

## Supplementary Information.

### Materials and Methods.

**Antibodies.** Antibodies were used at working dilution of 1:1000 unless otherwise noted. The following primary antibodies were purchased from Cell Signaling: rabbit polyclonal anti-Mono-Methyl-Histone H3 (Lys4)(#5326) , anti-Di-Methyl-Histone H3 (Lys4)(#9725), anti-Tri-Methyl-Histone H3 (Lys4)(#9751), anti-Mono-Methyl-Histone H3 (Lys36)(#5928), anti-Di-Methyl-Histone H3 (Lys36)(#2901), anti-Tri-Methyl-Histone H3 (Lys36)(1:500)(#4909), anti-Tri-Methyl-Histone H4 (Lys20)(#5737), anti-Di-Methyl-Histone H3 (Lys79)(#9757), anti-Tri-Methyl-Histone H3 (Lys27)(#9733), anti-Di-Methyl-Histone H3 (Lys27)(#9728), anti-Di-Methyl- Histone H3 (Lys9)(#4658), anti-Mono-Methyl- Histone H3 (Lys9)(#7538), anti- Histone H3(#4499), anti-MEF2C (#5030), anti-NCOR1(#5948), anti-HDAC3(#2632), anti-PAF1(#12883), anti-CTR9(#12619), anti-CDC73(#8126), anti-OGT(#5368) and an anti-RUVBL1(#12300). We also used rabbit polyclonal anti-Histone H3 (acetyl K9+K14+K18+K23+K27) (ab47915, Abcam), anti-TBL1X(13540-1-AP, Proteintech group) and an anti-Ki67 (RM-9106-S1, ThermoFisher (1:500 dilution)) antibodies. In addition, we used a rat monoclonal anti-BrdU (MCA2060T, from AbD Serotech), mouse monoclonal anti-activated CASP3 (C8487, Sigma-Aldrich (1:500 dilution)), rabbit polyclonal anti-SETD5 (#2256.00.02, Strategic Diagnostics (at 1:500 dilution, Western blot), rabbit polyclonal anti-SETD5 (ab 204363, Abcam, 1:200 immunofluorescence), rat monoclonal anti-PECAM 1(#550274), anti-CD41 (#550539, both from BD Biosciences), mouse monoclonal anti-myosin heavy chain (clone MF20) (MAB4470,R&D Systems) and mouse monoclonal anti-FLAG HRP-conjugated (A8592, Sigma-Aldrich (1:500 dilution)) antibodies. The secondary antibodies used were a mouse anti-rabbit light chain specific HRP conjugated (#211-032-171), donkey anti-rabbit HRP conjugated (711-035-152) donkey anti-mouse HRP conjugated (715-035-151) and donkey anti-rat HRP conjugated (712-035-150, all from Jackson ImmunoResearch); donkey-anti-rabbit Alexa-555 conjugated (A-31572, ThermoFisher). ChIP was performed using rabbit anti-Histone H3 (acetyl K9+K14+K18+K23+K27)(ab47915) and normal rabbit IgGs (ab27472, both from Abcam).

**Embryo isolation, whole mount staining and imaging.** Timed matings were performed where noon of the day of identification of a vaginal plug was considered to be embryonic (E) day 0.5. Embryos at E8.5 to 11.5 were dissected out and imaged using a Leica MZ 16 FA fluorescent stereoscope equipped with a QImaging RETIGA 4000R camera and genotyped using embryonic or yoke sac tissue. For whole mount staining embryos were fixed in 4% paraformaldehyde for 2 hours, permeabilized in 1% Triton X-100/ PBS for 2 hours, blocked in staining buffer (3% BSA, 1% Triton X-100 in PBS) with 0.3% H<sub>2</sub>O<sub>2</sub> at 4°C overnight, stained with primary antibodies in staining buffer overnight, washed with 1% Triton X-100 in PBS and stained with HRP-conjugated secondary antibodies overnight. Detection of HRP activity was done using DAB Peroxidase Substrate Kit (Vector Labs).

**Northern blot and RT-qPCR analysis.** RNA was isolated from embryos at E9.5 using TRIzol Reagent (Thermo Fisher). Northern blot analysis was performed using standard procedures. 5 µg aliquots of total RNAs were separated by 1% agarose formaldehyde gel electrophoresis in MOPS buffer (Thermo Fisher), blotted to a nylon membrane, and probed with a <sup>32</sup>P-labeled probe (652 bp EcoRI fragment containing exons 2-6 of *Setd5*). For RT-qPCR, cDNA was prepared using a High Capacity cDNA Reverse Transcription Kit (Life Technologies). 2 ng of cDNA were used in real-time qPCR with Power SYBR Green PCR master mix (Thermo Fisher) using ABI 7900HT Real Time PCR system (Applied Biosystems). Primer sequences are listed in Table S1. Relative expression was determined from at least three independent assays by 2<sup>-ΔCt</sup> method (Winer et al., 1999). Mouse *Actb* gene was used as an endogenous control.

**Immunohistochemistry and immunofluorescence.** Embryos were dissected at E9.5, fixed in 4% paraformaldehyde, embedded in paraffin, cut into 5 µm serial sections and select sections were stained with haematoxylin and eosin stain (Sigma). To assess cell proliferation in embryos, pregnant females were injected IP with 100 µg of 5-bromodeoxyuridine (BrdU, Sigma) per gram of body weight and sacrificed after 3 hours. For BrdU staining, rehydrated slides were treated with 2 M HCl/0.3% Triton X-100 for 30 min then neutralized with 0.1 M sodium borate for 10 min. Otherwise, after heat antigen retrieval in sodium citrate buffer (pH 6), slides were washed with PBS,

blocked with staining buffer (3% BSA in PBST (0.02% Tween in PBS)) for 1 hour at room temperature, and then incubated with primary antibodies in the staining buffer at 4°C overnight. After washing with PBST the HRP-conjugated secondary antibodies were applied in the staining buffer for 2 hours at room temperature. Following final washes with PBST, slides were processed with DAB Peroxidase Substrate Kit, counterstained with haematoxylin, dehydrated and mounted in Cytoseal mounting media (Thermo Fisher). Mouse ES cells were grown in 8-well chamber slides (Thermo Fisher) and, for proliferation assay, incubated with 20  $\mu$ M of BrdU for 30 min, then washed with PBS and fixed with 4% paraformaldehyde for 15 min.

Immunofluorescence staining was performed as described above using fluorochrome-conjugated secondary antibodies. Slides were mounted with ProLong Gold antifade with DAPI reagent (Thermo Fisher). Images were acquired using an Axioplan2 fluorescent microscope (Zeiss) with 20X and 40X objectives and QImaging RETIGA EXi camera. Confocal images were taken with Zeiss LSM 510 META confocal laser scanning microscope using Zeiss imaging software. For display, images were converted into JPG format and processed using Adobe Photoshop software. Cell counts were done using ImageJ software. For tissues, step sections (50  $\mu$ m apart) from at least 3 different animals of the same age and genotype were counted. At least 1000 cells were counted for each genotype in each experiment.

**Dual luciferase assay.** A 439 bp DNA fragment containing the Setd5-Rosa26 promoter region (Fig. S1) was PCR-amplified and cloned into pLucRluc (Polson et al., 2011) in both orientations thereby generating pLucRluc-SR and pLucRluc-RS, respectively. Similarly, pLucRluc-SR $\Delta$ 181, was made to mimic the 181 bp deletion that occurs in the ROSA26228.3TF mice. These plasmids were transfected into mouse ES cells together with pCMV- $\beta$ -Gal (Thermo Fisher,) at a 2:1 ratio using Xfect Mouse Embryonic Stem Cell Transfection Reagent (Clontech). Cells were lysed, and luciferase activity was measured by using the Dual-Luciferase reporter Assay System (Promega) and a BioTek Microplate Reader. Luciferase activity was normalized to  $\beta$ -galactosidase activity detected in parallel using  $\beta$ -Galactosidase Assay System (Promega).

**ES cell line derivation.** Wild type (WT), heterozygous (Het) and knockout (KO) *Setd5* mES cell lines were derived from inner cells mass outgrowths obtained from blastocysts cultured in mouse ES cell media (Dulbecco's modified Eagle's medium supplemented with 15% FBS, 1X nonessential amino acids, 1X penicillin–streptomycin, 1× L-glutamine (all from Thermo Fisher)) containing MEK1 inhibitor PD98059 (Cell Signaling) following published protocol (Meissner et al., 2009). All mES cells were cultured on a feeder layer of mitotically inactivated mouse embryonic fibroblasts (MEFs) in mES cell culture medium supplemented with 1000 U/ml of leukemia inhibitory factor (ESGRO/LIF, Millipore) according to standard protocols. To obtain KO(+SETD5) ES cell line, KO cells were transfected with pEf1a-FLAG-SETD5 vector using Xfect Mouse Embryonic Stem Cell Transfection Reagent (Clontech).

**RNA-seq analysis.** RNA isolates (N=3) from heterozygous and knockout genotype of *Setd5* ES cells were subjected to RNA quality analysis and sequenced. Paired-end sequencing (75 bp length) was performed on an Illumina HiSeq3000 genome analyzer. Read alignment to the mouse genome mm10 (GRCm38) was done by Spliced Transcripts Alignment to a Reference (STAR) (Dobin et al., 2013) software using GENCODE comprehensive gene annotations (Release M8) as a reference. Differential gene expression analysis was done using DESeq2 (Love et al., 2014). Gene Ontology analysis was performed using DAVID Bioinformatics Resources v.6.7 (Huang da et al., 2009).

**Directed differentiation of mouse ES cells.** Cardiac differentiation in embryoid bodies (EBs) was induced in ES cell culture medium with 20% FBS without LIF at a final concentration of  $5 \times 10^4$  cells/ml following ES cell dissociation and suspension. Hanging drops (20  $\mu$ l) were plated on the inside lids of low attachment dishes. After 48 hours, EBs were transferred in 10 ml of medium to low attachment dishes. At day 4, the EBs were plated on tissue culture dishes, allowed to adhere and beating was scored at day 8. A portion of the EBs were plated on 8-well chamber slides coated with fibronectin (Sigma) at day 7, and fixed and processed for immunostaining at day 8.

**Cell cycle and apoptosis assays.** For cell cycle distribution analysis, mESCs were trypsinized, washed with PBS, resuspended in 300  $\mu$ l of PBS and fixed by addition of 100% ice cold ethanol and incubating at 4°C overnight. Cells were washed the next

day with PBS, incubated for 30 min in 1 ml of PBS containing 10 µg/ml of ribonuclease A (Sigma) and 20 µg/ml of propidium iodide. The cell suspension was then analyzed for DNA content on a LSRII/Fortessa flow cytometer (BD Biosciences) using FACS DIVA (BD Biosciences) software. For quantitative analysis of apoptosis, mESCs were trypsinized, washed with PBS and incubated with 5 µl of Alexa 555-conjugated annexin-V (Thermo Fisher) in 100 µl of annexin binding buffer (10 mM HEPES/NaOH, pH 7.4; 140 mM NaCl; 2.5 mM CaCl<sub>2</sub>) for 30 min at 37°C in the dark. The uptake of DAPI (Sigma), as measured by flow cytometry, was used to assess cell viability. The percentage of apoptotic cells was calculated on the basis of the proportion of Annexin V positive/DAPI negative cells.

**Immunoblotting.** Embryos or mES cells were lysed in RIPA buffer (10 mM TrisHCl, pH 7.5; 140 mM NaCl; 1 mM EDTA; 1% Nonidet P-40; 0.1% sodium deoxycholate; 0.1% SDS) containing protease inhibitors (300 µg/ml phenylmethylsulfonyl fluoride [PMSF], 1X protease inhibitor cocktail (Sigma)). Samples (5 µg of total protein) in 1X Laemmli sample buffer were resolved on a gradient 4-20% SDS-PAGE and transferred to polyvinylidene difluoride (PVDF) membranes (Millipore). Membranes were blocked with TBST (50 mM TrisHCl; 150 mM NaCl; 0.02 % Tween 20, pH 7.5) containing 5% non-fat dry milk and incubated with primary antibodies overnight. After washing with TBST, the membranes were then incubated with a secondary HRP-conjugated antibody, and the signals were detected with Western Lightning Plus ECL chemiluminescence kit (Perkin Elmer). Each Western blot experiment was done at least 3 times.

**Immunoprecipitation.** A FLAG-SETD5 expression plasmid was made by cloning *Setd5* coding sequences (from clone 30536831, ThermoFisher) into pCMV-FLAG-6a (Sigma). HEK293T cells were grown in DMEM supplemented with 10% FBS and antibiotics and transfected using PolyFect Reagent (Qiagen). After 48 hours, cells were lysed in RIPA buffer containing protease inhibitors and the lysates were cleared by centrifugation. The soluble fraction of lysates (2 mg of total protein) was used for immunoprecipitations with 40 µl of anti-FLAG agarose beads (Sigma) at 4°C overnight. FLAG-bound beads were washed four times in RIPA buffer and proteins were eluted with 200 µg/ml of FLAG peptide (Sigma) in 10 mM TrisHCl, pH 7.5, for 30 min at 4°C.

For reverse immunoprecipitations, lysates were incubated with 40  $\mu$ l of Protein G magnetic beads (Cell Signaling) pre-coupled to 10  $\mu$ g of specific or control Ig isotype antibodies. Eluates in 1X Laemmli sample buffer were resolved by 7.5% SDS-PAGE, and processed for immunoblotting with the indicated antibodies. Each IP experiment was done at least 3 times.

**Chromatin Immunoprecipitation.**  $10^7$  mESCs were cross-linked with 1% formaldehyde in PBS for 8 min, quenched with 125 mM glycine and washed with PBS. Cells were lysed in 100  $\mu$ l of ChIP lysis buffer (50 mM Tris-HCL, pH 8.0, 10 mM EDTA, 1% SDS, 1X protease inhibitor mix (Sigma), 1 mM PMSF, 20 mM sodium butyrate), sonicated for 25 cycles (Bioraptor Pico, Diagenode, Denville, NJ, USA) and lysates diluted to 1 ml with RIPA buffer (10 mM Tris-HCL, pH 7.5, 140 mM NaCl, 1 mM EDTA, 0.5 mM EGTA, 1% Triton X-100, 0.1% (wt/vol) SDS, 1X protease inhibitor mix (Sigma), 1 mM PMSF, 20 mM sodium butyrate). 20  $\mu$ g of sonicated chromatin were incubated with 10  $\mu$ g of antibodies coupled to Protein G magnetic beads (Cell Signaling) overnight at 4°C. The magnetic beads were washed 5 times with RIPA buffer and once with TE buffer (10 mM Tris, pH 8.0, 1 mM EDTA). After washing, bound DNA was eluted at 65°C in elution buffer (20 mM Tris-HCl, pH 7.5, 5 mM EDTA, 50 mM NaCl, 1% SDS, 50  $\mu$ g/ml Proteinase K) overnight. After cross-linking reversal, the immunoprecipitated DNA was purified by Qiagen columns and eluted in 30  $\mu$ L of TE buffer. Enrichment at target promoters was determined by real-time PCR with Power SYBR Green PCR master mix (Life Technologies) using primers specific for different areas of target genes (**Table S1**). Relative fold enrichment at different sites was calculated by using the  $2^{-\Delta C_t}$  method using data from 4 different ChIP experiments.

## References.

Dobin, A., Davis, C.A., Schlesinger, F., Drenkow, J., Zaleski, C., Jha, S., Batut, P., Chaisson, M., and Gingeras, T.R. (2013). STAR: ultrafast universal RNA-seq aligner. *Bioinformatics* 29, 15-21.

Huang da, W., Sherman, B.T., and Lempicki, R.A. (2009). Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nat Protoc* 4, 44-57.

Love, M.I., Huber, W., and Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol* 15, 550.

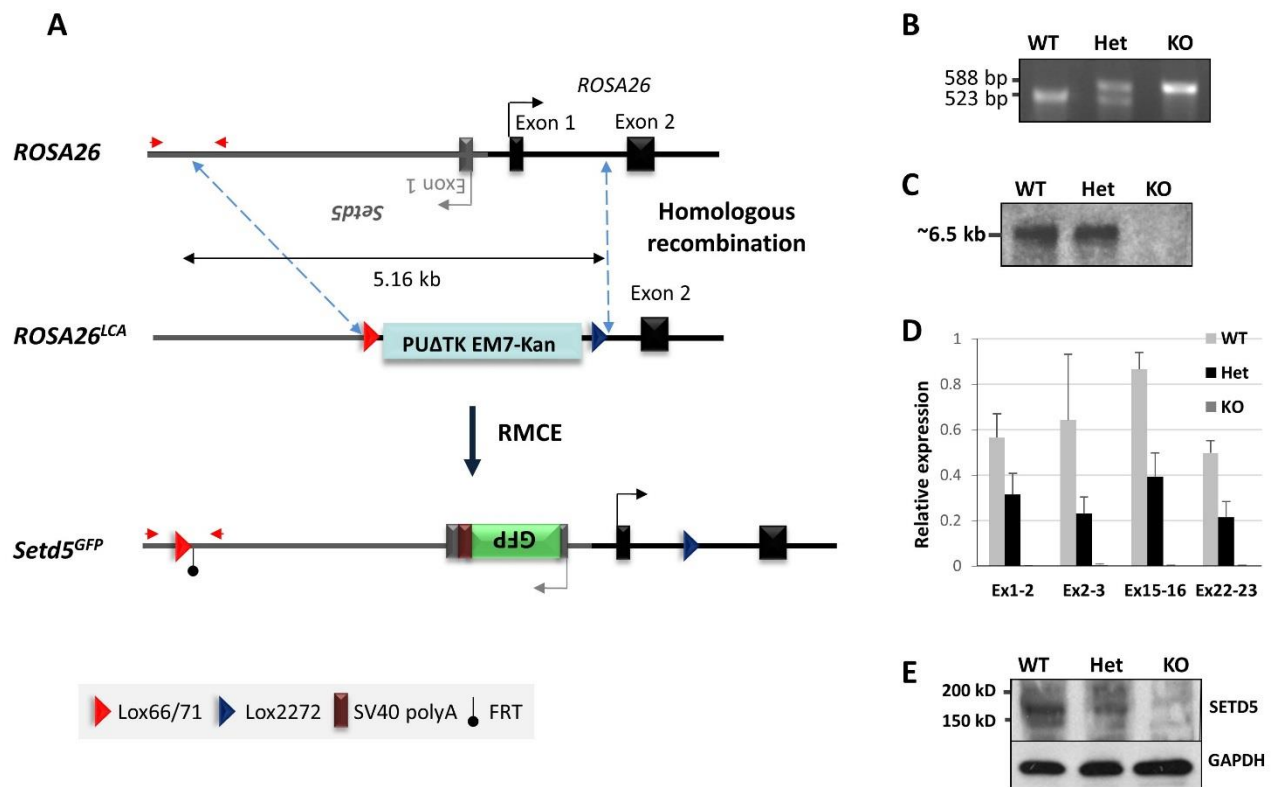
Meissner, A., Eminli, S., and Jaenisch, R. (2009). Derivation and manipulation of murine embryonic stem cells. *Methods in molecular biology* 482, 3-19.

Winer, J., Jung, C.K., Shackel, I., and Williams, P.M. (1999). Development and validation of real-time quantitative reverse transcriptase-polymerase chain reaction for monitoring gene expression in cardiac myocytes in vitro. *Analytical biochemistry* 270, 41-49.

**Figure S1.**

TTCCCCCGGGGCCCGGTTCGTGTGGTTCGGTGTCTCTTTTCTGTTGGACCCTTACCTTGACCCAGGCGCTG  
Setd5 exon 1  
CCGGGGCCTGGGCCCCGGGCTGCGGCGCACGGCACTCCCGGGAGGCAGCGAGACTCGAGTTAGGCCCAACG  
 CGGCGCCACGGCGTTTCCTGGCCGGGAATGGCCCGTACCCGTGAGGTGGGGGTGGGGGGCAGAAAAGGCG  
 GAGCGAGCCCGAGGCGGGGAGGGGAGGGCCAGGGGCGGAGGGGGCCGGCACTACTGTGTTGGCGGACTG  
 GCGGGACTAGGGCTGCGTGAGTCTCTGAGCGCAGGCGGGCGGCGGCCGCCCTCCCCCGGCGGCGGCAGC  
 GGCGGCAGCGGCGGCAGCTCACTCAGCCCGCTGCCCAGCGGAAACGCCACTGACCGCACGGGGATTCCC  
 AGTGCCGGCGCCAGGGGCACGCGGGACACGCCCCCTCCCGCCGCGCCATTGGCCTCTCCGCCACCGCCC  
 CACACTTATTGGCCGGTGCGCCGCCAATCAGCGGAGGCTGCCGGGGCCGCTAAAGAAGAGGCTGTGCTT  
Rosa26 exon 1  
TGGGGCTCCGGCTCCTCAGAGAGCCTCGGCTAGGTAGGGGATCGGGACTCTGGCGGGAGGGCGGCTTGGT  
GCGTTTGCGGGGATGGGCGGCCGCGGCAGGCCCTCCGAGCGTGGTGGAGCCGTTCTGTGAGACAGCCGGG  
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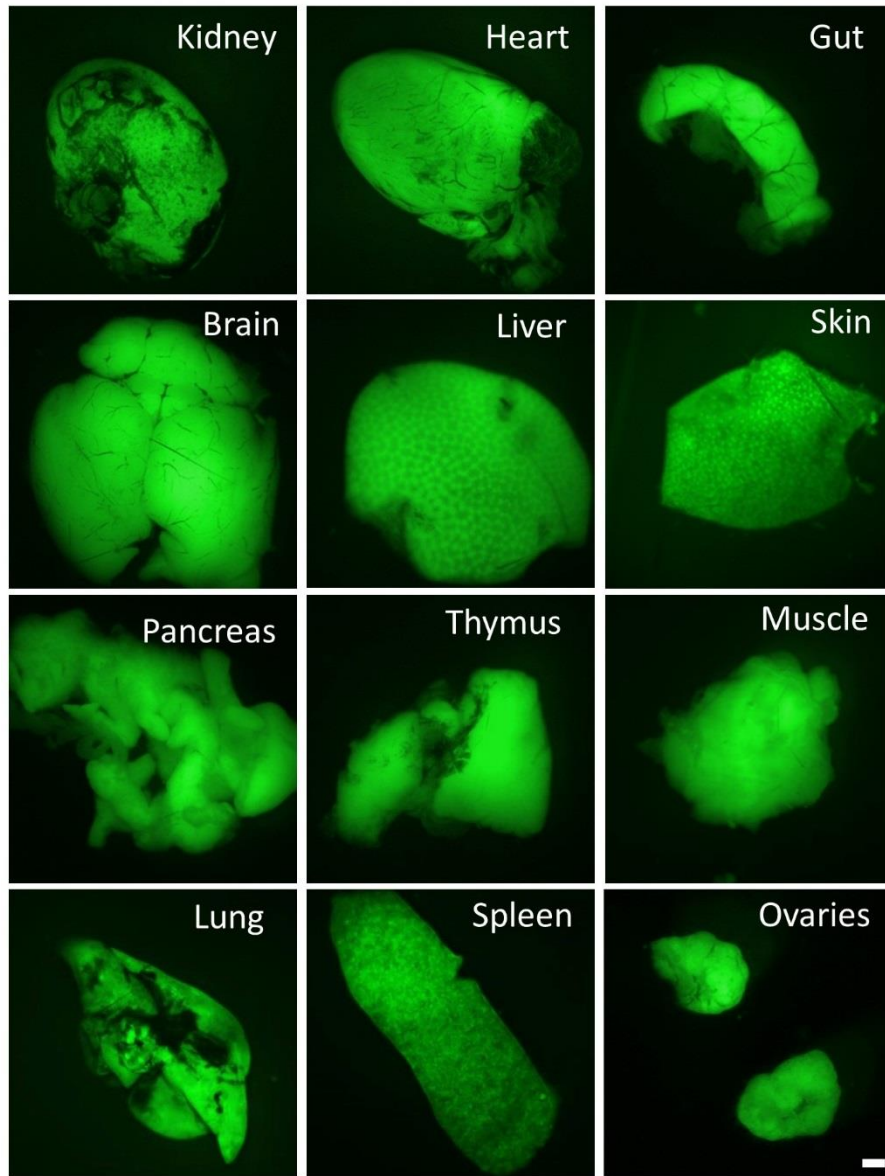
**Figure S1. *Setd5*-*ROSA26* (SR) promoter region.** The DNA sequence of the anti-sense strand (*ROSA26* direction) of *Setd5*-*ROSA26* promoter region is shown. Highlighted are *Setd5* exon1 (pink) and *ROSA26* exon 1 (green) according to the latest annotation of mouse genome at NCBI. The sequence of the portion of the SR promoter cloned into luciferase reporter vector to study bi-directional activity is underlined. Asterisk denotes the start position of *ROSA26* promoter deletion in *ROSA26*<sup>-228.3TF.GFP</sup> mice and in SRΔ181 promoter in luciferase reporter.

**Figure S2.**

**Figure S2. Generation of *Setd5<sup>GFP</sup>* allele.** (A) Schematic representation of the strategy used to make *Setd5<sup>GFP</sup>* allele. In the first step, the *Setd5/ROSA26* locus was modified using homologous recombination to make *ROSA26<sup>LCA</sup>* allele thereby removing 5.16 kb of genomic DNA and replacing it with the selection cassette flanked by heterotypic LoxP sites. In the second step, *Recombinase-Mediated Cassette Exchange* (RMCE) was used to derive the *Setd5<sup>GFP</sup>* knock-in allele where deleted portions of the locus were re-introduced together with the insertion of a GFP-polyA cassette into the first exon of *Setd5* gene. The positions of genotyping primers are indicated by red arrows. (B) PCR genotyping of *Setd5<sup>GFP</sup>* wild type (WT), heterozygous (Het) and knockout (KO) embryos. The wild-type and targeted alleles are represented by 523 and 588 base pair (bp)

bands, respectively. **(C)** Northern blot analysis of total RNA from WT, Het and KO embryos probed with *Setd5* cDNA probe (exons 2-6) shows complete disruption of *Setd5* mRNA (6.5kb) in the KO sample. **(D)** RT-qPCR analysis of total RNA from WT, Het and KO embryos confirms absence of *Setd5* transcripts in knockout tissues (N=3). **(E)** Western blot analysis of protein lysates of WT, Het and KO embryos probed with anti-*SETD5* antibodies shows the band corresponding to the predicted SETD5 protein size (~158 kb) is absent in KO sample. Error bars: SEM.

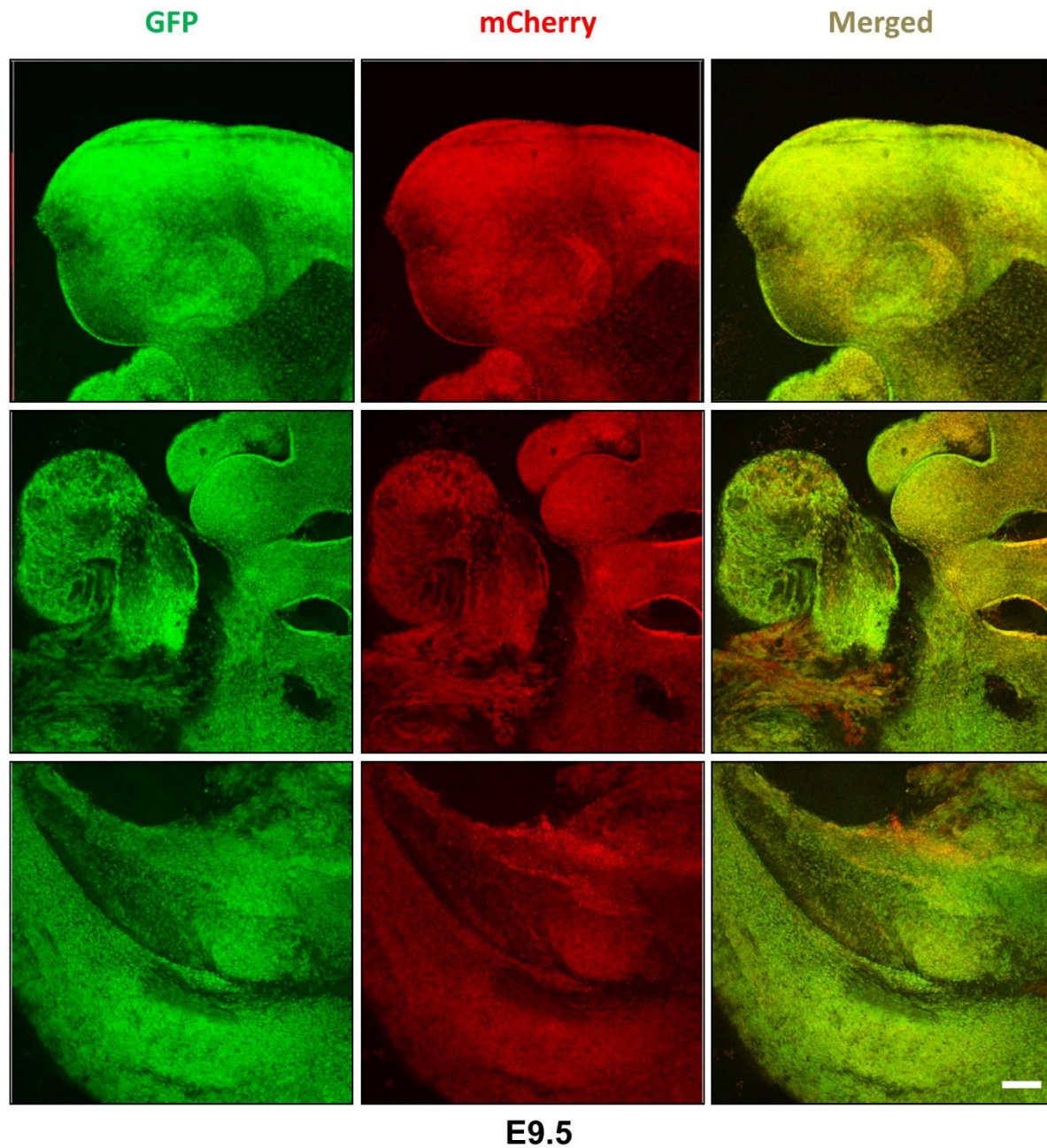
**Figure S3**



**Figure S3. Expression of GFP in adult tissues of *Setd5<sup>GFP</sup>* mice.**

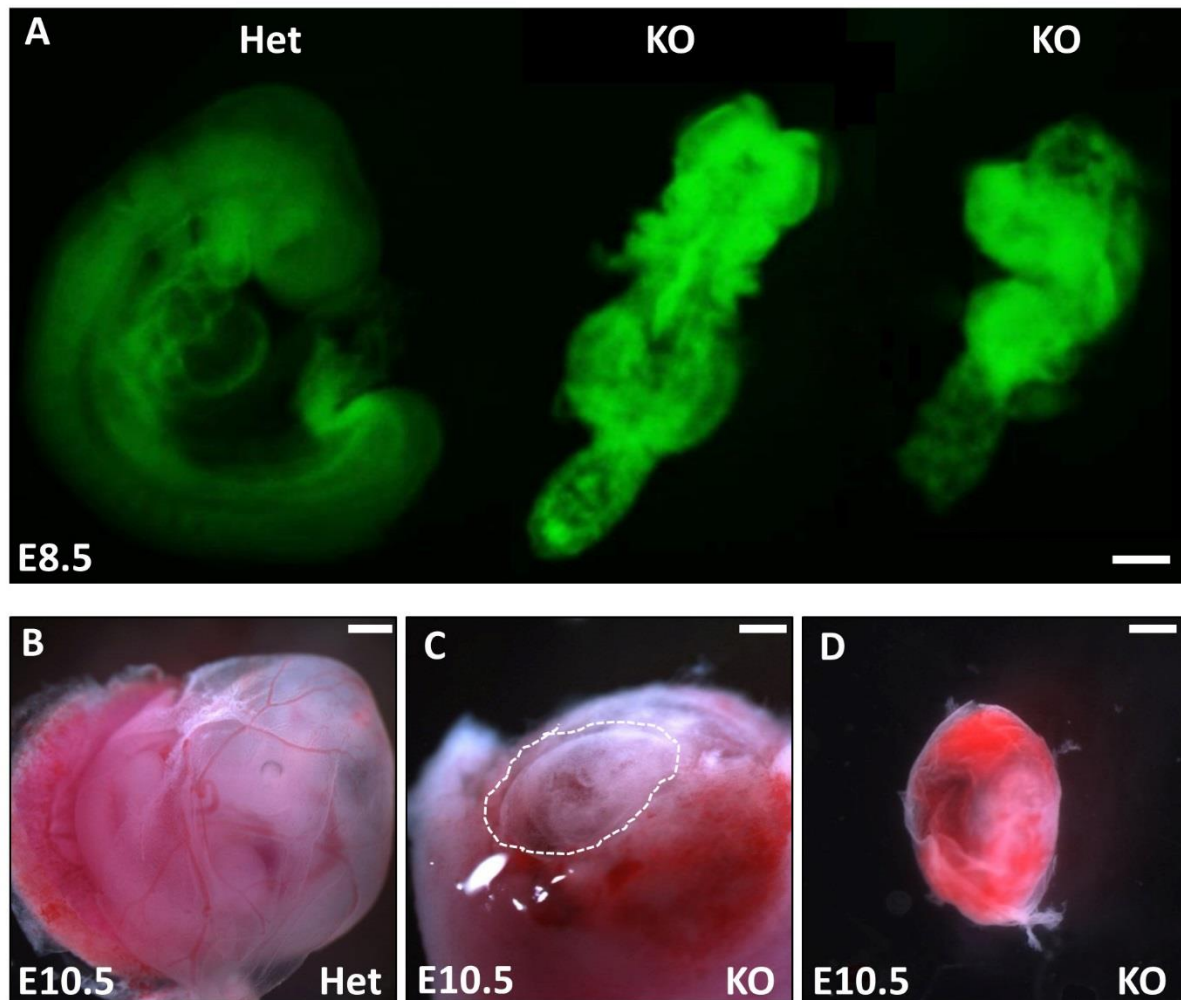
Stereoscope images of green fluorescence show ubiquitous distribution of GFP reporter expression in the adult tissues of *Setd5<sup>GFP</sup>* mice. Scale bar: 1mm.

**Figure S4**

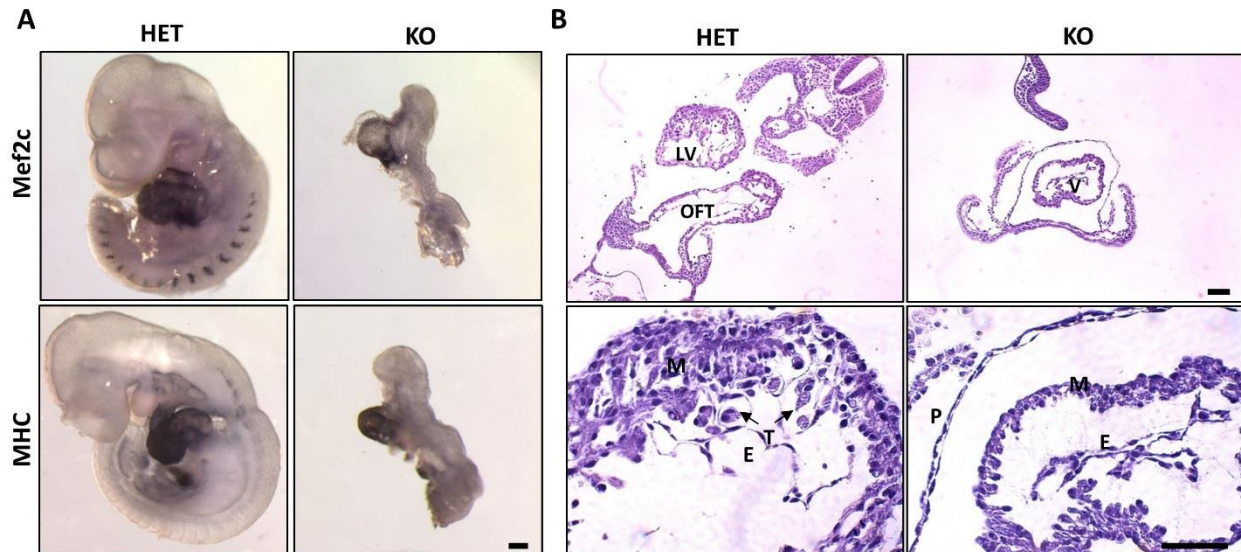


**Figure S4. Co-expression of fluorescent proteins driven by *Setd5* and *ROSA26* promoters.** Confocal imaging of direct fluorescence of *Setd5*<sup>GFP</sup> and *ROSA26*<sup>mCherry</sup> double heterozygous embryo (E9.5) shows co-localization of green (GFP) and red (mCherry) fluorescent proteins in different cell types. Scale bar: 100µm.

**Figure S5**

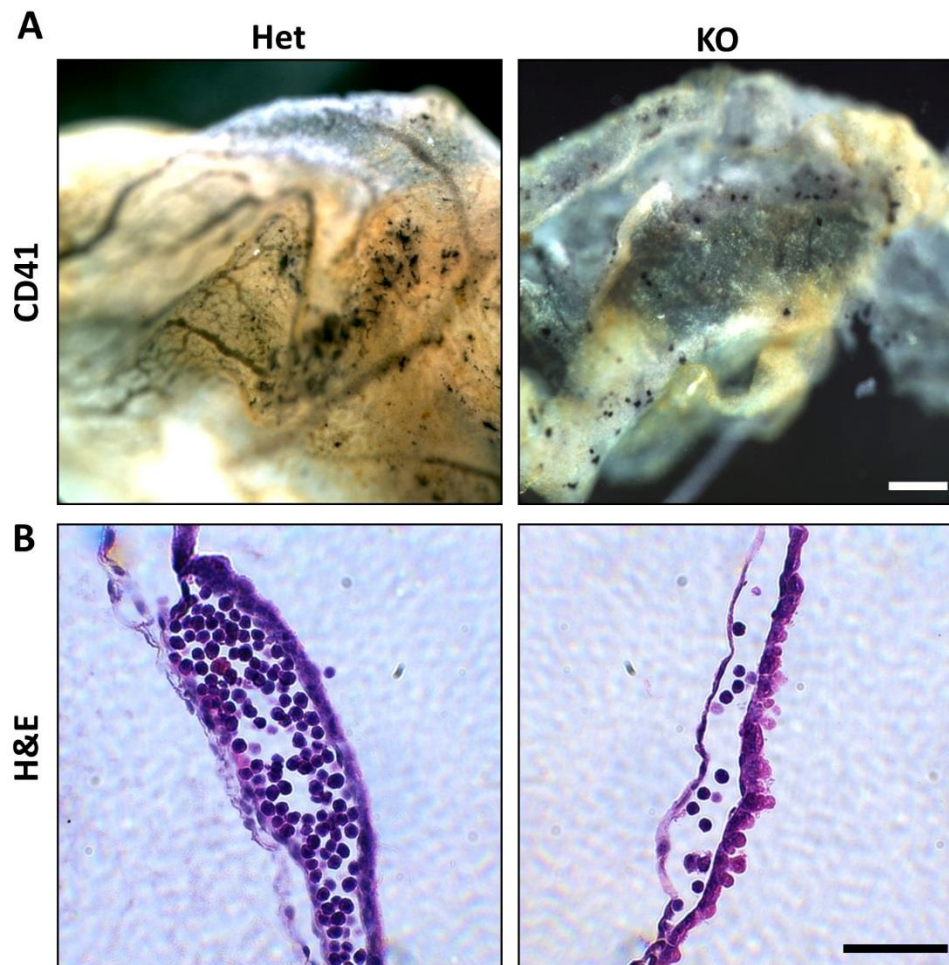


**Figure S5. Phenotype of *Setd5* knockout embryos at E8.5 and E10.5.** (A) Fluorescent (GFP) image shows gross morphology of *Setd5*<sup>GFP</sup> heterozygous (Het) and knockout (KO) embryos at E8.5. KO embryos appear to be underdeveloped. (B) Stereoscope images taken at the same magnification in reflected light show morphology of *Setd5*<sup>GFP</sup> Het and KO embryos in yolk sacs at E10.5. Dashed outline demarcates the size of KO yolk sac. (C) Many surviving KO embryos have widespread hemorrhaging in a yolk sac at E10.5. Scale bar: Scale bars: 500μm.

**Figure S6****Figure S6. Cardiac phenotype of *Setd5* knockout embryos at E9.5.**

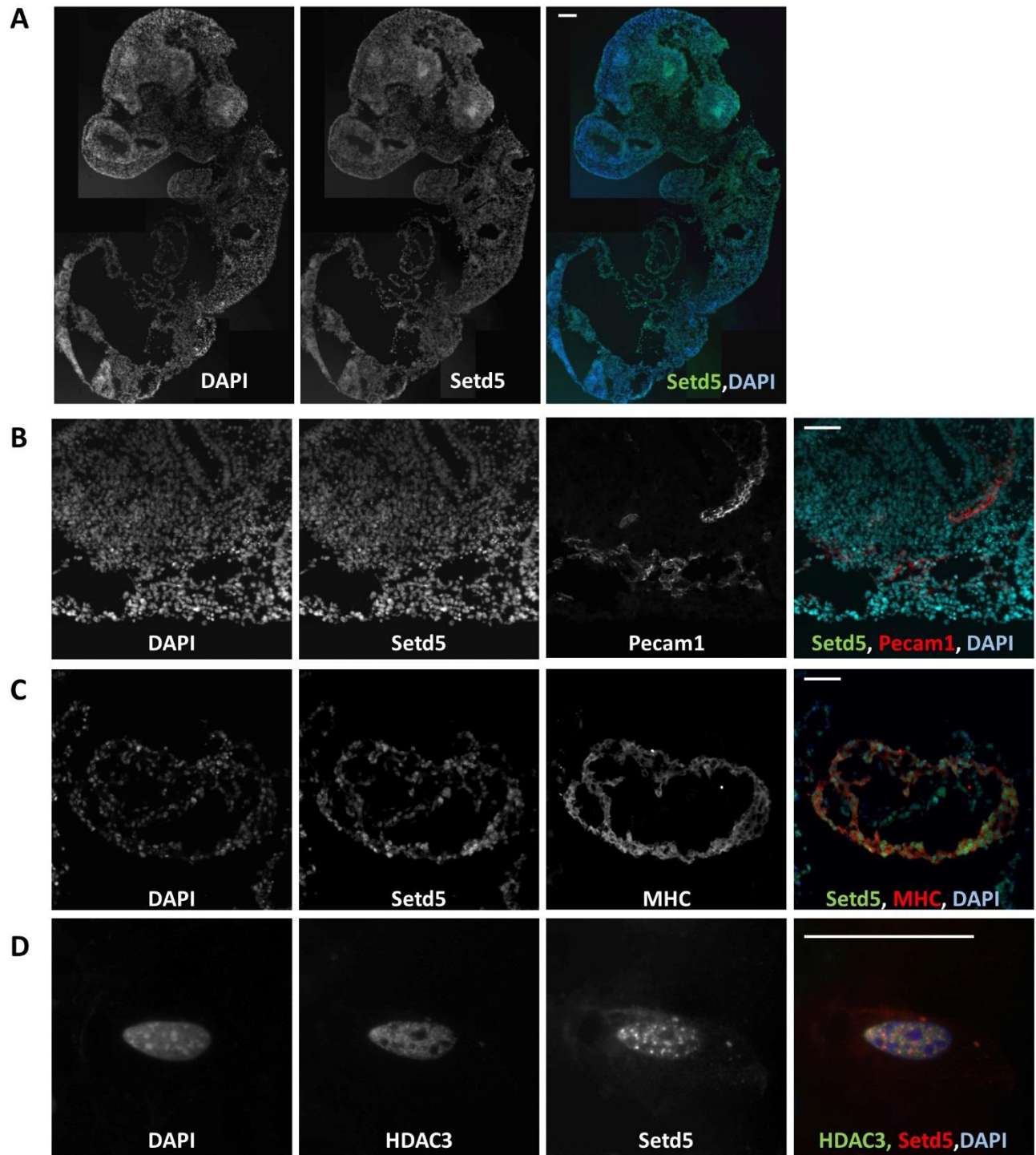
(A) Whole mount staining with cardiac specific markers MEF2C and myosin heavy chain (MHC) shows strong staining in looped heart in *Setd5*<sup>GFP</sup> heterozygous (Het) embryos, while in the knock-out (KO) embryos weaker expression is evident in the heart. Scale bar: 100µm. (B) H&E stained transverse sections of *Setd5*<sup>GFP</sup> Het and KO hearts. The KO heart did not undergo looping and has only one ventricle (V) (top panel). Moreover, KO hearts have thinner myocardium layer with reduced trabeculation and gaps between pericardium, myocardium, and endocardium. LV, left ventricle; OFT, outflow tract; M, myocardium; P, pericardium; E, endocardium; T, trabeculae. Scale bar: Scale bar: 50µm.

**Figure S7**



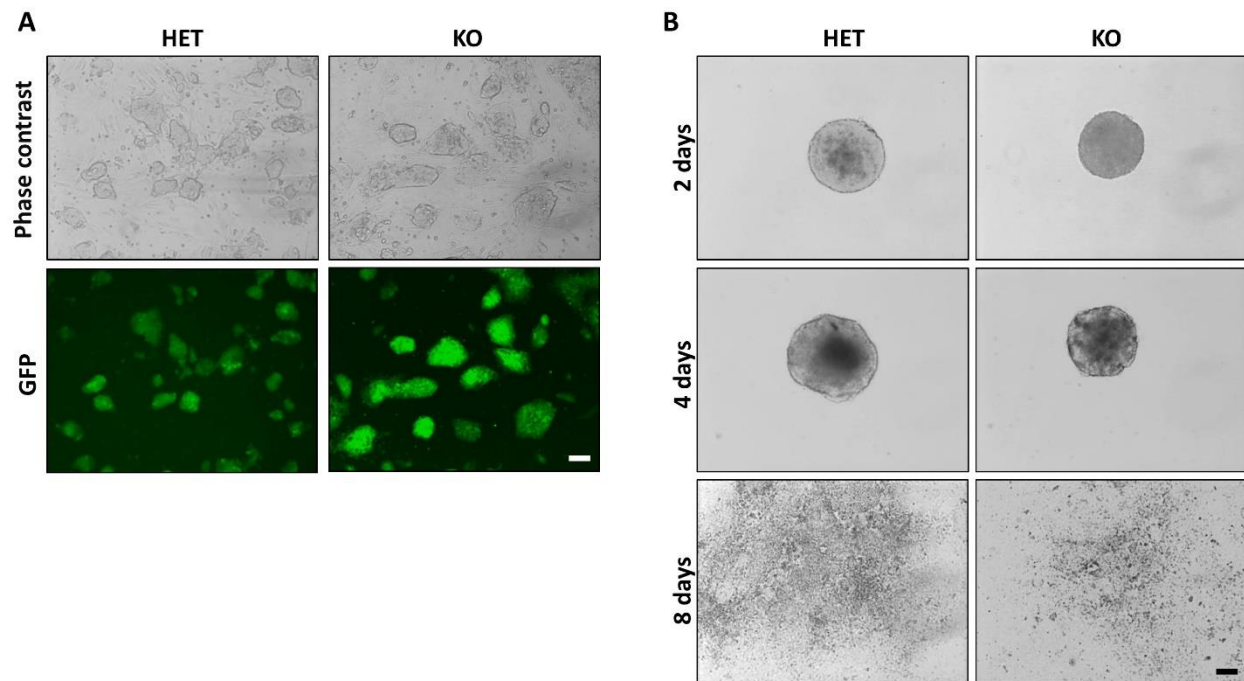
**Figure S7. Hematopoiesis in *Setd5* knockout embryos at E9.5.** (A) Whole mount staining with definitive hematopoiesis marker CD41 demonstrate visible blood islands in both *Setd5* heterozygous (Het) and knockout (KO) embryos. Scale bar: 500 $\mu$ m. (B) H&E stained cross-section through the yolk sac blood vessel shows the presence of blood cells including mature erythrocytes. Scale bar: 50 $\mu$ m.

**Figure S8.**

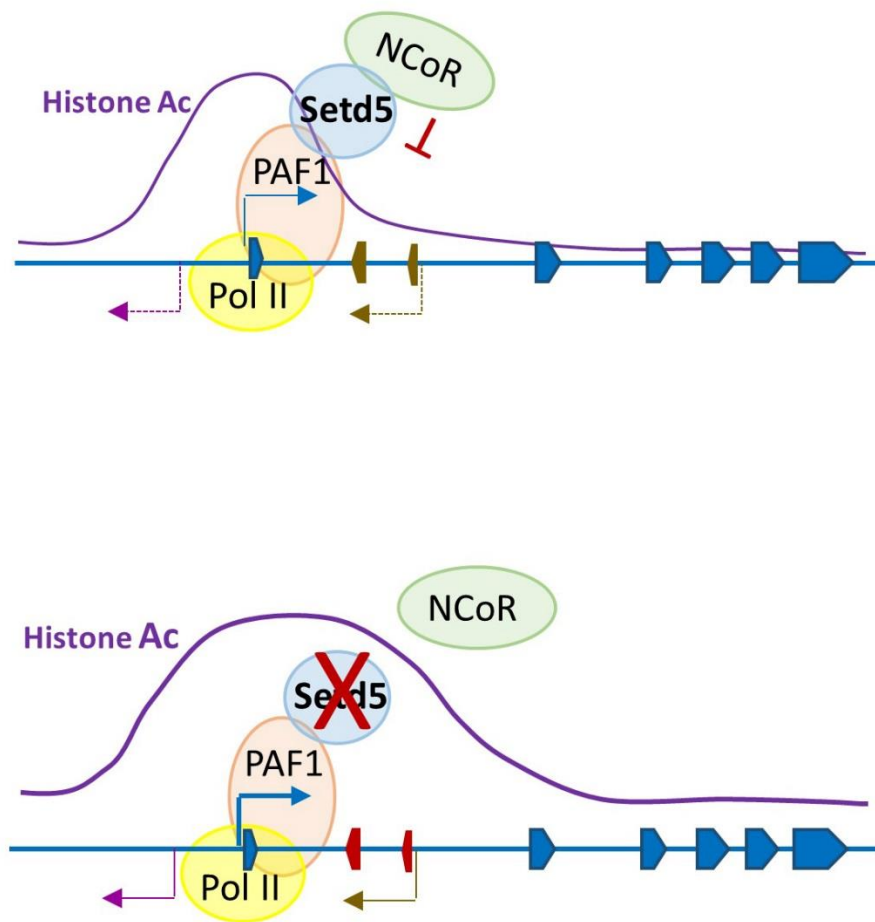


**Figure S8. Immunofluorescent staining for endogenous SETD5 protein.** (A) SETD5 protein is expressed at relatively similar levels in every cell in E9.5 wild type embryo.

Several images are stitched together to reveal sagittal view of the whole embryo. SETD5 expression in PECAM1 positive cells (**B**) and MHC positive cells (**C**) is not significantly different from surrounding tissues. (**D**) SETD5 is localized to nucleus but not nuclei of NIH3T3 cells and is partially co-localized with HDAC3. Scale bar: 50µm.

**Figure S9**

**Figure S9. Phenotype of *Setd5* ES cell lines.** (A) Microscope images of *Setd5*<sup>GFP</sup> heterozygous (Het) and knockout (KO) ES cell lines taken with phase contrast (top panel) and green fluorescence (GFP) filter. Both cell lines display typical ES cell line morphology. (B) Differentiation of *Setd5*<sup>GFP</sup> ES cells through EB formation. Appearance of EBs after 2 days (top), 4 days (middle) and EB outgrowths at 8 days of the differentiation protocol (bottom). Both, heterozygous and KO embryos formed EBs. Scale bar: 100µm.



**Figure S10. Model for *Setd5* involvement in co-transcriptional regulation of histone acetylation.** SETD5 protein may facilitate recruitment of histone deacetylase-containing NCoR complex to PAF1 elongation complex thereby restricting histone acetylation and chromatin accessibility around promoter/first exon regions. In the absence of SETD5, histone acetylation is increased around promoter and further into a gene region, possibly, due to failed recruitment of NCoR complex to PAF1. Changes in the chromatin landscape increase expression of antisense genes located in a locus.

**Table S1. PCR primers used in the study.**

| Primer                          | Sequence (5' to 3')                                | PCR product size             | Application  |
|---------------------------------|--|------------------------------|--|
| Rosa26.S1<br>Hygro5'            | AGACTTATCTACCTCATAGGTG<br>ACGAGACTAGTGAGACGTGCTACT | 649 bps                      | Rosa26 RMCE<br>screening   |
| Rosa26.S11                      | CGTGCTGAGCCAGACCTCCAT                              | Targeted: 588 bps            | Genotyping of<br><i>Setd5<sup>GFP</sup></i> ,<br><i>ROSA26<sup>EN.Cherry</sup></i> , and<br><i>ROSA26228.3TF</i><br>mice |
| Rosa26.S2                       | TCACAAGCAATAATAACCTGTAGT                           | Wild type: 523 bps           |  |
| Setd5D1<br>Setd5R1              | CTGGGTCAAGGATTCTCATG<br>GACGTCCAAGACAGCAGAAGG      | 126 bps<br>(exons 1-2)       | RT-qPCR of <i>Setd5</i>  |
| Setd5Ex2-3D<br>Setd5Ex2-3R      | GGCTGTCTTGACGTCATGAG<br>CTGCTGGACTAGCCTCCACAG      | 116 bps<br>(exons 2-3)       | RT-qPCR of <i>Setd5</i>  |
| Setd5Ex15-16D<br>Setd5 Ex15-16R | GATGGCACATTGAGCTCCTG<br>CTAGTCTCTTGGGGCACAGATG     | 92 bps<br>(exons 15-16)      | RT-qPCR of <i>Setd5</i>  |
| Setd5Ex22-23D<br>Setd5Ex22-23R  | GAGCCATTGAGCTCAGCACTC<br>GGACGAACCTCTGCTGAAGGAG    | 126 bps<br>(exons 22-23)     | RT-qPCR of <i>Setd5</i>  |
| SRpromF<br>SRpromR              | GAGACTCGAGTTAGGCCAAC<br>GCACAGCCTCTTCTTTAGGC       | 439 bps<br>(SR promoter)     | Cloning of <i>Setd5</i> -<br><i>Rosa26</i> promoter  |
| SRpromF<br>SRpromΔ181R          | GAGACTCGAGTTAGGCCAAC<br>GCCGGGGAGGGCGGC            | 222 bps<br>(SRΔ181 promoter) | Cloning of <i>Setd5</i> -<br><i>Rosa26</i> promoter  |
| R26mRNA-D<br>R26mRNA-R          | TCCTCAGAGAGCCTCGGCTAG<br>CATCATGCCTCTGCTTGCTTC     | 160 bps (exons 1-<br>2)      | RT-qPCR of<br><i>ROSA26</i>  |
| Eef1aPr1-F<br>Eef1aPr1-R        | GAATCAACCGGCATTGGATG<br>CTGGGAGAGGAACACAATGTTG     | 132bps (Area I)              | ChIP <i>Eef1a1</i>   |
| Eef1a1ex-F<br>Eef1a1ex-R        | GAACGGTATATAAGTGCGGCAG<br>GGGAATGCTCGCAGCTAATC     | 171bps (Area II)             | ChIP <i>Eef1a1</i>   |
| Eef1a1in-F<br>Eef1a1in-R        | GTCATGGTTGGGGAGGAATG<br>CTGGTTGCTTCGGGAAAAAC       | 103bps (Area III)            | ChIP <i>Eef1a1</i>   |
| Eef1a4ex-F<br>Eef1a4ex-R        | CCTGATTGTTGCTGCTGGTG<br>GACTGTATGGTGGCTCGGTG       | 152bps (Area IV)             | ChIP <i>Eef1a1</i>   |
| Eef1a8ex-F<br>Eef1a8ex-R        | GTCGCTTTGCTGTTCGTGAC<br>ACTGGGGTGGCAGGTGTTAG       | 154bps (Area V)              | ChIP <i>Eef1a1</i>   |
| Ip6kPr1-F<br>Ip6kPr1-R          | CAGGAACCTAACGGTGCTCTGAG<br>CACAGCCGTCTGTGCTACCTC   | 127bps (Area I)              | ChIP <i>Ip6k1</i>  |
| Ip6kPr2-F<br>Ip6kPr2-R          | CTCCACTGCTAGCAAACCTGAG<br>CTTCGCGTTGATTGCTCAAC     | 127bps (Area II)             | ChIP <i>Ip6k1</i>  |
| Ip6kEx1-F<br>Ip6kEx1-R          | GTTGACGAATCAACGCGAAG<br>CACTGGGTACGGATCAACAAC      | 164bps (Area III)            | ChIP <i>Ip6k1</i>  |
| Ip6kln1-F<br>Ip6kln1-R          | CTGCTTGCTCTGGCCCATAG<br>CTGACTGCTCTCCTGGAGGTC      | 156bps (Area IV)             | ChIP <i>Ip6k1</i>  |
| Ip6kEx2-F<br>Ip6kEx2-R          | GACTCTGGCCCAGAGTTCCCTC<br>GACGTGCTCTGCTCACACTTC    | 136 bps (Area V)             | ChIP <i>Ip6k1</i>  |
| Ip6kEx5-F<br>Ip6kEx5-R          | GACTCTGGCCCAGAGTTCCCTC<br>GACGTGCTCTGCTCACACTTC    | 155bps (Area VI)             | ChIP <i>Ip6k1</i>  |
| HaghPr1-F<br>HaghPr1-R          | CATTAACACCGGTGAGCCCTC<br>CTGCGTGGGGAGGAACCTATG     | 121bp (Area I)               | ChIP <i>Hagh1</i> ,<br>RT-qPCR <i>Fadh1</i>  |
| HaghPr2-F<br>HaghPr2-R          | CACTGGTTGGCTGTGAACTCTATG<br>CAGTGTCGTCTCCTACTGCTC  | 127bps (Area II)             | ChIP <i>Hagh1</i>  |
| HaghEx1-F<br>HaghEx1-R          | GAGCAGTAGGAGGACGACACTG<br>GACAGGCTCCGGAGACACAG     | 148bps (Area III)            | ChIP <i>Hagh1</i>  |
| Haghln1-F                       | CAGGGTTGTGACAGAGCACTTC                             | 127bps (Area IV)             | ChIP <i>Hagh1</i>  |

|           |                          |                    |                        |
|-----------|--------------------------|--------------------|------------------------|
| HaghIn1-R | CACCGAAGGATTGTCTTGGAG    |                    |                        |
| HaghEx4-F | CTGGTGGGAACGAGAAGCTG     | 110bps (Area V)    | ChIP <i>Hagh1</i>      |
| HaghEx4-R | CTGCAGTGTGGAGAGGTGTG     |                    |                        |
| HaghEx8-F | GACTGTGCAACAGCATGCTG     | 137bps (Area VI)   | ChIP <i>Hagh1</i>      |
| HaghEx8-R | CAGGCCAGTCGGTTAGAAGTC    |                    |                        |
| HaghRT-F  | GACGCTGGTGTTCGGACTC      | 140bps (exons 1-2) | ChIP <i>Hagh1</i>      |
| HaghRT-R  | GATAGTCCCAGCAGGGCCTG     |                    |                        |
| Ef1a1ex-F | GAACGGTATATAAGTGCGGCAG   | 171bps (exons 1-2) | RT-qPCR <i>Eef1a1</i>  |
| Ef1a1RT-R | GTGGTGGACTTGCCGGAATC     |                    |                        |
| GM38RT -R | ATGGGCTGGTGAGATGGCTC     | 189bps (exons 1-2) | RT-qPCR <i>GM38134</i> |
| Ip6kIn1-F | CTGCTTGCTCTGGCCCATAG     |                    |                        |
| Ip6kRT-F  | GATCCGTACCCAGTGGGCAG     | 212bps (exons 1-2) | RT-qPCR <i>Ip6k1</i>   |
| Ip6kRT-R  | GAGGCCAATGGTCACAGTCTG    |                    |                        |
| Mesp1-F   | TGTACGCAGAAACAGCATCC     | 144bps (exons 1-2) | RT-qPCR <i>Mesp1</i>   |
| Mesp1-R   | TTGTCCCCTCCACTCTTCAG     |                    |                        |
| Meox1-F   | GAGTTGAAGGTTAGGAAGTGGC   | 92bp (exon 3)      | RT-qPCR <i>Meox1</i>   |
| Meox1-R   | TTCCAAGGTCCACGTATCTC     |                    |                        |
| Actb-F    | ACGATGCTCCCCGGGCTGCATTC  | 115bp (exons2-3)   | RT-qPCR <i>Actb</i>    |
| Actb-R    | TCTCTTGCTCTGGGCCTCGTCACC |                    |                        |

**Table S2. Analysis of *Setd5*<sup>GFP</sup> matings at weaning and during embryogenesis.**

|          | Genotype |   |     |   |       |
|----------|----------|---|-----|---|-------|
|          | +/+      |   | +/- |   | -/-   |
| Weaning  | 27       |   | 42  |   | 0     |
|          | 39%      | : | 61% | : |       |
| E9.5     | 48       |   | 103 |   | 43(6) |
|          | 25%      | : | 53% | : | 22%   |
| E10.5    | 12       |   | 21  |   | 3(8)  |
|          | 33%      | : | 58% | : | 8%    |
| E11.5    | 8        |   | 14  |   | (10)  |
|          | 36%      | : | 63% | : |       |
| Expected | 25%      | : | 50% | : | 25%   |

The first row for each age shows the number of viable offspring of different genotypes derived from matings of animals heterozygous for *Setd5*. The number of dead/resorbed embryos are indicated in parentheses. The second row (shaded grey) shows the percentage of embryos of each genotype of the total live embryos analyzed for each age. Expected Mendelian ratios are shown in the bottom row.

**Table S3.**

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**Table S4.**

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