Induction of NLR genes in coronavirus infections.

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Nearly 20,000,000 people worldwide have now been infected with the novel Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)^{1, 2}. We mined published microarray data to identify genes of the inflammasome system whose expression was modulated by coronavirus infection *in vivo* and *in vitro*³⁻⁸. We observed unique and shared induction or repression of NLR genes by coronaviruses including SARS-CoV-1, Middle East Respiratory Syndrome (MERS)-CoV, and human coronavirus 229E (HCoV-229E). MERS-CoV infections resulted in differential expression of NLRX1 while SARS-CoV-1 infections resulted in differential expression of NLRC5 in the lung. Both of these NLR proteins function as negative regulators of downstream inflammasome signaling⁹⁻¹³. These data reveal unique modulation of innate immune pattern recognition receptor gene expression during coronavirus infections.

Keywords: NLR, Nod-like receptors, inflammasome, coronavirus, MERS-CoV, SARS-CoV-1, HCoV-229E, systems biology of viral infection.

1 The coronavirus family includes seven viruses that infect humans, including SARS-
2 CoV-1, human coronavirus 229E (HCoV-229E), the Middle East Respiratory Syndrome (MERS)
3 coronavirus (MERS-CoV) , as well as novel and pandemic public health problem SARS-CoV-2¹,
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5 ¹⁴⁻¹⁶. The innate immune system utilizes a network of pathogen recognition receptors, including
6 NLR (nucleotide-binding oligomerization domain (NOD) leucine-rich repeat-containing)
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8 proteins that respond to pathogen-associated molecular patterns to initiate rapid immune
9 signaling in response to stereotyped foreign insults^{17,18}. Inflammasomes are multi-protein
10 complexes that assemble in response to pattern recognition and most classically include an NLR-
11 containing protein (e.g., NLRP3) that recruits and utilizes caspase function (e.g., caspase-1) to
12 cleave and mature the pro-inflammatory cytokine interleukin-1beta and/or induce the
13
14 transactivation of innate immune response genes¹⁷⁻¹⁹. Our understanding of how NLR gene
15 expression is modulated during the course of coronavirus infections is limited²⁰⁻²⁴, and it is
16
17 unknown whether coronaviruses, as a family, induce or repress the expression of the same or
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19 different NLR proteins, whether as part of the host cell immune response or as a consequence of
20 viral manipulation of host cell gene expression. We observed unique differential expression of
21 NLRC5 by MERS-CoV *in vitro* and of NLRX1 by SARS-CoV-1 *in vivo*, while infection with
22 HCoV 229E and MERS-CoV London *in vitro* resulted in shared differential expression of
23 NLRP1 and NLRP3. Each of these insights was gained by study of published microarray
24 datasets. These data are an entry point to our understanding of the fundamental host-pathogen
25
26 interactions that underly the transcriptional immune response to coronavirus infection in humans.
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Methods

We used datasets GSE100509³, GSE56677⁴, GSE59185⁵, GSE68820⁶, GSE22581⁷, GSE89167⁸ for this analysis of gene expression changes following coronavirus infection. GSE100509 was generated using Agilent-026652 Whole Human Genome Microarray 4x44K v2 technology with $n=5$ each for primary human microvascular endothelial cells infected with MERS-CoV at 0 hours, 12 hours, 24 hours, 36 hours and 48 hours. GSE56677 was generated using Agilent-039494 SurePrint G3 Human GE v2 8x60K Microarray 039381 technology with $n=3$ each for CALU3 2B4 cells infected with MERS-CoV London (Eng1): at 0 hours, 3 hours, 7 hours, 12 hours, 18 hours and 24 hours post-infection. GSE59185 was generated using Agilent-028005 SurePrint G3 Mouse GE 8x60K Microarray technology with $n=3$ lung tissue from mock-infected BALB/c mice and $n=3$ lung tissue from SARS-CoV-1-infected BALB/c mice, both at 2 days post-infection. GSE68820 was generated using Agilent-014868 Whole Mouse Genome Microarray 4x44K G4122F technology with $n=5$ for lung tissue from mock-infected C57BL6/NJ mice and $n=5$ for lung tissue from SARS-CoV-1-infected C57BL6/NJ mice, both at 2 days post-infection. GSE22581 was generated using Affymetrix Canine Genome 2.0 Array technology with $n=3$ each for ferret lung on day 0, day 1 and day 2 post-infection through the intranasal route with SARS-CoV-1. GSE89167 was generated using 039494 SurePrint G3 Human GE v2 8x60K Microarray 039381 technology with $n=2$ HuH-7 cells and $n=2$ HCoV-229E infected HuH-7 cells. The Benjamini and Hochberg method of p -value adjustment was used for ranking of differential expression but raw p -values were used for assessment of statistical significance of global differential expression. Log-transformation of data was auto-

1 detected, and the NCBI generated category of platform annotation was used.

2 A statistical test was performed to evaluate the significance of difference in NLRC3
3 mRNA expression levels in CALU3 2B4 cells with MERS-CoV infection at 3 hours, 7 hours, 12
4 hours, 18 hours and 24 hours post-infection as compared to CALU3 2B4 cells at baseline (0
5 hours) using a one-way ANOVA with Dunnett's multiple comparisons test. Only *p*-values less
6 than 0.05 were considered statistically significant. We used PRISM for all statistical analyses
7 (Version 8.4.0)(455).
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10 11 **Results**

12 To determine whether significant transcriptional modulation of NLR genes could be
13 observed in any coronavirus infection model, we mined published microarray data from *in vitro*
14 and *in vivo* coronavirus infection models³⁻⁸.
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17 We observed significant induction of the NOD-like receptor NLRC3 following MERS-
18 CoV infection of primary human microvascular endothelial cells. At 12 hours post-infection
19 with MERS-CoV, the expression of NLRC3 increased and remained at that level until 36 hours
20 post-infection, when NLRC3 expression began to decrease; still, at 48 hours post-infection,
21 NLRC3 expression remained significantly higher than at baseline (Figure 1). At 12 hours, 24
22 hours, 36 hours and 48 hours post-infection with MERS-CoV, NLRC3 expression was
23 significantly higher than at baseline (Figure 1; $p < 0.0001$ for all comparisons to baseline); we
24 calculated mean fold increases in NLRC3 mRNA levels of 1.5125 ± 0.0123 at 12 hours, $1.5128 \pm$
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0.0071 at 24 hours, 1.2822 ± 0.0166 at 36 hours, and 1.2099 ± 0.0132 at 48 hours as compared to baseline (Table 1). NLRC3 was the 174th most differentially expressed gene following infection of primary human microvascular endothelial cells with MERS-CoV wild-type out of 34127 total transcripts, equaling differential expression more significant than 99.5% of the total transcriptome. Differential expression of NLRC3 following MERS-CoV infection was statistically significant (Table 1; $p=3.5E-25$).

NAIP and NLRX1 were also significantly differentially expressed following infection of primary human microvascular endothelial cells with MERS-CoV.

NAIP was the 1512th most differentially expressed gene following infection of primary human microvascular endothelial cells with MERS-CoV out of 34127 total transcripts, equaling differential expression more significant than 95.6% of the transcriptome. Differential expression of NAIP was statistically significant (Table 2; $p=3.61E-20$).

NLRX1 was the 3483th most differentially expressed gene following infection of human microvascular endothelial cells with MERS-CoV wild-type out of 34127 total transcripts, equaling differential expression more significant than 89.8% of the transcriptome. Differential expression of NLRX1 was statistically significant (Table 2; $p=1.63E-17$).

Next, we studied an *in vitro* model of human coronavirus 229E (HCoV-229E) infection in HuH-7 cells. We observed significant differential expression of NLRP3, NAIP and NLRP1.

NLRP3 was the 1372nd most differentially expressed gene following infection of human HuH-7 cells with HCoV-229E out of 62976 total transcripts, equaling differential expression more significant than 97.8% of the transcriptome. Differential expression of NLRP3 following

1 HCoV-229E infection was statistically significant (Table 3; $p=0.0172689$).

2 NAIP was the 3012th most differentially expressed gene following infection of human
3
4 HuH-7 cells with HCoV-229E out of 62976 total transcripts, equaling differential expression
5 more significant than 95.2% of the transcriptome. Differential expression of NAIP following
6 HCoV-229E infection was statistically significant (Table 3; $p=0.0360424$).

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8 NLRP1 was the 4449th most differentially expressed gene following infection of human
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10 HuH-7 cells with HCoV-229E out of 62976 total transcripts, equaling differential expression
11 more significant than 92.9% of the transcriptome. Differential expression of NLRP1 following
12 HCoV-229E infection was statistically significant (Table 3; $p=0.0523633$).

13 We studied a separate *in vitro* model of MERS-CoV infection, MERS-CoV London
14
15 (Eng1) infection of the CALU3 2B4 human cell line. We observed significant differential
16 expression of NLRP3, NLRP1 and NLRX1 following infection of CALU3 2B4 cells with
17 MERS-CoV London.

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19 NLRP3 was the 3116th most differentially expressed gene following infection of human
20
21 CALU3 2B4 cells with MERS-CoV London out of 28653 total transcripts, equaling differential
22 expression more significant than 89.1% of the transcriptome. Differential expression of NLRP3
23 following MERS-CoV London infection was statistically significant (Table 4; $p=1.07E-08$).

24 NLRP1 was the 4055th most differentially expressed gene following infection of human
25
26 CALU3 2B4 cells with MERS-CoV London out of 28653 total transcripts, equaling differential
27 expression more significant than 85.8% of the transcriptome. Differential expression of NLRP1
28 following MERS-CoV London was statistically significant (Table 4; $p=4.16E-08$).

1 NLRX1 was the 5217th most differentially expressed gene following infection of human
2 HuH CALU3 2B4 cells with MERS-CoV London out of 28653 total transcripts, equaling
3 differential expression more significant than 81.8% of the transcriptome. Differential expression
4 of NLRX1 following MERS-CoV London was statistically significant (Table 4; $p=1.7E-07$).
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6 We also studied three separate models of SARS-CoV-1 coronavirus infection *in vivo*; in
7 the lungs of ferrets, and in the lungs of C57BL6/NJ and BALB/c mice.
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9 NLRP10, NLRC5, and NLRP2 were all significant differentially expressed in the ferret
10 lung following intranasal infection with SARS-CoV-1.
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12 In the lungs of ferrets infected with SARS-CoV-1, NLRP10 was the 750th most
13 differentially expressed gene out of 43035 total transcripts, equaling differential expression more
14 significant than 98.3% of the transcriptome. Differential expression of NLRP10 following
15 SARS-CoV-1 infection was statistically significant (Table 5; $p=5.39E-06$).
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17 NLRC5 was the 2314th most differentially expressed gene in the ferret lung following
18 intranasal infection with SARS-CoV-1 out of 43035 total transcripts, equaling differential
19 expression more significant than 94.6% of the transcriptome. Differential expression of NLRC5
20 following SARS-CoV-1 infection was statistically significant (Table 5; $p=1.27E-04$).
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23 NLRP2 was the 5203rd most differentially expressed gene in the ferret lung following
24 intranasal infection with SARS-CoV-1 out of 43035 total transcripts, equaling differential
25 expression more significant than 87.9% of the transcriptome. Differential expression of NLRP2
26 following SARS-CoV-1 infection was statistically significant (Table 5; $p=9.89E-04$).
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NLRC5, NAIP2, and NLRC3 were all significant differentially expressed in the mouse

lung following *in vivo* infection of C57BL6/NJ mice with SARS-CoV-1.

NLRC5 was the 1009th most differentially expressed gene in the mouse lung following infection with SARS-CoV-1 out of 29649 total transcripts, equaling differential expression more significant than 96.6% of the transcriptome. Differential expression of NLRC5 following SARS-CoV-1 infection was statistically significant (Table 6; $p=4.52E-09$).

NAIP2 was the 1426th most differentially expressed gene in the mouse lung following infection with SARS-CoV-1 out of 29649 total transcripts, equaling differential expression more significant than 95.2% of the transcriptome. Differential expression of NAIP2 following SARS-CoV-1 infection was statistically significant (Table 6; $p=1.55E-08$).

NLRC3 was the 2145th most differentially expressed gene in the mouse lung following infection with SARS-CoV-1 out of 29649 total transcripts, equaling differential expression more significant than 92.8% of the transcriptome. Differential expression of NLRC3 following SARS-CoV-1 infection was statistically significant (Table 6; $p=7.16E-08$).

NLRC5, NLRP1b, NAIP2 and NLRP1a were all significant differentially expressed in the mouse lung following *in vivo* infection of BALB/c mice with SARS-CoV-1.

NLRC5 was the 278th most differentially expressed gene in the mouse lung following infection with SARS-CoV-1 out of 62976 total transcripts, equaling differential expression more significant than 99.6% of the transcriptome. Differential expression of NLRC5 following SARS-CoV-1 infection was statistically significant (Table 7; $p=2.7E-07$).

NLRP1b was the 2728th most differentially expressed gene in the mouse lung following infection with SARS-CoV-1 out of 62976 total transcripts, equaling differential expression more

1 significant than 95.7% of the transcriptome. Differential expression of NLRP1b following
2 SARS-CoV-1 infection was statistically significant (Table 7; $p=2.25E-04$).
3

4 NAIP2 was the 2946th most differentially expressed gene in the mouse lung following
5 infection with SARS-CoV-1 out of 62976 total transcripts, equaling differential expression more
6 significant than 95.3% of the transcriptome. Differential expression of NAIP2 following SARS-
7 CoV-1 infection was statistically significant (Table 7; $p=2.93E-04$).
8

9 NLRP1a was the 3458th most differentially expressed gene in the mouse lung following
10 infection with SARS-CoV-1 out of 62976 total transcripts, equaling differential expression more
11 significant than 94.5% of the transcriptome. Differential expression of NLRP1a following
12 SARS-CoV-1 infection was statistically significant (Table 7; $p=4.87E-04$).
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15 Thus, we found that NLRC5 was differentially expressed in the lungs of mice and ferrets
16 after SARS-CoV-1 infection, and that NLRX1 was differentially expressed following MERS-
17 CoV infection of human cells. Differential expression of NAIP was common to humans cells
18 following infection with both MERS-CoV wild-type and HCoV-229E, while differential
19 expression of NLRP1 and NLRP3 was common to human cells following infection with both
20 MERS-CoV London (Eng1) and HCoV-229E.
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23 **Discussion**

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25 We used published microarray data³⁻⁸ to discover differentially expressed NLR genes
26 following infection with a series of coronaviruses that have the ability to infect humans: SARS-
27 CoV-1, MERS-CoV wild-type and MERS-CoV London (Eng1), and human coronavirus 229E.
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1 While transcriptional modulation of NOD-like receptors *in vitro* and *in vivo* following human
2 coronavirus infections was generally divergent based on type of coronavirus and species
3 infected, there appeared to be patterns of transcriptional change. Two main conserved
4 transcriptional changes following coronavirus infection were the transcriptional modulation of
5 NLRX1 by MERS-CoV and the transcriptional modulation of NLRC5 by SARS-CoV-1.
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8 NLRX1 and NLRC5 both have the ability to restrict rather than propagate signaling
9 downstream of inflammasome activation. NLRX1 can control NF- κ B signaling induced by
10 lipopolysaccharide (LPS) by interactions with TRAF6; the interaction between TRAF6 and
11 NLRX1 can be detected within 15 minutes of LPS stimulation⁹. In *Nlrp1*^{-/-} mouse embryonic
12 fibroblasts, I κ B α phosphorylation is significantly diminished and bone marrow macrophages
13 from *Nlrp1*^{-/-} mice are largely dysregulated in their ability to produce cytokines in response to
14 LPS, secreting significantly higher quantities of IL-1 β , IL-6 and IFN- β as compared to wild-type
15 mouse macrophages¹⁰. NLRX1 can also control interferon signaling in response to viral
16 infection. MEFs from *Nlrp1*^{-/-} mice produce significantly higher quantities of IFN- β in response
17 to infection with Sendai virus, influenza A virus and vesicular stomatitis virus¹⁰. Sensing of
18 RNA viruses by NLRX1 appears to be dependent on RIG-I and MAVS but not MDA-5¹⁰.
19
20 Interaction between NLRX1 at the mitochondrial outer membrane and MAVS are another
21 important mechanism by which NLRX1 can control anti-viral innate immune responses: NLRX1
22 can block enhancement of interaction between MAVS and RIG-I induced by Sendai virus
23 infection¹¹. Another mechanism by which NLRX1 can negatively regulate innate immune
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1 signaling is through sequestration of STING, disrupting the interaction between STING and the
2 TANK-binding kinase TBK1; NLRX1 control of STING has been demonstrated in response to
3 DNA virus infection as well as HIV-1 infection¹⁰.

5 The other major conserved transcriptional change in NLR gene expression we observed
6 was NLRC5 modulation by SARS-CoV-1. NLRC5 can also regulate NF- κ B signaling, by
7 interacting with IKK α and IKK β to impair IKK α and IKK β phosphorylation and inhibit
8 NF- κ B activation¹². Interestingly, NLRC5 interacts with RIG-I and MDA-5 to inhibit anti-viral
9 interferon signaling, but not with MAVS¹². NLRC5 also functions at the transcriptional level as
10 an activator of major histocompatibility class I (MHC-I) gene expression¹³. Connecting NLRC5
11 transcriptional functions at MHC-I with anti-viral function for NLRC5 are data demonstrating
12 that T-cell specific NLRC5-deficiency results in significant loss of T-cells under conditions of
13 LCMV infection as loss of MHC-I results in targeting of self by natural killer cells²⁶. Changes in
14 NLRC5 expression have been observed in cytomegalovirus infection and respiratory syncytial
15 virus infection^{27,28}.

20 We also observed changes in Naip2 expression following SARS-CoV-1 infection *in vivo*.
21 Transcriptional modulation of Naip2 appeared to be specific to SARS-CoV-1 infection in mice,
22 as we did not observe significant differential expression of Naip2 in the lungs of ferrets
23 following SARS-CoV-1 infection. Naip2 is described as a component of the sensor for the PrgJ
24 inner rod protein of bacterial type III secretion systems, facilitating activation of the NLRC4
25 inflammasome¹⁴. It is unclear why Naip2 expression would change in the context of a viral
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1 infection and would be of interest to determine whether Naip2 is transcriptionally perturbed in
2 the human lung following SARS-CoV-2 infection.

3
4 We surveyed multiple microarray datasets and identified conserved and unique changes
5 in NLR gene expression following coronavirus infection in human cells and in the lungs of mice
6 and ferrets. Deletion of NLR genes in primary human cells and tissue-specific deletion of NLR
7 genes in mice can be used to assess the importance of NLRC5, NLRX1 and other NLR genes in
8 coronavirus infection.
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Figure 1: Transcriptional induction of NLRC3 following MERS-CoV infection *in vitro*.

The expression of NLRC3 in primary human microvascular endothelial cells at 12 hours, 24 hours, 36 hours and 48 hours post-infection with MERS-CoV is represented with mean mRNA values marked and the result of a statistical test evaluating significance of difference in NLRC3 mRNA levels as compared to baseline (0 hours), a *p*-value, marked above.

Rank	ID	p-value	F	FC	Gene	Gene name
174	A_23_P340019	3.5E-25	881.2393	12hr: 1.5125 ± 0.0123 24hr: 1.5128 ± 0.0071 36hr: 1.2822 ± 0.0166 48hr: 1.2099 ± 0.0132	NLRC3	NLR family, CARD domain containing 3

Table 1: NLRC3 is among the genes most differentially expressed following MERS-CoV infection of primary human microvascular epithelial cells.

The rank of differential expression, the probe ID, p-value for differential expression of this transcript, F statistic, the fold change in NLRC3 expression at 12 hours, 24 hours, 36 hours and 48 hours post-infection, gene and gene name are listed in this chart.

Rank	ID	p-value	F	Gene	Gene name
1512	A_23_P110473	3.61E-20	325.2675	NAIP	NLR family, apoptosis inhibitory protein
3483	A_24_P244356	1.63E-17	190.5255	NLRX1	NLR family member X1

Table 2: NAIP and NLRX1 are differentially expressed following MERS-CoV infection of primary human microvascular epithelial cells.

The rank of differential expression for each NLR gene, probe ID, p-value, F statistic, gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	Gene	Gene name
1372	30685	0.0172689	-3.1910744	-2.963	NLRP3	NLR family, pyrin domain containing 3
3012	59562	0.0360424	2.6461483	-3.427	NAIP	NLR family, apoptosis inhibitory protein
4449	21961	0.0523633	2.3786103	-3.672	NLRP1	NLR family, pyrin domain containing 1

Table 3: NLRP3, NAIP and NLRP1 are differentially expressed following HCoV-229E infection of HuH-7 cells.

The rank of differential expression for each NLR gene, probe ID, p-value, F statistic, gene and gene name are listed in this chart.

Rank	ID	p-value	F	Gene	Gene name
3116	A_23_P9883	1.07E-08	78.14128	NLRP3	NLR family, pyrin domain containing 3
4055	A_24_P83615	4.16E-08	62.14098	NLRP1	NLR family, pyrin domain containing 1
5217	A_24_P244356	1.7E-07	48.82447	NLRX1	NLR family member X1

Table 4: NLRP3, NLRP1 and NLRX1 are differentially expressed following MERS-CoV London infection of CALU3 2B4 cells.

The rank of differential expression for each NLR gene, probe ID, p-value, F statistic, gene and gene name are listed in this chart.

Rank	ID	p-value	F	Gene	Gene name
750	CfaAffx.11222.1.S1_at	5.39E-06	9.26E+01	NLRP10	NLR family, pyrin domain containing 10
2314	CfaAffx.14140.1.S1_s_at	1.27E-04	3.75E+01	NLRC5	NLR family, CARD domain containing 5
5203	CfaAffx.4813.1.S1_s_at	9.89E-04	2.01E+01	NLRP2	NLR family, pyrin domain containing 2

Table 5: NLRP10, NLRC5 and NLRP2 are differentially expressed in the ferret lung following intranasal SARS-CoV-1 infection.

The rank of differential expression for each NLR gene, probe ID, p-value, F statistic, gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	Gene	Gene name
1009	A_52_P382107	4.52E-09	17.61952	11.241449	Nlrc5	NLR family, CARD domain containing 5
1426	A_51_P265026	1.55E-08	15.58123	9.923219	Naip2	NLR family, apoptosis inhibitory protein 2
2145	A_51_P193743	7.16E-08	13.35636	8.281812	Nlrc3	NLR family, CARD domain containing 3

Table 6: NLRC5, NAIP2 and NLRC3 are differentially expressed in the mouse lung following SARS-CoV-1 infection in C57BL6/NJ mice.

The rank of differential expression for each NLR gene, probe ID, p-value, F statistic, gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	Gene	Gene name
278	31806	2.7E-07	1.74E+01	7.75447	Nlrc5	NLR family, CARD domain containing 5
2728	12958	2.25E-04	6.65	0.61063	Nlrp1b	NLR family, pyrin domain containing 1B
2946	37883	2.93E-04	6.38	0.32395	Naip2	NLR family, apoptosis inhibitory protein 2
3458	23770	4.87E-04	5.89	-0.23215	Nlrp1a	NLR family, pyrin domain containing 1A

Table 7: NLRC5, NAIP2 and NLRC3 are differentially expressed in the mouse lung following SARS-CoV-1 infection in BALB/c mice.

The rank of differential expression for each NLR gene, probe ID, p-value, F statistic, gene and gene name are listed in this chart.

Virus	Cell / Tissue	Gene	% Differential Expression
MERS-CoV wild-type	primary human microvascular epithelial cells	NLRC3	99.5
<i>in vitro</i>		NAIP	95.6
		NLRX1	89.8
MERS-CoV London	CALU3 2B4 human cell line	NLRP3	89.1
<i>in vitro</i>		NLRP1	85.8
		NLRX1	81.8
HCoV-229E	HuH-7 human cell line	NLRP3	97.8
<i>in vitro</i>		NAIP	95.2
		NLRP1	92.9
SARS-CoV-1	ferret lung	NLRP10	98.3
<i>in vivo</i>		NLRC5	94.6
		NLRP2	87.9
SARS-CoV-1	mouse lung (C57BL6/NJ)	NLRC5	96.6
<i>in vivo</i>		NAIP2	95.2
		NLRC3	92.8
SARS-CoV-1	mouse lung (BALB/c)	NLRC5	99.6
<i>in vivo</i>		NLRP1b	95.7
		NAIP2	95.3
		NLRP1a	94.5

Table 8: Transcriptional modulation of NLR genes in coronavirus infection.

A summary of each differentially expressed NLR gene following coronavirus infection is listed in this chart, with host cell or tissue for each coronavirus, name of NLR gene differentially expressed in that system, and the percent differential expression for that gene, calculated by ranking every transcript measured by microarray based on statistical significance of change in mRNA expression between uninfected or mock-infected and infected condition, and dividing that ranking by total number of transcripts whose expression was measured in that dataset.