Generation and Validation of miR-142 Knock Out Mice

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Abstract

miRNA-142 (miR-142) is an important regulator of many biological processes and associated signaling pathways during embryonic development, homeostasis and disease. The miR-142 hairpin gives rise to the "guide strand" miR-142-3p and the sister "passenger" strand miR-142-5p. miR-142-3p has been shown to play critical, non-redundant functions in the development of the hematopoietic lineage. We have recently reported that miR-142-3p is critical for the control of Wnt signaling in the mesenchyme of the developing lung. miR-142-5p has been proposed to control adaptive growth in cardiomyocytes postnatally and its increase is associated with extensive apoptosis and cardiac dysfunction in a murine heart failure model. Using homologous recombination, we now report the generation and validation of miR-142-null mice. miR-142-null mice show a significant decrease in the expression levels of both the 3p and 5p isoforms. The expression of Bzrap1, a gene immediately flanking miR-142 is not altered while the expression of a long non-coding RNA embedded within the miR-142 gene is decreased. miR-142-null newborn pups appear normal and are normally represented indicating absence of embryonic lethality. At embryonic day 18.5, miR-142-null lungs display increased Wnt signaling associated with the up-regulation of Apc and p300, two previously reported targets of miR-142-3p and -5p, respectively. Adult miR-142-null animals display impaired hematopoietic lineage formation identical to previously reported miR-142 gene trap knockdown mice. We report, for the first time, the homologous recombination-based miR-142-null mice that will be useful for the scientific community working on the diverse biological functions of miR-142.
Introduction

MicroRNAs (miRNAs) are 22 to 25 nucleotide-long, single stranded RNAs that are processed from hairpin transcripts. The maturation of the hairpin transcript gives rise to the 3p guide strand and 5p sister passenger strand, both of which allow the silencing of specific sets of genes through base pairing to a minimal recognition sequence [1,2]. We previously described that miR-142-3p is among a few miRs expressed at high level in the lung mesenchyme during early embryonic development [3]. Such restricted expression pattern suggested that miR-142-3p could play critical functions in controlling cell lineage formation in the mesenchyme. Using in-vitro approaches with embryonic lungs cultured in presence of morpholinos for miR-142-3p, we showed that knockdown of miR-142-3p leads to arrested proliferation and premature differentiation of smooth muscle progenitor cells. We established that miR-142-3p positively regulates Ctnnb1 (β-catenin) signaling during lung development by targeting Adenomatous polyposis coli (Apc) mRNA for degradation. Apc negatively regulates Ctnnb1 via direct binding to Ctnnb1 thereby antagonizing the interaction of Ctnnb1 with the transcription factor Tcf. In combination with Axin and Gsk3b, Apc induces ubiquitination and degradation of Ctnnb1 [4]. Using genetic tools, we showed that up-regulation of Ctnnb1 signaling specifically in the mesenchyme via the induced expression of a stable form of Ctnnb1 or the deletion of a copy of Apc is sufficient to rescue miR-142-3p morpholino-mediated loss-of-function and that Apc is a critical target of this miRNA.

Beyond its proposed function in lung development, miR-142-3p is one of the highest expressed miRs in various hematopoietic lineages [5,6]. miR-142-3p controls neutrophil development in zebrafish [7], orchestrates a network of actin cytoskeleton regulator during megakaryopoiesis [8] and regulates the specification of definitive hemangioblasts during organogenesis [9]. Increased levels of miR-142-3p in the serum are associated with recurrence of adenocarcinoma in humans [10]. miR-142-3p is a target of Interleukin 6 (IL6) in glioblastoma and it has been proposed that miR-142-3p blocks the expression of IL6, Hmga2 and Sox2 thereby suppressing the stem like properties of glioblastomas [11]. Supporting our in-vitro results obtained with miR-142-3p during lung development [3], it was reported that miR-142-3p, which is up-regulated in human breast cancer stem cells, activates the canonical WNT signaling pathway in a APC-suppression dependent manner [12], resulting in enhanced tumorigenicity. miR-142-3p regulates IL6 production upon lipopolysaccharide stimulation in dendritic cells [13]. RNA viruses can also bind miR-142-3p to suppress innate immunity promoting neurological disease manifestations [14]. While a significant amount of information is available for miR142-3p, our knowledge of miR-142-5p is still scarce. In the postnatal heart, miR-142-5p targets p300, a gene encoding a positive regulator of Wnt signaling and increased expression of miR-142-5p is associated with extensive apoptosis and cardiac dysfunction in murine heart failure model [15].

In order to unveil the role of miR-142 in organogenesis, homeostasis and disease in-vivo, we generated and validated the miR-142-null mouse. Our validation studies confirm that both miR-142-3p and miR-142-5p are no longer expressed in these mice. We also show that miR-142-null embryonic lungs display increased Wnt signaling confirming the functional role of miR-142 during lung development. miR-142-null adult mice display various hematological abnormalities such as decreased platelet and white blood cell count and increased mean platelet volume indicating that these mice suffer from thrombocytopenia. Similar results were obtained with the miR-142 knockdown mice developed by Chapnik et al., using exogenous gene trap technology [8]. In this paper, we report the generation and validation of a miR-142-null mouse line that will be useful for the scientific community working on miR-142.
Materials and Methods

**miR-142-null mouse line establishment**

The miR-142-null mutant mouse line was established at the MCI/ICS (Institut Clinique de la Souris, iCS, Infrastructure Nationale PHENOMIN, 1 rue Laurent Fries, 67404 Illkirch, France). A MCI proprietary vector containing a floxed neomycin resistance cassette and Protamine-Cre cassette was used (Fig A and B in S1 Fig). The use of Protamine-Cre cassette in the construction vector offers an efficient solution for the auto-excision of the floxed region in the male germ line of mice. In parallel, a 3.3kb fragment (corresponding to the 5’ homology arm) and 2.6kb fragment (corresponding to the 3’ homology arms) were amplified by PCR and sub-cloned into MCI proprietary vector to generate the final targeting construct. The linearized construct was electroporated in C57BL/6N mouse embryonic stem (ES) cells. After selection, targeted clones were identified by PCR using external primers and further confirmed by Southern blotting with 3’ external probe. Two positive recombinant ES clones were injected into BALB/CN blastocysts, and resulting male chimeras were crossed with wild type females. The genotype of mice with germ-line transmission was confirmed using the primers described below. Generated mice were transferred to Mfd Diagnostics (Wendelsheim/Rheinland-Pfalz, Germany), and housed in a SPF environment. Harvesting organs and tissues from wild type and mutant mice following euthanasia using CO2 was approved at Justus Liebig University Giessen by the federal authorities for animal research of the Regierungspräsidium Giessen, Hessen, Germany (Approved Protocol No. 452_M).

**PCR Genotyping**

miR-142 heterozygous and null mutant mice were identified by performing PCR on tail genomic DNA using four primers (P1-4). The P2/P3 primers allow detecting the miR-142 wild type allele (174 bp). The P1/P4 primers allow detecting the presence of the LoxP cassette (105 bp) and the P1/P3 primers allow detecting the deletion of the endogenous miR-142 gene (294 bp).

(P1) forward: GAA GAA CGA GAT CAG CAG CCT CTG TTC C; (P2) forward: ACG CTA GCA CAG TGT GTG CCC A; (P3) reverse: ACC CAT ATG ATA CAC CAG GCA CGT C; (P4) reverse: GAA GTT ATA CTA GAG CGG CCG TTC AC.

The PCR program consists of a denaturation step at 95°C for 4 min, followed by 34 cycles of denaturation (94°C for 30 s), annealing (62°C for 30 s) and extension steps (72°C for 60 s). The program ends with a completion step at 72°C for 420 s. Each PCR tube contains 2.6 U of Taq polymerase in 5 μL of reaction buffer (Qiagen Master Mix), 15 pmol of each primer, 0.5 mM dNTPs and 10 ng of genomic DNA in final volume of 10 μL.

**Murine Peripheral Blood Counts**

In the Mfd diagnostics facility, about 250 μL of whole blood was retro-orbitally drawn from 8 weeks-old sex-matched miR-142-null and wild type littermates into glass capillary tubes containing 5 μL of 0.5 M EDTA, to prevent coagulation. ADVIVA2120 Hematology System (Siemens Healthcare, Germany) was used to perform complete blood count measurements. Blood count measurement was performed at Justus-Liebig Universität Klinik für Kleintiere (Central laboratory for small animals), Giessen Germany.

**miRNA computational analysis**

The target prediction and pathway intersection for miR-142-3p and miR-142-5p were performed using the software mirPath. [16]
Quantitative Real-time PCR and Statistical Analysis

Freshly isolated embryonic lungs were lysed and total RNA was extracted using miRNeasy Mini or Micro kit (Qiagen, Hilden Germany). 1 μg of RNA was used for cDNA synthesis and RT-PCR for mRNA was carried out using Quantitative Reverse Transcription kit (Qiagen). RT-PCR for miRNA was carried out using Taqman MicroRNA Reverse Transcription kit (Applied Biosystem). In both cases, reactions were assembled following the manufacturer’s recommendations. qPCR was performed using the Light Cycler 480 system (Roche Applied Science). The TaqMan microRNA assay (Applied Biosystem) was used for screening the differential expression of miRNAs whereas SYBR Green (Platinum SYBR Green qPCR Super-Mix-UDG Invitrogen) was used for the analysis of mRNA expression. U6 and Hprt (Hypoxanthine phosphoribosyl transferase1) were used as reference genes for normalization of miRNA and mRNA abundance respectively. Results were collected from at least three lung samples and each reaction was run in triplicate. Data were assembled using Graph Pad Prism Software (Graph Pad software, USA) and statistical analyses were performed using Student’s t-test. Data were significant if p<0.05. Primers for miR142-3p, miR142-5p and U6 were obtained from Applied Biosystems. Primers for Bzrap1: Fwd: 5’ AGA GAG CCC TGG GTA CAG C 3’, Rev: 5’ CCC GAA GCC TAT GTT GAA CT 3’. Primers for LncRNA, Fwd: 5’ CCT CCT GCC TGA TAC TG 3’, Rev: 5’ CCC ATA TCC TCA CGG ACG 3’. Primers for Apc, Fwd: 5’ - CAT GGA CCA GGA CAA AAA CC -3’, Rev: 5’ - GAA CAC ACA CAG CAG CAC AGA - 3’. Primers for for p300, Fwd: 5’-ACA TGA TGC TCT GGA TGA TGA CT -3’, Rev: 5’- TAG GGG GCT GTG GCA TAT T -3’. Primers for beta-catenin/Ctnnb1, Fwd: 5’- GCA GCA GCA GGT TGT GTA -3, Rev: 5’- TGT GGA GAG CTC CAG TAC ACC-3.

Immunofluorescence and In-situ hybridization

Tissues were fixed in 4% PFA, gradually dehydrated in ethanol, impregnated with xylene, embedded in paraffin and sectioned into 5 μm slices on poly-L-Lysine-coated slides. Antigen retrieval was performed by treating the sample with Proteinase K for 3 min at 37°C. Slides were blocked twice for 5 min with Dako (DAB Emission +Dual Linksystem HRP, Life Technologies) and then incubated with digoxigenin labeled LNA probes (Exiqon, miRCURY LNA Detection probe, Vedbaek, Denmark) specific for miR142-3p and miR142-5p. For immunofluorescence staining the slides were de-paraffinized, blocked with 3% bovine serum albumin (BSA) and 0.4% Triton X-100 (in Tris-buffered saline (TBS) at room temperature (RT) for 1 hour and then incubated with primary antibodies against Apc (#Ab15270, Abcam; 1:200), p-532β-catenin (#9566, Cell Signaling; 1:200) and p300 (# sc-585, Santa Cruz; 1:200) at 4°C overnight. After incubation with primary antibodies, slides were washed three times in TBST (Tris buffer saline + 0.1% Tween 20) for 5 minutes, incubated with secondary antibodies at RT and washed three times with TBST before being mounted with Prolong Gold Anti-fade Reagent with DAPI (4’,6-diamidino-2-phenylindole;Invitrogen). Photomicrographs of immunofluorescence staining were taken using a Leica DMRA fluorescence microscope with a Leica DFC360 FX camera (Leica, Wetzlar, Germany). Figures were assembled using Adobe Illustrator. The data are representative of at least three lungs from independent experiments.

Results

Generation of miR-142-null mice

In mice, the miR-142 gene (ENSMUSG00000065420) is located in chromosome 11 adjacent to the second exon of a long non-coding RNA (A430104N18Rik ENSMUSG00000084796) (Fig 1). The function is still LncRNA is still unknown. Benzodiazepine receptor (peripheral)
**associated protein 1** (Bzrap1, ENSMUSG00000034156) is also another gene found downstream of miR-142. The miR-142 locus also contains many GC-rich repeat regions, which render PCR amplification and screening difficult. The constitutive and complete deletion of the miR-142 gene was therefore carried out and miR-142 heterozygous mice were generated. Fig 2B shows the genotyping results of E18.5 embryos arising from crossing miR-142 heterozygous mice. Fig 2C shows the validation of our primer sets to amplify either the wild type miR-142 allele (P2/P3), the presence of the LoxP site (P1/P4) or the miR-142-null allele (P1/P3).

**Fig 1. Schematic Representation of the miR-142 locus in the mouse genome.** miR-142 gene is located in the vicinity of an exon belonging to a LncRNA (A430104N18Rik ENSMUSG00000084796) on chromosome 11. Deletion of miR-142 led to the deletion of the LncRNA exon as well as part of the 5' UTR of Bzrap1. LncRNA: Long non-coding RNA. Bzrap1: Benzodiazepine receptor (peripheral) associated protein 1.

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**Fig 2. Genotyping Strategy for miR-142 KO allele.** A) Schematic diagram showing the WT, targeted and KO alleles for the miR-142 locus. P1, P2, P3 and P4 represent the primers used to genotype wild type and KO alleles. B-C) PCR analysis of the genomic DNA obtained from the embryos at E18.5 from miR-142+/− male and female intercrossing. 254 bp band size for KO allele (Primers used: P1 and P3) and 174 bp band size for WT allele (Primers used: P2 and P3) was determined. A 105 bp band was observed using LoxP specific primers (P1 and P4).

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miR-142-null pups are born alive and do not display obvious abnormalities

Litters from miR-142 heterozygous crosses were genotyped at E12.5, E18.5 and postnatally. Although the expected Mendelian ratio for generating knockout (KO) pups from crossing two hemizygous mice is 25%, this ratio appeared higher when embryos were harvested at E12.5 (39%) and E18.5 (40%) (Table 1). The likely explanation for this observation is the low number of harvested embryos (eighteen and fifteen respectively). Another important conclusion from these data is that miR-142-null embryos are not dying in utero. In addition, individual monitoring and genotyping of all the neonates born from different litters up to 4 months of age indicate a normal representation of miR-142-null mice (20% of the overall postnatal mice (n = 79)). In the miR-142-null group, only one 4 month-old mouse died while three animals died in the miR-142 heterozygous group between the ages of 3 and 4 months. None of the mice died in the control wild type group. Overall, our data indicate very low death rate in the miR-142-null group postnatally.

miR-142 deletion abolishes the expression of both miR-142-3p and miR-142-5p

Our experimental approach was designed to carry out the complete deletion of miR-142. In the process of deleting miR-142, one exon for the LncRNA, close to miR-142, was also deleted. In addition, part of the 5’ region of Bzrap1, a gene encoding a regulator of synaptic transmission, was also deleted. To confirm the successful deletion of miR-142, RNA from E18.5 miR142+/+, miR-142+/− and miR-142−/− lungs from littermate embryos (n = 3 for each genotype) were extracted. qPCR analysis showed that the expression of both miRNAs, miR-142-3p and miR-142-5p, was reduced in miR-142 heterozygous lungs compared to wild type lungs. In addition, the expression of both isoforms was completely abolished in miR-142-null lungs (Fig 3A and 3B). The expression levels of Bzrap1, a gene positioned 3.5kb downstream of miR142, were unchanged in miR-142 heterozygous or null lungs compared to wild type littermates (Fig 3C), indicating that the promoter region of Bzrap1 was not impaired for the expression in the lung. Interestingly, we observed a statistically significant decrease in Bzrap1 expression in tissues of interest. As expected, exon 2 of the LncRNA, which is located within the deleted region of miR142, was not detected in both miR-142 heterozygous and null embryos (Fig 3D). It still remains to be demonstrated whether the deletion of this exon leads to the complete loss of function the LncRNA.

Using specific digoxigenin-labeled probes for these two microRNAs, in-situ hybridization showed that miR-142-3p and miR-142-5p are expressed in both the mesenchyme and the epithelium of the E18.5 lung (Fig 4A, 4B, 4E and 4F). The level of expression of both microRNAs was significantly decreased in miR142-null samples compared to WT samples (Fig 4C, 4D, 4G, 4H).
4H vs. 4A, 4B, 4E and 4F). Blinded quantification of the expression of the respective isoforms of miR-142 confirms the very low level of expression of both miR-142 isoforms (Fig 4I and 4J).

Embryonic miR-142-null lungs display increased Wnt signaling associated with up-regulation of Apc and p300

We previously reported that attenuation of miR-142-3p in lung explants grown in vitro leads to impaired Wnt signaling in the mesenchyme via the up-regulation of Apc [3]. We therefore used immunofluorescence to examine the status of Wnt signaling and Apc in miR-142-null versus wild type lungs at E18.5. Surprisingly, our results indicate increased expression of the activated form of Ctnnb1/B-catenin (p-S552) in the nuclei of miR-142-null (Fig 5C and 5D) versus control (Fig 5A and 5B) lungs, suggesting increased Wnt signaling upon loss of miR-142. However, and in agreement with our previous report [3], miR-142-null lungs display increased expression of Apc (Fig 5G and 5H vs. 5E and 5F). Next, we investigated the expression of p300, a previously validated target of miR-142-5p [15]. p300 is a positive regulator of Ctnnb1 that synergistically activates Ctnnb1/TCF transcription [17]. Our results indicate increased expression of p300 in miR-142-null versus wild type lungs (Fig 5K and 5L vs. 5I and 5J). The
respective increase in Apc and p300 in miR-142-null lungs was also validated at the mRNA level (Fig 5N and 5O). Ctnnb1 mRNA expression is not changed between miR-142-null and control lungs (Fig 5M).

miR-142-null mice display a wide range of hematological disorder

miR-142 is highly expressed in hematopoietic cells belonging to both the myeloid and lymphoid lineages. Using an exogenous gene trap technology, Chapnik and colleagues recently reported the phenotypic analysis of miR-142 knockdown mice. Their mutant mice displayed an array of hematological defects [8]. In order to validate our mouse model, we performed total blood count on 8 week-old miR-142+/+ and miR-142−/− mice (n = 3 for each genotype). Our results indicate a significant decrease in the number of white blood cells, lymphocytes, eosinophils, monocytes and platelets in miR-142-null vs. wild type animals (Fig 6B–6E and 6G). A significant increase in mean platelet volume was also observed (Fig 6D and 6F).
Discussion

We are the first group to report the generation and validation of classical miR-142-null mice. We show that both isoforms of miR-142, the 3p and 5p, are no longer expressed in miR-142-null mice. The expression of Bzrap1, a gene immediately flanking miR-142, is not altered while the expression of a LncRNA embedded within the miR-142 gene is abolished. E18.5 miR-142-null lungs display increased Wnt signaling associated with the up-regulation of Apc and p300, two previously reported targets of miR-142-3p and -5p, respectively. miR-142-null pups are born alive and are normally represented indicating absence of embryonic lethality. Adult miR-142-null animals are viable and display impaired hematopoietic lineage formation. This novel, homologous recombination-based, miR-142-null mouse line will be useful for the scientific community.

A miR-142 gene-trap allele was recently created by insertion of an exogenous gene trap sequence 50 bp upstream of the murine pre-miR-142 [8]. In these mice, the expression of both 3p and 5p was reduced to very low levels but not completely abolished. The authors describe that at E14.5, embryos homozygous for the mutant allele display a normal Mendelian distribution. However, one third of the postnatal homozygous mutant mice died within 3 weeks. Our results support these observations with the exception of the lack of perinatal death. Even though this remains to be shown, perinatal death may be associated with heart failure due to the absence of adaptive growth of the cardiomyocytes [15]. Similarly to the homozygous miR-
Fig 6. miR-142 KO mice display hematological abnormalities. (A) Schematic representation of hematopoietic stem cells differentiated into different blood cell types. Peripheral blood cell count in 8 week old miR-142^-/- mice (n = 3) showing a significant decrease in circulating white blood cells (B), lymphocytes (C), platelets (D), eosinophils (E) and monocytes (G) in addition to an increase in mean platelet volume (F).

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gene-trap mice, our miR-142-null mice also display decreased white blood cells, decreased platelets and increased mean platelet volume. However, we did not observe the decrease in red blood cell numbers as well as the increased level of basophils and reticulocytes (data not shown) initially reported in the homozygous miR-142 gene-trap mice. The basis for such differences is not known so far and could be linked to the genetic background.

In order to predict the potential defects observed in miR-142-null animals, the predicted targets for miR-142-3p and 5p were characterized using Diana-MicroT and Targetscan (Fig 7A). Among the processes and pathways potentially affected, we identified endocytosis, regulation of actin cytoskeleton, pathways in cancer, TGFβ signaling and Wnt signaling.

As the miR-142 conventional KO leads to the deletion of both miR-142-3p and miR-142-5p, it might be challenging to tease out the activity of each isoform. Others and we have previously reported that Apc is a direct target of miR-142-3p [3,12]. Fig 7B describes the common targets (in orange) for miR-142-3p and 5p as well as unique targets (yellow) for each isoform in regard to the Wnt pathway. Reduction in Apc leads to the activation of canonical Wnt signaling by preventing the recruitment of Ctnnb1 to the degradation complex. Increased Apc expression upon miR-142-3p silencing causes down-regulation of Wnt signaling [3]. On the other hand Sharma et al. found that p300 is a target of miR-142-5p. Interestingly, this target was not identified as a main putative target through in-silico analysis and demonstrates the importance of identifying and confirming interacting partners through pull-down assays followed by gene arrays [3] or next generation sequencing. A mutual inhibitory loop between p300 and miR-142-5p has also been described and under mechanical stress, p300 is accumulated in the cardiac cells, which results in the down-regulation of miR-142-5p. The loss of miR-142-5p expression activates genes required for myocyte survival and function [15]. p300 is a positive regulator of Ctnnb1, which synergistically activates Ctnnb1/TCF transcription [17]. It is therefore not surprising to find that the loss of miR-142 leads to increased expression of both Apc and p300 (Fig 5). As Apc is a negative regulator and p300 is a positive regulator of Wnt signaling, we expect that upon deletion of miR-142, the balance between Apc and p300 will lead to either no change in Wnt signaling, an increase or a decrease in Wnt signaling. In the E18.5 lung, our results indicate that the Apc/p300 balance favors Wnt signaling. The regulation of the relative expression of both isoforms in specific tissues over time is still unknown. In the embryonic lung at E18.5, both the -5p and -3p isoforms are normally co-expressed in the epithelium and mesenchyme while -3p appears to be more abundantly expressed in the mesenchyme at E12.5 [3]. In the hematopoietic system, -3p is the predominant isoform expressed. In the heart, it appears that -5p is the major isoform. Our mouse model will allow determining the overall contribution of -3p and -5p to Wnt signaling in different tissues and at different developmental stages.

Another aspect related to the reported miR-142-null mouse is the deletion of the LncRNA together with the miR-142 gene. LncRNAs contribute also to gene regulation [18] and in the future, the generation of new mouse models allowing the tissue-specific rescue of miR142-3p/5p expression in the context of the miR-142 KO animals will allow determining the role of this LncRNA. Interestingly, the expression of this LncRNA has not been investigated in the miR-142 gene-trap line. More recently, a conditional KO for miR-142 was generated by deleting 900 bp of the genomic sequence that encompasses the miR-142 precursor in the germ line [19]. The authors reported that miR-142-null mice are born at the expected Mendelian ratio and appear healthy and fertile with no apparent internal organ defects, similarly to the findings described in this report. They also described a highly penetrant splenomegaly that is also observed in our miR142-null mice (data not shown).

In conclusion, we report the generation and validation of a novel homologous recombination-based miR-142 KO mouse model. Interestingly, these mice display differences as compared to the previously described gene-trap model, which will need to be analyzed further to
Fig 7. Predicted targets of miR-142-3p and miR-142-5p using Diana-MicroT and Target Scan software prediction tools. (A) Pathway intersection between miR-142-3p and miR-142-5p. (B) Predicted targets of miR-142-3p and miR-142-5p in the Wnt signaling pathway. Orange boxes: common predicted targets for both isoforms; yellow boxes: predicted targets for both isoforms; green boxes: experimentally validated target of miR-142-3p; red box: experimentally validated target of miR-142-5p.

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validate the proposed function of miR-142 in hematopoiesis. Together with the recently reported conditional miR-142 KO, our mouse model will be the first generation of tools designed to decipher the role of miR-142 in development and disease.

Supporting Information
S1 Fig. Schematic representation of mir-142 targeting vector. (A) Map of mir-142 targeting vector plasmid. (B) Deletion of mir-142 locus in the genome. (TIF)

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Author Contributions
Conceived and designed the experiments: AS GC EEA RM AR GB SB. Performed the experiments: AS RM CMC. Analyzed the data: AS GC CMC RM AR GB SB. Wrote the paper: AS EEA SB.

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