

SUPPLEMENTARY INFORMATION

Supplementary Tables and Figures supporting the results of the study.

Supplementary Table S1. Summary data of calibration parameters for LLQV and LEMP peptides.

	Slope	Intercept	R ²
LLQV, <i>n</i> = 6			
Mean	0.00249	0.0155	0.9953
SD	0.000253	0.00690	0.0023
%CV	10.2	44.5	0.2
LEMP, <i>n</i> = 5			
Mean	0.00273	0.0211	0.9925
SD	0.000407	0.00485	0.0049
%CV	14.9	23.0	0.5

%CV, percent coefficient of variation; *n*, number of runs; SD, standard deviation. Data are from the clinical sample runs only.

Supplementary Table S2. Calibration parameters for LLQV and LEMP peptides.

Peptide	Month ^a	Curve number	Slope	Intercept	R ²	LLOQ, pM	ULOQ, pM
LLQV	1	11	0.00221	0.00651	0.9964	20.0	3330
	2	15	0.00230	0.0100	0.9918	20.0	3330
	9	17	0.00265	0.0124	0.9940	20.0	3330
	12	19	0.00284	0.0224	0.9949	20.0	3330
	13	21	0.00262	0.0232	0.9986	20.0	3330
	21	23	0.00229	0.0187	0.9960	20.0	3330
LEMP	1	11	0.00229	0.0274	0.9887	20.0	3330
	2	15	0.00247	0.0191	0.9890	20.0	3330
	9	17	0.00272	0.0246	0.9889	20.0	3330
	12	19	0.00336	0.0152	0.9980	20.0	3330
	21	23	0.00280	0.0193	0.9978	20.0	3330

Data are shown for each individual run. For summary data see main body of the text.

^a Taken from the start of the validation runs. Data are from the clinical sample runs only.

LLOQ, lower limit of quantification; ULOQ, upper limit of quantification.

Supplementary Table S3. Summary of back-calculated calibration standards for LLQV and LEMP peptides.

	Calibration standard, pM									
	20.0	26.0	52.1	104	208	417	833	1670	2500	3330
LLQV										
<i>n</i>	11	12	11	12	12	12	12	12	12	12
Inter-run mean, pM	19.8	26.3	51.9	106	205	425	851	1640	2470	3270
Inter-run SD	1.54	2.25	2.82	3.35	10.8	14.3	41.8	104	112	126
Inter-run %CV	7.8	8.6	5.4	3.2	5.3	3.4	4.9	6.3	4.5	3.9
Inter-run %RE	-1.0	1.2	-0.4	1.9	-1.4	1.9	2.2	-1.6	-1.2	-1.9
LEMP										
<i>n</i>	9	7	9	10	10	10	10	10	10	10
Inter-run mean, pM	19.2	27.4	51.8	107	212	422	843	1610	2460	3270
Inter-run SD	1.77	3.07	3.37	4.94	20.2	16.3	52.1	117	119	127
Inter-run %CV	9.2	11.2	6.5	4.6	9.5	3.9	6.2	7.3	4.8	3.9
Inter-run %RE	-4.0	5.4	-0.6	2.9	1.9	1.2	1.2	-3.6	-1.6	-1.8

%CV, percent coefficient of variation; %RE, percent relative error; CS=calibration standard; *n*, number of samples; SD, standard deviation. Data are from the clinical sample runs only.

Supplementary Table S4. Back-calculated calibration standards for LLQV and LEMP peptides.

Peptide	Month ^a	Calibration standard, pM									
		20.0	26.0	52.1	104	208	417	833	1670	2500	3330
LLQV	1	19.2	25.4	49.9	102	217	433	907	1580	2300	3310
		20.7	26.8	55.2	102	197	444	768	1670	2560	3440
	2	20.9	27.8	40.1 ^b	107	205	426	876	1360	2630	3270
		16.5	27.9	54.0	109	186	430	798	1730	2600	3340
	9	10.7 ^c	28.5	57.5	104	197	452	875	1700	2310	3300
		18.5	24.6	51.9	104	224	408	900	1550	2360	3040
	12	19.9	30.3	49.0	110	212	422	889	1650	2500	3110
		19.0	23.4	53.0	108	211	407	840	1700	2380	3130
	13	18.7	25.7	52.3	105	205	413	818	1730	2480	3360
		21.6	25.9	50.5	107	215	412	852	1640	2500	3210
	21	21.4	22.3	48.4	99.9	197	436	841	1690	2570	3380
		20.9	27.4	49.7	110	199	422	849	1680	2490	3400
LEMP	1	18.1	36.4 ^b	124 ^b	111	246	435	911	1480	2300	3190
		20.9	53.5 ^b	54.3	99.5	245	420	714	1560	2340	3300
	2	20.1	23.4	47.1	113	210	433	863	1340	2590	3210
		18.0	31.1	56.9	112	192	418	810	1670	2620	3350
	9	7.89 ^c	29.8	54.8	102	191	458	856	1690	2330	3370
		15.4	29.9	49.6	106	221	405	869	1620	2430	3040
	12	20.8	26.8	53.3	105	213	415	869	1640	2520	3220
		19.6	23.6	52.6	109	212	402	855	1690	2390	3170
	21	19.7	27.5	48.7	101	196	421	839	1710	2590	3400
		20.4	33.7 ^b	48.6	111	196	416	840	1670	2490	3460

Data are shown for each individual run.

^a Taken from the start of the validation runs. Data are from the clinical sample runs only.

^b Relative error >20%.

^c Relative error >25%.

Supplementary Table S5. Quality control samples for LLQV and LEMP peptides.

Peptide	Month ^a	QCL (50.0 pM)	QCM1 (200 pM)	QCM2 (800 pM)	NP-3 (1470 pM)	QCH (2400 pM)
LLQV	1	49.6	195	595 ^b	1380	2290
		51.6	207	816	1530	2130
		51.5	207	788	1460	2240
		54.9	203	785	1500	2240
		—	—	—	1490	—
		—	—	—	1370	—
	2	53.5	228	878	1400	2750
		49.8	215	831	1470	2560
		50.6	195	908	1560	2640
		45.4	202	872	1470	2460
		—	—	—	1540	—
		—	—	—	1590	—
	9	50.5	181	817	1410	2410
		48.2	200	838	1160	2550
		49.5	200	771	1140	2140
		52.3	207	830	1120	2450
		—	—	—	1180	—
		—	—	—	1170	—
	12	46.4	195	818	1480	2450
		49.2	205	856	1380	2440
		42.1	178	772	1360	2380
		43.9	197	810	1370	2420
		—	—	—	1360	—
		—	—	—	1350	—
	13	49.3	206	826	1420	2370
		47.7	201	801	1360	2360
		43.6	202	777	1360	2310
		51.3	183	792	1340	2380
		—	—	—	829 ^c	—

		—	—	—	1340	—
	21	50.1	203	797	1510	2440
		52.6	232	895	1340	2510
		53.9	190	839	1430	2510
		49.5	190	857	1430	2490
		—	—	—	1420	—
		—	—	—	1530	—
LEMP	1	36.4 ^b	176	537 ^b	—	1990
		43.8	176	762	—	2060
		34.8 ^b	193	724	—	2010
		41.1	185	630 ^b	—	2170
	2	46.9	234	847	—	2740
		40.9	208	808	—	2560
		46.9	186	871	—	2600
		40.9	198	847	—	2380
	9	48.0	184	836	—	2420
		44.0	199	849	—	2610
		47.0	204	752	—	2180
		51.7	208	847	—	2540
	12	47.7	195	799	—	2480
		47.4	206	823	—	2440
		46.5	180	767	—	2440
		44.1	196	794	—	2490
	21	47.0	204	793	—	2440
		49.6	230	883	—	2500
		51.8	188	829	—	2520
		46.4	189	844	—	2490

^a Taken from the start of the validation runs. Data are from the clinical sample runs only.

^b Relative error >20%.

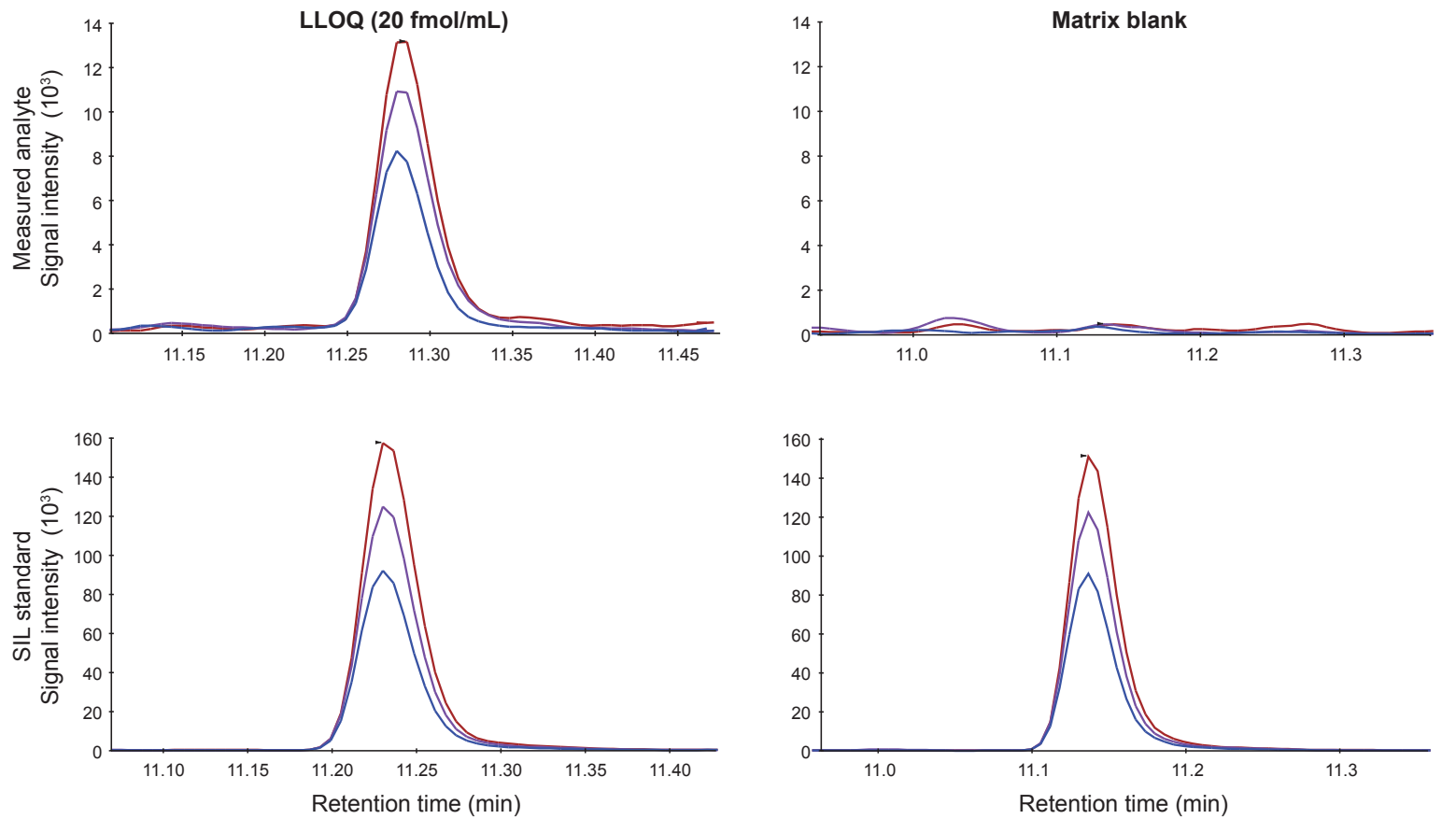
^c Relative error >25%.

NP, normal pooled; QCH, high quality control; QCL, low quality control; QCM1, medium quality control 1; QCM2, medium quality control 2.

Supplementary Figure S1. DMD skeletal muscle lysate (matrix blank) samples for LLQV and LEMP analyte signal.

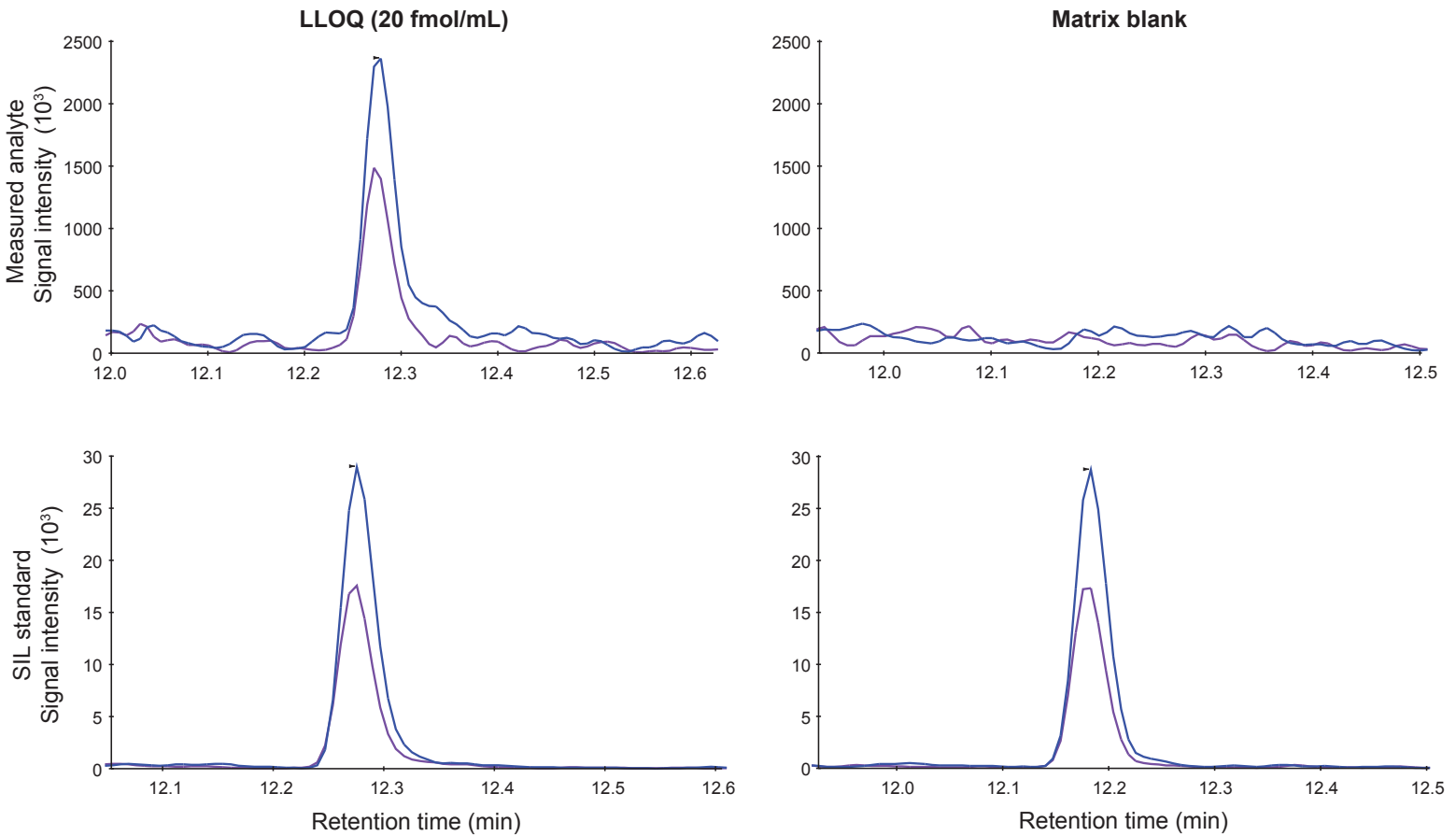
A

LLQV Peptide



B

LEMP Peptide



DMD skeletal muscle lysate samples (matrix blank) were spiked only with SIL mini-dystrophin standard and scaled to the LLOQ standard (20 fmol/mL) for comparison. Due to the high SIL amino acid incorporation efficiency into the mini-dystrophin internal standard, the matrix blank samples showed no observable signal in the light channels for either LLQV (A) or LEMP (B) peptides.

LLOQ, lower limit of quantification; SIL, stable isotope labeled.