

***Secreted frizzled related-protein 2 (Sfrp2) deficiency decreases adult skeletal stem cell function in mice***

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**Supplementary Materials**

**Supplementary Table 1 - Primers used for qRT-PCR**

| Gene             | Primer                                 |
|------------------|----------------------------------------|
| <i>Axin2</i>     | Forward 5'-CCGATTGCTGAGAGGAACTGGAAG-3' |
|                  | Reverse 5'-GCTGCTGCTGGGATCTGGAAGG-3'   |
| <i>C-myc</i>     | Forward 5'-TCCCACCCCGCCCCTGTC-3'       |
|                  | Reverse 5'-CCACCGCCGCCGTCATCG-3'       |
| <i>Cyclin D1</i> | Forward 5'-GCGTGGTGGCTGCGATGC-3'       |
|                  | Reverse 5'-GAAAGAAAGTGCGTTGTGCGGTAG-3' |
| <i>GAPDH</i>     | Forward 5'-TGCACCACCAACTGCTTAGC-3'     |
|                  | Reverse 5'-GGCATGGACTGTGGTCATGAG-3'    |
| <i>Osterix</i>   | Forward 5-AGCACCAATGGACTCCTCTC-3       |
|                  | Reverse 5-GGGTGGGTAGTCATTTGCAT-3       |
| <i>Runx2</i>     | Forward 5'-CGCCCCTCCCTGAACTCT-3'       |
|                  | Reverse 5'-TGCCTGCCTGGGATCTGTA-3'      |
| <i>Sfrp1</i>     | Forward 5'-CTAGTGGCCTTGGATTGACC-3      |
|                  | Reverse 5'-GTTGGCATGGTGAGTTTTCA-3      |

|              |                                         |
|--------------|-----------------------------------------|
| <i>Sfrp2</i> | Forward 5'-AAACCCTTTGTAAAAATGACTTCG-3'  |
|              | Reverse 5'-CAGCTTGTAATGGTCTTGCTC-3'     |
| <i>Sfrp4</i> | Forward 5'-ACCTGAGCAAAAACACTACAGCTATG-3 |
|              | Reverse 5'-CTACCACAGTTGTGACCTCATTG-3    |

11 **Supplementary Table 2 – Sex, age and number of animals or culture replicates used per**  
12 **experiment**

| In vivo | Assay                               | Figure          | Sex                     | Age   | Genotype | N    |     |   |
|---------|-------------------------------------|-----------------|-------------------------|-------|----------|------|-----|---|
|         | X-ray and $\mu$ CT characterization | 1A, SF 1, SF 2A | Males                   | 3 m   | +/+      | 6    |     |   |
|         |                                     |                 |                         |       | -/-      | 8    |     |   |
|         |                                     |                 |                         | 11 m  | +/+      | 7    |     |   |
|         |                                     |                 |                         |       | -/-      | 6    |     |   |
|         |                                     |                 | Females                 | 3 m   | +/+      | 5    |     |   |
|         |                                     |                 |                         |       | -/-      | 6    |     |   |
|         |                                     |                 |                         | 11 m  | +/+      | 5    |     |   |
|         |                                     |                 |                         |       | -/-      | 5    |     |   |
|         |                                     |                 | Calvarial TRAP staining | SF 2B | Females  | 3 m  | +/+ | 3 |
|         |                                     |                 |                         |       |          |      | -/- | 3 |
|         |                                     |                 | Knee joint H&E          | 1B,C  | Males    | 3 m  | +/+ | 5 |
|         |                                     |                 |                         |       |          |      | -/- | 5 |
|         | 11 m                                | +/+             |                         |       |          | 3    |     |   |
|         |                                     | -/-             |                         |       |          | 3    |     |   |
|         | Females                             | 3 m             |                         |       | +/+      | 2    |     |   |
|         |                                     |                 |                         |       | -/-      | 2    |     |   |
|         |                                     | 11 m            |                         |       | +/+      | 4    |     |   |
|         |                                     |                 |                         |       | -/-      | 5    |     |   |
|         | Drilled defect regeneration         | 3               |                         |       | Males    | 12 m | +/+ | 4 |
|         |                                     |                 |                         |       |          |      | -/- | 4 |
|         |                                     |                 | Females                 | +/+   | 2        |      |     |   |
|         |                                     |                 |                         | -/-   | 2        |      |     |   |
|         | BMSC ectopic bone formation         | 2B-E            | Males                   | 6 w   | +/+      | 6    |     |   |
|         |                                     |                 |                         |       | -/-      | 5    |     |   |

| <i>In vitro</i> | Assay                                                                   | Figure  | Replicates (N) | Experiment (N) |
|-----------------|-------------------------------------------------------------------------|---------|----------------|----------------|
|                 | CFE, <i>Sfrp 1</i> , 2 and 4 expression in in <i>Sfrp2</i> KO BMSCs     | 2A, SF4 | 3              | 3              |
|                 | Osteogenesis in <i>Sfrp2</i> KO BMSCs and <i>Sfrp2-siRNA</i> DM-5 cells | 4       |                |                |
|                 | Wnt pathway in <i>Sfrp2</i> KO BMSCs and <i>Sfrp2-siRNA</i> DM-5 cells  | 5       |                |                |

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## Supplementary Figure Legend

### **Supplementary Fig. 1. $\mu$ CT analysis of femora of *WT* and *Sfrp2* KO littermates show no**

**significant differences.**  $\mu$ CT parameters of distal trabecular bone and mid-diaphyseal cortical bone measured in young (3 months) and old (11 months) males and females. Tb BV/TV (%) = Trabecular Bone Volume/Total Volume (%), Tb.Th = Trabecular Thickness, Tb.N = Trabecular Number, Tb.Sp = Trabecular Separation, C.Po = Cortical Porosity, C.Th = Cortical Thickness. Individual values of 5 or more are shown as white or black circles and blue bars represent the mean.

### **Supplementary Fig. 2. Skulls from *WT* and *Sfrp2* KO littermates show no significant**

**anatomical differences, or differences in osteoclastic activity.** *A* Representative  $\mu$ CT reconstructions of the skull of 4-month-old male mice showing no anatomical differences. *B* Representative photographs of TRAP staining of 3-month-old female calvariae show no increase in TRAP staining in the sutures between *WT* and *Sfrp2* KO mice.

### **Supplementary Fig. 3. Transfection of immortalized DM5 cells with *Sfrp2*-siRNA**

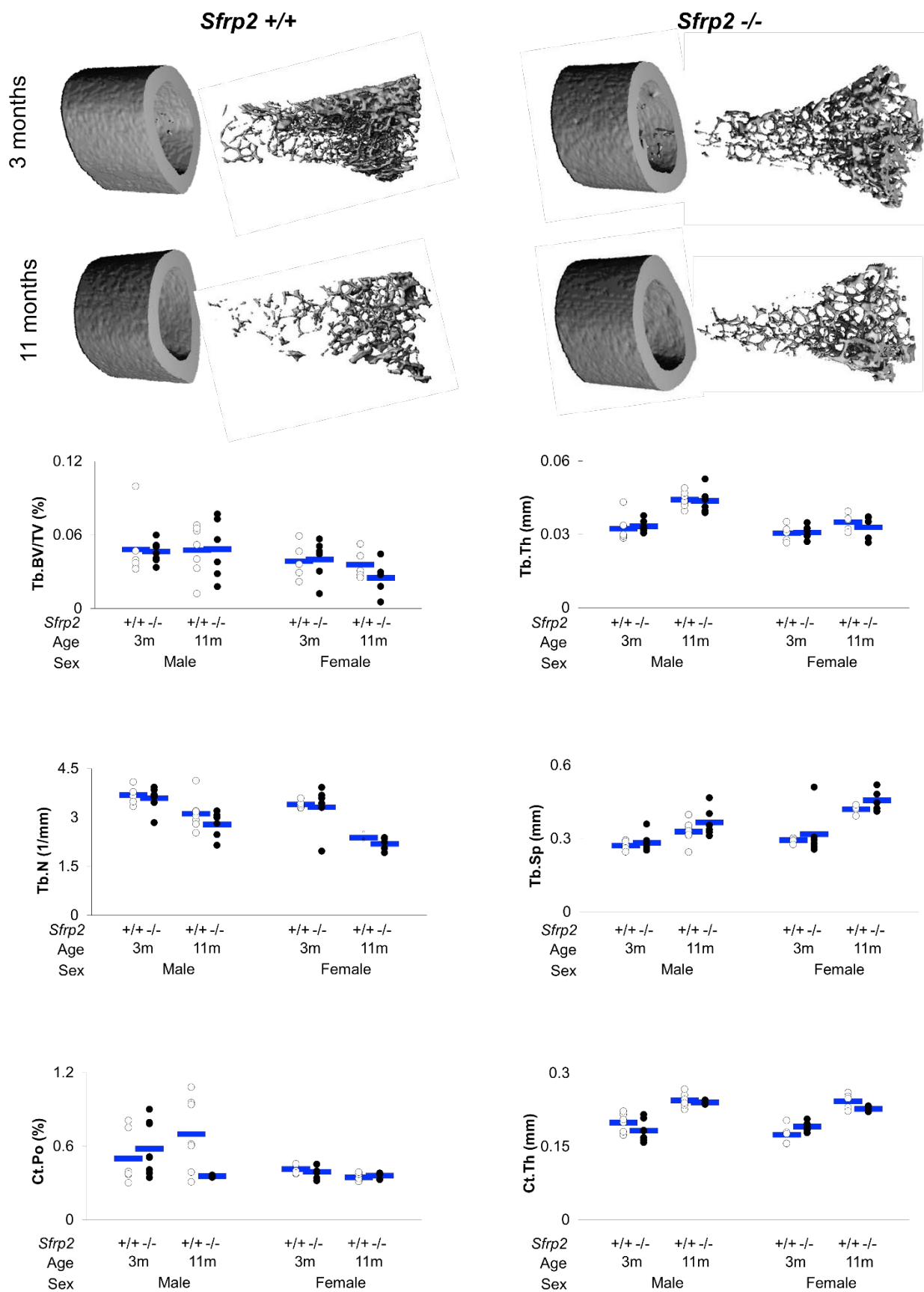
**successfully decreases *Sfrp2* RNA levels for at least 7 days.** qRT-PCR was used to assess the relative expression of *Sfrp2*. *Gapdh* was used as housekeeping gene for normalization.

\*\*p<0.01, \*\*\*p<0.001 vs. untreated control.

### **Supplementary Fig. 4. *Sfrp2*-deficient BMSCs/SSCs exhibit an increased expression of**

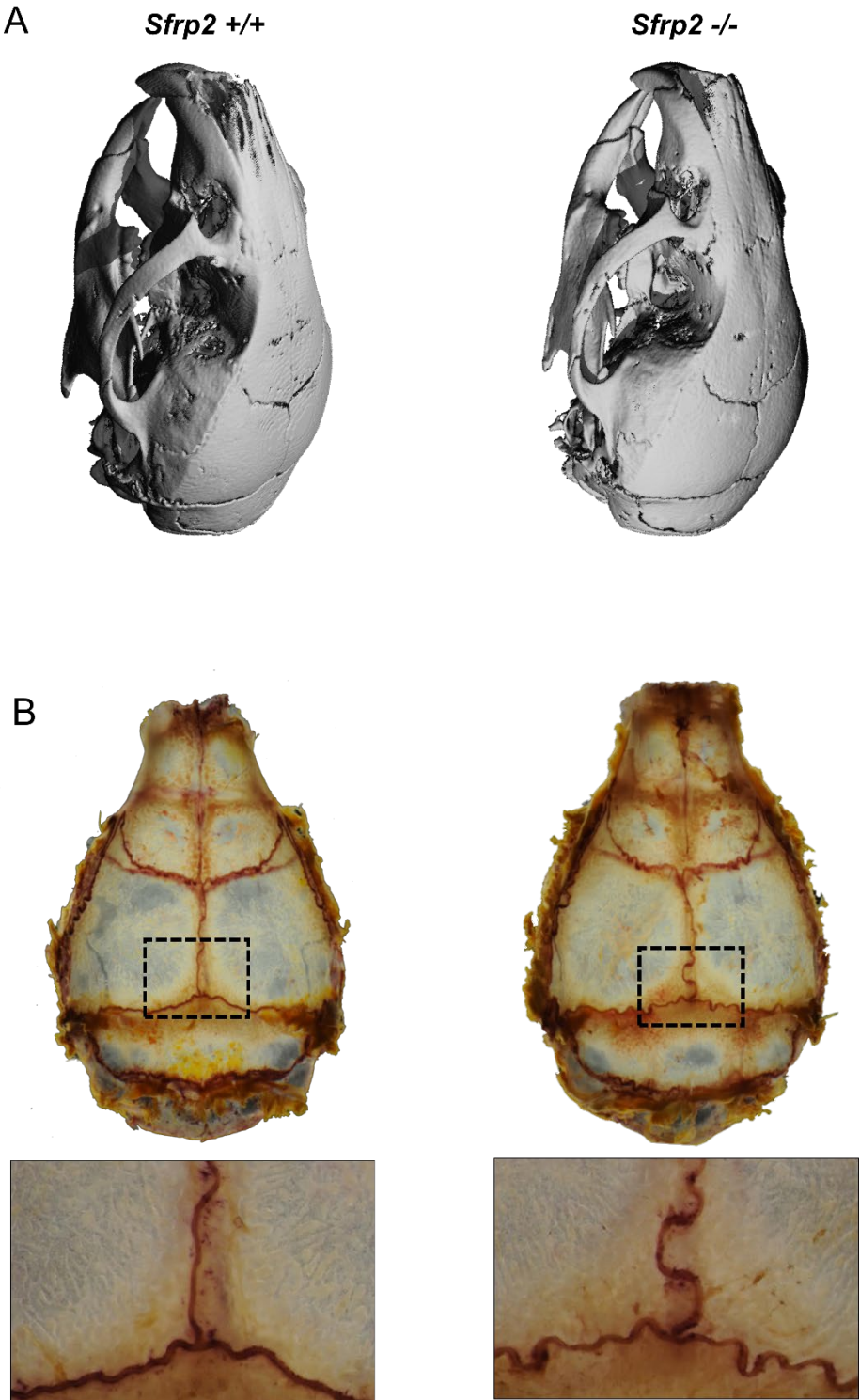
***Sfrp1* and *Sfrp4*.** Expression levels of *Sfrp1*, *Sfrp2* and *Sfrp4* in BMSC/SSC cultures from *Sfrp2* KO and *WT* mice after 15 days of culture. \*p<0.05, \*\*p<0.01, \*\*\*p<0.001.

## Supplementary Fig. 1

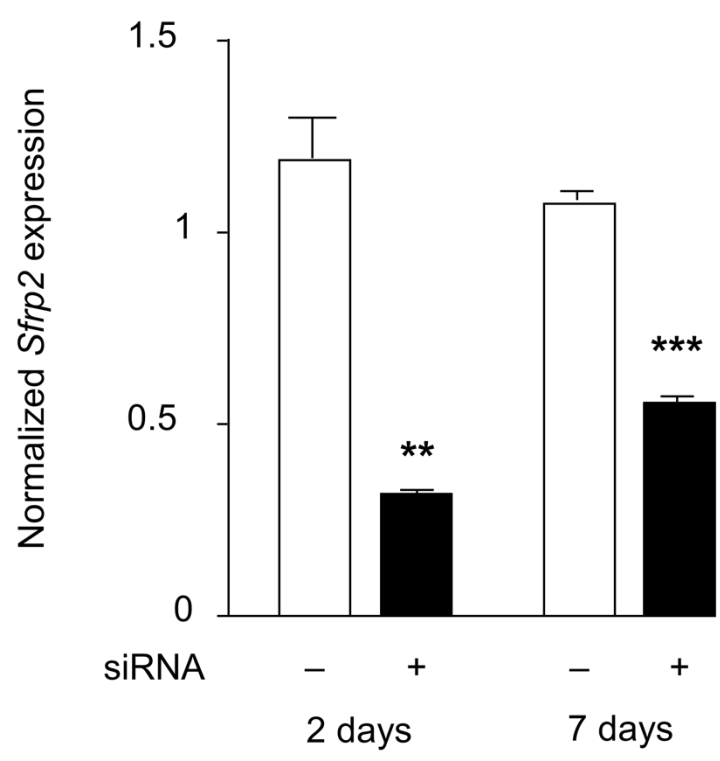


Supplementary Fig. 2

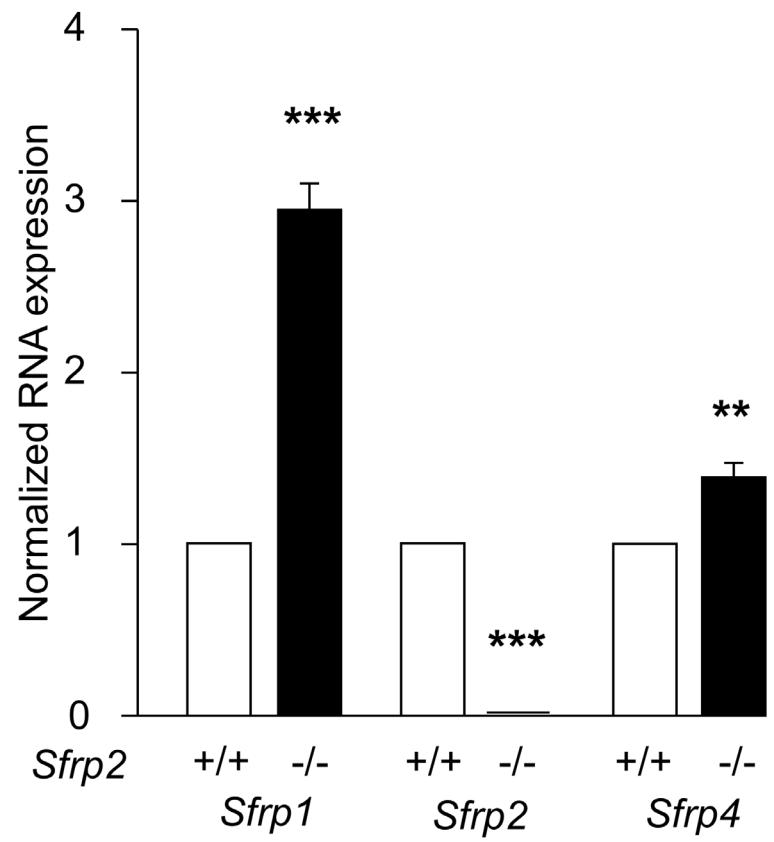
3 month



Supplementary Fig. 3



## Supplementary Fig. 4



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