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Supplementary Materials

CRISPR-Cas13-mediated RNA editing in the silkworm *Bombyx mori*

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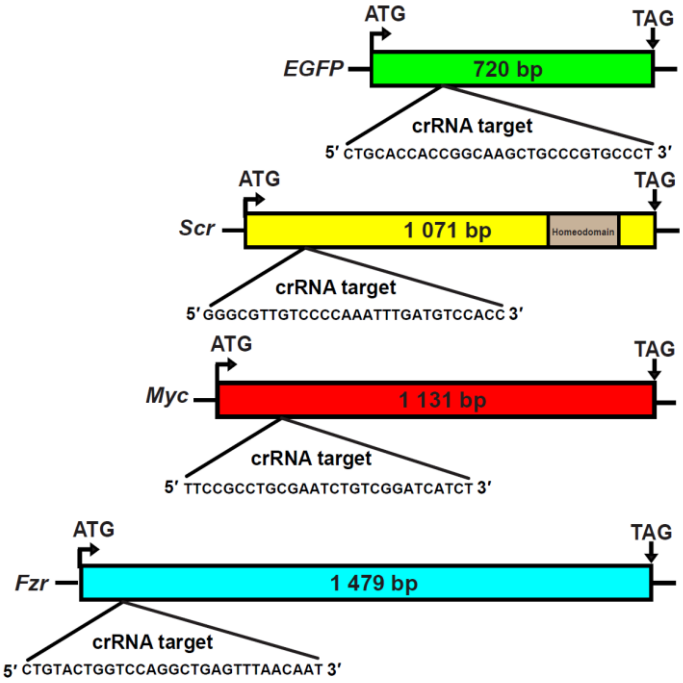
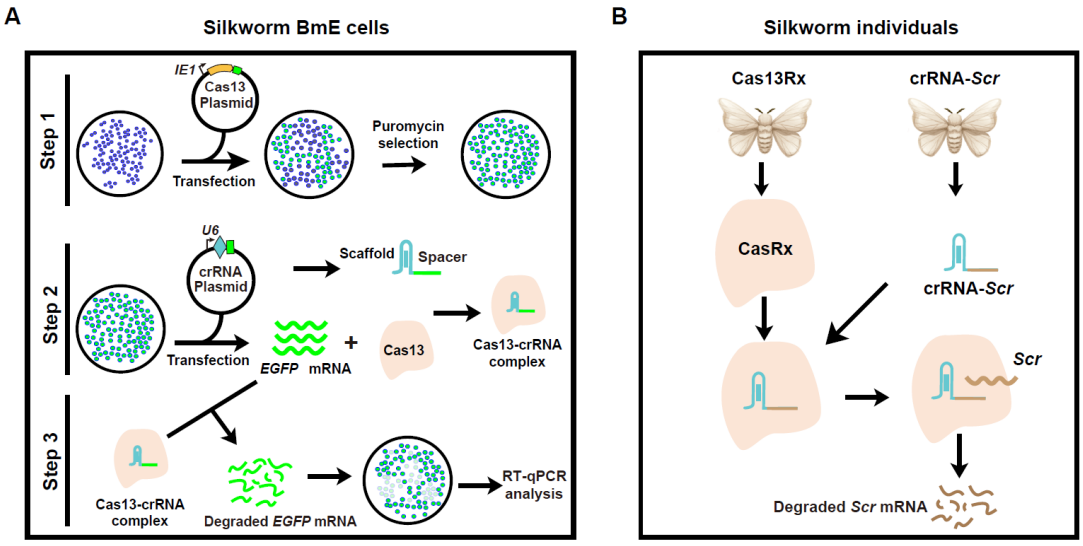
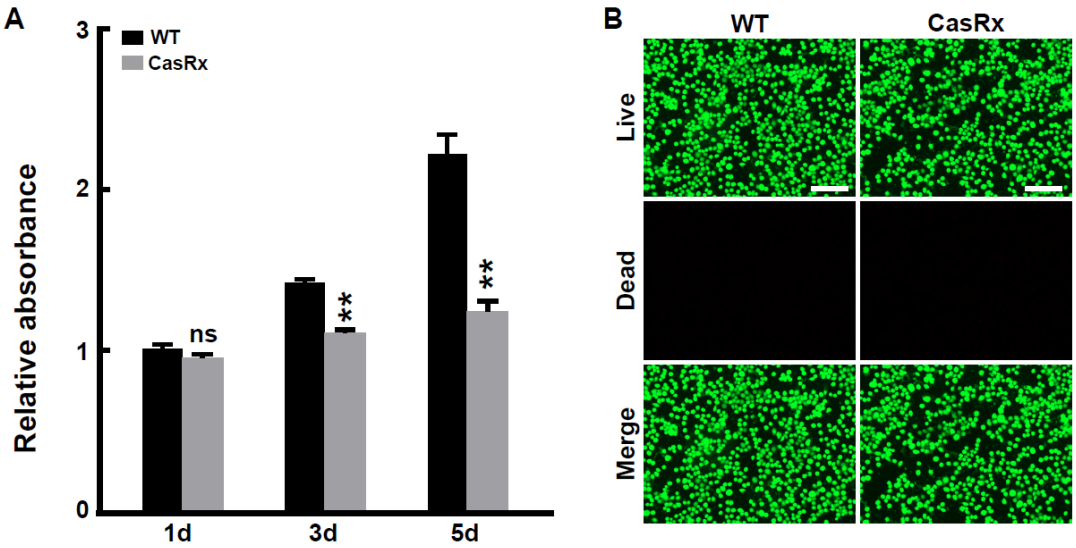


Supplementary Figure S1. Schematic of target regions for different crRNAs in the transcripts of *EGFP*, *Scr*, *Myc*, and *Fzr*

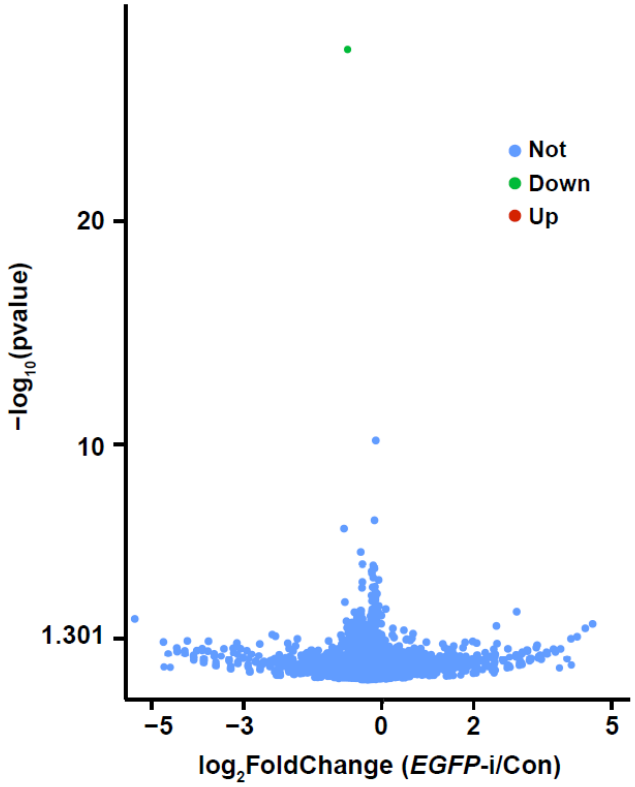


Supplementary Figure S2. Procedures for CRISPR-Cas13-mediated RNA editing analyses in silkworm BmE cells (A) and living individuals (B)

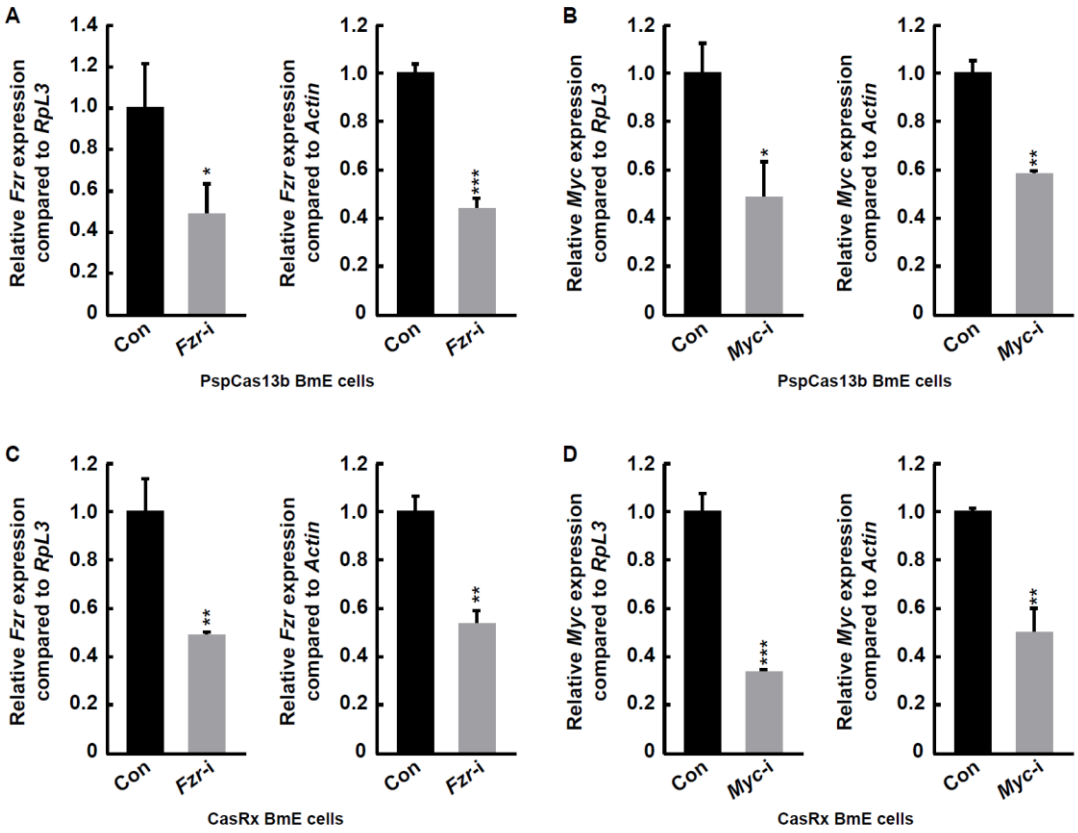


Supplementary Figure S3. The effect of overexpressed CasRx protein on the viability of silkworm BmE cells

A: CCK-8 assay showed that overexpressed CasRx protein moderately inhibited the proliferation of BmE cells. B: Live/Dead staining showed that no dead cell was detected following stable CasRx expression, indicating that CasRx protein had no effect on the viability of BmE cells. Scale bar, 200 μm. Values are mean±SE (error bars) from three independent biological replicates. d, day. Significant difference was determined using Student's *t*-test; ns: not significant; **: $P<0.01$ versus WT as the control. WT, wild-type.

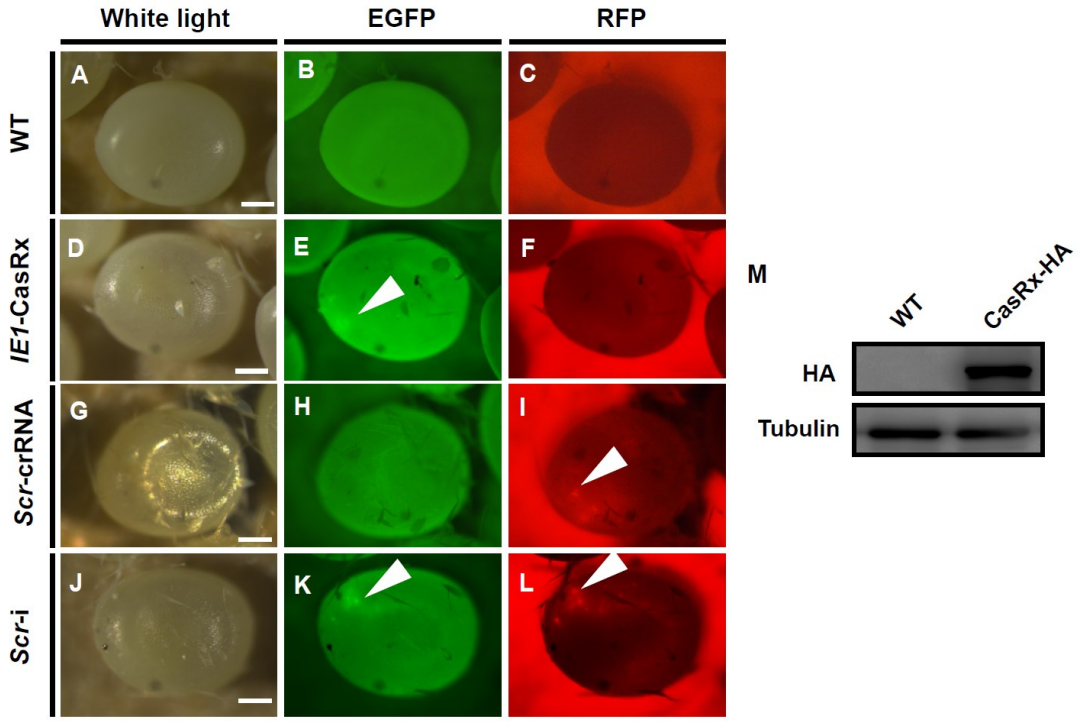


Supplementary Figure S4. RNA-seq analysis of transcriptome change following *EGFP* knockdown in the CasRx BmE cells



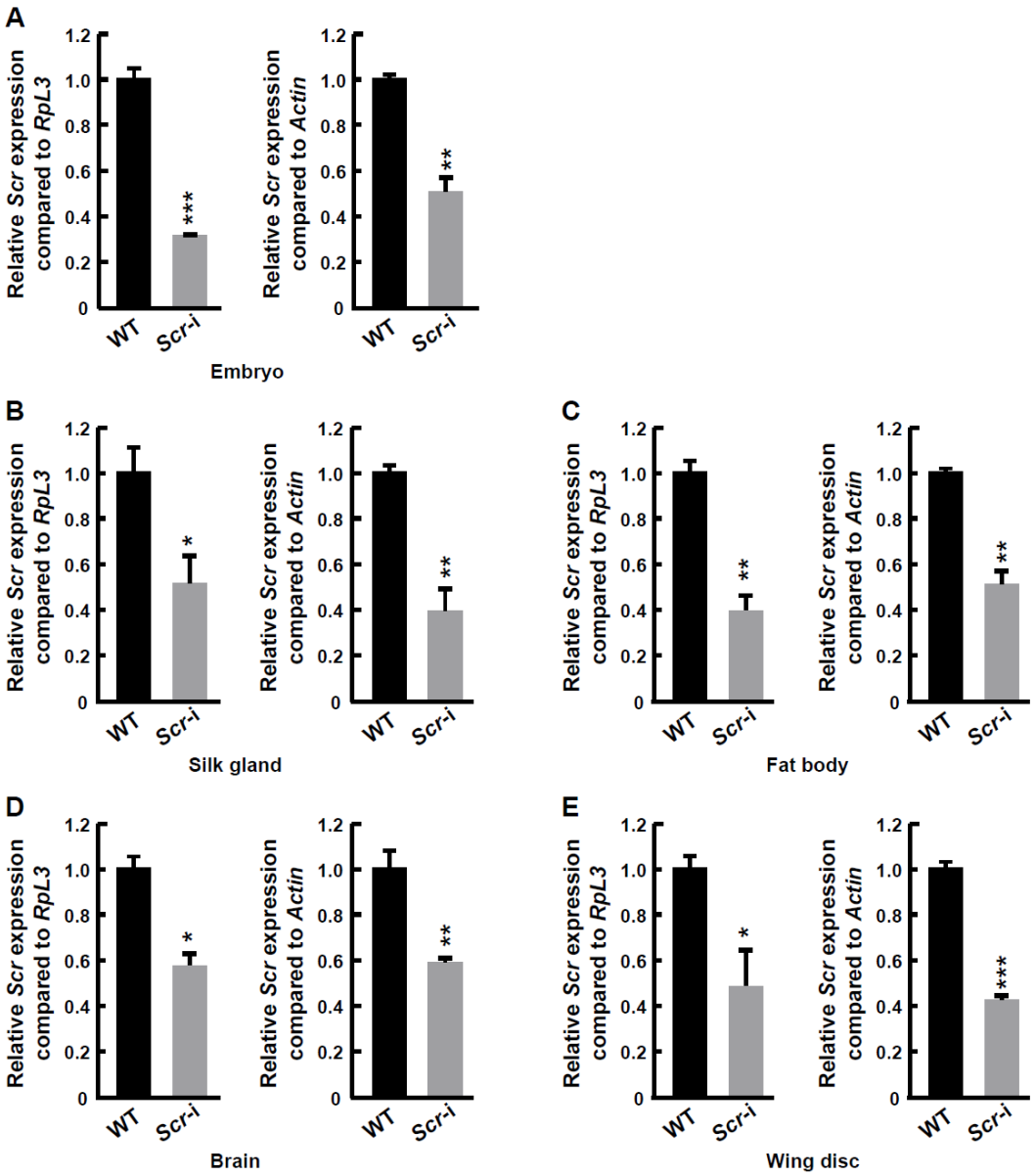
Supplementary Figure S5. CRISPR-Cas13-mediated RNA editing of several genes in silkworm BmE cells

A–D: RT-qPCR analysis of CRISPR-Cas13-mediated knockdown of *Fzr* and *Myc* in silkworm BmE cells. *RpL3* and *Actin* genes were used as internal controls. Values are mean±SE (error bars) from three independent biological replicates. Significant difference was determined using Student's *t*-test; *: $P<0.05$; **: $P<0.01$; ***: $P<0.001$ versus the control.



Supplementary Figure S6. Fluorescent screening of positive transgenic silkworm strains

A–L: Positive transgenic silkworm eggs were screened by the expression of fluorescent EGFP and DsRed (red fluorescent protein, RFP) markers in the eye driven by the *3×P3* promoter. Scale bar, 0.2 mm. M: Western blotting validation of stable CasRx expression in silkworm embryo. WT, wild-type; RFP, red fluorescent protein.



Supplementary Figure S7. Effects of CRISPR-CasRx-meditated *Scr* knockdown on mRNA expression of *Scr*

A: CRISPR-CasRx-mediated editing of *Scr* led to the knockdown of *Scr* transcripts in silkworm embryos on the sixth day after oviposition. B–E: RT-qPCR analysis of *Scr* knockdown-induced change of *Scr* mRNA expression in several tissues from silkworm larvae on the third day of the fifth larval instar. *RpL3* and *Actin* genes were used as internal controls. Values are mean±SE (error bars) from three independent biological replicates. Significant difference was determined using Student's *t*-test, *: $P<0.05$; **: $P<0.01$; ***: $P<0.001$ versus WT as the control. WT, wild-type.

Supplementary Table S1. The primers used in this study

Purpose	Primer name	Primer sequence
Construction of the Cas13 expression plasmid	*IE1* promoter	Forward: 5' GCTTATCGATACGCGTACGGAAGCTTTTGCAGTTCGGGAC 3'
		Reverse: 5' CCAGAGCGGGGATGTTCATGGTAGATCCCTAGTCGTTTGGTTGTT 3'
	U6 promoter	Forward: 5' TACCCATACGATGTTCCAGATTACGCT TATCCCTACGACGTGCCTGATTATGCA 3'
		Reverse: 5' ATTCGGCCGGCCTAGGAAAAAAGTTGTAATAGCCCCTCAAAACTGGACCTTCCACAACAGATGAT CCGACAGATTCGCAGGCGGAACTTGTAGAGCACGATATTTT 3'
	Cas13b	Forward: 5' GCTTATCGATACGCGTACGGTTGGCGCGCCATGAACATCCCCGCTCTGG 3'
		Reverse: 5' TAGGCTGCCGCGTCCTTCGGTTAGGCATAGTCGGGGACATCATA 3'
	CasRx	Forward: 5' GCTTATCGATACGCGTACGGTTGGCGCGCCATGAGCCCCAAGAAGAAGAGAAA 3'
		Reverse: 5' TAGGCTGCCGCGTCCTTCTTATGCATAATCAGGCACGTCGTAGGGATATGCATAATCAGGCACGTC GTAGGGATAAGCGTAATCTGGAACATCGTAT 3'
	EGFP	Forward: 5' GCTTATCGATACGCGTACGGTTGGCGCGCCGAAGGACGCGGCAGCCTACT 3'
		Reverse: 5' TCGAATTCGGCCGGCCTAGGTTCGATGCTAGACGATCCAGACAT 3'
	Puro	Forward: 5' ATGGCAACCACAGTCACCACAGTTACCGGCACT 3'
		Reverse: 5' TCAGTCCAGTACACTAAGACTATCTGCCGCCGGAACATT 3'
	SV40	Forward: 5' TAAAGCTTATCGATACGCGTACTTGGCGCGCCCTTCGAGCAACTTGTTTATTGCAG 3'
		Reverse: 5' CCATTCGAATTCGGCCGGCCTATTCGATGCTAGACGATCCAGACAT 3'
	2A	Forward: 5' ATCCGGGCCCCATGCGCTTCGTATTATGCTGTACCCTGATTGCTTTGGCTGC 3'
		Reverse: 5' CACCATGGTGGTGGGGCCCGGATTCTCTTCGACGTCTCCAGCCT 3'
CrRNA for Cas13b	*EGFP*	Forward: 5' CAAGCTGCACCACCGGCAAGCTGCCCGTGCCCT 3'
		Reverse: 5' CAACAGGGCACGGGCAGCTTGCCGGTGGTGCAG 3'
	Fzr	Forward: 5' CAAGCTGTACTGGTCCAGGCTGAGTTTAACAAT 3'
		Reverse: 5' CAACATTGTTAAACTCAGCCTGGACCAGTACAG 3'
	Scr	Forward: 5' AAACGGTCGTACCGGGACACCCGTAGAACAAAC 3'
		Reverse: 5' AAAAGTTTGTTCTACGGGTGTCCCGGTACGACC 3'

	Myc	Forward: 5' CAAGTTCCGCCTGCGAATCTGTCGGATCATCT 3'
		Reverse: 5' CAACAGATGATCCGACAGATTCGCAGGCGGAA 3'
	RFP	Forward: 5' CAAGTCCAAGGTGTACGTGAAGCACCCCGCCGAC 3'
		Reverse: 5' CAACGTCGGCGGGGTGCTTCACGTACACCTTGGA 3'
CrRNA for CasRx	*EGFP*	Forward: 5' AAACCTGCACCACCGGCAAGCTGCCCGTGCCCT 3'
		Reverse: 5' AAAAGAGGGCACGGGCAGCTTGCCGGTGGTGCAG 3'
	Fzr	Forward: 5' AAACCTGTACTGGTCCAGGCTGAGTTTAACAAT 3'
		Reverse: 5' AAAAATTGTTAAACTCAGCCTGGACCAGTACAG 3'
	Scr	Forward: 5' AAACGGGCGTTGTCCCCAAATTTGATGTCCACC 3'
		Reverse: 5' AAAAGGTGGACATCAAATTTGGGGACAACGCCC 3'
	Myc	Forward: 5' AAACTTCCGCCTGCGAATCTGTCGGATCATCT 3'
		Reverse: 5' AAAAAGATGATCCGACAGATTCGCAGGCGGAA 3'
	RFP	Forward: 5' AAACTCCAAGGTGTACGTGAAGCACCCCGCCGAC 3'
		Reverse: 5' AAAAGTCGGCGGGGTGCTTCACGTACACCTTGGA 3'
RT-qPCR	*Myc*	Forward: 5' GTCGAGCACACCGTATCAGA 3'
		Reverse: 5' AGATAAACGCTGCTGGAGGA 3'
	Fzr	Forward: 5' TACTCGTTGTCACCCGTCAG 3'
		Reverse: 5' GCGCTCCACAGATAGACACA 3'
	Scr	Forward: 5' AACTCCAACTCAAACAACACCAAG 3'
		Reverse: 5' TCCTTTTCGAGCTCCAGCGT 3'
	EGFP	Forward: 5' GGTGAACTTCAAGATCCGCC 3'
		Reverse: 5' CTTGTACAGCTCGTCCATGC 3'
	RpL3	Forward: 5' TGGCACACAAAGAAGCTACCC 3'
		Reverse: 5' TGACCAGCACGAGCTACAGTG 3'
	Actin	Forward: 5' AACACCCCGTCCTGCTCACTG 3'
		Reverse: 5' GGGCGAGACGTGTGATTTCCT 3'
Mutant spacer	*Cas13b-EGFP-Mis3*	Forward: 5' CAAGAAACACCACCGGCAAGCTGCCCGTGCCCT 3'

	Reverse: 5' CAACAGGGCACGGGCAGCTTGCCGGTGGTGTTT 3'
Cas13b-EGFP-Mis6	Forward: 5' CAAGCTGTTTCACCGGCAAGCTGCCCGTGCCCT 3'
	Reverse: 5' CAACAGGGCACGGGCAGCTTGCCGGTGAAACAG 3'
Cas13b-EGFP-Mis9	Forward: 5' CAAGCTGCACGGGCGGCAAGCTGCCCGTGCCCT 3'
	Reverse: 5' CAACAGGGCACGGGCAGCTTGCCGCCCGTGCAG 3'
Cas13b-EGFP-Mis12	Forward: 5' CAAGCTGCACCACTTTCAAGCTGCCCGTGCCCT 3'
	Reverse: 5' CAACGAGGGCACGGGCAGCTTGAAAGTGGTGCAG 3'
Cas13b-EGFP-Mis15	Forward: 5' CAAGCTGCACCACCGGGGGGCTGCCCGTGCCCT 3'
	Reverse: 5' CAACAGGGCACGGGCAGCCCCCCGGTGGTGCAG 3'
Cas13b-EGFP-Mis18	Forward: 5' CAAGCTGCACCACCGGCAAAAAGCCCGTGCCCT 3'
	Reverse: 5' CAACAGGGCACGGGCTTTTTGCCGGTGGTGCAG 3'
Cas13b-EGFP-Mis21	Forward: 5' CAAGCTGCACCACCGGCAAGCTTTTCGTGCCCT 3'
	Reverse: 5' CAACAGGGCACGAAAAGCTTGCCGGTGGTGCAG 3'
Cas13b-EGFP-Mis24	Forward: 5' CAAGCTGCACCACCGGCAAGCTGCTTTTGCCCT 3'
	Reverse: 5' CAACAGGGCAAAAGCAGCTTGCCGGTGGTGCAG 3'
Cas13b-EGFP-Mis27	Forward: 5' CAAGCTGCACCACCGGCAAGCTGCCCGAAACCT 3'
	Reverse: 5' CAACAGGTTTCGGGCAGCTTGCCGGTGGTGCAG 3'
Cas13b-EGFP-Mis30	Forward: 5' CAAGCTGCACCACCGGCAAGCTGCCCGTGCGGG 3'
	Reverse: 5' CAACCCCGCACGGGCAGCTTGCCGGTGGTGCAG 3'
CasRx-EGFP-Mis3	Forward: 5' AAACAAACACCACCGGCAAGCTGCCCGTGCCCT 3'
	Reverse: 5' AAAAAGGGCACGGGCAGCTTGCCGGTGGTGTTT 3'
CasRx-EGFP-Mis6	Forward: 5' AAACCTGTTTCACCGGCAAGCTGCCCGTGCCCT 3'
	Reverse: 5' AAAAAGGGCACGGGCAGCTTGCCGGTGAAACAG 3'
CasRx-EGFP-Mis9	Forward: 5' AAACCTGCACGGGCGGCAAGCTGCCCGTGCCCT 3'
	Reverse: 5' AAAAAGGGCACGGGCAGCTTGCCGCCCGTGCAG 3'
CasRx-EGFP-Mis12	Forward: 5' AAACCTGCACCACTTTCAAGCTGCCCGTGCCCT 3'
	Reverse: 5' AAAAGAGGGCACGGGCAGCTTGAAAGTGGTGCAG 3'

CasRx-EGFP-Mis15	Forward: 5' AAACCTGCACCACCGGGGGGCTGCCCGTGCCCT 3'
	Reverse: 5' AAAAAGGGCACGGGCAGCCCCCCGGTGGTGCAG 3'
CasRx-EGFP-Mis18	Forward: 5' AAACCTGCACCACCGGCAAAAAGCCCGTGCCCT 3'
	Reverse: 5' AAAAAGGGCACGGGCTTTTTGCCGGTGGTGCAG 3'
CasRx-EGFP-Mis21	Forward: 5' AAACCTGCACCACCGGCAAGCTTTTCGTGCCCT 3'
	Reverse: 5' AAAAAGGGCACGAAAAGCTTGCCGGTGGTGCAG 3'
CasRx-EGFP-Mis24	Forward: 5' AAACCTGCACCACCGGCAAGCTGCTTTTGCCCT 3'
	Reverse: 5' AAAAAGGGCAAAAGCAGCTTGCCGGTGGTGCAG 3'
CasRx-EGFP-Mis27	Forward: 5' AAACCTGCACCACCGGCAAGCTGCCCGAAACCT 3'
	Reverse: 5' AAAAAGGTTTCGGGCAGCTTGCCGGTGGTGCAG 3'
CasRx-EGFP-Mis30	Forward: 5' AAACCTGCACCACCGGCAAGCTGCCCGTGCGGG 3'
	Reverse: 5' AAAACCCGCACGGGCAGCTTGCCGGTGGTGCAG 3'