ORIGINAL RESEARCH

In Vivo Human Single-Chain Fragment Variable Phage Display-Assisted Identification of Galectin-3 as a New Biomarker of Atherosclerosis

Audrey Hemadou, PhD; Alexandre Fontayne, PhD; Jeanny Laroche-Traineau, PhD; Florence Ottones, PhD; Philippe Mondin, PhD; Stéphane Clavier, PhD; Éric Ducasse, MD, PhD; Stéphane Sanchez, HSD; Sarah Mohamad, MSc; Cyril Lorenzato, PhD; Martine Duonor-Cerutti, PhD; Gisèle Clofent-Sanchez, PhD*; Marie-Josée Jacobin-Valat, PhD*

BACKGROUND: Atherosclerosis is a complex pathology in which dysfunctional endothelium, activated leucocytes, macrophages, and lipid-laden foam cells are implicated, and in which plaque disruption is driven by many putative actors. This study aimed to identify accurate targetable biomarkers using new in vivo approaches to propose tools for improved diagnosis and treatment.

METHODS AND RESULTS: Human scFv (single-chain fragment variable) selected by in vivo phage display in a rabbit model of atherosclerosis was reformatted as scFv fused to the scFv-Fc (single-chain fragment variable fused to the crystallizable fragment of immunoglobulin G format) antibodies. Their reactivity was tested using flow cytometry and immunocassays, and aorta sections from animal models and human carotid and coronary artery specimens. A pool of atherosclerotic proteins from human endarterectomies was co-immunoprecipitated with the selected scFv-Fc followed by mass spectrometry for target identification. Near-infrared fluorescence imaging was performed in Apoe−/− mice after injection of an Alexa Fluor 647–labeled scFv-Fc-2c antibody produced in a baculovirus system with 2 additional cysteine residues (ie, 2c) for future coupling to nano-objects for theranostic applications. One scFv-Fc clone (P3) displayed the highest cross-reactivity against atherosclerotic lesion sections (rabbit, mouse, and human) and was chosen for translational development. Mass spectrometry identified galectin-3, a β-galactoside-binding lectin, as the leader target. ELISA and immunofluorescence assays with a commercial anti-galectin-3 antibody confirmed this specificity. P3 scFv-Fc-2c specifically targeted atherosclerotic plaques in the Apoe−/− mouse model.

CONCLUSIONS: These results provide evidence that the P3 antibody holds great promise for molecular imaging of atherosclerosis and other inflammatory pathologies involving macrophages. Recently, galectin-3 was proposed as a high-value biomarker for the assessment of coronary and carotid atherosclerosis.

Key Words: biomarkers ■ flow cytometry ■ human antibodies ■ imaging ■ in vivo phage display

Atherosclerosis, the main cause of death in Western countries, is a chronic and inflammatory disease characterized by the buildup of lipid-rich plaques that clog the arteries. Atherosclerotic lesions result from the local accumulation of lipids, immune and nonimmune cells, and cellular debris.1 Endothelial cells, activated by lipoproteins, express chemokines and adhesion molecules that contribute to the recruitment of...
monocytes. Monocytes then differentiate into macrophages that express scavenger receptors and CD36 molecules and uptake oxidized low-density lipoprotein (LDL), leading to the formation of foam cells, the precursors of plaque instability and vulnerability.\(^2,3\)

To improve atherosclerosis diagnosis and treatment, the identification of new targetable biomarkers is crucial. Currently, the diagnosis of atheroma plaques is invasive and is often done only at advanced disease stages. Recently, we developed contrast agents functionalized with antibody fragments to target platelets and loaded with iron oxide for noninvasive magnetic resonance molecular imaging. These targeting objects can specifically recognize the atheroma plaque in Apoe\(^{-/-}\) mice ex vivo and in vivo.\(^4\)

To identify new targetable proteins that are overexpressed in the atheroma plaque, we recently set up an in vivo phage display method to select antibody fragments from a human scFv (single-chain fragment variable) library (MG-Umb\(^5\)) in an hypercholesterolemic rabbit model.\(^6\) The in vivo discovery of biomarkers by this approach should allow better understanding atherosclerosis pathogenesis and development of new tools for imaging modalities. Moreover, human antibodies show limited immunogenicity when used in the clinic. After high-throughput flow cytometry analysis, this method led to the selection and retrieval of 142 scFv-phages with complete and in-frame VH and VL germline genes (Sanger sequencing of the whole scFv fragment). Immunohistochemistry experiments confirmed the ex vivo reactivity of 60% of these scFv-phages in sections of rabbit aorta with atheroma.\(^6\)

Here, we characterized some of these selected in vivo human antibodies produced in HEK293 cells (scFv-Fc [scFv fused to the crystallizable fragment of immunoglobulin G format]) to confirm their reactivity by flow cytometry assays with rabbit atheroma protein extracts and by immunohistochemistry using rabbit and mouse aorta sections and human endarterectomy specimens. Importantly, we selected only the antibodies that recognized atheroma in arterial tissue sections from 2 preclinical models and in human endarterectomy biopsies.

We then focused on P3 scFv-Fc because of its high cross-reactivity against protein extracts from atherosclerotic lesions in all tested species, and because it was highly represented during the in vivo phage-display selection, as reported in our previous work using third-generation next-generation sequencing.\(^7\) By mass spectrometry and ELISA assays, we identified galectin-3 as the P3 scFv-Fc target. Galectin-3, a member of \(\beta\)-galactoside-binding proteins, plays key roles in several physiological and pathophysiological processes.\(^8\) Besides its expression in endothelial cells, galectin-3 is also overexpressed by macrophages, the main inflammatory cells in the atheromatous plaque,\(^9,10\) and is involved in monocyte attraction and macrophage activation.\(^11\) In agreement, we found that P3 scFv-Fc and an antibody against the macrophage receptor LOX1 (lectin-type oxidized LDL receptor 1) colocalized in human endarterectomy sections.

Finally, we showed the ex vivo binding of P3 scFv-Fc to its target in atherosclerotic aorta of Apoe\(^{-/-}\) mice by...
fluorescence. Because it has been suggested that the expression of galectin-3 fluctuates during plaque progression,6,9 our findings could lead to the development of novel specific contrast agents functionalized with the P3 human antibody for noninvasive preclinical imaging of atherosclerosis and other inflammatory diseases.

**METHODS**

The data and analytical methods will be made available to other researchers for purposes of reproducing the results or replicating the procedure. However, because of a patent, study materials will not be made available to other researchers.

**Animal Models**

All animal experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals (NIH publication no. 85-23, revised 1996) and were approved by the Bordeaux Ethics Committee (CEEA50). All preclinical experiments described in this publication were approved by the Animal Care and Use Committee of Bordeaux, France (no. 50120192-A).

Adult male New Zealand White rabbits, weighing between 2.5 and 3.0 kg, were obtained from Charles Rivers Laboratories (St. Germain sur l’Arbresle, France). For 6 to 8 months, rabbits were fed a fat atherogenic diet that included 0.3% (w/w) cholesterol. To promote the development of complicated plaques, rabbits were subject to a surgical inflammatory injury 1 to 2 months after the beginning of the diet. De-endothelialization of thoracic and abdominal aortic areas was mechanically induced by 3 inflations and retractions of a 4-F Fogarty balloon catheter (Edwards Lifesciences, Maurepas, France). Surgery was performed under anesthesia induced by intramuscular injection of 50 mg/kg ketamine (Merial, France) and 5 mg/kg xylazine (Bayer Healthcare, France) and maintained by mask inhalation of 1.5% to 2% of isoflurane. Preventive antithrombotic treatment was heparin sodium solution (1000 IU) (Heparin Choay; Sanofi Synthelabo, Paris, France). Preoperative and postoperative analgesia were performed by administration of 100 mg of aspirin (Injectable Aspegic; Sanofi Synthelabo), and tolfedine (4 mg/kg; 2 subcutaneous injections 48 hours apart), respectively.12 Rabbits were euthanized by a single intravenous injection of pentobarbital in the marginal ear vein (120 mg/kg) (CEVA Santé Animale, France).

Female, 6-week-old Apoe−/− mice (17–18 g in weight) were purchased from Charles River Laboratories (Saint Germain Nuelles, France) and housed under a 12-hour light/dark cycle with food and water provided ad libitum. Mice were fed a high cholesterol diet (0.15% cholesterol) for 24 weeks to promote the development of atherosclerotic lesions. Animals were cared for in accordance with the institutional guidelines, and they were familiarized with their environment for at least 7 days before initiation of any experiments. For ex vivo studies and aorta isolation, animals were euthanized by intraperitoneal injection of Exagon (Axience, France) (300 mg/kg), diluted with 0.9% NaCl (1:4) under general anesthesia with 5% isoflurane.

**Human Specimen Collection**

Human tissue specimens were provided by Dr Ducasse, vascular surgeon at CHU Pellegrin Hospital (Bordeaux, France). Human samples were from patients who underwent endarterectomy after an acute vascular event. Human coronary artery samples were harvested from patients with end-stage heart failure during heart transplantation. All clinical interventions took place at CHU Pellegrin (Bordeaux, France) and at Haut-Lévêque Hospital (Pessac, France). All work with human tissues was approved by the Bordeaux Ethics Committee (Committee for the Protection of Persons Southwest and Overseas) and by the Research Ministry in France (authorization no. DC –2016-2724). The Bordeaux ethics committee waived the need for the patient written consent because surgical waste no longer attached to the person is considered res nulius. Patients were informed by the clinicians; if they did not express their opposition to research, the deidentified samples were immediately processed (paraffin embedding or protein extraction). All study procedures complied with the ethical standards of the Declaration of Helsinki.

**In Vivo Selection of scFv-Phages and Screening by Flow Cytometry of Individual Clones**

**In Vivo Selection**

ScFv-phages from the optimized (in-frame scFv selection) MG-Umb library (fully human scFv) were selected by in vivo phage display in the rabbit model of atherosclerosis. The scFv-phages were isolated from atherosclerotic lesions, located from the aortic arch to the iliac bifurcations. Briefly, they were recovered from the endothelial layer and then from the intartissular (ie, subendothelial layer in the intima) and intracellular (ie, cells in the plaque, such as macrophages, foam cells, T cells) fractions after extensive washes between each fraction. This process was repeated for each round of biopanning. Three cycles of selection were performed to enrich for specific antibodies, as described in our previous studies.6,7 Then, the in vivo–selected scFv-phages were screened by flow cytometry for the identification of clones of interest.6
Flow Cytometry Screening

Rabbit Protein Extraction and Coupling to Magnetic Beads

Protein extraction from rabbit atherosclerotic lesions was performed as previously described. Briefly, proteins were solubilized with the T-PER lysis buffer (Thermo Fisher Scientific, France) complemented with protease inhibitor cocktail (Thermo Fisher Scientific, France) and a Polytron TP-20 Homogenizer 8 (Kinematica, Lucerne, Switzerland). After 2 centrifugation steps at 13 000g at 4°C for 45 minutes to discard the insoluble material in the supernatant, the protein concentration of every soluble extract was determined using the Bradford Assay Kit, according to the manufacturer’s instructions (Thermo Fisher Scientific).

Fifty micrograms of protein extracts were covalently coupled to 300 nm of magnetic Carboxyl Adembeads according to the manufacturer’s instructions (Ademtech, France). Three batches of protein-coupled beads per sample were used.

Phage Antibody Preparation

Individual phage-infected XL1 blue bacteria were grown in 96-well plates (Greiner Bio One, France) in 500 µL triptone/yeast extract (2TY)/ampicillin/5% glucose supplemented with 10 µg/mL tetracycline. After overnight incubation, 25 µL of bacterial culture were inoculated in 500 µL 2xTYGAT medium and incubated for 3 hours. Phage production was induced by adding 25 µL of 2TY containing 3.10⁸ M13K07 helper phage particles (Stratagene, France). After infection at 37°C for 1 hour, bacteria were pelleted, resuspended in 500 µL 2TY/ampicillin/5% glucose medium supplemented with 40 µg/mL kanamycin, and grown at 26°C under rotation (New Brunswick Scientific, Edison, NJ) overnight. Bacteria were spun down at 10 000g for 10 minutes, and supernatants were used immediately for flow cytometry assay.

scFv-Phage Screening by Flow Cytometry

Binding of scFv-phages or scFv-Fc fusion antibodies to atherosclerotic protein extracts was determined by flow cytometry. Forty microliters of atherosclerotic rabbit protein-coated beads (5 µg/mL) were added to 100 µL of scFv-phages and incubated at 4°C under rotation for 3 hours. This was followed by incubation with mouse anti-pVIII (Abcam, France) or rabbit anti-human Fcy (Jackson Immunoresearch, USA) primary antibodies (1:10000) at 4°C under rotation overnight, and by incubation with Alexa Fluor 488– labeled anti-mouse antibodies (Life Technologies, France) (1:40) for scFv-phae detection.

Validation of the scFv Fragment VH-VL Sequences by Sequencing

Sanger sequencing was performed using primers for the scFv flanking regions in the phagemid (5'-TGCAATTCTATTCCAAGGAG-3' and 5'-AGA ATCATCAGATAAAGTAATCC-3'). Antibody gene fragments were analyzed using the IMGT/V-QUEST database (www.imgt.org/IMGT_vquest/vquest) for V germline determination and Complementarity-Determining Region analyses.

Production of Recombinant scFv-Fc Antibodies in HEK-293 FreeStyle and Baculovirus Expression Systems

Antibody Production and Purification From HEK-293 Cells

ScFv-Fc was produced by transient transfection using the FreeStyle 293-F expression system (Invitrogen, France). Expression vectors were prepared by producing (using Invitrogen GeneArt) the scFv-encoding cDNA fragments as linear fragments with optimized codons for Homo sapiens. HEK-293 FreeStyle cells were transfected with the purified expression vectors, according to the supplier instructions, with PEI 250 kDa (Sigma-Aldrich) at 1:2 ratio (DNA:transfection reagent). After 7 days of production at 37°C and 8% CO₂ in F17 medium supplemented with 8 mmol/L glutamine, scFv-Fc antibodies were purified by 1-step affinity chromatography with HiTrap FF protein A (GE Healthcare, France) on an ÄKTA avant 80 chromatography system. Molecules were eluted with 25 mmol/L citrate, pH3.0, and dialyzed against PBS, filtered (0.2 µm), and stored at 4°C until use.

Antibody Production and Purification From Insect Cells

The fusion ScFv-Fc P3 with 2 cysteine residues (scFv-Fc-2c) was produced using the baculovirus expression system. Briefly, the cDNA encoding the P3 scFv was polymerase chain reaction–amplified with the following forward and reverse primers: 5'-GCTACTTAAAGG GTGTCCAGTGCAAGTGAAGCTGACGCTGG ACCCGG-3' and 5'-GCTACGTACGCTTGATTTCCA GCTTGTTGCGCTGCT- 3'. The polymerase chain reaction fragment was inserted into a specific transfer vector in frame with the sequence encoding the immunoglobulin (lg) G1 signal peptide at the 5’ end and with a cDNA encoding the human IgG1 fragment variable domain with 2 extra cysteine residues at the C-terminus. Sf9 cells were cotransfected by lipofection with the transfer vector and purified viral DNA in the presence of 40 µL of DOTAP liposomal transfection reagent (Roche). Recombinant viruses were isolated by plaque assay. ScFv-Fc-2c was produced by infecting Sf9 cells, adapted to grow in serum-free medium, with the selected recombinant virus at a multiplicity of infection of 3 plaque-forming units per cell. At day 3 after infection, cell culture supernatant was harvested, and scFv-Fc-2c was purified using HiTrap FF, protein A, as
recommended by the manufacturer (GE Healthcare), and an ÄKTA purifier system. ScFv-Fc-2c was filter-sterilized through a 0.22 µm filter (Millipex GP; Millipore) and stored at 4°C until use.

Reactivity Analysis of scFv-Fc Clones by Flow Cytometry and Immunohistochemistry

**Flow Cytometry Analyses**

Maintenance of reactivity after scFv-Fc reformattting was first assessed by flow cytometry using the same protocol described for scFv-phages. Briefly, scFv-Fc was diluted at 10 µg/mL in PBS buffer and incubated with beads coated with protein extracts from rabbit atherosclerotic lesions (5 µg/mL) at 4°C under rotation for 3 hours. This was followed by incubation with rabbit anti-human Fcγ primary (1:65 dilution; Jackson Immunoresearch) and Alexa Fluor 488–labeled anti-rabbit (1:30 dilution, Life Technologies) secondary antibodies.

**Immunohistochemistry Analysis of Rabbit and Mouse Aorta Tissue Sections and of Human Endarterectomy Specimens**

The immunoreactivity of scFv-Fc antibodies was evaluated using tissue sections prepared from paraffin-embedded human, mouse, and rabbit atheromatous plaque specimens. Tissue sections were deparaffinized and rehydrated. Blocking steps (H2O2 blocking and unspecific site blocking with PBS/1% BSA/0.2% Triton X-100) and a retrieval step (10 mmol/L Tris, 1 mmol/L EDTA, 0.05% Tween 20, pH9) were performed. For human tissue sections, another blocking step was performed by incubation with 5% goat serum and the F(ab)′2 fragment goat anti-human IgG (Heavy +Light chains) (Jackson Immunoresearch) and goat anti-human IgG (Fcγ specific) (Jackson Immunoresearch), diluted at 100 µg/mL. After washes with PBS/1% BSA, sections were incubated with the scFv-Fc antibodies at 10 µg/mL at 4°C overnight. After washes with PBS/1% BSA/0.025% Triton X-100, sections were incubated with the secondary horseradish peroxidase–conjugated goat anti-human (Fcγ specific) antibody (1:1000) (Jackson Immunoresearch). Negative control samples were incubated with only the secondary antibody. Antibody binding was revealed using the 3,3’-diaminobenzidine system (DAKO, France).

Target Identification by Immunoprecipitation and Mass Spectrometry Analysis

**Com Immunoprecipitations**

The Invitrogen Dynabeads co-immunoprecipitation kit (14321D; Thermo Fisher Scientific) was used following the manufacturer’s instructions. Briefly, 20 µg of P3 scFv-Fc or C4 scFv-Fc (control antibody) or 10 µg of a commercial Gal3 Ab antibody (anti-galectin-3) (Abcam) was added to 1 mg of Dynabeads in coupling buffer and incubated at 37°C under rotation overnight. Proteins from human endarterectomy specimens were extracted and solubilized following the same protocol used for proteins from rabbit atherosclerotic lesions. After extraction, proteins were pooled and stored at −80°C. Dynabeads/antibody (P3 or C4) complexes were equilibrated in extraction buffer and then incubated with 1 mg of pooled human protein homogenates at 4°C under rotation for 1 hour. The Dynabeads/commercial Gal3 Ab complexes were incubated with 3 µg of Gal3R (recombinant human galectin-3). Then, beads were washed, and the co-immunoprecipitated proteins were eluted in the elution buffer (2x SDS Laemmli buffer supplied with the kit). One aliquot of each eluate was directly analyzed by SDS-PAGE polyacrylamide gel (4%–10%) and stained with silver nitrate. The rest of each sample was sent to a mass spectrometry facility for further processing.

Mass Spectrometry Analysis

**Sample Preparation and Protein Digestion**

Samples were solubilized in Laemmli buffer and were concentrated and cleaned on SDS-PAGE gels. Separation was stopped when proteins entered the resolving gel. After colloidal blue staining, all of the bands present in the relevant lane were extracted from the SDS-PAGE gel and cut in 1x1-mm pieces. Gel pieces were destained in 25 mmol/L ammonium bicarbonate 50% acetonitrile (ACN), rinsed twice in ultrapure water, and shrunk in ACN for 10 minutes. After ACN removal, gel pieces were dried at room temperature, covered with trypsin solution (10 ng/µL in 50 mmol/L NH4HCO3), rehydrated at 4°C for 10 minutes, and incubated at 37°C overnight. They were then incubated in 50 mmol/L NH4HCO3 at room temperature with rotary shaking for 15 minutes. Supernatants were collected, and the H2O/ACN/HCOOH (47.5:47.5:5) extraction solution was added to the gel slices for 15 minutes. The extraction step was repeated twice. Supernatants were pooled and dried in a vacuum centrifuge. Digests were solubilized in 0.1% HCOOH.16,17

**Nano-Scale Liquid Chromatographic Tandem Mass Spectrometry Analysis and Label-Free Quantitative Data Analysis**

Peptide mixtures were analyzed with an UltiMate 3000 nano—liquid chromatography system (Dionex, Amsterdam, the Netherlands) coupled to an Electrospray Orbitrap Fusion Lumos Tribid Mass Spectrometer (Thermo Fisher Scientific). Ten microliters of peptide digests were loaded onto a 300-µm-ID×5-mm C18
PepMap trap column (LC Packings) at a flow rate of 10 µL/min. Peptides were eluted from the trap column onto an analytical 75-mm-ID×50-cm C18 PepMap column (LC Packings) with a 4% to 40% linear gradient of solvent B in 48 minutes (solvent A was 0.1% formic acid and solvent B was 0.1% formic acid in 80% ACN). The separation flow rate was set at 300 nL/min. The mass spectrometer operated in positive ion mode at 1.8-kV needle voltage. Data were acquired using the Xcalibur 4.1 software in data-dependent mode. Mass spectrometry (MS) scans (m/z 375–1500) were recorded at a resolution of R=120,000 (at m/z 200) and an automatic gain control target of 4×10^5 ions collected within 50 milliseconds. Dynamic exclusion was set to 60 seconds, and top speed fragmentation in higher-energy C-trap dissociation (HCD) mode was performed over a 3-second cycle. Tandem Mass Spectrometry (MS/MS) scans with a target value of 3×10^3 ions were collected in the ion trap with a maximum fill time of 300 milliseconds. Additionally, only +2 to +7 charged ions were selected for fragmentation. Other settings were as follows: no sheath, no auxiliary gas flow, heated capillary temperature=275°C, normalized HCD collision energy=30%, and isolation width=1.6 m/z. The monoisotopic precursor selection was set to peptide and the intensity threshold to 5×10^5.

**Database Search and Result Processing**

The obtained data were analyzed with SEQUEST and Proteome Discoverer 2.3 (Thermo Fisher Scientific) using the Homo sapiens Reference Proteome Set (from Uniprot 2019-05; 73 645 entries). Spectra from peptides higher than 5000 Da or lower than 350 Da were rejected. The search parameters were as follows: mass accuracy of the monoisotopic peptide precursor and of peptide fragments was set to 10 ppm and 0.6 Da, respectively. Only b- and y-ions were considered for mass calculation. Methionine oxidation (+16 Da) and N-terminal acetylation (+42 Da) were considered as variable modifications and cysteine carbamidomethylation (+57 Da) as fixed modification. Two missed trypsin cleavages were allowed. Peptide validation was performed with the Percolator algorithm, and only high-confidence peptides were retained (ie, false positive rate=1% at the peptide level). Peaks were detected and integrated using the Minora algorithm embedded in Proteome Discoverer.

**ELISA Assay**

Three independent ELISA assays were performed in triplicate. ELISA plates were coated (at 4°C overnight) with recombinant human galectin-3 (Abcam), galectin-3BP (Abcam), galectin-1 (Abcam), GPαllβ3 integrin (Enzyme Research Laboratory, UK), or BSA (all at 5 µg/mL diluted in carbonate buffer). Then, each well was blocked with PBS/5% milk at 37°C for 1 hour. A mouse antibody against galectin-1 (Abcam) and rabbit antibodies against human galectin-3 and galectin-3BP (Abcam) and the mouse AP2 antibody (a gift from Dr Nurden) against the glycoprotein dllβ3 (GPαllβ3) integrin were used as positive controls. The human P3 scFv-Fc and control negative (CN) scFv-Fc (irrelevant antibody, a gift from Laboratoire Français du Fractionnement et des Biotechnologies antibodies (diluted to 50 µg/mL), and the commercial antibodies (diluted to 10 µg/mL) were added to the respective wells at room temperature for 2 hours, followed by horseradish peroxidase–conjugated secondary antibodies. Each step was followed by extensive washes in PBS/0.1% Tween 20. The final wash was with PBS alone, and the antibody reaction was evaluated with the o-phenylenediamine dihydrochloride system for ELISA (Sigma-Aldrich). Color absorbance was immediately read at 405 nm in a plate reader (Chameleon; Thermo Fisher Scientific).

**Co-staining Experiments**

**Human Tissue Sections**

Paraffin-embedded atherosclerotic lesions from human endarterectomy and coronary artery specimens were used in co-staining experiments. All of the steps including the retrieval process and classical blocking steps were as described for immunohistochemistry. The following antibody combinations were used: (1) P3 scFv-Fc diluted to 50 µg/mL in PBS/1% BSA with the anti-human galectin-3 antibody (10 µg/mL) (Abcam), and (2) P3 scFv-Fc diluted to 50 µg/mL in PBS/1% BSA with the rabbit anti-human LOX1 antibody (5 µg/mL). After incubation with these antibody combinations at 4°C overnight, sections were washed and then incubated (room temperature for 1 hour) with the following secondary fluorescent antibodies: Alexa Fluor 568–labeled anti-human (1:200) for P3 scFv-Fc, Alexa Fluor 488–labeled anti-rabbit (1:1000) for the anti-LOX1 antibody, or Alexa Fluor 488–labeled anti-mouse (1:200) for the anti-galectin-3 antibody. After washes, sections were mounted with Vectashield (VWR, France).

For all experiments, adjacent sections incubated only with secondary antibodies were used as a negative control.

Images were acquired with a Nanozoomer 2.0 HT slide scanner and the fluorescence imaging module (Hamamatsu Photonics, France) using an UPS APO 20X NA 0.75 objective and an additional 1.75× lens, leading to a final magnification of 35×. Virtual slides were acquired with a TDI-3CCD camera. Fluorescent images were acquired with a mercury lamp (LX2000 200W; Hamamatsu Photonics, Massy, France), and the filter was set for DAPI and/or GFP (green fluorescent protein)/Alexa Fluor 488, and/or Alexa Fluor 568, and/or Alexa Fluor 647/Cy5 fluorescence.
Mouse Tissue Sections

For immunofluorescence co-staining of atherosclerotic lesions from Apoe−/− mouse aorta samples, the following antibodies were tested (at 4°C overnight): P3 scFv-Fc (diluted to 50 µg/mL in PBS/1% BSA) and rabbit anti-LOX1 antibody (5 µg/mL). After washes and incubation (room temperature for 1 hour) with the secondary fluorescent antibodies Alexa Fluor 647–labeled anti-human (1:100) for scFv-Fc and Alexa Fluor 488 anti-rabbit (1:1000) for the anti-LOX1 antibody, sections were washed, stained with DAPI (Thermo Fisher Scientific), and mounted with ProLong Gold (Life Technologies).

For all experiments, adjacent sections incubated only with secondary antibodies were used as negative controls.

Ex Vivo Fluorescence Imaging of P3 scFv-Fc-2c

The chests of 3 mice (n=2 Apoe−/− and n=1 control) were opened by thoracotomy, the heart exposed, and the right atrium cut. A 30-gauge needle was inserted in the left ventricle. PBS/heparin (50 IU/mL per 2.5 mL; Sanofi Aventis, France) was inoculated, followed by 10 mL of PBS. Perfusion was continued with 2 mL of PBS containing the human P3 scFv-Fc-2c or the control human IgG antibody (100 µg for both) coupled to Alexa Fluor 647 according to the manufacturer’s instructions and using the Alexa Fluor 647 Antibody Labeling Kit (Thermo Fisher Scientific). After 20 minutes, mice were perfused with 5 mL of 4% v/v paraformaldehyde. The aorta was removed and embedded in an 80-mm Petri dish containing 0.8% p/v high-grade, 245 low-melting-point agarose.

Two sets of images of the same aortas were acquired. The first set of images was taken with a fluorescent ultramicroscope (light sheet imaging macroscopy; LaVision Biotech, France), equipped with a light cube for Alexa Fluor 647 acquisition. For the second set of images, aortas were imaged with 2 different confocal microscopes. The first was a Leica TCS SP8 mounted on an upright stand DM6 FS (Leica Microsystems, Mannheim, Germany) equipped with 405, 488, 552, and 638 lasers. The scanning was done using a conventional scanner (10–1800 Hz). The microscope was equipped with a galvanometric stage to do fast z acquisition and a motorized xy stage.

Then, a more resolutive confocal microscope was used (a Leica TCS SP5 on an upright stand DM6000 (Leica Microsystems), controlled by the software LAS AF and using objective HC PL FLUOTAR 10× dry NA 0.30). The fluorescent molecule was excited with a laser Helium-Neon 633 nm, and the emission of fluorescence was collected on a conventional Photomultiplier Tube (PMT) from 650 to 720 nm. The transmitted light image was done at the same time on a PMT on transmission pathway. The 3-dimensional mosaic was done automatically on 4x2 positions by using the Tile Scan module included in the software controlling the motorized stage (Märzhäuser, Wetzlar, Germany). Finally, a maximum intensity projection was applied to represent the localization of the fluorescence signal through the thickness of the aorta.

The microscopy experiments were performed at the Bordeaux Imaging Center of the Neurosciences Institute of the University of Bordeaux, France.

Image Quantification

Immunofluorescence Image Quantification

Image processing and analysis were done automatically with a Fiji-ImageJ macro. After opening the images from the ndpis source file with the Bio-formats plugin, a median filter was applied to remove noise. Then, for each tissue section, a different automatic threshold was applied for the red (P3 scFv-Fc) and green (mouse anti-galectin-3 and rabbit anti-LOX1 antibodies) channels to measure the area of positive pixels. Then, a logical AND was applied to create the colocalization image from the 2 separated channels and to determine the percentage of colocalization.

Ex Vivo Fluorescence Image Quantification

Image processing and analysis were done automatically with a Fiji-ImageJ macro. After applying a median filter to remove noise, the tissue contour was automatically drawn using an automatic threshold in a brightfield image. This contour was then transferred to the fluorescence image (P3 scFv-Fc-2c or IgG antibodies) to measure the intensity of all the pixels inside the contour and the intensity of the pixels above a fixed threshold.

Statistical Analysis

All ELISA measurements were performed in triplicate and repeated in 3 independent experiments. Triplicate values were averaged, and differential analyses were then conducted using the Kruskal-Wallis methodology. The Dunn test method was then used to identify significant difference between proteins. Analyses were performed using the R environment (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

In Vivo Selection and Individual Screening by Flow Cytometry of scFv-Phages and Reformattinng Into scFv-Fc Fragments

After the third round of in vivo biopanning of the MG-Umab library, >800 different scFv-phages were
screened using protein extracts from rabbit atherosclerotic lesions. Overall, ≈200 scFv-phages (24%) recognized the atherosclerotic protein extracts (binding at least 2-fold above the background noise of wild-type phages) in flow cytometry experiments. Figure 1A and 1B show the results of a typical experiment with 4 positive scFv-phages (P3, D4, C8, and C4). The E9 clone was chosen as a negative control for all of the experiments. The gating strategy to analyze the binding of scFv-phages and scFv-Fc clones on rabbit atheromatous proteins coupled to beads is illustrated in Figure 1A.

On the basis of the immunohistochemistry results in rabbit aorta sections with atheromatous lesions and the antibody sequence integrity, 142 scFv-phages were finally considered for further investigation. Fifty of them were successfully produced in HEK-293 FreeStyle cells as soluble scFv-Fc fragments with a concentration >5 µg/mL (ie, threshold value for screening tests in supernatants). After scFv-Fc engineering, analysis of the antibody reactivity in supernatants by flow cytometry using protein extracts from rabbit atherosclerotic lesions showed that 60% of the selected scFv-Fc maintained their reactivity.

### In Vitro Validation of the Bioreactivity of Reformatted Clones by Immunohistochemistry Assays

ScFv-Fc capacity to recognize their targets in artery sections with atheromatous lesions from different mammalian species was assessed, as done for scFv-phages. Figure 2 shows the results obtained with the P3, C4, D4, and C8 scFv-Fc antibodies. In sections of human endarterectomy specimens, the P3 scFv-Fc signal was strong in clusters of foam cells (arrowheads) and in areas with a necrotic core. In aorta sections from hypercholesterolemic rabbits, P3 staining was observed in the adventitia (A), in the intima (I) subendothelial area, and in some spindle-shaped cells in the disorganized media layer (M). In sections from Apoe−/− mice, P3 scFv-Fc with atheroma plaques, the necrotic area (arrowhead) and adventitia (A) were clearly stained. In sections of the human endarterectomy sample, the C4 scFv-Fc antibody detected foam cells in necrotic cores of the subendothelial area and near to the tunica media (M) layer (arrowhead). Similarly, in atherosclerotic rabbit sections, specific C4 labeling was observed near the tunica media/intima (M/I) interface and in the necrotic core (arrowhead). In Apoe−/− mouse aorta sections, C4 scFv-Fc recognized the tunica intima (I) (arrowhead), although some unspecific background signal was observed because of degradation and detachment of the adventitia tissue on the slide (asterisks). D4 scFv-Fc only labeled a few groups of cells (arrowhead) in the atheroma plaque of the human carotid specimen. However, this signal might not be specific when compared with the background signal obtained by incubation with only the secondary antibody (upper panel, horseradish peroxidase–goat anti-human Fcγ). This is because of the presence of human antibodies in the plaque, despite the different specific blocking steps with goat serum, Fab′/Fab′ fragment goat anti-human IgG (H+L), and goat anti-human IgG (Fcγ specific). Conversely, in rabbit aorta sections, D4 scFv-Fc clearly labeled the intima (I) and the endothelium (arrowhead). In the Apoe−/− mouse aorta section, D4 scFv-Fc only labeled the adventitia (A) (arrowhead). C8 scFv-Fc did not show any significant labeling in all section types, with the exception of an area close to the tunica media (M, arrowhead) in rabbit aorta sections with atheromatous lesions, and the adventitia (A) in Apoe−/− mouse atheroma sections (arrowhead). Because the final aim of the study was to use in vivo–selected human antibodies as targeting moieties for the detection of atheromatous plaques in patients, the best translatable scFv-Fc antibodies were clearly P3 and C4, which displayed strong labeling of atheroma plaques in both animal models and also in human carotid sections. Moreover, the sequence of P3 was chosen as the reference for a recently published third-generation sequencing project to study clone enrichment during the in vivo phage display selection. Specifically, it was among the best enriched sequences, and clones belonging to the same P3 clonotype with well-characterized somatic mutations in the VL gene were identified. On the basis of these previous results and because P3 reformatted as scFv-Fc showed the highest binding by flow cytometry and immunohistochemistry experiments, the rest of the study focused on the identification of the P3 scFv-Fc target, and C4 scFv-Fc was used as a comparison in LC-MS/MS analyses.

### Antigen Identification by Immunoprecipitation and Proteomic Analyses

To identify the antigen recognized by P3 scFv-Fc among the proteins extracted from endarterectomy specimens, after immunoprecipitation of human endarterectomy proteins with the P3 and C4 scFv-Fc clones (P3-PH [proteins from human biopsies], C4-PH), samples were separated by SDS-PAGE followed by silver staining to visualize the major bands. In samples immunoprecipitated with P3 scFv-Fc, a single band with a relative molecular mass of 60 kDa was observed on the SDS-PAGE gel (Figure 3, SDS-PAGE 1). Conversely, after immunoprecipitation with C4 scFv-Fc, multiple bands were detected (Figure 3, SDS-PAGE 2). Extraction and analysis by LC-MS/MS of the different bands (Table S1) showed that in both cases, the most represented proteins in the MS/MS spectra
were keratin, filamin, vimentin, desmoglein, desmoplakin, and junction plakoglobin. However, galectin-3, a new biomarker of atherosclerosis, was the highest represented protein in the P3-PH sample compared with C4-PH extract (abundance=35 326 405 in P3-PH and 1 266 731 in C4-PH; C4-PH/P3-PH abundance ratio=0.037 (Table S1). Because monomeric galectin-3 is subject to modifications, such as self-association (dimerization or oligomerization), which increase its range of biological activity, this might explain the band detected at 60 kDa (theoretical molecular weight of galectin-3=30 kDa). To test this hypothesis, Gal3R was immunoprecipitated with a commercial anti-Gal3 Ab, and the immunoprecipitate (Gal3 Ab-Gal3R) was separated by SDS-PAGE. As observed with P3 scFv-Fc, a single band was detected (Figure 3, SDS-PAGE 3).
Figure 2. Immunohistochemical analysis of arterial tissue sections from atheromatous rabbits and Apoe$^{-/-}$ mice and human endarterectomy specimens using the indicated scFv-Fc (single-chain fragment variable fused to the crystallizable fragment of immunoglobulin G).

The different areas in transversal sections are identified: adventitia (A), media (M), and intima (I). Sections were incubated with the indicated scFv-Fc antibodies (P3, C4, D4, and C8), followed by the HRP-conjugated goat anti-human Fcγ antibody, and the DAB substrate kit reagent. The yellow-brown staining indicates the presence of the antigen recognized by the scFv-Fc. No staining was observed in mouse and rabbit sections incubated with the secondary antibody alone, and there is low background noise in the human sections (upper panels). Scale bars, 100 µm. Nuclei were counterstained with hematoxylin. DAB indicates 3,3'-diaminobenzidine; and HRP, horseradish peroxidase.
Moreover, the Gal3R protein was identified by LC-MS/MS analysis for the Gal3 Ab-Gal3R immunoprecipitate, with an abundance of 30,552,241. Comparison of galectin-3 abundance in Gal3Ab-Gal3R and P3-PH gave a ratio close to 1 (Gal3Ab-Gal3R/P3-PH ratio=0.739), indicating a comparable galectin-3 amount in the 2 immunoprecipitates (Table S1).

Then, to assess the specificity of P3 scFv-Fc binding to galectin-3, an ELISA assay was performed using recombinant galectin-3 and galectin-3BP proteins, which is the main galectin-3 ligand, and also recombinant galectin-1, another β-galactoside lectin family member, and other irrelevant proteins such as BSA and the GPαIIbβ3 integrin (Figure 4A). Globally, average OD values tended to significantly differ ($P_{\text{Kruskals-Wallis}}=0.0503$) between studied proteins. Deeper analyses revealed that OD was significantly higher in galectin-3 than in galectin-3BP ($P=0.022$), BSA ($P=0.018$), and galectin-1 ($P=0.002$) (Figure 4A). These results highlight that P3 scFv-Fc bound specifically to galectin-3, and did not recognize galectin-3BP, galectin-1 protein, and BSA. Some cross-reactivity was observed with GPαllβ3, with a $P$ value=0.1087 for galectin-3 versus GPαllβ3. This could be explained by the fact that integrins, such as GPα3β1 and GPαvβ3, are among the galectin-3 ligands. Commercial antibodies against all these recombinant proteins and the AP2 (AP2 clone) antibody were used as positive controls, and the irrelevant CN antibody (scFv-Fc format) as a negative control (Figure 4B).

To further validate the identified target in human endarterectomy samples, immunofluorescence experiments were performed with P3 scFv-Fc and a commercial murine antibody against human galectin-3. Image analysis (Figure 5A through 5D) showed that P3 scFv-Fc labeled tunica intima areas that were recognized also by the commercial anti-galectin-3 antibody as indicated by the enlarged merge image (64.62% of colocalization with the commercial anti-galectin-3 antibody) (Figure 5C).

Figure 3. SDS-PAGE analysis of immune complexes.

After immunoprecipitate elution in 2x SDS Laemmli buffer, immune complexes were separated on polyacrylamide gels (4%-10%) and stained with silver nitrate. SDS-PAGE 1 and 2: proteins extracted from human endarterectomy specimens were immunoprecipitated with the P3 and C4 scFv-Fc (single-chain fragment variable fused to the crystallizable fragment of immunoglobulin G) (from mammalian cells), respectively. SDS-PAGE 3: Gal3R (recombinant galectin-3 protein) was immunoprecipitated with a commercial anti-GAL3 Ab (anti-galectin-3 antibody). MW indicates molecular weight; M, marker; NA, non attributed; PH, proteins from human biopsies.
Because galectin-3 is overexpressed by macrophages,26 human coronary biopsies were analyzed using P3 scFv-Fc and a rabbit antibody against LOX1 (the macrophage receptor for oxidized LDL) (Figure 5E through 5H). The enlarged merge image shows the colocation between P3 scFv-Fc and LOX1, especially in the subendothelial and lipid core areas (79.31%) (Figure 5G). Because galectin-3 is expressed also by
Figure 5. Colocalization of P3 scFv-Fc (single-chain fragment variable fused to the crystallizable fragment of immunoglobulin G) with an anti-galectin-3 antibody and an anti-LOX1 (lectin-type oxidized LDL receptor 1) antibody in the intima of human endarterectomy specimens and coronary sections.

A through D, Co-staining of P3 scFv-Fc (single-chain fragment variable fused to the crystallizable fragment of immunoglobulin G) and an anti-galectin-3 antibody in human endarterectomy sections by immunofluorescence. An Alexa Fluor 488 anti-mouse antibody (+488 anti-Ms [mouse]) was used to reveal the specific binding of the commercial anti-human galectin-3 antibody (Ms anti-galectin-3) (A and C). The Alexa Fluor 568 anti-human antibody (+568 anti-Hum) was used to reveal the specific binding of P3 scFv-Fc (Hum P3 scFv-Fc) (B and C). Before image merging, the red and green fluorescence signals were adjusted to comparable levels. The yellow color indicates colocalization of the antigens recognized by P3 scFv-Fc and the anti-human galectin-3 antibody (C). Secondary antibodies alone were used as negative controls (D). Size bars: 100 µm.

E through H, The Alexa Fluor 488 anti-rabbit antibody (+488 anti-Rb [rabbit]) was used to reveal the specific binding of the commercial anti-LOX1 antibody (Rb anti-LOX1) (E and G). The Alexa Fluor 647 anti-human antibody (+568 anti-Hum) was used to reveal the specific binding of P3-scFv-Fc (Hum P3 scFv-Fc) (F and G). Before image merging, the red and green fluorescence signals were adjusted to comparable levels. The yellow color indicates colocalization of the antigens labeled by P3 scFv-Fc and anti-LOX1 antibody (G). As galectin-3 is also expressed also by other cell types (ie, mast cells, eosinophils, neutrophils, endothelial cells, and activated T and B cells8,27), areas outside the macrophage location also are stained by P3 scFv-Fc (G). Size bars: 100 µm.
other cell types (eg, mast cells, eosinophils, neutrophils, endothelial cells, and activated T and B cells),
areas outside the macrophage location were also stained by P3 scFv-Fc (Figure 5F and 5G).

P3 ScFv-Fc Labels Atheroma Plaques in Apoe−/− Mice
To assess whether P3 scFv-Fc could be used for atheroma diagnostic imaging, first we costained aorta sections from Apoe−/− mice (a model of atherosclerosis) with P3 scFv-Fc and the anti-LOX1 antibody (Figure 6). The colocalization (in yellow) of the P3 scFv-Fc and anti-LOX1 antibodies (84.24%) indicated the presence of galectin-3 in the area of foam cells. Next, the P3 scFv-Fc binding profile was characterized ex vivo in the aorta of Apoe−/− and wild-type mice (Figure 7 and Figure S1). For this experiment, the P3 antibody with 2 extra cysteines at the C-terminus (P3 scFv-Fc-2c) was used. This modification is crucial for coupling the antibody to nanoparticles in future magnetic resonance imaging experiments. P3 scFv-Fc-2c specificity was assessed and validated by flow cytometry (data not shown). Compared with the signal obtained from control human IgG in Apoe−/− mouse aorta, macroscale fluorescence imaging revealed a significant signal of P3 scFv-Fc-2c in the aortic root (83.33%) (yellow arrowheads in Figure 7). Conversely, P3 scFv-Fc-2c and the human IgG control did not give any significant signal in the aorta of wild-type mice.

In addition to this first set of images, the specific binding of P3 scFv-Fc-2c on atheromatous plaques was confirmed by the confocal microscopy images shown in Figure S1 (yellow arrowheads, Figure S1B and S1F).

DISCUSSION
Nowadays, there is a rising interest in accessing and characterizing the molecular and cellular components of atheroma plaques to find strategies for reducing the associated risk of stroke and myocardial infarction. It is acknowledged that the plaque composition more than the narrowing of arteries defines the condition of plaque rupture. Imaging technologies are frequently used to determine the molecular composition of atheroma plaques, and specific contrast agents are urgently needed. This specificity can be offered by human antibodies that can target atherosclerosis biomarkers for diagnostic purposes.

Phage display is an efficient tool for biomarker identification for several tissues and cell types. Keller et al
developed the direct cell phage display approach to select human scFv that targets blood and lymphatic cells in cancer.28 In atherosclerosis, the combination of this method and fresh human tissues allowed the selection of peptides against CD100.29

In 1998, Arap et al set up the first in vivo phage display method to map endothelial cells in cancer.30 Later on, Arap et al,31 Staquicini et al,32 and Krag et al33 initiated a human vascular mapping project and identified peptides that recognize tumor cells in patients with cancer. In atherosclerosis, in vivo phage selection in animal models led to the discovery of peptides34 and antibodies35,36 that bind to a large panel of targets in their microenvironment, thus allowing studying the atheroma plaque composition. One important limitation of in vivo phage display using antibodies is the identification of the target among all of the overexpressed proteins extracted from tissues. Unlike peptides that can be compared with protein databases,32 in vivo antibody discovery requires more complex methodologies for target identification to better understand the pathogenesis and for biotherapeutic innovation. However, the myriad of unknown targets, which is a drawback of in vivo phage display using antibodies, also represents a large panel of antibody targets in their microenvironment. Here, immunoprecipitation and mass spectrometry analyses were used to show that galectin-3, a protein of 25 to 30 kDa, is the target of P3 scFv-Fc in proteins extracted from human endarterectomy samples.

Galectin-3 is a member of a family of 15 β-galactoside-binding proteins, named galectins, characterized by the presence of conserved carbohydrate recognition domains. Galectin-3 is the only chimeratype galectin in vertebrates, with a single carbohydrate recognition domain and a nonlectin N-terminal domain. Galectins are synthesized in the cytoplasm and secreted through a nonclassical exocytic pathway37 to interact with cell surface glycans. They regulate the immune system by modulating monocyte/macrophage functions.38 Galectin-3 is implicated in different biological processes, including cell activation, anti-apoptotic activity, cytokine secretion, and cell migration.22,39–42 Galectin-3 also strongly induces P-selectin, which interacts with PSGL1 (P-selectin glycoprotein ligand 1) on leukocyte to form platelet-leukocyte aggregates.43 The

Figure 7. Ex vivo imaging of P3 scFv-Fc (single-chain fragment variable fused to the crystallizable fragment of immunoglobulin G) in Apoe<sup>−/−</sup> and wild-type (WT) mice using a fluorescent ultramicroscope.

Fluorescence macroscopy analysis of P3 scFv-Fc coupled to Alexa Fluor 568 dye after ex vivo injection in Apoe<sup>−/−</sup> and WT mice. A human immunoglobulin (Ig)G coupled to Alexa Fluor 568 was used as negative control antibody. P3 scFv-Fc shows specific labeling of the atheroma in the Apoe<sup>−/−</sup> mouse (yellow arrowheads). Size bars: 500 μm.
interaction between platelets and leukocytes promotes their activation, which is crucial for triggering inflammation, vascular remodeling, and thrombosis. Galectin-3 implication in different processes in cancer and inflammation led to the development of molecules to block the underlying mechanisms.48

Currently, much interest is focused on the role of galectin-3 in cardiovascular diseases, particularly in atherosclerosis.20,24,42,49–51 Galectin-3 protein was first detected in carotid samples from endarterectomies.26 Galectin-3 has been considered as an inflammation amplifier during atherosclerotic plaque progression because of its close interrelation with macrophages and foam cells. Zhu et al showed that 125I-oxidized LDL and 125I-acetylated LDL are actively endocytosed by galectin-3-expressing Chinese hamster ovary (CHO) cells. Moreover, incubation with acetylated-LDL led to intracellular accumulation of cholesteryl esters, highlighting galectin-3 role in endocytosis of advanced glycation end-proteins and modified LDLs.52 More recently, Madrigal-Matute et al reported that galectin-3 can modulate oxidative stress by stimulating superoxide production in monocytes and regulates the adhesion of monocytes/macrophages to endothelial cells.53 Because of its implication in various diseases, galectin-3 therapeutic potential has been evaluated using inhibitors and animal knock-out models.53 In atherosclerosis, inactivation of Lgals3 (the gene encoding galectin-3) in Apoe−/− mice reduces the atheroma thickness.54,55 Moreover, in patients on chronic statin treatment, galectin-3 level was elevated, and the macrophage number was reduced within plaques, suggesting that this protein is a biomarker of plaque inflammation severity.56 Dysfunction of the endothelial barrier is the starting point of atherosclerosis; however, the exact role of galectin-3 in endothelial cells is unclear. A recent study showed that the interaction between galectin-3 and integrin β1 promotes different inflammatory factors, leading to endothelial cell stress and apoptosis.57 Galectin-3 might have both pro- and antiatherosclerotic roles. For instance, increased accumulation of galectin-3-negative macrophages has been observed in advanced human, rabbit, and mouse plaques compared with early lesions.58 Although many studies reported high galectin-3 expression in plaque macrophages, the functional heterogeneity of the macrophage population needs to be taken into account when assessing galectin-3 expression. Single-cell technologies, such as single-cell RNA sequencing and cytometry by time-of-flight, allowed identifying different macrophage clusters with different gene expression profiles and phenotypes. Besides the resident-like and proinflammatory subsets, the newly described anti-inflammatory TREM2 subset of macrophages has been linked to lipid uptake and foam cell formation, which are mainly associated with plaque progression. However, in agreement with the recent publication by Di Gregoli et al emphasizing that galectin-3 identifies a subset of macrophages with a beneficial role in plaque regression, the gene expression profile of foamy macrophages was also associated with plaque-resolving parameters, such as efferocytosis and tissue repair.59

Moreover, during atherosclerosis progression, foamy macrophage apoptosis may contribute to disease worsening. Although in the present study we did not compare plaque composition and phenotype in early and advanced atherosclerotic lesions in animal models and in human samples, we did observe significant differences in galectin-3 expression in human samples (data not shown). Additional analyses of macrophages from plaques at different disease stages would be useful to characterize galectin-3 expression level in nonfoamy, foamy, and apoptotic macrophages and to determine whether our anti-galectin-3 antibody P3 scFv-Fc could be used as a marker of plaque stability or progression. All of these studies indicate that galectin-3 is an ideal target for imaging modalities and for developing biotherapeutics to regulate its role in atherosclerosis by directly targeting this protein or one of its ligands (eg, galectin-3 binding protein, integrins GPa3β1,24 and GPa3β25), which are all overexpressed in atherosclerosis. Because P3 scFv-Fc can bind to galectin-3 ex vivo, future studies should determine whether this antibody can act as an antagonist or agonist on one of the mechanisms implicated in the inflammation process that characterizes atherosclerosis. If necessary, random mutations could be introduced in P3 scFv-Fc using human polymerases to increase its affinity for galectin-3. Moreover, our recent study performed by third-generation sequencing of scFv clones issued from the in vivo selection highlighted sequences related to the P3 scFv clone with point mutations.

In conclusion, our study shows that the combination of in vivo antibody selection and in vitro characterization of the target by considering the pathological microenvironment is a good starting point for developing diagnostic and biotherapeutic molecules that can easily be transferred to the clinic.

ARTICLE INFORMATION
Received April 14, 2020; accepted March 10, 2021.

Affiliations
CRMSB (Centre de Resonance Magnétique des Systèmes Biologiques), UMR5536 CNRS (Centre National de Recherche Scientifique), INSB (Institut National des Sciences Biologiques), Bordeaux, France (A.H., J.L., F.O., S.S., S.M., C.L., G.C.-S., M.-J.J.-V.); LFB (Laboratoire Français de Fractionnement et de Biotechnologies) Biotechnologies, Lille, France (A.F., P.M.); Protéome
Galectin-3, a Target of Human scFv-Phages in Atheroma

Pole, CGFB (Centre de Génomique Fonctionnelle de Bordeaux), Bordeaux, France (S.C.); CHU Pellegrin, Bordeaux, France (E.D.); UPS3044, CNRS (Centre National de Recherche Scientifique), Saint-Chrïost-Lés-Alés, France (M.D.-C.); and BE4S (Bio-Experts for Success), Croix, France (A.F.).

Acknowledgments
The microscopy work was done at Bordeaux Imaging Center, a service unit of CNRS-INSPERM and Bordeaux University, a member of the national France Biomaging infrastructure supported by the French National Research Agency (ANR-10-INBS-04). The help of C. Poujol and S. Marais is acknowledged. The statistical analysis was done by D.-A. Tregouet, Centre Bordeaux Population Research, Inserm U1219.

Sources of Funding
This work was supported by grant ANR-13-BSVS-0018 from the French National Research Agency Program named ATERANOS and a public grant from the French National Research Agency in the context of the Investments for the Future Program, reference ANR-10-LABX-57 named TRAIL and ANR-10-LABX-53 named Mablimprove.

Disclosures
None.

Supplementary Material
Table S1
Figure S1

REFERENCES

1. Hansson GK, Libby P. The immune response in atherosclerosis: a double-edged sword. Nat Rev Immunol. 2006;6:508–519.
2. Virmani R, Kolodgie FD, Burke AP, Farb A, Schwartz SM. Lessons from sudden coronary death: a comprehensive morphological classification scheme for atherosclerotic lesions. Arterioscler Thromb Vasc Biol. 2000;20:1262–1275. DOI: 10.1161/01.ATV.20.5.1262.
3. Junjathoor VV, Febrero MA, Podrez EA, Moore KJ, Andersson L, Koehn S, Rhee JS, Silverstein R, Hoff HF, Freeman MW. Scavenger receptors class A-I/II and CD36 are the principal receptors responsible for the uptake of modified low density lipoprotein leading to lipid loading in macrophages. J Biol Chem. 2002;277:49982–49988. DOI: 10.1074/jbc.M209649200.
4. Lariviere M, Lorenzo CS, Adumeau L, Bonnet S, Hemadou A, Alamyar E, Duroux P, Lefranc MP, Giudicelli V. IMGT® tools for the nucleotide analysis of immunoglobulin (IG) and T cell receptor (TR) V-(D)-J repertoires, polymorphisms, and IG mutations: IMGT/V-QUEST and IMGT/High-V-QUEST for NGS. Methods Mol Biol. 2012;882:569–604.
5. Juliant S, Leveque M, Cerutti P, Ozil A, Choblet S, Violet ML, Slemmrich MC, Harduin-Lepers A, Cerutti M. Engineering the baculovirus genome to produce galactosylated antibodies in lipoprotein cells. Methods Mol Biol. 2013;988:59–77.
6. Fassart D, Martin-Negrier ML, Claverol S, Thiolat ML, Crevel H, Toussaint C, Bonneau M, Muller B, Savineau JP, Delom F. Proteomic remodeling of proteasome in right heart failure. J Mol Cell Cardiol. 2014;66:41–52. DOI: 10.1016/j.yjmcc.2013.10.015.
7. Kall L, Canterbury JD, Weston J, Noble WS, MacCosas MJ. Semi-supervised learning for peptide identification from shotgun proteomics datasets. Nat Methods. 2007;4:923–925. DOI: 10.1038/nmeth1113.
8. Spivak M, Weston J, Bottou L, Kall L, Noble WS. Improvements to the percolator algorithm for peptide identification from shotgun proteomics data sets. J Proteome Res. 2009;8:3737–3745. DOI: 10.1021/pr900601k.
9. Schindelin J, Arganda-Carreras I, Frise E, Kaynig V, Longair M, Pietzsch T, Preibisch S, Rueden C, Saalfeld S, Schindel I, et al. Fiji: an open-source platform for biological-image analysis. Nat Methods. 2012;9:676–682. DOI: 10.1038/nmeth.2019.
10. Aksan G, Gedikli O, Keskin K, Nar G, Inci S, Yildiz SS, Kaplan O, Soylu K, Kilickesmez KO, Sahin M. Is galectin-3 a biomarker, a player-or both-in the presence of coronary atherosclerosis? J Invest Med. 2016;64:764–770. DOI: 10.1136/jim-2015-000041.
11. Falcone C, Lucibello S, Mazzucchelli I, Boffini S, D’Angelo A, Schirinzi S, Totaro R, Falcone R, Bondeens M, Pelosiro G. Galectin-3 plasma levels and coronary artery disease: a new possible biomarker of acute coronary syndrome. Int J Immunopathol Pharmacol. 2011;24:905–913. DOI: 10.1177/0394632011024010.
12. Suthahar N, Meijers WC, Sillje HHW, Ho JE, Liu FT, Panjwani N. Galectin-3 activation and inhibition in heart failure and cardiovascular disease: an update. Theranostics. 2018;8:593–609. DOI: 10.7150/thno.22196.
13. DeRoos EP, Wrobleski SK, Shea EM, Al-Khail RK, Hawley AE, Henke PK, Myers DD Jr, Wakefield TW, Diaz JA. The role of galectin-3 and galectin-3-binding protein in venous thrombosis. Blood. 2015;125:1813–1821. DOI: 10.1182/blood-2014-04-569939.
14. van der Hoeven NW, Hollander MR, Yildirim C, Jansen MF, Teunissen PF, Horrevoets AJ, van der Pouw Kraan TC, van Royen N. The emerging role of galectins in cardiovascular disease. Vascu Pharmcol. 2016;81:31–41. DOI: 10.1016/j.vph.2016.02.006.
15. Markowska AI, Liu FT, Panjwani N. Galectin-3 is an important mediator of VEGF- and bFGF-mediated angiogenic response. J Exp Med. 2010;207:1981–1993. DOI: 10.1084/jem.20090121.
16. Nachtigal M, Al- Assaad Z, Mayer EP, Kim K, Monsigny M. Galectin-3 expression in human atherosclerotic lesions. Am J Pathol. 1998;152:1199–1208.
17. Liu FT. Regulatory roles of galectins in the immune response. Int Arch Allergy Immunol. 2005;136:385–400. DOI: 10.1159/000084545.
18. Keeler T, Kalt R, Raab I, Schwarzner H, Mayrhofer C, Kerjaschi D, Hantusch B. Selection of scFv antibody fragments binding to human blood versus lymphatic endothelial surface antigens by direct cell phage display. PLoS One. 2015;10(5):e0127169. DOI: 10.1371/journ al.pone.0127169.
19. Luque MC, Gutierrez PS, Debias V, Martins WK, Puech-Leao P, Porto G, Coelho V, Boumsell L, Kall L, Stoff B. Phage display identification of...
SUPPLEMENTAL MATERIAL
| Accession | Description                                                                 | MW [kDa] | Abundance Ratio: (Gal3Ab-Gal3R) / (P3-PH) | Abundance Ratio: (C4-PH) / (P3-PH) | Abundances: P3-PH | Abundances: Gal3Ab-Gal3R | Abundances: C4-PH |
|-----------|------------------------------------------------------------------------------|----------|----------------------------------------|----------------------------------|-----------------|------------------------|----------------|
| P04264    | Keratin. type II cytoskeletal 1 OS=Homo sapiens OX=9606 GN=KRT1 PE=1 SV=6     | 66       | 2.33                                   | 1.225                             | 45 381 190     | 105 509 281            | 55 462 114     |
| P35527    | Keratin. type I cytoskeletal 9 OS=Homo sapiens OX=9606 GN=KRT9 PE=1 SV=3     | 62       | 2.616                                  | 1.106                             | 526 112 111    | 1 368 298 427          | 826 012 151    |
| P13645    | Keratin. type I cytoskeletal 10 OS=Homo sapiens OX=9606 GN=KRT10 PE=1 SV=6   | 58.8     | 1.188                                  | 1.332                             | 807 546 518    | 923 347 631            | 1 097 290 637  |
| P13647    | Keratin. type II cytoskeletal 5 OS=Homo sapiens OX=9606 GN=KRT5 PE=1 SV=3    | 62.3     | 1.701                                  | 1.701                             | 58 165 573    | 117 840 313            | 111 121 533    |
| P15924    | Desmoplakin OS=Homo sapiens OX=9606 GN=DSP PE=1 SV=3                        | 331.6    | 2.345                                  | 1.134                             | 22 454 072    | 52 833 781             | 23 573 337    |
| P02538    | Keratin. type II cytoskeletal 6A OS=Homo sapiens OX=9606 GN=KRT6A PE=1 SV=3 | 60       | 1.454                                  | 0.375                             | 10 841 863    | 15 352 283             | 3 805 985     |
| P04259    | Keratin. type II cytoskeletal 6B OS=Homo sapiens OX=9606 GN=KRT6B PE=1 SV=5 | 60       | 0.859                                  | 0.588                             | 5 179 109     | 4 525 792              | 2 750 493     |
| P02533    | Keratin. type I cytoskeletal 14 OS=Homo sapiens OX=9606 GN=KRT14 PE=1 SV=4  | 51.5     | 1.374                                  | 1.24                              | 32 955 718    | 50 877 777             | 38 641 899    |
| P08779    | Keratin. type I cytoskeletal 16 OS=Homo sapiens OX=9606 GN=KRT16 PE=1 SV=4  | 51.2     | 1.266                                  | 0.551                             | 28 412 636    | 35 940 365             | 15 478 307    |
| P21333    | Filamin-A OS=Homo sapiens OX=9606 GN=FLNA PE=1 SV=4                         | 280.6    | 0.837                                  | 31.76                             | 2 331 699     | 113 268                | 184 168 581   |
| P78385    | Keratin. type II cuticular Hb3 OS=Homo sapiens OX=9606 GN=KRT83 PE=1 SV=2   | 54.2     | 0.001                                  | 0.001                             | 8 831 388     |                        |               |
| Q86YZ3    | Hornerin OS=Homo sapiens OX=9606 GN=HRNR PE=1 SV=2                           | 282.2    | 3.61                                   | 1.218                             | 3 849 321     | 12 238 252             | 4 518 731     |
| P01857    | Immunoglobulin heavy constant gamma 1 OS=Homo sapiens OX=9606 GN=IGHG1 PE=1 SV=1 | 36.1 | 0.787                                 | 166.4                             | 10 092 989   | 8 868 397              | 1 783 465 295 |
| P14923    | Junction plakoglobin OS=Homo sapiens OX=9606 GN=JUP PE=1 SV=3               | 81.7     | 2.038                                  | 1.186                             | 12 809 734    | 27 080 217             | 15 755 277    |
| Q02413    | Desmoglein-1 OS=Homo sapiens OX=9606 GN=DSG1 PE=1 SV=2                       | 113.7    | 1.072                                  | 1.394                             | 8 753 901    | 11 331 669             | 12 730 080    |
| Accession | Name                                      | Species                        | Transcript Name | Protein  | RefSeq  | Protein  | Ensembl | Gene  | RefSeq  | Protein  | RefSeq  |
|----------|-------------------------------------------|-------------------------------|----------------|----------|---------|----------|---------|-------|---------|----------|---------|
| Q04695   | Keratin, type I cytoskeletal 17          | Homo sapiens                  | KRT17          | E=1 SV=2 | 48.1    | 4.332    | 1.145   | 2988 213 | 4.15    | 3406 516 |
| P01834   | Immunoglobulin kappa constant            | Homo sapiens                  | IGLK1          | E=1       | 11.8    | 0.392    | 124.2   | 7322 950 | 3.76    | 1031 345 |
| Q72794   | Keratin, type II cytoskeletal 1b        | Homo sapiens                  | KRT77          | E=2 SV=3 | 61.9    | 0.808    | 0.813   | 46801 707 | 1.07    | 77872 103 |
| P60709   | Actin, cytoplasmic 1                     | Homo sapiens                  | ACTB            | E=1 SV=1 | 41.7    | 2.303    | 23.77   | 4824 083 | 1.09    | 127722 848 |
| P62736   | Actin, aortic smooth muscle              | Homo sapiens                  | ACTA2           | E=1 SV=1 | 42.0    | 2.389    | 24.64   | 4088 968 | 2.06    | 168336 149 |
| P07355   | Annexin A2                               | Homo sapiens                  | ANXA2           | E=1 SV=2 | 38.6    | 1.416    | 12.17   | 7486 298 | 1.14    | 96256 829 |
| P35749   | Myosin-11                                | Homo sapiens                  | MYH11           | E=1 SV=3 | 227.2   | 0.757    | 13.97   | 125171 226 | 1.02    | 96454 844 |
| P02751   | Fibronectin                              | Homo sapiens                  | FN1             | E=1 SV=4 | 262.5   | 1.323    | 39.27   | 554226 68  | 1.65    | 69405 208 |
| P12035   | Keratin, type II cytoskeletal 3           | Homo sapiens                  | KRT3            | E=1 SV=3 | 64.4    | 1.684    | 1.922   | 160742 60  | 1.62    | 683572  |
| P35579   | Myosin-9                                 | Homo sapiens                  | MYH9            | E=1 SV=4 | 226.4   | 1.000    | 1000    | 502752 15  | 1.00    | 15683738 |
| P04114   | Apolipoprotein B-100                     | Homo sapiens                  | APOB            | E=1 SV=2 | 515.3   | 1.000    | 1000    | 49359 26  | 1.00    | 26384 937 |
| O95678   | Keratin, type II cytoskeletal 75          | Homo sapiens                  | KRT75           | E=1 SV=2 | 59.5    | 0.001    | 0.001   | 459243 59  | 1.00    | 39436 191 |
| P01860   | Immunoglobulin heavy constant gamma 3    | Homo sapiens                  | IGHG3           | E=1 SV=2 | 41.3    | 1000     |         | 39436 191 | 1.00    | 7946357  |
| Q8N1N4   | Keratin, type II cytoskeletal 78          | Homo sapiens                  | KRT78           | E=1 SV=2 | 56.8    | 1.64     | 1.527   | 5945 200 9 | 1.00    | 54255233 |
| P01859   | Immunoglobulin heavy constant gamma 2    | Homo sapiens                  | IGHG2           | E=1 SV=2 | 35.9    | 2.117    | 77.93   | 795076 14  | 1.00    | 44126 712 |
| P35580   | Myosin-10                                | Homo sapiens                  | MYH10           | E=1 SV=3 | 228.9   | 0.001    | 3.44    | 147192 10  | 1.00    | 10681 021 |
| P08670   | Vimentin                                 | Homo sapiens                  | VIM              | E=1 SV=4 | 53.6    | 0.048    | 6.615   | 10735669 87 | 1.00    | 75379085 |

**Note:** The table lists various proteins with their respective accession numbers, species, gene names, protein names, Ensembl gene ID, gene names, and RefSeq protein and transcript accessions. The values represent protein weights in kilodaltons (kDa) and are followed by protein accession numbers and gene names.
| P19013 | Keratin. type II cytoskeletal 4 OS=Homo sapiens OX=9606 GN=KRT4 PE=1 SV=4 | 57.3 | 1.97 | 0.274 | 8 086 080 | 16 872 053 | 1 770 839 |
| P13646 | Keratin. type I cytoskeletal 13 OS=Homo sapiens OX=9606 GN=KRT13 PE=1 SV=5 | 49.6 | 2.499 | 0.765 | 2 982 381 | 7 565 975 | 1 542 532 |
| P12111 | Collagen alpha-3(VI) chain OS=Homo sapiens OX=9606 GN=COL6A3 PE=1 SV=5 | 343.5 | 0.524 | 17.83 | 648 511 | 79 816 | 47 866 537 |
| Q92764 | Keratin. type I cuticular Ha5 OS=Homo sapiens OX=9606 GN=KRT35 PE=2 SV=5 | 50.3 | 2.236 | 0.001 | 7 709 838 | 8 583 421 |
| Q05707 | Collagen alpha-1(XIV) chain OS=Homo sapiens OX=9606 GN=COL14A1 PE=1 SV=3 | 193.4 | 79.17 | 31 329 | 78 467 | 41 524 411 |
| Q9Y490 | Talin-1 OS=Homo sapiens OX=9606 GN=TLN1 PE=1 SV=3 | 269.6 | 1000 | 16 445 393 |
| P46940 | Ras GTPase-activating-like protein IQGAP1 OS=Homo sapiens OX=9606 GN=IQGAP1 PE=1 SV=1 | 189.1 | 3.172 | 6.531 | 299 520 | 792 296 | 28 319 279 |
| P04406 | Glyceraldehyde-3-phosphate dehydrogenase OS=Homo sapiens OX=9606 GN=GAPDH PE=1 SV=3 | 36 | 1.366 | 3.607 | 3 766 910 | 6 067 009 | 18 817 508 |
| Q5D862 | Filaggrin-2 OS=Homo sapiens OX=9606 GN=FLG2 PE=1 SV=1 | 247.9 | 1.166 | 1.126 | 1 865 274 | 2 525 897 | 1 940 336 |
| P29508 | Serpin B3 OS=Homo sapiens OX=9606 GN=SERPINB3 PE=1 SV=2 | 44.5 | 4.519 | 1.273 | 2 665 624 | 15 747 547 | 3 565 204 |
| P26038 | Moesin OS=Homo sapiens OX=9606 GN=MSN PE=1 SV=3 | 67.8 | 1000 | 1000 | 28 527 | 14 284 505 |
| A0A286YFJ8 | Immunoglobulin heavy constant gamma 4 (Fragment) OS=Homo sapiens OX=9606 GN=IGHG4 PE=1 SV=1 | 43.8 | 1000 | 1000 | 32 076 | 6 207 931 |
| P05089 | Arginase-1 OS=Homo sapiens OX=9606 GN=ARG1 PE=1 SV=2 | 34.7 | 1.338 | 1.118 | 2 450 206 | 2 929 382 | 2 874 411 |
| Q08554 | Desmocollin-1 OS=Homo sapiens OX=9606 GN=DSC1 PE=1 SV=2 | 99.9 | 1.212 | 2.119 | 3 353 097 | 4 710 847 | 7 365 633 |
| Q5T749 | Keratinocyte proline-rich protein OS=Homo sapiens OX=9606 GN=KPRP PE=1 SV=1 | 64.1 | 1.095 | 1.608 | 4 589 295 | 7 546 455 | 10 630 003 |
| P17931 | Galectin-3 OS=Homo sapiens OX=9606 GN=LGALS3 PE=1 SV=5 | 26.1 | 0.739 | 0.037 | 35 326 405 | 30 552 241 | 1 266 731 |
| Accession | Description                                      | OS   | OX    | GN    | PE  | SV  | MW (kDa) | pI     | Molar Extinction Coefficient (Molar) |  |  |
|-----------|--------------------------------------------------|------|-------|-------|-----|-----|----------|--------|-------------------------------------|---|---|
| E9PKE3    | Heat shock cognate 71 kDa protein OS=Homo sapiens | 9606 | 9606  | HSPA8 | 1   | 1   | 68.8     | 1000   | 1000                                | 535 457 | 2 435 736 |
| Q08188    | Protein-glutamine gamma-glutamyltransferase E OS=Homo sapiens | 9606 | 9606  | TGM3  | 1   | 4   | 76.6     | 1.256  | 0.907                               | 4 132 727 | 5 165 512 | 3 745 956 |
| P04083    | Annexin A1 OS=Homo sapiens Ox=9606 GN=ANXA1 PE=1 SV=2 | 9606 | 9606  | ANXA1 | 1   | 1   | 38.7     | 1.51   | 2.346                               | 3 110 045 | 4 500 463 | 8 648 641 |
| P12814    | Alpha-actinin-1 OS=Homo sapiens Ox=9606 GN=ACTN1 PE=1 SV=2 | 9606 | 9606  | ACTN1 | 1   | 1   | 103      | 0.001  | 18.98                               | 54 288  | 2 606 953 |
| Q723Y8    | Keratin. type I cytoskeletal 27 OS=Homo sapiens Ox=9606 GN=KRT27 PE=1 SV=2 | 9606 | 9606  | KRT27 | 1   | 1   | 49.8     | 0.001  | 2.116                               | 690 173 | 1 986 529 |
| Q13813    | Spectrin alpha chain. non-erythrocytic 1 OS=Homo sapiens Ox=9606 GN=SPTAN1 PE=1 SV=3 | 9606 | 9606  | SPTAN1| 1   | 3   | 284.4    | 0.592  | 3.895                               | 32 298  | 68 760  | 5 824 759 |
| P01024    | Complement C3 OS=Homo sapiens Ox=9606 GN=C3 PE=1 SV=2 | 9606 | 9606  | C3    | 1   | 2   | 187      | 1.11   | 14.65                               | 238 945 | 658 899 | 9 816 719 |
| Q3SY84    | Keratin. type II cytoskeletal 71 OS=Homo sapiens Ox=9606 GN=KRT71 PE=1 SV=3 | 9606 | 9606  | KRT71 | 1   | 1   | 57.3     | 0.001  | 2.108                               | 1631 769 | 1 702 343 |
| P31944    | Caspase-14 OS=Homo sapiens Ox=9606 GN=CASP14 PE=1 SV=2 | 9606 | 9606  | CASP14| 1   | 1   | 27.7     | 2.237  | 1.565                               | 1508 967 | 3 673 264 | 2 994 961 |
| P02675    | Fibrinogen beta chain OS=Homo sapiens Ox=9606 GN=FGB PE=1 SV=2 | 9606 | 9606  | FGB   | 1   | 1   | 55.9     | 1.682  | 21.6                                | 204 267 | 81 373  | 14 895 512 |
| P11021    | Endoplasmic reticulum chaperone BiP OS=Homo sapiens Ox=9606 GN=HSPA5 PE=1 SV=2 | 9606 | 9606  | HSPA5 | 1   | 1   | 72.3     | 2.581  | 5.152                               | 372 169 | 1 822 958 | 4 027 552 |
| O43707    | Alpha-actinin-4 OS=Homo sapiens Ox=9606 GN=ACTN4 PE=1 SV=2 | 9606 | 9606  | ACTN4 | 1   | 1   | 104.8    | 2.448  | 5.214                               | 74 939  | 281 232 | 2 933 348 |
| P25311    | Zinc-alpha-2-glycoprotein OS=Homo sapiens Ox=9606 GN=AZGP1 PE=1 SV=2 | 9606 | 9606  | AZGP1 | 1   | 1   | 34.2     | 0.618  | 1.454                               | 4 171 075 | 2 627 848 | 6 422 387 |
| P0DOY3    | Immunoglobulin lambda constant 3 OS=Homo sapiens Ox=9606 GN=IGLC3 PE=1 SV=1 | 9606 | 9606  | IGLC3 | 1   | 1   | 11.3     | 1000   | 1000                                | 63 879  | 23 435 315 |
| P06702    | Protein S100-A9 OS=Homo sapiens Ox=9606 GN=S100A9 PE=1 SV=1 | 9606 | 9606  | S100A9| 1   | 1   | 13.2     | 2.549  | 1.011                               | 3 260 708 | 9 956 036 | 4 538 845 |
| Q71U36    | Tubulin alpha-1A chain OS=Homo sapiens Ox=9606 GN=TUBA1A PE=1 SV=1 | 9606 | 9606  | TUBA1A| 1   | 1   | 50.1     | 2.052  | 27.53                               | 362 570 | 745 243 | 10 901 197 |
| Accession | Description | OS            |OX | GN       | PE | SV | MW  | p-value | E-value | Length |
|-----------|-------------|---------------|----|----------|----|----|-----|---------|---------|--------|
| P02649    | Apolipoprotein E | Homo sapiens | 9606 | APOE    | 1  | 1  | 36.1| 0.001   | 24.7    | 889 811 |
| Q6KB66    | Keratin. type II cytoskeletal 80 | Homo sapiens | 9606 | KRT80   | 1  | 2  | 50.5| 1.725   | 1.046   | 2 250 313 |
| Q15063    | Periostin | Homo sapiens | 9606 | POSTN   | 1  | 2  | 93.3| 0.001   | 70.78   | 35 600 |
| A0A1B0GUU9 | Immunoglobulin heavy constant mu (Fragment) | Homo sapiens | 9606 | IGHM   | 1  | 1  | 51.9| 0.171   | 11.48   | 2 232 212 |
| P55072    | Transitional endoplasmic reticulum ATPase | Homo sapiens | 9606 | VCP | 1  | 4  | 89.3| 0.123   | 0.099   | 8 671 478 |
| Q14CN4    | Keratin. type II cytoskeletal 72 | Homo sapiens | 9606 | KRT72  | 1  | 2  | 55.8| 0.438   | 1 825 028 | 1 574 891 |
| A0A087WVV2 | Ribosome-binding protein 1 | Homo sapiens | 9606 | RRPB1  | 1  | 1  | 102.7| 1000    | 1000    | 3 987 261 |
| P04004    | Vitronectin | Homo sapiens | 9606 | VTN    | 1  | 1  | 54.3| 0.638   | 85.16   | 810 878 |
| Q8IU7     | Adipocyte enhancer-binding protein 1 | Homo sapiens | 9606 | AEBP1  | 1  | 1  | 130.8| 1000    | 11 142 930 |
| P68371    | Tubulin beta-4B chain | Homo sapiens | 9606 | TUBB4B | 1  | 1  | 49.8| 5.825   | 14.72   | 19 223 |
| Q6UWP8    | Suprabasin | Homo sapiens | 9606 | SBSN   | 1  | 2  | 60.5| 1.615   | 1.485   | 246 320 |
| A0A0B4J231 | Immunoglobulin lambda-like polypeptide 5 | Homo sapiens | 9606 | IGLL5  | 1  | 1  | 23.1| 2.034   | 121.9   | 133 573 |
| A0A087X055 | Collagen alpha-1(VI) chain | Homo sapiens | 9606 | COL6A1 | 1  | 1  | 108.3| 0.001   | 66.33   | 242 097 |
| Q09666    | Neuroblast differentiation-associated protein AHNAK | Homo sapiens | 9606 | AHNAK  | 1  | 2  | 628.7| 0.761   | 7.562   | 469 657 |
| Q13867    | Bleomycin hydrolase | Homo sapiens | 9606 | BLMH   | 1  | 1  | 52.5| 1.957   | 1.198   | 1 304 594 |

### Additional EC Number Information
- **P02649**: 3.4.1.25, 3.3.2.1, 3.2.1.103
- **Q6KB66**: 3.2.1.42, 3.4.13.1
- **Q15063**: 3.4.1.46
- **A0A1B0GUU9**: 3.4.1.4
- **P55072**: 3.4.1.5
- **Q14CN4**: 3.4.1.17
- **A0A087WVV2**: 3.4.1.5
- **P04004**: 3.4.1.17
- **Q8IU7**: 3.4.1.17
- **P68371**: 3.4.1.5
- **Q6UWP8**: 3.4.1.17
- **A0A0B4J231**: 3.4.1.17
- **A0A087X055**: 3.4.1.5
- **Q09666**: 3.4.1.46
- **Q13867**: 3.4.1.46
| Accession  | Description                                                                 | Species       | Chromosome | Start | End   | Log2Fold | p-Value  | FDR    | Log10 p-Value | Log10 FDR |
|------------|------------------------------------------------------------------------------|---------------|------------|-------|-------|---------|----------|--------|--------------|-----------|
| Q99715     | Collagen alpha-1(XII) chain OS=Homo sapiens                                | Homo sapiens | 9          | 332.9 | 0.001 | 5.481   | 25 882   | 4 187 082 |
| P02545     | Prelamin-A/C OS=Homo sapiens                                               | Homo sapiens | 22         | 74.1  | 3.833 | 10.01   | 572 827  | 2 978 452 |
| P21810     | Biglycan OS=Homo sapiens                                                    | Homo sapiens | 19         | 41.6  | 0.001 | 93.09   | 164 572  | 50 722 913 |
| P02647     | Apolipoprotein A-I OS=Homo sapiens                                         | Homo sapiens | 17         | 30.8  | 0.752 | 8.986   | 1 093 580| 1 009 112 |
| A0A0G2JIW1 | Heat shock 70 kDa protein 1B OS=Homo sapiens                               | Homo sapiens | 16         | 70.1  | 1.364 | 8.382   | 84 517   | 481 548 |
| P51888     | Prolargin OS=Homo sapiens                                                   | Homo sapiens | 15         | 43.8  | 0.001 | 30.13   | 219 197  | 20 257 631 |
| P15311     | Ezrin OS=Homo sapiens                                                       | Homo sapiens | 6          | 69.4  | 1000  | 1000    | 41 623   | 436 838 |
| A0A2R8Y5S7 | Radixin OS=Homo sapiens                                                     | Homo sapiens | 10         | 69.3  | 1000  | 1000    | 549 735  |         |
| Q5JP53     | Tubulin beta chain OS=Homo sapiens                                         | Homo sapiens | 14         | 47.7  | 0.94  | 53.18   | 22 422   | 21 076  |
| C9JEU5     | Fibrinogen gamma chain OS=Homo sapiens                                     | Homo sapiens | 12         | 50.3  | 0.001 | 25.87   | 122 868  | 14 365 894 |
| P10909     | Clusterin OS=Homo sapiens                                                   | Homo sapiens | 11         | 52.5  | 0.348 | 40.32   | 361 040  | 95 260  |
| J3QSU6     | Tenascin OS=Homo sapiens                                                    | Homo sapiens | 9          | 220.7 | 1000  | 1000    | 6 574 638 |         |
| Q01082     | Spectrin beta chain. non-erythrocytic 1 OS=Homo sapiens                   | Homo sapiens | 8          | 274.4 | 1000  | 1000    | 2 599 602 |         |
| P01876     | Immunoglobulin heavy constant alpha 1 OS=Homo sapiens                     | Homo sapiens | 7          | 37.6  | 0.713 | 3.226   | 3 276 026| 2 484 800 |
| P14618     | Pyruvate kinase PKM OS=Homo sapiens                                        | Homo sapiens | 5          | 57.9  | 2.228 | 7.358   | 645 019  | 1 894 485 |
| P68104     | Elongation factor 1-alpha 1 OS=Homo sapiens                               | Homo sapiens | 4           | 50.1  | 3.596 | 7.059   | 1 380 167| 6 111 838 |

**Note:** The provided information includes accession numbers, protein descriptions, species, chromosome, start and end positions, log2 fold change, p-value, and FDR values for various proteins. The data is presented in a tabular format.
| P22735 | Protein-glutamine gamma-glutamyltransferase K OS=Homo sapiens OX=9606 GN=TGM1 PE=1 SV=4 | 89.7 | 1.205 | 1.033 | 791 100 | 1 005 590 | 818 794 |
| Q13835 | Plakophilin-1 OS=Homo sapiens OX=9606 GN=PKP1 PE=1 SV=2 | 82.8 | 3.662 | 1.336 | 943 686 | 3 864 503 | 1 185 744 |
| P04792 | Heat shock protein beta-1 OS=Homo sapiens OX=9606 GN=HSPB1 PE=1 SV=2 | 22.8 | 3.509 | 11.81 | 785 763 | 3 944 150 | 18 569 316 |
| P20930 | Filaggrin OS=Homo sapiens OX=9606 GN=FLG PE=1 SV=3 | 434.9 | 2.074 | 1.084 | 612 555 | 1 573 109 | 816 305 |
| P23284 | Peptidyl-prolyl cis-trans isomerase B OS=Homo sapiens OX=9606 GN=PPIB PE=1 SV=3 | 23.7 | 1.016 | 24.38 | 59 248 | 112 122 | 62 051 286 |
| A0A0C4DGN4 | Zymogen granule protein 16 homolog B OS=Homo sapiens OX=9606 GN=ZG16B PE=1 SV=1 | 19.6 | 4.517 | 2.153 | 1 827 338 | 8 034 713 | 3 421 855 |
| P06733 | Alpha-enolase OS=Homo sapiens OX=9606 GN=ENO1 PE=1 SV=2 | 47.1 | 4.655 | 3.312 | 921 269 | 4 564 445 | 2 969 887 |
| P98160 | Basement membrane-specific heparan sulfate proteoglycan core protein OS=Homo sapiens OX=9606 GN=HSPG2 PE=1 SV=4 | 468.5 | 1000 | | | 1 922 225 |
| P00734 | Prothrombin OS=Homo sapiens OX=9606 GN=F2 PE=1 SV=2 | 70 | 1000 | 1000 | 68 460 | 6 723 894 |
| P62805 | Histone H4 OS=Homo sapiens OX=9606 GN=HIST1H4A PE=1 SV=2 | 11.4 | 4.228 | 0.481 | 2 814 698 | 16 733 421 | 1 202 205 |
| P04196 | Histidine-rich glycoprotein OS=Homo sapiens OX=9606 GN=HRG PE=1 SV=1 | 59.5 | 57.81 | 15 439 | 99 841 | 11 061 712 |
| C9J406 | MICOS complex subunit MIC60 OS=Homo sapiens OX=9606 GN=IMMT PE=1 SV=1 | 73.2 | 1000 | | | 4 407 636 |
| P35442 | Thrombospondin-2 OS=Homo sapiens OX=9606 GN=THBS2 PE=1 SV=2 | 129.9 | 1000 | | | 3 436 183 |
| P01023 | Alpha-2-macroglobulin OS=Homo sapiens OX=9606 GN=A2M PE=1 SV=3 | 163.2 | 7.245 | 170 500 | 43 721 | 3 469 375 |
| Q08431 | Lactadherin OS=Homo sapiens OX=9606 GN=MFGF8 PE=1 SV=3 | 43.1 | 0.001 | 27.93 | 154 872 | 19 943 799 |
| E9PF17 | Versican core protein OS=Homo sapiens OX=9606 GN=VCAN PE=1 SV=2 | 176.7 | 0.712 | 0.465 | 4 688 793 | 3 339 429 | 2 629 844 |
| Accession | Description | OS | SACC | Length | Mw (kDa) | pI | FW (Da) | GC | ORF | Database |
|-----------|-------------|----|------|--------|----------|----|--------|----|------|----------|
| Q01469    | Fatty acid-binding protein 5 | Homo sapiens | 9606 | 15.2   | 2.276    | 1.236 | 1 063 748 | 2 030 899 | 1 052 884 | Uniprot |
| P31947    | 14-3-3 protein sigma | Homo sapiens | 9606 | 27.8   | 5.962    | 0.001 | 194 048 | 2 695 048 | Uniprot |
| Q5QNW6    | Histone H2B type 2-F | Homo sapiens | 9606 | 13.9   | 5.335    | 1.15  | 1 374 217 | 8 101 146 | 1 992 132 | Uniprot |
| P05109    | Protein S100-A8 | Homo sapiens | 9606 | 10.8   | 2.245    | 1.816 | 3 413 420 | 8 180 162 | 6 171 888 | Uniprot |
| P32119    | Peroxiredoxin-2 | Homo sapiens | 9606 | 21.9   | 2.373    | 1.736 | 475 631 | 840 660 | 803 651 | Uniprot |
| P12273    | Prolactin-inducible protein | Homo sapiens | 9606 | 16.6   | 0.487    | 1.866 | 1 561 619 | 693 561 | 3 541 678 | Uniprot |
| Q96P63    | Serpin B12 | Homo sapiens | 9606 | 46.2   | 1.144    | 1.483 | 2 160 855 | 2 699 507 | 3 658 133 | Uniprot |
| P00338    | L-lactate dehydrogenase A chain | Homo sapiens | 9606 | 36.7   | 2.311    | 6.299 | 637 766 | 2 201 239 | 6 423 825 | Uniprot |
| Q5JX18    | Four and a half LIM domains protein 1 | Homo sapiens | 9606 | 29.1   | 0.001    | 42.77 | 283 968 | 44 626 126 | Uniprot |
| P02671    | Fibrinogen alpha chain | Homo sapiens | 9606 | 94.9   | 0.001    | 13.01 | 242 044 | 9 308 706 | Uniprot |
| Q6NZI2    | Caveolae-associated protein 1 | Homo sapiens | 9606 | 43.5   | 1000     | 1000 | 8 956 870 | Uniprot |
| P81605    | Dermcidin | Homo sapiens | 9606 | 11.3   | 0.878    | 1.349 | 1 597 868 | 1 409 168 | 2 108 953 | Uniprot |
| P07996    | Thrombospondin-1 | Homo sapiens | 9606 | 129.3  | 1000     | 1000 | 4 314 638 | Uniprot |
| P07900    | Heat shock protein HSP 90-alpha | Homo sapiens | 9606 | 84.6   | 1000     | 1000 | 224 887 | 2 127 037 | Uniprot |
| Q8WWA0    | Intelectin-1 | Homo sapiens | 9606 | 34.9   | 0.014    | 0.001 | 12 452 942 | 24 658 | Uniprot |
| P06576    | ATP synthase subunit beta mitochondrial | Homo sapiens | 9606 | 56.5   | 1.736    | 3.654 | 348 341 | 927 933 | 2 719 680 | Uniprot |
| P01042    | Kininogen-1 | Homo sapiens | 9606 | 71.9   | 0.204    | 58.59 | 148 106 | 14 553 | 14 828 856 | Uniprot |
| P18206 | Vinculin OS=Homo sapiens OX=9606 GN=VCL PE=1 SV=4 | 123.7 | 0.682 | 6.079 | 209 980 | 80 825 | 2 489 326 |
| P60174 | Triosephosphate isomerase OS=Homo sapiens OX=9606 GN=TP1 PE=1 SV=3 | 30.8 | 2.343 | 0.92 | 275 550 | 779 269 | 347 355 |
| B72624 | cDNA FLJ6329. highly similar to Myosin light polypeptide 6 OS=Homo sapiens OX=9606 GN=MYL6 PE=1 SV=1 | 26.7 | 1000 | 1000 | 151 145 | 8 869 697 |
| J3QR68 | Haptoglobin (Fragment) OS=Homo sapiens OX=9606 GN=HP PE=1 SV=3 | 45 | 0.923 | 3.686 | 296 485 | 214 197 | 1 595 809 |
| P12110 | Collagen alpha-2(VI) chain OS=Homo sapiens OX=9606 GN=COL6A2 PE=1 SV=4 | 108.5 | 1.22 | 31.02 | 303 644 | 132 739 | 13 859 775 |
| P51884 | Lumican OS=Homo sapiens OX=9606 GN=LUM PE=1 SV=2 | 38.4 | 0.001 | 13.52 | 556 747 | 9 128 102 |
| P21291 | Cysteine and glycine-rich protein 1 OS=Homo sapiens OX=9606 GN=CSRP1 PE=1 SV=3 | 20.6 | 0.001 | 17.55 | 284 626 | 15 167 061 |
| P08133 | Annexin A6 OS=Homo sapiens OX=9606 GN=ANXA6 PE=1 SV=3 | 75.8 | 0.001 | 7.254 | 59 216 | 2 881 655 |
| P08238 | Heat shock protein HSP 90-beta OS=Homo sapiens OX=9606 GN=HSP90AB1 PE=1 SV=4 | 83.2 | 1000 | 1000 | 116 746 | 1 181 134 |
| O75342 | Arachidonate 12-lipoxygenase. 12R-type OS=Homo sapiens OX=9606 GN=ALOX12B PE=1 SV=1 | 80.3 | 1.134 | 1.661 | 290 418 | 312 236 | 519 182 |
| Q16527 | Cysteine and glycine-rich protein 2 OS=Homo sapiens OX=9606 GN=CSRP2 PE=1 SV=3 | 20.9 | 1000 | 8 578 199 |
| Q01995 | Transgelin OS=Homo sapiens OX=9606 GN=TAGLN PE=1 SV=4 | 22.6 | 0.001 | 2.437 | 1 301 022 | 3 375 481 |
| Q14574 | Desmocollin-3 OS=Homo sapiens OX=9606 GN=DSC3 PE=1 SV=3 | 99.9 | 1.207 | 1.717 | 536 353 | 725 441 | 930 781 |
| P14625 | Endoplasmin OS=Homo sapiens OX=9606 GN=HSP90B1 PE=1 SV=1 | 92.4 | 1000 | 3 495 217 |
| P00747 | Plasminogen OS=Homo sapiens OX=9606 GN=PLG PE=1 SV=2 | 90.5 | 1000 | 2 546 712 |
| P02743 | Serum amyloid P-component OS=Homo sapiens OX=9606 GN=APCS PE=1 SV=2 | 25.4 | 0.001 | 40.22 | 1 163 541 | 63 837 550 |
| P31151 | Protein S100-A7 OS=Homo sapiens OX=9606 GN=S100A7 PE=1 SV=4 | 11.5 | 1.801 | 1.167 | 434 969 | 1 507 674 | 420 937 |
| Accession   | Description                                                                 | Start | End   | Score  | E-value | spectrum_length | Length |
|-------------|------------------------------------------------------------------------------|-------|-------|--------|----------|-----------------|--------|
| A0A0A0MSV6  | Complement C1q subcomponent subunit B (Fragment) OS=Homo sapiens OX=9606 GN=C1QB PE=1 SV=6 | 24    | 24    | 428.154 | 8.474E+15 | 9.824           | 428.154 |
| Q15582      | Transforming growth factor-beta-induced protein ig-h3 OS=Homo sapiens OX=9606 GN=TGFBI PE=1 SV=1 | 74.6  | 1000  | 504.499 | 2.506E+07 | 7.126           | 504.499 |
| P02748      | Complement component C9 OS=Homo sapiens OX=9606 GN=C9 PE=1 SV=2              | 63.1  | 7.126 | 504.499 | 2.506E+07 | 7.126           | 504.499 |
| P01040      | Cystatin-A OS=Homo sapiens OX=9606 GN=CSTA PE=1 SV=1                        | 11    | 2.506 | 996.644 | 4.641E+07 | 2.506           | 996.644 |
| Q6ZN40      | Tropomyosin 1 (Alpha) isoform CRA_f OS=Homo sapiens OX=9606 GN=TPM1 PE=1 SV=1 | 37.4  | 2.366 | 68.650  | 1.172E+09 | 47.232          | 68.650  |
| M0QZK8      | Uncharacterized protein OS=Homo sapiens OX=9606 PE=4 SV=1                  | 11.6  | 1.305 | 735.058 | 7.899E+07 | 1.083           | 735.058 |
| P50995      | Annexin A11 OS=Homo sapiens OX=9606 GN=ANXA11 PE=1 SV=1                   | 54.4  | 23.31 | 47.232  | 3.238E+07 | 8.363           | 47.232  |
| Q15149      | Plectin OS=Homo sapiens OX=9606 GN=PLEC PE=1 SV=3                          | 531.5 | 1000  | 831.389 | 3.303E+07 | 1000            | 831.389 |
| P01009      | Alpha-1-antitrypsin OS=Homo sapiens OX=9606 GN=SERPINA1 PE=1 SV=3         | 46.7  | 4.59  | 487.599 | 3.303E+07 | 5.233           | 487.599 |
| P51911      | Calponin-1 OS=Homo sapiens OX=9606 GN=CNN1 PE=1 SV=2                      | 33.2  | 10.09 | 165.932 | 4.661E+07 | 1.001           | 165.932 |
| Q16270      | Insulin-like growth factor-binding protein 7 OS=Homo sapiens OX=9606 GN=IGFBP7 PE=1 SV=1 | 29.1  | 1000  | 3.480E+07 | 1.480E+07 | 1000            | 3.480E+07 |
| P19823      | Inter-alpha-trypsin inhibitor heavy chain H2 OS=Homo sapiens OX=9606 GN=TIH2 PE=1 SV=2 | 106.4 | 1000  | 2.145E+07 | 2.145E+07 | 1000            | 2.145E+07 |
| G8JLG2      | CDSN OS=Homo sapiens OX=9606 GN=CDSN PE=1 SV=1                            | 51.6  | 2.676 | 859.709 | 1.593E+07 | 1.406           | 859.709 |
| Q16853      | Membrane primary amine oxidase OS=Homo sapiens OX=9606 GN=AOC3 PE=1 SV=3   | 84.6  | 12.45 | 50.740  | 7.642E+07 | 1.142           | 50.740  |
| P40926      | Malate dehydrogenase mitochondrial OS=Homo sapiens OX=9606 GN=MDH2 PE=1 SV=3 | 35.5  | 0.952 | 1.304E+07 | 2.564E+04 | 4.425           | 1.304E+07 |
| Accession | Description | OS | OX | GN | PE | SV | Mass (kDa) | pI | Length (aa) |
|-----------|-------------|----|----|----|----|----|------------|----|-------------|
| E9PFZ2    | Ceruloplasmin | Homo sapiens | 9606 | CP | 1 | 1 | 108.8 | 2.636 | 461703 |
| Q9NZT1    | Calmodulin-like protein 5 | Homo sapiens | 9606 | CALML5 | 1 | 2 | 15.9 | 4.925 | 501469 |
| P00558    | Phosphoglycerate kinase 1 | Homo sapiens | 9606 | PGK1 | 1 | 3 | 44.6 | 0.99 | 1043848 |
| P02792    | Ferritin light chain | Homo sapiens | 9606 | FTL | 1 | 2 | 20 | 0.001 | 3631057 |
| P07384    | Calpain-1 catalytic subunit | Homo sapiens | 9606 | CAPN1 | 1 | 2 | 81.8 | 1.426 | 2052887 |
| P02747    | Complement C1q subcomponent subunit C | Homo sapiens | 9606 | C1QC | 1 | 3 | 25.8 | 0.001 | 8785534 |
| P61160    | Actin-related protein 2 | Homo sapiens | 9606 | ACTR2 | 1 | 1 | 44.7 | 1000 | 1738341 |
| P07476    | Involucrin | Homo sapiens | 9606 | IVL | 1 | 2 | 68.4 | 1000 | 831200 |
| A0A087WVQ6 | Clathrin heavy chain | Homo sapiens | 9606 | CLTC | 1 | 1 | 191.9 | 1000 | 933697 |
| F5GX50    | Complement C4-B | Homo sapiens | 9606 | C4B | 1 | 1 | 187.6 | 0.001 | 1269947 |
| H0Y7A7    | Calmodulin-2 (Fragment) | Homo sapiens | 9606 | CALM2 | 1 | 1 | 20.7 | 1000 | 51075 |
| P07237    | Protein disulfide-isomerase | Homo sapiens | 9606 | P4HB | 1 | 1 | 57.1 | 3.062 | 1129350 |
| Q16610    | Extracellular matrix protein 1 | Homo sapiens | 9606 | ECM1 | 1 | 2 | 60.6 | 2.306 | 214816 |
| P08603    | Complement factor H | Homo sapiens | 9606 | CFH | 1 | 4 | 139 | 1000 | 788827 |
| P46821    | Microtubule-associated protein 1B | Homo sapiens | 9606 | MAP1B | 1 | 2 | 270.5 | 1000 | 2261574 |
| P52943    | Cysteine-rich protein 2 | Homo sapiens | 9606 | CRIP2 | 1 | 1 | 22.5 | 1000 | 6350152 |
| Q5T750    | Skin-specific protein 32 | Homo sapiens | 9606 | XP32 | 1 | 1 | 26.2 | 1.082 | 2974391 |
| Accession | Protein Name                                                                 | Organism       | OS          | OX          | GN         | PE | SV | Width   | Height   | Area     | Enlarged Area | Score |
|-----------|------------------------------------------------------------------------------|----------------|-------------|-------------|------------|----|----|---------|----------|----------|---------------|-------|
| P30101    | Protein disulfide-isomerase A3 OS=Homo sapiens OX=9606 GN=PDIA3 PE=1 SV=4   |                |             |             |            |    |    | 56.7    | 1000     | 1000     | 64 313        | 1 374 169 |
| Q9NZU5    | LIM and cysteine-rich domains protein 1 OS=Homo sapiens OX=9606 GN=LIMCD1 PE=1 SV=1 |                |             |             |            |    |    | 40.8    | 1000     | 20.91    | 41 215        | 4 172 391 |
| P04003    | C4b-binding protein alpha chain OS=Homo sapiens OX=9606 GN=C4BPA PE=1 SV=2  |                |             |             |            |    |    | 67      | 0.001    | 20.91    | 41 215        | 4 607 305 |
| P00491    | Purine nucleoside phosphorylase OS=Homo sapiens OX=9606 GN=PNP PE=1 SV=2    |                |             |             |            |    |    | 32.1    | 2.457    | 1.41     | 89 423        | 346 441 | 126 090 |
| A0A0AOMTH3| Integrin-linked protein kinase OS=Homo sapiens OX=9606 GN=ILK PE=1 SV=1     |                |             |             |            |    |    | 54.6    | 1000     |          |               |        |        |
| P09382    | Galectin-1 OS=Homo sapiens OX=9606 GN=LGALS1 PE=1 SV=2                      |                |             |             |            |    |    | 14.7    | 1000     | 1 819 743 |               |        |        |
| P30837    | Aldehyde dehydrogenase X. mitochondrial OS=Homo sapiens OX=9606 GN=ALDH1B1 PE=1 SV=3 |                |             |             |            |    |    | 57.2    | 1000     |          |               |        | 420 189 |
| C9JF17    | Apolipoprotein D (Fragment) OS=Homo sapiens OX=9606 GN=APOD PE=1 SV=1      |                |             |             |            |    |    | 24.1    | 0.21     | 3.809    | 827 521       | 48 247 | 2 980 241 |
| X6RJP6    | Transgelin-2 (Fragment) OS=Homo sapiens OX=9606 GN=TAGLN2 PE=1 SV=1        |                |             |             |            |    |    | 21.1    | 8.921    | 51 188   | 53 473        | 2 778 755 |
| P01833    | Polymeric immunoglobulin receptor OS=Homo sapiens OX=9606 GN=PIGR PE=1 SV=4 |                |             |             |            |    |    | 83.2    | 1.263    | 1.477    | 1 054 297     | 1 054 565 | 1 240 193 | 1 240 193 |
| G3V3U4    | Proteasome subunit alpha type OS=Homo sapiens OX=9606 GN=PSMA6 PE=1 SV=1    |                |             |             |            |    |    | 11.6    | 1.361    | 1.508    | 345 184       | 439 769 | 548 912 |
| P09525    | Annexin A4 OS=Homo sapiens OX=9606 GN=ANXA4 PE=1 SV=4                       |                |             |             |            |    |    | 35.9    | 1000     | 1000     | 371 153       | 418 889 |
| P0DP42    | V-set and immunoglobulin domain-containing protein 8 OS=Homo sapiens OX=9606 GN=VSIG8 PE=2 SV=1 |                |             |             |            |    |    | 43.9    | 0.062    | 0.001    | 1 071 003     | 44 472 |
| Q08380    | Galectin-3-binding protein OS=Homo sapiens OX=9606 GN=LGALS3BP PE=1 SV=1   |                |             |             |            |    |    | 65.3    | 0.001    | 11.32    | 40 712        | 1 175 630 |
| P17655    | Calpain-2 catalytic subunit OS=Homo sapiens OX=9606 GN=CAPN2 PE=1 SV=6     |                |             |             |            |    |    | 79.9    | 1000     |          |               |        | 1 258 940 |
| P48745    | CCN family member 3 OS=Homo sapiens OX=9606 GN=CCN3 PE=1 SV=1               |                |             |             |            |    |    | 39.1    | 1000     |          |               |        | 1 585 307 |

**Note:** The table includes protein accessions, protein names, organism, organism abbreviation, organism ID, gene name, protein ID, protein ID extension, protein ID score, protein ID width, protein ID height, protein ID area, and protein ID enlarged area.
| ID     | Description                                                                 | OS          | OX     | GN      | PE | SV | M    | L    | Q    | M    | P    |
|--------|------------------------------------------------------------------------------|-------------|--------|---------|----|----|------|------|------|------|------|
| P03950 | Angiogenin OS=Homo sapiens OX=9606 GN=ANG PE=1 SV=1                          |             |        |         |    |    | 16.5 | 1000 |      |      | 7 008 451 |
| B7ZKJ8 | ITIH4 protein OS=Homo sapiens OX=9606 GN=ITIH4 PE=1 SV=1                    |             |        |         |    |    | 103.8| 13.54| 27 611| 28 165| 1 166 765 |
| O14818 | Proteasome subunit alpha type-7 OS=Homo sapiens OX=9606 GN=PSMA7 PE=1 SV=1|             |        |         |    |    | 27.9 | 1.39 | 1.49  | 166 918| 331 016 | 456 350 |
| A0A0C4DH38 | Immunoglobulin heavy variable 5-51 OS=Homo sapiens OX=9606 GN=IGHV5-51 PE=3 SV=1 |             |        |         |    |    | 12.7 | 1000 |      |      | 3 613 847 |
| P01011 | Alpha-1-antichymotrypsin OS=Homo sapiens OX=9606 GN=SERPINA3 PE=1 SV=2     |             |        |         |    |    | 47.6 | 0.792| 2.341 | 524 467| 339 828 | 1 961 292 |
| P07737 | Profilin-1 OS=Homo sapiens OX=9606 GN=PFN1 PE=1 SV=2                        |             |        |         |    |    | 15   | 2.002| 10.76 | 73 160 | 200 657 | 1 187 235 |
| E9PK25 | Cofilin-1 OS=Homo sapiens OX=9606 GN=CFL1 PE=1 SV=1                         |             |        |         |    |    | 22.7 | 3.301| 18.5  | 73 612 | 291 701 | 1 902 475 |
| P06727 | Apolipoprotein A-IV OS=Homo sapiens OX=9606 GN=APOA4 PE=1 SV=3             |             |        |         |    |    | 45.4 | 1000 |      |      | 1 254 604 |
| Q15046 | Lysine--tRNA ligase OS=Homo sapiens OX=9606 GN=KARS PE=1 SV=3              |             |        |         |    |    | 68   | 1000 |      |      | 728 628 |
| P00325 | Alcohol dehydrogenase 1B OS=Homo sapiens OX=9606 GN=ADH1B PE=1 SV=2        |             |        |         |    |    | 39.8 | 0.001| 8.389 | 92 282 | 1 151 690 |
| K7EK77 | ATP synthase subunit alpha mitochondrial (Fragment) OS=Homo sapiens OX=9606 GN=ATPSF1A PE=1 SV=1 |             |        |         |    |    | 22.2 | 1.867| 7.849 | 95 222 | 253 308 | 1 919 224 |
| Q9UI42 | Carboxypeptidase A4 OS=Homo sapiens OX=9606 GN=CPA4 PE=1 SV=2              |             |        |         |    |    | 47.3 | 1.011| 1.191 | 436 078| 489 788 | 518 292 |
| Q9NR12 | PDZ and LIM domain protein 7 OS=Homo sapiens OX=9606 GN=PDLIM7 PE=1 SV=1   |             |        |         |    |    | 49.8 | 1000 |      |      | 2 352 668 |
| P21980 | Protein-glutamine gamma-glutamyltransferase 2 OS=Homo sapiens OX=9606 GN=ARPC4 PE=1 SV=3 |             |        |         |    |    | 77.3 | 1000 |      |      | 599 576 |
| P59998 | Actin-related protein 2/3 complex subunit 4 OS=Homo sapiens OX=9606 GN=ARPC4 PE=1 SV=3 |             |        |         |    |    | 19.7 | 1.714| 23.86 | 72 430 | 229 689 | 3 163 160 |
| A0A0A0MSQ0 | Plastin-3 OS=Homo sapiens OX=9606 GN=PLS3 PE=1 SV=1                     |             |        |         |    |    | 69.3 | 1000 | 1000 | 44 104 | 78 282 |
| P00624 | HK0139  | Alpha-parvin OS=Homo sapiens OX=9606 GN=PARVA PE=1 SV=1 | 46.6 | 1000 | 226 091 |
| P47929 | Galectin-7 OS=Homo sapiens OX=9606 GN=LGALS7 PE=1 SV=2 | 15.1 | 10.594 | 0.829 | 656 160 | 6 022 365 | 568 841 |
| P29536 | Leiomodin-1 OS=Homo sapiens OX=9606 GN=LMOD1 PE=1 SV=3 | 67 | 1000 | 3 109 431 |
| P20774 | Mimetcan OS=Homo sapiens OX=9606 GN=OGN PE=1 SV=1 | 33.9 | 0.001 | 15.44 | 469 785 | 6 874 894 |
| Q8WX93 | Palladin OS=Homo sapiens OX=9606 GN=PALLD PE=1 SV=3 | 150.5 | 1000 | 716 729 |
| Q9H3U7 | SPARC-related modular calcium-binding protein 2 OS=Homo sapiens OX=9606 GN=SMOC2 PE=2 SV=2 | 49.6 | 1000 | 646 965 |
| P48735 | Isocitrate dehydrogenase [NADP] mitochondrial OS=Homo sapiens OX=9606 GN=IDH2 PE=1 SV=2 | 50.9 | 1000 | 410 840 |
| P10301 | Ras-related protein R-Ras OS=Homo sapiens OX=9606 GN=RRAS PE=1 SV=1 | 23.5 | 1000 | 625 649 |
| P08294 | Extracellular superoxide dismutase [Cu-Zn] OS=Homo sapiens OX=9606 GN=SOD3 PE=1 SV=2 | 25.8 | 1000 | 2 150 059 |
| P62140 | Serine/threonine-protein phosphatase PP1-beta catalytic subunit OS=Homo sapiens OX=9606 GN=PPP1CB PE=1 SV=3 | 37.2 | 1000 | 346 858 |
| P18669 | Phosphoglycerate mutase 1 OS=Homo sapiens OX=9606 GN=PGAM1 PE=1 SV=2 | 28.8 | 1000 | 168 058 | 415 579 |
| Q96QA5 | Gasdermin-A OS=Homo sapiens OX=9606 GN=GSDMA PE=1 SV=4 | 49.3 | 1.357 | 1.406 | 660 379 | 879 530 | 912 017 |
| Q07960 | Rho GTPase-activating protein 1 OS=Homo sapiens OX=9606 GN=ARHGP1 PE=1 SV=1 | 50.4 | 1000 | 671 085 |
| A0A3B3IRNS | Fibromodulin OS=Homo sapiens OX=9606 GN=FMOD PE=1 SV=1 | 32.7 | 0.001 | 11.33 | 75 912 | 1 829 809 |
| P07225 | Vitamin K-dependent protein S OS=Homo sapiens OX=9606 GN=PROS1 PE=1 SV=1 | 75.1 | 1000 | 2 039 996 |
| E9HK0 | Tetranclectin OS=Homo sapiens OX=9606 GN=CLEC3B PE=1 SV=1 | 17.8 | 1000 | 822 733 |
| Accession  | Description                                                                 | OS     | OX     | GN      | PE | SV | Start | Stop | Score |
|------------|-------------------------------------------------------------------------------|--------|--------|---------|----|----|-------|------|-------|
| P06312     | Immunoglobulin kappa variable 4-1 OS=Homo sapiens OX=9606 GN=IGKV4-1 PE=1 SV=1| 13.4   | 1000   |         |     |    |       |      |       |
| P13796     | Plastin-2 OS=Homo sapiens OX=9606 GN=LCP1 PE=1 SV=6                          | 70.2   | 1000   |         |     |    |       |      |       |
| E9PK52     | Band 4.1-like protein 2 OS=Homo sapiens OX=9606 GN=EPB41L2 PE=1 SV=1         | 90.9   | 1000   |         |     |    |       |      |       |
| Q03135     | Caveolin-1 OS=Homo sapiens OX=9606 GN=CAV1 PE=1 SV=4                         | 20.5   | 1000   |         |     |    |       |      |       |
| Q9ULV4     | Coronin-1C OS=Homo sapiens OX=9606 GN=CORO1C PE=1 SV=1                       | 53.2   | 1000   |         |     |    |       |      |       |
| Q9HC84     | Mucin-5B OS=Homo sapiens OX=9606 GN=MUC5B PE=1 SV=3                          | 596    | 0.925  | 0.001   | 119,972 | 110,987 | 605,229 |
| B7Z4L4     | Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit 1 OS=Homo sapiens OX=9606 GN=RPN1 PE=1 SV=1 | 49.9   | 2.441  | 15.9    | 19,587 | 47,819 |       |
| P20851     | C4b-binding protein beta chain OS=Homo sapiens OX=9606 GN=C4BPB PE=1 SV=1   | 28.3   | 1000   |         |     |    |       |      |       |
| P27824     | Calnexin OS=Homo sapiens OX=9606 GN=CANX PE=1 SV=2                          | 67.5   | 1000   |         |     |    |       |      |       |
| P00740     | Coagulation factor IX OS=Homo sapiens OX=9606 GN=FP9 PE=1 SV=2              | 51.7   | 1000   |         |     |    |       |      |       |
| P05164     | Myeloperoxidase OS=Homo sapiens OX=9606 GN=MPO PE=1 SV=1                   | 83.8   | 1.143  | 1.747   | 154,701 | 368,143 | 358,838 |
| Q96CG8     | Collagen triple helix repeat-containing protein 1 OS=Homo sapiens OX=9606 GN=CTHRC1 PE=1 SV=1 | 26.2   | 1000   |         |     |    |       |      |       |
| P02760     | Protein AMBP OS=Homo sapiens OX=9606 GN=AMBP PE=1 SV=1                      | 39     | 0.122  | 15.73   | 149,814 | 18,263 | 2,842,627 |
| Q14195     | Dihydropyrimidinase-related protein 3 OS=Homo sapiens OX=9606 GN=DPYSL3 PE=1 SV=1 | 61.9   | 1000   | 1000    | 89,536 | 542,310 |
| P07858     | Cathepsin B OS=Homo sapiens OX=9606 GN=CTSB PE=1 SV=3                       | 37.8   | 0.001  | 10.65   | 61,154 | 1,562,756 |
| A0A0U1RQV3 | EGF-containing fibulin-like extracellular matrix protein 1 (Fragment) OS=Homo sapiens OX=9606 GN=EFEMP1 PE=1 SV=1 | 31.7   | 0.001  | 2.283   | 331,532 | 1,133,040 |
| Accession | Name                                      | Species | Organism | Gene Symbol | Protein | Transcript | 1000 | 1000 | 2000 | 1000 |
|---------|-------------------------------------------|---------|----------|-------------|---------|------------|------|------|------|------|
| Q9C075 | Keratin, type I cytoskeletal 23 OS=Homo sapiens OX=9606 GN=KRT23 PE=1 SV=2 | Homo sapiens | Homo sapiens | KRT23 | 48.1 | 1000 | 1000 | 2000 | 1000 |
| P60842 | Eukaryotic initiation factor 4A-I OS=Homo sapiens OX=9606 GN=EIF4A1 PE=1 SV=1 | Homo sapiens | Homo sapiens | EIF4A1 | 46.1 | 7.99 | 3.315 | 23958 | 327983 | 215830 |
| Q8IZP2 | Putative protein FAM10A4 OS=Homo sapiens OX=9606 GN=ST13P4 PE=5 SV=1 | Homo sapiens | Homo sapiens | ST13P4 | 27.4 | 1000 | 423965 |
| Q9Y6C2 | EMILIN-1 OS=Homo sapiens OX=9606 GN=EMILIN1 PE=1 SV=3 | Homo sapiens | Homo sapiens | EMILIN1 | 106.6 | 1000 | 290750 |
| H0YD13 | CD44 antigen OS=Homo sapiens OX=9606 GN=CD44 PE=1 SV=2 | Homo sapiens | Homo sapiens | CD44 | 22.7 | 1000 | 190818 | 441593 |
| D6R9Z1 | Receptor of-activated protein C kinase 1 (Fragment) OS=Homo sapiens OX=9606 GN=RACK1 PE=1 SV=8 | Homo sapiens | Homo sapiens | RACK1 | 26.3 | 1000 | 190818 | 441593 |
| Q6A163 | Keratin, type I cytoskeletal 39 OS=Homo sapiens OX=9606 GN=KRT39 PE=2 | Homo sapiens | Homo sapiens | KRT39 | 55.6 | 0.001 | 0.001 | 941702 |
| P07360 | Complement component C8 gamma chain OS=Homo sapiens OX=9606 GN=C8G PE=1 SV=3 | Homo sapiens | Homo sapiens | C8G | 22.3 | 1000 | 1430389 |
| P04899 | Guanine nucleotide-binding protein G(i) subunit alpha-2 OS=Homo sapiens OX=9606 GN=GNAI2 PE=1 SV=3 | Homo sapiens | Homo sapiens | GNAI2 | 40.4 | 1000 | 867424 |
| Q9Y6R7 | IgGFc-binding protein OS=Homo sapiens OX=9606 GN=FGCBP PE=1 SV=3 | Homo sapiens | Homo sapiens | FGCBP | 571.6 | 1000 | 605189 |
| P13639 | Elongation factor 2 OS=Homo sapiens OX=9606 GN=EEF2 PE=1 SV=4 | Homo sapiens | Homo sapiens | EEF2 | 95.3 | 1000 | 443046 | 102939 |
| P29279 | Connective tissue growth factor OS=Homo sapiens OX=9606 GN=CTGF PE=1 SV=2 | Homo sapiens | Homo sapiens | CTGF | 38.1 | 1000 | 102939 |
| P13489 | Ribonuclease inhibitor OS=Homo sapiens OX=9606 GN=RNH1 PE=1 SV=2 | Homo sapiens | Homo sapiens | RNH1 | 49.9 | 1000 | 45571 | 172496 |
| Q15404 | Ras suppressor protein 1 OS=Homo sapiens OX=9606 GN=RSU1 PE=1 SV=3 | Homo sapiens | Homo sapiens | RSU1 | 31.5 | 1000 | 412358 |
| O94905 | Erlin-2 OS=Homo sapiens OX=9606 GN=ERLIN2 PE=1 SV=1 | Homo sapiens | Homo sapiens | ERLIN2 | 37.8 | 1000 | 783840 |
| O14773 | Tripeptidyl-peptidase 1 OS=Homo sapiens OX=9606 GN=TPP1 PE=1 SV=2 | Homo sapiens | Homo sapiens | TPP1 | 61.2 | 1000 | 873519 |
| Accession | Description                                      | Species       | Oxidation | Peptide   | Sequences | Width (AA) | Height (KDa) | Mw (KDa)  |
|-----------|--------------------------------------------------|---------------|-----------|-----------|-----------|------------|--------------|-----------|
| P55290    | Cadherin-13 OS=Homo sapiens OX=9606              | Homo sapiens  | 9606      | 1         | 1         | 78.2       | 1000         | 2967442   |
| P49908    | Selenoprotein P OS=Homo sapiens OX=9606          | Homo sapiens  | 9606      | 1         | 3         | 43.2       | 1000         | 1089297   |
| P27169    | Serum paraoxonase/arylesterase 1 OS=Homo sapiens| Homo sapiens  | 9606      | 1         | 3         | 39.7       | 1000         | 1639039   |
| P55058    | Phospholipid transfer protein OS=Homo sapiens    | Homo sapiens  | 9606      | 1         | 1         | 54.7       | 1000         | 642557    |
| P61158    | Actin-related protein 3 OS=Homo sapiens          | Homo sapiens  | 9606      | 1         | 3         | 47.3       | 1000         | 29954     |
| E9PDU6    | Calponin (Fragment) OS=Homo sapiens              | Homo sapiens  | 9606      | 1         | 1         | 20.2       | 1000         | 152572    |
| P05154    | Plasma serine protease inhibitor OS=Homo sapiens | Homo sapiens  | 9606      | 1         | 3         | 45.6       | 1000         | 561747    |
| P33176    | Kinesin-1 heavy chain OS=Homo sapiens            | Homo sapiens  | 9606      | 1         | 1         | 109.6      | 1000         | 491424    |
| Q07065    | Cytoskeleton-associated protein 4 OS=Homo sapiens| Homo sapiens  | 9606      | 1         | 2         | 66         | 1000         | 481910    |
| P38646    | Stress-70 protein mitochondrial OS=Homo sapiens  | Homo sapiens  | 9606      | 1         | 2         | 73.6       | 1000         | 20792     |
| O00159    | Unconventional myosin-Ic OS=Homo sapiens         | Homo sapiens  | 9606      | 1         | 2         | 121.6      | 1000         | 134782    |
| B4DPQ0    | Complement C1r subcomponent OS=Homo sapiens      | Homo sapiens  | 9606      | 1         | 4         | 81.8       | 1000         | 60192     |
| P35555    | Fibrillin-1 OS=Homo sapiens OX=9606              | Homo sapiens  | 9606      | 1         | 1         | 312.1      | 1000         | 406352    |
| Q9Y277    | Voltage-dependent anion-selective channel protein| Homo sapiens  | 9606      | 1         | 1         | 30.6       | 1.034        | 26367     |
| Q96PD5    | N-acetylmuramoyl-L-alanine amidase OS=Homo sapiens| Homo sapiens  | 9606      | 1         | 1         | 62.2       | 1000         | 363275    |
| Q15084    | Protein disulfide-isomerase A6 OS=Homo sapiens   | Homo sapiens  | 9606      | 1         | 1         | 48.1       | 1000         | 15615     |
| Accession   | Description                                                                 | GO            | E-Value | Score  | Log10(P-Value) | MAF   |
|-------------|------------------------------------------------------------------------------|---------------|---------|--------|----------------|-------|
| A0A0A0MS15  | Immunoglobulin heavy variable 3-49                                          | OX=9606 GN=IGHV3-49 PE=3 SV=1 | 13   | 1000   | 1 744 048       |       |
| P24539      | ATP synthase F(0) complex subunit B1 mitochondrial                           | OX=9606 GN=ATP5PB PE=1 SV=2 | 28.9 | 1000   | 431 898         |       |
| O75083      | WD repeat-containing protein 1                                               | OX=9606 GN=WDR1 PE=1 SV=4 | 66.2 | 1000   | 374 808         |       |
| Q9UBC9      | Small proline-rich protein 3                                                 | OX=9606 GN=SPRR3 PE=1 SV=2 | 18.1 | 1.549  | 0.001 179 655 257 193 |       |
| P15144      | Aminopeptidase N                                                             | OX=9606 GN=ANPEP PE=1 SV=4 | 109.5| 1000   | 279 343         |       |
| Q15019      | Septin-2                                                                     | OX=9606 GN=SEPT2 PE=1 SV=1 | 41.5 | 1000   | 377 432         |       |
| P20073      | Annexin A7                                                                   | OX=9606 GN=ANXA7 PE=1 SV=3 | 52.7 | 1000   | 228 191         |       |
| Q8N436      | Inactive carboxypeptidase-like protein X2                                   | OX=9606 GN=CPXM2 PE=2 SV=3 | 85.8 |        |                   |       |
| Q5JR08      | Rho-related GTP-binding protein RhoC (Fragment)                              | OX=9606 GN=RHOC PE=1 SV=8 | 21.5 | 1000   | 905 779         |       |
| Q99536      | Synaptic vesicle membrane protein VAT-1 homolog                             | OX=9606 GN=VAT1 PE=1 SV=2 | 41.9 |        |                   |       |
| Q9NZN4      | EH domain-containing protein 2                                               | OX=9606 GN=EHD2 PE=1 SV=2 | 61.1 | 1000   | 1 898 281       |       |
| B4DT28      | Heterogeneous nuclear ribonucleoprotein R. isoform CRA_a                     | OX=9606 GN=HNRNPR PE=1 SV=1 | 55.7 | 1000   | 651 320         |       |
| B5MDF5      | GTP-binding nuclear protein Ran                                                | OX=9606 GN=RAN PE=1 SV=1 | 26.2 | 1000   | 102 675 1 558 912 |       |
| Q8TF66      | Leucine-rich repeat-containing protein 15                                     | OX=9606 GN=LRRC15 PE=2 SV=2 | 64.3 | 0.001  | 214 171 674 082 |       |
| Accession | Description | MW (kDa) | Molecular Weight in kDa | Gal3Ab/Gal3R | C4-PH | P3-PH |
|-----------|-------------|----------|--------------------------|--------------|--------|--------|
| A0A0U1RRM8 | Fermitin family homolog 2 (Fragment) OS=Homo sapiens OX=9606 GN=FERMT2 PE=1 SV=1 | 61.9 | 1000 | | | |
| Q92765 | Secreted frizzled-related protein 3 OS=Homo sapiens OX=9606 GN=FRZB PE=1 SV=2 | 36.2 | 1000 | | | 7 510 093 |
| Q08722 | Leukocyte surface antigen CD47 OS=Homo sapiens OX=9606 GN=CD47 PE=1 SV=1 | 35.2 | 1000 | | | 2 008 365 |
| P07093 | Gli-derived nexin OS=Homo sapiens OX=9606 GN=SERPINE2 PE=1 SV=1 | 44 | 1000 | | | 582 320 |
| J3QRN6 | Unconventional myosin-IId OS=Homo sapiens OX=9606 GN=MYO1D PE=1 SV=1 | 111.2 | 1000 | | | 151 508 |
| P02763 | Alpha-1-acid glycoprotein 1 OS=Homo sapiens OX=9606 GN=ORM1 PE=1 SV=1 | 23.5 | 1000 | 1000 | | 263 871 |
| Q8IWU6 | Extracellular sulfatase Sulf-1 OS=Homo sapiens OX=9606 GN=SULF1 PE=1 SV=1 | 101 | 1000 | | | 398 478 |
| Q15746 | Myosin light chain kinase. smooth muscle OS=Homo sapiens OX=9606 GN=MYLK PE=1 SV=4 | 210.6 | 1000 | | | 719 644 |
| P84243 | Histone H3.3 OS=Homo sapiens OX=9606 GN=H3F3A PE=1 SV=2 | 15.3 | 5.924 | 0.74 | 872 765 | 5 212 878 |
| Q86VP6 | Cullin-associated NEDD8-dissociated protein 1 OS=Homo sapiens OX=9606 GN=CAND1 PE=1 SV=2 | 136.3 | 2.101 | 4 458 635 | 9 368 130 | 28 020 |

Galactin-3 protein is highlighted in yellow

MW [kDa]: Molecular Weight in kDa, Gal3Ab-Gal3R: immunoprecipitate of anti-galactin-3 antibody with recombinant human galactin-3, C4-PH: immunoprecipitate of C4 scFv-Fc antibody with human endarterectomy proteins, P3-PH: immunoprecipitate of P3 scFv-Fc antibody with human endarterectomy protein
Figure S1. *Ex vivo* imaging of P3 scFv-Fc in Apoe-/- (B, C, D, E, F) and wild-type (WT) mice (A) using confocal microscopes.

Confocal microscopy analysis was performed using P3 scFv-Fc coupled to Alexa-Fluor 648 dye after ex vivo injection in Apoe-/- and WT mice. A human IgG coupled to Alexa-Fluor 648 was used as negative control antibody. The presence of atheromatous plaques in the lumen of the aorta was observed in the Apoe-/- mouse (yellow arrowheads) (B, F). The yellow dotted square corresponds to the enlarged view of P3 binding (C). The merged image was performed to show the localization of the fluorescence signal through the thickness of the aorta (F). Size bars (A, B, D, E, F): 500 μm; Size bars (C): 200 μm.