Bone & Joint Research

Supplementary Material

10.1302/2046-3758.116.BJR-2021-0436.R1

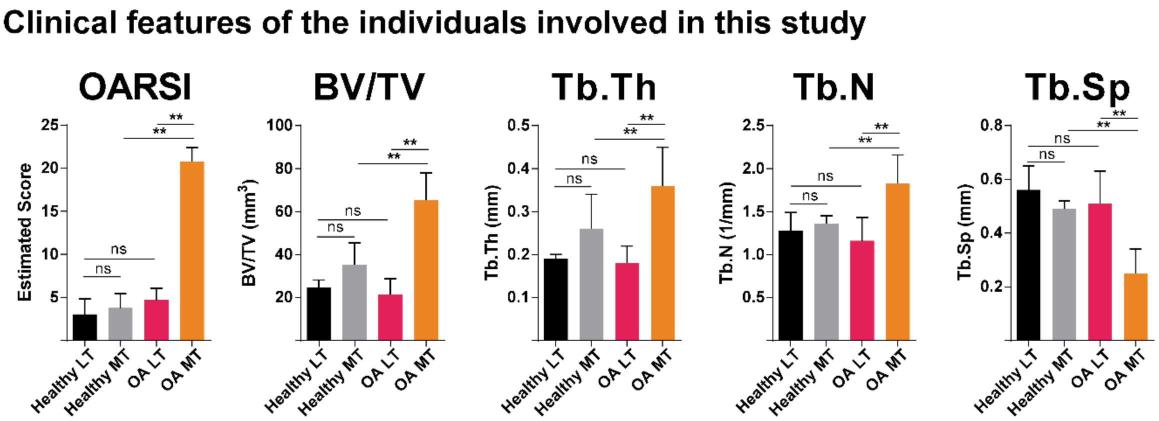

Fig. a. The summary of the clinical features of the individuals involved in this study. This study contains human osteoarthritis (OA) (n = 20) and non-OA (n = 5) knee lateral or medial tibial plateaus (LT and MT). OARSI, Osteoarthritis Research Society International scoring assessment system; BV/TV, bone volume/tissue volume fraction; Tb.Th, trabecular thickness; Tb.N, trabecular number; Tb.Sp, trabecular separation; ns, not significant. *p < 0.05, ** p < 0.01; all p-values estimated by one-way analysis of variance (ANOVA). Data were obtained from original paper of dataset GSE51588.

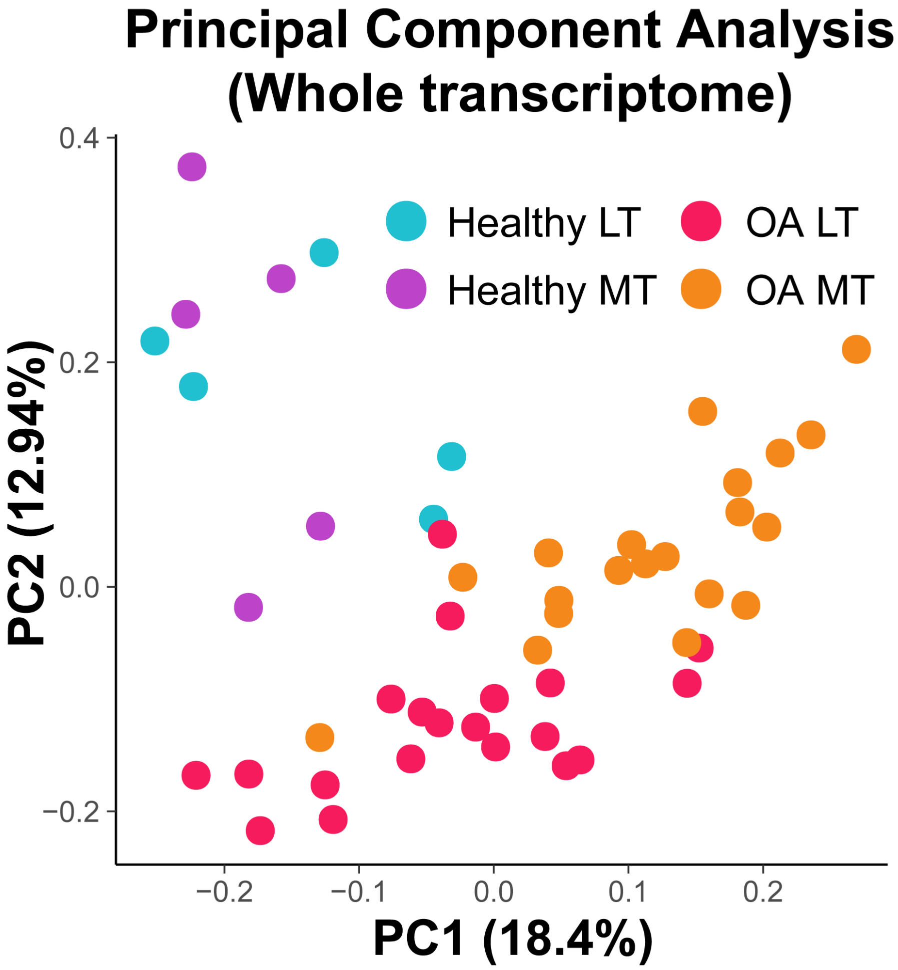

Fig. b. The principal component analysis based on whole transcriptome of subchondral bone transcriptome. LV, lateral tibial plateau; MV, medial tibial plateau; OA, osteoarthritis.

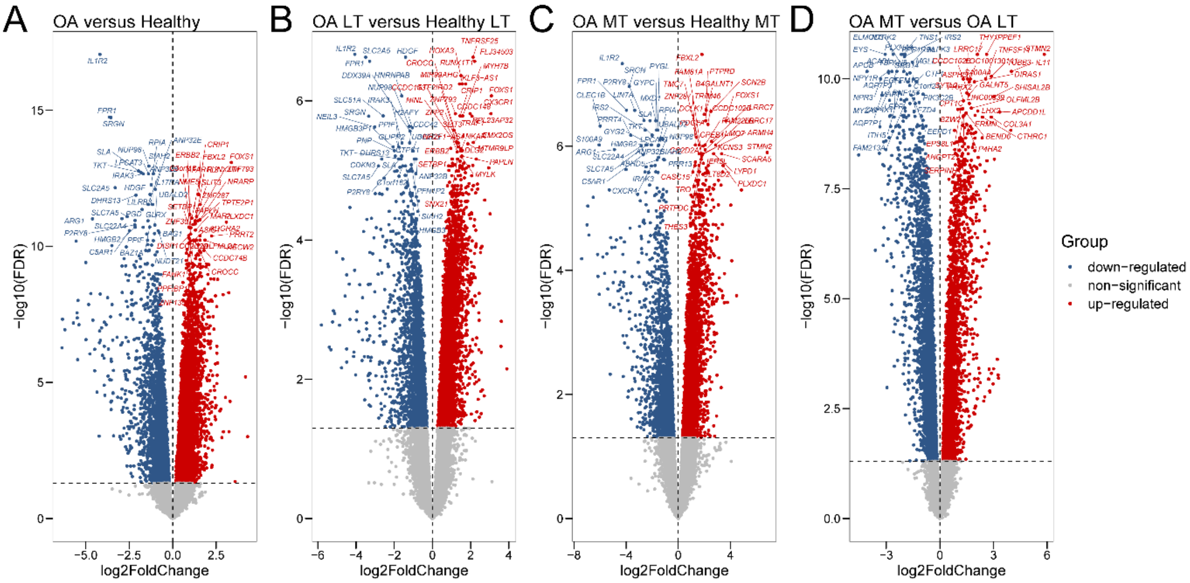

Fig. c. The volcano plots of the differentially expressed genes (DEGs) in the comparisons of the osteoarthritis (OA) versus the healthy samples, the OA lateral tibial plateau (LT) versus the healthy LT, the OA medial tibial plateau (MT) versus the healthy MT, and the OA MT versus the paired LT. The top 30 upregulated or downregulated genes are highlighted in the volcano plots based on false discovery rate (FDR). FDR, p-value adjusted by Benjamini & Hochberg method. FDR < 0.05 was considered statistically significant. Red and blue dots represent upregulated and downregulated genes, respectively.

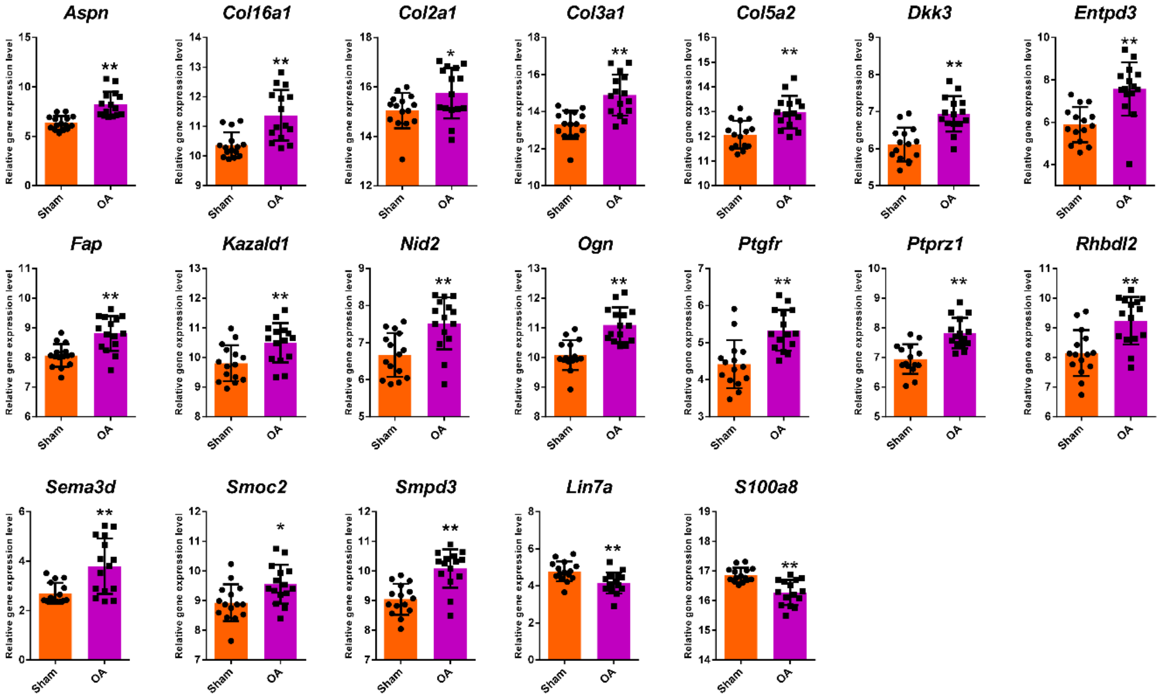

Fig. d. Independent dataset validation of 19 persistent genes. Out of 77 persistent genes, 68 rat genes were identified via homologous mapping. A total of 47 genes were expressed in the validation dataset GSE30322. Overall 23 genes were significant, among which 19 genes were consistent with human data. OA versus sham; *p < 0.05; **p < 0.01; all p-values estimated by unpaired two-tailed *t*-test.

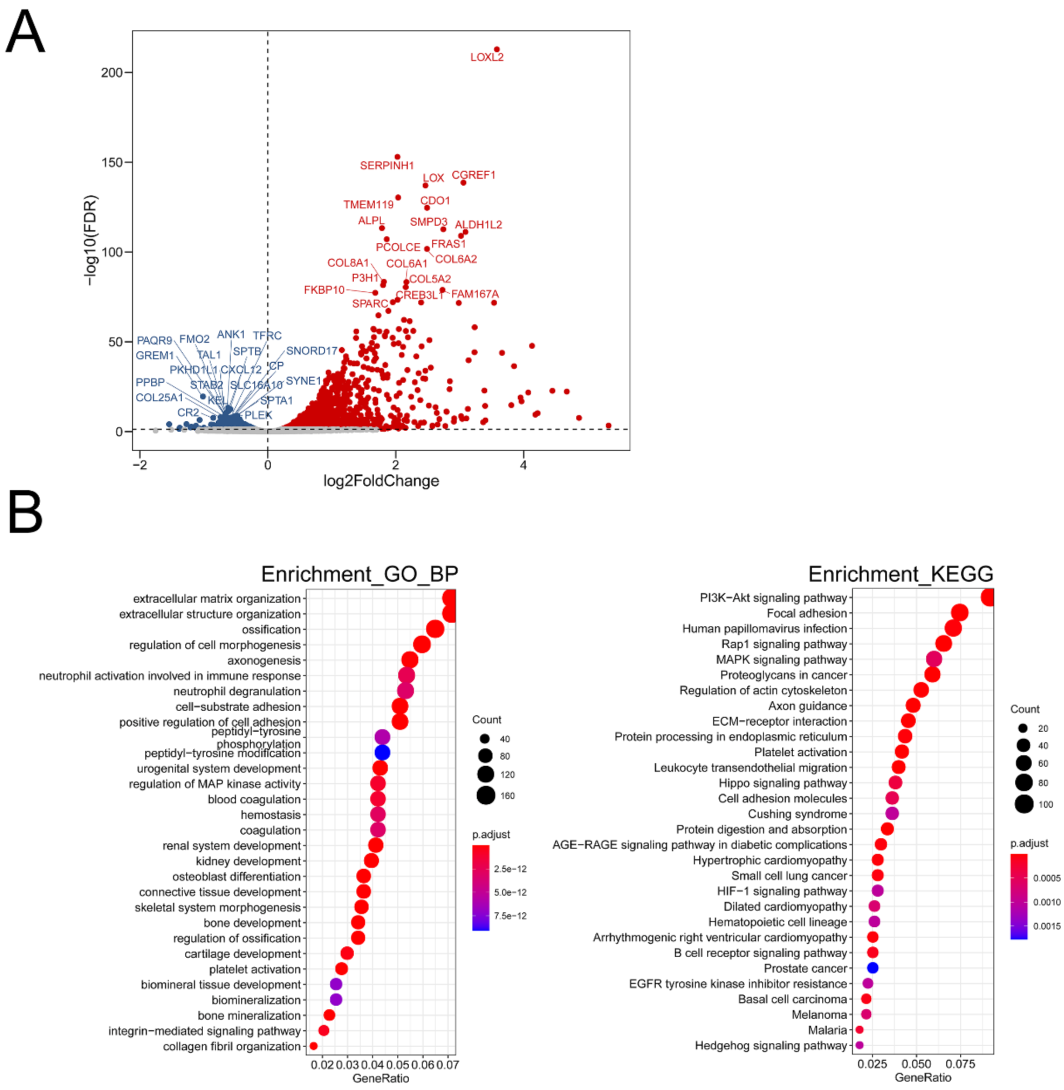

Fig. e. The bone loading-responsive gene set defined by the comparison of the gene expression profiles of four loading and four paired non-loading tibias. a) A total of 2,632 genes were identified as the transcriptome response to mechanics. FDR, p-value adjusted by Benjamini & Hochberg method. FDR < 0.05 was considered statistically significant. The top 20 upregulated or downregulated genes are highlighted in the volcano plot. b) Gene Ontology (GO) terms and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways of the persistent genes. GeneRatio indicates the gene number ration in each GO term or KEGG pathway. The colour and size of each dot represents a p-value adjusted by Benjamini & Hochberg method and gene number assigned to the corresponding GO term and KEGG pathway, respectively.

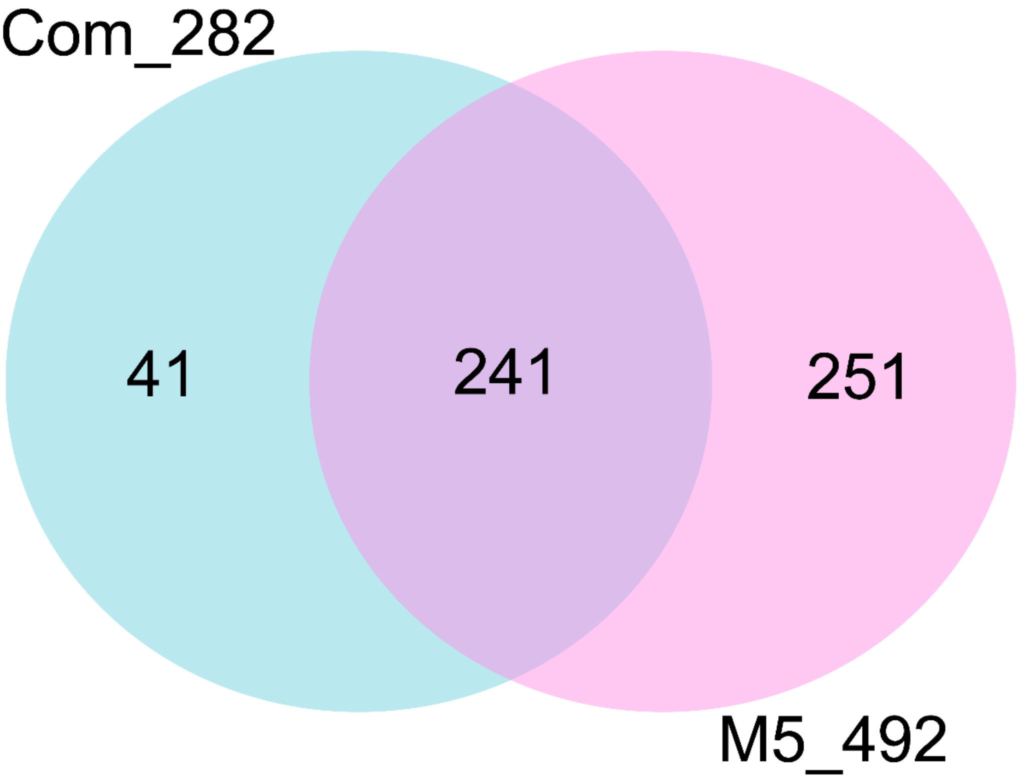

Fig. f. The overlapping of the 282 common genes and Module 5. More than 85% of the 282 common genes were assigned to this module. The overlapped 241 co-expressing common genes were identified as the osteocyte mechanics-responsive genes.

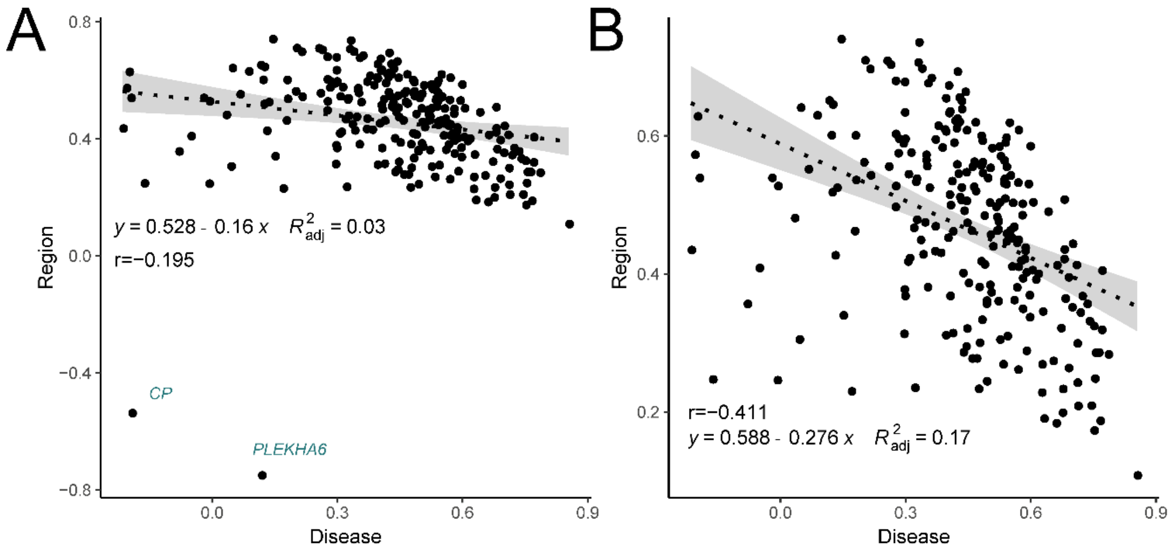

Fig. g. The scatter plot of the Pearson correlation coefficient of the correlations of the genes in Module 5 and disease or region. The correlation between gene-disease and gene-region was poor (A), even though two outlier genes (CP and PLEKHA6) were removed from the analysis (B).

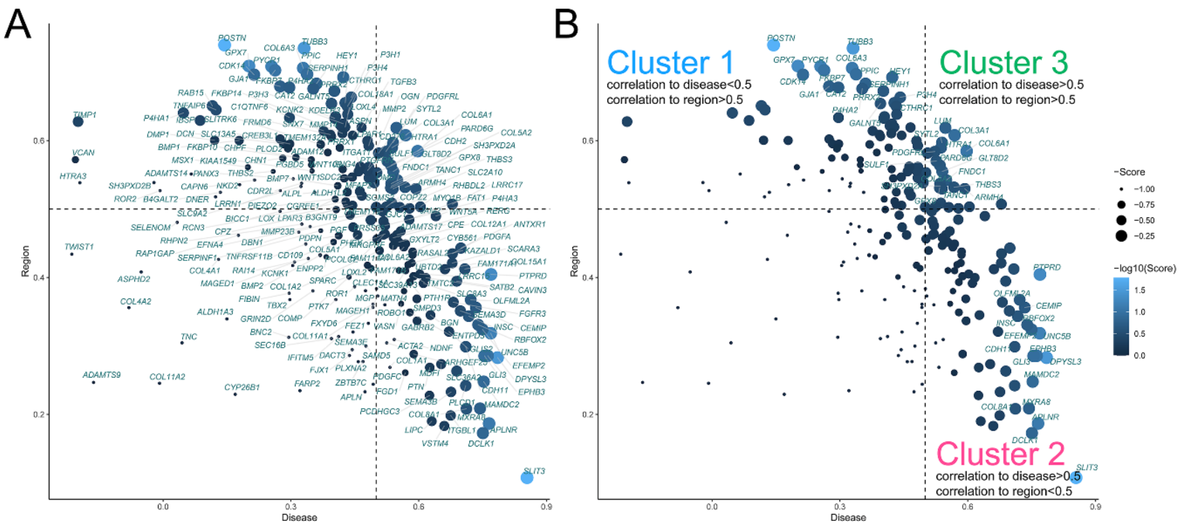

Fig. h. The three clusters of mechanics-responsive genes relevant to osteocyte function. Pearson correlation coefficient *r* = 0.5 was applied as a cut-off to separate the genes in Module 5 into three clusters (A): cluster 1, the late-responsive genes; cluster 2, the early-responsive genes; and cluster 3, the persistently responsive genes. Estimated score was calculated by robust rank aggregation (RRA) algorithm to integrate the gene-disease and gene-region correlations (B).

Table i. Validation of persistent genes by an independent rat osteoarthritis (OA) subchondral bone dataset GSE30322.

ID	**logFC**	**p-value**	**Conclusion**
Lrrc15	-2.04	6E-07	conflict
Ogn	1.02	2E-05	validated
Dkk3	0.83	4E-05	validated
Smpd3	1.03	5E-05	validated
Ptprz1	0.88	5E-05	validated
Aspn	1.87	6E-05	validated
S100a8	-0.59	1E-04	validated
Col3a1	1.60	1E-04	validated
Entpd3	1.68	2E-04	validated
Ptgfr	0.91	3E-04	validated
Col5a2	0.91	4E-04	validated
Fap	0.75	4E-04	validated
Col16a1	1.01	5E-04	validated
Rhbdl2	1.09	7E-04	validated
Nid2	0.85	1E-03	validated
Sema3d	1.10	2E-03	validated
Lin7a	-0.62	4E-03	validated
Kazald1	0.69	6E-03	validated
Smoc2	0.62	1E-02	validated
Fcar	0.42	1E-02	conflict
Ssc5d	-1.15	2E-02	conflict
Col2a1	0.71	4E-02	validated
Fasn	0.30	4E-02	conflict
Col1a2	-0.41	8E-02	not significant
Slc8a3	-0.35	9E-02	not significant
Nampt	-0.46	1E-01	not significant
Lrrc17	0.50	1E-01	not significant
Olfml2a	0.31	1E-01	not significant
Dapl1	-0.26	2E-01	not significant
Olfm4	0.47	2E-01	not significant
Fn1	0.18	2E-01	not significant
Bfsp1	0.15	3E-01	not significant
Cst7	-0.17	4E-01	not significant
Thy1	0.30	4E-01	not significant
Plac8	-0.09	4E-01	not significant
Mmrn1	-0.22	5E-01	not significant
Angptl4	-0.15	5E-01	not significant
Prss57	-0.17	5E-01	not significant
Anxa3	0.07	6E-01	not significant
Adamtsl2	0.19	6E-01	not significant
Irgm	-0.23	6E-01	not significant
Slc4a1	-0.21	6E-01	not significant

Tnnt3	-0.22	7E-01	not significant
Gpx3	-0.12	8E-01	not significant
Timp4	0.08	8E-01	not significant
Grin2a	-0.04	8E-01	not significant
Slc2a3	-0.01	1E+00	not significant

FC, fold-change value (OA versus sham). All p-values estimated by unpaired two-tailed *t*-test.

Table ii. Validation of the critical bone loading-responsive genes by an independent rat osteoarthritis subchondral bone dataset GSE30322.

ID	logFC	p-value	Conclusion
Fzd1	0.88	8.71E-07	validated
Srpx	0.71	3.35E-06	validated
Ogn	1.02	2.24E-05	validated
Smo	-1.58	3.60E-05	conflict
Aspn	1.87	5.98E-05	validated
Thbs2	1.43	8.99E-05	validated
Col3a1	1.60	1.09E-04	validated
Fbn2	-0.77	2.03E-04	conflict
Epha4	0.97	2.59E-04	validated
Col5a2	0.91	3.68E-04	validated
Dpt	1.49	4.76E-04	validated
Mfap5	0.79	4.85E-04	validated
Col6a1	0.81	7.81E-04	validated
Efemp2	0.62	8.79E-04	validated
Postn	1.59	1.13E-03	validated
Nid2	0.85	1.26E-03	validated
Tgfb2	0.76	1.27E-03	validated
Dcn	0.83	1.51E-03	validated
Col15a1	0.76	1.91E-03	validated
Col11a1	1.10	2.08E-03	validated
Sema3d	1.10	2.36E-03	validated
Cthrc1	1.23	3.09E-03	validated
Bgn	0.53	3.32E-03	validated
Pcolce	0.75	3.78E-03	validated
Ephb2	-0.62	3.83E-03	conflict
Lum	0.68	4.96E-03	validated
Col18a1	0.82	5.18E-03	validated
Mgp	0.67	1.13E-02	validated
Tgfb3	0.62	1.37E-02	validated
Col4a1	0.57	1.45E-02	validated
Bmp7	-0.42	1.92E-02	conflict
Acvr1	0.61	2.10E-02	validated
Col5a3	0.52	2.41E-02	validated
Col8a1	0.72	2.88E-02	validated
Ephb3	-0.53	3.45E-02	conflict
Col12a1	0.87	4.03E-02	validated
Inhba	-0.78	5.88E-02	not significant
Comp	0.72	6.48E-02	not significant
Vwa1	0.36	7.69E-02	not significant
Col1a2	-0.41	8.28E-02	not significant
Col1a1	0.38	1.06E-01	not significant
Serpinf1	0.58	1.17E-01	not significant

Sparc	0.49	2.04E-01	not significant
Mfap2	0.37	2.19E-01	not significant
Fn1	0.18	2.25E-01	not significant
Pik3r2	-0.18	2.42E-01	not significant
Col6a2	0.32	2.70E-01	not significant
Ror2	-0.24	2.79E-01	not significant
Emilin1	-0.28	2.83E-01	not significant
Tnc	0.32	3.45E-01	not significant
Eln	-0.22	3.83E-01	not significant
Vcan	-0.24	4.17E-01	not significant
Nkd2	0.17	4.57E-01	not significant
Col4a2	0.19	5.79E-01	not significant
Slit3	0.09	5.94E-01	not significant
Fyn	-0.09	6.83E-01	not significant
Bmp2	0.08	7.26E-01	not significant
Aebp1	-0.14	7.59E-01	not significant
Camk2b	0.01	9.57E-01	not significant
Tcf7	0.00	9.85E-01	not significant

FC, fold-change value (osteoarthritis versus sham). All p-values estimated by unpaired two-tailed *t*-test.