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Supplementary information

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Exercise enhances aged chondrocyte health through microRNA-29-enriched extracellular vesicles

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Table S1. CellProfiler shape and size features measured by the MeasureObjectSizeShape module.
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Table S1. CellProfiler shape and size features measured by the MeasureObjectSizeShape module.

Variable	Description
Area	Total number of pixels in the object. Reflects the overall size of the object.
BoundingBoxArea	Area of the smallest rectangle that completely encloses the object. Larger values indicate elongated or irregular shapes.
BoundingBoxMaximum_X	Maximum X coordinate of the bounding box. Represents the rightmost boundary of the object.
BoundingBoxMaximum_Y	Maximum Y coordinate of the bounding box. Represents the bottom boundary of the object.
BoundingBoxMinimum_X	Minimum X coordinate of the bounding box. Represents the leftmost boundary of the object.
BoundingBoxMinimum_Y	Minimum Y coordinate of the bounding box. Represents the top boundary of the object.
Center_X	X coordinate of the object centroid. Indicates the center of mass along the X-axis.
Center_Y	Y coordinate of the object centroid. Indicates the center of mass along the Y-axis.
Compactness	Measure of object shape compactness, calculated as $Perimeter^2$ / (4π * Area). Values approaching 1 indicate a more circular shape, while higher values indicate elongated or irregular shapes.
Eccentricity	Ratio of the distance between the foci of the ellipse and its major axis length. Ratio approaching 1 indicates a more elliptical shape.
EquivalentDiameter	Diameter of a circle with the same area as the object. Provides a linear measure of object size.
Extent	Ratio of object area to bounding box area. Ratio of 1 indicates the object fully fills its bounding box; lower values indicate gaps or irregular shapes.
FormFactor	4π * Area / $Perimeter^2$. Ratio approaching 1 indicates a circular shape, while smaller values indicate irregular or elongated objects.
MajorAxisLength	Length of the major axis of the ellipse that best fits the object. Represents the longest dimension of the object.
MaxFeretDiameter	Maximum distance between any two points along the object boundary. Indicates the largest linear extent of the object in any direction.
MaximumRadius	Greatest distance from any pixel inside the object to the nearest pixel outside the object. Reflects the furthest point from the centroid.
MeanRadius	Mean distance from any pixel inside the object to the nearest pixel outside the object. Provides an average radius measure of the object.
MedianRadius	Median distance from any pixel inside the object to the nearest pixel outside the object. Robust measure of object size less sensitive to outliers.
MinFeretDiameter	Minimum distance between any two points along the object boundary. Indicates the smallest width of the object.
MinorAxisLength	Length of the minor axis of the ellipse that bounds the object. Represents the shortest dimension of the object.
Orientation	Angle between the major axis of the ellipse and the X-axis. Reflects the alignment of the object within the image.
Perimeter	Total length of the object boundary. Sum of all edges surrounding the object.
Solidity	Measure of holes or concave boundaries in the object. Calculated as area / convex hull area. The convex hull is fitted onto the object by drawing straight lines across concave regions while expanding outward along protrusions. Ratio of 1 indicates an object without any concavities, while values less than 1 indicate holes or an irregular boundary.
Zernike_0_0 – Zernike_9_9	Set of polynomial coefficients used to describe object shape with increasing detail. The smallest circle that encloses the object is used to calculate Zernike features. Objects that are more closely related to the Zernike polynomial in question are reflected in higher values. These features are rotationally invariant and capture fine-scale shape details.

Descriptions were adapted from the CellProfiler documentation[1] and Cutiongco.[2]

Table S2. Participants characteristics (n = 10).

ID	Sex	Age, years
480007	Male	78
480008	Female	65
480011	Female	69
480027	Female	71
480039	Female	68
480047	Female	66
480055	Female	69
480059	Female	66
480060	Female	71
480063	Male	71

Table S3. Summary of EV characterization based on MISEV recommendations.[*1]

MISEV recommendation	Characterization performed	Experimental method	Results reported	Contribution to EV characterization
Quantification of EV preparations	Starting biofluid volume	Human serum collection and processing	0.150 mL human serum	Reports EV source and starting material.
	Total EV protein	BCA protein assay	3.21 ± 0.20 mg	Quantifies total EV-associated protein recovered.
	Particle concentration	NTA	$(2.3 \pm 0.2) \times 10^{10}$ particles/mL	Quantifies EV abundance.
	Protein-to-particle ratio	BCA + NTA	0.14 pg protein/particle	Assesses EV preparation consistency and purity.
Characterization of EV protein composition Single-particle characterization	Particle size	NTA	92.20 ± 16.85 nm	Characterizes EV biophysical properties by determining particle size distribution.
	Total RNA content	Quant-iT miRNA Assay kit	644.62 ± 55.84 ng	Quantifies EV-associated nucleic acid cargo.
	Transmembrane or GPI-anchored proteins associated with plasma membrane and/or endosomes	Western blot	CD9 and CD63	Confirms enrichment of canonical EV membrane proteins.
	Assessment of non-EV contaminants	Western blot	Albumin	Evaluates serum protein contamination.
	Transmembrane, lipid-bound and soluble proteins associated with intracellular compartments other than PM/endosomes	Western blot	Calnexin	Assesses contamination from intracellular components.
Single-particle characterization[*2]	Particle size distribution	NTA	Predominant particle size 70–100 nm	Confirms particle size consistent with small EVs.
EV biological activity	Cellular uptake	Fluorescent EV uptake assays (*in vitro* and *ex vivo*)	Efficient uptake by human chondrocytes *in vitro* and mouse cartilage *ex vivo*	Demonstrates interaction of EVs with recipient cells.
	EV cargo transfer	miRNA-engineered EV studies	Successful transfer of EV-associated miRNA into chondrocytes	Demonstrates functional delivery of EV cargo.
	Functional activity	*In vitro* chondrogenesis assays	Enhanced chondrogenesis by post-exercise and miRNA-engineered EVs	Demonstrates biological activity of isolated EVs.
	Therapeutic efficacy	Mouse KOA model	Improved cartilage integrity and reduced OARSI scores following EV treatment	Confirms *in vivo* functional activity of EV preparations.

[*1] MISEV recommendations: MISEV 2018 and/or MISEV 2023.[3,4]

[*2] According to MISEV2018 recommendations,[3] single-particle characterization can be assessed using complementary approaches, including high-resolution imaging techniques (e.g., TEM/cryo-TEM) and single-particle tracking methods that provide biophysical parameters (e.g., NTA). In this study, NTA was performed to characterize particle concentration and size distribution as part of the single-particle characterization of the EV preparations.

SUPPLEMENTARY REFERENCES

1. Kamentsky, L.*, et al.* Improved structure, function and compatibility for CellProfiler: modular high-throughput image analysis software. *Bioinformatics* **27**, 1179-1180 (2011).
2. Cutiongco, M.F., Jensen, B.S., Reynolds, P.M. & Gadegaard, N. Predicting gene expression using morphological cell responses to nanotopography. *Nature communications* **11**, 1-13 (2020).
3. Théry, C.*, et al.* Minimal information for studies of extracellular vesicles 2018 (MISEV2018): a position statement of the International Society for Extracellular Vesicles and update of the MISEV2014 guidelines. *J Extracell Vesicles* **7**, 1535750 (2018).
4. Welsh, J.A.*, et al.* Minimal information for studies of extracellular vesicles (MISEV2023): From basic to advanced approaches. *J Extracell Vesicles* **13**, e12404 (2024).