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Supplementary Materials for

In vivo hematopoietic stem cell modification by mRNA delivery

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The PDF file includes:

Other Supplementary Material for this manuscript includes the following:

Materials and Methods

RNA synthesis and preparation of targeted LNP-mRNA

Gene sequences for Firefly Luciferase (luc2), Cre Recombinase (cre), and enhanced Green Fluorescent Protein (eGFP) were sourced from SnapGene (www.snapgene.com/resources) software plasmids pGL4.10[luc2], pCMV6-Entry-Cre, and pEGFP, respectively, then codon-optimized. The PUMA gene was codon-optimized from the NCBI reference sequence NM_133234.3. Sequences for murine BAK1, CASP3, and CASP7 were codon-optimized from NCBI reference sequences NM_007523.3, NM_009810.3, and NM_007611 respectively. Constructs incorporating three miR-122 binding sites in the 3'UTR were directly reproduced from Jain *et al (19)*. The ABE sequence (ABE8e-NRCH) and HBB^S-targeting sgRNA sequences were replicated from Newby *et al* (*16*) and the sgRNA was purchased from Synthego (Redwood City, CA, USA). All sequences are in supplementary table 1. Each gene coding sequence was cloned into an IVT-mRNA production template plasmid carrying a T7 promoter, 5' and 3' UTR elements, Kozak consensus sequence, and 101 poly(A) tail. DNA synthesis, cloning and industrial grade endotoxin-free plasmid preparation service was provided by GenScript (Piscataway, NJ, USA). IVT-mRNA was produced using linearized IVT template plasmid and the MEGAScript T7 kit (Thermo Fisher Scientific, AMB13345, Waltham, MA, USA) and formulated with nucleoside-modified m1Ψ-5'-triphosphate (TriLink, N-1081, San Diego, CA) instead of UTP. 5' Capping of the IVT-mRNAs were performed co-transcriptionally using the trinucleotide cap1 analog, CleanCap® Reagent AG (3' OMe) (TriLink, N-7413, San Diego, CA, USA). Single-stranded IVT-mRNA was the purified by cellulose purification, as previously described (*25*). All mRNAs were analyzed by agarose gel electrophoresis and were stored at -20°C. Cellulose purified m1Ψ-containing RNAs were encapsulated in LNP using a self-assembly process as previously described (*26*), briefly an ethanolic lipid mixture of ionizable cationic lipid, phosphatidylcholine, cholesterol, and polyethylene glycol-lipid was rapidly mixed with an aqueous solution containing the mRNA at acidic pH. The RNA-loaded particles were characterized by dynamic light scattering using a Zetasizer Nano ZS (Malvern Instruments, Malvern, UK) and a Ribogreen assay. The mean hydrodynamic diameter of these LNP- mRNAs was approximately 80nm with a polydispersity index of 0.02-0.06 and an encapsulation efficiency of ~95%. LNP used in this study are proprietary to Acuitas Therapeutics (Vancouver, BC, Canada). The ionizable cationic lipid and LNP composition are described in US patent US10,221,127. To prepare antibody-targeted LNP-mRNA, LNP-mRNA were conjugated with purified rat anti-mouse CD117 (c-kit), clone 2B8 (BioLegend, 93235, San Diego, CA, USA) or mouse anti-human CD117 (c-kit), clone 104D2 (BioLegend, 95747, San Diego, CA, USA), mouse anti-mouse CD45.2, clone 104 (Biolegend, 92176, San Diego, CA, USA), and control isotype-matched IgG (Thermo Fisher Scientific, Rat IgG Isotype 31933, Mouse IgG Isotype 10400C, Waltham, MA, USA) via SATA–maleimide chemistry, as described previously (*9*). Briefly, LNP was modified with maleimide functioning groups (DSPE-PEG-mal) by a post-insertion technique. The antibody was functionalized with SATA (N-succinimidyl *S*-acetylthioacetate, 26102) from Thermo Fisher (Burlington, MA, USA) to introduce sulfhydryl groups allowing conjugation to maleimide. SATA was deprotected using 0.5 M hydroxylamine followed by removal of the unreacted components by Zeba spin desalting columns (Thermo Fisher Scientific, 89890, Waltham, MA, USA). The reactive sulfhydryl group on the antibody was then conjugated to maleimide moieties using thioether conjugation chemistry. Purification was carried out using Sepharose CL-4B gel filtration columns

(MilliporeSigma, GE17-0150-01, Burlington, MA, USA). mRNA content was calculated by performing a modified Quant-iT RiboGreen RNA assay (Thermo Fisher Scientific, R11490, Waltham, MA, USA). After addition of the targeting ligand, all the targeted and non-targeted LNP preparations were kept at 4°C and were used within three days of preparation. A complete list of LNP preparations and utilization for *ex vivo/in vivo* treatments is provided in table S2.

***In vitro* cell transfection studies and luciferase assay**

LNP carrying reporter luciferase IVT-mRNA were added at increasing concentrations to the cells and incubated for 24 h. Plates were then washed with PBS, lysed in luciferase cell culture lysis reagent (Promega, E1531, Madison, WI, USA). The cell lysate was mixed with Firefly Luciferase Assay System substrate (Promega, E1500, Madison, WI, USA) and measured on a MiniLumat LB 9506 luminometer (Berthold/EG&G; Wallac, Bad Wildbad, Germany).

Bioluminescence imaging

C57BL/6J mice were i.v. injected with control IgG/LNP-Luc, CD117/LNP-Luc or CD117/LNP-Luc-miRts formulations. At 5h post-injection, bioluminescence imaging was carried out as described previously (*5*) using an IVIS Spectrum imaging system (Caliper Life Sciences, Waltham, MA, USA). D-luciferin sodium salt (Regis Technologies, 1-360243-200, Morton Grove, IL, USA) dissolved in PBS was administered to mice intraperitoneally at a dose of 150 mg/kg. After 5 min, the mice were euthanized; desired tissues were harvested, washed with PBS, and immediately placed on the imaging platform. Harvested femurs were slightly crushed by spatula to expose the bone marrow for imaging. Tissue luminescence was measured on the IVIS imaging system using an exposure time of 5s or longer to ensure that the signal obtained was within operative detection range.

Animal husbandry and regulatory

Animal treatments were approved by the Institutional Animal Care and Use Committee at the University of Pennsylvania (IACUC protocol #803941) and the Children's Hospital of Philadelphia (IACUC protocol #1173). Reporting of animal studies have been provided in accordance with ARRIVE guidelines.

Human subjects

Specimens from patients with sickle cell disease were collected upon consent, which was obtained according to the Declaration of Helsinki, following the approval by the Children's Hospital of Philadelphia, Institutional Review Board (IRB; #15-012123 and #04-004078). Deidentified apheresis waste product from patients with SCD at the Children's Hospital of Philadelphia and $CD34^+$ progenitor cells were isolated with the MACS MicroBead kit (Miltenyi).

In vitro cell treatment

Bone marrow cells were isolated from femurs of animals, after removal of muscle and connective tissues, by mechanical crushing, which maximizes cell recovery. BM cells were resuspended in 4% FBS (SH3007103) from Hyclone (Logan, UT) and PBS (10010023) from Gibco (Waltham, MA) and RBC were lysed using ACK lysis buffer (A1049201) from Gibco (Waltham, MA) at room temperature, according to manufacturer protocol, filtered through a 40μM sterile strainer (431750) from Corning (Glendale, AZ) and washed with 4% FBS PBS solution. After lysis, cells were counted and assessed for viability by AOPI staining (CS2-0106), using a Cellometer Auto

2000 cell viability counter (Nexcelom Bioscience, Lawrence, MA) and seeded at a 1.5E+06/mL concentration in Stemspan SFEM (096000) from Stem Cell Technologies (Vancouver, BC, Canada) supplemented with 50 ng/mL mSCF (250-03), 6ng/mL mIL-3 (213-13), and 10 ng/mL mIL-6 (216-16), all supplied by Peprotech (Cranbury, NJ), LNP formulations were added at the time of seeding and for up to 18 hours, depending upon assay.

Human erythroid progenitor cells (ErPC) editing and differentiation

Human CD34+ cells were isolated from blood products by immunomagnetic-separation using the CD34 MicroBead Kit (130-046-702) from Miltenyi Biotec Inc. (Auburn, CA). ErPC were obtained through expansion of CD34+ cells, using a culture system previously described (*27*) and frozen after 6–10 days. Upon thawing cells were let recover for 48hrs and exposed to anti-human CD117/LNP-ABE and CD117/LNP-sgRNA formulation (3 to 10pg/cells dose, at 1:1 weight ratio) at a 1.5E+06/mL cell concentration for 6 hours. Cell viability was measured before and after treatment by AOPI staining. ErPC were let expand 24 prior to inducing differentiation. Differentiated erythroblasts were collected after 7 days, using a protocol previously described *(27*).

Sickling Assay

The degree of cell sickling was measured using a modified version of a method already described (*28*). Briefly, 1E+06 differentiated erythroblasts were suspended in 100µL of isotonic TES, supplemented with 10 mM glucose and 0.2% bovine serum albumin, in individual wells of a Costar polystyrene 96-well microplate (№ 9017; Corning, Corning, NY). The microplate was then transferred to a Thermomixer R shaker-incubator (Eppendorf, Enfield, CT), and maintained under hypoxia (2.5% Oxygen gas, balance Nitrogen gas), with continuous agitation at 900 rpm, at 37° C for 2 hours. At conclusion, aliquots (~ 20 µL) of each sample were collected in 2% glutaraldehyde solution for immediate fixation without exposure to air. Subsequently, fixed cell suspensions were introduced into specialized glass microslides (Dawn Scientific, Inc., Newark, NJ) (*29*) for acquisition of bright field images (at 20x magnification) of single layer cells on a Zeiss Axiovert 200M inverted microscope (Carl Zeiss Microscopy, LLC, White Plains , NY), fitted with an Infinity 2 camera (Teledyne Luminera, Ottawa, ON, Canada) and the coupled Image Capture software.

Quantification of $Hbb^{G\text{-}Makassar}$ protein

Single chain quantification of individual globins was assessed by reverse-phase HPLC on clarified cell lysates obtained by disrupting cell pellets in water. Hemoglobin samples were injected in a Nexera apparatus from Shimadzu Scientific Instruments, Inc. (Columbia, MD) using a 250 mm x 4.6 mm Aeris 3.6 um C4 200A column from Phenomenex (Torrance, CA) and a gradient from 32% to 47% of 0.1% trifluoroacetic acid in acetonitrile in water over 60 minutes, with UV detection at 215 nm. Serial dilutions of a solution with known concentrations of Hbb A-F-S-C (Helena Laboratories, Beaumont, TX) were used to generate a calibration curve, where the peak areas were plotted against the concentration values. Types and relative quantity of Hbs in samples were assessed by comparison to standard hemoglobin controls.

Quantification of base editing

Genomic DNA was extracted with QIAamp DNA Mini Kit (56304) from Qiagen (Hilden, Germany) or QuickExtract™ DNA Extraction Solution (QE09050 and QE0905T), from Lucigen (Middelton, WI). Quantification of base editing was performed on 50 ng of genomic DNA upon

amplification of the region that includes the binding site of the gRNA using KAPA2G Fast ReadyMix from Kapa Biosystem (Wilmington, MA, USA). Primers for amplification were HBB3-F 5'-TCTGGAGACGCAGGAAGAGA-3' and HBB3-R 5'-CTGTCTCCACATGCCCAGTT-3'. After Sanger sequencing (Azenta), the editing percentage was calculated using EditR (for A>G conversion, Adenine Base editor targeting), following a workflow previously described (*30*).

CFU assay

CFU assay was conducted using reagents from Stem Cell Technologies. Bone marrow harvested from animals was seeded at 30,000 cells/well in complete Methocult media (M3434), in meniscus-free 6-well SmartDish plates (27371), using 16-gauge blunt end needles (28110) per directions from Stem Cell Technologies. Colonies were incubated for 2 weeks at 37^{o}C in CO2 incubators. Colonies were imaged using Evos FL Auto (AMAFD1000) manufactured by Life Technologies (Waltham, MA) microscope and analyzed using superimposed bright field and fluorescent (Texas Red or GFP filter as appropriate) images.

Animal treatments for ex vivo/competitive transplants, PUMA preconditioned transplants, i.v. injections, and perfusion

C57BL/6 CD45.1 (002014) recipients were obtained from The Jackson Laboratory (Bar Harbor, MA) lethally irradiated with 2 consecutive cycles of 5 Gy each, 4 hours apart from X-RAD 320 manufactured by Precision X-RAY Irradiation (Madison, CT). Each recipient received 2-3 million donor RBC-lysed bone marrow cells after *ex-vivo* treatment with LNP formulations (18hr). CD117/LNP-PUMA pre-conditioning was provided by i.v. injection 6.5 days prior to bone marrow transplant of 10E+06 RBC-depleted GFP^{+} C57BL/6 CD45.2 BM cells. In vivo injections of LNP formulations and BM infusions were provided by retroorbital vein on mice under isoflurane (NDC #66794-017-10, from Piramal Enterprises Ltd, Telangana, India)-induced anesthesia, using a precision vaporizer (#V7015, from SurgiVet, St. Paul, MN). Primers for amplification of cre-recombinase genome edited bone marrow and spleen from Ai9 and Ai14 animals were: Cre-F: 5'-GCT GGT TAT TGT GCT GTC TCA TC-3'and Cre-R: 5'-CAT GAA CTC TTT GAT GAC CTC CTC-3'.

Lung and Liver Perfusion

In preparation for organ perfusion mice were induced to general anesthesia by injected by IP injection of a 200 mg/kg ketamine (NDC #13985-584-10 From Vet One, Boise, ID) and 20 mg/kg xylazine (NDA #139-236, from Akorn Inc., Lake Forest, IL) solution in PBS. Upon reaching complete loss of footpad reflexes, the abdominal cavity was cut open and the ribcage was dissected to open the chest cavity. The heart was slowly infused with 10mL of 1% FBS PBS solution using a 27 Gx ½ inch needle (305109) supplied by Beckton Dickinson (Franklin Lakes, NJ) upon interruption of portal vein flow. Lungs and liver were rapidly removed and first incubated in 1X Buffer S, provided with GentleMACS Lung Dissociation Kit (130-095-927), or Corning DMEM (10-017-CV) media, respectively. After initial homogenization, lung and liver were processed following manufacturing instructions for GentleMACS Lung or Liver (130-105-807) Dissociation Kits, purchased from Miltenyi Biotec (Bergisch Gladbach, North Rhine-Westphalia, Germany). Tissues were homogenized using GentleMACs Dissociator system (130-093-235), following the recommended programs. Cell pellets underwent 1-2 cycles of RBC lysis with ACK buffer, followed by ice cold PEB (phosphate-buffered saline (PBS) pH 7.2, 0.5% bovine serum albumin

(12659) from Sigma Aldrich (St. Louis, MO), and 2 mM EDTA (Invitrogen)) buffer washes. Cell number and viability was assessed by AOPI staining with Cellometer, as described.

Flow cytometry for analyses for BM and peripheral blood

Monthly assessments of peripheral blood cell tdTomato marking were carried out by direct measurement of tdTomato expression in whole blood for the RBC compartment, or after RBC lysis, using ACK lysis buffer at room temperature, for the WBCs analyses. tdTomato expression in WBC was assessed using the following antibodies: violetFluor™ 450 CD3 (clone 17A2, 75-0032-U025) from Tonbo Biosciences (San Diego, CA), CD45R/B220-FITC (clone RA3-6B2, 103205) from BioLegend (San Diego, CA), Ly-6G/Gr1 PE- Cyanine7 (clone RB6-8C5, 565033) from BD Biosciences (Franklin Lakes, NJ), for detection of T, B cells and Granulocytes, respectively, while CD45.2 PerCP-Cyanine5.5 (clone 104, 45-0454-82) from eBioscience (Waltham, MA), CD45.1 APC (clone A20, 110713) BioLegend antibodies were used to discriminate proportion of donor versus recipient chimerism, respectively. Bone marrow samples obtained after crushing were treated with ACK lysis buffer to remove RBC prior to analyses . The following biotinylated antibodies were used to discriminate lineage committed cells: CD45R (13-0452-82)/CD8 (13-0081-82)/CD4 (3-0042-82)/CD127 (13-1271-82)/Gr1-Ly6G (13-5931-82)/Ter119 (13-5921-82) from eBiosciences. An APC-eFluor 780 streptavidin (47-4317-82) from Invitrogen was used to bind lineage committed cells pre-incubated with biotinylated lineage antibody cocktail. To discriminate LSK cells we utilized an APC anti-CD117 (clone 2B8, 17-1171-82) from Invitrogen and a ScaI-PE-Cyanine7 antibody (clone D7, 25-5981-82) from eBioscience. LT-HSC (LSK CD150+ CD48-) were gated using a Pacific Blue anti-CD48 antibody (clone HM48-1, 103418) and a BV650 anti-CD150 antibody (clone TC15-12F12.2, 115931), both from BioLegend. The EPCR+ (or CD201+) LT-HSC subpopulation was gated using a PE anti-CD201 antibody (clone RCR-16) from BioLegend. Peripheral blood and spleen samples were acquired on a CytoFLEX S analyzer with 4 lasers (405, 488, 561, 638 nm) made by Beckman Coulter (Brea, CA), while bone marrow cells were acquired on a CytoFLEX LX analyzer with 6 lasers (375, 405, 488, 561, 638, 808 nm) also manufactured by Beckman Coulter. All acquisition data was analyzed using FlowJo software from Tree Star Inc (Ashland, OR).

S**upplemental Figures:**

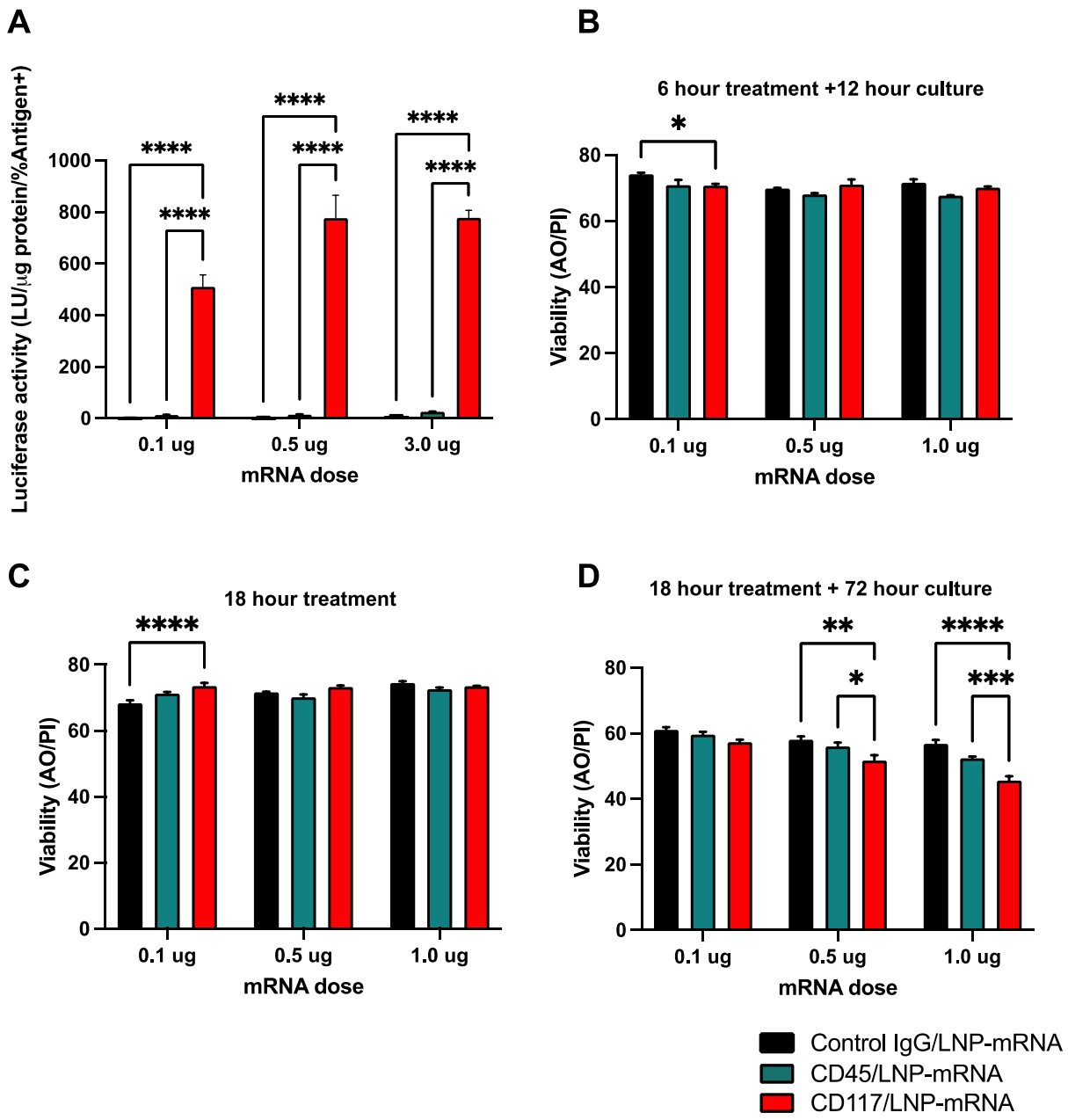

Fig. S1. Delivery to antigen positive cells in whole bone marrow and viability in LNP-Cre treated bone marrow cells.

(**A**) Luciferase activity normalized to protein in cell lysate and to the frequency of antigen positive cells in whole bone marrow (WBM) cells (anti-CD45 [85%], antiCD117 [2.8%], or unnormalized for control IgG/LNP (N=3 replicates). Mean +/- SEM shown. (**B**) Viability of *Ai6* WBM cells after 6, (**C**) 18 and (**D**) 18 hour + 72 hours culture *in vitro* exposure to increasing doses of LNP (up to 1 μg) and assessed by AO/PI staining. Mean +/- SEM are shown for B-D (N=3 independent replicates). P-value calculated with Dunnett's multiple comparison test after 2way ANOVA.* $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$.

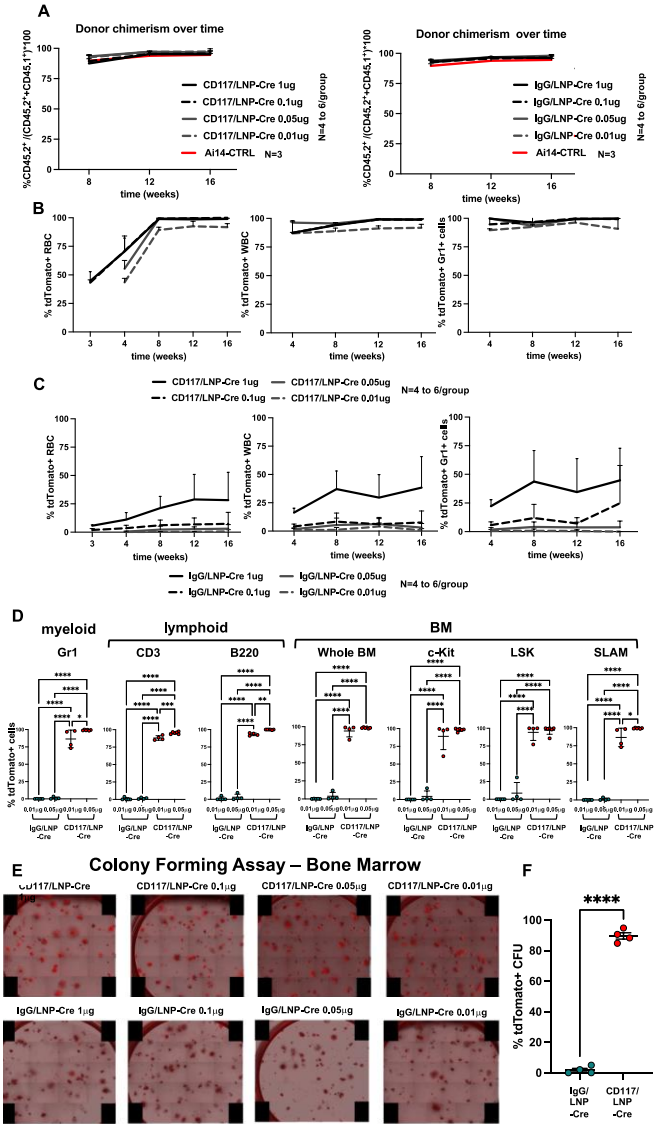

Fig. S2. Ex vivo CD117-LNP/Cre treated HSC retain multi-lineage engraftment

(**A**) Peripheral blood donor chimerism in lethally irradiated congenic (CD45.1) recipients of e*x vivo* treated of bone marrow cells from Ai14 donor mice (CD45.2) with CD117/LNP-Cre (left) or Control IgG/LNP-Cre (right) across a 100-fold range in dose. (**B**) tdTomato+ cell frequency in red blood cells (RBC), white blood cells (CD45.2+), and granulocytes (Gr1+) cells from 8-16 weeks post-transplant after ex vivo treatment of Ai14 bone marrow cells with (**B**) CD117/LNP-Cre or (**C**) control IgG/LNP-Cre. (**D**) tdTomato^{+} cell frequency in peripheral blood myeloid (Gr1+) and lymphoid cells (CD3+ [T-cells], B220+ [B-cells]) and in bone marrow (BM) subsets (c-Kit, Lin^{-}c-Kit^{+} subset, LSK, Lin^{-}c-Kit^{+}Sca1^{+}, SLAM, LSK CD150^{+} CD48^{-}) at 4 months after 0.01 or 0.05 mg of CD117/LNP-Cre or control IgG/LNP-Cre. For the groups in A-D, N=4 to 6, except untreated Ai14 (N=3). Mean +/- SEM are shown in A-D. P-values are Tukey's multiple comparison test after one-way ANOVA *p<0.05, **p<0.01 *** p<0.001, **** p<0.0001 **(E)** Colony forming unit assay from bone marrow at 4 months after transplantation with ex vivo treated Ai14 BM at the doses shown. (**F**) Percentage of total CFU that are tdTomato+ 16 weeks post-transplant with ex vivo treated BM. Quantification of images in E. N=4 independent samples for each group. Shown are the mean +/- SEM. P-value by paired t-test **** p<.0001

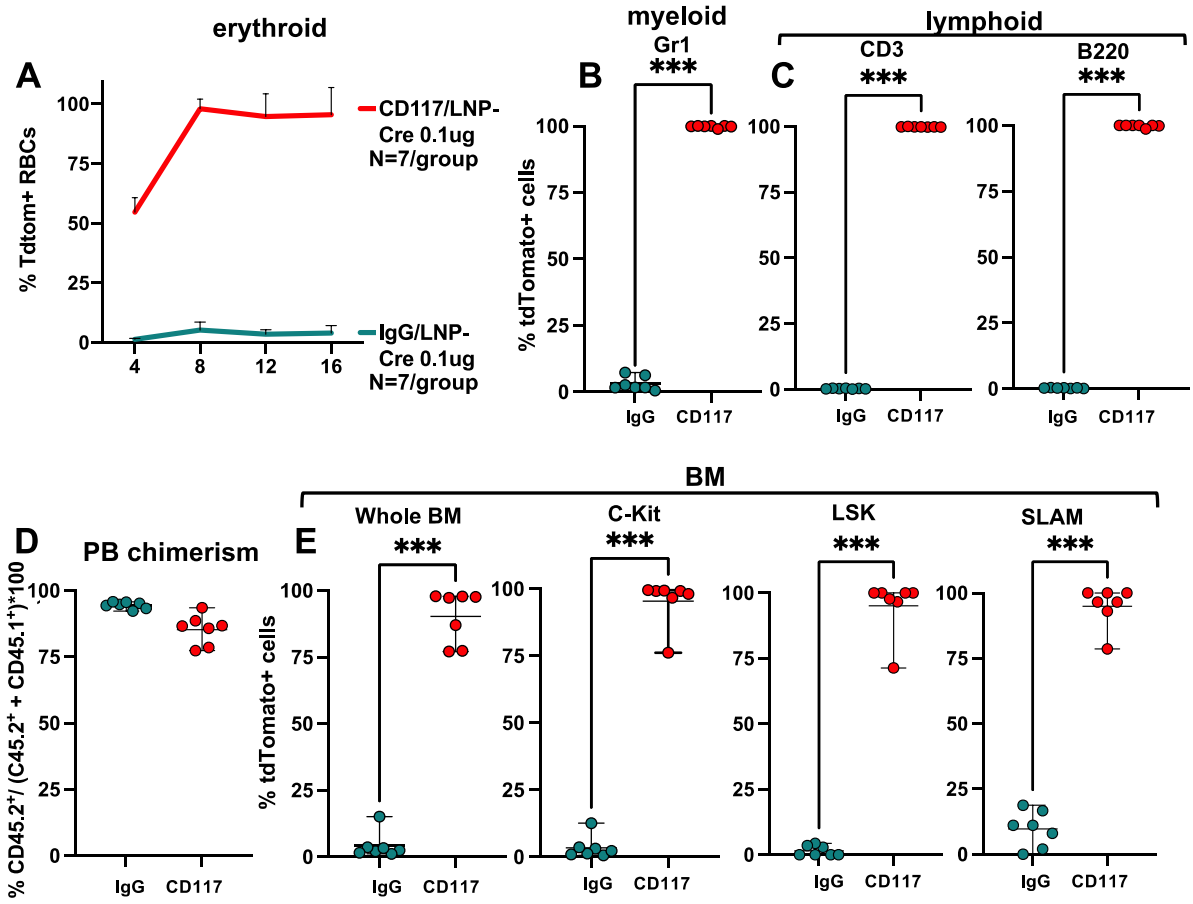

Fig. S3. Ex vivo CD117/LNP-Cre edited HSC persist upon secondary transplantation
tdTomato^{+} cell frequency in peripheral blood (**A**) red blood cells, (**B**) myeloid cells (Gr1+), and (**C**) lymphoid cells (CD3+ [T-cells], B220+ [B-cells]) 4 months after transplantation with bone marrow from primary chimeras of ex vivo CD117 or control IgG/LNP-Cre (0.01 μg mRNA) treated bone marrow (1 mouse from each in vivo treated cohort was used to engraft 7 secondary recipient mice). (**D**) Donor chimerism in peripheral blood at 4 months in secondary transplants. (**E**) Frequency of gene edited cells in whole bone marrow (BM) and bone marrow subsets (c-Kit, Lin^{-}c-Kit^{+} subset, LSK, Lin^{-}c-Kit^{+}Sca1^{+}, SLAM, LSK CD150^{+} CD48^{-}) in secondary chimeras at 16 weeks posttransplant. N=7 for each group in all panels. Mean +/- range are shown. P-value by Mann-Whitney test. ***p<0.001.

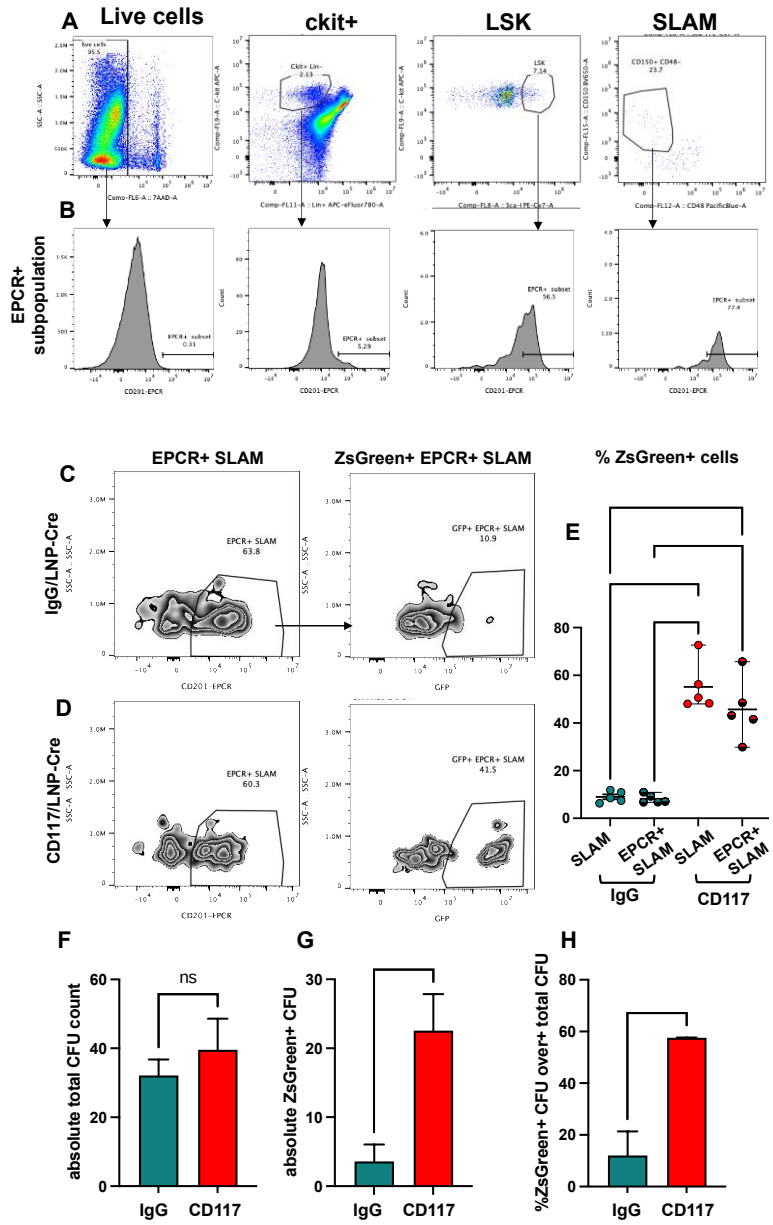

Fig. S4 CD117/LNP-Cre mediated in vivo editing of the dormant EPCR+ HSC population and colony forming cells

(**A**) Gating scheme used to quantitate live whole bone marrow, cKit+ Lin-, LSK, and SLAM cells and (**B**) relative frequency of EPCR+ cells within each subpopulation. (**C**) Representative contour plots of the EPCR+ subpopulation within the SLAM population (left) and relative ZsGreen expression in the EPCR+ population (right) one month after *in vivo* treatment with control IgG or CD117/LNP-Cre (**D**). (**E**) Percentages of *in vivo* edited LT-HSC SLAM and LT-HSC EPCR+ SLAM in Control IgG or CD117/LNP-Cre in Ai6 mice. N=5 experimental mice in each group. Mean +/- range shown. P-values are Tukey's multiple comparison test after one-way ANOVA **** p<0.0001. **(F)** Absolute number of CFU and (**G**) ZsGreen+ CFU of BM from primary chimeras (N=3 per group) generated from *in vivo* treated *Ai6* donors. Results are the average of three technical replicates for each animal (nine total CFU per group). (**H**) Percentage of ZsGreen+ CFU in each cohort. p-value by t-test. * p-value <0.05, ** p-value <0.01.

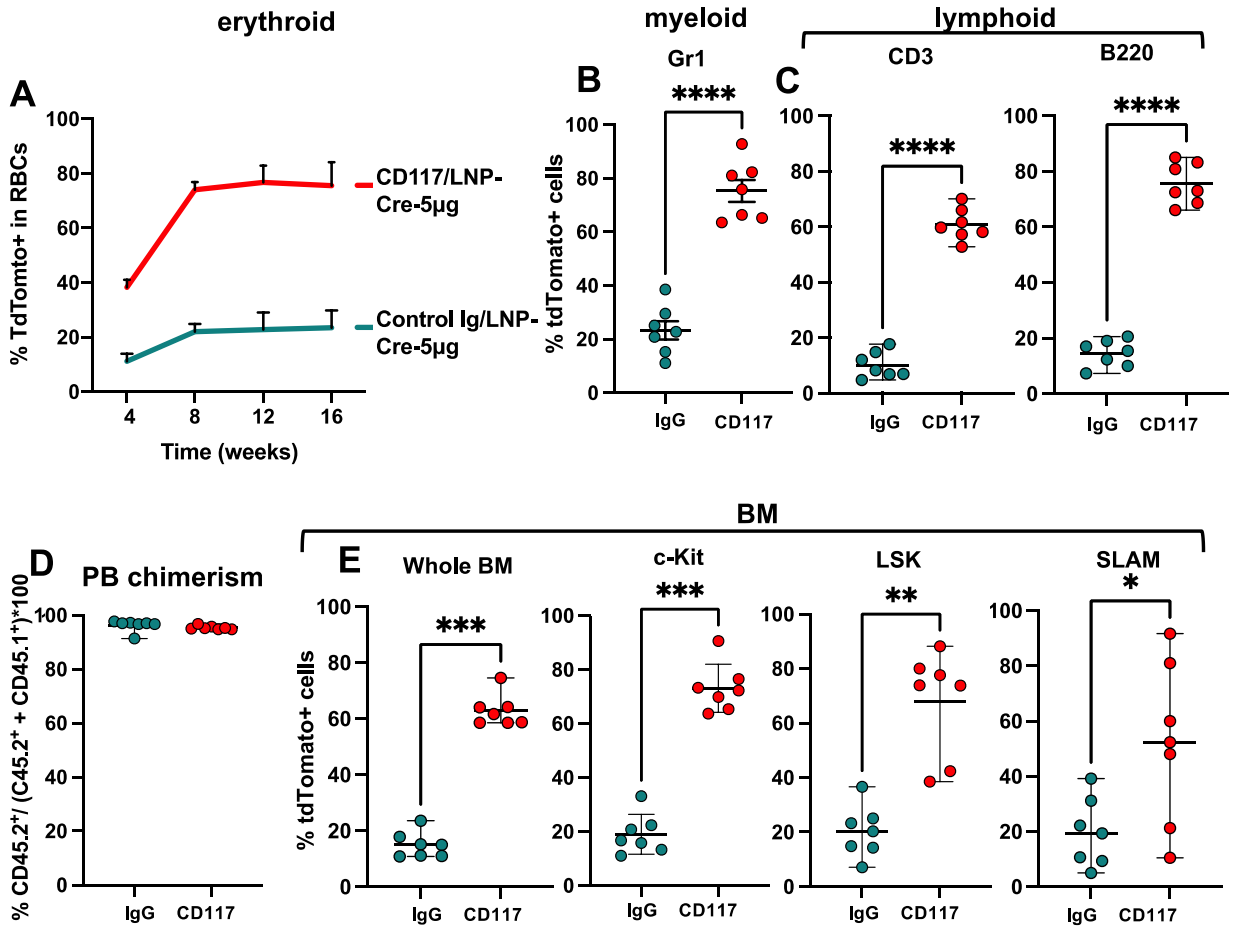

Fig. S5. CD117/LNP-Cre edited HSC persist upon primary transplantation of BM from in vivo treated donors

tdTomato^{+} cell frequency in peripheral blood (**A**) red blood cells, (**B**) myeloid cells (Gr1+), and (**C**) lymphoid cells (CD3+ [T-cells], B220+ [B-cells]) 4 months after transplantation with bone marrow from Ai9 mice injected *in vivo* with control IgG/LNP-Cre or CD117/LNPCre formulations (1 mouse from each in vivo treated cohort was used to engraft 7 primary recipient mice). (**D**) Donor chimerism in the primary chimeras at 16 weeks posttransplant. (**E**) Frequency of gene edited cells in whole bone marrow (BM) and bone marrow subsets (c-Kit, Lin^{-}c-Kit^{+} subset, LSK, Lin^{-}c-Kit^{+}Sca1^{+}, SLAM, LSK CD150^{+} CD48^{-}) in primary chimeras at 16 weeks posttransplant. N=7 experimental mice per group for A-E. Shown is mean +/- SEM. p-value calculated t-test. *p<0.05, ** p<0.01 *** p<0.001.

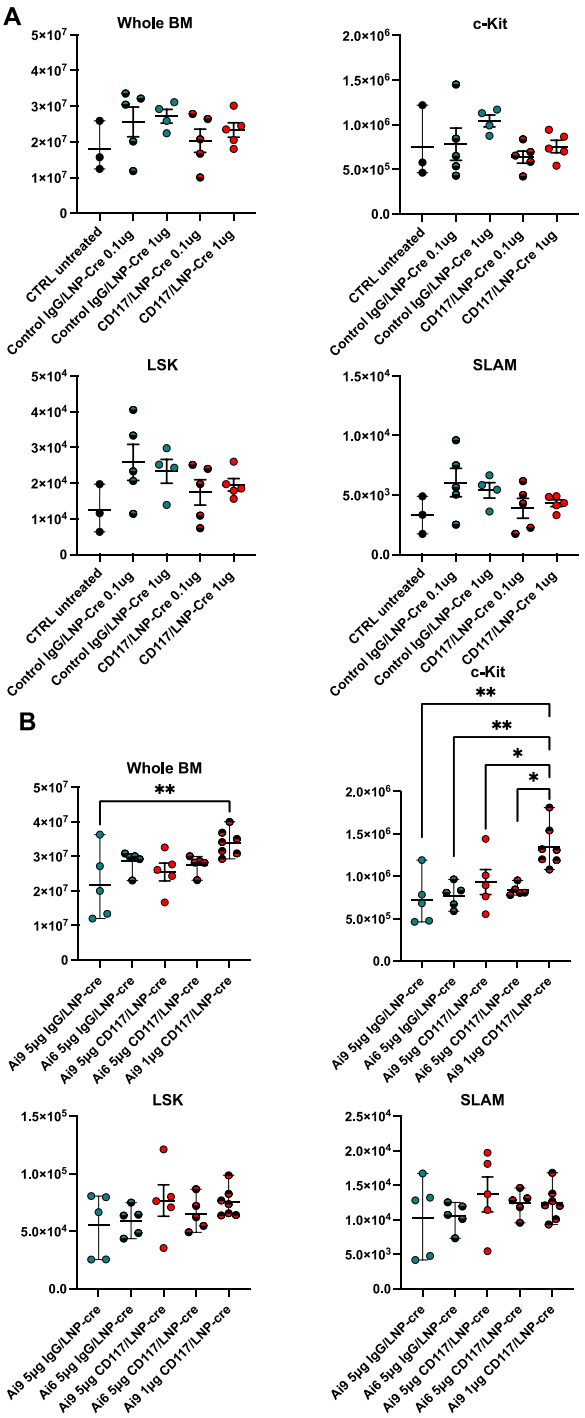

Fig. S6 Absolute cell counts of BM subpopulation in *ex vivo* and *in vivo* treated animals are consistent among cohorts

(**A**) Absolute cell number in BM after red blood cell lysis (upper left), cKit+Lin- (upper right), LSK (lower left) and SLAM (lower right) cells quantified by flow cytometry, upon exclusion of dead (7AAD +) cells. Samples obtained upon crushing one femur and leg from each mouse in *ex vivo* and (**B**) *in vivo* treated mice. N=5 to 7 animals per group. Mean +/- standard deviation shown. P-values are Tukey's multiple comparison test after one-way ANOVA *p<0.05 ** p<0.01.

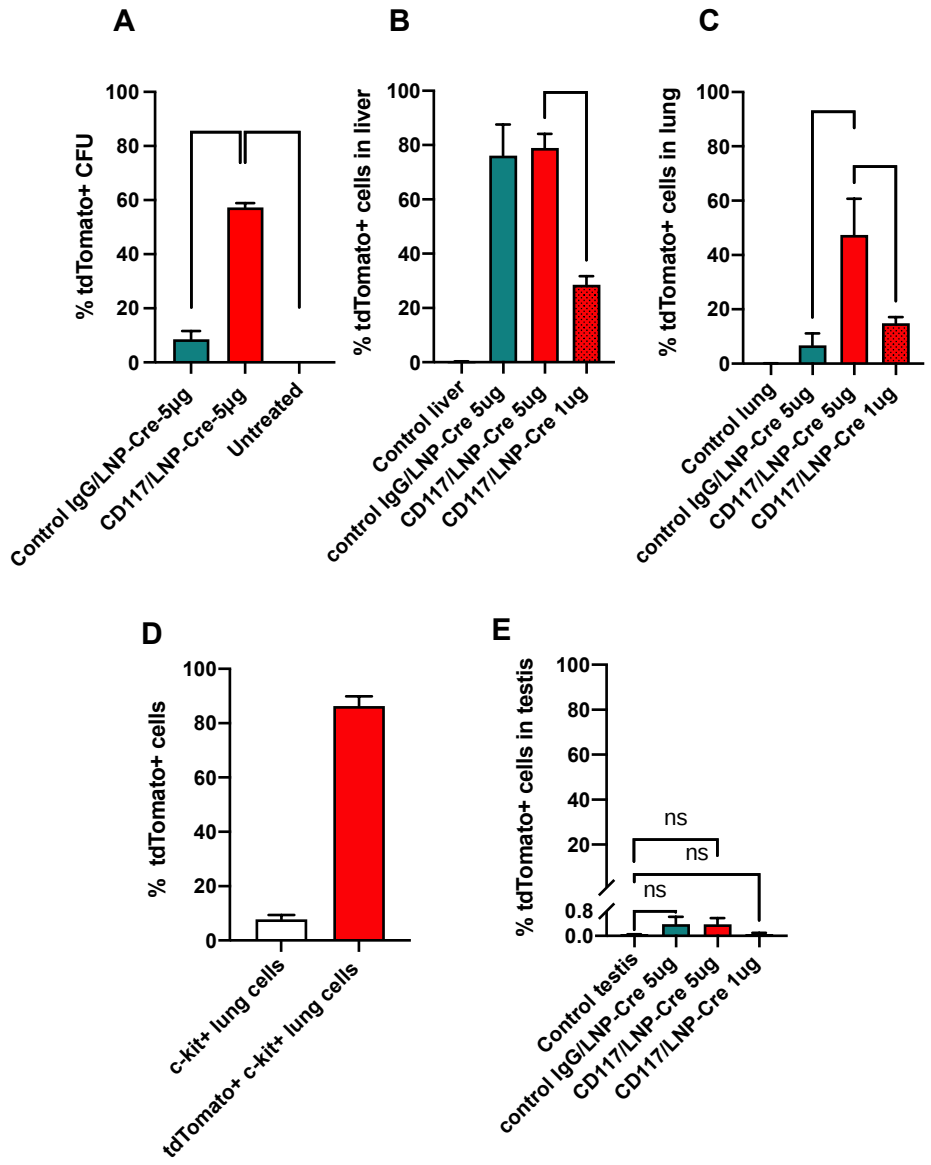

Fig S7. *in vivo* editing after CD117/LNP treatment

(**A**) Percentage of total CFU that are tdTomato+ at 4 months post i.v. treatment. N=2 technical replicates for each group. Mean +/- standard deviation shown. p-value by t-test. *p<0.05. (**B**) Gene editing frequency in non-hematopoietic organs/cells of the liver and (**C**) lung 16 weeks post *in vivo* treatment with CD117/LNP-Cre (1 µg and 5 µg) and control IgG/LNP-Cre (5 µg) assessment by flow cytometry. Shown mean +/- standard deviation (N=5 to 7); p-value calculated using Tukey's multiple comparison test after one-way ANOVA (p<0.05) for (A) and (B). Comparisons to control liver not shown. ****p<0.0001. (**D**) Frequency of cKit$^+$ cells in the lung and frequency of gene editing in those cKit$^+$ lung cells 6d after *in vivo* CD117/LNP-Cre treatment (3.5 µg) N=5. Shown mean +/- SD. (**E**) Gene editing frequency in non-hematopoietic organs/cells of the testis 16 weeks post *in vivo* treatment with CD117/LNP-Cre (5 µg and 1 µg) and control IgG/LNP-Cre (5 µg) assessment by flow cytometry. Shown mean +/- standard deviation (N=5 to 7); p-value calculated using Dunn's multiple comparison test after one-way ANOVA (p<0.05).

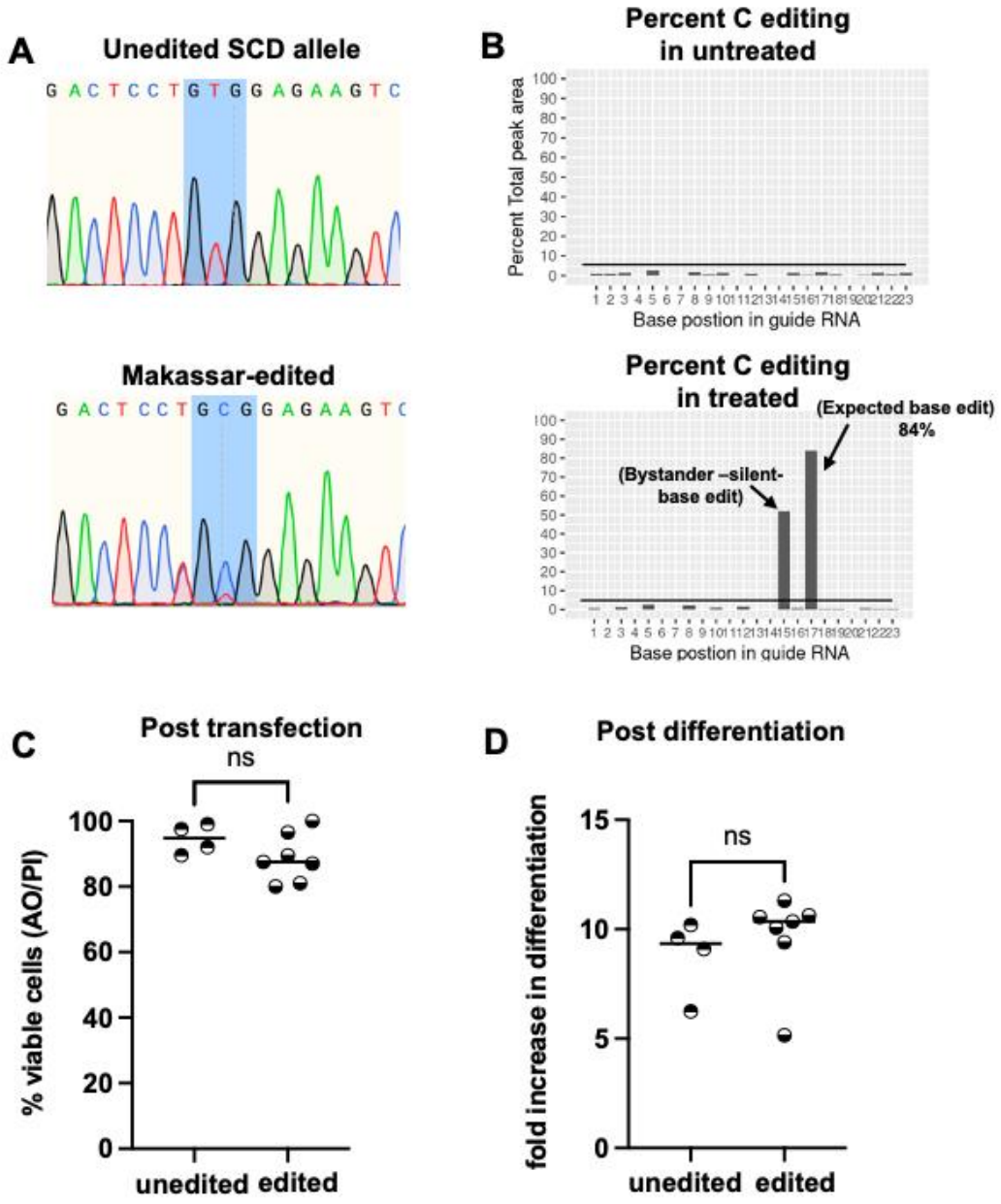

Fig. S8. Analyses of genome editing, cell viability and proliferation in human erythroid cells treated with CD117/LNP-ABE and CD117/LNP-sgRNA.
(**A)** Representative sequences and (**B**) quantification of C editing of control unedited (top) and edited (bottom) genomic DNA extracted from SCD cells after treatment with anti-human CD117/LNP formulations carrying an adenine base editor and a sgRNA aimed at converting the pathogenic codon 6 (highlighted in blue, GAG,) to non-pathogenic variant (->GCG) named $HBB^{G\text{-}Makassar}$. (**C**) Quantification of viability (AO/PI staining) from unedited (n=4) and edited (n=7) early erythroid progenitors cultured independently. (**D**) Proliferation rate of specimens in **C** calculated by measuring the cell count fold increase from D0 to D7/8 in differentiation media. Shown is mean. P-value by Mann-Whitney; alpha=0.05.

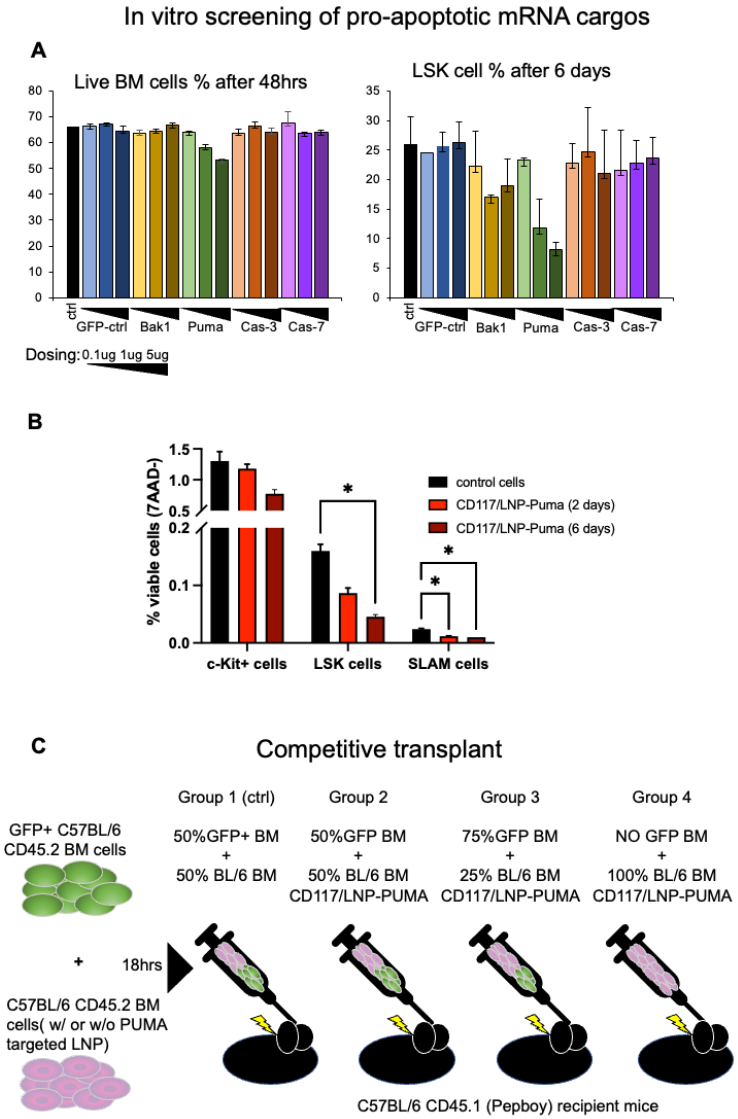

Fig S9. Identification of human PUMA as the most effective pro-apoptotic mRNA in HSC (**A, left**) Viability of mouse bone marrow cells 48 hours after *in vitro* treatment with CD117/LNP carrying pro-apoptotic mRNAs and GFP mRNA at escalating doses. Assessment with flow cytometry and 7-AAD stain. Shown mean +/- SEM (n=3). (**A**, **right**) Frequency of $Lin^{-}Sca1^{+}c\text{-}Kit^{+}$ subset of bone marrow cells six days after *in vitro* treatment with LNP-mRNA. (**B**) Frequency of viable c-Kit^{+} cells, LSK cells, and SLAM ($CD150^{+}CD48^{-}$ subset of LSK) cells two or six days after *in vivo* treatment of C57BL/6 mice with CD117/LNP-PUMA (n=3). Shown are mean +/- SEM. p-value calculated with Bonferroni's multiple comparison test after two-way ANOVA ($p<0.05$). *$p<0.05$. (**C**) Competitive transplantation schema for comparing residual engraftment capacity of CD45.2 bone marrow cells treated *ex vivo* with CD117/LNP-PUMA for 18 hours against varying ratios of untreated GFP^{+}, $CD45.2^{+}$ donor cells. Control groups of 50:50 mixture of untreated cells (GFP^{+} and GFP^{-} cells) (group 1) and 100% CD117/LNP-PUMA treated cells without any untreated GFP^{+} cells (group 4). Recipients are lethally irradiated $CD45.1^{+}$ mice.

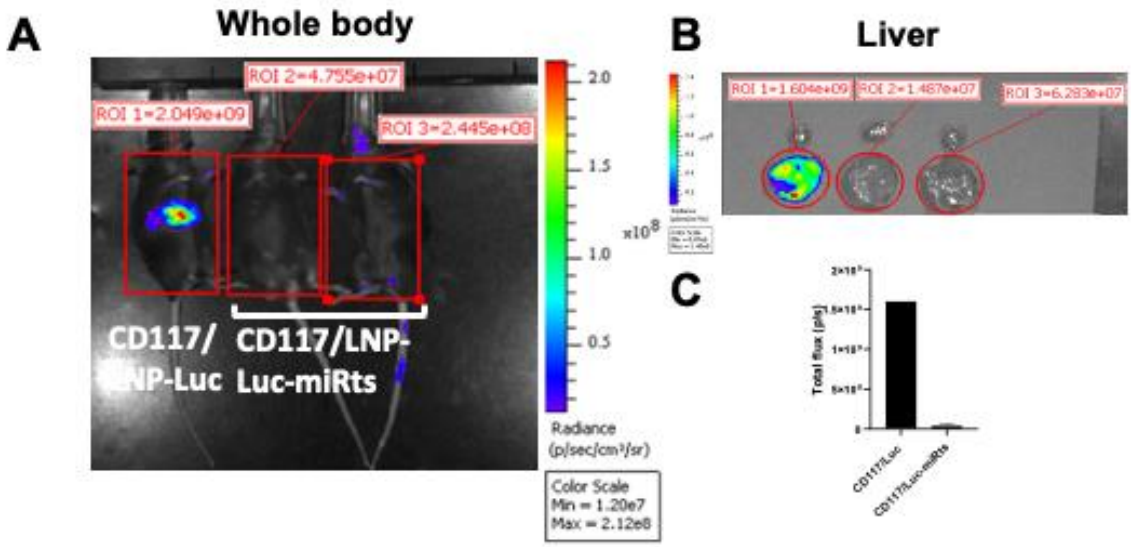

Fig. S10. **Imaging of mice and livers upon i.v. administration of CD117/LNP-Luc versus CD117/LNP-Luc-miRt formulations**

Biodistribution upon i.v. injection of 1 µg of targeted LNP-mRNA expression *in vivo* by luminescence imaging at 24 hours. A representative sample set of mice **(A)** and corresponding livers dissected from these animals **(B)** were analyzed 5 min after the administration of D-luciferin. **(C)** Quantification of liver bioluminescence in B.

Supplement Table 1

eGFP	ATGGTGTCTAAGGGCGAGGAATTGTTTACAGGTGTGGTGCCCATCCT GGTGGAGCTTGATGGCGATGTAAATGGACACAAATTCTCCGTTAGTG GGGAAGGCGAAGGGGATGCCACCTACGGTAAGCTTACGCTGAAATT CATCTGCACCACTGGTAAACTCCCCGTGCCATGGCCAACCCTGGTCA CGACCCTTACTTATGGGGTGCAGTGTTTTTCAAGGTACCCCGACCATA TGAAACAACATGATTTCTTCAAGTCCGCCATGCCGGAGGGGTACGTC CAGGAGAGAACAATCTTTTTCAAAGATGACGGGAACTACAAGACTCG CGCAGAAGTCAAGTTTGAGGGAGACACTCTCGTAAACCGAATTGAAC TGAAAGGAATTGACTTTAAGGAAGACGGTAATATACTGGGCCACAA GCTGGAGTATAATTATAACAGCCATAATGTGTATATCATGGCAGACA AGCAAAAGAACGGCATTAAAGTGAACTTCAAGATCCGTCACAATATC GAGGATGGCAGCGTCCAGCTGGCTGACCACTACCAGCAGAACACAC CTATTGGAGACGGCCCAGTTTTACTACCTGACAACCACTATCTCAGTA CACAGAGCGCCCTCTCTAAGGACCCTAATGAAAAGCGGGATCATATG GTTTTACTGGAGTTTGTCACAGCTGCGGGAATAACCTTGGGCATGGA TGAGTTGTACAAATGA
Luc2	ATGGAAGACGCCAAGAACATTAAGAAGGGCCCTGCACCCTTCTACCC ACTGGAAGACGGTACTGCAGGGGAGCAGCTGCACAAGGCCATGAAG CGGTATGCCCTCGTTCCTGGCACTATCGCCTTCACAGATGCCCACATC GAAGTAGATATCACCTATGCTGAGTACTTTGAGATGAGTGTGAGACT GGCAGAGGCAATGAAACGTTATGGACTGAACACCAACCATAGAATC GTAGTGTGCTCTGAGAACAGCTTGCAGTTCTTCATGCCTGTCTTAGGA GCACTGTTCATAGGCGTCGCCGTGGCACCAGCCAATGACATTTACAA TGAGAGGGAGCTCCTGAATAGTATGGGCATAAGCCAGCCAACAGTG GTTTTCGTTTCCAAGAAAGGGCTTCAAAAAATCCTGAATGTGCAAAA GAAGCTCCCTATCATCCAGAAGATCATTATAATGGACTCAAAGACTG ATTACCAGGGCTTCCAGTCCATGTATACATTTGTCACCAGCCACCTTC CACCAGGCTTCAATGAATATGATTTCGTGCCTGAGTCATTTGACAGG GACAAAACAATTGCACTCATTATGAATTCTTCCGGGTCAACAGGTTT GCCCAAGGGGGTGGCGCTACCACACAGAACGGCGTGTGTGCGCTTTA GCCATGCTCGAGACCCCATCTTCGGGAATCAGATCATTCCCGACACT GCCATCTTGTCTGTCGTCCCTTTTCACCATGGTTTCGGTATGTTCACCA CGTTGGGCTACCTGATCTGTGGTTTCCGGGTAGTACTGATGTACAGGT TTGAAGAAGAGCTCTTCCTGCGGAGCCTACAGGACTACAAGATCCAG AGCGCACTGCTGGTGCCCACCCTTTTTTCGTTCTTTGCCAAATCCACC CTGATTGATAAATATGACCTATCCAACCTTCATGAGATAGCATCTGG AGGTGCTCCTCTGAGTAAAGAAGTCGGAGAAGCTGTAGCCAAGAGG TTCCACCTGCCAGGCATTCGCCAAGGATATGGCCTGACAGAGACTAC AAGTGCCATTTTAATAACTCCAGAGGGAGATGACAAGCCTGGGGCTG TGGGCAAAGTTGTTCCGTTCTTCGAAGCTAAGGTGGTTGACCTGGAC ACAGGAAAAACCCTGGGCGTCAACCAGCGTGGGGAACTCTGCGTCC GAGGGCCCATGATCATGTCTGGCTACGTGAACAACCCCGAGGCCACC AATGCCCTCATTGACAAGGATGGCTGGCTCCATTCAGGAGACATTGC

	CTACTGGGACGAGGATGAACACTTTTTTATTGTGGACAGGCTCAAGT CGCTTATCAAGTACAAAGGCTACCAGGTGGCTCCTGCTGAATTGGAA TCCATCTTACTTCAGCACCCCAACATATTTGATGCGGGTGTGGCCGGT CTACCGGATGATGATGCAGGAGAGCTGCCCGCTGCTGTTGTTGTGCT GGAGCATGGTAAGACCATGACTGAGAAGGAGATTGTGGACTATGTA GCGTCTCAAGTCACGACCGCTAAAAAACTAAGAGGGGGTGTGGTCTT TGTGGATGAGGTCCCAAAAGGATTGACTGGGAAGCTGGATGCTCGCA AAATAAGAGAAATCCTCATCAAAGCAAAGAAGGGAGGGAAAATTGC TGTCTGA
Cre	ATGTCTAATCTCCTCACTGTGCATCAGAATCTTCCAGCTTTACCGGTA GACGCCACGTCTGATGAAGTGCGCAAAAATCTCATGGACATGTTCAG GGACCGGCAAGCCTTCAGTGAGCACACATGGAAGATGTTGTTGTCTG TGTGTCGCTCCTGGGCTGCCTGGTGCAAACTTAACAACAGGAAGTGG TTCCCTGCAGAGCCTGAGGACGTCAGAGACTATCTGCTCTACTTGCA AGCACGAGGACTCGCGGTAAAGACCATCCAGCAGCACCTGGGCCAG CTGAACATGCTGCACAGGAGGTCTGGGCTGCCCCGACCAAGTGACTC AAATGCTGTGTCTCTGGTCATGAGACGCATCCGCAAGGAGAATGTGG ATGCCGGAGAACGAGCCAAGCAGGCTCTGGCTTTTGAACGGACAGA CTTTGATCAGGTGAGATCCCTGATGGAGAACTCAGATAGATGCCAGG ACATCCGGAACCTGGCCTTTCTTGGGATAGCCTACAACACCTTGCTG AGGATAGCAGAAATTGCCAGAATTCGGGTCAAGGACATTAGCAGGA CAGATGGGGGCAGGATGCTCATCCACATTGGCCGGACTAAAACCCTT GTTTCAACTGCAGGCGTGGAAAAAGCCTTGAGCTTAGGTGTCACCAA GCTGGTGGAGAGATGGATCAGCGTCTCCGGAGTTGCAGACGACCCAA ATAATTATCTCTTCTGTCGTGTTCGGAAGAACGGAGTTGCAGCGCCCT CGGCTACCAGCCAACTAAGCACGAGAGCTCTGGAGGGCATTTTTGAG GCCACTCATCGCCTGATCTATGGAGCAAAAGATGACTCCGGGCAGAG ATACCTGGCATGGAGTGGTCATAGTGCTCGTGTCGGTGCTGCAAGAG ATATGGCCCGGGCTGGGGTTTCCATACCTGAAATCATGCAGGCTGGT GGCTGGACAAACGTGAACATTGTGATGAACTACATCAGGAATCTAGA TTCTGAGACAGGAGCCATGGTGCGATTACTGGAAGATGGCGATTGA
mPUMA	ATGGCCAGAGCAAGGCAAGAAGGCAGCAGTCCAGAACCTGTGGAAG GATTGGCCAGGGACTCCCCGAGACCATTTCCTCTCGGCCGGCTCATG CCCAGTGCTGTCAGCTGCTCATTGTGTGAGCCAGGCCTTCCAGCAGC ACCAGCTGCACCCGCCCTGTTGCCAGCAGCTTACCTGTGCGCCCCTAC TGCCCCCCCTGCCGTGACCGCGGCACTTGGTGGACCCCGCTGGCCTG GTGGGCACAGAAGCAGACCGCGAGGCCCCAGACCAGATGGCCCCCA GCCTTCCCTGTCTCCCGCGCAGCAGCACCTGGAGTCCCCTGTACCATC AGCCCCGGAAGCTCTGGCAGGGGGTCCTACACAAGCCGCTCCTGGGG TTCGGGTGGAGGAAGAAGAGTGGGCCCGAGAGATCGGGGCTCAGCT GCGTAGGATGGCTGATGACCTCAATGCTCAGTATGAACGCCGGCGTC AGGAGGAGCAGCATAGGCATCGCCCCTCTCCCTGGAGGGTCATGTAC AACCTATTCATGGGATTACTGCCTCTCCCTCGGGACCCAGGAGCCCC AGAGATGGAGCCCAACTGA
mBAK1	ATGGCCTCCGGCCAGGGCCCCGGCCCCCCCAAGGTGGGCTGCGACGA GTCCCCCTCCCCCTCCGAGCAGCAGGTGGCCCAGGACACCGAGGAGG

	TGTTCCGCTCCTACGTGTTCTACCTGCACCAGCAGGAGCAGGAGACC CAGGGCGCCGCCGCCCCCGCCAACCCCGAGATGGACAACCTGCCCCT GGAGCCCAACTCCATCCTGGGCCAGGTGGGCCGCCAGCTGGCCCTGA TCGGCGACGACATCAACCGCCGCTACGACACCGAGTTCCAGAACCTG CTGGAGCAGCTGCAGCCCACCGCCGGCAACGCCTACGAGCTGTTCAC CAAGATCGCCTCCTCCCTGTTCAAGTCCGGCATCTCCTGGGGCCGCGT GGTGGCCCTGCTGGGCTTCGGCTACCGCCTGGCCCTGTACGTGTACC AGCGCGGCCTGACCGGCTTCCTGGGCCAGGTGACCTGCTTCCTGGCC GACATCATCCTGCACCACTACATCGCCCGCTGGATCGCCCAGCGCGG CGGCTGGGTGGCCGCCCTGAACTTCCGCCGCGACCCCATCCTGACCG TGATGGTGATCTTCGGCGTGGTGCTGCTGGGCCAGTTCGTGGTGCAC CGCTTCTTCCGCTCCTAG
mCASP3	ATGGAGAACAACAAGACCTCCGTGGACTCCAAGTCCATCAACAACTT CGAGGTGAAGACCATCCACGGCTCCAAGTCCGTGGACTCCGGCATCT ACCTGGACTCCTCCTACAAGATGGACTACCCCGAGATGGGCATCTGC ATCATCATCAACAACAAGAACTTCCACAAGTCCACCGGCATGTCCTC CCGCTCCGGCACCGACGTGGACGCCGCCAACCTGCGCGAGACCTTCA TGGGCCTGAAGTACCAGGTGCGCAACAAGAACGACCTGACCCGCGA GGACATCCTGGAGCTGATGGACTCCGTGTCCAAGGAGGACCACTCCA AGCGGTCCTCCTTCGTGTGCGTGATCCTGTCCCACGGCGACGAGGGC GTGATCTACGGCACCAACGGCCCCGTGGAGCTGAAGAAGCTGACCTC CTTCTTCCGCGGCGACTACTGCCGCTCCCTGACCGGCAAGCCCAAGC TGTTCATCATCCAGGCCTGCCGCGGCACCGAGCTGGACTGCGGCATC GAGACCGACTCCGGCACCGACGAGGAGATGGCCTGCCAGAAGATCC CCGTGGAGGCCGACTTCCTGTACGCCTACTCCACCGCCCCCGGCTACT ACTCCTGGCGCAACTCCAAGGACGGCTCCTGGTTCATCCAGTCCCTGT GCTCCATGCTGAAGCTGTACGCCCACAAGCTGGAGTTCATGCACATC CTGACCCGCGTGAACCGCAAGGTGGCCACCGAGTTCGAGTCCTTCTC CCTGGACTCCACCTTCCACGCCAAGAAGCAGATCCCCTGCATCGTGT CCATGCTGACCAAGGAGCTGTACTTCTACCACTAG
mCASP7	ATGACCGACGACCAGGACTGCGCCGCCGAGCTGGAGAAGGTGGACT CCTCCTCCGAGGACGGCGTGGACGCCAAGCCCGACCGCTCCTCCATC ATCTCCTCCATCCTGCTGAAGAAGAAGCGCAACGCCTCCGCCGGCCC CGTGCGCACCGGCCGCGACCGCGTGCCCACCTACCTGTACCGCATGG ACTTCCAGAAGATGGGCAAGTGCATCATCATCAACAACAAGAACTTC GACAAGGCCACCGGCATGGACGTGCGCAACGGCACCGACAAGGACG CCGGCGCCCTGTTCAAGTGCTTCCAGAACCTGGGCTTCGAGGTGACC GTGCACAACGACTGCTCCTGCGCCAAGATGCAGGACCTGCTGCGCAA GGCCTCCGAGGAGGACCACTCCAACTCCGCCTGCTTCGCCTGCGTGC TGCTGTCCCACGGCGAGGAGGACCTGATCTACGGCAAGGACGGCGTG ACCCCCATCAAGGACCTGACCGCCCACTTCCGCGGCGACCGCTGCAA GACCCTGCTGGAGAAGCCCAAGCTGTTCTTCATCCAGGCCTGCCGCG GCACCGAGCTGGACGACGGCATCCAGGCCGACTCCGGCCCCATCAAC GACATCGACGCCAACCCCCGCAACAAGATCCCCGTGGAGGCCGACTT CCTGTTCGCCTACTCCACCGTGCCCGGCTACTACTCCTGGCGCAACCC CGGCAAGGGCTCCTGGTTCGTGCAGGCCCTGTGCTCCATCCTGAACG

	AGCACGGCAAGGACCTGGAGATCATGCAGATCCTGACCCGCGTGAA CGACCGCGTGGCCCGCCACTTCGAGTCCCAGTCCGACGACCCCCGCT TCAACGAGAAGAAGCAGATCCCCTGCATGGTGTCCATGCTGACCAAG GAGCTGTACTTCTCCCGCTAG
miRts	TGATAATAGCAAACACCATTGTCACACTCCAGCTGGAGCCTCGGTGG CCATGCTTCTTGCCCCTTGGGCCCAAACACCATTGTCACACTCCATCC CCCCAGCCCCTCCTCCCCTTCCTGCACCCGTACCCCCCAAACACCATT GTCACACTCCAGTGGTCTTTGAATAAAGTCTGAGTGGGCGGC
ABE8e-NRCH	ATGAAACGGACAGCCGACGGAAGCGAGTTCGAGTCACCAAAGAAGA AGCGGAAAGTCTCTGAGGTGGAGTTTTCCCACGAGTACTGGATGAGA CATGCCCTGACCCTGGCCAAGAGGGCACGGGATGAGAGGGAGGTGC CTGTGGGAGCCGTGCTGGTGCTGAACAATAGAGTGATCGGCGAGGGC TGGAACAGAGCCATCGGCCTGCACGACCCAACAGCCCATGCCGAAAT TATGGCCCTGAGACAGGGCGGCCTGGTCATGCAGAACTACAGACTGA TTGACGCCACCCTGTACGTGACATTCGAGCCTTGCGTGATGTGCGCC GGCGCCATGATCCACTCTAGGATCGGCCGCGTGGTGTTTGGCGTGAG GAACTCAAAAAGAGGCGCCGCAGGCTCCCTGATGAACGTGCTGAACT ACCCCGGCATGAATCACCGCGTCGAAATTACCGAGGGAATCCTGGCA GATGAATGTGCCGCCCTGCTGTGCGATTTCTATCGGATGCCTAGACA GGTGTTCAATGCTCAGAAGAAGGCCCAGAGCTCCATCAACTCCGGAG GATCTAGCGGAGGCTCCTCTGGCTCTGAGACACCTGGCACAAGCGAG AGCGCAACACCTGAAAGCAGCGGGGGCAGCAGCGGGGGGTCAGACA AGAAGTACAGCATCGGCCTGACCATCGGCACCAACTCTGTGGGCTGG GCCGTGATCACCGACGAGTACAAGGTGCCCAGCAAGAAATTCAAGG TGCTGGGCAACACCGACCGGCACAGCATCAAGAAGAACCTGATCGG AGCCCTGCTGTTCGACAGCGGCGAAACAGCCGAGGCCACCCGGCTGA AGAGAACCGCCAGAAGAAGATACACCAGACGGAAGAACCGGATCTG CTATCTGCAAGAGATCTTCAGCAACGAGATGGCCAAGGTGGACGACA GCTTCTTCCACAGACTGGAAGAGTCCTTCCTGGTGGAAGAGGATAAG AAGCACGAGCGGCACCCCATCTTCGGCAACATCGTGGACGAGGTGGC CTACCACGAGAAGTACCCCACCATCTACCACCTGAGAAAGAAACTGG TGGACAGCACCGACAAGGCCGACCTGCGGCTGATCTATCTGGCCCTG GCCCACATGATCAAGTTCCGGGGCCACTTCCTGATCGAGGGCGACCT GAACCCCGACAACAGCGACGTGGACAAGCTGTTCATCCAGCTGGTGC AGACCTACAACCAGCTGTTCGAGGAAAACCCCATCAACGCCAGCGGC GTGGACGCCAAGGCCATCCTGTCTGCCAGACTGAGCAAGAGCAGAC GGCTGGAAAATCTGATCGCCCAGCTGCCCGGCGAGAAGAAGAATGG CCTGTTCGGAAACCTGATTGCCCTGAGCCTGGGCCTGACCCCCAACTT CAAGAGCAACTTCGACCTGGCCGAGGATGCCAAACTGCAGCTGAGC AAGGACACCTACGACGACGACCTGGACAACCTGCTGGCCCAGATCG GCGACCAGTACGCCGACCTGTTTCTGGCCGCCAAGAACCTGTCCGAC GCCATCCTGCTGAGCGACATCCTGAGAGTGAACACCGAGATCACCAA GGCCCCCCTGAGCGCCTCTATGGTGAAGAGATACGACGAGCACCACC AGGACCTGACCCTGCTGAAAGCTCTCGTGCGGCAGCAGCTGCCTGAG AAGTACAAAGAGATTTTCTTCGACCAGAGCAAGAACGGCTACGCCGG CTACATTGACGGCGGAGCCAGCCAGGAAGAGTTCTACAAGTTCATCA

	AGCCCATCCTGGAAAAGATGGACGGCACCGAGGAACTGCTCGTGAA GCTGAACAGAGAGGACCTGCTGCGGAAGCAGCGGACCTTCGACAAC GGCATTATCCCCCACCAGATCCACCTGGGAGAGCTGCACGCCATTCT GCGGCGGCAGGGCGATTTTTACCCATTCCTGAAGGACAACCGGGAAA AGATCGAGAAGATCCTGACCTTCCGCATCCCCTACTACGTGGGCCCT CTGGCCAGGGGAAACAGCAGATTCGCCTGGATGACCAGAAAGAGCG AGGAAACCATCACCCCCTGGAACTTCGAGGAAGTGGTGGACAAGGG CGCTTCCGCCCAGAGCTTCATCGAGCGGATGACCAACTTCGATAAGA ACCTGCCCAACGAGAAGGTGCTGCCCAAGCACAGCCTGCTGTACGAG TACTTCACCGTGTATAACGAGCTGACCAAAGTGAAATACGTGACCGA GGGAATGAGAAAGCCCGCCTTCCTGAGCGGCGAGCAGAAAAAGGCC ATCGTGGACCTGCTGTTCAAGACCAACCGGAAAGTGACCGTGAAGCA GCTGAAAGAGGACTACTTCAAGAAAATCGAGTGCTTCGACTCCGTGG AAATCTCCGGCGTGGAAGATCGGTTCAACGCCTCCCTGGGCACATAC CACGATCTGCTGAAAATTATCAAGGACAAGGACTTCCTGGACAATGA GGAAAACGAGGACATTCTGGAAGATATCGTGCTGACCCTGACACTGT TTGAGGACAGAGAGATGATCGAGGAACGGCTGAAAACCTATGCCCA CCTGTTCGACGACAAAGTGATGAAGCAGCTGAAGCGGCTGAGATAC ACCGGCTGGGGCAGGCTGAGCCGGAAGCTGATCAACGGCATCCGGG ACAAGCAGTCCGGCAAGACAATCCTGGATTTCCTGAAGTCCGACGGC TTCGCCAACAGAAACTTCATGCAGCTGATCCACGACGACAGCCTGAC CTTTAAAGAGGACATCCAGAAAGCCCAGGTGTCCGGCCAGGGCGAT AGCCTGCACGAGCACATTGCCAATCTGGCCGGCAGCCCCGCCATTAA GAAGGGCATCCTGCAGACAGTGAAGGTGGTGGACGAGCTCGTGAAA GTGATGGGCGGCCACAAGCCCGAGAACATCGTGATCGAAATGGCCA GAGAGAACCAGACCACCCAGAAGGGACAGAAGAACAGCCGCGAGA GAATGAAGCGGATCGAAGAGGGCATCAAAGAGCTGGGCAGCCAGAT CCTGAAAGAACACCCCGTGGAAAACACCCAGCTGCAGAACGAGAAG CTGTACCTGTACTACCTGCAGAATGGGCGGGATATGTACGTGGACCA GGAACTGGACATCAACCGGCTGTCCGACTACGATGTGGACCATATCG TGCCTCAGAGCTTTCTGAAGGACGACTCCATCGACAACAAGGTGCTG ACCAGAAGCGACAAGAACCGGGGCAAGAGCGACAACGTGCCCTCCG AAGAGGTCGTGAAGAAGATGAAGAACTACTGGCGGCAGCTGCTGAA CGCCAAGCTGATTACCCAGAGAAAGTTCGACAATCTGACCAAGGCCG AGAGAGGCGGCCTGAGCGAACTGGATAAGGCCGGCTTCATCAAGAG ACAGCTGGTGGAAACCCGGCAGATCACAAAGCACGTGGCACAGATC CTGGACTCCCGGATGAACACTAAGTACGACGAGAATGACAAGCTGAT CCGGGAAGTGAAAGTGATCACCCTGAAGTCCAAGCTGGTGTCCGATT TCCGGAAGGATTTCCAGTTTTACAAAGTGCGCGAGATCAACAACTAC CACCACGCCCACGACGCCTACCTGAACGCCGTCGTGGGAACCGCCCT GATCAAAAAGTACCCTAAGCTGGAAAGCGAGTTCGTGTACGGCGACT ACAAGGTGTACGACGTGCGGAAGATGATCGCCAAGAGCGAGCAGGA AATCGGCAAGGCTACCGCCAAGTACTTCTTCTACAGCAACATCATGA ACTTTTTCAAGACCGAGATTACCCTGGCCAACGGCGAGATCCGGAAG CGGCCTCTGATCGAGACAAACGGCGAAACCGGGGAGATCGTGTGGG ATAAGGGCCGGGATTTTGCCACCGTGCGGAAAGTGCTGAGCATGCCC

	CAAGTGAATATCGTGAAAAAGACCGAGGTGCAGACAGGCGGCTTCA GCAAAGAGTCTATCCTGCCCAAGGGCAACAGCGATAAGCTGATCGCC AGAAAGAAGGACTGGGACCCTAAGAAGTACGGCGGCTTCAACAGCC CCACCGTGGCCTATTCTGTGCTGGTGGTGGCCAAAGTGGAAAAGGGC AAGTCCAAGAAACTGAAGAGTGTGAAAGAGCTGCTGGGGATCACCA TCATGGAAAGAAGCAGCTTCGAGAAGAATCCCATCGACTTTCTGGAA GCCAAGGGCTACAAAGAAGTGAAAAAGGACCTGATCATCAAGCTGC CTAAGTACTCCCTGTTCGAGCTGGAAAACGGCCGGAAGAGAATGCTG GCCTCTGCCGGCGTGCTGCAGAAGGGAAACGAACTGGCCCTGCCCTC CAAATATGTGAACTTCCTGTACCTGGCCAGCCACTATGAGAAGCTGA AGGGCTCCCCCGAGGATAATGAGCAGAAACAGCTGTTTGTGGAACA GCACAAGCACTACCTGGACGAGATCATCGAGCAGATCAGCGAGTTCT CCAAGAGAGTGATCCTGGCCGACGCTAATCTGGACAAAGTGCTGTCC GCCTACAACAAGCACCGGGATAAGCCCATCAGAGAGCAGGCCGAGA ATATCATCCACCTGTTTACCCTGACCAATCTGGGAGCCCCTGCCGCCT TCAAGTACTTTGACACCACCATCAACCGGAAGCAATACAACACGACC AAAGAGGTGCTGGACGCCACCCTGATCCGTCAGAGCATCACCGGCCT GTACGAGACACGGATCGACCTGTCTCAGCTGGGAGGTGACTCTGGCG GCTCAAAAAGAACCGCCGACGGCAGCGAATTCGAGCCCAAGAAGAA GAGGAAAGTCTAA
HBB$^{G\text{-}}$ Makassar sgRNA	5’-UUCUCCACAGGAGUCAGGUG-3’

Supplemental Table 2

Used for in vivo-primary animals			
IgG_CD117/LNP-cre preps	number of animal treated with IgG/LNP-cre	number of animal treated with CD117/LNP-cre	BM strain
3/26/21	3	2	Ai9
5/21/21	2	3	Ai9
8/26/21		7	Ai9
1/19/2023*	5	5	Ai6
Used for ex vivo-primary animals			
IgG_CD117/LNP-cre preps	number of animal treated with IgG/LNP-cre	number of animal treated with CD117/LNP-cre	
3/23/21	5	6	Ai14
5/20/21	13	15	Ai14

All formulations were freshly conjugated 1 to 2 days prior to the indicated dates.
*Indicates different batch of encapsulated LNP.

Supplemental Table 3

Pups derived from IgG/LNP-cre in vivo treated Ai9 mice				
Cage Number	**Birth Date**	**Test Number**	**Sex**	**ID**
561279	6/24/21	42	F	N
		43	F	R
		44	F	L
561282	6/22/21	14	M	N
		15	M	R
		16	M	L
		17	M	RL
		18	M	RR
561149	6/24/21	11	M	N
		12	M	R
		13	M	L
561148	6/24/21	45	F	N
		46	F	R
		47	F	L
561281	6/22/21	32	F	N
		33	F	R
		34	F	L
568834	7/22/21	53	M	N
		54	M	R
		55	M	L
568835	7/22/21	56	F	N
		57	F	R
		58	F	L
569157	8/13/21	A	F	N
		B	F	R
		C	F	L
		D	F	RL
569153	8/20/21	K	M	N
		L	M	R
		M	M	L
		N	M	RL
569154	8/20/21	R	F	N
		S	F	R
		T	F	L
		U	F	RL
		V	F	RR
568861	8/12/21	O	M	N
		P	M	R

		Q	M	L
Pups derived from CD117/LNP-cre in vivo treated Ai9 mice				
Cage Number	**Birth Date**	**Test Number**	**Sex**	**ID**
561276	6/23/21	21	M	N
		22	M	R
		23	M	L
		24	M	RL
		25	M	RR
561278	6/23/21	40	F	N
561277	6/23/21	35	F	N
		36	F	R
		37	F	L
		38	F	RL
		39	F	RR
561134	6/24/21	48	F	N
		49	F	R
		50	F	L
		51	F	RL
		52	F	RR
561285	6/28/21	41	F	N
561131	5/27/21	27	F	N
		28	F	RRL
		29	F	RL
561129	5/27/21	30	M	L
		31	M	R
561130	5/.27/21	19	M	R
		20	M	L
561275	5/27/21	26	M	N
561135	6/24/21	10	M	N
561101	6/25/21	5	F	N
		6	F	R
		7	F	L
		8	F	RL
		9	F	RR
561107	6/25/21	1	M	R
		2	M	N
		3	M	L
		4	M	RL
569134	7/16/21	59	M	N
		60	M	R
		61	M	L

		62	M	RL
569156	8/20/21	E	M	N
		F	M	R
		G	M	L
		H	M	RL
569155	8/20/21	I	F	N
		J	F	R
568859	8/23/21	W	M	N
		X	M	R
		Y	M	L
		Z	M	RL
		AA	M	RR

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