Figure S1. Ddx18 loss results in reduced numbers of l-plastin expressing cells
(A–D) 27 hpf embryos, lateral views, head to the left and dorsal upwards. Two-color WISH for mpx (black) and l-plastin (red) show that these genes mark and overlapping but distinct population of myeloid cells in zebrafish embryos at 27 hpf. Loss of Ddx18 in ddx18<sup>hi1727/hi1727</sup> mutants results in near absence of l-plastin and mpx expression (A) and (B) compared to (C) and (D). (E–H) 52 hpf embryos, lateral view, head to the left and dorsal upwards. In situ hybridization for mpx (Black dots) showing that ddx18<sup>hi1727/hi1727</sup> mutants (G, H) continue to show reduced myeloid cell numbers compared to siblings (E, F) after the onset of definitive hematopoiesis. The reduction in myeloid cell number is observed throughout the embryo.

Figure S2. ddx18 exon2-intron2(E2–I2) splice donor morpholino phenocopies the ddx18<sup>hi1727/hi1727</sup> mutant phenotype
(A,B) 27 hpf embryos, lateral views, head to the left and dorsal upwards. WISH for mpx expression in control (A) and ddx18 E2–I2 (B) morpholino injected embryos (4ng). (C) quantification of myeloid cell numbers/embryos at different concentrations of control and ddx18 E2–I2 morpholino injected embryos shows a dose-dependent effect on myeloid cell numbers. (D) RT-PCR from embryos injected with control of ddx18 E2–I2 morpholino show WT ddx18 transcript (arrowhead) and an increasing amounts of an aberrantly spliced product (arrow) suggesting intron retention.

Figure S3. Expression pattern of early markers of hematopoiesis and vasculogenesis
(A–D) genotyped ddx18 WT-siblings. (E–F) genotyped ddx18<sup>hi1727/hi1727</sup> mutants. (A), (E), (D) and (H) are posterior views, (B) and (F) are anterior views and (C) and (G) are lateral views head to the left dorsal upwards. WISH showing expression of the hemangioblast marker lmo-2 (A, E), and master myeloid regulator pu.1 (B, F) at 14 somites; and master erythroid regulator gata-1 at 20 somites. No difference is discernable between the siblings and mutants.

Figure S4. Definitive HSCs and intersegmental vessels are reduced in ddx18<sup>hi1727/hi1727</sup> mutants
(A, B) WISH for the HSC marker c-myb. Ddx18<sup>hi1727/hi1727</sup> mutants have markedly reduced c-myb expression compared to siblings. Close-up of the trunk is shown in 32 hpf embryos, lateral views, head to the left and dorsal upwards. (C, D) Lateral views, head to the left and dorsal upwards. WISH for the vascular marker flk1. Ddx18<sup>hi1727/hi1727</sup> mutants have markedly reduced development of intersegmental blood vessels marked by flk1 expression compared to siblings at 24hpf.

Figure S5. ddx18 is expressed in zebrafish hematopoietic cells
Quantitative RT-PCR for ddx18 in sorted erythroid cells (Tg(gata1:rfp)) and myeloid (Tg(pu.1:EGFP)) cells at 24hpf and HSC (Tg(c-myb:EGFP)) at 32hpf. Light grey bars show expression in ddx18<sup>hi1727+</sup> siblings, dark grey bars show expression in ddx18<sup>hi1727+</sup> mutants. Data is shown relative to beta actin and normalized to siblings from three experiments. Error bars indicate standard deviation.

Figure S6. ddx18<sup>hi1727/hi1727</sup> mutants show an increase in apoptosis
(A, B) TUNEL stain shows an increase in TUNEL positive cells in ddx18<sup>hi1727/hi1727</sup> mutants (A) compared to siblings (B). (C, D) Annexin V binding in Tg(pu.1:EGFP); ddx18<sup>hi1727+</sup>
homozygous mutants (D) and siblings (C). The cell population is gated on live cells by forward and side scatter. GFP is on the x-axis and PE on the y-axis. Samples were compensated post-acquisition using Flow-jo. Annexin V binding is markedly increased in the mutants compared to siblings. No increase in the proportion of Annexin V binding cells expressing GFP is observed relative to total the Annexin V binding observed in the whole embryo compared to controls, indicating that while apoptosis is increased it is not more apparent in the GFP-expressing myeloid cells in the Tg(pu.EGFP) mutants.

**Figure S7.** *p53<sup>e7/e7</sup> homozygous mutants rescue many but not all of the effects of Ddx18 loss* (A, D) *p53<sup>e7/e7</sup>;ddx18<sup>hi1727</sup> sibling shows normal development at 52 hpf. (B, E) *p53<sup>e7/e7</sup>;ddx18<sup>hi1727/hi1727</sup> double mutants show some cerebral edema (arrow) and an underdeveloped eye (arrowhead) compared to the *p53<sup>e7/e7</sup>;ddx18<sup>hi1727</sup> sibling. (C, F) *p53<sup>wt/wt</sup>;ddx18<sup>hi1727/hi1727</sup> mutants show far more marked developmental defects with small necrotic brain and eye and absent blood.
Figure S1

*mpx/l-plastin* in situ hybridization 27hpf

*mpx* in situ hybridization 52hpf
Figure S3

<table>
<thead>
<tr>
<th>14 somites</th>
<th>20 somites</th>
</tr>
</thead>
<tbody>
<tr>
<td>ddxdd18+/+ sibling</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>ddxdd18+/+hi1727/hi1727</td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>F</td>
</tr>
</tbody>
</table>

Images show expression of genes in embryos at 14 and 20 somites stages.

- A and B: Expression of lmo2 and pu.1 in ddxdd18+/+.
- C and D: Expression of gata1 in ddxdd18+/+.
- E and F: Expression of lmo2 and pu.1 in ddxdd18+/+hi1727/hi1727.
- G and H: Expression of gata1 in ddxdd18+/+hi1727/hi1727.
Figure S4

WISH for c-myb at 32hpf

A

ddx18\textsuperscript{hi1727} sibling

B

ddx18\textsuperscript{hi1727/hi1727} sibling

WISH for flk1 at 24hpf

C

ddx18\textsuperscript{hi1727} sibling

D

ddx18\textsuperscript{hi1727/hi1727} sibling

YE

flk1
Figure S5

Expression level of *ddx18*

Expression relative to β-Actin

- *gata1+ RBC*
- *pu.1+ Myeloid*
- *cmyb+ HSC*

*ddx18* (light grey) and *ddx18* (dark grey) relative to sibling.
Figure S6

TUNEL staining at 24hpf

A: ddx18hi1727 siblings
B: ddx18hi1727/hi1727 siblings

C: Annexin V-PE
D: GFP
Figure S7

52 hpf

A B C
D E F