SUPPLEMENTAL METHODS

Biodistribution of PHA-408 following Intra-articular Injection

Mice (20-25 weeks, n=2-5 group/timepoint) in cohort 4 underwent compressive joint injury followed by intra-articular injections of either PHA-408 (3 µg/5 µL) or vehicle (5 µL, sterile saline + 0.1% DMSO) immediately post-joint injury and 48 h post-joint injury. At terminal timepoints (30 min, 2 h, 3 d, 7 d) 1 mL of blood was collected by cardiac puncture and serum was obtained following centrifugation. Lymph nodes (inguinal and lumbar), knees, and urine were collected at the time of sacrifice. Lymph nodes were suspended in 500 µL acetonitrile (ACN), and homogenized (Mini-BeadBeater™, BioSpec, Bartlesville, OK). Knees were flash frozen in liquid nitrogen, homogenized (BioPulverizer™, BioSpec), and suspended in 2 mL of ACN. Samples were then vortexed for 2 h and centrifuged to collect clarified ACN. Drug concentration in serum, urine, lymph nodes, and knee tissue was estimated via LC-MS upon an Agilent 1200 series HPLC instrument and an Agilent 6410 triple-quadruple mass spectrometer operated in positive electrospray ionization mode (collision gas flow rate: 11 L/min, source block temperature: 300°C). Samples (20 µL) were injected into a C18 column (4.6×100 mm, 3.5 µm, Agilent) at 37°C using a gradient elution method (Water and ACN mix) at a flow rate of 0.65 mL/min. PHA-408 was quantified by multiple reaction monitoring (Q1/Q3, 560.246/514.113) with a 51V collision energy.

Hindpaw Thermal Sensitivity

Mice were gently scruffed and the plantar side of the tested paw was placed on a hot/cold plate (50/0°C, BIO-CHP, Bioseb) while the other paw was placed on a room temperature plexiglass surface at the same height. Mice were held stationary until a clear paw withdrawal occurred or a 20 or 30 sec threshold was reached on the hot and cold plate respectively. Latency to withdrawal was recorded, three independent measures were collected for each hindpaw at each temperature. Animals were allowed a one minute recovery period between consecutive hindpaw measurements.

Longitudinal NF-κB-driven Luminescence and Allodynia Correlation

To test for relationships between NF-κB luminescence and allodynia Pearson correlation coefficients were calculated. In vivo imaging and 50% withdrawal threshold data were log-transformed (base 10). All data were then normalized by each animals' individual baseline value, represented as the difference from baseline, and the area under the curve was calculated in an early (0 to 7/8 d) study window. Data were then z-scored and the Pearson correlation coefficient was calculated. This early window was chosen based on observed NF-κB luminescence temporal dynamics and appropriately accounts for repeated measures taken from the same animal over time and is more telling of prolonged changes to behavior than correlating changes observed at any one point in time. Temp and colleagues have tested for similar relationships between pain-sensitivity measures and endpoint histology[29].

Joint Laxity Testing

Murine cadavers (male, 9-12 weeks of age, n=10) underwent cyclic compressive loading of a randomly assigned knee joint to induce knee joint injury. Hindlimbs were then dissected and scored by three blinded investigators for anterior-posterior laxity (0=naive, 1=increased anterior-posterior laxity). Summed scores for each joint were calculated and CTRL and INJ joint scores were compared via a Mann-Whitney test.