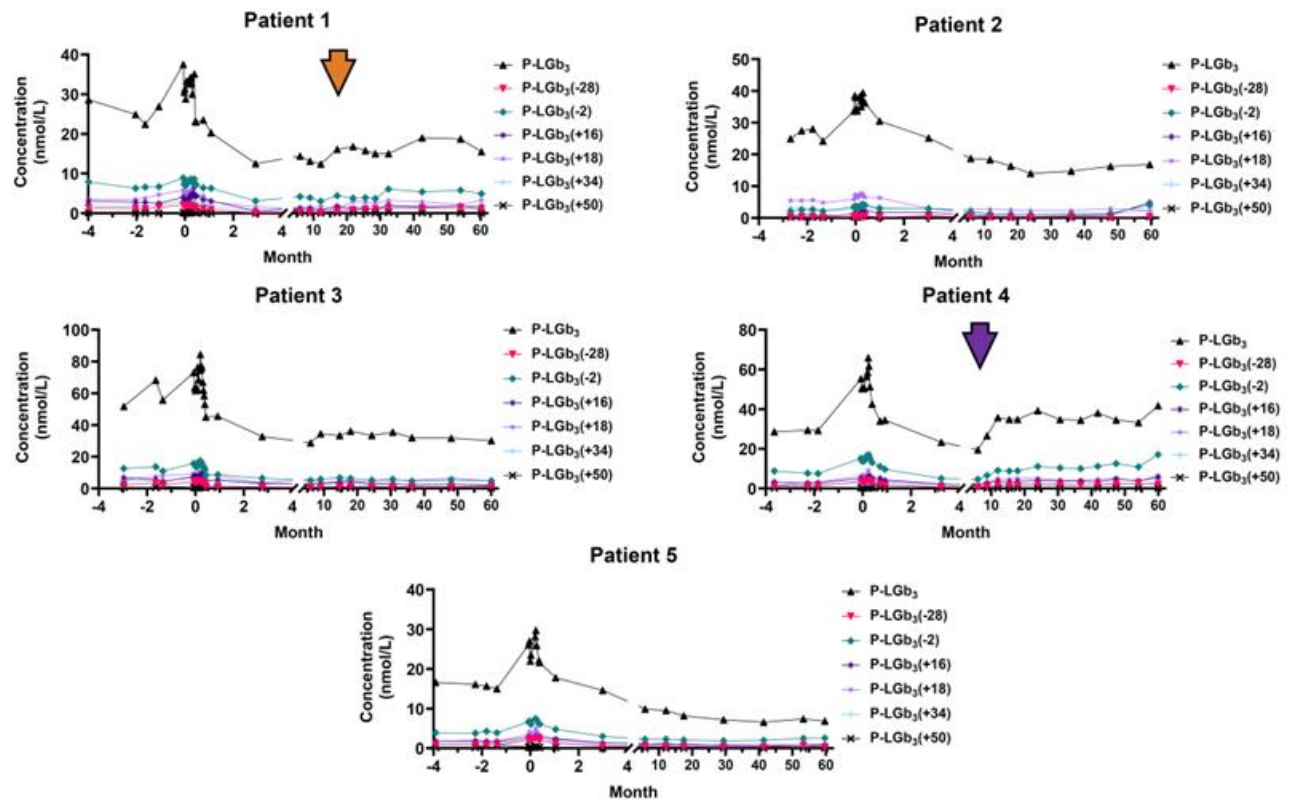
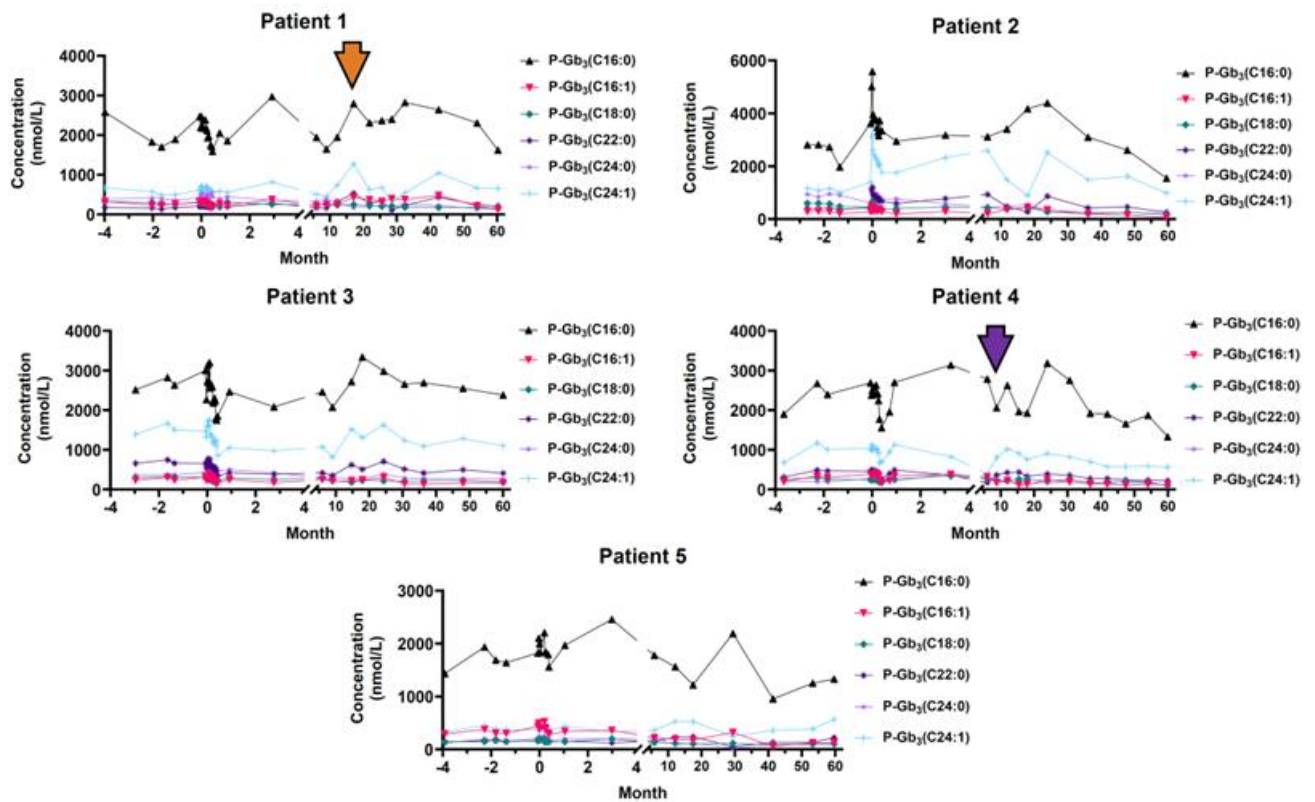


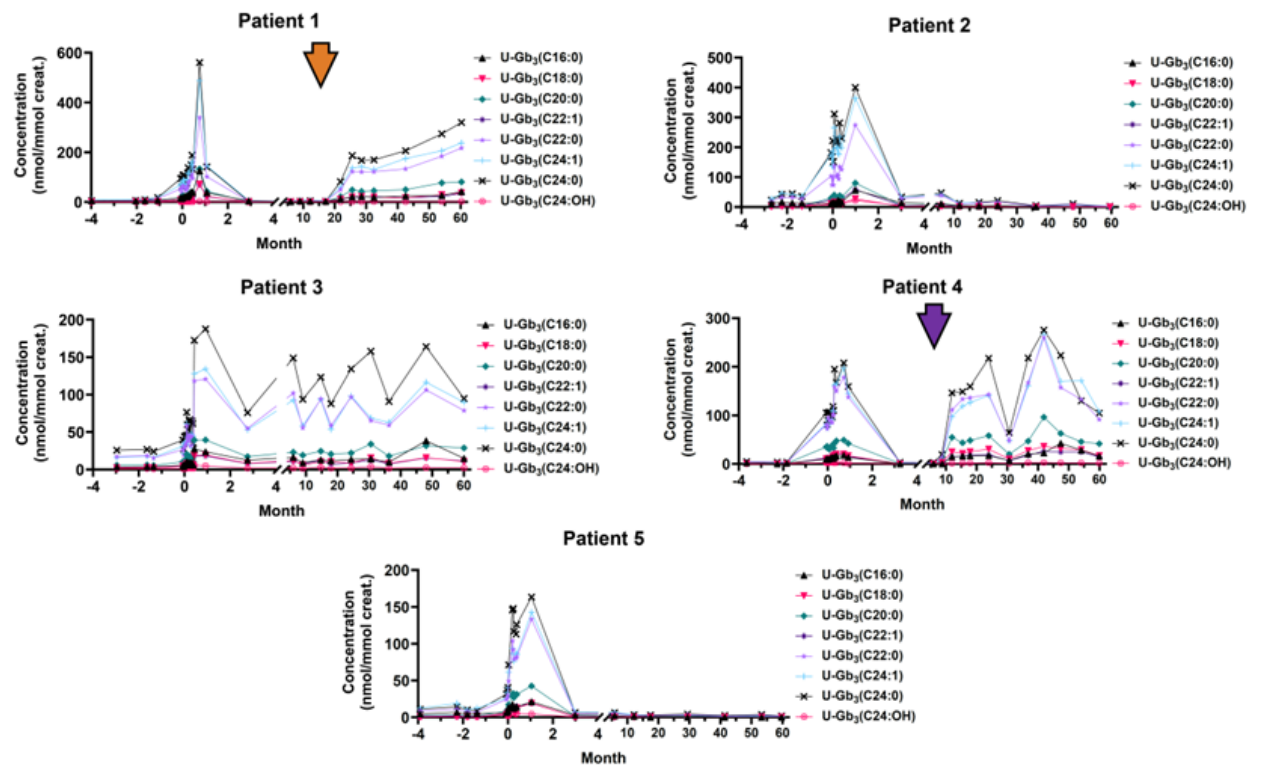
**Supplementary Figure 1.** This figure shows the 60-month longitudinal follow-up of urine (U-) levels of Lyso-Gb<sub>3</sub> and analogues normalized to creatinine for each patient. ERT was stopped before mobilization at Month -1 and resumed at Month 1 after gene therapy for patients 1, 2, 4, and 5. The orange arrow shows when Patient 1 stopped ERT at Month 18 and the violet arrow shows when Patient 4 stopped ERT at Month 7. Patient 3 chose not to restart ERT.



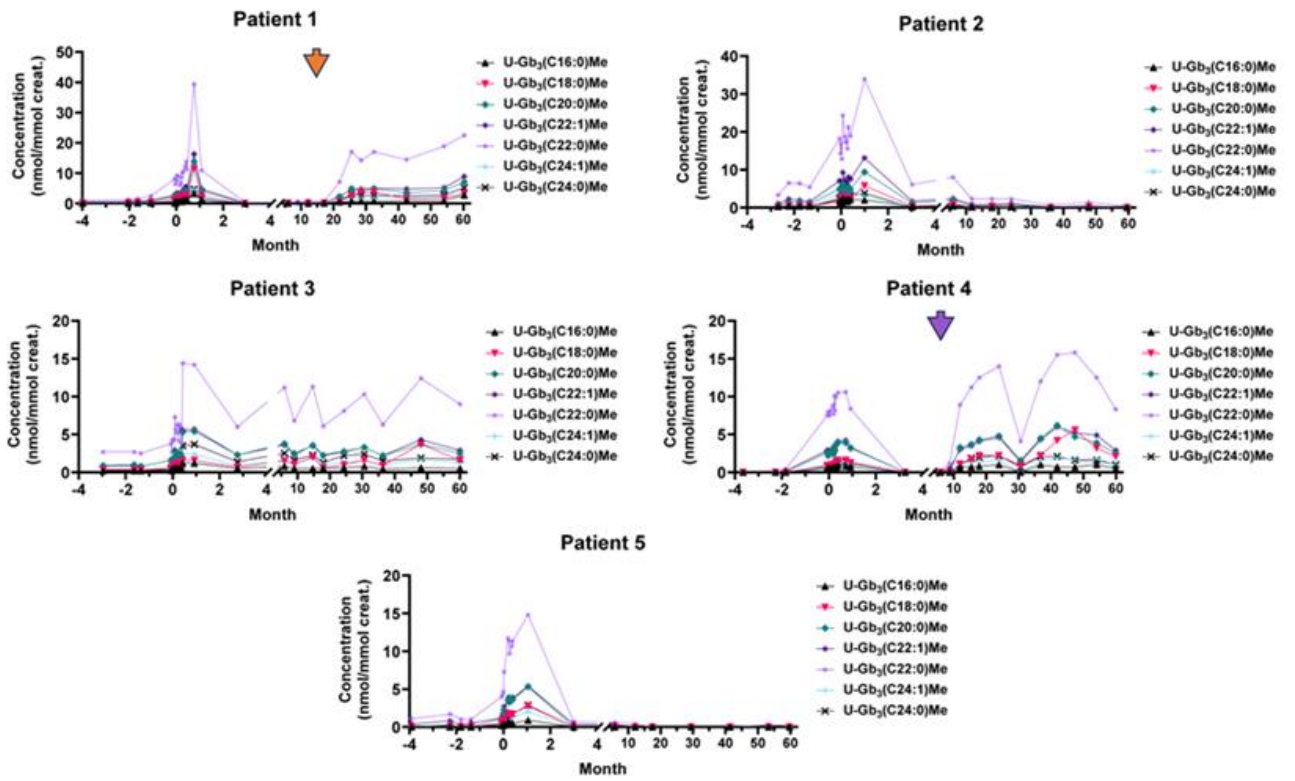
**Supplementary Figure 2.** This figure shows the 60-month longitudinal follow-up of plasma (P-) levels of Lyso-Gb<sub>3</sub> and analogues for each patient. ERT was stopped before mobilization at Month -1 and resumed at Month 1 after gene therapy for patients 1, 2, 4, and 5. The orange arrow shows when Patient 1 stopped ERT at Month 18 and the violet arrow shows when Patient 4 stopped ERT at Month 7. Patient 3 chose not to restart ERT.



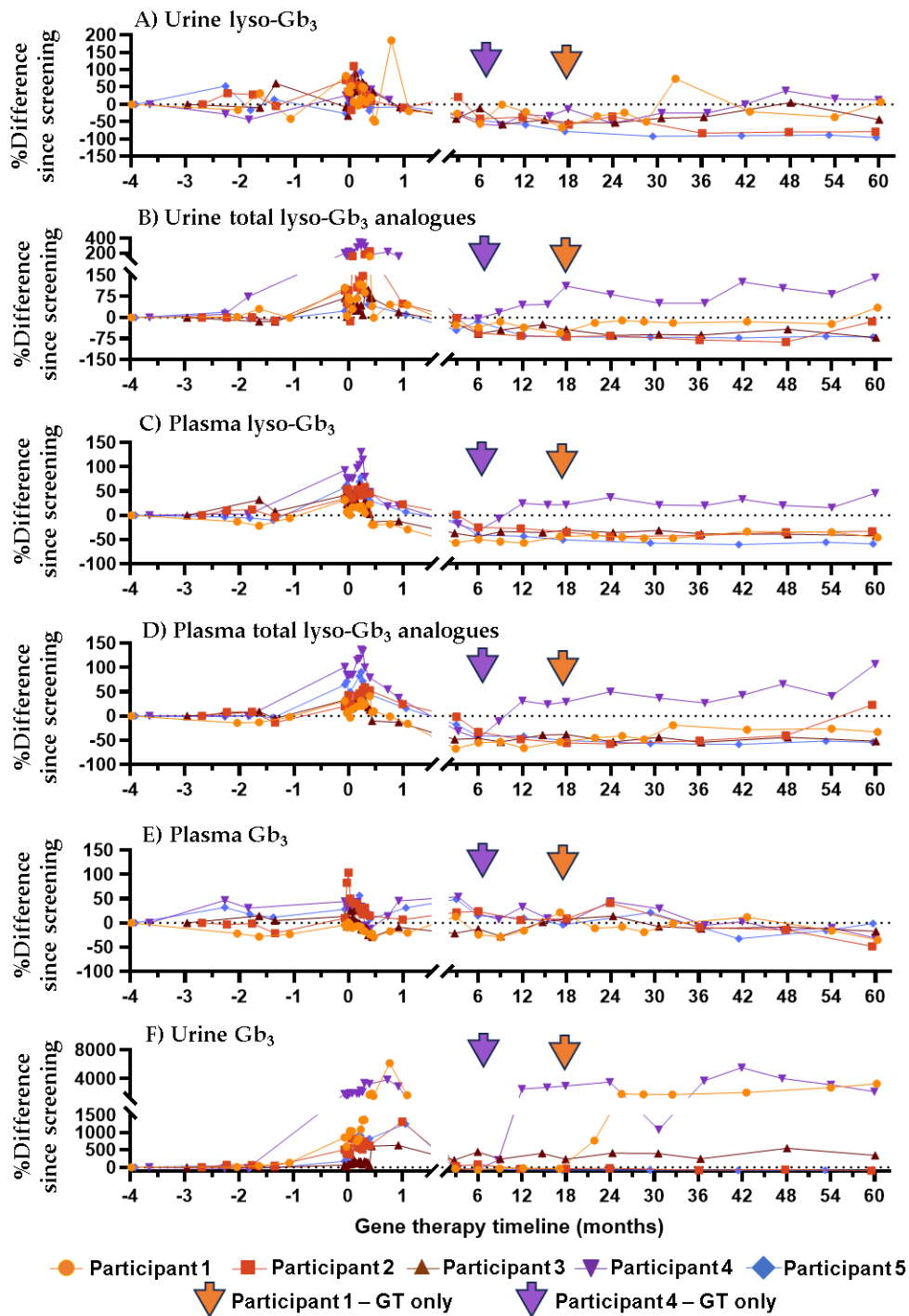
**Supplementary Figure 3.** This figure shows the 60-month longitudinal follow-up of plasma (P-) levels of Gb<sub>3</sub> isoforms for each patient. ERT was stopped before mobilisation at Month -1 and resumed at Month 1 after gene therapy for patients 1, 2, 4, and 5. The orange arrow shows when Patient 1 stopped ERT at Month 18 and the violet arrow shows when Patient 4 stopped ERT at Month 7. Patient 3 chose not to restart ERT.



**Supplementary Figure 4.** This figure shows the 60-month longitudinal follow-up of urine (U-) levels of Gb<sub>3</sub> non-methylated isoforms normalized to creatinine for each patient. ERT was stopped before mobilisation at Month -1 and resumed at Month 1 after gene therapy for patients 1, 2, 4 and 5. The orange arrow shows when Patient 1 stopped ERT at Month 18 and the violet arrow shows when Patient 4 stopped ERT at Month 7. Patient 3 chose not to restart ERT.



**Supplementary Figure 5.** This figure shows the 60-month longitudinal follow-up of urine (U-) levels of Gb<sub>3</sub> methylated isoforms normalized to creatinine for each patient. ERT was stopped before mobilisation at Month -1 and resumed at Month 1 after gene therapy for patients 1, 2, 4, and 5. The orange arrow shows when Patient 1 stopped ERT at Month 18 and the violet arrow shows when Patient 4 stopped ERT at Month 7. Patient 3 chose not to restart ERT.



**Supplementary Figure 6.** 60-month longitudinal biomarker follow-up for all 5 participants. This figure shows the percentage difference between the screening period and each individual longitudinal time point measured for 60 months. Urine lyso-Gb<sub>3</sub> (A); Urine total lyso-Gb<sub>3</sub> analogues (B); Plasma lyso-Gb<sub>3</sub> (C); Plasma total lyso-Gb<sub>3</sub> (D); Plasma Gb<sub>3</sub> (E); and Urine Gb<sub>3</sub> (F) levels are illustrated for each patient. ERT was stopped prior to mobilization at month 1 and was restarted at month 1 after the gene therapy for Patients 1, 2, 4, and 5. The orange arrow shows when Patient 1 stopped ERT at month 18 and the violet arrow shows when Patient 4 stopped ERT at month 7. Patient 3 chose not to restart ERT.

**Supplementary Table 1. Summary of mass spectrometry multiple reaction monitoring transitions.**

	Precursor ions [M+H] <sup>+</sup> ( <i>m/z</i> )	Product ions [M+H] <sup>+</sup> ( <i>m/z</i> )
<b>Gb<sub>3</sub> isoforms</b>		
<i>Methylated isoforms</i>		
Gb <sub>3</sub> (d18:1)(C16:0)Me	1038.69	534.53
Gb <sub>3</sub> (d18:1)(C18:0)Me	1066.73	562.56
Gb <sub>3</sub> (d18:1)(C20:0)Me	1094.76	590.59
Gb <sub>3</sub> (d18:1)(C22:1)Me	1120.77	616.60
Gb <sub>3</sub> (d18:1)(C22:0)Me	1122.79	618.62
Gb <sub>3</sub> (d18:1)(C24:1)Me	1148.80	644.63
Gb <sub>3</sub> (d18:1)(C24:0)Me	1150.82	646.65
<i>Non-methylated isoforms</i>		
Gb <sub>3</sub> (d18:1)(C16:1)	1022.66	518.49
Gb <sub>3</sub> (d18:1)(C16:0)	1024.68	520.51
Gb <sub>3</sub> (d18:1)(C17:0) ( <b>CS</b> )	1038.69	534.53
Gb <sub>3</sub> (d18:1)(C18:0)	1052.71	548.54
Gb <sub>3</sub> (d18:1)(C18:0)D <sub>3</sub> ( <b>IS</b> )	1055.73	551.56
Gb <sub>3</sub> (d18:1)(C20:0)	1080.74	576.57
Gb <sub>3</sub> (d18:1)(C22:1)	1106.76	602.59
Gb <sub>3</sub> (d18:1)(C22:0)	1108.77	604.60
Gb <sub>3</sub> (d18:1)(C23:0) ( <b>CS</b> )	1122.79	618.62
Gb <sub>3</sub> (d18:1)(C24:1)	1134.79	630.62
Gb <sub>3</sub> (d18:1)(C24:0)	1136.80	632.63
Gb <sub>3</sub> (d18:1)(C24:OH)	1152.80	648.63
<b>Lyso-Gb<sub>3</sub> and analogues</b>		
Lyso-Gb <sub>3</sub> -28 Da	758.42	254.25
Lyso-Gb <sub>3</sub> -12 Da	774.41	252.23
Lyso-Gb <sub>3</sub> -2 Da	784.43	280.26
Lyso-Gb <sub>3</sub> ( <b>CS</b> )	786.45	282.28
Lyso-Gb <sub>3</sub> +14 Da	800.43	278.25
Lyso-Gb <sub>3</sub> +16 Da	802.44	280.26
Lyso-Gb <sub>3</sub> +18 Da	804.46	318.30
Lyso-Gb <sub>3</sub> +34 Da	820.45	334.30
Lyso-Gb <sub>3</sub> +50 Da	836.45	350.29
Lyso-Gb <sub>3</sub> -Gly ( <b>IS</b> )	843.47	264.27

CS: Calibration standard; IS: Internal standard.