Nucleic Acids Research, 2025, gkaf1189
https://doi.org/10.1093/nar/gkaf1189
Database issue

SVAtlas: a comprehensive single extracellular vesicle omics resource

Zhonghui Wei[1,2,3,†], Na Zhou[1,2,†], Ming Jing[4,†], Yanling Cai[5], Yingxin Zhang[1,2], Xinyu Wang[1,2], Linxinyu Wang[1,2], Di Wu[5], Fuzhong Xue[1,2,*], Qingzhen Hou[1,2,*]

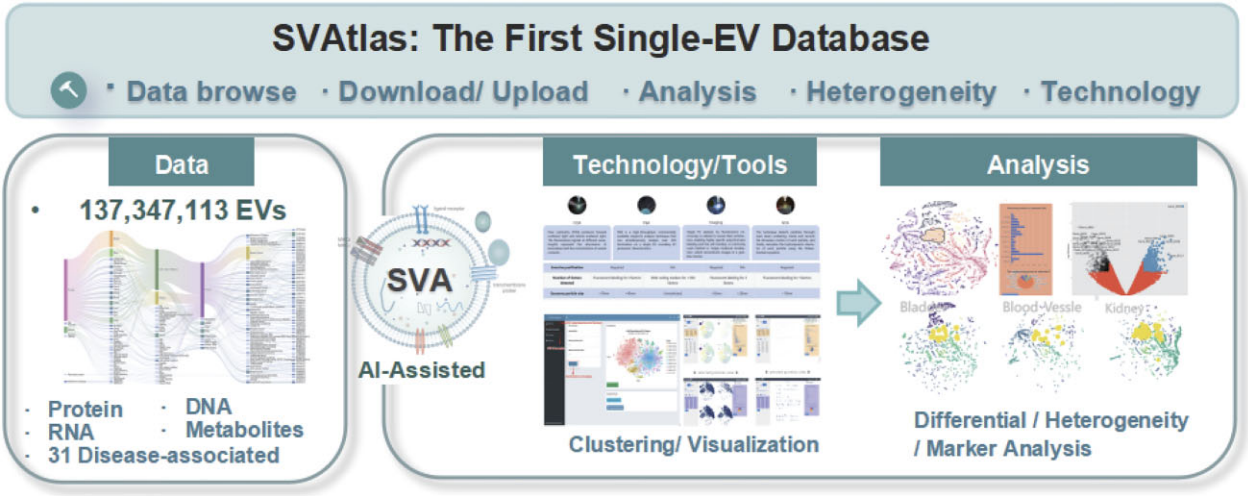
[1]Department of Biostatistics, School of Public Health, Cheeloo College of Medicine, Shandong University, Jinan 250100, China
[2]National Institute of Health Data Science of China, Shandong University, Jinan 250100, China
[3]Medical Integration and Practice Center, Cheeloo College of Medicine, Shandong University, Jinan, Shandong 250012, China
[4]Department of Software Engineering, School of Data and Computer Science, Shandong Women's University, Jinan 250300, China
[5]Shanghai Secretech Co., Ltd.,Shanghai201112,China

*To whom correspondence should be addressed. Email: houqingzhen@sdu.edu.cn
Correspondence may also be addressed to Fuzhong Xue. Email: xuefzh@sdu.edu.cn
†The first three authors should be regarded as Joint First Authors.

Abstract

Extracellular vesicles (EVs) are nanoscale particles released by cells, carrying proteins, nucleic acids, lipids, and metabolites. These vesicles mediate intercellular communication and modulate disease progression across various conditions. Owing to their molecular heterogeneity and the stable bilayer protecting their cargo, EVs serve as valuable tools for early disease detection and personalized therapies. However, traditional bulk EV studies aggregate data, which can mask the distinct molecular profiles of individual EVs critical for targeted diagnostics and treatments, diminishing diagnostic precision. Recent advances in single-EV analysis, leveraging high-resolution sequencing and imaging technologies, have revealed unique molecular signatures. However, a comprehensive database integrating multi-omics data from single EVs remains lacking. To address this, we developed SVAtlas, the first database dedicated to integrating single-EV datasets (2015–2025). SVAtlas incorporates 8120 protein entries, 106 RNA entries (miRNA, mRNA, circRNA, and lncRNA), 2 DNA entries, and 8 lipid/metabolite entries across 276 EV projects, spanning 31 diseases, 32 tissues/organs, and 10 biofluids from five species. SVAtlas offers single-EV datasets with experimental parameters, heterogeneity analyses, disease-specific marker exploration, built-in analysis/clustering/visualization pipelines, and an LLM-based question-answering tool, empowering researchers to explore single-EV omics in detail. Free and accessible at https://www.svatlas.org/, SVAtlas accelerates the clinical translation of single-EV analysis and biomarker discovery.

Graphical abstract



Received: August 15, 2025. **Revised:** October 13, 2025. **Accepted:** October 13, 2025

Introduction

Extracellular vesicles (EVs) are membrane-bound nanoscale particles secreted by nearly all cell types, carrying a diverse range of proteins, nucleic acids, lipids, and metabolites [1, 2]. These vesicles mediate key biological processes, including intercellular communication, cellular homeostasis, and pathogenesis, with implications in cancer progression and metastasis [3–6]. EVs exhibit unique properties, such as a stable phospholipid bilayer that protects their cargo from degradation in biofluids [7], abundant presence in easily accessible fluids like plasma, urine, and saliva [8], and the ability to cross biological barriers such as the blood-brain barrier [9]. These features make EVs promising tools for non-invasive biomarker discovery and liquid biopsy applications in diseases like cancer and neurodegenerative disorders [10–13]. However, EVs exhibit significant heterogeneity, with diverse subpopulations differing in biogenesis, size, and molecular composition, which complicates their diagnostic use [14–17].

Traditional bulk EV studies aggregate data from entire populations, which masks subpopulation-specific signatures and diminishes diagnostic sensitivity [18–21]. For instance, rare disease-associated EVs, such as L1CAM$^+$ neuronal EVs that make up only 3.7% of plasma EVs, are often overlooked [22]. Methods like quantitative reverse transcription polymerase chain reaction (qRT-PCR) fail to capture this heterogeneity, reducing sensitivity and specificity [23]. Additionally, bulk approaches involve complex isolation techniques (e.g. ultracentrifugation), which may risk contamination and ignore crucial molecular transfer mechanisms [24, 25].

In contrast, single-EV analysis enables profiling of individual vesicles, overcoming the limitations of bulk EV studies by revealing disease-specific subpopulations with high sensitivity and multiplexing capabilities [26–29]. Recent advancements in high-resolution, single-EV technologies, such as nanoscale flow cytometry (nFCM) [30, 31], proximity barcoding assays (PBA) [32], nanoparticle tracking analysis (NTA) [33–35], stochastic optical reconstruction microscopy (STORM) [36], single-molecule localization microscopy (SMLM) [37], droplet-based single-EV sequencing [21, 38], and digital PCR-based EV profiling [39], enable detailed profiling of individual vesicles and have significantly improved sensitivity.

These methods resolve heterogeneity (e.g. 30%–63% L1CAM$^+$ EVs carrying neuronal markers) [22] and enable multi-omics integration, enhancing diagnostic accuracy (e.g. 95% for breast cancer via CD9$^+$/HER2$^+$/ERBB2$^+$ EVs) [40]. Consequently, these methods are pivotal in clinical applications, including early disease detection and prognostic assessment [41, 42]. For example, PBA identified ITGB3$^+$ exosomes as markers for colorectal cancer progression, aiding noninvasive early detection [43], while TACSTD2$^+$ sEVs were shown to outperform traditional biomarkers like CEA in cancer susceptibility, especially in the elderly [44]. In liver cancer, miR-122 in exosomes demonstrated superior sensitivity compared to RT-qPCR [45], and DLL3 in exosomes, combined with ASCL1 and POU2F3 mRNA, significantly improved small cell lung cancer diagnosis [46]. However, MISEV (Minimal Information for Studies of Extracellular Vesicles) guidelines highlight the lack of standardized protocols for single-EV isolation and characterization, which has led to fragmented datasets and hindered clinical application [47].

To address this gap, we developed the Single Vesicle Atlas (SVAtlas), the first multi-omics database integrating single-EV data across diseases, biofluids, and species. SVAtlas curates 276 EV projects spanning 31 diseases, sourced from 75 published studies (January 2015–June 2025) and unpublished data. It includes data from 32 tissues/organs and 10 biofluids across five species (human, mouse, canine, hamster, and bacteria). Key features of SVAtlas include: (i) single-EV datasets along with experimental parameters; (ii) heterogeneity analyses to construct EV atlases for subpopulation analysis; (iii) identification of subgroup-specific markers in human disease landscapes; (iv) built-in tools for single-EV analysis, clustering, and interactive visualization; and (v) an AI-driven question-answering tool for cataloging characterization methods. Its intuitive interface, with navigations for Home, Atlas, Technology, Diseases, Heterogeneity, Analysis, Download, Share-your-study, and About/Help, supports researchers in exploring EV heterogeneity and identifying disease-specific markers.

Presented at the 2024 APSEV and CSEV meetings, SVAtlas received highly positive feedback, amassing over 700 views. By providing a robust, accessible platform for single-EV data, SVAtlas represents a key resource for EV researchers, facilitates deeper insights into EV biology, and advances the development of precise diagnostic and therapeutic strategies.

Materials and methods

Data collection

We manually collected single EV data from 121 studies indexed in PubMed (January 2015 to June 2025) using keywords such as “single extracellular vesicle,” “single membrane particle,” “single vesicle,” and “single exosome.” After rigorous screening for relevance and quality, we curated data from 75 single EV (sEV) studies, encompassing over 130 million single EVs across 31 distinct disease types. For each study, we systematically summarized and double-checked associated sample information (e.g. biofluid type, species), experimental procedures (e.g. isolation methods like ultracentrifugation or droplet microfluidics), and datasets (e.g. proteomic and transcriptomic profiles) for accuracy. Following MISEV 2023-guided quality control and filtration, the data were incorporated into the database (details in Fig. 1).

Data organization and molecular annotation

Each study in SVAtlas was assigned a unique accession identifier (e.g. PRJ20250616_1), where PRJ20250616 denotes the study and _1 indicates the sample source. In addition to curating sample information, we recorded biofluid preprocessing and single-vesicle isolation steps and parameters, given their impact on EV yield and purity. For subsequent analyses, we organized details on single EV localization molecules, analytical principles, techniques, and equipment to help users select suitable research methods.

More importantly, we collected single EV raw data, reported clustering subgroups, differentially expressed biomarkers between subpopulations, and properties of the obtained EVs (such as size, density, and distribution). Electron microscopy images, MS/MS spectra, and other supporting data are deposited when available. Additionally, all molecules identified in single EVs are annotated with molecular function, biological processes, signaling pathways, and associated diseases using gene ontology from NCBI [48] and GeneCards [49]. We performed preliminary analyses on proteins critical

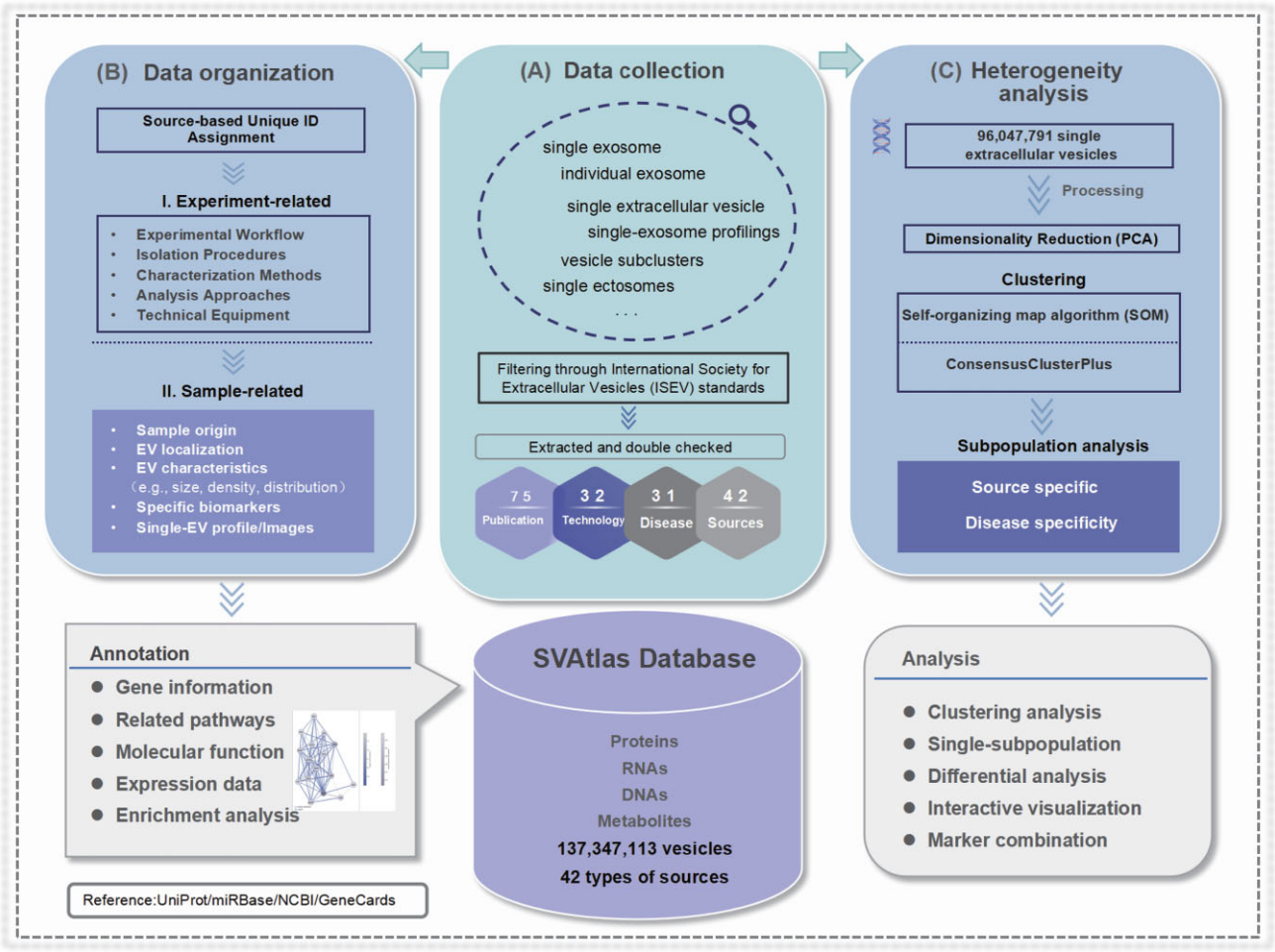


Figure 1. The construction of SVAtlas database. (**A**) Data Sourcing: SVAtlas collected and curated data from single-EV publications (Jan 2015–Jun 2025) using keyword searches. (**B**) Data Organization: each study is assigned a unique project ID. Its experimental protocols, sample information, and EV biomarkers were systematically curated and annotated. (**C**) Analysis Examples: SVAtlas performed comprehensive clustering, annotation, and visualization on single EV datasets from 23 projects. All the data used is publicly accessible on the SVAtlas website.

to single EV secretion in diseases, using functional/pathway enrichment and protein–protein interaction (PPI) networks.

Preprocessing and heterogeneity analysis of single-EV data

SVAtlas provides standardized workflows for single-vesicle data preprocessing and analysis, exemplified by datasets from 23 projects. Any single-EV data conforming to a matrix format with columns "symbol" (sample name), "EV" (single-vesicle probe sequence), "variable" (biomarker name), "value" (count), and "group" (disease/control) can be uploaded and processed.

The raw single-vesicle data undergo systematic preprocessing. The workflow begins with quality control, followed by filtering to retain high-quality vesicles expressing at least two distinct markers, each with a count of three or greater. Random subsampling is applied to each sample to reduce redundancy, with library sizes normalized using the counts per million (CPM) algorithm. Data features are z-score normalized to account for differences in units and scales. Dimensionality reduction is then performed using principal component analysis (PCA) and multidimensional scaling (MDS) to identify primary sources of variation.

Based on the reduced dimensions, single-EV subpopulation heterogeneity is characterized across cohorts, groups, and individual samples. The unsupervised flow cytometry self-organizing maps (FlowSOM) algorithm identifies subpopulations, which are visualized by t-distributed stochastic neighbor embedding (t-SNE) and uniform manifold approximation and projection (UMAP). The custom EVisualizer platform enables interactive parameter adjustment, multidimensional exploration, and visual validation of clustering at the single-vesicle level.

Construction of an online platform for SVAtlas data exploration

The SVAtlas (SVA) platform delivers an autonomous, end-to-end pipeline for single EV data analysis, facilitating unrestricted exploration of both repository-held and user-generated datasets via an automated, user-friendly, and robust workflow.

Architected as an integrated web application, it ensures seamless analytics through a user-facing front-end composed of three interconnected tools: (i) the core single-EV analysis pipeline, with open-source code available also on GitHub, processes raw expression matrices through normalization, di-

mensionality reduction, and unsupervised clustering to produce structured JSON files and t-SNE plots of EV subclusters; it is also supplemented by a Docker-based alternative on Hugging Face for handling large datasets with enhanced computational resources; (ii) the primary visualization component, EVisualizer, developed using R Shiny, parses JSON outputs (or user-uploaded files) to create dynamic Plotly visualizations, featuring modules for heterogeneity analysis (e.g. subgroup annotation and top marker identification), subpopulation distribution comparisons, marker abundance quantification, and source composition charting, complete with customizable options for plotting (total/group/sample) and styles; and (iii) the differential analysis interface enables users to specify comparison groups and statistical thresholds (e.g. logFC, *P*-value) for calculations, yielding downloadable volcano plots and data tables.

In addition to these tools, an LLM-powered question-answering system was integrated into SVAtlas, leveraging a comprehensive knowledge base derived from ∼121 curated single-EV research articles using retrieval-augmented generation (RAG) technology. Accessible via a Q&A shortcut in the lower-right corner of the website, this intelligent module functions as a responsive knowledge agent, providing context-aware, literature-grounded answers to user queries to enhance research exploration and decision-making. To ensure user awareness, the results from this tool are explicitly labelled as GAI-based and to be treated with caution.

This modular framework empowers users to advance from raw data to in-depth interactive exploration within a unified, self-contained platform, enhanced by demo files for testing and extensive customization capabilities.

Platform architecture and implementation

The SVAtlas system consists of three core components: a user interface developed using the Bootstrap framework for responsive design, a backend server implemented in PHP for handling logic and data processing, and a database managed via MySQL Community Edition for storing and querying EV-related datasets. The platform is powered by an Nginx web server running on Ubuntu Linux, which efficiently handles resource retrieval requests and routes data analysis tasks to the backend computing module. Additionally, the R programming environment is integrated to enable advanced data analysis interfaces, such as those in EVisualizer. For enhanced frontend visualization, the D3.js library—an open-source JavaScript tool for data-driven documents—is employed to create interactive effects and dynamic graphical support.

Additionally, a list of abbreviations used in the manuscript is provided in Supplementary Table 1.

Results

Data summary

SVAtlas encompasses 276 single-EV projects across 31 diseases, sourced from 75 published articles (January 2015–June 2025) and unpublished data. It includes over 130 million single vesicles from 31 disease categories and five species (human, mouse, hamster, canine, and microbial). The database includes 8236 standardized entries, structured by a checklist of 30 core parameters covering sample source (32 tissues/organs and 10 biofluids), isolation methods, pre-analytical variables, detection strategies, and molecular annotations. The marker library comprises 358 proteins, 18 RNAs, and 5 lipids/metabolites. The database integrates a human body map for dynamic image-text interactions, allowing users to explore data by molecular type, sample source, or species. SVAtlas provides three online tools for automated annotation, processing, and visualization, alongside an LLM-based query system for enhanced usability. Users can access vesicle heterogeneity visualizations (including clustering of over 96 million EVs into subpopulations using our analysis pipeline) and browse a curated summary of 32 single-EV detection technologies (2015–2025). All data are openly available at https://www.svatlas.org/. The procedure for using the SVAtlas dataset is detailed below, with the key steps summarized in Fig. 2.

User-friendly data browsing and download

SVAtlas offers intuitive browsing modalities: (i) The "Home" module provides a multi-layered Sankey diagram visualizing metadata (species, organs, biofluids, diseases, marker types, and PubMed IDs) for rapid dataset overview. (ii) The "Atlas" module enables searches via an interactive human anatogram or filters for molecular type and sample source (Fig. 3A). (iii) The "Disease" module displays exosomal marker distributions across pathologies, integrating pre-computed PPI networks [50], KEGG (Kyoto Encyclopedia of Genes and Genomes) [51, 52], and Gene Ontology (GO) [53, 54] annotations for visualization and download (Fig. 3A). Selecting a dataset from "Atlas" or "Disease" navigates to a "Project" page with a unique ProjectID, detailing marker types, validation methods, isolation protocols, vesicle features, and associated diseases. Clicking a gene symbol reveals experimental and annotation metadata, with data matrices and electron microscopy images available where provided (Fig. 3B).

High-throughput proteomics/transcriptomics data are available for download as shown in Fig. 4, including raw files and precalculated JSON clustering files for organs/tissues, body fluids, and diseases. We also categorize diseases into 13 systems (e.g. Digestive System: 57 projects, 296 markers; Nervous System: 39 projects, 298 markers).

Heterogeneity analysis and visualization

SVAtlas presents examples of single-EV heterogeneity analysis using 23 curated datasets in our database (details can be seen on the "Summary" page). The analysis integrates 96 047 791 EVs from 211 samples. Two analysis types are performed based on sample sources: tissue/organ heterogeneity (45 samples) and disease-specific heterogeneity (166 samples). Each includes (i) overall clustering (Analysis 1), (ii) selected highly heterogeneous subpopulations (Analysis 2), and (iii) differential expression heatmaps of marker combinations (Analysis 3), as shown in Fig. 5A.

As shown in Fig. 5A, the "Summary" page shows the overall clustering results derived from single EV in tissue/organ, body fluid, or disease modules. A "RE-ANALYSIS" button enables users to perform online heterogeneity analysis with advanced visualization features. Detailed instructions are provided in the "Help" section. Raw data, FlowSOM-clustered JSON files, and visualization results are downloadable at the bottom of the page.

In the "Heterogeneity" page, highly specific subpopulations of two analysis types were selected for further analysis to investigate their distribution across samples, EV source compo-

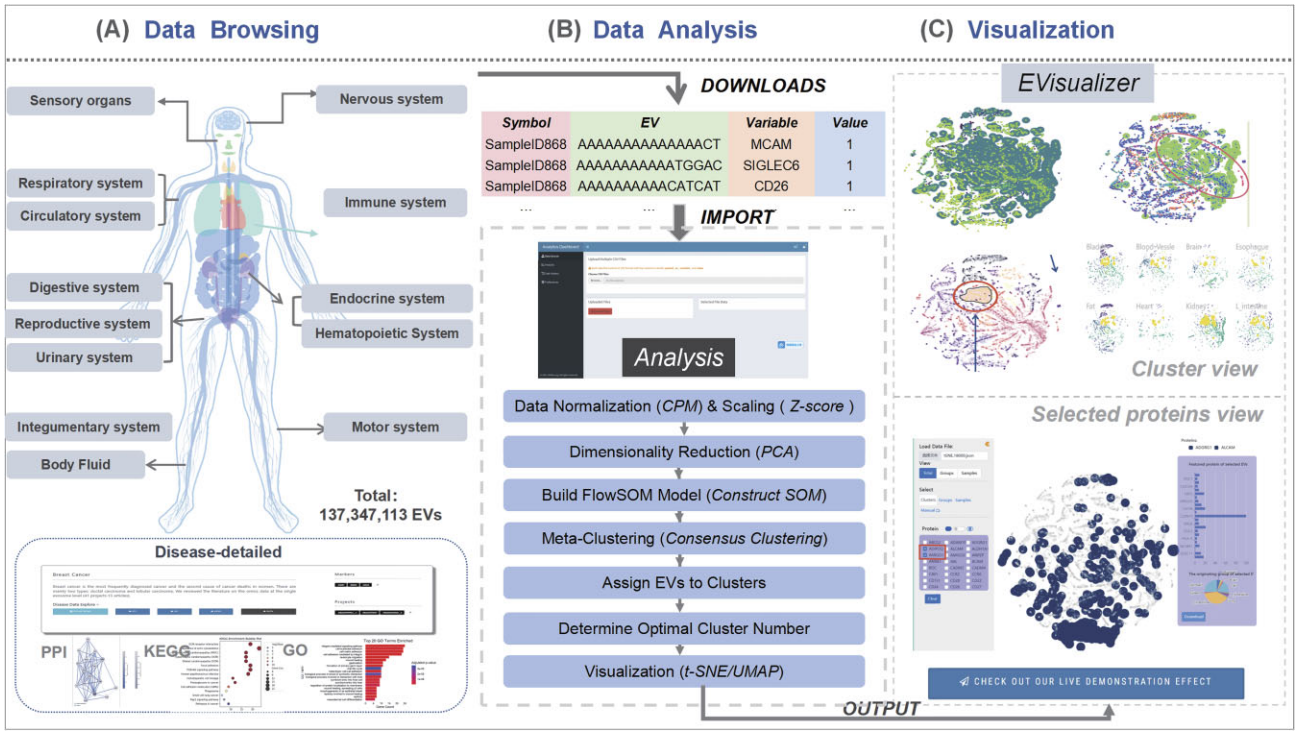


Figure 2. Core procedures for SVAtlas usage. (**A**) Data Browsing: Interactive human body map categorizes EVs by systems and diseases, totaling 137 347 113 EVs. (**B**) Data Analysis: Pipeline processes imported data via normalization (CPM/*z*-score), PCA reduction, FlowSOM modeling, consensus clustering, EV assignment, optimal cluster determination, and t-SNE/UMAP visualization, with import/download options. (**C**) Result Visualization: EVisualizer shows cluster scatter plots, protein heatmaps, organ icons, expression bars, and EV origin pies for interactive exploration. Our platform enables EV data browsing, analysis, and visualization across various systems and diseases.

sition, and highly expressed markers. Heatmaps and t-SNE plots are implemented to show this heterogeneity.

The "Combined Markers" page shows dual-, triple-, and quadruple-marker combinations for subpopulation discrimination, as single-marker approaches might limit specificity for distinguishing EV subpopulations.

Automated analytical pipeline

SVAtlas provides the first fully automated single-EV analysis pipeline, supporting platform-provided and user-uploaded datasets (format: group/sample/EV identifier/molecule/value) without the need of external software. As shown in Fig. 5B, the integrated platform for single-EV analysis includes: (i) the single-EV analysis pipeline, which processes raw matrices through preprocessing, clustering, and visualization, generating t-SNE plots and JSON-formatted clustering results; (ii) EVisualizer, built on R Shiny and Plotly, which parses JSON outputs or user-uploaded files for dynamic visualizations, supporting flexible grouping, subpopulation comparisons, and user-defined gating with displayed marker/source compositions; and (iii) the differential analysis tool, which generates volcano plots and tables based on user-defined thresholds, highlighting differentially expressed molecules.

Emerging single-EV technologies

To support experimental method selection, SVAtlas summarizes 32 single-EV analysis techniques (2015–2025), such as (i) enhanced microscopy (e.g. atomic force microscopy) for high-resolution EV imaging, (ii) nanoparticle tracking analysis (NTA) with fluorescence for size/biomarker detection, (iii) nanoscale flow cytometry (nFCM) for high-throughput protein/RNA profiling, and (iv) PBA for DNA-encoded antibody labeling. The "Main Technology" webpage summarizes their characteristics and parameters. Additionally, a tree diagram summarizing nearly all measurement methods from 2015 to 2025 can be found on the "Technical Diagram" page.

Data submission

The "Share your study" page offers users a convenient way to upload data that are not recorded in SVAtlas, including published or unpublished data. Based on the latest MISEV2023 recommendations, we have refined the collection criteria for isolating, identifying, and characterizing single EVs, such as uploading EV diameter distributions rather than just average values. The page tailors different data collection entries based on the characterization methods, allowing for personalized uploads by selecting different methods. Users can submit studies via a form capturing project details, author info, sample/experimental metadata, and analytical methods, with submission history tracked.

Discussion

SVAtlas advances single extracellular vesicle research by addressing key challenges in heterogeneity resolution and biomarker discovery. Single-EV analysis is crucial for uncovering disease-specific subpopulations, as it overcomes bulk methods' limitations in sensitivity and specificity, enabling precise diagnostics.

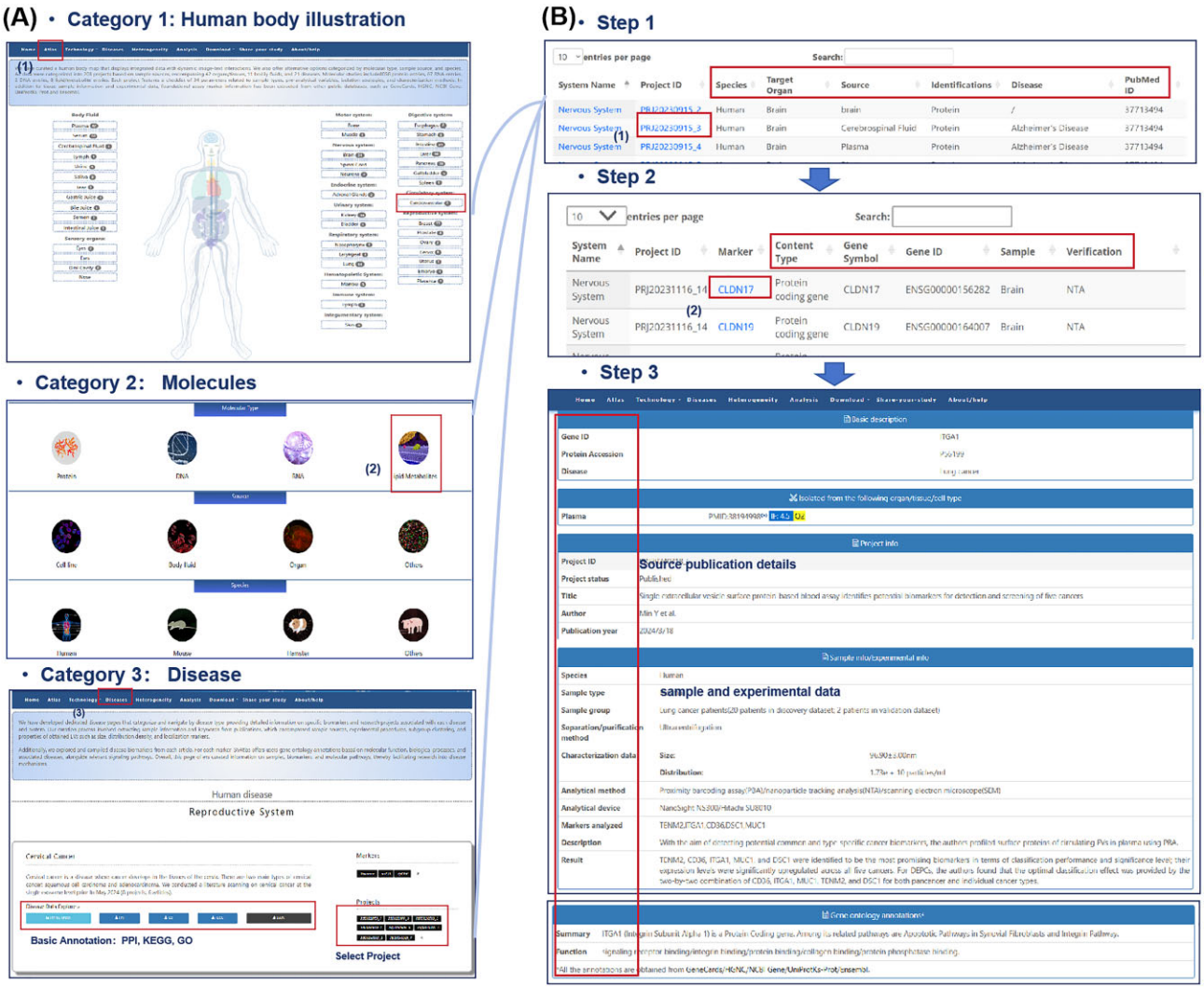


Figure 3. SVAtlas Data Browsing. (**A**) Browse: Users could explore single-EV studies through the interactive human body map, EV-expressed molecules, or EV-related diseases modules. (**B**) Access project information: Sample details and a PubMed ID are shown by clicking a project entry (Step 1); EV biomarkers are also deposited in each project (Step 2); one can also obtain the project's experimental parameters, data processing workflow, and the biomarkers' function annotation by clicking each biomarker entry (Step 3).

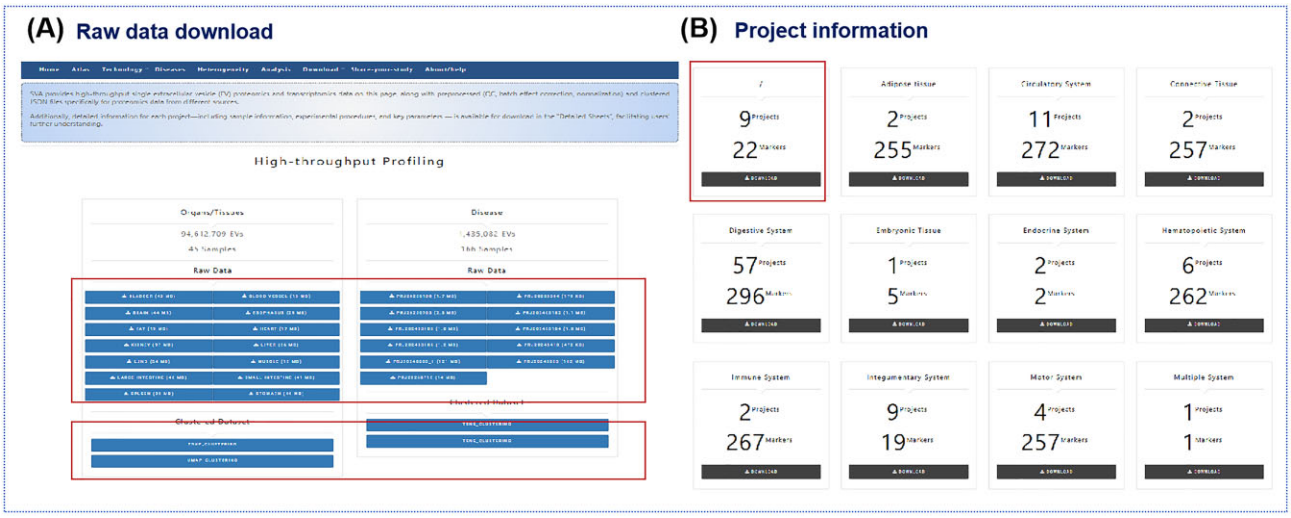


Figure 4. Datasets download. (**A**) This page offers high-throughput single-EV proteomics and transcriptomics data for downloading, including pre-processed results and clustering JSON files. (**B**) Users can download project sheets with detailed information containing sample information, experimental protocols, and key parameters.

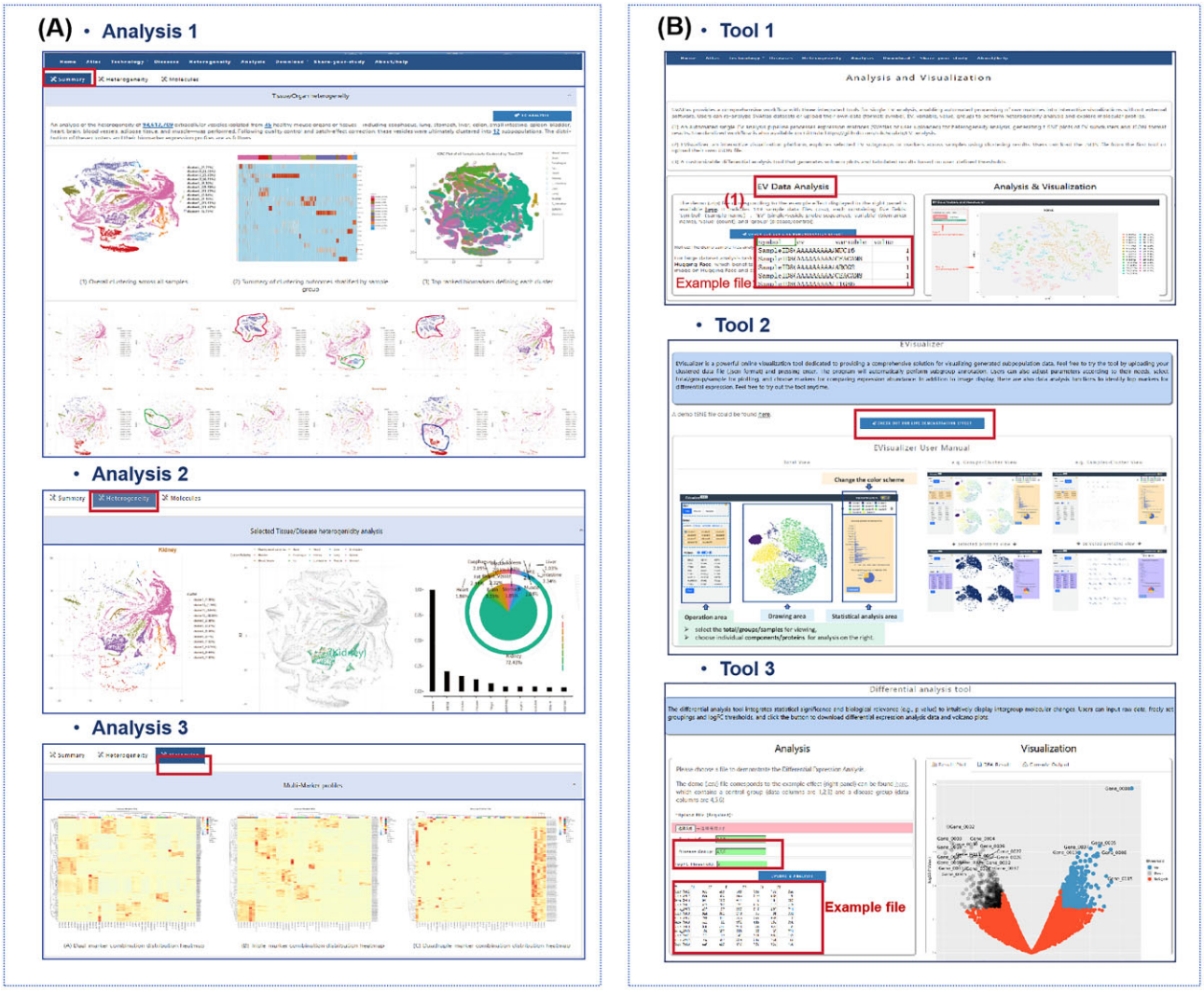


Figure 5. Heterogeneity examples and online analysis workflow. (**A**) shows the examples of single-EV heterogeneity analysis, including global clustering (Analysis 1), selected highly heterogeneous subpopulations (Analysis 2), and marker combination heatmaps (Analysis 3); (**B**) represents an integrated platform for single-EV analysis, comprising three modules: a single-EV data analysis pipeline (Tool 1), EVisualizer for visualization (Tool 2), and differential expression analysis (Tool 3).

The clinical potential of the single-vesicle approach is already demonstrated by a new generation of cutting-edge technologies that are delivering breakthroughs in sensitivity, multiplexing, and unbiased analysis. For instance, platforms for high sensitivity are now a reality; a plasmon-enhanced fluorescence assay (FLEX) achieved an area under the curve (AUC) of 0.93 for diagnosing cholangiocarcinoma, significantly outperforming the current clinical biomarker CA19-9 (AUC = 0.69) [55]. Similarly, CRISPR-based digital assays (ddSEE) reached 92% accuracy in breast cancer detection by co-analyzing protein and miRNA on single EVs [23]. In parallel, high-multiplex profiling methods like PBA can analyze over 100 proteins simultaneously to discover novel, functionally distinct subpopulations, such as $CD35^{+}$ urinary EVs, which serve as a biomarker for sepsis-associated acute kidney injury (SA-AKI) with high diagnostic accuracy (AUC 0.89) [56]. Finally, label-free techniques like surface-enhanced Raman spectroscopy (SERS), when combined with machine learning, provide a holistic biochemical "fingerprint" of each vesicle, enabling the identification of glioblastoma mutations with 87% accuracy [57].

Despite the power of these individual technologies, single-EV research as a whole faces persistent barriers. First, the lack of dominant standardized methods leads to variability in isolation and characterization, as highlighted by MISEV 2023 guidelines [47]. Second, algorithms often adapted from single-cell analysis overlook EV-specific features like membrane protein dynamics, reducing applicability to diverse omics [16, 58–60]. Third, fragmented datasets hinder data sharing and re-analysis, impeding cross-study validation and clinical translation. These defects limit the field's progress, particularly in integrating multi-omics for broad disease applications.

SVAtlas mitigates these field-wide issues by providing a unified platform for single-EV data curation and analysis as the first comprehensive single-EV multi-omics database. It centralizes single-EV multi-omics data using MISEV 2023-compliant protocols, offers EV-specific tools like EVisualizer for interactive clustering, and enables AI-driven queries

for method details. Through this unique platform, SVAtlas will facilitate efficient data sharing, validation, and the development of computational methods specialized for single-EV studies.

Despite its strengths, SVAtlas has limitations, such as reliance on published data quality and small cohorts in some studies, potentially limiting generalizability. SVAtlas will continue updating data to support comprehensive single-EV analysis. Future expansions will include larger datasets, novel omics layers (e.g. glycans, as well as important non-coding RNAs like lncRNAs and circRNAs as more single-vesicle data becomes available), and enhanced AI for predictive modeling, positioning SVAtlas as a central hub for single EV research, accelerating liquid biopsy and precision medicine in diseases.

Acknowledgements

We thank the research community for contributing published and unpublished single-EV datasets to SVAtlas.

Author contributions: Zhonghui Wei (Conceptualization [equal], Data curation [equal], Formal analysis [equal], Methodology [equal], Writing—original draft [equal]), Na Zhou (Data curation [equal], Formal analysis [equal], Visualization [equal], Writing—original draft [equal]), Ming Jing (Conceptualization [equal], Methodology [equal], Validation [equal], Writing—original draft [equal]), Yanling Cai (Data curation [equal], Methodology [equal], Writing—review & editing [equal]), Yingxin Zhang (Formal analysis [equal], Software [equal], Visualization [equal]), Xinyu Wang (Data curation [equal], Software [equal], Validation [equal]), Linxinyu Wang (Formal analysis [equal], Visualization [equal], Writing—review & editing [equal]), Di Wu (Methodology [equal], Validation [equal], Writing—review & editing [equal]), Fuzhong Xue (Conceptualization [supporting], Funding acquisition [equal], Supervision [equal], Writing—review & editing [supporting]), and Qingzhen Hou (Conceptualization [lead], Funding acquisition [equal], Project administration [lead], Supervision [equal], Writing—original draft [lead], Writing—review & editing [lead]).

Supplementary data

Supplementary data is available at NAR online.

Conflict of interest

None declared.

Funding

This work was supported by the National Natural Science Foundation of China (82473733) and the National Natural Science Foundation Key Program (82330108). Funding to pay the Open Access publication charges for this article was provided by National Natural Science Foundation of China.

Data availability

The data underlying this article are available in SVAtlas, at https://www.svatlas.org/.

References

1. Kalluri R, LeBleu VS. The biology, function, and biomedical applications of exosomes. *Science* 2020;367:eaau6977.
2. Jeppesen DK, Zhang Q, Franklin JL *et al.* Extracellular vesicles and nanoparticles: emerging complexities. *Trends Cell Biol* 2023;33:667–81. https://doi.org/10.1016/j.tcb.2023.01.002
3. Rak J. Extracellular Vesicles – biomarkers and effectors of the cellular interactome in cancer. *Front Pharmacol* 2013;4:21. https://doi.org/10.3389/fphar.2013.00021
4. Mashouri L, Yousefi H, Aref AR *et al.* Exosomes: composition, biogenesis, and mechanisms in cancer metastasis and drug resistance. *Mol Cancer* 2019;18:75. https://doi.org/10.1186/s12943-019-0991-5
5. Zhu L, Sun H-T, Wang S *et al.* Isolation and characterization of exosomes for cancer research. *J Hematol Oncol* 2020;13:152. https://doi.org/10.1186/s13045-020-00987-y
6. Rajagopal C, Harikumar KB. The origin and functions of exosomes in cancer. *Front Oncol* 2018;8:66. https://doi.org/10.3389/fonc.2018.00066
7. Rani S, O'Brien K, Kelleher FC *et al.* Isolation of Exosomes for Subsequent mRNA, MicroRNA, and Protein Profiling. In: O'Driscoll L (ed.), *Gene Expression Profiling: Methods and Protocols*. Totowa, NJ: Humana Press, 2011, 181–95.
8. Raposo G, Stoorvogel W. Extracellular vesicles: exosomes, microvesicles, and friends. *J Cell Biol* 2013;200:373–83. https://doi.org/10.1083/jcb.201211138
9. Morad G, Carman CV, Hagedorn EJ *et al.* Tumor-derived extracellular vesicles breach the intact blood–brain barrier via transcytosis. *ACS Nano* 2019;13:13853–65. https://doi.org/10.1021/acsnano.9b04397
10. Xu R, Rai A, Chen M *et al.* Extracellular vesicles in cancer—implications for future improvements in cancer care. *Nat Rev Clin Oncol* 2018;15:617–38. https://doi.org/10.1038/s41571-018-0036-9
11. Hornung S, Dutta S, Bitan G. CNS-derived blood exosomes as a promising source of biomarkers: opportunities and challenges. *Front Mol Neurosci* 2020;13:38. https://doi.org/10.3389/fnmol.2020.00038
12. Tofaris GK. A critical assessment of exosomes in the pathogenesis and stratification of Parkinson's disease. *J Park Dis* 2017;7:569–76.
13. Yu D, Li Y, Wang M *et al.* Exosomes as a new frontier of cancer liquid biopsy. *Mol Cancer* 2022;21:56. https://doi.org/10.1186/s12943-022-01509-9
14. Carney RP, Mizenko RR, Bozkurt BT *et al.* Harnessing extracellular vesicle heterogeneity for diagnostic and therapeutic applications. *Nat Nanotechnol* 2025;20:14–25. https://doi.org/10.1038/s41565-024-01774-3
15. van de Wakker SI, Meijers FM, Sluijter JPG *et al.* Extracellular vesicle heterogeneity and its impact for regenerative medicine applications. *Pharmacol Rev* 2023;75:1043–61. https://doi.org/10.1124/pharmrev.123.000841
16. Silva TF, Hutchins E, Zhao W *et al.* Extracellular vesicle heterogeneity through the lens of multiomics. *Cell Rep Med* 2025;6:102161. https://doi.org/10.1016/j.xcrm.2025.102161
17. Willms E, Cabañas C, Mäger I *et al.* Extracellular vesicle heterogeneity: subpopulations, isolation techniques, and diverse functions in cancer progression. *Front Immunol* 2018;9:738. https://doi.org/10.3389/fimmu.2018.00738
18. Bordanaba-Florit G, Royo F, Kruglik SG *et al.* Using single-vesicle technologies to unravel the heterogeneity of extracellular vesicles. *Nat Protoc* 2021;16:3163–85. https://doi.org/10.1038/s41596-021-00551-z
19. Qiu L, Liu X, Zhu L *et al.* Current advances in technologies for single extracellular vesicle analysis and its clinical applications in cancer diagnosis. *Biosensors* 2023;13:129. https://doi.org/10.3390/bios13010129

20. Morales R-TT, Ko J. Future of digital assays to resolve clinical heterogeneity of single extracellular vesicles. *ACS Nano* 2022;16:11619–45. https://doi.org/10.1021/acsnano.2c04337
21. Walker SN, Lucas K, Dewey MJ *et al.* Rapid assessment of biomarkers on single extracellular vesicles using "catch and display" on ultrathin nanoporous silicon nitride membranes. *Small* 2025;21:2405505. https://doi.org/10.1002/smll.202405505
22. Nogueras-Ortiz CJ, Eren E, Yao P *et al.* Single-extracellular vesicle (EV) analyses validate the use of L1 cell adhesion molecule (L1CAM) as a reliable biomarker of neuron-derived EVs. *J Extracell Vesicles* 2024;13:e12459. https://doi.org/10.1002/jev2.12459
23. Xu X, Zhang Y, Liu J *et al.* Concurrent detection of protein and miRNA at the single extracellular vesicle level using a digital dual CRISPR–Cas assay. *ACS Nano* 2025;19:1271–85. https://doi.org/10.1021/acsnano.4c13557
24. Li P, Kaslan M, Lee SH *et al.* Progress in exosome isolation techniques. *Theranostics* 2017;7:789–804. https://doi.org/10.7150/thno.18133
25. Bai J-J, Zhang X, Wei X *et al.* Dean-flow-coupled elasto-inertial focusing accelerates exosome purification to facilitate single vesicle profiling. *Anal Chem* 2023;95:2523–31. https://doi.org/10.1021/acs.analchem.2c04898
26. Chiang C, Chen C. Toward characterizing extracellular vesicles at a single-particle level. *J Biomed Sci* 2019;26:9. https://doi.org/10.1186/s12929-019-0502-4
27. Banijamali M, Höjer P, Nagy A *et al.* Characterizing single extracellular vesicles by droplet barcode sequencing for protein analysis. *J Extracell Vesicles* 2022;11:12277.
28. Yin L, Han X, Guo F *et al.* An all-in-one nanohole array for size-exclusive trapping and high-throughput digital counting of single extracellular vesicles for non-invasive cancer screening. *Angew Chem Int Ed Engl* 2025;64:e202506744.
29. Wang D, Shen Y, Qian H *et al.* Emerging advanced approaches for liquid biopsy: *in situ* nucleic acid assays of extracellular vesicles. *Theranostics* 2024;14:7309–32.
30. van der Vlist EJ, Nolte-'t Hoen ENM, Stoorvogel W *et al.* Fluorescent labeling of nano-sized vesicles released by cells and subsequent quantitative and qualitative analysis by high-resolution flow cytometry. *Nat Protoc* 2012;7:1311–26.
31. Choi D, Montermini L, Jeong H *et al.* Mapping subpopulations of cancer cell-derived extracellular vesicles and particles by nano-flow cytometry. *ACS Nano* 2019;13:10499–511.
32. Wu D, Yan J, Shen X *et al.* Profiling surface proteins on individual exosomes using a proximity barcoding assay. *Nat Commun* 2019;10:3854. https://doi.org/10.1038/s41467-019-11486-1
33. Cho S, Yi J, Kwon Y *et al.* Multifluorescence single extracellular vesicle analysis by time-sequential illumination and tracking. *ACS Nano* 2021;15:11753–61. https://doi.org/10.1021/acsnano.1c02556
34. Dragovic RA, Gardiner C, Brooks AS *et al.* Sizing and phenotyping of cellular vesicles using nanoparticle tracking analysis. *Nanomedicine* 2011;7:780–8. https://doi.org/10.1016/j.nano.2011.04.003
35. Filipe V, Hawe A, Jiskoot W. Critical evaluation of nanoparticle tracking analysis (NTA) by NanoSight for the measurement of nanoparticles and protein aggregates. *Pharm Res* 2010;27:796–810. https://doi.org/10.1007/s11095-010-0073-2
36. McNamara RP, Zhou Y, Eason AB *et al.* Imaging of surface microdomains on individual extracellular vesicles in 3-D. *J Extracellular Vesicle* 2022;11:e12191. https://doi.org/10.1002/jev2.12191
37. Wei J, Zhang S, Yuan J *et al.* Nanoscale imaging of tumor cell exosomes by expansion single molecule localization microscopy (ExSMLM). *Talanta* 2023;261:124641. https://doi.org/10.1016/j.talanta.2023.124641
38. Ortega-Sanchez FG, Valero T, Widmann T *et al.* . Microfluidic systems in extracellular vesicles single analysis. A systematic review. *TrAC Trends in Analytical Chemistry* 2023;159:116920. https://doi.org/10.1016/j.trac.2023.116920
39. Ko J, Wang Y, Carlson JC *et al.* Single EV protein analysis using immuno ddPCR (iddPCR) amplification. *Adv Biosys* 2020;4:e1900307. https://doi.org/10.1002/adbi.201900307
40. Lin H, Li B, Guo J *et al.* Simultaneous detection of membrane protein and mRNA at single extracellular vesicle level by droplet microfluidics for cancer diagnosis. *JAdv Res* 2024;73:645–57. https://doi.org/10.1016/j.jare.2024.08.026
41. Wang S, Khan A, Huang R *et al.* Recent advances in single extracellular vesicle detection methods. *Biosen Bioelectron* 2020;154:112056. https://doi.org/10.1016/j.bios.2020.112056
42. Ferguson S, Yang KS, Weissleder R. Single extracellular vesicle analysis for early cancer detection. *Trends Mol Med* 2022;28:681–92. https://doi.org/10.1016/j.molmed.2022.05.003
43. Guo W, Cai Y, Liu X *et al.* Single-exosome profiling identifies ITGB3+ and ITGAM+ exosome subpopulations as promising early diagnostic biomarkers and therapeutic targets for colorectal cancer. *Research* 2023;6:0041. https://doi.org/10.34133/research.0041
44. Ning N, Lu J, Li Q *et al.* Single-sEV profiling identifies the TACSTD2+ sEV subpopulation as a factor of tumor susceptibility in the elderly. *J Nanobiotechnol* 2024;22:222. https://doi.org/10.1186/s12951-024-02456-x
45. Sun Z, Chen X, Niu R *et al.* Liposome fusogenic enzyme-free circuit enables high-fidelity determination of single exosomal RNA. *Mater Today Bio* 2023;19:100613. https://doi.org/10.1016/j.mtbio.2023.100613
46. Li H, Chiang C, Kwak KJ *et al.* Extracellular vesicular delta-like ligand 3 and subtype transcription factors for small cell lung cancer diagnosis. *Adv Sci* 2025;12:2416711. https://doi.org/10.1002/advs.202416711
47. Welsh JA, Goberdhan DCI, O'Driscoll L *et al.* Minimal information for studies of extracellular vesicles (MISEV2023): from basic to advanced approaches. *J Extracellular Vesicle* 2024;13:e12404. https://doi.org/10.1002/jev2.12404
48. Sayers EW, Beck J, Bolton EE *et al.* Database resources of the National Center for Biotechnology Information in 2025. *Nucleic Acids Res* 2024;53:D20–9. https://doi.org/10.1093/nar/gkae979
49. Stelzer G, Rosen N, Plaschkes I *et al.* The GeneCards Suite: from gene data mining to disease genome sequence analyses. *Curr Protoc Bioinformatics* 2016;54:1.30.1–1.30.33. https://doi.org/10.1002/cpbi.5
50. Szklarczyk D, Kirsch R, Koutrouli M *et al.* The STRING database in 2023: protein–protein association networks and functional enrichment analyses for any sequenced genome of interest. *Nucleic Acids Res* 2023; 51:D638–46.
51. Kanehisa M, Furumichi M, Sato Y *et al.* KEGG: biological systems database as a model of the real world. *Nucleic Acids Res* 2025; 53:D672–7.
52. Kanehisa M. Toward understanding the origin and evolution of cellular organisms. *Protein Sci* 2019;28:1947–51. https://doi.org/10.1002/pro.3715
53. Ashburner M, Ball CA, Blake JA *et al.* Gene ontology: tool for the unification of biology. The Gene Ontology Consortium. *Nat Genet* 2000;25:25–9. https://doi.org/10.1038/75556
54. Ontology Consortium G, Aleksander SA, Balhoff J *et al.* The Gene Ontology knowledgebase in 2023. *Genetics* 2023;224:iyad031. https://doi.org/10.1093/genetics/iyad031
55. Jeong MH, Son T, Tae YK *et al.* Plasmon-enhanced single extracellular vesicle analysis for cholangiocarcinoma diagnosis. *Adv Sci* 2023;10:e2205148.
56. Li N, Tang TT, Gu M *et al.* Single urinary extracellular vesicle proteomics identifies complement receptor CD35 as a biomarker for sepsis-associated acute kidney injury. *Nat Commun* 2025;16:6960. https://doi.org/10.1038/s41467-025-62229-4
57. Jalali M, Del Real Mata C, Montermini L *et al.* MoS2-plasmonic nanocavities for Raman spectra of single extracellular vesicles

reveal molecular progression in glioblastoma. *ACS Nano* 2023;17:12052–71. https://doi.org/10.1021/acsnano.2c09222
58. Shaba E, Vantaggiato L, Governini L *et al.* Multi-omics integrative approach of extracellular vesicles: a future challenging milestone. *Proteomes* 2022;10:12. https://doi.org/10.3390/proteomes10020012
59. Liu C-J, Xie G-Y, Miao Y-R *et al.* EVAtlas: a comprehensive database for ncRNA expression in human extracellular vesicles. *Nucleic Acids Res* 2022; 50:D111–7. https://doi.org/10.1093/nar/gkab668
60. Spitzberg JD, Ferguson S, Yang KS *et al.* Multiplexed analysis of EV reveals specific biomarker composition with diagnostic impact. *Nat Commun* 2023;14:1239. https://doi.org/10.1038/s41467-023-36932-z

Received: August 15, 2025. **Revised:** October 13, 2025. **Accepted:** October 13, 2025