SUPPLEMENTAL DATA

SUPPLEMENTAL RESULTS

The heart of the mutant embryos was enlarged

Live $^{-/-}$*mz* (M^-Z^-) embryonic day 14.5 (E14.5) embryos appeared to have a larger heart than littermate $^{-/+}$ (M^-Z^+) embryos (SI Appendix, Figures S2a-S2b). Similarly, live (SI Appendix, Figure S2d) or dead (SI Appendix, Figure S2e) $^{-/-}$***z*** (M^+Z^-) neonatal pups had a larger heart than littermate $^{+/-}$ (M^+Z^+) ones (SI Appendix, Figure S2c). This may be similar to the enlarged heart caused by the dilated cardiomyopathy symptom (1).

SUPPLEMENTAL METHODS

Whole Heart Isolation

Whole hearts were isolated from the live embryos with a beating heart under a dissecting microscope. Dead neonatal pups were collected every day until postnatal day 2 (P2) after they were just born. At P2, all live littermates were collected as well. Whole hearts were isolated from all live and dead neonatal pups under a dissecting microscope.

Combined bisulphite restriction analysis (COBRA) analysis

Heart samples were isolated from live E14.5 embryos derived from the cross between a homozygous female mouse and a heterozygous male mouse. Genomic DNA was extracted from the whole heart samples and subjected to bisulfite mutagenesis with the EZ DNA methylation-Gold^{TM} Kit (Zymo Research #D5006). Bisulfite-treated DNA samples were then amplified by PCR with the primers covering a portion of the DMR region of the imprinted

domains. The PCR product was then subject to restriction enzyme digestion recognizing the CpG sites within the DMR region (2, 3).

BrdU Incorporation Assay

Pregnant female mice were injected with 30 μg of BrdU (Invitrogen) per gram of body weight one hour (h) before live embryos were collected. The isolated embryos were fixed in 4% (wt/vol) of paraformaldehyde (PFA) solution for 4 h at 4 ^{o}C, and embedded in OCT compound (Sakura). Transverse serial sections of 8 μm in thickness obtained from the OCT-embedded embryos were pre-treated with HCl and stained with anti-BrdU (1:50, Invitrogen) primary antibody overnight at 4 ^{o}C followed by Alexa Fluor 488-conjugated Goat Anti-Mouse IgG1 (1:500, Invitrogen) secondary antibody for 1 h at room temperature (RT). Fluorescent images were taken under Axioplan2IE microscope with Apotome (Zeiss). Immunofluorescent cells were counted on comparable sections by using ImageJ software. At least three separate embryos for each genotype were quantified with the ImageJ software. The percentage was calculated based on the ratio of the number of BrdU-positive cells relative to the total number of DAPI-positive cells in the heart sections.

Immunostaining on cryo-sections with antigen retrieval

For the N1ICD staining in SI Appendix, Figure S4d, the embryonic heart samples were fixed in 4% (wt/vol) of PFA solution at 4 ^{o}C overnight and embedded in OCT. Transverse serial sections of 8 μm in thickness were air-dried for 15 minutes at RT. The slides were incubated with 10 mM of sodium citrate (pH=6.0) and heated in a microwave oven twice for 5 minutes each time. After cooling to RT, endogenous biotin was blocked by incubation of the sections with the ABC reagent in the VECTASTAIN ABC Kit (Vector Laboratories #PK-4001) for 30 minutes. Then the endogenous peroxidase and the horseradish peroxidase of the ABC reagent were quenched with 1.2% (vol/vol) of hydrogen peroxide (H_2O_2) in 100% of methanol for 40

minutes. To minimize non-specific binding, the slides were incubated with a blocking solution containing 5% (vol/vol) of fetal bovine serum (FBS) and 3% of bovine serum albumin (BSA). Subsequently, they were incubated with rabbit monoclonal antibodies against N1ICD (1:100, Cell Signaling #4147) in the blocking solution at 4 °C overnight. Next day the slides were incubated with biotinylated goat anti-rabbit secondary antibody in 5% (wt/vol) of BSA for 1 h at RT followed by the incubation with the ABC reagent for 1 h at RT. Finally, they were subjected to the signal amplification with TSA Plus Cy3 System (Perkin Elmer) and DAPI staining before microscopy.

Real-time RT-PCR

Whole hearts were isolated from live embryos and snap frozen in TRIzol Reagent (Invitrogen). Total RNA was extracted from each heart sample. Approximately 1 μg of total RNA for each of three independent heart samples of each genotype was converted into cDNA with Transcriptor First Strand cDNA Synthesis Kit (Roche) according to the manufacturer's suggested protocols. Quantitative PCR (qPCR) reactions were carried out for these cDNA samples in triplicate with 2X Maxima SYBRGreen qPCR Master Mix (Thermo Scientific) and qPCR was run on a 7500 Real-Time PCR system (Applied Biosystems). The sequences for the primers used in the qPCR reactions for each gene are listed in the Supp. Table 1 below. Values represent mean ± s.e.m of three biological and three technical replicates. The expression level of an analyzed gene was normalized to the expression level of the house-keeping *Gapdh* gene in the same cDNA sample.

Supp. Table 1. Quantitative RT-PCR primers

Gene	Primer set (5' to 3')	Annealing temperature (°C)
c-Kit	CAAGAGTTCCGCCTTCTTTA GACCTTCAGTTCCGACATTAG	60
Nkx2.5	GACAGGTACCGCTGTTGCTT AGCCTACGGTGACCCTGAC	60
Gata4	GAATAAATCTAAGACGCCAGCAGGTCC GACAGCTTCAGAGCAGACAGCA	62
Bmp10	ACCAAGCTGAGGACACCGGAAGG CTTCGTGGGCACACAGCAGGCTTT	62
Hey2	GCGTCGGGATCGAATAAATAAC CAAGAGCATGGGCATCAAAG	62
N-Myc	TCCTAATCCGGTCATCCTT TTCCTCCTCTTCATCTTCCT	60
Gapdh	ACGACCCCTTCATTGACC AGACACCAGTAGACTCCACG	60

REFERENCES:

1. Seidman JG & Seidman C (2001) The genetic basis for cardiomyopathy: from mutation identification to mechanistic paradigms. *Cell* 104(4):557-567.
2. Li X*, et al.* (2008) A maternal-zygotic effect gene, *Zfp57*, maintains both maternal and paternal imprints. *Dev Cell* 15(4):547-557.
3. Zuo X*, et al.* (2012) Zinc finger protein ZFP57 requires its co-factor to recruit DNA methyltransferases and maintains DNA methylation imprint in embryonic stem cells via its transcriptional repression domain. *J Biol Chem* 287(3):2107-2118.

Supplemental Figure Legends

Figure S1. A diagram is shown for the crosses to generate zygotic and maternal-zygotic mutants. *Zfp57* zygotic mutant, $^{-/-}$***z*** (M^+Z^-), embryos are derived from the mating between *Zfp57* heterozygous (*Zfp57*$^{+/-}$) female mice and *Zfp57* homozygous (*Zfp57*$^{-/-}$) male mice (shown here) or from the mating between *Zfp57* heterozygous female and *Zfp57* heterozygous male mice (not shown here). Zygotic mutant, $^{-/-}$***z*** (M^+Z^-), embryos lack the zygotic *Zfp57* product (Z, indicated by red dots) that is expressed from the wild-type allele of *Zfp57* after fertilization. But they have inherited the maternal *Zfp57* product (M, indicated by green dots) from the oocyte that is produced from the wild-type allele of *Zfp57* in the female germline. The heterozygous, $^{+/-}$ (M^+Z^+), embryos derived from the heterozygous (*Zfp57*$^{+/-}$) female mice have both maternal and zygotic *Zfp57* products (M^+Z^+, indicated by both red and green bots). In contrast, *Zfp57* maternal-zygotic mutant, $^{-/-}$***mz*** (M^-Z^-), embryos are derived from the mating between *Zfp57* homozygous (*Zfp57*$^{-/-}$) female mice and *Zfp57* heterozygous (*Zfp57*$^{+/-}$) male mice (shown here) or from the mating between *Zfp57* homozygous female and *Zfp57* homozygous male mice (not shown here). Maternal-zygotic mutant, $^{-/-}$***mz*** (M^-Z^-), embryos lack both maternal and zygotic *Zfp57* products (M^-Z^-, no green or red dots). The heterozygous, *Zfp57*$^{-/+}$ (M^-Z^+), embryos derived from the homozygous (*Zfp57*$^{-/-}$) female mice lack the maternal *Zfp57* product but contain the zygotic *Zfp57* product that is expressed after fertilization from the wild-type allele of *Zfp57* (red dots only, no green dots).

Figure S2. Gross morphological change was seen in the heart of *Zfp57* mutants. a-b, representative images of the whole hearts isolated from live E14.5 embryos. a, three live *Zfp57* heterozygous, $^{-/+}$ (M^-Z^+), embryos derived from a homozygous female mouse. b, three littermate live *Zfp57* maternal-zygotic mutant, $^{-/-}$***mz*** (M^-Z^-), embryos derived from a homozygous female

mouse. c-e, representative images of the whole hearts isolated from neonatal pups. c, live *Zfp57* heterozygous, $^{+/-}$ (M^+Z^+), P2 pups from a heterozygous female mouse. d, live *Zfp57* zygotic mutant, $^{-/-}$*z* (M^+Z^-), P2 pups from a heterozygous female mouse. e, dead *Zfp57* zygotic mutant, $^{-/-}$*z* (M^+Z^-), P0 pups from a heterozygous female mouse. f and g, hematoxylin and eosin (H&E) stained transverse sections through the hearts of *Zfp57* zygotic mutant, $^{-/-}$*z* (M^+Z^-), and littermate heterozygous, $^{+/-}$ (M^+Z^+), E18.5 embryos derived from a heterozygous female mouse. ra, right atrium; la, left atrium; as, atrial septum (arrow); ASD, atrial septal defect (*). h, percentage (%) of live $^{+/-}$ (M^+Z^+) or $^{-/-}$*z* (M^+Z^-) E18.5 embryos with ASD. The number above each bar indicates the number of embryos analyzed for each genotype.

Figure S3. *Zfp57* was expressed in the embryonic heart of wild-type embryos based on RNA in situ hybridization. RNA in situ hybridization (purple blue) was performed on the cross sections of the E10.5 and E13.5 embryos with digoxigenin (DIG)-labeled riboprobes for *Zfp57* transcribed from a plasmid containing the full-length *Zfp57* cDNA of 1.35 kb. ra, right atrium. rv, right ventricle. la, left atrium. lv, left ventricle. as, atrial septum. ct, cushion tissue. vs, ventricular septum. h, heart. lu, lung. nt, neural tube. cc, central canal. Arrows, *Zfp57*-expressing cells (dark purple blue dots) in the heart. Arrowheads, *Zfp57*-expressing cells at the epicardium. a, RNA in situ hybridization of wild-type E10.5 embryos with the antisense probe (left panel) or sense probe (right panel) of *Zfp57.* b, RNA in situ hybridization of wild-type E13.5 embryos with the antisense probe (left panel) or sense probe (right panel) of *Zfp57.* c, four magnified areas of the heart of wild-type E10.5 embryos hybridized with antisense probe of *Zfp57* shown in Fig. S2a. d, four magnified areas of the heart of wild-type E13.5 embryos hybridized with the antisense probe of *Zfp57* shown in Fig. S2b. e, RNA in situ hybridization of *Zfp57* maternal-zygotic mutant, $^{-/-}$*mz* (M^-Z^-), E13.5 embryos hybridized with the antisense probe of *Zfp57.* The whole cross

section of a $^{-/-}$*mz* (M^-Z^-) E13.5 embryo is shown on the left. Four magnified areas of the heart of a $^{-/-}$*mz* (M^-Z^-) E13.5 embryo are shown on the right.

Figure S4. Loss of ZFP57 resulted in loss of DNA methylation imprint, DLK1 expression and NOTCH1 activation in the heart of *Zfp57* mutant embryos.

a, COBRA analysis of DNA methylation imprint in the heart. Genomic DNA was isolated from the hearts of live E13.5 embryos and subjected to COBRA analysis on the *H19* DMR, *Peg1* DMR, *Peg3* DMR, *Peg5* DMR and the IG-DMR of the *Dlk1-Dio3* imprinted region. 1 and 2, two maternal-zygotic mutant, $^{-/-}$*mz* (M^-Z^-), embryos derived from homozygous female mice. 3 and 4, two heterozygous, $^{-/+}$ (M^-Z^+), embryos derived from homozygous female mice. u, unmethylated product after restriction digestion. m, methylated product after restriction digestion.

b, DLK1 expression in the embryonic heart. Immunostaining (green) was performed with antibodies against DLK1 on the heart cryo-sections of the E13.5 embryos derived from pregnant *Zfp57* homozygous female mice. $^{-/+}$ (M^-Z^+), heterozygous embryos derived from homozygous female mice. $^{-/-}$*mz* (M^-Z^-), homozygous embryos derived from homozygous female mice. ra, right atrium. rv, right ventricle. la, left atrium. lv, left ventricle. vs, ventricular septum. as, atrial septum. Blue signal, DAPI staining. The small box in white dotted lines is shown as a magnified inset image (the large box in white dotted lines) inside the original image of lower magnification in the same panel.

c, NOTCH1 expression in the embryonic heart. Immunostaining was performed with monoclonal antibodies against NOTCH1 (red) on the heart cryo-sections of the E13.5 embryos. $^{+/-}$ (M^+Z^+), heterozygous embryos derived from heterozygous female mice. $^{-/-}$*mz* (M^-Z^-), homozygous embryos derived from homozygous female mice. rv, right ventricle. lv, left ventricle. vs,

ventricular septum. Blue signal, DAPI staining. The small box in white dotted lines in vs is shown as a separate magnified image on the right next to the image with lower magnification.

d, NOTCH1 activation in the embryonic heart. Immunostaining was performed with monoclonal antibodies against N1ICD (red) on the heart cryo-sections of the E13.5 embryos. $^{+/-}$ (M^+Z^+), heterozygous embryos derived from heterozygous female mice. $^{-/-}$***z*** (M^+Z^-), homozygous embryos derived from heterozygous female mice. $^{-/+}$ (M^-Z^+), heterozygous embryos derived from homozygous female mice. $^{-/-}$***mz*** (M^-Z^-), homozygous embryos derived from homozygous female mice. rv, right ventricle. lv, left ventricle. vs, ventricular septum. Blue signal, DAPI staining. Red-stained nuclei of the N1ICD-positive ($N1ICD^+$) cells inside the boxed areas in white dotted lines are shown on the right with a higher magnification (40X), next to the heart image with lower magnification. Arrows, clustered blood cells in the heart. Arrowheads, N1ICD-positive nuclei.

e, co-immunostaining of DLK1 and NOTCH1 in the embryonic heart. Co-immunostaining was performed for NOTCH1 (red) and DLK1 (green) on the heart cryo-sections of E13.5 embryos. $^{-/+}$ (M^-Z^+), heterozygous embryos derived from homozygous female mice. $^{-/-}$***mz*** (M^-Z^-), homozygous embryos derived from homozygous female mice. rv, right ventricle. Blue signal, DAPI-stained nuclei. Arrow, NOTCH1 staining (red) on the cell surface. Arrowhead, DLK1 staining (green) on the cell surface. a and c, images with lower magnification. b and d, images with higher magnification (63X object lens) for the boxed area with white dotted lines in Panel a and Panel c, respectively.

Figure S5. Expression of *Nkx2.5* and *Hey2* was slightly reduced in the heart of E10.5 embryos without ZFP57.

a, RNA in situ hybridization (purple blue) was performed on the heart cryo-sections of E10.5 embryos. $^{-/+}$ (M^-Z^+), heterozygous embryos derived from homozygous female mice. $^{-/-}$***mz*** (M^-Z^-),

homozygous embryos derived from homozygous female mice. The probes used for RNA in situ are digoxigenin (DIG)-labeled riboprobes for *Hey1*, *Hey2*, *Nkx2.5* and *Bmp10*. ra, right atrium. la, left atrium. rv, right ventricle. lv, left ventricle.

b, RNA in situ hybridization (purple blue) on the heart cryo-sections of E10.5 embryos. $^{+/-}$ (M^+Z^+), heterozygous embryos derived from heterozygous female mice. $^{-/-}$***z*** (M^+Z^-), homozygous embryos derived from heterozygous female mice. $^{-/-}$***mz*** (M^-Z^-), homozygous embryos derived from homozygous female mice. The whole heart images are shown on the top panels. A portion of lv and a portion of rv are shown at high magnification in the middle and bottom panels, respectively. The probes used for RNA in situ are digoxigenin (DIG)-labeled riboprobes for *Nkx2.5* and *Hey2*. ra, right atrium. la, left atrium. rv, right ventricle. lv, left ventricle.

c, quantitative RT-PCR (qRT-PCR) analysis of the NOTCH1 target genes in the heart of E10.5 embryos. Black filled bar, *Zfp57* $^{-/+}$ (M^-Z^+) embryos. Grey bar, $^{-/-}$***mz*** (M^-Z^-) embryos. Three independent embryos were analyzed for each group. Values are mean ± s.e.m. Student's t-test: *, $P<0.05$; n.s., statistically not significant.

Figure S6. Expression of some NOTCH1 target genes was down-regulated in the heart of E13.5 embryos without ZFP57.

a, RNA in situ hybridization (purple blue) was performed on the heart cryo-sections of E13.5 embryos. $^{-/+}$ (M^-Z^+), heterozygous embryos derived from homozygous female mice. $^{-/-}$***mz*** (M^-Z^-), homozygous embryos derived from homozygous female mice. The probes used for RNA in situ are digoxigenin (DIG)-labeled riboprobes for *Hey1*, *Hey2*, *Nkx2.5* and *Bmp10*. ra, right atrium. la, left atrium. rv, right ventricle. lv, left ventricle. vs, ventricular septum.

b, RNA in situ hybridization (purple blue) on the heart cryo-sections of E13.5 embryos. $^{-/+}$ (M^-Z^+), heterozygous embryos derived from homozygous female mice. $^{-/-}$*mz* (M^-Z^-), homozygous embryos derived from homozygous female mice. A portion of rv or a portion of lv of the corresponding images in Panel a is shown here at high magnification. The probes used for RNA in situ are digoxigenin (DIG)-labeled riboprobes for *Hey1*, *Hey2*, *Nkx2.5* and *Bmp10*. ra, right atrium. vm, ventricular myocardium. rv, right ventricle. lv, left ventricle. trab, trabeculae.

c, quantitative RT-PCR (qRT-PCR) analysis of the NOTCH1 target genes in the heart of E13.5 embryos. Black filled bar, *Zfp57* $^{-/+}$ (M^-Z^+) embryos. Grey bar, $^{-/-}$*mz* (M^-Z^-) embryos. Three independent embryos were analyzed for each group. Values are mean ± s.e.m. Student's t-test: **, P<0.01; n.s., statistically not significant.

Figure S7. Loss of ZFP57 caused reduced proliferation but no increase in apoptosis in the embryonic heart. Immunostaining was performed on the heart cryo-sections of E13.5 embryos. $^{-/+}$ (M^-Z^+), heterozygous embryos derived from homozygous female mice. $^{-/-}$*mz* (M^-Z^-), homozygous embryos derived from homozygous female mice.

a, BrdU-labeling was performed on the pregnant female mice. The heart cryo-sections of E13.5 embryos derived from BrdU-labeled pregnant female mice were stained with antibodies against BrdU (green). Blue signal, DAPI staining. ra, right atrium. vs, ventricular septum. rv, right ventricle. lv, left ventricle.

b, cleaved Caspase-3 immunostaining (red) on paraffin-embedded sections. Boxes in white dotted lines, the areas of vs and rv that are shown as the magnified images underneath the heart images of lower magnification. lv, left ventricle. vs, ventricular septum. rv, right ventricle. ec, endocardial cushion.

c, percentage (%) of BrdU-labeled cells in the heart relative to DAPI-positive cells. Black filled bars, $^{-/+}$ (M^-Z^+) embryos. Grey filled bars, $^{-/-}$*mz* (M^-Z^-) embryos. BrdU-labeled cells and DAPI-positive cells were quantified with the ImageJ software to measure the entire heart section of each embryo. At least three separate embryos were used for each genotype. Values are mean ± s.e.m. Student's t-test: **, P<0.01.

d, percentage of Caspase-3-positive (Caspase-3^+) cells in the heart relative to DAPI-positive cells. ImageJ software was used to quantify the numbers of immuno-stained cells or DAPI-stained cells. Black filled bar, *Zfp57* $^{-/+}$ (M^-Z^+) embryos. Grey filled bar, $^{-/-}$*mz* (M^-Z^-) embryos. At least three independent embryos were analyzed for each group. Values are mean ± s.e.m.. Student's t-test: n.s., statistically not significant.

Figure S8. Quantitative RT-PCR analysis of potential target genes of ZFP57 in the heart of E10.5 or E13.5 embryos. Total RNA samples were isolated from the hearts of live E10.5 or E13.5 embryos. $^{+/-}$ (M^+Z^+), heterozygous embryos derived from heterozygous female mice. $^{-/-}$*z* (M^+Z^-), homozygous embryos derived from heterozygous female mice. $^{-/+}$ (M^-Z^+), heterozygous embryos derived from homozygous female mice. $^{-/-}$*mz* (M^-Z^-), homozygous embryos derived from homozygous female mice. Three independent heart RNA samples for each genotype were subjected to quantitative RT-PCR (qRT-PCR) analysis. The qRT-PCR data from three biological and three technical replicates were analyzed for each experimental group. Values are mean ± s.e.m. Student's t-test: *, P<0.05; **, P<0.01; n.s., statistically not significant. a-b, qRT-PCR analysis of *N-Myc* in the heart of E13.5 embryos.

c, qRT-PCR analysis of *c-Kit, Nkx2.5* and *Gata4* in the heart of E10.5 embryos.

d, qRT-PCR analysis of *c-Kit, Nkx2.5* and *Gata4* in the heart of E13.5 embryos.

e, qRT-PCR analysis of *c-Kit* in the heart of E13.5 embryos.

Figure S9. Loss of ZFP57 causes increased expression of c-KIT and decreased expression of both NKX2.5 and GATA4 in the embryonic heart. Immunostaining (red) was performed on the heart cryo-sections of E10.5 or E13.5 embryos. $^{+/-}$ (M^+Z^+), heterozygous embryos derived from heterozygous female mice. $^{-/-}$***z*** (M^+Z^-), homozygous zygotic mutant embryos derived from heterozygous female mice. $^{-/+}$ (M^-Z^+), heterozygous embryos derived from homozygous female mice. $^{-/-}$***mz*** (M^-Z^-), homozygous maternal-zygotic mutant embryos derived from homozygous female mice. ra, right atrium. rv, right ventricle. la, left atrium. lv, left ventricle. vs, ventricular septum. Blue signal, DAPI staining. The small box in white dotted lines in Panels b-d is shown as a magnified inset image (the large box in white dotted lines) inside the original image of lower magnification in the same panel. ImageJ software was used to quantify the number of immunostained cells or measure the intensity of immunostaining on the heart sections. Values are mean ± s.e.m. Student's t-test: *, $P<0.05$; **, $P<0.01$.

a, NKX2.5 immunostaining (red) of E10.5 embryos. Top panels, whole-heart images. Bottom panels, magnified images of the ventricles.

b, c-KIT immunostaining (red) of E13.5 embryos.

c, NKX2.5 immunostaining (red) of E13.5 embryos.

d, GATA4 immunostaining (red) of E13.5 embryos.

e, the magnified images of ra, rv and vs regions of c-KIT-immunostained (red) heart sections in Panel b.

f, percentage (%) of c-KIT$^+$, NKX2.5$^+$ or GATA4$^+$ cells on the heart sections relative to DAPI-positive cells. Black filled bars, $^{-/+}$ (M$^-$Z$^+$) E13.5 embryos. Light grey bars, $^{-/-}$*mz* (M$^-$Z$^-$) E13.5 embryos.

g, average immunostaining intensity of NKX2.5$^+$ cells on the heart sections. Black filled bars, $^{-/+}$ (M$^-$Z$^+$) E13.5 embryos. Light grey bars, $^{-/-}$*mz* (M$^-$Z$^-$) E13.5 embryos.

Figure S10. Cell proliferation was reduced in the NKX2.5-positive (NKX2.5$^+$) population of cardiac cells without ZFP57. EdU-labeling was performed on the pregnant *Zfp57* homozygous female mice mated with *Zfp57* heterozygous male mice. VS, ventricular septum. Co-immunostaining was carried out on the heart cryo-sections of E13.5 embryos for NKX2.5 (red) and EdU (green). $^{-/+}$ (M$^-$Z$^+$), heterozygous embryos derived from homozygous female mice. $^{-/-}$*mz* (M$^-$Z$^-$), homozygous embryos derived from homozygous female mice. ImageJ software was used to quantify EdU-positive (EdU$^+$) or NKX2.5-positive (NKX2.5$^+$) cells. Percentage (%) of EdU$^+$ cells in the NKX2.5$^+$ population of cardiac cells, i.e. # of NKX2.5$^+$EdU$^+$ cells/ # of NKX2.5$^+$ cells, was quantified for the entire heart section (b) or a part of the heart section (c). Three $^{-/+}$ (M$^-$Z$^+$) and three $^{-/-}$*mz* (M$^-$Z$^-$) E13.5 embryos were used for these analyses. Unfilled bars, $^{-/+}$ (M$^-$Z$^+$) embryos. Black filled bars, $^{-/-}$*mz* (M$^-$Z$^-$) embryos. Values are mean ± s.e.m.. Student's t-test: *, $P<0.05$; n.s., statistically not significant.

a, the images of the heart cryo-sections stained with antibodies against EdU (green) and NKX2.5 (red). Blue signal, DAPI staining. Arrows, NKX2.5$^+$EdU$^+$ cells with white nuclei.

b, % of EdU$^+$ cells in the NKX2.5$^+$ population of cardiac cells was quantified for the entire heart section.

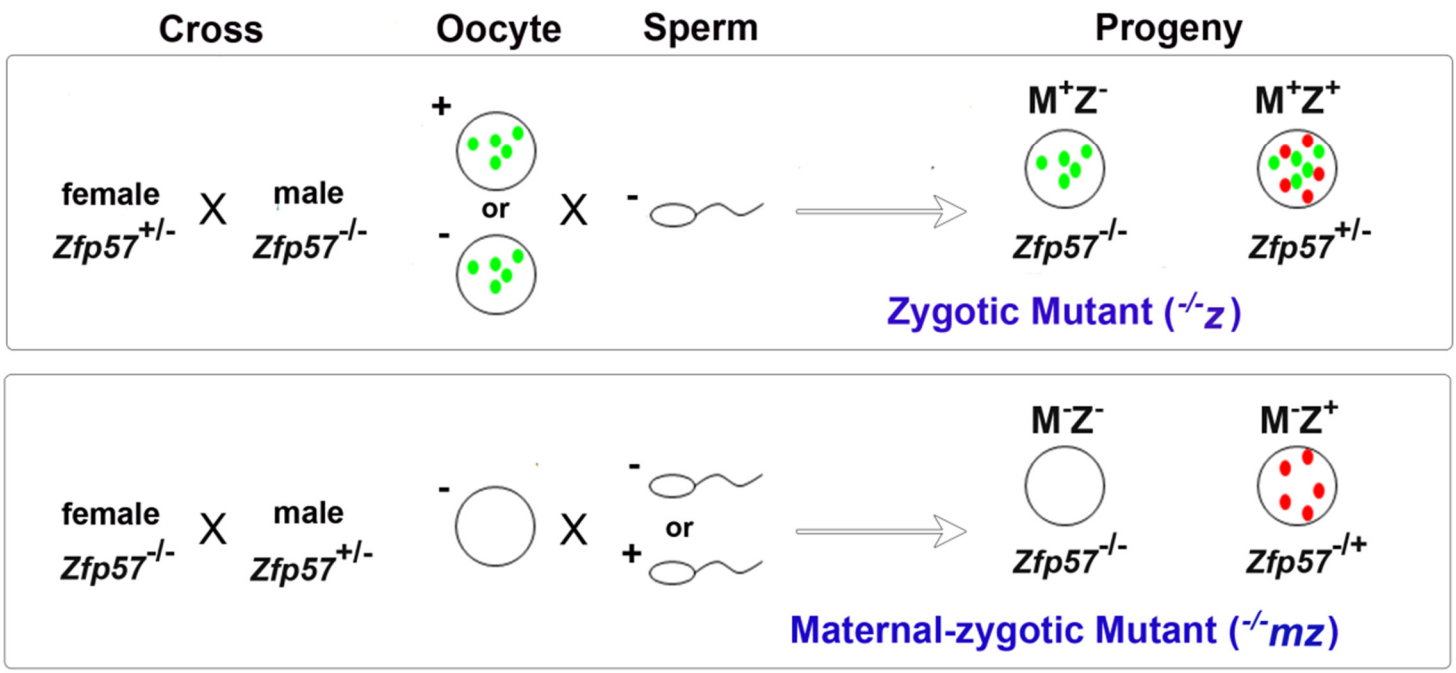
c, % of EdU^+ cells in the $NKX2.5^+$ population of cardiac cells was quantified separately in the atria, ventricle and VS.

Figure S11. Cell proliferation was increased in the c-KIT-positive (c-KIT^+) population of the cells in the heart without ZFP57. EdU-labeling was performed on the pregnant *Zfp57* homozygous female mice mated with *Zfp57* heterozygous male mice. VS, ventricular septum. Co-immunostaining was carried out on the heart cryo-sections of E13.5 embryos for c-KIT (red) and EdU (green). ra, right atrium. lv, left ventricle. vs, ventricular septum. A portion of ra, lv or vs is shown as a magnified image on the right, next to the image of lower magnification. $^{-/+}$ (M^-Z^+), heterozygous embryos derived from homozygous female mice. $^{-/-}$*mz* (M^-Z^-), homozygous embryos derived from homozygous female mice. ImageJ software was used to quantify EdU-positive (EdU^+) or c-KIT-positive (c-KIT^+) cells. Percentage (%) of EdU^+ cells in the c-KIT^+ population of cardiac cells, i.e. # of c-KIT^+EdU^+ cells/ # of c-KIT^+ cells, was quantified for the entire heart section (b) or a part of the heart section (c). Three $^{-/+}$ (M^-Z^+) and three $^{-/-}$*mz* (M^-Z^-) E13.5 embryos were used for these analyses. Unfilled bars, $^{-/+}$ (M^-Z^+) embryos. Black filled bars, $^{-/-}$*mz* (M^-Z^-) embryos. Values are mean ± s.e.m.. Student's t-test: **, P<0.01; n.s., statistically not significant.

a, the images of the heart cryo-sections stained with antibodies against EdU (green) and c-KIT (red). Blue signal, DAPI staining. Arrows, c-KIT^+EdU^+ cells with green-stained (EdU^+) and blue-stained ($DAPI^+$) nuclei inside red-colored cell surface (c-KIT^+).

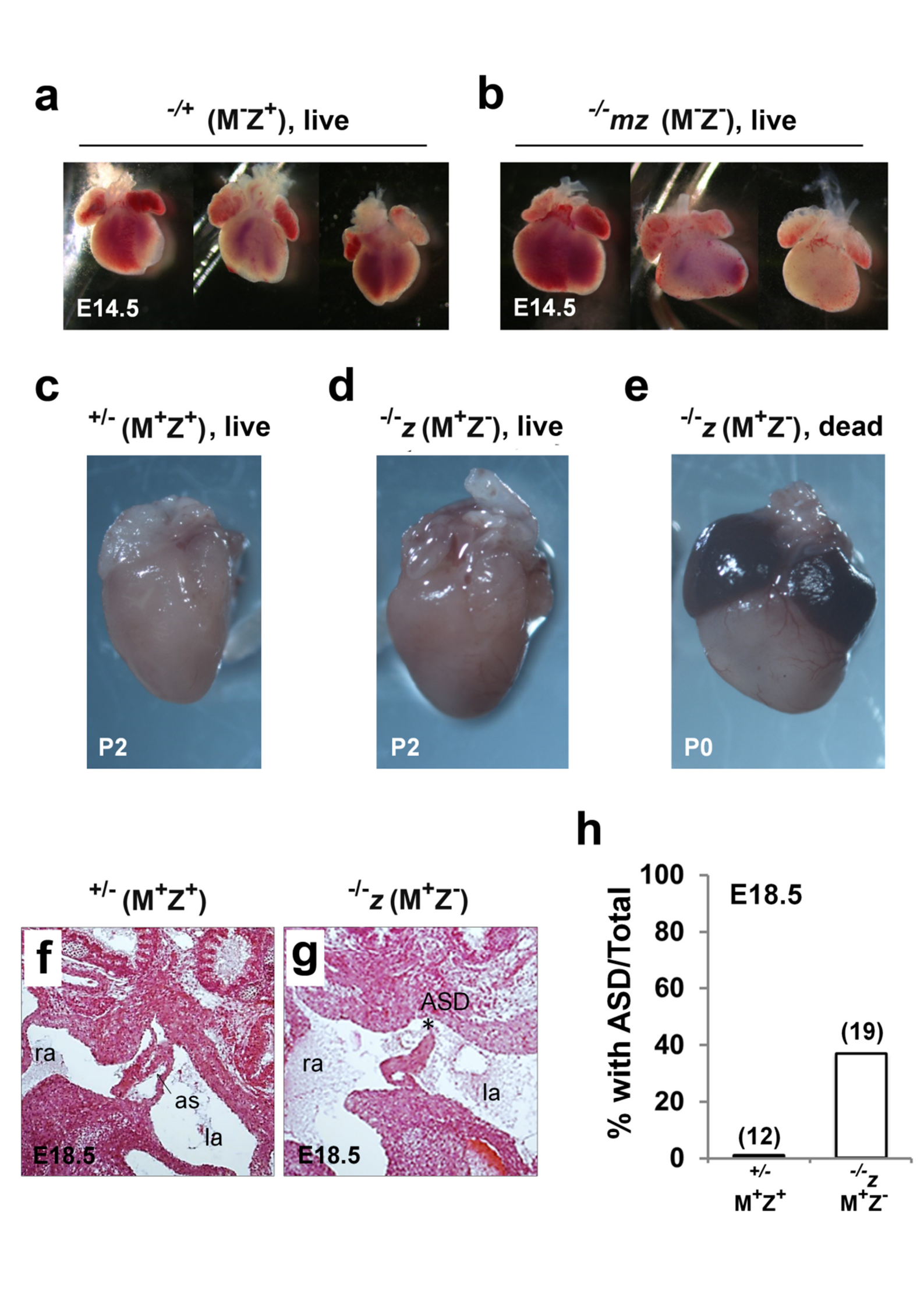
b, % of EdU^+ cells in the c-KIT^+ population of cardiac cells was quantified for the entire heart section.

c, % of EdU^+ cells in the c-KIT^+ population of cardiac cells was quantified separately in the atria, ventricle and VS.



- Maternal *Zfp*57 product (M)

- Zygotic *Zfp*57 product (Z)


a
-/+ (M-Z+), live
E14.5
b
-/-mz (M-Z-), live
E14.5
c
+/- (M+Z+), live
P2
d
-/-z (M+Z-), live
P2
e
-/-z (M+Z-), dead
P0
+/- (M+Z+)
-/-z (M+Z-)
f
ra
as
la
E18.5
g
ASD
*
ra
la
E18.5
h
% with ASD/Total
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(12)
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-/-z
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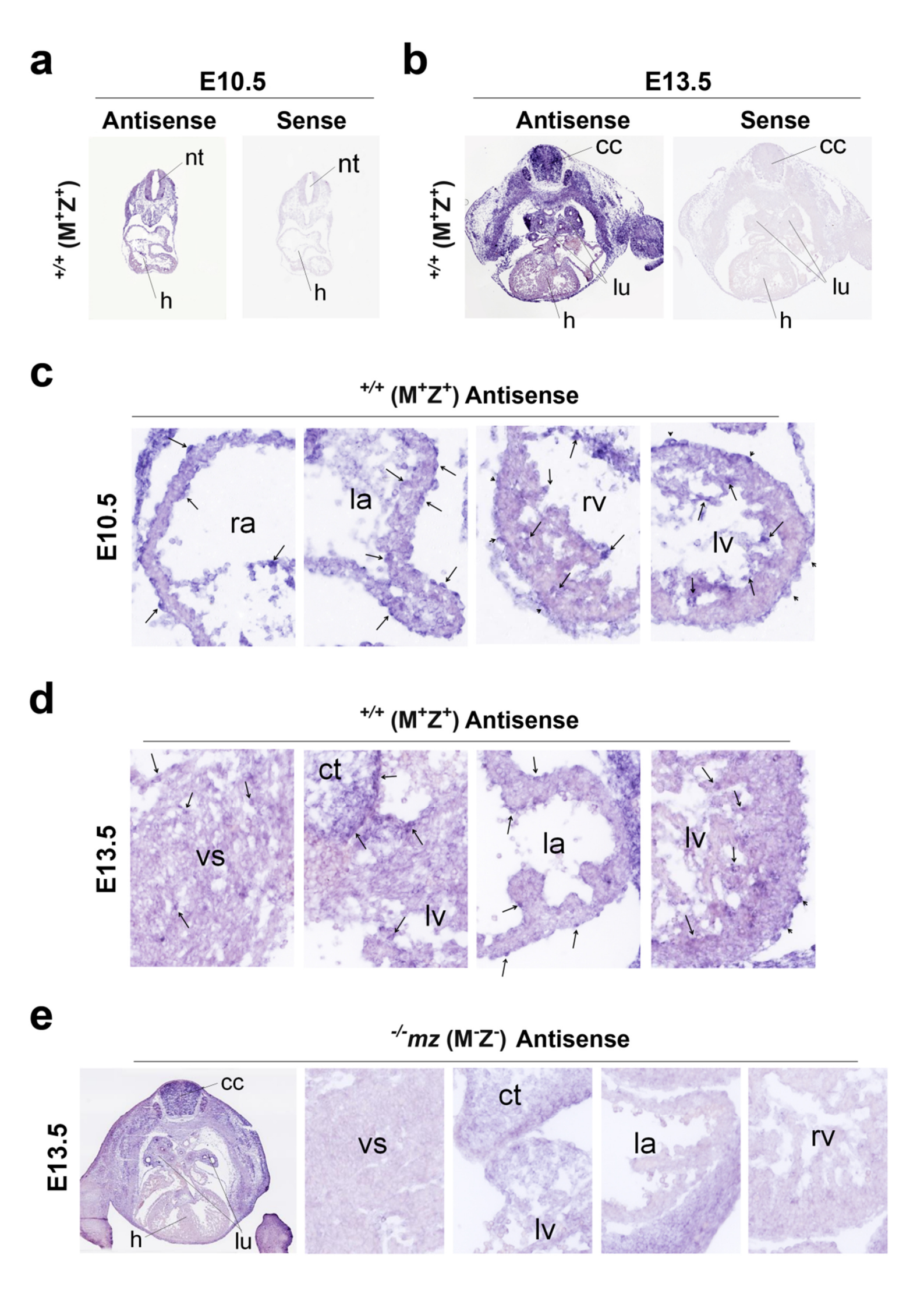
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E10.5
Antisense
Sense
+/+ (M+Z+)
nt
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b
E13.5
Antisense
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cc
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+/+ (M+Z+) Antisense
E10.5
ra
la
rv
lv
d
+/+ (M+Z+) Antisense
E13.5
vs
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la
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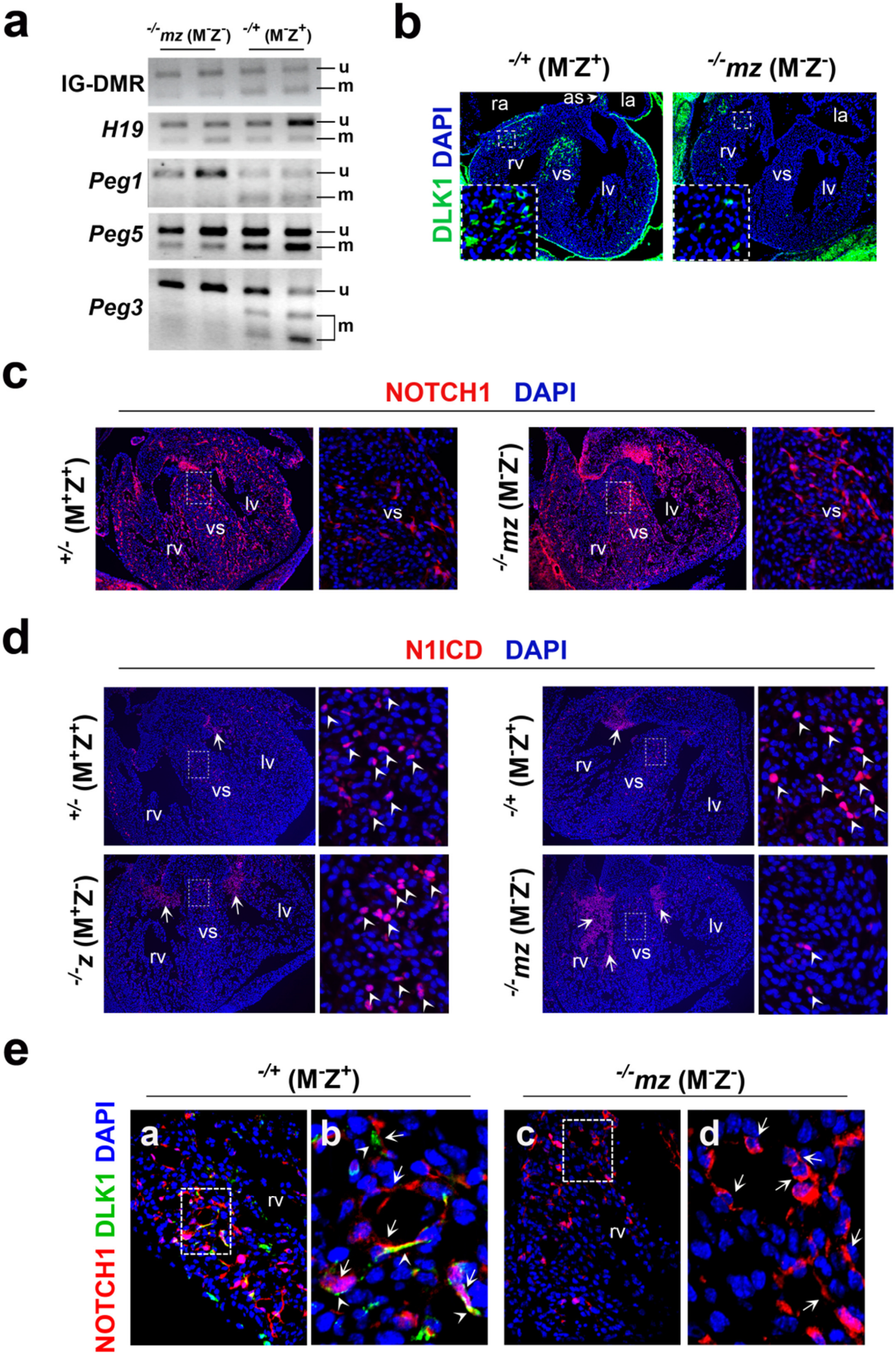
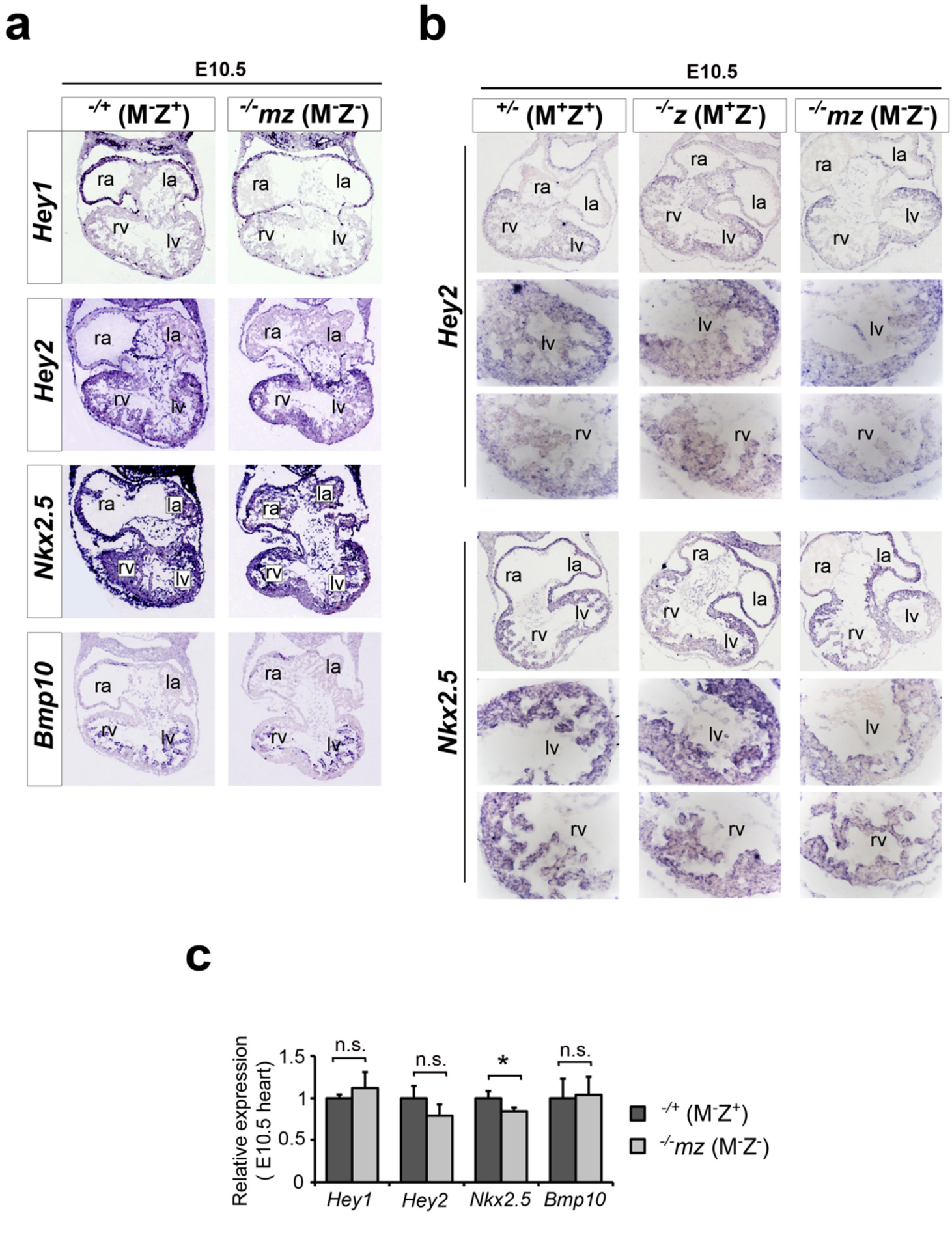
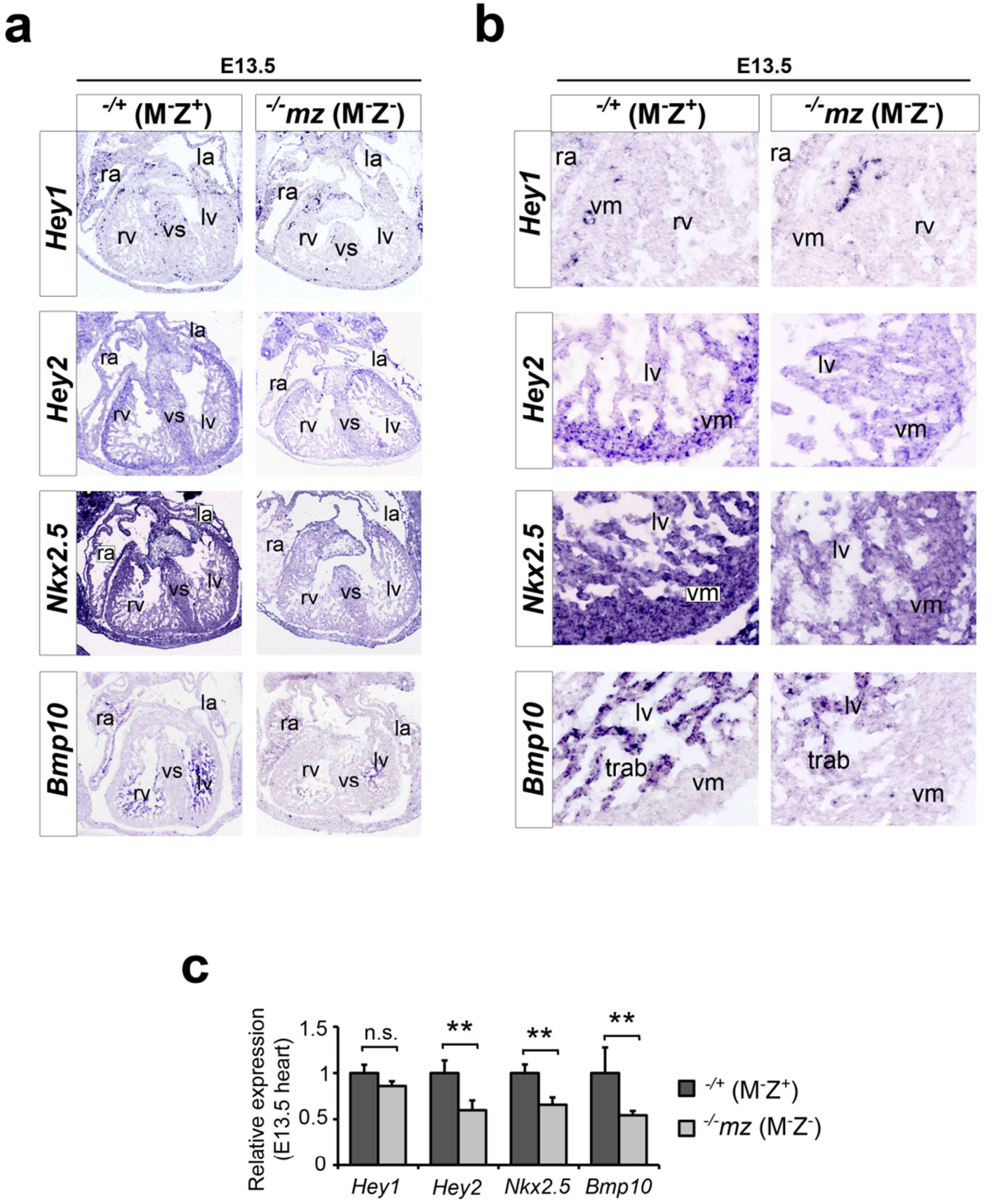
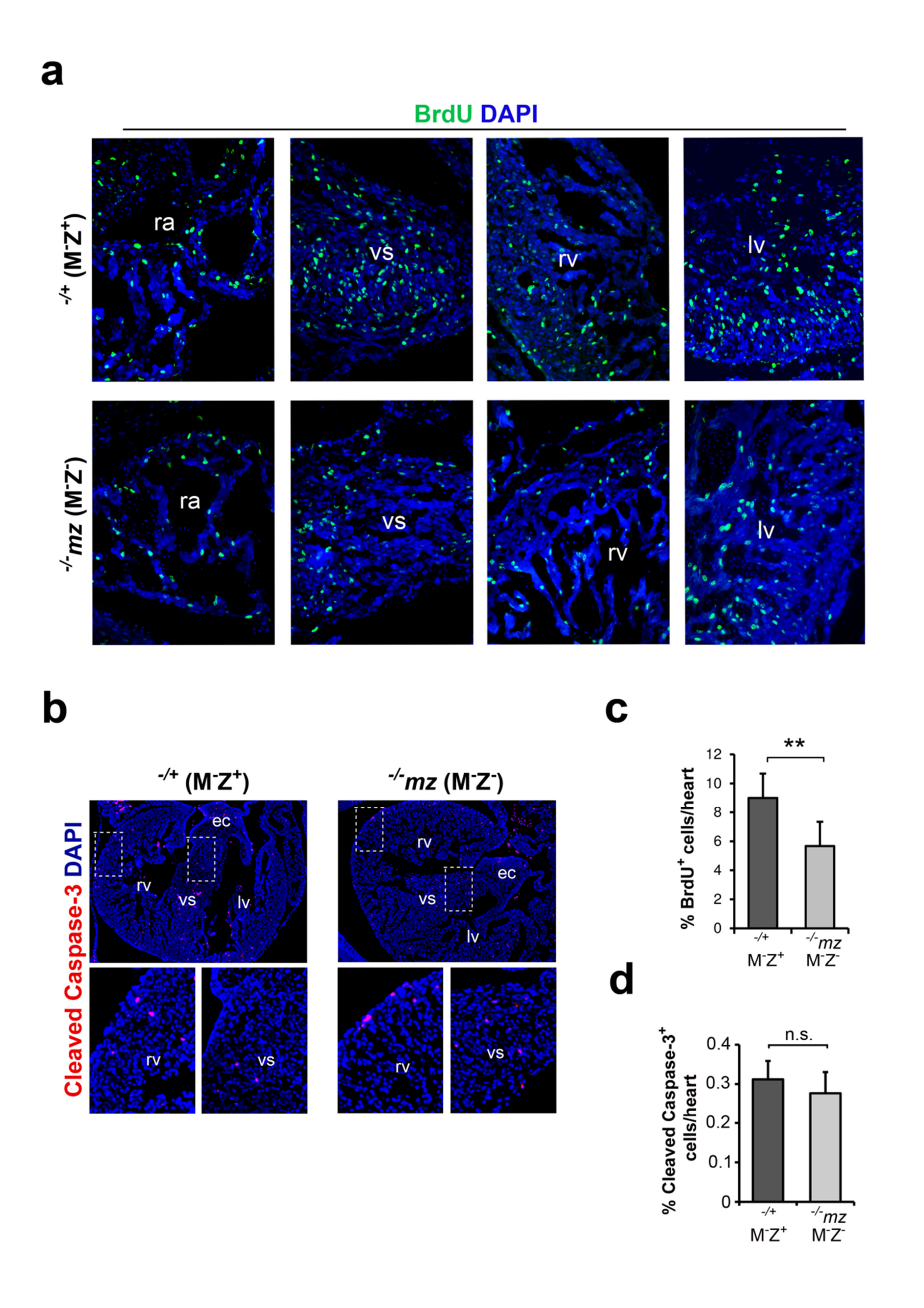
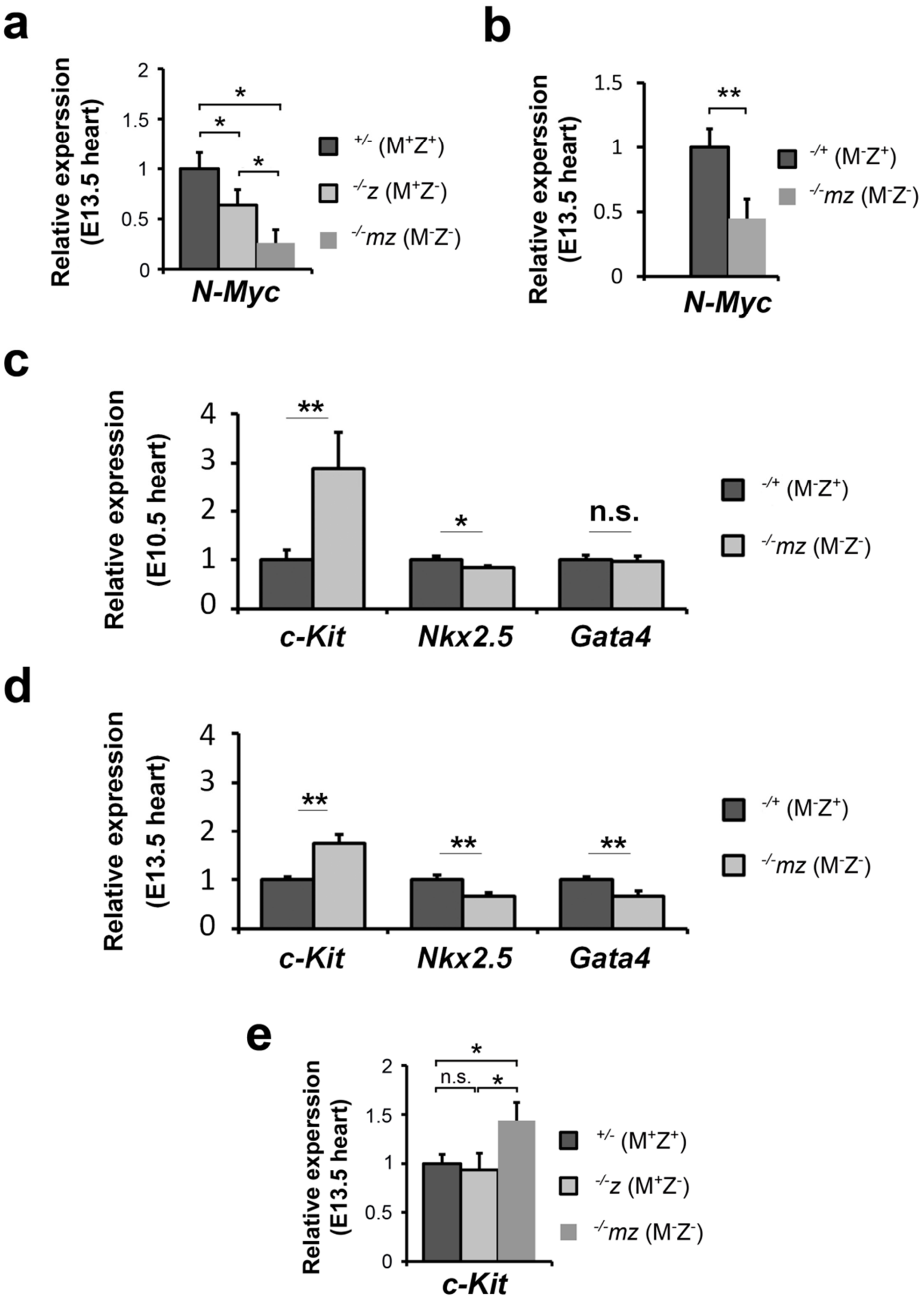
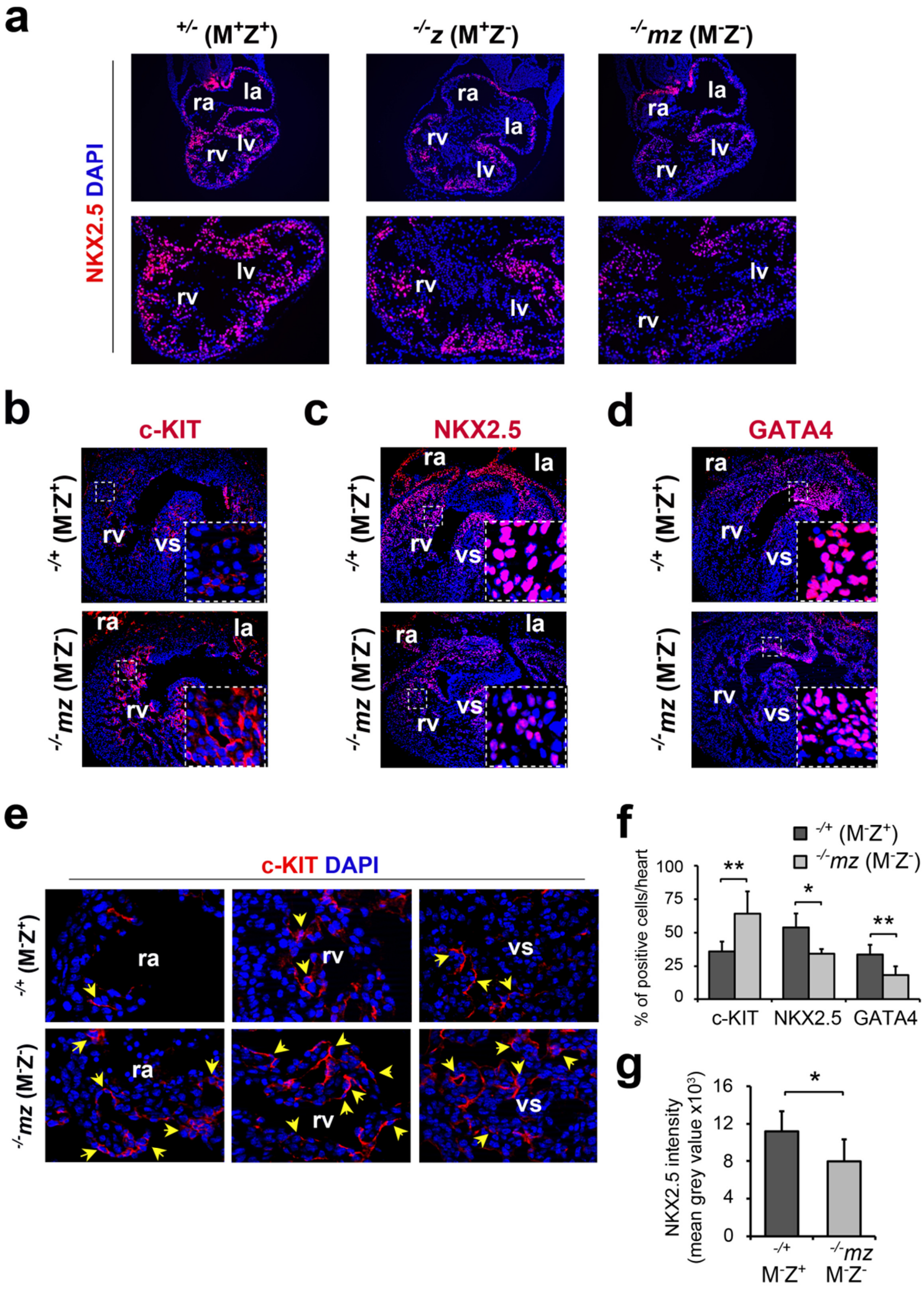

a
-/-mz (M-Z-)
-/+ (M-Z+)
IG-DMR
H19
Peg1
Peg5
Peg3
u
m
b
-/+ (M-Z+)
-/-mz (M-Z-)
DLK1 DAPI
ra
as
la
rv
vs
lv
c
NOTCH1 DAPI
+/- (M+Z+)
-/-mz (M-Z-)
d
N1ICD DAPI
+/- (M+Z+)
-/+ (M-Z+)
-/-z (M+Z-)
-/-mz (M-Z-)
e
-/+ (M-Z+)
-/-mz (M-Z-)
NOTCH1 DLK1 DAPI
a
b
c
d
rv

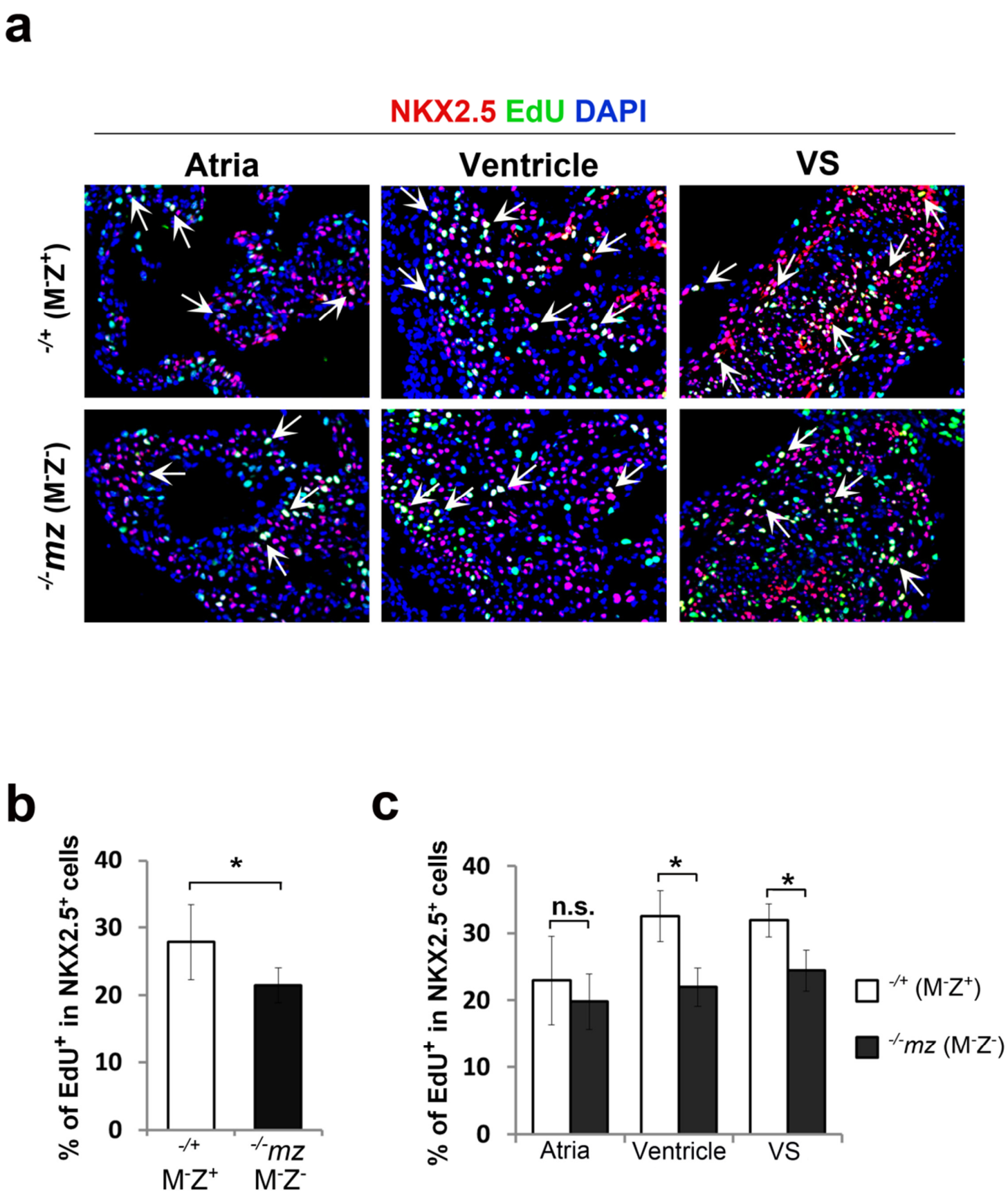

a
E10.5
-/+ (M-Z+)
-/-mz (M-Z-)
Hey1
Hey2
Nkx2.5
Bmp10
ra
la
rv
lv
b
E10.5
+/- (M+Z+)
-/-z (M+Z-)
-/-mz (M-Z-)
Hey2
Nkx2.5
c
Relative expression (E10.5 heart)
1.5
1
0.5
0
n.s.
n.s.
*
n.s.
Hey1
Hey2
Nkx2.5
Bmp10
-/+ (M-Z+)
-/-mz (M-Z-)

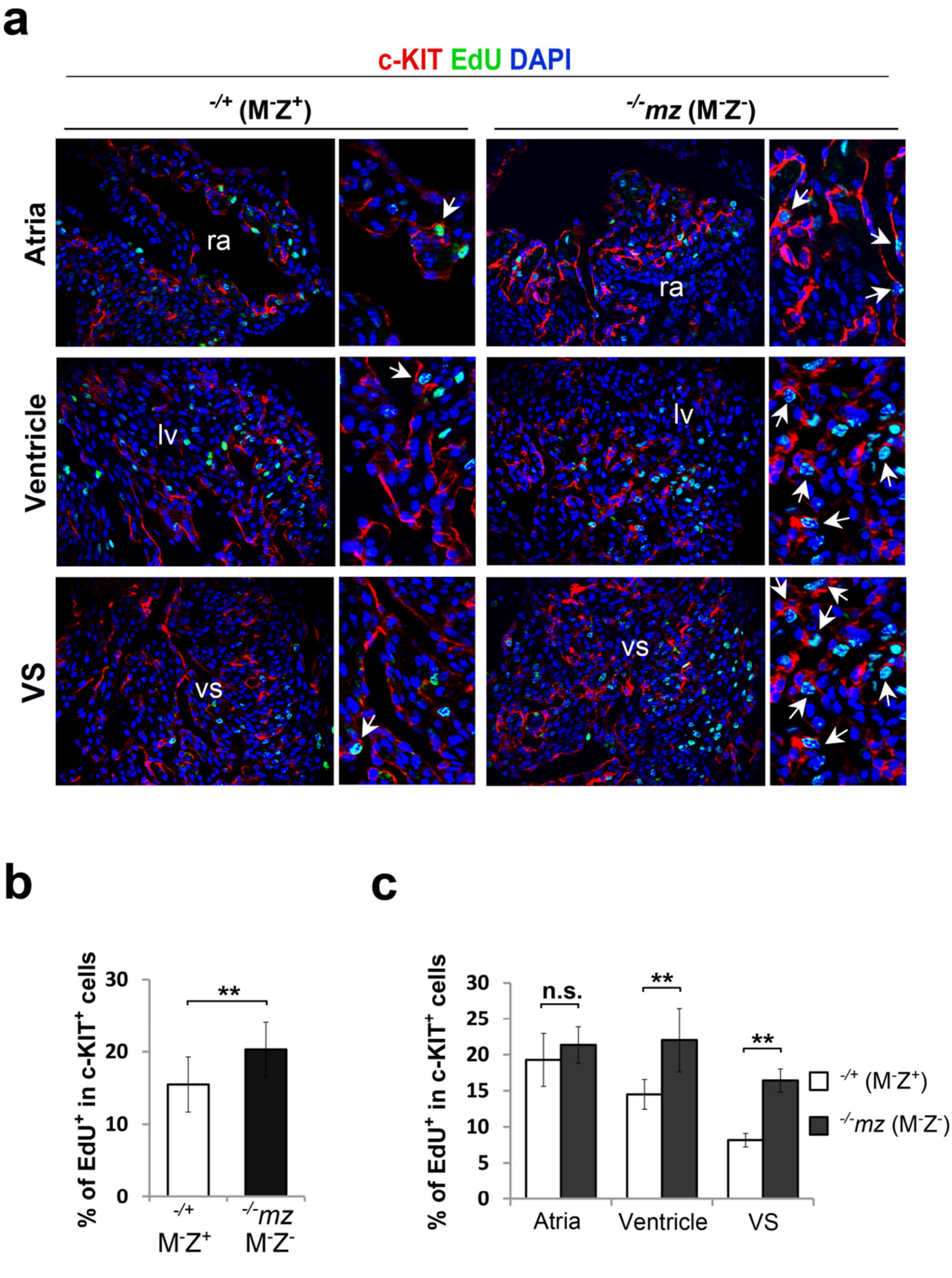

a
E13.5
-/+ (M-Z+)
-/-mz (M-Z-)
Hey1
Hey2
Nkx2.5
Bmp10
la
ra
lv
rv
vs
b
E13.5
-/+ (M-Z+)
-/-mz (M-Z-)
Hey1
Hey2
Nkx2.5
Bmp10
ra
vm
rv
lv
trab
c
Relative expression (E13.5 heart)
1.5
1
0.5
0
n.s.
**
**
**
Hey1
Hey2
Nkx2.5
Bmp10
-/+ (M-Z+)
-/-mz (M-Z-)


a
BrdU DAPI
-/+ (M-Z+)
-/-mz (M-Z-)
ra
vs
rv
lv
b
-/+ (M-Z+)
-/-mz (M-Z-)
Cleaved Caspase-3 DAPI
ec
rv
vs
lv
c
% BrdU+ cells/heart
**
12
10
8
6
4
2
0
-/+ M-Z+
-/-mz M-Z-
d
% Cleaved Caspase-3+ cells/heart
n.s.
0.4
0.3
0.2
0.1
0
-/+ M-Z+
-/-mz M-Z-


a
Relative experssion (E13.5 heart)
2
1.5
1
0.5
0
*
*
*
N-Myc
+/- (M+Z+)
-/-z (M+Z-)
-/-mz (M-Z-)
b
Relative experssion (E13.5 heart)
1.5
1
0.5
0
**
N-Myc
-/+ (M-Z+)
-/-mz (M-Z-)
c
Relative expression (E10.5 heart)
4
3
2
1
0
**
*
n.s.
c-Kit
Nkx2.5
Gata4
-/+ (M-Z+)
-/-mz (M-Z-)
d
Relative expression (E13.5 heart)
4
3
2
1
0
**
**
**
c-Kit
Nkx2.5
Gata4
-/+ (M-Z+)
-/-mz (M-Z-)
e
Relative experssion (E13.5 heart)
2
1.5
1
0.5
0
*
n.s.
*
c-Kit
+/- (M+Z+)
-/-z (M+Z-)
-/-mz (M-Z-)


a
+/- (M+Z+)
-/-z (M+Z-)
-/-mz (M-Z-)
NKX2.5 DAPI
ra
la
rv
lv
b
c-KIT
c
NKX2.5
d
GATA4
-/+ (M-Z+)
-/-mz (M-Z-)
vs
e
c-KIT DAPI
f
-/+ (M-Z+)
-/-mz (M-Z-)
% of positive cells/heart
100
75
50
25
0
**
*
**
c-KIT
NKX2.5
GATA4
g
NKX2.5 intensity
(mean grey value x10³)
16
12
8
4
0
*
-/+
M-Z+
-/-mz
M-Z-


a
NKX2.5 EdU DAPI
Atria
Ventricle
VS
-/+ (M-Z+)
-/-mz (M-Z-)
b
% of EdU+ in NKX2.5+ cells
40
30
20
10
0
*
-/+
M-Z+
-/-mz
M-Z-
c
% of EdU+ in NKX2.5+ cells
40
30
20
10
0
n.s.
*
*
Atria
Ventricle
VS
-/+ (M-Z+)
-/-mz (M-Z-)


a
c-KIT EdU DAPI
-/+ (M-Z+)
-/-mz (M-Z-)
Atria
Ventricle
VS
ra
lv
vs
b
% of EdU+ in c-KIT+ cells
30
20
10
0
**
-/+
M-Z+
-/-mz
M-Z-
c
% of EdU+ in c-KIT+ cells
30
25
20
15
10
5
0
n.s.
**
**
Atria
Ventricle
VS
-/+ (M-Z+)
-/-mz (M-Z-)