

## **Supplementary Information**

### **Articular Tissue-Mimicking Organoids Derived from Mesenchymal Stem Cells and Induced Pluripotent Stem Cells**

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**Table S1.** Composition of media used for cell maintenance and tri-lineage differentiation assay.

Medium	Composition
<b>GM-MS<sup>1</sup></b>	High-glucose Dulbecco's Modified Eagle's Medium (DMEM, Gibco) 10% (v/v) fetal bovine serum (FBS, Gemini Bioproducts) 1X antibiotic-antimycotic (Gibco) 1.5 ng/mL fibroblast growth factor-2 (FGF2, RayBiotech)
<b>GM-iMPC</b>	DMEM/F-12 (Gibco) 10% (v/v) fetal bovine serum 1X antibiotic-antimycotic 1.5 ng/mL FGF2
<b>OIM</b>	High-glucose DMEM 10% (v/v) FBS 1X antibiotic-antimycotic 0.1 $\mu$ M dexamethasone (Sigma-Aldrich) 10 mM $\beta$ -glycerophosphate (Sigma-Aldrich) 50 $\mu$ g/mL L-ascorbic acid (Sigma-Aldrich)
<b>CIM<sup>2</sup></b>	High-glucose DMEM 1X antibiotic-antimycotic 0.1 $\mu$ M dexamethasone 40 $\mu$ g/mL L-proline (Sigma-Aldrich) 1X Insulin-Transferrin-Selenium-Ethanolamine (ITS, Gibco) 50 $\mu$ g/mL L-ascorbic acid 10 ng/mL transforming growth factor beta-3 (TGF $\beta$ 3, Peprotech)
<b>AIM</b>	DMEM 10% (v/v) FBS 1X antibiotic-antimycotic 1 $\mu$ M dexamethasone 0.1X ITS 0.5 mM 3-isobutyl-1-methylxanthine (IBMX, Sigma-Aldrich)

<sup>1</sup>GM-MS without FGF2 was used for the control group for osteo- and adipo-induction in 2D cultures.

<sup>2</sup>CIM without TGF $\beta$ 3 was utilized for the control group for chondro-induction in 2D cultures.

**Table S2.** Composition of media used to induce MSC and iMPC differentiation to form different organoids.

<b>Medium</b>	<b>Composition</b>
<b>Control medium for MSC<sup>1</sup></b>	High-glucose DMEM 10% (v/v) FBS 1X antibiotic-antimycotic
<b>Control medium for iMPC<sup>2</sup></b>	DMEM/F-12 10% (v/v) FBS 1X antibiotic-antimycotic 1.5 ng/mL FGF2
<b>OM-MSC / OM-iMPC</b>	High-glucose DMEM 10% (v/v) FBS 1X antibiotic-antimycotic 0.1 $\mu$ M dexamethasone 10 mM $\beta$ -glycerophosphate 50 $\mu$ g/mL L-ascorbic acid 10 nM 1,25-dihydroxy vitamin D3 (Sigma-Aldrich)
<b>CM-MSC</b>	High-glucose DMEM 1X antibiotic-antimycotic 0.1 $\mu$ M dexamethasone 40 $\mu$ g/mL L-proline 1X ITS 50 $\mu$ g/mL L-ascorbic acid 10 ng/mL TGF $\beta$ 3
<b>CM-iMPC</b>	High-glucose DMEM 1X antibiotic-antimycotic 0.1 $\mu$ M dexamethasone 40 $\mu$ g/mL L-proline 1X ITS 50 $\mu$ g/mL L-ascorbic acid 10 ng/mL TGF $\beta$ 3 100 ng/mL bone morphogenetic protein 6 (BMP6, PeproTech)
<b>AM-MSC / AM-iMPC</b>	Minimum Essential Medium $\alpha$ 10% (v/v) FBS 1X antibiotic-antimycotic 0.1 $\mu$ M dexamethasone 0.2 mM indomethacin (Sigma-Aldrich) 0.1X ITS 0.45 mM IBMX

<sup>1</sup>Used as the control medium for OM-MSC and AM-MSC; CM-MSC with 10  $\mu$ M kartogenin (Sigma-Aldrich) but without TGF $\beta$ 3 was utilized as the control medium for CM-MSC.

<sup>2</sup>Used as the control medium for OM-iMPC and AM-iMPC; CM-iMPC without TGF $\beta$ 3 and BMP6 was utilized as the control medium CM-iMPC.

**Table S3.** Primer sequences used for RT-qPCR.

<b>Gene</b>	<b>Forward primer (5'-&gt;3')</b>	<b>Reverse primer (5'-&gt;3')</b>
<i>RPL13A</i>	GCCATCGTGGCTAAACAGGTA	GTTGGTGTTTCATCCGCTTGC
<i>OCN</i>	TCACACTCCTCGCCCTATTG	GAAGAGGAAAGAAGGGTGCC
<i>OPN</i>	TCACCAGTCTGATGAGTCTCACCATTC	TAGCATCAGGGTACTGGATGTCAGGTC
<i>BSP2</i>	CGAATACACGGGCGTCAATG	GTAGCTGTACTCATCTTCATAGGC
<i>BMP2</i>	ACTACCAGAAACGAGTGGGAA	GCATCTGTTCTCGGAAAACCT
<i>ALP</i>	ATCTTTGGTCTGGCCCCCATG	AGTCCACCATGGAGACATTCTCTC
<i>RUNX2</i>	GTGATAAATTCAGAAGGGAGG	CTTTTGCTAATGCTTCGTGT
<i>COL2</i>	GGATGGCTGCACGAAACATACCGG	CAAGAAGCAGACCGGCCCTATG
<i>ACAN</i>	AGTCACACCTGAGCAGCATC	AGTTCTCAAATTGCATGGGGTGTC
<i>SOX9</i>	GGCGGAGGAAGTCGGTGAAGAA	GCTCATGCCGGAGGAGGAGTGT
<i>PPARG1</i>	GACAGGAAAGACAACAGACAAATC	GGGGTGATGTGTTTGAACCTG
<i>LPL</i>	GAGATTTCTCTGTATGGCACTG	CTGCAAATGAGACACTTTCTC
<i>ADIPOQ</i>	AACATGCCCATTCGCTTTAC	AGAGGCTGACCTTCACATCC
<i>ADIPSIN</i>	GACACCATCGACCACGACC	GCCACGTCGCAGAGAGTTC

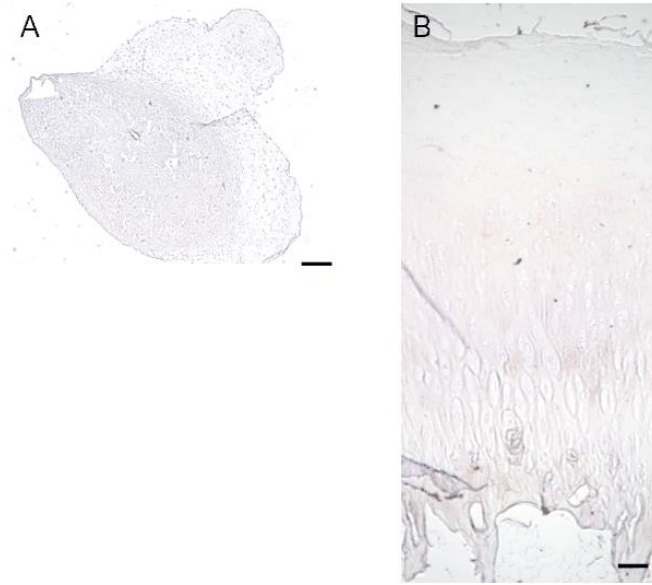


Figure S1. Negative controls for IHC, using mouse immunoglobulin G (IgG) isotypes (0.5  $\mu\text{g/mL}$ ) in place of the primary antibodies. (A) iMPC-derived osteochondral organoid. (B) Native osteochondral tissue. Scale bar = 200  $\mu\text{m}$ .