Article

VLDLR and ApoER2 are receptors for multiple alphaviruses

Lars E. Clark1,2, Sarah A. Clark1,2, ChieYu Lin1,2, Jianying Liu1,2,3, Adrian Coscia1, Katherine G. Nabel1, Pan Yang1, Dylan V. Neel4, Hyo Lee1, Vesna Brusic6, Iryna Stryapunina6, Kenneth S. Plante6,7, Asim A. Ahmed8, Flaminia Catteruccia6, Tracy L. Young-Pearse5, Isaac M. Chiou4, Paula Montero Llopis9, Scott C. Weaver2,3,7 & Jonathan Abraham1,10,11

Alphaviruses, like many other arthropod-borne viruses, infect vertebrate species and insect vectors separated by hundreds of millions of years of evolutionary history. Entry into evolutionarily divergent host cells can be accomplished by recognition of different cellular receptors in different species, or by binding to receptors that are highly conserved across species. Although multiple alphavirus receptors have been described1–3, most are not shared among vertebrate and invertebrate hosts. Here we identify the very low-density lipoprotein receptor (VLDLR) as a receptor for the prototypic alphavirus Semliki forest virus. We show that the E2 and E1 glycoproteins (E2–E1) of Semliki forest virus, eastern equine encephalitis virus and Sindbis virus interact with the ligand-binding domains (LBDs) of VLDLR and apolipoprotein E receptor 2 (ApoER2), two closely related receptors. Ectopic expression of either protein facilitates cellular attachment, and internalization of virus-like particles, a VLDLR LBD–Fc fusion protein or a ligand-binding antagonist block Semliki forest virus E2–E1-mediated infection of human and mouse neurons in culture. The administration of a VLDLR LBD–Fc fusion protein has protective activity against rapidly fatal Semliki forest virus infection in mouse neonates. We further show that invertebrate receptor orthologues from mosquitoes and worms can serve as functional alphavirus receptors. We propose that the ability of some alphaviruses to infect a wide range of hosts is a result of their engagement of evolutionarily conserved lipoprotein receptors and contributes to their pathogenesis.

Alphaviruses are enveloped RNA viruses that cause disease in humans ranging from acute febrile illness with rash and arthralgia to lethal encephalitis. Their genomes encode four nonstructural proteins, nsP1–nsP4, and structural proteins, capsid and E3–E2–(6K/TF)–E1. The viral envelope proteins are arranged with icosahedral symmetry and E2–E1 glycoproteins form heterodimers that assemble as 80 trimers that mediate receptor binding and fusion of viral and cellular membranes4–6. To use a system that accurately mimics E2–E1 organization, we converted an alphavirus replicon system7 into a DNA-based reporter virus particle (RVP) system in which one plasmid encodes heterologous E3–E2–(6K/TF)–E1 proteins and a second plasmid encodes Ross River virus (RRV) nonstructural proteins, capsid and a reporter (Extended Data Fig. 1a, b). We also generated a library of single guide RNAs (sgRNAs) that target membrane-associated proteins in the human genome (Extended Data Fig. 1c, Supplementary Table I). We used the library to perform a CRISPR–Cas9 screen for cellular viral receptors using HEK 293T (human kidney epithelial) cells expressing Cas9 (HEK 293T-Cas9) infected with Semliki forest virus (SFV) RVPs. The screen identified VLDLR as the top candidate (Fig. 1a, Supplementary Table 2). VLDLR is a part of the low-density lipoprotein receptor (LDLR) family and mediates endocytosis of lipoproteins and other ligands8.

Guide RNAs targeting HSP90B1 and STT3A were also enriched in the screen (Fig. 1a). HSP90B1 encodes an endoplasmic reticulum-resident chaperone that binds the proprotein convertase subtilisin/kinin type 9 serine protease (PCSK9) and prevents PCSK9 from inducing the degradation of LDLR family members9. STT3A encodes the catalytic subunit of the N-oligosaccharyltransferase complex and is also involved in cellular infection by flaviviruses, another group of arthropod-borne viruses that, like alphaviruses, carry positive-sense RNA genomes10. STT3A has a role in flavivirus RNA replication and binds to viral nonstructural proteins11,12. Genetic disruption of STT3A in our screen may have, therefore, affected replication through the RRV component of the RVP system and may act downstream of SFV E2–E1-mediated entry.

1Department of Microbiology, Blavatnik Institute, Harvard Medical School, Boston, MA, USA. 2Institute for Human Infections and Immunity, University of Texas Medical Branch, Galveston, TX, USA. 3Department of Microbiology and Immunology, University of Texas Medical Branch, Galveston, TX, USA. 4Ann Romney Center for Neurologic Diseases, Department of Neurology, Brigham & Women’s Hospital, Harvard Medical School, Boston, MA, USA. 5Department of Microbiology, Blavatnik Institute, Harvard Medical School, Boston, MA, USA. 6Department of Immunology and Infectious Diseases, Harvard T.H. Chan School of Public Health, Boston, MA, USA. 7World Reference Center for Emerging Viruses and Arboviruses, University of Texas Medical Branch, Galveston, TX, USA. 8Division of Infectious Diseases, Boston Children’s Hospital, Boston, MA, USA. 9MicRoN Core, Harvard Medical School, Boston, MA, USA. 10Department of Medicine, Division of Infectious Diseases, Brigham and Women’s Hospital, Boston, MA, USA. 11Broad Institute of Harvard and MIT, Cambridge, MA, USA. 12These authors contributed equally: Lars E. Clark, Sarah A. Clark, ChieYu Lin, Jianying Liu. 13e-mail: jonathan_abraham@hms.harvard.edu

https://doi.org/10.1038/s41586-021-04326-0

Received: 26 November 2020
Accepted: 9 December 2021
Published online: 20 December 2021

Check for updates

Nature | Vol 602 | 17 February 2022 | 475
We focused on exploring the role of human VLDLR as a cellular receptor for SFV. Clonal VLDLR-knockout HEK 293T cells became resistant to infection by GFP-expressing SFV RVPs, and this resistance could be reverted by VLDLR overexpression (Fig. 1b, Extended Data Fig. 2a, b). An antibody against VLDLR, but not a control antibody, blocked infection of HEK 293T cells by SFV RVPs (Fig. 1c). In an experiment using African green monkey kidney (Vero) cells, an anti-VLDLR antibody inhibited the entry of SFV, but not of control RVPs for Chikungunya virus (CHIKV) (Fig. 1d), an alphavirus that uses MXRA8 as a cellular receptor. The anti-VLDLR antibody also blocked SFV RVP infection of immortalized human cell lines derived from brain, lung, liver, lymphoid, bone and kidney tissues (Extended Data Fig. 2c, d). We generated replication-competent chimeric alphaviruses expressing Sindbis virus (SINV) nonstructural proteins with heterologous structural proteins (capsid and E3–E2–(6K/TF)–E1) and GFP as a reporter. The anti-VLDLR antibody, but not a control antibody, inhibited chimeric SINV–SFV infection of Vero cells (Fig. 1e). Receptor-associated protein (RAP) is a chaperone that binds to some LDLR-related receptors in the endoplasmic reticulum and blocks ligand engagement. Addition of RAP blocked SFV RVP infection of HEK 293T cells, whereas addition of a control protein did not (Fig. 1f).

VLDLR knockdown HEK 293T cells showed reduced infection compared to wild-type cells (Extended Data Fig. 2c, d). This suggested that some alphasviruses may be able to bind multiple LDLR family members.
ApoeR2 are highly conserved and have superimposable structures, indicating that their corresponding genes evolved from a single ancestor\(^{14}\). ApoeR2 and VLDLR have critical roles in brain development and modulate synaptic plasticity in adults\(^{14–16}\). ApoER2 is enriched in the brain and can undergo a large number of alternative splicing events\(^{14}\), indicating that their corresponding genes evolved from a single ancestor\(^{14}\).

We cloned an ApoER2 isoform that contains all seven possible LA repeats (LA1–LA7) (ApoER2iso\(_{1}\)) and another that contains only LA1–LA3 (ApoER2iso\(_{2}\)), which is thought to be the predominant form\(^{14,20}\) (Extended Data Fig. 5c, d). Addition of RAP or a soluble RVP blocked SFV, SINV and EEEV RVP infection (Extended Data Fig. 5e). VLDLR or ApoER2 expression did not reveal substantial amounts of lipoprotein-associated peptides in purified VLP samples as compared to a VDLR control (Supplementary Table 3). In biolayer interferometry (BLI)-based experiments, VLDLR\(_{\text{LBD}}–\text{Fc}, \text{ but not MXRA8}_{\text{ect}}–\text{Fc, captured SFV, SINV and EEEV VLPs (Fig. 3b, Extended Data Fig. 4c). We also generated an ApoER2iso\(_{2}\)–F fusion protein (ApoER2iso\(_{2}\)–F) (Extended Data Fig. 4a). ApoER2iso\(_{2}\)–F captured SFV, EEEV and SINV VLPs (Fig. 3b, Extended Data Fig. 4c).}

Addition of RAP, but not a control protein, specifically blocked VLP binding to VLDLR\(_{\text{LBD}}–\text{Fc} and ApoER2iso\(_{2}\)–F (Fig. 3b, Extended Data Fig. 4c). Thus the LBDs of VLDLR and ApoER2 interact directly with alalphavirus E2–E1 proteins.

We next turned to confocal microscopy to determine whether the expression of VLDLR or ApoER2iso\(_{2}\) (chosen because this shorter form is predominant\(^{14,20}\)) enables cell surface binding and internalization of fluorescently labelled VLPs. We incubated labelled VLPs with transduced K562 cells that had also been treated with heparinase and stained with wheat germ agglutinin (WGA) to visualize cell membranes (Fig. 3c, Extended Data Figs. 7, 8). Expression of VLDLR, but not MXRA8, promoted the binding of labelled SFV VLPs to cell surface membranes, and more particles were detected in the cytoplasm of cells at 37 °C than at 4 °C (Extended Data Fig. 8). Expression of VLDLR LBD–Fc or MXRA8ect–Fc after pre-dipping into buffer or solution containing RAP or transferrin (TF). The maximal response value is plotted. Sensorgrams are shown in Extended Data Fig. 4c. The number of VLPs bound to individual cell membranes (membr.) or found in the cytoplasm (cyto.) of individual cells at the indicated temperatures (see Extended Data Fig. 8). Data are mean ± s.d. from two experiments performed in triplicate (n = 6); two-way ANOVA with Šidák’s multiple comparison test, ****P < 0.0001 (a). Mean of values obtained from two experiments; one-way ANOVA with Tukey’s multiple comparisons test, ****P < 0.0001; ***P = 0.0003 (d).

Fig. 3 | Human VLDLR and ApoER2 support E2–E1-mediated entry of divergent alphaviruses. a, Cell surface expression of VLDLR\(_{\text{LBD}}–\text{Fc} or MXRA8\(_{\text{ect}}–\text{Fc in HEK 293T cells transfected with plasmids encoding alalphavirus E3–E2–(6K/TF)–E1 proteins. PE, R-phycocyanin. B, BLI-based binding analysis of VLPs to sensor tips coated with VLDLR\(_{\text{LBD}}–\text{Fc} or MXRA8\(_{\text{ect}}–\text{Fc after pre-dipping into buffer or solution containing RAP or transferrin (TF). The maximal response value is plotted. Sensorgrams are shown in Extended Data Fig. 4c. c, xy slice and 3D volume renderings of representative images of WGA (green)-stained transduced K562 cells incubated with fluorescently labelled VLPs (pink) imaged by live-cell confocal microscopy after co-incubation of cells and VLPs at the indicated temperatures (see Extended Data Fig. 6). Scale bars, 5 μm. d, Number of VLPs bound to individual cell membranes (membr.) or found in the cytoplasm (cyto.) of individual cells at the indicated temperatures (see Extended Data Fig. 8). Data are mean ± s.d. from two experiments performed in triplicate (n = 6); two-way ANOVA with Šidák’s multiple comparison test, ****P < 0.0001 (a). Mean of values obtained from two experiments; one-way ANOVA with Tukey’s multiple comparisons test, ****P < 0.0001; ***P = 0.0003 (d).
at 4 °C, suggesting internalization (Fig. 3c, d). We also observed an increased number of SFV VLPs in the cytoplasm of cells expressing ApoER2 at 37 °C. The expression of VLDLR and ApoER2 promoted cell surface binding of EEEV VLPs, and more particles were again detected in the cytoplasm of cells expressing VLDLR or ApoER2 at 37 °C (Fig. 3d).

We detected cell surface binding and internalization of SINV VLPs on cells expressing VLDLR and ApoER2, but the magnitude of the effects was more modest than those observed with SFV and EEEV VLPs (Fig. 3d).

SFV is neuropathogenic in young laboratory mice and has also caused fatal encephalitis in an exposed laboratory worker, although this individual had chronic purulent bronchitis and may have been immunocompromised. We sought to determine whether VLDLR–Fc or RAP could prevent SFV RVP infection of mouse primary cortical neurons and of human neurons differentiated from induced pluripotent stem (iPS) cells; VLDLR–Fc and RAP, but not a control protein, blocked SFV RVP infection of mouse cortical cells and human iPS cell-derived neurons (Fig. 4a, b, Extended Data Fig. 9a–d).

We next tested wild-type, replication-competent strains of SFV (A774), EEEV (FL-939-39, and SINV (Ar M812) in a multi-step viral replication assay using transduced K562 cells. Ectopic expression of VLDLR and ApoER2 isoforms resulted in faster kinetics and increased levels of viral replication, and we observed a three-log increase in viral replication for SFV and an almost five-log increase with EEEV (Extended Data Fig. 9e). The effect was less pronounced but nonetheless significant with SINV infection, consistent with the moderate phenotype we observed with SINV VLPs in infectivity assays with K562 cells expressing VLDLR and ApoER2 (Extended Data Fig. 3c, d) or VLP cell binding and internalization assays (Fig. 3c, d).

When infected with SFV strain A774, neonatal mice, but not adult mice, rapidly die from fulminant encephalitis. Because VLDLR and ApoER2 are important for the development of the central nervous system, mice that are deficient in both receptors have ataxia, severe cognitive deficits and early lethality, thus limiting our ability to carry out in vivo studies in double-knockout mice. We instead used VLDLR–Fc as a blocking agent for in vivo studies. This protein should block VLDLR- and ApoER2-dependent cellular entry, as VLDLR–Fc blocking experiments suggest that alphasivirus E2–E1 proteins use the same surface to bind to both receptors (Extended Data Fig. 3e). VLDLR–Fc neutralized SFV A774 in a plaque assay (Extended Data Fig. 5g). When ten-day-old mice were inoculated with SFV A774 six hours after receiving phosphate-buffered saline or an isotype control IgG, all mice rapidly succumbed to infection within three days of viral challenge. However, 100% of mice treated with VLDLR–Fc 6 h before viral challenge were still alive 3 days after challenge (Fig. 4c, d). Although all VLDLR–Fc-treated mice eventually succumbed to infection (became moribund, meeting euthanasia criteria) on day 8 (100 plaque-forming units (PFU) challenge dose group) or day 7 (1,000 PFU challenge dose group), the isotype control-treated mice all became moribund significantly faster, by day three. Because VLDLR–Fc could have, in principle, been cleared from circulation by associating with lipoproteins (in addition to binding virus), further studies will be required to determine whether repeated administration would provide additional therapeutic benefit in this model system for otherwise rapidly fatal viral encephalitis.

The LBDs of VLDLR and ApoER2 are mostly conserved even among highly divergent species (Extended Data Figs. 6, 10). We transduced K562 cells with various VLDLR and ApoER2 orthologues and—taking advantage of the ability of RAP to interact with highly divergent lipoprotein receptor orthologues—but not with LDR when added exogenously—we used RAP to monitor orthologue cell surface expression (Extended Data Fig. 3c). SFV RVPs infected K562 cells that expressed equine (Eques aegypti) and avian (Sturnus vulgaris) VLDLR orthologues (Fig. 4e). SFV and EEEV infected cells that expressed mosquito (Aedes aegypti and Aedes albopictus) VLDLR orthologues (lipophorin receptor 1) (Fig. 4e). Remarkably, SFV RVPs could also infect cells over-expressing a Caenorhabditis elegans VLDLR orthologue, a receptor with a role in regulating the worm’s intestinal lipid content and murine, equine and avian ApoER2 orthologues supported entry of SFV, EEEV and SINV VLPs to varying degrees (Fig. 4f, Extended Data Fig. 10c). ApoER2 is almost exclusively expressed in the central nervous system. Accordingly, we did not detect ApoER2 on the surface of HEK 293T, Vero or K562 cells (Extended Data Fig. 3b). These findings may explain why inoculation with an antibody against VLDLR is sufficient to block SFV E2–E1-mediated infection of multiple cell lines (Fig. 1c–e, Extended Data Fig. 2d). Whereas SFV primarily depends on VLDLR for entry into several cell types, EEEV and SINV—although they can bind VLDLR and ApoER2—can enter HEK 293T and Vero cells through independent pathways, as genetic disruption of VLDLR in HEK 293T cells, or treatment of Vero cells with an antibody against VLDLR, did not decrease EEEV or SINV RVP infection of these cells (Extended Data Fig. 5a, b). NRAMP2 is a possible alternative receptor for SINV. Other LDR-family members that we did not test, including LRPl, LRPlb, LRPlP2 and LRPlP4, could also have roles in alphasivirus entry. An anti-VLDLR antibody had a modest effect on SFV entry into U2OS cells, a human bone-derived cell line (Extended Data Fig. 2d), also suggesting the presence of alternative SFV receptors on this cell type. SFV is reported to partially depend on MXRA8 for entry into mouse cells. Human MXRA8,
however, is an unlikely alternative receptor for SFV on U2OS cells, as we did not detect SFV RVP entry into K562 cells overexpressing human MXRAS8 (Fig. 2a, b. Extended Data Fig. 5c, d), nor did we detect SFV VLP binding to these cells (Fig. 3d) or to human MXRAS β-β in BLI experiments (Fig. 3b).

SFV causes encephalitis in horses, mice, rats, rabbits and guinea pigs, and SINV also causes age-dependent encephalitis in mice.33,34 Because SFV, SINV and EEEV can cause encephalitis in humans or animals, binding to VLDLR or ApoER2 could contribute to their neuropathogenesis. VEEV has recently been shown to bind low-density lipoprotein receptor class A domain-containing 3 (LDLRAD3) as a receptor.1 While LDLRAD3 is found in vertebrates, it does not have an apparent orthologue in mosquitos.3 However, every VLDLR orthologue we tested supported SFV E2–E1-mediated infection, including that of C. elegans, which is separated by 10^9 years of evolutionary divergence from humans (Fig. 4e, Extended Data Fig. 10c). Furthermore, only the entry of VEEV RVPs, but not that of SFV, EEEV or SINV RVPs, was enhanced by overexpression of LDLRAD3 on K562 cells, suggesting that the VEEV E2–E1 proteins evolved the ability to only recognize this specific receptor that is structurally homologous to LDLR family members (Extended Data Fig. 5h). Our data, therefore, reveal that similarities in alphavirus E2–E1 protein structure correlate with structural homology in receptors from organisms as evolutionarily distant from humans as worms.

The lipoprotein receptor gene family appeared in an evolutionary burst at the advent of multicellular life and has maintained a remarkable degree of conservation throughout evolutionary history.35 The ability of some alphaviruses to bind LDLR family members probably accounts for their extensive tissue and species tropism. Many viruses, including vesicular stomatitis virus,36 minor group rhinoviruses,37,38 subgroup A Rous sarcoma virus,39 several Flaviviridae family members,33,34 VEEV and Rift Valley fever virus,40 have been reported to bind LDLR-repeat-containing receptors to enter cells. LDLR is also a receptor for Clostridium difficile toxin A.41 LDLR family members may, therefore, represent evolutionary conserved ‘hotspots’ for interfacing with pathogens. Pathogens, in turn, may be driving sequence divergence of the receptor LBDs. A similar evolutionary arms race has been described with the iron-uptake protein transferrin receptor 1, which is also recurrently targeted by pathogens for cellular entry.27,42,43

Our studies help answer, in part, the longstanding question of how some alphaviruses can infect a wide range of organisms. They further suggest that strategies targeting multiple cellular receptors, and possibly multiple receptor binding sites on virions, may be required to effectively limit the cellular entry of some pathogenic alphaviruses.

Online content
Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41586-021-04326-0.
44. Agnello, V., Abel, G., Elfahal, M., Knight, G. B. & Zhang, Q. X. Hepatitis C virus and other Flaviviridae viruses enter cells via low density lipoprotein receptor. Proc. Natl Acad. Sci. USA 96, 12766–12771 (1999).

45. Ganaie, S. S. et al. Lrp1 is a host entry factor for Rift Valley fever virus. Cell 184, 5163–5178. e5124 (2021).

46. Tao, L. et al. Sulfated glycosaminoglycans and low-density lipoprotein receptor contribute to Clostridium difficile toxin A entry into cells. Nat. Microbiol. 4, 1760–1769 (2019).

47. Demogines, A., Abraham, J., Choe, H., Farzan, M. & Sawyer, S. L. Dual host-virus arms races shape an essential housekeeping protein. PLoS Biol. 11, e1001571 (2013).

48. Gruszczynk, J. et al. Transferrin receptor 1 is a reticulocyte-specific receptor for Plasmodium vivax. Science 359, 48–55 (2018).

49. Li, W. et al. MAGeCK enables robust identification of essential genes from genome-scale CRISPR/Cas9 knockout screens. Genome Biol. 15, 554 (2014).

Publisher’s note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

© The Author(s), under exclusive licence to Springer Nature Limited 2021
Methods

Cells and viruses
We maintained HEK 293T (human kidney epithelial, ATCC CRL-11268), 293FT (Thermo Fisher Scientific R70007), Vero (Cercopithecus aethiops kidney, ATCC CCL-81), U2OS (human bone, ATCC HTB-96), A549 (human lung epithelial, ATCC CCL-185), SV-G (human astroglial, provided by T. Kirchhausen), and HuH7 cells (provided by F. Zhang) in Dulbecco’s modified Eagle’s medium (DMEM, Gibco) supplemented with 10% (v/v) fetal bovine serum (FBS), 25 mM HEPES (Thermo Fisher Scientific), and 1% (v/v) penicillin-streptomycin (Thermo Fisher Scientific). We maintained Jurkat clone E6-1 (human lymphoblast, ATCC TIB-152) and K562 (human chronic myelogenous leukemia, ATCC CCL-243) cells in RPMI (RPMI, Thermo Fisher Scientific) supplemented with 10% (v/v) FBS, 25 mM HEPES, and 1% (v/v) penicillin-streptomycin. We maintained SK-N-SH (human brain, ATCC HTB-11) and EBC-1 (human squamous cell lung carcinoma, provided by T. Kirchhausen) in Eagle’s minimum essential medium (EMEM, Sigma) supplemented with 10% (v/v) FBS, 25 mM HEPES, and 1% (v/v) penicillin-streptomycin. We maintained mEF293 cells (Thermo Fisher Scientific A14527) in Exp293 Expression Medium (Thermo Fisher Scientific) supplemented with 1% (v/v) penicillin-streptomycin. We maintained BHK-21 cells (ATCC CCL-10) in DMEM supplemented with 10% (v/v) fetal bovine serum and 1% (v/v) penicillin-streptomycin. Cell lines were not authenticated. We confirmed the absence of mycoplasma in all cell lines through monthly testing using an e-Myclo PCR detection kit (Budowl Bio 25234).

The following wild-type replication-competent alphaviruses were used: SINV (strain DAK Ar Mg812) and EEEV (strain FL-93-939). These viruses were propagated in Vero CCL-81 cells and titrated by standard plaque assays. For work with unmodified replication-competent SFV, we rescued a molecular clone using a plasmid encoding SFV A774 (pCMV-A774wt) obtained from A. Merits. The SFV clone was rescued by electroporating 10 μg of pCMV-A774wt plasmid into BHK-21 cells (220 V, 975 μF, one pulse in a cuvette with a 4-mm electrode gap) using a BTX-Harvard Apparatus ECM830 Square Wave Electroporator (Harvard Apparatus). After incubation for 24 h at 37 °C, the stock of rescued virus (P0 stock) was collected and titrated in a plaque assay on Vero cells. To obtain P1 stock, confluent BHK-21 cells grown on T75 flasks were infected with P0 stock at an MOI of 5. At 24 h post infection, the supernatant (P1 stock) was collected, and virus titre was determined on Vero cells.

Primary mouse cortical neuron culture and infection assays
Mouse experiments were approved at Harvard Medical School under the Harvard Medical School Institutional Animal Care and Use Committee protocol number ISO00000054. Mouse (C57BL/6j) primary cortical neurons were dissociated and cultured using the Papain Dissociation System (Worthington Biochemical LK00313) as previously described10. In brief, postnatal day 0 (P0) C57BL/6j mice were euthanized and mouse cortices were collected in cold Earle’s balanced salt solution (EBSS) and resuspended in 2.5 ml of warmed EBSS supplemented with papain (20 units ml−1) and Dnase (2000 units ml−1). Following a 12 min incubation at 37 °C, cortices were triturated using fire polished glass Pasteur pipettes. Samples were centrifuged (2,000g for 5 min) to pellet cells, and then resuspended in 1.6 ml of suspension media (1.375 ml EBSS, 150 μl albumin-ovomucoid inhibitor (10 mg ml−1 in EBSS), and 75 μl Dnase (2,000 units ml−1)). This solution was then layered on top of a 2.5 ml solution of albumin-ovomucoid inhibitor (10 mg ml−1 in EBSS) to create a continuous density gradient and the samples were centrifuged at 1,000 rpm for 5 min. The supernatant (gradient) was discarded, and pelleted neurons were collected in pre-warmed Neurobasal Plus medium (Thermo Fisher Scientific) supplemented with 200 mM L-glutamine (Thermo Fisher Scientific) and 1× B-27 (Thermo Fisher Scientific) with 1% (v/v) penicillin-streptomycin (Thermo Fisher Scientific). Cells were plated in 24 well glass bottom dishes (Cellvis 24 well plate 1.5 glass bottom cover dish) (P241.5HN) for imaging experiments at a density of 4 × 105 cells per well. After neurons had been cultured for 7 d, we pre-incubated SFV RVPs with VLDLRm1-Fc fusion proteins or RAP in culture media containing 5 μg ml−1 polybrene for 30 min at 37 °C. We then added SFV RVP and Fc fusion proteins or SFV RVP and RAP mixtures to cells. Cells were imaged every 2 h for 24 h using the Incucyte S3 Live Cell Imaging system (Sartorius) with Incucyte S3 Software v2018B (Sartorius) using the following objectives: 20×/0.45 Plan Fluor (4465), 10×/0.3 Plan Fluor (4464). GFP-positive neurons were scored as cells with a threshold signal greater than 5 green calibrated units (GCU) above background, using a Top-hat background subtraction method. To calculate the percent positive cells, at the time point of 22 h post-infection, the area of GFP signal above background was divided by the total area covered by cells under phase contrast and was multiplied by 100. We calculated relative infection as follows: Relative infection (%) = (% GFP-positive cells in the presence of antibody or Fc fusion protein or RAP)/(% GFP-positive cells in the absence of antibody or Fc fusion or RAP) × 100%.

Induced pluripotent stem cell lines
The generation of cell lines from human iPS cells was approved by the institutional review board (IRB) of Brigham & Women’s Hospital (IRB protocol 2015P0001676). iPSCs were generated from peripheral blood mononuclear cells (PBMCs) from the Religious Order Study (ROS) and Memory and Aging Project (MAP) cohort using the Sendai virus reprogramming method as previously described25. iPSCs underwent a rigorous quality check procedure that includes a sterility check, mycoplasma testing, karyotyping and pluripotency assays performed by the New York Stem Cell Foundation (NYSCF). iPSCs were maintained using StemFlex Medium (Thermo Fisher Scientific). For this study, two cell lines (one male and one female) were used for induced neuron differentiation.

Induced neuron differentiation
Induced neurons were generated as previously described25 with minor modifications that have also been previously described25. In Brief, iPSCs were plated at a density of 95,000 cells per cm² on a growth factor reduced Matrigel basement membrane matrix (Corning) coated plate, then were transduced with three lentiviruses: pTet-O-NGN2-puro (Addgene plasmid #52047, a gift from M. Wernig)25, Tet-O-FUW-EGFP (Addgene plasmid 30130, a gift from M. Wernig)25, and FDLuGower-tTA (Addgene plasmid 19780, a gift from K. Hockendlinger)24. The cells were then dissociated with StemPro Accutase Cell Dissociation Reagent (Thermo Fisher Scientific) plated at 200,000 cells per cm² using StemFlex and ROCK inhibitor Y-27632 (Stemcell Technologies) (10 μM) (day 0). From day 1 to day 3, the media was gradually switched from KSR media (KnockOut DMEM (Thermo Fisher Scientific), 15% (v/v) KnockOut Serum Replacement (Thermo Fisher Scientific), 1× MEM non-essential amino acids solution (Thermo Fisher Scientific), 55 μM β-mercaptoethanol (Thermo Fisher Scientific), 1× GlutaMAX (Life Technologies) to KSR media (KnockOut DMEM (Thermo Fisher Scientific), 1× MEM non-essential amino acids solution (Thermo Fisher Scientific), 55 μM β-mercaptoethanol (Thermo Fisher Scientific), 1× GlutaMAX (Life Technologies), 0.3% (v/v) dextrose (d- (+)-glucose) (Sigma)). Day 1 media contained 100% (v/v) KSR, day 2 media contained 50% (v/v) KSR and 50% (v/v) KSR, and day 3 media contained 100% (v/v) KSR. Doxycycline (2 μg ml−1) (Sigma) was added from day 1 to the end of the differentiation, and puromycin (5 μg ml−1) (Gibco) was added from day 2 to the end of the differentiation. On day 3, B27 supplement (1:100) (Life Technologies) was added. On day 4, cells were replated at 50,000 cells per cm² and from day 4 to the end of differentiation (day 21), cells were cultured with NBM media (Neurobasal medium (Thermo Fisher Scientific), 0.5× MEM-NEAA (Thermo Fisher Scientific), 1× GlutaMAX 0.3% dextrose (d- (+)-glucose) (Sigma)) supplemented with 1:50 B27 + BDNF, GDNF,CNTF (10 ng ml−1) (PeproTech) with media replaced every 2 to 3 days. After neurons had been cultured for an additional 14 d, we pre-incubated SFV RVPs with VLDLRm1-Fc
fusion proteins or RAP in culture media containing 5 μg ml−1 polybrene for 30 min at 37 °C. We then added SFV RVP and Fc fusion proteins or SFV RVP and RAP mixtures to cells. Cells were imaged every 2 h for 24 h using the Incucyte S3 Live Cell Imaging system and relative infection based on GFP expression at the time point of 22 h post-infection was calculated as described above in experiments performed with primary mouse cortical neuron cultures.

**Reporter virus particle generation**

To create a two-component RVP system, we used elements from an RRV replicon plasmid (pRR64) provided by R. Kuhn (Purdue University). We also removed RRV E3–E2–(6K/TF)–E1 sequence from pRR64 and replaced it with reporter gene (CD20) or turbo GFP downstream of the capsid gene sequence and preceded by a 2A ‘self-cleaving’ peptide derived from porcine teschovirus-1, and included a BGH termination signal after the 3’ UTR poly-A tail. RVPs were generated by providing in trans the modified pRR64 plasmid and a pCAGGS vector expressing the heterologous alphavirus E3–E2–(6K/TF)–E1 proteins with a start codon upstream of E3. pCAGGS E3–E2–(6K/TF)–E1–E1 expressor plasmids for CHIKV (strain 37997; GenBank A7276732.1) and EEEV (Florida 91-469, 4Q4X7.1) have been previously described. pCAGGS E3–E2–(6K/TF)–E1–E1 expressor plasmids for SFV (SFV4, AKC01681.1), SINV (Toto101 T6144, AKZ17594.1), WEEV (71V-1658, NP_640331.1), and VEEV (GC-83, AAB25171.1) were subcloned for this study. We used Lipofectamine 3000 (Invitrogen) to transfect 293FT cells using the manufacturer’s protocol and replaced media with Opti-MEM (Thermo Fisher Scientific) supplemented with 5% (v/v) FBS, 25 mM HEPES, and 5 mM sodium butyrate 1 d post-transfection. We collected supernatants 3 d post-transfection. Supernatants were then centrifuged at 1,000 rpm for 5 min, filtered using a 0.45 μm filter, and frozen at −80 °C for storage.

To purify RVPs for SDS–PAGE analysis, we collected supernatants 24 and 48 h (EEEV and SINV) or 48 h (SFV) post-transfection. We clarified supernatants by centrifugation at 3,000 g for 10 min. We then performed polyethylene glycol (PEG)-precipitation by mixing clarified supernatants to a final concentration of 7% (v/v) PEG 6000 and 2.3% (v/v) NaCl and incubated samples at 4 °C for 4 h. Precipitates were pelleted by centrifugation at 4,000 g for 30 min and resuspended in phosphate-buffered saline (PBS). We loaded resuspended RVPs onto a 20–70% continuous sucrose gradient and centrifuged samples at 210,000 g for 1.5 h. We collected VLP bands and then used 100-kDa Amicon filters (Sigma UFC910096) to buffer exchange samples into PBS and to concentrate samples. Samples were analysed by SDS–PAGE using a 4–15% Mini-ProTEAN TGX Stain-Free Protein Gel (Bio-Rad 4568083), with proteins visualized using a stain-free gel imaging system (Bio-Rad ChemiDoc). Uncropped, unprocessed images of scanned gels are provided in Supplementary Fig. 1.

**sgRNA library design, screening, and data analysis**

We generated a list of membrane or membrane-associated proteins by examining list of proteins determined to be on the cell surface by mass spectrometry or predicted bioinformatically to be cell surface associated. We also obtained a list of genes that encode proteins predicted to be on endosomes, lysosomes, vesicles or the cell surface by UniProt (https://www.uniprot.org). To compile a comprehensive list of genes using these resources, we included genes encoding proteins that were identified to be cell surface associated by mass spectrometry and then added any other gene that was predicted to be on cell surface by at least one of the other resources. The final list of genes is included in Supplementary Table 1. A CRISPR knockout library containing 10 sgRNAs per gene was generated by Desktop Genetics. We amplified the library in Endura ElectroCompetent cells (Lucigen 60242) as previously described. We packaged the sgRNA plasmid library in HEK 293T cells through co-transfection of the lentiGuide-Puro vector (provided by F. Zhang, Addgene #52963) and pSAX2 (provided by D. Trono, Addgene #12260) and pMD2.G (provided by D. Trono, Addgene #12259) using Lipofectamine 3000 (Invitrogen) and following the manufacturer’s instructions. Supernatants were collected 1 d and 2 d post-transfection, pooled, clarified by centrifugation (1,200 rpm for 5 min), filtered through a 0.45 μm membrane, and stored at −80 °C.

To generate a clonal HEK 293T cell line that expresses *Streptococcus pyogenes* Cas9 (HEK 293T-Cas9), we transduced cells with lentCas9-blast (provided by F. Zhang, Addgene #52962), selected transduced cells with blasticidin, and isolated clones by limiting dilution. We selected a HEK 293T-Cas9 clone with high Cas9 activity after clonal dilution by transfecting cells with plxPR_011 vector (provided by J. Doench and D. Root, Addgene #59702), which expresses GFP and an sgRNA against GFP, and monitoring for low GFP expression by FACS. We selected a clone that had roughly a 80% decrease in GFP signal as compared to WT HEK 293T cells. We expanded the HEK 293T-Cas9 cells and transduced cells (10 × 106) with the CRISPR sgRNA lentivirus library at a multiplicity of infection (MOI) of 0.3. We began selection of sgRNA containing cells with puromycin at 1 μg ml−1 d post-transduction.

For disrupting genes using CRISPR–Cas9, we used paired sgRNAs with a nuclease to introduce ~200-bp deletions in target genes. We used Lipofectamine 3000 (Invitrogen) and followed the manufacturer’s protocol to co-transfect HEK 293T cells with the lentiGuide-Puro vector (Addgene #52963) expressing sgRNAs and the lentCas9-blast plasmid (Addgene #52962). Two days post-transfection, we split cells and started selection with blasticidin at 10 μg ml−1, and puromycin at 1 μg ml−1. We returned cells to normal media for replication 3 d post-transfection and isolated individual clones using clonal dilution. We isolated genomic DNA from clonal cells and used a genotyping PCR to confirm successful deletion and confirmed lack of cell surface VLDLR expression using the mouse anti-human VLDLR antibody IH10 (Genetex GTX79552).

**Genetic knockout validation**

sgRNA sequences for VLDLR disruption were as follows: *VLDLR* sgRNA-1: 5′-CACCGGACCACTTGATGAGTCCC-3′; *VLDLR* sgRNA-2: 5′-AACCGGACTCTACATCAGTGTTGCG-3′; *VLDLR* sgRNA-3: 5′-CACCAGTGGACGGAGTGACTCCTG-3′; *VLDLR* sgRNA-4: 5′-AACACAGTTACCTGCTTGCTACGTC-3′.

Genotyping primer sequences were as follows: primer 1: 5′-CCATGG TAGCCTTTAAGTTGGG-3′; primer 2: 5′-TCCTCACCATTCAAATTGGTG-3′; primer 3: 5′-CACTTGCTCCATCGACGTC-3′.

**SINV chimera generation**

A plasmid encoding infectious SINV expressing GFP (pTE3′2J) was provided by R. Andino. To generate plasmid launched SINV chimeric
recombinant alphaviruses, we replaced the Sp6 promoter located at the 5' end of the SINV insert with a CMV/T7 promoter cassette and introduced at the 3' end the hepatitis delta virus (HDV) ribozyme and SV40 poly(A) sequences. To generate replication-competent SINV chimeric viruses, we then replaced the genes encoding SINV structural proteins (capsid and E3–E2–(6K/TF)–E1) with the structural proteins of CHIKV (strain 37997, GenBank AY726732.1) or SFV (SFV4, AK016681.1). We transfected HEK 293T cells using Lipofectamine 3000 (Invitrogen) following the manufacturer's protocol. We replaced media 12 h post-transfection with Opti-MEM (Thermo Fisher Scientific) for 5% (v/v) FBS and 25 mM HEPES. We collected the supernatant 48 h after transfection, filtered these through a 0.45 μm filters, and froze supernatants at −80 °C for storage. Chimeras were titred on Vero (CCL-81) cells by plaque assay.

**Expression and purification of virus-like particles**

To produce SINV and SFV VLPs, we subcloned the structural proteins (capsid and E3–E2–(6K/TF)–E1) of SINV (Strain T6P144, GenBank AK17594.1) and SFV (SFV4, AK016681.1) into the mammalian expression vector pHSeC23. We used previously described vectors to produce CHIKV22 and EEEV23 VLPs. We transfected HEK 293T cells with Lipofectamine 3000 (Invitrogen) according to the manufacturer's instructions. We purified VLPs as previously described26. In brief, we collected supernatant 24 h and 48 h post-transfection and clarified these by centrifugation at 3,000g for 10 min. We then performed PEG precipitation by mixing clarified supernatants to a final concentration of 7% (v/v) PEG 6000 and 2.3% (v/v) NaCl and incubated at 4 °C overnight. Precipitates were pelleted by centrifugation at 4,000g for 30 min and resuspended in PBS. We loaded resuspended VLPs onto a 20–70% continuous sucrose gradient and centrifuged samples at 210,000g for 1.5 h. We collected VLP bands and buffer exchanged using a 20–70% sucrose/0.1 M NaCl buffer. We confirmed particle integrity and the absence of degradation products using SDS–PAGE (Extended Data Fig. 1d) in addition to negative-stain electron microscopy (Extended Data Fig. 4b). VLPs were always used within seven days of purification.

**Protein sequence analysis by LC–MS/MS**

Protein sequence analysis by LC–MS/MS of purified alphavirus VLPs and human VLDL (Sigma LPI) was performed at the Taplin Biological Mass Spectrometry Facility at Harvard Medical School. Prior to analysis, samples were digested in trypsin followed by a reverse phase clean up. Samples were then dried in a Vacufuge (Eppendorf) concentrator for 1 h and stored at 4 °C until analysis. On the day of analysis, samples were reconstituted in 10 μl of high-performance liquid chromatography (HPLC) solvent A and B (18% (v/v) acetonitrile, 0.1% (v/v) formic acid). A nano-scale reverse-phase HPLC capillary column was created by packing Accucore 2.6 μm C18 spherical silica beads (Thermo Fisher Scientific) into a fused silica capillary (100 μm inner diameter × ~30 cm length) (Polymicro Technologies) with a flame-drawn tip26. After equilibrating the column each sample was loaded using a FAMOS autosampler (Polymicro Technologies) with a flame-drawn tip66. After equilibrating into a fused silica capillary (100 μm inner diameter × ~30 cm length) Accucore 2.6 μm C18 spherical silica beads (Thermo Fisher Scientific) onto the column. A gradient was formed, and peptides were eluted with increasing concentrations of solvent B (97.5% (v/v) acetonitrile, 0.1% (v/v) formic acid). As peptides eluted, they were subjected to electrospray ionization and then entered into an LTQ Orbitrap Velos Pro ion-trap mass spectrometer (Thermo Fisher Scientific). Peptides were detected, isolated, and fragmented to produce a tandem mass spectrum of specific fragment ions for each peptide. Peptide sequences (and hence, protein identity) were determined by matching protein databases with the acquired fragmentation pattern using the software program SEQUEST version 28 rev 13 (Thermo Fisher Scientific)26. All databases include a reversed version of all the sequences and the data was filtered to between a one and two percent peptide false discovery rate.

**Labelling of virus-like particles**

We purified VLPs as described above with the exception that we first buffer exchanged particles into 0.1 M Sodium Bicarbonate (pH 8.3) and diluted them to a concentration of 1 mg ml−1 for labelling. Immediately before use, Alexa Fluor 647 (AF647) NHS ester (succinimidyl ester) (Invitrogen A37573) was dissolved into dimethyl sulfoxide (DMSO) at a final concentration of 1 mg ml−1. While stirring, we added 25 μg of the amine reactive dye to 1 mg of VLP and incubated the mixture for 1 h at room temperature. We removed excess dye from the solution with a Zeba Spin Desalting Column (Thermo Fisher Scientific) and buffer exchanged labelled VLPs into PBS and stored these at 4 °C. Labelled VLPs were used for confocal microscopy experiments within 12 h of labelling.

**Ectopic expression experiments**

cDNA encoding full length human VLDLR (GenBank NP_003374.3), human LDLR (GenBank AAP88892) and MRX8 (clone ID: NM_032348.3) were obtained from GenScript. cDNA encoding human NRP2 (GenBank NM_201267.2)22 was provided by S. Whelan. Codon-optimized versions of the following H. sapiens VLDLR (GenBank NP_003374.3), H.sapiensApoER2isofrom1 (GenBank NM_004631.5, H.sapiensApoER2 isofrom 2 (GenBank NM_004631.5), M. musculus ApoER2 (GenBank XP_056019651), E. carabellusVLDLR (GenBank XP_023483037), E. carabellus A poER2 (GenBank XP_023485352), S. vulgaris VLDLR (GenBank XM_0148805991), S. vulgaris ApoER2 (GenBank XM_0148706081), A. aegypti lipophorin receptor 1 (GenBank JN411069.1), A. albopticus lipophorin receptor 1 (GenBank JAC13440) and C. elegans VLDLR (GenBank NM_182223.6). For constructs containing a Flag tag, we used the SignalP server27 to predict signal peptide processing sites and introduced sequence encoding a glutamic acid residue following a Flag tag (DYKDDDDK) and a short linker (GSG) at the N-terminus of each construct to monitor cell surface expression. We subcloned untagged or Flag-tagged versions of these constructs into the backbone of the lentivGuide–Puro vector (Addgene #52963)27. We packaged lentivirus in HEK 293T cells by co-transfecting this vector with psPAX2 (Addgene #12260), and a previously described pCAGGs vector expressing vesicular stomatitis virus (VSV) G28 in a ratio of 3:2:1 using Lipofectamine 3000 (Invitrogen). We exposed K562 cells to filtered (0.45 μm) supernatants containing lentivirus for 48 h. We selected transduced cell populations with puromycin (2 μg ml−1). For all Flag-tagged constructs, we used a FACS sorting step to select subpopulations of positive cells and confirmed construct cell surface expression using cell surface antibody staining (Extended Data Figs. 2b, 3a).

**Generation of Fc fusion proteins and RAP**

We subcloned the LBD of human VLDLR (residues 31–355, GenBank NP_003374.3), the LBD of human ApoER2 isoform 1 (46–334, GenBank NM_004631.5), the ectodomain of human MRX8 (residues 20–337, GenBank NP_001269511.1), or the α1 subdomain of NRP2 (NRP2α1, residues 32–146, GenBank NM_182223.6). For constructs containing a Flag tag, we used the signal sequence (DYKDDDDK) and a short linker (GSG) at the N-terminus of each construct to monitor cell surface expression. We subcloned untagged or Flag-tagged versions of these constructs into the backbone of the lentivGuide–Puro vector (Addgene #52963)27. We expressed these Fc fusion proteins in HEK 293T cells by co-transfecting this vector with psPAX2 (Addgene #12260), and a previously described pCAGGs vector expressing vesicular stomatitis virus (VSV) G28 in a ratio of 3:2:1 using Lipofectamine 3000 (Invitrogen). We exposed K562 cells to filtered (0.45 μm) supernatants containing lentivirus for 48 h. We selected transduced cell populations with puromycin (2 μg ml−1). For all Flag-tagged constructs, we used a FACS sorting step to select subpopulations of positive cells and confirmed construct cell surface expression using cell surface antibody staining (Extended Data Figs. 2b, 3a).
improved expression yields. VLDLR_{res}–Fc fusion protein and RAP co-eluted as a stable complex when purified using protein A affinity chromatography and subsequent size-exclusion chromatography on a Superdex 200 Increase column. We separated the VLDLR_{res}–Fc from RAP by binding the complex to protein A resin and washing with 100 column volumes of 10 mM EDTA in TBS, followed by a wash with 50 column volumes of 10 mM EDTA and 500 mM NaCl in TBS. The washes were collected, buffer exchanged in TBS containing 2 mM CaCl₂, and concentrated. RAP eluted as a single peak by size-exclusion chromatography using a Superdex 200 Increase column. The VLDLR_{res}–Fc fusion protein was refolded on the column by washing with 100 column volumes of TBS containing 2 mM CaCl₂, and eluted using the manufacturer’s protocol.

Prior to mouse studies and after size exclusion, the VLDLR_{res}–Fc fusion protein was bound to a protein A resin and washed with 100 column volumes of 10 mM EDTA in TBS, followed by a wash with 100 column volumes of 0.5 M L-Arginine in TBS. The VLDLR_{res}–Fc fusion protein was washed with 100 column volumes of TBS containing 2 mM CaCl₂, and eluted. Endotoxin levels were <4 endotoxin units ml⁻¹ for both the VLDLR_{res}–Fc fusion protein and the control IgG used in mouse studies as quantified using a Pierce Chromogenic Endotoxin Quantification Kit (Thermo Fisher Scientific).

To generate Flag-tagged RAP (RAP_{Flag}), we cloned RAP (residues 1–353) with a C-terminal Flag tag in place of the endoplasmic reticulum site (IEGR) followed by a SGSG linker into the pHLsec vector. We co-transfected HEK 293T cells in suspension with vectors encoding VLDLR_{res}–Fc and human RAP_{Flag} in a 1:1 ratio and purified the protein as described earlier.

To generate soluble VLDLR-LBD (sVLDLR_{res}), we cloned VLDLR residues 31–355 with an N-terminal twin-Strep tag (WSHPQFEKGF) using an iQue3 Screener PLUS (Intellicyt) with Intellicyt ForeCyt Olympus UPLFNL Semi-Apo Phase objectives, with a 470 nm LED fluoro-}

**Cell surface Fc fusion protein binding assays**

Wetransfected HEK293T cells with pCAGGS vSVLPAVirusE3–E2–(6K/TF)–E1 and tagged plasmids using Lipofectamine 3000 (Invitrogen) or an empty pCAGGS vector. We detached cells 48 h post-transfection with TrypLE Express (Thermo Fisher Scientific) and washed them in 50 mM Tris HCL pH 7.5, 150 mM NaCl, 2 mM CaCl₂, 2% (v/v) bovine serum albumin (BSA) (binding buffer) followed by incubation in 50 mM Tris HCL pH 7.5, 150 mM NaCl, 2 mM CaCl₂, 4% (v/v) BSA (blocking buffer). Cells were then incubated with increasing concentrations of MRXRA8ect–Fc or VLDLR_{res}–Fc fusion protein in binding buffer with 3% (v/v) goat serum (BSA) (binding buffer) followed by incubation in 50 mM Tris HCL pH 7.5, 150 mM NaCl, 2 mM CaCl₂, 4% (v/v) BSA (blocking buffer). Cells were then incubated with increasing concentrations of MRXRA8ect–Fc or VLDLR_{res}–Fc fusion protein in binding buffer with 3% (v/v) goat serum for 1 h at 4 °C. We then washed cells three times in binding buffer and incubated them with a phycocerythrin (PE)-coupled goat anti-human F(ab')₂ fragment (Jackson ImmunoResearch, 109-116-098) at 1:200 dilution in binding buffer for 1 h at 4 °C. We washed cells three times with binding buffer then twice with binding buffer without BSA, and fixed cells with 2% (v/v) formalin. We measured cell binding by monitoring PE-intensity and percent positivity using Flow Cytometer (BD Biosciences) with BD FACS Diva (BD Biosciences) software. 30 h post infection for RVPs and 24 h post infection for SINV chimeras, after washing cells twice with PBS then fixing with PBS containing 2% (v/v) formalin. We used Intracellular Cyt ForeCyt Standard Edition version 8.1.7524 (Sartorius) or Flowjo (BD Biosciences) version 10.6.2 to analyze data. An example of the flow cytometry gating scheme used to quantify GFP-expressing RVP infection is provided in Extended Data Fig. lb. We calculated relative infection as follows: Relative infection (%) = (%GFP positive cells in the presence of antibody or Fc fusion protein or RAP) / (%GFP positive cells in the absence of antibody or Fc fusion or RAP) × 100%.

For plaque-neutralization assays with infectious SFV A774, the VLDLR_{res}–Fc fusion protein or an isotype control IgG (CIA-H12, a previously described SARS-CoV-2 non-neutralizing IgG1 antibody) were serially diluted tenfold in PBS with a starting concentration of 100 µg ml⁻¹. Fifty PFUs of SFV A774 were mixed with the serially diluted VLDLR_{res}–Fc fusion protein, isotype control antibody, or PBS. After incubation at 37 °C for 1 h, the protein–virus mixtures were inoculated onto 12-well plates with a monolayer of Vero cells (which had been seeded the previous day). The cells were incubated at 37 °C with 5% CO₂ for 1 h with gentle shaking every 15 min. After 1 h, 1 ml of overlay medium (DMEM, 2% (v/v) FBS, 0.8% (v/v) methyl cellulose, and 1% penicillin/streptomycin) was added onto each well. The plates were cultured at 37 °C with 5% CO₂ for 2 d until clear plaques formed. The plates were fixed in 4% (v/v) formaldehyde solution for 2 h and stained with 1% (v/v) crystal violet. Plaques were counted. We calculated relative infection as follows: Relative infection (%) = (number of plaques in the presence of antibody or Fc fusion protein)/(number of plaques in the absence of antibody or Fc fusion) × 100%.

Confocal microscopy with labelled virus-like particles

We spun down 10 × 10⁵ KS62 cells transduced for ectopic expression of membrane proteins in a 15 ml conical tube at 1200 RPM for 3 min. We removed supernatant and treated the cells with a Heparinase I and III mixture (Sigma H3917) at 2 units ml⁻¹ and Heparinase II (Sigma H8891) at 1 unit ml⁻¹ for 1 h at 37 °C. Cells were washed and resuspended in 1.5 ml microcentrifuge tube at a concentration of 0.5 × 10⁵ cells ml⁻¹ in culture medium. Twenty-five micrograms of VLPs were added to 0.5 × 10⁴ cells. For cells kept at 4 °C, after virus was added, 1.5 ml microcentrifuge tubes were immediately placed on ice and incubated for 15 min. Cells were then washed twice with PBS and kept at 4 °C before imaging. For the 37 °C condition, after adding virus to 1.5 ml microcentrifuge tubes, these were immediately placed at 37 °C and incubated for 15 min. Cells were then washed twice with PBS, and then kept at 4 °C.
of the measurements. Nikon NIS-Elements Advanced Research (AR) sequentially at each using the piezo drive. Fluorescence from each fluorophore was acquired from the bottom of the cell, using WGA-AF488 fluorescence as a reference. The scan unit, respectively. Z was acquired sequentially with either a hard-coated Chroma ET525/36m dichroic mirror was used for both channels. Signal from each channel was acquired sequentially with either a hard-coated Chroma ET125/36m or Chroma ET700/75m emission filters in a filter wheel placed within the scan unit, respectively. Z-stacks were set by determining the top and bottom of the cell, using WGA-AF488 fluorescence as a reference. The approximate volume was 20 μm, and the step size was set to 0.2 μm, using the piezo drive. Fluorescence from each fluorophore was acquired sequentially at each z-step of the confocal to improve the precision of the measurements. Nikon NIS-Elements Advanced Research (AR) 5.02 acquisition software was used to acquire the data, and the files were exported in ND2 file format. Figures were generated in Fiji. A median filter of 1.0 pixels was applied to both channels (VLP and WGA). Gamma of 0.7 or 1.2 gamma was set for WGA and VLP filtered images, respectively, before adjusting brightness and contrast. Top views of stacks were created by using the (Stacks>Reslice>Top) function with an output spacing of 0.3 μm. 3D renderings were created by using the 3D projection function (Stacks>3D Project). The starting angle was set to 30° with 10° increments and interpolation selected to smooth the 3D rendering.

For VLP quantification of confocal images, 3D image analysis was performed using custom pipelines built in Arivis 4Dfusion 3.4 analysis software. Viral particles were detected using a particle enhancement filter of 0.65 μm followed by a dilation morphology filter of diameter 0.52 μm (sphere shaped) and a Blob Finder segmentation filter set to 0/52 μm diameter, 3% probability threshold and split sensitivity of 70%. To segment cells, the signal from WGA-488 was first enhanced using the enhance edges filter within the membrane detection operation, selecting a membrane width of 0.6 μm and a gap size of 0.13 μm. A discrete gaussian filter of diameter 0.65 μm was applied to the resulting enhanced image. Finally, the membrane-based segmentation operation was used to segment the processed image to obtain the whole cell masks. The two compartments, cytoplasm and membrane, were created by eroding the cell mask by two pixels (cytoplasm) and by performing object math between the cell masks and the eroded cytoplasm masks, producing the membrane masks. The number of viruses in each compartment was then calculated by combining all masks.

Bilayer interferometry binding assays
We performed BLI experiments with an Octet RED96e (Sartorius) and analyzed data using ForteBio Data Analysis HT version 12.0.1.55 software. MXRA8ect–Fc, VLDLRsh–Fc, or ApoER2sh–Fc were loaded onto Anti-Human IgG Fc Capture (AHC) Biosensors (Sartorius 18-5063) at 250 nM in kinetic buffer (TBS containing 2 mM CaCl₂, and 0.1% (w/v) BSA) for 80 s. After a baseline measurement for 60 s in kinetic buffer, Fc fusion protein coated sensor-tips surfaces were incubated with RAP or transferrin at 100 μg ml⁻¹ for 50 s, or kinetic buffer alone for 50 s. After an additional baseline measurement for 20 s in kinetic buffer, VLPs were associated for 300 s at 20 nM. We plotted total response nm change at the end of the 300 s association in Fig. 3b, and raw sensorgrams are provided in Extended Data Fig. 4c.

Cell surface staining of receptors and receptor orthologues
We incubated cells for 30 min at 4 °C in PBS containing 5% (v/v) goat serum (blocking buffer), prior to incubating them with an anti-human VLDLR monoclonal antibody 1H10 (Genetex GTX79552) at 10 μg ml⁻¹, anti-human ApoER2 (LRP8) antibody (clone 3H2) (Sigma WH0007804M1-100) at 10 μg ml⁻¹, anti-human LDLR monoclonal antibody (R&D Systems MAB2148-100) at 10 μg ml⁻¹, anti-human MXRA8 antibody (clone 2H12-G12A) (MBL International W040-3) at 10 μg ml⁻¹, or no antibody in PBS containing 2% (v/v) goat serum (binding buffer) for 1 h. Following incubation, we washed cells three times in binding buffer and then incubated cells for 30 min with a PE-conjugated donkey anti-mouse F(ab'), fragment (Jackson ImmunoResearch 715-116-150) at 1:200 dilution according to the manufacturer's recommendation. We washed cells three times with binding buffer, fixed them with 2% (v/v) formalin, and detected cell surface receptor expression by FACS using an iQue3 Screener PLUS (InteliCyt) with ForeCyt (Sartorius) software.

For staining of cells expressing Flag-tagged receptors, we added an APC conjugated anti-DYKDDDDK (Flag) antibody (BioLegend 637307) at 1:200 dilution in binding buffer, according to the manufacturer's recommendation. We washed cells three times with binding buffer, fixed them with 2% (v/v) formalin, and detected cell surface receptor expression by FACS. For staining using Flag-tagged RAP, we incubated cells with RAPFlag at 10 μg ml⁻¹, or no protein in binding buffer for 30 min. Following incubation, we washed cells three times with binding buffer and added an APC conjugated anti-DYKDDDDK (BioLegend 637307) at 1:200 dilution and carried out the steps described above for antibody staining of Flag-tagged receptors.

Negative-stain electron microscopy
We collected negative-stain micrographs of VLPs at the Molecular Electron Microscopy Core Facility at Harvard Medical School. We buffer-exchanged VLPs into buffer containing 50 mM Tris HCl pH 7.4, 100 mM NaCl, 1 mM EDTA using a 100-kDa Amicon filter (Sigma). VLPs were adsorbed to glow-discharged Formvar carbon film grids (Electron Microscopy Sciences), rinsed twice with water, and stained with 1.5% uranyl formate. Multiple fields of view were inspected to confirm VLP integrity and the absence of associated lipoproteins. Representative micrographs shown in Extended Data Fig. 4b were collected using a Tecnai T12 (ThermoScientific) at 120 kV with a Gatan UltraScan 895 4k CCD camera.

Replication-competent virus replication kinetics assays
Transduced K562 cells (2.5 × 10⁵) were spun in a 15 ml polypropylene conical tube at 1200 RPM for 5 mins; SFV (A774), SINV (Dak Ar Mg812), or EEEV (FL93-939) were used to inoculate cells at a multiplicity of infection (MOI) of 0.01. Cells were incubated with virus for 2 h in a 15 ml polypropylene conical tube in a CO₂ incubator at 37 °C, washed three times with D-PBS (Lonza), and resuspended to a final concentration of 5 × 10⁵ cells ml⁻¹ with culture medium in T25 flasks (Corning). At indicated time points (0, 6, 12, 24 or 48 h), 500 μl of culture supernatants were collected for plaque assays, with 500 μl of fresh culture medium added back to the flask. Virus titres were then determined on Vero cells by plaque assay.

In vivo study
Mouse studies were performed in accordance with the NIH Guidance for the Care and Use of Laboratory Animals. The study protocol was approved by the University of Texas Medical Branch Institutional Animal Care and Use Committee under protocol 1708051. Pregnant mice were received by the dedicated animal research personnel at the University...
of Texas Medical Branch, who randomly assigned animals to one mouse per cage with no additional knowledge of the study design. Mice were fed a 19% protein diet (Teklad, 2919. Irradiated), had a 12 h light-dark cycle (0600–1800 h), and were housed in a facility maintained at a temperature range of 20 to 26 °C with a humidity range of 30 to 70%. Food and water were provided ad libitum. The offspring were too young to be randomly separated into different cages, and no further randomization was performed by study personnel. Pups were taken as mixed groups and were not sexed (that is, both sexes were used). All mouse manipulations were performed under anesthesia with isoflurane. Ten-day-old CD-1 mice (Charles River) were administered 125 μg VLDLRads-Fc fusion protein or IgG1 isotype control monoclonal antibody through the intraperitoneal route 6 h before intraperitoneal inoculation with 100 PFU or 1,000 PFU of SFV A774. Survival rate of mice was monitored daily.

Statistical analysis
Data were deemed statistically significant when P values were < 0.05 using version 9 of GraphPad Prism. Experiments were analysed by one- or two-way ANOVA with multiple comparison correction or by log-rank (Mantel–Cox) test in GraphPad Prism. P values are indicated in each of the figure legends.

Reporting summary
Further information on research design is available in the Nature Research Reporting Summary linked to this paper.

Data availability
All data that support the findings of this study are available within the Article and its Supplementary Information. Confocal microscopy images that support the findings of this study are available at https://omero.hms.harvard.edu/webclient/?show=project-8752. Any other relevant data are available from the corresponding author upon reasonable request. Source data are provided with this paper.

Code availability
Custom pipelines built in Arivis 4DFusion 3.4 analysis software used for this study are available at https://github.com/paulamonterollopis/Viral_Particle_on_Cells_Arivis.

Acknowledgements J.A. is a recipient of a William Randolph Hearst Foundation and Bringham and Women’s Hospital Young Investigator in Medicine Award, and a Burroughs Wellcome Fund Career Award for Medical Scientists. This work was also supported by a Harvard Milton Fund Award (J.A.), van Leeuwen Scholar Award (J.A.), NIH grant T32 AO07061 (I.A.), NIH grant R24 AI120942 (S.C.W.), NIH grant T32 GM007575 (A.C., K.G.N. and D.V.N.), NIH grant R01 DK127257 (I.M.C.), Burroughs Wellcome Fund Pathogenesis Award (I.M.C.), and NIH T32 CA09216-40 (C.L.), and in part by a grant to Harvard Medical School from the Howard Hughes Medical Institute through the James H. Gilliam Fellowships for Advanced Study program (L.E.C.). The authors acknowledge the MicroRN (Microscopy Resources on the North Quad) Core at Harvard Medical School and the Molecular Electron Microscopy Core Facility at Harvard Medical School for their support and assistance in this work. Additionally, the authors thank A. Burdyukin and M. Burdyukin for support in building custom image analysis pipelines, and R. Tomaino from the Taplin Biological Mass Spectrometry Facility at Harvard Medical School for assistance with mass spectrometry of VLPs and data analysis.

Author contributions C.L. designed the sgRNA library and the RVP system and performed the CRISPR-Cas9 genetic screen and initial validation. L.E.C. generated cell lines, VLPs and recombinant proteins, and performed infectivity studies for validation with VLPs with assistance from S.A.C., A.C., K.G.N., D.V.N., H.L. and V.B. S.A.C. produced recombinant proteins, and generated cell lines, SINV chimeras and VLPs, and performed experiments with VLPs and SINV chimeras. S.A.C. additionally performed mass spectrometry experiments, B.L. experiments and confocal microscopy experiments, the latter of which were performed with assistance from P.M.L. P.M.L. developed the imaging workflow and analysed confocal microscopy data with S.A.C, A.C., K.G.N. provided purified RVPs and VLPs for characterization, and A.C. performed negative-stain electron microscopy with VLPs. J.L., K.S.P. designed and executed experiments with wild-type, replication-competent viruses including in vitro and in vivo studies. D.V.N. and I.M.C. provided mouse cortical neurons and assisted with RVP infectivity studies of mouse and human cortical neurons. H.L. and T.L.-P. provided human iPS cell-derived neurons. I.S., A.A.A. and F.C. participated in study conceptualization or provided critical reagents. I.M.C., S.C.W. and J.A. acquired funding. J.A. wrote the original draft of the manuscript and all authors participated in reviewing and editing.

Competing interests The authors declare no competing interests.

Additional information
Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41586-021-04326-0.
Correspondence and requests for materials should be addressed to Jonathan Abraham.
Peer review information Nature thanks Laurie Silva and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.
Reprints and permissions information is available at http://www.nature.com/reprints.
Extended Data Fig. 1 | See next page for caption.
Extended Data Fig. 1 | Screening strategy, reporter virus particle system, and gating strategy. 

a. Ross River (RRV) reporter virus particle (RVP) system. Cells are transfected with two plasmids. CD20 or GFP is included as a reporter downstream of the capsid (C) after a 2A peptide sequence. The arrow indicates a subgenomic promoter. b. SDS-PAGE gel of purified RVPs imaged with a stain-free imaging system. The experiment was performed twice independently, and a representative gel image is shown. 

c. Screening strategy. HEK 293T-Cas9 cells are first transduced with the guide (sgRNA) library using vesicular stomatitis virus (VSV) glycoprotein G pseudotyped lentiviruses and are then infected with RVPs expressing CD20. Infected cells are depleted using magnetic beads against CD20. Selection is repeated iteratively to improve the signal-to-noise ratio of the screen. Non-infected, CD20 negative cells are sequenced using next generation sequencing at the final step. See Methods for additional details.

d. Coomassie-stained SDS-PAGE gel of purified virus-like particles (VLPs). The experiment was performed twice independently, and a representative gel image is shown. 

e. Flow cytometry gating strategy for quantification of GFP-expressing cells after RVP infection. K562 cells expressing human VLDLR (top panels) or wild-type (WT) K562 cells (bottom panels) were infected with GFP-expressing SFV RVPs. The percentage of cells falling within each gate is shown. The example is from an experiment shown in Fig. 4e. f. Flow cytometry gating strategy for detection of receptor cell surface staining. K562 cells overexpressing VLDLR (top panels) or WT K562 cells (bottom panels) were stained with RAPFLAG and a FLAG-APC antibody was used for detection. In the rightmost panel, the staining of each cell type is overlaid to allow for comparison. The example is from an experiment shown in Extended Data Fig. 3c. M: molecular weight marker. For gel source data, see Supplementary Fig. 1.
Extended Data Fig. 2 | Knockout cell line validation and antibody blocking of SFV E2-E1-mediated entry into multiple cell lines. 

**a**, Genotyping DNA gel (left panel) and anti-VLDLR (α-VLDLR) antibody cell surface staining of WT HEK 293T (middle panel) or HEK 293T VLDLR clonal knockout (K.O.) cells (right panel) as monitored by FACS. The experiment was performed at least twice independently, and a representative gel image is shown.

**b**, Anti-VLDLR (α-VLDLR) cell surface staining of WT HEK 293T, HEK 293T VLDLR K.O., and HEK 293T VLDLR K.O. cells transiently transfected with cDNA encoding VLDLR-Flag (VLDLR.FLAG) as monitored by FACS.

**c**, α-VLDLR cell surface staining of the indicated cell types as monitored by FACS.

**d**, The indicated cell types were infected with GFP-expressing SFV single-cycle RVPs in the presence or absence of a α-VLDLR or an anti-HLA control antibody (α-HLA) and infection was measured by FACS. Means ± standard deviation from two experiments performed in triplicate (n = 6) are shown. One-way ANOVA with Tukey’s multiple comparisons test, ****P < 0.0001 (d). For gel source data, see Supplementary Fig. 1.
Extended Data Fig. 3 | Immunostaining to monitor cell surface receptor expression. **a**, Anti-FLAG (α-FLAG) and anti-MXRA8 (α-Mxra8), staining of WT K562 cells or K562 cells expressing the indicated constructs as monitored by FACS. **b**, Anti-ApoER2 (α-ApoER2) and anti-LDLR (α-LDLR) staining of the indicated cell types as monitored by FACS. **c**, RAPFLAG staining of WT K562 cells or K562 cells transduced with the indicated constructs as monitored by α-FLAG tag staining and FACS.
Extended Data Fig. 4 | VLDLR and ApoER2 ligand binding domains directly bind alphavirus E2–E1 proteins. a, Size exclusion chromatography traces of the indicated purified proteins. Insets are SDS-PAGE gels of the peak fraction. Molecular weight markers are indicated. Each experiment was performed at least twice, and representative traces are shown. b, Electron micrographs of negatively stained purified VLPs. Scale bar is 100 nm. The experiment was performed twice, and representative micrographs are shown. c, Sensorgrams for binding of the indicated alphavirus VLPs to tips coated with VLDLR<sub>Iso</sub>-Fc, ApoER2<sub>LBD</sub>-Fc, or Mxra8<sub>ect</sub>-Fc fusion proteins as measured by biolayer interferometry. Fc fusion protein coated sensor-tips surfaces were incubated with RAP or transferrin, or kinetic buffer alone, and VLPs were associated followed by dissociation. The experiment was performed twice and representative results from one experiment are shown.
Extended Data Fig. 5 | See next page for caption.
Extended Data Fig. 5 | Role of VLDLR and ApoER2 in E2–E1-mediated cellular infection by divergent alphaviruses. a, Wild-type (WT) or clonal VLDLR knockout (K.O.) HEK 293T cells were infected with GFP-expressing single-cycle alphavirus RVPs with relative infection measured by FACS. EEEV RVPs more efficiently entered VLDLR K.O. cells, which we suspect could be related to clonal variability, as the cell line was generated by clonal dilution. b, Vero cells were infected with GFP-expressing alphavirus single-cycle RVPs in the presence of the indicated antibodies with relative infection measured by FACS. c, Infection of WT or transduced K562 cells with GFP-expressing single-cycle RVPs. Cells were imaged by fluorescence microscopy. Scale bar is 100 μm. The experiment was performed twice, and representative images are shown. d, Infection of WT or transduced K562 cells with GFP-expressing single-cycle RVPs measured by FACS. NRP2 is a control membrane protein. e, K562 cells expressing VLDLR or ApoER2 iso2 were infected with the indicated single-cycle RVPs in the presence of RAP, soluble VLDLR LBD (sVLDLR(LBD)), or a control protein (transferrin, Tf) with infection measured by FACS. f, WT or transduced K562 cells were infected with the indicated GFP-expressing single-cycle RVPs with infection measured by FACS. g, SFV A774 plaque reduction neutralization test with the indicated proteins performed on Vero cells. h, WT K562 cells or K562 cells transduced to express LDLRAD3 were infected with the indicated GFP-expressing single-cycle RVPs with infection measured by FACS. Means ± standard deviation from an experiment performed once in triplicate (n = 3) (a), or experiments performed twice in triplicate (n = 6) with similar results (b, d–h). One-way ANOVA with Tukey’s multiple comparisons test, ****P < 0.0001 (a, b, d–h). Two-way ANOVA with Šidák’s multiple comparison test, ****P < 0.0001 (g). Cell surface expression of constructs used in (c), (d), and (f) was confirmed with immunostaining (see Extended Data Fig. 3).
Extended Data Fig. 6 | Ligand-binding domain sequence alignment and domain organization of ApoER2 constructs. a, Sequence alignment of the Homo sapiens, Mus musculus, Equus caballus, and Sturnus vulgaris ApoER2 ligand binding domains. The LDLR class A (LA) repeats contained in each protein are shown in parentheses. The domain numbering is based on the human sequence shown. b, Schematic representation of the ectodomains of ApoER2 constructs used in this study. In mammals, exon regions encoding LA repeats 4-6 are almost exclusively spliced out, while the predominant avian isoforms retain these repeats14. Panel (a) was generated using ESPript 3.014.
Extended Data Fig. 7 | Representative confocal microscopy images for virus-like particle cell binding and internalization. K562 cells transduced with human VLDLR, human ApoER2, or human MXRA8 were incubated with fluorescently labeled VLPs at 4 °C or 37 °C and then imaged by live cell confocal microscopy. WGA: wheat germ agglutinin. Scale bar is 10 μm. The experiment was performed twice independently, and representative images are shown.
Extended Data Fig. 8 | Workflow diagram of the 3-dimensional quantification of virus-like particle cell surface membrane binding and internalization. a, 3D analysis of multi-colored stacks (pink, VLPs; green, cell membranes) using Arivis 4DFusion. Two custom-made pipelines were used to detect VLPs and cellular compartments. b, VLPs: left panel shows 3D rendering of VLP stacks, and right panel shows 3D rendering of detected VLPs. c, Cellular compartments: left panel shows 3D rendering of cellular membranes stacks; right, top panel shows 3D rendering of the detected cytoplasms (red) overlayed with an enhanced-membrane filter (white); right, bottom panel shows 3D rendering of the detected membranes (yellow). Objects obtained in each pipeline were combined to quantify the number of VLPs in each cellular compartment. d, Top: single plane representation of the detected objects, showing VLPs in the cytoplasm and the membrane. Bottom: 3D-view of the same cell. Related to Fig. 3c and 3d.
Extended Data Fig. 9 | Effects of VLDLR LBD -Fc and RAP on E2–E1-mediated neuron infection and viral replication assays. 

**a**, Infection of human neurons derived from induced pluripotent stem cell (iPSCs) with GFP-expressing SFV single-cycle RVPs in the presence of the indicated proteins. Cells were imaged by fluorescence microscopy. The experiment was performed twice with representative images shown.

**b**, Quantification of single-cycle SFV RVP infection of human iPSC-derived neurons for the experiment shown in (**a**) using a live cell imaging system (see Methods for additional details).

**c**, Merged phase contrast and fluorescent microscopy for the experiment with mouse cortical neurons shown in Fig. 4a. Scale bars are 100 μm. Magnification is 20X.

**d**, Merged phase contrast and fluorescent microscopy images for the experiment with human neurons shown in (**a**). Scale bars are 100 μm. Magnification is 10X.

**e**, Viral replication curve for SFV, EEEV, and SINV strains in transduced K562 cells. Means ± standard deviation from two experiments done in triplicate (n = 6) with one-way ANOVA with Tukey’s multiple comparisons test, ****P < 0.0001 (**b**). Means ± standard deviation from two experiments done in triplicate (n = 6) with two-way ANOVA with Tukey’s multiple comparisons test, *P = 0.0233, ****P < 0.0001 (**e**).
Extended Data Fig. 10 | Sequence alignment and domain organization of VLDLR constructs and summary of observed effects with alphavirus RVPs. 

**a**, Sequence alignment of the *Homo sapiens*, *Mus musculus*, *Equus caballus*, *Sturnus vulgaris*, *Aedes aegypti*, *Aedes albopictus*, and *C. elegans* VLDLR ortholog ligand binding domains. The LDLR class A (LA) repeats contained in each protein are shown in parentheses. The domain numbering is based on the human sequence shown. 

**b**, Schematic representation of the ectodomains of VLDLR constructs used in this study. 

**c**, Summary of effects observed with GFP-expressing RVP infection of K562 cells transduced to express various VLDLR or ApoER2 orthologs derived from data shown in Extended Data Fig. 5d and Fig. 4e and 4f. +++: RVP infection with greater than 50% GFP positive cells achieved with overexpression. ++: RVP infection with 20–50% GFP positive cells achieved with overexpression. +: RVP infection with 5–20% GFP positive cells achieved with overexpression. +/-: RVP infection with less than 5% GFP positive cells of unclear biological significance. -: no enhancement. 

Panel (a) was generated using ESPrit 3.0."
Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a

☐ Confirmed

☐ The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement

☐ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly

☐ The statistical test(s) used AND whether they are one- or two-sided

Only common tests should be described solely by name; describe more complex techniques in the Methods section.

☐ A description of all covariates tested

☐ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons

☐ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)

☐ For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted

Give P values as exact values whenever suitable.

☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings

☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes

☐ Estimates of effect sizes (e.g. Cohen's d, Pearson's r), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection

IntelliCyt ForeCyt Standard Edition Version 8.1.7524, ForteBio Data Analysis HT Version 12.0.1.55, Nikon NIS-Elements Advanced Research (AR) 5.02, BD FACSDiva, Incucyte S3 Software (v2018B)

Data analysis

FlowJo version 10.6.2, GraphPad Prism (version 8.4.3), MAGeCK, Arvis Vision4D, SEQUEST ver 28 rev 13, Incucyte S3 Software (v2018B)

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

All data that support the findings of this study are available within the Article and its Supplementary Information. Source data are provided with this paper. Confocal microscopy images that support the findings of this study are available at https://omero.hms.harvard.edu/webclient/?show=project-8752. Any other relevant data are available from the corresponding author upon reasonable request.
Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

- [ ] Life sciences
- [ ] Behavioural & social sciences
- [x] Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size
Sample sizes for mouse studies were determined based on previously published results for similar in vivo experiments (PMID: 33208938). No sample size calculations were performed to power each study and no statistical methods were used to predetermine sample size.

Data exclusions
No data were excluded from analysis.

Replication
All experiments with statistical analysis were repeated at least two independent times with the exception of Extended Data Fig. 3a, which was performed once in triplicate. n values are defined and provided in each figure legend. All attempts to replicate results were successful.

Randomization
For in vivo studies, pregnant mice were received by the dedicated animal research personnel at the University of Texas Medical Branch, who randomly assigned animals to one mouse per cage with no additional knowledge of the study design. The offspring were too young to be randomly separated into different cages, and no further randomization was performed by study personnel. For in vitro studies, sample allocation was not randomized because the results are quantitative and did not require subjective judgment or interpretation. This practice is standard in the field (PMID: 33208938).

Blinding
The investigators were not blinded to the allocation during experiments or to outcome assessment for in vivo or in vitro studies. Blinding was not deemed necessary because the results are quantitative and did not require subjective judgment or interpretation. Blinding is also not typically used in the field for similar in vitro and in vivo studies (PMID: 33208938).

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

- Antibodies
- Eukaryotic cell lines
- Palaeontology and archaeology
- Animals and other organisms
- Human research participants
- Clinical data
- Dual use research of concern

Methods

- ChIP-seq
- Flow cytometry
- MRI-based neuroimaging

Antibodies

1H10 (GeneTex #GTX793552), Phycoerythrin-conjugated donkey anti-mouse F(ab')2 fragment (Jackson ImmunoResearch #715-116-150), APC conjugated anti-DYKDDDDK (FLAG) antibody (BioLegend #637307), Anti-human ApoER2 (LRP8) antibody (clone 3H2) (Sigma #WH0007804M1-100), anti-human Mxra8 antibody (clone 2H2G12A) (MBL International #W040-3), anti-human LDLR monoclonal antibody (R&D Systems #MAB2148-100), horseradish peroxidase (HRP)-conjugated goat anti-mouse secondary antibody (Thermo Fisher Scientific #31430), anti-CD20 APC conjugate antibody (Miltenyi Biotec, Clone LT20 #130-113-370).

Validation

1. 1H10 - commercially validated, application western blot, ELISA, and neutralizing/inhibition, with human reactivity.
2. Phycoerythrin-conjugated donkey anti-mouse F(ab')2 fragment - commercially validated, tested by ELISA and/or solid-phase adsorbed to ensure minimal cross-reaction with bovine, chicken, goat, guinea pig, syrian hamster, horse, human, rabbit and sheep serum proteins, application multiple labeling.
3. APC conjugated anti-DYKDDDDK (FLAG) - commercially validated, generated against DYKDDDDK-tagged mouse Langerin, purified by affinity chromatography, quality control tested by intracellular staining with flow cytometric analysis.
4. Anti-human ApoER2 (LRP8) antibody (clone 3H2) - commercially validated, purified from hybridoma culture supernatant, application indirect ELISA and western blot, with human reactivity.
5. Anti-human Mxra8 antibody (clone 2H2G12A) - commercially validated, application flow cytometry, human reactivity.
6. Anti-human LDLR monoclonal antibody - commercially validated, protein G or A purified from culture supernatant, detects human LDLR in ELISAs and western blot (no cross-reactivity to recombinant mouse LDLR, recombinant human LRPS, or recombinant mouse...
LRP6 observed); applications western blot, flow cytometry, immunoprecipitation: human reactivity.

7. Horseradish peroxidase (HRP)-conjugated goat anti-mouse secondary antibody - commercially validated, purified by antigen affinity chromatography, has been successfully used in western blot, immunohistochemistry, and immunoprecipitation applications, reacts with the light chains common to most mouse immunoglobulins, but does not react against non-immunoglobulin serum proteins, with mouse reactivity.

8. Anti-CD20 APC conjugate antibody (Miltenyi Biotec, Clone LT20 #130-113-370) - commercially validated, extended validation performed through epitope competition assays with other known clones recognizing the same antigen, application staining of formaldehyde-fixed cells, immunofluorescence, immunohistochemistry, immunocytochemistry, reactivity human.

Eukaryotic cell lines

| Policy information about cell lines | HEK293T (human kidney epithelial, ATCC CRL-11268), 293FT (Thermo Fisher Scientific), Vero (Cercopithecus aethiops kidney, ATCC CCL-81), U2OS (human bone, ATCC HTB-96), A549 (human lung epithelial, ATCC CCL-185), SVG-A (human astroglial, provided by Thomas Kirchhausen), Jurkat clone E6-1 (human lymphoblast, ATCC TIB-152), K562 (human chronic myelogenous leukemia, ATCC CCL-243), SK-N-SH (human brain, ATCC HTB-11), EBC-1 (human squamous cell lung carcinoma, provided by Tomas Kirchhausen), HuH7 cells (provided by Feng Zhang), Epxi293F cells (Thermo Fisher Scientific #A14527), BHK-21 cells (Mesocricetus auratus kidney, ATCC CCL-10). |

Authentication

| Cell lines in almost all cases were obtained directly from ATCC and were not authenticated; exceptions were; SVG-A, EBC-1, and HuH7. All cell lines grew as expected and had the expected morphology when inspected by microscopy. |

Mycoplasma contamination

| We confirmed the absence of mycoplasma in all cell lines through monthly testing using an e-Mycoplasma PCR detection kit (Bulldog Bio). |

Commonly misidentified lines

| None. |

Animals and other organisms

| Policy information about studies involving animals | ARRIVE guidelines recommended for reporting animal research |

Laboratory animals

| For in vivo protection studies, ten-day old CD-1 mice were used. Pups were taken as mixed groups and were not sexed (e.g., both sexes were used). Mice were fed a 19% protein diet (Teklad, 2919, Irradiated), had 12 h light/dark cycle (0600-1800), and were housed in a facility maintained at a temperature range of 20-26 °C with a humidity range of 30–70. Food and water were provided ad libitum. For mouse cortical neuron isolation, postnatal day 0 (P0) C57BL/6J mice were used, we pooled cortices derived from P0 pups from one litter, and we did not keep track of exact sexes of each pup, which is challenging to do in neonatal ages (therefore, analysis likely included both male and female mice). |

Wild animals

| No wild animals were involved in the study. |

Field-collected samples

| No field samples were collected in this study. |

Ethics oversight

| The in vivo study protocol was approved by the University of Texas Medical Branch Institutional Animal Care and Use Committee under protocol 1708051. Mouse experiments for cortical neuron primary culture were approved at Harvard Medical School under the Harvard Medical School Institutional Animal Care and Use Committee protocol number ISO0000054. |

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Flow Cytometry

Plots

| Confirm that: |

| The axis labels state the marker and fluorochrome used (e.g. CD4-FITC). |
| The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers). |
| All plots are contour plots with outliers or pseudocolor plots. |
| A numerical value for number of cells or percentage (with statistics) is provided. |

Methodology

Sample preparation

| We incubated cells for 30 min at 4 °C in PBS containing 5% (v/v) goat serum (“blocking buffer”), prior to incubating them with an anti-human VLDLR monoclonal antibody 1H10 (GeneTex #GTX79552), anti-human ApoER2 (LRP8) antibody (clone 3H2) (Sigma #WH0007804M1-100), anti-human LDLR monoclonal antibody (R&D Systems #MAB2148-100), anti-human Mxra8 antibody (clone 2H2G12A) (MBL International #W040-3) at 10 µg ml-1, or no antibody in PBS containing 2% (v/v) goat serum (“binding buffer”) for 1 h. Following incubation, we washed cells three times in binding buffer and then incubated cells for 30 min with a phycoerythrin-conjugated donkey anti-mouse F(ab')2 fragment (Jackson ImmunoResearch #715-116-150) according to the manufacturer’s recommended binding buffer. We washed cells twice with binding buffer, fixed them with 2% (v/v) formalin, and detected cell surface receptor expression by FACS. For staining of cells expressing FLAG-tagged
receptors, we added an APC conjugated anti-DYKDDDDK (FLAG) antibody (BioLegend #637307) in binding buffer, according to the manufacturer’s recommendation. We washed cells three times with binding buffer, fixed them with 2% (v/v) formalin, and detected cell surface receptor expression by FACS. For staining of using FLAG-tagged RAP, we incubated cells with RAPFLAG at 10 μg ml⁻¹, or no protein in binding buffer for 30 min. Following incubation, we washed cells three times with binding buffer and added an APC conjugated anti-DYKDDDDK (BioLegend #637307) and carried out the steps described above for antibody staining of FLAG-tagged receptors. For infectivity studies with chimeric alphaviruses or RVPs, cells were harvested 30 h post infection, washed in PBS, and fixed with 2% (v/v) formalin prior to FACS to detect GFP expression.

| Instrument       | Intellicyt iQue3, BD LSR-II Analyser |
|------------------|--------------------------------------|
| Software         | IntelliCyt ForeCyt Standard Edition Version 8.1.7524 (iQue3), BD FACSDiva (BD LSR-II) |
| Cell population abundance | For sorted cell stable lines, purity was confirmed by cell surface staining after expansion in media containing puromycin. |
| Gating strategy  | Gated for live cells with FSC-H and SSC-H. Gated for single cells with FSC-H and FSC-A. Then gated for GFP positive or fluorophore (PE or APC) positive cells. |

☐ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.