

Dual Role of Interleukin-20 in Different Stages of Osteoclast Differentiation and Its Osteoimmune Regulation during Alveolar Bone Remodeling

Bowen Meng^{1,2,3,†}, Benyi Yang^{1,2,3,†}, Yan Qu^{1,2,3}, Yuanbo Liu^{1,2,3}, Dongle Wu^{1,2,3}, Chaoran Fu^{1,2,3}, Yifan He^{1,2,3}, Xi Chen^{1,2,3}, Chufeng Liu⁴, Xiaoxing Kou^{1,2,3,*} and Yang Cao^{1,2,3,*}

¹ Hospital of Stomatology, Sun Yat-sen University, Guangzhou 510055, China.

² Guangdong Provincial Key Laboratory of Stomatology, Guangzhou 510055, China.

³ South China Center of Craniofacial Stem Cell Research, Guanghua School of Stomatology, Sun Yat-sen University, Guangzhou 510055, China

⁴ Department of Orthodontics, Stomatological Hospital, Southern Medical University, No.366 Jiangnan Da Dao South Haizhu District, Guangzhou 510260, China

* Correspondence:

kouxiaoxing@mail.sysu.edu.cn (X.K.); caoyang@mail.sysu.edu.cn (Y.C.)

Correspondences

Prof. Yang Cao, Hospital of Stomatology, Guanghua School of Stomatology, Sun Yat-sen University, Guangdong Provincial Key Laboratory of Stomatology, Guangzhou 510055, China.

Tel: +8613922278766

Email: caoyang@mail.sysu.edu.cn

Prof. Xiaoxing Kou, South China Center of Craniofacial Stem Cell Research, Hospital of Stomatology, Guanghua School of Stomatology, Sun Yat-sen University, Guangdong Provincial Key Laboratory of Stomatology, Guangzhou 510055, China.

Tel: +8613581814339

Email: kouxiaoxing@mail.sysu.edu.cn

† These authors contributed equally to this work.

Supplementary Figures and figure legends

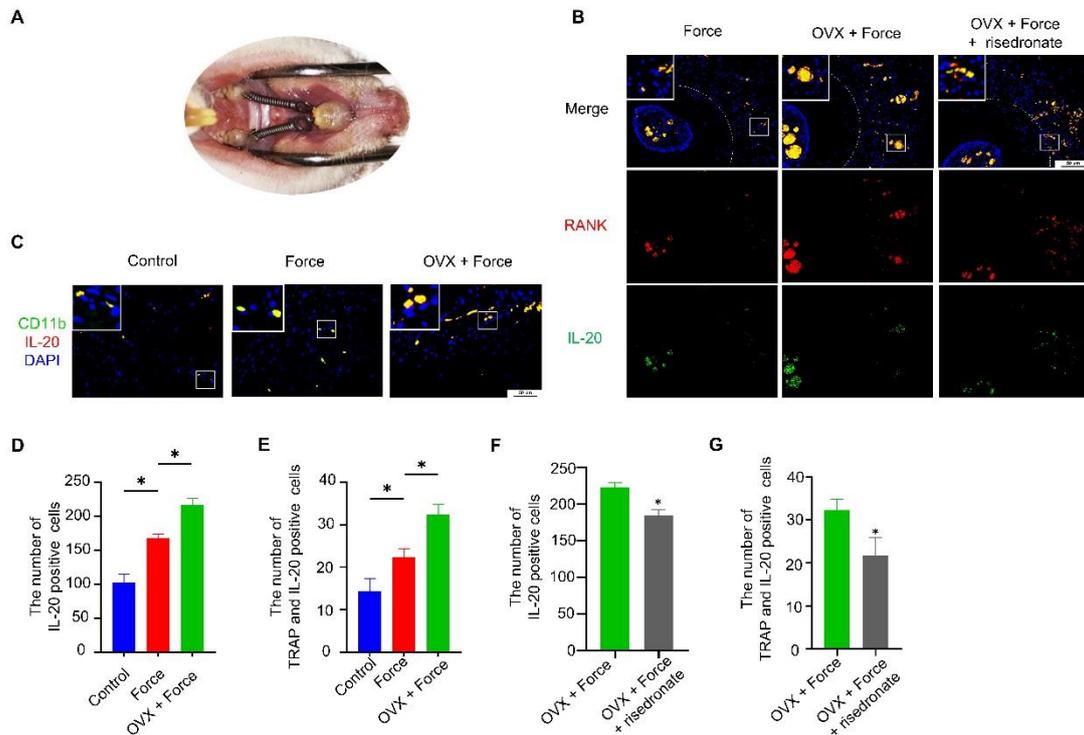


Figure S1. IL-20 accelerated orthodontic tooth movement. **(A)** The rat model of orthodontic tooth movement. **(B)** Double-labelled immunofluorescence staining showed that, in the context of orthodontic force, the expression levels of IL-20 and osteoclast marker protein RANK increased in the first molar periodontal ligament. **(C)** Immunofluorescence staining showed that the expression levels of IL-20 and CD11b in the first molar periodontal ligament after the application of orthodontic force. **(D)** The statistical analysis of IL-20 positive cells in the Control group, Force group, and OVX + Force group. **(E)** The statistical analysis of TRAP and IL-20 positive cell number of the first molar periodontal ligament in the Control group, Force group and OVX + Force group. **(F)** The statistical analysis of IL-20 positive cells in the OVX + Force group and OVX + Force + risedronate group. **(G)** The statistical analysis of TRAP and IL-20 positive cell number of the first molar periodontal ligament in the OVX + Force group and OVX + Force + risedronate group. * $p < 0.05$ vs. the control group. $n = 6$.

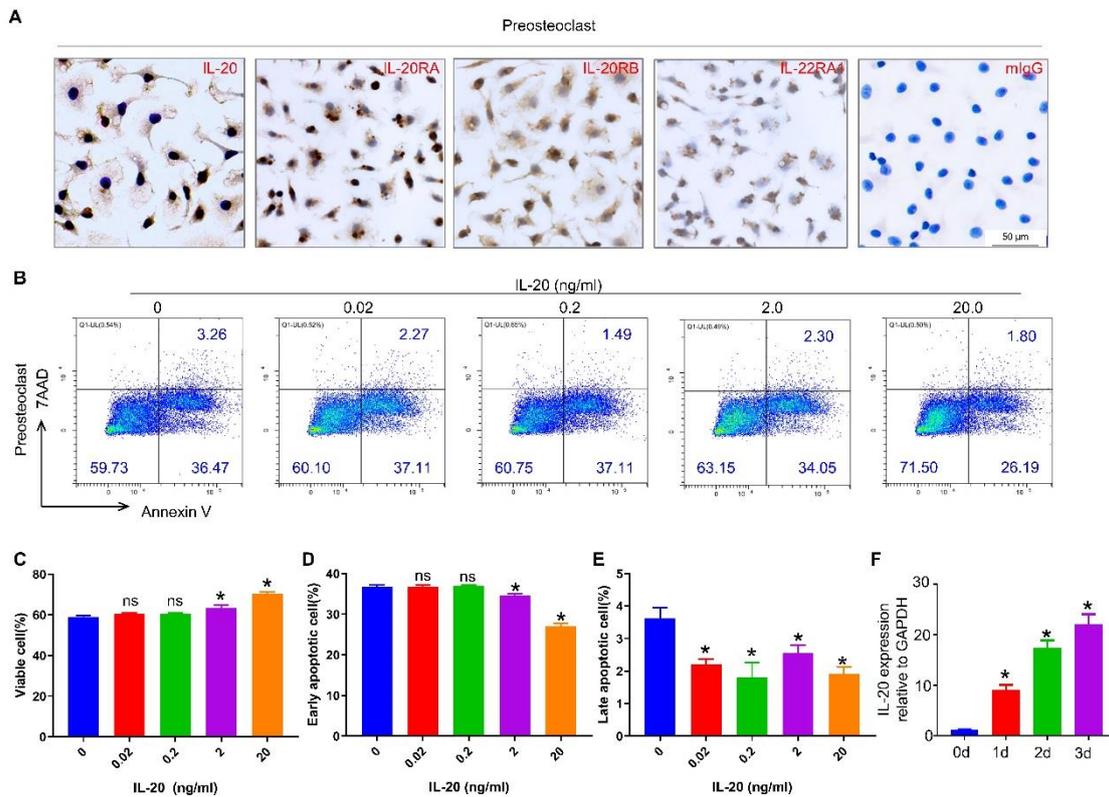


Figure S2. IL-20 inhibited preosteoclast apoptosis. **(A)** The expression of IL-20, IL-20RA, IL-20RB, IL-22RA1 and isotype control (mIgG) in M-CSF-induced preosteoclasts was determined by immunohistochemical staining. **(B)** Cell apoptosis were examined in M-CSF-induced preosteoclasts by flow cytometry assay. **(C-E)** Apoptotic cells were detected using an Annexin V-APC/7-AAD apoptosis kit after 3 days of IL-20 treatment. **(F)** The mRNA expression level of IL-20 in BMMs was evaluated by qRT-PCR after 30 ng/ml M-CSF treatment. * $p < 0.05$ vs. control group. ns $p > 0.05$ vs. control group. $n = 6$.

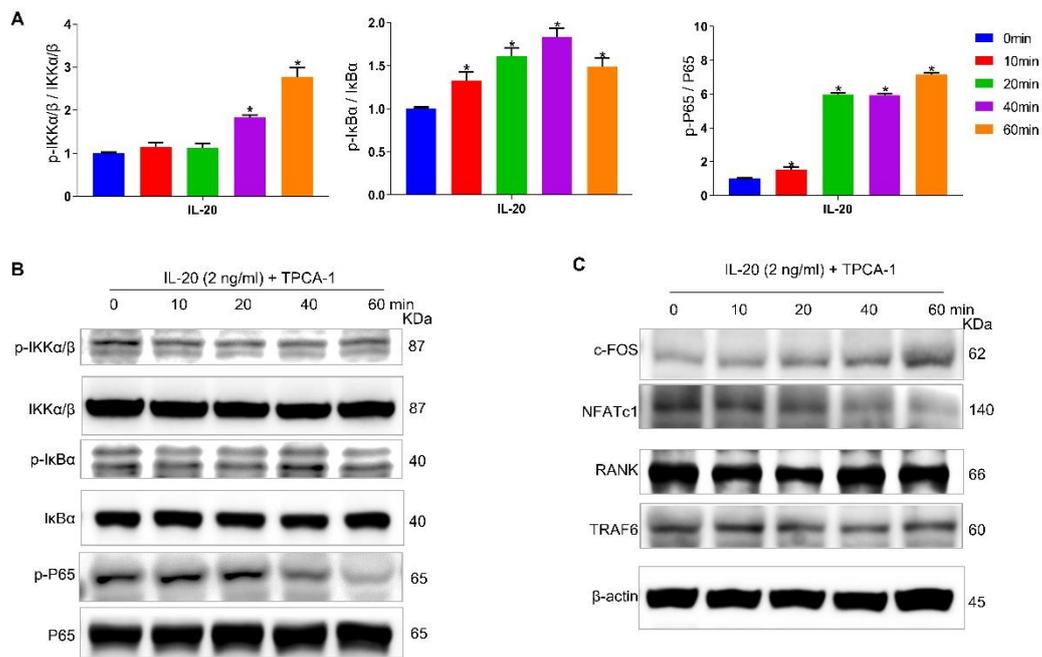


Figure S3. IL-20-mediated activation of NF- κ B pathway was blocked by TPCA-1. Preosteoclasts were stimulated with IL-20 and NF- κ B pathway inhibitor TPCA-1. **(A)** The levels of phosphorylation for proteins in the NF- κ B pathway, including the IKK α/β , I κ B- α , and P65 proteins in IL-20 treated preosteoclasts, were detected using Western blotting. **(B)** The levels of phosphorylation for proteins in the NF- κ B pathway, including the IKK α/β , I κ B- α , and P65 proteins in IL-20 + TPCA-1 treated preosteoclasts, were detected using Western blotting. **(C)** The levels of activated proteins in signaling pathways, including the RANK, TRAF6, c-Fos and NFATc1 proteins without RANKL, were detected using Western blotting. * $p < 0.05$ vs. the 0 ng/ml IL-20 group. $n = 6$.

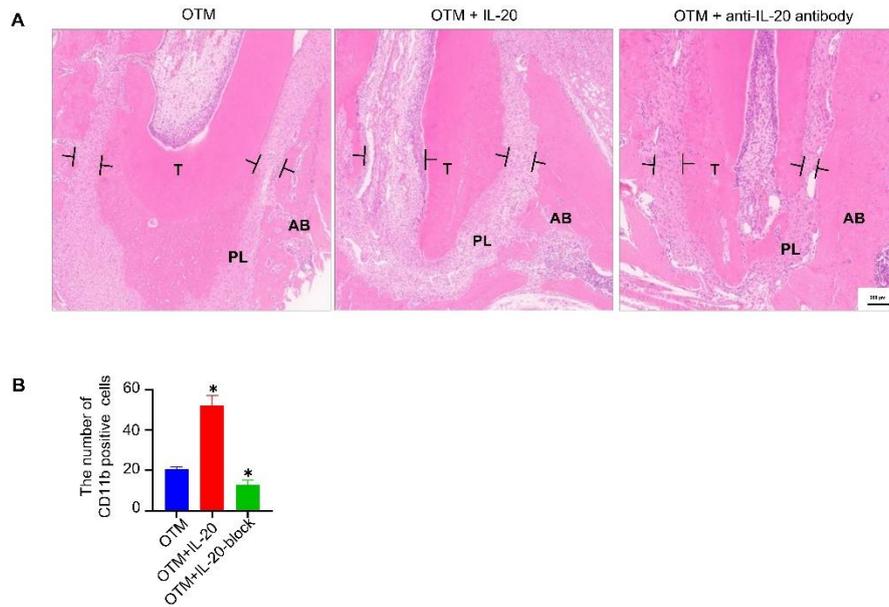


Figure S4. The effect of IL-20 on orthodontic tooth movement and osteoclast differentiation. **(A)** HE staining showed significant changes in first molar periodontal ligament thickness. **(B)** Immunofluorescence staining showed that the expression levels of MCP-1 and CD11b in the first molar periodontal ligament after the application of orthodontic force. OTM + IL-20-block group meant that rats were locally infused with anti-IL-20 antibody. * $p < 0.05$ vs. the control group. $n = 6$.

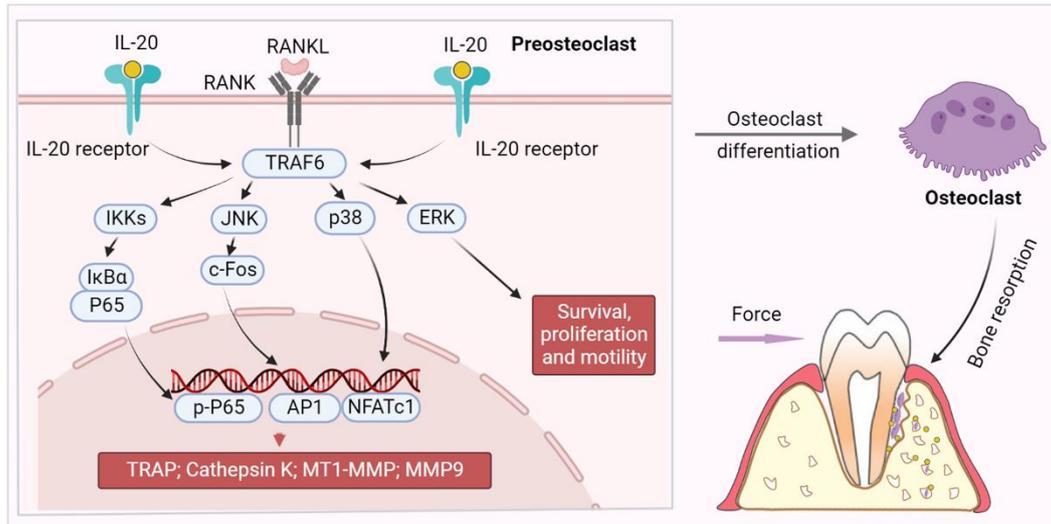


Figure S5. A schematic diagram shows the IL-20 – mediated regulation of osteoclast formation and function through the NF- κ B and MAPK signaling pathways.



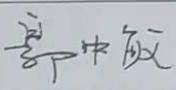
中山大学
SUN YAT-SEN UNIVERSITY

中山大学实验动物管理与使用委员会
Institutional Animal Care and Use Committee,
Sun Yat-Sen University

中山大学动物实验伦理审查同意书
Affidavit of Approval of Animal Use Protocol, IACUC, SYSU

申请编号 Application No.	2018000294	批准编号 Approval No.	SYSU-IACUC-2018-000099
-------------------------	------------	----------------------	------------------------

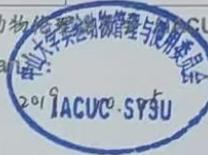
本动物实验方案经过中山大学实验动物伦理委员会审核,符合动物保护、动物福利和伦理原则,符合国家实验动物福利伦理的相关规定。The animal use protocol listed below has been reviewed and approved by the Institutional Animal Care and Use Committee (IACUC), Sun Yat-Sen University.

实验名称 Protocol Title	利塞膦酸钠对卵巢切除 SD 大鼠正畸牙移动的影响和机制研究 The effect and mechanism of risedronate sodium in OVX SD rats following orthodontic tooth movement			
实验申请人 Applicant	吴冬乐 Donald	职称/学位 Title/Degree	硕士 Master	邮箱 Email wudongle@qq.com
实验负责人 Principle Investigator (PI)	曹阳 Yang Cao	职称/学位 Title/Degree	教授 Professor	邮箱 Email caoyang34@163.com
院系(部门) Department	中山大学光华口腔医学院 Guanghua School of stomatology, Hospital of stomatology, Sun Yat-sen University		申请日期 Application date	2018/7/12
动物种系 Species or Strains	大鼠 CD(SD)IGS CD(SD)IGS		动物数量 Quantity	75
计划执行时间 Period of Protocol	2018/7/13 ~2020/7/13		实验动物使用许可证 Number of Animal use permit	中山大学(实验动物中心北校园) (SYXK(粤)2017-0081)
审查意见 Results of inspection	<input checked="" type="checkbox"/> 符合动物福利伦理要求,同意实验 Agree <input type="checkbox"/> 调整方案后,可进行实验 Agree after modification			
兽医师 Chief Veterinary Officer	 日期 Date		2019.10.25	

中山大学实验动物伦理委员会(IACUC, SYSU)

主席(Chairman):

日期(Date):



地址:广州中山二路74号中山大学实验动物中心 邮编:510080

Add: Laboratory Animal Center, SUN YAT-SEN UNIVERSITY, No. 74, Zhongshan Road II, Guangzhou, 510080, P.R. China