Figure S1

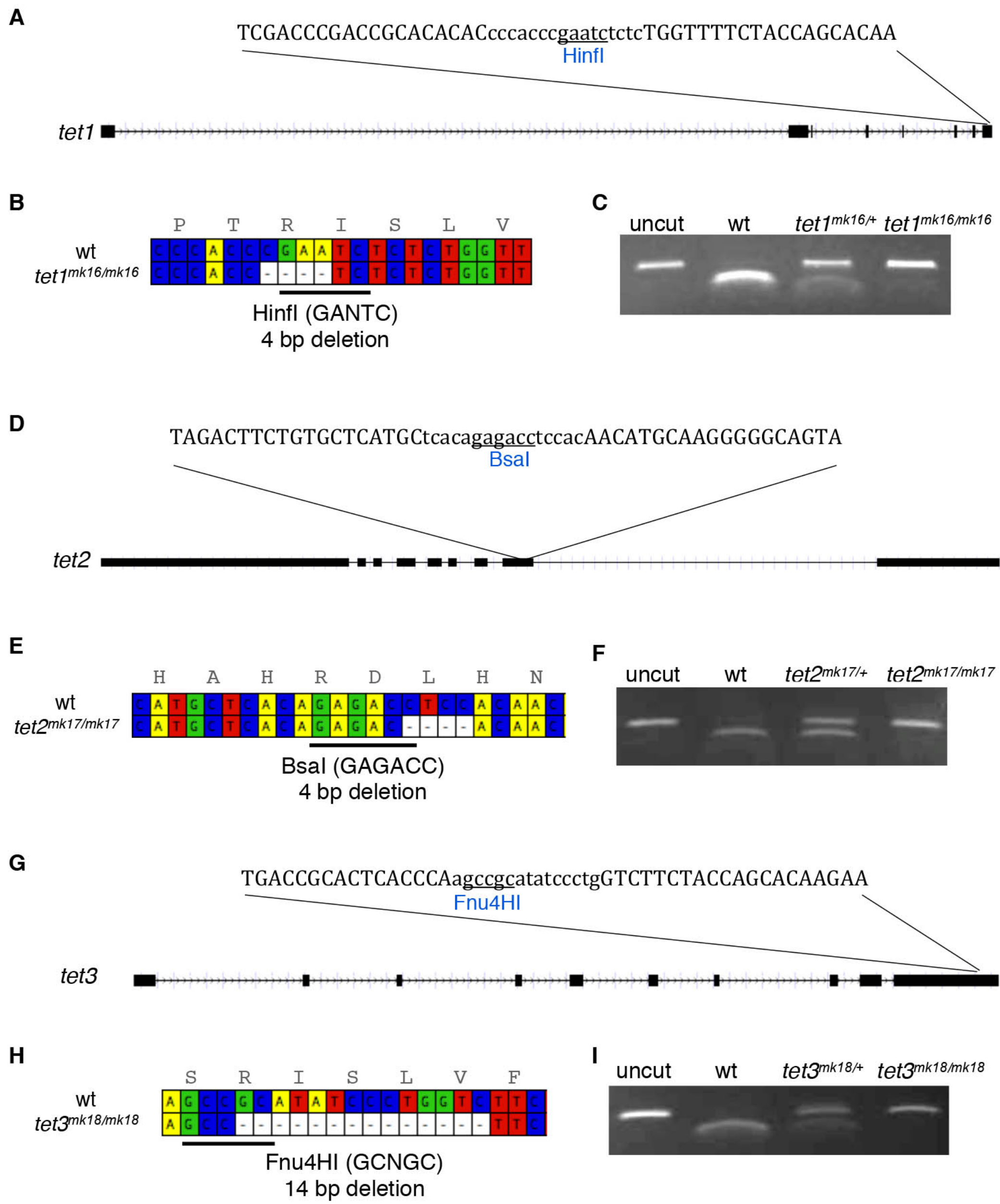


Figure S2

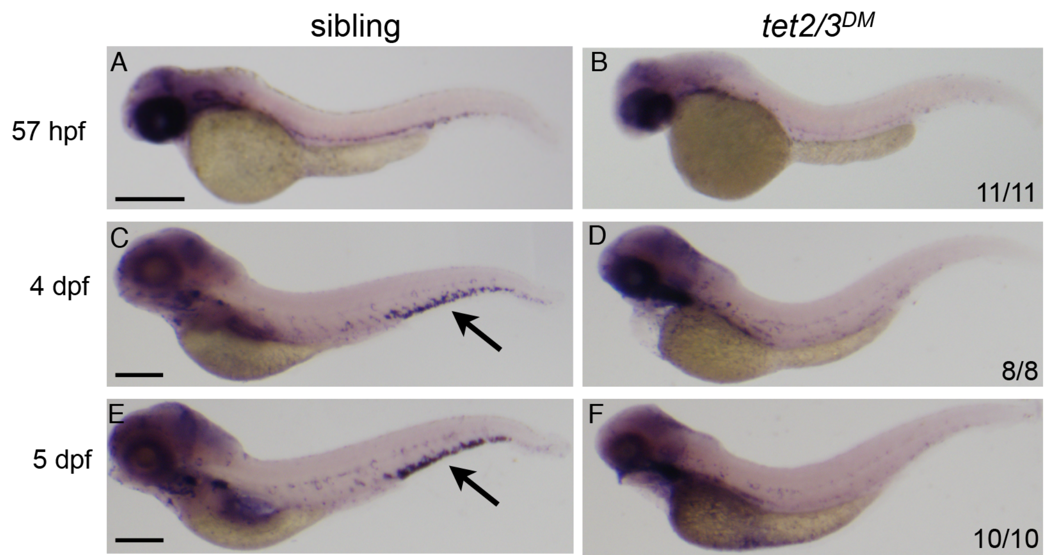
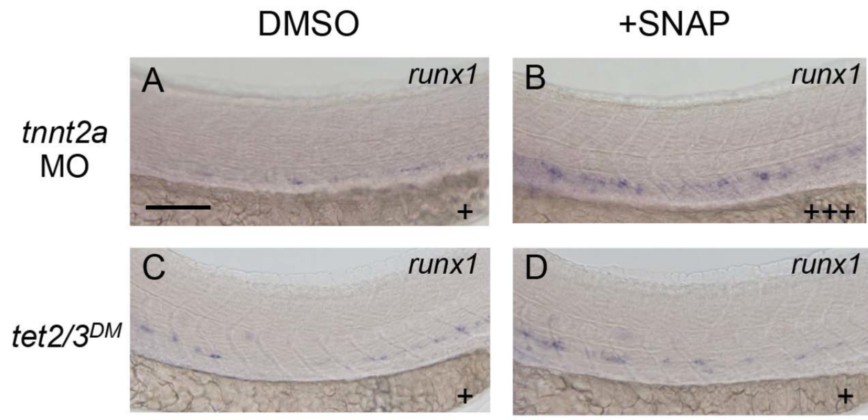


Figure S3



E

	DMSO			SNAP		
	+++	++	+	+++	++	+
WT	97% 34/35	3% 1/35	0% 0/35	91% 33/36	8% 3/36	0% 0/36
tnnt2a MO	0% 0/37	54% 20/37	46% 17/37	39% 11/28	54% 15/28	7% 2/28
tet2/3^{DM}	13% 3/23	34% 8/23	52% 12/23	9% 2/21	24% 5/21	66% 14/21

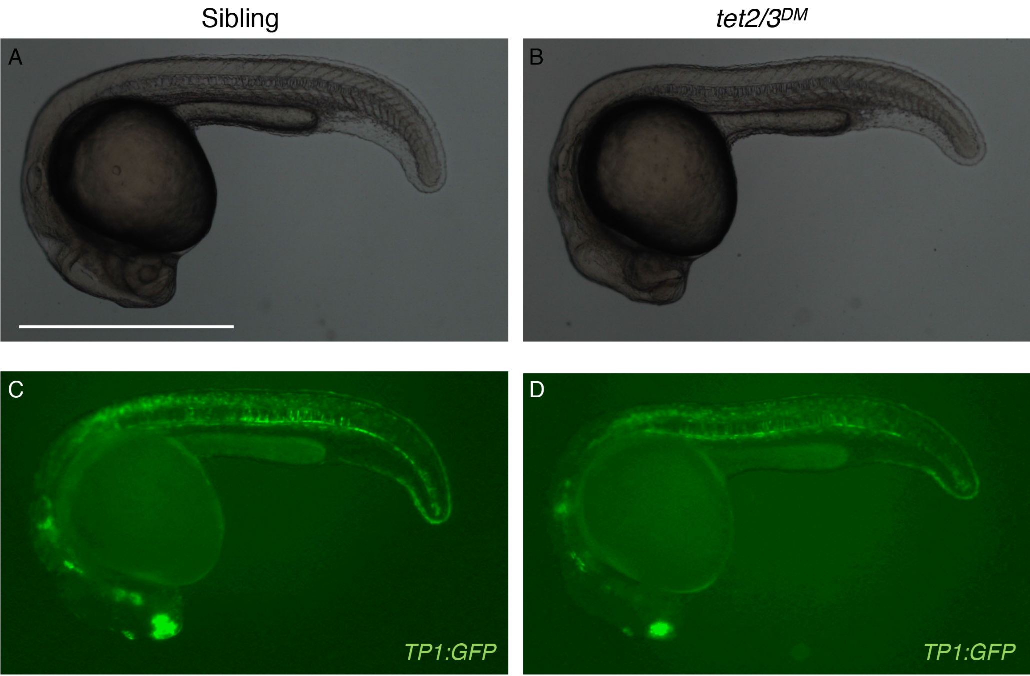
Figure S4

runx1 | scl | gata2b

sibling

$tet2/3^{DM}$

Figure S5



Supplemental Data

Figure S1. Generation and validation of TALEN induced mutations in *tet1*, *tet2*, *tet3,* Related to Figure 1

(A) *tet1* TALEN design. Sequences targeted by the left and right TALEN monomers are included in capitals. The *HinfI* diagnostic restriction site located within the spacer region of the wild-type sequence is underlined.

(B) Schematic indicating the 4 base pair deletion in the *tet1*^{mk16} allele. Amino acids encoded by the wild-type sequence are indicated at the top. The *Hinf1* restriction site, which is destroyed in *tet1*^{mk16}, is underlined.

(C) Representative gel genotyping larvae for the *tet1*^{mk16} mutation. Following PCR amplification of the targeted region, only the wild-type *tet1* allele can be digested by Hinf1.

(D) *tet2* TALEN design. Sequences targeted by the left and right TALEN monomers are included in capitals. The *BsaI* diagnostic restriction site located within the spacer region of the wild-type sequence is underlined.

(E) Schematic indicating the 4 base pair deletion in the *tet2*^{mk17} allele. Amino acids encoded by the wild-type sequence are indicated at the top. The *BsaI* restriction site, which is destroyed in *tet2*^{mk17}, is underlined.

(F) Representative gel genotyping larvae for the *tet2*^{mk17} mutation. Following PCR amplification of the targeted region, only the wild-type *tet2* allele can be digested by BsaI.

(G) *tet3* TALEN design. Sequences targeted by the left and right TALEN monomers are included in capitals. The *Fnu4HI* diagnostic restriction site located within the spacer region of the wild-type sequence is underlined.

(H) Schematic indicating the 14 base pair deletion in the *tet3*^{mk18} allele. Amino acids encoded by the wild-type sequence are indicated at the top. The *Fnu4HI* restriction site, which is destroyed in *tet3*^{mk18}, is underlined.

(I) Representative gel genotyping larvae for the *tet3*^{mk18} mutation. Following PCR amplification of the targeted region, only the wild-type *tet3* allele can be digested by Fnu4HI.

Figure S2. Reduced labeling of *c-myb* positive hematopoietic stem and progenitor cells in *tet2/3*^{DM} larvae, Related to Figure 3

(A-B) WISH for *c-myb* at 57 hpf.

(C-D) WISH for *c-myb* at 4 dpf. Arrow indicates the caudal hematopoietic tissue (CHT).

(E-F) WISH for *c-myb* at 5 dpf. Arrow indicates the caudal hematopoietic tissue (CHT).

The scale bar represents 500 μm.

Figure S3. SNAP does not rescue HSC development in *tet2/3^{DM}* larvae, Related to Figure 4

(A-B) WISH for *runx1* in the DA of 36 hpf *tnnt2a* morpholino-injected embryos following exposure to DMSO or SNAP.

(C-D) WISH for *runx1* in the DA of 36 hpf *tet2/3^{DM}* embryos following exposure to DMSO or SNAP.

(E) Number of *tet2/3^{DM}* larvae exhibiting high (+++), low (++) or negligible (+) *runx1* expression in the DA at 36 hpf following exposure to DMSO or SNAP.

The scale bar represents 50 μm.

Figure S4. Promoter methylation at *runx1*, *scl* and *gata2b* is unaffected in *tet2/3^{DM}* larvae, Related to Figure 6

Bisulfite sequencing of the region upstream of the transcriptional start site for *runx1*, *scl* and *gata2b.* DNA was isolated from FACs sorted *kdrl:mCherry* positive cells at 28 hpf. Open circles indicate unmethylated CpGs, filled circles indicate CpGs in which the cytosine residue carries either the methyl or hydroxymethyl modification. Each horizontal row indicates an individual bisulfite clone.

Figure S5. *TP1:GFP* expression reveals similar overall patterns of Notch signaling in sibling and *tet2/3^{DM}* larvae, Related to Figure 7

(A-B) Bright-field images of a representative sibling and *tet2/3^{DM}* larvae at 26 hpf.

(C-D) GFP expression in *TP1:GFP* transgenic larvae at 26hpf.

The scale bar represents 500 μm.

Supplemental Movies

Movie S1. Example of an HSC emerging from the DA of a phenotypically wild-type sibling larva, Related to Figure 5

Movie S2. Example of a nuclear fragmentation event in the DA of a *tet2/3^{DM}* larva, Related to Figure 5

Supplemental Experimental Procedures

Recovery of mutant zebrafish

Embryos injected with mRNA encoding TALEN pairs that targeted each *tet* gene were separately raised to adulthood and crossed to wild type. Founders carrying germline transmissible mutations were identified by isolating DNA from pooled larval progeny. DNA was PCR amplified and digested using diagnostic restriction enzymes within the region targeted for mutation (See Supplemental Figure S1). The presence of a resistant band indicated that some larval progeny from the tested founder were heterozygous for a mutation in the targeted region. Additional progeny from the identified founder were then raised to adulthood and heterozygotes were identified using the same genotyping strategy on DNA isolated from caudal fin tissue. The PCR fragment was then cloned and sequenced to identify the exact lesion. Adults carrying mutations that generated predicted frame-shift events were crossed to wild-type adults to generate stable lines.

*tet1*F	ACAGCGAGCACAATTTCCTT
*tet1*R	TTCTCTCTGGCCTTCTCTGC
*tet2*F	GCTGGGCCTAAAAGAAGGAC
*tet2*R	TTTTGTGGCCACTACTTTGTT
*tet3*F	TCCCAACATCGGAGGAGTAG
*tet3*R	GCTCTGCCAGGATTTTCATC

Genotyping primers used in this study

TUNEL staining

TUNEL staining was performed on larvae at 28 hpf, 32 hpf and 40 hpf using the *In Situ* Cell Death Detection Kit, TMR red (Roche).

5mC analysis

Genomic DNA was prepared from *kdrl:mCherry* positive FACs sorted endothelial cells isolated from pools of roughly 40 *tet2/3*^{DM} or sibling embryos at 28 hpf. DNA was bisulfite converted using the EZ methylation kit (Zymo-Research). The target DNA regions were amplified using nested primers (see Supplemental Table for complete list of primers used). PCR products were cloned into P-GEM easy, sequenced and analyzed by QUMA (Kumaki et al., 2008).

scl-F1	ATAATAGTAAATAATAGAATAAATA
scl-F2	TTAATTTAGTTATTAGGTATTTATA
scl-R1	ACCCATTATTTATAAAATAATTCA
scl-R2	ATATACATAAAAAAATAACTCAAC
runx1-F1	TTTTATATTATTGAGTTGGTTTGGA
runx1-F2	TGGGATGGTTGAGTTTATTTATT
runx1-R1	TATTCCTAAATCAAATAATACGCTA
runx1-R2	CCAACCAAAACAACCAATTTACATA
gata2b-F1	AGTTGTAATTAATATTGGTGGTAAG
gata2b-F2	TAAAGTGATGGATAGATTTTATGAGAGTTA
gata2b-R1	TTCAAAAAATAAATTTCTAACCAAC
gata2b-R2	TTACTCAAAATAAAATAAATATTTTT

Bisulfite primers used in this study

Supplemental Reference

Kumaki, Y., Oda, M., and Okano, M. (2008). QUMA: quantification tool for methylation analysis. Nucleic acids research *36*, W170-175.