nature COMMUNICATIONS

ARTICLE

DOI: 10.1038/s41467-018-03432-4 OPEN

Structural basis for the recognition of LDL-receptor family members by VSV glycoprotein

Jovan Nikolic [1], Laura Belot[1], Hélène Raux[1], Pierre Legrand [2], Yves Gaudin [1] & Aurélie A. Albertini[1]

Vesicular stomatitis virus (VSV) is an oncolytic rhabdovirus and its glycoprotein G is widely used to pseudotype other viruses for gene therapy. Low-density lipoprotein receptor (LDL-R) serves as a major entry receptor for VSV. Here we report two crystal structures of VSV G in complex with two distinct cysteine-rich domains (CR2 and CR3) of LDL-R, showing that their binding sites on G are identical. We identify two basic residues on G, which are essential for its interaction with CR2 and CR3. Mutating these residues abolishes VSV infectivity even though VSV can use alternative receptors, indicating that all VSV receptors are members of the LDL-R family. Collectively, our data suggest that VSV G has specifically evolved to interact with receptor CR domains. These structural insights into the interaction between VSV G and host cell receptors provide a basis for the design of recombinant viruses with an altered tropism.

[1] Institute for Integrative Biology of the Cell (I2BC), CEA, CNRS, Univ. Paris-Sud, Université Paris-Saclay, 91198 Gif-sur-Yvette cedex, France. [2] Synchrotron SOLEIL, 91192 Gif-sur-Yvette cedex, France. These authors contributed equally: Jovan Nikolic, Laura Belot. Correspondence and requests for materials should

Vesicular stomatitis virus (VSV) is an enveloped, negative-strand RNA virus that belongs to the Vesiculovirus genus of the Rhabdovirus family. It is an arbovirus which can infect insects, cattle, horses, and pigs. In mammals, its ability to infect and kill tumor cells, although sparing normal cells makes it a promising oncolytic virus for the treatment of cancer[1–3]. VSV genome encodes five structural proteins among which a single-transmembrane glycoprotein (G). G plays a critical role during the initial steps of virus infection[4]. First, it is responsible for virus attachment to specific receptors. After binding, virions enter the cell by a clathrin-mediated endocytic pathway[5,6]. In the acidic environment of the endocytic vesicle, G triggers the fusion between the viral and endosomal membranes, which releases the genome in the cytosol for the subsequent steps of infection. Fusion is catalyzed by a low-pH-induced large structural transition from a pre toward a post-fusion conformation, which are both trimeric[7,8].

The polypeptide chain of G ectodomain folds into three distinct domains which are the fusion domain (FD), the pleckstrin homology domain (PHD), and the trimerization domain (TrD). During the structural transition, the FD, the PHD, and the TrD retain their tertiary structure. Nevertheless, they undergo large

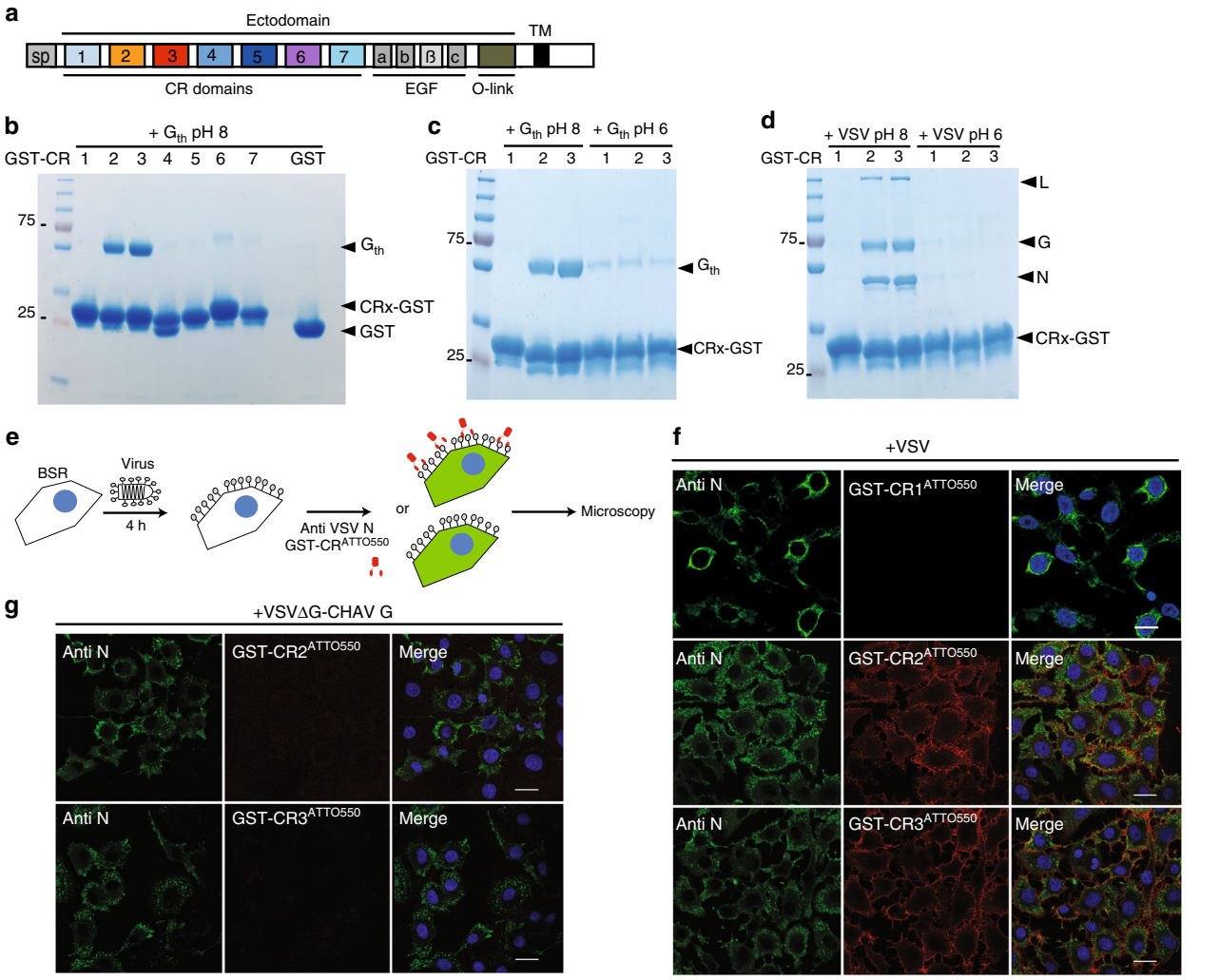

Fig. 1 VSV G interacts specifically with CR2 and CR3 in its pre-fusion conformation. **a** Scheme of the modular organization of the LDL-R indicating the 7 CR modules (1–7), the 3 EGF repeats (a,b and c) , the seven-bladed β-propeller domain (β) of the epidermal growth factor precursor like domain (EGF), and the C-terminal domain containing O-linked oligosaccharides (O-link). SP signal peptide, TM transmembrane domain. **b** SDS-PAGE analysis of interaction experiments between the 7 GST-CR proteins, bound to GSH magnetic beads, and G_{th} at pH 8. **c**, **d** Coomassie-stained SDS-PAGE of interaction experiments between GST-CR1, GST-CR2 and GST-CR3, bound to GSH magnetic beads, and G_{th} (**c**) or VSV (**d**) at pH 8 and 6, respectively. Purified GST-CR bound to GSH magnetic beads were incubated with either G_{th} or VSV in the appropriate pH condition in presence of Ca^{2+} for 20 min at 4 °C. Then, after wash, the beads were directly loaded on a gel. As a control in **b**, GST alone bound to the GSH coated beads was incubated in presence of G_{th}. **e** Cartoon that illustrates the experiments presented in **f** and **g**. After 4 h of infection, BSR cells were labeled with an antibody directed against VSV nucleoprotein (anti-VSV N) to visualize the infection (green fluorescence) and a GST-$CR^{ATTO550}$ to probe CR domain recognition by the surface displayed glycoprotein (red fluorescence). **f** Labeling of G at the surface of BSR cells infected with VSV using fluorescent GST-$CR1^{ATTO550}$, GST-$CR2^{ATTO550}$, and GST-$CR3^{ATTO550}$. At 4 h post-infection (p.i.), cells were incubated with the appropriate GST-$CR^{ATTO550}$ at 4 °C during 30 min prior fixation and permeabilization and then immuno-labeled using an anti-VSV N antibody to visualize the infection. **g** Labeling of CHAV G at the surface of BSR cells infected with a recombinant VSV expressing CHAV G (VSVΔG-CHAVG) using fluorescent GST-$CR2^{ATTO550}$ and GST-$CR3^{ATTO550}$. Infected cells are

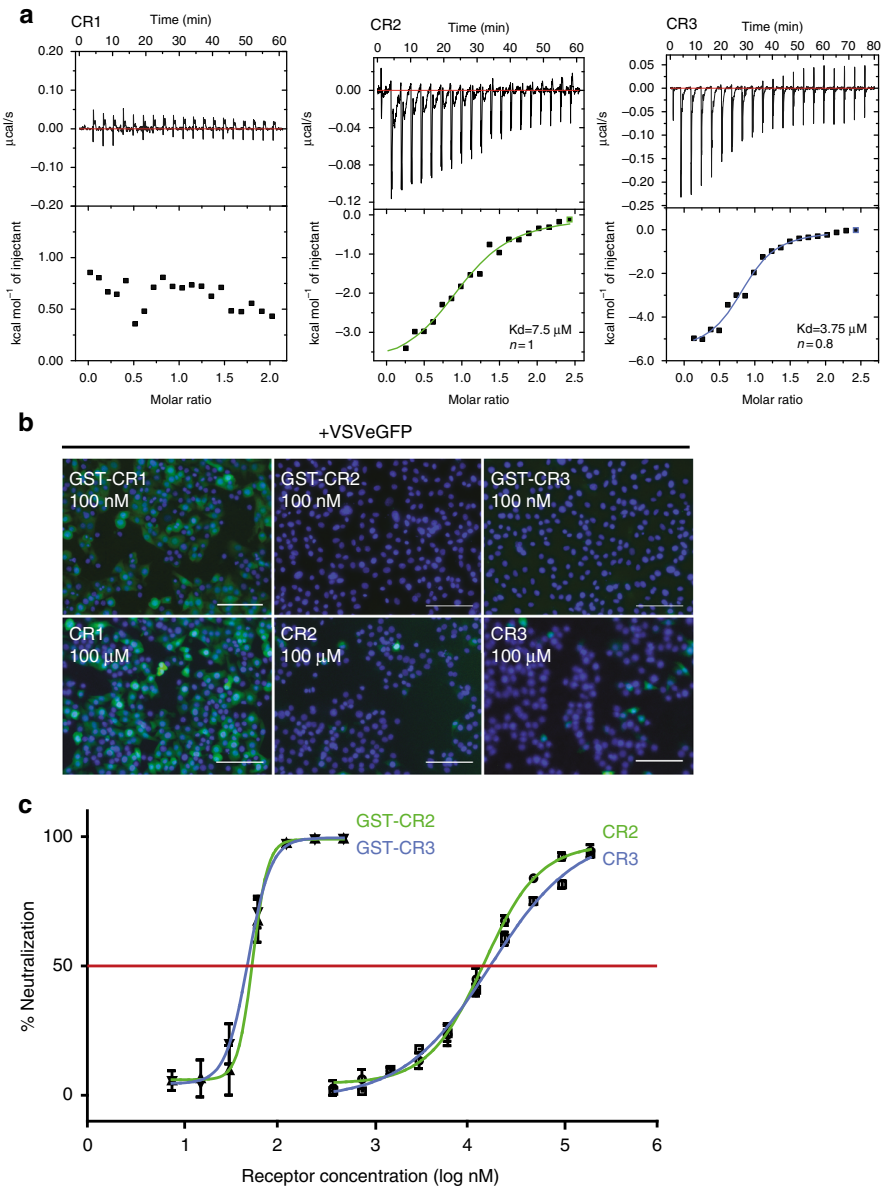

Fig. 2 Characterization of VSV G-CR2 and VSV G-CR3 interaction **a** Isothermal titration calorimetry (ITC) analysis between G_{th} and CR1, G_{th} and CR2, G_{th} and CR3 at 20 °C. Representative plots of each ITC experiments are illustrated with raw data in the upper panel. Binding parameters were determined by curve fitting analysis with the single-site binding model. The values indicated in the panel are those corresponding to the curves that are presented. K_d values given in the text are means of three independent experiments±standard errors. **b**, **c** Inhibition of VSV infection by soluble forms of CR domains. **b** BSR cells were infected with VSV-eGFP preincubated with GST-CR1, GST-CR2, GST-CR3 (upper part), CR1, CR2, or CR3 monovalent domains (lower part) at the indicated concentrations. Cells were fixed 4 h p.i. Only infected cells are expressing eGFP. Note that neither CR1 nor GST-CR1 construction protect cells from infection. DAPI was used to stain the nuclei. Scale bars=100 µm. **c** VSV-eGFP was preincubated with increasing concentrations of GST-CR2, GST-CR3, CR2, or CR3 monovalent domains. At 4 h p.i., the percentage of infected cells was determined by counting the number of cells expressing eGFP using a flow cytometer. The percentage of neutralization was equal to 100 × [1−(% of infected cells in presence of CR)/(% of infected cells in the absence of CR domains)]. Data depict the mean with standard error for experiments performed in triplicate

rearrangements in their relative orientation due to secondary changes in hinge segments (S1 to S5), which refold during the low-pH induced conformational change[7–10].

VSV G has been widely used for pseudotyping other viruses[11–13] and VSV-G-pseudotyped lentiviruses (VSV-G-LVs) exhibit the same broad tropism as VSV. Recently it has been shown that low-density lipoprotein receptor (LDL-R) and other members of this receptor family serve as VSV receptors[14]. This result explains why T cells, B cells, and hematopoietic stem cells, as they have a very low expression level of LDL-R[15].

The LDL-R is a type I transmembrane protein which regulates cholesterol homeostasis in mammalian cells[16]. LDL-R removes cholesterol carrying lipoproteins from plasma circulation. Ligands bound extracellularly by LDL-R at neutral pH are internalized and then released in the acidic environment of the endosomes leading to their subsequent lysosomal degradation. The receptor

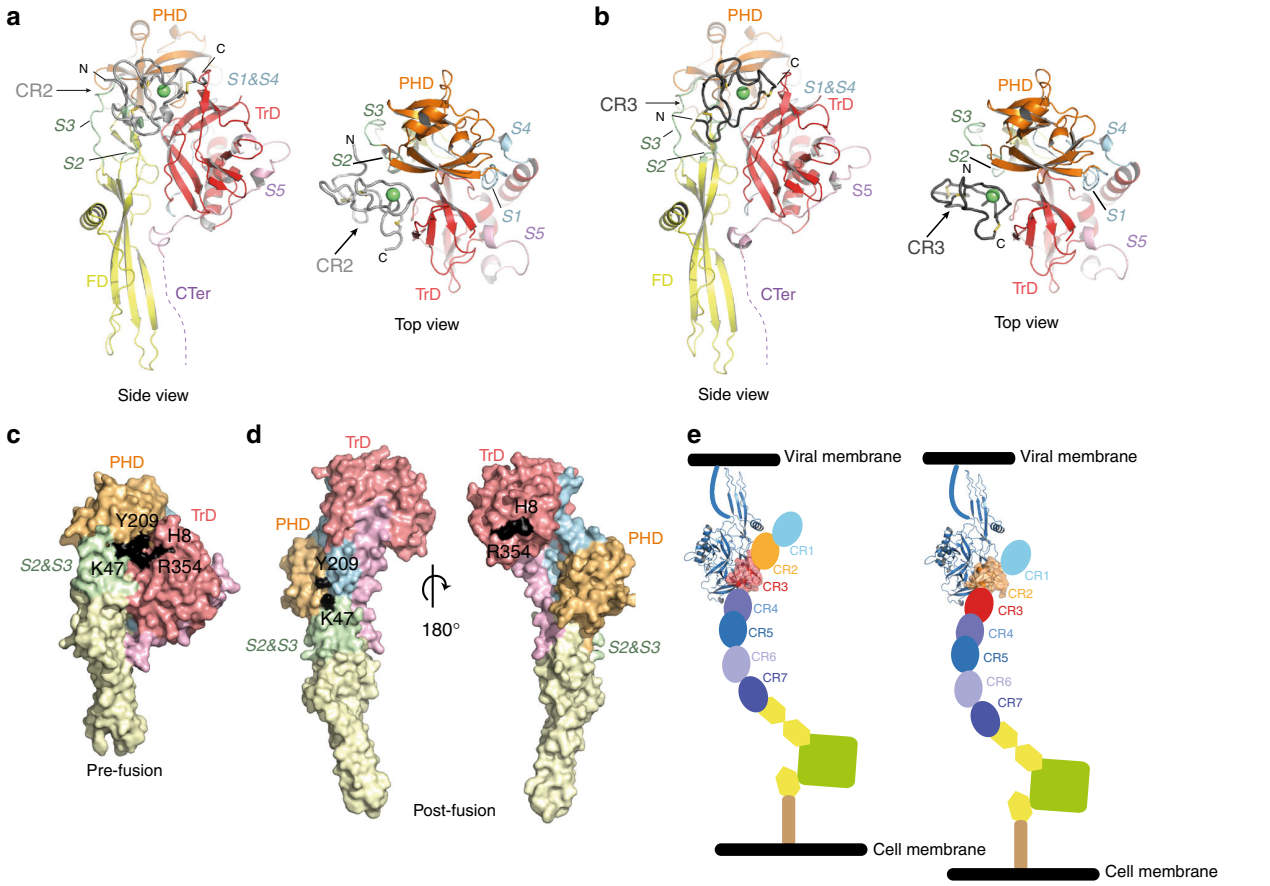

Fig. 3 X-ray structures of G_{th}-CR2 and G_{th}-CR3 complexes **a**, **b** Overview of G_{th}CR2 (**a**) and G_{th} CR3 (**b**) crystalline structures in ribbon representation. G is depicted by domains and CR domains are in two shades of gray. The conserved disulfides bonds of each CR that maintain their secondary structure are in yellow. In both complexes the CR domain is nested in the same cavity of G. N and C-terminal extremities of each CR are indicated. Color code for G_{th}: the trimerization domain (TrD) is in red, the pleckstrin homology domain (PHD) is in orange, the fusion domain (FD) is in yellow. Those domains are connected by segments (S1 to S4) which refold during conformational change: segments S1 and S4 are in cyan, segments S2 and S3 are in green, S5 and the C-terminal segment (CTer) are in purple. The calcium ion of the CR domains is depicted as a green sphere. **c** Footprint of CR2 domain on G pre-fusion conformation. G is in full atoms view and depicted by domains. Residues of G that establish contacts with CR are shown in black on the surface of the protein. **d** Location of residues interacting with CR domains on G post-fusion conformation. Two views at 180° are shown. Note that the interaction patch is scattered when G is in this conformation. **e** Scheme showing the two complexes that can be formed between VSV G and LDL-R. At the cell surface, at neutral pH, the LDL-R adopts an open extended conformation[19] and VSV G can bind either CR2 or CR3. Note that the LDL-R in this extended conformation has the appropriate orientation to interact with G anchored in the viral membrane

composed of a ligand-binding domain, an epidermal growth factor (EGF) precursor homology domain and a C-terminal domain enriched in O-linked oligosaccharides. The ligand binding domain is made of 7 cysteine-rich repeats (CR1 to CR7, Fig. 1a and Supplementary Fig. 1). Each repeat is made of approximately 40 amino acids and contains 6 cysteine residues, engaged in 3 disulfide bridges, and an acidic residues cluster that coordinates a Ca^{2+} ion[17]. The intracellular release of the cargo is driven by a low-pH-induced conformational change of LDL-R from an open to a closed conformation (Supplementary Fig. 1)[17–19].

The LDL-R gene family consists of trans-membrane receptors that reside on the cell-surface, are involved in endocytic uptake of lipoproteins, and require Ca^{2+} for ligand binding. All these receptors have in common several CR repeats (up to several tens), EGF precursor-like repeats, a membrane-spanning region and an intracellular domain containing at least one internalization signal sequence[20]. They are found ubiquitously in all animals including

Here we show that VSV G is able to independently bind two distinct CR domains (CR2 and CR3) of LDL-R and we report crystal structures of VSV G in complex with those domains. The structures reveal that the binding sites of CR2 and CR3 on G are identical. We show that HAP-1 cells in which the LDL-R gene has been knocked out are still susceptible to VSV infection confirming that VSV G can use receptors other than LDL-R for entry. However, mutations of basic residues, which are key for interaction with LDL-R CR domains, abolish VSV infectivity in mammalian, as well as insect cells. This indicates that the only receptors of VSV in mammalian and in insect cells are members of the LDL-R family and that VSV G has specifically evolved to interact with their CR domains.

Results

LDL-R CR2 and CR3 bind G and neutralize viral infectivity. We have expressed individually each LDL-R CR domain in fusion with the glutathione S-transferase (GST) in *Escherichia coli*

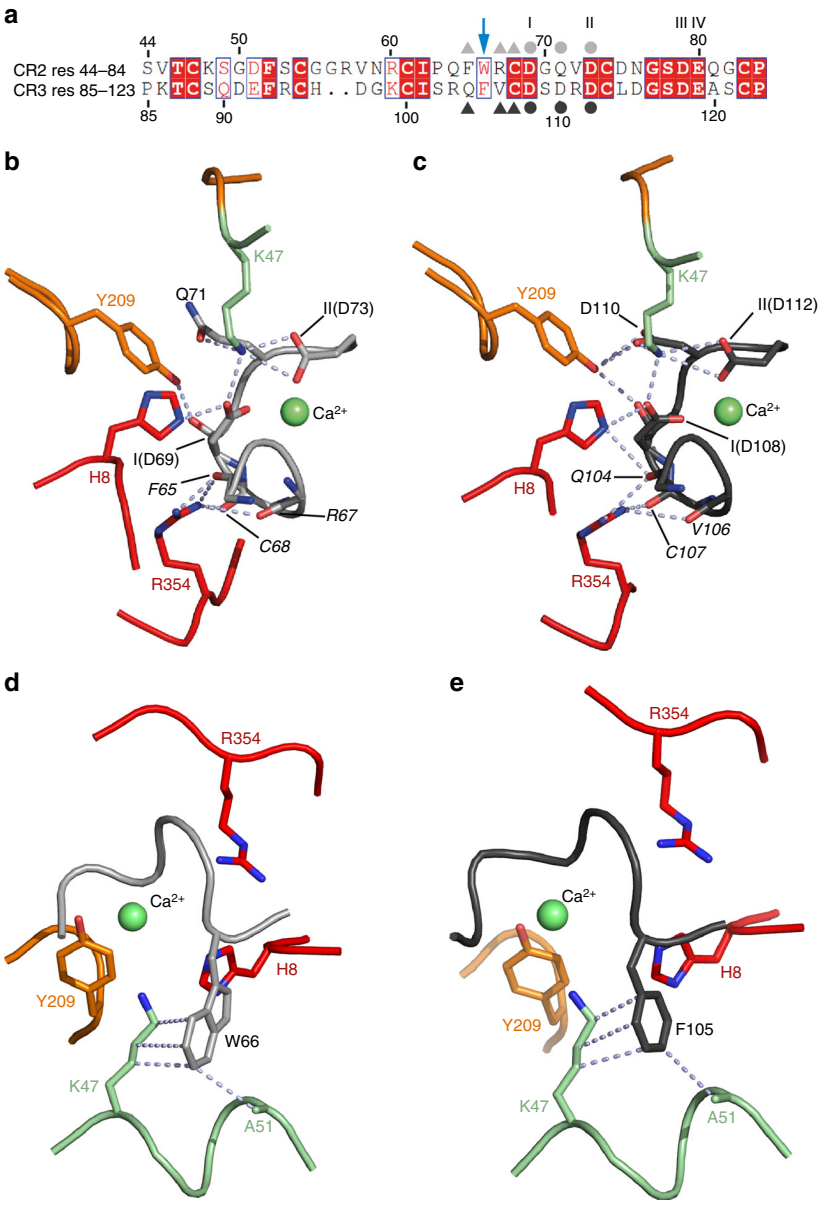

Fig. 4 Molecular basis of G/CR interaction. **a** Sequence alignment of LDL-R CR2 and CR3. Conserved residues are in a red box and similar residues are shown by red letters boxed in blue. Acidic residues involved in the binding of the Ca^{2+} ion are indicated by I, II, III, and IV. CR residues involved in polar contacts with G are labeled with gray symbols (light gray for CR2 and dark gray for CR3; dots when the contact is established via the lateral chain and triangles when the contact is established via the main chain) on each CR sequence. The aromatic residue which protrudes from the CR modules and establishes hydrophobic interactions with G is indicated by a blue arrow. **b**, **c** Close-up view on the G_{th}-CR interface showing the docking of G basic residues on the acidic patch of both CR2 (**b**) and CR3 (**c**). In both cases, the same G residues (H8, K47, Y209, and R354) are involved in the interaction. Residues labels on each CR domain are in italic letters when the contact is established via the main chain; putative bonds are shown as light blue dashed lines. **d**, **e** Close-up view on the G_{th}-CR interface showing the hydrophobic interactions between the aromatic residue W66 of CR2 (**d**) and F105 of CR3 (**e**) and residues K47 and A51 of G.The color code is the same as in Fig. 3a and b

mild denaturing agent), DTT and Ca^{2+} and renatured by dilution in a Ca^{2+} containing buffer. The presence of Ca^{2+} was mandatory for correct folding of the proteins. Individual CR domains were then obtained by cleavage of the GST tag by prescission protease. All purified CR domains behave similarly in gel filtration experiments (Supplementary Fig. 2).

Each fusion protein was incubated at pH 8 with magnetic beads coated with glutathione before addition of a soluble form of the ectodomain of G (VSV G, amino acid (AA) residues 1–422 (Fig. 1b). After 20 min of incubation at 4 °C, the beads were washed and the associated proteins were analyzed by SDS/PAGE followed by Coomassie blue staining. This revealed that only CR2 and CR3 domains are able to directly bind VSV G (Fig. 1b) at pH 8. These results are consistent with previous data indicating that a monoclonal antibody (Mab) directed against LDL-R CR3 almost completely inhibited the VSV-triggered cytopathic effect which was not the case with a MAb directed against LDL-R CR6[14]. The binding of G, or VSV to fusion proteins GST-CR2 and GST-CR3

DOCKET ALARM

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS
Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS
Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS
Sync your system to PACER to automate legal marketing.