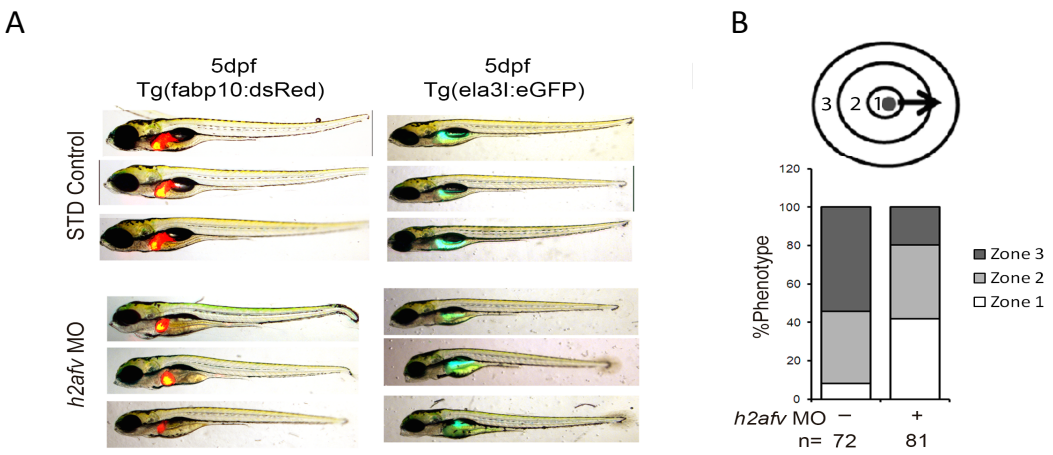
Supplementary Figures

Supplementary Figure 1. *h2afv* morpholino targets both of the zebrafish *h2afv* variants

A. The RNA of both the *h2afv* variants (*h2afva* and *h2afvb)* have 83% sequence homology. The h2afv morpholino (the targeting region indicated in the red box) was designed to target the ATG of both the isoforms.

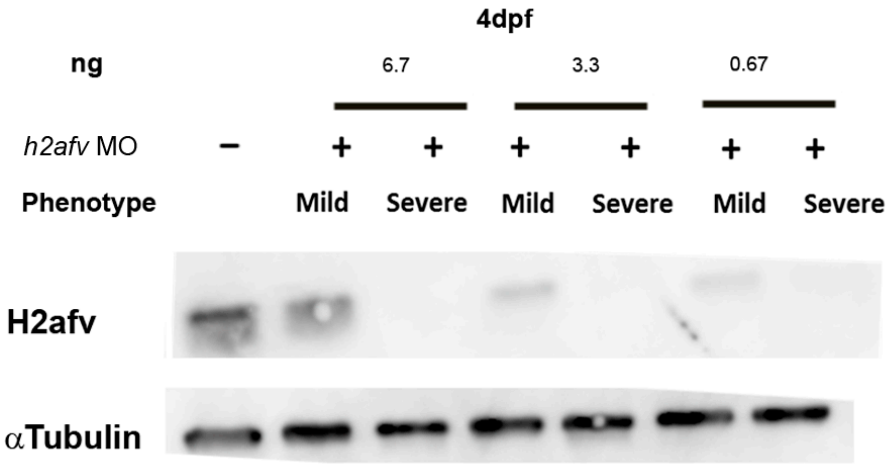
https://www.ncbi.nlm.nih.gov/Class/NAWBIS/Modules/DNA/dna13.html

B. Analysis of publically available RNAseq data (http://www.ebi.ac.uk/gxa/home/) from WT zebrafish embryos shows both *h2afv* paralogs (*h2afva* and *h2afvb)* are expressed during early zebrafish development. While *h2afva* expression is decreased by 70% epiboly, *h2afvb* is stably and strongly expressed during segmentation and till 4 dpf.



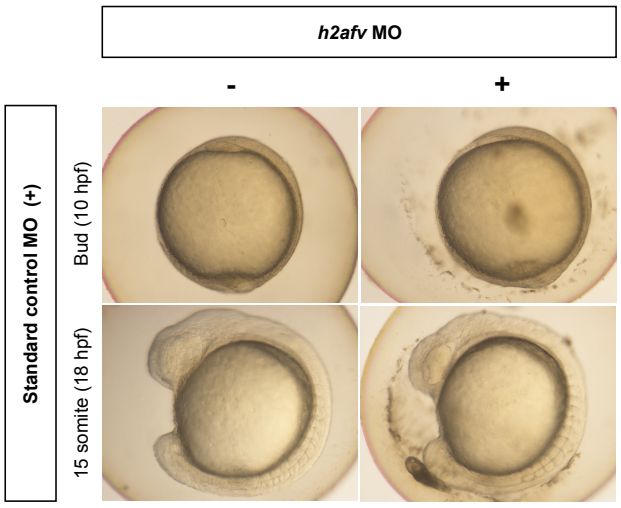
Supplementary Figure 2. *h2afv* morphants display multi- systemic abnormalities

(A) Bright field images of 5 dpf (120 hpf) *Tg(fabp10:dsRed)* and *Tg(ela31:eGFP)* control uninjected and *h2afv* MO injected into single-cell embryos. (B) Motility is severely affected in the *h2afv* morphants at 48 hpf.



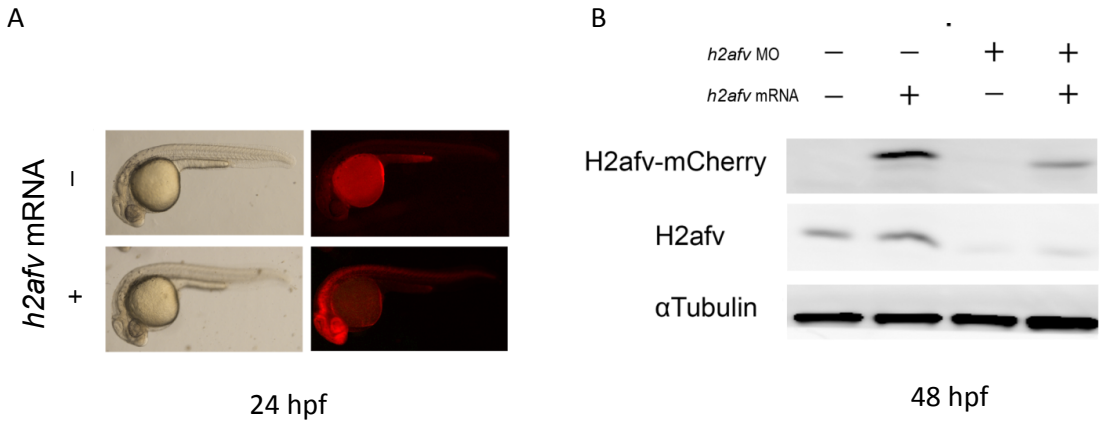
Supplementary Figure 3. H2afv protein depletion is sustained severely affected *h2afv* morphants.

Western Blot analysis of H2afv expression in 4 dpf lysates from *h2afv* MO injected embryos at increasing amounts (0.67, 3.3 and 6.7ng). Embryos were separated based on their phenotype scored as severe and mild (see Materials and Methods).



Supplementary Figure 4. *h2afv* morphants do not exhibit any overt morphological phenotype by 18 hpf.

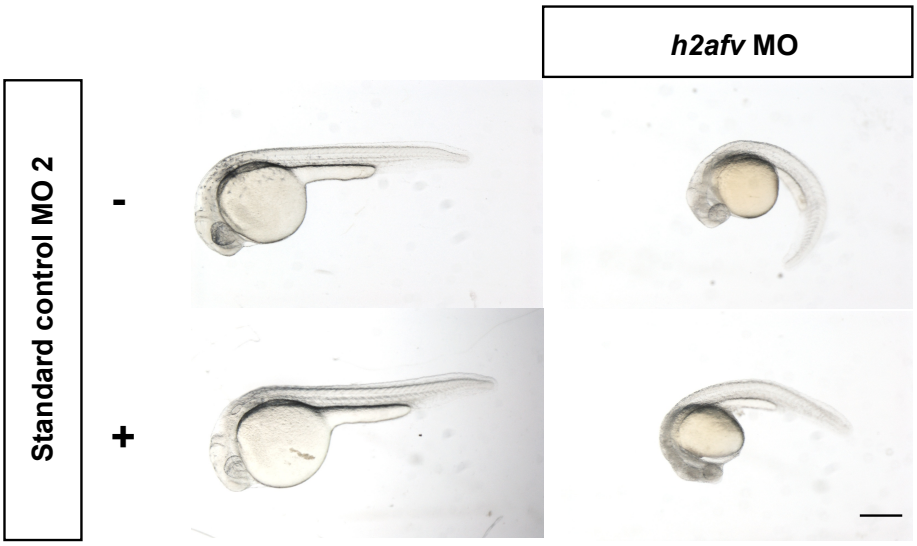
Embryos injected with standard control MO 1, *h2afv* MO or standard control MO 1 + *h2afv* MO were examined for morphological changes at the bud stage (10 hpf) and at the 13-15 somite stage (16-18 hpf) by bright field microscopy. At both the early stages examined, the morphants were phenotypically indistinguishable from their control morpholino injected controls, indicating that the onset of the phenotype occurs later than 18 hpf. Images are representative of over 300 embryos observed from over 5 clutches.



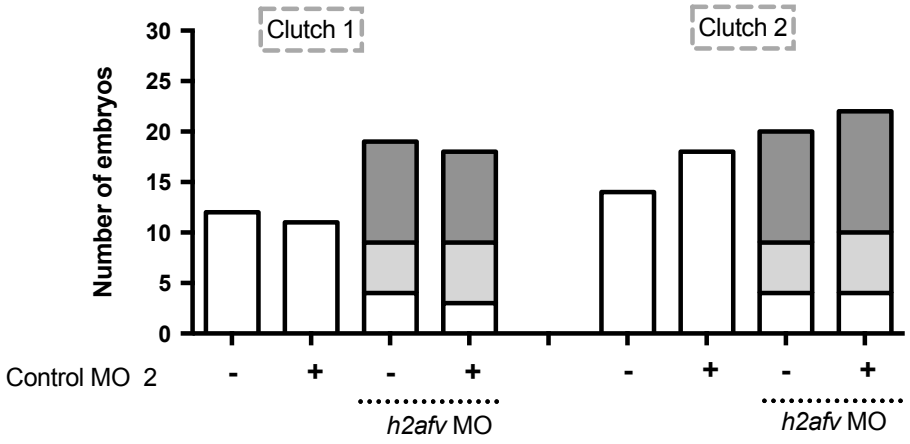
Supplementary Figure 5. *h2afva* mRNA rescues the *h2afv* morphant phenotype.

(A) Fish were injected with mCherry tagged *h2afva* mRNA that has been mutated to a 4 base mis-match with the morpholinos sequence and visualized by fluorescent microscopy at 24 hours in comparison to non-injected controls. (B) Western blot analysis of H2afv and H2afv-mCherry in 48 hpf lysates from fish injected with *h2afv* MO, *h2afv* mRNA, *h2afv* MO + mRNA or standard control MO 1.

A

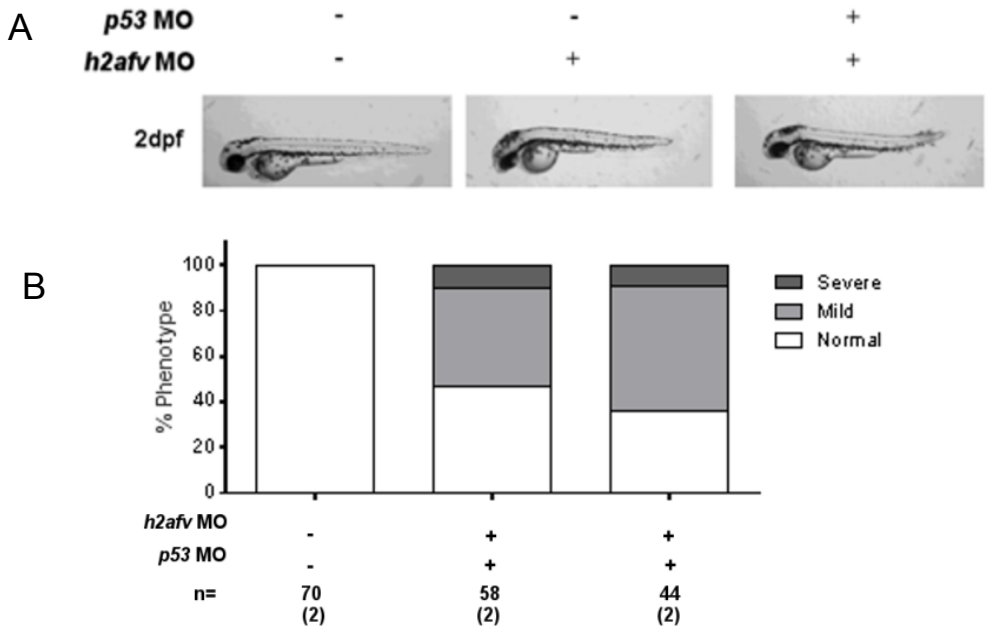


B



Supplementary Figure 6. The *h2afv* morphant phenotype is unaffected by co-injection with standard control morpholino.

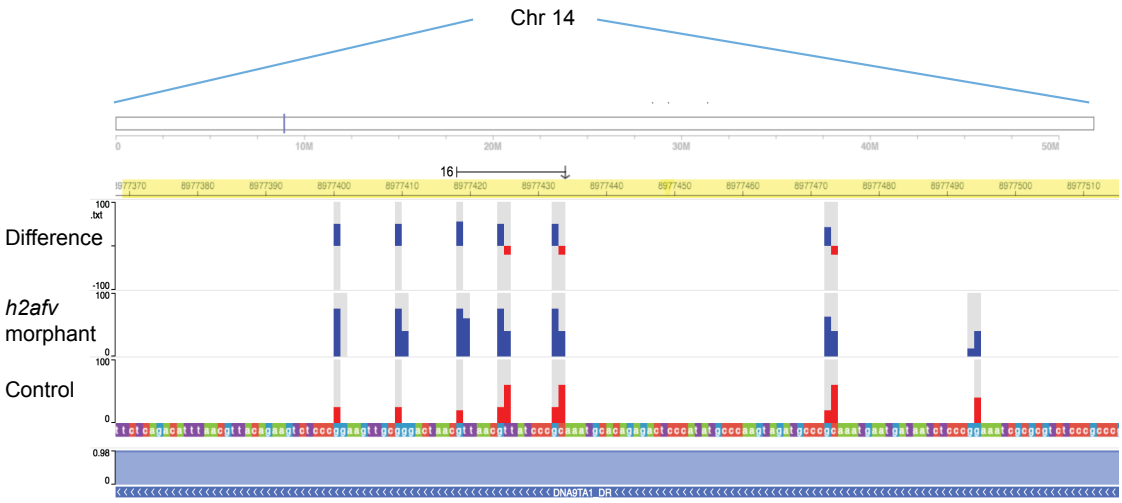
(A) Fish embryos were injected with standard control MO 2, *h2afv* MO or standard control MO 2 + *h2afv* MO and were scored for phenotype at 24 hpf by bright field microscopy. Both the uninjected and standard control MO 2 were identical. Injection of *h2afv* MO along with the standard control MO 2 elicited the same phenotype as seen with *h2afv* MO alone, confirming that the phenotype is specifically due to the loss of H2afv protein. Scale bar: 1000 μm. (B) Analysis of the number of the *h2afv* morphants with and without addition of standard control MO 2 at 24 hpf. The control MO injection (0.67 ng) alone does not elicit any overt phenotype and its addition in *h2afv* morphants does not cause a change the phenotype caused by the h2afv morpholino.



Supplementary Figure 7. The *h2afv* morphant phenotype is unaffected by co-injection with a morpholino targeting *tp53*.

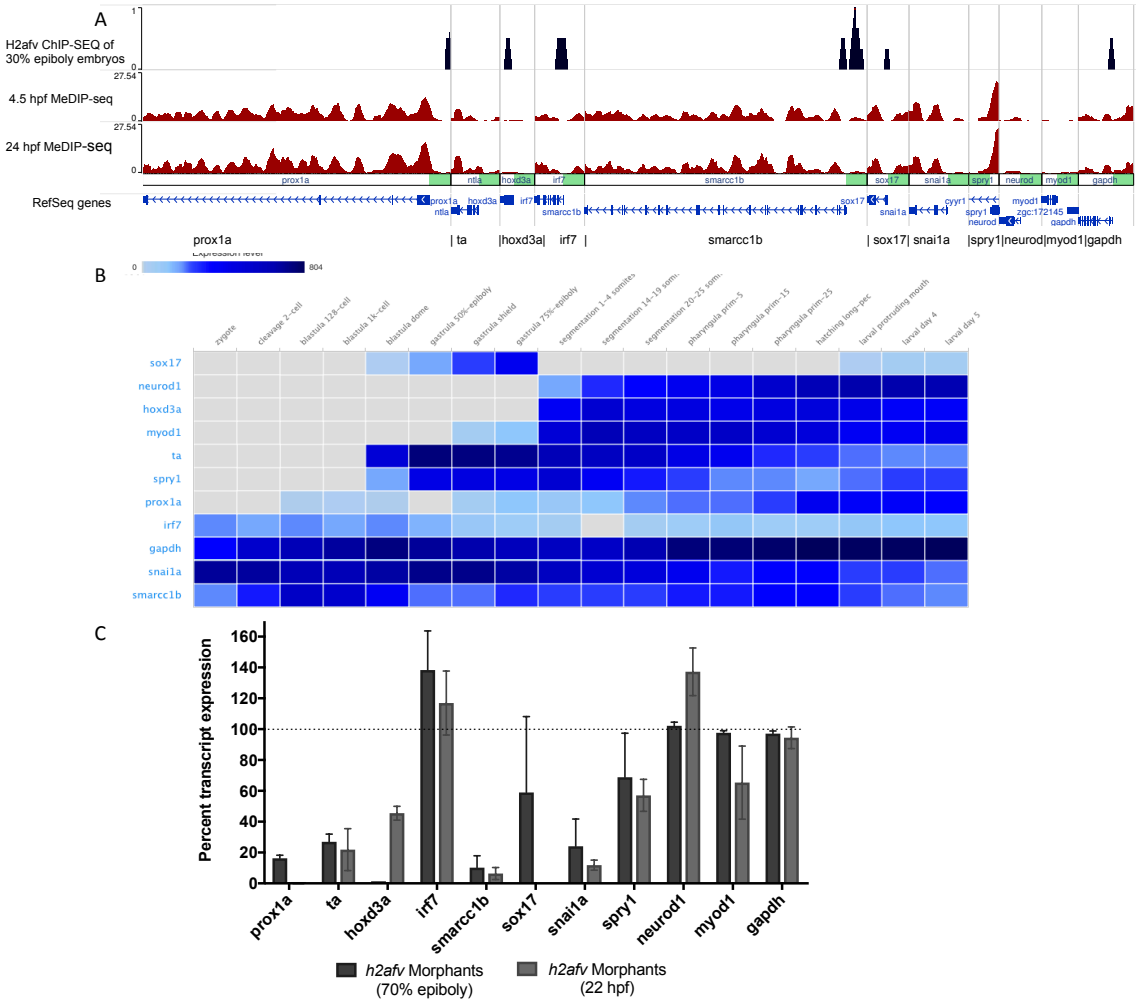
(A) Fish injected with standard control MO 1, *h2afv* MO or *h2afv* + *p53* MO were scored for phenotype at 2 dpf by bright field microscopy. (B)

Phenotypes were scored at 2 dpf. n indicates total number of embryos scored and the number of clutches analyzed is indicated in parenthesis.



Supplementary Figure 8. Comparative analysis of genomic methylation status between the control and *h2afv* morphants at base-pair resolution on a DMR.

A hyper-methylated region of chromosome 14 in epigenome browser view, top panel shows methylation difference at common CpG sites between *h2afv* morphants and control embryos, displayed in blue is increased methylation relative to control in *h2afv* morphants, and in red, decreased methylation in *h2afv* morphants relative to control. Individual methylation status for *h2afv* morphants and control is displayed. Sequence information and annotation can be found at the bottom.



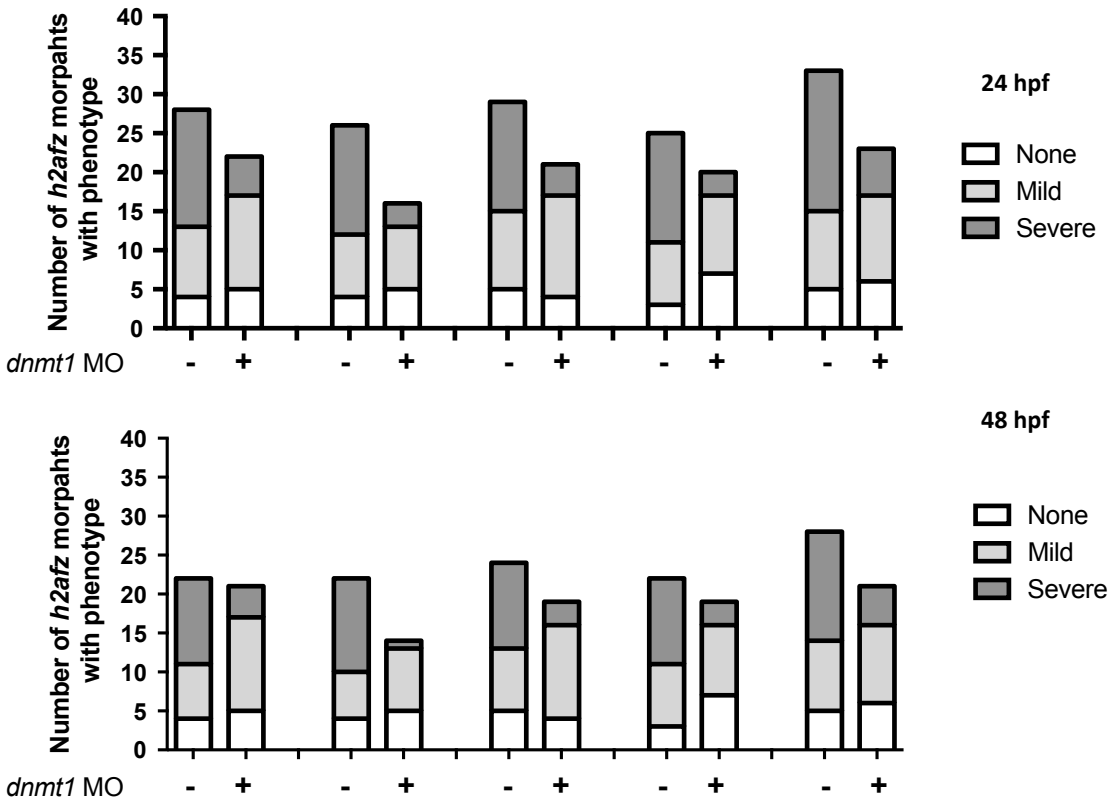
Gene	Control and early embryo	Control and late embryo	Early and late embryo
prox1a	****	****	****
ta	****	****	ns
hoxd3a	ns	****	****
irf7	ns	ns	ns
smarcc1b	***	***	ns
sox17	ns	*	ns
snai1a	***	***	ns
spry1	ns	ns	ns
neurod1	ns	**	**
myod1	ns	*	ns
gapdh	ns	ns	ns

Adjusted p-value
****= <0.0001
***=>0.0001 and <0.001
**= >0.001 and <0.005
*= >0.005 and <0.01
Adjusted p-value

Supplementary Figure 9. H2afv occupancy in 30% epiboly (4.5 hpf) embryos have an overall inverse correlation with DNA methylation on specific genes which are suppressed in *h2afv* morphants.

(A) Publically available H2afv ChIP from 30% epiboly stage embryos[1] shows peaks that are inversely correlated with DNA methylation. Genes are

displayed in a gene set view with 3kb promoter and gene body, upstream of gene body was marked with green. Some of the genes associated with H2afv near the TSS during 30% epiboly were selected based on the analysis of the ChIP-seq data and based on literature[2] (*prox1a, ta, hoxd3a, irf7, smarcc1b, sox17*) and other key genes involved in development are displayed using epigenome browser (http://epigenomegateway.wustl.edu) (B) Expression of the genes associated with H2afv near the TSS from A displayed using expression atlas (http://www.ebi.ac.uk/gxa/home/) (C) Expression of genes with H2afv occupancy in the promoters at 30% epiboly (*prox1a, ntla, hoxd3a, irf7, smarcc1b, sox17)*[1,2] and key genes involved in development (*spry1, snai1a, neurod1, myod1*) and a house-keeping control (*gapdh*) were assessed by qPCR at 30% epiboly and 22 hpf. The average expression in 3 clutches of *h2afv* MO were normalized to uninjected age matched siblings and expressed as the fold change. (D) Quantitative real-time PCR of RNA expression of *h2afv* morphants at both 70% epiboly and at 22 hpf show changes in expression of key genes involved in development. Statistical analysis using an ANOVA to generate an adjusted p-value between control uninjected and early *h2afv* morphants (70% epiboly), between control uninjected and 22 hpf *h2afv* morphants and also between the 70% epiboly and 22 hpf *h2afv* morphants.



Supplementary Figure 10. *dnmt1* morpholino injection *partially* rescues the phenotype of *h2afv* morphants.

Analysis of the number of *h2afv* morphants with and without co-injection of *dnmt1* MO in every clutch scored for either exhibiting mild, severe or no phenotype at both 24 hpf and 48 hpf. Average based on these data are shown in main Figure 5B.

Supplementary Table 1: Table S1 excel spread sheet

Table S2. Primers used for real-time q-PCR

Gene name	Sequence
Zirf7-Fq	GTCCCTCTGATTTGCcG
Zirf7-Rq	GGCAGACCCAGAGAATTGATG
Zntla fq1	TCACCAAGACTGGGAGACGA
Zntla Rq1	ACCCATTCACCGTTCACGTA
Zhoxd3a fq1	CTAATGGATCCAGCACTGCCA
Zhoxd3a rq1	GTCATCGCAGGTTTCTCCTGCT
Zprox1a Fq1	AGCAACTTCCGCGAGTTCTA
Zprox1a Rq1	GAATCGCTCCGGAACCTCAA
Zneurod1 Fq1	CACACCCTAGAGTTCCGACA
Zneurod1 Rq1	GGTCTTGTCCACGTCTCGTT
Zmyod1 Fq1	GACACACCAAATGCTGACGC
Zmyod1 Rq1	ATCCCTCATGCGGAGAACAC
Zspry1 Fq1	CTCTCCACAACACCACAGCA
Zspry1 Rq1	TTTACACTTCCCGCAGCTCT
Zsnai1aFq	TGCATTTCACATCAGCGTTCA
Zsnai1aRq	TCTGTCTGGTGAAGTGTCGTT
Zsmarcc1bFq1	TTCACCAAGGTCGTCCAGTG
Zsmarcc1bRq1	ACCTAACCCTCGGTGAGGAC

Supplemental materials and methods:

Transgenic zebrafish maintenance

Tg(fabp10:dsRed) and Tg(ela31:eGFP) transgenic embryos were collected immediately after spawning and incubated at 28^{o} C in fish water as described in materials and methods. Larvae were imaged at 5 dpf for liver and pancreas size and morphology. Representative images are shown from at least 4 clutches in Figure S2A.

Quantitative real-time PCR

h2afv –fluorescein tagged morpholino was injected into fertilized single cells zebrafish embryos and collected at specific stages of embryonic development (70% epiboly and 22 hpf). Only well-injected *h2afv* morphants were screened and selected based on the expression of the fluorescein tag attached to the morpholino and further processed for RNA extraction. Hence, the data in Figure S9 represents the embryos that belong to the "severe" *h2afv* MO phenotype.

Motility Assay

Motility of 48 hpf embryos was analyzed by placing a single embryo in the center of a Petri dish marked with concentric rings. Swimming distance in response to manual stimulation of the head using a fine needle was measured by scoring the zone in which the embryo ended its escape response.

Bioinformatic analysis of H2afv occupancy and DNA methylation

H2afv Chip-seq data at dome-30%-epiboly embryo was from NCBI SRA database (ID SRA055273), which was mapped to Zv9/danRer7 reference genome using Bowtie aligner. Final conversion to SGA files and call peaks were done using ChIP-Seq [3]. DNA methylation data at 4.5hpf, 6hpf and 24hpf were

incubated in WashU Epigenome Browser, also H2afv peaks were visualized in a gene set view with epigenome browser (PMC3643794).

REFERENCES

1. Nepal C, Hadzhiev Y, Previti C, Haberle V, Li N, Takahashi H, Suzuki AM, Sheng Y, Abdelhamid RF, Anand S and others. Dynamic regulation of the transcription initiation landscape at single nucleotide resolution during vertebrate embryogenesis. Genome Res 2013;23(11):1938-50.
2. Wu S-F. Epigenomes of Zebrafish Mature Sperm and Early Embryos. ProQuest: The University of Utah; 2013.
3. Ambrosini G, Dreos R, Kumar S, Bucher P. The ChIP-Seq tools and web server: a resource for analyzing ChIP-seq and other types of genomic data. BMC Genomics 2016;17(1):938.