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.
| 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                        |
| UY356    | *tra-2(zen145) II*                           | Rsa only, no blocking            |
| UY362    | *tra-2(zen151) II*                           | P2 + Rsa                         |
| UY364    | *tra-2(zen153) II*                           | P11 + Rsa                        |
| UY370    | *tra-2(zen157) II*                           | P20 + Rsa                        |
| 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              |
| Oligonucleotide       | Sequence (5’-3’)                                                                                                                                                                                                 |
|-----------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| **tra-2 crRNA**       | AUUUUACUAACAGAUAUAA                                                                                                           |
tra-2 P8 ssODN
CCTACAAATTATTTTGAGTAATATTTTTTATTTTACGATATTATTT
TACTAACA\_CATAATAATGG\_TACAATGAAATTGAAATACAATA
AACTTCTCGTTTCGGGTG

tra-2 P11 ssODN
CCTACAAATTATTTTGAGTAATATTTTTTATTACGATATTATTTT
TACTA\_CAGATAATAATGG\_TACAATGAAATTGAAATACAATA
AACTTCTCGTTTCGGGTG

tra-2 P11+PAM ssODN
CCTACAAATTATTTTGAGTAATATTTTTTATTACGATATTATTTT
TACTA\_CAGATAATAATCG\_TACAATGAAATTGAAATACAATA
AACTTCTCGTTTCGGGTG

tra-2 P14 ssODN
CCTACAAATTATTTTGAGTAATATTTTTTATTACGATATTATTTT
TAGTAACAGATAATAATGG\_TACAATGAAATTGAAATACAATA
AACTTCTCGTTTCGGGTG

tra-2 P17 ssODN
CCTACAAATTATTTTGAGTAATATTTTTTATTACGATATTATTTT
A\_TACTAACAGATAATAATGG\_TACAATGAAATTGAAATACAATA
AACTTCTCGTTTCGGGTG

tra-2 P20 ssODN
CCTACAAATTATTTTGAGTAATATTTTTTATTACGATATTATTTT
TACTAACAGATAATAATGG\_TACAATGAAATTGAAATACAATA
AACTTCTCGTTTCGGGTG

tra-2 P20+PAM ssODN
CCTACAAATTATTTTGAGTAATATTTTTTATTACGATATTATTTT
TACTAACAGATAATAATCG\_TACAATGAAATTGAAATACAATA
AACTTCTCGTTTCGGGTG

let-7 PAM ssODN
GATTGGTGGACGGTCTACACTGTTGATCCCGGTGAGGTAGT
ACGTGTATAGTTTTGGAATATTACACCACGGTGAACATGCA
|       | DNA Sequence                                      |
|-------|--------------------------------------------------|
| let-7 no-Block | GATTGGTGACGGTCTACACTGTGGATCCGGTGAGGTA CGT    |
| ssODN | AGGTTCTATAGGGGAATATTACCACCGGTGTAAGT            |
| let-7(n2853) ssODN | GATTGTGGACGGTCTACACTGTGGATCGGTAGT            |
|       | AGGTTGTATAGGGGAATATTACCACCGGTGTAAGT            |
| tra-2 RsaI Reversion | CCTACAAATTATTTTGAAGTAATATATTTTTTTTACTGATATTATTT |
| ssODN | TACTAAGAGATAATAATGGGAACAAATGGAATATGAAAAATA    |
|       | ACTTCTCGTTTCTGGGTGG                                  |
| dpy-10(cn64) ssODN | CACTTGAACTTCAATACCGCAAGATGAGAATGACTGGAAAC          |
|       | CGTACCACCATGCCGATGTAGGCTATGGTAGGGAGGCTACATGA        |
|       | GCTTCAGACCAACAGCCTAT                                   |
Table S3 (Related to Figure 2). Editing Rates of *tra-2* in F2 Generation Dumpy (top) and Non-dumpy (bottom) Animals.

| Genotypes of F2 Dumpy | No-blocking | PAM | P2 | P2+PAM | P5 | P8 | P11 | P11+PAM | P14 | P17 | P20 | P20+PAM |
|-----------------------|-------------|-----|----|--------|----|----|-----|---------|-----|-----|-----|---------|
| HDR Edited (%)        | 19 (21.8)   | 42 (95.4) | 40 (80.0) | 45 (93.8) | 50 (75.8) | 22 (67.6) | 28 (75.7) | 31 (88.6) | 39 (59.1) | 23 (54.8) | 28 (66.6) | 34 (89.5) |
| Indel (%)             | 8 (9.2)     | 1 (2.3) | 1 (2.0) | 0 (0.0) | 1 (1.5) | 4 (11.8) | 3 (8.1) | 2 (5.7) | 8 (12.1) | 5 (11.8) | 4 (9.6) | 0 (0.0) |
| Not Edited (%)        | 60 (69.0)   | 1 (2.3) | 9 (18.0) | 3 (6.2) | 15 (22.7) | 7 (20.6) | 6 (16.2) | 2 (5.7) | 19 (28.8) | 14 (33.4) | 10 (23.8) | 4 (10.5) |

| Genotypes of F2 non-Dumpy | No-blocking | PAM | P2 | P2+PAM | P5 | P8 |
|----------------------------|-------------|-----|----|--------|----|----|
| HDR Edited (%)             | 2 (2.3)     | 3 (6.8) | 7 (14.0) | 5 (10.4) | 4 (6.1) | 2 (5.9) |
| Indel (%)                  | 6 (6.9)     | 6 (13.6) | 9 (18.0) | 5 (10.4) | 5 (7.6) | 2 (5.9) |
| Not Edited (%)             | 79 (90.8)   | 35 (79.6) | 34 (68.0) | 38 (79.2) | 57 (86.3) | 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.

| Genotype Block | Dpy | No-Dpy | P2 | P2+PAM | P14 | P17 | P20 | P20+PAM |
|----------------|-----|--------|----|--------|-----|-----|-----|---------|
| HDR Editted     | 0   (0.0) | 3   (7.0) | 3   (5.4) | 3   (5.7) | 2   (3.0) | 1   (2.8) | 1   (1.5) | 4 (10.3) |
| HDR Not Editted | 16  (19.0) | 33  (76.7) | 32  (57.1) | 40  (75.5) | 42  (63.6) | 22  (61.1) | 28  (56.1) | 4 (15.4) |

Indel Editted:

| HDR Editted     | 0   (0.0) | 1   (2.3) | 0   (0.0) | 0   (0.0) | 0   (0.0) | 1   (2.8) | 0   (0.0) | 1   (2.3) |
| HDR Not Editted | 1   (1.2) | 4   (9.3) | 0   (0.0) | 0   (0.0) | 0   (0.0) | 1   (2.8) | 0   (0.0) | 1   (1.5) |

Block Editted:

| HDR Editted     | 0   (0.0) | 1   (1.8) | 5   (10.4) | 4 (10.3) |
| HDR Not Editted | 1   (1.8) | 5   (7.6) | 1   (1.5) | 4 (10.3) |
|          | 7.7 | 16.7 | 38.2 | 26.3 | 16.3 | 22.2 | 22.7 | 3.8 | 12.5 | 3.3 | 61.9 |
|----------|-----|------|------|------|------|------|------|-----|------|-----|------|
| 3        | 52  | 7    | 15   | 8    | 17   | 21   | 8    | 3   | 1    | 1   | 4    |
| 2        | 0.0 | 4.2  | 1.5  | 2.8  | 0.0  | 1.5  | 3.6  | 1   | 2    | 2   | 1    |
| 0        | 0   | 1    | 1    | 0    | 1    | 1    | 2    | 1   | 1    | 1   | 1    |

|          | 2.8 | 3.6 | 15.2 | 22.7 | 3.8 | 1.5 | 0.0 | 0.0 | 1.5 | 3.6 | 2.8 |
|----------|-----|-----|------|------|-----|-----|-----|-----|-----|-----|-----|
| 2        | 1   | 2   | 2    | 0    | 0   | 1   | 1   | 1   | 1   | 1   | 1   |

|          | 6.0 | 0.0 | 8.3 | 9.1 | 10.6 | 2.3 | 5.6 | 1.5 | 3.8 | 0.0 | 5.0 |
|----------|-----|-----|-----|-----|------|-----|-----|-----|-----|-----|-----|
| 0        | 5   | 0   | 4   | 7   | 1    | 2   | 1   | 2   | 0   | 0   | 0   |

| HDR      | Not Edited | Indel | Edited | Not Edited |
|----------|------------|-------|--------|------------|
| 2        | 0          | 2     | 0      | 0          |

Not Edited
Table S5 (Related to Figure 4A). HDR Incorporation Rates of Blocking Mutations and Non-blocking RsaI Restriction Site Among HDR-edited Chromosomes

| Blocking Mutation | Rsa Only (%) | Blocking Only (%) | Blocking + Rsa (%) |
|-------------------|--------------|-------------------|-------------------|
| 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 + Rsal (%) |
|-------------------|--------------|-------------------|--------------------|
| 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)       |