

### Supplementary Table 1

UPLC method for the measurement of enzyme activities for 6 LSDs.

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Column temperature	40°C
Weak wash solvent	MeOH/CH <sub>3</sub> CN/IPA/0.1%aqueous formic acid
Strong wash solvent	MeOH/CH <sub>3</sub> CN/IPA/0.1%aqueous formic acid
Mobile phase	CH <sub>3</sub> CN/H <sub>2</sub> O/0.2% formic acid in H <sub>2</sub> O
Flow rate	0.3 mL/min
Injection volume	10 µL
Injection mode	Direct injection
Autosampler temperature	4°C

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LSD, lysosomal storage disorder.

**Supplementary Table 2**

Instrument parameters for the measurement of substrates, internal standards and products of 6 LSD enzyme activities using LC-MS/MS.

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Interface	ESI
Polarity	Positive
Capillary voltage	2.0 kV
Source temperature	120°C
Desolvation temperature	450°C
Flow rate of nebulizing gas	800L/h
Flow rate of drying gas	50 L/h
Analyzing mode	MRM
Scan time	0.2 s
Data format	Centroid

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**Supplementary Table 3**

Product ion, precursor ion, cone voltage and collision energy for the fragmentation of substrates, products and internal standards using LC-MS/MS.

Species	Precursor ion ( <i>m/z</i> )	Product ion ( <i>m/z</i> )	Cone (V)	Collision energy (V)
GAA-S	660.4	560.3	25	22
GAA-P	498.3	398.3	10	20
GAA-IS	503.4	403.3	10	16
GLA-S	646.4	546.3	23	19
GLA-P	484.3	384.2	22	12
GLA-IS	489.3	389.3	22	12
IDUA-S	602.3	317.2	35	14
IDUA-P	426.2	317.2	20	12
IDUA-IS	431.3	322.2	20	15
ABG-S	546.4	264.2	20	15
ABG-P	384.4	264.3	15	12
ABG-IS	391.4	271.3	15	12
ASM-S	563.4	184.0	15	30
ASM-P	398.4	264.3	15	20
ASM-IS	405.4	264.3	15	20
GALC-S	574.5	264.3	20	25
GALC-P	412.4	264.3	20	15
GALC-IS	417.4	264.3	20	18

GAA,  $\alpha$ -glucosidase; GLA,  $\alpha$ -galactosidase A ; IDUA,  $\alpha$ -L-iduronidase; ABG, glucocerebrosidase; ASM, acid sphingomyelinase; GALC, galactosylceramidase.

**Supplementary Table 4**

Retention times for the substrates, products and internal standards of assay for lysosomal enzyme activities using LC-MS/MS.

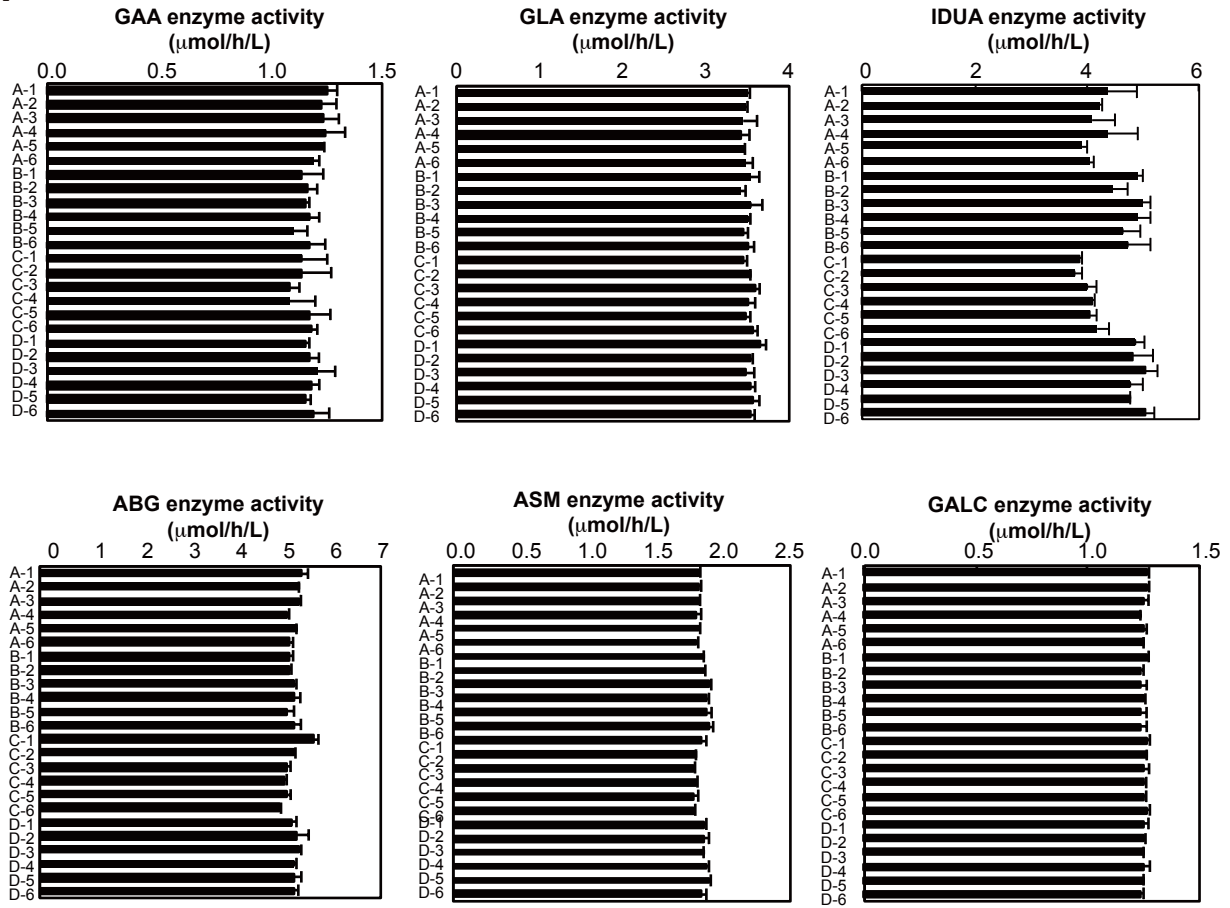
Column	Column 1 (ACQUITY BEH C18)	Column 2 (Chromolith)	Column 3 (Mono Tower C18)
Flow rate (mL/min)	0.3	0.3	0.6
Species	Retention time (min)		
GAA-S	0.63	0.74	1.16
GAA-P	0.99	1.04	1.59
GAA-IS	0.98	1.04	1.59
GLA-S	0.57	0.70	1.10
GLA-P	0.85	0.94	1.48
GLA-IS	0.86	0.94	1.48
IDUA-S	0.50	0.63	0.95
IDUA-P	0.53	0.66	1.07
IDUA-IS	0.53	0.66	1.05
ABG-S	2.73	2.36	2.81
ABG-P	3.85	3.30	3.71
ABG-IS	3.85	3.30	3.70
ASM-S	2.49	2.40	2.61
ASM-P	4.21	3.58	2.09
ASM-IS	4.20	3.58	2.10
GALC-S	3.42	2.89	3.27
GALC-P	4.56	3.84	2.80
GALC-IS	4.56	3.84	2.78

**Supplementary Table 5**Intraday and interday assay precision for multiple analyses of the CDC quality control samples.<sup>a</sup>

CDC QC	Intraday CV (%)						Interday CV (%)					
	GAA	GLA	IDUA	ABG	ASM	GALC	GAA	GLA	IDUA	ABG	ASM	GALC
High (100% whole blood)	12.1	8.3	12.5	0.6	8.9	6.8	7.4	5.5	14.1	18.0	17.3	10.5
Middle (50% whole blood)	18.2	9.6	16.5	10.1	2.9	8.9	11.9	4.0	19.8	22.1	11.1	14.7
Low (5% whole blood)	25.8	15.7	4.0	5.7	12.9	10.1	10.9	27.0	47.0	17.8	6.7	2.9
Base (0% whole blood)	52.9	36.5	18.9	9.5	12.0	7.3	17.1	38.1	56.0	19.0	4.6	7.6
Replicate ( <i>n</i> )	3	3	3	3	3	3	4	4	4	4	4	4

<sup>a</sup>Data obtained using a Chromolith column.

**A**



**B**

