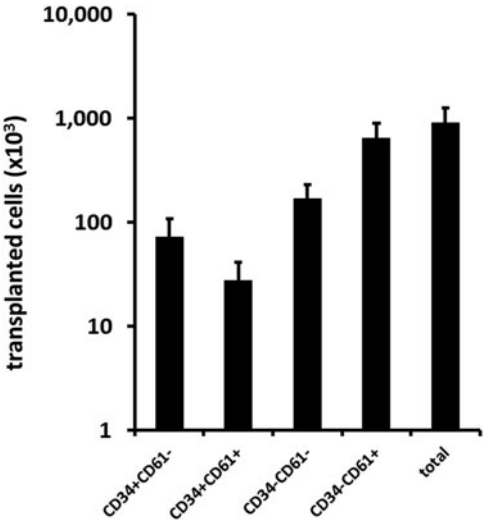
Supplementary Data

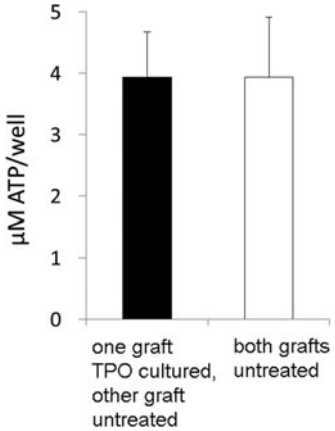
Supplementary Materials and Methods

Hematopoietic progenitor cell assays

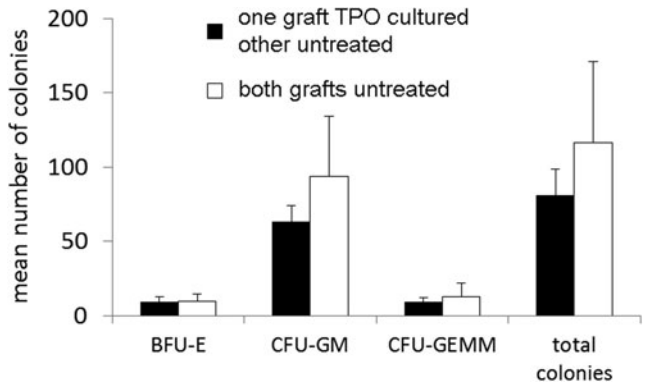
To investigate whether bone marrow cells harvested 20 weeks after transplantation contained human hematopoietic progenitor cells (HPC), three different in vitro assays were used; HALO assay (Hemogenix, Colorado Springs, CO), myeloid colony forming unit (CFU) cultures, and cobblestone area-forming cell (CAFC) assays. The HALO assay (HemoGenix) was used to enumerate the number of colony-forming cells. In short, 1.25×10^4 human $CD45^+$ cells from the bone marrow of each mouse were suspended in the HALO master mix (containing EPO, GM-CSF, IL-2, IL-3, IL-6, IL-7, SCF, TPO, and Flt3-L) and incubated in a 96-well plate for 6 days at 37°C and 5% CO_2 in a humidified incubator. After 6 days, the cells were harvested from the wells and lysed, after which the ATP content was measured with a luciferase/luciferin assay using a standard ATP curve to determine the ATP concentration. Methocult CFU assays (H4434 Classic; Stemcell) were used to determine the capacity to differentiate into different myeloid lineages. In this study, 2×10^4 cells were suspended in classic Methocult medium and cultured for 2 weeks at 37°C and 5% CO_2 in a humidified incubator, after which different differentiated colonies were counted with an inverted microscope. Human burst-forming unit erythroid (BFU-E)-derived colonies were counted as defined by Stem Cell Technologies and these are clearly distinguishable from mouse BFU-E. CAFC assays were used to determine whether the cells were primitive HPCs. In short, 6.4×10^4 cells from one representative mouse of each group for each experiment were seeded onto an irradiated NIH3T3 monolayer, growing on a collagen coated 24-well plate in the CAFC medium containing 3% (v/v) fetal calf serum, 3% (v/v) horse serum, glutamine, PenStrep, hydrocortisone, and β-mercaptoethanol. Five wells/mouse were seeded with bone marrow cells. Cells were cultured for 5 weeks at 37°C and 5% CO_2 in a humidified incubator and colonies were counted at week 4 and 5 with an inverted microscope.



SUPPLEMENTARY FIG. S1. Number of cells and composition of the thrombopoietin (TPO) treated graft of the six different donor grafts transplanted into the mice that received a TPO treated graft and an untreated graft. The transplanted cell number was based on equivalent $CD34^+$ cell numbers in the original graft before culturing with TPO and in the untreated cord blood (CB) unit. Thus, the total number of cells in the double CB transplant was variable between all donors that received one TPO treated graft. Although all transplants included a fixed number of $CD34^+$ cells from the untreated donor graft, the cell numbers that were culture derived from the same number of $CD34^+$ cells of the other donor graft varied between 2.6×10^5 and 24.0×10^5 cells. Moreover, the composition of the grafts was also variable, although all grafts contained high numbers of expanded megakaryocytic cells, ranging from 2.0×10^5 to 17.3×10^5 cells and relatively low numbers of $CD34^-$ $CD61^+$ cells.



SUPPLEMENTARY FIG. S2. Mean (±SEM) ATP levels produced by expanded cells harvested from the bone marrow of the mice 20 weeks after transplantation. Shown is the mean ATP concentration/well of all mice of the groups that received one TPO treated graft and an untreated graft (*black bar*) and the mice that received two untreated grafts (*white bar*). There was no difference in ATP production after HALO culture between the N+X and the N+N group.



SUPPLEMENTARY FIG. S3. Mean number of different myeloid colonies formed by the engrafted bone marrow cells of a mouse of each representative group of each experiment after 2 weeks of culture in Methocult. Cells from the N + N group produced more colonies than cells from the N + X group, but this difference was not significant.

SUPPLEMENTARY TABLE S1. GRAFT COMPOSITIONS OF TPO CULTURED CD34$^+$ CELLS FROM THE SIX DIFFERENT DONORS USED IN THE THREE DIFFERENT TRANSPLANTATION EXPERIMENTS

CD34$^+$CD61$^-$	*nr CD34$^+$ d0*	*nr CD34$^+$CD61$^-$ d10*	*Fold expansion from CD34$^+$*
Graft 1	188,520	78,732	0.42
Graft 2	195,260	249,080	1.28
Graft 3	92,420	12,164	0.13
Graft 4	96,890	50,922	0.53
Graft 5	97,450	31,715	0.33
Graft 6	93,200	9,846	0.11
CD34$^+$CD61$^+$	*nr CD34$^+$ d0*	*nr CD34$^+$CD61$^+$ d10*	*Fold expansion from CD34$^+$*
Graft 1	188,520	12,636	0.07
Graft 2	195,260	91,010	0.47
Graft 3	92,420	4,871	0.05
Graft 4	96,890	7,424	0.08
Graft 5	97,450	14,030	0.14
Graft 6	93,200	35,373	0.38
CD34$^-$CD61$^-$	*TNC d0*	*nr CD34$^-$CD61$^-$ d10*	*Fold expansion from TNC*
Graft 1	200,000	377,136	1.89
Graft 2	200,000	318,535	1.59
Graft 3	100,000	34,629	0.35
Graft 4	100,000	82,276	0.82
Graft 5	100,000	141,126	1.41
Graft 6	100,000	64,592	0.65
CD34$^-$CD61$^+$	*TNC d0*	*nr CD34$^-$CD61$^+$ d10*	*Fold expansion from TNC*
Graft 1	200,000	503,496	2.52
Graft 2	200,000	1,736,375	8.68
Graft 3	100,000	203,337	2.03
Graft 4	100,000	135,378	1.35
Graft 5	100,000	992,129	9.92
Graft 6	100,000	287,190	2.87

Shown are the results of each population after CD34 and CD61 analysis with flow cytometry (grafts 1 and 2, grafts 3 and 4, and grafts 5 and 6 were respectively combined in the different experiments).

nr, number of; TNC, total nucleated cells.