(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
24 January 2013 (24.01.2013)

(10) International Publication Number
WO 2013/013118 A1

(51) International Patent Classification:
C12N 7/06 (2006.01)

(21) International Application Number:
PCT/US2012/047534

(22) International Filing Date:
20 July 2012 (20.07.2012)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/510,298 21 July 2011 (21.07.2011) US

(71) Applicant *(for all designated States except US)*: **THE TRUSTEES OF PRINCETON UNIVERSITY** [US/US]; 4 New South Building, Princeton, NJ 08544 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **SHENK, Thomas** [US/US]; 12 Boudinot Street, Princeton, NJ 08540 (US). **GUDLESKI, Nicole** [US/US]; 1601 Aspen Drive, Plainsboro, NJ 08536 (US). **GRECO, Todd** [US/US]; 226 West Rittenhouse Sq., Apt 216, Philadelphia, PA 19103 (US). **CRISTEA, Ileana** [US/US]; 73 Coppermine Road, Princeton, New Jersey 08540 (US).

(74) Agent: **WILLIAMS, JR., Joseph, A.**; Marshall, Gerstein & Borun LLP, 233 S. Wacker Drive, 6300 Willis Tower, Chicago, IL 60606-6357 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

WO 2013/013118 A1

(54) **Title**: METHOD TO INCREASE THE INFECTIVITY OF VIRUS PARTICLES

(57) **Abstract**: A method for enhancing infectivity of HCMV virus particles is provided.

METHOD TO INCREASE THE INFECTIVITY OF VIRUS PARTICLES

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application claims priority of U.S. Provisional Patent Application No. 61/510,298, filed July 23, 2011, the disclosure of which is incorporated herein by reference in its entirety.

[0002] This invention was made with government support under grant numbers CA82396 and DP1DA026192 awarded by the National Institutes of Health. The government has certain rights in the invention.

BACKGROUND

[0003] Since the ability to obtain adequate yields of infectious virus can limit vaccine manufacturing, improved methods of virus production are always needed to meet an important industrial and medical need. Previous work (Munger et al., *PLoS Pathog* 2:e132, 2006; Munger et al., *Nat Biotech* 26:1179-86, 2008)) has demonstrated that human cytomegalovirus (HCMV) induces the synthesis of fatty acids, and, importantly, that the virus requires the *de novo* synthesis of fatty acids to generate an optimal yield of infectious progeny. Despite this understanding, US Patent No. 5,360,736 discloses that the addition of lipids during growth of certain viruses, and in particular after initiation of infection of the cultured cells, inhibits virus production.

[0004] Preparation of stock virus is necessary for development of therapeutic methods and materials. Accordingly, improved methods for virus production are useful for improving the yield of infectious virus, and more specifically for vaccine production.

DESCRIPTION OF THE INVENTION

[0005] A method for enhancing the infectivity of HCMV virus particles is described. The method of the disclosure is useful to increase infectivity of HCMV particles in a "live" virus preparation, and in particular, a vaccine, providing a manufacturing advantage over methods known in the art. In general, a method is provided for production of virus particles in a virus-infected host cell comprising the step of contacting the cell with a compound that induces the production of virus particles with increased infectivity, wherein the progeny virus particles produced in the cell in the presence of the compound display increased ability to successfully initiate infection of cells compared to virus particles produced in the cells in the absence of the compound. It will be readily apparent that each aspect of each embodiment of the method described herein is embraced and attaches to this general method. Production of virus is measured, in various aspects, by (i) the number of infectious virus particles, (ii) the number of virus particles, infectious and non-infectious, (iii) an amount of a specific viral antigen, and/or (iv) combinations of (i)-(iii).

[0006] In one embodiment, a method is provided for production of virus particles in a virus-infected host cell comprising the step of contacting the cell with a compound that decreases activity of low density lipoprotein related receptor 1 (LRP1), wherein the virus particles produced in the cell in the presence of the compound display increased infectivity compared to virus particles produced in the cell in the absence of the compound. In various aspects, the compound decreases LRP1 binding activity, the compound binds LRP1 and blocks LRP1 binding activity, the compound is a blocking antibody, and/or the compound is an extracellular fragment of LRP1. In various aspects, the compound decreases transcription and/or translation of a polynucleotide encoding LRP1, the compound decreases surface expression of LRP1, the compound decreases mobilization of LRP1 to a cell membrane, and/or the compound induces an inactive conformation of LRP1 at a cell membrane. LRP1 is also known as alpha-2-macroglobulin receptor (A2MR), apolipoprotein E receptor (APOER) or cluster of differentiation 91 (CD91). LRP1 has been shown to interact with, at least, apolipoprotein E, lipoprotein lipase, urokinase receptor, MAPK8IP2, tissue plasminogen activator, thrombospondin 1, SYNJ2BP, DLG4, NOS1AP, calreticulin, APBB1, ITGB1BP1, MAPK8IP1, GIPC1 and SHC1. Accordingly, LRP1 binding activity includes interaction with one or more of these proteins and any other LRP1 binding partner.

[0007] Also provided is a method for production of virus particles in a virus-infected host cell comprising the step of contacting the cell with a compound that increases intracellular cholesterol, wherein the virus particles produced in the cell in the presence of the compound display increased infectivity compared to virus particles produced in the cell in the absence of the compound. In various aspects, the compound increases cholesterol import into a cell, decreases cholesterol export from a cell, or both. In various aspects of the method, the compound modulates LDL-cholesterol receptor (LDLR) activity, the compound modulates ABCA1 activity, the compound is cholesterol, a cholesterol derivative or a cholesterol ester at an amount effective to increase intracellular levels of cholesterol. In various aspects, the compound decreases LRP1 activity, the compound decreases LRP1 binding activity, the compound binds LRP1 and blocks LRP1 binding activity, the compound is a blocking antibody, the compound is an extracellular fragment of LRP1, the compound decreases transcription and/or translation of a polynucleotide encoding LRP1, and/or the compound decreases surface expression of LRP1. In aspects utilizing cholesterol, a cholesterol derivative, or a cholesterol ester, the cholesterol, cholesterol derivative, or cholesterol ester is present at a concentration of at least 5 μM, at least 10 μM, at least 15 μM, at least 20 μM, at least 25μM, at least 30 μM, at least 35 μM, at least 40 μM, at least 45 μM, at least 50 μM, at least 55 μM, at least 60 μM, at least 65 μM, at least 70 μM, at least 75 μM, at least 80 μM, at least 85μM, at least 90 μM, at least 95 μM, at least 100 μM, at least 110 μM, at least 120 μM, at least 130 μM, at least 140 μM, at least 150 μM or more but at a concentration that is not toxic to the host cell. In various aspects, the method utilizes cholesterol that is present at a concentration of no more than 5 μM, no more than 10 μM, no more than 15 μM, no more than 20 μM, no more than 25μM, no more than 30 μM, no more than 35 μM, no more than 40 μM, no more than 45 μM, no more than 50 μM, no more than 55 μM, no more than 60 μM, no more than 65 μM, no more than 70 μM, no more than 75 μM, no more than 80 μM, no more than 85μM, no more than 90 μM, no more than 95 μM, no more than 100 μM, no more than 110 μM, no more than 120 μM, no more than 130 μM, no more than 140 μM, or no more than 150 μM.

[0008] In various aspects of each embodiment of the method provided, the cell is contacted with the compound prior to virus infection, the cell is contacted with the compound after virus infection or the cell is contacted with the compound concurrently with virus infection.

[0009] In various aspects of each embodiment of the method, the virus is an enveloped virus, the virus is an enveloped DNA virus or an enveloped RNA virus, the virus is a herpes virus, the virus is an alpha family herpes virus, the virus is a beta family herpes virus, the virus is an gamma family herpes virus, the virus is CMV, and/or the virus is a pox virus, a non-envelope picorna virus, an RNA virus influenza virus, herpes simplex virus, Epstein Barr virus, hepatitis C virus, Dengue virus, HIV, mumps virus, measles virus, and/or parainfluenza virus.

[0010] In various aspects of each embodiment of the method, the cell is a eukaryotic cell, the cell is a mammalian cell or the cell is a human cell.

[0011] In each embodiment of the method provided, in various aspects, the method optionally further comprises the step of isolating the virus from medium of cell growth. In various aspects, the method further comprises the step of isolating the virus from the host cell.

[0012] In each embodiment of the method provided, in various aspects, the method optionally, comprises the step of infecting the host cells with the virus. In various aspects, the host cell is infected at a multiplicity of infection (MOI), including e.g. and without limitation, 0.0001, 0.001, 0.01, 0.1, 1.0, 3, 5, 10 or more infectious units per cell.

[0013] In each embodiment of the method provided, in various aspects, the method optionally further comprises the step of growing the host cells to confluence, to about 90% , about 80% confluence, about 70% confluence, about 60% confluence, about 50% confluence, or less than 50% confluence prior to infecting the host cells with the virus.

[0014] In each embodiment of the method provided, in various aspects, the method optionally further comprises the step of culturing the host cells after infecting the host cells with the virus.

[0015] In each embodiment of the method provided, in various aspects, the method optionally further comprises the step of adding or changing medium of growth for the host cells prior to isolating the virus.

[0016] In each embodiment of the method provided, in various aspects, the method optionally further comprises the step of incubating the host cells with an infecting virus for an adsorption period.

DESCRIPTION OF THE DRAWINGS

[0017] Fig. 1. Strategy to identify PM protein alterations in HCMV-infected cells.

[0018] Fig. 2. LRP1 levels are induced after infection, and LRP1 modulates the production of HCMV progeny.

[0019] Fig. 3. LRP1 knockdown has little effect on the accumulation viral proteins and DNA, but causes a dramatic increase in virion infectivity.

[0020] Fig. 4. Cholesterol content is increased in LRP1 knockdown cells via increased cholesterol import, not de novo synthesis.

[0021] Fig. 5. Cholesterol content in virus particles regulates virus infectivity.

[0022] Fig. 6. Infectivity of cholesterol-depleted virus can be restored by circumventing the fusion step.

EXAMPLES

[0023] The invention is further described in the following examples. The examples serve only to illustrate the invention and are not intended to limit the scope of the invention in any way.

Example 1

[0024] The majority of disease biomarkers and drug targets are membrane proteins, and previous studies have demonstrated changes occur at the surface of virus-infected cells.

[0025] Consequently, an experiment was performed to identify changes in cellular plasma membrane (PM) protein expression following virus infection. The PM proteome of uninfected fibroblasts was compared to that of HCMV-infected fibroblasts using mass spectrometry (MS) analysis of affinity purified PM proteins. The experimental design is outlined in Figure 1.

[0026] In short, MRC5 fibroblasts were mock infected or infected with HCMV strain AD169 at a multiplicity of 5 pfu/cell. After 6, 24, or 72 hours, cell surface proteins were biotinylated, lysed in detergents and sonicated. Biotinylated proteins were bound to an avidin matrix, and samples were eluted with DTT. Filter-aided sample preparation (FASP) was used to concentrate the sample, remove detergents, and unfold the proteins. Isolated proteins were digested with trypsin, and peptides were analyzed by mass spectrometry (MS).

[0027] Results from mass spectrometry analysis identified multiple PM proteins whose levels changed after infection. One of proteins was the low density lipoprotein related receptor 1 – LRP1, for recent reviews of LRP1 function see (Lillis, Van Duyn et al. 2008; Boucher and Herz 2010; Gaultier, Simon et al. 2010).

Example 2

[0028] Western blot analysis was then carried out to confirm these initial results. Fibroblasts were mock infected or infected at a multiplicity of 5 pfu/cell with HCMV strain AD169, and the level of LRP1 in whole cell extracts (WCL) and plasma membrane (PM) were determined by western blot at 6, 12, 24, 48 and 72 hours later. β-actin and insulin receptor (IR) levels were assayed as controls.

[0029] Results from the western blot analysis confirmed that total cellular and PM-associated LRP1 was elevated at 6, 24 and 48 hours after infection (Fig. 2A).

[0030] Since protein level changed, the possibility that LRP1 performs an important function during HCMV infection was tested using siRNA knockdown to reduce protein level or a blocking/neutralizing antibody to decrease protein activity.

[0031] At 24 hours after siRNA transfection or 1 hour after antibody treatment, fibroblasts were infected at a multiplicity of 0.1 PFU/cell with HCMV strain AD169. 96 hours later, virus in culture medium was quantified by $TCID_{50}$ assay. IE2-specific siRNA served as a positive control; scrambled (Scr) and GFP-specific siRNAs served as negative controls; tubulin-specific antibody served as a negative control. ** P<0.001 (t-test, compared to scrambled siRNA or tubulin antibody).

[0032] Knockdown of LRP1 protein levels with siRNA increased the production of HCMV by more than a factor of 10 (Fig. 2B), and a similar increase was observed when LRP1 antibody (Orr, Pedraza et al. 2003) was used to neutralize LRP1 activity (Fig. 2C). Error bars represent the standard errors of the means from three independent experiments, each performed in triplicate. These experiments demonstrate that LRP1 restricts or inhibits the production of HCMV infectivity, and infectivity can be increased if the level of LRP1 protein or its activity is reduced.

Example 3

[0033] In order to determine whether the observed change in infectivity of the virus results from a change in actual virus particle number, the following experiment was carried out.

[0034] LRP1-specific siRNA-treated fibroblasts were infected with HCMV strain AD169 at a multiplicity of 5 PFU/cell. Cells were harvested at the indicated times, and the accumulation of IE1 (HCMV immediate-early protein), pUL44 (HCMV early protein), pUL99 (HCMV late protein), β-actin (loading control), and LRP1 were analyzed by Western blot. hpi=hours post infection.

[0035] Under conditions where the production of HCMV infectivity was enhanced by siRNA-mediated knockdown of LRP1, little difference was observed in the accumulation of a representative immediate-early (IE1), early (pUL44) or late (pUL99) viral protein (Fig. 3A).

[0036] Particle to PFU ratios were calculated for partially purified virions by dividing the amount of DNA in virus particles (quantified by qPCR with UL123-specific primers) by the virus titer (quantified by $TCID_{50}$). Error bars represent the standard errors of the means from two independent experiments, each performed in triplicate. ** P<0.001 (t-test).

[0037] The particle/PFU ratio decreased by a factor of about 10 (Fig. 3B), demonstrating that viral particles are more infectious when made in cells with reduced LRP1 activity.

Example 4

[0038] LRP1 has been shown to prevent intracellular accumulation of free cholesterol and cholesterol esters by stimulating a Wnt5a signaling pathway (Terrand, Bruban et al. 2009). In order to determine whether the change in LRP1 activity seen with virus infection alters cellular cholesterol, the following experiment was carried out.

[0039] MRC5 fibroblasts were left untreated, transfected with siRNAs (LRP1 or scrambled control), or treated with antibodies (LRP1 or tubulin), then left uninfected or infected with HCMV strain AD169 at a multiplicity of 0.1 or 5 pfu/cell. At 24 (A) or 96 (B) hpi, the cholesterol content of MRC5 cells was quantified using the amplex red (Invitrogen) specrofluorometric assay. Total cholesterol corresponds to free + esterified cholesterol per 100,000 MRC5 cells.

[0040] Results demonstrated that cellular cholesterol levels were increased by treatment of infected cells with LRP1-specific siRNA or LRP1 antibody (Fig. 4A and B).

[0041] In order to assess the mechanism by which cellular cholesterol changes, the following experiments were carried out.

[0042] In a first experiment, MRC5 cells were left untreated, treated with Mevastatin or mock treated. At 90 minutes post Mevastatin treatment, cells were treated with antibody to LRP1 or tubulin or left untreated. 1hour post Mevastatin treatment, cells were infected at a multiplicity of 0.1 PFU/cell. At 96 hpi, released virus was used to infect new cells.

[0043] In a second experiment, cells were serum starved for 48 hours then treated with indicated antibodies. 1 hour post antibody treatment, serum-starved, confluent MRC5 cells were treated with various concentrations of cholesterol (+ fatty acid-free BSA carrier) for 1 hour then infected at a multiplicity of 0.1 PFU/cell. At 96 hpi, released virus was used to infect new cells. “N” indicates no added cholesterol. Cell-free virus was quantified by a $TCID_{50}$ assay. Data represents the mean and standard deviation from three independent experiments with three replicates each.

[0044] Results showed that intracellular cholesterol levels influenced the production of HCMV infectivity in two ways. First, cells were treated with Mevastatin to inhibit HMG-CoA reductase and thereby block *de novo* cholesterol biosynthesis within infected cells; and, although the effect was modest, the drug inhibited the production of infectivity in a dose-dependent manner (Fig. 4C). Second, cultures were supplemented with cholesterol and the supplementation substantially enhanced the production of HCMV infectivity by cells that were also treated with LRP1-specific antibody (Fig. 4D). Mevastatin reversed the increased infectivity caused by inhibition of LRP1 activity to a limited extent and cholesterol supplementation demonstrated that LRP1 had little effect in the absence of extracellular cholesterol. Thus, cholesterol import is at least partly responsible for increased infectivity produced when LRP1 function is antagonized.

Example 5

[0045] Previous studies have shown that ABCA1 (a cellular cholesterol export receptor) expression is positively regulated by LRP1 (Zhou, Choi et al. 2009) and the low density lipoprotein receptor (LDLR) PM localization is negatively regulated by LRP1 (Zhou, Takayama et al. 2009). Accordingly, experiments were designed to test whether the increased cholesterol import and overall increase in intracellular cholesterol content in LRP1 knockdown cells was due to altered expression of these proteins.

[0046] Protein levels of LDLR were upregulated and ABCA1 were downregulated upon LRP1 knockdown (data not shown). Virus infection alone has the same effect as LRP1 knockdown, but to a lesser degree (data not shown). Further, it was observed that siRNA knockdown of LDLR partially reversed the increase in infectivity and intracellular cholesterol content resulting from LRP1 knockdown. Together, these results suggest that altered expression of LDLR and ABCA1 caused by LRP1 knockdown result in an overall increase in intracellular cholesterol levels with an increase in virion infectivity.

Example 6

[0047] Due to the ability to sequester cholesterol in its hydrophobic pocket, methyl-ß-cyclodextrin (MCβD) removes cholesterol from membranes (Christian, Haynes et al. 1997). Thus, in order to directly test the role of virion envelope cholesterol in HCMV infection, purified virions were treated with various concentrations of MCβD to deplete cholesterol.

[0048] Gradient purified HCMV strain AD169 virions were either mock treated or treated with increasing concentrations of MCβD for 30 min at 37°C. MCβD (50mM) treated virus was incubated in varying concentrations of cholesterol for 30 min at 37°C. Virus (no MCβD treatment) was incubated in varying concentrations of cholesterol for 30min at 37°C. In all experiments, infectivity (Fig. 5, left panels) and cholesterol content (Fig. 5, right panels) were determined for the treated samples. Infectivity of untreated virus was set to 100%. Virus cholesterol content was determined using the amplexred (Invitrogen) specrofluorometric assay.

[0049] Results showed that incubation of virus particles with MCβD resulted in a dose-dependent inhibition of HCMV infectivity (Fig. 5A, left).

[0050] To confirm that MCβD treatment depleted virus of envelope cholesterol, the cholesterol content of MCβD-depleted virus was determined (Fig. 5A, right). Treatment achieved a dose-dependent decrease in cholesterol content, with ~90% depletion obtained with 50 mM MCβD, thus confirming that MCβD treatment produces efficient depletion of virion envelope cholesterol which results in decreased virus infectivity.

[0051] To determine whether the effect of MCβD could be rescued (and is thus reversible), and moreover, to determine if the loss of infectivity was due to cholesterol depletion, exogenous cholesterol was used to replenish the envelopes of MCβD-treated virions by incubating the virus particles with various amounts of free cholesterol.

[0052] Results showed that incubation with cholesterol partially restored HCMV infectivity (Fig. 5B, left) and envelope cholesterol (Fig. 5B, right) content in a dose-dependent manner.

[0053] To determine if virus infectivity could be increased by increasing virion cholesterol content alone, gradient purified HCMV virions were incubated with increasing concentrations of cholesterol then gradient purified for a second time.

[0054] Results show that incubation with cholesterol increased virus infectivity (Fig. 5C, left) and overall cholesterol content (Fig. 5C, right) in a dose dependent manner, demonstrating that enhanced virion cholesterol content increases the infectivity of virions.

Example 7

[0055] It is known that infection of host cells by enveloped viruses relies on the fusion of the viral envelope with either an endosomal membrane or the plasma membrane of the cell. Therefore, experiments were designed to test whether infectivity of MCβD-treated virus can be restored by using an "acid bypass assay" (Mercer and Helenius 2008) to circumvent the normal membrane fusion process during virus entry.

[0056] Cells were infected with MCβD (50mM)-treated or mock-treated HCMV strain AD169 at a multiplicity of 3 pfu/cell, allowed to sit at 4°C for 30 min to allow virus binding, then treated with buffer at a neutral (7.4) or acidic (4.7) pH for 3 min, washed with PBS, and examined for IE1 expression 24 hours later.

[0057] HCMV particles, either treated with MCβD or left untreated, were bound to cells at 4°C, then incubated in a low pH buffer (pH 4.7) to artificially induce the fusion event, thus allowing fusion-deficient viruses to enter the cell and replicate. A neutral pH buffer was used as a control. Virus replication after acid bypass was visualized by IE1 expression at 24 hpi (Fig. 6).

[0058] Results showed that acid bypass of the fusion event caused a dramatic difference in the ability of MCβD-treated viruses to enter the cell and express IE1 protein. These data demonstrate that the level of envelope cholesterol is important for HCMV fusion with host cells.

[0059] All publications, patents and patent applications cited in this specification are herein incorporated by reference as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference. Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to those of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

References:

Boucher, P. and J. Herz (2011). "Signaling through LRP1: Protection from atherosclerosis and beyond." Biochem.Pharmacol., in press.

Christian, A. E., M. P. Haynes, et al. (1997). "Use of cyclodextrins for manipulating cellular cholesterol content." J Lipid Res **38**(11): 2264-2272.

Gaultier, A., G. Simon, et al. (2010). "LDL Receptor-Related Protein 1 Regulates the Abundance of Diverse Cell-Signaling Proteins in the Plasma Membrane Proteome." J.Proteome.Res.

Lillis, A. P., L. B. Van Duyn, et al. (2008). "LDL receptor-related protein 1: unique tissue-specific functions revealed by selective gene knockout studies." Physiol Rev. **88**(3): 887-918.

Mercer, J. and A. Helenius (2008). "Vaccinia virus uses macropinocytosis and apoptotic mimicry to enter host cells." Science. **320**(5875): 531-535.

Orr, A. W., C. E. Pedraza, et al. (2003). "Low density lipoprotein receptor-related protein is a calreticulin coreceptor that signals focal adhesion disassembly." J Cell Biol **161**(6): 1179-1189.

Terrand, J., V. Bruban, et al. (2009). "LRP1 controls intracellular cholesterol storage and fatty acid synthesis through modulation of Wnt signaling." J.Biol.Chem. **284**(1): 381-388.

Zhou, L., H. Y. Choi, et al. (2009). "LRP1 controls cPLA2 phosphorylation, ABCA1 expression and cellular cholesterol export." PLoS One **4**(8): e6853.

Zhou, L., Y. Takayama, et al. (2009). "LRP1 regulates architecture of the vascular wall by controlling PDGFRbeta-dependent phosphatidylinositol 3-kinase activation." PLoS.One. **4**(9): e6922.

What is claimed is;

1. A method for production of virus particles in a virus-infected host cell comprising the step of contacting the cell with a compound that decreases activity of LRP1, wherein the virus particles produced in the cell in the presence of the compound display increased infectivity compared to virus particles produced in the cell in the absence of the compound.

2. The method of claim 1 wherein the compound decreases LRP1 binding activity.

3. The method of claim 1 or 2 wherein the compound binds LRP1 and blocks LRP1 binding activity.

4. The method of claim 3 wherein the compound is a blocking antibody.

5. The method of claim 3 wherein the compound is an extracellular fragment of LRP1.

6. The method of any one of claims 1 and 2 wherein the compound decreases transcription and/or translation of a polynucleotide encoding LRP1.

7. The method of any one of claims 1 and 2 wherein the compound decreases surface expression of LRP1.

8. The method of any one of claims 1 and 2 wherein the compound decreases mobilization of LRP1 to a cell membrane.

9. The method of any one of claims 1and 2 wherein the compound induces an inactive conformation of LRP1 at a cell membrane.

10. A method for production of virus particles in a virus-infected host cell comprising the step of contacting the cell with a compound that increases intracellular cholesterol, wherein the virus particles produced in the cell in the presence of the compound display increased infectivity compared to virus particles produced in the cell in the absence of the compound.

11. The method of claim 10 wherein the compound increases cholesterol import into a cell, decreases cholesterol export from a cell, or both.

12. The method of claim 11 wherein the compound modulates LDL-cholesterol receptor (LDLR) activity.

13. The method of claim 11 wherein the compound modulates ABCA1 activity.

14. The method of claim 10 wherein the compound is cholesterol at an amount effective to increase intracellular levels of cholesterol.

15. The method of claim 14 wherein cholesterol is a cholesterol derivative.

16. The method of 14 wherein cholesterol is a cholesterol ester.

17. The method of any one of claims 14, 15 and 16 wherein cholesterol is present at a concentration of at least 5 μM, at least 10 μM, at least 15 μM, at least 20 μM, at least 25μM, at least 30 μM, at least 35 μM, at least 40 μM, at least 45 μM, at least 50 μM, at least 55 μM, at least 60 μM, at least 65 μM, at least 70 μM, at least 75 μM, at least 80 μM, at least 85μM, at least 90 μM, at least 95 μM, at least 100 μM, at least 110 μM, at least 120 μM, at least 130 μM, at least 140 μM, at least 150 μM or more but at a concentration that is not toxic to the host cell.

18. The method of claim 10 wherein the compound decreases LRP1 activity.

19. The method of claim 18 wherein the compound decrease LRP1 binding activity.

20. The method of claim 18 and 19 wherein the compound binds LRP1 and blocks LRP1 binding activity.

21. The method of claim 20 wherein the compound is a blocking antibody.

22. The method of claim 20 wherein the compound is an extracellular fragment of LRP1.

23. The method of any one of claims 18 and 19 wherein the compound decreases transcription and/or translation of a polynucleotide encoding LRP1.

24. The method of any one of claims 18, 19 and 23 wherein the compound decreases surface expression of LRP1.

25. A method for production of virus particles in a virus-infected host cell comprising the step of contacting the cell with a compound that facilitates virus fusion with cells, wherein the virus particles produced in the cell in the presence of the compound display increased ability to fuse with cells compared to virus a[particles produced in the cells in the absence of the compound.

26. The method of any of the preceding claims wherein the cell is contacted with the compound prior to virus infection.

27. The method of any of the preceding claims wherein the cell is contacted with the compound after to virus infection.

28. The method of any of the preceding claims wherein the virus is an enveloped virus

29. The method of any of the preceding claims wherein the virus is an enveloped DNA virus or an enveloped RNA virus.

30. The method of any of the preceding claims wherein the virus is a herpes virus.

31. The method of any of the preceding claims wherein the virus is an alpha family herpes virus.

32. The method of any of the preceding claims wherein the virus is a beta family herpes virus.

33. The method of any of the preceding claims wherein the virus is an gamma family herpes virus.

34. The method of any of the preceding claims wherein the virus is CMV.

35. The method of any of the preceding claims wherein the virus is a pox virus, a non-envelope picorna virus, an RNA virus influenza virus, herpes simplex virus, Epstein Barr virus, hepatitis C virus, Dengue virus, HIV, mumps virus, measles virus, and/or parainfluenza virus.

36. The method of any of the preceding claims wherein the cell is a eukaryotic cell.

37. The method of any of the preceding claims wherein the cell is a mammalian cell.

38. The method of any of the preceding claims wherein the cell is a human cell.

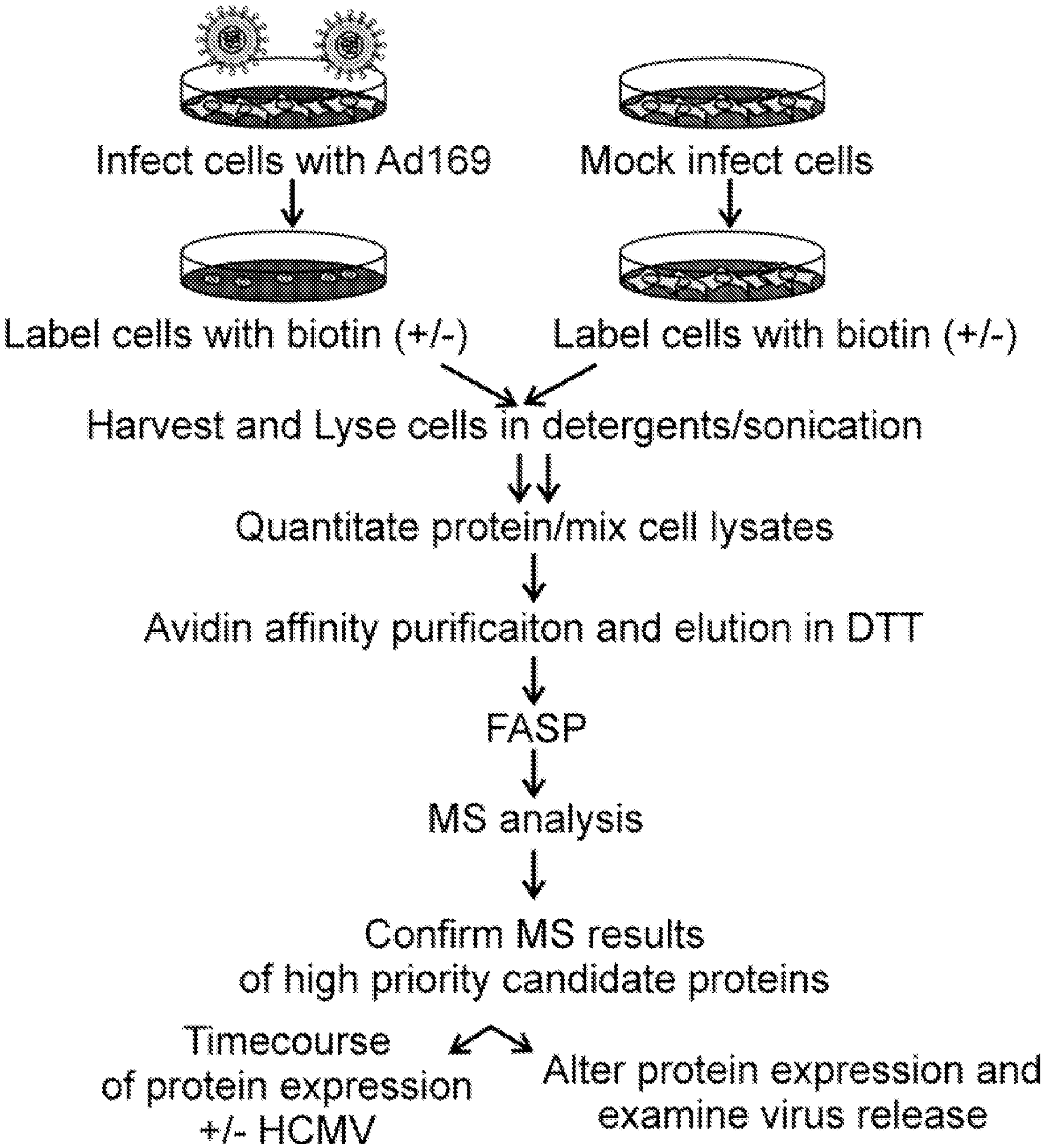

Infect cells with Ad169
Mock infect cells
Label cells with biotin (+/-)
Label cells with biotin (+/-)
Harvest and Lyse cells in detergents/sonication
Quantitate protein/mix cell lysates
Avidin affinity purificaiton and elution in DTT
FASP
MS analysis
Confirm MS results
of high priority candidate proteins
Timecourse
of protein expression
+/- HCMV
Alter protein expression and
examine virus release

FIGURE 1

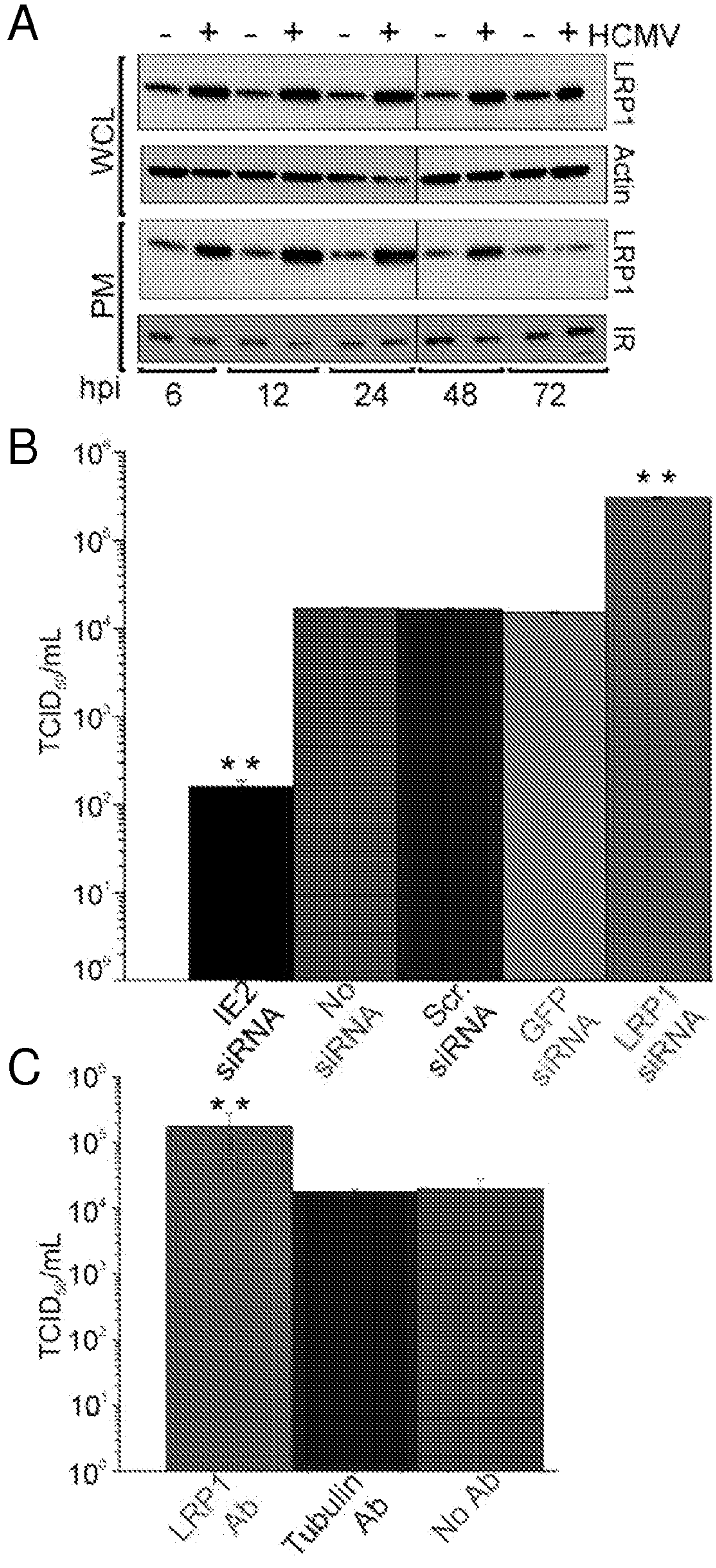
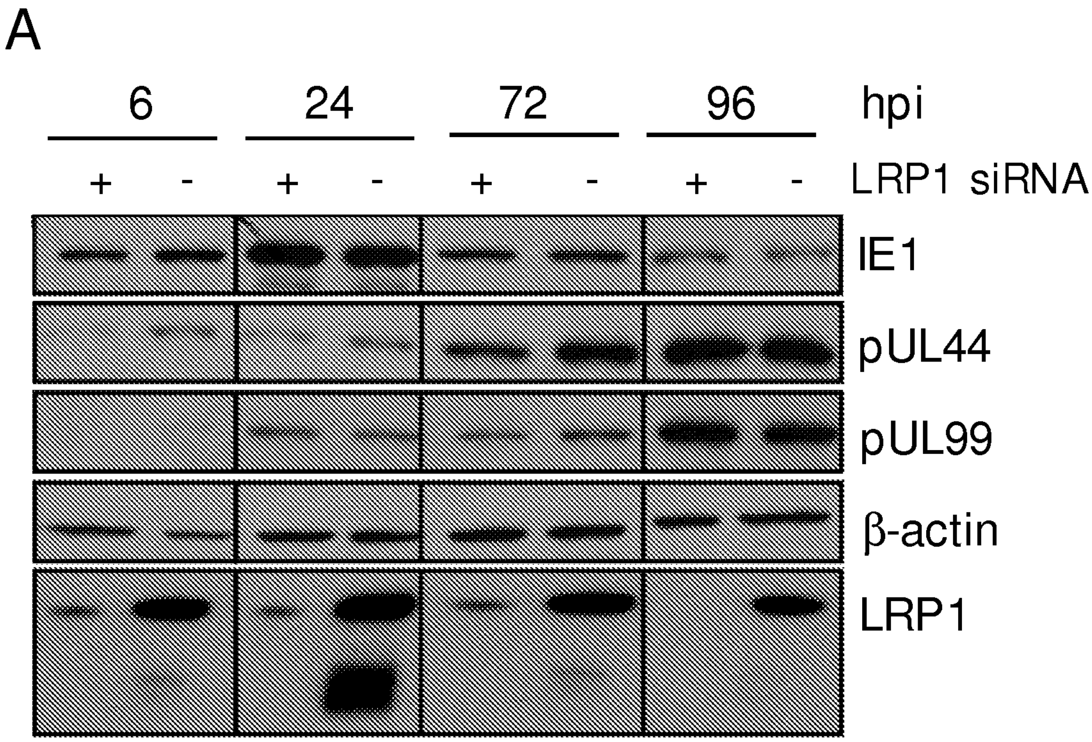

A
HCMV
WCL
PM
LRP1
Actin
LRP1
IR
hpi
6
12
24
48
72
B
TCID50/mL
IE2 siRNA
No siRNA
Scr. siRNA
GFP siRNA
LRP1 siRNA
C
TCID50/mL
LRP1 Ab
Tubulin Ab
No Ab

FIGURE 2


A
6
24
72
96
hpi
+ - + - + - + -
LRP1 siRNA
IE1
pUL44
pUL99
β-actin
LRP1

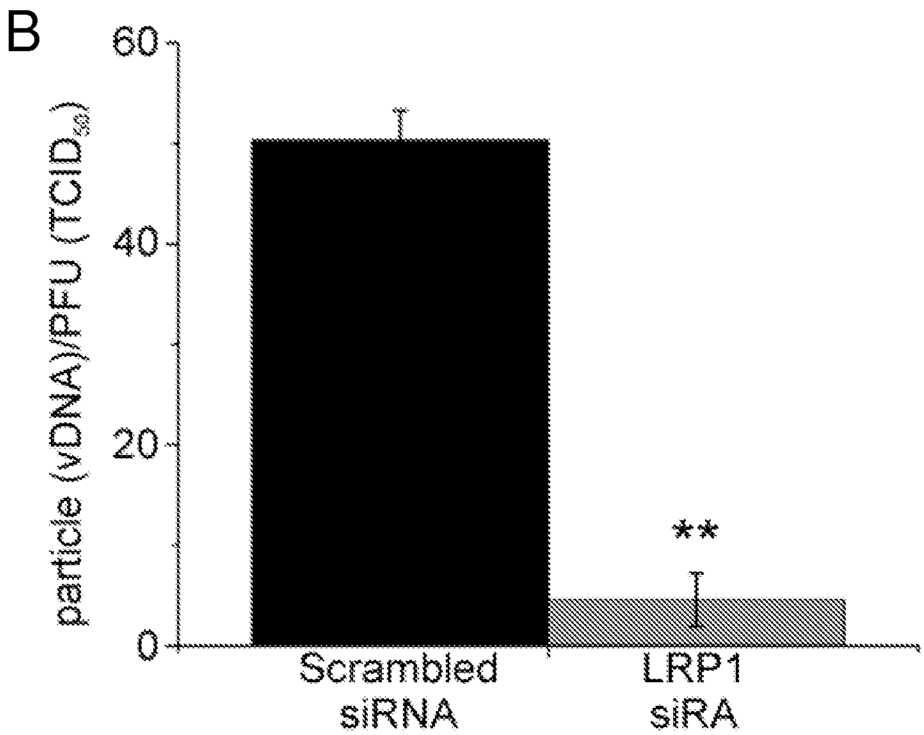

B
particle (vDNA)/PFU (TCID50)
60
40
20
0
**
Scrambled siRNA
LRP1 siRA

FIGURE 3

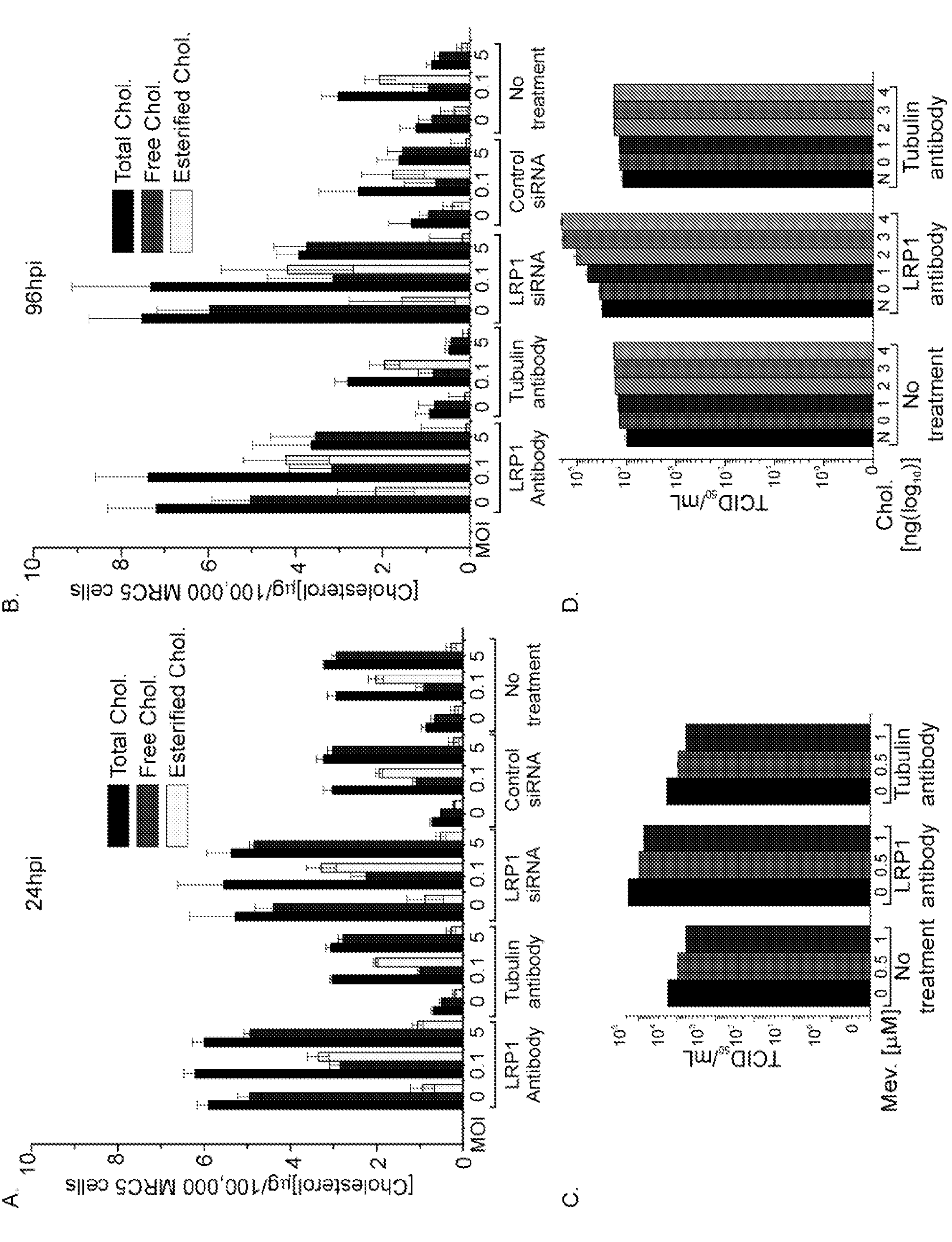

A.
24hpi
Total Chol.
Free Chol.
Esterified Chol.
[Cholesterol]µg/100,000 MRC5 cells
MOI
LRP1 Antibody
Tubulin antibody
LRP1 siRNA
Control siRNA
No treatment
B.
96hpi
C.
Mev. [µM]
LRP1 antibody
D.
Chol. [ng(log10)]

FIGURE 4

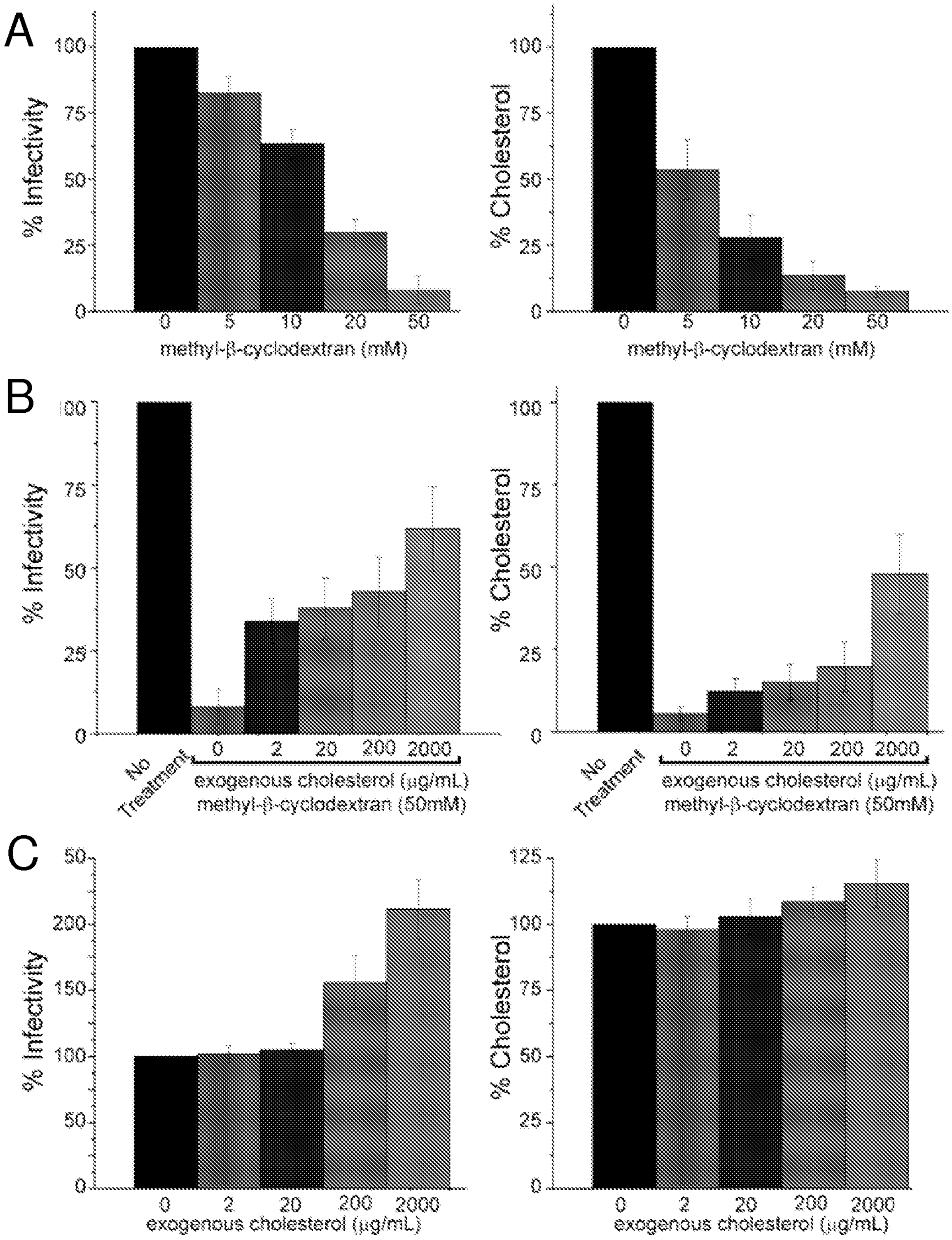

A
% Infectivity
100
75
50
25
0
0 5 10 20 50
methyl-β-cyclodextran (mM)
% Cholesterol
0 5 10 20 50
methyl-β-cyclodextran (mM)
B
% Infectivity
No Treatment
0 2 20 200 2000
exogenous cholesterol (μg/mL)
methyl-β-cyclodextran (50mM)
% Cholesterol
No Treatment
0 2 20 200 2000
exogenous cholesterol (μg/mL)
methyl-β-cyclodextran (50mM)
C
% Infectivity
250
200
150
100
50
0
0 2 20 200 2000
exogenous cholesterol (μg/mL)
% Cholesterol
125
100
75
50
25
0
0 2 20 200 2000
exogenous cholesterol (μg/mL)

FIGURE 5

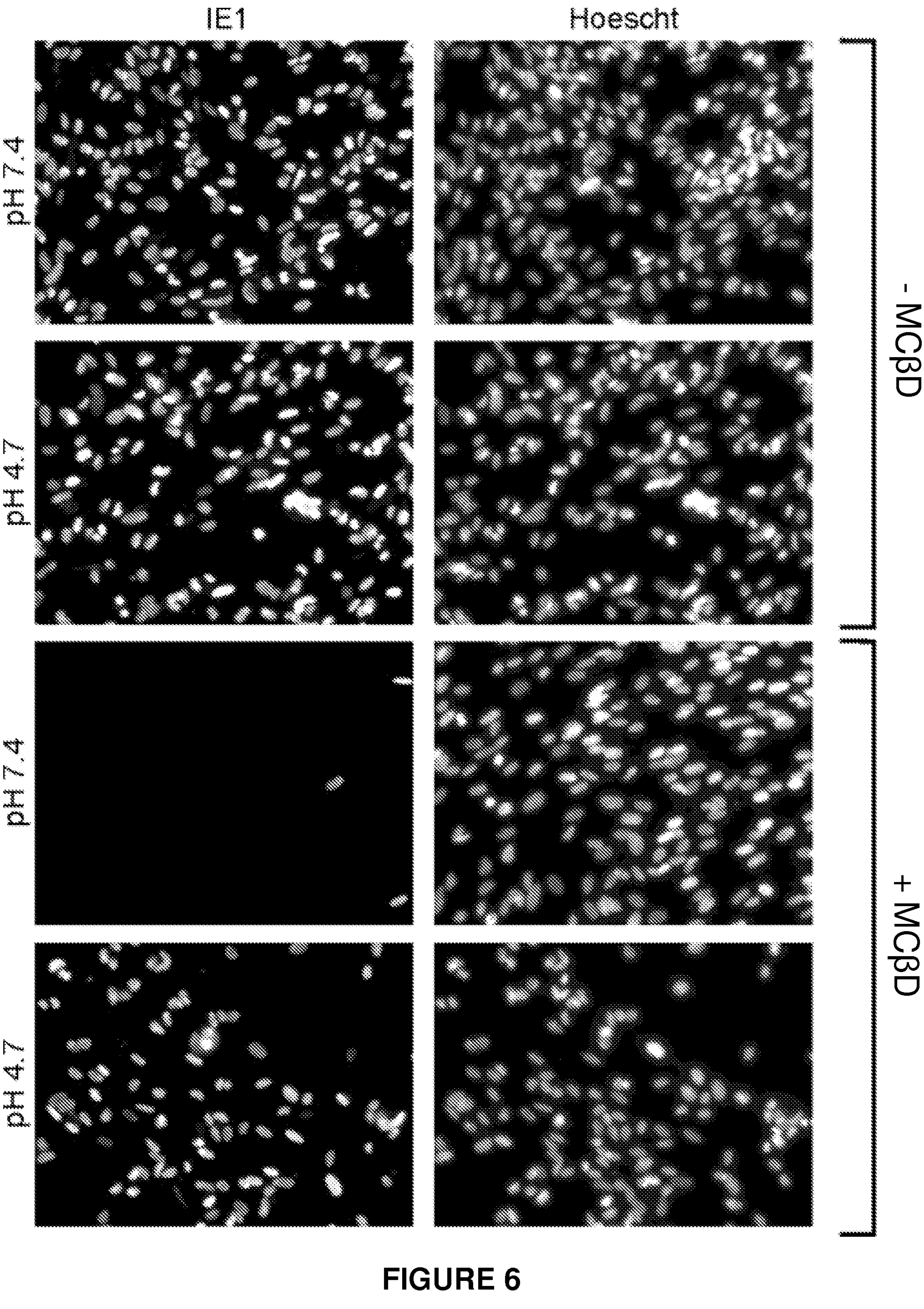


FIGURE 6

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 12/47534

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.: 24 and 26-38
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:

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 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.:

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.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 12/47534

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C12N 7/06 (2012.01)
USPC - 435/238
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)
USPC:435/238

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC:435/235.1; 435/236; 435/948 (see search terms below)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PUBWEST: PGPB, USPT, USOC, EPAB, JPAB; Google: Scholar/Patents
Search terms: lrp1 blocking increase infectivity virus hcmv infection enhance production inhibitor antibody cytomegalovirus "virus production" anti-lrp1 antagonist decreased expression induces inactive conformation cholesterol concentration intracellular levels

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	VIARD et al., Role of Cholesterol in Human Immunodeficiency Virus Type 1 Envelope Protein-Mediated Fusion with Host Cells. Journal of Virology, November 2002, Vol 76, No 22, pp 11584-11595. Especially abstract	25
Y	KONECSNI et al., Low pH-Triggered Beta-Propeller Switch of the Low-Density Lipoprotein Receptor Assists Rhinovirus Infection. Journal of Virology, November 2009, Vol 83, No 21, pp 10922-10930. Especially pg 10922, abstract	1-23
Y	US 7,169,559 B2 (REID et al.) 30 January 2007 (30.01.2007) col 1, ln 14-16, ln 20-24; col 2, ln 61 to col 3, ln 3; col 5,ln 1-5; col 13, ln 17-22; col 16, ln 6-9; col 17, ln 22-27; col 26, ln 50-64; col 27, ln 1-5	1-23
Y	ZHOU et al., LRP1 Controls cPLA2 Phosphorylation, ABCA1 Expression and Cellular Cholesterol Export. PLOS ONE, 31 August 2009, Vol 4, No 8, pp 1-9. Especially pg 1, col 2, para 3 to pg 2, col 1, para 1; pg 4, col 2, para 1; pg 8, col 2, para 2	10-23

☐ Further documents are listed in the continuation of Box C. ☐

* 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 September 2012 (17.09.2012)	04 OCT 2012

Name and mailing address of the ISA/US	Authorized officer:
Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774