# nature portfolio

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# **Reporting Summary**

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our <u>Editorial Policies</u> and the <u>Editorial Policy Checklist</u>.

Please do not complete any field with "not applicable" or n/a. Refer to the help text for what text to use if an item is not relevant to your study. For final submission: please carefully check your responses for accuracy; you will not be able to make changes later.

#### Statistics

For	all st	atistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.
n/a	Cor	ifirmed
	$\mathbf{\nabla}$	The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
		A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
		The statistical test(s) used AND whether they are one- or two-sided Only common tests should be described solely by name; describe more complex techniques in the Methods section.
		A description of all covariates tested
		A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
		A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
		For null hypothesis testing, the test statistic (e.g. <i>F</i> , <i>t</i> , <i>r</i> ) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable</i> .
$\checkmark$		For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
$\checkmark$		For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
$\checkmark$		Estimates of effect sizes (e.g. Cohen's d, Pearson's r), indicating how they were calculated
		Our web collection on <u>statistics for biologists</u> contains articles on many of the points above.

## Software and code

Policy information about availability of computer code

Data collection Skanlt Software 6.1.1.7; LAS X Life Science Microscope Software Lite 4.0; EnSpire; Primer-BLAST

Data analysis

GraphPad Prism 8.2.1; Skanlt Software 6.1.1.7; SPSS 21.0; LAS X Life Science Microscope Software

For manuscripts utilizing custom algorithms of software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

#### 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 description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

Raw data are available in the Data Source fille, associated to this paper

## Research involving human participants, their data, or biological material

Policy information about studies with <u>human participants or human data</u>. See also policy information about <u>sex, gender (identity/presentation)</u>, <u>and sexual orientation</u> and <u>race, ethnicity and racism</u>.

Reporting on sex and gender	Not applicable
Reporting on race, ethnicity, or other socially relevant groupings	Not applicable
Population characteristics	Not applicable
Recruitment	Not applicable
Ethics oversight	Not applicable

Note that full information on the approval of the study protocol must also be provided in the manuscript.

# Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

For a reference copy of the document with all sections, see <u>nature.com/documents/nr-reporting-summary-flat.pdf</u>

## Life sciences study design

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

Sample size	Chickens, n=100, divided into 4 groups, 25 animals each (determined by Power Analysis)
Data exclusions	No data were excluded (all obtained results were appopriate for analyses)
Replication	In laboratory experiments, at least 3 biological repeats (independent experiments) were conducted.
Randomization	Animals in the group were of the same age, and they were randomly ascribed to each experimental group
Blinding	Different persons collected the samples, coded them, and provided for analysis by other persons. Then, the results were de-coded

# Behavioural & social sciences study design

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

Study description	Not applicable
Research sample	Not applicable
Sampling strategy	Not applicable
Data collection	Not applicable
Timing	Not applicable
Data exclusions	Not applicable
Non-participation	Not applicable
Randomization	Not applicable

# Ecological, evolutionary & environmental sciences study design

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

Study description	Not applicable	
Research sample	Not applicable	
Sampling strategy	Not applicable	
Data collection	Not applicable	
Timing and spatial scale	Not applicable	
Data exclusions	Not applicable	
Reproducibility	Not applicable	
Randomization	Not applicable	
Blinding	Not applicable	
Did the study involve field work? Yes No		

## Field work, collection and transport

Field conditions	Not applicable
Location	Not applicable
Access & import/export	Not applicable
Disturbance	Not applicable

# Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems	Methods	
n/a Involved in the study	n/a Involved in the study	
Antibodies	ChIP-seq	
Eukaryotic cell lines	Flow cytometry	
Palaeontology and archaeology	MRI-based neuroimaging	
Animals and other organisms		
Clinical data		
Dual use research of concern		
Plants		

#### Antibodies

Antibodies were purchesed from companies listed in the Validation section (below), with catalogue numbers indicated. Antibodies used Certificate of quality from Shanghai Coon koon Biotech Co. Ltd. is attached. Validation by calibration curves with the target antigen is also attached. For ELISA, the commercial reagent kits were used (Shanghai Coon Koon Biotech Co., Ltd., Shanghai, China; Wuhan Xinqidi Biological Tec hnology Co., Ltd, Wuhan, China; Enlibio, Wuhan, China; Cell Signaling Technology, Massachuse tts, USA; Elabscience, Texas, USA; and MyBiosource, San Diego, USA). For determination of levels of TLR4 (#EIA06452Ch), IFN $\beta$  (#CK-bio-27460), TNF $\alpha$  (#CK-bio-18240), TBKBP1 (#CK-bio-22537), cGAS (#CK-bio-27451), IKKe (CK-bio-22816), IF116 (#CK-bio-22673), IRF3 (#CK-bio-22057), phospho-IRF3 (Ser 396) (#CK-bio-29556), phospho-IRF7 (Ser 477) (#CK-bio-22459), and TANK (#CK-bio-25271), a 1:1 dilution was used. For determination of levels of IRAK1 (#CK-bio-27453), IRAK4 (#CK-bio-27452), MyD88 (#CK-bio-27461), DDX41 (#CK-bio-22537), IRF7 (#CK-bio-26639), NAP1 (#CK-bio-26749), cGMP (#CK-bio-24454), IFNα (#CK-bio-18168), NF-кВ (#CK-bio-22695), phospho- NF-кВ (S932) (#CK-bio-29204), phospho-TBK1 (Ser172) (#CK-bio-29739), TBK1 (#CK-bio-28646), TLR9 (#CK-bio-23737), TMEM 173/STING (#CK-bio-23792), TLR6 (#CK-bio-23254), TLR3 (#CK-bio-23730), and TRAF6 (#CK-bio-26795 samples were not diluted. For dsRNA, anti-dsRNA clone rJ2 antibody (Sigma-Aldrich, #MABE1134) at the 1:100 dilution, and secondary Goat Anti-Mouse IgG (H+L), Highly Cross-Adsorbed antibody (Biotium, #20231-1) (1:500 ratio)

## Eukaryotic cell lines

Policy information about <u>cell lines and Sex and Gender in Research</u>		
Cell line source(s)	The DT40 line of chicken lymphoblasts was purchased from American Type Culture Collection (AT CC)	
Authentication	ATTCC guarantees the authenticy of the cell line; cells were checked for morphology and Western-blotting profile for TLR2.1, TLR2.2 and TLR4.	
Mycoplasma contamination	We confirm that the cell line was tested negatively for mycoplasma contamination	
Commonly misidentified lines (See <u>ICLAC</u> register)	We confirm that no commonly misidentified cell line was used in the study	

## Palaeontology and Archaeology

Specimen provenance	Not applicable	
Specimen deposition	Not applicable	
Dating methods	Not applicable	
Tick this box to confirm that the raw and calibrated dates are available in the paper or in Supplementary Information.		
Ethics oversight Not applicable		

Note that full information on the approval of the study protocol must also be provided in the manuscript.

## Animals and other research organisms

Policy information about studies involving animals; <u>ARRIVE guidelines</u> recommended for reporting animal research, and <u>Sex and Gender in</u> <u>Research</u>

Laboratory animals	Chickens (Gallus gallus domesticus), wild-type, age of 7 days, delivered by the breeder (registration no. PL28036602). Mice (Mus musculus), line C57BL/6J, age of 9 months, purchased from the Tri-City Central Animal Laboratory, Gdansk, Pola
Wild animals	The study did not involve wild animals.
Reporting on sex	Both males and females were used in experiments. No differences in results related to sex were noted.
Field-collected samples	The study did not involve samples collected in the field
Ethics oversight	Local Ethical Committee for Experimental Animals in Olsztyn, Poland (permission no. 62/2019); Local Ethics Committee for Experimental Animals in Bydgoszcz (Poland) (permission no. 02/2022).

Note that full information on the approval of the study protocol must also be provided in the manuscript.

## Clinical data

 Policy information about clinical studies

 All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

 Clinical trial registration
 Not applicable

 Study protocol
 Not applicable

 Data collection
 Not applicable

 Outcomes
 Not applicable

## Dual use research of concern

Policy information about dual use research of concern

#### Hazards

Could the accidental, deliberate or reckless misuse of agents or technologies generated in the work, or the application of information presented in the manuscript, pose a threat to:

No	Yes
$\checkmark$	Public health
$\checkmark$	National security
$\checkmark$	Crops and/or livestock
$\checkmark$	Ecosystems
$\checkmark$	Any other significant area

#### Experiments of concern

Does the work involve any of these experiments of concern:

No	Yes
$\checkmark$	Demonstrate how to render a vaccine ineffective
$\checkmark$	Confer resistance to therapeutically useful antibiotics or antiviral agents
$\checkmark$	Enhance the virulence of a pathogen or render a nonpathogen virulent
$\checkmark$	Increase transmissibility of a pathogen
$\checkmark$	Alter the host range of a pathogen
$\checkmark$	Enable evasion of diagnostic/detection modalities
$\checkmark$	Enable the weaponization of a biological agent or toxin
$\checkmark$	Any other potentially harmful combination of experiments and agents

#### Plants

Seed stocks	Not applicable
Noval plant gapatypas	
Novel plant genotypes	Not applicable
Authentication	Not applicable

## ChIP-seq

#### Data deposition

Confirm that both raw and final processed data have been deposited in a public database such as GEO.

Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

Data access links May remain private before publication.	Not applicable
Files in database submission	Not applicable
Genome browser session (e.g. <u>UCSC</u> )	Not applicable

#### Methodology

Replicates	Not applicable
Sequencing depth	Not applicable
Antibodies	Not applicable
Peak calling parameters	Not applicable
Data quality	Not applicable

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Not applicable

## Flow Cytometry

#### Plots

Confirm that:

The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

All plots are contour plots with outliers or pseudocolor plots.

A numerical value for number of cells or percentage (with statistics) is provided.

#### Methodology

Sample preparation	Not applicable
Instrument	Not applicable
Software	Not applicable
Cell population abundance	Not applicable
Gating strategy	Not applicable

Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.

#### Magnetic resonance imaging

#### Experimental design

Design type	Not applicable
Design specifications	Not applicable
Behavioral performance measures	Not applicable
Imaging type(s)	Not applicable
Field strength	Not applicable
0	
Sequence & imaging parameters	Not applicable
A 6	
Area of acquisition	Not applicable
Diffusion MRI Used	Not used

## Preprocessing

Preprocessing software	Not applicable
Normalization	Not applicable
Normalization template	Not applicable
Noise and artifact removal	Not applicable
Volume censoring	Not applicable

#### Statistical modeling & inference

Model type and settings	Not applicable
Effect(s) tested	Not applicable

Specify type of analysis: 🗌 Whole brain 🗌 ROI-based 🗌 Both		
Statistic type for inference Not applicable		
(See <u>Eklund et al. 2016</u> )		
Correction	Not applicable	
Models & analysis		
n/a Involved in the study		
Functional and/or effective connectivity		
Graph analysis		
Multivariate modeling or pre	dictive analysis	
Functional and/or effective connection	ctivity Not applicable	
Graph analysis	Not applicable	
Multivariate modeling and predicti	ive analysis Not applicable	

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