Supplemental Methods

Generation of CK alleles (in detail).

Construction of the CK allele for any gene of interest simply requires the amplification and ligation of the targeting arms into the appropriate pCK plasmid. As discussed in the methods section an exon can be included within the region of the genome to be inverted but this is not required for the system to work. If an exon is included then three separate genomic regions need to be amplified and sub-cloned into the targeting vector. If no exon included then only clone cloning steps are required and you can skip step 1:

1. To insert the exon region, amplify the desired region and sub-clone it into the "MCS for Exon" (**Supplementary Fig. 1**; BglII.ApaI.BsiWI.NdeI). For the *Hmga2*^{CK} allele, the exon region was inserted with a 5' BglII site and a 3' NdeI site.
2. To insert the right hand 3' arm, amplify the desired region and clone it into the "MCS for right arm" (**Supplementary Fig. 1**; PacI.ZraI.AatII.AvrII.BstXI). For the *Hmga2*^{CK} allele, the right arm was inserted with a 5' PacI and 3'AvrII compatible site (XbaI).
3. To insert the left hand 5' arm, amplify the desired region and clone it into the "MCS for left arm" (**Supplementary Fig. 1;** SalI.AscI.FseI.NotI). For the *Hmga2*^{CK} allele, the left arm was inserted with a 5' AscI site and a 3' FseI site.

Prior to electroporation into ES cells the plasmid can be linearized with either PmeI or SwaI.

Hmga2 **and mir-763 qPCR.**

For *Hmga2* qPCR total RNA was purified from MEFs and embryos using the miRNeasy Mini Kit (Qiagen). cDNA was synthesized from using the High Capacity cDNA Reverse Transcription kit (Applied Biosystems) with random-hexamers. qPCR reactions were performed in triplicate using the SYBR green Jumpstart Taq kit (Sigma; 40 cycles at 95°C for 10 seconds and at 60°C for 1 min), and real-time detection was performed on an ABI StepOneplus (Applied Biosystems). The specificity of the PCR amplification procedures was determined with a heat-dissociation step (from 60°C to 95°C) at the end of the run and by agarose gel electrophoresis. *Hmga2* expression (primers: mHmga2-exon1F 5'acatcagcccagggacaa3' and mHmga2-exon2R 5'ggctcacaggttggctctt3'), was normalized to *Gapdh* (primers: mGapdh-F 5'tttgatgttagtggggtctcg3' and mGapdh-R 5'agcttgtcatcaacgggaag3').

For mmu-miR-763 qPCR, small RNA quantification was performed using the TaqMan® MicroRNA Assays System (Applied Biosystems). miRNA cDNA was synthesized from 2µg of RNA using the TaqMan MicroRNA Reverse Transcription Kit using snoRNA202 and mmu-miR-763 specific RT primers (Applied Biosystems). qPCR reactions were performed on ABI StepOnePlus in triplicate using the TaqMan mmu-miR-763 and snoRNA202 probe sets (Applied Biosystems; 50 cycles of 95°C for 5 seconds and 60°C for 1 minutes). mmu-miR-763 expression was normalized to snoRNA202.

Immunohistochemistry, direct fluorescence analysis, and fluorescence quantification

Immunohistochemical analysis of Hmga2 was performed using an anti-Hmga2 primary antibody (59170AP; BioCheck). Direct fluorescence visualization of the embryos was achieved using a fluorescent dissecting microscope. Quantitative fluorescence imaging was performed on embryos using an IVIS® Spectrum imager (Caliper Life Sciences) measuring GFP signal with an excitation wavelength of 465 nm, an emission wavelength of 520 nm, and an exposure time of 5 seconds. Data analysis was then performed using Living Image™ Software 4.2 (Caliper Life Sciences), and embryo genotypes were confirmed from genomic DNA prepared from tissue collected subsequent to imaging.

Western blotting

MEFs (murine embryonic fibroblasts) were trypsinized and pelleted before being lysed. For the preparation of embryo lysates, samples were homogenized before lysis, which was performed on ice for 10 minutes in RIPA buffer (1% sodium deoxycholate, 25 mM Tris-HCl (pH 7.6), 150 mM NaCl, 1% NP-40, 0.1% SDS, and protease inhibitor cocktail (Sigma-Aldrich Corporation)). The supernatants were boiled with Laemmli buffer and β-mercaptoethanol at 95°C for 5-10 minutes. Samples were then separated on a SDS-PAGE, followed by transfer to PVDF membranes. After transfer, membranes were blocked with 5% skim milk at room temperature for one hour. Membranes were stained with indicated primary antibodies (rabbit anti-Hmga2-P1 (59170AP), BioCheck; rabbit anti-GFP (2956), Cell Signaling Technology) overnight at 4°C on a shaker. Horseradish peroxidase (HRP)-conjugated anti-mouse (sc-2005, Santa Cruz Biotechnology) and anti-rabbit (sc-2004, Santa Cruz Biotechnology) secondary IgG antibodies were used, and signal was visualized using the ECL Plus Western Blotting Detection System (GE Healthcare Biosciences). For input normalization, membranes were stripped (2% SDS, 62.5 mM Tris-HCl, pH 6.8, and 0.8% β-mercaptoethanol) for 30 minutes at 50°C before an overnight incubation with anti-β-actin antibody (A5441, Sigma-Aldrich). For LI-COR imaging, membranes were first stained with indicated primary antibodies followed by the staining with either the goat anti-mouse-IRDye 800CW (926-32210, LI-COR, Inc.) or the goat anti-rabbit-Alexa Fluor 680 (A-21109, Life Technologies, Inc.) for 1 hour at room temperature in the dark. After 3 washes (1X PBS, 0.05% Tween20), the membranes were imaged with the LI-COR imager LI-COR Odyssey.

Flow cytometry

Fluorophore-conjugated antibodies were used according to the manufacturer's protocols. Briefly, fetal liver cells were prepared from E12.5-16 embryos. The Fc receptors were blocked by incubation with a purified, rat anti-mouse CD16/CD32 antibody (2.4G2, BD Biosciences) for 30 minutes on ice. Fluorophore-conjugated antibodies (lineage markers: anti-γδ T cell receptor-Pacific Blue or anti-γδ TCR-PB (GL-3), anti-αβ (H57-597) TCR-PB, anti-B220 (RA3-6B2)-PB, anti-CD19 (6D5)-PB, anti-CD11c (N418)-PB, anti-Gr-1 (RB6-8C5)-PB, anti-F4/80 (BM8)-PB, and anti-TER119 (TER-119)-PB antibodies; anti-Sca-1-PerCP-Cy5.5 (D7) and anti-CD11b (M1/70)-PE were used as markers for fetal liver-resident progenitor/stem cells; all antibodies were purchased from BioLegend) were then applied to the samples and incubated for another 30 minutes on ice in the dark. After washing, cells underwent FACS analysis. For the

compensation of multiple fluorescent colors, splenocytes stained with individual anti-CD19 antibodies conjugated with FITC, PE, pacific blue, PerCP-Cy5.5, or APC were used. For exclusion of dead cells, DAPI was used on a LSR II equipped with an UV laser. All data analyses were performed on FlowJo software (Tree Star).

MEFs preparation and adenoviral infection

Embryos were collected between E12.5 and E16.5. To determine the genotypes, a piece of the amnionic sac from each fetus was collected for genomic DNA preparation, and the head and fetal liver were removed prior to preparing MEFs from the embryo. Briefly, the tissue was homogenized with a sterilized scalpel, trypsinized on a petri dish, and allowed to grow for 3-5 days (one 15 cm plate/fetus). Cells were subsequently split into 5x15 cm plates per embryo to reach near confluency before cryopreservation. To express Cre-recombinase in MEFs, cells were infected with either Adeno-Cre or Adeno-FLPo (control) purchased from Gene Transfer Vector Core at the University of Iowa. Analysis of the cells was performed at least 3 days after infection.

***Let7* transfection and retroviral Lin28 expression**

To deliver control (mirVana negative control mimic) and *let7* miRNA mimics (mirVana mmu-let7a-5p miRNA mimic) into MEFs, we used Lipofectamine RNAiMAX according to manufacturer's description (Life Technologies). MEFs were plated in a 12-well plate 24 hours prior to transfection. Analysis was performed 3 days after transfection. To generate murine stem cell virus (MSCV), 293T cells were co-transfected with the retroviral plasmid and the packaging pCL-Eco plasmid (1:1) using the calcium phosphate method. Supernatants of the 293T transfectants were collected 48-72 hours post-transfection. MEFs of the indicated genotypes were then cultured in the filtered supernatants. Subsequently, transduced MEFs were cultured in the presence of G418 (0.5 mg/ml) for 3 days to allow complete enrichment of transductants. Selected MEFs were assessed for GFP and Hmga2 levels using flow cytometry and western blot analysis.

Northern blot hybridization (in detail)

Radiolabeled probes for Northern hybridization were made by random hexamer-primed synthesis using a PCR-amplified cDNA sequences from plasmid. 50-150 ng template DNA was denatured for 5 min at 95˚C and quenched on ice for 3 min, then incubated at 37˚C for 30 min with 1.25x Cocktail C (63 mM Tris-HCl pH 7.5 (Gibco), 6.3 mM $MgCl_2$ (Sigma-Aldrich), 13 mM beta-mercaptoethanol (Sigma-Aldrich), and 15 µM each dGTP, dATP, and dTTP (New England Biolabs, N0442S, N0440S, N0443S)), 0.3 units/µL of DNA polymerase I Klenow fragment (New England Biolabs, M0210S), 0.3 ng/µL of random hexamers (Invitrogen, 51709), and 6.25 µCi/µL (1 µM), 6000 Ci/mmol, alpha-32P-dCTP (Perkin-Elmer, EasyTide, BLU513Z500UC). Probe was purified from excess nucleotide using a spin column kit (Nucleotide Removal Kit, QIAGEN) and stored frozen in TE (10 mM Tris-HCl pH 8.0, 1 mM EDTA (Promega)) until use. Extent of probe labeling was quantified by liquid scintillation counting using EconoSafe cocktail (RPI Corp.). Membranes were pre-washed for 1 hour at 42˚C with 50 mM Tris pH 8.0, 1 M NaCl (Sigma-Aldrich), 1 mM EDTA, and 0.1% SDS (Invitrogen). 1.35 µCi (3 x 10^6 DPM) of radiolabeled probe was mixed with 1 mg of salmon sperm DNA (Invitrogen, 15632-011) in a final volume of

~500 µL. The probe was denatured with 50 µL of 10N NaOH, then quenched with 150 µL of 2M Tris-HCl pH 7.6 and 500 µL 1N HCl. It was then mixed immediately with 20-25 mL pre-warmed hybridization buffer (50% deionized formamide (American Bioanalytical), 5x SSC, 10% dextran sulfate (Amresco), 20 mM Tris pH 7.6, 1X Danhardt's reagent (0.02% Ficoll, 0.02% polyvinylpyrrolidone, 0.02% BSA Fraction V; gift from Crabtree Lab, Stanford University), and 0.1% SDS) and added to a hybridization tube containing the pre-washed membrane. Hybridization was carried out overnight for 16-20 hours at 42˚C with constant rotation. The membranes were then washed twice in pre-warmed 100 mL 2x SSC and 0.1% SDS at 42˚C for 20 minutes, and twice in pre-warmed 100 mL 0.1X SSC and 0.1% SDS at 55˚C for 20 minutes. Membranes were wrapped in plastic wrap and exposed for 3-6 days to a Molecular Dynamics phosphor screen (GE Healthcare), which was imaged on a Molecular Dynamics Typhoon 9400 Imager (Amersham/GE Healthcare).