Supplementary Information

**Methods**

**Behavioural testing**

Rats were habituated to testing equipment prior to baseline testing.

After intra-articular injection, rats were maintained under the same conditions as during the preoperative period. The posture and behaviour of the rats were carefully monitored following recovery from the anaesthesia and then on the first post-injury day. Baseline measurements were taken immediately prior to intra-articular injection (day 0) and then from day 3 onwards. Behavioural tests assessing changes in weight-distribution and sensitivity to mechanical stimuli were performed for up to 28 days post-injection. The weight gain and general behaviour of the rats was monitored throughout the post-injury period.

Effects of joint damage on weight-distribution through the left (ipsilateral) and right (contralateral) knee were assessed using an incapacitance tester (Linton Instrumentation, U.K.). The two hind-paws were placed on separate sensors and the force (in grams) exerted by each hind limb was calculated and averaged over a period of 3 seconds as previously described \(^1\,^2\). Each data point was taken as the mean of three 3 sec readings. Effects of an intra-articular injection of MIA or saline on weight distribution were assessed between post-operative days 3-28. Naïve or saline-treated rats distribute their weight evenly between both paws, however, following joint injury, changes in weight distribution can be used an indicator of joint discomfort and associated pain in the injured knee \(^3\,^4\,^5\). The development of tactile allodynia was assessed using von Frey monofilaments (Semmes-Weinstein monofilaments of bending forces 1, 1.4, 2, 4, 6, 8, 10 and 15 g). The rats were placed in transparent plastic cubicles on a mesh floored table and a period of acclimatization was allowed prior to testing. Von Frey monofilaments were applied, in ascending order of bending force, to the plantar surface of both hind-paws. Each von Frey was applied for a 3 sec period. Once a withdrawal reflex was established, the paw was re-tested with the next descending von Frey monofilament until no response occurred. The lowest weight of monofilament which elicited a withdrawal reflex was noted as the paw withdrawal threshold (PWT).

**Pre-emptive OPG-Fc in neuropathic rats:**

In a separate study the effect of OPG-Fc was assessed in a model of a different type of chronic pain, neuropathic pain to determine whether the analgesic effects of OPG-Fc are generalised to other types of chronic pain. Rats were habituated to hindpaw withdrawal threshold testing prior to surgery. Following spinal nerve ligation (SNL) or sham surgery,
rats received subcutaneous injections of OPG-Fc (3mgkg⁻¹) or vehicle (phosphate buffered saline; PBS) every other day from days 1-13 post-surgery, based on⁶. Sham-operated rats received vehicle. At the end of the pharmacological study (day 14), rats were killed by anaesthetic overdose and tissues were fixed by transcardiac perfusion with 4% paraformaldehyde (PFA) solution. N= 8 rats per group. Due to the shorter timeframe for the development of pain behaviour and associated glial activation⁷,⁸ following spinal nerve ligation, rats were followed for 2 weeks post-injury.
Figures and Legends

**Supplementary Figure 1**: Time-line of the interventions in the MIA model of OA pain and the SNL model of neuropathic pain

**Arthritic Rats**

- **Weightbearing and von Frey testing**

**Pre-emptive**

- OPG-Fc (30mg/kg) or PBS every other day s.c.

**Therapeutic**

- PBS (2ml/kg) every other day s.c.

- OPG-Fc (30mg/kg) or PBS every other day s.c.

**Pre-emptive**

- Zoledronate (100µg/kg) or PBS every 3rd day s.c.

**Neuropathic Rats**

- von Frey testing

**Pre-emptive**

- OPG-Fc (30mg/kg) or PBS every other day s.c.

- OPG-Fc (30mg/kg) or PBS every other day s.c.

**WEEK**

- 0 1 2 3 4

**Tissue Collection**
**Supplementary Table 1:** The number of rats used in each intervention group in the MIA model of OA pain and SNL model of neuropathic pain

<table>
<thead>
<tr>
<th>Drug</th>
<th>Arthritis (MIA) Study</th>
<th>Neuropathic (SNL) Study</th>
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<tbody>
<tr>
<td></td>
<td>MIA-Drug</td>
<td>MIA-vehicle</td>
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<tr>
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</tr>
<tr>
<td>Pre-emptive zoledronate</td>
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**TOTAL RATS** 104
Supplementary Figure 2. Representative images of subchondral TRAP-positive osteoclasts in the medial tibial plateau of an MIA-treated rat at (A) 10X, (B) 20X and (B) 40X magnification. Boxes indicate area of further magnification.
Supplementary Figure 3. Therapeutic OPG-Fc did not alter the development of histopathological changes in the knee joints of MIA-treated rats. MIA-injected rats treated with vehicle had thicker synovial lining (arrows) and increased cellularity throughout the synovia (A). Osteophyte growth (circle) at joint margin (D) accompanied with chondrocyte hypocellularity and severe loss of proteoglycan staining (red asterisk), as well as increased number of channels (black asterisks) crossing the osteochondral junction (black line) were observed in MIA-injected rats treated with vehicle (D).

Thicker synovial lining (B, arrows) and increased cellularity was observed in MIA-injected rats which received therapeutic OPG-Fc. Channels (black asterisks) crossing the osteochondral junction (black line), osteophytes at joint margins (circle), chondrocyte hypocellularity and proteoglycan loss (red asterisk) (E) were evident in MIA therapeutic OPG treated rats.

Non-arthritic saline injected control rats (C, F) had normal synovia, with 1-2 cell thick synovial lining (C; arrows), normal smooth cartilage and joint margins with normal chondrocyte distribution and proteoglycan staining (F; arrows) and fewer channels crossing the osteochondral junction (black asterisks). Coronal sections stained with haematoxylin and Eosin (synovia: A-C) and Safranin-O (D-F) stains. Bars = 100µm.
Supplementary Figure 4: Mechanical withdrawal thresholds of the hind paw were significantly decreased in neuropathic vehicle-treated rats, compared to sham-operated vehicle-treated rats. OPG-Fc treatment did not alter the decrease in withdrawal thresholds of the hind paw in neuropathic rats. Statistical analyses were performed using a Kruskall Wallis test. *p<0.05, **p<0.01, ***p<0.001 for vehicle-treated neuropathic rats versus vehicle-treated sham-operated rats; +p<0.05, ++p<0.01 for OPG-treated neuropathic rats versus vehicle-treated sham-operated rats. N=8 rats per group