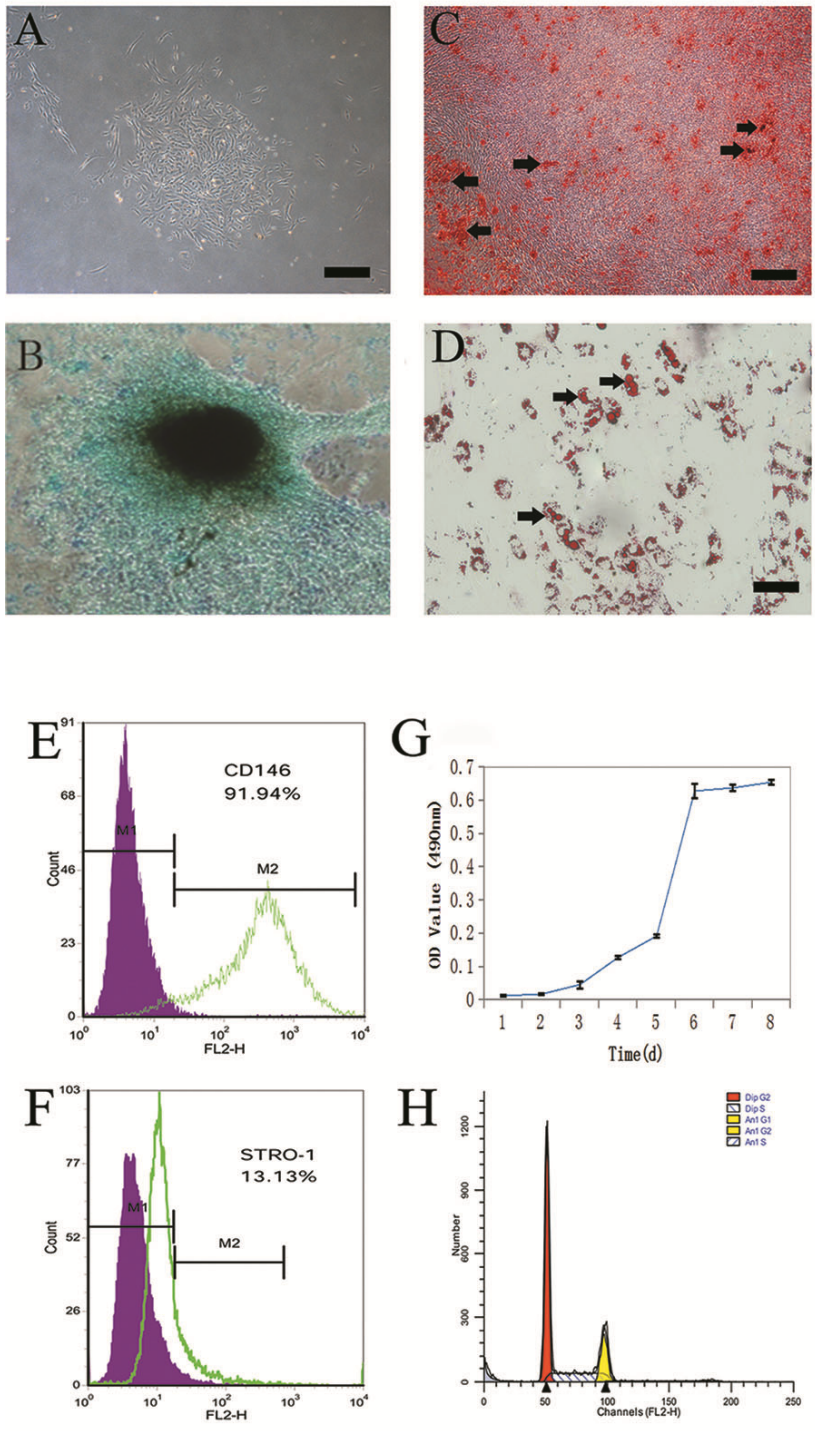
## Additional information

Supplementary data (S) accompanies this paper:

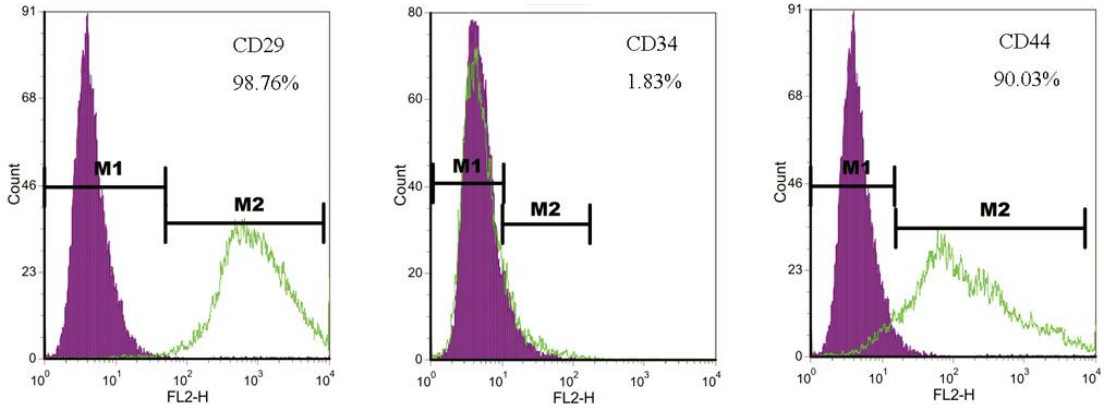
S1:




S1. Characterization of dental pulp stem cells (DPSCs). (A) The cultured DPSCs from single colonies showed typical fibroblast-like cells under a light microscope. (B) Cultured in

chondrogenic induction media, DPSCs pellets were positive to Alcian Blue staining. (C) When DPSCs were cultured in osteogenic inductive conditions for 4 weeks, mineralized nodules (arrows) were found by Alizarin red staining. (D) Cultured DPSCs formed Oil red O-positive lipid clusters (arrows) after 4 weeks of adipogenic induction, Original magnification: X200; Bar=100 μm. (E&F) Flow cytometric analysis of DPSCs revealed expression of CD146 (91.94%) and Stro-1 (13.13%). (G) Growth curves showed the cell proliferation potential of DPSCs. (H) Representative histograms of cell cycle analysis. (Figs. 1A, B, and C: Original magnification: X40; Bar=500 μm)

S2




S2. Flow cytometric analysis of DPSCs. More than 90% of DPCs positively expressed mesenchymal stromal progenitor markers, such as CD29, CD44, but displayed negative expression of hematopoietic markers, such as CD34.

S3.

Statistical analysis of results in the gene expressions of tendon specific and related matrix genes in engineered tendon scaffolds with or without loading after 1, 2, and 3 days.

S3 Table 1. Relative Quantity (Col1/β-actin) in different groups at different time points

| | 1d | 2d | 3d |
|---|---|---|---|
| loaded(n=3) | 0.915±0.067 | 1.874±0.045 | 2.315±0.067 |
| non-loaded(n=3) | 0.637±0.026 | 0.977±0.034 | 1.234±0.17 |
| p *value* | 0.002 | < 0.0001 | < 0.0001 |

S3 Table2. Relative Quantity (ColVI/β-actin) in different groups at different time points

| | 1d | 2d | 3d |
|---|---|---|---|
| loaded(n=3) | 0.981±0.068 | 1.724±0.1 | 2.578±0.107 |
| non-loaded(n=3) | 0.823±0.03 | 0.969±0.009 | 1.289±0.175 |
| p *value* | 0.148 | 0.029 | 0.001 |

S3 Table3. Relative Quantity (SCX/β-actin) in different groups at different time points

| | 1d | 2d | 3d |
|---|---|---|---|
| loaded(n=3) | 5.91±1.45 | 13.09±13.09 | 5.82±1.36 |
| non-loaded(n=3) | 2.36±1.36 | 9.18±0.36 | 4.82±0.89 |
| p *value* | < 0.0001 | 0.001 | 0.09 |

S3 Table4. Relative Quantity (TNC/β-actin) in different groups at different time points

| | 1d | 2d | 3d |
|---|---|---|---|
| loaded(n=3) | 0.95±0.52 | 1.48±0.05 | 2.14±0.29 |
| non-loaded(n=3) | 0.62±0.14 | 0.71±0.05 | 0.95±0.57 |
| p *value* | < 0.0001 | < 0.0001 | < 0.0001 |

S3 Table5. Relative Quantity (EYA2/β-actin) in different groups at different time points

| | 1d | 2d | 3d |
|---|---|---|---|
| loaded(n=3) | 1.268±0.085 | 1.426±0.072 | 1.653±0.099 |
| non-loaded(n=3) | 0.958±0.006 | 1.188±0.026 | 1.388±0.072 |
| p *value* | 0.015 | 0.122 | 0.022 |

S3 Table 6. The different expression of Col-1 in two groups among the different time points (p *value*)

| | loaded(n=3) | non-loaded(n=3) |
|---|---|---|
| 1d vs 2d | < 0.0001 | 0.002 |
| 1d vs 3d | < 0.0001 | 0.002 |
| 2d vs 3d | < 0.0001 | < 0.0001 |

S3 Table 7. The different expression of Col-6 in two groups among the different time points (p *value*)

| | loaded(n=3) | non-loaded(n=3) |
|---|---|---|
| 1d vs 2d | 0.001 | 0.007 |
| 1d vs 3d | 0.001 | 0.007 |
| 2d vs 3d | < 0.0001 | 0.001 |

S3 Table 8. The different expression of SCX in two groups among the different time points (p *value*)

| | loaded(n=3) | non-loaded(n=3) |
|---|---|---|
| 1d vs 2d | < 0.0001 | < 0.0001 |
| 1d vs 3d | 0.434 | < 0.0001 |
| 2d vs 3d | < 0.0001 | < 0.0001 |

S3 Table 9 The different expression of EYA2 in two groups among the different time points(p *value*)

| | loaded(n=3) | non-loaded(n=3) |
|---|---|---|
| 1d vs 2d | 0.01 | 0.018 |
| 1d vs 3d | 0.01 | 0.018 |
| 2d vs 3d | 0.021 | 0.002 |

S3 Table 10. The different expression of TNC in two groups among the different time points(p *value*)

| | loaded(n=3) | non-loaded(n=3) |
|---|---|---|
| 1d vs 2d | < 0.0001 | < 0.0001 |
| 1d vs 3d | < 0.0001 | < 0.0001 |
| 2d vs 3d | < 0.0001 | < 0.0001 |

S3 Table1-10: Quantitative real-time PCR confirmed the gene expression of tendon specific and related matrix genes in engineered tendon scaffolds with or without loading after 1, 2, and 3 days in vitro (n=3, mean±SD).

All data were expressed as mean and standard deviation. Student-t test was applied to analyze the difference between loaded and non-loaded groups at a particular time point (Table1-5). Additionally, one-way ANOVA test was perform to analyze the differences in the expression of tendon related genes, as well as the differences in tendon width and thickness among different time points(Table6-10). A p-value less than 0.05 was considered statistically significant.

S4.

Statistical analysis showed a significant difference in thickness (S4a) and width (S4b) among all groups.

**S4a.** Central thickness of engineered tendon (mm) in Different groups at different time points

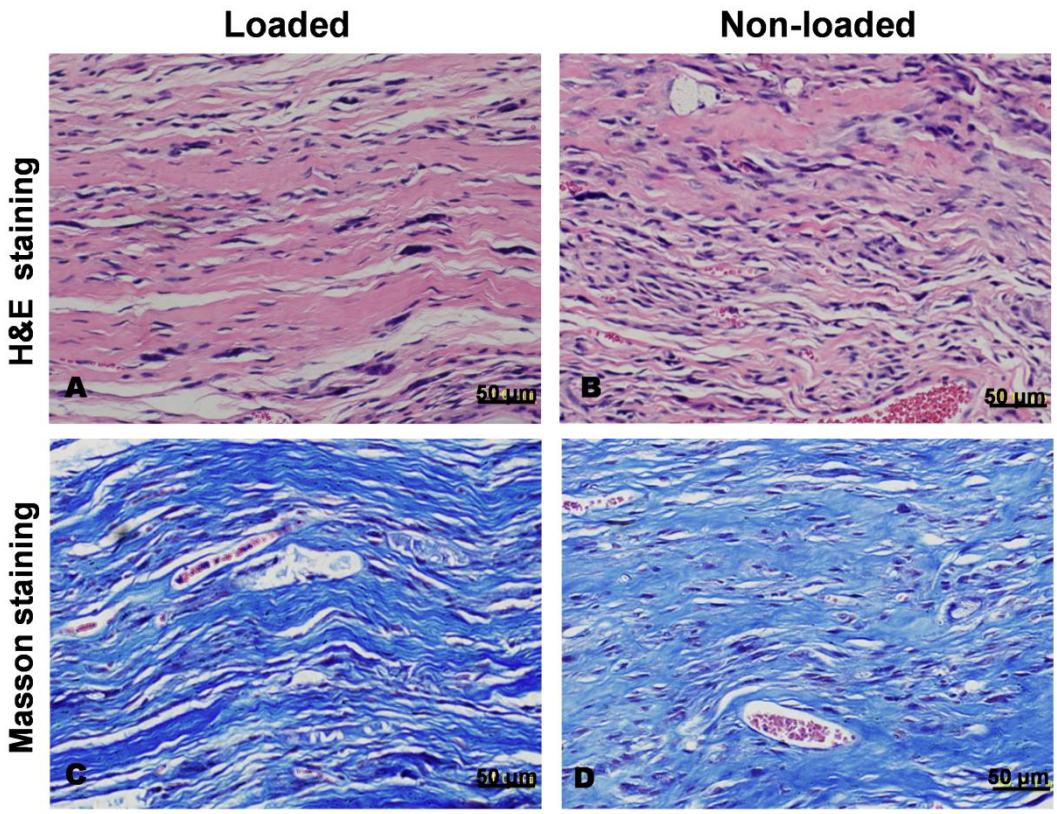
| | 8W | 14W | P value |
|---|---|---|---|
| loaded(n=5) | 1.292±0.2369 | 2.496±0.1721 | 0.0006 |
| non-loaded(n=5) | 0.798±0.08927 | 1.31±0.19161 | 0.011 |
| P value | 0.0161 | 0.0009 | |

**S4b.** Central width of engineered tendon (mm) in Different groups at different time points

| | 8W | 14W | P value |
|---|---|---|---|
| loaded(n=5) | 0.81±0.0254 | 3.058±0.1094 | < 0.0001 |
| non-loaded(n=5) | 0.592±0.02863 | 1.522±0.10709 | < 0.0001 |
| P value | < 0.0001 | < 0.0001 | |

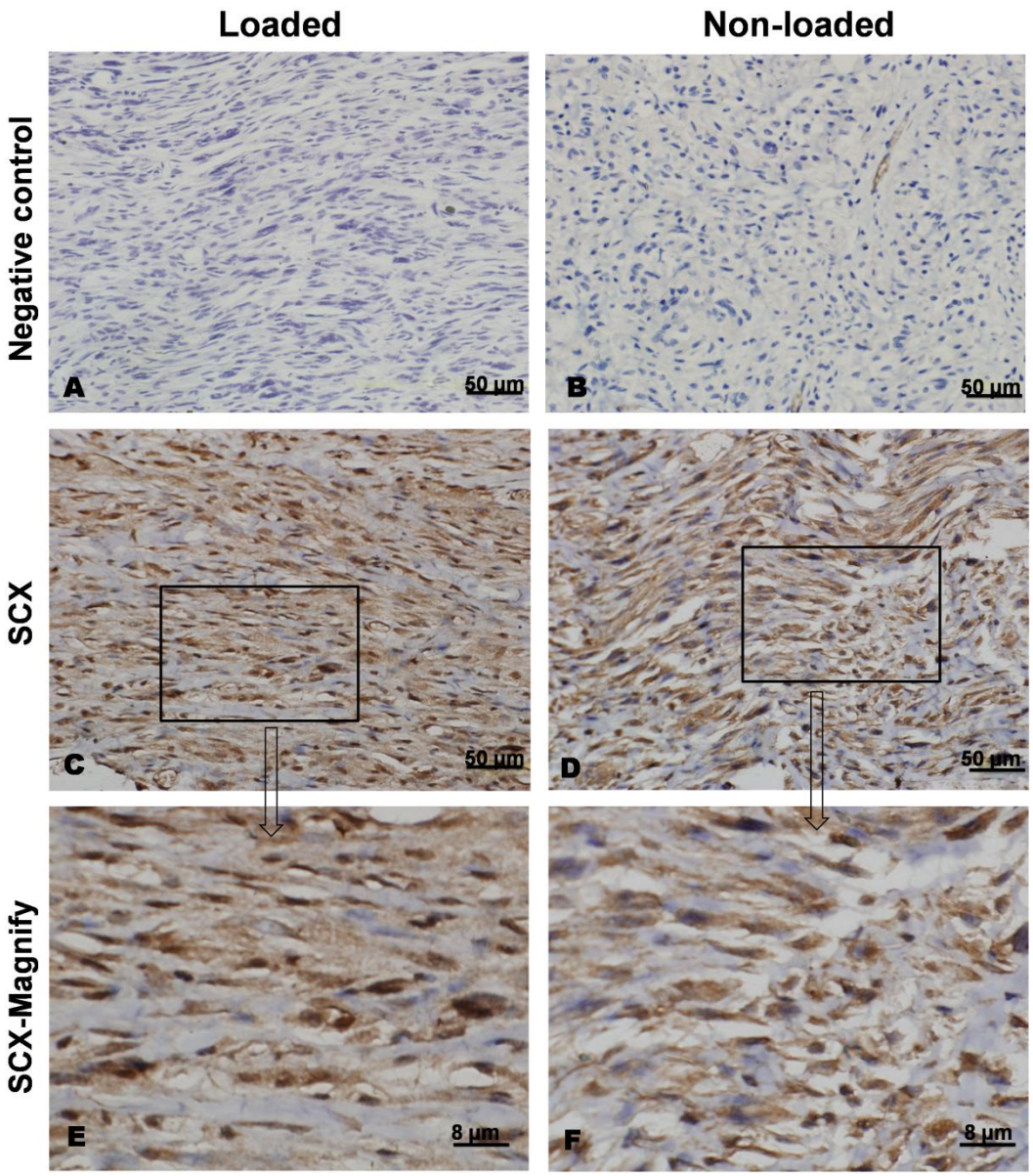
**S4a&b**: All data were expressed as mean and standard deviation. One-way ANOVA analysis of week 8, and 14 specimens finds significant difference in thickness and central width of engineered tendon in two groups(vertical), and ANOVA analysis of two group specimens finds significant difference at each time points (horizontal).

**S5.**

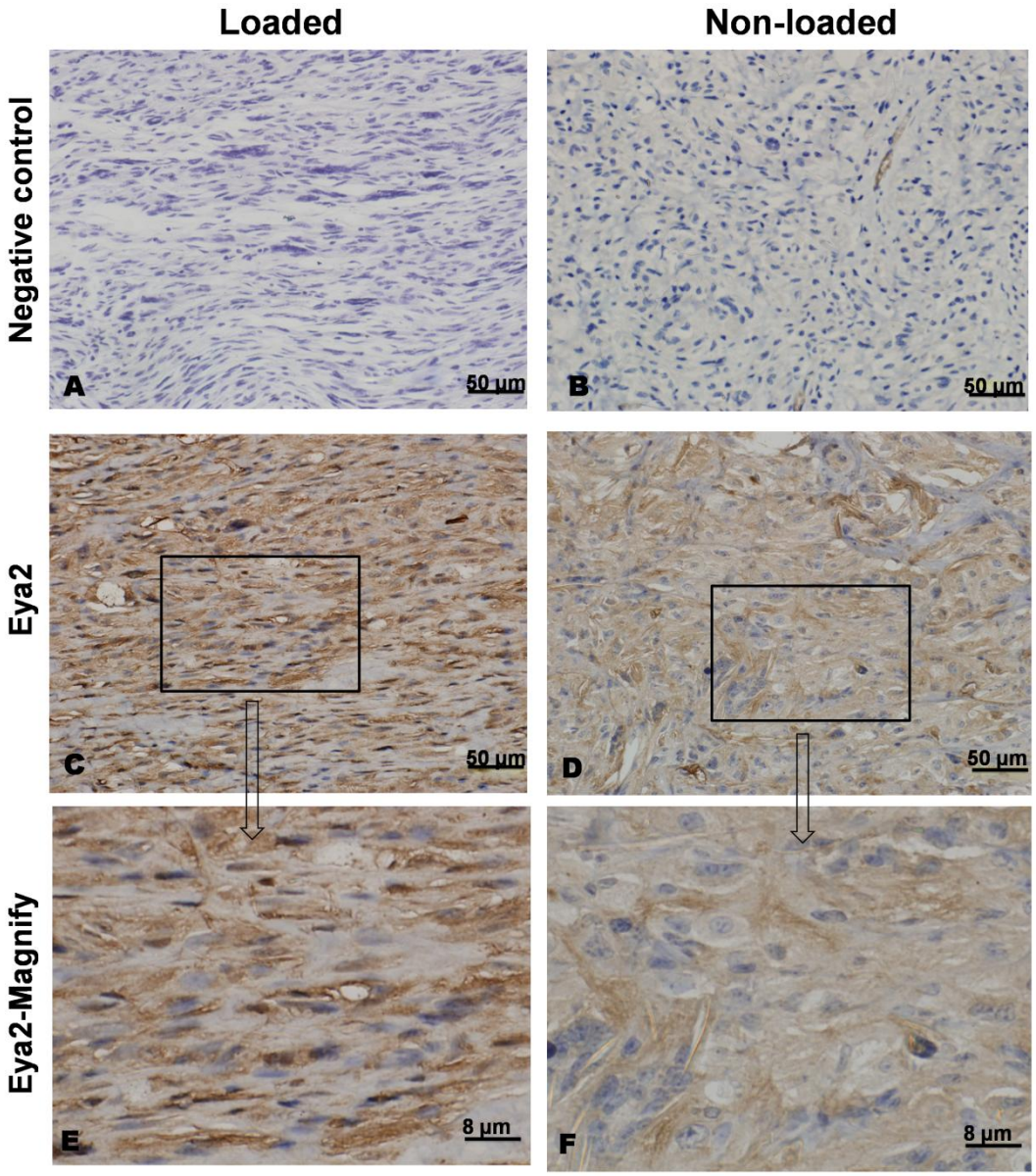



**S5** (A-D) Histological evaluations of tendons engineered with DPSCs. H&E staining(A,B), and Masson staining(C, D) images of the tendons engineered at 14 weeks post-implantation. Original magnifications: X400; Bar=50 µm; Loaded=with mechanical load, Non-Loaded=without mechanical load.

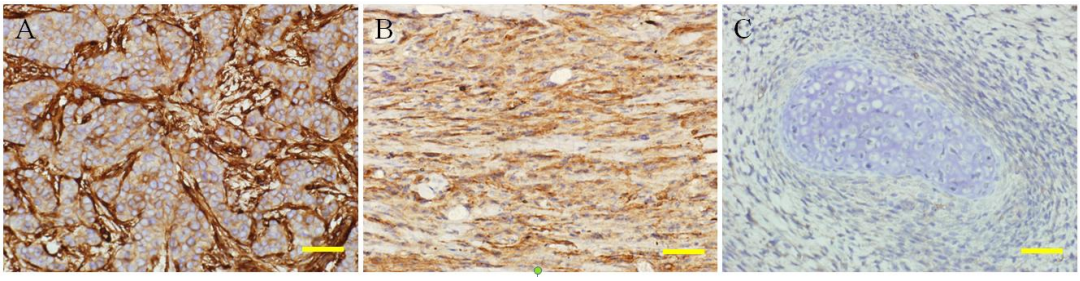
**S6**




**S6.** Immunohistochemical staining shows the expression of SCX in tendons engineered by DPSCs at 14 weeks. The engineered tendon was transplanted into the mice with (Loaded) and without (Non-Loaded) mechanical loading. (A, B) Images of negative control of the tendons engineered at 14 weeks post-implantation. PBS was used as replacer for anti-SCX antibody. (C, D) Images of the tendons engineered at 14 weeks post-implantation. Original magnification: X400; Bar=50 μm. W=week. (E, F) Higher magnification of the palatal side of C&D (The arrows showed some collagens without immunostaining of SCX).




**S7.** Immunohistochemical staining shows the expression of Eya2 in tendons engineered by DPSCs at 14 weeks. The engineered tendon was transplanted into the mice with (Loaded) and without (Non-Loaded) mechanical loading. (A, B) Images of negative control of the tendons engineered at 14 weeks post-implantation. PBS was used as replacer for anti-Eya2 antibody. (C, D)Images of the tendons engineered at 14 weeks post-implantation. Original magnification: X400; Bar=50 μm. (E, F) Higher magnification of the palatal side of C&D.




S8. Immunohistochemical staining shows the expression of HLA Class I ABC in human lymph node, tendons engineered by DPSCs and fetal mouse cartilage. (A) HLA Class I ABC was strongly positive expression in the cytoplasm of human lymph node. (B) It's showed that the positive expression in the tendons engineered by DPSCs after 14 weeks post-implantation. (C) HLA Class I ABC-positive cells were not observed in peripheral areas of the fetal mouse cartilage. Original magnification: ×400; Bar=50 μm.