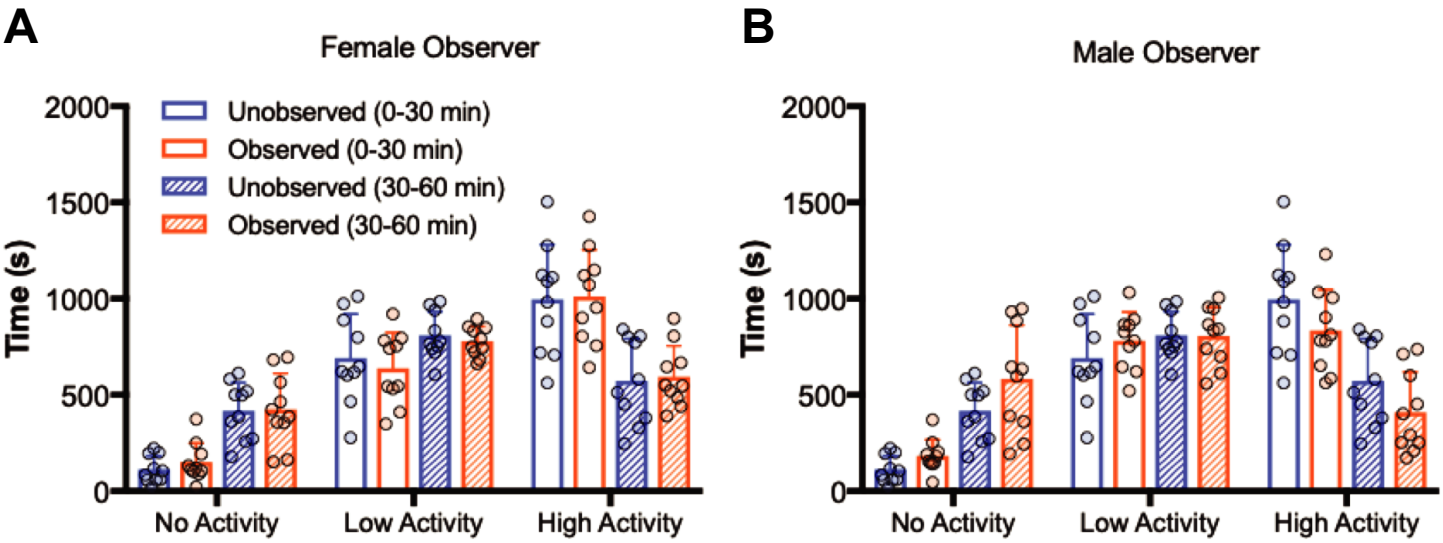
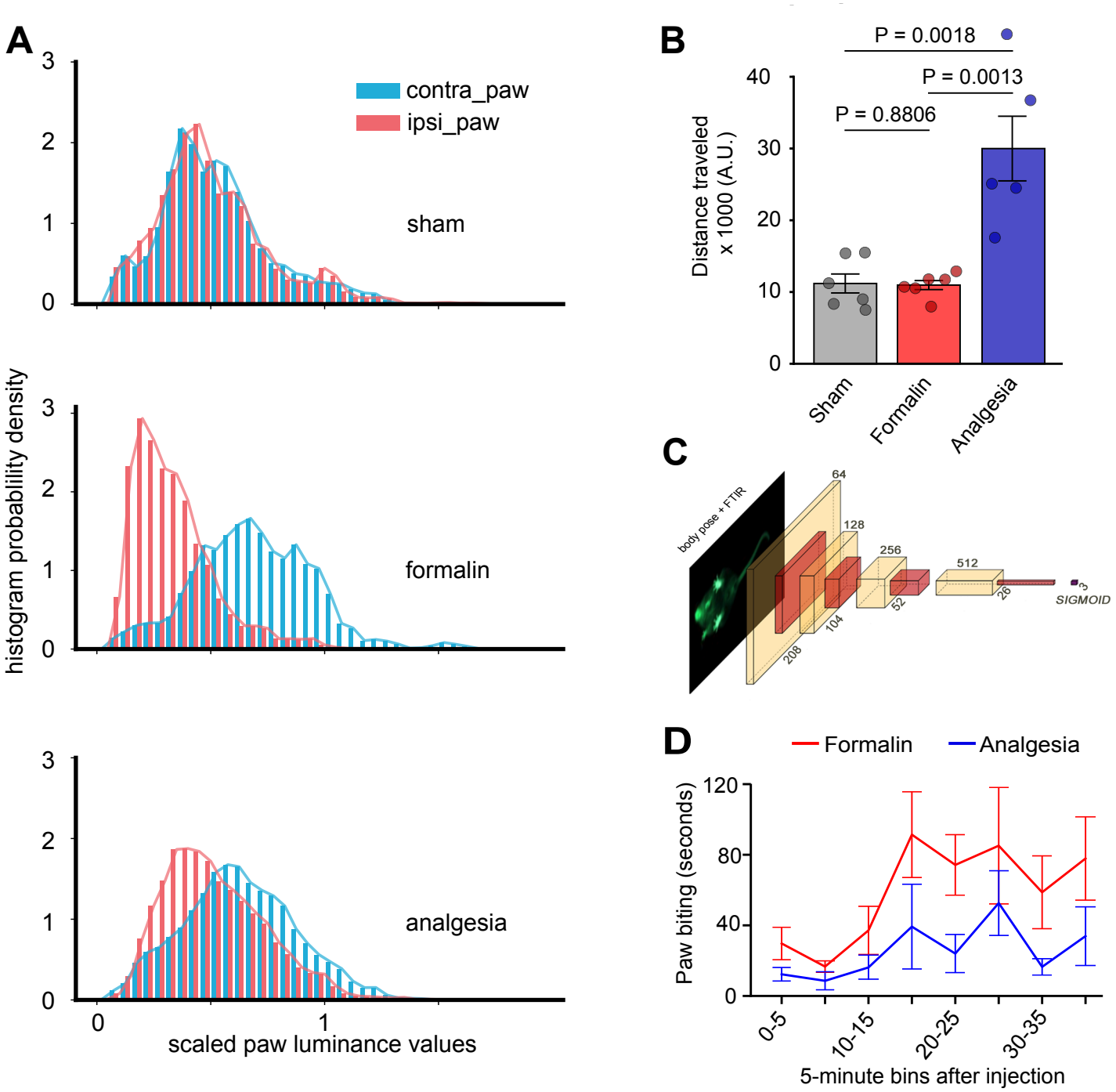
Supplemental Figure 1



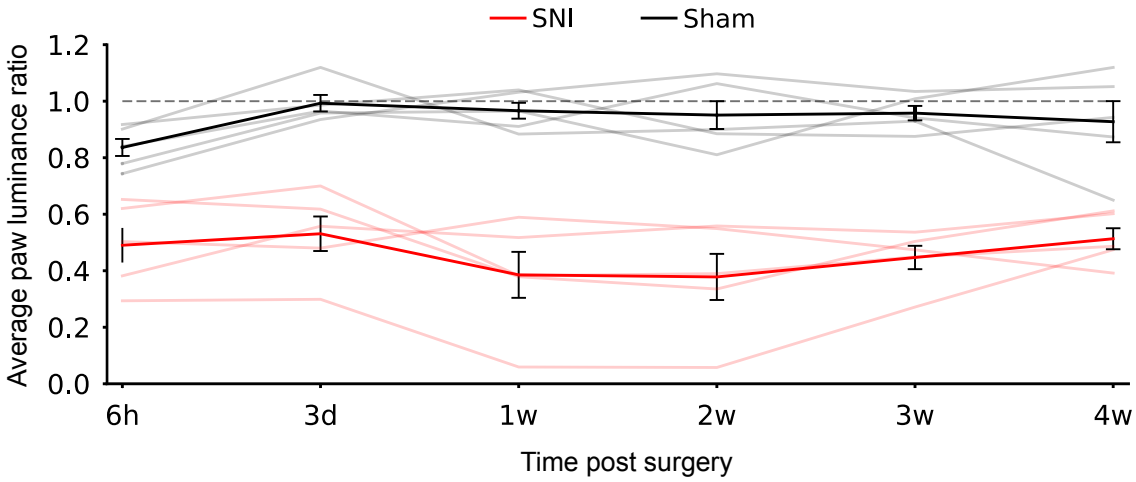
Supplemental Figure 1.: Animals were placed in individual enclosed chambers and recorded with a camera for one hour at 30 frames per second. General activity levels were divided into no activity, low activity, and high activity, with the time spend in each quantified. The presence of either a male **(A)** or female **(B)** observer did not impact overall activity of the mice in the enclosure. Data presented as mean ± SEM.

Supplemental Figure 2



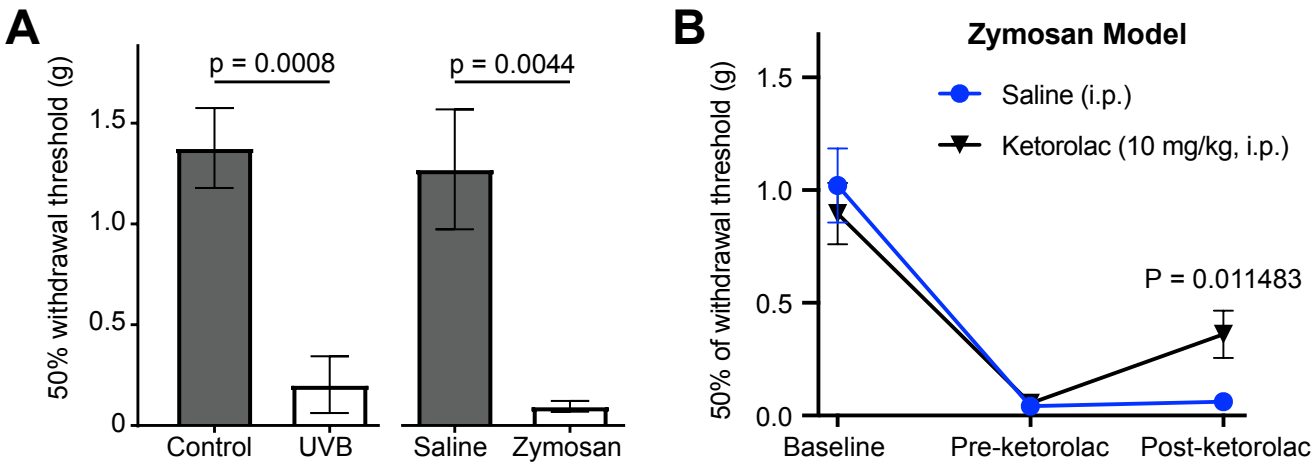
Supplemental Figure 2.: **(A)** Density histograms of the scaled paw luminance values from ipsilateral and contralateral hindpaws over 30-minute recording of mice from sham group (top, n = 3), formalin group (middle, n=3), and analgesia group (bottom, n=3). Formalin reduced ipsilateral paw luminance and increased contralateral paw luminance, and this is rescued towards normal levels by morphine. **(B)** The centroid of the mice was tracked using DeepLabCut and used to calculate distance traveled in pixel unit. Mice treated with morphine (3 mg/kg) show hyper-locomotion (n=5), compared to sham and formalin groups. **(C)** Schematic diagram of the convolutional neural network for behavior classification with supervised learning. Body frame with superimposed FTIR signal is used as input. Convolutional layers (yellow), max-pooling layers (red), and a sigmoid activation function (purple) are shown with their respective dimensions. **(D)** Automated scoring of paw-biting behavior in mice following intraplantar injection of formalin, with (blue line, n=4) and without (red line, n=4) concomitant morphine (3 mg/kg) treatment, captures the analgesic effect of morphine as a decrease of paw-biting after formalin injection. Values represent total duration of licking or biting per 5-minute interval. Data presented as mean ± SEM. Statistical significance for panel a determined by two-tail unpaired Student's t-test.

Supplemental Figure 3



Supplemental Figure 3.: Average paw luminance ratio over 20-minute recording captures absence of recovery in 4 weeks in SNI model (red, n=5), compared to the sham group (black, n=5). The readouts of individual mice are shown as shaded lines. Data presented as mean ±SEM.

Supplemental Figure 4



Supplemental Figure 4.: (A) Mechanical hypersensitivity detected by von Frey withdrawal thresholds of UVB (n=6) or zymosan (n=5) treated mice compared to naïve (n=5) or saline (n=5) treated animals. **(B)** Effect of ketorolac (10 mg/kg and saline control; n=10 for each group) on von Frey mechanical thresholds in the zymosan tonic pain model. Baseline readouts were conducted before zymosan injection, the “pre” readouts were 4 hours after zymosan injection but before ketorolac administration, and the “post” 1 hour after ketorolac administration. Data presented as mean ± SEM. Statistical significance determined by two-tail unpaired Student’s t-test. Data presented as mean ± SEM. Statistical significance determined by two-tail unpaired Student’s t-test.

Supplemental Table 1

	von Frey assay	The bottom-up imaging technology
Punctate stimulus	Yes	No
Evoked response	Yes	No
Quantitative mechanical threshold measurement	Yes	No
Measure mechanical pain in different body locations	Yes (e.g., abdomen, hindpaw, cheek pad)	No, restricted to single hindlimb pain
The temporal resolution of the measurement	discrete measurement every 30 mins	Continuous (every 40 ms) over tens of minutes
Number of animals needed for detecting analgesia	>8	As small as 5
Concurrent detection of hyperlocomotion/sedation	No	Yes
In the dark	No	Yes
Observer-free	No	Yes
Objective readout	No	Yes
Automated readout	No	Yes
Scalability in the application of in vivo analgesic efficacy validation	limited by available human work time	Parallelizable with sufficient computation resource

Supplemental Table 1.: Differences between the von Frey assay and the bottom-up imaging technology.