1 **USP18 is differentially expressed in SARS and MERS coronavirus infection models.**

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6 COVID19 is caused by infection with the seventh virus of the coronavirus family capable of
7 infecting humans, now known as Severe Acute Respiratory Syndrome 2, or SARS-CoV-2 (1-3).
8 We mined published and public microarray data from coronavirus infection models to identify
9 genes whose expression changed most significantly following infection with coronaviruses
10 (4-9). In two *in vivo* mouse models of SARS coronavirus infection, the ubiquitin-specific
11 protease USP18 was among the genes whose expression changed most significantly in the
12 lungs of mice infected with SARS-CoV-1. In the blood of ferrets after intranasal infection with
13 SARS-CoV-1, and in human cells *in vitro* after infection with the Middle Eastern Respiratory
14 Syndrome (MERS) coronavirus, Usp18 was also found to be differentially expressed when
15 compared to mock-infected tissues or uninfected cells. Differential expression of Usp18
16 following infection with coronaviruses can be observed in experimental models *in vivo* and *in*
17 *vitro*, and across species.

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24 Keywords: COVID19, Usp18, SARS-CoV-2, systems biology of viral infection, coronaviruses.
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Coronavirus infection is a global health crisis (2) and over 80,000 people in the United States have thus far lost their lives after infection with the novel coronavirus SARS-CoV-2 (10). We leveraged published microarray data from *in vivo* infection models following infection mice and ferrets with SARS-CoV-1 (or SARS-CoV), and integrated these findings with similar differential gene expression analyses of the transcriptional response of human cells *in vitro* after infection with MERS-CoV or human coronavirus 229E (HCoV 229E) (4-9). In the lung tissues of mice infected with SARS-CoV, and in the blood of ferrets infected with SARS-CoV through the intranasal route, the ubiquitin-specific protease 18, Usp18 was among the genes whose expression changed most significantly (4-6). *In vitro*, Usp18 was differentially expressed after MERS coronavirus infection of primary human cells and in cell culture (7). Usp18 may be a target of interest in understanding the biology of coronavirus infection, in the host cell response to SARS and MERS coronavirus infection, and in understanding of the most significant gene expression changes after infection of mammalian cells with coronaviruses like SARS-CoV-2.

Methods

We used datasets GSE68820 (4), GSE59185 (5), GSE56677 (6), GSE22581 (7), GSE100509 (8), and GSE89167 (9) for this systems-level analysis of coronavirus infection. GSE68820 was generated using Agilent-014868 Whole Mouse Genome Microarray 4x44K G4122F technology. GSE59185 was generated using Agilent-028005 SurePrint G3 Mouse GE 8x60K Microarray technology. GSE56677 was generated using Agilent-039494 SurePrint G3 Human GE v2 8x60K Microarray 039381 technology. GSE22581 was generated using Affymetrix Canine Genome 2.0 Array technology. GSE100509 was generated using Agilent-026652 Whole Human Genome Microarray 4x44K v2 technology. GSE89167 was generated using 039494 SurePrint G3 Human GE v2 8x60K Microarray 039381 technology.

The Benjamin 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

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global differential expression. Log-transformation of data was auto-detected, and the NCBI generated category of platform annotation was used.

A statistical test was performed to evaluate the significance of difference between mRNA expression levels of USP18 in the lungs of C57BL6/NJ mice and BALB/c mice in mock-infected and SARS-infected mice using a two-tailed, unpaired t-test with Welch's correction. Only p-values less than 0.05 were considered statistically significant. We used PRISM for all statistical analyses (Version 8.4.0)(455).

Results

We mined published microarray datasets to understand how gene expression was most significantly different following coronavirus infection using *in vivo* infection models of the mouse and ferret (4-6). We integrated this data with analysis of datasets from *in vitro* infection models of MERS coronavirus (7, 8) and human coronavirus 229E (9) to identify genes whose expression was modulated across the coronavirus family.

USP18 is differentially expressed in the lungs of C57BL6/NJ mice following infection with SARS coronavirus (SARS-CoV-1).

We identified the ubiquitin-specific protease Usp18 as differentially expressed in the lungs of C57BL6/NJ mice infected with SARS coronavirus. When sorting all of the genes expressed in the mouse lung based on change in expression between the lungs of mock infected mice and the lungs of mice infected with SARS-CoV-1 at 2 days post-infection, Usp18 ranked 15 out of 29649 total transcripts. Differential expression of Usp18 in the lungs of C57BL6/NJ mice following SARS-CoV-1 infection was statistically significant (Table 1; $p=2.3E-14$).

1 USP18 is differentially expressed in the lungs of BALB/c mice following infection with SARS
2 coronavirus (SARS-CoV-1).

3 Analysis of a second dataset revealed that Usp18 was also differentially expressed in
4 the lungs of BALB/c mice infected with SARS coronavirus. When sorting all of the genes
5 expressed in the mouse lung based on change in expression between the lungs of mock
6 infected mice and the lungs of mice infected with SARS-CoV-1, Usp18 ranked 31 out of 62976
7 total transcripts. Differential expression of Usp18 in the lungs of BALB/c mice following SARS-
8 CoV-1 infection was statistically significant (Table 2; $p=2.28E-09$).
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11 USP18 is differentially expressed in the blood of ferrets after respiratory tract infection with
12 SARS coronavirus (SARS-CoV-1).

13 We further identified Usp18 as a differentially expressed gene in the blood of ferrets
14 infected through the respiratory tract with SARS-CoV-1. When sorting all of the genes
15 expressed in ferret blood based on change in expression between the blood of mock infected
16 ferrets and the blood of ferrets infected with SARS-CoV-1, Usp18 ranked 906 out of 43035
17 total transcripts. Differential expression of Usp18 in the ferret blood following respiratory tract
18 SARS-CoV-1 infection was statistically significant (Table 3; $p=1.38E-02$). Usp18 was not
19 differentially expressed in the lung tissues of ferrets as we observed in mice (Table 4; rank
20 12973 out of 43035 total transcripts).
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23 USP18 is differentially expressed in the CALU3 2B4 human liver cell line after infection with
24 MERS coronavirus.

25 We sought to determine whether differential expression of Usp18 following coronavirus
26 infection would be observed in different coronavirus types, in coronavirus infection models in
27 vitro and in human cells. In CALU3 2B4 cells infected with MERS-CoV London, Usp18 was
28 among the genes most differentially expressed. When sorting all of the genes expressed in
CALU3 2B4 cells based on change in expression between MERS-CoV London infected cells

1 and uninfected cells, Usp18 ranked 909 out of 28653 total transcripts. Differential expression
2 of Usp18 in human cell culture following MERS-CoV London infection *in vitro* was statistically
3 significant (Table 5; $p=2.22E-09$). While Usp18 was also differentially expressed following
4 infection of primary human microvascular endothelial cells with MERS-CoV wildtype, with a
5 change in expression greater than 85% of the transcriptome, this was less significant than
6 observed with MERS-CoV London (Table 6; $p=3.28E-16$).

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8 Usp18 was not differentially expressed following infection of HuH-7 cells with human
9 coronavirus 229E (Table 7; $p=0.50542853$).

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11 USP18 is transcriptionally induced following infection with SARS coronavirus in the lungs of
12 both C57BL6/NJ and BALB/c mice.

13 We obtained exact mRNA expression values for Usp18 in the lung tissues of mice
14 infected with SARS-CoV-1. In both C57BL6/NJ mice and in BALB/c mice, Usp18 was
15 expressed at significantly higher levels following SARS-CoV-1 infection as compared to mock
16 infected mice (Figure 1 and Figure 2; $p<0.0001$).

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18 Thus, we found that Usp18 was differentially expressed in the lungs of mice infected
19 with SARS-CoV-1, in the blood of ferrets infected with SARS-CoV-1 through the respiratory
20 tract, and in human cell culture following infection with MERS-CoV London and MERS-CoV
21 wild-type. Usp18 gene expression was significantly induced following infection *in vivo* in the
22 lungs of mice infected with SARS-CoV-1.
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24 25 Discussion

26 We mined multiple published and public microarray datasets to identify genes
27 differentially expressed across the coronavirus infection models, *in vivo* and *in vitro*. We
28 identified USP18 as among the genes whose expression changed most significantly in the
lungs of mice infected with SARS-CoV, in the blood of ferrets infected with SARS-CoV-1
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1 through the respiratory tract, and in cultured and primary human cells in *vitro* after infection
2 with MERS-CoV.

3 The specific function of USP18 (also known as Ubp43) is to remove the ISG15 post-
4 translational modification (11). ISG15 is induced in the setting of viral infection, type I interferon
5 responses, and after exposure to the TLR4 ligand lipopolysaccharide (12). Usp18 has a
6 developmental role in the immune system as it is important for the development of CD11b+
7 dendritic cells (13). Usp18 is important for the control of viral infection multiple contexts: in
8 Vesicular Stomatitis Virus (VSV), in Lymphocytic Choriomeningitis Virus (LCMV) , and in Sindbis
9 Virus (SNV) infections of mice (13). Usp18^{-/-} mice displayed enhanced interferon responses to
10 VSV and SNV, indicating a role for Usp18 in restraining the antiviral response (14). Similar
11 regulatory properties of Usp18 were found in the host responses to Hepatitis C virus (15).
12 USP18, when deficient in humans, can lead to pseudo-TORCH syndrome, an inherited
13 encephalopathy with genetic susceptibility to congenital infections including viral infections like
14 Herpes Simplex Virus or Rubella virus (16). Viral titers in the lung and in the spleen of
15 enzymatically deficient C61A USP18 knock-in mice after infection with VACV were significantly
16 lower than that found after VACV infection of wild-type mice, demonstrating that Usp18
17 antagonism of ISG15-mediated interferon signaling through its catalytic site was important for
18 reduction of viral titers by Usp18 (17). Enzymatically deficient Usp18 C61A mice displayed
19 elevated ISGylation as well as enhanced resistance to vaccinia and influenza B virus (17).
20 These mice did not manifest the morphological abnormalities of the brain seen in wild-type
21 Usp18 mice, demonstrating that functions of Usp18 could be separated and that the enzymatic
22 activity of Usp18 was not required for all Usp18 functions. A separate study showed that
23 Usp18 negatively regulated the JAK-STAT signaling pathway by interactions with STAT2 (18).
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25 We found that Usp18 was among the genes most differentially expressed following
26 SARS coronavirus infection of the mouse lung, and in the blood of ferrets after intranasal
27 infection with SARS-CoV-1. Usp18 was also among the genes most differentially expressed
28 following MERS-CoV infection of primary and cultured human cells. Usp18 mRNA levels

1 significantly increased in the mouse lung following infection with SARS-CoV-1. The blood and
2 tissues of patients with the novel coronavirus SARS-CoV-2 can be assessed to determine if
3 Usp18 differential expression and induction is conserved across the coronavirus family.
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Rank	ID	p-value	t	B	Fold change	Gene	Gene name
15	A_51_P164219	2.3E-14	57.78361	23.38869	1.4357 ± 0.0156	Usp18	ubiquitin specific peptidase 18

Table 1: USP18 is differentially expressed in the lungs of C57BL6/NJ mice following infection with SARS coronavirus (SARS-CoV-1).

The rank of differential expression globally, the probe/transcript ID, the p-value of differential expression globally, t, a moderated t statistic, B, the log-odds of differential expression between the groups compared, the gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	Fold change	Gene	Gene name
31	7388 NM_011909	2.28E-09	3.34E+01	12.04542	1.5186 ± 0.0250	Usp18	ubiquitin specific peptidase 18

Table 2: USP18 is differentially expressed in the lungs of BALB/c mice following infection with SARS coronavirus (SARS-CoV-1).

The rank of differential expression globally, the probe/transcript ID, the p-value of differential expression globally, t, a moderated t statistic, B, the log-odds of differential expression between the groups compared, the gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	Gene	Gene name
906	CfaAffx. 24933.1.S1_at	1.38E-02	3.329695	-2.5995	USP18	ubiquitin specific peptidase 18

Table 3: USP18 is differentially expressed in the blood of ferrets after intranasal infection with SARS coronavirus (SARS-CoV-1).

The rank of differential expression globally, the probe/transcript ID, the p-value of differential expression globally, t, a moderated t statistic, B, the log-odds of differential expression between the groups compared, the gene and gene name are listed in this chart.

Rank	ID	p-value	F	Gene	Gene name
12973	CfaAffx. 24933.1.S1_at	1.29E-02	8.25	USP18	ubiquitin specific peptidase 18

Table 4: USP18 is not differentially expressed in the lungs of ferrets after intranasal infection with SARS-CoV-1.

The rank of differential expression globally, the probe/transcript ID, the p-value of differential expression globally, F, a statistic used when more than two groups are compared in differential gene expression analysis (rather than the moderated t-statistic “t”), the gene and gene name are listed in this chart.

Rank	ID	p-value	F	Gene	Gene name
909	A_23_P132159 NM_017414	2.22E-09	59.3636	USP18	ubiquitin specific peptidase 18

Table 5: USP18 is differentially expressed in the CALU3 2B4 human liver cell line after infection with MERS coronavirus London.

The rank of differential expression globally, the probe/transcript ID, the p-value of differential expression globally, F, a statistic used when more than two groups are compared in differential gene expression analysis (rather than the moderated t-statistic “t”), the gene and gene name are listed in this chart.

Rank	ID	p-value	F	Gene	Gene name
4890	A_23_P132159 NM_017414	3.28E-16	146.0393	USP18	ubiquitin specific peptidase 18

Table 6: USP18 is differentially expressed in primary human microvascular endothelial cells following infection with MERS-CoV wild-type.

The rank of differential expression globally, the probe/transcript ID, the p-value of differential expression globally, F, a statistic used when more than two groups are compared in differential gene expression analysis (rather than the moderated t-statistic “t”), the gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	Gene	Gene name
40315	19270 A_23_P132159 NM_017414	0.50542853	7.1E-01	-5.917765	USP18	ubiquitin specific peptidase 18

Table 7: USP18 is not differentially expressed in the HuH-7 cells after infection with HCoV 229E (human coronavirus 229E).

The rank of differential expression globally, the probe/transcript ID, the p-value of differential expression globally, t, a moderated t statistic, B, the log-odds of differential expression between the groups compared, the gene and gene name are listed in this chart.

Figure 1: USP18 is transcriptionally induced and expressed at significantly higher levels after infection with SARS-CoV-1 in the lungs of C57BL6/NJ mice.

Expression of USP18 at the mRNA level is graphically represented with the mean value marked and the p-value listed for each comparison relative to mock infection at 2 days post-infection.

Figure 2: USP18 is transcriptionally induced and expressed at significantly higher levels after infection with SARS-CoV-1 in the lungs of BALB/c mice.

Expression of USP18 at the mRNA level is graphically represented with the mean value marked and the p-value listed for each comparison relative to mock infection at 2 days post-infection.