Autologous genome-edited hematopoietic stem cells correct Gaucher disease and establish a platform for clinical translation

Author list

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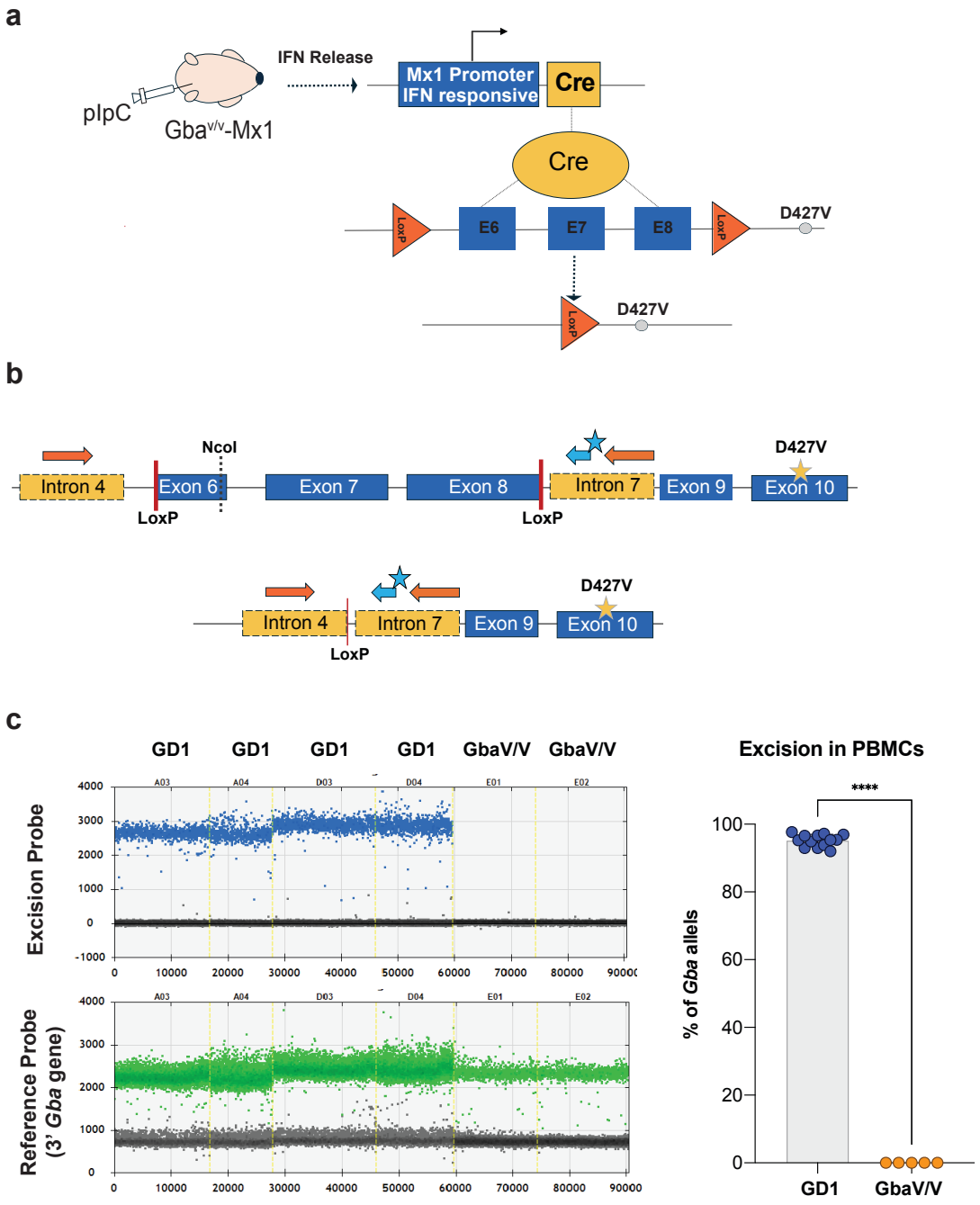
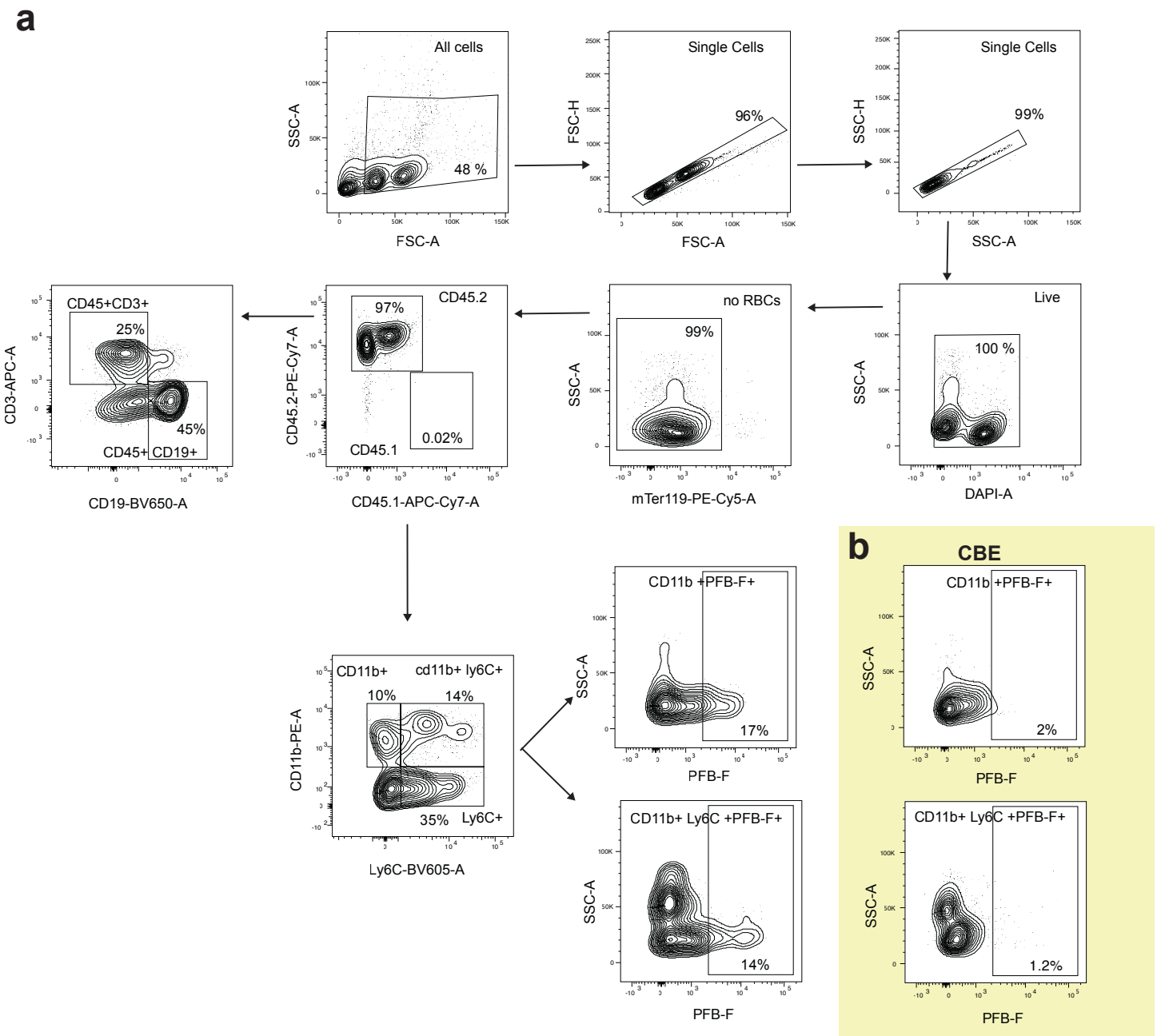
Supplementary Figures



Supplementary Figure 1. Description of the ddPCR assay to measure *Gba1* excision

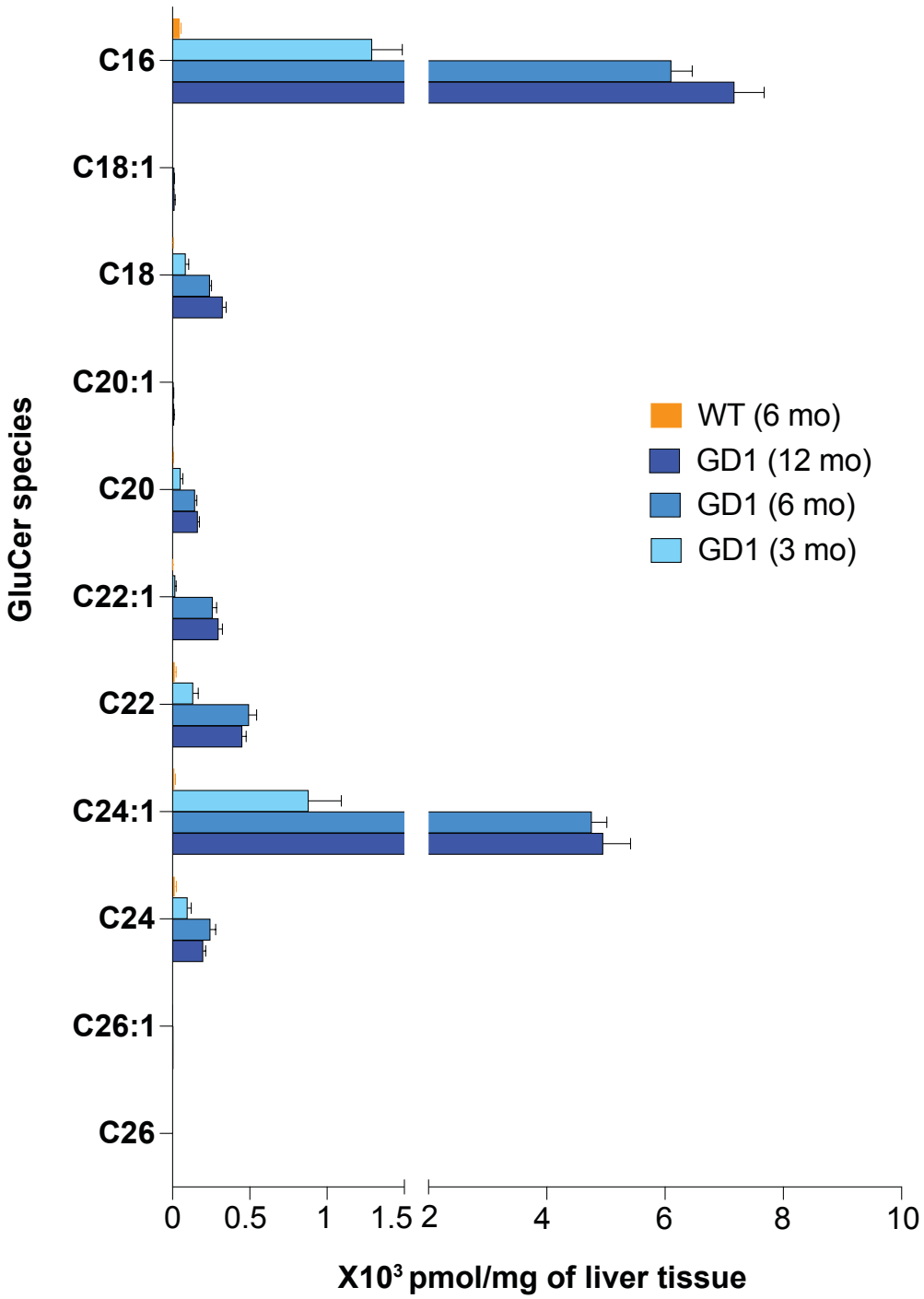
a, Schematic representation of the Cre-mediated deletion of *Gba1* exons 6-8, induced by an interferon response and activation of the MX1 promoter. **b,** Schematic representation of the digital droplet PCR (ddPCR) assays developed to accurately measure the excision and the input *Gba1* gene. The excision assay (light blue/orande) specifically spans the DNA sequence boundary created after excision. The reference assay detects unmodified exon 12 (not shown). Dark blue rectangles represent *Gba1* exons, the red lines indicate loxP sites and the yellow star denotes the D427V mutation. The black dashed line represents the restriction enzyme restriction used for the assay. **c,** Representative ddPCR one-dimensional plots showing probe amplification in peripheral blood mononuclear cells (PBMCs). The first four columns show pIpC-injected Gba1$^{f/f;D427V/D427V}$-Tg(Mx1-Cre) (GD1) and the last two homozygous Gba1$^{f/f;D427V/D427V}$ lacking the Mx1-Cre transgene (Gba$^{V/V}$). Blue dots are positive droplets for the excision probe and green dots for the reference probe. **d,** Percentage of excised alleles measured as a ratio of the number of positive excised droplets over the number of reference droplets. GD1 (blue, n=13) and Gba$^{V/V}$ (green, n=5). Data represented as mean ±SD. Statistical analysis was performed using unpaired one-tailed t-test with significance levels indicated as ****$p < 0.0001$.



Supplementary Figure 2. Gating scheme to measure GCase activity in peripheral blood cells.

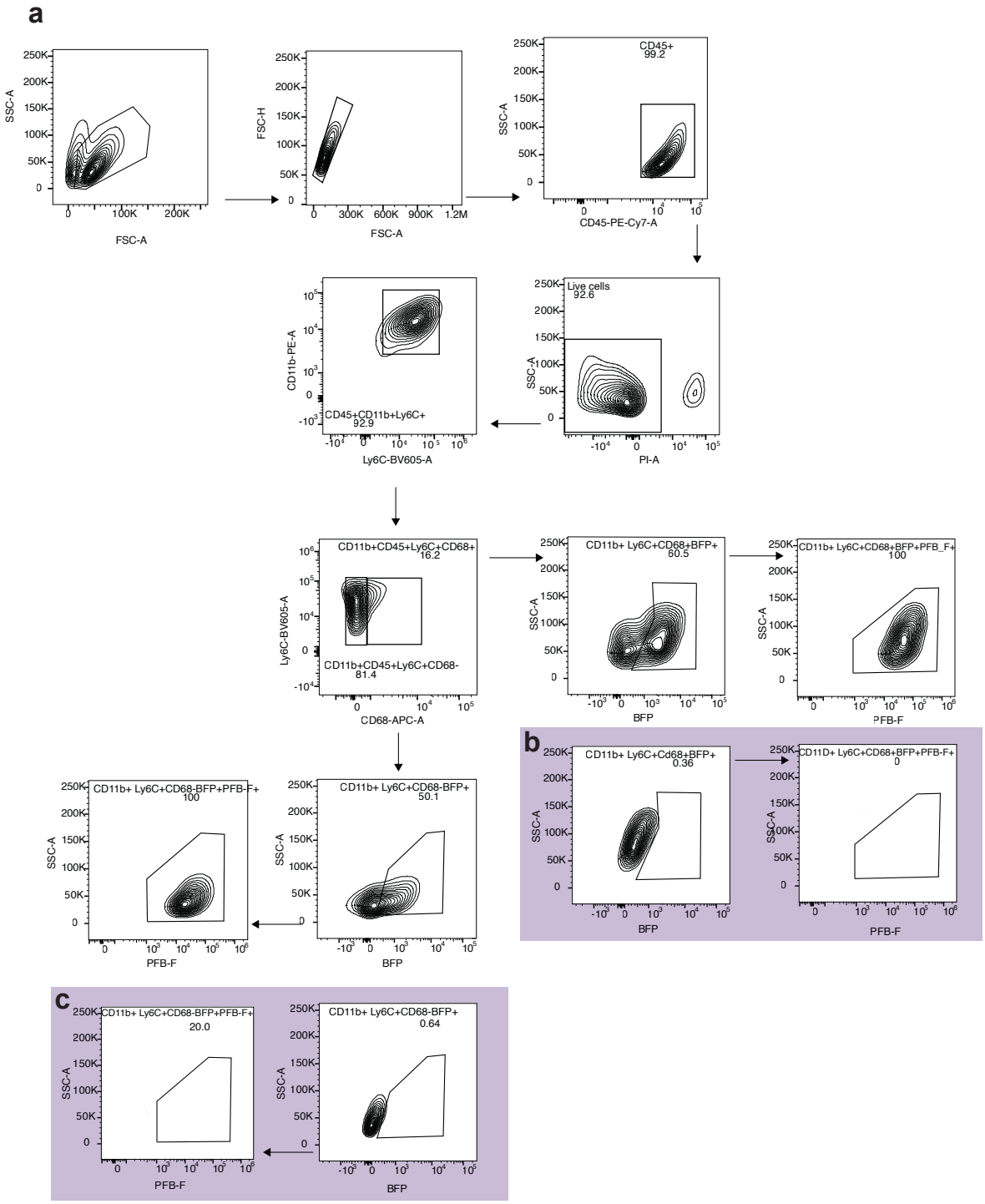
a, Representative gating strategy of cells in the peripheral blood analyzed by flow cytometry. The flow plots show the gating strategy used to determine the percentage of hematopoietic cells (CD45.1 or CD45.2), myeloid cells (CD11b), and monocytes (Ly6C).

b, GCase activity was measured at the single-cell level using the membrane-permeable fluorogenic substrate PFB-FDGlu (5-(Pentafluorobenzoylamino) fluorescein di-β-D-glucopyranoside), which releases the fluorescent product PFB-F upon cleavage by GCase. Flow plots show the PFB-F signal in CD11b+ and CD11b+/Ly6C+ cells. Conduritol B epoxide (CBE), an irreversible inhibitor of GCase, was used to establish the positive gate for GCase activity.



Supplementary Figure 3. Accumulation of GluCer species in the liver of GD1 mice over time.

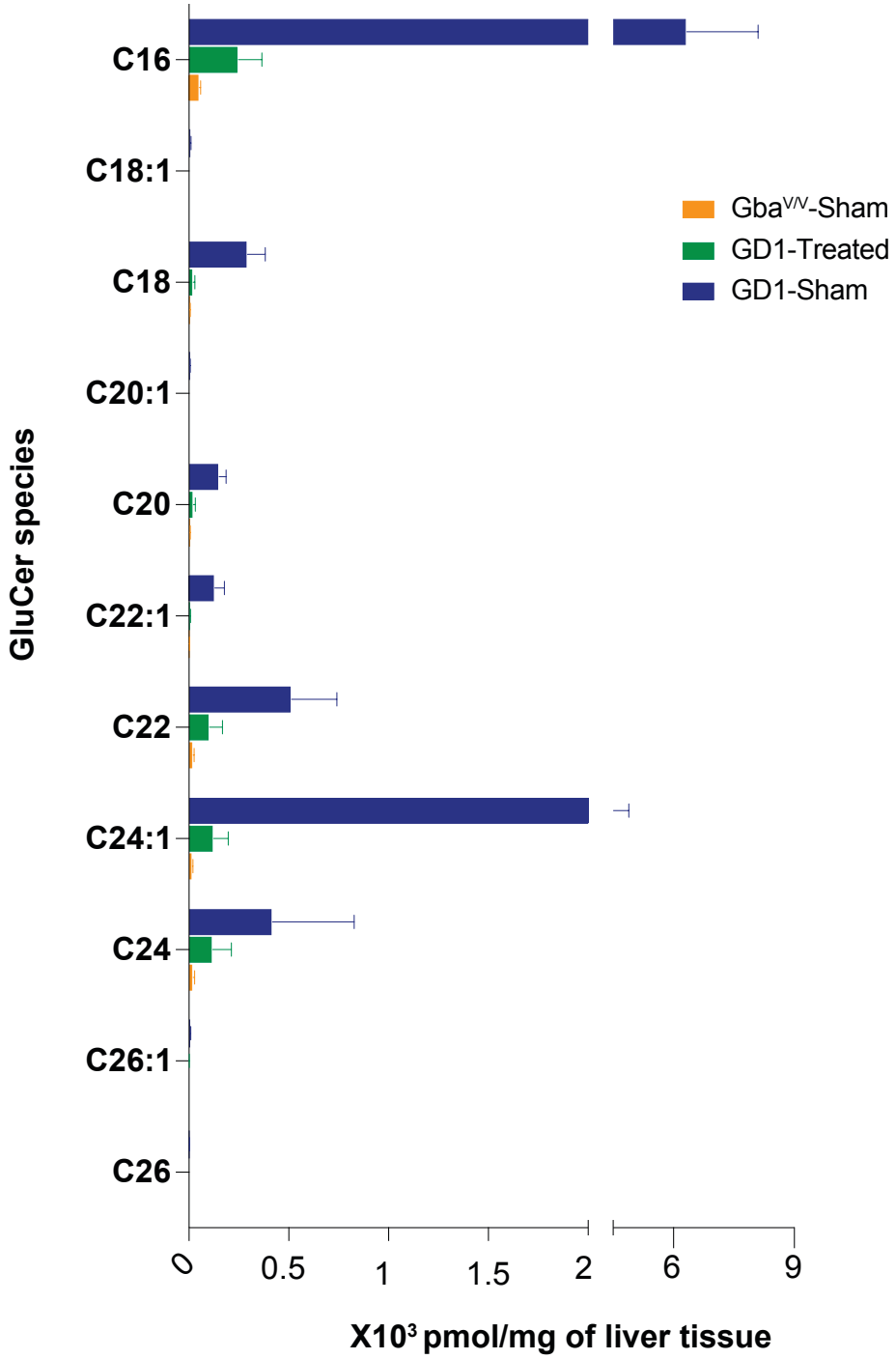
Quantification of individual glucosylceramide (GluCer) species in liver tissue from wild-type (WT, orange; 6 months), and GD1 mice at 3 months (light blue), 6 months (blue), and 12 months (dark blue) of age. Values are expressed as $\times 10^3$ pmol GluCer per mg of liver tissue. GD1 mice exhibited progressive accumulation of GluCer species, particularly C16 and C24:1, with age. Data are presented as mean ± SEM.



Supplementary Figure 4. Gating strategy for analysis of macrophages in vitro.

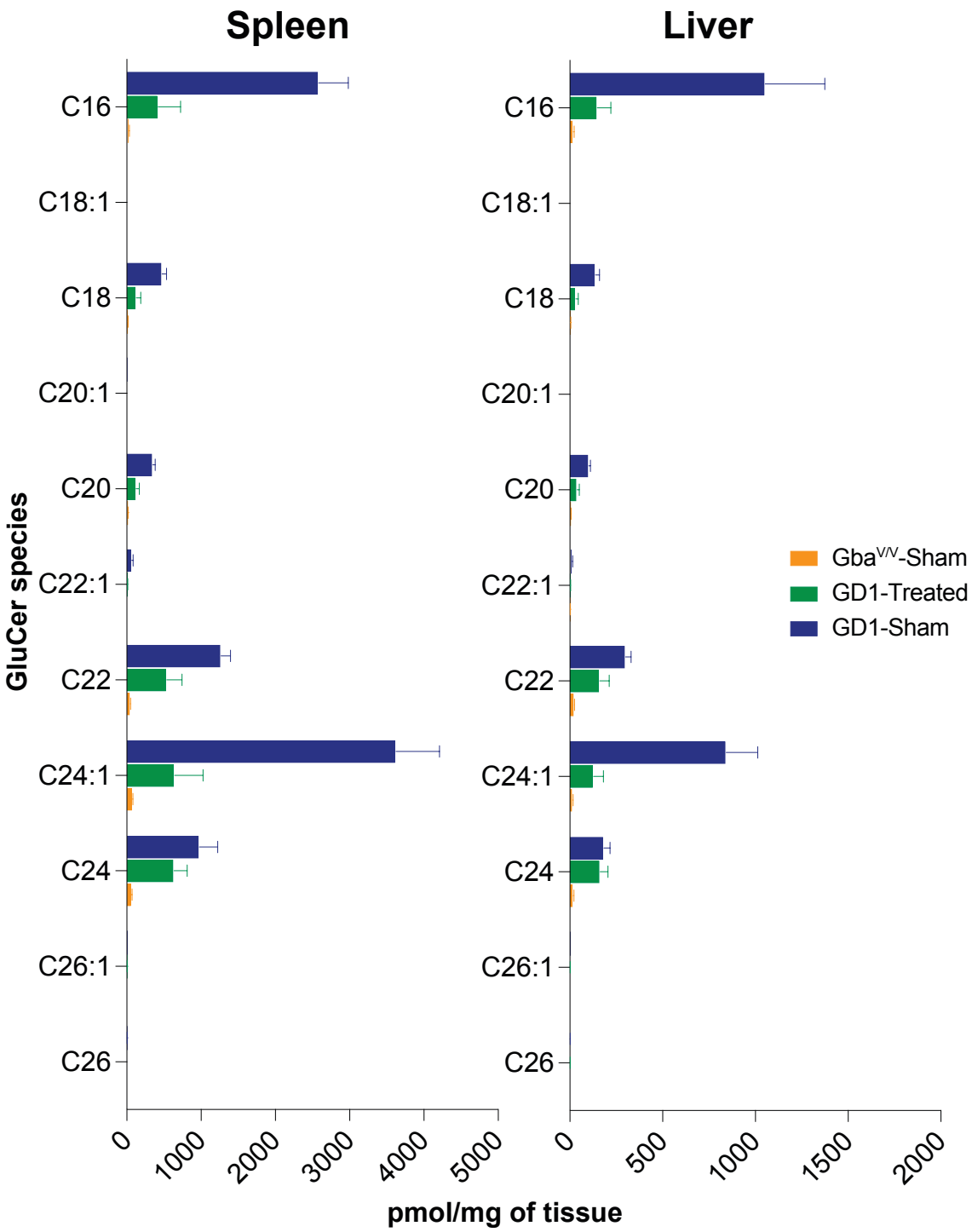
a, Representative gating strategy used to analyze HSPC-derived cells during in vitro differentiation into macrophages in the presence of M-CSF and GM-CSF. GCase activity was measured at the single-cell level using the membrane-permeable fluorogenic substrate PFB-FDGlu (5-(Pentafluorobenzoylamino) fluorescein di-β-D-glucopyranoside), which releases the fluorescent product PFB-F upon cleavage by GCase. Flow cytometry plots depict the gating used to quantify $CD11b^{+}Ly6C^{+}CD68^{+}BFP^{+}$ cells and assess GCase activity based on PFB-F signal.

b–c, Flow plot in the purple panels show PFB-F signal in $CD11b^{+}/Ly6C^{+}$ macrophages expressing either CD68 or blue fluorescent proteint (BFP). Conduritol B epoxide (CBE), an irreversible inhibitor of GCase, was used to define the negative population and establish the threshold for GCase activity gating.



Supplementary Figure 5. GluCer species in the liver of GD1 mice transplanted with edited HSPCs following TBI-based myeloblation.

Quantification of individual glucosylceramide (GluCer) species in liver tissue from Gba$^{V/V}$-Sham (*n* = 5), GD1-Sham (*n* = 6); and GD1-Treated (*n* = 6). Values are expressed as ×10³ pmol GluCer per mg of liver tissue. GD1-Treated mice exhibited reduction in all GluCer species. Data are presented as mean ± SD.



Supplementary Figure 6. GluCer species in the liver and spleen of GD1 mice transplanted with edited HSPCs following Busulfan-based conditioning.

Quantification of individual glucosylceramide (GluCer) species in liver and spleen tissue from Gba$^{V/V}$-Sham (*n* = 5), GD1-Sham (*n* = 5); and GD1-Treated (*n* = 5). Values are expressed as ×10³ pmol GluCer per mg of tissue. GD1-Treated mice exhibited reduction in all GluCer species. Data are presented as mean ± SD.