For gene therapy of gain-of-function autosomal dominant diseases, either correcting or deleting the disease allele is potentially curative. To test whether there may be an advantage of one approach over the other for WHIM (warts, hypogammaglobulinemia, infections and myelokathexis) syndrome — a primary immunodeficiency disorder caused by gain-of-function autosomal dominant mutations in chemokine receptor CXCR4 — we performed competitive transplantation experiments using both lethally irradiated wild-type (Cxcr4+/+) and unconditioned WHIM (Cxcr4+/w) recipient mice. In both models, hematopoietic reconstitution was markedly superior using bone marrow (BM) cells from donors hemizygous for Cxcr4 (Cxcr4+/o) compared with BM cells from Cxcr4+/+ donors. Remarkably, only ~6% Cxcr4+/o hematopoietic stem cell (HSC) chimerism post-transplantation in unconditioned Cxcr4+/w recipient BM supported >70% long-term donor myeloid chimerism in blood and corrected myeloid cell deficiency in blood. Donor Cxcr4+/o HSCs differentiated normally and did not undergo exhaustion as late as 465 days post-transplantation. Thus, disease allele deletion resulting in Cxcr4 haploinsufficiency was superior to disease allele repair in a mouse model of gene therapy for WHIM syndrome, allowing correction of leukopenia without recipient conditioning.
Cxcr4-haploinsufficient Bone Marrow Transplantation Corrects Leukopenia in an Unconditioned WHIM Syndrome Model

**Short title:** Modeling gene therapy in WHIM syndrome without conditioning

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JG, MS, EY, QL, DHM and PMM are listed as inventors on United States patent application 20170196911
Abstract

For gene therapy of gain-of-function autosomal dominant diseases, either correcting or deleting the disease allele is potentially curative. To test whether there may be an advantage of one approach over the other for WHIM (warts, hypogammaglobulinemia, infections and myelokathexis) syndrome—a primary immunodeficiency disorder caused by gain-of-function autosomal dominant mutations in chemokine receptor CXCR4—we performed competitive transplantation experiments using both lethally irradiated wild-type (Cxcr4+/+) and unconditioned WHIM (Cxcr4+/w) recipient mice. In both models, hematopoietic reconstitution was markedly superior using bone marrow (BM) cells from donors hemizygous for Cxcr4 (Cxcr4+/o) compared with BM cells from Cxcr4+/+ donors. Remarkably, only ~6% Cxcr4+/o hematopoietic stem cell (HSC) chimerism post-transplantation in unconditioned Cxcr4+/w recipient BM supported >70% long-term donor myeloid chimerism in blood and corrected myeloid cell deficiency in blood. Donor Cxcr4+/o HSCs differentiated normally and did not undergo exhaustion as late as 465 days post-transplantation. Thus, disease allele deletion resulting in Cxcr4 haploinsufficiency was superior to disease allele repair in a mouse model of gene therapy for WHIM syndrome, allowing correction of leukopenia without recipient conditioning.
Introduction

WHIM syndrome is a rare combined primary immunodeficiency disease caused by gain-of-function autosomal dominant mutations in CXCR4, a G protein-coupled receptor for the chemokine CXCL12 (1, 2). Among other functions, CXCR4 normally promotes BM homing and retention of neutrophils and HSCs, as well as HSC quiescence (3-6). The WHIM mutation increases BM retention of neutrophils causing neutropenia, a hematologic picture referred to as myelokathexis (7-9). G-CSF, which is commonly used to treat neutropenia in WHIM patients, selectively increases the neutrophil count in part by inducing degradation of CXCL12, thereby reducing CXCR4 signaling (2, 10).

Discovery of CXCR4 as the disease gene in WHIM syndrome has provided a precise target for development of novel therapeutic strategies. Regarding drug development, two specific CXCR4 antagonists, plerixafor (Mozobil™, AMD3100) and X4P-001 (AMD11070), are currently in clinical trials (11-13). With regard to cure, several patients have been cured by allogeneic BM transplantation (14, 15), and one patient has been cured by spontaneous deletion of the WHIM allele in a single HSC by chromothripsis (chromosome shattering). Remarkably, the chromothriptic HSC in this patient acquired a selective growth advantage leading to ~100% chimerism with CXCR4-haploinsufficient (CXCR4<sup>+/o</sup>) myeloid cells (16). The genetic mechanism responsible for myeloid repopulation remains undefined, in large part because 163 other genes were also deleted by the chromothriptic event. Nevertheless, a potential contribution from CXCR4 haploinsufficiency alone is supported by a mouse model of the patient in which the Cxcr4<sup>+/o</sup> genotype conferred a strong long-term engraftment advantage over both Cxcr4<sup>+/w</sup> and Cxcr4<sup>+/+</sup> genotypes after competitive BM transplantation in lethally
irradiated congenic recipient mice (16).

Importantly, cure of these patients demonstrates that the hematologic phenotypes and infection susceptibility in WHIM syndrome are driven by expression of the WHIM allele in hematopoietic cells, which suggests that both correcting and deleting the disease allele in patient HSCs are rational curative gene therapy strategies. The Cxcr4<sup>+/o</sup>/Cxcr4<sup>+/+</sup> competitive BM transplantation experiments in lethally irradiated mice suggested that disease allele deletion may actually be superior to disease allele correction as a gene therapy strategy because of the potential for enhanced engraftment of Cxcr4<sup>+/o</sup> HSCs. Here we test this hypothesis directly in mouse models of gene therapy for WHIM syndrome.
Results and Discussion

Cxcr4 genotype is a major determinant of hematopoietic reconstitution during competitive BM transplantation in lethally irradiated mice

We first conducted competitive transplantation experiments with lethally irradiated wild-type (Cxcr4\(^{+/+}\)) recipient mice and 1:1 BM mixtures from all three possible pairings of congenic donor mice distinguished by the following Cxcr4 genotypes: Cxcr4\(^{+/+}\), Cxcr4\(^{+/-}\) (Cxcr4 hemizygous/haploinsufficient) and Cxcr4\(^{+/w}\) (WHIM model mice). The Cxcr4\(^{+/-}\)/Cxcr4\(^{+/+}\) and Cxcr4\(^{+/-}\)/Cxcr4\(^{+/w}\) competitions confirmed our previously published results (16) and are included as contemporaneous comparators for the Cxcr4\(^{+/+}\)/Cxcr4\(^{+/w}\) competition, which has not been previously tested. In all three competitions, recipient blood reconstitution with donor-derived leukocytes was strongly polarized with the rank order Cxcr4\(^{+/-}\) > Cxcr4\(^{+/+}\) > Cxcr4\(^{+/w}\) (Figure 1A, Supplemental Figure 1A). The rank order was stable out to 300 days, HSC-intrinsic (Figure 1B, Supplemental Figures 1B & 2), and independent of the CD45 genetic background of the donor mice (Supplemental Figure 3).

For the Cxcr4\(^{+/-}\)/Cxcr4\(^{+/w}\) competition, the absolute numbers of Cxcr4\(^{+/-}\) donor-derived mature leukocytes rapidly increased to the average value for each subset for Cxcr4\(^{+/+}\) mice, whereas the absolute numbers of Cxcr4\(^{+/w}\) donor-derived mature leukocytes remained below the average value for Cxcr4\(^{+/w}\) mice (Figure 1C). In contrast, when each donor BM was transplanted independently into lethally irradiated Cxcr4\(^{+/+}\) recipients, the steady state absolute numbers of donor-derived peripheral blood leukocytes in the recipients consistently tracked the average values for the corresponding subset in donor mice (Supplemental Figure 4). Thus, the results identify competitive
suppression of Cxcr4+/- leukocyte reconstitution in peripheral blood by Cxcr4+/- hematopoiesis in the same mouse.

**The superiority of Cxcr4+/- BM for reconstituting peripheral blood leukocytes after competitive transplantation in lethally irradiated mice involves an early HSC proliferative advantage and superior long-term HSC engraftment**

We next evaluated potential mechanisms for the hematopoietic reconstitution rank order conferred by Cxcr4 genotype. We first tested HSC homing to BM, which is known to be mediated by CXCR4 (7, 17). Consistent with this, four hours after a 50:50 mixture of Cxcr4+/- and Cxcr4+/-w lineage-negative BM cells was co-injected into lethally-irradiated mice, Cxcr4+/-w HSCs and hematopoietic progenitor cells (HPCs) outnumbered Cxcr4+/- HSCs and HPCs in the BM by a ~4:1 margin (Figure 2A). However, this ratio was inverted when BM was analyzed 465 days after competitive transplantation, aligning with the Cxcr4 genotype rank order for blood reconstitution by mature donor-derived leukocytes in the same animals (Figure 2B). This provides evidence for a post-homing BM engraftment advantage conferred by Cxcr4 haploinsufficiency to transplanted HSCs.

CXCR4 is also known to mediate retention of HSCs and neutrophils in BM (4, 18). Thus, biased BM retention of mature Cxcr4+/-w leukocytes must be considered as a potential explanation for the blood results. We found no evidence for this in the Cxcr4+/o/Cxcr4+/- competition (Figure 2C). However, in the Cxcr4+/o/Cxcr4+/-w competition, we observed significantly higher frequencies of mature Cxcr4+/-w leukocytes, especially B and T cells, in BM than in blood, and, conversely, higher frequencies of Cxcr4+/-o mature leukocytes in blood than in BM (Figure 2C). Thus, biased BM retention
of certain mature leukocytes in this competition may contribute to the net hematopoietic reconstitution activity rank order conferred by Cxcr4 genotype to transplanted BM.

CXCR4 is also known to inhibit cell proliferation (5). Consistent with this, in our previous report, we found that Cxcr4<sup>+/o</sup> HSCs were hyperproliferative compared to Cxcr4<sup>+/+</sup> HSCs (16). Likewise, here we found that BrdU<sup>+</sup> cell frequency was ~20% greater for Cxcr4<sup>+/o</sup> HSCs than for Cxcr4<sup>+/+</sup> HSCs 20 hours after BrdU injection 7 days after competitive transplantation (Figures 2D, Supplemental Figure 5). Thus, the superiority of Cxcr4<sup>+/o</sup> BM for reconstituting peripheral blood leukocytes after competitive transplantation in lethally irradiated mice may involve an early HSC proliferative advantage.

Although enhanced HSC proliferation has been reported to possibly result in HSC exhaustion and depletion, and maintenance of HSCs in quiescence has been reported to favor long-term hematopoiesis (19-21), we did not observe any evidence of BM failure as late as 465 days after competitive transplantation of Cxcr4<sup>+/o</sup> HSCs (Figures 1B & 2C, Supplemental Figure 6). Furthermore, in a formal aging study we found no difference in survival and health of Cxcr4<sup>+/o</sup> mice compared to Cxcr4<sup>+/+</sup> littermates (Figure 2E).

**Leukopenia can be corrected in WHIM mice by Cxcr4<sup>+/o</sup> BM transplantation without recipient conditioning**

We next tested whether Cxcr4 genotype also affects engraftment after competitive BM transplantation in unconditioned congenic recipient mice. Overall, the Cxcr4 genotype rank order for donor leukocyte reconstitution in peripheral blood was the same as when lethally irradiated Cxcr4<sup>+/+</sup>mice were tested as the recipients: Cxcr4<sup>+/o</sup> > Cxcr4<sup>+/+</sup> >
Cxcr4\textsuperscript{+/w} (compare Figures 1A & 3A). In the Cxcr4\textsuperscript{+/o}/Cxcr4\textsuperscript{+/w} competition, the frequency of differentially marked Cxcr4\textsuperscript{+/w} donor-derived cells was only 1-4%, whereas the frequencies of donor-derived Cxcr4\textsuperscript{+/o} myeloid and B cells reached a plateau of 20-40% by 100 days post-transplantation that remained stable out to 315 days when the experiment was arbitrarily terminated. Cxcr4\textsuperscript{+/+} BM also had increased engraftment activity relative to Cxcr4\textsuperscript{+/w} BM, but the difference was only ~2-fold and declined towards the end of the experiment for neutrophils and B cells. In the direct Cxcr4\textsuperscript{+/o}/Cxcr4\textsuperscript{+/+} competition, Cxcr4\textsuperscript{+/o} donor-derived cell frequency for each leukocyte subset in the peripheral blood was ~2-3-fold greater than for the corresponding Cxcr4\textsuperscript{+/+} subsets and this advantage was sustained long-term for CD11b\textsuperscript{+}, CD19\textsuperscript{+} and CD3\textsuperscript{+} subsets, but not for neutrophils (Figure 3A). Overall, for each BM donor, myeloid cells reconstituted the best and CD3\textsuperscript{+} cells least well.

Since chimerism in an unconditioned congenic transplantation system is donor BM cell dose-dependent (22), we tested different doses and found that transplantation of 5x10\textsuperscript{7} Cxcr4\textsuperscript{+/o} BM cells alone into unconditioned Cxcr4\textsuperscript{+/w} recipients (Figure 3B), which established 60-70% donor chimerism at steady state for total leukocytes, myeloid cells and B cells (Figure 3B), restored the absolute myeloid cell counts to the normal range for Cxcr4\textsuperscript{+/+} mice (Figure 3B). In contrast, transplantation of 5x10\textsuperscript{7} Cxcr4\textsuperscript{+/w} BM cells alone resulted in no greater than 15% Cxcr4\textsuperscript{+/w} donor-derived CD45\textsuperscript{+} cells in peripheral blood (Figure 3B), and did not significantly increase the absolute cell number (Figure 3B).

Low level Cxcr4\textsuperscript{+/o} HSC and HPC engraftment is sufficient to correct leukopenia in unconditioned WHIM mice
At termination 385 days after co-transplantation of unconditioned Cxcr4+/w recipients with 10^7 Cxcr4+/o and 10^7 Cxcr4+/w donor BM cells (time course described in Figure 3A), the frequencies of mature Cxcr4+/w donor-derived leukocytes were very low and similar for each subset in BM and peripheral blood (Figure 4A). The BM frequencies of Cxcr4+/w donor-derived HSCs and HPCs were also very low (Figure 4A). In contrast, the frequencies of mature Cxcr4+/o donor-derived leukocytes were much higher in peripheral blood for each subset than in BM (Figure 4A). Surprisingly, the frequencies of Cxcr4+/o donor-derived HSCs and HPCs in BM were also low and similar to the frequencies of Cxcr4+/w donor-derived HSCs and HPCs in BM (Figure 4A). The same pattern of results was obtained when unconditioned Cxcr4+/w recipients were transplanted separately with 5x10^7 BM cells from either Cxcr4+/o or Cxcr4+/w donors (time course described in Figure 3B), and analyzed 348 days post-transplantation (Figure 4B). These data are consistent with a BM egress advantage for donor-derived mature leukocytes conferred by Cxcr4 haploinsufficiency over the Cxcr4+/w genotype.

Taken together, our results provide evidence in two transplantation models that WHIM allele silencing, as represented by the Cxcr4+/o genotype, is superior to WHIM allele correction, as represented by the Cxcr4+/+ genotype, as a cure strategy in a mouse model of WHIM syndrome. Importantly, transplantation of Cxcr4+/o BM, but not Cxcr4+/w BM, in unconditioned Cxcr4+/w recipients corrected myeloid cell deficiency in the blood, a major phenotype in WHIM syndrome that predisposes patients to bacterial infections. Even low levels of donor Cxcr4+/o LT-HSCs (~6% of total HSCs) in the BM of unconditioned Cxcr4+/w mice after transplantation resulted in correction of the blood.

In lethally irradiated recipients, the major mechanism involves markedly
enhanced BM engraftment of $Cxcr4^{+/-}$ HSCs. This was associated with early enhanced $Cxcr4^{+/-}$ HSC proliferation which may overcome the BM homing disadvantage of these cells relative to $Cxcr4^{+/+}$ HSCs. The proliferation data in the lethally irradiated recipient transplantation model are consistent with previous reports indicating that wild-type CXCR4 negatively regulates HSC proliferation (5). At steady state in the model, the great majority of myeloid cells are from the $Cxcr4^{+/-}$ BM donor in both recipient BM and blood, suggesting that differential retention of mature donor-derived $Cxcr4^{+/-}$ versus $Cxcr4^{+/+}$ leukocytes in the BM plays little if any role in explaining the almost complete reconstitution of the blood with $Cxcr4^{+/-}$ myeloid cells. In contrast, differential BM retention of lymphoid cells may contribute substantially to the predominance of $Cxcr4^{+/-}$ lymphoid cells in the blood.

The quite different BM results in the two transplantation models tested here suggest that the increased proliferative potential of $Cxcr4^{+/-}$ HSCs over $Cxcr4^{+/+}$ HSCs may only become manifest when stem cell niches are present in great excess over available donor HSCs or when it is needed for an immune response. The number of HSCs in BM is thought to be limited by the total number of specific niches available (5, 23). We speculate that in the lethally irradiated recipient model, the proliferative advantage of $Cxcr4^{+/-}$ HSCs is not limited by niche availability. In contrast, in unconditioned WHIM mice, most niches are already occupied. Therefore, HSC engraftment is limited regardless of the $Cxcr4$ genotype. In unconditioned $Cxcr4^{+/+}$ recipients, leukopenia may stimulate production of mature leukocytes from both $Cxcr4^{+/+}$ and $Cxcr4^{+/-}$ HSCs. However, differential HSC proliferation and mature myeloid cell egress from BM may provide the selective mechanism for restoring the peripheral
myeloid cell counts to normal with donor-derived Cxcr4+/o but not Cxcr4+/w cells. A limitation of this interpretation is that it is difficult to directly evaluate donor-derived HSC proliferation in vivo in non-conditioned recipients due to the paucity of these cells in BM and the slow time course of engraftment.

Importantly, we found that reconstitution of unconditioned WHIM mice with Cxcr4+/o leukocytes was durable after BM transplantation, and that survival in mice with a single copy of Cxcr4 in their hematopoietic cells is normal. Although our results are not consistent with CXCR4 haploinsufficiency being the sole genetic explanation for the WHIM patient cured by chromothripsis (16), they do suggest that CXCR4 haploinsufficiency may have contributed. The patient is also haploinsufficient for 163 other genes, so many other possible genetic mechanisms must be considered. Nevertheless, our results support further development of a cure strategy for WHIM syndrome focused on syngeneic transplantation of patient HSCs in which the WHIM allele has been deleted by gene editing and patient conditioning is limited or even unnecessary.
Methods

Detailed methods are described in the Supplemental Methods online.

Statistics. All data are presented as mean ± SEM. Two-tailed Student’s t test for single comparisons and 2-way ANOVA for multiple comparisons were used. $P$ less than 0.05 was considered significant.

Study approval. All animal experiments were approved by the NIAID Animal Care and Use Committee.
AUTHOR CONTRIBUTIONS

Experimental design was provided by J.G., D.H.M. and P.M.M. Generation and analysis of experimental data were provided by J.G., E.Y., M.S., A.Y., Q.L., A.A. and A.O.-A. with supervision and analysis by J.G., D.H.M. and P.M.M. J.G. and P.M.M. wrote the manuscript.
ACKNOWLEDGMENTS

WHIM mice were graciously provided by Francoise Bacherie and Karl Balabanian.

This work was supported by the Division of Intramural Research of the National Institute of Allergy and Infectious Diseases, NIH.
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Figure 1. The Cxcr4 genotype rank order for peripheral blood reconstitution after competitive BM transplantation in lethally irradiated mice is Cxcr4<sup>+/o</sup> > Cxcr4<sup>+/+</sup> > Cxcr4<sup>+/w</sup>. (A and B) Donor-derived leukocyte frequencies after transplantation with total BM cells (A) or FACS-purified Lin<sup>-</sup>Sca-1<sup>-</sup>Kit<sup>-</sup>CD48<sup>-</sup>CD34<sup>-</sup>CD150<sup>+</sup> HSCs (B). Experimental design is to the left of each row of graphs. Data are the percentage (mean ± SEM) of total donor-derived cells for each subset indicated at the top of the corresponding column of graphs. See Supplemental Figures 1A & 1B for representative flow cytometry plots and transplantation conditions. (C) Donor-derived absolute leukocyte counts after competitive BM transplantation. Experimental design is to the left. Data are absolute numbers of donor-derived cells per ml (mean ± SEM) of the subset indicated at the top of each graph. The average absolute blood counts of naive Cxcr4<sup>+/+</sup> (black dashed lines, n=58) and Cxcr4<sup>+/w</sup> (blue dotted lines, n=38) mice from our colony are also presented. Each recipient was transplanted with 5 million BM cells (A and C) or 2000 HSCs (B). n was at least 5 mice for all conditions. SEM was < 5% of the mean at all timepoints lacking visible error bars. Results were verified in three additional independent experiments for A. +/o, +/+ and +/w denote Cxcr4<sup>+/o</sup>, Cxcr4<sup>+/+</sup> and Cxcr4<sup>+/w</sup> donors, respectively. p values, 2-way ANOVA.
Figure 2. The superiority of Cxcr4+/o BM for blood reconstitution after competitive transplantation in lethally irradiated mice involves an early HSC proliferative advantage and superior long-term engraftment, but not an HSC BM-homing advantage. (A) Homing. Equal numbers of Cxcr4+/o and Cxcr4+/w lineage-negative BM cells were co-transplanted into lethally-irradiated mice. Four hours later, the recipient BM was analyzed for donor-derived cells. (B) Long-term engraftment of HSCs and HPCs. (C) Mature leukocyte retention. Blood and BM cells of the mice in Figure 1B were analyzed on day 465 post transplantation. Data (n=5) are % (mean ± SEM) of total donor-derived cells specific for the indicated Cxcr4 genotype (abbreviated as +/o, +/+ and +/w) for the indicated HSCs and HPCs (A and B) and mature leukocytes (C) from individual experiments and are representative of 2 independent experiments in A, B and C. (D) HSC proliferation. (see Supplemental Figure 5 for details). Data (n=5) are % (mean ± SEM) of BrdU+ HSCs for each donor from a single experiment representative of two independent experiments. (E) Survival. Cxcr4+/+ (n=15) and Cxcr4+/o (n=27) littermates were observed in specific pathogen-free conditions. Abbreviations: Lin−, lineage-negative leukocytes; LT-HSC, long-term engrafting HSCs; ST-HSC, short-term engrafting HSCs; LSK, lin-Sca1+c-kit; MPP, multipotent progenitors. * p<0.05; ** p<0.01; *** p<0.005; **** p<0.001; n.s., not significant, student’s t test.
Figure 3. Correction of myeloid cytopenia in unconditioned WHIM mice by congenic Cxcr4<sup>+/o</sup> BM transplantation. (A) Cxcr4<sup>+/o</sup> BM is superior to Cxcr4<sup>+/+</sup> BM for establishing durable hematopoietic chimerism in unconditioned congenic Cxcr4<sup>+/w</sup> recipients. **Left panels**: Experimental design; **Right panels**: Donor-derived cell frequencies for each Cxcr4 genotype for the leukocyte subsets indicated at the top. Data are % (mean ± SEM) of total cells for each subset (n=5-8 mice per data point). Donor genotypes abbreviated as +/o, +/+ and +/w. See Supplemental Figure 7 for representative flow cytometry plots and transplantation conditions. **(B) Correction of myeloid cytopenia.** Non-competitive transplantation of 5x10<sup>7</sup> total BM cells from Cxcr4<sup>+/o</sup> (red) or Cxcr4<sup>+/w</sup> (blue) donor mice to unconditioned Cxcr4<sup>+/w</sup> recipients. **Upper-left**: Experimental design; **Lower-panels**: Donor-derived cell frequencies for the leukocyte subsets indicated at the top, presented as % (mean ± SEM) of total cells for each subset (n=5 mice per data point). Donor genotypes are indicated to the right of the corresponding graphs and abbreviated as +/o and +/w. **Upper-right**: Leukocyte subset counts at day 120 post-transplantation of unconditioned Cxcr4<sup>+/w</sup> mice receiving Cxcr4<sup>+/o</sup> (red) or Cxcr4<sup>+/w</sup> (blue) BM. Dotted lines, average values of blood counts for each subset of naive Cxcr4<sup>+/+</sup> (red, n=58) or Cxcr4<sup>+/w</sup> (blue, n=38) mice in our colony. Symbols +/o→+/w and +/w→+/w to the right are the abbreviations of Cxcr4<sup>+/w</sup> mice receiving Cxcr4<sup>+/o</sup> BM (red) and Cxcr4<sup>+/w</sup> mice receiving Cxcr4<sup>+/w</sup> BM (blue), respectively. * p<0.05; ** p<0.01; *** p<0.005. Single comparisons, student’s t test; multiple comparisons, 2-way ANOVA.
Figure 4. Low level Cxcr4+/o HSC engraftment after BM transplantation is sufficient to correct leukopenia in unconditioned WHIM mice. (A) Competitive model. $10^7$ BM cells from each of the indicated donors were mixed (final donor BM cell ratio=47:53, Cxcr4+/o:Cxcr4+/w, Supplemental Figure 7) and transplanted into each unconditioned Cxcr4+/w mouse. Upper-left: Experimental design. Recipient mice were euthanized on day 385 post-transplantation for mature leukocyte subset analysis in blood and BM (Lower-panels), and HSC and HPC analysis in BM (Upper-right). (B) Non-competitive model. 5x10^7 total BM cells from each of the indicated donors were transplanted separately into unconditioned Cxcr4+/w mice. Upper-left: Experimental design. Mice were euthanized on day 348 post-transplantation for mature leukocyte subset analysis in blood and BM (Lower-panels), and HSC and HPC analysis in BM (Upper-right). For both A and B, data are % (mean ± SEM) of total cells contributed by the indicated donor (Cxcr4 genotype indicated to the right of each panel and abbreviated as +/o or +/w) for each indicated HSC/HPC subset in BM and mature leukocyte subset in BM and blood (n=5 mice per data point). * p<0.05; ** p<0.01; *** p<0.005; **** p<0.001, student’s t test. n.s., not significant.