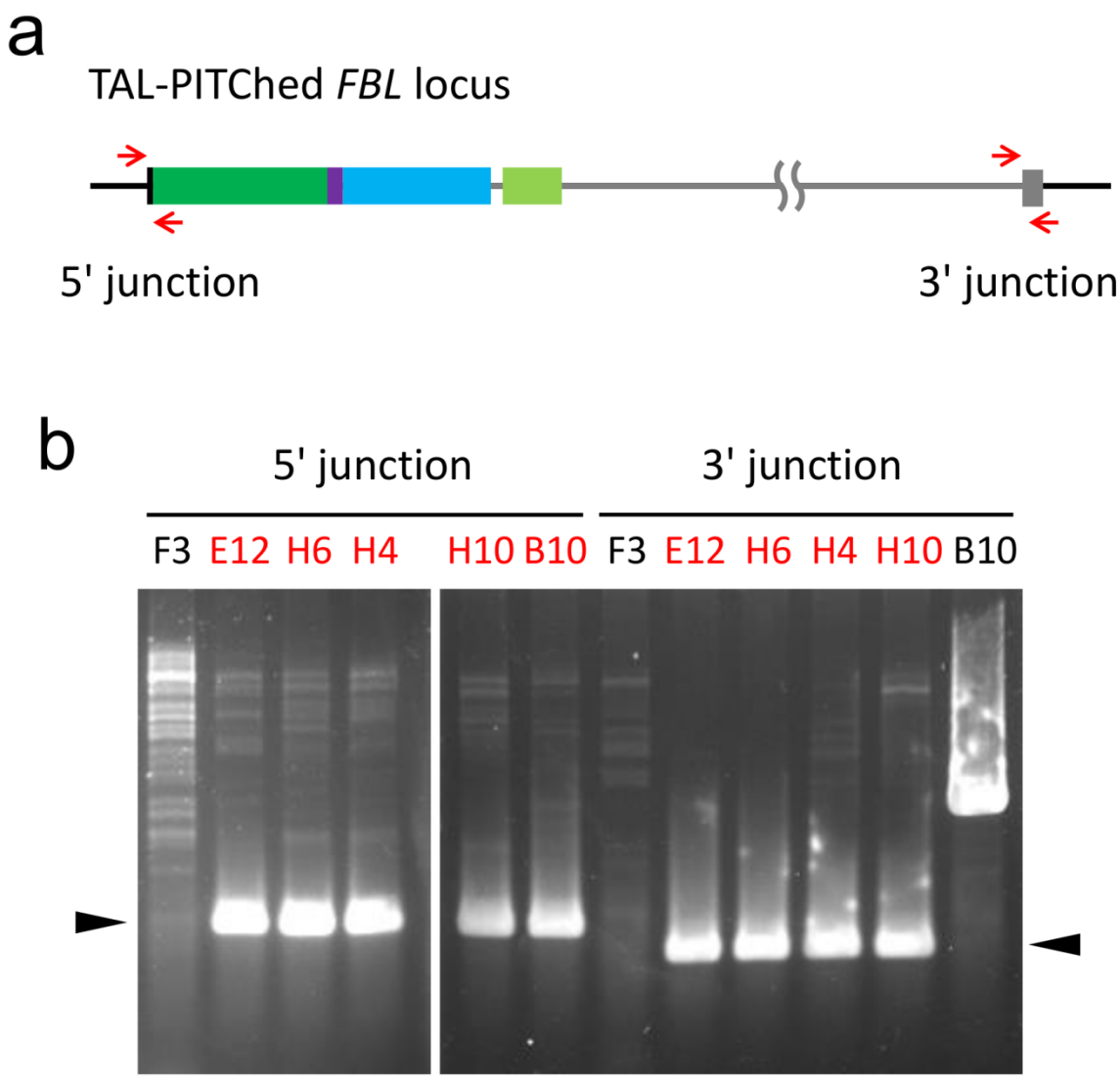
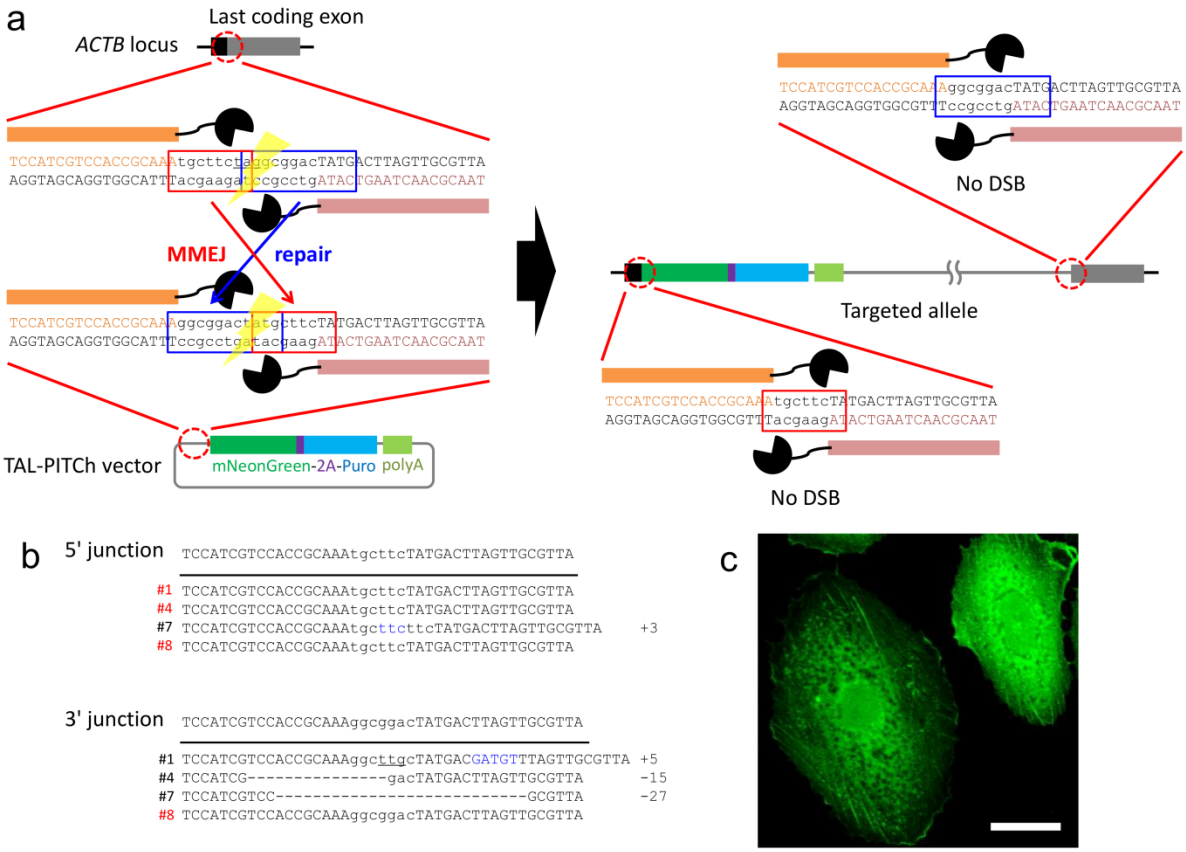
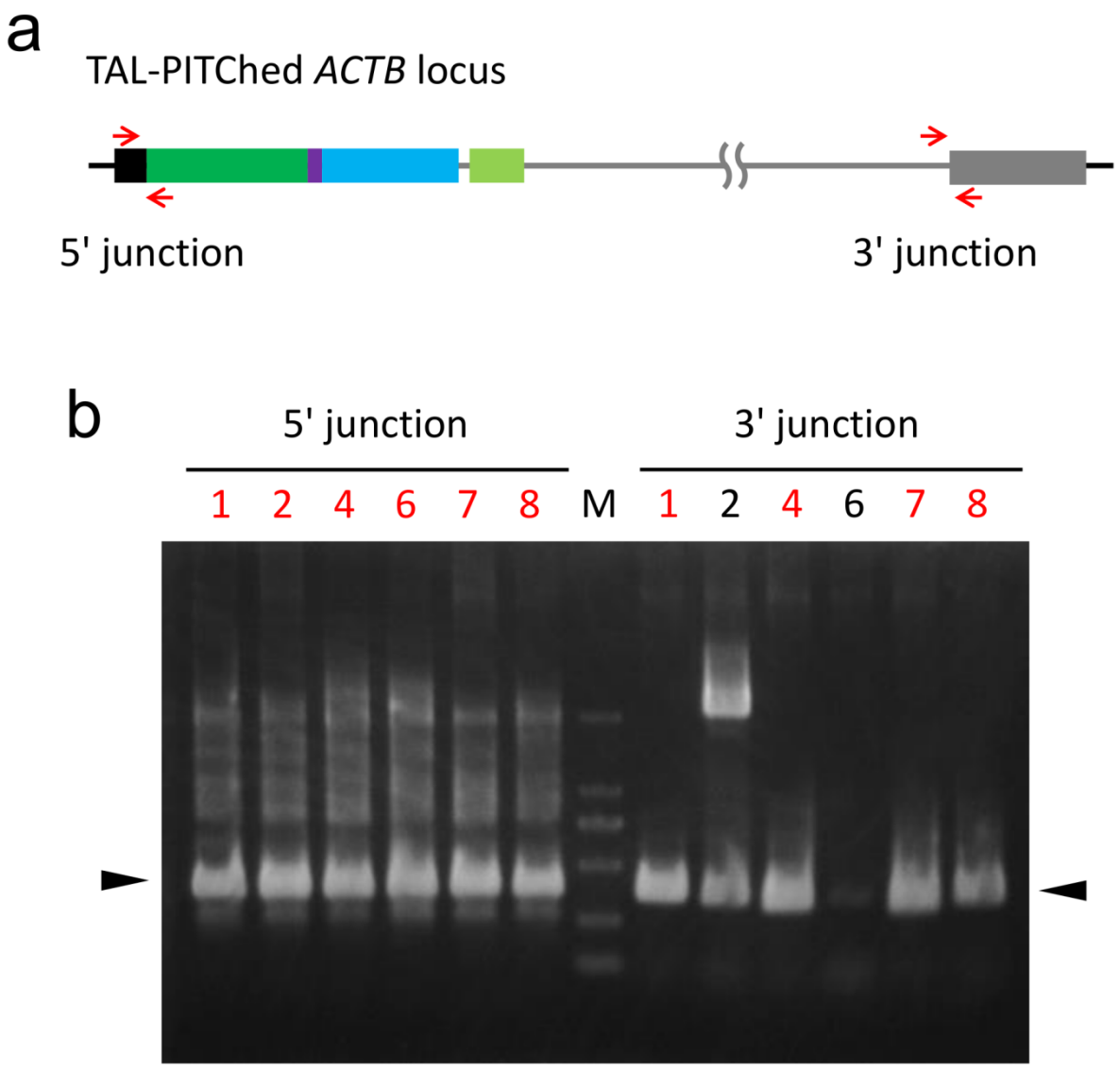


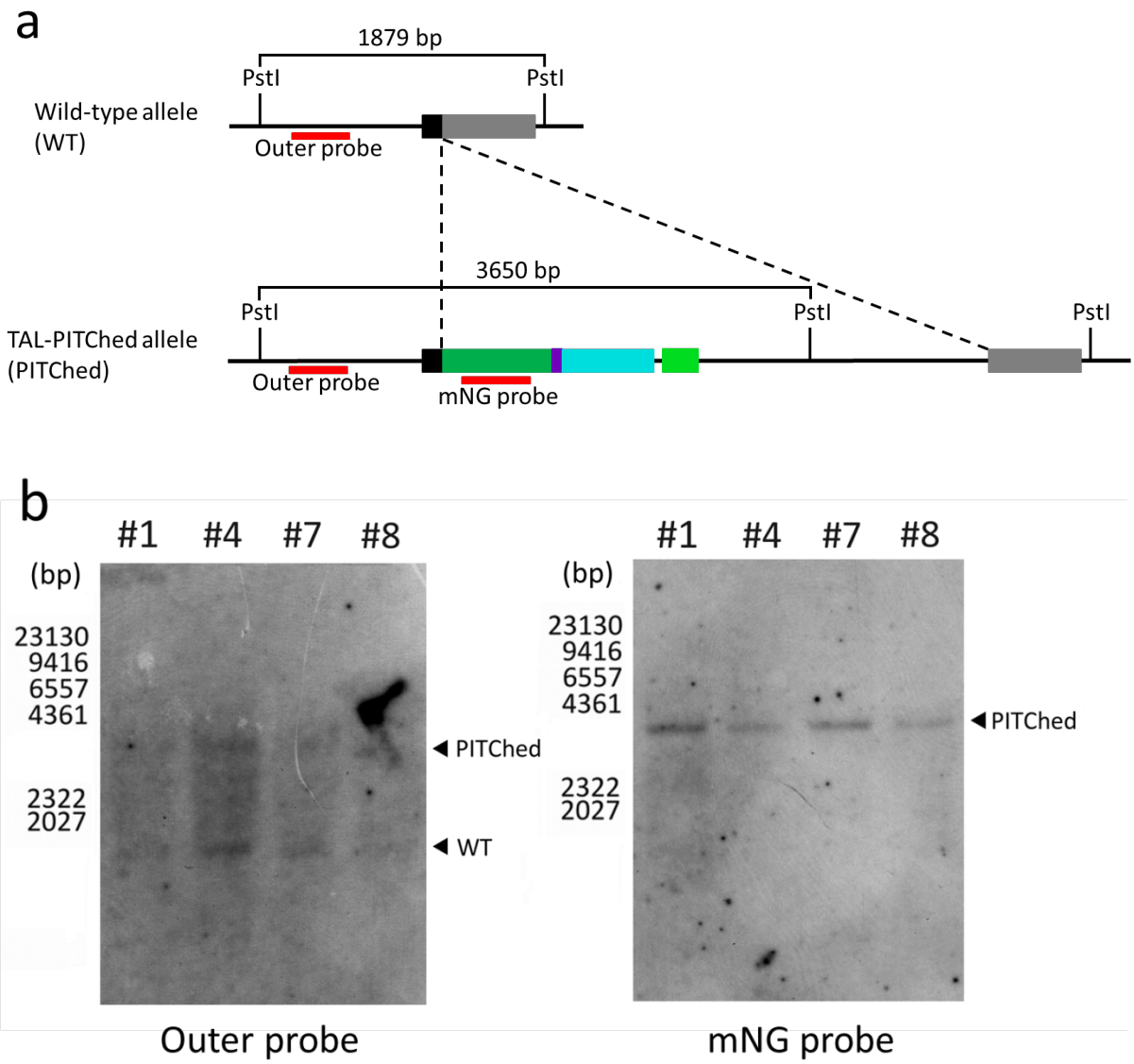
Supplementary Figure 1 Genomic PCR analysis of TAL-PITChed cell clones at the *FBL* locus. (**a**) Schematic illustration of the amplified genomic regions. Red arrows indicate primer annealing sites. (**b**) Gel images of the PCR products. Arrowheads indicate the expected positions of the amplicons from knocked-in alleles. Red letters indicate clone IDs showing the correct knockin bands.



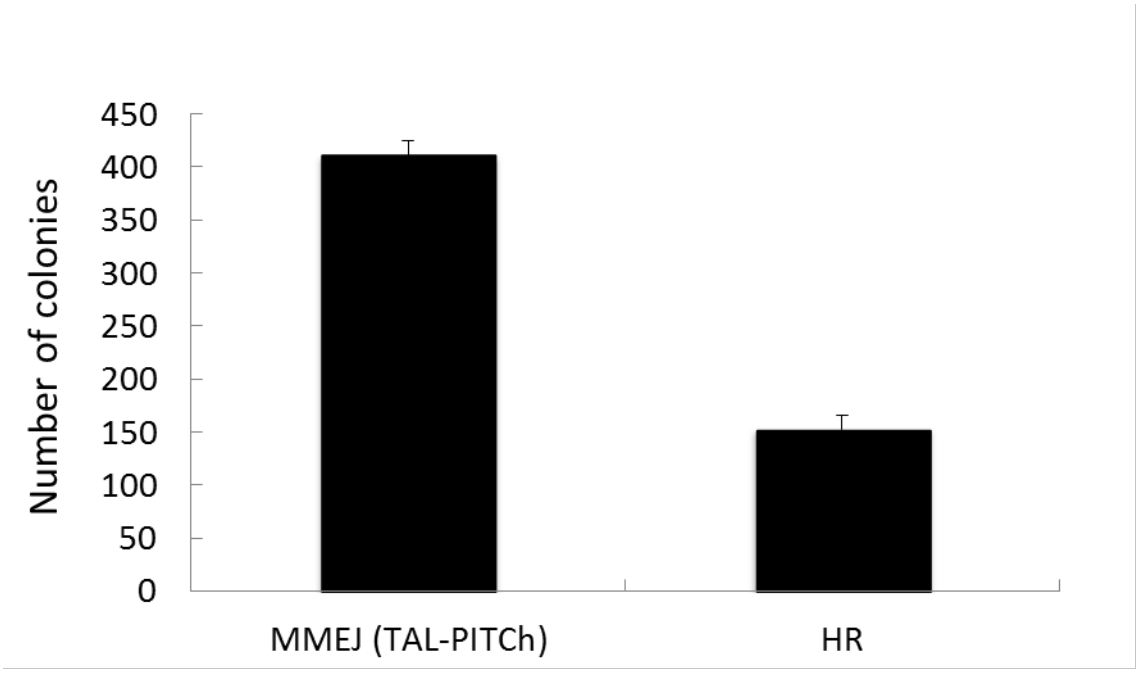
Supplementary Figure 2 TAL-PITCh at the *ACTB* locus in HeLa cells. (**a**) Schematic illustration of TAL-PITCh at the *ACTB* locus. Orange and pink letters indicate the left and right TALEN target sites, respectively. Red and blue boxes indicate the microhomologous sequences. The stop codon is underlined. (**b**) Sequences of knocked-in clones. The intended knocked-in sequence is shown at the top. TALEN target sites are shown in capital letters. Dashes indicate deletions. Blue letters indicate insertions. Substitutions are underlined. Red letters indicate correctly knocked-in clones. (**c**) Confocal laser scanning microscopy image of knocked-in cells (clone #8). Scale bar: 30 μm.



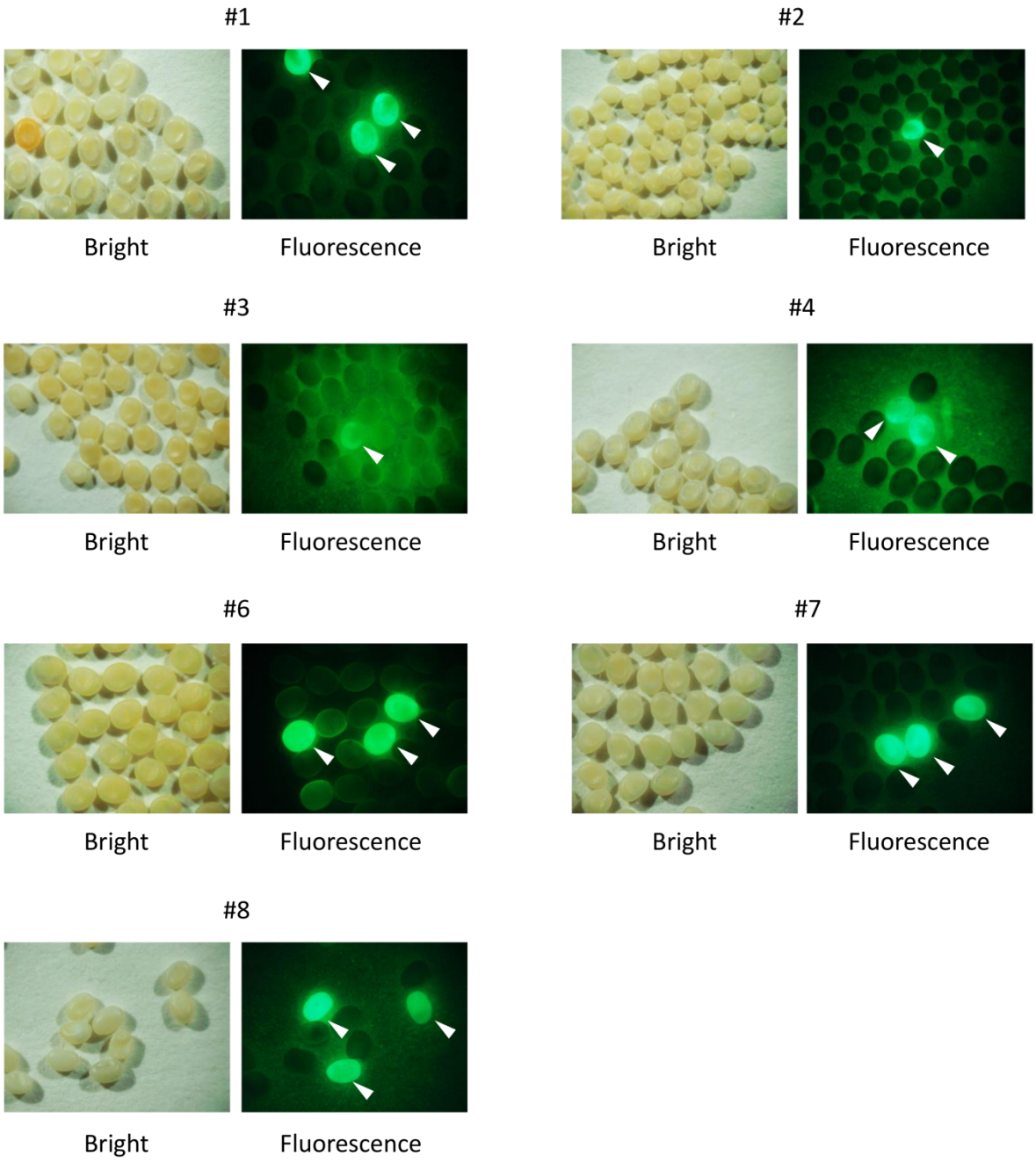
Supplementary Figure 3 Genomic PCR analysis of TAL-PITChed cell clones at the *ACTB* locus. (**a**) Schematic illustration of the amplified genomic regions. Red arrows indicate primer annealing sites. (**b**) A gel image of the PCR products. Arrowheads indicate the expected positions of the amplicons from knocked-in alleles. Red letters indicate clone IDs showing the correct knockin bands. M, Wide-Range DNA Ladder (100–2,000 bp) (Takara).



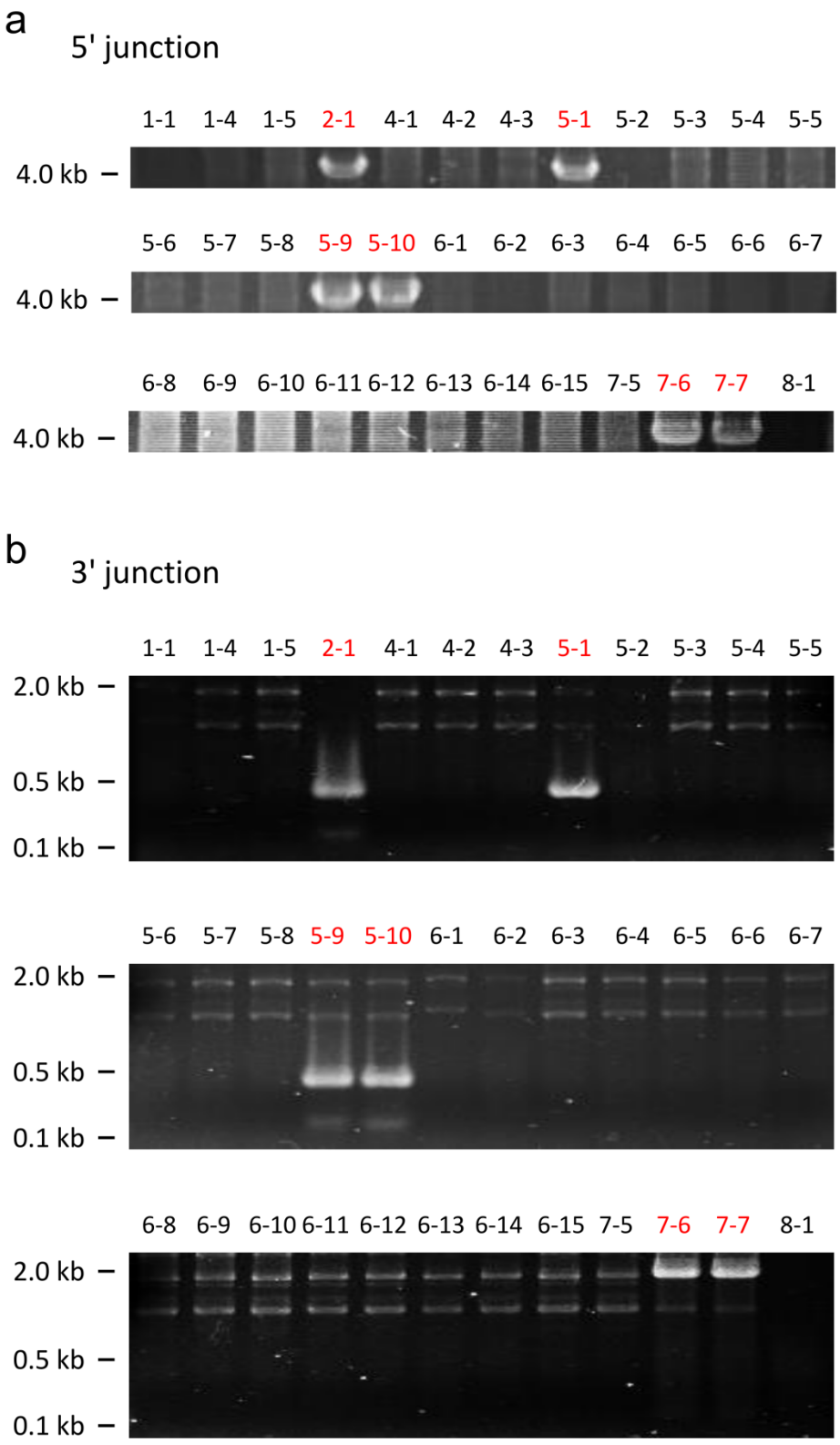
Supplementary Figure 4 Southern blot analysis for TAL-PITChed cell clones at the *ACTB* locus. (**a**) Schematic illustration of wild-type and TAL-PITChed *ACTB* locus. Two probe sites for endogenous (Outer probe) and exogenous (mNG probe) sequences are shown as red bars. mNG, mNeonGreen. (**b**) Chemiluminescent images of Southern blotting experiments. Clone IDs shown on the top of each panel are identical to Supplementary Figs. 2 and 3.



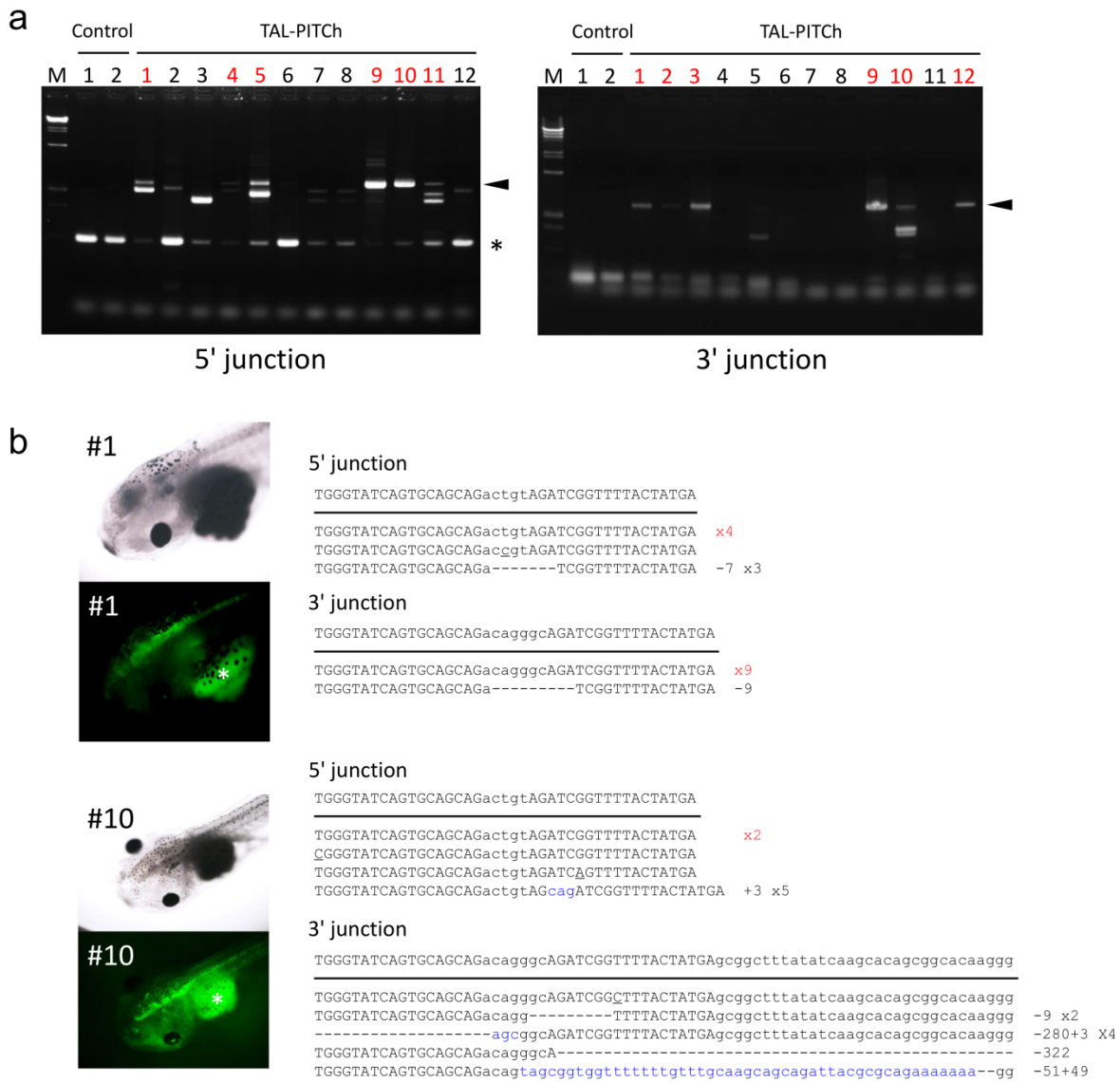
Supplementary Figure 5 Comparison of colony-forming efficiencies between MMEJ- and HR-mediated gene knockin at the *ACTB* locus in HeLa cells. For the MMEJ- or HR-mediated knockin, TALENs and TAL-PITCh vector or HR vector containing 1-kb homology arms were cotransfected and selected by puromycin. Numbers of all the colonies formed in 100-mm dishes showing fluorescence were counted. Data are expressed as means ±SEM (n=3).



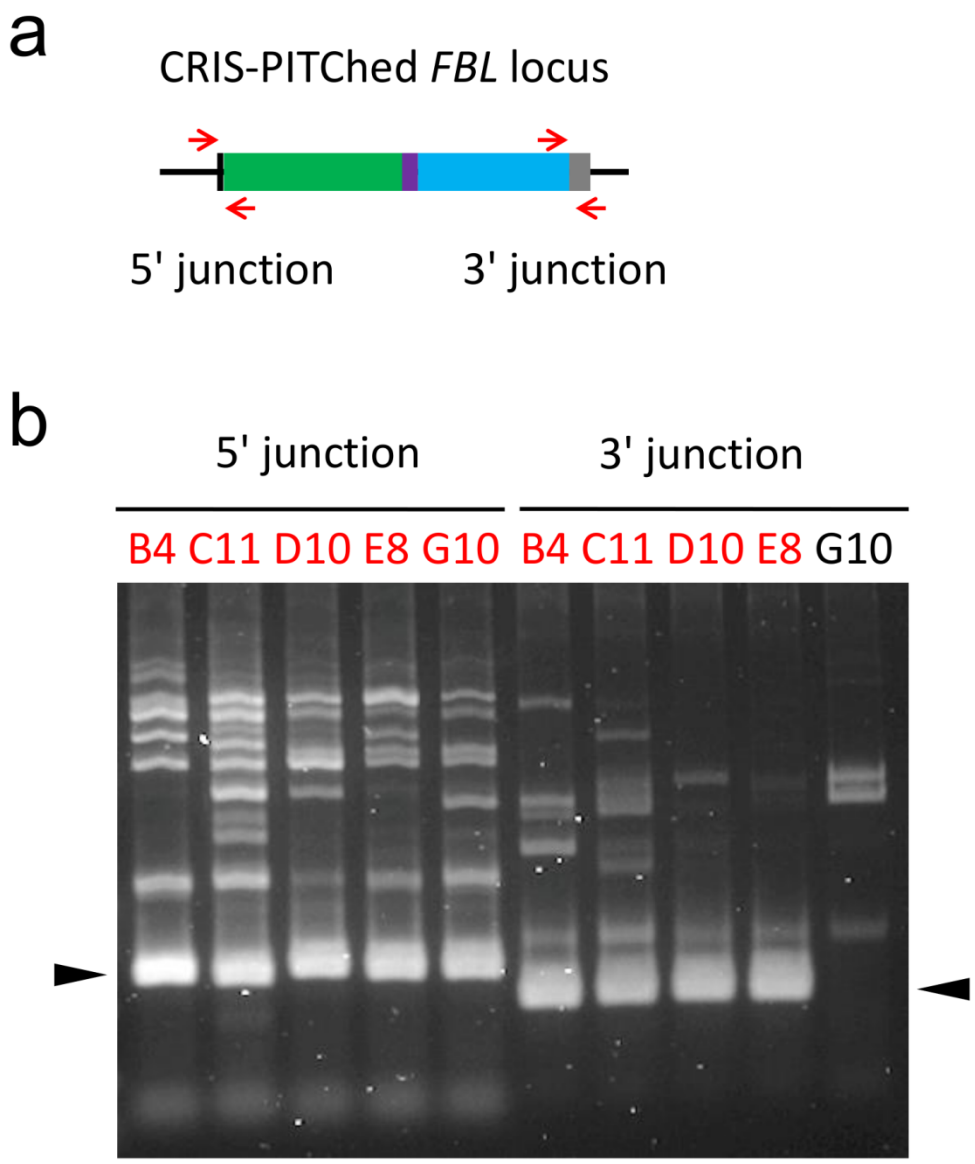
Supplementary Figure 6 EGFP expression in the G_1 embryos of TAL-PITChed silkworms. Batches with EGFP-positive embryos, excluding #5, are shown. White arrowheads indicate EGFP-positive embryos.



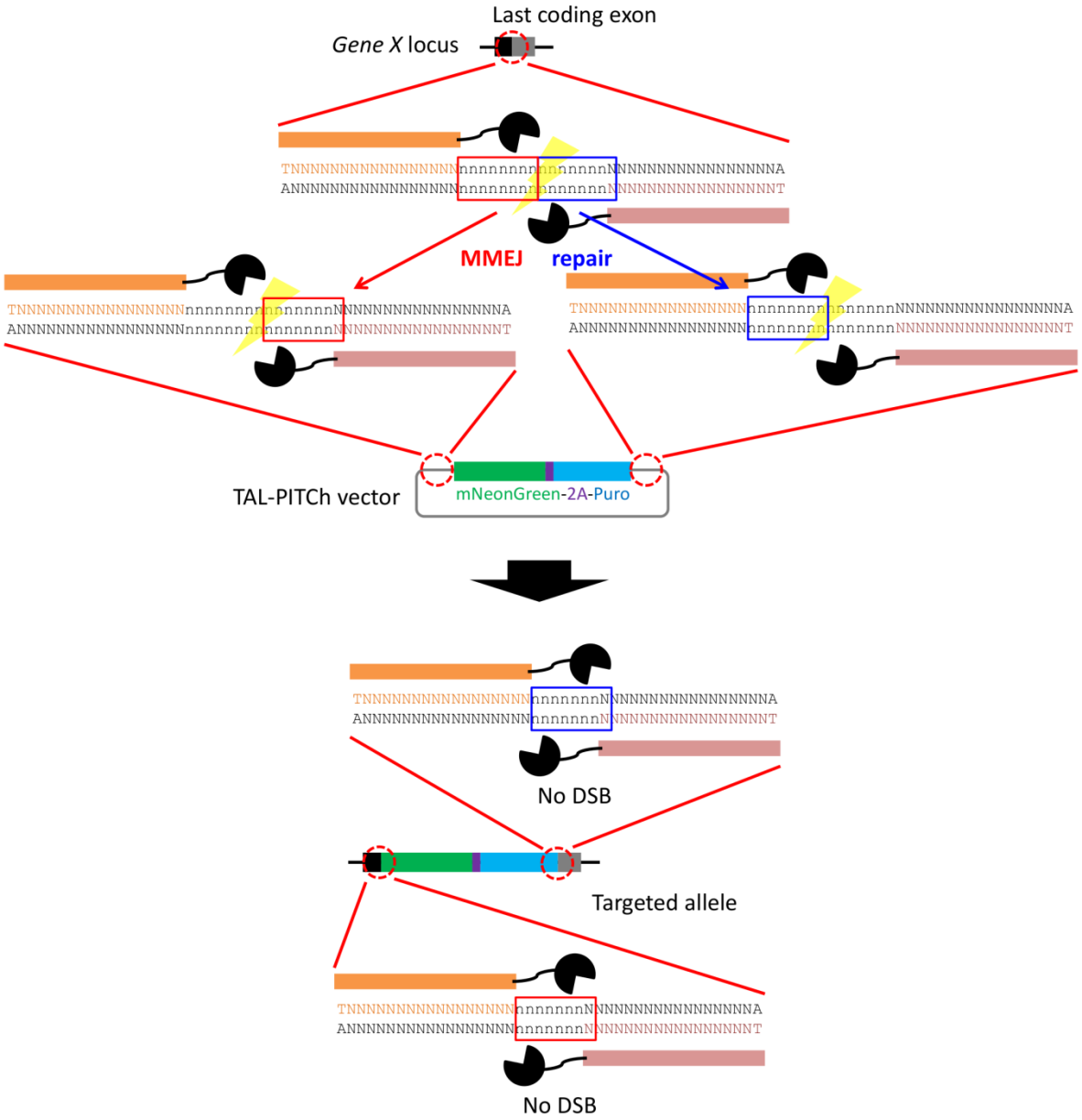
Supplementary Figure 7 Genomic PCR analysis of TAL-PITChed worms. Gel images of the PCR products at the 5′ (**a**) and 3′ (**b**) junctions. Red letters indicate worm IDs showing the knockin bands.



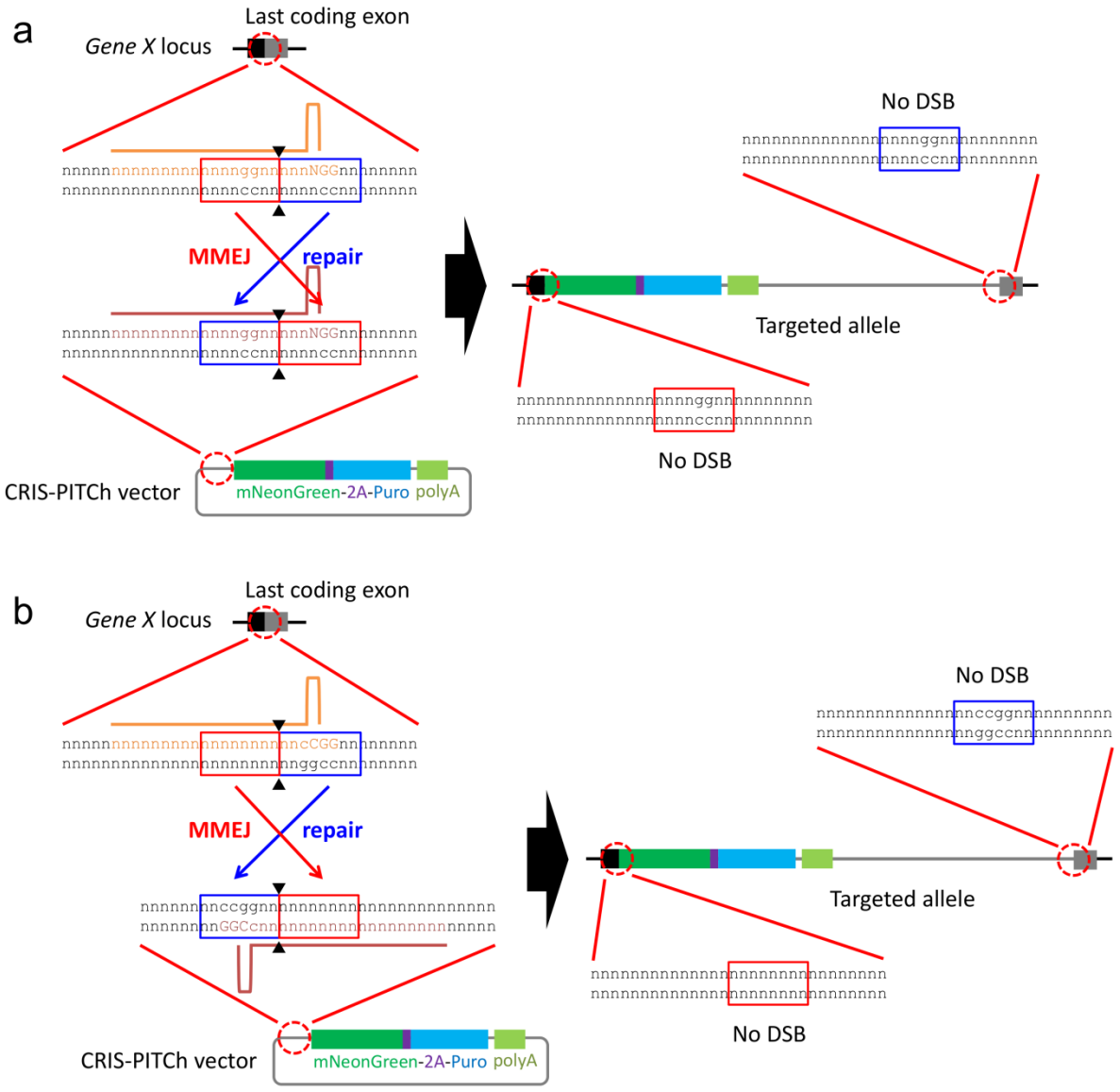
Supplementary Figure 8 Genomic PCR and sequencing analysis of TAL-PITChed embryos at the *no29* locus. (**a**) Gel images of the PCR products. Arrowheads indicate the expected positions of the amplicons from knocked-in alleles. Asterisks indicate non-specific bands. Red letters indicate clone IDs showing the correct knockin bands. M, λDNA/HindIII+pUC/HinfI marker. (**b**) Microscopy images and sequences of the embryos #1 and #10. Asterisks indicate yolk autofluorescence. Red letters indicate correctly knocked-in alleles. Blue letters indicate insertions. Dashes indicate deletions. Substitutions are underlined.



Supplementary Figure 9 Genomic PCR analysis of CRIS-PITChed cell clones at the *FBL* locus. (**a**) Schematic illustration of the amplified genomic regions. Red arrows indicate primer annealing sites. (**b**) A gel image of the PCR products. Arrowheads indicate the expected positions of the amplicons from knocked-in alleles. Red letters indicate clone IDs showing the correct knockin bands.



Supplementary Figure 10 Possible design of TAL-PITCh for cassette integration. For TAL-PITCh-mediated cassette integration, two TALEN target sites should be added at both ends of the cassette in the TAL-PITCh vector. The same TALEN set can be used to target three loci on the genome and on the TAL-PITCh vector. Orange and pink letters indicate the left and right TALEN target sites, respectively. Red and blue boxes indicate the microhomologous sequences.



Supplementary Figure 11 Possible design of CRIS-PITCh for full plasmid integration. For CRIS-PITCh-mediated full plasmid integration, a single CRISPR/Cas9 site targeting the sense (**a**) or antisense (**b**) strand should be added to the CRIS-PITCh vector. The limitation of the genomic target sequence depends on which strand is targeted. Orange and pink letters indicate the gRNA target sites. Red and blue boxes indicate the microhomologous sequences.

>TAL-PITCh vector for the *FBL* gene

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>TAL-PITCh vector for the *ACTB* gene

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>CRIS-PITCh vector for the *FBL* gene

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>HR vector for the *ACTB* gene

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>TAL-PITCh vector for the *BLOS2* gene

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>TAL-PITCh vector for the *no29* gene
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>TAL-PITCh vector for the *fgk* gene
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TAGCC

Supplementary Figure 12 Full plasmid sequences of the TAL-PITCh, CRIS-PITCh, and HR vectors. Green, purple, blue and red letters indicate the coding sequences of mNeonGreen/EGFP, 2A peptide, the puromycin resistance gene and no29, respectively. Light blue letters indicate the *hsp90* promoter. Target sites for TALENs and CRISPR/Cas9 are underlined.

Supplementary Table 1. TAL-PITCh and CRIS-PITCh efficiencies in human cells.

	Gene	Cell line	PCR positive clone/total clone			Knockin with precise 5′ junction	Knockin with precise 3′ junction
			5′ junction	3′ junction	Both junctions		
TAL-PITCh	*FBL*	HEK293T	5/6	4/6	4/6	4/4 (100%)	4/4 (100%)
	ACTB	HeLa	6/6	4/6	4/6	3/4 (75%)	1/4 (25%)
CRIS-PITCh	*FBL*	HEK293T	5/5	4/5	4/5	2/4 (50%)	0/4 (0%)

Supplementary Table 2. Summary of silkworm experiments.

G_0		
Eggs injected	Hatched larvae	Adults crossed with non-injected individuals
181	62	35

G_1					
Batches with EGFP-positive embryos	Total number of EGFP-positive embryos	Genotyped individuals	Knockin into *BLOS2* locus	Knockin with precise 5′ junction	Knockin with precise 3′ junction
8	92	36	6	6 (100%)	4 (67%)

Supplementary Table 3. Summary of the off-target analyses for TAL-PITCh.

Name	Sequence*	TALEN score**	Coordinate	Genomic region	Mutation
On-target	5′-TCCTGACAGCGCTGAACTtcagttcttcaccttGGGGGGTGGCCTGTGAGA-3′ 3′-AGGACTGTCGCGACTTGAagtcaagaagtggaaCCCCCCACCGGACACTCT-5′	100	chr19:40325160 -40325210	Exon	
TAL-OT1	5′-TCTCCCAGGCCACCCCTCggtcccagagtctcctcccCGTCCAGAGCTCTCAGGA-3′ 3′-AGAGGGTCCGGTGGGGAGccagggtctcagaggagggGCAGGTCTCGAGAGTCCT-5′	61.72	chr5:42953443-42953497	Intergenic	Not detected
TAL-OT2	5′-TCTCACATGCCACCCCACtcaatgggtggtgcttCTTTGAGCACTGTAAGGT-3′ 3′-AGAGTGTACGGTGGGGTGagttacccaccacgaaGAAACTCGTGACATTCCA-5′	58.44	chr10:98256567 -98256618	Intron	Not detected
TAL-OT3	5′-TCCTGACTGCACAGAACTggacaagagctagttGCTGGGTGGGTTGGGGGA-3′ 3′-AGGACTGACGTGTCTTGAcctgttctcgatcaaCGACCCACCCAACCCCCT-5′	56.82	chr16:70751469 -70751519	Intron	Not detected
TAL-OT4	5′-TCCTGACCTCCCTGAACTccacccaccttggcctcccaaAGTGCTGGGATTACAGGA-3′ 3′-AGGACTGGAGGGACTTGAggtgggtggaaccggagggttTCACGACCCTAATGTCCT-5′	56.56	chr5:61711213-61711269	Intron	Not detected
TAL-OT5	5′-TCACAAAAACACTGAACTtgagcaaatattgtcAGTTCTGGGGTGGCAGGA-3′ 3′-AGTGTTTTTGTGACTTGAactcgtttataacagTCAAGACCCCACCGTCCT-5′	56.3	chr3:178104774 -178104824	Intergenic	Not detected
TAL-OT6	5′-TCTCACAGGCCACCACCAaggtgtcagccaggcCTGGGGTCTCCTTTGATA-3′ 3′-AGAGTGTCCGGTGGTGGTtccacagtcggtccgGACCCCAGAGGAAACTAT-5′	56.11	chr7:153319981 -153320031	Intergenic	Not detected

*Red and blue letters indicate left and right TALEN target sequences, respectively. Mismatches are underlined.

**TALEN scores were calculated by the PROGNOS tool (http://baolab.bme.gatech.edu/cgi-bin/prognos/prognos.cgi).

Supplementary Table 4. Summary of the off-target analyses for CRIS-PITCh.

Name	Sequence*	Score**	Coordinate	Strand	Mutation
On-target (gRNA on the genome)	CTCTCACAGGCCACCCCCCAAGG				
CRIS-OT1A	CCCTCCCAGGCCACCCCCCAAGG	3.6	chr18:46206217	-	Not detected
CRIS-OT2A	TGATCACAGGCCACCCCCCATAG	2.3	chr6:30796131	-	Not detected
CRIS-OT3A	CTCTCTCAGGCCACCCTCCAGAG	2.2	chr11:76938544	+	Not detected
On-target (gRNA at the 5' junction)	TGGATCCGTGGGGTGGCCCCGGG				
CRIS-OT1B	TGGCTCCGTGGGGTGGCCGCTGG	4.3	chr10:29645541	-	Not detected
CRIS-OT2B	TTGCTCCTTGGGGTGGCCCCAGG	2.5	chr22:38619312	+	Not detected
CRIS-OT3B	TGGATCTGGGGGGTGGCCCCTGG	2.3	chr3:133419488	+	Not detected
On-target (gRNA at the 3' junction)	GGTGCCTGACCAAGGTGCCCGGG				
CRIS-OT1C	TGTGTCTGAGCAAGGTGCCCAGG	2.5	chr7:29849668	+	Not detected
CRIS-OT2C	GCTGGCTGGCCAAGGTGCCCGAG	1.6	chr15:61098465	+	Not detected
CRIS-OT3C	GGAGCCTGGGCAAGGTGCCCAAG	1.4	chr17:79974949	+	Not detected

* Red letters indicate PAM sequence. Mismatches are underlined.

**Scores were calculated by the CRISPR design tool (http://crispr.mit.edu/).

Supplementary Table 5. Oligonucleotides for gRNA templates.

Gene	Target locus	Sense oligonucleotide (5′->3′)	Antisense oligonucleotide (5′->3′)
FBL	Genome	caccgctctcacaggccacccccca	aaactgggggtggcctgtgagagc
	5' junction	caccgtggatccgtggggtggcccc	aaacggggccaccccacggatccac
	3' junction	caccggtgcctgaccaaggtgccc	aaacgggcaccttggtcaggcacc

Supplementary Table 6. Primers for PCR at the on-target sites.

	Gene	Locus	Forward primer (5′->3′)	Reverse primer (5′->3′)
TAL-PITCh in human cells	*FBL*	5' junction	acaccaagacagacatctctgtcccttg	atccgtatccaatgtggggaac
		3' junction	gcccttaattgtgagcggataac	tcagcaggtcaaggggaggaatg
	ACTB	5' junction	tggcctcgctgtccaccttc	tgcagcctgaaatgggctcatc
		3' junction	tcgcccttaattgtgagcggataac	gatgctcgctccaaccgactg
CRIS-PITCh in human cells	*FBL*	5' junction	acaccaagacagacatctctgtcccttg	atccgtatccaatgtggggaac
		3' junction	ccgcaacctccccttctacgag	tcagcaggtcaaggggaggaatg
TAL-PITCh in *B. mori*	*BLOS2*	5' junction	agcaaattgttgcaagggac	ccgctcgagttaattcacacaaaatgac
		3' junction	ataacgaccgcgtgagtcaa	agctagccacagtgtttatc
TAL-PITCh in *X. laevis*	*no29*	5' junction	agcataggcatctccctgatgtggt	ggtcttcatcatcactggctacaacg
		3' junction	gaaaggcggacaggtatccggtaag	ggcttccaaaagctgcctgtctcta

	fgk	5' junction	gtcaagaccattgttgaagaagt	tcagcttgccgtaggtggcatcgc
		3' junction	ggcgcgtcccattcgccatt	agacctcaagactccaaggcaacg
Southern blotting	*ACTB*	Outer probe	cgtgttctttgcactttctgcatgtc	tctccttaatgtcacgcacgatttcc
		mNG probe	gcaccggcaatcccaatgac	gtttgccgtttccggtggtg

Supplementary Table 7. Primers for PCR at the off-target sites.

	Locus	Forward primer (5′->3′)	Reverse primer (5′->3′)
TAL-PITCh at the *FBL* locus in HEK293T cells	TAL-OT1	cgtgctgggagggaagtctgt	ggtcaggcctgaagtcgaggt
	TAL-OT2	ggcgaccaccttgcttaaagcag	gccactgcacccggccatttt
	TAL-OT3	ctcgcctctgcctgcacactac	atgcgcctgtggtctcagctactc
	TAL-OT4	tgtccctgctaactgctcctg	tgggcaaaaagagcagaactc
	TAL-OT5	cggcaatgtgttttgggccc	cagcagactctgggggaggat
	TAL-OT6	atgatggtttccagcttcatcc	gaagcagatcctgtccaaaacc
CRIS-PITCh at the *FBL* locus in HEK293T cells	CRIS-OT1A	cttctgaatcctccttccatcc	ttgattctaggccaattttgagg
	CRIS-OT2A	atgtcatgcaggattggaagac	tataagccaaacaaggcttcctg
	CRIS-OT3A	gccatctcctaactgccaatatg	atcatacagccactgagtccaca
	CRIS-OT1B	atggctgttgatctgagagtcc	ttctcttggaaaaatgcctgtg
	CRIS-OT2B	agctctgcacctaagcctatcc	gtgggggaagacctactctgtg
	CRIS-OT3B	tagggtgatcagatggcttgag	atcctgagtcgcggaaaaatac
	CRIS-OT1C	gcacatggactgtgagagtcag	tatgctgggaatggagatgaac
	CRIS-OT2C	gctttgtaaagcctccctcttg	tggtctggcctgatttagtctg
	CRIS-OT3C	cactcaccactctgtgtcttcg	acacatcagggtgaccacacac