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(54) Title: METHODS AND PHARMACEUTICAL COMPOSITIONS FOR THE TREATMENT OF VIRAL INFECTIONS

(57) Abstract: The present invention relates to methods and pharmaceutical compositions for the treatment of viral infection. In particular, the present invention relates to a method of treating a viral infection in a subject in need thereof comprising administering to the subject a therapeutically effective amount of an inhibitor of glycogen synthase kinase 3 (GSK3).

METHODS AND PHARMACEUTICAL COMPOSITIONS FOR THE TREATMENT OF VIRAL INFECTIONS

FIELD OF THE INVENTION:

The present invention relates to methods and pharmaceutical compositions for the treatment of viral infection.

BACKGROUND OF THE INVENTION:

Production of interferon-β (IFN-β) plays a central role in the induction of the innate antiviral response (1,2). Rapid up regulation of IFN-β gene expression occurs after recognition of viral nucleic acids by Pathogen Pattern Recognition Receptors (PPRs) either cytosolic such as retinoic acid-inducible gene I (RIG-I) and melanoma differentiation-associated antigen 5 (MDA-5) or membrane-associated Toll-like Receptors such as TLR3 (3). After sensing single or double stranded RNA of viral origin, these receptors activate signaling pathways implicating the phosphorylation and nuclear translocation of several transcription factors among which the Interferon Regulatory Factor 3 (IRF3), rapidly leading downstream to a robust activation of the expression of the IFN-β gene. After being secreted, IFN-β protein binds to the type I interferon receptor and triggers the JAK-STAT1/2 signal transduction pathway. This pathway leads to the activation and inhibition of the expression of a large set of genes that constitute the type I IFN response mounted to antagonize viral infection at different levels (4).

Mice lacking IFN-β [5] or the subunit of the type I interferon receptor (6,7) are highly susceptible to viral infections. They succumb to sub-lethal doses of a variety of viruses thus confirming the main role of IFN-β in the establishment of an innate antiviral response. However, beyond the antiviral response, IFN-β affects a wide range of other biological functions for the most related to modulation of the immune (innate and adaptive) and inflammatory responses as well as to cell proliferation and differentiation. Even though IFN-β has been described of anti-inflammatory benefit, it has also been implicated in the development of several inflammatory and autoimmune diseases (8-10). Hence, the beneficial or detrimental outcome of IFN-β expression for the organism will depend on the timing, the kinetics and the amount of IFN-β being synthesized (11,12). If a marked activation of IFN-β gene expression is required to efficiently set up the appropriate response to an external

aggression such as virus infection, this response needs to be adjusted in order to limit its pathological side effects.

As expected for a gene with pleiotropic functions, its transcriptional state is regulated at different levels. At the cellular level, only a stochastic fraction of the infected cells produce IFN-β (13,14) as a way to avoid an exacerbated and uncontrolled IFN response. At the nuclear level, one allele of the IFN-β locus localizes within interchromosomal regions rich in NF-κB DNA binding sites before and after infection (15) whereas the other allele localizes next to pericentromeric heterochromatin (PCH) clusters in the absence of infection and dissociates from PCH clusters during infection (16). The monoallelic characteristic of these particular subnuclear localizations suggests that a yet non-deciphered regulatory mechanism exists at the chromosome level. Finally, at the promoter level, the coordinated action of several transcription factors and chromatin remodeling complexes (17-21) regulate the IFN-β promoter transcriptional capacity. Among transcription factors, IRF3 plays an essential role during pathogen dependent activation of IFN-β gene expression in most cell types (22). Alongside with IRF3, are recruited over the promoter transcription factors such as NF-κB (15,23), ATF2/c-Jun and YY1 (20,24,25) that participate in the recruitment of chromatin remodeling complexes associated with histone acetyltransferase CBP. Some of these factors play dual roles, acting not only as activators but also as repressors of IFN-β expression. This is the case for NF-kB (26) and YY1 (27). Specially, YY1 participates in the transcriptional activation through recruitment of CBP and in the establishment of the repressive state of the IFN-β promoter through recruitment of co-repressor SAP30 (21) and association with pericentromeric heterochromatin (16).

Even though the IFN-β gene has been considered as repressed in naïve cells, low levels of IFN-β, regarded as constitutive, have been detected in different types of non-infected cells in the central nervous system (28,29), splenocytes and MEFs (30) implying the existence of mechanisms capable to regulate the production of limited amounts of IFN-β in the absence of infection. Using anti-IFN-α/β antibodies, Haller et al. (31) evidenced a role of such constitutive IFN-β production with respect to the establishment of an active antiviral response. Using a similar strategy, Vogel and Fertsch (32) showed that constitutive IFN-β production had an autostimulatory role upon macrophage differentiation. More recently, results obtained with mice lacking either IFN-β or its receptor have confirmed the physiological role of constitutive amounts of IFN-β produced by healthy animals in relation with several biological functions such as immune cell function, antiviral defense, bone

remodeling and modulation of homeostatic balance (reviewed in 33). As in the case of virus-induced IFN-β production, deregulation of such constitutive IFN-β expression can lead to pathological effects. However, mechanisms capable to affect constitutive IFN-β expression in absence of infection remain to be clarified.

In the cytoplasm, β-catenin is found within a degradation complex associated with Adenomatous Polyposis Coli (APC), Axin and GSK3β as well as CK1A Ser/Thr kinases that control the level of free β-catenin by a phosphorylation-dependent targeting of β-catenin towards proteasome degradation (34,35). Disruption of the degradation complex through inhibition of GSK3β kinase leads to the increase of β-catenin and its subsequent nuclear accumulation. In the nucleus, β-catenin physically interacts with T-cell factor (TCF) to regulate the expression of target genes through the recruitment of a transcriptional activator complex containing B-Cell Lymphoma 9 (BCL9) protein and CBP over promoter regions carrying binding sites for TCF factors (36-38).

SUMMARY OF THE INVENTION:

The present invention relates to methods and pharmaceutical compositions for the treatment of viral infection. In particular, the present invention is defined by the claims.

DETAILED DESCRIPTION OF THE INVENTION:

Rapid up regulation of interferon-β (IFN-β) expression following virus infection is essential to set up an efficient innate antiviral response. Biological roles related to the antiviral and immune response have also been associated to the constitutive production of IFN-β in naïve cells. However, mechanisms capable to modulate constitutive IFN-β expression in the absence of infection remain largely unknown.

Now the inventors demonstrate that inhibition of kinase GSK3β leads to the up-regulation of the constitutive level of IFN-β expression in non-infected cells, provided that GSK3β inhibition be correlated with binding of β-catenin to the IFN-β promoter. Under these conditions, IFN-β expression occurred through the T-cell factor (TCF) binding sites present on the IFN-β promoter, independently of IRF3. Enhancement of the constitutive level of IFN-β was *per se* capable to confer an efficient antiviral state to naïve cells and acted in synergy with virus infection to stimulate virus-induced IFN-β expression. Further emphasizing the role of beta-catenin in the innate antiviral response, the inventors show here that highly pathogenic Rift Valley fever virus (RVFV) targets the Wnt/β-catenin pathway and the

formation of active TCF/β-catenin complexes at the transcriptional and protein level in RVFV-infected cells and mice.

Accordingly one object of the present invention relates to a method of treating a viral infection in a subject in need thereof comprising administering to the subject a therapeutically effective amount of an inhibitor of glycogen synthase kinase 3 (GSK3).

Treatment may be for any purpose, including the therapeutic treatment of subjects suffering from a viral infection, as well as the prophylactic treatment of subjects who do not suffer from a viral infection (e.g., subjects identified as being at high risk a viral infection). As used herein, the terms "treatment," "treat," and "treating" refer to reversing, alleviating, inhibiting the progress of a disease or disorder as described herein (i.e. a viral infection), or delaying, eliminating or reducing the incidence or onset of a disorder or disease as described herein, as compared to that which would occur in the absence of the measure taken. The terms "prophylaxis" or "prophylactic use" and "prophylactic treatment" as used herein, refer to any medical or public health procedure whose purpose is to prevent the disease herein disclosed (i.e. a viral infection). As used herein, the terms "prevent", "prevention" and "preventing" refer to the reduction in the risk of acquiring or developing a given condition (i.e. a viral infection), or the reduction or inhibition of the recurrence or said condition (i.e. a viral infection) in a subject who is not ill, but who has been or may be near a subject with the condition (i.e. a viral infection).

In some embodiments, the viral infection comprises infection by one or more viruses selected from the group consisting of Arenaviridae, Astroviridae, Birnaviridae, Bromoviridae, Bunyaviridae, Caliciviridae, Closteroviridae, Comoviridae, Cystoviridae, Flaviviridae, Flexiviridae, Hepevirus, Leviviridae, Luteoviridae, Mononegavirales, Mosaic Viruses, Nidovirales, Nodaviridae, Orthomyxoviridae, Picobirnavirus, Picornaviridae, Potyviridae, Reoviridae, Retroviridae, Sequiviridae, Tenuivirus, Togaviridae, Tombusviridae, Totiviridae, Tymoviridae, Hepadnaviridae, Herpesviridae, Paramyxoviridae or Papillomaviridae viruses. Relevant taxonomic families of RNA viruses include, without limitation, Astroviridae, Birnaviridae, Bromoviridae, Caliciviridae, Closteroviridae, Comoviridae, Cystoviridae, Flaviviridae, Flexiviridae, Hepevirus, Leviviridae, Luteoviridae, Mononegavirales, Mosaic Viruses, Nidovirales, Nodaviridae, Orthomyxoviridae, Picobirnavirus, Picornaviridae,

Potyviridae, Reoviridae, Retroviridae, Sequiviridae, Tenuivirus, Togaviridae, Tombusviridae, Totiviridae, and Tymoviridae viruses.

In some embodiments, the viral infection comprises infection by one or more viruses selected from the group consisting of adenovirus, rhinovirus, hepatitis, immunodeficiency virus, polio, measles, Ebola, Coxsackie, Rhino, West Nile, small pox, encephalitis, yellow fever, Dengue fever, influenza (including human, avian, and swine), lassa, lymphocytic choriomeningitis, junin, machuppo, guanarito, hantavirus, Rift Valley Fever, La Crosse, California encephalitis, Crimean-Congo, Marburg, Japanese Encephalitis, Kyasanur Forest, Venezuelan equine encephalitis, Eastern equine encephalitis, Western equine encephalitis, severe acute respiratory syndrome (SARS), parainfluenza, respiratory syncytial, Punta Toro, Tacaribe, pachindae viruses, adenovirus, Dengue fever, influenza A and influenza B (including human, avian, and swine), junin, measles, parainfluenza, Pichinde, punta toro, respiratory syncytial, rhinovirus, Rift Valley Fever, severe acute respiratory syndrome (SARS), Tacaribe, Venezuelan equine encephalitis, West Nile and yellow fever viruses, tick-borne encephalitis virus, Japanese encephalitis virus, St. Louis encephalitis virus, Murray Valley virus, Powassan virus, Rocio virus, louping-ill virus, Banzi virus, Ilheus virus, Kokobera virus, Kunjin virus, Alfuy virus, bovine diarrhea virus, and Kyasanur forest disease.

As used herein the term “GSK3” has its general meaning in the art and refers to glycogen synthase kinase 3. GSK3 is a protein- serine/threonine kinase whose activity is inhibited by Akt phosphorylation. GSK3 phosphorylates a broad range of substrates including glycogen synthase, several transcription factors, and translation initiation factor. GSK3 is involved in multiple cellular processes including metabolism, cell survival, proliferation, and differentiation. As used herein the term “inhibitor of GSK3” refers to any compound that is able to inhibit the activity or expression of GSK3. The term encompasses any GSK3 inhibitor that is currently known, and/or any GSK3 inhibitor that can be subsequently discovered or created, can be employed with the presently disclosed subject matter.

Several GSK3 inhibitors have been identified and are well known in the art:

- Arfeen M, Bharatam PV. Design of glycogen synthase kinase-3 inhibitors: an overview on recent advancements. Curr Pharm Des. 2013;19(26):4755-75. Review.

- Osolodkin DI, Palyulin VA, Zefirov NS. Glycogen synthase kinase 3 as an anticancer drug target: novel experimental findings and trends in the design of inhibitors. Curr Pharm Des. 2013;19(4):665-79. Review.
- García I, Fall Y, Gómez G. QSAR, docking, and CoMFA studies of GSK3 inhibitors. Curr Pharm Des. 2010;16(24):2666-75. Review.
- Eldar-Finkelman H, Licht-Murava A, Pietrokovski S, Eisenstein M. Substrate competitive GSK-3 inhibitors - strategy and implications. Biochim Biophys Acta. 2010 Mar;1804(3):598-603.
- Takahashi-Yanaga F, Sasaguri T. Drug development targeting the glycogen synthase kinase-3beta (GSK-3beta)-mediated signal transduction pathway: inhibitors of the Wnt/beta-catenin signaling pathway as novel anticancer drugs. J Pharmacol Sci. 2009 Feb;109(2):179-83.
- Duchowicz PR, Castro EA. QSAR studies for the pharmacological inhibition of glycogen synthase kinase-3. Med Chem. 2007 Jul;3(4):393-417. Review.

Example of inhibitors of GSK3 include lithium, in particular lithium chloride, AR-A014418, 4-Acylamino-6-arylfuro[2,3-d]pyrimidines, lithium, SB-415286, P24, CT98014, CHIR98023, ARA014418, AT7519, DM204, Evocapil, LY2090314, Neu120, NP01139, NP03, NP060103, NP07, NP103, SAR502250, VX608 and Zentylor.

Other examples of inhibitors of GSK3 include those described in EP2433636, WO2007031878, WO2007016539, WO2009007457 and WO2005051392.US7595319, US20090041863, US20090233993, EP1739087A1, WO2001070729, WO 03/004472, WO 03/055492, WO 03/082853, WO 06/001754, WO 07/040436, WO 07/040438, WO 07/040439, WO07/040440, WO08/002244 and WO08/992245, WO 00/21927, EP 470490, WO 93/18766, WO 93/18765, EP 397060, WO 98/11103, WO 98/11102, WO 98/04552, WO 98/04551, DE 4243321, DE 4005970, DE 3914764, WO 96/04906, WO 95/07910, DE 4217964, U.S. Pat. No. 5,856,517, U.S. Pat. No. 5,891,901, WO 99/42100, EP 328026, EP 384349, EP 540956, DE 4005969, and EP 508792 which are hereby incorporated by reference.

Particular inhibitors of GSK3 are compounds selected from the group consisting of 3-[7-(2- morpholin-4-ylethoxy)quinazolin-4-yl]-2-oxo- 1 ,3-dihydroindole-5-carbonitrile; 3-[7-(2-methoxyethoxy)quinazolin-4-yl]-2-oxo-l,3-dihydroindole-5-carbonitrile; 3-[7-[2-(2-

methoxyethoxy)ethoxy]quinazolin-4-yl]-2-oxo-l,3-dihydroindole-5- carbonitrile; 3-[7-(3-morpholin-4-ylpropoxy)quinazolin-4-yl]-2-oxo-l,3-dihydroindole-5-carbonitrile; 2-hydroxy-3-[5-(4-methylpiperazine-l-carbonyl)pyridin-2-yl]-lH-indole-5-carbonitrile; 1 -[(4-methoxyphenyl)methyl]-3-(5-nitro 1 ,3-thiazol-2-yl)urea; 2-hydroxy-3-[5-[(4-phenylpiperazin-l-yl)methyl]pyridin-2-yl]-lH-indole-5-carbonitrile; 2-hydroxy-3-[5-(morpholin-4-ylmethyl)pyridin-2-yl]-lH-indole-5-carbonitrile; 2-hydroxy-3-[5-(4-methylpiperazin-l-yl)sulfonylpyridin-2-yl]-lH-indole-5-carbonitrile; 2-hydroxy-3-[5-[(4-methylpiperazin-l-yl)methyl]pyridin-2-yl]-lH-indole-5-carbonitrile; 3-[5-(morpholin-4-ylmethyl)pyridin-2-yl]-5-nitro-lH-indol-2-ol; 2-hydroxy-3-[5-(pyrrolidin-l-ylmethyl)pyridin-2-yl]-lH-indole-5-carbonitrile; [6-(2-hydroxy-5-nitro-lH-indol-3-yl)pyridin-3-yl]-(4-methylpiperazin-l-yl)methanone; 2-hydroxy-3-[5-(l-piperidylmethyl)pyridin-2-yl]-lH-indole-5-carbonitrile; 2-hydroxy-3-[5-(morpholin-4-ylmethyl)pyridin-2-yl]-lH-indole-6-carbonitrile; 2-hydroxy-3-[5-(4-methylpiperazin-l-yl)sulfonylpyridin-2-yl]-lH-indole-6-carbonitrile; 3-fluoro-3-[5-(morpholin-4-ylmethyl)pyridin-2-yl]-2-oxo-lH-indole-6-carbonitrile; [4-[5-(4-methoxyphenyl)-2,7,9-triazabicyclo[4.3.0]nona-l,3,5,7-tetraen-8-yl]phenyl]-(4-methylpiperazin- 1 -yl)methanone; 3-(4-methoxyphenyl)-N-(2-morpholin-4-ylethyl)-5,7-diazabicyclo[4.3.0]nona-l,3,5,8- tetraene-9-carboxamide; 3-(4-chlorophenyl)-N-(2-morpholin-4-ylethyl)-5,7-diazabicyclo[4.3.0]nona-l,3,5,8- tetraene-9-carboxamide; 5-fluoro-N-[4-(4-methylpiperazin-l-yl)sulfonylphenyl]-4-(2-methyl-3-propan-2-yl- imidazol-4-yl)pyrimidin-2-amine; [4-[[5-fluoro-4-(2-methyl-3-propan-2-yl-imidazol-4-yl)pyrimidin-2-yl]amino]phenyl]-(4- methylpiperazin- 1 -yl)methanone; [4-[[5-fluoro-4-(2-methyl-3-propan-2-yl-imidazol-4-yl)pyrimidin-2-yl]amino]phenyl]- phenyl-methanone; N-(3-methoxypropyl)-8-[4-(trifluoromethyl)phenyl]-2,7,9-triazabicyclo[4.3.0]nona-1 ,3,5,7-tetraene-5-carboxamide; 5-(4-methoxyphenyl)-8-[4-(l-piperidylmethyl)phenyl]-2,7,9-triazabicyclo[4.3.0]nona-1,3,5,7-tetraene; N-(3-methoxypropyl)-8-[4-(morpholin-4-ylmethyl)phenyl]-2,7,9-triazabicyclo[4.3.0]nona-1 ,3,5,7-tetraene-5-carboxamide; [4-[[5-fluoro-4-(2-methyl-3-propan-2-yl-imidazol-4-yl)pyrimidin-2-yl]amino]phenyl]- pyridin-2-yl-methanone; [4-[[4-(3-cyclohexyl-2-methyl-imidazol-4-yl)-5-fluoro-pyrimidin-2-yl]amino]phenyl]-(4- i o methylpiperazin- 1 -yl)methanone; [4-[[5-fluoro-4-[2-methyl-3-(oxan-4-yl)imidazol-4-yl]pyrimidin-2-yl]amino]phenyl]-(4- methylpiperazin- 1 -yl)methanone; 5-fluoro-4-[2-methyl-3-(oxan-4-yl)imidazol-4-yl]-N-[4-(4-methylpiperazin-l- yl)sulfonylphenyl]pyrimidin-2-amine; 4-(2,3-dimethylimidazol-4-yl)-5-fluoro-N-[4-(morpholin-4-ylmethyl)phenyl]pyrimidin-2- amine; [4-[5-(4-chlorophenyl)-2,7,9-triazabicyclo[4.3.0]nona-l,3,5,7-tetraen-8-yl]phenyl]-(4- methylpiperazin- 1 -yl)methanone; N-(3-methoxypropyl)-8-

[3-(2,2,3,3-tetrafluoropropoxymethyl)phenyl]-2,7,9- triazabicyclo[4.3.0]nona-l,3,5,7-tetraene-5-carboxamide; [4-[[5-fluoro-4-[2-methyl-3-(oxan-4-yl)imidazol-4-yl]pyrimidin-2-yl]amino]phenyl]- pyridin-2-yl-methanone; [4-[[4-(2,3-dimethylimidazol-4-yl)-5-fluoro-pyrimidin-2-yl]amino]phenyl]-pyridin-2-yl- methanone; 5-fluoro-4-[2-methyl-3-(oxan-4-yl)imidazol-4-yl]-N-[4-(morpholin-4- ylmethyl)phenyl]pyrimidin-2-amine; [4-[[4-(2,3-dimethylimidazol-4-yl)-5-fluoro-pyrimidin-2-yl]amino]phenyl]-(4- methylpiperazin- 1 -yl)methanone; 4-(2,3-dimethylimidazol-4-yl)-5-fluoro-N-[4-[(4-methylpiperazin-l- yl)methyl]phenyl]pyrimidin-2-amine; 5 -fluoro-4- [2-methyl-3 -(oxan-4-yl)imidazol-4-yl] -N-(4-methylsulfonylphenyl)pyrimidin-2-amine; 5 -fluoro-4- [2-methyl-3-(oxan-4-yl)imidazol-4-yl]-N-(6-methylpyridin-3-yl)pyrimidin-2- amine; [4-[[4-(2,3-dimethylimidazol-4-yl)-5-fluoro-pyrimidin-2-yl]amino]-2-(trifluoromethoxy)phenyl]-(4-methylpiperazin- 1 -yl)methanone; 5-fluoro-N-[4-(4-methylpiperazin-l-yl)sulfonylphenyl]-4-[3-(oxan-4-yl)-2-(trifluoromethyl)imidazol-4-yl]pyrimidin-2-amine; azetidin-l-yl-[4-[[5-fluoro-4-[2-methyl-3-(oxan-4-yl)imidazol-4-yl]pyrimidin-2- yl]amino]phenyl]methanone; 5-fluoro-N-[3-methyl-4-(morpholin-4-ylmethyl)phenyl]-4-[2-methyl-3-(oxan-4- yl)imidazol-4-yl]pyrimidin-2-amine; 5-fluoro-N-[4-(morpholin-4-ylmethyl)phenyl]-4-[3-(oxan-4-yl)-2-(trifluoromethyl)imidazol-4-yl]pyrimidin-2-amine; 5-fluoro-N-[4-(4-methylpiperazin-l-yl)sulfonylphenyl]-4-[3-(oxan-4-yl)imidazol-4- yl]pyrimidin-2-amine; [4-[[5-fluoro-4-[3-(oxan-4-yl)-2-(trifluoromethyl)imidazol-4-yl]pyrimidin-2- yl] amino]phenyl] -(4-methylpiperazin- 1 -yl)methanone; 5-[5-fluoro-2-[[4-(4-methylpiperazin-l-yl)sulfonylphenyl]amino]pyrimidin-4-yl]-l-(oxan- 4-yl)imidazole-2-carbonitrile; 5-fluoro-N-[4-(4-methylpiperazin-l-yl)sulfonylphenyl]-4-[3-methyl-2-(trifluoromethyl)imidazol-4-yl]pyrimidin-2-amine; 5-fluoro-4-[3-methyl-2-(trifluoromethyl)imidazol-4-yl]-N-[4-(morpholin-4-ylmethyl)phenyl]pyrimidin-2-amine; [4-[[5-fluoro-4-[3-methyl-2-(trifluoromethyl)imidazol-4-yl]pyrimidin-2-yl]amino]phenyl]-(4-methylpiperazin- 1 -yl)methanone; N-(2-cyanoethyl)-3-[5-(4-methoxyphenyl)-2,7,9-triazabicyclo[4.3.0]nona-l,3,5,7-tetraen-8-yl]benzamide; 5-fluoro-4-[2-methyl-3-(oxan-4-yl)imidazol-4-yl]-N-[4-methylsulfonyl-3-(trifluoromethyl)phenyl]pyrimidin-2-amine; azetidin- 1 -yl-[4-[8-[4-(morpholin-4-ylmethyl)phenyl]-2,7,9-triazabicyclo[4.3.0]nona- l,3,5,7-tetraen-5-yl]phenyl]methanone; 8-[4-(morpholin-4-ylmethyl)phenyl]-N-pyridin-3-yl-2,7,9-triazabicyclo[4.3.0]nona-1 ,3,5,7-tetraene-5-carboxamide; 2-[3-[8-[4-(4-methylpiperazine-l-carbonyl)phenyl]-2,7,9-triazabicyclo[4.3.0]nona-l,3,5,7- tetraen-5-yl]phenoxy]acetonitrile; 5 -fluoro-N-(4-methylsulfbnylphenyl)-4- [3 -propan-2-yl-2-(trifluoromethyl)imidazol-4- yl]pyrimidin-2-amine; 5-fluoro-N-[4-(4-methylpiperazin-l-yl)sulfonylphenyl]-4-[3-propan-2-yl-2-

(trifluoromethyl)imidazol-4-yl]pyrimidin-2-amine; [4-[[5-fluoro-4-[3-propan-2-yl-2-(trifluoromethyl)imidazol-4-yl]pyrimidin-2- yl] amino]phenyl] -(4-methylpiperazin- 1 -yl)methanone; 4- [2-methyl-3 -(oxan-4-yl)imidazol-4-yl] -N-(4-methylsulfonylphenyl)pyrimidin-2-amine; 4-[2-methyl-3-(oxan-4-yl)imidazol-4-yl]-N-[4-(4-methylpiperazin-l-yl)sulfonylphenyl]pyrimidin-2-amine; [4-[[4-[2-methyl-3-(oxan-4-yl)imidazol-4-yl]pyrimidin-2-yl]amino]phenyl]-(4- methylpiperazin- 1 -yl)methanone; 4-[2-methyl-3-(oxan-4-yl)imidazol-4-yl]-N-[4-(morpholin-4-ylmethyl)phenyl]pyrimidin-2- amine; 5-(4-methoxyphenyl)-8-(3-methylsulfonylphenyl)-2,7,9-triazabicyclo[4.3.0]nona-l,3,5,7-tetraene; [4-[5-(3-fluoro-4-methoxy-phenyl)-2,7,9-triazabicyclo[4.3.0]nona-l,3,5,7-tetraen-8-yl]phenyl]-(4-methylpiperazin- 1 -yl)methanone; 5-fluoro-4-[2-methyl-3-(oxan-4-yl)imidazol-4-yl]-N-(6-methylsulfonylpyridin-3- yl)pyrimidin-2-amine; (2,6-dimethylmorpholin-4-yl)-[4-[[5-fluoro-4-[2-methyl-3-(oxan-4-yl)imidazol-4- yl]pyrimidin-2-yl]amino]phenyl]methanone; [5-[[5-fluoro-4-[2-methyl-3-(oxan-4-yl)imidazol-4-yl]pyrimidin-2-yl]amino]pyridin-2-yl]-(4-methylpiperazin- 1 -yl)methanone; 5-fluoro-4-[2-methyl-3-(oxan-4-yl)imidazol-4-yl]-N-[4-(l-morpholin-4- ylethyl)phenyl]pyrimidin-2-amine; 5 -fluoro-N-(4-methylsulfbnylphenyl)-4- [3 -(oxan-4-yl)-2-(trifluoromethyl)imidazol-4-yl]pyrimidin-2-amine; azetidin- 1 -yl-[2-chloro-4-[[4-(2,3-dimethylimidazol-4-yl)-5-fluoro-pyrimidin-2- yl] amino]phenyl]methanone; azetidin- l-yl-[4-[[4-(2,3-dimethylimidazol-4-yl)-5-fluoro-pyrimidin-2-yl]amino]-2- methyl-phenyl]methanone; azetidin- l-yl-[5-[[4-(2,3-dimethylimidazol-4-yl)-5-fluoro-pyrimidin-2-yl]amino]pyridin-2- yljmethanone; azetidin- l-yl-[4-[[4-(2,3-dimethylimidazol-4-yl)-5-fluoro-pyrimidin-2-yl]amino]-2-(trifluoromethoxy)phenyl]methanone; azetidin- 1 -yl- [3 -chloro-5 - [[4-(2,3 -dimethylimidazol-4-yl)-5 -fluoro-pyrimidin-2- yl]amino]pyridin-2 -yljmethanone; 5-fluoro-4-[2-methyl-3-(oxan-4-yl)imidazol-4-yl]-N-[6-(morpholin-4-ylmethyl)pyridin-3- yl]pyrimidin-2-amine; 5-fluoro-4-[2-methyl-3-(oxan-4-yl)imidazol-4-yl]-N-(6-propan-2-ylsulfonylpyridin-3- yl)pyrimidin-2-amine; azetidin- l-yl-[3-chloro-5-[[5-fluoro-4-[2-methyl-3-(oxan-4-yl)imidazol-4-yl]pyrimidin-2- yl]amino]pyridin-2 -yljmethanone; N-(6-ethylsulfonylpyridin-3-yl)-5-fluoro-4-[2-methyl-3-(oxan-4-yl)imidazol-4- yl]pyrimidin-2-amine; [3-chloro-5-[[5-fluoro-4-[3-(oxan-4-yl)-2-(trifluoromethyl)imidazol-4-yl]pyrimidin-2- yl]amino]pyridin-2-yl]-(4-methylpiperazin-l-yl)methanone; 5-[[5-fluoro-4-[2-methyl-3-(oxan-4-yl)imidazol-4-yl]pyrimidin-2-yl]amino]-N-methyl-N- propan-2-yl-pyridine-2-carboxamide; N-ethyl-5-[[5-fluoro-4-[2-methyl-3-(oxan-4-yl)imidazol-4-yl]pyrimidin-2-yl]amino]-N- methyl-pyridine-2-carboxamide; 3-[4-[8-[4-(morpholin-4-ylmethyl)phenyl]2,7,9-triazabicyclo[4.3.0]nona-l,3,5,7-tetraene- 5-carbonyl]piperazin-l-yl]propanenitrile; [3-chloro-5-[[5-fluoro-4-[3-methyl-

2-(trifluoromethyl)imidazol-4-yl]pyrimidin-2- yl]amino]pyridin-2-yl]-(4-methylpiperazin-l-yl)methanone; [3-chloro-5-[[5-fluoro-4-[3-methyl-2-(trifluoromethyl)imidazol-4-yl]pyrimidin-2- yl]amino]pyridin-2-yl]-(l-piperidyl)methanone; azetidin- 1 -yl-[3-chloro-5-[[5-fluoro-4-[3-methyl-2-(trifluoromethyl)imidazol-4- yl]pyrimidin-2-yl]amino]pyridin-2-yl]methanone; 5-fluoro-4-[2-methyl-3-(oxan-4-yl)imidazol-4-yl]-N-[6-(4-methylpiperazin-l-yl)sulfonylpyridin-3-yl]pyrimidin-2-amine; N-[6-[(4,4-difluoro-l-piperidyl)methyl]pyridin-3-yl]-5-fluoro-4-[2-methyl-3-(oxan-4- yl)imidazol-4-yl]pyrimidin-2-amine; 5-fluoro-4-[2-methyl-3-(oxan-4-yl)imidazol-4-yl]-N-(oxan-4-yl)pyrimidin-2-amine; l-[4-[[5-fluoro-4-[2-methyl-3-(oxan-4-yl)imidazol-4-yl]pyrimidin-2-yl]amino]-l- piperidyl]ethanone; [4-[[5-fluoro-4-[2-methyl-3-(oxan-4-yl)imidazol-4-yl]pyrimidin-2-yl]amino]-l-piperidyl]- phenyl-methanone; l-[4-[[5-fluoro-4-[2-methyl-3-(oxan-4-yl)imidazol-4-yl]pyrimidin-2-yl]amino]-l-piperidyl]-2-phenyl-ethanone; benzyl 4-[[5-fluoro-4-[2-methyl-3-(oxan-4-yl)imidazol-4-yl]pyrimidin-2- yl]amino]piperidine- 1 -carboxylate; 5-fluoro-4-[2-methyl-3-(oxan-4-yl)imidazol-4-yl]-N-(l-methylsulfonyl-4- piperidyl)pyrimidin-2-amine; N-[l-(benzenesulfonyl)-4-piperidyl]-5-fluoro-4-[2-methyl-3-(oxan-4-yl)imidazol-4- yl]pyrimidin-2-amine; 5-[[5-fluoro-4-[2-methyl-3-(oxan-4-yl)imidazol-4-yl]pyrimidin-2-yl]amino]-N,N-dimethyl-pyridine-2-sulfonamide; an isomer, a metabolite , a prodrug or a pharmaceutically acceptable salt thereof, or a solvate or a solvate of a pharmaceutically acceptable salt thereof.

By a "therapeutically effective amount" of the inhibitor of GSK3 as above described is meant a sufficient amount of the compound. It will be understood, however, that the total daily usage of the compounds and compositions of the present invention will be decided by the attending physician within the scope of sound medical judgment. The specific therapeutically effective dose level for any particular subject will depend upon a variety of factors including the disorder being treated and the severity of the disorder; activity of the specific compound employed; the specific composition employed, the age, body weight, general health, sex and diet of the subject; the time of administration, route of administration, and rate of excretion of the specific compound employed; the duration of the treatment; drugs used in combination or coincidential with the specific polypeptide employed; and like factors well known in the medical arts. For example, it is well within the skill of the art to start doses of the compound at levels lower than those required to achieve the desired therapeutic effect and to gradually increase the dosage until the desired effect is achieved. However, the daily dosage of the products may be varied over a wide range from 0.01 to 1,000 mg per adult per day. Typically, the compositions contain 0.01, 0.05, 0.1, 0.5, 1.0, 2.5, 5.0, 10.0, 15.0, 25.0,

50.0, 100, 250 and 500 mg of the active ingredient for the symptomatic adjustment of the dosage to the subject to be treated. A medicament typically contains from about 0.01 mg to about 500 mg of the active ingredient, preferably from 1 mg to about 100 mg of the active ingredient. An effective amount of the drug is ordinarily supplied at a dosage level from 0.0002 mg/kg to about 20 mg/kg of body weight per day, especially from about 0.001 mg/kg to 7 mg/kg of body weight per day.

In some embodiments, the inhibitor of GSK3 is administered to the subject in combination or conjunction with, without limitation, adamantane inhibitors, neuraminidase inhibitors, alpha interferons, non-nucleoside or nucleoside polymerase inhibitors, NS5A inhibitors, antihistamines, protease inhibitors, helicase inhibitors, P7 inhibitors, entry inhibitors, IRES inhibitors, immune stimulators, HCV replication inhibitors, cyclophilin A inhibitors, A3 adenosine agonists, and microRNA suppressors. Cytokines that could be administered in combination or conjunction with inhibitor of the present invention, without limitation, IL-2, IL-12, IL-23, or IL-27. Examples of anti-viral compounds that can be used in combination with the inhibitor of the present invention typically include but are not limited to ACH-1625 (Achillion); Glycosylated interferon (Alios Biopharma); ANA598, ANA773 (Anadys Pharm); ATI-0810 (Arisyn Therapeutics); AVL-181 (Avila Therapeutics); LOCTERON® (Biolex); CTS-1027 (Conatus); SD-101 (Dynavax Technologies); Clemizole (Eiger Biopharmaceuticals); GS-9190 (Gilead Sciences); GI-5005 (GlobalImmune BioPharma); Resiquimod/R-848 (Graceway Pharmaceuticals); Albinterferon alpha-2b (Human Genome Sciences); IDX-184, IDX-320, IDX-375 (Idenix); IMO-2125 (Idera Pharmaceuticals); INX-189 (Inhibitex); ITCA-638 (Intarcia Therapeutics); ITMN-191/RG7227 (Intermune); ITX-5061, ITX-4520 (iTherx Pharmaceuticals); MB11362 (Metabasis Therapeutics); Bavituximab (Peregrine Pharmaceuticals); PSI-7977, RG7128, PSI-938 (Pharmasset); PHX1766 (Phenomix); Nitazoxanide/ALINIA® (Romark Laboratories); SP-30 (Samaritan Pharmaceuticals); SCV-07 (SciClone); SCY-635 (Scynexis); TT-033 (Tacere Therapeutics); Viramidine/taribavirin (Valeant Pharmaceuticals); Telaprevir, VCH-759, VCH-916, VCH-222, VX-500, VX-813 (Vertex Pharmaceuticals); neuraminidase inhibitors (Peramivir, Laninamivir); triple therapy—neuraminidase inhibitors ribavirin, amantadine (ADS-8902); polymerase inhibitors (Favipiravir); reverse transcriptase inhibitor (ANX-201); inhaled chitosan (ANX-211); entry/binding inhibitors (Binding Site Mimetic, Flucide™); entry inhibitor, (Fludase®; NexBio, Inc., San Diego, Calif.); fusion inhibitor,

(MGAWN1 for West Nile); host cell inhibitors (lantibiotics); cleavage of RNA genome (RNAi, RNAse L); and TG21.

Typically, the inhibitor of GSK3 is combined with pharmaceutically acceptable excipients, and optionally sustained-release matrices, such as biodegradable polymers, to form pharmaceutical compositions. "Pharmaceutically" or "pharmaceutically acceptable" refer to molecular entities and compositions that do not produce an adverse, allergic or other untoward reaction when administered to a mammal, especially a human, as appropriate. A pharmaceutically acceptable carrier or excipient refers to a non-toxic solid, semi-solid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type. Typically, the pharmaceutical compositions contain vehicles, which are pharmaceutically acceptable for a formulation capable of being injected. These may be in particular isotonic, sterile, saline solutions (monosodium or disodium phosphate, sodium, potassium, calcium or magnesium chloride and the like or mixtures of such salts), or dry, especially freeze-dried compositions which upon addition, depending on the case, of sterilized water or physiological saline, permit the constitution of injectable solutions. The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions; formulations including sesame oil, peanut oil or aqueous propylene glycol; and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases, the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms, such as bacteria and fungi. Sterile injectable solutions are prepared by incorporating the inhibitor of GSK3 in the required amount in the appropriate solvent with several of the other ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum-drying and freeze-drying techniques which yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

The invention will be further illustrated by the following figures and examples. However, these examples and figures should not be interpreted in any way as limiting the scope of the present invention.

FIGURES:

Figure 1. LiCl treatment enhances the constitutive and virus-induced IFN-β expression. (**A** and **F**) Immunolocalisation of β-catenin in L929. Cells were either non-treated (NT), treated with 20mM LiCl (LiCl) during 24h, or infected with NDV, labeled with an anti-β-catenin antibody (a, d) and a DNA intercalating agent to visualize the nucleus (ToPro3; b, e). Nuclei were outlined as shown in merge images (c, f), and total green pixel intensity corresponding to β-catenin labeling in the nucleus was quantified (**B** and **G**). β-catenin was analyzed by Western blot (**C**) in nuclear extracts from L929 cells either non treated (NT) or treated with 20 mM LiCl (LiCl) during 24h. IFN-β mRNA (**D** and **H**) and Oas1b or IRF7 mRNA (**E**) were analyzed by RT-qPCR: in non-infected L929 cells either non-treated (NT) or LiCl treated (LiCl) (**D** and **E**); at different times post-infection (p.i) in L929 cells mock- or NDV-infected, either non-treated (NT) or pre-treated with LiCl (LiCl) (**H**). In (**D** and **E**), the corresponding fold inductions were calculated with respect to non treated cells; and in (**H**), the corresponding fold inductions were calculated with respect to non-infected and non-treated cells. **B** and **G**) n=33 (minimum) to 71 (maximum) counted nuclei; **D**) n=42 from 13 independent experiences; **E**) n=18 from 6 independent experiences; **H**) n=6. Student test: p-value less than 0.001 (***), 0.01 (**) and 0.05 (*). All images correspond to one confocal section. Scale bar = 10μM.

Figure 2. LiCl enhancement of IFN-β expression is mediated by β-catenin. (**A-D**) AML12 cells were either non-treated (NT) or treated with 20mM LiCl (LiCl) during 24h and either non-infected (**A-C**) or NDV-infected (**D**). (**A** and **B**) Cells were labeled with an anti-β-catenin antibody (a, d) and a DNA intercalating agent to visualize the nucleus (ToPro3; b, e). Nuclei were outlined as shown in merge images (c, f), and total green pixel intensity corresponding to β-catenin labeling in the nucleus was quantified (**B**). IFN-β mRNA (**C** and **D**) was analyzed by RT-qPCR in non-infected (**C**) and NDV-infected (6h p.i.) (**D**) cells either non-treated (NT) or pre-treated with LiCl (LiCl). The corresponding fold inductions were calculated with respect to non-treated (**C**) or non-infected and non-treated (**D**) cells. (**E-G**) L929 cells were either mock-transfected (mock) or transfected with β-catenin specific (siβcat) or control (siCtrl) siRNA for 72h. β-catenin mRNA (**E**) and protein level (**F**) were analyzed by RT-qPCR and WB respectively. (**G**) Post-siRNA, cells were either non-treated (NT) or

treated with 20mM LiCl (LiCl) during 24h before being NDV-infected. IFN-β mRNA was analyzed by RT-qPCR. (**E** and **G**) the corresponding fold inductions were calculated with respect to mock-transfected, non-treated and NDV-infected cells (n=3). Student test: p-value less than 0.001 (***) and 0.01 (**). All images correspond to one confocal section. Scale bar = 10μM.

Figure 3. Interaction of β-catenin with the IFN-β promoter region containing a TCF binding site is necessary for LiCl enhancement of IFN-β promoter activity. (A and B) β-catenin binding to WT330 and WT110 promoters and corresponding CAT activities were analysed in L929 WT330 and WT110 cells either non-treated (NT) or treated with LiCl 20mM (LiCl). **(A)** Cells were collected post-LiCl treatment and their CAT activities quantified. The corresponding fold inductions were calculated with respect to non-treated cells (n=12 for WT330 and n=6 for WT110 cell lines). **(B)** Post-LiCl treatment, cells were further mock- or NDV-infected, collected at different times post-infection and the corresponding CAT activities were quantified. The corresponding fold inductions were calculated with respect to non-infected non-treated cells (n=4). **(C and D)** CAT activities of L929 cells either non-treated (NT) or treated with 50 μM of SB216763 (**C**) or 30μM of IX inhibitor during 24h (**D**). Cells were further mock- or NDV-infected, collected 8h post-infection and the corresponding CAT activities quantified. The corresponding fold inductions were calculated with respect to non-infected non-treated cells.

Figure 4. Non-pathogenic and pathogenic strains of RVFV have opposite effects on the Wnt/β-catenin pathway. Genes participating in the Wnt/β-catenin pathway whose promoter regions were identified as significantly interacting with RVFV NSs protein during ChIP-on-chip experiments, listed in Table I, are shown in gray. Genes whose expressions were affected after infection with either the non-pathogenic ΔNSs or the pathogenic ZH strain of RVFV with respect to mock-infected cells are indicated by blue (upregulated) or red (downregulated) arrows.

EXAMPLE:

Materials and Methods

Virus, Cells and Mice

Stocks of RVFV ZH548 and RVFV ZHΔNSs were produced under BSL3 conditions by infecting Vero cells at m.o.i. of 10^{-3} and by harvesting the medium at 72 hr p.i. Murine fibroblastic L929 cell line and NDV infections were described previously (45). Murine hepatocyte AML12 cell line was from ATCC (ref. CRL-2254). For preparation of bone marrow-derived macrophages (BMDM) preparation, bone marrow from tibiae and femora of 8-12 weeks B6 mices was flushed and cultured for 7 days in DMEM containing 10% heat-inactivated fetal bovine serum, 100 U/ml penicillin/streptomycin, 2 mM glutamine and 10 ng/ml murine M-CSF (Peprotech). Infection of mice was performed as previously described (46). When indicated, LiCl, iCRT3 (Sigma) or LiCl+iCRT3 were directly added to the culture medium.

Antibodies

Mouse anti-NSs and rabbit anti-N polyclonal antibodies were raised respectively against the entire NSs or N protein (47). Anti-β-catenin from BD Transduction laboratories (Cat#610154) was used for immunofluorescence, Western blot and gel retardation. Secondary antibodies used for immunofluorescence were Alexa 488 fluor-conjugated chicken anti-mouse from Invitrogen (A21200) and Alexa 555 fluor-conjugated donkey anti-rabbit from Invitrogen (A31572). Secondary antibodies used for Western blot were ECL Mouse IgG, HRP-linked whole Ab (NA931) an ECL Rabbit IgG, HRP-linked whole Ab (NA934) from GE Healthecare Life Sciences.

Immunofluorescence

For immunofluorescence, cells grown in 6-well plates on coverslips were fixed with 4% formaldehyde in PBS for 15 minutes and permeabilized with 1% Triton X-100 in PBS for 20 minutes. Then cells were incubated for 1 hour at room temperature with the corresponding primary antibodies diluted in PBS/BSA 5%. Cells were then washed with PBS and incubated for 1 hour at room temperature with corresponding secondary antibodies.

Image acquisition and manipulation

Samples were analyzed at room temperature by confocal laser scanning microscopy using a Zeiss LSM710 confocal system at the *Service Commun de Microscopie* (SCM) of

Université Paris Descartes. This system is equipped with a 63x, 1.4 O.N oil immersion lens (Plan Neofluor). For oil immersion microscopy, we used oil with refractive index of 1.518 (Zeiss). Image capture was carried out with a definition of 1024x1024 pixels with 8 bit data for each color. Images were analyzed using Image J software. Total pixel intensity was quantified using Image J software.

Chromatin immunoprecipitation

Chromatin immunoprecipitation experiments were carried out as previously described (21). PCR analysis of inputs or immunoprecipitated DNAs was performed using oligonucleotides F-40 and CAT to reveal the integrated WT330 and WT110 IFN-β promoter. For the F-40, CAT set of primers, PCR conditions were as follows: 1 cycle of 94°C for 5 minutes; 20 cycles of 94°C for 30 seconds, 53°C for 30 seconds and 72°C for 30 seconds; 1 cycle of 72 °C for 10 minutes. A first "cold" PCR was carried out in the presence of 25 pmol of each primer; 1.5 µl of the product of the first PCR was subjected to a second "hot" PCR carried out in the presence of 0.1 µl a-^{32}PdATP (6000 Ci/mmol) and 25 pmol of each primer.

RT-qPCR

Total RNA was extracted using Tri Reagent (Sigma) according to the manufacturer's protocol. 1 µg of total RNA was reversely transcribed using High Capacity cDNA Reverse Transcription Kit (Applied Biosystems) according to the manufacturers' recommendations using Random Primers. qPCR was performed using SYBR Green (Thermo Scientific) reagents: 95°C 15 minutes, then 40 cycles at 95°C 15 seconds, 60°C 1 minute, followed by a dissociation step. Relative quantification of mRNA expression was calculated using the $\Delta\Delta C_T$ method using three reference genes among Ppib, Hprt1, Utp6c and Rplp0). For fold changes lower than 1 (translating a repression of the gene's expression), the inverted values were determined such that a 0.5 fold change corresponds to -2. Statistical analysis was carried out using Student T-test or the REST software that uses a Pair-Wise Fixed Reallocation Randomization Test© to determine a P-value.

RNA interference experiments

L929 cells were transfected using Lipofectamine 2000 (Invitrogen Life Technologies) with a pool of siRNA oligos specific for β-catenin (Thermo Scientific Dharmacon®

L04062800) or with a pool of control siRNA sequences (Thermo Scientific Dharmacon® D-001810-10-05) at a final concentration of 50nM each during 72 h.

Gel retardation assay

Nuclear extracts of murine L929 cells either non-treated or treated with LiCl for 24 h were incubated with the corresponding 5' ^{32}P-labeled probes in 20 µl (final volume) of 50 mM Tris-HCl (pH 7.5), 50 mM NaCl, 5 mM EDTA, 10% glycerol and 5 mM dithiothreitol. When indicated, anti-β-catenin antibody was incubated with the nuclear extracts 1 h at 4°C prior to the addition of the labeled probe.

Cytopathic effect assays.

Monolayer cultures of L929 cells in 96-well plates were incubated with LiCl for 24 h before VSV infection. Before VSV infection, the medium containing LiCl was removed. Viruses were diluted in medium with serum (2% final concentration) and added directly to the culture medium. The monolayers were stained with crystal violet as vital dye 24 h after VSV infection or fixed 8h after VSV infection for immunofluorescence analysis. For measurement of the cytopathic effect of RVFV, L929 cells either non-treated or treated with LiCl for 24 h were infected with RVFVZH548 and incubated under an overlay consisting of DMEM, 2% fetal calf serum, antibiotics and 1% agarose at 37°C. At 3 day p.i., the lytic plaques were counted after staining with a solution of crystal violet.

Western blot

For Western blot, total protein extracts were prepared in RIPA buffer and resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) in 4-12% precast gels (Life Technologie). Relative quantification of proteins was carried out using Image Quant software on scanned WB films.

Ethics Statement

Experiments on live mice were conducted according to the French and European regulations on care and protection of laboratory animals (EC Directive 86/609, French Law 2001-486 issued on June 6, 2001) and the National Institutes of Health Animal Welfare (Insurance #A5476-01 issued on 02/07/2007). Experimental protocols were approved by the Animal Ethics Committee #1 of the *Comité Régional d'Ethique pour l'Expérimentation*

Animale (CREEA), Ile de France (N°2012-0025), and carried out in compliance with Institut Pasteur Biosafety Committee.

Results

LiCl treatment enhances constitutive IFN-β gene expression.

The proximal region of the IFN-β promoter, made of negative and positive regulatory domains, is highly conserved between murine and human cells. The Virus Responsive Element (VRE) that contains binding sites for transcription factors IRF3, ATF2/c-Jun, NF-κB and YY1 is surrounded by two Negative Regulatory Domains (NRDI and II) described as regions of nucleosome positioning (18). Sequence analysis of the murine IFN-β promoter revealed the presence in the NDRII of two potential DNA-binding sequences for TCF transcription factors. A first site, TCFa, present between positions -374 and -367 that perfectly matches the consensus TCF DNA binding sequence (38). A second site, TCFb, present between positions -233 and -226 that differs from the consensus TCF DNA binding sequence by one base. Two sequences homologous to the murine TCFb sequence are present in the human IFN-β promoter, between positions -260 and -253 and +26 and +33 respectively.

The presence of potential TCF binding sites within the proximal region of the IFN-β promoter suggested that expression of the IFN-β gene could be modulated in response to pathways inducing nuclear accumulation of β-catenin and formation of TCF/β-catenin complexes over the IFN-β promoter region. In order to test this hypothesis, we analyzed IFN-β gene expression in non-infected cells, corresponding to an IRF3-NF-κB-ATF2/c-Jun independent expression that we have considered as constitutive IFN-β expression, before and after nuclear accumulation of β-catenin. Experiments were carried out in murine fibroblastic L929 cells, which are potent IFN-β producer cells. In the absence of GSK3β inhibition, little if any β-catenin was detected in the nucleus of L929 cells whereas the nuclear distribution of β-catenin was significantly enhanced after treatment of the cells with LiCl, an inhibitor of GSK3β (Fig. 1A-C). IFN-β gene expression measured by RT-qPCR in LiCl-treated cells compared to untreated cells, showed that LiCl treatment significantly enhanced the constitutive level of IFN-β mRNAs ($p<10^{-5}$) (Fig. 1D) independently of any virus infection. Even though the level of IFN-β expression measured after LiCl treatment was much lower than that induced during viral infection (16), it was sufficient to trigger an IFN-β response as

reflected by a statistically significant induction of the expression of two major Interferon Stimulated Genes (ISGs) such as the *Oas1b* and *Irf7* genes (Fig. 1E).

Low constitutive levels of IFN-β have been previously shown to synergize with virus infection for efficient virus-induced IFN-β production (30,48). In order to test if the enhancement of the constitutive level of IFN-β gene expression induced by LiCl in naïve cells could affect virus induced IFN-β expression, L929 cells were pretreated or not with LiCl before being infected with Newcastle Disease Virus (NDV) that, contrary to LiCl, does not induce the nuclear accumulation of β-catenin (Fig. 1F and G). As shown in Fig. 1H, pretreatment of the cells with LiCl significantly potentiated NDV-induced IFN-β expression.

The effect of LiCl treatment on IFN-β expression is mediated by β-catenin.

In order to confirm that the effect of LiCl treatment on constitutive IFN-β expression was not due to LiCl treatment *per se* but required the nuclear accumulation of β-catenin, we made use of murine hepatocyte AML12 cells that produce IFN-β in response to viral infection but, by contrast to L929 cells, do not accumulate nuclear β-catenin after LiCl treatment (Fig. 2A and B). Measurement of the expression of IFN-β mRNA in AML12 cells either treated or not with LiCl, before (Fig. 2C) and after NDV (Fig. 2D) infection, showed that the inability of LiCl to induce the accumulation of nuclear β-catenin in this particular cell type correlated with the inability of LiCl treatment to enhance IFN-β expression either in the absence (Fig.2C) or presence (Fig. 2D) of virus infection.

The role of β-catenin during LiCl enhancement of IFN-β expression was further confirmed using siRNAs. For this, L929 cells were either mock-transfected or transfected with siRNAs directed against β-catenin or with control siRNAs (Fig. 2E-G) before being treated with LiCl for 24h. Under conditions of diminution of β-catenin mRNA (Fig. 2E) and protein (Fig. 2F) level, we observed that LiCl treatment had no significant effect on IFN-β expression compared to cells treated with control siRNAs (Fig. 2G) confirming therefore that the capacity of LiCl treatment to activate IFN-β expression was mediated by β-catenin.

LiCl treatment stimulates the recruitment of β-catenin on the IFN-β promoter through the NRDII promoter region containing a TCF binding site.

IRF3 transcription factor is known to promote IFN-β expression, yet its activation, nuclear translocation and subsequent IFN-β promoter binding, require the previous activation

of PPRs. The capacity of LiCl treatment to affect IFN-β gene expression in the absence of infection showed that activation of the transcriptional capacity of the IFN-β promoter could occur independently of IRF3, through a mechanism that we hypothesized to be TCF/β-catenin dependent. In order to test this hypothesis, we made use of previously established L929 cell lines carrying integrated into their genome the proximal IFN-β promoter region fused to the CAT reporter gene either from position -330 to +20 (L929 WT330 cells) or from position -110 to +20 (L929 WT110 cells) (45). The NRDI region and the entire VRE region, containing the IRF3 binding site, are present in both promoters whereas the NRDII region, containing a TCF binding site, is only present on the WT330 promoter. Therefore, contrary to the WT330 promoter that contains a TCF binding site, no TCF binding site is present in promoter WT110.

ChIP analysis indicated that LiCl treatment induced β-catenin binding to the WT330 promoter but not to the WT110 promoter, demonstrating that β-catenin binding to the IFN-β promoter that occurred in the absence of virus infection required the NRDII region containing a TCF binding site.

If, as hypothesized, the activation of IFN-β expression induced after LiCl treatment required the recruitment of β-catenin on the IFN-β promoter, then only the WT330 promoter was expected to respond to LiCl treatment. In order to test this hypothesis, we pretreated L929 WT330 and WT110 cells with LiCl and assayed the corresponding CAT activities (Fig. 3A). As expected, LiCl treatment enhanced by 4-folds the constitutive transcriptional capacity of the WT330 promoter, but not that of the WT110 promoter. Thus reinforcing the indispensable role of the promoter region positioned 5' of the VRE, containing a TCF binding site, for LiCl treatment to enhance the constitutive transcriptional capacity of the IFN-β promoter.

The role of the NRDII region containing a TCF binding site during LiCl-dependent promoter activation was also analyzed after virus infection. As shown in Fig. 3B, LiCl treatment enhanced the virus-induced activity of the WT330 promoter but not that of the WT110 promoter. Therefore, the presence of the NRDII region containing a TCF-binding site was also required for the LiCl-dependent enhancement of the virus-induced transcriptional capacity of the IFN-β promoter. The presence of only the VRE region, containing the IRF3 binding site (promoter WT110), was not sufficient to either recruit β-catenin or mediate LiCl enhancement of the constitutive as well as virus-induced transcriptional capacity of the IFN-β promoter.

LiCl that is considered as an inhibitor of GSK3β can also affect the activity of other kinases. In order to further investigate if the effect of LiCl on IFN-β expression was the consequence of the inhibition of GSK3β, L929wt330 and wt110 cells were treated with two other inhibitors of GSK3β corresponding to SB216763 and inhibitor IX. As shown in Fig. 2E and F, treatment with SB216763 as well as inhibitor IX had the same effect than LiCl, activating the transcriptional capacity of the WT330 promoter but not that of the WT110 promoter. Since GSK3β is a common target for these three inhibitors, the fact that LiCl, SB216763 and inhibitor IX had the same effect on the transcriptional capacity of the IFN-β promoter strongly supports the hypothesis that LiCl dependent enhancement of IFN-β expression is the consequence of the inhibition of GSK3β.

The effect of LiCl treatment on IFN-β expression is mediated by TCF.

Gel retardation assays were used to analyze the capacity of TCF binding sites present on the NRDII region of the IFN-β promoter to form TCF-DNA complexes. For this, we used oligonucleotides containing the sequence of either wild type or mutated TCFa and b sites. TCF binding sites were mutated in their stretches of A required for the HMG box of TCF factors to interact with DNA. The TCFa site was mutated to give rise to the TCFmutA (5'-TTC*gg*AGG-3') site and the TCFb site was mutated to give rise to the TCFmutB (5'-CC*cc*TGcT-3') site. The corresponding radioactively labeled, double-stranded DNA probes were incubated with nuclear extracts prepared from L929 cells either non-treated or treated by LiCl during 24h. Whereas almost no complex formation was observed in the presence of nuclear extracts from non-treated cells, a protein-DNA complex was clearly formed when the DNA probes containing the wild type TCFa or b sites were incubated with nuclear extracts from LiCl treated cells. As expected for a TCF-DNA complex, complex formation was disrupted when the core region of the TCF DNA binding sequence was mutated as in TCFmutA and mutB probes. Also, incubation of the nuclear extracts with anti-β-catenin antibodies affected the formation of the protein-DNA complex that in the presence of antibodies was less intense and supershifted, indicative of the presence of β-catenin within the complex.

To confirm the role of TCF binding sites in the LiCl dependent activation of the IFN-β promoter transcriptional capacity, the IFN-β promoter region from position -458 to +36 either wild type or mutated in one or both TCF binding sites was cloned in front of the luciferase reporter gene. After transfecting the corresponding plasmids into L929 cells, the transfected

cells were either treated or not with LiCl before being infected with NDV. The corresponding luciferase activities were measured. Contrary to wild type and mutB promoters whose corresponding transcriptional capacities could be activated following LiCl treatment, promoter mutA partially lost its capacity to be activated by LiCl and no activation was observed in the case of the promoter mutAB. Therefore, results obtained here with TCF mutated promoters demonstrated that the presence of at least one TCF binding site was required for LiCl to activate the transcriptional capacity of the IFN-β promoter. In the context of the promoter region cloned in these constructions (from -458 to +36), the TCFa that perfectly matches the consensus sequence site appeared more efficient than the TCFb site.

In order to definitively confirm the role of TCF/β-catenin complexes during LiCl enhancement of the transcriptional capacity of the endogenous IFN-β promoter, we used iCRT3, which is a potent inhibitor of TCF4/β-catenin complexes (49). The presence of IFN-β mRNAs was measured in NDV-infected L929 cells that were either non-treated or pretreated with LiCl during 24 or 48 h in the absence or presence of different amounts of iCRT3. Whereas LiCl treatment significantly enhanced IFN-β expression by 2.5 or 6 folds after 24 or 48 h of LiCl treatment respectively, a dose-dependent inhibition of the effect of LiCl was observed in the presence of iCRT3, and this in the case of 24h as well as 48h of LiCl treatment. Thus demonstrating that the capacity of LiCl to enhance IFN-β expression was mediated by TCF/β-catenin complexes.

LiCl treatment confers an efficient antiviral response.

In Fig. 1D we showed that LiCl dependent enhancement of the constitutive level of IFN-β expression was sufficient to lead to a significant activation of the expression of two major ISGs: *Oas1b* and *Irf7* genes, responsible for the RNaseL-dependent degradation of viral ARN and for the amplification of the IFN-β response respectively. In order to analyze the efficiency of such LiCl-induced interferon response with respect to the establishment of an antiviral state, we compared the cytopathic effect (CPE) of VSV in murine L929 cells either pretreated or not with LiCl. Indeed, following infection of murine L929 cells with VSV, cell viability is expected to decrease as the amount of VSV increases unless an efficient IFN-β response had been mounted previously to VSV infection (50).

L929 cells pretreated or not with LiCl were infected with increasing titers of VSV. Twenty-four hours after infection, cells were stained with crystal violet, which detects CPE as a decrease in staining intensity. As shown previously, at low multiplicity of infection (MOI),

cells pretreated with LiCl displayed less VSV-induced CPE than untreated cells highlighting the capacity of LiCl treatment to induce an efficient antiviral response protecting cells against VSV-induced cell death. The capacity of LiCl treatment to confer an efficient antiviral response at low MOI is further depicted in photographs of culture fields of LiCl-treated versus untreated cells 24h after post infection (p.i.). Whereas the majority of non-treated cells died 24h p.i., LiCl-treated cells grew to confluence displaying resistance to infection.

Even though at conditions of higher MOI all cells died by 24 hr p.i. regardless of LiCl treatment, at earlier times after infection (8h p.i.), the percentage of cells detected as infected by fluorescent staining at MOI=1 was significantly lower among LiCl-treated cells than among untreated cells. This is indicative of the capacity of LiCl-treated cells to dampen the kinetics of virus multiplication.

The canonical Wnt/β-catenin pathway is a major target of Rift Valley fever virus.

Cellular pathways participating in the establishment of an effective antiviral response are expected to be affected during viral infections: either "positively", leading to the activation of the host antiviral response, or "negatively", in the case of viruses capable to efficiently inhibit the cellular antiviral response. Therefore, if as suggested in this work a TCF/β-catenin transcription complex participates in the establishment of an efficient antiviral response then pathways regulating active TCF/β-catenin complex formation are expected to be targeted during viral infection.

The effect of a viral infection upon the canonical Wnt/β-catenin pathway, which is a major regulator of the formation of active TCF/β-catenin complexes, was analyzed here during infection with Rift Valley fever virus (RVFV) for which two different strains, pathogenic and non-pathogenic are available. The wild type RVFV ZH548 strain (ZH) of the virus that codes for a non-structural NSs protein, suppresses IFN-β expression and is highly pathogenic causing severe illness in men and animals (51-53), whereas the RVFV ZH548ΔNSs strain (ΔNSs), deleted for the region coding for NSs protein, strongly activates IFN-β expression and is fully avirulent (54).

The viral NSs protein, which is a major factor responsible of RVFV pathogenicity, has the characteristic to form filamentous structures in nuclei of infected cells abnormally trapping within these structures transcription factors and co-factors of the host (21,55). During a recent genome wide search of regulatory DNA regions of the host interacting with RVFV NSs protein, several genes associated with the canonical Wnt/β-catenin pathway were

identified as interacting with NSs (Table I) (56). Among these were genes coding for several WNT ligands (*Wnt1*, *Wnt2*, *Wnt6* and *Wnt8b*), for antagonists of Wnt signaling (*Apcdd1*, *Dkk1* and *Kremen2*), for members of the multiprotein complex regulating the degradation of β-catenin (*Gsk3b*, *Gsk3a* and *Axin2*), for β-catenin itself (*Ctnnb1*) as well as for members of the TCF/β-catenin complex (*Tcf7l2*, *Lef1* and *Bcl9*). Also, DNA regulatory sequences associated with genes coding for casein kinases (*Csnk1d*, *Csnk1g2*, *Csnk1g3* and *Csnk2a2*) known to positively regulate the canonical Wnt/β-catenin signaling pathway at different levels were present among cellular DNA regions targeted by NSs.

Using RT-qPCR, we analyzed the effect of RVFV infection, either pathogenic (ZH strain) or non-pathogenic (ΔNSs strain), upon the expression of the genes related with canonical Wnt/β-catenin pathway identified as significantly interacting with NSs. For this, RNA was purified from three different cell types targeted by RVFV (L929 fibroblasts, AML12 hepatocytes and bone marrow derived macrophages (BMDM)) either non-, ΔNSs- or ZH-infected. After reverse transcription, the fold change in the expression level of genes of interest (including the gene coding for IFN-β) was calculated with respect to non-infected cells using three different reference genes.

As expected, infection with the avirulent ΔNSs strain strongly activated IFN-β gene expression in the three cell types tested, whereas infection with the virulent ZH strain maintained the IFN-β expression in an abnormally repressed state.

Both strains affected the Wnt/β-catenin pathway but with opposite effects. Infection with the non-pathogenic ΔNSs strain led to i) the activation of the expression of genes coding for casein kinases, with *Csnk1d* and *Csnk2a2* genes activated in the three cell types tested, and *Csnk1g3* activated in fibroblasts and BMDM; ii) the activation of the expression of genes coding for factors essentials for the formation of an active β-catenin transcription complex such as *Bcl9* and *Tcf7l2* in hepatocytes and BMDM; and iii) the repression of the expression of the *Axin2* gene, a negative regulator of the canonical Wnt/β-catenin pathway (57), in hepatocytes. On the contrary, infection with ZH led in BMDM to the inhibition of the expression of *Csnk1d* and *Csnk2a2* genes, and the activation of the expression of *Axin2* gene. Also, a slight inhibition of the expression of *Bcl9* gene was observed in fibroblasts and hepatocytes.

As shown in Fig. 4, all the effects observed after non-pathogenic ΔNSs infection synergized towards the formation of an active nuclear β-catenin complex. On the contrary, the

effects observed after infection with the pathogenic ZH strain of the virus synergized against the formation of a nuclear β-catenin transcription complex.

The level of β-catenin protein was affected in organs of RVFV infected mice.

In order to test the effect of RVFV infection upon the level of β-catenin *in vivo*, the presence of β-catenin was analyzed in the liver and brain of mice that were either non-infected, or infected with ΔNSs or ZH strains. Of note, the liver and brain are two main organs targeted during RVFV infection (58). Mice were euthanized at days 3 and 5 p.i. and the liver and brain were removed; one half of each organ was used for total protein purification and Western blot analysis and the other half for RNA purification and RT-qPCR analysis. Whereas no variation was observed for β-catenin at the transcriptional level (data not shown), Western blot analysis showed significant variations at the protein level in the liver of ZH-infected mice at day 5 p.i.. Under these conditions, the average relative level of β-catenin significantly diminished in the liver of all ZH-infected mice compared to non-infected and ΔNSs-infected mice.

Viral targeting of β-catenin was further analyzed with respect to the presence of the virus (as translated by the presence of viral mRNAs coding for viral proteins N and NSs) in the liver and the brain of each mouse under each condition.

As expected in the case of an infection that induces IFN-β expression and mounts an efficient antiviral response, the virus remained for the most undetectable in the liver and the brain of mice infected with the ΔNSs strain at day 3 and 5 p.i. (data not shown). In the case of ZH-infected mice, viral N and NSs mRNAs were clearly detected at day 5 p.i. in the liver of mouse#1, 2 and 3 and by day 3 p.i. in the liver of mouse#1 but remained essentially undetectable in the brain of infected mice. Therefore, the effect of RVFV infection upon the level of β-catenin appeared correlated with the presence of the virus in these organs.

Results shown here indicate that *in vivo*, β-catenin is targeted during RVFV infection with the level of β-catenin diminished in the liver of mice that were unable to efficiently inhibit virus production. Virus-induced IFN-β expression depends on the presence of several transcription factors and co-factors that synergize to give rise to a maximum of expression (16-27). In previous work, we have shown that RVFV infection counteracts the transcriptional activation of the IFN-β expression by abnormally maintaining the IFN-β promoter region associated to a transcriptionally repressive environment (16, 21). Therefore, even though RVFV targeting of TCF/β-catenin complex formation could be participating in the RVFV-

induced inhibition of IFN-β expression, it certainly could not be considered as by itself responsible of the almost complete lack of IFN-β expression in RVFV infected cells. However, the fact that avirulent and virulent strains of RVFV have opposite, mirror, effects upon the Wnt/β-catenin pathway strengthens the hypothesis that this pathway could be playing a role in mounting an efficient innate antiviral response against RVFV infection. If this was the case, then it is expected that LiCl treatment should at least partly protect against the cytopathic effect induced by RVFV. In order to test this, L929 cells were either non-treated or pretreated with LiCl during 24 h before infection with the virulent ZH strain of RVFV. As shown previously pretreatment with LiCl significantly diminished the cytopathic effect (number of lytic plaques) induced by RVFV.

Discussion

In this work we have demonstrated that cell treatment with LiCl, an inhibitor of GSK3β, leads to the enhancement of the constitutive (namely IRF3-NFκB-ATF2/cJUN-independent) as well as virus-induced level of IFN-β gene expression, provided that LiCl treatment be correlated with the IFN-β promoter recruitment of β-catenin. The effect of LiCl on the constitutive as well as virus-induced transcriptional capacity of the IFN-β promoter required the presence of the IFN-β promoter region containing TCF-binding sites, positioned 5' of the VRE region. Results obtained in this work with siRNA directed against β-catenin added to results obtained with iCRT3, an inhibitor of TCF/β-catenin complexes (49), demonstrated that the capacity of LiCl to enhance IFN-β expression was mediated through TCF/β-catenin complexes rather than through IRF3 as suggested until now in the literature.

Contrary to results obtained by Wang et al. (59) that have observed a LiCl-dependent attenuation of IFN-β production caused by a LiCl-dependent inhibition of kinase TBK1, we never observed under our experimental conditions an inhibitory effect of LiCl upon IFN-β expression. Not even under conditions when the activatory effect of LiCl was not detected, such as when LiCl was unable to induce accumulation of nuclear β-catenin (i.e.: AML12 murine hapatocytes) or unable to induce β-catenin recruitment over the IFN-β promoter (i.e.: WT110 promoter). The effect we have observed in the presence of LiCl was also observed after treatment with SB216763 and with inhibitor IX, which are two different inhibitors of GSK3β, suggesting that the capacity of LiCl to enhance IFN-β expression could be the consequence of the inhibition of GSK3β that is targeted by these three inhibitors.

Several reports in the literature point at physiological roles for constitutive IFN-β expression in the absence of viral infection (33). However, little is known concerning the mechanism(s) capable to directly modulate IFN-β expression in naïve cells except for results obtained with *c-Jun*$^{-/-}$ murine embryonic fibroblasts that identified transcription factor c-Jun as a potential regulator of the constitutive level of IFN-β expression (60). Since inhibition of GSK3β, leading to the formation of nuclear TCF/β-catenin complexes, can be reached not only physiologically through pathways associated with Wnt ligands and Akt phosphorylation but also pharmaceutically, results shown here open the possibility of a wide range of cellular and extra-cellular mechanisms susceptible to modulate the level IFN-β expression in the absence of infection.

In this work we have demonstrated that LiCl treatment induced an effective antiviral response, protecting naïve murine fibroblasts from the cytopathic effect induced by VSV at a low MOI, which mimics physiological conditions of viral infection. While these results are in contradiction with those of Wang et al. (49), they are in agreement with the antiviral effects associated to LiCl treatment described by Hillesheim et al. (43) with respect to H7N7 influenza A virus and Hao et al. (61) on Porcine Reproductive and Respiratory Syndrome (PRRS) virus. Further confirming a role for TCF/β-catenin complexes in the establishment of an efficient innate antiviral response, we show here that the Wnt/β-catenin pathway is targeted during infection with RVFV: positively in the case of infection with the avirulent strain, and negatively following infection with the virulent strain of RVFV. In agreement with a potential role for the Wnt/β-catenin pathway mounting an efficient antiviral response during RVFV infection, we have shown here that LiCl treatment of L929 cells protected these cells against RVFV cytopathic effect. Viral targeting of the formation of active nuclear β-catenin transcription complexes has been observed in the context of infections with other viruses such as influenza H1N1 virus, Human Cytomegalovirus (HCMV), Venezuelan Equine Encephalitis Virus (VEEV) and HIV-1 (40,62-64). Interestingly, the recent work of Hillesheim et al. (43) on the capacity of β-catenin to regulate the innate cellular immune response to influenza A virus (IAV) infections in human cells, show results indicative of a role for TCF during β-catenin-dependent regulation of IFN-β expression. Overall, these observations indicate a general role for TCF/β-catenin complexes with respect to the establishment of an efficient innate antiviral response.

However, if we consider that TCF/β-catenin complexes can directly affect constitutive levels of IFN-β expression in the absence of pathogens as described here, then the existence

of cross-talks between β-catenin and IFN-β should also be considered beyond the issue of pathogen infection. For example, the capacity of β-catenin to modulate inflammatory and immune responses (64) could be related to its ability to modulate the expression of IFN-β, which is a major regulator of immunity and inflammatory responses. Also, a cross-talk between β-catenin and IFN-β during the establishment and/or treatment of neurological disorders such as Alzheimer disease and multiple sclerosis should be considered since both, Wnt/β-catenin pathway and IFN-β response have been associated to these neurological pathologies (65-67).

Although modulation of the constitutive level of IFN-β expression has probably been observed in the past by many researchers, it has often been neglected because it seemed only minor compared to pathogen-induced IFN-β expression. Nevertheless, even though weak, the biological significance of constitutive IFN-β expression is no longer to be demonstrated (33). Therefore, mechanisms capable to modulate constitutive IFN-β expression, such as the one our results demonstrate here, should gain interest in the future.

Table I. List of canonical Wnt/β-catenin genes whose regulatory DNA regions were identified as interacting with RVFV NSs protein during ChIP-on-chip assays (55).

Gene	**Chromo-some**	**NSs interacting Region Start**	**Region Length** (bps)	**Probes in Region**	**p-value**
Apcdd1	Chr18	63078268	563	16	0.007658
Axin2	Chr11	108827835	502	14	0.00618531
Bcl9	Chr3	97004948	751	21	0.00823024
Csnk1d	Chr11	120859171	651	18	0.0033914
Csnk1g2	Chr10	80096623	865	24	0.00136329
Csnk1g2	Chr10	80082196	920	22	0.00671548
Csnk1g3	Chr18	54109583	850	18	0.00104351
Csnk1g3	Chr18	54156237	499	10	0.00828074
Csnk2a2	Chr8	97965333	511	12	0.00827232
Ctnnb1	Chr9	120803511	517	15	0.00766641
Gsk3a	Chr7	26016588	488	14	0.00998065
Gsk3b	Chr16	38213326	554	16	0.006564
Kremen2	Chr17	23882003	542	14	0.00559623
Lef1	Chr3	130837713	488	13	0.00916435

Tcf7l2	Chr19	55894582	531	15	0.00931583
Wnt1	Chr15	98620114	407	11	0.00822183
Wnt2	Chr6	17981380	579	17	0.00595809
Wnt6	Chr1	74823318	824	23	0.00226374
Wnt6	Chr1	74830849	550	14	0.00750652
Wnt6	Chr1	74828942	494	14	0.00782631
Wnt6	Chr1	74818891	394	11	0.00812085
Wnt6	Chr1	74816494	489	13	0.00885298
Wnt8b	Chr19	44545480	754	20	0.00226374
Wnt8b	Chr19	44544618	808	23	0.00506606

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Throughout this application, various references describe the state of the art to which this invention pertains. The disclosures of these references are hereby incorporated by reference into the present disclosure.

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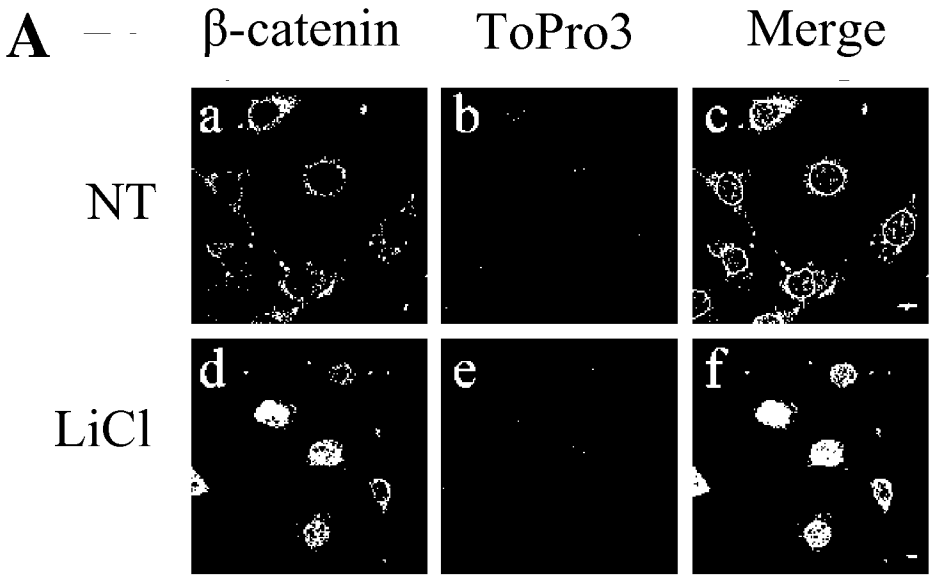
CLAIMS:

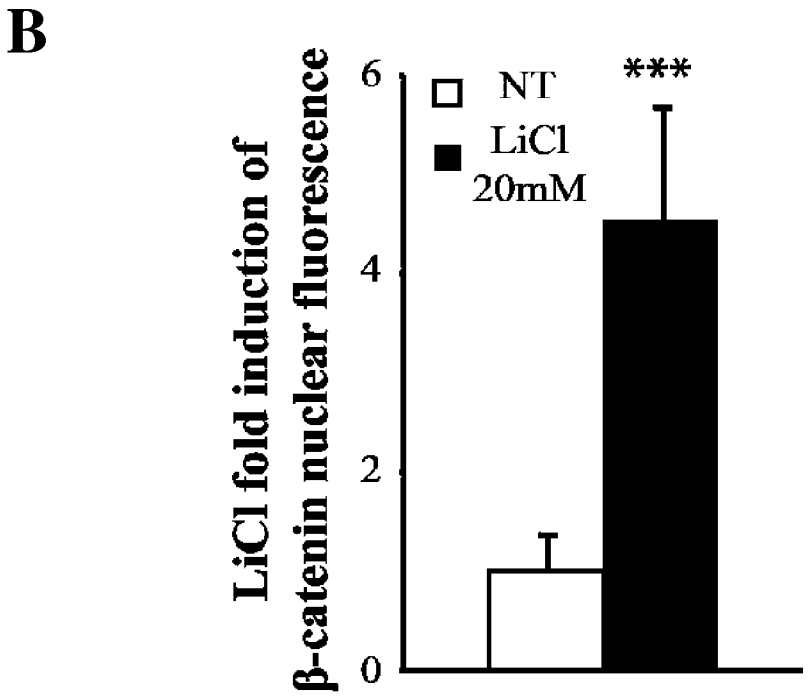
1. A method of treating a viral infection in a subject in need thereof comprising administering to the subject a therapeutically effective amount of an inhibitor of glycogen synthase kinase 3 (GSK3).

2. The method of claim 1 wherein the viral infection comprises infection by one or more viruses selected from the group consisting of Arenaviridae, Astroviridae, Birnaviridae, Bromoviridae, Bunyaviridae, Caliciviridae, Closteroviridae, Comoviridae, Cystoviridae, Flaviviridae, Flexiviridae, Hepevirus, Leviviridae, Luteoviridae, Mononegavirales, Mosaic Viruses, Nidovirales, Nodaviridae, Orthomyxoviridae, Picobirnavirus, Picornaviridae, Potyviridae, Reoviridae, Retroviridae, Sequiviridae, Tenuivirus, Togaviridae, Tombusviridae, Totiviridae, Tymoviridae, Hepadnaviridae, Herpesviridae, Paramyxoviridae or Papillomaviridae viruses.

3. The method of claim 1 wherein the viral infection comprises infection by one or more viruses selected from the group consisting of adenovirus, rhinovirus, hepatitis, immunodeficiency virus, polio, measles, Ebola, Coxsackie, Rhino, West Nile, small pox, encephalitis, yellow fever, Dengue fever, influenza (including human, avian, and swine), lassa, lymphocytic choriomeningitis, junin, machuppo, guanarito, hantavirus, Rift Valley Fever, La Crosse, California encephalitis, Crimean-Congo, Marburg, Japanese Encephalitis, Kyasanur Forest, Venezuelan equine encephalitis, Eastern equine encephalitis, Western equine encephalitis, severe acute respiratory syndrome (SARS), parainfluenza, respiratory syncytial, Punta Toro, Tacaribe, pachindae viruses, adenovirus, Dengue fever, influenza A and influenza B (including human, avian, and swine), junin, measles, parainfluenza, Pichinde, punta toro, respiratory syncytial, rhinovirus, Rift Valley Fever, severe acute respiratory syndrome (SARS), Tacaribe, Venezuelan equine encephalitis, West Nile and yellow fever viruses, tick-borne encephalitis virus, Japanese encephalitis virus, St. Louis encephalitis virus, Murray Valley virus, Powassan virus, Rocio virus, louping-ill virus, Banzi virus, Ilheus virus, Kokobera virus, Kunjin virus, Alfuy virus, bovine diarrhea virus, and Kyasanur forest disease.

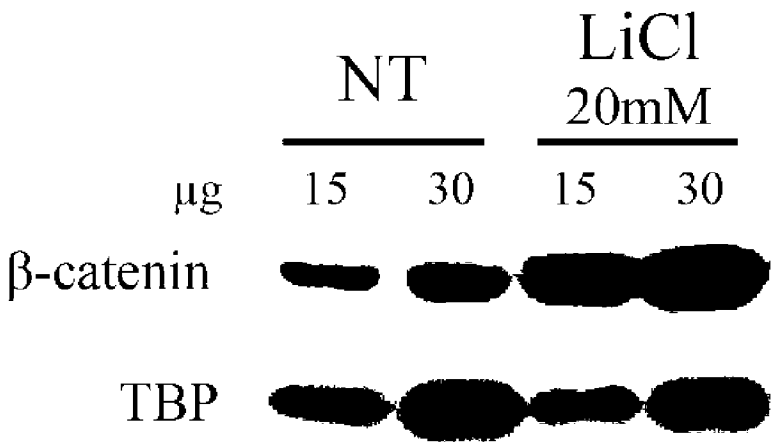
4. The method of claim 1 wherein the inhibitor of GSK3 is lithium chloride





Figures 1A and 1B

C



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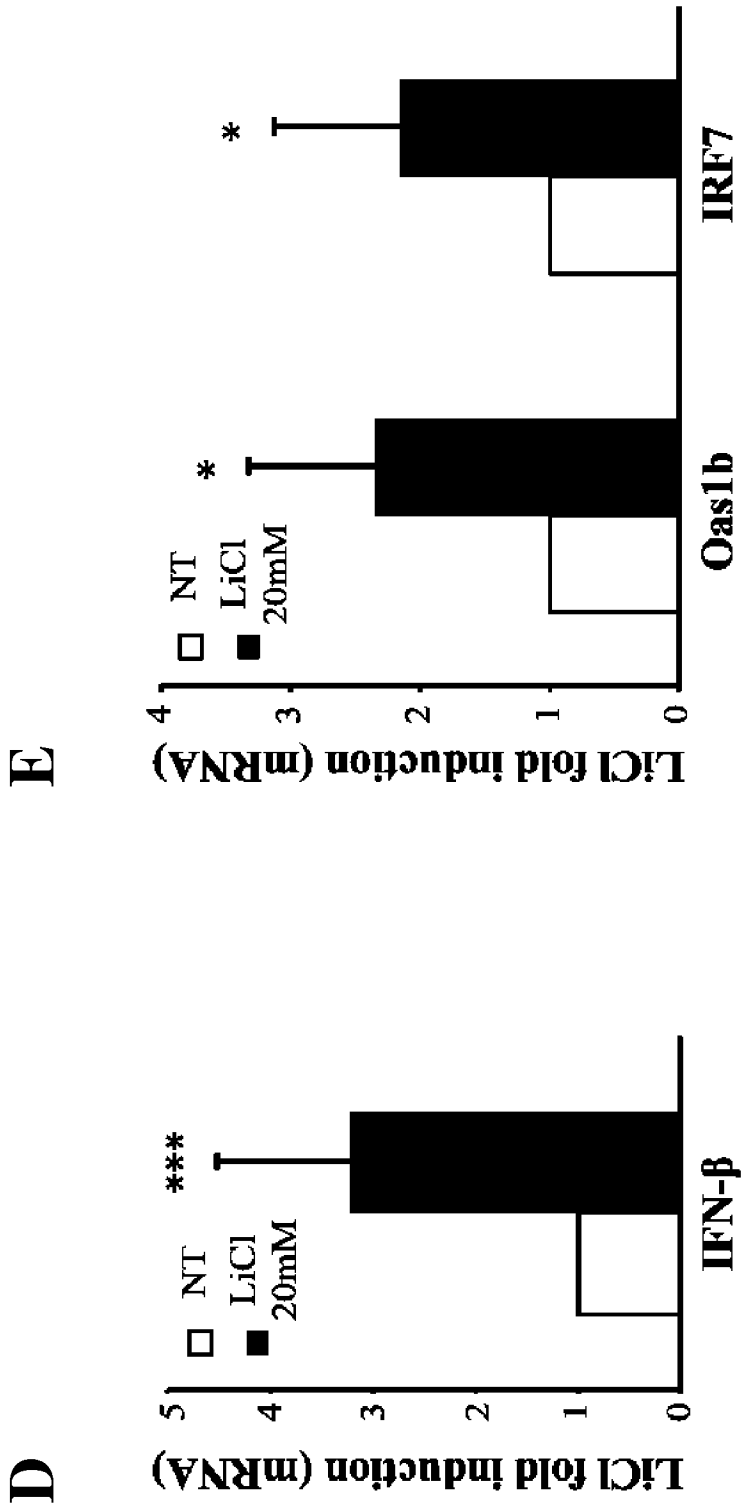
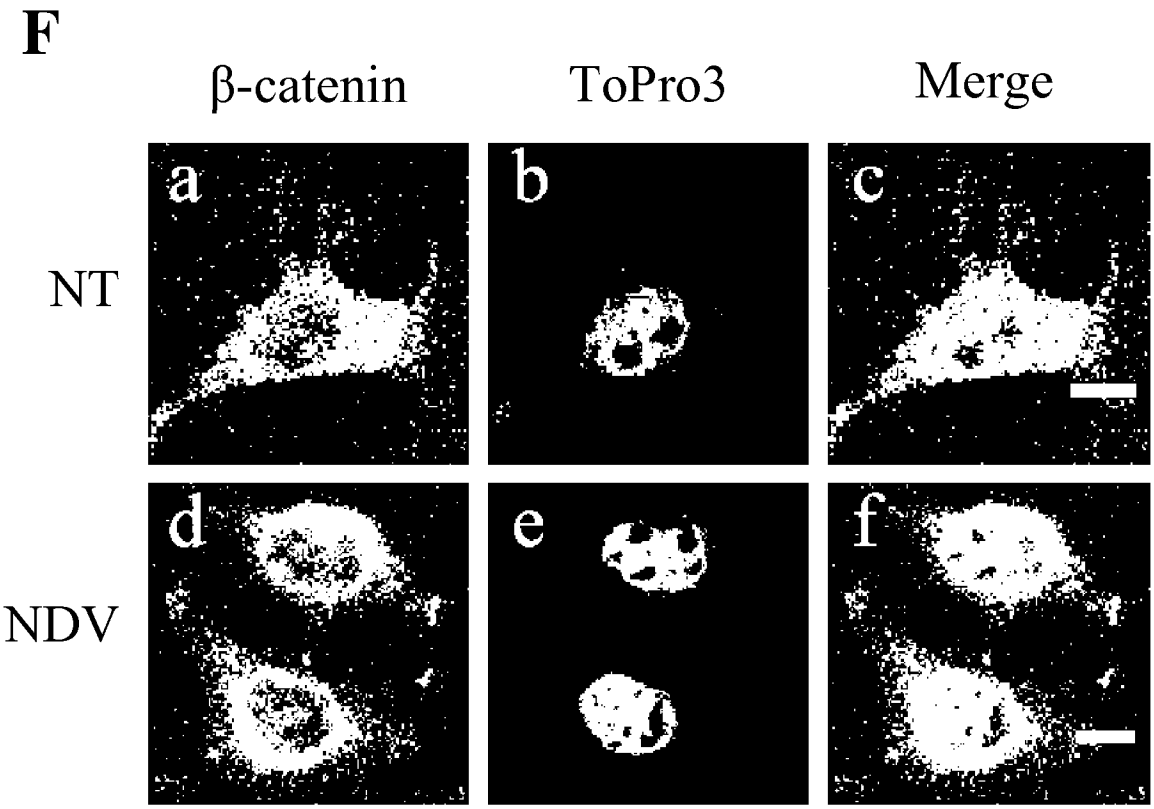


Figures 1D and 1E



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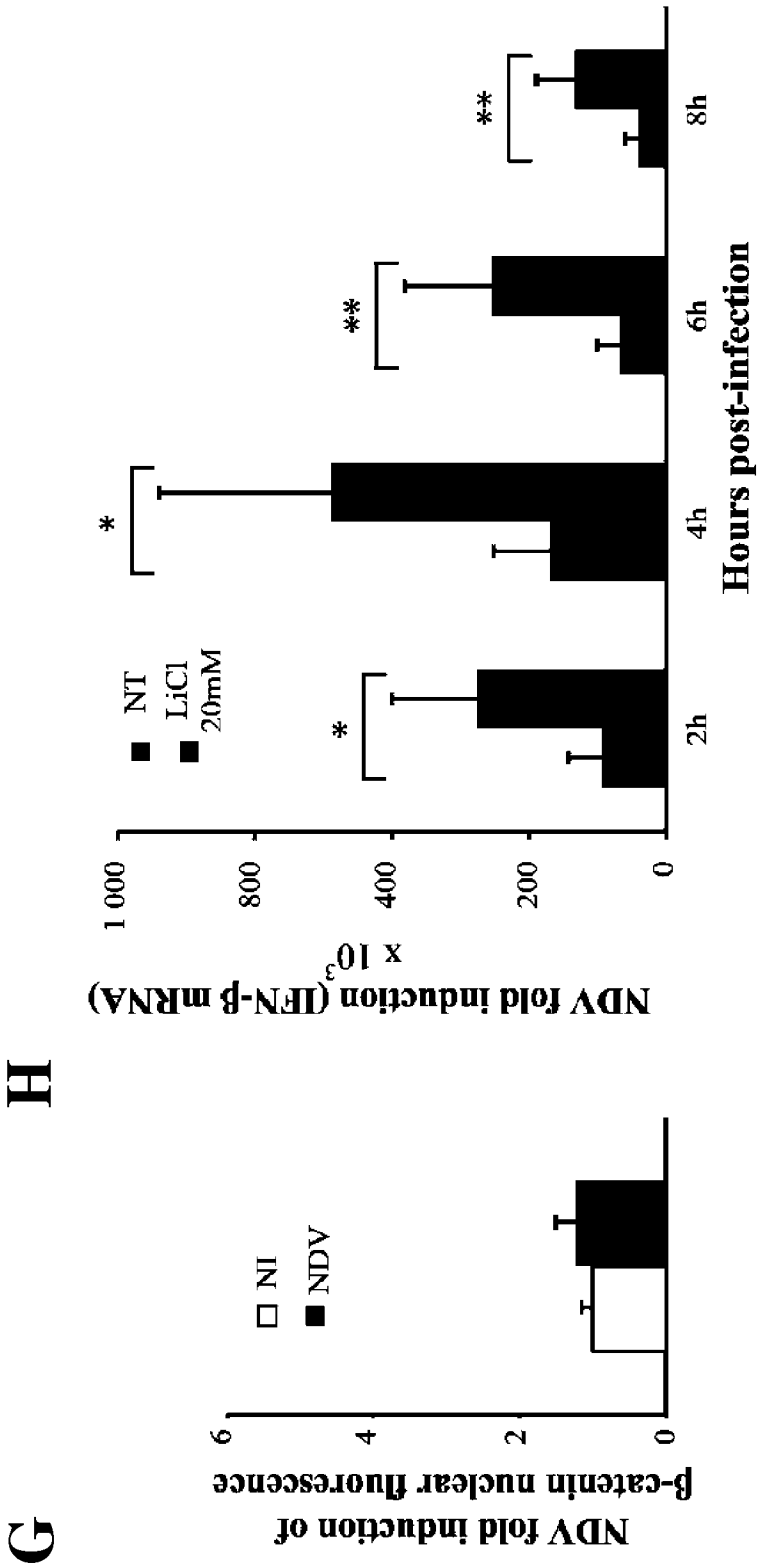
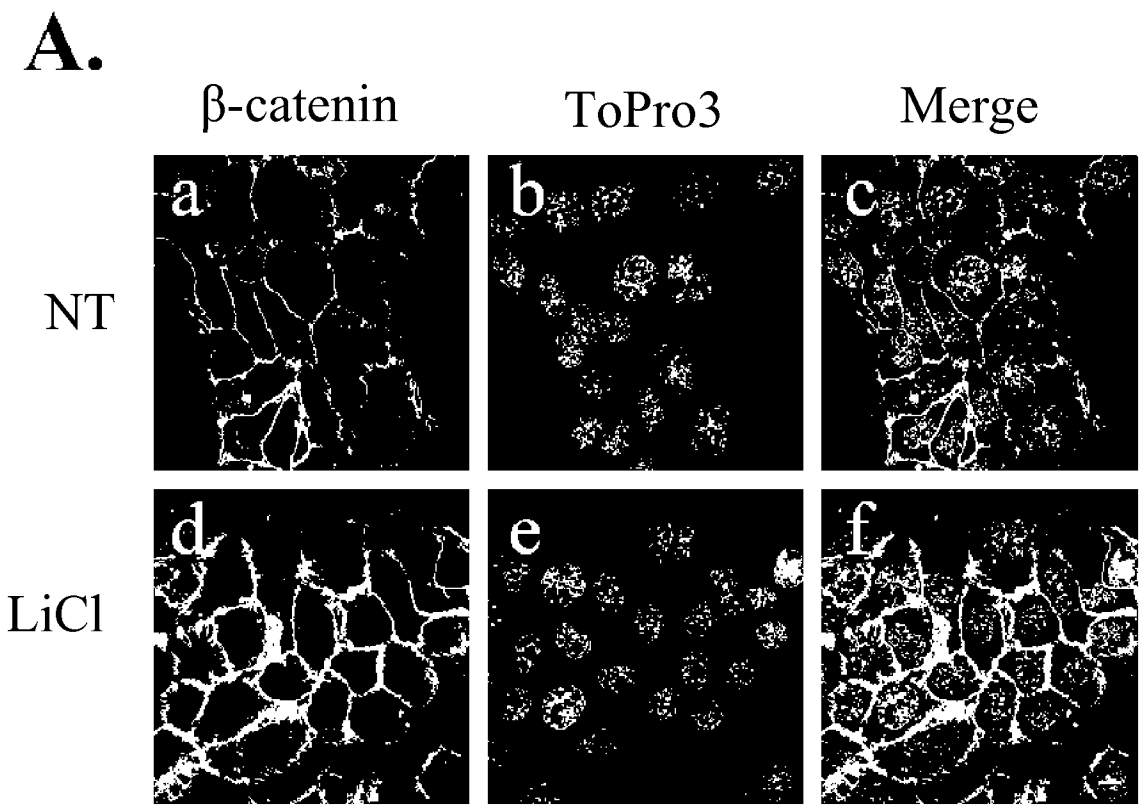


Figures 1G and 1H



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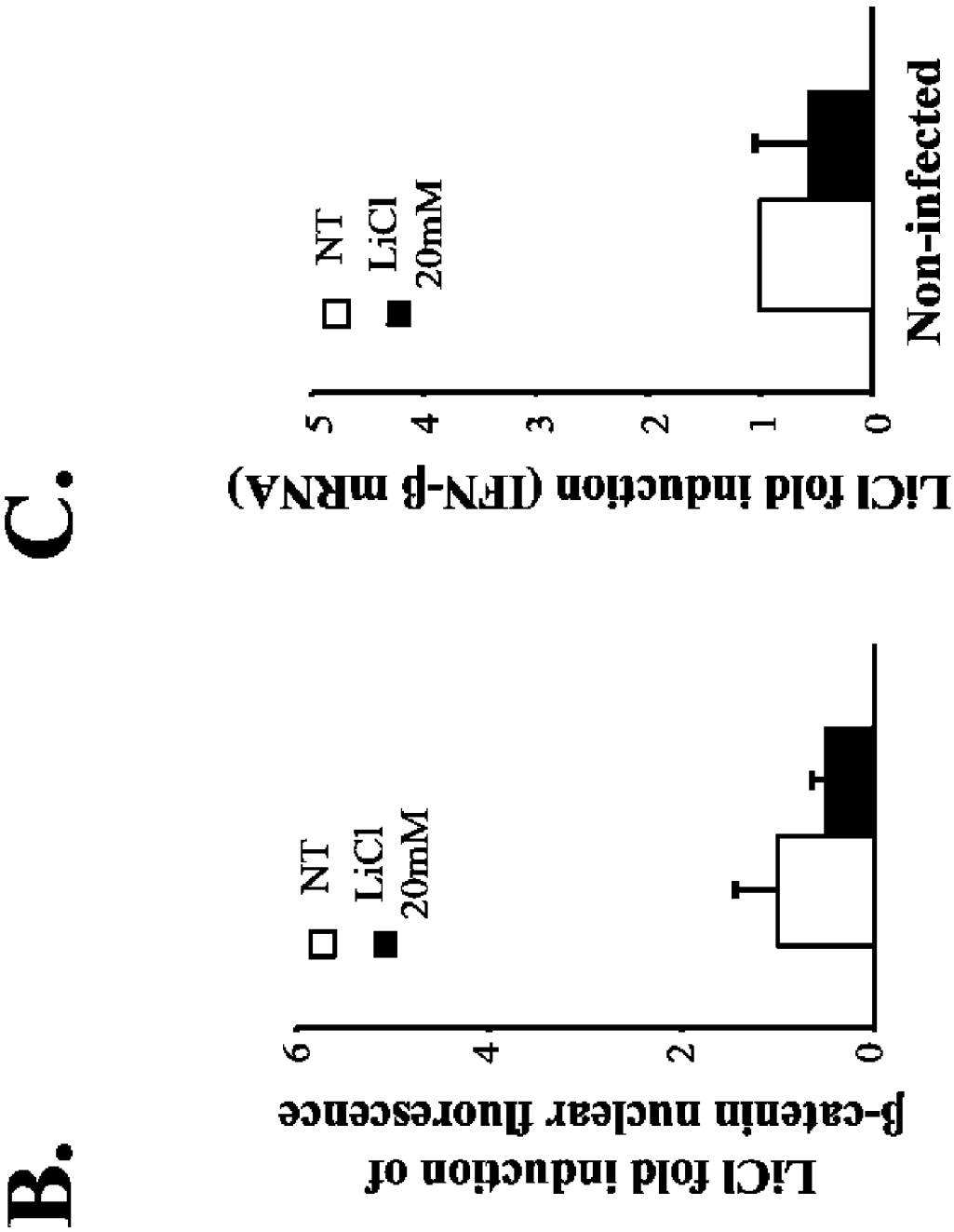
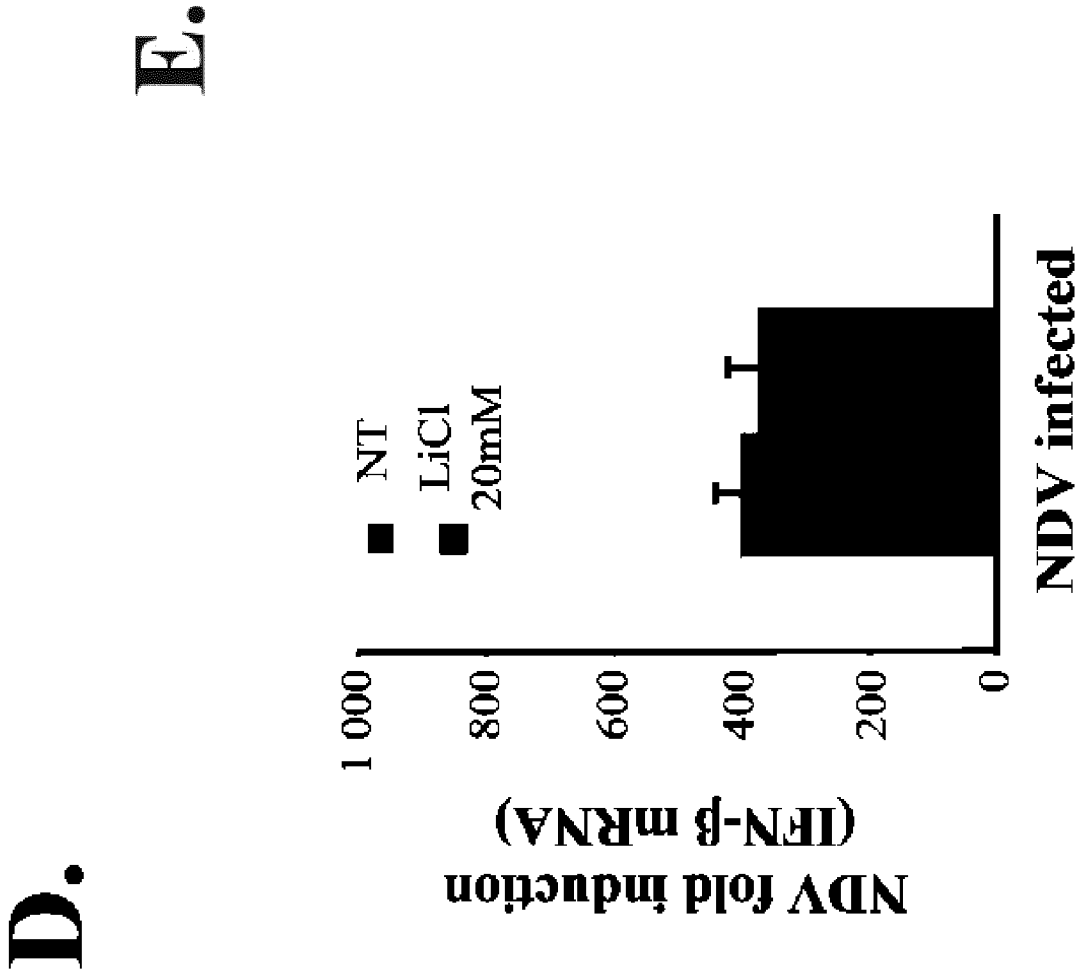
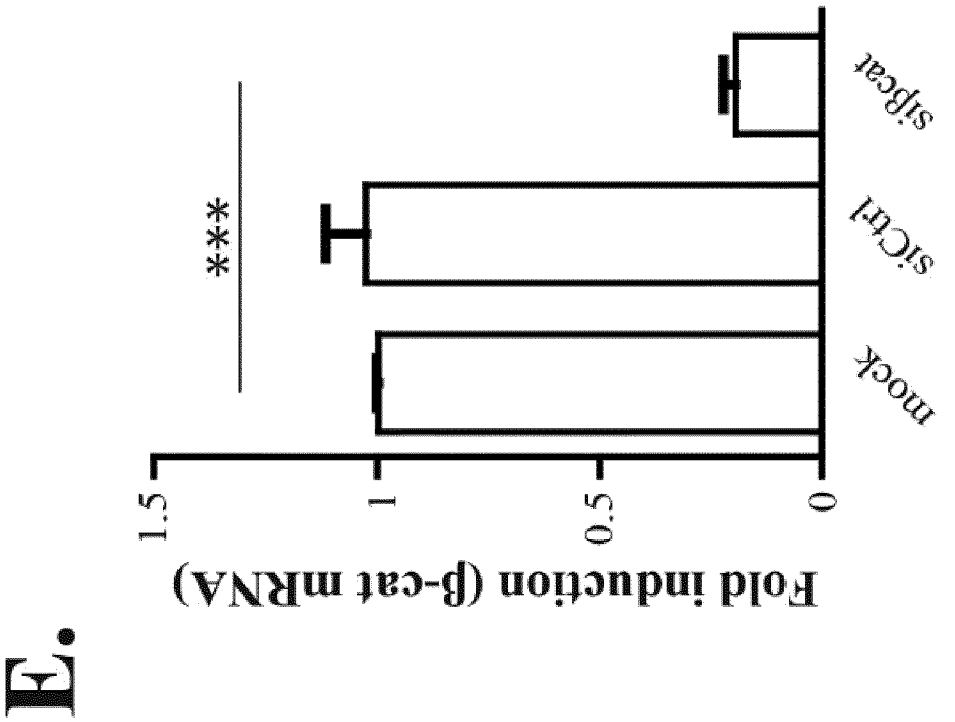
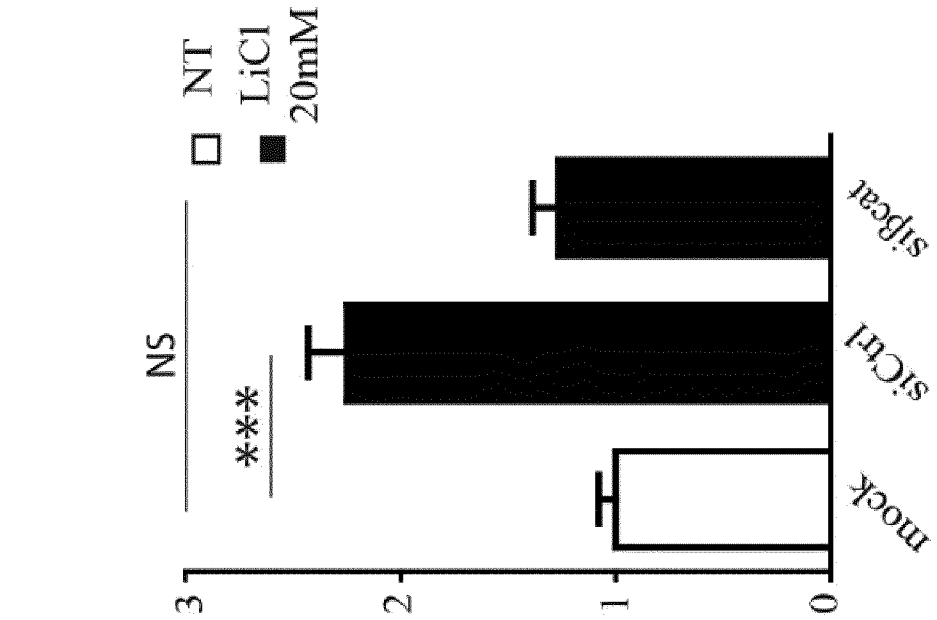


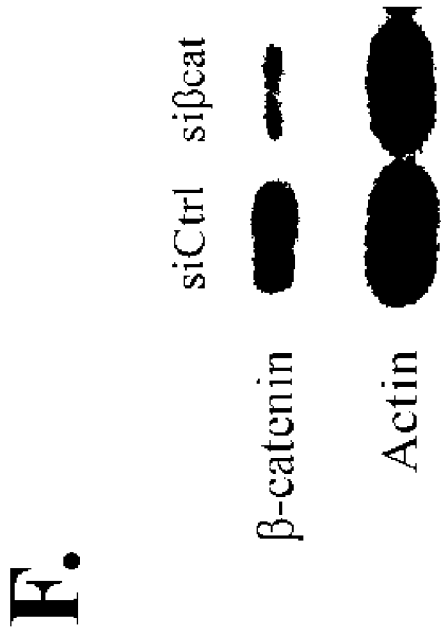
Figures 2B and 2C



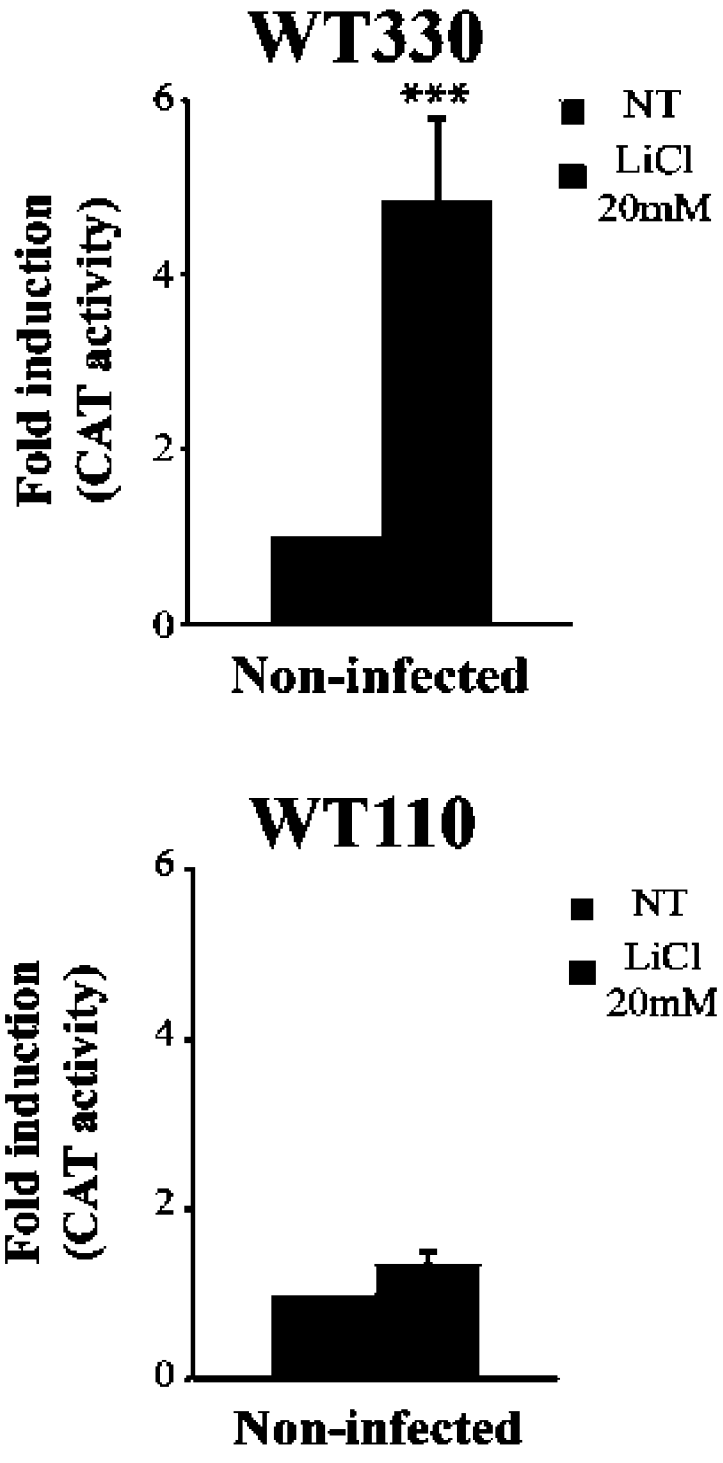


Figures 2D and 2E





Figures 2F and 2G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LiCl
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Fold induction
(CAT activity)
6
4
2
0
Non-infected
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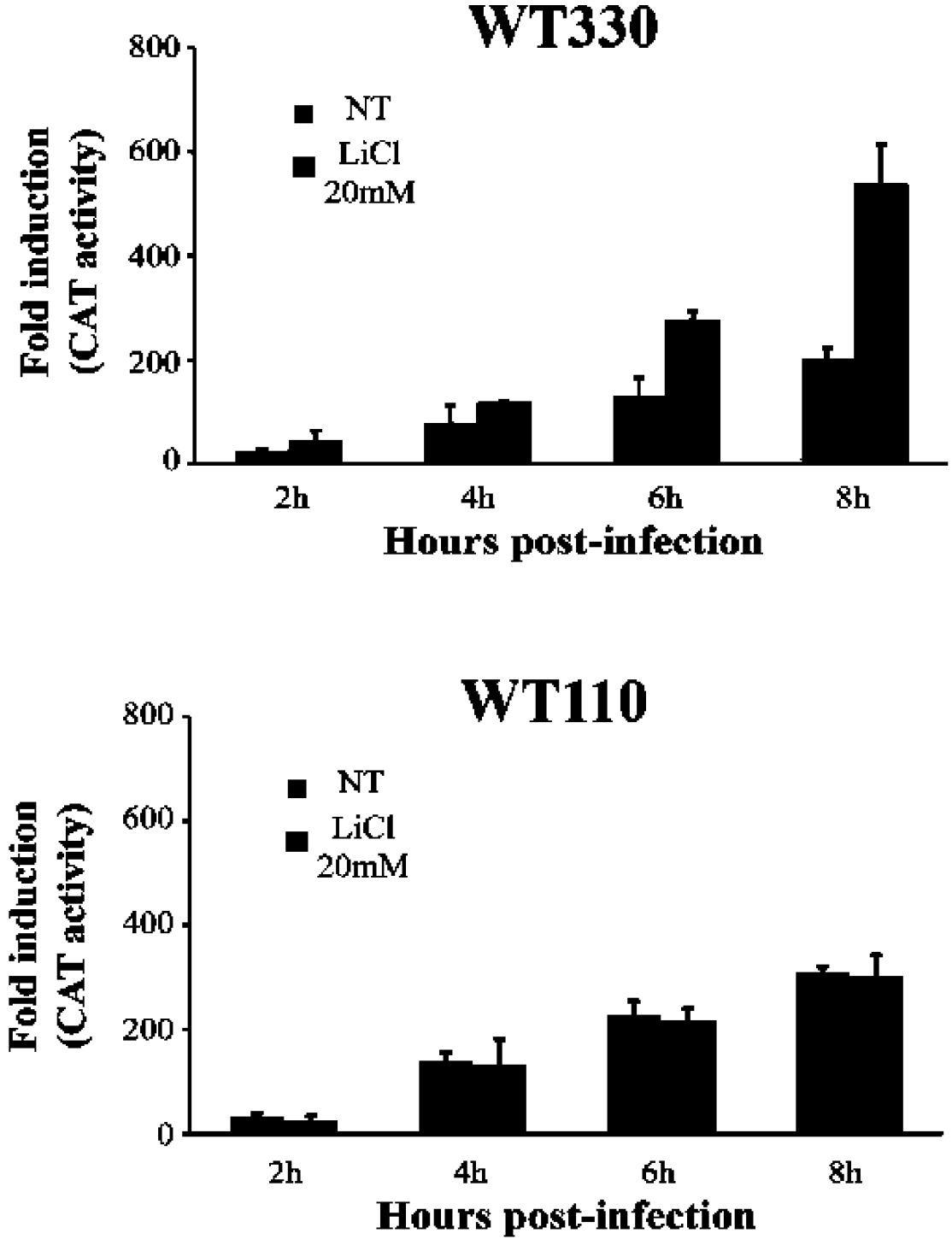
20mM
Fold induction
(CAT activity)
6
4
2
0
Non-infected

Figure 3A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Fold induction
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NT
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4h
6h
8h
Hours post-infection
WT110
800
600
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Fold induction
(CAT activity)
NT
LiCl
20mM
2h
4h
6h
8h
Hours post-infection

Figure 3B

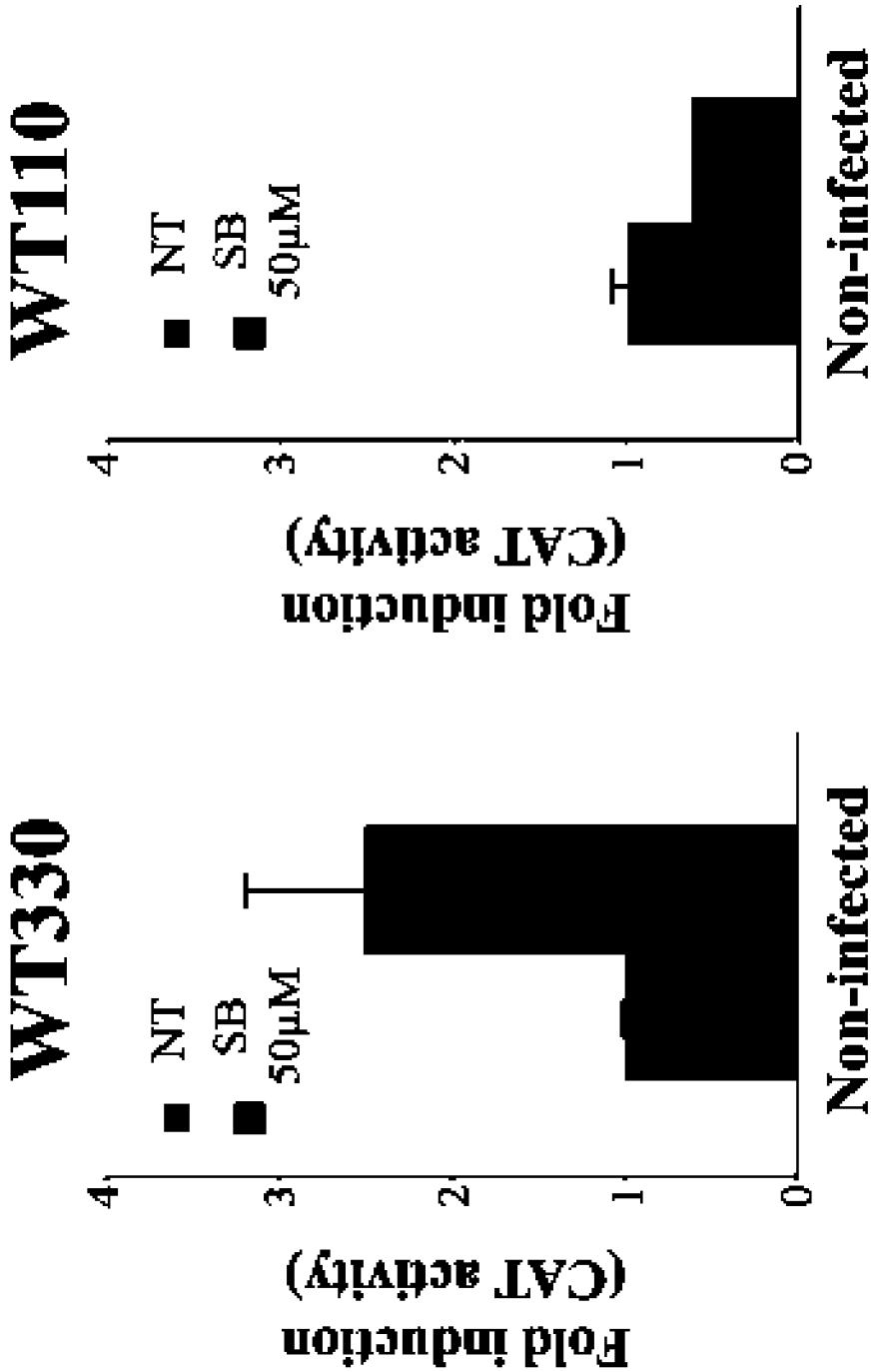
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Figure 3C

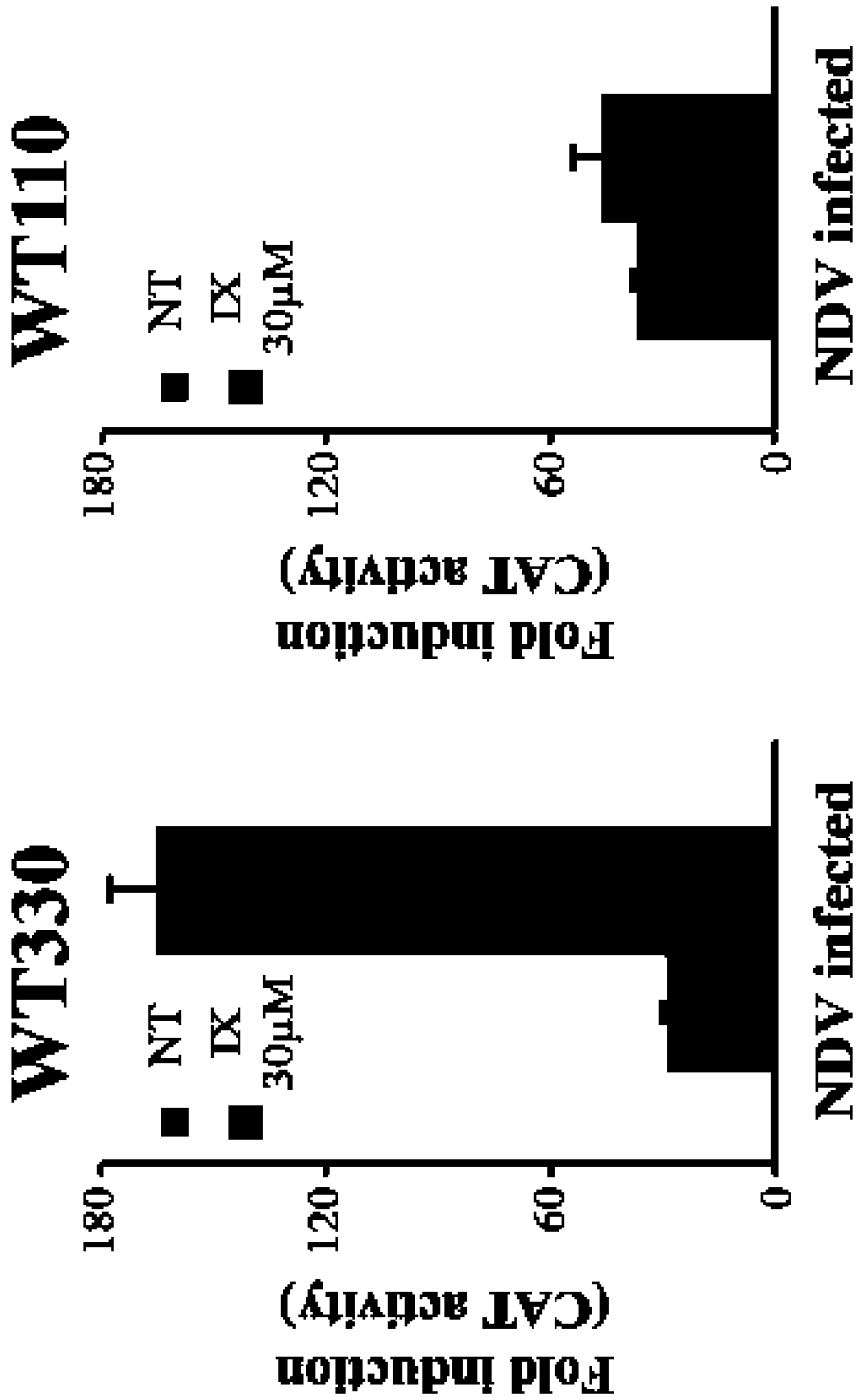
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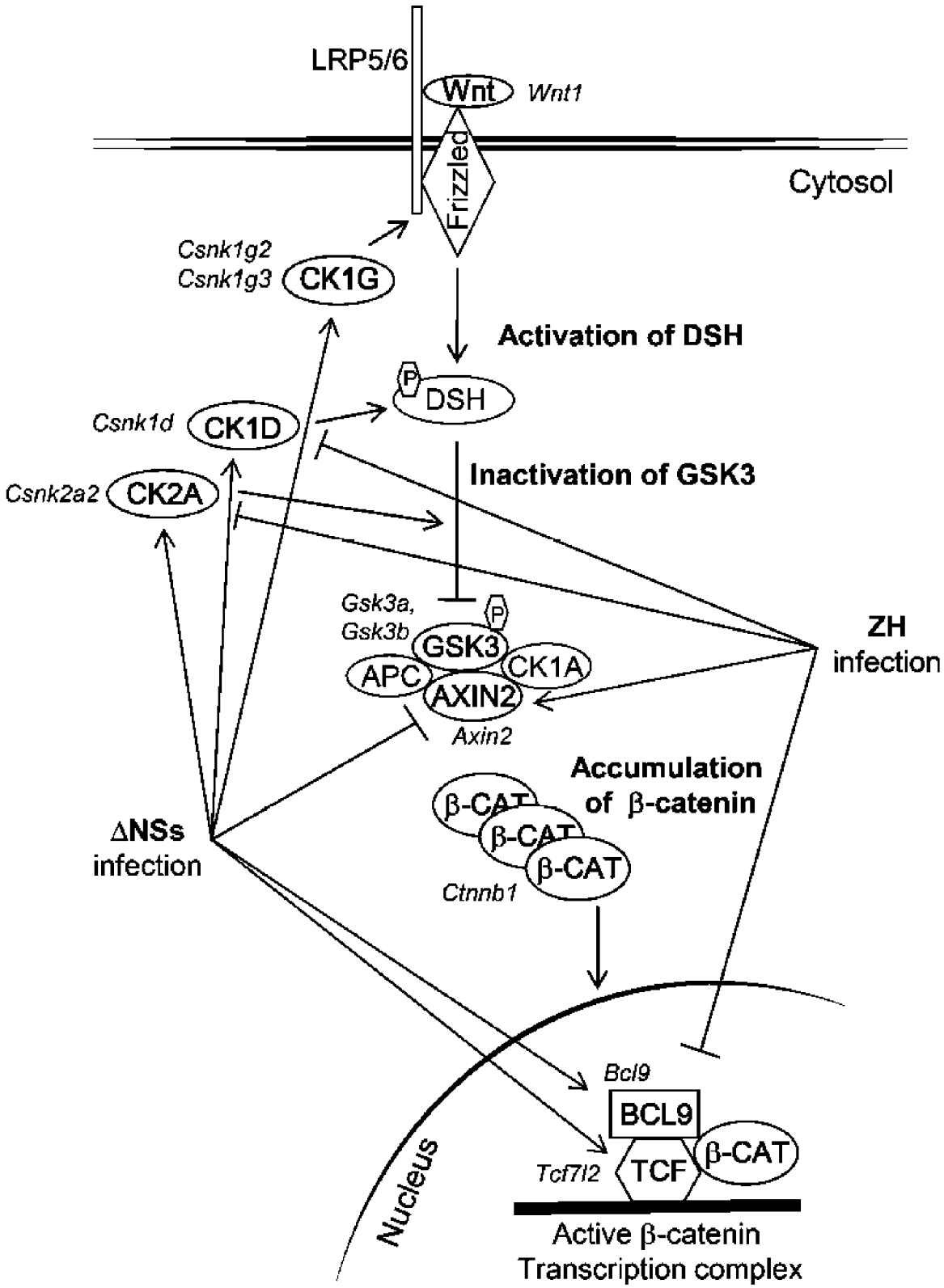
Figure 3D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Wnt1
Frizzled
Cytosol
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CK1G
Activation of DSH
P
DSH
Csnk1d
CK1D
Inactivation of GSK3
Csnk2a2
CK2A
Gsk3a,
Gsk3b
P
GSK3
CK1A
APC
AXIN2
Axin2
ZH
infection
Accumulation
of β-catenin
β-CAT
β-CAT
β-CAT
Ctnnb1
ΔNSs
infection
Bcl9
BCL9
β-CAT
Tcf7l2
TCF
Nucleus
Active β-catenin
Transcription complex

Figure 4

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2016/064696

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K31/404 A61K33/14
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, CHEM ABS Data, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CH 695 757 A5 (HEINZ FORSTER [CH]) 31 August 2006 (2006-08-31)	1-3
Y	example 5	4

Y	WO 2008/016994 A2 (UNIV RUSH MEDICAL CENTER [US]; STERN LENA [US]) 7 February 2008 (2008-02-07) the whole document, in particular the claims	4

Y	WO 98/17288 A1 (SCOTIA HOLDINGS PLC [GB]; GILBURT SIMON JOHN ARTHUR [GB]; HORROBIN DAV) 30 April 1998 (1998-04-30) the whole document, in particular the claims	4

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
17 August 2016	21/11/2016

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Albrecht, Silke

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Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	HONG-PING HAO ET AL: "LiCl inhibits PRRSV infection by enhancing Wnt/[beta]-catenin pathway and suppressing inflammatory responses", ANTIVIRAL RESEARCH, vol. 117, 5 March 2015 (2015-03-05), pages 99-109, XP055228441, NL ISSN: 0166-3542, DOI: 10.1016/j.antiviral.2015.02.010 figures 1,4-7 Chapter "Discussion" -----	4

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2016/064696

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-4(partially)

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-4(partially)

   A method of treating a viral infection in a subject in need thereof comprising administering to the subject a therapeutically effective amount of a GSK3 inhibitor, wherein the GSK3 inhibitor is lithium chloride and the viral infection is caused by a virus belonging to the group of Arenaviridae

   ---

2. claims: 1, 2, 4(all partially)

   A method of treating a viral infection in a subject in need thereof comprising administering to the subject a therapeutically effective amount of a GSK3 inhibitor, wherein the GSK3 inhibitor is lithium chloride and the viral infection is caused by a virus belonging to the group of Astroviridae

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3. claims: 1, 2, 4(all partially)

   A method of treating a viral infection in a subject in need thereof comprising administering to the subject a therapeutically effective amount of a GSK3 inhibitor, wherein the GSK3 inhibitor is lithium chloride and the viral infection is caused by a virus belonging to the group of Birnaviridae

   ---

4. claims: 1, 2, 4(all partially)

   A method of treating a viral infection in a subject in need thereof comprising administering to the subject a therapeutically effective amount of a GSK3 inhibitor, wherein the GSK3 inhibitor is lithium chloride and the viral infection is caused by a virus belonging to the group of Bromoviridae

   ---

5. claims: 1-4(partially)

   A method of treating a viral infection in a subject in need thereof comprising administering to the subject a therapeutically effective amount of a GSK3 inhibitor, wherein the GSK3 inhibitor is lithium chloride and the viral infection is caused by a virus belonging to the group of Bunyaviridae

   ---

6. claims: 1, 2, 4(all partially)

A method of treating a viral infection in a subject in need thereof comprising administering to the subject a therapeutically effective amount of a GSK3 inhibitor, wherein the GSK3 inhibitor is lithium chloride and the viral infection is caused by a virus belonging to the group of Caliciviridae

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7. claims: 1, 2, 4(all partially)

A method of treating a viral infection in a subject in need thereof comprising administering to the subject a therapeutically effective amount of a GSK3 inhibitor, wherein the GSK3 inhibitor is lithium chloride and the viral infection is caused by a virus belonging to the group of Closteroviridae

---

8. claims: 1, 2, 4(all partially)

A method of treating a viral infection in a subject in need thereof comprising administering to the subject a therapeutically effective amount of a GSK3 inhibitor, wherein the GSK3 inhibitor is lithium chloride and the viral infection is caused by a virus belonging to the group of Comoviridae

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9. claims: 1, 2, 4(all partially)

A method of treating a viral infection in a subject in need thereof comprising administering to the subject a therapeutically effective amount of a GSK3 inhibitor, wherein the GSK3 inhibitor is lithium chloride and the viral infection is caused by a virus belonging to the group of Cystoviridae

---

10. claims: 1-4(partially)

A method of treating a viral infection in a subject in need thereof comprising administering to the subject a therapeutically effective amount of a GSK3 inhibitor, wherein the GSK3 inhibitor is lithium chloride and the viral infection is caused by a virus belonging to the group of Flaviviridae

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11. claims: 1, 2, 4(all partially)

A method of treating a viral infection in a subject in need thereof comprising administering to the subject a therapeutically effective amount of a GSK3 inhibitor, wherein the GSK3 inhibitor is lithium chloride and the viral infection is caused by a virus belonging to the group of

Flexiviridae

---

12. claims: 1-4(partially)

A method of treating a viral infection in a subject in need thereof comprising administering to the subject a therapeutically effective amount of a GSK3 inhibitor, wherein the GSK3 inhibitor is lithium chloride and the viral infection is caused by a virus belonging to the group of Hepevirus

---

13. claims: 1, 2, 4(all partially)

A method of treating a viral infection in a subject in need thereof comprising administering to the subject a therapeutically effective amount of a GSK3 inhibitor, wherein the GSK3 inhibitor is lithium chloride and the viral infection is caused by a virus belonging to the group of Leviviridae

---

14. claims: 1, 2, 4(all partially)

A method of treating a viral infection in a subject in need thereof comprising administering to the subject a therapeutically effective amount of a GSK3 inhibitor, wherein the GSK3 inhibitor is lithium chloride and the viral infection is caused by a virus belonging to the group of Luteoviridae

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15. claims: 1-4(partially)

A method of treating a viral infection in a subject in need thereof comprising administering to the subject a therapeutically effective amount of a GSK3 inhibitor, wherein the GSK3 inhibitor is lithium chloride and the viral infection is caused by a virus belonging to the group of Mononegavirales including Paramyxoviridae

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16. claims: 1, 2, 4(all partially)

A method of treating a viral infection in a subject in need thereof comprising administering to the subject a therapeutically effective amount of a GSK3 inhibitor, wherein the GSK3 inhibitor is lithium chloride and the viral infection is caused by a virus belonging to the group of Mosaic Viruses

---

17. claims: 1-4(partially)

A method of treating a viral infection in a subject in need thereof comprising administering to the subject a therapeutically effective amount of a GSK3 inhibitor, wherein the GSK3 inhibitor is lithium chloride and the viral infection is caused by a virus belonging to the group of Nidovirales

---

18. claims: 1, 2, 4(all partially)

A method of treating a viral infection in a subject in need thereof comprising administering to the subject a therapeutically effective amount of a GSK3 inhibitor, wherein the GSK3 inhibitor is lithium chloride and the viral infection is caused by a virus belonging to the group of Nodaviridae

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19. claims: 1-4(partially)

A method of treating a viral infection in a subject in need thereof comprising administering to the subject a therapeutically effective amount of a GSK3 inhibitor, wherein the GSK3 inhibitor is lithium chloride and the viral infection is caused by a virus belonging to the group of Orthomyxoviridae

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20. claims: 1, 2, 4(all partially)

A method of treating a viral infection in a subject in need thereof comprising administering to the subject a therapeutically effective amount of a GSK3 inhibitor, wherein the GSK3 inhibitor is lithium chloride and the viral infection is caused by a virus belonging to the group of Picobirnavirus

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21. claims: 1-4(partially)

A method of treating a viral infection in a subject in need thereof comprising administering to the subject a therapeutically effective amount of a GSK3 inhibitor, wherein the GSK3 inhibitor is lithium chloride and the viral infection is caused by a virus belonging to the group of Picornaviridae

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22. claims: 1, 2, 4(all partially)

A method of treating a viral infection in a subject in need thereof comprising administering to the subject a therapeutically effective amount of a GSK3 inhibitor, wherein the GSK3 inhibitor is lithium chloride and the viral infection is caused by a virus belonging to the group of

Potyviridae

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23. claims: 1, 2, 4(all partially)

A method of treating a viral infection in a subject in need thereof comprising administering to the subject a therapeutically effective amount of a GSK3 inhibitor, wherein the GSK3 inhibitor is lithium chloride and the viral infection is caused by a virus belonging to the group of Reoviridae

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24. claims: 1-4(partially)

A method of treating a viral infection in a subject in need thereof comprising administering to the subject a therapeutically effective amount of a GSK3 inhibitor, wherein the GSK3 inhibitor is lithium chloride and the viral infection is caused by a virus belonging to the group of Retroviridae

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25. claims: 1, 2, 4(all partially)

A method of treating a viral infection in a subject in need thereof comprising administering to the subject a therapeutically effective amount of a GSK3 inhibitor, wherein the GSK3 inhibitor is lithium chloride and the viral infection is caused by a virus belonging to the group of Sequiviridae

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26. claims: 1, 2, 4(all partially)

A method of treating a viral infection in a subject in need thereof comprising administering to the subject a therapeutically effective amount of a GSK3 inhibitor, wherein the GSK3 inhibitor is lithium chloride and the viral infection is caused by a virus belonging to the group of Tenuivirus

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27. claims: 1-4(partially)

A method of treating a viral infection in a subject in need thereof comprising administering to the subject a therapeutically effective amount of a GSK3 inhibitor, wherein the GSK3 inhibitor is lithium chloride and the viral infection is caused by a virus belonging to the group of Togaviridae

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28. claims: 1, 2, 4(all partially)

A method of treating a viral infection in a subject in need thereof comprising administering to the subject a therapeutically effective amount of a GSK3 inhibitor, wherein the GSK3 inhibitor is lithium chloride and the viral infection is caused by a virus belonging to the group of Tombusviridae

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29. claims: 1, 2, 4(all partially)

A method of treating a viral infection in a subject in need thereof comprising administering to the subject a therapeutically effective amount of a GSK3 inhibitor, wherein the GSK3 inhibitor is lithium chloride and the viral infection is caused by a virus belonging to the group of Totiviridae

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30. claims: 1, 2, 4(all partially)

A method of treating a viral infection in a subject in need thereof comprising administering to the subject a therapeutically effective amount of a GSK3 inhibitor, wherein the GSK3 inhibitor is lithium chloride and the viral infection is caused by a virus belonging to the group of Tymoviridae

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31. claims: 1-4(partially)

A method of treating a viral infection in a subject in need thereof comprising administering to the subject a therapeutically effective amount of a GSK3 inhibitor, wherein the GSK3 inhibitor is lithium chloride and the viral infection is caused by a virus belonging to the group of Hepadnaviridae

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32. claims: 1, 2, 4(all partially)

A method of treating a viral infection in a subject in need thereof comprising administering to the subject a therapeutically effective amount of a GSK3 inhibitor, wherein the GSK3 inhibitor is lithium chloride and the viral infection is caused by a virus belonging to the group of Herpesviridae

---

33. claims: 1, 2, 4(all partially)

A method of treating a viral infection in a subject in need thereof comprising administering to the subject a therapeutically effective amount of a GSK3 inhibitor, wherein the GSK3 inhibitor is lithium chloride and the viral infection is caused by a virus belonging to the group of

Papillomaviridae

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34. claims: 1, 3, 4(all partially)

A method of treating a viral infection in a subject in need thereof comprising administering to the subject a therapeutically effective amount of a GSK3 inhibitor, wherein the GSK3 inhibitor is lithium chloride and the viral infection is caused by a virus belonging to a group other than the virus groups mentioned in inventions 1-33

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35. claims: 1-3(partially)

A method of treating a viral infection in a subject in need thereof comprising administering to the subject a therapeutically effective amount of a GSK3 inhibitor, wherein the GSK3 inhibitor is an organic compound mentioned on pages 5-10 of the description of the present application

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2016/064696

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
CH 695757 A5	31-08-2006	NONE	
WO 2008016994 A2	07-02-2008	US 2008193563 A1	14-08-2008
		WO 2008016994 A2	07-02-2008
WO 9817288 A1	30-04-1998	AU 4715397 A	15-05-1998
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