

RESEARCH ARTICLE

Hydroxyapatite/BMP-2–mineralized decellularized amniotic membrane scaffolds for orbital defect repair

Supplementary file
Table S1. Primer sequences

Gene	Primer sequences
mouse <i>Gapdh</i> F	TGCCCCATGTTTGTGATG
mouse <i>Gapdh</i> R	TGTGGTCATGAGCCCTTC
mouse <i>Fgfr2</i> F	GTCTCCGAGTATGAGTTGCCAG
mouse <i>Fgfr2</i> R	CCACTGCTTCAGCCATGACTAC
mouse <i>Fgfr3</i> F	ACAGGTGGTCATGGCAGAAGCT
mouse <i>Fgfr3</i> R	CTCCATCTCAGATACCAGGTCC
mouse <i>Bmpr1a</i> F	ATCCGATGGCTGGTTGTGCTCA
mouse <i>Bmpr1a</i> R	CCAAATCACGGTTGTAACGACCC
mouse <i>Smad9</i> F	GAAAACACCAGGAGGCACATTGG
mouse <i>Smad9</i> R	GCTCTGGACAAAGATGCTGCTG
mouse <i>Wnt4</i> F	GAGAACTGGAGAAGTGTGGCTG
mouse <i>Wnt4</i> R	CTGTGAGAAGGCTACGCCATAG
mouse <i>Irs1</i> F	TGTCACCCAGTGGTAGTTGCTC
mouse <i>Irs1</i> R	CTCTCAACAGGAGGTTTGGCATG
mouse <i>Tgfb1</i> F	TGCTCCAAACCACAGAGTAGGC
mouse <i>Tgfb1</i> R	CCCAGAACTAAGCCATTGC
mouse <i>Fbxo32</i> F	CTTCTCGACTGCCATCCTGGAT
mouse <i>Fbxo32</i> R	TCTTTGGGCGATGCCACTCAG
mouse <i>Atm</i> F	CCAAGATGGCAGTGAACCAGAC
mouse <i>Atm</i> R	ATGCTGGACAGCTATGGTGGAG
mouse <i>Sirt1</i> F	GGAGCAGATTAGTAAGCGGCTTG
mouse <i>Sirt1</i> R	GTTACTGCCACAGGAACTAGAGG
mouse <i>Runx2</i> F	CCATCCATCCACTCCACCAC
mouse <i>Runx2</i> R	AGTTCTGAAGCACCTGCCTG
mouse <i>COL1</i> F	CGATGGATTCCCGTTTCGAGT
mouse <i>COL1</i> R	GAGGCCTCGGTGGACATTAG
mouse <i>OPN</i> F	GCTGCTGACCTGAAGAAGAC
mouse <i>OPN</i> F	CAGGCTTGTCACATCTCTGG

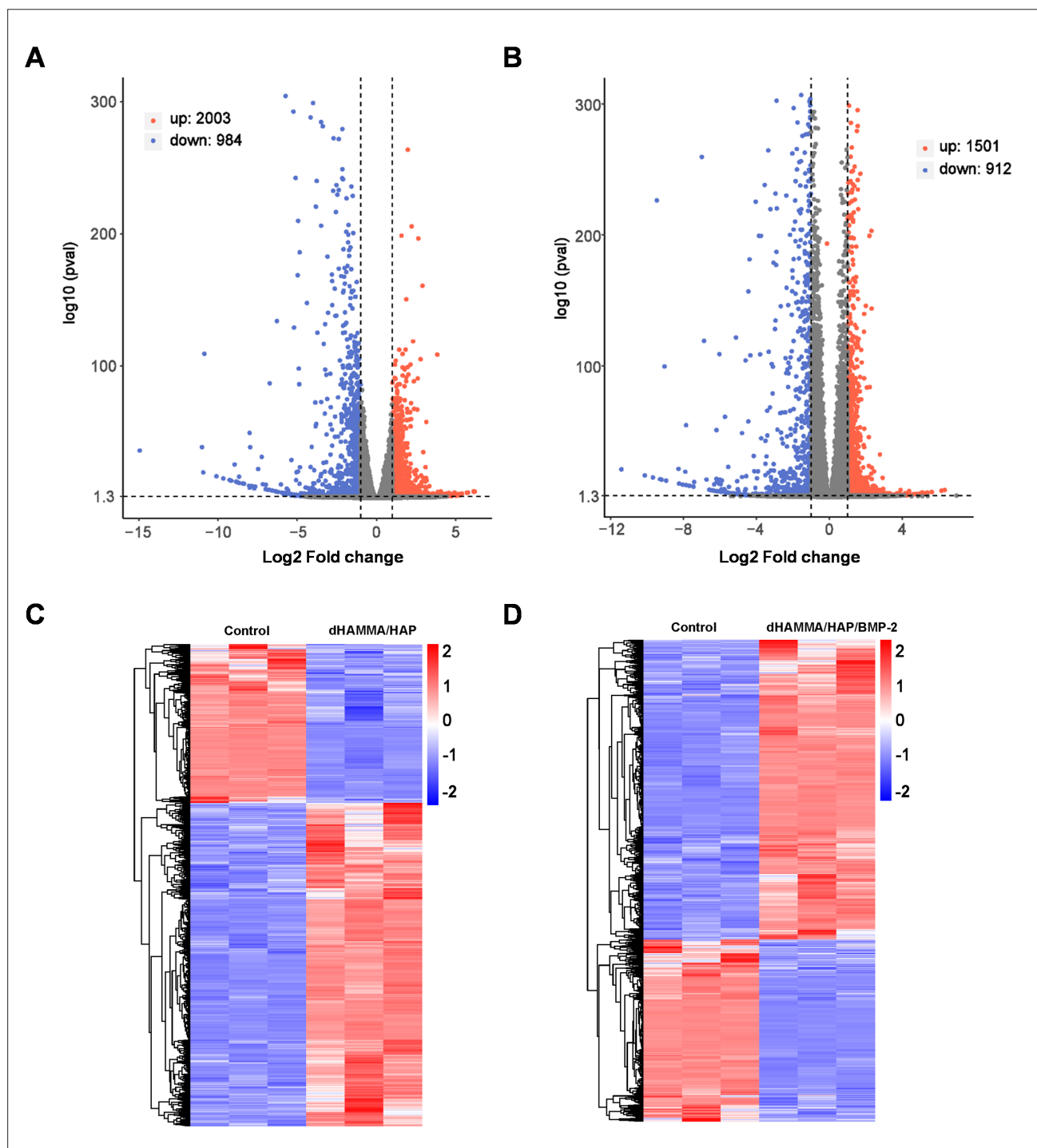


Figure S1. Transcriptome quantitative analysis of the osteogenesis mechanism. Volcano plot of DEGs between the control and (A) HAMMA/HAP group or (B) HAMMA/HAP/BMP-2 group. Heatmap of DEGs between the control and (C) HAMMA/HAP group or (D) HAMMA/HAP/BMP-2 group. Abbreviations: BMP-2, bone morphogenetic protein 2; HAMMA, methacrylate-functionalized human acellular amniotic membrane matrix; HAP, hydroxyapatite.

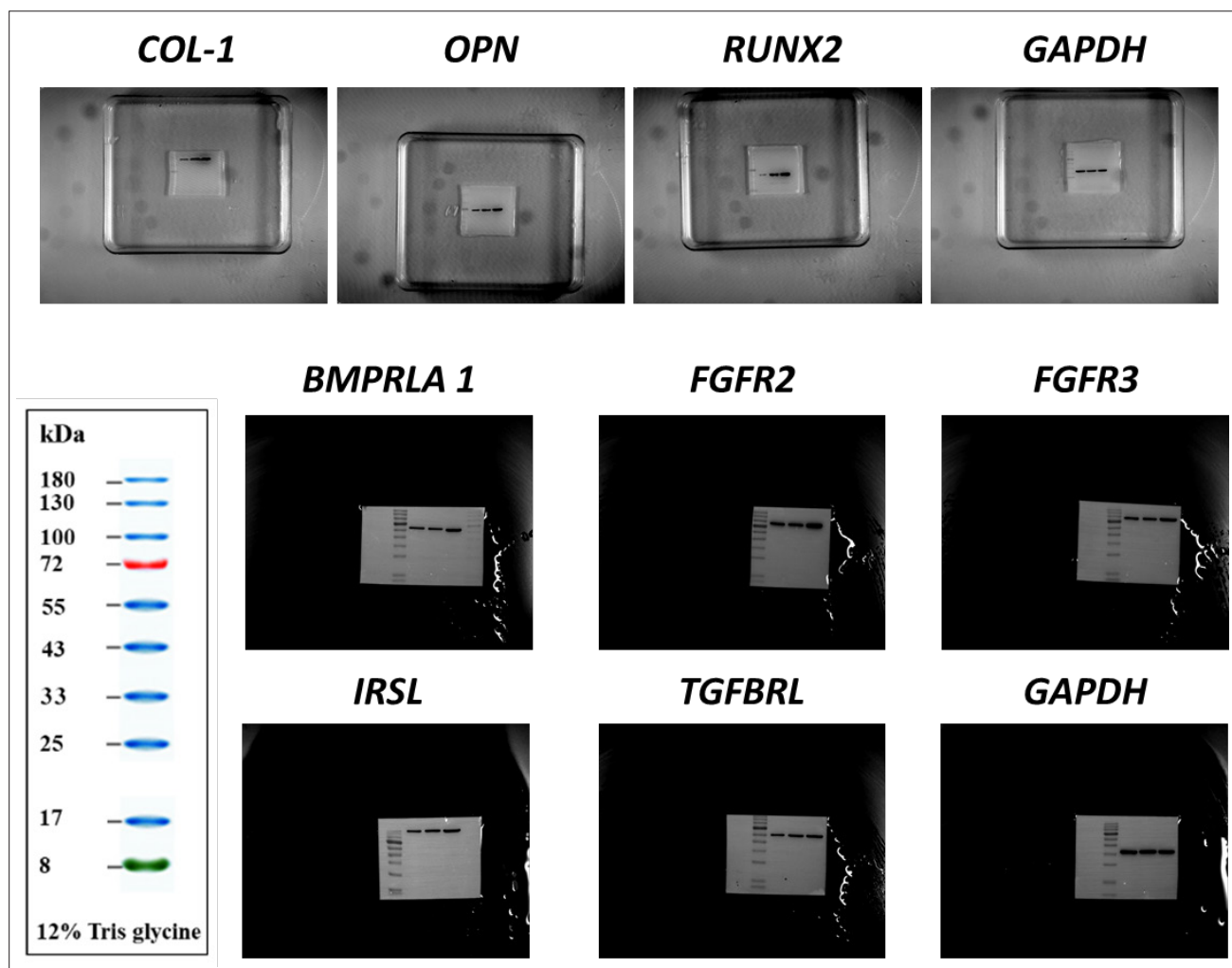


Figure S2. The raw data of Western blot of OPN, RUNX2, collagen type I (Coll-1), BMPRLA1, FGFR2, FGFR3, IRS1, TGFBR1, and GAPDH in **Figures 3 and 5**. Abbreviations: BMPRLA, bone morphogenetic protein receptor type 1A; FGFR2/3, fibroblast growth factor receptor 2/3; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; IRS1, insulin receptor substrate 1; OPN, osteopontin; TGFBR1, transforming growth factor beta receptor type 1.

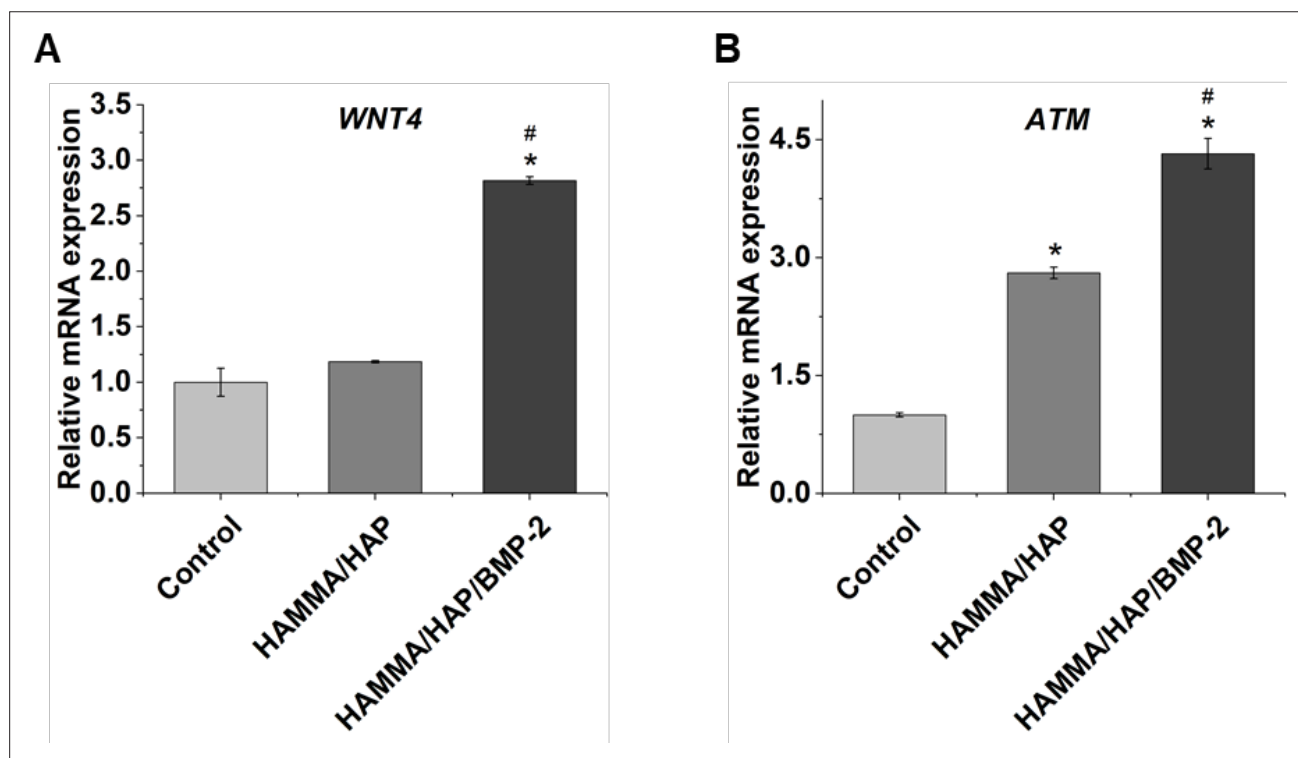


Figure S3. Real-time quantitative polymerase chain reaction analysis to assess the expression of differentially expressed genes (A) *Wnt4* and (B) *Atm*. Notes: $n=3$; * $p < 0.05$ compared to control; # $p < 0.05$ compared to HAMMA/HAP. Abbreviations: BMP-2, bone morphogenetic protein 2; HAMMA, methacrylate-functionalized human acellular amniotic membrane matrix; HAP, hydroxyapatite.

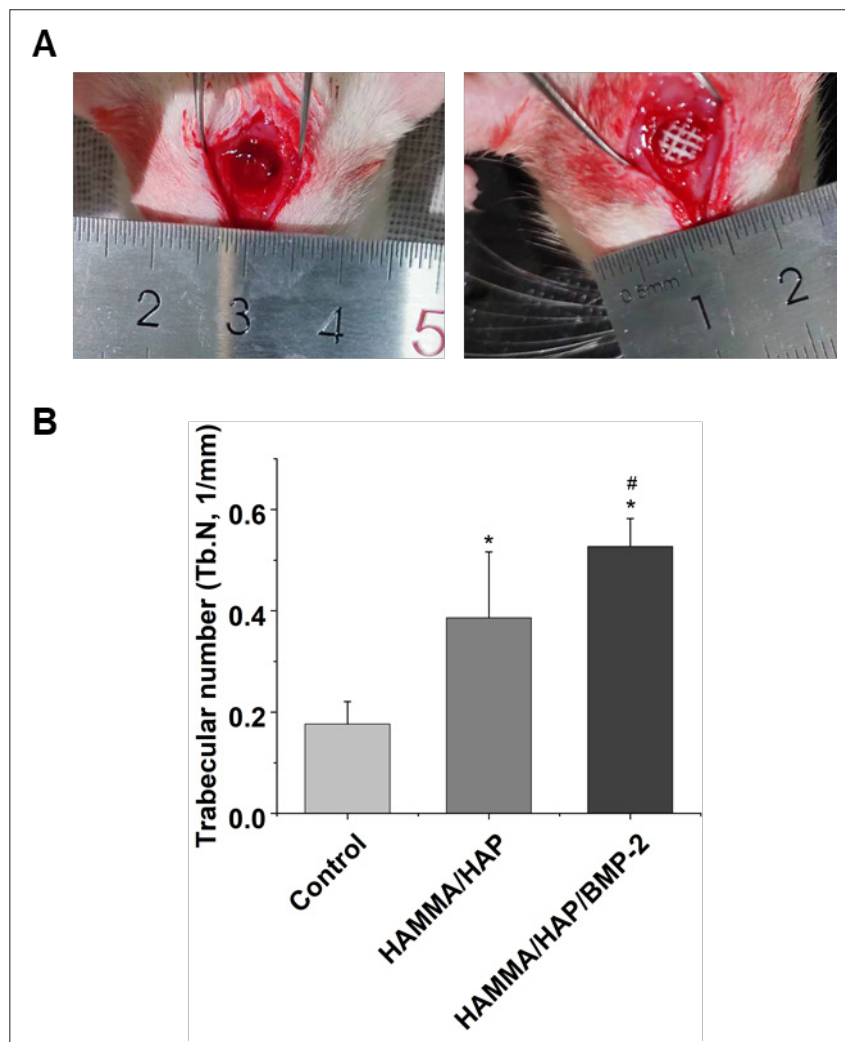


Figure S4. Enhanced boner egeneration by HAMMA/HAP scaffolds in a rat calvarial defect. (A) The rat calvarial defect and implantation of scaffolds. (B) Micro-computed tomography quantification of the calvarial defects. Abbreviations: BMP-2, bone morphogenetic protein 2; HAMMA, methacrylate-functionalized human acellular amniotic membrane matrix; HAP, hydroxyapatite.

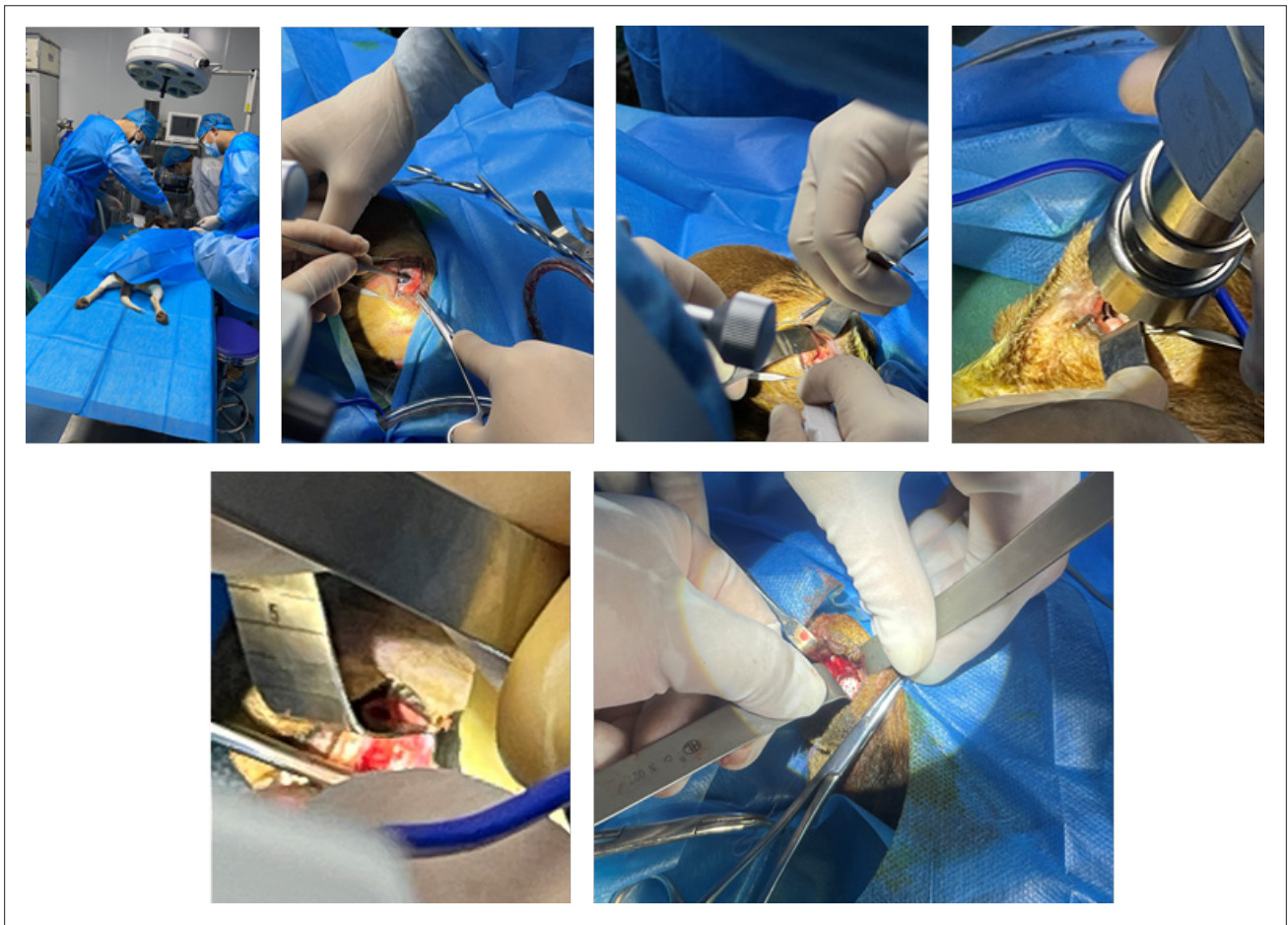


Figure S5. Establishment of orbital bone defects and implantation of scaffolds.

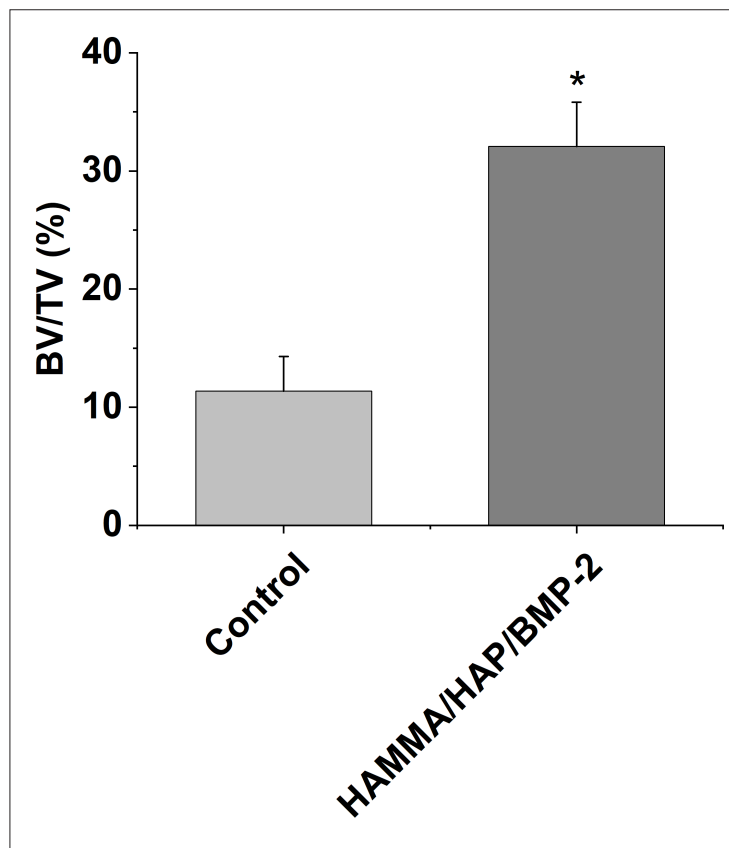


Figure S6. Micro-computed tomography quantification of the orbital bone defects. Notes: $n=3$; $*p < 0.05$ compared to control. Abbreviations: BMP-2, bone morphogenetic protein 2; BV/TV, bone volume/total volume; HAMMA, methacrylate-functionalized human acellular amniotic membrane matrix; HAP, hydroxyapatite.