

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

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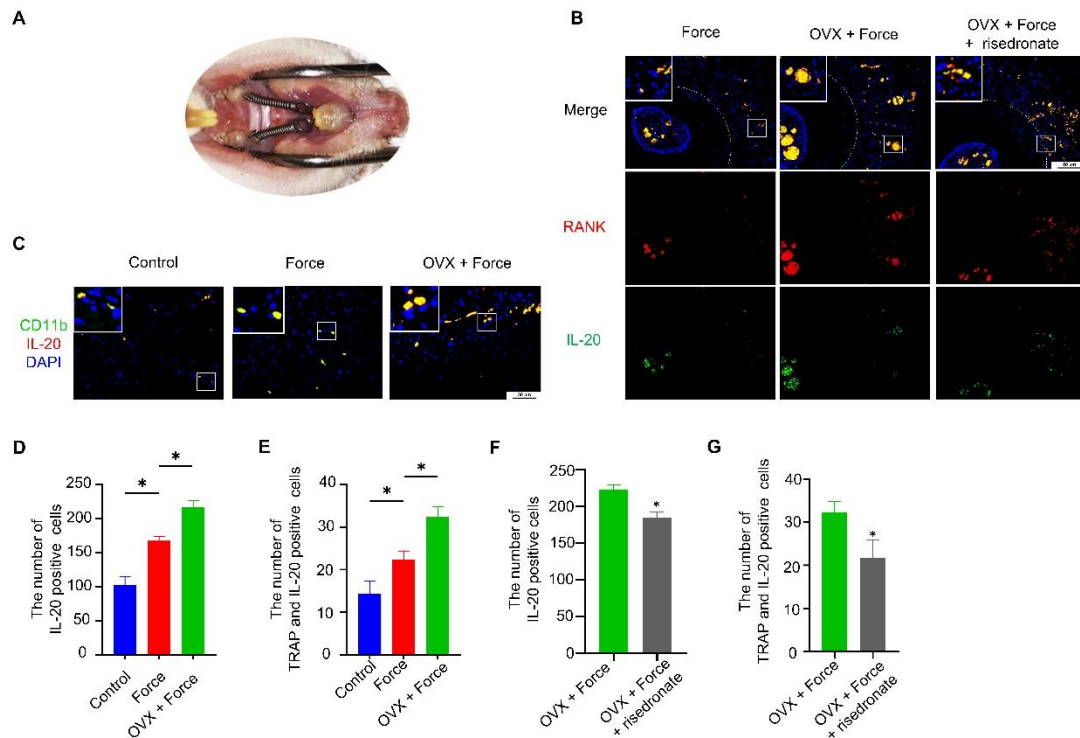
Prof. Xiaoxing Kou, South China Center of Craniofacial Stem Cell Research, Hospital of Stomatology, Guanghua School of Stomatology, Sun Yat-sen University, Guangdong Provincial Key Laboratory of Stomatology, Guangzhou 510055, China.

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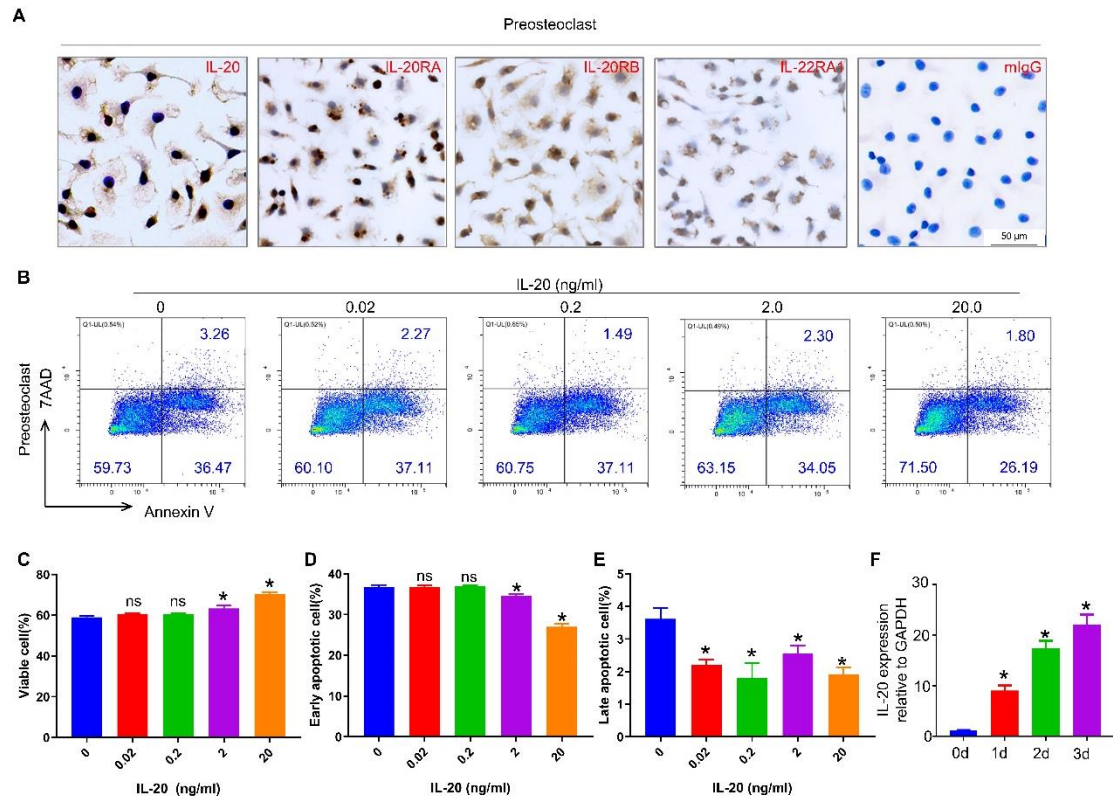
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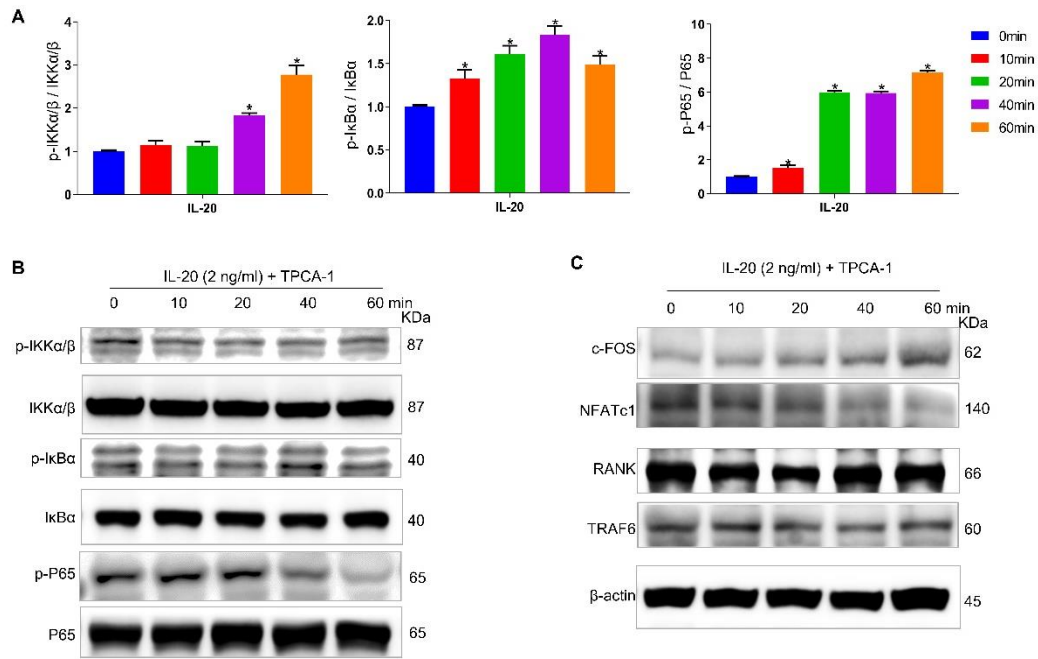
## Supplementary Figures and figure legends



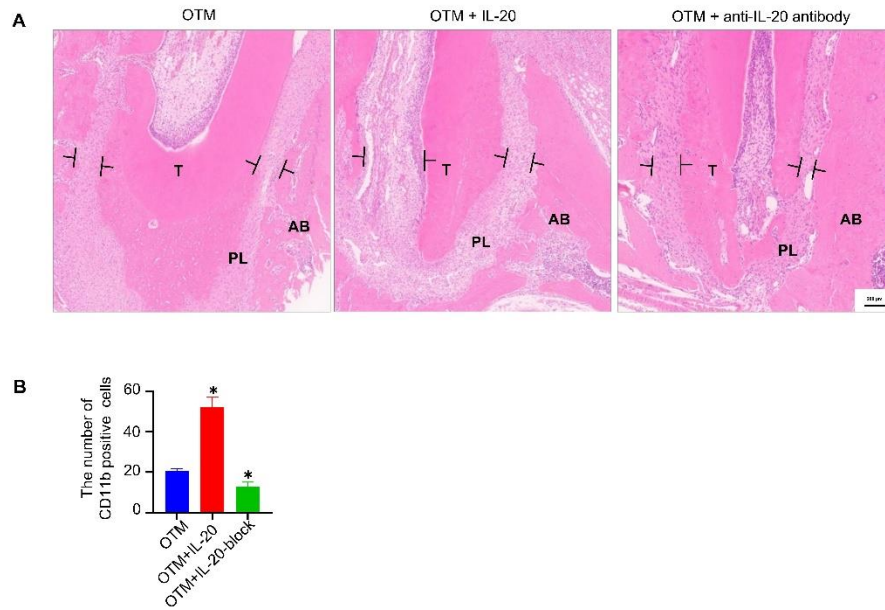
**Figure S1.** IL-20 accelerated orthodontic tooth movement. **(A)** The rat model of orthodontic tooth movement. **(B)** Double-labelled immunofluorescence staining showed that, in the context of orthodontic force, the expression levels of IL-20 and osteoclast marker protein RANK increased in the first 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.  $n = 6$ .



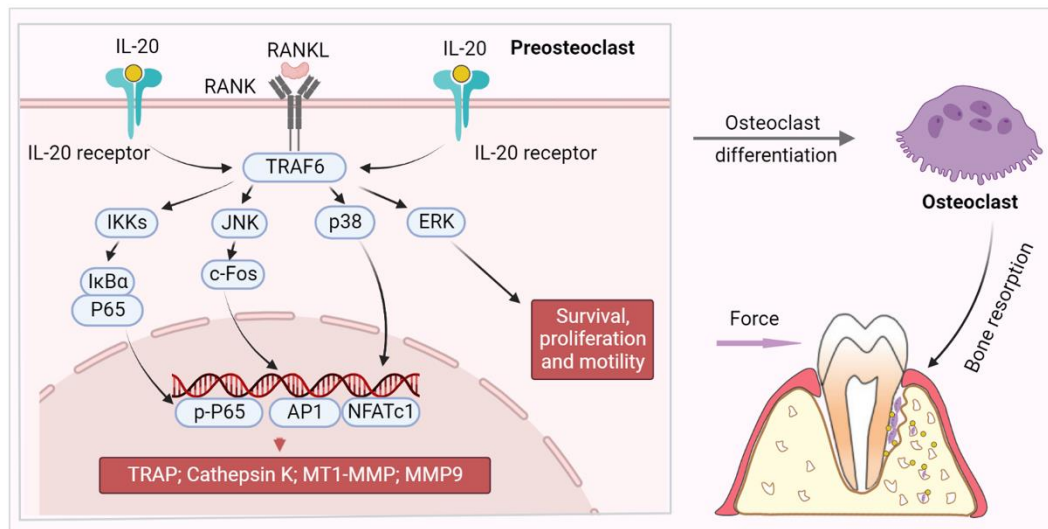
**Figure S2.** IL-20 inhibited preosteoclast apoptosis. **(A)** The expression of IL-20, IL-20RA, IL-20RB, IL-22RA1 and isotype control (mIgG) in M-CSF-induced preosteoclasts was determined by immunochemical staining. **(B)** Cell apoptosis were examined in M-CSF-induced preosteoclasts by flow cytometry 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-κB pathway was blocked by TPCA-1. Preosteoclasts were stimulated with IL-20 and NF-κB pathway inhibitor TPCA-1. **(A)** The levels of phosphorylation for proteins in the NF-κB pathway, including the IKKα/β, IκB-α, and P65 proteins in IL-20 treated preosteoclasts, were detected using Western blotting. **(B)** The levels of phosphorylation for proteins in the NF-κB pathway, including the IKKα/β, IκB-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-  $\kappa$  B and MAPK signaling pathways.



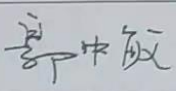


## 中山大学动物实验伦理审查同意书

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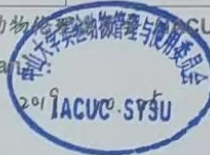
本动物实验方案经过中山大学实验动物伦理委员会审核,符合动物保护、动物福利和伦理原则,符合国家实验动物福利伦理的相关规定。The animal use protocol listed below has been reviewed and approved by the Institutional Animal Care and Use Committee (IACUC), Sun Yat-Sen University.

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审查委员会 (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

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