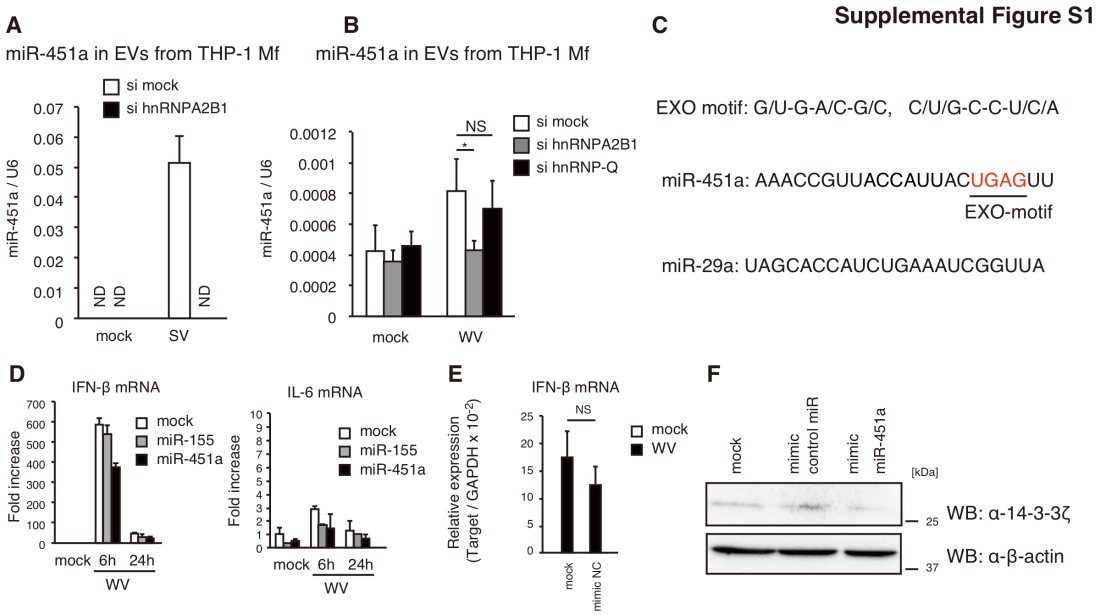
Supporting Information



Supplemental Figure S1. Role of miR-451a in type I IFN and IL-6 expression by macrophages

(**A**) siRNA for hnRNPA2B1 or negative control was transfected into THP-1 macrophages for 2 days. Cells were then stimulated with 40 μg/ml of SV for 24 hr. The expression levels of miR-451a in EVs were determined by RT-qPCR. ND represents "not detected" (A).

(**B**) siRNA for hnRNPA2B1, hnRNP-Q, and negative control were transfected into THP-1 macrophages for 2 days. Cells were then stimulated with 20 μg/ml of inactivated WV for 24 hr. The expression levels of miR-451a in EVs were determined by RT-qPCR. ND represents "not detected".

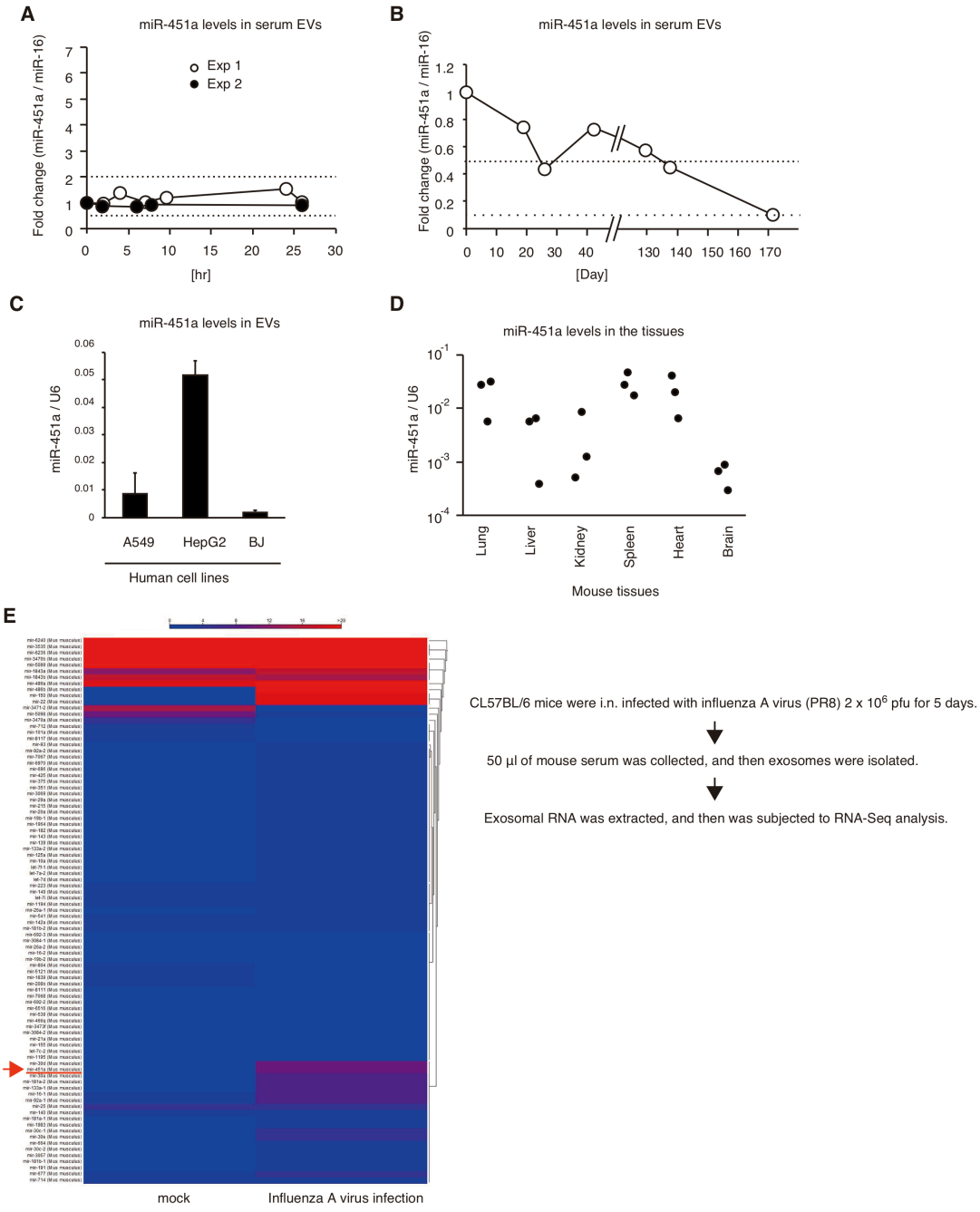
(**C**) Consensus sequence of EXO motif and sequences of miR-451a and miR-29a. There is an EXO-motif in miR-451a (shown in red color).

(**D**) Mock, miR-155 mimic, and miR-451a mimic RNAs were transfected into THP-1 macrophages for 2 days. Cells were stimulated with WV. Total RNA was extracted at indicated time points, and the expression of IFN-β and IL-6 was determined by RT-qPCR and normalized to GAPDH. Fold increase was calculated by dividing each value by that of mock-infected and mock-treated sample.

(**E**) THP-1 cells were transfected with mock and control miRNA (mimic NC) for 2 days. Cells were then stimulated with or without inactivated WV. The expression of IFN-β was determined by RT-qPCR and normalized to GAPDH.

(**F**) Mock, control microRNA (mimic control miRNA), and mimic miR-451a were transfected into THP-1 cells for 2 days. Whole cell lysate was prepared, and were subjected to SDS-PAGE. The proteins were detected by western blotting with anti-14-3-3ζ and β-actin antibodies.

Supplemental Figure S2



Supplemental Figure S2. Expression profile of miR-451a in human and mouse

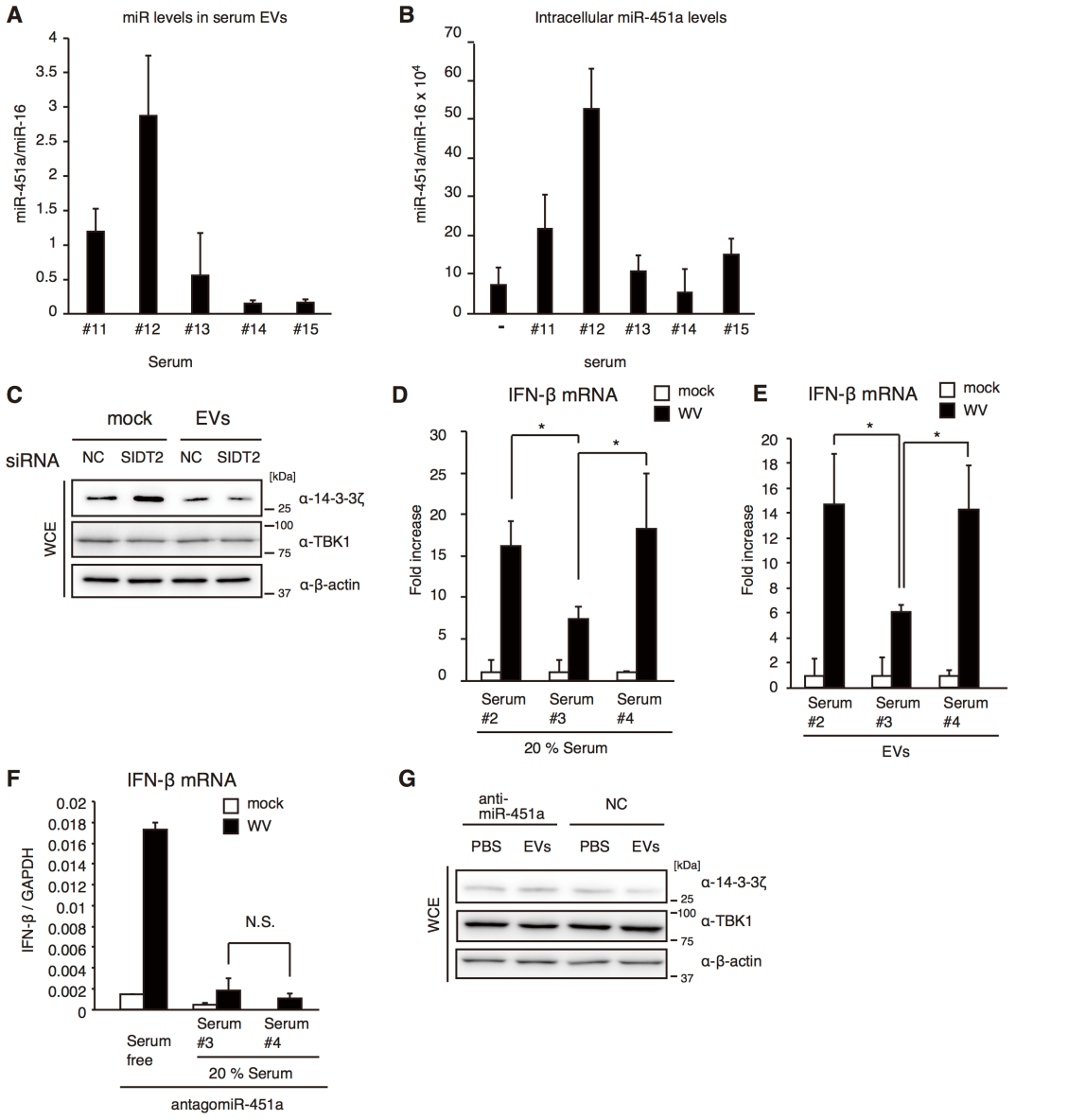
(**A**, **B**) Sera were collected from human healthy subjects over 1day (A) and 170 days (B). miR-451a levels in human serum EVs were determined by RT-qPCR and was normalized to miR-16 levels.

(**C**) A549, HepG2, and BJ cells were cultured in serum free medium for 2 days. EVs were collected from cell culture supernatants, and miR-451a levels in EVs were determined by RT-qPCR and were normalized to U6 levels.

(**D**) Mouse tissues were excised from C57BL/6 mice, and total RNAs were isolated.

miR-451a levels in each tissue were determined by RT-qPCR and were normalized to U6 levels.
(**E**) C57BL/6 mice were intranasally infected with mock or influenza A virus (2 x 10^6 pfu) for 5 days. EVs were collected from mock or flu-infected mouse serum, and total RNA was subsequently extracted from EVs. Small RNA libraries were prepared, according to manufacture's instructions (Ilummina) and were sequenced by Mi-Seq. Obtained data was analyzed with CLC genomics workbench software. RNA-Seq data has been deposited to DRA in DDBJ (Accession number: DRA005817).

Supplemental Figure S3



Supplemental Figure S3. miR-451a levels in serum EVs and cytokine expression of macrophages cultured with serum

(**A**) Sera (#11–#15) were collected five times from a human healthy subject. EVs were collected from each serum, and their miRNA levels were determined by RT-qPCR.

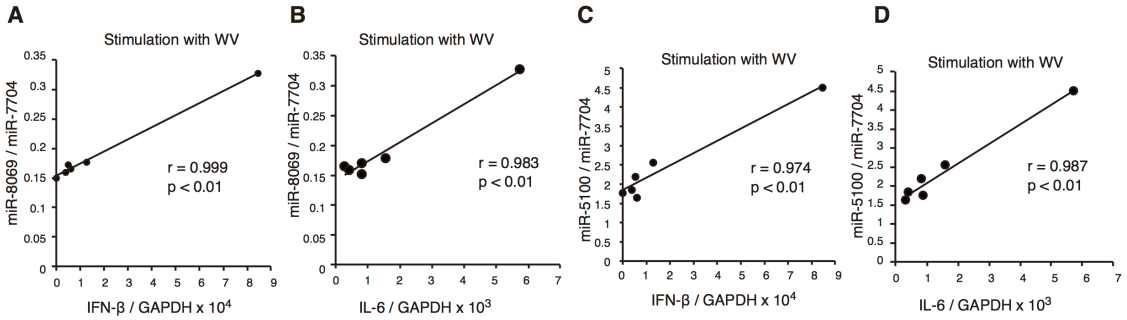
(**B**) THP-1 macrophages were cultured in medium with each human sera for 2 days. Intracellular miR-451a levels were determined by RT-qPCR and normalized to miR-16 levels.

(**C**) THP-1 macrophages were transfected with siRNA for negative control (NC) and SIDT2 for 2 days. Whole cell extracts (WCE) were prepared and were subsequently subjected to SDS-PAGE. The proteins were detected by western blotting with anti-14-3-3ζ, anti-TBK-1, and anti-β-actin antibodies.

(**D**) THP-1 macrophages were cultured with each human serum (#2, #3, and #4), which were used in Figure 5, for 2 days. Cells were stimulated with inactivated WV for 6 hr, and the expression of IFN-β mRNA was determined by RT-qPCR. Data represent means

± SD (n = 3) ($p < 0.05$, t-test).
(**E**) EVs were collected from 0.1 ml of human sera (#2, #3, and #4). Cells were cultured in serum-free medium with collected EVs in 24-well plates for 2 days, and were subsequently stimulated with inactivated WV for 6hr. The expression of IFN-β mRNA was determined by RT-qPCR. Data represent means ± SD (n = 3) ($p < 0.05$, t-test).
(**F**) AntagomiR-451a was transfected into THP-1 macrophages, and cells were then cultured with or without human serum. IFN-β mRNA expressions in response to inactivated WV were determined by RT-qPCR. Data represent means ± SD (n = 3). "ns" represents "not significant" ($p > 0.05$, t-test).
(**G**) AntagomiR-451a was transfected into THP-1 macrophages, and cells were then cultured in medium with or without EVs from human serum for 2 days. Whole cell extracts (WCE) were prepared and subjected to SDS-PAGE. The proteins were detected by western blotting with anti-14-3-3ζ, anti-TBK-1, and anti-β-actin antibodies.

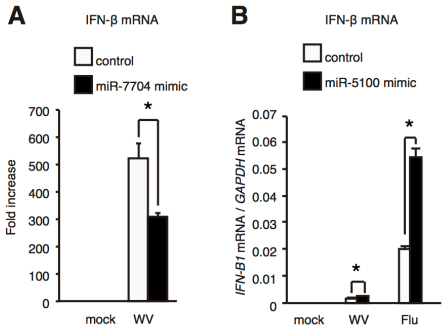
Supplemental Figure S4



Supplemental Figure S4. Correlation coefficients between miRNA ratio and cytokine expression in response to inactivated WV

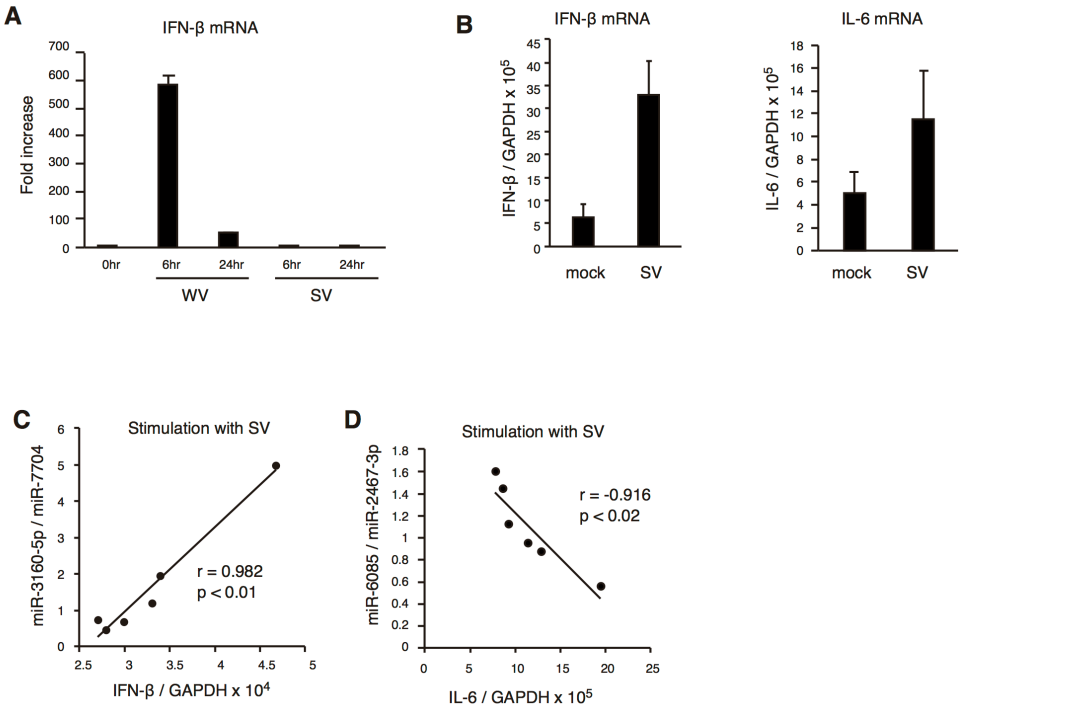
(A–D) THP-1 macrophages were cultured with serum collected from the 6 healthy subjects for 2 days, and were stimulated with inactivated WV for 6 hr. Total RNA was extracted from cells, and the expression of IFN-β (A, C) and IL-6 (B, D) were determined by RT-qPCR and normalized to GAPDH. Y axis shows miRNA ratios determined by the microarray analysis, and X axis shows IFN-β or IL-6 mRNA expression. Correlation coefficient (r) was calculated, and statistic analysis was performed (n = 6).

Supplemental Figure S5



Supplemental Figure S5. Roles of miR-7704 and miR-5100 in the innate immune response to WV.
(A) miR-7704 mimic RNA (miR-7704 mimic) was transfected into THP-1 macrophages for 2 days. EVs released from cells transfected with miR-7704 mimic or control miRNA (control) were collected to transfer other cells. Independently prepared THP-1 macrophages were treated with mock EVs or miR-7704 mimic EVs for 2 days, and then were stimulated with inactivated WV for 6 hr. IFN-β mRNA expression was determined by RT-qPCR.
(B) miR-5100 mimic or control miRNA were transfected into THP-1 macrophages for 2 days. Cells were stimulated with inactivated WV for 6 hr or infected with influenza A virus. IFN-β mRNA expression was determined by RT-qPCR.

Supplemental Figure S6



Supplemental Figure S6. Correlation coefficients between miRNA ratio and cytokine expression in response to SV

(A) THP-1 macrophages were stimulated with 20 μg/ml of inactivated WV and split vaccine (SV). Total RNA was extracted at indicated time points, and the expression of IFN-β was determined by RT-qPCR.

(B) THP-1 macrophages were treated with IFN-γ for 1 day, and then stimulated with 20 μg/ml of SV for 6 hr. Total RNA was extracted at indicated time points, and the expression of IFN-β and IL-6 was determined by RT-qPCR.

(C, D) THP-1 macrophages were cultured with serum collected from the 6 healthy subjects for 2 days, and were stimulated with SV for 6 hr. Total RNA was extracted from cells, and the expression of IFN-β (C) and IL-6 (D) were determined by RT-qPCR and normalized to GAPDH. Y axis shows miRNA ratios determined by the microarray analysis, and X axis shows IFN-β or IL-6 mRNA expression. Correlation coefficient (r) was calculated, and statistic analysis was performed (n = 6).