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Supplemental Information

sEVs^{RVG} selectively delivers antiviral siRNA to fetus brain, inhibits ZIKV infection and mitigates ZIKV-induced microcephaly in mouse model

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Supplemental information

Figure S1

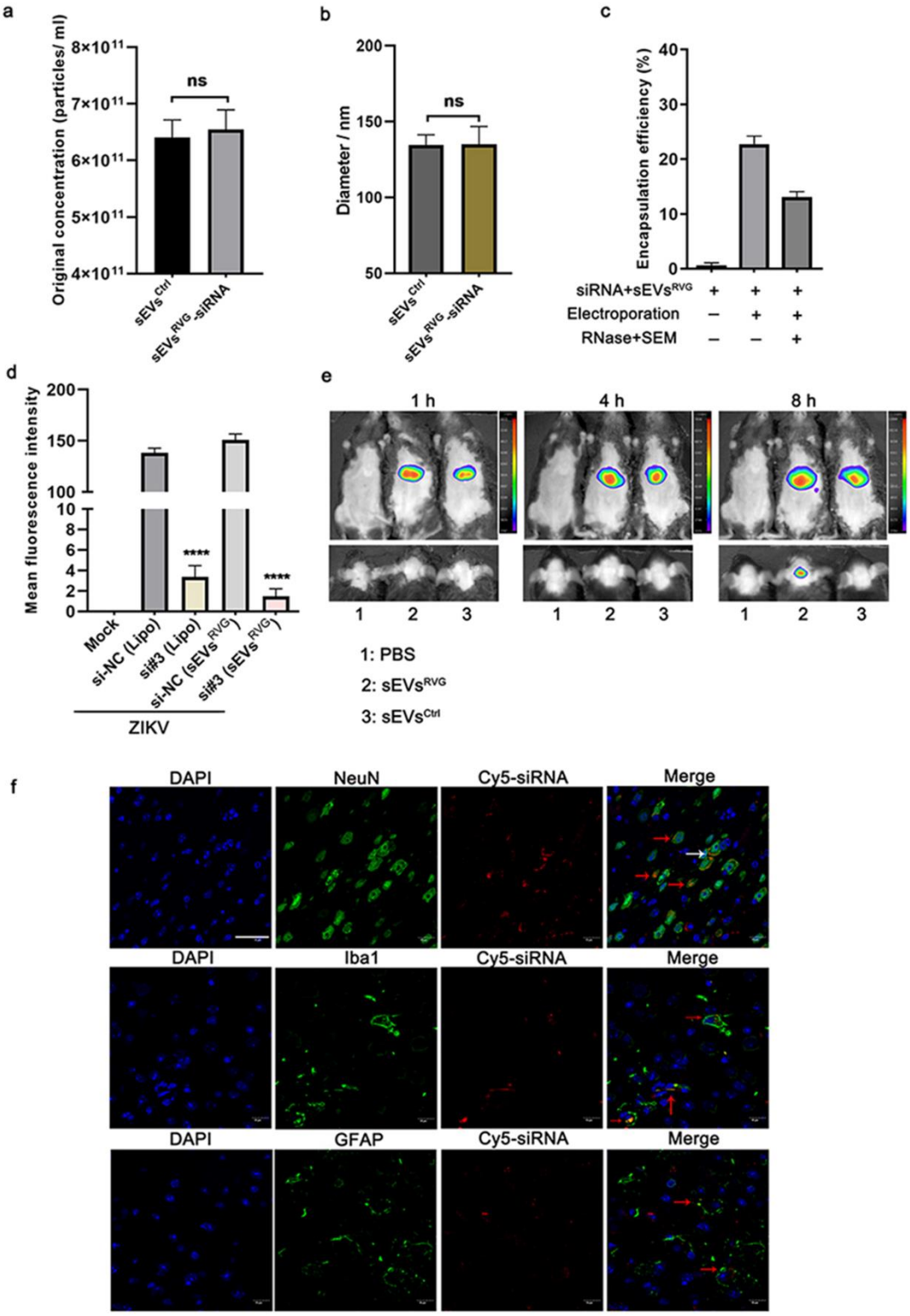

Figure S1 legend:

(a): Statistical analysis of particle concentrations in sEVs^{Ctrl} and sEVs^{RVG}-siRNA groups (ns represent no

significant). (b): Statistical analysis of particle diameter in $sEVs^{Ctrl}$ and $sEVs^{RVG}$-siRNA groups (ns represent no significant). (c): Encapsulation efficiency of siRNA under different treatment conditions (RNase + SEM: RNase treatment and size exclusion chromatography filtration). (d): The mean fluorescence intensity (green) in Figure 2g was measured using Image J. Each group was taken from three random images for statistics (****P < 0.0001). (e): Imaging of sEVs in mice injected with DiR-labeled $sEVs^{Ctrl}$/ $sEVs^{RVG}$ at 1, 4, 8 h. (f): Representative images of cerebral cortex stained with primary antibodies against NeuN (neurons), GFAP (astrocytes) and Iba1 (microglias), a secondary antibody (green), cy5-siRNA (red) (scale bar: 30 μm).

Figure S2

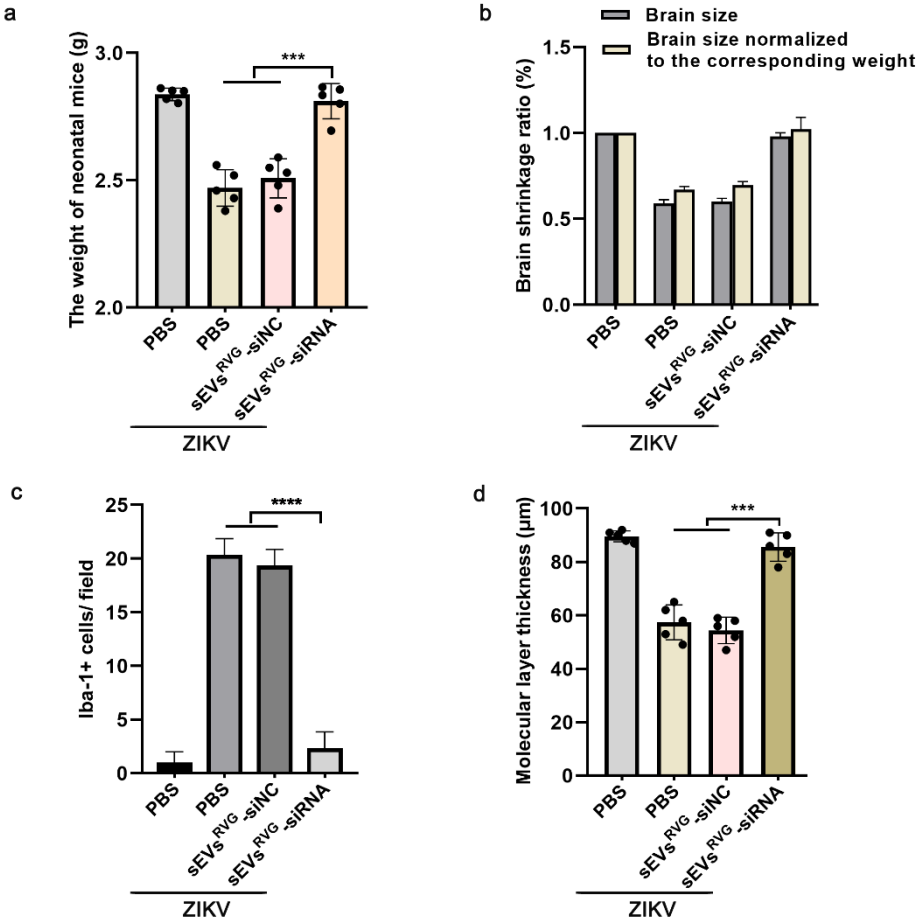

Figure S2 legend:

(a): The weight of neonatal mice (*P5*) from different groups in Figure 6a was measured (***P < 0.001). (b): Brain size of neonates in Figure 6a was calculated based on length * width * height. Brain shrinkage ratio was determined by comparison with the PBS group (grey column), and brain shrinkage ratio was corrected for the weight of each group (yellow column). (c): The number of $Iba1^{+}$ cells per 20

magnification field was calculated, 3 different fields of each group were taken for the statistics (****P < 0.0001). (d): Thickness of molecular layers was measured by Image J (***P < 0.001). The analysis was analyzed using One-way ANOVA with Bonferroni's multiple comparisons test. All the data showed mean ± SD from three experimental replicates.

Figure S3

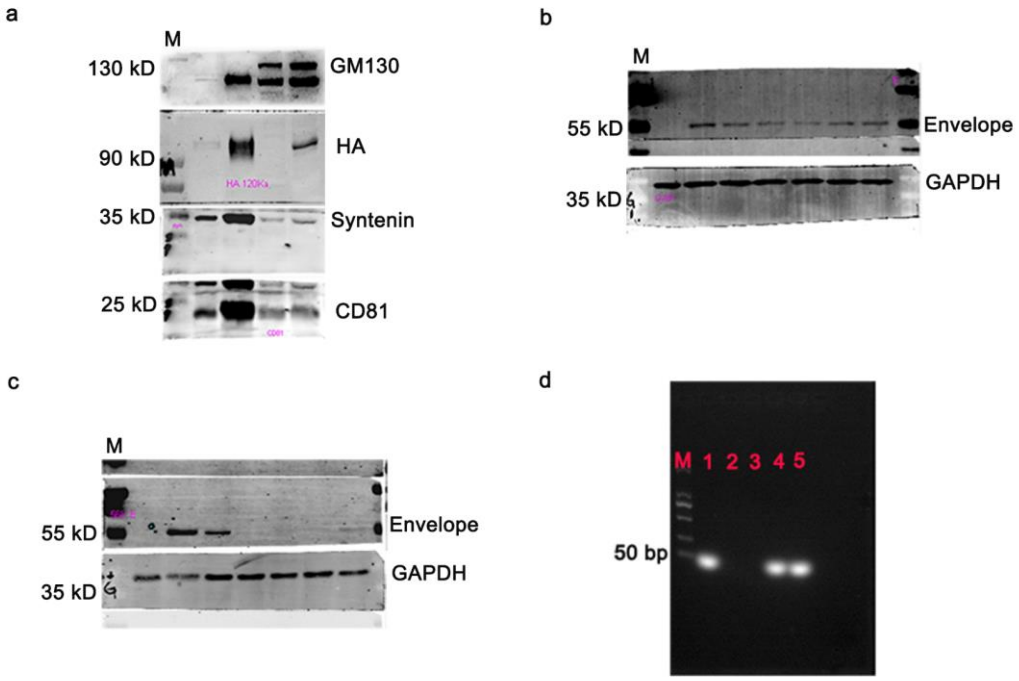

Figure S3 legend:

(a): The original image of Figure 1e. (b- c): Original images of Figure 2f and 2g. (d): The original DNA gel image of Figure 3e.

Supplemental Tables

Table S1: Primers for Q-PCR experiments

Primers	Sequence (5’-3’)
Forward	GAACATGGAGGTTGTGTCAC
Reverse	AGGTAGGCTTCACCTTGTGT

Table S2: Primers required for cloning and confirmation of plasmid

Primers	Sequence (5’-3’)
Forward	GCTAGCCGCAAATGGGCGGTAGGCGTG
Reverse	GGATCCTAGAAGGCACAGTCGAGG

Table S3: Stem-loop-specific reverse primers (RT reaction).

Primers	Sequence (5’-3’)
Reverse_stemloop (si#3)	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGA TACGACAATTGAAA
Forward_si#3	AGCCGGGATCTCCTCTGT
Reverse_stemloop	AGTGCAGGGTCCGAGGTATT