**Supplementary Information for**

**Noggin combined with dental pulp stem cells repair muscle injury through Smad/Pax7 signaling pathway**

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This file contains Supplementary Sections 1-5, including **Fig. S1-3** and **Table S1-2**. Isolation, culture and identification of DPSCs was described. Donor information was provided. It is proved that 5-Aza can induce myogenic differentiation of DPSCs, and noggin has no effect on cell proliferation of DPSCs. The primer sequences for PCR amplification were also listed.

**Fig. S1** Isolation, culture and identification of human dental pulp stem cells. **A** Schematic diagram of DPSCs extraction from dental pulp tissue and isolation by collagenase digestion. **B** Microscopic picture of DPSCs from dental pulp tissue. **C** Cell morphology of DPSCs cultured 24 h, 48 h and 72 h. Bar, 50 μm. **D** Flow cytometric analysis of the surface markers of DPSCs: positive for CD29, CD44, CD90 and negative for CD34, CD45

**Fig. S2** 5-Aza induces myogenic differentiation of human dental pulp stem cells. **A** Cell morphology of DPSCs induced by different concentrations of 5-Aza for 21 day-myogenic differentiation. Scale bar, 20 µm. **B-D** The mRNA expression of (**B**) Myf5, (**C**) Desmin and (**D**) MYH4 was assessed with or without 1 μM 5-Aza induction using quantitative PCR. Data are presented as the fold‑change of the control group without 5-Aza treatment(*n* = 4). **E** Protein expression of MyHC, Desmin and Myf5 was assessed using western blotting with or without 1 μM 5-Aza induction.\**p* < 0.05, \*\**p* < 0.01, and \*\*\**p* < 0.001

**Fig. S3** Noggin has no effect on cell proliferation of DPSCs. **A** Cell morphology of DPSCs cultured 24h and 48h with different concentrations of Noggin (50 ng/ml, 100 ng/ml, 200 ng/ml or 400 ng/ml). Scar bar, 100 μm. **B** Cell cycle distribution was monitored using flow cytometry. **C** Statistical analysis of the cell cycle (*n* = 3)

The donors sought for dental treatment in Shanghai Stomatological Hospital were recruited. The extracted teeth were those needed be removed for orthodontic treatment, or the wisdom teeth for dental preventive care. It has been approved by Ethics Committee (No. 2017-0004). The age of the patients ranged from 18 to 25 years old (see Table below). Collected teeth were free of caries or periodontal diseases.

**Table S1.** Donor information

|  |  |  |  |
| --- | --- | --- | --- |
| Age/years | Gender | Recruitment date | Tooth position |
| 18 | Male | 30/04/2018 | Right upper third molar |
| 18 | Female | 28/04/2018 | Right upper third molar |
| 25 | Female | 28/04/2018 | Right upper third molar |
| 25 | Female | 28/04/2018 | Right lower third molar |
| 25 | Female | 21/04/2018 | Right lower third molar |
| 22 | Male | 18/09/2019 | Left upper third molar |
| 23 | Male | 18/09/2019 | Left upper third molar |
| 18 | Female | 09/11/2019 | Right upper first premolar |
| 18 | Female | 09/11/2019 | Left upper third molar |
| 21 | Male | 17/11/2019 | Right upper first premolar |
| 25 | Female | 17/11/2019 | Right upper first premolar |

**Table S2.** Sequence of primers used for PCR amplification

|  |  |  |
| --- | --- | --- |
| Gene | Forward (5'‑3') | Reverse (5'‑3') |
| Desmin | GACCATCGCGGCTAAGAAC | GTGTAGGACTGGATCTGGTG |
| MRF4 | GGAGCGCCATCAGCTATATTG | ATCCGCACCCTCAAGATTTTC |
| MyoD1 | GCAACGCCATCCGCTATATC | TGTAGTCCATCATGCCGTCG |
| Pax7 | ACCCCTGCCTAACCACATC | AGCGGCAAAGAATCTTGGAG |
| Pax3 | AGCTCGGCGGTGTTTTTATCA | CTGCACAGGATCTTGGAGAC |
| Six1 | CTGCCGTCGTTTGGCTTTAC | GCTCTCGTTCTTGTGCAGG |
| Eya2 | AGCGATTGTCTGGATAAACTG | AGGTGGGTAAGCTGTATAGG |
| ID1 | CTGCTCTACGACATGAACGG | GAAGGTCCCTGATGTAGTCG |
| MSX1 | TCAAGCTGCCAGAAGATGCG | GGCTTACGGTTCGTCTTGTG |
| MYH4 | CTTGAAGTAGTTGTCTGCTTTGAGC | TTTCGGAGGAAAGGAGCAGC |
| Myf5 | AACCCTCAAGAGGTGTACCAC | GGACTGTTACATTCGGGCATG |
| β-actin | CCAACCGCGAGAAGATGA | CCAGAGGCGTACAGGGATAG |