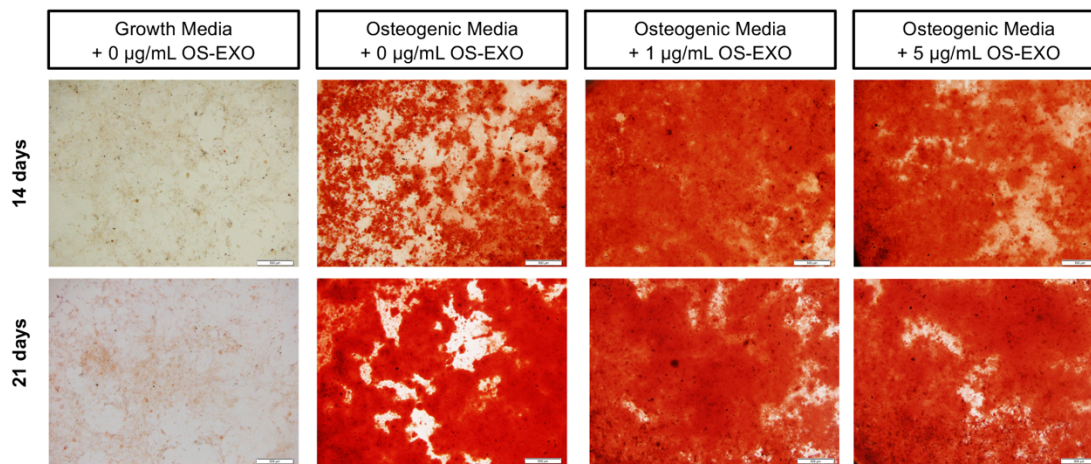


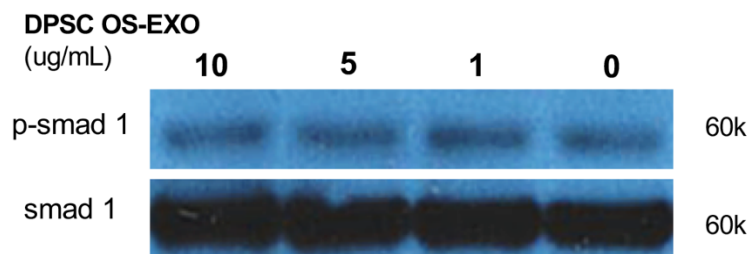
## Supplementary Information

### Scaffolds with Controlled Release of Pro-Mineralization Exosomes Promoting Craniofacial Bone Healing without Cell Transplantation

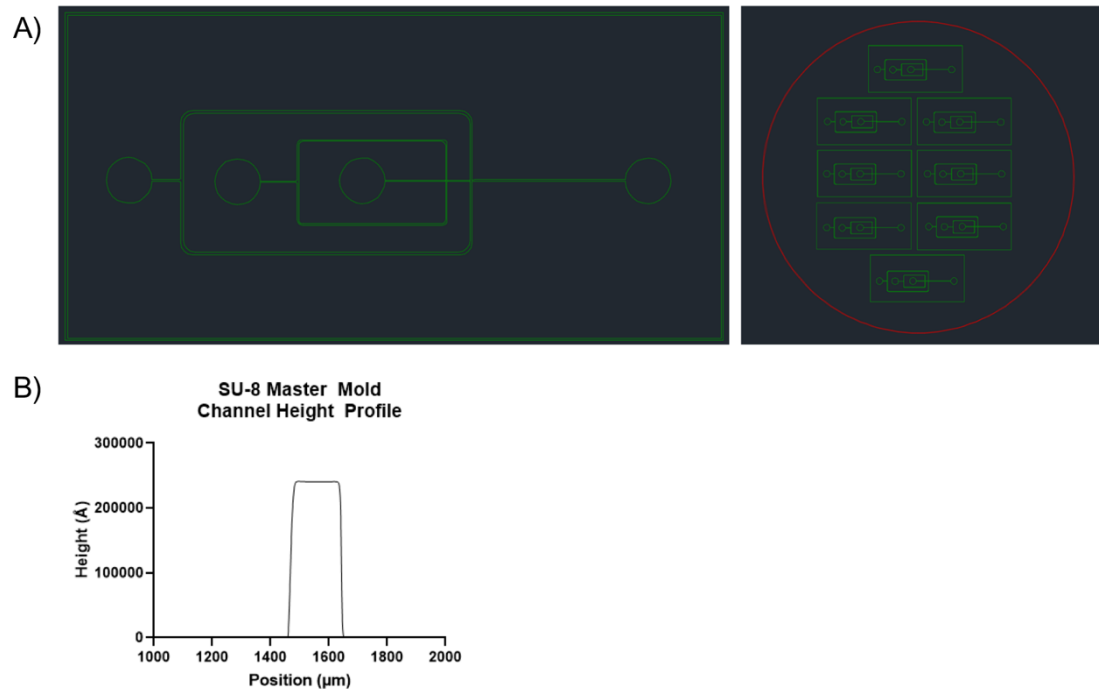
W. Benton Swanson, Zhen Zhang, Kemao Xiu, Ting Gong, Miranda Eberle, Ziqi Wang, Peter X. Ma



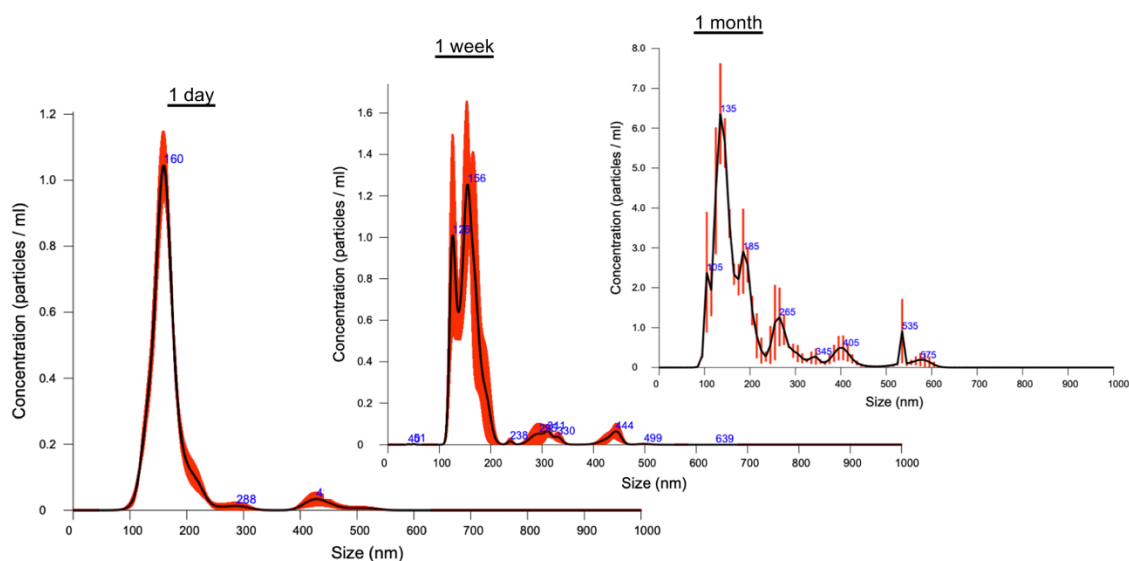
**Sup. Fig. 1.** Evaluating the osteogenic potential of hDPSC-derived exosomes (OS-EXO) in primary human bone marrow stromal cells (hBMSCs). hBMSCs cultured in the presence of exogenous OS-EXO (1 and 5 μg/mL) mineralized significantly more than those cultured in growth or osteogenic media alone, determined by Alizarin Red staining, at 14 and 21 days *in vitro*. Scale bars: 500μm for all images.



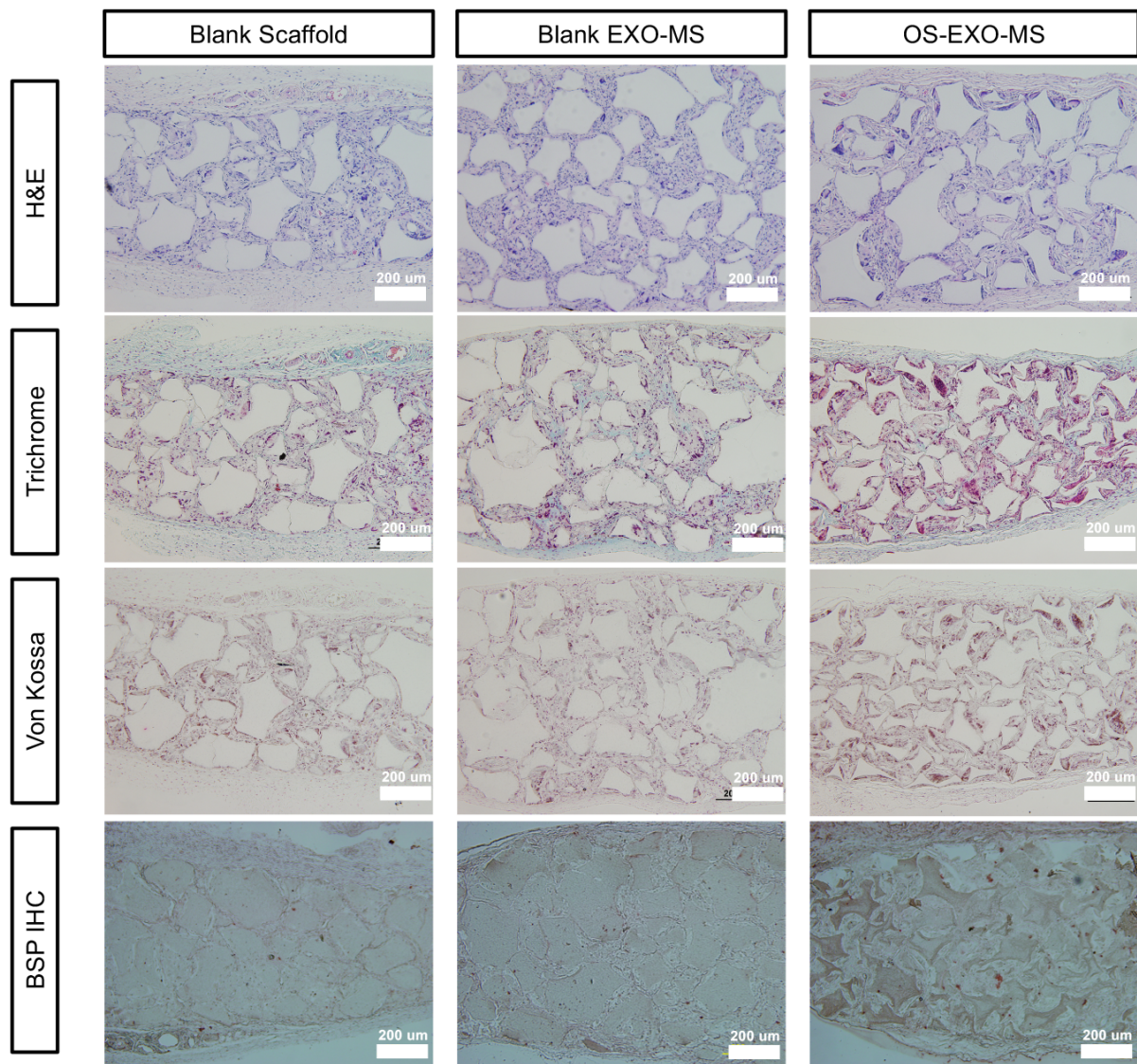
**Sup. Fig. 2.** DPSC OS-EXO treatment of mBMSCs does not upregulate Smad1 phosphorylation, characteristic of canonical BMP signaling.



**Sup. Fig. 3.** Computer aided design (CAD) drawing of the dual flow-focusing junction microfluidic device (A) used to generate a mask for photolithography. After exposing and developing SU-8 photoresist, the height of the resulting pattern was measured by profilometer (B), which demonstrates straight side walls and height of  $25\ \mu\text{m}$ .



**Sup. Fig. 4.** Nanoparticle tracking analysis at various time points during release: OS-EXO released at 1 day, 1 week, and 1 month. The average hydrodynamic diameter of OS-EXO is similar to freshly isolated virgin OS-EXO (Fig. 1A) and does not change significantly over time, indicating that the exosomes remain stable throughout release.



**Sup. Fig. 5.** Low magnification histologic images of subcutaneous explants. Scale bars: 200  $\mu\text{m}$ .

**Sup. Table 1.** List of primer sequences used in quantitative polymerase chain reaction experiments.

<u>Gene</u>	<u>Forward Sequence</u>	<u>Reverse Sequence</u>	<u>Reference</u>
GAPDH	AGGTCGGTGTGAACGGATTTG	TGTAGACCATGTAGTTGAGGTCA	Harvard Primer Bank
RUNX2	ATGCTTCATTGCGCTCACAAA	GCACTCACTGACTCGGTTGG	Harvard Primer Bank
BSP	CAGGGAGGCAGTGACTCTTC	AGTGTGGAAAGTGTGGCGTT	Harvard Primer Bank
OCN	GAACAGACAAGTCCCACACAG	AGCAGAGTGAGCAGAAAGATG	IDT Company

**Sup. Table 2.** List of antibodies used in immunoprecipitation and immunohistochemistry experiments.

<u>Antigen</u>	<u>Manufacturer</u>	<u>Lot</u>
$\beta$ -actin	Cell Signaling Technology	8457S
Runx2	Cell Signaling Technology	12556S
OCN	Thermo Fisher Scientific	PA5-86886
BSP	Thermo Fisher Scientific	PA5-79423
Erk1/2	Cell Signaling Technology	9102S
p-Erk1/2	Cell Signaling Technology	9101S
Smad 1	Cell Signaling Technology	6944S
p-Smad 1	Cell Signaling Technology	5753S
2° Ab (for Western Blot)	Santa Cruz Biotechnology	SC-2004

**Attached separately:**

**Sup. Movie 1** – Particle formation on the microfluidic chip, observed by light microscope.