VIRAL IMMUNITY

An interferon-independent lncRNA promotes viral replication by modulating cellular metabolism

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Viruses regulate host metabolic networks to improve their survival. The molecules that are responsive to viral infection and regulate such metabolic changes are hardly known, but are essential for understanding viral infection. Here we identify a long noncoding RNA (lncRNA) that is induced by multiple viruses, but not by type I interferon (IFN-I), and facilitates viral replication in mouse and human cells. In vivo deficiency of lncRNA-ACOD1 (a lncRNA identified by its nearest coding gene *Acod1*, aconitate decarboxylase 1) significantly attenuates viral infection through IFN-I–IRF3 (interferon regulatory factor 3)–independent pathways. Cytoplasmic lncRNA-ACOD1 directly binds the metabolic enzyme glutamic-oxaloacetic transaminase (GOT2) near the substrate niche, enhancing its catalytic activity. Recombinant GOT2 protein and its metabolites could rescue viral replication upon lncRNA-ACOD1 deficiency and increase lethality. This work reveals a feedback mechanism of virus-induced lncRNA-mediated metabolic promotion of viral infection and a potential target for developing broad-acting antiviral therapeutics.

Metabolism is the source of energy and materials for all biological processes. The productive viral infection requires changes to host cellular metabolic networks to obtain the building blocks for the viral life cycle (*1*, *2*). Positive-strand RNA viruses and enveloped viruses rewire lipid metabolism for replication (*3*, *4*), and hepatitis C virus (HCV) triggers tumorlike glutamine metabolism in hepatocytes (*5*). Although some host- and virus-encoded microRNAs have been shown to partly mediate these alterations of metabolic pathways (*6*), the molecular mechanisms involved are still largely elusive—in particular, how viruses target and regulate the host metabolic network for replication and escape from host defenses.

Thousands of long noncoding RNAs (lncRNAs) have been identified in mammalian genomes (*7*), of which only a minority, but increasingly more, have been functionally characterized in different processes and diseases, including infection (*8*, *9*) and innate and adaptive immunity (*10*–*12*). Interferon, especially type I interferon (IFN-I), are vital cytokines that control viral infection through activation of interferon-stimulated genes (*13*, *14*). Although some host lncRNAs have been reported to regulate expression of these genes or be regulated by interferon in viral infection (*15*, *16*), our knowledge about the function of lncRNAs in infected cells is still very limited, especially in regard to the mechanism by which viruses hijack host metabolism for replication. Currently, reported patterns of lncRNA function are mainly focused on regulating gene expression in the nucleus (*17*, *18*) and influencing signaling transduction or absorbing microRNAs in the cytoplasm (*19*–*21*). However, the mode of action of lncRNAs remains to be fully understood. Whether there are other unknown mechanisms of lncRNA activity needs to be further explored.

To investigate the role of lncRNAs in viral infection and their relationship with the IFN-I

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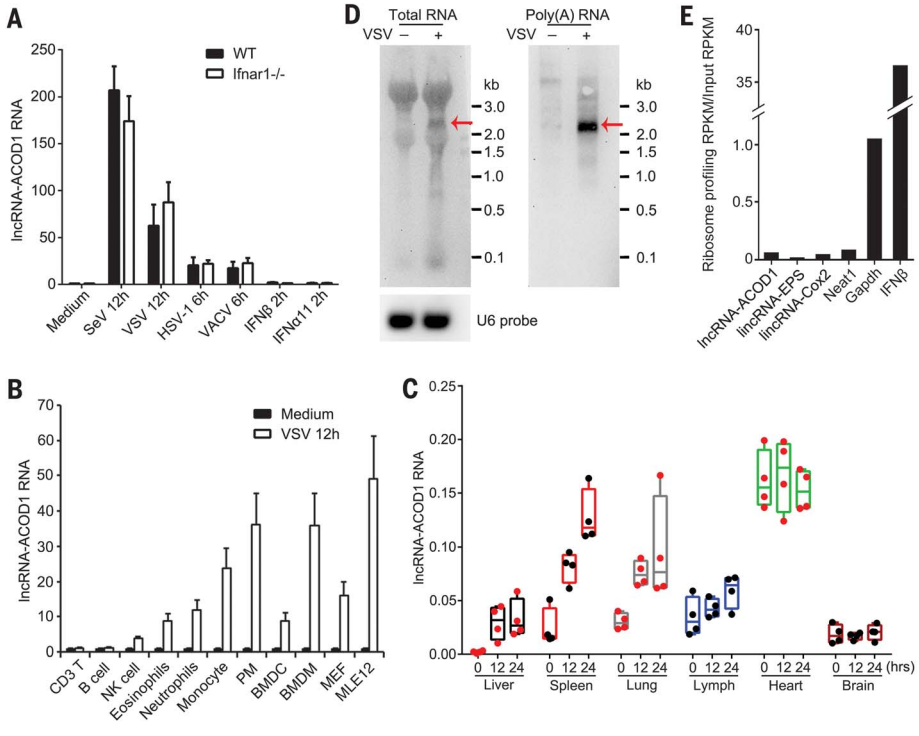

Fig. 1. Identification and characterization of viral infection–induced IFN-I–independent lncRNA-ACOD1. (**A**) Quantitative real-time fluorescence PCR (QPCR) analysis of lncRNA-ACOD1 in *Ifnar1*$^{-/-}$ or WT peritoneal macrophages infected with the indicated virus or stimulated with IFN subtypes for the indicated times (SeV, Sendai virus). Data are normalized to hypoxanthine-guanine phosphoribosyltransferase (HPRT) expression levels, and the WT medium group is set to a value of 1. Unless noted otherwise, the error bars in this and all other panels indicate SD. $n = 3$. (**B**) QPCR analysis of lncRNA-ACOD1 in indicated primary cells or cell lines infected with or without VSV for 12 hours. Data are normalized to HPRT and the medium group of each cell is set to a value of 1. $n = 3$. NK, natural killer; PM, mouse peritoneal macrophages; BMDC, bone marrow–derived dendritic cell; BMDM, bone marrow–derived macrophages; MEF, mouse embryonic fibroblast; MLE12, mouse lung epithelial cell line. (**C**) QPCR analysis of lncRNA-ACOD1 in organs from mice intraperitoneally injected with VSV [1×10^7 plaque-forming units (PFU)/g)] for the indicated time. RNA expression levels are normalized to HPRT expression for each sample. $n = 4$. (**D**) Northern blotting of lncRNA-ACOD1 with total RNA or poly(A)$^+$ RNA from macrophages infected with or without VSV for 12 hours. *U6* RNA serves as a loading control. These data were representative of at least three independent experiments. kb, kilobase. Red arrows indicate lncRNA-ACOD1. (**E**) Ratio of reads per kilobase million (RPKM) values from ribosomal profiling and input-RNA profiling of each indicated gene in peritoneal macrophages stimulated with VSV for 12 hours. lincRNA-EPS, lincRNA-Cox2, and *Neat1* were used as noncoding RNA control. *Ifnb* and *Gapdh* (glyceraldehyde-3-phosphate dehydrogenase) were used as coding RNA control.

system, we profiled lncRNA expression in wild-type (WT) and IFN-I receptor–deficient (*Ifnar*$^{-/-}$) macrophages with or without vesicular stomatitis virus (VSV) infection. Interestingly, we identified a group of viral-induced, IFN-I–independent lncRNAs: virus infection induced their expression regardless of IFN-I–receptor deficiency, whereas IFN-I stimulation alone could not induce their expression (fig. S1A). By performing RNA interference (RNAi)–mediated functional screening of these IFN-I–independent lncRNAs, we found that virus titer in macrophages was significantly downregulated by knockdown of an intergenic lncRNA (gene symbol LOC102637961) (fig. S1B), whose nearest coding gene is *Acod1* (aconitate decarboxylase 1), named as lncRNA-ACOD1 hereafter. We confirmed that lncRNA-ACOD1 could be stimulated by many types of viruses independent of IFN-I (Fig. 1A), in contrast to IFN-I–dependent lncRNA LOC625033 (fig. S1C). Furthermore, lncRNA-ACOD1 is induced by viral infection in most of the cells and organs studied (Fig. 1, B and C). Expression of lncRNA-ACOD1 with a polyadenylate [poly(A)] tail was confirmed by Northern blot (Fig. 1D). Rapid amplification of cDNA ends and RNA-sequencing data revealed that lncRNA-ACOD1 has three exons and two transcript variants (2330 and 2259 nucleotides, respectively) (fig. S2, A to C). lncRNA-ACOD1 has a moderate expression level of about 100 transcript copies per cell (fig. S2D), and our ribosomal profiling data revealed that it has very low ribosome occupancy (Fig. 1E), suggesting that lncRNA-ACOD1 lacks coding potential.

The transcription factor interferon regulatory factor 3 (IRF3) plays a vital role in IFN-I production and function in viral infection (*22*). Using IRF3-deficient (*Irf3*$^{-/-}$) or *Ifnar1*$^{-/-}$ cells and mice, we found that lncRNA-ACOD1 expression was independent of IFN-I–IRF3 signaling (fig. S3). However, our pharmacological inhibitor assay revealed that lncRNA-ACOD1 expression was largely dependent on nuclear factor κB (NF-κB) (fig. S4A), a transcription factor complex involved in many types of cellular processes, including cellular metabolism and inflammation (*23*). Accordingly, we identified three binding sites for subunit RelA of the NF-κB complex in the lncRNA-ACOD1 promoter sequence. Then, the promoter regions either with or without a RelA-motif mutation were cloned upstream of the firefly luciferase coding region (fig. S4, B and C, left). Luciferase expression was high only when WT RelA motifs were present and could be further enhanced by RelA overexpression (fig. S4, B and C, right), suggesting that NF-κB, but not the IFN-I–IRF3 pathway, participates in the induction of lncRNA-ACOD1.

Next, we investigated the role of lncRNA-ACOD1 in viral infection. We found that knockdown of lncRNA-ACOD1 (Fig. 2A) significantly reduced

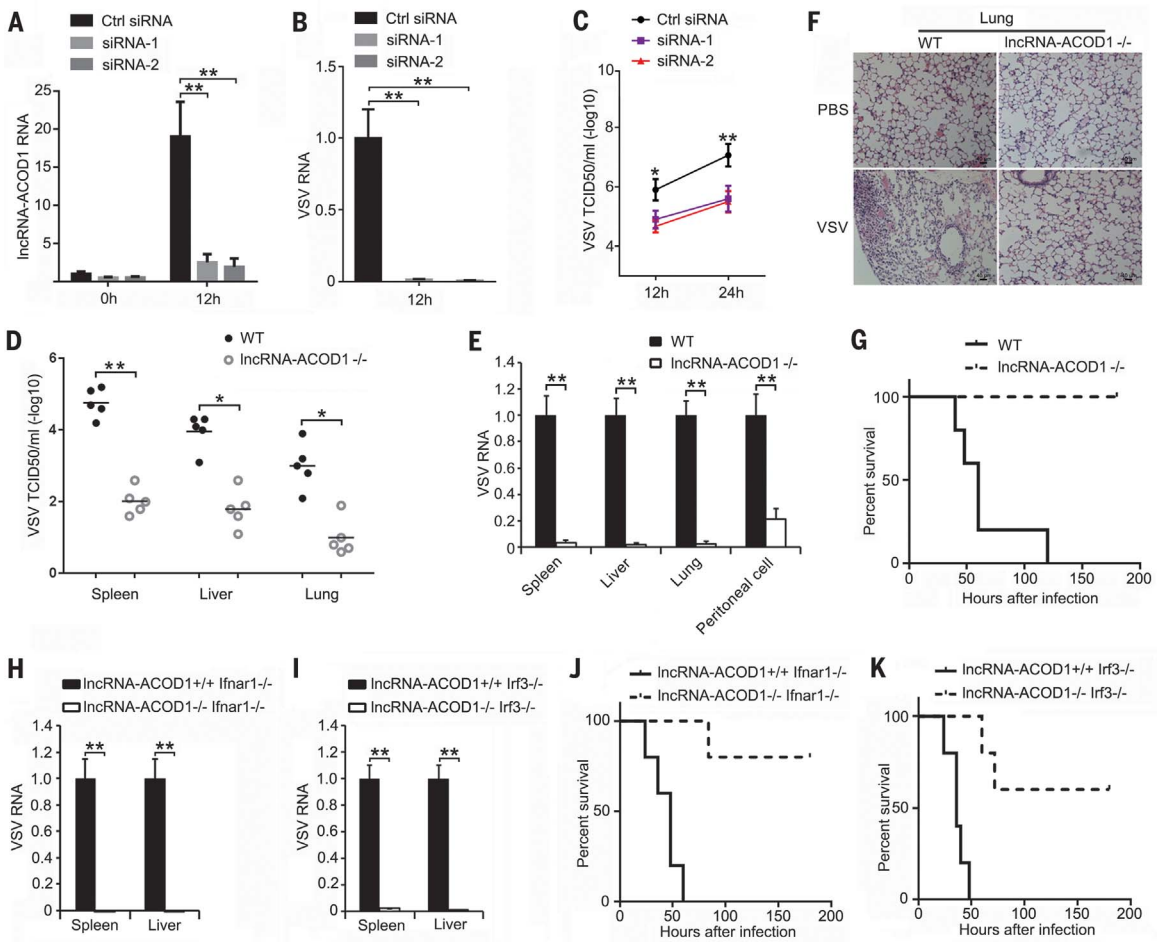

Fig. 2. lncRNA-ACOD1 promotes viral infection in vitro and in vivo through an IFN-I–IRF3–independent pathway. (**A** and **B**) QPCR analysis of lncRNA-ACOD1 levels (A) and VSV RNA levels (B) in peritoneal macrophages transfected with lncRNA-ACOD1 small interfering RNA (siRNA) followed 24 hours later with or without VSV infection for 12 hours. Data are normalized to HPRT and the control (Ctrl) siRNA group is set to a value of 1. $n = 3$. (**C**) Determination of VSV titers in culture medium supernatants of macrophages transfected and infected as in (A) for the indicated time by median tissue culture infectious dose ($TCID_{50}$) assay. $n = 3$. (**D**) Determination of VSV loads in organ homogenate supernatants by $TCID_{50}$ assay 36 hours after *lncRNA-ACOD1*$^{-/-}$ and WT mice were intraperitoneally injected with VSV (1×10^7 PFU/g). $n = 5$. (**E**) QPCR analysis of VSV RNA expression in organs from mice as in (D). Data are normalized to HPRT, and WT group is set to a value of 1. $n = 3$. (**F**) Hematoxylin and eosin staining of lung sections from mice in (D). PBS, phosphate-buffered saline; scale bars, 40 μm. (**G**) Survival of 7-week-old *lncRNA-ACOD1*$^{-/-}$ and WT mice intraperitoneally injected with VSV (1×10^7 PFU/g). $n = 5$. Log-rank (Mantel-Cox) test, $P < 0.01$. (**H** and **I**) QPCR analysis of VSV RNA levels in organs from *lncRNA-ACOD1*$^{-/-}$*Ifnar*$^{-/-}$ and *lncRNA-ACOD1*$^{+/+}$*Ifnar*$^{-/-}$ mice (H) or *lncRNA-ACOD1*$^{-/-}$*Irf3*$^{-/-}$ and *lncRNA-ACOD1*$^{+/+}$*Irf3*$^{-/-}$ mice (I) 36 hours after intraperitoneal injection with VSV (1×10^6 PFU/g). Data are normalized to HPRT, and the *lncRNA-ACOD1*$^{+/+}$ group is set to a value of 1. $n = 3$. (**J**) Survival of 7-week-old *lncRNA-ACOD1*$^{-/-}$*Ifnar*$^{-/-}$ and *lncRNA-ACOD1*$^{+/+}$*Ifnar*$^{-/-}$ mice intraperitoneally injected with VSV (1×10^6 PFU/g). $n = 5$. Log-rank (Mantel-Cox) test, $P < 0.01$. (**K**) Survival of 7-week-old *lncRNA-ACOD1*$^{-/-}$*Irf3*$^{-/-}$ and *lncRNA-ACOD1*$^{+/+}$*Irf3*$^{-/-}$ mice treated as in (J). Log-rank (Mantel-Cox) test, $P < 0.01$. *$P < 0.05$ and **$P < 0.01$ (two-tailed Student's *t* test).

VSV burden in macrophages (Fig. 2, B and C). This decrease in viral load was not due to a heightened host innate immune response, as IFN-I and interleukin 6 production and effector-gene expression levels were also decreased by knockdown of lncRNA-ACOD1 (fig. S5). Similar reductions of viral load and innate response were also observed in two other virus infections—DNA virus herpes simplex virus type 1 (HSV-1) and vaccinia virus (VACV) (fig. S6)—indicating that lncRNA-ACOD1 promotes viral infection through a general mechanism. We performed short-term infection assays when viral entry had just completed, but before replication had started, and showed that lncRNA-ACOD1 had no effect on viral entry (fig. S7A). Then, we used ultraviolet (UV)–irradiated VSV (UV-VSV), which can infect cells but not proliferate, and poly(I:C), which mimics virus RNA, to stimulate cells. We found that lncRNA-ACOD1 had no effect on UV-VSV and poly(I:C) responses (fig. S7, B and C), indicating that lncRNA-ACOD1 acts at the stage of viral replication in host cells. In addition, we used CRISPR-Cas9 (*24*) to knock out lncRNA-ACOD1 in the macrophage cell line RAW264.7 and obtained similar results (fig. S8), further confirming that lncRNA-ACOD1 is vital to viral replication.

To explore the role of lncRNA-ACOD1 in vivo, we constructed lncRNA-ACOD1 knockout (*lncRNA-ACOD1*$^{-/-}$) mice (fig. S9, A and B). We found that VSV replication in vivo was significantly reduced in lncRNA-ACOD1–deficient mice (Fig. 2, D and E), along with attenuated pathological changes (Fig. 2F and fig. S9C) and decreased innate immune responses (fig. S9, D and E). Moreover, all lncRNA-ACOD1–deficient mice survived when challenged with a lethal dose of VSV infection, whereas most of the sibling controls died (Fig. 2G). We also used a viral intranasal infection model to test lncRNA-ACOD1 function in vivo and observed similar results (fig. S10). In humans, a lncRNA-ACOD1 ortholog was also upregulated in response to viral infection by influenza A/PR/8/34 virus, and its knockdown by RNA interference resulted in a lower viral load in A549 human alveolar basal epithelial cells (fig. S11), suggesting that the function of lncRNA-ACOD1 in promoting viral replication is conserved, at least in human and mouse.

Because lncRNA-ACOD1 expression is independent of the IFN-I–IRF3 axis, we analyzed whether lncRNA-ACOD1 function is also independent of the IFN-I–IRF3 axis in vivo. Therefore, we generated double-knockout mice by breeding lncRNA-ACOD1–deficient mice with *Ifnar1*$^{-/-}$ or *Irf3*$^{-/-}$ mice. We found that, in the absence of IFN-I signaling, deficiency of lncRNA-ACOD1 led to a dramatic suppression of VSV proliferation, along with alleviated immune responses (Fig. 2, H and I, and fig. S12, A and B) and significantly lower lethal rates (Fig. 2, J and K). Therefore, we propose that lncRNA-ACOD1 promotes viral replication, possibly through a previously unidentified mechanism rather than through the canonical IFN-I–IRF3 pathway.

To check if lncRNA-ACOD1 functions through apoptosis and autophagy, which are reported to be involved in viral clearance (*25*, *26*), we tested the effect of lncRNA-ACOD1 with flow cytometry and immunoblot assay and found that host-cell apoptosis and autophagy were not influenced by lncRNA-ACOD1 (fig. S13). We then examined whether lncRNA-ACOD1 acts in cis, affecting nearby gene expression (fig. S14A). We found that knockdown or deficiency of lncRNA-ACOD1 did not affect the expression of nearby genes (fig. S14, B and C) and vice versa (fig. S14D). We next checked the location of lncRNA-ACOD1 in cells. RNA fluorescence in situ hybridization and reverse transcription–polymerase chain reaction (RT-PCR) of nuclear and cytoplasmic fractions (fig. S15) suggested that lncRNA-ACOD1 is located in cytoplasm, indicating that it might interact with cytoplasmic molecules or proteins.

To gain clues into lncRNA-ACOD1 activity, we performed microarray transcriptome analysis and found that lncRNA-ACOD1 deficiency led to changes in the expression levels of many metabolism-related genes (fig. S16A). Pathway enrichment analysis with Gene-Cloud of Biotechnology Informa (GCBI) revealed that metabolic pathways were the ones most significantly affected by loss of lncRNA-ACOD1 (fig. S16, B and C), indicating the possible role of lncRNA-ACOD1 in metabolic regulation during viral infection. Therefore, we performed whole metabolomics surveys using liquid chromatography–tandem mass spectrometry (LC-MS/MS) and found that viral infection led to large metabolic changes in WT cells, which was abolished by lncRNA-ACOD1 deficiency, as shown by the cluster map of identified negative and positive ions of intermediates (fig. S17).

To search for lncRNA-ACOD1–interacting molecules in vivo, we purified endogenous lncRNA-ACOD1 complexes using modified chromatin isolation by RNA purification. After confirming that lncRNA-ACOD1, rather than *U6* RNA and *Actb* mRNA (which served as negative controls), was selectively retrieved (fig. S18), the lncRNA-ACOD1–associated proteins were separated by gel electrophoresis and identified by mass spectrometry (MS). Glutamic-oxaloacetic transaminase (GOT2), a key metabolic enzyme involved in many processes—including amino acid metabolism, long-chain fatty acid uptake, and the tricarboxylic acid cycle (*27*, *28*)—was identified as a lncRNA-ACOD1–binding protein, which was further confirmed by independent immunoblot assay (Fig. 3A). RNA immunoprecipitation (RIP) assays with a GOT2-specific antibody under native conditions

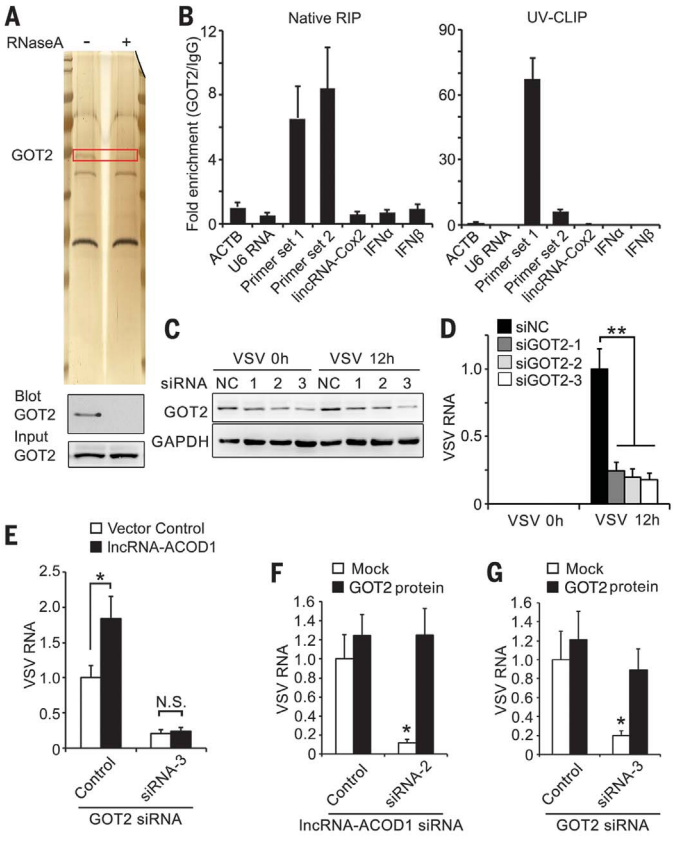

Fig. 3. lncRNA-ACOD1 directly binds GOT2 and functions through GOT2. (**A**) Specific bands retrieved by lncRNA-ACOD1 were identified by MS (top) or immunoblot of GOT2 (bottom). (**B**) QPCR detection of the indicated RNAs retrieved by GOT2-specific antibody compared with those retrieved by IgG in native RIP and UV-CLIP assays of peritoneal macrophages infected with VSV for 12 hours. $n = 3$. Primer sets 1 and 2 are two different QPCR primer sets of lncRNA-ACOD1. (**C** and **D**) Immunoblot detection of GOT2 (C) and QPCR analysis of VSV RNA levels (D) in peritoneal macrophages transfected with *Got2* siRNA followed 24 hours later with or without infection with VSV for 12 hours. $n = 3$. (**E**) QPCR analysis of VSV RNA levels in macrophages cotransfected with lncRNA-ACOD1 expression vector and *Got2* siRNA and infected 24 hours later with VSV for 12 hours. $n = 3$. (**F** and **G**) QPCR analysis of VSV RNA levels in macrophages transfected with lncRNA-ACOD1 siRNA (F) or *Got2* siRNA (G) followed 24 hours later with transfection with or without active recombinant GOT2 protein then infected with VSV for 12 hours. $n = 3$. (D) to (G) Data are normalized to HPRT, and the control group is set to a value of 1. $^*P < 0.05$ and $^{**}P < 0.01$ (two-tailed Student's t test).

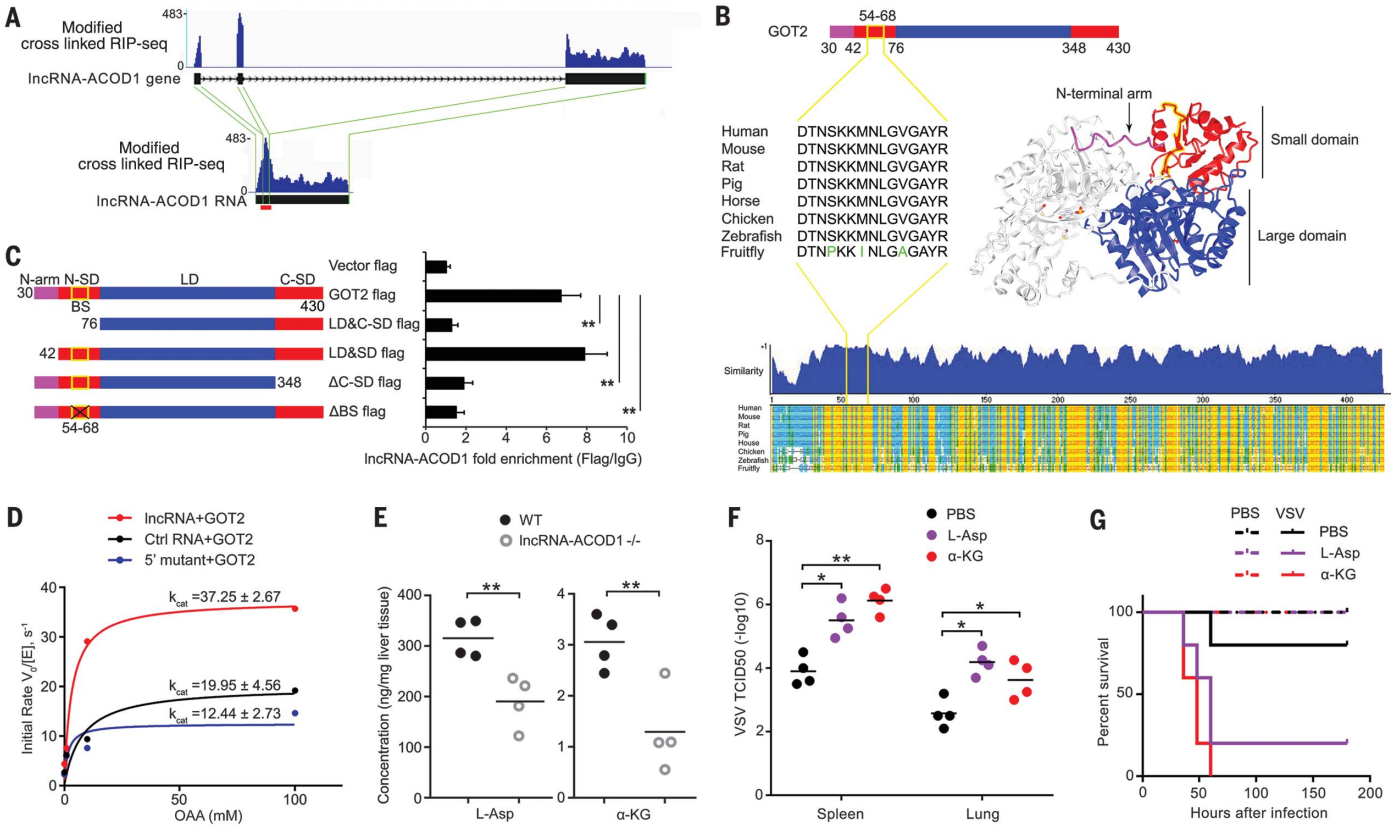

Fig. 4. lncRNA-ACOD1 promotes GOT2 enzymatic activity and functions through GOT2-catalyzed metabolites. (**A**) GOT2-binding RNAs were analyzed by RNA sequencing of GOT2 antibody-retrieved complexes. Data are shown as read density around lncRNA-ACOD1 loci (upper) and lncRNA-ACOD1 RNA (lower). Red bar indicates the protein-binding site immune to ribonuclease I (RNase I) digestion. (**B**) Top: lncRNA-ACOD1–retrieved GOT2 peptide identified by MS analysis and its position in the GOT2 protein linear structure and spatial structure (Protein Data Bank 3PDB) (highlighted in yellow). Bottom: GOT2 protein sequence conservation in vertebrates. (**C**) QPCR detection of lncRNA-ACOD1 retrieved by full-length or domain-truncated GOT2-FLAG using FLAG antibody in the RIP assay with human embryonic kidney (HEK) 293T cells transfected with indicated vectors. BS, binding site; C-SD, C-terminal part of the small domain; LD, large domain; N-arm, N-terminal arm; N-SD, N-terminal part of the small domain; Δ, deletion. $n = 3$. (**D**) Michaelis-Menten plots for GOT2 enzymatic reactions with oxaloacetic acid (OAA, 0 to 100 mM, $n = 4$) and L-glutamic acid (L-Glu, 100 mM), in the presence of 10 nM RNA (lncRNA-ACOD1, antisense control, or 5′ end–binding site mutant). [E], enzyme concentration. (**E**) Targeted metabolomics detection of L-aspartate (L-Asp) and α-ketoglutarate (α-KG) through LC-MS with liver tissues from *lncRNA-ACOD1*$^{-/-}$ and WT mice 36 hours after intraperitoneal injection with VSV (1×10^6 PFU/g). (**F**) Determination of VSV loads by $TCID_{50}$ assay in organ homogenate supernatants of *lncRNA-ACOD1*$^{-/-}$ mice intraperitoneally injected with L-Asp (5 μg/g body weight) or α-KG (5 μg/g body weight) and, 2 hours later, intraperitoneally injected with VSV (1×10^7 PFU/g) for 36 hours. $n = 4$. $^*P < 0.05$ and $^{**}P < 0.01$ (two-tailed Student's t test). (**G**) Survival of 7-week-old *lncRNA-ACOD1*$^{-/-}$ mice treated as in (F). $n = 5$. Log-rank (Mantel-Cox) test, $P < 0.01$.

(native RIP) and UV cross-linking and immunoprecipitation (UV-CLIP) assays both showed that lncRNA-ACOD1, but not other tested RNAs, was highly enriched in the GOT2 antibody-retrieved complexes compared with the immunoglobulin G (IgG)–retrieved sample, verifying the specificity of the interaction in both mouse and human (Fig. 3B and fig. S19). Our subcellular-fraction assay further revealed that their interaction occurred in the cytoplasm rather than in the mitochondria (fig. S20). The dissociation constant of lncRNA-ACOD1 and GOT2 is $1.51 \pm 0.05 \times 10^{-8}$ M (fig. S21). We also found that lncRNA-ACOD1 had no effect on GOT2 expression levels (fig. S22).

We then checked whether lncRNA-ACOD1 functions through GOT2. After ruling out a possible role in cell viability or other vital processes (fig. S23), we found that GOT2 knockdown significantly reduced VSV replication (Fig. 3, C and D) and immune response (fig. S24, A and B) and phenocopied lncRNA-ACOD1 deficiency in viral infection. We also found that lncRNA-ACOD1 overexpression promoted viral replication and immune response in control cells, whereas it had no effect in GOT2 knockdown cells (Fig. 3E and figs. S2 and S4C), indicating that knockdown of GOT2 blocks lncRNA-ACOD1 activity. Furthermore, supplementing cells with recombinant active GOT2 rescued the effects of lncRNA-ACOD1 knockdown (Fig. 3F) and GOT2 knockdown (Fig. 3G) to promote viral replication. Based on these data, we propose that virus-induced lncRNA-ACOD1 feedback facilitates viral replication by directly binding metabolic enzyme GOT2.

RNA sequencing of the GOT2 complex revealed that a 5′ segment of lncRNA-ACOD1 (around nucleotides 165 to 390) interacted with GOT2 (Fig. 4A). Then, using full-length or 5′-binding site–deleted lncRNA-ACOD1 to complement lncRNA-ACOD1 knockout macrophages, we found GOT2 binding site–dependent rescue of viral replication (fig. S25). GOT2 protein structure is highly conserved, with a large domain, a small domain, and an amino-terminal tail (Fig. 4B, top) (*29*). Two GOT2 protein molecules form a biological homodimer, and two homodimers construct an asymmetric unit (*29*), which catalyzes reversible transamination between metabolites, such as oxaloacetic acid and L-glutamic acid into L-aspartate and α-ketoglutarate (*30*). To understand how binding of lncRNA-ACOD1 affects GOT2 function, we investigated which part of GOT2 interacts with lncRNA-ACOD1 by binding-peptide MS identification in the protease-digested lncRNA-ACOD1 complex, and we found a 15-residue

peptide (residues 54 to 68) from the small domain of GOT2 as the binding site of lncRNA-ACOD1 (Fig. 4B, middle), which is relatively highly conserved among vertebrates (Fig. 4B, bottom). By using intact or fragment-deleted GOT2, our RIP assay confirmed that this 15-residue region was critical for lncRNA-ACOD1 binding (Fig. 4C).

Structurally, the binding peptide was spatially close to the substrate site (4.2 Å between the Gly65 residue and oxaloacetic acid) (fig. S26), indicating that lncRNA-ACOD1 may affect enzymatic activity of GOT2. To test this hypothesis, we used purified GOT2 protein and T7 RNA polymerase–transcribed lncRNA to perform kinetic assays of enzymatic reactions in vitro. By detecting the rate of L-aspartate output through high-performance LC-MS, we found that GOT2 catalytic activity was enhanced by lncRNA-ACOD1, which is dependent on its 5′ binding site, as the catalytic rate constant (k_{cat}) values show (Fig. 4D). Furthermore, we also tried to determine the effect of lncRNA-ACOD1 on GOT2 activity in vivo. We found that GOT2 metabolites, L-aspartate and α-ketoglutarate, were substantially reduced in lncRNA-ACOD1–deficient mice in metabolite-targeted LC-MS detection (Fig. 4E) and also in our metabolomics data (table S1), indicating that GOT2 enzymatic activity is depressed by lncRNA-ACOD1 deficiency in vivo. We then checked if GOT2 metabolites could rescue the depressed viral replication in lncRNA-ACOD1–deficient mice. We supplemented *lncRNA-ACOD1*$^{-/-}$ mice intraperitoneally with L-aspartate or α-ketoglutarate before VSV challenge and found that they promoted viral replication in vivo and increased lethality (Fig. 4, F and G). Taken together, our results demonstrate that viral infection–induced lncRNA-ACOD1 feedback facilitates viral replication by promoting GOT2 catalytic activity and production of its metabolites.

Although metabolic enzymes have been identified as potential RNA-binding proteins in systematic approaches (*31*, *32*), little is known about their functional relevance and molecular mechanisms. We found that the aminotransferase GOT2 interacts with virus-induced lncRNA-ACOD1 at its most conserved 15-residue section, which is spatially close to the substrate niche, and, through simulating GOT2 activity and production of its metabolites, lncRNA-ACOD1 facilitates viral replication and infection. In comparison to the reported lncRNA working models (*9*, *33*), we identified a viral infection–induced lncRNA regulated through NF-κB rather than the IFN-I–IRF3 pathway, which operates through a metabolic enzyme–binding mechanism to promote viral infection. This represents a new way by which viruses use host metabolic networks for survival, which provides insights into metabolic regulation and viral infection that may have relevance for clinical diseases involving metabolic dysfunction and viral infection, where lncRNA-ACOD1 could be a useful target for intervention.

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SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/358/6366/1051/suppl/DC1
Materials and Methods
Figs. S1 to S26
Table S1
References (*34*–*39*)

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An interferon-independent lncRNA promotes viral replication by modulating cellular metabolism

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Host RNA helps promote viral replication

Viruses exploit host metabolic networks for survival. Wang *et al.* identified a long noncoding RNA (lncRNA) that enhances replication of multiple viruses in both mouse and human cells (see the Perspective by Kotzin *et al*). The expression of this cytoplasmic lncRNA was induced by viruses and independent of type I interferon. The lncRNA directly bound to and stimulated the metabolic enzyme glutamic-oxaloacetic transaminase. This viral strategy may have relevance for clinical diseases involving metabolic dysfunction and viral infection.

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