The activating transcription factor ATF3 is differentially expressed and transcriptionally induced in multiple models of coronavirus infection.

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The coronavirus COVID19 pandemic is an emerging biosafety threat to the nation and the world (1). There are no treatments approved for coronavirus infection in humans (2) and there is a lack of information available regarding the basic transcriptional behavior of human cells and mammalian tissues following coronavirus infection. We mined four independent datasets (3-6), public (3) and published (4-6) containing transcriptome data from infection models of the Middle East respiratory syndrome (MERS) coronavirus, severe acute respiratory syndrome (SARS) coronavirus and human coronavirus (HCoV) to discover genes that are differentially expressed in coronaviruses and identify potential therapeutic targets and host cell vulnerabilities. We identified the activating transcription factor ATF3 (7) as a conserved differentially expressed gene following coronavirus infection. Importantly, ATF3 is a negative regulator of the pattern recognition receptor TLR4 as well as the cytokines IL6 and IL12b (8). ATF3 induction following coronavirus infection represents either a host cell response to viral infection or a pathogenic co-opting of a host cell transcriptional pathway. In either case, ATF3 may be a relevant therapeutic target for intervention in coronavirus infections across multiple coronavirus types and potentially for COVID19 infections.

Keywords: coronavirus, MERS coronavirus, SARS coronavirus, human coronavirus, HCoV, systems biology of viral infection, COVID19.

Viruses are classified according to a system known as the “Baltimore” classification of viruses (9) wherein the characteristics of the viral genome, including whether it is plus or minus strand, also known as positive-sense or negative-sense, whether the genome is single-stranded or double-stranded, whether their genome is composed of RNA or DNA, and whether or not they use a reverse transcriptase to replicate are used to place viruses into categories. Coronaviruses are plus-strand RNA viruses that contain an envelope surrounding their viral particle (10). Their genome is largest of all RNA viruses, ranging from 27 to 33 kb in size (11). They obtain their name from the crown-like appearance of the viral particle imparted by the structure of the large-surface glycoprotein (11). The coronaviridae family includes seven viruses capable of infecting humans, including the severe acute respiratory distress syndrome, or SARS coronavirus (12), the Middle East respiratory syndrome coronavirus, or MERS coronavirus (13), the human coronaviruses (HCoV) 229E, OC43, HKU and NL63 (14-16), and the novel human coronavirus now known as COVID19 (17-18). As of March 19, 2020, the World Health Organization reported 209,839 cases of COVID19 and 8778 deaths from COVID19 infection world-wide (19). There are no FDA-approved treatments for human coronavirus infection.

We used a systems-level approach to identify the genes whose expression changes most significantly following infection with multiple types of coronavirus, including MERS-CoV, SARS-CoV, and HCoV 229E using four independent datasets (3-6). From these datasets were mined transcriptome data following coronavirus infection in human cell culture, in primary human endothelial cells, and in two mouse models of coronavirus infection of the lungs. Across each of these datasets, we identified the activating transcription factor ATF3 as among the genes most differentially expressed following coronavirus infection. ATF3 represents a transcriptional target of the host cell gene expression program following infection with coronaviruses *in vitro* and *in vivo*.

Methods

We used datasets GSE100509 (3), GSE89167 (4), GSE68820 (5) and GSE59185 (6) for this systems-level differential gene expression analysis of coronavirus infections, all in conjunction with GEO2R.

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 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 ATF3 in the lungs of C57BL6/NJ and BALB/C mice with SARS-CoV infection as compared to mock infection using a two-tailed, unpaired t-test with Welch's correction. A statistical test was performed to evaluate the significance of difference between mRNA expression levels of ATF3 in HuH-7 cells with and without HCoV 229E infection using a two-tailed, unpaired t-test. A statistical test was performed to evaluate the significance of difference between mRNA expression levels of ATF3 in human primary microvascular endothelial cells using a one-way ANOVA with multiple comparisons, all compared to baseline infection at 0 hours. 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 four independent microarray datasets, public (3) and published (4-6) containing transcriptome data from multiple models of coronavirus infection in cells and tissues from human and mice. Across species and experimental models, we identified the activating transcription factor ATF3 as differentially expressed following coronavirus infection.

1 ATF3 is differentially expressed in primary human microvascular endothelial cells when
2 comparing cells infected with wild-type Middle East respiratory syndrome coronavirus (MERS-
3 CoV), icMERS-CoV EMC2012 and uninfected cells.

4 We identified ATF3 as differentially expressed following infection of primary human
5 microvascular endothelial cells with wild-type Middle East respiratory syndrome coronavirus
6 (MERS-CoV), icMERS-CoV EMC2012 when compared to non-infected cells (Table 1) (3). When
7 sorting all of the transcripts expressed in human microvascular endothelial cells measured by
8 microarray based on change in expression with and without infection, ATF3 ranked 38 out of
9 34127 transcripts. Differential expression of ATF3 in primary human microvascular endothelial
10 cells following infection with MERS-CoV was statistically significant (Table 1; $p=1.68E-28$).

11 ATF3 is differentially expressed in the human cell line HuH-7 when comparing cells with human
12 coronavirus 229E and uninfected cells.

13 We also identified ATF3 as differentially expressed in the human cell line HuH-7 when
14 comparing cells with human coronavirus 229E with uninfected cells (Table 2) (4). When sorting
15 all of the transcripts expressed in human HuH-7 cells measured by microarray based on
16 change in expression with and without infection with human coronavirus 229E, ATF3 ranked 15
17 out of 62976 transcripts. Differential expression of ATF3 in HuH-7 following infection with
18 HCoV 229E was statistically significant (Table 2; $p= 0.00002468$).

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20 ATF3 is differentially expressed in the lungs *in vivo* when comparing C57BL/6 mice infected
21 with severe acute respiratory distress syndrome (SARS) coronavirus and mock-infected
22 C57BL6/NJ mice.

23 We also identified ATF3 as differentially expressed *in vivo* when comparing the lungs of
24 C57BL6/NJ mice infected with severe acute respiratory distress syndrome (SARS) coronavirus
25 with that of mock-infected C57BL6/NJ mice (Table 3) (5). When sorting all of the transcripts
26 expressed in the lungs of C57BL6/NJ mice measured by microarray based on change in
27 expression with and without infection with SARS coronavirus, ATF3 ranked 115 out of 29649
28 transcripts. Differential expression of ATF3 in the lungs of C57BL6/NJ mice infected with SARS
coronavirus was statistically significant (Table 3; $p= 3.16E-12$).

1 ATF3 is differentially expressed in the lungs *in vivo* when comparing BALB/c mice infected with
2 severe acute respiratory distress syndrome (SARS) coronavirus in and mock infected BALB/c
3 mice.

4 We also identified ATF3 as differentially expressed *in vivo* when comparing the lungs of
5 BALB/c mice infected with severe acute respiratory distress syndrome (SARS) coronavirus with
6 that of mock-infected BALB/c mice (Table 4) (6). When sorting all of the transcripts expressed
7 in the lungs of BALB/c mice measured by microarray based on change in expression with and
8 without infection with SARS coronavirus, ATF3 ranked 149 out of 62976 transcripts.

9 Differential expression of ATF3 in the lungs of BALB/c mice infected with SARS coronavirus
10 was statistically significant (Table 4; $p= 3.16E-12$).
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12 ATF3 is transcriptionally induced following infection of primary human microvascular
13 endothelial cells with wild-type Middle East respiratory syndrome coronavirus (MERS-CoV).
14 icMERS-CoV EMC2012.

15 We extracted exact mRNA expression values for ATF3 from primary human
16 microvascular endothelial cells infection with wild-type Middle East respiratory syndrome
17 coronavirus (MERS-CoV), icMERS-CoV EMC201, and from uninfected primary human
18 microvascular endothelial cells in order to compare expression levels of ATF3 between these
19 two groups rather than relative to the rest of the transcriptome as assessed in differential gene
20 expression analysis. This dataset contained transcriptome information from infection of
21 primary human microvascular endothelial cells at 12 hours, 24 hours, 36 hours and 48 hours
22 post-infection at compared to baseline (0 hours). We also performed a statistical test to
23 evaluate whether the difference in expression of ATF3 in primary human microvascular
24 endothelial cells infection with and without MERS-CoV infection was statistically significant.
25 ATF3 was expressed at significantly lower levels at 12 hours post-infection as compared to
26 cells infected at baseline (0 hours) (Figure 1; $p<0.0001$). In contrast, ATF3 was expressed at
27 significantly higher levels higher levels at 24 hours, 36 hours, and 48 hours post-infection with
28 MERS-CoV as compared to cells infected at baseline (0 hours) (Figure 1; $p<0.0001$ for all
comparisons).

1 ATF3 is transcriptionally induced in the human cell line HuH-7 following infection with human
2 coronavirus 229E.

3 We also extracted exact mRNA expression values for ATF3 from HuH-7 cells following
4 infection with human coronavirus 229E and from uninfected HuH-7 cells in order to compare
5 expression levels of ATF3 between these two groups rather than relative to the rest of the
6 transcriptome as assessed in differential gene expression analysis. We also performed a
7 statistical test to evaluate whether the difference in expression of ATF3 in HuH-7 cells following
8 infection with human coronavirus 229E as compared to uninfected HuH-7 cells was statistically
9 significant. ATF3 was expressed at significantly higher levels in HuH-7 cells following infection
10 with HCoV 229E (Figure 2; $p=0.0127$).
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12 ATF3 is transcriptionally induced in the lungs *in vivo* after infection with severe acute respiratory
13 distress syndrome (SARS) coronavirus in C57BL6/NJ mice.

14 We also extracted exact mRNA expression values for ATF3 from the lungs of C57BL6/
15 NJ mice following infection with SARS coronavirus and from the lungs of mock-infected
16 C57BL6/NJ mice in order to compare expression levels of ATF3 between these two groups
17 rather than relative to the rest of the transcriptome as assessed in differential gene expression
18 analysis. We also performed a statistical test to evaluate whether the difference in expression
19 of ATF3 between the lungs of SARS-infected and mock-infected C57BL6/NJ mice was
20 statistically significant. ATF3 was expressed at significantly higher levels in the lungs of
21 C57BL6/NJ mice infected with the MA15 SARS coronavirus as compared to the lungs of
22 mock-infected C57BL6/NJ mice 2 days post-infection (Figure 3; $p<0.0001$).
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24 ATF3 is transcriptionally induced in the lungs *in vivo* after infection with severe acute respiratory
25 distress syndrome (SARS) coronavirus in BALB/c mice.

26 We also extracted exact mRNA expression values for ATF3 from primary human
27 microvascular endothelial cells infection with wild-type Middle East respiratory syndrome
28 coronavirus (MERS-CoV), icMERS-CoV EMC201, and from uninfected primary human
microvascular endothelial cells in order to compare expression levels of ATF3 between these

two groups rather than relative to the rest of the transcriptome as assessed in differential gene expression analysis. We also performed a statistical test to evaluate whether the difference in expression of ATF3 between the lungs of SARS-infected and mock-infected BALB/c mice was statistically significant. ATF3 was expressed at significantly higher levels in the lungs of BALB/c mice infected with the SARS coronavirus as compared to the lungs of mock-infected BALB/c mice (Figure 4; $p=0.0005$).

Discussion

COVID19 is a newly discovered coronavirus that infects humans and whose spread has lead to a global pandemic (19) with zero available therapeutic strategies. To facilitate target discovery and to further understanding of the basic transcriptional program of cells and tissues following coronavirus infection, we mined multiple microarray datasets containing transcriptome data of human cells infected with MERS-CoV and HCoV 229E, in primary cells and in cell culture, and in the lungs of two separate mouse strains infected with SARS-CoV. Across each dataset and experimental model, we identified the activating transcription factor ATF3 as one of the genes whose expression changed most significantly following infection with a coronavirus. In every experimental model analyzed, ATF3 expression significantly increased after infection with coronavirus, except for the 12 hour timepoint in primary human microvascular endothelial cells infected with MERS-CoV where ATF3 expression dropped significantly from its baseline expression level at 0 hours, and then rebounded at 24 hours to a level above its baseline expression at 0 hours.

ATF3 is important for preventing LPS-induced inflammation by inhibiting expression of the LPS pattern recognition receptor TLR4 and through inhibiting expression of the cytokines IL-6 and IL-12b (8). This can represent a host strategy to mitigate pathology associated with a cytokine storm as seen in the lethal Spanish influenza (20, 21), or it could represent a pathogen-induced transcriptional event in order to promote viral replication by restricting influx of cells following a bacterially-induced co-infection based inflammatory reaction following viral

1 infection (22-25). If part of a host defense strategy, ATF3 induction can be achieved
2 therapeutically using NSAIDs, troglitazone, diallyl disulfide, and resveratrol, all of which lead to
3 induction of ATF3 mRNA and protein in HCT116 cells *in vitro* (26). If part of a pathogen-
4 induced host cell transcriptional program to promote viral replication, ATF3 inhibition can be
5 achieved using the compounds GW5074 as well as the RNA-dependent protein kinase
6 inhibitors SK4 and SK6, all of which lead to inhibition of ATF3 expression (27).
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8 ATF3 expression should be evaluated in the cells and tissues of patients infected with
9 the novel coronavirus COVID19 to assess whether COVID19 infection similarly leads to
10 induction of ATF3 as seen after infection with MERS-CoV, SARS-CoV, and HCoV 229E.
11 Modulation of ATF3 may represent a potential therapeutic strategy in coronavirus infection
12 broadly and specifically in COVID19 infection.
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References

1. World Health Organization, 2020. Coronavirus disease 2019 (COVID-19): situation report, 49.
2. Wang, Y. and Zhu, L.Q., 2020. Pharmaceutical care recommendations for antiviral treatments in children with coronavirus disease 2019. *World Journal of Pediatrics*, pp.1-4.
3. GSE100509. Baric R, Sims A, Heller N, Waters KM, Einfeld AJ, Kawaoka Y. PNNL. 902 Battelle Blvd. Richland, USA.
4. 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- κ B-dependent and-independent transcriptome and chromatin landscapes of human coronavirus 229E-infected cells. *PLoS pathogens*, 13(3), p.e1006286.
5. 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.
6. 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.
7. Hai, T.W., Liu, F., Coukos, W.J. and Green, M.R., 1989. Transcription factor ATF cDNA clones: an extensive family of leucine zipper proteins able to selectively form DNA-binding heterodimers. *Genes & development*, 3(12b), pp.2083-2090.
8. Gilchrist, M., Thorsson, V., Li, B., Rust, A.G., Korb, M., Kennedy, K., Hai, T., Bolouri, H. and Aderem, A., 2006. Systems biology approaches identify ATF3 as a negative regulator of Toll-like receptor 4. *Nature*, 441(7090), pp.173-178.
9. Baltimore, D., 1971. Expression of animal virus genomes. *Bacteriological reviews*, 35(3), p. 235.
10. Sawicki, S.G., Sawicki, D.L. and Siddell, S.G., 2007. A contemporary view of coronavirus transcription. *Journal of virology*, 81(1), pp.20-29.

11. Lai, M.M. and Cavanagh, D., 1997. The molecular biology of coronaviruses. In *Advances in virus research* (Vol. 48, pp. 1-100). Academic Press.
12. Peiris, J.S.M., Lai, S.T., Poon, L.L.M., Guan, Y., Yam, L.Y.C., Lim, W., Nicholls, J., Yee, W.K.S., Yan, W.W., Cheung, M.T. and Cheng, V.C.C., 2003. Coronavirus as a possible cause of severe acute respiratory syndrome. *The Lancet*, 361(9366), pp.1319-1325.
13. Lu, G., Hu, Y., Wang, Q., Qi, J., Gao, F., Li, Y., Zhang, Y., Zhang, W., Yuan, Y., Bao, J. and Zhang, B., 2013. Molecular basis of binding between novel human coronavirus MERS-CoV and its receptor CD26. *Nature*, 500(7461), pp.227-231.
14. van der Hoek, L., Pyrc, K., Jebbink, M.F., Vermeulen-Oost, W., Berkhout, R.J., Wolthers, K.C., Wertheim-van Dillen, P.M., Kaandorp, J., Spaargaren, J. and Berkhout, B., 2004. Identification of a new human coronavirus. *Nature medicine*, 10(4), pp.368-373.
15. van der Hoek, L., Pyrc, K., Jebbink, M.F., Vermeulen-Oost, W., Berkhout, R.J., Wolthers, K.C., Wertheim-van Dillen, P.M., Kaandorp, J., Spaargaren, J. and Berkhout, B., 2004. Identification of a new human coronavirus. *Nature medicine*, 10(4), pp.368-373.
16. Woo, P.C., Lau, S.K., Chu, C.M., Chan, K.H., Tsoi, H.W., Huang, Y., Wong, B.H., Poon, R.W., Cai, J.J., Luk, W.K. and Poon, L.L., 2005. Characterization and complete genome sequence of a novel coronavirus, coronavirus HKU1, from patients with pneumonia. *Journal of virology*, 79(2), pp.884-895.
17. Zhu, N., Zhang, D., Wang, W., Li, X., Yang, B., Song, J., Zhao, X., Huang, B., Shi, W., Lu, R. and Niu, P., 2020. A novel coronavirus from patients with pneumonia in China, 2019. *New England Journal of Medicine*.
18. Bai, Y., Yao, L., Wei, T., Tian, F., Jin, D.Y., Chen, L. and Wang, M., 2020. Presumed asymptomatic carrier transmission of COVID-19. *Jama*.
19. World Health Organization, 2020. Coronavirus disease 2019 (COVID-19): situation report, 59.
20. Salomon, R. and Webster, R.G., 2009. The influenza virus enigma. *Cell*, 136(3), pp. 402-410.
21. Liu, Q., Zhou, Y.H. and Yang, Z.Q., 2016. The cytokine storm of severe influenza and development of immunomodulatory therapy. *Cellular & molecular immunology*, 13(1), pp. 3-10.

- 1 22. Rice, T.W., Robinson, L., Uyeki, T.M., Vaughn, F.L., John, B.B., Miller III, R.R., Higgs, E.,
2 Randolph, A.G., Smoot, B.E., Thompson, B.T. and NHLBI ARDS Network, 2012. Critical
3 illness from 2009 pandemic influenza A (H1N1) virus and bacterial co-infection in the
4 United States. *Critical care medicine*, 40(5), p.1487.
- 5 23. Hoffmann, J., Machado, D., Terrier, O., Pouzol, S., Messaoudi, M., Basualdo, W., Espínola,
6 E.E., Guillen, R.M., Rosa-Calatrava, M., Picot, V. and Bénet, T., 2016. Viral and bacterial
7 co-infection in severe pneumonia triggers innate immune responses and specifically
8 enhances IP-10: a translational study. *Scientific reports*, 6, p.38532.
- 9 24. Beadling, C. and Slifka, M.K., 2004. How do viral infections predispose patients to
10 bacterial infections?. *Current opinion in infectious diseases*, 17(3), pp.185-191.
- 11 25. Bakaletz, L.O., 2017. Viral–bacterial co-infections in the respiratory tract. *Current opinion in*
12 *microbiology*, 35, pp.30-35.
- 13 26. Bottone, F.G., Moon, Y., Kim, J.S., Alston-Mills, B., Ishibashi, M. and Eling, T.E., 2005. The
14 anti-invasive activity of cyclooxygenase inhibitors is regulated by the transcription factor
15 ATF3 (activating transcription factor 3). *Molecular cancer therapeutics*, 4(5), pp.693-703.
- 16 27. Chen, H.M., Wang, L. and D’Mello, S.R., 2008. Inhibition of ATF-3 expression by B-Raf
17 mediates the neuroprotective action of GW5074. *Journal of neurochemistry*, 105(4), pp.
18 1300-1312.
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Rank	ID	p-value	F	Gene	Gene name
GSE100509					
38	A_23_P34915/ NM_001040619	1.68E-28	1696.6111	ATF3	activating transcription factor 3

Table 1: ATF3 is differentially expressed in primary human microvascular endothelial cells when comparing cells infected with wild-type Middle East respiratory syndrome coronavirus (MERS-CoV), icMERS-CoV EMC2012 and non-infected cells.

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.

	ID	p-value	t	B	Gene	Gene name
GSE89167						
15	46825/ NM_001040619	0.00002468	1.21E+01	2.496443	ATF3	activating transcription factor 3

Table 2: ATF3 is differentially expressed in the human cell line HuH-7 when comparing cells with human coronavirus-229E and non-infected cells.

The rank of differential expression globally, the probe/transcript ID, the p-value of differential expression globally, the moderated t-statistic “t”, the gene and gene name are listed in this chart.

	ID	p-value	t	B	Gene	Gene name
GSE68820						
115	A_52_P452689	3.16E-12	35.8825	18.779264	Atf3	activating transcription factor 3

Table 3: ATF3 is differentially expressed in the lungs *in vivo* when comparing C57BL/6 mice infected with severe acute respiratory distress syndrome (SARS) coronavirus and mock-infected C57BL6/NJ mice.

The rank of differential expression globally, the probe/transcript ID, the p-value of differential expression globally, the moderated t-statistic “t”, the gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	Gene	Gene name
GSE59185						
149	35279 / NM_007498	4.76E-08	2.21E+01	9.43664	Atf3	activating transcription factor 3

Table 4: ATF3 is differentially expressed in the lungs *in vivo* when comparing BALB/c mice infected with severe acute respiratory distress syndrome (SARS) coronavirus in and mock infected BALB/c mice.

The rank of differential expression globally, the probe/transcript ID, the p-value of differential expression globally, the moderated t-statistic “t”, the gene and gene name are listed in this chart.

Figure 1: ATF3 is transcriptionally induced following infection of primary human microvascular endothelial cells with wild-type Middle East respiratory syndrome coronavirus (MERS-CoV), icMERS-CoV.

Expression of ATF3 at the mRNA level is graphically represented with the mean value marked and the p-value listed for each comparison relative to baseline infection at 0 hours.

Figure 2: ATF3 is transcriptionally induced in the human cell line HuH-7 following infection with human coronavirus HCoV 229E.

Expression of ATF3 at the mRNA level is graphically represented with the mean value marked and the p-value listed, the result of a test evaluating the significance of difference in ATF3 expression with and without HCoV infection.

Figure 3: ATF3 is transcriptionally induced in the lungs *in vivo* after infection with severe acute respiratory distress syndrome (SARS) coronavirus in C57BL/6 mice.

Expression of ATF3 at the mRNA level is graphically represented with the mean value marked and the p-value listed, the result of a test evaluating the significance of difference in ATF3 expression between SARS infected and mock-infected lung tissue 2 days post-infection.

Figure 4: ATF3 is transcriptionally induced in the lungs *in vivo* after infection with severe acute respiratory distress syndrome (SARS) coronavirus in BALB/c mice.

Expression of ATF3 at the mRNA level is graphically represented with the mean value marked and the p-value listed, the result of a test evaluating the significance of difference in ATF3 expression between SARS infected and mock-infected lung tissue.