

Developing an enhanced chimeric permuted intron-exon system for circular RNA therapeutics

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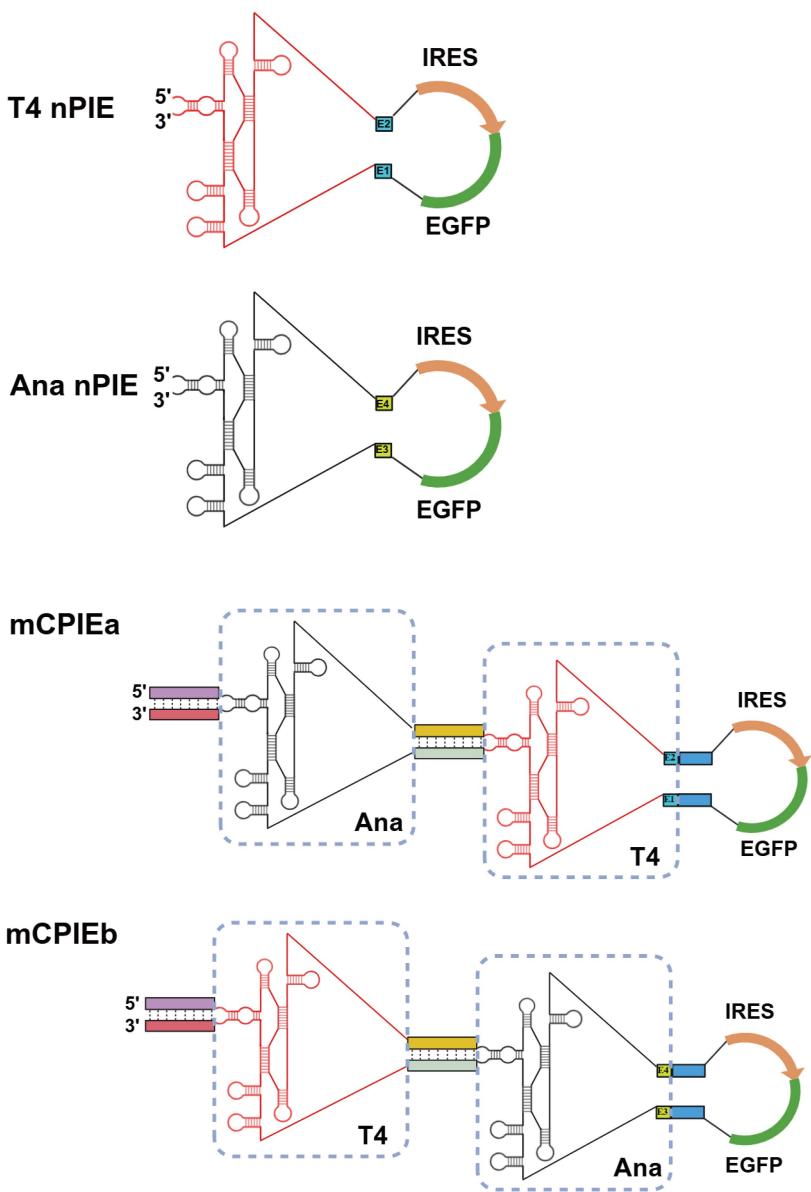


Figure S1. A schematic diagram depicts the components of the T4-nPIE, Ana-nPIE, mCPIEa, and mCPIEb systems.

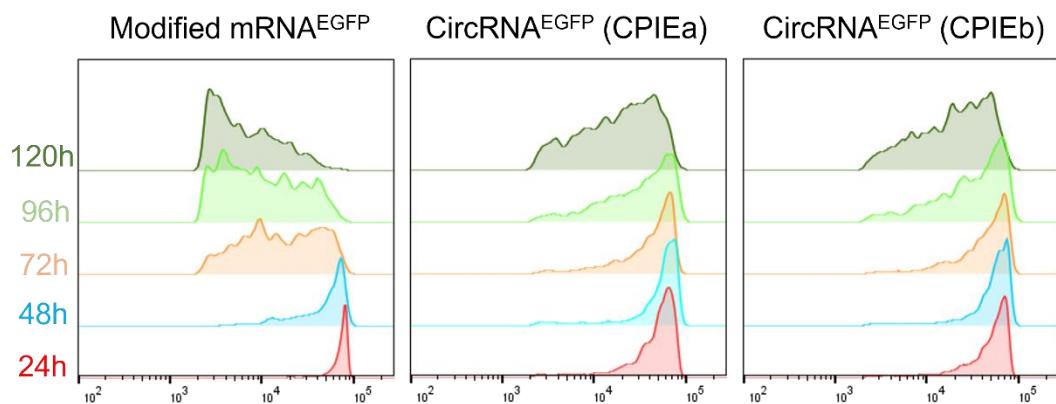


Figure S2. Assessment of EGFP expression in 293F cells transfected with circRNA^{EGFP} (CPIEa), circRNA^{EGFP} (CPIEb) and modified mRNA^{EGFP} over 5 days by flow cytometry.

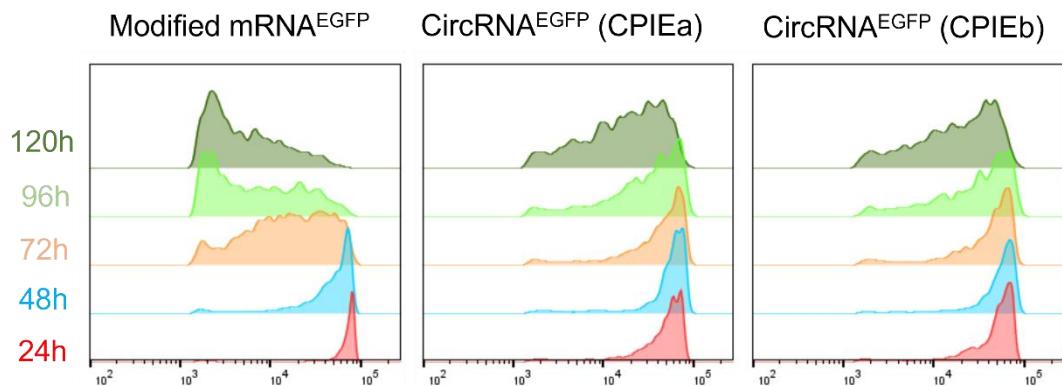


Figure S3. Assessment of EGFP expression in 293T cells transfected with circRNA^{EGFP} (CPIEa), circRNA^{EGFP} (CPIEb) and modified mRNA^{EGFP} over 5 days by flow cytometry.

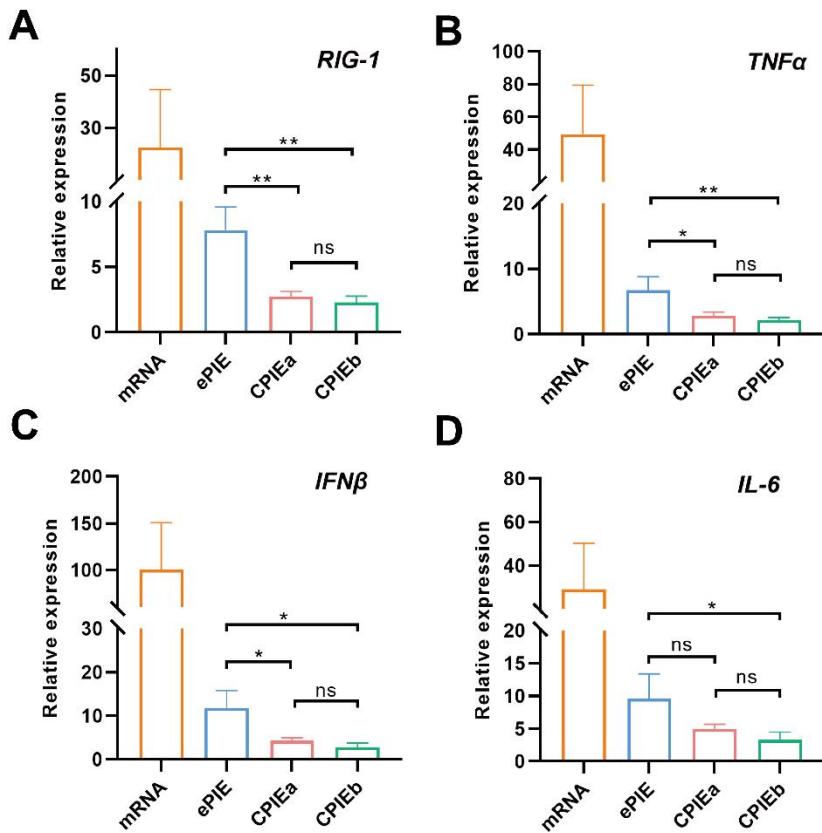


Figure S4. RT-qPCR analysis was performed to evaluate the relative expression of specific genes, *RIG-1* (a), *TNF α* (b), *IFN β* (c) and *IL-6* (d) in A549 cells transfected with ePIE- and CPIE-generated circRNA^{EGFP} at 12 h post-transfection. Notably, the unmodified linear mRNA^{EGFP} served as control. The mRNA expression levels were normalized not only using actin as a reference gene, but also by considering intracellular mRNA or circRNA quantities to account for variations in transfection efficiencies and RNA stability.

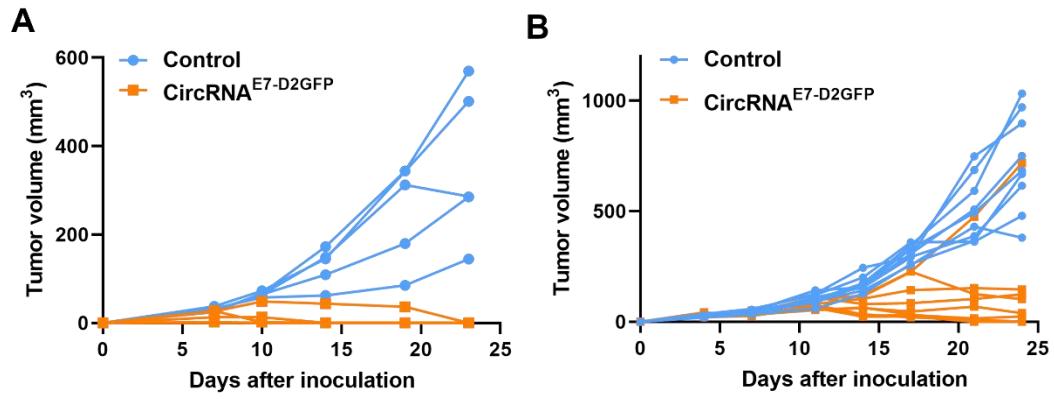


Figure S5. Individual tumor growth curves of the mice from control and vaccinated groups in Figure 6B and Figure 6D.

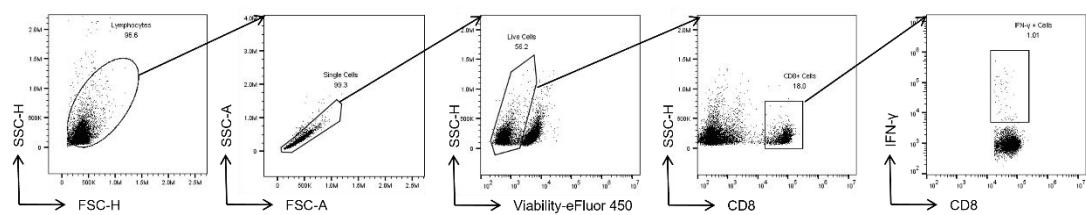


Figure S6. Flow cytometric gating strategies for the identification of $\text{IFN-}\gamma^+\text{CD8}^+$ T cells subset.

Table S1

IVT templete	
CircRNA ^{EGFP} templete (T4 nPIE)	GGTTCTACATAAATGCCAACGACTATCCCTGGGGAGTAGGGTCAAGTGACTCGA AACGATAGACAACCTGCTTAACAAGTGGAGATATAGTCTGCTCTGCATGGTACA TGAGCTGGATATAATTCCGGGGTAAGATTAACGACCTTATGAAACATAATGCTACC GTTAATATTGCGTCATTAAAACAGCCTGTGGTTGATCCCACCCACAGGCCATT GGCGCTAGCACTCTGGTATCACGGTACCTTGTGCGCCTGTTTATACCCCTCCC CCAACGTAACTTAGAAGTAACACACACCGATCAACAGTCAGCGTGGCACACCAG CCACGTTTGATCAAGCACTCTGTTACCCCGACTGAGTATCAATAGACTGCTCAC GCGGTTGAAGGAGAAAGCGTTGTTACCGGCCAACTACTTCGAAAAACCTAGTAA CACCGTGAAGTTGAGGTGTTGCTCAGCACTACCCAGTGTAGATCAGGTCG ATGAGTCACCGCATTCCCCACGGCGACCGTGGCGGTGGCTGCGTTGGCGCCCTGC CCATGGGAAACCCATGGGACGCTTAATACAGACATGGTGAAGAGTCTATTGA GCTAGTTGGTAGTCCTCCGGCCCTGAATGCGGCTAACCTAACTGCGGAGCACAC ACCTCAAGCCAGAGGGCAGTGTGCGTAACGGCAACTCTGCAGCGAACCGAC TACTTGGGTGTCGTGTTCATTTATTCTATACTGGCTGTTATGGTACAATTG AGAGATCGTACCATATAGCTATTGGATTGCCATCCGGTACTAATAGAGCTATTAT ATATCCCTTGTGGTTTATACCACTTAGCTGAAAGAGGTTAAACATTACAATT ATTGTTAAGTTGAATACAGCAAAGCCACCATGGTGAGCAAGGGCAGGGAGCTGTT ACCGGGGTGGTGCCCACCTGGTGAGCTGGACGGCAGCTAACGGCCACAAGT TCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCGTGA GTTCATCTGCACCACCGCAAGCTGCCGTGCCCTGGCCCACCCCTGACCGAC TGACCTACGGCGTGCAGTGTCTCAGCCGTACCCGACCATGAAGCAGCACGAC TTCTCAAGTCCGCCATGCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTCAA GGACGACGGCAACTACAAGACCCGCCAGGTGAAGTTCGAGGGCAGACCCCT GGTGAACCGCATCGAGCTGAAGGGCATCGACTCAAGGAGGACGGCAACATCCTG GGCACAAGCTGGAGTACAACACTACAAGCCACAACGTCTATATCATGGCCGACAA GCAGAAGAACGGCATCAAGGTGAACCTCAAGATCCGCCACAACATCGAGGACGGC AGCGTGCAGCTGCCGACCAACTACCAGCAGAACACCCCCATGGCGACGGCCCCG TGCTGCTGCCGACAACCAACTACCTGAGCACCCAGTCCGCCCTGAGCAAAGACCC CAACGAGAACGGCGATCACATGGCCTGCTGGAGTTGACCGCCGCCGGATC ACTCTCGGCATGGACGAGCTGTACAAGTAAAGATTTCTGGGTTAATTGAGGC CTGAGTATAAGGTGACTTATACTTGTAACTATCTAAACGGGAACCTCTAGTAG ACAACCCGTGCTAAATTGTAGGACT
CircRNA ^{EGFP} templete (Ana nPIE)	TGACTTACAACATAATCGGAAGGTGCAGAGACTCGACGGGAGCTACCTAACGTCAA GACGAGGGTAAAGAGAGAGTCCAATTCTCAAAGCCAATAGGCACTAGCGAAAGCT GCAAGAGAATGAAACCGTTAAACAGCCTGTGGTTGATCCCACCCACAGGCC CATTGGCGCTAGCACTCTGGTATCACGGTACCTTGTGCGCCTGTTTATACCCCT CCCCCAACTGTAACCTAGAAGTAACACACACCGATCAACAGTCAGCGTGGCACACC AGCCACGTTGATCAAGCACTCTGTTACCCCGACTGAGTATCAATAGACTGCTC ACGCGGTTGAAGGAGAAAGCGTTGTTACCGGCCAACTACTTCGAAAAACCTAGT AACACCGTGGAAAGTTGACAGTGTTGCTCAGCACTACCCAGTGTAGATCAGGT CGATGAGTCACCGCATTCCCCACGGCGACCGTGGCGGTGGCTGCGTTGGCGCCCT GCCATGGGAAACCCATGGGACGCTTAATACAGACATGGTGAAGAGTCTATT

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CircRNA ^{EGFP} templete (ePIE)	GGGAGACCCTCGACCGTCGATTGTCCACTGGTCAACAATAGATGACTTACAACATAAT CGGAAGGTGCAGAGACTCGACGGGAGCTACCTAACGTCAAGACGAGGGTAAAGA GAGAGTCCAATTCTCAAAGCCAATAGGCAGTAGCGAAAGCTGCAAGAGAATGAAA ATCCGTTGACCTTAAACGGTCGTGGGTTCAAGTCCCTCACCCCCACGCCGGAA ACGAAATAGCGAAAAAAACAAAAACAAAAACAAAACAAAATTAAAACAGCCT GTGGGTTGATCCCACCCACAGGCCATTGGCGCTAGCACTCTGGTATCACGGTACC TTTGTGCGCCTGTTTATACCCCTCCCCAACGTAACTTAGAAGTAACACACACC GATCAACAGTCAGCGTGGCACACCAGCCACGTTGATCAAGCACTCTGTTACCC CGGACTGAGTATCAATAGACTGCTCACCGGGTGAAGGAGAAAGCGTCGTTATCC GGCCAACTAATCGAAAAACCTAGTAACACCGTGAAGTTGCAAGTGTTGCTC AGCACTACCCAGTGTAGATCAGGTCGATGAGTCACCGCATTCCCCACGGCGACC GTGGCGGTGGCTGCGTGGCGCCTGCCATGGGAAACCCATGGGACGCTCTAAT ACAGACATGGTGCAGAGTCTATTGAGCTAGTTGGTAGTCCTCCGGCCCTGAAT GCGGCTAATCTAACCGGAGCACACACCCCTAACGCCAGAGGGCAGTGTGCGTA ACGGGCAACTCTGCAGCGGAACCGACTACTTGGGTGTCGTGTTCATTTATTCC TATACTGGCTGTTATGGTACAATTGAGAGATCGTTACCATATAGCTATTGGATTGG CCATCCGGTACTAATAGAGCTATTATATCCCTTGTGGGTTATACCACTAGCT TGAAAGAGGTTAAACATTACAATTCTGTTAAGTTGAATACAGCAAAGCCACCAT GGTGAGCAAGGGCGAGGAGCTGTTCACCGGGTGGTGCCATCCTGGTCAGCTG GACGGCGACGTAACCGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATG CCACCTACGGCAAGCTGACCCCTGAAGTTCATCTGCACCACCGCAAGCTGCCGTG CCCTGGCCCACCCCTCGTACCACCCCTGACCTACGGCGTCAGTGCTCAGCCGCTA CCCCGACCACATGAAGCAGCACGACTCTTCAAGTCCGCCATGCCGAAGGCTACG TCCAGGAGCGCACCATCTTCTCAAGGACGACGGCAACTACAAGACCCGCGCCGA GGTGAAGITCGAGGGCGACACCCCTGGTAACCGCATCGAGCTGAAGGGCATCGAC TTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACACTACAAGCC ACAACGTCTATATCATGGCCACAAGCAGAAGAACGGCATCAAGGTGAACCTCAAG ATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGA ACACCCCCATCGCGACGGGGCGTGTGCTGCCGACAACCAACTACCTGAGCACC CAGTCCGCCCTGAGCAAAGACCCCAACGAGAACGGCGATCACATGGCCTGCTGG AGTCGTGACCGCCGCCGGGATCACTCTGGCATGGACGAGCTGTACAAGTAAAAAA AAAAAAAAAAAAAAACGGCTATTATGCGTTACCGGCAGACGCTACGGACTTA ATAATTGAGCCTTAAAGAAGAAATTCTTAAGTGGATGCTCTAAACTCAGGGAA ACCTAAATCTAGTTATAGACAAGGCAATCTGAGCCAAGCCGAAGTAGTAGTAATTAGTA AGACCAAGTGGACAATCGACGGATAACAGCATATCTAG
CircRNA ^{E7-D2GFP} templete (CPIEb)	TTATAATGTGGATGTATATAAGGTTACATATAATGCCAACGACTATCCCTTGGGG GTAGGGTCAAGTGACTCGAAACGATAGACAACCTGCTTAACAAGTTGGAGATATA GTCGCTCTGCATGGTGACATCGCAGCTGGATATAATTCCGGGTAAGATTAACGACC TTATCTGAACATAATGCTACCGTTAATATTGCGTCATTGGATTGGCAGCCAGACTGC TGACTTACAACATAATCGGAAGGTGCAGAGACTCGACGGGAGCTACCTAACGTCAA GACGAGGGTAAAGAGAGAGTCAATTCTAACGCCAACAGGCCATTGGCGCTAGCACTCTGGTAT GCAAGAGAATGAAAATCCGAAAAAAACAAAAACAAAAACAAAACAAAATTAA AACAGCCTGTGGGTTGATCCCACCCACAGGCCATTGGCGCTAGCACTCTGGTAT CACGGTACCTTGTGCGCCTGTTTATACCCCTCCCCAACGTAACTTAGAAGTA

	TCGTTATCCGGCCAACCTACTCGAAAAACCTAGTAACACCGTGGAAAGTTGCAGAGT GTTTCGCTCAGCACTACCCAGTGTAGATCAGGTGATGAGTCACCGCATTCCCCAC GGCGACCGTGGCGGTGGCGCCTGCCCATGGGAAACCCATGGGA CGCTCTAATACAGACATGGTGCAGAGAGTCTATTGAGCTAGTTGGTAGTCCTCCGGC CCCTGAATCGGGCTAACCTAACTCGGGAGCACACACCCTCAAGCCAGAGGGCAGT GTGTCGAACGGCAACTCTGCAGCGAACCGACTACTTGGGTGTCGTGTTCA TTTATTCCCTAACTGGCTGTTATGGTACAATTGAGAGATCGTACCATATAGCTAT TGGATTGCCATCCGGTACTAATAGAGCTATTATATCCCTTGTGGTTATACC ACTTAGCTGAAAGAGGTTAAACATTACAATTGTTAAGTTGAATACAGCAA GCCACCATGATCCACAGCGTGTCCCTGCTGATGTTCCCTGACCCCTACCGAGAGC AGAGTCAGCCCACAGAGTCCATCGTGCCTTCCAATATTACAAACCTGTGTC TTCGGGAAAGTGTCAACGCCACAAGATTGCTAGCGTGTATGCTGGAATAGAAA AAGAATTCCAACCGTGGCTGACTACAGCGTGTGACAACCTCGCTAGCTTCA GCACCTCAAGTGCTATGGGTGAGCCCCACCAAGCTGAACGATCTGCTTAC AATGTGTATGCCAGCTCGTGTAGAGGGATGAGGTCCGGCAGATCGCTCC CGGCCAACCGGCAAGATCGCCGACTACAATTACAAGCTCCCGATGACTTACCG GCTGCGTGTACGCCCTGGAATAGCAACAACCTGGACTCCAAGGTGGCGGCAATTAC AATTACAGATAACAGACTGTTCGGAAGTCCAACCTGAAACCCCTGAGCGGGATAT CTCCACCGAAATCTACCAAGCCGGAGCAAGCCCTGTAACGGGTGGAGGGCTTC AACTGCTACTTCCCTGCAAGAGTACCGCTTCAAGCTGCCACCAACGGCTGGGCTA TCAGCCCTACAGAGTGGTCGTGCTCAGCTCGAGCTGCTCCATGCTCCGCTACCGT GTGCGGCCCAAGAAATCCACCAACCTGGTGAAGAACACAAGCGGGTGCAGCCCACC GAGAGCATCGTCAGATTCCCCAACATACCAACCTGTGCCCCCTCGACGAGGTGTT CAACGCTACAAGATTGCTAGCGTGTACGCGCTGGAATAGAAAAAGAATTAGCAATT GTGTGGCTGATTATAGCGTCTGTACAACCTCGCCCCCTCTCGCCTCAAGTGCTA CGGCGTCTCCCCAACAAAGCTGAACGACCTGTGCTTCACCAACGTGTACGCCGATA GCTTTGTGATCAGAGGCAACGAAGTGAAGCCAATCGCCCCGGCAGACCGGCAA CATTGCCACTACAACACTACAAGCTGCCGACGACTTCACCGGCTGCGTCATCGCTT GGAACAGCAATAAGCTGGACAGCAAGGTGGCGCAACTATAACTACCTCTACAGA CTGTTAGAAAGCAACCTGAAGCCTTCAGACCCACCTACGGCGTGGCTTCAACTGTTACTCCCC TGCGCAGCTACGGCTTCAGACCCACCTACGGCGTGGCTTCAACTGTTACTCCCC GTCGTGCTGCTTCAGACCCACCTACGGCGTGGCTTCAACTGTTACTCCCC AAGCACCAATCTGGTCAAGAACACAAGCATACCCATACCGAGATTAA AAAAAAAAAAACGGACTTAAATAATTGAGCCTAAAGAAGAAATTCTTAAGTGG ATGCTCTCAAACCTACGGAAACCTAAATCTAGTTATAGACAAGGCAACCTGAGCC AAGCCGAAGTAGTAATTAGTAAGTTAGCAGTCTGGCTGCCAATCCAAGATGTTCT TGGGTTAATTGAGGCCTGAGTATAAGGTGACTTACTTGTAACTCTAAACGGGG AACCTCTAGTAGACAATCCCGTGCTAAATTGAGGACTTATATGCGTTCATATTATA G
qPCR primers	
EGFP-qF	CCACATGAAGCAGCACGACTT
EGFP-qR	CGATGTTGTGGCGGATCTTGA
Circ-qF	GATCACTCTCGGCATGGACGA

Circ-qR	GCAGTCTATTGATACTCAGTC
RIG-1-qF	TGTGGCAATGTCATCAAA
RIG-1-qR	GAAGCACTTGCTACCTCTTGC
IL-6-qF	AGACAGCCACTCACCTCTTCAG
IL-6-qR	TTCTGCCAGTGCCTCTTGCTG
TNF α -qF	CTCTTCTGCCTGCTGCACTTG
TNF α -qR	ATGGGCTACAGGCTTGTCACTC
IFN β -qF	CTTGGATTCCCTACAAAGAACAGC
IFN β -qR	TCCTCCTCTGGAAGTGCAGCA
β -actin-qF	CACCATTGGCAATGAGCGGTT
β -actin-qR	AGGTCTTGCGGATGTCCACGT