



Haematopoietic stem cell-dependent Notch transcription is mediated by p53 through the Histone chaperone Supt16h

Sophia G. Espanola¹, Hyemin Song^{1,2}, Eunjin Ryu³, Aditya Saxena⁴, Eun-Sun Kim², Jennifer E. Manegold¹, Chanond A. Nasamran⁵, Debashis Sahoo⁶, Chang-Kyu Oh², Cara Bickers¹, Unbeom Shin³, Stephanie Grainger⁴, Yong Hwan Park², Lauren Pandolfo⁴, Mi-Sun Kang², Sukhyun Kang², Kyungjae Myung^{2,3}, Kimberly L. Cooper⁴, Deborah Yelon⁴, David Traver¹✉ and Yoonsung Lee^{1,2,3}✉

Haematopoietic stem and progenitor cells (HSPCs) have been the focus of developmental and regenerative studies, yet our understanding of the signalling events regulating their specification remains incomplete. We demonstrate that *supt16h*, a component of the Facilitates chromatin transcription (FACT) complex, is required for HSPC formation. Zebrafish *supt16h* mutants express reduced levels of Notch-signalling components, genes essential for HSPC development, due to abrogated transcription. Whereas global chromatin accessibility in *supt16h* mutants is not substantially altered, we observe a specific increase in p53 accessibility, causing an accumulation of p53. We further demonstrate that p53 influences expression of the Polycomb-group protein PHC1, which functions as a transcriptional repressor of Notch genes. Suppression of *phc1* or its upstream regulator, p53, rescues the loss of both Notch and HSPC phenotypes in *supt16h* mutants. Our results highlight a relationship between *supt16h*, p53 and *phc1* to specify HSPCs via modulation of Notch signalling.

Haematopoiesis is an evolutionarily conserved process that allows for a small population of haematopoietic stem and progenitor cells (HSPCs) to generate trillions of blood cells over a lifetime. Despite being one of the best characterized stem-cell systems, the ontogeny of HSPCs is not completely understood¹. Many studies have focused on events required for HSPC emergence, whereby HSPCs bud from the dorsal aorta into circulation, but little is known about the previous signals needed to determine HSPC fate^{2,3}. To fill these gaps in our understanding, we employed a forward genetic screen in zebrafish to detect genes required for HSPC specification and identified *supt16h*, a component of the Facilitates chromatin transcription (FACT) complex that forms a heterodimer with SSRP1. The FACT complex has been described in two roles: (1) a histone chaperone that promotes H2A–H2B dimer dissociation from the nucleosome, allowing RNA polymerase II (RNAP2) to access chromatin templates⁴ and (2) an initiation and elongation factor that colocalizes with RNAP2 to allow for transcriptional activity^{5,6}.

Despite what might be considered a globally essential function in genome regulation, we found *supt16h* primarily affects the expression of Notch-pathway components essential for HSPC specification. An absence of Notch receptors (*notch1* and *notch3*) and ligands (*jagged1*, *deltaC* and *deltaD*) results in a loss of HSPCs in mouse and zebrafish^{7–10}. Moreover, we demonstrated that these ‘Notch genes’, defined here as Notch receptors and ligands, are transcriptionally affected by the levels of polyhomeotic homologue 1 (PHC1),

a Polycomb-group protein whose expression is controlled by p53. Although canonically described in the induction of apoptosis, cell-cycle arrest and senescence, recent findings show p53 involvement in genomic stability, transcriptional regulation and epigenetic modifications^{11–13}. We demonstrate that p53 controls *phc1* expression through direct binding at the *phc1* locus, which influences transcriptional repression of Notch genes. This work highlights a previously uncharacterized method of Notch-gene regulation and elucidates an unrecognized relationship between Polycomb-group proteins, p53 and FACT in modulating Notch-gene transcription during HSPC development.

Results

A forward genetic screen identifies *supt16h*^{−/−} mutants lacking HSPCs. Zebrafish *supt16h* mutants (*supt16h*^{−/−}) were obtained through a forward genetic screen for animals defective in HSPC specification (Extended Data Fig. 1a) and identified using RNA sequencing (RNA-seq)-based single nucleotide polymorphism (SNP) linkage mapping¹⁴. Our candidate gene mapped to Chromosome 7 and corresponded to a premature stop codon in *supt16h* (Extended Data Fig. 1b–d and Supplementary Table 1). Mutants exhibited enlarged hindbrains and curved tails beginning at 32 h post fertilization (h.p.f.), leading to lethality at 3 d post fertilization (Fig. 1a,b). These traits were linked to a complete loss of HSPCs, as assessed by whole-mount in situ hybridization (WISH) for *runx1* and *cmyb*

¹Department of Cellular and Molecular Medicine and Section of Cell and Developmental Biology, University of California, San Diego, La Jolla, CA, USA.

²Center for Genomic Integrity, Institute for Basic Science (IBS), Ulsan, Republic of Korea. ³School of Life Sciences, Ulsan National Institute of Science and Technology (UNIST), Ulsan, Republic of Korea. ⁴Section of Cell and Developmental Biology, Division of Biological Sciences, University of California, San Diego, La Jolla, CA, USA. ⁵Center for Computational Biology and Bioinformatics, University of California, San Diego, La Jolla, CA, USA. ⁶Department of Pediatrics and Department of Computer Science and Engineering, University of California, San Diego, La Jolla, CA, USA. ✉e-mail: dtraver@ucsd.edu; yoonsunglee@ibs.re.kr

HSPC markers along the aortic floor at 28 and 36 h.p.f., respectively, as well as RNA-seq and quantitative reverse transcription PCR (RT-qPCR) analyses (Fig. 1c–f and Extended Data Fig. 1e,f). In addition, *supt16h*^{−/−} mutants on a Tg(*cmyb:eGFP*; *kdr1*; *mCherry*) background, where double-positive cells along the aortic floor represent HSPCs, exhibited a significantly reduced number of HSPCs compared with their wild-type (WT) siblings (Fig. 1g–i)². Moreover, injection of WT *supt16h* messenger RNA into mutant embryos rescued the hindbrain, tail and HSPC phenotypes (Fig. 1j–q), and injecting *supt16h* antisense Morpholino oligonucleotide (MO) phenocopied the reduced HSPCs in *supt16h*^{−/−} mutants (Extended Data Fig. 1g–m)². Together, these data indicate that Supt16h is required for HSPC formation.

The *supt16h* gene was broadly expressed throughout development (Extended Data Fig. 2a–c and Fig. 1r). Higher-magnification and cross-sectional views of *supt16h* WISH embryos showed its expression along the dorsal aorta floor, where HSPCs emerge (Fig. 1s–u and Extended Data Fig. 2d,e). Moreover, RT-qPCR of HSPCs purified using fluorescence-activated cell sorting (FACS) demonstrated enrichment of *supt16h*, providing further evidence of expression in HSPCs. In addition, the transcription of *supt16h* was substantially reduced in our mutant line—as observed by WISH, RNA-seq and RT-qPCR—due to nonsense-mediated decay (Fig. 1v–z and Extended Data Fig. 2f–k). To further characterize the specific requirement of *supt16h* on HSPC formation, we examined tissues essential for HSPC specification. The expression of markers of the posterior lateral mesoderm, somites, sclerotome, vascular endothelium, dorsal aorta, vein, primitive erythrocytes and primitive leukocytes was normal in mutants (Extended Data Fig. 2f–t). Overall, these results indicate that the specific loss of HSPCs in *supt16h*^{−/−} is not due to improper formation of upstream or adjacent tissues.

The Notch pathway is downregulated in *supt16h*^{−/−}. To explore the contribution of *supt16h* during haematopoiesis, we performed Gene Ontology (GO) analysis using transcripts that were significantly downregulated in mutants (Extended Data Fig. 3a,b)^{15,16}. Although the molecular processes that were most affected were associated with transcriptional regulation and binding of RNAP2 to DNA, we observed no bias in the number of upregulated and downregulated genes in *supt16h*^{−/−} (Extended Data Fig. 3c). We did observe that the only affected signalling pathway and top downregulated biological process was Notch (Fig. 2a and Extended Data Fig. 3b), which has been associated with HSPC specification and emergence^{7–9,17–21}. The transcriptional levels of Notch genes were reduced in mutant embryos at 28–32 h.p.f., based on RNA-seq, RT-qPCR and WISH analyses (Fig. 2b–g and Extended Data Fig. 3d–g). The *notch2* gene was reduced to a lesser extent, but it is known to be dispensable for

HSPC formation^{7,22}. Furthermore, mutants harbouring the Notch reporter line *Tp1:GFP* displayed significantly downregulated Notch activity in the dorsal aorta at 22 and 28 h.p.f. (Fig. 2h–j and Extended Data Fig. 3h–j). Sorted *Tp1:GFP*⁺; *fli1:DsRed*⁺ cells, which have been shown to have high *runx1* expression, were present at lower levels in *supt16h* morphants²¹ (Extended Data Fig. 3k,l). Interestingly, there was no alteration on Notch-gene activity in mutants during early somitogenesis at 14 h.p.f. (Extended Data Fig. 3m,n). Therefore, *supt16h*^{−/−} mutants have impaired late (28 h.p.f.), but not early (14 h.p.f.), Notch-gene expression. To determine whether the reduction in Notch-gene transcripts in *supt16h*^{−/−} mutants led to HSPC loss, we forced global expression of Notch intracellular domain 1 (NICD) using a Tg(*hsp70*) driver and endothelial expression using a Tg(*cdh5:gal4ff*) driver²³. Restoration of Notch activity using these transgenes in *supt16h*^{−/−} mutants rescued HSPC development (Fig. 2k–r and Extended Data Fig. 3o–v). Together, these data indicate that Notch signalling is downstream of *supt16h* function during HSPC specification.

Induction of p53 in *supt16h*^{−/−} mutants perturbs HSPC formation. The FACT complex colocalizes with RNAP2 to initiate transcription and can associate with Tif1γ to promote transcriptional elongation of erythroid genes²⁴. We therefore examined the connection between Supt16h and initiation and elongation of Notch-gene transcripts using RT-qPCR, assaying for changes between the 3′- and 5′-end transcript levels. We found that, although transcriptional initiation was substantially altered in all but three Notch genes, elongation of *supt16h*^{−/−} transcripts was unaffected in most genes, which suggests that the Notch genes are inefficiently transcribed as a result of aberrant Supt16h activity as part of the transcriptional initiation complex (Extended Data Fig. 4a).

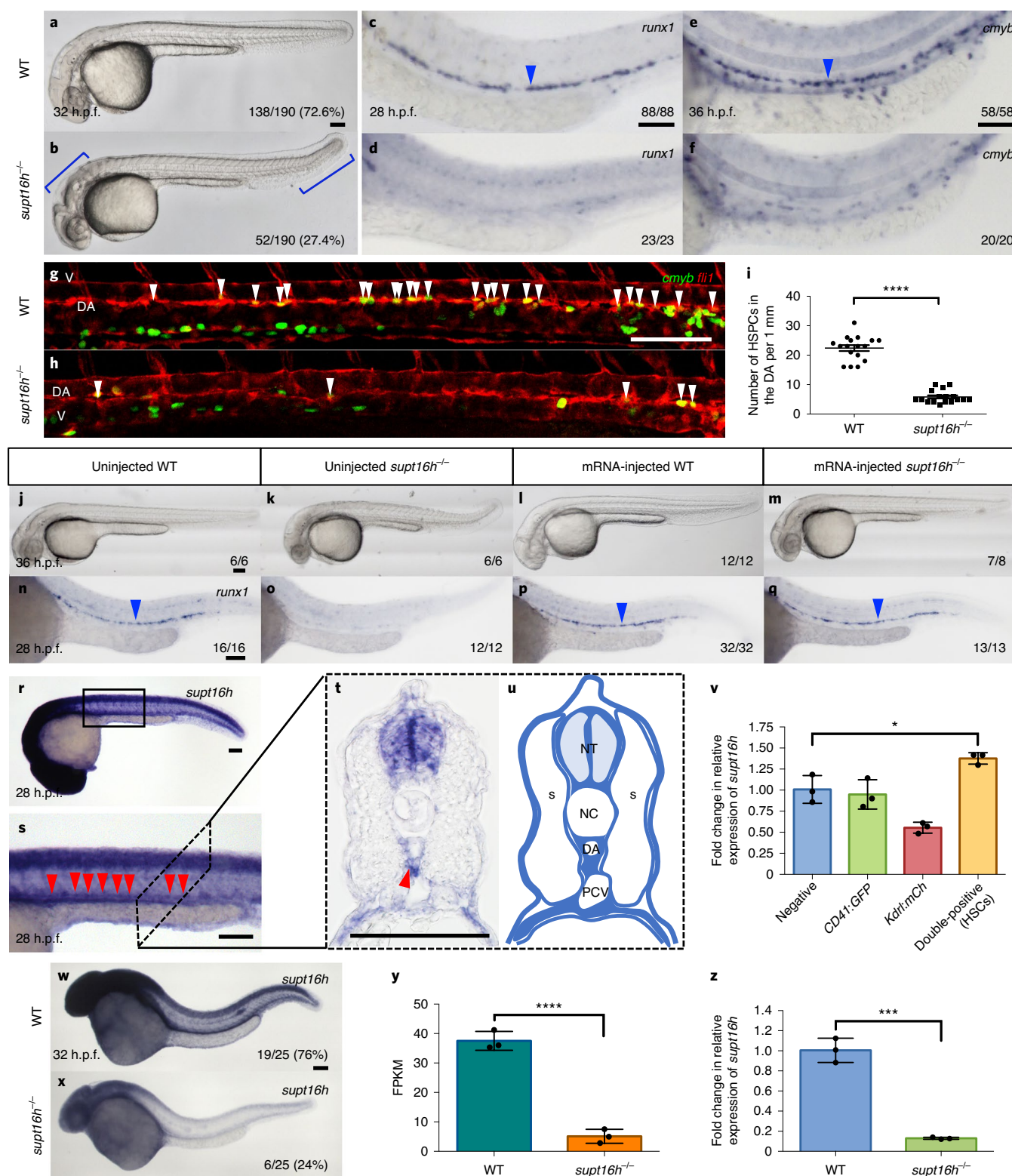
Due to the role of Supt16h as a histone chaperone to remodel chromatin, we conducted an assay for transposases accessible chromatin with sequencing (ATAC-seq) to determine whether chromatin accessibility of the Notch-gene coding sequences was affected in the *supt16h*^{−/−} mutants. We observed no difference in accessibility at transcriptional start sites or on Notch genes globally, and no correlation between DNA accessibility and Notch transcription (Extended Data Fig. 4b–g). As there was no obvious connection between Notch-gene transcription levels and accessibility, we evaluated genes with the most differentially accessible peaks in the *supt16h*^{−/−} mutants and found *p53* to be the most accessible gene (Fig. 3a and Extended Data Fig. 4h). We observed notable increases in both *p53* transcript and protein expression in mutants (Fig. 3b–d and Extended Data Fig. 4i,j). WISH analyses demonstrated *p53* increases throughout *supt16h*^{−/−} embryos, including the dorsal aorta, commencing at 18 h.p.f. (Fig. 3e–j and Extended Data Fig. 4k–r). To determine whether there is a specific haematopoietic

Fig. 1 | A forward genetic screen identifies *supt16h*^{−/−} mutants that specifically lack HSPCs. a,b, Images of WT (**a**) and *supt16h*^{−/−} (**b**) siblings at 32 h.p.f. The blue brackets highlight the morphological defects of the heads and tails of the mutants. **c–f**, WISH of the HSPC markers *runx1* at 28 h.p.f. (**c,d**) and *cmyb* at 36 h.p.f. (**e,f**) of WT (**c,e**) and *supt16h*^{−/−} (**d,f**) siblings. **g,h**, Representative confocal images of WT (**g**) and *supt16h*^{−/−} (**h**) siblings crossed with Tg(*cmyb:GFP*; *fli1:DsRed*) embryos. Double-positive HSPCs (yellow fluorescence) at 48 h.p.f. are indicated by white arrowheads. DA, dorsal aorta; V, vein. **i**, Number of double-positive HSPCs from **g,h**. Data are represented as the mean ± s.e.m.; *n* = 17. Two-tailed Student's *t*-test, *****P* < 0.0001. **j–q**, Injection of WT *supt16h* mRNA in WT and *supt16h*^{−/−} sibling embryos. Bright-field images at 36 h.p.f. show morphological rescue of injected *supt16h*^{−/−} embryos (**j–m**) and WISH of *runx1* at 28 h.p.f. (**n–q**). **r–u**, WISH of *supt16h* at 28 h.p.f. (**r**), with a magnified view of the boxed region in **r** along the dorsal aorta (**s**), a cross-sectional view (**t**) and a representative cartoon of **t** with the regions of expression coloured (**u**). NT, neural tube; S, somites; NC, notochord; DA, dorsal aorta; and PCV, post cardinal vein. The red arrowheads indicate the DA; *n* = 4. **v**, RT-qPCR analysis of *supt16h* in FACS-purified cells—double-positive (HSPCs), single-positive and negative cells—from Tg(*CD41:GFP*; *kdr1:mCherry*) embryos at 48 h.p.f. Data are represented as the mean ± s.d.; *n* = 3. One-way analysis of variance (ANOVA) with Tukey's post-hoc test, **P* = 0.0331. **w,x**, WISH of *supt16h* on WT (**w**) and *supt16h*^{−/−} (**x**) sibling embryos at 32 h.p.f. The percentages correlate with Mendelian segregation. **y,z**, Expression of *supt16h* in WT and *supt16h*^{−/−} (pooled) sibling embryos at 32 h.p.f. determined through RNA-seq (**y**) and RT-qPCR (**z**). Data are represented as the mean ± s.d.; *n* = 3. Two-tailed Student's *t*-test, *****P* = 0.0001 (**y**) and ****P* = 0.0002 (**z**). FPKM, fragments per kilobase of transcript per million mapped reads. For RT-qPCR, the expression values are relative to those of the WT siblings. The blue arrowheads indicate HSPCs. Scale bars, 100 μm. For **a,b,w,x** the fractions represent the number of genotypic embryos over the clutch total. For **c–f, j–q** the fractions are the representative outcome for each genotypic group. Source data are provided.

consequence of reducing the levels of *p53* in the absence of Supt16h, we examined *supt16h*^{-/-} crossed with *p53* mutants (*tp53*^{zdf1/zdf1})²⁵. The *supt16h*;*p53* double mutants rescued HSPC formation, probably due to an inability of *p53* to activate the downstream targets (Fig. 3k–m)²⁵. Morpholino oligonucleotide knockdown of *supt16h* in the *p53* mutants or *p53* in the *supt16h*^{-/-} mutants similarly rescued HSPCs (Fig. 3n–q and Extended Data Fig. 3s–v). This was further

highlighted by RT–qPCR, where we detected a significant increase in *runx1* transcripts in the *p53*-MO-injected mutants (Fig. 3r).

To examine whether a reduction in *p53* prevents HSPC death to rescue specification in *supt16h*^{-/-} mutants, we conducted terminal deoxynucleotidyl transferase dUTP nick-end labelling (TUNEL) assays and acridine orange staining on *supt16h*^{-/-} embryos harbouring a *fli1*:GFP endothelial reporter that marks the shared vascular



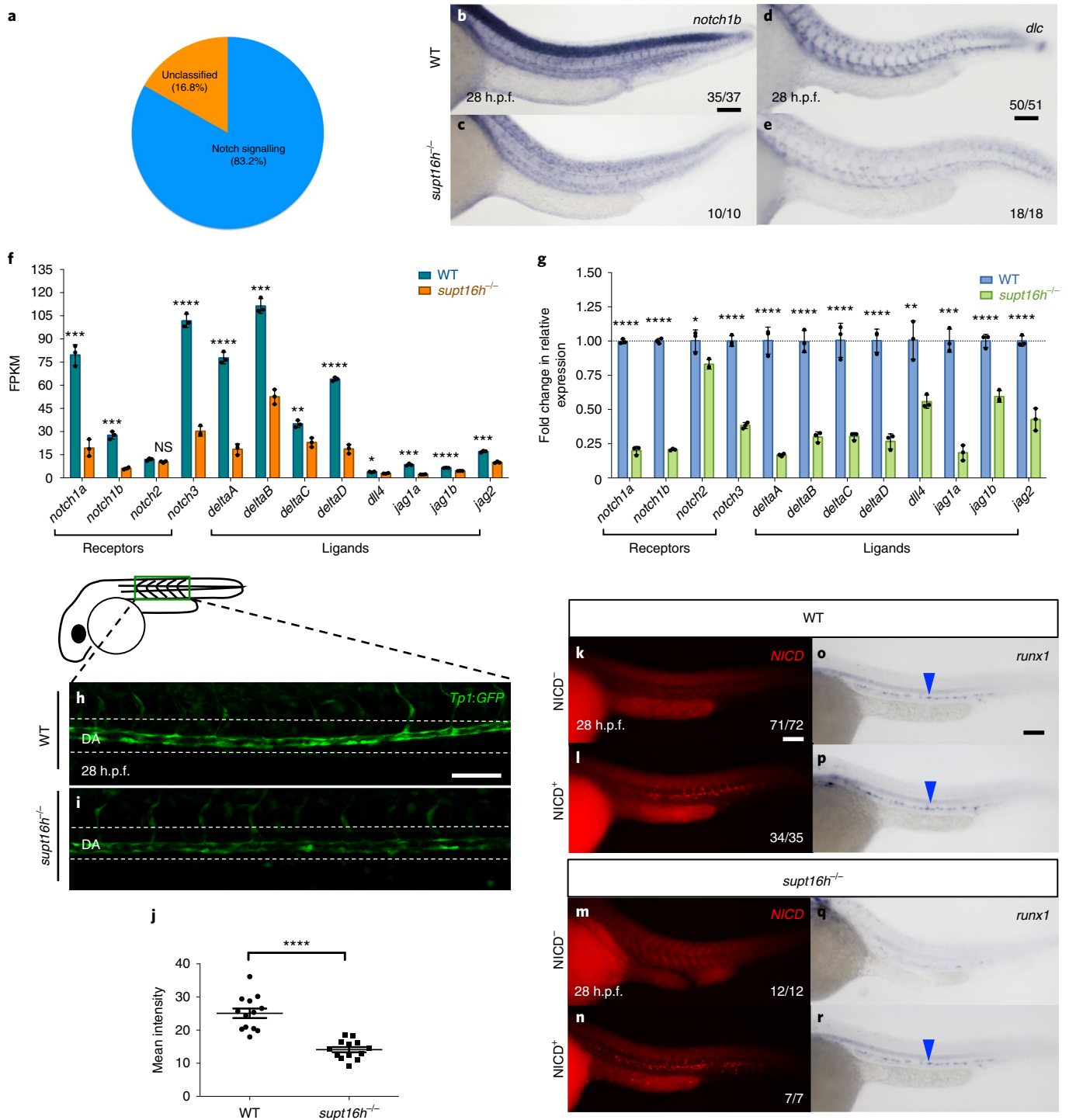


Fig. 2 | The Notch pathway is downregulated in *supt16h*^{-/-} mutants. **a**, Pie chart of the downregulated pathways in *supt16h*^{-/-} mutants based on Gene Ontology analysis ($\log_2[\text{fold change}] > -1$) of RNA-seq data. Fisher's exact test with Bonferroni correction, $P = 0.0334$. **b–e**, WISH of *notch1b* (**b,c**) and *dlc* (**d,e**) in WT (**b,d**) and *supt16h*^{-/-} (**c,e**) sibling embryos. **f**, Expression levels of Notch genes based on RNA-seq. **g**, Expression levels, determined using RT-qPCR, of Notch genes in pooled embryos at 32 h.p.f. where expression is relative to the WT siblings (horizontal dotted line). FPKM, fragments per kilobase of transcript per million mapped reads. **f,g**, Data are represented as the mean \pm s.d.; $n = 3$. Two-tailed Student's *t*-test with Holm–Sidak correction for multiple comparisons; **** $P < 0.0001$, *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$ and NS, not significant. **h,i**, Representative confocal images along the dorsal aorta (DA) of *supt16h*^{-/-} (**i**) and WT (**h**) sibling embryos on a Tg(*Tp1:GFP*) background at 28 h.p.f. **j**, Mean fluorescence intensity of *Tp1:GFP* along the DA from **h,i** calculated using ImageJ. Data are represented as the mean \pm s.e.m.; $n = 13$. Two-tailed Student's *t*-test; **** $P < 0.0001$. **k–r**, Vascular-specific expression of NICD⁺ (**l,n,p,r**) and NICD⁻ (**k,m,o,q**) WT (**k,l,o,p**) and *supt16h*^{-/-} (**m,n,q,r**; *supt16h*^{-/-}; *cdh5:gal4ff* \times *supt16h*^{+/+}; UAS-*myc:NICD*) sibling embryos analysed at 28 h.p.f. by NICD immunohistochemistry (**k–n**) and *runx1* WISH (**o–r**). Representative images from two independent experiments. The blue arrowheads indicate HSPCs. For **b–e**, **k–r** the fractions are the representative outcome for each genotyped group. Scale bars, 100 μ m. Source data are provided.

precursors of HSPCs. We discerned no difference in the number of double-positive TUNEL⁺*fli1*⁺ cells at 14, 18 and 28 h.p.f., despite being able to distinguish between global apoptotic events in WT and mutants from 18 h.p.f. (Fig. 3s–w and Extended Data Fig. 5a–r). This demonstrates that a loss of HSPCs in *supt16h*^{−/−} mutants is not a result of their death in the endothelium. We further characterized the role of p53-independent apoptosis and found that p53 loss in the *supt16h*^{−/−} mutants did not resolve their abnormal morphologies or extend survival (Extended Data Fig. 5s–w). In addition, we observed that although general genotoxic stress through ionizing radiation increased p53 expression and cell death, it did not affect HSPC specification (Extended Data Fig. 5x–e). Overall, these results suggest that elevated p53, caused by a global loss of *supt16h*, perturbs HSPC formation independent of cell-mediated apoptosis.

The transcription levels of the Notch genes are influenced by p53 abundance. It is known that p53 is a transcriptional regulator of numerous genes by direct stimulation of the transcription of RNAP2-transcribed genes or activation of the associated pathways¹³. To address whether ectopically elevated p53 is related to the reduced transcript levels of Notch genes in *supt16h*^{−/−} mutants, we examined the effect of p53 downregulation on Notch-pathway activation using the *Tp1:GFP* Notch reporter line and found that p53-MO injection into *supt16h*^{−/−} mutants restored Notch activity (Fig. 4a–e). Both double mutants and double morphants of *supt16h*;p53 also rescued *notch1b*, which we previously demonstrated is required for HSPC specification, and *runx1* expression (Fig. 4f–m and Extended Data Fig. 6a–h)⁸. We next tested the effect of differences in p53 levels on Notch-gene transcription, given that previous studies have linked p53 abundance to impaired RNAP2 activity and suppressed mRNA synthesis^{26,27}. We observed reduced expression of *p21*—a downstream target of p53—in *supt16h*^{−/−} mutants (WT, heterozygous and homozygous for p53), which was relative to the genetic dosing of the p53 mutants (Fig. 4n). Remarkably, we observed the converse trend with *runx1* and Notch genes, where the transcript levels increased following p53 reduction (Fig. 4o,p). These data indicate that p53 and Supt16h coordinate to activate the transcription of Notch genes during HSPC specification.

To further examine the epistasis between p53 and Notch signalling during HSPC formation, we co-injected *notch1b*- and p53-MOs to determine whether p53 regulation of Notch genes is confined to *supt16h*^{−/−}. We observed an absence of HSPCs in *notch1b* morphants and, notably, a lack of HSPC rescue in double *notch1b*;p53 morphants (Extended Data Fig. 6i–l). In addition, p53 knockdown in *mindbomb* (*mib*) mutants with defective Notch-pathway signalling and HSPC formation resulted in continued aberrant HSPCs (Extended Data Fig. 6m–t)²⁸. These data indicate that a reduction of p53 alone is not sufficient to restore HSPCs in embryos when Notch genes are downregulated and further suggests that Notch signalling is downstream of p53 function.

HSPC specification is unaffected by *ssrp1*. As part of the larger FACT complex, Supt16h forms a heterodimer with SSRP1 to effectively alter chromatin accessibility²⁹. We examined the consequences of *ssrp1* knockdown on HSPC formation to address whether the effect of Supt16h on HSPC development was independent of the FACT complex or associated with the role of FACT as a transcriptional regulator. Given that the zebrafish paralogue *ssrp1a* can compensate for the role of *ssrp1b*^{30,31}, we mainly focused on *ssrp1a* in future experiments. HSPC development was normal in both single *ssrp1a* and double *ssrp1a*; *ssrp1b* morphants (Fig. 5a–h). Similar results were observed for *ssrp1a*^{−/−}, characterized by a premature stop codon on exon 11 and reduced *ssrp1a* transcripts (Fig. 5i–k)³². In addition, we observed no effect on the dorsal aorta, early red blood cell or vasculature in morphants (Extended Data Fig. 7a–f). WISH analysis showed both *ssrp1a* and *ssrp1b* maintained a similar global expression pattern to *supt16h* from 14 to 32 h.p.f., and that these genes are present along the floor of the dorsal aorta, which corresponded to their detectable upregulation in HSPCs (Fig. 5l–p and Extended Data Fig. 8g–o).

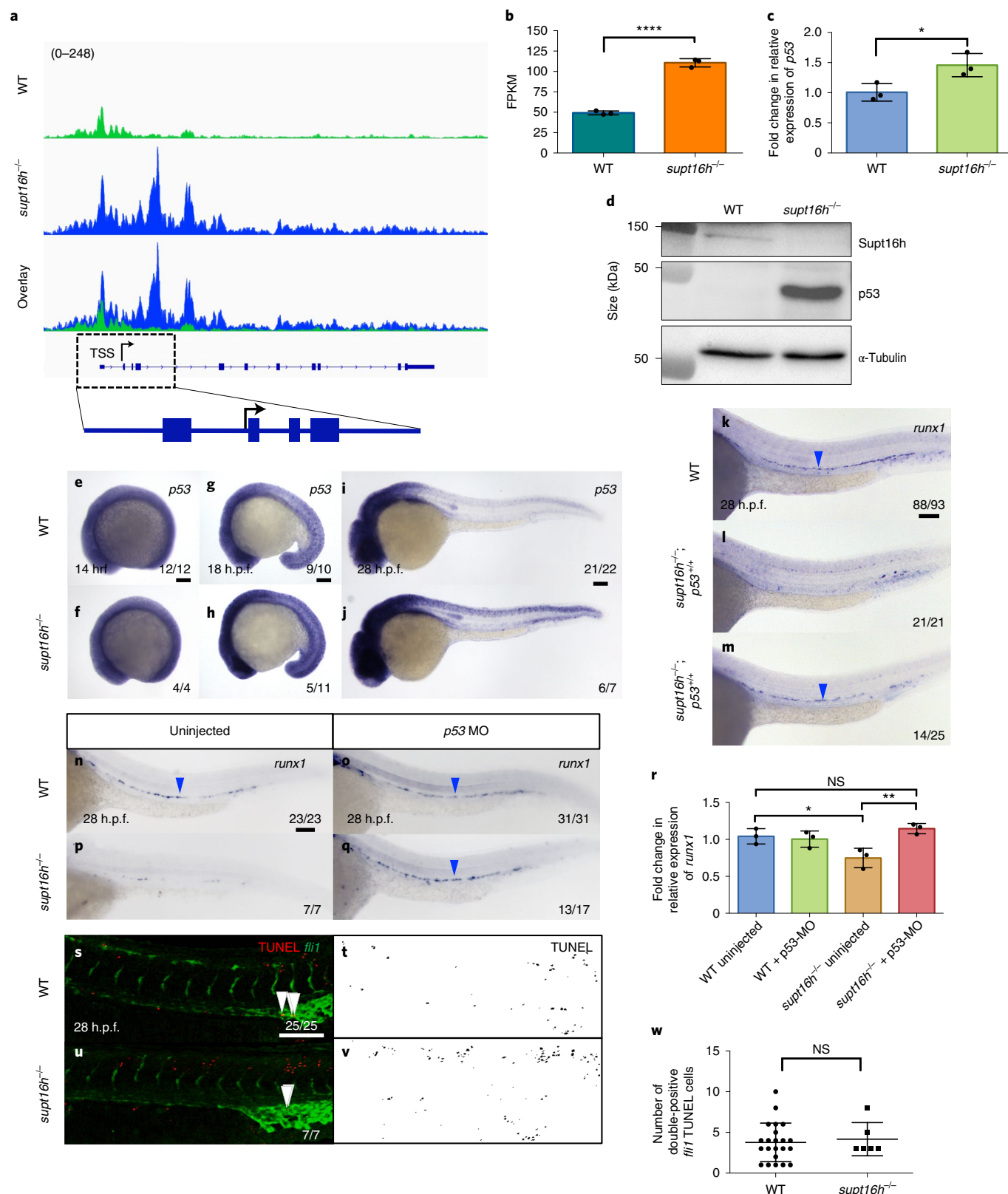
To further understand of the disparate effect of SSRP1 and Supt16h on HSPC development, we examined the influence of SSRP1 on p53 and Notch abundance. We saw no discernable difference in p53 expression or cell-death events in the *ssrp1a* mutants or morphants (Fig. 5q and Extended Data Fig. 8p–x). Furthermore, we observed no altered Notch signalling activity in our morphants following *ssrp1a* knockdown in the *Tp1:GFP* reporter line (Fig. 5r,s and Extended Data Fig. 8y). Consistent with these findings, *notch1b* expression was unchanged in both *ssrp1a* mutants and morphants based on WISH (Fig. 5t,u and Extended Data Fig. 8z,a'). Overall, these results demonstrate that, despite sharing similar expression profiles to *supt16h*, *ssrp1a* is dispensable for proper Notch activity, p53 activation and HSPC formation, thereby suggesting that Supt16h and SSRP1 maintain independent roles during HSPC specification.

p53 regulates *phc1* expression to control Notch-gene transcription and HSPC specification. To assess the mechanism by which p53 regulates Notch-gene expression in *supt16h*^{−/−} mutants, we conducted chromatin immunoprecipitation–sequencing (ChIP-seq) to investigate whether p53 binds directly to Notch genes to influence their activity or to known transcriptional regulators to indirectly affect Notch expression. Following p53-based ChIP-seq, we observed through GO analysis that the most highly expressed genes were associated with apoptotic processes and p53 signalling, including *p21* expression (Extended Data Fig. 8a–c). We then examined the direct interaction between p53 and Notch genes, and found no substantial increase in binding in *supt16h*^{−/−} mutants (Extended Data Fig. 8d–f), indicating that p53 does not mediate Notch-gene expression directly.

Fig. 3 | Induction of p53 in *supt16h*^{−/−} mutants perturbs HSPC formation. **a**, Peaks of WT and *supt16h*^{−/−} sibling embryos for p53 ATAC-seq. The transcriptional start site (TSS), introns (blue lines) and exons (blue boxes) are shown (bottom). Value in parenthesis represents track height in pixels. **b,c**, Expression levels of p53 in *supt16h*^{−/−} and WT sibling embryos at 32 h.p.f. based on RNA-seq (**b**) and RT-qPCR (**c**). Data are represented as the mean ± s.d.; n = 3. Two-tailed Student's t-test, ****P < 0.0001 (**b**) and *P = 0.0317 (**c**). **d**, Supt16h, p53 and α-tubulin western blots of *supt16h*^{−/−} and WT sibling embryos at 32 h.p.f. Representative images from three biological replicates. Raw unprocessed blots are available in the source data. **e–j**, WISH for p53 expression in WT and *supt16h*^{−/−} sibling embryos at 14 (**e,f**), 18 (**g,h**) and 28 h.p.f. (**i,j**). **k–m**, WISH for *runx1* of WT (**k**), *supt16h*^{−/−}; p53^{+/+} (**l**) and *supt16h*^{−/−}; p53^{−/−} (**m**) sibling embryos at 28 h.p.f. Representative of four biological replicates. **n–q**, WISH for *runx1* of WT (**n,o**) and *supt16h*^{−/−} (**p,q**) sibling embryos injected with p53-MO (**o,q**) as well as uninjected controls (**n,p**). **r**, Expression levels of *runx1*, determined using RT-qPCR, in pooled embryos from **n–q** at 28 h.p.f. Data are represented as the mean ± s.d.; n = 3. One-way ANOVA with Tukey's post-hoc test; *P = 0.039, **P = 0.0078 and NS, not significant. **s–v**, TUNEL analysis of WT and *supt16h*^{−/−} siblings crossed with Tg(*fli1*:GFP) at 28 h.p.f. Confocal images of TUNEL, *fli1* and double-positive TUNEL⁺*fli1*⁺ (yellow; indicated by white arrowheads; left), and TUNEL-only cells are shown (right). **w**, Number of double-positive TUNEL⁺*fli1*⁺ cells at 28 h.p.f. from **s–v**. Data are represented as the mean ± s.e.m.; n = 22 (WT) and 6 (*supt16h*^{−/−}). Two-tailed Student's t-test; NS, not significant. For the RT-qPCR, the expression values are relative to those of the WT siblings. The blue arrowheads indicate HSPCs. For **e–q**, **s** and **u** the fractions are the representative outcome for each genotyped group. Scale bars, 100 μm. Source data are provided.

We thus explored the option that p53 indirectly influences Notch signalling by increasing the expression of a Notch transcriptional repressor, given that correlative analysis of our ChIP-seq results with our RNA- and ATAC-seq datasets showed a majority of the ChIP genes that were differentially expressed

were upregulated and highly accessible (Extended Data Fig. 8g,h). We discovered a known Notch transcriptional repressor, *phc1*, had elevated p53 binding and increased accessibility in *supt16h*^{-/-} mutants (Fig. 6a,b). In addition, we observed elevated *phc1* expression in *supt16h* mutants and morphants, paralleling



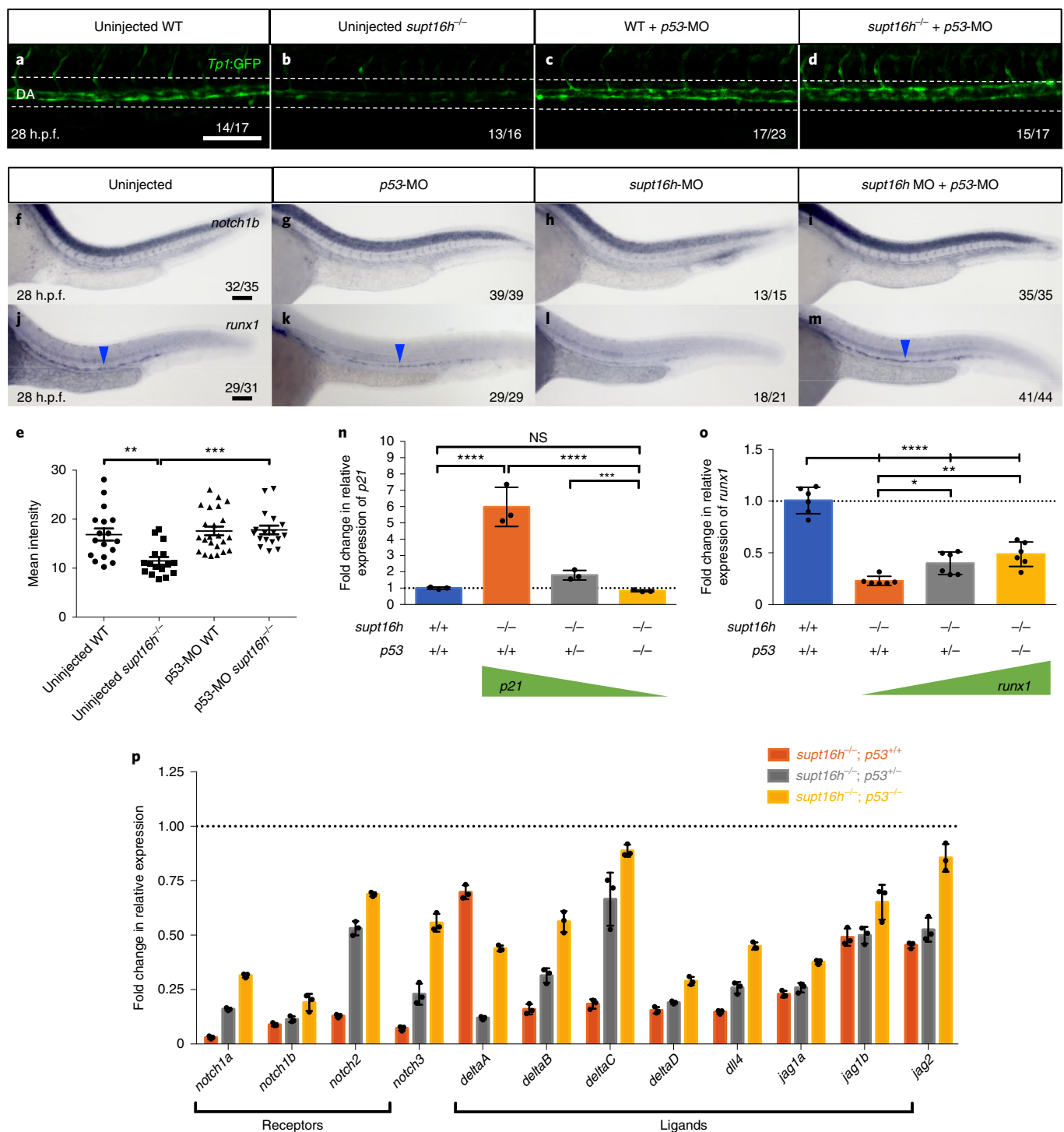


Fig. 4 | The transcription levels of the Notch genes are influenced by p53 abundance. **a–d**, Representative confocal images of the dorsal aorta (DA) of *supt16h*^{-/-} and WT sibling embryos on a Tg(*Tp1:GFP*) background injected with *p53*-MO (**c,d**) at 28 h.p.f. as well as uninjected controls (**a,b**). The white dotted lines represent the location of DA; *n* > 16. Scale bar, 25 μ m. **e**, Mean fluorescence intensity of *Tp1:GFP* of the DA from **a–d** calculated in ImageJ based on Integrated Density. Data are represented as the mean \pm s.e.m.; *n* = 17 (uninjected WT and *p53*-MO *supt16h*^{-/-}), 16 (uninjected *supt16h*^{-/-}) and 23 (*p53*-MO WT). One-way ANOVA with Tukey's post-hoc test, $^{**}P = 0.0021$ and $^{***}P = 0.0002$. **f–m**, WISH for *notch1b* (**f–i**) and *runx1* (**j–m**) at 28 h.p.f. of uninjected embryos (**f,j**) and embryos injected with *p53*-MO (**g,k**), *supt16h*-MO (**h,l**) and *supt16h*-MO + *p53*-MO (**i,m**). The blue arrowheads indicate HSPCs. **n,o**, Expression levels at 32 h.p.f. of *p21* (**n**) and *runx1* (**o**), determined by RT-qPCR, in WT and *supt16h*^{-/-} sibling embryos that are *p53*^{+/+}, *p53*^{+/-} or *p53*^{-/-}. Data are represented as the mean \pm s.d.; *n* = 3 (**n**) and 6 (**o**). One-way ANOVA with Tukey's post-hoc test; $^{****}P < 0.0001$, $^{***}P = 0.0002$, $^{**}P = 0.0021$, $^{*}P = 0.0492$ and NS, not significant. **p**, Expression levels at 32 h.p.f., determined by RT-qPCR (3' ends), of Notch receptors and ligands in *supt16h*^{-/-} embryos that are *p53*^{+/+}, *p53*^{+/-} or *p53*^{-/-}. Data are represented as the mean \pm s.d.; *n* = 3). **n–p**, The expression values are relative to those of the WT siblings (horizontal dotted line). Scale bar, 100 μ m. Source data are provided.

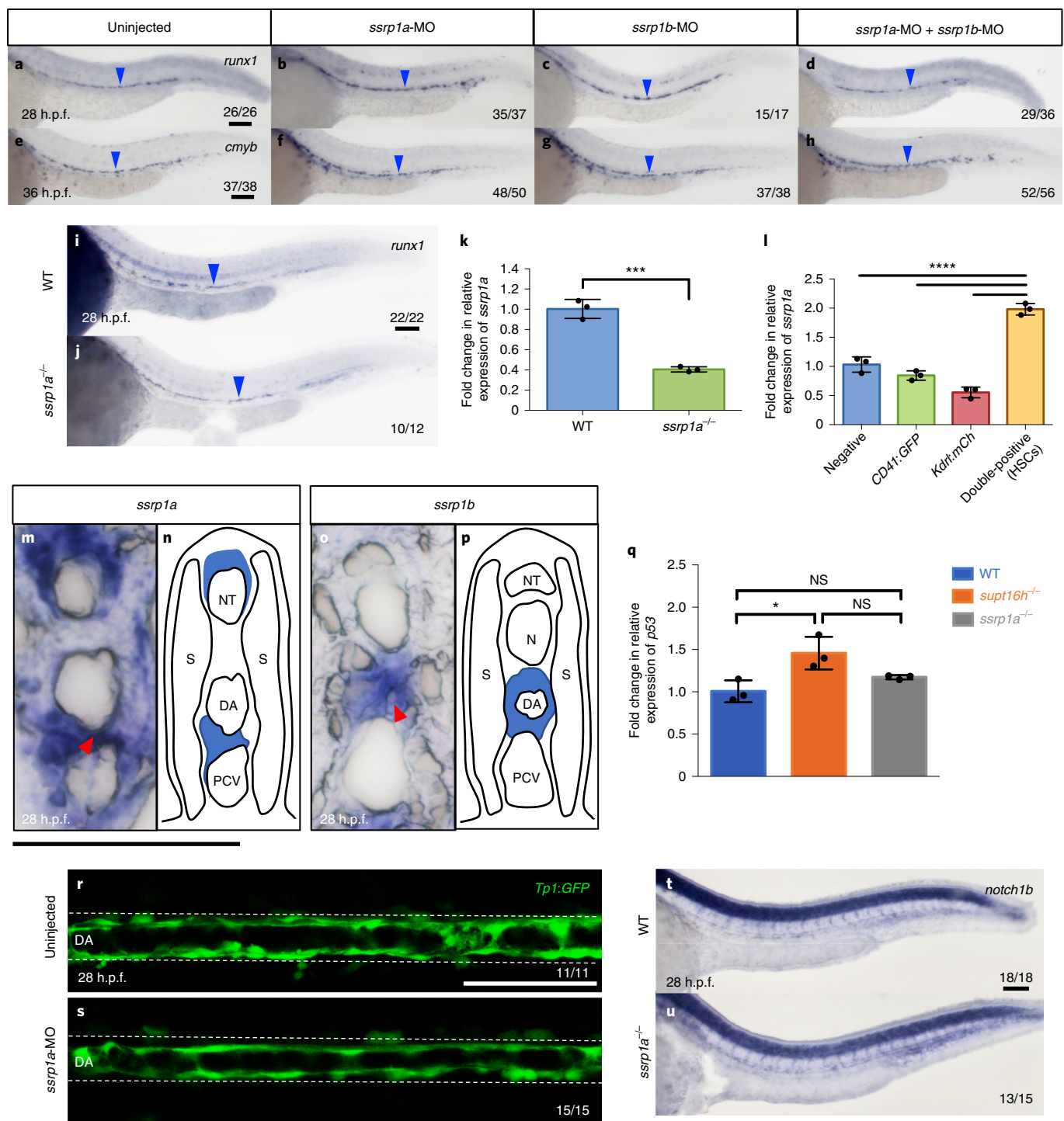


Fig. 5 | HSPC specification is unaffected by *ssrp1*. **a–h**, WISH for *runx1* (**a–d**) and *cmyb* (**e–h**) at 28 and 36 h.p.f., respectively, of embryos injected with *ssrp1a*-MO, *ssrp1b*-MO or both. **i,j**, WISH for *runx1* of WT (**i**) and *ssrp1a*^{-/-} (**j**) sibling embryos at 28 h.p.f. **a–j**, The blue arrowheads indicate HSPCs. **k**, Expression of *ssrp1a* in WT and *ssrp1a*^{-/-} sibling embryos at 32 h.p.f. based RT-qPCR. Data are represented as the mean ± s.d.; n = 3. Two-tailed Student's *t*-test, ****P* = 0.0004. **l**, Expression of *ssrp1a* in FACS-purified cells (double-positive (HSCs), single-positive and negative cells) from Tg(*CD41:GFP*; *kdr1:mCherry*) embryos at 48 h.p.f. determined by RT-qPCR. Data are represented as the mean ± s.d.; n = 3. One-way ANOVA with Tukey's post-hoc test; *****P* < 0.0001, ***P* = 0.0019 and **P* = 0.0328. **m–p**, Cross section of *ssrp1a* (**m,n**) and *ssrp1b* (**o,p**) WISH at 28 h.p.f. The red arrowheads indicate expression at the floor of the dorsal aorta. **n,p**, Representative cartoons showing expression from **m** and **o**, respectively. NT, neural tube; S, somites; DA, dorsal aorta; and PCV, post cardinal vein. **q**, Expression of *p53* in WT, *supt16h*^{-/-} and *ssrp1a*^{-/-} embryos at 32 h.p.f. determined by RT-qPCR. Data are represented as the mean ± s.d.; n = 3. One-way ANOVA with Tukey's post-hoc test; **P* = 0.0147; and NS, not significant. **r,s**, Representative confocal images along the dorsal aorta (DA) of Tg(*Tp1:GFP*) embryos injected with *ssrp1a*-MO (**s**), as well as uninjected controls (**r**), at 28 h.p.f. based on one independent experiment. **t,u**, WISH for *notch1b* expression in WT (**t**) and *ssrp1a*^{-/-} sibling (**u**) embryos at 28 h.p.f. **k,l,q**, The expression values are relative to those of the WT siblings. For **a–j**, **r–u** the fractions are the representative outcome for each genotyped group. Scale bars, 100 μm. Source data are provided.

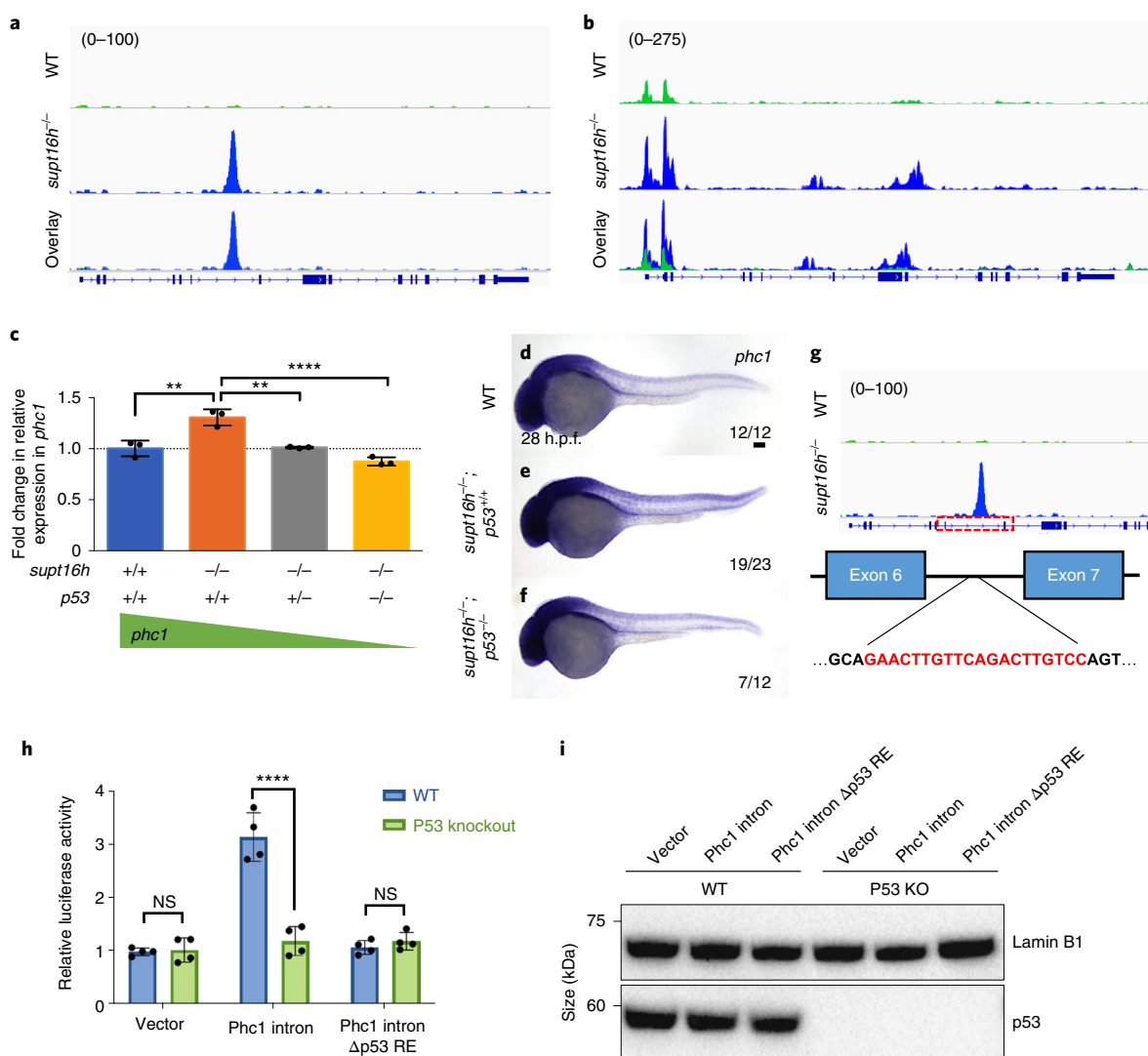


Fig. 6 | p53 augments *phc1* expression in *supt16h* mutants. **a**, Image of *phc1* peaks in WT (top) and *supt16h*^{-/-} (middle) siblings based on p53 ChIP-seq, with the location of the introns (blue lines) and exons (blue boxes) indicated (bottom). **b**, ATAC-seq peaks for *phc1* in WT and *supt16h*^{-/-} sibling embryos. **c**, Expression of *phc1*, determined by RT-qPCR, at 32 h.p.f. in WT and *supt16h*^{-/-} sibling embryos that are *p53*^{+/+}, *p53*^{+/-} or *p53*^{-/-}. The expression values are relative to those of the WT siblings (horizontal dotted line). Data are represented as the mean \pm s.d.; $n = 3$. One-way ANOVA with Tukey's post-hoc test, **** $P < 0.0001$ and ** $P = 0.001$. **d–f**, WISH analysis of *phc1* in WT (**d**), *supt16h*^{-/-}; *p53*^{+/+} (**e**) and *supt16h*^{-/-}; *p53*^{-/-} (**f**) sibling embryos at 28 h.p.f. Scale bar, 100 μ m. **g**, Predicted p53 responsive element in the *phc1* allele of *Danio rerio*. The schematic has been overlaid with p53 ChIP-seq peaks of *phc1*. **h**, Relative luciferase activity of a HCT116 p53-knockout (Δ p53 RE) cell line to assess the p53-dependent transactivation of the predicted responsive element in *phc1*. Data are represented as the mean \pm s.d.; $n = 4$. Two-way ANOVA with Holm–Sidak correction for multiple comparisons; **** $P < 0.00001$ and NS, not significant. **i**, Western blot of the whole-cell lysates of Δ p53 RE and WT HCT116 cell lines probed for p53. Raw unprocessed blots and quantification data are available. For **a–c** value in parenthesis represents track height in pixels.

the upregulation of *p53* levels to suggest p53 enhances the expression of *phc1* in these embryos (Fig. 6c–f and Extended Data Fig. 8i–w). To further determine transcriptional regulation of *phc1* by p53, we performed a luciferase enhancer assay³³ in HCT116 p53-knockout cells using a 611-base pair intronic sequence of zebrafish *phc1* based on p53 ChIP binding and a deletion of the 20-base pair p53 responsive element³⁴ (Δ p53 RE) found in this sequence (Fig. 6g). We observed a notable increase in luciferase activity at the *phc1* intron sequence compared with the controls and Δ p53 RE mutants, whereas no change in luciferase activity was observed in the absence of p53 (Fig. 6h,i), suggesting that p53 has the ability to regulate *phc1* transcription.

We knocked down *phc1* in *supt16h*^{-/-}; *Trp1*:GFP mutants to determine the effect of *phc1* on Notch expression and HSPC formation,

and observed a detectable elevation in Notch activity at 28 h.p.f. (Fig. 7a–d). We obtained similar results through WISH, where *notch1b*, *notch3*, *dlc* and *dll4* expression along with the HSPC marker *runx1* were rescued specifically within the dorsal aorta in *supt16h*^{-/-} mutants injected with *phc1*-MO (Fig. 7e–x). However, the rescue was not as pronounced in *notch1a*, *dla* and *dld*, whose expression pattern was contained mostly to neural tissues (Extended Data Fig. 9a–l). In addition, we performed complementary experiments overexpressing *phc1* via injection of mRNA or plasmid containing endothelial-specific *fli1a* promoter driving *phc1* and found no pronounced decrease in Notch transcripts or effect on HSPC formation with sole induction of *phc1* expression in WT embryos (Extended Data Fig. 9m–j). These data highlight the ability of p53 to modulate *phc1* expression and suggest PHC1 influences specific Notch genes,

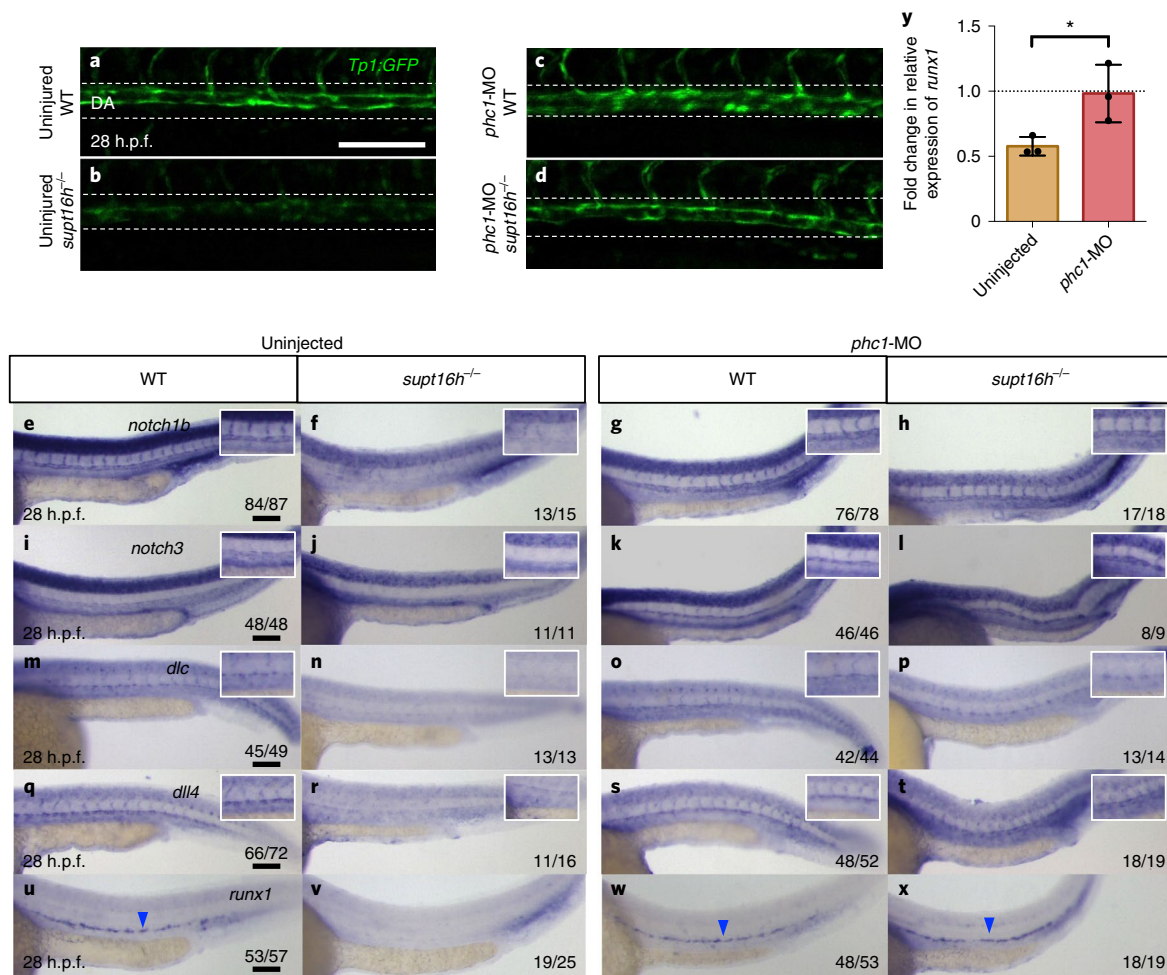


Fig. 7 | Expression of *phc1* influences Notch-gene transcription and HSPC specification. **a–d**, Representative confocal images, at 28 h.p.f., along the dorsal aorta (DA) of Tg(*Tp1:GFP*) embryos on WT (**a,c**) and *supt16h*^{−/−} (**b,d**) sibling backgrounds injected with *phc1*-MO (**c,d**) as well as uninjected controls (**a,b**). $n = 15$ (WT), 5 (*supt16h*^{−/−}), 16 (WT + *phc1*-MO) and 4 (*supt16h*^{−/−} + *phc1*-MO). **e–x**, WISH of WT and *supt16h*^{−/−} sibling uninjected controls (**e,f,i,j,m,n,q,r,u,v**) and embryos injected with *phc1*-MO (**g,h,k,l,o,p,s,t,w,x**) probed for *notch1b* (**e–h**), *notch3* (**i–l**), *dlc* (**m–p**), *dll4* (**q–t**) and *runx1* (**u–x**) at 28 h.p.f. Insets: magnified views of the dorsal aorta and intersomitic vessels. The blue arrowheads indicate HSPCs. **y**, Expression of *runx1*, determined by RT-qPCR, of *supt16h*^{−/−} embryos injected with *phc1*-MO at 28 h.p.f. Data are represented as the mean \pm s.d.; $n = 3$. Two-tailed Student's t-test, $P = 0.0393$. The expression values are relative to those of the WT siblings (horizontal dotted line). For **e–x** the fractions are the representative outcome for each genotyped group. Scale bars, 100 μ m. Source data provided.

notably those expressed along the dorsal aorta—such as *notch1b*, *notch3*, *dlc* and *dll4*—to affect HSPC formation.

Discussion

We report the characterization of a *supt16h* vertebrate mutant, highlighting the complex mechanism by which the Notch pathway is regulated to control HSPC development. Our findings have several implications, notably: (1) a histone chaperone plays an essential role in haematopoietic development, (2) vertebrate Supt16h functions as a transcriptional regulator but not as a global chromatin remodeller, (3) Supt16h has independent roles from SSRP1 and the FACT complex, (4) p53 can mediate cell differentiation and stem-cell fate through specific gene activation, and (5) PHC1 influences the transcription of Notch genes to allow HSPC formation. Ultimately, our study has elucidated a highly precise and complex mechanism for the regulation of the expression of Notch gene components.

We suggest a model to describe this complex regulation where in a WT setting, p53 and *phc1* exhibit baseline levels of accessibility and expression that result in normal transcription of Notch genes and proper HSPC specification (Extended Data Fig. 10). In

the absence of *supt16h*, the p53 locus becomes highly accessible, resulting in elevated p53 levels. Subsequently, p53 binds to *phc1* chromatin to allow for enhanced *phc1* expression. PHC1, as part of the Polycomb repressive complex 1 (PRC1), then inhibits Notch signalling by acting as a transcriptional repressor or co-repressor of Notch genes. Due to the absence of Notch expression, HSPCs fail to specify.

Notch has multiple iterative roles during HSPC development^{3,7–9,17,21,35}. Here, we propose that the levels of p53, through Supt16h-mediated accessibility, influence the transcriptional abundance of Notch genes by activating *phc1* expression. PHC1 influences Notch-gene expression as part of PRC1, possibly as a co-factor with another protein based on our knockdown and overexpression studies. Research by Martinez and colleagues support the role of the Ph locus as a direct Notch transcriptional regulator with their studies in *Drosophila* showing that knockout of the Ph locus (*ph-p* and *pd-d*) upregulates Notch genes (*notch*, *serrate* and *eyegone*), whereas overexpression reduces expression³⁶. Similar work by Boyer and colleagues demonstrate Phc1 binds to *dll4* and *dll1* along with 920 other genes in mouse ES cells to influence

differentiation through dynamic repression³⁷. Additional studies, both in *Drosophila* and human cell lines, have shown that many target genes of Polycomb-group proteins are components of the Notch signal-transduction pathway^{38–40}.

An alternative possibility is that PHC1, as part of PRC1, regulates HSPC formation independently of Notch signalling by instead binding to core transcription factors that affect HSPC expression. Yu and colleagues demonstrated two core PRC1 components, Bmi1 and Ring1b, directly bind to the Runx1–CBF β transcription factor complex, which is highly involved in haematopoietic development. Following knockdown of Bmi1 or Ring1b, zebrafish HSPC formation was markedly impaired, highlighting the necessary recruitment of PRC1 by Runx1–CBF β to affect haematopoiesis. Moreover, studies of *phc1*-deficient mice show impaired haematopoietic activity in the fetal liver due to its role in maintaining HSPC self-renewal and proliferation capabilities^{41,42}. Cumulatively, these data implicate a role of PRC1 regulation during HSPC formation.

The influence of Supt16h on p53 expression has been highlighted in several studies that link FACT to p53 activation in response to DNA damage^{43,44}. On forming a complex with FACT, the protein kinase CK2 undergoes a conformational change that allows it to preferentially recognize and activate p53 (ref. 44). In addition, drug-induced ‘chromatin trapping’ of the FACT complex by curaxin in tumour lines results in p53 pathway activation, NF- κ B suppression and tumour-cell death⁴⁵. Further work is required to determine the mechanism by which Supt16h influences p53 expression, whether it is through transcriptional regulation, as we have suggested based on p53 chromatin accessibility, or through a direct protein interaction with FACT, based on the work of Keller and colleagues^{43,44}.

Despite being characterized as a histone chaperone, global chromatin accessibility was not substantially affected in *supt16h*^{−/−} embryos. This could be attributed to the heterogeneity of using whole embryos, which makes it difficult to accurately assess global impacts, especially when transcriptional changes may be more subtle in certain tissues. Kolundzic and colleagues have compelling studies on FACT in more homogenous systems, suggesting accessibility correlates with gene expression⁴⁶. In addition, they have shown in *C. elegans* and human fibroblasts that FACT can both positively and negatively influence gene regulation and it does not notably alter chromatin accessibility genome wide, which provides support for our observation in zebrafish. Other studies have shown FACT increases chromatin accessibility in certain regions or only changes DNA shape in a weak and transient manner, which parallels the minor changes we observed in the chromatin accessibility and tissue-specific alteration of Notch transcription in *supt16h*^{−/−} animals⁴⁷. Further research on the temporal and tissue-specific function of Supt16h, using our vertebrate animal model, can expand our understanding of the highly complex and tightly regulated role of the FACT complex during gene regulation.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at <https://doi.org/10.1038/s41556-020-00604-7>.

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Methods

Zebrafish strains. Zebrafish were maintained and propagated as previously described⁴⁸ in accordance with the guidelines of the University of California, San Diego Institutional Animal Care and Use Committee (IACUC). Embryos and adult fish were raised in a circulating aquarium system (Aquaneering) at 28°C. The following lines were used: WT AB*, WT WIK, Tg(*cmyb:GFP*)^{z169} (ref. 49), Tg(*kdr1:mCherry*) (ref. 18), Tg(*flil:DsRed*)^{um13} (ref. 50), Tg(*CD41:GFP*) (ref. 51), Tg(*Trp1:GFP*)^{um14} (ref. 52), UAS:*NICD-myc* Tg(5×UAE-*E1b*:6×MYC-*notch1a*)^{kca3} (ref. 17), TgBAC(*cdh:gal4ff*)^{mu101} (ref. 53), Tg(*hsp70l:gal4*)^{1.5kca4} (ref. 54), Tg(*flil1a:GFP*)^{y1} (ref. 55), *tp53*^{zdf1/zdf3} (ref. 25) and *ssrp1a*^{sa31984} (ref. 32). Mutant *supt16h* animals were generated through a forward genetic screen described in the 'Forward genetic screen' section of Methods. The allele number for *supt16h*^{−/−} zebrafish is SD45. Heat shock was performed at 14 h.p.f. for 45 min at 37°C as previously described¹⁷. Details on the strains and ages are noted for each experiment. Gender was not selected in any of the studies conducted.

Forward genetic screen. Wild-type AB* strain males were mutagenized through treatment with 3.3 mM ENU (Sigma) weekly for three weeks. The mutagenized males were crossed to WT WIK females to produce the F₁ generation. These were outcrossed to WIK or AB animals and the resulting F₂ siblings were subjected to random sibling incrosses. The F₃ embryos were screened using WISH at 26–30 h.p.f. with the *runx1* HSPC marker.

Mutant mapping and differential expression analysis by RNA-seq.

Approximately 40 *supt16h*^{−/−} and 40 WT embryos were collected at 32 h.p.f. and RNA was extracted using TRIzol (Ambion). RNA libraries were generated using a TruSeq stranded mRNA kit and run on an Illumina HiSeq2500 PE50 system. The RNA-seq data underwent quality assessments using FastQC 0.11.2 and MultiQC 1.5 and were trimmed using Trimmomatic 0.32 (refs. 56,57). Reads were aligned to the zebrafish genome Zv9.69 and the assembled transcripts were inputted into the RNAmapper pipeline to map the mutants through linkage analysis of SNP haplotype blocks. Candidate mutations were identified using the Ensembl Variant Effect Predictor¹⁴. A list of candidate SNPs causal for the mutation was then generated using the RNAidentifieR custom R script¹⁴.

Three additional biological replicates to assess differential expression were sequenced on the Illumina NovaSeq PE100 system. Quality-control checks were performed using RSeQC⁵⁸ and FastQC⁵⁶. The reads were aligned using the Burrows–Wheeler transform⁵⁹, and aligned with TopHat⁶⁰. Cufflinks was used to determine differential expression and the DEGseq R package was used to identify genes that were differentially expressed⁶¹.

WISH. Embryos were fixed overnight in 4% paraformaldehyde (PFA) in PBS at 4°C, washed with PBS containing 0.1% Tween-20 (PBST) and transferred stepwise into methanol in PBST (25, 50, 75 and 100%). The embryos were hydrated stepwise into PBST (25, 50, 75 and 100%) and incubated with 100% acetone at −20°C for 10 min for embryos at 10–32 h.p.f. and 15 min for older embryos. The samples were washed with PBST, prehybridized at 65°C for 1 h in hybridization buffer (Hyb; 50% formamide, 5×SSC, 500 µg ml^{−1} torula (yeast) tRNA, 50 µg ml^{−1} heparin, 0.1% Tween-20 and 9 mM citric acid (pH 6.5)) and hybridized overnight with digoxigenin (DIG)- or fluorescein-labelled RNA probe in Hyb. The samples were washed stepwise at 65°C for 15 min in Hyb containing a 2×SSC mix (75, 50 and 25%), followed by two washes with 0.2×SSC for 30 min at 65°C. Subsequent washes were performed at room temperature for 5 min with 0.2×SSC in PBST (75%, 50%, 25%). The embryos were placed in PBST containing WISH block solution (2% heat-inactivated goat serum and 2 mg ml^{−1} BSA) for 1 h and incubated overnight at 4°C in anti-DIG-AP (1:5,000; Roche) diluted in WISH block solution. To visualize, the samples were washed 3× in AP (alkaline phosphatase) reaction buffer (100 mM Tris, pH 9.5, 50 mM MgCl₂, 100 mM NaCl, 0.1% Tween-20 and 1 mM tetramisole hydrochloride) for 5 min and incubated in AP reaction buffer with NBT/BCIP substrate (Promega).

Antisense RNA probes for the following genes were prepared using DIG- or FITC-labelled UTP (Roche) as previously described: *cdh5*, *cmyb*, *dla*, *dlb*, *dlc*, *dlf*, *dll4*, *efnb2a*, *etsrp*, *flt4*, *foxc1b*, *gata1*, *jag1a*, *kdr1*, *l-plastin*, *lmo2*, *notch1a*, *notch1b*, *notch2*, *notch3*, *rag1*, *runx1*, *scf* and *twist1b*^{2,63}. The probes for *phc1*, *ssrp1a*, *ssrp1b* and *supt16h* were generated from the full-length complementary DNA.

Immunohistochemistry. For staining of Myc in UAS:*NICD-myc* after WISH, the samples were placed in block solution (150 mM maleic acid, pH 7.5, 100 mM NaCl and 2% Boehringer blocking reagent) for 1 h at room temperature and incubated with anti-c-myc antibody (1:1,000; BioLegend) in block solution overnight at 4°C. These were washed 4× with block solution at room temperature for 30 min, incubated with secondary donkey anti-mouse AlexaFluor-488 antibody (1:1,000; ThermoFisher) at 4°C overnight and washed 4× with blocking solution at room temperature for 30 min.

Double fluorescence in situ hybridization. Double fluorescence in situ hybridization embryos were fixed with 4% PFA, dehydrated and rehydrated as described earlier for WISH. The embryos were then washed twice with PBST for 5 min, fixed again with 4% PFA for 20 min at room temperature, digested with

Proteinase K (Sigma) for 3 min at room temperature, rinsed with PBST and fixed again with 4% PFA for 20 min at room temperature. These were then washed twice with PBST, incubated in Hyb buffer at 65°C for 1 h, and incubated with anti-DIG *gfp* and anti-FITC *etsrp* probes diluted in Hyb at 65°C for 2 d. After incubation, the following washes were performed at 65°C: twice with Hyb for 30 min, twice with SSC for 15 min and once with 0.2×SSC for 30 min. The embryos were incubated in block solution (150 mM maleic acid, pH 7.5, 100 mM NaCl and 2% Boehringer blocking reagent) for 1 h at room temperature. Diluted anti-fluorescein-POD (1:500; Roche) in block solution was then added overnight at 4°C. The following washes were performed at room temperature: 4× with maleic acid buffer (150 mM maleic acid, pH 7.5 and 100 mM NaCl) for 20 min and 2× with PBS for 5 min. The samples were incubated for 1 h in TSA plus fluorescein solution (PerkinElmer), dehydrated stepwise into methanol in PBS (30, 50, 75 and 100%), incubated in 2% H₂O₂ in methanol for 30 min and rehydrated into methanol in PBS (75, 50 and 30%). The embryos were washed 2× with PBS, fixed with 4% PFA for 20 min, washed 2× with PBS, blocked for 1 h at room temperature with blocking solution and incubated with anti-DIG-POD (1:1,000; Roche) overnight at 4°C. The following washes were then done at room temperature: 4× with maleic acid buffer for 20 min and 2× with PBS for 5 min. The samples were incubated for 1 h in TSA plus Cy5 solution (PerkinElmer), washed 3× with PBST, fixed with 4% PFA at room temperature and placed stepwise in glycerol (25 and 50%).

Western blotting. Approximately 400 WT and 400 *supt16h*-mutant sibling embryos were deyolked (manual pipetting) at 32 h.p.f. in Ginzburg fish Ringer solution (55 mM NaCl, 1.8 mM KCl and 1.25 mM NaHCO₃). Whole-cell lysates were prepared by lysing the cells with Buffer X (100 mM Tris–HCl, pH 8.5, 250 mM NaCl, 1 mM EDTA, 1% Nonidet P-40 and 5 mM MgCl₂) with PhosSTOP phosphatase inhibitor (Roche), complete EDTA-free protease inhibitor cocktail (Roche) and 500 units of benzonase for 1 h at 4°C. The digested lysates were sonicated at 80% amplitude for 3 min (3 s on, 3 s off) and centrifuged at 13,000g for 5 min at 4°C. The collected supernatant was measured using a Bradford assay. Approximately 20 µg of protein was boiled for 3 min at 100°C, loaded onto a 7.5% SDS–PAGE gel and transferred onto a nitrocellulose membrane. The membrane was blocked (5% milk) and probed with anti-Supt16h (1:500; Cell Signaling Technology), anti-P53 (1:500; GeneTex), anti-α-tubulin (1:20,000; Sigma Aldrich) or anti-Lamin B1 antibody (1:2,000; Abcam) in TBST. Amersham ECL anti-mouse IgG (horseradish peroxidase-linked; 1:5,000; GE Healthcare) and Amersham ECL anti-rabbit IgG (horseradish peroxidase-linked; 1:5,000; GE Healthcare) secondary antibody was used. Protein was detected using Supersignal west pico luminol/enhancer solution (ThermoFisher) and Supersignal west femto maximum sensitivity substrate (ThermoFisher). Images were taken on a ChemiDoc XRS+ system using Image Lab (Bio-Rad).

GO analysis. The Gene Ontology Consortium's GO enrichment analysis (PANTHER classification system)^{15,16} was used to perform GO analysis. Downregulated genes with a log₂[fold change] > 1.0 based on Cufflinks were used for analysis of the RNA-seq data. Significant (adjusted *P* > 0.05) p53 ChIP peaks based on DESeq2 were used to analyse the ChIP-seq data.

Microscopy and image analysis. An SP5 inverted confocal microscope (Leica Microsystems) was used to image fluorescent transgenic embryos, double fluorescence in situ hybridization and TUNEL assays. Fluorescein and GFP were excited by a 488-nm laser, DsRed and Cy3 by a 543-nm laser, Streptavidin-647 by a 633-nm laser and 4,6-diamidino-2-phenylindole (DAPI) by a 405-nm laser. Sequential images were overlaid using ImageJ (NIH) or Imaris (Bitplane). A Zeiss AxioZoom.V16 microscope was used to image the immunohistochemistry samples using the AxioVision software 4.8. Visible-light imaging for sections or WISH was performed on a BX-51 Olympus or Leica MZ16 microscope (Leica FireCam Software 3.4.1).

Microinjections of mRNA, Morpholino and plasmid. Embryos were injected at the one-cell stage with 1 nl of antisense MOs (GeneTools) and/or mRNA. The MOs were diluted from 25 mM stocks in DEPC-treated H₂O and were used at concentrations of 2.5 ng *supt16h*-MO²⁴, 3.75 ng *p53*-MO⁶⁴, 2.5 ng *ssrp1a*-MO³¹, 2.5 ng *ssrp1b*-MO³¹ and 2.5 ng *phc1*-MO-5'-GGCTTCTGACCCACCTGAACAG-3'. Capped mRNAs were synthesized from linearized pCS2+ constructs using an mMessage mMachine SP6 transcription kit (Ambion). Full-length *supt16h* or *phc1* mRNA was injected into embryos at 200 ng µl^{−1} and 100 ng µl^{−1}, respectively. Transient *phc1* expression was conducted using the 478 p5Efl1ep plasmid (Addgene, 31160), described here as *fliep:phc1*. A PM1000 cell microinjector (MicroData Instrument) was used to inject the embryos using borosilicate glass needles (Sutter Instrument) made on the PMP102 micropipette puller (MicroData Instrument).

Cell preparation and flow cytometry. Tg(*CD41:GFP*; *kdr1:mCherry*) embryos (*n* = 100–200) were collected at 48 h.p.f., dissociated and digested with 1×Liberase TM (Roche) in PBS at 32°C for 30 min. Cells were centrifuged at 300g at 4°C for 10 min and resuspended in PBSF (1% heat-inactivated fetal bovine serum). The cells were filtered through a 40-µm filter by centrifugation and washed with PBSF.

SYTOX red (ThermoFisher) was added to exclude the non-viable cells. Sorting was performed on a FACSaria II (BD Biosciences).

Tg(*flil1a:dsRed*; *TP1:eGFP*) embryos ($n=100-200$) were dissociated at 22 h.p.f., with shaking at 120 r.p.m. in dissociation buffer (0.9×PBS, Liberase TM (1:50; Roche) and 10 µM EDTA) at 37 °C for 1–2 h. Cells were pelleted at 500g for 5 min, resuspended in buffer (0.9×PBS, 1% FBS, 1 mM EDTA and 0.1 µg ml⁻¹ DAPI) and filtered through an 80-µm filter. The cells were quantified using a BD LSR Fortessa and analysed using FlowJo. DAPI was used for dead-cell discrimination; 3–5 × 10⁶ cells were analysed per biological replicate.

RT-qPCR. RNA was extracted from whole embryos using TRIzol (Ambion) or an RNeasy mini kit (Qiagen). Complementary DNA was synthesized using an iScript gDNA clear cDNA synthesis kit (Bio-Rad). RT-qPCR was performed on a Bio-Rad CFX96 system according to the manufacturer's instructions. *Tbp* expression was used to normalize the amount of the investigated transcripts using $\Delta\Delta C_t$. The primers are listed in Supplementary Table 2.

ATAC-seq. Embryos were collected at 32 h.p.f. and deyolked in Ginzburg fish Ringer solution. Cells ($n=50,000$) were collected, washed with PBS and pelleted at 500 × g for 5 min at 4 °C. Nuclei were extracted by adding 50 µl of cold lysis buffer (10 mM Tris-HCl, pH 7.4, 10 mM NaCl, 3 mM MgCl₂ and 0.1% (vol/vol) Igepal CA-630) and centrifuging at 500 × g for 10 min at 4 °C. The pellets were resuspended in 50 µl transposition reaction (Nextera Tn5 transposase kit). The nuclei were incubated at 37 °C for 30 min and DNA was purified using an Omega MicroElute DNA cleanup kit. The DNA was PCR amplified and barcoded with NEBNext high-fidelity 2×PCR master mix using the following protocol: (1) 72 °C for 5 min, (2) 98 °C for 30 s, (3) 98 °C for 10 s, (4) 63 °C for 30 s, (5) 72 °C for 1 min, (6) five repeats of steps (3)–(5), and (6) hold at 4 °C (ref. ⁶⁵). RT-qPCR was performed to determine additional cycles. Libraries were size selected using Mag-Bind RxnPure plus (Omega), with the first selection eliminating the small products (0.4:1 ratio) and the second selection (1.4:1 ratio) to select the desired products. The samples were quantified using a BioAnalyzer and KAPA library quantification kit before sequencing at PE100 on a HiSeq4000 system (Illumina).

Two technical and three biological replicates were performed for the WT and *supt16h*^{-/-} embryos. OLEgo 1.1.5 was used for sequence alignment⁶⁶ on the zebrafish genome GRCz10 and HOMER 4.9.1 was used to call and analyse the peaks⁶⁷. The peaks were visualized using Integrative Genomics Viewer (IGV) 2.3.65 (ref. ⁶⁸).

Genotyping. DNA was extracted from embryos or fin clips by digesting with DNA extraction buffer (25 mM NaOH and 0.2 mM EDTA) for 1 h at 95 °C and stopped with Neutralization buffer (40 mM Tris-HCl). Genomic DNA was amplified by PCR using the primers: *Supt16h*-F, 5'-ATGAAGACGAGGACGAGGAA-3' and *Supt16h*-R, 5'-TTATGTGGGTGGGGAACACT-3'; or *Ssrp1a*-F, 5'-TTGTCTGCAGCACCTTGTC-3' and *Ssrp1a*-R, 5'-TTTGCTGCTGGGAATTTGT-3'. The amplified products were submitted for sequencing for SNP detection.

Cryosection. Following WISH, the embryos were washed with PBST and placed overnight in 30% sucrose at 4 °C. The samples were equilibrated 1:1 with Tissue Tek O.C.T. medium (Sakura) for 30 min at room temperature, mounted on cryoblocks containing O.C.T., and frozen on dry ice. Sections (7 µm) were cut on a Leica CM1860 Cryostat.

Detection of apoptotic cell death by TUNEL labelling. TUNEL was performed on rehydrated, fixed embryos that were permeabilized with PBSTx + dimethylsulfoxide (1×PBS, 0.5% Triton-X100 and 1% dimethylsulfoxide) for 3 h at room temperature. The samples were washed with PBSTw (1×PBS and 0.1% Tween-20), post-fixed with ethanol:acetic acid (2:1) at -20 °C for 20 min and washed with PBSTw. The embryos were blocked overnight at 4 °C with 5% BSA, washed with PBSTw, blocked with Avidin D solution (Vector Laboratories) for 30 min at room temperature, and washed with PBSTw. They were then blocked with Biotin solution (Vector Laboratories) for 30 min at room temperature, washed with PBSTw and incubated with equilibration buffer (1×TdT reaction buffer, 1×CoCl₂ and 1×PBS; Roche) for 1 h at room temperature. The samples were incubated in TdT reaction mix (600 units of Terminal Transferase, Biotin-16 UTP, Equilibration Buffer; Roche) at 37 °C for 4 h and washed 6 × 15 min with PBSTw. Tg(*flil1:GFP*) embryos were incubated overnight with anti-GFP antibody (1:500; Aves Lab) at 4 °C. These were washed 6 × 15 min with PBSTw and incubated with anti-streptavidin-Alexa647 antibody (1:500; ThermoFisher), goat anti-chicken Alexa Fluor 488 secondary antibody (1:500; ThermoFisher) and DAPI (1:1,000; Life Technologies) at room temperature for 3 h. The embryos were washed 6 × 15 min with PBSTw and mounted in Vectashield (Vector Laboratories).

Detection of apoptotic cell death by acridine orange staining. Dechorionated embryos were incubated in 50 µg ml⁻¹ acridine orange solution (Sigma) in E3 water for 1 h, protected from light. After three washes in 1×E3 water, the embryos were imaged by confocal microscopy.

ChIP-seq. Approximately 2,000 WT or mutant *supt16h* embryos were collected for two technical replicates and deyolked in Ginzburg fish Ringer solution. The embryos were resuspended in 1 ml PBS with 1 mM PMSF, 1×Complete EDTA-free protease inhibitor cocktail tablet (Roche) and 1×phosSTOP phosphatase inhibitor tablet (Roche), and pushed through a 21 G needle. The embryos were crosslinked with 4 mM DSG (ProteoChem) for 30 min, followed by 1% PFA for 8 min and stopped with 0.125 M glycine. The fixed embryos were resuspended in 1 ml Lysis buffer 1 (0.05 M HEPES, 140 mM NaCl, 1 mM EDTA, 1% glycerol, 0.5% NP40 and 0.25% Triton-X100) and lysed on the TissueRuptorII (Qiagen) for 20 s, low. The tissues were rocked for 10 min at 4 °C, pelleted and resuspended in 1 ml Lysis buffer 2 (10 mM Tris, pH 8, 200 mM NaCl, 1 mM EDTA and 0.5 mM EGTA). They were rocked again for 10 min at room temperature, pelleted and resuspended in 1.5 ml Lysis buffer 3 (10 mM Tris, pH 8, 100 mM NaCl, 1 mM EDTA, 0.5 mM EGTA, 0.1% sodium deoxycholate and 0.05% SDS). The tissues were sonicated using a Diagenode Bioruptor 300 (30 s on, 30 s off; high; 34 cycles). Triton-X100 (0.8%) was added and the cells were spun down. An aliquot of the lysate (20 µl) was saved to be used as an input control and the remainder was incubated overnight with Dynabeads bound with 10 µg p53 antibody (GeneTex) at 4 °C with rotation. The beads were collected on a magnet and washed 1× with Wash buffer 1 (20 mM Tris, pH 8, 150 mM NaCl, 2 mM EDTA, 0.1% SDS and 1% Triton-X100), 1× with Wash buffer 2 (20 mM Tris, pH 8, 500 mM NaCl, 2 mM EDTA, 0.1% SDS and 1% Triton-X100), 1× with Wash buffer 3 (10 mM Tris, pH 8, 250 mM LiCl, 1 mM EDTA, 1% sodium deoxycholate and 1% NP40), 3× with TET buffer (1×TE and 0.2% Tween-20) and 1× with TE-NaCl (1×TE and 50 mM NaCl). The beads were resuspended in 500 µl Elution buffer (50 mM NaHCO₃ and 1% SDS) and incubated overnight with 0.2 M NaCl at 65 °C. The lysate was incubated for 2 h at 37 °C with 5 µl RNase A (10 mg ml⁻¹) and 2 h at 55 °C with 5 µl proteinase K (20 mg ml⁻¹). DNA was purified using phenol-chloroform-isoamyl alcohol and resuspended in 50 µl TE (10 mM Tris, pH 8 and 1 mM EDTA). A TruSeq ChIP library preparation kit (Illumina) was used to construct SR75 libraries run on an Illumina HiSeq4000.

ChIP-seq data underwent quality assessments using FastQC 0.11.2 and MultiQC 1.5, and subsequent trimming of Illumina adaptors using Trimmomatic. STAR was used for sequence alignment using GRCz10 (ref. ⁶⁹) and HOMER was used to call and analyse the peaks⁶⁷. Differential gene expression was analysed using DESeq2 1.18.1. The peaks were visualized using Integrative Genomics Viewer (IGV) 2.3.65 (ref. ⁶⁸).

p53 Transactivation luciferase assay. HCT116 (ATCC CCL-247) cells knocked out for p53 were maintained at 37 °C in McCoy's 5a medium modified with 10% heat-inactivated FBS. The *Danio rerio* *phc1* intron was cloned into pGL4.23 (5'-GAGACGGATCCATACCATACATCAGTGGAAAGC-3' and 5'-GTCTCGTCGACTGCTAGGAATGCACCGATAA-3') and the 20-bp responsive element was deleted by Gibson assembly (5'-GCGTCGCACTTGCCTGGGCAAGTGCACCTATAATGGAGCTTTTC-3' and 5'-AAGCTCCATTATAGTGCATTCGCCACGCAAGTGCACGC-3'). The HCT116 cells were transiently transfected with pGL4.23 constructs by X-tremeGENE HP DNA transfection reagent (Roche). The cells were harvested after 48 h and the luciferase activity was measured using a Dual-luciferase reporter assay system (Promega) and SYNERGY Neo2 multi-mode reader (BioTek).

Statistics and reproducibility. In all figures: NS, not significant ($P>0.05$); * $P<0.05$; ** $P<0.01$; *** $P<0.001$ and **** $P<0.0001$. Data were analysed using Prism 6 and 7 (GraphPad). A two-tailed Student's *t*-test was conducted to compare two sample groups. To test for significance in larger groups, one-way or two-way ANOVAs, corrected for multiple comparisons using Tukey's or Sidak's statistical hypothesis testing, respectively, were used. In addition, comparisons for multiple *t*-tests were corrected for using the Holm-Sidak method. All tests used a confidence interval of 0.05. *P* values less than 0.05 were considered significant. Sample sizes were chosen based on the number of independent experiments required for statistical significance and technical feasibility. The statistical methods used for comparisons are indicated in the relevant figure legends.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

Raw and processed RNA-seq (linkage mapping), RNA-seq (differential expression), ATAC-seq and ChIP-seq data have been deposited into the public functional genomics data repository Gene Expression Omnibus. The accession numbers for these data are GSE106342, GSE127555, GSE106341 and GSE116088 for RNA-seq (linkage mapping), RNA-seq (differential expression), ATAC-seq and ChIP-seq, respectively. All other data supporting the findings of this study are available from the corresponding author on reasonable request. Source data are provided with this paper.

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Author contributions

Conceptualization and methodology: Y.L., D.Y. and D.T. Validation: Y.L. and S.G.E. Formal analysis and visualization: S.G.E. Investigation: Y.L., S.G.E., H.S., E.R., A.S., E.-S.K., J.E.M., C.-K.O., C.B., U.S., S.G., Y.H.P., L.P., M.-S.K., S.K. and K.M. Software: S.G.E., D.S. and C.A.N. Writing of the original draft: S.G.E., Y.L., D.T., D.Y. and K.L.C. Supervision: Y.L. and D.T.

Competing interests

All authors declare no competing interests.

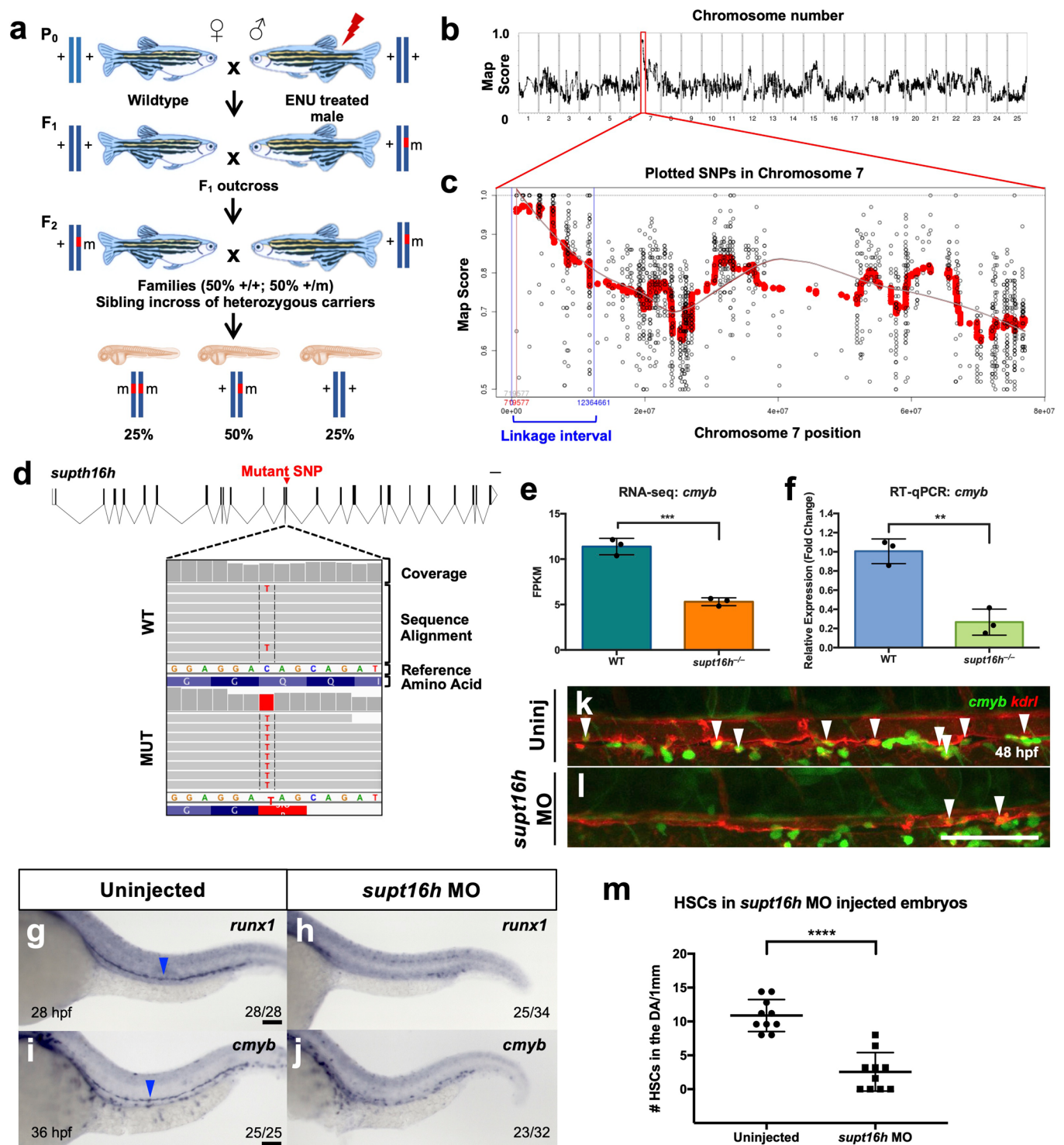
Additional information

Extended data is available for this paper at <https://doi.org/10.1038/s41556-020-00604-7>.

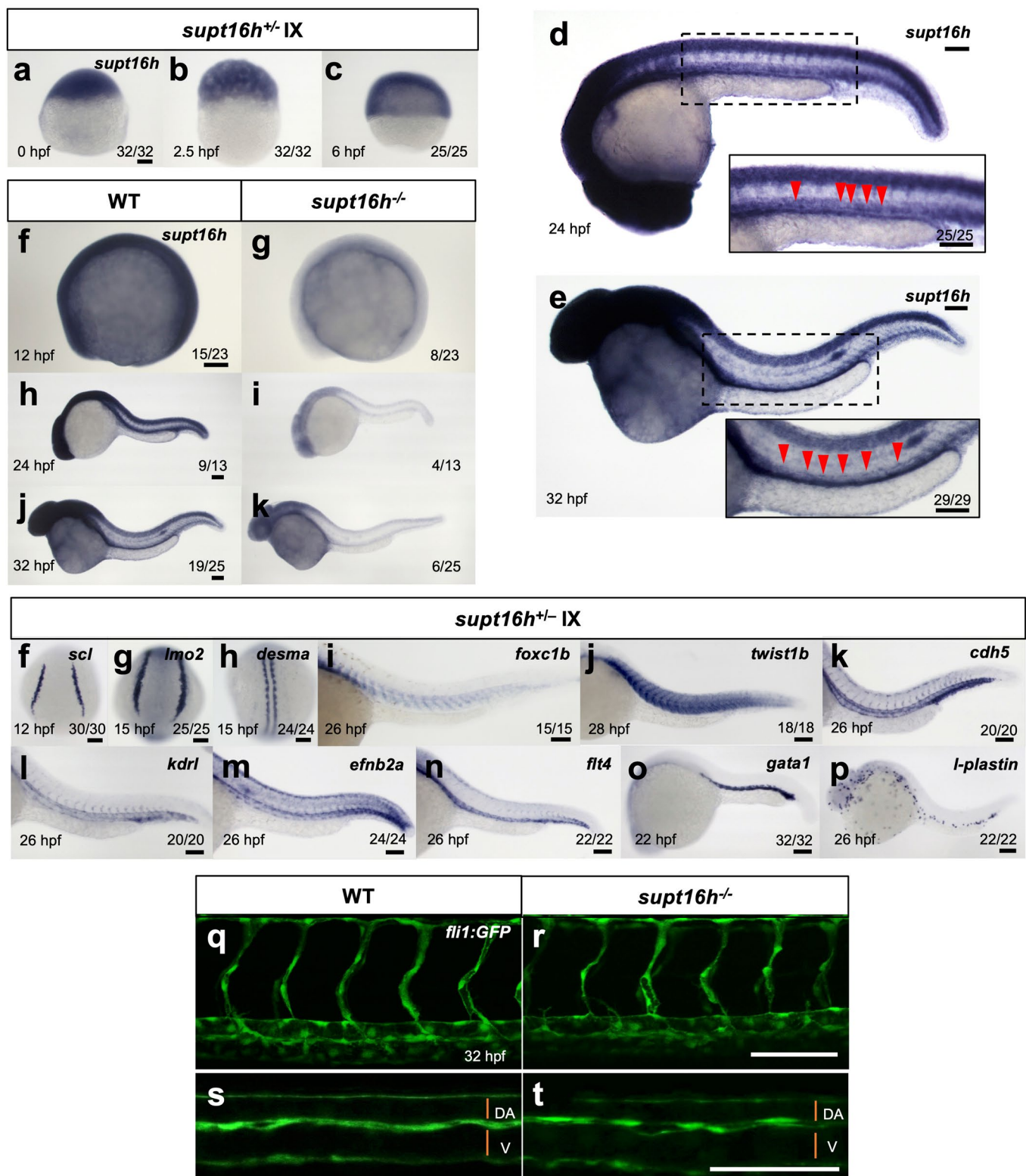
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Correspondence and requests for materials should be addressed to D.T. or Y.L.

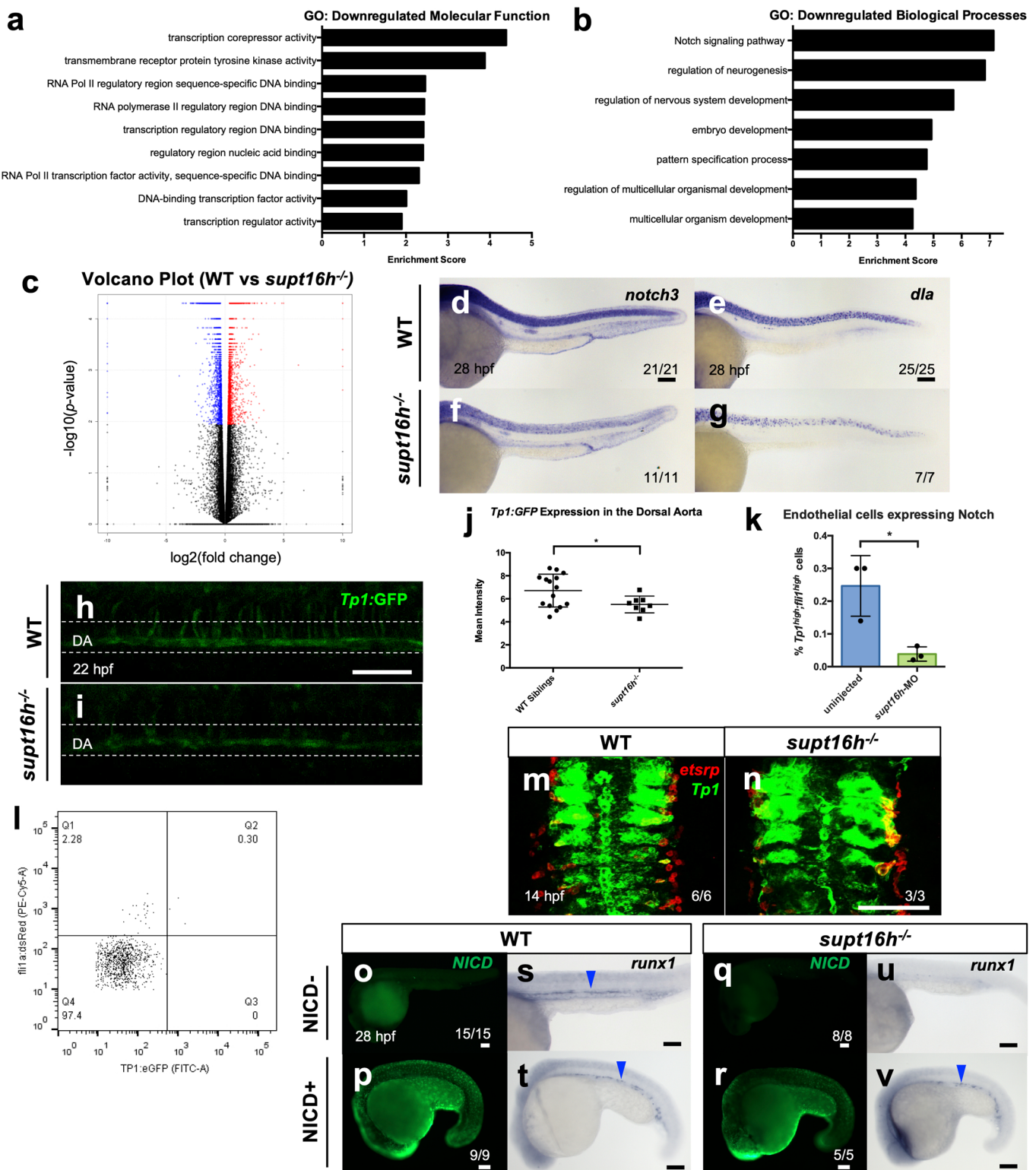
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Extended Data Fig. 1 | Characterizing the causal mutation from our forward genetic screen. **a**, Diagram of the forward genetic screen strategy. **b,c**, Mapping of RNA-seq using RNaMapper with whole genome view (**b**) and specifically looking at the linked interval on Chr 7 (**c**). **d**, Position and RNA-seq coverage of SNP on *supt16h* resulting in a premature stop codon. **e,f**, Expression of *supt16h*^{-/-} based on RNA-seq (**e**) (Represented as mean \pm s.d., two-tailed Student's *t*-test, $n = 3$, $P = 0.0005$) and RT-qPCR (**f**) (Represented as mean \pm s.d., two-tailed Student's *t*-test, $n = 3$, $P = 0.0024$) for *cmyb*. For RT-qPCR, expressions are relative to WT sibling. **g-j**, WISH of WT embryos injected with *supt16h*-MO for *runx1* (blue arrowheads) at 28 hpf and *cmyb* at 36 hpf. **k,l**, Representative confocal of Tg(*cmyb:GFP*; *kdr1:mCherry*) embryos injected with *supt16h*-MO from one independent experiment. Double positive HSPCs indicated by white arrowheads at 48 hpf. DA = dorsal aorta; V = vein. **m**, Quantification of double positive HSPCs from (**g** and **h**) (Represented as mean \pm s.e.m., two-tailed *t*-test, $n = 10$, $P < 0.0001$). Bar, 100 μ m. Source data provided in Supplementary Table 5.

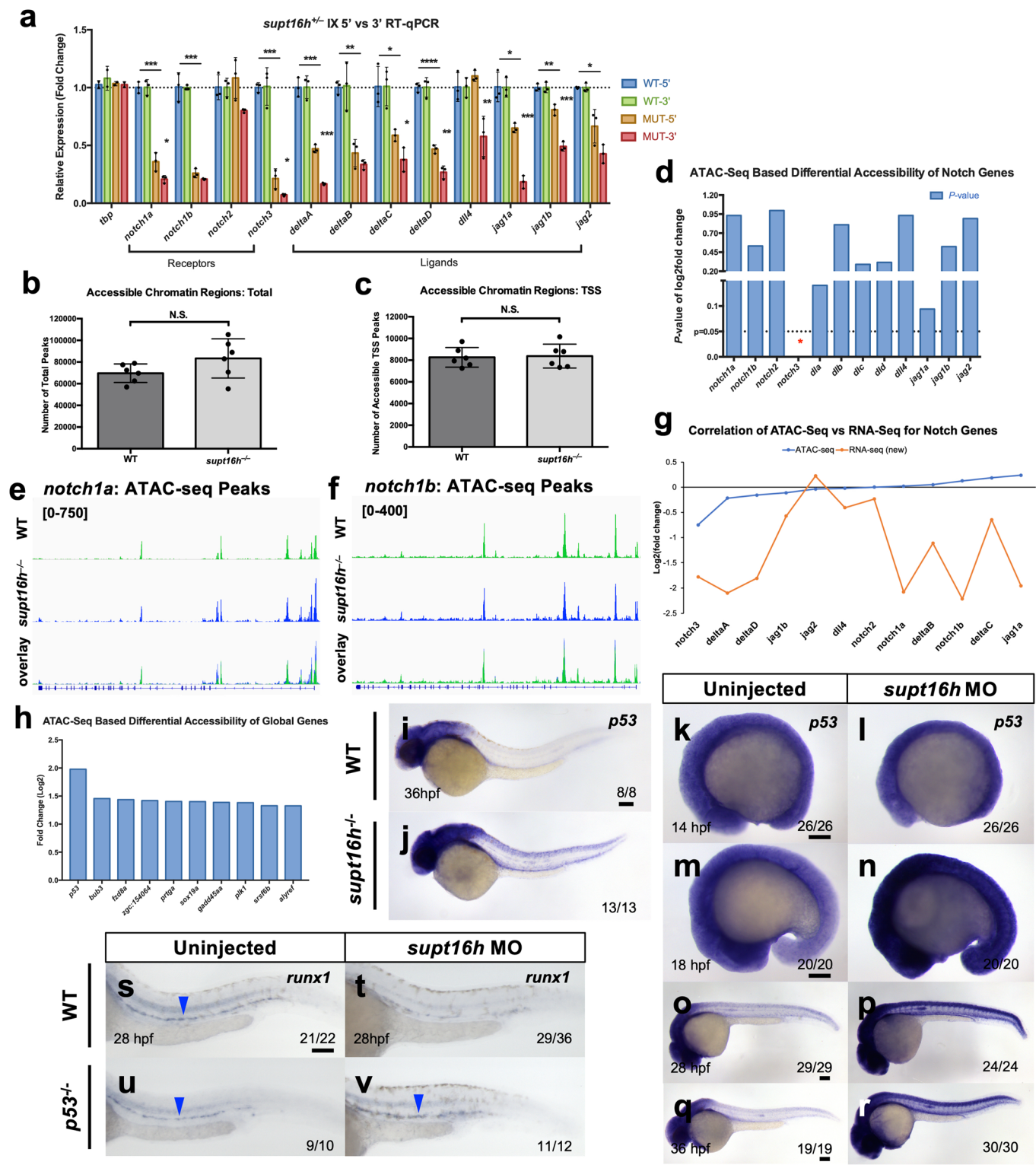


Extended Data Fig. 2 | The expression pattern of *supt16h* and the effect of its knockout on HSPC relevant tissues. a–c, WISH of embryos from a *supt16h^{+/-}* incross (IX) for *supt16h* expression at 0, 2.5, and 6 hpf. **d,e,** WISH of WT embryos for *supt16h* expression at 24 and 32 hpf. Insets zoom magnify the DA (red arrowheads). **f–k,** WISH of WT sibling and *supt16h^{-/-}* embryos for *supt16h* expression at 12, 24, and 32 hpf. **f–p,** WISH of *supt16h^{+/-}* incross (IX) using probes for posterior lateral mesoderm (PLM) makers *scl*, and *lmo2* (**f,g**), somitic marker *desma* (**h**), sclerotome marker *foxc1b* and *twist1b* (**i,j**), endothelial markers *cdh5* and *kdrl* (**k,l**), arterial marker *efnb2a* (**m**), venous marker *flt4* (**n**), primitive erythroid marker *gata1* (**o**), and primitive leukocyte marker *l-plastin* (**p**). **q,r,** Representative confocal of *supt16h^{-/-}* and WT sibling embryos on *Tg(fli1:GFP)* background examining vasculature development. Based on one independent experiment. **s,t,** Magnified images of (**q,r**) highlight vein (V) and dorsal aorta (DA) formation. Bar, 100 μ m.



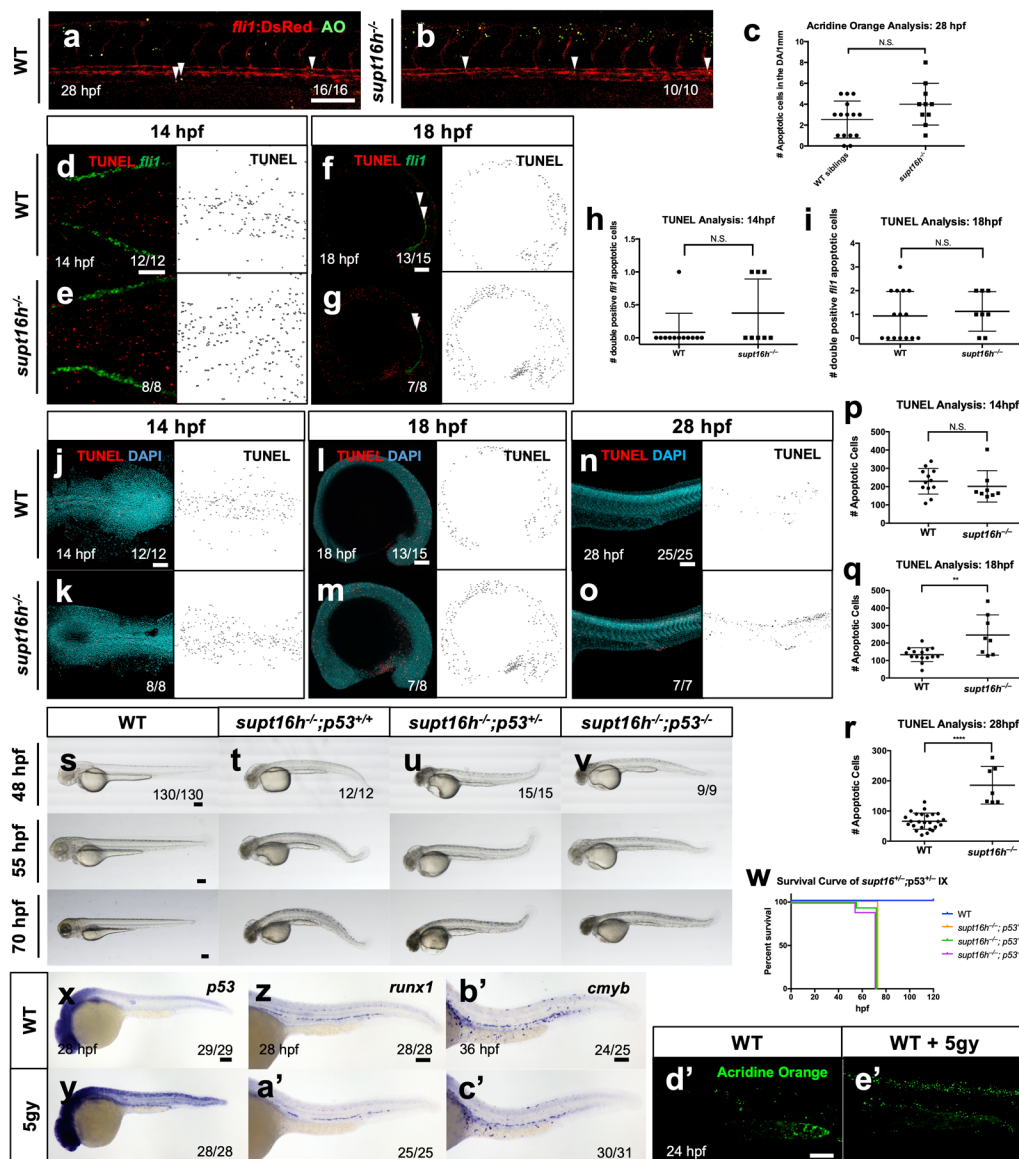
Extended Data Fig. 3 | See next page for caption.

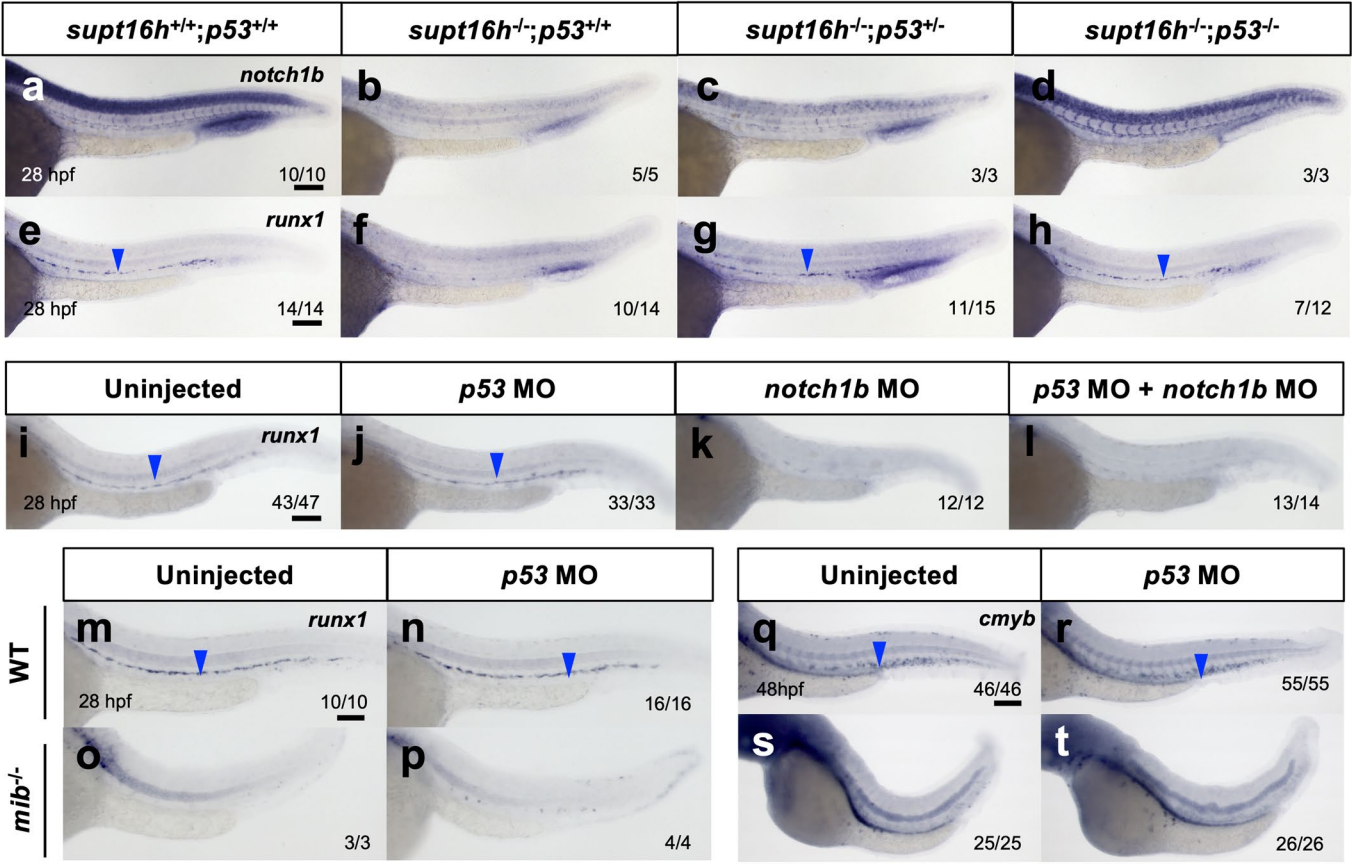
Extended Data Fig. 3 | Characterizing the effect of *supt16h* on Notch gene expression. **a,b**, Gene Ontology Analysis of Biological Components (**a**) and Molecular Components (**b**) shows downregulated genes in *supt16h*^{-/-} embryos based on a log2fold change >1. **c**, Volcano plot of the differentially expressed genes between WT sibling and *supt16h*^{-/-} embryos based on RNA-seq. Data representative of 3 (**a-c**) biological replicates. **d-g**, WISH of *supt16h*^{-/-} and WT sibling embryos using probes for *notch3* and *dla*. **h,i** Representative confocal images along the DA of *supt16h*^{-/-} and WT sibling embryos on *Tg(Tp1:GFP)* background at 22 hpf. Bar, 50 μ m. **j**, Mean fluorescence level from (**h,i**) of *Tp1:GFP* along the DA calculated in ImageJ (Represented as mean \pm s.e.m., two-tailed Student's *t*-test, $n_{WT}=15, n_{MUT}=8, P=0.0373$). **k**, Sorted double positive *Tp1:GFP*⁺; *fli1:DsRed*⁺ cells from *supt16h* morphants and uninjected controls at 22 hpf by flow cytometry (Represented as mean \pm s.d, two-tailed Student's *t*-test, $n=3, P=0.0192$). **l**, Gating strategy used to quantify TP1 (Notch- FITC) + endothelial (PE-Cy5) cells. **m,n**, DFISH of *Notch-active* (green) and *etsrp* (red) tissues in *supt16h*^{-/-} and WT sibling embryos on a *Tg(Tp1:GFP)* background at 14 hpf. Based on one independent experiment. **o-v**, Global expression of NICD⁺ and NICD⁻ embryos that are WT siblings or *supt16h*^{-/-} (*supt16h*^{-/-}; *hsp70:gal4* x *supt16h*^{-/-}; *UAS-myc:NICD*) analysed at 28 hpf by NICD immunohistochemistry (IHC) (**l-o**) and *runx1* WISH (**p-s**). Representative images from two independent experiments. Bar, 100 μ m. Blue arrowheads indicate HSPCs. Bar, 100 μ m. Source data provided in Supplementary Table 5.



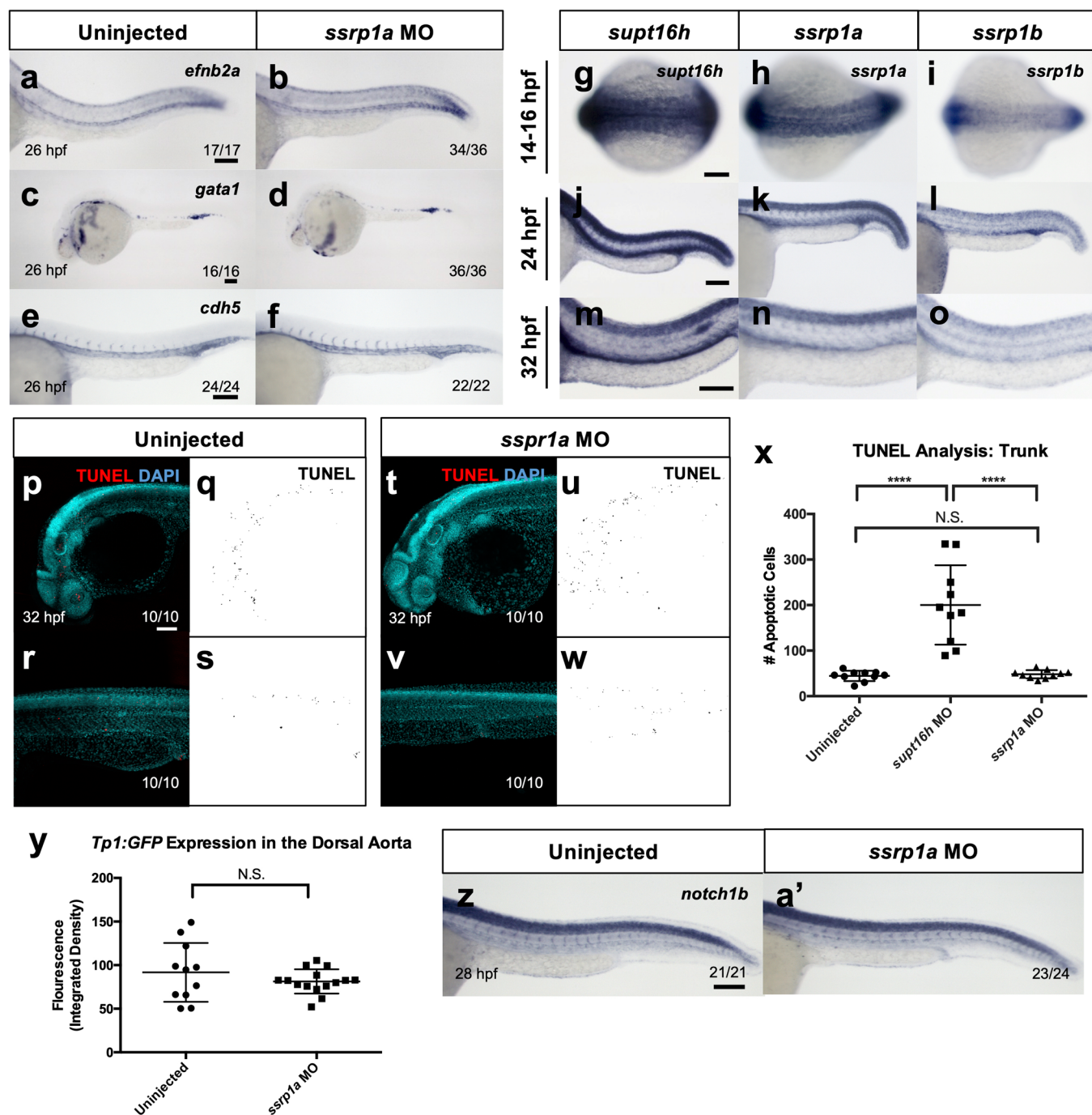
Extended Data Fig. 4 | See next page for caption.

Extended Data Fig. 4 | The effect of *supt16h* on transcript elongation and chromatin accessibility. **a**, RT-qPCR of 5' vs 3' initiation/elongation of Notch genes in WT sibling and *supt16h*^{-/-} embryos at 32 hpf. Expressions relative to WT sibling (horizontal dotted line) (Represented as mean \pm s.d, two-tailed Student's *t*-test with Holm-Sidak correction for multiple comparisons, *n* = 3, ****P* < 0.001, ***P* < 0.01, **P* < 0.05 lined significance compares WT 5' to MUT 3', significance over MUT 5' compares MUT 5' to MUT 3'). **b**, ATAC-seq results plotting the total number of accessible peaks in WT and *supt16h*^{-/-} embryos at 32 hpf (Represented as mean \pm s.d, two-tailed *t*-test, *n* = 6, N.S. = not significant). **c**, ATAC-seq results plotting the number of accessible TSS peaks in WT sibling and *supt16h*^{-/-} embryos (Represented as mean \pm s.d, two-tailed Student's *t*-test, *n* = 6, N.S. = not significant). **d**, *p*-values of the differential accessibility of Notch genes based on ATAC-seq log2fold change of *supt16h*^{-/-} vs WT sibling. **e,f**, ATAC-seq peak plot of chromatin accessibility in *supt16h*^{-/-} and WT sibling embryos for *notch1a* and *notch1b*. Bottom panel shows peak overlay. **g**, Plot of ATAC-seq log2fold change by increasing accessibility vs. corresponding RNA-seq log2fold change values. **h**, Rank order of top 10 differentially accessible genes based on ATAC-seq of *supt16h*^{-/-} and WT sibling embryos at 32 hpf. **i,j**, WISH of *p53* in WT sibling and *supt16h*^{-/-} embryos at 36 hpf. **k-r**, WISH of *p53* in embryos injected with *supt16h*-MO at 14, 18, 28, and 36 hpf. **s-v**, WISH of *runx1* (blue arrowheads) for WT sibling and *p53*^{-/-} injected with *supt16h*-MO. Data representative of 2 (**i-r**) and 3 (**a,h**) biological replicates. Bar, 100 μ m. Source data provided in Supplementary Table 5.





Extended Data Fig. 6 | Characterizing the effect of *p53* on Notch gene expression and HSPC formation. **a–h**, WISH of WT sibling (**a,e**) and *supt16h*^{-/-} embryos that are *p53*^{+/+} (**b,f**), *p53*^{+/-} (**c,g**), or *p53*^{-/-} (**d,h**) for *notch1b* (**a–d**) and *runx1* (**e–h**) at 28 hpf. **i–l**, WISH for *runx1* of embryos injected with *p53*-MO, *notch1b*-MO, or both MOs at 28 hpf. **m–t**, WISH of *runx1* (**m–p**) and *cmyb* (**q–t**) for WT sibling or *mib*^{-/-} embryos injected with *p53*-MO. Blue arrowheads indicate HSPCs. Bar, 100 μm.

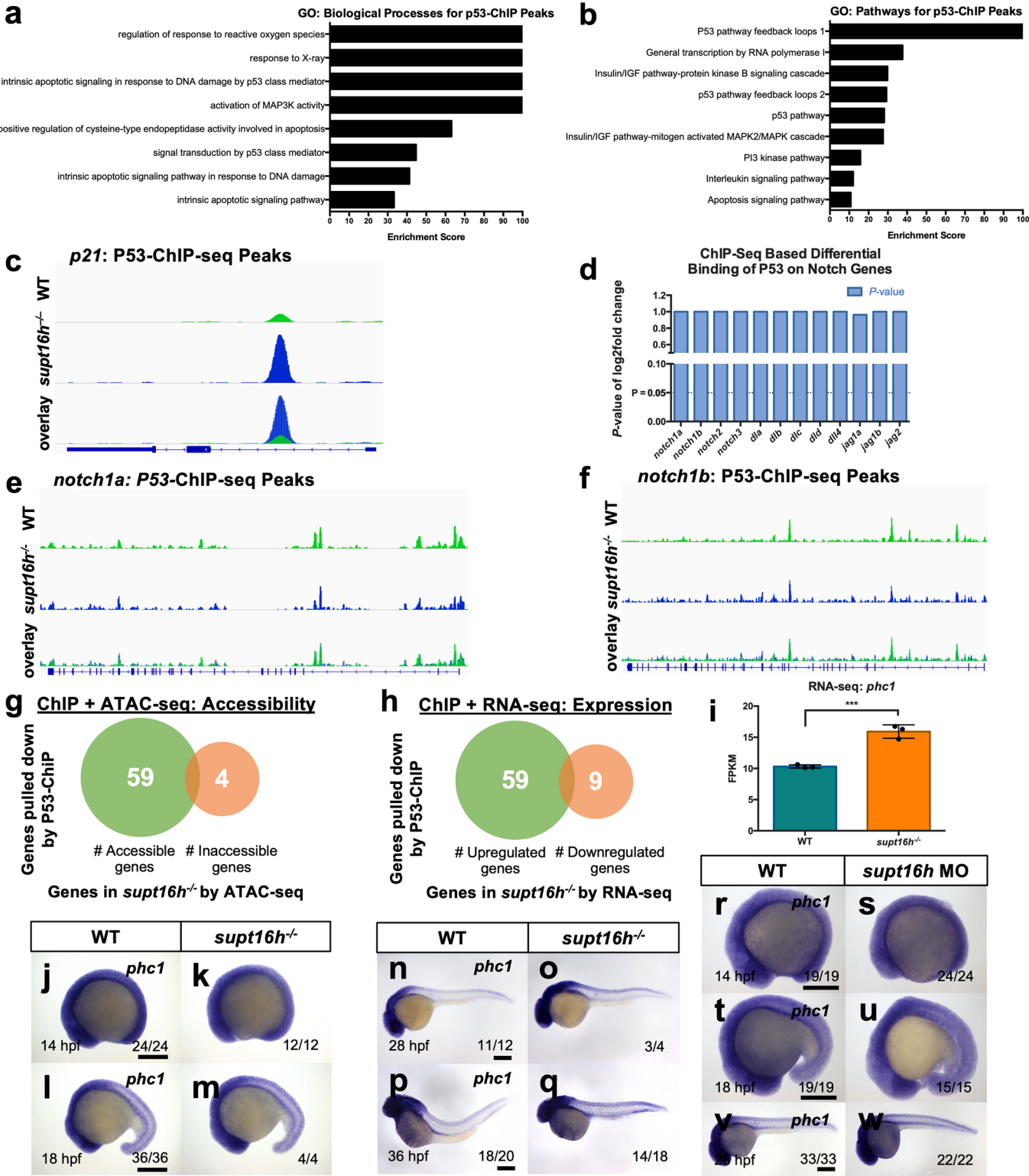


Extended Data Fig. 7 | Characterizing the expression profile and effect of *ssrp1a* on HSPCs. **a-f**, WISH of embryos injected with *ssrp1a*-MO and probed with *efnb2a* (**a,b**), *gata1* (**c,d**), and *cdh5* (**e,f**) at 26 hpf. No effect is seen on dorsal aorta, red blood cell, or vasculature formation upon *ssrp1a* knockdown.

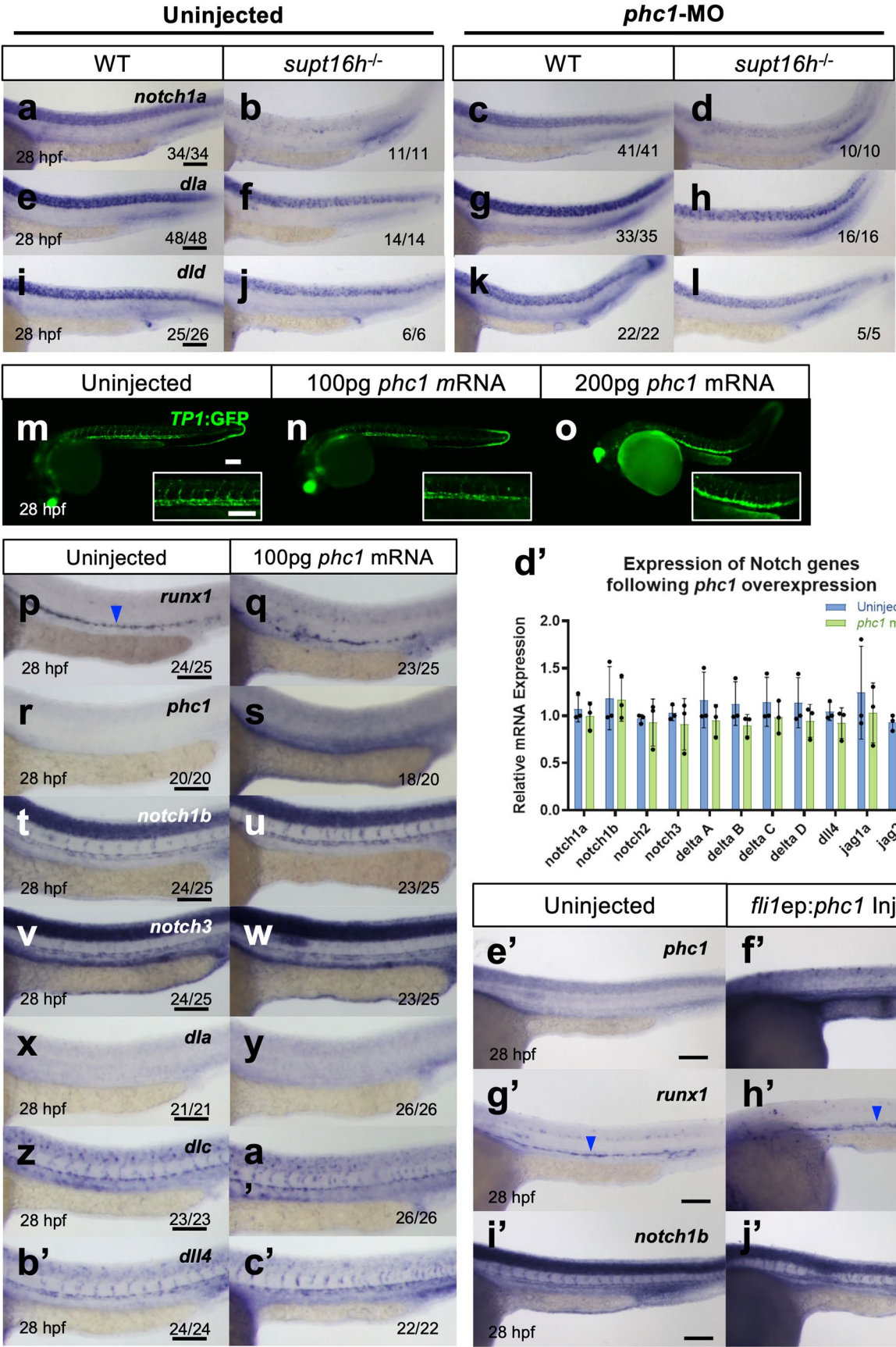
g-o, WISH of *supt16h*, *ssrp1a*, and *ssrp1b* for WT embryos at 14-16 hpf with a dorsal view (**g-i**), and 24 hpf (**j-l**) and 32 hpf with a posterior lateral view (**m-o**).

p-w, TUNEL of uninjected (**p-s**) and *ssrp1a*-MO (**t-w**) embryos at 32 hpf. Confocal images of TUNEL and DAPI (left) and TUNEL only (right) are shown.

x, Quantification of the number of apoptotic cells in *ssrp1a* morphants from (**p-w**) based on TUNEL for the trunk region (Represented as mean \pm s.d., one-way ANOVA with post-hoc Tukey, $n = 10$, $P < 0.0001$, N.S. = not significant). **y**, Mean fluorescence level of *Tp1:GFP* of the DA calculated in ImageJ based on Integrated Density (Represented as mean \pm s.d., two-tailed t-test, $n_{WT} = 11$, $n_{MO} = 15$, $*P = 0.0147$, N.S. = not significant). **z,a'**, WISH of *notch1b* in WT sibling and *ssrp1a*^{-/-} embryos at 28 hpf. Data representative of 2 (**g-o**) biological replicates. Bar, 100 μ m. Source data provided in Supplementary Table 5.

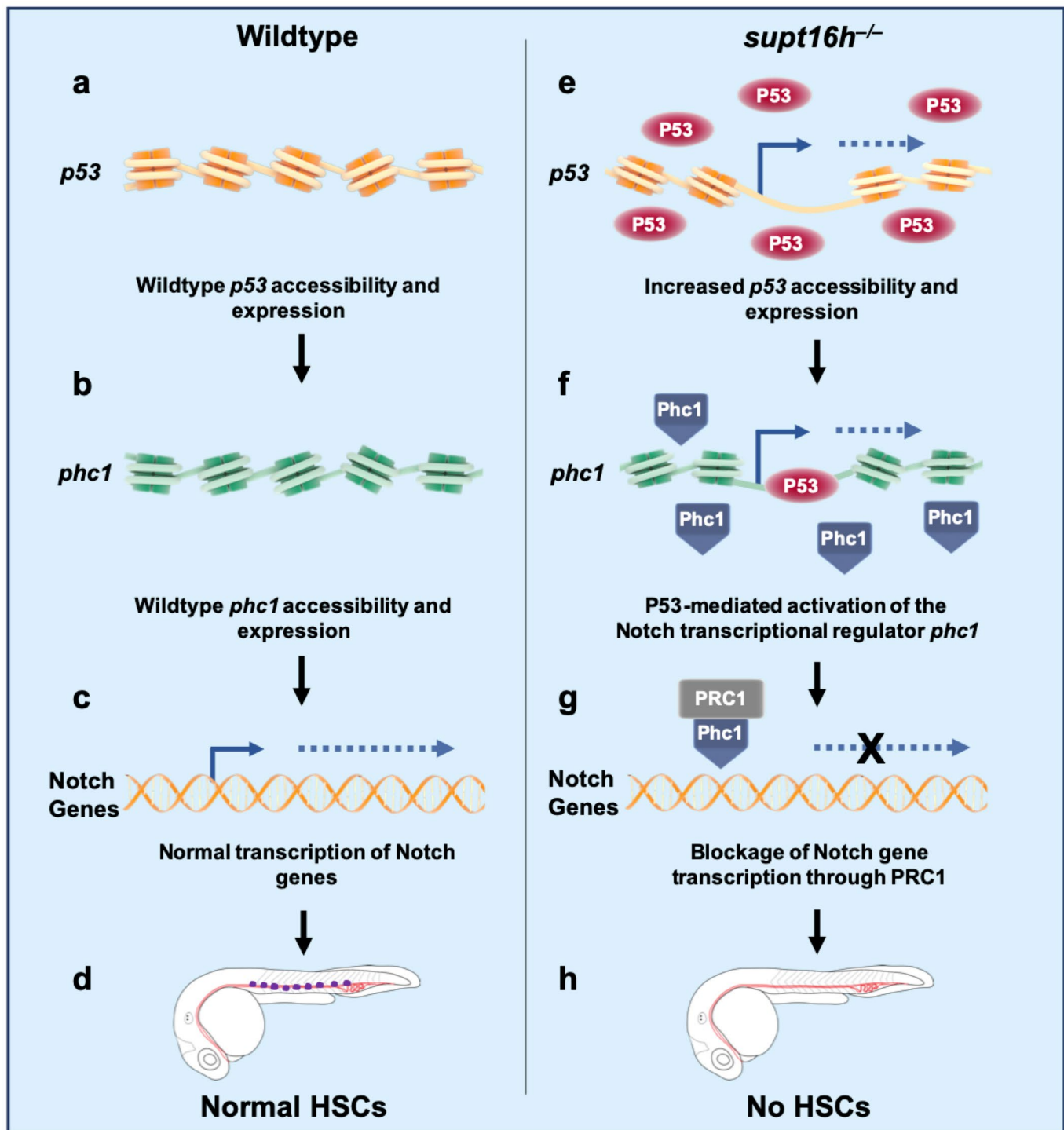


Extended Data Fig. 8 | Characterizing the role of P53 on modulating Notch expression. **a,b**, Gene Ontology analysis of the significant p53-ChIP-peaks for the Biological Processes and Pathways affected. **c**, Plot of p53-ChIP-seq peaks for a known p53-target gene, *p21*. **d**, Graph of *P*-values representing the log2 fold change of the differential binding of p53 to Notch genes between WT sibling and *supt16h*^{-/-} embryos. No significant effect is observed in binding to Notch genes. **e,f**, p53-ChIP-seq plots for *notch1a* and *notch1b*, graphing WT sibling and *supt16h*^{-/-} peaks. **g**, Venn diagram depicting the number of accessible (green) and inaccessible (orange) p53-bound genes based on p53-ChIP-seq and ATAC-seq of WT sibling and *supt16h*^{-/-} embryos at 32 hpf. Genes possessing significant p53-bound peaks ($n = 2$ for ChIP-seq, $n = 6$ for ATAC-seq, two-tailed Student's *t*-test, adjusted $P > 0.05$) were assessed for their accessibility based on ATAC-seq (two-tailed *t*-test, adjusted $P > 0.05$), where genes had at least >1 Log2 fold change between WT and *supt16h*^{-/-} samples. **h**, The number of upregulated (green) and downregulated (orange) p53-bound genes based on p53-ChIP-seq and RNA-seq of WT sibling and *supt16h*^{-/-} embryos at 32 hpf. Genes possessing significant p53-bound peaks ($n = 2$ for ChIP-seq, $n = 3$ for RNA-seq, two-tailed Student's *t*-test, adjusted $P > 0.05$), where genes had at least >1 Log2 fold change between WT and *supt16h*^{-/-} samples, were assessed for their expression based on RNA-seq (two-tailed *t*-test, adjusted $P > 0.05$). p53 binding stimulates increased gene expression. **i**, Expression of *phc1* in *supt16h* mutants and WT siblings based on RNA-seq (Represented as mean \pm s.d, two-tailed Student's *t*-test, $n = 3$, $P = 0.0009$). **j-q**, WISH of *phc1* expression in WT sibling and *supt16h*^{-/-} embryos at 14 (**j,k**), 18 (**l,m**), 28 (**n,o**), and 36 hpf (**p,q**). r-w, WISH of *phc1* expression in uninjected and *supt16h*-MO injected embryos at 14 (**r,s**), 18 (**t,u**), and 28 (**v,w**). Bar, 200 μ m. Source data provided in Supplementary Table 5.



Extended Data Fig. 9 | See next page for caption.

Extended Data Fig. 9 | Characterizing the effect of *phc1* overexpression on Notch signalling. **a-l**, WISH of WT sibling and *supt16h*^{-/-} embryos injected with *phc1*-MO probed for *notch1a*, *dla*, and *dlc* at 28 hpf. **m-o**, Representative confocal images of wild-type embryos on a *Tg(Tp1:GFP)* background injected with 100 and 200 pg of *phc1* mRNA at 28 hpf. Insets are magnified images of the DA and intersomitic vessels. Representative images from one independent experiment. **p-q**, WISH of wild-type embryos injected with 100 pg of *phc1* mRNA probed for at 28 hp for *runx1* (**p-q**), *phc1* (**r-s**), *notch1b* (**t-u**), *notch3* (**v-w**), *dla* (**x-y**), *dlc* (**z-a'**), and *dll4* (**b-c'**). **d'**, RT-qPCR of Notch genes in WT sibling and embryos injected with 100 pg (r) of *phc1* mRNA collected at 28 hpf. No significant changes observed between samples. (Represented as mean \pm s.d., two-tailed Student's *t*-test with Holm-Sidak correction for multiple comparisons, *n* = 3). **e'-j'**, WISH at 28 hpf for *phc1* (**e'-f'**), *runx1* (**g'-h'**), and *notch1b* (**i'-j'**) of wild-type embryos injected with *fli1ep:phc1* plasmid for transient expression of *phc1* in the vasculature. Blue arrowheads indicate HSPCs. Bar, 100 μ m.



Extended Data Fig. 10 | Model of how Supt16h and P53 regulate HSPC specification through notch transcription. **a,b**, In a wild-type setting, *p53* (**a**) and *phc1* (**b**) maintain baseline levels of accessibility and transcriptional expression. **c,d**, This results in normal transcription of Notch genes (**c**) and allows for proper specification of HSCs (**d**). **e**, In *supt16h* mutants, the *p53* gene locus is highly accessible, resulting in increased *p53* mRNA and protein levels. **f**, *p53* then binds to *phc1*, a repressor of Notch gene expression, to allow for its enhanced expression. **g**, PHC1, as part of PRC1, inhibits Notch signalling by acting as direct or indirect a transcriptional repressor of Notch genes. **h**, In the absence of Notch expression, HSCs fail to specify.

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Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a Confirmed

- ☒ ☐ The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
- ☒ ☐ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- ☒ ☐ The statistical test(s) used AND whether they are one- or two-sided
Only common tests should be described solely by name; describe more complex techniques in the Methods section.
- ☒ ☐ A description of all covariates tested
- ☒ ☐ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- ☒ ☐ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- ☒ ☐ For null hypothesis testing, the test statistic (e.g. F , t , r) with confidence intervals, effect sizes, degrees of freedom and P value noted
Give P values as exact values whenever suitable.
- ☒ ☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- ☒ ☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- ☒ ☐ Estimates of effect sizes (e.g. Cohen's d , Pearson's r), indicating how they were calculated

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

Policy information about [availability of computer code](#)

Data collection

Image Lab™ Software Version 6.0, BioRad (used to collect Western Blot data)
FireCam Version 3.4.1, Leica (used for brightfield imaging of whole embryos)
AxioVision Version 4.8, Zeiss (used for imaging whole fluorescent embryos)

Data analysis

R Statistical Package Version 3.4.0, R Core Team (used to generate graphs and perform statistical analysis)
Prism 6 and 7, GraphPad (used to generate graphs and perform statistical analysis)
ImageJ Version 1.52a, NIH (used for analyzing mean fluorescence intensity)
Imaris Version 7.7, Bitplane (used for analyzing fluorescence images)
RNAmapper (used to perform linkage mapping analysis)
FastQC Version 0.11.2 (used to examine the quality of Next Generation Sequencing reads)
Trimmomatic Version 0.32 (used to trim sequencing reads)
MultiQC Version 1.5 (used to examine the quality of Next Generation Sequencing reads)
TopHat Version 2.1.1, Cufflinks Version 2.2.1, CummeRbund Version 2.7.2 (used to analyze RNAseq data)
HOMER Version 4.9.1 (used to analyze ChIP-seq data)
OLego Version 1.1.5 (used for mapping reads)
STAR Version 2.5.4b (used to align RNA-seq data)
DESeq2 Version 1.18.1 (used to analyze ATAC-seq peak data)
ApE Plasmid Editor Version 2.0.51 (used for cloning strategy design)
Integrated Genomics Viewer (IGV) Version 2.3.65 (used to view peak data)
4 Peaks Version 1.8 (used to examine Sanger sequencing chromatograms)
FlowJo Software X.0.7 (used to analyze FACS data)

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research [guidelines for submitting code & software](#) for further information.

Data

Policy information about [availability of data](#)

All manuscripts must include a [data availability statement](#). This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

Our raw and processed RNA-seq (linkage mapping), RNA-seq (differential expression), ATAC-seq, and ChIP-seq data have been deposited into the public functional genomics data repository, Gene Expression Omnibus (GEO). The accession number for these data are GSE106342, GSE127555, GSE106341, and GSE116088 for RNA-seq (linkage mapping), RNA-seq (differential expression), ATAC-seq, and ChIP-seq respectively.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see [nature.com/documents/nr-reporting-summary-flat.pdf](https://www.nature.com/documents/nr-reporting-summary-flat.pdf)

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	Sample sizes (approximately n=10 embryos) were chosen based on garnering sufficient statistical power based on Mead's Resource Equation. Additionally, sample sizes were influenced by the amount of individual embryos that could feasibly be screened for each experiment. Since our breeding pairs were heterozygous for the <i>supt16h</i> mutation, roughly 25% of embryos are expected to be mutants based on Mendelian ratio. As a result, blinded experiments examined 40 embryos, where we would expect roughly 10 embryos to be mutants. For <i>supt16h</i> and <i>p53</i> double mutants, a heterozygous breeding pair would produce roughly 12.5% double mutants, limiting the number of embryos that can be examined.
Data exclusions	One wildtype replicate for the ChIP-seq assay was excluded due to contamination from a mutant sample during sample preparation. This wildtype sample had roughly mean peak value of the remaining wildtype and two mutant samples. Additionally, it was a prominent outlier in the Multidimensional scaling (MDS) plots generated, indicating a contamination. The exclusion criteria established are an IP efficiency of >2%. Samples were also excluded as outliers in the case of obvious error in instrument detection. The exclusion criteria was pre-established as a zero-read on the instrument. Zebrafish cell counts were only excluded in case of staining failure.
Replication	All experiments (i.e. morpholino/mRNA injections and subsequent ISH, RT-qPCR, TUNEL, U2OS transfection) were reproduced at least two times using three biological replicates (3 different mating pairs to produce an experimental clutch of embryos or 4 separate transfections for U2OS cells). For single experiments requiring greater than one clutch size of embryos (i.e. ChIP-seq, Western Blot), multiple clutches were combined. For linkage mapping by RNA-seq, only one replicate was performed as this was sufficient for mutant mapping purposes. For differential expression analysis by RNA-seq, 3 replicates for each WT and mutant sample were used. All relevant RNA-seq based expression data was validated using RT-qPCR. For ChIP-seq, two replicates were performed for <i>supt16h</i> ^{-/-} and a single replicate for wildtype embryos (Refer to "Data exclusions" above for reasoning for WT ChIP-seq replicates). See figure legends for specific details.
Randomization	Randomization of wildtype and <i>supt16h</i> ^{-/-} mutants was performed for all experiments conducted prior to 32 (hours post fertilization) hpf, as this is the earliest time point that can distinguish between the two genotypes based on an observable phenotype. In order to assess between wildtype or <i>supt16h</i> ^{-/-} embryos at developmental stages earlier than 32hpf, samples were genotyped by PCR and Sanger sequencing at the mutated <i>supt16h</i> locus.
Blinding	For zebrafish genetic experiments, heterozygous animals were crossed, and clutchmates were genotyped after experimental analysis (blinded). For MO injected fish, blinding was not performed. In reporter experiments, data was collected and analyzed blindly and labeled post analysis following genotyping by PCR and Sanger sequencing.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
<input type="checkbox"/>	<input checked="" type="checkbox"/> Antibodies
<input type="checkbox"/>	<input checked="" type="checkbox"/> Eukaryotic cell lines
<input checked="" type="checkbox"/>	<input type="checkbox"/> Palaeontology and archaeology
<input type="checkbox"/>	<input checked="" type="checkbox"/> Animals and other organisms
<input checked="" type="checkbox"/>	<input type="checkbox"/> Human research participants
<input checked="" type="checkbox"/>	<input type="checkbox"/> Clinical data
<input checked="" type="checkbox"/>	<input type="checkbox"/> Dual use research of concern

Methods

n/a	Involved in the study
<input type="checkbox"/>	<input checked="" type="checkbox"/> ChIP-seq
<input type="checkbox"/>	<input checked="" type="checkbox"/> Flow cytometry
<input checked="" type="checkbox"/>	<input type="checkbox"/> MRI-based neuroimaging

Antibodies

Antibodies used	Refer to Supplementary Table 3 for a list of antibodies used with their corresponding catalogue number and dilutions.
Validation	<p>For Western Blots, p53, spt16, a-tubulin, and LaminB1 antibodies were assayed for the appropriate signal using HEK293T or HCT116 cells. Once a working antibody was obtained, they were used on wildtype and supt16h^{-/-} zebrafish embryos and examined for the appropriate size and expected signal based on obtained RNA expression results. For ChIP-seq, the p53 antibody was validated for protein expression using Western Blot and co-immunoprecipitation experiments in both zebrafish and HEK293T cells.</p> <p>Manufacturer validations: P53 (GeneTex, Cat# GTX128135)- western blot analysis using zebrafish embryos treated and untreated with UVC (8000J/m²) SPT16 (supt16h) (Cell Signaling, Cat# 12191S)- western blot analysis of total lysates from HeLa and HCT116 cell lines a-tubulin (Sigma Aldrich, Cat# T9026)- western blot analysis in human osteosarcoma and in breast cancer cell lines LaminB1 (Abcam, Cat# ab16048)- western blot analysis in HeLa cell lysates Anti-c-myc (9E10) (Biolegend, Cat#626802)- western blot analysis of total cell lysates from 293E cells transfected with multi-tag or non-transfected Anti-GFP Chicken IgY (Aves Lab, Cat#GFP-1020)- western blot analysis (1:5000 dilution) and immunohistochemistry (1:500 dilution) using transgenic mice expressing the GFP gene product</p>

Eukaryotic cell lines

Policy information about [cell lines](#)

Cell line source(s)	HCT116 ATCC ® CCL-247™ Homo sapiens colorectal carcinoma (commercial available from ATCC)
Authentication	HCT116 cell line was not authenticated
Mycoplasma contamination	HCT116 cell line was not tested for mycoplasma contamination
Commonly misidentified lines (See ICLAC register)	No commonly misidentified lines were used in this study.

Animals and other organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research

Laboratory animals	<p>Zebrafish were maintained and propagated as previously described⁴⁵ in accordance with University of California at San Diego Institutional Animal Care and Use Committee (IACUC) guidelines. Embryos and adult fish were raised in a circulating aquarium system (Aquaneering) at 28°C. The following lines were used: wildtype AB*, wildtype WIK, Tg(cmyb:GFP)zf169 (ref.46), Tg(kdrl:mCherry) (ref.18), Tg(fli1:DsRed)um13 (ref.47), Tg(CD41:GFP) (ref.48), Tg(Tp1:GFP)um14 (ref.49), UAS:NICD-myc Tg(5xUAE-E1b:6xMYC-notch1a)kca3 (ref.17), TgBAC(cdh:gal4ff)mu101 (ref.50), Tg(hsp70l:gal4)1.5kca4 (ref.51), Tg(fli1a:GFP)jy1 (ref.52), tp53zdf1/zdf1 (ref.25), and ssrp1asa31984 (ref.32). Mutant supt16h were generated through a forward genetic screen described in Method Details. The allele number for supt16h^{-/-} zebrafish is #SD45. Heat shock was performed at 14 hpf for 45 min at 37°C as previously described (Burns et al, 2005).</p> <p>Adult fish were mated between 3-15 months of age. Offspring were generated with a male x female cross; the sex of zebrafish embryos is not readily detectable, but a 50:50 distribution is assumed. Offspring ages are noted in each figure.</p>
Wild animals	The study did not involve wild animals.
Field-collected samples	The study did not involve samples collected from the field.
Ethics oversight	Zebrafish were maintained and propagated in accordance with University of California at San Diego Institutional Animal Care and Use Committee (IACUC) guidelines.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

ChIP-seq

Data deposition

- ☒ Confirm that both raw and final processed data have been deposited in a public database such as [GEO](#).
- ☒ Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

Data access links

May remain private before publication.

ChIP-seq GEO Accession Number: GSE116088
Reviewer Key: ilejsgkazncrpyh

Files in database submission

WT_input.fastq.gz
WT_p53-ChIP.fastq.gz
MUT_1_input.fastq.gz
MUT_1_p53-ChIP.fastq.gz
MUT_2_input.fastq.gz
MUT_2_p53-ChIP.fastq.gz
WT_p53-ChIP_Tagdir.ucsc.bedGraph
WT_input_Tagdir.ucsc.bedGraph
MUT_1_input_Tagdir.ucsc.bedGraph
MUT_1_p53-ChIP_Tagdir.ucsc.bedGraph
WT_2_p53-ChIP_Tagdir.ucsc.bedGraph
WT_2_input_Tagdir.ucsc.bedGraph

Genome browser session (e.g. [UCSC](#))

[https://genome.ucsc.edu/cgi-bin/hgTracks?](https://genome.ucsc.edu/cgi-bin/hgTracks?hgS_doOtherUser=submit&hgS_otherUserName=sdespanola&hgS_otherUserSessionName=danRer10_p53%2DChIP)
[hgS_doOtherUser=submit&hgS_otherUserName=sdespanola&hgS_otherUserSessionName=danRer10_p53%2DChIP](https://genome.ucsc.edu/cgi-bin/hgTracks?hgS_doOtherUser=submit&hgS_otherUserName=sdespanola&hgS_otherUserSessionName=danRer10_p53%2DChIP)

Methodology

Replicates

Two replicates for supt16h/- and one replicate for wildtype was performed for p53-ChIP and input samples. Each replicate contained approximately 1000 zebrafish embryos at 32hpf.

Sequencing depth

Sample, Read Depth, Uniquely Mapped, Read Length, Single/Paired-End
WT_input 15,374,049 12,201,863 75 single-end
WT_p53-ChIP 30,907,761 20,941,740 75 single-end
MUT_1_input 18,845,806 15,147,191 75 single-end
MUT_1_p53-ChIP 45,550,916 29,557,858 75 single-end
MUT_2_input 12,400,353 9,808,642 75 single-end
MUT_2_p53-ChIP 42,362,794 26,237,702 75 single-end

Antibodies

P53-antibody (GeneTex, GTX128135, Lot#42263)

Peak calling parameters

We used the findPeaks function with default parameters from the HOMER suite (4.9.1) for peak calling.

Data quality

Data quality was evaluated based on peak calling statistics from HOMER. Using the HOMER software to calculate IP efficiency, which describes the fraction of tags found in peaks versus genomic background (i.e. how well the ChIP worked), we obtained efficiencies within the acceptable range of 1-20%.

WT: 3.66% IP efficiency
MUT1: 4.73% IP efficiency
MUT2: 6.38% IP efficiency

In addition, we evaluated the number of putative peaks based on strict guidelines of an FDR of 0.1% with at least a 4-fold increase over input control. The number of resulting putative peaks for each sample are listed below.

WT: 23,120 putative peaks
MUT1: 8,939 putative peaks
MUT2: 17,853 putative peaks

Software

Prior to mapping, reads were trimmed using Trimmomatic (0.32) with the following parameters: LEADING: 3, TRAILING: 3, SLIDINGWINDOW:4:15, and MINLEN 27. We mapped the trimmed reads to the reference genome GRCz10.85 using STAR (2.5.4b) with the parameters: --outFilterScoreMinOverLread 0 --outFilterMatchNminOverLread 0. We used the HOMER suite (4.9.1) for creating Tag directories (makeTagDirectory) using the -tbp1 option to limit the number of tags per basepair to 1, peak calling (findPeaks) using default parameters, and differential expression analysis using default parameters (getDiffExpression.pl). DESeq2 1.18.1. was used to analyze differential gene expression.

Flow Cytometry

Plots

Confirm that:

- ☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- ☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
- ☒ All plots are contour plots with outliers or pseudocolor plots.
- ☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation

Clutches of 100 Tg(fli1a:dsRed; TP1:eGFP) zebrafish embryos were dissociated at 22 hours post fertilization with gentle shaking at 120 rpm in dissociation buffer (0.9X PBS, Liberase TM (1:50, Roche), 10uM EDTA) at 37°C for 1-2 hours. The resultant cells were pelleted gently at 500 g for 5 minutes, resuspended in flow cytometry buffer (0.9X PBS, 1% FBS, 1mM EDTA, 0.1ug/mL DAPI), and filtered through an 80um filter. The cells were quantified using a BD LSR Fortessa, and analyzed using FlowJo software. DAPI was used for dead cell discrimination. 3-5 million total cells were analyzed in each biological replicate, which each contained a minimum of 100 larvae.

Instrument

BD LSR Fortessa II

Software

FlowJo Software X.0.7

Cell population abundance

A minimum of 10,000 cells per sample were analyzed

Gating strategy

FSC and SSC were cutoff at 50K, live cells were DAPI- ($<10^2$). 5×10^2 and above was set as + fluorescent signal for GFP and dsRed, as shown in Extended Data Fig. 3I.

- ☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.