Α

## Supplementary Material Keisuke Nakajima and Yoshio Yaoita doi: 10.1242/bio.201410009

AY341764 GTTGACTTTGCCCATGAAGCTCCAGCCTTTGTACCCTGGCACCGGTACTTCTTGCTGCACT WT-A [%]

#1 GTTGACTTTGCCCATGAAGCTCCAGCCTTTGTACCCTGGCACCGGTACTTCTTGCTGCACT WT-A [%]

#1 GTTGACTTTGCCCATGAAGCTCCAGCCTTTGTACCCTGGCACCGGTACTTCTTGCTGCACT WT-A [%]

#1 GTTGACTTTGCCCATGAAGCTCCAGCCTTTGTACCCTGGCACCGGTACTTCTTGCTGCACT WT-A [%]

#2 GTTGACTTTGCCCATGAAGCTCCAGCCTTTGTACCCTGGCACCGGTACTTCTTGCTGCACT WT-A [%]

#2 GTTGACTTTGCCCATGAAGCTCCAGCCTTTGTACCCTGGCACCGGTACTTCTTGCTGCACT WT-A [%]

#3 ATTGACTTTGCCCATGAAGCTCCAGCCTTTGTACCCTGGCACCGGTACTTCTTGCTGCACT WT-A [%]

#4 GTTGACTTTGCCCATGAAGCTCCAGCCTTTGTACCCTGGCACCGGTACTTCTTGCTGCACT WT-A [%]

#5 ATTGACTTTGCCCATGAAGCTCCAGCCTTTGTACCCTGGCACCGGTACTTCTTGCTGCACT WT-B [%]

#6 GTTGACTTTGCCCATGAAGCTCCAGCCTTTGTACCCTGGCACCGGTACTTCTTGCTGCACT WT-B [%]

#7 ATTGACTTTGCCCATGAAGCTCCAGCCTTTGTACCCTGGCACCGGTACTTCTTGCTGCACT WT-B [%]

#7 GTTGACTTTGCCCATGAAGCTCCAGCCTTTGTACCCTGGCACCGGTACTTCTTGCTGCACT WT-B [%]

#7 ATTGACTTTGCCCATGAAGCTCCAGCCTTTGTACCCTGGCACCGGTACTTCTTGCTGCACT WT-B [%]

#7 GTTGACTTTGCCCATGAAGCTCCAGCCTTTGTACCCTGGCACCGGTACTTCTTGCTGCACT WT-B [%]

#7 GTTGACTTTGCCCATGAAGCTCCAGCCTTTGTACCCTGGCACCGGTACTTCTTGCTGCACT WT-B [%]

#7 GTTGACTTTGCCCATGAAGCTCCAGCCTTTGTACCCTGGCACCGGTACTTCTTGCTGCACT WT-B [%]

#7 ATTGACTTTGCCCATGAAGCTCCAGCCTTTGTACCCTGGCACCGGTACTTCTTGCTGCACT WT-B [%]

W H R

A F V P

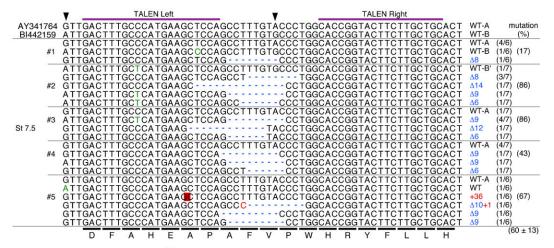
#3; GCCCTG

Tyr-TALEN: fertilized eggs - Stage 6

B Tyr-TALEN: fertilized eggs - Stage 7.5

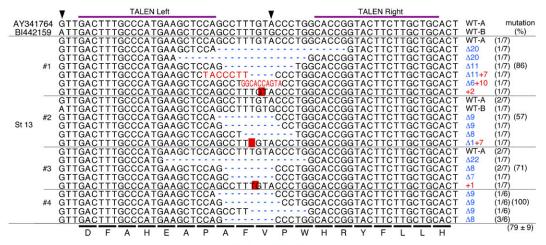
H E A

P



■ #5; ATGGCCTCTGGAGCTCCGTTGACTTTGCCCATGAAG

C Tyr-TALEN: fertilized eggs - Stage 13

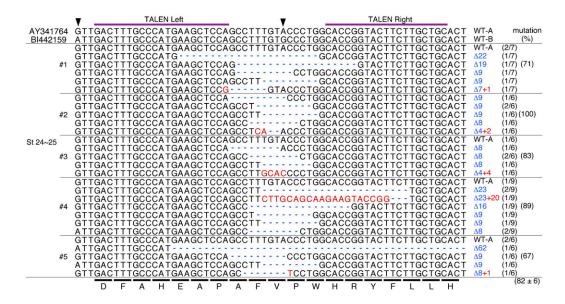


#1; TG, #2; GGCACCG, #3; G

continued

Fig. S1. Analysis of mutations induced by microinjection with Tyr-TALEN or Tyr-TALEN-DS mRNAs into fertilized eggs. (A-G) Genotype analysis of embryos derived from fertilized eggs injected with Tyr-TALEN (A-F) or Tyr-TALEN-DS (G) mRNAs. The target DNA was amplified from the genomic DNA purified from the embryos of the indicated stage and was recloned for sequence determination. The wild-type target DNA and amino acid sequences are indicated on the top and bottom of the panels, respectively. The pair of purple bars represents TALEN binding sites. # refers to a specific embryo. The gaps resulting from deletion ( $\Delta$ ), inserted nucleotides (+), and exchanged nucleotides are denoted as blue dashes, red characters (red squares) and green characters, respectively. The mutation types and frequencies are indicated on the right. Arrowheads indicate the different nucleotide sequences between two tyrosinase gene sequences, the accession numbers AY341764 and BI442159.

D Tyr-TALEN: fertilized eggs - Stage 24~25



E Tyr-TALEN: fertilized eggs - Stage 40

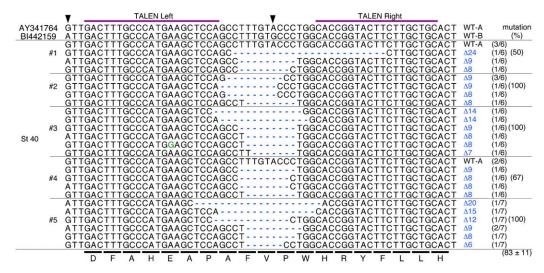
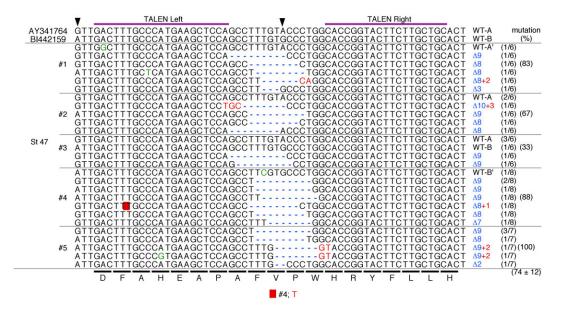


Fig. S1. continued

F

Tyr-TALEN: fertilized eggs - Stage 47



G Tyr-TALEN-DS: fertilized eggs - Stage 8

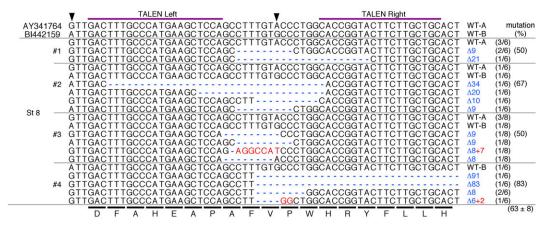


Fig. S1. continued

Fig. S2. Analysis of mutations

induced by microinjection with Tyr-TALEN or Tyr-TALEN-DS mRNAs into oocytes. (A-H) Genotype analysis of embryos derived from oocytes injected with Tyr-TALEN (A-E) or Tyr-TALEN-DS (F-H) mRNAs. The target DNA was amplified from the genomic DNA purified from the embryos of the indicated stage and was recloned for sequence determination. The alignment is labeled as described in the

legend of supplementary material Fig. S1.

В

C

Tyr-TALEN: Oocytes - Stage 4 Α

		<b>v</b>		TA	LEN Lef	t				•				TAL	EN Rig	ht				
AY341	764	GTTGAC	TTT	GCC	CATGA	AGC	TCCA	GCC	TTT	GTÁ	CCC.	TGGC/	ACCGG	TAC	TTC.	TTGO	CTGC	ACT	WT-A	mutation
BI442	159	ATTGAC	TTT	GCC	CATGA	AGC'	TCCA	GCC	TTT	GTG	CCC	TGGC/	ACCGG	TAC	TTC.	TTG	CTGC	ACT	WT-B	(%)
		GTTGAC	TTT	GCC	CATGA	AGC	TCCA	GCC	TTT	GTA	CCC	TGGC/	ACCGG	TAC	TTC.	TTGC	CTGC	ACT	WT-A	(3/7)
	#1	ATTGAC	TTT	GCC	CATGA	AGC	TCCA	GCC	TTT	GTG	CCC	TGGC/	ACCGG	TAC	TTC:	TTG	CTGC	ACT	WT-B	(2/7) (29)
		ATTGAC	TTT	GCC	CATGA	AGC'	TCCA							TAC	TTC:	TTGC	CTGC	ACT	Δ20	(2/7)
St 4	#2	GTTGAC	TTT	GCC	CATGA	AGC	TCCA	GCC	TTT(	GTA	CCC.	TGGC/	ACCGG	TAC	TTC:	TTGC	CTGC	ACT	WT-A	(4/7) (0)
314		ATTGAC	TTT	GCC	CATGA	AGC	TCCA	GCC	TTT	GTG	CCC	TGGC/	ACCGG	TAC	TTC:	TTGC	CTGC	ACT	WT-B	(3/7)
	#3	GTTGAC	TTT	GCC	CATGA	AGC'	TCCA	GCC	TTT	GTA	CCC.	TGGC/	ACCGG	TAC	TTC.	TTGO	CTGC	ACT	WT-A	(4/7) (0)
	100	ATTGAC	TTT	GCC	CATGA	AGC	TCCA	GCC	TTT	GTG	CCC	TGGC/	ACCGG	TAC	TTC.	TTG	CTGC	ACT	WT-B	(3/7)
	#4	GTTGAC	TTT	GCC	CATGA	AGC	TCCA	GCC	TTT	GTA	CCC.	TGGC/	ACCGG	TAC	TTC.	TTGC	CTGC	ACT	WT-A	(1/7) (0)
		ATTGAC	TTT	GCC	CATGA	AGC	TCCA	GCC	TTT	GTG	CCC	TGGC/	ACCGG	TAC	TTC:	TTG	CTGC	ACT	WT-B	(6/7)
			F	Δ	н г	Δ	Р	A	F	V	D	w	H B	$\overline{}$	F	1	_	н		$(7 \pm 7)$

Tyr-TALEN: Oocytes - Stage 6

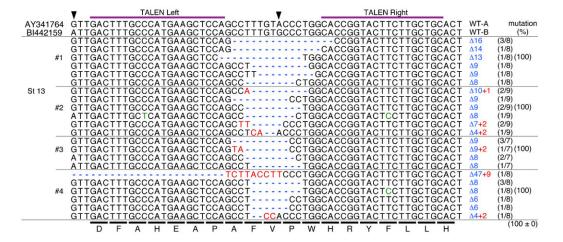
	v	T/	ALEN Left				▼	_		TALE	N Righ	it			
AY341764	GTTGACT	TTGCC	CATGA	GCTC	CAGCC	TTTGT	ACCC	TGGCA	CCGG	TACT	TCT	TGC	TGCACT	WT-A	mutation
BI442159	ATTGACT	TTGCC	CATGAA	GCTC	CAGCC	TTTGT	GCCC	TGGCA	CCGG	TACT	TCT	TGCT	TGCACT	WT-B	(%)
	GTTGACT													WT-A	(3/6)
#1	GTTGACT													Δ9	(2/6) $(50)$
	GTTGACT													Δ9	(1/6)
	GTTGACT													WT-A	(3/6)
#2	GTTGACC													WT-A'	(1/6) (17)
	ATTGACT	TTGCC	CATGA	AGCTC	CAGCC	TTTGT	GCCC	TGGCA	CCGG	TACT	TCT	TGC	TGCACT	WT-B	(1/6)
	ATTGACT													Δ78	(1/6)
#3	GTTGACT													WT-A	(2/6) (0)
	ATTGACT													WT-B	(4/6)
St 6	GTTGACT													WT-A	(2/8)
	ATTGACT												TGCACT	Δ20	(3/8)
#4														Δ9	(1/8) (75)
	ATTGACT													Δ9	(1/8)
	ATTGACT													Δ7	(1/8)
	GTTGACT													WT-A	(1/7)
	ATTGACT													WT-B	(1/7)
#5														WT-B'	(1/7) $(57)$
	ATTGACT													Δ12	(2/7)
	GTTGACT													Δ8	(1/7)
	ATTGACT	TTGCC	CATGA	AGCTC	CAG		-CCC	TGGCA	CCGG	TACT	TCT	TGC	TGCACT	Δ8	(1/7)
		FΔ	H E	Δ	Δ .	F V	Р	W H	R		F	-	. н		$(40 \pm 14)$
	D			^	_			** 11	п				- 11		

Tyr-TALEN: Oocytes - Stage 8

		▼TALEN Left	•	TALEN Right	
AY341	764	GTTGACTTTGCCCATGAAGCTCCAGCCTT	STACCCTGG	CACCGGTACTTCTTGCTGCACT	NT-A mutation
BI4421		ATTGACTTTGCCCATGAAGCTCCAGCCTT			NT-B (%)
		GTTGACTTTGCCCATGAAGCTCCAGCCTT	STACCCTGG	GCACCGGTACTTCTTGCTGCACT	NT-A (1/8)
		ATTGACTTTG	G	CACCGGTACTTCTTGCTGCACT	1/8)
		ATTGACTTTGCCCATGAAGCTCCAGC			120 (1/8)
	#1	GTTGACTTTGCCCATGAAGCTC		CACCGGTACTTCTTGCTGCACT	(1/8) (88)
		GTTGACTTTGCCCATGAAGCTCCAGCCT-	GG	SCACCGGTACTTCTTGCTGCACT	(1/8)
		ATTGACTTTGCCCATGAAGCTCCAGCCT-			(2/8)
		ATTGACTTTGCCCATGAAGCGCCAGCCT-			(1/8)
		ATTGACTTTGCCCATGAAGCT			(1/6)
	#2	ATTGACTTTGCCCATGAAGCTCCAG			(3/6) (100)
St 8		ATTGACTTTGCCCACGAAGCTCCAG			(1/6)
		ATTGACTTTGCCCATGAAGCTCCAGCC			12+5 (1/6)
		GTTGACTTTGCCCATGAAGCTCCAG			110 (1/7)
	#3	GTTGACTTTGCCCATGAAGCTCCAG			(2/7)
		GTTGACTTTGCCCATGAAGCTCCAG			(1/7) (100)
		ATTGACTTTGCCCATGAAGCTCCAG			18 (1/7)
		GTTGACTTTGCCCATGAAGCTCCAGCCT-			18 (1/7)
	-	ATTGACTTTGCCCATGAAGCTCCAGCCTT			18 (1/7)
		GTTGACTTTGCCCATGAAGCTCCAG			122 (1/7)
		GTTGACTTTGCACATGAAGCTCC			112 (1/7)
	#4	ATTGACTTTGCCCATGAAGCTCCAG			(3/7) (100)
		GTTGACTTTGCCCATGAAGCTCCAG			18 (1/7)
		<u>ATTGACTTTGCCCATGAAGCTCCAGCCTT</u>	TGG	CACCGG FACT ICTTGCTGCACT	7 (1/7)
		DFAHEAPAE	V P W	HBYFIIH	$(97 \pm 3)$

continued

**D** Tyr-TALEN: Oocytes - Stage 13



E Tyr-TALEN: Oocytes - Stage 24~25

	<b>v</b>		TALE	N Left					•				TALE	N Rig	ht				
AY341764	GTTGAC	TTTG	CCCA	TGA	AGCT	CCAC	CCT	TTG	TÁC	CCTC	GCA	CCGG	TAC	TTC.	TTG	CTG	CACT	WT-A	mutation
BI442159	ATTGAC	TTTG	CCCA	TGAA	AGCT	CCAG	CCT	TTG	TGC	CCTC	GCA	CCGG	TAC	TTC	TTG	CTG	CACT	WT-B	(%)
	GTTGAC	TTTG	CCCA	TGAA	GC -						A	CCGG	TAC	TTC	TTG	CTG	CACT	Δ20	(1/7)
#1	GTTGAC	TTTG	CCCA	TGA	AGCT	CCAC	SCCT					CCGG	TAC	TTC	TTG	CTG	CACT	Δ13	(1/7)(100)
7.50	GTTGAC	TTTG	CCCA	TGAA	AGCT	CCAG	GCC -			-CTC	GCA	CCGG	TAC	TTC	TTG	CTG	CACT	Δ8	(5/7)
	GTTGAC	TTTG	CCCG	TGA	AGCT	CCAC	GC							TTC	TTG	CTG	CACT	Δ22	(1/7)
	GTTGAC	CTTG	CCCA	TGAA	AGC -				(	CCTC	GGCA	CCGG	TAC	TTC	TTG	CTG	CACT	Δ14	(1/7)
#2	ATTGAC	TTTG	CCCA	TGAA	AGCT				C	CCTC	GGCA	CCGG	TAC	TTC:	TTG	CTG	CACT	Δ12	(1/7)(100)
St 24~25	ATTGAC	TTTG	CTCA	TGAA	AGCT	CCAC	à		(	CCTC	GCA	CCGG	TAC	TTC	TTG	CTG	CACT	Δ9	(1/7)
	GTTGAC	TTTG	CCCA	TGA	AGCT	CCAC	GCC -		- AG	CCTC	GGCA	CCGG	TAC	TTC	TTG	CTG	CACT	$\Delta 7+2$	(1/7)
	GTTGAC																	$\Delta 6 + 4$	(2/7)
	GTTGAC	TTTG	CCCA	TGAA	AGCT	CCAG	GCC -			TO	GCA	CCGG	TAC	TTC	TTG	CTG	CACT	Δ9	(4/7)
#3	ATTGAC																	Δ9	(2/7)(100)
	GTTGAC	TTTG	CCCA	TGA	AGCT	CCAC	SCCT		A	CTC	GGCA	CCGG	TAC	TTC	TTG	CTG	CACT	$\Delta 7+2$	(1/7)
	GTTGAC	TT									-CA	CCGG	TAC	TTC:	TTG	CTG	CACT	Δ31	(3/6)
#4	ATTGAC	TTTG	CCCA	TGAA	AGCT	CCAC	CCT	TTG			GCT	CCGG	TAC	TTC	TTG	CTG	CACT	Δ7	(1/6) (100)
	GTTGAC	TTTG	CCCA	TGAA	AGCT	CCAC	CCT			TC	GCA	CCGG	TAC	TTC	TTG	CTG	CACT	Δ8	(2/6)
	D	F	А Н	F	Δ	P	Δ	FΙ	/ [	> V	V H	B	$\overline{}$	F	To the	T.	н		$(100 \pm 0)$

F Tyr-TALEN-DS: Oocytes - Stage 4

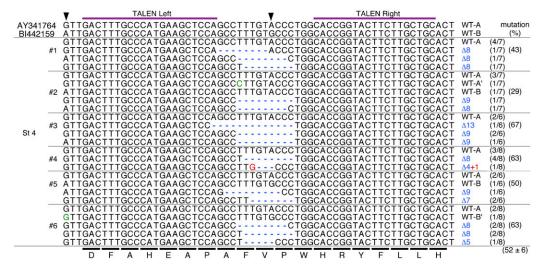
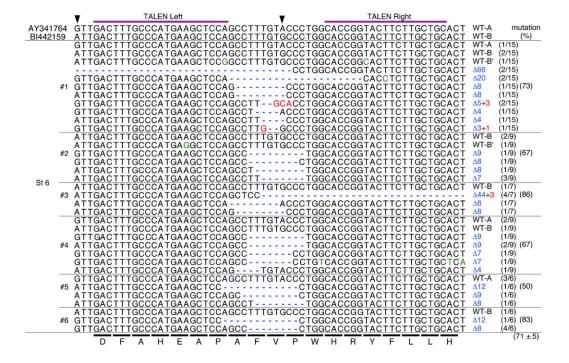


Fig. S2. continued

G Tyr-TALEN-DS: Oocytes - Stage 6



Tyr-TALEN-DS: Oocytes - Stage 8

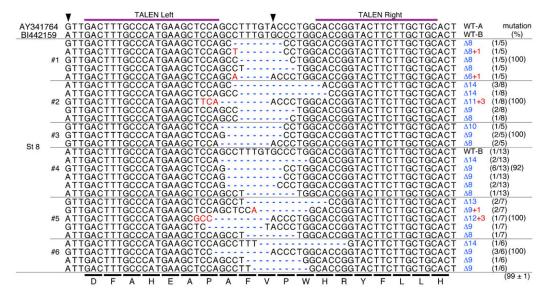


Fig. S2. continued

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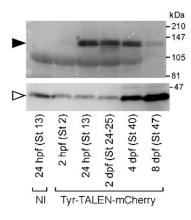


Fig. S3. Time course of TALEN-mCherry fusion protein expression in embryos and tadpoles. Fertilized eggs were injected with Tyr-TALEN-mCherry mRNAs. Five embryos or tadpoles were homogenized at the indicated time and examined by Western blot analysis using anti-DsRed and anti-actin antibodies. The closed and open arrowheads indicate the positions of the Tyr-TALEN-mCherry and actin proteins, respectively. The stage 13 embryos (NI) in the left-most lane were not injected with mRNAs. Note that levels of TALEN-mCherry fusion proteins were not reduced till 4 dpf. A level of actin protein increased at 4 and 8 dpf of tadpoles, which may be ascribed to the development of muscle tissue.

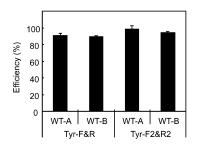


Fig. S4. Amplification efficiency of two homoeologs of *tyrosinase* genes during PCR. Two types of *tyrosinase* genes, WT-A and WT-B, were cloned. The amplification efficiencies using primer sets, Tyr-F and -R and Tyr-F2 and -R2, were determined by Thermal Cycler Dice Real Time System TP850 (TaKaRa). The data are expressed as the mean value  $\pm$  standard error (n=3).