Cell Reports, Volume *26*

Supplemental Information

TETs Regulate Proepicardial Cell Migration through Extracellular Matrix Organization during Zebrafish Cardiogenesis

Yahui Lan, Heng Pan, Cheng Li, Kelly M. Banks, Jessica Sam, Bo Ding, Olivier Elemento, Mary G. Goll, and Todd Evans

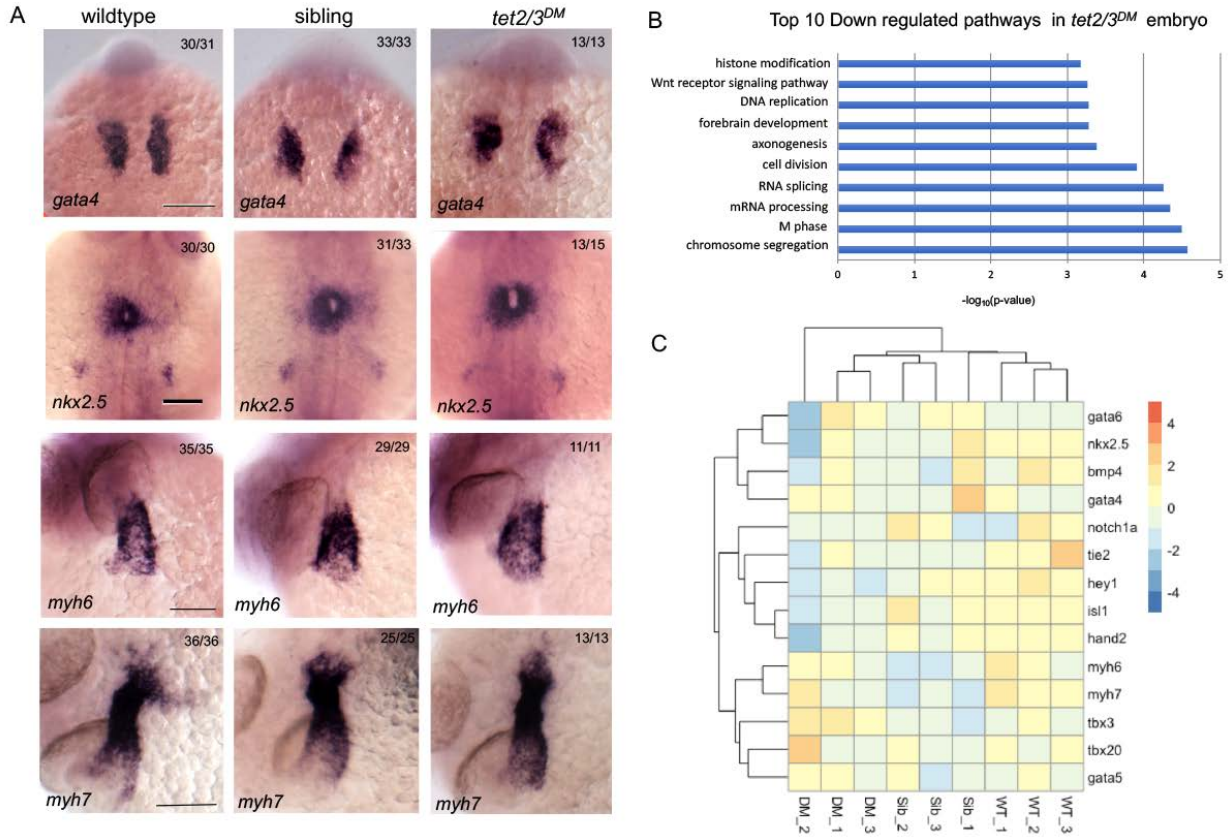

Figure S1: *tet2/3*^{DM} Larvae Show Neuronal but not Cardiac Defects at 28 hpf. Related to Figure 1.

(A) Markers of cardiac progenitors are similarly expressed in wildtype, sibling and *tet2/3*^{DM} larvae. WISH for *gata4* was performed at 18 hpf, *nkx2.5* was performed at 22-hpf, atrial myosin marker *myh6* and myosin marker *myh7* were performed at 28-hpf. Scale bar: 100 μm.
(B) Gene ontology (GO) analysis shows top ten down-regulated biological pathways in 28-hpf *tet2/3*^{DM} larvae by RNA sequencing.
(C) Heatmap of RNA sequencing data illustrating similar transcriptional expression of cardiac genes in *tet2/3*^{DM} (DM) compared with wildtype (WT) or sibling (Sib) larvae.

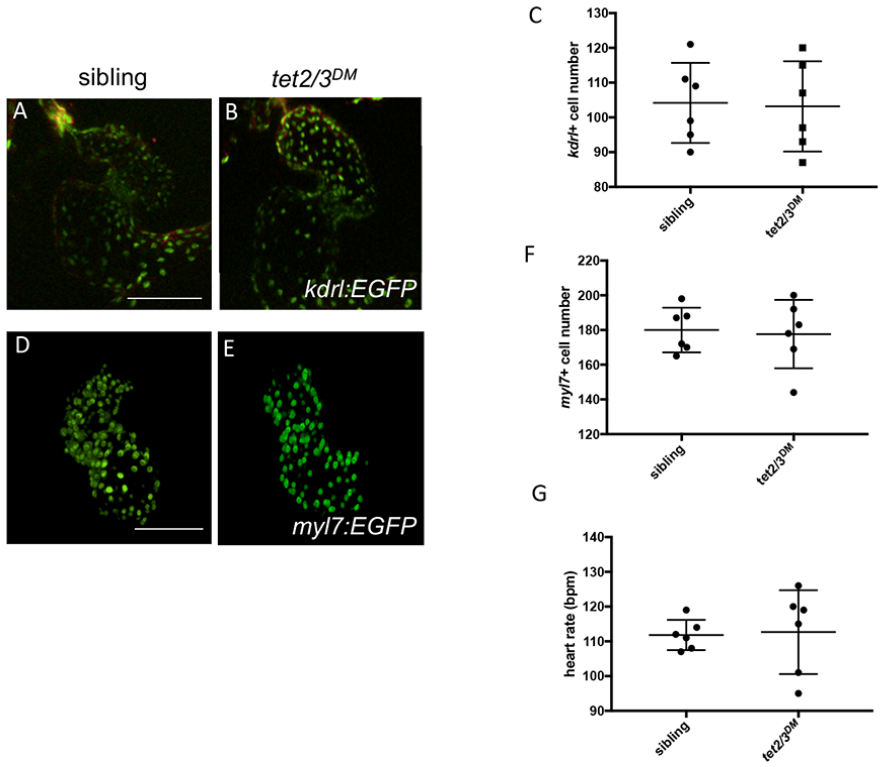

Figure S2: Normal Myocardium and Endocardium in 2-dpf *tet2/3^{DM}* Larvae. Related to Figure 1.
(A and B) GFP labeled endocardium in 2-dpf larvae carrying the Tg(*kdrl:EGFP-NLS)* transgene.
(C) Graph indicates the number of endocardial cells in 48-hpf sibling and *tet2/3^{DM}* larvae. Data are presented as the mean ± SD.
(D and E) GFP labeled myocardium in 2-dpf larvae carrying the Tg(*myl7:EGFP)* transgene.
(F) Graph indicates the number of myocardial cells in 48-hpf sibling and *tet2/3^{DM}* larvae. Data are presented as the mean ± SD.
(G) Graph indicates heart rate in 48-hpf sibling and *tet2/3^{DM}* larvae. Heart rate was measured in beats per minute. Data are presented as the mean ± SD.
Scale bars: 100 μm.

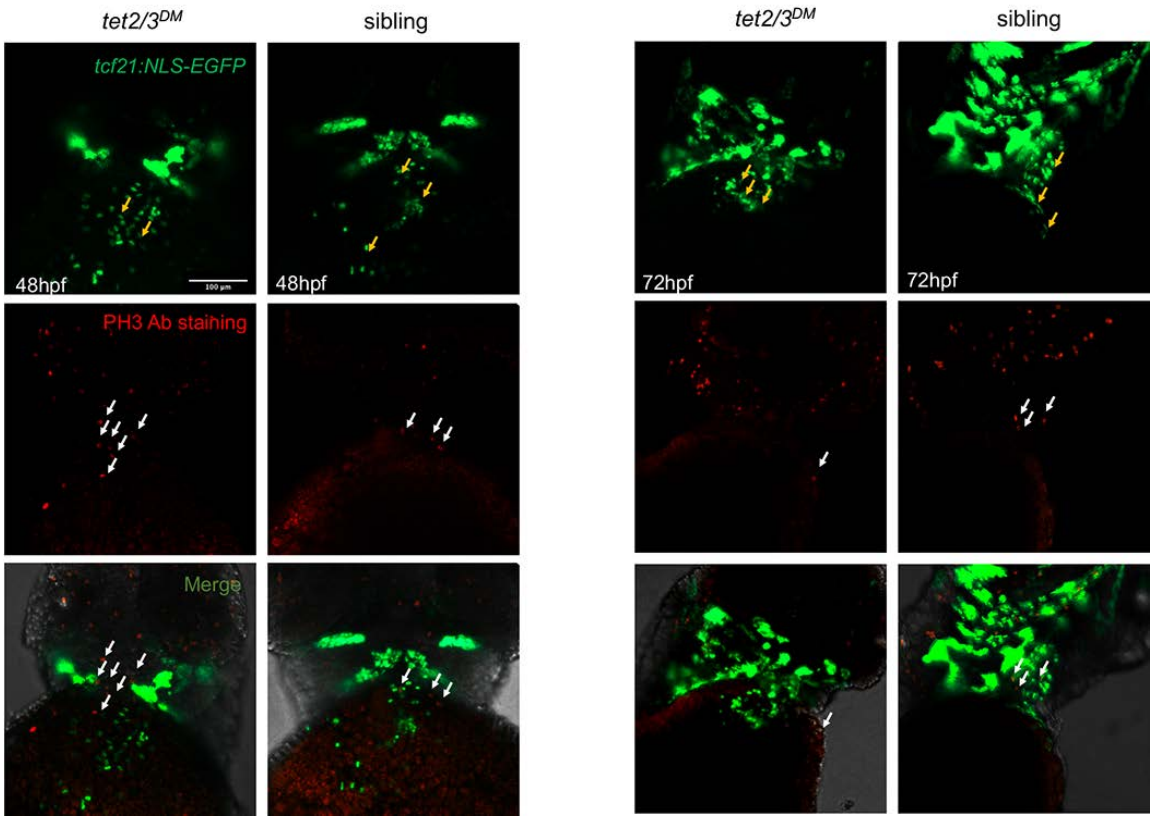

Figure S3: No PE Cell Proliferation at 48 and 72 hpf Sibling and *tet2/3^{DM}* Larvae. Related to Figure 1.
Confocal images showing sibling and *tet2/3^{DM}* larvae carrying the Tg(*tcf21:NLS-EGFP*) transgene stained by anti-GFP antibody (Green) plus anti-pH3 antibody (Red). Yellow arrows indicate PE cells. White arrows indicate pH3+ proliferating cells. Co-staining shows essentially no cell proliferation of *tcf21*+ PE cells at 48-hpf or 72-hpf.
Scale bar: 100 μm.

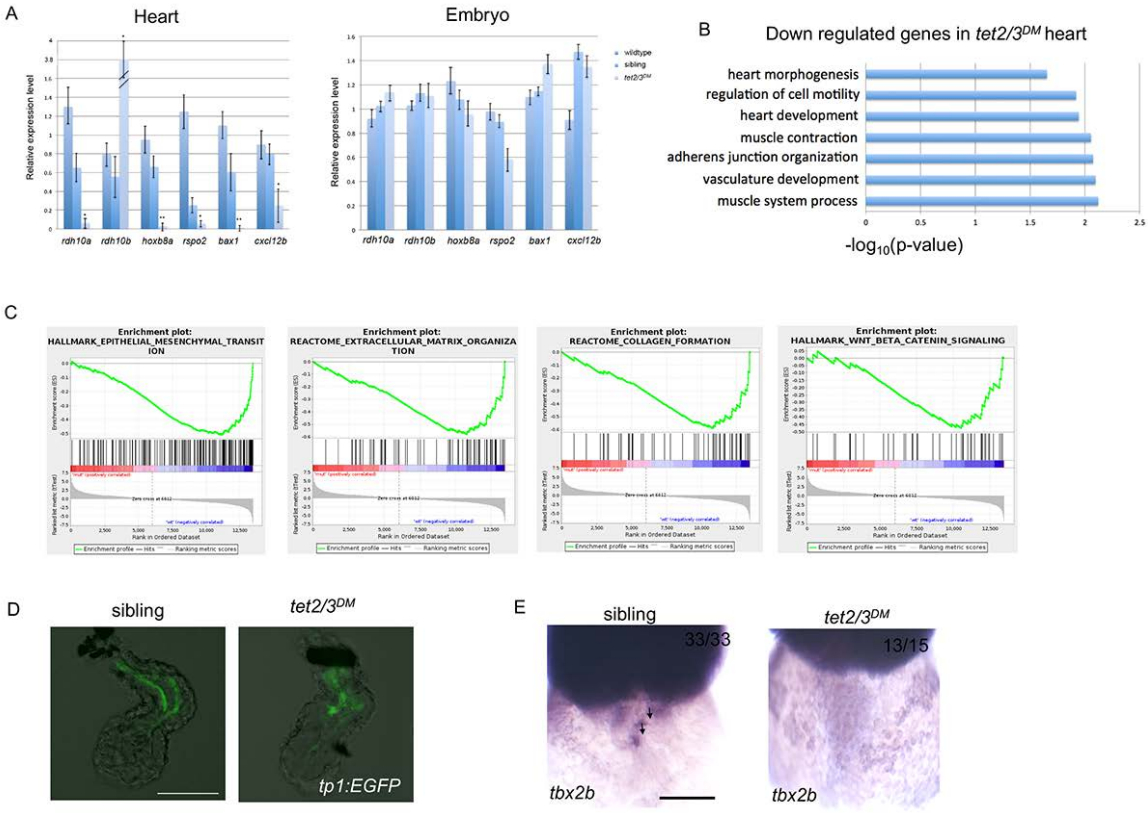

Figure S4: *tet2/3^{DM}* Larvae Show Cardiac AVC Defects at 48-hpf. Related to Figure 3 and Figure 4.
(A) Quantitative RT-PCR validation of top down-regulated genes from RNA sequencing. RT-PCR analysis of *rdh10a, hoxb8a, rspo2, bax1, cxcl12b* transcripts in 2-dpf embryonic heart or whole embryo. Notably, *rdh10b* apparently compensates for the *rdh10a* loss, with 4-fold increase in *tet2/3^{DM}* hearts, which is consistent with RNA sequencing results.
(B) GO analysis shows down-regulated biological pathways in 48 hpf *tet2/3^{DM}* hearts compared to wildtype hearts by RNA sequencing using isolated hearts.
(C) Gene set enrichment analysis shows down-regulated biological pathways in 48-hpf tet2/3DM hearts compared to wildtype hearts by RNA sequencing using isolated hearts.
(D) Normal Notch Activity in tet2/3DM endocardium. GFP-labeled atrial endocardium indicates Notch activity in sibling hearts as well as tet2/3DM hearts. Hearts were dissected from 48 hpf larvae carrying the Tg(*tp1:EGFP*) Notch reporter transgene.
(E) WISH for AVC markers *tbx2b* at 48 hpf. Black arrows indicate AVC specific expression of *tbx2b* in sibling but not *tet2/3^{DM}* heart. Scale bars: 50µm.

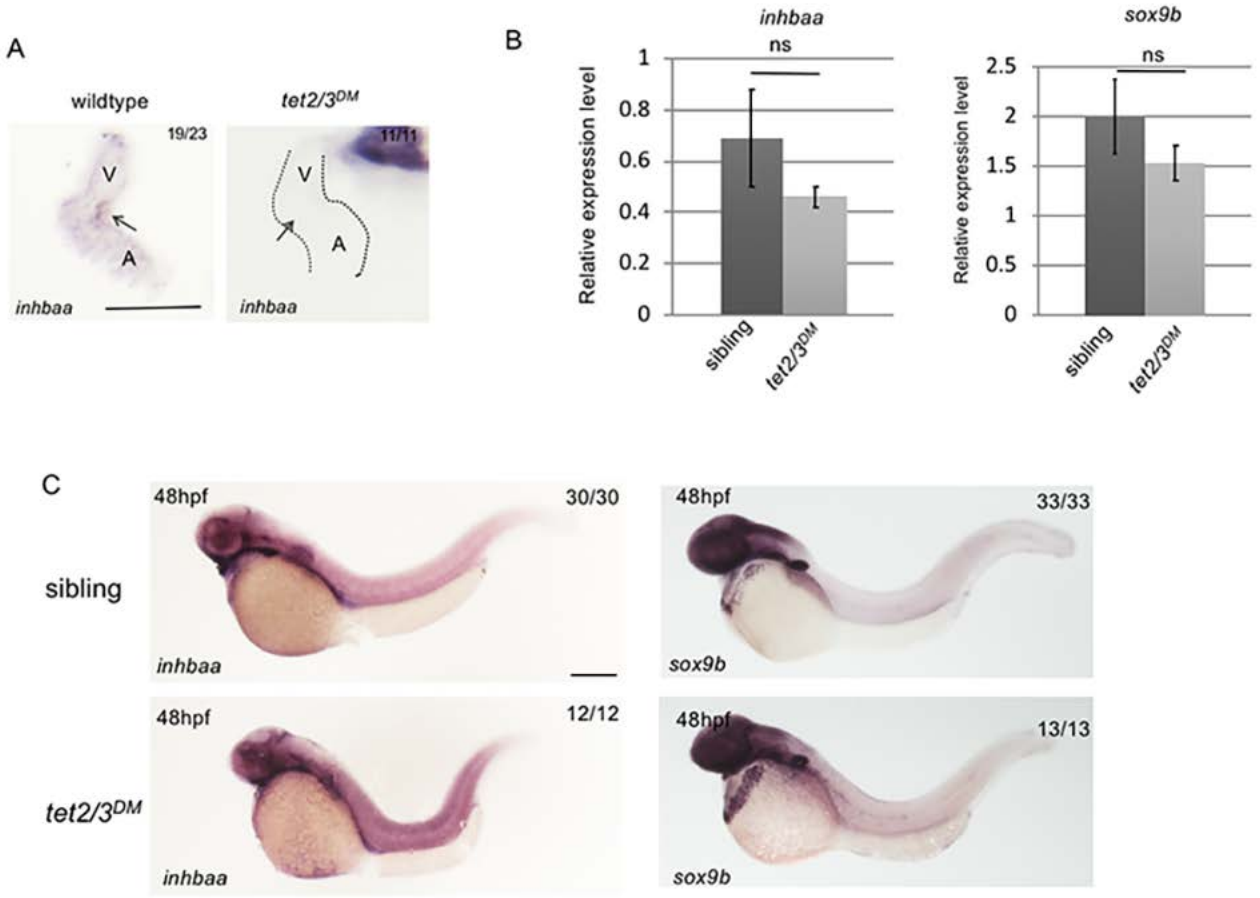

Figure S5: *inhbaa* and *sox9b* Expression Shows Cardiac Specific Defects in *tet2/3*^{DM} Larvae. Related to Figure 5.

(A) WISH for *inhbaa* at 48-hpf isolated hearts. Arrows indicate AVC endocardium with *inhbaa* transcripts. Scale bar: 100 µm.

(B) RT-PCR analysis of *inhbaa* and *sox9b* transcripts in representative 48-hpf embryos.

(C) WISH for *inhbaa* and *sox9b* in representative 48-hpf embryos. Scale bar: 300 µm.

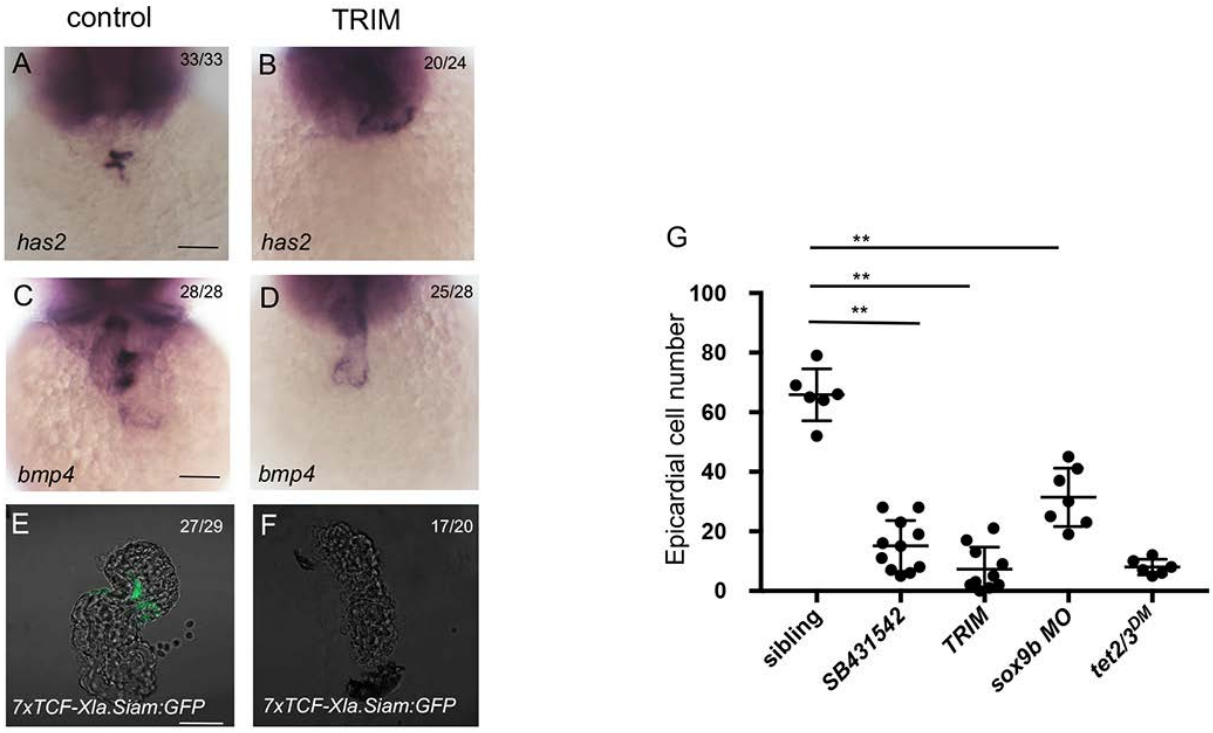

Figure S6: Inhibiting the Activin-A Pathway or *sox9b* Causes AVC Disruption and PE Migration Defect. Related to Figure 5.

(A-D) WISH for AVC markers *has2* and *bmp4* at 48-hpf in wildtype or TRIM treated larvae.

(E-F) GFP-labeled AVC endocardium represents Wnt activity in wildtype but not TRIM treated heart. Hearts were dissected from 48-hpf larvae carrying the Tg(*7xTCF-Xla.Siam:GFP*) transgene.

(G) Number of epicardial cells in the heart of 4-dpf sibling, sibling exposed to SB431542 or TRIM from 24 hpf, *sox9b* morphant and *tet2/3*^{DM} larvae carrying the Tg(*tcf21:NLS-EGFP*) transgene. Data are presented as the mean ± SD. The significance is indicated as **P < 0.01.

Scale bar: 50μm.

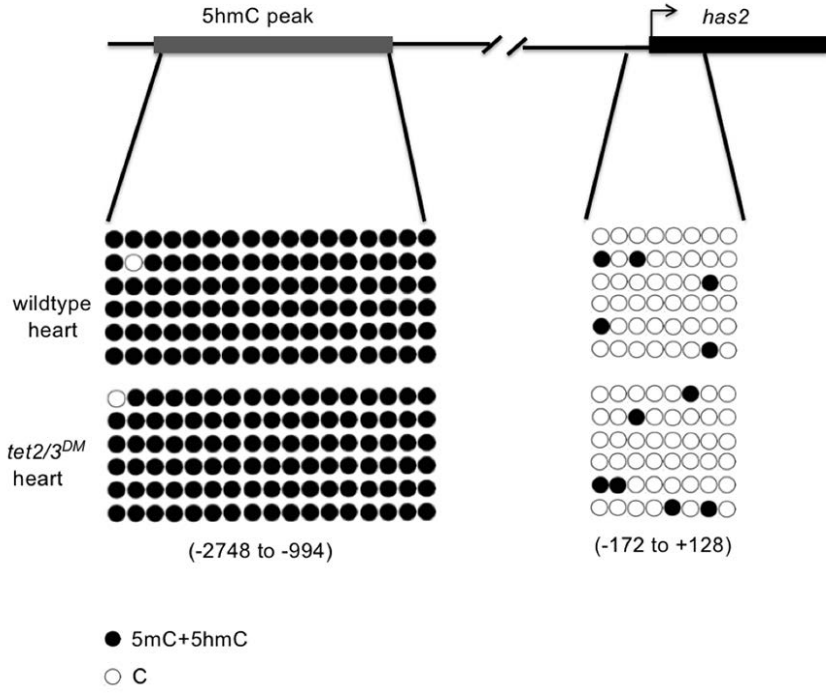

Figure S7. No Hypermethylation at the Promoter of the *has2* Gene in *tet2/3^{DM}* Hearts. Related to Figure 6.

Diagram indicates *has2* locus and the associated regulatory regions. Gray box represents a 5hmC peak. Black box represents the coding sequence. Profiles of 5mC + 5hmC in the putative promoter regions revealed by bisulfite sequencing. Numbers indicate regions analyzed relative to the translation start site.

bis-sequence primer		5'--3'
inhbaa	F	TTTGGGGGGGGGGTTTTTATTG
	R	AATAAAAAAACACATACTTAACATACTAC
sox9b	F	TGTGTTTATTTGTGTTGGTGATGTTGTGG
	R	ACAAAAAACTACAATAACCATACTAAAATCTC
has2-promoter	F1	GTATGTTTGTATTGTGGGGGAAATTTGAGGGTTTGGAG
	R1	TCATTATTTCATTCATTTTCTTTTCAACTTAATCCC
	F2	AAAAGATTGTTGAGGTGTTGAAGG
	R2	CAAAATCAATTACACATAACCTCTCAATAATAC
has2-exon1	F	ATTTGAAGGGGTAGTATTTTTTTTTTTTAGATGTTG
	R	CATTCAAATTTTTTTTTTCCCCAAAC

qRT-PCR primer		5'---3'
inhbaa	F	GCACATTCAGAAGCCGACTGCC
	R	TAAAGTCCGTCTGCCTGTGCGC
sox9b	F	AGGTGCTGAAGGGCTACGACTGGT
	R	GATTTCCTCCGTCTGGGCTGGTATT
cxcl12b	F	CAAGTCATTGCCAAGCTGAAG
	R	CTCGTCTGTTTACTCTGAGCG
rdh10a	F	AGTTCACTGGGCCTTTTCAG
	R	ACACCAGCGTCATCTTGATAC
hoxb8a	F	ACAACTGTTCCCGTGGATG
	R	TGTGACACTTCAATCCGACG
rspo2	F	TCCAACCATCGCTGAATCTAG
	R	TCGCTGTGTTGGTTCTGAG
barx1	F	CCGGATCAGAAGGTATCAAGTC
	R	GGAAACTTCAGGATACCCGTC
b-actin	F	CGAGCAGGAGATGGGAACC
	R	CAACGGAAACGCTCATTGC
rdh10b	F	GAGAGCCATACTAACAGACCAG
	R	AGGGTACATGCACTTATCAGC

mRNA generation primer	5'--3'		
EcoRI-inhbaa-F	CG GAATTC AGTCCCAGACCTCTTACGAG		
XhoI-inhbaa-R	CTAACG CTCGAG ttacgagcagccgcattctt		
ECORI-sox9b-F	CG GAATTC ttataacacacacgcgtgcg		
XhoI-sox9b-R	CTAACG CTCGAG TCAGGGTCTGGACAGCTGTG		
BamHI-has2-F	CTG**GGATCC**ATGAGATGTGATAAAGCGGTCAGC		
XhoI-has2-R	GTCA**CTCGAG**CTATACGTCAAGAACCATGTC		

Table S1. PCR Primer Sequences Used in the Paper. Related to Figure 5 and Figure 6.

alpi.1
amer2
ets2
foxa3
g6pc3
gmpr
inhbaa
lama4
nog2
pecam1
pisd
pts
rtn4rl2a
slit1a
sox9b
spag1a
srl
trim69
tyr
zgc:153184

Table S2. Genes have hyper-DMR in 2 kb upstream and downstream of transcription start sites as well as transcriptional downregulation in *tet2/3^{DM}* hearts (fold change >2). Related to Figure 5.