Appendix S1

SUPPLEMENTARY METHODS

Wild type C57BL/6 and NPPB knockout and TRPV1/TRPA1 double knockout mice were generated and maintained on a C57BL/6 background. NOD and NOD/SCID mice were purchased from the Jackson Laboratory. Mice used in this study were age-matched (10–14 weeks old). All experiments using mice were approved by NC State University's laboratory animal care policies.

In situ hybridization (ISH) was performed at high stringency (washed 30 min, 0.2 × SSC, 70°C) as described previously (6). Calcium imaging was performed as described previously using a Nikon Eclipse TE200 microscope (7). Dissociated mouse DRGs cells were used to investigate NPPB release. Cells were acutely cultured for 2 h and then stimulated for 8 h with IL-31 (0.3 μM). NPPB release was quantified by ELISA (RayBiotech, GA, USA).

For behavior analysis, IL-31 was reconstituted in 1X PBS (pH 7.4), and 1.5 nmol was injected subcutaneously to NPPB knockout mice and in a separate experiment to mice, we killed NPRA-receptors expressing neurons in the spinal cord using NPPB conjugated with saporin (6). For NOD and NOD/SCID mice, we injected 1.5 nmol of IL-31. For double knockout mice behavior, we used 0.3 nmol of IL-31 (5). Itch behavior was recorded and counted on a computer screen by investigators that were blinded to the mouse video.

Data were presented as mean ± standard error of mean (SEM), and a student's *t*-test was used to calculate *p* values. $p \leq 0.05$ was considered significant.