Know Your Vesicles:
How Cell Source Shapes Extracellular Vesicle Attributes

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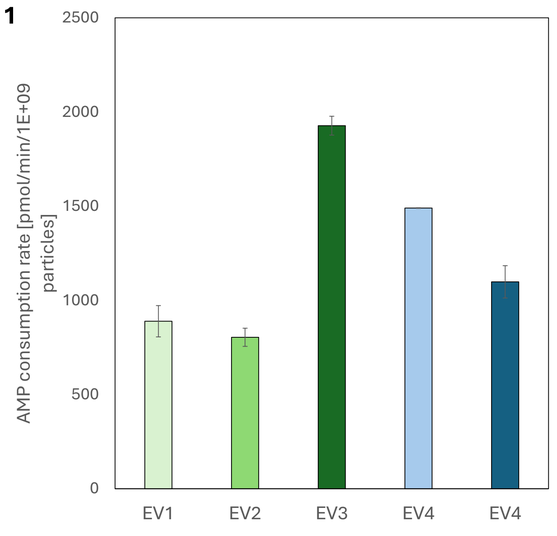
EV*scale*™
SCALABLE PRODUCTION OF EXTRACELLULAR VESICLES

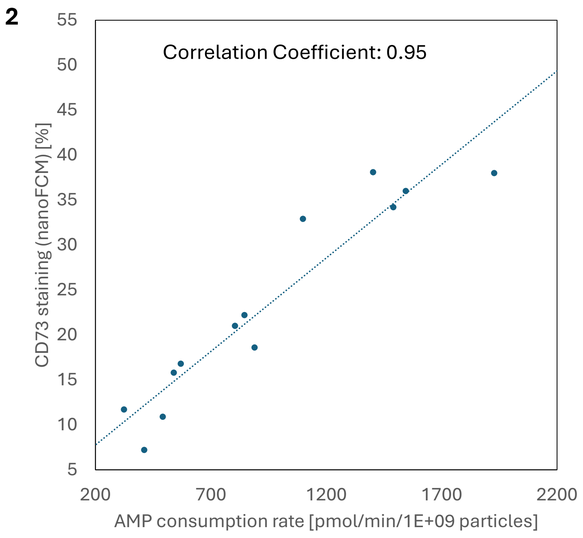
Standardized EV Production to Drive Functional Insights

EV*scale*™ provides the foundation for functional EV analysis by enabling the production of EVs with reproducible quality. This consistency is achieved through the use of telomerized mesenchymal stromal cell (MSC) lines, xeno-, serum- and hPL-free media, and controlled, scalable processing. Our strategy allows the establishment of a robust and reproducible data foundation for product definition and supports the identification of true cell line-specific commonalities and differences in EV characteristics.

CD73 Activity

CD73 is a hallmark cell surface protein to identify MSCs. It is an ecto-5'-nucleotidase that catalyzes the conversion of AMP to adenosine, a known regulator of immune responses. Measuring CD73 activity on EVs may provide functional insights into their immunomodulatory potential.

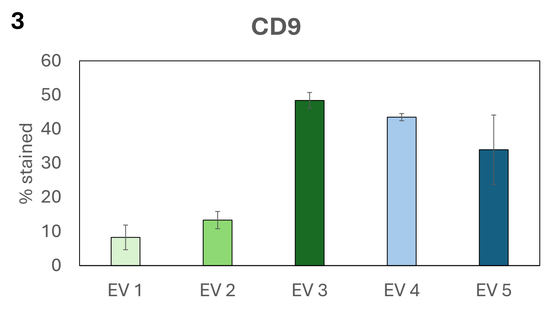


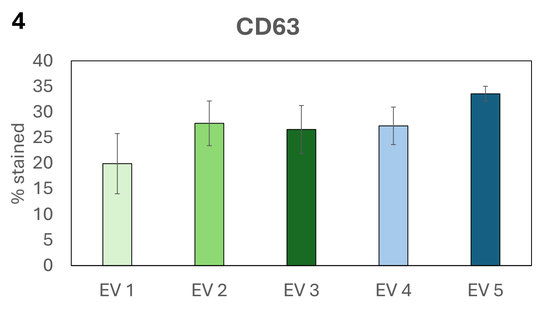


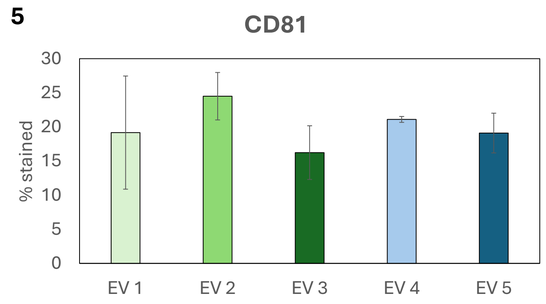
1 Comparison of different EV sources based on their CD73 activity (AMP hydrolysis via AMP Glo™, Promega), normalized on particles. **2** Correlation plot: positive correlation between CD73-positive particles (via nanoFCM, antibody labelling) and AMP consumption rate.

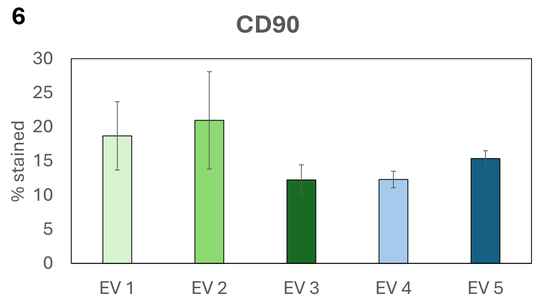
EV Phenotyping

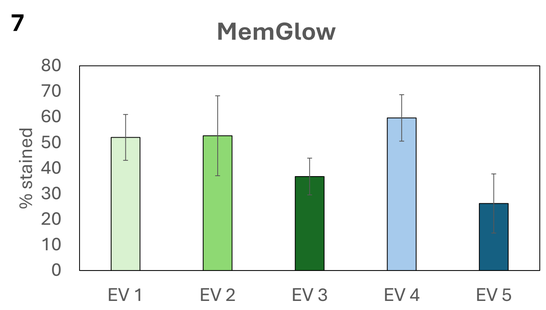
EVs from varying cell sources were probed for multiple markers and analyzed using the NanoFCM NanoAnalyzer. Distinct marker profiles were observed across the five EV populations, highlighting source-specific EV (surface) signatures.

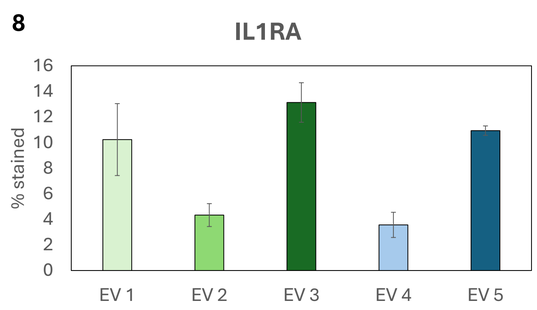












3-8: The proportion of particles stained positively for CD9, CD81, CD63, CD90, MemGlow™, or Interleukin 1 receptor antagonist (IL1RA) was determined using the NanoAnalyzer (NanoFCM). Data represent the percentage of marker-positive events relative to the total number of detected EV-sized particles. Values are represented as mean from different timepoints from representative cultivations. CD9, CD81, and CD63 are canonical EV markers (tetraspanins), CD90 is associated with mesenchymal origin (besides CD73), the lipid-dye MemGlow™ labels the membrane, and IL1RA reflects potential functional cargo.

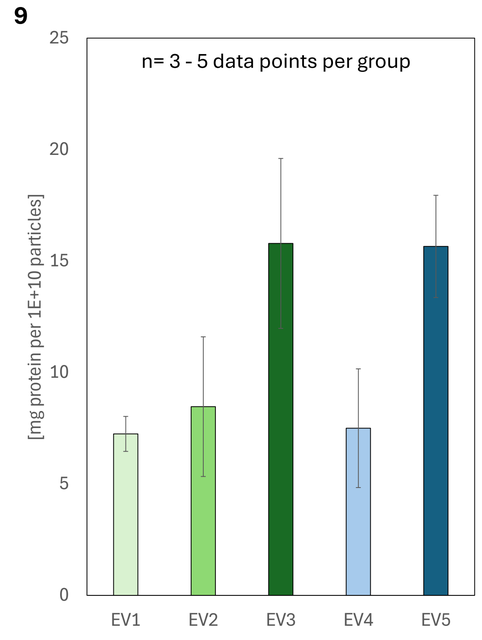
miRNA profile analysis

Different MSC-EV sources reproducibly share similar miRNAs among their top five most abundant species. While some of these miRNAs are consistently detected across different sources, their ranking within the top five varies, suggesting source-specific regulation or enrichment mechanisms.

Top 5 – most abundant	EV 3	EV 4	EV 5	EV 1	EV 2
1	miRNA 1	miRNA 1	miRNA 1	miRNA 3	miRNA 2
2	miRNA 2	miRNA 2	miRNA 2	miRNA 2	miRNA 3
3	miRNA 3	miRNA 3	miRNA 4	miRNA 1	miRNA 1
4	miRNA 5	miRNA 7	miRNA 3	miRNA 6	miRNA 5
5	miRNA 6	miRNA 4	miRNA 8	miRNA 9	miRNA 10

Protein to particle ratio

Conditioned media from different cell sources was processed equally: A depth filtration step is followed by tangential-flow filtration (TFF), in which conditioned media is concentrated and buffer exchanged with six diavolumes, achieving an exchange rate of >98%.
Depending on the cell source, a different amount of protein per particle is retained, as observed in multiple processing runs. Since the buffer exchange rate remained consistent across all processes, and the same media was used for the cultivation of all cell lines, it can be concluded that this effect is likely dependent on the cell source. This variability in protein retention may impact the functional properties of the extracellular vesicles.



9 Protein-to-particle ratio after tangential flow filtration differs between cell sources.

Other Functional Assays & Conclusion

EVs from different cell sources show unique profiles and functional behaviors. The table below highlights the multitude of promising results in various biological assays. By building a comprehensive database with our reproducible process platform, we can purposefully select the most suitable EV preparation for a given application — moving closer towards uncovering specific modes of action.

Assay	EV1	EV2	EV3	EV4	EV5	Comments
Anti-Fibrosis 2D	+++	++	++	++	-	human skin fibroblasts
Anti-Fibrosis 3D	++	+++	+*	+*	+++	human liver organoids
Anti-Inflammation	+/++	++	++	++	+++	mouse macrophage/microglia
Proliferation - Kidney	++	+++	+++	++	+++	human tubular cells
Proliferation - Nerve	++	+	n.a.	+++	n.a.	human Schwann cells
Wound Healing	+++	n.a.	n.a.	n.a.	n.a.	HACAT cell scratch assay
Vasculogenesis 2D	+++	+++	++	+++	+++	HUVEC Matrigel assay
Vaculogenesis 3D	+++	+++	+++	++	+*	HUVEC 3D Fibrin clot assay
Cardioprotection	+++	n.a.	n.a.	+++	n.a.	Rat cardiac myoblast assay

*: not significant; n.a.: not analyzed

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