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Supplementary information

Nanoparticle-encapsulated siRNAs for gene silencing in the haematopoietic stem-cell niche

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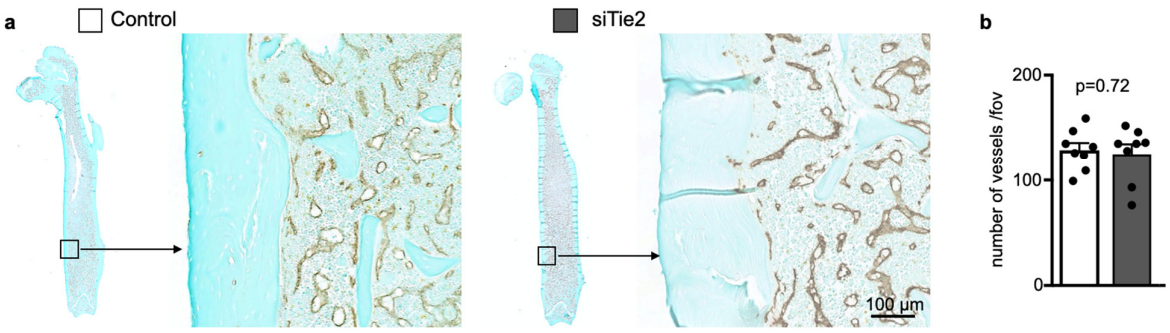
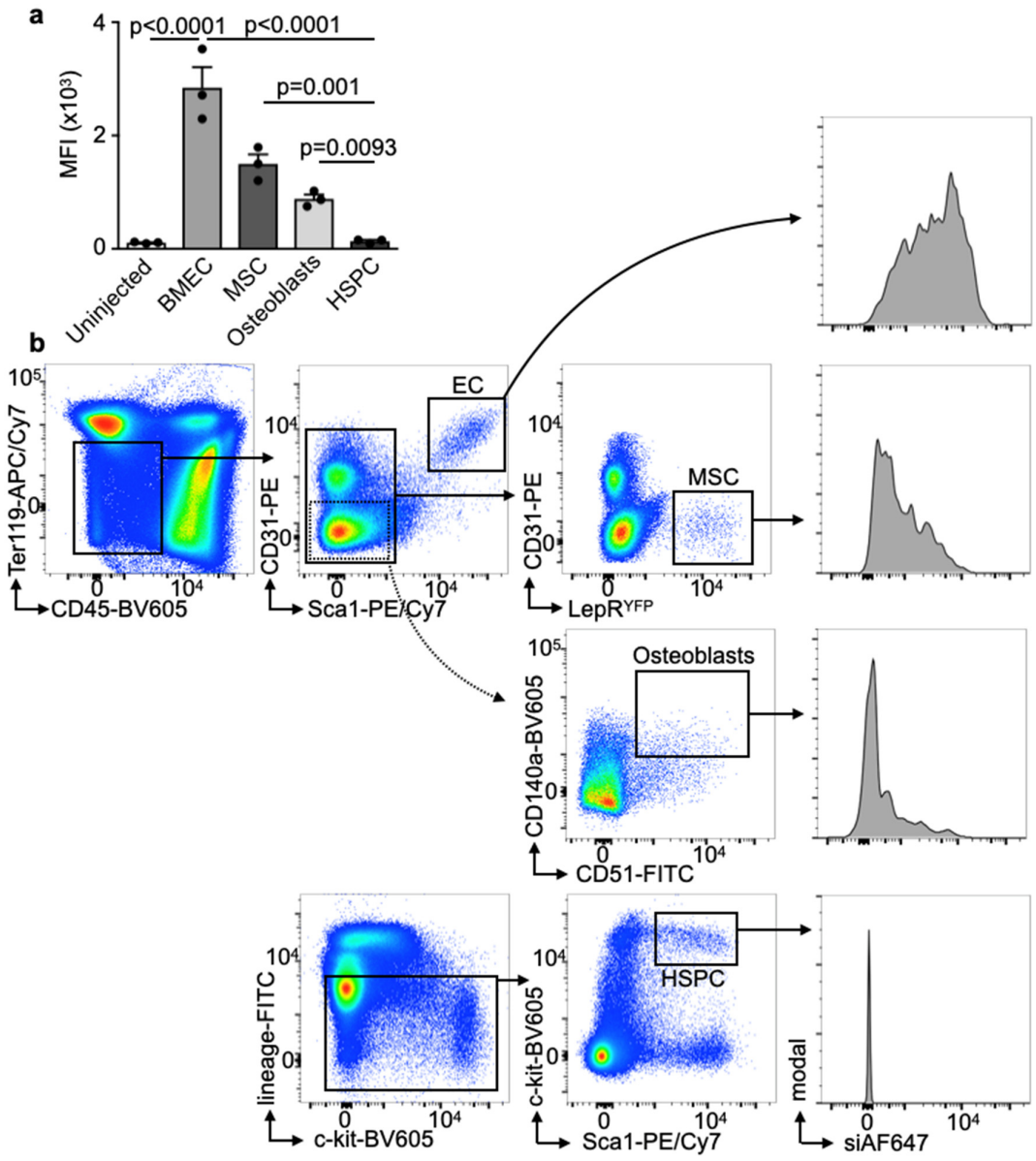
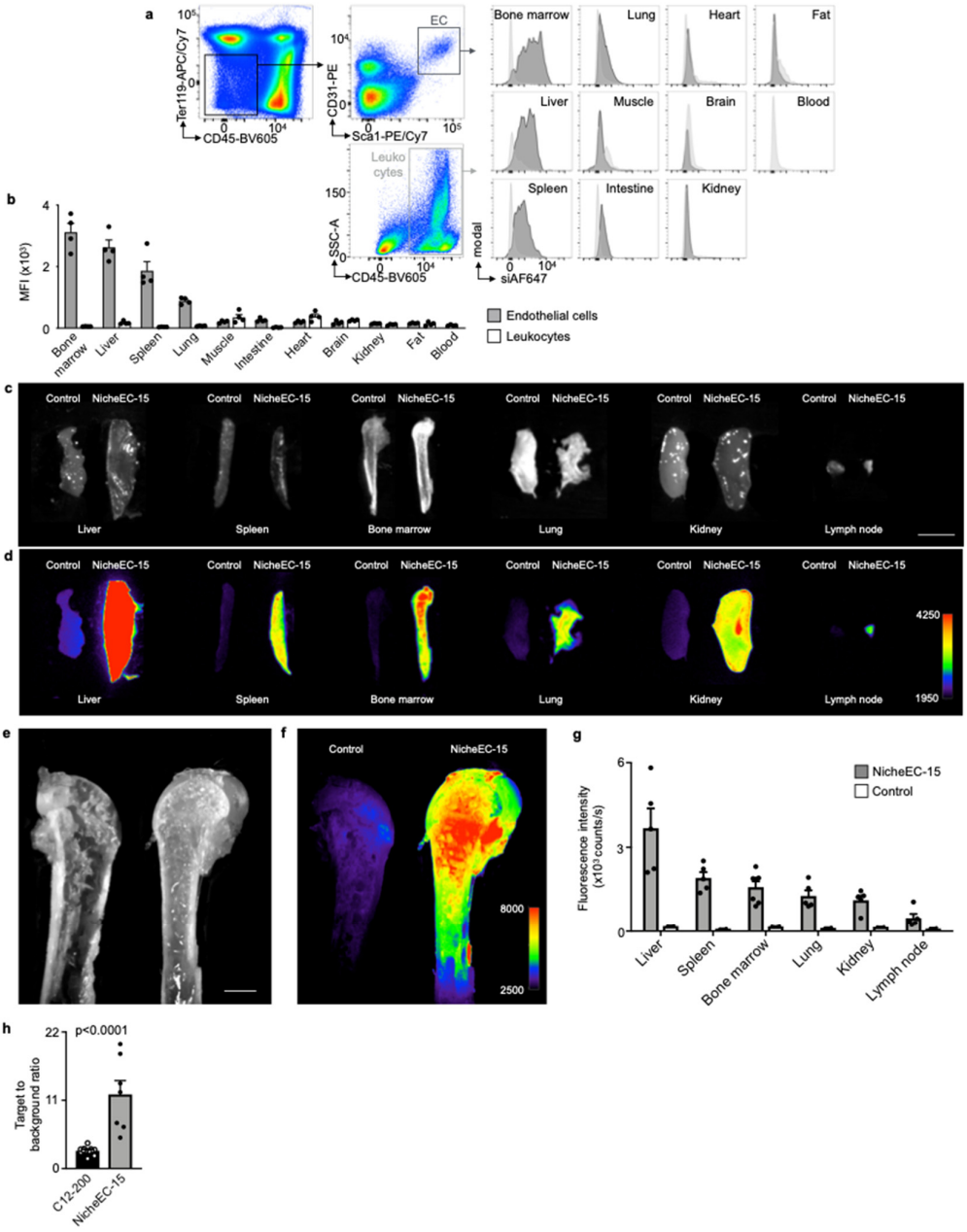


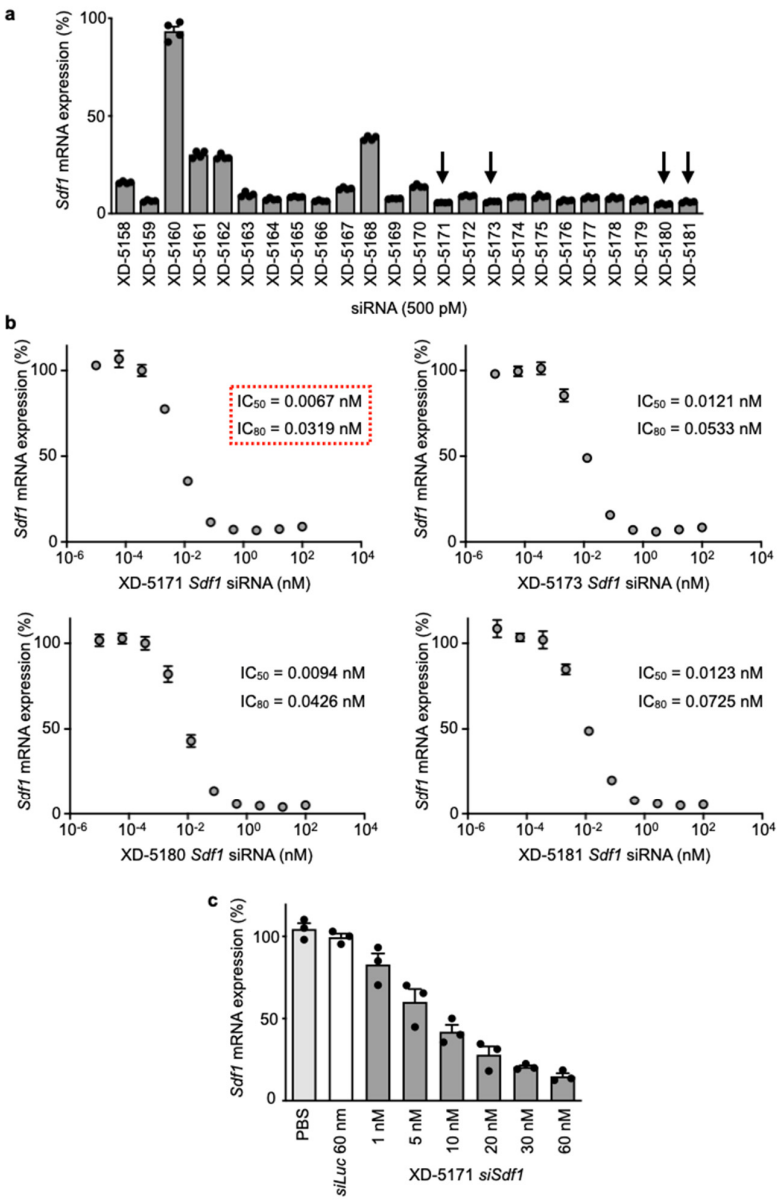
Supplementary Figure 1 | Bone marrow vessel density 7 days after siTie2. Mice were harvested 7 days after treatment with 2 mg/kg of siTie2 and compared to PBS injected controls. **a**, Low and high magnification view of endomucin staining of the femur. **b**, Number of vessels per field of view (n=4 mice per group, and two sections per mouse; two-sided t-test). Data are shown as mean ± s.e.m.



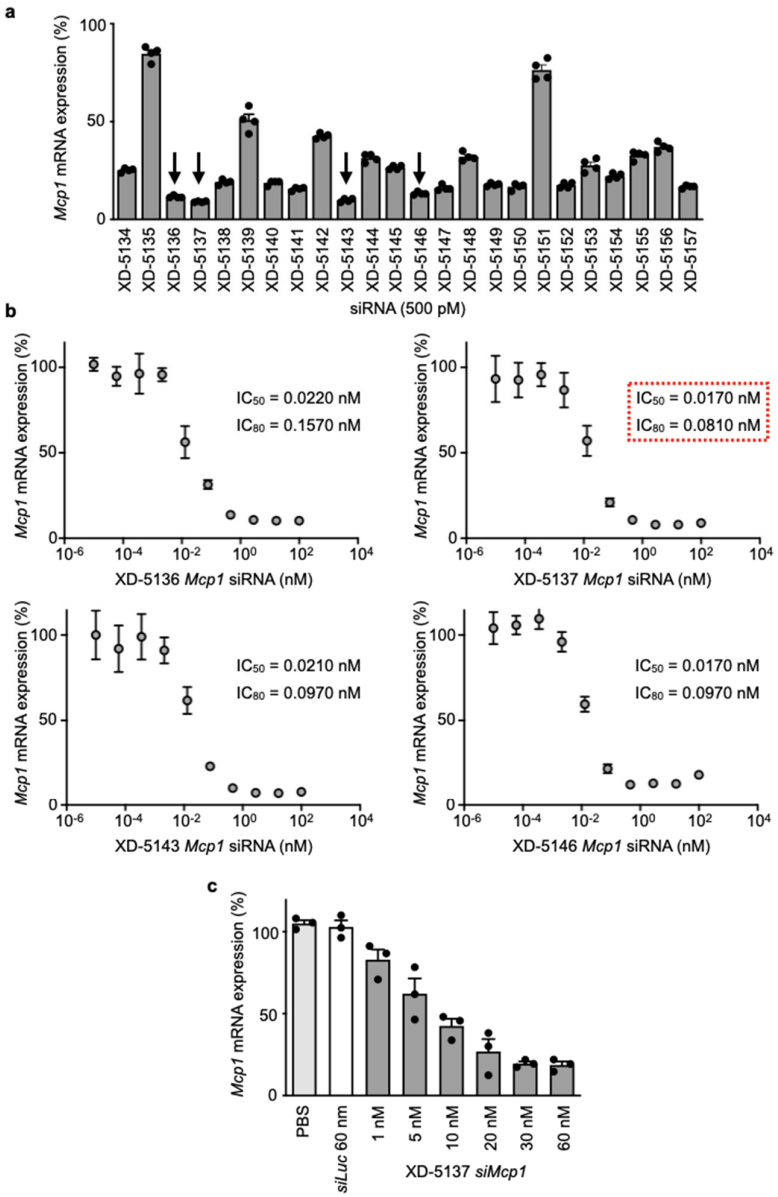
Supplementary Figure 2 | Uptake of AF647-labeled siRNA in bone marrow cells. **a**, Mean fluorescence intensity of uninjected control mice, bone marrow endothelial cells (BMEC), perivascular stromal cells (MSC), osteoblasts and hematopoietic stem and progenitor cells (HSPC) 2 hrs after injection of 2 mg/kg siRNA labeled with AF647 (n=3 mice per group each; one-way ANOVA with multiple comparisons). **b**, Gating strategy and histograms of AF647 signal in specific cells. Gating was performed in the sequence indicated by the arrows. Data are shown as mean ± s.e.m.



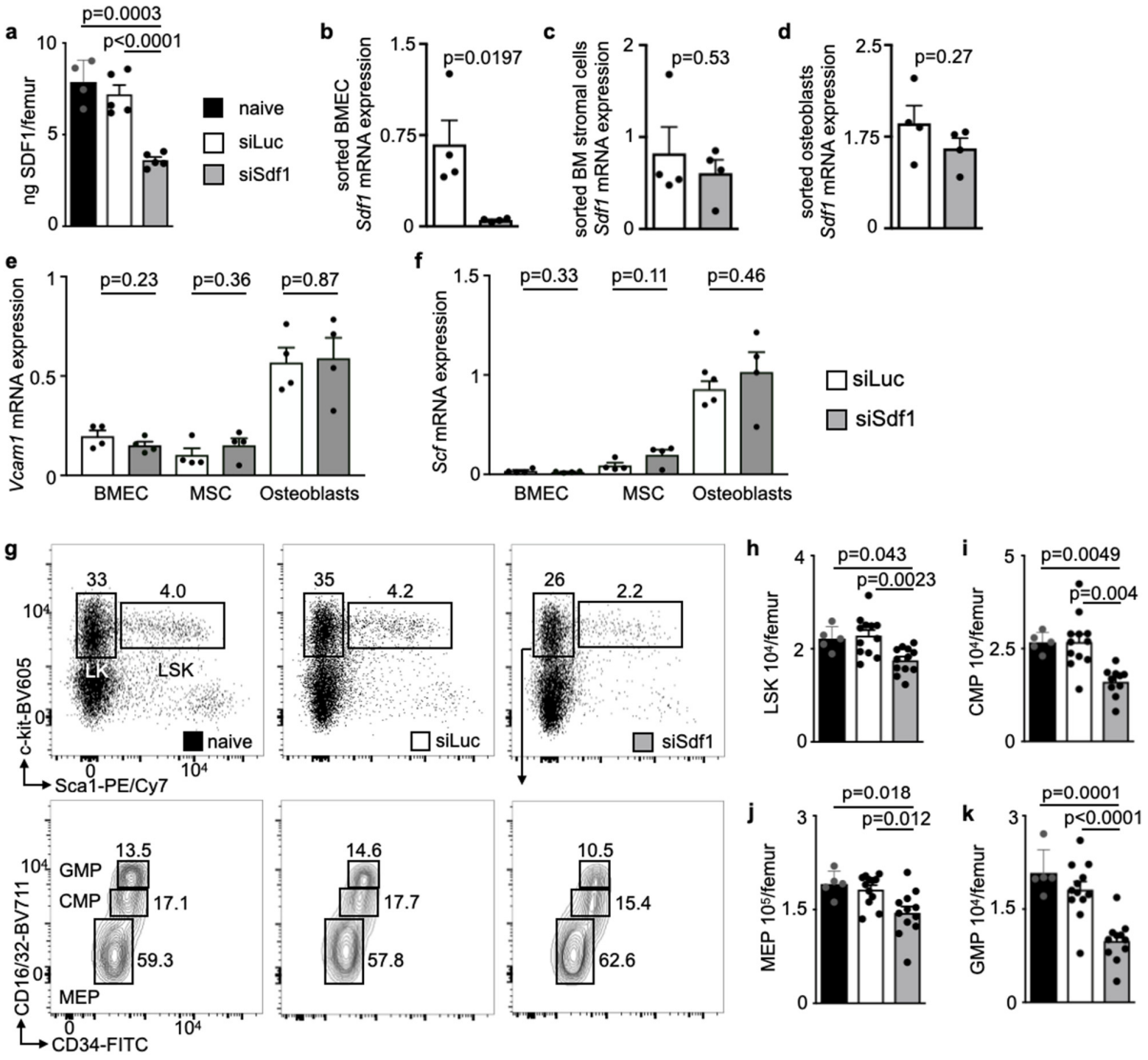
Supplementary Figure 3 | Biodistribution of NicheEC-15 nanoparticles. a, Gating strategy and histograms for biodistribution of NicheEC-15 encapsulating AF647-labeled siRNA (2 hrs after injection, dose 1 mg/kg, n=4 mice). Gating was performed in the sequence indicated by the arrows. **b**, Mean fluorescence intensity measured by flow cytometry. **c-f**, Fluorescence reflectance imaging 2 hrs after injection of NicheEC-15 encapsulating AF647-labeled siRNA (2 hrs after injection, dose 2mg/kg, n=5 mice). Two naive control mice served as autofluorescence controls. **c**, White light image of explanted organs. Scale bar equals 4 mm. **d**, Near infrared images of explanted organs. **e**, Magnified white light image of femur bone with opened cortex. Scale bar equals 0.5 mm. **f**, Magnified near infrared image. **g**, Quantification of fluorescence in key organs. **h**, Fluorescence reflectance imaging of bone marrow. Mice were injected i.v. with 2mg/kg AF647-labeled siRNA encapsulated in C12-200 or NicheEC-15 nanoparticles and femurs harvested 2 hours later. Bar graph shows quantification of target to background ratio in femur marrow. Femurs from non-injected control mice were used to quantify background autofluorescence. Two-sided t-test. Data are shown as mean ± s.e.m.



Supplementary Figure 4 | siRNA screen for *Sdf1* gene silencing. **a**, *Sdf1* gene expression in Hepa1-6 cells after treatment with 500 pM of each of 24 different siRNAs for 24 hrs (n=4 each). Results with target-specific siRNAs were normalized to mean activity of two unspecific control siRNAs. The best four siRNAs are highlighted with arrows. **b**, *Sdf1* knockdown dose response curves in Hepa1-6 after treatment with XD-5171, XD-5173, XD-5180 and XD-5181 siRNAs for 24 hrs (n=4). The best siRNA (XD-5171) which was used for subsequent experiments is highlighted with a red box. **c**, *Sdf1* silencing in endothelial cells in vitro (doses 1-60 nM). bEnd.3 cells were treated with NicheEC-15 containing the XD-5171 siRNA for 24 hrs prior to qPCR analysis (n=3 independent experiments each). Data are shown as mean ± s.e.m.

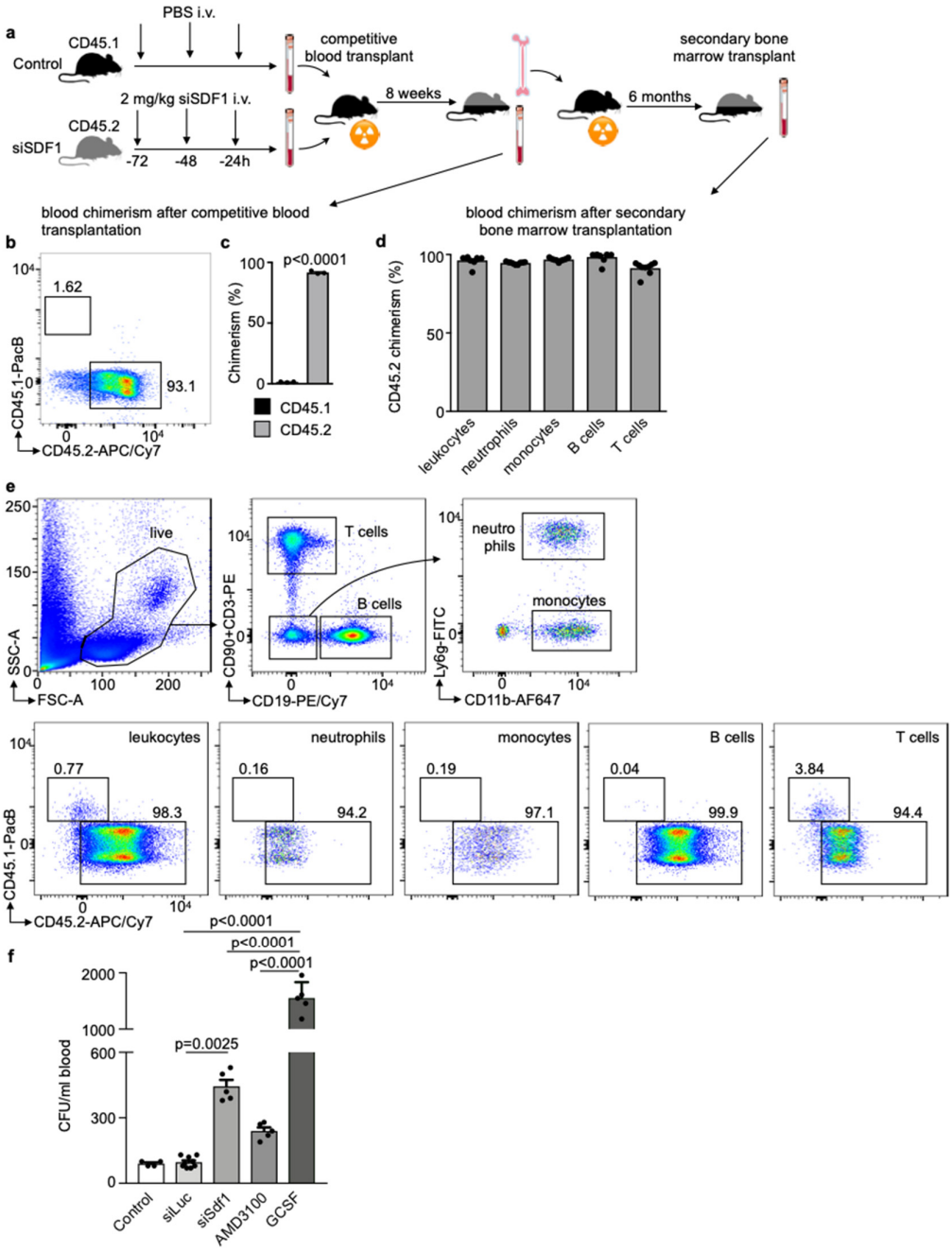


Supplementary Figure 5 | siRNA screen for *Mcp1* gene silencing. **a**, *Mcp1* gene expression in Hepa1-6 cells after treatment with 500 pM of each of 24 different siRNAs for 24 hrs (n=4 each). Results with target-specific siRNAs were normalized to mean activity of two unspecific control siRNAs. The best four siRNAs are highlighted with arrows. **b**, *Mcp1* knockdown dose response curves in Hepa1-6 after treatment with XD-5136, XD-5137, XD-5143 and XD-5146 siRNAs for 24 hrs (n=4). The best siRNA (XD-5137), which was used for subsequent experiments, is highlighted with a red box. **c**, *Mcp1* silencing in endothelial cells in vitro (doses 1-60 nM). bEND.3 cells were treated with NicheEC-15 containing the XD-5137 siRNA for 24 hrs prior to qPCR analysis (n=3 independent experiments each Data are shown as mean ± s.e.m.



Supplementary Figure 6 | Sdf*1* gene silencing in BM niche cells.

a, Sdf1 protein by ELISA in bone marrow plasma. Mice were either injected with 2 mg/kg siSdf1 siLuc control or not (n=4 mice for naive, n=5 for siLuc and siSdf1; one-way ANOVA with multiple comparisons.). **b-f**, Endothelial cells, mesenchymal stromal cells and osteoblasts were sorted from the femoral bone marrow side by side. qPCR for *Sdf1* in **b,** BMEC, **c**, mesenchymal stromal cells or **d**, osteoblasts. **e**, Evaluation for unspecific knock down of *Vcam1* or **f,** *Scf*. Mice received 2 mg/kg siSdf1 or siLuc i.v. and 48 hrs later bone marrow endothelial cells (BMEC), perivascular mesenchymal stromal cells (MSC) and osteoblasts were FACS isolated from the femur, followed by qPCR. Values are relative to GAPDH expression. **b-f,** n=4 mice per group each; two-sided t-test. **g**, Representative dot plots for LSK, CMP, MEP and GMP. Gating was performed in the sequence indicated by the arrow from top to bottom. **h-k**, Number of LSK, CMP, MEP and GMP in the femoral bone marrow (n=5 mice for naive and n=12 for siLuc and siSDF1; one-way ANOVA with multiple comparisons). Data are shown as mean ± s.e.m.



Supplementary Figure 7 | Primary and secondary transplantation assays following RNAi. a, Experimental design of the primary competitive peripheral blood stem cell transplantation and the subsequent secondary bone marrow transplantation. **b**, Gating for chimerism of blood leukocytes. **c**, Bar graph for leukocyte chimerism obtained in the blood after competitive peripheral blood transplantation (n=3 mice each; two-sided t-test). **d**, Chimerism for CD45.2 of blood leukocytes and leukocyte subpopulations 6 months after the second transplantation (n=5 mice each; one-way ANOVA with multiple comparisons). **e**, Gating strategy for (d) and FACS plots showing CD45.2 and CD45.1 chimerism. Gating was performed in the sequence indicated by the arrows. **f**, Blood CFU assay reporting the release of hematopoietic stem cells after treatment with siSdf1, AMD3100 and GCSF compared to untreated controls and mice injected with control siRNA (=siLuc; n=5 mice each, except siLuc n=10; one-way ANOVA with multiple comparisons). Data are shown as mean ± s.e.m.