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Supplemental information

The scaffolding function of LSD1/KDM1A reinforces a negative feedback loop to repress stem cell gene expression during primitive hematopoiesis

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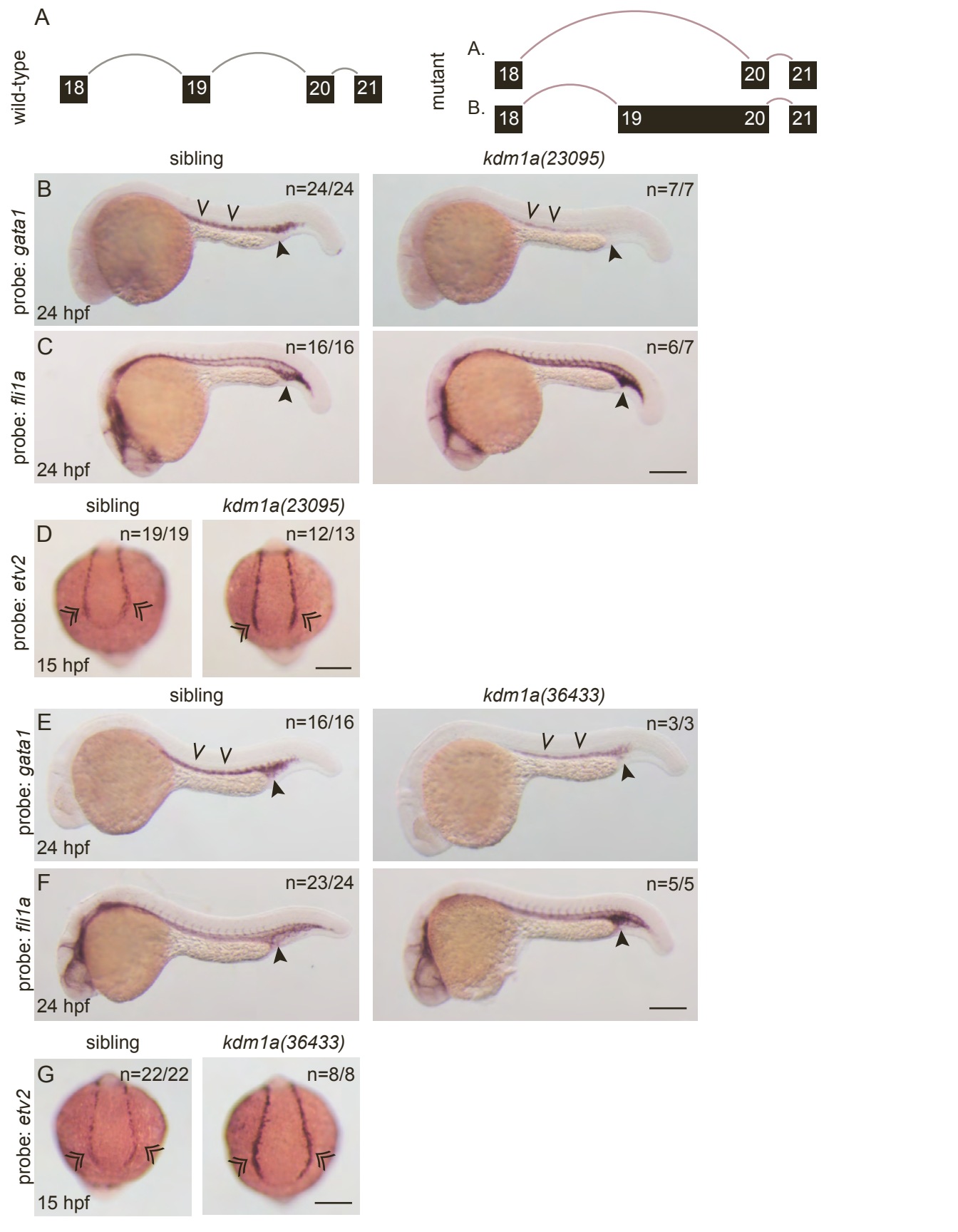
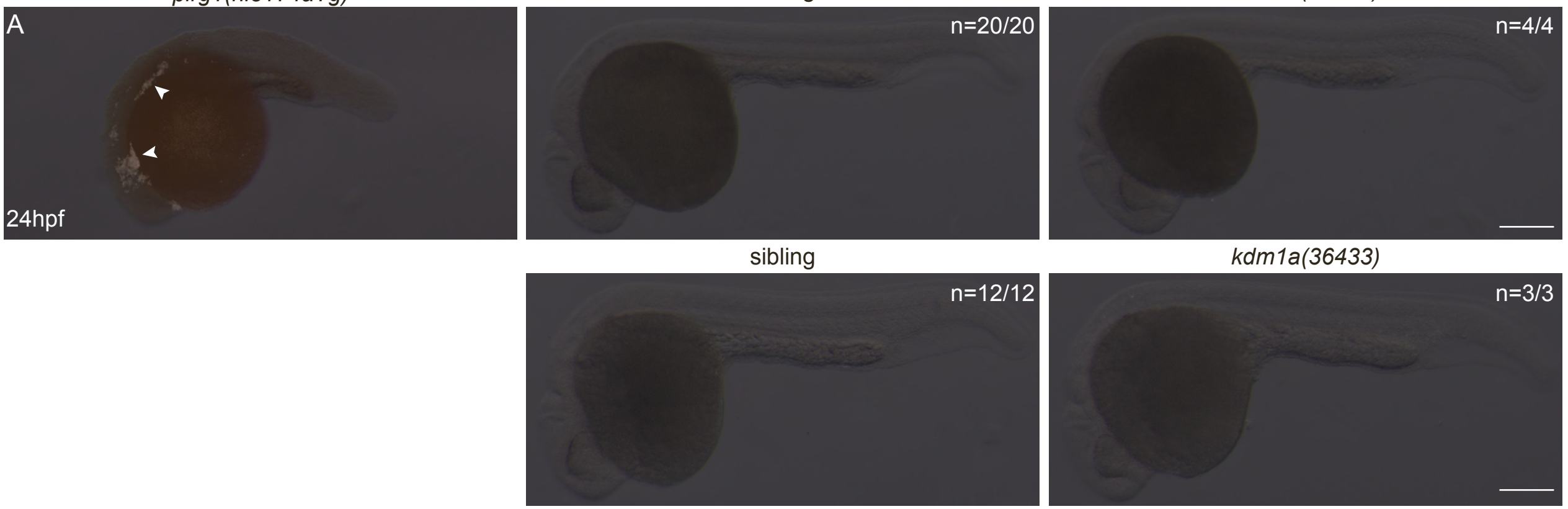

A
wild-type
18
19
20
21
mutant
A.
18
20
21
B.
18
19
20
21
sibling
kdm1a(23095)
B
n=24/24
n=7/7
probe: gata1
24 hpf
C
n=16/16
n=6/7
probe: fli1a
24 hpf
sibling
kdm1a(23095)
D
n=19/19
n=12/13
probe: etv2
15 hpf
sibling
kdm1a(36433)
E
n=16/16
n=3/3
probe: gata1
24 hpf
F
n=23/24
n=5/5
probe: fli1a
24 hpf
sibling
kdm1a(36433)
G
n=22/22
n=8/8
probe: etv2
15 hpf

Figure S1. Hematopoietic transcription factors are dysregulated in *lsd1* mutants, related to Figure 1. (A) Wild-type and mutant *kdm1a(23095)* splice variations between exons 18-21 are shown in the schematic. (B-G) WISH of embryos derived from an incross of *kdm1a(23095)* heterozygotes (B-D) or *kdm1a(36433)* heterozygotes (E-G) using probes to detect *gata1* (B,E), *fli1a* (C,F), and *etv2* (D,G). Representative images are shown with the number of animals with similar staining patterns for the indicated genotype shown in the upper right corner of each image. The lower left corner indicates the approximate stage of the animal at the time of fixation. Embryos were imaged, blindly scored by expression and then genotyped by HRMA. Carons indicate ICM expression; closed arrowheads indicate posterior blood island/caudal hematopoietic tissue; double carons indicate posterior lateral mesoderm. Scale bar, 200 μm.

Activated Caspase-3



Phospho-histone H3

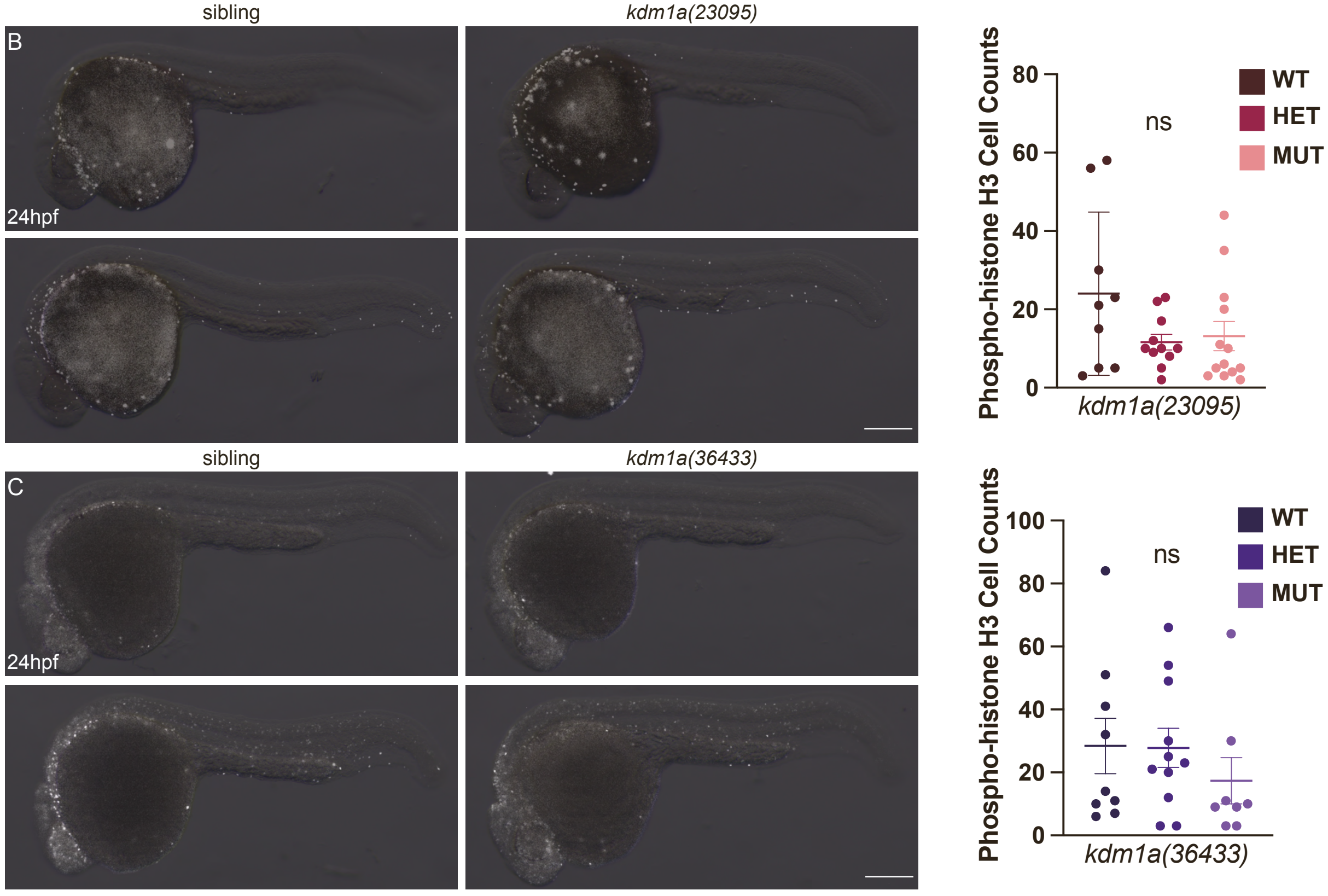


Figure S2. Cell death is not increased in *lsd1* mutants, related to Figure 1. (A) Immunofluorescence of activated Caspase-3 in 24-hpf *kdm1a(23095)* and *kdm1a(36433)* mutants. As a positive control for the assay, *plrg1(hi3174aTg)* mutants were used. Representative images are shown with the number of animals with similar staining patterns for the indicated genotype shown in the upper right corner of each image. (B-C) Immunofluorescence of phospho-histone H3 in 24-hpf *kdm1a(23095)* (B) and *kdm1a(36433)* (C) mutants. Staining variability is shown for both siblings and mutants. Quantification of the number of phospho-histone-H3-positive cells in the tail is shown on the right. Each point represents an individual animal. The number of phospho-histone-H3-positive cells in the tail were measured using the Analyze Particles tool on ImageJ without size exclusion. Data is not statistically significant. The brightness of raw images of both siblings and mutants were adjusted to the same level using Photoshop (Adobe 2017). ns, not significant. Scale bar, 200 μm.

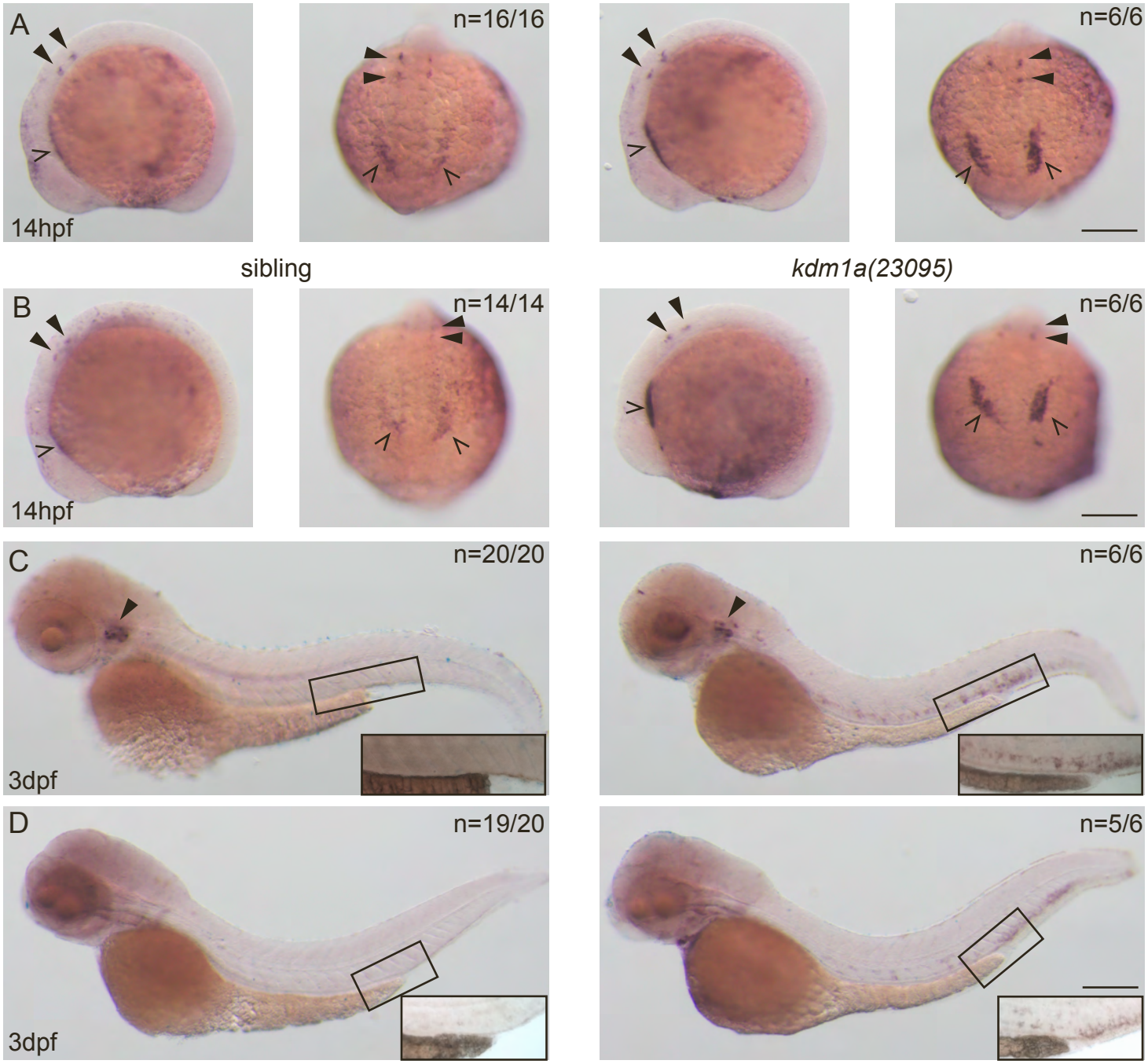

sibling
kdm1a(36433)
probe: gfi1ab
A
n=16/16
n=6/6
14hpf
sibling
kdm1a(23095)
probe: gfi1ab
B
n=14/14
n=6/6
14hpf
probe: gfi1ab
C
n=20/20
n=6/6
3dpf
probe: gfi1b
D
n=19/20
n=5/6
3dpf

Figure S3. GFI-family genes are dysregulated throughout development, related to Figure 2. (A-B) WISH of *gfi1ab* of 14-hpf embryos from an incross of *kdm1a(36433)* (A) or *kdm1a(23095)* (B) heterozygotes. Lateral (left) and dorsal (right) views are shown. (C-D) WISH of embryos derived from an incross of *kdm1a(23095)* heterozygotes using probes to detect *gfi1ab* (C) or *gfi1b* (D) at 3 dpf. Representative images are shown with the number of animals with similar staining patterns for the indicated genotype shown in the upper right corner of each image. The lower left corner indicates the approximate stage of the animal at the time of fixation. Embryos were imaged, blindly scored by expression levels and then genotyped by HRMA. Narrow triangles indicate inner-hair-cell expression. Scale bar, 200 μm.

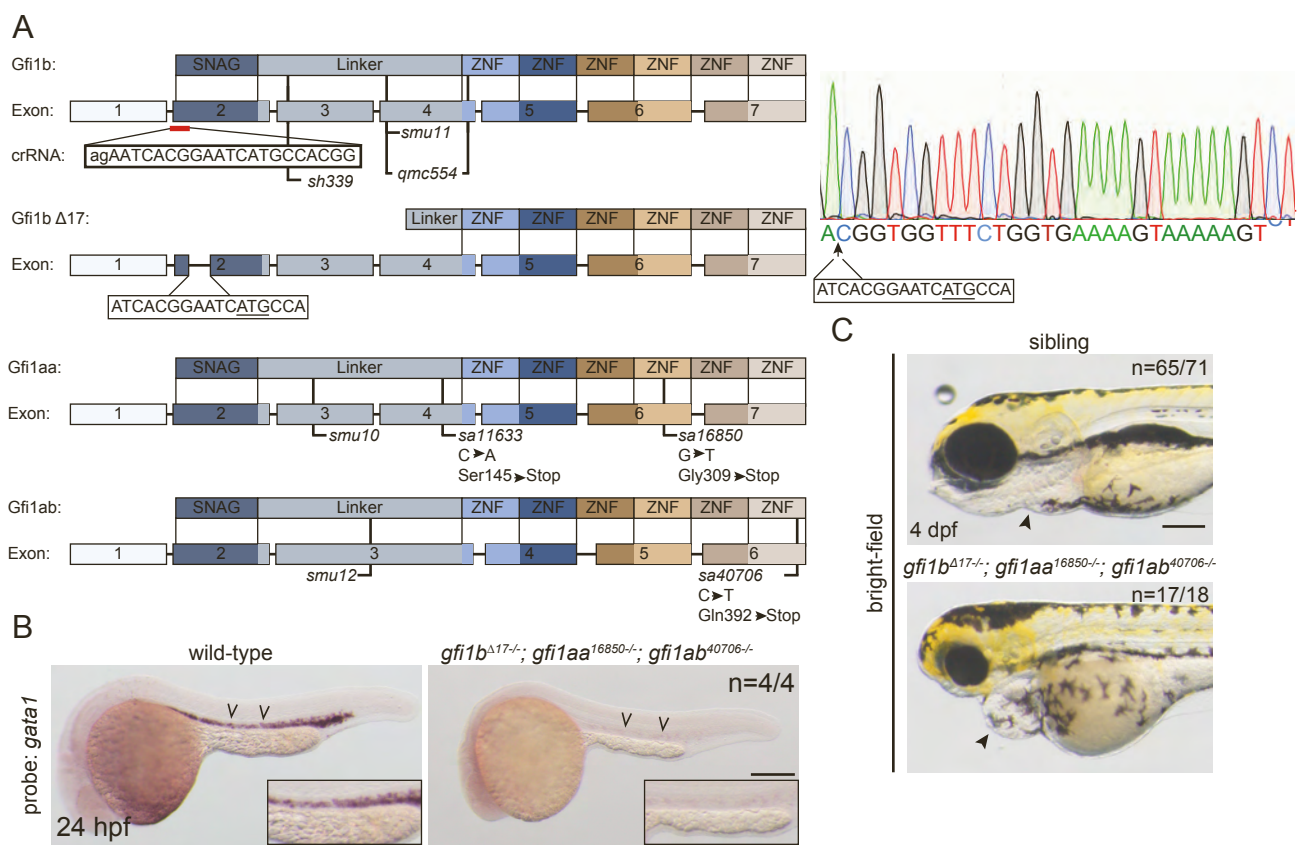

A
Gfi1b:
SNAG
Linker
ZNF
Exon:
1
2
3
4
5
6
7
crRNA:
agAATCACGGAATCATGCCACGG
smu11
qmc554
sh339
ACGGTGGTTTCTGGTGAAAAGTAAAAAGT
ATCACGGAATCATGCCA
Gfi1b Δ17:
ATCACGGAATCATGCCA
Gfi1aa:
smu10
sa11633
C➤A
Ser145➤Stop
sa16850
G➤T
Gly309➤Stop
Gfi1ab:
smu12
sa40706
C➤T
Gln392➤Stop
B
wild-type
gfi1b^Δ17-/-; gfi1aa^16850-/-; gfi1ab^40706-/-
n=4/4
probe: gata1
24 hpf
C
sibling
n=65/71
4 dpf
bright-field
gfi1b^Δ17-/-; gfi1aa^16850-/-; gfi1ab^40706-/-
n=17/18

Figure S4. Triple-*gfi*-mutant zebrafish phenocopy *lsd1* mutants, related to Figure 2. (A) Schematic of the structure of zebrafish *Gfi1b, Gfi1aa*, and *Gfi1ab* proteins and corresponding genetic mutations relevant to this study. The mRNA sequence of *gfi1b*Δ17 is shown on the right. (B) WISH to detect *gata1* in embryos derived from an incross of either *gfi1b*$^{\Delta 17/\Delta 17}$; *gfi1aa*$^{16850/16850}$; *gfi1ab*$^{40706/+}$ triple mutants or wild-type zebrafish. A representative image of a *gfi1b*$^{\Delta 17/\Delta 17}$; *gfi1aa*$^{16850/16850}$; *gfi1ab*$^{40706/40706}$ mutant animal is shown with the number of animals with similar staining patterns for that genotype indicated in the upper right corner. The lower left corner indicates the approximate stage of the animal at the time of fixation. Embryos were imaged and genotyped by HRMA. Wild-type embryos were included within each well to control for staining. Carons, ICM expression. (C) Brightfield image of 4-dpf embryos from a *gfi1b*$^{\Delta 17/\Delta 17}$; *gfi1aa*$^{16850/16850}$; *gfi1ab*$^{40706/+}$ triple-mutant incross. Embryos with small heads and edema were genotyped via HRMA, and the number of animals with the phenotype shown and corresponding genotype is indicated. Scale bar, 200 μm.

A

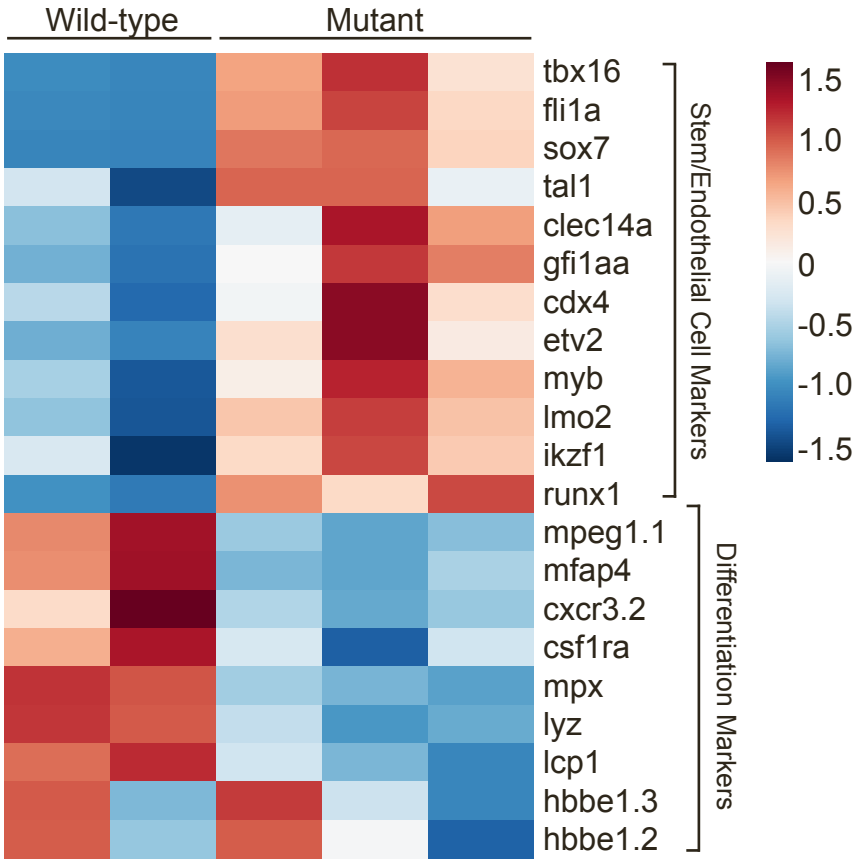
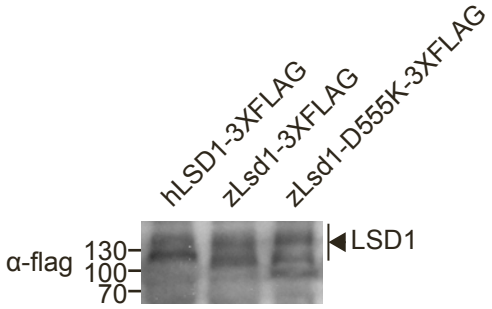

Wild-type
Mutant
tbx16
fli1a
sox7
tal1
clec14a
gfi1aa
cdx4
etv2
myb
lmo2
ikzf1
runx1
mpeg1.1
mfap4
cxcr3.2
csf1ra
mpx
lyz
lcp1
hbbe1.3
hbbe1.2
Stem/Endothelial Cell Markers
Differentiation Markers
1.5
1.0
0.5
0
-0.5
-1.0
-1.5

Figure S5. Hematopoietic progenitor markers are upregulated at the expense of differentiation markers, related to Figure 3. (A) Heatmap of *kdm1a(23095)* mutants compared to wild-type siblings for the differential expression of stem/endothelial-cell-specific genes and myeloid- and erythroid-cell-specific genes (“Differentiation markers”).

A


hLSD1-3XFLAG
zLsd1-3XFLAG
zLsd1-D555K-3XFLAG
α-flag
130
100
70
LSD1

B

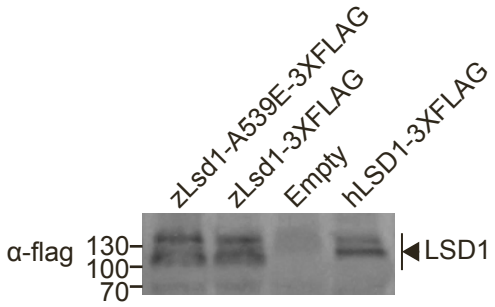

zLsd1-A539E-3XFLAG
zLsd1-3XFLAG
Empty
hLSD1-3XFLAG
α-flag
130
100
70
LSD1

Figure S6. FLAG-tagged rescue constructs are translated to protein *in vivo,* related to Figure 4 and Figure 6. (A-B) Immunoblotting with an anti-Flag antibody of protein lysate from wild-type zebrafish injected with the indicated 3XFLAG-tagged mRNA constructs.

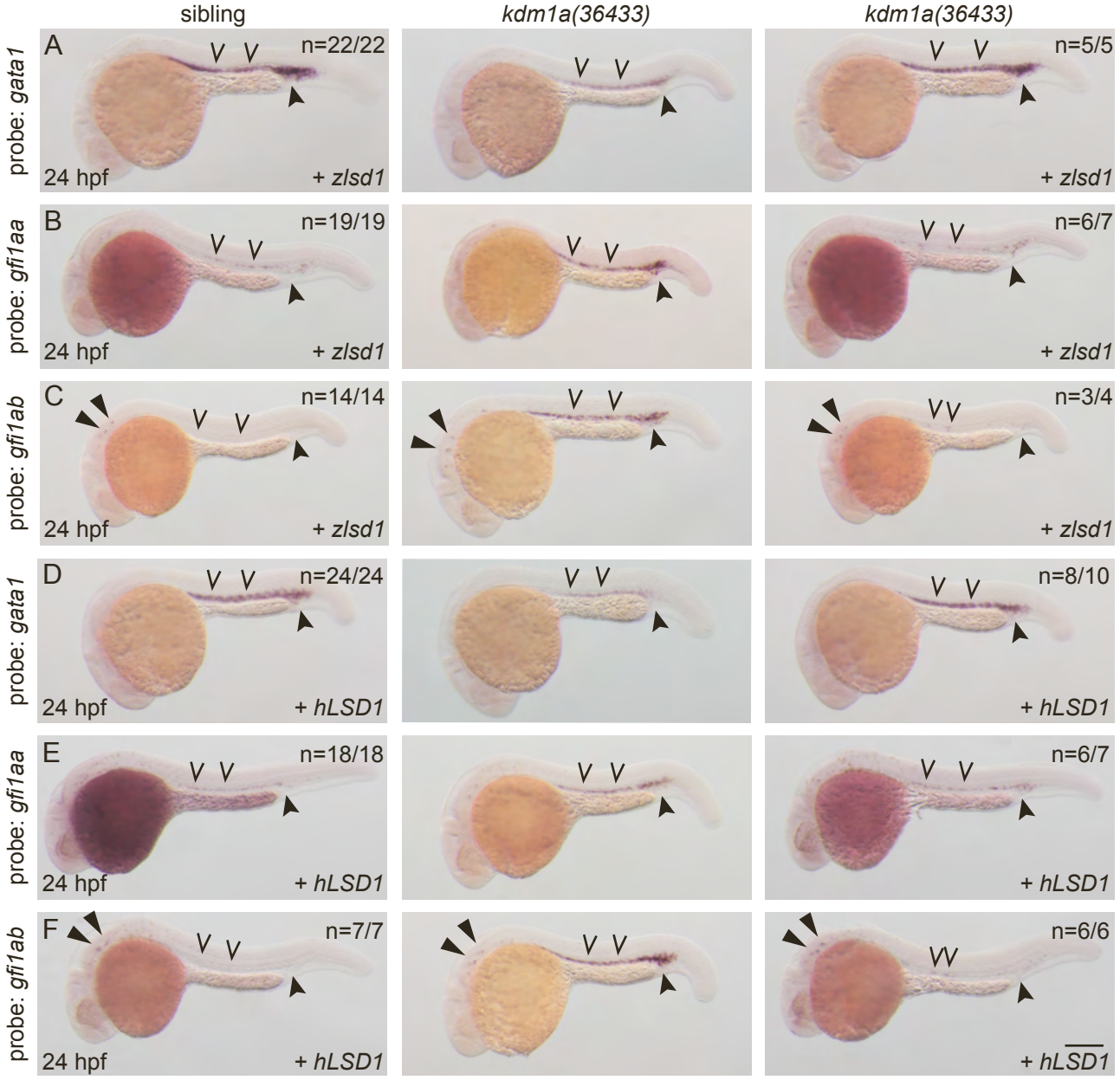

sibling
kdm1a(36433)
kdm1a(36433)
A
probe: gata1
n=22/22
24 hpf
+ zlsd1
n=5/5
+ zlsd1
B
probe: gfi1aa
n=19/19
24 hpf
+ zlsd1
n=6/7
+ zlsd1
C
probe: gfi1ab
n=14/14
24 hpf
+ zlsd1
n=3/4
+ zlsd1
D
probe: gata1
n=24/24
24 hpf
+ hLSD1
n=8/10
+ hLSD1
E
probe: gfi1aa
n=18/18
24 hpf
+ hLSD1
n=6/7
+ hLSD1
F
probe: gfi1ab
n=7/7
24 hpf
+ hLSD1
n=6/6
+ hLSD1

Figure S7. Wild-type *lsd1* rescues hematopoietic transcription factor gene expression in *kdm1a(36433)* mutants, related to Figure 4. Single-cell zebrafish embryos derived from an incross of *kdm1a(36433)* heterozygotes were injected with either *zlsd1* (A-C) or *hLSD1* mRNA (D-F). WISH was then performed at 24 hpf with the indicated probes. Representative images are shown with the number of animals with similar staining patterns for the indicated genotype shown in the upper right corner. The lower right corner indicates the mRNA injection conditions. Uninjected mutant embryos are shown in the middle column for comparison. Embryos were imaged, blindly scored by expression and then genotyped by HRMA. Carons indicate ICM expression; closed arrowheads indicate posterior blood island/caudal hematopoietic tissue; narrow triangles indicate inner-hair-cell expression. Scale bar, 200 μm.

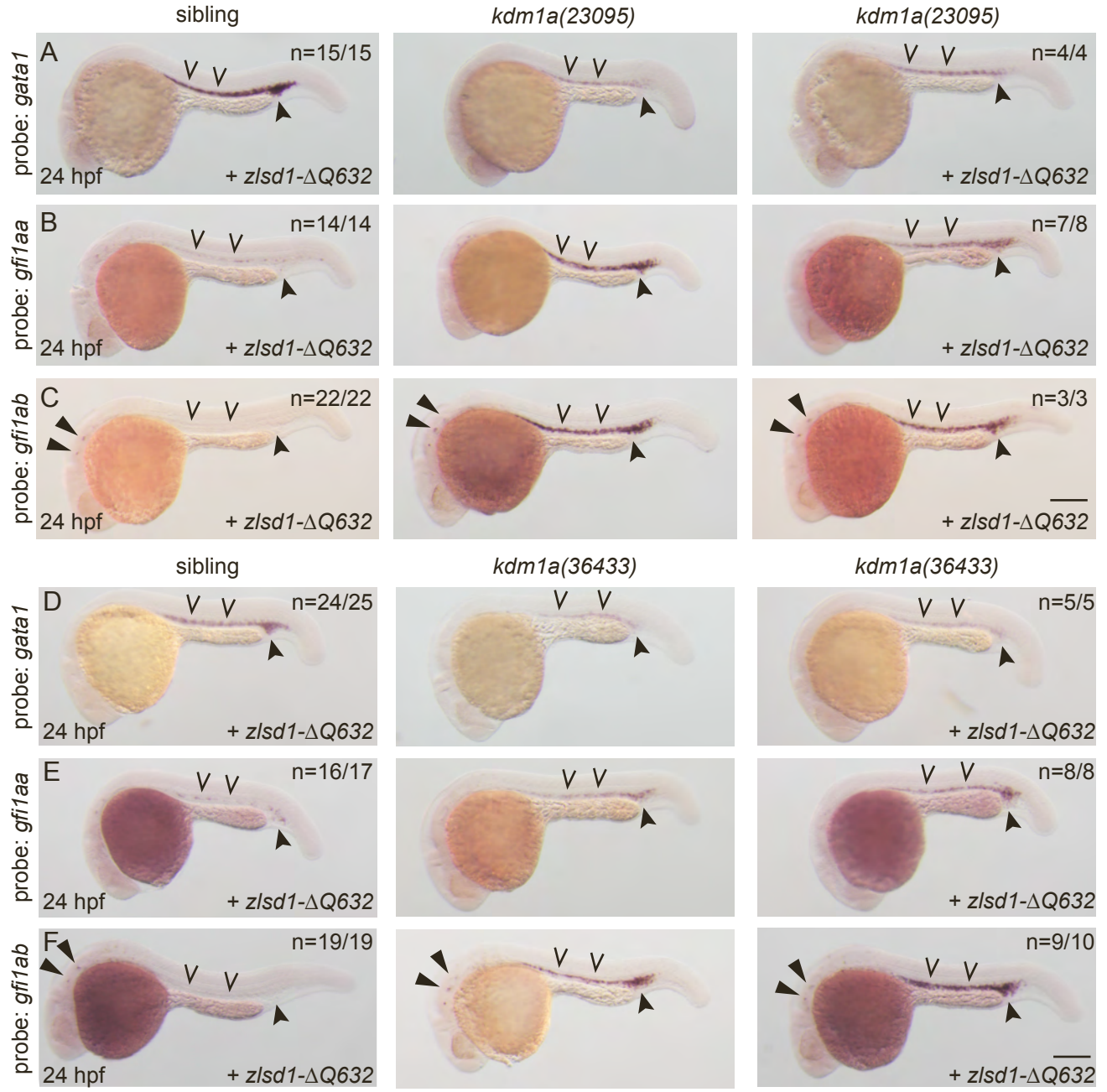

sibling
kdm1a(23095)
kdm1a(23095)
A
n=15/15
n=4/4
probe: gata1
24 hpf
+ zlsd1-ΔQ632
B
n=14/14
n=7/8
probe: gfi1aa
C
n=22/22
n=3/3
probe: gfi1ab
sibling
kdm1a(36433)
kdm1a(36433)
D
n=24/25
n=5/5
E
n=16/17
n=8/8
F
n=19/19
n=9/10

Figure S8. Truncated *lsd1* is unable to rescue gene expression in *lsd1* mutants, related to Figure 4. Single-cell zebrafish embryos from an incross of *kdm1a(23095)* heterozygotes (A-C) or *kdm1a(36433)* heterozygotes (D-F) were injected with *zlsd1-ΔQ632* mRNA. WISH was then performed at 24 hpf with the indicated probes. Representative images are shown with the number of animals with similar staining patterns for the indicated genotype shown in the upper right corner. The lower right corner indicates the mRNA injection conditions. Uninjected mutant embryos are shown in the middle column for comparison. Embryos were imaged, blindly scored by expression and then genotyped by HRMA. Carons indicate ICM expression; closed arrowheads indicate posterior blood island/caudal hematopoietic tissue; narrow triangles indicate inner-hair-cell expression. Scale bar, 200 μm.

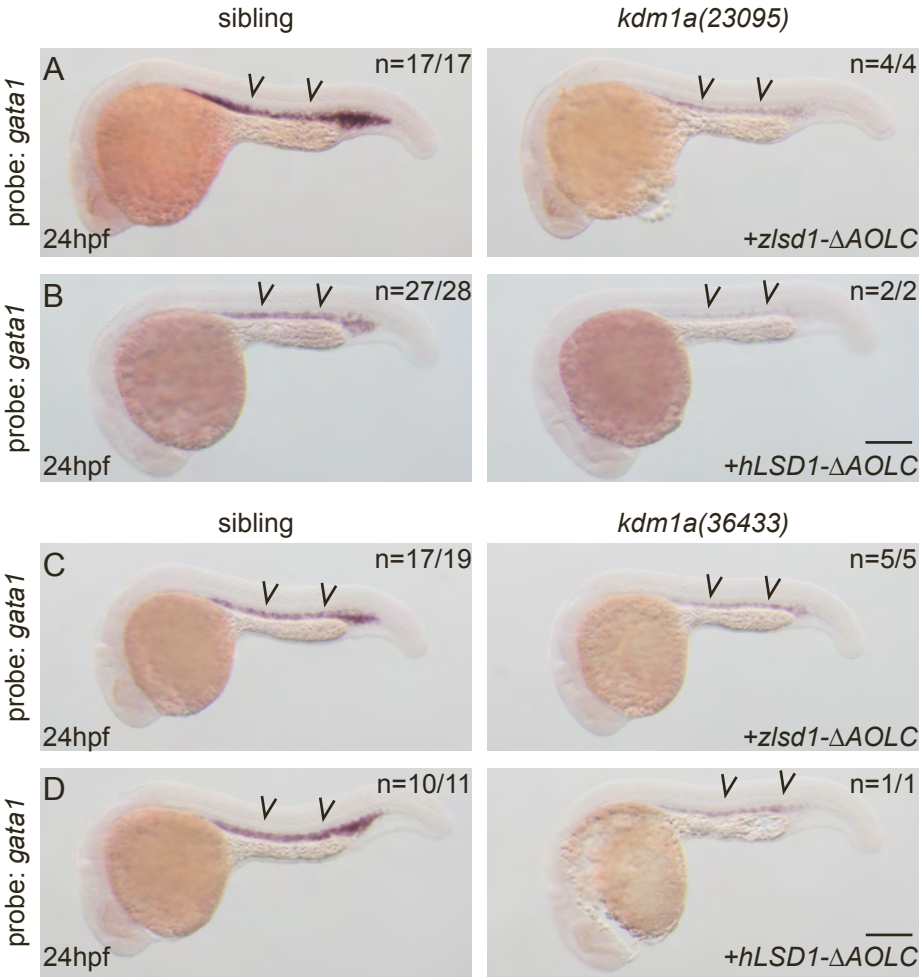

sibling
kdm1a(23095)
A
n=17/17
n=4/4
probe: gata1
24hpf
+zlsd1-ΔAOLC
B
n=27/28
n=2/2
probe: gata1
24hpf
+hLSD1-ΔAOLC
sibling
kdm1a(36433)
C
n=17/19
n=5/5
probe: gata1
24hpf
+zlsd1-ΔAOLC
D
n=10/11
n=1/1
probe: gata1
24hpf
+hLSD1-ΔAOLC

Figure S9. *Lsd1* lacking the AOLC domain is unable to rescue gene expression in *lsd1* mutants, related to Figure 4. (A-D) Single-cell zebrafish embryos from an incross of *kdm1a(23095)* (A-B) or *kdm1a(36433)* (C-D) heterozygotes were injected with *zlsd1-ΔAOLC* (A, C) or *hLSD1-ΔAOLC* (B, D) mRNA. WISH was then performed at 24 hpf with the indicated probes. Representative images are shown with the number of animals with similar staining patterns for the indicated genotype shown in the upper right corner. The lower right corner indicates the mRNA injection conditions. Embryos were imaged, blindly scored by expression and then genotyped by HRMA. Carons indicate ICM expression. Scale bar, 200 μm.

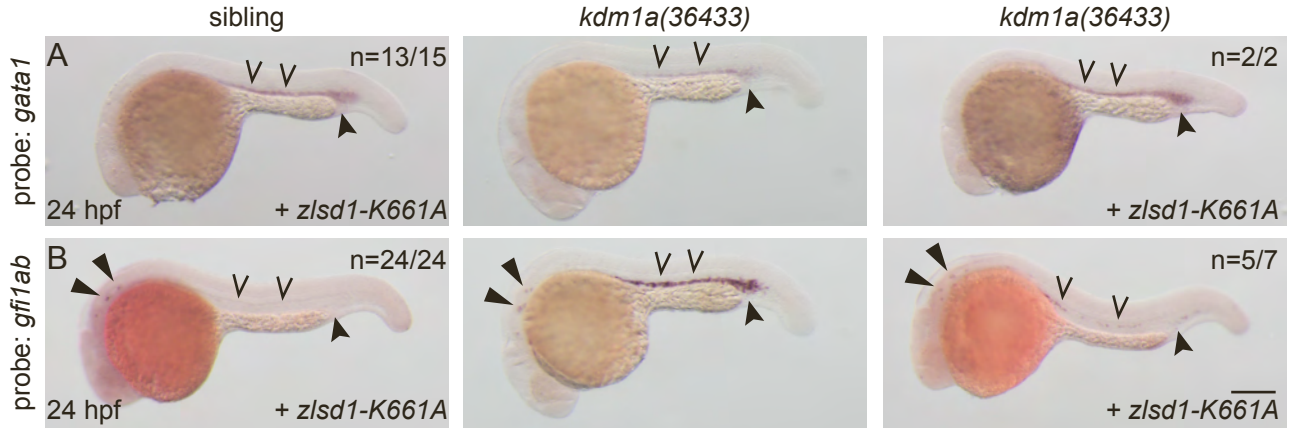

sibling
kdm1a(36433)
kdm1a(36433)
A
probe: gata1
n=13/15
n=2/2
24 hpf
+ zlsd1-K661A
B
probe: gfi1ab
n=24/24
n=5/7
24 hpf
+ zlsd1-K661A

Figure S10. Demethylase-deficient *lsd1* rescues gene expression in *kdm1a(36433)* mutants, related to Figure 5. Single-cell zebrafish embryos from an incross between *kdm1a(36433)* heterozygotes were injected with mRNAs encoding *zlsd1-K661A*. WISH was then performed at 24 hpf for *gata1* (A) and *gfi1ab* (B) expression. Representative images are shown with the number of animals with similar staining patterns for the indicated genotype shown in the upper right corner. The lower right corner indicates the mRNA injection conditions. Uninjected mutant embryos are shown in the middle column for comparison. Embryos were imaged, blindly scored by expression and then genotyped by HRMA. Carons indicate ICM expression; closed arrowheads indicate posterior blood island/caudal hematopoietic tissue; narrow triangles indicate inner-hair-cell expression. Scale bar, 200 μm.

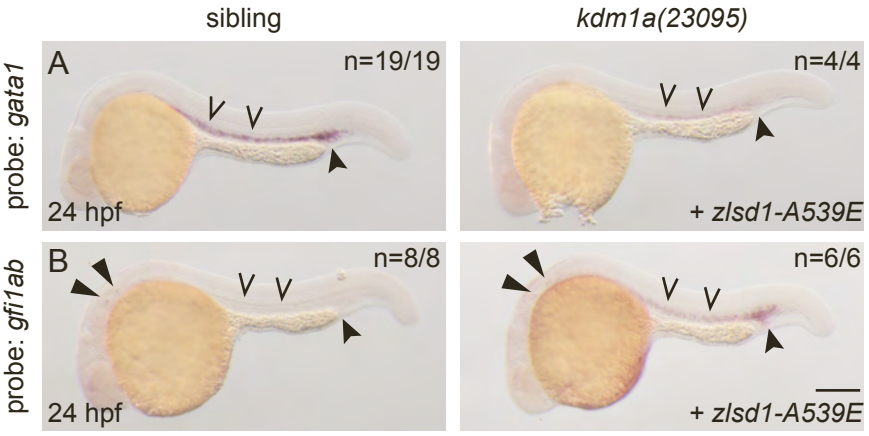

sibling
kdm1a(23095)
probe: gata1
A
n=19/19
n=4/4
24 hpf
+ zlsd1-A539E
probe: gfi1ab
B
n=8/8
n=6/6
24 hpf
+ zlsd1-A539E

Figure S11. Substrate-binding-mutant *lsd1* does not rescue gene expression, related to Figure 5. (A-B) Single-cell zebrafish embryos from an incross between *kdm1a(23095)* heterozygotes were injected with *zlsd1-A539E* mRNA. WISH was performed for *gata1* (A) and *gfi1ab* (B). Representative images are shown with the number of animals with similar staining patterns for the indicated genotype shown in the upper right corner. Embryos were imaged, blindly scored by expression and then genotyped by HRMA. Carons indicate ICM expression; closed arrowheads indicate posterior blood island/caudal hematopoietic tissue; narrow triangles indicate inner-hair-cell expression. Scale bar, 200 μm.

Table S1: LSD1 residues within 4Å of SNAIL, Histone H3, or FAD

4Å of SNAG	4Å of H3	4Å of FAD	4Å of SNAG; no overlap with FAD	4Å of SNAG proline
T335	V333	I284	T335	A539*
L386	T335	G285	L386	N540*
N535*	I356	S286	N535*	D555*
L536	Q358	G287	L536	A809
F538*	L386	V288	F538*	
A539*	N535*	S289	A539*	
N540*	L536	L307	N540*	
W552	A539*	E308	W552	
D553**	N540*	A309	D553**	
D555*	W552	R310	D555*	
D556*	D553**	G314	D556*	
H564	D555*	G315	H564	
L677	D556*	R316	L677	
L693	H564	V317	L693	
Y761*	W695	L329		
A809	Y761*	G330		
T810	A809	A331		
	T810	M332*		
		V333*		
		T588		
		A589		
		V590		
		T624		
		L625		
		P626		
		V637		
		L659*		
		K661*		
		W751		
		W756		
		S760*		
		Y761*		
		G800		
		E801		
		A809		
		T810		
		V811		
		A814		

Table S1. LSD1 residues within 4Å of SNAIL, histone H3, or FAD, related to Figure 6. Residues that are within 4Å of the first 6 amino acids of the SNAIL1 SNAG domain (Baron et al., 2011), the histone-H3 tail (Forneris et al., 2007) or the location of FAD are listed. Residues that are specifically within 4Å of the SNAG domain but not within 4Å of FAD are also listed, as well as residues that are within 4Å of the second proline residue of the SNAG domain. *Residues that have been previously shown to impact LSD1 catalytic activity to any extent. **Asp553 was shown to impact catalytic activity when mutated to Lys but not when mutated to Ala (Chen et al., 2006).

Table S2: List of primers

Primers		
Primer Name	**Sequence**	**Description**
sa23095_HRMA_fwd	cgatgatgtcatcgttggtcgttgt	For HRMA genotyping
sa23095_HRMA_rev	aatgaagcaagcgcctccga	For HRMA genotyping
sa36433_HRMA_fwd	gccgtgttgtgtaccctacctt	For HRMA genotyping
sa36433_HRMA_rev2	gtcttccattctggcagaggag	For HRMA genotyping
sa23095_fwd2	tctgacctttgcatcttcaca	For PCR and sequencing
sa23095_rev2	tttaaggccggtgactgaac	For PCR and sequencing
sa36433_fwd	gtctcaggttgcgaggtgat	For PCR and sequencing
sa36433_rev2	tttgacctaaccagcccaag	For PCR and sequencing
kdm1a_cr3_fwd	agctgaagcagggctaggtt	For PCR and sequencing
kdm1a_exon21_ qpcr_rev	gcgtggcatagtgtacatgg	For PCR and sequencing
sa16850_HRMA_rev1	cctgtatgaatgaacgtgtgctt	For HRMA genotyping
sa16850_HRMA_fwd2	actcggacaccagaccgtatc	For HRMA genotyping
sa40706_HRMA_fwd1	gtttggatgtgacctttgtgga	For HRMA genotyping
sa40706_HRMA_rev1	gacgcatgtttcctcacttcag	For HRMA genotyping
zGfi1B-REV-E2	tcgtttggtcgatgcacattat	For HRMA genotyping
gfi1b_HRMAfwd_MC2	cgaaagcatctcagtaaacaaatctc	For HRMA genotyping
zgfi1aa_sa16850_fwd2	ttgcaggaaagggtcttcag	For PCR and sequencing
zgfi1aa_sa16850_rev1	cctattgaaggcaagcctaatg	For PCR and sequencing
zgfi1ab_sa40706_fwd2	tgtcgcagttgagaggaaaa	For PCR and sequencing
zgfi1ab_sa40706_rev1	acatgaaagctgccagacct	For PCR and sequencing
zgfi1b_e2fwd_seq	tcgtagcctattgcatttgtca	For PCR and sequencing

zGfi1b-REV-E2-a	gcgcgggagtttttgtggagcttgtt	For PCR and sequencing
gfi1aa_ish_fwd	attggtgaccctgaagctga	To clone from cDNA
gfi1aa_ish_rev	agtgtctcacgtgcaagcaa	To clone from cDNA
gfi1ab_ish_fwd	gggacatatgaggcagagga	To clone from cDNA
gfi1ab_ish_rev2	aatctcgtggacgcatgttt	To clone from cDNA
gfi1b_ish_fwd1	atgccacggtcgtttctggt	To clone from cDNA
gfi1b_ish_rev1	aacaaccatctgtcgccttc	To clone from cDNA
fli1a_ish_fwd1	cgcaaaatggacggaactat	To clone from cDNA
fli1a_ish_rev1	agctccagtatggggttgtg	To clone from cDNA
zkdm1a_k661a_ sdm_fwd	atgggctttggaaatctcaacgcggtggtgttgtgttttgata g	For SDM of zLSD1 to K661A
zkdm1a_k661a_ sdm_rev	ctatcaaaacacaacaccaccgcgttgagatttccaaag cccat	For SDM of zLSD1 to K661A
zlsd1_36433sdm_fwd	gggagtgatgaaatagcagccgccggcc	For SDM of zLSD1 to Q632Stop
zlsd1_36433sdm_rev	ggccggcggctgctatttcatcactccc	For SDM of zLSD1 to Q632Stop
zlsd1_d535k_fwd	tcactcaaacactgggatcagaaggatgattttgagtttac gggc	For SDM of zLSD1 to D555K
zlsd1_d535k_rev	gcccgtaaactcaaaatcatccttctgatcccagtgtttgag tga	For SDM of zLSD1 to D555K
zlsd1_a539e_fwd	tgcaaacctggagtttgagaatgccacaccgctgtc	For SDM of zLSD1 to A539E
zlsd1_a539e_rev	gacagcggtgtggcattctcaaactccaggtttgca	For SDM of zLSD1 to A539E
zDeltaTower_fwd	gctcaagagaccgtcagattcttgactgg	To delete zLSD1 Tower domain

zDeltaTower_rev	ggtctcttgagctgtatgaccacttccagtgcttg	To delete zLSD1 Tower domain
Gfi1b_ish_mc2fwd	gccactcaaacaatcaaagga	For RT-PCR of gfi1b
Gfi1b_ish_rev1	aacaaccatctgtcgccttc	For RT-PCR of gfi1b
Gfi1b_rev_e3b	gcgcgagtgatgggattgggcagt	For sequencing RT-PCR product
Gfi1b_e2_s2	ggatcctaatacgactcactata**ggaatcacggaatcatgcca**gttttagagctagaa	For generating sgRNA

Table S3: List of RNA probes

WISH probe	Reference
gata1	Detrich et al., 1995
fli1a	This paper
etv2	Sumanas et al., 2005
gfi1b	This paper
gfi1aa	This paper
gfi1ab	This paper
snail1a	Thisse et al., 1993
snail1b (sna2)	Thisse et al., 1995
snai2 (slug)	Thisse et al., 2001

Table S4. List of differentially expressed genes, related to Figure 3. Genes were filtered to include those that were protein-coding and with padj<0.05.