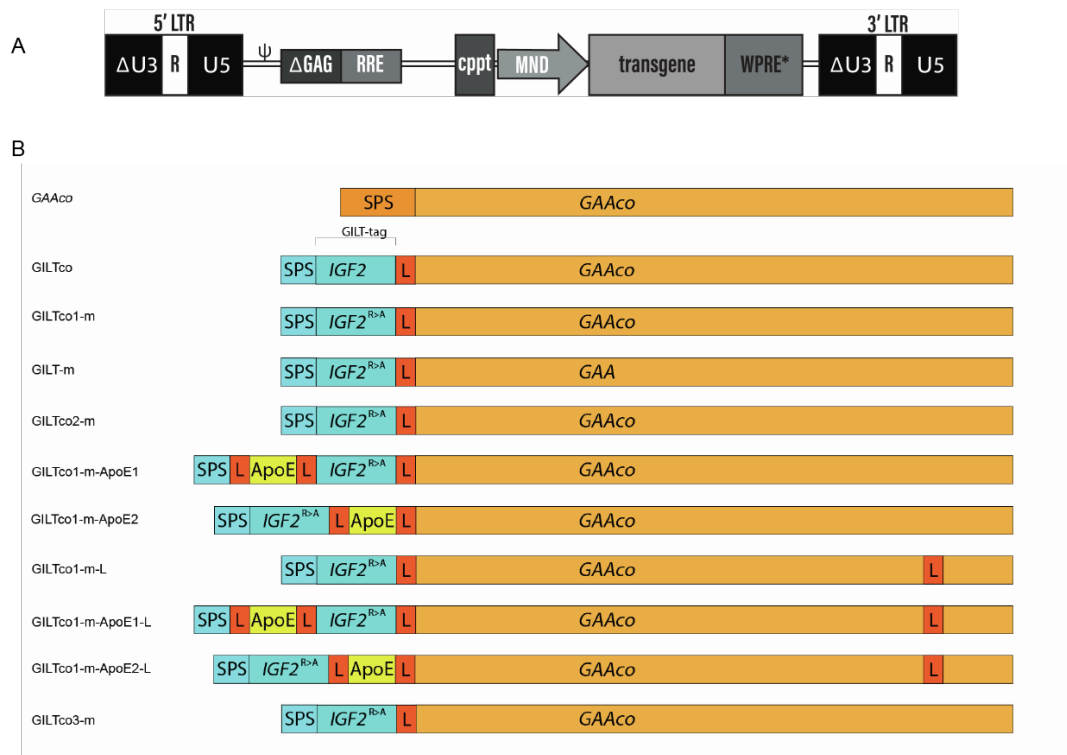


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**Supplemental information**

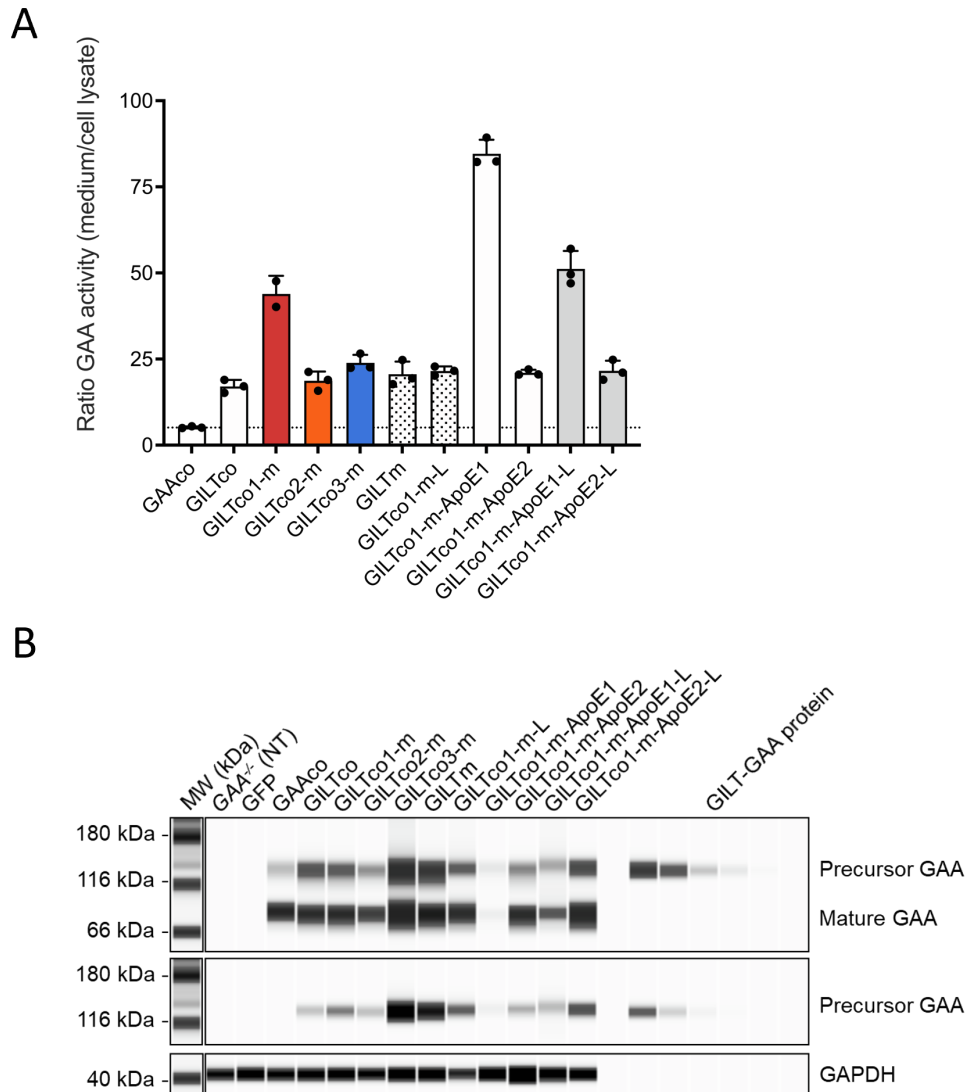
**Screening chimeric GAA variants in preclinical  
study results in hematopoietic stem cell gene  
therapy candidate vectors for Pompe disease**

**Yildirim Dogan, Cecilia N. Barese, Jeffrey W. Schindler, John K. Yoon, Zeenath Unnisa, Swaroopa Guda, Mary E. Jacobs, Christine Oborski, Tim Maiwald, Diana L. Clarke, Axel Schambach, Richard Pfeifer, Claudia Harper, Chris Mason, and Niek P. van Til**



**Figure S1. Graphical depiction of the lentiviral vector-mediated proviral and GAA sequences.**

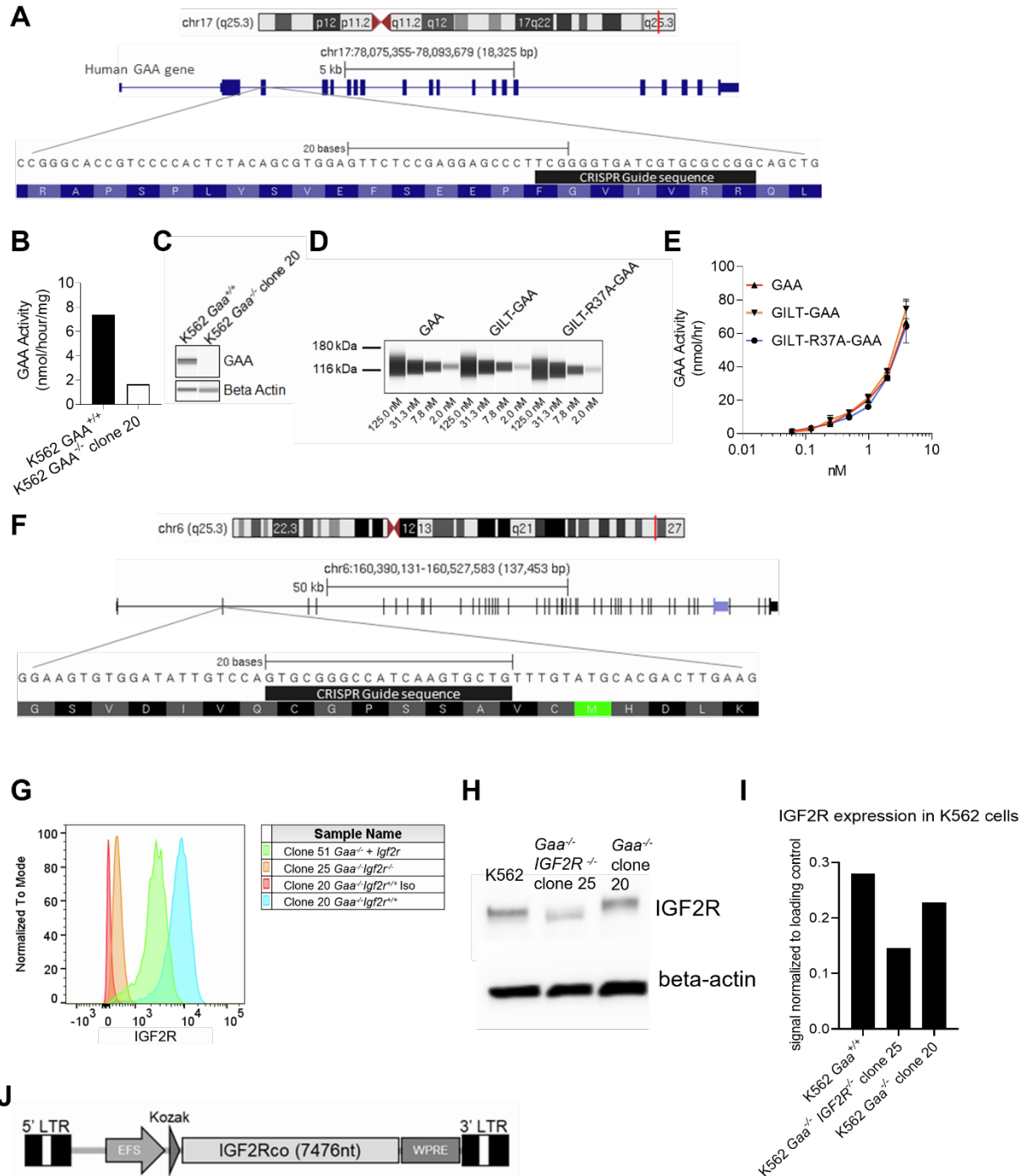
(A) Schematic of proviral vector: 5' LTR = HIV 5' long terminal repeat with inactivated (delta) Unique 3', Repeat, Unique 5';  $\psi$  = packaging signal; delta GAG = truncated HIV GAG sequence; RRE = Rev-Response Element; cppt = central polypurine tract; MND = myeloproliferative sarcoma virus enhancer, negative control region deleted, *d1587rev* primer-binding site substituted (MND) promoter; Transgene, cDNA of interest listed under Fig. S1B, WPRE\* = mutated Woodchuck hepatitis virus posttranscriptional regulatory element, and 3' LTR= inactivated (delta) Unique 3', Repeat, Unique 5'. (B) Overview of modified acid alpha-glucosidase (*GAA*) sequences. IGF2 = insulin-like growth factor 2; SPS = signal peptide sequence (orange, native *GAA* SPS and blue, IGF2 SPS); 'co' = codon optimized; GILT = glycosylation independent lysosomal targeting; 'L' = Gly-Ala-Pro peptide linker; R>A = Arginine to Alanine substitution; ApoE = Apolipoprotein E tag. Note, cDNA sequences were codon optimized using Genscript's or ThermoFisher's GeneArt algorithm.



**Figure S2. Relative secretion and Western blot analysis of GAA and GILT-tag in HAP1 cells.**

(A) The ratio of GAA enzyme activity in medium divided by the GAA enzyme activity in HAP *GAA*<sup>-/-</sup> cell lysates.

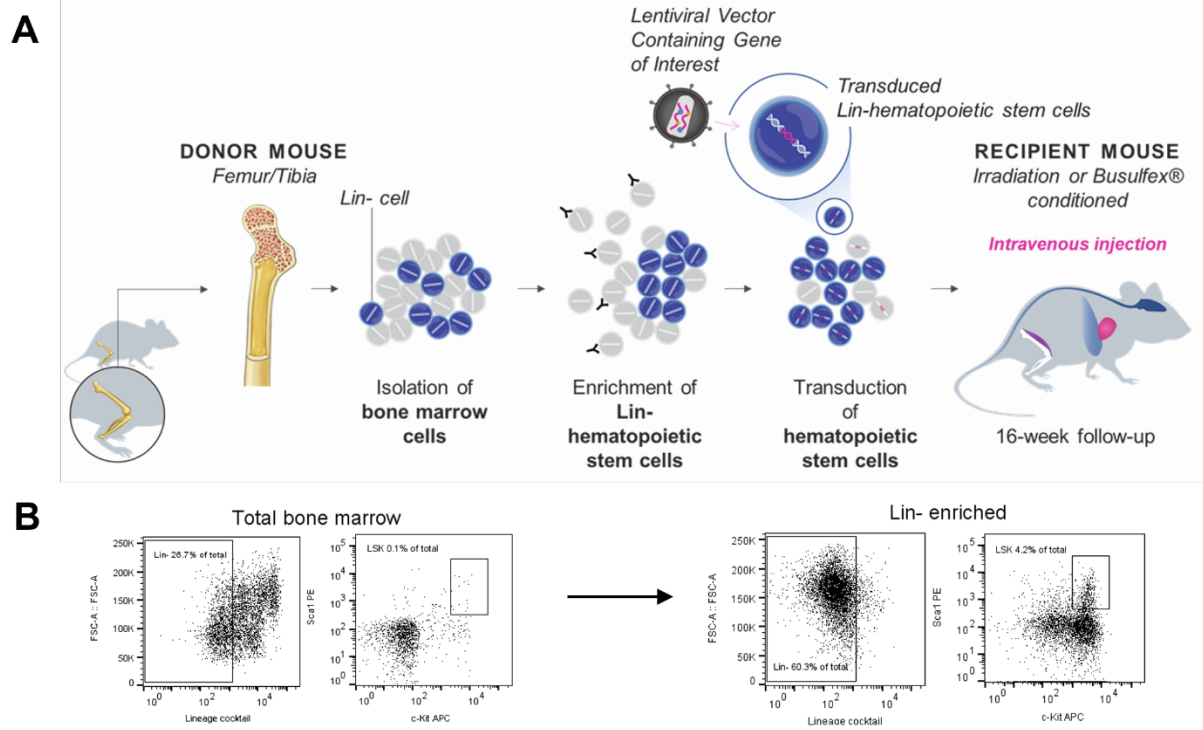
(B) Top: Western blot analysis of transduced HAP cell lysates (MOI 3) with all *GAA* containing vectors, using an anti-GAA monoclonal antibody. Middle: WB analysis of transduced HAP cell lysates with all *GAA* containing vectors, using an anti-GILT monoclonal antibody. An anti-GAPDH antibody was used for loading control. All three blots were run using the same cell lysate master mix preparation (NT = not transduced).



**Figure S3. Characterization of gene-edited K562 *GAA* and *IGF2R* knockout cells.**

(A) Representation of the human *GAA* gene exon 3 specific CRISPR/Cas9 guide sequence aligned to the human reference genome hg19. (B) *GAA* activity of wildtype *GAA*<sup>+/+</sup> K562 cell lysates (ATCC) and *GAA*<sup>-/-</sup> K562 clone 20. (C) WB analysis of wildtype *GAA*<sup>+/+</sup> K562 cells and *GAA*<sup>-/-</sup> K562 clone 20 lysates using anti-*GAA* antibody (clone 1C12C11/F9). (D) WB of *GAA*, *GILT-GAA* and *GILT-R37A-GAA* protein preparations are presented in Fig. 1C and

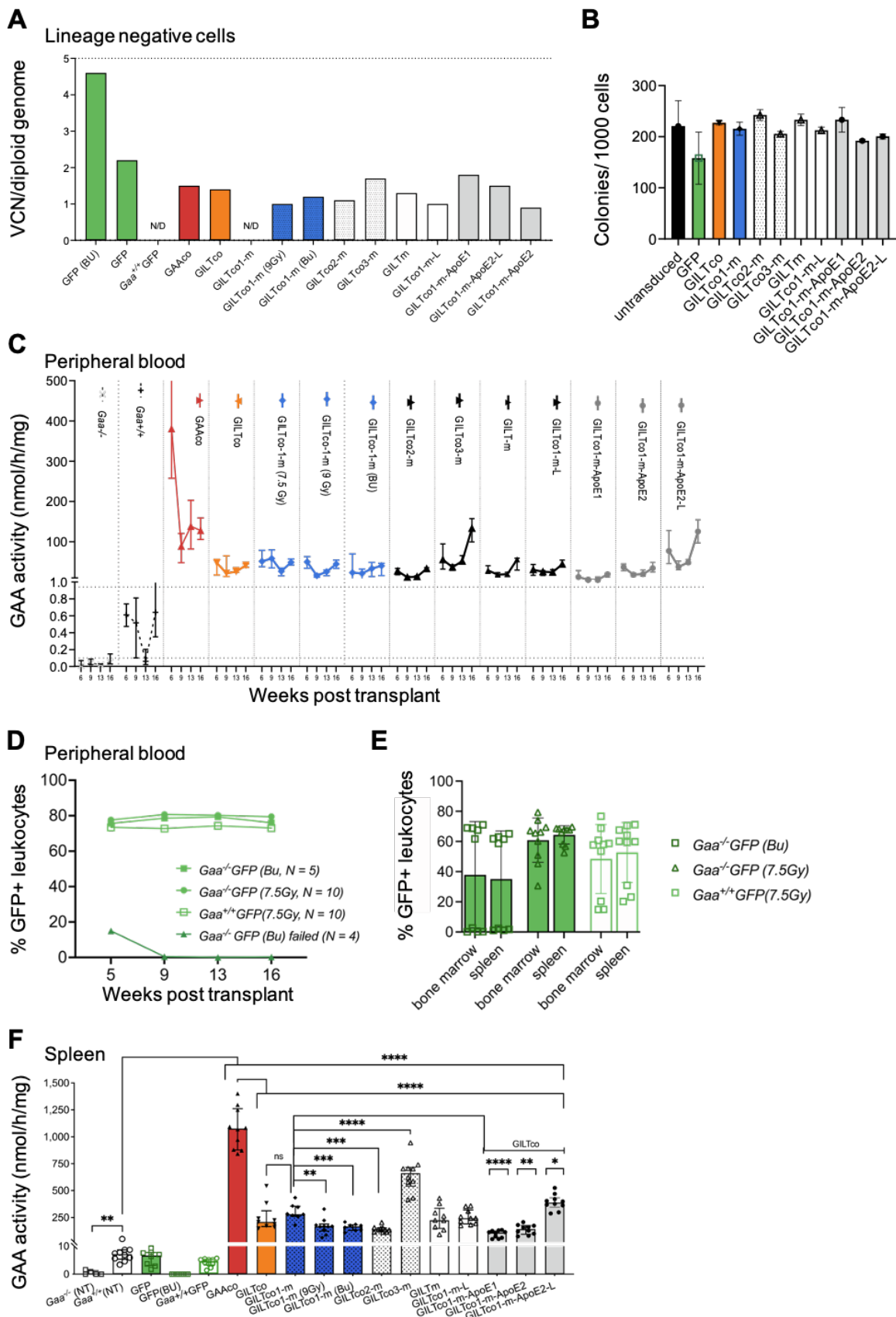
D. **(E)** GAA activity of the three purified GAA, GILT-GAA and GILT-R37A-GAA protein preparations mentioned under Fig. S3E. **(F)** Representation of the human *IGF2R* gene exon 2 specific CRISPR/Cas9 guide sequence aligned to the human reference genome hg19. **(G)** Flow cytometry analysis of wild-type (WT) parental, *GAA*<sup>-/-</sup> clone 20, *GAA*<sup>-/-</sup> *IGF2R*<sup>-/-</sup> clone 25 and *GAA*<sup>-/-</sup> + *IGF2R* vector clone 51 K562 cells. **(H)** WB of WT, *GAA*<sup>-/-</sup> clone 20, *GAA*<sup>-/-</sup> *IGF2R*<sup>-/-</sup> clone 25 using an IGF2R specific monoclonal antibody. **(I)** Quantification of IGF2R protein via WB normalized to beta-actin loading control. **(J)** Representative schema of a lentiviral vector containing the codon optimized *IGF2R* transgene driven by the EFS promoter used to transduce *GAA*<sup>-/-</sup> *IGF2R*<sup>-/-</sup> clone 25 at MOI 10. The resulting *GAA*<sup>-/-</sup> + *IGF2R* add-back cell line was noted as clone 51.



**Figure S4. Illustration of experimental enrichment and transplant model.**

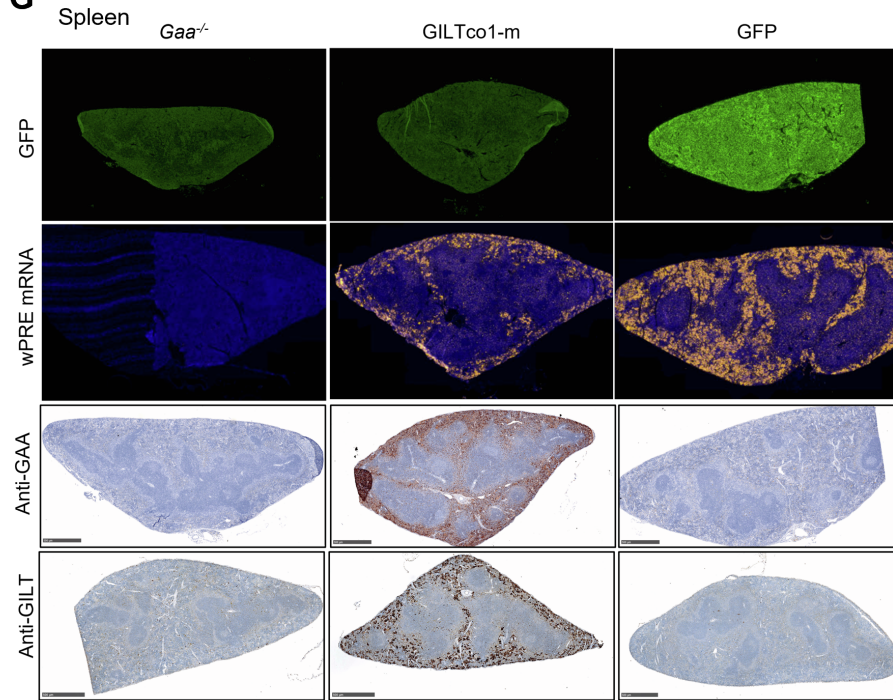
(A) Graphical depiction of the study design. Harvested donor bone marrow cells from femurs and tibias were Lin depleted using Robosep™ (StemCell Technologies), transduced with lentiviral vectors and infused into irradiation or Busulfex® conditioned recipient mice. (B) Representative dot plot of Lin- bone marrow cells enrichment. Frequencies of cell subpopulations are indicated as a percentage of total cells.

Supplementary figure 5

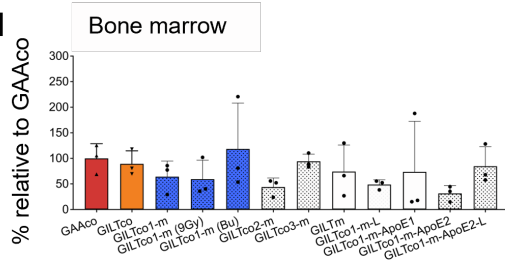


Supplementary figure 5

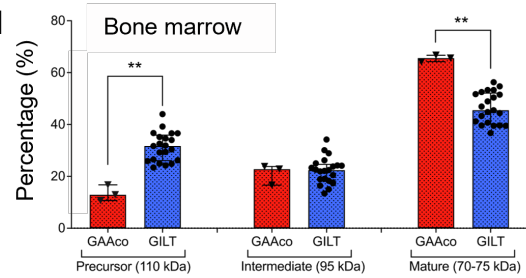
**G**



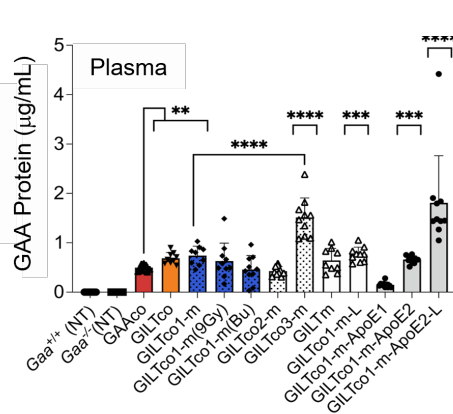
**H**



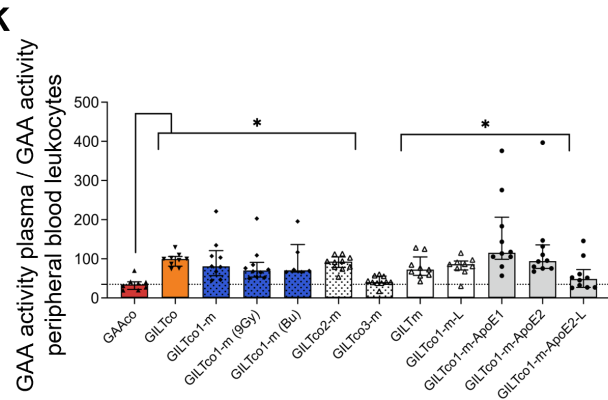
**I**



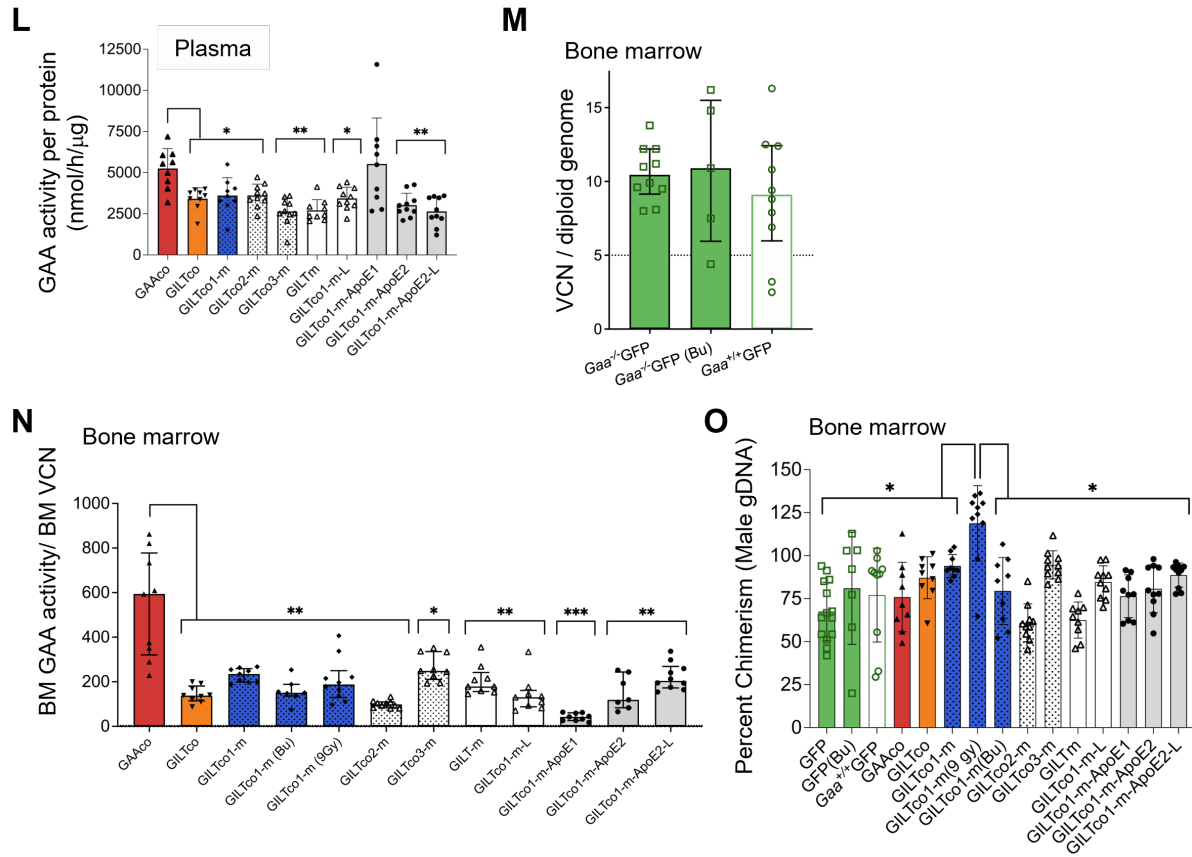
**J**



**K**



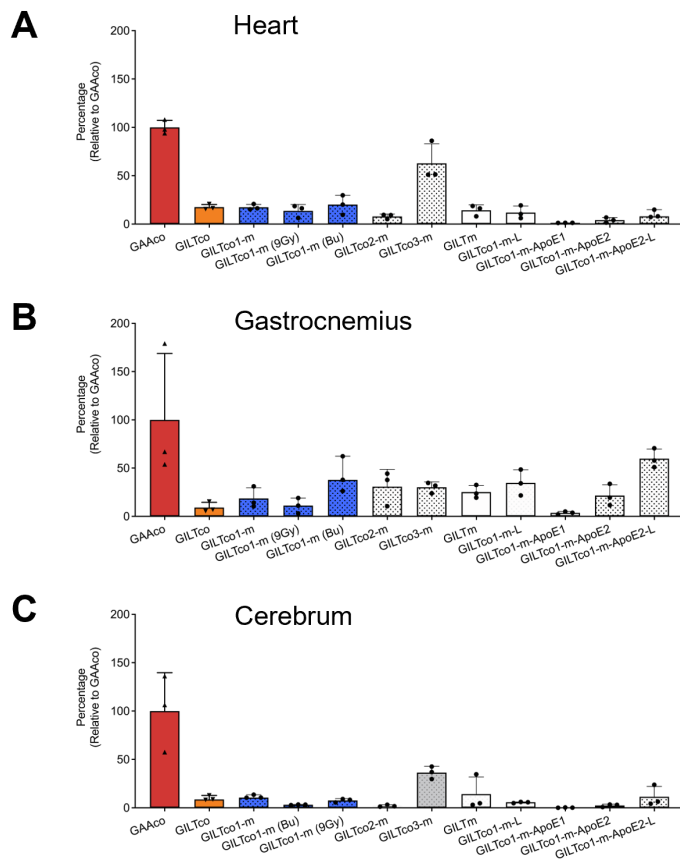




**Figure S5. Characterization of genetically modified cultured donor cells and recipient hematopoietic compartment.**

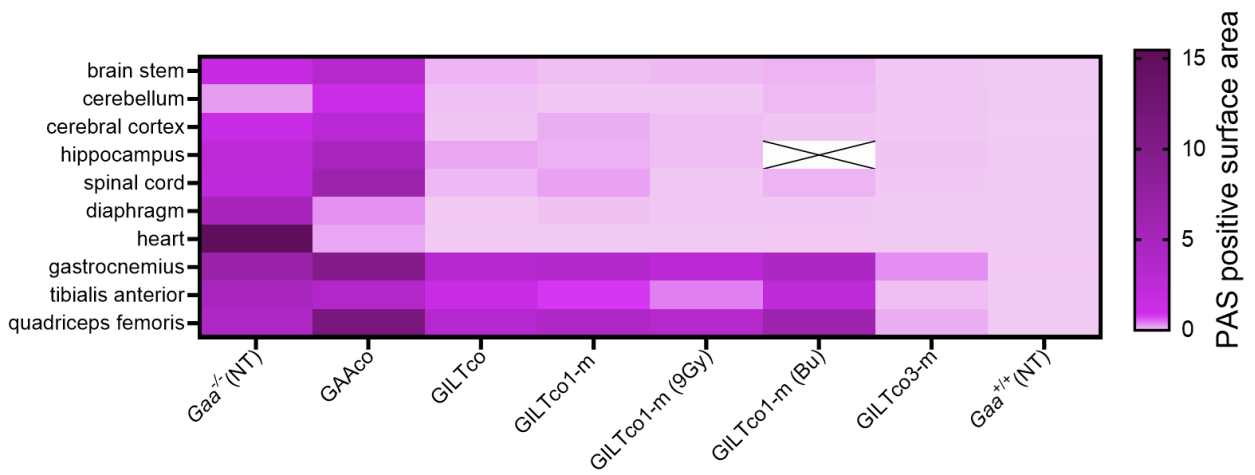
Cultured transduced  $Gaa^{-/-}$  Lin- donor bone marrow cells on day 7 were used to determine (A) VCN (duplicate measure of single samples; group  $Gaa^{-/-}$  GFP and GILTco-1-m contained insufficient material) (B) and HSPC differentiation potentials in CFU assays (n=2). (C) GAA activity in PB leukocytes of  $Gaa^{-/-}$  mice at weeks 6, 9, 13 and 16, group medians and interquartile ranges (n = 10). (D) % GFP+ cells in PB CD45+ leukocytes, at weeks 5, 9, 13 and 16 post-transplant, group medians shown (n = 8-13). (E) % GFP+ CD45+ leukocytes in bone marrow and spleen of the GFP vector groups at 16 weeks post-transplant, individual values, means, and SD shown (n = 9-10). (F) GAA activity in spleen at week 16 post-transplant, individual values, group medians, and interquartile ranges shown. Exact Wilcoxon Rank Sum p-values  $Gaa^{-/-}$  group with treatment groups, and GAAco and GILTco1-m with treatment groups (n = 8-10). (G) GFP immunofluorescence (top), FISH *WPRES* (second row), GAA (third row) immunohistochemistry and GILT (bottom) transcripts in spleens. (H) Bone marrow cells GAA protein normalized to beta-actin loading control reported as % of GAAco group. (I) Relative presence of precursor, intermediate and mature

GAA species in bone marrow lysates in the GAAco group (n = 3) and the groups containing only GILT/R37A (GILT, GILTm, GILTco1-m (7.5 Gy, 9 Gy, Bu), GILTco2-m and GILTco3-m; n = 21). **(J)** Final plasma GAA protein quantification per mL of mouse plasma ( $\mu\text{g/mL}$ ). **(K)** Ratio of GAA activity in the plasma and PB leukocytes at 16 weeks post-transplant, medians and interquartile ranges shown. Dotted line is the average of the GAAco group used as a reference (n = 8-10). Exact Wilcoxon Rank Sum p-values for comparing GAAco group to other treatment groups. **(L)** GAA activity calculated per protein in plasma. Exact Wilcoxon Rank Sum p-values for comparing GAAco group to other treatment groups. **(M)** VCN in bone marrow cell pellets measured in the GFP groups at week 16 post-transplant, individual values, group medians and interquartile ranges (n = 5-10). **(N)** Bone marrow GAA activity normalized to bone marrow VCN (n = 9-10) **(O)** Donor cells (male) chimerism via Y-chromosome PCR quantification, individual values, group means, and SD (n = 7-10). Kruskal Wallis p-values GILTco1-m comparison to all treated groups, significant, where p-value \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$  and \*\*\*\* $p < 0.0001$ .



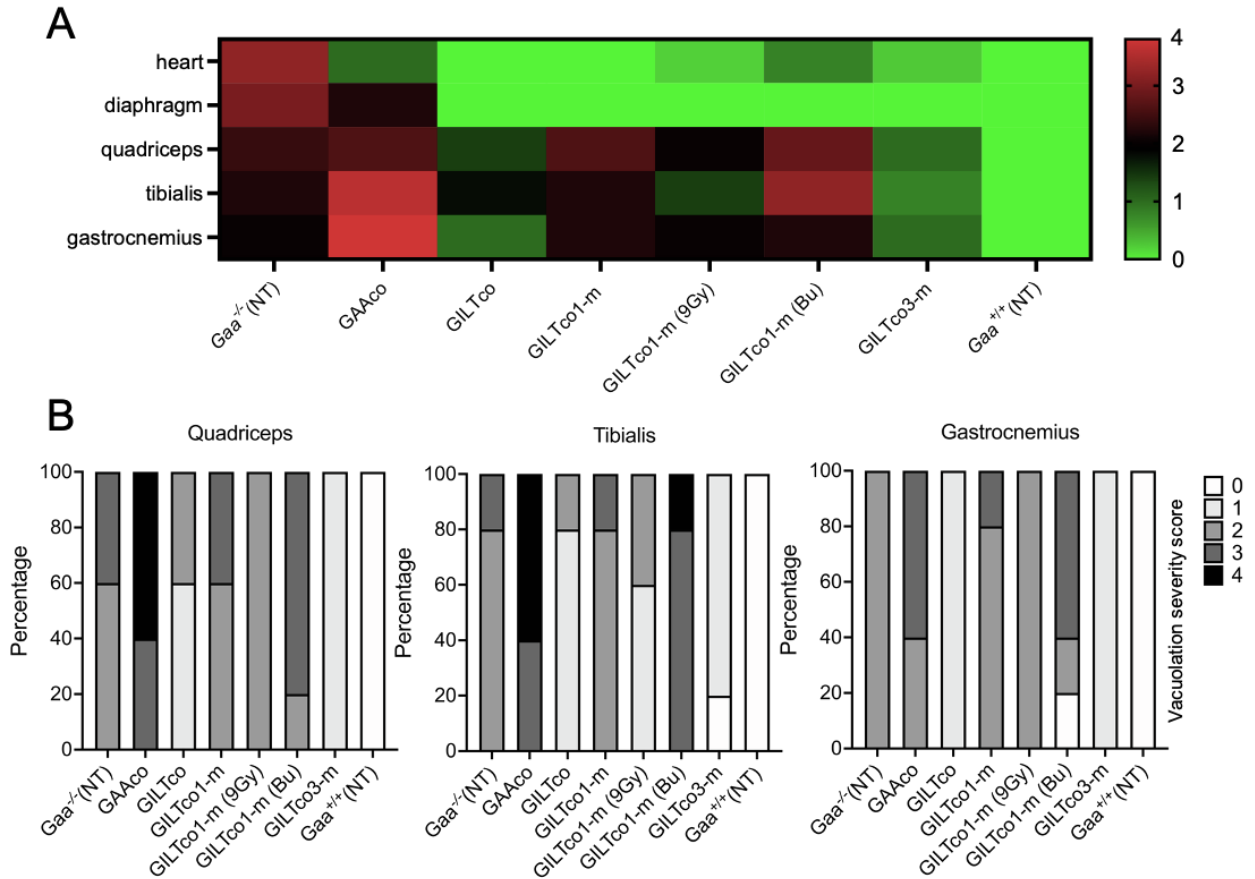
**Figure S6. Relative GAA protein levels in heart, gastrocnemius and cerebrum in treatment groups.**

GAA protein quantification in WB from (A) heart, (B) gastrocnemius and (C) cerebrum lysates normalized to GAPDH or beta-actin controls and reported as percentages of the respective GAAco group value. Data presented as mean  $\pm$  SD (n = 3).



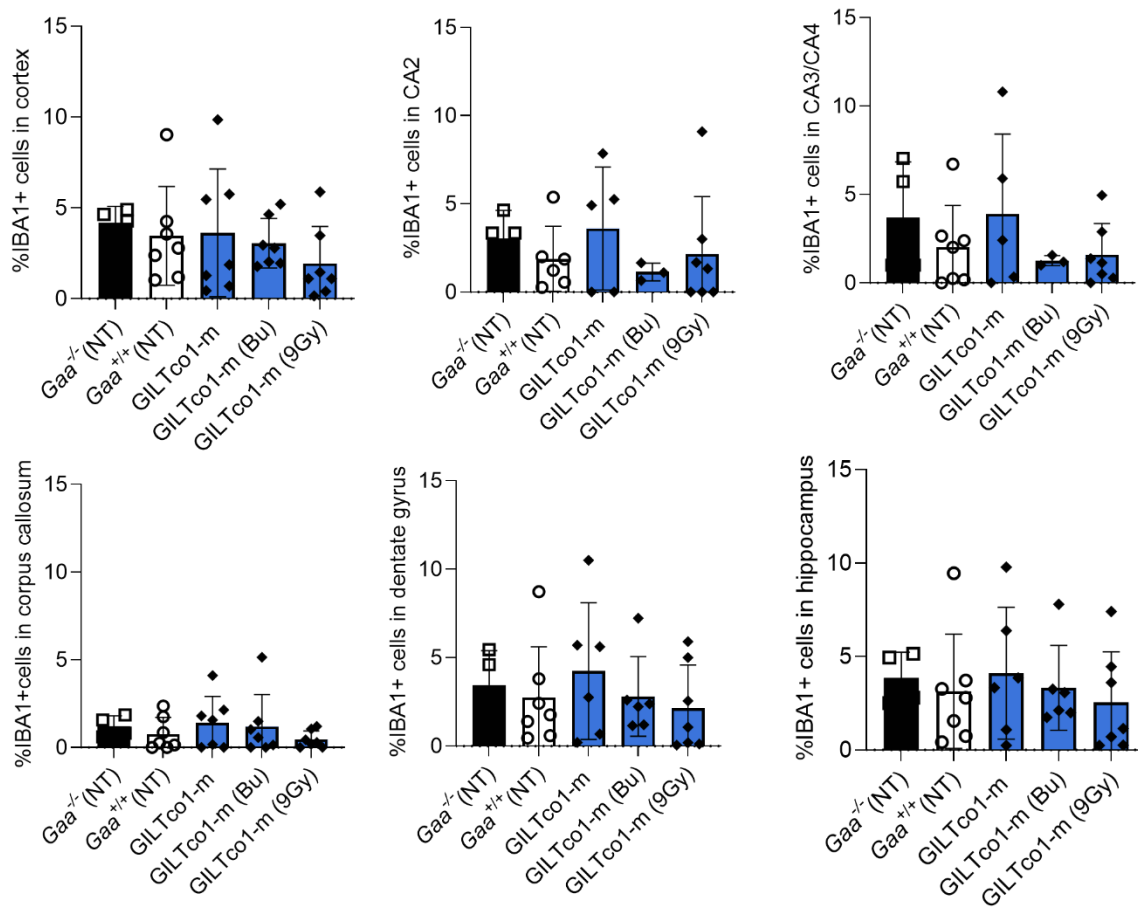
**Figure S7. Quantification of glycogen via PAS staining in heart, skeletal muscles and CNS.**

Glycogen accumulation in tissues analyzed by PAS staining at 16 weeks post-transplant. Intensity (light to solid) of purple color depicts means of PAS+ surface area in representative tissue samples (n = 3).



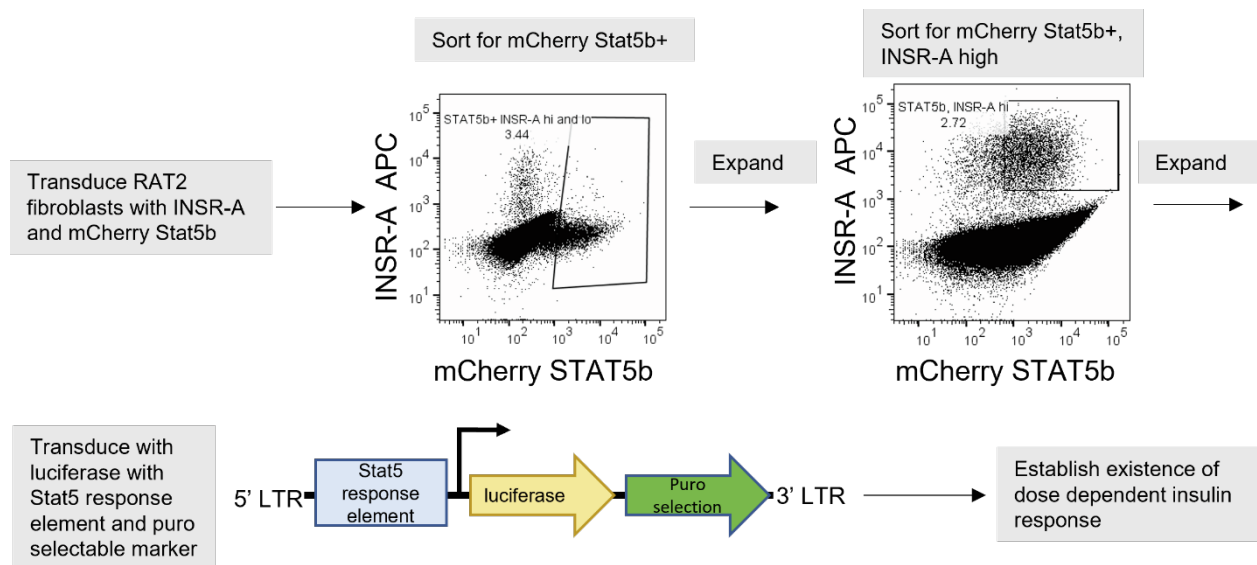
**Figure S8. Quantification of myofiber vacuolation in heart and skeletal muscle.**

(A) Assessment of selected groups for myofiber vacuolation in tissues stained with H&E. Severity scores assigned 0 to 4 from low to high, stained by H&E. (B) Percentages severity scores per group for quadriceps, tibialis and gastrocnemius (n = 4-5).



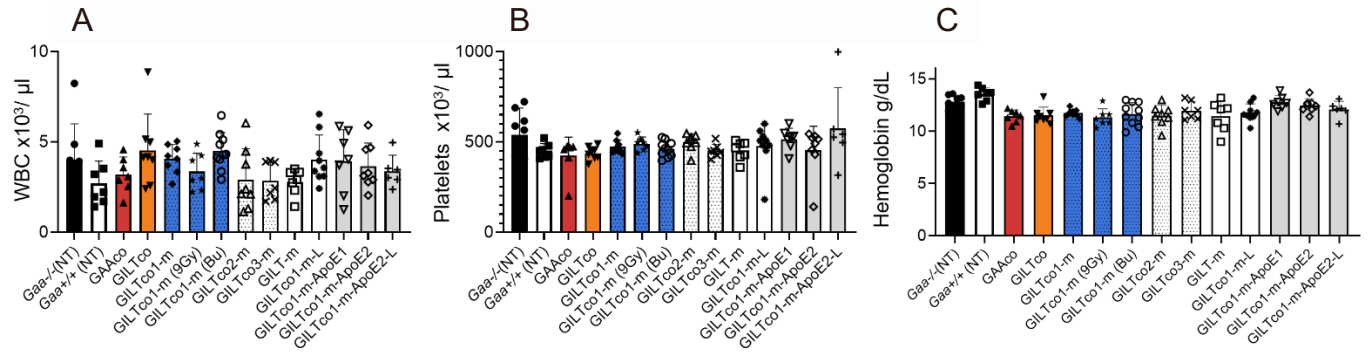
**Figure S9. Quantification of total Iba1+ cells in multiple brain regions.**

Brain tissue was stained for Iba1 and scanned slides were quantified by defining regions of interest where Iba1+ cells were counted as % of DAPI+ total cells per region. Group means with SD shown (n = 4-7).



**Figure S10. Diagram presenting the generation of insulin reporter cell line.**

RAT2 fibroblasts were transduced with a lentiviral vector expressing insulin receptor isoform A (INSR-A) and another expressing STAT5b, and sorted for INSR-A and mCherry positive cells. After expansion, the double positive INSR-A high/STAT5b high cells were collected, expanded, and transduced with a luciferase STAT5 reporter containing a puromycin selectable marker. Puromycin positive cells were selected and tested in a dose dependent luciferase response to insulin assay.



**Figure S11. Complete blood count results in transplanted mice.**

Absolute counts of (A) white blood cells (WBC) (B) platelets (C) hemoglobin at week 16 post-transplant. Individual values, group means, and SD shown (n = 6-9).



**Table S1. Study Design of the Vector Comparison Study.**

N of Mice	Donor Phenotype	Recipient Phenotype	Abbreviated Transduced Cell Identity	Donor Cell Dose	Conditioning
16	N/A	<i>Gaa</i> <sup>-/-</sup>	<i>Gaa</i> <sup>-/-</sup> (NT)	N/A	N/A
16	N/A	<i>Gaa</i> <sup>-/-</sup>	<i>Gaa</i> <sup>+/+</sup> (NT)	N/A	N/A
13	<i>Gaa</i> <sup>-/-</sup>	<i>Gaa</i> <sup>-/-</sup>	GFP	5 x 10 <sup>5</sup>	7.5 Gy
10	<i>Gaa</i> <sup>-/-</sup>	<i>Gaa</i> <sup>-/-</sup>	GFP (Bu)	5 x 10 <sup>5</sup>	Busulfex®
10	<i>Gaa</i> <sup>+/+</sup>	<i>Gaa</i> <sup>+/+</sup>	<i>Gaa</i> <sup>+/+</sup> GFP	5 x 10 <sup>5</sup>	7.5 Gy
10	<i>Gaa</i> <sup>-/-</sup>	<i>Gaa</i> <sup>-/-</sup>	GAACO	5 x 10 <sup>5</sup>	7.5 Gy
10	<i>Gaa</i> <sup>-/-</sup>	<i>Gaa</i> <sup>-/-</sup>	GILTco	5 x 10 <sup>5</sup>	7.5 Gy
10	<i>Gaa</i> <sup>-/-</sup>	<i>Gaa</i> <sup>-/-</sup>	GILTco1-m	5 x 10 <sup>5</sup>	7.5 Gy
10	<i>Gaa</i> <sup>-/-</sup>	<i>Gaa</i> <sup>-/-</sup>	GILTco1-m (9Gy)	5 x 10 <sup>5</sup>	9.0 Gy
10	<i>Gaa</i> <sup>-/-</sup>	<i>Gaa</i> <sup>-/-</sup>	GILTco1-m (Bu)	5 x 10 <sup>5</sup>	Busulfex®
10	<i>Gaa</i> <sup>-/-</sup>	<i>Gaa</i> <sup>-/-</sup>	GILTco2-m	5 x 10 <sup>5</sup>	7.5 Gy
10	<i>Gaa</i> <sup>-/-</sup>	<i>Gaa</i> <sup>-/-</sup>	GILTco3-m	5 x 10 <sup>5</sup>	7.5 Gy
10	<i>Gaa</i> <sup>-/-</sup>	<i>Gaa</i> <sup>-/-</sup>	GILTm	5 x 10 <sup>5</sup>	7.5 Gy
10	<i>Gaa</i> <sup>-/-</sup>	<i>Gaa</i> <sup>-/-</sup>	GILTco1-m-L	5 x 10 <sup>5</sup>	7.5 Gy
10	<i>Gaa</i> <sup>-/-</sup>	<i>Gaa</i> <sup>-/-</sup>	GILTco1-m-ApoE1	5 x 10 <sup>5</sup>	7.5 Gy
10	<i>Gaa</i> <sup>-/-</sup>	<i>Gaa</i> <sup>-/-</sup>	GILTco1-m-ApoE2	5 x 10 <sup>5</sup>	7.5 Gy
10	<i>Gaa</i> <sup>-/-</sup>	<i>Gaa</i> <sup>-/-</sup>	GILTco1-m-ApoE2-L	5 x 10 <sup>5</sup>	7.5 Gy

**Table S2. PAS+ stain quantification in CNS and skeletal muscle.**

	<i>Gaa</i> <sup>-/-</sup> (NT)	<i>Gaa</i> <sup>+/+</sup> (NT)	GAAco	GILTco	GILTco1-m	GILTco1-m (9Gy)	GILTco1-m (Bu)	GILTco3-m
<b>Brain Stem, N</b>	<b>3</b>	<b>3</b>	<b>3</b>	<b>3</b>	<b>3</b>	<b>3</b>	<b>3</b>	<b>3</b>
Percent PAS+, %	1.6683	0.0011	3.6396	0.1558	0.0744	0.0816	0.7902	0.0140
(SD)	(0.3424)	(0.0012)	(0.5917)	(0.0960)	(0.0788)	(0.0205)	(1.1699)	(0.0060)
<b>Cerebellum, N</b>	<b>3</b>	<b>3</b>	<b>3</b>	<b>3</b>	<b>3</b>	<b>3</b>	<b>3</b>	<b>2</b>
Percent PAS+, %	0.2819	0.0016	1.2362	0.0756	0.0126	0.0174	0.2930	0.0223
(SD)	(0.1137)	(0.0008)	(0.6042)	(0.0421)	(0.0119)	(0.0180)	(0.3941)	(0.0249)
<b>Cerebral Cortex, N</b>	<b>3</b>	<b>3</b>	<b>3</b>	<b>3</b>	<b>3</b>	<b>3</b>	<b>3</b>	<b>3</b>
Percent PAS+, %	1.1863	0.0002	3.3613	0.0675	0.1259	0.0555	0.6911	0.0092
(SD)	(0.4570)	(0.0002)	(1.1267)	(0.0603)	(0.1090)	(0.0094)	(1.1698)	(0.0044)
<b>Diaphragm, N</b>	<b>3</b>	<b>3</b>	<b>3</b>	<b>3</b>	<b>3</b>	<b>3</b>	<b>2</b>	<b>3</b>
Percent PAS+, %	4.8662	0.0240	0.3120	0.0073	0.0309	0.0384	0.0106	0.0072
(SD)	(2.7952)	(0.0404)	(0.1690)	(0.0045)	(0.0248)	(0.0358)	(0.0040)	(0.0097)
<b>Gastrocnemius, N</b>	<b>3</b>	<b>3</b>	<b>3</b>	<b>3</b>	<b>3</b>	<b>3</b>	<b>2</b>	<b>3</b>
Percent PAS+, %	6.8983	0.0111	10.0793	2.5953	3.8556	4.2678	4.3241	0.3160
(SD)	(4.5706)	(0.0090)	(1.1000)	(1.6335)	(1.3688)	(4.1667)	(3.1180)	(0.0928)
<b>Heart, N</b>	<b>3</b>	<b>3</b>	<b>3</b>	<b>3</b>	<b>3</b>	<b>3</b>	<b>3</b>	<b>3</b>
Percent PAS+, %	14.9640	0.0016	0.3090	0.0025	0.0037	0.0026	1.7620	0.0005
(SD)	(9.1151)	(0.0021)	(0.2585)	(0.0018)	(0.0034)	(0.0006)	(3.0408)	(0.0004)
<b>Hippocampus, N</b>	<b>1</b>	<b>2</b>	<b>2</b>	<b>3</b>	<b>3</b>	<b>3</b>	<b>0</b>	<b>2</b>
Percent PAS+, %	2.5743	0.0009	4.7405	0.1680	0.1584	0.0675	N/A	0.0295
(SD)	N/A	(0.0012)	(2.6534)	(0.0829)	(0.1703)	(0.0327)	N/A	(0.0102)
<b>Quadriceps Femoris, N</b>	<b>3</b>	<b>3</b>	<b>3</b>	<b>3</b>	<b>3</b>	<b>3</b>	<b>3</b>	<b>3</b>
Percent PAS+, %	8.8374	0.0725	10.9931	2.1886	3.7669	2.4127	7.2275	0.1554
(SD)	(10.3491)	(0.1227)	(0.8839)	(1.8304)	(0.6523)	(1.9177)	(5.5191)	(0.0356)
<b>Spinal Cord, N</b>	<b>3</b>	<b>3</b>	<b>3</b>	<b>3</b>	<b>3</b>	<b>3</b>	<b>3</b>	<b>3</b>
Percent PAS+, %	2.2401	0.0028	5.8312	0.1205	0.2225	0.0256	1.8864	0.0142
(SD)	(0.4483)	(0.0017)	(0.9861)	(0.0706)	(0.0742)	(0.0131)	(3.1222)	(0.0041)
<b>Tibialis Anterior, N</b>	<b>3</b>	<b>3</b>	<b>3</b>	<b>3</b>	<b>3</b>	<b>3</b>	<b>3</b>	<b>3</b>
Percent PAS+, %	5.5348	0.0086	3.5480	0.9759	1.2340	0.4417	6.9388	0.0570
(SD)	(4.4465)	(0.0094)	(3.1285)	(0.7629)	(0.8260)	(0.4188)	(8.7839)	(0.0368)

**Table S3. Vacuolation scores in heart and skeletal muscle.**

	<i>Gaa</i> <sup>-/-</sup> (NT)	<i>Gaa</i> <sup>+/+</sup> (NT)	GAAco	GILTco	GILTco1-m	GILTco1-m (9Gy)	GILTco1-m (Bu)	GILTco3-m
<b>Heart (# examined)</b>	5	5	5	5	5	5	5	5
<b>Vacuolation, myofiber</b>	5	0	5	0	0	0	3	1
Minimal	0	0	5	0	0	0	2	1
Mild	0	0	0	0	0	0	1	0
Moderate	4	0	0	0	0	0	0	0
Marked	1	0	0	0	0	0	0	0
<b>Vacuolation, vascular</b>	5	0	3	0	0	0	1	0
Minimal	2	0	3	0	0	0	1	0
Mild	3	0	0	0	0	0	0	0
<b>Quadriceps femoris (# examined)</b>	5	5	5	5	5	5	5	5
<b>Vacuolation, myofiber</b>	5	0	5	5	5	5	5	5
Minimal	0	0	0	3	0	0	0	5
Mild	3	0	0	2	2	5	1	0
Moderate	2	0	3	0	3	0	4	0
Marked	0	0	2	0	0	0	0	0
<b>Vacuolation, vascular</b>	1	0	2	0	0	0	0	0
Minimal	1	0	2	0	0	0	0	0
<b>Diaphragm (# examined)</b>	5	5	5	5	5	5	4	5
<b>Vacuolation, myofiber</b>	5	0	5	0	0	0	0	0
Minimal	0	0	0	0	0	0	0	0
Mild	0	0	4	0	0	0	0	0
Moderate	5	0	1	0	0	0	0	0
<b>Vacuolation, vascular</b>	4	0	1	0	0	0	0	0
Minimal	0	0	0	0	0	0	0	0
Mild	2	0	1	0	0	0	0	0
Moderate	2	0	0	0	0	0	0	0
<b>Gastrocnemius (# examined)</b>	5	5	5	5	5	5	5	5
<b>Vacuolation, myofiber</b>	5	0	5	5	5	5	4	5
Minimal	0	0	0	0	0	0	0	5
Mild	5	0	0	5	4	5	1	0
Moderate	0	0	1	0	1	0	3	0
Marked	0	0	4	0	0	0	0	0
<b>Vacuolation, vascular</b>	4	0	2	0	0	0	0	0
Minimal	0	0	2	0	0	0	0	0
Mild	4	0	0	0	0	0	0	0
<b>Tibialis anterior (# examined)</b>	5	5	5	5	5	5	5	5
<b>Vacuolation, myofiber</b>	5	0	5	5	5	5	5	4
Minimal	0	0	0	1	0	3	0	4
Mild	4	0	0	4	4	2	0	0
Moderate	1	0	2	0	1	0	4	0
Marked	0	0	3	0	0	0	1	0
<b>Vacuolation, vascular</b>	5	0	2	0	0	0	1	0
Minimal	0	0	0	0	0	0	1	0
Mild	5	0	0	0	0	0	0	0
Moderate	0	0	0	0	0	0	0	0
Marked	0	0	2	0	0	0	0	0

**Table S4 Vacuolation and degeneration scores in CNS**

	<i>Gaa</i> <sup>-/-</sup> (NT)	<i>Gaa</i> <sup>+/+</sup> (NT)	GAAco	GILTco	GILTco1-m	GILTco1-m (Bu)	GILTco1-m (9Gy)	GILTco3-m
<b>Brain (# examined)</b>	5	5	5	5	5	5	5	5
<b>Vacuolation, neuronal/axonal</b>	5	5	5	5	5	5	5	3
Minimal	0	0	0	5	3	2	4	3
Mild	0	0	0	0	2	1	1	0
Moderate	1	0	0	0	0	2	0	0
Marked	4	0	5	0	0	0	0	0
<b>Degeneration/necrosis neuronal/axonal</b>	5	0	1	4	3	4	4	4
Minimal	3	0	0	1	2	3	2	2
Mild	2	0	1	3	1	1	2	2
<b>Vacuolation, vascular</b>	5	0	5	1	4	4	0	0
Minimal	1	0	1	1	3	0	0	0
Mild	3	0	4	0	1	4	0	0
Moderate	1	0	0	0	0	0	0	0
<b>Vacuolation, meninges</b>	4	0	0	0	0	1	0	0
Minimal	3	0	0	0	0	1	0	0
Mild	1	0	0	0	0	0	0	0
<b>Vacuolation, choroid plexus</b>	2	0	2	3	1	4	0	0
Minimal	2	0	2	3	1	4	0	0
<b>Spinal Cord (# examined)</b>	5	5	5	5	5	5	5	5
<b>Vacuolation, neuronal/axonal</b>	5	0	5	1	5	5	0	1
Minimal	0	0	0	1	5	4	0	1
Mild	0	0	0	0	0	0	0	0
Moderate	1	0	0	0	0	0	0	0
Marked	4	0	5	0	0	0	0	0
Severe	0	0	0	0	0	1	0	0
<b>Degeneration/necrosis neuronal/axonal</b>	3	0	1	2	2	2	0	1
Minimal	1	0	1	2	2	1	0	1
Mild	2	0	0	0	0	1	0	0
<b>Vacuolation, vascular</b>	2	0	1	0	0	0	0	0
Mild	2	0	1	0	0	0	0	0

**Table S5. Blood Glucose over study time points.**

**Table S6. Flow cytometry immunophenotyping of the PB leukocytes at week 16 post-transplant.**

PB leukocytes 16 weeks							
Group name		% total leukocytes	% GR1+ Mac1+	% B220+	%CD3+	%CD4+	%CD8+
<i>Gaa</i> <sup>-/-</sup>	n	6	6	6	6	6	6
	Mean	91.61	29.04	33.98	24.39	16.46	7.21
	SD	3.01	8.68	6.85	5.57	4.38	1.70
<i>Gaa</i> <sup>+/+</sup>	n	9	9	9	9	9	9
	Mean	91.45	31.20	32.14	30.15	20.00	9.30
	SD	3.85	9.26	10.09	8.07	6.27	2.09
GFP	n	7	7	7	7	7	7
	Mean	96.10	15.43	34.67	40.75	29.41	10.57
	SD	1.40	4.14	2.81	2.94	2.53	1.69
GFP (Bu)	n	5	5	5	5	5	5
	Mean	94.87	23.17	30.97	38.02	28.88	8.51
	SD	2.35	9.25	5.04	6.66	5.42	1.63
GFP <i>Gaa</i> <sup>+/+</sup>	n	6	6	6	6	6	6
	Mean	94.42	12.87	35.14	44.03	32.17	10.89
	SD	1.84	3.83	4.96	5.77	4.91	1.74
GAAco	n	6	6	6	6	6	6
	Mean	92.97	36.55	28.45	28.57	21.25	6.83
	SD	1.98	11.21	8.29	3.82	2.52	1.81
GILTco	n	7	7	7	7	7	7
	Mean	93.08	33.22	35.50	25.13	17.36	7.23
	SD	1.08	11.05	10.17	6.88	4.90	2.05
GILTco1-m	n	9	9	9	9	9	9
	Mean	94.26	32.07	28.62	32.89	23.04	9.19
	SD	2.01	10.75	9.87	3.63	3.68	1.00
GILTco1-m (9Gy)	n	6	6	6	6	6	6
	Mean	92.20	25.94	35.77	30.61	23.29	6.81
	SD	0.89	4.19	4.85	2.58	2.54	0.29
GILTco1-m (Bu)	n	10	10	10	10	10	10
	Mean	93.26	43.96	23.24	27.83	19.47	7.77
	SD	1.88	7.94	8.00	4.86	3.11	2.01
GILTco2-m	n	8	8	8	8	8	8
	Mean	91.32	25.68	32.91	31.59	23.77	7.25
	SD	2.11	6.53	8.80	4.90	4.38	1.28
GILTco3-m	n	7	7	7	7	7	7
	Mean	89.47	29.87	29.73	31.13	23.50	7.01
	SD	2.92	8.73	4.41	5.34	4.14	1.28
GILT-m	n	6	6	6	6	6	6
	Mean	88.97	27.40	32.64	30.14	23.50	6.07
	SD	2.96	7.81	8.40	5.59	4.81	0.98
GILTco1-m-L	n	8	8	8	8	8	8
	Mean	91.63	23.73	35.86	31.12	23.61	6.98
	SD	2.54	3.98	3.09	1.60	1.61	0.70
GILTco1-m-ApoE1	n	7	7	7	7	7	7
	Mean	92.97	25.17	39.41	28.80	20.88	7.36
	SD	2.31	5.27	6.94	4.82	3.73	1.14
GILTco1-m-ApoE2	n	5	5	5	5	5	5
	Mean	88.62	22.39	32.60	35.80	27.23	8.03
	SD	3.90	1.70	3.19	1.59	1.89	0.63
GILTco1-m-ApoE2-L	n	3	3	3	3	3	3
	Mean	83.85	26.37	29.91	35.21	26.64	7.94
	SD	7.21	6.13	4.97	2.17	2.77	0.65

**Table S7. Flow cytometry immunophenotyping of the bone marrow at week 16 post-transplant.**

Group name		%total leukocytes	% GR1+ Mac1+	% B220+	% CD3+	% CD4+	% CD8+
<b>Gaa<sup>-/-</sup></b>	n	10	10	10	10	10	10
	Mean	97.70	58.99	22.86	3.44	1.16	1.59
	SD	0.61	5.67	4.56	1.45	0.44	0.87
<b>Gaa<sup>+/+</sup></b>	n	10	10	10	10	10	10
	Mean	97.37	48.80	28.11	6.92	2.82	3.10
	SD	2.29	5.24	5.02	1.73	0.82	1.05
<b>GFP</b>	n	10	10	10	10	10	10
	Mean	97.82	33.20	41.95	8.83	3.38	4.34
	SD	0.38	7.46	6.59	2.17	1.05	1.16
<b>GFP (Bu)</b>	n	5	5	5	5	5	5
	Mean	97.70	38.13	38.80	7.91	3.28	3.62
	SD	0.59	6.32	5.58	1.23	0.58	0.67
<b>GFP Gaa<sup>+/+</sup></b>	n	10	10	10	10	10	10
	Mean	98.53	37.33	35.76	10.56	4.72	4.47
	SD	0.31	13.78	9.70	4.83	2.16	2.28
<b>GAAco</b>	n	10	10	10	10	10	10
	Mean	97.90	57.21	23.89	4.35	1.58	1.89
	SD	0.46	2.37	2.12	0.79	0.44	0.42
<b>GILTco</b>	n	9	9	9	9	9	9
	Mean	97.53	54.59	23.48	5.71	1.74	2.10
	SD	0.76	5.74	4.24	1.54	0.61	0.58
<b>GILTco1-m</b>	n	9	9	9	9	9	9
	Mean	97.33	55.94	23.94	4.39	1.60	1.86
	SD	0.51	2.31	2.28	0.88	0.43	0.43
<b>GILTco1-m (9Gy)</b>	n	10	10	10	10	10	10
	Mean	97.62	53.39	26.92	5.16	2.02	2.59
	SD	0.44	6.41	5.63	1.24	0.50	0.72
<b>GILTco1-m (Bu)</b>	n	10	10	10	10	10	10
	Mean	97.52	54.41	24.53	4.87	1.70	2.03
	SD	0.42	4.89	4.32	1.56	0.31	0.55
<b>GILTco2-m</b>	n	10	10	10	10	10	10
	Mean	97.92	53.35	27.75	4.52	1.72	2.26
	SD	0.39	4.34	4.10	1.13	0.48	0.60
<b>GILTco3-m</b>	n	10	10	10	10	10	10
	Mean	97.15	54.84	24.80	5.09	1.90	2.62
	SD	2.21	5.41	4.09	1.23	0.52	0.69
<b>GILT-m</b>	n	9	9	9	9	9	9
	Mean	97.94	52.91	27.11	5.43	2.10	2.71
	SD	0.26	2.39	2.69	0.95	0.39	0.56
<b>GILTco1-m-L</b>	n	10	10	10	10	10	10
	Mean	97.77	48.52	29.72	4.68	1.75	2.40
	SD	0.49	3.35	3.32	0.83	0.45	0.39
<b>GILTco1-m-ApoE1</b>	n	10	10	10	10	10	10
	Mean	98.04	52.80	28.36	4.70	1.91	2.28
	SD	0.35	4.94	4.59	0.61	0.31	0.32
<b>GILTco1-m-ApoE2</b>	n	10	10	10	10	10	10
	Mean	97.61	49.89	28.16	5.56	2.20	2.80
	SD	0.28	1.98	1.43	1.16	0.56	0.59
<b>GILTco1-m-ApoE2-L</b>	n	10	10	10	10	10	10
	Mean	97.66	51.64	26.90	5.78	2.23	2.94
	SD	0.37	4.57	3.54	1.33	0.52	0.76

**Table S8. Flow cytometry immunophenotyping of the spleen at week 16 post-transplant.**

Group name		% total leukocytes	% GR1+ Mac1+	% B220+	%CD3+	%CD4+	%CD8+
<b>Gaa-/-</b>	n	10	10	10	10	10	10
	Mean	96.13	5.96	55.63	26.35	13.60	10.73
	SD	3.16	3.56	4.63	5.53	3.41	2.47
<b>Gaa+/+</b>	n	10	10	10	10	10	10
	Mean	96.83	4.34	49.95	37.75	19.66	16.29
	SD	1.10	1.31	4.77	3.80	2.14	1.84
<b>GFP</b>	n	10	10	10	10	10	10
	Mean	98.58	2.80	57.80	31.40	18.44	10.87
	SD	0.45	0.63	3.37	3.63	2.39	1.19
<b>GFP (Bu)</b>	n	5	5	5	5	5	5
	Mean	98.85	2.73	56.92	32.95	19.64	11.32
	SD	0.24	0.85	3.03	3.52	2.09	1.59
<b>GFP Gaa+/+</b>	n	10	10	10	10	10	10
	Mean	98.83	1.98	58.12	33.09	20.00	11.27
	SD	0.68	1.52	4.62	3.31	2.92	1.13
<b>GAAco</b>	n	10	10	10	10	10	10
	Mean	97.29	6.31	49.55	34.51	22.07	11.02
	SD	1.16	2.32	3.93	4.69	3.31	1.43
<b>GILTco</b>	n					9	9
	Mean	97.66	5.58	56.10	30.47	18.13	10.94
	SD	0.77	2.53	5.67	6.04	4.41	1.92
<b>GILTco1-m</b>	n	9	9	9	9	9	9
	Mean	96.66	7.83	47.74	34.39	21.36	11.33
	SD	1.11	2.32	3.67	3.87	3.00	1.22
<b>GILTco1-m (9Gy)</b>	n	10	10	10	10	10	10
	Mean	97.48	5.19	55.50	31.03	19.56	10.02
	SD	0.94	1.16	2.06	2.03	1.64	0.95
<b>GILTco1-m (Bu)</b>	n					10	10
	Mean	98.40	4.70	54.65	32.35	19.55	11.45
	SD	0.32	1.20	3.11	3.34	2.33	1.24
<b>GILTco2-m</b>	n	10	10	10	10	10	10
	Mean	97.69	5.11	56.59	29.01	18.01	9.72
	SD	0.69	1.59	3.76	4.13	2.91	1.37
<b>GILTco3-m</b>	n	10	10	10	10	10	10
	Mean	96.95	5.13	54.31	30.79	19.25	10.26
	SD	0.73	2.00	2.81	3.18	2.36	1.56
<b>GILT-m</b>	n	9	9	9	9	9	9
	Mean	97.02	4.05	4.82	54.21	19.55	10.06
	SD	0.58	0.81	1.45	2.73	3.45	1.35
<b>GILTco1-m-L</b>	n	10	10	10	10	10	10
	Mean	97.74	5.20	56.72	28.91	18.13	9.52
	SD	0.65	1.47	3.56	3.65	2.68	1.04
<b>GILTco1-m-ApoE1</b>	n	10	10	10	10	10	10
	Mean	97.79	5.52	54.68	30.71	19.85	9.56
	SD	0.39	1.67	3.10	2.74	1.47	1.30
<b>GILTco1-m-ApoE2</b>	n	10	10	10	10	10	10
	Mean	98.24	3.69	55.57	31.14	19.96	9.85
	SD	0.41	0.79	4.12	4.02	3.05	1.03
<b>GILTco1-m-ApoE2-L</b>	n	10	10	10	10	10	10
	Mean	96.91	4.99	54.18	31.22	19.57	10.24
	SD	1.25	1.71	2.31	2.45	1.94	0.85

**Table S9. Primer and probe sequences**

Target	Purpose	Primer	Sequence
<i>Gtdc1</i>	Housekeeping	Forward	GAAGTTCAGGTTAATTAGCTGCTG
<i>Gtdc1</i>	Housekeeping	Reverse	GGCACCTTAACATTTGGTTCTG
<i>Gtdc1</i>	Housekeeping	Probe	ACGAACTTCTTGGAGTTGTTTGCT
Psi element	Vector copy number	Forward	CAGGACTCGGCTTGCTGAAG
Psi element	Vector copy number	Reverse	TCCCCCGCTTAATACTGACG
Psi element	Vector copy number	Probe	CGCACGGCAAGAGGCGAGG
<i>Zfy1</i>	Donor cell chimerism	Forward	TGGAGAGCCACAAGCTAACCA
<i>Zfy1</i>	Donor cell chimerism	Reverse	CCCAGCATGAGAAAGATTCTTC
<i>Bcl2</i>	Housekeeping	Forward	AAGCTGTCACAGAGGGGCTA
<i>Bcl2</i>	Housekeeping	Reverse	CAGGCTGGAAGGAGAAGATG