Supplemental figures and tables

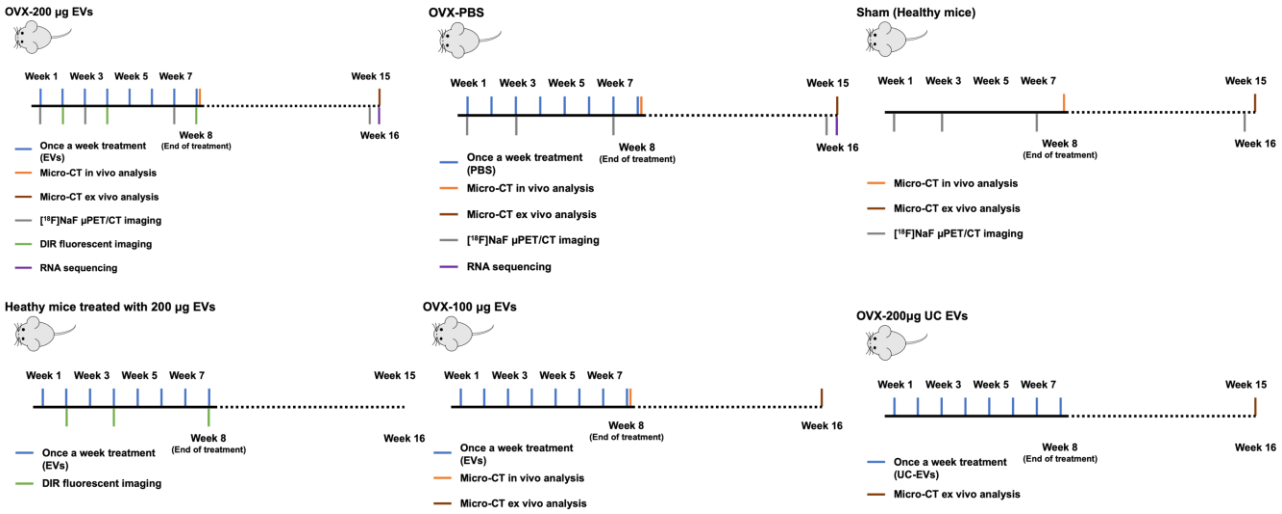

Figure S1. The design of the experiment. Different procedures used in animal studies were illustrated.

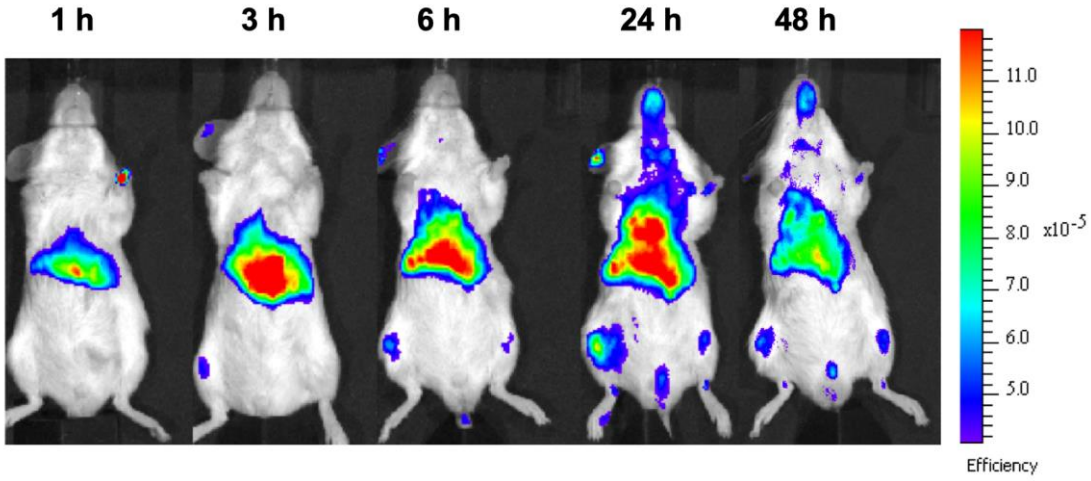

Figure S2. Images of WJ-MSC-EVs labeled with DIR fluorescent. The 200 μg EVs labeled with DIR were systemically administrated into healthy BALB/c mice. The mice were imaged at 1, 3, 6, 24, 48 h after injection.

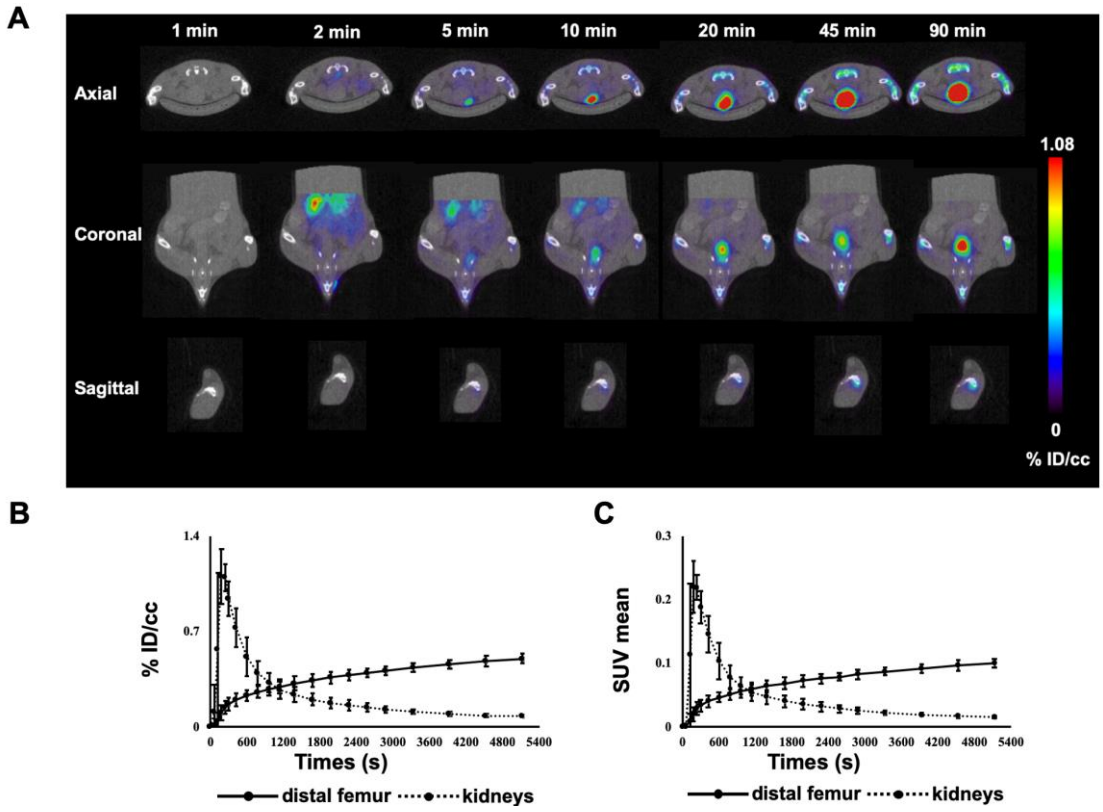

Figure S3. Dynamic acquisition of [^{18}F]NaF. (A) Dynamic imaging was performed by μPET/CT after injection, and images were shown in axial, coronal, sagittal views. **(B, C)** The time-activity relationship in ROIs at distal femur and kidneys of mice were analyzed and presented by %ID/cc and SUVmean.

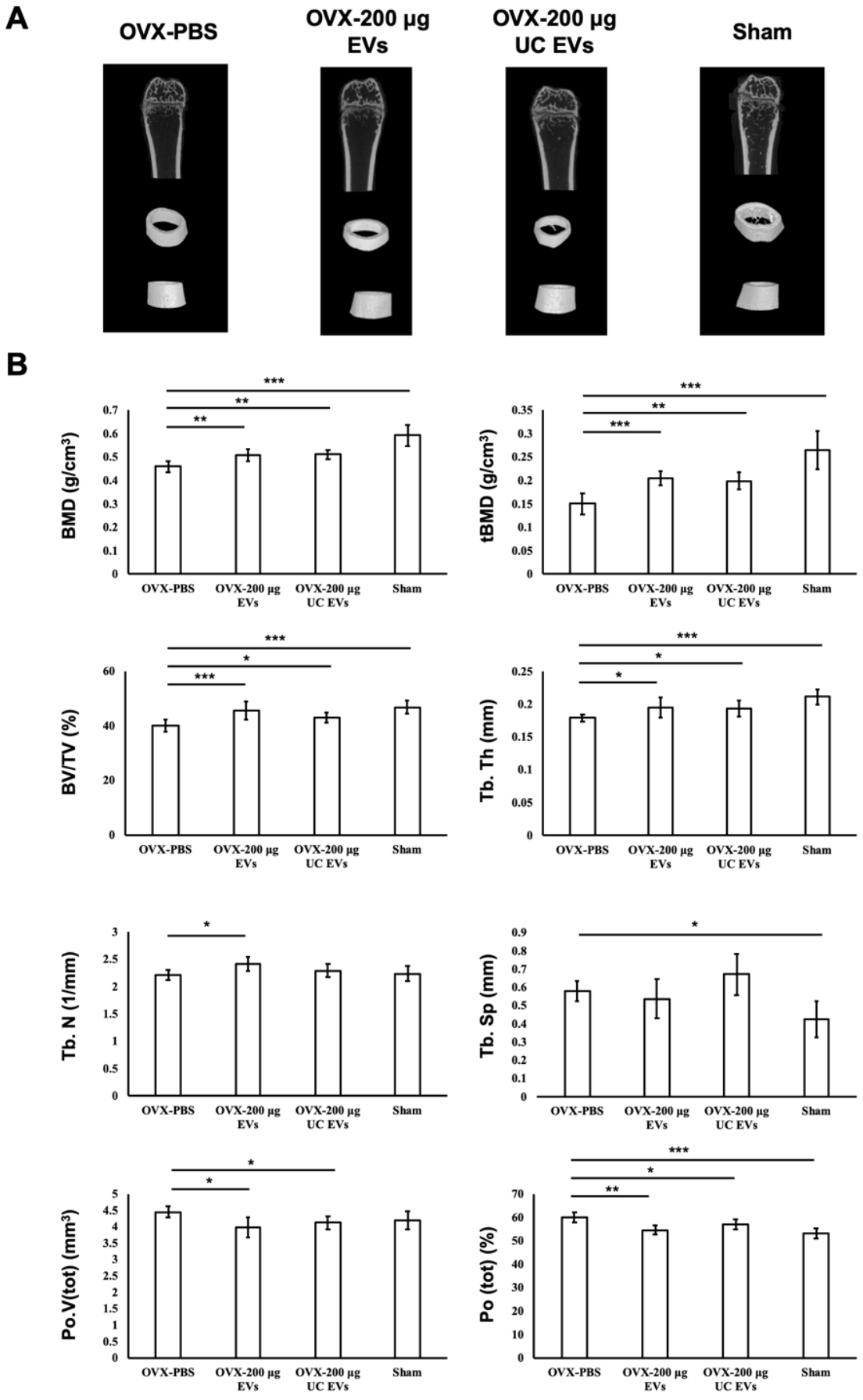

Figure S4. ***Ex vivo*** **μCT imaging and quantitative analysis in OVX-200 μg UC EVs group. (A)**

3D-μCT *in vivo* and *ex vivo* images of trabecular and cortical bone microstructure in OVX-PBS, OVX-200 μg EVs, OVX-200 μg UC EVs group, and Sham groups. **(B)** Images were further quantitatively analyzed by μCT. The parameters including BMD, tBMD, BV/TV, Tb.N, Tb.Th, Tb.Sp, Po.V (tot) , and Po (tot) were acquired. Data are expressed as mean ± SD (*$p < 0.05$, **$p < 0.01$, ***$p < 0.005$).

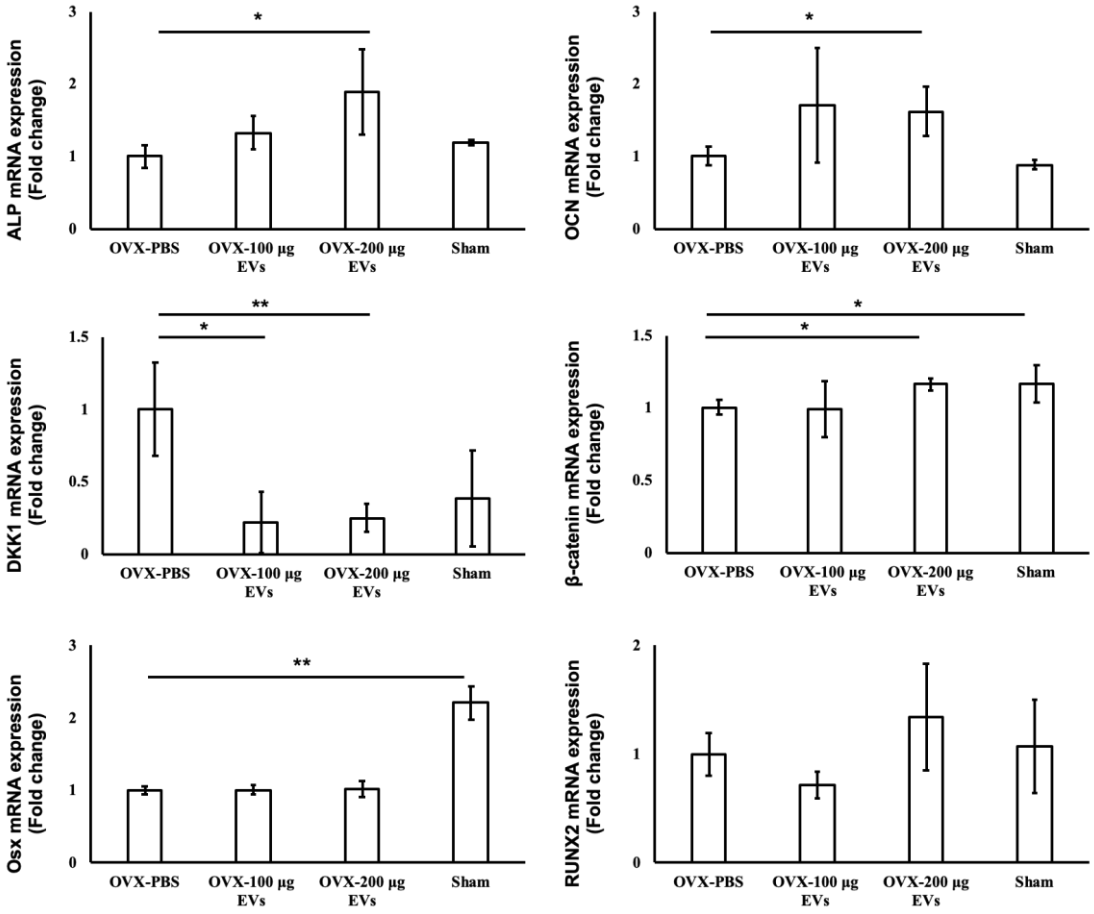

Figure S5. Gene regulation by treatment using exogenous WJ-MSC-EVs in osteoporotic mice.

The mRNA expression levels of ALP, OCN, DKK1, β-catenin, Osx, RUNX2 in bone tissues were calculated by qRT-PCR. Data are expressed as mean ± SD (*$p < 0.05$, **$p < 0.01$, ***$p < 0.005$).

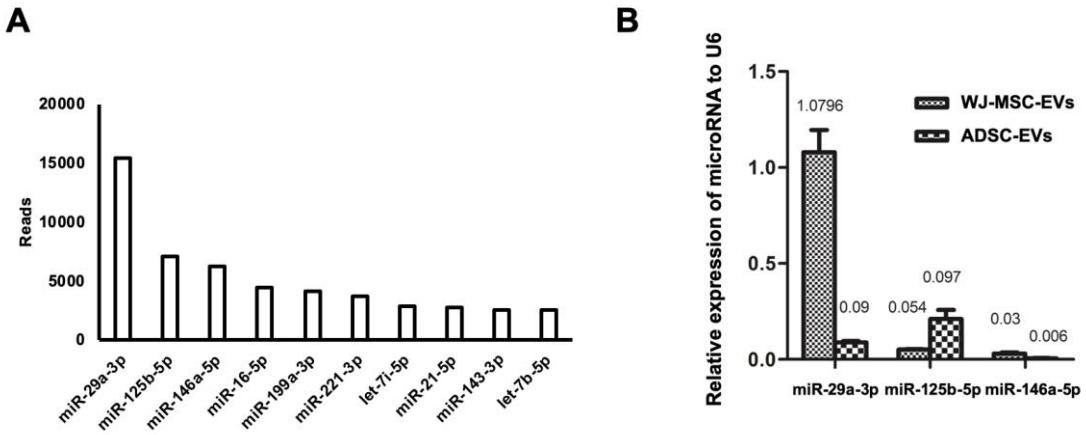

Figure S6. miRNA NGS analysis of WJ-MSC-EVs. (A) Top 10 most highly enriched miRNAs in WJ-MSC-EVs. (B) Comparing the top 3 enriched miRNAs in WJ-MSC-EVs with ADSC-EVs (Adipose tissue-derived stem cells secreted extracellular vesicles) by qPCR. Adapted from Chen YA, Lu CH, Ke CC, et al. Mesenchymal Stem Cell-Derived Exosomes Ameliorate Alzheimer's Disease Pathology and Improve Cognitive Deficits. Biomedicines. 2021;9(6). Creative Commons license and disclaimer available from: http://creativecommons.org/licenses/by/4.0/legalcode. [1]

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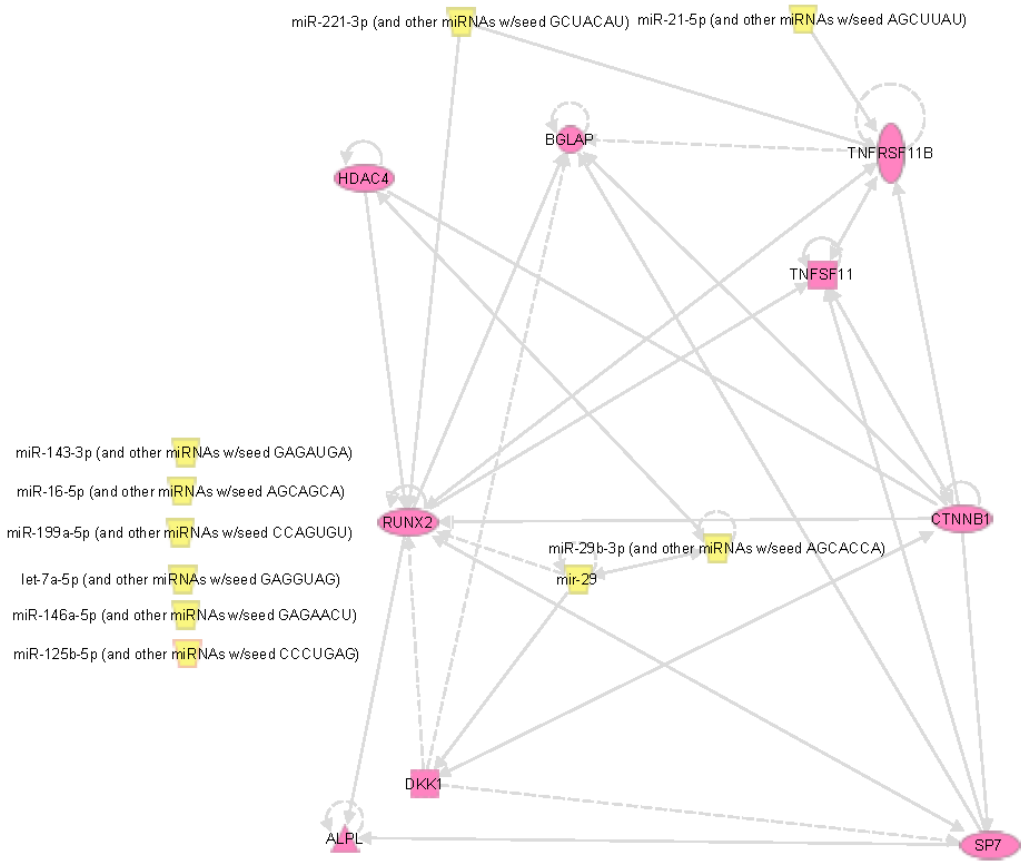
miR-221-3p (and other miRNAs w/seed GCUACAU)
miR-21-5p (and other miRNAs w/seed AGCUUAU)
BGLAP
TNFRSF11B
HDAC4
TNFSF11
miR-143-3p (and other miRNAs w/seed GAGAUGA)
miR-16-5p (and other miRNAs w/seed AGCAGCA)
miR-199a-5p (and other miRNAs w/seed CCAGUGU)
let-7a-5p (and other miRNAs w/seed GAGGUAG)
miR-146a-5p (and other miRNAs w/seed GAGAACU)
miR-125b-5p (and other miRNAs w/seed CCCUGAG)
RUNX2
CTNNB1
miR-29b-3p (and other miRNAs w/seed AGCACCA)
mir-29
DKK1
ALPL
SP7

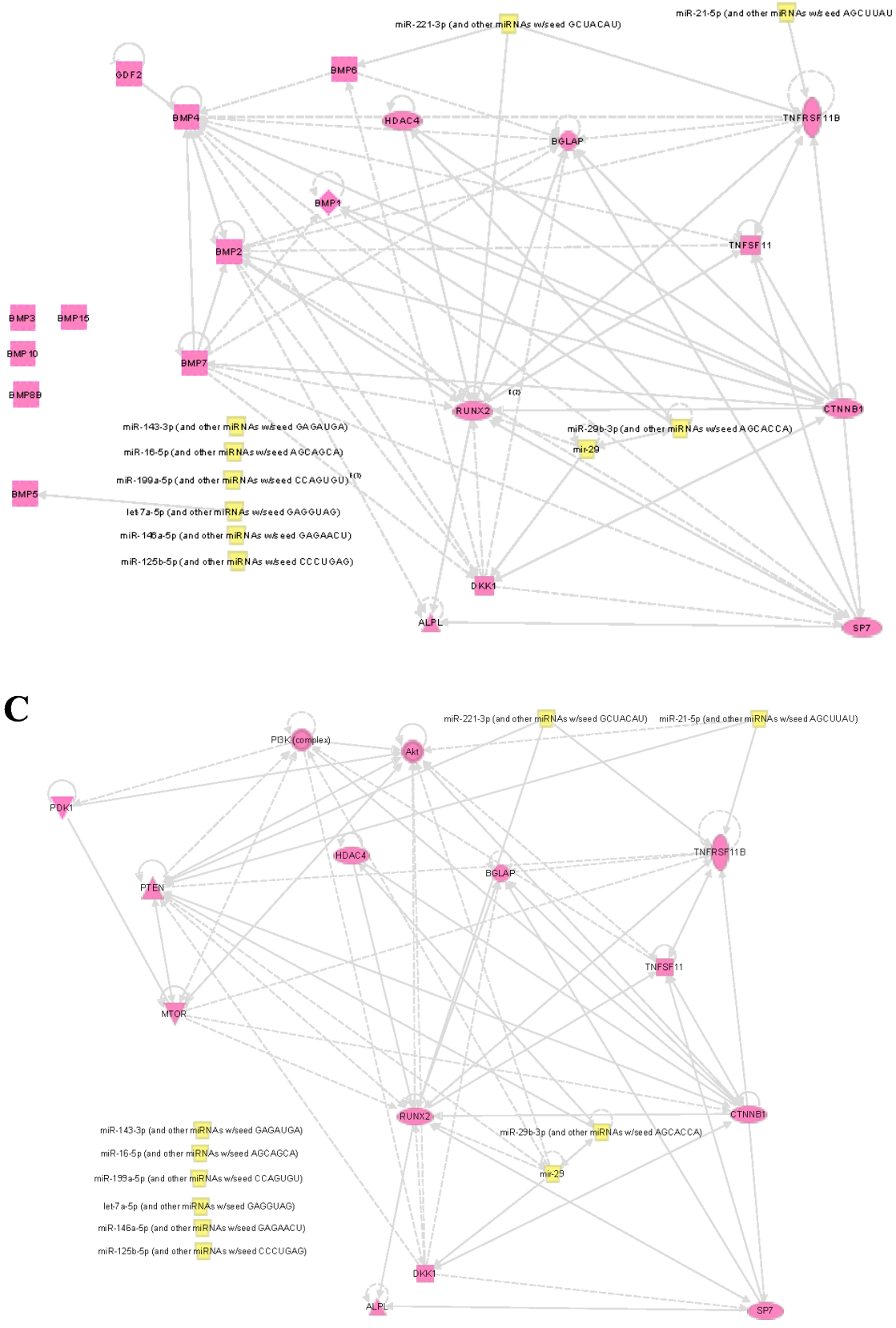

Figure S7. IPA analysis of possible mechanism. (A) The connections between top 10 enriched EV-

miRNAs and general markers of bone remodeling were illustrated. **(B)** In addition of canonical markers, supplementary analysis of connections between EV-miRNAs and BMP signaling pathway was illustrated. **(C)** Replacement of BMP signaling pathway, PI3K/AKT was selected in the analysis.

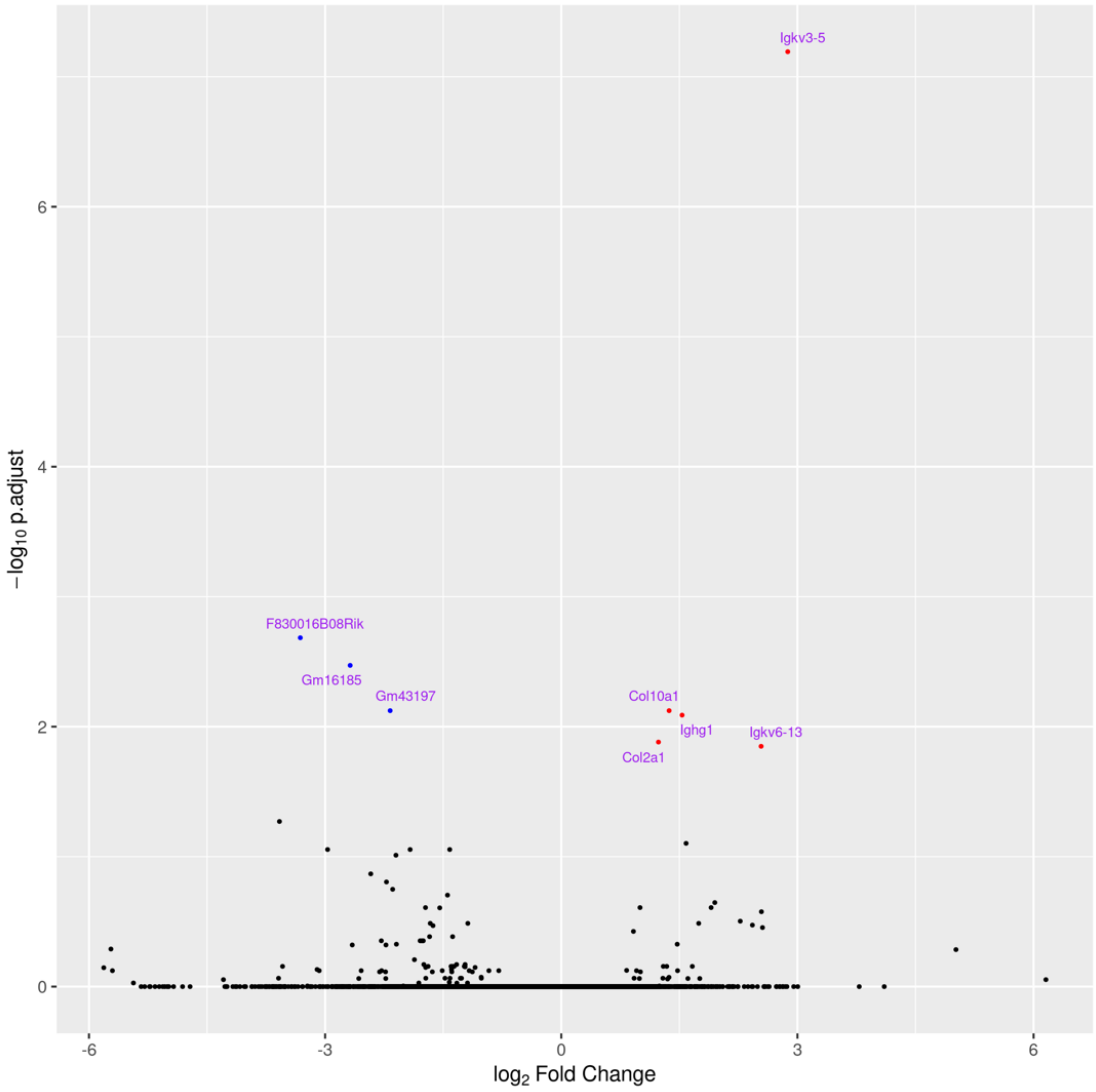

Figure S8. Volcano plot of fold change of gene expression. The screened DEGs illustrated by volcano map, the p-value was used to discriminate the significant change.

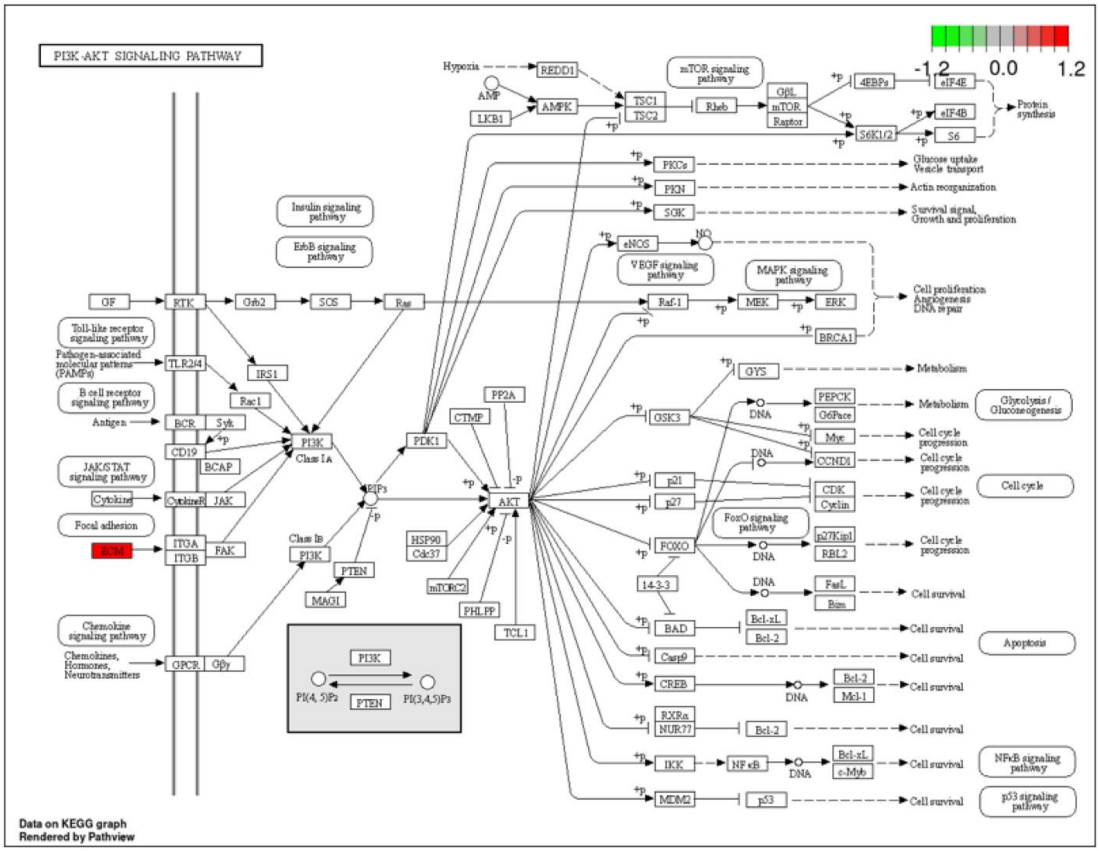

Figure S9. Prediction of signaling pathways. Use of the KEGG database to predict the significant alteration of potential molecules in OVX mice treated with exogenous WJ-MSC-EVs. Red: Up-regulated genes.

Table S1. ROI analysis of μPET/CT images.

%ID/cc	1 week	3 weeks	7 weeks	15 weeks
OVX-PBS	0.44 ± 0.03	0.39 ± 0.06	0.31 ± 0.02	0.33 ± 0.03
OVX-200 μg EVs	0.5 ± 0.01	0.57 ± 0.09	0.47 ± 0.08	0.35 ± 0.01
Sham	0.5 ± 0.03	0.49 ± 0.04	0.51 ± 0.05	0.40 ± 0.05

SUVmean	1 week	3 weeks	7 weeks	15 weeks
OVX-PBS	0.09 ± 0.01	0.08 ± 0.01	0.06 ± 0.01	0.06 ± 0.01
OVX-200 μg EVs	0.1 ± 0.01	0.12 ± 0.02	0.09 ± 0.02	0.07 ± 0.01
Sham	0.1 ± 0.01	0.11 ± 0.01	0.1 ± 0.01	0.08 ± 0.01

Quantitative ROIs of [^{18}F]NaF in distal femur at 1,3,7, and 15 weeks after OVX. Each value represents mean ± SD. Unit: %ID per cubic centimeter (%ID/cc); SUVmean, mean activity of standardized uptake values.

Table S2. List of specific primers for genes used in RT-qPCR.

Gene (human)	Direction	Sequence (5'–3')
ALP	Forward	GCCTGGCTACAAGGTGGTG
	Reverse	GGCCAGAGCGAGCAGC
OPG	Forward	GGCAACACAGCTCACAAGAA
	Reverse	CTGGGTTTGCATGCCTTTAT
DKK1	Forward	GATCATAGCACCTTGGATGGG
	Reverse	GGCACAGTCTGATGACCGG
RUNX2	Forward	GGTACCAGATGGGACTGTGG
	Reverse	GAGGCGGTCAGAGAACAAAC
HDAC4	Forward	GGCCCACCGGAATCTGAAC
	Reverse	GAACTCTGGTCAAGGGAACTG
GAPDH	Forward	GAGTCAACGGATTTGGTCGT
	Reverse	TTGATTTTGGAGGGATCTCG

Gene (mouse)	Direction	Sequence (5'–3')
ALP	Forward	TGTGGAATACGAACTGGATGAG
	Reverse	ATAGTGGGAATGCTTGTGTCTG
OCN	Forward	GAGGACCATCTTTCTGCTCACT

	Reverse	CGGAGTCTGTTCACTACCTTATTG
Osx	Forward	GAAAGGAGGCACAAAGAAG
	Reverse	CACCAAGGAGTAGGTGTGTT
DKK1	Forward	GAGGTCCCGAAGTTGAGGTT
	Reverse	GCAGAGAGCCATCATTGTAAAC
β-catenin	Forward	GCAGTCTTACTTGGATTCTGGA
	Reverse	AGCCTTGCTCCCATTCATAA
RUNX2	Forward	GCCGGGAATGATGAGAACTA
	Reverse	GGTGAAACTCTTGCCTCGTC
GAPDH	Forward	GGAGATTGTTGCCATCAACGA
	Reverse	GAAGACACCAGTAGACTCCACGACA

1. Chen YA, Lu CH, Ke CC, et al. Mesenchymal Stem Cell-Derived Exosomes Ameliorate Alzheimer's Disease Pathology and Improve Cognitive Deficits. *Biomedicines.* 2021;9(6).