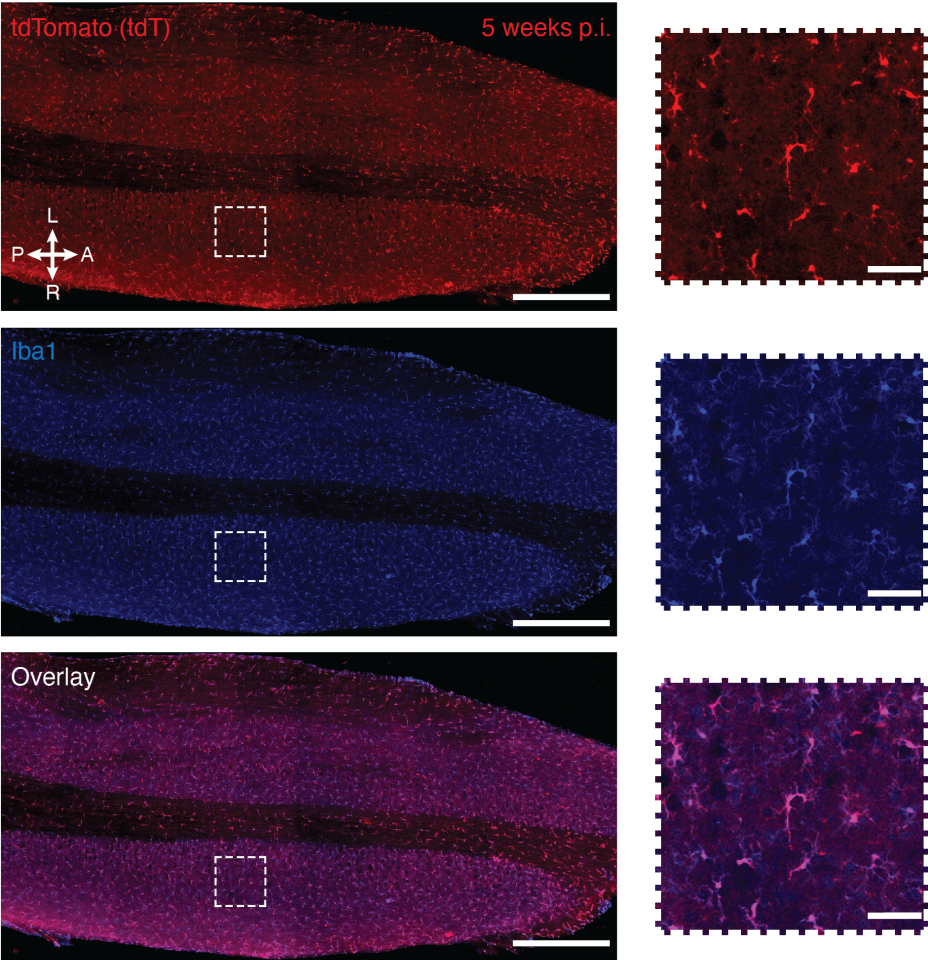
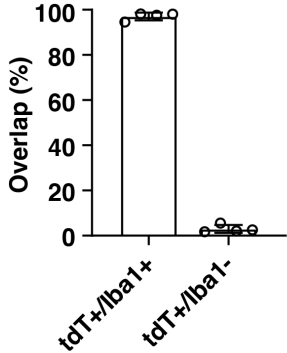


Supplementary Figures

a Spinal cord tangential slice (CX3CR1-GCaMP5g-tdTomato) **b**

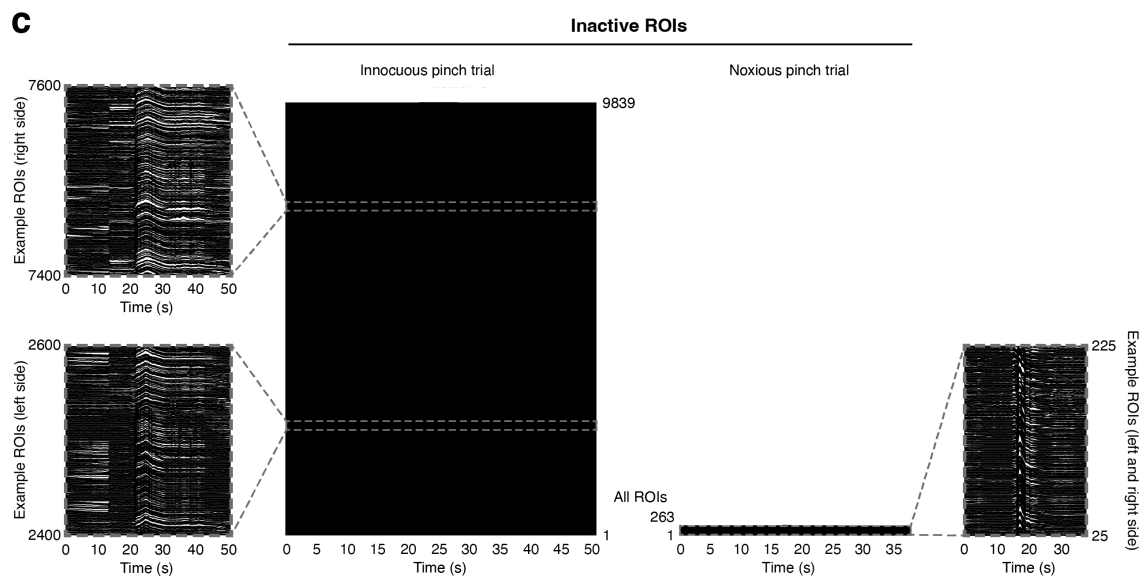
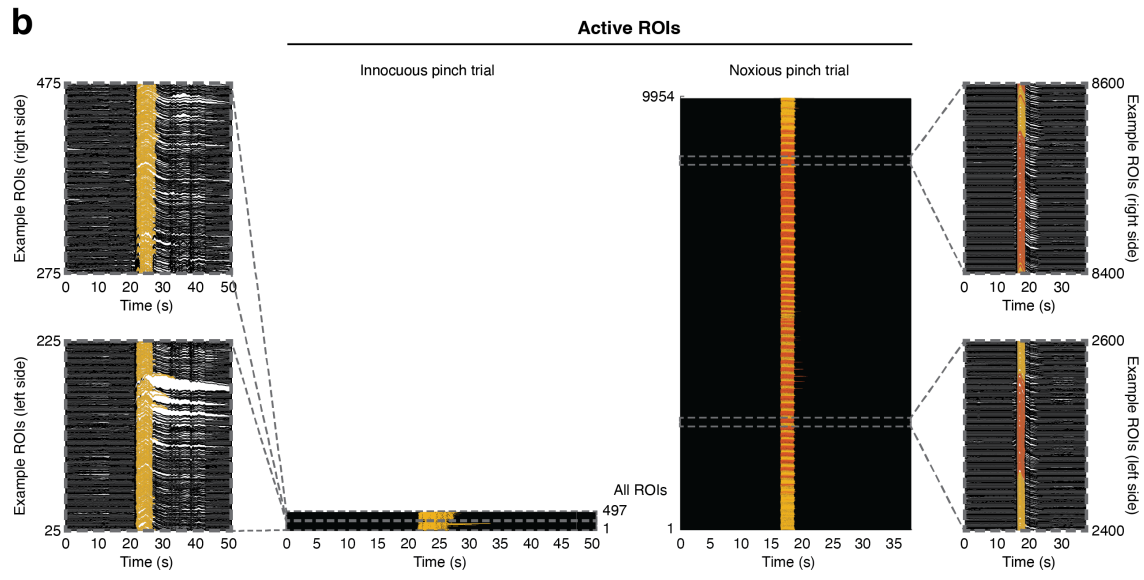
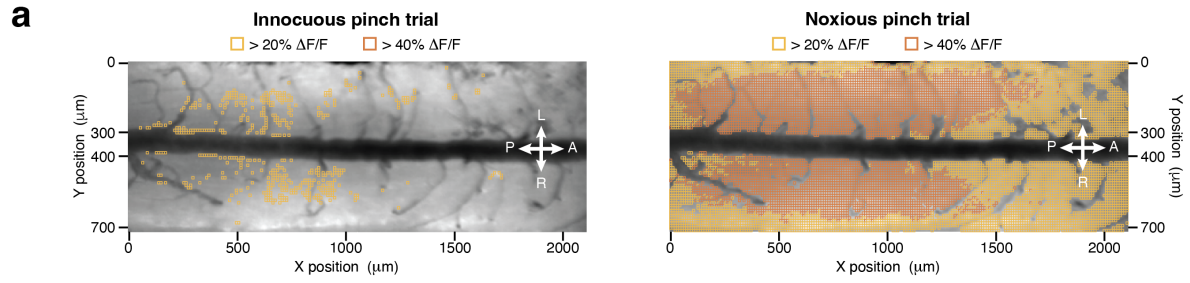


c Recombination efficiency and specificity



Supplementary Fig. 1 | See next page for caption.

Supplementary Fig. 1 | Conditional indicator expression in spinal microglia of CX3CR1-GCaMP5g-tdTomato mice. **a**, Fluorescence images of a tangential spinal cord section (50 μ m thickness including the dorsal surface) from a CX3CR1-GCaMP5g-tdTomato mouse five weeks after Tamoxifen injection (**Methods**). *Top*, tdTomato (tdT) expression. *Center*, Iba1 immunostain. *Bottom*, overlay. Scale bars, 500 μ m. **b**, Zoom-ins of the indicated regions in **a**. Scale bars, 50 μ m. **c**, Population analysis of conditional indicator expression extent (tdT-positive/Iba1-positive cells) and specificity (tdTomato-positive/Iba1-negative cells). The data are from two representative mice and two tissue sections per animal presented as the mean \pm s.e.m.



Supplementary Fig. 2 | See next page for caption.

Supplementary Fig. 2 | High-speed trans-segmental imaging of sensory-evoked calcium activity in focally restrained behaving GFAP-GCaMP6f mice. **a**, Activity maps for the innocuous ($p < 200$ g) and noxious pinch trials ($p > 500$ g) of **Fig. 2** from the same focally restrained animal. **b**, All active ROI calcium transients (defined as $>20\%$ $\Delta F/F$ and >6 s.d. above baseline; **Methods**) from the innocuous (center left) and noxious pinch trials (center right) in **a**. The ROI transients are ordered line by line according to their X and Y positions, starting from the top left. Each zoom-in shows 200 calcium transients from the left and right sides of the spinal cord in more detail. **c**, All inactive ROI transients (defined as $<20\%$ $\Delta F/F$ or <6 s.d. above baseline; **Methods**) from the innocuous (center left) and noxious pinch trials (center right) in **a**. Each zoom-in shows 200 transients. Locomotion (**Extended Data Fig. 8**) and blood vessel diameter changes (**Supplementary Videos 6-7**) can cause artifactual transients. Of the 9,839 inactive innocuous pinch trial transients, 85.4% and 14.6% were $<20\%$ $\Delta F/F$ or <6 s.d. above baseline, respectively. Of the 263 inactive noxious pinch trial transients, 11.4% and 88.6% were $<20\%$ $\Delta F/F$ or <6 s.d. above baseline, respectively.

Supplementary Tables

Surface	Type	Radius	Thickness	Glass	Diameter
OBJ	STANDARD	Infinity	0.40000	N-BK7	6.00000
1	STANDARD	Infinity	3.83988		5.95166
2	STANDARD	-5.74334	1.60002	N-SF66	5.36800
3	STANDARD	15.70964	0.81873		6.41800
4	STANDARD	-32.74580	1.60104	S-LAH97	6.89400
5	STANDARD	-8.05357	0.21700		7.61200
6	STANDARD	Infinity	1.60035	N-LAF34	8.46000
7	STANDARD	-12.51519	0.52205		8.77400
8	STANDARD	21.51710	1.60150	S-LAH55V	9.00000
9	STANDARD	-47.46657	0.50000		9.00000
10	STANDARD	Infinity	1.00000	FSILICA	8.92000
11	STANDARD	Infinity	5.50000		8.81600
12	COORDBRK	-	0.00000		-
13	STANDARD	Infinity	0.00000	MIRROR	12.21416
14	COORDBRK	-	5.50000		-
STO	STANDARD	-33.34728	1.98940	S-LAH97	7.12400
16	STANDARD	6.13755	1.00000	N-SF66	7.23800
17	STANDARD	17.95736	0.10000		7.75400
18	STANDARD	-6.35500	1.89487	S-LAH58	8.06200
19	STANDARD	-35.88850	1.60925		7.63800
20	STANDARD	Infinity	0.74100		6.07952
21	STANDARD	15.94777	0.99962	N-SF66	5.53400
22	STANDARD	-21.55705	2.80317		5.09800
23	STANDARD	Infinity	0.10000	N-BK7	4.08338
IMA	STANDARD	Infinity			4.05844

Supplementary Table 1 | Wearable microscope optical design. The specified values reflect the optical model parameters. For design tolerances and tolerancing analysis, see **Supplementary Tables 2-3**.

Operand	Surface	Nominal	Min.	Max.
COMP	19	0.037	-0.5	0.5
TWAV	-	-	0.6328	-
TFRN	2	0	-5	5
TFRN	3	0	-5	5
TFRN	4	0	-5	5
TFRN	5	0	-5	5
TFRN	6	0	-5	5
TFRN	7	0	-5	5
TFRN	8	0	-4	4
TFRN	9	0	-4	4
TFRN	12	0	-5	5
TFRN	13	0	-5	5
TFRN	14	0	-4	4
TFRN	15	0	-5	5
TFRN	16	0	-4	4
TFRN	17	0	-5	5
TFRN	18	0	-5	5
TTHI	2	1.6	-5.00E-03	5.00E-03
TTHI	3	0.8187	-5.00E-03	5.00E-03
TTHI	4	1.601	-0.025	0.025
TTHI	5	0.217	-1.00E-02	1.00E-02
TTHI	6	1.6003	-0.025	0.025
TTHI	7	0.5221	-0.025	0.025
TTHI	8	1.6015	-0.025	0.025
TTHI	9	0.5	-0.025	0.025
TTHI	12	1.9894	-0.025	0.025
TTHI	13	1	-0.025	0.025
TTHI	14	0.1	-5.00E-03	5.00E-03
TTHI	15	1.8949	-1.00E-02	1.00E-02
TTHI	16	2.3502	-5.00E-03	5.00E-03
TTHI	17	0.9996	-0.025	0.025
TIRR	2	0	-1	1
TIRR	3	0	-1	1
TIRR	4	0	-1	1
TIRR	5	0	-1	1
TIRR	6	0	-1	1
TIRR	7	0	-1	1
TIRR	8	0	-1	1
TIRR	9	0	-1	1
TIRR	12	0	-1	1
TIRR	13	0	-1	1
TIRR	14	0	-1	1
TIRR	15	0	-1	1
TIRR	16	0	-1	1
TIRR	17	0	-1	1

TIRR	18	0	-1	1
TIRX	2	0	-1.00E-02	1.00E-02
TIRX	3	0	-5.00E-03	5.00E-03
TIRX	4	0	-5.00E-03	5.00E-03
TIRX	5	0	-1.00E-02	1.00E-02
TIRX	6	0	-5.00E-03	5.00E-03
TIRX	7	0	-1.00E-02	1.00E-02
TIRX	8	0	-5.00E-03	5.00E-03
TIRX	9	0	-5.00E-03	5.00E-03
TIRX	12	0	-1.00E-02	1.00E-02
TIRX	13	0	-1.00E-02	1.00E-02
TIRX	14	0	-5.00E-03	5.00E-03
TIRX	15	0	-1.00E-02	1.00E-02
TIRX	16	0	-5.00E-03	5.00E-03
TIRX	17	0	-5.00E-03	5.00E-03
TIRX	18	0	-5.00E-03	5.00E-03
TIRY	2	0	-1.00E-02	1.00E-02
TIRY	3	0	-5.00E-03	5.00E-03
TIRY	4	0	-5.00E-03	5.00E-03
TIRY	5	0	-1.00E-02	1.00E-02
TIRY	6	0	-5.00E-03	5.00E-03
TIRY	7	0	-1.00E-02	1.00E-02
TIRY	8	0	-5.00E-03	5.00E-03
TIRY	9	0	-5.00E-03	5.00E-03
TIRY	12	0	-1.00E-02	1.00E-02
TIRY	13	0	-1.00E-02	1.00E-02
TIRY	14	0	-5.00E-03	5.00E-03
TIRY	15	0	-1.00E-02	1.00E-02
TIRY	16	0	-5.00E-03	5.00E-03
TIRY	17	0	-5.00E-03	5.00E-03
TIRY	18	0	-5.00E-03	5.00E-03

Supplementary Table 2 | Wearable macroscope optical design tolerances. Abbreviations: COMP, moves the object plane to find the best focus before calculating RMS spot radius. Units are in mm. In real-world applications, the device is placed at the best focus. TWAV, is the test wavelength (632.8 nm). Although this wavelength is outside our lens system's achromatic range, it is matched to the lens fabricator's metrology interferometer, which uses a HeNe laser. Note that the HeNe laser has an extremely narrow bandwidth (0.002 nm) and, therefore, chromatic aberration is negligible during metrology. TFRN, is the radius of curvature tolerance defined in fringes. TTHI, is the thickness tolerance of both the glass and air spaces defined in mm. TIRR, is the surface irregularity tolerance (i.e., the deviation from, in our case, a spherical surface profile) defined in fringes. TIRX and TIRY, are x- and y-axis tolerances, respectively, on the surface wedge (i.e., the surface tilt between the two surfaces of a lens), defined as twice the sag in mm. Because our lens system is radially symmetric, the x- and y-axis wedge tolerances are the same for every surface.

Condition	Distance from FOV center (mm):	±0	±0.8	±1.4	±1.6	±2
Nominal		2.13	2.76	3.38	4.37	7.81
Best	(Trial 2,293)	1.97	2.37	3.02	3.86	6.50
Worst	(Trial 8,009)	6.99	7.80	7.47	7.81	11.6
Mean		2.88	3.56	3.98	4.85	8.09
Std. dev.		0.62	0.70	0.55	0.47	0.63
Yield						
98%		4.56	5.47	5.52	6.06	9.64
90%		3.71	4.49	4.70	5.47	8.92
80%		3.31	4.04	4.34	5.20	8.58
50%		2.72	3.39	3.86	4.77	8.02
20%		2.37	2.99	3.54	4.46	7.56
10%		2.25	2.84	3.41	4.32	7.35
2%		2.13	2.66	3.23	4.13	7.01

Supplementary Table 3 | Wearable macroscope optical design tolerancing analysis. RMS spot radius across the FOV, calculated by the Monte Carlo tolerance analysis after 10,000 trials. Units are in μm . The top half of the table shows Nominal, Best, and Worst cases and the Mean and Standard Deviation of RMS spot radii across all trials. The bottom half of the table shows the RMS spot radii at a given yield, which is equivalent to the statistical percentile (i.e., 50% yield means 50% of the runs had an RMS spot radius smaller or equal to the values given in the table).