

## SUPPLEMENTARY INFORMATION FOR

### **ER $\alpha$ /KDM6B Regulatory Axis Modulates Osteogenic Differentiation in Human Mesenchymal Stem Cells**

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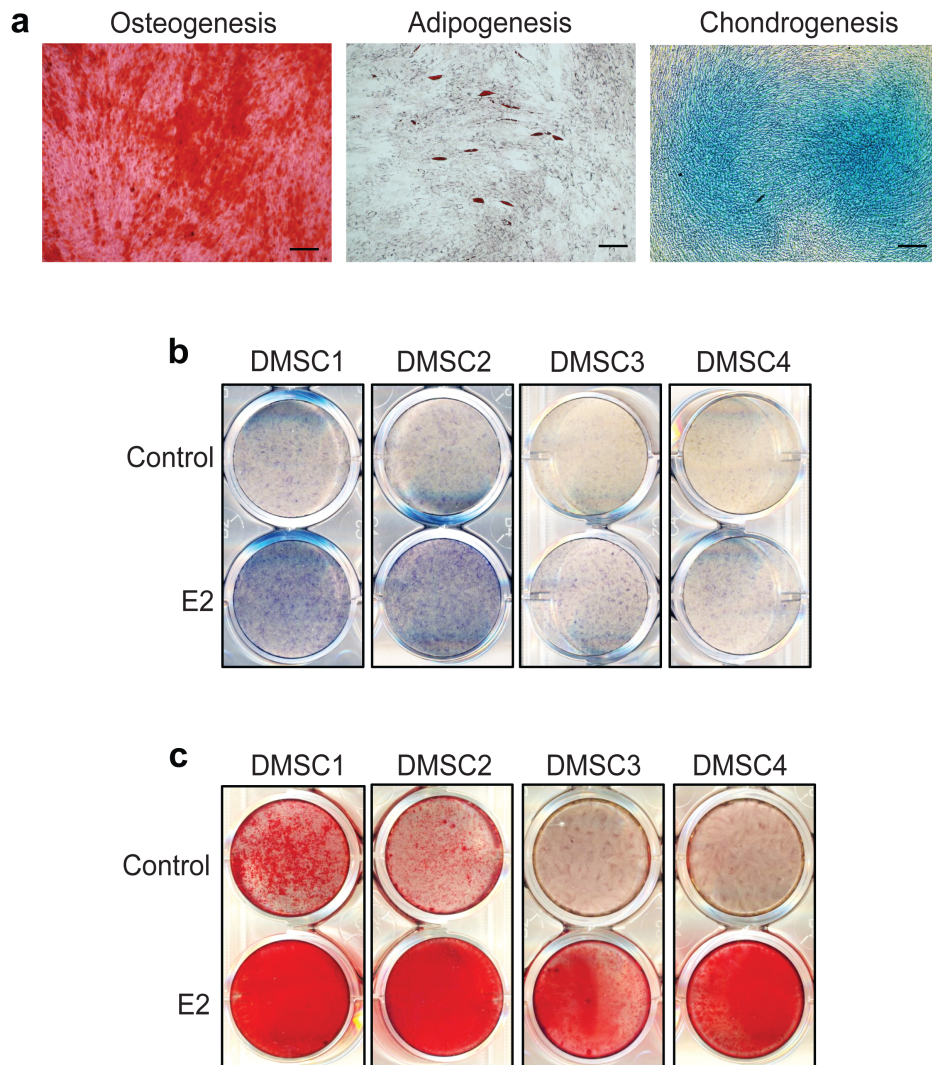
## Supplementary Methods

### Multi-lineage differentiation potential of DMSCs

DMSCs at passage 5-7 ( $6 \times 10^4$  cells) were seeded into 24-well plates. For osteogenic differentiation, DMSCs were differentiated in  $\alpha$ -MEM with 10% FBS containing 100  $\mu$ M ascorbic acid, 2 mM  $\beta$ -glycerophosphate, and 10 nM dexamethasone. for 14 days. Differentiated cells were fixed in 10% Neutral buffered formalin for 10 min and stained with 2% Alizarin Red solution for 20 min at room temperature to visualize calcium deposits. For adipogenic differentiation, DMSCs were differentiated in  $\alpha$ -MEM with 10% FBS containing 0.5 mM isobutylmethylxanthine, 0.5  $\mu$ M hydrocortisone, and 60  $\mu$ M indomethacin (Sigma-Aldrich) for 21 days. Differentiated cells were fixed in 10% Neutral buffered formalin for 10 min and stained with 0.5% Oil Red O solution for 15 min at room temperature to detect lipid vacuoles. For chondrogenic differentiation, DMSCs were differentiated in  $\alpha$ -MEM with 10% FBS containing 100 mM sodium pyruvate, 40  $\mu$ g/ml proline, 100 nM dexamethasone, 200  $\mu$ M ascorbic acid (all from Sigma-Aldrich), and 10 ng/mL TGF- $\beta$  (R&D systems) for 21 days. Differentiated cells were fixed in 10% Neutral buffered formalin for 10 min and stained with 1% Alcian Blue 8GX solution (Sigma-Aldrich) for 30 min at room temperature to observe the proteoglycan content. The differentiation assay was performed two times with triplicated samples.

Supplementary Fig. 1

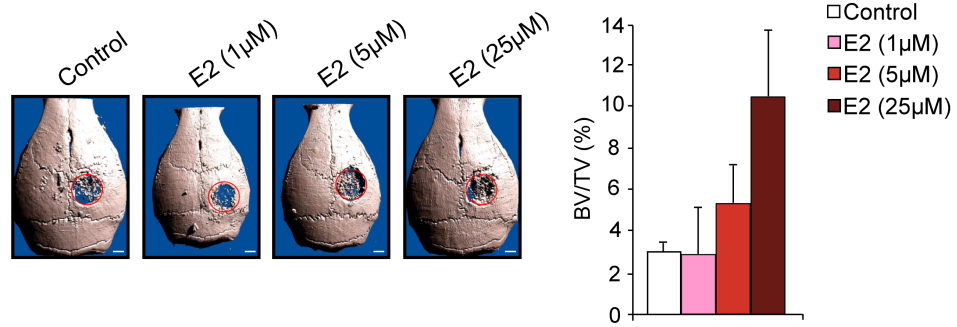
Supplementary Fig. 1



**Supplementary Figure 1.** E2 increases osteogenic differentiation on DMSCs. **a** Multipotency assessment of DMSCs. Left: Alizarin Red S staining after 14 days treatment of osteogenic media; Middle: Oil red O staining after 21 days treatment of adipogenic media; Right: Alcian blue staining after 21 days treatment of chondrogenic media. Scale bar: 100 $\mu$ m. **b** Alkaline phosphatase staining and quantification after 5 days treatment of osteogenic media with and without E2 on DMSCs from four different donors. **c** Alizarin Red S staining and quantification after 14 days treatment of osteogenic media with and without E2 on DMSCs from four different donors.

Supplementary Fig. 2

Supplementary Fig. 2



**Supplementary Figure 2.** E2 increased DMSC bone formation *in vivo*. Micro-CT images and bone volume quantification (BV/TV) of calvarial bone defects with scaffolds containing DMSCs with different concentration of E2 (1µM, 5µM, 25µM) and control. Data are presented as means  $\pm$  SEMs ( $n = 3$ ). Scale bars, 1mm.

### Supplementary Table 1

The primers for epigenetic regulator genes are as follows:

| Genes  | Forward                  | Reverse                 |
|--------|--------------------------|-------------------------|
| KDM2A  | CAAGGAGAGTGTGGTGTGTTGCC  | ACCTCTCCACAGAGGGAACATG  |
| KDM2B  | CATGGAGTGCTCCATCTGCAATG  | ACTTCGGACACTCCCAGCAGTT  |
| KDM3A  | GCCAACATTGGAGACCACTTCTG  | CTCGAACACCTTTGACAGCTCG  |
| KDM3B  | GCTCGTAATGTCTGAGAAGGAGG  | CACATTTGCGACAAACCCAGTGG |
| KDM3C  | TCCTGTCAGACCTTCCAGTGCA   | GTGGATGCAACAGACCGTAATGG |
| KDM4A  | TGCGGCAAGTTGAGGATGGTCT   | GCTGCTTGTTCCTCCTCATC    |
| KDM4B  | GCCGAGAGGAAGTTCAACGCAG   | TGCCTCCTTCTCAGTCTGTAGG  |
| KDM4C  | CCGATGACTCTTGTGAAGCAGC   | GACTTCGTCTGCCAAAGGTGGA  |
| KDM4D  | CCTGAACGCTATGACCTGTGGA   | TCTCCTGGGTAACCTGGACTTCC |
| KDM5A  | GCTAAGGTCTGCCTACAGGCAA   | CCACTTTAGCGGTCCATTCTCG  |
| KDM5B  | AGCCAGAGACTGGCTTCAGGAT   | AGCCTGAACCTCAGCTACTAGG  |
| KDM5C  | ACTGCTGACCATTGCTGAACGC   | CCTCCTTGAGAGCCTGGATGTT  |
| KDM5D  | GGCTGAGTCTTTTGACACCTGG   | CAGGCAGTTCCTCAGTCGCTGA  |
| KDM6A  | AGCGCAGAGGAGCCGTGGAAAA   | GTCGTTACCATTAGGACCTGC   |
| KDM6B  | CCTCGAAATCCCATCACAGT     | GTGCCTGTCAGATCCCAGTT    |
| KDM6C  | GAGAAAACAGCTATCTAACTCCGC | CAGTGCTAGTTGCCTGGTGATAC |
| KDM7A  | CGGTGGAACCTTCAGTCTGGTAC  | CTCCAAAGAACACCTCACTCTGG |
| KDM7B  | GGACACATACAGTCATCAGGCAC  | GGCTCTCATTTCCATCAAGGTCC |
| KDM7C  | TGCGTGAAGGACAGTTACACCG   | CATCTCGCTGTGGTTAGAGGCA  |
| HR     | TCTGCGAACTGCTGGCTTCTAC   | GTGCGATAATGCTGTCCAGGATG |
| JARID2 | GGACAAAGGCGTCCTCAATGAC   | GCAGGCTCCTTGCTGAAACACA  |
| JMJD4  | ACTTCTCGTCCGACTGGCTGAA   | AGCTGAAGGAGCGGAAGATGTC  |
| JMJD5  | CACAGATGAGGAATGGTCCCAG   | GCTGATGTCCTGCTTCAACTCC  |
| JMJD6  | CCAACTTCCCTGTGGTATGGCA   | TCCTGAAGGTCAACCGAGTCTG  |
| JMJD7  | GGAGTCCTCTATGTGCAGAAGC   | CAGCCAGAAGTTCACAGCATCG  |