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Supplemental information

An empirical pipeline for personalized diagnosis of Lafora disease mutations

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Supplemental Figures

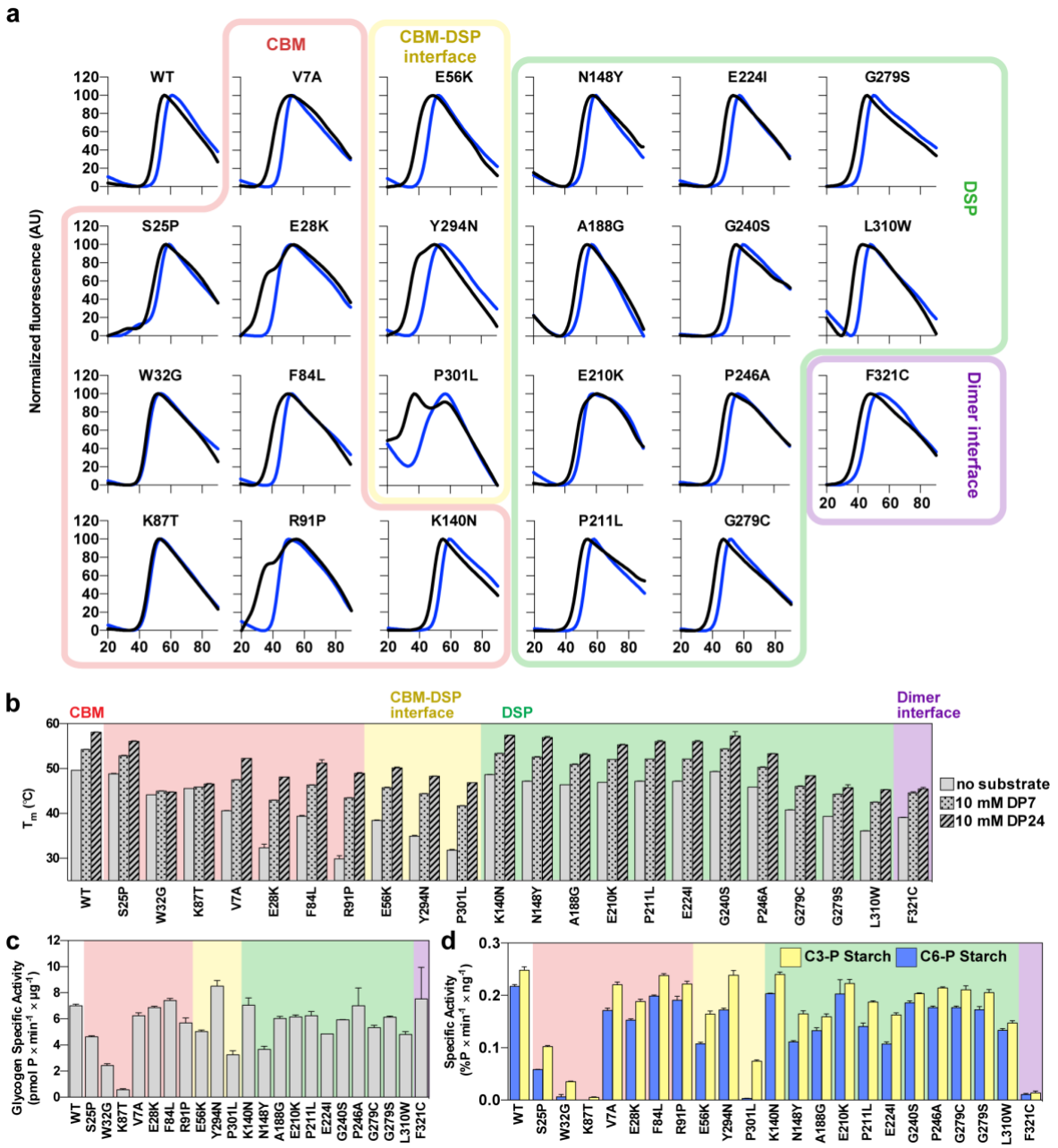


Figure S1. Stability, carbohydrate binding, and specific phosphatase activity of WT and mutant laforin, related to Figure 2. (a) Melting profiles of WT laforin and mutants in the absence of substrate are shown in black. Melting profiles in the presence of 10 mM DP7 are shown in blue. Data are representative 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 ± SD.

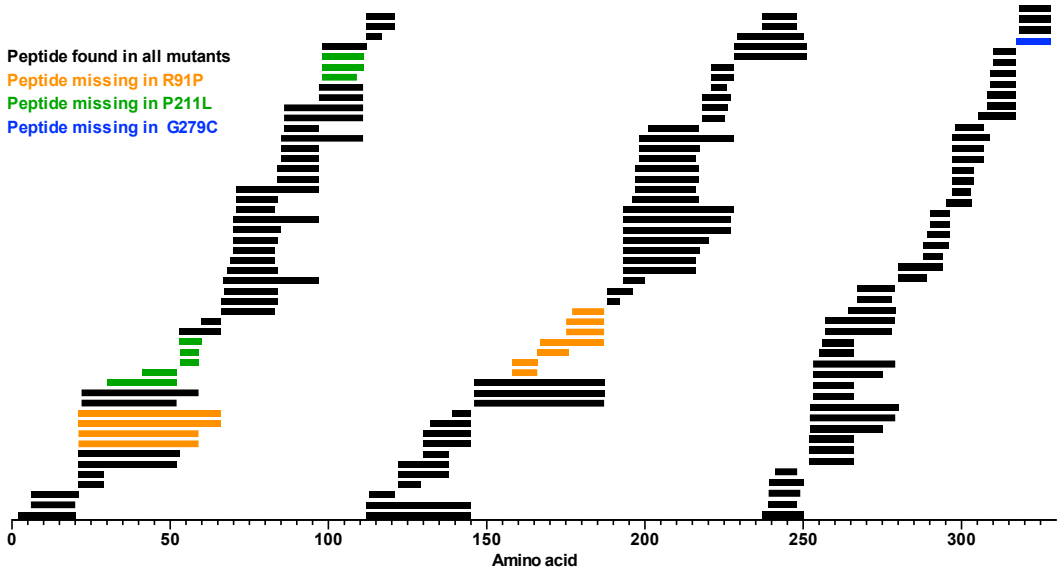


Figure S2. HDX peptide coverage of selected laforin mutants, related to Figure 4. Sequence coverage of WT laforin by pepsin-digested peptides. In all 4 mutants, 100% of residues 2 – 228 were covered by at least one peptide. A total of 148 high-quality peptides were identified. Digestion patterns of mutants 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.

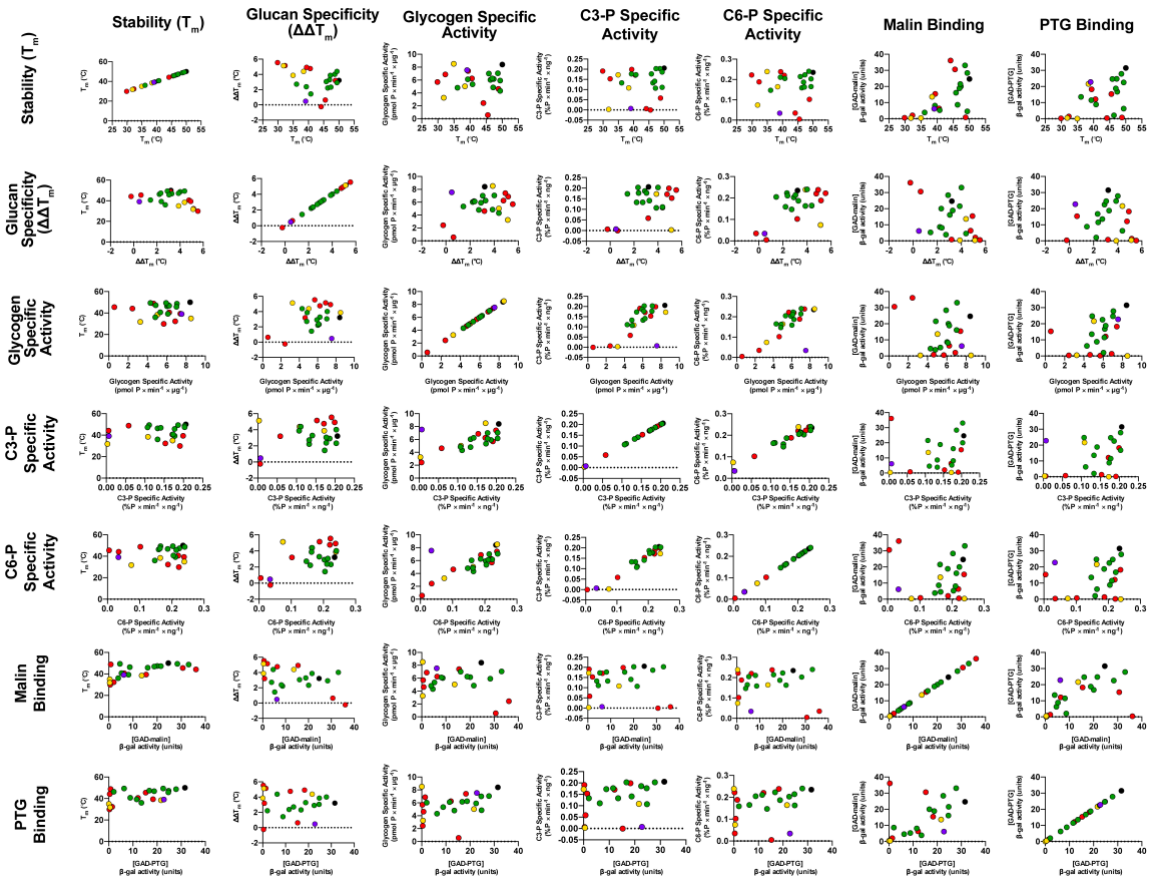


Figure S3. Pairwise correlation plots of all laforin biochemical and functional measurements, related to Figure 5 and Table S1. Nonparametric spearman correlations were used to compare laforin properties. Correlation coefficient (r) and p-values are in Table S1. Datapoints are color coded by structural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

		Stability (T_m)	Glucan specificity ($\Delta\Delta T_m$)	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 ($\Delta\Delta T_m$)	Spearman r	-0.2955	1	0.165	0.2195	0.414	-0.417	-0.1433
	P (two-tailed)	0.1711		0.4518	0.3142	0.0495	0.0478	0.5143
	P value summary	ns		ns	ns	*	*	ns
Glycogen specific activity	Spearman r	-0.005929	0.165	1	0.6507	0.6897	0.01087	0.3646
	P (two-tailed)	0.9786	0.4518		0.0008	0.0003	0.9607	0.0872
	P value summary	ns	ns		***	***	ns	ns
C3-P specific activity	Spearman r	0.2141	0.2195	0.6507	1	0.9177	0.1637	0.3051
	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 activity	Spearman r	0.09289	0.414	0.6897	0.9177	1	0.03656	0.1789
	P (two-tailed)	0.6734	0.0495	0.0003	< 0.0001		0.8685	0.4142
	P value summary	ns	*	***	****		ns	ns
Malin binding (β-gal activity)	Spearman r	0.6087	-0.417	0.01087	0.1637	0.03656	1	0.6462
	P (two-tailed)	0.0021	0.0478	0.9607	0.4556	0.8685		0.0009
	P value summary	**	*	ns	ns	ns		***
PTG binding (β-gal activity)	Spearman r	0.499	-0.1433	0.3646	0.3051	0.1789	0.6462	1
	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 to Figures 5 and S3. Analyses were performed using nonparametric Spearman correlations in Prism Graphpad software Spearman correlation coefficients (r), p-values, and p-value summaries are shown (* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, *** $p \leq 0.0001$).

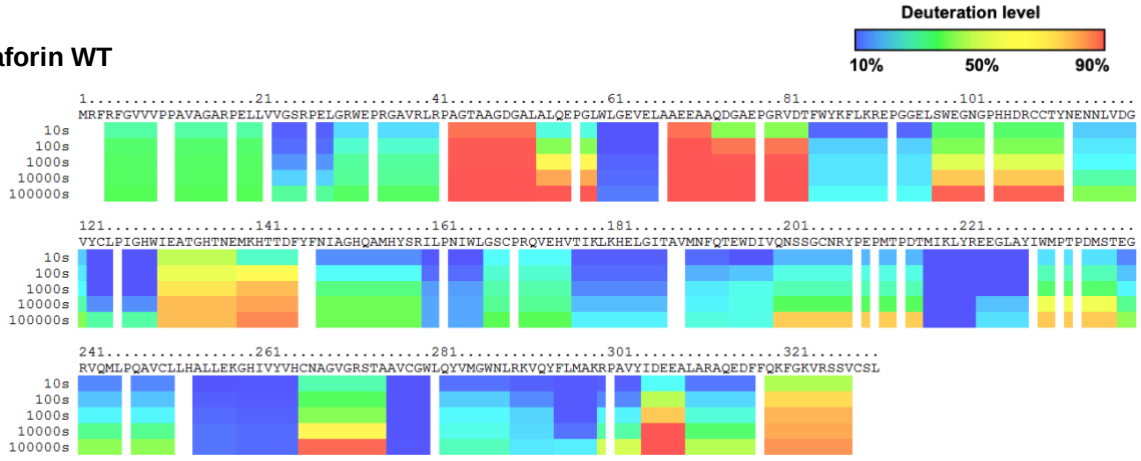
Supplemental Data

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

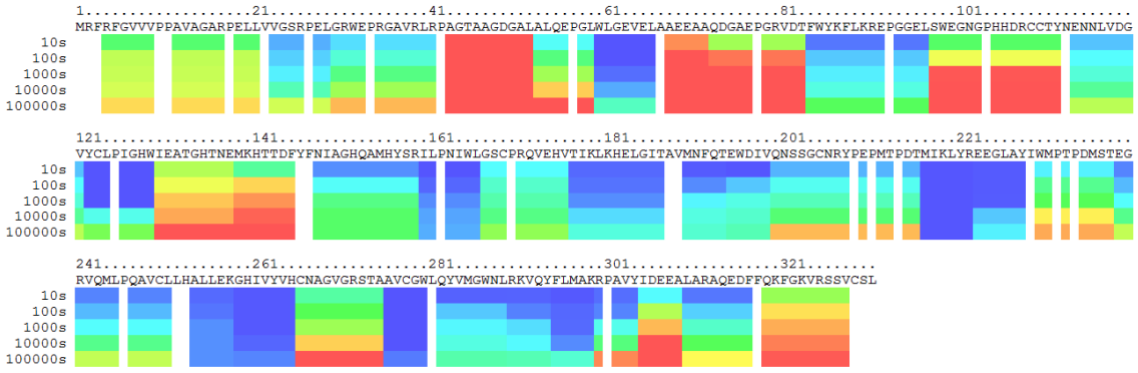
Data S2. **Difference maps of HDX results, related to Figure 4.** Change in deuteration in LD mutants compared to WT laforin. Blue indicates regions with reduced exchange in mutant, red indicates increased exchange in mutant.

Data S1

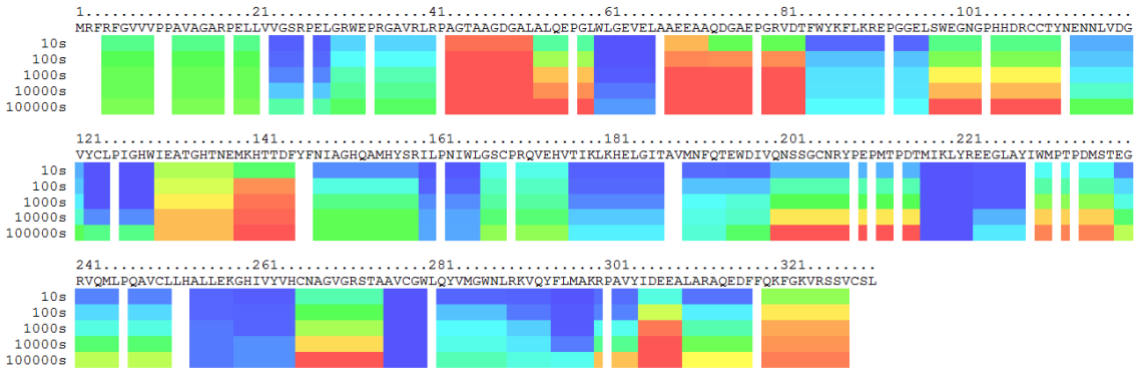
Laforin WT



Laforin R91P

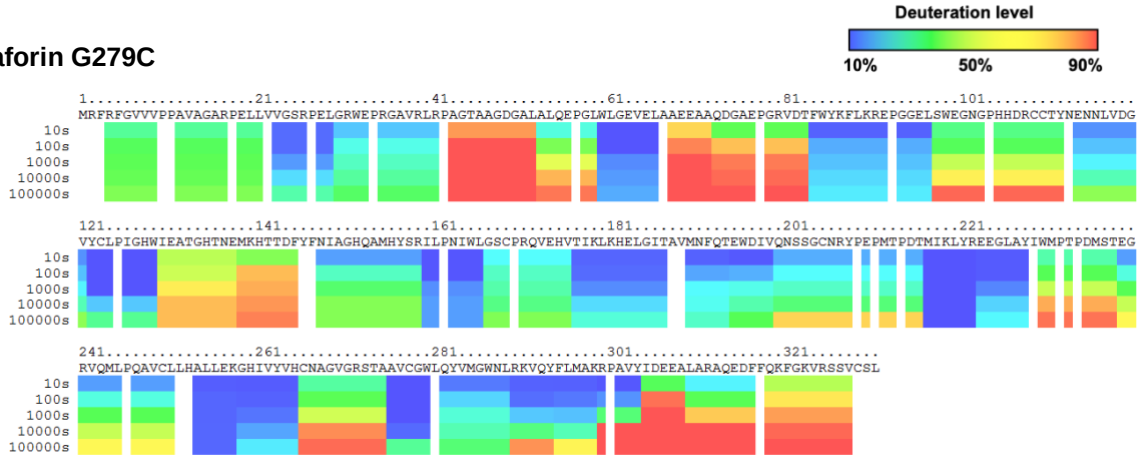


Laforin P211L

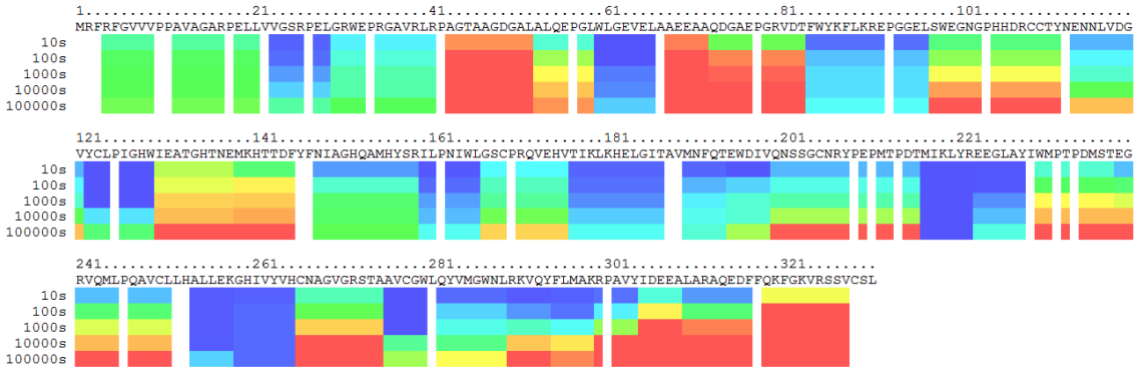


Data S1

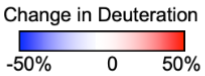
Laforin G279C



Laforin F321C



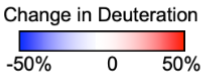
Data S2



Laforin R91P

Laforin P211L

Data S2



Laforin G279C

Laforin F321C