Supporting Online Material
Material and Methods

Mouse lines and mating paradigms

All animal work was conducted in accordance with Baylor College of Medicine and Case Western Reserve University IACUC guidelines.

The generation of *Atoh1*^{flox}, *Atoh1*^{LacZ}, *Hoxb1*^{Cre} and *ROSA*^{R26R} mice were described previously *(S1-4)*. All animals used for these experiments were maintained on mixed genetic backgrounds except for *Atoh1*^{LacZ} and *ROSA*^{R26R} mice, which are congenic on the C57Bl/6J strain background.

Atoh1 conditional knockout (*Atoh1*^{CKO}) mice were generated by first crossing *Hoxb1*$^{Cre/+}$ mice with *Atoh1*$^{LacZ/+}$ mice to generate *Hoxb1*$^{Cre/+}$; *Atoh1*$^{LacZ/+}$ double transgenic animals. These animals were mated with *Atoh1*$^{flox/flox}$ mice to generate triple transgenic mice of four genotypes: *Hoxb1*$^{+/+}$; *Atoh1*$^{+/flox}$, *Hoxb1*$^{Cre/+}$; *Atoh1*$^{+/flox}$, *Hoxb1*$^{+/+}$; *Atoh1*$^{LacZ/flox}$ and *Hoxb1*$^{Cre/+}$; *Atoh1*$^{LacZ/flox}$. Only animals with *Hoxb1*^{Cre} and *Atoh1*^{LacZ} alleles lack *Atoh1* expression in the *Hoxb1*^{Cre} distribution (*Atoh1*^{CKO}). Mice of the other three genotypes (*Hoxb1*$^{+/+}$*; Atoh1*$^{+/flox}$, *Hoxb1*$^{Cre/+}$*; Atoh1*$^{+/flox}$, and *Hoxb1*$^{+/+}$*; Atoh1*$^{LacZ/flox}$) are collectively referred to as "wildtype" because they display no abnormal phenotypes and are indistinguishable based on the testing reported here. *Hoxb1*$^{+/+}$*; Atoh1*$^{LacZ/flox}$ mice are alternately designated as "*Atoh1*$^{LacZ/+}$".

Tissue preparation and sectioning

Timed matings of the appropriate genotypes were arranged and the day of conception was designated as E0. Pregnant dams were euthanized and embryos dissected from the uterus. Embryonic tails were collected from each of the embryos for genotyping.

For wholemount Xgal staining, embryos and adult tissue were immersion fixed for 15-30 minutes in ice-cold 10% neutral buffered formalin (NBF; Fisher), washed three times in 1X PBS, and then Xgal (Gold Biotechnology) stained at 37^{o}C for 16-20 hours. They were then washed three times in 1X PBS and fixed overnight in 10% NBF at 4^{o}C. The tissue was then rinsed three times in 1X PBS, equilibrated in 30% sucrose/1X PBS, and embedded in OCT (Sakura) for cryostat sectioning. Wholemount photographs were taken prior to equilibration in sucrose.

For immunostaining, embryos were decapitated and immersion fixed in 10% NBF overnight at 4^{o}C. They were then washed three times in 1X PBS, equilibrated in 30% sucrose/1x PBS, and embedded in OCT for cryostat sectioning.

All tissues were sectioned at 25 μm on a Leica cryostat and collected on Superfrost Plus slides (Fisher). Slides were dried at room temperature overnight and then stored at -80^{o}C.

Counterstaining and immunostaining

Xgal stained sections were counterstained for 3 minutes in 0.1% nuclear fast red solution (Sigma), dehydrated and mounted using Cytoseal 60 (Richard Allan Scientific).

The following antibodies and dilutions were used: rat anti-Keratin 8 (TROMA1, DSHB) 1:20; chicken anti-β-galactosidase (AbCam, cat #ab9361) 1:1000; mouse anti-NF200 (Sigma, cat #N0142) 1:500; and rabbit anti-VGLUT2 (Synaptic Systems, cat #135402) 1:3000. Secondary antibodies conjugated to various fluorophores (Jackson Immunochemicals) were all used at a 1:500 dilution.

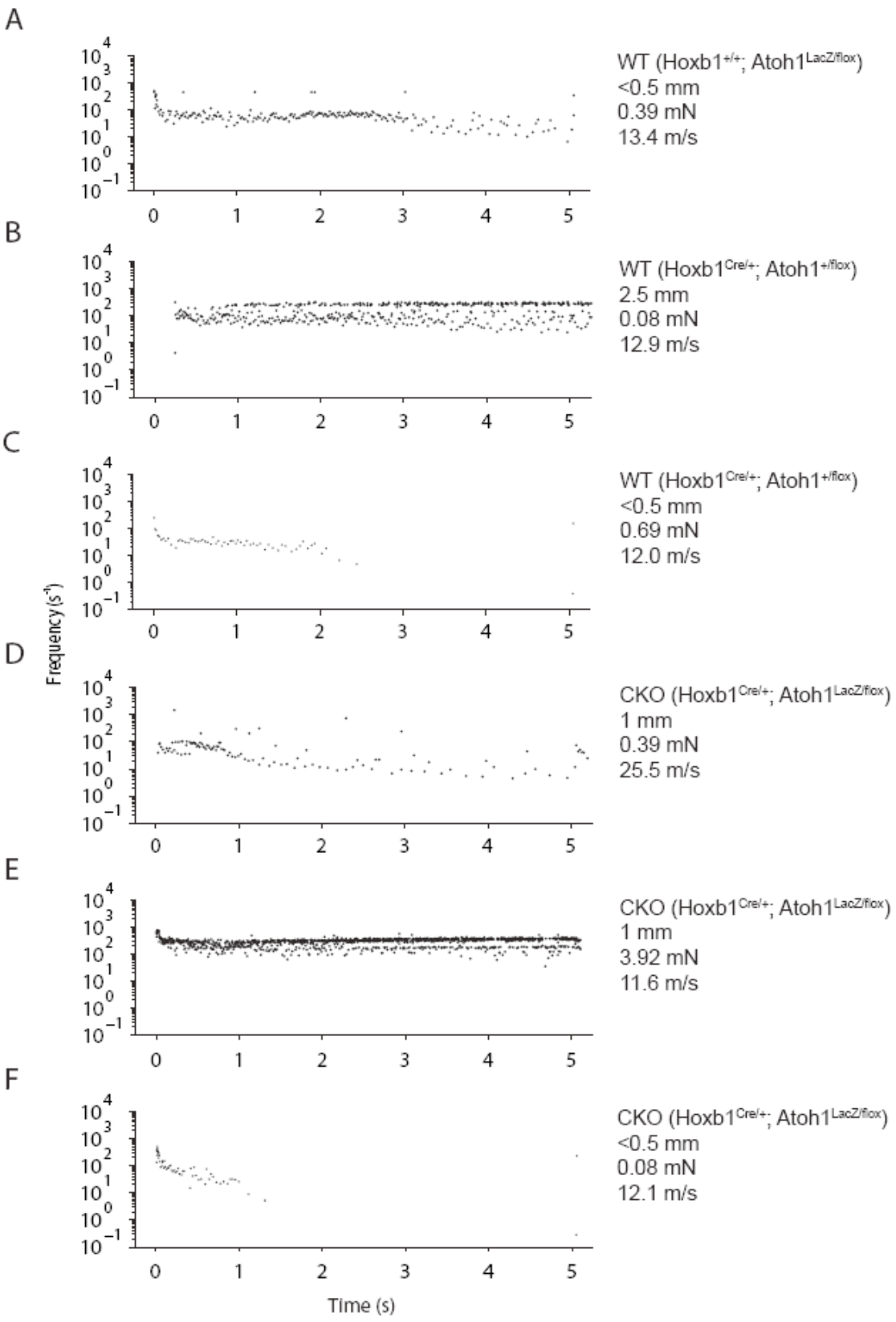
All images were obtained on a Zeiss LSM 510 confocal microscope as z-stacks. Projections of the z-stack images were created in Image J, and montages were assembled in Adobe Photoshop CS2.

FM 1-43 dye injections
Adult mice were injected subcutaneously with FM 1-43 (Biotium) in sterile PBS at a dosage of 4mg/kg and then sacrificed 24 hours later. Back and belly skin was harvested and imaged in wholemount using a Zeiss LSM 510 confocal microscope.

Skin-nerve preparations and electrophysiology
Mechanosensitive responses in the skin were recorded after dissecting the skin of the hindlimb and the dorsal aspect of the foot in continuum with the saphenous and femoral nerves to the lumbar plexus. During recordings, the skin was kept epidermis-up and the underside perfused via nylon wick with synthetic interstitial fluid (SIF) containing (in mM) 108 NaCl, 3.5 KCl, 0.7 $MgSO_4$, 26.0 $NaHCO_3$, 1.7 NaH_2PO_4, 9.5 sodium gluconate, 5.5 glucose, 27.5 sucrose and 1.5 $CaCl_2$. SIF was maintained at 32°C and saturated with 95%O_2/5%CO_2. The nerve was bathed in mineral oil in an adjacent recording chamber and teased apart. Teased nerve bundles were placed on a gold electrode and responses were recorded using a model 1800 differential amplifier (A-M Systems, Sequim WA) and Sci-Works Experimenter software (Datawave, Boulder CO). Receptive fields and mechanical thresholds were identified with calibrated von Frey monofilament fibers. Conduction velocity was determined by electrically stimulating the receptive field, and mechanical responses were elicited by a 3 mm diameter cylindrical ceramic probe driven by a custom-built, computer-controlled indenter. Mechanical stimuli were held for 5-s at a constant displacement of 0.5 to 2.0 mm. Afferents were categorized as Aβ-, Aδ- or C-fibers based on conduction velocity, and then further subdivided based on mechanical threshold, receptive field size, adaptation properties and firing-frequency variability *(S5)*. Afferents failing to satisfy criteria for any of these categories were designated as "ambiguous". All recordings and classifications were made blind to genotype. Statistics were done in Microsoft Excel, MatLab and R *(S6)*.

Supplementary material
Fig. S1. Instantaneous firing frequency plotted against time for mechanosensory responses categorized as "ambiguous." A-C) Ambiguous responses from wildtype fibers. D-F) *Atoh1*^{CKO} responses. Two fibers had receptive field sizes and mechanical thresholds that could not be easily reconciled with their firing properties (A and D). The fiber response graphed in (A) fits the profile of either an SAI or SAII fiber and displays spike couplets that are atypical of any fiber. The fiber response shown in (D) is typical of a D-hair fiber but its receptive field size is too small and the conduction velocity is too high. Two other fibers (B and E) displayed a similar but extremely atypical bursting phenotype,

but despite firing property similarities they had extremely disparate receptive field sizes and thresholds. The remaining five uncategorized fibers (two examples shown in C and F) showed an intermediately-adapting phenotype that does not match well with either the rapidly- or slowly-adapting categories. To the right of each plot are genotypes, receptive field sizes, von Frey thresholds and conduction velocities.



Maricich et al - Supplemental Figure S1

Table S1. Mechanosensory afferent response properties. Median and quartiles of conduction velocity (CV) and von Frey threshold (vF) in milli-Newtons (mN) for all mechanosensory afferents and Aβ, Aδ and C-fiber populations. The number of fibers tested (N) is shown at the right. Each group displays data for fibers from all four genotypes: *Atoh1*^{CKO} animals, pooled littermate controls, and each of the three wildtype genotypes.

All Fibers

	CV (m/s)		vF (mN)		
	median	quartiles	median	quartiles	*N*=
All genotypes	6.2	(1.4, 10.2)	0.69	(0.08, 3.92)	135
Atoh1^{CKO} (*Hoxb1*$^{Cre/+}$; *Atoh1*$^{LacZ/flox}$)	6.1	(1.8, 11.3)	0.39	(0.08, 3.92)	38
Control (combined)	6.2	(1.3, 10.0)	0.69	(0.08, 3.92)	97
Hoxb1$^{+/+}$; *Atoh1*$^{+/flox}$	5.8	(2.2, 9.1)	0.30	(0.08, 1.57)	42
Hoxb1$^{+/+}$; *Atoh1*$^{LacZ/flox}$	3.5	(0.4, 9.6)	1.57	(0.39, 3.92)	16
Hoxb1$^{Cre/+}$; *Atoh1*$^{+/flox}$	7.3	(4.9, 11.2)	0.69	(0.14, 3.92)	39

Aβ

	CV (m/s)		vF (mN)		
	median	quartiles	median	quartiles	*N*=
All genotypes	12.8	(10.7, 14.2)	0.39	(0.08, 1.57)	39
Atoh1^{CKO} (*Hoxb1*$^{Cre/+}$; *Atoh1*$^{LacZ/flox}$)	12.6	(11.4, 15.0)	0.30	(0.08, 0.69)	12
Control (combined)	12.8	(10.5, 13.9)	0.39	(0.08, 1.57)	27
Hoxb1$^{+/+}$; *Atoh1*$^{+/flox}$	13.0	(10.6, 13.5)	0.08	(0.08, 0.2)	9
Hoxb1$^{+/+}$; *Atoh1*$^{LacZ/flox}$	12.4	(10.9, 13.9)	0.98	(0.39, 2.65)	4
Hoxb1$^{Cre/+}$; *Atoh1*$^{+/flox}$	12.6	(10.8, 14.0)	1.57	(0.32, 3.33)	14

Aδ

	CV (m/s)		vF (mN)		
	median	quartiles	median	quartiles	*N*=
All genotypes	5.9	(4.1, 7.8)	0.20	(0.08, 3.92)	64
Atoh1^{CKO} (*Hoxb1*$^{Cre/+}$; *Atoh1*$^{LacZ/flox}$)	5.0	(3.7, 7.3)	0.20	(0.08, 5.88)	18
Control (combined)	6.0	(4.4, 7.9)	0.20	(0.08, 1.57)	46
Hoxb1$^{+/+}$; *Atoh1*$^{+/flox}$	5.9	(3.9, 7.9)	0.20	(0.08, 1.13)	23
Hoxb1$^{+/+}$; *Atoh1*$^{LacZ/flox}$	4.3	(3.3, 5.7)	2.16	(0.16, 3.92)	6
Hoxb1$^{Cre/+}$; *Atoh1*$^{+/flox}$	6.2	(5.7, 8.1)	0.20	(0.08, 1.57)	17

C-fibers

	CV (m/s)		vF (mN)		
	median	quartiles	median	quartiles	*N*=
All genotypes	0.41	(0.38, 0.5)	3.92	(1.57, 3.92)	32
Atoh1^{CKO} (*Hoxb1*$^{Cre/+}$; *Atoh1*$^{LacZ/flox}$)	0.50	(0.48, 0.66)	3.92	(3.04, 6.37)	8
Control (combined)	0.39	(0.35, 0.45)	3.92	(1.57, 3.92)	24
Hoxb1$^{+/+}$; *Atoh1*$^{+/flox}$	0.40	(0.36, 0.43)	3.92	(1.57, 3.92)	10
Hoxb1$^{+/+}$; *Atoh1*$^{LacZ/flox}$	0.37	(0.35, 0.40)	2.75	(0.91, 3.92)	6
Hoxb1$^{Cre/+}$; *Atoh1*$^{+/flox}$	0.41	(0.38, 0.48)	3.92	(1.35, 5.88)	8

References and Notes

S1. B. R. Arenkiel, G. O. Gaufo, M. R. Capecchi, *Dev Dyn* **227**, 379 (Jul, 2003).
S2. N. Ben-Arie *et al.*, *Nature* **390**, 169 (Nov 13, 1997).
S3. N. F. Shroyer *et al.*, *Gastroenterology* **132**, 2478 (Jun, 2007).
S4. P. Soriano, *Nat Genet* **21**, 70 (Jan, 1999).
S5. C. J. Woodbury, H. R. Koerber, *J Comp Neurol* **505**, 547 (Dec 10, 2007).
S6. R. D. C. Team. (R Foundation for Statistical Computing, Vienna, Austria, 20