**Supplementary**

**Materials**

**Reagents**

* Albumax I: Gibco, 11020-021
* Alizarin red S: Sigma, A5533
* Anti-beta actin horseradish peroxidase-conjugated antibody: Abcam, ab49900
* Araldite 6005: EMS, 10920
* Ascorbic acid: Sigma, A4544
* B-27: Gibco, 17504-044
* bFGF: Sigma, F0291
* BMP7: Sigma, B1434
* BSA: Sigma, A2153
* Collagenase I: Sigma, C0130
* Complete lysis-M: Roche, 04719956001
* Dexamethasone: Sigma, D4902
* DMEM/F12: Sigma, D8437
* DMEM/HG: Lonza, BE12-604F
* DMEM/LG: Lonza, BE12-707F
* DMSO: Sigma, D2650
* DPBS: Sigma, D8662
* ECL (LumiGLO® reagent and peroxide): CellSignaling, 7003
* EGF: Sigma, E9644
* Eosin: Sigma, E6003
* FBS: Hyclone, SH30071.03HI
* Formalin: Sigma, HT501128
* FSH: Sigma, F4021
* Gelatin: Sigma, G1820
* Glutamine: Sigma, G7513
* Goat serum: Sigma, G9023
* Hematoxylin: Sigma, H9627
* Horseradish peroxidase-conjugated secondary antibody: BioRad, 1706515
* ITS: Sigma, I3146
* LIF: Sigma, L5158
* LightCycler® 480 SYBR Green I Master: Roche, 4707516001
* Mounting medium (ProLong® gold antifade mountant with DAPI): Thermofisher, P36931
* N-2: Gibco, 17502-048
* PBS: Lonza, BE17-516F
* PFA: Sigma, 158127
* PCR primers: Integrated DNA Technologies
* PSA: Lonza, 17-745E-20
* PVDF membrane (iBlot® transfer stack, PVDF): Invitrogen, IB401001
* RA: Sigma, R2625
* SDS-PAGE (Novex™ 4-20% Tris-glycine mini protein gels, 1.0 mm): Invitrogen, EC6025BOX
* SP: Sigma, P5280
* Taq DNA polymerase dNTPack kit: Roche, 4728874
* TGF- β1: Sigma, T7039
* Toluidine blue: Sigma, 89640
* Transcriptor first strand cDNA synthesis kit: Roche, 4897030001
* Triton X-100: Sigma, X-100
* TRIzol: Thermofisher, 15596-026
* Trypsin-EDTA: Hyclone, SV30031.01
* β-Estradiol: Sigma, E2758
* β-Glycerophosphate: Sigma, G9422
* β-Mercaptoethanol: Sigma, M3148

**Equipment**

* Cell strainer: BD Falkon™, 352340
* Digital camera of fluorescence/light microscope: Zeiss Axiocam, ERc 5s
* Digital camera of inverted microscope: Olympus, U-LH100L-3
* Fluorescence/light microscope: Carl Zeiss, Axioskop 451485
* iBlot dry blotting system: Invitrogen
* Inverted microscope: Olympus, IX71
* LightCycler® 480/384 system: Roche, Switzerland
* NanoDrop spectrophotometer: NanoDrop technologies, ND-1000
* Stereomicroscope: Nikon, SMZ745T
* Thermal gradient cycler: Bio-Rad, ALS1296
* Transmission electron microscope: Oberkohen-Germany, Leo 906 E
* Western blot imaging system: Kodak Image Station, 4000MM
* Western blot spectrophotometer: BioRad, SmartSpec 3000

**Follicular fluid preparation**

20 ovaries were dissected, rinsed gently in PBS and completely cleaned out from the surrounding tissues under a stereomicroscope. Ovaries were entirely minced and smashed by sterile disposable surgical scalpel in 5 ml DMEM/F12 (supplemented only with 1% PSA) under a laminar flow hood. Prepared suspension was centrifuged at 1200 g for 10 min, then supernatant was filtered through 0.2 µm pore size sterile filter and heat inactivation was performed at 56°C for 30 min. Follicular fluid aliquoted and stored at - 20°C.

**Table S1** List of primary and secondary antibodies

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Antibodies** | | **Brand, Cat. No.** | **Working concentration** | **Molecular weight (KD)** |
| **Primary Antibodies** | Rabbit polyclonal to Oct-4 | Abcam, ab137427 | 1:200 | 38 |
| Rabbit polyclonal to Nanog | Abcam, ab80892 | 1:100 | 35 |
| Rabbit polyclonal to Sox2 | Abcam, ab97959 | 1:200 | 43 |
| Mouse monoclonal to SSEA1 | Abcam, ab16285 | 1:100 | 59 |
| Rabbit polyclonal to DDX4 | Abcam, ab13840 | 1:200 | 76 |
| Rabbit polyclonal to DAZL | Abcam, ab34139 | 1:200 | 33 |
| Rabbit polyclonal to ZP3 | Santa Cruz, 25802 | 1:200 | 47 |
| Rabbit polyclonal to Nestin | Abcam, ab27952 | 1:200 | 177 |
| Rabbit polyclonal to GFAP | Abcam, ab7260 | 1:500 | 55.5 |
| Rabbit polyclonal to Collagen II | Abcam, ab34712 | 1:200 | 142 |
| **Secondary Antibodies** | Goat anti-Rabbit IgG, Alexa Fluor® 488 | Thermo Fisher Scientific, A11034 | 1:500 |  |
| Goat anti-Mouse IgG, Alexa Fluor® 594 | Thermo Fisher Scientific, A11032 | 1:500 |  |

**Table S2** Primers used for RT-PCR and qRT-PCR

|  |  |  |  |
| --- | --- | --- | --- |
| **Gene (NCBI RefSeq)** | **Primer sequence (5ʹ→3ʹ)** | **Tm (ºC)** | **Product size (bp)** |
| *Oct-4* (NM\_013633.3) | F: AGAAGGATGTGGTTCGAGTATGGT | 60-63 | 204 |
| R: AAGGGACTGAGTAGAGTGTGGTG |
| *Sox2* (NM\_011443.3) | F: AGTGGTACGTTAGGCGCTTC | 63 | 206 |
| R: CCCAGCAAGAACCCTTTCCT |
| *Nanog*  (NM\_028016.3) | F: TGAGCTATAAGCAGGTTAAGAC | 63 | 139 |
| R: CAATGGATGCTGGGATACTC |
| *SSEA1* (NM\_010242.3) | F: TCCTCTTTGGGGCTCCTACA | 60 | 123 |
| R: GTGCGGGTCCCAGTTTCAT |
| *DDX4* (NM\_010029.2) | F: AGGCAGAAATACTCAAACCTCAC | 58 | 203 |
| R: AGACTCGCCAATATCTCTGCT |
| *DAZL* (NM\_010021.5) | F: TATAACTACCAGATGCCACC | 58-63 | 229 |
| R: CAGGGTTAAACAGACAAGAG |
| *NOBOX* (NM\_130869.3) | F: GAATCATGGTGTGGTTTCAG | 58 | 146 |
| R: AGGCATAGGGTCTAGGAG |
| *ZP2* (NM\_011775.6) | F: TCCTACAAACATACCCAGACC | 60 | 222 |
| R: GTTCATATTCACAGCCATCCA |
| *ZP3* (NM\_011776.1) | F: CACCTTCCTACTCCACGAC | 58 | 208 |
| R: ATTCCAGTTCTCCTCCATCAG |
| *Nestin* (NM\_016701.3) | F: TCGGGAGAGTCGCTTAGAGGT | 62 | 266 |
| R: CCAGCAGAGTCCTGTATGTAGCC |
| *GFAP* (NM\_010277.3) | F: TACAGACTTTCTCCAACCTCC | 58 | 127 |
| R: TCCTTAATGACCTCACCATCC |
| *Osteocalcin* (NM\_007541.3) | F: CTGACCTCACAGATGCCAAGC | 62 | 195 |
| R: AGCCATACTGGTCTGATAGCTCG |
| *Osteopontin* (NM\_001204201.1) | F: TGATGAACAGTATCCTGATGCC | 58 | 205 |
| R: CTCTTTGGAATGCTCAAGTCTG |
| *ALP* (NM\_001204201.1) | F: AACCCAGACACAAGCATTCCC | 60 | 263 |
| R: GTCAATCCTGCCTCCTTCCAC |
| *Aggrecan* (NM\_007424.2) | F: CTATTTCCACACGCTACACCC | 60 | 293 |
| R: AAAGACCTCACCCTCCATCTC |
| *Collagen II* (NM\_031163.3) | F: ACAGTACCTTGAGACAGCACG | 60 | 164 |
| R: CCAGTAGTCTCCGCTCTTCCA |
| *DMC1* (NM\_010059.3) | F: ACAAGCTTATTGAACCAGGA | 60 | 162 |
| R: TTCCAGTACGAAATTCTCCA |
| *PRDM1* (NM\_007548.4) | F: GACGCTGATATGACTTTGTGG | 58 | 179 |
| R: ACGCCAATAACCTCTTTGCT |
| *STRA8* (NM\_009292.1) | F: GAGAAGGCAACCAACCCA | 60 | 194 |
| R: ATTCAGTACCTGCCACTTTG |
| *SCP3* (NM\_011517.2) | F: CGAGCAGTTCATAAAGAGTTTGGA | 58 | 154 |
| R: AGACTTTCGAACATTTGCCATCTC |
| *HPRT* (NM\_013556.2) | F: AAAGGACCTCTCGAAGTG | 58-63 | 121 |
| R: CAAACGTGATTCAAATCCC |

**Figure S1** Chondrogenic differentiation was performed in 15 ml polypropylene centrifuge tubes. Pellet of chondrocytes aggregate at the third week of differentiation process (arrows)

**Movie** **S1** Pulsing in one of the EBs that lasted 8 days continuously.