OMTO, Volume *12*

Supplemental Information

Extremely Low Organ Toxicity and Strong Antitumor Activity of miR-34-Regulated Oncolytic Coxsackievirus B3

Yang Jia, Shohei Miyamoto, Yasushi Soda, Yuto Takishima, Miyako Sagara, Jiyuan Liao, Lisa Hirose, Yasuki Hijikata, Yoshie Miura, Kenichiro Hara, Atsufumi Iwanaga, Yasunori Ota, and Kenzaburo Tani

Figure S1

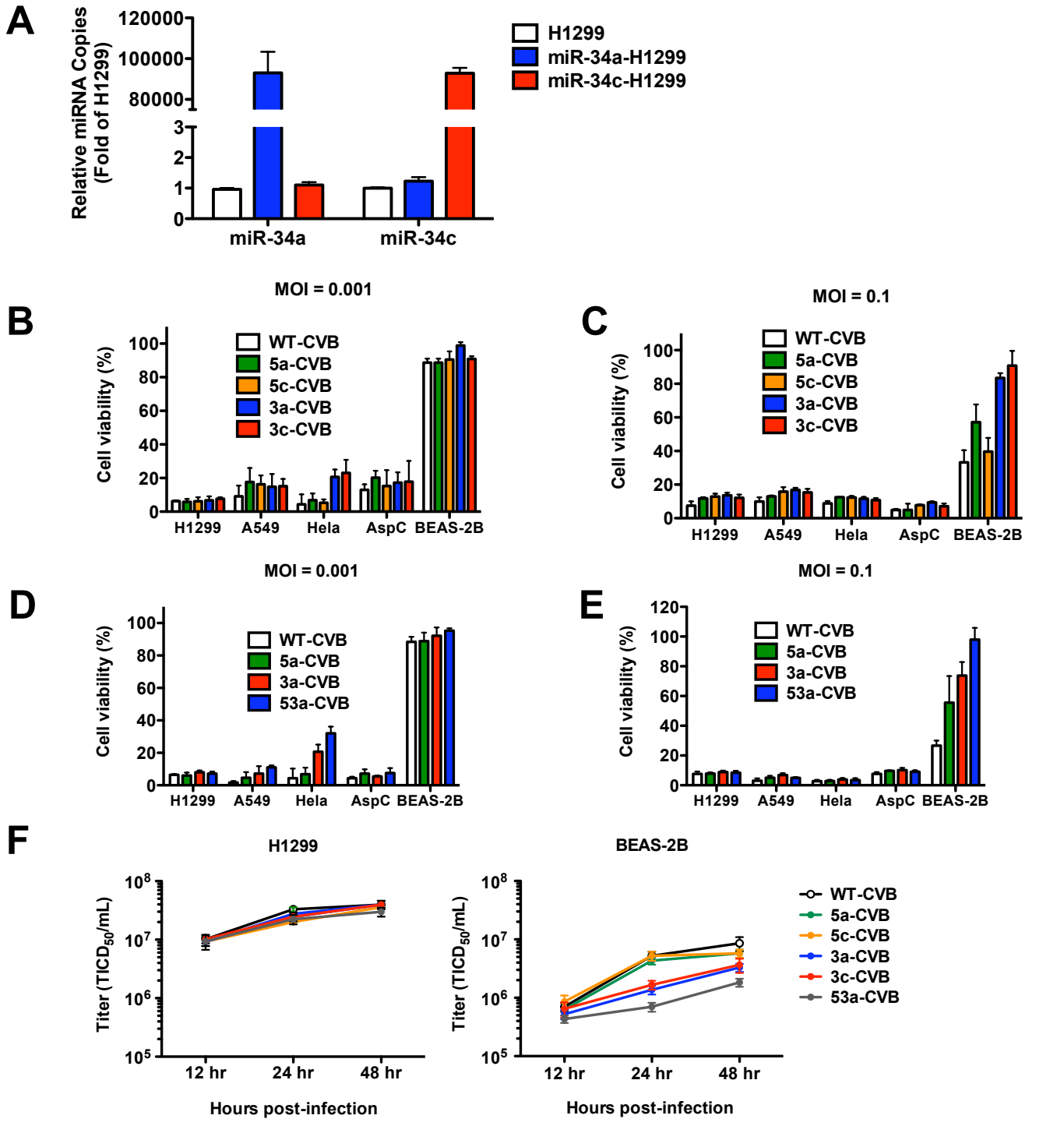

Figure S1. Relative copy numbers of miR-34 mimics in transfected H1299 cells and cytotoxicity of miRT-CVBs in various cell lines.

(A) H1299 cells were transfected with 10 μM miR-34a (miR-34a-H1299) or miR-34c (miR-34c-H1299) mimics. At 24 hrs later, relative copy numbers of miR-34a and miR-34c were measured by RT-qPCR and normalized against U6 snRNA. Data represent means ± SD of triplicate assays. (B-E) The cCell viability of various cell lines was determined by MTS assay 72 hrs after inoculation with 5-CVBs and 3-CVBs at a concentration of MOI = 0.001 (B) and MOI = 0.1 (C), and 53a-CVBs at MOI = 0.001 (D) and MOI = 0.1 (E). (F) Replication kinetics of miRT-CVBs were determined by single-step growth curve analysis (MOI = 3) in H1299 cells and BEAS-2B cells. Data are represented as mean virus titer ± SD.

Figure S2

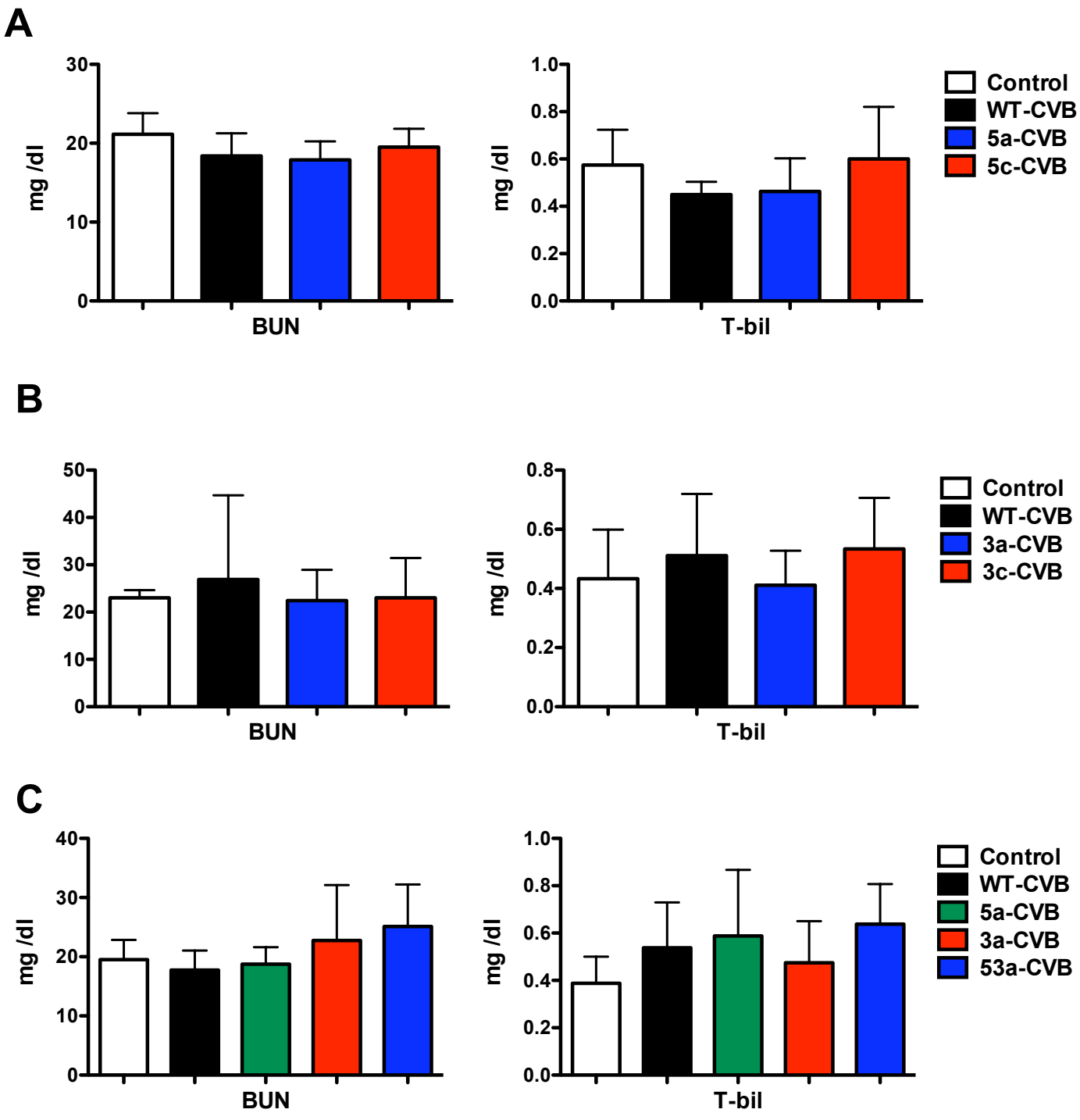

Figure S2. Serum BUN and T-bil levels of mice treated with CVBs.
Serum BUN and T-bil levels of mice treated with 5-CVBs (A), 3-CVBs (B), or 53a-CVB (C). Data represent means ± SD of eight or nine mice in each group.

Figure S3

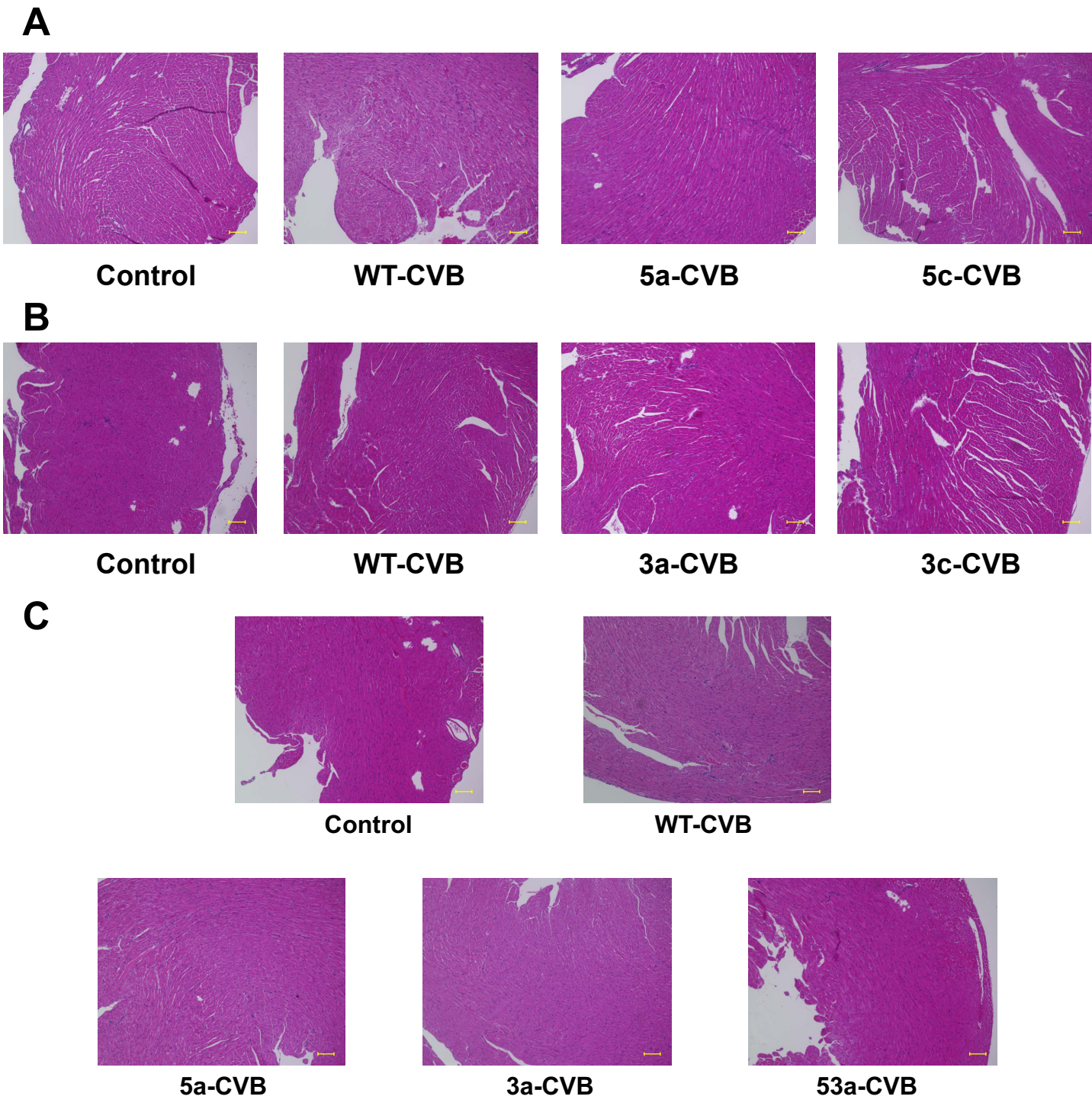

Figure S3. Histological examination of mouse hearts.
H-E images of the hearts of mice treated with 5-CVBs (A), 3-CVBs (B), or 53a-CVB (C) 2 two days after inoculation with the indicated viruses. Scale bars, 100 μm.

Figure S4

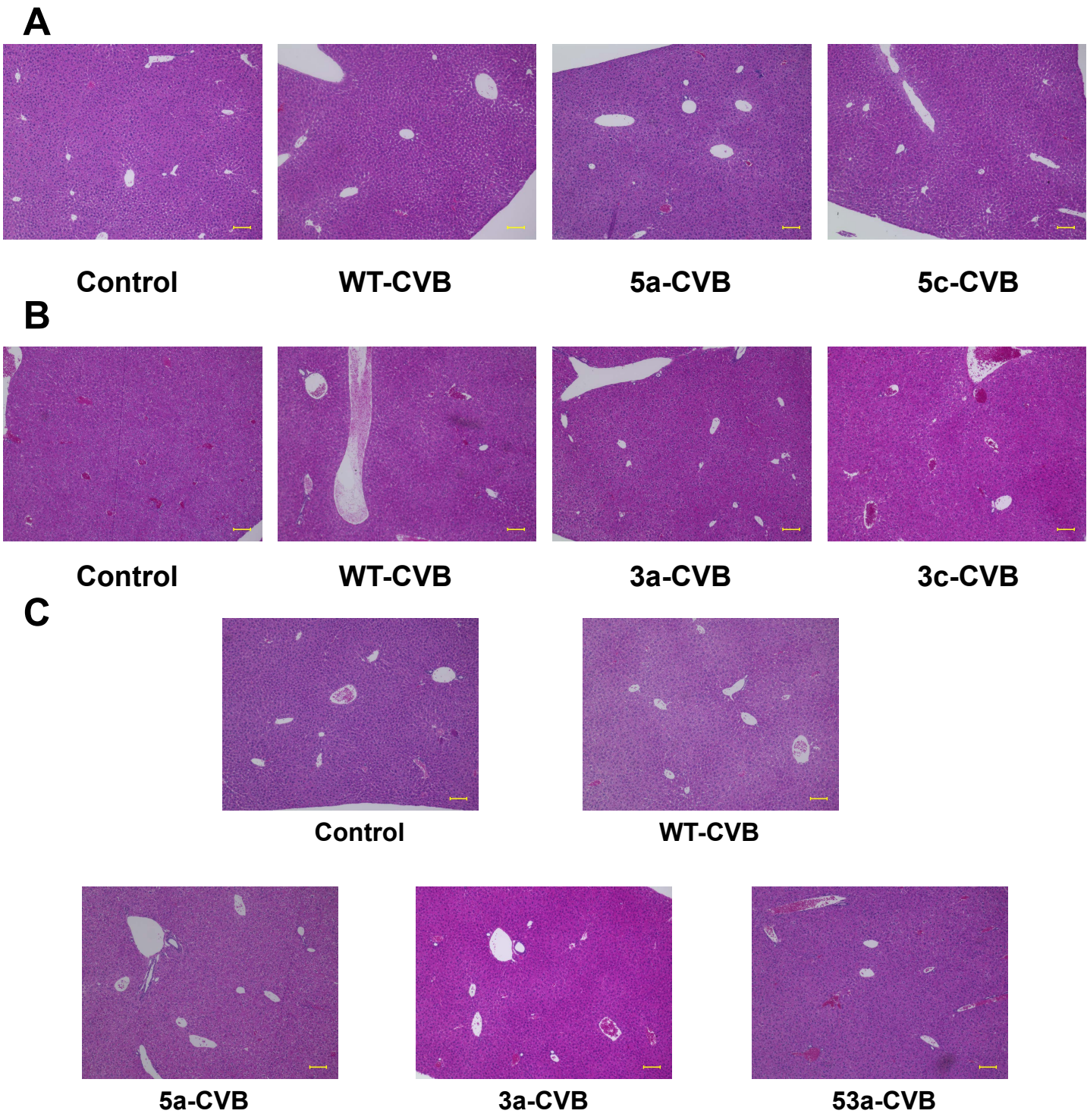

Figure S4. Histological examination of mouse livers.
H-E images of the livers of mice treated with 5-CVBs (A), 3-CVBs (B), or 53a-CVB (C) 2 two days after inoculation with the indicated viruses. Scale bars, 100 μm.

Figure S5

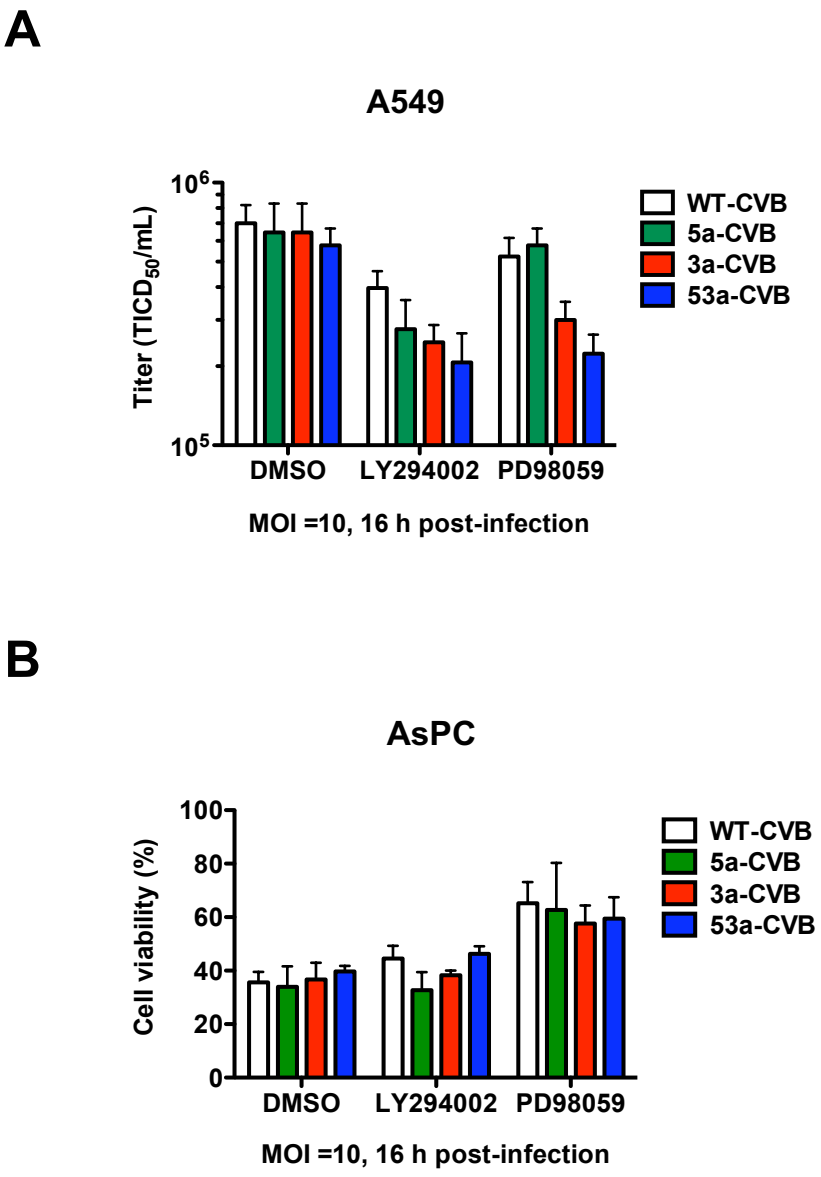

Figure S5. Attenuated cytotoxicity of miR-34T-CVBs in miR-34a-high A549 cells with PI3K inhibitor LY294002 or MEK inhibitor PD98059.

A549 cells (A) and AsPC cells (B) were treated with 10 μM LY294022, 10 μM PD0335901, or DMSO for 1 hour, and then infected with viruses for 16 hrs. Supernatants from A549 cells were collected and a viral titer was determined (A). MTS assay was performed to determine the cell viability of AsPC cells (B). Data represent means ± SD.

Figure S6

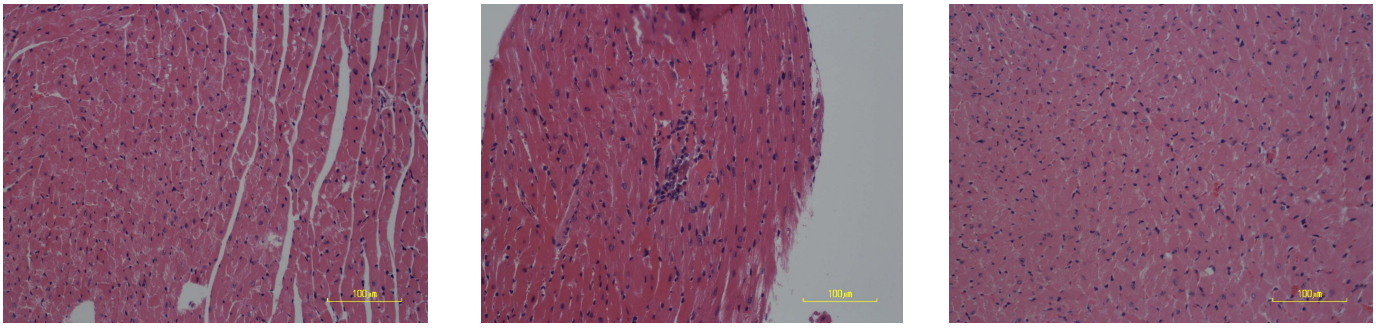

Figure S6. Histological examination of hearts in a myocarditis model.
Two days after three doses of virus were injectedinjection, mouse hearts were collected for histological examination. H-E images of the hearts of mice treated with vehicle control (A), WT-CVBs (B), or 53a-CVB (C). Magnification: 20x. Scale bar, 100 μM.

Figure S7

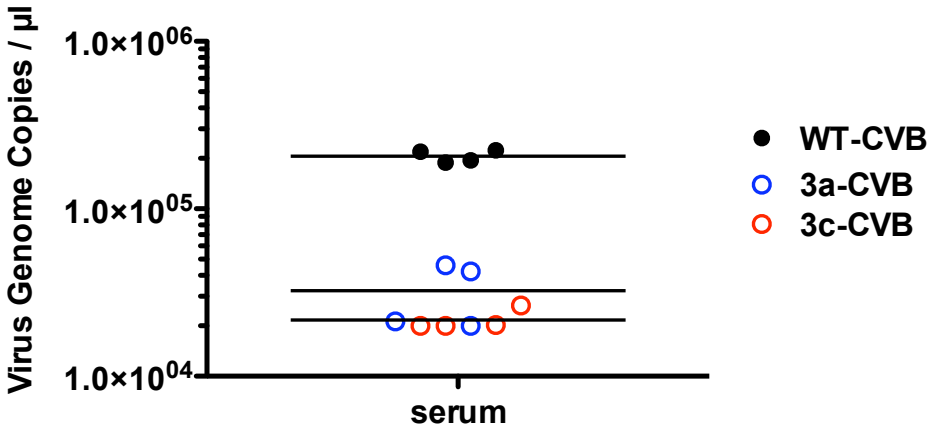

Figure S7. Virus loads of 3-CVBs in mouse serum.
Serum samples were collected two days after CVB injection in H1299 xenografts, and copy numbers of the CVB3 genome were quantified by RT-qPCR. Data represent means ± SD of triplicated experiments.

Figure S8

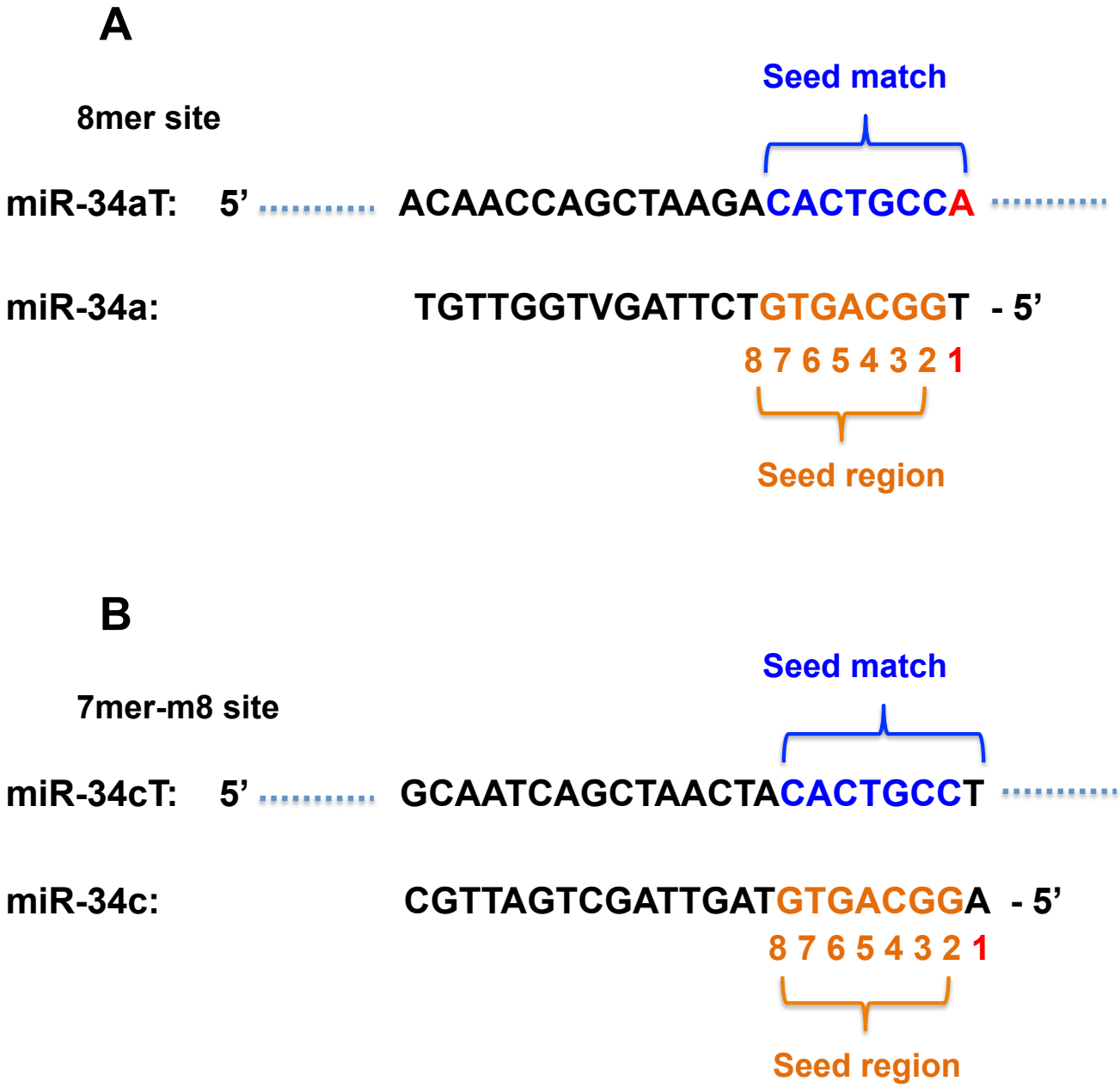

Figure S8. Nucleotide sequences of miR-34a, miR-34c, and their target sites.
(A) miR-34a target site has seven contiguous Watson-Crick pairs complementary to the seed region (positions 2–8) of miR-34a, with an A at position 1. (B) miR-34c target site has a perfect match to the miRNA seed (positions 1-8).