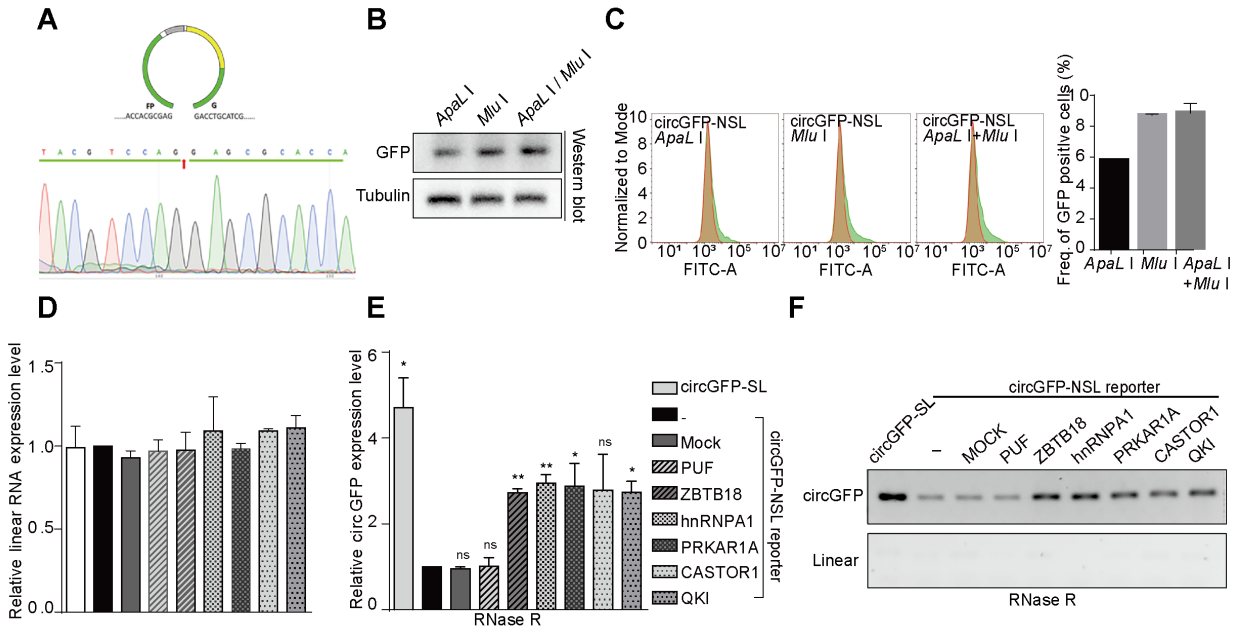
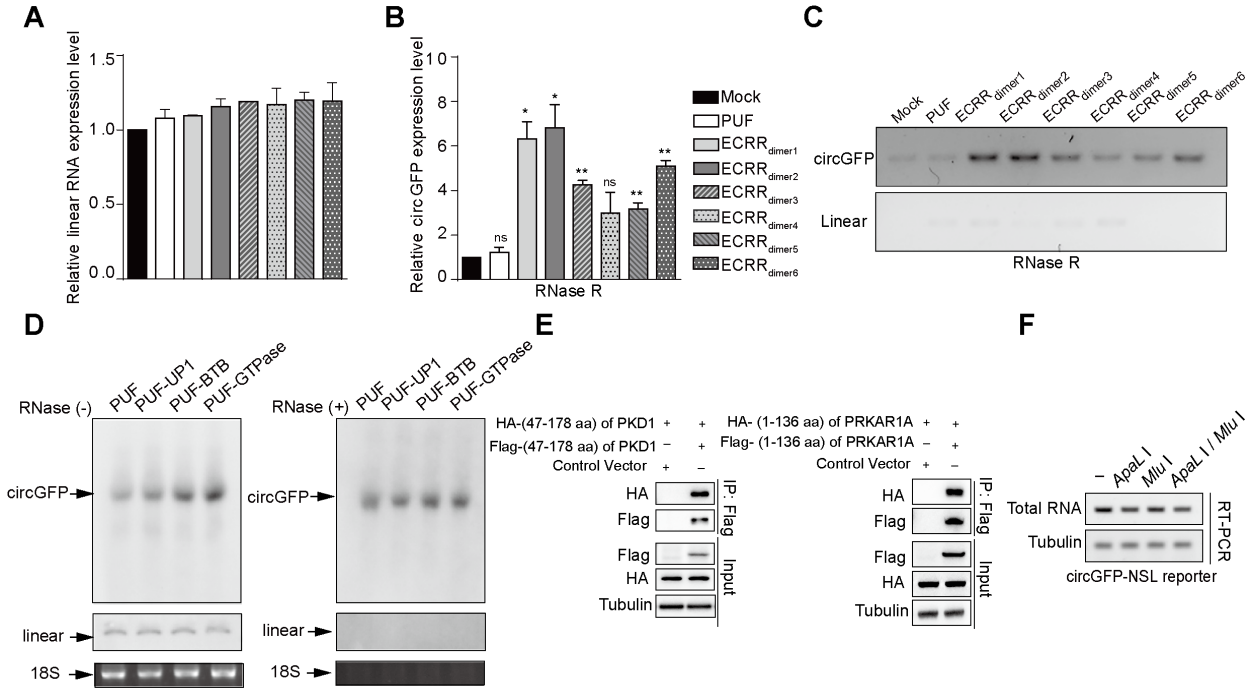
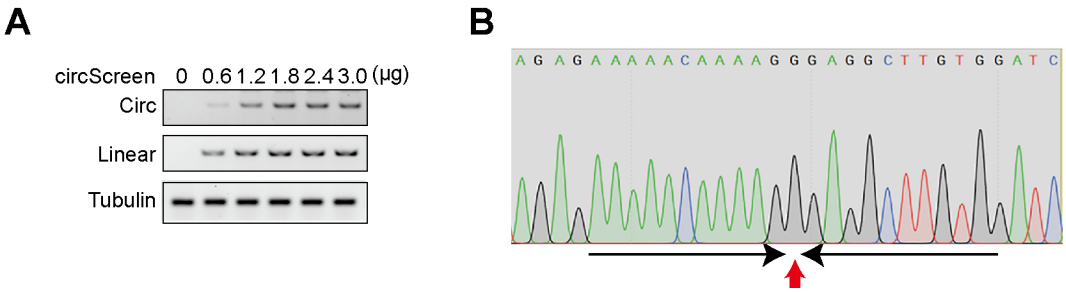



**Supplementary Figure S1.** ECRRs promote the production of circGFP. **(A)** The junction sequences of circGFP were validated with Sanger sequencing. **(B-C)** The circGFP reporter vector has single *ApaL* I and *Mlu* I enzyme sites in the plasmid backbone. The circGFP vector was linearized with *ApaL* I, *Mlu* I, or *ApaL* I/*Mlu* I digestion, and the resulting linearized DNA was transiently transfected into 293T cells to examine GFP expression at 36 h after transfection. The GFP protein was detected with western blot assay (B) and flow cytometry assay (C). **(D)** The gray-scale value of straps densities was quantified by Image J to detect linear RNA abundance from circGFP reporter in Fig1E. Three experiments were carried out with mean +/- S D plotted in each group. **(E-F)** The regulatory effects of different ECRRs on the biogenesis of circGFP in the presence of RNase R were determined using RT-PCR assay. The densities of signals were determined by Image J and three experiments were carried out with mean +/- SD of relative fold change of circGFP expression plotted in (E). The representative gel was shown in (F). * indicates $p < 0.05$, ** indicates $p < 0.01$.

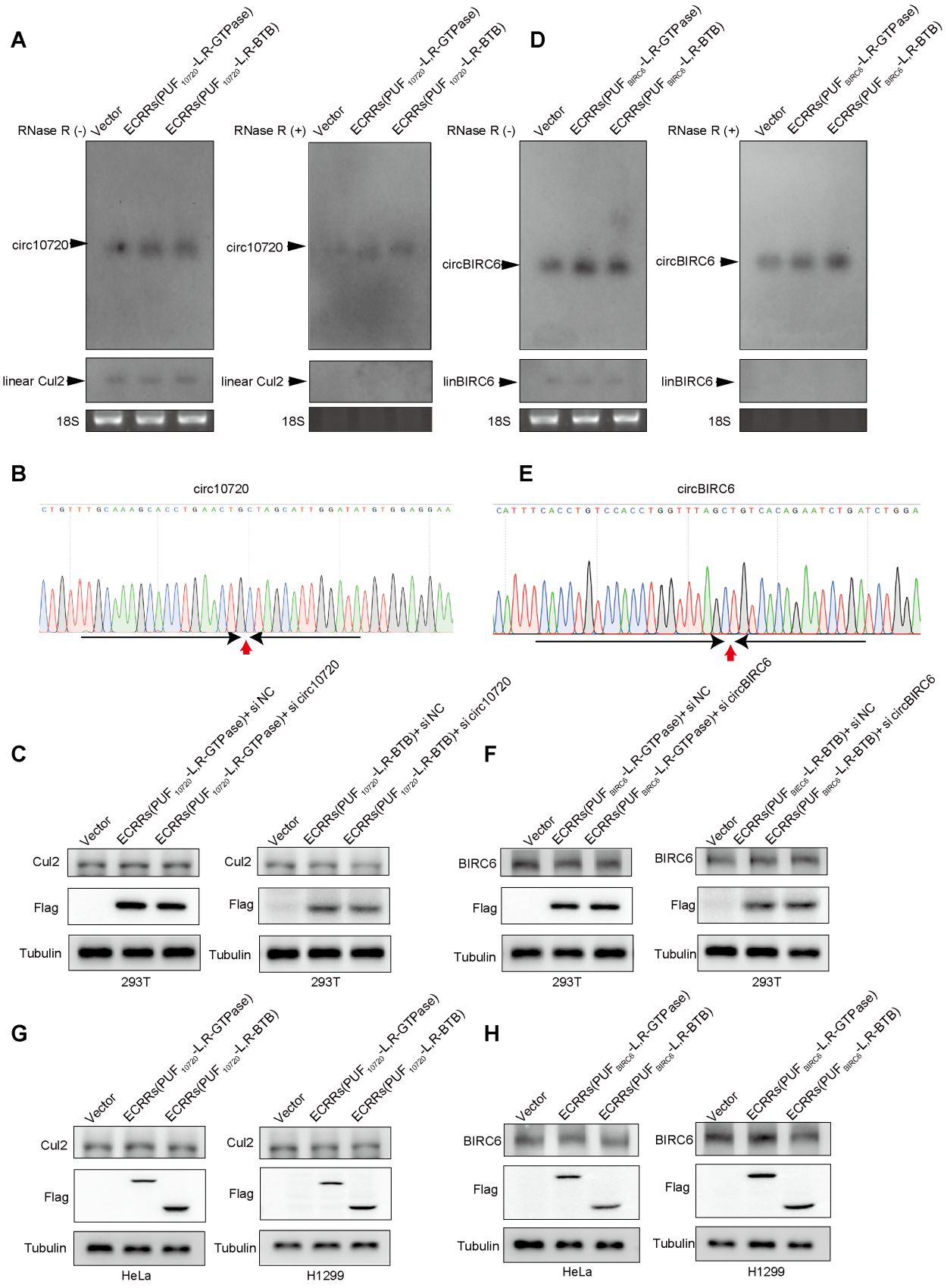



**Supplementary Figure S2.** Dimerization domain containing ECRRs stimulate the generation of circGFP. **(A)** The densities of RT-PCR bands were quantified by Image J to detect linear RNA abundance from circGFP reporter in Fig2B. Three experiments were carried out with mean +/- SD plotted in each group. **(B-C)** The regulatory effects of different ECRRs containing GTPase domain of ATL1, BTB domain of ZBTB18, DH domain of ITSN1, 47-178 aa of PKD1, 1-136 aa of PRKAR1A, and RRM domain of HNRNPA1 in the presence of RNase R on the biogenesis of circGFP were determined using RT-PCR assay. The densities of signals were determined by Image J and three experiments were carried out with mean +/- SD of relative fold change of circGFP expression plotted in (B). The representative gel was shown in (C). * indicates $p < 0.05$, ** indicates $p < 0.01$. **(D)** The circGFP-NSL reporter was transiently co-transfected with different ECRRs or control vector. The production of circGFP and linear RNA was examined with the northern blot assay in the absence of presence of RNase R treatment. **(E)** The dimerization ability of 47-178 aa of PKD1, 1-136 aa of PRKAR1A were examined with a co-

immunoprecipitation assay. **(F)** 1 μg of the linearized plasmids (*ApaL* I or/and *Mlu* I) were transfected into 293T cells in 12-well plates to examine circGFP expression. To determine the circular and linear transfection efficiency, RNAs were isolated from the transfected cells to measure the efficiency by RT-PCR.




**Supplementary Figure S3**. ECRRs function in another circular RNA reporter. **(A)** The production of circular RNA and linear RNA of circScreen reporter was analyzed with the RT-PCR approach. The representative gels were shown. **(B)** The junction sequence of the circScreen reporter was verified with Sanger sequencing.


A
RNase R (-)
Vector
ECRRs(PUF10720-L,R-GTPase)
ECRRs(PUF10720-L,R-BTB)
RNase R (+)
circ10720
linear Cul2
18S
D
circBIRC6
linBIRC6
B
circ10720
E
circBIRC6
C
Cul2
Flag
Tubulin
293T
ECRRs(PUF10720-L,R-GTPase)+ si NC
ECRRs(PUF10720-L,R-GTPase)+ si circ10720
ECRRs(PUF10720-L,R-BTB)+ si NC
ECRRs(PUF10720-L,R-BTB)+ si circ10720
F
BIRC6
ECRRs(PUFBIRC6-L,R-GTPase)+ si NC
ECRRs(PUFBIRC6-L,R-GTPase)+ si circBIRC6
ECRRs(PUFBIRC6-L,R-BTB)+ si NC
ECRRs(PUFBIRC6-L,R-BTB)+ si circBIRC6
G
HeLa
H1299
H

**Supplementary Figure S4.** ECRRs promote the production of endogenous circular RNAs. (**A**) The ECRRs($PUF_{10720}$-L, R-GTPase) or ECRRs($PUF_{10720}$-L, R-BTB) were used to promote the biogenesis of endogenous circ10720. The production of circ10720 and linear Cul2 RNA was examined with a northern blot assay in the absence (left) or presence (right) of RNase R. **(B)** The junction sequences of the endogenous circ10720 were verified with Sanger sequencing. (**C**) The protein levels of Cul2 and exogenously expressed ECRRs in 293T cells were determined with a western blot assay. (**D**) The ECRRs($PUF_{BIRC6}$-L, R-GTPase) or ECRRs($PUF_{BIRC6}$-L, R-BTB) were used to promote the biogenesis of endogenous circBIRC6. The production of circBIRC6 and linear BIRC6 RNA was examined with a northern blot assay in the absence (left) or presence (right) of RNase R. **(E)** The junction sequences of the endogenous circBIRC6 were verified with Sanger sequencing. (**F**) The protein levels of BIRC6 and exogenously expressed ECRRs in 293T cells were determined with a western blot assay. **(G)** The protein levels of Cul2, ECRR($PUF_{10720}$-GTPase) and ECRR($PUF_{10720}$-BTB) in HeLa and H1299 cells were examined with a western blot assay. **(H)** The protein levels of BIRC6, ECRR($PUF_{BIRC6}$-GTPase) and ECRR($PUF_{BIRC6}$-BTB) in HeLa and H1299 cells were examined with a western blot assay.

Supplementary Table S1

| Primer | Name | Sequence | Notes |
|---|---|---|---|
| 1 | Gexon1f | AGTGCTTCAGCCGCTACCC | The primer pair used for testing circRNA from GFP reporter |
| 2 | Gexon3r | GTTGTACTCCAGCTTGTGCC | |
| 3 | Linear-F | ACGTAAACGGCCACAAGTTC | The primer pair used for testing linear RNA from GFP reporter |
| 4 | Linear-R | CTGAGGGCATCTTATTTGGG | |
| 5 | circScreen-F | TGTGAGTTGGATAGTTGTGGAA | The primer pair used for testing circRNA from circScreen reporter |
| 6 | circScreen-R | GGGTGTTTACCTTACGCTTC | |
| 7 | Dendra2-F | CAGAAGAAGACCCTGAAGTGG | The primer pair used for testing linear RNA from circScreen reporter |
| 8 | Dendra2-R | TTGTAGTCGCTGTCGTTGC | |
| 9 | circ-10720-F | ATGCCAACACTATTTGTGGA | The primer pair used for testing for endogenous circ-10720 |
| 10 | circ-10720-R | CCCCATGGATTACTTTCTGGTTTGG | |
| 11 | Cul2-F | GGAATACGTCGAAAGAG | The primer pair used for testing cul2 mRNA |
| 12 | Cul2-R | AGCAAGGGTGCAGACTAT | |
| 13 | si circ-10720 | UAUCCAAUGCUAGCAGUUCAGG | siRNA used for circ10720 knockdown |
| 14 | circBIRC6-F | TCAAGGAGACCAACTTTGGC | The primer pair used for testing endogenous circBIRC6 |
| 15 | circBIRC6-R | CTGGAGTTTGCAGAGCAGTG | |
| 16 | linBIRC6-F | ATCAGGAGACCCAAGCTCAG | The primer pair used for testing for endogenous circBIRC6 |
| 17 | linBIRC6-R | ACTTCCATGGCTTCCTTCTG | |
| 19 | si circBIRC6 | UCAGAUUCUGUGACAGCUAAA | siRNA used for circBIRC6 knockdown |
| 20 | QKI-F | CCGACGCGTACCGGCAACGGCTCTC AGATGGTCGGGGAAATGGAAACG | The primer pair used for cloning Quaking into pCI-neo vector |
| 21 | QKI-R | CACGCGGCCGCTTAGTTGCCGGTGG CGGCTC | |
| 22 | CASTOR1-F | CCGACGCGTACCGGCAACGGCTCTC AGATGGAGCTGCACATCCTAG | The primer pair used for cloning CASTOR1 into pCI-neo vector |
| 23 | CASTOR1-R | CACGCGGCCGCTCAGGAAGCCAGGC CTTCC | |
| 23 | PKD1-F | CCGACGCGTACCGGCAACGGCTCTCA GTGCCGCGTCAACTGCTC | The primer pair used for cloning 47-178 aa of PKD1 into pCI-neo vector |
| 25 | PKD1-R | CACGCGGCCGCTTACTCACCACAGCC ACTGTC | |

| | | | |
|---|---|---|---|
| 26 | PRKAR1A-F | CCGACGCGTACCGGCAACGGCTCTCAGATGGAGTCTGGCAGTACC | The primer pair used for cloning PRKAR1A into pCI-neo vector |
| 27 | PRKAR1A-R | CACGCGGCCGCTCAGACAGACAGTGACAC | |
| 28 | DH-F | CCGACGCGTACCGGCAACGGCTCTCAGAAGCGACAAGGATACATCC | The primer pair used for cloning DH domain of ITSN1 into pCI-neo vector |
| 29 | DH-R | CACGCGGCCGCTTACCCTTCGTTCACCTGG | |
| 30 | ZBTB18-F | CCGACGCGTACCGGCAACGGCTCTCAGATGTGTCCTAAAGGTTATGAAG | The primer pair used for cloning ZBTB18 into pCI-neo vector |
| 31 | ZBTB18-R | CACGCGGCCGCTTATTTCCAAAGTTCTTG | |
| 32 | BTB-F | CCGACGCGTACCGGCAACGGCTCTCAGTGTGACTGCACTGTTCTG | The primer pair used for cloning the BTB domain of ZBTB18 into pCI-neo vector |
| 33 | BTB-R | CACGCGGCCGCTTAGTCTTTGAACTGGAGTTTC | |
| 34 | HNRNPA1-F | CCGACGCGTACCGGCAACGGCTCTCAGATGTCTAAGTCAGAGTCTCC | The primer pair used for cloning HNRNPA1 into pCI-neo vector |
| 35 | HNRNPA1-R | CACGCGGCCGCTTAAAATCTTCTGCCACTGCC | |
| 36 | UP1-R | CACGCGGCCGCTTACTCTTGCTTTGACAGGGCTTTTC | The primer with HNRNPA1-F used for cloning the UP1 domain of HNRNPA1 |
| 37 | GTPase-F | CCGACGCGTACCGGCAACGGCTCTCAGACATATGAATGGAGCTCAGAAG | The primer pair used for cloning the GTPase domain of ATL1 into pCI-neo vector |
| 38 | GTPase-R | CACGCGGCCGCTTAGGTACGAGCTGCATGGAAG | |
| 39 | flagnls-F | CCGCTCGAGCCATGGACTACAAGGACGACGATGACAAGCCCAAGAAAAAGAGGAAG | The primer pair used for cloning FLAG-NLS-PUF into pCI-neo vector |
| 40 | EcoPUF-F | CCCAAGAAAAAGAGGAAGGTAGAATTCGGCCGCAGCCGCCTTTTGGAAG | |
| 41 | PUF-Mlu-R | CCGACGCGTCCCTAAGTCAACACCGTTCTT | |
| 42 | pLVX -F | CCGCCCGGGCCATGGACTACAAGGACGACG | The primer pair with GTPase-R or BTB-R used for clone $ECRR_{10720/\ BIRC6}$ into pLVX-Puro vector. |
| 43 | HAnls-F | ccgCTCGAGccatgTACCCATACGACGTCCCAGACTACGCTCCCAAGAAAAAGAGGAAG | The primer with reverse primers of different dimerized protein or domains used for cloning HA-tag ECRRs into pCI-neo vector. |

| | | | |
|---|---|---|---|
| 44 | T7-circGFP-R | TAATACGACTCACTATAGGGTTACTTGTAC AGCTCGTCC | Northern probes |
| 45 | GFP-probe-F | ATGGTGAGCAAGGGCGAGG | Northern probes |
| 46 | T7-linear-R | TAATACGACTCACTATAGGG GCTGTCCCAGTCCTCTATCC | Northern probes |
| 47 | linear-probe-F | ATCACATCTTTAAGCCTTCCAT | Northern probes |
| 48 | T7-circBIRC6-R | TAATACGACTCACTATAGGGCT GGAGTTTGCAGAGCAGTG | Northern probes |
| 49 | BIRC6-probe-F | TCAAGGAGACCAACTTTGGC | Northern probes |
| 50 | T7-preBIRC6-R | TAATACGACTCACTATAGGGAC CAAGCCTGGCCTAATTCT | Northern probes |
| 51 | T7-cul2-R | TAATACGACTCACTATAGGGGTGGACACA GCACGGAGTAAG | Northern probes |
| 52 | cul2-probe-F | CGTGGTGGAGAAGACCCAAACC | Northern probes |
| 53 | T7-circ10720-R | TAATACGACTCACTATAGGGTGTTTCAGT CAGAAAGGGAGA | Northern probes |
| 54 | circ10720-probe-F | GCCAACACTATTTGTGGAGTC | Northern probes |