

Figure S1. Plasmid map of p3E_polyA_U6:universalgRNA. The plasmid contains attR2 and attL3 recombination sites (dark turquoise), SV40 poly(A) signal (grey), U6 promoter (orange), BseR1 recognition site (red) where the specific gRNA will be placed, the gRNA scaffold (light blue), and the sequencing primer (Forward direction).

TGGCGGGCGTCCTGCCCGCCACCCTCCGGGCGGTTGCTTCACAACGTTCAAATCCGCTCCCCGGCG
GATTTGTCCTACTCAGGAGAGCGTTCACCGACAAACAACAGATAAAACGAAAGGCCAGTCTCCGA
CTGAGCCTTTTCGTTTTATTTGATGCCTGGCAGTTCCTACTCTCGCGTTAACGCTAGCATGGATGTTT
TCCCAGTCACGACGTTGTA AACGACGGCCAGTCTTAAGCTCGGGCCCTGCAGCTCTAGAGCTCGA
ATTCTACAGGTCATAATACCATCTAAGTAGTTGGTTTACAGGTCACTAATACCATCTAAGTAGTTGGT
TCATAGTGA CTGCATATGTTGTGTTTTACAGTATTATGTAGTCTGTTTTTTATGCAAAATCTAATTTAAT
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AATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAGTTAACACAACAATTG
CATTCATTTTATGTTTCAGGTT CAGGGGAGGTGTGGGAGGTTTTTTCATCATCGATGCGTCTTTTGT
TCTGGTCATCAAGGAGGGGGGAATGTTCTGCGCATGCCTGTGGGGGAGGGAGAAGGACACGTCAC
TGAAAACGTCCCTGCATCACACCGAGACACCCAATCACTCAAGCCGAGACCAGATAATTTTGCATAT
GCTTTACAGTTTGAAAAATACCACGGTAAACCTCACACAACTCTGGATTGAGATCTTT CAGGTTT
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CATGGTCATAGCTGTTTCCTGGCAGCTCTGGCCCGTGTCTCAAATCTCTGATGTTACATTGCACAA
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AGCCATATTCAACGGGAAACGTCGAGGCCGCGATTAAATCCAACATGGATGCTGATTTATATGGGT
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ATGCGCCAGAGTTGTTTCTGAAACATGGCAAAGGTAGCGTTGCCAATGATGTTACAGATGAGATGGT
CAGACTAACTGGCTGACGGAATTTATGCCTCTTCCGACCATCAAGCATTTTATCCGTA CTCTGATG
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TCTTGCCATCCTATGGAAGTGCCTCGGTGAGTTTTCTCCTTCATTACAGAAACGGCTTTTTCAAAAAT
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AGCGGTGGTTTGTGGCCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAAGTGGCTTCAGCAGA
GCGCAGATACCAATACTGTCCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAAGTCTGTAG
CACCGCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTG
TCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCCGGCTGAACGGGGG
GTTTCGTGCACACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGC
ATTGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTC
GGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCCTGTCCG
GTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGGAGCCTATGGAA
AAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGTT

Figure S2. P3E_polyA_U6:universalgRNA sequence.

attR2 and attL3 (green), SV 40 polyA (grey), U6 promoter (orange), primer (purple),
BseR1 enzyme site (red), gRNA scaffold (blue).

A F0 transmission frequencies

Founders	Transmission freq - GH positive
Tg[pGH:Ziwi:cas9T2AGFP_U6kif5Ba gRNA] 3073-1 male	0.27
Tg[pGH:Ziwi:cas9T2AGFP_U6kif5Ba gRNA] 3590-1 male	0.01
Tg[pGH:Ziwi:cas9T2AGFP_U6kif5Ba gRNA] 3590-5-20 male	0.03

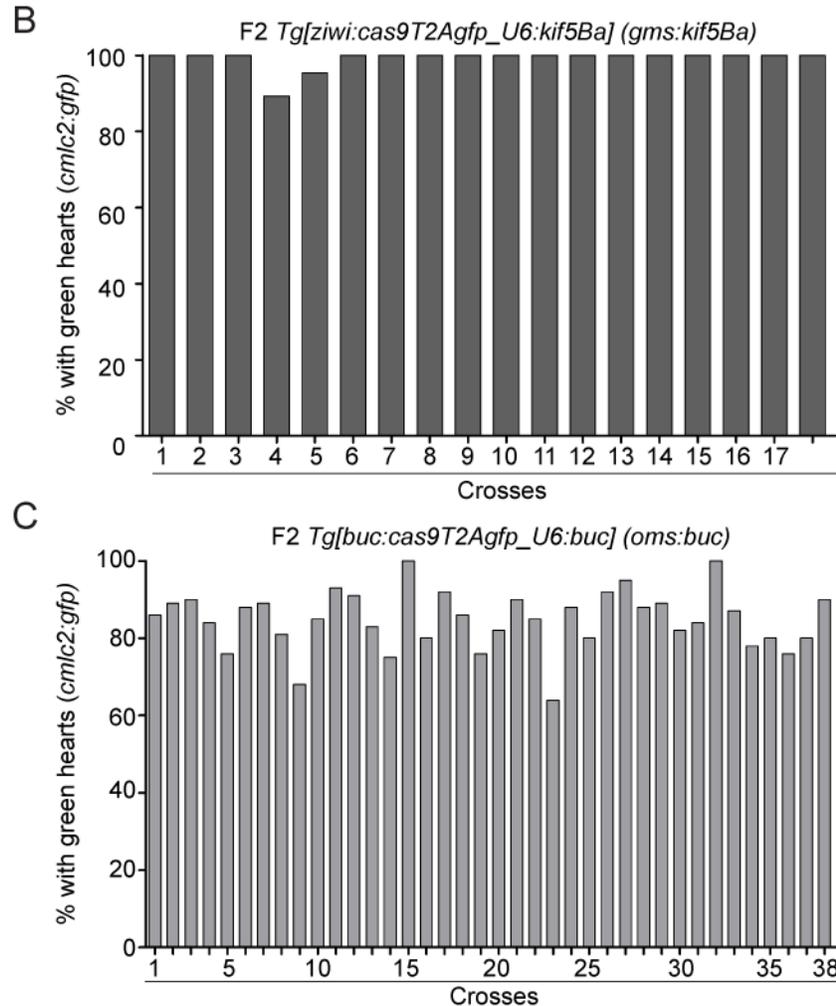


Figure S3. Germline transgene transmission. A) Representative F0 transmission frequencies (GH positive indicates - GFP positive hearts). B) Quantification of positive green heart expression among *Tg[buc:cas9T2Agfp_U6:kif5B]* (*gms:kif5B*) embryos (n=18). C) Quantification of positive green heart expression among *Tg[ziwi:cas9T2Agfp_U6:buc]* (*gms:buc*) embryos on d3 (n=38).

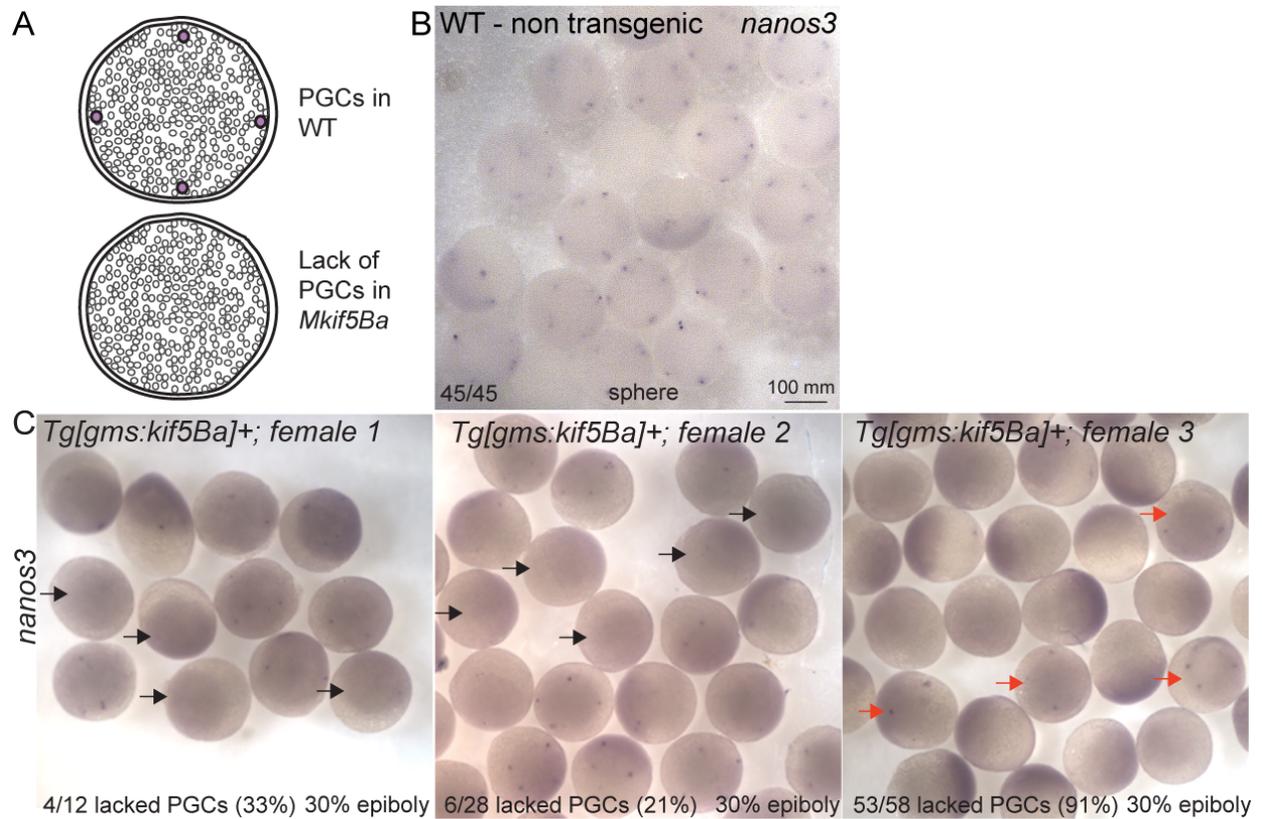


Figure S4. Phenocopy of maternal *kif5Ba* PGC phenotype. A) Schematic of expected wild-type and *Mkif5Ba* PGC phenotypes. B,C) *nanos3* staining in (B) non-transgenic control and (C) three independent clutches of F1 *gms:kif5Ba* transgenic mothers – the panels correspond to those in main Figure 3E. Black arrows indicate progeny lacking germ cells and red arrows indicate the few embryos with germ cells in female 3.



Figure S5. Sequence and chromatogram from recovered *buc* germline mutations from *gms:buc* mothers. (A) Sequence of the region flanking the *buc* gRNA site and recovered sequences after Cas9 cleavage. (B) Chromatogram of the WT sequence and the mutated sequences in eggs from *gms:buc* mothers.

Table S1. Plasmids generated/used in this study

Marlow Lab Database Number	Plasmid Name	Other Info.
#60	<i>p5E pBD119 ziwi promoter</i>	(Leu and Draper, 2010)
#190	<i>2K Buc promoter in PDONR (p5E-Buc)</i>	(Heim et al., 2014)
#878	<i>Tol2-R4/R3 cmcl2:gfp</i>	Tol2 sites surrounding R4/R3 att sites, with cmcl2:gfp transgenesis marker, Tol2 v1.0 (Kwan et al., 2007)
#1190	<i>pME-Cas9</i>	Addgene 63154 (Zon lab) (Ablain et al., 2015)
#1191	<i>pME-cas9-T2A-GFP</i>	Addgene 63155 (Zon lab) (Ablain et al., 2015)
#1195	<i>p3E_pA_U6:kif5Ba_gRNA</i>	Guide from (Campbell et al., 2015)
#1244	<i>pGH ziwi:cas9-T2A-GFP u6:kif5Ba gRNA (GMS)</i>	Guide from (Campbell et al., 2015)
#1245	<i>pGH buc:cas9-T2A-GFP u6:kif5Ba gRNA (OMS)</i>	Guide from (Campbell et al., 2015)
#1246	<i>p3E_pA_u6:universal gRNA</i>	
#1268	<i>pGHziwiCas9T2AGFPU6bucgRNA</i>	
#1269	<i>pGHbucCas9T2AGFPU6bucgRNA</i>	
#1272	<i>p3E-pA-U6bucgRNA</i>	

Table S2

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Supplementary materials and methods

Protocol for germline-specific gene disruption in zebrafish

Adapted from (Ablain et al., 2015)

Overview

This protocol is based on (Ablain et al., 2015) tissue-specific gene disruption protocol in zebrafish. The method allows gene inactivation in zebrafish in a germline-specific manner. It can be used to analyze maternal effect genes in F1 embryos and generate stable tissue-specific knock-out lines to analyze maternal effect genes. It takes advantage of the Tol2 transposase technology to integrate in the fish genome a vector expressing a guide RNA (gRNA) from a ubiquitous zebrafish U6 promoter and Cas9 under the control of a tissue-specific promoter. This protocol comprises 5 steps: 1) the identification of efficient CRISPR target sequences in the gene of interest; 2) the annealing of gene-specific oligonucleotides; 3) the construction of the germline-specific CRISPR vector; 4)

the injection of the CRISPR construct in zebrafish embryos; 5) the phenotypic analysis and generation of stable lines (Fig. P1).

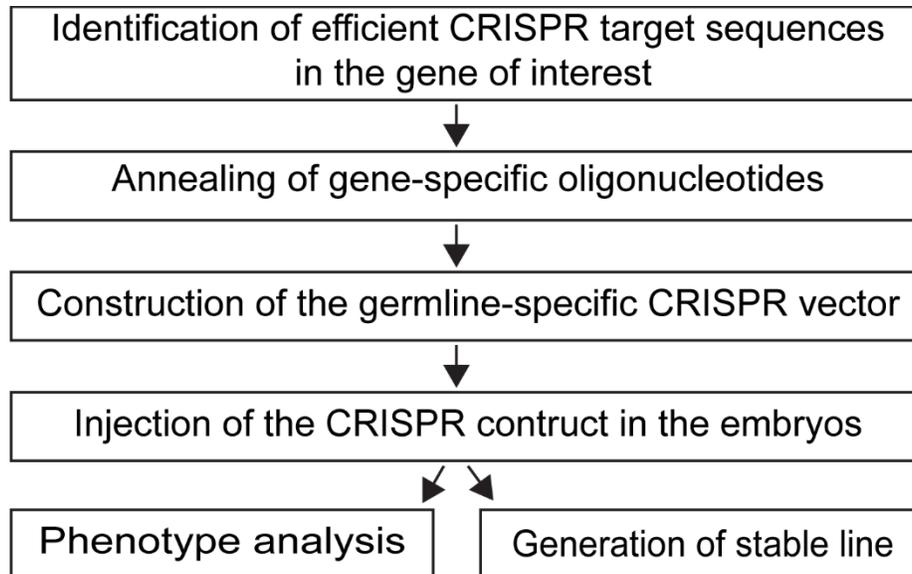


Figure P1 – Workflow of germline-specific gene disruption

Reagents

- Destination vector for Gateway (e.g. pDestTol2CG2)
- Middle entry vector for Gateway containing Cas9 (e.g. pME-Cas9 or pME-Cas9-T2A-GFP, available through Addgene)
- 5' entry vector for Gateway containing the germline-specific promoter of interest (p5E_*ziwi* promoter or p5E_*buc* promoter)
- 3' entry vector for Gateway containing a polyA sequence (p3E_polyA_U6:universalgRNA)
- Gene-specific oligonucleotides (see below)
- BseRI enzyme (New England Biolabs)
- Gateway LR clonase II (Invitrogen)
- T4 DNA ligase (New England Biolabs)
- Gel extraction kit or PCR-purification kit (Qiagen)

Procedure

All the steps except step 3 are identical to Ablain et al procedure. (Ablain et al., 2015)

1. Identification of efficient CRISPR target sequences in the gene(s) of interest

Pick several (3 to 6) CRISPR target sequences in each gene of interest using available tools (Hsu et al., 2013; Montague et al., 2014). Produce the gRNAs *in vitro* as per usual procedures (Gagnon et al., 2014b; Hwang et al., 2013). Inject the gRNAs along with Cas9 protein or mRNA into one-cell stage embryos of a WT strain. Extract DNA from injected embryos at 24 or 48 hpf by the HotSHOT method (Meeker et al., 2007) and assess mutation rates at target loci by sequencing or enzymatic assays (e.g. T7E1 assay, Surveyor assay) (Gagnon et al., 2014b; Kim et al., 2009). Proceed with the target sequences that have shown effective targeting (we usually only keep target sequences for which the mutation rates exceed 10%).

Note: alternatively, it is possible to test the efficiency of target sequences directly in the context of the Tol2 vector by cloning various target sequences in the U6:gRNA cassette of a vector expressing Cas9 under the control of a ubiquitous promoter.

2. Annealing of gene-specific oligonucleotides

Order 22-mer, unmodified oligonucleotides as follows:

Forward: target sequence (20 bases)-GT

Reverse: reverse complement of target sequence-GA

Note: the target sequence must start with a G. If not, replace the first base by a G. This will introduce a mismatch but most mismatches at the 5' end of the target sequence are well tolerated.

Example: for CRISPR target sequence GGTGGGAGAGTGGATGGCTG, order GGTGGGAGAGTGGATGGCTGGT (forward) and CAGCCATCCACTCTCCCACCGA (reverse).

Anneal the two oligos in thermocycler (5 min. at 95°C, -1°C/min. down to 20°C).

3. Construction of the germline-specific CRISPR vector

Clone the gene-specific seed sequence into the p3E_polyA_U6:universal gRNA predigested with BseRI and then perform the Gateway reaction with the germline-specific promoter (ziwi or buc) and Cas9 (Fig. 1B).

- Digest the p3E_polyA_U6:universalgRNA with BseRI enzyme.
- Purify on 1% agarose gel or column.
- Ligate the gene-specific seed sequence into BseRI-digested p3E_polyA_U6:universalgRNA vector (1:20 vector:insert molar ratio).
- Transform chemically competent bacteria, plate on LB Agar and select with Kanamycin

- Extract plasmid DNA from 2-3 distinct colonies and sequence using the following primer: CCTCACACAAACTCTGGATT to check the insertion of the specific gRNA.
- Perform the Gateway reaction (Hartley et al., 2000) with the destination vector, a 5' entry vector containing a tissue-specific promoter of interest, a middle entry vector containing zebrafish codon-optimized Cas9 and p3E-polyA, according to manufacturer's protocol.
- Transform chemically competent bacteria, plate on LB Agar and select with ampicillin.
- Extract plasmid DNA from 2-3 colonies and check correct recombination by digestion or sequencing (*ziwi* or *buc*, *Cas9*, *U6* promoter primer listed in the table).

4. Injection of the CRISPR construct in zebrafish embryos

Mix and inject 20-30 pg of CRISPR vector and 20 pg of Tol2 mRNA into one-cell stage embryos (Kawakami et al., 2004). For reliable phenotypic analyses, we recommend injecting >50 embryos per construct. Vectors expressing gRNAs targeting an irrelevant gene or driving Cas9 expression in a different tissue can be used as negative controls. Allow injected embryos to develop at 28.5°C.

5. Phenotypic analysis and generation of stable lines

Sort injected F0 embryos based on the expression of a transgenesis marker present either in the destination vector (e.g. *cmlc2*:GFP) or in the middle entry part of the vector (e.g. T2A-GFP). Only positive embryos should be considered for further analysis. For the generation of stable lines, raise positive F0 fish to adulthood. Back-cross them to the strain used for injection and sort positive F1 embryos (according to the expression of the transgenesis marker). F1 embryos can be analyzed phenotypically or raised to adulthood.

Note: depending on the transgenesis marker used, it may be possible to evaluate the level of mosaicism in injected embryos. In that case, injected embryos could be further sorted according to their level of mosaicism.

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