Supplementary Information

Osteoporosis limits neurological recovery after spinal cord injury through PDGF-BB-MMP14 signalling

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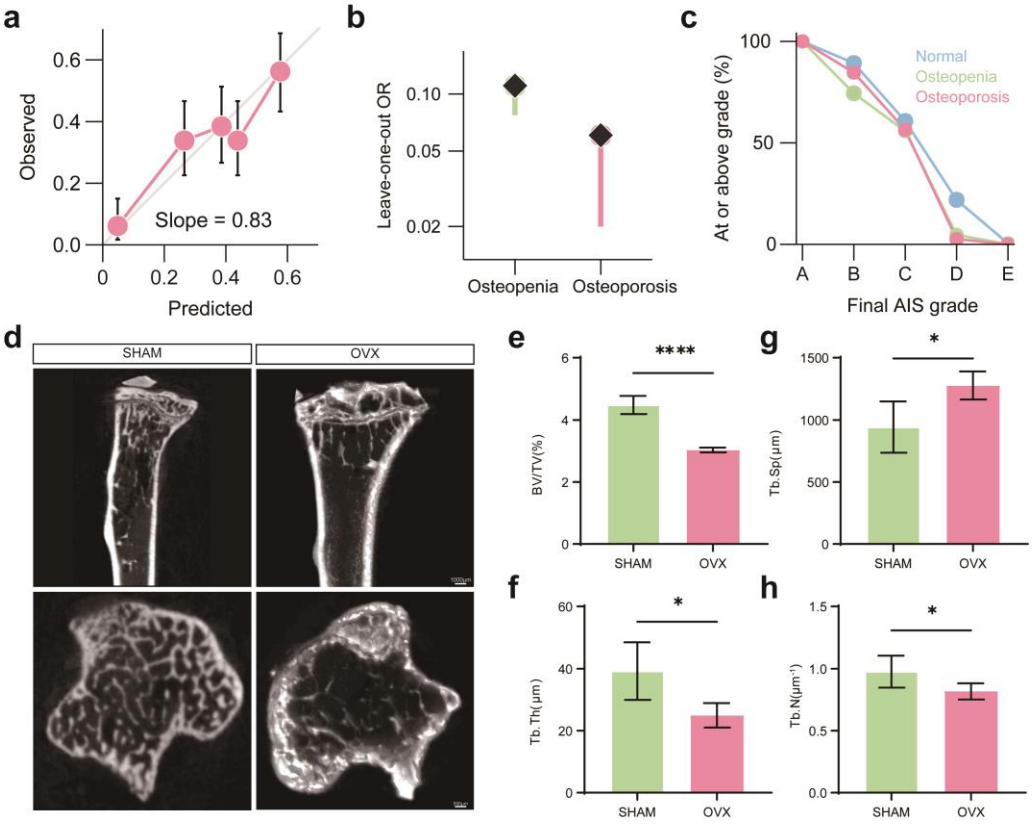
Contents summary: This file contains six supplementary figures and four supplementary tables supporting clinical-model validation, transcriptomic quality control, single-nucleus annotation, pharmacological and skeletal assessments, exploratory structural modelling, and the eligibility screening of public GEO samples.

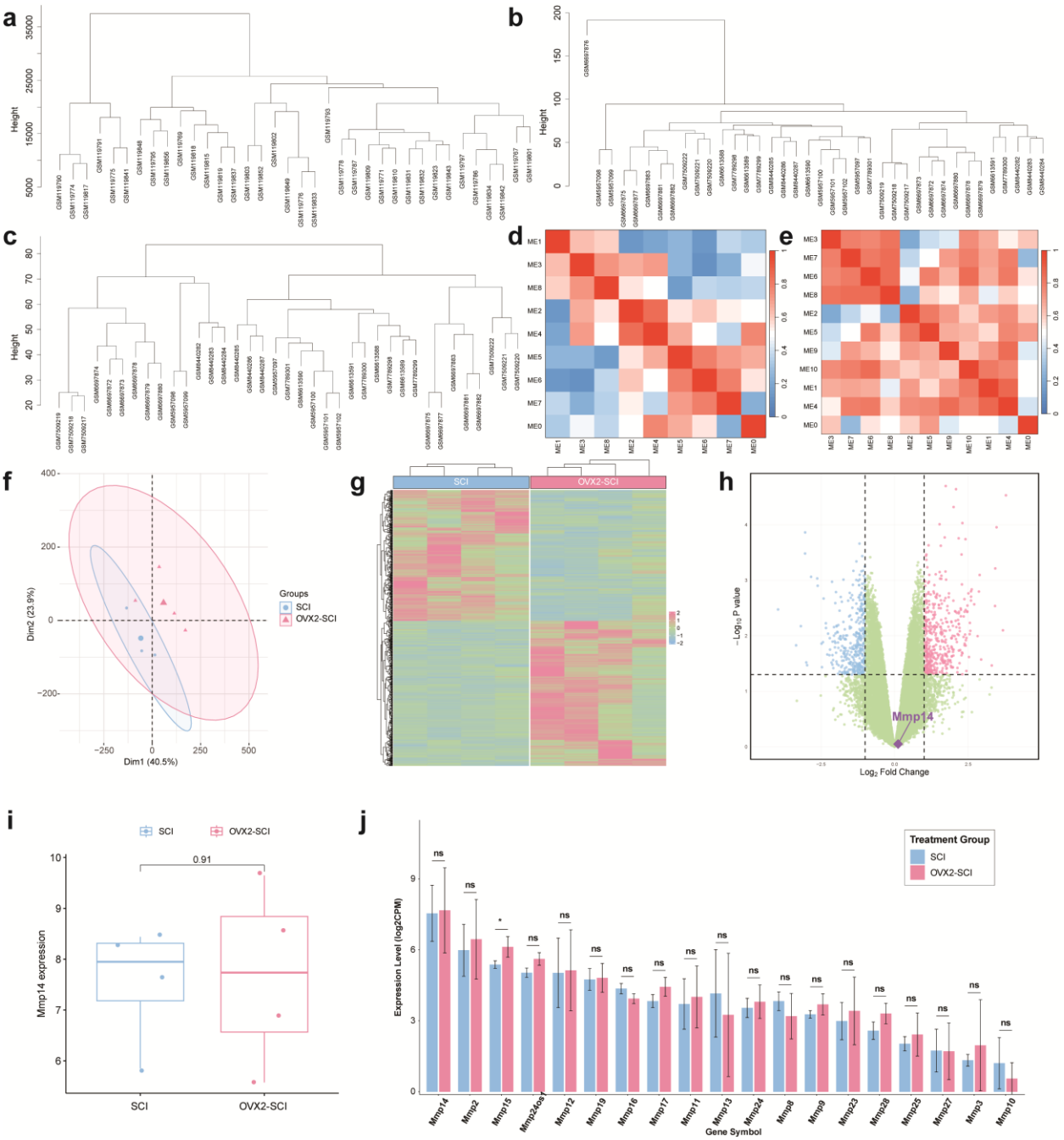
Figure S1 | Internal validation of the primary clinical model and verification of the OVX osteoporosis model



a, Bootstrap out-of-bag calibration plot for the primary Firth penalised logistic regression model, showing agreement between the observed probability of 1-year AIS grade improvement and the model-predicted probability. b, Leave-one-out refitting analysis assessing the stability of bone-status odds ratio estimates. Black diamonds indicate the full-cohort model estimates, and coloured line segments indicate the range of estimates obtained after sequentially excluding individual patients. c, Unadjusted cumulative distribution of final 1-year AIS grades by bone-status group, showing the proportion of patients reaching each AIS grade or a higher grade. d, Representative proximal tibial micro-CT images from SHAM and OVX mice, including longitudinal and transverse reconstructions. e–h, Micro-CT quantification of proximal tibial cancellous bone in SHAM and OVX mice, including bone volume fraction (BV/TV, e),

trabecular thickness (Tb.Th, f), trabecular separation (Tb.Sp, g), and trabecular number (Tb.N, h). Statistical analysis: e–h, two-sided unpaired Student's t-test. Data are mean ± SEM. *P < 0.05, ****P < 0.0001.

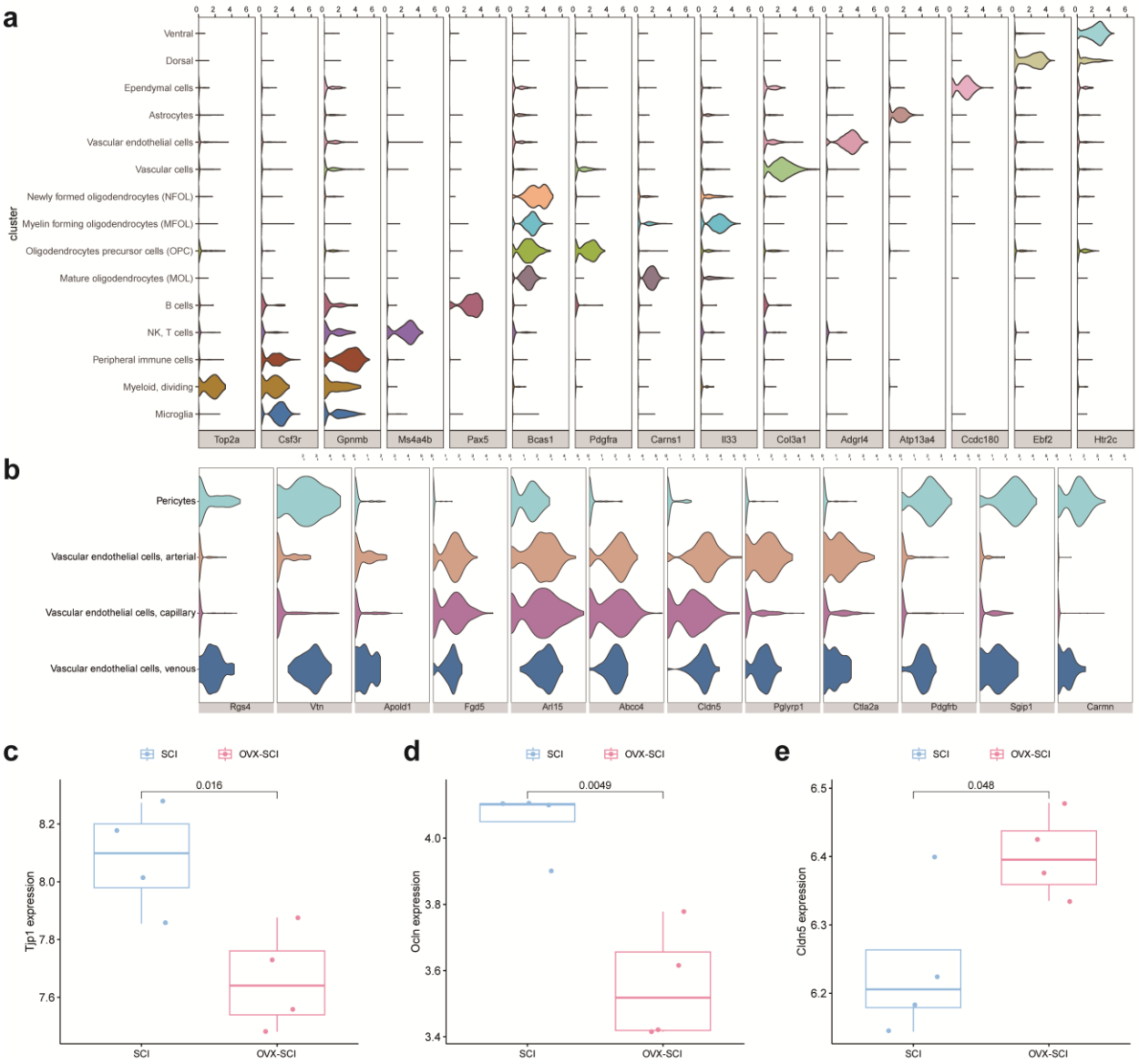
Figure S2 | Public dataset quality control and RNA-sequencing analysis 2 weeks after OVX



a–c, Hierarchical clustering of samples from osteoporosis (OP) and SCI transcriptomic datasets obtained from GEO; the sample GSM6697876 was initially included but was subsequently identified as an outlier by hierarchical sample clustering and excluded from downstream analyses. d,e, Module eigengene adjacency heat maps from WGCNA. f, Principal component analysis of

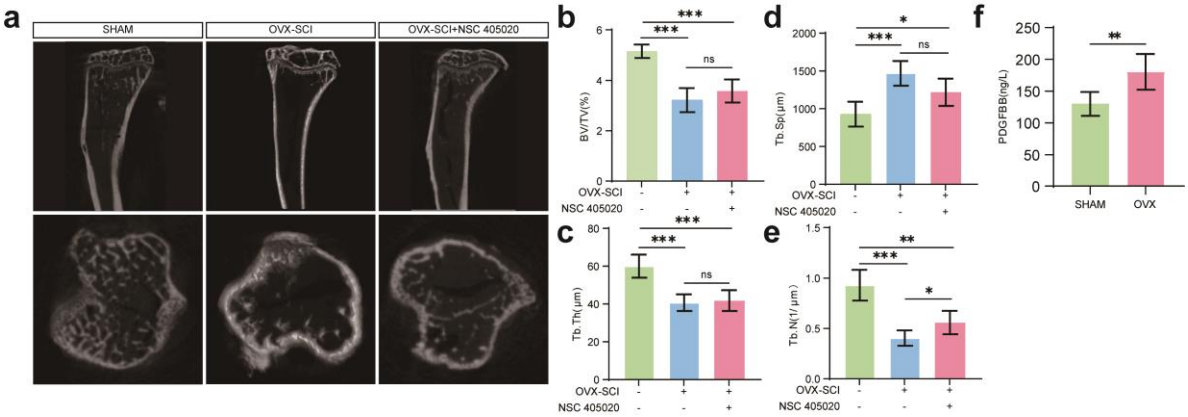
the OVX2-SCI versus SCI dataset. g, Heat map of differentially expressed genes in OVX2-SCI versus SCI. h, Volcano plot for the OVX2-SCI versus SCI comparison. i, Mmp14 expression in OVX2-SCI and SCI spinal cord RNA-sequencing samples. j, Differential expression of MMP family members in OVX2-SCI versus SCI. Statistical analysis: h, nominal $P < 0.05$ and |log2 fold change| > 1 were used for exploratory volcano-plot thresholding. i, two-tailed unpaired Student's t-test. j, DESeq2 with Benjamini–Hochberg-adjusted P values. Unless otherwise indicated, data are mean ± SEM. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$; ns, not significant.

Figure S3 | Marker-gene violin plots in single-nucleus RNA-sequencing data and tight-junction transcripts in OVX-SCI



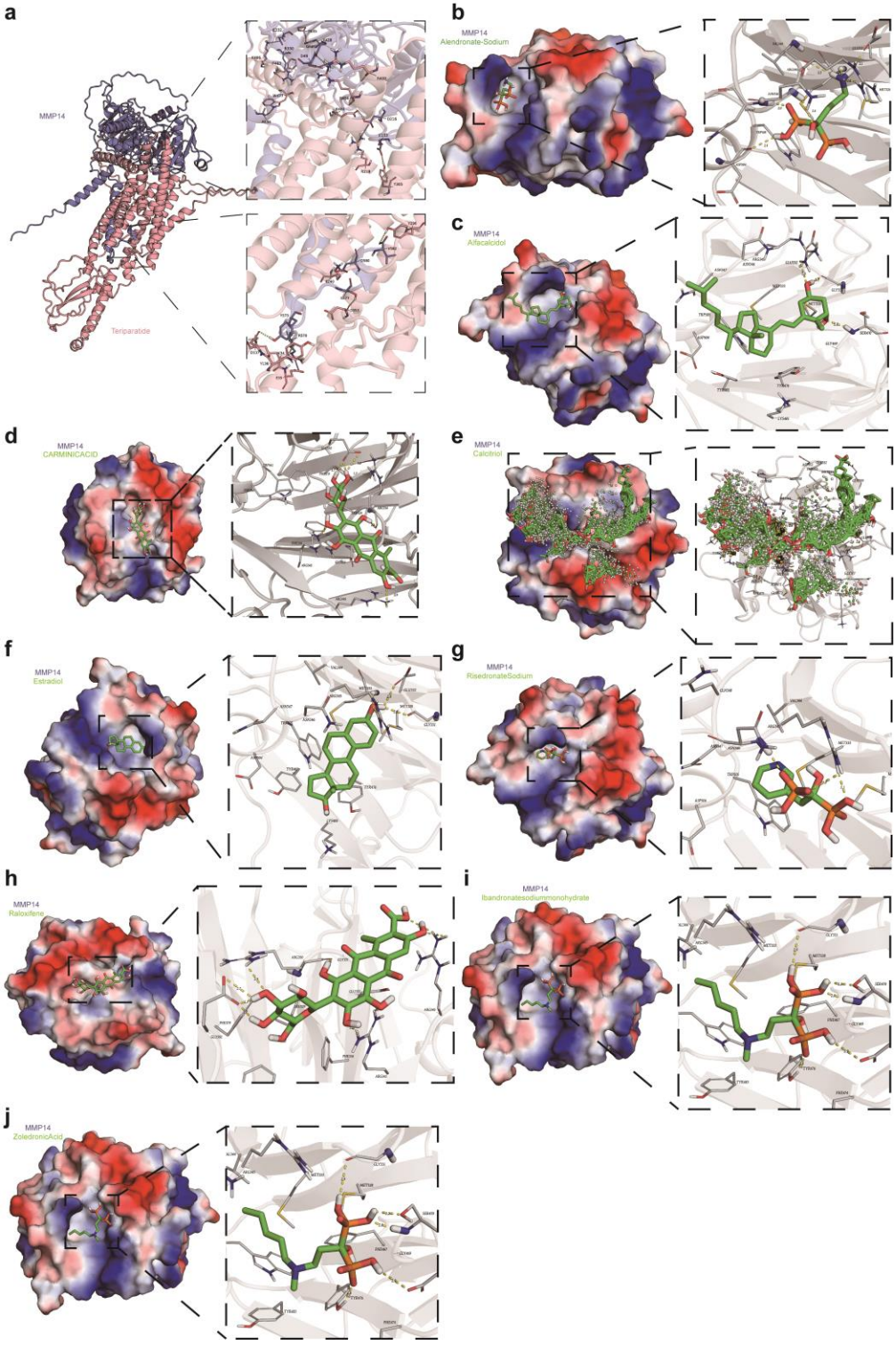
a, Violin plots of canonical marker genes across major spinal cord single-nucleus identities. b, Violin plots of vascular-associated marker genes across endothelial-cell, pericyte, fibroblast and microglial subclusters. c–e, Expression of Tjp1, Ocln, and Cldn5 in OVX-SCI and SCI spinal cord RNA-sequencing samples. Statistical analysis: c–e, two-tailed unpaired Student's t-test based on log2(CPM + 1) values. Data are mean ± SEM. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.

Figure S4 | Bone microarchitecture after NSC 405020 treatment and serum PDGF-BB concentration in OVX mice



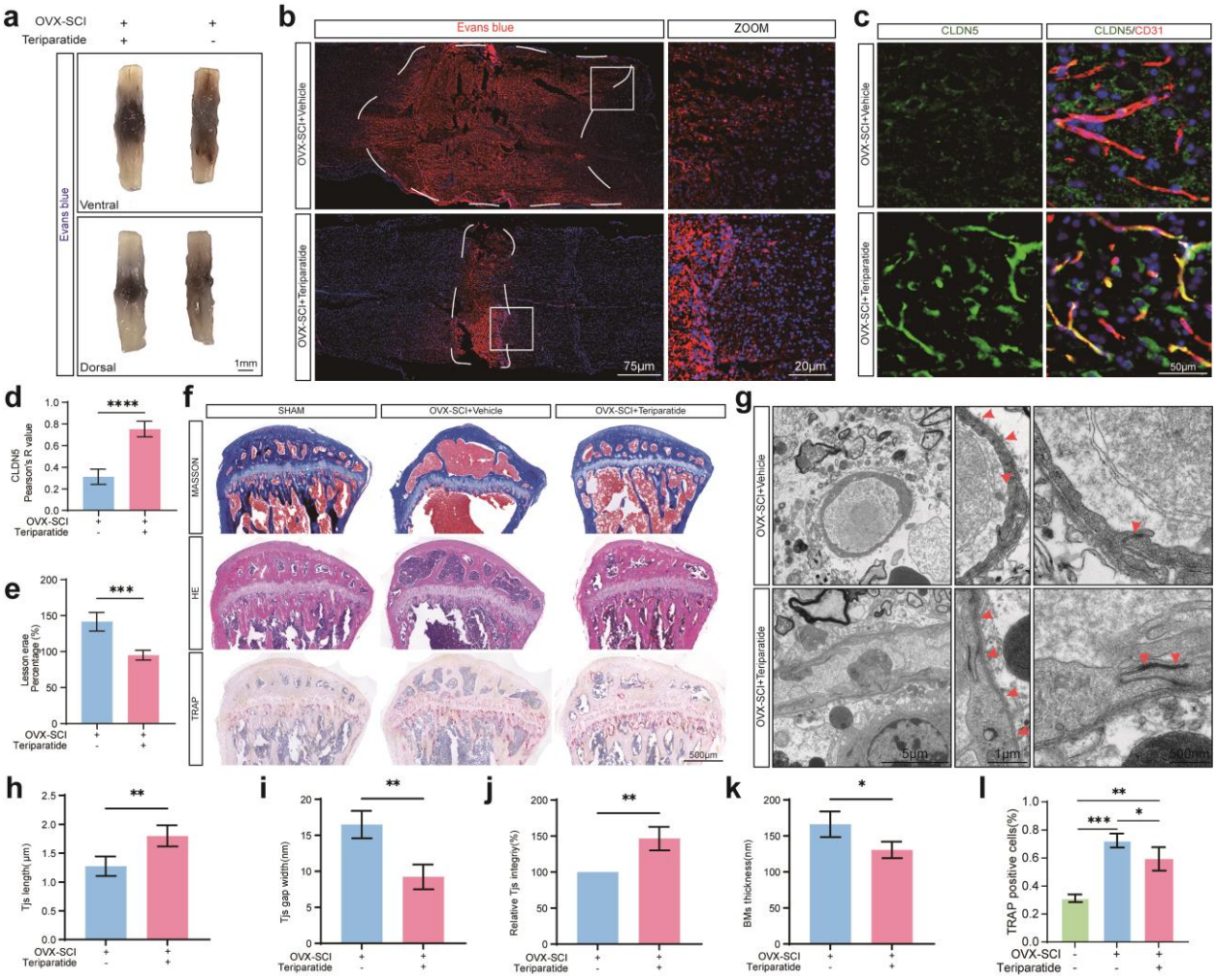
a, Representative proximal tibial micro-CT reconstructions from sham, vehicle-treated, and NSC 405020-treated OVX-SCI mice 28 days after treatment. b–e, Quantification of BV/TV, Tb.Th, Tb.Sp, and Tb.N (n = 5). f, Serum PDGF-BB concentration in SHAM and OVX mice (n = 6). Statistical analysis: b–e, one-way ANOVA followed by post hoc multiple-comparison tests. f, two-tailed unpaired Student's t-test. Data are mean ± SEM. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001; ns, not significant.

Figure S5 | Exploratory structural illustrations of MMP14 with teriparatide and selected compounds



a, Exploratory AlphaFold model of the MMP14–teriparatide complex. b–j, SwissDock-derived poses showing predicted interactions between MMP14 and selected anti-osteoporosis or related compounds. These structural visualisations were descriptive; no formal statistical testing was performed.

Figure S6 | Neurovascular, histological, and skeletal readouts after teriparatide treatment



a,b,e, Gross Evans blue extravasation, section-level Evans blue fluorescence, and lesion-area quantification in injured spinal cord after teriparatide treatment (n = 6). c,d, Representative CLDN5/CD31 immunofluorescence and Pearson colocalisation analysis (n = 6). f, Representative Masson's trichrome, haematoxylin and eosin (H&E), and TRAP staining of femoral sections. g–k, Representative transmission electron microscopy and quantification of tight junction length, tight junction cleft width, tight junction integrity, and basement membrane thickness (n = 6). l, Quantification of TRAP-positive osteoclasts in tibial sections (n = 5). Statistical analysis: d,e and h–k, two-tailed unpaired Student's t-test. l, one-way ANOVA

followed by post hoc multiple-comparison tests. Data are mean ± SEM. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001; ns, not significant.

Supplementary tables

Supplementary Table 1. Antibodies used for immunofluorescence staining

Antibody type	Antibody name	Manufacturer	Catalogue no.	Antibody dilution
Primary antibody	Goat anti-CD31	Servicebio	GB12063	1:200
Primary antibody	Rabbit anti-GFAP	Abcam	ab68428	1:400
Primary antibody	Guinea pig anti-NeuN	Abcam	ab104224	1:800
Primary antibody	Rabbit anti-IBA1	Abcam	ab178846	1:400
Primary antibody	Rabbit anti-PDGFRB	Abcam	ab32570	1:200
Primary antibody	Goat anti-CD13	Bio-Rad	MCA2183	1:200
Primary antibody	Rabbit anti-MMP14	Abcam	ab51074	1:200
Primary antibody	Rabbit anti-ZO-1	Invitrogen	33-9100	1:400
Primary antibody	Rabbit anti-PDGF-B	Abcam	ab23914	1:400
Fluorophore-conjugated primary antibody	Alexa Fluor 488-conjugated Mouse anti-CLDN5	Invitrogen	352588	1:200
Secondary antibody	Goat anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor Plus 488	Invitrogen	A32731TR	1:1000
Secondary antibody	Goat anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor Plus 647	Invitrogen	A32733TR	1:1000
Secondary antibody	Goat anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor Plus 488	Invitrogen	A32723TR	1:1000
Secondary antibody	Goat anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor Plus 647	Invitrogen	A32728TR	1:1000
Secondary antibody	Donkey anti-Goat IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 488	Invitrogen	A-11055	1:1000
Secondary antibody	Donkey anti-Goat IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 647	Invitrogen	A-21447	1:1000
Secondary antibody	Goat anti-Guinea Pig IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 488	Invitrogen	A-11073	1:1000
Secondary antibody	Goat anti-Guinea Pig IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 647	Invitrogen	A-21450	1:1000

Supplementary Table 2. Antibodies used for western blotting

Antibody type	Antibody name	Manufacturer	Catalogue no.	Antibody dilution
Primary antibody	Rabbit anti-MMP14	Abcam	ab51074	1:1000
Primary antibody	HRP-conjugated anti-GAPDH antibody	HuaBio	HA721131	1:10000
Primary antibody	Rabbit anti-ZO-1	Invitrogen	33-9100	1:1000
Primary antibody	Mouse anti-CLDN5	Invitrogen	352588	1:1000
Primary antibody	Rabbit anti-PDGF-B	Abcam	ab178409	1:1000
HRP-conjugated secondary antibody	HRP-linked anti-mouse IgG antibody	CST	#7076	1:10000
HRP-conjugated secondary antibody	HRP-linked anti-rabbit IgG antibody	CST	#7074	1:10000

Supplementary Table 3. Antibodies and reagents used for histological and immunohistochemical staining

Antibody type	Antibody name	Manufacturer	Catalogue no.	Antibody dilution
Primary antibody	Rabbit anti-MMP14	Abcam	ab51074	1:100
Histological staining reagents	H&E staining kit	Servicebio	No. G1005	Not applicable
Histological staining reagents	Masson's trichrome staining kit	Servicebio	No. G1006	Not applicable
Histological staining reagents	TRAP Staining Kit	Servicebio	No. G1050-50T	Not applicable
Histological staining reagents	Oil Red O Stain	Servicebio	No. G1015-100ML	Not applicable

Manufacturer locations for Supplementary Tables 1–3: Servicebio, Wuhan, China; Abcam, Cambridge, UK; Bio-Rad, Hercules, CA, USA; Invitrogen/Thermo Fisher Scientific, Waltham, MA, USA; HuaBio, Hangzhou, China; and Cell Signaling Technology (CST), Danvers, MA, USA.

Supplementary Table 4. Initial eligibility screening of public GEO samples before analytical quality control

GEO accession	GSM or sample ID	Species	Tissue or cell type	Sex or age	Original condition	Drug, genotype, or other manipulation	Decision	Reason for decision
GSE198806	GSM5957097	Mus musculus	bone marrow-derived macrophages; limbs	Age: 8 weeks	sham_rep1; sham	sham; C57BL/6	Included	sham
GSE198806	GSM5957098	Mus musculus	bone marrow-derived macrophages; limbs	Age: 8 weeks	sham_rep2; sham	sham; C57BL/6	Included	sham
GSE198806	GSM5957099	Mus musculus	bone marrow-derived macrophages; limbs	Age: 8 weeks	sham_rep3; sham	sham; C57BL/6	Included	sham
GSE198806	GSM5957100	Mus musculus	bone marrow-derived macrophages; limbs	Age: 8 weeks	ovx_rep1; ovariectomised (OVX)	ovariectomised (OVX); C57BL/6	Included	OP-related condition
GSE198806	GSM5957101	Mus musculus	bone marrow-derived macrophages; limbs	Age: 8 weeks	ovx_rep2; ovariectomised (OVX)	ovariectomised (OVX); C57BL/6	Included	OP-related condition
GSE198806	GSM5957102	Mus musculus	bone marrow-derived macrophages; limbs	Age: 8 weeks	ovx_rep3; ovariectomised (OVX)	ovariectomised (OVX); C57BL/6	Included	OP-related condition
GSE198806	GSM6085319	Mus musculus	mesenchymal stem cell; limbs	Age: 8 weeks	msc_rep1; control	control; C57BL/6	Excluded	Mesenchymal stem cells
GSE198806	GSM6085320	Mus musculus	mesenchymal stem cell; limbs	Age: 8 weeks	msc_rep2; control	control; C57BL/6	Excluded	Mesenchymal stem cells
GSE198806	GSM6085321	Mus musculus	mesenchymal stem cell; limbs	Age: 8 weeks	MRL_rep1; co-cultured with LPS stimulated RAW cells	co-cultured with LPS stimulated RAW cells; C57BL/6	Excluded	LPS stimulation

GEO accession	GSM or sample ID	Species	Tissue or cell type	Sex or age	Original condition	Drug, genotype, or other manipulation	Decision	Reason for decision
GSE198806	GSM6085322	Mus musculus	mesenchymal stem cell; limbs	Age: 8 weeks	MRL_rep2; co-cultured with LPS stimulated RAW cells	co-cultured with LPS stimulated RAW cells; C57BL/6	Excluded	LPS stimulation
GSE198806	GSM6085323	Mus musculus	mesenchymal stem cell; limbs	Age: 8 weeks	MR_rep1; co-cultured with RAW cells	co-cultured with RAW cells; C57BL/6	Excluded	Co-culture with RAW cells
GSE198806	GSM6085324	Mus musculus	mesenchymal stem cell; limbs	Age: 8 weeks	MR_rep2; co-cultured with RAW cells	co-cultured with RAW cells; C57BL/6	Excluded	Co-culture with RAW cells
GSE214645	GSM6613588	Mus musculus	skeletal stem cells	Not reported in GEO	sham-SSC-1.control; sham	sham; WT	Included	sham
GSE214645	GSM6613589	Mus musculus	skeletal stem cells	Not reported in GEO	sham-SSC-2.control; sham	sham; WT	Included	sham
GSE214645	GSM6613590	Mus musculus	skeletal stem cells	Not reported in GEO	Ovx-SSC-1.control; ovariectomy	ovariectomy; WT	Included	OP-related condition
GSE214645	GSM6613591	Mus musculus	skeletal stem cells	Not reported in GEO	Ovx-SSC-2.control; ovariectomy	ovariectomy; WT	Included	OP-related condition
GSE216929	GSM6697872	Mus musculus	Bone Marrow; Macrophage	Sex: Male	Male Macrophage 1; 25 ng/mL MCSF	25 ng/mL MCSF; WT; C57BL/6	Included	sham
GSE216929	GSM6697873	Mus musculus	Bone Marrow; Macrophage	Sex: Male	Male Macrophage 2; 25 ng/mL MCSF	25 ng/mL MCSF; WT; C57BL/6	Included	sham
GSE216929	GSM6697874	Mus musculus	Bone Marrow; Macrophage	Sex: Male	Male Macrophage 3; 25 ng/mL MCSF	25 ng/mL MCSF; WT; C57BL/6	Included	sham

GEO accession	GSM or sample ID	Species	Tissue or cell type	Sex or age	Original condition	Drug, genotype, or other manipulation	Decision	Reason for decision
GSE216929	GSM6697875	Mus musculus	Bone Marrow; Osteoclast	Sex: Male	Male Osteoclast 1; 25 ng/mL MCSF + 100 ng/mL RANKL	25 ng/mL MCSF + 100 ng/mL RANKL; WT; C57BL/6	Included	OP-related condition
GSE216929	GSM6697876	Mus musculus	Bone Marrow; Osteoclast	Sex: Male	Male Osteoclast 2; 25 ng/mL MCSF + 100 ng/mL RANKL	25 ng/mL MCSF + 100 ng/mL RANKL; WT; C57BL/6	Included	OP-related condition
GSE216929	GSM6697877	Mus musculus	Bone Marrow; Osteoclast	Sex: Male	Male Osteoclast 3; 25 ng/mL MCSF + 100 ng/mL RANKL	25 ng/mL MCSF + 100 ng/mL RANKL; WT; C57BL/6	Included	OP-related condition
GSE216929	GSM6697878	Mus musculus	Bone Marrow; Macrophage	Sex: Female	Female Macrophage 1; 25 ng/mL MCSF	25 ng/mL MCSF; WT; C57BL/6	Included	sham
GSE216929	GSM6697879	Mus musculus	Bone Marrow; Macrophage	Sex: Female	Female Macrophage 2; 25 ng/mL MCSF	25 ng/mL MCSF; WT; C57BL/6	Included	sham
GSE216929	GSM6697880	Mus musculus	Bone Marrow; Macrophage	Sex: Female	Female Macrophage 3; 25 ng/mL MCSF	25 ng/mL MCSF; WT; C57BL/6	Included	sham
GSE216929	GSM6697881	Mus musculus	Bone Marrow; Osteoclast	Sex: Female	Female Osteoclast 1; 25 ng/mL MCSF + 100 ng/mL RANKL	25 ng/mL MCSF + 100 ng/mL RANKL; WT; C57BL/6	Included	OP-related condition
GSE216929	GSM6697882	Mus musculus	Bone Marrow; Osteoclast	Sex: Female	Female Osteoclast 2; 25 ng/mL MCSF + 100 ng/mL RANKL	25 ng/mL MCSF + 100 ng/mL RANKL; WT; C57BL/6	Included	OP-related condition

GEO accession	GSM or sample ID	Species	Tissue or cell type	Sex or age	Original condition	Drug, genotype, or other manipulation	Decision	Reason for decision
GSE216929	GSM6697883	Mus musculus	Bone Marrow; Osteoclast	Sex: Female	Female Osteoclast 3; 25 ng/mL MCSF + 100 ng/mL RANKL	25 ng/mL MCSF + 100 ng/mL RANKL; WT; C57BL/6	Included	OP-related condition
GSE235773	GSM7509217	Mus musculus	BMM	Not reported in GEO	BMM biol rep 1; No treatment	No treatment; WT	Included	sham
GSE235773	GSM7509218	Mus musculus	BMM	Not reported in GEO	BMM biol rep 2; No treatment	No treatment; WT	Included	sham
GSE235773	GSM7509219	Mus musculus	BMM	Not reported in GEO	BMM biol rep 3; No treatment	No treatment; WT	Included	sham
GSE235773	GSM7509220	Mus musculus	Mature Osteoclast	Not reported in GEO	Mature Osteoclast biol rep 1; Osteoclastogenic differentiation for 4 days	Osteoclastogenic differentiation for 4 days; WT	Included	OP-related condition
GSE235773	GSM7509221	Mus musculus	Mature Osteoclast	Not reported in GEO	Mature Osteoclast biol rep 2; Osteoclastogenic differentiation for 4 days	Osteoclastogenic differentiation for 4 days; WT	Included	OP-related condition
GSE235773	GSM7509222	Mus musculus	Mature Osteoclast	Not reported in GEO	Mature Osteoclast biol rep 3; Osteoclastogenic differentiation for 4 days	Osteoclastogenic differentiation for 4 days; WT	Included	OP-related condition
GSE243475	GSM7789298	Mus musculus	Bone marrow	Age: 3 months	3-month-old C57BL/6 mouse 1	WT; C57BL/6	Included	sham
GSE243475	GSM7789299	Mus musculus	Bone marrow	Age: 3 months	3-month-old C57BL/6 mouse 2	WT; C57BL/6	Included	sham

GEO accession	GSM or sample ID	Species	Tissue or cell type	Sex or age	Original condition	Drug, genotype, or other manipulation	Decision	Reason for decision
GSE243475	GSM7789300	Mus musculus	Bone marrow	Age: 24 months	24-month-old C57BL/6 mouse 1	WT; C57BL/6	Included	OP-related condition
GSE243475	GSM7789301	Mus musculus	Bone marrow	Age: 24 months	24-month-old C57BL/6 mouse 2	WT; C57BL/6	Included	OP-related condition
GSE273951	GSM8440282	Mus musculus	bone marrow-derived macrophages; macrophage	Not reported in GEO	M1; MCSF	MCSF; C57BL/6J	Included	sham
GSE273951	GSM8440283	Mus musculus	bone marrow-derived macrophages; macrophage	Not reported in GEO	M2; MCSF	MCSF; C57BL/6J	Included	sham
GSE273951	GSM8440284	Mus musculus	bone marrow-derived macrophages; macrophage	Not reported in GEO	M3; MCSF	MCSF; C57BL/6J	Included	sham
GSE273951	GSM8440285	Mus musculus	osteoclast; macrophage	Not reported in GEO	M_R1; MCSF and RANKL	MCSF and RANKL; C57BL/6J	Included	OP-related condition
GSE273951	GSM8440286	Mus musculus	osteoclast; macrophage	Not reported in GEO	M_R2; MCSF and RANKL	MCSF and RANKL; C57BL/6J	Included	OP-related condition
GSE273951	GSM8440287	Mus musculus	osteoclast; macrophage	Not reported in GEO	M_R3; MCSF and RANKL	MCSF and RANKL; C57BL/6J	Included	OP-related condition
GSE273951	GSM8440288	Mus musculus	osteoclast; macrophage	Not reported in GEO	M_R_B1; MCSF, RANKL, BMS309403	MCSF, RANKL, BMS309403; C57BL/6J	Excluded	Treated with BMS309403
GSE273951	GSM8440289	Mus musculus	osteoclast; macrophage	Not reported in GEO	M_R_B2; MCSF, RANKL, BMS309403	MCSF, RANKL, BMS309403; C57BL/6J	Excluded	Treated with BMS309403

GEO accession	GSM or sample ID	Species	Tissue or cell type	Sex or age	Original condition	Drug, genotype, or other manipulation	Decision	Reason for decision
GSE273951	GSM8440290	Mus musculus	osteoclast; macrophage	Not reported in GEO	M_R_B3; MCSF, RANKL, BMS309403	MCSF, RANKL, BMS309403; C57BL/6J	Excluded	Treated with BMS309403
GSE273951	GSM8440291	Mus musculus	osteoclast; macrophage	Not reported in GEO	M_R_R1; MCSF, RANKL, RHC80267	MCSF, RANKL, RHC80267; C57BL/6J	Excluded	Treated with RHC80267
GSE273951	GSM8440292	Mus musculus	osteoclast; macrophage	Not reported in GEO	M_R_R2; MCSF, RANKL, RHC80267	MCSF, RANKL, RHC80267; C57BL/6J	Excluded	Treated with RHC80267
GSE273951	GSM8440293	Mus musculus	osteoclast; macrophage	Not reported in GEO	M_R_R3; MCSF, RANKL, RHC80267	MCSF, RANKL, RHC80267; C57BL/6J	Excluded	Treated with RHC80267

Note: Decision refers to initial eligibility screening before analytical quality control. GSM6697876 met the eligibility criteria and was included at this stage; hierarchical sample clustering subsequently identified it as an outlier, and it was excluded from downstream analyses (Fig. S2a–c).

Abbreviations: BMM, bone marrow-derived macrophage; GEO, Gene Expression Omnibus; LPS, lipopolysaccharide; MCSF, macrophage colony-stimulating factor; OP, osteoporosis; OVX, ovariectomy; RANKL, receptor activator of nuclear factor-κB ligand; WT, wild type.