A

# of m6A sites

2 GAAUUCUGGACUAAAGCCGGACUUGUGAUAUCAUG

1 GAAUUCUGGACUAAAGCCGG-CUUGUGAUAUCAUG

0 GAAUUCUGGUCUAAAGCCGG-CUUGUGAUAUCAUG

N GAAUUCCGGTGCCGGTGCGAUAUCAUG

# of m6A site: 2 1 0 N

Protein
GFP
GAPDH

RNA
GFP
GAPDH

B

RSV
ACGAGUCC UGGAC U
UGUUCAGG C A A A G

RSV-mut
ACGAGUCC UGGA U
UGUUUAGG C A A A G

RSVns
UACACUCGAUCUGGACUAAAGCUGCUC

RSVns-mut
UACACUCGAUCUGGAUUAAAGCUGCUC

RSV RSV-mut RSVns RSVns-mut

Protein
GFP
GAPDH

RNA
GFP
GAPDH

Figure S3. Translation of circRNA in different cell types.

(A) Translation of circRNA can be driven by m⁶A motifs in HeLa cells. 0-2 copies of m⁶A motifs (GGACU) were inserted at upstream of AUG in circRNA reporters, and each reporter was transfected into HeLa cells to detect protein production with western blot 48 hours after transfection.

(B) Known m⁶A sites (RSV and RSVns) and their mutations were tested for the activity of driving translation. Experimental procedure is same as panel a.