced in infected *Adar1^{mZα/mZα}* lungs (Figure 5C). Concomitantly, mRNA levels of *Ifnb1*, *Ifng*, *Il1b*, *Tnfa* and *Il6*, as well as IL6 protein, were strongly induced in infected WT lungs, and these effects were curtailed in *Adar1^{mZα/mZα}* lungs (Figure 5D, E). In sum, IAV replication and virus-induced inflammation were reduced in *Adar1^{mZα/mZα}* animals.

ISG induction in *Adar1^{mZα/mZα}* mice is MAVS-dependent

We next investigated which nucleic acid sensing pathway triggered spontaneous ISG expression in *Adar1^{mZα/mZα}* mice. Since ADAR1-deficiency results in activation of the MDA5-MAVS pathway (Liddicoat et al., 2015; Mannion et al., 2014; Pestal et al., 2015), we hypothesised that the ISG signature in *Adar1^{mZα/mZα}* mice was driven by MAVS. To test this, we crossed *Adar1* mutant mice with MAVS knock-out animals to generate *Adar1^{mZα/mZα}; Mavs^{-/-}* mice. Loss of MAVS prevented the ISG induction observed in *Adar1^{mZα/mZα}* lungs, livers and spleens (Figure 6A). Because of the neuropathology observed in patients with AGS, we also analysed brain tissue. *Adar1^{mZα/mZα}* mice showed elevated *Ifnb1* expression and a pronounced ISG signature in the brain (Figure 6B). Akin to the situation in other tissues, these effects were fully MAVS-dependent (Figure 6B). Taken together, the Zα domain of ADAR1-p150 was involved in preventing MAVS-mediated IFN induction, implicating a role in limiting activation of MDA5 or RIG-I.

The Zα domain of ADAR1-p150 is required for editing of a subset of RNAs

Human ADAR1-p150 bearing the p.Pro193Ala mutation shows reduced editing activity in a reporter assay (Mannion et al., 2014). To identify natural RNA substrates edited by ADAR1-p150 in a Zα domain-dependent manner, we analysed our RNA sequencing data from WT and *Adar1^{mZα/mZα}* lungs for A→G transitions. These are indicative of A-to-I RNA editing as inosine pairs with

cytosine during reverse transcription. The most sensitive and specific method for annotating this mutational profile compares the fit of Dirichlet models of observed base frequencies between test and control samples (Piechotta et al., 2017). We extended this methodology to allow comparisons of biological replicates, to incorporate the orientation of the originating RNA (revealed by our stranded RNAseq protocol), to include fine-grained filtering of potential editing sites, and to add a ‘detection’ step, whereby observed base frequencies at potential editing sites are additionally compared to a model of the per-base error rate obtained *de novo* from the dataset.

Considered individually, for each of the three WT and three *Adar1^{mZα/mZα}* lung samples analysed, we detected ~35,000-40,000 editing sites (Figure 7A). Considered as biological replicates, increasing statistical robustness, 40,342 and 46,164 sites were identified that were shared amongst all three WT or *Adar1^{mZα/mZα}* lung samples, respectively. These ‘detected’ sites had a median editing level of ~10% in both WT and *Adar1^{mZα/mZα}* samples (Figure 7B). This shows that there was no global defect in RNA editing in *Adar1^{mZα/mZα}* mice.

We further defined ‘differential’ sites as those that were ‘detected’ in the WT samples and showed higher (>2-fold) levels of editing than in *Adar1^{mZα/mZα}* mice. 3,249 sites (8% of sites ‘detected’ in WT) were ‘differential’ and displayed ~9% median editing in WT lungs, which was strongly reduced for the majority of sites in *Adar1^{mZα/mZα}* mice (Figure 7B). Read depth was equivalent between ‘detected’ and ‘differential’ sites (data not shown), excluding the possibility that lack of editing in *Adar1^{mZα/mZα}* samples was simply due to reduced sequence coverage. We then attempted to understand whether these Zα domain-dependent sites were characterised by unique properties. First, we annotated editing sites to 5’ untranslated regions (UTRs), coding sequences (CDSs), 3’UTRs, intronic and intergenic regions (Figure 7C). The majority of editing sites ‘detected’ in WT samples were found in intronic regions (~83%). 3’UTRs

accounted for ~11% of edits and intergenic regions contained ~5% of sites. Less than 1% of sites mapped to 5'UTRs and CDSs. The annotation of 'differential' sites was similar with a small increase in intronic sites (~86%), while 8% of 'differential' edits were found in 3'UTRs.

Given that many A-to-I RNA editing events are known to occur in repetitive elements (REs) (Eisenberg and Levanon, 2018), we analysed the enrichment of editing events in WT samples, by computing observed versus expected numbers, for each RE class (Figure 7D). This analysis showed that editing events were greatly enriched within SINEs (Figure 7D) and further showed that, with the exception of the B4 family, all SINE sub-families exhibited enrichment, albeit to varying degrees (Figure S6A). Furthermore, there were no large-scale differences between the SINEs represented when comparing 'detected' and 'differential' sites (Figure S6B), suggesting that all SINEs had equal potential to contribute sites that were differentially edited between WT and *Adar1^{mZα/mZα}* mice.

We next analysed the genomic distance of edited SINEs to another SINE in inverted orientation. In human, transcripts spanning inverted repeat *Alu* elements have been suggested to form duplex RNA structures recognised by ADAR1 and MDA5 (Ahmad et al., 2018; Mehdipour et al., 2020). Compared to all SINEs, we found that edited SINEs were closer to another SINE in inverted orientation (Figure 7E), although there was no difference between SINEs containing 'detected' (in WT samples) and 'differential' sites. Taken together, these data demonstrate that a subset consisting of about 8% of editing sites required a functional ADAR1-p150 Zα domain for efficient A-to-I conversion.

Discussion

Although Z-nucleic acids were discovered ~40 years ago, little is known to this date about their biological activities. This is in part due to their thermodynamic properties: the B- and A-conformations of dsDNA and dsRNA, respectively, are energetically favoured compared to the Z-conformation, making Z-DNA and Z-RNA difficult to study (Herbert, 2019). The formation of Z-DNA has been proposed to release torsional strain induced by the movement of polymerases (Wittig et al., 1992; Wolfl et al., 1995). Physiological functions of Z-RNA have remained enigmatic until recently. We and others proposed that Z-RNA is recognised by ZBP1 in settings of viral infection and autoinflammation, resulting in the induction of programmed cell death (Devos et al., 2020; Jiao et al., 2020; Maelfait et al., 2017; Sridharan et al., 2017; Wang et al., 2020; Zhang et al., 2020b).

Here, we studied ADAR1-p150 that like ZBP1 contains a $Z\alpha$ domain specialised in binding to Z nucleic acids. We report spontaneous induction of type I IFNs *in vivo* upon introduction of mutations into the ADAR1-p150 $Z\alpha$ domain that prevent binding to Z-DNA/RNA. This effect was observed in multiple organs and cell types from *Adar1*^{mZ α /mZ α} mice and required MAVS. These data show that RIG-I-like receptors were activated by endogenously generated Z-RNAs, and that editing of these Z-RNAs by ADAR1-p150 limited the response. We therefore reveal type I IFN induction as a new biological function of Z-RNA.

Our computational analysis mapped ~40,000 editing sites in lung RNA samples from WT mice. These sites were enriched in SINEs but not in other classes of REs. Furthermore, we found that edited SINEs were more likely to be in proximity to another SINE in inverted orientation. These results agree with previous findings demonstrating that editing sites are enriched in non-coding sequences containing self-complementary regions predicted to form duplex

RNA structures (Eisenberg and Levanon, 2018; Porath et al., 2017; Solomon et al., 2017; Tan et al., 2017).

Importantly, we revealed that ~8% of editing sites detected in WT samples required a functional ADAR1-p150 Z α domain. This subset of Z α -dependent sites mapped to genomic features and REs similarly to all detected sites. To characterise whether the sequences surrounding Z α -dependent editing sites have a propensity to form Z-RNA, we analysed sequences 500 nt up- and down-stream of editing sites. We tested GC content, given that GC-repeats have a higher tendency to adopt the Z-conformation (Davis et al., 1986). There was no enrichment of GC dinucleotides, or other motifs, around differentially edited sites compared to all detected sites (data not shown). We also used the Z-hunt algorithm (Champ et al., 2004; Ho et al., 1986) to predict the likelihood of sequences around editing sites to form the Z-conformation. In parallel, we calculated the distance of editing sites to genomic regions predicted by SIBZ to form the Z-conformation (Zhabinskaya and Benham, 2011). Neither computational approach revealed differences between ‘detected’ and ‘differential’ sites. It is noteworthy that both algorithms were developed for dsDNA and may be unsuitable for predicting the Z-conformation in RNA. Future studies will be required to dissect the properties the Z α -dependent RNA editing sites.

We also found diminished editing in WT samples at ~14% of the sites detected as edited in *Adar1*^{mZ α /mZ α} mice (data not shown). ADAR1-p150 is encoded by an ISG and *Adar1* transcript levels increased by 1.6-fold in *Adar1*^{mZ α /mZ α} lungs (data not shown). Thus, it is likely that type I IFN-induced expression of ADAR1-p150 in mutant mice and recruitment of A-form dsRNAs via the dsRBDs explains editing at these sites.

ADAR1 mutations in human cause AGS and include the missense p.Pro193Ala mutation in the Z α domain. Interestingly, this *ADAR1* allele is

common with frequencies of up to ~1/160 (www.ensembl.org) (Mannion et al., 2014). In AGS patients, homozygous p.Pro193Ala mutation has not been observed; instead, this mutation occurs together with other *ADAR1* mutations (Rice et al., 2012). It is therefore likely that *ADAR1* p.Pro193Ala is hypomorphic and does not cause disease when present homozygously. Consistent with this notion, we found that *Adar1*^{mZα/mZα} mice did not have any gross abnormalities and were fertile. These mice nonetheless displayed type I IFN and ISG induction in multiple organs. This included the lung and bestowed protection against IAV infection at early stages. It is therefore conceivable that the *ADAR1* p.Pro193Ala variant has been maintained in humans by providing a selective advantage during viral infections due to elevated expression of antiviral factors at baseline.

It is noteworthy in this context that anti- and pro-viral functions of ADAR1 have been reported. ADAR1 limits or controls replication of several viruses, including measles virus, members of *Paramyxoviridae* family, IAV, HIV-1, vesicular stomatitis virus and Hepatitis delta virus (Casey, 2012; Li et al., 2010; Vogel et al., 2020; Ward et al., 2011; Weiden et al., 2014). However, for other viruses such as Zika and KSHV, ADAR1 has been shown to facilitate replication (Zhang et al., 2020a; Zhou et al., 2019). It will be interesting for future studies to determine the role of the *ADAR1* variants unable to bind Z nucleic acids in these viral infections.

ADAR1 has recently emerged as a promising target for cancer treatment. If expressed by transformed cells *in vitro* or by tumours *in vivo*, ADAR1 protects against both cell death and anti-cancer immune responses (Gannon et al., 2018; Ishizuka et al., 2019; Liu et al., 2019). Loss of ADAR1 in cancer cells results in death or reduced growth and sensitises to immunotherapy. Interestingly, the protective effects appear to depend on ADAR1-p150 (Gannon et al., 2018; Ishizuka et al., 2019). It is therefore possible that endogenous Z-RNAs induce

anti-cancer effects upon ADAR1 loss. Future studies should test this, for example by reconstitution of ADAR1-p150 mutants unable to bind Z-RNA. Furthermore, development of inhibitors that target the Z α domain of ADAR1, the Z α - Z-RNA interaction or Z-RNA formation should be considered. Compared to deaminase inhibitors, such 'Z-inhibitors' would have the advantage of specifically targeting the p150 isoform, avoiding possible detrimental consequences of targeting ADAR1-p110 (Pestal et al., 2015).

In addition to the activation of MDA5, loss of ADAR1 also results in activation of the OAS-RNaseL system and PKR. The latter may be particularly important in cancer settings (Gannon et al., 2018; Ishizuka et al., 2019; Liu et al., 2019). Although PKR activation results in a global shutdown of translation, some proteins are selectively made and mediate the integrated stress response (Pakos-Zebrucka et al., 2016). These include the transcription factor ATF4 that induces stress-response genes. We observed moderate induction of some ATF4-dependent genes (Harding et al., 2003) including *Asns* (1.3-fold), *Slc7a5* (1.3-fold), *Slc7a11* (1.9-fold) and *Mthfd2* (1.7-fold) (data not shown). It is therefore possible that editing of Z-RNA by ADAR1-p150 limits not only type I IFN induction but also PKR-dependent stress responses.

In conclusion, we discovered MAVS-dependent type I IFN induction as a biological function of Z-RNA that is curtailed by ADAR1-p150. These insights are not only of fundamental value but also have important implications for understanding and modulating detrimental and beneficial type I IFN responses in autoinflammation and cancer.

Materials and Methods

Mice

Adar1^{+/*fl-mZα*} mice were generated by Cyagen. In brief, genomic fragments containing homology arms were amplified from a BAC and were sequentially assembled into a targeting vector together with recombination sites and selection markers as shown in Figure S1A. Successful assembly of the targeting vector was verified by restriction digest and sequencing. The linearised vector was subsequently delivered to ES cells (C57BL/6) via electroporation, followed by drug selection, PCR screening and sequencing. After confirming correctly targeted ES clones via Southern blotting, we selected clones for blastocyst microinjection, followed by chimera production. Founders were confirmed as germline-transmitted via crossbreeding with WT animals. The Neo cassette was flanked by Rox sites and contained a Dre recombinase controlled by a promoter active in the germline, resulting in deletion of the Neo cassette in F1 animals (Figure S1A). These *Adar1*^{+/*fl-mZα*} mice were further crossed with *Pgk-Cre* mice provided by Samira Lakhali-Littleton to produce *Adar1*^{+/*mZα*} animals (Figure S1A). The following PCR primers were used for genotyping:

F, 5'-TGACGAGAGACTTGTTTTCTAGCATG-3',

R1, 5'-TGCCTCAATGAGACCTCCAACCTTAAGTC-3',

R2^{WT}, 5'-CAGGGAGTACAAAATACGATT-3', and

R2^{MUT}, 5'-CAGGGAGGCCAAAATACGAGC-3'.

PCR with primers F and R1 yielded a product of 357 bp for the WT *Adar1* allele and a 421 bp product for both '*fl-mZα*' and '*mZα*' alleles. PCR with primers F and R2^{WT} resulted in 1095 and 1158 bp products for the WT and '*fl-mZα*' alleles, respectively, and no product for the '*mZα*' allele. Finally, PCR with primers F and R2^{MUT} resulted in a 1158 bp product for the '*mZα*' allele only.

Mavs^{-/-} mice were a gift from C. Reis e Sousa and were originally from J. Tschopp (Michallet et al., 2008). All mice were on the C57BL/6 background. This work was performed in accordance with the UK Animal (Scientific Procedures) Act 1986 and institutional guidelines for animal care. This work was approved by project licenses granted by the UK Home Office (PPL numbers PC041D0AB, PBA43A2E4 and P79A4C5BA) and was also approved by the Institutional Animal Ethics Committee Review Board at the University of Oxford.

Cell culture

Lung fibroblasts and MEFs were grown in DMEM and BMMCs in RPMI, as described previously (Li et al., 2013; Maelfait et al., 2017). Media were supplemented with 10% heat-inactivated FCS and 2 mM L-glutamine; for BMMCs, 200 U/ml recombinant mouse GM-CSF (Peprotech) was added. MEFs were cultured at 3% oxygen.

RNA extraction and RT-qPCR

Organs collected from freshly killed mice (8-10 weeks of age) were snap frozen in liquid nitrogen immediately after dissection and stored at -80°C until further processing. Organs were homogenised with glass beads (425-600 µm, Sigma-Aldrich) in TRIzol (Thermo Fisher Scientific) using a FastPrep F120 instrument (Thermo Savant). RNA was extracted following the manufacturer's instructions and further purified using RNeasy Plus columns (Qiagen) including a gDNA eliminator column step. cDNA synthesis was performed with SuperScript II reverse transcriptase (Thermo Fisher Scientific) with random hexamer (Qiagen) or oligo (dT)₁₂₋₁₈ (Thermo Fisher Scientific) as primers. qPCR was done using Taqman Universal PCR Mix (Thermo Fisher Scientific) and Taqman probes (Applied Biosystems). Alternatively, qPCR was performed

using EXPRESS SYBR GreenER qPCR Supermix (Thermo Fisher Scientific) and DNA oligonucleotides (Sigma Aldrich). qPCR was performed on a QuantStudio 7 Flex real-time PCR system (Applied Biosystem). The qPCR probes and primers used in this study are listed in Table S2.

In vivo infection

WT and *Adar1^{mZα/mZα}* mice were used at 8-10 weeks of age. Mice were intranasally inoculated with 50 µl X31 (0.04 haemagglutination units (HAU)) diluted in viral growth medium (VGM; DMEM with 1% bovine serum albumin (Sigma-Aldrich A0336), 10 mM HEPES buffer, penicillin (100 U/ml) and streptomycin (100 µg/ml)) or mock infected with 50 µl VGM under light isoflurane anaesthesia. Animals were assessed daily for weight loss and signs of disease. Mice reaching 20% weight loss were euthanised.

Western Blot

Cells were lysed in RIPA buffer (50 mM Tris.HCl, pH7.4; 150 mM NaCl; 1% NP-40 (Sigma-Aldrich), 0.5% Deoxycholate, 0.1% SDS and Complete protease inhibitor (Roche)) at 4°C for 10 minutes. Protein lysates were then cleared by centrifugation at 13000 rpm for 10 minutes. Samples were mixed with NuPAGE SDS-PAGE sample loading buffer (ThermoFisher) containing 10% 2-mercaptoethanol. A primary antibody against ADAR1 was purchased from Santa Cruz (sc-73408). The antibody recognising ISG15 was a gift from Klaus-Peter Knobeloch. HRP-coupled secondary antibodies were from GE Healthcare.

Flow cytometry

Lungs from 8-10 week old mice were dissected and mechanically disrupted using scissors before incubation in RPMI containing 1 µg/ml type II collagenase

(Worthington Biochemical Corporation) and 40 U/ml DNase I (Sigma Aldrich) at 37°C for 60 minutes, with resuspension after 30 minutes to facilitate tissue dissociation. Cells were filtered through a 70 µm cell strainer (BD Falcon), rinsed with RPMI and pelleted at 400 x g for 5 minutes. The cell pellet was resuspended in 5 ml RBC lysis buffer (Qiagen), incubated at room temperature for 5 minutes and then washed twice with 45 ml RPMI. Cells were resuspended in 500 µl FACS buffer (PBS containing 10% (v/v) FCS and 2 mM EDTA) and passed through a 70 µm cell strainer. Viable cells were counted using a haemocytometer. Cells were washed with PBS before incubation with LIVE/DEAD Fixable Aqua Dead Cell Stain (Invitrogen) diluted 1:200 in PBS for 30 minutes at room temperature. Cells were washed once with FACS buffer and then stained with surface antibodies diluted 1:200 in Brilliant buffer (BD Biosciences) for 30 minutes. Cells were sorted directly into TRIzol-LS Reagent (Thermo Fisher Scientific) on BD FACSAria II and III machines (BD Biosciences). Alternatively, 1.5×10^6 cells were stained and analysed using an Attune NxT Flow Cytometer (Thermo Fisher Scientific). Data were analysed using FlowJo (v10.6.2).

Magnetic cell fraction

Cells from lungs were prepared as described above. 10^7 cells were resuspended in 90 µl of MACS buffer (PBS containing 0.5% BSA and 2 mM EDTA) and then incubated with 10 µl of CD45 microbeads (Miltenyi) for 15 minutes. The mixture was then washed with MACS buffer and resuspend in 500 µl MACS buffer for magnetic separation on MACS LS columns (Miltenyi) according to the manufacturer's instructions. Cells were recovered from the flow-through (CD45-) and column (CD45+). 10% of cells were stained and analysed by FACS to confirm purity. The remaining cells were pelleted, resuspend in TRIzol (ThermoFisher) and processed for RT-qPCR.

615

616 Bone marrow chimera

617 B6.SJL-CD45.1 mice were used as bone marrow recipients and were
618 lethally irradiated twice (4.5 Gy for 300 seconds, separated by a ~3 hour rest).
619 Mice were then injected intravenously with bone marrow from either WT
620 (CD45.2) or *Adar1*^{mZα/mZα} mice. Recipient mice received antibiotics (0.16
621 mg/mL, Enrofloxacin (Baytril), Bayer Corporation) in drinking water for four
622 weeks following irradiation and were rested for >8 weeks before tissue
623 collection.

624

625 RNA-seq and data processing

626 Stranded Illumina sequencing libraries were prepared with the RNA-Seq
627 Ribozero kit from isolated RNAs and submitted for PE150 sequencing using an
628 Illumina NovaSeq6000 machine, yielding ~100M reads per sample.
629 Sequencing data was processed using a Nextflow v20.07 (Di Tommaso et al.,
630 2017) pipeline automating quality control using FastQC v0.11.8
631 (bioinformatics.babraham.ac.uk/projects/fastqc/), quality and adapter trimming
632 using cutadapt v1.18 (Martin, 2011), contaminant detection using screen.sh
633 (within BBMap v36.20, sourceforge.net/projects/bbmap/), strand-aware
634 alignment using HISAT2 v2.1.0 (Kim et al., 2019) and STAR v2.7.1a (Dobin
635 et al., 2013), post-alignment quality-assurance using 'gene body coverage',
636 'transcript integrity', and 'inner distance' metrics from RSeQC v2.6.4 (Wang et
637 al., 2012), and strand-specific counting of uniquely-mapping reads using
638 featureCounts (within Subread v1.6.4, (Liao et al., 2014)) against Ensembl
639 GRCm38.100 annotations. Additional, unstranded counts were obtained with
640 featureCounts against a database of repetitive elements previously prepared
641 for GRCm38 (Attig et al., 2017) using reads unassigned to features during the
642 previous step.

643

644 Differential expression analysis

645 Downstream differential expression analysis was conducted using counts
646 obtained for STAR read mappings using DESeq2 (Love et al., 2014) (v1.22.1)
647 within R (v4.0.2). Gene ontology analysis was performed using goseq (Young
648 et al., 2010) (1.34.1). Heatmaps were generated using the pheatmap package
649 (v1.0.12).

650

651 Detection of A-to-I editing

652 A Python 3.8 program, edlted (github.com/A-N-Other/pedestal), was
653 produced to identify editing sites. edlted performs stranded assessments of
654 RNA editing from samtools mpileup (Li, 2011) data, building on the Dirichlet-
655 based models implemented in ACCUSA2 and JACUSA (Piechotta and
656 Dieterich, 2013; Piechotta et al., 2017). When run with test data alone, edlted
657 runs in 'detect' mode, finding base modifications by comparing the goodness of
658 fit of Dirichlet models of the base error (derived from the Phred quality data in
659 the mpileup input) and the background sequencing error to the base
660 frequencies recorded at a specific position. With an additional control dataset,
661 edlted runs in 'differential' mode, performing the above analysis to determine
662 significantly edited sites before additionally testing for differential editing by
663 comparing the goodness of fit of Dirichlet models of the base error from the test
664 and control datasets to their own and each other's base frequencies. When
665 biological replicates are provided, edlted adjusts the reported Z scores to reflect
666 the proportion of test dataset samples displaying editing. edlted was run in both
667 modes with samtools mpileup files (supplemented with TS tag metadata)
668 separately for HISAT2 and STAR alignments of the data with the flags '--
669 min_depth 5 --min_alt_depth 2 --min_edited 0.01 --max_edited 0.9 --z_score
670 2.58'. For differential analyses the '--min_fold 2' flag was used and, where

considering biological replicates the '--reps 3' flag, such that editing is required in all three samples. All analyses were conducted supplying BED files of ENCODE blacklisted regions (Amemiya et al., 2019) and known splice sites (regions set to splice site +/- 2 nts) to the '--blacklist' flag. Sites that were found in common between the HISAT2- and STAR-mapped data were retained for further analysis.

Analysis of A-to-I editing sites

Sites obtained from edlited were assigned to genomic features using annotatr v1.16 within R (Cavalcante and Sartor, 2017). Assessments of editing enrichment within repetitive elements were conducted using regioneR v1.22 within R (Gel et al., 2016) using randomization-based permutation tests with 100 bootstraps. Assessment of distance to neighbouring inverted SINE superfamily (B1, B2, B3, B4) members was conducted with bedtools v2.29.2 closest (Quinlan and Hall, 2010) using the '-io -S' flags. Outputs and the detailed statistics were produced with GraphPad Prism v8.

ELISA

Mouse IL-6 was quantified by uncoated ELISA Kit (ThermoFisher) according to manufacturer's instruction.

Author contributions (using the CRediT taxonomy)

Conceptualisation: Q.T. and J.R.; Methodology: Q.T., R.E.R. and G.Y.; Software: R.E.R. and G.Y.; Validation: Q.T. and J.R.; Formal analysis: Q.T., R.E.R., G.Y. and J.R.; Investigation: Q.T., R.E.R., G.Y., A.K.H., T.K.T. and A.B.; Resources: A.T. and G.K.; Data curation: Q.T., R.E.R. and G.Y.; Writing – Original Draft: Q.T., G.Y. and J.R.; Writing – Review & Editing: all authors; Visualisation: Q.T., R.E.R., G.Y. and J.R.; Supervision: J.R., A.T., and G.K.; Project administration: Q.T.; Funding acquisition: J.R.

Acknowledgments

The authors thank P. Shing Ho and Craig Benham for providing access to the Z-hunt and SIBZ codes, respectively. We further thank Daniel Stetson, Andrew Oberst, Jonathan Maelfait, Caetano Reis e Sousa, David Ron, Annemarthé van Der Veen and members of the Rehwinkel lab for discussion. The authors thank Ziqi Long and Oliver Bannard for their help with generating BM chimeras.

This work was funded by the UK Medical Research Council [MRC core funding of the MRC Human Immunology Unit; J.R.], the Wellcome Trust [grant number 100954; J.R.], the Lister Institute [J.R.] and the Francis Crick Institute [G.K.], which receives its core funding from Cancer Research UK, the UK Medical Research Council, and the Wellcome Trust. T.K.T. was funded by the Townsend-Jeantet Charitable Trust (charity number 1011770) and the EPA Cephalosporin Early Career Researcher Fund. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Declaration of interests

The authors have declared that no conflict of interest exists.

References

- Ahmad, S., Mu, X., Yang, F., Greenwald, E., Park, J.W., Jacob, E., Zhang, C.Z., and Hur, S. (2018). Breaching Self-Tolerance to Alu Duplex RNA Underlies MDA5-Mediated Inflammation. *Cell* 172, 797-810 e713.
- Amemiya, H.M., Kundaje, A., and Boyle, A.P. (2019). The ENCODE Blacklist: Identification of Problematic Regions of the Genome. *Sci Rep* 9, 9354.
- Ashley, C.L., Abendroth, A., McSharry, B.P., and Slobedman, B. (2019). Interferon-Independent Upregulation of Interferon-Stimulated Genes during Human Cytomegalovirus Infection is Dependent on IRF3 Expression. *Viruses* 11.
- Athanasiadis, A. (2012). Zalpha-domains: at the intersection between RNA editing and innate immunity. *Seminars in cell & developmental biology* 23, 275-280.
- Athanasiadis, A., Placido, D., Maas, S., Brown, B.A., 2nd, Lowenhaupt, K., and Rich, A. (2005). The crystal structure of the Zbeta domain of the RNA-editing enzyme ADAR1 reveals distinct conserved surfaces among Z-domains. *Journal of molecular biology* 351, 496-507.
- Attig, J., Young, G.R., Stoye, J.P., and Kassiotis, G. (2017). Physiological and Pathological Transcriptional Activation of Endogenous Retroelements Assessed by RNA-Sequencing of B Lymphocytes. *Frontiers in microbiology* 8, 2489.
- Bajad, P., Ebner, F., Amman, F., Szabo, B., Kapoor, U., Manjali, G., Hildebrandt, A., Janisiw, M.P., and Jantsch, M.F. (2020). An internal deletion of ADAR rescued by MAVS deficiency leads to a minute phenotype. *Nucleic acids research* 48, 3286-3303.
- Bartok, E., and Hartmann, G. (2020). Immune Sensing Mechanisms that Discriminate Self from Altered Self and Foreign Nucleic Acids. *Immunity* 53, 54-77.
- Brown, B.A., 2nd, Lowenhaupt, K., Wilbert, C.M., Hanlon, E.B., and Rich, A. (2000). The zalpha domain of the editing enzyme dsRNA adenosine deaminase binds left-handed Z-RNA as well as Z-DNA. *Proceedings of the National Academy of Sciences of the United States of America* 97, 13532-13536.
- Casey, J.L. (2012). Control of ADAR1 editing of hepatitis delta virus RNAs. *Curr Top Microbiol Immunol* 353, 123-143.
- Cavalcante, R.G., and Sartor, M.A. (2017). annotatr: genomic regions in context.

753 Bioinformatics 33, 2381-2383.

754 Champ, P.C., Maurice, S., Vargason, J.M., Camp, T., and Ho, P.S. (2004).
755 Distributions of Z-DNA and nuclear factor I in human chromosome 22: a model
756 for coupled transcriptional regulation. *Nucleic acids research* 32, 6501-6510.

757 Chung, H., Calis, J.J.A., Wu, X., Sun, T., Yu, Y., Sarbanes, S.L., Dao Thi, V.L.,
758 Shilvock, A.R., Hoffmann, H.H., Rosenberg, B.R., and Rice, C.M. (2018).
759 Human ADAR1 Prevents Endogenous RNA from Triggering Translational
760 Shutdown. *Cell* 172, 811-824 e814.

761 Chuong, E.B., Elde, N.C., and Feschotte, C. (2016). Regulatory evolution of
762 innate immunity through co-option of endogenous retroviruses. *Science* 351,
763 1083-1087.

764 Davis, P.W., Hall, K., Cruz, P., Tinoco, I., Jr., and Neilson, T. (1986). The
765 tetranucleotide rCpGpCpG forms a left-handed Z-RNA double-helix.
766 *Nucleic Acids Res* 14, 1279-1291.

767 DeFilippis, V.R., Robinson, B., Keck, T.M., Hansen, S.G., Nelson, J.A., and
768 Fruh, K.J. (2006). Interferon regulatory factor 3 is necessary for induction of
769 antiviral genes during human cytomegalovirus infection. *Journal of virology* 80,
770 1032-1037.

771 Devos, M., Tanghe, G., Gilbert, B., Dierick, E., Verheirstraeten, M., Nemegeer,
772 J., de Reuver, R., Lefebvre, S., De Munck, J., Rehwinkel, J., *et al.* (2020).
773 Sensing of endogenous nucleic acids by ZBP1 induces keratinocyte
774 necroptosis and skin inflammation. *The Journal of experimental medicine* 217.

775 Di Tommaso, P., Chatzou, M., Floden, E.W., Barja, P.P., Palumbo, E., and
776 Notredame, C. (2017). Nextflow enables reproducible computational workflows.
777 *Nat Biotechnol* 35, 316-319.

778 Dias Junior, A.G., Sampaio, N.G., and Rehwinkel, J. (2019). A Balancing Act:
779 MDA5 in Antiviral Immunity and Autoinflammation. *Trends in microbiology* 27,
780 75-85.

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

784 Eisenberg, E., and Levanon, E.Y. (2018). A-to-I RNA editing - immune protector
785 and transcriptome diversifier. *Nature reviews. Genetics* 19, 473-490.

786 Feng, S., Li, H., Zhao, J., Pervushin, K., Lowenhaupt, K., Schwartz, T.U., and

787 Droge, P. (2011). Alternate rRNA secondary structures as regulators of
788 translation. *Nature structural & molecular biology* 18, 169-176.

789 Gannon, H.S., Zou, T., Kiessling, M.K., Gao, G.F., Cai, D., Choi, P.S., Ivan, A.P.,
790 Buchumenski, I., Berger, A.C., Goldstein, J.T., *et al.* (2018). Identification of
791 ADAR1 adenosine deaminase dependency in a subset of cancer cells. *Nature*
792 *communications* 9, 5450.

793 Gel, B., Diez-Villanueva, A., Serra, E., Buschbeck, M., Peinado, M.A., and
794 Malinverni, R. (2016). regioneR: an R/Bioconductor package for the association
795 analysis of genomic regions based on permutation tests. *Bioinformatics* 32,
796 289-291.

797 Goubau, D., Romieu-Mourez, R., Solis, M., Hernandez, E., Mesplede, T., Lin,
798 R., Leaman, D., and Hiscott, J. (2009). Transcriptional re-programming of
799 primary macrophages reveals distinct apoptotic and anti-tumoral functions of
800 IRF-3 and IRF-7. *Eur J Immunol* 39, 527-540.

801 Grandvaux, N., Servant, M.J., tenOever, B., Sen, G.C., Balachandran, S.,
802 Barber, G.N., Lin, R., and Hiscott, J. (2002). Transcriptional profiling of
803 interferon regulatory factor 3 target genes: direct involvement in the regulation
804 of interferon-stimulated genes. *Journal of virology* 76, 5532-5539.

805 Guallar, D., Fuentes-Iglesias, A., Souto, Y., Ameneiro, C., Freire-Agulleiro, O.,
806 Pardavila, J.A., Escudero, A., Garcia-Outeiral, V., Moreira, T., Saenz, C., *et al.*
807 (2020). ADAR1-Dependent RNA Editing Promotes MET and iPSC
808 Reprogramming by Alleviating ER Stress. *Cell stem cell* 27, 300-314 e311.

809 Hall, K., Cruz, P., Tinoco, I., Jr., Jovin, T.M., and van de Sande, J.H. (1984). 'Z-
810 RNA'--a left-handed RNA double helix. *Nature* 311, 584-586.

811 Harding, H.P., Zhang, Y., Zeng, H., Novoa, I., Lu, P.D., Calton, M., Sadri, N.,
812 Yun, C., Popko, B., Paules, R., *et al.* (2003). An integrated stress response
813 regulates amino acid metabolism and resistance to oxidative stress. *Molecular*
814 *cell* 11, 619-633.

815 Hartner, J.C., Schmittwolf, C., Kispert, A., Muller, A.M., Higuchi, M., and
816 Seeburg, P.H. (2004). Liver disintegration in the mouse embryo caused by
817 deficiency in the RNA-editing enzyme ADAR1. *J Biol Chem* 279, 4894-4902.

818 Hartner, J.C., Walkley, C.R., Lu, J., and Orkin, S.H. (2009). ADAR1 is essential
819 for the maintenance of hematopoiesis and suppression of interferon signaling.
820 *Nature immunology* 10, 109-115.

821 Heraud-Farlow, J.E., and Walkley, C.R. (2020). What do editors do?

822 Understanding the physiological functions of A-to-I RNA editing by adenosine
823 deaminase acting on RNAs. *Open Biol* 10, 200085.

824 Herbert, A. (2019). Z-DNA and Z-RNA in human disease. *Commun Biol* 2, 7.

825 Ho, P.S., Ellison, M.J., Quigley, G.J., and Rich, A. (1986). A computer aided
826 thermodynamic approach for predicting the formation of Z-DNA in naturally
827 occurring sequences. *The EMBO journal* 5, 2737-2744.

828 Ishizuka, J.J., Manguso, R.T., Cheruiyot, C.K., Bi, K., Panda, A., Iracheta-Vellve,
829 A., Miller, B.C., Du, P.P., Yates, K.B., Dubrot, J., *et al.* (2019). Loss of ADAR1
830 in tumours overcomes resistance to immune checkpoint blockade. *Nature* 565,
831 43-48.

832 Jiao, H., Wachsmuth, L., Kumari, S., Schwarzer, R., Lin, J., Eren, R.O., Fisher,
833 A., Lane, R., Young, G.R., Kassiotis, G., *et al.* (2020). Z-nucleic-acid sensing
834 triggers ZBP1-dependent necroptosis and inflammation. *Nature* 580, 391-395.

835 Kesavardhana, S., and Kanneganti, T.D. (2020). ZBP1: A STARGTE to decode
836 the biology of Z-nucleic acids in disease. *The Journal of experimental medicine*
837 217.

838 Kim, D., Paggi, J.M., Park, C., Bennett, C., and Salzberg, S.L. (2019). Graph-
839 based genome alignment and genotyping with HISAT2 and HISAT-genotype.
840 *Nat Biotechnol* 37, 907-915.

841 Kim, S.H., Lim, S.H., Lee, A.R., Kwon, D.H., Song, H.K., Lee, J.H., Cho, M.,
842 Johner, A., Lee, N.K., and Hong, S.C. (2018). Unveiling the pathway to Z-DNA
843 in the protein-induced B-Z transition. *Nucleic acids research* 46, 4129-4137.

844 Kim, Y.G., Muralinath, M., Brandt, T., Percy, M., Hauns, K., Lowenhaupt, K.,
845 Jacobs, B.L., and Rich, A. (2003). A role for Z-DNA binding in vaccinia virus
846 pathogenesis. *Proceedings of the National Academy of Sciences of the United*
847 *States of America* 100, 6974-6979.

848 Lazear, H.M., Lancaster, A., Wilkins, C., Suthar, M.S., Huang, A., Vick, S.C.,
849 Clepper, L., Thackray, L., Brassil, M.M., Virgin, H.W., *et al.* (2013). IRF-3, IRF-
850 5, and IRF-7 coordinately regulate the type I IFN response in myeloid dendritic
851 cells downstream of MAVS signaling. *PLoS Pathog* 9, e1003118.

852 Li, H. (2011). A statistical framework for SNP calling, mutation discovery,
853 association mapping and population genetical parameter estimation from
854 sequencing data. *Bioinformatics* 27, 2987-2993.

855 Li, H., Xiao, J., Li, J., Lu, L., Feng, S., and Droge, P. (2009). Human genomic

856 Z-DNA segments probed by the Z alpha domain of ADAR1. *Nucleic acids*
857 *research* 37, 2737-2746.

858 Li, X.D., Wu, J., Gao, D., Wang, H., Sun, L., and Chen, Z.J. (2013). Pivotal roles
859 of cGAS-cGAMP signaling in antiviral defense and immune adjuvant effects.
860 *Science* 341, 1390-1394.

861 Li, Y., Banerjee, S., Goldstein, S.A., Dong, B., Gaughan, C., Rath, S., Donovan,
862 J., Korennykh, A., Silverman, R.H., and Weiss, S.R. (2017). Ribonuclease L
863 mediates the cell-lethal phenotype of double-stranded RNA editing enzyme
864 ADAR1 deficiency in a human cell line. *Elife* 6.

865 Li, Z., Wolff, K.C., and Samuel, C.E. (2010). RNA adenosine deaminase
866 ADAR1 deficiency leads to increased activation of protein kinase PKR and
867 reduced vesicular stomatitis virus growth following interferon treatment.
868 *Virology* 396, 316-322.

869 Liao, Y., Smyth, G.K., and Shi, W. (2014). featureCounts: an efficient general
870 purpose program for assigning sequence reads to genomic features.
871 *Bioinformatics* 30, 923-930.

872 Liddicoat, B.J., Piskol, R., Chalk, A.M., Ramaswami, G., Higuchi, M., Hartner,
873 J.C., Li, J.B., Seeburg, P.H., and Walkley, C.R. (2015). RNA editing by ADAR1
874 prevents MDA5 sensing of endogenous dsRNA as nonself. *Science* 349, 1115-
875 1120.

876 Liu, H., Golji, J., Brodeur, L.K., Chung, F.S., Chen, J.T., deBeaumont, R.S.,
877 Bullock, C.P., Jones, M.D., Kerr, G., Li, L., *et al.* (2019). Tumor-derived IFN
878 triggers chronic pathway agonism and sensitivity to ADAR loss. *Nature*
879 *medicine* 25, 95-102.

880 Love, M.I., Huber, W., and Anders, S. (2014). Moderated estimation of fold
881 change and dispersion for RNA-seq data with DESeq2. *Genome Biol* 15, 550.

882 Luo, H., Wang, D., Che, H.L., Zhao, Y., and Jin, H. (2012). Pathological
883 observations of lung inflammation after administration of IP-10 in influenza
884 virus- and respiratory syncytial virus-infected mice. *Exp Ther Med* 3, 76-79.

885 Maelfait, J., Liverpool, L., Bridgeman, A., Ragan, K.B., Upton, J.W., and
886 Rehwinkel, J. (2017). Sensing of viral and endogenous RNA by ZBP1/DAI
887 induces necroptosis. *The EMBO journal* 36, 2529-2543.

888 Maelfait, J., Liverpool, L., and Rehwinkel, J. (2020). Nucleic Acid Sensors and
889 Programmed Cell Death. *Journal of molecular biology* 432, 552-568.

890 Mannion, N.M., Greenwood, S.M., Young, R., Cox, S., Brindle, J., Read, D.,
891 Nellaker, C., Vesely, C., Ponting, C.P., McLaughlin, P.J., *et al.* (2014). The RNA-
892 editing enzyme ADAR1 controls innate immune responses to RNA. *Cell reports*
893 9, 1482-1494.

894 Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput
895 sequencing reads. 2011 17, 3.

896 Mehdipour, P., Marhon, S.A., Ettayebi, I., Chakravarthy, A., Hosseini, A., Wang,
897 Y., de Castro, F.A., Loo Yau, H., Ishak, C., Abelson, S., *et al.* (2020). Epigenetic
898 therapy induces transcription of inverted SINEs and ADAR1 dependency.
899 *Nature* 588, 169-173.

900 Michallet, M.C., Meylan, E., Ermolaeva, M.A., Vazquez, J., Rebsamen, M.,
901 Curran, J., Poeck, H., Bscheider, M., Hartmann, G., Konig, M., *et al.* (2008).
902 TRADD protein is an essential component of the RIG-like helicase antiviral
903 pathway. *Immunity* 28, 651-661.

904 Pakos-Zebrucka, K., Koryga, I., Mnich, K., Lujic, M., Samali, A., and Gorman,
905 A.M. (2016). The integrated stress response. *EMBO reports* 17, 1374-1395.

906 Perng, Y.C., and Lenschow, D.J. (2018). ISG15 in antiviral immunity and
907 beyond. *Nature reviews. Microbiology* 16, 423-439.

908 Pestal, K., Funk, C.C., Snyder, J.M., Price, N.D., Treuting, P.M., and Stetson,
909 D.B. (2015). Isoforms of RNA-Editing Enzyme ADAR1 Independently Control
910 Nucleic Acid Sensor MDA5-Driven Autoimmunity and Multi-organ Development.
911 *Immunity* 43, 933-944.

912 Pfaller, C.K., Donohue, R.C., Nersisyan, S., Brodsky, L., and Cattaneo, R.
913 (2018). Extensive editing of cellular and viral double-stranded RNA structures
914 accounts for innate immunity suppression and the proviral activity of
915 ADAR1p150. *PLoS Biol* 16, e2006577.

916 Pichlmair, A., Lassnig, C., Eberle, C.A., Gorna, M.W., Baumann, C.L., Burkard,
917 T.R., Burckstummer, T., Stefanovic, A., Krieger, S., Bennett, K.L., *et al.* (2011).
918 IFIT1 is an antiviral protein that recognizes 5'-triphosphate RNA. *Nature*
919 *immunology* 12, 624-630.

920 Piechotta, M., and Dieterich, C. (2013). ACCUSA2: multi-purpose SNV calling
921 enhanced by probabilistic integration of quality scores. *Bioinformatics* 29, 1809-
922 1810.

923 Piechotta, M., Wyler, E., Ohler, U., Landthaler, M., and Dieterich, C. (2017).
924 JACUSA: site-specific identification of RNA editing events from replicate

- 925 sequencing data. *BMC Bioinformatics* 18, 7.
- 926 Porath, H.T., Knisbacher, B.A., Eisenberg, E., and Levanon, E.Y. (2017).
- 927 Massive A-to-I RNA editing is common across the Metazoa and correlates with
- 928 dsRNA abundance. *Genome Biol* 18, 185.
- 929 Quinlan, A.R., and Hall, I.M. (2010). BEDTools: a flexible suite of utilities for
- 930 comparing genomic features. *Bioinformatics* 26, 841-842.
- 931 Reich, D.P., and Bass, B.L. (2019). Mapping the dsRNA World. *Cold Spring*
- 932 *Harb Perspect Biol* 11.
- 933 Rice, G.I., Kasher, P.R., Forte, G.M., Mannion, N.M., Greenwood, S.M.,
- 934 Szykiewicz, M., Dickerson, J.E., Bhaskar, S.S., Zampini, M., Briggs, T.A., *et*
- 935 *al.* (2012). Mutations in ADAR1 cause Aicardi-Goutieres syndrome associated
- 936 with a type I interferon signature. *Nature genetics* 44, 1243-1248.
- 937 Schade, M., Turner, C.J., Lowenhaupt, K., Rich, A., and Herbert, A. (1999).
- 938 Structure-function analysis of the Z-DNA-binding domain Zalpha of dsRNA
- 939 adenosine deaminase type I reveals similarity to the (alpha + beta) family of
- 940 helix-turn-helix proteins. *The EMBO journal* 18, 470-479.
- 941 Schoggins, J.W. (2019). Interferon-Stimulated Genes: What Do They All Do?
- 942 *Annu Rev Virol* 6, 567-584.
- 943 Schwartz, T., Rould, M.A., Lowenhaupt, K., Herbert, A., and Rich, A. (1999).
- 944 Crystal structure of the Zalpha domain of the human editing enzyme ADAR1
- 945 bound to left-handed Z-DNA. *Science* 284, 1841-1845.
- 946 Solomon, O., Di Segni, A., Cesarkas, K., Porath, H.T., Marcu-Malina, V., Mizrahi,
- 947 O., Stern-Ginossar, N., Kol, N., Farage-Barhom, S., Glick-Saar, E., *et al.* (2017).
- 948 RNA editing by ADAR1 leads to context-dependent transcriptome-wide
- 949 changes in RNA secondary structure. *Nature communications* 8, 1440.
- 950 Song, Y., Yang, W., Fu, Q., Wu, L., Zhao, X., Zhang, Y., and Zhang, R. (2020).
- 951 irCLASH reveals RNA substrates recognized by human ADARs. *Nature*
- 952 *structural & molecular biology* 27, 351-362.
- 953 Sridharan, H., Ragan, K.B., Guo, H., Gilley, R.P., Landsteiner, V.J., Kaiser, W.J.,
- 954 and Upton, J.W. (2017). Murine cytomegalovirus IE3-dependent transcription
- 955 is required for DAI/ZBP1-mediated necroptosis. *EMBO reports* 18, 1429-1441.
- 956 Tan, M.H., Li, Q., Shanmugam, R., Piskol, R., Kohler, J., Young, A.N., Liu, K.I.,
- 957 Zhang, R., Ramaswami, G., Ariyoshi, K., *et al.* (2017). Dynamic landscape and
- 958 regulation of RNA editing in mammals. *Nature* 550, 249-254.

959 Uggenti, C., Lepelley, A., and Crow, Y.J. (2019). Self-Awareness: Nucleic Acid-
960 Driven Inflammation and the Type I Interferonopathies. *Annual review of*
961 *immunology* 37, 247-267.

962 Vogel, O.A., Han, J., Liang, C.Y., Manicassamy, S., Perez, J.T., and
963 Manicassamy, B. (2020). The p150 Isoform of ADAR1 Blocks Sustained RLR
964 signaling and Apoptosis during Influenza Virus Infection. *PLoS Pathog* 16,
965 e1008842.

966 Wang, A.H., Quigley, G.J., Kolpak, F.J., Crawford, J.L., van Boom, J.H., van der
967 Marel, G., and Rich, A. (1979). Molecular structure of a left-handed double
968 helical DNA fragment at atomic resolution. *Nature* 282, 680-686.

969 Wang, L., Wang, S., and Li, W. (2012). RSeQC: quality control of RNA-seq
970 experiments. *Bioinformatics* 28, 2184-2185.

971 Wang, Q., Miyakoda, M., Yang, W., Khillan, J., Stachura, D.L., Weiss, M.J., and
972 Nishikura, K. (2004). Stress-induced apoptosis associated with null mutation of
973 ADAR1 RNA editing deaminase gene. *J Biol Chem* 279, 4952-4961.

974 Wang, R., Li, H., Wu, J., Cai, Z.Y., Li, B., Ni, H., Qiu, X., Chen, H., Liu, W., Yang,
975 Z.H., *et al.* (2020). Gut stem cell necroptosis by genome instability triggers
976 bowel inflammation. *Nature* 580, 386-390.

977 Ward, S.V., George, C.X., Welch, M.J., Liou, L.Y., Hahm, B., Lewicki, H., de la
978 Torre, J.C., Samuel, C.E., and Oldstone, M.B. (2011). RNA editing enzyme
979 adenosine deaminase is a restriction factor for controlling measles virus
980 replication that also is required for embryogenesis. *Proceedings of the National*
981 *Academy of Sciences of the United States of America* 108, 331-336.

982 Weiden, M.D., Hoshino, S., Levy, D.N., Li, Y., Kumar, R., Burke, S.A., Dawson,
983 R., Hioe, C.E., Borkowsky, W., Rom, W.N., and Hoshino, Y. (2014). Adenosine
984 deaminase acting on RNA-1 (ADAR1) inhibits HIV-1 replication in human
985 alveolar macrophages. *PloS one* 9, e108476.

986 Wittig, B., Wolfl, S., Dorbic, T., Vahrson, W., and Rich, A. (1992). Transcription
987 of human c-myc in permeabilized nuclei is associated with formation of Z-DNA
988 in three discrete regions of the gene. *The EMBO journal* 11, 4653-4663.

989 Wolfl, S., Wittig, B., and Rich, A. (1995). Identification of transcriptionally
990 induced Z-DNA segments in the human c-myc gene. *Biochim Biophys Acta*
991 1264, 294-302.

992 Young, M.D., Wakefield, M.J., Smyth, G.K., and Oshlack, A. (2010). Gene
993 ontology analysis for RNA-seq: accounting for selection bias. *Genome Biol* 11,

- 994 R14.
- 995 Zhabinskaya, D., and Benham, C.J. (2011). Theoretical analysis of the stress
996 induced B-Z transition in superhelical DNA. PLoS Comput Biol 7, e1001051.
- 997 Zhang, H., Ni, G., and Damania, B. (2020a). ADAR1 Facilitates KSHV Lytic
998 Reactivation by Modulating the RLR-Dependent Signaling Pathway. Cell
999 reports 31, 107564.
- 1000 Zhang, T., Yin, C., Boyd, D.F., Quarato, G., Ingram, J.P., Shubina, M., Ragan,
1001 K.B., Ishizuka, T., Crawford, J.C., Tummers, B., *et al.* (2020b). Influenza Virus
1002 Z-RNAs Induce ZBP1-Mediated Necroptosis. Cell 180, 1115-1129 e1113.
- 1003 Zhou, S., Yang, C., Zhao, F., Huang, Y., Lin, Y., Huang, C., Ma, X., Du, J., Wang,
1004 Y., Long, G., *et al.* (2019). Double-stranded RNA deaminase ADAR1 promotes
1005 the Zika virus replication by inhibiting the activation of protein kinase PKR. J
1006 Biol Chem 294, 18168-18180.
- 1007

Figures and figure legends

Figure 1.

Figure 1. Mutation of ADAR1-p150's Z α domain triggers spontaneous type I IFN responses in multiple organs.

A. Levels of the indicated mRNAs were analysed by RT-qPCR in RNA samples extracted from tissues of WT and *Adar1*^{mZ α /mZ α} animals and are shown relative to *Gapdh*. Each dot represents an individual mouse. *N.D.*, not detectable.

B. Protein extracts from whole lungs from animals of the indicated genotypes were used for western blot with an α -ISG15 antibody. β -Actin served as a loading control. Each lane represents a sample from an individual mouse.

C-E. mRNA levels of the indicated ISGs were analysed by RT-qPCR from cultured lung fibroblasts (C), BMMCs (D), and MEFs (E) of the indicated genotypes and are shown relative to *Gapdh*. Each dot represents cells derived from an individual mouse.

Pooled data from biological replicates are shown with mean (A, D, E) or mean $\pm$ SD (C) and were analysed by unpaired t test (****p<0.0001, **p<0.01, *p<0.05). See also Figure S1.

Figure S1.

Figure S1, related to Figure1. Generation of *Adar1*^{mZα/mZα} animals.

A. Schematic representation of the *Adar1* WT allele, targeting vector, targeted allele, *Adar1*^{fl-mZα} allele and the two-step Cre-mediated recombination process that resulted in the *Adar1*^{mZα} allele. Please see text for details. cKI, conditional knock-in.

B. Genomic DNA was prepared from WT and *Adar1*^{mZα/mZα} animals and the mutated region in exon 2 was sequenced.

C. *Adar1*^{+/fl-mZα} mice were bred with the *Pgk-Cre* line. *Adar*^{+/mZα} offspring were then mated to generate *Adar1*^{mZα/mZα} animals. The numbers and percentages of animals obtained with the indicated genotypes are shown.

D. BMMCs were grown from bone marrow from five mice of the indicated genotypes. Protein extracts were used for western blot with α-ADAR1 antibody. β-Actin served as a loading control. *, non-specific band

Figure 2.

Figure 2. *Adar1*^{mZα/mZα} lungs display a type I IFN gene signature.

Total RNA was extracted from lungs of three WT and three *Adar1*^{mZα/mZα} mice.

Ribosomal RNAs were depleted before random-primed library preparation and

RNA sequencing. About 100 million reads were obtained per sample.

A. Differentially expressed genes were defined as displaying a fold change of

≥2 with an adjusted p-value <0.01. The 89 upregulated and 10 downregulated

1052 genes were ordered by decreasing fold change and the data were clustered by
1053 sample. ISGs are indicated in bold.

1054 **B.** GO analysis of up-regulated genes. The top 20 GO terms (biological
1055 processes), ranked and ordered by p-value, are shown. Diameters indicate the
1056 number of induced genes assigned to the GO term and colours show the p-
1057 value.

1058 **C.** Detected and differentially expressed REs were assigned to the indicated
1059 classes and are shown as pie charts. Differentially expressed REs were
1060 identified as having a minimum fold change of 2 and an adjusted p value of less
1061 than 0.01.

1062 See also Table S1.

Figure 3.

Figure 3. Stromal and haematopoietic cells upregulate ISGs in *Adar1*^{mZα/mZα} lungs.

A. The proportion of haematopoietic (CD45+) and stromal (CD45-) cells in WT and *Adar1*^{mZα/mZα} lungs is shown.

B. mRNA levels of the indicated ISGs were analysed by RT-qPCR using RNA extracted from whole lung, or from CD45+ or CD45- cells, and are shown relative to *Actb*. Fold increases relative to WT samples were calculated.

Data points represent individual animals. In (A), data from a representative experiment are shown with mean ± SD. In (B), pooled data from two independent experiments including a total of six animals are shown with mean and were analysed by unpaired t test (****p < 0.0001, ***p < 0.001, **p < 0.01).

See also Figure S2.

Figure S2.

Figure S2, related to Figure 3. MACS separation of lung cells.

A. Cell surface levels of CD45 were analysed by flow cytometry in single cell suspensions obtained from lung tissue before MACS (left) and in CD45- and CD45+ cell fractions obtained after MACS (middle and right). Data are from a representative WT animal.

B. The percentage of CD45-expressing cells is shown for CD45+ and CD45- MACS fractions. Data points represent individual animals (n=4) from a representative experiment and bars indicate the mean.

Figure 4.

Figure 4. Multiple haematopoietic and non-haematopoietic cell types display ISGs upregulation in *Adar1*^{mZα/mZα} lungs.

A-C. mRNA levels of the indicated ISGs were analysed by RT-qPCR using RNA extracted from cell populations sorted from lungs of WT and *Adar1*^{mZα/mZα} mice and are shown relative to *Actb*. B, B cells; T, T cells; DC, dendritic cells; Mono, monocytes; Mph, macrophages; NK, natural killer cells; Neut, neutrophils; Eos, eosinophils; Total, whole lung.

D. ISG mRNA levels were analysed as in (A-C) in cell populations sorted from lungs of BM chimeric mice and are shown relative to *Gapdh*.

Each data point represents an individual mouse. Due to the small number of epithelial cells recovered, samples were pooled from multiple mice before RNA extraction (C). Pooled data from two (A,B) or three (D) independent experiments are shown with mean (****p < 0.0001, ***p<0.001, **p<0.01, *p<0.05, unpaired t test). See also Figures S3, S4 and S5.

Figure S3.

A

CD45.2-AF488; CD11c-APC; CD11b-BV785
MHCII-AF700; CD24-BV605; CD64-PE

B

CD45.2-AF488; CD24-BV605; CD11c-APC
MHCII-e780; Ly6G-AF700; SiglecF-BV421

1102

Figure S3, related to Figure 4A and 4B. Gating strategy for sorting of haematopoietic lung cells.

Two staining panels were used to identify and isolate haematopoietic cell populations by FACS. Panel (A) is related to Figure 4A and panel (B) to Figure 4B. Antibodies and conjugated fluorophores are shown in boxes. Gating strategies are shown for a representative WT (A) and *Adar1^{mZα/mZα}* (B) animal. Bar graphs show the proportion of each cell population as a percentage of CD45+ cells. Each dot represents an individual mouse and data from two independent experiments were pooled (**p<0.01, unpaired t test).

Figure S4.

1112

1113

1114 Figure S4, related to Figure 4C. Gating strategy for sorting of stromal lung cells.

1115 The staining panel used to identify and isolate non-haematopoietic cell

1116 populations by FACS is shown. Antibodies and conjugated fluorophores are

1117 shown in the box. The gating strategy is shown for a representative WT animal.

1118 The bar graph shows the proportion of each cell population as a percentage of

1119 CD45- cells. Each dot represents an individual mouse.

Figure S5.

Figure S5, related to Figure 4D. Analysis of BM chimeras.

A. Schematic representation of the generation of BM chimeric animals.

B. White blood cells from BM chimeric mice and, as control, from an untreated B6.SJL-CD45.1 animal, were analysed by FACS. Cells were gated on single, live cells. Representative FACS plots from a WT→WT animal (left) and pooled data from two independent experiments involving a total seven WT→WT and eight *Adar1*^{mZα/mZα}→WT BM chimeric animals (right) are shown. Bars show the mean and error bars represent SD.

1130 **C.** Lung cells from BM chimeric mice were analysed by FACS. Data from a
1131 representative WT→WT animal are shown.

Figure 5.

Figure 5. *Adar1*^{mZα/mZα} mice are protected from early IAV infection.

A. WT or *Adar1*^{mZα/mZα} mice were infected intranasally with 0.04 HAU of IAV strain A/X31. Body weight was monitored daily and is shown as a percentage of starting body weight.

B-E. WT or *Adar1*^{mZα/mZα} mice were infected as in (A) or mock infected using viral growth medium. On day 3 post infection, lungs and sera were collected.

B. A 'lung index' was calculated (lung weight/body weight x100).

C. Levels of the viral *NP* and *M* transcripts were analysed by RT-qPCR in RNA samples extracted from total lung. Data are shown relative to *Actb* (*NP*) or *Gapdh* (*M*).

1144 **D.** Levels of the indicated mRNAs were determined as in (C).

1145 **E.** Serum IL-6 concentrations were analysed by ELISA.

1146 In (A), data from three independent experiments including a total of 15 mice per
 1147 genotype were pooled (mean + SD; ****p<0.0001, ***p<0.001, **p<0.01,
 1148 *p<0.05, mixed-effects analysis). In (B-E), pooled data from two independent
 1149 experiments (mock infected: n=4 mice per genotype; X31-infected: n=16 WT
 1150 and n=13 *Adar1*^{mZα/mZα} mice) are shown. Each dot represents an individual
 1151 mouse and the mean is indicated (****p < 0.0001, ***p<0.001, **p<0.01,
 1152 unpaired t test).

Figure 6.

Figure 6. ISG induction in *Adar1*^{mZα/mZα} mice is MAVS-dependent.

Levels of the indicated mRNAs were analysed by RT-qPCR in RNA samples extracted from tissues of WT and *Adar1*^{mZα/mZα} animals that were either MAVS-sufficient or -deficient. Data are shown relative to *Gapdh*.

Each dot represents an individual mouse. Pooled data from biological replicates are shown (****p < 0.0001, ***p < 0.001, **p < 0.01, *p < 0.05, unpaired t test).

Figure 7.

Figure 7. ADAR1-p150's Z α domain is required for editing of a subset of RNAs.

A. Editing sites were mapped in RNA sequencing reads from three WT and three *Adar1*^{mZα/mZα} lung samples (Z>2.58). The numbers of edited sites were scaled to the total number of reads per sample. Each data point corresponds to an animal and the mean is shown (ns, not significant; unpaired t test).

B. Editing frequencies for sites detectable in all three WT or *Adar1*^{mZα/mZα} samples (left; Z>2.58) and for differentially edited sites (right; Z>2.58, >2-fold) are shown as violin plots. Solid horizontal lines show the median and dotted lines indicate quartiles.

1172 **C.** Editing sites detected in WT samples and differentially edited sites were
 1173 matched to annotated genomic features. The percentage of sites is shown for
 1174 each category.

1175 **D.** The number of expected and observed editing sites in WT samples are
 1176 shown for families of REs for which either value exceeded 500. Please see text
 1177 for details.

1178 **E.** The distances of SINEs to their nearest inverted super-family member were
 1179 determined for all SINEs and for SINEs harbouring an editing site in WT
 1180 samples (Detected) or containing a differentially edited site. Results are shown
 1181 as proportions of SINEs with the maximum count set to 100.

1182 See also Figure S6.

Figure S6.

Figure S6. Analysis of RNA editing in SINEs. Related to Figure 7.

A. Sub-families of SINEs were analysed as in Figure 6D, but are plotted to include those where observed or expected values exceeded 250.

B. Fold enrichments of editing events were calculated relative to expected numbers of edits for SINE sub-families using editing events detected in WT samples and differentially edited sites.

Table S1. Differentially expressed REs.

REs differentially expressed in *Adar1*^{mZ α /mZ α} lungs are shown with their log2 fold change and adjusted p-value. The RE class, family and chromosomal location are indicated. REs were sorted by adjusted p-value. The cut-offs used were: log2 fold-change >1 or <-1, and adjusted p-value <0.01.

Class Family Chr Start Stop	log2 fold change	padj
Satellite GSAT_MM JH584304.1 60177 60876	3.3	0
LTR/ERV1-MaLR MTD JH584304.1 61136 61478	3.3	6.80E-259
LTR/ERV1-MaLR MTD JH584304.1 25102 25465	3.0	4.93E-157
LTR/ERV1-MaLR MTD-int 5 120880478 120881813	3.3	1.00E-100
LTR/ERV1 ETnERV2-int 18 60283765 60287191	2.1	4.51E-93
LINE/L1 L1MdA_VII 18 60302236 60308420	1.9	4.12E-82
LTR/ERV1-MaLR MTD-int 5 120900459 120901797	2.3	9.73E-59
LINE/L1 L1M5 5 120744115 120744500	2.8	5.45E-56
LINE/L1 Lx8b 18 60290416 60292037	1.9	1.30E-51
LTR/ERV1 ERV4_1-I_MM~RLTR50B 18 60288796 60289913	2.1	1.67E-46
LTR/ERV1 APEY3_LTR~IAPEY-int 1 173572498 173580638	1.1	6.52E-44
LINE/L1 Lx7 3 151733082 151735087	2.7	1.69E-42
SINE/B4 B4A 5 120879672 120879973	3.9	2.34E-38
SINE/B4 B4A 5 120743868 120744091	2.5	6.13E-38
LINE/L1 L1MdTf_III 7 104475378 104477900	1.6	1.40E-37
SINE/B2 B3 JH584304.1 61870 61954	2.8	7.81E-37
SINE/B2 B2_Mm2 5 120744880 120745045	2.8	5.86E-36
LTR/ERV1 ERV2_1-I_MM~RLTR13D2 1 173566173 173572495	1.0	3.47E-35
LTR/ERV1 RLTR33 12 103438085 103438897	1.7	9.56E-35
LINE/L1 L1MdN_I 18 60219113 60220489	1.7	1.00E-33
LTR/ERV1 RLTR6-int 12 103440882 103441504	1.9	2.23E-32
SINE/B4 RSINE1 5 120744507 120744635	3.5	7.38E-32
LINE/L1 Lx8 5 114904191 114904607	1.9	1.11E-26
SINE/B2 B3 5 120744692 120744879	2.9	3.76E-26
LINE/L1 L1MdA_I 7 104502396 104504479	1.5	4.46E-25
LTR/ERV1 RMER12B 18 60308716 60309841	1.7	4.59E-25
LTR/ERV1 ETnERV2-int 18 60287718 60288163	2.1	5.34E-25
SINE/B4 B4A 5 120899645 120899943	2.3	9.68E-25
LTR/ERV1 RLTR1A2_MM 18 60287192 60287717	2.2	3.30E-24
LINE/L1 L1MdF_I 7 104489329 104493378	1.6	5.47E-24
LTR/ERV1-MaLR MTD~MTD-int 5 120867256 120868952	2.7	2.72E-23
LINE/L1 Lx8b 18 60292356 60292908	2.1	7.39E-23
LINE/L1 Lx3_Mus 1 173476327 173479976	1.8	3.80E-22
LINE/L1 L1MdF_III 7 104412877 104415655	1.0	8.14E-22
LINE/L1 Lx7 3 151735621 151736718	2.5	1.26E-21
LINE/L1 L1MdF_IV 18 60258255 60262863	1.8	4.41E-21
LTR/ERV1 RLTR10-int 7 104417226 104418855	1.0	5.22E-21
LTR/ERV1-MaLR MTA_Mm 5 114907656 114908049	2.1	5.94E-21
LTR/ERV1 RMER19B 11 119395887 119396564	1.3	7.35E-21
LTR/ERV1 MurERV4-int 11 119398983 119400690	1.1	4.62E-20
LTR/ERV1 RLTR13B1 5 120894364 120895346	2.3	1.73E-19
SINE/B4 B4A 5 120732706 120732910	2.6	2.17E-19
SINE/ID ID2 5 114901484 114901562	1.8	7.16E-19
SINE/B2 B2_Mm2 5 114910369 114910572	2.2	1.60E-18
LINE/L1 L1MdF_III 7 104456010 104461764	1.4	6.23E-18
LINE/L1 L1MdF_IV 8 62028789 62032657	1.4	6.98E-18
LTR/ERV1 RLTR11A2 15 77730371 77730874	2.9	8.25E-18
LTR/ERV1 RLTR10~RLTR10-int 7 104415825 104417145	1.1	1.24E-17
LINE/L1 L1MdA_III 18 60218363 60219112	1.5	3.04E-17
LINE/L1 Lx8 11 48893355 48894122	2.5	4.36E-17
LINE/L1 Lx5 18 60255846 60257256	1.8	5.14E-17
LINE/L1 Lx9 11 48988698 48990056	1.6	8.90E-17
LTR/ERV1 LTR72_RN 18 60217070 60217447	1.6	1.33E-16

LINE/L1 L1MC5 3 151741122 151741823	2.9	1.94E-16
LTR/ERV ERV2_1A-I_MM~RLTR13D5 18 60282851 60283792	1.9	3.57E-16
LTR/ERV RLTR17D_MM 18 60255056 60255845	2.1	4.71E-16
LINE/L1 L1MdF_I 1 85299575 85304519	2.8	5.46E-16
SINE/B2 B3 18 60247949 60248146	1.9	1.13E-15
LINE/L1 L1MEc 16 97458642 97459049	3.0	2.18E-15
LTR/ERV L-MaLR ORR1C2 5 120748110 120748423	2.8	5.32E-15
LINE/L1 L1MdTf_III 6 57628358 57634538	1.1	6.33E-15
LINE/L1 Lx6 2 173195005 173200024	1.8	7.37E-15
LTR/ERV L-MaLR ORR1A2 18 60292038 60292355	2.4	7.76E-15
LINE/L1 L1MdV_III 5 120882900 120884828	4.1	9.71E-15
SINE/B4 B4A 5 120737450 120737698	2.8	1.24E-14
LTR/ERV L-MaLR MTD X 112371164 112371262	2.3	1.28E-14
LTR/ERV L-MaLR MTD 11 72304177 72304543	1.2	1.44E-14
SINE/Alu B1_Mur2 5 120731990 120732119	3.1	2.38E-14
LTR/ERV L-MaLR MTD~MTD-int 5 120817871 120818905	1.4	2.75E-14
LINE/L1 L1M5 6 3341749 3341957	1.1	3.15E-14
LTR/ERV L-MaLR MTEb 19 34585573 34585953	2.0	4.45E-14
LINE/L1 L1MdV_III 7 104493403 104495643	1.5	4.59E-14
SINE/B2 B3 5 120745046 120745085	2.7	1.17E-13
LINE/L1 L1MdN_I 7 104477902 104478732	1.3	1.60E-13
LINE/L1 Lx8 18 60212692 60213382	1.6	1.71E-13
LINE/L1 L1MdTf_III 18 60236091 60238578	1.6	2.06E-13
LTR/ERV ERV5_1-I_MM 11 83007973 83009733	1.7	3.34E-13
LINE/L1 L1_Mur3 8 61931617 61933065	1.3	4.13E-13
LINE/L1 L1MdF_IV 18 60231803 60232768	1.7	5.15E-13
LTR/ERV MLTR14 7 104421879 104422356	1.2	5.39E-13
LTR/ERV L-MaLR MTC 16 97454326 97454700	2.7	6.93E-13
LTR/ERV L-MaLR MTB_MM-int 17 78862042 78863246	1.0	7.35E-13
DNA/hAT-Charlie MER58A 5 120899366 120899528	2.4	1.55E-12
LTR/ERV RMER15-int 18 60388388 60389084	1.4	2.49E-12
LINE/L1 L1MdF_III 1 52127797 52128672	1.2	3.84E-12
LTR/ERV L-MaLR ORR1E 11 72305310 72305625	1.2	3.84E-12
LTR/ERV RMER19C 5 120739285 120739711	3.8	3.85E-12
LINE/L1 L1MdV_III 7 104450903 104454061	1.4	4.92E-12
SINE/Alu PB1D9 19 34586260 34586378	2.0	5.01E-12
LINE/L1 Lx5 8 61953216 61953896	1.6	5.84E-12
LINE/L1 L1MdA_III 7 104497785 104499051	1.6	6.75E-12
LTR/ERV RLTR10F 5 120745593 120745641	2.3	9.12E-12
LTR/ERV MT2C_MM 12 103431337 103431828	3.1	1.01E-11
LINE/L1 L1MdF_IV 18 60263356 60264933	2.1	1.21E-11
SINE/Alu B1_Mus1 5 114898644 114898789	2.0	1.28E-11
LINE/L1 Lx8 5 114904021 114904128	2.4	1.34E-11
LTR/ERV L-MaLR ORR1C1~ORR1C1-int 8 62019273 62020492	1.8	1.82E-11
LTR/ERV L-MaLR MTD 16 97459784 97460166	2.5	2.57E-11
LTR/ERV MER65-int 18 60215493 60215799	1.7	2.61E-11
LINE/L1 Lx7 8 61930625 61931615	1.5	2.77E-11
LINE/L1 L1MdV_I 7 104473898 104475381	1.4	3.00E-11
LTR/ERV L-MaLR ORR1A4~ORR1A4-int 8 61959410 61960410	1.7	3.12E-11
LTR/ERV L-MaLR ORR1E 16 97455751 97456075	3.0	3.63E-11
LTR/ERV L-MaLR MTC 3 151744695 151745110	2.6	4.72E-11
DNA/TcMar-Tigger Tigger13a 19 28642966 28643654	9.3	5.06E-11
LINE/L1 L1MEc 16 97460167 97460362	3.3	7.07E-11
LTR/ERV RMER19B 17 78878705 78879529	1.2	8.11E-11
SINE/Alu B1F2 5 114899111 114899246	2.1	8.42E-11
LINE/L1 Lx8 8 61978737 61979085	1.7	8.42E-11
LTR/ERV RLTR17D_MM 18 60228369 60229159	1.5	1.01E-10
LINE/CR1 X6A_LINE 6 127449631 127450147	1.7	1.08E-10
SINE/B4 B4 11 119418705 119418955	1.3	1.62E-10
LTR/ERV RMER10B 12 103434622 103435041	1.8	2.64E-10
LINE/L1 Lx4A 7 104462257 104463184	1.7	2.88E-10
SINE/Alu B1_Mur1 11 119424502 119424651	1.2	2.88E-10
LTR/ERV L-MaLR ORR1A4-int 8 61961707 61963155	1.3	3.22E-10

LTR/ERV1 RLTR1B 16 97457671 97458166	2.2	3.52E-10
SINE/B2 B3A 11 119404723 119404878	1.3	3.77E-10
LTR/ERV1-MaLR MTC 18 60381338 60381622	1.7	3.77E-10
LINE/L1 L1MdV_I 5 105283637 105285975	1.6	3.97E-10
LTR/ERV1 RLTR25B 19 34650691 34651330	1.4	4.78E-10
SINE/B2 B3 5 114899419 114899545	2.0	6.53E-10
LTR/ERV1 RMER15 19 28639198 28639693	8.9	6.63E-10
LINE/L1 L1_Mur3 16 97548067 97549012	1.7	7.03E-10
LINE/L1 L1MEI 19 28631069 28632121	8.8	8.12E-10
LTR/ERV1 RLTR25A 8 61972997 61973607	1.4	8.16E-10
LINE/L1 L1MdA_VII 18 60288431 60288795	1.8	1.45E-09
LINE/L1 L1Lx_III 3 151742481 151742961	2.4	1.67E-09
LTR/ERV1 MurERV4-int 11 119401805 119402501	1.3	1.86E-09
SINE/B2 B3 X 112370657 112370771	2.0	2.38E-09
LTR/ERV1 MurERV4-int 11 119400881 119401263	1.3	2.38E-09
LINE/L1 Lx9 JH584304.1 46386 46861	1.1	2.46E-09
LTR/ERV1 RMER17B 5 120869622 120870528	2.9	2.74E-09
LTR/ERV1-MaLR MTB_Mm 15 77733815 77734092	3.4	3.12E-09
LTR/ERV1 ERV2_1-I_MM 1 173580639 173582295	1.1	3.35E-09
LTR/ERV1-MaLR ORR1B2 5 114908516 114908882	1.5	4.45E-09
LTR/ERV1 MYSERV-int 6 57638599 57641256	1.0	6.35E-09
SINE/B4 B4 11 119446380 119446625	1.0	7.11E-09
LTR/ERV1 ERV4_1-I_MM 18 60288165 60288430	1.8	9.39E-09
LTR/ERV1-MaLR MLT1A 18 60223904 60224219	1.5	1.15E-08
SINE/B2 B2_Mm2 19 34584659 34584843	2.2	1.27E-08
LTR/ERV1-MaLR MTE2b 8 61970047 61970414	1.6	1.28E-08
LTR/ERV1 ERV5_1-I_MM 11 83010068 83011537	1.5	1.51E-08
LTR/ERV1 MLTR14 7 104480250 104480718	1.3	1.59E-08
LTR/ERV1-MaLR MTE2b 1 52136423 52136770	1.1	1.59E-08
SINE/B4 B4A 5 120741648 120741943	2.5	1.60E-08
LINE/L1 Lx8 19 28632845 28633192	8.4	1.81E-08
LINE/L1 Lx2A1 18 60277986 60280175	2.3	1.82E-08
LINE/L1 Lx3_Mus 18 60295395 60298143	1.8	1.91E-08
LINE/L1 L1MEC 16 97459476 97459783	2.7	2.30E-08
LTR/ERV1-MaLR MTD 5 120900388 120900472	3.2	2.76E-08
LINE/L1 L1M4 16 97553934 97554356	2.3	2.81E-08
LTR/ERV1 IAPLTR2_Mm 3 151740656 151741121	2.1	3.42E-08
LINE/L1 L1MdV_III 7 104488452 104489331	1.6	3.71E-08
LTR/ERV1 MERVK26-int~RLTR26_Mus 7 104448877 104450693	1.2	4.13E-08
SINE/Alu B1_Mus2 18 60251725 60251812	2.6	4.69E-08
LTR/ERV1-MaLR ORR1B1 11 119404156 119404499	1.0	6.92E-08
SINE/B2 B2_Mm1t 5 114909495 114909661	2.2	7.44E-08
SINE/B4 RSINE1 11 119425189 119425383	1.2	7.93E-08
SINE/B2 B3A 18 60380840 60381056	1.7	8.13E-08
SINE/B4 B4 19 28629227 28629484	8.2	9.75E-08
LTR/ERV1 RLTR42-int 17 36238528 36239128	4.8	1.06E-07
LTR/ERV1-MaLR MTC 18 60382046 60382401	1.4	1.18E-07
SINE/B4 ID_B1 11 119407311 119407556	1.0	1.18E-07
DNA/hAT-Charlie Charlie16a 1 52132936 52133086	1.3	1.23E-07
LTR/ERV1-MaLR MTB 16 97458167 97458530	2.0	1.37E-07
LINE/L1 L1MC5 18 60271813 60272228	2.2	1.40E-07
SINE/B4 RSINE1 5 120877997 120878148	3.8	1.42E-07
LINE/L1 Lx3_Mus 1 173583337 173589127	1.4	1.42E-07
LINE/L1 L1M2 18 60252957 60253392	2.0	1.47E-07
LTR/ERV1 LTRIS4A 5 114905222 114905693	2.1	1.84E-07
LINE/L1 Lx8 8 61926037 61926661	2.5	1.95E-07
SINE/B2 B3 11 119408463 119408535	1.4	2.06E-07
SINE/Alu PB1D11 5 114900810 114900897	2.0	2.39E-07
LINE/L1 Lx5 1 85293799 85295584	3.7	2.48E-07
LTR/ERV1 RLTR13D3 18 60232779 60233438	1.8	2.54E-07
LTR/ERV1-MaLR MTD 5 120737214 120737449	3.1	2.65E-07
LTR/ERV1-MaLR MTEb 16 97450859 97451049	3.4	2.73E-07
SINE/B4 B4A 18 60257733 60257992	1.7	2.89E-07

LTR/ERVJ MLTR25A 8 61960825 61961601	1.7	2.89E-07
LTR/ERVJ RLTR20A2B_MM 1 52124850 52125329	1.2	3.05E-07
SINE/Alu B1_Mus 1 1735395764 35395900	1.1	3.22E-07
LINE/L1 L1Lx_III 7 104442758 104443886	1.4	3.32E-07
LTR/ERVL RMER15-int 18 60217650 60217971	1.2	3.36E-07
DNA/hAT-Charlie MER20 1 52158511 52158655	1.3	3.50E-07
LINE/L1 L1_Mur3 18 60226049 60226282	2.1	3.64E-07
LTR/ERVJ RLTR20C1_MM 5 120734599 120734859	4.1	4.08E-07
LTR/ERVL-MaLR MTEb 11 119419503 119419749	1.3	4.17E-07
DNA/hAT-Charlie MER33 19 28641324 28641627	7.9	4.22E-07
LTR/ERVL-MaLR MLT1A 18 60250995 60251268	1.7	4.23E-07
LINE/L1 L1MdF_I 11 119397535 119397973	1.3	4.23E-07
SINE/Alu B1_Mur4 5 114900337 114900479	1.6	4.29E-07
LINE/L1 L1_Mur2 5 105281882 105283598	1.7	4.42E-07
Other RMER1A 8 61979640 61980291	1.5	4.58E-07
LTR/ERVJ ETnERV2-int 8 62007507 62009017	1.6	4.83E-07
LINE/L1 L1MC5 3 151743348 151743525	2.7	5.34E-07
LTR/ERVL-MaLR MTD 12 26472066 26472478	2.0	5.39E-07
LINE/L1 L1MB1 18 60220588 60220785	1.4	5.59E-07
SINE/B4 ID_B1 2 173207281 173207474	1.9	5.93E-07
SINE/B4 ID_B1 11 119425805 119425924	1.3	6.35E-07
LTR/ERVL-MaLR ORR1A2 5 120740579 120740914	3.0	6.88E-07
LINE/L1 Lx4A 7 104437169 104438548	1.4	7.31E-07
LTR/ERVJ ERV5_1-I_MM 11 83006799 83007974	1.4	9.02E-07
LINE/L1 L1M5 1 78402205 78403427	1.1	1.01E-06
SINE/B2 B2_Mm2 5 114910612 114910685	2.4	1.06E-06
SINE/B4 B4A 1 52129374 52129536	1.4	1.22E-06
SINE/B2 B2_Mm2 19 28642281 28642465	7.7	1.38E-06
SINE/B2 B3 19 28633498 28633654	7.7	1.38E-06
LINE/L1 Lx2B2 11 49062198 49062461	1.2	1.47E-06
LTR/ERV1 MER34A 6 57582287 57582506	1.1	1.49E-06
LINE/L1 L1MdF_I GL456221.1 2915 7856	2.8	1.54E-06
LTR/ERVL MLT2D 11 48885340 48885618	2.4	1.54E-06
LTR/ERVJ MMERVK9E_I-int 8 62004675 62005632	1.5	1.72E-06
LINE/L1 Lx8 18 60239540 60240228	1.8	1.84E-06
LTR/ERVJ BGLI 7 104482055 104482477	1.4	2.15E-06
LTR/ERVJ RMER6C 2 173214417 173215067	2.6	2.19E-06
LTR/ERVL-MaLR MTEa-int 1 85289885 85291027	7.6	2.30E-06
LTR/ERVL-MaLR ORR1C2 2 131068596 131068990	1.1	2.40E-06
LTR/ERVL-MaLR ORR1F 18 60253720 60254029	2.1	2.42E-06
LTR/ERVJ RMER12 5 120762281 120763522	3.3	2.49E-06
SINE/B4 RSINE1 4 99841078 99841236	7.6	2.59E-06
LINE/L1 Lx4A 7 104504433 104505979	1.4	2.59E-06
LTR/ERVL-MaLR MTD-int 5 120819453 120820125	1.2	2.78E-06
LINE/L1 L1MC 11 58190640 58190990	1.4	3.05E-06
LINE/L1 L1MdFanc_I 8 62027358 62028774	1.4	3.18E-06
LINE/L1 L1MdF_V 6 57656263 57657234	1.6	3.19E-06
SINE/B4 B4A 11 119428315 119428565	1.8	3.55E-06
LTR/ERVJ RLTR31B2 11 48886943 48887241	2.0	3.61E-06
LINE/L1 L1MEg 14 22926181 22927055	1.2	3.61E-06
LTR/ERVL-MaLR MLT1A 18 60248622 60248960	1.5	3.84E-06
LTR/ERVL-MaLR MLT1E1A 8 61928913 61929235	1.9	4.17E-06
LINE/L1 Lx8 18 60383318 60384031	1.1	4.25E-06
LTR/ERVL-MaLR MTA_Mm-MTA_Mm-int 19 56257034 56258929	1.3	4.57E-06
LINE/L1 L1MCC 1 173466324 173466705	2.3	4.62E-06
LINE/L1 L1Lx_IV 11 82998919 83000309	1.6	4.81E-06
SINE/B4 B4A 5 120741975 120742193	2.7	4.96E-06
SINE/B4 B4A 19 28635419 28635576	7.6	4.96E-06
SINE/B4 ID_B1 11 119403020 119403132	1.2	5.58E-06
LINE/L1 L1M5 18 60226313 60226492	2.0	5.89E-06
LTR/ERVL-MaLR ORR1A3 11 119407950 119408239	1.1	6.40E-06
LINE/L1 L1MC 11 58189736 58190050	1.3	7.01E-06
SINE/B2 B3A 8 61987073 61987211	1.7	7.39E-06

LTR/ERVJ RMER20B 3 151742296 151742480	2.7	8.09E-06
SINE/B4 RSINE1 16 97453740 97453952	2.7	8.54E-06
LTR/ERVJ MaLR ORR1G 18 60226675 60226792	2.8	8.94E-06
LTR/ERVJ L1 L1 18 60387696 60387959	1.2	9.12E-06
SINE/B4 ID_B1 12 26447776 26447975	4.2	1.01E-05
LTR/ERVJ MaLR ORR1B1-int 7 104412596 104412775	1.4	1.03E-05
SINE/Alu PB1D10 5 114903889 114904001	2.0	1.07E-05
DNA/hAT-Charlie MER20 5 120732243 120732442	2.4	1.21E-05
LTR/ERVJ MLTR18D_MM 19 34642526 34643041	1.5	1.27E-05
LTR/ERVJ MaLR ORR1F 16 97539474 97539795	2.5	1.37E-05
LTR/ERVJ MaLR MTE2a 18 60222383 60222695	1.5	1.38E-05
LTR/ERVJ MLTR25A 8 61972378 61972814	1.4	1.49E-05
LTR/ERVJ MaLR MTD-MTD-int 11 119438208 119439017	1.3	1.60E-05
LINE/L1 Lx8b 7 104422425 104422813	1.0	1.60E-05
SINE/Alu B1_Mur2 11 58189251 58189397	1.2	1.60E-05
SINE/B2 B2_Mm2 5 114900909 114901081	1.9	1.64E-05
LTR/ERVJ RMER6D 8 61937912 61938757	1.4	1.74E-05
LINE/L1 L1MdF_IV 14 59239026 59244038	1.0	1.75E-05
LTR/ERVJ MaLR MTB_MM 15 77406091 77406368	4.2	1.89E-05
SINE/Alu B1_Mus1 18 60224405 60224548	2.0	1.89E-05
SINE/Alu B1_Mur3 11 119403373 119403524	1.5	1.89E-05
LTR/ERVJ RMER17D 18 60376370 60377162	1.3	2.06E-05
LINE/L1 L1MdGf_II 8 61952074 61952803	1.6	2.07E-05
SINE/B2 B3A 5 120736482 120736718	2.9	2.09E-05
LTR/ERVJ RMER20B 3 151742962 151743316	1.9	2.12E-05
SINE/B4 RSINE1 4 99840780 99840864	7.3	2.22E-05
LTR/ERVJ RLTR10 7 104415656 104415786	1.3	2.23E-05
LTR/ERVJ MaLR MTD 6 121255828 121256203	1.2	2.24E-05
LTR/ERVJ RMER6C 8 62032655 62033307	2.1	2.33E-05
LINE/L1 L1MdFanc_II 11 48895925 48896912	2.5	2.39E-05
LTR/ERVJ MaLR MTD 1 52138546 52138957	1.1	2.40E-05
LINE/L1 Lx2B2 11 48990324 48990587	1.8	2.56E-05
SINE/B4 B4A 11 72310037 72310328	1.2	2.56E-05
LINE/L1 Lx8b 18 60275235 60275895	3.0	2.59E-05
LTR/ERVJ MMERVK9E_I-int 8 62005696 62006980	1.4	2.65E-05
LINE/L1 Lx8b 6 57648031 57648834	1.0	2.83E-05
SINE/B2 B3A 5 120906530 120906727	2.1	2.97E-05
LINE/L1 L1M2 6 121257693 121258500	1.2	3.22E-05
LINE/L1 L1MdF_V 7 105888905 105891419	1.0	3.33E-05
SINE/B4 B4A 18 60222757 60223022	1.4	3.54E-05
LINE/L1 L1MdMus_I 7 105905000 105906394	1.1	3.88E-05
SINE/B2 B3 18 60223226 60223431	1.4	3.95E-05
LINE/L1 L1MEc 16 97540878 97541130	2.8	3.98E-05
LTR/ERVJ MaLR ORR1B1 10 128274867 128275232	1.1	3.98E-05
SINE/B2 B2_Mm2 5 120736758 120736943	3.2	4.16E-05
LTR/ERVJ RMER6A 8 61944108 61944875	2.1	4.30E-05
SINE/B2 B2_Mm2 17 78880852 78881043	1.4	4.30E-05
LINE/L1 L1M5 8 61946379 61947158	1.8	4.69E-05
SINE/Alu B1_Mus1 5 114909326 114909471	1.9	4.70E-05
LINE/L1 Lx5 11 48881306 48882584	2.4	4.97E-05
LINE/L1 Lx8 11 78973853 78974577	1.0	5.04E-05
SINE/B2 B2_Mm2 19 28632419 28632602	7.2	5.25E-05
LTR/ERVJ ERVJ4_1C-LTR_MM 1 52125995 52126510	1.1	5.62E-05
LTR/ERVJ MaLR MTC 14 59357742 59357949	1.4	5.66E-05
LTR/ERVJ ERVJ5_1-I_MM-ERVJ5_2-LTR_MM 11 83005040 83006189	1.7	6.18E-05
SINE/B4 RSINE1 18 60213658 60213808	1.6	6.18E-05
LINE/L1 L1MdF_II 6 57667427 57673682	1.1	6.18E-05
LTR/ERVJ RLTR20A3_MM 7 104463189 104463682	1.4	6.53E-05
SINE/Alu PB1D7 5 120876099 120876194	4.1	6.65E-05
LTR/ERVJ MaLR MTD 19 34649682 34650059	1.8	6.96E-05
SINE/Alu B1_Mus2 11 119397366 119397508	1.5	7.02E-05
LTR/ERVJ MT2B1 18 60266284 60266703	2.0	7.08E-05
LTR/ERVJ MT2B2 16 23610543 23610701	1.8	7.08E-05

LINE/L1 L1M5 8 62026273 62026678	1.9	7.22E-05
SINE/B2 B3 11 119431991 119432188	1.5	8.27E-05
LINE/L1 L1MA9 18 60389514 60389752	1.2	8.52E-05
LTR/ERV K RMER16B2 19 34643495 34643896	1.6	9.10E-05
SINE/B2 B3 11 48885122 48885339	2.9	9.26E-05
LTR/ERV K RLTR31D_MM 8 61934369 61934756	1.5	9.26E-05
SINE/B4 B4A 1 52121302 52121516	1.5	9.44E-05
LINE/L1 Lx6 2 173200965 173202050	2.1	9.82E-05
LINE/L1 L1MC3 5 100554042 100554239	1.8	0.000109224
LTR/ERV K ERV4_1-I_MM~RLTR43C 8 62002691 62003827	1.4	0.000109224
LINE/L1 L1_Rod 6 57584670 57585726	1.1	0.000110408
LTR/ERV K RLTR33 7 104482478 104483095	1.1	0.000112452
LINE/L1 Lx2A1 18 60275890 60277983	1.7	0.000114821
LTR/ERV L-MaLR MT 18 60235502 60235795	1.8	0.000116123
LINE/L1 Lx8 18 60380013 60380364	1.5	0.000116123
LTR/ERV L-MaLR ORR1F 5 120760326 120760690	2.6	0.00012678
LTR/ERV 1 MER65-int 18 60386130 60386423	1.1	0.000133868
LTR/ERV L-MaLR ORR1A2 1 173467096 173467424	1.9	0.00013703
LINE/L1 Lx8 8 94438097 94440818	1.0	0.000152871
SINE/B2 B3 11 119448108 119448296	1.1	0.000154092
SINE/Alu PB1 11 119422838 119422954	1.2	0.000158146
LINE/L1 L1ME3A 1 85672489 85672941	1.0	0.000158146
SINE/B2 B2_Mm1t 11 78980069 78980247	1.7	0.000159374
LINE/L1 L1MdFanc_I 8 62026867 62027358	1.8	0.000168661
LTR/ERV L-MaLR ORR1D1 8 61993767 61994083	1.9	0.000168999
LINE/L1 L1MdF_III 5 109141850 109144085	1.7	0.00017257
LTR/ERV K RLTR50B 18 60281383 60281838	1.7	0.000172929
LINE/L1 L1MB3 16 97449241 97449562	2.5	0.000177
SINE/Alu B1_Mur3 16 35844933 35845040	1.2	0.000177668
LTR/ERV K RLTR17 11 173482685 173483219	2.2	0.000179126
SINE/B2 B3 GL456211.1 227084 227304	3.2	0.000182121
LINE/L1 Lx8 8 61988622 61989015	1.6	0.000186362
LTR/ERV K RMER17A2 11 48885977 48886924	1.6	0.000186858
LTR/ERV L-MaLR ORR1D1 8 62016352 62016678	1.9	0.000207871
DNA/TcMar-Tigger Tigger17a 1 52148302 52148747	1.1	0.000209188
SINE/B4 RSINE1 11 119421171 119421274	1.8	0.000217129
SINE/B4 B4A 5 120866302 120866579	3.2	0.000219053
SINE/B2 B2_Mm1t 11 58190313 58190486	1.4	0.000220304
SINE/B2 B3 14 59352375 59352576	1.4	0.000223123
SINE/Alu B1_Mus1 18 60249131 60249275	1.6	0.00023365
SINE/B4 ID_B1 1 78411074 78411271	1.5	0.00023459
LTR/ERV K ERV4_1-I_MM 18 60282151 60282841	1.8	0.000240016
LINE/L1 Lx7 11 49092819 49093419	1.2	0.000244381
LINE/L1 L1MA9 6 57597772 57598304	1.1	0.000249114
SINE/Alu B1_Mus1 12 103436139 103436281	1.9	0.00027349
LINE/L1 L1MdA_III 16 36223021 36229214	1.2	0.000274576
SINE/B4 RSINE1 5 120872117 120872273	3.3	0.000287785
LTR/ERV L-MaLR MTD 11 72309643 72309975	1.1	0.000289508
SINE/B4 B4A 1 173484993 173485286	2.2	0.000290371
SINE/B4 B4A 18 60231038 60231308	1.5	0.000327918
LTR/ERV L-MaLR MTA_Mm 16 97552450 97552832	1.9	0.000331455
LINE/L1 L1MdV_I 11 119469230 119469675	1.0	0.000339371
SINE/B2 B3A 8 61937106 61937279	1.7	0.000348467
SINE/B2 B3A 17 6453751 6453921	2.1	0.000353282
LTR/ERV L-MaLR ORR1A2 7 104497343 104497650	1.6	0.00035773
LTR/ERV L-MaLR ORR1D2-int 7 105893142 105893563	1.0	0.00035773
LINE/L1 Lx8 8 61959169 61959373	1.9	0.000360775
LINE/L1 Lx8 19 12528103 12528695	1.8	0.000365486
LINE/L1 L1MdF_III 7 104454974 104455697	1.4	0.000367396
LINE/L1 L1MdA_IV 11 118381899 118388191	1.3	0.000368207
SINE/Alu PB1 16 35845564 35845645	1.0	0.000374836
LINE/L1 L1MdA_III 18 60406432 60412549	1.2	0.00038205
LTR/ERV L RMER10B 16 23611518 23611704	1.8	0.00040784

LINE/L1 Lx7 1 173470250 173470764	1.9	0.000408506
LTR/ERV RLTR25A 8 61960411 61960742	2.2	0.000410879
LINE/L1 Lx6 7 104440983 104441613	1.3	0.000418042
SINE/Alu PB1D10 11 119403173 119403281	1.2	0.000428833
LTR/ERV L-MaLR ORR1A2 5 120893806 120893895	3.5	0.000439001
LTR/ERV L-MaLR MLT1A0 3 151744445 151744691	2.5	0.000451551
LTR/ERV1 RLTR23 5 120818906 120819376	1.1	0.000453883
LINE/L1 L1MC5 3 151740389 151740655	2.6	0.000462351
LINE/L1 Lx2B 8 61987536 61987650	2.2	0.000462351
SINE/Alu B1_Mur3 11 72309443 72309576	1.8	0.000466519
SINE/B2 B3A 5 120886654 120886852	2.8	0.000474244
LTR/ERV L-MaLR ORR1B2-int 18 60308447 60308715	1.8	0.000476449
LINE/L1 L1Lx_IV 11 83024253 83025643	1.3	0.000478685
SINE/Alu B1_Mur4 18 60388241 60388387	1.5	0.000504818
LINE/L1 L1MEg 14 22924975 22925754	1.1	0.000521911
LINE/L1 L1MC3 19 12525661 12526010	2.0	0.000523155
SINE/B2 B3 11 119396967 119397132	1.2	0.000532394
LINE/L1 L1MdV_I 6 57655972 57656262	1.8	0.00058615
LTR/ERV1 MER65-int 18 60242375 60242677	2.0	0.000593172
LINE/L1 L1Lx_IV 7 104447255 104447947	1.2	0.000595394
LTR/ERV L RMER15-int 18 60388050 60388240	1.2	0.000595471
SINE/Alu B1_Mus1 11 119422309 119422456	1.3	0.000597017
LINE/L1 Lx8 14 59362138 59362787	1.3	0.000608974
LINE/L1 L1MEc 16 97542889 97543268	2.3	0.000613872
LINE/L1 L1Lx_I 8 61950817 61951123	1.7	0.000627706
LINE/L1 Lx3B 18 60271246 60271457	2.0	0.000635352
LTR/ERV RLTR33 7 104481788 104482054	1.2	0.000639421
LINE/L1 L1MdA_IV 15 75013194 75019358	1.0	0.000654485
LTR/ERV K MMERV10D3_I-int 19 12537134 12538136	1.6	0.00066349
SINE/B4 RSINE1 18 60384304 60384453	1.2	0.000669838
LINE/L1 L1MdA_II Y 90726495 90727209	6.2	0.000676413
LTR/ERV K RMER17C2 11 119394660 119395025	1.1	0.000679554
LINE/L1 L1ME2 19 34584395 34584639	1.8	0.000701496
LTR/ERV L MT2B2 5 114912527 114913101	1.5	0.000762304
LTR/ERV L-MaLR ORR1C2 11 118395751 118396103	1.6	0.000762944
LTR/ERV1 MMVL30-int 18 60249553 60250002	1.4	0.000823898
SINE/B4 B4 1 173475932 173476222	1.8	0.000849572
SINE/B4 B4A 1 52133585 52133834	1.1	0.000870689
LINE/L1 L1_Mur2 7 104496617 104497342	1.3	0.00094743
SINE/B2 B3A 1 85660064 85660261	1.1	0.000971813
LTR/ERV K RMER19B 1 85214688 85215577	3.1	0.000987718
SINE/B2 B3A 11 58190051 58190291	1.6	0.001005606
LINE/L2 L2 4 128922527 128922668	2.6	0.001051836
LINE/L1 Lx8b 18 60375408 60375552	2.8	0.001063904
SINE/Alu B1_Mur1 11 72310329 72310474	1.5	0.00107883
LTR/ERV1 MER34 8 61991169 61991412	1.3	0.001089669
SINE/Alu B1_Mus1 18 60251484 60251628	2.4	0.00110542
LINE/L1 L1_Rod 16 97549085 97549369	1.8	0.001150467
SINE/B4 B4A 5 120742594 120742675	2.3	0.001173077
SINE/B4 ID_B1 5 120736246 120736457	2.4	0.001203452
LINE/L1 L1MdGf_I 4 96209256 96210247	5.5	0.001205117
LINE/L1 Lx7 11 48898552 48898950	3.0	0.001264124
SINE/B4 B4A 8 61938958 61939194	1.9	0.001273237
SINE/B4 B4A 12 26455259 26455616	3.1	0.001297065
SINE/B4 RSINE1 3 151741825 151741992	2.3	0.001354579
LINE/L2 L2c 17 78881711 78882045	1.3	0.001408036
SINE/Alu B1_Mur3 11 119427174 119427277	1.3	0.001415298
LINE/L1 L1M5 6 57664127 57664870	1.4	0.001441351
LTR/ERV L RMER10A 11 58191702 58192067	1.4	0.001493181
SINE/B2 B3 1 78405712 78405917	1.4	0.001633648
LINE/L1 L1M2 18 60245064 60245319	1.7	0.001694221
SINE/Alu PB1D10 11 119474405 119474499	1.6	0.001812519
LINE/L1 L1M1 1 78410131 78410363	1.3	0.001823318

LTR/ERV1 LTR53-int 8 61923911 61924391	2.0	0.001837723
DNA/TcMar-Tc1 EutTc1-N2 7 104499725 104499945	1.4	0.001884216
SINE/B2 B3A 8 61970949 61971157	1.6	0.001896582
LINE/L1 L1MC4 6 121243607 121243843	2.9	0.001905609
LTR/ERV1 RMER15-int 18 60243994 60244664	1.5	0.001914539
LINE/L1 L1MdF_I GL456221.1 185129 190073	2.1	0.00193405
LINE/L1 L1MdGf_II 7 104461765 104462256	1.8	0.001965446
SINE/B2 B2_Mm1t 5 120742377 120742561	2.4	0.001970116
SINE/B2 B3A 18 60234288 60234503	1.9	0.001990529
Unknown YREP_Mm 18 60224643 60224761	1.7	0.002034878
LTR/ERV1 RodERV21-int 5 38628522 38629464	2.3	0.002074407
LTR/ERV1-MaLR ORR1G 18 60227122 60227332	1.4	0.002074407
LTR/ERV1 ERV5_2-LTR_MM 11 83011538 83011994	1.7	0.002105801
LINE/L1 L1MC5a 5 120729622 120729973	3.3	0.002120947
LINE/L1 L1MdA_VII 14 22550685 22551432	1.3	0.002120947
SINE/B2 B3A 8 61929572 61929765	1.8	0.002135782
SINE/B4 RSINE1 1 52121688 52121837	1.5	0.002244338
SINE/B2 B3 18 60250421 60250516	1.8	0.00227286
SINE/Alu B1_Mus1 5 120737774 120737916	2.2	0.002313263
LINE/L1 L1MdV_III 1 173472409 173472805	2.1	0.002313263
LINE/L1 L1MdTf_I 11 11287224 11294492	1.4	0.002358409
SINE/B4 B4A 5 120869392 120869621	3.0	0.002427648
LINE/L1 L1MD2 1 78415831 78416284	1.1	0.002580399
DNA/hAT-Charlie URR1A 8 61989152 61989341	1.8	0.002588266
SINE/B4 B4A 10 128280206 128280475	1.1	0.002608665
SINE/B4 ID_B1 6 121257175 121257271	2.3	0.0026213
SINE/B2 B2_Mm2 11 58205520 58205716	1.8	0.002654082
LINE/L1 L1M3 7 104480884 104481245	1.2	0.002675589
SINE/B2 B2_Mm2 4 128922392 128922528	2.7	0.002693458
LTR/ERV1-MaLR MLT1A0 16 97552073 97552408	1.8	0.002912433
LINE/L1 L1M3 18 60225894 60226042	2.4	0.002922993
SINE/Alu B1_Mur4 11 119432213 119432355	1.4	0.002957208
SINE/B4 B4A 11 119454023 119454233	1.1	0.003132828
LTR/ERV1 RLTR25A 1 85292929 85293798	2.8	0.003188085
SINE/B4 RSINE1 5 120734442 120734572	3.4	0.003210838
SINE/B2 B3A 8 62017361 62017545	2.3	0.003228318
SINE/B4 B4 5 120814775 120815080	1.3	0.003330175
SINE/B2 B3 2 122154363 122154562	1.0	0.003385239
SINE/B4 B4A 12 103430589 103430874	2.6	0.003428791
DNA/hAT-Charlie URR1B 6 57634600 57634757	1.5	0.003486983
LINE/L1 Lx5c 3 151730142 151730853	2.7	0.003555802
LTR/ERV1 MMERV10D3_I-int-RLTR10D2 4 156157848 156161930	1.2	0.003555802
SINE/B2 B3 11 119403552 119403719	1.1	0.003661118
SINE/B2 B2_Mm2 11 119474900 119475066	1.4	0.003666262
SINE/Alu B1_Mur1 11 119426938 119427074	1.2	0.003667705
LTR/ERV1 RMER17C2 11 48891203 48891735	2.0	0.003754317
LINE/L1 L1MdA_VI 18 60412548 60416252	1.5	0.003804298
SINE/Alu PB1 11 119415833 119415919	1.3	0.00384182
LTR/ERV1 RMER4A 1 52145655 52146094	1.1	0.003931657
SINE/B4 ID_B1 1 78408347 78408481	1.3	0.004223362
LTR/ERV1 RLTR53_Mm 3 7605461 7605702	1.2	0.004487373
SINE/B2 B3A 14 59352033 59352172	1.1	0.004525402
LTR/ERV1 LTR33 8 61980345 61980747	1.4	0.004585898
LINE/L1 Lx8 11 48897140 48897506	2.9	0.00469754
SINE/MIR MIR 11 78984217 78984391	1.2	0.004703329
SINE/Alu B1_Mur4 1 85678728 85678875	1.1	0.004703329
SINE/B2 B3 1 85699200 85699407	1.1	0.004748338
SINE/B2 B3A 11 119453072 119453230	1.1	0.004748338
LTR/ERV1 LTR72_RN 18 60243439 60243666	1.9	0.004758321
SINE/Alu B1_Mm JH584304.1 45633 45776	1.0	0.004811999
SINE/Alu B1F 19 34589599 34589737	1.8	0.004941696
LTR/ERV1 MurERV4-int-RMER4B 5 38640610 38642621	1.8	0.005052178
SINE/Alu B1F 17 36016700 36016833	1.0	0.005108486

LTR/ERVJ RLTR11A2 14 74841714 74841956	1.5	0.005149262
LINE/L1 L1M3 5 105287249 105287488	2.1	0.005207743
LINE/L1 L1ME2 19 34608766 34609012	1.6	0.005229814
LTR/ERVJ MaLR ORR1B1 1 85206513 85206871	2.0	0.00524806
SINE/B2 B3 12 103430943 103431152	2.7	0.005295518
SINE/B2 B3 5 120742194 120742376	2.2	0.005295518
LTR/ERVJ MLTR11B 8 61922797 61923291	2.5	0.005350656
SINE/B4 B4A 3 151732580 151732796	2.2	0.005350656
LTR/ERVJ RMER17A2 11 83025644 83026552	1.4	0.005414877
SINE/Alu B1_Mur1 5 120741326 120741462	2.2	0.005446161
LINE/L1 Lx9 8 61996144 61997073	1.4	0.005583858
LTR/ERVJ RLTR12BD_Mm 11 119413650 119414012	1.4	0.005603168
LINE/L1 Lx7 5 81690438 81691087	1.2	0.00570399
LINE/L1 Lx5b 16 97541515 97541575	3.7	0.00581336
LTR/ERVJ MaLR MTC 18 60234785 60235077	1.8	0.005929058
LINE/L1 L1MC4 1 105984514 105984997	1.5	0.006181554
LINE/L1 Lx6 3 151725975 151728003	2.7	0.006490343
LTR/ERVJ MLTR14 11 119393861 119393938	1.2	0.006490343
LINE/L1 L1MdFanc_I 8 61966298 61966923	1.6	0.006798172
Unknown MurSatRep1 8 61986589 61986618	2.1	0.006944851
LTR/ERVJ RMER6C 1 173466744 173467049	1.9	0.007173117
LTR/ERVJ RMER15 16 97552833 97553254	1.7	0.007459961
SINE/Alu B1F1 10 128273839 128273957	1.2	0.007468964
LINE/L1 L1MdA_V 1 147487927 147488645	1.1	0.007581135
SINE/Alu B1_Mur2 11 119422475 119422589	1.1	0.007632876
SINE/B2 B3 1 52161968 52162165	1.3	0.007756351
SINE/B2 B3 14 59244430 59244631	2.6	0.00778006
SINE/B4 RSINE1 16 35862636 35862773	1.1	0.007852115
LTR/ERVJ MaLR ORR1D1 11 78966330 78966666	1.1	0.007852374
SINE/Alu PB1D10 1 52157797 52157904	1.1	0.00786182
LTR/ERVJ MaLR ORR1E 16 97545892 97546219	1.6	0.007932381
LTR/ERVJ RLTR10D2 11 83014754 83015108	1.8	0.00795139
LINE/L1 Lx4B 18 60254283 60254608	1.4	0.008019575
SINE/Alu PB1 JH584304.1 46147 46243	1.3	0.008096681
SINE/B4 RSINE1 11 119454707 119454839	1.2	0.008275749
SINE/B2 B2_Mm2 11 48892662 48892842	2.2	0.008551993
SINE/B4 ID_B1 10 128280630 128280828	1.1	0.008555514
LTR/ERVJ MaLR MTC 5 114931510 114931937	4.4	0.008616601
LTR/ERVJ RMER15-int 5 38610880 38612957	1.6	0.008698384
LINE/L1 Lx3_Mus 5 109113342 109113831	2.3	0.008784429
LINE/L1 L1_Mus3 16 35876707 35879416	2.1	0.008815001
LINE/L1 L1MdMus_II 5 109131054 109134304	1.3	0.009424916
SINE/B2 B3A 11 119419727 119419826	1.1	0.009454442
SINE/B2 B3A 16 35855574 35855739	1.0	0.009590665
LTR/ERVJ MaLR ORR1B1 11 119397240 119397294	1.5	0.009627717
LINE/L1 Lx4B 18 60227587 60227901	1.5	0.009650305
SINE/Alu B1_Mur2 4 42698174 42698309	-1.2	0.006343316
SINE/Alu B1_Mus2 14 52188546 52188712	-1.3	0.006282691
SINE/Alu B1_Mur4 4 42702271 42702324	-1.0	0.00577895
LTR/ERVJ ERV7_1-LTR_MM~ETnERV-int 7 133528843 133536216	-4.1	0.005738875
LINE/L1 Lx6 5 109930987 109932960	-1.1	0.004709914
LTR/ERVJ MaLR ORR1A2 4 42198416 42198743	-1.8	0.003795357
LTR/ERVJ MaLR MTC 4 41860499 41860704	-1.2	0.002380466
LTR/ERVJ MaLR ORR1D1 5 81438765 81439074	-1.3	0.001738738
LINE/L1 Lx8 5 81442017 81442485	-1.1	0.001387036
SINE/Alu B1_Mus1 4 42701837 42701939	-1.3	0.001332738
LINE/L1 L1MB4 GL456350.1 213136 214351	-1.3	0.001244243
LINE/L1 Lx3B 1 38194814 38195586	-1.1	0.001203452
SINE/Alu B1_Mus1 4 42211905 42212047	-3.1	0.000987718
LINE/L1 L1Lx_III 14 22888262 22889657	-1.3	0.00085488
LTR/ERVJ MaLR MTD 4 42470347 42470605	-1.4	0.000541809
LTR/ERVJ MaLR MTD 4 42206462 42206627	-1.2	0.000526551

LTR/ERV1 LTRIS2 4 41882251 41882738	-1.9	0.000395352
SINE/B2 B3 4 42006940 42007096	-1.6	0.000368207
LTR/ERV1-MaLR MTC 4 41859834 41859870	-5.7	0.000364103
LTR/ERV1 RMER17B2 4 42199930 42200852	-1.6	0.000331455
LINE/L1 Lx9 2 106407857 106408375	-1.2	0.000292512
LINE/L1 L1M4b 4 42207136 42207346	-5.0	0.00029125
SINE/Alu B1_Mus1 14 52189113 52189250	-1.3	0.000225471
LINE/L1 L1MB4 GL456350.1 215333 215759	-1.7	0.000179446
LTR/ERV1 ERV7_1-LTR_MM~ETnERV-int 3 78552311 78559462	-2.2	0.000126775
LTR/ERV1-MaLR MTC 4 42209075 42209111	-7.0	8.34E-05
SINE/B4 B4A 4 42205385 42205658	-1.3	7.74E-05
SINE/Alu B1_Mur4 4 42005473 42005619	-1.2	7.08E-05
SINE/Alu B1_Mus2 4 42202263 42202409	-1.5	6.34E-05
LTR/ERV1-MaLR ORR1A3 4 42201549 42201891	-1.4	5.86E-05
DNA/hAT-Tip100 Arthur2 4 41855107 41855330	-1.1	4.99E-05
LTR/ERV1 MMERV10C-int~RLTR10B2 8 3945964 3951863	-1.1	4.34E-05
LTR/ERV1 RMER17B2 4 41944961 41945909	-1.2	3.08E-05
SINE/B2 B3 4 41846277 41846489	-1.1	2.22E-05
LTR/ERV1 ERV7_1-LTR_MM~ETnERV-int 3 8383520 8390993	-3.2	1.63E-05
SINE/Alu B1_Mus2 4 42699410 42699556	-1.1	5.87E-06
SINE/Alu B1_Mur1 4 42699613 42699730	-1.1	4.11E-06
scRNA BC1_Mm JH584293.1 158471 158531	-7.4	2.78E-06
LINE/L2 L2 18 78192885 78194073	-3.5	1.06E-06
LTR/ERV1-MaLR ORR1B2 4 42210920 42211264	-3.1	9.23E-07
SINE/Alu B1_Mur3 4 41845780 41845916	-1.2	2.66E-07
LTR/ERV1-MaLR ORR1E 4 42488823 42489144	-1.6	2.57E-07
SINE/ID ID4 4 42689431 42689510	-1.2	2.13E-07
SINE/Alu B1_Mur2 4 42006829 42006920	-1.4	2.08E-07
SINE/Alu B1_Mur4 4 42693249 42693395	-1.3	1.76E-07
SINE/B4 RSINE1 4 42698000 42698095	-1.3	8.46E-08
LTR/ERV1-MaLR ORR1A3 4 42698696 42699038	-1.6	4.88E-08
LINE/L1 Lx5 4 42692601 42692763	-1.2	2.87E-08
SINE/B2 B3 4 42677410 42677598	-1.3	1.91E-08
LINE/L1 L1MdTf_I 10 106206469 106213575	-2.0	1.75E-08
LINE/L1 L1MdF_I 4 42699736 42700028	-1.3	1.60E-08
LTR/ERV1-MaLR ORR1A3 4 42010910 42011252	-1.1	1.44E-08
SINE/B2 B3 4 42694716 42694872	-1.1	1.35E-08
SINE/Alu B1_Mur2 4 42690321 42690460	-1.1	8.84E-09
LTR/ERV1-MaLR MTC 4 42208669 42208881	-3.3	5.97E-09
LTR/ERV1 MERV1_I-int~RLTR41 4 41997400 42000692	-1.1	5.26E-09
Unknown YREP_Mm 4 42698157 42698308	-1.5	3.92E-09
LTR/ERV1-MaLR MTD 4 42212252 42212563	-3.6	2.84E-09
LTR/ERV1-MaLR MTD 4 42211419 42211544	-3.1	1.45E-09
LTR/ERV1-MaLR ORR1E 4 42693396 42693452	-1.4	1.39E-09
LTR/ERV1-MaLR MTD 4 42703609 42703774	-1.2	1.26E-09
LINE/L1 L1M4b JH584293.1 158632 158842	-6.4	1.20E-09
SINE/B2 B2_Mm2 4 21873292 21873426	-1.4	9.41E-10
SINE/B2 B2_Mm1t 4 42487831 42488007	-1.5	7.00E-11
SINE/B2 B3 4 42206861 42207067	-2.8	7.19E-12
LTR/ERV1-MaLR ORR1E 4 42005151 42005472	-1.3	3.84E-12
SINE/B2 B3 4 42692346 42692558	-1.2	1.67E-12
SINE/B2 B2_Mm1t 4 41859871 41860047	-5.8	8.98E-13
LTR/ERV1 LTR72_RN 4 41863323 41863472	-2.5	8.63E-13
SINE/B4 B4A 4 42696105 42696301	-1.5	1.93E-13
LTR/ERV1-MaLR ORR1D2 4 41866797 41866870	-6.0	3.33E-14
LTR/ERV1 MERV1_I-int~RLTR41 4 42493603 42496895	-1.4	2.28E-14
LTR/ERV1-MaLR ORR1E 4 42692927 42693248	-1.3	1.71E-14
LTR/ERV1 RLTR1B-int 4 41881485 41882110	-1.4	4.25E-15
LTR/ERV1 IAPEz-int~IAPLTR1_Mm 2 154354263 154359592	-1.0	3.35E-15
LTR/ERV1 ETnERV-int 4 41882740 41882895	-2.4	2.39E-15
LTR/ERV1-MaLR MTD 4 42703056 42703249	-1.3	1.37E-15
LINE/L1 L1M5 4 42688781 42688992	-1.3	6.88E-16
SINE/Alu B1_Mur2 4 21872875 21873007	-1.9	9.59E-17

SINE/B2 B3A 4 42688563 42688744	-1.2	6.68E-17
DNA/hAT-Tip100 Arthur2 4 42701104 42701325	-1.3	2.80E-17
SINE/B2 B2_Mm1t 4 42006288 42006464	-1.5	1.95E-17
LINE/L1 Lx9 4 42696517 42697053	-1.3	3.62E-19
SINE/B4 B4A 4 42702532 42702805	-1.3	2.99E-20
SINE/B4 B4 4 41861171 41861469	-1.5	2.73E-20
LTR/ERV1-MaLR MTD 4 41862178 41862303	-6.4	6.78E-21
SINE/B2 B2_Mm2 4 42006123 42006221	-1.5	1.95E-21
LINE/L1 L1MD2 4 42689041 42689329	-1.3	8.59E-22
LTR/ERV1 LTRIS2 4 41877124 41877612	-1.8	7.03E-22
LTR/ERV1-MaLR MTEa 4 42689524 42689847	-1.1	4.80E-22
LTR/ERV1-MaLR MTC 4 41859428 41859640	-7.2	2.79E-22
LINE/L1 L1MD2 4 42689848 42690320	-1.2	6.12E-23
LTR/ERV1-MaLR ORR1A2 4 42695575 42695902	-1.4	3.42E-23
LTR/ERV1-MaLR ORR1B2 4 41861679 41862023	-4.9	5.00E-24
LINE/L1 L1MdV III 4 42682716 42684974	-1.2	4.51E-25
LTR/ERV1-MaLR ORR1D2 4 41869118 41869231	-5.5	2.52E-27
LINE/L1 Lx5 4 41869274 41869392	-4.0	5.24E-28
LINE/L1 Lx9 4 41864119 41864457	-3.1	1.55E-28
LTR/ERV1 RMER17B2 4 42697077 42697999	-1.5	1.29E-28
SINE/Alu B1_Mus1 4 41862664 41862806	-6.0	6.72E-32
LINE/L1 Lx5 4 41860065 41860499	-6.5	2.14E-32
LINE/L1 L1MD2 4 42690461 42691435	-1.2	6.41E-34
LTR/ERV1 RMER16-int 4 41880620 41881663	-2.0	1.18E-38
LTR/ERV1-MaLR MTD 4 41863011 41863322	-6.8	1.33E-40
LTR/ERV1 MERV1 I-int~RLTR41 4 42685154 42688451	-1.2	8.05E-43
LTR/ERV1 RLTR40 4 21871708 21872365	-1.8	2.50E-45
LTR/ERV1 ERV7 1-LTR MM~ETnERV-int 4 41870907 41877123	-3.1	2.95E-48
LINE/L1 L1MEg 4 41866400 41866748	-5.6	2.14E-50
LTR/ERV1-MaLR MTD 4 41864735 41864993	-4.3	1.66E-52
SINE/B2 B3 4 41865957 41866165	-5.1	8.07E-64
LTR/ERV1-MaLR ORR1E 4 41868720 41868968	-4.6	1.22E-74
LTR/ERV1 RLTR1B-int 4 41877612 41880640	-2.1	4.96E-98

Table S2. qPCR probes and primers.

Taqman Probes	Assay Probe ID
<i>Ifnb1</i>	Mm00439552_s1
<i>Ifng</i>	Mm01168134_m1
<i>Tnfa</i>	Mm00443258_m1
<i>Il1b</i>	Mm00434228_m1
<i>Il6</i>	Mm00446191_m1
<i>Ifit1</i>	Mm00515153_m1
<i>Ifit2</i>	Mm00492606_m1
<i>Ifi44</i>	Mm00505670_m1
<i>Isg15</i>	Mm01705338_s1
<i>Isg20</i>	Mm00469585_m1
<i>Zbp1</i>	Mm01247052_m1
<i>Oas1a</i>	Mm00836412_m1
<i>NP</i>	Custom probe based on NC_002019.1 Assay ID:AIX02UC
<i>Actin</i>	Mm02619580_g1
<i>Gapdh</i>	Mm99999915_g1 (4352932E)
SYBR Green oligos	Sequence
<i>M</i>	F: 5'-CTTCTAACCGAGGTCGAAACGTA R: 5'-GGTGACAGGATTGGTCTTGTCTTTA from: Shin et al. 2013, Virology Journal 10:303
<i>Gapdh</i>	F: 5'-CATGGCCTTCCGTGTTTCCTA R: 5'-CCTGCTTCACCACCTTCTTGAT from: Tsujita et al. 2006, PNAS 103:11946