## Supplemental Figures

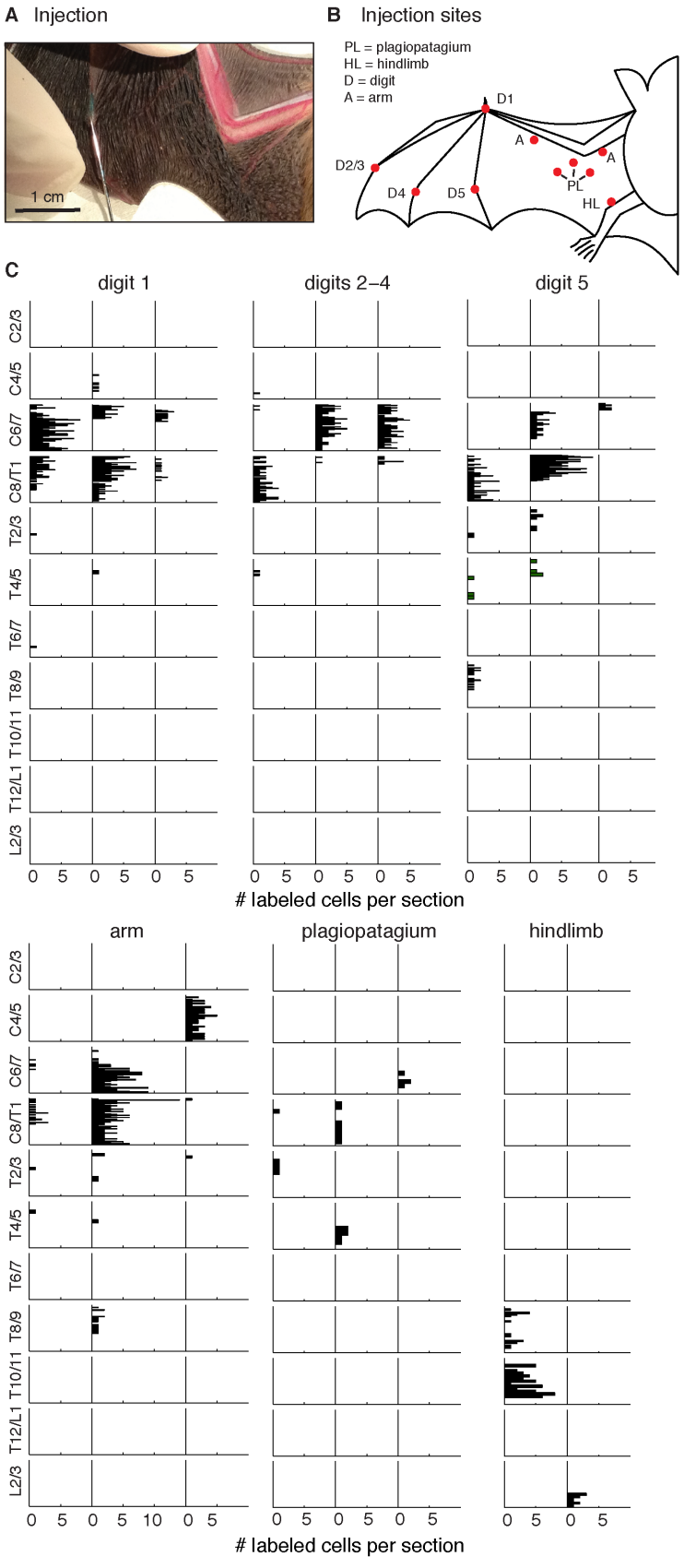


**Figure S1. Quantification of DRG neurons innervating the wing, related to Figure 1.**

(A) Image of a subcutaneous CTB-647 injection on the fifth digit of an anesthetized bat.

(B) Map of CTB injection sites on the wing used to map sensory and motor innervation.

(C) Histograms show the number of neurons labeled within each spinal level. Each column represents an individual injection at that wing site. Each row within the histograms represents the number of neurons labeled in a single section of DRG tissue.

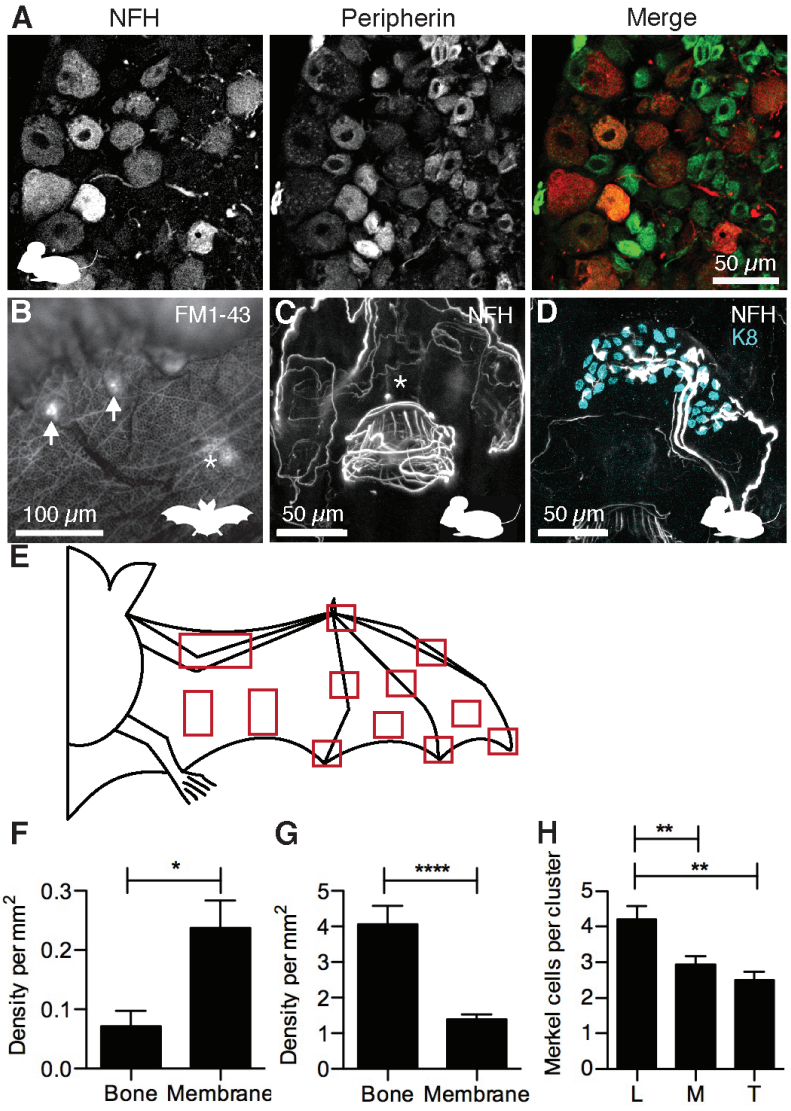


**Figure S2. Comparison of mouse and bat sensory neurons show conserved receptor types and differential densities across the bat wing, related to Figure 2.**

(A) Immunohistochemistry of a mouse DRG labeled with antibodies against Neurofilament H (NFH; red), which marks myelinated neurons, and peripherin (green) which labels small-diameter neurons in mouse DRGs. By contrast, peripherin uniformly labeled all somatosensory neurons in bat DRGs (Figure 2b).

(B) FM1-43 labeling of a diffuse ending (asterisk) compared with two punctate hair receptors (arrows) in an intact bat wing.

(C) Whole-mount immunohistochemistry of lanceolate and circumferential endings, visualized with NFH antibodies, around a mouse tylotrich hair (arrowhead).

(D) A cluster of mouse Merkel cells and their afferent (labeled with Krt8 and NFH antibodies, respectively) adjacent to a hair follicle.

(E) Twelve defined wing areas were imaged for receptor density measurements.

(F) Quantification of FM1-43 labeled diffuse receptors (left; *P=0.017) and punctate hair receptors (middle; ****P<0.0001) and on skin overlying bone versus skin membrane. [*N*=4 wings from 4 bats]. Analysis performed using Students two-tailed *t* test. (right) Quantification of the size of Merkel-cell clusters by anatomical area on the wing was analyzed by a one-way ANOVA, P=0.0008. L: leading edge, M: mid-wing, T: trailing edge. [*N*=4 wings from 3 bats], Asterisks indicate significance between groups after Bonferroni's Multiple Comparison Test, **P≤0.01. Bars: mean ± SEM.


**Figure S3. Plots of single-unit responses as a function of airflow intensity from 12 representative SI neurons, related to Figure 3.**

(A) Bubble plots of spike counts. The size of each circle represents the number of trials with corresponding number of spikes.

(B) Scatter plots of spike latency with reference to stimulus onset. Note that points were jittered along both axes for ease of visualization.

## Supplemental Experimental Procedures

### Experimental Animals

Twenty adult insectivorous bats (*Eptesicus fuscus*) weighing 16–22 g were used for this study. Bats were wild-caught in Maryland under a permit issued by the Maryland Department of Natural Resources (#55440), housed in a vivarium at the University of Maryland under reversed 12 h light/dark conditions and given food and water *ad libitum*. Mouse tissue was harvested from four female *Atoh1/nGFP* transgenic mice (8–10 weeks of age). All experimental procedures followed National Institute of Health guidelines and were approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Maryland or Columbia University Medical Center.

### Cholera toxin B labeling

Cholera toxin B conjugated to AlexaFluor 488, 594 or 647 (Molecular Probes) was dissolved in sterile PBS at 4 mg/ml. Six bats were anesthetized using isoflurane and depth of anesthesia was assessed by toe pinch. While subjects were under anesthesia, focal injections of 0.7–1 µl were made into one of nine wing sites using a Hamilton syringe. Up to four injections were performed on each wing. Injections were monitored under a dissection microscope to ensure the tracer remained in the tissue. Seven days later, bats were sacrificed via transcardial perfusion with 4% PFA and spinal columns were removed and fixed in 4% PFA for 20 minutes. Spinal cords and DRG from cervical through lumbar levels were removed, post-fixed again in 4% PFA for 20 minutes, cryopreserved in 30% sucrose, and sectioned at 25–30 µm. Every section from each recovered DRG was imaged and at least every other section from cervical through the entire thoracic spinal cord was visually inspected at appropriate wavelengths. Images were captured in three dimensions with laser scanning confocal microscopy (Zeiss exciter confocal microscope equipped with a 20X, 0.8NA objective lens).

Spinal levels were grouped in twos to represent our minimum level of resolution when DRGs were removed from the vertebral column. DRGs were assigned the vertebral level from which they were dissected. Spinal cords were transected at C2, T2, T6 and T11 to yield three partitions (C2–T1, T2–T5, T6–T11). After fixation and cryoprotection, each partition was serially sectioned onto *n* microscope slides. The vertebral level of motor neurons on each slide was estimated as follows:

$$L_i = \left(\frac{i * p}{n}\right)$$

Where $L_i$ is the vertebral level of slide *i*, *p* is the number of vertebral levels in the partition, and *n* is the total number of slides from that partition. For example, consider a labeled neuron located in the second spinal partition (T2–T5; *p*=4). If this labeled neuron was on the second slide of ten (*i*=2, *n*=10), the estimated vertebral level is ≤1, so the labeling was assigned as T2, the first vertebral level represented. Thus, our spinal level estimates assumed that levels within a partition were of equal size.

### Immunohistochemistry

Mouse skin was shaved, depilated (Surgi-cream) and dissected from the proximal hind limb. DRGs were freshly dissected from lumbar levels. Mouse skin and

DRGs were fixed in 4% paraformaldehyde (PFA) for 20 min. Bat wing skin was fixed in 4% PFA for 15 min. For H&E staining, tissue with futher fixed in 70% ethanol. For immunohistochemistry in tissue sections, tissue was cryoprotected in 30% sucrose, frozen and sectioned at 20-µm thickness. Cryosectioned tissue was labeled overnight with primary antibodies against Krt20 (Abcam: ab15205, 1:50), Krt8 (Developmental Studies Hybridoma Bank: TROMA-I, 1:100), Peripherin (Abcam; ab4666, 1:500) and/or Neurofilament H (Abcam: ab4680, 1:1000). Secondary goat AlexaFluor-conjugated antibodies (Invitrogen) directed against rat (Alexafluor 594; A11007), chicken (Alexafluor 647; A21449) or rabbit (Alexafluor 488; A11008) IgG were used for 1 h at room temperature.

We used whole-mount immunostaining to visualize Merkel cells and sensory end organs in the intact skin as previously described (Lesniak et al., 2014). Briefly, tissue was permeabilized with 0.1% TritonX-100 and incubated in primary antibodies from 48-72 h and secondary antibodies for 48 h.

Immunofluorescence imaging was performed according to published methods (Lesniak et al., 2014) with laser scanning confocal microscopy (Zeiss exciter equipped with 20X, 0.8 NA and 40X, 1.3 NA objective lenses). H&E sections were imaged by brightfield microscopy (Zeiss Axioplan2 equipped with 10X, 0.45 NA lens).

### *In vivo* FM1-43 labeling

FM1-43 (Biotium; #70020) was diluted at 2 mM in sterile PBS and injected subcutaneously at 2.5 µl/g body weight (Meyers et al., 2003). Tissue was harvested 18 h after injection, fixed in 4% PFA for 15 min and visualized in whole-mount by epifluorescence microscopy using 2X, 0.08 NA and 10X, 0.3 NA objective lenses.

### Receptor Density Analysis

To quantify hair receptors and diffuse endings, epifluorescent images were taken of four whole-mount bat wings from four separate animals after *in vivo* FM 1-43 injections. Four wings from three bats were used for whole-mount immunostaining of Merkel cells. All statistics reported are from student's two-tailed *t* tests.

Twelve locations across the wing were used as representative areas to investigate receptor density **(Figure S2E)**. For Merkel-cell staining, specimens measuring approximately 40 $mm^2$ were taken from each defined location. Bones were removed to facilitate mounting skin tissue on slides. One to ten images were taken of each skin specimen. For FM1-43 labeled receptors, one to ten images were taken in each of the 12 defined skin locations on the intact wing. We quantified hair receptors by their association with FM1-43 puncta. End organs were quantified using *Image J* by two independent observers to calculate density. Images from all bats were grouped together by location and averaged to obtain a mean for each of the 12 locations. These means were used to formulate density maps for each receptor type **(Figure 4D–F)**. For quantification by gross anatomical area, images were grouped by their location on the leading edge, mid-wing, or trailing edge **(Figure 2G).** Receptor density from all of these images was averaged for each anatomical location to obtain density across gross wing areas for each receptor type **(Figure 2M)**. In a separate analysis, densities for all areas on either bone or membrane were averaged. Densities of bone and membrane were both included when calculating by anatomical area.

**Surgery and neurophysiology**

At least 1–3 days prior to initiating neural recordings, a custom stainless steel headpost (diameter 2 mm, length, 20 mm) was glued to the skull using cyanoacrylate glue (Loctite 4161) along the midline just rostral to bregma. Surgeries were performed under Isoflurane anesthesia (1–3%, 750 cc/min $O_2$). Most neural recordings were done under Isoflurane anesthesia except for a subset of data obtained using Ketamine-Xylazine (intramuscular, standard dosage of 52.4 mg/kg). Body temperature was maintained at 37°C by placing the animal on a heating pad, and breathing was monitored at 15-min intervals. Standard sterile surgical procedures were followed throughout the experiment.

The skull-attached head-post was used to secure the bat to a vibration isolation table (Kinetic Systems) and a small craniotomy (approximately 1.5 mm x 1.5 mm, enlarged as needed) over the parietal cortex allowed access to SI (Big brown bat stereotaxic brain atlas, E. Covey, University of Washington). The dura mater was left intact, and sterile saline/silicone oil (DC 200, Fluka Analytical) was used to prevent desiccation.

Single neuron recordings from SI were acquired using a 16-channel linear probe with four recordings sites separated by 50 µm and arranged vertically on four shanks (Neuronexus Inc.). A silver wire inserted under the scalp/muscles of mastication served as a reference electrode. The recording probe was attached to a micromanipulator (Mitutoyo America Corporation), oriented perpendicularly and lowered into the cortex until all the recording sites were in the grey matter (~400 µm). Recordings lasted 2–6 hours and each animal underwent 4–12 recording sessions spread over a period of 1–4 weeks. At the conclusion of recordings, bats were euthanized with an intraperitoneal injection of sodium pentobarbital (0.1 ml, 390 mg/ml), and wings and vertebral column harvested for immunohistochemistry and microscopy.

Multiunit recordings from SI neurons were as described (Chadha et al., 2011). Receptive fields were mapped with von Frey filaments to estimate mechanical thresholds and receptive field sizes. The receptive fields of SI neurons, which varied from punctate on the thumb to large on wing membranes, might reflect innervation of multiple hair follicles by single afferents (Li et al., 2011), multiunit responses, or cortical integration of many sensory neurons.

**Tactile and airflow stimulation**

With the recording electrode(s) in SI, tactile receptive fields on the wing were localized by means of handheld calibrated monofilaments (von Frey filaments, North Coast). Stimulation consisted of pressing the monofilaments perpendicular to the dorsal wing membrane until they bent, at which point they were removed. Neuronal thresholds were determined based on the lowest weighted filaments that elicited a cortical response.

Airflow stimuli were generated using a fluid dispensing station (Ultra 2400, Nordson EFD) that allowed control over the duration and intensity of air puffs. The dispensing station was connected to a tank of compressed nitrogen gas and stimuli were delivered using a series of flexible tubes connected to a 14-g blunt needle, placed 3 mm away at 45° inclination from the dorsal wing surface. Stimuli were repeated 20–26 times at 5-s intervals. Similarly to tactile stimulation, neural thresholds were determined by the lowest airflow intensity that elicited a response.

**Electrophysiology data collection and analysis**

Activity from single neurons was acquired using 16-channel linear electrode arrays, connected via a unity gain head-stage to a data acquisition system (Omniplex D System, Plexon Inc.). Neural data was digitized at 16-bit resolution, sampled at 40 kHz, amplified 200-1000X and band-pass filtered at 500-5000Hz. Start of data recording was done via a dedicated PC terminal, and a TTL pulse was used to trigger and time-stamp the onset of stimulus delivery.

With the animal under Isoflurane anesthesia, spontaneous activity was rarely observed, which allowed us to precisely delineate and quantify the spike trains evoked in response to stimulation. Spike waveforms and timestamps of extracellularly recorded potentials were extracted using commercially available software (Offline Sorter v.3, Plexon Inc.). Single unit discrimination was achieved using thresholding, template matching, principal-component analysis of waveforms and the presence of absolute refractory period in inter-spike interval histograms. Further analysis on spike timestamps was done in MATLAB (version R2012a, The Mathworks Inc.).

A subset of neurons ($N$=11) were recorded under Ketamine-Xylazine anesthesia to rule out Isoflurane-specific effects on neural response profiles. Variable spontaneous firing was evident with Ketamine-Xylazine but airflow responses of SI neurons were similar to those observed under Isoflurane anesthesia. While anesthesia may have an impact on cortical firing, data from awake, behaving rats confirms that tactile encoding in S1 is sparse even without anesthesia. For rats scanning a complex texture, slip-stick motion events from their whiskers were encoded by only one or two low-probability spikes in S1 neurons (Jadhav et al., 2009).