SUPPLEMENTAL MATERIAL

Histone demethylases *kdm6ba* and *kdm6bb* redundantly promote cardiomyocyte proliferation during zebrafish heart ventricle maturation

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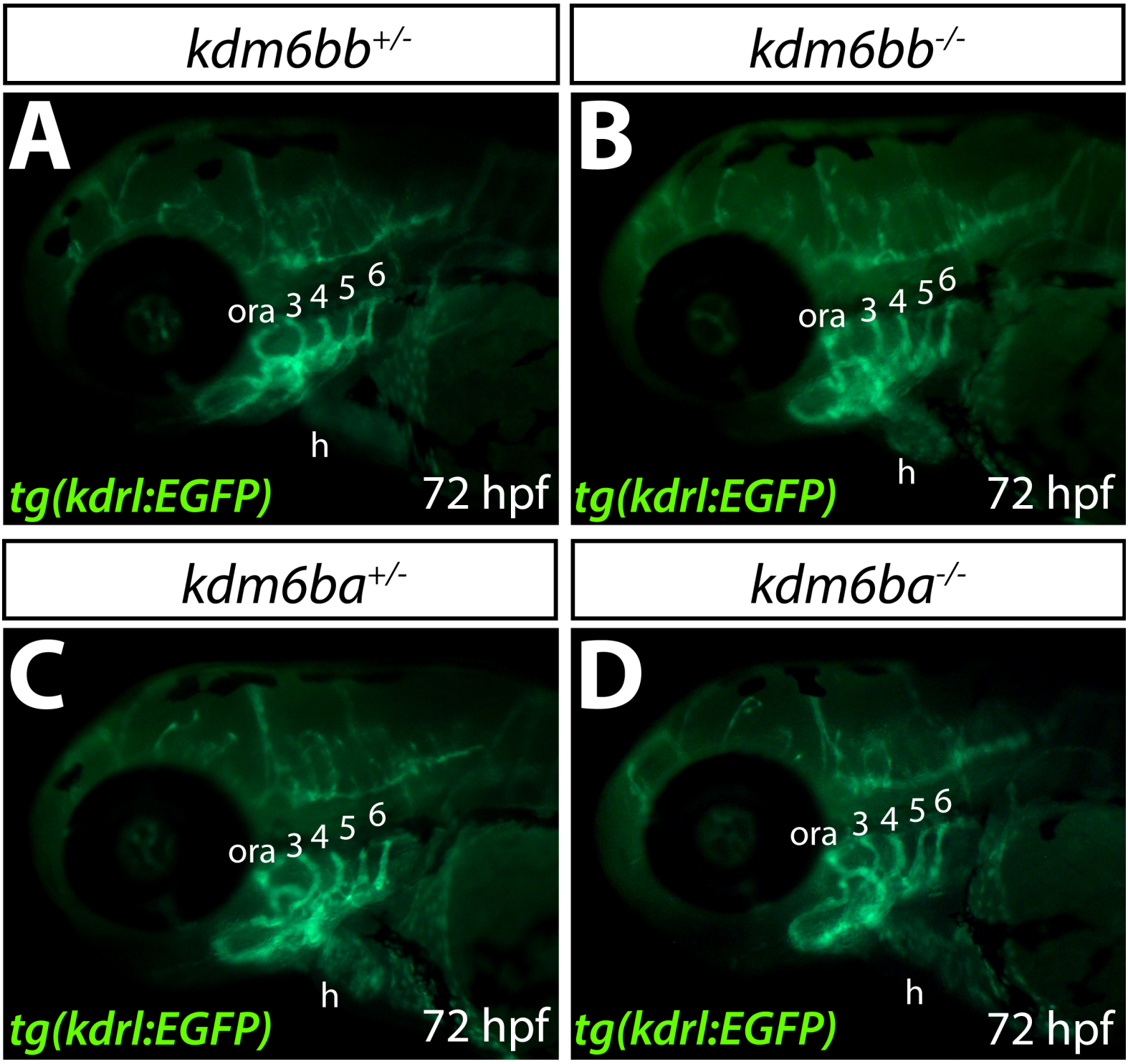
kdm6bb+/-
kdm6bb-/-
A
B
ora 3 4 5 6
h
tg(kdrl:EGFP)
72 hpf
kdm6ba+/-
kdm6ba-/-
C
D

Figure S1. Aortic artery organization is unperturbed in *kdm6bb*$^{-/-}$ and *kdm6ba*$^{-/-}$ zebrafish.

(**A-D**) Fluorescent whole mount images of 72 hpf *Tg(kdrl:EGFP)* zebrafish larvae highlighting the aortic arch arteries of control (A, C), k*dm6bb*$^{-/-}$ (B), and *kdm6ba*$^{-/-}$ (D) animals.

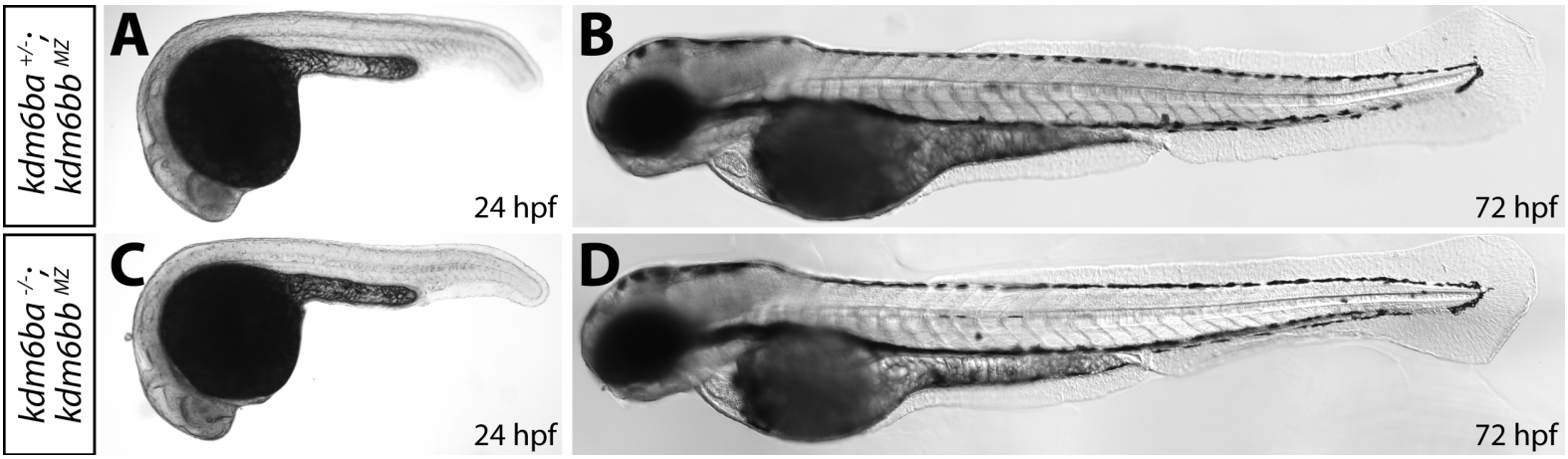
kdm6ba +/-; kdm6bb MZ
A
24 hpf
B
72 hpf
kdm6ba -/-; kdm6bb MZ
C
24 hpf
D
72 hpf

Figure S2. *kdm6b*-deficient zebrafish complete embryonic development without overt defects.

(**A-D**) Whole mount DIC microscopy images of 24 hpf and 72 hpf control (A, B) and *kdm6ba*/*kdm6bb* homozygous mutant (C, D) embryos.

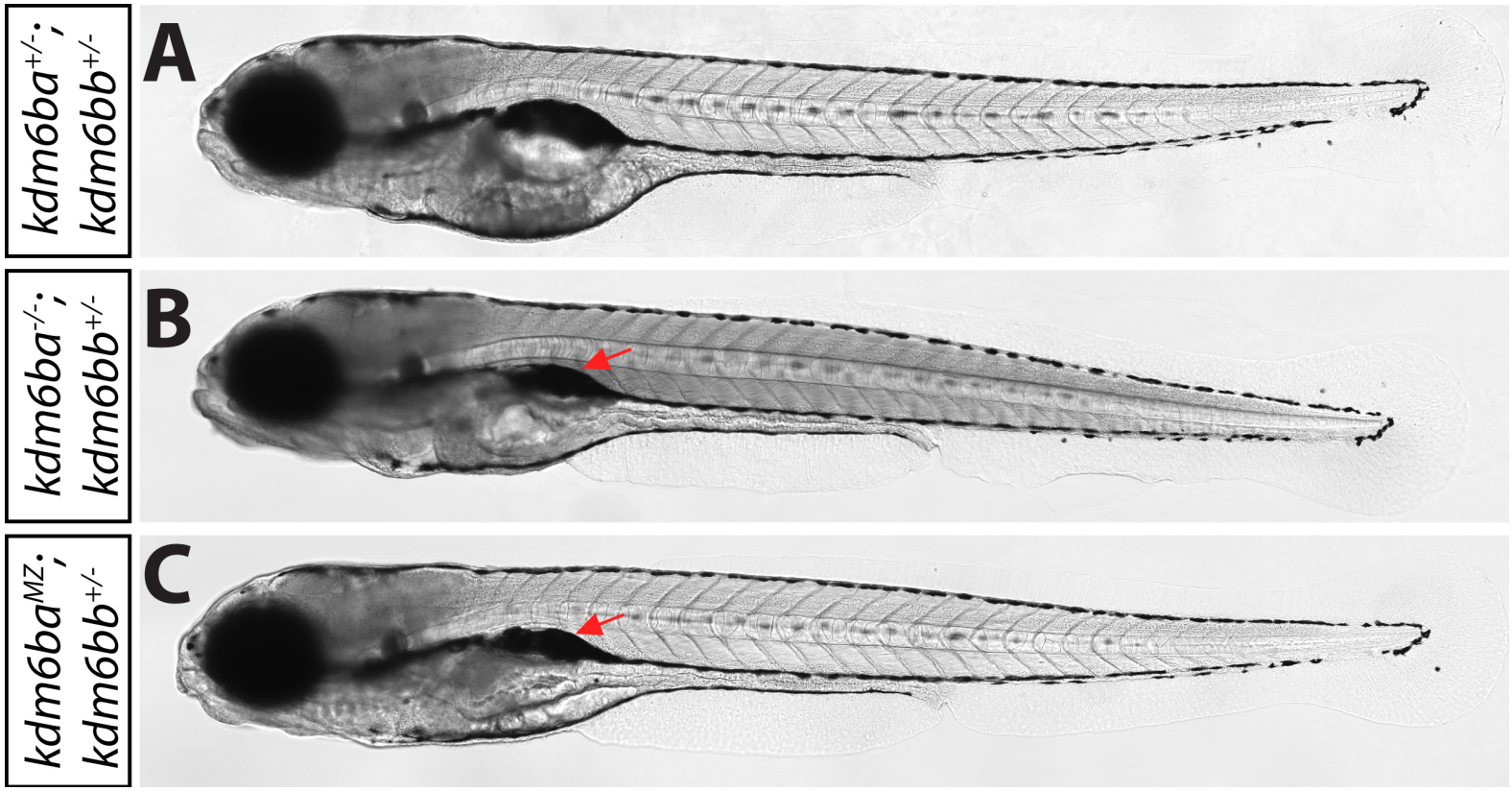
kdm6ba+/-; kdm6bb+/-
A
kdm6ba-/-; kdm6bb+/-
B
kdm6baMZ; kdm6bb+/-
C

Figure S3. Depletion of maternal and zygotic *kdm6ba*/*kdm6bb* transcripts does not affect early embryonic development.

(**A-C**) DIC imaged 5 dpf progeny of the indicated genotypes from a *kdm6bb*$^{+/-}$; *kdm6bb*$^{+/-}$ in-cross (A, B) and a cross between a *kdm6ba*$^{-/-}$; *kdm6bb*$^{+/+}$ female and *kdm6ba*$^{+/-}$; *kdm6bb*$^{-/-}$ male (C). Arrows indicate an un-inflated swim bladder.

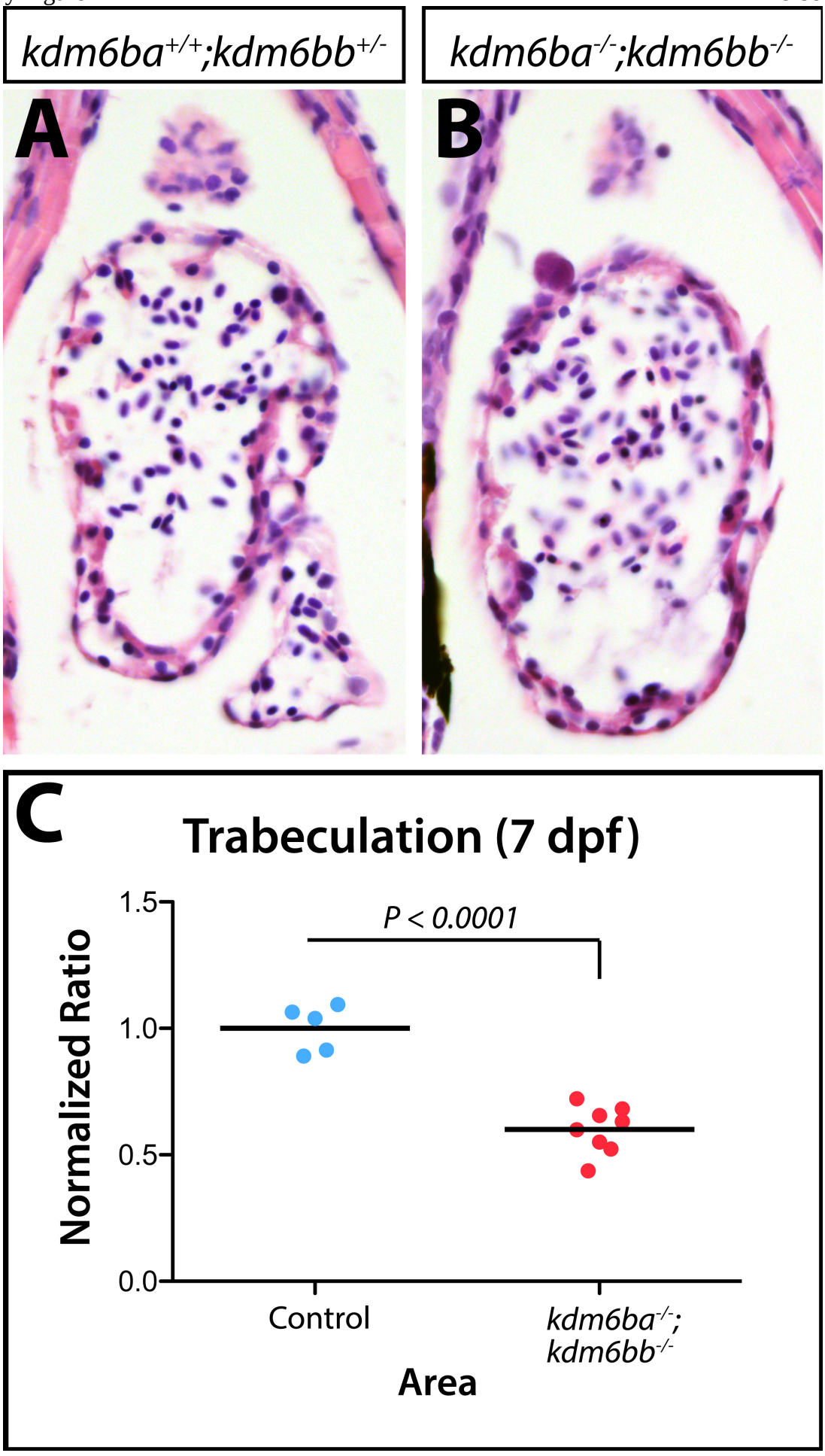
kdm6ba+/+;kdm6bb+/-
kdm6ba-/-;kdm6bb-/-
A
B
C
Trabeculation (7 dpf)
P < 0.0001
Normalized Ratio
1.5
1.0
0.5
0.0
Control
kdm6ba-/-;
kdm6bb-/-
Area

Figure S4. Decreased ventricular area in coronal sections of 7 dpf *kdm6b*-deficient embryos.

(**A-B**) H&E-stained paraffin coronal sections showing the ventricle of 7 dpf control and *kdm6b*-deficient larvae. (**C**) Scatterplot graphs showing the clutch-normalized trabeculated ventricle area measured on matched ventricular coronal sections from 7 dpf control and *kdm6ba*$^{-/-}$; *kdm6bb*$^{-/-}$ double mutant larvae. Each point represents an individual fish. The p-value is from a two-tailed Student's *t*-test.

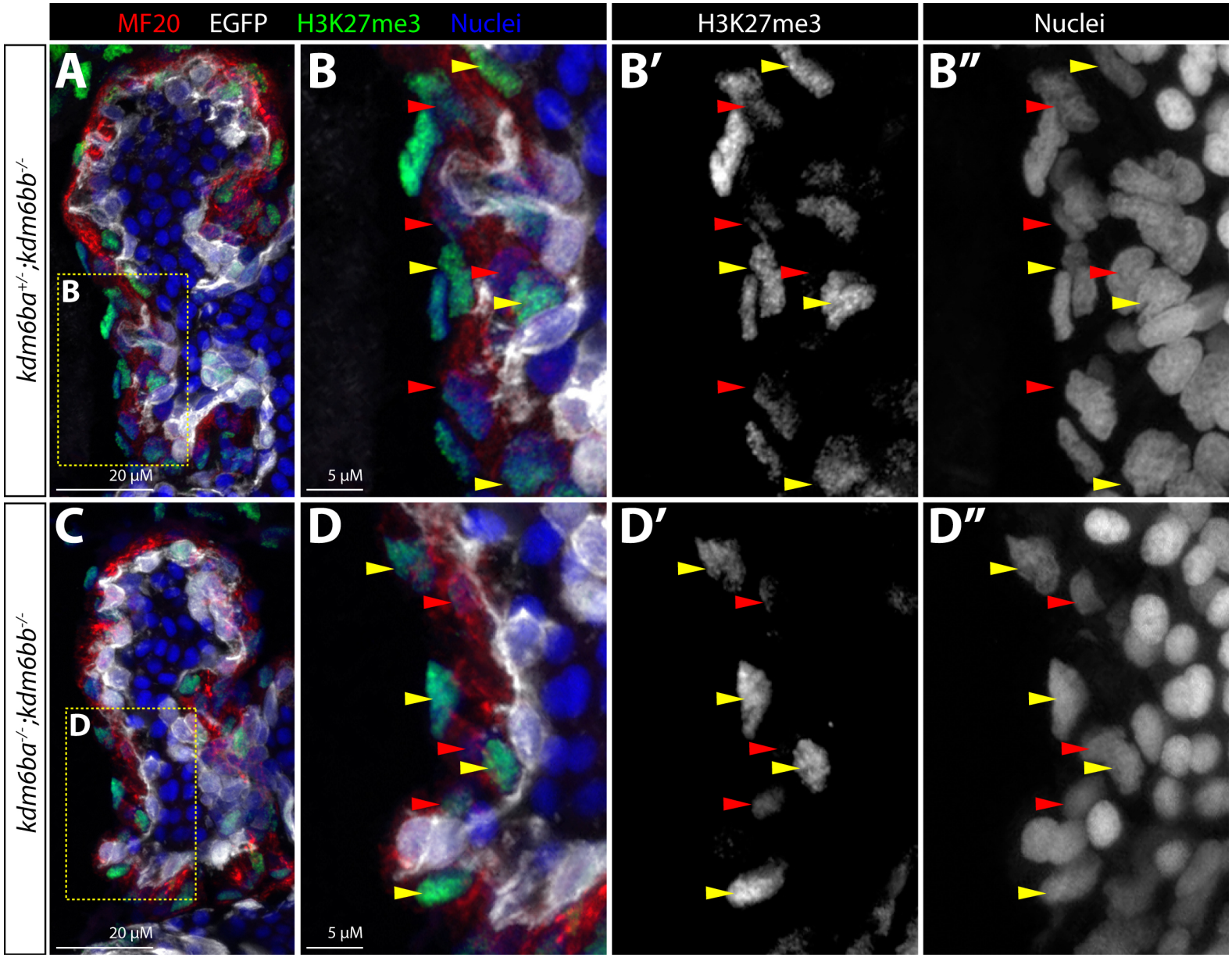
MF20 EGFP H3K27me3 Nuclei
H3K27me3
Nuclei
A
B
B′
B″
C
D
D′
D″
kdm6ba+/-;kdm6bb-/-
kdm6ba-/-;kdm6bb-/-
20 μM
5 μM
20 μM
5 μM

Figure S5. *kdm6ba/bb* are not required for the transient depletion of bulk H3K27me3 levels in trabeculating cardiomyocytes.

(**A-D"**) Confocal microscopy immunofluorescence images of sections through the hearts of 3 dpf *Tg(kdrl:EGFP)* control and *kdm6ba/bb*-deficient embryos stained with anti-myosin heavy chain (red, MF20, myocardium), anti-EGFP (white, endocardium), and anti-H3K27me3 (green) antibodies with Hoechst-stained nuclei in blue (A, B, C, D). Yellow boxed areas in A and C are shown zoomed in B-B" and D-D" respectively with grey-scale single channel images of H3K27me3 staining (B', D') and nuclei (B", D"). Arrowheads indicate myocardial cells with robust (yellow) or depleted (red) bulk H3K27me3 levels. 20 μM and 5 μM scale bars are shown.

Movie S1. *kdm6b-deficient* zebrafish have malformed but functioning heart ventricles.

Whole mount DIC microscopy movie showing blood flow through the hearts and nearby tissue of live 5 dpf *kdm6ba*$^{+/-}$*; kdm6bb*$^{+/-}$ (left) and *kdm6ba*$^{-/-}$*; kdm6bb*$^{-/-}$ (right) clutch mate larvae. Larvae are imaged ventrally and oriented anterior up.