

Supplemental Data

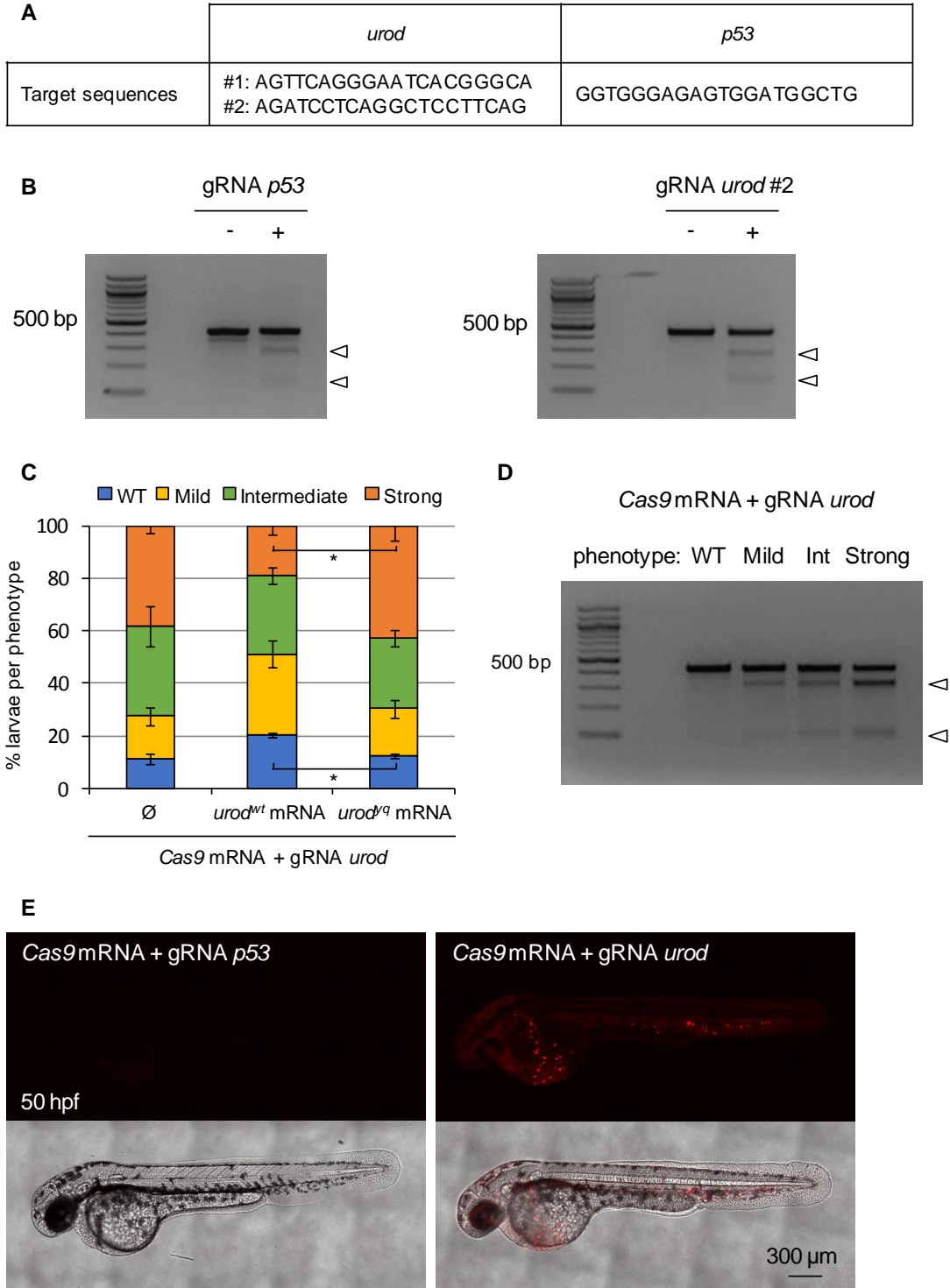


Figure S1. CRISPR targeting of *urod*, but not *p53*, elicits a fluorescent phenotype in zebrafish embryos (related to Figure 1)

(A) CRISPR target sequences used to knockout the *urod* and *p53* zebrafish genes.

(B) T7E1 mutagenesis assay at the *p53* CRISPR target site (left) and at the second *urod* site (right). The assay was performed on genomic DNA from 2 dpf embryos injected at the one-cell stage with *Cas9* mRNA and either the gRNA against *p53* or the gRNA against *urod*. Arrows point to cleavage bands.

(C) Quantification of the fluorescent phenotype obtained after co-injection of *Cas9* mRNA and a gRNA against *urod* ± wt *urod* mRNA (*urod^{wt}*) or *yquem urod* mRNA (*urod* mRNA bearing the inactivating mutation found in the *yquem* mutant, *urod^{yq}*). WT: no fluorescent blood cell. Mild: <10 fluorescent blood cells. Intermediate: <50 fluorescent blood cells. Strong: >50 fluorescent blood cells. Data represented as mean percentage ± SD of 3 independent experiments. *: p<0.05, paired t-test.

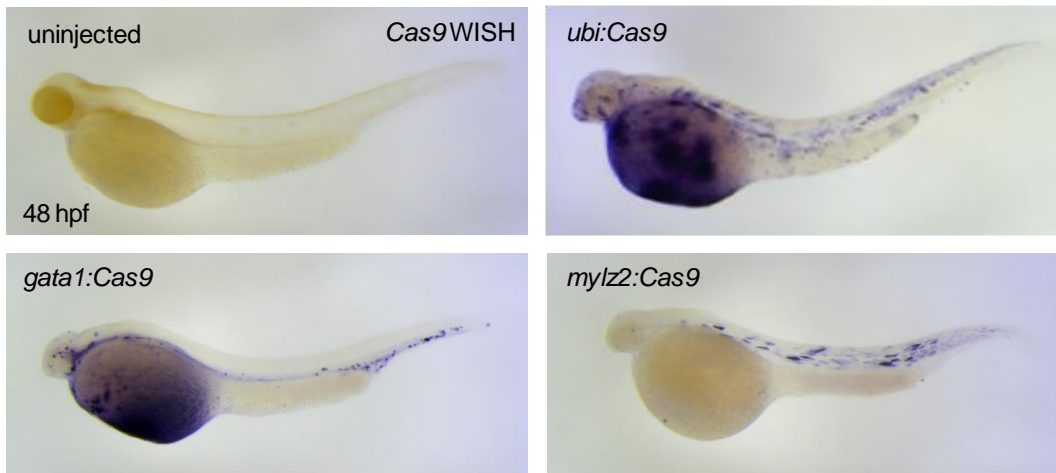
(D) T7E1 mutagenesis assay at the CRISPR target site in the *urod* gene. AB embryos were injected at the one-cell stage with *Cas9* mRNA and a gRNA against *urod*, and sorted into 4 groups (WT, mild, intermediate, high) according to their fluorescent phenotypes (see Figure S1C). The assay was performed on genomic DNA from 8 embryos from each group at 2 dpf. Arrows point to cleavage bands.

(E) Confocal images at 50 hpf of embryos injected at the one-cell stage with *Cas9* mRNA and either the gRNA against *p53* (negative control) or a gRNA against *urod*.

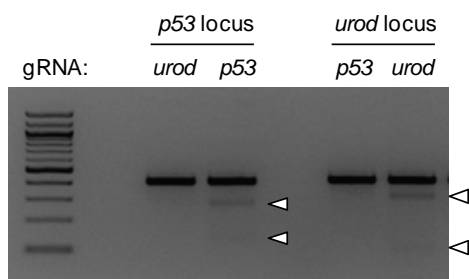
A

GCGCTTTTGTCTGGTCA TCAAGGAGGGGGGAATGTTCTGCGCATGCCTGTGGGGGAGGGAGAAGGA
 CACGTCACTGAAAACGTCCCTGCATCACA CCGAGACA CCAATCACTCAAGCCGAGACCAGATAA TTTT
 GCATATGCTTTACAGTTTGAAAAATACCA CGGTAACCTCA CACAACTCTGGATTTGAGA TCTTTCA GG
 TTTTATCAGTTTGCAGGTTTATGTCA CCATGATATAGGGTCAGACTTGA TCTAAGGGAGCTGAATAAGTG
 GTTTAGTCACTCA CCACTCCCAAAAACATA CCGAGAAGTCCCTGGTATATATA GCTCTCCCTCCAGCTC
 TTGGTTCGCA TATGA CTCTCAGTGAC GAGGAGTCAGTAGCG TTTTAGAGCTAGAAATAGCAAGTTAAAA
 TAAGGCTAGTCCGTTA TCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTT
 U6-3 promoter BseRI sites gRNA scaffold

B



C *pcmlc2:GFP, U6:gRNA, gata1:Cas9*



Blood

D

urod target locus

AACAGAGTTCAGGGAATCACGGGCAGGGAAAGATT	WT
AACAGAGTTCAGGGAA ----- AGATT	-14 del
AACAGAGTTCAGGGAATCACGGGC -----	del
AACAGAGTTCAGGGAATCAC ----- GGGAAAGATT	-5
AACAGAGTTCAGGGAATCACGG -CAGGGAAAGATT	-1
AACAGAGTTCAGGGAATCACGGG ----- GAAAGATT	-4
AACAGAGTTCAGGGAATCA ----- GGGAAAGATT	-6
AACAGAGTTCAGGGAATCACGG ----- AAAGATT	-6

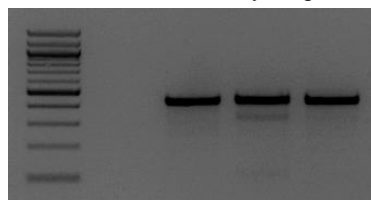
p53 target locus

ATGTTGGTGGGAGAGTGGATGGCTGAGGCTGTTCT	WT
ATGTTGGTGGGAGAGTGGATGGCTG -----	del
----- GCTGAGGCTGTTCT	del
----- CTGAGGCTGTTCT	del
ATGTTGGTGGGAGAGTGGATG ----- AGGCTGTTCT	-4
ATGTTGGTGGGAGAGTGGATGGCTG ----- TTCT	-6

E

pcmlc2:GFP
U6:gRNA urod

ctrl *mylz2* *gata1:Cas9*



Sorted muscle

Figure S2. Tissue-specific expression of Cas9 and gene targeting with the CRISPR vector (related to Figure 2).

(A) Sequence of the portion of the vector presented in Figure 2A encompassing the *U6* promoter and the gRNA scaffold.

(B) Representative images of whole mount *in situ* hybridization (WISH) using an anti-sense RNA probe against *Cas9* mRNA in 48 hpf embryos injected with *Tol2* mRNA and the *pcmlc2:GFP*, *U6:gRNA* vectors expressing *Cas9* under the control of the indicated promoters.

(C) T7E1 mutagenesis assay at the CRISPR target sites in the *p53* or *urod* genes. The assay was performed on genomic DNA from the blood of 2 dpf embryos injected at the one-cell stage with *Tol2* mRNA and the *pcmlc2:GFP*, *U6:gRNA*, *gata1:Cas9* vectors containing a gRNA against *p53* or *urod*. Arrows point to cleavage bands.

(D) Most frequent (>2%) mutant *urod* and *p53* alleles found by deep-sequencing in the blood of embryos injected with *Tol2* mRNA and the *pcmlc2:GFP*, *U6:gRNA*, *gata1:Cas9* vectors containing gRNAs against *urod* and *p53* respectively. The CRISPR target sequence is in green, mutations in red. The type of mutation is indicated (del: large deletion).

(E) T7E1 mutagenesis assay at the CRISPR target site in the *urod* gene. The assay was performed on genomic DNA from sorted mCherry-positive cells from 2 dpf Tg(*mylz2:mCherry*) embryos injected at the one-cell stage with *Tol2* mRNA and the *pcmlc2:GFP*, *U6:gRNA urod*, *mylz2:Cas9* or *pcmlc2:GFP*, *U6:gRNA urod*, *gata1:Cas9* vectors.

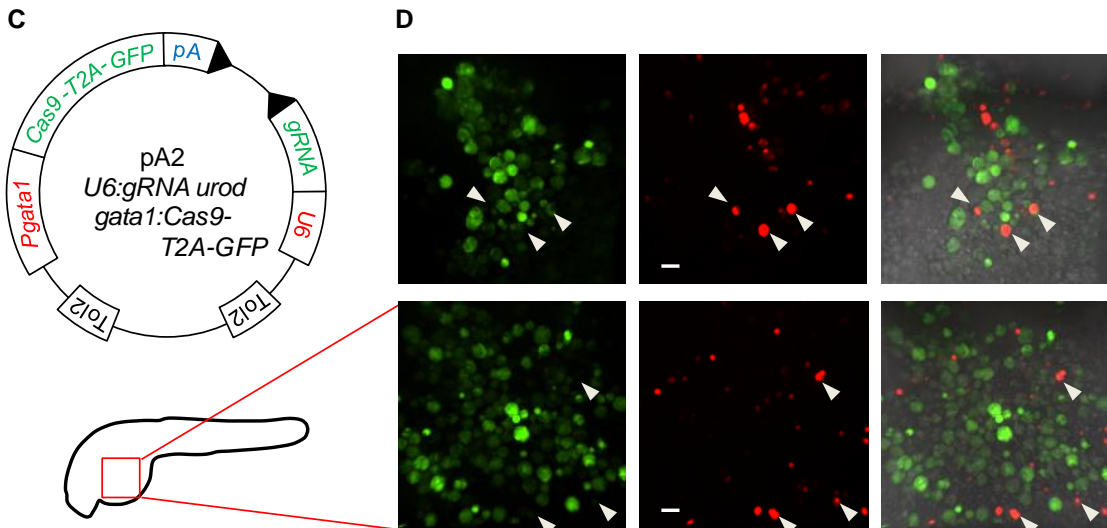
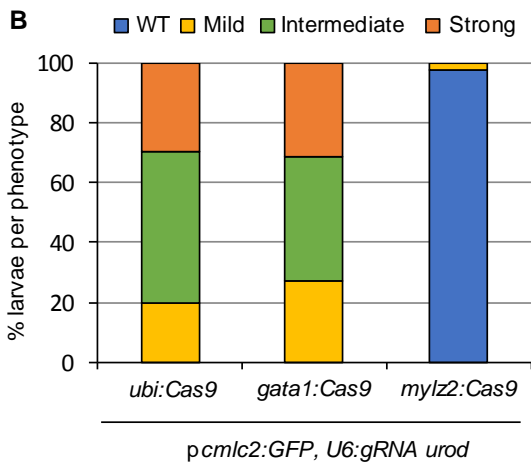
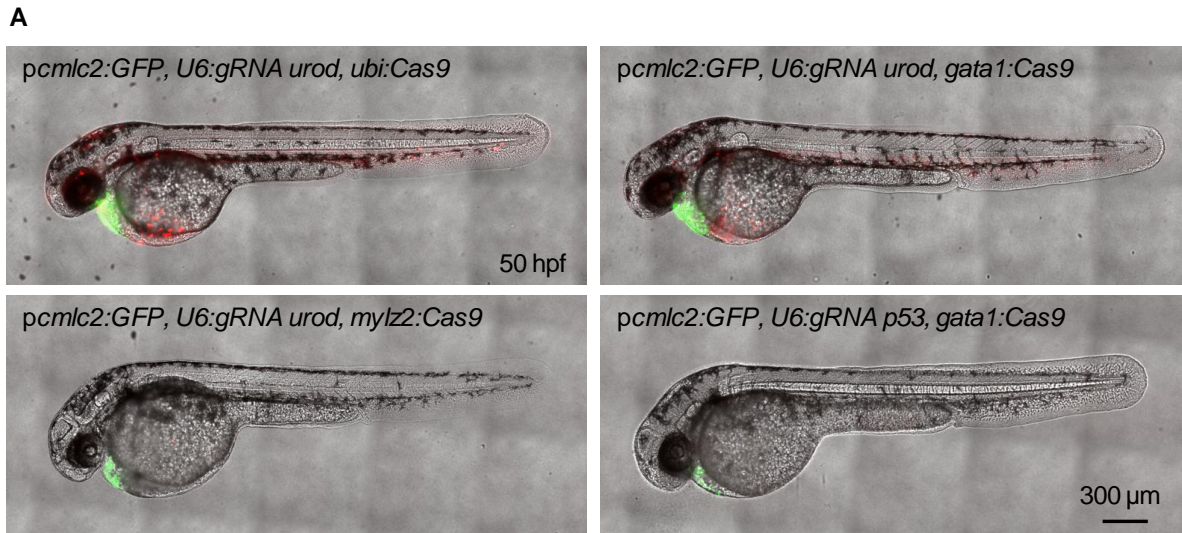


Figure S3. Fluorescent phenotype induced by CRISPR vectors targeting *urod* (related to Figure 3).

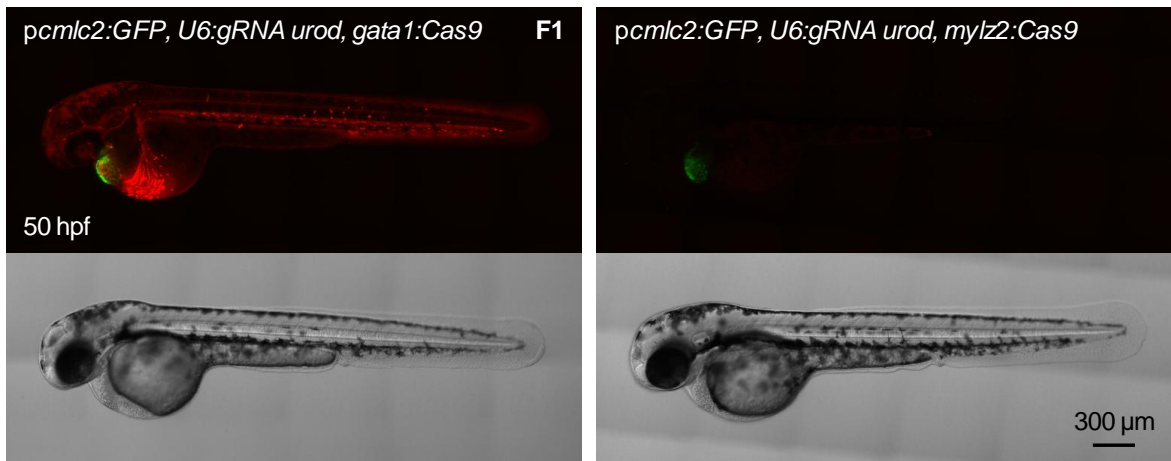
(A) Confocal images of 50 hpf embryos injected with *Tol2* mRNA and *pcmlc2:GFP*, *U6:gRNA urod* vectors expressing *Cas9* under the control of the indicated promoters.

(B) Quantification of the fluorescent phenotype presented in Figure 3A. WT: no fluorescent blood cell. Mild: <10 fluorescent blood cells. Intermediate: <50 fluorescent blood cells. Strong: >50 fluorescent blood cells. *Ubi:Cas9* (n=61), *gata1:Cas9* (n=51), *mylz2:Cas9* (n=43).

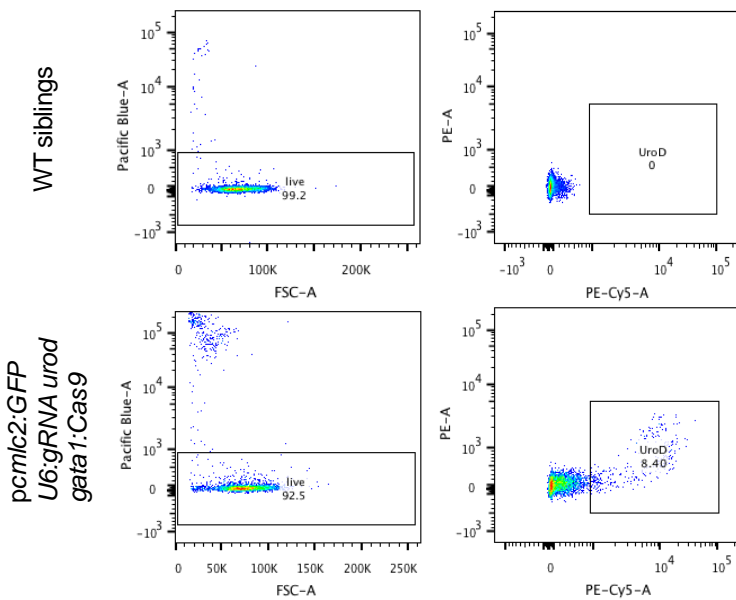
(C) Schematic representation of a variant tissue-specific CRISPR vector. *Gata1* promoter (*Pgata1*) drives both *Cas9* and *GFP* expression through a self-cleaving T2A peptide. Any gRNA of interest can be produced ubiquitously off the *U6* promoter. Tol2 indicates transposition sites for the Tol2 transposase. pA: SV40 polyA sequence.

(D) Confocal images of the yolk region of 30 hpf embryos injected with *Tol2* mRNA and the pA2, *U6:gRNA urod*, *gata1:Cas9-T2A-GFP* vector. Arrows point to cells that are both green and red. Scale bar: 20 μ m. Note the small size of red-only cells.

A



B F1, 36 hpf

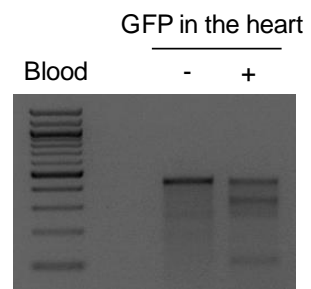


D

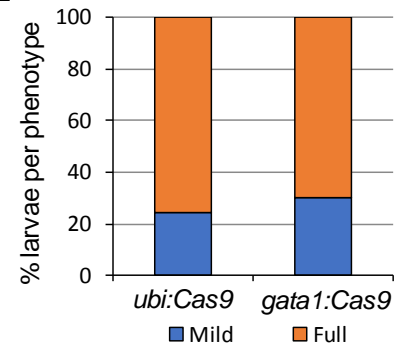
14 mutant alleles out of 20 sequenced alleles

CAGAGTTCAGGGAATCACGGGCA	GGGAAAGATT	WT	x6
CAGAGTTCAGGGAATCACGG	- CAGGAAAGATT	-1	x4
CAGAGTTCAGGGAATCAC	----- GGGAAAGATT	-5	x2
CAGAGTTCAG	----- CAGGAAAGATT	-11	x2
CAGAGTTCAGGGAA	----- AGATT	-14	
CAGAGTTCAGGGAATCAC	----- AGGAAAGATT	-4	
CAGAGTTCAGGGAATCACGGGC	-----	del	
CAGAGTTCAGGGAATCACG	-- CAGGAAAGATT	-2	
CAGAGTTCAGGGAATCACGG	----- AAAGATT	-6	
CAGAGTTCAGGGAAT	- A ----- GAAAGATT	-10;+1	

C



E



F

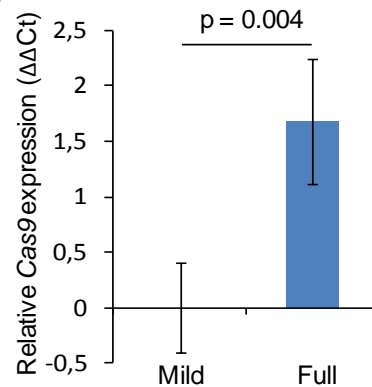


Figure S4. Analysis of the *urod* phenotype in stable F1 embryos (related to Figure 4).

(A) Confocal images of 50 hpf embryos from the F1 generation of fish stably expressing the indicated vectors.

(B) FACS analysis of the blood of 36 hpf F1 embryos stably expressing the *pcmlc2:GFP*, *U6:gRNA urod*, *gata1:Cas9* vector (bottom panels). Blood from F1 siblings not presenting green hearts was used as a negative control (top panels). Red fluorescence due to *urod* inactivation was detected in the PE-Cy5 channel (right panels). Cytox blue was used to mark dead or dying cells (left panels).

(C) T7E1 mutagenesis assay at the CRISPR target site in the *urod* gene. The assay was performed on genomic DNA from the blood of 2 dpf F1 embryos stably expressing the *pcmlc2:GFP*, *U6:gRNA urod*, *gata1:Cas9* vector. Blood from F1 siblings not presenting green hearts was used as a negative control.

(D) Sequences of the mutant *urod* alleles found in the blood of embryos stably expressing the *pcmlc2:GFP*, *U6:gRNA urod*, *gata1:Cas9* vector. The CRISPR target sequence is in green, mutations in red. The type of mutation and the associated number of detected alleles are indicated.

(E) Quantification of the fluorescent phenotype observed in F1 embryos. Mild: <10 fluorescent blood cells. Full denotes a number of fluorescent cells comparable to that observed in age-matched *LCR:GFP* embryos. *Ubi:Cas9* (n=37), *gata1:Cas9* (n=80).

(F) qPCR analysis of *Cas9* expression in F1 embryos stably expressing *pcmlc2:GFP*, *U6:gRNA urod*, *ubi:Cas9* vector. Four embryos showing no or few fluorescent cells (mild) and four embryos showing a full phenotype were compared. Data represented as mean \pm SD.

Mutation index	F0 ubi promoter (whole embryos)	F0 gata1 promoter (blood)	F1 ubi promoter (whole embryos)	F1 gata1 promoter (blood)
Deep-sequencing	36%	25%	NA	NA
TA cloning	40%	33%	82%	70%

Table S1. Mutation indexes for the *pcmlc2:GFP*, *U6:gRNA urod*, *ubi:Cas9* and *pcmlc2:GFP*, *U6:gRNA urod*, *gata1:Cas9* vectors (related to Figure 4).

The index was calculated as the number of mutated alleles over the total number of sequenced alleles using either of two methods: deep-sequencing or TA cloning followed by Sanger sequencing of PCR amplicons around the CRISPR target site in the *urod* gene. Mutation indexes for both injected F0 and stable F1 embryos are indicated.

Movie S1. Circulating fluorescent erythrocytes after tissue-specific *urod* targeting (related to Figure 3).

Live confocal imaging of a 50 hpf embryo injected with *Tol2* mRNA and the *pcmlc2:GFP*, *U6:gRNA urod*, *gata1:Cas9* vector at the one-cell stage.