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Activation of the SARS-CoV-2 Receptor Ace2 through JAK/STAT-Dependent Enhancers during Pregnancy

Graphical Abstract

Highlights
- Ace2 expression is induced in the mammary glands of pregnant and lactating mice
- Gene enhancers are activated by the prolactin-activated transcription factors STAT5A/B
- Deletion of the Stat5a gene mitigates enhancer formation and Ace2 expression
- Ace2 levels also increase in lung tissue during lactation

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In Brief
Hennighausen and Lee find that increased expression of the SARS-CoV-2 receptor Ace2 in mammary tissue of mice during pregnancy and lactation is controlled through the hormone-activated JAK/STAT5 signaling pathway. Their findings suggest the possibility of vertical transmission of SARS-CoV-2 through breast milk and non-pulmonary tissue damage.
Activation of the SARS-CoV-2 Receptor Ace2 through JAK/STAT-Dependent Enhancers during Pregnancy

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SUMMARY

ACE2 binds the coronavirus SARS-CoV-2 and facilitates its cellular entry. Interferons activate ACE2 expression in pneumocytes, suggesting a critical role of cytokines in SARS-CoV-2 target cells. Viral RNA was detected in breast milk in at least seven studies, raising the possibility that ACE2 is expressed in mammary tissue during lactation. Here, we show that Ace2 expression in mouse mammary tissue is induced during pregnancy and lactation, which coincides with the activation of intronic enhancers. These enhancers are occupied by the prolactin-activated transcription factor STAT5 and additional regulatory factors, including RNA polymerase II. Deletion of Stat5a results in decommissioning of the enhancers and an 83% reduction of Ace2 mRNA. We also demonstrate that Ace2 expression increases during lactation in lung, but not in kidney and intestine. JAK/STAT components are present in a range of SARS-CoV-2 target cells, opening the possibility that cytokines contribute to the viral load and extrapulmonary pathophysiology.

INTRODUCTION

Angiotensin-converting enzyme 2 (ACE2), the receptor for SARS-associated coronavirus (SARS-CoV) (Imai et al., 2005) and SARS-CoV-2 (Hoffmann et al., 2020), has been detected in a range of target cells, including absorptive enterocytes (Lamers et al., 2020), colon organoids (Stanifer et al., 2020), small intestine and colonic epithelial cells (Lee et al., 2020), the olfactory system (Brann et al., 2020) and several epithelial cell types (Brann et al., 2020; Lukassen et al., 2020; Qi et al., 2020). Although SARS-CoV-2 infection of lung epithelium is a critical driver of disease, extrapulmonary manifestations of coronavirus disease 2019 (COVID-19) infection (Gupta et al., 2020) have been associated with direct viral damage of tissues that carry the ACE2 receptor, such as intestinal enterocytes and renal tissue (Monteil et al., 2020). Deciphering the regulation of the ACE2 gene in SARS-CoV-2 target cells is a step forward in linking ACE2 levels with viral damage and COVID-19 pathology. This is relevant not only in the context of cytokine storms but also in patients with different physiological conditions, such as pregnancy and lactation.

Although a body of work has focused on the pathology of COVID-19 during pregnancy (Khalil et al., 2020), the extent to which hormones control ACE2 expression during pregnancy and lactation, and possibly the susceptibility of cells to SARS-CoV-2 infection, has not been investigated. One hallmark of pregnancy is the development of functional mammary tissue that produces large quantities of milk during lactation. Both mammary development and milk production are governed by the cytokine prolactin and the downstream Janus kinase (JAK)/signal transducer and activator of transcription (STAT) signaling pathway (Liu et al., 1997). STAT5 is activated by prolactin and is essential for both mammary development and milk production. Its role in the activation of transcriptional enhancers during pregnancy is well established (Lee et al., 2018; Yamaji et al., 2013). SARS-CoV-2 RNA has been detected in breast milk of infected individuals (Bastug et al., 2020; Buonsenso et al., 2020; Costa et al., 2020; Grob et al., 2020; Kirtsman et al., 2020; Tam et al., 2020; Wu et al., 2020), suggesting the possibility of vertical transmission. However, the presence of ACE2 in mammary tissue and its regulation during pregnancy has not been investigated. Because interferons (IFNs) can activate ACE2 expression in pneumocytes (Ziegler et al., 2020), the question arose whether other cytokines can regulate ACE2 in mammary cells through the JAK/STAT pathway. In addition, the widespread expression of JAK/STAT components and associated receptors, their overlapping activities, and potential redundancy might affect ACE2 expression in a range of epithelial cell types. Based on the presence of SARS-CoV-2 RNA in milk, we investigated the presence and regulation of Ace2 in mammary tissue throughout pregnancy and lactation in mice. Specifically, we asked whether the hormonal milieu results in the activation of enhancer structures that induce Ace2 expression and the genetic role of the transcription factor STAT5 during pregnancy and lactation.
Ace2 expression in mammary tissue during pregnancy

Expression of the ACE2 gene in type II pneumocytes is activated by IFNs (Ziegler et al., 2020), opening the possibility that the cytokine storm in COVID-19 patients, and peptide hormones in general, might lead to increased levels of ACE2 in a range of putative SARS-CoV-2 target tissues. This in turn could result in elevated viral load and subsequent tissue damage. To explore the possibility that Ace2 gene expression is regulated by cytokines through the family of STAT transcription factors, we initially mined scRNA-seq data (Ziegler et al., 2020). The abundant presence of interferon receptor (IFNAR) and its downstream mediators JAK1, JAK2, and TYK2, as well as STAT1, STAT3, and STAT5, supports a pivotal contribution of the JAK/STAT pathway in the activation of ACE2 by IFN-α/β and IFN-γ. STAT1 levels are increased sharply in cells treated with IFNs, supporting the notion that an autoregulatory loop (Yuasa and Hiji-kata, 2016) is needed to activate IFN target genes.

Ace2 expression in mammary tissue during pregnancy

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The presence of a range of cytokine receptors and JAK/STAT components suggests that ACE2 might be activated by a selection of cytokines including prolactin, which controls mammary development and other physiological parameters during pregnancy and lactation. In a first step, we determined whether Ace2 expression in lung, kidney, and intestine, well-established SARS-CoV-2 target tissues, is regulated during pregnancy and lactation in female mice (Figure 1A). Although Ace2 levels in kidney and intestine were equivalent between non-parous and day 10 of lactation (L10) mice, a 2-fold increase was observed in lung tissue, which harbors the prolactin receptor and all necessary downstream signaling components. Following up on reports of SARS-CoV-2 RNA in milk, we explored Ace2 expression in mammary tissue. Ace2 mRNA was present in mammary tissue, and an approximately 13-fold increase was observed during lactation (Figure 1A). Gene expression in mammary tissue during pregnancy and lactation is activated by prolactin through STAT5 (Liu et al., 1997), and increased expression of Stat5a during lactation is the result of an autoregulatory enhancer (Metszer et al., 2016). Next, we mined RNA sequencing (RNA-seq) data from our lab and demonstrated increased Ace2 expression throughout pregnancy and lactation (Figure 1B) with a pattern similar to that of other prolactin-regulation genes (Lee et al., 2018; Yamaji et al., 2013). In concordance, ACE2 protein levels increased during pregnancy and lactation (Figure 1C). In contrast, expression of the ACE2-associated serine protease Tmprss2 and the novel putative SARS-CoV-2 receptor neuropilin-1 (Nrp1) was not further induced, suggesting that they are not under overt control of the JAK/STAT pathway. Surfactant protein D (SFTPD) is a secreted protein expressed in both lung tissue and mammary tissue, and its gene is induced during pregnancy and lactation (Figure 1B). Expression of Stat5a, a key driver of prolactin signaling in mammary tissue, increases during pregnancy and lactation (Figure 1D).

Activation of STAT5 Enhancers

The Ace2 expression pattern during pregnancy and lactation mirrored that of mammary-specific prolactin-regulated genes, suggesting a key role of STAT5 in its regulation. To explore this further, we dug deeper and analyzed chromatin immunoprecipitation sequencing (ChIP-seq) profiles aimed at identifying mammary regulatory elements at L10 (Figure 2). ChIP-seq for histone H3 lysine 4 monomethylation (H3K4me1) and histone H3 lysine 27 acetylation (H3K27ac) suggested the presence of several enhancers in the extended locus (Figure 2A), with two putative intronic enhancers in the Ace2 gene (Figure 2B). STAT5 binding coincided with two GAS motifs, which constitute bona fide STAT binding sites. STAT5 binding was at sequences void of H3K27ac marks, suggesting direct transcription factor binding to histone-free areas. In addition to STAT5, co-occupancy of several other transcription factors, including the glucocorticoid receptor (GR), nuclear factor 1 B (NFIB) and mediator complex subunit 1 (MED1), was observed (Figure 2B). Because of the absence of bona fide binding motifs for these factors, we propose that they bind by contacting STAT5, rather than binding independently to DNA. RNA polymerase II (RNA Pol II) occupancy supports the validity of this regulatory region. No STAT3 occupancy was observed, suggesting a predominance of STAT5.

To directly link the activation of the intronic enhancer to increased Ace2 expression, we analyzed ChIP-seq datasets throughout pregnancy, lactation, and involution (Figure 3). Although signs of enhancers were detected at day 6 of pregnancy (p6), complete occupation with transcription factors and activation occurred only at L10 (Figure 3A). Upon cessation of lactation, the enhancers were decommissioned within 24 h of involution (I24), similar to what was observed with other pregnancy-regulated genes (Willi et al., 2016). Notably, coinciding with the loss of STAT5 occupancy at the enhancers, limited STAT3 binding was observed, similar to that of other mammary genes (Willi et al., 2016). The Stat1 enhancer served as a control (Figure 3B).

Ace2 Is under Direct STAT5 Control

Although the ChIP-seq experiments strongly suggest that Ace2 expression is under the control of STAT5, experimental genetics is required to prove a direct relationship. To directly address the contribution of STAT5, we analyzed mammary tissue from two lines of Stat5 mutant mice (Yamaji et al., 2013). One line lacked the two Stat5a alleles (Stat5a<sup>−/−</sup>; Stat5b<sup>+/−</sup>), and the other lacked both Stat5 genes. In lactating mammary tissues, Stat5a accounts for at least 75% of total STAT5 (Yamaji et al., 2013). In the absence of Stat5a, Ace2 expression at day 1 of lactation (L1) was reduced by 83% (Figure 4A). A similar reduction was observed in the absence of both Stat5 genes, suggesting an insignificant contribution of STAT5b.

ChIP-seq experiments validated the absence of STAT5a binding in mice lacking both Stat5a alleles (Figure 4B). In addition, STAT5b binding was impaired in these mutant mice, suggesting that the presence of Stat5a is required for Stat5b binding or that a specific threshold of STAT5 is needed. The Cish gene served as a control for STAT5b binding in mutant mice (Figure 4B).

DISCUSSION

Our study directly demonstrates that the Ace2 gene is expressed in mammary tissue and activated during pregnancy and lactation through intronic enhancers built on the transcription factor STAT5. Our findings built a framework needed to assess and understand the contributions of a range of cytokines faced under various physiological conditions in extrapulmonary manifestations of COVID-19 (Gupta et al., 2020). The hormonal milieu associated with pregnancy and lactation is unique, and high levels of prolactin, a cytokine that activates the pan-JAK/STAT signaling pathway, control hundreds of target genes. Although these target genes have been well characterized in mammary
tissue (Yamaji et al., 2013), less is known about them in other cell types. Because cytokine receptors and downstream JAK/STAT components are present in a range of cell types, it can be predicted that pregnancy hormones significantly, and possibly cell-preferentially, affect genetic programs. Our finding that Ace2 expression was increased in lung tissue during lactation is significant, because it might place women during pregnancy and lactation at a higher risk. A retrospective study would be warranted.

Although SARS-CoV-2 has been detected in breast milk in at least seven studies and our research has demonstrated that its receptor ACE2 is present in mammary tissue and highly induced during lactation, the impact of these findings on COVID-19 requires further investigation. Interrogating the mechanism of Ace2 regulation observed in mammary tissue during pregnancy through the use of cell lines will likely be futile, because cell lines do not mimic the complexity of functional mammary tissue. The use of primary tissue and organoids from pregnant women cannot be pursued. Vertical transmission of SARS-CoV-2 through breast feeding (Lackey et al., 2020) would depend on the infectivity of the virus in milk and upon passing through the gastrointestinal tract. Because human colonic organoids and gut enterocytes can be productively infected \textit{in vitro} (Lamers et al., 2020; Stanifer et al., 2020), the transmission of milk-borne SARS-CoV-2 to the infant needs further examination. Clearly, other viruses are vertically transmitted through milk (Reid et al., 1984).

Based on a pneumocyte study (Ziegler et al., 2020) and our results, we predict that cytokines regulate ACE2 levels in a range of SARS-CoV-2 target cells by drawing on JAKs and STAT transcription factors. Although the impact on virus-induced non-pulmonary pathology remains to be determined, the effectiveness of JAK/STAT pathway inhibitors in mitigating ACE2 levels needs to be evaluated. Interfering with individual STATs results in compensational recruitment of other STAT members to cytokine receptors (Cui et al., 2007), with all its transcriptional consequences (Hennighausen and Robinson, 2008). In contrast, JAK inhibitors could prove effective. They are used to suppress cytokine storms induced by the pan-JAK/STAT pathway, and inflammation in COVID-19 patients treated with the JAK1/2 inhibitor ruxolitinib is reduced (La Rosée et al., 2020). Similarly, baricitinib, which inhibits the proinflammatory signal of several cytokines by suppressing JAK1/JAK2, has a beneficial impact in COVID-19 patients (Cantini et al., 2020).

Future investigations aimed at understanding the mechanism of ACE2 gene regulation in SARS-CoV-2 target tissues, such as kidney and intestine, need to address the range of cytokines and all components of the pan-JAK-STAT pathway. The presence of JAK/STAT components and their respective receptors is likely not sufficient to activate Ace2 expression, as shown in kidney and intestine of this study, and additional cell-specific transcription factors and receptors might be required. Candidates are mammary-enriched transcription factors NFIB, ELF5, and GR. Based on our study and previous data (Ziegler et al., 2020) it is likely that IFNs and prolactin can activate Ace2 expression through STAT1 and STAT5. By including steroid hormones and males and females of different ages in future studies, insight into the sex differences seen in COVID-19 morbidity and mortality (Galbadage et al., 2020) might emerge.

### STAR METHODS

Detailed methods are provided in the online version of this paper and include the following:

- **KEY RESOURCES TABLE**
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  - Data and Code Availability
- **EXPERIMENTAL MODEL AND SUBJECT DETAILS**
  - Animals
- **METHOD DETAILS**
  - Chromatin immunoprecipitation sequencing (ChIP-seq) analysis
  - RNA-seq analysis

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**Figure 4. STAT5 Is Required for Ace2 Expression and Enhancer Activation in Mammary Epithelium**

(A) Ace2 mRNA levels in mouse mammary tissue from wild-type and Stat5 mutant mice were measured by RNA-seq (Yamaji et al., 2013). One-way ANOVA was used to evaluate the statistical significance of differences in WT and mutants. ***p < 0.0001.

(B) STAT5 enhancers were profiled using ChIP-seq data from wild-type and Stat5 mutant tissue.

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Western blot
- RNA isolation and quantitative real-time PCR (qRT-PCR)
- QUANTIFICATION AND STATISTICAL ANALYSIS

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AUTHOR CONTRIBUTIONS

H.K.L. and L.H. designed the study. H.K.L. performed experiments, data analysis, and computational analysis. H.K.L. and L.H. supervised the study and wrote the manuscript. Both authors approved the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

REFERENCES

Anders, S., Pyl, P.T., and Huber, W. (2015). HTSeq—a Python framework to work with high-throughput sequencing data. Bioinformatics 31, 166–169.

Bastug, A., Hanifehnezhad, A., Tayman, C., Ozkul, A., Ozbay, O., Kazancioglu, S., and Bodur, H. (2020). Virolactia in an Asymptomatic Mother with COVID-19. Breastfeed Med. 31, 2114–2120.

Bolger, A.M., Lohse, M., and Usadel, B. (2014). Trimmomatic: a flexible trimmer for Illumina sequencing data. Bioinformatics 30, 419–420.

Brann, D.H., Tsukahara, T., Weinreb, C., Lipovsek, M., Van den Berge, K., Brann, D.H., Tsukahara, T., Weinreb, C., Lipovsek, M., Van den Berge, K., Gong, B., Chance, R., Macaulay, I.C., Chou, H.-J., Fletcher, R.B., et al. (2020). Non-neuronal expression of SARS-CoV-2 entry genes in the olfactory system suggests mechanisms underlying COVID-19-associated anosmia. Sci. Adv. 6, eabc5801.

Buonsenso, D., Costa, S., Sanguinetti, M., Cattani, P., Posteraro, B., Marchetti, T., Carducci, B., Lanzone, A., Tamburini, E., Vento, G., et al. (2020). Neonatal Late Onset Infection with Severe Acute Respiratory Syndrome Coronavirus 2. Am. J. Perinatol. 37, 869–872.

Cantini, F., Niccoli, L., Nannini, C., Matarese, D., Natale, M.E.D., Lotti, P., Aquilini, D., Landini, G., Cimolato, B., Pietro, M.A.D., et al. (2020). Beneficial impact of Baricitinib in COVID-19 moderate pneumonia; multicentre study. Gastroenterology 159, R25.

Costa, S., Posteraro, B., Marchetti, S., Tamburini, E., Carducci, B., Lanzone, A., Valentini, P., Buonsenso, D., Sanguinetti, M., Vento, G., and Cattani, P. (2020). Excretion of SARS-CoV-2 in human breast milk. Clin. Microbiol. Infect. Published online June 2, 2020. https://doi.org/10.1016/j.cmi.2020.06.052.

Cui, Y., Hosui, A., Sun, R., Shen, K., Gavrilova, O., Chen, W., Cam, M.C., Gao, B., Robinson, G.W., and Hennighausen, L. (2007). Loss of signal transducer and activator of transcription 5 leads to hepatosteatosis and impaired liver regeneration. Hepatology 46, 504–513.

Dobin, A., Davis, C.A., Schlesinger, F., Drenkow, J., Zaleski, C., Jha, S., Batut, P., Chaisson, M., and Gingeras, T.R. (2013). STAR: ultrafast universal RNA-seq aligner. Bioinformatics 29, 15–21.

Galbadage, T., Peterson, B.M., Awada, J., Buck, A.S., Ramirez, D.A., Wilson, J., and Gunasekera, R.S. (2020). Systematic Review and Meta-Analysis of Sex-Specific COVID-19 Clinical Outcomes. Front. Med. (Lausanne) 7, 348.

Groß, R., Conzelmann, C., Müller, J.A., Stenger, S., Steinhart, K., Kirchhoff, F., and Münch, J. (2020). Detection of SARS-CoV-2 in human breastmilk. Lancet 395, 1757–1758.

Gupta, A., Madhavan, M.V., Seghal, K., Nair, N., Mahajan, S., Sehrawat, T.S., Bikkeli, B., Aihuwalia, N., Ausiello, J.C., Wan, E.Y., et al. (2020). Extrapolatory manifestations of COVID-19. Nat. Med. 26, 1017–1032.

Heinz, S., Benner, C., Spahn, N., Bertolino, E., Lin, Y.C., Laslo, P., Cheng, J.X., Murre, C., Singh, H., and Glass, C.K. (2010). Simple combinations of lineage-determining transcription factors prime cis-regulatory elements required for macrophage and B cell identities. Mol. Cell 38, 576–589.

Hennighausen, L., and Robinson, G.W. (2008). Interpretation of cytokine signaling through the transcription factors STAT5A and STAT5B. Genes Dev. 22, 711–721.

Hoffmann, M., Kleine-Weber, H., Schroeder, S., Krüger, N., Herrler, T., Erichsen, S., Schiergens, T.S., Herrler, G., Wu, N.-H., Nitsche, A., et al. (2020). SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. Cell 181, 271–280.

Huber, W., Carey, V.J., Gentleman, R., Carlson, L., Carvalho, B.S., Bravo, H.C., Davis, S., Gatto, L., Girke, T., et al. (2015). Orchestrating high-throughput genomic analysis with Bioconductor. Nat. Methods 12, 115–121.

Imai, Y., Kuba, K., Rao, S., Huan, Y., Guo, F., Guan, B., Yang, P., Sarao, R., Wada, T., Leong-Poi, H., et al. (2005). Angiotensin-converting enzyme 2 protects from severe acute lung failure. Nature 436, 112–116.

Khalil, A., Kalafat, E., Benioglu, C., O’Brien, P., Morris, E., Draycott, T., Thangaratinam, S., Doare, K.L., Heath, P., Ladhani, S., et al. (2020). SARS-CoV-2 infection in pregnancy: A systematic review and meta-analysis of clinical features and pregnancy outcomes. EclinicalMedicine 25, 100446.

Kirtsman, M., Diambomba, Y., Poutanen, S.M., Malinowski, A.K., Vilachodimotropoulou, E., Parks, W.T., Erdman, L., Morris, S.K., and Shah, P.S. (2020). Probable congenital SARS-CoV-2 infection in a neonate born to a woman with active SARS-CoV-2 infection. CMAJ 192, E647–E650.

La Rosée, F., Bremer, H.C., Gehrke, I., Kehr, A., Hochhaus, A., Birndt, S., Fellhauer, M., Henkes, M., Krum, B., Russo, S.G., and La Rosée, P. (2020). The Janus kinase 1/2 inhibitor ruxolitinib in COVID-19 with severe systemic hyper-inflammation. Leukemia 34, 1805–1815.

Lackey, K.A., Pace, R.M., Williams, J.E., Bode, L., Donovan, S.M., Järvinen, K.M., Seppo, A.E., Raiten, D.J., Meehan, C.L., McGuire, M.A., and McGuire, M.K. (2020). SARS-CoV-2 and human milk: What is the evidence? Matern. Child Nutr. Published online May 30, 2020. https://doi.org/10.1111/mcn.13032.

Lamers, M.M., Beumer, J., van der Vaart, J., Knoops, K., Puschhof, J., Breugem, T.I., Ravelli, R.B.G., Paul van Schaayck, J., Mykytyn, A.Z., Duimel, H.O., et al. (2020). SARS-CoV-2 productively infects human gut enterocytes. Science 369, 50–54.

Langmead, B., Trapnell, C., Pop, M., and Salzberg, S.L. (2009). Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. Genome Biol. 10, R25.

Lee, H.K., Willi, M., Shin, H.Y., Liu, C., and Hennighausen, L. (2018). Progressing super-enhancer landscape during mammary differentiation controls tissue-specific gene regulation. Nucleic Acids Res. 46, 10796–10809.

Lee, J.J., Kopetz, S., Vilar, E., Shen, J.P., Chen, K., and Maitra, A. (2020). Relative Abundance of SARS-CoV-2 Entry Genes in the Enterocytes of the Lower Gastrointestinal Tract. Genes (Basel) 11, 645.

Liu, X., Robinson, G.W., Wagner, K.U., Garrett, L., Wynshaw-Boris, A., and Hennighausen, L. (1997). Stat5a is mandatory for adult mammary gland development and lactogenesis. Genes Dev. 11, 179–186.

Love, M.I., Huber, W., and Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol. 15, 550.

Lukassen, S., Chua, R.L., Trefzer, T., Kahn, N.C., Schneider, M.A., Muley, T., Winter, H., Meister, M., Veith, C., Boots, A.W., et al. (2020). SARS-CoV-2 receptor ACE2 and TMPRSS2 are primarily expressed in bronchial transient secretory cells. EMBO J. 39, e105114.
Metser, G., Shin, H.Y., Wang, C., Yoo, K.H., Oh, S., Villarino, A.V., O’Shea, J.J., Kang, K., and Hennighausen, L. (2016). An autoregulatory enhancer controls mammary-specific STAT5 functions. Nucleic Acids Res. 44, 1052–1063.

Monteil, V., Kwon, H., Prado, P., Hagelkruys, A., Wimmer, R.A., Stahl, M., Leopoldi, A., Garreta, E., Hurtado Del Pozo, C., Prosper, F., et al. (2020). Inhibition of SARS-CoV-2 Infections in Engineered Human Tissues Using Clinical-Grade Soluble Human ACE2. Cell 171, 905–913.

Qi, F., Qian, S., Zhang, S., and Zhang, Z. (2020). Single cell RNA sequencing of 13 human tissues identify cell types and receptors of human coronaviruses. Biochem. Biophys. Res. Commun. 526, 135–140.

Reid, H.W., Buxton, D., Pow, I., and Finlayson, J. (1984). Transmission of loping-ill virus in goat milk. Vet. Rec. 114, 163–165.

Risso, D., Ngai, J., Speed, T.P., and Dudoit, S. (2014). Normalization of RNA-seq data using factor analysis of control genes or samples. Nat. Biotechnol. 32, 896–902.

Stanifer, M.L., Kee, C., Cortese, M., Zumaran, C.M., Triana, S., Mukenheim, M., Kraeusslich, H.G., Alexandrov, T., Bartenschlager, R., and Boulant, S. (2020). Critical Role of Type III Interferon in Controlling SARS-CoV-2 Infection in Human Intestinal Epithelial Cells. Cell Rep. 32, 107863.

Tam, P.C.K., Ly, K.M., Kernich, M.L., Spurrier, N., Lawrence, D., Gordon, D.L., and Tucker, E.C. (2020). Detectable severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in human breast milk of a mildly symptomatic patient with coronavirus disease 2019 (COVID-19). Clin. Infect. Dis. Published online May 30, 2020. https://doi.org/10.1093/cid/ciaa673.

Thorvaldsdóttir, H., Robinson, J.T., and Mesirov, J.P. (2013). Integrative Genomics Viewer (IGV): high-performance genomics data visualization and exploration. Brief. Bioinform. 14, 178–192.

Willi, M., Yoo, K.H., Wang, C., Trajanoski, Z., and Hennighausen, L. (2016). Differential cytokine sensitivities of STAT5-dependent enhancers rely on Stat5 autoregulation. Nucleic Acids Res. 44, 10277–10291.

Yuasa, K., and Hijikata, T. (2016). Distal regulatory element of the STAT1 gene potentially mediates positive feedback control of STAT1 expression. Genes Cells 21, 25–40.

Zhao, Y., Zhao, Z., Wang, Y., Zhou, Y., Ma, Y., and Zuo, W. (2020). Single-cell RNA expression profiling of ACE2, the receptor of SARS-CoV-2. Am. J. Respir. Crit. Care Med. 202, 756–759.

Ziegler, C.G.K., Allon, S.J., Nyquist, S.K., Mbano, I.M., Miao, V.N., Tzouanas, C.N., Cao, Y., Yousif, A.S., Bals, J., Hauser, B.M., et al. (2020). SARS-CoV-2 Receptor ACE2 Is an Interferon-Stimulated Gene in Human Airway Epithelial Cells and Is Detected in Specific Cell Subsets across Tissues. Cell 171, 1016–1035.
# STAR METHODS

## KEY RESOURCES TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| **Antibodies**      |        |            |
| Rabbit polyclonal anti-ACE2 | Proteintech | Cat# 21115-1-AP; RRID: AB_10732845 |
| Rabbit monoclonal anti-GAPDH | Cell signaling | Cat# 5174; RRID: AB_10622025 |
| **Chemicals, Peptides, and Recombinant Proteins** |        |            |
| SsoAdvanced Universal Probes Supermix | Bio-rad | Cat# 172-5281 |
| **Critical Commercial Assays** |        |            |
| PureLink RNA Mini Kit | Invitrogen | Cat# 12183018A |
| SuperScript III First-Strand Synthesis SuperMix | Invitrogen | Cat# 18080-400 |
| **Deposited Data** |        |            |
| ChIP-seq and RNA-seq data | NCBI GEO dataset | GSE115370 |
| ChIP-seq data | NCBI GEO dataset | GSE121438 |
| ChIP-seq data and RNA-seq data | NCBI GEO dataset | GSE127193 |
| ChIP-seq data | NCBI GEO dataset | GSE40930 |
| ChIP-seq data | NCBI GEO dataset | GSE37646 |
| RNA-seq data | NCBI GEO dataset | GSE148829 |
| Mouse reference genome UCSC, mm10 | UCSC Genome Browser | http://hgdownload.soe.ucsc.edu/downloads.html#mouse |
| **Experimental Models: Organisms/Strains** |        |            |
| C57BL/6 mice | Charles River | N/A |
| **Oligonucleotides** |        |            |
| mouse Ace2 probe (Mm01159006_m1) | Thermo Fisher scientific | Cat# 4351370 |
| mouse Stat5a probe (Mm03053818_s1) | Thermo Fisher scientific | Cat# 4351370 |
| mouse Stat5b probe (Mm00839889_m1) | Thermo Fisher scientific | Cat# 4351370 |
| mouse Cish probe (Mm01230623_g1) | Thermo Fisher scientific | Cat# 4351370 |
| mouse Gapdh probe | Applied Biosystems | Cat# 4352339E |
| **Software and Algorithms** |        |            |
| Trimmomatic (version 0.36) | Bolger et al., 2014 | http://www.usadellab.org/cms/?page=trimmomatic |
| Bowtie (version 1.1.2) | Langmead et al., 2009 | http://bowtie-bio.sourceforge.net/manual.shtml |
| Picard | | http://broadinstitute.github.io/picard/ |
| Homer (version 4.8.2) | Heinz et al., 2010 | http://homer.ucsd.edu/homer/ |
| Integrative Genomics Viewer | Thorvaldsdóttir et al., 2013 | http://software.broadinstitute.org/software/igv/ |
| STAR (2.5.4a) | Dobin et al., 2013 | https://anaconda.org/bioconda/star/files?version=2.5.4a |
| HTSeq | Anders et al., 2015 | https://htseq.readthedocs.io/en/master/ |
| R (3.6.3) | https://www.R-project.org/ |
| Bioconductor | Huber et al., 2015 | https://www.bioconductor.org/ |
| DESeq2 | Love et al., 2014 | https://bioconductor.org/packages/release/bioc/html/DESeq2.html |
| RUVSeq | Risso et al., 2014 | https://bioconductor.org/packages/release/bioc/html/RUVSeq.html |

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RESOURCE AVAILABILITY

Lead Contact
Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact Lothar Hennighausen (lotharh@nih.gov).

Materials Availability
This study did not generate new unique reagents.

Data and Code Availability
RNA-seq data shown in Figures 1B and 1D and ChIP-seq data shown in Figures 2, 3, and 4 were generated in our lab and deposited in the Gene Expression Omnibus (GEO). ChIP-seq and RNA-seq data of mouse lactating tissue were obtained from GEO: GSE115370, GSE121438 and GSE127139. ChIP-seq and RNA-seq data from lactating tissue from \( \text{Stat5} \) mutant mice were obtained from GEO: GSE40930 and GSE37646. RNA-seq data of human bronchial cell line (BEAS-2B) and airway basal cells from human donors treated with IFNa2, IFNg, IL4 or IL17A were obtained from GEO: GSE148829.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Animals
All animals were housed and handled according to the Guide for the Care and Use of Laboratory Animals (8th edition) and all animal experiments were approved by the Animal Care and Use Committee (ACUC) of National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK, MD) and performed under the NIDDK animal protocol K089-LGP-17. Two-month-old C57BL/6 female mice (Charles River) were bred and the mammary gland tissue was harvested at days 6, 13 and 19 of pregnancy (p6, p13 and p18), at day 1 and 10 (L1 and L10) after parturition, and after 24 hours of involution (I24). For the I24 time point, pups were separated from lactating dams at day 10 of lactation and tissue was harvested after 24 hours.

METHOD DETAILS

Chromatin immunoprecipitation sequencing (ChIP-seq) analysis
Quality filtering and alignment of the raw reads was done using Trimmomatic (Bolger et al., 2014) (version 0.36) and Bowtie (Langmead et al., 2009) (version 1.1.2), with the parameter '-m 1' to keep only uniquely mapped reads, using the reference genome mm10. Picard tools (Broad Institute, Picard, http://broadinstitute.github.io/picard/, 2016) was used to remove duplicates and subsequently, Homer (Heinz et al., 2010) (version 4.8.2) software was applied to generate bedGraph files, seperately. Integrative Genomics Viewer (Thorvaldsdóttir et al., 2013) (version 2.3.81) was used for visualization.

RNA-seq analysis
mRNA-seq read quality control was done using Trimmomatic (Bolger et al., 2014) (version 0.36) and STAR RNA-seq (Dobin et al., 2013) (version STAR 2.5.4a) using 50bp paired-end mode was used to align the reads (mm10). HTSeq (Anders et al., 2015) was to retrieve the raw counts and subsequently, R (https://www.R-project.org/), Bioconductor (Huber et al., 2015) and DESeq2 (Love et al., 2014) were used. Additionally, the RUVSeq (Risso et al., 2014) package was applied to remove confounding factors. The data were pre-filtered keeping only those genes, which have at least ten reads in total. Genes were categorized as significantly differentially expressed with an adjusted p value (pAdj) below 0.05 and a fold change > 2 for upregulated genes and a fold change of < 0.5 for downregulated ones. The visualization was done using dplyr (https://cran.r-project.org/web/packages/dplyr/index.html) and ggplot2 (Wickham, 2009).

Western blot
Proteins (100 µg) from mouse mammary tissues were extracted with lysis buffer (50 mM Tris-Cl pH 8.0, 150 mM NaCl, 0.5% Na-DOC, 1% NP-40, 0.1% SDS, 5 mM EDTA, 1 mM PMSF, and protease inhibitor cocktail), separated on a 4%–12% NuPage gradient gel...
(Invitrogen) and transferred to a PVDF membrane (Invitrogen). Membranes were blocked for 1 h with 5% nonfat dry milk in PBS-T buffer (PBS containing 0.1% Tween 20) and incubated for 1.5 hr at 4°C with the primary antibody against ACE2 (Proteintech, 21115-1-AP) and GAPDH (Cell signaling, #5174). After washing, membranes were incubated for 1 h with HRP-conjugated secondary antibodies (Cell signaling). Labeled protein bands were detected using an enhanced chemiluminescence system (Thermo scientific) and Amersham Imager 600 (GE healthcare). Band density was analyzed using this imager.

RNA isolation and quantitative real-time PCR (qRT–PCR)
Mammary tissues were harvested from non-parous and day 10 of lactating female mice and homogenized using an electronic homogenizer. RNA was extracted using the PureLink RNA Mini Kit (Invitrogen) according to the manufacturer’s instructions. cDNA was synthesized from total RNA using Superscript II (Invitrogen). Quantitative real-time PCR (qRT-PCR) was performed using TaqMan probes (Ace2, Mm01159006; Stat5a, Mm03053818; Stat5b, Mm00839889; Cish, Mm01230623, Thermo Fisher scientific; mouse Gapdh, 4352339E, Applied Biosystems) on the CFX384 Real-Time PCR Detection System (Bio-Rad) according to the manufacturer’s instructions. PCR conditions were 95°C for 30 s, 95°C for 15 s, and 60°C for 30 s for 40 cycles. All reactions were done in triplicate and normalized to the housekeeping gene Gapdh. Relative differences in PCR results were calculated using the comparative cycle threshold (C_{T}) method.

QUANTIFICATION AND STATISTICAL ANALYSIS
For comparison of samples, data were presented as standard deviation in each group and were evaluated with a t test and ANOVA multiple comparisons using PRISM GraphPad. Statistical significance was obtained by comparing the measures from wild-type or control group, and each mutant group. A value of *p < 0.05, **p < 0.001, ***p < 0.0001, ****p < 0.00001 was considered statistically significant.