Supporting Information

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SI Methods

LV Construction, Packaging, and Concentration. Three bicistronic self-inactivating LVs were constructed by insertion into a third-generation LV backbone pLV-TW (1) at the Afe I restriction sites (between cppt and WPRE) with EF-1α (GenBank database, accession no. AF403737, 1–1192); an LTR promoter/enhancer from SF (2); or an erythroid-specific hybrid promoter containing a human ALAS2 intron 8 erythroid-specific enhancer, HS40 core element from human α-globin locus control region, and human ankyrin-1 promoter (3). The transfer LVs were packaged by cotransfection of 293T cells with 3 helper plasmids: p2NRF for *gag-pol*, pEF1.Rev for *Rev*, and pMD.G for VSVG *env* function as described in ref. 1. The potency of viral stocks (typical 10^8–10^9 TU/mL) was determined by FACS analysis for GFP$^+$ percentage on 293T cells or MEL cells (for LV-KIiG vector) exposed to serial LV dilutions using a FACS Canto Flow Cytometer. Less than 30% of GFP$^+$ cells were considered reliable for titer calculation when most transduced cells contained 1 transgene copy.

Cell Line Manipulation. MEL cells were cultured at the concentration of 0.5–2.0 $\times$ 10^6 cells/mL in DMEM with 10% FBS (v/v) and antibiotics. Cells were transduced with each vector stock at 2–3 MOI. To induce erythroid differentiation, MEL cells were subcultured at 10^6 cells/mL in DMEM containing 20% FBS and 1 mg/mL HMBA with medium change every other day for a total of 8 days. To monitor erythroid differentiation, cytospins were prepared at 500 rpm $\times$ 5 min in a Cytocentrifuge (Cytospin-4, Thermo Shandon, Inc.) and stained with Wright's stain (Harleco EMD).

LCL cell lines were prepared by transformation of PBLs from a normal individual or an MPS I patient with Epstein-Barr virus (4). Cells were routinely cultured in RPMI medium with 10% (v/v) FBS, 2 mM glutamine, and antibiotics. All cells were maintained at 37 °C in a humidified atmosphere containing 5% (v/v) CO_2 and were routinely tested for *Mycoplasma* infection.

Uptake Assay and Lysosomal Staining in LCL Cells. To evaluate enzyme uptake in enzyme-deficient cells, 1 $\times$ 10^6 LCLmps cells were incubated for 3 h at 37 °C with 1 mL of medium that was preconditioned by a 24-h culture of MEL-KIiG at day 7 of induction culture and contained 30 U/mL IDUA enzyme. Control medium, which was preconditioned by a 24-h culture of MEL cells at day 7 of induction and contained undetectable IDUA, was applied to untreated LCLmps and LCLnormal cells. To inhibit IDUA uptake, 1 mM M6P (Sigma) was added 30 min before subculture with enzyme-containing medium as well as during uptake incubation. Each experiment was performed in triplicate wells.

To study lysosomal morphology change, an aliquot of the treated cells described above was washed 3 times and incubated for 1 h with 75 nM LysoTracker Red (Invitrogen). After 3 washing steps with PBS and 1% FBS, we then fixed cells with 4% (w/v) paraformaldehyde, followed by cytospin at 500 rpm $\times$ 5 min in a Cytospin 4 Centrifuge at 1 $\times$ 10^5 cells/spot. The slides were then mounted using Vectashield mounting medium with DAPI (Vector Laboratories, Inc.) and observed using an Olympus inverted fluorescence microscope.

Isolation, Transduction, and Transplantation of Lin$^-$ Cells. To enrich HSC, low-density bone marrow cells were stained with a set of biotinylated antibodies including anti-CD3e, B220, CD4, CD8, CD11b, Gr-1, and Ter119, followed by lineage depletion using antibiotin microbead mediated MACS LS column (Miltenyi Biotec, Inc.). Ex vivo transduction of Lin$^-$ cells was conducted by culturing cells for a 12-h prestimulation period in serum-free StemSpan medium (StemCell Technologies) supplemented with 40 μ/ml LDL, 50 ng/ml stem cell factor (SCF), 20 ng/ml thrombopoietin (TPO), 10 ng/ml IL3, 50 ng/ml IL6. Cells were then transduced twice within 24-h at the presence of 8 μg/ml protamine sulfate. Lin$^-$ cells were then injected into lethally irradiated (split dosage of 700 and 475 cGy) mice at 10^5 cells/mouse. All animal procedures were approved by Institutional Animal Care and Use Committee of Cincinnati Children's Hospital Medical Center.

Immunochemical Staining and Flow Cytometry Analysis. LCL cells were stained with Lyso Tracker Red (Invitrogen), and evaluated using mounting medium with DAPI (Vector Laboratories Inc.). Fresh bone marrow or CFU-S cells were immunostained with PE-conjugated anti-CD71 and PE-Cy7-conjugated anti-Ter119 (BD Biosciences) as described in ref. 5, with concurrent staining for 7-amino-actinomycin D (BD Biosciences) to gate out apoptotic cells. Single cell suspensions were analyzed using a FACS Canto with FacsDiva software v6.1 (Becton Dickson).

Spleen Colony-Forming Unit Assay. CFU-S assay was performed by transplanting 1 $\times$ 10^5 bone marrow cells from a primary recipient into each irradiated (950 cGy) C57BL/6J mouse. Discrete spleen colonies were collected 12 days after transplantation. Aliquots from each colony were analyzed by enzyme assay for IDUA expression, and by qPCR for copy number analysis.

IDUA Enzyme Assay. The catalytic activity of IDUA was measured with a fluorometric enzyme assay as previously described, with modifications (6). Cell pellets were homogenized in distilled water using an Ultrasonic Processor (GE-130, Sonics and Materials). Aliquots of cleared lysate, plasma, or culture medium were incubated with 2.5 mM fluorogenic substrate and 4-methylumbelliferyl (4MU) α-L-idopyranosiduronic acid sodium salt (Toronto Research Chemicals, Inc.), together with no sample blank controls in parallel. All samples were assayed in duplicate reactions, and each reaction was quantified twice using a fluorescent plate reader (SPECTRA MAX Gemini XS; Molecular Devices). Protein concentration was measured by a Coomassie blue dye-binding assay (BioRad). One unit of enzyme activity is defined as the release of 1 nmol 4MU in a 1-h reaction at 37 °C. The intracellular IDUA specific activity was calculated as U/mg of protein, and extracellular IDUA activity was measured as U/mL medium.

Real-Time qPCR. We quantified both GFP transgene and endogenous murine ApoB simultaneously in the same 25-μL reaction by real-time PCR as described previously, with minor modifications (7). Genomic DNA was isolated from PBLs, bone marrow, or CFU-S colonies with a Gentra Puregene Blood Kit (Qiagen). The multiplex reaction contained 5–20 ng of genomic DNA, 200 nM each of GFP primer, 200 nM TaqMan GFP probe, 40 nM each of ApoB primer, 200 nM ApoB probe, and 12.5 μL of TaqMan 2X Universal Master Mix (Applied Biosystems). Unknown samples were run in triplicate, and standard samples were run in duplicate. A standard curve (ranging from 0.1–100%) was established from a series of genomic DNA mixtures (10 ng) of a murine myeloid cell line (32Dp210) with a GFP-

containing cell line (32Dp210-LNChRGFP) (1 copy per genome as determined by Southern blot analysis). The amplification conditions were 2 min at 50 °C and 10 min at 95 °C for the initial cycle, followed by 45 cycles at 95 °C for 15 sec and 60 °C for 1 min.

Quantification of Urinary GAG. Urine samples were obtained by bladder palpation. We quantified GAG excretion based on methods described in ref. 8, with modifications. Briefly, urine aliquots were serially diluted with sodium formate buffer (pH 3.0) and mixed in duplicate with freshly prepared 1,9-dimethylmethylene blue solution (0.35 μM in sodium formate buffer, pH 3.0). Absorbance of the color reaction was measured at 535 nm within 30 min on a DU50 spectrophotometer (Beckman Coulter) and compared with the standard curve generated with heparan sulfate standard solutions (Sigma). To normalize urine concentration, urinary creatinine was quantified by incubating diluted samples with freshly made picric acid/sodium hydroxide solution (10%-v/v saturated picric acid and 0.09 M NaOH) for 20 min, measuring absorbance at 535 nm, and calculated using the standard curve established with creatinine reference solutions (Sigma).

Chemical Staining and Pathology Evaluation. At the end of observation period, mice were euthanized by i.p. administration of an overdose of sodium Nembutal (Abbott Laboratories). After blood collection and removal of hind legs for marrow harvest, each mouse was perfused transcardially through the aorta with cold normal saline briefly, followed by 4% (w/v) paraformaldehyde. Tissue samples were fixed by 2% (w/v) glutaraldehyde in 0.175 M sodium cacodylate buffer (pH 7.4) at 4 °C. The tissue was then treated with 1% osmium tetroxide, washed in 0.175 M sodium cacodylate buffer, dehydrated by a graded ethanol series, and embedded in LX112. Sections (0.5–1 μm) were prepared and stained with 1% Toluidine blue in 1% sodium borate, followed by examination for the presence of pathological storage vacuoles. Two animals per group were analyzed, with 6 sections randomly selected from 3 slides for each organ. For brain pathology scoring, cells containing <5 cytoplasmic vacuoles were considered normal, whereas those with >30 vacuoles were considered positive. More than 500 microvessels were scored for each animal from 9 sections randomly picked from 3 slides. The mean of scoring data from 6 slides is shown for each group.

Behavioral Test. The repeated open-field test was performed 5 months after BMT at the age of 7 months, as described in ref. 9. The open-field apparatus (60×60 cm) consisted of a white Plexiglas box with 25 squares (12×12 cm) painted on the floor (16 outer and 9 inner). Briefly, the mouse was placed in one of the 4 corners of the apparatus and allowed to explore the whole field freely for 5 min. Activity was monitored and quantified for ambulation (number of inner and outer squares crossed), rearing frequency, and time spent grooming by 2 observers who did not know the genotype or treatment of the animal during testing. Each mouse was tested for 3 repeated trials with 30-min intertrial intervals.

Statistical Analysis. All quantitative assays were performed in duplicate or triplicate from at least 2 individual experiments. Data are presented as mean $\pm$ SD unless specified. Comparisons between the 2 groups were performed using two-tailed Student's t tests. Probability values of <0.05 were considered to be statistically significant.

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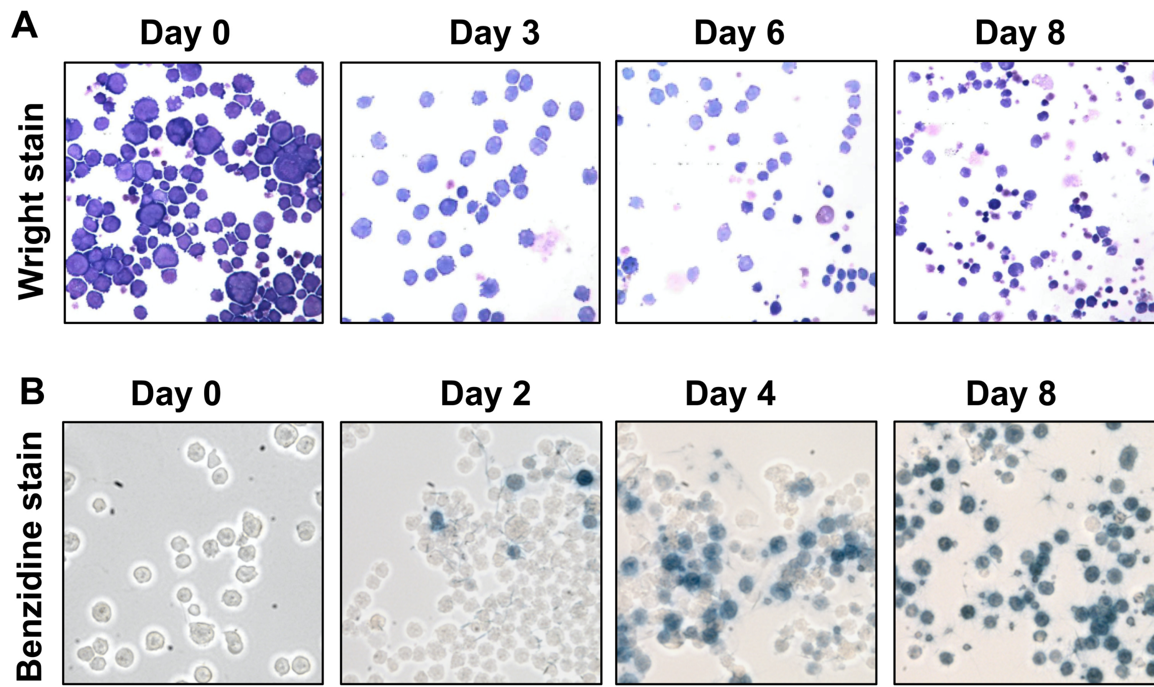

Fig. S1. Erythroid differentiation of MEL cells during HMBA induction. (*A*) Morphology changes in MEL cells during inductive culture with 1 mg/mL HMBA. Cytospin slides were counterstained with Wright's dye, demonstrating a gradual reduction of cell volume. (*B*) Detection of hemoglobin-expressing cells by histochemical staining with benzidine-hydrogen peroxide solution.

Fig. S2. Erythroid-derived IDUA is functional in correcting a patient's cells. LCLmps cells were exposed for 3 h to medium preconditioned by a 24-h culture of enzyme-overexpressing MEL-KIiG at day 7 of induction (30 U/ml IDUA), with or without the presence of 1 nM M6P inhibitor. (*A*) IDUA-specific activity in cell lysate. Data were derived from 2 experiments in triplicate. (*B*) Normalization of lysosomal abundancy in treated LCLmps cells. Cells were labeled with lysosome-specific probe (red) and counterstained with DAPI (blue) for nuclei.

Fig. S3. Cellular composition of CFU-S and GFP expression in clonal progeny of GFP^{+} CFU-S colonies. (*A*) Discrete CFU-S colonies were collected from 6 mice (1 KIiG donor into 5 recipients and 1 normal donor into 1 control recipient) 12 days after transplantation, followed by immunostaining with erythroid markers CD71 (C) and Ter119 (T). A representative cytogram is shown to define nonerythroid cells (C^{-}T^{-}), early-stage erythroblasts (C^{+}T^{-}), mid/late-stage erythroblasts (C^{+}T^{+}), and reticulocytes/mature RBCs (C^{-}T^{+}). (*B*) Cellular compositions of all individual colonies collected (except 1 lost colony with significantly fewer amounts of cells) are shown. (*C*) GFP expression in 4 subpopulations of a GFP^{+} CFU-S colony (dark lines) and a colony from a control animal (light dotted lines). Arrows indicate MFI of GFP.

Table S1. Erythrocyte parameters in mice

Parameters	MPS (n = 7)	MPS/KIiG (n = 6)	MPS/WT (n = 7)	Normal (n = 9)
RBC, $\times 10^6$ cells/μL	8.8 ± 0.7	8.6 ± 0.4	8.8 ± 0.4	8.6 ± 1.0
HGB, g/dL	12.6 ± 1.0	12.0 ± 0.5*	11.9 ± 0.7†	12.9 ± 1.1
HCT, %	42.2 ± 4.7	41.8 ± 2.7	41.7 ± 1.5	42.2 ± 4.6
MCV, fL	47.3 ± 2.3	48.1 ± 3.3	47.6 ± 1.4	49.0 ± 2.9
MCH, pg	14.0 ± 0.4	14.5 ± 1.3	13.7 ± 0.7‡	15.1 ± 1.6
MCHC, g/dL	29.7 ± 1.4	29.0 ± 2.3	28.9 ± 1.5	30.8 ± 2.5
RDW, %	18.5 ± 0.8	18.4 ± 3.1	17.8 ± 0.6	18.7 ± 1.6

Complete blood cell count was performed in primary or secondary MPS I chimeras 5–6 months after transplantation as well as in age-matched MPS I or normal controls. Data are presented as mean ± SD. HGB, hemoglobin; HCT, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RDW, RBC distribution width.

*P = 0.074 in comparison to normal controls.

†P = 0.065 in comparison to normal controls.

‡P = 0.055 in comparison to normal controls.