Supplementary figures

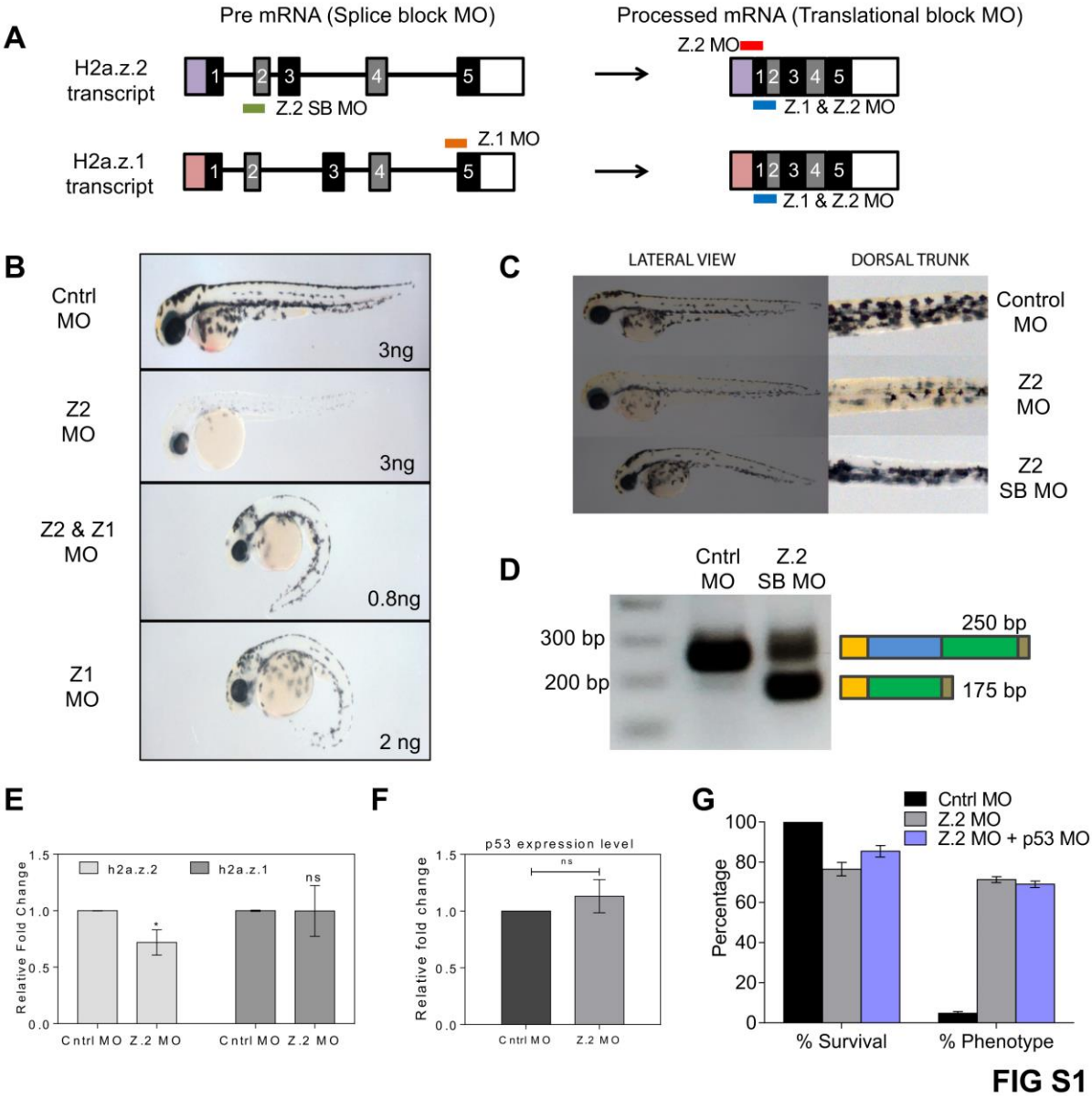

Fig S1: Validating H2a.z.2 specific pigmentation phenotype using multiple silencing approaches

(A) Schematic representing the target region for H2a.z.2 MO (Z2), H2a.z.1 MO (Z1), H2a.z.2 splice block (Z2 SB MO) and Z2 & Z1 MO.

(B) Brightfield images representing 48 hpf embryos injected with Control MO, Z2 MO, Z1 MO, Z1 & Z2 MO.

(C) Brightfield images showing lateral and dorsal view of Control MO, Z2 MO and Z2 SB MO embryos at 48 hpf.

(D) H2a.z.2 RT-PCR amplicons from control and Z2 SB MO injected embryos depicting the mis-spliced product.

(E) Bar graphs representing mRNA levels of h2a.z.1 and h2a.z.2 upon Z2 MO injection. Bars represent mean ± SEM across n=3 biological replicates.
(F) Bar graphs representing relative p53 mRNA levels in Z2 MO as compared to control MO. Bars represent mean ± SEM across n=5 biological replicates.
(G) Grouped bar plots representing percentage survival and pigmentation phenotype observed across control MO, Z2 MO and Z2 + p53 MO. Bars represent mean ± SEM across n=6 biological replicates.

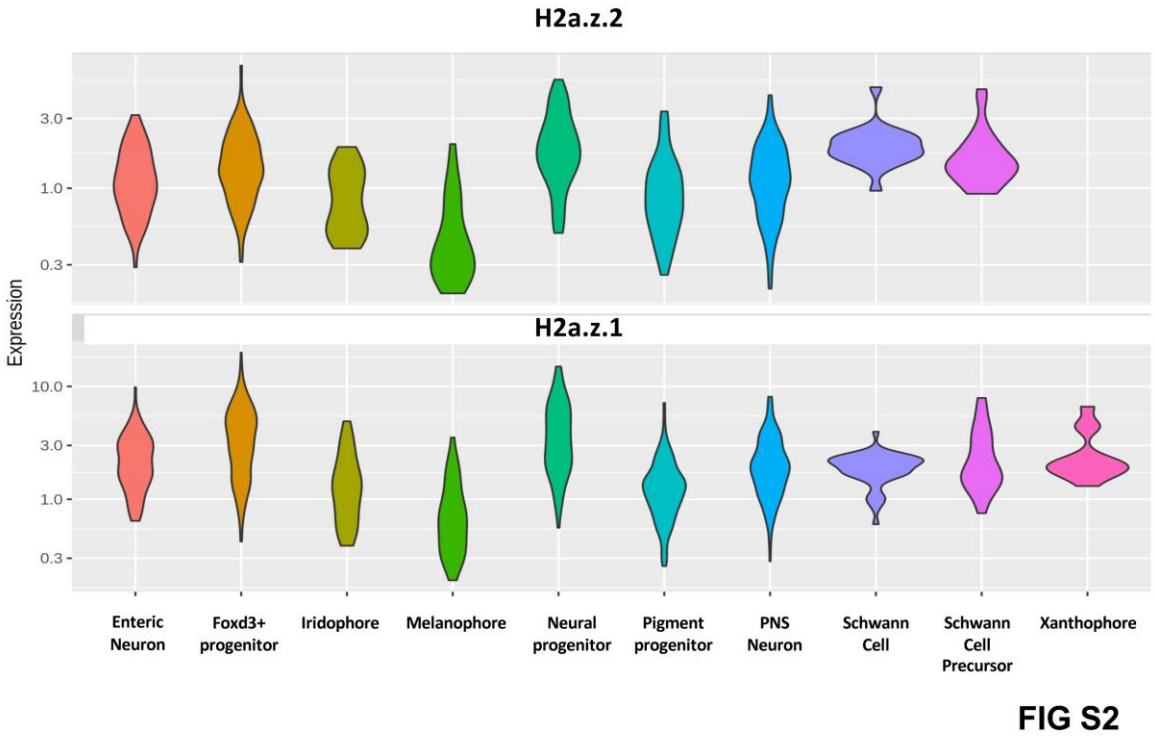

Fig S2: Single cell meta-analysis of zebrafish sox10 positive cells and their derivatives. Expression of H2a.z.2 (Adameyko et al.) and H2a.z.1 (bottom) from zebrafish single cell sequencing data (GSE131136), X-axis different cell types are indicated, Y-axis represents gene expression values in UMI counts.

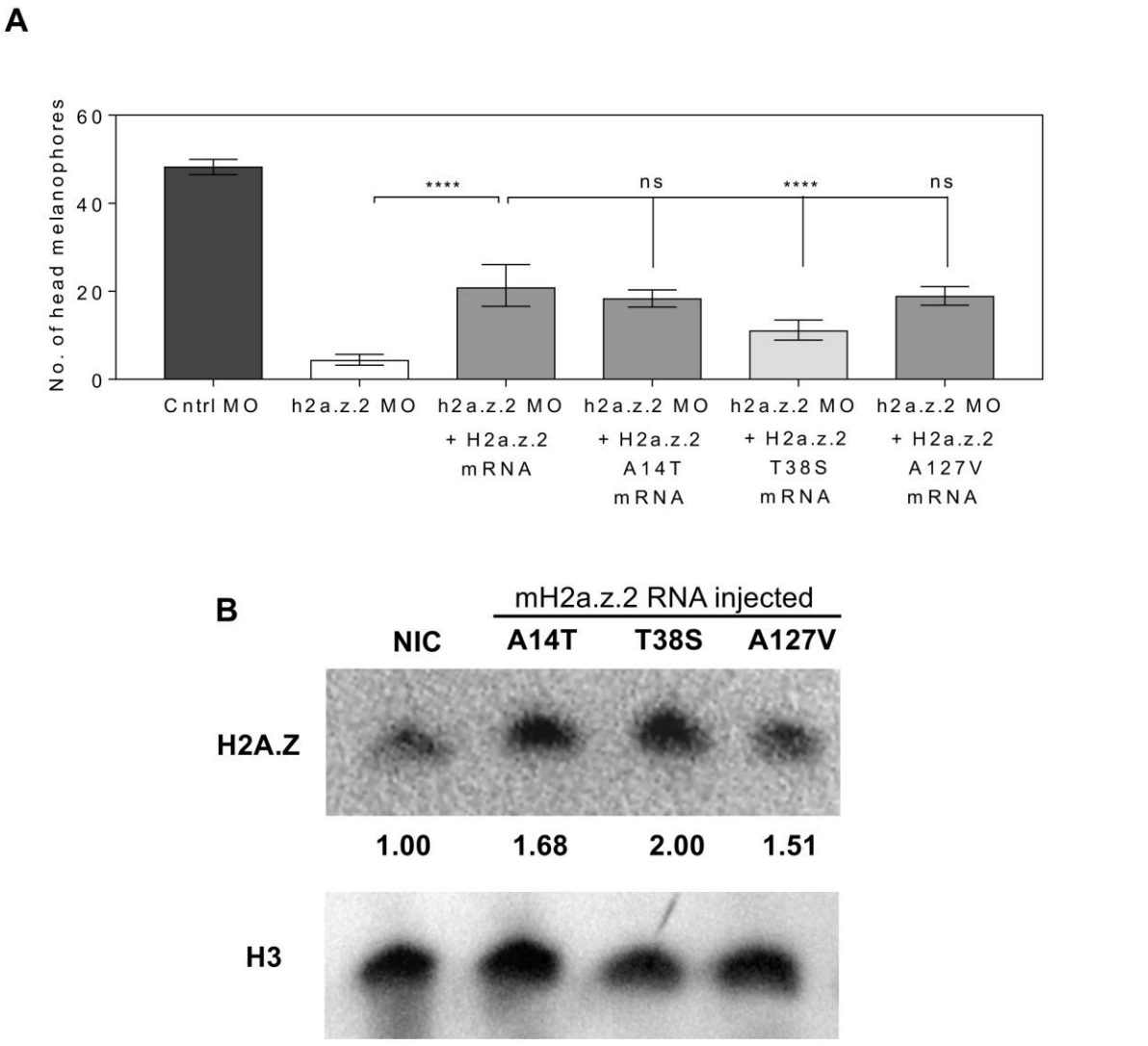

FIG S3

Fig S3: A single amino acid substitution of T38S in mouse H2A.Z.2 contributes largely to the observed pigmentation effects

(A) Bar graphs representing the number of head melanophores, denote geometric mean with 95% CI, representing data of n ≥ 50 embryos.

(B) Western blot analysis using H2AZ antibody in RNA injected embryos with H2a.z.2 mutants, normalised to H3. Numbers represent H3 normalized fold change compared to non-injected control (Adameyko et al.).

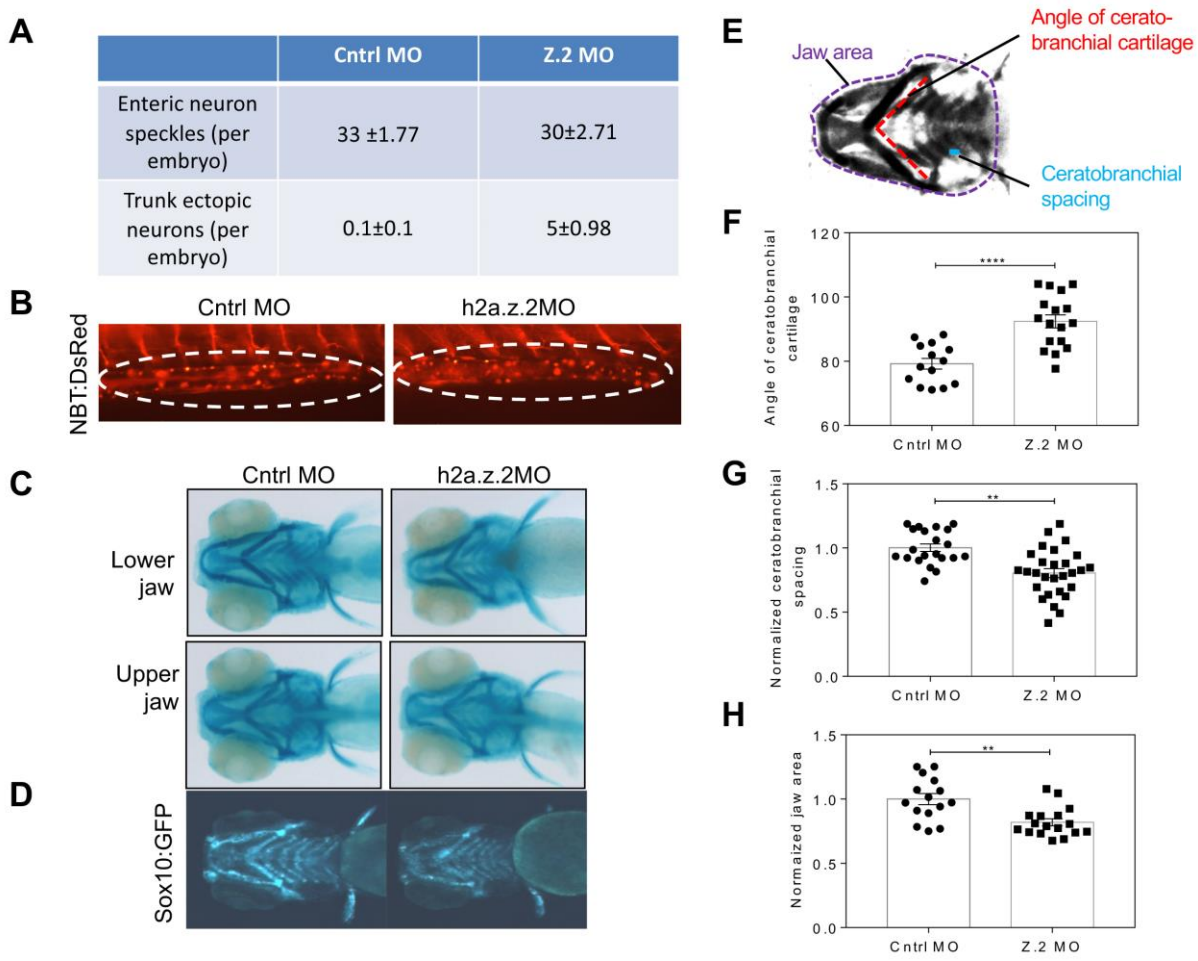

FIG S4

Fig S4: Status of neural crest derivatives in H2a.z.2 morphants

(A) Table enumerating the number of enteric, and trunk ectopic neurons observed in control and Z2 morphants. Numbers represent mean ± SD counted manually across ~50 embryos.

(B) Fluorescence images of enteric neurons in control and Z2 morphants in *Tg(NBT:DsRed)* fish embryos at 5 dpf.

(C) Alcian blue staining highlighting the craniofacial cartilage and fin cartilage in control and Z2 morphants.

(D) Fluorescence images of *Tg(sox10:EGFP)* head region representing the craniofacial cartilage system in control and Z2 morphants.

(E) Image representing the parameters utilized for jaw defect quantification in Z2 morphants represented in panels F to H.

(F) Bar graphs representing angle of the ceratobranchial cartilage in Z2 MO as compared to control. Bars represent mean ± SEM across $n \geq 15$ embryos.

(G) Bar graphs representing ceratobranchial spacing in Z2 MO as compared to control. Bars represent mean ± SEM across $n \geq 15$ embryos.

(H) Bar graphs representing area of the jaw in Z2 MO as compared to control. Bars represent mean ± SEM across $n \geq 15$ embryos.

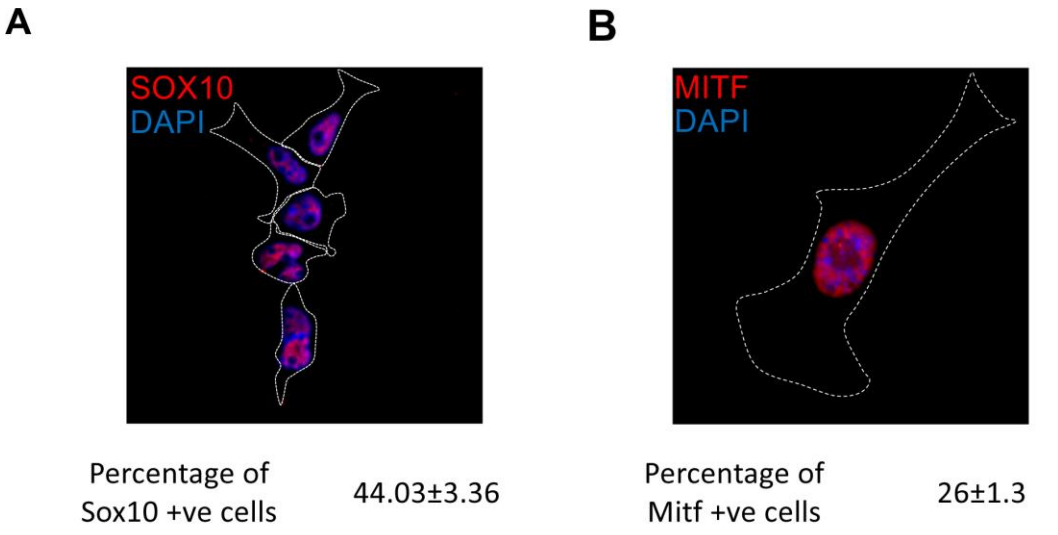

Fig S5: R1E to melanocyte differentiation generates SOX10 and MITF positive cells

(A) Immunocytochemistry for SOX10 counterstained with DAPI in Day10 after differentiation in melanocyte promoting medium.

(B) Immunocytochemistry for MITF counterstained with DAPI in Day10 differentiated cells.

Numbers at the bottom of the image represents percentage of cells positive for SOX10 and MITF staining respectively. At least 500 cells were taken for analysis.

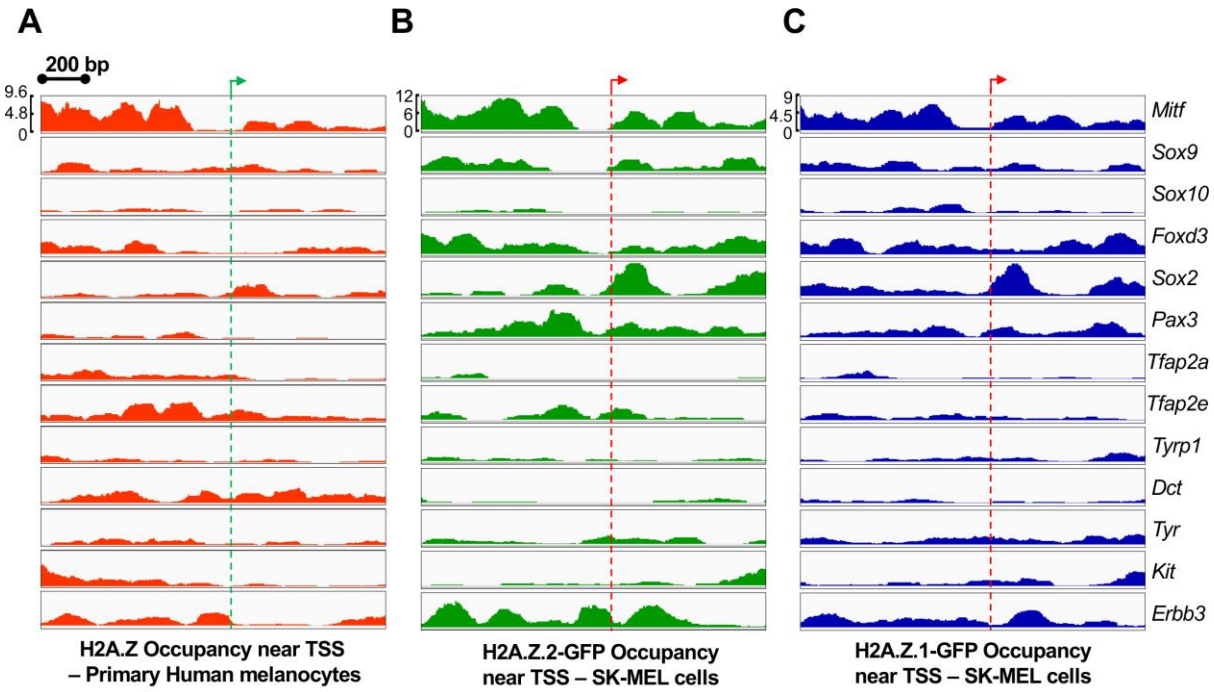

Fig S6: H2A.Z occupancy in primary melanocytes and SKMEL cells

(A-C) Metanalysis of chromatin immunoprecipitaton (ChIP) data in melanocyte derived lines from GSE68223.

(A) H2A.Z (Z1 + Z2) occupancy near transcription start site (TSS) in primary human melanocytes.

(B) H2A.Z.2-GFP occupancy near TSS in SK-Mel 147 metastatic melanoma cells.

(C) H2A.Z.1-GFP occupancy near TSS in SK-Mel 147 metastatic melanoma cells.

Dotted line indicates the TSS. Gene names are displayed on right corresponding to their ChIP seq profiles. Percent enrichment relative to input in the ChIP-seq data is displayed to the left for the top panel.

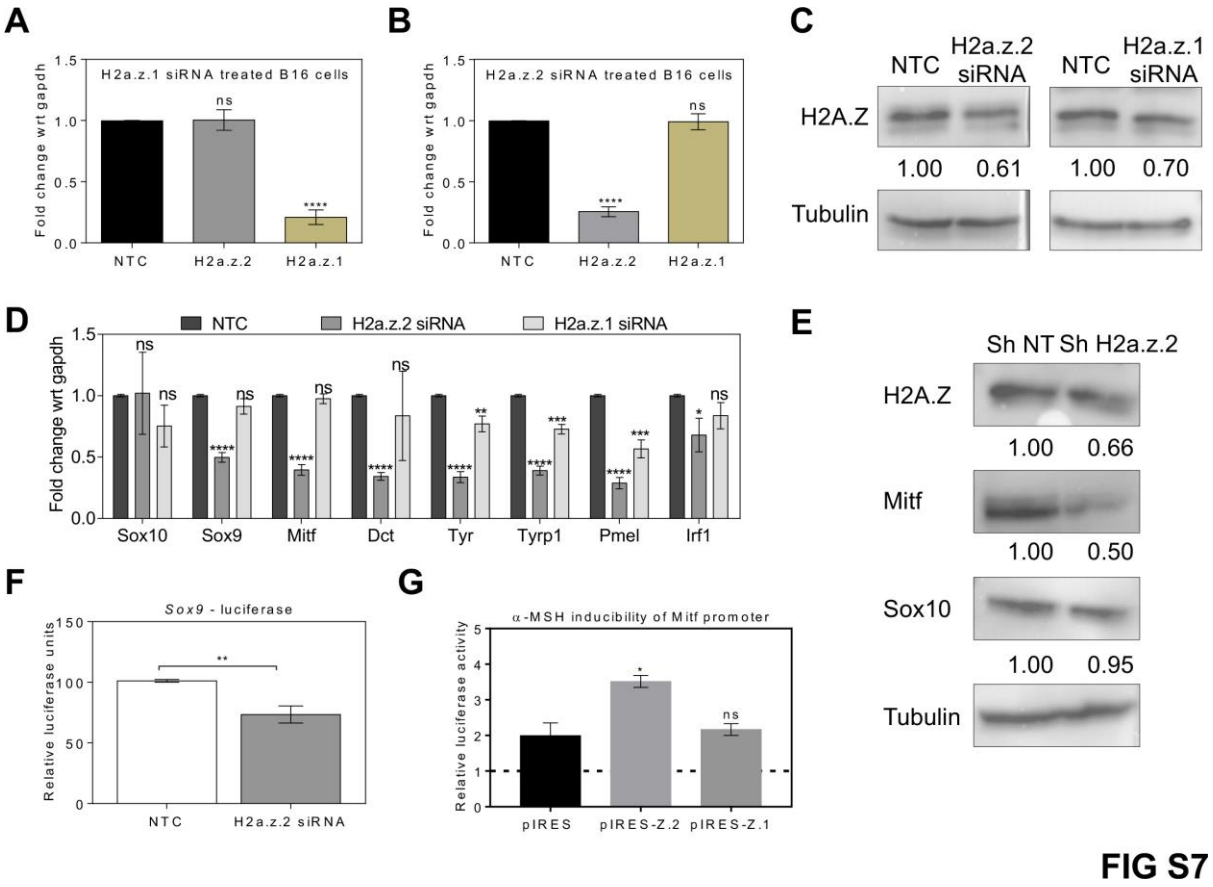

FIG S7

Fig S7: H2A.Z mediates gene expression changes in B16 melanoma cells

(A) Bar graph representing mRNA levels of *H2a.z.1* and *H2a.z.2* upon *Z1* silencing (mean ± SEM, n=3).

(B) Bar graph representing mRNA levels of *H2a.z.1* and *H2a.z.2* upon *Z2* silencing (mean ± SEM, n=3).

(C) Western blot analysis of total H2A.Z levels upon H2a.z.1 and H2a.z.2 siRNA treatment, normalized to tubulin.

(D) Bar plot representing relative mRNA levels of pigmentation related genes upon Z2 and Z1 silencing in B16 cells (mean ± SEM, n=3).

(E) Western blot analysis of total H2A.Z, MITF and SOX10 levels upon H2a.z.2 shRNA treatment in B16 melanoma cells, normalized to tubulin. Numbers represent tubulin normalized values with respect to shNT condition.

(F) Bar graphs representing the relative Sox9 luciferase activity of cells treated with non targeting control and Z2 siRNA (mean±SEM, N=4).

(G) Bar graphs representing the relative Mitf luciferase activity of B16 stable cells transfected with empty pIRES vector or pIRES containing H2a.z.1 or H2a.z.2 upon α-MSH treatment (mean ± SEM, n=4).

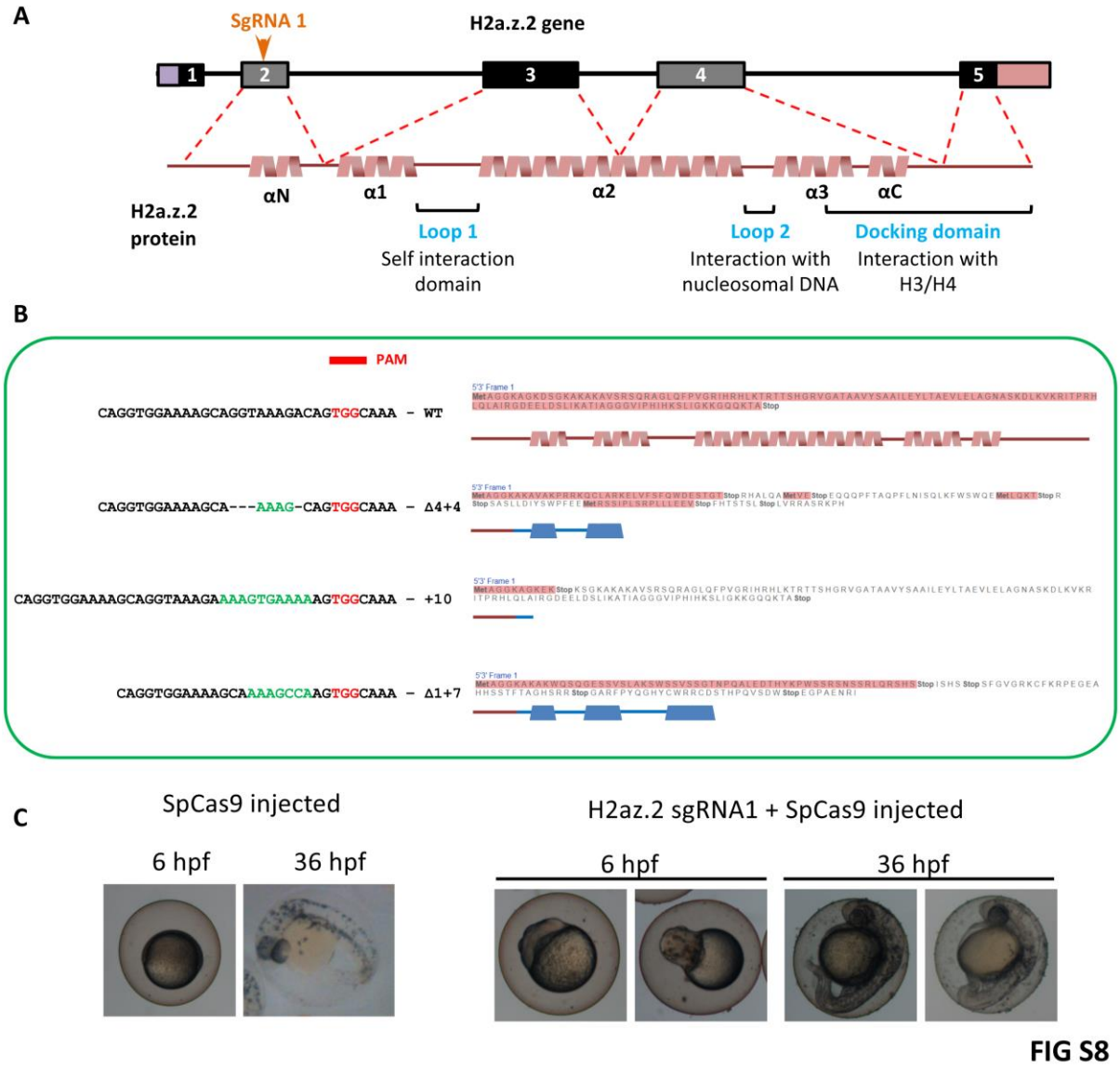

FIG S8

Fig S8: CRISPR mediated targeting of *h2a.z.2* N-terminus region is lethal in zebrafish

(A) Schematic representing the region targeted by *h2a.z.2* sgRNA1.

(B) Sequences displaying representative mutations occurred upon *h2a.z.2* sgRNA1 injections leading to embryonic lethality; predicted protein sequences are displayed on the right hand side.

(C) Bright field images of SpCas9 and SpCas9 + sgRNA1 injected embryos at 6 and 36 hpf.

TABLE S1

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Anti-Histone H2A.Z antibody	Abcam	Cat No: ab4174; RRID:AB_304345
Anti-Histone H3 antibody	Abcam	Cat No:ab1791; RRID:AB_302613
Anti-Beta tubulin antibody-HRP	Abcam	Cat No: ab21058; RRID:AB_727045
Anti-MITF antibody	Abcam	Cat No: ab12039; RRID:AB_298801
Anti-SOX10 antibody	Abcam	Cat No: ab155279; RRID:AB_2650603
Anti-Digoxigenin-AP, Fab fragments from sheep	Sigma	Cat No: **11093274910** Roche ; RRID:AB_514497
Anti-Rabbit IgG	Thermo Fisher Scientific	Cat No: 02-6102; RRID:AB_2532938
Goat anti-Mouse IgG (H+L) Cross-Adsorbed ReadyProbes™ Secondary Antibody, Alexa Fluor 594, Thermo Fisher Scientific	Thermo Fisher Scientific	Cat No: R37121; RRID: AB_2556549
Goat anti-Rabbit IgG (H+L) Cross-Adsorbed ReadyProbes™ Secondary Antibody, Alexa Fluor 594, Thermo Fisher Scientific	Thermo Fisher Scientific	Cat No: A-11037; RRID: AB_25344095
Chemicals, Peptides, and Recombinant Proteins		
N-Phenylthiourea	Sigma	Cat No: P7629
Pronase	Sigma	Cat No: P8811
TrypLE Express	Thermo Fisher Scientific	Cat No: 12604039
DMEM-High glucose media	Sigma	Cat No: D5648
Fetal Bovine Serum	Thermo Fisher Scientific	Cat No: 10270-106
DMEM-Glutamax supplemented with sodium pyruvate	Thermo Fisher Scientific	Cat No: 10569-010
MEM-NEAA	Thermo Fisher Scientific	Cat No: 11140-050
Beta-mercaptoethanol	Thermo Fisher Scientific	Cat No: 31350-010
PANSERA	Pan Biotech	Cat No: P30-2602
Collagen	Thermo Fisher Scientific	Cat No: A10483-01
M254	Thermo Fisher Scientific	Cat No: M254-CF
SCF	Peprotech	Cat No: 300-07-10

Endothelin-3	Sigma	Cat No: E9137
Cholera toxin	Sigma	Cat No: C8052
Dexamethasone	Sigma	Cat No: D1756
WNT-3a	Peprotech	Cat No: 315-20-10
b-FGF	Thermo Fisher Scientific	Cat No: RFGFB50
PMA	Sigma	Cat No: P1585
N2-Supplement	Thermo Fisher Scientific	Cat No: 17502-048
Anti-Anti	Thermo Fisher Scientific	Cat No: 15240-062
Protein A agarose beads	G-Biosciences	Cat No: 786-283
SYBR Green	KAPA biosystems	Cat No: KK4601
Alpha-MSH	Sigma	Cat No: M4135
Critical Commercial Assays		
Zero Blunt TOPO vector	Thermo Fisher Scientific	Cat No: K287540
T7-ULTRA mRNA synthesis kit	Thermo Fisher Scientific	Cat No: AM1345
Nucleospin RNA XS kit	Macherey Nagel	Cat No: 740902
Non targeting control siRNA	Dharmacon ON-TARGETplus	Cat No: D-001810-10-05
Mouse *H2a.z.2* siRNA	Dharmacon ON-TARGETplus	Cat No: L-063612-01-0005
Mouse *H2a.z.1* siRNA	Dharmacon ON-TARGETplus	Cat No: L-042994-01-0005
Dharmafect transfection reagent	Dhamacon	Cat No: T-2001
Lipofectamine 2000	Thermo Fisher Scientific	Cat No: 11668019
Nucleospin Triprep	Macherey Nagel	Cat No: 740966
Superscript III cDNA synthesis kit	Thermo Fisher Scientific	Cat No: 1800051
GIPZ *H2a.z.2* shRNA	Dharmacon	Cat No: RMM4532-EG77605
BCA Kit	Thermo Fisher Scientific	Cat No: 23225
QUBIT ds HS DNA estimation kit	Thermo Fisher Scientific	Cat No: Q32851
Dual luciferase assay system	Promega	Cat No: E1960
T7 Megashortscript kit	Thermo Fisher Scientific	Cat No: AM1354

Deposited Data		
Raw and analyzed microarray data	This paper	GSE133141
Experimental Models: Cell Lines		
B16 melanoma cell		RRID: CVCL_0158
ES-R1 cell line		RRID: CVCL_2167
Experimental Models: Organisms/Strains		
ASWT	Patowary A et al; 2013.	ZFIN ID: ZDB-PUB-130423-9
Tg(–4.9Sox10:egfp)^{ba2}	Carney T J et al; 2006.	ZFIN ID: ZDB-ALT-050913-4
Tg(mitfa:GFP)^{w47}	Curran K et al; 2009.	ZFIN ID: ZDB-ALT-081203-1
Tg(NBT-dsRed)	Peri F et al; 2008.	ZFIN ID: ZDB-TGCONSTRCT-081023-2
Tg(foxd3:GFP)	Gilnour DT et al; 2002	ZFIN ID: ZDB-TGCONSTRCT-070117-95
Tg(ftyrp1:GFP)	Zou J et al; 2006.	N/A
Oligonucleotides		
T7 promoter-Mouse H2a.z.1 CDS FP: TAATACGACTCACTATAGGGAGAGCA AACATGGCTGGCGGTAAGGC	This paper; Sigma	N/A
Mouse H2a.z.1 CDS RP: TTAAACAGTCTTCTGTTGTCCTTTC	This paper; Sigma	N/A
T7 promoter-Mouse H2a.z.2 CDS FP: TAATACGACTCACTATAGGGAGAGCA AACATGGCTGGAGGCAAAGCTG	This paper; Sigma	N/A
Mouse H2a.z.2 CDS RP: CTAAGCAGTTTTCTGCTGCCCC	This paper; Sigma	N/A
Zebrafish mitfa CDS FP: ATGTTGGAGATGCTCGAGTA	This paper; Sigma	N/A
Zebrafish mitfa CDS RP: CTAACAGCCATTGTCATGTT	This paper; Sigma	N/A
Zebrafish *h2a.z.2* WISH FP: GTGCAGACATGACTCAAGGACT	This paper; Sigma	N/A
Zebrafish *h2a.z.2* WISH RP: ATAAAACAGCTCCACGGCTC	This paper; Sigma	N/A
Z.2 Splice block check primer FP: ACCTCCCAGGATCCCATTCA	This paper; Sigma	N/A
Z.2 Splice block check primer RP: TGCCAACTCCAAAACTTCAGC	This paper; Sigma	N/A
Z.1 MO TGTGAGGAATGACTCCTGCGGACGC	Sivasubbu S et al; 2006	N/A

Z.2 MO CCACCTGCCATTTCAGCGATGT	This paper; Genetools	N/A
Z.1 – Z.2 MO CTTTACCTGCTTTTCCACCTGCCAT	This paper; Genetools	N/A
Z.2 Splice block MO TCCACCTGCCTGCAAAACAATAATT	This paper; Genetools	N/A
Control MO CCTCTTACCTCAGTTACAATTTATA	Genetools	N/A
P53 MO GCGCCATTGCTTTGCAAGAATTG	Genetools	N/A
Zebrafish *h2a.z.2* sgRNA 1 GGAAAAGCAGGTAAAGACAG	This paper; Sigma	N/A
Zebrafish *h2a.z.2* sgRNA 2 GGGAGCTCCTCATCTCCTCGAA	This paper; Sigma	N/A
Zebrafish *h2a.z.2* sgRNA 3 GGAGCTCGATTCCCTTATCA	This paper; Sigma	N/A
Zebrafish *h2a.z.2* sgRNA 4 GGAACTATGCGGTTTTCTGC	This paper; Sigma	N/A
Zebrafish *h2a.z.2* sgRNA 1 target region amplification FP TGTTTGCTTGCATTGGATTGAGT	This paper; Sigma	N/A
Zebrafish *h2a.z.2* sgRNA 1 target region amplification RP CAGCTGTGAGATATTCAAGAATGG	This paper; Sigma	N/A
Zebrafish *h2a.z.2* sgRNA 2&3 target region amplification FP CATTGAAGCTGATATGGCAAACTT	This paper; Sigma	N/A
Zebrafish *h2a.z.2* sgRNA 2&3 target region amplification RP AGGGGTCAATTTTGAAGCTCTTG	This paper; Sigma	N/A
Zebrafish *h2a.z.2* sgRNA 4 target region amplification FP CCCGAGCTGTCTTAATGTGC	This paper; Sigma	N/A
Zebrafish *h2a.z.2* sgRNA 4 target region amplification RP TGGATTAAAGGATGAACACAAGC	This paper; Sigma	N/A
Mitf ChIP FP TTCTGGTCCAAGTCCCAAGC	This paper; Sigma	N/A
Mitf ChIP RP ACTTCGAAATCCGCCACGAT	This paper; Sigma	N/A
Sox9 ChIP FP CTCGGAACTGCCTGGAAACTT	This paper; Sigma	N/A
Sox9 ChIP RP AAAACAGAGAACGAAACCGGG	This paper; Sigma	N/A
Sox10 ChIP FP TTCAGGCTCCGTCCAGACAAG	This paper; Sigma	N/A
Sox10 ChIP RP CAAGGTGTGCGGTCCAGC	This paper; Sigma	N/A
Foxd3 ChIP FP CGTAGAGAAGCGTCGAGGAC	This paper; Sigma	N/A

Foxd3 ChIP RP GTCAGCTCACACGAGGAGG	This paper; Sigma	N/A
Pax3 ChIP FP GTCTCCTCCTCGGCCAATCG	This paper; Sigma	N/A
Pax3 ChIP RP GGGATCCGGACTAGGGAGC	This paper; Sigma	N/A
Sox2 ChIP FP GGCAGAGAAGAGAGTGTTTGC	This paper; Sigma	N/A
Sox2 ChIP RP CTTAAGCCTCGGGCTCCAAA	This paper; Sigma	N/A
Tfap2a ChIP FP TGTGATTCGCCAGACGCC	This paper; Sigma	N/A
Tfap2a ChIP RP GAGACAAAAAGCGAGCGACG	This paper; Sigma	N/A
C-kit ChIP FP ATCTGCTCTGCGTCCTGTTG	This paper; Sigma	N/A
C-kit ChIP RP GGGTGCAGTCCTCTTGTCTG	This paper; Sigma	N/A
Tfap2e ChIP FP CACCCGTTGCCCGACTTTTT	This paper; Sigma	N/A
Tfap2e ChIP RP AAGGTACGGGGTACTCAGCG	This paper; Sigma	N/A
Dct ChIP FP GGGAGCTTTCGTCTTGCTCT	This paper; Sigma	N/A
Dct ChIP RP TCCATTAAGGGCGCATAGCC	This paper; Sigma	N/A
Tyr ChIP FP GGGAGGAAAGGGTGCTTGAG	This paper; Sigma	N/A
Tyr ChIP RP AGGCTTGGGTTGTAATGCCA	This paper; Sigma	N/A
Tyrp1 ChIP FP CCAGTGTGAGGAATCTGGCTTG	This paper; Sigma	N/A
Tyrp1 ChIP RP TGCCAGCTGTTAATTGCCCG	This paper; Sigma	N/A
mH2a.z.2 A14T oligo GACAGTGGGAAGGCCAAGACTAAGGCG GTGTCTCGTTCC	This paper; Sigma	N/A
mH2a.z.2 A14T oligo complement GGAACGAGACACCGCCTTAGTCTTGGCC TTCCCACTGTC	This paper; Sigma	N/A
mH2a.z.2 T38S oligo CACAGACACTTGAAGAGTCGCACCACAA GCCATG	This paper; Sigma	N/A
mH2a.z.2 T38S complement CATGGCTTGTGGTGCGACTCTTCAAGTG TCTGTG	This paper; Sigma	N/A
mH2a.z.2 A127V oligo GGGCAGCAGAAAACTGTTCCCGGGCCC GC	This paper; Sigma	N/A

mH2a.z.2 A127V oligo complement GCGGGCCCGGGAACAGTTTTCTGCTGCCC	This paper; Sigma	N/A
Recombinant DNA		
Mouse H2a.z.2 CDS – Zero TOPO Blunt	This paper	N/A
Mouse H2a.z.1 CDS – Zero TOPO Blunt	This paper	N/A
Zebrafish mitfa CDS – Zero TOPO Blunt	This paper	N/A
pIRES-mouse H2a.z.2 CDS	This paper	N/A
Zebrafish *h2a.z.2*-3ÚTR WISH probe construct	This paper	N/A
Zebrafish *foxd3* WISH probe construct	Stewart R.A et al; 2006; Dev Biol	N/A
Zebrafish *sox10* WISH probe construct	Stewart R.A et al; 2006; Dev Biol	N/A
Zebrafish *tfap2a* WISH probe construct	Stewart R.A et al; 2006; Dev Biol	N/A
Zebrafish *crestin* WISH probe construct	Stewart R.A et al; 2006; Dev Biol	N/A
Zebrafish *sox9a* WISH probe construct	Yan YL et al; 2005; Development	N/A
Zebrafish *sox9b* WISH probe construct	Yan YL et al; 2005; Development	N/A
Zebrafish *krox20* WISH probe construct	Fiejoo CG et al; 2009; Mol Cell Neuro	N/A
Zebrafish *mbp* WISH probe construct	Brosamle C et al; 2002; Glia	N/A
Zebrafish *neurod1* WISH probe construct	Rauch G; 2003	N/A
Zebrafish *mitfa* WISH probe construct	Lister JA et al; 1999; Development	N/A
Zebrafish *dct* WISH probe construct	Lister JA et al; 1999; Development	N/A
Zebrafish *c-kit* WISH probe construct	Parichy DM et al; 1999; Development	N/A
Zebrafish *isl2a* WISH probe construct	Asad Z et al; 2016; Hum Mol Genetics	N/A
Softwares and Algorithm		

R Studio i386 3.5.3		N/A
Prism 7.0	Graphpad Software	N/A
Reactome PA	Yu G et al; 2016, Mol Biosystem	https://github.com/GuangchuangYu/ReactomePA
ImageJ	Schneider et al., 2012	https://imagej.nih.gov/ij/