Supplementary Figures

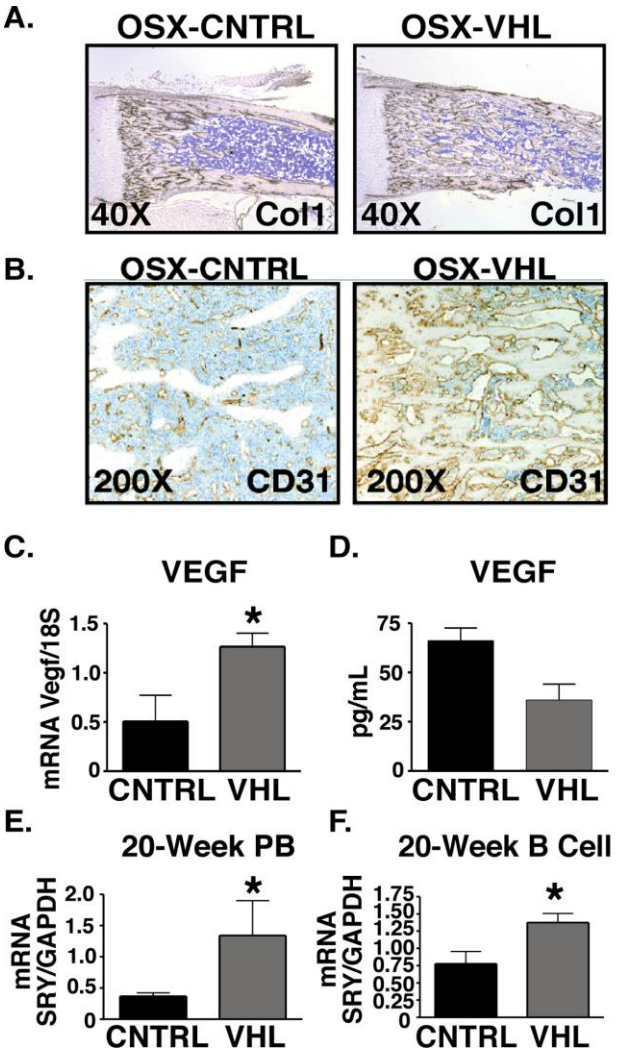

Figure S1, related to Figure 2. HSC niche expansion in OSX-VHL mice is associated with increased local VEGF expression and vascularity. A. OSX-VHL mutant tibias have increased numbers of trabecular osteoblasts as shown by increased expression of collagen type I (Col1) as determined by in situ hybridization. **B.** OSX-VHL tibias show increased vascularization as shown by increased staining for the endothelial cell marker CD31. **C.** Local VEGF expression is enhanced in OSX-VHL bone as primary bone marrow stromal cells isolated from OSX-VHL mice express increased levels of VEGF mRNA compared to osteoblasts isolated form OSX-Control mice. **D.** Systemic levels of VEGF are not affected in OSX-VHL mutants as serum levels of VEGF were not statistically different between 8 week old OSX-Control and OSX-VHL mutant mice. **E-F.** Real time PCR analysis of SRY gene expression in peripheral blood (PB, E) and B cells (F) from mice 20 weeks following lethal irradiation and transplantation with OSX-CNTRL (CNTRL, n = 5) or OSX-VHL (VHL, n = 4) bone marrow.

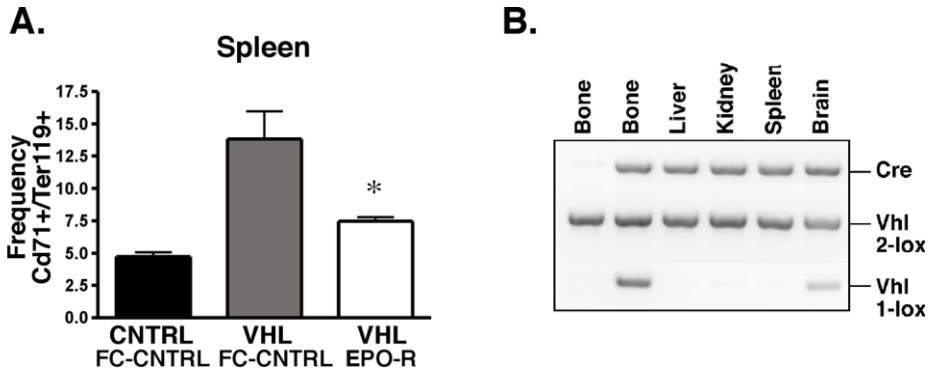

Figure S2, related to Figure 4. A. FACS analysis of the erythroid lineage in the spleen of OSX-VHL mice treated with adenovirus expressing FC control or soluble Epo-receptor (EPO-R) for 23 days (n = 4 in Control-FC treated, n = 3 in EPO-R treated groups). Note a significant decrease in CD71+/Ter119+ erythroid progenitors in the spleen of OSX-VHL mice treated with EPO-R compared to OSX-VHL mice treated with Fc-Control adenovirus. **B.** Genomic PCR analysis for Cre, VHL floxed allele (2-lox) and VHL recombined allele (1-lox) in DNA isolated from OSX-VHL tissues.

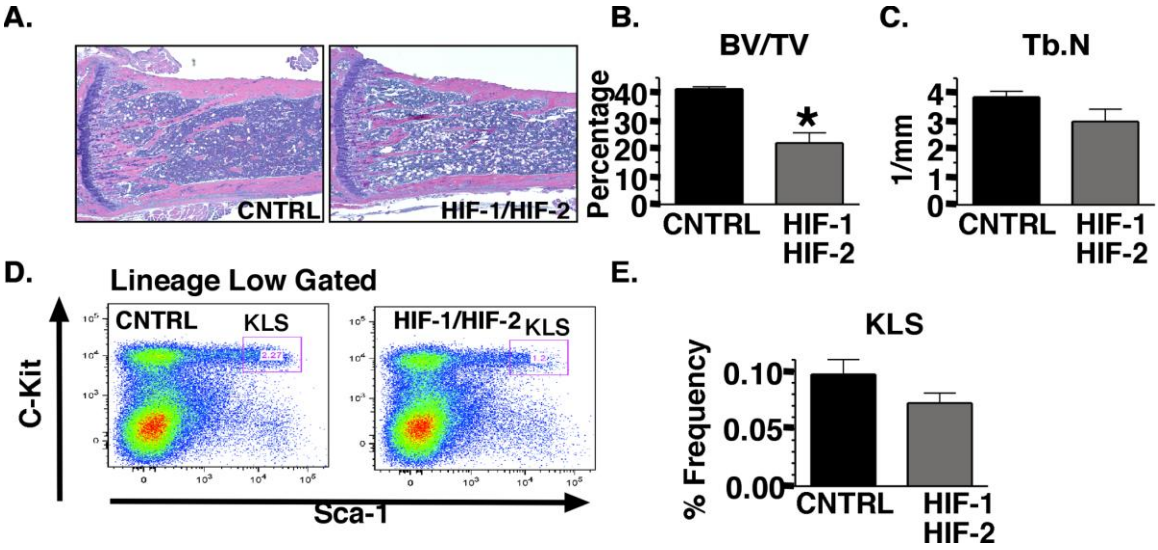

Figure S3, related to Figure 5. HIF-1 and HIF-2 deletion in osteoblasts reduces trabecular bone volume, but does not impair the number of HSCs. A. H&E stained OSX-CNTRL (CNTRL) and OSX-HIF-1/HIF-2 tibias at 8-weeks of age. **B-C.** Histomorphometric analysis of trabecular bone volume (BV/TV) and trabecular number (Tb.N) in OSX-CNTRL and OSX-HIF-1/HIF-2 tibias. For each group analyzed n = 4. **D.** Flow cytometric analysis (FACS) of c-Kit^{+} Lineage^{low} Sca-1^{+} (KLS) progenitors in OSX-CNTRL and OSX-HIF-1/HIF-2 bone marrow. **E.** Frequency of KLS in OSX-Cre mutant bone marrow shown as percentage of total bone marrow cells at 8-weeks-of age **(**K, n = 7 in each group; L, n = 3 in each group).

Supplemental Experimental Procedures

Bone Marrow Transplantation

The VHL and OSX-Cre mouse strains used in bone marrow transplantation studies were backcrossed >10 generations onto the FVB/N background. $1X10^6$ bone marrow mononuclear cells from OSX-VHL or OSX-CNTRL male mice were mixed with $1X10^6$ bone marrow mononuclear cells from wild-type female FVB/N mice. $1X10^6$ cells were transplanted into lethally irradiated (8.5 Gy) female FVB/N mice. 20 weeks following transplantation, peripheral blood was collected and B220+ B cells were isolated from the bone marrow. For this purpose, $4X10^6$ bone marrow cells were incubated with Rat anti-mouse B220 antibodies (ebioscience). Cells were washed and B220 positive cells were purified using sheep anti-Rat IgG dynabeads according to manufacturers protocols (Invitrogen). DNA was isolated using the DNeasy Blood and Tissue Qiagen kit. The relative male chimerism between transplanted mice was determined by Real Time PCR of SRY gene expression. Genomic DNA (25-75ng) was amplified using primers specific to SRY ((van Til et al., 2010), see below). GAPDH was utilized to normalize DNA content. Known dilutions of male DNA mixed with female DNA was used to generate standard curves as a control.

Real Time PCR

Real time PCR was performed using the following primers; *Epo*: FWD 5'-CAT CTG CGA CAG TCG AGT TCT G-3' , Rev 5'-CAC AAC CCA TCG TGA CAT TTT C-3' ; *18S:* FWD: 5-GCC CGA AGC GTT TAC TTT GA-3', REV: 5-TCC ATT ATT CCT AGC TGC GGT ATC-3; *Sry*: FWD 5'-CAT CGG AGG GCT AAA GTG TCA C-3',

REV 5’-TGG CAT GTG GGT TCC TGT CC-3’; *Gapdh*: FWD 5’-CAT CAC TGC CAC CCA GAA GAC-3’, REV 5’-TGA CCT TGC CCA CAG CCT TG-3’.

Supplemental References

van Til, N.P., Stok, M., Aerts Kaya, F.S., de Waard, M.C., Farahbakhshian, E., Visser, T.P., Kroos, M.A., Jacobs, E.H., Willart, M.A., van der Wegen, P.*, et al.* (2010). Lentiviral gene therapy of murine hematopoietic stem cells ameliorates the Pompe disease phenotype. Blood *115*, 5329-5337.