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Sample-size estimation

- You should state whether an appropriate sample size was computed when the study was being designed
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- If no explicit power analysis was used, you should describe how you decided what sample (replicate) size (number) to use

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Sample size: This is a genome-wide study, therefore all data that passed their respective thresholds as described in the Methods section was used.

Replicates

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- If you encountered any outliers, you should describe how these were handled
- Criteria for exclusion/inclusion of data should be clearly stated
- High-throughput sequence data should be uploaded before submission, with a private link for reviewers provided (these are available from both GEO and ArrayExpress)

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Our analyses exclusively used publicly available datasets. All IDs of datasets used referenced in Supplementary Table 1. Only RNA-seq samples with sufficient sequencing depth (Supplementary Table 1) and good sequencing quality (assessed by FastQC, see Methods section) were included in this study. We only included physiologically equivalent tissues across species analysed. Only tissues with samples available in majority of species were included in study.

Our study used two background datasets: one biological (species-specific) and one technical (defined as background; see Methods section at Background Datasets). Analyses with these datasets can be found in the Results section and described in Figures 2-4.



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Statistical reporting

- Statistical analysis methods should be described and justified
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- For each experiment, you should identify the statistical tests used, exact values of N, definitions of center, methods of multiple test correction, and dispersion and precision measures (e.g., mean, median, SD, SEM, confidence intervals; and, for the major substantive results, a measure of effect size (e.g., Pearson's r, Cohen's d)
- Report exact p-values wherever possible alongside the summary statistics and 95% confidence intervals. These should be reported for all key questions and not only when the p-value is less than 0.05.

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Non-parametric statistical tests were used to ensure no assumptions were made about underlying distribution of data. All statistical tests used are quoted in text and figure legends alongside p-value. Specific parameters of the statistical tests used can be found in the Methods section. Multiple test corrections was performed when appropriate (See Results and Methods sections).

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Group allocation

- Indicate how samples were allocated into experimental groups (in the case of clinical studies, please specify allocation to treatment method); if randomization was used, please also state if restricted randomization was applied
- Indicate if masking was used during group allocation, data collection and/or data analysis

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N/A – as genome-wide analysis performed

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- We encourage you to upload relevant additional data files, such as numerical data that are represented as a graph in a figure, or as a summary table
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- Include model definition files including the full list of parameters used
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Please indicate the figures or tables for which source data files have been provided:

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Information of the datasets used can be found in the Supplementary Table 1.

We have deposited our code at https://github.com/WeatherittLab/Evolution-ofcircRNAs-in-Primates

R version (4.0.5 --- "Shake and Throw") python 2.7.15 cutadapt 1.18 bedtools v2.29.2 julia version 0.6.4 STAR_2.6.0c_mod1 Whippet v0.11.1 FastQC v0.11.8 samtools 1.3.1 CIRI_simulator (2014-08-08, https://sourceforge.net/projects/ciri/files/) circExplorer2 (2.3.8) CLEAR/circExplorer3 (https://github.com/YangLab/CLEAR) find_circ (1.99) **CIRIquant (1.1.2)** HISAT2 version by Daehwan Kim (www.ccb.jhu.edu/people/infphilo)