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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>.

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For	all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.
n/a	Confirmed
	\square 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
\boxtimes	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
\boxtimes	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>
\boxtimes	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
\boxtimes	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
\boxtimes	Estimates of effect sizes (e.g. Cohen's <i>d</i> , Pearson's <i>r</i>), 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 This study does not use any computer software codes for data collection

Data analysis

Statistical analysis of each data were performed using Graphpad Prism (version 9.0) software. Flow cytometry data were analyzed by FlowJo software (version 10.8.0). Chromatography data were analyzed by Waters Empower (version 3.0) software

For manuscripts utilizing custom algorithms or 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 <u>data availability statement</u>. 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 <u>policy</u>

All data generated during preparatoin of this manuscript are available from the corresponding author on request.

Research	involving	human r	participants.	their data.	or biological	material
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Policy information about and sexual orientation		ith <u>human participants or human data</u> . See also policy information about <u>sex, gender (identity/presentation),</u> <u>hnicity and racism</u> .	
Reporting on sex and gender n/a		n/a	
Reporting on race, ethnicity, or other socially relevant groupings		n/a	
Population characte	eristics	n/a	
Recruitment		n/a	
Ethics oversight n/a		n/a	
Note that full informatio	n on the appro	val of the study protocol must also be provided in the manuscript.	
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Field-spec		·	
	below that is	the best fit for your research. If you are not sure, read the appropriate sections before making your selection.	
∑ Life sciences	☐ Be	ehavioural & social sciences	
For a reference copy of the	document with a	Il sections, see <u>nature.com/documents/nr-reporting-summary-flat.pdf</u>	
Life scienc	ces stu	dy design	
All studies must disclo	ose on these p	points even when the disclosure is negative.	
Sample size Th	he size of samp	ble was determined base on the former references in which similar experiments were performed	
Data exclusions Th	There are no data exclusions for analysis		
Replication	All experiments are replicated for statistical analysis. The replication number were indicated in the main text, figures and methods section		
	For sample acquisition and analysis from chicken serum and eggs, more than three chickens and eggs at each age were randomly selected. For in vivo analysis, animals were randomly assigned for the different treatment group		
so	For experiments of producing genome edited chickens, researchers are not blinded but accumulation of foreign protein in eggs was confirmed so that we did not expect any errors in producing genome edited chickens. For N-glycosylation pattern and SPR analysis, we send samples to independent laboratory out of the university so that we did not expect any bias in data interpretation. For in vivo study, two researchers conduct experiments independently and collected data. In interpreting in vivo data, investigators were not blinded to group allocation		
We require information	from authors a	bout some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.	
Materials & expe	Materials & experimental systems Methods		
n/a Involved in the s	study	n/a Involved in the study	
Antibodies		ChIP-seq	
Eukaryotic cell lines		Flow cytometry	
Palaeontology	Palaeontology and archaeology MRI-based neuroimaging		
_ _	Animals and other organisms		
Clinical data			
Dual use resea	Dual use research of concern		
Plants			
Antihodies			

Antibodies used

goat-anti human IgG primary antibody (10319, Alpha diagnostic), horseradish peroxidase (HRP)-conjugated donkey anti-goat IgG

Antibodies used secondary antibody (sc-2020, Santa Cruz Biotechnology), Sambucus Nigra Lectin (SNA, EBL), Biotinylated (B-1305-2, Vector

Laboratories), Maackia Amurensis Lectin II (MAL II), Biotinylated (B-1265-1, Vector Laboratories), horseradish peroxidase-conjugated

avidin (VECTASTAIN ABC kit, Vector Laboratories)

Validation All primary and secondary antibodies and lectins used in this study are commercially available and validated by manufacturers

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s) Chicken primordial germ cells, WIL2-S, Raji , Jurkat T cell

Authentication Chicken primordial germ cell lines are established in our laboratory. WIL2-S, Raji and Jurkat T cells are provided by ADCC and

ADCP reporter bioassay kits (PROMEGA)

Mycoplasma contamination We confirmed that all cells used in this manuscript are free of mycoplasma contamination

Commonly misidentified lines (See ICLAC register)

There are no commonly misidentified cell lines used in this study

Animals and other research organisms

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

Laboratory animals C57BL/6 mouse, Female, 8 weeks of age

White Leghorn chickens, Both sexes, 12~40 weeks after hatch

Wild animals Wild animals were not involved in this study

Reporting on sex Female mice were used for in vivo experiments. Analysis of recombinant Fc was performed from both sexes of chicken

Field-collected samples Field-collected samples were not involved in this study

Ethics oversight The management and experimental uses of animals were approved by the Institutional Animal Care and Use Committee (IACUC),

Seoul National University

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

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 Spleen of mouse in each experimental groups were extracted after 6 hour of sample treatment. Spleens were dissociated

into single cells by using Trypsin-EDTA. Dissociated spleen cells were immunostained using a mouse Treg detection kit (130-120-674, Miltenyi Biotec). After that, the spleen cells were stained with APC labeled anti mouse CD25 antibody and Vio-Blue-labeled CD4 antibody for 30 min under refrigeration. Cells were then washed with PBS, permeabilized, and fixed using a fixation/permeabilization solution for 30 min in dark under refrigeration. After washing twice, the cells were treated with PE-

labeled anti-mouse Foxp3 antibody for 30 min in dark under refrigeration

Instrument Flow Cytometer (BD bioscience)

Software FlowJo (version 10.8.0) Software (Tree Star)

Cell population abundance More than 10,000 cells were counted per sample. No sorting was conducted

Gating strategy

Analysis was performed with FSC/SSC gating to remove debris and dead cells. To remove doublets and other aggregated particels, single cell gating was performed by FSC-A x FSC-H parameter. Gating was determined using unstained control and single-stained controls (CD4 and FOXP3) to set up flow cytometer compensatio and quadrants.

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