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## Mixed-species RNA-seq for elucidating non-cell-autonomous control of gene transcription

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- The exact sample size ( $n$ ) for each experimental group/condition, given as a discrete number and unit of measurement
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*Give  $P$  values as exact values whenever suitable.*
- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- Estimates of effect sizes (e.g. Cohen's  $d$ , Pearson's  $r$ ), indicating how they were calculated
- Clearly defined error bars  
*State explicitly what error bars represent (e.g. SD, SE, CI)*

*Our web collection on [statistics for biologists](#) may be useful.*

### Software and code

Policy information about [availability of computer code](#)

Data collection

No software was used.

Data analysis

We analysed the data in this study using version 1.1 of Sargasso <https://github.com/statbio/Sargasso>, doi:10.5281/zenodo.260123 (using version 2.7.6 of Python <https://www.python.org>), version 2.4.0i of STAR <https://github.com/alexdobin/STAR> and version v0.5.4 of sambamba <https://github.com/biod/sambamba>), and version 1.5.1 of featureCounts <http://subread.sourceforge.net>), version 1.16.1 of DESeq2 <https://bioconductor.org/packages/release/bioc/html/DESeq2.html>), version 0.8.2 of Salmon <https://combine-lab.github.io/salmon/>), and version 1.4.0 of tximport <https://bioconductor.org/packages/release/bioc/html/tximport.html>).

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## Data

Policy information about [availability of data](#)

All manuscripts must include a [data availability statement](#). This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

The RNA-seq data produced and used in the Anticipated Results section of this protocol can be downloaded from the European Nucleotide Archive (accession number E-MTAB-5987). Other publicly-available RNA-seq data sets that were used are downloadable from Gene Expression Omnibus series GSE85839 and ArrayExpress accession E-MTAB-5489.

## Field-specific reporting

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Life sciences       Behavioural & social sciences       Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see [nature.com/authors/policies/ReportingSummary-flat.pdf](https://www.nature.com/authors/policies/ReportingSummary-flat.pdf)

## Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	Example data for the Anticipated Results section was based on n=3, found to be sufficient in our prior publication Hasel et al (2017). Nat Commun.
Data exclusions	No data were excluded
Replication	This is a Protocol paper, experimental finding and their replication is not a facet of the manuscript.
Randomization	All experimental conditions (con vs LPS) were applied to culture material derived from the same animals, therefore the allocation of animals to one or another treatment is not applicable.
Blinding	RNA-seq and analysis of reads was done blind to the experimental condition

## Reporting for specific materials, systems and methods

### Materials & experimental systems

n/a	Involvement in the study
<input checked="" type="checkbox"/>	<input type="checkbox"/> Unique biological materials
<input checked="" type="checkbox"/>	<input type="checkbox"/> Antibodies
<input checked="" type="checkbox"/>	<input type="checkbox"/> Eukaryotic cell lines
<input checked="" type="checkbox"/>	<input type="checkbox"/> Palaeontology
<input type="checkbox"/>	<input checked="" type="checkbox"/> Animals and other organisms
<input checked="" type="checkbox"/>	<input type="checkbox"/> Human research participants

### Methods

n/a	Involvement in the study
<input checked="" type="checkbox"/>	<input type="checkbox"/> ChIP-seq
<input checked="" type="checkbox"/>	<input type="checkbox"/> Flow cytometry
<input checked="" type="checkbox"/>	<input type="checkbox"/> MRI-based neuroimaging

## Animals and other organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research

Laboratory animals	P1 Long Evans rat pups were used of both sexes. E17.5 CD1 mouse embryos were used of both sexes
Wild animals	n/a
Field-collected samples	n/a