DNA mismatch repair is required for the host innate response and controls cellular fate after influenza virus infection

Benjamin S. Chambers\(^1\), Brook E. Heaton\(^1\), Keiko Rausch\(^2\), Rebekah E. Dumm\(^1\), Jennifer R. Hamilton\(^3\), Sara Cherry\(^*\)\(^2\) and Nicholas S. Heaton\(^*\)\(^1\)

Despite the cytopathic nature of influenza A virus (IAV) replication, we recently reported that a subset of lung epithelial club cells is able to intrinsically clear the virus and survive infection. However, the mechanisms that drive cell survival during a normally lytic infection remained unclear. Using a loss-of-function screening approach, we discovered that the DNA mismatch repair (MMR) pathway is essential for club cell survival of IAV infection. Repair of virally induced oxidative damage by the DNA MMR pathway not only allowed cell survival of infection, but also facilitated host gene transcription, including the expression of antiviral and stress response genes. Enhanced viral suppression of the DNA MMR pathway prevented club cell survival and increased the severity of viral disease in vivo. Altogether, these results identify previously unappreciated roles for DNA MMR as a central modulator of cellular fate and a contributor to the innate antiviral response, which together control influenza viral disease severity.

Influenza A virus causes an acute respiratory infection, and neither viral replication nor transmission requires long-term persistence of an infected cell. As a result, IAV infection typically proceeds with a characteristic cytopathic phenotype culminating in the death of infected cells through a variety of cell death pathways, including necrosis, necroptosis and pyroptosis\(^1\). Additionally, part of the innate immune response to viruses includes the induction of apoptosis, a pathway of controlled cell death\(^2,3\). While cellular viability must be maintained for a long enough time to facilitate sufficient production of viral progeny (and indeed, IAV has evolved mechanisms to block maintenance for a long enough time to facilitate sufficient production of viral progeny (and indeed, IAV has evolved mechanisms to block early apoptosis of the host cell\(^4,5\)), the long-term fate of the infected cells is thought to be irrelevant for viral replication/transmission. In addition to virally induced death of infected cells, professional immune cells can effectively recognize and clear infected cells from the host\(^6\). Thus, until recently, it had been assumed that viral clearance from the host is dependent on the death of all infected cells through one of the processes described above.

It was therefore surprising when we and others reported that a subset of respiratory epithelial cells, named club cells, can survive direct infection with IAV and persist in the host long term\(^7,8\). We have shown that these cells are not persistently infected, but rather that these cells have been infected and have cleared the virus through a non-lytic process\(^7\). Non-lytic clearance of Sindbis virus from neurons\(^9\) and hepatitis B virus and lymphocytic choriomeningitis virus (LCMV) from hepatocytes\(^10,11\) have also been reported, with the underlying hypothesis that, in some tissues, extensive cell death would compromise organ architecture and function\(^12,13\). While autophagy plays a role in cell-intrinsic control of some viral infections\(^14\), the mechanisms that promote non-lytic viral clearance remain incompletely understood.

To identify genes and pathways that promote non-lytic viral clearance after IAV infection, we performed a short interfering RNA (siRNA) screen to identify modulators of cellular survival. This screen revealed a number of genes that promote club cell survival following IAV infection, including a gene involved in the DNA MMR pathway. Since IAV is not generally thought to lead to cellular death via lethal genomic DNA damage, it was unclear how this pathway would promote survival from IAV infection. We found that the DNA MMR pathway was required to prevent the accumulation of oxidative DNA lesions in antiviral gene loci, which probably contributes to the non-lytic viral clearance phenotype in club cells. Additionally, we engineered viruses that artificially enhance suppression of the DNA MMR pathway and showed that, under these conditions, cells were unable to survive IAV infection and the overall severity of viral disease was increased in vivo. Together, these data have revealed an unappreciated role for a DNA repair mechanism that is required for the innate cellular response and controls cellular fate during IAV infection.

Results

Differential cellular fate after influenza A virus infection can be modelled in vitro. To observe cellular fate after IAV infection in vitro, we previously utilized LoxP-Stop-LoxP-reporter-containing NCI-H441 cells, which activate a fluorescent reporter after infection with a Cre-expressing influenza virus (IAV-Cre)\(^15\). For this study, we generated an analogous reporter in an alveolar epithelial cell line (A549) to compare rates of survival after infection (Fig. 1a). Following IAV-Cre infection, we found that, while both cell types initially activate the ZsGreen reporter, the infected A549-CR cells ultimately succumbed while the H441-CR cells survived (Fig. 1b). A549-CR cells divide much more rapidly than H441-CR cells (Supplementary Fig. 1), discounting the possibility that higher numbers of survivors at later time points are simply due to rapid growth of surviving H441-CR cells.

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\(^{\text{1}}\)Department of Molecular Genetics and Microbiology, Duke University School of Medicine, Durham, NC, USA. \(^{2}\)Department of Microbiology, University of Pennsylvania, Philadelphia, PA, USA. \(^{3}\)Department of Molecular and Cell Biology, University of California, Berkeley, Berkeley, CA, USA. 
\(^*\)e-mail: cherrys@pennmedicine.upenn.edu; nicholas.heaton@duke.edu
To better understand the nature of infection in both A549 and H441 cells, we monitored infection and haemagglutinin (HA) surface trafficking via microscopy, as well as the activity of the viral RNA-dependent RNA polymerase (RdRP); however, no substantial differences were observed (Fig. 1c,d). We also conducted a multicell growth curve and found that infectious progeny viruses are released from H441 cells, albeit at lower levels than A549 cells (Fig. 1e). These data suggested that H441 cells have the ability to clear actively replicating virus rather than being incompletely permissive. To monitor the kinetics of viral infection and clearance in H441 cells, we conducted an infection time course and visualized viral protein production (red) and induction of the ZsGreen reporter (green) over time on a per cell basis. Despite early viral protein expression, we observed rapid clearance of replicating virus from infected cells, which subsequently survive (Fig. 1f,g). H441 cells, however, were killed after infection with an alphavirus (Sindbis virus), indicating that these cells are not just generally resistant to virus-dependent cytotoxicity (Fig. 1h).

siRNA screening identifies a DNA mismatch repair protein as required for H441 cell survival after IAV infection. To identify the genes that allow H441 cells to clear and survive IAV infection, we conducted an RNAi screen using a library targeting the druggable genome (23,349 siRNAs targeting 7,783 genes). We transfected H441 cells containing our ZsGreen reporter with siRNAs arrayed in 384-well plates, infected with IAV-Cre virus for 120 h and used automated imaging and analysis to determine genes that, when targeted by siRNA, altered H441 survival (Fig. 2a). We treated cells with type I interferon (IFN) as a negative control (which blocks infection and prevents ZsGreen signal) and transfected cells with an siRNA targeting the viral nucleoprotein (NP) as a positive control (which increases cell survival by suppressing viral replication). We performed the screen in duplicate, with two unique siRNAs per gene per well in two wells (for a total of four unique siRNAs per gene). A Z-score was calculated based on the ability of the gene to either substantially increase or decrease H441 cell survival (percentage of ZsGreen+ cells) when knocked down, and a ‘hit’ was defined as having a log_{10}[Z-score] of greater than 1.0 or less than −1.0 in both replicates of the screen (Fig. 2b,c and Supplementary Table 1).

Using these criteria, we identified 87 candidate genes. In addition to those 87 genes, we also selected 9 additional genes that had large phenotypes (log_{10}[Z-score] of greater than 1.3) in only one of the two replicate screens, giving a total of 96 candidate genes for further testing. We obtained two additional siRNAs per gene and performed an RNAi validation screen (Fig. 2d,e and Supplementary Table 2). Because genes that directly affect viral infection could also indirectly impact cellular survival, we infected siRNA-transfected H441 cells with a luciferase-expressing influenza virus (IAV-Luc) and eliminated genes that modulated luciferase levels at 16 h postinfection (Fig. 2f,g and Supplementary Fig. 2). Finally, because some siRNAs can induce cellular toxicity irrespective of viral treatment, we focused on genes that did not decrease total cell numbers by more than 50% relative to control. Together, these analyses revealed 15 genes that substantially affected H441 survival in both the primary and validation screens, with only minor effects on virus replication levels and cell viability (Table 1).

Many of the validated genes play roles in cellular stress response pathways. Examples include genes involved in protein degradation (PSMA2, PSMA5), host transcription (POLR2C, POLR2E, CKD12) and protein folding (PPIAL4A, TCP1). One of the more unexpected hits was MSH6, a core member of the DNA MMR pathway. This pathway is responsible for excising and repairing mismatched nucleotides that arise during DNA replication or some types of DNA damage16. As an RNA virus, IAV infection is not generally thought to affect host DNA metabolic processes, and thus it was unclear why this gene would be required for cellular survival from IAV infection; we therefore selected this gene for further study.

DNA MMR is differentially regulated in A549 and H441 cells during IAV infection. First, we determined whether MSH6 was required for H441 cell survival in the context of DNA MMR, or whether it had an alternative ‘moonlighting’ activity. Knockdown of other key proteins involved in the canonical DNA MMR pathway (MSH2, MLH1, PMS2, EXO1) revealed that each member of the pathway was required for H441 cell survival (Fig. 3a–c and Supplementary Fig. 3). We next explored whether differences in the regulation of the DNA MMR pathway could explain the differential survival phenotypes of H441 and A549 cells. We therefore monitored expression of DNA MMR genes in cells mock-infected or infected with wild-type A/Puerto Rico/8/1934 (WT PR8), a laboratory-adapted H1N1 strain of IAV. At 9 h postinfection, PR8-infected A549 cells display a substantial reduction in the gene expression of all core DNA MMR genes compared with uninfected cells, whereas PR8-infected H441 cells maintain relatively high levels of expression of each DNA MMR gene, comparable to uninfected cells (Fig. 3d and Supplementary Table 3). Western blot analysis of two main DNA MMR proteins (MSH2 and MSH6) also displayed a reduction at 24 h postinfection in A549 cells, but not in H441 cells (Fig. 3e).

To determine whether differences in MMR protein levels impacted DNA MMR activity, we adapted a previously described17 DNA MMR assay by generating a NanoLuc plasmid with a single nucleotide mismatch (G-G) in the start codon (Fig. 3f). We compared NanoLuc expression from the ‘mismatch plasmid’ to the ‘WT plasmid’ to measure the level of DNA MMR activity. Cells transfected with non-targeting control siRNAs repaired and expressed the ‘mismatch plasmid’ in an MSH2/6-dependent manner (Fig. 3g). After infection of A549 and H441 cells, we observed that A549 cells lost >25% of their MMR activity while H441 cells maintained levels equal to mock-infected cells (Fig. 3h).

DNA MMR is required to repair reactive oxygen species (ROS)-induced DNA damage during IAV infection. Since we identified...
a DNA repair pathway as critical for surviving viral infection, we next verified that DNA damage was induced during IAV infection. We infected H441 cells with WT PR8 and monitored phospho-H2AX levels, a histone modification associated with DNA repair (Fig. 4a,b). We observed strong induction of phospho-H2AX 8–12 h after PR8 infection, which is on a par with treatment with the control DNA-damaging agents etoposide and hydrogen peroxide (H2O2).
Fig. 2 | An siRNA screen of the druggable genome reveals host factors that control H441 cell survival during IAV infection. 

**a.** Experimental diagram of the siRNA screen. 

**b, c.** Results of the two independent siRNA screens. The log₁₀ transformation of the Z-scores is plotted for each gene targeted by siRNA and their effect on survival (two siRNA per gene per independent screen). Highlighted are those genes that substantially increase H441 survival (green boxes) when targeted and those that substantially decrease H441 survival (red boxes) when targeted (n = 4 independent samples per gene per screen). Average log₁₀[Z-score] for all targeted genes are reported in Supplementary Table 1.

**d.** Validation of the hits (96 genes) from the high-throughput screen with two additional siRNAs (n = 4 independent samples per siRNA). Mock-infected, no Cre present, represents the negative control. NP, siRNA targeting the IAV NP gene, represents the positive control. Boxes label targeted genes that reproducibly increased survival (green) or decreased survival (red). P values listed in Supplementary Table 2.

**e.** Representative images of the screen validation controls and MSH6, one of the screen hits. Survivor cells (green) were visualized in H441 monolayers following transfection with the specified siRNA and then infection with IAV-Cre for 120 h. Scale bar, 200 μm. Representative of two independent experiments.

**f.** Experimental protocol used for the viral replication counter-screen.

**g.** Results of the viral replication counter-screen. The average luciferase values of both individual siRNAs are plotted. The grey shaded box indicates two standard deviations above and below the control siRNA. All replication values were normalized to the non-targeting siRNA control (blue bar). Data shown as mean ± s.e.m., n = 6 independent samples (three for each siRNA). A detailed version of this graph is available in Supplementary Fig. 2.
Apart from its major role in repairing spontaneous nucleotide mismatches following DNA replication, the DNA MMR pathway also plays a role in repairing ROS-induced DNA damage\(^1\). IAV infection is known to alter the redox state of infected cells\(^19,20\) and increase intracellular ROS levels and oxidative damage in a variety of cell lines, including A549 and MDCK cells\(^21–26\). These data suggested a possible link between IAV infection and the DNA MMR pathway. Although total ROS levels have been reported to increase during IAV infection, it was unknown whether this leads to ROS-induced DNA damage. We therefore employed an assay to measure 8-hydroxy-2'-deoxyguanosine (8-OHdG), a common biomarker of ROS-induced DNA damage\(^27\). We found increased levels of 8-OHdG following PR8 infection, which suggested that IAV infection increases ROS-induced DNA damage (Fig. 4e,f). Our data suggested that maintaining DNA MMR during infection controls ROS-induced DNA damage. Therefore, artificially decreasing ROS levels could potentially increase the rate of cellular survival. When we infected A549-CR cells (which normally succumb to IAV infection) with IAV-Cre in the presence of the ROS scavenger Trolox\(^16\), cell survival roughly doubled (Fig. 4g). However, the same treatment of H441-CR cells had no effect on the percentage of survivors (Fig. 4h), probably due to their maintenance of DNA MMR activity.

### DNA MMR impacts expression of innate antiviral genes

We hypothesized that the avoidance of lethal oxidative genomic damage was part of a larger programme required for epithelial cells to survive viral infection. ROS-induced DNA damage increases stall of RNA polymerase II during transcription\(^31,32\) and DNA MMR is associated with transcription-coupled repair\(^33–35\). Although DNA MMR and antiviral gene expression have not been previously linked, we tested whether the reduction in DNA MMR activity and the subsequent accumulation of DNA damage in IAV-infected cells also attenuated transcription of host antiviral genes.

We first tested whether ROS-induced DNA damage affects cellular transcription in club cells. Hydrogen peroxide treatment

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**Table 1 | Genes required for H441 cell survival after influenza virus infection**

| NCBI gene ID | Gene symbol | Official full gene name | Gene function | Average log\(_{10}\) [Z-score]\(^a\) Screen 1 | Average log\(_{10}\) [Z-score]\(^a\) Screen 2 | Validation screen siRNA 1 \(P\) value\(^b\) | Validation screen siRNA 2 \(P\) value\(^b\) | Effect on virus replication\(^c\) | Total cell number (% of overall median)\(^d\) |
|--------------|-------------|-------------------------|---------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| 1133         | CHRM5       | Cholinergic receptor muscarinic 5 | GPCR signalling | −1.72                       | −1.20                       | 0.0038                      | 0.0016                      | 1.11-fold change            | 55.6                        |
| 51755        | CDK12       | Cyclin-dependent kinase 12 | Transcription regulation | −1.35                       | −1.10                       | 0.0002                      | <0.0001                     | 0.97-fold change            | 67.3                        |
| 8880         | FUBP1       | Far upstream element (FUSE) binding protein 1 | Nucleic acid binding | −1.01                       | −1.32                       | <0.0001                     | 0.0033                      | 1.19-fold change            | 95.6                        |
| 2821         | GPI         | Glucose-6-phosphate isomerase | Protein metabolism | −1.11                       | −1.38                       | 0.0002                      | <0.0001                     | 1.02-fold change            | 80.4                        |
| 115330       | GPR146      | G protein-coupled receptor 146 | Plasma membrane signalling | −1.30                       | −1.07                       | <0.0001                     | 0.0002                      | 1.00-fold change            | 75.1                        |
| 3783         | KCNN4       | Potassium-calcium-activated channel subfamily N member 4 | Ion channel | −1.21                       | −1.38                       | 0.0076                      | 0.0008                      | 1.02-fold change            | 132.6                       |
| 2956         | MSH6        | MutS homologues 6 | DNA repair | −1.30                       | −1.02                       | 0.0078                      | 0.0345                      | 1.34-fold change            | 86.6                        |
| 5432         | POLR2C      | RNA polymerase II subunit C, 33 kDa | Transcription | −1.07                       | −2.00                       | <0.0001                     | <0.0001                     | 1.20-fold change            | 88.2                        |
| 5434         | POLR2E      | RNA polymerase II subunit E, 25 kDa | Transcription | −1.49                       | −2.33                       | <0.0001                     | <0.0001                     | 1.07-fold change            | 76.1                        |
| 653505       | PPIAL4A     | Peptidylprolyl isomerase A like 4A | Protein folding | −1.39                       | −1.04                       | <0.0001                     | 0.0155                      | 1.09-fold change            | 96.1                        |
| 5521         | PP2R2B      | Protein phosphatase 2 regulatory subunit Bbeta 2 | Cell growth/division | −1.41                       | −1.57                       | <0.0001                     | <0.0001                     | 1.01-fold change            | 64.8                        |
| 5683         | PSMA2       | Proteasome subunit alpha 2 | Protease activity | −2.56                       | −1.11                       | <0.0001                     | <0.0001                     | 0.88-fold change            | 57.5%                       |
| 5686         | PSMAS5      | Proteasome subunit alpha 5 | Protease activity | −5.06                       | −1.80                       | 0.0005                      | <0.0001                     | 0.97-fold change            | 59.0%                       |
| 5870         | RAB6A       | RAB6A, member RAS oncogene family | Intracellular trafficking | −2.44                       | −1.20                       | 0.0034                      | <0.0001                     | 1.24-fold change            | 79.8%                       |
| 6950         | TCFP1       | T-complex 1 | Protein folding | −2.75                       | −1.04                       | <0.0001                     | <0.0001                     | 1.06-fold change            | 94.2%                       |

The 15 genes that substantially decreased H441 cell survival in both the primary siRNA screen and the validation screen, with only minor effects on viral replication levels and cell viability. \(^a\)Average \(\log_{10}\) [Z-scores] for each gene in the primary siRNA screen (n=4 samples per gene per screen replicate). \(^b\) \(P\) values (unpaired two-tailed t-tests) for two new siRNAs targeting each gene in the validation screen (n=4 samples per siRNA). \(^c\)Average fold change in IAV-Luc replication in the viral replication counter-screen when the indicated gene was targeted by two separate siRNAs (n=3 samples per siRNA). \(^d\)Total cell number for each gene when targeted by siRNA compared to the overall median cell number for all genes on the same 384-well plate in the primary siRNA screen (n=4 samples per gene).

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reduced expression of a cellular NanoLuc reporter by ~80% compared with PBS-treated control cells (Fig. 5a). The decrease in NanoLuc expression, however, was not due to cell death, as cell viability in both samples was comparable (Fig. 5b). We also monitored the effects of oxidative damage on expression of endogenous antiviral genes. Expression of the interferon-stimulated gene (ISG)

Fig. 3 | DNA MMR genes are important for survival and their expression is maintained in H441 cells during infection. a, Visualization of survivor cells (green) and nuclei (blue) in H441-CR cell monolayers following siRNA transfection and infection with IAV-Cre for 120 h. Scale bar represents 200 μm. Representative of two independent experiments. b, Quantification of survivor cells (normalized to nuclei) in the samples described in a. Data shown as mean ± s.d., n = 5 independent experiments; **P < 0.001. Representative of two independent experiments. c, Validation of siRNA knockdown of each of the DNA MMR genes from a. Data shown as mean ± s.d., n = 3 independent samples. Representative of two independent experiments. d, Heat map displaying the level of RNA expression for each of the DNA MMR genes at 9 h postinfection with WT PR8 in A549 and H441 cells, each normalized to mock-infected cells (averaged across replicates). Inset bar graphs highlight the difference in MSH2 and MSH6 RNA levels between cell types at 9 h postinfection. Data shown as mean ± s.d., n = 3 independent samples. Representative of two independent experiments. e, Western blot comparing MSH2 and MSH6 protein levels at 24 h postinfection with WT PR8 in A549 and H441 cells. Tubulin was used as the loading control. Representative of two independent experiments. f, Schematic showing the engineered ‘mismatch-reporter’ NanoLuc plasmid used to determine the level of DNA MMR activity. g, Validation of the MMR activity assay using A549 cells with or without depletion of MSH2+MSH6. Luciferase was measured following transfection with the WT, mismatch (G-G) or an unrepairable NanoLuc plasmid. Data shown as mean ± s.d., n = 3 independent samples. Representative of two independent experiments. h, Comparison of DNA MMR activity in A549 and H441 cells at 24 h postinfection with WT PR8. Each bar shown is the ratio of mismatch plasmid luciferase/WT plasmid luciferase, normalized to the mock-infected control for each cell type. Data shown as mean ± s.d., n = 3 independent samples. Representative of two independent experiments. For all panels, P values were calculated using unpaired two-tailed Student’s t-tests.
MXI was substantially reduced following treatment with \( \text{H}_2\text{O}_2 \) at both the RNA (Fig. 5c) and protein level (Fig. 5d).

Previous reports have shown that DNA repair pathways, in general, are frequently downregulated during IAV infection. To our knowledge, the contribution of virally induced oxidative DNA damage to host shutoff, however, is unexplored. Infection of our NanoLuc reporter club cells led to the expected reduction in luciferase levels (Fig. 5e,f). To test whether the DNA MMR-mediated effects on host translation could extend to antiviral gene expression, we made use of an interferon reporter construct (ISRE-GFP). Reporter expression was significantly reduced following knockdown of DNA MMR genes (Fig. 5g). Together, these data are consistent with a model in which DNA MMR activity repairs oxidative DNA damage to allow for transcriptional induction of antiviral genes (Fig. 5h).

To more broadly investigate the impact of DNA MMR on antiviral gene expression, we performed RNA-seq on H441 cells infected with control or DNA MMR siRNA and then infected with WT PR8 for 24 h (Fig. 5i and Supplementary Table 4; NCBI GEO Series GSE130189). We confirmed depletion of the MMR genes, as well as viral infection (Supplementary Fig. 4a–c). Out of the 282 genes significantly reduced following knockdown of DNA MMR genes (Fig. 5g). Together, these data are consistent with a model in which DNA MMR activity repairs oxidative DNA damage to allow for transcriptional induction of antiviral genes (Fig. 5h).
Fig. 5 | Loss of DNA MMR activity reduces the innate antiviral transcriptional response against influenza A virus. a, b, NanoLuc reporter expression (a) and relative cell viability (b) in H441 cells that have been treated with PBS or H\textsubscript{2}O\textsubscript{2}, (for 30 min). Data shown as mean ± s.d., n = 4 independent samples. c, Fold change of MX1 RNA levels in H441 cells following treatment with PBS or IFN-α ± H\textsubscript{2}O\textsubscript{2} treatment (for 30 min). Data shown as mean ± s.d., n = 4 independent samples. d, Western blot for MX1 in H441 cells following the specified treatments. Tubulin was used as a loading control. e, f, NanoLuc reporter expression (e) and relative cell viability (f) in H441 cells following the specified treatments. Data shown as mean ± s.d., n = 4 independent samples. h, Model depicting the role of DNA MMR in preserving antiviral gene expression. i, RNA-seq data showing fold change of mRNA levels in H441 cells, comparing PR8-infected cells transfected with non-targeting siRNA (black) or MSH2 + MSH6 siRNA (blue) to mock-infected cells. Inset is a magnified view of all genes induced >5-fold in PR8-infected cells on the basis of the effect MMR knockdown has on their mRNA levels. k, Heat map displaying the effect of MMR knockdown (genes induced >5-fold by WT PR8) on the basis of the effect MMR knockdown has on their mRNA levels.

n | o
--- | ---
1.5 | 1.5
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1.0 | 1.0
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0.5 | 0.5
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0.0 | 0.0

P values were calculated using unpaired two-tailed Student’s t-tests; representative of two independent experiments, unless otherwise indicated.
induced >5-fold by PR8 infection, the expression of 115 genes was reduced (defined as a log$_2$ fold change $<-0.2$) in cells transfected with MSH2 + MSH6 siRNA (Fig. S1j and Supplementary Table 5). More specifically, out of the 51 ISG and stress response genes induced >5-fold, the expression of 28 of those genes was reduced >10% by MSH2 + MSH6 depletion (Fig. 5k and Supplementary Table 5). We confirmed that induction of two of the ISGs identified in the RNA sequencing (IFI44L and IFIT1) was significantly attenuated in cells depleted of DNA MMR genes (Fig. 5l–o). We also verified that the reduction of IFIT1 mRNA led to reduced IFIT1 protein in infected H441 cells depleted of DNA MMR genes (Fig. 5p).

Enhanced viral suppression of the DNA MMR pathway decreases cellular survival and increases the severity of viral disease. We wanted to generate a recombinant IAV strain that would suppress the DNA MMR pathway in all infected cells. To accomplish this goal, we inserted artificial microRNAs (amiRNAs) into an extension of the 3’ untranslated region (UTR) of segment 6 (Fig. 6a). Processing of amiRNAs leads to the generation of small RNAs that are functionally equivalent to siRNAs. We generated two separate viruses: one with an amiRNA designed to target GFP as a control (GFP-amiRNA virus), and a second targeting mouse Msh6 to suppress DNA MR activity (MSH6-amiRNA virus). We also inserted Cre recombinase into segment 8 of both viruses (Fig. 6a) to facilitate measurement of cellular survival. We verified functional Cre recombinase expression and monitored processing of the cleaved, mature amiRNAs via northern blot (Fig. 6b,c). We also confirmed that the mature amiRNAs reduced MSH6 RNA levels in mouse epithelial cells (Fig. 6d). In chicken cells where the amiRNA against mouse Msh6 is non-functional (Fig. 6e), we performed growth curves and observed that the growth of the GFP-amiRNA and MSH6-amiRNA viruses was identical (Fig. 6f).

To evaluate the effect of targeting MSH6 in primary cells, we generated air–liquid interface (ALI) epithelial cultures (Fig. 6g) from transgenic mice harbouring a LoxP-Stop-LoxP-dTomato cassette and infected them with our amiRNA viruses. An equal number of cells were initially infected with the GFP-amiRNA and MSH6-amiRNA viruses, but, at later time points, we observed a significant reduction in cellular survival when Msh6 was targeted by the virally encoded amiRNA (Fig. 6b.i). Next, we set out to determine the roles of DNA MMR and club cell survival in viral pathogenesis. We infected 8-week-old female C57BL/6j mice intranasally with a range of doses of the parental WT PR8, GFP-amiRNA or MSH6-amiRNA viruses. Mice that were infected with the MSH6-amiRNA virus experienced increased morbidity and mortality relative to GFP-amiRNA-infected mice (Fig. 6j–l), although both modified viruses were attenuated relative to parental PR8. The MSH6-amiRNA virus also replicated to higher titres in the mouse lungs at 4 d postinfection (Supplementary Fig. 5), which is consistent with dampened antiviral gene expression.

Discussion
Until recently, it was assumed that IAV clearance from the host was the result of the elimination of all infected cells. However, it is now clear that some cells can clear the virus and survive infection. In this report, we demonstrate that DNA MMR is critical for club cell survival of IAV infection. We found that IAV infection generally leads to a reduction in DNA MMR, while club cells are uniquely able to maintain normal levels of DNA MMR activity. The higher level of DNA MMR activity allows club cells to repair virus-induced oxidative DNA damage, facilitating the transcriptional expression of antiviral genes that, together, probably contribute to viral clearance and cell survival. This has important consequences in vivo, as loss of DNA MMR led to decreased cell survival and increased severity of viral disease.

Our observation of IAV-induced oxidative DNA damage is consistent with previous reports of DNA damage after IAV infection. In addition to IAV, many other RNA viruses are known to cause significant DNA damage during infection. This includes RNA viruses that exclusively replicate in the cytoplasm, such as hepatitis C virus and alphaviruses, which have been found to induce DNA damage and activate DNA damage response elements in infected cells. Altogether, these data suggest a broader role for DNA MMR and the innate cellular response against many RNA viruses. Although the sites of oxidative DNA damage were not mapped in our study, it is known that DNA damage preferentially accumulates at sites of actively transcribed DNA. It is therefore likely that, during viral infection, DNA damage accumulates at genetic loci involved in the antiviral and stress response pathways that are highly upregulated after viral infection.

In addition to inducing oxidative DNA damage, infection with a number of RNA viruses is linked to the general downregulation of DNA MMR pathways in infected cells. While it is currently unclear whether DNA repair pathway suppression is the result of a host response, or of subversion by viruses, we propose that DNA MMR is a key host–pathogen interface that impacts infection by a range of pathogens. In support of this, we mined publicly available transcriptomic studies and found many DNA viruses and bacterial pathogens whose infection is also associated with downregulation of DNA MMR, including Bombyx mori nucleopolyhedrovirus, Vaccinia virus, Escherichia coli, Yersinia enterocolitica and Helicobacter pylori. Given that this response to infection appears to be more common than previously appreciated, determining how the DNA MMR pathway and other DNA repair pathways are controlled during infection is an important area of future study.

Fig. 6 | DNA MMR is required for cellular survival and protection from virulence in mice. a, Diagram of the engineered IAV PR8 Seg8-Cre+Seg6-amiRNA viruses. b, PR8-Cre+amiRNA viruses activate Cre-reporter (ZsGreen) expression in H441-CR cells (nuclei, blue). Scale bars, 200 µm. Representative of two independent experiments. c, Northern blot of total RNA from MLE15 cells infected with the indicated PR8-Cre+amiRNA virus. Probes are specific for the GFP-amiRNA, MSH6-amiRNA or U6-snRNA loading control. Red arrows indicate precursor-amiRNA; black arrows indicate mature amiRNA. Representative of two independent experiments. d, Relative MSH6 RNA levels in MLE15 cells cotransfected with a pLEX plasmid (for puromycin selection) and the indicated Seg6-amiRNA plasmid. Data shown as mean±s.d., n = 3 samples. Representative of three independent experiments. e, Alignment of the antisense MSH6-amiRNA sequence and the MSH6 gene target sequence (both mouse and chicken). Bases are coloured green (complementary) or red (non-complementary). f, Growth curve in fertilized chicken eggs comparing WT PR8 and PR8-Cre+amiRNA viruses. Data shown as mean±s.e.m., n = 3 samples; *P<0.05. NS, not significant. Representative of two independent experiments. g, Experimental timeline for differentiation and infection of ALI cultures and validation of differentiation by staining for mature ciliated cells (ACTUB, green), basal cells (Krt5+, red) and nuclei (blue). Scale bar, 25 µm. Representative of two independent experiments. h, ALI cultures derived from tdTomato-Cre-reporter transgenic mice were infected with the indicated viruses. Quantification of the number of HA+ (PY102+) cells per field of view at 1d postinfection (n=5), and the number of tdTomato+ cells per field of view at 8d postinfection (n=10). Data shown as mean±s.d. Representative of two independent experiments. i, Nuclei staining (DAPI, blue) and survivor cells (tdTomato, red) in ALI cultures at 8d postinfection. Scale bars, 50 µm. Representative of two independent experiments. j–l, Weight loss (top) and survival (bottom) curves for mice infected with 4,000 (j), 1,200 (k) or 400 (l) PFU of the indicated virus. Data shown as mean±s.d., n = 4 mice per group. Representative of two independent experiments. For all panels, P values were calculated using unpaired two-tailed Student’s t-tests.
One question raised by this study is why the DNA MMR pathway is essential for survival when other oxidative DNA damage repair pathways exist in the cell? Nucleotide excision repair, base excision repair and MMR are all used by the cell to repair damage restricted to a single strand of the DNA, but show different specificity based on the type of damage that they recognize and repair\textsuperscript{56–58}.

\begin{figure}
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\end{figure}
While we did not identify these genes in our screen, future studies will dissect the role of these pathways in cellular survival. Another question is how does IAV inhibit DNA MMR activity in most cells? Possible mechanisms probably relate to host shutoff, which is largely implemented by the viral polymerase, NS1 and PA-X proteins. We hypothesize that club cells are able to partially evade the IAV shutoff mechanisms, and the pathways used to accomplish this will be explored in the future. Finally, it will also be important to determine the relative DNA MMR activity and magnitude of oxidative DNA damage after infection with a number of different influenza virus subtypes to define any strain-specific effects on this process.

In this study, we found that IAV-induced oxidative DNA damage impacts both cellular survival and antiviral gene expression. Therefore, we propose that in addition to promoting the maintenance of cellular genomic integrity, an important aspect of DNA MMR is to also promote strong induction of antiviral genes, perhaps including those genes responsible for non-lytic viral clearance from infected cells. Determining the pathways required for non-lytic clearance, and their mechanisms of action, will be important not only to understand viral infection but also as a novel strategy to boost viral clearance from cells that cannot be replaced. Altogether, our study has identified a previously unappreciated interface between the virus and host. DNA MMR, and DNA repair pathways in general, are commonly attenuated during infection with diverse pathogens, and this may be the result of common regulatory mechanisms. Preventing DNA MMR pathway suppression in host cells, or inducing repair pathways, may represent a general antimicrobial strategy for the future.

Methods

Cells. All cells were obtained from ATCC and grown at 37 °C in 5% CO₂. H441 cells were grown in RPMI medium supplemented with 10% foetal bovine serum, HEPES, glucose, sodium pyruvate and penicillin–streptomycin. The 293T and A549 cells (ATCC) were grown in DMEM supplemented with 5% foetal bovine serum, GlutaMAX and penicillin–streptomycin. Madin–Darby canine kidney (MDCK) cells were grown in MEM supplemented with 5% foetal bovine serum, HEPES, NaHCO₃, GlutaMAX and penicillin–streptomycin. MLE15 cells were grown in DMEM supplemented with 10% foetal bovine serum, GlutaMAX and penicillin–streptomycin. The Cre-reporter cassette was constructed and transduced into H441 and A549 cells as previously described. The growth curve comparing division rates of A549-CR and H441-CR cells was conducted in 6-well plates. Cells were seeded at 150,000 cells per well and were collected and counted at 24, 48, 72 and 96 h.

Viruses. Recombinant viruses were generated using reverse genetics methods, as previously described, using the bicistronic pdZ rescue plasmid system. All viruses were based on the A/Puerto Rico/8/1934 (PR8) H1N1 influenza virus background. The WT PR8 virus contains all WT PR8 segments. The NEBuilder HiFi DNA assembly kit (NEB) was used to assemble DNA fragments to create the desired recombiant DNA gene segments. The IAV-Cre recombinant virus was engineered to express Cre recombinase within the PR8 PB2 segment (segment 1), as previously described. The time course measuring ZsGreen expression during IAV-Cre infection in A549-CR and H441-CR cells was conducted by infecting cells at multiplicity of infection (MOI) = 1. Every 24 h, cells were fixed with 4% paraformaldehyde (PFA) in PBS and nuclei were stained with Hoechst dye before images were taken with the ZOE fluorescent cell imager (Bio-Rad). The IAV-Luc recombinant virus was also engineered to expressing luciferase in the PR8 PB2 segment (segment 1). For the PR8-Cre-amiRNA viruses, the Cre recombinase was inserted into the PR8 NS segment (segment 8) following separation of the NS1 and NEP coding regions, and the amiRNA expression sequence was inserted into the PR8 NA segment (segment 6). After assembly and transformation of the constructs into high-efficiency competent cells (NEB), the plasmids were collected and used in separate rescue transfections in 293T/MDCK cocultures. Rescued virus was expanded in day 10 fertilized chicken eggs and then plaque-purified on MDCK cells. Individual plaques were picked and expanded in day 10 fertilized chicken eggs before being validated via PCR with reverse transcription (RT–PCR) and sequencing of the segment 2A. For the viruses containing Cre recombinase, Cre activity was confirmed by infecting H441-CR or MLE15 cells (segment 5) and observing the expression of the Cre-controlled ZsGreen reporter in cell monolayers at 24–48 h postinfection.

siRNA screen. Single siRNAs (two sites per target, 25 nM final concentration, Qiagen) were spotted in collagen-coated 384-well plates complexed with HiPerFect (0.6 μl per well in 9.4 μl OptiMEM). Followingcomplexing, H441-CR cells (1,625 cells per well) were added to each well and infected 48 h later with IAV-Cre at an MOI = 5. After 120 h, cells were fixed with 4% PFA, washed and stained with Hoechst stain. All solutions were added using automated liquid handling (WellMate, ThermoFisher) to limit well-to-well variability. Images were captured (four sites per well) at ×10 using an ImagoXpress Microscope (Molecular Devices). Automated image analysis (MetaXpress) was used to calculate the total number of cells (Hoechst⁺) and the number of survivor cells (ZsGreen⁺). These values were used to calculate the mean and calculate the median (using log transformed data), which were then used to calculate robust Z-scores. The median of each plate in the screen was used to calculate baseline. Hits were identified as those wells that exhibited a change in survivor cell percentage of total cells by the indicated standard deviations from calculated Z-scores within each plate.

siRNA survivor cell assays. H441-CR cells were transfected with 0.2 μM siRNA using HiPerFect reagent (Qiagen) in OptiMEM. After 48 h, cells were infected with IAV-Cre at MOI = 1 for 1 h before virus was removed and replaced with complete media. Cells were incubated at 37 °C for 5 d before being fixed with 4% PFA and stained with Hoescht dye for nucleus labelling. Images were then collected using the ZOE fluorescent cell imager (Bio-Rad) to identify the level of ZsGreen⁺ cells indicating survival of direct infection. Image analysis was done using ImageJ (NIH).

Viral replication counter-screen. H441-CR cells were transfected with the siRNA and then infected after 48 h with IAV-Luc at MOI = 0.5. At 16 h postinfection, cells were lysed and luciferase activity was measured using a luciferase assay system (Promega) as a readout of viral replication. All siRNAs were compared relative to the non-targeting control siRNA and those that altered viral replication greater than two standard deviations were eliminated from the final list of screen hits.

Viral growth comparisons. The RdRP replication assay was conducted as previously described. For the multiplicity growth assay comparing A549 and H441 cells, WT PR8 was added to cells at MOI = 1 for 1 h before being maintained in OptiMEM with 0.3 μg/ml T7-PCK-treated trypsin protease at 37 °C. For the experiment comparing IAV and Sindbis survival, H441-CR cells were infected with MOI = 1 of WT PR8 influenza virus or Sindbis virus (gift from B. tenOever). After 48 h, the total number of remaining cells was quantified compared to mock-infected controls using flow cytometry and counting beads. The amiRNA virus growth curves, eggs were injected with 1,000 plaque-forming units (PFU) virus and incubated at 37 °C for 24–32 h. MLE15 cells (2,000 TCID₅₀ per well) were infected at MOI = 0.1 for 1 h before being maintained in OptiMEM with 0.4 μg/ml T7-PCK-treated trypsin protease for 24–72 h at 37 °C. All samples were then titrated using plaque assays.

Plaque assays. Viral titres were determined using standard plaque assay procedures on MDCK cells. Serial virus dilutions were incubated on cells for 1 h before removing the virus and adding the agar overlay. Cells were then incubated at 37 °C for 48 h before fixing in 4% PFA for at least 12 h. The 4% PFA was then aspirated, and the agar layer was removed before washing with PBS. Serum from WT PR8-infected mice was diluted 1:2,000 in antibody dilution buffer (5% (w/v) non-fat dried milk and 0.05% Tween-20 in PBS) and incubated on wells at 4 °C for 2 h. Cells were then washed three times with PBS and then incubated for 2 h in anti-mouse IgG horseradish peroxidase (HRP)-conjugated sheep (GE Healthcare) diluted 1:4,000 in antibody dilution buffer. Assays were then washed three times with PBS and exposed to 0.5 ml of True Blue peroxidase substrate (KPL) for 15 min. Plates were then washed with water and dried before plaques were counted.

Cell infections. For experiments measuring RNA and protein levels of MMR genes and ISGs, H441-CR cells were infected with WT PR8 at MOI = 5 for 1 h before virus was removed and complete media was added. Cells were incubated at 37 °C for 3–24 h before total RNA was collected, or for 24–48 h before total protein was collected for experiments using siRNA-treated cells. For experiments using siRNA transfection to ensure maximum knockdown of target genes, qRT–PCR assays. For cell culture-based qRT–PCR experiments, total RNA was collected using Monarch Total RNA Miniprep Kits (NEB). For the mouse lung IAV NP qRT–PCR, lungs were collected at 4 d postinfection (with 1,200 PFU virus) and homogenized directly in 1×TLZ, before using chloroform and isopropanol alcohol to complete the RNA extraction. For the confirmation of siRNA knockdown, measurement of ISG RNA levels, the comparison of relative MSH6 RNA levels in amiRNA-transfected cells and the quantification of mouse lung IAV NP levels, samples were analysed using the EXPRESS One-Step Superscript Universal qRT–PCR Kit (ThermoFisher) and gene-specific TaqMan Expression Assay Probes (ThermoFisher) (Supplementary Table 6). For the MMR gene panel, the qRT–PCR was performed in two separate steps. First, single-stranded complementary DNA was synthesized using the High-Capacity cDNA Reverse Transcription Kit with RNaseInhibitor (ThermoFisher). Second, cDNA was loaded into Custom TaqMan Array 96-well Plates (ThermoFisher) that we designed to include all major genes.
directly involved in the DNA MMR pathway, as well as two housekeeping gene controls for normalization.

**Western blotting.** Equal amounts of protein were loaded into 4–20% acrylamide gels (Bio-Rad) and transferred to nitrocellulose membrane. PBS with 5% (w/v) non-fat dried milk and 0.1% Tween-20 were used to block for 2 h at 4°C. Primary antibodies were then incubated with the membrane overnight at 4°C. Antibodies used were rabbit anti-MSH2 (Cell-Signaling Technology, D24B5), rabbit anti-MSH6 (Abcam, EPR3945), mouse anti-HA (PY102, gift from T. Moran at Mt. Sinai), rabbit anti-MX1 (Abcam, 95926) and mouse anti-α-Tubulin (Sigma, T5168). Membranes were washed five times in PBS with 0.1% Tween-20 and then mouse-HPR or anti-rabbit-HPR secondary antibody was added for 1 h. The membrane was then washed five times and Clarity or Clarity Max ECL substrate (Bio-Rad) was added before being exposed to film and developed. Uncropped scans of all western blots are displayed in Supplementary Fig. 6.

**DNA MMR activity assay.** The assay was designed as previously described17, with some adjustments. In short, we digested our synthesized plCMV-Nano Luc plasmid with NheI and PflFII to remove the NanoLuc start codon region. We then ligated the resulting linear DNA with our own primer set of annealed oligonucleotides with a WT start codon to generate the NanoLuc start codon; this was referred to as our ‘Mismatch plasmid’. A set of annealed oligonucleotides with a WT start codon was also ligated in, to be used as a normalization control. The annealed oligonucleotides also contained a disrupted HpaI restriction site, allowing us to specifically eliminate any uncut parental plasmid. These grafted sequences were ligated to the 5′-ends of pGL2-MV-FLuc (Promega, which has an intact HpaI site, located upstream of the start codon) following plasmid ligation. Validation of the MMR activity assay was done using A549 cells transfected with MSH2 and MSH6 siRNA. For the comparison of A549 and H441 cells, the cells were infected with MOI = 10, transfected with 0.5 μg of the WT or Mismatch plasmid at 24 h postinfection and then analysed at 16 h posttransfection. To measure NanoLuc expression, cells were lysed with Lysis Buffer and then evaluated using the Nano-Glo Luciferase Assay Reagent Kit (Promega).

**DNA damage assays.** For phospho-H2AX staining, H441 cells were plated on collagen-coated cover slides and infected with WT PR8 (MOI = 5) for indicated lengths of time before fixation with 4% PFA. Positive controls were treated with 1 μM etoposide (Abcam) or 5 μM H2O2 (EMD Millipore) for 30 min before fixation. Cells were stained using the Histone H2A.X.S139ph antibody (Active Motif, catalogue no. 39117). 4,6-Diamidino-2-phenylindole (DAPI) was added for nuclear staining. Cells were then fixed and counterstained with Fluoroshield with DAPI (Millipore). For 8-OHdG staining, H441 cells were stained with the INSEL-1 antibody (Trevigen) and then stained with 4′,6-diamidino-2-phenylindole (DAPI) to visualize the nucleus. Images were captured using an Axioskop microscope using a ×40 oil objective. Images within the same panel were collected at the same time using the same settings and analysed using ImageJ (NIH).

**RNA sequencing.** For RNA-seq expression, H441 cells transfected with siRNA were incubated for 48 h before being infected with WT PR8 (MOI = 10). Total RNA was collected at 24 h posttransfection using Monarch Total RNA Miniprep Kits (NEB). RNA was then purified using the NEBNext Poly(A) mRNA Magnetic Isolation Module (NEB), NEBNext Ultra II RNA Library Prep Kit for Illumina (NEB) and NEBNext Multiplex Oligos for Illumina (NEB). Mapping of raw reads to the human hg19 reference genome was accomplished using a custom application on the Illumina BaseSpace Sequence Hub. After data normalization, average read values were compared across samples. For comparisons in which some samples had zero reads detected for a specific gene, one read was added to all values in the sample to complete analyses that required non-zero values. Raw data are available at NCBI GEO (GSE130189).

**Expressing ISG reporter experiments.** For the comparison of A549 and H441 cells, the cells were infected with MOI = 10, transfected with 0.5 μg of the WT or Mismatch plasmid at 24 h postinfection and then analysed at 16 h posttransfection. To measure NanoLuc expression, cells were lysed with Lysis Buffer and then evaluated using the Nano-Glo Luciferase Assay Reagent Kit (Promega).

**Expression of NanoLuc and ISRE-GFP reporters.** All experiments measuring ISG expression were conducted in H441 cells. For experiments including ROS treatment, cells were transfected with siRNA and after 48 h, 5 mM H2O2 was added to the media for 30 min. Media was then aspirated and replaced with media containing 500 μM 1′IFN-α for activation of ISG expression. Cells were then incubated at 37°C for 3 h before total RNA was collected, and for 24 h before total protein samples were collected. For IF14L and IFIT1 experiments, cells were transfected with siRNA and incubated for 48 h before infecting with WT PR8 at MOI = 10 and collecting total RNA at 48 h postinfection.

**Northern blot.** Total RNA samples were collected from either MLE15 cells infected in a 10-cm dish with the PR8-Cre-amiRNA viruses for 24 h, or from MLE15 cells transfected in a 10-cm dish with 15 μg of the Seg6-amiRNA plasmids for 24 h. Equal amounts of RNA were loaded into a 15% TBE-urea gel (Bio-Rad) and transferred to a nitrocellulose membrane. The dried membrane was UV-cross-linked and then exposed to prehybridization solution for 1 h. Probes specific for the GFP-amiRNA, MSH6-amiRNA and U6 control short nuclear RNA (snRNA) were labelled with [32P]ATP and then added to the membrane for 1 h. Membrane was washed twice with SSC buffer plus 0.05% SDS and then exposed to film for 96 h before developing. Uncropped scans are displayed in Supplementary Fig. 6.

**amiRNA activity confirmation.** MLE15 cells were cotransfected in 24-well plates with 300 ng of the Seg6-amiRNA plasmids and 500 ng of a PLEX helper plasmid with puromycin resistance marker using Lipofectamine 2000 reagent (ThermoFisher). After 24 h, cotransfected cells were selected with 2.5 μM puromycin. After 48 h, between the 36th and 38th posttransfection, total RNA was collected using TRIzol reagent. MSH6 RNA levels were then quantified in each of the samples using one-step qRT-PCR.

**All culture infections.** Tracheal epithelial cells were isolated from transgenic Cre-tdTomato-reporter mice and cultured in ALI for 14 d to allow formation of a pseudo-stratified epithelium. This was monitored using markers for mature differentiated cell types (KRT5, basal stem cells; ACTUB, mature ciliated cells). These cultures were then infected with 500,000 PFU of WT PR8, GFP-amiRNA or MSH6-amiRNA virus. At 1 and 8 d postinfection, cultures were collected, rinsed twice with PBS and fixed with 2% PFA at room temperature for 15 min. Cultures were stained with anti-GFP antibody (1:100 in PBS, 5 min) and then exposed to prehybridization solution for 1 h. Probes specific for the GFP-amiRNA, MSH6-amiRNA and U6 control short nuclear RNA (snRNA) were labelled with [32P]ATP and then added to the membrane for 1 h. Membrane was washed twice with SSC buffer plus 0.05% SDS and then exposed to film for 96 h before developing. Uncropped scans are displayed in Supplementary Fig. 6.

**Mouse infections.** Eight-week-old female C57BL/6j mice were used for all experiments, with a sample size of four mice per dose of virus. Prior to infection, mice were anaesthetized with a 100-μl injection of a ketamine–xylazine mixture. After being tail-marked and weighed, mice were intranasally infected with 40 μl of virus diluted in pharmaceutical grade PBS. Mice were weighed daily and euthanized once their body weight reached <80% of the starting weight as a humane endpoint. Euthanasia was performed via CO2 as the primary method and a bilateral thoracotomy was performed as the secondary method. All procedures were approved by the Duke University IACUC.
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**Author contributions**

B.S.C., S.C. and N.S.H. designed the study and experiments. B.S.C. generated many of the reagents and performed and analysed the majority of the biochemical and mouse experiments. B.E.H. performed some of the interferon gene expression experiments. K.R. and S.C. performed and analysed the siRNA screen data. R.E.D. performed and analysed the phospho-H2AX and ALI culture experiments. J.R.H. and N.S.H. generated the Cre-reporter assays and optimized screening conditions. N.S.H. performed and analysed the 8-OHdG experiment, most of the screen validation experiments and most of the experiments characterizing H441 cells as models for cell survival. B.S.C., S.C. and N.S.H. wrote the manuscript.

**Competing interests**

Duke University has filed a provisional patent for targeting DNA MMR as a method to enhance the growth of influenza vaccine strains.

**Additional information**

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Correspondence and requests for materials should be addressed to S.C. or N.S.H.

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Reporting Summary

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Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

| n/a | Confirmed |
|-----|-----------|
| ☐   | ☒         |
|     | The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement |
| ☒   | A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly |
| ☐   | The statistical test(s) used AND whether they are one- or two-sided |
| ☒   | Only common tests should be described solely by name; describe more complex techniques in the Methods section. |
| ☐   | A description of all covariates tested |
| ☒   | A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons |
| ☒   | A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals) |
| ☒   | For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted |
| ☒   | Give P values as exact values whenever suitable. |
| ☒   | For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings |
| ☒   | For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes |
| ☒   | Estimates of effect sizes (e.g. Cohen’s d, Pearson’s r), indicating how they were calculated |

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection

| StepOne Software v2.3 was used for qPCR data collection. Leica LAS AF 2.6 software was used for multiple confocal microscopy experiments as specified in methods. Bio-Rad ZOE software was used for image acquisition. BD FACSDiva v8.0.1 was used for flow cytometry. Cellomics Scan Software v5.6.2.1 was used for image acquisition and analysis. |

Data analysis

| Analysis for multiple experiments was done using: Prism 7.0a, Prism 8.0, NIH ImageJ 1.50i, NIH Fiji, FlowJo 10.5 |

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

The raw RNAseq data files from Fig. 5i-k are available at NCBI GEO (Series GSE130189). The raw data for Fig. 2b-d, 3d, and 5i-k are available in Supplementary Tables 1-5. Raw data for all other figures are available from the authors upon request.
Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

- Life sciences
- Behavioural & social sciences
- Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size
Sample size for all experiments was set at a minimum of three to ensure statistical tests could be completed. The sample size for mouse experiments was determined based on previous work in the lab that obtained statistical significance.

Data exclusions
No data was excluded from analysis.

Replication
All experiments were repeated at least two times to ensure reproducibility and significance. All results were able to be replicated.

Randomization
For all experiments, compared samples were all based on the same cell line, etc., so no randomization was needed. For mouse experiments, all groups consisted of same age, similar starting weight females. Image analysis was done on multiple images across replicate samples to ensure no bias.

Blinding
No blinding approaches were used for the analysis in this study.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

| n/a | Involved in the study |
| --- | --- |
| | Antibodies |
| | Eukaryotic cell lines |
| | Palaeontology |
| | Animals and other organisms |
| | Human research participants |
| | Clinical data |

Methods

| n/a | Involved in the study |
| --- | --- |
| | ChIP-seq |
| | Flow cytometry |
| | MRI-based neuroimaging |

Antibodies

WT PR8 infected mice primary sera (collected in lab)
anti-Mouse IgG HRP conjugated Sheep Ab (GE Healthcare, catalog #NA931V)
anti-MSH2 XP Rabbit mAb (Cell Signaling Technology, clone D24B5, catalog #2017S)
anti-MSH6 Rabbit Ab (Abcam, clone D24B5, catalog #2017S)
Mouse anti-HA antibody (clone PY102, gift of Thomas Moran from Mt. Sinai)
Rabbit anti-Mx1 polyclonal antibody (Abcam, catalog #95926)
Mouse anti-alpha-tubulin mAb (Sigma-Aldrich, clone B-5-1-2, catalog #T1568)
Goat anti-Mouse IgG (H+L) Cross-absorbed secondary Ab, HRP (ThermoFisher, catalog #A16072)
Goat anti-Rabbit IgG (H+L) Cross-absorbed secondary Ab, HRP (ThermoFisher, catalog #A16104)
Histone H2A.XS139ph polyclonal Ab (Active Motif, catalog #39118)

Validation
We did not do any particular validation, manufacturer's recommendations were followed.

Eukaryotic cell lines

Policy information about cell lines

| Cell line source(s) | All cell lines used (A549, H441, MDCK, MLE15) were obtained from ATCC. |
| Authentication | No authentication of the cell lines was performed |
| Mycoplasma contamination | H441, A549, and MDCK cell lines were ordered Myco-free from ATCC or treated for mycoplasma using Invivogen's |

n/a Not applicable
Mycoplasma contamination Plasmocure, and then maintained in Invivogen Plasmocin prophylactic to ensure no Mycoplasma contamination. MLE15 cell line was mycoplasma positive.

Commonly misidentified lines (See ICLAC register) n/a

Animals and other organisms
Policy information about studies involving animals. ARRIVE guidelines recommended for reporting animal research

Laboratory animals Eight-week old C57BL/6J female mice were used.
Wild animals Study did not involve wild animals.
Field-collected samples Study did not involve field-collected samples.
Ethics oversight All animal procedures were approved by the Duke University IACUC.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Flow Cytometry

Plots
Confirm that:
☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
☒ All plots are contour plots with outliers or pseudocolor plots.
☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology
Sample preparation Cell lines (ATCC) were collected and fixed in 4% PFA, and then resuspended in PBS before analysis.
Instrument BD Biosciences FACSCanto II and BD Biosciences Fortessa X-20 were used for data collection.
Software BD Biosciences FACSDiva v8.0.1 software used for data collection.
Cell population abundance The entire population of cells is shown.
Gating strategy Cell debris was excluded but the only gate included all cells.

☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.