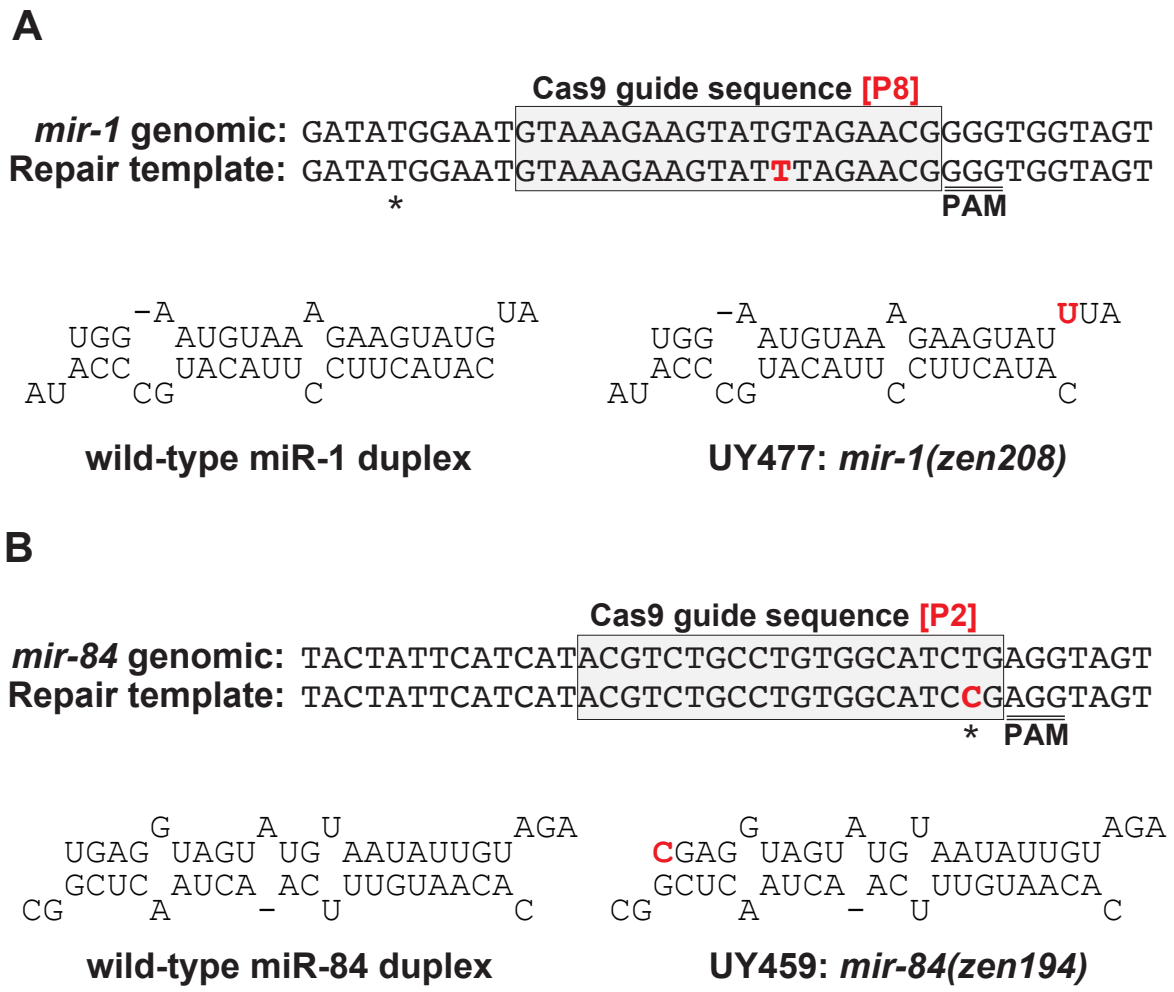

Figure S1: Examples of CRISPR-generated alleles using single nucleotide substitutions in the guide-binding region. (A) Partial alignment of the ssODN repair template used to mutate *mir-1* compared to the wild-type *mir-1* genomic sequence (Top). The resulting strain (UY477) carries a single nucleotide change of the 8th nucleotide from the 3' end of the guide sequence. (Bottom) Comparison of the wild-type *mir-1* duplex and the UY477 mutated *mir-1* duplex. (B) Partial alignment of the ssODN repair template used to mutate *mir-84* compared to the wild-type *mir-84* genomic sequence (Top). The resulting strain (UY459) contains a single nucleotide change at the 2nd nucleotide from the 3' end of the guide sequence. (Bottom) Comparison of the wild-type *mir-84* duplex and the UY459 mutated *mir-84* duplex. (A-B) Changes to the wild-type sequence are indicated by red text. The PAM sequence is double underlined, and the guide sequence is highlighted by a gray box. Asterisk indicates the 5' nucleotide of the mature microRNA guide strand. Duplexes were derived from www.mirbase.org.

Table S1. List of Strains Used in This Study

Strain	Genotype	Information
FX30240	*tmc24 [F23D12.4(tmIs1240)] X*	Dejima et al., 2018
N2	wild-type	From CGC
UY352	*tra-2(zen142) II*	PAM + *Rsa*I
UY356	*tra-2(zen145) II*	*Rsa*I only, no blocking
UY362	*tra-2(zen151) II*	P2 + *Rsa*I
UY364	*tra-2(zen153) II*	P11 + *Rsa*I
UY370	*tra-2(zen157) II*	P20 + *Rsa*I
UY386	*let-7(zen162)/tmc24 X*	PAM
UY389	*let-7(zen165)/tmc24 X*	No blocking
UY392	*let-7(zen168)/tmc24 X*	*let-7(n2853*-equivalent)
UY440	*ndf51 V; let-7(zen171) mir-84(n4037)/tmc24 X*	*let-7* family mutant
UY459	*mir-84(zen194) X*	P2, see Figure S1
UY477	*mir-1(zen208) I*	P8, see Figure S1
VT1066	*ndf51 V; mir-84(n4037) X*	Abbott et al., 2005

Table S2. List of Oligonucleotides Used in This Study

Oligonucleotide	Sequence (5'-3')
tra-2 crRNA	AUUUUACUAACAGAUAAUAA
let-7 crRNA	UGUGGAUCCGGUGAGGUAGU
dpy-10 crRNA	UUCUGCUGUCUUGAUUGACG
tra-2 PAM ssODN	CCTACAAATTATTTTGAGTAATATTTTTATTTACGATATTATTTTACTAACAGATAATAATCGTACAATGAAATTGAAATACAATAAACTTCTCGTTTCGGTGG
tra-2 No-Block ssODN	CCTACAAATTATTTTGAGTAATATTTTTATTTACGATATTATTTTACTAACAGATAATAATGGTACAATGAAATTGAAATACAATAAACTTCTCGTTTCGGTGG
tra-2 P2 ssODN	CCTACAAATTATTTTGAGTAATATTTTTATTTACGATATTATTTTACTAACAGATAATTATGGTACAATGAAATTGAAATACAATAAACTTCTCGTTTCGGTGG
tra-2 P2+PAM ssODN	CCTACAAATTATTTTGAGTAATATTTTTATTTACGATATTATTTTACTAACAGATAATTATCGTACAATGAAATTGAAATACAATAAACTTCTCGTTTCGGTGG
tra-2 P5 ssODN	CCTACAAATTATTTTGAGTAATATTTTTATTTACGATATTATTTTACTAACAGATTATAATGGTACAATGAAATTGAAATACAATAAACTTCTCGTTTCGGTGG

tra-2 P8 ssODN	CCTACAAATTATTTTGAGTAATATTTTTATTTACGATATTATTT TACTAACACATAATAATGGTACAATGAAATTGAAATACAATA AACTTCTCGTTTCGGTGG
tra-2 P11 ssODN	CCTACAAATTATTTTGAGTAATATTTTTATTTACGATATTATTT TACTATCAGATAATAATGGTACAATGAAATTGAAATACAATA AACTTCTCGTTTCGGTGG
tra-2 P11+PAM ssODN	CCTACAAATTATTTTGAGTAATATTTTTATTTACGATATTATTT TACTATCAGATAATAATCGTACAATGAAATTGAAATACAATA AACTTCTCGTTTCGGTGG
tra-2 P14 ssODN	CCTACAAATTATTTTGAGTAATATTTTTATTTACGATATTATTT TAGTAACAGATAATAATGGTACAATGAAATTGAAATACAATA AACTTCTCGTTTCGGTGG
tra-2 P17 ssODN	CCTACAAATTATTTTGAGTAATATTTTTATTTACGATATTATT ATACTAACAGATAATAATGGTACAATGAAATTGAAATACAAT AAACTTCTCGTTTCGGTGG
tra-2 P20 ssODN	CCTACAAATTATTTTGAGTAATATTTTTATTTACGATATTITTT TACTAACAGATAATAATGGTACAATGAAATTGAAATACAATA AACTTCTCGTTTCGGTGG
tra-2 P20+PAM ssODN	CCTACAAATTATTTTGAGTAATATTTTTATTTACGATATTITTT TACTAACAGATAATAATCGTACAATGAAATTGAAATACAATA AACTTCTCGTTTCGGTGG
let-7 PAM ssODN	GATTGGTGGACGGTCTACACTGTGGATCCGGTGAGGTAGT ACGTTGTATAGTTTGGAATATTACCACCGGTGAACTATGCA

let-7 no-Block ssODN	GATTGGTGGACGGTCTACACTGTGGATCCGGTGAGGTAGTAGGTTCTATAGTTTGGAATATTACCACCGGTGAACTATGCA
let-7(n2853) ssODN	GATTGGTGGACGGTCTACACTGTGGATCCGGTGAGATAGTAGGTTGTATAGTTTGGAATATTACCACCGGTGAACTATGCA
tra-2 RsaI Reversion ssODN	CCTACAAATTATTTTGAGTAATATTTTTATTTACGATATTATTTTACTAACAGATAATAATGGAACAATGAAATTGAAATACAATAAACTTCTCGTTTCGGTGG
dpy-10(cn64) ssODN	CACTTGAACTTCAATACGGCAAGATGAGAATGACTGGAAACCGTACCGCATGCGGTGCCTATGGTAGCGGAGCTTCACATGGCTTCAGACCAACAGCCTAT

Table S3 (Related to Figure 2). Editing Rates of *tra-2* in F2 Generation Dumpy (top) and Non-dumpy (bottom) Animals.

	Blocking Mutation	HDR Edited (%)	Indel (%)	Not Edited (%)
Genotypes of F2 Dumpy	No-blocking	19 (21.8)	8 (9.2)	60 (69.0)
	PAM	42 (95.4)	1 (2.3)	1 (2.3)
	P2	40 (80.0)	1 (2.0)	9 (18.0)
	P2+PAM	45 (93.8)	0 (0.0)	3 (6.2)
	P5	50 (75.8)	1 (1.5)	15 (22.7)
	P8	22 (67.6)	4 (11.8)	7 (20.6)
	P11	28 (75.7)	3 (8.1)	6 (16.2)
	P11+PAM	31 (88.6)	2 (5.7)	2 (5.7)
	P14	39 (59.1)	8 (12.1)	19 (28.8)
	P17	23 (54.8)	5 (11.8)	14 (33.4)
	P20	28 (66.6)	4 (9.6)	10 (23.8)
	P20+PAM	34 (89.5)	0 (0.0)	4 (10.5)
Genotypes of F2 non-Dumpy	No-blocking	2 (2.3)	6 (6.9)	79 (90.8)
	PAM	3 (6.8)	6 (13.6)	35 (79.6)
	P2	7 (14.0)	9 (18.0)	34 (68.0)
	P2+PAM	5 (10.4)	5 (10.4)	38 (79.2)
	P5	4 (6.1)	5 (7.6)	57 (86.3)
	P8	2 (5.9)	2 (5.9)	30 (86.4)

P11	5 (13.5)	1 (2.7)	31 (83.8)
P11+PAM	5 (14.3)	4 (11.4)	26 (74.3)
P14	1 (1.5%)	7 (10.6)	58 (87.9)
P17	3 (7.2%)	7 (16.6)	32 (76.2)
P20	6 (14.3)	6 (14.3)	30 (71.4)
P20+PAM	6 (15.8)	6 (15.8)	26 (68.4)

Table S4 (Related to Figure 2D). Paired-Genotype Analysis of F2 Dumpy and Non-dumpy Animals from Single F1 Rollers.

Dpy Genotype	Non-Dpy Genotype	No-block (%)	PAM (%)	P2 (%)	P2+PAM (%)	P5 (%)	P8 (%)	P11 (%)	P14 (%)	P17 (%)	P20 (%)	P20+PAM (%)
	HDR Edited	0 (0.0)	3 (7.0)	3 (5.4)	3 (5.7)	2 (3.0)	1 (2.8)	4 (9.3)	1 (1.5)	1 (1.8)	5 (10.4)	4 (10.3)
HDR Edited	Indel	4 (4.8)	1 (2.3)	8 (14.3)	4 (7.5)	4 (6.1)	0 (0.0)	2 (4.7)	5 (7.6)	1 (1.8)	2 (4.2)	6 (15.4)
	Not Edited	16 (19.0)	33 (76.7)	32 (57.1)	40 (75.5)	42 (63.6)	22 (61.1)	28 (56.1)	34 (51.5)	19 (34.5)	24 (50.0)	24 (61.5)
	HDR Edited	0 (0.0)	1 (2.3)	1 (1.8)	0 (0.0)	0 (0.0)	1 (2.8)	1 (2.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Indel	Indel	1 (1.2)	4 (9.3)	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.8)	0 (0.0)	1 (1.5)	4 (7.3)	2 (4.2)	0 (0.0)

	Not Edited	5	0	0	2	1	2	1	7	5	4	0
		(6.0)	(0.0)	(0.0)	(3.8)	(1.5)	(5.6)	(2.3)	(10.6)	(9.1)	(8.3)	(0.0)
	HDR	2	0	2	2	1	0	0	0	2	1	1
	Edited	(2.4)	(0.0)	(3.6)	(3.8)	(1.5)	(0.0)	(0.0)	(0.0)	(3.6)	(2.1)	(2.6)
Not Edited	Indel	4	0	3	0	1	1	0	1	2	2	1
		(4.8)	(0.0)	(5.4)	(0.0)	(1.5)	(2.8)	(0.0)	(1.5)	(3.6)	(4.2)	(2.6)
	Not Edited	52	1	7	2 (	15	8	7	17	21	8	3
		(61.9)	(2.3)	(12.5)	3.8)	(22.7)	(22.2)	(16.3)	(25.8)	(38.2)	(16.7)	(7.7)

Table S5 (Related to Figure 4A). HDR Incorporation Rates of Blocking Mutations and Non-blocking *Rsa*I Restriction Site Among HDR-edited Chromosomes

Blocking Mutation	*Rsa*I Only (%)	Blocking Only (%)	Blocking + *Rsa*I (%)
No-blocking	21 (100.0)	0 (0.0)	0 (0.0)
PAM	1 (2.2)	0 (0.0)	44 (97.8)
P2	15 (31.9)	15 (31.9)	17 (36.2)
P2+PAM	0 (0.0)	0 (0.0)	52 (100.0)
P5	1 (1.8)	40 (74.1)	13 (24.1)
P8	2 (8.0)	13 (56.0)	8 (36.0)
P11	0 (0.0)	21 (63.6)	12 (36.4)
P11+PAM	0 (0.0)	10 (27.8)	26 (72.2)
P14	8 (20.5)	22 (56.4)	9 (23.1)
P17	4 (15.4)	16 (61.6)	6 (23.0)
P20	4 (12.5)	17 (53.1)	11 (34.4)
P20+PAM	0 (0.0)	11 (28.2)	28 (71.8)

Table S6 (Related to Figure 3B). Effect of Distance to Cut Site on Incorporation of Single Nucleotide Guide Substitutions.

Blocking Mutation	PAM Only (%)	Blocking Only (%)	Blocking + *Rsa*I (%)
PAM	45 (100.0)	0 (0.0)	0 (0.0)
P2+PAM	0 (0.0)	0 (0.0)	52 (100.0)
P11+PAM	26 (72.2)	4 (11.2)	6 (16.6)
P20+PAM	28 (71.8)	0 (0.0)	11 (28.2)

Table S7 (Related to Figure 4). Blocking Efficacy of Single Nucleotide Substitutions

	Blocking Mutation	Both Reverted (%)	Heterozygous (%)	Not Reverted (%)
F1 Rol	No-blocking	0 (0.0)	25 (50.0)	25 (50.0)
	PAM	0 (0.0)	0 (0.0)	56 (100.0)
	P2	0 (0.0)	1 (1.8)	56 (97.2)
	P11	0 (0.0)	3 (5.4)	53 (94.6)
	P20	0 (0.0)	7 (12.7)	48 (87.3)
F1 Dpy	No-blocking	5 (10.0)	22 (44.0)	23 (46.0)
	PAM	0 (0.0)	0 (0.0)	50 (100.0)
	P2	0 (0.0)	3 (5.3)	53 (94.7)
	P11	0 (0.0)	3 (5.3)	53 (94.7)
	P20	1 (1.8)	12 (21.8)	42 (76.4)

Table S8 (Related to Figure 6). Editing Rates of *let-7* in F2 Generation non-Venus Animals.

Blocking Mutation	HDR Edited (%)	Indel (%)	Not Edited (%)
No-blocking	1 (3.7)	11 (40.7)	15 (55.6)
PAM	17 (60.7)	3 (10.7)	8 (28.6)
n2853 [P6]	17 (56.7)	7 (23.3)	6 (20.0)