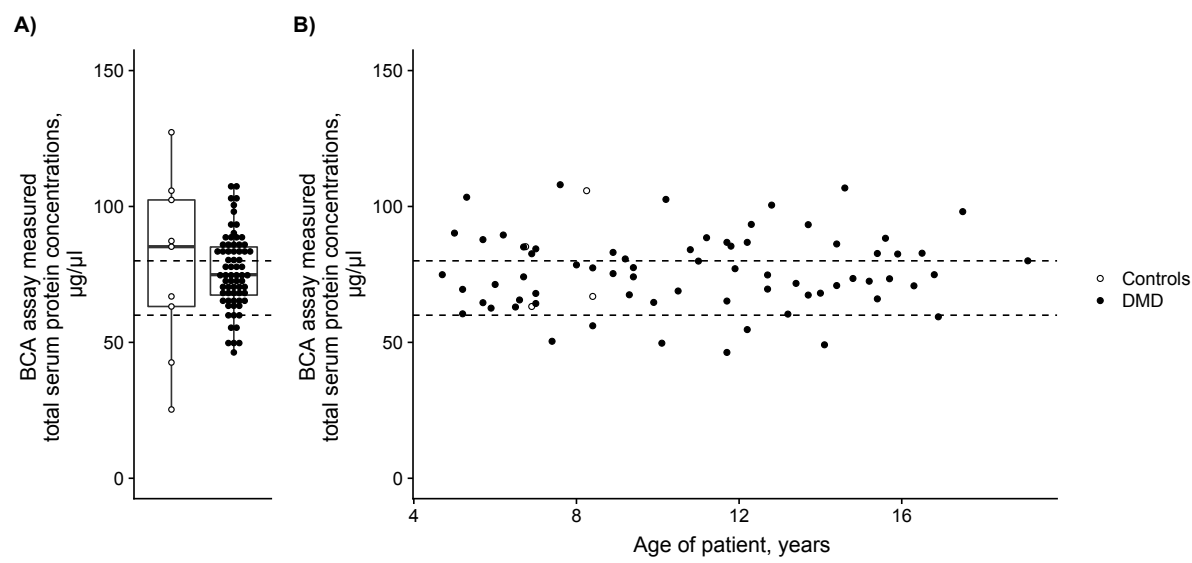


Orthogonal proteomics methods warrants development of Duchenne Muscular
Dystrophy biomarkers

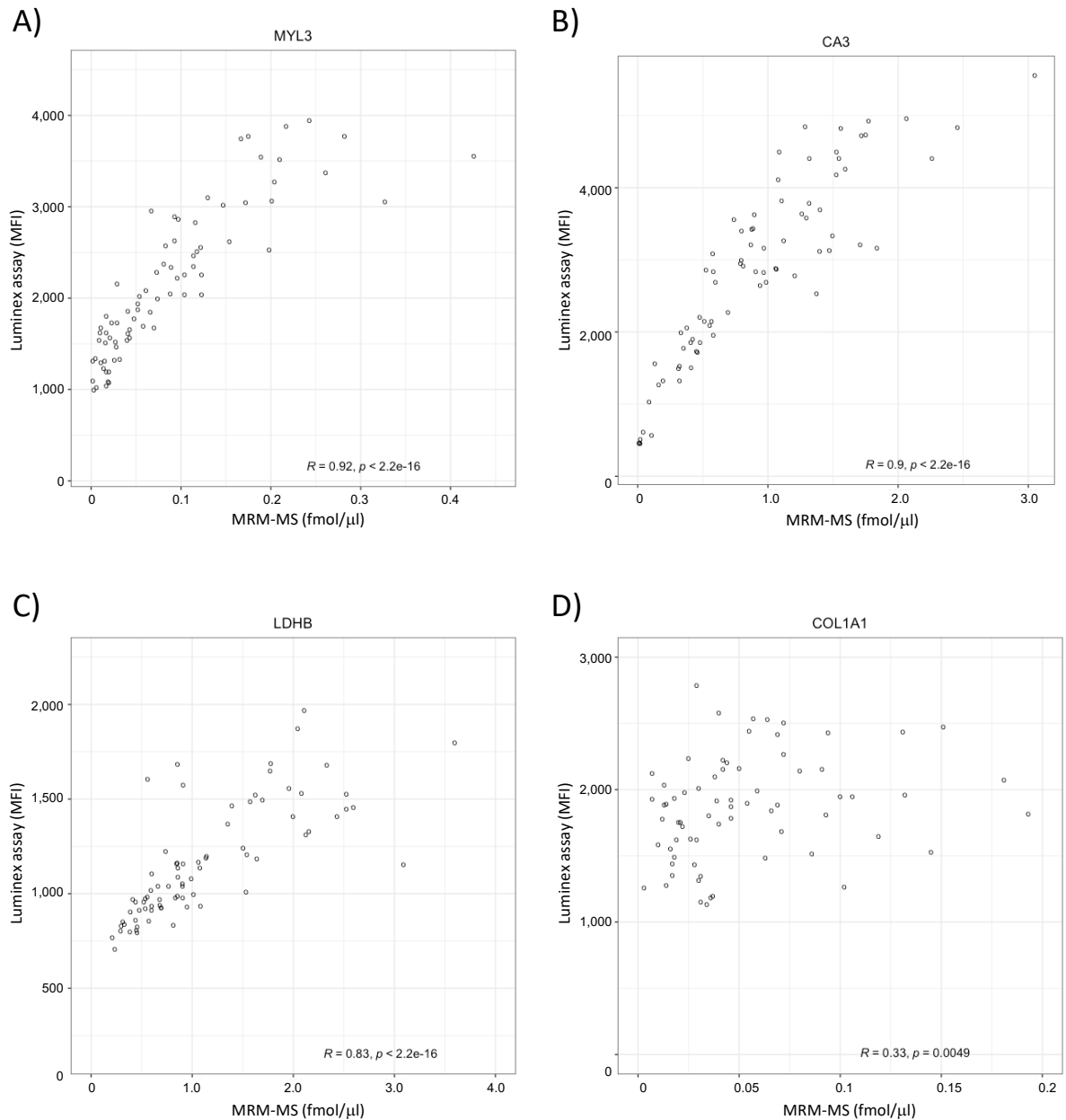
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Supplementary figures and tables

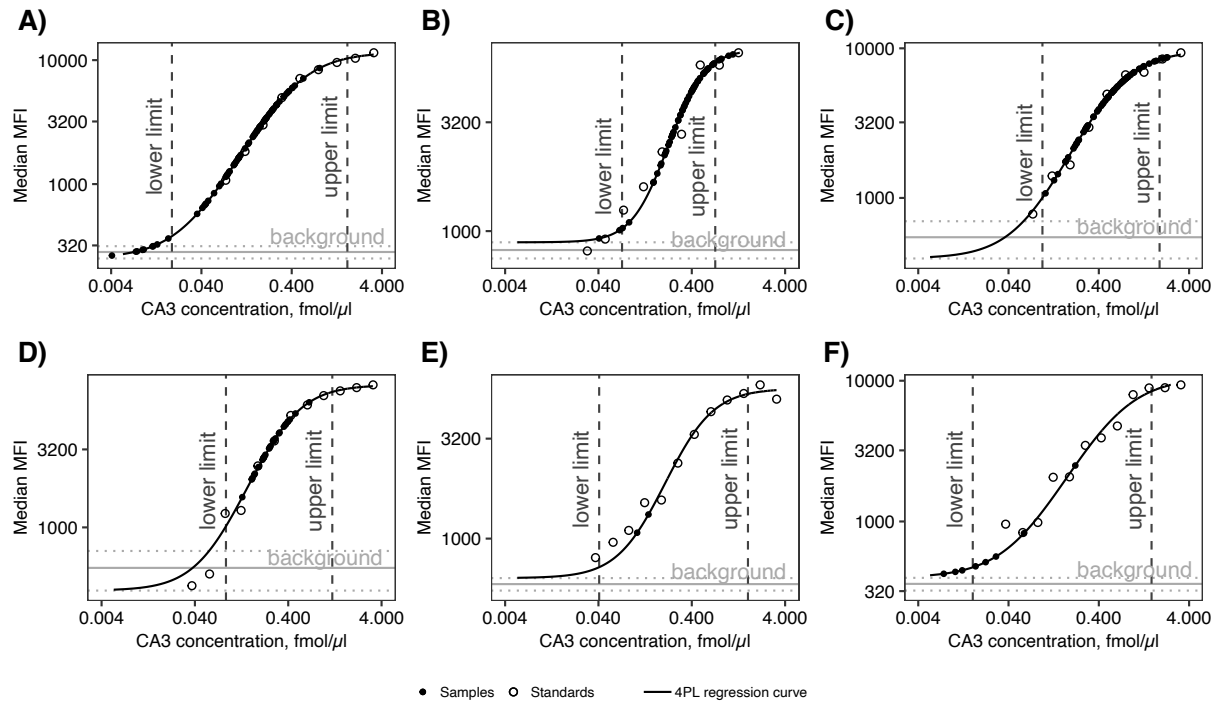
Supplementary figure 1. Total protein concentration in serum samples.



Supplementary figure 2. Spearman correlation between biomarker abundance measured using the suspension bead array platform and quantification by MRM-MS. Abundance is measured as Median Fluorescent Intensities for MYL3 (A), CA3 (B), LDHB (C) and COL1A1 (D) whereas protein concentrations are measured in fmol/ μ l. Spearman's correlation and correlation coefficients are shown in graphs.



Supplementary figure 3. Standard curves and quantified samples for each replicate of the carbonic anhydrase 3 sandwich immunoassay. Standard diluents are denoted with open circles, four-parametric log-logistic regression curve (4PL) model shown with a line, and calculated CA3 concentrations for each quantifiable sample denoted with closed circles.



Curve	No. Quantified	Sample Dilution	Highest CA3 standard diluent, fmol/μl	Lowest CA3 standard diluent, fmol/μl	Number of standard diluents	Background MFI	Bead stock batch	Bead stock dilution	Biotinylated antibody batch	Biotinylated antibody dilution	Diluted SAPE batch	Dilution guinea-pig serum	Batch standard diluents
	Samples												
A)	71 DMD+CONT	~500x	3,2	0,068	9 283 ± 32	A	8x	A	150x	A	200x	A	
B)	53 DMD+CONT	~200x	1,3	0,003	9 817 ± 70	B	7x	B	120x	B	200x	A	
C)	71 DMD	~200x	3,2	0,068	9 546 ± 152	B	12x	B	120x	B	200x	A	
D)	29 DMD with CA3 > 130 fmol/μl	~500x	3,2	0,003	12 547 ± 157	C	7x	C	150x	C	500x	B	
E)	2 CONT	~100x	3,2	0,003	12 589 ±43	C	7x	C	150x	C	100x	C	
F)	5 CONT	~50x	3,2	0,003	12 360 ± 37	D	8x	D	150x	D	50x	D	