Supplementary information

Supplementary table 1. Proteins and antibodies in the bead array

Suffix indicate that several antibodies were included towards the same protein.

Gene	Gene desc	Uniprot	Antibody
A2M	alpha-2-macroglobulin	P01023	HPA002265
CFI	complement factor I	P05156	HPA001143
AGT	angiotensinogen	P01019	HPA001557
CFB	complement factor B	P00751	HPA001817
APOA1	apolipoprotein A1	P02647	HPA046715
APOA4.1	apolipoprotein A4	P06727	HPA001352
APOA4.2	apolipoprotein A4	P06727	HPA002549
APOA4.3	apolipoprotein A4	P06727	HPA005149
APOC1	apolipoprotein C1	P02654	HPA051518
APOE	apolipoprotein E	P02649	HPA065539
APOE	apolipoprotein E	P02649	HPA068768
APP	amyloid beta precursor protein	P05067	HPA031303
B4GAT1	beta-1,4-glucuronyltransferase 1	O43505	HPA015484
BTD.1	biotinidase	P43251	HPA040225
BTD.2	biotinidase	P43251	HPA052275
C4orf48	chromosome 4 open reading frame 48	Q5BLP8	HPA052447
C7	complement C7	P10643	HPA067450
CDH2.1	cadherin 2	P19022	HPA004196
CDH2.2	cadherin 2	P19022	HPA058574
CFH.1	complement factor H	P08603	HPA005551
CFH.2	complement factor H	P08603	HPA049176
CFH.3	complement factor H	P08603	HPA053326
CHGB.1	chromogranin B	P05060	HPA008759
CHGB.2	chromogranin B	P05060	HPA012602
CHGB.3	chromogranin B	P05060	HPA012872
CHL1	cell adhesion molecule L1 like	O00533	HPA003345
CLSTN1.1	calsyntenin 1	O94985	HPA012412
CLSTN1.2	calsyntenin 1	O94985	HPA012749
CLU	clusterin	P10909	HPA000572

CNTN1.1	contactin 1	Q12860	HPA041060
CNTN1.2	contactin 1	Q12860	HPA070467
COCH	cochlin	O43405	HPA065086
COL6A1.1	collagen type VI alpha 1 chain	P12109	HPA019142
COL6A1.2	collagen type VI alpha 1 chain	P12109	HPA029401
COL6A1.3	collagen type VI alpha 1 chain	P12109	HPA029402
CR1.1	complement C3b/C4b receptor 1 (Knops blood group)	P17927	HPA043579
CR1.2	complement C3b/C4b receptor 1 (Knops blood group)	P17927	HPA042455
DAG1.1	dystroglycan 1	Q14118	HPA044662
DAG1.2	dystroglycan 1	Q14118	HPA061826
DAG1.3	dystroglycan 1	Q14118	HPA070883
DKK3.1	dickkopf WNT signaling pathway inhibitor 3	Q9UBP4	HPA011164
DKK3.2	dickkopf WNT signaling pathway inhibitor 3	Q9UBP4	HPA011868
DKK3.3	dickkopf WNT signaling pathway inhibitor 3	Q9UBP4	HPA063769
EFEMP1.1	EGF containing fibulin extracellular matrix protein 1	Q12805	HPA062231
EFEMP1.2	EGF containing fibulin extracellular matrix protein 1	Q12805	HPA071588
ENPP2.1	ectonucleotide pyrophosphatase/phosphodiesterase 2	Q13822	HPA000434
ENPP2.2	ectonucleotide pyrophosphatase/phosphodiesterase 2	Q13822	HPA023759
ENPP2.3	ectonucleotide pyrophosphatase/phosphodiesterase 2	Q13822	HPA053652
FGFR2.1	fibroblast growth factor receptor 2	P21802	HPA010693
FGFR2.2	fibroblast growth factor receptor 2	P21802	HPA010710
FGFR2.3	fibroblast growth factor receptor 2	P21802	HPA035305
GAB2.1	GRB2 associated binding protein 2	Q9UQC2	HPA000979
GAB2.2	GRB2 associated binding protein 2	Q9UQC2	HPA001368
GM2A	GM2 ganglioside activator	P17900	HPA008063
GSN	gelsolin	P06396	HPA070538
HPX.1	hemopexin	P02790	HPA065318
HPX.2	hemopexin	P02790	HPA068847
KRT1.1	keratin 1	P04264	HPA017917
KRT1.2	keratin 1	P04264	HPA019797
KRT1.3	keratin 1	P04264	HPA062908
LSAMP.1	limbic system-associated membrane protein	Q13449	HPA054051
LSAMP.2	limbic system-associated membrane protein	Q13449	HPA065923
MEGF8.1	multiple EGF like domains 8	Q7Z7M0	HPA007691
MEGF8.2	multiple EGF like domains 8	Q7Z7M0	HPA008178
MEGF8.3	multiple EGF like domains 8	Q7Z7M0	HPA067494
NELL2.1	neural EGFL like 2	Q99435	HPA035715
NELL2.2	neural EGFL like 2	Q99435	HPA035714
NELL2.3	neural EGFL like 2	Q99435	HPA057385
NEO1.1	neogenin 1	Q92859	HPA003913
NEO1.2	neogenin 1	Q92859	HPA027806

NRCAM	neuronal cell adhesion molecule	Q92823	HPA061433
NRXN1.1	neurexin 1	P58400;Q9ULB1	HPA053886
NRXN1.2	neurexin 1	P58400;Q9ULB1	HPA059963
NRXN1.3	neurexin 1	P58400;Q9ULB1	HPA067588
NUCB1.1	nucleobindin 1	Q02818	HPA007689
NUCB1.2	nucleobindin 1	Q02818	HPA008176
OGN	osteoglycin	P20774	HPA015482
PCSK1N	proprotein convertase subtilisin/kexin type 1 inhibitor	Q9UHG2	HPA064734
PLG.1	plasminogen	P00747	HPA021602
PLG.2	plasminogen	P00747	HPA048823
PTGDS.1	prostaglandin D2 synthase	P41222	HPA004938
PTGDS.2	prostaglandin D2 synthase	P41222	HPA067456
SCG3.1	secretogranin III	Q8WXD2	HPA006880
SCG3.2	secretogranin III	Q8WXD2	HPA007213
SCG3.3	secretogranin III	Q8WXD2	HPA053715
SEBOX	SEBOX homeobox	Q9HB31	HPA045689
SERPIND1	serpin family D member 1	P05546	HPA055767
SOD1	superoxide dismutase 1	P00441	HPA001401
SOD3	superoxide dismutase 3	P08294	HPA042110
SPARCL1.1	SPARC like 1	Q14515	HPA059084
SPARCL1.2	SPARC like 1	Q14515	HPA067641
TIMP1	TIMP metallopeptidase inhibitor 1	P01033	HPA053417
VGF.1	VGF nerve growth factor inducible	O15240	HPA055177
VGF.2	VGF nerve growth factor inducible	O15240	HPA058371
VGF.3	VGF nerve growth factor inducible	O15240	HPA072505
VTN	vitronectin	P04004	HPA068011
WFIKKN2.1	WAP, follistatin/kazal, immunoglobulin, kunitz and netrin domain containing 2	Q8TEU8	HPA010953
WFIKKN2.2	WAP, follistatin/kazal, immunoglobulin, kunitz and netrin domain containing 2	Q8TEU8	HPA010954

4

Supplementary table 2. Results from the multivariate analysis

2A) Proteins with absolute p(corr)≥0.5 in the model discriminating NP1 to C1 (model 1). Suffix indicate that multiple antibodies were included towards the same protein.

Protein	Predictive loading	P(corr)
APOC1	-0,21	-0.57
KRT1.1	0,20	0.56
APOA1	0,19	0.52
NRXN1.2	0,185	0.51

2B) Proteins with absolute p(corr)≥0.5 in the model discriminating NP2 to C3 (model 2). Suffix indicate that multiple antibodies were included towards the same protein.

Protein	Predictive loadings	p(corr)
PTGDS.1	0.18	0.67
SOD1	0.18	0.66
SOD3	0.17	0.63
CHGB.3	-0.17	-0.62
DKK3.1	0.16	0.59
CHGB.2	-0.16	-0.59
LSAMP.1	0.16	0.58
APOA1	0.16	0.58
WFIKKN2.1	0.16	0.58
PTGDS.2	0.15	0.57
GAB2.1	0.15	0.56
LYNX1	0.15	0.56
APP	0.15	0.54
DAG1.1	0.14	0.53
ENPP2.1	0.14	0.52
DAG1.2	0.14	0.51

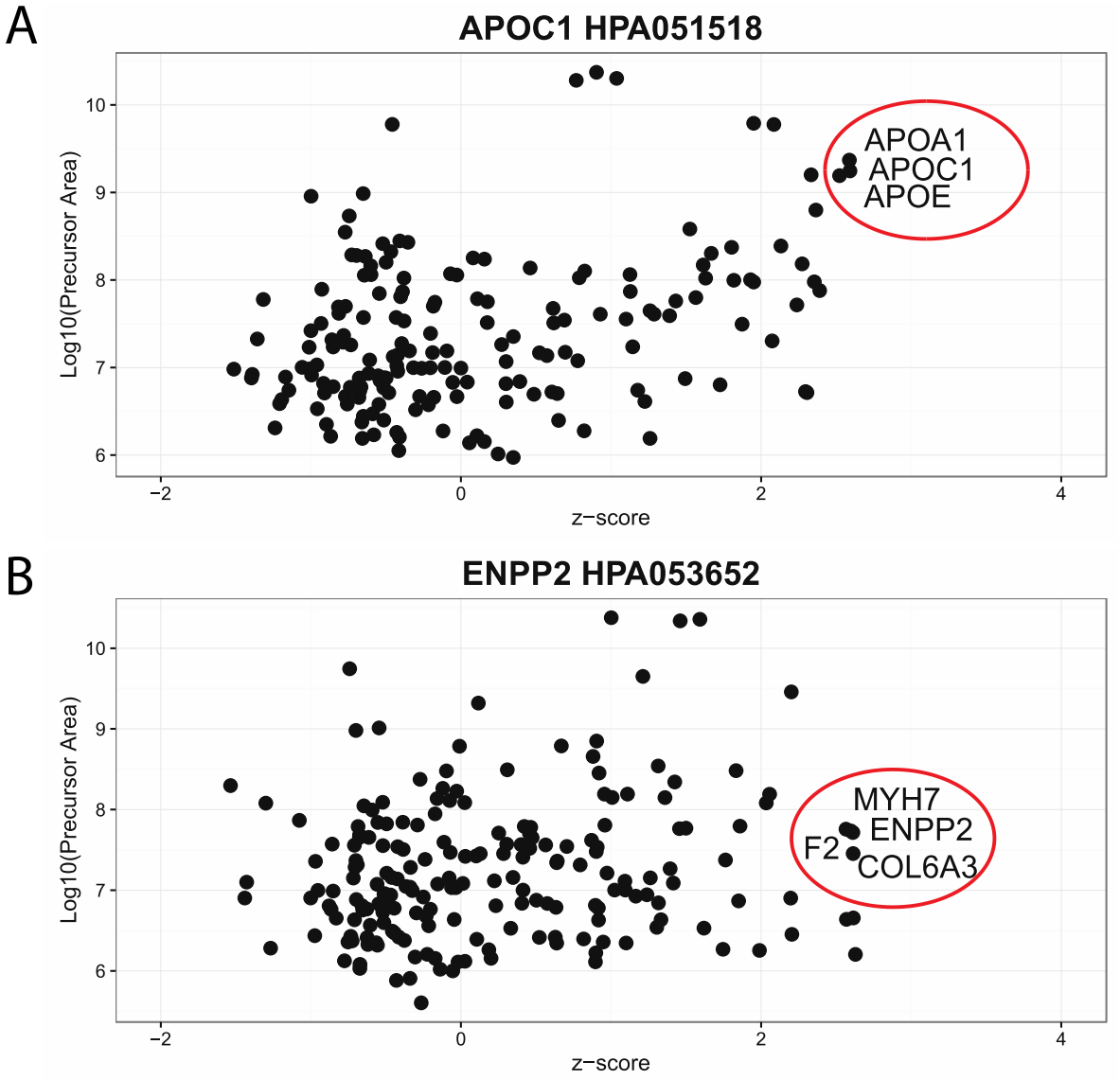
2C) Proteins with absolute p(corr)≥0.5 in the model discriminating FM to C2. Suffix indicate that multiple antibodies were included towards the same protein.

Protein	Predictive loadings	p(corr)
ENPP2.3	0.22	0.69
SOD1	0.21	0.65
CLU	0.19	0.58
HPX.2	0.18	0.56
SOD3	0.18	0.55
PCSK1N	0.17	0.54
PTGDS.2	0.16	0.50

Supplementary table 3. Results from the IC-MS

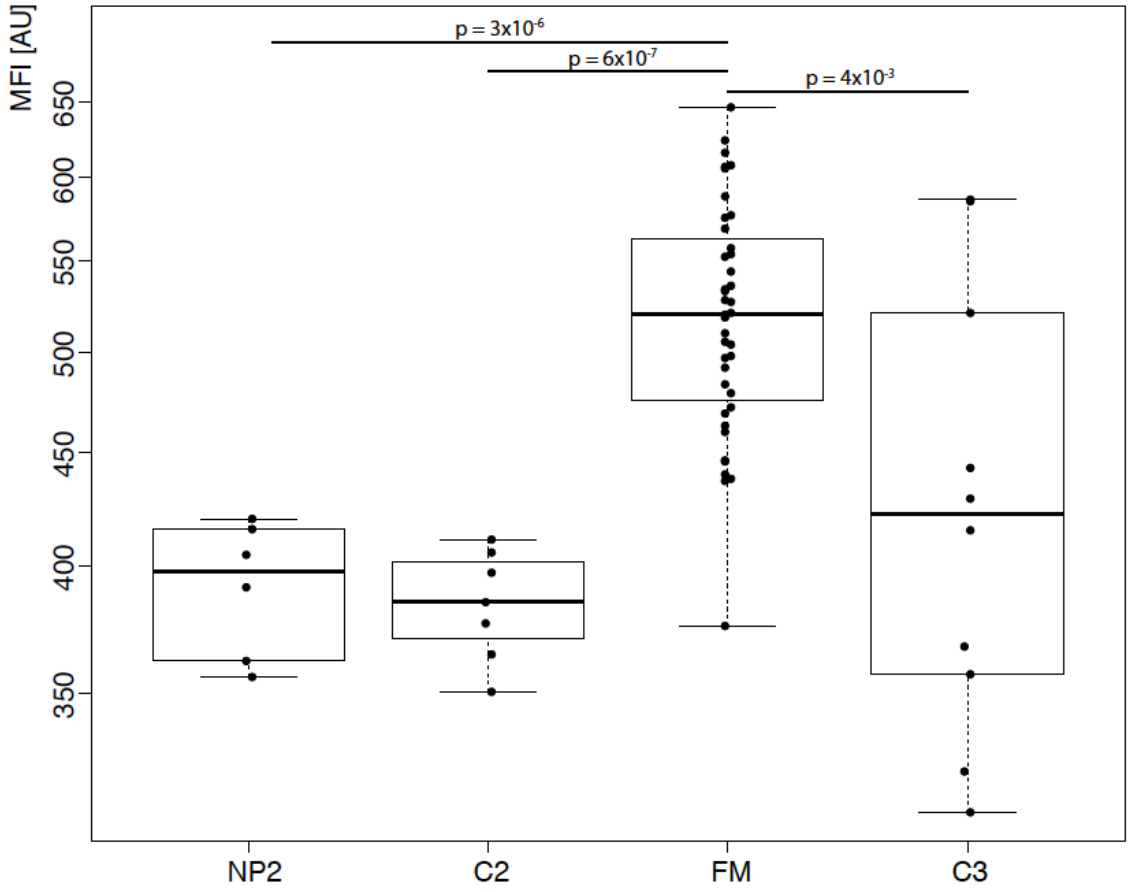
Proteins detected in immunocapture-mass spectrometry for antibodies targeting APOC1 and ENPP2

Antibody	Captured protein	Z-score	LFQ Intensity
HPA051518	APOC1	2.59	1765450000
	APOA1	2.59	2345550000
	APOE	2.59	1558700000
HPA053652	ENPP2	2.60	54462500
	COL6A3	2.61	28598500
	MYH7	2.61	52166000
	F2	2.57	57414000



Supplementary figure 1. Results from the IC-MS

Z-score/LFQ-intensity plots for specificity and selectivity of the anti-APOC1 and anti-ENPP2 antibodies.



Supplementary figure 2.

ENPP2 levels in female samples of controls (C2, n=7; C3, n = 9), neuropathic pain patients (NP2, n = 6) and fibromyalgia patients (FM, n = 40). FM patient levels were significantly higher than all other groups.

Supplementary methods: Patient inclusion and sample collection

Fibromyalgia patient samples (FM)

Patients were recruited by study information which was distributed to members by the Norwegian Fibromyalgia Association. Forty females (ages 20-60) suffering from FM as defined by the 1990 criteria of the American College of Rheumatology (ACR) were included. The diagnosis was confirmed or established by a consultant in rheumatology. Patients were excluded from participation if any verified organic cause of the pain condition was found, or if there was an organic condition probably influencing the symptoms of FM. Moreover, any history of serious medical illness, or current or previous DSM-IV diagnosis of mood disorders (last 12 months), anxiety disorders, psychotic disorder, dementia, epilepsy, seizure disorders, alcohol or drug abuse led to exclusion. All patients underwent a structured psychiatric interview for DSM-IV and Montgomery-Aasberg Depression Rating Scale.

Lumbar puncture was performed fasting between eight and nine in the morning. Ten ml of CSF was obtained and immediately put on dry ice for cooling and freezing, and afterwards stored in an ultra-freezer. None of the patients received any psychotropic medication at the time of the lumbar puncture.

Neuropathic pain patient samples

CSF samples were collected from fasting patients underwent lumbar puncture by a thin low-traumatic spinal needle. After removal of the first sample aliquots to avoid blood contamination of the CSF from potential puncture bleeding, the samples were collected in

polypropylene tubes, which were sealed, gently mixed and put on ice. The samples were centrifuged at 1,300 g for 10 min at 4˚C and decanted to remove cells, visually inspected for blood contamination, aliquoted in 1 mL cryotubes and stored at -70°C until analysis.

NP1

14 patients from Uppsala Academic Hospital (mean age 57 (47-68), 4 males) with long lasting neuropathic pain (median 10 years, range 3-23 years), and permanently implanted SCS since more than three months (median 3 years, range 1-10 years) with self-reported good pain relief, were included in the study. Pain diagnoses were radiculopathy (n=10), post-surgical pain (n=1), phantom limb pain (n=1), low back pain (n=1) and polyneuropathy (n=1). For patient characteristics see Table 1. Neuropathic pain patients underwent two consecutive lumbar punctures, resulting in 28 paired CSF samples. Before the first lumbar puncture their stimulator was turned off for 48 hours (except for one patient who chose 24h), and medications were kept constant. After three weeks of normal SCS use the patients returned for the second lumbar puncture.

NP2

Eleven pain patients participating in a clinical trial of intrathecal bolus injections of the analgesic ziconotide (ClinicalTrials.gov identifier NCT01373983) were recruited. Inclusion criteria were: 1) patient, at least 18 years of age, suffering from chronic (≥6 months) neuropathic pain due to trauma or surgery, who had failed on conventional pharmacological treatment; 2) average visual analog scale pain intensity last week ≥40 mm; 3) patient capable

of judgment, ie, able to understand information regarding the drug, the mode of administration, and evaluation of efficacy and side effects; and 4) signed informed consent.

Exclusion criteria were: 1) limited life expectancy (investigator's judgment); 2) intrathecal chemotherapy; 3) known or suspected intracranial hypertension; 4) known liver or kidney disease, defined as serum transaminases, total bilirubin, alkaline phosphatase or creatinine >1.2× upper limit of normal; 5) advanced cardio-pulmonary disease (investigator's judgment); 6) ongoing infection, whether systemically or locally in the lumbar area; 7) coagulopathy (including medication with warfarin, clopidogrel, and heparin); 8) allergy to ziconotide or any of the excipients in the ziconotide vial; 9) history of psychiatric disorders, which in the investigator's opinion would put the patient at risk; 10) pregnant or lactating woman; and/or 11) participation in another clinical trial during the last 30 days.

After informed consent was received, the following data were registered: basic demographic data; pain diagnosis; pain duration; present and past medical history; and concomitant medication. A medical examination was performed. All patients had at least probable neuropathic pain according to the criteria published by Treede et al [9] and all were or had been candidates for SCS. After CSF sampling, the patients received an intrathecal bolus injection of ziconotide according to the protocol of the clinical trial (ClinicalTrials.gov NCT01373983).

Control samples

C1 and C3

CSF was collected at the Cluj Hospital, Romania from 124 individuals without neurological complaints undergoing spinal anesthesia for planned minor urology surgeries, these were analyzed in two separate runs making them two samples sets, the first one with 96 samples (age range 21-81 (mean = 60; median = 65), 92 males) and the second one with 28 samples (age range 21-89 (mean = 51; median = 55), males 19). Samples were collected according to the same protocol as NP1.

C2

For one of the neuropathic pain patient sample sets (n=11) there was a dedicated control sample set (n=11) collected simultaneous to the pain samples at the same site from healthy volunteers recruited via a local at the Faculty of Health Sciences, Linköping University, Sweden, and by contacting healthy subjects from earlier studies. After informed consent, a structured interview covering multiple physical and psychological aspects was conducted to ensure the absence of any significant medical condition (for further details see publication [32]. A medical examination was performed, including assessment for fibromyalgia tender points. Intrathecal access was obtained by lumbar puncture and a 10 ml sample of CSF was taken. The sample was immediately cooled on ice and transported to the Painomics® laboratory, Linköping University Hospital, where it was visually checked for blood contamination, centrifuged and divided in aliquots and stored at -70◦ C until analysis.

111