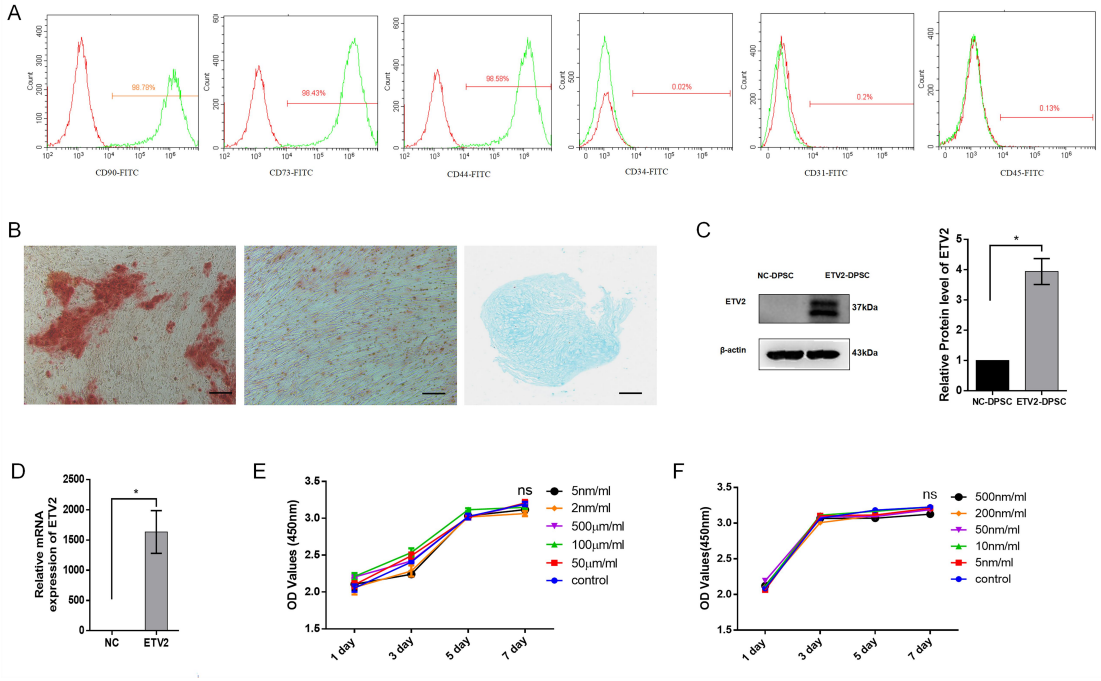
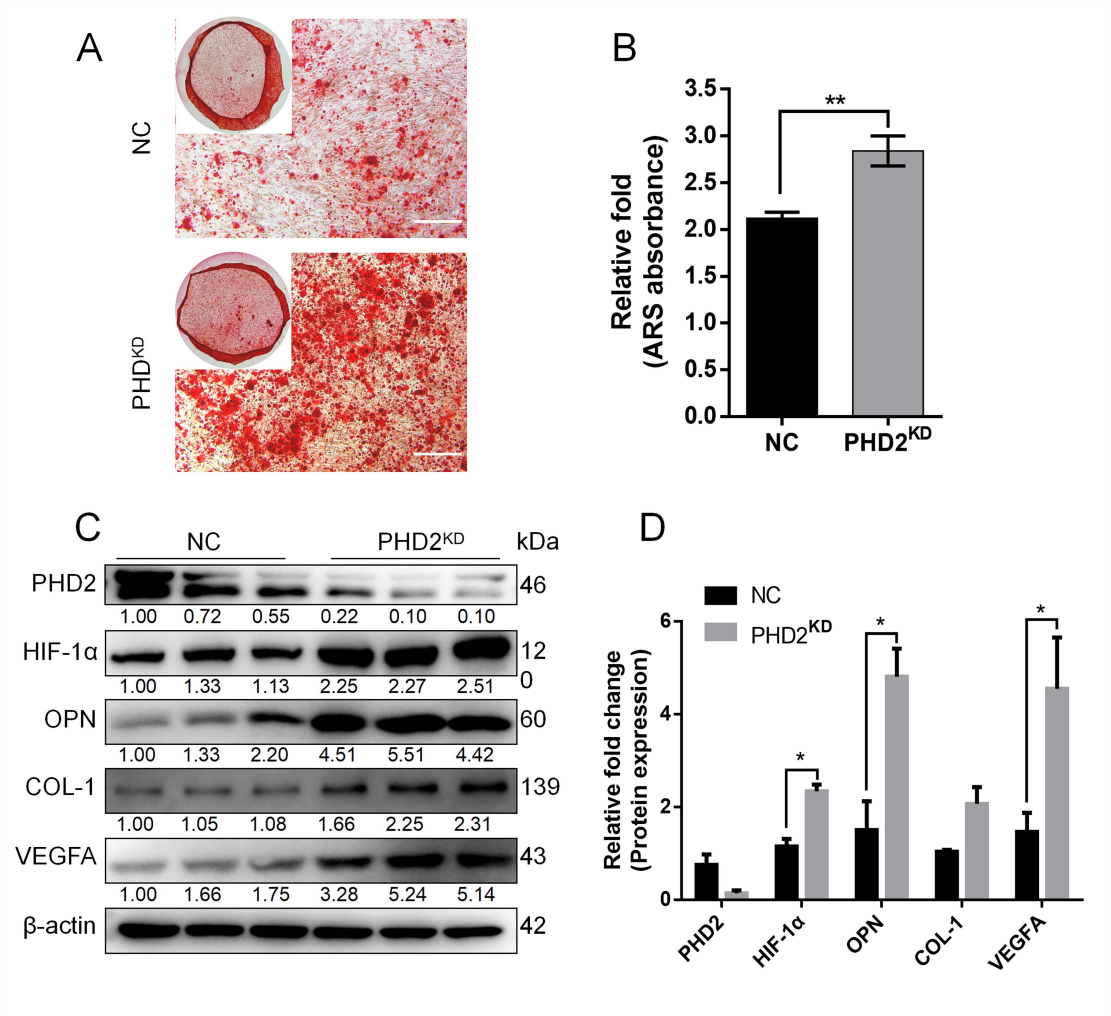
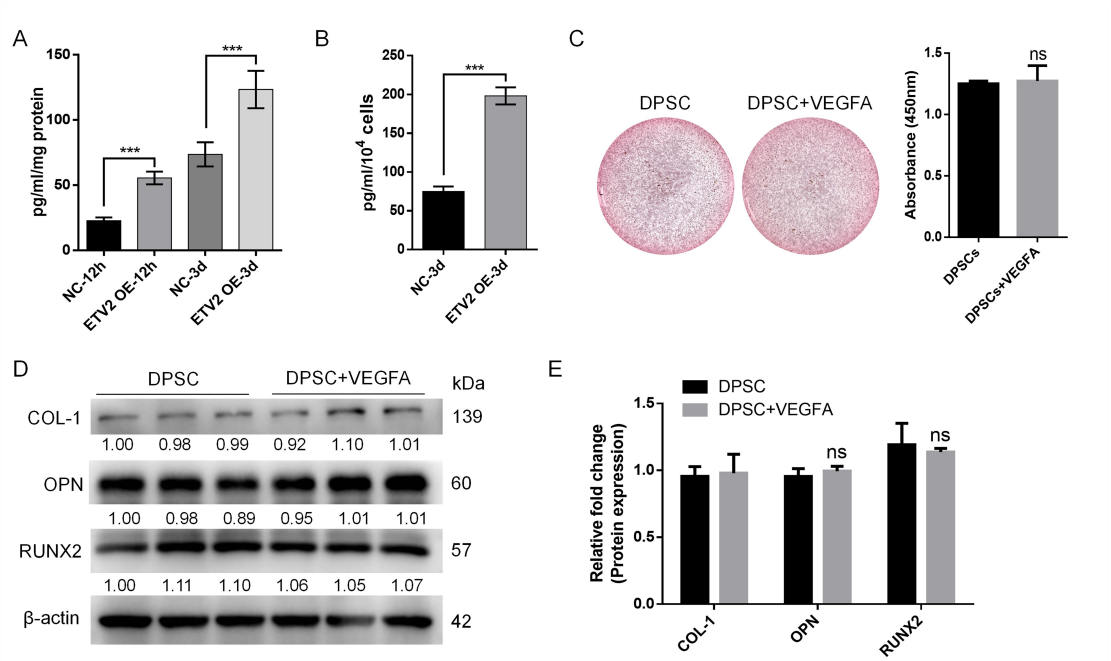
ETV2 Regulating PHD2-HIF-1α Axis Controls Metabolism Reprogramming Promotes Vascularized Bone Regeneration



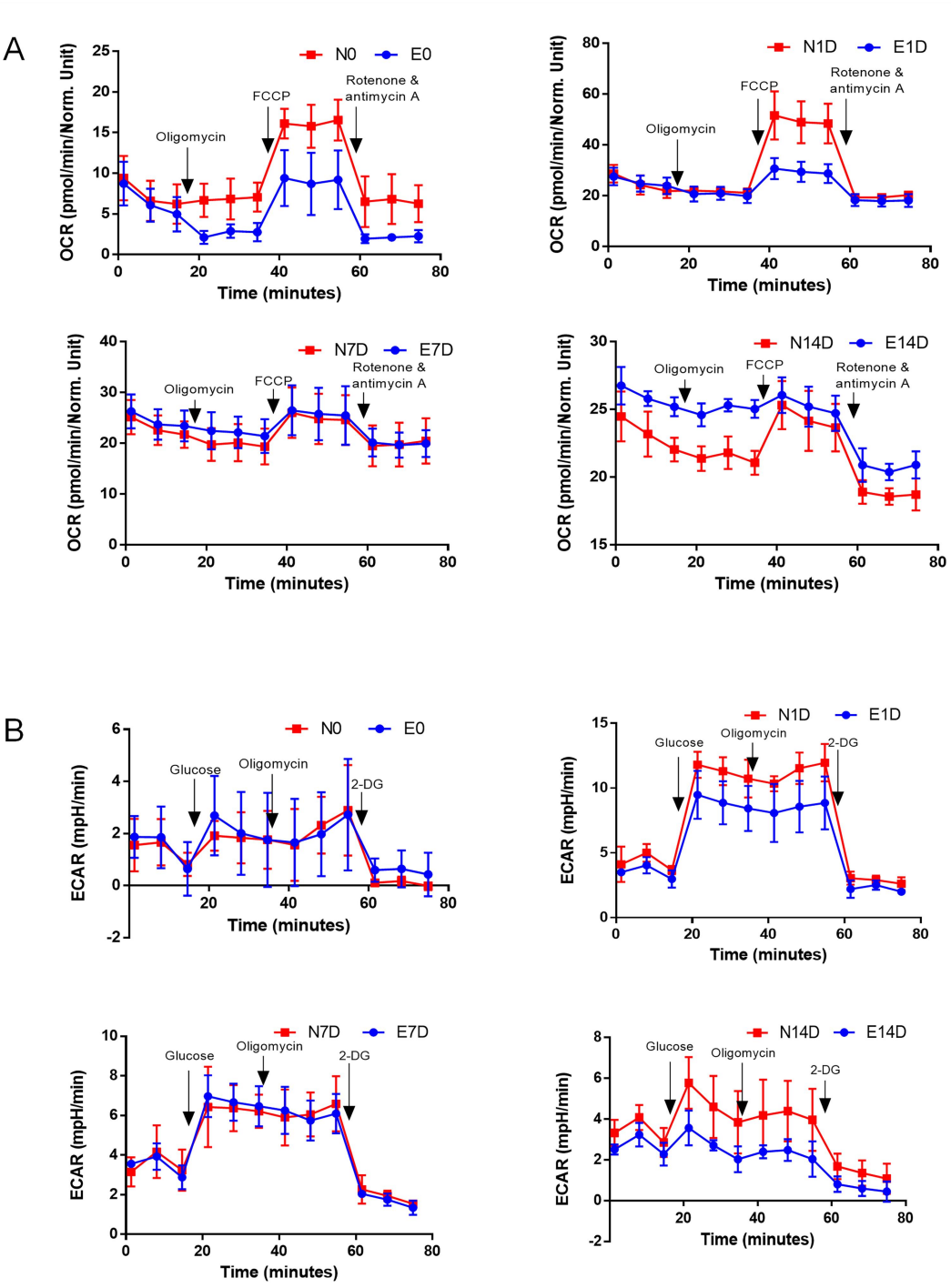
Supplementary Figure 1. (A) Flow cytometry was used to detect the expression of stem cell surface markers on DPSCs (B) Tri-lineage differentiation assays of DPSCs demonstrated the potential to differentiate into osteogenic, adipogenic, and chondrogenic lineages from left to right, as indicated. Scale bars: 200 μm (C, D) The protein and mRNA expression levels of ETV2 were assessed following ETV2 overexpression (E) The CCK-8 assay was performed on HUVECs treated with α-KG at various time points (1, 3, 5, and 7 days) (F) The CCK-8 assay was conducted on HUVECs treated with IOX4 at various time points (1, 3, 5, and 7 days) (NS, no significant difference, NC, negative control; OE, overexpression. Data are presented as the mean of >3 independent experiments ± SD. *P < 0.05, **P < 0.01, and ***P < 0.001).



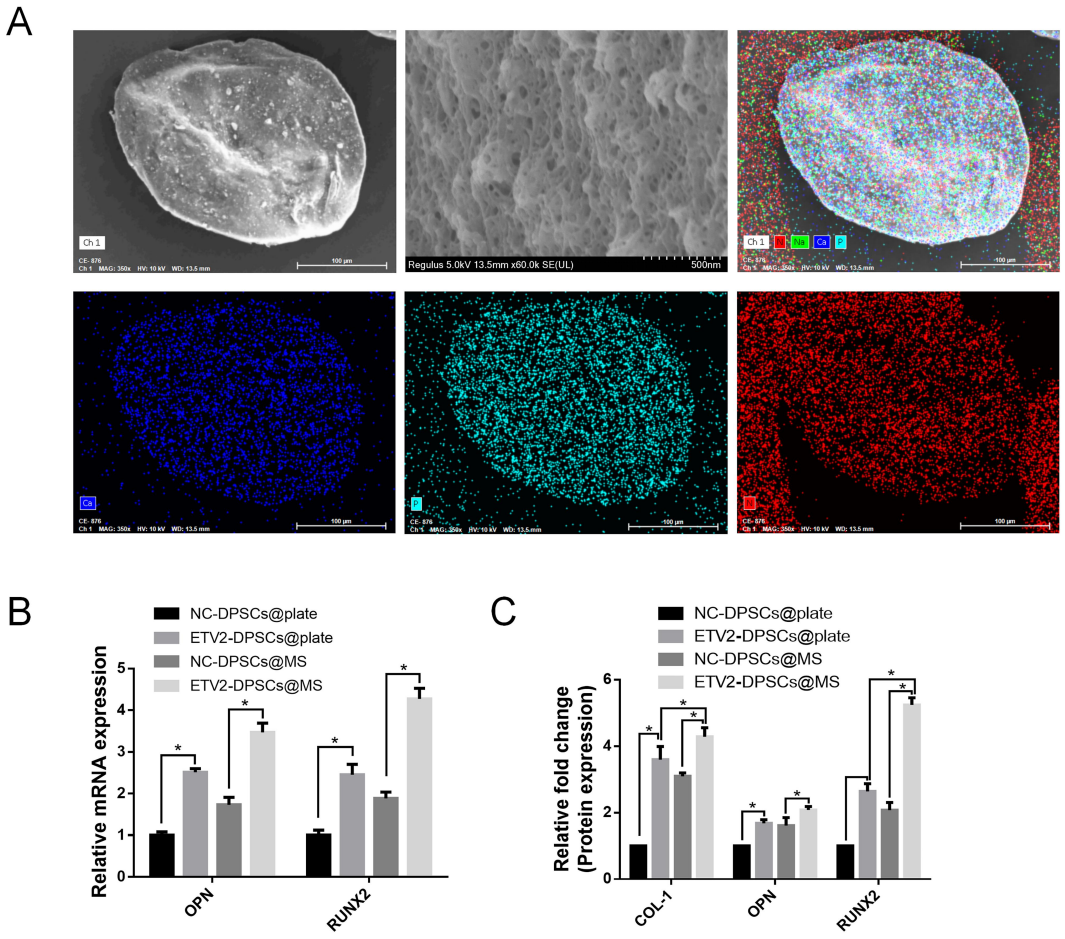
Supplementary figure 2. (A, B) Representative image and semi-quantitative analysis of ARS staining after PHD2 knockdown. Scale bar: 100 μm (C, D) The protein expression and semi-quantitative analysis of HIF-1 signaling (PHD2, HIF-1α, VEGFA) and osteogenic makers (COL-1, OPN) after PHD2 knockdown (KD knockdown, NC negative control, OE overexpression. Data are presented as the mean of >3 independent experiments ± SD. *P < 0.05, **P < 0.01, and ***P < 0.001).



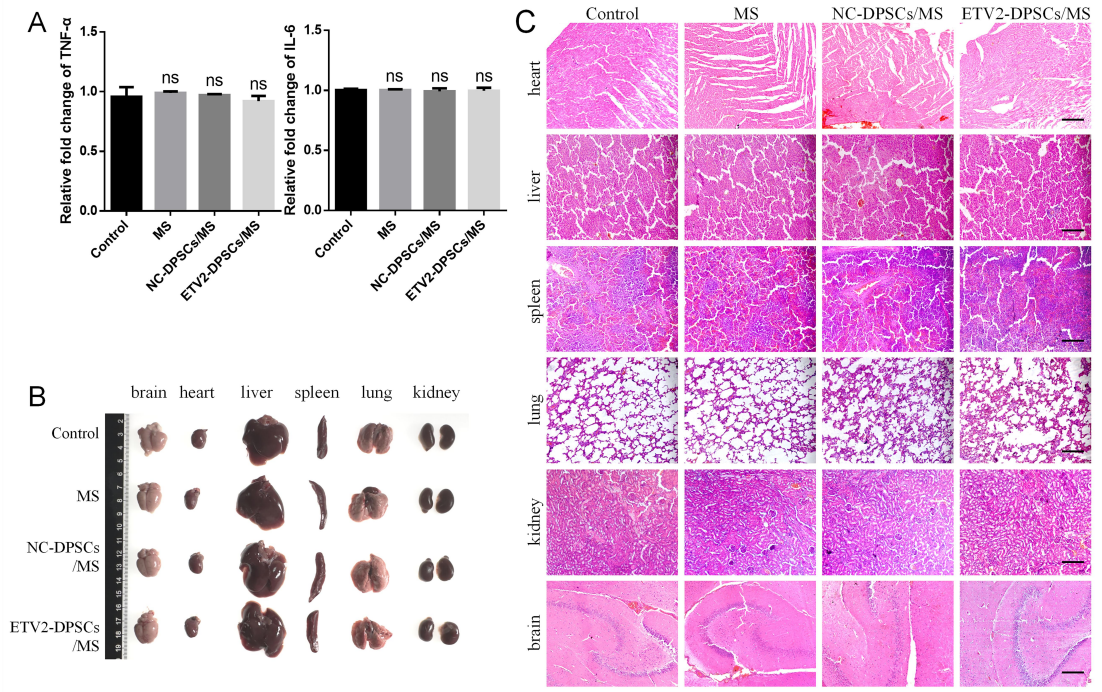
Supplementary figure 3. (A) ELISA was used to measure intracellular VEGFA levels in ETV2-DPSCs after osteogenic induction for 12 hours and 3 days (B) Secretory VEGFA levels in ETV2-DPSCs were determined by ELISA after osteogenic induction for 3 days (C) Alizarin Red staining and semi-quantitative analysis of DPSCs treated with 40ng/ml VEGFA (D, E) Protein expression and quantitative analysis of COL-1, OPN, and RUNX2 in DPSCs treated with 40ng/ml VEGFA (NS, no significant difference, NC, negative control; OE, overexpression. Data are presented as the mean of >3 independent experiments ± SD. *P < 0.05, **P < 0.01, and ***P < 0.001).



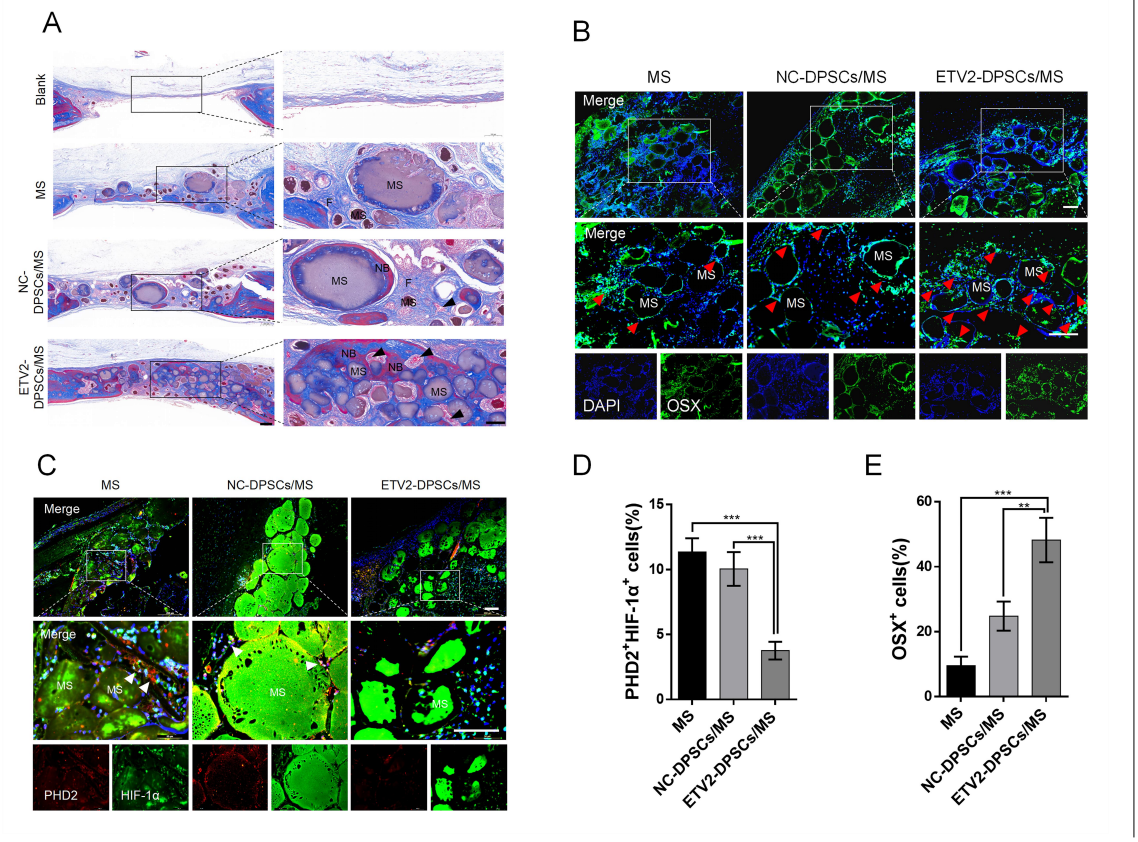
Supplementary figure 4. (A) Mitochondrial respiration of ETV2-DPSCs is measured using OCR detection at various time points after osteogenic induction (B) Glycolysis stress of ETV2-overexpressing DPSCs is measured using ECAR detection at different time points after osteogenic induction. (Data are presented as the mean of >3 independent experiments ± SD.)



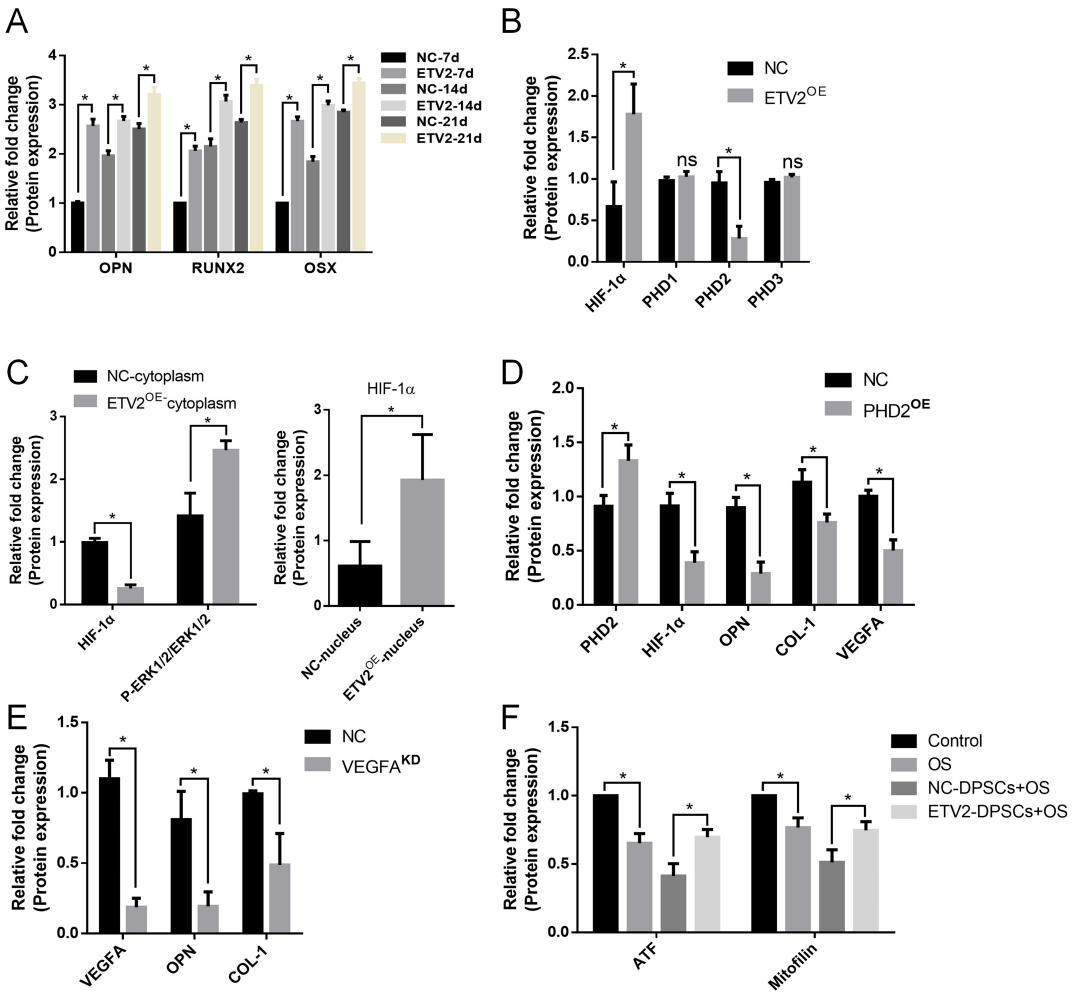
Supplementary figure 5. (A) Elemental distribution mapping of Ca, P, and N on the surface of HA/CS microspheres (B) The mRNA expression of OPN and RUNX2 after seven days of osteogenic induction in cells cultured on well plates or microsphere surfaces (C) Quantitative statistics of the protein expression of COL-1, OPN, and RUNX2 after seven days of osteogenic induction in cells cultured on well plates or microsphere surfaces (NC, negative control; OE, overexpression. Data are presented as the mean of >3 independent experiments ± SD. *P < 0.05).



Supplementary figure 6. (A) ELISA analysis of TNF-α and IL-6 levels in mouse ocular blood three days after modeling.(B) Macroscopic images of vital organs (C) H&E staining of vital organs (MS, microsphere; NS, no significant difference; NC, negative control. Data are presented as the mean of >3 independent experiments ± SD.)



Supplementary figure 7. (A) Masson staining of the defect area. The white arrow indicates the microsphere. The black arrow indicates neovasculars. Scale bar: 200 μm (B) Representative images of immunofluorescence labeling OSX in tissue sections of defect area at 3 d post-modeling. Red arrows indicate OSX^+ cells. Scale bar: 200 μm (C) Representative images of immunofluorescence co-staining PHD2 (red) and HIF-1α (green) in tissue sections of defect area at 3 d post-modeling. Scale bar: 200 μm (D, E) Quantification of the proportion of $PHD2^+HIF\text{-}1\alpha^+$ cells and OSX^+ cells (MS, microsphere; NC, negative control. Data are presented as the mean of >3 independent experiments ± SD. *P < 0.05, **P < 0.01, and ***P < 0.001)



Supplementary figure 8. (A-F) Quantitative statistics of the protein expression of figure 1E (A), 2C (B), 2G (C), 3D (D), 3F (E), 4E (F).

Table 1 Primer sequences

Gene	Forward sequence	Reverse sequence
ETV2	GAAGGAGCCAAATTAGGCTTCT	GAGCTTGTACCTTTCCAGCAT
RUNX2	TGGTTACTGTCATGGCGGGTA	TCTCAGATCGTTGAACCTTGCTA
OSX	CCTCTGCGGGACTCAACAAC	AGCCCATTAGTGCTTGTAAAGG
COL1A1	GAGGGCCAAGACGAAGACATC	CAGATCACGTCATCGCACAAC
OPN	CTCCATTGACTCGAACGACTC	CAGGTCTGCGAAACTTCTTAGAT
PHD1	TGGCCCTGGACTATATCGTG	GGCACCAATGCTTCGACAG
PHD2	GAAGGCGAACCTGTACCCC	TTCATGCACGGCACGATGTA
PHD3	CTGGGCAAATACTACGTCAAGG	GACCATCACCGTTGGGGTT
HIF-1α	GAACGTCGAAAAGAAAAGTCTCG	CCTTATCAAGATGCGAACTCACA

Table 2 Plasmid sequences

gene	sense（5'-3'）	antisense（5'-3'）
Human-si-VEGFA	CAAGAUCCGCAGACGUGUA(dT)(dT)	UACACGUCUGCGGAUCUUG(dT)(dT)
Human-si-PHD2	CAAGGUAAGUGGAGGUAUA(dT)(dT)	UAUACCUCCACUUACCUUG(dT)(dT)