

Supplementary information

Table S1 – Output of the enrichment analysis for the GO cellular component, biological process and molecular function categories

p-value	corr p-value	*x	*n	*X	*N	Description	set of significant proteins	Uniprot entries	enrichment
<i>GO Cellular component</i>									
4,07E-23	7,94E-21	55	2196	89	16949	extracellular region	0,618	0,130	4,770
9,97E-10	9,72E-08	28	1005	89	16949	extracellular region part	0,315	0,059	5,306
6,85E-08	4,46E-06	23	772	89	16949	extracellular space	0,258	0,046	5,674
1,88E-02	7,32E-01	11	345	89	16949	extracellular matrix	0,124	0,020	6,072
7,51E+00	6,10E+01	11	670	89	16949	cytoplasmic membrane-bounded vesicle	0,124	0,040	3,127
2,08E-01	5,79E+00	10	364	89	16949	cell surface	0,112	0,021	5,232
1,78E-01	5,79E+00	8	216	89	16949	apical part of cell	0,090	0,013	7,053
1,11E+00	1,84E+01	8	280	89	16949	lysosome	0,090	0,017	5,441
7,11E-01	1,54E+01	6	133	89	16949	anchored to membrane	0,067	0,008	8,591
2,90E+00	2,98E+01	6	172	89	16949	apical plasma membrane	0,067	0,010	6,643
5,96E+00	5,54E+01	6	197	89	16949	stored secretory granule	0,067	0,012	5,800
4,61E-01	1,12E+01	5	75	89	16949	basement membrane	0,056	0,004	12,696
1,22E+00	1,84E+01	5	92	89	16949	melanosome	0,056	0,005	10,350
3,34E+00	3,25E+01	5	114	89	16949	extracellular matrix part	0,056	0,007	8,353
8,29E+00	6,46E+01	4	80	89	16949	sarcolemma	0,045	0,005	9,522
2,30E+00	2,80E+01	3	23	89	16949	cornified envelope	0,034	0,001	24,840
7,44E+00	6,10E+01	3	34	89	16949	protein-lipid complex	0,034	0,002	16,803
9,58E+00	6,92E+01	2	9	89	16949	lysosomal lumen	0,022	0,001	42,320
<i>GO Biological process</i>									
79E-06	3,93E-03	37	2342	82	14881	response to stress	0,451	0,157	2,867
3,67E-01	2,15E+01	30	2638	82	14881	positive regulation of biological process	0,366	0,177	2,064
2,28E-02	3,21E+00	22	1344	82	14881	immune system process	0,268	0,090	2,971
5,51E-05	3,88E-02	21	865	82	14881	response to wounding	0,256	0,058	4,406
7,91E-04	3,72E-01	19	825	82	14881	defense response	0,232	0,055	4,179
3,12E-03	6,27E-01	17	721	82	14881	cell adhesion	0,207	0,048	4,279
5,47E-02	5,59E+00	16	792	82	14881	regulation of immune system process	0,195	0,053	3,666
8,75E-02	7,71E+00	16	822	82	14881	immune response	0,195	0,055	3,532
2,84E-03	6,27E-01	14	475	82	14881	cell activation	0,171	0,032	5,349
3,15E+00	8,70E+01	14	886	82	14881	regulation of cell proliferation	0,171	0,060	2,868
4,59E-01	2,49E+01	12	551	82	14881	regulation of body fluid levels	0,146	0,037	3,952
5,94E-01	2,72E+01	12	566	82	14881	wound healing	0,146	0,038	3,848
6,50E-02	6,10E+00	11	376	82	14881	innate immune response	0,134	0,025	5,309
5,98E-01	2,72E+01	11	478	82	14881	coagulation	0,134	0,032	4,176
1,88E+00	6,61E+01	11	544	82	14881	positive regulation of immune system process	0,134	0,037	3,670
3,73E+00	9,63E+01	9	404	82	14881	negative regulation of cell death	0,110	0,027	4,043
9,33E-02	7,73E+00	8	189	82	14881	lymphocyte mediated immunity	0,098	0,013	7,682
3,96E-01	2,23E+01	8	231	82	14881	platelet activation	0,098	0,016	6,285
2,71E-01	1,73E+01	7	159	82	14881	extracellular structure organization	0,085	0,011	7,989
4,99E-01	2,61E+01	7	175	82	14881	immunoglobulin mediated immune response	0,085	0,012	7,259
6,86E-01	2,93E+01	7	184	82	14881	protein activation cascade	0,085	0,012	6,904

5,05E-02	5,59E+00	6	80	82	14881	platelet degranulation	0,073	0,005	13,611
2,27E+00	6,90E+01	6	157	82	14881	lipid localization	0,073	0,011	6,935
2,87E+00	8,36E+01	6	164	82	14881	complement activation	0,073	0,011	6,639
9,83E-01	4,07E+01	4	44	82	14881	cellular response to reactive oxygen species	0,049	0,003	16,498
2,04E+00	6,85E+01	4	53	82	14881	acute inflammatory response	0,049	0,004	13,696
1,32E-01	1,04E+01	3	9	82	14881	regulation of cholesterol esterification	0,037	0,001	60,492
3,83E+00	9,63E+01	3	26	82	14881	peptide cross-linking	0,037	0,002	20,939
3,83E+00	9,63E+01	3	26	82	14881	plasma lipoprotein particle organization	0,037	0,002	20,939

GO Molecular function

1,22E-02	6,14E-01	18	910	87	16254	enzyme regulator activity	0,207	0,056	3,695
3,55E-04	9,08E-02	12	284	87	16254	enzyme inhibitor activity	0,138	0,017	7,894
1,75E-03	2,24E-01	9	159	87	16254	peptidase inhibitor activity	0,103	0,010	10,575
6,60E-03	4,43E-01	9	186	87	16254	peptidase regulator activity	0,103	0,011	9,040
4,44E+00	8,74E+01	9	425	87	16254	lipid binding	0,103	0,026	3,956
1,45E-02	6,14E-01	8	151	87	16254	endopeptidase inhibitor activity	0,092	0,009	9,898
1,68E-02	6,14E-01	8	154	87	16254	endopeptidase regulator activity	0,092	0,009	9,705
6,92E-03	4,43E-01	7	94	87	16254	serine-type endopeptidase inhibitor activity	0,080	0,006	13,913
4,69E-01	1,33E+01	7	178	87	16254	antigen binding	0,080	0,011	7,347
3,15E-01	1,01E+01	3	12	87	16254	phospholipase inhibitor activity	0,034	0,001	46,707
6,43E-01	1,65E+01	3	15	87	16254	lipase inhibitor activity	0,034	0,001	37,366
8,47E-01	1,97E+01	2	3	87	16254	phospholipase A2 inhibitor activity	0,023	0,000	124,552

***x**, urinary proteins annotated to each GO term; **X**, UniProtKB/Swiss-Prot proteins annotated to each GO term; **n**, urinary proteins linked to at least one annotation term within the indicated GO categories; **N**, UniProtKB/Swiss-Prot proteins linked to at least one annotation term within the indicated GO categories.

Table S2 – Proteins differentially expressed in the urines of naïve patients with respect to healthy subject

N	Protein IDs	Protein Descriptions	Sequence Coverage	Mol. Weight [kDa]	P/C ratio	t-test
			[%]			
1	P63104	14-3-3 protein zeta/δ	40.8	27.745	3.13	0.047123
2	P43652	Afamin	26.5	69.068	3.57	0.027293
3	P02763	α-1-acid glycoprotein 1	51.2	23.511	2.58	0.026458
4	P19652	α-1-acid glycoprotein 2	39.3	23.602	3.08	0.028027
5	P05067	Amyloid β A4 protein	4.5	86.942	2.61	0.009813
6	P01019	Angiotensinogen	10.9	53.154	0.34	0.001529
7	P04083	Annexin A1	48.8	38.714	4.78	0.013495
8	P07355	Annexin A2	44.8	40.411	6.31	0.005963
9	P02647	Apolipoprotein A-I	39.0	30.777	0.50	0.012096
10	P06727	Apolipoprotein A-IV	48.2	45.398	2.68	0.023815
11	P02749	β-2-glycoprotein 1	32.5	38.298	4.31	0.029697
12	P61769	β-2-microglobulin	37.0	13.714	0.47	0.000036
13	P60022	β-defensin 1	44.1	7.4197	8.79	0.015387
14	P16278	β-galactosidase	16.2	76.074	2.95	0.030313
15	P07686	β-hexosaminidase	4.7	63.111	2.48	0.037685

16	P27482	Calmodulin-like protein 3	57.7	16.891	7.15	0.013228
17	P00915	Carbonic anhydrase	16.9	28.870	2.33	0.019143
18	P07858	Cathepsin B	23.3	37.821	3.52	0.002542
19	P26842	CD27 antigen	11.2	29.137	2.45	0.005251
20	Q9NPF0	CD320 antigen	8.5	28.991	3.00	0.041232
21	Q8NFZ8	Cell adhesion molecule 4	33.0	42.785	2.13	0.017387
22	P26992	Ciliary neurotrophic factor Receptor subunit α	8.9	40.633	2.40	0.010440
23	Q9UGN4	CMRF35-like molecule 8	7.0	33.200	3.09	0.009352
24	Q6UXG3	CMRF35-like molecule 9	13.3	36.060	2.74	0.020334
25	Q2UY09	Collagen α -1(XXVIII) chain	2.7	116.66	4.68	0.039802
26	P08572	Collagen α -2(IV) chain	1.5	167.55	2.83	0.003999
27	Q5KU26	Collectin-12	6.3	81.514	2.42	0.022040
28	P15924	Desmoplakin	1.7	331.77	9.21	0.041175
29	O95967	EGF-containing fibulin-like extracellular matrix protein 2	12.0	49.405	4.20	0.001338
30	P19957	Elafin	54.7	12.269	10.50	0.006814
31	P15311	Ezrin	8.4	69.412	3.91	0.023543
32	P35555	Fibrillin-1	7.5	312.24	2.05	0.021818
33	Q9UBX5	Fibulin-5	14.7	50.180	3.80	0.008267
34	P51654	Glypican-3	2.9	65.562	0.49	0.021562
35	P28799	Granulins	10.8	63.544	6.01	0.035560
36	P04792	Heat shock protein β -1	30.2	22.782	13.67	0.032606
37	P68871	Hemoglobin subunit β	63.9	15.998	0.23	0.005090
38	P69905	Hemoglobin subunit α	42.3	15.257	0.27	0.017533
39	P62805	Histone H4	29.1	11.367	4.17	0.014935
40	P01857	Ig gamma-1 chain C region	46.7	36.105	2.75	0.003321
41	P01766	Ig heavy chain V-III region BRO	25.0	13.227	2.58	0.003449
42	P01779	Ig heavy chain V-III region TUR	26.7	12.188	3.37	0.004080
43	P01613	Ig kappa chain V-I region Ni	30.4	11.745	11.17	0.036036
44	P06309	Ig kappa chain V-II region GM607 (Fragment)	37.6	12.664	2.81	0.016287
45	P01620	Ig kappa chain V-III region SIE	59.6	11.775	3.30	0.029641
46	P06314	Ig kappa chain V-IV region B17	31.3	14.966	4.14	0.001567
47	P17936	Insulin-like growth factor-binding protein 3	8.6	31.674	2.29	0.008376
48	Q14624	Inter- α -trypsin inhibitor heavy chain H4	9.7	103.36	2.26	0.024468
49	P18510	Interleukin-1 receptor antagonist protein	41.5	17.888	6.25	0.000925
50	O75144	Isoform 2 of ICOS ligand	12.3	34.225	4.04	0.002729
51	P15941	Isoform B of Mucin-1	1.7	122.94	4.40	0.000368
52	P07900	Isoform HSP90AA1-2 of Heat shock protein HSP 90- α	3.0	98.16	4.51	0.009825
53	Q9HCN6	Isoform VI-3 of Platelet glycoprotein VI	6.1	67.308	3.75	0.000750
54	O43278	Kunitz-type protease inhibitor	7.4	58.397	2.46	0.002371
55	Q14767	Latent-transforming growth factor beta-binding protein 2	1.9	195.05	2.09	0.010793
56	P00338	L-lactate dehydrogenase A	9.0	36.688	2.30	0.010086

57	Q9Y5Y7	Lymphatic vessel endothelial hyaluronic acid receptor 1	15.2	35.213	2.64	0.014817
58	Q14210	Lymphocyte antigen 6D	10.9	13.286	4.70	0.000005
59	O95460	Matrilin-4	4.2	68.486	2.72	0.014591
60	Q13201	Multimerin-1	1.6	138.11	0.50	0.005666
61	P24158	Myeloblastin	8.2	27.807	0.44	0.011049
62	P60660	Myosin light polypeptide 6	31.1	16.93	5.49	0.018268
63	Q7Z3B1	Neuronal growth regulator 1	11.9	38.719	3.48	0.021043
64	P10153	Non-secretory ribonuclease	23.0	18.354	7.48	0.008605
65	Q14982	Opioid-binding protein/cel adhesion molecule	11.6	38.007	2.56	0.009059
66	P00995	Pancreatic secretory trypsin inhibitor	53.2	8.507	3.61	0.002369
67	Q06830	Peroxiredoxin-1	25.1	22.110	2.40	0.003888
68	P07602	Proactivator polypeptide	21.2	58.112	3.64	0.021461
69	P12273	Prolactin-inducible protein	14.4	16.572	0.50	0.000079
70	P41222	Prostaglandin-H2 d-isomerase	37.9	21.029	4.93	0.012138
71	Q8N114	Protein shisa-5	15.0	25.582	2.16	0.019159
72	P49221	Protein-glutamine γ - glutamyltransferase 4	4.5	77.144	7.26	0.026851
73	P14618	Pyruvate kinase isozymes M1/M2	14.3	57.936	6.32	0.000136
74	P07998	Ribonuclease pancreatic	43.6	17.644	6.39	0.002200
75	P55000	Secreted Ly-6/uPAR-related protein 1	44.7	11.186	2.66	0.003509
76	P02787	Serotransferrin	68.5	77.049	2.99	0.022646
77	P02768	Serum albumin precursor	56.8	69.366	4.21	0.001105
78	Q9HAT2	Sialate O-acetyltransferase	11.5	58.314	4.04	0.014911
79	P22531	Small proline-rich protein 2E	61.1	7.8553	4.84	0.010623
80	Q14515	SPARC-like protein 1	3.5	75.207	0.33	0.000139
81	Q8TB96	T-cell immunomodulatory protein	4.1	68.107	0.49	0.049350
82	P10599	Thioredoxin	42.9	11.737	7.02	0.031122
83	Q9H665	Transmembrane protein 149	5.9	37.894	2.71	0.011154
84	Q92956	Tumor necrosis factor receptor superfamily member 14	14.1	30.391	14.43	0.000920
85	P08138	Tumor necrosis factor receptor superfamily member 16	9.4	45.183	2.35	0.006670
86	P07911	Uromodulin	37.7	69.76	4.10	0.011963
87	P11684	Uteroglobin	30.8	9.9937	0.22	0.000190
88	P19320	Vascular cell adhesion protein 1	4.7	81.275	6.51	0.030800
89	P02774	Vitamin D-binding protein	37.1	52.963	2.11	0.026877
90	P04004	Vitronectin	23.4	54.305	2.82	0.036068
91	Q9Y4E5	Zinc finger protein 451	1.6	121.48	8.17	0.013310

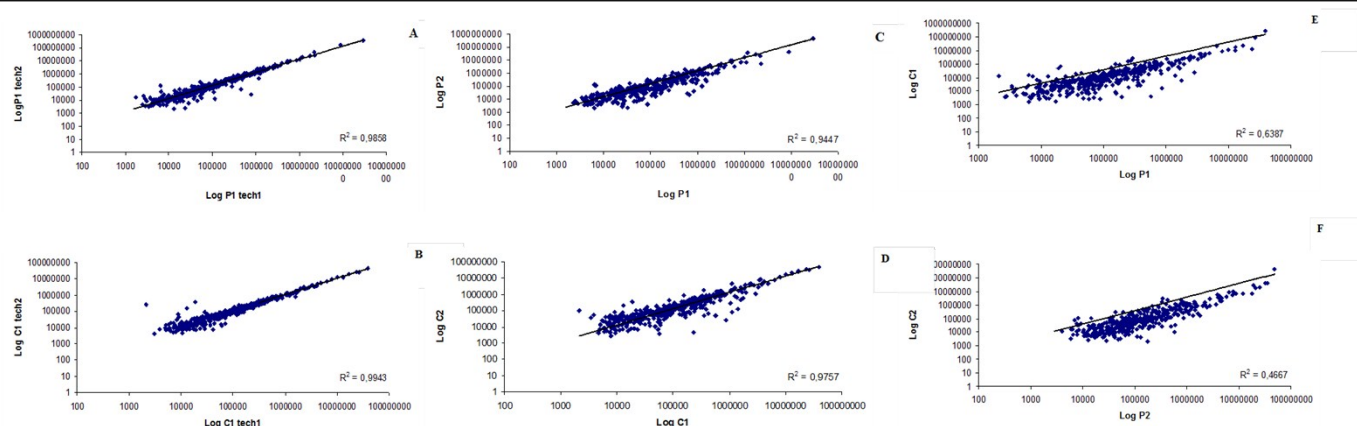


Fig. S1. Reproducibility of the label free data and difference between patients' and controls. Protein intensity was reported in logarithmic scale for each of the 557 proteins quantified by MaxQuant software and compared in two different LC-MS/MS runs (x and y axes) of the same sample. As an example, technical reproducibility of protein intensity measurement is reported for one naïve patient and one healthy control (panel A and B, respectively). Biological reproducibility is reported for two different naïve patients (panel C), two different pools of control subjects (panel D), and for one patient respect to control (panels E and F). R^2 values are also reported.

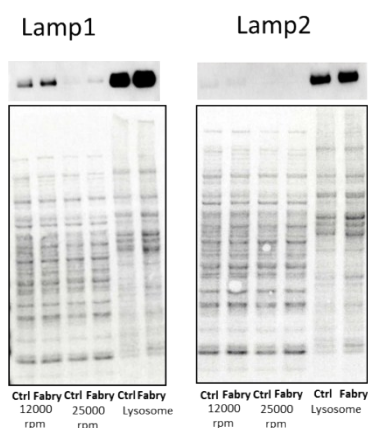


Fig. S2. Mouse liver lysosomal proteins purification. Protein extracts from Fabry and control (Ctrl) mice were subjected to sequential centrifugation steps at 12000 and 25000 rpm for lysosome enrichment. 20 micrograms of proteins coming from each steps were loaded on a gel, electro-blotted on a nitrocellulose membrane and probed with two lysosomal markers such as Lamp1 and Lamp2. Both proteins are clearly enriched in lysosomal fraction confirming the efficiency and specificity of the lysosomal enrichment protocol.

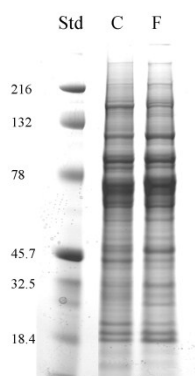


Fig. S3. 1D Gel Electrophoresis of mouse liver lysosomal proteins. C, protein extract from control sample; F, protein extract from Fabry mouse.

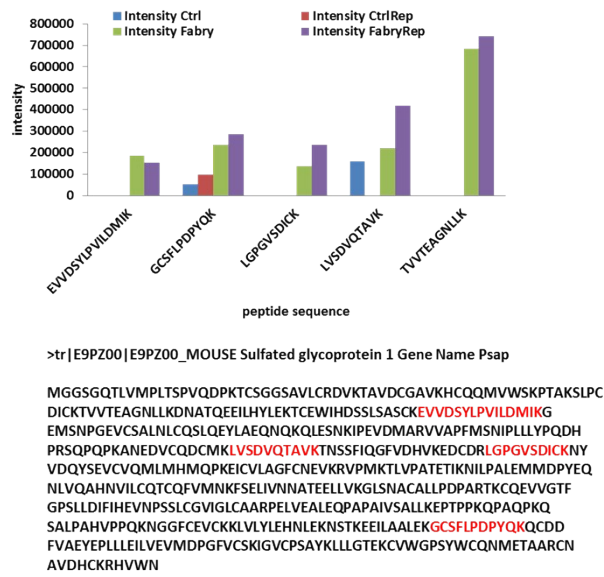


Fig. S4. Identified peptides of PSAP. Above panel: relative intensities of the five different peptides from prosaposin are reported for controls (Ctrl, two technical replicates), and Fabry samples (Fabry, two technical replicates). Lower panel: The sequenced peptides are depicted in red into the FASTA sequence of prosaposin.