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

Author list

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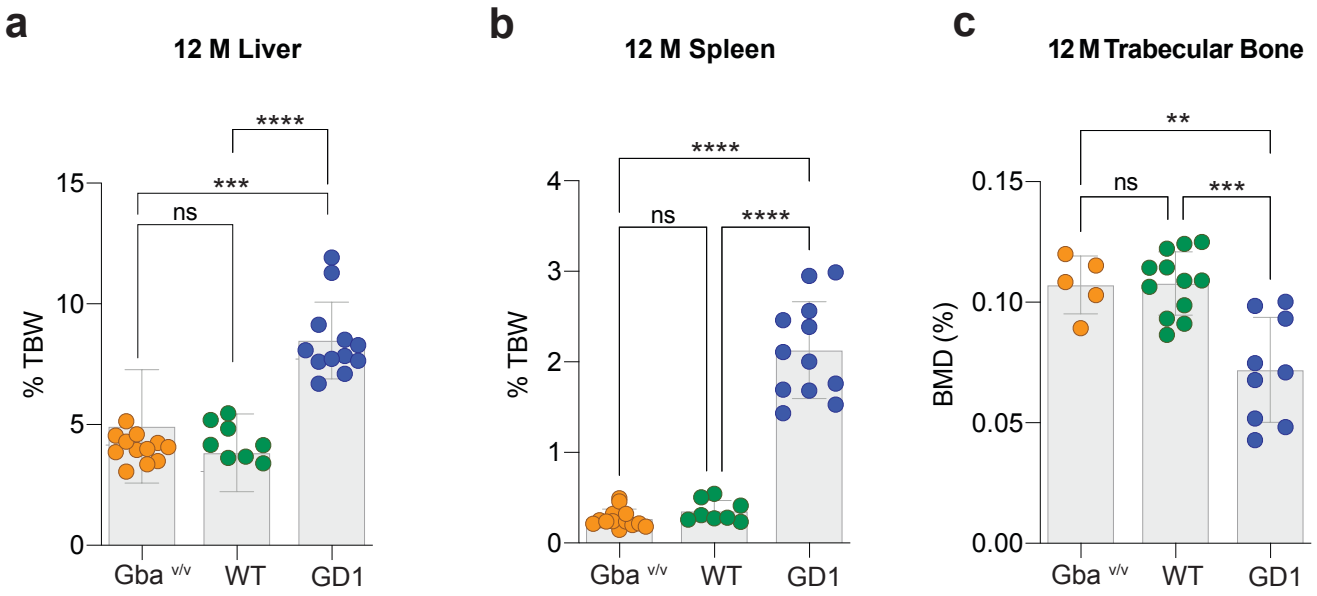
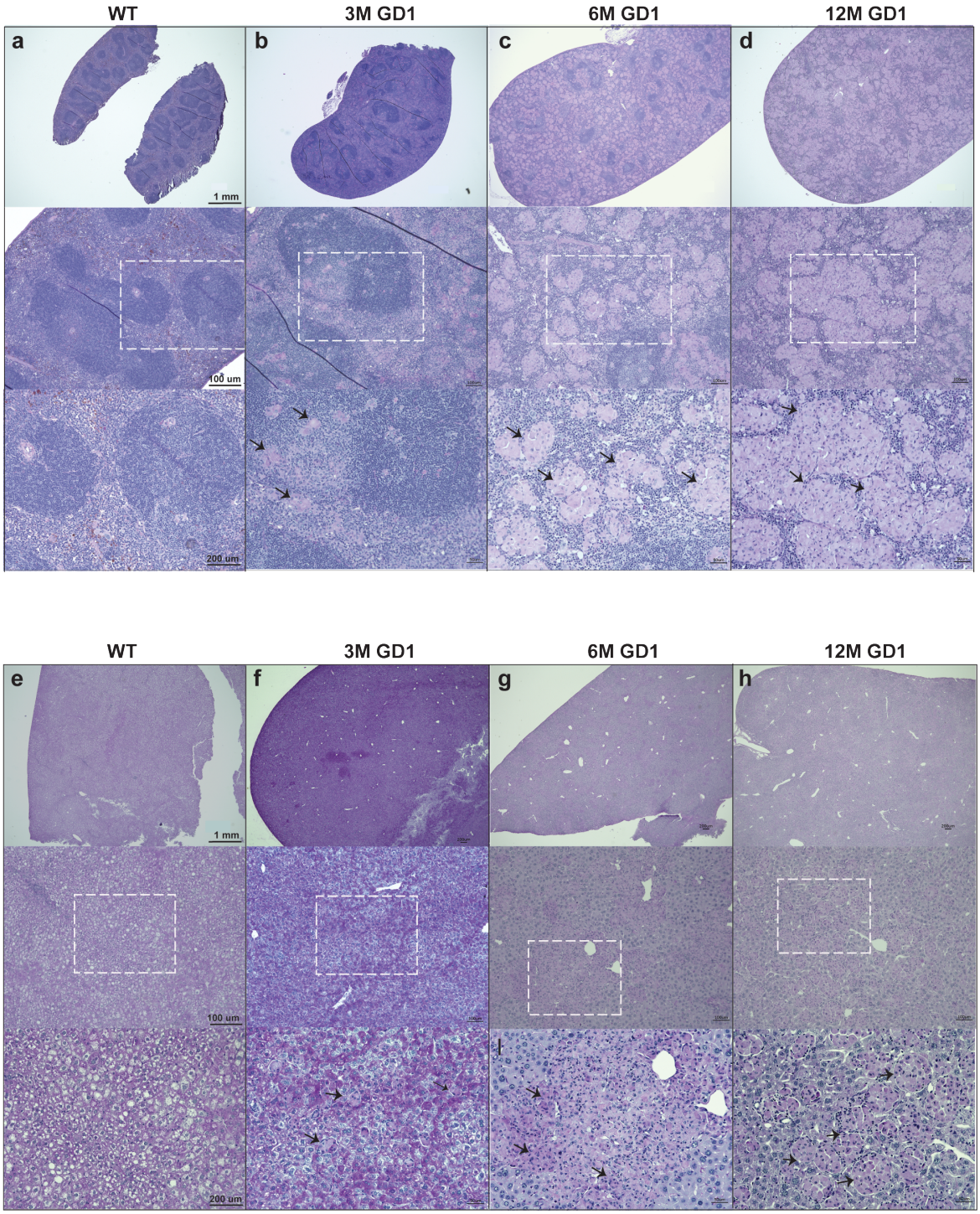
Extended Data



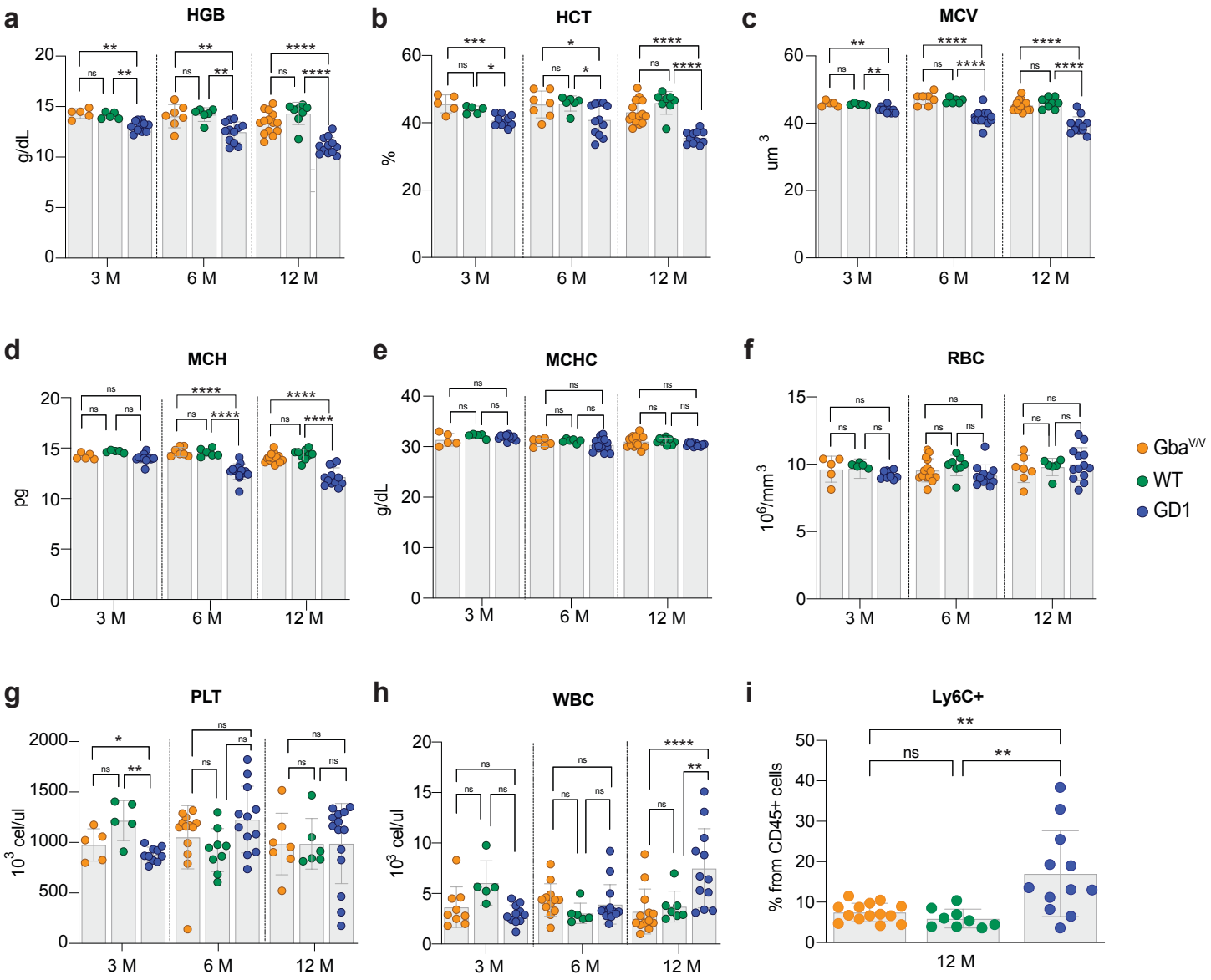
Extended Data Figure 1. Comparison of hematologic and visceral parameters in wildtype, D427V knock-in, and GD1 mice at 12 months

a, and b, Spleen and liver weights as a percentage of total body weight for 12-month-old mice ($Gba^{V/V}$ n=13, GD1 n=12, and WT n=11). **c,** Trabecular bone mineral density in distal femurs at 12 months ($Gba^{V/V}$ n=12 and GD1 n=8). Data are shown as mean ± SD. Statistical analysis was performed using one-way ANOVA with Dunnett's multiple comparison test. Significance levels indicated as $^{**}p < 0.01$, $^{***}p < 0.001$, and $^{****}p < 0.0001$.



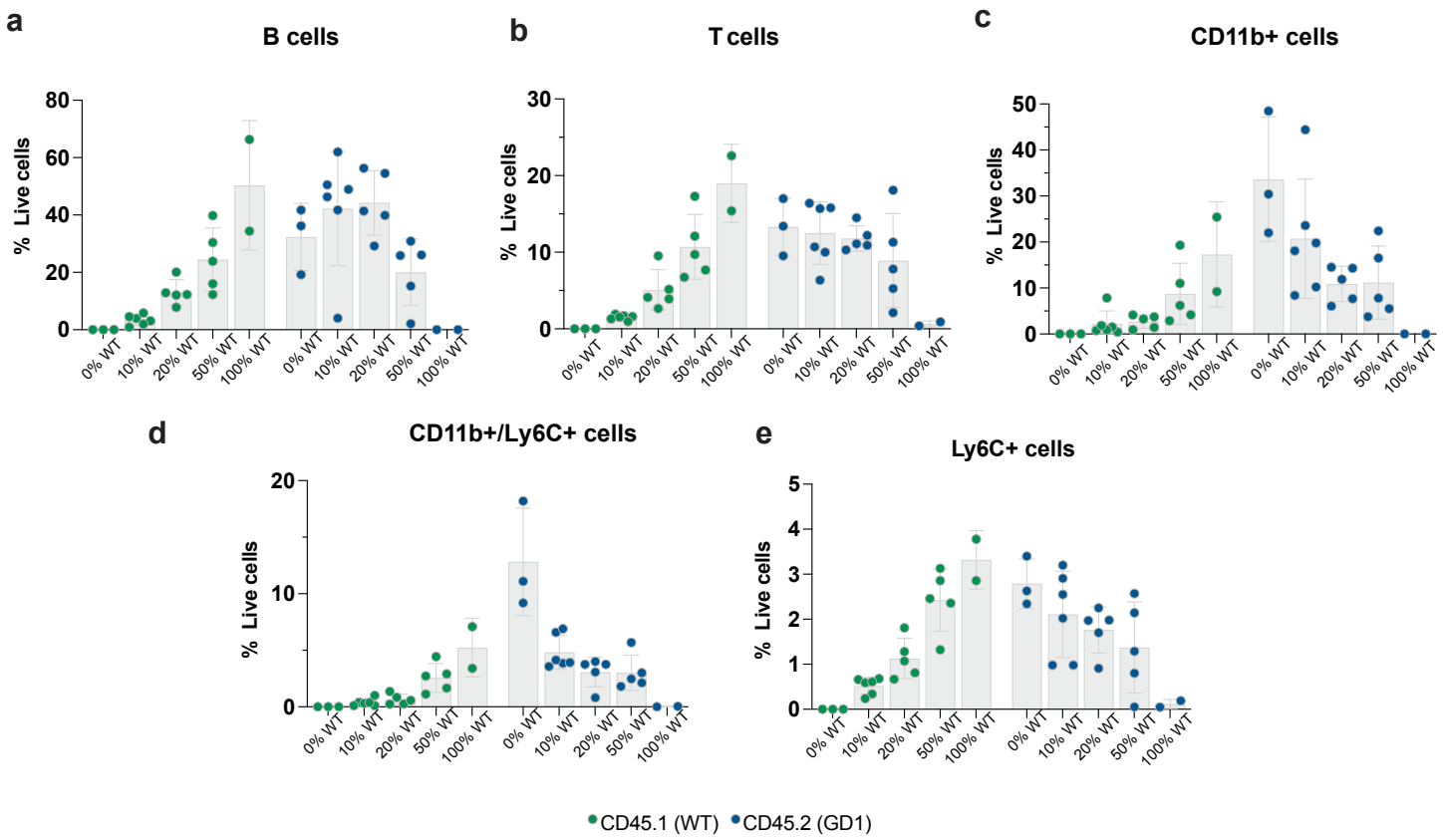
Extended Data Figure 2. Gaucher cells infiltrate the spleen and liver following the conditional deletion of *Gba1* exons 6-8.

Periodic acid-Schiff staining reveals focal collections of classical foamy Gaucher cells (indicated by black arrows) in representative sections of the spleen (**a-d**) and liver (**e-h**) of WT and GD1 mice at 3, 6, and 12 months of age (from left to right). Sections from three different magnifications are presented from top to bottom. Scale bars: 1 mm; 200μm and 100μm. The sections are representative from a total of 5 sections from 3 independent mice per condition.



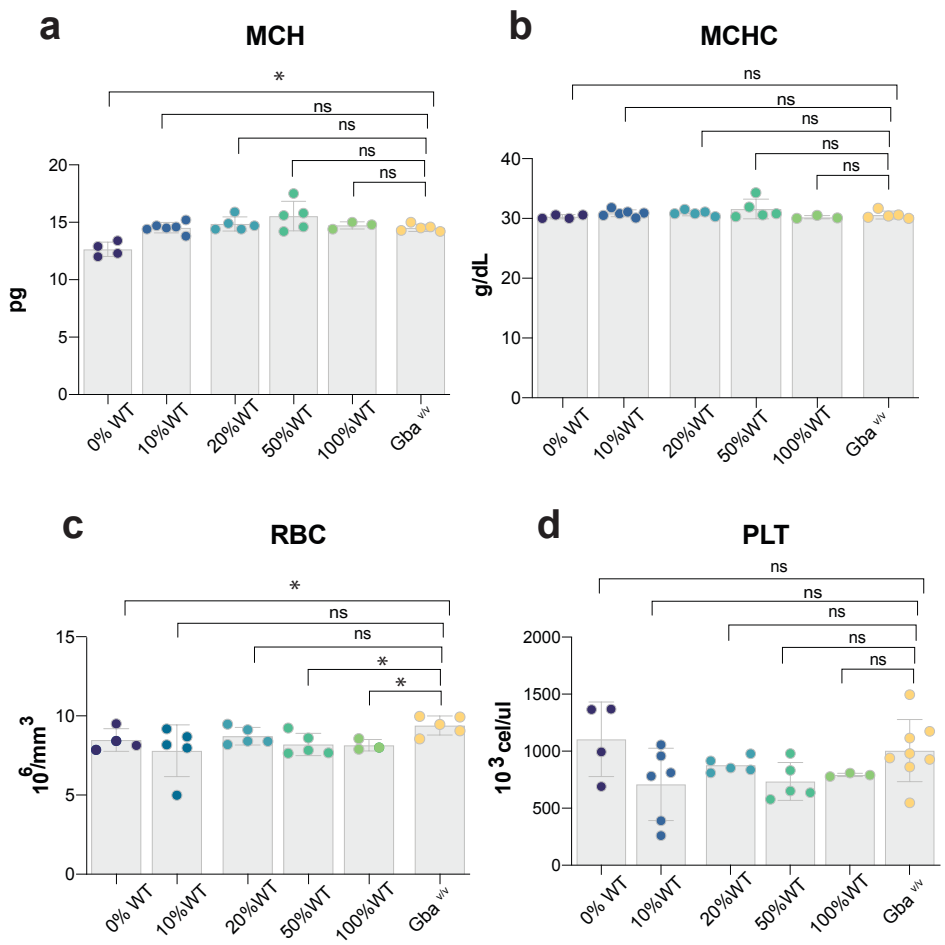
Extended Data Figure 3. Complete blood cell counts for all *Gba* genotypes at 3, 6, and 12 months.

Data were collected from wild-type mice ($Gba^{+/+}$, green, n=5-9), homozygous mice lacking the Cre transgene ($Gba^{f/f;D427V/D427V,}$ or $Gba^{V/V}$, yellow, n=5-14), and pIpC-injected homozygous mice carrying the Cre transgene ($Gba^{f/f;D427V/D427V}$-Tg(Mx1-CRE), or GD1, blue, n=9-14) at 3, 6, and 12 months of age. **a,** Hemoglobin (HGB). **b,** Hematocrit (HCT). **c,** Mean red blood cell volume (MCV). **d,** Mean corpuscular hemoglobin (MCH). **e,** Mean corpuscular hemoglobin concentration (MCHC). **f,** Red blood cell (RBC) count. **g,** Platelet count (PLT). **h,** White blood cell (WBC). **i,** Percent LyC6+ cells in the peripheral blood of 12-month-old mice for the same genotypes measured by flow cytometry. Data are shown as mean ± SD. Statistical analysis from **a-i** was performed using One-way ANOVA with Sidak's multiple comparison test for the three genotypes in each time point. Significance levels indicated as **p < 0.01, ***p < 0.001, and ****p < 0.0001.



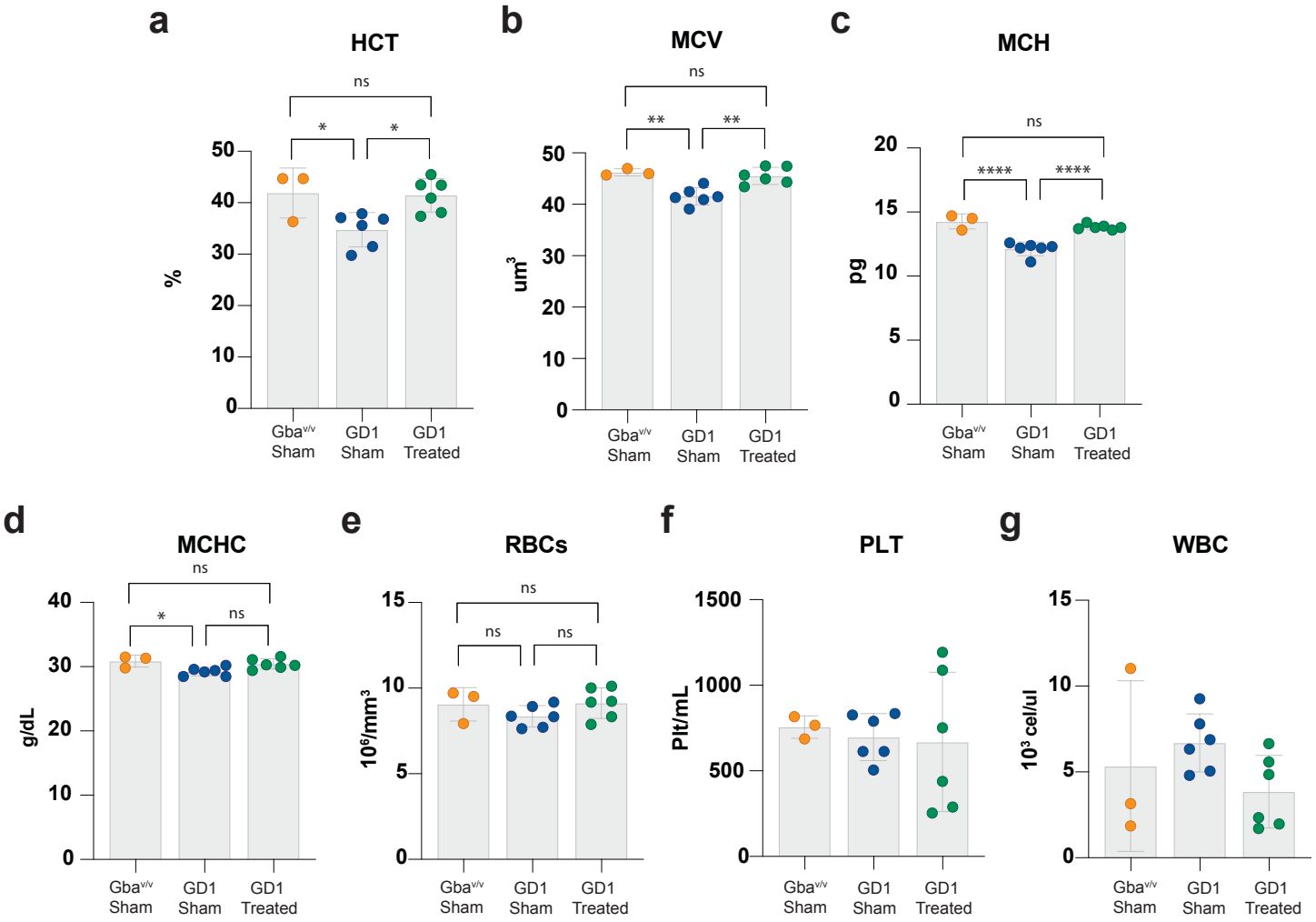
Extended Data Figure 4. In vivo multilineage differentiation of WT Gba$^{+/+}$ and GD1 Gba$^{-/-}$ HSCs

Frequency and engraftment of **a,** B cells (CD19+), **b,** T cells (CD3+) and **c-e,** myeloid cells (CD11b+, Ly6C+) in peripheral blood twenty weeks post-transplantation. Each dot represents data from one mouse. Data are shown as mean ± SD.



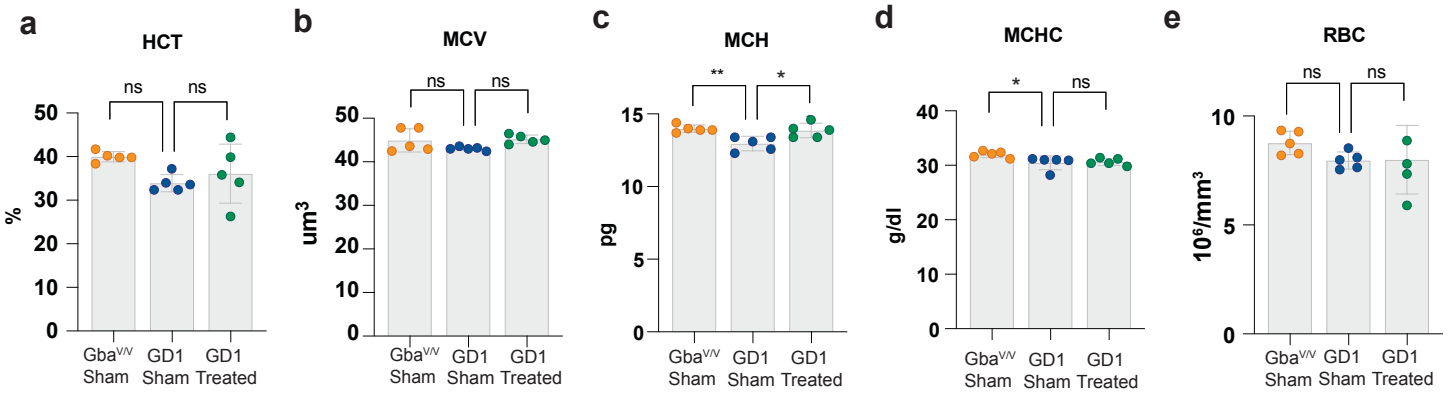
Extended Data Figure 5. Complete blood cell counts in GD1 mice with mixed grafts containing 0%, 10%, 20%, 50%, or 100% WT cells.

a, Mean corpuscular hemoglobin (MCH). **b,** Mean corpuscular hemoglobin concentration (MCHC); **c,** red blood cell (RBC) count. **d,** Platelet count (PLT). All data are presented as mean ± SD. Statistical analysis for **a** was performed using two-way ANOVA with Sidak's multiple comparisons test; for **b, c,** and **d,** one-way ANOVA with Dunnett's multiple comparison test. Significant differences are indicated by asterisks (*p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001).



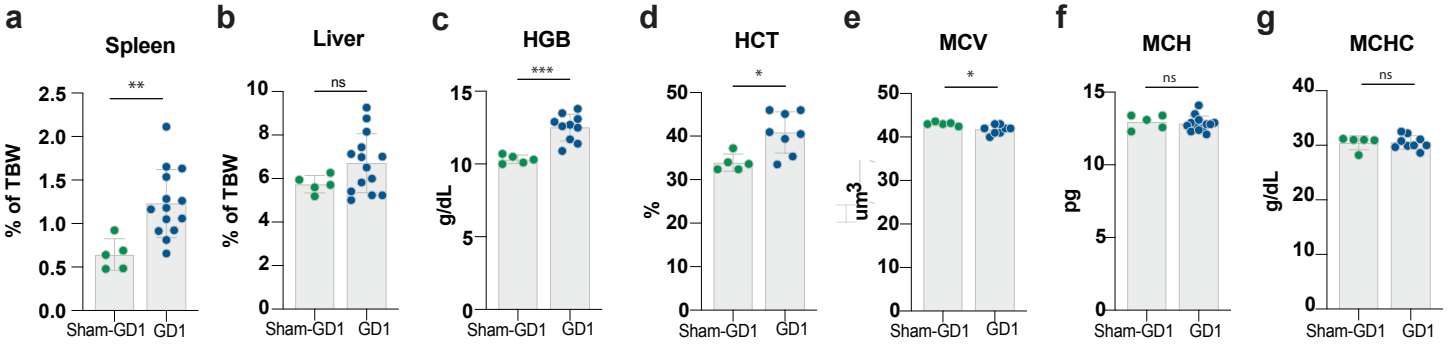
Extended Data Figure 6. Additional hematologic parameters in TBI-conditioned GD1 mice transplanted with genome edited HSPCs.

a, Hematocrit. **b,** Mean red blood cell volumes. **c**, Mean corpuscular hemoglobin (MCH). **d**, Mean corpuscular hemoglobin concentration (MCHC). **e**, Red blood cell (RBC) count. **f**, Platelet count; and **b**, White blood cell (WBC) count measured in blood. Data were collected from Sham Gba$^{V/V}$ (n = 3), Sham-GD1 (n = 6), and Treated-GD1 (n = 6) mice. All data are presented as mean ± SD. Statistical analysis was one-way ANOVA with Tukey multiple comparison test. Significant differences are indicated by asterisks (*p < 0.05; **p < 0.01; ***p < 0.001; and ****p < 0.0001).



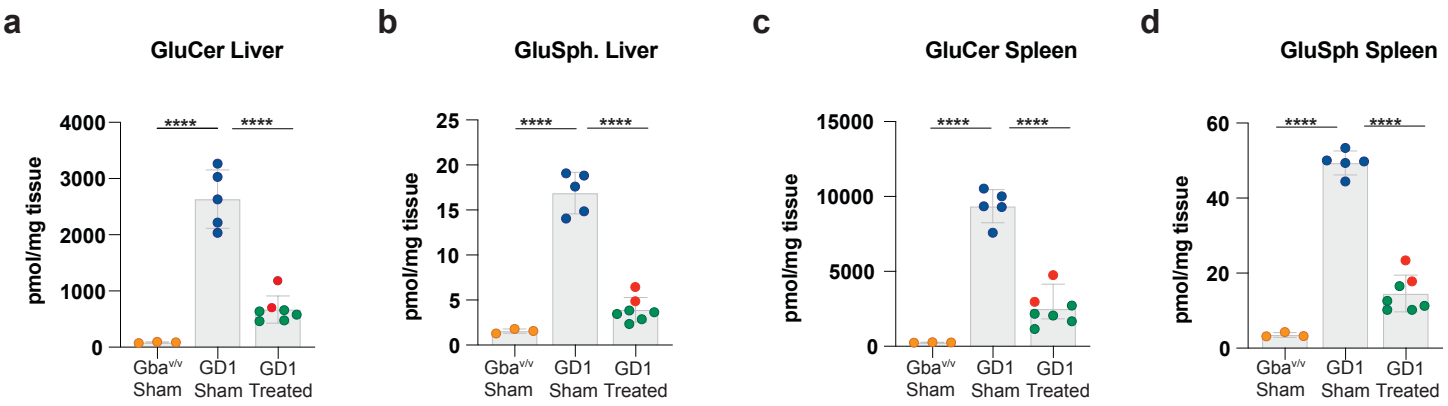
Extended Data Figure 7. Additional hematologic parameters in Busulfan-condition GD1 mice transplanted with genome edited HSPCs.

a, Hematocrit. **b,** Mean red blood cell volumes. **c**, Mean corpuscular hemoglobin (MCH). **d**, Mean corpuscular hemoglobin concentration (MCHC). **e**, Red blood cell (RBC) count. Data were collected from Sham Gba$^{V/V}$ (n = 5), Sham-GD1 (n = 5), and Treated-GD1 (n = 5) mice. All data are presented as mean ± SD. Statistical analysis was one-way ANOVA with Tukey multiple comparison test. Significant differences are indicated by asterisks (*p < 0.05; **p < 0.01; ***p < 0.001; and ****p < 0.0001).



Extended Data Figure 8. Hematologic and organ size parameters in GD1-Sham mice following Busulfan conditioning compared to age-matched unmanipulated GD1 mice.

a. Spleen weight. **b.** Liver weight. **c.** Hemoglobin (HGB). **d.** Hematocrit (HCT). **e.** Mean corpuscular volume (MCV). **f.** Mean corpuscular hemoglobin (MCH). **g.** Mean corpuscular hemoglobin concentration (MCHC). Comparisons were made between Sham-GD1 mice (busulfan-conditioned GD1 mice transplanted with GD1 cells) and age-matched untreated GD1 mice. Data are presented as mean ± SD; individual values are shown as dots. Statistical significance was determined using unpaired t-tests.



Supplementary Figure 9. Tissue reduction of glucosylceramide and glucosylsphingosine in treated GD1 mice, including those with low edited cell engraftment.

Levels of glucosylceramide (GluCer) and glucosylsphingosine (GluSph) in the liver (**a–b**) and spleen (**c–d**) of busulfan-conditioned GD1 mice transplanted with HSPCs. Data include mice with low levels of edited allele engraftment (~1%, red dots), compared to untreated controls and other treated mice. These results demonstrate that even minimal engraftment of edited cells leads to a measurable reduction in substrate accumulation.