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Rheumatoid arthritis-autoantibody induced pain-like behavior and bone erosion

– links to osteoclast activity and ASIC3-mediated sensitization

Alexandra Jurczak¹, Lauriane Delay³, Julie Barbier³, Emerson Krock¹, Gustaf Wigerblad¹, Katalin Sandor¹, Katarzyna Rogó¹, Arnaud Briat⁴, Elisabeth Miot-Noirault⁴, Arisai Martinez⁵, Preety Panwar⁶, Dieter Bromme⁶, Juan Miguel Jiménez-Andrade⁵, Anca I. Catrina², Lars Klareskog², Fabien Marchand³, Camilla I. Svensson¹

Department of ¹Physiology and Pharmacology and ²Medicine, Karolinska Institutet, Stockholm, Sweden, ³Université Clermont Auvergne, Inserm U1107 Neuro-Dol, Pharmacologie Fondamentale et Clinique de la Douleur and ⁴Inserm U990 Imagerie Moléculaire et Thérapie Vectorisée, Clermont-Ferrand, France, ⁵Unidad Academica Multidisciplinaria Reynosa Aztlan, Universidad Autonoma de Tamaulipas, Reynosa, Tamaulipas, Mexico, ⁶Dept. Biochemistry and Molecular Biology, University of British Columbia, Canada



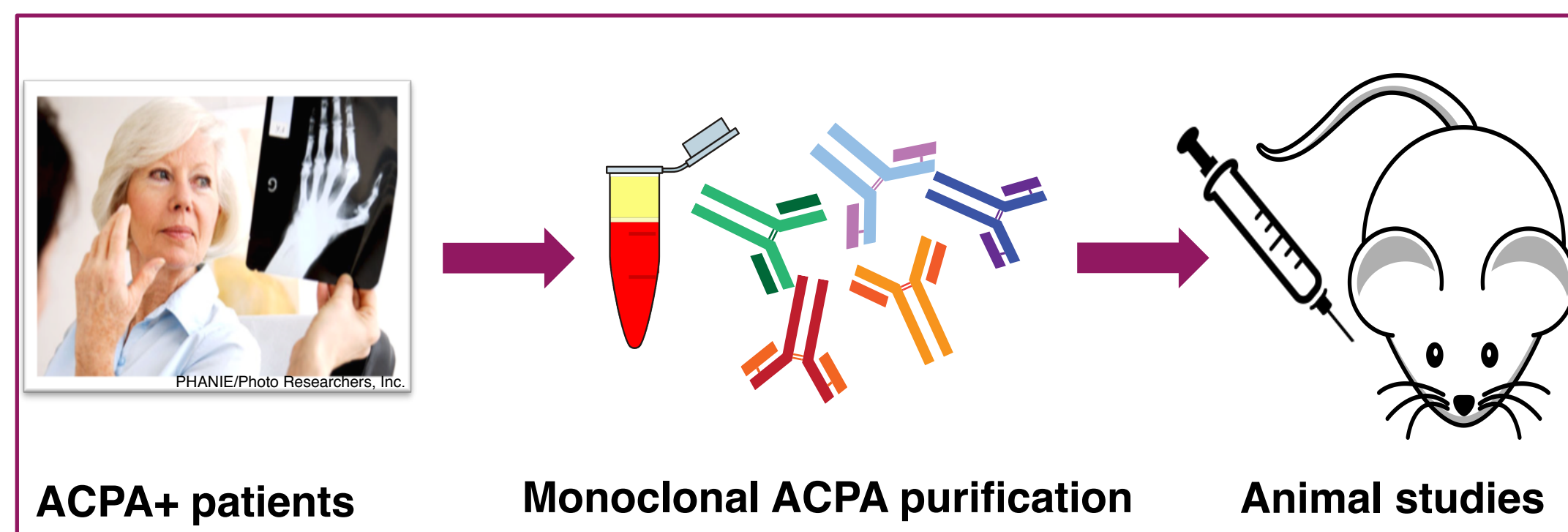
INTRODUCTION

Joint pain is one of the most debilitating symptoms of rheumatoid arthritis (RA) and up to 70% of patients rate pain improvement as their priority. A major subset of RA patients is seropositive for anti-citrullinated protein antibodies (ACPA), which together with other autoantibodies, are often detected in serum or synovial fluid before joint inflammation and RA diagnosis. Seropositivity is often associated with pre-arthritis symptoms which include arthralgia (joint pain) and bone loss. We previously found that transferring ACPA IgG purified from RA patients to mice induces pain-like behavior and bone erosion, independent of joint inflammation¹.

AIM

The aim of the current study was to further characterize the mechanisms behind RA-associated autoantibody-induced hypersensitivity by investigating if monoclonal RA-associated antibodies (mAbs) with particular epitope specificities differ in their abilities to induce pain-like behavior and bone-erosion.

METHODS



Animals

Experiments were performed using female Balb/c and ASIC3 knock-out (KO) mice on C57/Bl6 background² (>12 weeks old).

Monoclonal ACPA preparation and administration

Monoclonal antibodies (mAbs) 1103:01B02, 1325:01B09, 1276:01G09 (control mAb) were generated from CD19+ B cells sorted from RA patients³. Each mAb was injected intravenously alone (2mg) or in combination (B09/B02; 1mg each).

Mechanical and thermal hypersensitivity

Withdrawal thresholds were assessed using von Frey filaments, heat sensitivity with a modified Hargreaves box and cold sensitivity with the acetone drop test.

Pharmacological treatment

The following analgesics were tested at different time points: naproxen, acetaminophen, buprenorphine, morphine, zoledronic acid (osteoclast inhibitor), T06 (cathepsin K inhibitor) and APTx2 (ASIC3 inhibitor).

Joint inflammation

Inflammation was measured *in vivo* using 2-DeoxyGlucosone-750 (2-DG-75) fluorescent imaging and by assessing lipocalin-2 (low-grade inflammation marker) levels in the tibiotarsal joint with ELISA.

Bone morphology

Bone remodeling was evaluated by micro-CT and *in vivo* scintigraphic imaging using ^{99m}Tc-HMDP-labelled hydroxymethylene diphosphonate. Increased osteoclast activity in the joint was assessed on a molecular level with qPCR.

RESULTS

1. RA-derived monoclonal antibodies induce pain-like behavior in mice and prolonged effect when combined

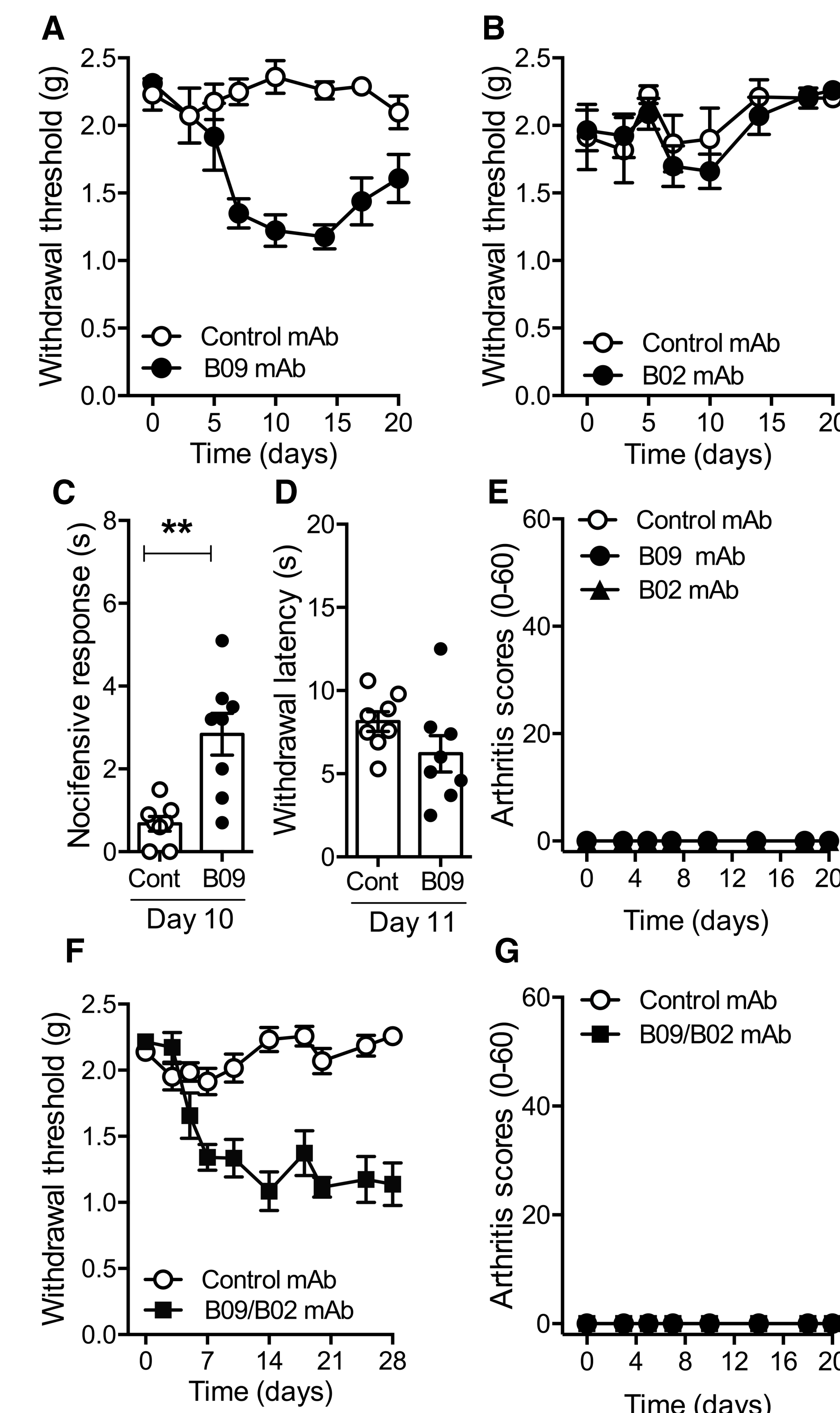


Fig 1. (A) Single i.v. injection of 2mg B09 mAb, but not 2mg B02 mAb (B) induces mechanical hypersensitivity that lasts approx. 21 days, compared to control G09 mAb; n=8/group. (C) B09 mAb induces cold allodynia measured 10 days post mAb injection, but (D) no significant heat hyperalgesia tested on day 11. (E) When combined, B09 and B02 (1mg each) cause long-lasting pain-like behavior compared to control mAb. None of the mAb injected alone (E) or in combination (G) caused visual signs of inflammation. Data presented as mean±SEM, ** p<0.01.

2. B09/B02 – induced pain-like behavior is not associated with joint inflammation

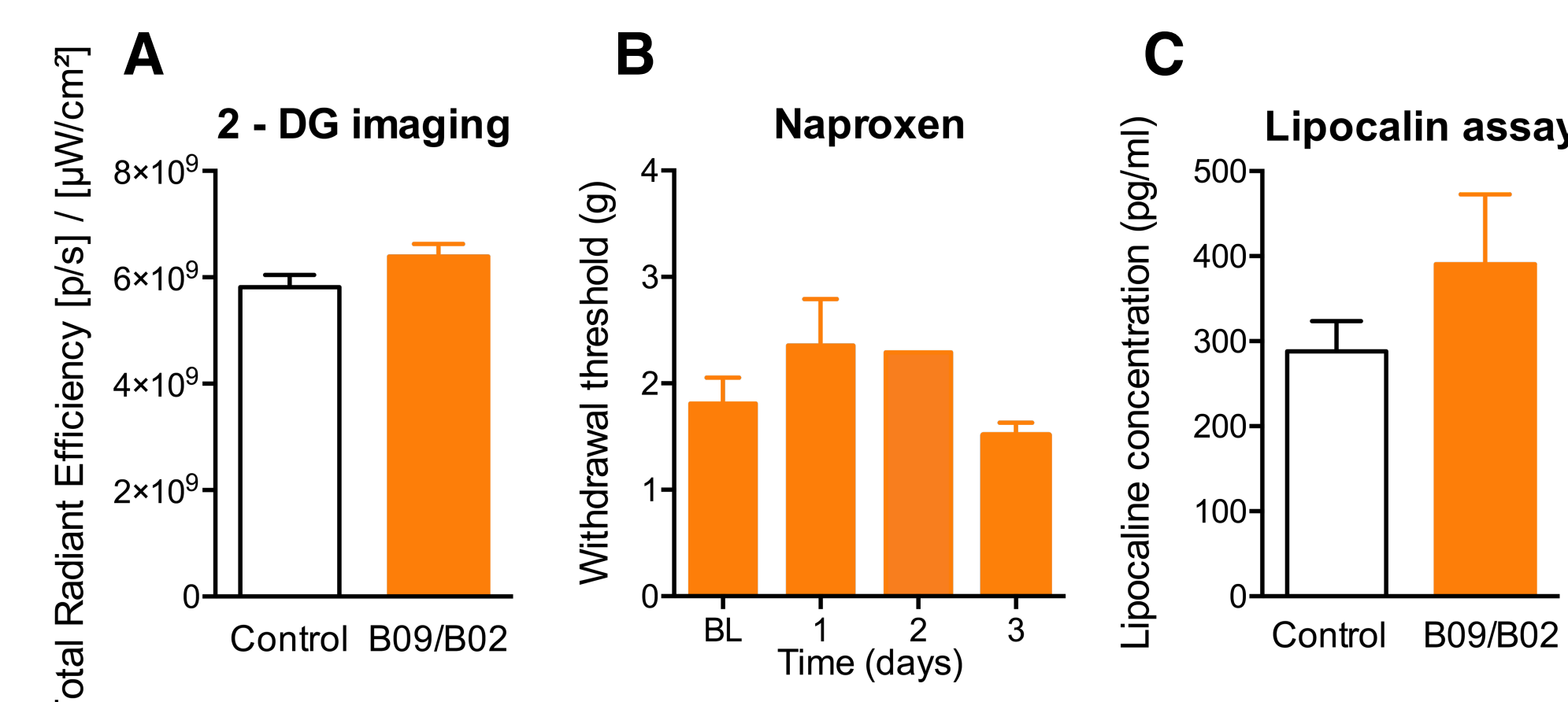


Fig 2. (A) B09/B02-injected mice do not show increased metabolic activity in the tibiotarsal joints, measured *in vivo* 3 hours after i.v. injection of 2-DG-750 luminescent probe (100nmol/100µl); day 14 post-mAb injection; n=8-11/group; results are presented as total counts of radiant efficiency/incident excitation power. (B) Repeated treatment with naproxen (50mg/kg, i.p.) on days 12,13,14 post-mAb injection does not affect mechanical hypersensitivity observed after B09/B02 administration; n=3/group. (C) Lipocalin-2 levels measured by ELISA in the tibiotarsal joint homogenates on day 36 post-mAb injection, were not significantly elevated in the B09/B02 group, compared to control; n=8/group. Data presented as mean±SEM.

3. B02/B09 – induced mechanical hypersensitivity is reversed by opioids but not acetaminophen

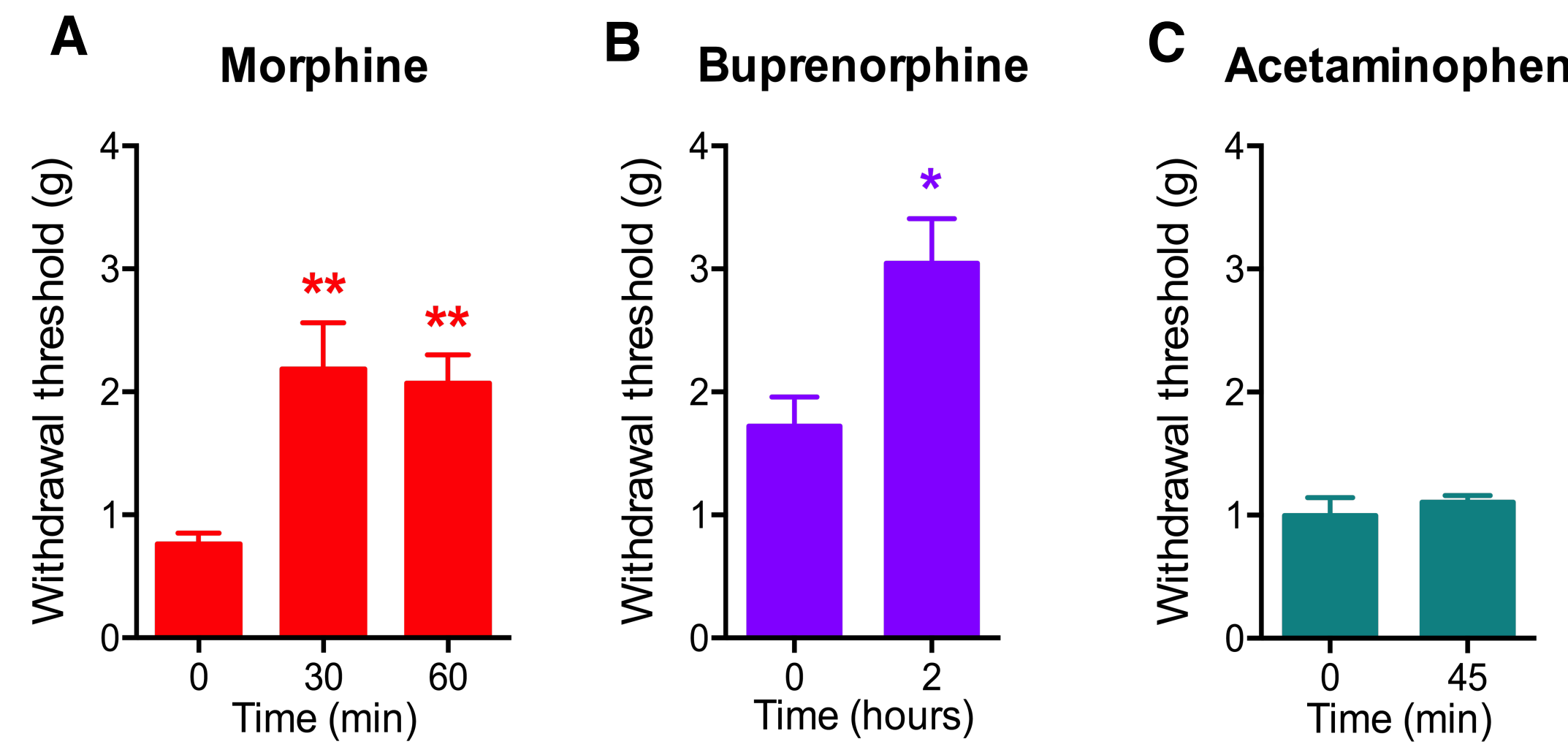


Fig 3. (A) Bolus injection of morphine (3mg/kg, i.p.) and (B) buprenorphine (0.1 mg/kg, i.p.) administered 17 and 10 days post-mAb injection, respectively, reverse mechanical hypersensitivity induced by B09/B02. (C) Acetaminophen (100mg/kg; i.p.) administered on day 22 post-mAb injection has no analgesic effect in B09/B02-injected mice. Data presented as mean±SEM, * p<0.05, ** p<0.01.

4. Intravenous injection of B02/B09 alters bone homeostasis and induces bone erosion

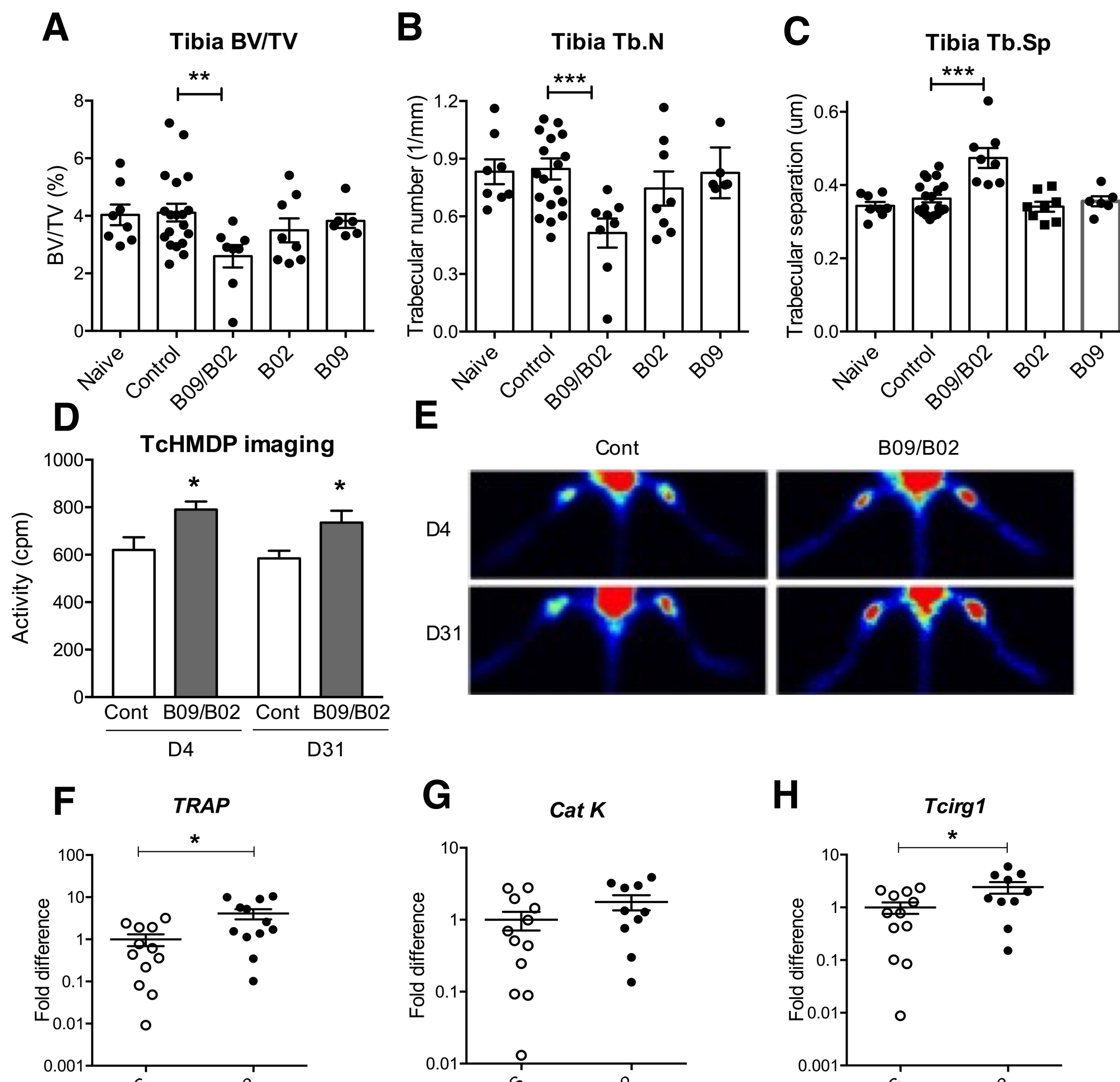


Fig 4. B09 and B02 mAbs induce bone erosion only when combined. Proximal tibia parameters (A) trabecular bone volume (BV/TV), (B) trabecular number (Tb.N) and (C) trabecular separations (Tb.Sp) were quantified using micro-CT imaging; n=6-19/group (D) B09/B02 combination significantly increases osteoblast activity in the knee joint detectable on day 4 and day 31 post-mAb injection, measured by means of *in vivo* scintigraphy (E), n=8-11/group. Expression of osteoclast: *Trap*, *CatK* (F-G) and acidification markers: *Tcrg1* (H) is increased in tibiotarsal joints of B09/B02 mice, compared to control; n=10-12/group. Data presented as mean±SEM, * p<0.05; ** p<0.01; *** p<0.001.

5. B02/B09-induced pain-like behavior is reversed by osteoclast inhibitors

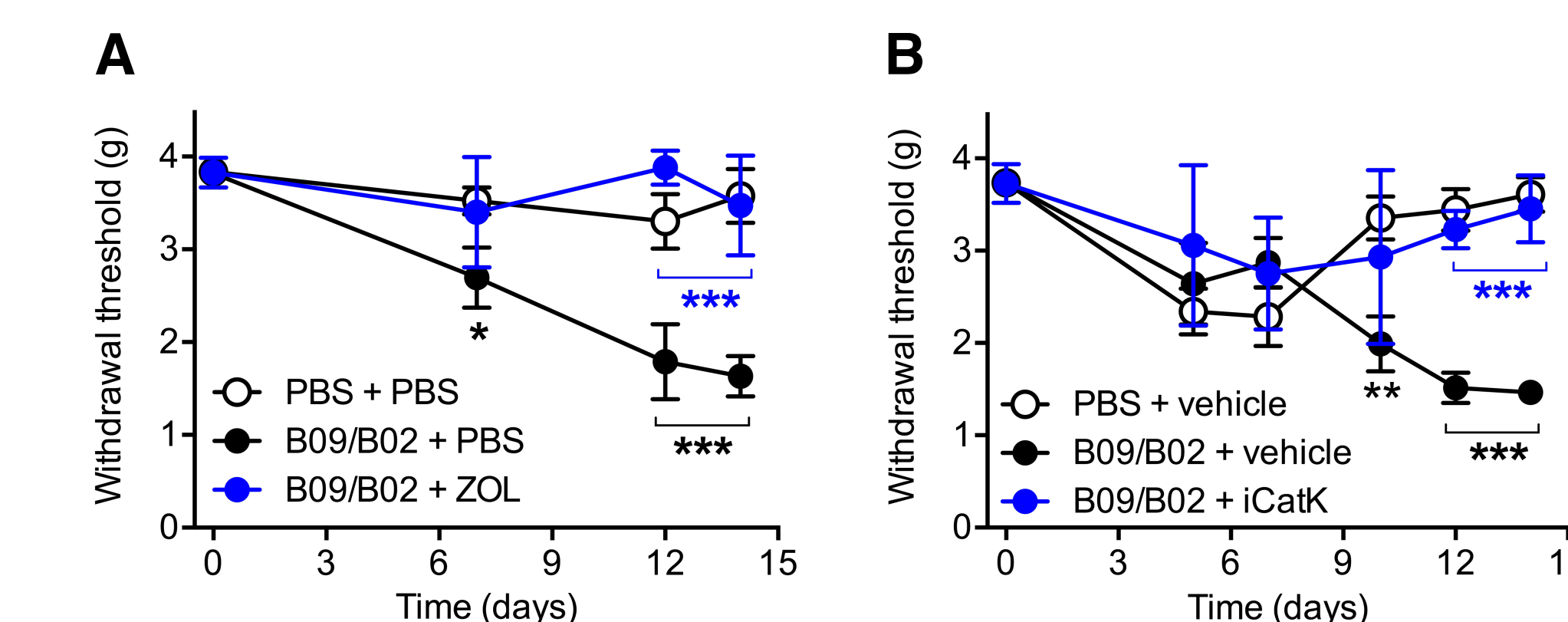


Fig 5. Mice treated with osteoclast inhibitors do not develop B09/B02-induced pain-like behavior. (A) Zolodronate (100µg/kg) was administered i.p. on days 1,4,7 post-mAb injection; n=6/group. (B) T06 (40mg/kg) was given by oral gavage once a day for the whole duration of the study (1-14 days); n=6-8/group. Data presented as mean±SEM; ** p<0.01, *** p<0.001.

6. Intra-articular ASIC3 inhibitor attenuates B09/B02-induced mechanical hypersensitivity

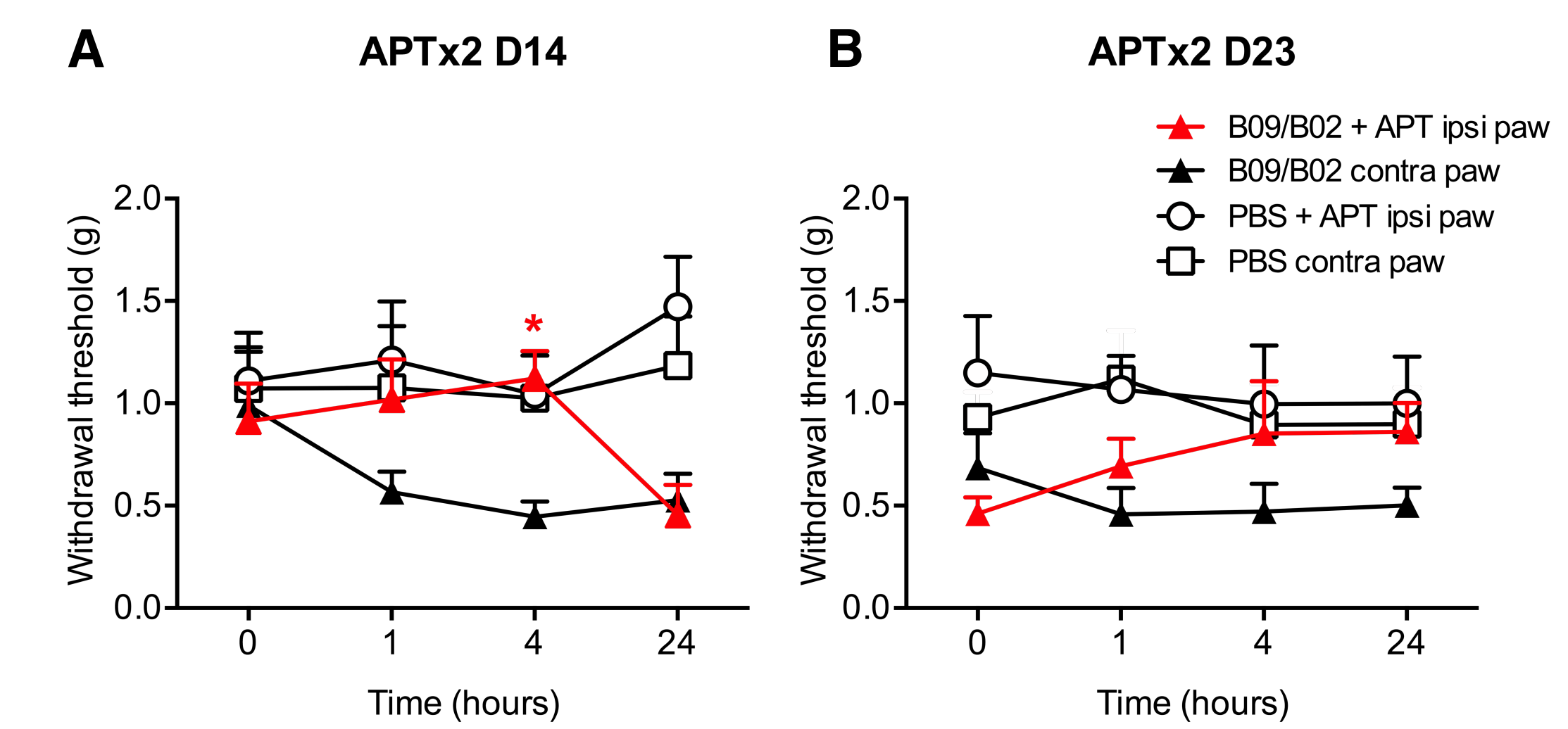


Fig 6. Local administration of APTx2 (20µM) into the tibiotarsal joint, on day 14 (A) and 23 (B) post-mAbs injection, transiently reverses mechanical hypersensitivity in the injected paw of B09/B02 mice; n=6-8/group. Data presented as mean±SEM, * p<0.05.

7. ASIC3 KO mice are protected from B09/B02 – induced pain-like behavior

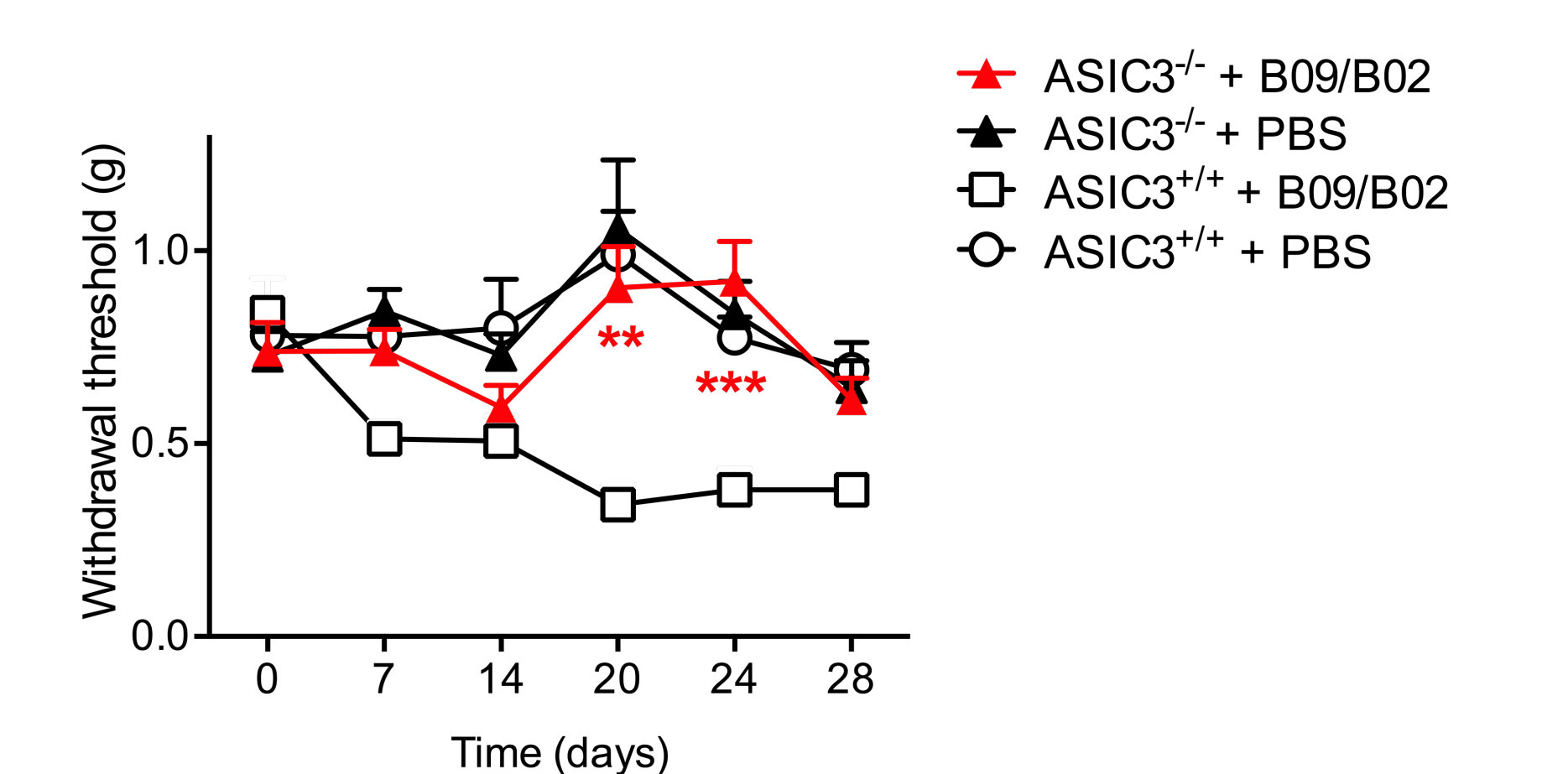
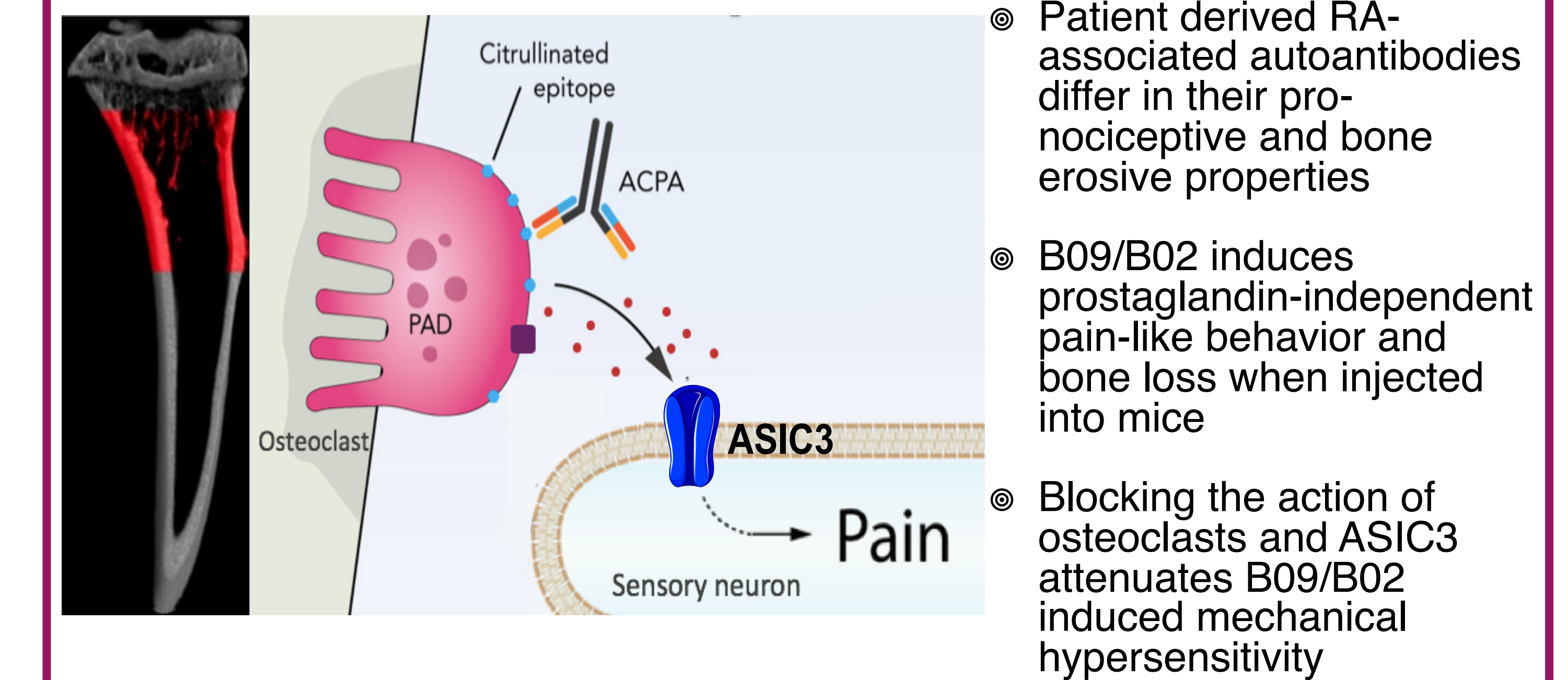


Fig 7. ASIC3 KO mice do not develop mechanical hypersensitivity following B09/B02 injection, in comparison with WT mice; n=26-29/group until day 14; n=9-19/group until day 28. Data presented as mean±SEM, ** p<0.01, *** p<0.001.

CONCLUSIONS



⊙ Patient derived RA-associated autoantibodies differ in their pronociceptive and bone erosive properties
 ⊙ B09/B02 induces prostaglandin-independent pain-like behavior and bone loss when injected into mice
 ⊙ Blocking the action of osteoclasts and ASIC3 attenuates B09/B02 induced mechanical hypersensitivity

This study points towards a possible role of ASIC3 sensitization in B09/B02-induced pain-like behavior. We suggest that as a result of autoantibody-induced osteoclast activation, certain factors are released, which sensitize ASIC3 located on sensory neurons innervating the joint.

Based on current results, ongoing experiments are searching for osteoclast-derived ligands of ASIC3.

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Alexandra Jurczak • PhD student
 Karolinska Institute • Stockholm • Sweden
 E-mail: alexandra.jurczak@ki.se