Supplementary information for

Needle-Free Injection of Exosomes Derived from Human Dermal Fibroblast Spheroids Ameliorates Skin Photoaging

Shiqi Hu, [†, ‡] *Zhenhua Li,* [†, ‡] *Jhon Cores,* [†, ‡] *Ke Huang,* [†] *Teng Su,* [†, ‡] *Phuong-Uyen Dinh* [†, ‡] *and Ke Cheng* *[, †, ‡]

[†] Department of Molecular Biomedical Sciences and Comparative Medicine Institute, North Carolina State University, Raleigh, North Carolina 27607, United States.

[‡] Joint Department of Biomedical Engineering, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599, and North Carolina State University, Raleigh, North Carolina 27606, United States.

*Corresponding author. E-mail: kcheng3@ncsu.edu

SUPPLEMENTARY METHODS

Immunohistochemistry Assessment: Monolayer cells or spheroids were fixed with 4% paraformaldehyde (20 min at room temperature), blocked with Protein Block Solution (DAKO) containing 0.1% saponin (1 h at room temperature) and then incubated with primary antibodies diluted in the blocking solution overnight at 4 °C. After washing with PBS, samples were stained with fluorescent secondary antibodies (1 h at room temperature). After washing with PBS again, slides were mounted with ProLong™ Diamond Antifade Mountant with DAPI (P36962, Thermo Fisher Scientific) and imaged with a Zeiss LSM 710 confocal microscope.

Primary antibodies used: CD34 (ab81289, Abcam) and vimentin (ab8978, Abcam). Secondary antibodies used: goat anti-rabbit IgG-Alexa Fluor 594 conjugate (1:400, ab150080, Abcam) and goat anti-mouse IgG-Alexa Fluor 488 conjugate (1:400, ab150113, Abcam).

Cellular Senescence Assay. SA-beta-gal activity was assessed using a SA-beta-gal staining kit (Cell Signaling Technology, Boston, MA, USA). HDFs with different treatment were fixed and stained at 37 °C overnight in freshly prepared SA-beta-gal staining solution.

Histological Analysis. Dorsal skin specimens of nude mice were obtained and fixed in 4% paraformaldehyde for at least 24 h. Next, they were embedded in paraffin and sectioned at 5 μm thicknesses. Briefly, H&E staining was conducted by deparaffinization, hydration, hematoxylin staining, eosin staining, and dehydration. To carry out the Masson's trichrome staining, the paraffin-embedded skin specimens were stained with Bouin's solution and Weigert's iron hematoxylin working solution, phosphomolybdic-phosphotungstic acid solution, aniline blue solution and dehydrated in series.

Western Blot Analysis. For western blot analysis, skin tissues were lysed by T-PER™ Tissue Protein Extraction Reagent (Fisher) and centrifuged at 12,000 × g for 20 min at 4℃. Skin lysates were then homogenized to yield equivalent amounts of protein based on protein concentration measurements carried out with BCA protein assay kit (Thermo Scientific). Samples were electrophoresed through sodium dodecyl sulfate – polyacrylamide gel (SDS-PAGE), transferred to a nitrocellulose membrane (Amersham Pharmacia Biotech, Buckinghamshire, UK), blocked with 5% milk for 1 h under room temperature conditions, and primary antibodies were used for incubation with the membrane overnight at 4 °C. The membrane was then washed three times and incubated with secondary antibody for 1 h at room temperature.

Primary antibodies used in this study: anti-Pro-Collagen Type 1, A1/COL1A1, (ABT257) (Sigma-Aldrich), MMP 1 (ab137332, Abcam), TNF-α (ab8348, Abcam), IL-1β (TE271712, invitrogen), TGF-β (ab29769, Abcam), GAPDH (ab9835, Abcam). Secondary antibodies used: Goat anti-Rabbit IgG (H+L) Secondary Antibody, HRP (65-6120) (Invitrogen, Carlsbad, CA) and Goat anti-Mouse IgG (H+L) Secondary Antibody, HRP (31430, invitrogen).

SUPPLEMENTARY FIGURES

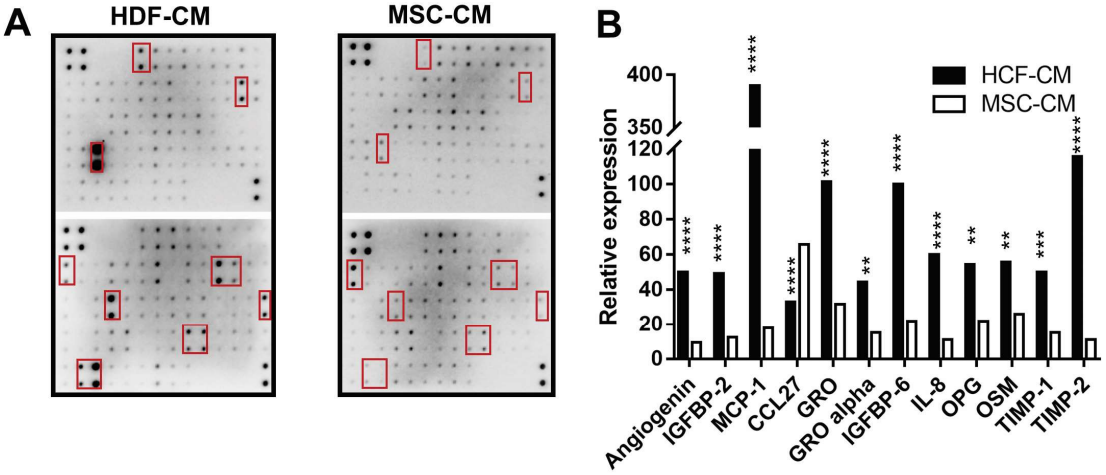

Figure S1. A) Comparison of growth factors secreted by HDFs (P1) and MSC by B) densitometric analysis, n=3. **p<0.01, ***p<0.001, ****p<0.0001.

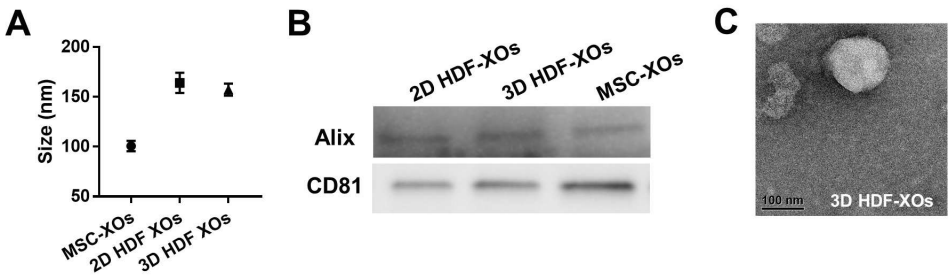

Figure S2. A) Size measurement of exosomes using DLS, n=3. B) Western blot showing Alix and CD81 blotting on exosome lysate. C) Transmission electron microscopy (TEM) image of 3D HDF-XOs.

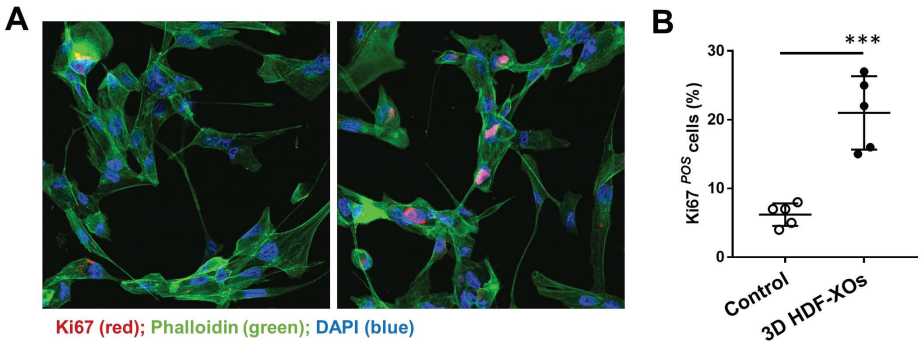

Figure S3. The effects of 3D HDF-XOs on the ki67 expression of UVB-irradiated HDFs, n=5. ***p<0.001.

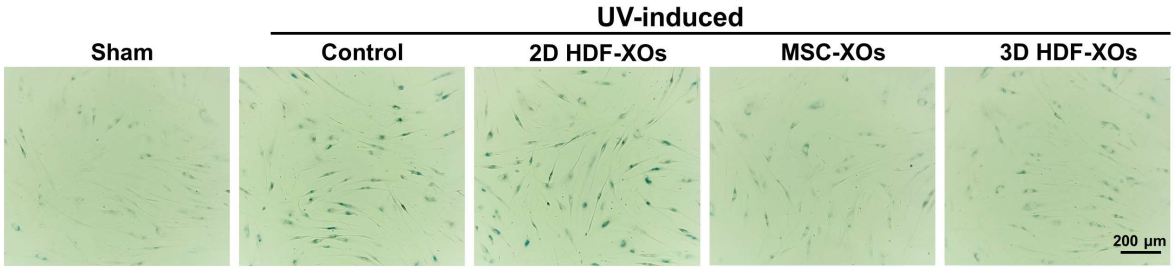

Figure S4. The effects of exosomes on senescent phenotype of UVB-irradiated HDFs by SA-β-Gal staining, n=3. Scale bar: 200 μm.

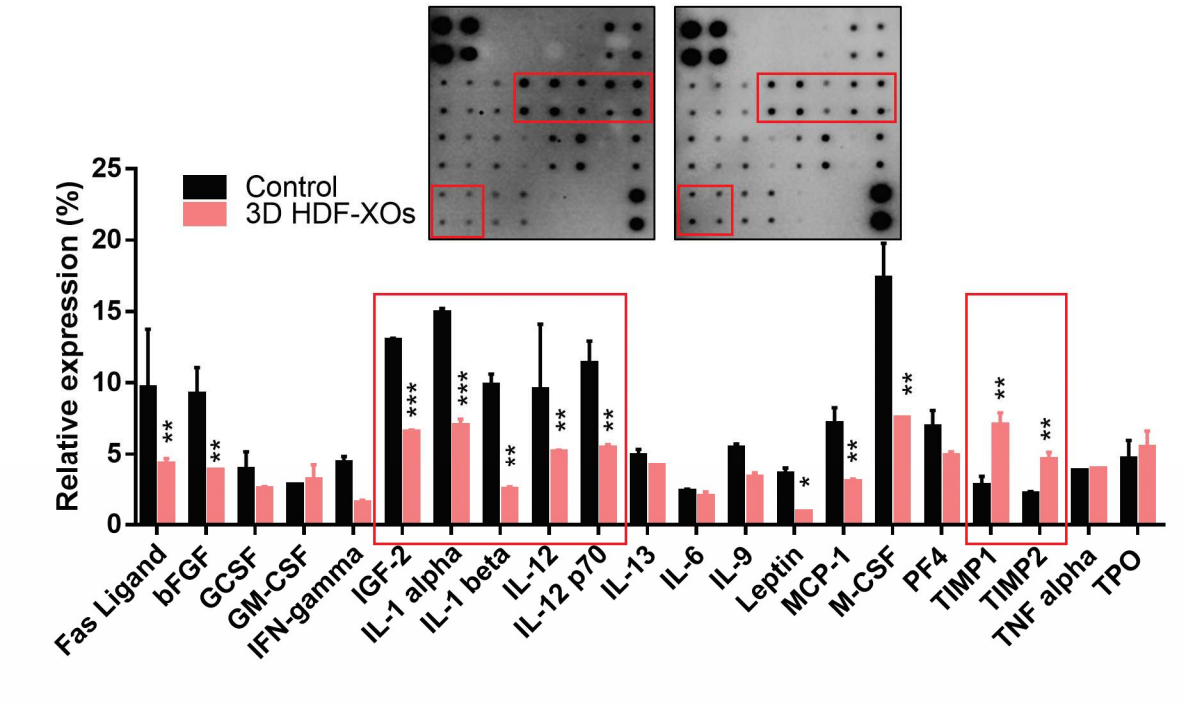

Figure S5. Cytokine analysis of skin samples from the control group and the 3D HDF-XOs treated group, n=3. *p<0.05, **p<0.01, ***p<0.001.

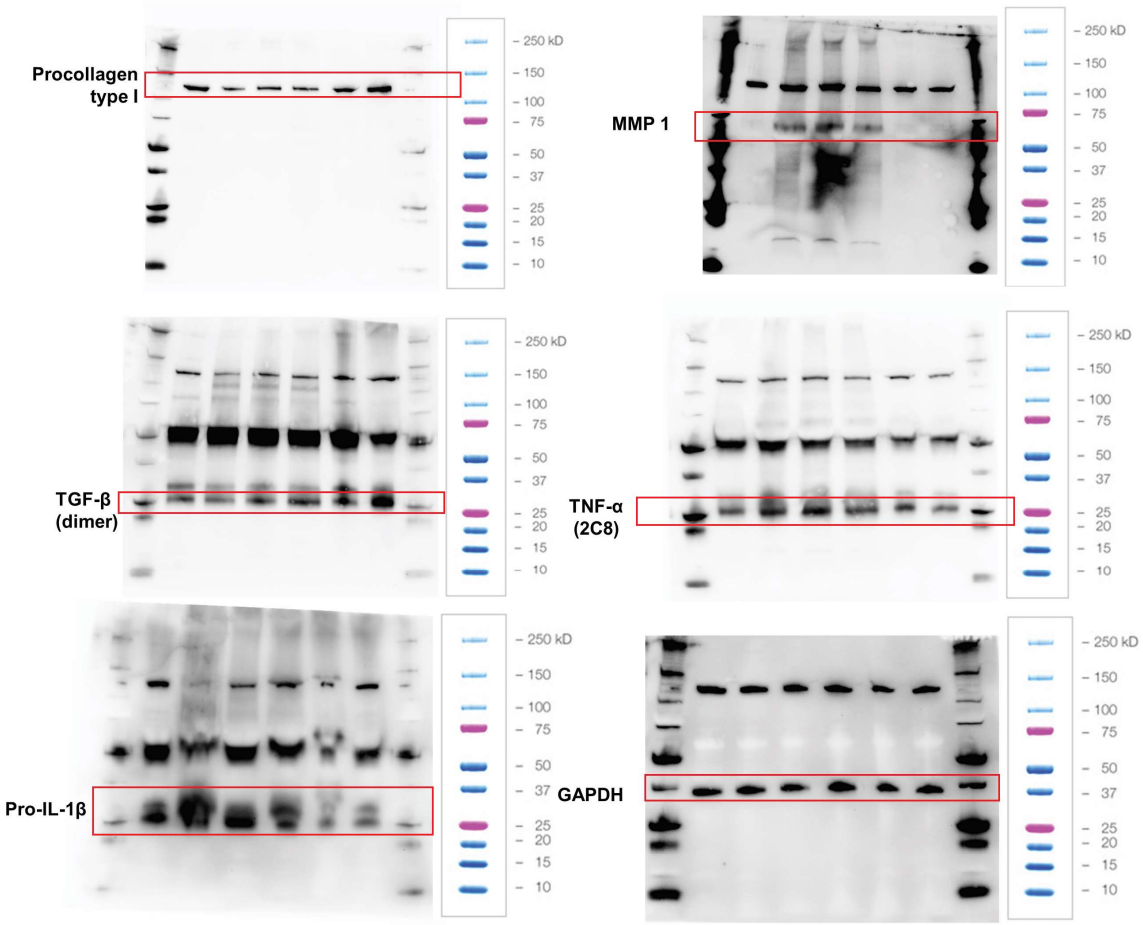

Figure S6. Western blot showing Procollagen type I, MMP1, TGF-β, TNF-α, IL-1β and GAPDH from the skin lysis of different groups. n=3.

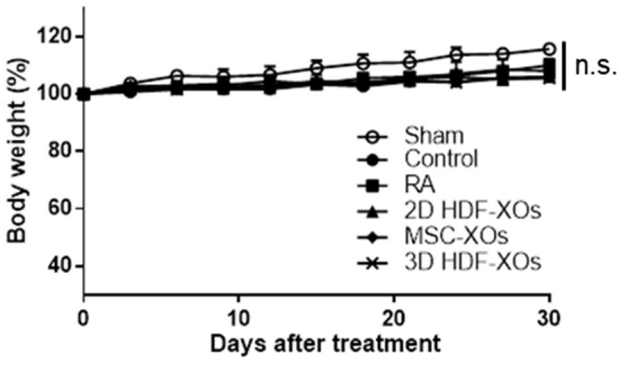

Figure S7. Mice body weight changes during treatment. n=3, n.s. means no significant difference.

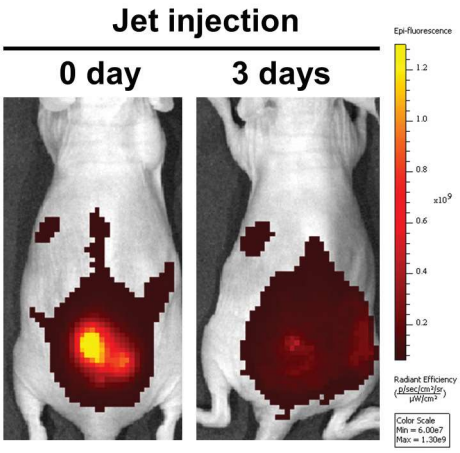

Figure S8. IVIS imaging of DiR-labeled exosomes injected *via* DERMOJET.

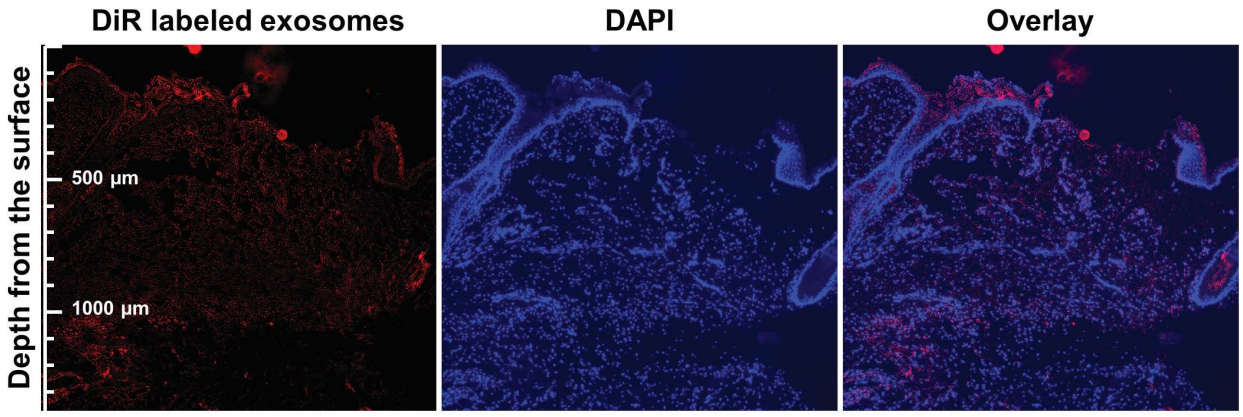

Figure S9. Distribution of exosomes delivered by DERMOJET in porcine skin. 50 µL DiR labeled exosomes were injected to the abdominal skin of Yorkshire piglets *via* DERMOJET. Then the skin was harvested and sectioned. From left to right are DiR labled exosomes (red), DAPI (blue) and overlay.

A 3D HDF-XOs VS. MSC-XOs

Layout	01	02	03	04	05	06
A	hsa-let-7d-5p17.08C	hsa-miR-1-3p17.08C	hsa-miR-101-3p17.08C	hsa-miR-10717.08C	hsa-miR-10a-5p-1.87B	hsa-miR-10b-5p-1.87B
B	hsa-miR-141-3p17.08C	hsa-miR-142-3p17.08C	hsa-miR-143-3p-7.65B	hsa-miR-145-5p-3.85A	hsa-miR-146a-5p-1.87B	hsa-miR-146b-5p-1.87B
C	hsa-miR-18a-5p17.08C	hsa-miR-192-5p-1.87B	hsa-miR-194-5p-1.87B	hsa-miR-195-5p17.08B	hsa-miR-196a-5p-4.52B	hsa-miR-199a-5p-1.87B
D	hsa-miR-204-5p17.08C	hsa-miR-208a-3p17.08C	hsa-miR-20a-5p17.08C	hsa-miR-211-5p17.08C	hsa-miR-215-5p-1.87B	hsa-miR-21-5p-9.89A
E	hsa-miR-26b-5p17.08C	hsa-miR-27a-3p-1.15B	hsa-miR-27b-3p-14.99B	hsa-miR-29a-3p-77.47A	hsa-miR-29b-3p-7.14B	hsa-miR-29c-3p-400.47A
F	hsa-miR-32-5p17.08C	hsa-miR-328-3p9.95B	hsa-miR-335-5p17.08B	hsa-miR-338-5p17.08C	hsa-miR-34a-5p546.64B	hsa-miR-372-3p17.08C
G	hsa-miR-451a17.08C	hsa-miR-491-5p-1.87B	hsa-miR-5011-5p546.64B	hsa-miR-503-5p546.64B	hsa-miR-5692a17.08C	hsa-miR-590-5p17.08C
H	cel-miR-39-3p17.08C	cel-miR-39-3p17.08C	SNORD6117.08B	SNORD6817.08B	SNORD7217.08C	SNORD957.49B
Layout	**07**	**08**	**09**	**10**	**11**	**12**
A	hsa-miR-122-5p17.08C	hsa-miR-125b-5p-36.14B	hsa-miR-126-3p17.08C	hsa-miR-129-5p-1.87B	hsa-miR-132-3p-1.26B	hsa-miR-133a-3p546.64B
B	hsa-miR-148a-3p17.08C	hsa-miR-150-5p17.08C	hsa-miR-155-5p-1.87B	hsa-miR-15b-5p17.08B	hsa-miR-16-5p-1.83B	hsa-miR-17-5p17.08B
C	hsa-miR-199b-5p546.64B	hsa-miR-19a-3p17.08C	hsa-miR-19b-3p-1.87B	hsa-miR-200a-3p17.08C	hsa-miR-200b-3p17.08C	hsa-miR-203a-3p17.08C
D	hsa-miR-216a-5p17.08C	hsa-miR-21717.08C	hsa-miR-223-3p546.64B	hsa-miR-23a-3p-8.55A	hsa-miR-25-3p-3.67A	hsa-miR-26a-5p1.52B
E	hsa-miR-302b-3p17.08C	hsa-miR-30a-5p-2.21A	hsa-miR-31-5p17.08B	hsa-miR-324-3p17.08B	hsa-miR-324-5p-1.87B	hsa-miR-325546.64B
F	hsa-miR-375-1.87B	hsa-miR-377-3p17.08C	hsa-miR-378a-3p-168.38A	hsa-miR-382-5p17.08B	hsa-miR-449a17.08C	hsa-miR-449b-5p17.08C
G	hsa-miR-6615.52B	hsa-miR-663a-1.95A	hsa-miR-744-5p-138.67A	hsa-miR-7-5p546.64B	hsa-miR-874-3p-1.87B	hsa-miR-92a-3p-1.90A
H	SNORD96A2.94B	RNU6-6P17.08C	miRTC-2.24A	miRTC-2.44A	PPC14.27	PPC16.16

B 3D HDF-XOs VS. 2D HDF-XOs

Layout	01	02	03	04	05	06
A	hsa-let-7d-5p-1.05C	hsa-miR-1-3p-1.05C	hsa-miR-101-3p-1.05C	hsa-miR-107-1.05C	hsa-miR-10a-5p-1.05C	hsa-miR-10b-5p-1.05C
B	hsa-miR-141-3p-1.05C	hsa-miR-142-3p-1.05C	hsa-miR-143-3p-1.05C	hsa-miR-145-5p30.39B	hsa-miR-146a-5p-1.05C	hsa-miR-146b-5p-1.05C
C	hsa-miR-18a-5p-1.05C	hsa-miR-192-5p-1.05C	hsa-miR-194-5p-1.05C	hsa-miR-195-5p30.39B	hsa-miR-196a-5p-33.69B	hsa-miR-199a-5p-1.05C
D	hsa-miR-204-5p-1.05C	hsa-miR-208a-3p-1.05C	hsa-miR-20a-5p-1.05C	hsa-miR-211-5p-1.05C	hsa-miR-215-5p-1.05C	hsa-miR-21-5p-1.05B
E	hsa-miR-26b-5p-1.05C	hsa-miR-27a-3p30.39B	hsa-miR-27b-3p-1.05C	hsa-miR-29a-3p-1.80B	hsa-miR-29b-3p-1.05C	hsa-miR-29c-3p-1.05C
F	hsa-miR-32-5p-1.05C	hsa-miR-328-3p30.39B	hsa-miR-335-5p30.39B	hsa-miR-338-5p-1.05C	hsa-miR-34a-5p30.39B	hsa-miR-372-3p-1.05C
G	hsa-miR-451a-1.05C	hsa-miR-491-5p-1.05C	hsa-miR-5011-5p30.39B	hsa-miR-503-5p30.39B	hsa-miR-5692a-1.05C	hsa-miR-590-5p-1.05C
H	cel-miR-39-3p-1.05C	cel-miR-39-3p-1.05C	SNORD61-1.05B	SNORD6830.39B	SNORD72-1.05C	SNORD95-1.05B
Layout	**07**	**08**	**09**	**10**	**11**	**12**
A	hsa-miR-122-5p-1.05C	hsa-miR-125b-5p-1.05C	hsa-miR-126-3p-1.05C	hsa-miR-129-5p-1.05C	hsa-miR-132-3p30.39B	hsa-miR-133a-3p30.39B
B	hsa-miR-148a-3p-1.05C	hsa-miR-150-5p-1.05C	hsa-miR-155-5p-1.05C	hsa-miR-15b-5p30.39B	hsa-miR-16-5p30.39B	hsa-miR-17-5p30.39B
C	hsa-miR-199b-5p30.39B	hsa-miR-19a-3p-1.05C	hsa-miR-19b-3p-1.05C	hsa-miR-200a-3p-1.05C	hsa-miR-200b-3p-1.05C	hsa-miR-203a-3p-1.05C
D	hsa-miR-216a-5p-1.05C	hsa-miR-217-1.05C	hsa-miR-223-3p30.39B	hsa-miR-23a-3p-1.05B	hsa-miR-25-3p30.39B	hsa-miR-26a-5p30.39B
E	hsa-miR-302b-3p-1.05C	hsa-miR-30a-5p-1.05B	hsa-miR-31-5p-1.05B	hsa-miR-324-3p-1.05B	hsa-miR-324-5p-1.05C	hsa-miR-32530.39B
F	hsa-miR-375-1.05C	hsa-miR-377-3p-1.05C	hsa-miR-378a-3p-1.05C	hsa-miR-382-5p-1.05B	hsa-miR-449a-33.69B	hsa-miR-449b-5p-1.05C
G	hsa-miR-6612.58B	hsa-miR-663a30.39B	hsa-miR-744-5p-33.69B	hsa-miR-7-5p30.39B	hsa-miR-874-3p-1.05C	hsa-miR-92a-3p-1.05B
H	SNORD96A30.39B	RNU6-6P-1.05C	miRTC-10.81B	miRTC-12.25B	PPC-1.87	PPC-1.86

Table S1. MiRNA array raw data of 3D HDF-XOs VS. MSC-XOs and 3D HDF-XOs VS. 2D-HDF-XOs.