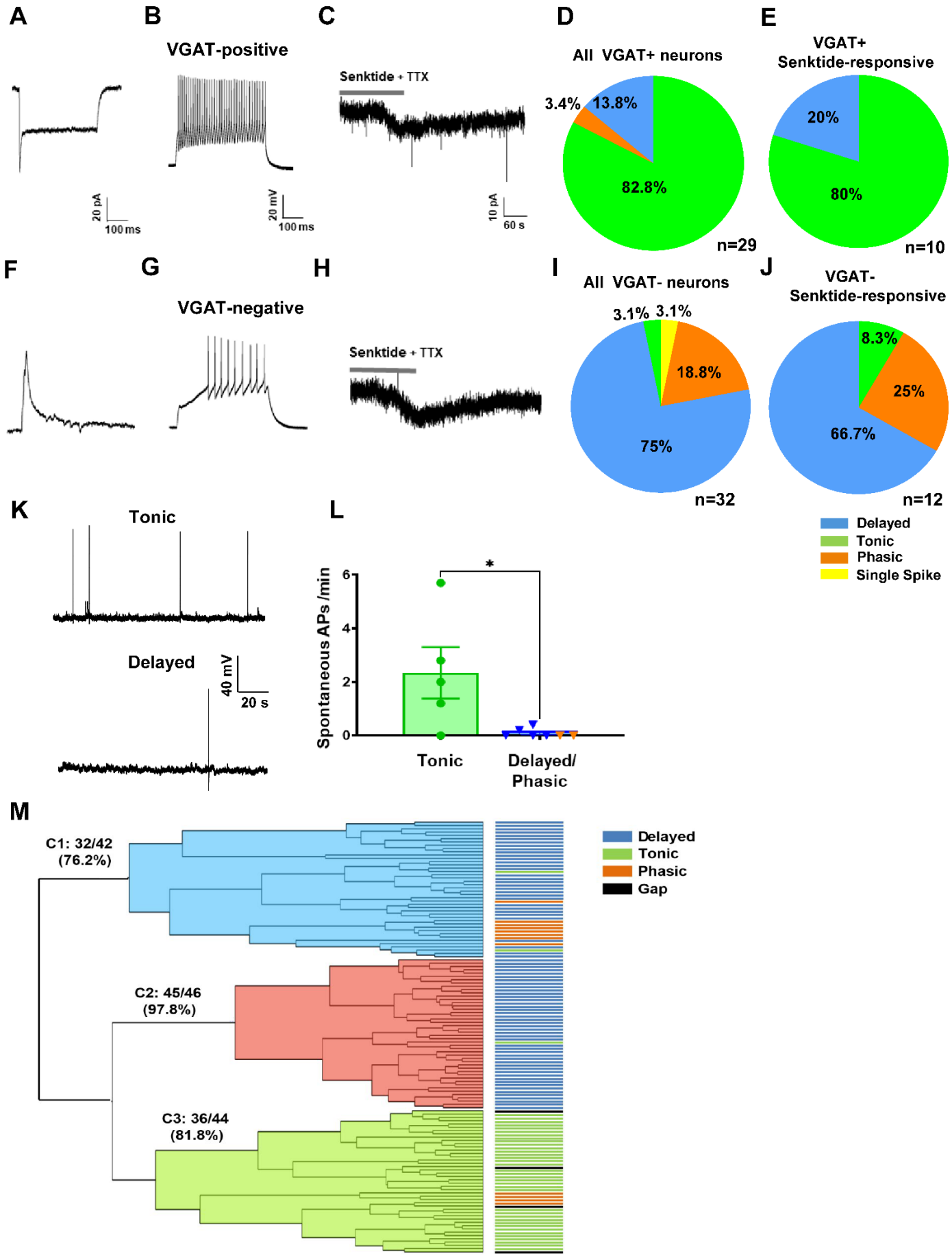

Figure S1.

Tonic-firing NK3R neurons exhibit distinct electrophysiological properties and predominantly correspond to inhibitory interneurons.

(A) Identification of VGAT-expressing inhibitory interneurons by the presence of a channelrhodopsin-2(ChR2)-mediated current in VGAT-ChR2-eYFP mice. **(B-C)** Observation of a tonic firing pattern (B), holding the neuron at hyperpolarized potentials, and a response to 1 μM senktide + 1 μM TTX (C) within the same VGAT-positive neuron. **(D-E)** Pie charts representing the percentages of the firing patterns observed across all recorded VGAT+ (inhibitory) neurons (D) and within the specific subset of neurons responsive to senktide (E), demonstrating a high prevalence of the tonic pattern. **(F)** Light-evoked IPSC recorded from a VGAT- excitatory interneuron lacking ChR2-mediated currents. **(G-H)** Traces representing a delayed firing pattern (G) and the response to senktide + TTX (H), recorded from the same neuron. **(I-J)** Pie charts illustrating the percentages of firing patterns determined across all recorded VGAT- neurons (I) and within the subset of senktide-responsive neurons (J), showing the prevalence of the delayed pattern. **(K)** Representative current-clamp traces from tonic and delayed NK3R neurons recorded at membrane potentials between –55 and –60 mV. **(L)** Tonic neurons (n=5) displayed a higher frequency of spontaneous action potentials (APs) compared to a sample of delayed (n=5) and phasic (n=2) firing neurons (*p<0.05, Mann –Whitney Rank sum test). **(M)** Hierarchical cluster analysis of NK3R neurons based on their electrophysiological attributes. The dendrogram represents the assignment of NK3R neurons (identified by their respective firing patterns) into three clusters, based on seven electrophysiological properties: resting potential and input resistance; rheobase, AP threshold and amplitude; delay to the first AP and AP number. Percentage values are referred to the neuron type that was in the majority (clusters C1 and C2: delayed; cluster C3: tonic) over the total numbers of cells in the cluster.

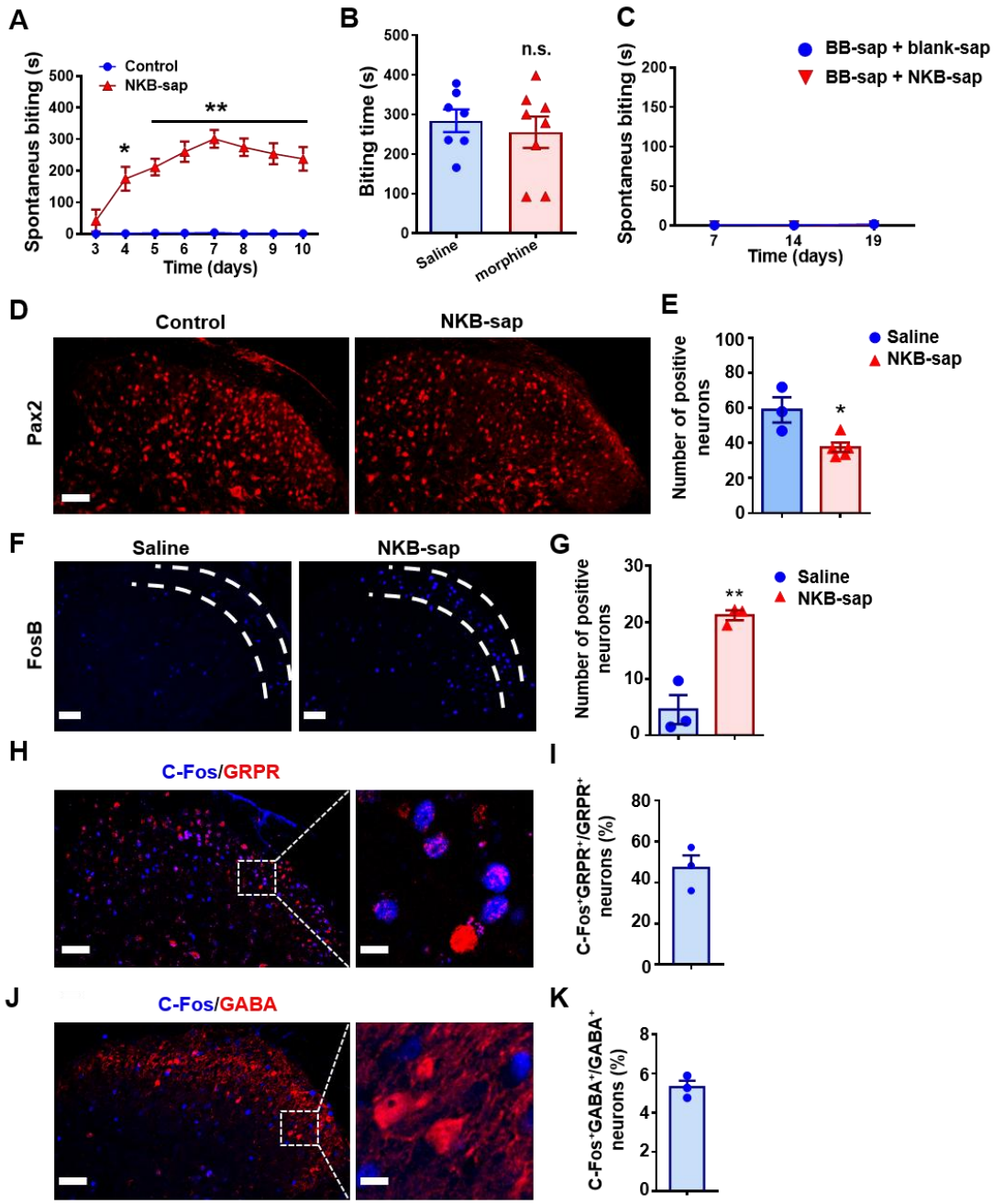

Figure S2.
GRPR neurons mediate neuropathic itch resulting from the loss of NK3R neurons.

(A) NKB-sap treatment caused robust spontaneous biting behaviors starting at day 4. n = 7-8 mice per group. *$p < 0.05$, **$p < 0.01$, two-way ANOVA with Bonferroni post-hoc test. **(B)** i.t. injections of morphine (0.3 nmol) did not affect spontaneous biting behaviors of NKB-sap treated mice. n = 7-8 mice per group. n.s., not significant. **(C)** After ablation of GRPR neurons with BB-sap (500 ng, i.t.), NKB-sap injections did not evoke spontaneous biting for up to 19 days. n = 6-7 mice per group. **(D-E)** Representative IHC images (d) and quantified data (e) showing a significant decrease of Pax2 neurons in mice treated with NKB-sap. Scale bar, 100 μm. n = 3-5 mice per group, *$p < 0.05$, unpaired t-test. **(F-G)** IHC images of C-Fos staining (f) and quantified data (g) demonstrating a significant increase in C-Fos-positive neurons in the superficial dorsal horn of NKB-sap-treated

mice. n = 3 mice per group, $^{**}p < 0.01$, unpaired t-test. Scale bar, 100 μm. **(H-I)** Double IHC images showing C-Fos expression (blue) in GRPR neurons (red in h), and statistics showing the percent C-Fos^{+}GRPR^{+} neurons (i). Scale bars: 100 μm for low magnification, 20 μm for high magnification. n = 3 mice. **(J-K)** Double IHC images and statistics showing barely C-Fos expression (blue) in GABA neurons (red in j). Scale bars: 100 μm for low magnification, 20 μm for high magnification. n = 3 mice.

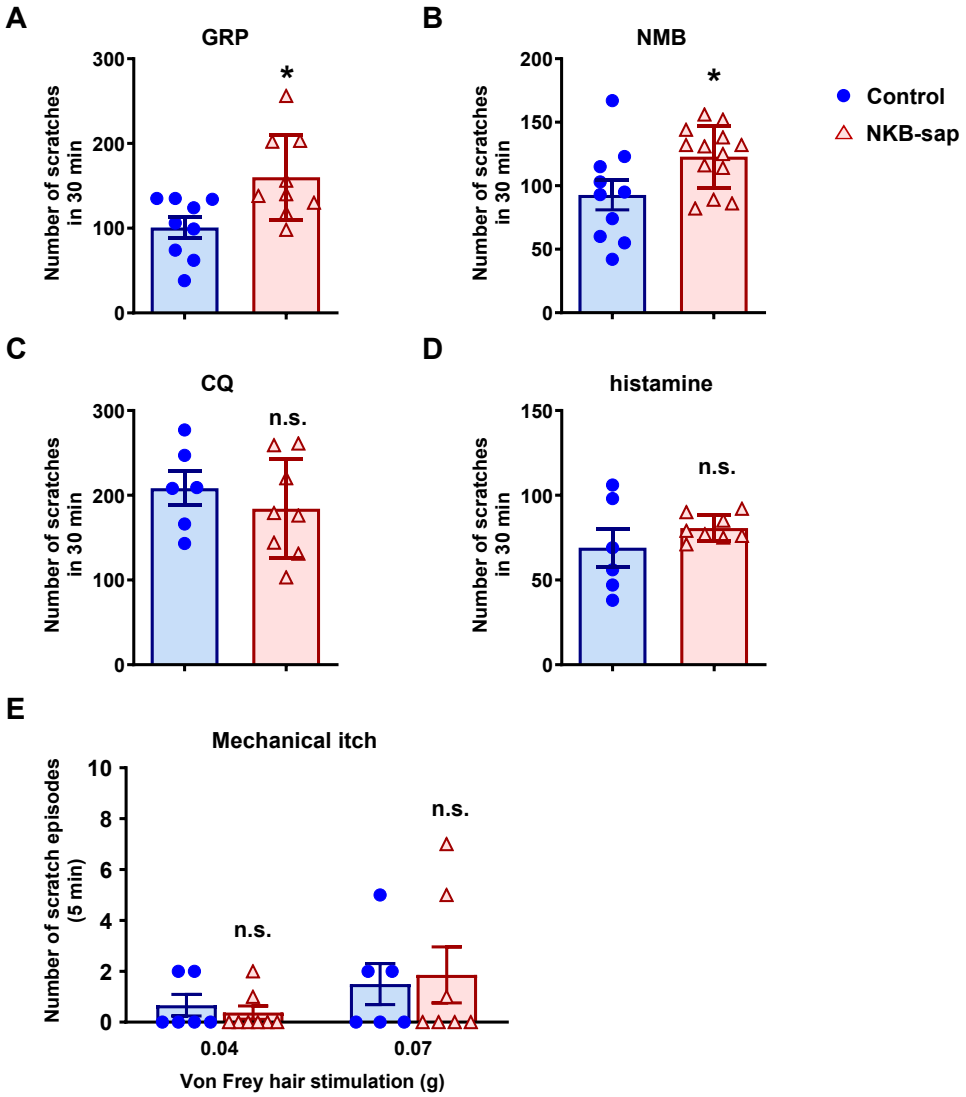

Figure S3.
Mice treated with NKB-sap exhibit enhanced itch responses upon GRP and NMB injection.

(A-B) The number of scratches was further increased in NKB-sap-treated mice following intrathecal injection of GRP (A) and NMB (B). n = 6-13 mice per group, $^{*}p < 0.05$, unpaired t-test. **(C-D)** The number of scratches was not altered in NKB-sap-treated mice after subcutaneous injection of CQ (C) or histamine (D). n = 6-8 mice per group, n.s., $p > 0.05$, unpaired t-test. **(E)** Mechanical itch induced by von Frey filament stimulation was comparable between control and

NKB-sap-treated mice. n = 6-8 mice per group. n.s., $p > 0.05$, two-way ANOVA with Bonferroni *post-hoc* test.

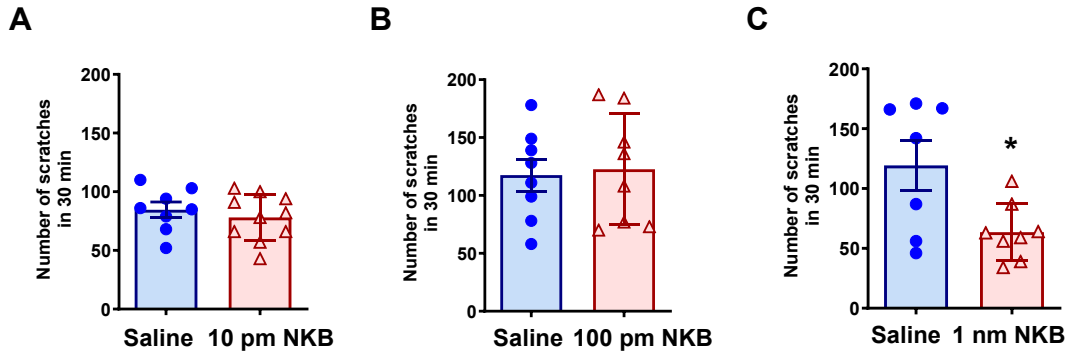

Figure S4.
NKB suppresses GRP-induced scratching in a dose-dependent manner.

(A–C) NKB at 10 pmol (A) and 100 pmol (B) did not alter the number of scratches induced by GRP injection, whereas 1 nmol NKB (C) significantly reduced the number of scratches following GRP injection. n = 7-10 mice per group, $*p < 0.05$, unpaired t-test.

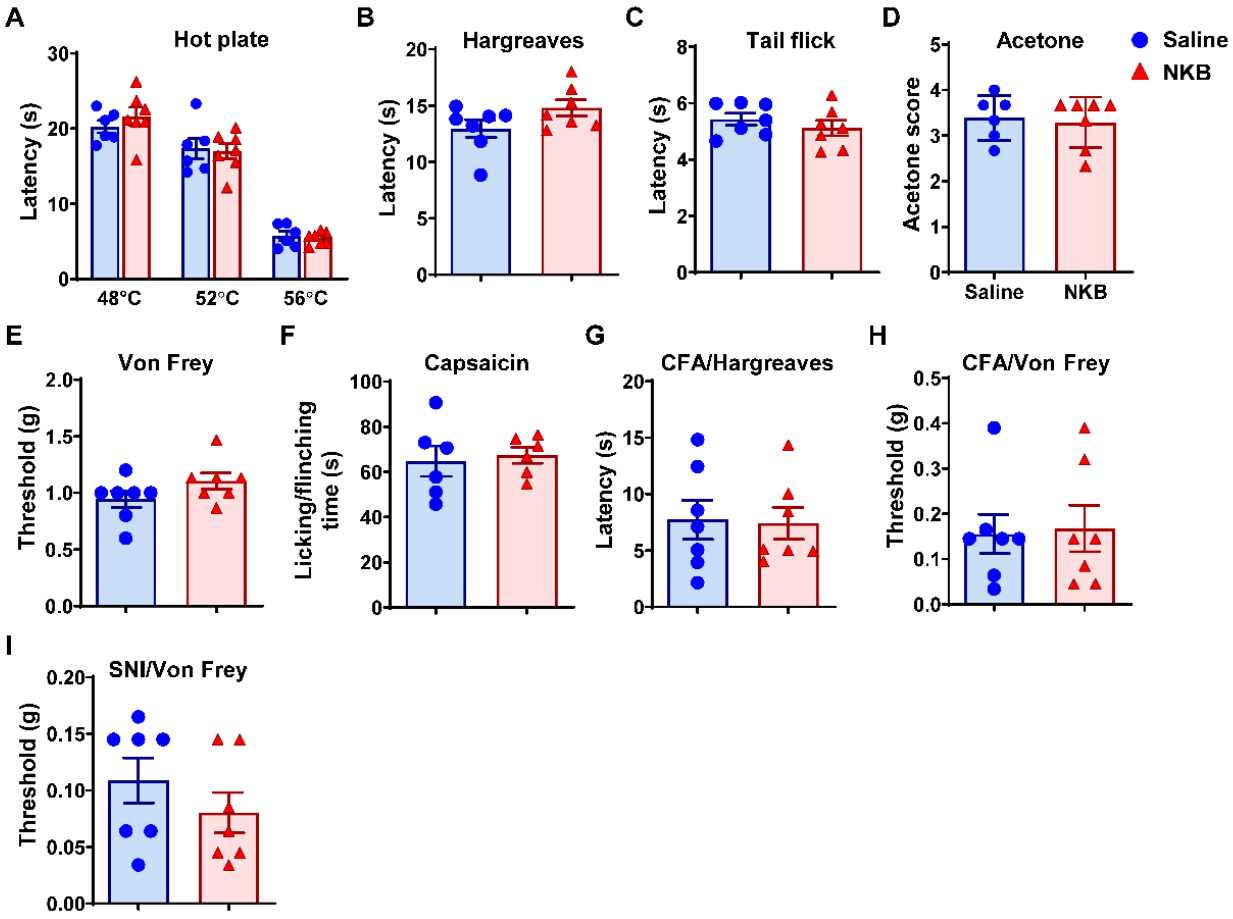

Figure S5.
NKB did not affect acute and chronic pain behaviors.

(A-E) Intrathecal injection of NKB (1 nmol) did not affect the baseline of nociceptive responses, as tested by the hot plate (A), Hargreaves (B), tail flick (C), acetone (D), and von Frey (E) tests. n = 6-7 mice per group. $p > 0.05$, unpaired *t*-test. **(F)** Licking/flinching behaviors induced by intraplantar (i.pl.) injection of capsaicin were not affected by pre-injection of NKB (1 nmol) for 10 min. $n = 6$ mice per group. $p > 0.05$, unpaired *t*-test. **(G-H)** Thermal hypersensitivity (G) and mechanical hypersensitivity (H) induced by i.pl. injection of CFA was not significantly affected by pre-injection of NKB (1 nmol) for 10 min. $n = 7$ mice per group. $p > 0.05$, unpaired *t*-test. **(I)** Mechanical hypersensitivity induced by SNI was not significantly affected by NKB injection. $n = 7$ mice per group. $p > 0.05$, unpaired *t*-test.

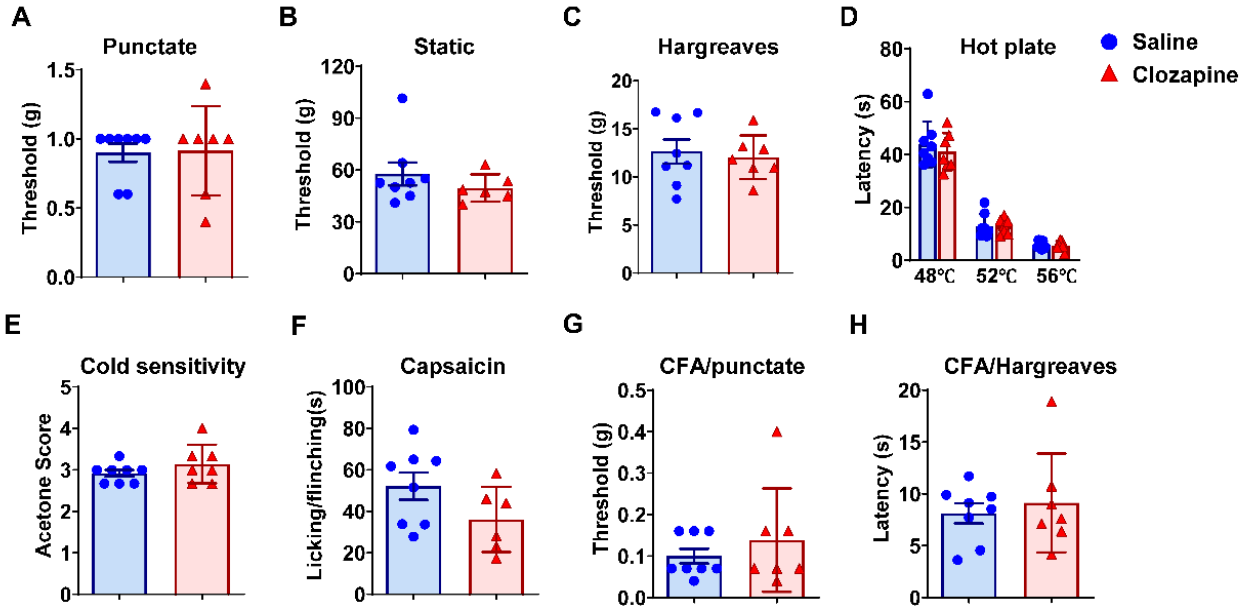

Figure S6.
Chemogenetic activation of NK3R inhibitory neurons did not alter pain behaviors.

(A-F) Activation of NK3R inhibitory neurons did not induce any detectable change in withdrawal thresholds to von Frey filament punctate stimulations (A), static (B), Hargreaves (C), hot plate (48 ℃, 52℃, 56℃, respectively) (D), cold sensitivity (E), or capsaicin test (F) $n = 6$-8 mice per group. $p > 0.05$, two-way ANOVA with Bonferroni *post-hoc* test or unpaired *t*-test. **(G-H)** Chemogenetic activation of NK3R inhibitory neurons did not impact on punctate hypersensitivity (G), and withdrawal responses to Hargreaves (H) in mice following CFA. $n = 6$-8 mice per group. $p > 0.05$, two-way ANOVA with Bonferroni *post-hoc* test or unpaired *t*-test.

Firing pattern	V resting (mV)	Input resistance (MOhm)	Rheobase (pA)	Threshold (mV)	AP amplitude (mV)	First AP latency (ms)	AP number
Delayed (n=77)	-59.1 ± 0.7	686 ± 22	65.1 ± 3.4 (*G ,***T)	-37.0 ± 0.5 (***T)	56.1 ± 1.1 (**T)	64.8 ± 4.2 (***T, **P, *G)	15.3 ± 0.5 (***P, **T)
Tonic (n=39)	-58.5 ± 1.0	761 ± 42	44.3 ± 3.0 (***D)	-41.0 ± 0.7 (***D)	62.5 ± 1.6 (**D)	27.1 ± 1.8 (***D)	17.8 ± 0.8 (***P, **D)
Phasic (n=12)	-58.2 ± 1.5	752 ± 110	60.0 ± 9.2	-41.7 ± 2.7	57.0 ± 2.7	35.9 ± 8.2 (**D)	3.6 ± 0.5 (***D,T,G)
Gap (n=4)	-58.7 ± 0.7	857 ± 148	30.0 ± 5.8 (*D)	-41.9 ± 2.7	59.5 ± 5.1	25.4 ± 9.6 (*D)	14.7 ± 2.8 (***P)

Table S1.
Electrophysiological properties of NK3R neurons exhibiting different firing patterns.

Comparisons between different classes of NK3R neurons were obtained by applying the non-parametric test Kruskal-Wallis One Way ANOVA on Ranks, followed by Dunn's test for pairwise multiple comparisons. For the action potential (AP) number, the one-way ANOVA test was performed, followed by the Holm-Sidak method. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ in the *post-hoc* test, followed by the abbreviation of the group the comparison was made with. n, number of neurons examined for each class.

Neuron type	Mice age	V resting (mV)	Input resistance (MOhm)	Threshold (mV)	AP amplitude (mV)	Firing patterns	Primary afferent input	Synaptic inhibition onto GRPR neurons	Capsaicin sensitivity	Menthol/ icilin sensitivity	References
NK3R	P20-P30	-58.5±1.0	761±42	-41.0±0.7	56.1±1.1	Tonic	Aδ>C, Aβ	Pharmacological activation (NKB) (GABA>> Glycine)	Responsive (100%) (TTX)	Responsive (71%) (TTX)	Present study
Galanin	4-6 weeks	n.d.	1142±226	n.d.	46.6±2.8	n.d.	n.d.	Optogenetic activation (GABA, Gly)	n.d.	n.d.	(1), (2)
Dynorphin (mostly inhibitory)	P21-P22	<-70	1156±190	-40/-45	n.d.	Phasic> Delayed> Tonic> Single	Aδ>C (LT +HT)>Aβ	n.d.	n.d.	n.d.	(3)
NPY	4-6 weeks	-51.1±1.0	1433.6±79.8	-33.6 ±0.5	57.4±1.7	Tonic> Phasic> Single	C>Aβ, Aδ	Optogenetic activation (GABA>>Gly) NPYR activation	Low response (17%; TTX)	Not responsive (TTX)	(4-6)

Table S2.

Electrophysiological properties of subsets of dorsal horn inhibitory interneurons in comparison with tonic-firing NK3R neurons

Sensitivity to capsaicin and menthol/icilin refers to the increase in the frequency of glutamatergic postsynaptic currents. Capsaicin or menthol/icilin were tested on mEPSCs in the presence of tetrodotoxin (TTX) to show direct connectivity. n.d.: not determined.

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