MiR-1-1: mutations in 64 of 66 sequences (~97.0%)

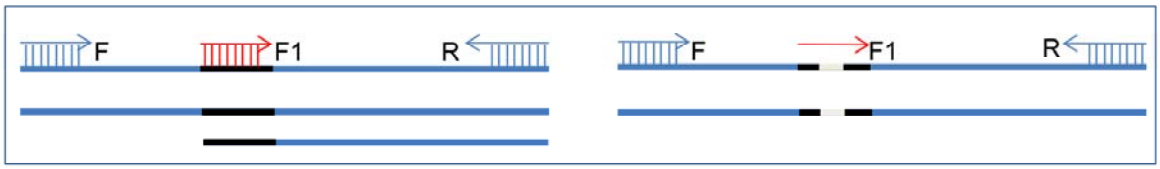
TATGAACAAGAGCAGCTAT**GGAATG**TAAAGAAGTATGTATCCCAGGTGA	WT	
TATGAACAAGAGCAGCTATGGAA---------->	Δ101	
TAT----------GAAGTATGTATCCCAGGTGA	Δ26	
TATG----------TAAAGAAGTATGTATCCCAGGTGA	Δ21	
TATGAACAAGA----------actatgttTCCCAGGTGA	Δ20(Δ28,+8)	
TATGAACAAGAGCAGCTATG----------TATCCCAGGTGA	Δ17	
TATGAACAAGAGCAGCTATGGA----------GTATGTATCCCAGGTGA	Δ10	X2
TATGAACAAGAGCAGC----------AAAGAAGTATGTATCCCAGGTGA	Δ10	
TATGAACAAGAGCAG----------TAAAGAAGTATGTATCCCAGGTGA	Δ10	
TATGAACAAGAGCAGCTATGGA---------AGTATGTATCCCAGGTGA	Δ9	X19
TATGAACAAGAGCAGCTAT---------AGAAGTATGTATCCCAGGTGA	Δ9	
TATGAACAAGAGCAGCTAT--------AAGAAGTATGTATCCCAGGTGA	Δ8	
TATGAACAAGAGCAGCTATGGA--------gAGTATGTATCCCAGGTGA	Δ8 (Δ9,+1)	
TATGAACAAGAGCAGCTATGG-------AGAAGTATGTATCCCAGGTGA	Δ7	X2
TATGAACAAGAGCAGCTATG-------AAGAAGTATGTATCCCAGGTGA	Δ7	
TATGAACAAGAGCAGCTAT-------AAAGAAGTATGTATCCCAGGTGA	Δ7	X2
TATGAACAAGAGCAGCTATGGAA------GAAGTATGTATCCCAGGTGA	Δ6	X13
TATGAACAAGAGCAGCTATGGA------tGAAGTATGTATCCCAGGTGA	Δ6 (Δ7,+1)	
TATGAACAAGAGCAGCTATGGA------AGAAGTATGAATCCCAGGTGA	Δ6	
TATGAACAAGAGCAGCTATG------AAAGAAGTATGTATCCCAGGTGA	Δ6	X2
TATGAACAAGAGCAGCTATGG-----aagaagaTATGTATCCCAGGTGA	Δ5 (Δ12,+7)	
TATGAACAAGAGCAGCTATGGAA----AAGAAGTATGTATCCCAGGTGA	Δ4	X2
TATGAACAAGAGCAGCTATGC----TAAAGAAGTATGTATCCCAGGTGA	Δ4	
TATGAACAAGAGCAGCTATGGAATGTAA-GAAGTATGTATCCCAGGTGA	Δ1	
TATGAACAAGAGCAGCTATGGAAgtatgtAAGAAGTATGTATCCCAGGT	+2 (Δ6,+8)	
TATGAACAAGAGCAGCTATGagaagtagaacctAAGAAGTATGTATCCC	+6 (Δ7,+13)	
TATGAACAAGAGCAGCTATGGAAagtatgaaaaGTAAAGAAGTATGTAT	+9 (Δ1,+10)	
TATGAACAAGAGCAGCTATGGAAgtatgtatcccaggtgaGAAGTATGT	+11(Δ6,+17)	
TATGAACAAGAGCAGCTATGGAtgtatgtagtatgtattgtatggatgt	+21(Δ6,+27)	
TATGAACAAGAGCAGCTAgtatgaagtatgtataattggagcagctagt	+21(Δ10,+31)	

Figure S1. Targeted disruption of zebrafish *miR-1-1*. The TALEN binding sites are shown in yellow background. DNA sequence encoding the mature miR-1-1 is underlined, with the seed sequence in bold. The sizes of the insertions (+) or deletions (Δ) and the number of times each mutant allele appearing are shown on the right side of the mutant allele.

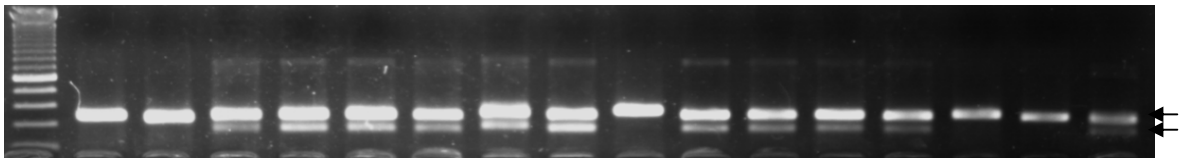
A Chromosome 1: 2805894-2807334

CGTGTGACATGTGCTTTGCCATGAGGCCCTCGCTTTTGTAGTATAGAAAGTCAACCCCATGTACCATCAATATCTATTG
TATTTGAATAATTCTACAGGTAACAAACTAAC----------1.2kb----------
TCATTTATTATCTTAACACTGTAATTTTTACACCATGCAAGGCATGATTAACATTTAGACACTTGTTTGCTATGCTGTT
AAGTGTGTTAATGACTTGGGTTTTATTAAACCAACATAATCGGACAAATCAACATAGTCTATCAGCTGCTCTTCAGATT
TATCAAATCCAATTATAAAAATGCAGAGTAAGCCGAGTAATCTAGC

B



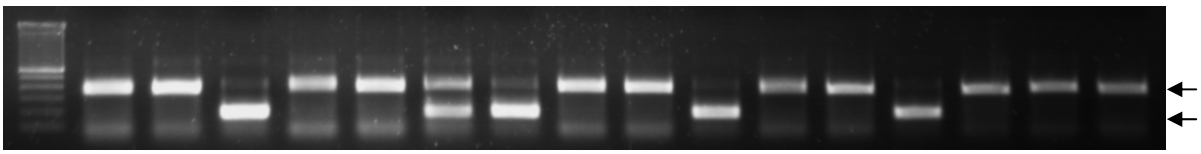
C



D

```
TCAACCCCATGTACCATCAATATCTATTGTATTTGAATAATTCTACAGGTA  WT
TCAACCCCATGTACCATCAAT----------TTGAATAATTCTACAGGTA  Δ11
TCAACCCCATGTACCATCAATAT---------TTGAATAATTCTACAGGTA  Δ9
TCAACCCCATGTACCATCAATAT-----GTATTTGAATAATTCTACAGGTA  Δ5  X2
TCAACCCCATGTACCATCAATATCTcaattgtattcaaATTGTATTTGAAT  +13 (Δ26, +13)
```

E



F

```
TATTATCTTAACACTGTAATTTTTACACCATGCAAGGCATGATTAACATTTAGA    WT
TATTATCTTA----------------------gGGCATGATTAACATTTAGA    Δ24
TATTATCTTAACACTGTAATT----------CAAGGCATGATTAACATTTAGA    Δ11
TATTATCTTAACACTGTAATTTTTA-------CAAGGCATGATTAACATTTAGA    Δ7    X3
TATTATCTTAACACTGTAATTTTT-------GCAAGGCATGATTAACATTTAGA    Δ7
TATTATCTTAACACTGTAATTTTT----CATGCAAGGCATGATTAACATTTAGA    Δ4
TATTGTCTTAACACTGTAATT----gcaaATGCAAGGCATGATTAACATTTAGA    Δ4 (Δ8,+4)
TATTATCTTAACACTGTAATTTTTACAC-ATGCAAGGCATGATTAACATTTAGA    Δ4
TATTATCTTAACACTGTAATTTTTACAaattatgaATGCAAGGCATGATTAACA    +6 (Δ2,+8)
TATTATCTTAACACTGTAATTTTTtctaaatgttaatCATGCAAGGCATGATTA    +9 (Δ4,+13)
```

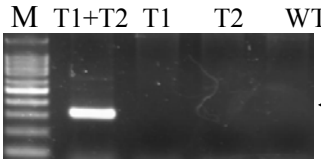
Figure S2. Precise large genomic deletion of *miR-17-92* cluster. (**A**) The target genomic region of the zebrafish *miR-17-92* cluster. The TALEN binding sites are shown in color. Primers to detect fragment deletion are underlined. (**B**) Schematic diagram of PCR-based mutation detection. Three primers were designed and used in the PCR reactions. F1 is located at the spacer region. In single clones containing the wild-type sequence, two bands would be amplified. Whereas in clones containing the mutated sequence, one single band would be amplified because changes in the spacer sequence destroy the binding site of the F1 primer. (**C** and **E**) Gel picture of PCR-based detection of T1 (**C**) and T2 (**E**) targeted loci mutations. The two expected bands in the wild-type clones are indicated by arrows. (**D** and **F**) Sequencing results confirming T1 (**D**) and T2 (**F**) targeted loci mutations. Deletions are indicated by dash lines and insertions are

indicated by lowercase letters. The sizes of the insertions (+) or deletions (Δ) and the number of times each mutant allele appearing are shown on the right side of the mutant allele.

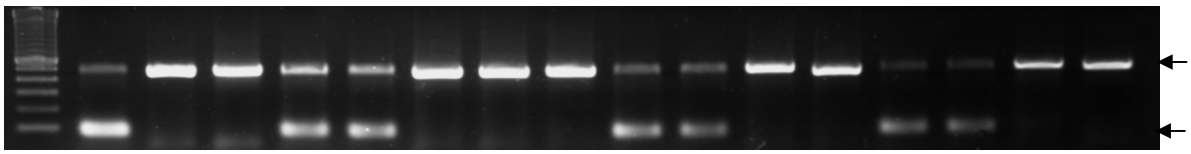
A Chrosome 4:27939580-28019747

GTCTGAAATGTCACATTAAAGGATTAATTTCAACAGCATCGCTGTAACAAGTCAATAGGCCATATGCTGTTTGCTGTCATAAGACT
ACAACCATGTTCTACATTTTGTTTTTTATCAACTTTGTGAATCTTGACACATATTGATCATTACTGCTAACGTTATACATATAACA
TGCAGGCTTCTGAATAGTCACAGTGTCTGTGTTTAATTAGAACAATGTTAAAATACAGATGTAAATAAATAAGCATGAGCCTTTCA
ATTACGGTTCATGTCCAGTAGAAAAACTAAACAAATGATTTG----------79.8kb----------
TAGATATATCAGCATTGGTCCCGATAGACTCTGCTAGAGGTTCAATAAATAATGTGAACTACAAGGGTGATAGGGGACAACCCTGT
CTGCAACCTATGCGTACTGGAAATCCTTAAGAAGTCAAACCATTTGTATTGACCGAGGCAACAGAATCTGCATAGAAGATACTTAA
TAACATTAATAAAATTAGAACTTAGTCCAAATTTTTTCAAAGCTAAAAACAAGAAAGGCCACTCTACACAGTAAAAATCCTTTTTG
GCTTCCAGAGATAATATCATTAATGTCTTTTTCTGTGTATGAAAATAATGTGGAGACGTACATTATCCAATCCATGCCTGGATTTC
ACAAAGTCTGCTATTTATAACCTTAGGAAGGATAGATTCCAATCTGCGAGCCA

B



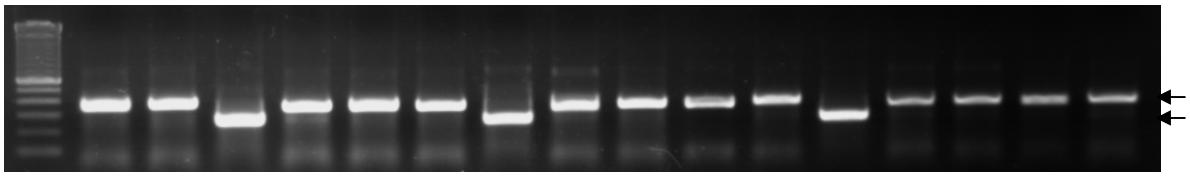
C miR-430 T1 locus



D

TACATCTGTATTTTAACATTGTTCTAATTAAAACACAGACACTGTGACTATTCAGA WT
TACATCTGTATTTTAACA----------CAGACACTGTGACTATTCAGA Δ16
TACATATGTATTTTAACATT----------AACAGACACTGTGACTATTCAGA Δ12
TACATCTGTATTTTAACATTGTTCT----------GACACTGTGACTATTCAGA Δ11
TACATCTGTATTTTAACATTGTTCTA----------GACACTGTGACTATTCAGA Δ10
TACATCTGTATTTTAACATTGTTT------AACACAGACACTGTGACTATTCAGA Δ6
TACATCTGTATTTTAACATTGTT------AAACACAGACACTGTGACTATTCAGA Δ6
TACATCTGTATTTTAACATTGTTCTgtcaatattttaACAGACACTGTGACTATT +4 (Δ8, +12)
TACATCTGTATTTTAACATTGTTCatctgtattttAACACAGACACTGTGACTAT +5 (Δ6, +11)
TACATCTGTATTTTAACATTgttcattgcaattccatgccaaatgggttcagtct +48(Δ8, +56)
tgcttgttgtacgtgtccttaTAAACACAGACACTGTGACTATTCAGA

E



F

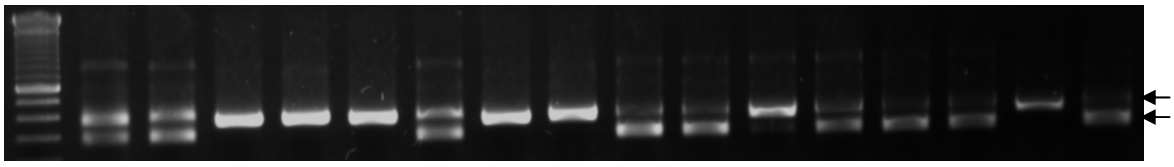
TCCTTAAGAAGTCAAACCATTTGTATTGACCGAGGCAACAGAATCTGCATAGA WT
TCCTTAAGAAGTCAAACCA----------GGCAACAGAATCTGCATAGA Δ14
TCCTTAAGAAGTCAAACC----------GAGGCAACAGAATCTGCATAGA Δ13
TCCTTAAGAAGTCAAACCATTTG----------GCAACAGAATCTGCATAGA Δ11
TCCTTAAGAAGTCAAACCAT----------CGAGGCAACAGAATCTGCATAGA Δ10
TCCTTAAGAAGTCAAACCA----------CCGAGGCAACAGAATCTGCATAGA Δ10
TCCTTAAGAAGTCAAACCA---------ACCGAGGCAACAGAATCTGCATAGA Δ9
TCCTTAAGAAGTCAAACCAT--------ctctatGCAACAGAATCTGCATAGA Δ9 (Δ15,+6)
TCCTTAAGAAGTCAAACCATTTGT-------ttGGCAACAGAATCTGCATAGA Δ7 (Δ10,+7)
TCCTTAAGAAGTCAAACCAT-----gttgCCGAGGCAACAGAATCTGCATAGA Δ5 (Δ9,+4)
TCCTTAAGAAGTCAAACCATTTGTAtTTGACCGAGGCAACAGAATCTGCATAG +1
TCCTTAAGAAGTCAAACCATTTGaggcaacacACCGAGGCAACAGAATCTGCA +4 (Δ5,+9)

Figure S3. Precise large genomic deletions of *miR-430* cluster. (**A**) The target genomic region of the zebrafish *miR-430* cluster. The TALEN binding sites are shown in color. Primers to detect fragment deletion are underlined. (**B**) Genomic PCR detection of the *miR-430* cluster deletion. Gel picture showing 40 cycles PCR amplification of genomic DNA isolated from the pooled zebrafish embryos microinjected with the dual TALEN or wildtype control. (**C** and **E**) Gel picture of PCR-based detection of T1(**C**) and T2 (**E**) targeted loci mutations. The two expected bands in the wildtype clones are indicated by arrows. (**D** and **F**) Sequencing results confirming T1 (**D**) and T2 (**F**) targeted loci mutations. Deletions are indicated by dash lines and insertions are indicated by lowercase letters. The sizes of the insertions (+) or deletions (Δ) and the number of times each mutant allele appearing are shown on the right side of the mutant allele.

A Chromsome 14: 48575037-48564858

ACACAAACAGGCTAAGATGGCTAGTCTTTCTTGTTTTTTAAGTGTATTTGTGTTCCCATCCCCCTTCCCTTCACATTAGC
AGTCTCGTCAGCACAAATACCAAAAGGAGACGTGCTGGAGGTCTCGTTTACCCCGCACCCACCCGTTTACTGTCCAAAGCGAC
CAAGAAACACAGTTTCCTTCAATATATCCCCATTTATTAACATTAAAATCACTTT----------9.0kb----------
CAAATCGTGCTCTGGTTCGTATACAAAAGTCAAATCTCTTTTGTAATCTTATACAACATTTATCTATTCTCTGCCTACTTGTT
TTAAGGTAACATTCATGTGAGAACATTTATTGGGGTTTGTTTGAATGTCCATGGGCATTGAAAATAATGGGAAATTGATAA
AGGGTTCGTTTCTCAGAACGATAACCTGTTACAAT

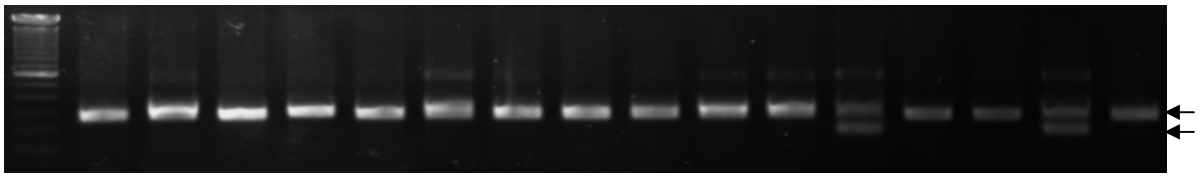
B



C

```
TAGCAGTCTCGTCAGCACAAATACCAAAAGGAGACGTGCTGGAGGTCTCGTTTA WT
TA----------------------------------------GTCTCGTTTA Δ42
TAGCAGTCTCGTCAGCACAAATACC-----------TGCTGGAGGTCTCGTTTA Δ11
TAGCAGTCTCGTCAGCACAAATA--------tgtCGTGCTGGAGGTCTCGTTTA Δ8
TAGCAGTCTCGTCAGCACAAATACCAA--GGAGACGTGCTGGAGGTCTCGTTTA Δ2
TAGCAGTCTCGTCAGCACAAATACCgtgcaataaACGTGCTGGAGGTCTCGTTT +1(Δ8,+9)
```

D



E

```
TATCTATTCTCTGCCTACTTGTTTTAAGGTAACATTCATGTGAGAACATTTA WT
TATCTATTCTCTGCCTACTTGT--------AACATTCATGTGAGAACATTTA Δ8 X2
TATCTATTCTCTGCCTACTTGTTT--------CATTCATGTGAGAACATTTA Δ8 X2
TATCTATTCTCTGCCTACTTGTTT-----TAACATTCATGTGAGAACATTTA Δ5
TATCTATTCTCTGCCaacatgtaatgtactctGGTAACATTCATGTGAGAAC +5 (Δ12,+17)
TATCTATTCTCTGCCTACTTGTTTcatgtttctctgcctacttCATTCATGT +11(Δ8,+19)
```

Figure S4. Precise large genomic deletions of *malat1*. (**A**) The target genomic region of the zebrafish *malat1*. The TALEN binding sites are shown in color. Primers to detect fragment deletion are underlined. (**B** and **D**) Gel picture of PCR-based detection of T1 (**B**) and T2 (**D**) targeted loci mutations. The two expected bands in the wildtype clones are indicated by arrows. (**C** and **E**) Sequencing results confirming T1 (**C**) and T2 (**E**) targeted loci mutations. Deletions are indicated by dash lines and insertions are indicated by lowercase letters. The sizes of the insertions (+) or deletions (Δ) and the number of times each mutant allele appearing are shown on the right side of the mutant allele.

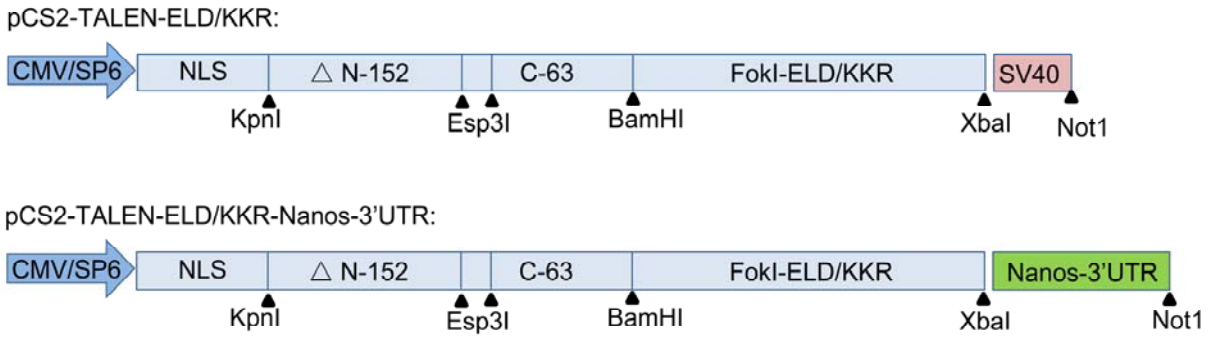


Figure S5. Incorporation of nanos-3'UTR into the pCS2-TALEN-ELD/KKR vector. Black triangles, endonuclease recognition sites; CMV/SP6, CMV/SP6 promoters; NLS, nuclear localization signal; SV40, SV40 polyadenylation signal sequence; △N152-Esp3I-C63, TALE scaffold with truncation of the N-terminal 152-aa and retention of C-terminal 63-aa, containing two Esp3I sites where the TALE repeats could be inserted.

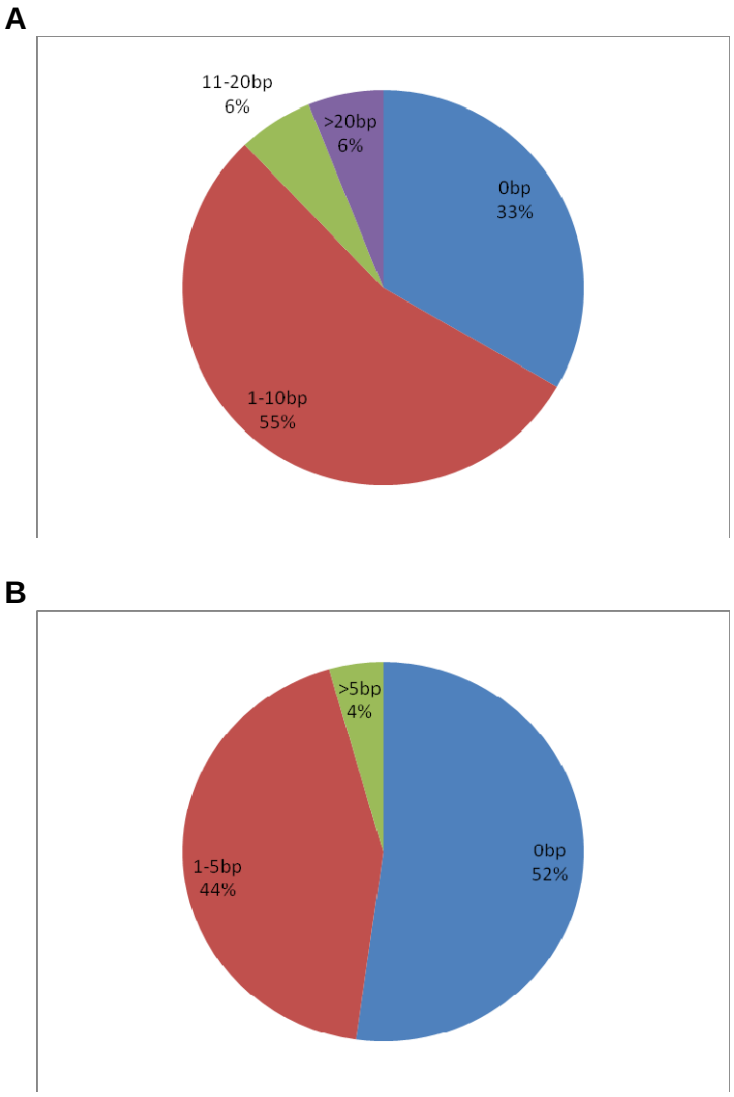


Figure S6. Proportion of the different ranges of alien nucleotides retained after large genomic deletions in somatic cells (total=33) (**A**) and germ cells (total=23) (**B**). Data were from the sequencing results of the *miR-17-92*, *miR-430* and *malat1*.

Table S1 TALEN injection records

TALEN targets	mRNA amount	Total embryos injected	Day1 #	Day2 #
MiR-1-1	200pg	33	29(0)	28(0)
MiR-1-2	200pg	41	37(5)	32(2)
MiR-430T1T2	100pg	174	156(14)	136(8)
MiR-430T1T2-nos1-3'UTR	100pg	152	146(6)	126(8)
Malat1T1T2	100pg	140	121(10)	112(9)
MiR-17-92T1T2	100pg	87	78(7)	68(4)

The total number of surviving embryos are shown, with the number of deformed embryos in brackets.

Table S2 Primers used in the present study

Primer name	Primer sequence (5′-3′)	Purpose
pCR8_F1	TTGATGCCTGGCAGTTCCCT	Amplification and sequencing of the assembled TALEs in the array plasmids.
pCR8_R1	CGAACCGAACAGGCTTATGT	
NTalF	GATGACAAGGGTACCGTG	Amplification and sequencing of the assembled TALENs.
CTalR	CTAGTTGGGATCCGGCAAC	
MiR-1-1F	GTCTAAATGCTCATATCTGAGG	*MiR-1-1* genomic fragment amplification.
MiR-1-1R	CATCAGGCCTGCGCATCACAC	
MiR-1-2F	CTAATCCACTGCATTGTGCAG	*MiR-1-2* genomic fragment amplification.
MiR-1-2R	GTGGACTGCTGGTGAAGTTACTG	
MiR-430F1	CACATATTGATCATTACTGCTAAC	MiR-430T1 and miR-430T2 genomic loci amplification. PCR-based mutation detection. MiR-430F1 + MiR-430R2 for detection of *miR-430* cluster deletion. MiR-430F3 + MiR-430R3 for amplification of reference fragment.
MiR-430R1	GTCAGGTAGGTCTTACGCACAC	
MiR-430M1	ACATTGTTCTAATTAAACACAG	
MiR-430F2	CTGACAGCAACGGGAACAGATG	
MiR-430R2	CTCGCAGATTGGAATCTATCCTTC	
MiR-430M2	CAAACCATTTGTATTGACCGAG	
MiR-430F3	CAGCATCGCTGTAACAAGTC	
MiR-430R3	GTATAACGTTAGCAGTAATG	
Malat1F1	CAAACAGGCTAAGATGGCTAGTC	Malat1T1 and Malat1T2 genomic loci amplification. PCR-based mutation detection. Malat1F1 + Malat1R2 for detection of cluster deletion; for amplification of reference fragment. Malat1F3 + Malat1R3 for amplification of reference fragment.
Malat1R1	AAGTTGTACAAACGCAGTACAG	
Malat1M1	CACAAATACCAAAAGGAGACG	
Malat1F2	AGGTGCATTTAGCTGATGTAGAG	
Malat1R2	GTAACAGGTTATCGTTCTGAG	
Malat1M2	CATGAATGTTACCTTAAAACAAG	
Malat1F3	GACTCACAACATCATGACGACAG	
Malat1R3	TCTACTGACCGGTACAAACCTG	
MiR-17-92F1	GTGACATGTGCTTTGCCATGAG	MiR-17-92T1 and miR-17-92T2 genomic loci amplification. PCR-based mutation detection. MiR-17-92F1 + MiR-17-92R2 for detection of cluster deletion; Mir-17-92F + Mir-17-92R2 for amplification of reference fragment.
MiR-17-92R1	GTTGGGTGTCTTGCCGAAGGATG	
MiR-17-92M1	CATCAATATCTATTGTATTTG	
MiR-17-92F2	GTAAAGGATTGTGGAGATTGTACC	
MiR-17-92R2	GACAAAACTTCAGCAGTGAACACAG	
MiR-17-92M2	GTAATTTTTACACCATGCAAG	
MiR-17-92F	GTACACATGCTTAATGCAGAGG	

Table S3 Mutation frequencies of the T1 and T2 targeted loci

TALEN name	Mutant alleles /total alleles	Mutation frequency (%)	TALEN name	Mutant alleles /total alleles	Mutation frequency (%)
MiR-430T1	14/32	43.8	MiR-430T2	27/32	84.4
MiR-430T1	17/32	53.1	MiR-430T2	26/32	81.3
MiR-430T1	12/32	37.5	MiR-430T2	20/32	62.5
Malat1T1	16/32	50	Malat1T2	26/32	81.3
Malat1T1	16/32	50	Malat1T2	29/32	90.6
Malat1T1	13/32	40.6	Malat1T2	29/32	90.6
MiR-17-92T1	8/32	25	MiR-17-92T2	24/31	77.4
MiR-17-92T1	3/27	11.1	MiR-17-92T2	23/31	74.2
MiR-17-92T1	4/32	12.5	MiR-17-92T2	23/32	71.9