LV production and titration
Vesicular stomatitis virus-pseudotyped LV were produced by transient transfection of the transfer constructs pCCLsin.cPPT.hPGK.GFP.Wpre or pCCLsin.cPPT.hPGK.hIDUA.mWpre, the third-generation packaging constructs pMDL.Lg/p and pRSV.Rev, and the pMD2.G envelope construct in 293T cells followed by ultracentrifugation of conditioned medium.[1] Stocks were titered by endpoint expression in HeLa cells.[2]

Auditory brainstem response measurement
Mice were anesthetized with 0.02ml/g body weight tribromoethanole (Avertine; Sigma) and placed under an infrared lamp to maintain body temperature above 34°C. We administered acoustic stimuli with earphones (intensity used was 100dB). The signal was recorded using a Myohandy electromyograph (Micromed, Mogliano Veneto, Italy) and filtered through a 100Hz–2 kHz band-pass filter, at a sweep velocity of 10ms and sensitivity of 500mV (for further details, please refer to supplemental methods). To record bioelectrical potentials we used monopolar 27G needle electrodes. The cathode tip was inserted at the midline of the interaural line through the scalp to contact the periosteum of the bregmatic suture; the anode tip was inserted 3–4 mm lateral and anterior to the cathode, just anterior to the ear to contact the temporal bone. Ground electrode was inserted on the back of the animal. We averaged 3 series of electrical signals (500 repetitions each). The custom-made software included an artifact rejection code (all waveforms with a peak to peak amplitude exceeding a defined voltage were rejected) to eliminate heart-beat and muscle activity.

Echocardiography
Trans-thoracic echocardiography (Aloka SSD-5500, Tokyo, Japan) was performed on conscious mice using a 13 MHz linear transducer at high frame rate imaging (102 Hz) and a 7.5 MHz phased array probe for pulsed-wave Doppler measurements. Para-sternal short- and long-axis views were recorded using two-dimensional (2D) and M-mode echocardiography on magneto-optic disk for off-line analysis by a sonographer, blind to study groups. Left atrial diameter, end-diastolic and end-systolic wall thicknesses and left ventricular internal dimensions were measured, as recommended by the American Society of Echocardiography. Fractional shortening was calculated from M-mode short-axis view. Left ventricular volumes and left ventricular ejection fraction were calculated by the modified simple plane Simpson's rule from the para-sternal long-axis view. Aortic outflow and trans-mitral left ventricular inflow velocities were measured from 5 and 4 apical chamber views respectively by pulsed-wave Doppler. All Doppler spectra were recorded for 5–10 cardiac cycles at a sweep speed of 100mm/s. The color Doppler preset was at a Nyquist limit of 0.44 m/s. The long-axis and 4 and 5 apical chamber views were used for 2D and color flow imaging and spectral Doppler interrogation of the mitral valve and/or aortic outflow tract.

FACS and hemocytometric analyses
Six months after the treatment, peripheral blood mononuclear cells from treated and control mice were incubated in blocking buffer (PBS with 1%BSA and 5% FBS) 15 minutes at 4°C in the dark and stained 20 minutes with anti-B220 mAb, anti CD3 mAb, and anti CD11b mAb (all from BD Biosciences Pharmigen, San Diego, CA) in the same buffer. Stained cells were washed and analyzed by flow cytometry (FACS CANTO). Results were analyzed by Flow-Jo 8.5.3 software.

For hemocytometric analysis, 50 µl of blood with 20 µl EDTA 45 mg/ml were analyzed with SYSMEX KX-21N.

Animal sacrifice modalities
Tissues were divided as follows: 1/3 of the spleen, liver and thymus were stored in formaline at room temperature for safety studies; 1/3 of spleen and liver, 1/2 of brain and heart and an entire kidney were fixed for histopathology (see below); 1/3 of spleen and liver, 1/2 of brain and of heart and an entire kidney were stored at −80°C for further activity and/or GAGs testing.

IDUA activity assay
Tissues were homogenized in lysis buffer (10mM NaH_2PO_4/$NaHPO_4$ pH5.8, 0.1mM dithiothreitol, 0.02% NaN3, 0.1%Triton X-100 and NaCl 0.9%). Samples were cleared by centrifugation at 16000× g for 20 minutes, and the supernatants collected for enzyme assay. Enzyme activity was measured fluorometrically,[3] following incubation of 10µl of sample for 1 hour at 37°C with 10µl of substrate (2mM 4-methylumbelliferyl α–L-iduronide, Glycosynth) in 0.1M Na formate buffer, pH 3.2. Fluorescence of the 4-methylumbelliferone released was measured after addition of 1ml of 0.5M carbonate buffer, pH10.7. Fluorescence was read at 365nm (excitation) and 448nm (emission) on a Perkin-Elmer fluorometer.

Analysis of glycosaminoglycans in tissues
Soluble GAGs were precipitated from tissue homogenates with Alcian blue for quantitative measurement. Briefly, frozen tissues were lyophilized and, after weighing, re-suspended in 0.9% NaCl/0.2% Triton X-100, (30ml/mg dry weight), rotated at 4°C overnight and centrifuged at 970g to remove debris. GAGs were precipitated with Alcian blue, the blue precipitate dissolved and absorbance measured at 600nm. Heparan sulfate from porcine intestinal mucosa was used as standard. Data (means of triplicates) were expressed as µg of GAGs per mg of dried pellet.

Analysis of glycosaminoglycans in brain extracts
Frozen samples were homogenized with a minimum volume of water (10% vol/weigth). After centrifugation at 970g, defatted pellets were dried and weighed. Dried tissues were digested at 65°C with papain (0.3% w:v) in 3ml of 100mM sodium acetate buffer pH5.5 containing 5mM cysteine and 5mM EDTA. After centrifugation, GAGs were measured in the supernatant with a dimethylmethylene blue dye-binding assay. Briefly, 200ml of the supernatant was added to 2.5ml of dimethylmethylene blue reagent[4] and the absorbance at 535nm was measured. Heparan sulfate was used as standard. Data (means of triplicates) were expressed as µg of GAGs per mg of dried pellet.

Quantification of urinary glycosaminoglycans
Twenty-four hour-urine collections were obtained in metabolic cages. Urinary GAGs were assayed by colorimetric assay using dimethylmethylene blue (Aldrich, Sigma) as described[4], with a chondroitin sulfate as standard. Urinary creatinine was measured (InterMedical CH-24), by mixing 100ml of urine (diluted 1:10) with a reagent solution of picric acid and sodium hydroxide. After reaction, the absorbance was measured at 492nm. Data (means of triplicates) were expressed as µg of GAGs *per* mg creatinine.

Western blot analysis
Western blot was performed on 40 µg of protein from mice serum. The nitrocellulose membranes were stained with anti-heparin co-factor II antibody (5 µg/ml) as described[5] or with mouse anti-GAPDH monoclonal antibody (2 µg, diluted 1:250, Chemicon).

Peripheral quantitative CT
pQCT measurements were performed *ex vivo* on mice tibiae and femurs fixed in 70% ethanol using a Stratec Research SA+ pQCT scanner (Stratec Medizintechnik, Pforzheim, Germany) with a voxel size of 0.070mm^3 and a scan speed of 3mm/s. All images were obtained with 360 projections, with a section thickness of 500µm. In order to orientate the long axes of the bones parallel to the image planes, retrieved bone specimens were held in place with manufacturer-made plastic holders for *ex vivo* measurements. The correct longitudinal positioning was determined by means of an initial scout scan. For each animal, the reference line was positioned between the tibia and the femur. To exactly determine the 2D scanning plane, a reference line was placed at the interface between tibia and femur after the scout view procedure. The measurement line was placed with the cursor keys at 1.5, 2.0, and 2.5, 4.5 and 5.0 mm distal to reference line in the tibia, and at −2.0, −2.5, −5.0 and −5.5 mm proximal to the reference line in the femur. Scanning was automatically performed at the established measurement points. Results are related to sections at 2.5 mm and 5 mm for tibia metaphysis and dyaphysis, respectively, and to sections at −2 mm and −5 mm for femur metaphysis and diaphysis, respectively. The scans were analyzed using contour mode 1 and peel mode 2 with a threshold of 280mg/cm³ for the calculation of trabecular and total bone parameters in metaphysis and with a threshold of 710mg/cm³ for cortical bone parameters in diaphysis. The different thresholds of 280 and 710mg/cm^3 for the metaphysis and diaphysis, respectively, were established to account for partial volume effect. The cortical bone density is lower at the metaphysis than at the diaphysis due to the thinner cortex. The threshold was therefore adjusted according to the cortical density to optimize accuracy.

Bone histopathology
The tibiae and femurs were first dehydrated in ethanol 70%, decalcified in EDTA 14% for 3 weeks with a daily change. Tissues were then processed in a Shandon Excelsior automated tissue processor (Thermo Fisher Scientific, Waltham) where they were embedded in paraffin wax and sectioned longitudinally with a Leica/Jung 2255 microtome in 4–8µm thick sections. Sections were stained with hematoxylin and eosin (H&E) and viewed on a Leica DM LB compound microscope (Leica Microsystems, Axioskope2) with a Q-Imaging Micropublisher Cooled CCD color digital camera (Vashaw Scientific Inc., Washington, DC). For morphometric analysis the region of interest (ROI) all around the perimeter, sized 54134X54134 pixel, was acquired at a magnification of 2.5×, 10×, and 20× with a transmission microscope (Axioplan, Jena Germany); measurements were performed using ImageJ software (Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA).

Histopathology of the retina
Upon eye collection, cornea and crystalline were removed, and retina was frozen. The measurement of retinal thickness (from retinal pigmented epithelium up to the ganglion cell layer) was performed on pictures at ×20 magnification of 20µm-thick sections after staining with DAPI (4′,6-diamidine-2′-phenylindone dihydrochloride, 1µg /ml, diluted 1:30, Roche).

Immunofluorescence studies
Brain tissues were frozen and cut in 40µm-thick slices. Sections were permeabilized for 5 minutes in PBS 0.3% Triton X-100 (PBS-T), and blocked with 10% goat serum in PBS-T. Primary rabbit anti–calbindin-D28k antibody (Swant) was diluted in blocking solution 1:500; after incubation for 2 hours at room temperature or 12 hours at 4°C, sections were washed in PBS and stained for 1 hour with secondary antibody (goat α-rabbit AlexaFluor488, Molecular Probes) diluted 1:1000 in PBS-T. Nuclei were stained for 5 min at room temperature with ToProIII (Molecular Probes T3605) 1:2000 in PBS. Sections were mounted with Mount-Quick "Aqueous" (Daido Sangyo, Electron Microscopy Sciences). Confocal microscopy was performed using a 3-laser confocal microscope (Radiance 2100; BioRad TCS SP2). Fluorescent signals from single optical sections were sequentially acquired and analyzed by Adobe Photoshop 7.0 software (Adobe Systems Inc., San Jose, California, USA). For computer aided image analysis, ImageJ software was used to score the number of Purkinje cells in the cerebellum, than normalized by the length of the Purkinje cell lane.

Isolation, transduction of human HSPC and transplantation in Rag2$^{-/-}$ γchain$^{-/-}$ mice
Human HSPC were isolated from cord blood by positive selection of CD34-expressing cells (CD34 progenitor cell isolation kit, MACS; Miltenyi Biotec, Bergisch Gladbach, Germany), upon obtainment of informed consent (TIGET 01), and transduced with IDUA- or GFP-LV at MOI 100.[6] After transduction, cells were transplanted into 3-day-old, sub-lethally irradiated (550cGy) Rag2$^{-/-}$ γ-chain$^{-/-}$ mice by temporal vein injection (2.5×10^5 cells/mouse). The transduced cells were also plated for clonogenic assay (800 cells/ml in methylcellulose medium [human MethoCult; StemCell Technologies]) and were cultured[6] for 14 days for IDUA activity evaluation.
Twelve weeks after transplantation, mice were sacrificed and BM, spleen, and thymus were collected for cytofluorimetric analysis (performed as described[6]) and IDUA activity measurement.

Southern blot analysis
Analysis was performed as described.[7]

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2. De Palma M, Naldini L. Transduction of a gene expression cassette using advanced generation lentiviral vectors. *Methods Enzymol*. 2002;346:514–529.
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4. Whitley CB, Ridnour MD, Draper KA, Dutton CM, Neglia JP. Diagnostic test for Mucopolysaccharidosis I. Direct method for quantifying excessive urinary glycosaminoglycan excretion. *Clin Chem*. 1989;35:374–379.
5. Randall DR, Colobong KE, Hemmelgarn H, et al. Heparin cofactor II-thrombin complex: a biomarker of MPS disease. *Mol Genet Metab*. 2008;94:456–461.
6. Capotondo A, Cesani M, Pepe S, et al. Safety of Arylsulfatase A Overexpression for Gene Therapy of Metachromatic Leukodystrophy. *Hum Gene Ther*. 2007;18:821–836.
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Figure S1. Correlation between VCN, IDUA activity and neurological phenotype of GT mice.
(A) IDUA activity measured in PBMCs (nmol/mg/h) was plotted against VCN (number of LV copies *per* genome) measured in the BM of gene therapy treated mice. (B, C) IDUA activity measured in the brain (nmol/mg/h) was plotted against the VCN measured in the BM (B) and the enzymatic activity measured in PBMCs (C) of gene therapy treated mice. Significant correlation for each of the tested pairs was observed. (D, E) The performance data of GT mice at open field test (% of change between the 1st and the 3rd day) were plotted against the VCN measured in the BM (D) and the enzymatic activity measured in the brain (E). Significant correlation for each of the tested pairs was observed. Statistical data are reported in the text boxes.

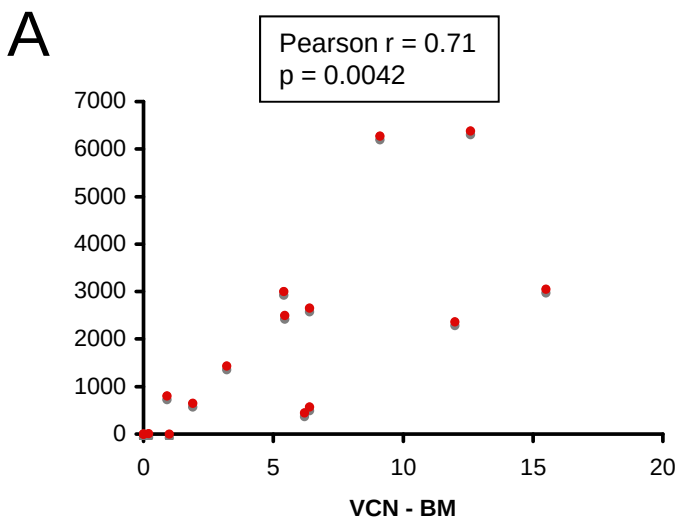
Figure S2. Morphometric analysis of the growth plate.
Representative images showing the details on the morphometric analysis of the growth plate in a MPS I mouse. (A) The perimeter and length of the growth plate were measured as shown by the black annotated lines. (B) The number of chondrocytes aligned in columns perpendicular to the major axis of the growth plate was counted as shown.

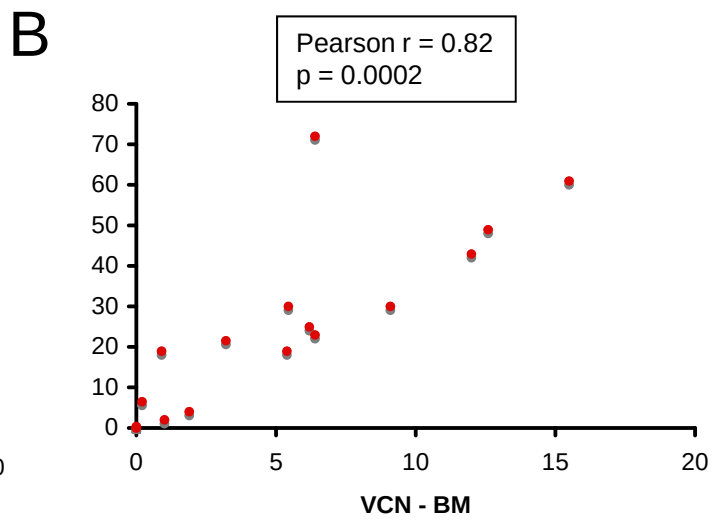
Figure S3. Effect of lethal irradiation on the cardiac phenotype and splenomegaly.
(A) CT scans performed on 8 month-old WT and MPS I untreated mice (left panels) and mice having received lethal irradiation and GFP LV-transduced HSPC transplantation from donors having the same genotype as recipients (mock-transplanted) at 2 months of age (right panels), showing the heart profile (depicted in red); please notice the enlarged heart present exclusively in untreated MPS I mice. (B) Representative pictures of the spleens retrieved from 8 month-old untreated mice (left panels) and mock-transplanted mice (right panels) are shown.

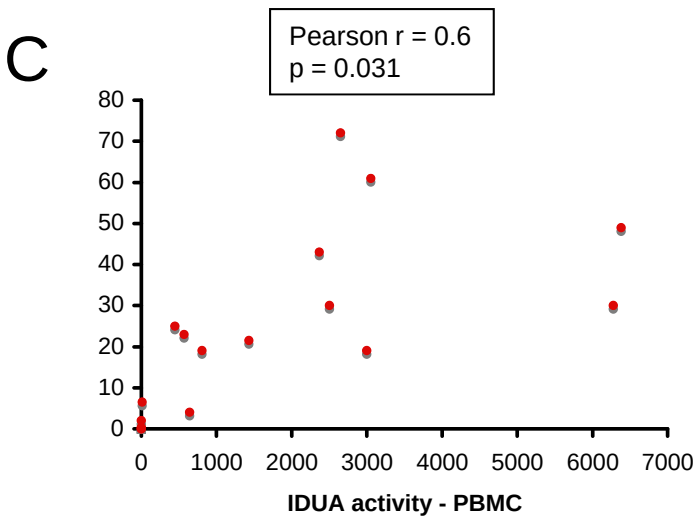
Figure S4. Hemocytometric and FACS analysis of treated and control mice.
Results from hemocytometric (A, C, D, E, F) and FACS analysis (for CD3, in B) performed 6 months after treatment on the peripheral blood of GT (n=17), HCT (n=18) and control mice (WT n=11, MPS I n=20) are shown. *=$p<0.05$ and **=$p<0.01$ at one-way Anova.

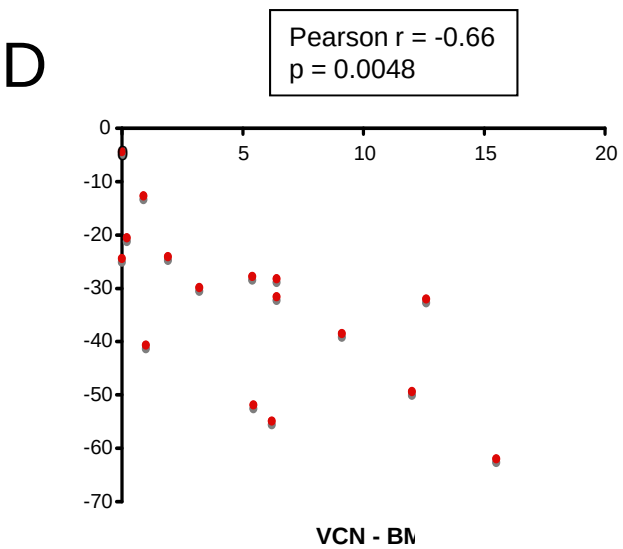
Figure S5. IDUA over-expression in human HSPC does not affect their function.
(A) Experimental scheme showing IDUA over-expression upon transduction of human HSPC with PGK.IDUA LV (measured on the in vitro culture progeny 14 days post-transduction). (B) Engraftment of un-transduced (black bars) and PGK.IDUA LV transduced (red bars) human HSPC is shown, expressed as % of human CD45^{+} cells in bone marrow (BM), spleen (Spl) and thymus (Thy) of Rag2$^{-/-}$ γchain$^{-/-}$ mice 12 weeks after transplantation (n=9 for un-transduced and n=13 for transduced HSPC). (C–E) Hematopoietic differentiation of the transplanted HSPC in BM (C), spleen (D) and thymus (E) of Rag2$^{-/-}$ γchain$^{-/-}$ mice is shown. (F) IDUA expression was measured in the BM, spleen and thymus of Rag2$^{-/-}$ γchain$^{-/-}$ mice un-transplanted (white bars) and transplanted with the abovementioned cells. Mean values and min/max are shown. (G) A correlation between IDUA activity (Y axis, nmol/mg/h) and human CD45+ cells engraftment (X axis, %human CD45+ cells) in tissues was observed ($p=0.012$ at Pearson correlation analysis).

Figure S1


A
Pearson r = 0.71
p = 0.0042
7000
6000
5000
4000
3000
2000
1000
0
0
5
10
15
20
VCN - BM


B
Pearson r = 0.82
p = 0.0002
80
70
60
50
40
30
20
10
0
0
5
10
15
20
VCN - BM


C
Pearson r = 0.6
p = 0.031
80
70
60
50
40
30
20
10
0
0
1000
2000
3000
4000
5000
6000
7000
IDUA activity - PBMC


D
Pearson r = -0.66
p = 0.0048
0
-10
-20
-30
-40
-50
-60
-70
5
10
15
20
VCN - BM

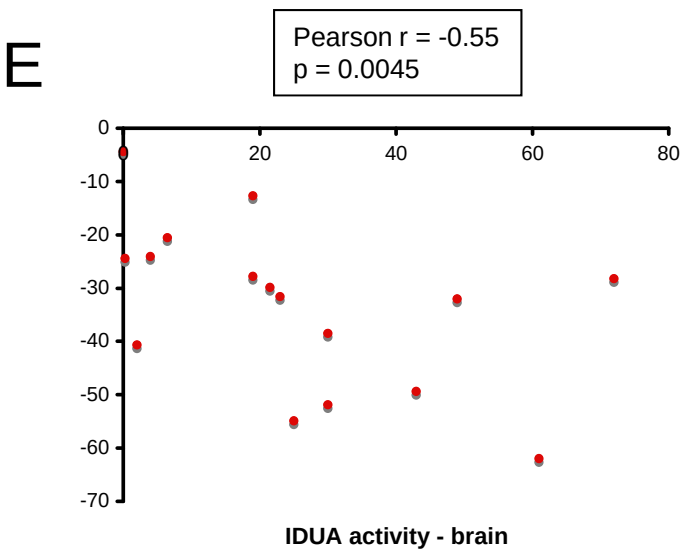
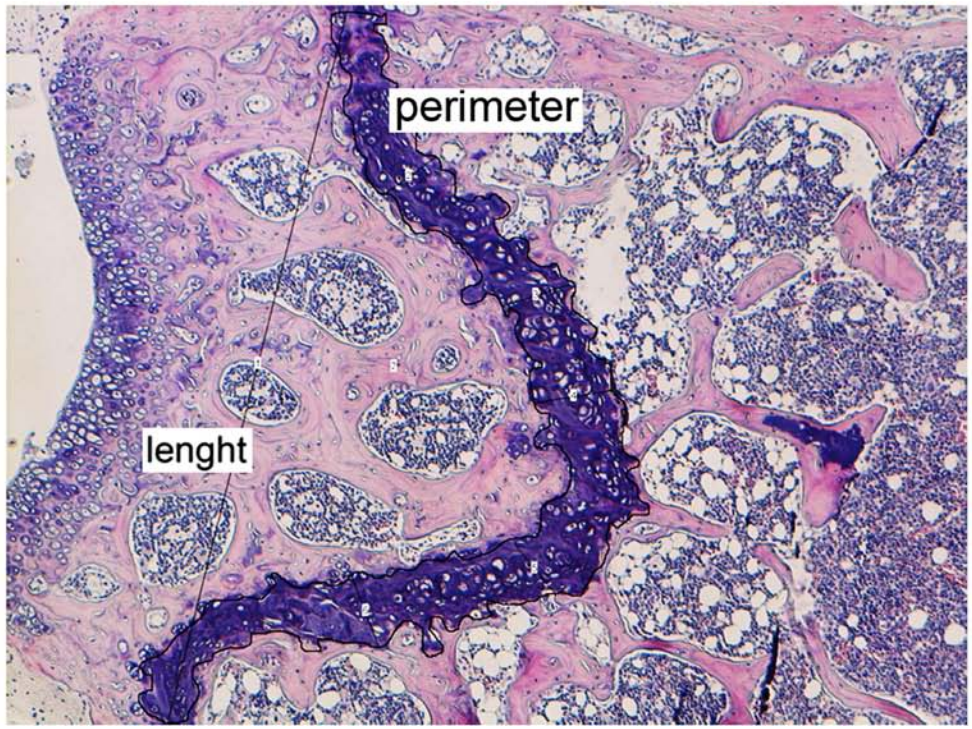

E
Pearson r = -0.55
p = 0.0045
0
-10
-20
-30
-40
-50
-60
-70
20
40
60
80
IDUA activity - brain

Figure S2

A



B

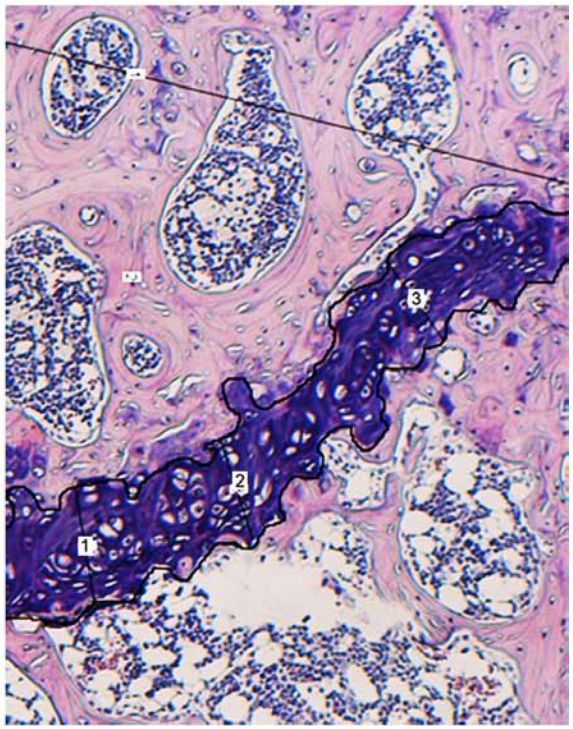
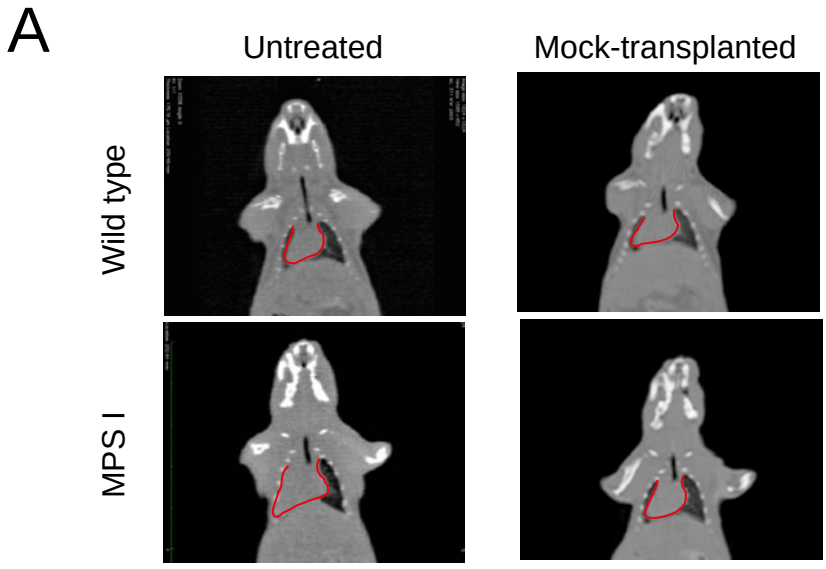


Figure S3


A
Untreated
Mock-transplanted
Wild type
MPS I

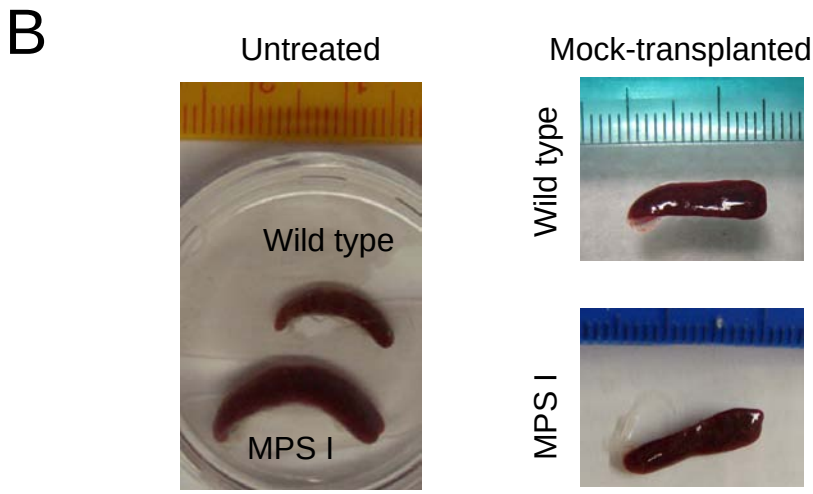

B
Untreated
Mock-transplanted
Wild type
MPS I
Wild type
MPS I

Figure S4

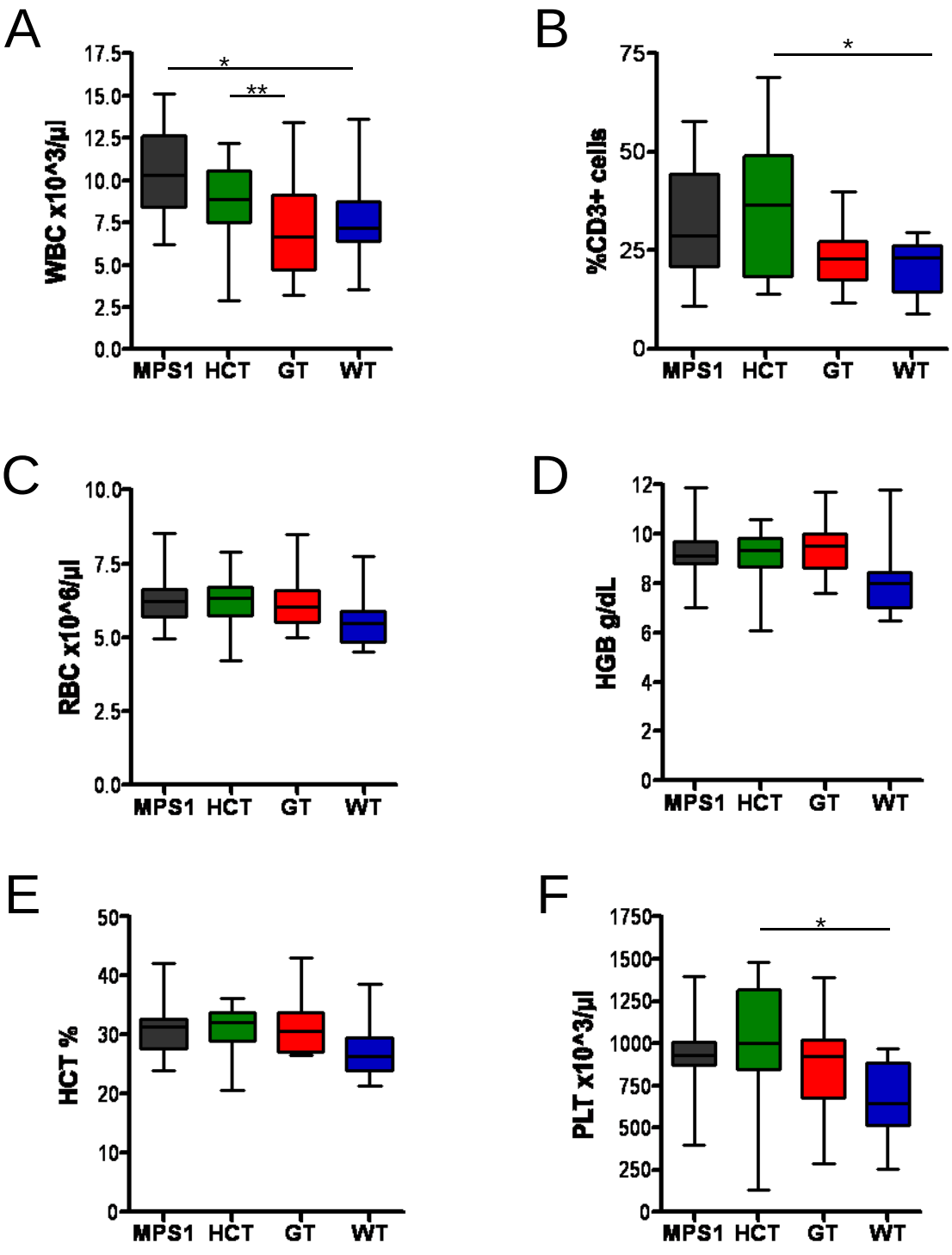
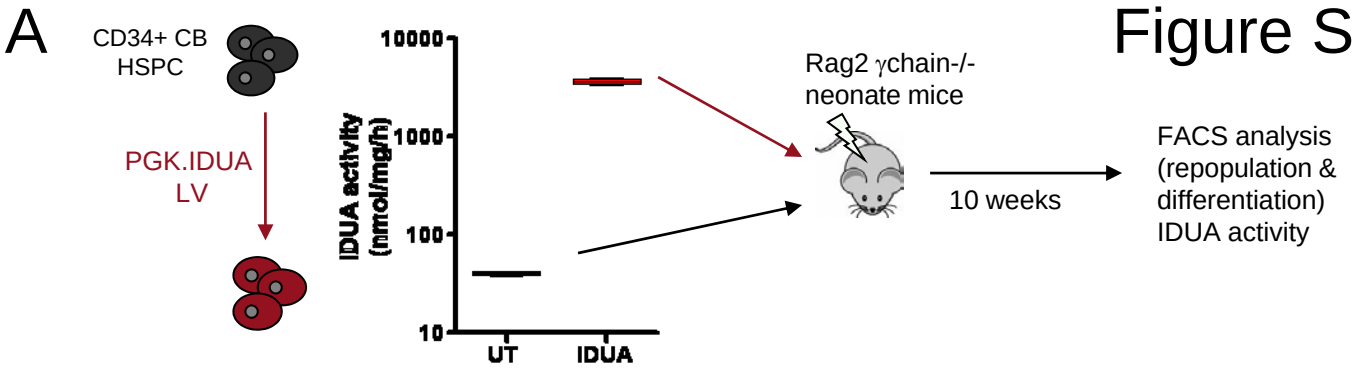
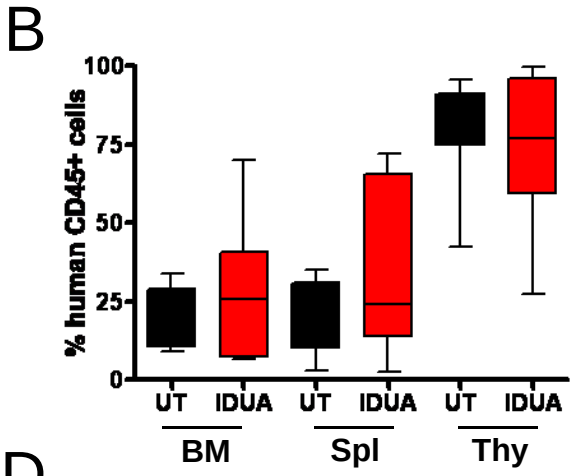
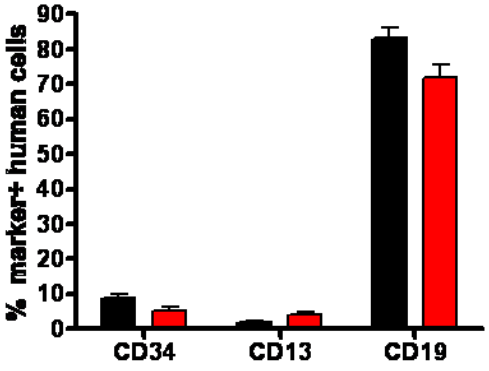
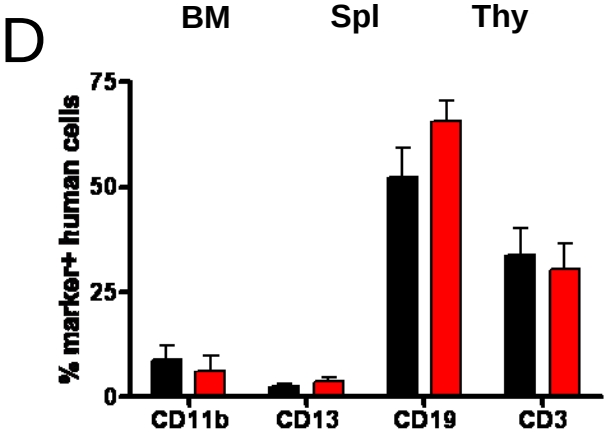

A
WBC x10^3/µl
17.5
15.0
12.5
10.0
7.5
5.0
2.5
0.0
*
**
MPS1 HCT GT WT
B
%CD3+ cells
75
50
25
0
*
MPS1 HCT GT WT
C
RBC x10^6/µl
10.0
7.5
5.0
2.5
0.0
MPS1 HCT GT WT
D
HGB g/dL
12
10
8
6
4
2
0
MPS1 HCT GT WT
E
HCT %
50
40
30
20
10
0
MPS1 HCT GT WT
F
PLT x10^3/µl
1750
1500
1250
1000
750
500
250
0
*
MPS1 HCT GT WT

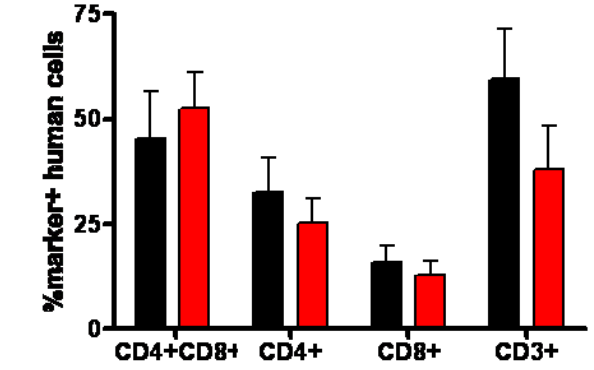
Figure S5

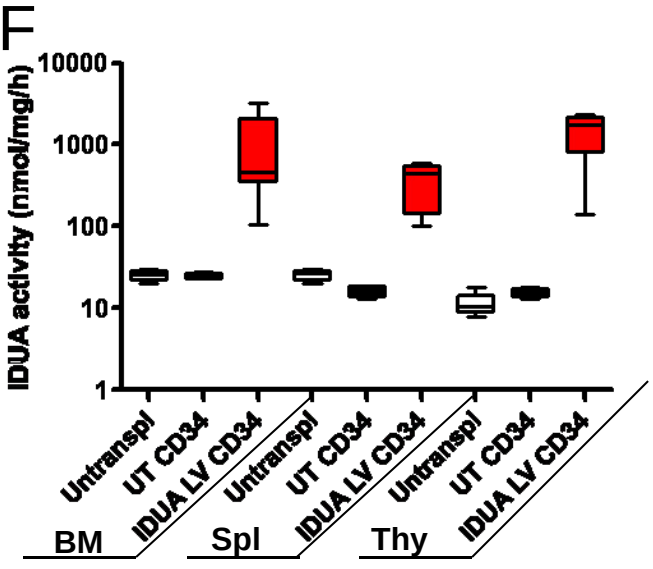

A
CD34+ CB HSPC
PGK.IDUA LV
IDUA activity (nmol/mg/h)
10000
1000
100
10
UT
IDUA
Rag2 γchain-/- neonate mice
10 weeks
FACS analysis (repopulation & differentiation) IDUA activity

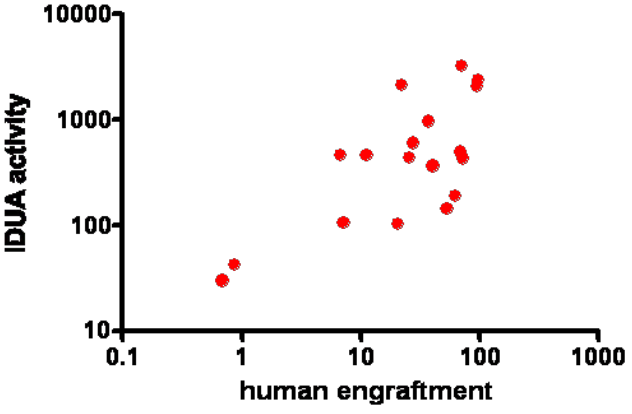

B
% human CD45+ cells
100
75
50
25
0
UT
IDUA
BM
Spl
Thy


C
% marker+ human cells
90
80
70
60
50
40
30
20
10
0
CD34
CD13
CD19


D
% marker+ human cells
75
50
25
0
CD11b
CD13
CD19
CD3


E
%marker+ human cells
75
50
25
0
CD4+CD8+
CD4+
CD8+
CD3+


F
IDUA activity (nmol/mg/h)
10000
1000
100
10
1
Untranspl
UT CD34
IDUA LV CD34
BM
Spl
Thy


G
IDUA activity
10000
1000
100
10
0.1
1
10
100
1000
human engraftment