Supplementary informations

Supplementary Results

LPC in patients with microcrystalline arthropathies

Although LPC16:0 did not appear to be the hallmark of a particular joint pathology, patients with microcrystalline arthropathies (CCA and gout) displayed lower levels of synovial LPC16:0 (**supplementary fig. 1**) than patients suffering from other inflammatory rheumatic diseases (RA + SPA + PA), suggesting that LPC16:0 might have a less prominent role in pain associated with these two particular joint pathologies, even if patients number is still too low to conclude.

LPC16:0 induces an ASIC3-dependent current associated to DRG neurons' depolarization

Extracellular application of LPC16:0 in HEK293 cells transfected with rat or human ASIC3 channels induced a progressive and non-inactivating inward current at resting pH 7.4, *i.e.* without any extracellular pH changes (**supplementary fig. 4A, C and D)**. Conversely, no significant LPC-activated currents were observed in ASIC1a-transfected cells, nor in non-transfected ones (**supplementary fig. 4B, D**). Furthermore, a LPC16:0-induced current was observed in primary cultured mouse DRG neurons (**fig. 4A-C**). This current had a significantly higher density in wild-type neurons that expressed a pH 6.6-induced native ASIC current (WT ASIC+) compared to neurons without native ASIC current (WT ASIC-), and to neurons isolated from ASIC3 knockout mice, expressing (KO ASIC+) or not-expressing (KO ASIC-) native ASIC currents (**fig. 4C**). Therefore, ASIC3 contributed largely to the LPC16:0-induced currents in mouse DRG neurons. A small ASIC3-independent current was still observed, suggesting an effect of LPC16:0 on other native ion channels, as expected [1-3]. Finally, the neuronal depolarization associated to a 30-sec application of LPC16:0 was significantly higher in WT ASIC+ neurons than in WT ASIC-, KO ASIC+ or KO ASIC- neurons, supporting a leading role of ASIC3 in the excitatory effect of LPC16:0 on DRG neuron membrane potential (**fig. 4D**).

Knee injections of LPC16:0 induces sensitization of spinal high threshold neurons

Recordings were obtained from spinal dorsal horn neurons receiving either non-noxious sensory inputs (low threshold neurons, LTNs) or noxious nociceptive inputs (high threshold neurons, HTNs), with receptive fields located in mice hindpaws (**fig. 6F-H**). Brushing-evoked activity of ipsilateral LTNs was not modified in LPC16:0-treated mice compared to vehicle or to contralateral LTNs (**fig. 6F**). Conversely, the evoked activities of HTNs in response to both pinch (**fig. 6G**) or von Frey filaments (**fig. 6H**) were significantly enhanced in mice injected with LPC16:0. Ipsilateral HTNs of LPC16:0-treated mice emitted on average twice as much APs in response to pinch as ipsilateral HTNs from vehicle-treated mice or contralateral HTNs (**fig. 6G**). Moreover, ipsilateral HTNs of LPC16:0-treated mice clearly displayed mechanical sensitization in response to the application of increasing von Frey filaments, with a response curve significantly shifted to the left compared to contralateral HTNs or to ipsilateral

HTNs from vehicle-treated mice (**fig. 6H**). *In vivo* recordings of spinal dorsal horn neurons clearly demonstrated a sensitization of spinal high threshold, but not low threshold neurons induced by LPC16:0 knee injections, which was correlated with the persistent secondary allodynia observed.

Supplementary References

[1] Andersson DA, Nash M, Bevan S. Modulation of the cold-activated channel TRPM8 by lysophospholipids and polyunsaturated fatty acids. J Neurosci 2007;27(12):3347-3355.

[2] Flemming PK, Dedman AM, Xu SZ, Li J, Zeng F, Naylor J, Benham CD, Bateson AN, Muraki K, Beech DJ. Sensing of lysophospholipids by TRPC5 calcium channel. J Biol Chem 2006;281(8):4977-4982.

[3] Gentry C, Stoakley N, Andersson DA, Bevan S. The roles of iPLA2, TRPM8 and TRPA1 in chemically induced cold hypersensitivity. Mol Pain 2010;6:4.

Supplementary tables and figures

patient number	Sexe	Age (year)	BMI	Joint pathology	LPC concentration in synovial fluid (µM)									
					LPC 16:0	LPC 18:2	LPC 18:1	LPC 18:0	LPC 20:5	LPC 20:4	LPC 20:3	LPC 20:2	LPC 22:6	Sum
1	F	67	26,8	OA	42,00	8,87	8,77	14,79	5,56	6,30	6,09	0,20	1,14	93,72
2	F	63		OA	51,74	15,40	12,32	18,84	11,63	11,42	11,33	0,16	1,74	134,58
3	F	71	21,8	OA	46,80	13,36	11,21	17,87	9,85	9,73	10,23	0,22	1,15	120,43
4	M	70	27,0	OA	36,04	9,03	7,54	13,91	6,64	7,10	8,02	0,12	1,59	89,98
5	F	67	26,6	OA	23,60	4,68	4,86	8,05	2,85	3,96	3,96	0,23	0,58	52,77
6	M	67	31,5	OA	18,42	4,53	3,97	6,65	2,02	2,24	2,40	0,18	0,48	40,90
7	F	68	26,3	OA	33,06	3,83	4,82	11,62	1,56	2,60	3,62	0,16	0,42	61,68
8	F	61	24,7	OA	12,40	2,92	2,49	4,03	1,54	1,72	1,71	0,19	0,50	27,50
9	M	67	25,2	OA	38,10	11,76	8,95	10,37	6,69	5,72	4,60	0,43	1,11	87,74
10	F	73	25,7	OA	27,52	5,42	4,50	9,48	4,28	4,31	4,95	0,40	1,17	62,02
11	F	54	34,2	OA	67,15	11,95	15,34	19,31	7,36	12,06	9,92	0,29	1,61	144,99
12	M	49	31,1	OA	20,92	5,69	4,25	5,47	2,39	2,90	2,03	0,14	0,37	44,15
13	F	67	27,0	OA	57,21	7,63	9,94	18,70	4,93	8,74	11,24	0,11	1,16	119,66
14	M	70		OA	43,87	8,50	8,24	14,12	5,41	6,92	6,04	0,24	0,98	94,32
15	M	68	27,3	OA	26,66	7,77	6,20	9,39	5,29	5,42	3,57	0,49	0,87	65,66
16	F	61	32,0	OA	17,13	4,93	4,27	5,60	3,05	2,92	2,55	0,31	0,53	41,29
17	M	73	28,0	OA	69,79	10,79	10,83	17,98	7,96	10,44	10,27	0,19	1,64	139,88
18	M	60	30,4	OA	25,30	5,38	6,62	8,07	3,43	4,74	3,48	0,20	0,48	57,69
19	F	52	23,2	OA	34,94	6,44	6,35	7,99	3,82	4,89	3,88	0,19	0,59	69,09
20	M	69	25,7	OA	47,57	8,13	10,88	13,14	5,27	8,76	4,68	0,14	0,83	99,42
21	M	60	27,2	OA	31,87	5,83	5,97	8,81	3,73	5,03	4,31	0,23	0,80	66,57
22	F	64		OA	26,02	5,19	6,18	12,93	3,33	5,30	5,32	0,38	1,00	65,65
23	F	61	27,4	OA	17,74	3,36	3,78	7,90	1,93	2,71	2,95	0,27	0,47	41,11
24	F	62	27,9	OA	36,81	8,08	7,62	9,86	4,39	5,33	3,84	0,19	0,95	77,07
25	M	58	24,3	OA	33,28	10,83	9,07	10,86	4,72	6,01	3,92	0,26	0,79	79,75
26	M	66	28,4	OA	38,14	7,23	7,22	14,54	3,51	4,34	4,69	0,36	0,88	80,91
27	M	69	31,9	OA	30,58	4,39	5,91	10,02	2,38	4,44	4,17	0,18	0,86	62,96
28	M	56	29,0	OA	19,70	5,91	5,65	7,01	3,71	4,42	3,40	0,30	0,68	50,77
29	M	72	24,6	OA	30,66	7,14	5,78	9,38	4,74	4,76	4,70	0,41	0,83	68,42
30	F	67	24,8	OA	27,97	5,53	5,63	8,66	3,03	4,33	3,78	0,16	0,78	59,86
31	M	66	36,3	OA	41,35	7,70	7,31	11,42	4,89	5,52	5,08	0,30	1,98	85,55
32	M	73	22,5	OA	23,46	4,84	5,74	6,76	2,90	5,15	3,52	0,15	0,71	53,21
33	M	68	30,1	OA	31,38	8,31	5,92	7,78	5,31	4,43	3,90	0,16	0,70	67,88
34	F	67	30,1	OA	44,50	6,86	6,22	13,01	4,37	5,85	6,92	0,12	0,97	88,81
35	M	63	33,2	OA	58,52	9,34	9,63	13,64	5,73	7,25	6,61	0,17	1,23	112,12
1				PM controls	25,74	5,20	5,03	7,21	2,64	3,91	2,33	0,40	0,77	53,23
2				PM controls	12,94	1,76	2,58	5,19	1,02	1,87	1,33	0,22	0,37	27,28
3				PM controls	12,31	2,65	3,28	6,34	1,60	2,69	1,45	0,35	0,59	31,26
4				PM controls	17,66	11,93	6,08	5,46	7,30	5,24	2,82	0,20	0,71	57,41
5				PM controls	13,71	2,65	3,85	3,80	1,97	4,47	2,15	0,39	0,65	33,64
6				PM controls	10,55	2,59	2,54	4,58	1,29	1,59	1,07	0,24	0,32	24,76
7				PM controls	21,58	4,33	4,54	5,74	2,05	2,93	1,81	0,26	0,54	43,77
8				PM controls	15,68	5,50	4,13	10,82	3,12	3,11	2,08	0,47	0,93	45,85
9				PM controls	18,83	3,69	3,49	6,32	2,23	2,51	2,44	0,23	0,89	40,64
10				PM controls	14,00	3,11	3,67	10,98	2,56	3,59	2,68	0,82	0,81	42,22

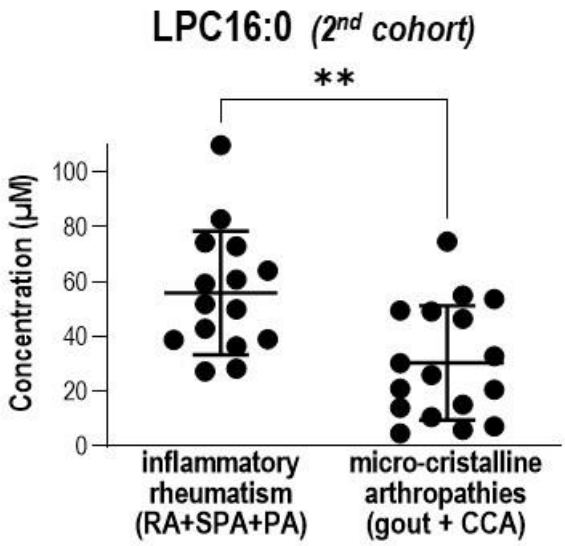
Supplementary Table 1. Subject characteristics and LPC concentrations in the knee synovial fluids of the first patients' cohort. Sexe, age and body mass index (BMI) are reported for each osteoarthritic (OA, n=35) patients. The concentrations of the different lysophosphatidyl-choline (LPC) species (LPC16:0, LPC18:2, LPC18:0, LPC20:5, LPC20:4; LPC20:3, LPC20:2, LPC 22:6) as well as the total concentrations of LPC (Sum) in the synovial fluid of each OA patient and postmortem control (n=10) are also reported in µM.

	Df	*Sum Sq*	*Mean Sq*	*F value*	*Pr(>F)*
VASknee	**1**	**268,6853147**	**268,6853147**	**6,681551328**	**0,016250218**
Gender	1	4,056135654	4,056135654	0,100866244	0,753539278
Age	1	29,27231263	29,27231263	0,727931333	0,401989662
BMI	1	41,38427425	41,38427425	1,029126407	0,32048253
log2IL6	1	76,66705017	76,66705017	1,906523367	0,180074114
Residuals	24	965,1123273	40,21301364	#N/A	#N/A
	Df	*Sum Sq*	*Mean Sq*	*F value*	*Pr(>F)*
VASglobal	**1**	**220,9059633**	**220,9059633**	**5,232414512**	**0,031281663**
Gender	1	3,462192865	3,462192865	0,082006062	0,777054523
Age	1	20,99892522	20,99892522	0,497383952	0,487440874
BMI	1	38,38032914	38,38032914	0,909082707	0,349863012
log2IL6	1	88,18017338	88,18017338	2,088649903	0,161328797
Residuals	24	1013,249831	42,21874295	#N/A	#N/A
	Df	*Sum Sq*	*Mean Sq*	*F value*	*Pr(>F)*
KOOS	**1**	**176,9162648**	**176,9162648**	**3,883437194**	**0,060404618**
Gender	1	6,938092967	6,938092967	0,15229605	0,699792949
Age	1	14,70660843	14,70660843	0,322820461	0,575195495
BMI	1	38,56010835	38,56010835	0,846421663	0,36672193
log2IL6	1	54,6975084	54,6975084	1,200649012	0,284066243
Residuals	24	1093,358832	45,55661799	#N/A	#N/A

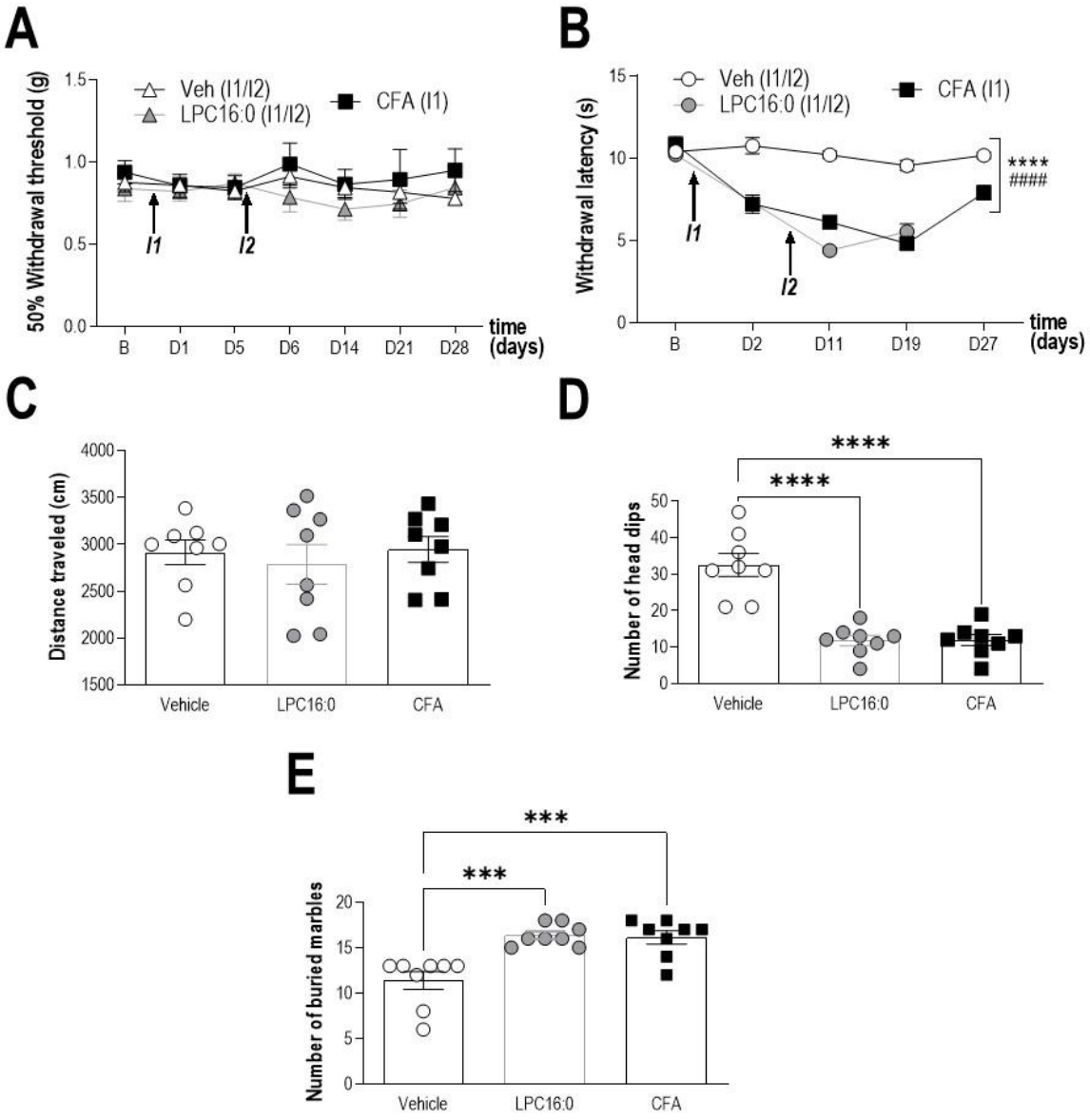
Supplementary Table 2. Statistical analysis of LPC16:0 concentrations in the synovial fluids of OA patients (first cohort) and of their pain outcomes (VAS knee, VAS global and KOOS). Analysis of variance (ANOVA) was made using age, gender, BMI and IL-6 as covariates.

Patient number	Sexe	Age (year)	BMI	Joint pathology	LPC concentration in synovial fluid (µM)									
					LPC 16:0	LPC 18:2	LPC 18:1	LPC 18:0	LPC 20:5	LPC 20:4	LPC 20:3	LPC 20:2	LPC 22:6	Sum
1	F	26	30,1	RA	82,78	8,62	10,53	23,77	4,66	11,14	10,04	0,30	2,07	153,91
2	F	71	18,1	RA	39,00	4,97	6,65	16,06	3,30	5,36	7,42	0,33	1,03	84,12
3	F	71	36,0	RA	28,29	3,32	5,85	14,05	1,65	4,10	5,46	0,69	1,11	64,52
4	F	47	35,2	RA	64,02	13,02	12,30	31,05	8,44	9,56	15,76	0,64	2,02	156,81
5	F	75	26,8	RA	27,19	8,43	7,07	8,72	4,80	6,03	4,01	0,25	0,82	67,31
6	F	36	ND	RA	36,40	4,24	7,03	13,00	2,67	6,35	5,87	0,31	1,09	76,97
7	F	63	32,0	OA	46,08	10,77	9,22	19,68	7,38	7,52	10,33	0,44	1,35	112,78
8	F	77	28,3	OA	28,75	5,58	6,26	9,16	3,96	5,83	5,59	0,27	0,90	66,30
9	F	74	25,4	OA	24,24	6,77	4,59	9,01	4,64	4,07	4,87	0,23	0,61	59,02
10	M	73	24,8	OA	29,93	5,58	4,68	11,79	4,27	5,66	7,64	0,22	1,04	70,81
11	M	87	27,2	OA	77,17	14,50	19,62	33,14	9,40	15,73	15,31	0,44	2,44	187,75
12	M	79	24,7	OA	50,03	9,37	9,89	15,93	3,06	5,12	4,19	0,19	0,78	98,56
13	F	71	ND	OA	26,94	7,73	7,08	10,64	4,32	4,65	4,55	0,25	0,71	66,88
14	M	80	27,3	OA	42,03	9,65	9,03	15,06	5,90	7,51	7,86	0,48	1,38	98,89
15	F	82		OA	55,82	19,32	14,32	24,93	16,76	16,00	17,45	0,47	2,46	167,54
16	M	82	32,3	OA	71,07	11,07	14,10	29,61	7,17	11,79	14,19	0,26	1,70	160,97
17	F	55	40,4	OA	35,54	8,19	10,68	14,48	5,44	8,66	8,13	0,31	1,65	93,08
18	M	70	29,9	OA	35,91	12,71	9,22	14,54	8,87	7,72	7,79	0,33	0,89	97,98
19	F	59	29,2	OA	36,06	8,77	7,40	13,52	4,64	5,27	5,56	0,38	1,00	82,61
20	F	94	24,5	OA	58,35	7,73	10,24	16,39	4,90	8,23	8,32	0,23	1,26	115,64
21	F	78	33,2	OA	14,36	5,09	4,05	5,50	4,02	4,06	3,57	0,13	0,64	41,42
22	F	70	29,0	OA	39,05	12,16	9,32	16,43	6,20	6,85	6,55	0,33	1,09	97,98
23	F	94	21,8	OA	25,00	4,49	4,86	9,74	2,83	3,75	4,72	0,23	0,51	56,14
24	F	57	33,9	OA	46,79	9,54	9,55	17,24	5,82	7,55	8,09	0,26	1,34	106,19
25	M	74	23,5	gout	10,59	1,51	2,60	3,90	0,93	2,19	2,10	0,27	0,49	24,58
26	F	63	32,6	gout	74,62	5,81	12,11	24,35	2,98	12,36	10,60	0,23	1,62	144,66
27	M	74	18,7	gout	20,53	1,62	3,18	5,49	0,81	2,94	2,22	0,30	0,74	37,82
28	M	91	24,3	gout	7,20	1,31	2,20	2,90	0,80	2,08	1,45	0,22	0,53	18,70
29	M	64	28,7	gout	49,48	4,16	7,59	14,21	2,43	6,71	7,21	0,33	1,27	93,38
30	F	81	23,4	CCA	13,88	1,45	2,97	6,26	0,86	2,35	2,60	0,39	0,50	31,26
31	M	83	ND	CCA	49,08	4,51	8,12	15,74	1,95	5,44	5,30	0,68	1,21	92,02
32	F	67	ND	CCA	25,93	8,53	5,05	9,52	5,18	3,78	4,51	0,24	0,87	63,60
33	F	83	17,8	CCA	53,61	9,02	12,10	18,99	5,09	8,78	7,80	0,44	1,03	116,86
34	F	66	22,6	CCA	46,49	4,72	8,72	20,89	2,84	6,95	10,06	0,29	1,07	102,04
35	F	84	24,7	CCA	5,84	0,81	1,66	2,27	0,41	1,09	0,95	0,23	0,29	13,55
36	M	69	24,6	CCA	15,05	1,97	2,68	7,07	1,24	2,54	4,30	0,37	0,61	35,81
37	M	79	21,0	CCA	30,21	3,46	5,73	9,41	2,08	4,84	5,51	0,40	0,64	62,28
38	M	71	40,2	CCA	20,90	2,39	5,34	7,53	1,55	5,25	4,68	0,29	0,68	48,61
39	M	75	24,8	CCA	54,96	7,73	11,26	15,24	5,98	11,12	10,47	0,44	1,69	118,88
40	M	73	ND	CCA	4,66	0,83	1,26	1,66	0,43	0,82	0,68	0,24	0,32	10,90
41	F	85	ND	CCA	32,75	4,69	6,30	9,59	3,29	5,45	5,59	0,19	0,82	68,66
42	F	50	20,7	PA	49,91	6,67	9,57	18,54	4,81	8,82	10,07	0,46	1,10	109,94
43	F	66	30,1	PA	59,22	14,56	13,30	16,07	11,02	11,60	9,82	0,18	1,52	137,29
44	M	68	26,1	PA	51,78	17,14	12,40	19,49	11,30	9,87	10,39	0,30	1,62	134,29
45	M	59	26,0	PA	42,83	4,87	7,19	16,81	2,66	5,13	7,63	0,30	0,92	88,35
46	F	55	34,7	SPA	72,94	6,49	9,31	24,59	4,04	7,56	10,83	0,35	1,59	137,69
47	F	41	20,8	SPA	109,87	9,82	16,43	44,07	6,96	15,16	21,20	0,38	2,34	226,23
48	F	33	28,0	SPA	38,73	6,59	6,66	17,12	4,50	6,63	9,08	0,24	0,97	90,52
49	F	48	22,5	SPA	74,26	13,76	13,14	32,42	6,99	8,68	11,51	0,42	0,98	162,17
50	F	57	26,4	SPA	60,79	6,54	9,34	21,62	4,10	10,07	12,85	0,36	1,66	127,33

Supplementary Table 3. Subject characteristics and LPC concentrations in the knee synovial fluids of the second patients' cohort. Sexe, age and body mass index (BMI) and joint pathology are reported for each patient (n=50). The concentrations of the different lysophosphatidyl-choline (LPC) species (LPC16:0, LPC18:2, LPC18:0, LPC20:5, LPC20:4; LPC20:3, LPC20:2, LPC 22:6) as well as the total concentrations of LPC (Sum) in the synovial fluid of each patient are also reported in µM. Rheumatoid arthritis (RA, n=6), osteoarthritis (OA, n=18), gout (n=5), chondrocalcinosis (CCA, n=12), psoriatic arthritis (PA, n=4) and spondyloarthritis (SPA, n=5).

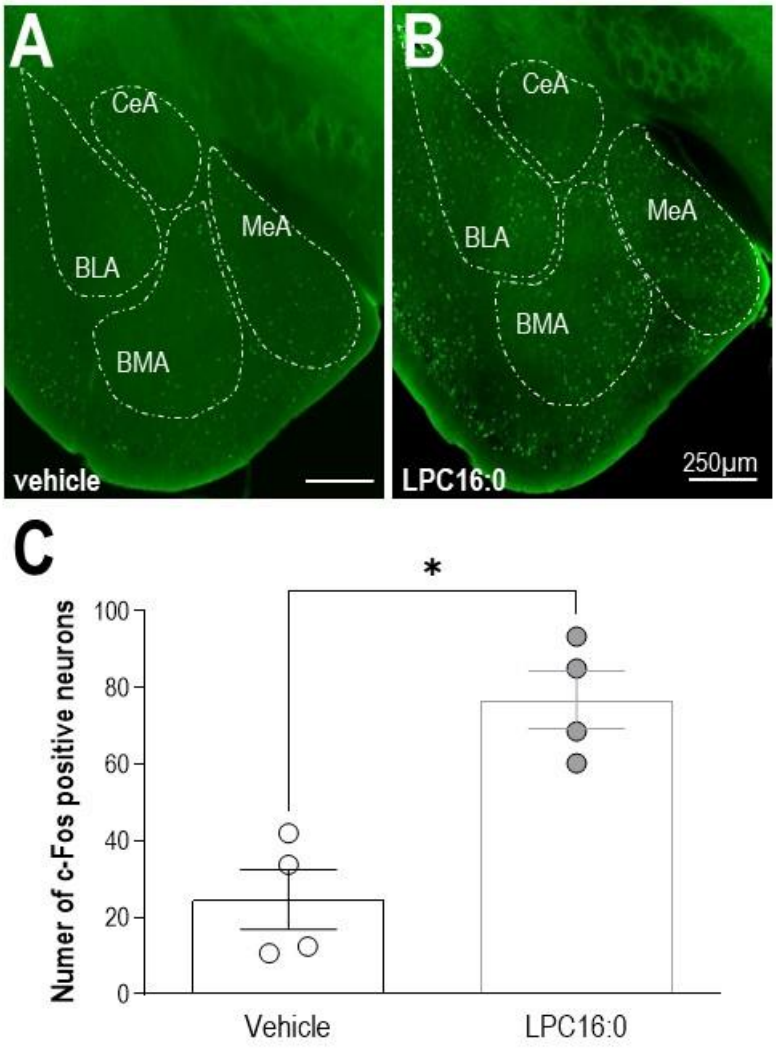


Supplementary Figure 1. LPC16:0 concentrations in knee synovial fluids from patients of the second cohort. LPC16:0 concentrations in the synovial fluids of patients from the second cohort grouped in two main joint pathologies, *i.e.,* inflammatory rheumatisms: rheumatoid arthritis (RA) + spondyloarthritis (SPA) + psoriatic arthritis (PA), and micro-cristalline arthropathies: gout + chondrocalcinosis (CCA); ** $p<0.01$, Unpaired t test).

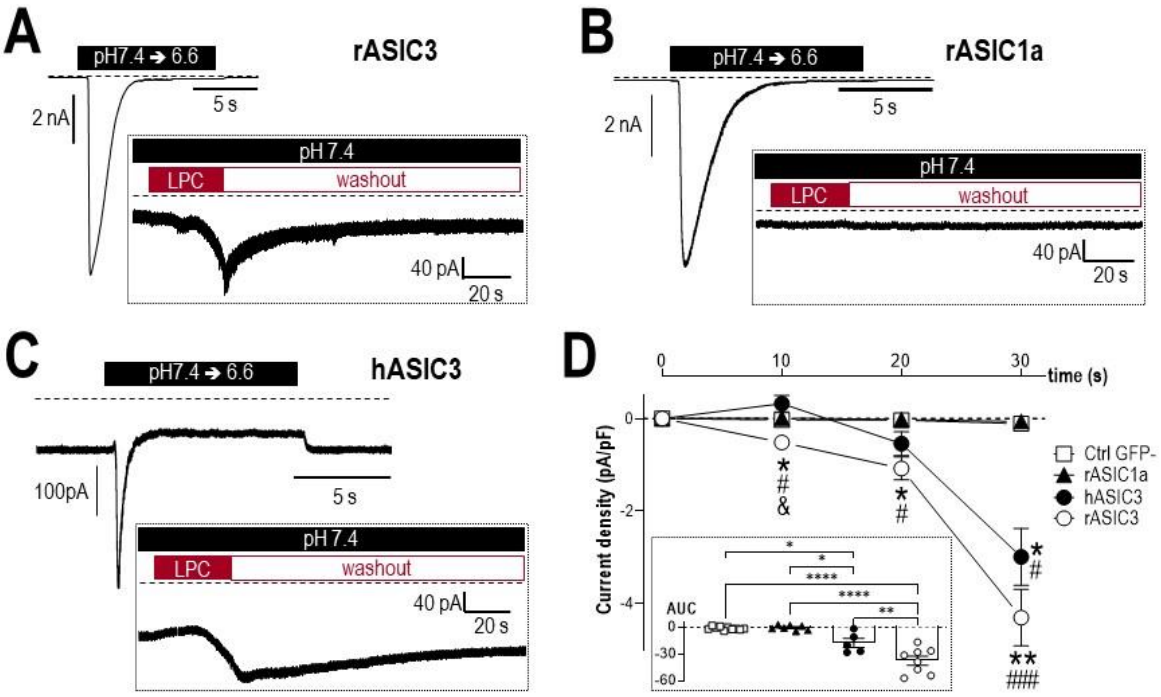


Supplementary Figure 2. LPC injections into mouse ankle produce long-lasting pain-like behaviors associated with anxiety. (A) Effect of intra-articular ankle administrations of LPC16:0 (10 nmol), complete freund adjuvant (CFA) or vehicle on mechanical allodynia of the contralateral paw of male mice. Mechanical paw threshold was assessed using the up and down method with von Frey filament on the contralateral hindpaw from D1 to D28**.** Results are expressed as 50% mechanical threshold (n=8-16 mice per group; no significant differences, two-way ANOVA followed by a Tukey's multiple comparison test). **(B)** Effect of intra-articular administrations of LPC16:0 (10 nmol), CFA or vehicle on thermal hyperalgesia in male mice. Thermal withdrawal threshold was assessed using the paw immersion test at 46°C (n=8-16 mice per group; ****p<0.0001, ####p<0.0001 and &&&&p<0.0001 for a main effect on CFA and LPC16:0 curves, respectively, as compared to Veh curve; mixed-effect analysis followed by a Tukey's multiple comparison test). **(C-E)** Effect of intra-articular administrations of LPC16:0 (10 nmol), CFA or vehicle in male mice on the distance travelled in the open field test at D20 **(C)**, on the number of head dips in the hole board test at D22 **(D)**, and on the number of buried marble in the marble burying test at D19 **(E)**. The open field test lasted 5 min and the distance travelled was automatically calculated using Ethovision XT 13 (Noldus). The

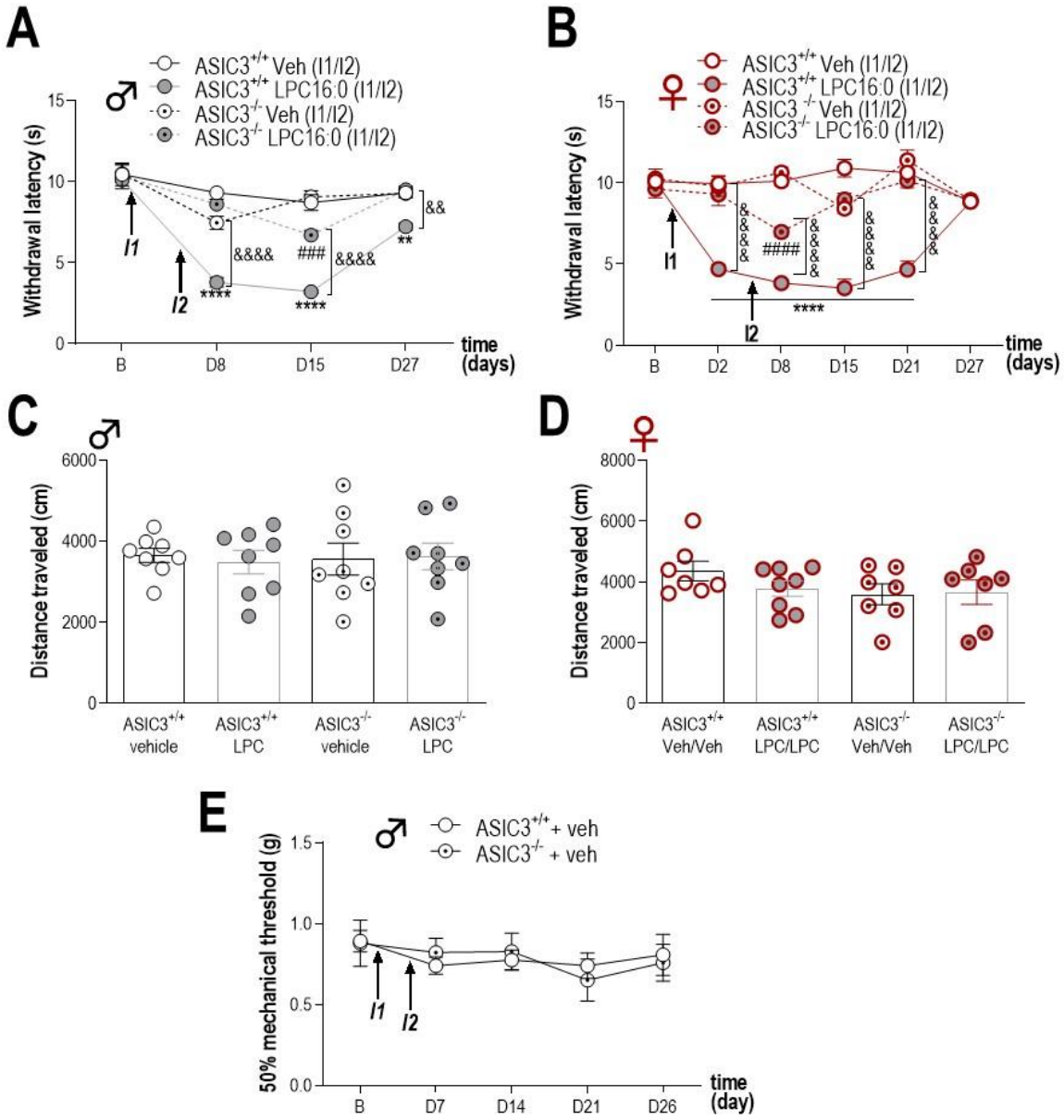
hole board and the marble burying tests lasted 5 and 30 min, respectively, and were analyzed by a blind experimenter (n=8 mice per group; ***p<0.001 and ****p<0.0001, one-way ANOVA followed by a Tukey's *post hoc* test)



Supplementary Figure 3. Intra-articular LPC16:0 injections in mice are associated to increased neuronal activity within the amygdala. **(A-B)** Representative photomicrographs of c-fos expression in the amygdala at D28, after intra-articular administrations of vehicle **(A)** or LPC16:0 (10 nmol, **B**) in the ankle joint of male mice**. (C)** Quantification of c-fos positive neurons in the basolateral nucleus of the amygdala (BLA). CeA: central nucleus of the amygdala; BMA: basomedial amygdala; MeA: medial amygdala (n= 4 in each group, *p<0.05, Mann-Whitney test).

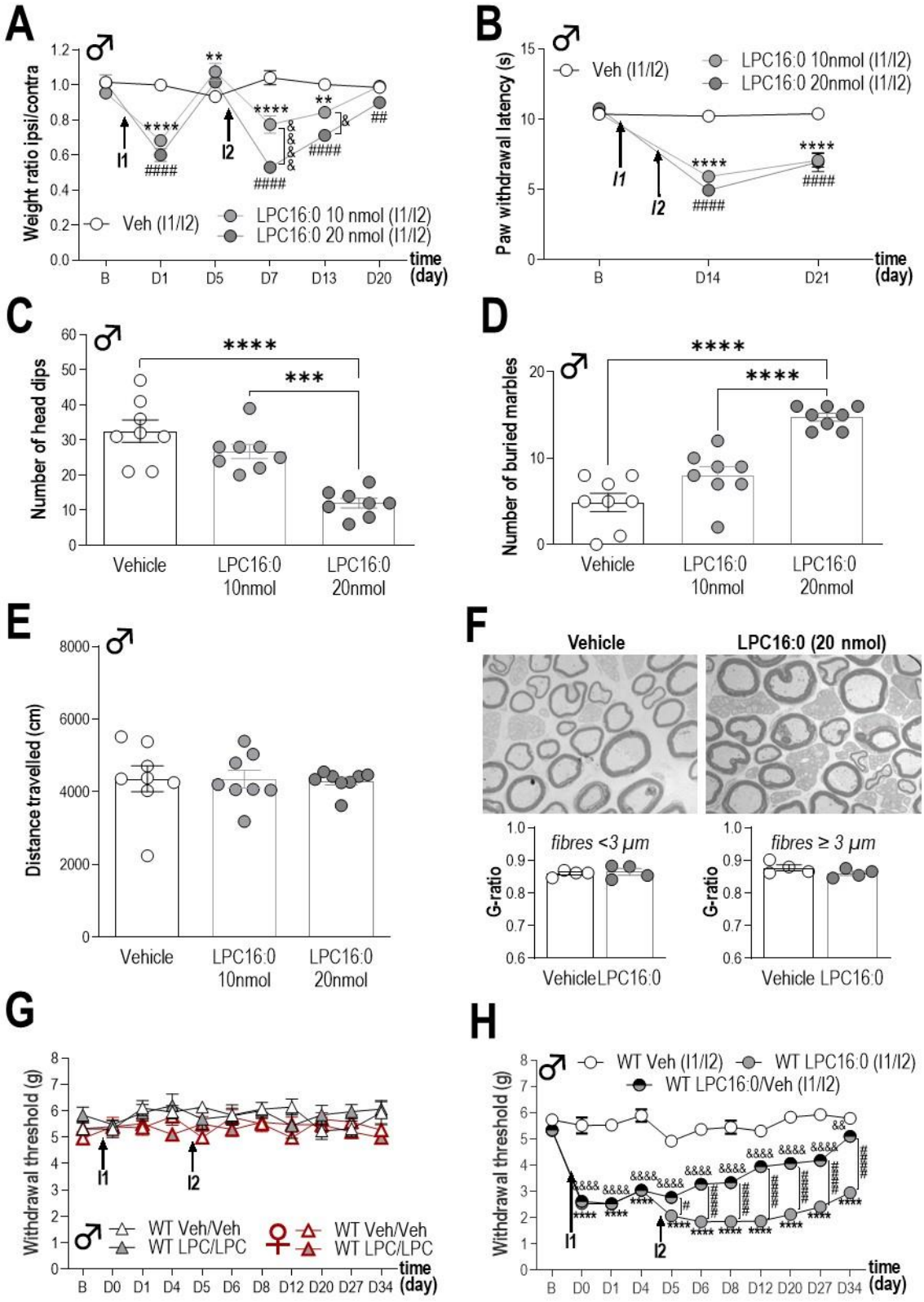


Supplementary Figure 4. LPC16:0 induces a non-inactivating ASIC3 current in a recombinant expression system. (A-C) Typical ASIC current traces recorded in patch-clamp (HP -50mV) from HEK293 cells transfected with rat ASIC3 (rASIC3, **A**), rat ASIC1a (rASIC1a, **B**) or human ASIC3 (hASIC3, **C**), following extracellular acidification from pH 7.4 to pH 6.6. *Insets* show the effects of LPC16:0 (5µM) applied for 30 seconds at the resting pH 7.4. **(D)** Analysis of the current densities measured after 10, 20 and 30 second-applications of LPC16:0 (5µM) onto HEK cells either non-transfected (Ctrl GFP-, n=10) or transfected with rASIC1a (n=7), hASIC3 (n=5) or rASIC3 (n=8). Two-way ANOVA followed by a Tukey's multiple comparison test was used (**p*<0.05 and ***p*<0.01 compared to Ctrl GFP- , $^{\#}p$<0.05 and $^{\#\#\#}p$<0.001 compared to ASIC1a, $^{\&}p$<0.05 compared to hASIC3. *Inset* shows the area under curve (AUC) obtained for the different transfection conditions (Ctrl GFP-, rASIC1a, hASIC3 and rASIC3). AUC were calculated using Prism software over 30 second periods from the baseline fixed at 0 (n=5-10, **p*<0.05, ***p*<0.01 and *****p*<0.0001, one-way ANOVA followed by a Tukey's multiple comparison test).



Supplementary Figure 5. ASIC3 is crucial for the development of both pain and anxiety-like behaviors induced by ankle LPC16:0 injections in male and female mice. **(A-B)** Effect of intra-articular administrations of LPC16:0 (10 nmol) or vehicle on thermal hyperalgesia in male **(A)** and female **(B)** ASIC3$^{+/+}$ and ASIC3$^{-/-}$ mice. Thermal withdrawal threshold was assessed using the paw immersion test at 46°C (n=7-8 mice per group; **p<0.01 and ****p<0.0001 for ASIC3$^{+/+}$ Veh *vs.* ASIC3$^{+/+}$ LPC16:0; ###p<0.001 and ####p< 0.0001 for ASIC3$^{-/-}$ Veh *vs.* ASIC3$^{-/-}$ LPC16/0;, &&p<0.01 and &&&&p< 0.0001 for ASIC3$^{+/+}$ LPC16:0 *vs.* ASIC3$^{-/-}$ LPC16:0; Two-way ANOVA followed by Tukey's multiple comparison tests). **(C-D)** Effect of intra-articular administrations of LPC16:0 (10 nmol) or vehicle on the distance travelled in the open field test at D20 in male **(C)** and female **(D)** ASIC3$^{+/+}$ and ASIC3$^{-/-}$ mice (n=7-8 mice per group, no significant difference, one-way ANOVA followed by Tukey's post hoc tests). The open field test lasted 5 min and the distance travelled was automatically calculated using Ethovision XT 13 (Noldus). **(E)** Time course effect of intra-articular ankle injections of vehicle in male ASIC3$^{+/+}$ and ASIC3$^{-/-}$ mice. Ipsilateral mechanical

paw withdrawal thresholds were assessed with von Frey filaments using the up and down method (n=8 mice per group, no significant differences, Two-way ANOVA followed by Tukey's multiple comparison tests).



Supplementary Figure 6. LPC knee injections generate dose-dependent weight bearing deficit, thermal hyperalgesia and anxiety-like behaviors, without apparent nerve demyelination. (A) Effect of intra-articular knee administrations of LPC16:0 (10 and 20 nmol) or vehicle on weight bearing in male mice. Results are expressed as the weight ratio between the ipsilateral and contralateral hindpaws (n=8 mice per group; $^{**}p<0.01$ and

****p<0.0001 for Veh *vs.* LPC16:0 10 nmol; ##p<0.01 and ####p<0.0001 for Veh *vs.* LPC16:0 20 nmol; &p<0.05 and &&&&p<0.0001 for LPC16:0 10 nmol *vs.* LPC16:0 20 nmol; Two-way ANOVA followed by a Tukey's multiple comparison test). **(B)** Effect of intra-articular knee administrations of LPC16:0 (10 and 20 nmol) or vehicle on thermal hyperalgesia in male mice. Thermal withdrawal threshold was assessed using the paw immersion test at 46°C (n= 8 mice per group; ****p<0.0001 and ####p<0.0001 for Veh *vs.* LPC16:0 10 nmol and Veh *vs.* LPC16:0 20 nmol, respectively; Two-way ANOVA followed by a Tukey's multiple comparison test). **(C-D)** Effect of intra-articular knee administrations of LPC16:0 (10 and 20 nmol) or vehicle on the number of head dips in the hole board test at D22 **(C)** and the number of buried marble in the marble burying test at D19 **(D),** in male mice. The hole board and marble burying tests lasted 5 and 30 min, respectively, and were analyzed by a blind experimenter (n=8 mice per group; ***p<0.001 and ****p<0.0001, one-way ANOVA followed by a Tukey's multiple comparison test). **(E)** Effect of intra-articular knee administrations of LPC16:0 (10 and 20 nmol) or vehicle on the distance travelled in the open field test in male mice. The open field test lasted 5 min and the distance travelled was automatically calculated using Ethovision XT 13 (Noldus; n=8 mice per group; no significant differences, one-way ANOVA test followed by a Tukey's multiple comparison test). **(F)** Representative electron photomicrographs of saphenous nerve sections from knee of male mice injected with vehicle (upper left) or LPC16:0 (20 nmol, upper right) at D7. G-ratios (lower panels) were calculated as a measure of myelin thickness and separated into small (left) and large (right) diameters based on internal axonal size (n=162 to 235 fibers per animal with 4 animals per group, no significant differences, Mann-Whitney tests). **(G)** Contralateral paw withdrawal thresholds (PWTs) of female and male WT mice injected twice in the knees with LPC16:0 (20 nmol) or vehicle. PWTs were assessed using a dynamic plantar aesthesiometer (n=6 per group; no significant differences; three-way ANOVA test followed by a Tukey's multiple comparison test). **(H)** Ipsilateral PWTs of male WT mice injected once or twice with LPC16:0 (20 nmol, n=8-14 per group, dynamic plantar aesthesiometer, ****p<0.0001 for WT LPC16:0 *vs.* WT Veh; &&p<0.01 and &&&&p<0.0001 for WT LPC16:0/Veh *vs.* WT Veh; #p<0.05 and ####p<0.0001 for WT LPC16:0 *vs.* WT LPC16:0/Veh; Two-way ANOVA test followed by a Tukey's multiple comparison test).