SUPPLEMENTARY INFORMATION

References

Prior publications using single-item UBDRS CGI scores in addition to reference #28 (Augustine *et al,* 2015) in main text.

Adams HR, Kwon J, Marshall FJ, de Blieck EA, Pearce DA, Mink JW. Neuropsychological symptoms of juvenile-onset batten disease: experiences from 2 studies. *J Child Neurol*. 2007;22(5):621-627. doi:10.1177/0883073807302603.

Adams HR, Mink JW; University of Rochester Batten Center Study Group. Neurobehavioral features and natural history of juvenile neuronal ceroid lipofuscinosis (Batten disease). *J Child Neurol*. 2013;28(9):1128-1136. doi:10.1177/0883073813494813.

Table S1. Demographics and clinical outcome measures for participants with CLN3 disease.

SP ID	Age	Sex	Height	Weight	UBDRS Scores[a]				Vineland ABC[b]	Magnetic Resonance Spectroscopy[c]					
	(years)		(cm)	(kg)	Physical	Capability	CGI[d]			Parietal Gray Matter			Left Centrum Semiovale		
							Wt	Overall		Cr	Glx	NAA	Cr	Chol	MyoI
SP1.2.2	13.3	M	159	40.5	4.0	10.0	11.7	2	88.0	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
SP2.2.3	9.0	M	124	24.9	3.0	7.0	12.8	2	72.0	-0.699	-0.254	-1.568	1.085	1.539	0.959
SP3.2.1	9.5	F	146	35.6	10.8	6.0	16.3	3	53.0	0.171	1.370	2.116	-1.674	0.998	0.363
SP4.2.2	7.3	M	132	25.8	4.0	8.0	9.3	2	77.0	0.317	0.423	-0.266	0.086	1.068	0.150
SP5.2.2	13.2	M	154	48.4	1.0	13.0	10.5	2	110.0	1.307	1.790	-0.570	0.033	0.430	-0.309
SP6.2.1	15.4	M	175	71.7	33.0	4.0	19.8	3	34.0	-2.649	-5.363	-5.646	-0.013	-1.696	-0.032
SP7.2.1	11.4	F	160	47.3	36.0	3.0	19.8	3	23.0	-0.291	-4.256	-3.256	0.494	2.464	0.074
SP8.2.1	16.6	F	164	52.2	18.0	4.0	19.8	4	35.0	0.358	-2.986	-3.624	1.097	0.741	0.586
SP9.2.1	16.6	M	173	70.2	20.0	6.0	18.7	3	42.0	-1.222	-3.253	-3.024	1.294	-0.772	-0.144
SP10.2.1	20.7	F	165	68.5	58.0	4.0	22.2	4	46.0	-4.294	-8.925	-7.714	0.460	4.868	0.564
SP10.2.5	6.8	M	120	21.3	5.0	14.0	10.5	2	83.0	2.378	3.948	2.181	-0.402	0.965	0.138
SP11.2.4	7.8	M	129	28.2	6.0	9.0	12.8	2	83.0	-0.576	-0.386	-0.903	-0.703	1.179	-0.401
SP12.2.1	9.3	F	138	33.5	10.0	9.0	11.7	2	70.0	-0.385	-0.546	-1.460	-0.169	0.846	-0.266
SP12.2.2	7.5	F	122	24.2	4.0	11.0	12.8	2	78.0	-0.134	-0.424	0.062	-0.476	0.041	0.302
SP13.2.3	7.8	M	122	21	19.0	5.0	16.3	3	66.0	-0.359	-0.011	-2.180	0.680	0.467	-0.124
SP14.2.2	6.8	M	122	23.7	3.0	11.0	9.3	2	81.0	0.290	0.561	-1.100	0.910	0.597	-0.340
SP15.2.1	10.0	F	145	44.3	2.0	10.0	14.0	3	84.0	0.256	-3.188	-1.442	-0.011	-0.004	0.070
SP16.2.1	16.2	F	161	71.5	9.0	6.0	18.7	3	50.0	-1.766	-8.831	-5.783	1.022	0.271	0.166
SP16.2.2	15.1	F	159	54.4	22.0	4.0	21.0	4	26.0	-1.657	-7.456	-6.664	0.959	-1.466	0.729
SP17.2.1	17.5	M	168	45.7	61.0	1.0	24.5	4	20.0	-2.148	-8.406	-7.438	1.136	3.640	0.341
SP18.2.1	11.6	F	151.7	84.9	37.3	6.0	24.5	4	35	1.743	-0.859	-0.933	0.862	2.855	0.133

[a] UBDRS: Unified Batten Disease Rating Scale. The Physical and Capability scores are weighted scores, calculated as described in Materials and Methods. [b] ABC: Adaptive Behavior Composite. [c] Values reflect the difference in tissue metabolite concentration between CLN3-Batten study participant and reference. [d] CGI: clinical global impression. The provided CGI scores include the weighted (Wt) scores, calculated as described in Materials and Methods, and the raw single-item overall score. Cr: creatinine. Glx: glutamine/glutamate/GABA. NAA: N-aspartylaspartate. N.D.: not done. Chol: choline. MyoI: myo-inositol.

Table S2. Genotype and neurofilament light chain levels for participants with CLN3 disease.

SP ID	**Genotype**[a]		**Neurofilament light chain levels**[b]		
	Allele 1	Allele 2	Cerebral Spinal Fluid		Serum
			PEA[c]	ELISA	Simoa™
SP1.2.2	c.461-280_677+382del[d]	c.1056+3A>C	8.15	2722.30	14.82
SP2.2.3	c.678-610_1057-846dup[e, h]	c.678-610_1057-846dup	9.62	1894.98	22.04
SP3.2.1	c.461-280_677+382del	c.461-280_677+382del	9.20	1765.05	30.80
SP4.2.2	c.461-280_677+382del	c.1056G>T [p.(Gln352His)]	8.44	1259.32	21.47
SP5.2.2	c.1001G>A [p.(Arg334His)]	c.1213C>T [p.(Arg405Trp)]	6.45	149.69	7.24
SP6.2.1	c.461-280_677+382del	c.461-280_677+382del	9.30	1977.60	12.69
SP7.2.1	c.944dup [p.(His315Glnfs*67)]	p.(His315Glnfs*67)	9.73	2235.23	32.26
SP8.2.1	c.461-280_677+382del	c.461-280_677+382del	10.40	3363.85	29.95
SP9.2.1	c.461-280_677+382del	c.461-280_677+382del	9.96	2467.34	14.21
SP10.2.1	p.(Arg334His)	NC_000016.9:g. (28211763_28486693)_(28488725_28488776)del[f,h]	10.79	4677.33	47.38
SP10.2.5	p.(Arg334His)	NC_000016.9:g. (28211763_28486693)_(28488725_28488776)del	8.29	794.68	13.98
SP11.2.4	c.461-280_677+382del	c.461-280_677+382del	10.31	4108.89	55.83
SP12.2.1	c.461-280_677+382del	c.461-280_677+382del	9.09	1205.64	20.97
SP12.2.2	c.461-280_677+382del	c.461-280_677+382del	8.63	999.17	20.29
SP13.2.3	c.678_838del[g,h]	c.678_838del	8.85	1143.14	40.20
SP14.2.2	c.461-280_677+382del	c.461-280_677+382del	8.13	798.42	21.88
SP15.2.1	c.461-280_677+382del	c.206C>A [p.(Ser69*)][h]	9.23	1246.49	15.90
SP16.2.1	c.461-280_677+382del	c.461-280_677+382del	9.77	2239.00	23.15
SP16.2.2	c.461-280_677+382del	c.461-280_677+382del	9.56	2120.36	46.47
SP17.2.1	c.461-280_677+382del	c.575G>A [p.(Gly192Glu)]	10.52	4065.41	84.51
SP18.2.1	c.461-280_677+382del	c.622dup [p.Ser208Phefs*28)]	10.14	2787.42	32.71

[a] RefSeq: NG_008654.2(CLN3_v001)/LRG_689t1, unless otherwise indicated. [b] The unit of measurement is normalized protein expression for PEA, and pg/mL for ELISA and Simoa™. [c] PEA: proximal extension assay. [d] Common 1-kb (966 bp; exon 7-8) deletion. [e] Exon 9-13 homozygous duplication, {arr[GRCh37] 16p11.2(28490044_28496049)x4 mat}. [f] Partial exon 15 deletion, {arr[GRCh37] 16p11.2(28486693_28488725)x1}. [g] Exon 9-10 deletion. [h] Variant not reported in: 1) Leiden Open Variation Database. CLN3 (ceroid-lipofuscinosis, neuronal 3). https://databases.lovd.nl/shared/variants/CLN3/unique. Accessed 8/27/2020. 2) ClinVar. CLN3. https://www.ncbi.nlm.nih.gov/clinvar/?term=cln3%5Bgene%5D. Accessed 9/2/2020. 3) gnomAD. CLN3. https://gnomad.broadinstitute.org/gene/ENSG00000188603?dataset=gnomad_r2_1. Accessed 8/27/2020.

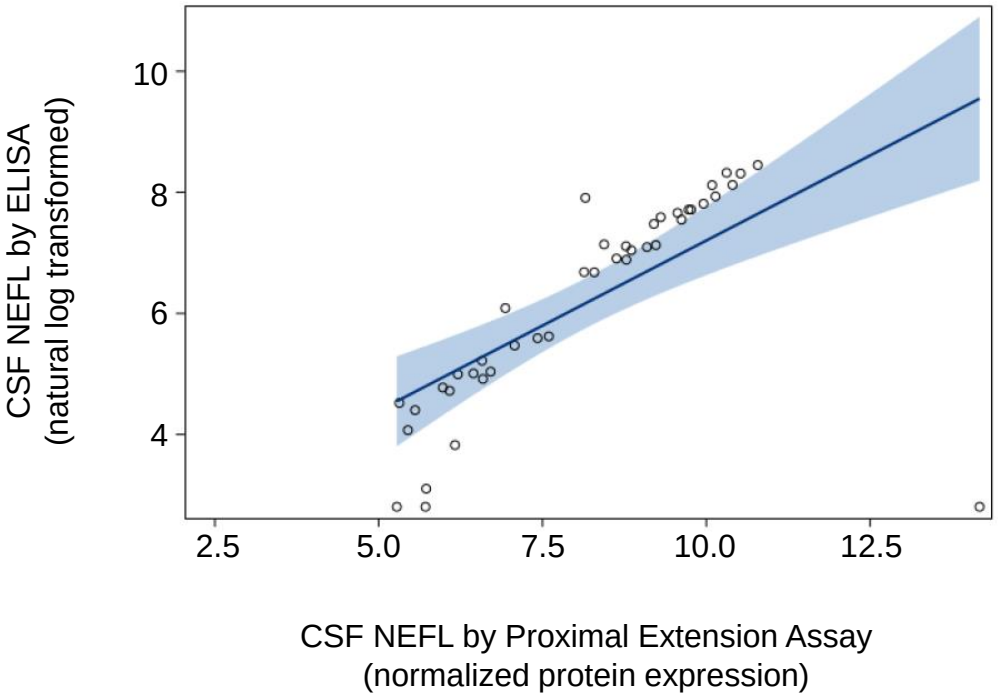


Figure S1. Correlation of CSF NEFL levels measured by ELISA (natural log transformed) and proximal extension assay. N=42. r_p=0.69; *p*<.0001. Shaded area: 95% confidence interval. The outlier [PEA NPX=14.17, ELISA (non-transformed)=16.5 pg/mL (below limit of detection)] belongs to an unaffected pediatric laboratory control sample. CSF: cerebrospinal fluid. NEFL: neurofilament light chain. NPX: normalized protein expression. PEA: proximal extension assay.

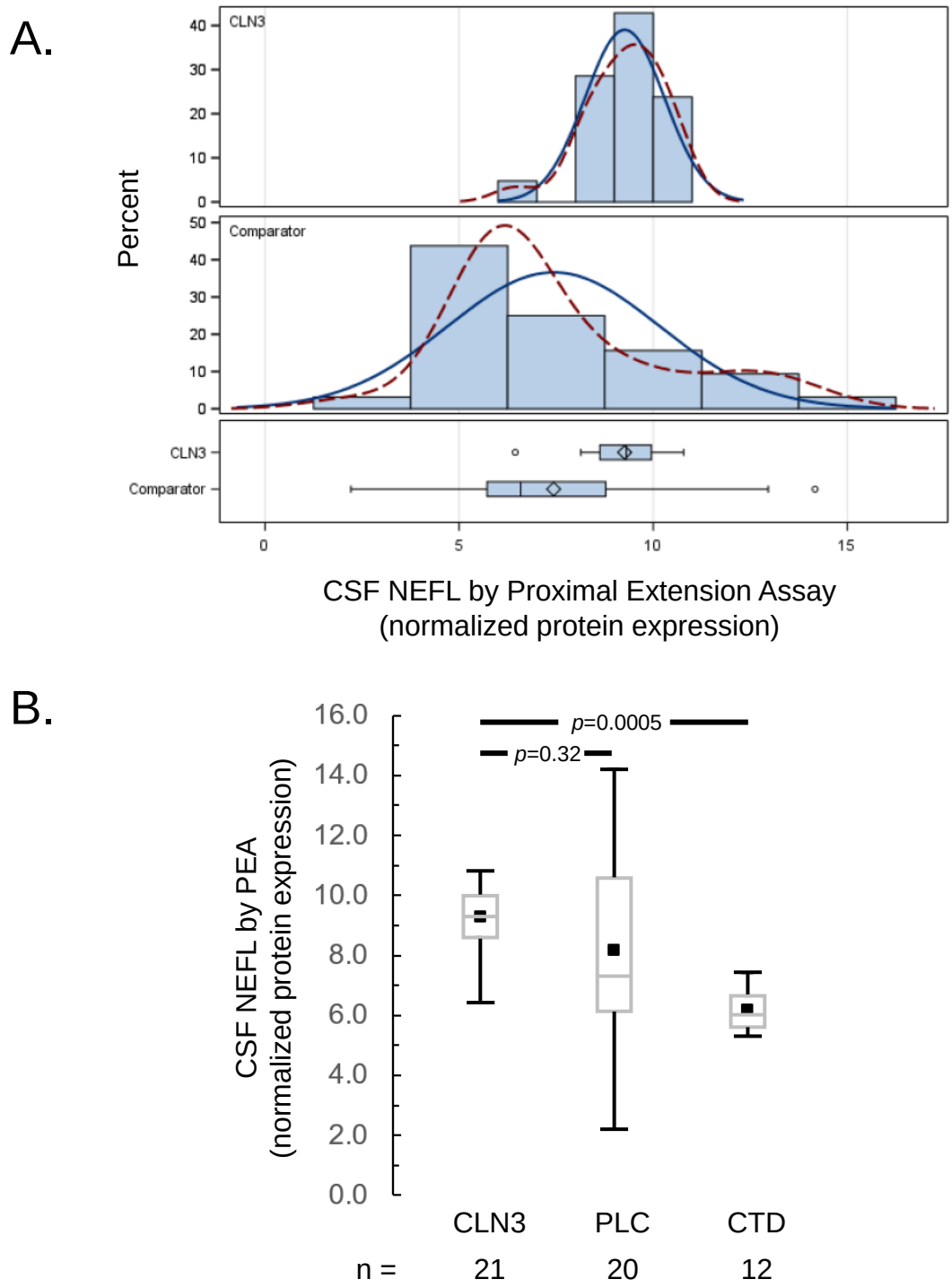
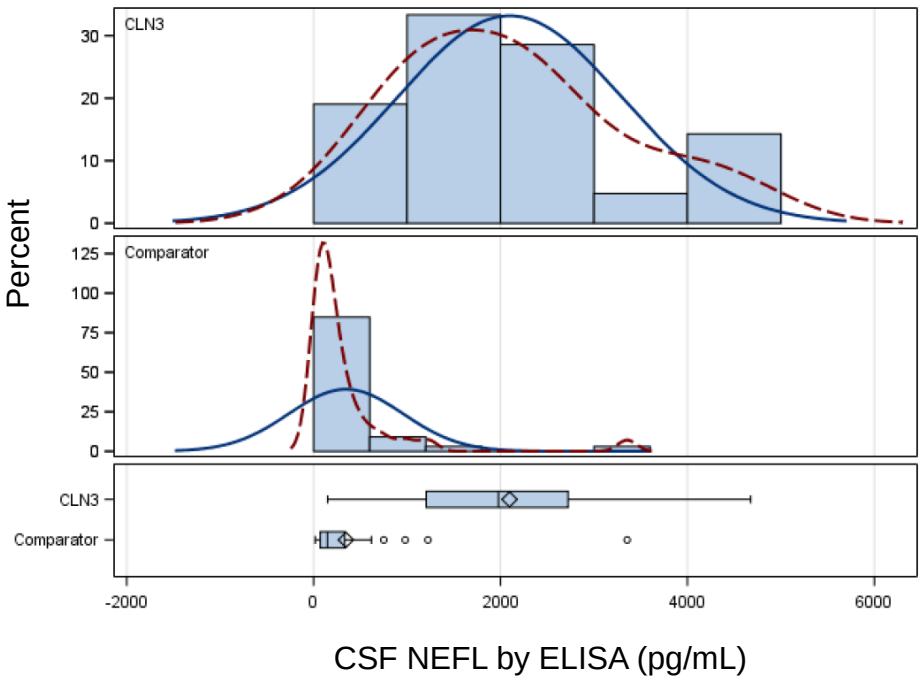


Figure S2. CSF NEFL level in samples from CLN3 versus non-CLN3 individuals measured by PEA. **A**. Top and middle panels: distribution of measured NEFL levels (solid line – normal; dashed line – kernel). Bottom panel: statistical values of measured NEFL levels {diamond – mean; box – median and interquartile range [IQR, Q1 (25th percentile) - Q3 (75th percentile)]; whiskers – minimum = Q1 – 1.5*IQR and maximum = Q3 + 1.5*IQR}. *p*=.0013. **B**. NEFL levels in individual cohort. *P* values are derived from ANOVA and Bonferroni adjustment for multiple comparisons. CSF: cerebrospinal fluid. CTD: creatine transport deficiency. PLC: pediatric laboratory controls.

A.



B.

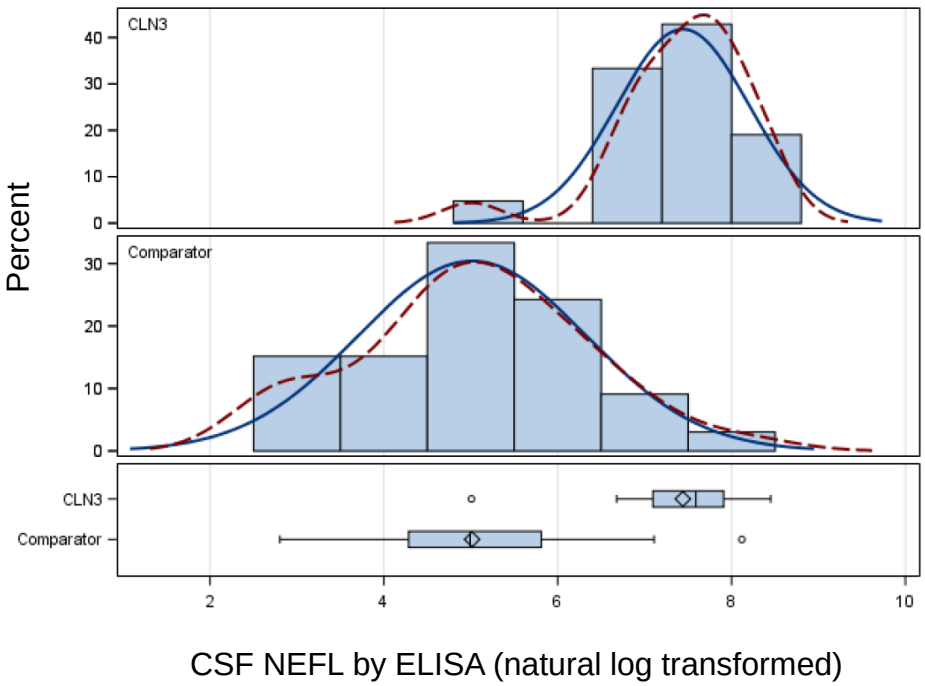


Figure S3. CSF NEFL level in samples from CLN3 versus non-CLN3 individuals measured by ELISA. **A**. Top and middle panels: distribution of measured NEFL levels (solid line – normal; dashed line – kernel). Bottom panel: statistical values of measured NEFL levels {diamond – mean; box – median and interquartile range [IQR, Q1 (25^{th} percentile) - Q3 (75^{th} percentile)]; whiskers – minimum = Q1 – 1.5*IQR and maximum = Q3 + 1.5*IQR}. *p*=<.0001. **B**. Same as A, except for NEFL values are log-transformed. CSF: cerebrospinal fluid. NEFL: neurofilament light chain.

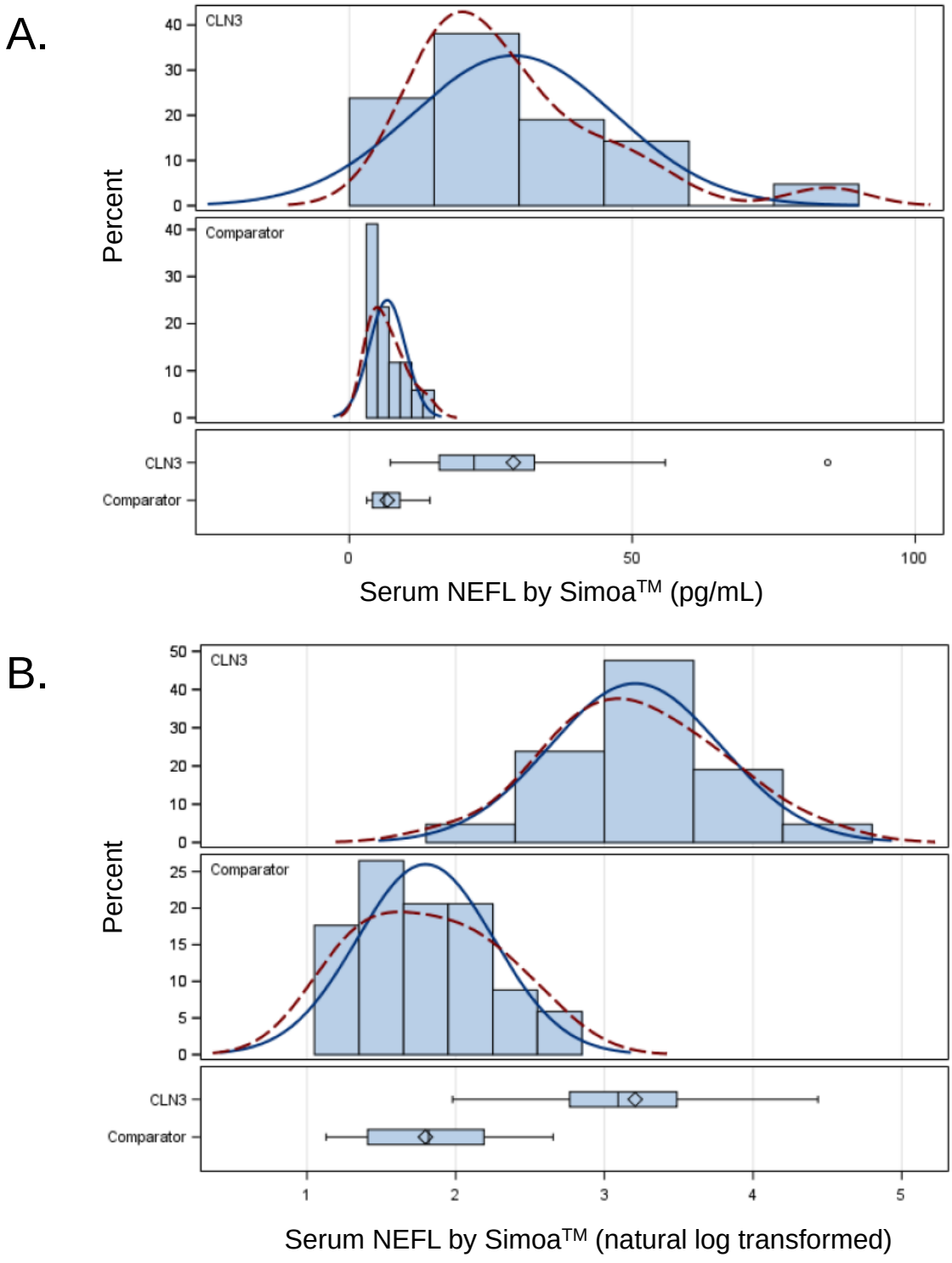
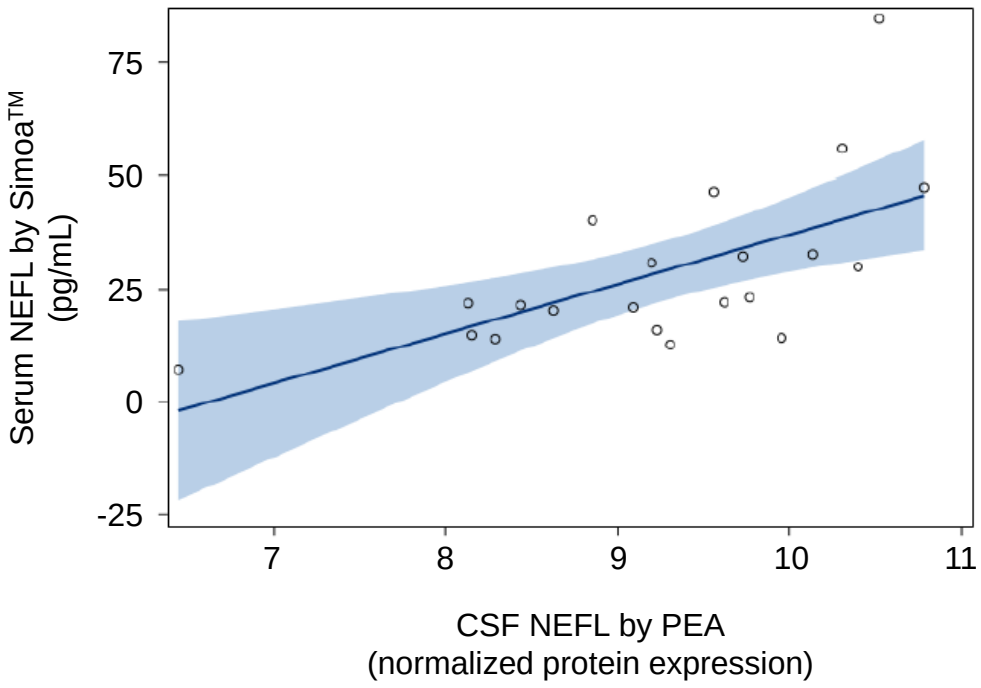


Figure S4. Serum NEFL level in samples from CLN3 versus non-CLN3 individuals. **A**. Top and middle panels: distribution of measured NEFL levels (solid line – normal; dashed line – kernel). Bottom panel: statistical values of measured NEFL levels {diamond – mean; box – median and interquartile range [IQR, Q1 (25th percentile) - Q3 (75th percentile)]; whiskers – minimum = Q1 – 1.5*IQR and maximum = Q3 + 1.5*IQR}. p<.0001. **B**. Same as A, except for NEFL values are log-transformed. NEFL: neurofilament light chain.

A.



B.

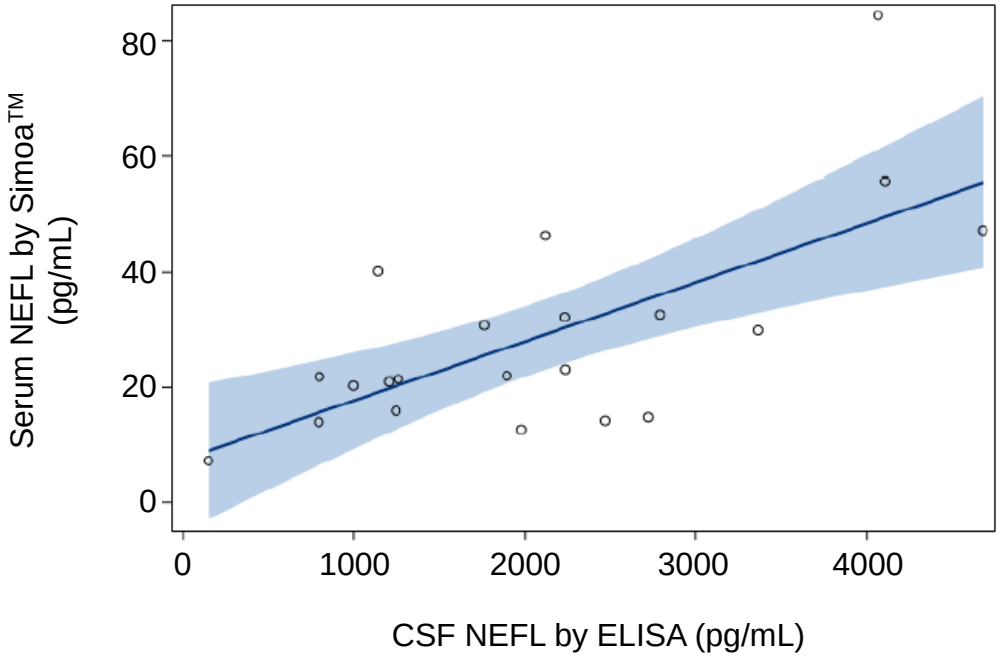


Figure S5. Correlation of NEFL levels in serum and CSF. **A**. Serum NEFL measured by Simoa™ versus CSF NEFL measured by PEA. N=21. r_p=0.75; *p*<.0001. **B**. Serum NEFL measured by Simoa™ versus CSF NEFL measured by ELISA. N=21. r_p=0.83; p<.0001 Shaded area: 95% confidence interval. CSF: cerebrospinal fluid. NEFL: neurofilament light chain. PEA: proximal extension assay. Simoa™: single molecule array.

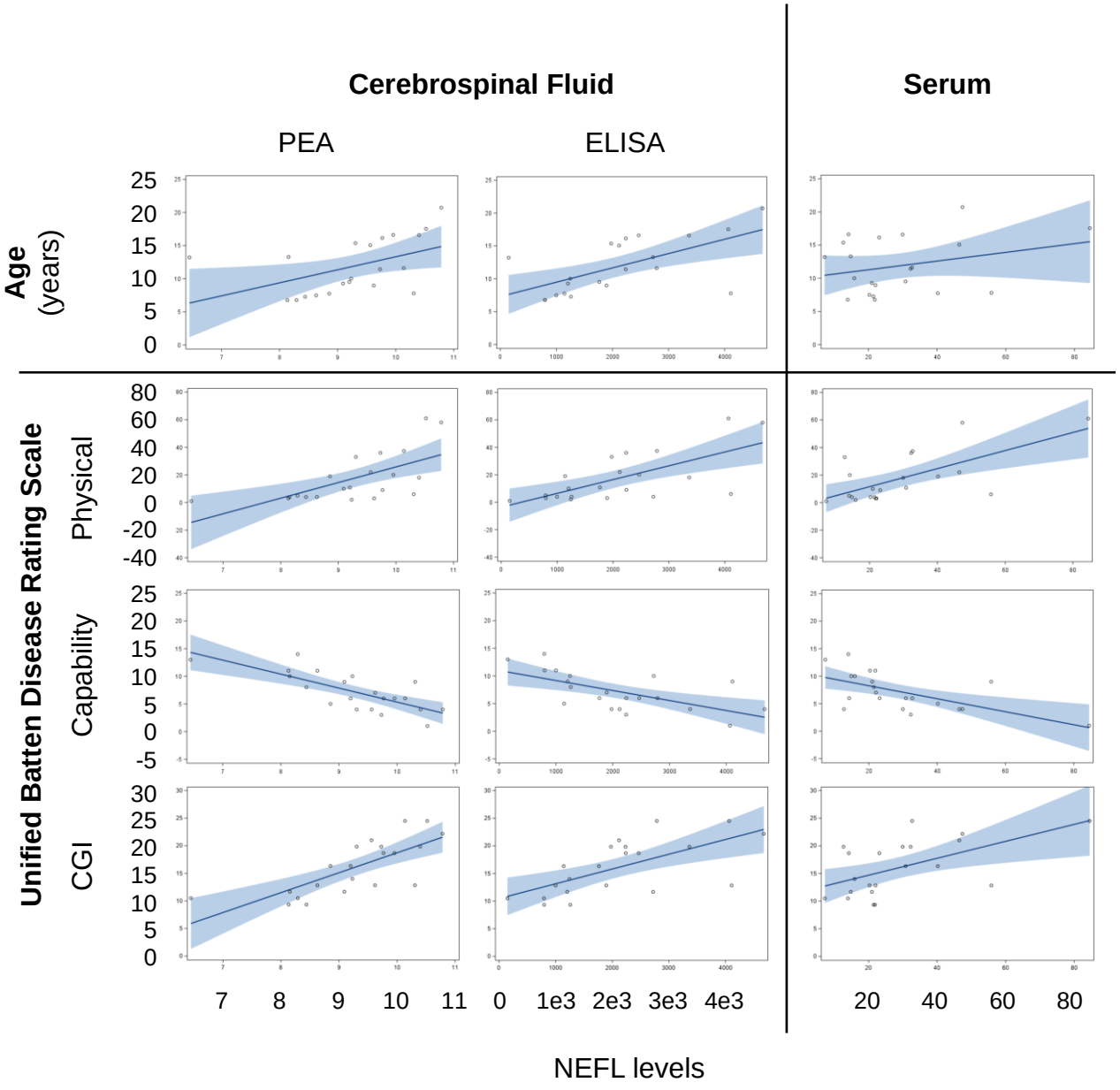


Figure S6. Correlation of NEFL levels with clinical outcome measures. Values on the x-axis are the same for all graphs in each respective column. Values on the y-axis are the same for all graphs in each respective row. Shaded area: 95% confidence interval. CGI: clinical global impression. ELISA: pg/mL. PEA: proximal extension assay (normalized protein expression). Simoa™: single molecule array (pg/mL).

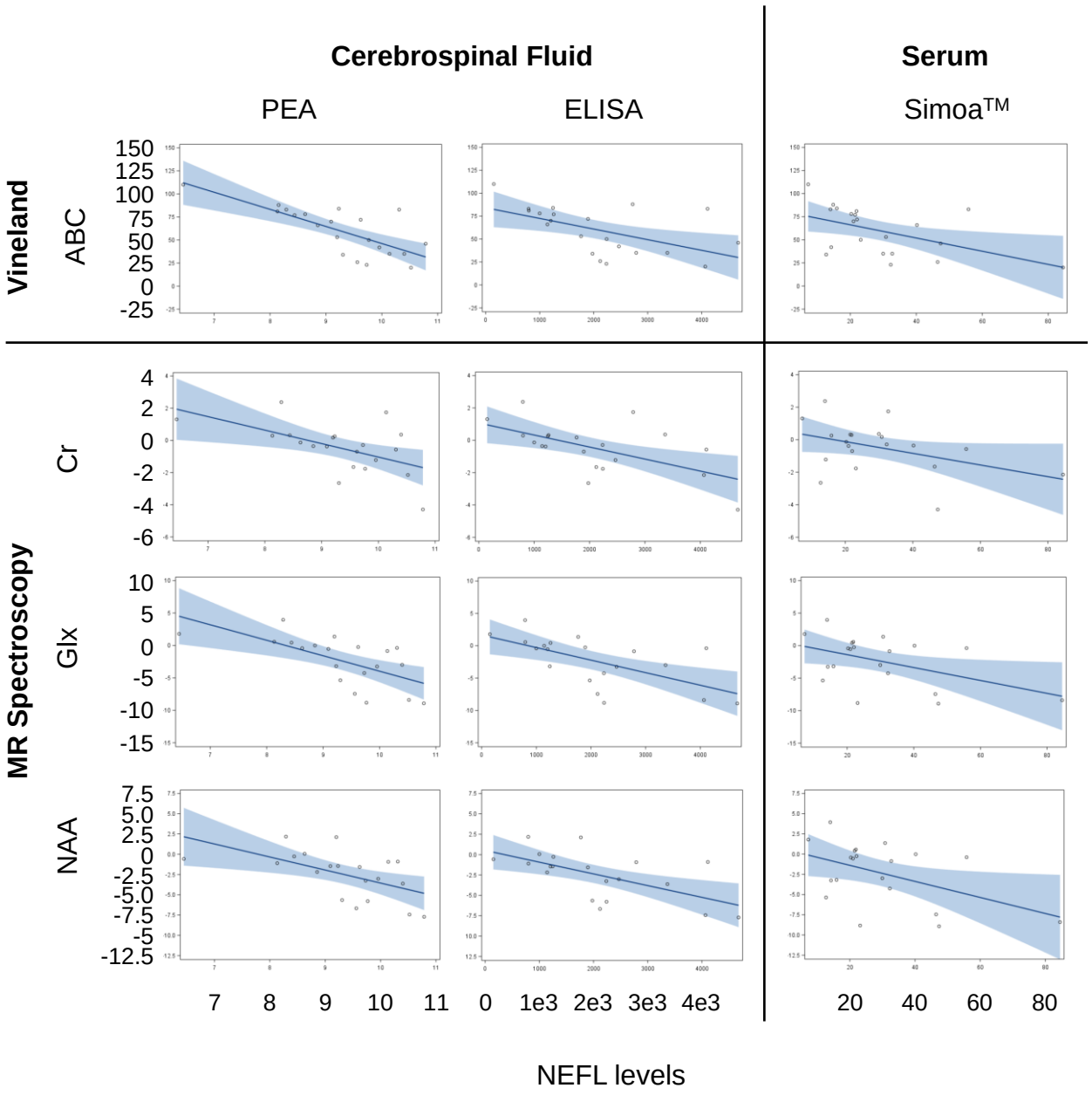


Figure S7. Correlation of NEFL levels with clinical outcome measures. Values on the x-axis are the same for all graphs in each respective column. Values on the y-axis are the same for all graphs in each respective row. Shaded area: 95% confidence interval. ABC: adaptive behavior composite. Cr: creatine. ELISA: pg/mL. Glx: glutamatergic metabolites. PEA: proximal extension assay (normalized protein expression). NAA: N-acetyl aspartate. Simoa™: single molecule array (pg/mL).

	r_p	95% confidence interval		*p* value	
		Lower	Upper	Raw	Adjusted
CSF by PEA					
Weighted	0.74	0.45	0.89	0.0001	0.0011
Overall	0.67	0.34	0.86	0.0004	0.0036
CSF by ELISA					
Weighted	0.64	0.29	0.84	0.0001	0.0011
Overall	0.58	0.19	0.81	0.0044	0.027
Serum by Simoa™					
Weighted	0.55	0.15	0.79	0.0073	0.066
Overall	0.53	0.13	0.78	0.010	0.092

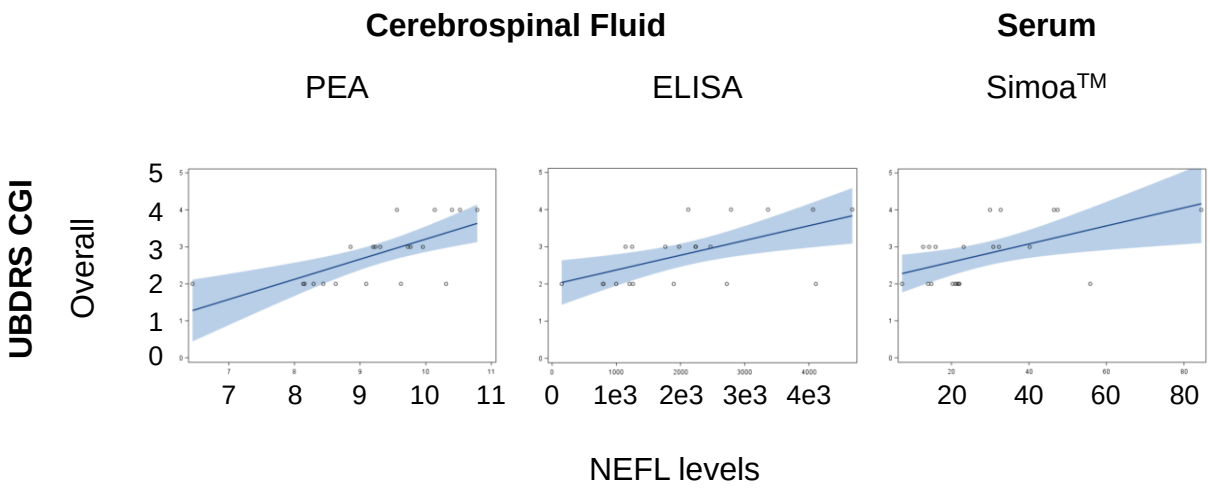


Figure S8. Correlation of NEFL levels with UBDRS CGI weighted and single-item overall scores. Upper panel table lists comparison values for both correlation methods, as prior publications **(Supplemental References**) only used single-item CGI scores**.** Lower panel graphs depict correlation of NEFL levels with UBDRS single-item overall CGI. Graphs of NEFL levels versus UBDRS weighted CGI are depicted in **Figure S6**. Shaded area: 95% confidence interval. CGI: clinical global impression. ELISA: pg/mL. PEA: proximal extension assay (normalized protein expression). UBDRS: Unified Batten Disease Rating Scale. r_p: correlation coefficient. Simoa™: single molecule array (pg/mL).