

Altered transition metal homeostasis in Niemann-Pick disease, Type C1

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Figure S1 Representative western blot of plasma CP in (A) P21 (n = 5) and (B) P49 (n = 18) *Npc1*^{-/-} and wild-type (WT) littermate control mice. Detection of total, apo- and holo-CP was achieved by differential sample preparation prior to separation by SDS-PAGE. For total CP detection, samples were heated 95°C, 5 min prior to separation by SDS-PAGE. For apo- and holo-CP differentiation, the samples were not heated. Samples from CP knockout and matched WT control were included as negative and positive controls, respectively.

Figure S2 Representative western blots of (A) CSF (n = 21) and (B) plasma CP from human NP-C patients (n = 40) and healthy controls (HC; n = 41). Detection of total, apo- and holo-CP was achieved by differential sample preparation prior to separation by SDS-PAGE. For total CP detection, samples were heated 95°C, 5 min prior to separation by SDS-PAGE. For apo- and holo-CP differentiation, the samples were not heated.

Table S1 Metal analysis of various tissues from P49 female *Npcl*^{-/-} (BALB/c *Npcl*^{nih}) and wild-type (WT) littermate control (BALB/c) mice (µg/g wet tissue weight). All data are presented as mean ± S.E.M. (n = 14/genotype). Unpaired *t*-test, two-tailed.

Tissue	Metal	Genotype		<i>p</i>
		WT	<i>Npcl</i> ^{-/-}	
Cerebellum	Mn	0.45 ± 0.01	0.56 ± 0.01	< 0.0001
	Fe	11.9 ± 0.3	13.9 ± 0.2	< 0.0001
	Cu	2.9 ± 0.07	2.50 ± 0.05	< 0.0001
	Zn	10.9 ± 0.3	12.5 ± 0.2	< 0.0001
Cerebrum (left hemisphere)	Mn	0.37 ± 0.01	0.45 ± 0.01	< 0.0001
	Fe	10.9 ± 0.2	13.2 ± 0.2	< 0.0001
	Cu	2.59 ± 0.04	2.21 ± 0.04	< 0.0001
	Zn	11.8 ± 0.2	13.3 ± 0.2	< 0.0001
Liver	Mn	1.15 ± 0.02	0.99 ± 0.02	< 0.0001
	Fe	79 ± 3	56 ± 1	< 0.0001
	Cu	5.0 ± 0.1	6.2 ± 0.2	< 0.0001
	Zn	24.8 ± 0.4	18.8 ± 0.3	< 0.0001
Heart	Mn	0.42 ± 0.01	0.44 ± 0.01	0.02
	Fe	32.7 ± 0.4	32.4 ± 0.6	0.7
	Cu	4.12 ± 0.06	3.89 ± 0.06	0.009
	Zn	11.3 ± 0.2	11.3 ± 0.3	0.8
Lung	Mn	0.11 ± 0.01	0.09 ± 0.01	0.02
	Fe	13.1 ± 0.8	14 ± 1	0.3
	Cu	0.93 ± 0.06	2.5 ± 0.1	< 0.0001
	Zn	8.0 ± 0.4	5.8 ± 0.3	< 0.0001
Spleen	Mn	0.15 ± 0.01	0.17 ± 0.01	0.03
	Fe	168 ± 6	137.3 ± 3.7	< 0.0001
	Cu	0.65 ± 0.02	0.59 ± 0.01	0.01
	Zn	11.9 ± 0.3	11.1 ± 0.2	0.03
Kidney	Mn	0.85 ± 0.01	0.86 ± 0.02	0.6
	Fe	24.0 ± 0.8	23.9 ± 0.4	0.9
	Cu	2.82 ± 0.04	2.43 ± 0.03	< 0.0001
	Zn	12.2 ± 0.2	11.1 ± 0.2	< 0.0001

Table S2 Metal analysis of heparinized plasma from P21 and P49 female *Npc1*^{-/-} (BALB/c *Npc1*^{nih}) and wild-type (WT) littermate control (BALB/c) mice (μM). All data are presented as mean ± S.E.M. (P21, n = 5/genotype; P49, n = 18/genotype). Two-way ANOVA with Bonferroni correction for multiple comparisons.

Metal	<u>Age</u>								<i>p</i> ¹ (Genotype)	<i>p</i> ¹ (Age)
	P21				P49					
	WT		<i>Npc1</i> ^{-/-}		WT		<i>Npc1</i> ^{-/-}			
Mn	0.11	± 0.02	0.16	± 0.02	0.12	± 0.01	0.15	± 0.01	0.04	0.8
Fe	62.7	± 5.6	80.4	± 20.8	103	± 7	101	± 6	0.4	0.004
Cu	7.5	± 1.2	9	± 1	9.3	± 0.5	12.5	± 0.5	0.002	0.0009
Zn	8.2	± 0.7	14	± 1	10.3	± 0.4	10.8	± 0.5	<0.0001	0.4

Table S3 Details of post-mortem human NP-C and non-NP-C control cases.

Case ID (UMB#)	Gender	Age, y	PMI*, h	NPC mutation	Tissue type	Clinical cause of death
NP-C cases						
4770	Female	2	8	Not available	Hippocampus	Complications of disorder
M4004M	Male	5	18	Not available	Hippocampus	Complications of disorder
5372	Female	11	10	NPC1 (D948N;I1061T)	Hippocampus Cerebellum	Complications of disorder
4237	Female	19	16	Not available	Cerebellum	Complications of disorder
M4002M	Male	20	18	Not available	Hippocampus Cerebellum	Complications of disorder
M4018M	Female	36	22	Not available	Hippocampus Cerebellum	Complications of disorder
Non-NP-C control cases						
1864	Female	2	24	—	Hippocampus	Laryngitis, bronchiolitis associated with beta-hemolytic Strep Group A infection
1500	Male	6	14	—	Hippocampus	Motor vehicle accident. Multiple injuries
5173	Female	10	18	—	Hippocampus Cerebellum	Asthma
1347	Female	19	24	—	Cerebellum	Motor vehicle accident. Multiple injuries
914	Male	20	24	—	Hippocampus Cerebellum	Motor vehicle accident. Multiple injuries
1406	Female	38	3	—	Hippocampus Cerebellum	Coronary Artery Thrombosis

* PMI = post-mortem interval

Table S4 Metal analysis of cerebellum and hippocampus from NP-C cases and matched non-NP-C control cases ($\mu\text{g/g}$ wet tissue weight). All data are presented as mean \pm S.E.M. Unpaired *t*-test, two-tailed.

Tissue	Metal	Diagnosis		<i>p</i>
		Non-NP-C control	NP-C	
Cerebellum	Mn	0.25 \pm 0.01	0.31 \pm 0.03	0.1
	Fe	29 \pm 5	47 \pm 6	0.06
	Cu	3.6 \pm 0.4	4.1 \pm 0.5	0.5
	Zn	7.9 \pm 0.4	10.2 \pm 0.7	0.03
Hippocampus	Mn	0.25 \pm 0.05	0.31 \pm 0.04	0.4
	Fe	36 \pm 7	30 \pm 3	0.4
	Cu	2.1 \pm 0.4	2.1 \pm 0.2	0.9
	Zn	8 \pm 1	10.4 \pm 0.5	0.09

Table S5 CSF samples from NP-C cases.

	Migulstat			
	-		+	
n	14		7	
Female (% of total)	4	(28.6)	5	(71.4)
	Mean	S.D.	Mean	S.D.
Age, Y	12	10	10	6
Severity score [#]	16	10	15	9
Metal, μM				
Mn [0.015 – 0.027]*	0.014	0.005	0.013	0.004
Fe [0.3 – 1.5]*	0.16	0.05	0.16	0.06
Cu [0.28 – 0.42]*	0.2	0.1	0.2	0.1
Zn [0.37 – 0.61]*	0.23	0.08	0.2	0.1
CP, nM				
Total [1.391– 5.828]*	6.6	2.0	6.1	1.3
Apo-CP	1.8	0.5	2.0	0.4
Holo-CP	5	2	4.1	1.4

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*Reference range values. Lentner, C., (Ed.) (1981). Geigy Scientific Tables: Units of Measurement, Body Fluids, Composition of the Body, Nutrition, 8th edn (Ciba-Geigy Corporation Medical Education Division).

Table S6 Heparinized plasma samples from NP-C cases and age-matched healthy controls (HC). Multiple comparison of means (Tukey contrasts).

	HC		NP-C				<i>P</i>
			Miglustat				
			-	+			
n	41		16		24		
Female (% of total)	21 (51.2)		7 (43.8)		13 (54.2)		0.807
	Mean	S.D.	Mean	S.D.	Mean	S.D.	<i>P</i>
Age, Y	16	10	24	20	18	10	0.1
Severity score [#]	-	-	26	8	15	10	0.02
Metal, μM							
Mn [0.007 – 0.25]	0.02	0.02	0.013	0.007	0.012	0.005	0.009
Fe [8.95 – 21.48] ^P [11.64 – 30.43] ^{A,δ} [8.95 – 30.43] ^{A,♀}	21	8	19	8	18	7	0.08
Cu [12.56 – 29.83] ^P [10.99 – 21.98] ^{A,δ} [12.56 – 24.34] ^{A,♀}	14	3	14	3	13	2	0.5
Zn [10.7 – 22.9]	12	3	10	2	11	2	< 0.001
CP, μM	Mean	S.D.	Mean	S.D.	Mean	S.D.	<i>P</i>
Total CP [1.67 – 4.39]	2.7	0.6	2.5	0.6	3	0.4	0.1
Holo-CP	2.4	0.5	2.2	0.6	2.8	0.5	0.02
Apo-CP	0.3	0.3	0.3	0.2	0.2	0.1	0.4
Cu:CP ratio							
Cu:Total CP	6	1	6	2	4.6	0.9	0.008
Cu:Holo-CP	6	1	7	3	5	1	0.005

	HC		NP-C				<i>P</i>
	Mean	S.D.	Miglustat		Mean	S.D.	
			-	+			
Oxidase activity, U/L							
Total	76.756	43.133	77.672	45.233	71.761	34.352	0.327
CP	74.683	42.478	74.648	43.878	69.703	33.84	0.377
Azide-resistant	2.072	1.759	3.024	3.268	2.058	1.195	0.020
Specific oxidase activity, U/mg CP							
Total CP	0.19	0.10	0.21	0.11	0.15	0.06	0.059
Holo-CP	0.21	0.11	0.23	0.12	0.16	0.07	0.038

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*Tietz, N.W. (1987). *Fundamentals of clinical chemistry*, 3rd edn (Philadelphia: Saunders).

^P Pediatric reference range.

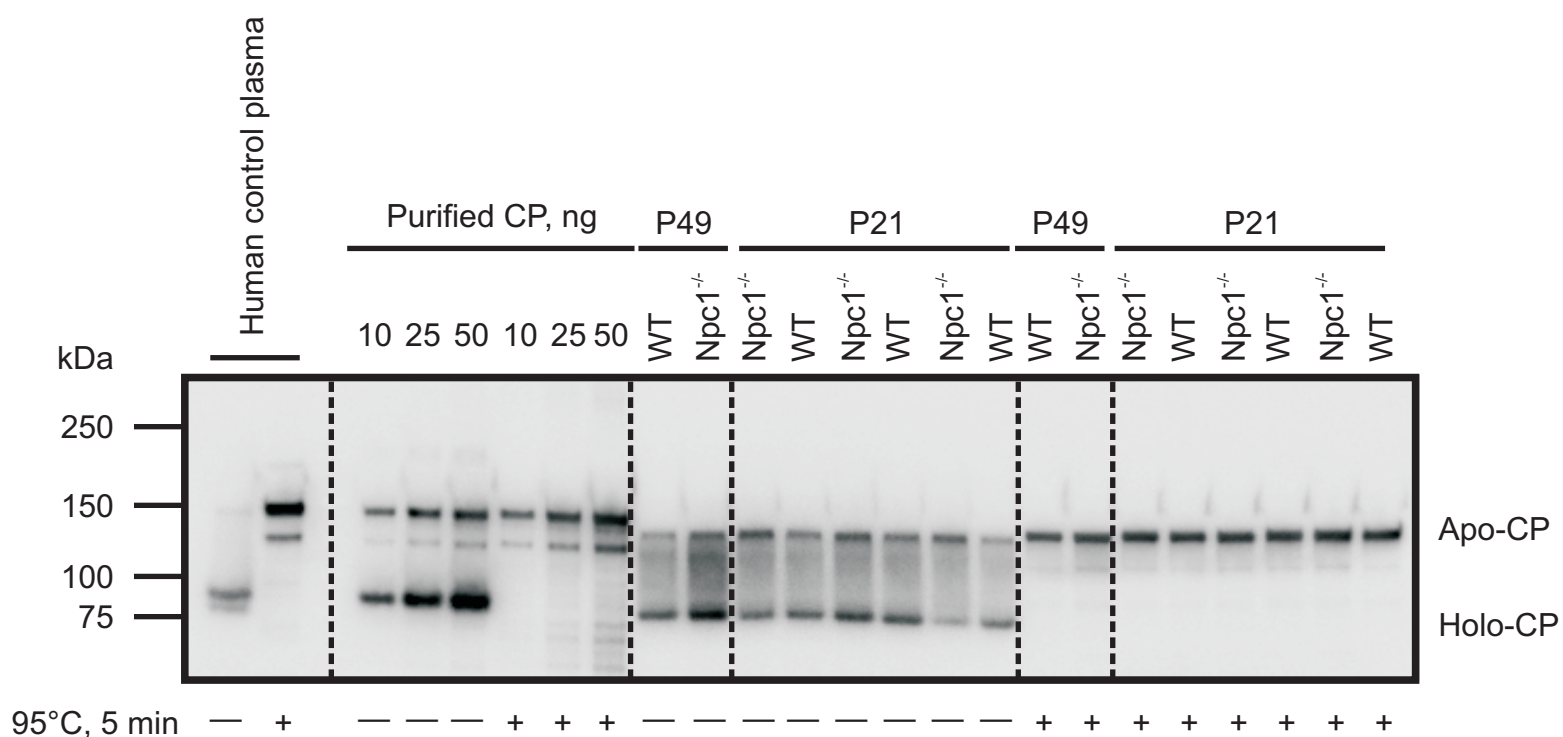
^A Adult reference range.

♂ Male.

♀ Female.

Figure S1

A



B

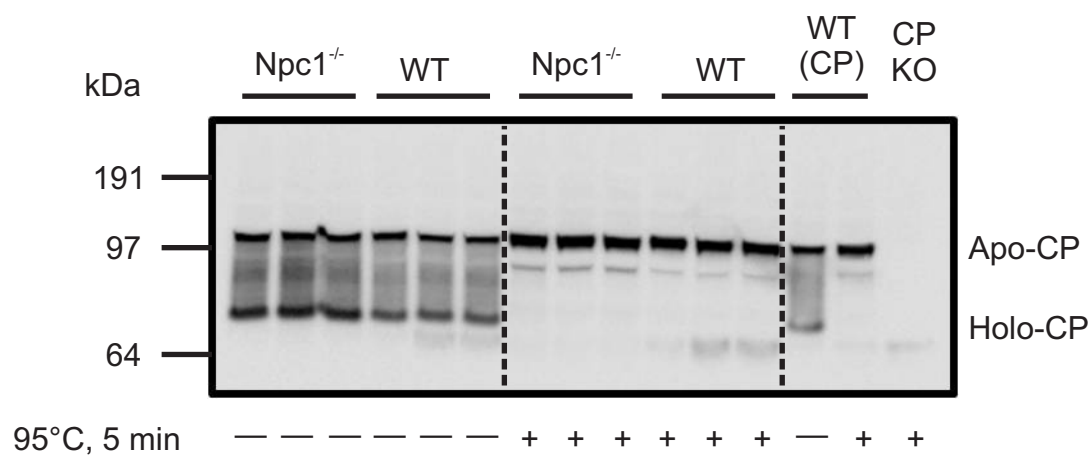
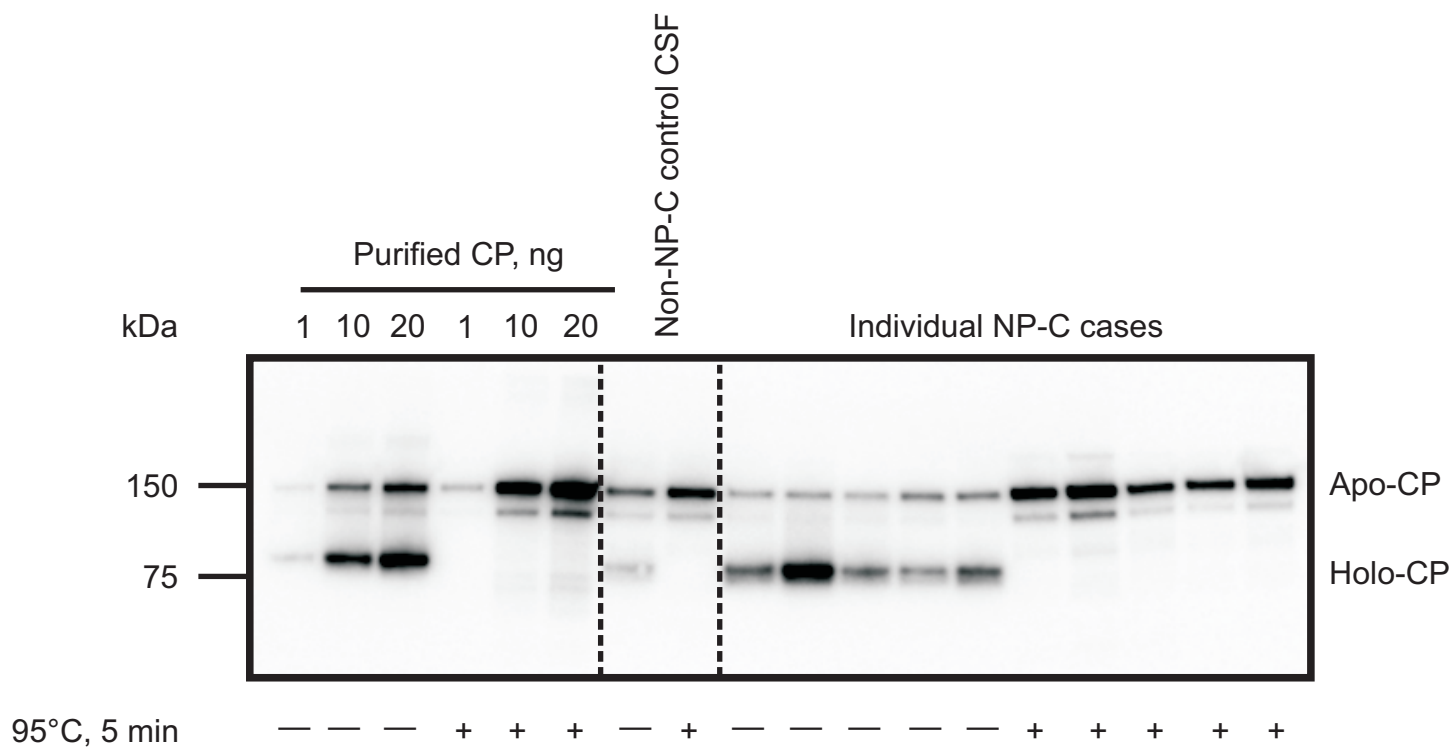


Figure S2

A



B

