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Supplemental information

An empirical pipeline for personalized

diagnosis of Lafora disease mutations

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Supplemental Figures



Figure S1. Stability, carbohydrate binding, and specific phosphatase activity of WT and mutant laforin, related to Figure 2. (a) Melting profiles of WT laforin and mutants in the absence of substrate are shown in black. Melting profiles in the presence of 10 mM DP7 are shown in blue. Data are representative of 3 replicates. (b) Absolute T_m for WT and mutants without substrate and in the presence of 10 mM DP7 or 10 mM DP24. For mutants with biphasic melting, only the T_m corresponding to the first transition is shown. (c) Specific activity toward glycogen of WT and mutant laforin. (d) Specific activity toward C3-P and C6-P starch substrates of WT and mutant laforin. In (b) through (d) bar graphs represent the average of triplicate reactions \pm SD.



Figure S2. HDX peptide coverage of selected laforin mutants, related to Figure 4. Sequence coverage of WT laforin by pepsin-digested peptides. In all 4 mutants, 100% of residues 2 – 228 were covered by at least one peptide. A total of 148 high-quality peptides were identified. Digestion patterns of mutants were almost identical to WT with the following exceptions: R91P lacked 11 peptides (orange), P211L lacked 8 peptides (green), and G279C lacked 1 peptide (blue). F321C contained all peptides.



Figure S3. Pairwise correlation plots of all laforin biochemical and functional measurements, related to Figure 5 and Table S1. Nonparametric spearman correlations were used to compare laforin properties. Correlation coefficient (r) and p-values are in Table S1. Datapoints are color coded by structural group: WT (black); CBM (red); CBM-DSP domain interface (yellow); DSP domain (green); dimer interface (purple).

		Stability (T _m)	Glucan specificity (ΔΔT _m)	Glycogen specific activity	C3-P specific activity	C6-P specific activity	Malin binding (β-gal activity)	PTG binding (β-gal activity)
Stability (T _m)	Spearman r	1	-0.2955	-0.005929	0.2141	0.09289	0.6087	0.499
(•)	P (two- tailed)		0.1711	0.9786	0.3266	0.6734	0.0021	0.0154
	P value summary		ns	ns	ns	ns	**	*
Glucan specificty	Spearman r	-0.2955	1	0.165	0.2195	0.414	-0.417	-0.1433
(ΔΔΤ _m)	P (two- tailed)	0.1711		0.4518	0.3142	0.0495	0.0478	0.5143
	P value summary	ns		ns	ns	*	*	ns
Glycogen specific	Spearman r	-0.005929	0.165	1	0.6507	0.6897	0.01087	0.3646
activity	P (two- 0.9786 tailed)		0.4518		0.0008	0.0003	0.9607	0.0872
	P value summary	ns	ns		***	***	ns	ns
C3-P specific	Spearman r	0.2141	0.2195	0.6507	1	0.9177	0.1637	0.3051
activity	P (two- tailed)	0.3266	0.3142	0.0008		< 0.0001	0.4556	0.1569
	P value summary	ns	ns	***		****	ns	ns
C6-P specific	Spearman r	0.09289	0.414	0.6897	0.9177	1	0.03656	0.1789
activity	P (two- tailed)	0.6734	0.0495	0.0003	< 0.0001		0.8685	0.4142
	P value summary	ns	*	***	****		ns	ns
Malin binding (β-	Spearman r	0.6087	-0.417	0.01087	0.1637	0.03656	1	0.6462
gal activity)	P (two- tailed)	0.0021	0.0478	0.9607	0.4556	0.8685		0.0009
	P value summary	**	*	ns	ns	ns		***
PTG bindina (ß-	Spearman r	0.499	-0.1433	0.3646	0.3051	0.1789	0.6462	1
gal activity)	P (two- tailed)	0.0154	0.5143	0.0872	0.1569	0.4142	0.0009	
	P value summary	*	ns	ns	ns	ns	***	

Table S1. Statistical results from pairwise analysis of properties of laforin mutants, related toFigures 5 and S3. Analyses were performed using nonparametric Spearman correlations in PrismGraphpad software Spearman correlation coefficients (r), p-values, and p-value summaries areshown (* $p \le 0.05$, ** $p \le 0.01$, *** $p \le 0.001$, *** $p \le 0.0001$).

Supplemental Data

Data S1. **Ribbon maps of HDX results, related to Figure 4.** Deuteration level for WT laforin and LD mutants.

Data S2. **Difference maps of HDX results, related to Figure 4.** Change in deuteration in LD mutants compared to WT laforin. Blue indicates regions with reduced exchange in mutant, red indicates increased exchange in mutant.

Data S1



Laforin R91P



Laforin P211L



Data S1



Laforin F321C



Data S2

Change in Deuteration												
-50%	0	50%										

Laforin R91P



Laforin P211L

	1						.41			61			81.				101			
	MRFRF	GVVVPI	PAVAGARP	ELLVVGSI	RPELGRWE	PRGAVRI	LRPAGTA	AGDGAL	ALQEPG	LWLGEVE	LAAEEA	AQDGAE	PGRVDTI	FWYKFI	KREPGO	SELSWE(GNGPH	HDRCCT	YNENNL	VDG
10s																				
100s																				
1000s																				
100005																				
100000s																				
	121			141			161						201				221			
	VYCLP	TCHWT	ATCHTNE	MKHTTDE	ENTACHO	AMHYSR	TT. DNTWT.	SCPRO	VEHVTT	KT.KHET.C	TTAVMN	FOTEND	VONSS	CONRYE	FDMTD	TMTKL	VREEC	TAYTWM	DTDDMS	TEG
100	VICIDE	1011111	orte Otte Here		i i i i i i i i i i i i i i i i i i i	man a bitt	LIFUTUD	BROENS	*			e grand.		JOINTE	LETILE L				E LE DEID	120
100-																				
1005																				
1000s																				
10000s																				
100000s																				
	241			261			.281						321							
	RVQML	PQAVCI	LLHALLEK	GHIVYVH	CNAGVGRS	TAAVCGI	VLQYVMG	WNLRKV	QYFLMA	KRPAVY1	DEEALA	RAQEDFI	FQKFGK	VRSSVC	SL					
10s																				
100s																				
10005																				
100000																				
1000000																				
1000008																				

Change in Deuteration												
-50%	0	50%										

Laforin G279C



Laforin F321C

10s 100s 1000s 10000s 100000s	1 MRFRF	GVVVP	PAVAGARI	PELLVVGSR	PELGRWEPF	GAVRLRPA	GTAAGDGA	LALQEPO	61 GLWLGEVEL2	AAEEAAQDG	AEPGRVDTF	WYKFLKREF	GGELSWEGN	1	NENNLVDG
10s 100s 1000s 10000s 100000s	121 VYCLP	PIGHWI	EATGHTNE	141 MKHTTDFY	FNIAGHQAM	161 MHYSRILPN	IWLGSCPR	QVEHVTI	181 IKLKHELGI?	TAVMNFQTE	201. WDIVQNSSG	CNRYPEPMI	PDTMIKLYR	1 EEGLAYIWME	PTPDMSTEG
10s 100s 1000s 10000s 100000s	241 RVQML	PQAVC	LLHALLEP	261 GHIVYVHC	NAGVGRSTA	281 AVCGWLQY	VMGWNLRK	VQYFLMA	301	EEALARAQE	321. DFFQKFGKV	RSSVCSL			