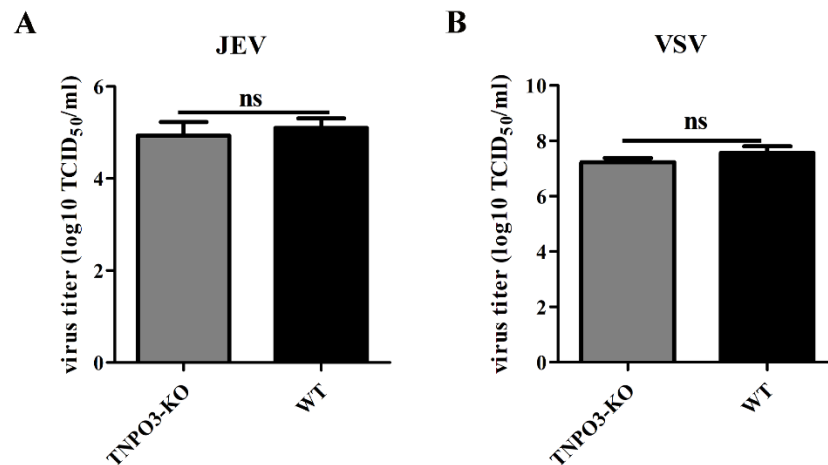


WT    CGCTC**TACCACGACCCAGATCCCAG**<sup>PAM</sup>**CGG**AAAGG  
 TNPO3-KO    CGCTC**TACCACGA**-----**CCCAGCGG**AAAGG    -7bp

**Figure S1.** Sanger sequencing of TNPO3-KO and WT cells. Gene fragments contained the sgRNA aimed at TNPO3 were amplified and proceeded to sanger sequencing. An example target region with CRISPR target sites highlighted in red, and the PAM highlighted in blue.



**Figure S2.** TNPO3 was not required for JEV and VSV replication. (A) TNPO3-KO and WT cells were challenged with JEV at MOI=0.1, and virus titers at 24 hpi were determined by TCID<sub>50</sub>. (B) TNPO3-KO and WT cells were challenged with VSV at MOI=0.01, and virus titers at 24 hpi were determined by TCID<sub>50</sub>. (mean ± SD of three independent experiments; \*P < 0.05; \*\*P < 0.01; \*\*\*P < 0.001; two-tailed Student's t test). hpi, hour post infection; WT, wild-type; KO, knockout.

		Epitope 1	Epitope 2	Epitope 3	
hnRNP A1	272	N <b>FGPM</b> KGGN <b>FGG</b> ---- <b>R</b> -----SSGPY			289
hnRNP D	329	<b>GYGKV</b> - <b>SRRGGHQ</b> -----NSYKPY			355
FUS	507	<b>GPGKMDSRGEHRQDRR</b> -----ERPY			526
Histone H3	11	<b>TGGKAPRKQLATKAARK</b>			27
IAV-M1	16	PS <b>G</b> PLK-----AE <b>I AQRL</b>			28
IAV-M2	32	I I <b>GILHL</b> -----ILW <b>I LDRL</b>			46
		Hydrophobic	Basic		

**Figure S3.** Sequence alignment of known PY-NLSs with the conserved IAV M1 and M2 sequences. Sequences for epitopes 1 (a hydrophobic patch) highlighted in light pink, 2 (a basic patch) highlighted in Blue and 3 (PY) highlighted in Gray.