Supplemental information

Enhanced genomic stability of new miRNA-regulated oncolytic coxsackievirus B3

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**miR-CVB3-2.1**

| miR-1-TS | miR-216-TS | miR-133-TS | miR-375-TS |
|----------|------------|------------|------------|
| atccggtgATACATACTTTTTACATCCATCACAGTTGCCAGCTGAGATTATAGCTGGTTGAAGGGGACCAATCACCGGAGCCTGA | TCACAGTTGCCAGCTGAGATTATAGCTGGTTGAAGGGGACCAATTACCCGAGCCGAACTTGAACTTAACAAATACCAATCTTTTCTT | TCACAGTTGCCAGCTGAGATTATAGCTGGTTGAAGGGGACCAATTACCCGAGCCGAACTTGAACTTAACAAATACCAATCTTTTCTT | TCACAGTTGCCAGCTGAGATTATAGCTGGTTGAAGGGGACCAATTACCCGAGCCGAACTTGAACTTAACAAATACCAATCTTTTCTT |

#1 atccggtgATACATACTTTTTACATCCATCACAGTTGCCAGCTGAGATTATAGCTGGTTGAAGGGGACCAATTACCCGAGCCGAACTTGAACTTAACAAATACCAATCTTTTCTT
#2 atccggtgATACATACTTTTTACATCCATCACAGTTGCCAGCTGAGATTATAGCTGGTTGAAGGGGACCAATTACCCGAGCCGAACTTGAACTTAACAAATACCAATCTTTTCTT
#3 atccggtgATACATACTTTTTACATCCATCACAGTTGCCAGCTGAGATTATAGCTGGTTGAAGGGGACCAATTACCCGAGCCGAACTTGAACTTAACAAATACCAATCTTTTCTT

**miR-CVB3-2.2**

| miR-1-TS | miR-216-TS | VP3 |
|----------|------------|-----|
| tcacagtgggatatccggtgttttcaacatag | TCACAGTTGCCAGCTGAGATTATAGCTGGTTGAAGGGGACCAATTACCCGAGCCGAACTTGAACTTAACAAATACCAATCTTTTCTT | TCACAGTTGCCAGCTGAGATTATAGCTGGTTGAAGGGGACCAATTACCCGAGCCGAACTTGAACTTAACAAATACCAATCTTTTCTT |

#1 tcacagtgggatatccggtgttttcaacatag | TCACAGTTGCCAGCTGAGATTATAGCTGGTTGAAGGGGACCAATTACCCGAGCCGAACTTGAACTTAACAAATACCAATCTTTTCTT |
#2 tcacagtgggatatccggtgttttcaacatag | TCACAGTTGCCAGCTGAGATTATAGCTGGTTGAAGGGGACCAATTACCCGAGCCGAACTTGAACTTAACAAATACCAATCTTTTCTT |
#3 tcacagtgggatatccggtgttttcaacatag | TCACAGTTGCCAGCTGAGATTATAGCTGGTTGAAGGGGACCAATTACCCGAGCCGAACTTGAACTTAACAAATACCAATCTTTTCTT |

**VP3**

| miR-133-TS | miR-375-TS |
|------------|------------|
| tcacagtgggatatccggtgttttcaacatag | TCACAGTTGCCAGCTGAGATTATAGCTGGTTGAAGGGGACCAATTACCCGAGCCGAACTTGAACTTAACAAATACCAATCTTTTCTT |

#1 tcacagtgggatatccggtgttttcaacatag | TCACAGTTGCCAGCTGAGATTATAGCTGGTTGAAGGGGACCAATTACCCGAGCCGAACTTGAACTTAACAAATACCAATCTTTTCTT |
#2 tcacagtgggatatccggtgttttcaacatag | TCACAGTTGCCAGCTGAGATTATAGCTGGTTGAAGGGGACCAATTACCCGAGCCGAACTTGAACTTAACAAATACCAATCTTTTCTT |
#3 tcacagtgggatatccggtgttttcaacatag | TCACAGTTGCCAGCTGAGATTATAGCTGGTTGAAGGGGACCAATTACCCGAGCCGAACTTGAACTTAACAAATACCAATCTTTTCTT |

**miR-CVB3-2.2**

| miR-375-TS |
|------------|
| tcacagtgggatatccggtgttttcaacatag |

**Original**

| Original | Original | Original |
|----------|----------|----------|
| atccggtgATACATACTTTTTACATCCATCACAGTTGCCAGCTGAGATTATAGCTGGTTGAAGGGGACCAATCACCGGAGCCTGA | TCACAGTTGCCAGCTGAGATTATAGCTGGTTGAAGGGGACCAATTACCCGAGCCGAACTTGAACTTAACAAATACCAATCTTTTCTT | TCACAGTTGCCAGCTGAGATTATAGCTGGTTGAAGGGGACCAATTACCCGAGCCGAACTTGAACTTAACAAATACCAATCTTTTCTT |

#1 atccggtgATACATACTTTTTACATCCATCACAGTTGCCAGCTGAGATTATAGCTGGTTGAAGGGGACCAATTACCCGAGCCGAACTTGAACTTAACAAATACCAATCTTTTCTT |
#2 atccggtgATACATACTTTTTACATCCATCACAGTTGCCAGCTGAGATTATAGCTGGTTGAAGGGGACCAATTACCCGAGCCGAACTTGAACTTAACAAATACCAATCTTTTCTT |
#3 atccggtgATACATACTTTTTACATCCATCACAGTTGCCAGCTGAGATTATAGCTGGTTGAAGGGGACCAATTACCCGAGCCGAACTTGAACTTAACAAATACCAATCTTTTCTT |

**Figure S1.** Three hearts (i.e., #1, #2, and #3) harvested from A/J mice from Figure 4, which were treated with miR-CVB3-2.1 or miR-CVB3-2.2 for 14 days, were subjected to RNA extraction and Sanger sequencing. The mutation is highlighted in red. “-” denotes missing nucleotide.
Figure S2. Total RNA was extracted from the hearts of miR-CVB3-treated NOD-SCID mice from Figure 5, which died prior to the experimental endpoint of day 150 (i.e., miR-CVB3-1.1 on day 47, miR-CVB3-2.1 on day 72 and miR-CVB3-2.2 on day 112) for examination of the integrity of miRNA target sequence by Sanger sequencing. The mutation is highlighted in red. “-” denotes missing nucleotide.