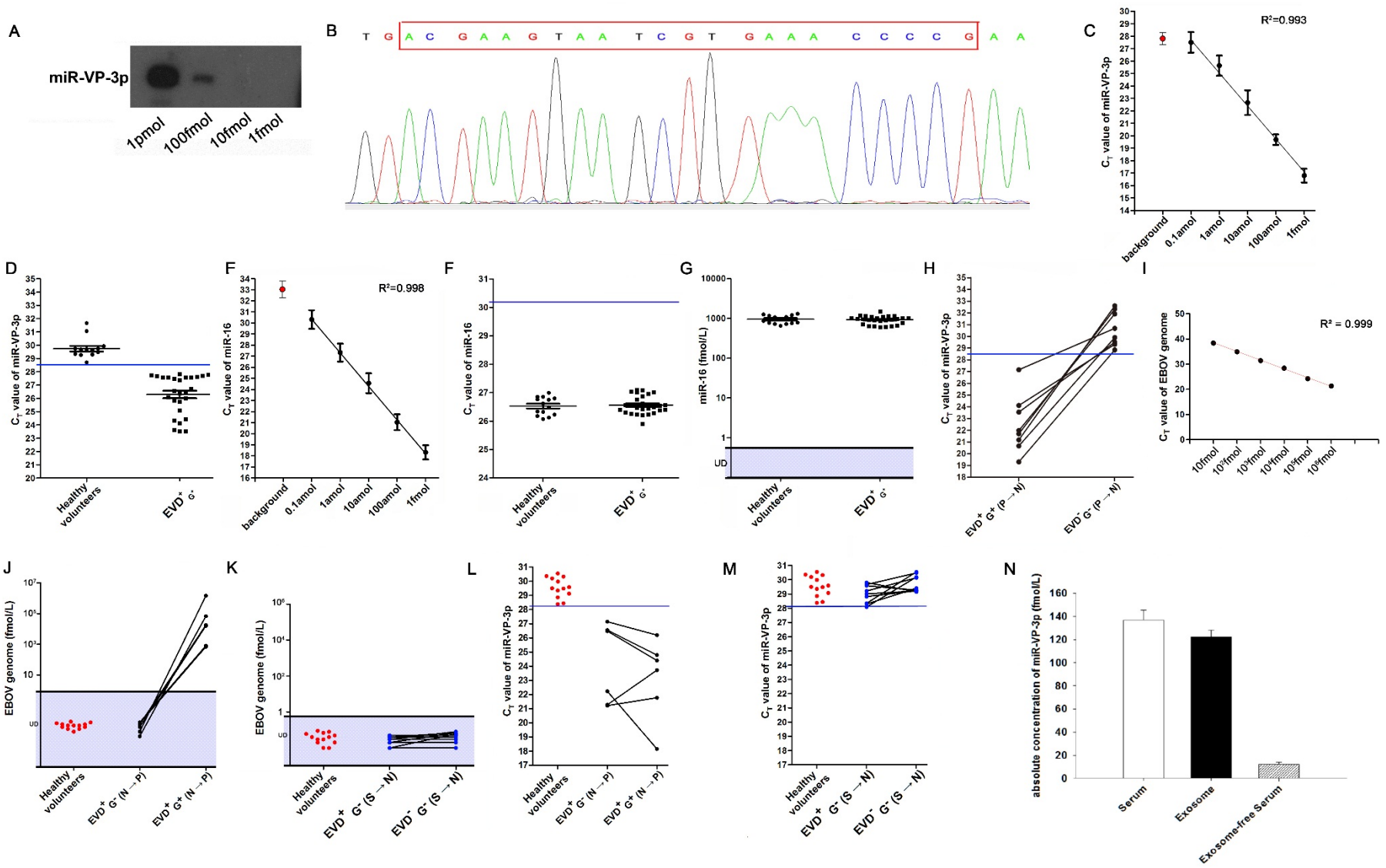
A
miR-VP-3p
1pmol
100fmol
10fmol
1fmol
B
T G A C G A A G T A A T C G T G A A A C C C C G A A
C
R²=0.993
C_T value of miR-VP-3p
background
0.1amol
1amol
10amol
100amol
1fmol
D
C_T value of miR-VP-3p
Healthy volunteers
EVD+ G-
F
R²=0.998
C_T value of miR-16
background
0.1amol
1amol
10amol
100amol
1fmol
F
C_T value of miR-16
Healthy volunteers
EVD+ G-
G
miR-16 (fmol/L)
UD
Healthy volunteers
EVD+ G-
H
C_T value of miR-VP-3p
EVD+ G+ (P→N)
EVD- G- (P→N)
I
R² = 0.999
C_T value of EBOV genome
10fmol
10²fmol
10³fmol
10⁴fmol
10⁵fmol
10⁶fmol
J
EBOV genome (fmol/L)
UD
Healthy volunteers
EVD+ G- (N→P)
EVD+ G+ (N→P)
K
EBOV genome (fmol/L)
UD
Healthy volunteers
EVD+ G- (S→N)
EVD- G- (S→N)
L
C_T value of miR-VP-3p
Healthy volunteers
EVD+ G- (N→P)
EVD+ G+ (N→P)
M
C_T value of miR-VP-3p
Healthy volunteers
EVD+ G- (S→N)
EVD- G- (S→N)
N
absolute concentration of miR-VP-3p (fmol/L)
Serum
Exosome
Exosome-free Serum

Supplementary information, Figure S2 The levels of miR-VP-3p in EVD patients. (A) The dynamic range and sensitivity of Northern blot for measuring putative miR-VP-3p. The synthetic single-stranded miR-VP-3p was serially diluted and assessed via Northern blot. **(B)** TA-cloning and sequencing of amplified PCR products. Small RNA isolated from sera of EVD patients was subjected to RT-PCR to amplify putative EBOV miRNA, and then the amplified product was ligated into a TA-vector and sequenced. **(C)** The dynamic range and sensitivity of the qRT-PCR assay for measuring putative miR-VP-3p. The synthetic single-stranded miR-VP-3p was serially diluted over several orders of magnitude, corresponding to levels ranging from 0.1 attomole to 1 femtomole and was assessed via the qRT-PCR assay. The resulting C_T values were plotted against the amount of input miR-VP-3p to generate a standard curve. Water was used in place of RNA as a no-template control (background) for the qRT-PCR assay. **(D)** The individual C_T values for miR-VP-3p in the serum from healthy volunteers and $EVD^{+}_{G^{+}}$. **(E)** The dynamic range and sensitivity of the qRT-PCR assay for measuring miR-16. The synthetic single-stranded miR-16 was serially diluted over several orders of magnitude, corresponding to levels ranging from 0.1 attomole to 1 femtomole and was assessed via the qRT-PCR assay. The resulting C_T values were plotted against the amount of input miR-16 to generate a standard curve. Water was used in place of RNA as a no-template control (background) for the qRT-PCR assay. **(F)** The individual C_T values for miR-16 in the serum from healthy volunteers and $EVD^{+}_{G^{+}}$. The lower boundary of the dynamic range is indicated by the blue line. **(G)** The concentrations of miR-16 in the serum from healthy volunteers and $EVD^{+}_{G^{+}}$. **(H)** The individual C_T values for miR-VP-3p in the serum from EBOV survivors in the acute phase (denoted as $EVD^{+}_{G^{+}(P\rightarrow N)}$) and the convalescence phase (denoted as $EVD^{-}_{G^{-}(P\rightarrow N)}$). **(I)** Dynamic range and sensitivity of the qRT-PCR assay for measuring EBOV genome. **(J and K)** Prospective qRT-PCR analysis of the EBOV genomic RNA in the serum samples from 15 patients with suspected EVD. These patients exhibited EBOV infection symptoms but tested negative for the EBOV genomic RNA based on RT-PCR. During the prospective observation period, 6 patients (black dots, denoted as $EVD^{+}_{G^{-}(N\rightarrow P)}$ and $EVD^{+}_{G^{+}(N\rightarrow P)}$) were confirmed to be EBOV positive (J); however, the other 9 patients (blue dots, denoted as $EVD^{+}_{G^{-}(S\rightarrow N)}$ and $EVD^{-}_{G^{-}(S\rightarrow N)}$) remained EBOV

negative (K). **(L and M)** The individual C_T values for miR-VP-3p in the serum from 15 patients with suspected EVD. These patients exhibited EBOV infection symptoms but tested negative for the EBOV genome based on RT-PCR at enrollment. During the prospective observation period, 6 patients (black dots, denoted as $EVD^{+}_{G^{-}(N\rightarrow P)}$ and $EVD^{+}_{G^{+}(N\rightarrow P)}$) were confirmed to be EBOV positive; however, the other 9 patients (blue dots, denoted as $EVD^{+}_{G^{-}(S\rightarrow N)}$ and $EVD^{-}_{G^{-}(S\rightarrow N)}$) remained EBOV negative. The lower boundary of the dynamic range is indicated by the blue line. **(N)** Comparison of the levels of miR-VP-3p in the exosome and exosome-free fractions derived from the serum of EVD patients. Exosomes were isolated from 100 μl of serum of EVD patients by using Total Exosome Isolation (from serum) Kit. After isolation, the exosome-free supernatant was transferred to a new tube and was subjected to RNA isolation and qRT-PCR analysis, and the exosomes that were contained in the pellet at the bottom of the tube were resuspended in 100 μl of 1 × PBS and were subjected to RNA isolation and qRT-PCR analysis.