Transcriptional induction of nucleic acid sensors during coronavirus infection.

Shahan Mamoor¹

¹shahanmamoor@gmail.com

East Islip, NY, USA

While adaptive immune responses involve antigen-specific responses, rapid innate immune responses involve detection of pathogen-associated molecular patterns such as the nucleic acids DNA and RNA¹. We mined five independent microarray datasets²⁻⁶ to discover in an unbiased and systematic fashion pattern recognition receptors associated with coronavirus infection across a series of coronaviruses capable of infecting humans. We describe here the transcriptional induction of four nucleic acid and PAMP sensors following coronavirus infection *in vitro* and *in vivo*: Z-DNA-binding protein ZBP1, the DExH-box helicase DHX58 (LGP2), PYHIN1 and the Mediterranean Fever gene PYRIN as among the genes most differentially expressed following infection with coronaviruses. These data reveal unprecedented mobilization of nucleic acid sensors during coronavirus infection.

Keywords: innate immunity, pattern recognition receptors, nucleic acid sensors, MEFV, PYRIN, PYHIN1, DHX58, ZBP1, MERS-CoV, HCoV-229E, SARS-CoV-1, SARS-CoV-2.

Human immunity can be viewed as either adaptive or innate depending on the nature of the response. While adaptive immune responses, like production of immunoglobulins (antibodies) by B-cells occurs on the time-scale of days to weeks, innate immune responses can occur on the time-scale of minutes to hours. Innate immune responses are essential to defense against pathogens including viruses such as coronaviruses like the novel human coronavirus SARS-CoV-2 which has now infected close to 20,000,000 people worldwide⁷.

We mined five independent published microarray datasets to determine in a blinded and systematic fashion the gene products whose expression changed most significantly following infection with a series of coronaviruses capable of infecting humans. Three nucleic acid sensors and a PAMP sensor were among the genes most differentially expressed following infection *in vivo* with the Severe Acute Respiratory Syndrome SARS-CoV-1 and *in vitro* with the Middle East Respiratory Syndrome MERS-CoV.

Methods

We used datasets GSE56677², GSE59185³, GSE68820⁴, GSE22581⁵ and GSE89167⁶ for this analysis of gene expression changes following coronavirus infection. 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, 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 $n=3$ mock-infected lung and $n=3$ SARS-CoV-1-infected lung at 2 days post-infection in BALB/c mice. GSE68820 was generated

1 using Agilent-014868 Whole Mouse Genome Microarray 4x44K G4122F technology with $n=5$
2 for lung from mock-infected C57BL6/NJ mice at 2 days post-infection and $n=5$ for lung from
3 SARS-CoV-1-infected mice at 2 days post-infection. GSE22581 was generated using Affymetrix
4 Canine Genome 2.0 Array technology with $n=3$ each for ferret lung on day 0, day 1 and day 2
5 post-infection with SARS-CoV-1. GSE89167 was generated using 039494 SurePrint G3 Human
6 GE v2 8x60K Microarray 039381 technology with $n=2$ HuH-7 cells and $n=2$ HuH-7 cells
7 infected with HCoV229E.
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11 The Benjamin and Hochberg method of p-value adjustment was used for ranking of
12 differential expression but raw p-values were used for assessment of statistical significance of
13 global differential expression. Log-transformation of data was auto-detected, and the NCBI
14 generated category of platform annotation was used.
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16 A statistical test was performed to evaluate the significance of difference in DHX58 and
17 ZBP1 mRNA expression levels in CALU3 2B4 cells with MERS-CoV infection as compared to
18 CALU3 2B4 cells at baseline (0 hours) using a one-way ANOVA with Dunnett's multiple
19 comparisons test. A statistical test was performed to evaluate the significance of difference
20 between mRNA expression levels of DHX58, ZBP1, PYHIN1 and PYRIN in the lungs of mock-
21 infected mice and in the lungs of mice 2 days post-infection with SARS-CoV-1 using a two-
22 tailed, unpaired t-test. We used PRISM for all statistical analyses (Version 8.4.0)(455).
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Results

We performed transcriptome-level screening of published microarray data²⁻⁶ to identify innate immune pathogen recognition receptors induced during coronavirus infection.

ZBP1 is differentially expressed following MERS-CoV infection of human cells.

We found that the Z-DNA binding protein ZBP1 was among the genes most differentially expressed following infection of CALU3 2B4 cells with MERS-CoV London (Eng1)². When sorting each of the transcripts measured by microarray based on significance of change in expression between infected and uninfected cells, ZBP1 ranked 228 out of 28653 total transcripts. Differential expression of ZBP1 in MERS-CoV-infected CALU3 cells was statistically significant (Table 1; $p=1.4E-11$).

ZBP1 is differentially expressed in the lungs of BALB/c mice following infection with SARS-CoV-1.

Analysis of a separate microarray dataset³ revealed that ZBP1 was among the genes most differentially expressed in the lungs of BALB/c mice infected with SARS-CoV-1. When sorting each of the transcripts measured by microarray based on significance of change in expression between the lungs of infected and mock-infected mice, ZBP1 ranked 19 out of 62976 total transcripts. Differential expression of ZBP1 in the lungs of BALB/c mice infected with SARS-CoV-1 was statistically significant (Table 2; $p=1.34E-09$).

DHX58 is differentially expressed in the lungs of BALB/c mice following infection with SARS-CoV-1.

A second nucleic acid sensor, DHX58 was also among the genes most differentially expressed in the lungs of BALB/c mice infected with SARS-CoV-1³. When sorting each of the transcripts measured by microarray based on significance of change in expression between the lungs of infected and mock-infected mice, DHX58 ranked 228 out of 62976 total transcripts. Differential expression of DHX58 was statistically significant in the lungs of BALB/c mice infected with SARS-CoV-1 (Table 3; $p=1.43E-07$).

DHX58 is differentially expressed in the lungs of C57BL6/NJ mice following infection with SARS-CoV-1.

Analysis of a third dataset⁴ revealed that DHX58 was also among the genes most differentially expressed in the lungs of C57BL6/NJ mice infected with SARS-CoV-1. When sorting each of the transcripts measured by microarray based on significance of change in expression between the lungs of infected and mock-infected mice, DHX58 ranked 21 out of 29649 total transcripts. Differential expression of DHX58 was statistically significant in the lungs of C57BL6/NJ mice infected with SARS-CoV-1 (Table 4; $p=3.28E-14$).

DHX58 is differentially expressed following MERS-CoV infection of human cells.

We found that DHX58 transcriptional induction was not unique to SARS-CoV-1 infections: DHX58 was also among the genes most differentially expressed following infection

of CALU3 2B4 cells with MERS-CoV London (Eng1)². When sorting each of the transcripts measured by microarray based on significance of change in expression between infected and uninfected cells, DHX58 ranked 28 out of 28653 total transcripts. Differential expression of DHX58 in MERS-CoV-infected CALU3 cells was statistically significant (Table 5; $p=3.39E-14$).

PYHIN1 is differentially expressed in the lungs of BALB/c mice following infection with SARS-CoV-1.

We identified a third nucleic acid sensor, PYHIN1, among the genes most differentially expressed in the lungs of BALB/c mice infected with SARS-CoV-1³. When sorting each of the genes measured by microarray based on significance of change in gene expression between the lungs of infected and mock-infected mice, PYHIN1 ranked 210 out of 62976 total transcripts. PYHIN1 differential expression in SARS-CoV-1-infected BALB/c mice was statistically significant (Table 6; $p=1.97E+01$).

PYHIN1 is differentially expressed in the lungs of C57BL6/NJ mice following infection with SARS-CoV-1.

PYHIN1 was also among the genes most differentially expressed in the lungs of C57BL6/NJ mice infected with SARS-CoV-1⁴. When sorting each of the transcripts measured by microarray based on significance of change in expression between the lungs of infected and mock-infected mice, PYHIN1 ranked 132 out of 29649 total transcripts. Differential expression of PYHIN1 was statistically significant in the lungs of C57BL6/NJ mice infected with SARS-CoV-1 (Table 7; $p=5.8E-12$).

1 The Mediterranean Fever gene PYRIN is differentially expressed in the lungs of BALB/c mice
2 infected with SARS-CoV-1.

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4 Finally, we identified a fourth nucleic acid sensor as among the genes most differentially
5 expressed in a model of coronavirus infection, in the lungs of BALB/c mice infected with SARS-
6 CoV-1: the Mediterranean Fever gene Mefv, also known as PYRIN³. When sorting each of the
7 transcripts measured by microarray based on significance of change in expression between the
8 lungs of infected and mock-infected mice, PYRIN ranked 234 out of 62976 total transcripts.
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10 Differential expression of PYRIN in the lungs of C57BL6/NJ mice infected with SARS-CoV-1
11 statistically significant (Table 8; $p=1.52E-07$).
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15 DHX58 is differentially expressed in the blood and lungs of ferrets infected with SARS-CoV-1.

16 We analyzed microarray data from a ferret intranasal SARS-CoV-1 infection model⁵;
17 among the four sensors we found differentially expressed in vitro and in the mouse lung, only
18 DHX58 was significantly differentially expressed in ferrets infected with SARS-CoV-1; both in
19 the blood (Table 9; $p=2.02E-02$) and in the lungs (Table 10; $p=2.96E-04$). When sorting each of
20 the transcripts measured by microarray based on significance of change in expression between
21 the blood of infected and mock-infected ferrets, DHX58 ranked 1341 out of 43035 total
22 transcripts (Table 9). When sorting each of the transcripts measured by microarray based on
23 significance of change in expression between the lungs of infected and mock-infected ferrets,
24 DHX58 ranked 3204 out of 43035 total transcripts (Table 10).
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None of these genes was differentially expressed in Huh-7 cells infected with the human coronavirus HCoV-229E (data not shown)⁶.

Transcriptional induction of ZBP1 in MERS-CoV and SARS-CoV-1 infections.

We observed rapid transcriptional induction of ZBP1 *in vitro* following infection of CALU3 cells with MERS-CoV London (Eng1) (Figure 1). We calculated a mean fold change in ZBP1 gene expression of 0.9736 ± 0.0168 at 3 hours, 0.9996 ± 0.0016 at 7 hours, 1.0103 ± 0.0260 at 12 hours, 1.2439 ± 0.0111 at 18 hours and 1.2345 ± 0.0162 at 24 hours.

Transcriptional induction of ZBP1 at 18 hours and at 24 hours was statistically significant (Figure 1; $p < 0.0001$ at 18 hours and $p < 0.0001$ at 24 hours).

In BALB/c mice infected with SARS-CoV-1, ZBP1 mRNA levels in the lung were significantly increased at day 2 post-infection when compared to mock-infected mice (Figure 2; $p = 0.0003$). We calculated a mean fold change of 1.716 ± 0.010 in ZBP1 expression when comparing the SARS-CoV-1 infected lung to mock-infected lung (Table 2).

Transcriptional induction of DHX58 in MERS-CoV and SARS-CoV-1 infections.

DHX58 was also rapidly transcribed *in vitro* following infection of CALU3 cells with MERS-CoV London (Eng1) (Figure 3). We calculated a mean fold change in DHX58 gene expression of 0.9766 ± 0.0110 at 3 hours, 0.9488 ± 0.0203 at 7 hours, 1.0144 ± 0.0111 at 12 hours, 1.3341 ± 0.0171 at 18 hours and 1.2425 ± 0.0124 at 24 hours. Transcriptional induction of DHX58 at 18 hours and at 24 hours was statistically significant (Table 3; $p < 0.0001$ at 18 hours and $p < 0.0001$ at 24 hours).

1 In BALB/c mice infected with SARS-CoV-1, DHX58 mRNA levels in the lung were
2 significantly increased at day 2 post-infection when compared to mock-infected mice (Figure 4;
3 $p=0.0001$). This was consistent with transcriptional induction of DHX58 in C57BL6/NJ mice
4 infected with SARS-CoV-1, with DHX58 mRNA levels in the lung significantly increased at day
5 2 post-infection when compared to mock-infected C57BL6/NJ mice (Figure 5; $p<0.0001$). We
6 calculated a mean fold change of 1.539 ± 0.042 and 1.4613 ± 0.0148 in DHX58 expression when
7 comparing the SARS-CoV-1 infected lung to mock-infected lung in BALB/c mice and C57BL6/
8 NJ mice (Table 4 and Table 5, respectively).

13 Induction of PYHIN1 mRNA in the mouse lung following SARS-CoV-1 infection.

15 In BALB/c mice infected with SARS-CoV-1, PYHIN1 mRNA levels in the lung were
16 significantly increased at day 2 post-infection when compared to mock-infected mice (Figure 6;
17 $p<0.0001$). This was consistent with transcriptional induction of PYHIN1 in C57BL6/NJ mice
18 infected with SARS-CoV-1, with PYHIN1 mRNA levels in the lung significantly increased at
19 day 2 post-infection when compared to mock-infected C57BL6/NJ mice (Figure 7; $p<0.0001$).
20 We calculated a mean fold change of 1.354 ± 0.019 and 1.4626 ± 0.0204 in PYHIN1 expression
21 when comparing the SARS-CoV-1-infected lung to mock-infected lung in BALB/c mice and
22 C57BL6/NJ mice (Table 6 and Table 7, respectively).

27 Induction of PYRIN mRNA in the mouse lung following SARS-CoV-1 infection.

28 In BALB/c mice infected with SARS-CoV-1, PYRIN mRNA levels in the lung were also

1 significantly increased at day 2 post-infection when compared to mock-infected mice (Figure 8;
2 $p<0.0001$). We calculated a mean fold change of 1.311 ± 0.014 in PYRIN expression when
3
4 comparing the SARS-CoV-1-infected lung to mock-infected lung in BALB/c mice (Table 8).

5 Thus, we discovered that pattern recognition receptors ZBP1, DHX58, PYRIN and
6
7 PYHIN1 were all among the genes most differentially expressed in the lungs of mice infected
8 with SARS-CoV-1; ZBP1 and DHX58 differential expression could also be found *in vitro*
9 following infection of human cells with MERS-CoV.
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12 Discussion

13 Virtually nothing is known about the transcriptional induction of nucleic acid sensors
14 following infection across the coronavirus family. By mining five independent microarray
15 datasets and screening transcriptome data from *in vitro* and *in vivo* infection models²⁻⁶, we
16 identified transcriptional induction of ZBP1, DHX58/LGP2, PYRIN and PYHIN1 by the
17 coronavirus family.
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20 ZBP1 functions as a sensor of DNA in the cytosol, including under conditions of viral
21 infection⁸⁻¹⁰ while LGP2, a RIG-I-like receptor is thought to function as a sensor of double-
22 stranded RNA, functioning in concert with MDA5¹¹⁻¹³. PYRIN is a sensor of changes in post-
23 translational glucosylation of the Rho GTPase executed by Rho-inactivating toxins from bacteria
24 such as *Clostridium botulinum* resulting in activation of caspase-1 inflammasome signaling¹⁴.
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27 PYHIN1/IFIX is poorly studied but thought to be a sensor of viral DNA¹⁵. We currently do not
28 understand how any of these molecules function in immune defense during coronavirus

infections but these data imply conserved nucleic acid sensing of the coronavirus family by

DHX58/LGP2; data demonstrating induction of PYRIN during coronavirus infection suggests cytoskeletal elements may perhaps be modulated during the course of coronavirus infection.

We describe here for the first time differential expression and transcriptional induction of four separate nucleic acid and PAMP sensors during infection by the coronavirus family. Future work is required to determine the relevance of each of these nucleic acid sensors in coronavirus outcomes and whether any of these genes are important for SARS-CoV-2 infection dynamics.

References

1. Iwasaki, A. and Medzhitov, R., 2015. Control of adaptive immunity by the innate immune system. *Nature immunology*, 16(4), pp.343-353., 26(4), pp.450-452.
2. Selinger, C., Tisoncik-Go, J., Menachery, V.D., Agnihothram, S., Law, G.L., Chang, J., Kelly, S.M., Sova, P., Baric, R.S. and Katze, M.G., 2014. Cytokine systems approach demonstrates differences in innate and pro-inflammatory host responses between genetically distinct MERS-CoV isolates. *BMC genomics*, 15(1), p.1161.
3. Regla-Nava, J.A., Nieto-Torres, J.L., Jimenez-Guardeño, J.M., Fernandez-Delgado, R., Fett, C., Castaño-Rodríguez, C., Perlman, S., Enjuanes, L. and DeDiego, M.L., 2015. Severe acute respiratory syndrome coronaviruses with mutations in the E protein are attenuated and promising vaccine candidates. *Journal of virology*, 89(7), pp.3870-3887
4. Totura, A.L., Whitmore, A., Agnihothram, S., Schäfer, A., Katze, M.G., Heise, M.T. and Baric, R.S., 2015. Toll-like receptor 3 signaling via TRIF contributes to a protective innate immune response to severe acute respiratory syndrome coronavirus infection. *MBio*, 6(3), pp.e00638-15.
5. Danesh, A., Cameron, C.M., León, A.J., Ran, L., Xu, L., Fang, Y., Kelvin, A.A., Rowe, T., Chen, H., Guan, Y. and Jonsson, C.B., 2011. Early gene expression events in ferrets in response to SARS coronavirus infection versus direct interferon-alpha2b stimulation. *Virology*, 409(1), pp.102-112.
6. Poppe, M., Wittig, S., Jurida, L., Bartkuhn, M., Wilhelm, J., Müller, H., Beuerlein, K., Karl, N., Bhujju, S., Ziebuhr, J. and Schmitz, M.L., 2017. The NF-κB-dependent and-independent transcriptome and chromatin landscapes of human coronavirus 229E-infected cells. *PLoS pathogens*, 13(3), p.e1006286.
7. World Health Organization. Coronavirus disease (COVID-19) pandemic. <https://www.who.int/emergencies/diseases/novel-coronavirus-2019>.
8. Takaoka, A., Wang, Z., Choi, M.K., Yanai, H., Negishi, H., Ban, T., Lu, Y., Miyagishi, M., Kodama, T., Honda, K. and Ohba, Y., 2007. DAI (DLM-1/ZBP1) is a cytosolic DNA sensor and an activator of innate immune response. *Nature*, 448(7152), pp.501-505.
9. Kuriakose, T., Man, S.M., Malireddi, R.S., Karki, R., Kesavardhana, S., Place, D.E., Neale, G., Vogel, P. and Kanneganti, T.D., 2016. ZBP1/DAI is an innate sensor of influenza virus triggering the NLRP3 inflammasome and programmed cell death pathways. *Science immunology*, 1(2).

- 1 10. Upton, J.W., Kaiser, W.J. and Mocarski, E.S., 2012. DAI/ZBP1/DLM-1 complexes with
2 RIP3 to mediate virus-induced programmed necrosis that is targeted by murine
3 cytomegalovirus vIRA. *Cell host & microbe*, 11(3), pp.290-297.
- 4 11. Li, X., Ranjith-Kumar, C.T., Brooks, M.T., Dharmiah, S., Herr, A.B., Kao, C. and Li, P.,
5 2009. The RIG-I-like receptor LGP2 recognizes the termini of double-stranded
6 RNA. *Journal of Biological Chemistry*, 284(20), pp.13881-13891.
- 7 12. Satoh, T., Kato, H., Kumagai, Y., Yoneyama, M., Sato, S., Matsushita, K., Tsujimura, T.,
8 Fujita, T., Akira, S. and Takeuchi, O., 2010. LGP2 is a positive regulator of RIG-I-and
9 MDA5-mediated antiviral responses. *Proceedings of the National Academy of
10 Sciences*, 107(4), pp.1512-1517.
- 11 13. Bruns, A.M., Leser, G.P., Lamb, R.A. and Horvath, C.M., 2014. The innate immune sensor
12 LGP2 activates antiviral signaling by regulating MDA5-RNA interaction and filament
13 assembly. *Molecular cell*, 55(5), pp.771-781.
- 14 14. Xu, H., Yang, J., Gao, W., Li, L., Li, P., Zhang, L., Gong, Y.N., Peng, X., Xi, J.J., Chen, S.
15 and Wang, F., 2014. Innate immune sensing of bacterial modifications of Rho GTPases by
16 the Pyrin inflammasome. *Nature*, 513(7517), pp.237-241.
- 17 15. Diner, B.A., Li, T., Greco, T.M., Crow, M.S., Fuesler, J.A., Wang, J. and Cristea, I.M., 2015.
18 The functional interactome of PYHIN immune regulators reveals IFIX is a sensor of viral
19 DNA. *Molecular systems biology*, 11(2), p.787.

Rank	ID	p-value	F	FC	Gene	Gene name
228	A_23_P259141	1.4E-11	119.6789	<u>3hr</u> : 0.9736 ± 0.0168 <u>7hr</u> : 0.9996 ± 0.0016 <u>12hr</u> : 1.0103 ± 0.0260 <u>18hr</u> : 1.2439 ± 0.0111 <u>24hr</u> : 1.2345 ± 0.0162	ZBP1	Z-DNA binding protein 1

Table 1: ZBP1 is among the genes most differentially expressed following infection of human cells with an isolate of MERS-CoV.

Rank of differential expression, probe ID, p-value of global differential expression, fold change in ZBP1 expression when comparing infected to non-infected cells, gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	FC	Gene	Gene name
19	43145	1.34E-09	3.59E+01	12.44186	1.716 ± 0.010	Zbp1	Z-DNA binding protein 1

Table 2: ZBP1 is among the genes most differentially expressed in the lungs of BALB/c mice following infection with SARS-CoV-1.

Rank of differential expression, probe ID, p-value of global differential expression, fold change in ZBP1 expression when comparing lungs of infected mice to that of mock-infected mice, gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	FC	Gene	Gene name
228	45822	1.43E-07	1.9E+01	8.38095	1.539 ± 0.042	Dhx58	DEXH (Asp-Glu-X-His) box polypeptide 58

Table 3: DHX58 (LGP2) is among the genes most differentially expressed following infection of BALB/c mice with SARS-CoV-1.

Rank of differential expression, probe ID, p-value of global differential expression, fold change in DHX58 expression when comparing lungs of infected mice to that of mock-infected mice, gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	FC	Gene	Gene name
21	A_52_P223809	3.28E-14	55.83308	23.078927	1.4613 ± 0.0148	Dhx58	DEXH (Asp-Glu-X-His) box polypeptide 58

Table 4: DHX58 (LGP2) is among the genes most differentially expressed following infection of C57BL6/NJ mice with SARS-CoV-1.

Rank of differential expression, probe ID, p-value of global differential expression, fold change in DHX58 expression when comparing lungs of infected mice to that of mock-infected mice, gene and gene name are listed in this chart.

Rank	ID	p-value	F	FC	Gene	Gene name
28	A_23_P38346	3.39E-14	270.3944	<u>3hr</u> : 0.9766 ± 0.0110 <u>7hr</u> : 0.9488 ± 0.0203 <u>12hr</u> : 1.0144 ± 0.0111 <u>18hr</u> : 1.3341 ± 0.0171 <u>24hr</u> : 1.2425 ± 0.0124	DHX58	DEXH (Asp-Glu-X-His) box polypeptide 58

Table 5: DHX58 (LGP2) is among the genes most differentially expressed following infection of human cells with a MERS-CoV isolate.

Rank of differential expression, probe ID, p-value of global differential expression, fold change in DHX58 expression when comparing infected to non-infected cells, gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	FC	Gene	Gene name
210	23366	1.11E-07	1.97E+01	8.63179	1.354 ± 0.019	Pyhin1	pyrin and HIN domain family, member 1

Table 6: PYHIN1 is among the genes most differentially expressed following infection of BALB/c mice with SARS-CoV-1.

Rank of differential expression, probe ID, p-value of global differential expression, fold change in PYHIN1 expression when comparing lungs of infected mice to that of mock-infected mice, gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	FC	Gene	Gene name
132	A_52_P193657	5.8E-12	33.82412	18.171795	1.4626 ± 0.0204	Pyhin1	pyrin and HIN domain family, member 1

Table 7: PYHIN1 is among the genes most differentially expressed following infection of C57BL6/NJ mice with SARS-CoV-1.

Rank of differential expression, probe ID, p-value of global differential expression, fold change in PYHIN1 expression when comparing lungs of infected mice to that of mock-infected mice, gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	FC	Gene	Gene name
234	47644	1.52E-07	1.89E+01	8.322	1.311 ± 0.014	Mefv	Mediterranean fever

Table 8: The Mediterranean fever gene, Mefv (PYRIN) is among the genes most differentially expressed following infection of BALB/c mice with SARS-CoV-1.

Rank of differential expression, probe ID, p-value of global differential expression, fold change in PYRIN expression when comparing lungs of infected mice to that of mock-infected mice, gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	Gene	Gene name
1341	CfaAffx. 24083.1.S1_s_at	2.02E-02	3.04219	-2.9151	DHX58	DEXH (Asp-Glu-X-His) box polypeptide 58

Table 9: DHX58 is among the genes most differentially expressed in the blood of ferrets infected intranasally with SARS-CoV-1.

Rank of differential expression, probe ID, p-value of global differential expression, fold change in PYHIN1 expression when comparing blood from infected ferrets to that of mock-infected ferrets, gene and gene name are listed in this chart.

Rank	ID	p-value	F	Gene	Gene name
3204	CfaAffx. 24083.1.S1_s_at	2.96E-04	2.92E+01	DHX58	DEXH (Asp-Glu-X-His) box polypeptide 58

Table 10: DHX58 is among the genes most differentially expressed in the lungs of ferrets following intranasal infection with SARS-CoV-1.

Rank of differential expression, probe ID, p-value of global differential expression, fold change in PYHIN1 expression when comparing the lungs of infected ferrets to that of mock-infected ferrets, gene and gene name are listed in this chart.

Figure 1: Transcriptional induction of the nucleic acid sensor ZBP1 in human cells following infection with a MERS-CoV isolate.

ZBP1 mRNA expression levels are graphically represented here following infection with MERS-CoV London with mean mRNA values marked and the results of statistical tests evaluating significance of difference in ZBP1 expression as compared to baseline (0 hours), *p*-values, listed above each infection time point.

Figure 2: Transcriptional induction of the nucleic acid sensor ZBP1 in the lungs of BALB/c mice infected with SARS-CoV-1.

ZBP1 mRNA expression levels are graphically represented here in the lungs of mock-infected BALB/c mice (left) and in the lungs of SARS-CoV-1 infected mice (right) at 2 days post-infection, with the result of a statistical test evaluating significance of difference in ZBP1 expression between the lungs of mock-infected and infected mice, a *p*-value, listed above.

Figure 3: Transcriptional induction of the nucleic acid sensor DHX58 (LGP2) in human cells following infection with a MERS-CoV isolate.

DHX58 mRNA expression levels are graphically represented here following infection with MERS-CoV London with mean mRNA values marked and the results of statistical tests evaluating significance of difference in DHX58 expression as compared to baseline (0 hours), *p*-values, listed above each infection time point.

DHX58 (LGP2) Lungs of BALB/C mice infected with SARS-CoV

Figure 4: Transcriptional induction of the nucleic acid sensor DHX58 (LGP2) in the lungs of BALB/c mice infected with SARS-CoV-1.

DHX58 mRNA expression levels are graphically represented here in the lungs of mock-infected BALB/c mice (left) and in the lungs of SARS-CoV-1 infected mice (right) at 2 days post-infection, with the result of a statistical test evaluating significance of difference in DHX58 expression between the lungs of mock-infected and infected mice, a *p*-value, listed above.

Figure 5: Transcriptional induction of DHX58 (LGP2) in the lungs of C57BL6/NJ mice infected with SARS-CoV-1.

DHX58 mRNA expression levels are graphically represented here in the lungs of mock-infected C57BL6/NJ mice (left) and in the lungs of SARS-CoV-1 infected mice (right) at 2 days post-infection, with the result of a statistical test evaluating significance of difference in C57BL6/NJ expression between the lungs of mock-infected and infected mice, a *p*-value, listed above.

Figure 6: Transcriptional induction of the nucleic acid sensor PYHIN1 in the lungs of BALB/c mice following infection with SARS-CoV-1.

PYHIN1 mRNA expression levels are graphically represented here in the lungs of mock-infected BALB/c mice (left) and in the lungs of SARS-CoV-1 infected mice (right) at 2 days post-infection, with the result of a statistical test evaluating significance of difference in PYHIN1 expression between the lungs of mock-infected and infected mice, a *p*-value, listed above.

Figure 7: Transcriptional induction of the nucleic acid sensor PYHIN1 in the lungs of C57BL6/NJ mice infected with SARS-CoV-1.

PYHIN1 mRNA expression levels are graphically represented here in the lungs of mock-infected C57BL6/NJ mice (left) and in the lungs of SARS-CoV-1 infected mice (right) at 2 days post-infection, with the result of a statistical test evaluating significance of difference in PYHIN1 expression between the lungs of mock-infected and infected mice, a *p*-value, listed above.

Figure 8: Transcriptional induction of the PAMP sensor PYRIN in the lungs of BALB/c mice following infection with SARS-CoV-1.

PYRIN mRNA expression levels are graphically represented here in the lungs of mock-infected BALB/c mice (left) and in the lungs of SARS-CoV-1 infected mice (right) at 2 days post-infection, with the result of a statistical test evaluating significance of difference in PYRIN expression between the lungs of mock-infected and infected mice, a *p*-value, listed above.