

Figure S1

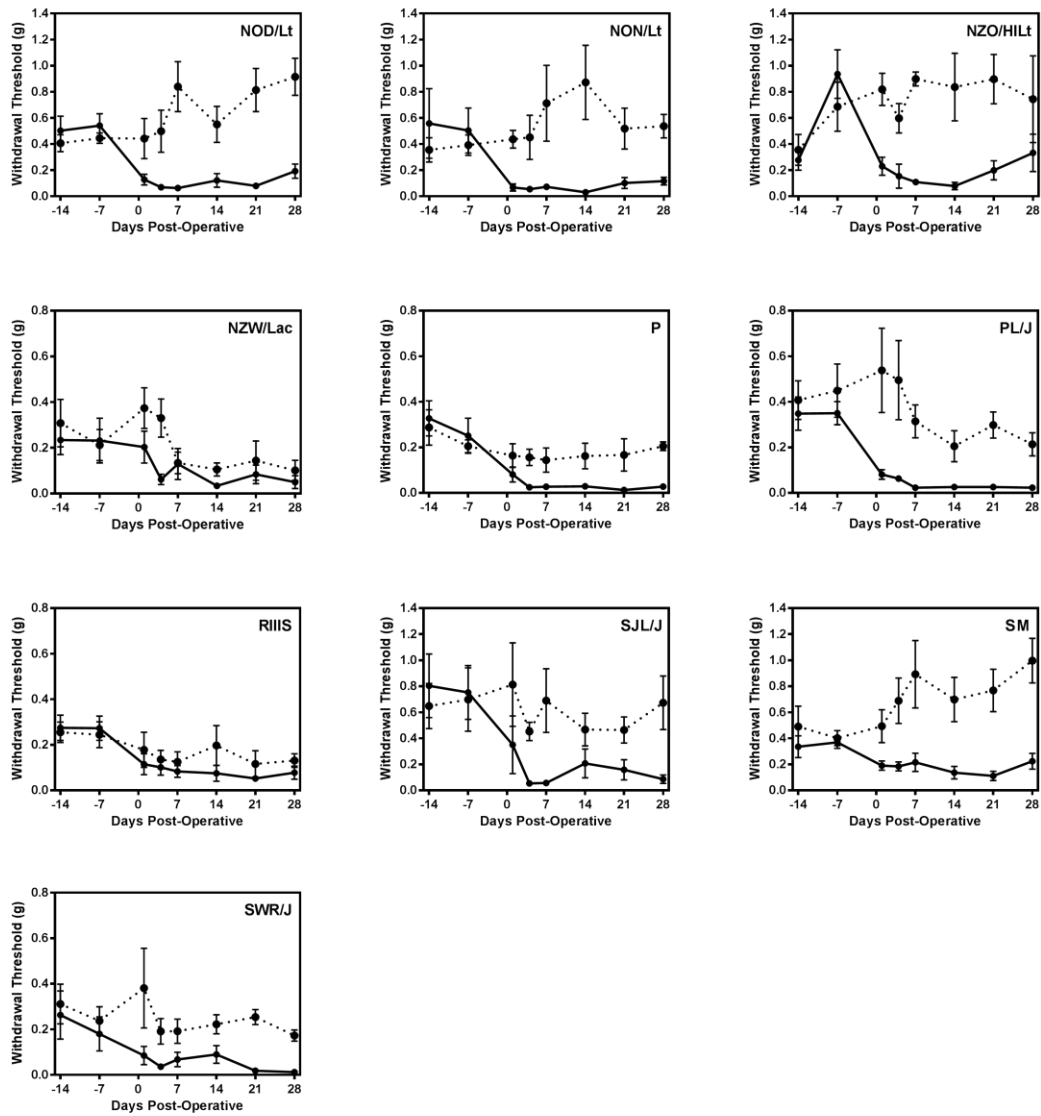


Fig. S1. Hind paw withdrawal thresholds to von Frey fiber stimulation measured before (-14, -7 days) and after (1, 4, 7, 14, 21 and 28 days) SNI surgery. Symbols ($n=4-6$ mice/strain) represent mean \pm S.E.M. withdrawal threshold (g); solid lines represent the ipsilateral hind paw and dotted lines the contralateral hind paw.

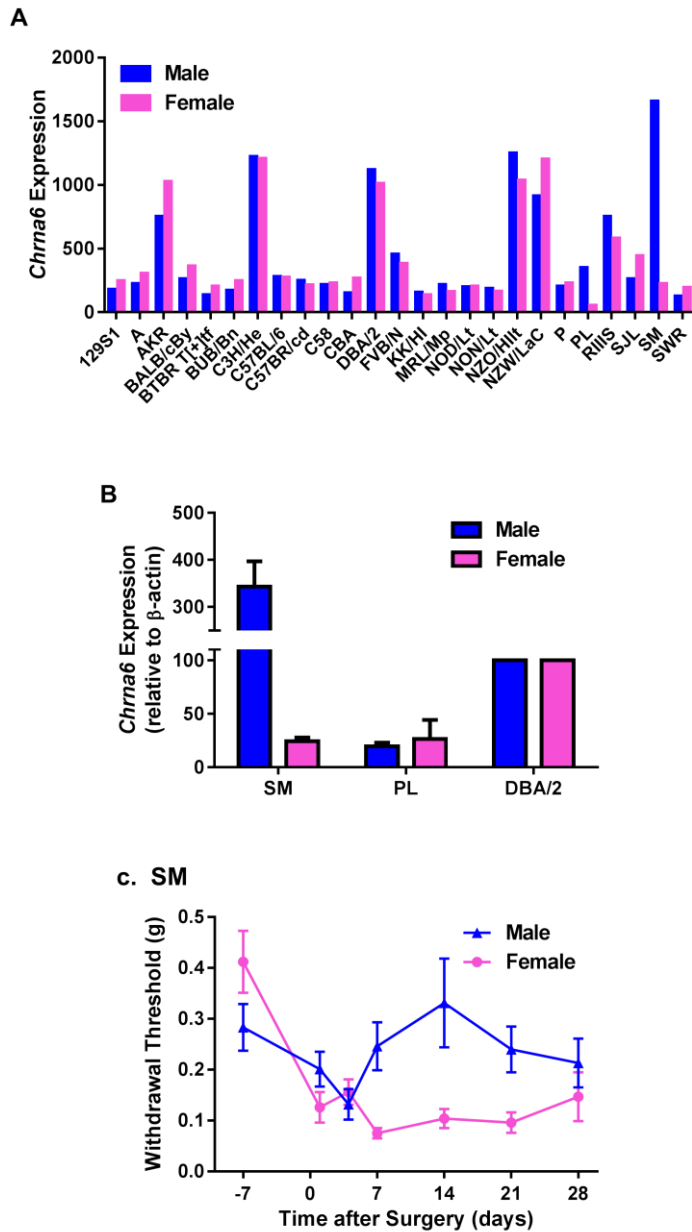


Fig. S2. Sex differences in *Chrna6* DRG mRNA expression in SM mice, and their correlation with sex differences in mechanical allodynia. A) Basal DRG expression of probeset 1450426_at (*Chrna6*) in male and female mice. B) Confirmation of microarray data using qPCR in three mouse strains, including SM. Bars represent mean \pm S.E.M. expression relative to β -actin (3 biological replicates; 2 technical replicates), and calibrated to DBA/2 males. **c)** Mechanical allodynia in SM mice of both sexes. Symbols ($n=8$ mice/sex) represent mean \pm S.E.M. withdrawal threshold (g).

A

Probeset	Gene	Protein	Fold Chg.
1426121_at	<i>Mrgpra3</i>	MAS-related GPR, member A3	-100.0
1450426_at	<i>Chrna6</i>	cholinergic receptor, nicotinic, $\alpha 6$	-67.3
1457651_x_at	<i>Rem2</i>	Rad and gem-related GTP binding protein 2	-52.0
1460663_at	<i>Cckbr</i>	cholecystokinin B receptor	43.0
1420573_at	<i>Hoxd1</i>	homeobox D1	-42.9
1450143_at	<i>Rasgrp1</i>	RAS guanyl releasing protein 1	-41.7
1450427_at	<i>Chrna6</i>	cholinergic receptor, nicotinic, $\alpha 6$	-41.6
1447812_x_at	<i>Flnc</i>	filamin C, γ (actin binding protein 280)	33.6
1429922_at		RIKEN clone: A230108N10	-30.2
1453060_at	<i>Rgs8</i>	regulator of G-protein signaling 8	-30.1

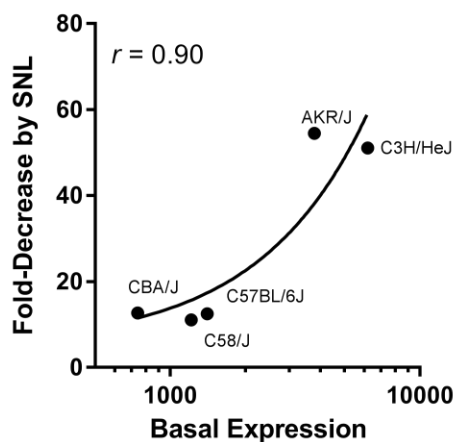
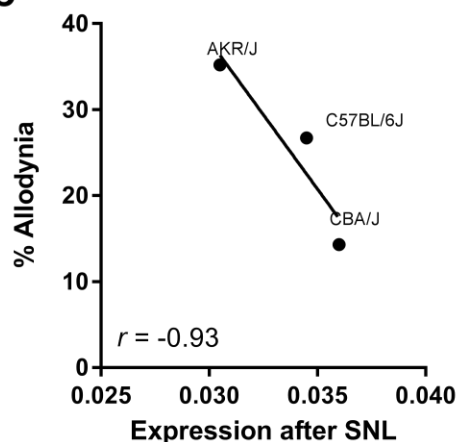
B**C**

Fig. S3. Downregulation of *Chrna6* by nerve injury, and correlation with mechanical allodynia. (A) The 10 probesets with greatest fold-changes in DRG gene expression after nerve injury (spinal nerve ligation; SNL) in AKR/J mice (1). (B) The correlation between basal DRG expression of *Chrna6* and fold-decrease in *Chrna6* expression 3 days after SNL in the five mouse strains studied by Persson et al. (2,3) (probeset 1450426_at). (C) The correlation between DRG *Chrna6* expression after SNL and mechanical allodynia in the three strains tested behaviorally by Persson et al. (2,3).

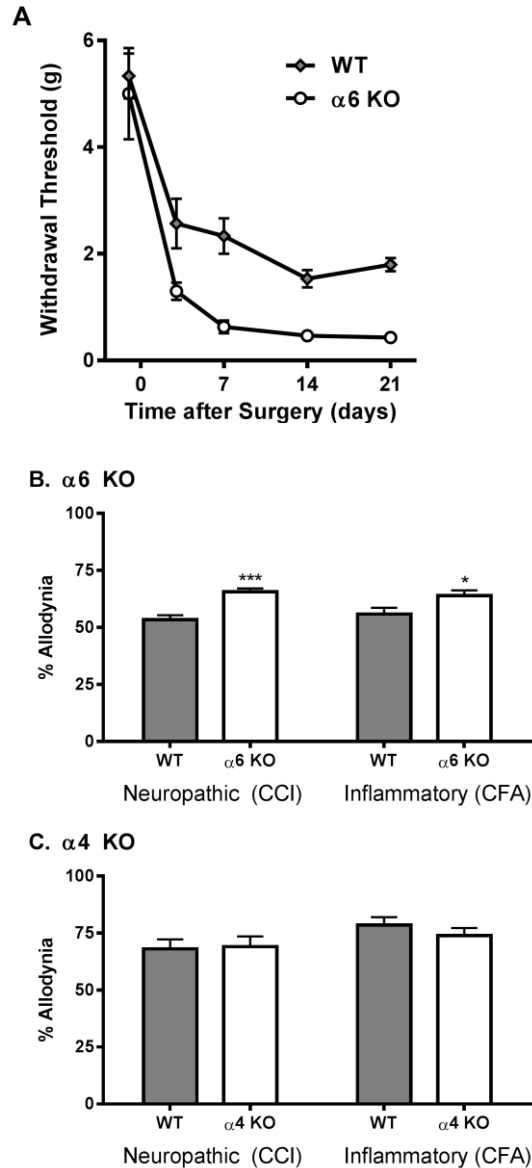


Fig. S4. Increased ipsilateral mechanical allodynia in *Chrna6* ($\alpha 6$ KO) but not *Chrna4* null mutants ($\alpha 4$ KO). A) Increased mechanical allodynia after chronic constriction injury (CCI) in $\alpha 6$ KO mice compared to wildtypes (WT); performed at Virginia Commonwealth University. Symbols represent mean \pm S.E.M. withdrawal threshold (g). Genotype differences were highly significant ($p < 0.001$) at all postoperative time points. B) Increased mechanical allodynia after CCI and CFA in $\alpha 6$ KOs, measured at the peak of allodynia at postoperative day 14 and post-injection day 3, respectively. Bars represent mean \pm S.E.M. percentage of maximum possible allodynia. * $p < 0.05$, *** $p < 0.001$ compared to WT by *t*-test. C) No CCI or CFA phenotype in $\alpha 4$ KOs; tested exactly as in graph B.

Figure S4

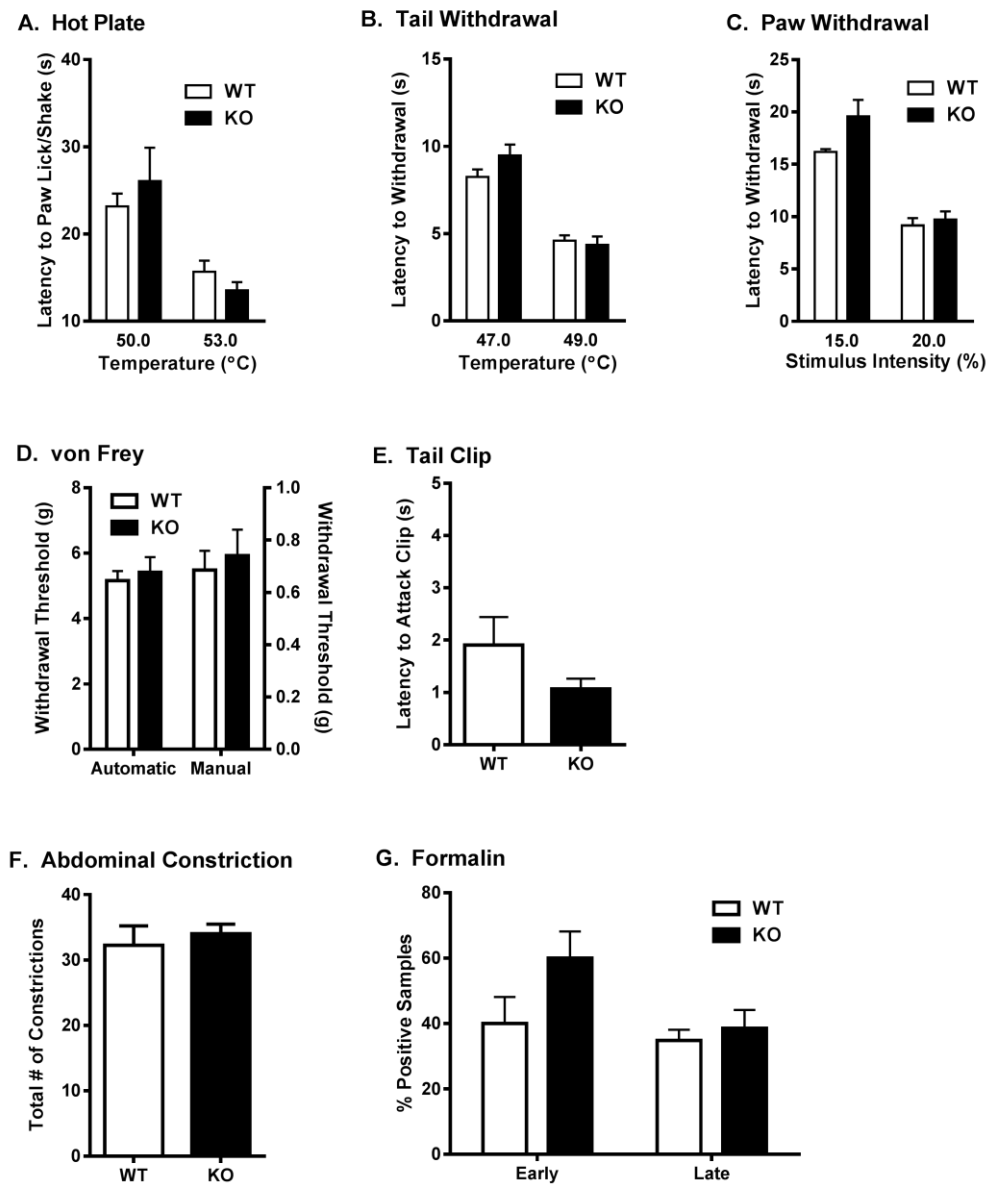


Fig. S5. No altered sensitivity of *Chrna6* KO mice in a battery of acute and tonic nociceptive assays. Bars represent mean \pm S.E.M. withdrawal latencies (A-C), withdrawal thresholds (D), latencies to nocifensive behavior (E), number of abdominal constrictions (F), or percentage of samples featuring licking behavior (G). See Mogil et al. (65) for methodological details.

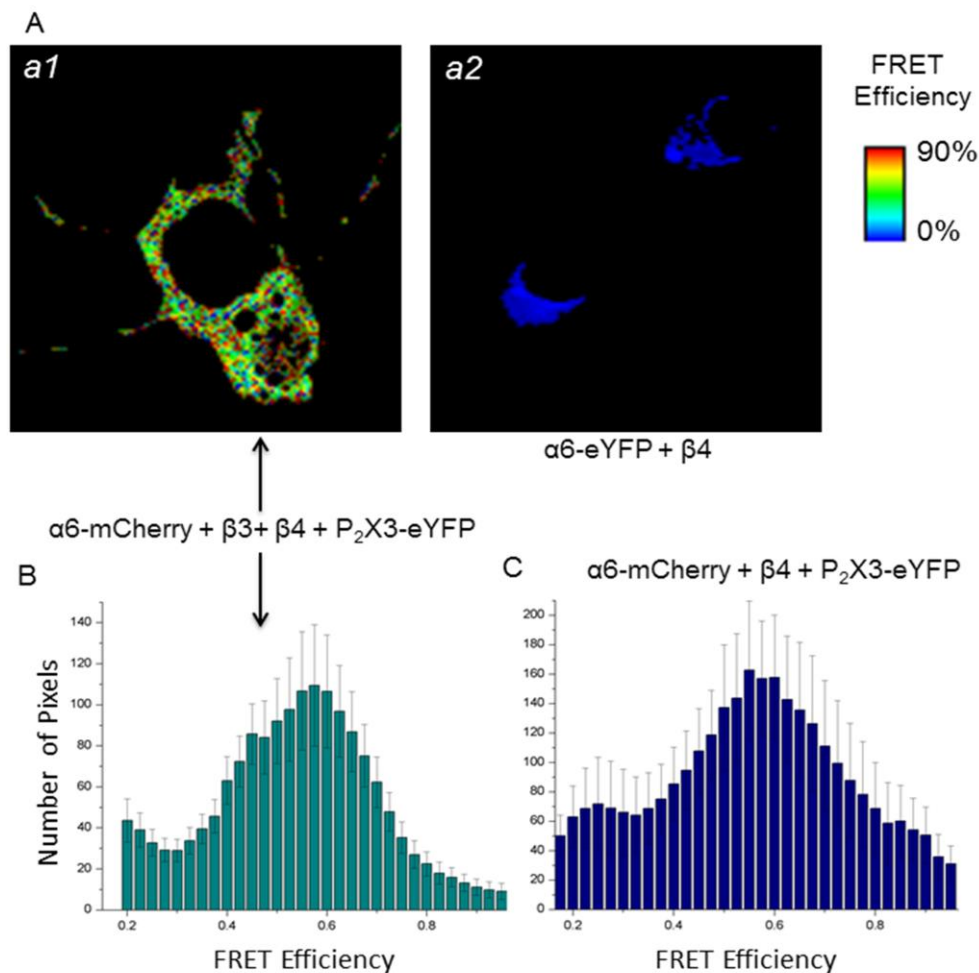


Fig. S6. Physical contacts between P2X3 and $\alpha 6\beta 4^*$ nAChRs revealed by fluorescence lifetime imaging microscopy. (A) Pixel-by-pixel analysis of FLIM data. *a1*: exemplar mouse cortical neuron transfected with $\alpha 6\text{-mCherry}\beta 3\beta 4 + P2X3\text{-eYFP}$. *a2*: exemplar neuron cortical neurons transfected with $\alpha 6\text{-eYFP}\beta 4$. (B,C) Analysis of data from 10 mouse cortical neurons transiently transfected with (B) $\alpha 6\text{-mCherry}\beta 4 + P2X3\text{-eYFP}$, (C) and $\alpha 6\text{-mCherry}\beta 3\beta 4 + P2X3\text{-eYFP}$. Error bars represent SEM.

Table S1. Affymetrix gene expression data of all Chr9* probes, and correlation with baseline nociceptive sensitivity and mechanical allodynia.

ProbeSet	Symbol	AKR/0	BALB/c/0	129S1/Sv	BTBR T+	1/SJL/0	C57BL/6J	NZW/LacJ	BUB/Brd	CBA/0	KK/Hrl	SM/0	PL/0	C58/0	RHS/0	NZO/HLU	DBA/2J	C3H/HeJ	NOJ/LU	C57BL/cd1	FVB/NJ	P/0	MRL/MpJ	SWR/0	A/0	NON/LU	Maximum	Average	SD	NCV	Baseline Corr.	Allodynia Corr.	
Baseline Nociceptive Sensitivity (g)		0.32	0.52	0.14	0.25	0.73	0.25	0.25	0.71	0.33	0.24	0.40	0.39	0.32	0.26	0.56	0.16	0.50	0.47	0.48	0.33	0.27	0.31	0.24	0.40	0.45							
Mechanical Allodynia (% max.)		79.7	71.5	80.8	83.8	82.9	71.4	85.6	79.2	76.1	74.5	80.3	89.9	75.2	74.8	69.8	83.3	84.5	77.8	75.1	74.1	86.2	84.9	69.8	80.0	69.3							
141882_at	Chr9a1	4.5	4.4	4.5	4.6	4.5	4.5	4.4	4.6	4.4	4.5	4.4	4.4	4.5	4.5	4.5	4.4	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.6	4.5	0.05	0.01	0.02	-0.08	
142500_at	Chr9a2	3.9	3.9	3.9	3.9	3.9	4.7	3.9	3.9	4.0	4.0	4.0	3.8	3.9	3.9	4.0	3.9	3.9	3.9	3.9	3.9	3.9	3.9	4.0	3.9	3.9	4.7	4.0	0.16	0.04	-0.36	-0.04	
1455931_at	Chr9a3	19.2	19.0	12.2	27.7	19.2	19.5	17.1	23.4	33.0	19.2	17.8	18.9	19.2	23.2	19.3	18.2	19.0	19.2	19.2	19.3	19.3	19.2	8.9	19.1	25.5	37.8	21.3	5.74	0.27	0.20	-0.27	
1452010_at	Chr9a3	18.3	17.9	14.3	11.8	27.7	14.5	15.0	21.1	20.1	21.1	39.5	17.5	16.0	26.0	22.5	12.3	19.0	18.3	18.3	18.4	27.8	12.1	12.1	15.2	21.8	39.5	19.9	6.23	0.31	0.36	-0.21	
1444388_at	Chr9a3	5.2	5.1	5.9	5.8	5.2	5.3	5.2	5.1	5.4	5.2	5.4	5.1	5.2	5.2	5.4	6.0	5.1	5.2	5.1	5.3	5.7	5.3	5.2	5.2	5.3	6.0	5.3	0.22	0.04	-0.46	-0.10	
Average	Chr9a3	14.3	14.0	10.7	15.1	17.4	11.1	12.4	16.5	19.5	15.2	27.6	11.8	13.5	18.1	15.7	12.2	14.3	14.2	14.2	14.3	17.6	12.2	8.8	13.2	17.5	27.8	15.5	3.56	0.21	0.31	-0.27	
1421203_at	Chr9a4	4.7	4.7	4.7	4.7	4.6	4.7	4.6	4.6	4.8	4.6	5.1	4.6	4.5	4.6	5.1	4.7	4.5	4.6	4.7	4.7	4.7	4.7	4.7	4.8	5.1	4.6	5.1	4.7	0.15	0.03	0.07	-0.21
1456354_at	Chr9a4	6.5	6.5	6.8	7.1	9.7	6.8	6.6	6.5	6.9	6.5	6.8	6.5	6.5	6.5	6.8	6.7	6.5	6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.7	6.9	0.63	0.09	0.41	0.18
1421202_at	Chr9a4	5.8	6.7	5.8	6.0	5.8	10.5	5.7	5.7	6.1	5.5	5.9	5.5	5.6	5.8	8.2	5.9	6.1	5.8	5.7	6.0	5.8	6.3	5.7	5.7	6.5	10.5	6.3	1.05	0.17	0.01	-0.12	
Average	Chr9a4	5.7	6.0	5.8	5.9	6.7	7.3	5.6	5.6	5.9	5.5	5.9	5.5	5.5	5.6	6.7	5.8	5.7	5.6	5.6	5.8	5.7	5.9	5.7	5.9	6.0	8.4	6.0	0.42	0.07	0.22	-0.04	
1442035_at	Chr9a5	9.4	9.2	9.9	10.2	10.0	9.9	9.5	9.1	9.9	9.4	10.7	8.7	9.3	9.4	10.0	10.1	9.1	9.6	9.3	9.6	9.8	9.8	9.5	9.5	9.6	10.7	9.7	0.42	0.04	-0.19	-0.28	
1427401_at	Chr9a5	5.5	5.2	5.6	5.6	5.5	5.6	5.6	5.5	5.7	5.5	5.6	5.1	5.5	5.6	6.4	5.6	5.3	5.6	5.4	5.6	5.6	5.6	5.5	5.5	5.5	5.5	5.6	0.22	0.04	-0.04	-0.16	
Average	Chr9a5	7.5	7.2	7.8	7.9	7.7	7.8	7.5	7.8	7.8	7.4	8.1	6.9	7.4	7.5	8.2	7.8	7.2	7.6	7.8	7.7	7.7	7.5	7.5	7.5	7.6	8.5	7.4	0.49	0.04	-0.15	-0.27	
1450427_at	Chr9a6	750.8	380.5	170.8	133.3	261.3	279.6	912.3	171.0	151.1	151.4	167.2	340.9	216.1	712.4	1240.0	1118.8	1211.4	186.5	247.2	456.2	203.8	215.6	121.2	222.0	185.6	1657.2	512.6	486.30	0.85	0.02	0.75	0.02
1450426_at	Chr9a6	344.3	128.0	69.8	31.0	113.1	113.1	312.1	93.1	73.8	62.8	1054.4	11.3	102.7	407.0	373.4	402.1	306.6	103.4	106.9	176.5	111.1	97.1	47.3	113.9	104.2	1054.4	228.5	217.63	0.95	0.01	0.72	0.02
Average	Chr9a6	547.6	194.2	124.3	82.2	187.2	196.4	612.2	132.1	112.5	108.1	1358.8	180.6	159.4	579.7	811.2	760.4	791.0	150.1	177.1	316.3	157.4	156.3	86.3	168.0	145.4	1355.8	371.1	319.77	0.86	0.01	-0.76	0.02
1440681_at	Chr9a7	508.7	449.4	564.9	287.7	475.7	660.3	673.3	636.7	432.9	257.6	714.8	387.4	472.1	903.5	770.6	223.2	427.7	566.3	585.4	721.5	649.8	467.7	490.8	482.2	385.0	903.5	542.3	164.09	0.30	0.05	0.00	
1449615_at	Chr9a9	13.0	13.3	15.5	32.3	19.3	13.5	12.3	13.7	13.8	16.2	14.8	10.3	10.4	11.4	13.8	13.2	16.7	14.2	12.2	13.5	20.6	13.6	11.1	14.6	14.1	32.3	15.4	4.39	0.29	0.04	0.24	
1447214_at	Chr9a9	6.9	6.9	7.3	7.6	6.9	7.1	6.9	6.8	7.1	6.8	7.1	6.8	6.8	6.7	7.0	7.0	6.8	6.9	6.9	7.0	7.0	7.0	6.9	6.9	6.9	7.6	7.0	0.18	0.03	-0.28	0.09	
1430036_at	Chr9a9	6.9	6.8	7.3	7.4	7.6	8.3	6.9	6.6	7.3	6.9	7.3	6.7	6.7	6.9	7.3	7.2	6.8	6.9	6.8	7.0	7.1	7.1	6.9	7.0	6.9	8.3	7.1	0.35	0.05	-0.14	-0.06	
Average	Chr9a9	8.9	9.0	10.0	15.8	11.3	9.7	8.7	9.1	9.4	10.0	9.7	7.9	7.9	8.3	9.4	9.1	10.1	9.3	8.6	9.2	11.5	9.2	8.3	9.5	9.3	16.1	9.8	1.55	0.16	0.02	0.22	
1420682_at	Chr9b1	5.3	5.2	5.3	6.8	5.3	5.4	5.2	5.2	5.4	5.2	5.6	5.2	5.2	5.3	5.3	5.4	5.2	5.3	6.1	5.3	5.3	5.3	5.3	5.3	5.3	5.3	6.8	5.4	0.34	0.06	-0.09	0.11
1436428_at	Chr9b2	177.1	194.7	203.7	215.3	182.3	292.0	207.0	153.4	187.8	221.1	269.9	168.8	182.1	228.7	273.8	205.3	165.1	166.8	198.0	291.5	248.0	158.5	159.8	144.7	190.2	292.0	266.8	42.85	0.21	-0.34	-0.21	
14535842_at	Chr9b3	4.6	4.5	4.6	4.6	4.6	4.7	4.6	4.5	4.7	4.6	4.6	4.6	4.6	4.6	4.6	4.6	4.6	4.6	4.6	4.6	4.6	4.6	4.6	4.6	4.6	4.6	4.6	4.6	4.6	4.6	4.6	4.6
1457028_at	Chr9b4	5.3	4.8	5.1	7.6	5.1	5.4	5.3	5.3	5.5	5.3	5.4	6.1	6.8	8.8	5.4	5.3	5.4	5.4	5.7	5.3	9.8	5.0	5.0	5.3	5.3	21.4	7.0	2.34	0.47	-0.02	-0.33	
1425849_at	Chr9b4	5.7	5.7	5.8	5.8	5.7	5.8	5.7	5.6	5.9	5.9	5.8	5.6	5.7	5.7	5.8	5.8	5.6	5.7	5.7	5.7	5.8	5.8	5.7	5.7	5.7	5.7	5.7	5.7	5.7	5.7	5.7	5.7
Average	Chr9b4	5.5	5.3	5.4	6.7	5.4	5.6	5.5	5.4	5.7	5.6	5.6	6.2	7.3	5.6	5.5	5.5	5.6	5.6	6.2	5.5	7.8	5.4	5.4	5.5	5.5	13.7	6.4	1.68	0.26	-0.03	-0.33	
1420761_at	Chr9b5	3.5	3.5	3.6	3.5	3.5	3.5	3.5	3.4	3.5	3.6	3.5	3.8	3.4	3.5	3.5	3.5	3.4	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.8	3.5	0.07	0.02	-0.24	0.32
1420590_at	Chr9b5	4.3	4.2	4.3	4.3	4.3	4.3	4.3	4.2	4.4	4.2	4.3	4.2	4.2	4.3	4.3	4.3	4.2	4.3	4.3	4.3	4.3	4.3	4.3	4.3	4.3	4.3	4.3	4.3	4.3	4.3	4.3	4.3
1425230_at	Chr9b5	5.0	5.0	5.1	5.3	5.0	5.2	5.0	4.9	5.3	4.9	5.2	4.9	5.0	5.0	5.2	5.1	4.9	5.1	5.0	5.1	5.1	5.1	5.1	5.1	5.0	5.0	5.3	5.1	0.11	0.02	-0.21	0.10
1449532_at	Chr9b5	4.3	4.2	4.3	4.4	4.3	4.4	4.3	4.2	4.4	4.3	4.4	4.2	4.2	4.6	4.3	4.4	4.2	4.2	4.2	4.2	4.3	4.3	4.3	4.3	4.3	4.3	4.3	4.3	4.3	4.3	4.3	4.3
1427728_at	Chr9b5	4.2	4.2	4.5	4.3	4.2	4.3	4.2	4.2	4.4	4.4	4.3	4.3	4.3	4.3	4.3	4.2	4.2	4.2	4.2	4.2	4.2	4.2	4.2	4.2	4.2	4.2	4.2	4.2	4.2	4.2	4.2	4.2
Average	Chr9b5	4.5	4.5	4.7	4.7	4.5	4.6	4.5	4.4	4.7	4.5	4.6	4.5	4.5	4.6	4.6	4.6	4.5	4.8	4.5	4.6	4.6	4.6	4.6	4.5	4.5	4.6	4.9	4.6	0.08	0.02	-0.27	-0.01

Table S2. Top 10 correlated haplotypes (by p -value), genome-wide, with SNI-induced mechanical allodynia in 25 mouse strains.

Chrom.	Interval (Mb) ^a	log p	Candidate Genes ^b
15	45.37–45.86	3.24	<i>Kcnn1</i> (potassium channel, V1)
5	126.31–126.53	3.10	<i>Ubc</i> (ubiquitin C)
18	56.01–56.22	2.99	<i>Aldh7a1</i> (aldehyde dehydrogenase, 7A1)
8	28.86–29.33	2.97	<i>Chrna6</i> (cholinergic receptor, nicotinic, $\alpha 6$) <i>Chrna3</i> (cholinergic receptor, nicotinic, $\alpha 3$)
5	125.08–125.52	2.96	–
14	71.54–71.77	2.95	<i>Gfra2</i> (glial cell line derived neurotrophic factor, $\alpha 2$)
5	127.24–127.45	2.94	–
8	28.26–28.47	2.87	<i>Chrna6</i> (cholinergic receptor, nicotinic, $\alpha 6$) <i>Chrna3</i> (cholinergic receptor, nicotinic, $\alpha 3$)
5	126.54–126.77	2.86	–
5	125.83–126.05	2.83	<i>Ubc</i> (ubiquitin C)

^amm8 database, NCBI Build 36.

^bGenes within 500 kb on either side of interval were identified using the Mouse Genome Informatics database (www.informatics.jax.org). A PubMed search was performed using the name of the gene (or gene class) and the search term "pain". Genes with non-zero hits are listed.

Table S3. Nicotine anti-allodynic ED₅₀s in all genotypes.

Assay	Route (dose units)	Genotype	ED ₅₀ (95% CI)
SNI	i.p. (mg/kg)	WT	2.7 (1.8–4.2)
		L9'S	1.1 (0.7–1.6)*
		KO	>100†
CFA	i.p. (mg/kg)	WT	1.3 (0.5–3.1)
		L9'S	0.3 (0.2–0.4)*
		KO	>100†
SNI	i.c.v. (μg)	WT	14.0 (9.5–20.8)
		L9'S	11.4 (2.9–45)
		KO	>100†
CFA	i.c.v. (μg)	WT	8.7 (2.0–37)
		L9'S	3.3 (0.2–47)
		KO	>100†
SNI	i.t. (μg)	WT	20.7 (6.4–68)
		L9'S	21.0 (4.1–107)
		KO	>100†
CFA	i.t. (μg)	WT	8.1 (2.0–33)
		L9'S	1.3 (0.2–7.4)*
		KO	>100†
SNI	i.pl. (μg)	WT	49 (32–74)
		L9'S	17.8 (7.7–41)
		KO	>100†
CFA	i.pl. (μg)	WT	12.6 (2.7–57)
		L9'S	3.9 (0.3–53)
		KO	>100†

*Significantly more sensitive than WT mice.

†Significantly less sensitive than other genotypes.

Table S4. Dose-response characteristics (EC₅₀ and Hill coefficients; mean ± S.E.M.) for various combinations of α6-containing nicotinic receptors and P2X receptors expressed in oocytes. *n*, number of cells.

Receptor(s)	Additional Agonist	ACh EC ₅₀ (μM)	ATP EC ₅₀ (μM)	Hill coefficient	<i>n</i>
α6(L9'S)β4		3.28 ± 0.11		1.36 ± 0.05	8
P2X ₂			23.9 ± 1.2	1.52 ± 0.10	18
α6(L9'S)β4 +P2X ₂		4.28 ± 0.10		1.30 ± 0.03	11
	ATP, 32 μM	4.52 ± 0.26		1.36 ± 0.09	14
	ATP, 100 μM	6.04 ± 0.82		1.46 ± 0.23	14
			22.2 ± 1.1	1.60 ± 0.11	11
	ACh, 100 μM		33.2 ± 3.6	1.32 ± 0.15	11
α6β4β3(V13'S)		1.25 ± 0.06		0.84 ± 0.03	10
α6β4β3(V13'S) +P2X ₂		1.57 ± 0.09		0.84 ± 0.03	12
	ATP, 32 μM	2.36 ± 1.09		0.75 ± 0.18	19
	ATP, 100 μM	1.59 ± 0.45		0.67 ± 0.09	8
			23.3 ± 1.7	1.58 ± 0.15	11
	ACh, 100 μM		24.5 ± 3.1	1.81 ± 0.35	12
P2X ₃ (K65A)			13.6 ± 1.3	1.41 ± .16	12
α6(L9'S)β4 +P2X ₃ (K65A)		3.27 ± 0.13		1.31 ± 0.05	8
			37.9 ± 6.1	0.94 ± 0.11	14
	ACh, 100 μM		32.8 ± 5.0	1.00 ± 0.04	11
α6β4β3(V13'S) +P2X ₃ (K65A)		1.09 ± 0.10		0.84 ± 0.05	7
			7.60 ± 0.33	1.55 ± 0.09	14
	ACh, 100 μM		11.5 ± 1.6	1.34 ± 0.21	11
α6β2 [‡]		0.121 ± 0.006		1.12±0.05	15
	ATP, 100 μM	0.121 ± 0.003		1.33±0.05	14
α6β2 [‡] +P2X ₂		0.15 ± 0.01		1.17±0.08	11
	ATP, 100 μM	0.3 ± 0.06		1.3±0.3	11
			26 ± 2	1.6±0.2	14
	ACh, 10 μM		29 ± 2	1.9±0.2	12
α6β2 [‡] +P2X ₃ (K65A)		0.127 ± 0.006		1.19±0.05	11
			44 ± 7	0.9±0.1	13

Table S4

Concentration-response data for each oocyte was normalized to the maximum current for that oocyte. The mean and S.E.M. for a series of oocytes were plotted against agonist or antagonist concentration and iteratively fitted to the following equation:

$$I_A = I_{\min} + \frac{I_{\max} - I_{\min}}{1 + 10^{n_H(\log A_{50} - \log A)}}$$

where A is the concentration of ligand present, I_A is the current in the presence of ligand concentration A , I_{\min} is the current when $A=0$; I_{\max} is the current when $A=\infty$, A_{50} is the concentration of A that evokes a current equal to $(I_{\max} + I_{\min})/2$, and n_H is the Hill coefficient.