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Supplementary information

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ADAR1 editing is necessary for only a small subset of cytosolic dsRNAs to evade MDA5-mediated autoimmunity

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Supplementary Note 1: Generation and characterization of ADAR1 mutant cell lines

To mimic the ADAR1 deaminase inactive E861A mutation in mice, we generated human ADAR1 E912A HEK293T cell line via CRISPR mediated homology directed repair. There are three *ADAR1* alleles in the HEK293T cell line we use. In the homozygous *ADAR1*$^{E912A/E912A/E912A}$ (hereafter referred to as ADAR1^{E912A}) cell line, RNA editing is almost entirely abolished and the residual amount of editing is presumably due to the lowly expressed ADAR2 (**Extended Data Fig. 1b–c**). In two heterozygous *ADAR1*$^{+/E912A/E912A}$ and *ADAR1*$^{+/+/E912A}$ lines we generated, we observed ADAR1 dosage-dependent levels of RNA editing (**Extended Data Fig. 1c–d**).

Supplementary Note 2: Comparative analysis of editing level at individual sites

We compared the editing level of known editing sites (documented in the RADAR database[1]) individually using the RNA-seq data from the MDA5-expressing *ADAR1*^{E912A} HEK293T cells complemented with p150 or p110 (**Extended Data Fig. 3a**). We found that most of the sites were similarly edited by p150 and p110, and overall, p150-expressing cells had even lower level of editing than p110-expressing cells, probably due to the higher expression level of p110 than p150.

Supplementary Note 3: Validation methods for immunogenic dsRNAs

To validate immunogenic dsRNAs as MDA5 substrates, we examined their ability to form MDA5 filaments *in vitro* using negative stain electron microscopy. We first tested perfect dsRNAs with different lengths. In agreement with previous findings[2], we observed that dsRNA under certain length could not support MDA5 filamentation and there was a correlation between dsRNA length and MDA5 filament length (**Extended Data Fig. 5c**). This provides critical references for the further characterization of newly identified dsRNAs.

To validate the cellular immunogenicity of identified dsRNAs, we first attempted to knockdown endogenous dsRNAs – either *cis*-NATs or IR*Alu*s – in ADAR1^{E912A} cells with Dox-inducible MDA5 and a luciferase IFN reporter (Methods). Despite high knockdown efficiency, we observed negligible difference between the knockdown and non-targeting controls in cellular immune response (**Extended Data Fig. 5f–g**). We reasoned that the 420 putatively immunogenic dsRNAs collectively contribute to the MDA5-dependent IFN response (**Table S1**), and such redundancy made it challenging to validate an individual or even mulitple dsRNAs by genetic perturbation.

We found that the genomic loci corresponding to immunogenic dsRNAs such as *cis*-NATs showed strong enrichment at the GWAS signals of inflammatory diseases. While we cannot rule out the possibility that the host genes for some of the immunogenic dsRNAs are regulating immune functions, majority of the host genes have no such function, suggesting the direct involvement of dsRNAs *per se* in inflammatory diseases.

Supplementary Note 4: Charaterization of the ADAR1$^{-/-}$ human neural progenitor cells

We performed RNA editing analysis using RNA-seq data from WT and *ADAR1*$^{-/-}$ NPCs at Day 29 after differentiation. Loss of *ADAR1* led to a significant reduction of RNA editing in a large number of editing clusters (**Extended Data Fig. 7f**), although a substantial amount of editing remained due to the ADAR2 expression in NPCs. ADAR2-mediated editing in NPCs was further confirmed by editing of *GRIA2* Q/R site, a known ADAR2-specific site (**Extended Data Fig. 7g)**. The presence of ADAR2, however, did not functionally compensate for the lack of ADAR1 to suppress the immune responses in NPCs, similar to our observations in HEK293T cells and previous *in vivo* mouse studies[3].

We investigated why the ISG induction and cell death of NPCs were observed at around Day 29, but not Day 15, after differentiation. By comparing the expression of genes involved in interferon signaling pathways (**Table S4**), we observed more than 2-fold increase in MDA5 and JAK1 expression and more than 2-fold decrease in ARL5B expression at Day 29 compared to Day 15 (**Fig. 3e**). Interestingly, ARL5B is a negative regulator of MDA5[4] and JAK1 is essential for type I and II interferon signaling[5], potentially contributing to elevated ISG expression in NPCs at Day 29. Second, by examining the expression level of NPC immunogenic dsRNAs at Day 15 and Day 29 we found that the total expression of immunogenic dsRNAs was 40% higher at Day 29 (**Fig. 3f**), as exemplified by *Alu*s (**Fig. 3g**), long intergenic LTRs (**Extended Data Fig. 8h**) and *cis*-NATs (**Extended Data Fig. 8i**). These data suggest that during NPC differentiation, the accumulation of immunogenic dsRNAs and/or the increased expression of interferon signaling proteins such as MDA5 activate the MDA5-mediated dsRNA sensing pathway and thus breach immune tolerance, implying a dose-dependent model.

We first chose an NPC-specific immunogenic dsRNA formed by IR-*Alu*s and overexpressed it in HEK293T cells, similar to the experiment described in **Fig. 2m** (see Methods). This IR-*Alu*s, compared to a single *Alu* as a negative control, led to an elevated IFN response that is dependent on MDA5 (**Fig. 4b, Extended Data Fig. 8c**). A similar effect was observed for an NPC-specific immunogenic dsRNA formed by *TAGLN:PCSK7 cis*-NAT when it was overexpressed in HEK293T cells (**Extended Data Fig. 8d–e**). To further test how the sequence and structure features may affect the immunogenicity of *cis*-NAT dsRNAs, we constructed a scrambled version of the *TAGLN:PCSK7 cis*-NAT that maintains the complementary structures of the overlapping region with randomized sequences (**Extended Data Fig. 8f**). We overexpressed this scrambled *cis*-NAT in the aforementioned HEK293T cells and observed elevated IFN response at comparable level to the original *cis*-NAT (**Extended Data Fig. 8d–e**). Our data suggests that the dsRNA structure, rather than its sequence, dictates the immunogenicity.

Supplementary Note 5: Conservation of ADAR1-dsRNA-MDA5 axis between human and mouse

We tested whether the mouse ADAR1 and MDA5 can substitute for their human counterparts in human cells. First, we transfected human or mouse ADAR1p150 into ADAR1^{E912A} HEK293T cells with Dox-inducible MDA5 and an IFN-responsive reporter (5xISRE-AP-NFkB-Lucia) (**Extended Data Fig. 10a–b**). Transfection of either human or mouse ADAR1p150 suppressed MDA5-dependent induction of the IFN-responsive reporter (**Fig. 5a**), suggesting that mouse ADAR1p150 can recognize and edit human immunogenic dsRNAs. Second, we stably integrated a human or mouse MDA5 in the human WT and *ADAR1*$^{-/-}$ HEK293T cells (**Extended Data Fig. 10c**) and measured the expression of signature ISGs. Both mouse and human MDA5 led to induction of ISGs in *ADAR1*$^{-/-}$ cells (**Fig. 5b**). Taken together, our data provides evidence of functional interchangeability of ADAR1 and MDA5 between human and mouse.

References

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