Supplementary Information for

Optimizing Tenogenic Differentiation of Equine Adipose-Derived Mesenchymal Stem Cells (eq-ASC) Using TGFB3 Along with BMP Antagonists

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Fig. S1: Concentration-dependent effect of GREM2 on tenogenic differentiation. A. eq-ASCs treated with 100 and 200 ng/ml GREM2 showed a little increase in expression of SCX in response to 200 ng/ml at day 3 while decreased compared to control group (1.3 vs. 2.8).

B. Morphological changes and qRT-PCR analysis for tenogenic-related genes (SCX, MKX, and COL1A1), and C. Osteogenic-related genes (CTNNB, RUNX2, BMPR2) of eq-ASCs in response to 200 ng/ml GREM2 on three-time points are shown (day 3, 7 and 10) (scale bar: 200 μm). Data were normalized to GAPDH and presented as mean ± SD.

*; Present significant changes vs. day 0 untreated cells (dashed line), P<0.05 and qRT-PCR; Real-time polymerase chain reaction.
Table S1: Culture medium composition for induction of tenogenesis in eq-ASCs

| Medium | Description |
|--------|-------------|
| Ctrl   | Basic medium: DMEM-high glucose, 10% FBS, 1% P/S          |
| T      | Basic medium+TGF-β3 (2.5 ng/ml)                          |
| T/G    | Basic medium+TGF-β3 (2.5 ng/ml)+GREM2 (200 ng/ml)        |
| T/G/S  | Basic medium+TGF-β3 (2.5 ng/ml)+GREM2 (200 ng/ml)+SOST (1250 ng/ml) |

Ctrl; Control, T; Treatment with TGF-β3 (2.5 ng/ml) for one day, T/G; Pretreatment with TGF-β3 (2.5 ng/ml) followed by GREM2 (200 ng), and T/G/S; Pretreatment with TGF-β3 (2.5 ng/ml) followed by GREM2 (200 ng/ml) and SOST (1250 ng/ml).

Table S2: Primer sequences which were used for real-time polymerase chain reaction

| Gene  | Accession Number | Primer sequence (5´-3´) Amplicon length (bp) |
|-------|------------------|-----------------------------------------------|
| GAPDH | NM_001163856.1   | F: GTGCTGAATATGTTGTGGAGT R: AGAAGGAGCAGAGATGATGAC 104 |
| SCX   | NM_001105150.1   | F:GAACGCCCAGGCAAACCA R:CATCCGCTCTAATCCGAATC 103 |
| MKX   | XM_014737017.1   | F:AATAATCCGTTTCACCATCCTG R:TTTGCTTTGCTTTCCATCAT 196 |
| COL1A1| XM_023652710.1   | F:CGGGTGTGGGAGAAAGTACG R:ACGAGGTAGTCTTTCCAGCAAC 140 |
| TNMD  | NM_001081822.1   | F:TCCTCACTCCCTCAACG R:AATAACCTCCTCTCAGCCAAC 179 |
| SOX9  | XM_014736619.1   | F:ATTTCAAGACAGCAACTCG R:ACACGGTTTCTCCATCT 157 |
| CTNNB1| NM_001122762.1   | F:ACTGTTCCTCGTGCTGGTAC R:AGTGTTAGGGGATGATTAGGA 163 |
| BMPR2 | XM_014732300.1   | F:GACCTCTGCTACTCTAATCC R:TCGGTCAAGGGTAAAT 158 |
| RUNX2 | XM_005603968.2   | F:ACGCATTCTACCTATGTCAG R:GGTGAAAGCAGACTAAAGGAC 133 |