Expression of ectopic heat shock protein 90 (eHsp90) in male and female primary afferent nociceptors regulates inflammatory pain

Supplemental Materials

Movie S1. 3D reconstruction of whole-body cryoimaging of a female mouse 6 hours after HS-131 administration on day 3 post CFA.

Supplemental Figure 1

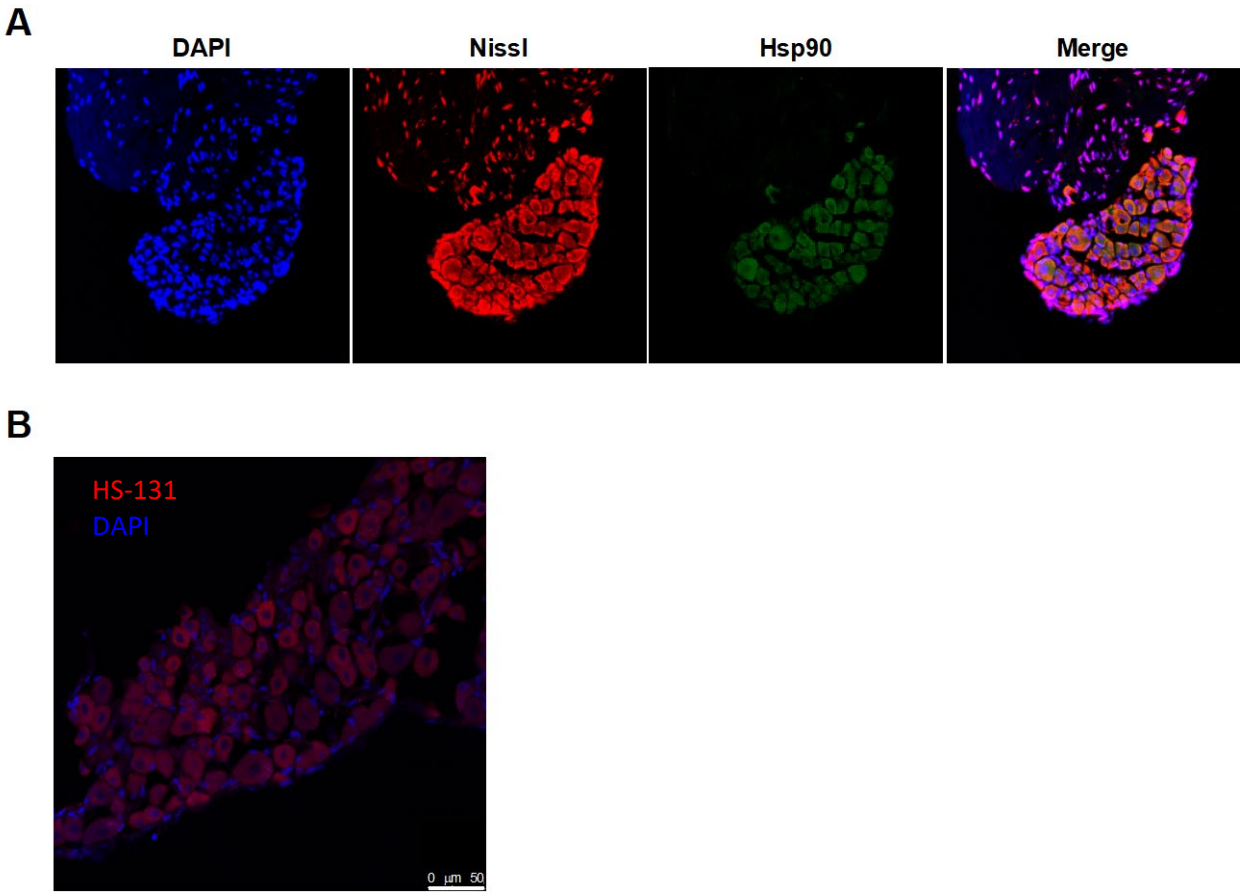

Fig S1. DRG neurons express Hsp90. (A) IHC analysis of DRG collected from IFA control mice show Hsp90 expression is localized to Nissl+ neurons. (B) Confocal analysis of DRG from mice in the CFA group collected on day 3, 6 hours following administration of the eHsp90 discriminate fluortagged inhibitor HS-131 show eHsp90 staining patterns similar to those observed in our IHC staining. Representative images from 4 mice (2M, 2F) are shown.

Supplemental Figure 2

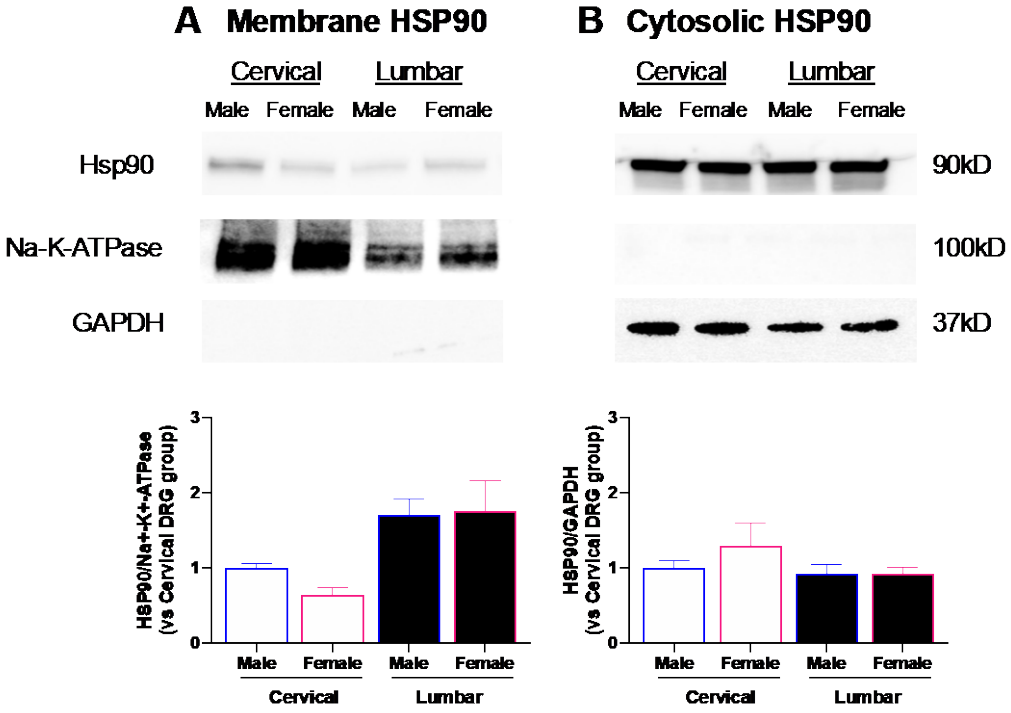

Fig S2. Intra-plantar IFA injection does not alter membrane or cytosolic Hsp90 expression in lumbar DRG. Male and female mice in the IFA control group exhibited comparable eHsp90 expression levels in (A) membrane bound and (B) cytosolic fractions isolated from lumbar and cervical DRG. N=12 (3M+3F) per group. Data are mean ± SEM.

Supplemental Figure 3

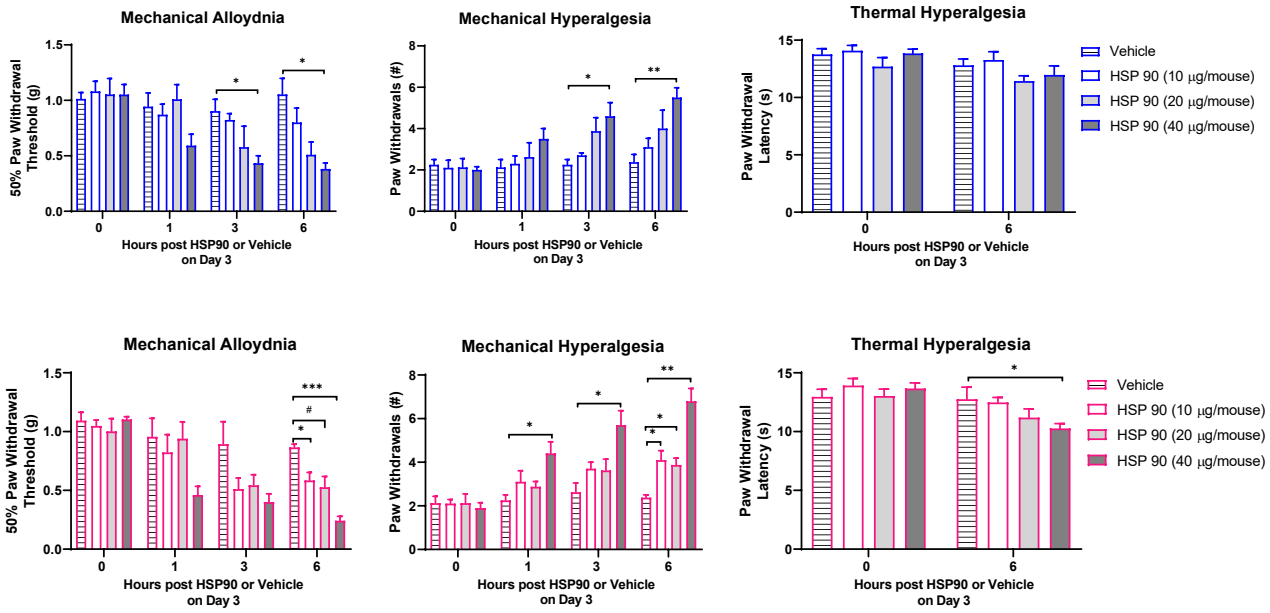

Fig S3. Systemic Hsp90 administration directly induces pain, to a greater degree in female versus male mice. Administration of Hsp90 dose-dependently produced mechanical allodynia and hyperalgesia in both male and female mice, and thermal hyperalgesia in female mice. N=8-10 (4-5M+4-5F) 4mice per group. Data are mean ± SEM. $^{\#}P$=0.06, *P<0.05, **P<0.01, ***P<0.001 different from baseline on day 0.

Supplemental Figure 4

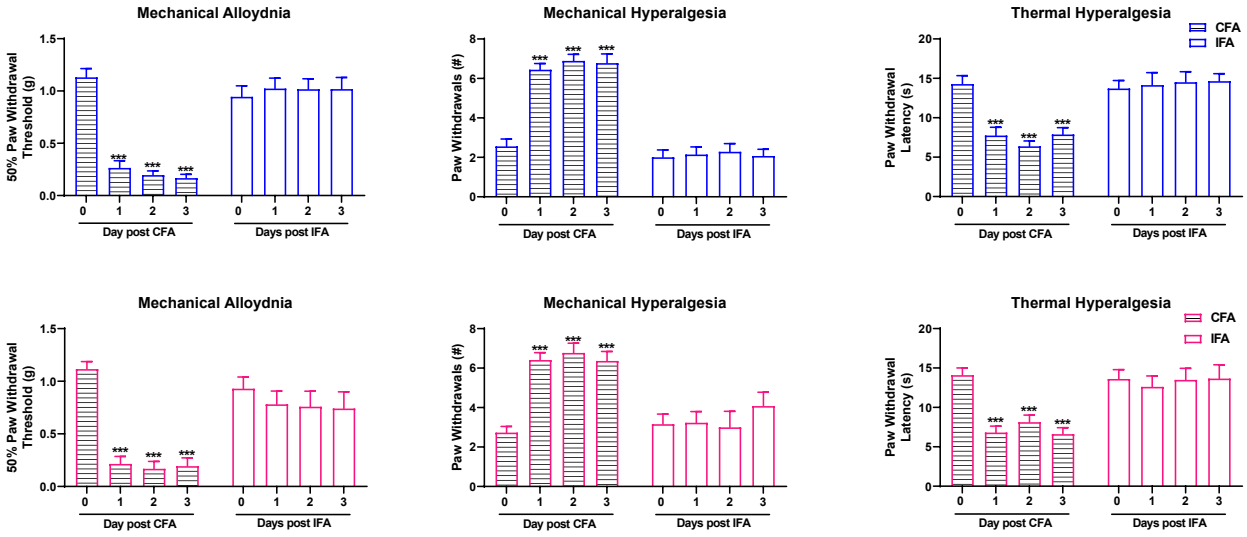

Fig S4. CFA, but not IFA, induces mechanical and thermal heat pain in the injected hindpaw. Intraplantar CFA induced mechanical allodynia, mechanical hyperalgesia, and thermal heat hyperalgesia on days 1-3 in both male and female mice. In contrast, IFA had no effect on mechanical or thermal pain. CFA group N=40 (18M, 22F) and IFA group N=27 (14M, 13F) mice. N=3-4 experimental replicates for behavioral assays. Data are mean ± SEM. ***P<0.001, different from baseline on day 0.

Supplemental Figure 5

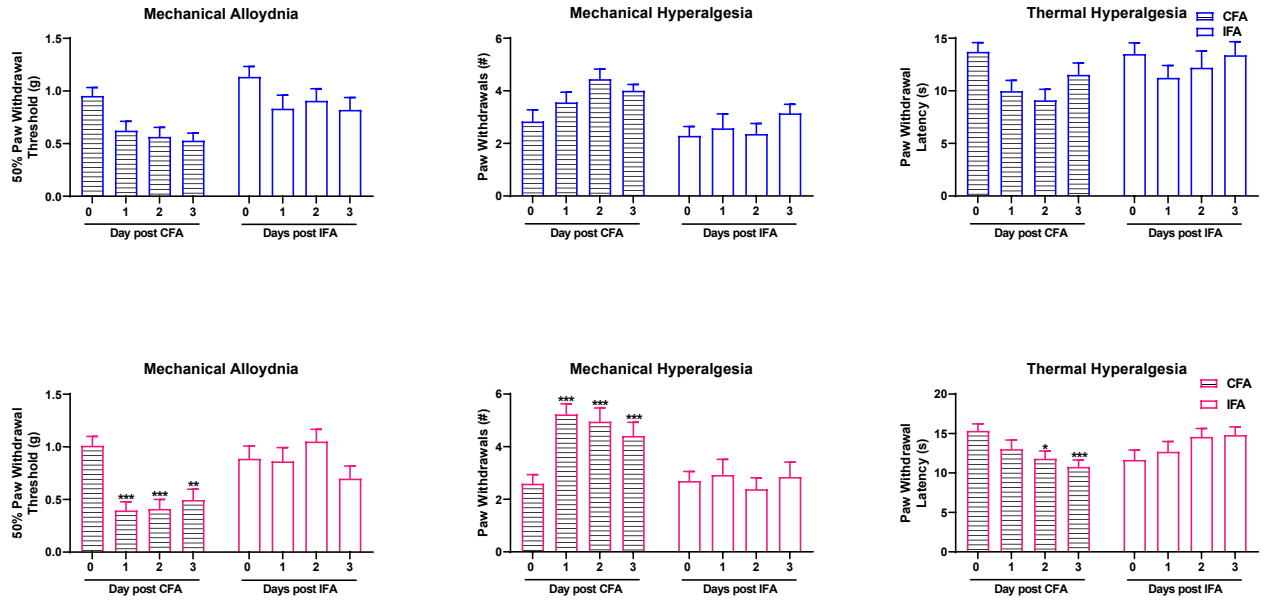

Fig S5. CFA, but not IFA, induces mechanical and thermal heat pain in the contralateral hindpaw. Intraplantar CFA induced mechanical allodynia, mechanical hyperalgesia, and thermal heat hyperalgesia on days 1-3 in female mice. Male mice showed similar trends towards CFA-dependent increases in mechanical and thermal pain in their non-injected hindpaws, although not significant. IFA had no effect on mechanical or thermal pain. CFA group N=40 (18M, 22F) and IFA group N=27 (14M, 13F) mice. N=3-4 experimental replicates for behavioral assays. Data are mean ± SEM. **P*<0.05, ***P*<0.01, ****P*<0.001, different from baseline on day 0.

Supplemental Figure 6

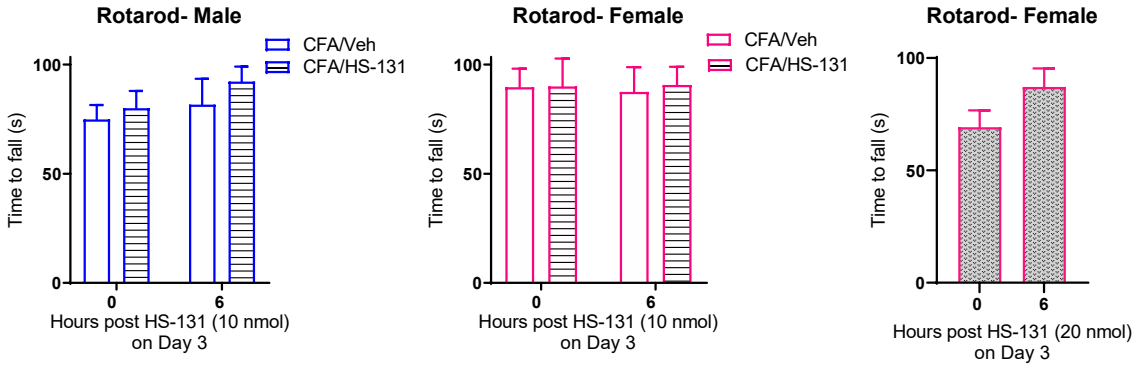

Fig S6. eHsp90 inhibition does not alter motor function. Administration of HS-131 (10 nmol or 20 nmol) had no effect on rotarod fall time in male or female mice 6 hours after treatment on day 3 post-CFA. CFA/Veh N=20 (9M, 11F), CFA/HS-131 (10 nmol) N=20 (9M, 11F), and CFA/HS-131 (20 nmol) N=10F mice. Data are mean ± SEM.