Supplementary Material

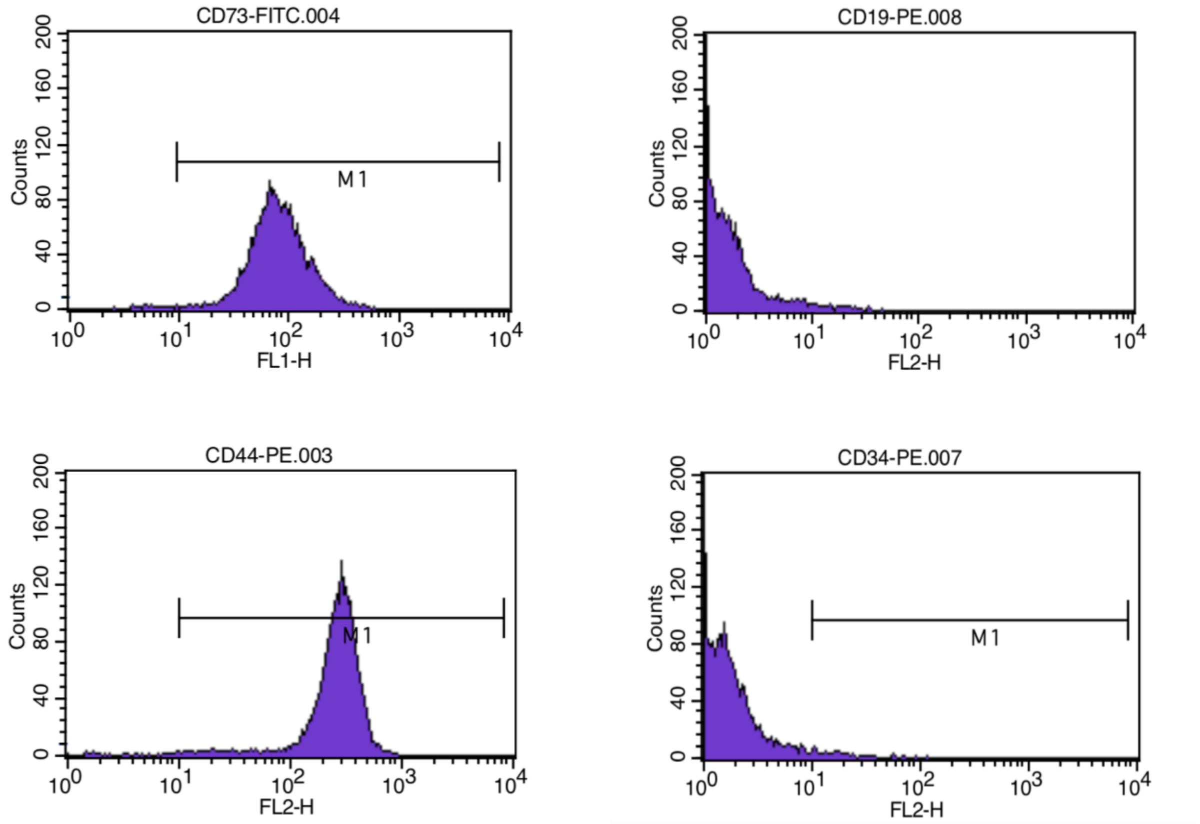

Figure S1. Flow cytometric analysis of hBMSC with specific antibodies against positive marker (CD44, CD73) and negative marker (CD19, CD34).

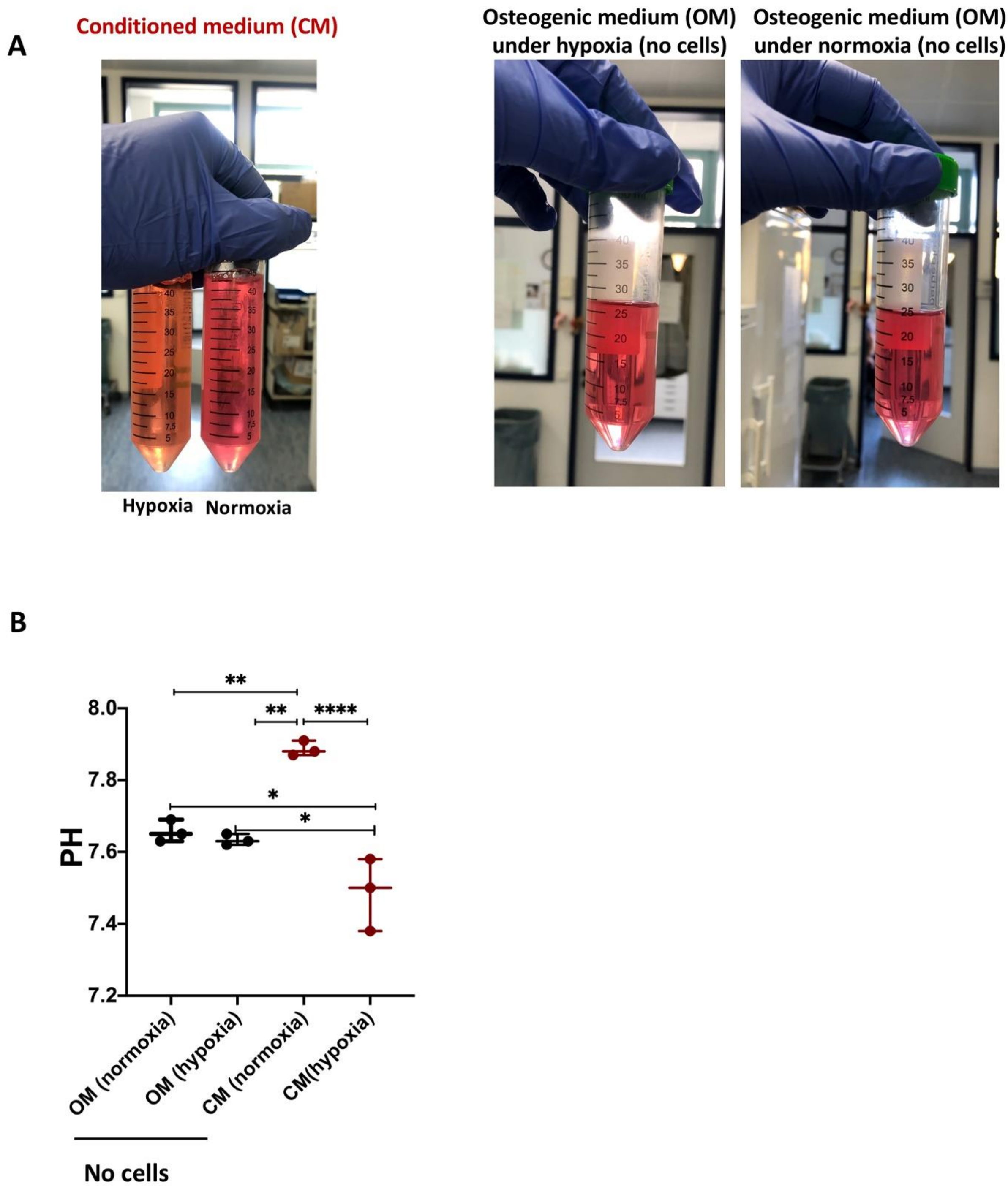

Figure S2. Conditioned medium from hypo-osteo EVs and norm-osteo EVs.

(A) Conditioned medium from hBMSCs undergoing osteogenic differentiation at 33-35 days under hypoxia and normoxia (CM). Osteogenic medium (OM, without subjecting to cell culture) was kept for two days under hypoxic and normoxic conditions.

(B) PH determination of the two conditioned media (CM) and the two osteogenic media (OM); n=3.

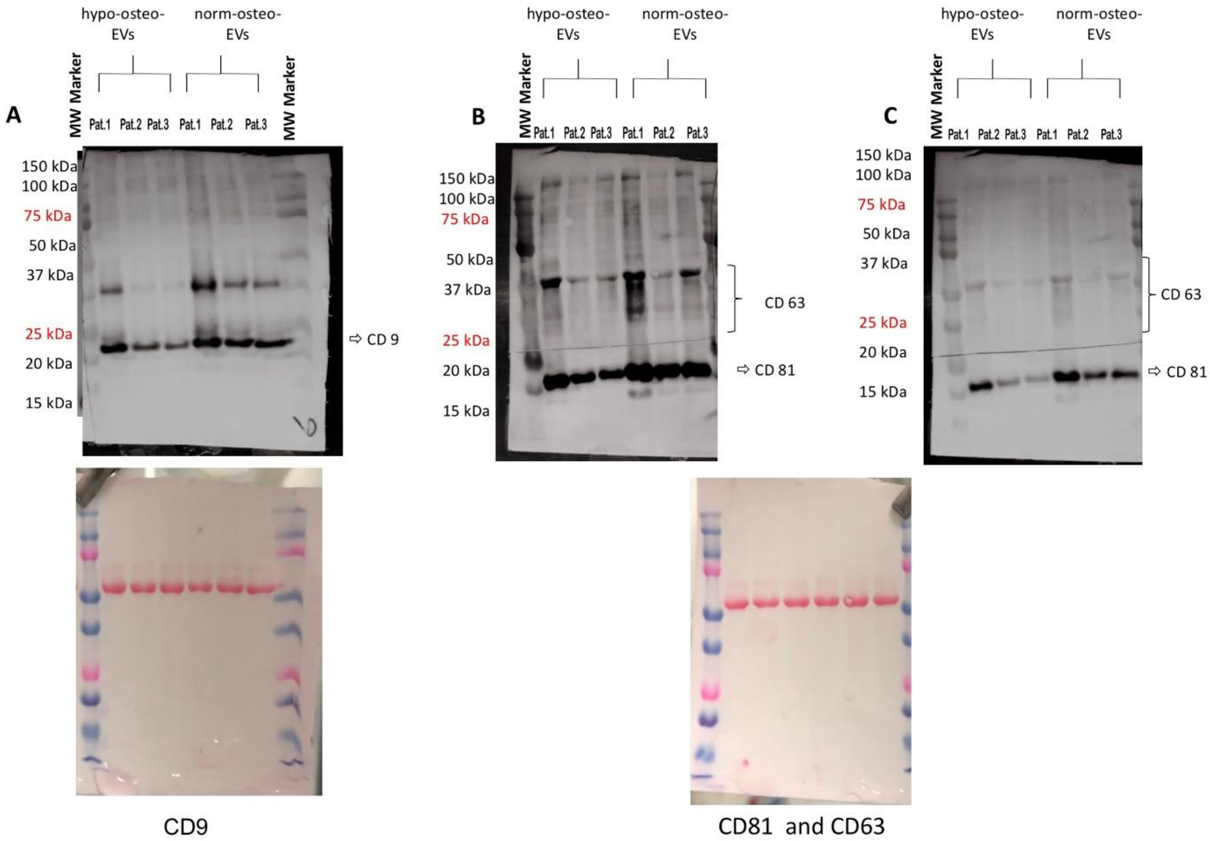

Figure S3. The uncropped western blot membranes of figure 2G and respective Ponceau Red stained loading control images below; n=3.

(A, C) Protein bands were detected using ECL detection reagents. （B）Protein bands were detected using SuperSignal West Femto Maximum Sensitivity Substrate.

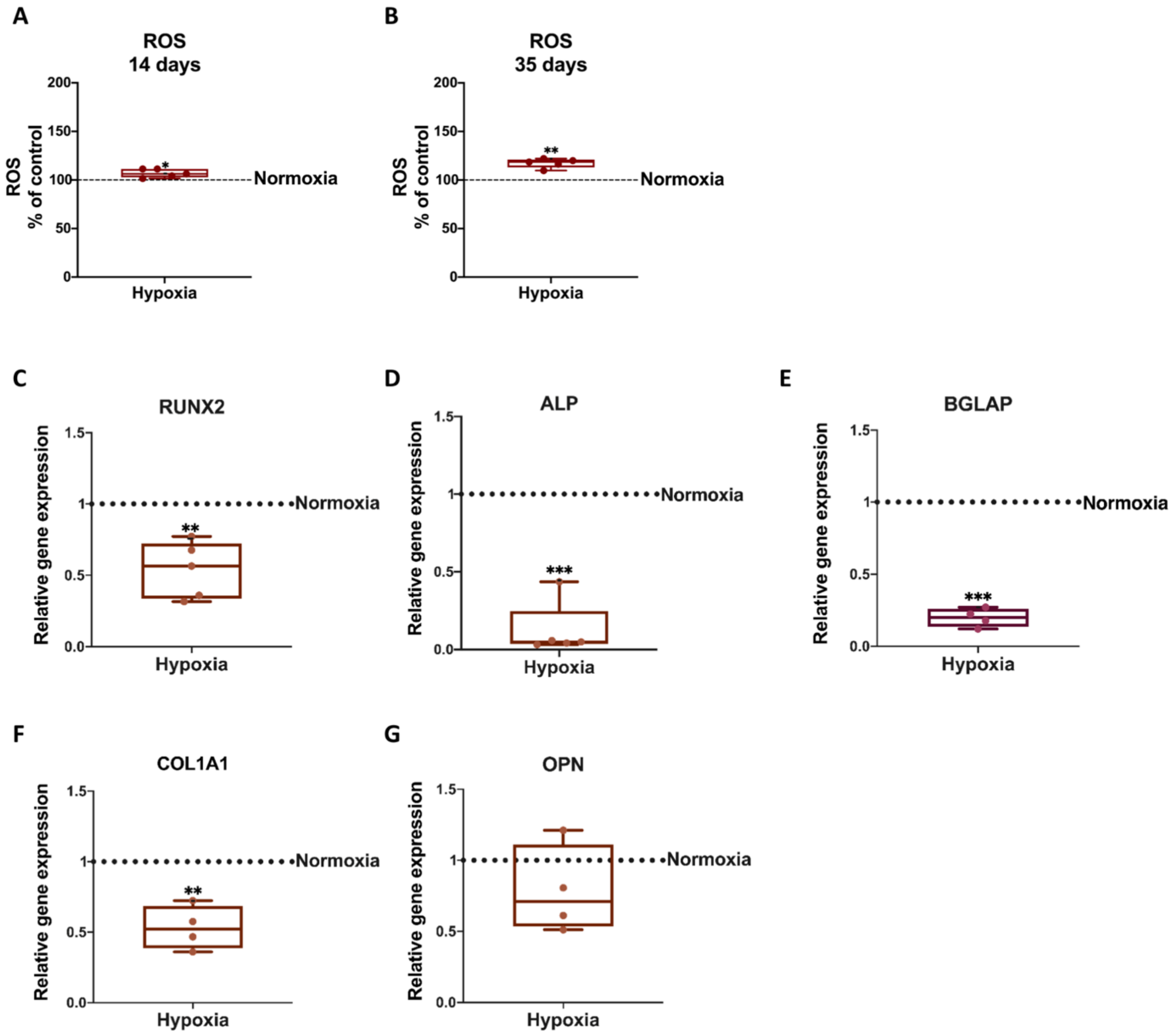

Figure S4. Evaluation of reactive oxygen species (ROS) secreted, and osteogenic marker gene expression in osteogenic differentiated hBMSCs under hypoxia and normoxia.

(A) Reactive oxygen species (ROS) secreted from hBMSCs after **14** days of osteogenic differentiation under hypoxia and normoxia; n=5.

(B) Reactive oxygen species (ROS) secreted from hBMSCs after **35** days of osteogenic differentiation under hypoxia or normoxia; n=5.

(C-G) Gene expression level of the osteogenic marker genes (OPN, BGLAP, ALP, RUNX2, and COL1A1) after 14 days of osteogenic differentiation of hBMSCs under hypoxia and normoxia; n=4-5.

Results were calculated as percentage to the control group (osteogenic differentiation of hBMSCs under normoxia, shown by the dotted line); *p < 0.05; **p < 0.01; ***p < 0.001;

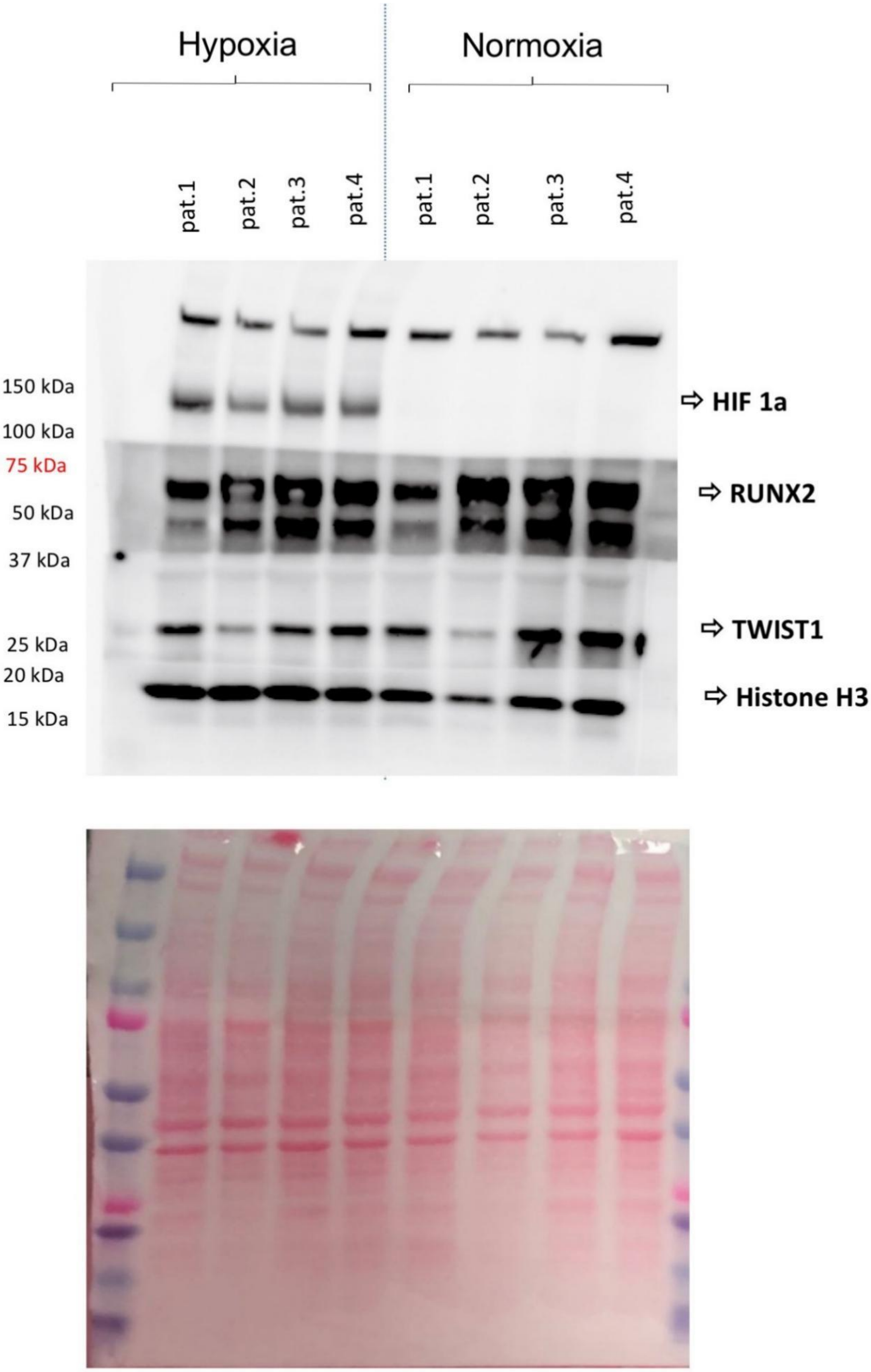

Figure S5. The uncropped western blot membrane of figure 4F and respective Ponceau Red stained loading control images below; n=4.

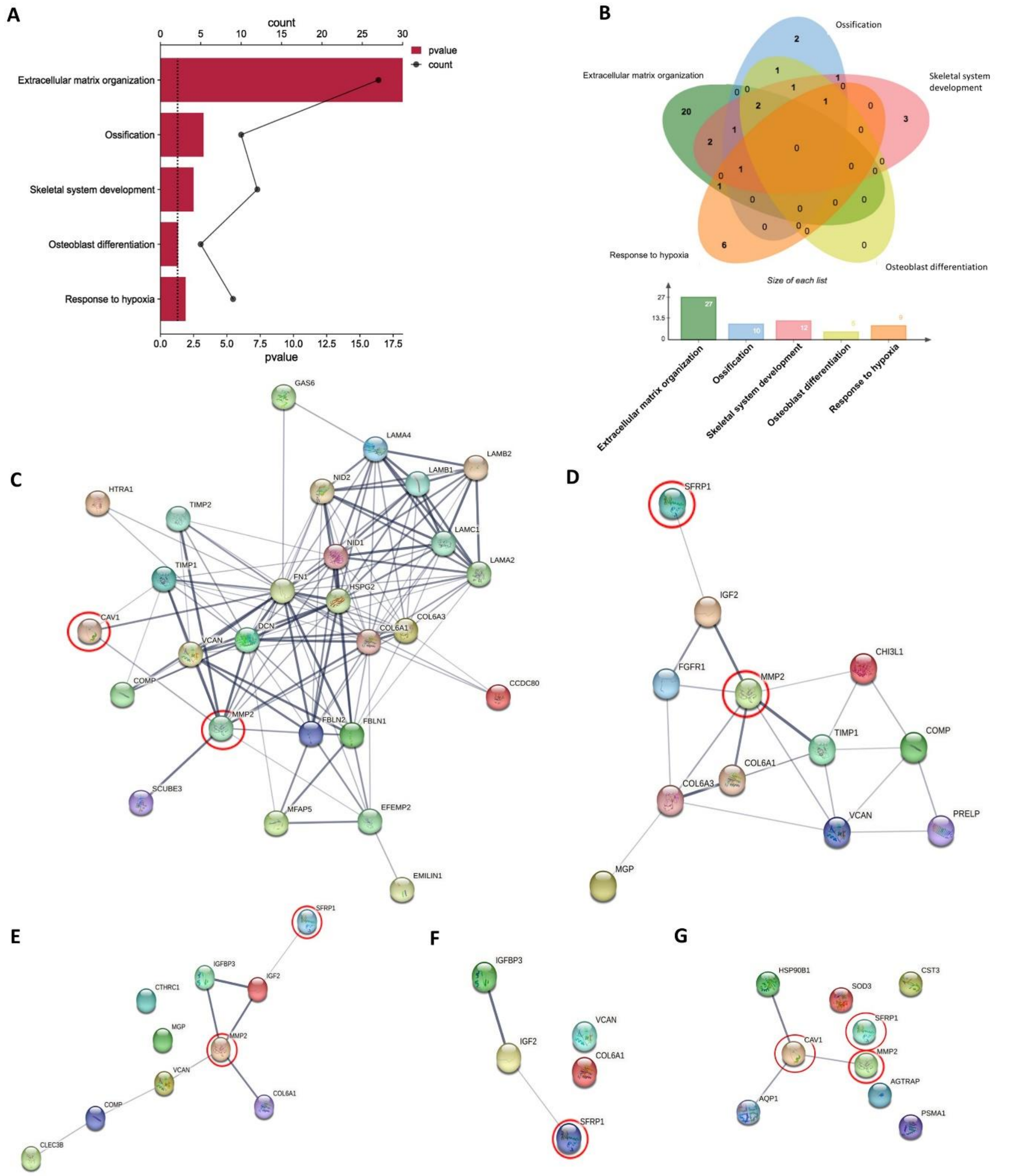

Figure S6. GO Enrichment analyses and protein-protein interaction (PPI) network analyses of norm-naïve EVs and norm-osteo EVs

(A) GO analysis for the “upregulated biological processes” related to hypoxia and osteogenesis in norm-osteo EVs compared with that in norm-naïve EVs.

(B) Venn diagram of ‘response to hypoxia associated”, ‘ossification’, ‘skeletal system development’, ‘osteoblast differentiation’ and ‘extracellular matrix (ECM) organization’. PPI networks of ‘ECM organization’ (C), ‘ossification’ (D), ‘skeletal system development’ (E), ‘osteoblast differentiation’ (F), and ‘response to hypoxia’ (G). The three proteins of ‘response to hypoxia’ (SFRP1, MMP2 and CAV1) in red circles are associated with ECM synthesis and osteogenesis; n=3.

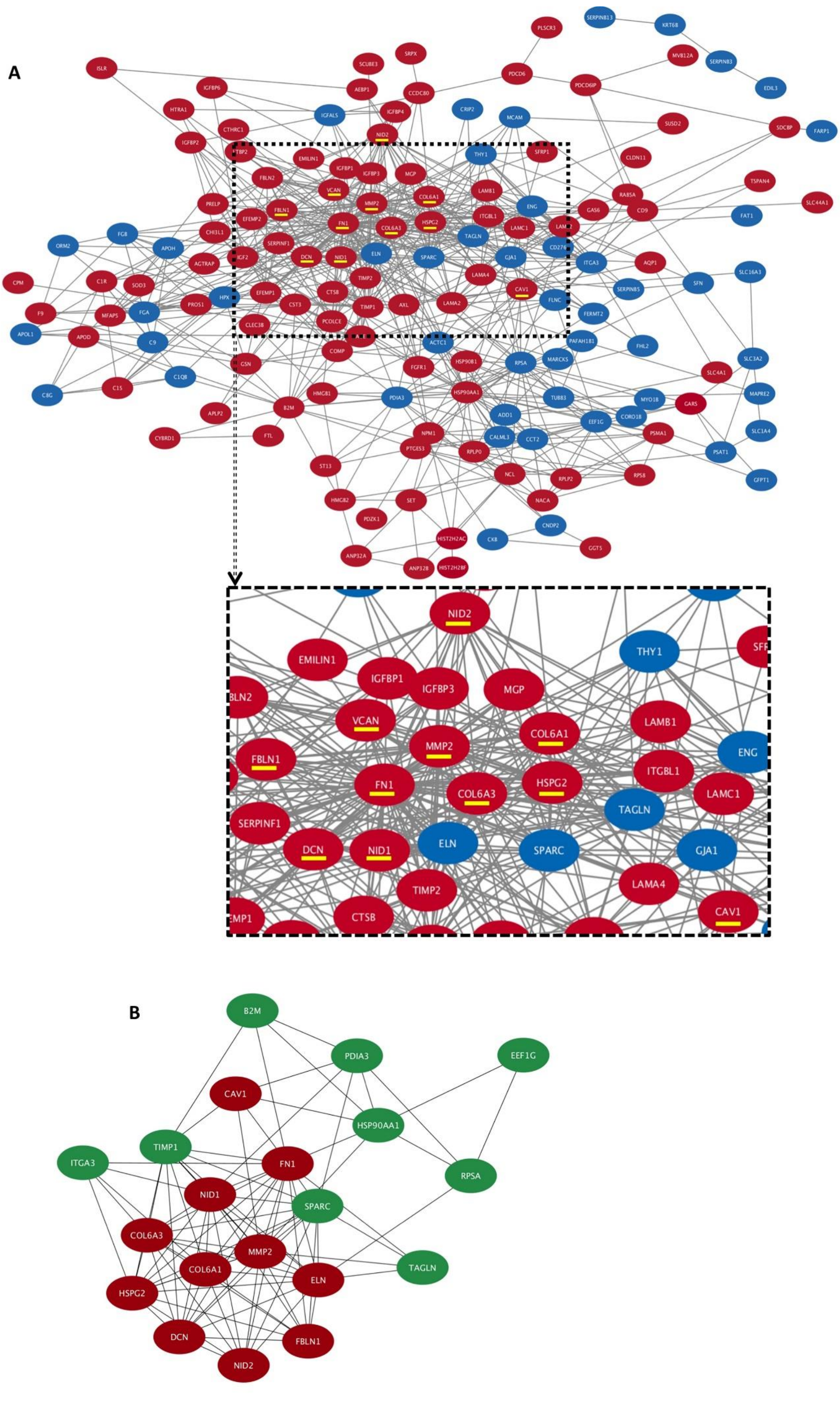
A
NID2
EMILIN1
IGFBP1
IGFBP3
MGP
THY1
FBLN2
VCAN
MMP2
COL6A1
LAMB1
ENG
FBLN1
FN1
COL6A3
HSPG2
ITGBL1
LAMC1
SERPINF1
TAGLN
DCN
NID1
ELN
SPARC
GJA1
TIMP2
LAMA4
CTSB
CAV1
B
B2M
PDIA3
EEF1G
CAV1
HSP90AA1
TIMP1
ITGA3
FN1
RPSA
NID1
SPARC
COL6A3
MMP2
TAGLN
COL6A1
ELN
HSPG2
DCN
FBLN1
NID2

Figure S7. PPI network of the distinct proteins and hub proteins identified in norm-osteo EVs compared with that in norm-naïve EVs .

(A) Interactions between upregulated and downregulated proteins in norm-osteo EVs compared with that in norm-naïve EVs; n=3; Red nodes indicate upregulated proteins, and blue nodes indicate downregulated proteins.

(B) The 20 most highly correlated hub proteins in PPI network. The red nodes indicate the 11 hub proteins related to hypoxia or osteogenesis. The 11 hub proteins are marked with a yellow line.

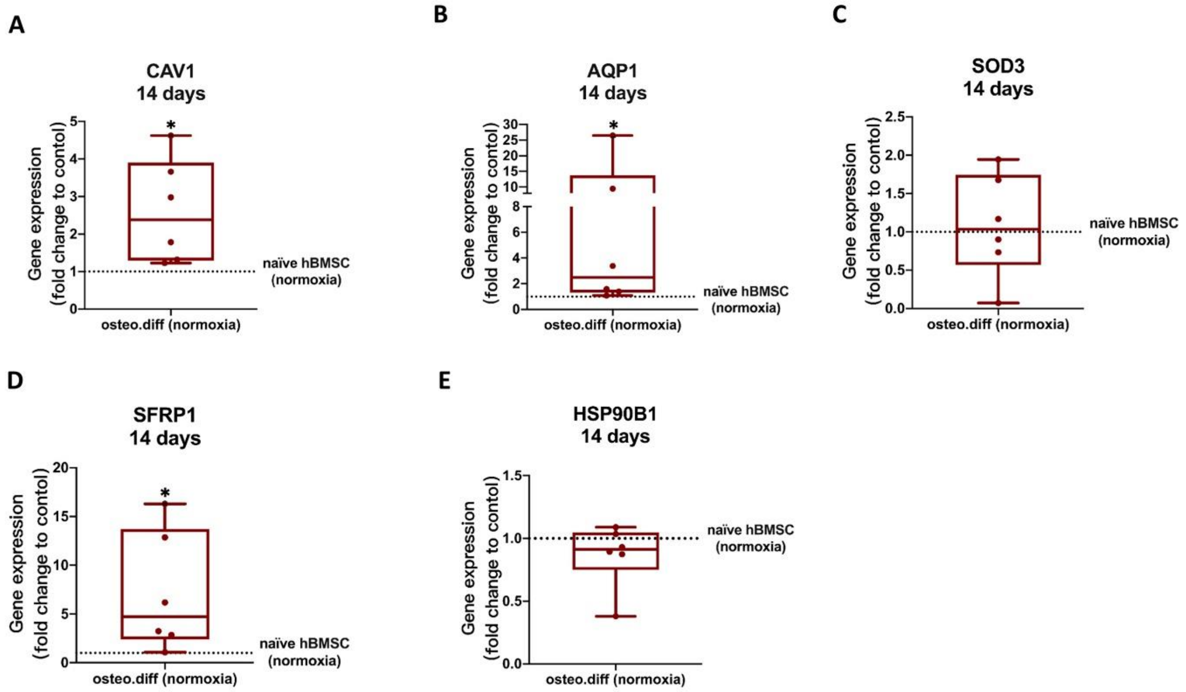

Figure S8. Validation of the proteomic data obtained from norm-naïve EVs and norm-osteo EVs by gene expression analysis in hBMSC.

(A-E) Expression level of the five anti-hypoxic genes were analysed in hBMSCs after **14 days** of osteogenic differentiation under normoxia and naïve hBMSCs under normoxia; n=6.

Tables

Table S1. Distinct proteins identified in hypo-osteo EVs and norm-osteo EVs (attached excel file).

Table S2. Primer sequences for qPCR.

Gene	**Primer sequences (5'–3')**
GAPDH	Fwd, CTGACTTCAACAGCGACACC
	Rev, CCCTGTTGCTGTAGCCAAAT
TBP	Fwd, TTGTACCGCAGCTGCAAAAT
	Rev, TATATTCGGCGTTTCGGGCA
ALP	Fwd, CCTCCTCGGAAGACACTCTG
	Rev, CCTCCTCGGAAGACACTCTG
BGLAP	Fwd, GTGCAGAGTCCAGCAAAGGT
	Rev, TCAGCCAACTCGTCACAGTC
COL1A1	Fwd, ACGTCCTGGTGAAGTTGGTC
	Rev, ACCAGGGAAGCCTCTCTCTC
RUNX2	Fwd, CGGAATGCCTCTGCTGTTATG
	Rev, GCTTCTGTCTGTGCCTTCTG
OPN	Fwd, TGAAACGAGTCAGCTGGATG
	Rev, TGAAATTCATGGCTGTGGAA
SOD3	Fwd, ATGCTGGCGCTACTGTGTT
	Rev, CTCCGCCGAGTCAGAGTTG
CAV1	Fwd, GCGACCCTAAACACCTCAAC
	Rev, ATGCCGTCAAAACTGTGTGTC

SFRP1	Fwd, ACGTGGGCTACAAGAAGATGG
	Rev, CAGCGACACGGGTAGATGG
AQP1	Fwd, CTGGGCATCGAGATCATCGG
	Rev, ATCCCACAGCCAGTGTAGTCA
HSP90B1	Fwd, CTGGGCATCGAGATCATCGG
	Rev, ATCCCACAGCCAGTGTAGTCA