Microglia and macrophages contribute to the development and maintenance of sciatica in lumbar disc herniation

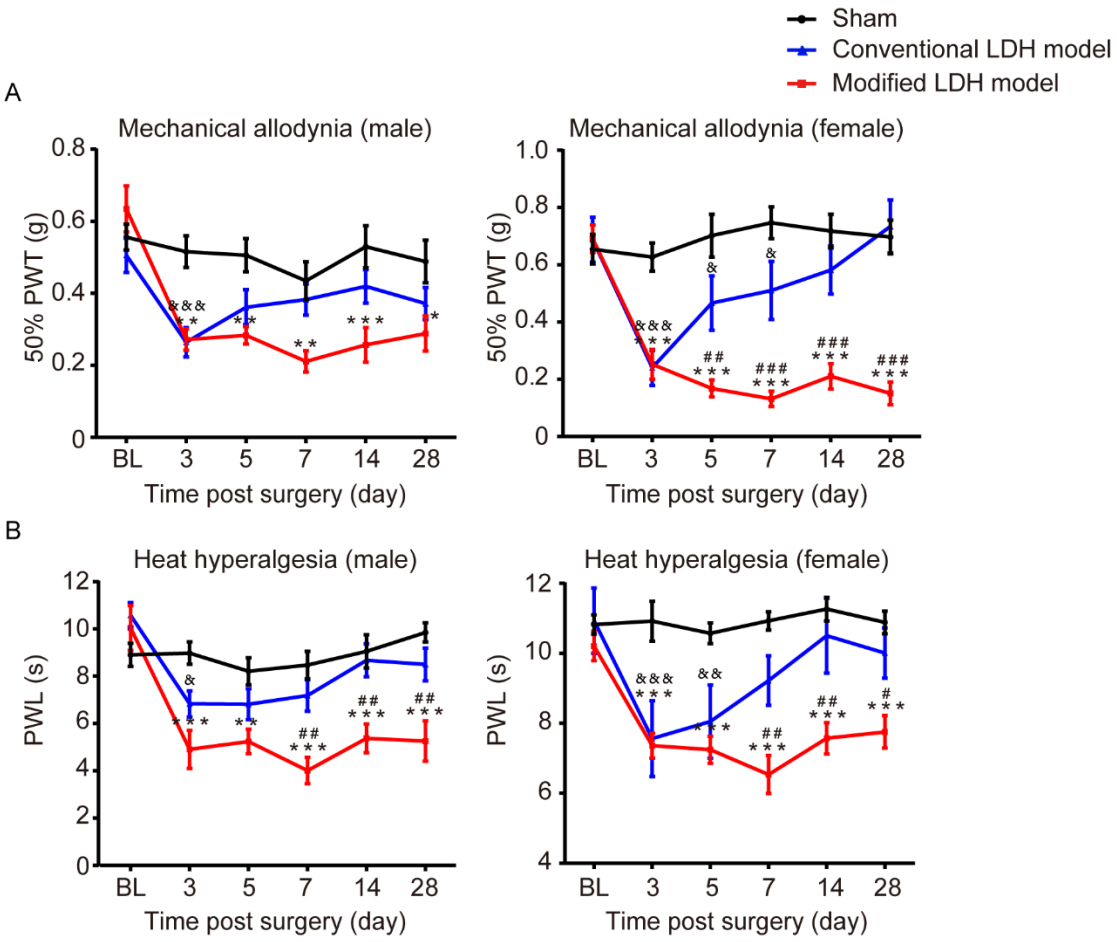
Supplementary figure 1

Supplementary figure 2

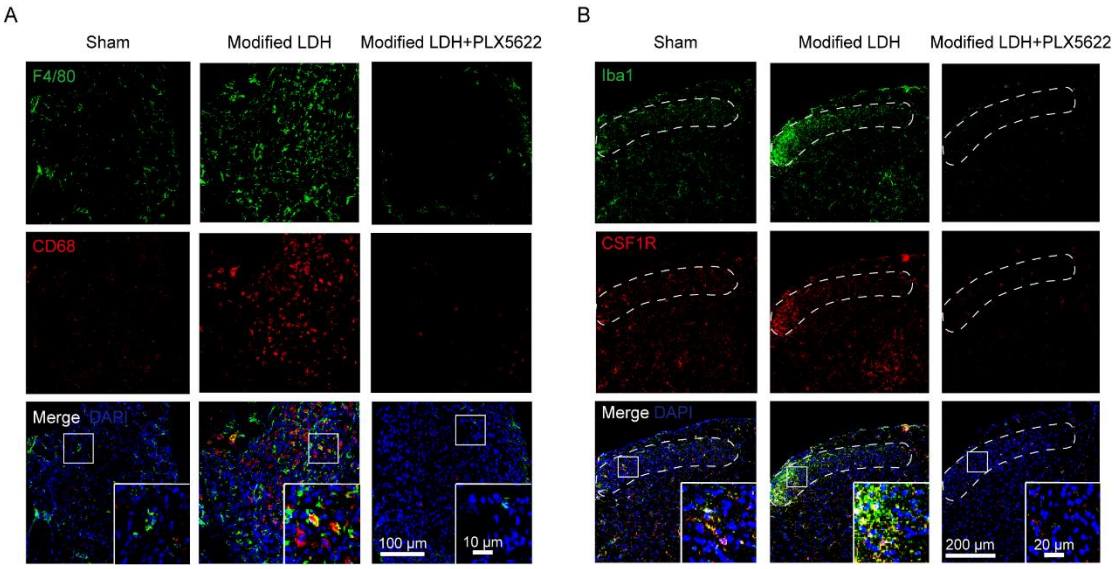
Supplementary figure 3

Supplementary table 1

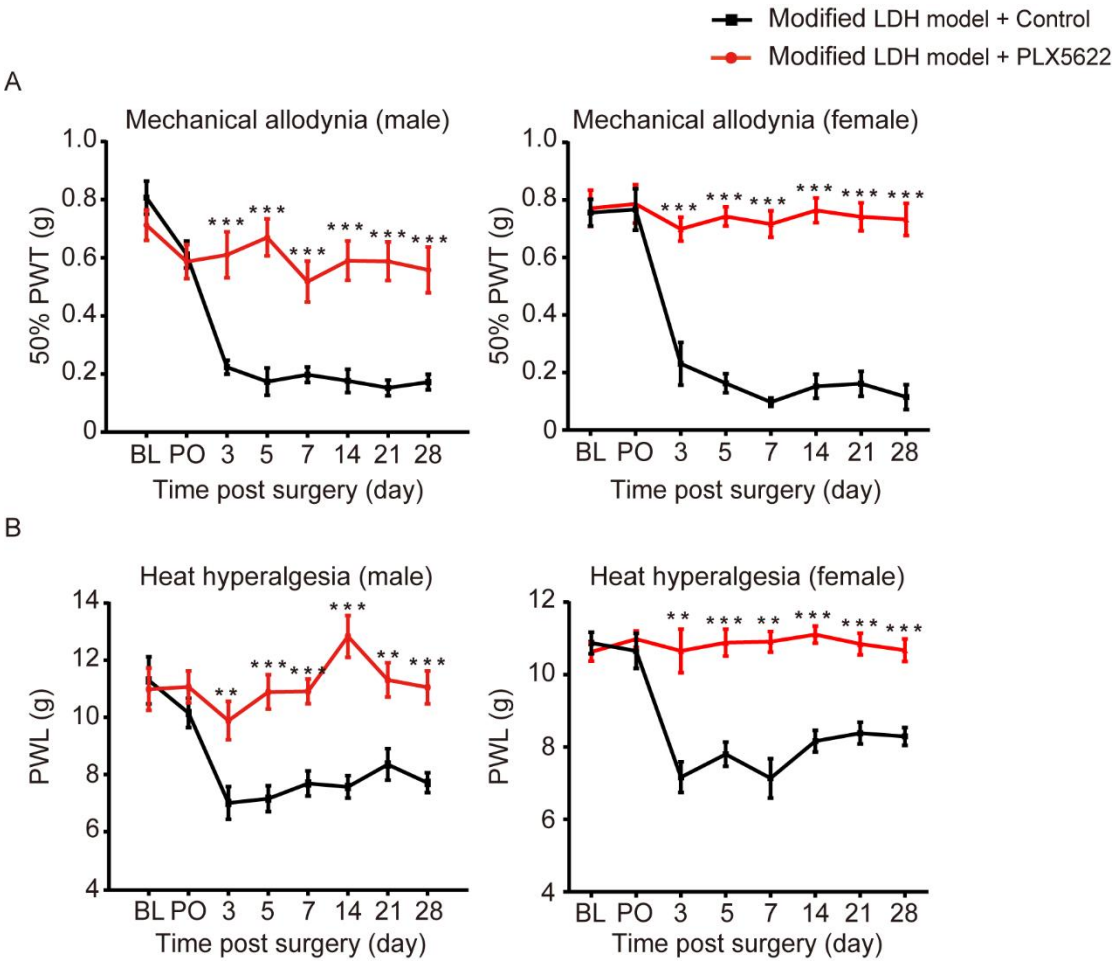
Supplementary table 2



Supplementary figure 1. Measurements of mechanical allodynia and heat hyperalgesia in male and female LDH models. (**A-B**) Nerve root and DRG compression with degenerated NP tissues, but not non-degenerated NP tissues, induced persistent mechanical allodynia (**A**) and heat hyperalgesia (**B**) in both male and female mice (n = 8-17 mice/group). Values are means ± SEM. $^{*/\&}p<0.05$, $^{**/\&\&}p<0.01$, $^{***/\&\&\&}p<0.001$ *vs.* sham group, and $^{\#}p<0.05$, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$, *vs.* conventional LDH model, two-way ANOVA with Bonferroni's *post hoc* tests among groups. NP: nucleus pulposus; DRG, dorsal root ganglia; BL, baseline; PWT, paw withdrawal threshold; PWL, paw withdrawal latency.



Supplementary figure 2. Changes of DRG macrophages and SDH microglia 7 days after LDH modeling in female mice. (**A**) CD68 was upregulated in F4/80^{+} macrophages of ipsilateral DRG at day 7 post surgery in female modified LDH model mice, but not in DRG of sham group. Macrophages can also be eliminated by continuous treatment of PLX5622 in female mice. Scale bar, 100 μm for outer panels and 10 μm for inner panels. (**B**) CSF1R^{+} Iba1^{+} microglia significantly activated and accumulated in ipsilateral L4 SDH at day 7 post surgery in female modified LDH model mice, but not in SDH of sham group. Microglia can also be eliminated by continuous treatment of PLX5622 in female mice. Scale bar, 200 μm for outer panels and 20 μm for inner panels. Dotted lines indicate the superficial region of SDH. SDH, spinal dorsal horn.



Supplementary figure 3. Persistent ablation of macrophages and microglia prevents LDH induced sciatica in male and female mice. (**A-B**) Using PLX5622, persistent ablation of microglia and macrophages significantly attenuates mechanical allodynia (**A**) and heat hyperalgesia (**B**) in both male and female modified LDH model mice (n = 7-10 mice/group). Values are means ± SEM. $^{**}p<0.01$, $^{***}p<0.001$ *vs.* Modified LDH model + Control, two-way ANOVA with Bonferroni's *post hoc* tests among groups. BL, baseline; PO: Pre-operation; PWT, paw withdrawal threshold; PWL, paw withdrawal latency.

Supplementary table 1. Fold changes of differentially expressed genes (DEGs) in DRGs at 7 days after modified LDH versus sham surgery

Gene	log_2Fold Change	Adjusted p value	Gene	log_2Fold Change	Adjusted p value
Saa3	7.8285	0.0193	Ncaph	1.4607	0.0315
Srd5a2	4.5079	0.0449	Mmp8	1.4476	0.0321
Mmp12	4.0767	0.0205	Ms4a6d	1.4313	0.0077
Cxcl14	3.3554	0.0018	Pdk4	1.4110	0.0235
Sprr1a	3.3452	< 0.0001	Itgam	1.3957	0.0034
Crlf1	2.9862	< 0.0001	Ctss	1.3837	0.0015
Timp1	2.5821	< 0.0001	Xdh	1.3676	0.0394
Mt2	2.5004	0.0022	Igsf6	1.3647	0.0125
Btc	2.3571	0.0006	S100a9	1.2954	0.0431
Trem2	2.3405	0.0034	Pirb	1.2873	0.0058
Gfap	2.2432	0.0004	Glipr1	1.2815	0.0321
Dio2	2.1649	0.0150	Anxa1	1.2768	0.0315
Ch25h	2.1632	0.0298	Gmfg	1.2688	0.0334
Npy	2.1022	0.0246	Fosl2	1.2616	0.0169
Ifitm6	2.0948	0.0145	Cd68	1.2178	0.0333
Ccnb2	2.0430	0.0069	Fbxo5	1.1889	0.0394
Gpnmb	2.0025	< 0.0001	Cenpe	1.1611	0.0101
Plac8	1.9052	0.0040	Emp1	1.1475	0.0193
Ecel1	1.8657	0.0234	Galnt6	1.1255	0.0022
Thbs2	1.7734	0.0321	Fos	1.1238	0.0169
Hrct1	1.7400	0.0460	Knstrn	1.1220	0.0321
Fgd3	1.7100	0.0311	Cyr61	1.1011	0.0153
Eln	1.6803	0.0006	D17H6S56E-5	1.0817	< 0.0001
Atf3	1.5890	0.0016	Sgk1	1.0808	0.0043
Cdc20	1.5497	< 0.0001	Prc1	1.0692	0.0015
Fgr	1.5428	0.0321	Alox5ap	1.0554	0.0142
Retnlg	1.5362	0.0270	Ckap2	1.0463	0.0135
Lgals3	1.5323	< 0.0001	Fam107a	1.0379	0.0056
Fyb	1.5288	0.0002	Hmgb2	1.0264	0.0325
Tlr13	1.5230	0.0075	Itgb2	1.0056	0.0460
Tnfaip2	1.5157	0.0129	Hspa1b	-1.0568	0.0006
S100a8	1.5000	0.0284	Hes5	-1.1262	0.0022
Ccr1	1.4882	0.0460	Xlr3b	-1.4378	0.0040
Mpeg1	1.4818	0.0022	Wdfy1	-1.5792	0.0003
Lilr4b	1.4798	0.0064	Dlk1	-1.8649	0.0315
Cd177	1.4705	0.0307			

Supplementary table 2. Fold changes of DEGs at 14 days after modified LDH versus sham surgery

Gene	log_2Fold Change	Adjusted p value	Gene	log_2Fold Change	Adjusted p value
Mmp12	6.2647	< 0.0001	Thbs2	1.4910	< 0.0001
Srd5a2	4.9478	0.0012	Cd300c2	1.4383	0.0195
Hoxd10	2.8965	0.0330	Mpeg1	1.4230	< 0.0001
Cxcl14	2.7186	< 0.0001	Lyz2	1.3021	0.0138
Ptgs2	2.3282	0.0428	Casp4	1.2399	0.0264
Trem2	2.2667	< 0.0001	Pmp2	1.1899	0.0101
Btc	2.1301	< 0.0001	Meox1	1.1844	0.0359
Dio2	2.1048	0.0106	Lyz1	1.1840	0.0337
Itgax	1.9572	< 0.0001	C3ar1	1.1710	0.0232
Ms4a7	1.9455	0.0015	Aldh1a3	1.1708	0.0002
Gfap	1.9381	0.0003	Cd68	1.1705	0.0318
Bcl2a1b	1.8832	0.0101	Fyb	1.1439	0.0003
Timp1	1.8221	0.0024	Fcgr2b	1.1400	0.0029
Crlf1	1.7891	< 0.0001	Atf3	1.0762	0.0044
Gpnmb	1.7293	< 0.0001	Fam177a	1.0174	0.0024
Clec7a	1.5902	0.0037	Npr3	1.0086	0.0479
Ctss	1.5607	< 0.0001	Wdfy1	-1.4275	0.0011
Corin	1.5321	0.0009			