Supplemental Material:

Gene therapy by allele selection in a mouse model for beta-thalassemia

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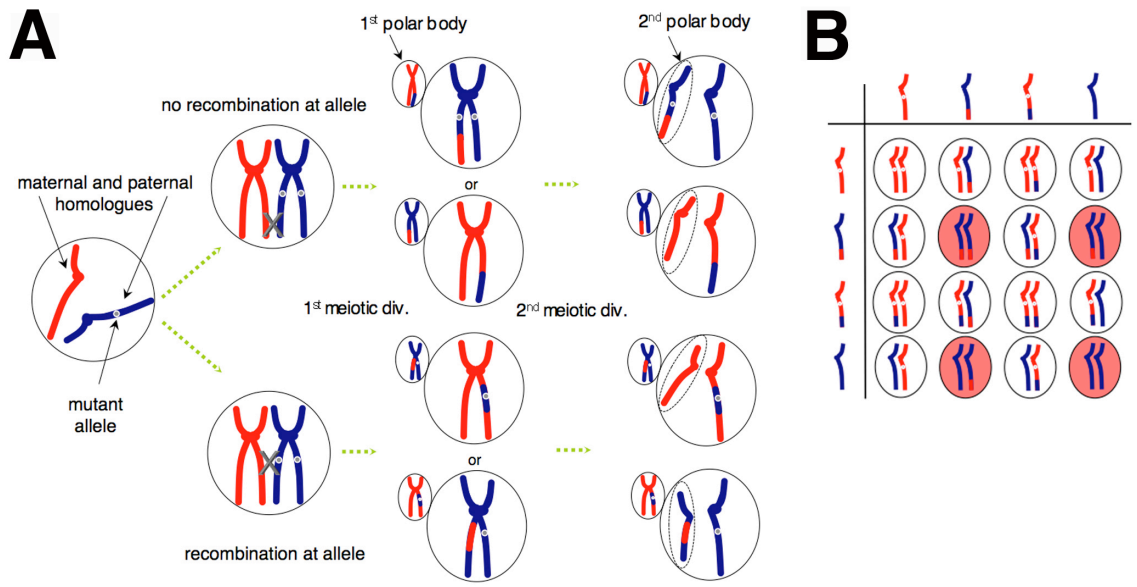
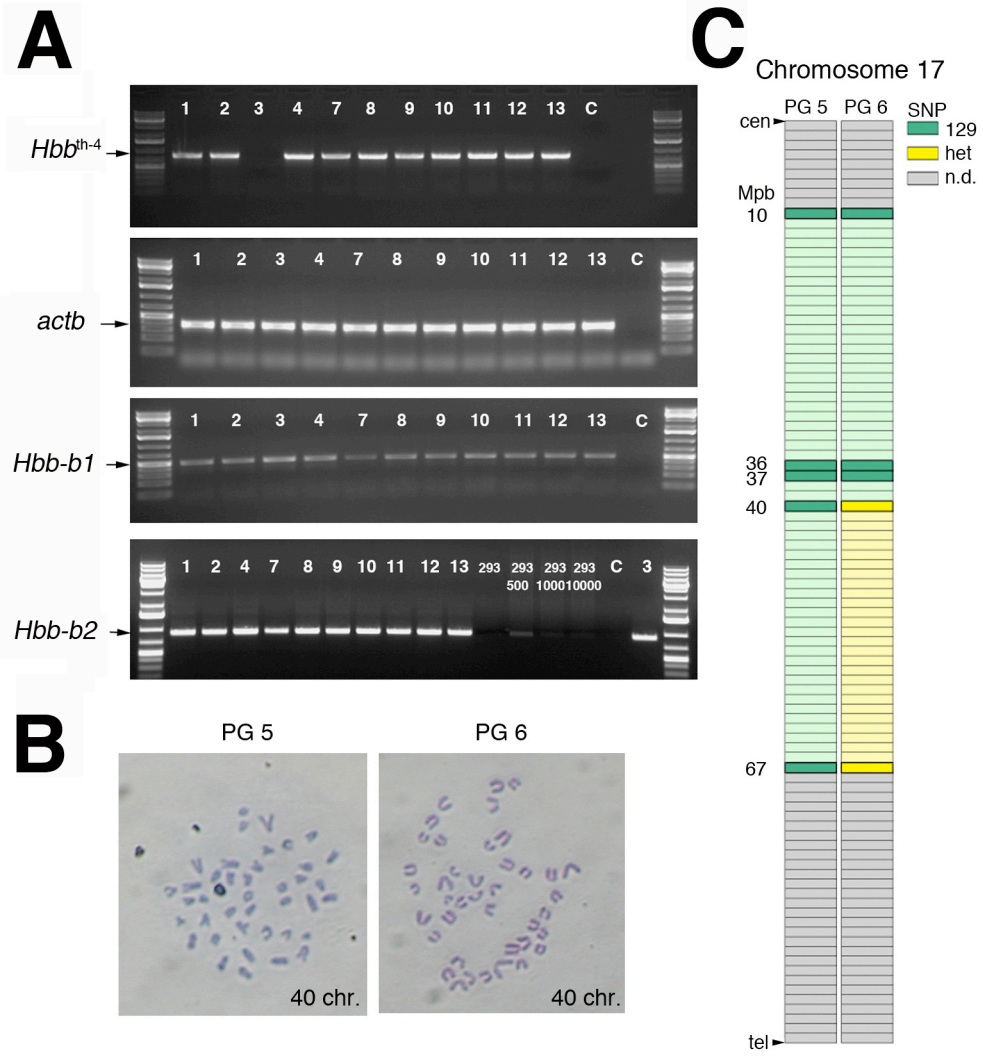


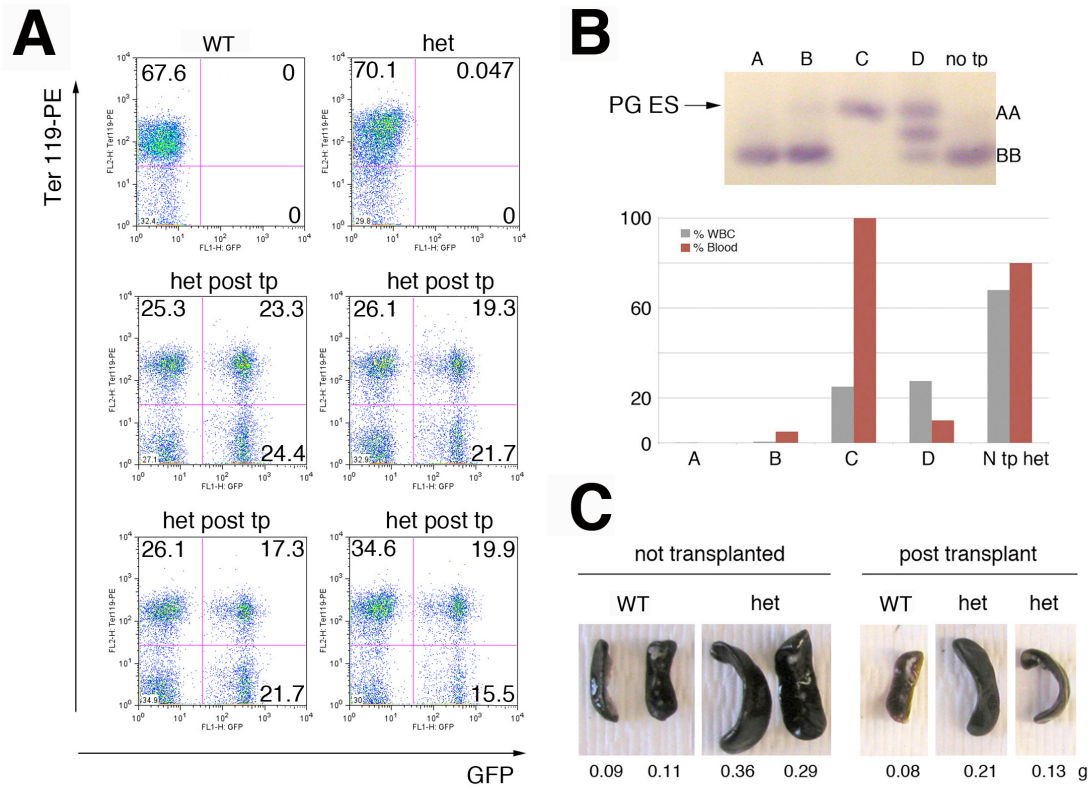
Supplemental Figure 1. Schematic representation of the distribution of a disease allele in gamete-derived uniparental embryos. A. PG Derivation. Simplified outline of meiosis with only one pair of homologous chromosomes shown (blue and red; disease allele marked). In the absence of meiotic recombination at the mutant locus (top), half of all PG embryos rendered diploid via suppression of second polar body extrusion would not carry the mutant allele. If recombination occurs at the allele (bottom), diploid PG embryos would be heterozygous. **B.** Androgenetic (AG) derived from two sperm, and gynogenetic (GG) embryos with two maternal genomes from different oocytes. The table illustrates possible combinations of alleles in diploid AG or GG embryos derived from

the haploid gametes of a heterozygote. Haploid gametes are indicated on the axes. Mutant-allele free embryos are highlighted pink.



Supplemental Figure 2. Characterization of PG ES cell lines derived from Hbb^{th-4} heterozygous mice. A. Genotyping for presence of the *Hbb*$^{th-4}$ allele, and analysis of *Hbb*$^{th-4}$ hetero- or homozygosity ascertained by PCR genotyping for the presence of the murine hemoglobin adult beta major (*Hbb-b1*) and beta minor (*Hbb-b2*) genes that are deleted in the *Hbb*$^{th-4}$ allele. Genomic DNA was isolated from PG ES cell lines 1 to 13 (indicated by respective number; C = water control) after expansion for 3 passages without feeder cells, and PCR amplified with primers specific for the amplicons

indicated. **Top:** *Hbb*$^{th-4}$ allele-specific amplicon. Lines 3, 5 and 6 are *Hbb*$^{th-4}$ allele-free, 5 and 6 not shown. **Upper middle**: Beta-actin (*actb*) amplicon as amplification control. **Lower middle:** *Hbb-b1* amplicon. All lines are positive, i.e. none of the *Hbb*$^{th-4}$ positive lines is *Hbb*$^{th-4}$ homozygous. **Bottom:** *Hbb-b2* amplicon. 293 = genomic DNA from human 293 cells, to verify lack of amplification from the human transgene present in the *Hbb*$^{th-4}$ allele. 293:500; 293:1000; 293:10000 = 293 genomic DNA spiked with 1:500,1:1000 and 1:10000 murine genomic DNA, to visualize potential amplification of genomic DNA from feeder cells (with intact beta globin locus) persisting after 3 passages on gelatin. The total genomic DNA input was the same for all reactions. All lines are positive, i.e. none of the *Hbb*$^{th-4}$ positive lines is homozygous for the allele. **B.** Metaphase spreads of chromosomes of two *Hbb*$^{th-4}$ allele-free PG ES cell lines derived from *Hbb*$^{th-4}$ heterozygous mice showing normal chromosome number. **C.** Chromosome 17 recombination patterns of two *Hbb*$^{th-4}$ allele free PG ES cell lines derived from *Hbb*$^{th-4}$ heterozygous F1 hybrid mice. Genomic DNA from ES cells was genotyped by direct sequencing of PCR amplicons encompassing a strain specific SNP distinct between 129S1 and C57/Bl6. Numbers indicate the distance from the centromere in Mpb of the SNPs analyzed (thick borders). Dark green – homozygous (129 allele); bright yellow - heterozygous for 129 and B6 alleles; grey- not determined.



Supplemental Figure 3. A. Erythroid engraftment of PG ES cell derivatives in peripheral blood of *Hbb*$^{th-4}$ heterozygotes post transplantation**.** *Hbb*$^{th-4}$ heterozygous recipients exhibit substantial contribution of GFP positive = ES cell derived cells to the Ter119+ population. FACS analysis of peripheral blood was performed 5 weeks post transplant. Numbers in quadrants indicate % of gated wbc. **B.** Differences in contribution of ES cell derivatives to wbc and whole blood (predominantly rbc). Top panel: GPI-1 isozyme analysis. PG ES cell lines are homozygous for the allele encoding the A electrophoretic form of GPI-1 (AA homodimer of GPI-1 marked by arrow), and recipients were either homozygous for the allele encoding the B electrophoretic form (recipients A, B, C; BB homodimer marked) or heterozygous for both alleles (recipient D), permitting detection and quantification of ES cell-derived cells by GPI-1 isoenzyme electrophoresis. Recipient C exhibits almost entirely PG derived peripheral blood (AA

only). Lower panel: Comparison of contribution of ES cell derived cells in heterozygous recipients of PG (A, B, C, D) and N (last column) derived transplants to white blood cells (FACS analysis of GFP positive cells; grey bars) and whole blood (GPI-1 isozyme analysis; red bars). **C.** Correction of splenomegaly post transplantation. Spleens of WT mice compared to *Hbb*$^{th-4}$ heterozygous mice without and after PG ES cell derivative transplantation. Weight shown in g.

Supplemental methods.

ES cell genotyping. Genotyping for the *Hbb*$^{th-4}$ allele was performed using primers oIMR801 5'-ggc aaa gga tgt gat acg tgg aag-3' and oIMR802 5'- cca gtt tca cta atg aca caa aca tg-3', amplifying an 830 bp product from the *Hbb*$^{th-4}$ allele. To distinguish ES cell lines hetero- or homozygous for the *Hbb*$^{th-4}$ allele, the presence or absence of the murine hemoglobin beta major (*Hbb-b1*) and beta minor (*Hbb-b2*) genes was ascertained by genotyping using primers 5'-cca atc tgc tca cac agg ata gag agg gca gg-3' and 5'-cct tga ggc tgt cca agt gat tca ggc cat cg-3' for *Hbb-b1;* and 5'-tca gag gga gta ccc aga gc-3' and 5'-gct cct gcc ctc tct atc ct-3' for *Hbb-b2* that are absent in the targeted allele. Cells were expanded for 3 passages without feeder cells prior to genomic DNA isolation (DNeasy Blood & Tissue Kit, Qiagen).

Metaphase spreads. Confluent ES cells on 6 cm dish (Nunc) were cultured in the presence of Colcemid (0.2 μg/ml) for 1h, treated with trypsin (0.25 % Trypsin [Sigma], 1 mM EDTA [Invitrogen], 1 x DPBS [Invitrogen), washed and resuspended in 1.5 ml fresh culture medium (32). 10 ml of warmed 0.075M KCl (Sigma) were added drop by drop with gentle flicking and cells incubated at 37ºC for 18 min. After addition of 2-3 drops of ice cold fixative (methanol:acetic acid 3:1 [Sigma]) cells were collected by centrifugation and resuspended in 1 ml of the supernatant. Cells were washed 3x by drop-wise addition of 10 ml ice-cold fixative and subsequent collection by centrifugation, resuspended in 0.5 ml fixative, and several drops of suspension applied to clean slides. Slides were dried at room temperature for 30 min and stained in Giemsa stain (Sigma) for 5-10 min.

Genotyping of chromosome 17. SNPs (IDs per MGI database, and genomic location according to NCBI Build 37) that distinguish the parental mouse strains 129S1 and C57/Bl/6, and primers used for amplification are listed below. PCR products from genomic DNA isolated from ES cell lines were sequenced directly.

SNP ID	Primer 1	Primer 2	Loc. Chr 17 (Mpb)
rs33483096	GAAATGCAACCCTCCCACT	CCAGAAACTGGAGTGGCTTC	10246903
rs33080598	CAGCTCTGAGCCATGACAAA	ACATGGAGCTTGTGGAGACC	36218286
rs3151023	AGAGAGGAAAGGGTGGCAAT	CAAATGATCCACGGTGTCAG	37405915
rs33427721	CATGTTTCCCTCTGCCATTT	GGAACCAACTTGGGAGACAA	40932068
rs3684212	CCAGCCAGAAGAGAACATGC	GCTCTGTTTCTTTGGGGTCTT	67451022

Lineage analysis. Peripheral blood was obtained from the retro-orbital sinuses of recipients and wbc were enriched by centrifugation subsequent to lysis of red blood cells in 0.155 M ammonium chloride, 10 mM potassium bicarbonate, 0.1 mM EDTA. Cells were stained with phycoerythrin (PE) and PE-Cy5-conjugated monoclonal antibodies specific for CD4 (L3T4), CD8 (Ly-2), CD45R/B220, and Ter119/Ly-76. All antibodies were obtained from BD Pharmingen. Cells were analyzed on a FACSCAN (BD Biosciences).

GPI-1 isozyme analysis. Blood samples (diluted 1:15 in water) were applied to Titan III cellulose acetate plates (Helena Laboratories) pre-soaked in running buffer (12 g Tris base and 5.76 g Glycine in 1000 ml H_2O) using the Super Z-12 applicator (Helena Laboratories). After electrophoresis at 200 V for 1 hour, plates were stained in the dark with 1.5% agarose in 25 mM Tris-HCl pH 8.0 supplemented with 75 microliter each of D-fructose-6-phosphate (200 mg/ml), beta-NADP (20 mg/ml), Phenazine methosulfate (5 mg/ml), Thiazolyl Blue Tetrazolium Bromide (20 mg/ml) and 5-10 units Glucose-6-phosphate dehydrogenase (all from Sigma).