Chroogomphus rutilus Regulates Bone Metabolism to Prevent Periodontal Bone Loss during Orthodontic Tooth Movement in Osteoporotic Rats

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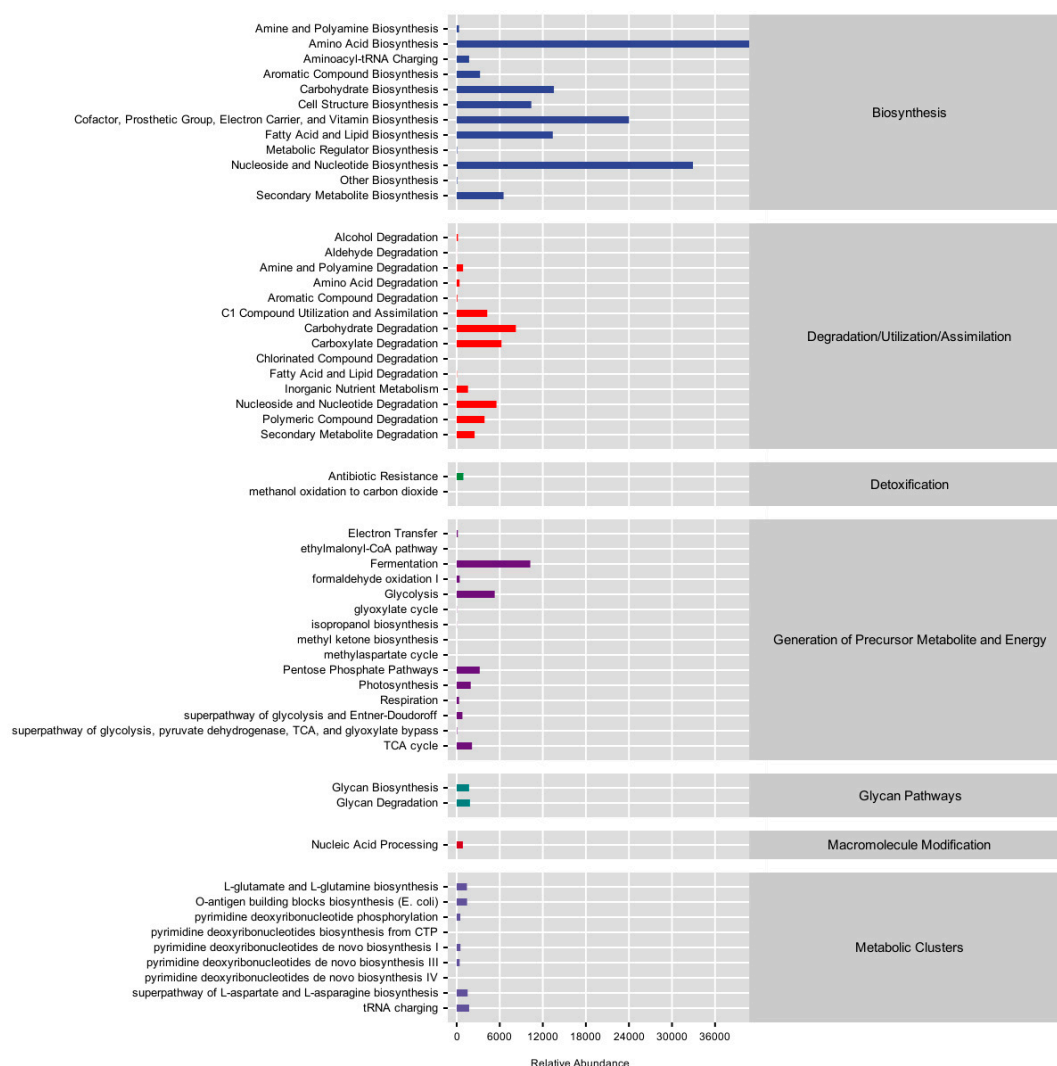* Correspondence: jluwangdi@jlu.edu.cn (D.W.); humin@jlu.edu.cn (M.H.)
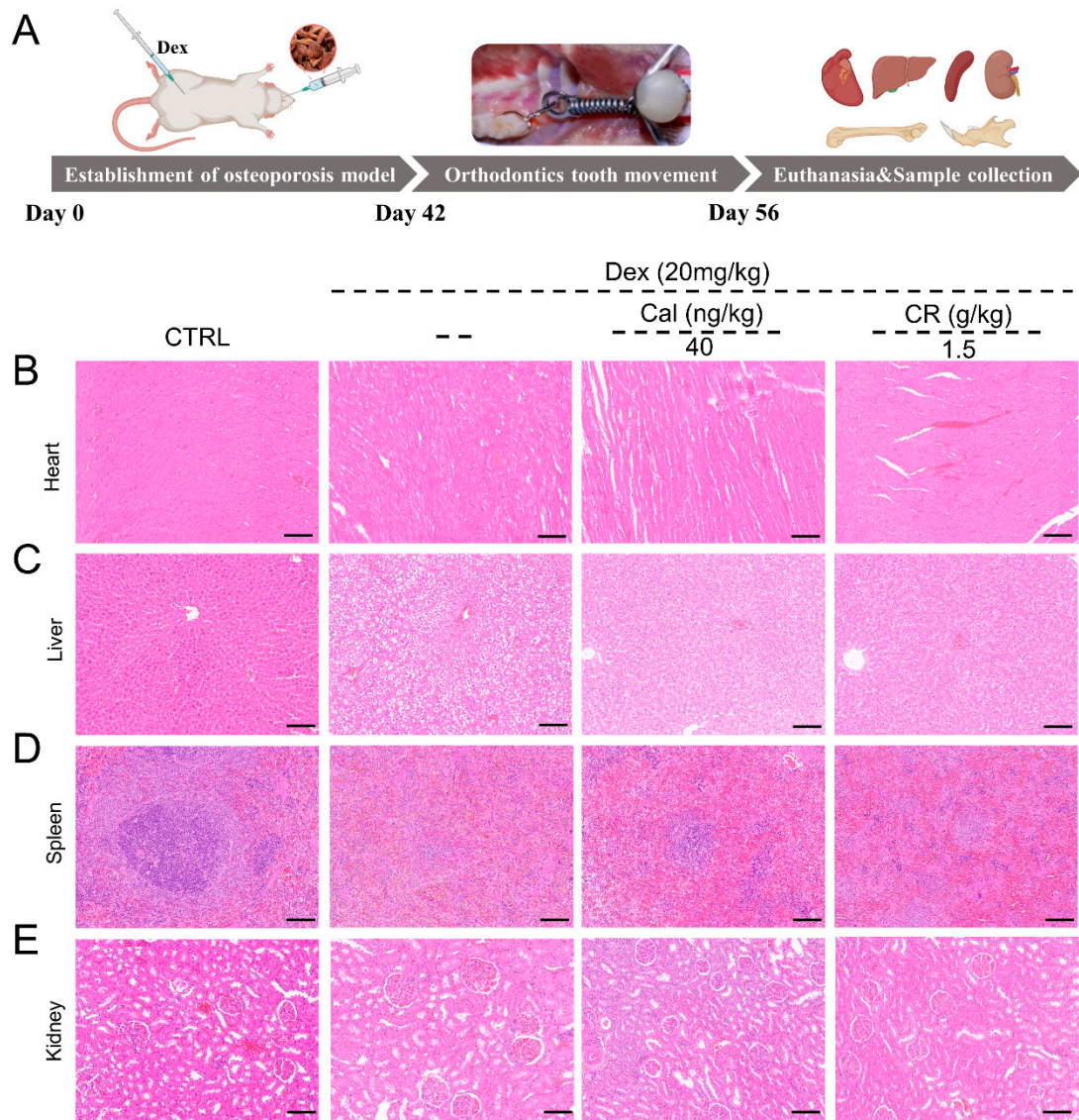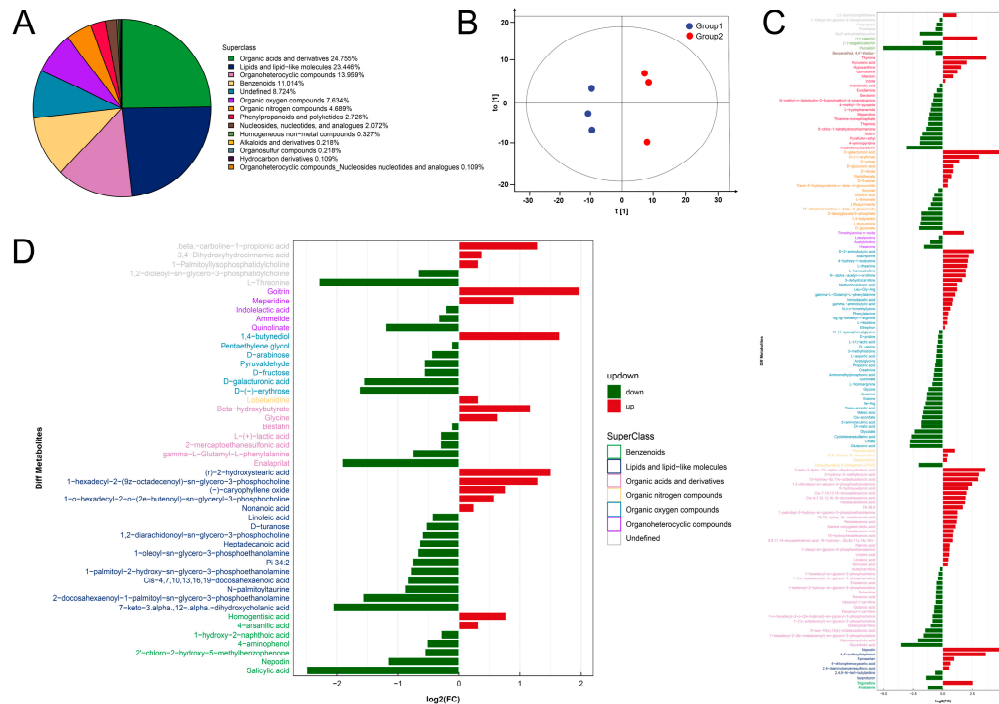
Figure S1. (A) Flow chart of animal experiment. H&E staining analysis of the **(B)** heart, **(C)** liver, **(D)** spleen, and **(E)** kidney of experimental rats (n = 3/group, 200×, scale bar: 50 µm
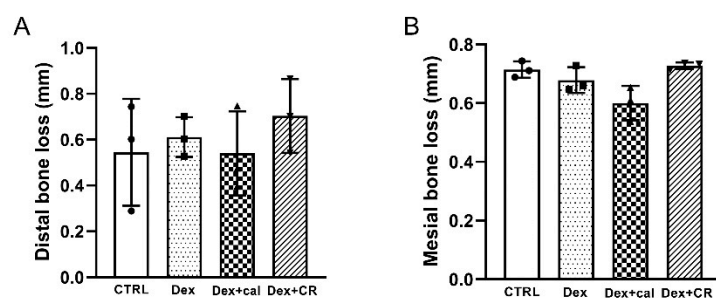
Figure S2. Mesial **(A)** and distal **(B)** bone loss for each group (*n* = 3/group).

Figure S3. MetaCyc database-based functional pathway analysis.

Figure S4. CR modified the metabolite levels in serum of osteoporosis (OP) rats. **(A)** Proportion of identified metabolites in each chemical class. **(B)** OPLS-DA score plots for the CTRL and vehicle-treated OP rats. **(C)** Differential fold analysis of significantly different metabolites between CTRL rats and vehicle-treated OP rats. **(D)** Differential fold analysis of significantly different metabolites between vehicle-treated and CR-treated OP rats ($n = 3$/group).
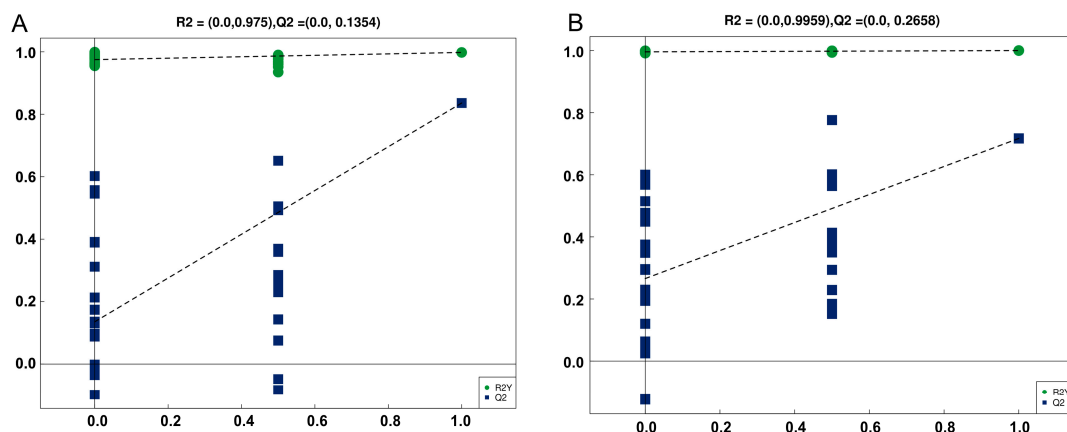
Figure S5. (A) OPLS-DA permutation test for the CTRL and vehicle-treated OP rats; **(B)** OPLS-DA permutation test for the vehicle-treated and CR-treated OP rats

Table S1. Details of antibodies used in IHC

Antibody	Molecular	Catalog number	Dilution for IHC	Company	Area
Runx2	57 kDa	AF5186	1:200	Affinity	Jiangsu, China
Osterix	45 kDa	DF7731	1:200	Affinity	Jiangsu, China
OPG	46 kDa	DF6824	1:200	Affinity	Jiangsu, China
Rankl	35 kDa	AF0313	1:200	Affinity	Jiangsu, China
Wnt1	49 kDa	A2475	1:200	Abclonal	Wuhan, China
GSK-3β	46 kDa	A2081	1:200	Abclonal	Wuhan, China
β-catenin	86 kDa	Bs-1165R	1:500	Bioss	Beijing, China
TNF-α	48 kDa	A0277	1:200	Abclonal	Wuhan, China
IL-1β	32 kDa	Bs-0812R	1:500	Bioss	Beijing, China
IL-6	24 kDa	DF6087	1:200	Affinity	Jiangsu, China

Table S2. The effect of CR on bodyweight and organ indexes in OP rats.

	Days	CTRL	Dex (20 mg/kg)		
			--	Cal (40 ng/kg)	CR (1.5 g/kg)
Body weight (g)	0 day	220.11 ± 11.05	224.09 ± 7.84	226.53 ± 5.94	227.91 ± 7.47
	7th day	267.30 ± 13.70	184.13 ± 9.12###	191.07 ± 16.63	193.36 ± 14.85
	14th day	323.56 ± 24.16	217.66 ± 53.17###	194.35 ± 18.78	193.05 ± 20.67
	21st day	342.69 ± 31.46	216.24 ± 54.80###	201.98 ± 15.72	189.89 ± 25.48
	28th day	323.59 ± 23.59	187.71 ± 7.67###	196.09 ± 18.30	189.18 ± 15.84
	35th day	354.61 ± 22.33	206.91 ± 52.48###	194.08 ± 15.17	189.49 ± 9.44
	42nd day	361.57 ± 21.07	199.44 ± 54.73###	177.86 ± 22.08	209.96 ± 13.58
	49th day	334.35 ± 19.17	199.14 ± 46.87###	183.62 ± 24.18	209.68 ± 19.42
	56th day	320.51 ± 22.19	189.14 ± 44.04###	193.79 ± 19.47	204.58 ± 17.47
Organ Index (%)	Heart	0.39 ± 0.58	0.58 ± 0.78###	0.51±0.72	0.65±1.01
	Liver	2.45 ± 0.25	5.84 ± 0.87###	3.82±0.53***	3.81±0.54***
	Spleen	0.14 ± 0.34	0.16 ± 0.34	0.19±0.42	0.16±0.46
	Kidney	0.68 ± 0.33	1.08 ± 0.93###	1.03±0.89	1.05±2.12

The data were analyzed using a one-way ANOVA and expressed as mean $\pm$ SEM (n = 15/group) [###]$P < 0.001$ *versus* CTRL rats; [***]$P < 0.001$ *versus* vehicle-treated OP rats.

Table S3. Relative abundance of top 20 genera.

Taxa	Group1	Group2	Group3
Faecalibaculum	0.1208	0.3410	0.2241
Muribaculum	0.0552	0.0753	0.1067
Lactobacillus	0.0064	0.1702	0.0181
Akkermansia	0.1216	0.0000	0.0000
Desulfovibrio	0.0156	0.0042	0.0115
Bacteroides	0.0067	0.0032	0.0170
Helicobacter	0.0073	0.0062	0.0120
Lachnoclostridium	0.0071	0.0044	0.0135
Ruminococcus	0.0115	0.0015	0.0050
Escherichia	0.0016	0.0119	0.0028
Turicibacter	0.0092	0.0025	0.0025
Prevotella	0.0022	0.0008	0.0092
Clostridium	0.0078	0.0034	0.0008
Streptococcus	0.0007	0.0079	0.0012
Faecalibacterium	0.0074	0.0000	0.0018
Aerococcus	0.0073	0.0008	0.0000
Alistipes	0.0039	0.0010	0.0030
Eisenbergiella	0.0010	0.0005	0.0046
Oscillibacter	0.0026	0.0000	0.0030
Flavonifractor	0.0030	0.0001	0.0014

Data are presented as the mean (n = 3/group). Group 1: CTRL rats; group 2: vehicle-treated OP rats; group 3: CR-treated OP rats.

Table S4. The differential metabolites of serum among experimental rats.

Metabolites	Group1	Group2	Group3	Group1 *vs* Group2		Group2 *vs* Group3	
				VIP	*P*-value	VIP	*P*-value
D-(-)-erythrose	25577805	187907396	64982362	1.579	0.020	1.677	0.031
Linoleic acid	1190670335	1868923221	1293795241	3.113	0.002	3.340	0.033
D-fructose	1033144948	1597061782	1030726929	2.837	0.018	3.390	0.027
D-galacturonic acid	7062557	317184933	111059612	2.215	0.013	2.100	0.048
Pyruvaldehyde	169621683	255993382	164406299	1.120	0.037	1.331	0.042
(-)-caryophyllene oxide	15619361	9533056	15298358	1.246	0.008	1.398	0.002
O-LPE	24063556	34451194	21924960	1.571	0.014	1.877	0.007
Gamma-L-Glutamyl-L-phenylalanine	9673371	23064031	13855925	1.861	0.000	1.575	0.015
LPE (16:0)	108250428	250992724	155883221	6.013	0.000	5.116	0.018
C16-20:4 PC	122796151	65626677	101577835	3.826	0.046	3.284	0.035
PE (16:0/22：6)	1940802	10506550	4237365	1.467	0.001	1.311	0.037
DOPC	50772497	48838416	28472184	0.767	0.898	2.318	0.041
Gamma-glutamylvaline	36913530	29485709	37748683	1.584	0.021	1.681	0.021
Phosphocreatine	9776492	1430439	4445177	1.475	0.001	0.907	0.042
PE (18:0/22:6)	1400720	9318459	4047688	1.415	0.006	1.122	0.047

Data are presented as the mean (*n* = 3/group). Differences were considered statistically significant at *P* < 0.05 and VIP > 1. Group 1: CTRL rats; group 2: vehicle-treated OP rats; group 3: CR-treated OP rats.